Design and Investigation of a Four Mirror Scan Engine Incorporating Parabolic Reflectors

Galiya Sharafutdinova
M. Sc.(Opt.and Spectr.), B.Sc (Phys.)

A thesis submitted for the degree of Doctor of Philosophy in the School of Mathematical and Physical Science
University of Newcastle, AUSTRALIA
July, 2010
This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to this copy of my theses, when deposited in the University Library, being made available for loan and photocopying subject to the provisions of the Copyright Act 1968.

(Signed)__________________________________________
Galiya Sharafutdinova
Acknowledgement of Authorship

I hereby certify that the work embodied in this thesis contains a published papers work of which I am a joint author and which are attached to this thesis.

(Signed) ______________________________________

Galiya Sharafutdinova
To the memory of my parents
and to the future of my daughter
Acknowledgements

This research would have not been completed without support from many people. I thank the University of Newcastle for providing me with scholarship support. I would like to thank the Physics academic staff for assistance.

I wish to express my sincere gratitude to my supervisors, Assoc. Prof. Dirk van Helden and Dr. John Holdsworth, for their continuous support, patience and encouragement through my PhD study. Their extensive discussions about my work and the way through this research, detailed review of drafts and excellent advice helped me to complete this thesis. I would like to thank my principal supervisor, John Holdsworth, for the motivation, inspiration and passion during this time.

I wish to express my warm and sincere thanks to Jim Cleary for his time and the sharing of his great knowledge and experience in Physics. It is beneficial for students when people like him are around.

Thanks to Drs. Ponomarenko, Alena and Pasha, who had guided me toward Physics at the University of Newcastle, and assisted during my acclimatisation.

I wish to thank the Physics staff, John Pearson, George Piszczuk, John Foster and Michael Cvetanovski, who contributed greatly by encouragement, advice in laboratory, friendly assistance and humour which saved me during this journey. Additional thanks to John Foster who found time for proof reading.

I thank Phil Greig and Ben Guest who manufactured components for my experiments.

I am grateful to my Russian friends in Newcastle, who have been a source of fantastic support and friendship through some very stressful and difficult times.

A special mention is to my family. I thank my wonderful sister Sania and her family, for providing emotional support and care. I thank my family in Russia who support from afar. I thank my husband’s relatives in Australia for involving us in their family life and creating a sense of warmth and respect.

Last of all, I would like to thank the most important people in my life, my husband Ilia and my daughter Liliya, for their love, support and understanding through this time. Without their encouragement it would be impossible for me to finish this work.
List of Abbreviations

2D - Two-Dimension
3D – Three Dimension
CCD – Charge-Coupled Device
DOE - Diffractive Optical Element
EMCCD - Electron Multiplying Charge-Coupled Device
FWHM – Full Width at Half Maximum
GVD - Group Velocity Dispersion
MMM - Multi-Photon Multi-Beam Microscopy
MPE - Multi-Photon Excitation
MPM - Multi-Photon Microscopy
MTF - Modulation Transfer Function
NA - Numerical Aperture
NIR - Near-Infrared
OPE - One-Photon Excitation
OSLO - Optical Software for Layout and Optimization
OTF - Optical-Transfer Function
PSE - Parabolic Scan Engine
SSE - Spherical Scan Engine
CCE - Close Coupled Mirror Scan Engine
RS - A Single Mirror Scan Engine Used As A Reference Scan Engine
OPD - Optical Path Difference
OPL - Optical Path Length
PSF - Point Spread Function
P-V OPD - Peak-To-Valley Optical Path Difference
Ti:S – Titanium-Sapphire Laser
TPE - Two-Photon Excitation
TPM - Two-Photon Microscopy
T/R – Transmission/Reflection
UV - Ultra-Violet
TABLE OF CONTENTS

Acknowledgements ........................................................................................................................................ vi
List of Abbreviations .................................................................................................................................. vii
Abstract ..................................................................................................................................................... 1

Introduction ............................................................................................................................................... 2

Chapter 1 Literature Review .................................................................................................................. 4

1.1 Introduction ......................................................................................................................................... 4

1.2 Scanners and Two-Dimensional Scan Engines .................................................................................. 5

1.2.1 Galvanometric and Resonant Scanners ......................................................................................... 5

1.2.1.1 Galvanometric Scanners ........................................................................................................... 6

1.2.1.2 Resonant Scanners .................................................................................................................... 6

1.2.1.3 Mirrors ....................................................................................................................................... 8

1.2.2 Scan Engine Designs for Two Dimensional Scanning in Two-Photon Microscopy ......................... 9

1.2.2.1 Scan Engine Position within Microscope Optical Path and Intermediate Optics ................. 10

1.2.2.2 Single Mirror Simultaneously Scanning in Two Directions .................................................... 11

1.2.2.3 Two Close-Coupled Mirror Scan Engine .................................................................................. 12

1.2.2.3.1 The Basic Design ................................................................................................................... 12

1.2.2.3.2 Paddle Mirror Variant .......................................................................................................... 14

1.2.2.3.3 Golf Club Mirror Variant ..................................................................................................... 15

1.2.2.4 Fast Scanning and Control of Scan Region ............................................................................... 16

1.2.2.5 Linearization of a Spot Motion Deflected by a Resonant Scanner ........................................ 17

1.2.2.6 Scan Engine with Three Moving Optical Elements ................................................................. 18

1.2.2.7 Polygon Mirror ......................................................................................................................... 19

1.2.2.8 More Complex Scan Engine Configurations ............................................................................ 20

1.2.2.8.1 Two-Dimensional Scan engine for Deflecting a Light Beam ............................................ 20

1.2.2.8.2 Compact Design of Two-Dimensional Scan Engine ............................................................ 21

1.2.2.8.3 Optical-Scanning Microscope Apparatus ............................................................................ 22

1.2.2.8.4 Micro-electromechanical systems ......................................................................................... 23

1.2.2.9 Scan Engine with Two Reflectors as Relay Optics .................................................................. 24

1.2.2.9.1 Spherical Relay Reflectors ................................................................................................. 24

1.2.2.9.2 Imaging System Comprising a Concave Mirror ............................................................... 25
Chapter 2 Capability and Limitations of OSLO Optical Modelling Software
2.3.8 Scan Lens ........................................................................................................... 90
2.3.9 Scan Field Evaluation Points ............................................................................. 92
2.3.10 Programming in OSLO .................................................................................... 92
2.4 Summary ................................................................................................................... 92

Chapter 3 An Improved Field Scan Engine Design Incorporating Parabolic Optics .........................................................................................................................94
3.1 Introduction ............................................................................................................... 94
3.2 Aberrations in the Optical System ........................................................................... 95
3.3 Advantage of Off-Axis Parabolic Reflector Compared to Spherical ......................... 99
3.4 Method and Modelling Design ................................................................................ 103
  3.4.1 Spherical Scan Engine ...................................................................................... 103
  3.4.2 Parabolic Scan Engine ...................................................................................... 105
  3.4.3 Other Modelled Specifications ......................................................................... 106
3.5 Results and Discussion ............................................................................................ 107
  3.5.1 The Shape of the Scan Lines ............................................................................ 107
  3.5.2 The Beam Spot Size ......................................................................................... 109
  3.5.3 Spot Diagrams and Point Spread Functions ..................................................... 111
  3.5.4 Positioning Error .............................................................................................. 112
  3.5.5 Comparison of Modelling Results with Specific Literature Results ................ 115
3.6 Summary ................................................................................................................. 118

Chapter 4 Experimental Verification of New Scan Engine Performance .120
4.1 Introduction ............................................................................................................. 120
4.2 Method .................................................................................................................... 120
4.3 Experimental setup ................................................................................................. 121
4.4 Results and Discussions .......................................................................................... 126
  4.4.1 The Investigation of Afocal Scan Engines ....................................................... 126
    4.4.1.1 Beam Spot Measurements ..................................................................... 126
    4.4.1.2 Comparison of the experimental and simulated results ....................... 132
    4.4.1.3 The Spot Size Ratio across the Scan Field Compare to an Ideal Single Mirror Scan Engine (RS) ................................................................. 132
  4.4.2 The Effect of the Scan Engines on Beam ......................................................... 136
    4.4.2.1 Evaluation of the Beam Collimation ..................................................... 136
    4.4.2.2 OSLO simulation of the PSE optical relay ........................................... 138
Chapter 5 Calculated Two-Photon Fluorescence Correction Factors for Reflective Scan Engines

Chapter 6 Summary of Thesis and Future Work

Appendix A. Pulsed Laser Beam Parameters

Appendix B. Database for Scan Engines
In this thesis, a novel optical design for an afocal scan engine based on two 90° off-axis parabolic optical elements acting as reflective relay optics between two deflecting mirrors is proposed. The characteristics of this scan engine such as linearity of scan lines, spot sizes, spot intensity profiles, field curvature, and temporal distortion across the scan field have been investigated. The new scan engine was also evaluated as an afocal relay, with scan optics and for optical zoom performance.

The engine was modelled and investigated using OSLO, commercial optical software. Its performance was evaluated and compared to known scan engines with results showing superior linearity of scan lines and intensity distribution across the scan field. The advantage of using it with a fast resonant scan mirror is highlighted.

The new scan engine prototype and known scan engines were assembled in the laboratory and their performance was evaluated and compared in order to verify the OSLO prediction. The experimentally measured results were found to be in agreement with the OSLO predicted results. A physical explanation for the difference in results has been provided.

Using OSLO optical software, a study of temporal distortion across the scan field was then conducted. The calculated results predict that the use of the new scan engine in two-photon microscopy may result in improved contrast in two-photon images across the scan field. The new two-photon excitation correction factor for reflective scan engines was introduced and its evaluation confirmed that the new engine is the best choice for fast scanning two-photon microscopy (TPM).
Introduction

Since the invention of the laser fifty years ago, optical scan engines have been widely applied for imaging and display purposes and there is a high demand for efficient scan engines in fields ranging from military, through commerce (bar code scan engines) and atmospheric science to the high-performance microscopes applied in biomedical science. Linearity of scan, a wide-field and fast scanning ability are important for many applications and the design of scan engines continues to be an active area of research within which there are numerous approaches. Specific applications have required new optical scan engine designs to meet specialised needs and to improve performance. The increased sophistication of computer control over the mirror movement has stimulated further improvements in scanner products and led to new applications. There are many scanner manufacturers such as General Scanning GSI Group, Cambridge Technology Inc., RAYLASE, Nutfield along with others who now produce galvanometer and resonant mirror scanners commercially.

The performance of the two-dimensional laser scan system applied to biomedical microscopes is very important and may determine the whole microscope system’s performance. In the past decade, there has been great progress in optical scanning microscopy particularly in the life science area. Development of new instruments has driven research to seek further improvements in microscopy and to expand the area of its application. Modern two-photon microscopy, which comes from an idea proposed more than 7 decades ago, has emerged in the last two decades as the most adventurous and widely applied biological instrument of our time. This emergence and the “success of two-photon excitation microscopy has been due to developments in two still-evolving areas: laser scanning microscopy and mode-locked lasers” [1].

This thesis investigates scan engines and their performance, particularly in relation to two- and multi-photon microscopy applied in biomedical science. The relatively new two-photon microscopy technique has opened up the possibility of deep imaging inside live biological tissue. Together with increased imaging rates, this type of microscopy has led to a greater understanding of three dimensional cell structures and their internal communication. It is now also widely used in neuroscience and cancer cell research.

As this is a laser scanning system, the imaging rate is dependent on the scanner rate used. Despite intensive theoretical and experimental research, scan engines for microscopy including two-photon excitation, many questions related to increasing the
imaging speed and improving imaging resolution are still under investigation. The effort in this area therefore, can be distilled to the design of a high performance scan engine operating at a fast scan rate with minimal wavefront distortion and consequent time distortion of ultra-short pulsed laser excitation used in a two-photon microscope to maintain the two-photon excitation advantage.

In this thesis an investigation into and modelling of scan engine designs and their performance across the scan field using commercial optical software OSLO is presented as the experimental measurement of system performance. A special investigation related to the pulse time distortion introduced by a scan engine across the scan field is also presented within the six chapters.

Chapter 1 is the literature review, which consists of three sections. In the first section, optical scanners and scan engine designs currently used in microscopy are reviewed. The location of a scan engine in two-photon microscope and its role in the whole microscope system performance is also discussed. In the second section, the concept and theoretical investigation of the two-photon excitation process, the advantages of applying it to biomedical microscopy and its characteristics are detailed. In the third section, the number of practical approaches to increase the imaging rate in two-photon microscopy is investigated.

In chapter 2, the capability and limitation of the OSLO optical software used to evaluate the scan engines performance is presented. The data for simulated scan engines, used in this thesis, are also summarised in chapter 2.

In chapter 3, the new scan engine design is introduced, and its characteristics and the modelling results described along with comparison to known engines.

In chapter 4, experimental results of the new scan engine and known engines are presented and compared. The measurements also verify the OSLO results.

In chapter 5, using OSLO simulation, the new method of calculating the time distortion across the scan field induced by a scan engine is introduced. The time distortions and the spot area are used to calculate a newly introduced two-photon fluorescence correction factor for reflective scan engines. It is demonstrated that the new scan engine introduces less pulse time spreading and when applied to two-photon microscopy, will improve the image contrast.

In chapter 6, synopsis of the main results of the research is presented along with recommendation for future related research.
Chapter 1  Literature Review

1.1 Introduction

Optical scan engines are instruments that deflect an incident laser beam to repeatedly pass over a number of positions in a scan field. Scan engines are an application driven research field and there exist a number of scan engine configurations which have been developed for specific requirements [2-7]. In a scanning microscope, two-dimensional scan engines form an important part of the illumination, and often the detection, paths and require diffraction-limited performance for particular wavelengths, power and scan field characteristics. This is particularly important for two-photon microscopy [8-13].

Two-photon fluorescence scanning microscopy (TPM) is a technique employing a two-photon excitation process where the fluorescence molecule, usually excited by ultraviolet light, can be excited by two photons of near-infra-red light. Due to reduced scattering, small excitation volume and less damaging wavelength of the near-infra-red ultra-short pulsed laser used, two-photon excitation applied to biomedical microscopy allows longer, deeper imaging within live cells at better contrast compared to the previous confocal and conventional microscopy techniques [10, 14-16]. The improved sectioning ability of TPM allows the building of 3D volume images of the live tissue cells. The specifications for an ideal two-dimensional scan engine used in TPM are an achromatic one pivot point two-dimensional scan engine with no pulse distortion introduced to the propagated pulsed beam across the scan field. These requirements emerge as TPM performance is highly depend on the spatial and temporal concentration of the pulse at the microscope objective focal point.

The advantages of deep imaging within intact live tissue by TPM have caused it to be applied to the hot areas of biomedical research, including neurosciences and cancer research [17, 18]. A fast imaging rate to probe fast cellular activities within a three-dimensional cellular network is also desirable.

Resolving the spatio-temporal activity pattern of local three-dimensional (3D) networks is an unrealised goal. The imaging rate depends on the two-dimensional (2D) scan rate and the axial scanning mechanism. Using a fast resonant scanner and/or multi-beam scanning can increase the scan rate. Advantages and disadvantages of all methods are discussed in this chapter which consists of three sections reviewing modern optical scanners and current scan engine designs used in laser scanning microscopy, the two-

1.2 Scanners and Two-Dimensional Scan Engines

Scan engines are now a mature field with overviews of the scanning drivers to be found in the literature [1-6]. Specific applications have required new oscillating optical scan engine designs to meet specialised needs and to improve performance. The increased sophistication of computer control over the mirror movement has stimulated further improvements of scanner products and hence new applications.

The first deflector applied in scanning a laser across an object was a polygon scanning mirror where a number of mirrored facets are formed on a cylinder and rotated about the cylinder axis. The scan non-uniformities introduced by the facet to facet imprecision and shaft wobbling of the polygon deflector led to an alternative approach reflecting the beam from only one mirror surface using vibrating mirror scanners [4, 19, 20]. These vibrating scanners include galvanometric and resonant devices and these are more widely used in laser scanning microscopes. Other types of deflectors applied in scanning microscopy are acousto-optic and electro-optic [20, 21], however, because of the wavelength dependence of the active element and drive frequency, they are not commonly applied in fluorescence microscope apparatus and especially not for two-photon microscopy.

In this section, current optical scanners and scan engine designs that are used in microscopy will be reviewed and only oscillating deflectors will be discussed in detail.

1.2.1 Galvanometric and Resonant Scanners

The galvanometer was introduced in 1880 by the French biologist and physicist Jacques d’Arsonval as a static measuring instrument. Its potential for dynamic optical scanning was first recognised in writing sound tracks on talking movies. With the invention of the laser in 1960, the demand for galvanometer based optical deflectors for different applications increased [3] and drove progress in improving the galvanometric scanners speed and accuracy, and the evolution of the main components: torque motor, transducers and amplifier. Computer control over scanner motion simplified system integration as sophisticated movement and drive algorithms were encoded.
Development of high field permanent magnets has led to the development of fast rotating resonant scanners. The progress of scanners involves many disciplines in physics and engineering, depending on the application. Recent applications will be reviewed.

1.2.1.1 Galvanometric Scanners

The modern galvanometer (galvo) uses moving magnet technology to oscillate a mirror and features low inertia, high rigidity components with low sensitivity to temperature change. It provides limited mirror rotation but has micro-radian precision and very controlled motion with position feedback allowing closed loop servo control of the motor. Galvo scanners may be optimised for either large or small signal applications and have been improved by high-accuracy capacitive feedback control [4, 19, 20, 22, 23]. The vibrating frequency of a galvo scanner is variable within a broad range from zero to a value close to the mechanical resonance frequency of the structure. Galvanometers can be driven by a sawtooth wavefront with a long active linear section or a triangle signal providing beam motion structured into a series of small angular vectors or steps. The maximum scan angle provided by a galvanometric scan mirror is ±50° optical scanning, i.e. ±25° mirror rotation and with small offset drift $\frac{50 \mu \text{rad}}{\circ \text{C}}$ and step time < 200 $\mu$ s [22].

The advantages of using a galvo mirror is that the mirror position feedback allows a uniform spot velocity across the scan field and it can be used to access to a specific point or, in two dimensions, a specific area section within the scan field. The mechanical aspects including mass and size however limit the speed of a galvanometric scanner. The bearings and suspension systems required must provide long time scan uniformity with minimal cross-scan wobble.

1.2.1.2 Resonant Scanners

The resonant driver is designed for very high scanning frequencies and employs a torsion bar with a mirror and a counter-weight with the torsion bar tuned to resonate at a specific high frequency allowing the system to execute near-perfect sinusoidal deflection when oscillating [20]. The GSI Group produces modern resonant scanners rotating at 8 kHz with typical scanning angles at 15° optical peak-to-peak (OPTP) which
can approach up to $26^\circ$ OPTP [23]. During imaging, the central quasi linear portion of the sinusoid is usually used.

Figure 1-1 shows the comparison between the waveform characteristics for a typical galvo (solid line) and resonant scanner (dashed line) with the same period. The galvanometer scanner provides a longer part of wavefront for a linear scan in one direction.

**Figure 1-1. Galvo and resonant scanner oscillating waveform detailing rotational angle $\Phi$ versus time. $T$ is the duty cycle. The galvo scanner provides a linear scan over of the 70% duty cycle while the resonant has an approximate linear section of 33% for scan in one direction. $\tau$ is the retrace time (adapted from [20]).**

Using a resonant scanner opens the possibility of collecting imaging data during forward and backward scanner motion, effectively doubling the scan line frequency up to 16 kHz and increasing the active scan for up to 88% of total scan period [23]. Resonant scanners consume very little energy, employ a small mirror, are optimised dynamically and operate without transmitting vibration to the rest of the system.

The disadvantage of a resonant scanner is that it does not provide the ability to access a specific point in a scan field. Additionally, image distortions created by the sinusoidal movement of the scanner needs attention and can be sometimes corrected computationally by linear scaling in position and/or sinusoidal variation of intensity signal [24].
1.2.1.3 Mirrors

The requirements for the mirror itself in terms of reflectivity, balance, inertia, thermal and dynamic deformation, mounting and so on, are the same for the galvo and resonant scanners. For high-power laser applications, the thermal conductivity of the mirror substrate also becomes an issue. Optimal scanner design requires successful integration of the mirror material, mirror-to-motor mounting and the motor itself.

For excellent scanning performance, the mirror in a scanner has to be small, have negligible inertia, be infinitely rigid, flat and appropriately reflective. Usually they are from 4 mm to 15 mm clear aperture diameter. The GSI Group uses optical grade beryllium for resonant mirrors due to its superior stiffness-to-weight ratio. Silver coated mirrors are used as ultra short pulse reflectors and provide excellent reflectivity, typically 90 % at 400 nm and 95 % above 550 nm [22].

The simplest shape of the mirror for scan application is usually a plane with the corners cut off. This design however, has the highest inertia which can be minimised with the precise shape of mirror and its weight (Figure 1-2 (a)). The optimal shape is pyramidal and the weight can be reduced by drilling holes through the body behind the reflective surface (Figure 1-2 (b)).

![Figure 1-2. A high-performance scanning mirrors: (a) the mirrors for galvo and resonant scanners (adapted from [25]); (b) the optimal mirror design in beryllium with nickel plating support for 2 kHz application [26].](image)

The mirror is mounted close to the front bearing of the scanner driver and is balanced in the mount to minimise wobble induced by angular acceleration. Mirror alignment,
critical to any scan engine design, is verified along both axes as misalignment can create error in beam position leading to line scan image distortion.

In summary, the major advantages of galvanometer and resonant scanners are their simplicity, small size and long life. The advantages of a galvanometer mounted mirror is that it provides a linear uniform motion over the rotating range, allows random access over the scan area, and has a variable range of scan frequencies. The disadvantages of the resonant scanning mirror are its fixed scanning frequency, no random access to the specific area and its sinusoidal motion which requires additional adaptation to form an undistorted image. Hybrids, combining the fast scanning advantage of a resonant scanner with the positioning abilities of galvo, have been developed and will be shown later in this section. The loud noise produced by scanning deflectors, especially resonant scanners, with operating frequencies in the audible high kHz range needs attention and the scanner is better positioned in a soundproof housing [27].

Usually two-dimensional scan engines include dual galvo mirrors to scan in both X and Y directions with the Y direction moving in a step waveform to provide the desired number of lines per frame. To increase imaging speed, the resonant mirror can replace one galvo for line scanning, the X direction.

1.2.2 Scan Engine Designs for Two Dimensional Scanning in Two-Photon Microscopy

In two-photon laser scanning microscope the image is constructed by collecting fluorescence signal point-by-point from a sample. Commercial two-photon microscopes are built using confocal microscopes [28] or laboratories construct them on conventional microscopes by adapting lasers and scanners to the microscope entrance port [29]. There are different scan engines for microscopes and the choice depends on the required performance characteristics including speed, scan linearity and ability to sample discrete points in the sample field image. The choice also can be determined by specific application: particular wavelengths, radiation duration, laser power as well as damage thresholds of the optical elements.

In this section, the position of a scan engine in the microscope will be defined and known scan engines for conventional, confocal and two-photon microscopes will be described and evaluated.
1.2.2.1 Scan Engine Position within Microscope Optical Path and Intermediate Optics

The illumination path in a typical laser scanning confocal and two-photon microscope is schematically illustrated in Figure 1-3. The incoming laser beam traverses the scan system, the intermediate optics including an eyepiece or f-theta scan lens [30-32] and the tube lens, and then enters the back focal aperture of the infinity-corrected objective [2, 30] which focuses it into the sample [31]. For the undeflected scan position, the incident laser beam is on the objective optical axis and illuminates the back entrance of the objective lens with a plane wavefront.

![Figure 1-3. Simplified diagram of the optical components of a laser scanning microscope. Intermediate image plane is conjugated to the objective focal plane. The centre of the scanner movements (pivot point) must be positioned at the plane conjugated to the objective telecentric plane.](image)

The scan pattern created is focused by the scan lens to the microscope intermediate image plane and projected to the objective focal plane where the sample is positioned. The position of the sampling spot in the objective focal plane depends on the incident beam angle at the telecentric plane (i.e. the back aperture of the objective) therefore, the scan engines’ centre of all deflecting movement (pivot point) must be positioned in the conjugated telecentric plane of the objective lens [30, 32]. The pivot point of a two-dimensional scan engine will therefore determine the diffraction-limited performance of the microscope.
1.2.2.2 Single Mirror Simultaneously Scanning in Two Directions

A single mirror simultaneously rotated about two orthogonal axes as shown in Figure 1-4(a) [3] was implemented by Leica TCS [30]. The scan engine position in the microscope setup and optical performance is detailed in Figure 1-4(b) [33]. It has two drivers, two encoders and rotates about one pivot point. The pivot point can easily be placed in the conjugated telecentric plane of the objective and aligned to the objective back entrance pupil and as such it is the optimum scan engine to use in light scanning microscopy providing precise movement of the scanning spot in the focal plane of the objective. As the output beam is reflected by only one scanning surface, the one mirror scan engine provides also the smallest possible beam intensity loss compared to all other scanning devices.

However, due to high rotational inertia, this type of scan engine can not function at high speed and in high precision applications. The performance of this scanning system is limited by double pincushion distortion and by the offset between the mirror surface and the pivot point.

Figure 1-4. (a) Single mirror scanning in two directions [3]. (b) Projection of the scan engine pivot point to the objective telecentric plane and forming the scan pattern in the focal plane [33].
1.2.2.3 Two Close-Coupled Mirror Scan Engine

This class of scan engine employs two independently driven mirrors separated by a small distance. There are several different optical arrangements of close-coupled scan engines and three of the most common designs [3] are reviewed here.

1.2.2.3.1 The Basic Design

The basic design of a close-coupled mirror scan engine and its performance is shown in Figure 1-5 (a) [34] and (b) [33] respectively. This design is the most widely used scan engine due to its simplicity and has been implemented in confocal and two-photon microscopy [30, 35].

From Figure 1-5 (a), the first scanning mirror 1 moves the beam across the second scan mirror’s (surface 2) rotational axis and describes a line on its surface as well as forming a line scanning across the object surface. The second mirror 2 moves the beam coming from the first scan mirror 1 incrementally in the Y direction by a step waveform to offset the next scan line and so on until a rectangular scan pattern 3 on the object surface is produced.

![Diagram of close coupled mirror scan engine](image)

**Figure 1-5.** The close coupled mirror scan engine. (a) The performance and image distortions in the created scan pattern [34]. (b) The field curvature in the intermediate plane and two telecentric planes due to separation distance between two scanning mirrors [33].

The disadvantage of this system is that the first scan mirror 1 does not deflect the incident beam to a single point on the rotational axis of the second mirror 2 thus requiring the two mirrors be of different size, inertia, and therefore speed. Additionally,
the separation distance between the two scan mirrors induces a geometrical image distortion [3, 36] as shown in Figure 1-5 (a). At the back aperture of the objective lens, the image planes for the first and second mirror are different and the pivot point is out of the objective telecentric plane and therefore the objective is not completely illuminated. The result of the telecentric plane shift (Figure 1-5(b)) causes loss of resolution [37].

This shift has been evaluated using the magnifying factor of the intermediate optics, the scan lens and the tube lens, as [33]:

\[
V = A \times \left( \frac{f_{SL}}{f_{TL}} \right)^2,
\]

where \( V \) is the resulting shift of the telecentric plane in mm, \( A \) is the distance between the two scan mirrors, \( f_{SL} \) and \( f_{TL} \) are the focal length of the scan and tube lenses respectively. For example, for two galvanometric mirrors separated by distance of 10 mm and the scan lens and tube lens at 20 mm and 200 mm focal length respectively, the shift in the telecentric plane is 100 μm.

Locating two mirrors as close as possible can improve telecentricity but the curved field scanning pattern created requires the objective be corrected for field flatness. Often, two close coupled scanners are designed to have the pivot point located at the mid point between the two mirrors.

The pincushion effect created in a close-coupled scan engine \( \varepsilon \) may be calculated from [3]:

\[
\varepsilon = \frac{X_{\theta_x} - X_{\theta_y}}{2X_{\theta_y}} = \frac{1 - \cos \theta_x}{2(1 + e/d)\cos \theta_y},
\]

where \( \theta_x \) and \( \theta_y \) are the X and Y optical scanners rotational angles respectively, \( e \) is the separation distance between two mirrors and \( d \) is the distance from the second scan mirror to the image (see Figure 1-5). Pawley in his book [30] specified the error, as defined by the magnification of the intermediate optical system, at around 4 mm over 100 mm. Hence it will have an effect at large scan angles.

When two galvanometric mirrors are employed in a close-coupled scan engine, the scan rate is typically 1 frame per second. To increase the imaging rate in this design, one mirror can be a 60 Hz galvanometric scanner and the second mirror a 15 kHz fast resonant scanner. This setup allows imaging at a video rate of ~16 frames per second [38].
The advantage of using this system is that it is small in size and can produce quite fast scanning over a small image field. Disadvantages are the image distortion introduced due to telecentricity, the field curvature and pincushion distortion all of which lead to a lower resolution.

1.2.2.3.2 Paddle Mirror Variant

A paddle scan engine is a variation of the close-coupled design in which one mirror is mounted on a paddle arm and interacts with incoming beam at 45°. The system configuration and paddle scanning motion are shown in Figure 1-6 (a) and (b) [3]. It has been applied in a custom-built two-photon microscopes [41-43] and implemented by Molecular Dynamics [30].

Usually, the first scanning mirror is in the paddle position. The paddle mirror size, position and rotation are designed to reflect the incoming beam to one point at the second mirror surface and hence minimise the amount of beam translation on the mirror Y as the mirror X rotates. The optical path length of the beam deflected by a mirror in paddle position varies. The amount of beam translation on the second mirror surface can be calculated (Figure 1-6 (a) [34]) as:

$$d = r(1/\cos 2\alpha -1),$$

where $r$ is the distance from the optical axis of the beam incident to the mirror surface to the scanner rotational axis, and $\alpha$ is the scanner deflection angle. For two mirrors separated by 10 mm and for $\pm 0.15$ radian angle rotation, the beam walk on the Y mirror surface is 0.33 mm [3].

The paddle mirror is physically large and its inertia is much greater in comparison to the inertia of the second scanning mirror. Therefore, the paddle mirror is generally driven by a galvo motor and the second scanning driver can be a fast resonant scanner with sinusoidal motion or a rotating polygon.

The combined movement in the paddle scanner compare to the basic close coupled design improves the beam position in the objective telecentric plane by a factor of 25 [30]. An imaging rate of 30 frames/second for a 512 line image with a fast resonant scanner operating at 9 kHz and sampled in both directions and a galvanometer in the paddle configuration has been reported [38].

For high angular speed the paddle mirror must be statically and dynamically balanced in both axes to avoid additional imaging distortion.
1.2.2.3.3 Golf Club Mirror Variant

A further variant of the close-coupled arrangement known as a golf club scan engine is shown in Figure 1-7 (a) and its movement characteristics in Figure 1-7 (b) [3]. Its features are similar to the paddle scanning design and it is employed in raster scanning.

The incoming beam hits the first mirror 1 at $45^\circ$. This mirror is mounted on an arm which has its rotational axis 2 normal to the reflected beam at the zero deflection position and intersects this line. The second mirror 3 is located approximately in the middle of the incident beam and the rotational axis of the mirror 1. The rotational axis of the second mirror 3 is in the plane of the page.

The beam walk at the second mirror surface is approximated in [3] as:

$$st = [\tan(\pi/4 + \theta) + \tan(\pi/4)][1 - \cos \theta]r/2,$$

where $\theta$ is the scan angle of the first scanning mirror and $r$ is the distance from the first mirror to its rotational axis. Compared to the paddle mirror scan engine, the beam walk on the surface of the second mirror, arising from rotation of the first scanner over $\pm 0.15$ radian and with a 10 mm separation distance between mirrors, is 0.24 mm which is smaller and hence, represents an improved system.
1.2.2.4 Fast Scanning and Control of Scan Region

A desirable feature of a scanning system is to have the ability to scan over any chosen area of a sample within the field of view of the microscope. The fastest scanning devices usually include one galvo and one resonant driving mirror. The galvanometric mirror can be scanned with variable frequency and amplitude, and can be given an angular offset to direct the beam to a particular area. However, because of their size and inertia the galvanometers have an upper oscillating frequency of a few hundred Hz [39]. Resonant drivers, in contrast, have a very high kHz oscillating frequency but can only operate at this frequency and cannot offset the scan from the zero position as was discussed previously. The apparatus for beam deflection patented by Storz et al. [39] shown schematically in Figure 1-8 (b), details a combination of two galvanometer mounted resonant scanners (Figure 1-8 (a)) in close coupled configuration, scan unit A and B. In Figure 1-8 (b), the first scan unit A has a mirror 3 mounted in paddle construction allowing its reflected beam to strike the same point 6 on the second scan mirror 4 regardless of its angular position.

The advantage of this system that it is can perform fast scanning about any desired position. However, using a mirror in paddle construction means increased mass and inertia which will affect the oscillating frequency and possibly cause additional beam wobbling and image distortion.
1.2.2.5 Linearization of a Spot Motion Deflected by a Resonant Scanner

Using a galvanometer driven mirror, the deflected beam moves with a constant speed and uniformly illuminates the sample. The resonant scanner reflects the illuminating spot sinusoidally with a periodic increase and decrease in acceleration. Montagu has patented [40] the design shown in Figure 1-9 which provides a fast scanning rate by using the resonant scanner and an arrangement which can provide a linear motion for the fast oscillating driver. Deflection in the Y axis is produced by a galvanometer mounted mirror 1 arranged in a paddle configuration to minimise the resultant motion of the beam at the following deflecting device. The deflection in the X axis is provided by the combination of two resonant driven mirrors 2 and 3 which oscillate about parallel axes at different frequencies, one of which is harmonic to the other, providing a constant speed of the beam across the target. One of the resonant scanners 5 is mounted on a galvanometer to provide the ability to scan over a chosen area in a sample. This design however is not particularly fast.

Figure 1-8. Schematic depiction of the (a) mutually independent rotary drive unit and (b) scan engine for variable oscillating frequency (adapted from [39]).
18 • Chapter 1

Figure 1-9. Improved scanning mechanism for providing a linear motion by resonant driver. 6 and 7 are galvanometric drivers, and 4 and 5 are resonant drivers. 6 provides the scanning in the Y direction and the assembly of 4, 5 and 7 provides deflection in the X direction (adapted from [40]).

1.2.2.6 Scan Engine with Three Moving Optical Elements

The design, patented by Goodman [41], is shown schematically in Figure 1-10. The movement of additional rotating glass wedge has synchronised to the Y scanner to ensure that the light reflected from the Y scanner mirror always hits the second X scanning mirror at its pivot point.

Figure 1-10. Three moving optical element scan engine setup (adapted from [41]).

The disadvantage of this system for application in two-photon microscopy is that the use of the additional refractive optical element leads to pulse time broadening from group velocity dispersion and as a result, a decrease in two-photon excitation signal.
1.2.2.7 Polygon Mirror

The rotatable polygon mirror, shown in Figure 1-11 finds many applications in different scanning instruments [20, 42]. It has several flat reflective surfaces (facets) symmetrically positioned around the circumference of a rotating disc. The maximum scan angle is $\frac{2\pi}{n}$, where $n$ is the number of facets. The active portion of a scan cycle decreases due to a blanking or retrace interval time $D/W$, where $D$ is the illuminating beam diameter and $W$ is the width of polygon facet. The scanner duty cycle is $\eta = 1 - \frac{D}{W}$.

![Rotating polygon mirror diagram](image)

Figure 1-11. Rotating polygon mirror.

It is mostly used for high amplitude scanning at high uniform velocity. The beam incident to and reflected from the polygon mirror is located in a plane perpendicular to the polygon rotational axis.

The first microscopy beam scanning system to use a polygon mirror was introduced by White in 1986 and included two separately rotating mirrors: one slow oscillating galvanometer reflector and one a fast polygonal mirror. A focusing telescope images the first scan mirror on the second scanning mirror to make both rotate about the same pivot point as shown in Figure 1-12. It could produce a relatively high scan rate of 4 video frames of 512 lines/second [43].

Problems with the design which include the noise of the polygon mirror, deviation from ideal movement, the fixed scan angle arising from the polygon face number and the chromatic aberrations from the refractive telescope elements, contribute to the limited acceptance of this system for fluorescence microscopy.
1.2.2.8 More Complex Scan Engine Configurations

1.2.2.8.1 Two-Dimensional Scan engine for Deflecting a Light Beam

Leica Microsystems patented a two-dimensional scan engine design [44] as shown in Figure 1-13. This arrangement is claimed to eliminate distortion errors. Two mirrors, 4 and 8, are rotated by drivers 3 and 7 about perpendicular axes 2 (X direction) and 6 (Y direction). The mirror 4 is fixed with respect to mirror 5 in the housing 10 so both mirrors 4 and 5 rotate as a unit about the Y axis 2 providing the rotation of the beam 1 about a pivot point 9 which lies on the rotational axis of the mirror 8. The optical axis of the beam between the mirrors 5 and 8 is always in a plane containing the second rotational axis 6 and perpendicular to the first rotational axis 2. In this way distortion errors are eliminated. The driver 3 is designed as a galvanometer at an oscillating frequency of from 10 to 800 Hz. The second driver 7 can be a resonant galvanometer which oscillates at a frequency of 4 kHz.

This is a sophisticated system requiring fine alignment and careful adjustment of all parts in the scan engine. Moreover, the housing 10 is directly connected to the galvanometric driver 3 which means that the driver’s job is to rotate the relatively heavy...
housing 10 with a large moment of inertia. As a result, this design is in unsuitable for fast scanning.

Figure 1-13. Optical arrangement for deflecting a light beam in two perpendicular directions [44]).

1.2.2.8.2 Compact Design of Two-Dimensional Scan Engine

To address the moment of inertia, a smaller compact design was patented by Widzgowski and assigned to Leica Microsystems CMS GmbH as shown in Figure 1-14. It comprises two deflection devices 2 and 3 for independent scanning in the Y and X directions respectively. Drivers 4 and 5 rotate devices 2 and 3 about mutually perpendicular axes.

The deflection device in the Y direction includes two mirrors 7 and 8 that are arranged in a specified angular position and are jointly rotated about the first axis. As a result the light beam 1 rotates around a centre point on the surface of the second scan mirror 6 which is on its rotational axis. The drivers 4 and 5 are configured as galvanometer drivers including a resonant galvanometer.

The entire arrangement is mounted on a plate 9 and glued into the housing. Housing size is reported to be in a range of height of 50 mm to 100 mm, width of 30 mm to 80 mm, and depth within a range of 30 mm to 80 mm. Those sizes however still represent a mass and inertia that is incompatible with fast scanning.
1.2.2.8.3 Optical-Scanning Microscope Apparatus

The scan engine design patented by Hara [46] and assigned to the Olympus Corporation claims to be a compact design with decreased astigmatism to produce high-quality imaging. Its schematic drawing and performance features are shown in Figure 1-15 (a)-(e). In Figure 1-15 (a), a first and a second scan mirror, 1 and 2 respectively, are secured on the tilted surfaces 3 and 4. These galvanometer mirrors constitute a proximity galvanometer mirror unit which is realised by electromagnetically driving micromachined mirrors (MEMS) 5, shown in Figure 1-15 (b).

A typical two-dimensional scan engine arrangement includes two galvo scanners which deflect the incoming beam at 90° to the input optical axis. This invention reduces the overall width of the scan engine as the beam is incident on the first scanning deflector at an angle θ smaller than 45° as shown in Figure 1-15 (c).

Because astigmatism, $\alpha$, depends on the angle of the incident beam to the flat mirror surface $\theta$, as well as on the mirror surface curvature $r$ (see Figure 1-15 (d)), a higher image quality can be produced for these smaller incident beam angles on flat surface mirrors. Ideally, the incident beam angle should be $0^\circ$.

Figure 1-15 (e) illustrates a geometrical restriction on the incident beam to the first scanning mirror 1 as it may vignette depending on the second scanning mirror size $W$ and distance between the two galvo mirrors $d$. 

---

Figure 1-14. Schematic diagram of compact arrangement of optical scan engine [45].
This scan engine is advantageous in terms of minimising the scan engine size and decreasing imaging astigmatism. Employing two galvanometric mirrors however, restricts the imaging speed.

### 1.2.2.8.4 Micro-electromechanical systems

MEMS (microelectromechanical system) optical scanners as first devised by Petersen [47] in the 1980s, find many applications including bar code scanners [48, 49], imaging devices [50] and displays [51]. There has been an investigation using MEMS scanners in a laser scanning microscope [52]. Their commercial usage is limited by the difficulties of combining optics and MEMS devices [52] which requires miniaturization of the whole optics together with the MEMS scanner. A single-fiber confocal microscope with a MEMS scan engine and a miniature objective lens was reported in 2007 [53] the schematic diagram of which is shown in Figure 1-16. An imaging rate of 8 frames per second over a 140 μm by 70 μm field-of-view was achieved.
1.2.2.9 Scan Engine with Two Reflectors as Relay Optics

1.2.2.9.1 Spherical Relay Reflectors

Amos proposed using two reflective focusing elements between two scan mirrors as an afocal achromatic design to create a one pivot point two-dimensional scan engine [54]. This design was employed by the BioRAD confocal microscope and further used by Denk in two-photon microscopy [8] to image a wide range of stained samples with sub-micron resolution. The design is shown in Figure 1-17 [54]. The incoming beam is reflected by four reflectors 1 to 4. The rotational axes of two scan mirrors 1 and 2 are perpendicular to each other. Two intermediate concave mirrors 3 and 4 face each other and transfer the beam reflected by the first scan mirror to a point on the rotational axis of the second scan mirror. This point is stationary for all angular beam displacement by the first scan mirror. The angular displacement of the second mirror 2 provides the scan for the second direction. The second flat scan mirror 2 is tilted on the direction of the outgoing beam. The radius of curvature of the two spherical reflectors is 75 mm. The plane scan mirrors are driven by galvo drivers for $+17.5\degree$ angular displacements at the line and frame rate of 525Hz and 1Hz respectively. The inventor reported that for two plane mirrors oriented non-parallel to one another, the scan line curvature can be minimized.
Using reflectors in relay optics instead of focusing lenses avoids the chromatic aberrations in the microscope system. The tilted spherical reflectors however, will introduce image optical distortions associated with coma and astigmatism, resulting in increased focused spot size, elliptical spot orientation, scan line curvature, scan linearity and reduce resolution [55].

### 1.2.2.9.2 Imaging System Comprising a Concave Mirror

An imaging system with tilted spherical surface produces imaging aberrations, such as spherical, astigmatism and coma as was shown in this chapter.

An arrangement introduced by Lindblom in 2005 [56] as shown in Figure 1-18 improves the optical collimation of the incident beam by positioning a negative lens 1 in the incident beam path before the spherical reflector 2 at an angle $\alpha$. To reduce and eliminate imaging aberrations in the focal plane, another lens 3 can be placed after the concave mirror. A mirror and lens collimate the exit beam.

---

**Figure 1-17. Schematic presentation of the achromatic scanning system [54].**

**Figure 1-18. Imaging system. The angle $\alpha$ of the lens 1 tilting is 45.6° [56].**
Figure 1-19 [56] shows the application of this invention for collimating and image correction. However the inclusion of thick glass elements is a disadvantage for two-photon work.

1.2.2.9.3 Hybrid Spherically Parabolic Reflector Relay

A scan engine with a hybrid spherical parabolic reflector relay between two flat scan mirrors, patented by Seel [57], is shown in Figure 1-20 (a) and (b). The first rotational mirror 2 and relay arrangement 5 image the deflection point (P) on surface 2 to the point (P’) at the surface of the second scan mirror 3. The imaging errors were reported to be independent of the deflecting angle of the first scan mirror and to be small as possible.

The reflective surface 4 is a surface of a parabolic torus which is formed by the rotation of a two-dimensional parabola around the z axis perpendicular to the optical axis of
parabola and shifted at 3f upwards from the parabola’s centre, where f is the focal distance of parabola (Figure 1-20 (b)). The parabola 4 is described by the formula \( r(z) = 3f - z^2 / 4f \). The parabolic torus surface is shown in Figure 1-21.

![Diagram](image)

**Figure 1-21.** (a) Parabolic torus surface. The blue sections show the section used for reflecting the light. (b) and (c) Optical design of scan engine by Seel along x and y axes. For every angle of the first scanner rotation, the beam rays in the YZ plane are reflected from a 90° off-axis parabolic surface and the rays in the YX plane are reflected from a 45° tilted spherical surface.

This design does not eliminate spherical aberration as the microscope beam encounters a tilted cylindrical surface. A model of this design has been constructed and is discussed in chapter 3.

### 1.2.3 Laser Scan Engines and Associated Distortions

This section summarizes the work of Sagan [58] covering the optical design requirements for preobjective scanning where the beam is scanned and then enters the objective via a scan lens. The entrance pupil of the scan lens is located near the final scanning mirror surface at a distance depending on the entrance pupil diameter, the input beam diameter and the scan angle. The complexity of the scan lens depends on the required correction to the field but in the TPM application must be a low group velocity dispersion lens.
The most common image distortions induced by a scan engine are described by Smith [59] and Montagu [3, 34] and an overview is presented here. Other errors, not included in this section, arise from variation in the index of air refraction, variation due to the local pressure, density and absolute temperature, air dynamics and turbulence, and the damping effect, created by mirrors scanning rapidly in air, which can reduce scanning speed and precision. Some advanced scan systems operate in a partial vacuum or in helium to avoid these problems.

1.2.3.1 Cosine Fourth Law
Distortion due to unequal illumination from different points of the incident beam can be seen from Figure 1-22 [34]. For a beam incident perpendicular to the image plane, the illumination at the off-axis point H is reduced compared to the on-axis point A by a factor of $\cos^3 \theta$. For other scanning beam positions, the beam strikes the image surface at angle $\theta$ from normal and there will be a fourth $\cos \theta$ factor:

$$I(H) = I(A) \times \cos^4 \theta,$$

where $I(H)$ and $I(A)$ are the irradiance of the on-axis point A and peripheral point H respectively.

![Figure 1-22. Cosine fourth off-axis image distortion [34].](image)

1.2.3.2 Off-set Mirror Surfaces
The scanner mirror reflective surface can be and is usually off-set from the rotational axis by a distance $T$. This causes an additional scan nonlinearity error. To minimise this
effect, the beam should be centred below the rotational axis by amount $K$ as illustrated in Figure 1-23 [34]. The associated error is defined by Montagu [34] as

$$Err = \frac{T - K \sin \alpha}{\cos \alpha},$$

where $\alpha$ is the angle of the tilted mirror surface to incident beam.

![Figure 1-23. Compensation for the mirror reflective surface being off-axis [34].](image)

1.2.3.3 Beam Path Distortion

Beam path distortion has the effect of blurring or defocusing the image. It is caused by a difference in the path length for different portions of the beam and is due to scan head misalignment, flat mirror surface quality and the imaging system. In a focused system, beam path distortion produces elongated or distorted spots with vertical and horizontal axes focused in different planes i.e. astigmatism. Defects of a lens system such as decentering and stress can cause image distortion as well.

1.2.3.4 Merit Function

Merit functions are used to compare the performance of one instrument to other instruments and are very important in the design step of a microscope construction. How much of the beam is delivered to the objective back entrance from a perfect planar wavefront depends on all the microscope elements including the scan engine and determines the performance of a microscope. These functions define how well the beam can be focused to achieve diffraction-limited performance and how much the objective
pupil must be overilluminated. A wavefront error of only $\lambda/4$ reduces the contrast by almost 50% [31].

There are two types of merit functions. The first is an object-dependent merit function which is the product of all transmission and all reflection coefficients of all optical elements including in the beam delivery to the focal point and depends on the emission and absorption spectra of the fluorophore used (see Table 1-1 and Table 1-2 [31]). The second type, object-independent merit functions, is the sum of overall surface errors including tilts and thickness deviations, and deviation from planarity of the optical elements.

1.2.4 Conclusion

In this section, modern optical scan engines, the galvanometers and resonant scanners, applied in the field are described. The increasing demand in biomedical research for imaging of live biological tissues has driven the development of the wide variety of scan engines and hybrid actuators reviewed. The advent of two-photon microscopy via ultra-short pulsed laser illumination has compounded the optical design requirements with the additional demand for low group velocity distortion. The quest for fast, low-distortion scanning remains an active endeavour demanding increased optical precision, lower manufacturing tolerances and better physical layout and design.

Table 1-1 Typical intensity losses in the illumination path of a laser-scanning fluorescence microscope.

<table>
<thead>
<tr>
<th>Element</th>
<th>Per stage, %</th>
<th>Remainder, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laser head exit</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Mirror</td>
<td>1-3</td>
<td>98</td>
</tr>
<tr>
<td>1-3 dichroic mirrors for several lasers</td>
<td>3-10 per mirror</td>
<td>88</td>
</tr>
<tr>
<td>Acousto-Optical Mechanism</td>
<td>10-15</td>
<td>75</td>
</tr>
<tr>
<td>Fiber</td>
<td>25</td>
<td>56</td>
</tr>
<tr>
<td>Collimator/beam expander</td>
<td>4</td>
<td>56</td>
</tr>
<tr>
<td>Mirrors</td>
<td>1-3 per mirror</td>
<td>52</td>
</tr>
<tr>
<td>Polarising beamsplitter and $\lambda/4$ plate</td>
<td>4</td>
<td>50</td>
</tr>
<tr>
<td>Dichroic mirror</td>
<td>10-20</td>
<td>42</td>
</tr>
<tr>
<td>Relay lens</td>
<td>10</td>
<td>37</td>
</tr>
<tr>
<td>Inside microscope</td>
<td>10</td>
<td>33</td>
</tr>
<tr>
<td>Overillumination of back entrance of an objective</td>
<td>80</td>
<td>7</td>
</tr>
<tr>
<td>Intensity entering sample</td>
<td></td>
<td>7</td>
</tr>
</tbody>
</table>
Table 1-2. Typical intensity losses in the detection path.

<table>
<thead>
<tr>
<th>Element</th>
<th>Loss, %</th>
<th>Remainder, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample emission</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Collection by high-NA lens</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>Inside microscope</td>
<td>10</td>
<td>23</td>
</tr>
<tr>
<td>Relay lens</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Scan engine</td>
<td>4</td>
<td>19</td>
</tr>
<tr>
<td>Dichroic mirror</td>
<td>25-30</td>
<td>15</td>
</tr>
<tr>
<td>Collimator/beam expander</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td>Dichroic mirror</td>
<td>20</td>
<td>11</td>
</tr>
<tr>
<td>Filter</td>
<td>40</td>
<td>7</td>
</tr>
<tr>
<td>Detector</td>
<td>85</td>
<td>1</td>
</tr>
<tr>
<td>Total intensity detected</td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

1.3 Two-Photon Excitation Process

In the past decade there has been great progress in optical microscopy particularly in the life sciences with further improvements in lasers and imaging approaches expanding the area of research application. Two-photon microscopy emerged from an idea proposed more than 7 decades ago and enabled by mode-locked lasers.

In this section the principle of two- and multi-photon excitation will be discussed along with the advantages of the two-photon excitation process and the excitation laser source required for fluorescence imaging microscopy.

1.3.1 History

The two-photon excitation (TPE) phenomenon was predicted by the Nobel laureate Maria Göppert-Mayer in 1931 [60] who introduced the possibility of one molecule being excited from the ground state to its first excitation state by simultaneous absorption of two photons during the same quantum event. This process is very rare in common circumstances because of two main factors: the time window and the photon concentration in any area. The time window for simultaneous absorption as defined by the Heisenberg uncertainly principle [61] is about $10^{-16}$ s (0.1 fs). The possibility of one molecule interacting in this time window with two photons in bright daylight was reported by Denk and Svoboda in 1997 [1] as one in every 10 million years.
The development of laser light sources [62-64] allowed generation of two-photon excitation, as was first achieved in the fluorescence of CaF$_2$:Eu$^{2+}$ by Kaiser and Garret in 1961 [65]. The second-harmonic generation in a crystalline quartz by Franken et al in 1961 [66] followed shortly. The Oxford group including Sheppard, Kompfner, Gannaway, and Choudhury later suggested employing the TPE process in scanning microscopy and using the non-linear excitation in the focal plane of the objective as a means to instantaneously achieve optical sectioning of the sample [67]. The invention of the femto-second ultra-short pulse mode-locked laser with kilowatt peak power per pulse and pulse repetition rates of 100 MHz [68, 69], and the application of it as an excitation source in a scanning microscope [70] enabled the nonlinear two-photon laser scanning microscopy technique [71], a technique that is now widely employed in the biomedical and life sciences.

### 1.3.2 One-, Two- and Multi-Photon Excitation

Jablonski diagrams showing the difference in the one, two- and multi-photon excitation are illustrated in Figure 1-24 (a)-(c).

![Figure 1-24. Jablonski diagram for (a) one-photon, (b) two-photon and (c) three-photon excitation: $S_0$ is the ground state, $S_1$ is the excited state and $V$ is the virtual state which exists only for a very short time.](image)

In a one-photon excitation (OPE) event, see Figure 1-24 (a), the fluorescent molecule which is normally in the ground state $S_0$ absorbs the energy of the single incident light photon, excites to a higher energy electronic state $S_1$, relaxes down the vibration levels to the lowest state, and then drops down to the $S_0$ ground electronic state with emission
of one fluorescence photon. Because of the energy lost during the relaxation, the emitted photon has a red-shifted wavelength relative to excitation photon. The shift can be understood from the energy and wavelength relation:

$$E = \frac{hc}{\lambda},$$

where $h = 6.6 \times 10^{-34}$ Js is Planck’s constant and $c = 3 \times 10^8$ m/s is the speed of the light in vacuum.

In two-photon excitation (TPE) Figure 1-24 (b), the same molecule can be excited to a virtual state $V$ by absorbing one photon and then to the higher energy state $S_1$ by absorbing a second photon. The virtual state is not an exciting allowed energy state of the molecule and exists only for a short time determined by Heisenberg’s time-energy uncertainly principle, in order not to violate energy conservation:

$$\Delta E \Delta t \geq \frac{\hbar}{2},$$

where $\Delta E$ is the difference between permitted state energy and absorbed photon energy and $\hbar = \frac{h}{2\pi}$. This time is extremely brief (about $10^{-16}$ s) resulting in effectively simultaneous absorption of the two photons. The combined energy of the two excitation photons must be enough for the $S_0-S_1$ transition in the fluorescent molecule:

$$E = E_1 + E_2 = \frac{hc}{\lambda_1} + \frac{hc}{\lambda_2} = hc \left( \frac{1}{\lambda_1} + \frac{1}{\lambda_2} \right).$$

There are no requirements that excitation photons have to be identical but the practical choice is to use the same photons. Hence, the illumination wavelength in TPE is approximately double the wavelength needed for OPE:

$$\lambda_i = \lambda_2 \approx 2\lambda_{OPE}.$$ 

As a result, near infra-red light of wavelength 750-950 nm can be used to excite an UV fluorophore by TPE. A fluorescent molecule in TPE excites to the same energy state $S_1$ as in OPE, hence it relaxes in the same way to the ground state $S_0$ with the emission photon with wavelength equal to the one emitted in OPE approach. Therefore, the emitted photon in a TPE event has a shorter wavelength than the excitation wavelength.

For three or more photons excitation (MPE) (Figure 1-24(c)), the molecule can simultaneously absorb three or several photons and be excited to the same energy state $S_1$. The subsequently emitted photon will have the same wavelength as in the OPE
process. The MPE wavelength is approximately n-times the wavelength needed for OPE:

\[ \lambda_{MPE} \approx n \lambda_{OPE}, \]

where \( n \) is the number of absorbed photons.

In summary, in all cases, of one-, two- and multi-photon excitation, the excited fluorescence molecule will relax from the excitation state to the ground state emitting a photon of the same wavelength. In TPE and MPE, the same fluorescence molecule as for OPE can be excited by a longer wavelength light source which has less energy so will produce less photodamage in the fluorescence molecule.

### 1.3.3 Non-Linear Two-Photon Excitation

A conceptual model and statistical treatment of TPE probability is presented in the literature [61, 72-74] and is summarised briefly in this section.

The probability that a molecule can simultaneously absorb two or n-photons is defined by the probability of finding \( n \)-photons within the molecule volume during the time window of the virtual state. A simple model assumes that the molecule occupies a cube with side \( s \) and an excitation laser beam of wavelength \( \lambda \) passes through this volume. Noting that the beam width is much larger than \( s \), the mean energy accumulated for a time in the cube can be defined by the mean number of photons \( m \) in there:

\[ E_m = m \frac{\hbar c}{\lambda}. \quad 1-4 \]

The cross-section area of the cube is \( s^2 \) and the time needed for all photons to cross the box is \( s/c \). Then the intensity of the laser beam inside the cube, i.e. energy per unit of area per unit time) is:

\[ I = \frac{E_m}{s^2 \times s/c} = \frac{m \hbar c^2}{\lambda \times s^3}, \quad 1-5 \]

where \( s^3 \) is the volume of the cube occupied by molecule. The molecule volume can be calculated using the molar volume \( V_m \) and Avogadro’s constant \( N_A \):

\[ V = V_m / N_A = s^3, \quad \text{where} \ N_A = 6.022 \times 10^{23} \text{mol}^{-1}. \]

Then the mean number of photons inside is:
Calculation shows that for a 780 nm laser beam at peak intensities of the order of \( GW \text{cm}^{-2} \) into a molecular volume \( 10^{-4} \text{ m}^3 \text{mol}^{-1} \), \( m \) is very small (in order of \( 10^{-5} \)).

Using a Poisson distribution [61], the probability can be presented as:

\[
p_n = \frac{m^n}{n!} e^{-m} .
\]

Using only the first term of an exponential Taylor series, the resulting probability for TPE becomes:

\[
p_2 = \frac{1}{2} m^2 .
\]

This conclusion shows that two-photon excitation is a non-linear process with a probability proportional to the square of the mean number of photons passing into the molecular volume or the instantaneous excitation beam intensity. TPE probability is very small due to a low photon concentration within the molecular volume.

### 1.3.4 Two-Photon Fluorescence Efficiency

The necessary photon flux for TPE can be provided by a powerful light source focused by a high-numerical aperture objective to a diffraction-limited point. The efficient excitation source for this purpose is a laser beam which can be either continuous wave (CW) or pulsed. The choice between them is defined by the fluorescence efficiency and requirements for live cell imaging.

The fluorescence emission intensity \( I_f(t) \) is described by molecular cross-section \( \delta_2 \) and relates the temporal characteristic of the excitation light, \( I(t) \), as:

\[
I_f(t) \approx \delta_2 I(t)^2 \approx \delta_2 P(t)^2 \left[ \frac{(NA)^2}{hc\lambda} \right] ,
\]

where \( P(t) \) is the laser power and \( NA \) is the numerical aperture. Hence, the time-averaged two-photon fluorescence intensity per molecule is defined as:

\[
\langle I(t) \rangle = \frac{1}{T} \int_0^T I_f(t)^2 dt = \delta_2 \left[ \frac{(NA)^2}{hc\lambda} \right]^2 \frac{1}{T} \int_0^T P(t)^2 dt .
\]

For continuous wave (CW) excitation \( P(t)=P_{\text{ave}} \) and Equation 1-9 becomes:
\[ \langle I_{f,CW}(t) \rangle = \delta_2 P_{ave}^2 \left[ \frac{\pi (NA)^2}{hc\lambda} \right]^2 \] \hspace{1cm} 1-10

For a pulsed laser beam the time averaged beam power is:

\[ P(t) = \begin{cases} \frac{P_{ave}}{\tau_p f_p} & \text{for } 0 < t < \tau_p \\ 0 & \text{for } \tau_p < t < \frac{1}{f_p} \end{cases} \] \hspace{1cm} 1-11

where \( \tau_p \) and \( f_p \) are the laser beam pulse width and pulse repetition rate. Hence Equation 1-9 is:

\[ I_{f,P(t)} = \delta_2 \frac{P_{ave}^2}{\tau_p^2 f_p^2} \left[ \frac{\pi (NA)^2}{hc\lambda} \right]^2 \int_0^{\tau_p} dt = \delta_2 \frac{P_{ave}^2}{\tau_p f_p} \left[ \frac{\pi (NA)^2}{hc\lambda} \right]^2 \] \hspace{1cm} 1-12

The result of comparison of Equations 1-10 and 1-12 evaluated in Equations 1-13, shows that the same TPE fluorescence signal can be achieved if the CW laser beam average power is kept higher by the factor of \( (\tau_p f_p)^{-0.5} \) compared to the average pulsed beam power. For a typical Ti:S pulsed laser beam with a time duration of 100 fs at a repetition rate of 100 MHz this factor is about 333 which means that a 10 W power CW laser beam can produce the same TPE fluorescence efficiency as a 30mW pulsed laser beam.

Using a low average power during live tissue imaging is preferable and the ultra-short pulsed laser beam is the choice for TPE imaging microscopy. Figure 1-25 shows the relative comparison of the temporal and spatial photons concentration in CW and pulsed laser beam [75].

Figure 1-25 Spatial and temporal compression of photons in (a) continuous and (b) 100 fs pulsed laser beam. If this beam has the same average power (i.e. mean number of photons per second) then the pulsed laser will cause a \( 10^5 \) temporal compression of photons. Hence nonlinear TPE fluorescence is enhanced by \( 10^{10} \) (adapted from [75]).
\[ P_{ave,CW} = \frac{P_{ave,pulsed}}{\sqrt{\tau_p f_p}} \]

\[ (\tau_p f_p)^{-1/2} = (100\text{fs} \times 100\text{MHz})^{-1/2} = (10^{-13})^{-1/2} \approx 0.003 \]

### 1.3.5 TPE Probability and Saturation

The probability that a certain fluorophore excited by a highly focused femtosecond laser beam pulse will simultaneously absorb two photons is given by Denk at al. [70] within the paraxial approximation:

\[ n_p \propto \frac{\delta \sigma P_{ave}^2}{\tau_p f_p^2} \left( \frac{(NA)^2}{2\eta c \lambda} \right)^2, \]

where \( \tau_p \) and \( f_p \) are the laser beam pulse width and pulse repetition rate respectively, \( \eta = \frac{h}{2\pi} \), \( h = 6.6 \times 10^{-34} \text{Js} \) is Planck’s constant, \( c = 3 \times 10^8 \text{m/s} \) is speed of the light in vacuum, \( P_{ave} \) is time-averaged beam power and \( \lambda \) is the excitation wavelength.

To avoid saturation or loss of fluorescence efficiency, every single fluorescent molecule has to be excited during one excitation event and have sufficient time to relax to the ground state. This is ensured by addressing two factors. Firstly, the 100 MHz excitation pulse train, corresponds to a pulse interval of 10 ns which matches with the typical fluorescence excited state lifetime of a few nanoseconds, see Figure 1-26.

![Figure 1-26. Timescale for TPE shown relation in the pulse duration, pulse repetition rate and fluorescence lifetime.](image)

The second factor to avoid saturation relates to limiting the excitation probability. From Equation 1-14 it can be seen that for the chosen fluorescence molecule, laser beam and
high-numerical aperture objective, the probability of absorption depends on the pulsed beam power. For example, in the case of a commonly used fluorescent molecule, such as fluorescein (two-photon cross-section $\delta_2 \approx 38 \text{ cm}^4 / \text{s}$ at 780 nm), excited by a laser pulsed beam (100 fs pulses at repetition rate 100 MHz) and focused by high-numerical aperture objective (NA=1.4), one can obtain:

$$n_a \approx 5900 \times P_{\text{ave}}^2.$$  

For $P_{\text{ave}} = 1, 10$ and 20 mW the probabilities are 0.0059, 0.59 and 2.4 respectively. This shows that with average power above 10 mW saturation of the fluorophore will occur. The TPE probability, Equation 1-13, shows also that to obtain a higher fluorescence signal a laser pulse arriving at the focal point has to be as short as possible.

### 1.3.6 TPE Cross-Section

The quantitative characteristic of TPE is the TPE cross-section. A theoretical description of TPE requires superposition of different theories such as quantum theory, laser field interaction and multi-photon transition. A simplified analysis can be found in [74] and is presented here.

A multi-photon excitation process requires a simultaneous interaction of $n$ photons with the same fluorescence molecule and the absorption to occur in the molecule volume. Therefore two-photon absorption cross-section $\delta_2$ can be estimated to be:

$$\delta_2 \approx \delta_1 \times \delta_1 \times \Delta \tau,$$

where $\delta_1$ is one molecule cross-section and $\Delta \tau$ is the time period for simultaneous absorption. The one molecule cross-section $\delta_1$ is estimated from the molecule dipole transition length to be $\delta_1 \approx 10^{-16} - 10^{-17} \text{ cm}^2$ [74]. The time interval for simultaneous absorption was defined earlier in this chapter and is estimated to be approximately to $10^{-15}-10^{-16}$ s. Therefore, the estimated two-photon cross-section $\delta_2$ is typically of the order of $10^{-49} \text{ cm}^4 / \text{s}$ or 10 $\text{GM}$, where a $\text{GM}$ is the unit for TPE cross-section defined as $1 \text{GM} = 10^{-58} \text{ m}^4 / \text{s} = 10^{-50} \text{ cm}^4 / \text{s}$.

Similarly, the cross-section for n-photon excitation can be derived to give:

$$\delta_n \approx \delta_1^n \Delta \tau^{n-1}.$$  

Selected fluorescent dyes and their one and two-photon excitation parameters are summarised in Table 1-3 (from literature [74, 76]).
From Table 1-3, it can be seen that knowledge of a one-photon excitation cross-section of a fluorescent molecule does not predict the exact value of the TPE cross-section [77, 78] and it has to be measured.

**Table 1-3. Excitation properties of selected fluorescent dyes.**

<table>
<thead>
<tr>
<th>Dyes</th>
<th>One-/Two-photon excitation/ Emission wavelength (nm)</th>
<th>Two-Photon cross-section ($10^{-50}$ cm$^4$s/photon)</th>
<th>Three-photon cross-section ($10^{-83}$ cm$^6$(s/photon)$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indo-I free</td>
<td>345/700/490</td>
<td>3.5</td>
<td>2</td>
</tr>
<tr>
<td>Indo-I Ca$^{2+}$</td>
<td>335/590/405</td>
<td>1.5</td>
<td>6</td>
</tr>
<tr>
<td>Fluorescein</td>
<td>498/782/518</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Fura-2 free</td>
<td>362/700/512</td>
<td>11</td>
<td>20</td>
</tr>
<tr>
<td>Fura-2 Ca$^{4+}$</td>
<td>335/700/505</td>
<td>12</td>
<td>30</td>
</tr>
</tbody>
</table>

Figure 1-27 [1] presents measured results for the TPE cross-section of common fluorophores at different excitation wavelengths. It is essential for an experiment that the peak of two-photon cross-section is not often exactly twice the one-photon absorption wavelength. Also, different dyes have broad excitation spectra which overlap for different fluorophores. This gives another advantage of TPE to be used in microscopy with the possibility of using multiple labelling [79, 80].

The problem in measuring a TPE cross-section is that the absorption rate strongly depends on the spatial and temporal coherence of the excitation light.

### 1.3.7 TPE Microscope: Optical Resolution and Advantages

The optical resolution of a TPE microscope should be worse than in conventional and OPE systems as the excitation wavelength is twice as long. Table 1-4 [81] shows that lateral and axial resolution in a confocal microscope is about 30 % smaller and in TPM is 15 % larger than in a conventional microscope respectively.
Figure 1-27. Measured TPE cross-section for common fluorescent molecules measured with commonly utilized tunable laser sources [1].

However, in practice, due to the non-linear nature of the TP fluorophore excitation and the temporal excitation occurring only at the focal point, the effective resolution of the two- and multi-photon microscopes is often superior [14, 82-84]. Figure 1-28 [14] shows the comparison between excited volumes in OPE and TPE. It is readily seen that the TPE microscope excitation happens only in a small voxel at the focal point of the objective whereas in OPE fluorescence molecules are also excited above and below the focal point [14].

Table 1-4. The lateral and axial resolution in conventional, confocal and two-photon fluorescence microscopes, where $\lambda_{em}$ is the emission wavelength, $NA$ is the numerical aperture of the objective lens and $n$ is refractive index of the specimen [81].

<table>
<thead>
<tr>
<th></th>
<th>Conventional</th>
<th>Confocal</th>
<th>Two-Photon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lateral resolution</td>
<td>$r_{xy} \approx 0.6 \frac{\lambda_{em}}{NA}$</td>
<td>$r_{xy,conf} \approx 0.4 \frac{\lambda_{em}}{NA}$</td>
<td>$r_{xy,TP} \approx 0.7 \frac{\lambda_{em}}{NA}$</td>
</tr>
<tr>
<td>Axial resolution</td>
<td>$r_{z} \approx 2 \frac{\lambda_{em} n}{NA^2}$</td>
<td>$r_{z,conf} \approx 1.4 \frac{\lambda_{em} n}{NA^2}$</td>
<td>$r_{z,TP} \approx 2.3 \frac{\lambda_{em} n}{NA^2}$</td>
</tr>
</tbody>
</table>
This unique advantage of TPE over OPE provides the advantages of applying TPE to microscopy. The depth resolution in TPM is provided by the nature of excitation process rather than the sophisticated detection system and TPM allows recording the optically sectioned images of live tissue without the knife.

This property of TPE reduces also the fluorescence background, which results in increased signal-to-noise ratio and increased image contrast. Also the absence of out-of-focus excitation helps to simplify the TPM detection arrangement compared to a confocal system, avoiding the need for a detection pin-hole as a discriminator and allowing the possibility of using a large area CCD as a detector.

As a result of limited excitation volume in TPM, there is no photobleaching and phototoxicity in the out-of-focus region, which increases imaging time with a more uniform statistical signal overall.

1.3.8 Theoretical Presentation of TPE Voxel

The great advantage of the TPE process for 3D imaging microscopy relates to the very small sub-femtolitre excitation region at the focal point of microscope objective. This is determined by optical diffraction theory [85-87] as per the following.

The intensity distribution at the focal point of the microscope objective with a numerical aperture \( NA = \sin \alpha \) and illuminated with the light at wavelength \( \lambda \) in the paraxial regime is:
\[ I(u, v) = \left| 2 \int_0^1 J_0(v \rho) e^{-i u \rho^2 / 2} \rho d\rho \right|^2, \]

where \( J_0 \) is the zeroth-order Bessel function, \( \rho \) is a radial coordinate in the pupil plane, \( u \) and \( v \) are respective dimensionless axial and radial coordinates normalized to the wavelength \([86, 87]\):

\[ u = \frac{8\pi}{\lambda^2} z \sin^2 \frac{1}{2} \alpha, \quad v = \frac{2\pi}{\lambda} r \sin \alpha. \]

These intensity distributions are called microscope point spread functions (PSF) \([85, 88]\). The fluorescence intensity distribution within the focal region for the OPE is proportional to \( I(u, v) \) and for TPE is proportional to \( I^2 \left( \frac{1}{2} u, \frac{1}{2} v \right) \). The arguments in the latter take into account the excitation wavelength being twice as long \([89]\).

Calculation of the integral in Equation 1-15, over lateral coordinate \( v \), keeping \( u \) constant, shows that the intensity distribution for OPE it is constant along the \( z \) axis and has a half-bell shape for TPE, Figure 1-29.

**Figure 1-29.** The comparison of fluorescence excitation area at the objective focal plane. The blue region together with the red region shows the area by one-photon excitation and the red area only illustrates two-photon excitation area.

According to the approximation of a conical illumination geometry \([1, 85]\) the maximum of the excitation power is at the focal point of the microscope objective and falls off as the square of the distance from the focal point. This means that in TPE the fluorescence intensity decreases as the fourth power of the distance from the focal point.
1.3.9 Calculation of TPE Voxel

The illuminated volume is described by the illumination intensity distribution or illumination point spread function (PSF) at the objective focal point. The TPE excitation voxel is described by the square of the illumination PSF due to the two-photon non-linear excitation process (Figure 1-30 [14]).

Figure 1-30. Illumination and square of illumination PSFs: (a) cross-section in lateral and axial planes, and (b) intensity profiles for OPE and TPE (adapted from [14]).

The approximate calculation for three-dimensional volume associated with TPE is presented in the literature by Zipfel etc [14] and is

\[ V_{\text{TPE}} = \pi^{\frac{3}{2}} \omega_x^2 \omega_z / 0.68, \]

where \( \omega_{xy} \) and \( \omega_z \) are the diffraction-limited lateral and axial \( 1/e \) radii of intensity-squared profile respectively which can be calculated as:

\[
\omega_{xy} = \begin{cases} 
\frac{0.320 \lambda}{\sqrt{2} NA} & \text{NA} \leq 0.7 \\
\frac{0.325 \lambda}{\sqrt{2} NA^{0.91}} & \text{NA} > 0.7 
\end{cases}
\]

\[
\omega_z = \frac{0.532 \lambda}{\sqrt{2}} \left[ \frac{1}{n - \sqrt{n^2 - NA^2}} \right],
\]

where NA is the objective lens numerical aperture and \( n \) is the refractive index of the medium.

From Equation 1-16, calculated TPE lateral and axial spot sizes and TPE volume are shown graphically in Figure 1-31. For example, for an NA=1.2 objective lens focusing 800 nm illumination beam, the effective TPE volume is 0.08 \( \mu m^3 \).
Figure 1-31. Two-photon excitation resolution for different excitation wavelengths. The vertical axis is the values for lateral and axial spot sizes (\(\omega_x\) and \(\omega_z\)) in \(\mu m\) and for the volume in \(\mu m^3\).

1.3.10 Excitation Source and TPE Efficiency

The ultra-short femtosecond pulsed lasers are routinely used in multi-photon biological and nano-structuring applications. The current sources are available in a very wide range of wavelengths, tunability and pulse duration [64, 90-95].

The pulse parameters define the TPE efficiency and are essential for TPM live biological tissue imaging [95, 96]. The TPE probability is proportional to the average power and inversely proportional to the pulse time. For the same average power, shorter pulses will have a higher peak power. To keep the peak power high enough to maximise the fluorescence excitation the pulse duration has to be as small as possible for a given average beam power. However, due to the group velocity dispersion (GVD) [97, 98] and lens curvature dispersion the pulse temporally and spatially broadens in the microscope system decreasing TPE efficiency.

1.3.10.1 Titanium-Sapphire Laser

The mode-locked Ti:S laser which can produced well collimated 100 femto-second pulses with a repetition rate of 80 MHz is the excitation source utilized in multi-photon fluorescence microscopy [91, 99].
A Ti:S (Titanium:Sapphire) laser is a solid state laser with a lasing medium of Al₂O₃ (sapphire) which is doped with titanium Ti³⁺ ions. In practise, this crystal is produced by introducing Ti₂O₃ into the melt of Al₂O₃. As a result some of the titanium ions take the place of the aluminium cations. An electronic state of the titanium ion has broad vibrational energy levels and can produce a wide emission spectrum. The meta-stable energy states of the titanium ion Ti:S crystal are excited by absorption of blue-green 400 nm to 650 nm photons, which results in emission of visible to near-infrared (NIR) photons [100]. Figure 1-32 [101] shows the absorption and emission spectrum of the Ti:S crystal.

![Figure 1-32. Ti:Sapphire energy level diagram with absorption and emission spectra. Interaction of the titanium electron with the Al₂O₃ produces a wide spreading of the energy levels and results in broad tuning (adapted from [101]).](image)

In the laser, the Ti:S crystal is initially pumped by an argon-ion laser with a wavelength of 514.5 nm and power between 5 and 20 W. The average output beam power is from 1W to 2W at a wide range of NIR wavelengths from 750 nm to 1050 nm as shown in Figure 1-33 [102].
1.3.10.2 Group Velocity Dispersion

As ultra-short pulses pass through optical elements in a microscope, they stretch spatially and temporarily due to the group velocity dispersion (GVD) caused by the large optical bandwidth of the ultra-short pulses and the wavelength dependency of the refractive index of the lens material [103]. Femto-second pulses have a certain spectral bandwidth $\Delta \lambda$ which determines the shortest possible pulse time duration [104]. The actual pulse duration is determined by the shape of each pulse. For Gaussian shaped mode-locked pulses, the minimum possible FWHM pulse duration $\tau_p$, is expressed by the time-bandwidth product as:

$$\tau_p \Delta \omega \approx 0.441,$$

where $\Delta \omega$ is the frequency width oscillating in the pulse. The spectral width of a bandwidth limited Gaussian pulse can be calculated by [97]:

$$\Delta \lambda = 0.441 \lambda^2 / c \tau_p,$$

where $\lambda$ is the central wavelength and $c$ is the speed of the light. Therefore, a 100 fs pulse centred on 800 nm wavelength has a spectral bandwidth of about $\Delta \lambda \approx 10$ nm.

The spectral bandwidth is significant and results in dispersion through the optical system. This occurs because the longer wavelength components travel faster through the media and vice versa (Figure 1-34).
Dispersion therefore results in a broadening and distortion of the temporal profile of the pulse and causes a decrease in the peak power which causes the decrease of the TPE fluorescence efficiency.

The expression for temporal dispersion of the bandwidth limited pulse is:

$$\tau = \tau_0 \sqrt{1 + \left[ \frac{4\ln(2)\beta_2 z}{\tau_0^2} \right]^2},$$

where $\tau_0$ is the initial unchirped FWHM input pulse duration, $\tau$ is the resultant pulse length, $\beta_2$ is the dispersion parameter of a material and $z$ is the length of the light path inside the material. The dispersive parameter $\beta_2$ is defined by the second derivative of the refractive index with respect to the wavelength:

$$\beta_2 = \frac{\lambda_0^3}{2\pi c^2} \left. \frac{d^2 n}{d\lambda^2} \right|_{\lambda_0}$$

where $c$ is the speed of the light and $\lambda_0$ is the centre wavelength for pulse. For example, 30 fs pulse at 800 nm after passing through 1 cm of fused silica with GVD dispersion of $362 \text{ fs}^2/\text{cm}$ will spread to 45 fs [106]. The GVD dispersion parameter depends on the type of the lens material and for example, dispersion for the Schott glasses BK7, LFS and SF2 are 453, 870 and 1193 $\text{ fs}^2/\text{cm}$ respectively [106].

From Equation 1-17, the shorter input pulses have a higher pulse broadening effect in the media. Figure 1-35 [107] demonstrates the width of a Gaussian pulse at 800 nm before and after propagation through 20 mm glass. For 100 fs to 200 fs pulses, the GVD broadening is relatively small and usually this range is used in TPM.
Figure 1-35. Broadening of a femtosecond pulse at 800 nm after propagation through 20 mm of BK7 glass [107].

For an optical system with many different optical components, the dispersion parameter of the whole system is the sum of all optical elements dispersion parameters. In a microscope, an objective is highly corrected optics which contain significant amount of glass and therefore disperse the pulse significantly. GVD can be compensated by a prechirping technique. There are various pulse compression techniques [108, 109] to reduce dispersion. One of these is pre-chirping the pulse: the initial pulse must be negatively pre-chirped in order to compress the pulse to its minimum value before entering the media. A negative chirp can be produced by passing the pulsed beam through an optical system containing prisms or gratings. Prism stretchers, prism-grating hybrid stretchers or grating stretchers can produce low, medium or high effective dispersion, respectively. In all pre-chirp elements shorter wavelength components travel faster than longer ones relative to the centre wavelength. When two prisms or gratings are used to prechirp the pulse, the distance between them will determine the amount of negative dispersion induced to balance the positive dispersion produced in the microscope system. In this case the pulselength delivered to the focal plane will be minimally temporally distorted.

In conclusion, as TPE probability is inversely proportional to the pulse duration at the focal point, special considerations for the pulse time broadening in the lens materials especially the objective lens have to be considered [104, 110, 111].
1.3.10.3 Pulsefront Distortion in Lenses

Another factor that can lead to pulse distortions and decreasing pulse peak power at the objective focal point is spherical and chromatic aberrations in lenses. Spherical aberration is due to the different radial portions of the beam across the pupil arriving at different times in the focal plane. Chromatic aberration is caused by different spectral components of the bandwidth limited pulse [97, 104].

The propagation-time difference can be estimated using the following expression:

$$\Delta \tau = -\frac{\lambda_0}{2c_0 f_o} r^2 \frac{df}{d\lambda \mid_{\lambda_0}}$$,

where $df/d\lambda$ is the chromatic aberration, and $f_o$ and $r$ are the focal length of the lens system and the radius of the pupil, respectively.

To minimise the propagation-time difference effect, a system should be achromatic and use less dispersive elements as possible.

1.3.11 Pulsed Laser Beam Parameters

The ultra-short laser pulse and high-numerical aperture objective used in TPM periodically creates a very high temporal and spatial photon concentration at the focal point. This arises from the very high peak power of any single pulse while maintaining comparatively low average power [112] due to the mode-locked laser behaviour. The calculation of beam parameters are presented in Appendix A.

For example, a Ti:S laser operating at 1W average power and emitting at a central wavelength of 800 nm with 100 fs pulse duration at an 80 MHz repetition rate, has an average of ~$10^5$ photons/pulse, 12.5 nJ energy per pulse and will produce a hundred kilowatt peak power. Focused by a high numerical aperture objective lens, the ultra-short pulse produces a hundred terawatts per cm$^2$ total radiance at the focal point.

1.3.12 Application of TPM

Since the introduction of two-and multi-photon excitation microscopy (MPM) [70] this technique has found many successful applications in biomedical research and clinical investigation [113-115]. The number of applications is growing constantly and stimulates developments of the technique and the technological components to enhance
resolution and increase imaging. With rapid development in fiber lasers and sub-20 fs pulse sources, the two- and multi-photon microscopy with optimized signals and reduced costs drive the growth of MPM micro-endoscope technologies [116, 117].

The areas of application of MPM in biomedical research include dermatology [71, 118-120], neuroscience [121-123], diverse ocular tissues [113, 124], cancer research and tumour biology [125, 126], cardiovascular systems [79, 99, 127], lung fibrosis [128] and immunology [129-132].

There are emerging technologies applying the two-photon excitation process including 3D optical data storage [133-135], femtosecond laser micromachining [136, 137] and micro-fabrication [138, 139].

1.3.13 Conclusion

TPM is the preferred method for imaging deep within live tissue with minimal disturbance to the sample. Two- and multi-photon fluorescence excitation is a non-linear process which requires a very high temporal and spatial photon concentration. It usually employs a mode-locked solid-state Ti:S pulsed laser which produces tunable wavelengths from 700 to 1050 nm, of ~100 fs pulse width at ~80 MHz repetition rate, and 1-2 W average power [12, 16, 90, 140]. A high numerical-aperture microscope objective lens focuses the beam to a ~300 nm diameter spot with power at the focal point in the order of 100 TW/cm² [115] which is dangerous for biological sample as well as for fluorophore itself [141]. Fortunately, it can scan biological samples with minimal damage due to the very short pulse time duration. TPE and MPE techniques in many respects give better performance than the OPE method. These are:

- NIR light used to excite UV fluorophores allows deeper penetration inside biological tissue due to less scattering. TPM can provide images from depths of 500 microns.
- TPE produces a tiny sub-femtolitre excitation voxel about the focal point which allows 3D imaging without a knife. Photobleaching and photodamage are restricted to this small volume. As near-infrared light photons have lower energy than ultra-violet photons TPE produces less photodamage to biological live cells.
• Due to the non-linear nature of TPE there is a very strong emitted signal from the focal point and no out-of-focus excitation. This simplifies image detection path as well as allows longer imaging times.

1.4 Fast Imaging Techniques in Multi-Photon Microscopy

The nervous system is organised in a three-dimensional cellular network and resolving the spatio-temporal activity pattern of local 3D networks is an unrealised goal. In this section, examples of some approaches to fast three-dimensional scanning will be shown.

Standard two- and multi-photon fluorescence microscopes (TPM and MPM) are single-beam scanning laser systems where an excitation focal spot is moved in a 2D raster pattern across a horizontal plane within a sample. Fluorescence signals from this plane are collected point-by-point by a detector and a computer builds a 2D image. The sample is then translated along the optical axis by a moving stage or an objective moving mechanism focuses to a different plane and the microscope system records the image of another sample slice. A 3D image of the sample volume is then reconstructed from the stack of 2D images. Usually, the lateral imaging speed is 2 frames per second for a 512 by 512 pixels for a 50-200 μm square area image [142] but this is not fast enough to image nerve conduction. For example, it will take 5 seconds to produce a 3D image of 10 layers and an observer will miss millisecond activities inside the live sample. Live tissue imaging therefore requires faster imaging to understand molecular dynamics in their functional context [143-145].

Another aspect of two-photon excitation is that the probability of two-photon excitation is low and the fluorescence signal is much less than in the linear excitation process. There could be several strategies to counter this. The fluorescence signal can be increased by increasing the excitation laser intensity however this is damaging to live tissue [96, 146, 147], or by increasing the fluorophore concentration in the sample but with the danger of chemical damage to living specimens [131, 146, 148].

The practical choice for increasing the scanning speed is to employ fast 8 kHz resonant scanner which will increase the line scanning time to 125 μsec or even to 62.5 μsec. Simultaneous signal acquisition from different fluorophores applied to label different cell structures allows best use of particular cell lines with multiple detectors in parallel and further increase the imaging rate. Parallelization of illumination is another approach to fast and light-efficient 3D imaging with good resolution with the advantage of also
decreasing the excitation time [9]. Multifocal imaging requires a fast detector capable of detecting the fluorescence signals of all foci.

In this section, a review of modern concepts towards, and practical results of, increased imaging rate in multi-photon microscopy is presented.

### 1.4.1 Using a Fast Resonant Mirror

A fast resonant scanner was used for the first time in a confocal microscope constructed by Tsien [149]. It was then used to construct a real-time imaging confocal [150] and two-photon excitation [151] microscope. Ian Parker from Irvine University gives the description and application of a home-made setup [38] and this has been duplicated by several labs including the Krummel Lab [152]. The resonant mirror increases the imaging rate to 30 Hz for 500 lines in a 2D image. The system is also constructed to collect up to four different fluorescent dye signals simultaneously with separate detectors which introduce another dimension into the fast imaging technique.

The overall design is illustrated in Figure 1-36 and consists of a scan head with two scanners orthogonally placed to each other, a detector head with four PMTs and a beam periscope. The scanning mirrors and drivers are GSI Lumonix. Acquisition is controlled by a personal computer with a video card and custom made software.

![Figure 1-36. Schematic diagram illustrating the two-photon excitation microscope with the resonant mirror scanning. The red coloured path represents the excitation beam path and the yellow is the fluorescence pathway [153].](image)

The horizontal scan is completed by a resonant scanner CRS (Counter Rotation Scanner) at 8 kHz frequency and a conventional galvanometer mirror is used for y-
scanning. The resonant mirror moves by sinusoidal angular displacement at the scanner’s resonant frequency which provides the ability to image a sample during the mirror’s flyback doubling the line scanning to 16 kHz frequency. The issue with the sinusoidal driven mirror scanner, described previously, is the different imaging speed at the edges compared to the middle line scan, which makes the image look longer than in reality. This is corrected in the custom software developed by Sanderson [154]. Simultaneous multi-focal imaging is achieved by Ian Parker using the same design and imaging using three cameras at three different distances from the focal plane of the microscope [155] and this is shown in Figure 1-37 [155].

![Multi-focal imaging system](image)

**Figure 1-37.** Multi-focal imaging system: three cameras Cam 1, Cam 2 and Cam 3 detect the two-photon fluorescence signal from three focal planes. The insert (a) shows the axial x-z point spread functions for the 3 cameras obtained by focusing through a sub-resolution (100 nm) fluorescent bead [155].

### 1.4.2 Multi-Beam Scanning

An excitation laser beam focused by a microscope objective produces only one excitation point in a fluorescent sample and hence only one emission signal from a sample. If the excitation beam could be split into \( n \) beams, the \( n \) beams will focus within a sample and excite \( n \) different points to produce \( n \) emissions. Therefore the image data
collection rate of a sample area scanned by \( n \) beams simultaneously can be increased by the number of beams used. In two-photon excitation microscopy, the maximum excitation power which can be safely used for scanning in a live specimen is 3 to 10mW. The laser typically produces 1 to 2 W average power, making the efficiency of the laser in TPM less than 5%. Multiple beam imaging increases the beam efficiency of the expensive Ti:S laser employed.

Many ways of multiplying the laser beam have been successfully developed in two-photon or Multifocal Multiphoton Microscopy (MMM). This section reports different approaches to MMM including employing a rotating microlens disk array, using a combination of beam splitter and reflective mirrors, and using a diffractive optical element.

### 1.4.2.1 Rotating Microlens Disk

One of the first approaches to MMM was reported by Bewersdorf, J., R. Pick, and S.W. Hell [142] whose microscope employed a microlens array as shown in Figure 1-38 [142].

![Figure 1-38](image)

Figure 1-38 Schematic presentation of multi-beam microscope: L1 and L2 form a beam expander, L3-L5 are intermediate optics to image the array of foci at the virtual prefocusing PFP plane into the focal plane of the objective; ST stop the outer part of Gaussian laser beam profile; ML-microlens disk; M- optional mirror; F- short-pass filter; DM-dichroic mirror. Inset shows the rotating microlens array. Each lens is 460 \( \mu \)m diameter and 6.0 mm focal length and are positioned in the rotating disk to form a 10 row spiral of ~6 \( \mu \)m beam waist spots in the prefocusing plane [142].
The disk is rotated at 75 Hz and completes three independent scans of the focal plane per cycle which gives $3 \times 75 = 225$ scans/sec or approximately 4 ms/image. The imaging rate is determined and restricted by the detector, a CCD camera. During the experiment, 12 layer optical sectioning of an autofluorescent pollen grain was demonstrated with 4 μm layer separation using an oil immersion lens and 780 nm excitation. The images, 384x384 pixels over 24 μm x 24 μm, were recorded in 100 ms. A maximum imaging depth of only 40 μm in scattering pollen grains was achieved due to long pulse duration of ~240 fs and these were stretched further due to group velocity dispersion by refractive optical elements in the path.

Straub and Hell [115] improved the microlens disk design to prevent light from the intermediate space between the microlenses in an array entering the microscope and introducing unwanted fluorescence excitation. Images of fluorescence spots for stationary and rotating microlens disk were taken (Figure 1-39) and it can be clearly seen that the rotating disk yields a dense coverage of the focal plane. Due to the elliptically shaped laser beam profile the images show that about 80% of the illumination laser beam intensity was dropped along the horizontal axis and ~ 60% along the vertical axis. Moreover, a ~50% non-uniformity between the emitted fluorescence foci was measured by imaging a uniform fluorescent plane.

![Figure 1-39](image)

*Figure 1-39 The images of fluorescence spots in a uniform sea of Rhodamine 6G dissolved in immersion oil of a (b) resting and (c) rotating microlens disk. A laser wavelength of 765 nm was used with a 100x NA 1.4 oil immersion objective and 1.6x tube lens [115].*

### 1.4.2.2 Time-Multiplexing MMM

A fundamental problem in parallelized MMM is the compromise between the number of the foci and the optical sectioning ability. Reducing the distance between the parallel
foci is limited due to possible interference between the neighbouring foci intensity point-spread function above and below the focal plane [156]. Wave optics calculations for an aberration free MMM show that for interfocal distances between foci smaller than \( \sim 7 \lambda \), or \( \sim 5.6 \mu m \), axial resolution decreases and this is observed as a haze in the image [156]. Therefore, the smallest possible interfocal distance recommended is from 5 to 10 \( \mu m \) and this was achieved in previous designs.

The problem between the parallelization of beamlets and the axial resolution in MMM can be solved by using the technique proposed by Anderson et al [157] of introducing a temporal separation of at least one pulse duration between the neighbouring foci. This microscope setup, known as a Time Multiplexing Multifocal Multiphoton Microscope (TMX), is shown in Figure 1-40 and is similar to the MMM systems with improved microlens design described above.

![Figure 1-40. Setup of Time Multiplexing Multifocal Multiphoton Microscope [156].](image)

On the back side of a rotating microlens array two rigid glass disks of 150 \( \mu m \) thickness each are mounted. A pattern of holes on these glass disks is organized in such a way that one third of the beamlets pass through both glass disks, while another third passes through one glass and the final third part does not pass through any glass at all. This produces neighbouring foci which are belong to different classes A, B and C as illustrated in Figure 1-41 [157]. Because of the introduced time delay the foci do not interfere with each other.
For a Ti:S pulse laser beam of 800 nm wavelength, 150 μm thickness of glass causes a delay of 750 fs duration and this is sufficient to separate neighbouring beamlets in time. The speed of light in vacuum is $3 \times 10^8$ m/sec = 300 nm/fsec. For light passing through glass, the speed decreases according to the refractive index of the glass used. For glass with $n=1.5$ the velocity in the glass becomes $200 \text{ nm/fsec}$.

$$
\Delta t = \frac{\text{thickness}}{\text{velocity}} = \frac{150 \mu m}{200 \text{nm/ fsec}} = 750 \text{fs}
$$

The distance between the neighbouring foci without TMX was measured at 5.12 μm (or 6.4λ) and after introducing TMX, the distance between the same neighbouring foci was increased by $\sqrt{3}$ times to 8.87 μm (or 11.1λ). The intensity amplitude for the beamlets of all three classes is nearly the same. The benefits of TMX for three dimensional imaging are shown by imaging spikes of a pollen grain, ~30 μm in diameter, by MMM and with TMX delay: Figure 1-42 (a) and (b) show x-y images taken at the same focal plane, and Figure 1-42 (d) and (e) compare 3D reconstructed images of the same spiky pollen grain. It is clear that the TMX method improves the image and eliminates the haze on images taken without TMX.

In conclusion, the multi-lens method of MMM imaging increases the imaging speed and the temporal separation of excitation foci in MMM and helps to resolve the problem of interfering neighbouring foci. It improves the axial resolution in 3D MMM as well as extends the lateral resolution of scanning microscopy.
1.4.2.3 Combination of Beam Splitter and Mirrors

Another approach to multi-beam scanning is to use a combination of mirrors and a beam splitter to give advantages, such as high light transmission, uniform intensity of the beamlets, flat optical surfaces limiting aberrations, and temporal separation due to different optical paths.

A multifocal beam generator proposed by Lévéque-Fort and Fontaine-Aupart for fluorescence lifetime imaging [158] uses four high-reflective mirrors and a 50 % beam splitter (Suprasil, 60x40x2 mm, p polarization) as shown in Figure 1-43. The incident beam is divided by a dichroic mirror into two beams which are then, reflected by two mirrors back to the dichroic mirror to produce four beams and finally reflected by the other mirrors to produce 8 beams in the same plane.
Figure 1-43. Beam generator including a 50 % p-polarisation beam splitter and four high reflective mirrors, to generate 8 beams in the same plane. The distance between beams depends on the distance between mirrors and the beamsplitter, and can be changed from 3 μm to 6 μm by the moving mirrors M1-M4 respectively to the beamsplitter [158].

Compared to the one beam scan method, the scanning rate increases but the imaging speed is limited by the CCD camera used (Hamamatsu, Orca ER) and recorded at 16.4 fr/sec.

The performance of the system was demonstrated by comparing intensity images and time-gated images acquired from autofluorescent urothelial cell imaging using the OPE and TPE method as shown in Figure 1-44. One image is acquired in 1.5 s. The whole 3D lifetime map of 10 images at a field of view 100 μm x 200 μm took 15 s.

Figure 1-44. Autofluorescence images of an urothelial cell: (a) by one-photon excitation and (b) by multifocal two-photon excitation [158].

The multifocal beam generator for up to 64 linearly array foci is illustrated in Figure 1-45 [159].

The beam generator consists of a beamsplitter and five mirrors that generate two sets of 32 beamlets, which combine into a linear array of 64 beamlets with two differently polarized states (s-p-s-p,…). The distance between foci is adjustable from 0.4 to 2 μm. The imaging rate is limited by the CCD camera read-out time. In this design, prechirp optics are used to compensate for pulse time dispersion in the optical elements.
Figure 1-45. Setup of MMM with 64 beamlets generator. In the right top corner – two-photon excited fluorescence of Rhodamine 6G dissolved in water [159].

The beamsplitter can generate either a linear or an area array of beamlets by coupling the output of the first stage beam splitter via a periscope to the input of the second stage. The number of beamlets produced may be varied from 64 to 32, 16, 8, 4, 2 or even a single beam by moving the beamsplitter mirror in and out of the optical path. The distance between foci can be decreased until the foci overlap each other and produce a short homogeneous line which can be used for line imaging without scanning. Scanning by a linear array has the additional advantage over conventional imaging in that it can scan in the XZ direction as well as in the XY direction (see Figure 1-46).

Figure 1-46 Fast scan imaging in the X-Z plane and X-Y plane [159].
A similar design using 8 x 8 array of foci to scan a sample reported by M. F. T. Nielsen, D. Hellweg and P. Andresen [160]. The acquired image of all focused beams in the homogeneous solution of laser dye (Coumarin 500) in immersion oil is shown in Figure 1-47 [160]. The uniformity of the beams was measured at 60 % maximum deviation of the mean value.

Figure 1-47. Image of the fluorescence of all beams in a homogeneous fluorescence solution [160].

In conclusion, there are several advantages in using the combination of reflectors and a beamsplitter. A temporal delay is introduced between the beams due to the different optical paths and this helps to avoid interference between the foci. The distance between excitation foci can be easily controlled by varying the distance between the mirrors employed and the beamsplitter. This is useful to avoid cross talk between scattered fluorescence light inside thick biological samples [156, 161] and is advantageous in placing all foci together to get an image of the sample without scanning.

The overall transmission of the laser beam intensity by the beam generator is up to 90 %. The power of each focus at a sample is 1-10 mW.

1.4.2.4 Diffractive Optical Element

The number of beams used for excitation can be increased by employing a diffractive optical element (DOE) as a beam generator. An evolution in DOE technology and particularly an improvement in the structure for fan-out diffractive elements has come
close to theoretical diffraction efficiency with minimal error in array uniformity giving a simple and cheap instrument to create a fast scanning system with uniform multi-beam. In the design developed by Sacconi shown in Figure 1-48 [162], a 4 x 4 fan-out diffractive element together with galvo scanners is used to scan a sample with 16 diffraction-limit foci. The distance between foci is 25 μm. The diffraction efficiency of the DOE was measured at 75 % and the uniformity of focal intensity was within 1 %, see Figure 1-49. The power of each focus was 1 mW. Due to DOE, the 150 fs pulse temporally spreads to 190 fs. The backscattered two-photon fluorescence emission light is collected by the CCD camera (Penta MAX, Princeton Instrument), the acquisition time of which is synchronised with the scanning time of galvo mirrors. The 100 μm x 100 μm size images of a cytoskeleton of a bovine pulmonary artery endothelial cell marking in Texas Red with 512 x 512 resolution were recorded at 100 ms per image which is much faster than a single beam excitation.

Figure 1-48. Experimental MMM setup, employed 4 x 4 fan-out diffractive element and two galvo mirrors (VM500, GSI Lumunics). L1, L1-telescope lens pair with 2x magnification; GX and GY – galvo scanning mirrors; L3 and L4 – telescope lens pair; L5 and tube lens TB – telescope lens pair with 4x magnification; DM-dichroic mirror; BF – IR blocking filter. Mode-locked Ti: Sa laser at 800 nm with 100 fs pulse duration at 80 MHz repetition rate [162].
1.4.3 Stochastic Wavefront Scanning Multi-photon Multifocal Microscopy

Conventional raster waveform scanning MMM gives oversampling at the edge of a scan units’ area due to the mechanical response of the scanner, and this leads to a grid-work appearance in the image. Scanning with a rotating microlens disk does not have this problem but random access to the scanning area is restricted. The Stochastic Scanning Multiphoton Multifocal Microscope (SS-MMM) claims to produce a more uniformly sampled image compared to a multi-beam raster scanning and acquires images 1000 times faster than a single beam raster scan engine.

The SS-MMM demonstrated by Jureller et al. [163] and schematically shown in Figure 1-50 [163], uses a diffractive optical element to create a 10 x10 hexagonal array of uniform <5 % 100 beamlets (see Figure 1-50 (b)). The excitation laser source is a Ti:S laser producing a 70 fs pulsed beam at 930 nm. The 6 μm distance between foci was selected to prevent undesirable interference effects [156]. The power of individual foci at the focal plane is from 0.5 mW to 10 mW.

The image was detected by an 1000 x 1000 pixel TE-cooled EMCCD camera (Andor DV885) at 10 MHz read-out rate or 9 fr/sec full-frame acquisition rate.
Figure 1-50. (a) SS-MMM setup. DOE-binary diffractive optical element; L1(f=20cm) and L2 (f=35cm) are NIR achromate lenses; MB- mechanical block to stop the residual undiffracted beam at the focus of the telescope; L1-L2; L3- NIR achromate scan lens(f=15cm); L4-tube lens (f=20 cm).
(b) two-photon fluorescence image of the multifocal array in a solution of Rhodamine 6G [163].

The two galvo scanners are driven by triangular raster or white noise waveforms synthesized by a NI-6052E DAQ card. The image was symmetrically sampled within a given integration time. The detector and scan engines are not synchronized and that allowed an asynchronous frame readout mode to be used. Two single images of the same area of a monolayer of 500 nm diameter fluorescent microspheres were obtained by a raster and stochastic scanning approaches and are shown Figure 1-51 (a) and (b) respectively.

The periodic bright boundaries in Figure 1-51 (a) clearly show the over-sampled areas of a single beam with raster scanning. At the same time, the stochastic scanning of beams, as shown in Figure 1-51 (b) does not display these artifacts. In both images, a very similar reduction of the intensity at the edges of whole frames can be seen and this is due to the increase of excitation spot size at field edges compared to the illumination focus resulting in a reduction of TPE.

The resulting brick effect in the image of raster scanning are explained by the simulation in Matlab, see Figure 1-52. In the raster scanning, the edges are oversampled two times more than the average but the stochastic histogram shows that the pixels in the centre are almost all sampled while those at the edges are not sampled enough.

The estimated 100 fr/s image rate is the limit of stochastic scanning with this galvanometer and could be increased with a faster detector.
1.4.4 Three-Dimensional Continuous Line Scanning Two-Photon System

Increasing the imaging speed for 2D requires fast scanning along the optical axis. Different devices such as piezoelectric focusing element [150], variable-focus lenses [164], deformable mirrors [165] and acousto-optical deflectors [166] have been applied for scanning along the third optical axis. But none of these approaches has yet produced 3D network imaging.

Göbel [167] introduced a two-photon 3D continuous line scanning microscope system which is shown schematically in Figure 1-53.
The axial scan is performed by two galvo scan mirrors and the scanning along the optical axis is based on piezo-electrically introduced mechanical vibration of the microscope objective where the piezo element is driven by a sinusoidal signal.

Amplitude of 200-300 μm in the sample is achieved by a 10 Hz vibration of the microscope objective lens. A Ti:S laser excitation source provides 100 fs pulses at 870 nm wavelength. The detector is a PMT and this sample signals at 10 MHz. TPE excitation reduces with focal depth due to the scattering properties of the sample [168] and a Pockel’s cell is used to compensate the excitation loss during 3D scanning in thick biological tissue.

Different possible ways to have a continuous 3D scan trajectory with sinusoidal movement of the objective were calculated and then tested in order to provide optimal volume coverage in measuring the distribution of cells in the sample volume. A pixel dwell time of 10 μs was constant for the entire scan line. These lines are shown in Figure 1-54 [167]. The simplest is a spiral pattern in the x-y plane; the others are square-spiral, Lissajous trajectory and “user-defined”. A user-define scan trajectory was calculated to ensure the acquisition of a signal from multiple points of interest.
The volume coverage using different 3D line scan modes was analysed using a scanning volume of $0.25 \times 0.25 \times 0.2 \, mm$ with 10 μm beads at a density of about 32000 beads/mm$^3$ and the fraction of cubes visited by the scan calculated. Results are shown in Table 1-5.

**Table 1-5. The results of scan by different scan modes**

<table>
<thead>
<tr>
<th>Scan mode</th>
<th>Spiral</th>
<th>Square-spiral</th>
<th>Lissajous</th>
<th>User-defined</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 μm cube</td>
<td>50 %</td>
<td></td>
<td></td>
<td>40 %</td>
</tr>
<tr>
<td>30 μm cube</td>
<td></td>
<td>90 %</td>
<td></td>
<td>80 %</td>
</tr>
<tr>
<td>Fraction of fluorescent beads hit by the 3D line scan</td>
<td>$71 \pm 3.5%$</td>
<td>$69 \pm 3%$</td>
<td>$44 \pm 1.5%$</td>
<td>$92 \pm 3.5%$</td>
</tr>
</tbody>
</table>

From these results it can be seen that the best sampling occurred during a spiral and user-defined line. Demonstrated results are from the calcium imaging of neuronal and glial dynamic networks from several hundred cells in the rat neocortex in vivo with 10 Hz temporal 3D resolution.

The complicated part of this fast scanning TPM is the creation of the 3D continuous scan line with synchronisation. A mechanically moving objective lens can give error...
due to mechanical component deviation in addition to possible aberrations due to mechanical wobbling of the microscope mechanical axis.

1.4.5 Conclusion

Fast imaging multi-photon microscopy is a new technique in biological and medical research. It helps to image dynamic processes in live cells, resolve communication mechanisms and look at the tiny cell structures within their natural environment. Biological events are very fast and to catch them the imaging rate has to be appropriate. Many different approaches to speeding the imaging have been taken.

Using a rotating microlens array disk increases the number of excitation foci and can increase the scanning speed up to 325 scan/sec. The microlens array suffered from low light transmission and lens aberrations. The cross-talk between the close foci can be eliminated by introducing the time-delay between foci but there is 50 % non-uniform fluorescence emission across the scan field. A combination of beamsplitter and mirrors produces a controlled number of up to 64 beamlets with uniform intensity of 0.26 of the mean signal, intrinsic time-multiplexing due to slightly different optical paths, absence of optical aberrations, and convenient control on the inter-focal distance and field of view. Diffractive optical beamsplitters offer compact, stable and efficient devices of producing >95 % uniformly illuminated multi-foci at the objective focal plane.

Scanning multi-beam with stochastic wavefronts can help to avoid this oversampling effect at the beamlets scan edges. Using a fast resonant scanner increases the imaging speed to video rate. A 3D continuous line scanning effect provides more uniform scanning and increases the imaging rate.

MMM has some limitations however. The overall average laser power of all multiple beams in a sample has to be carefully controlled particularly for a smaller scan area. In addition, cross-talking arises from the overlap of the focal fields of the multiple foci. Finally, the imaging speed is restricted by the CCD camera employed.

Proper function of any living organ is organized in a three-dimensional cellular network. Their dynamic interaction is particularly important in the brain where several hundred neurons and cells circuits process memory formation and generate motor commands. Direct measurements of local network activity in the intact brain have been limited. Optical microscopy, especially two-photon, allows functional imaging with cellular resolution. Recent advances in TPM allow imaging of some of the activities in
3D. This is possible by the reconstruction of a two dimensional images collected from different focal depths. The acquisition time of a stack of images is sufficient and depends on the scan area and number of planes. Typically it is 0.1 Hz and slows down due to the slow step focal-changing mechanism of the microscope. A continuously moving microscope objective mechanism increases imaging rates to 0.4 Hz. [169]. However it is still too slow to resolve neuronal activity which in spike-pattern detection based on calcium transients for example typically happens in few hundred milliseconds meaning the volume imaging rate must be more than 10 Hz.

1.5 Summary

In summary of Chapter 1, there are several scan engines arrangements applied in biomedical microscopes and their development have been driven by the requirements for increased optical performance of the instrument employed. A two-photon microscope is a favourite instrument in life sciences for high resolution imaging of thick intact tissues. It has very high optical performance requirements, enhanced contrast and sensitivity, and increased imaging rate which are highly dependant on advances in optical technology including scan engines in the illumination path of the microscope arrangement.

Distilling opportunities from this literature review point to device featuring speed of acquisition, excellent optical performance, flatness of field, linearly of scan, the preservation of diffraction limit across the scan field, low wavefront distortion and particularly, low temporal distortion.

These requisite features point towards further optical development of the scan engine and careful thought about properties brought parabolic optical elements forward as a solution. This thesis then took the direction of the design and investigation of a four mirror scan engine incorporating parabolic reflectors.
Chapter 2  Capability and Limitations of OSLO Optical Modelling Software

2.1 Introduction

Advances in computers and optical engineering software have seen dramatic increases in both speed and precision enabling new approaches to the design of optical systems. System characteristics including spot diagrams, point spread functions (PSF), modulation transfer functions (MTF), ray aberration and distortion may now be evaluated prior to device construction improving the precision of the overall outcome.

The first optical software prototypes were introduced in the late 1950’s and early 1960’s [170-174] and commercial programs were introduced in the 1970’s. Since then, the programmes have been continuously developed improving evaluation and optimization algorithms, the method and the user environment. Over the past few decades new applications of lasers and associated optics, fiber optics and devices in communication systems have come to the forefront [2, 175, 176]. The need to model and optimise these applications has driven the development of software such as TracePro, ZEMAX, CODE V and others. OSLO (Optics Software for Layout and Optimization) by the Lambda Research Corporation is the modelling program used in this work.

In this chapter, the capability and limitations of OSLO are discussed. The first section includes description of the optical ray construct, the way optical surfaces and lens systems, and the parameters and characteristics used for analysis. The second section covers OSLO modelling of a propagating laser beam, modelling of the scan engines and calculation of the optical path difference between peripheral and on-axis rays, all of which feature in this thesis.

2.2 Capability and Limitations of OSLO

A discussion of the capability and limitations requires an understanding of the approach to ray tracing software and the meaning of the data used to characterise an optical system.

OSLO mathematically computes the ray propagation based on matrix methods as described in the literature [176, 177]. The algorithm tracks the translation of a ray at an angle from one surface to the next, and refracting it at the surface, starting from the


2.2.2 Paraxial Ray Tracing

For paraxial ray tracing the optical system is considered to be rotationally symmetric and all refracting or reflecting surfaces have a common optical axis. Lenses usually, have a spherical surface specified by the radius of curvature \( r \). The two common approaches to matrix based paraxial ray propagation calculations, are known as \( ynu \) and \( yui \). These use different forms of paraxial translation and refraction equations and are evaluated by different repetitive calculations. The \( yui \) approach is the most common in computer programs and requires values for three characteristics of the propagated ray: (\( y \)) the ray height for the optical axis where the ray crosses the optical surface; (\( u \)) the ray slope; and (\( i \)) the incidence angle at which the ray arrives as shown in Figure 2-1 (a) and (b).

\[
y = y_{j-1}, \quad u = u_{j-1}, \quad y_j, \quad u_j, \quad y_j', \quad u_j'
\]

\[
t_{j-1} = t_j, \quad n_{j-1} = n_j, \quad n_j'
\]

\[
\text{Notations: } y \text{ is a ray height, } u \text{ and } u' \text{ - angles with the optical axis, and } t, \text{ thickness, - the distance between surfaces. (b) Ray refraction on a spherical surface.}
\]

For a paraxial ray, defined as one with a small angle to and distance from the optical axis, the sines of the angles can be approximated by the angles themselves to:

\[
ni = n'i',
\]

The angles are calculated from:
The refraction equation for paraxial ray propagation can be written:

\[ n_j u_j = n_{j-1} u_{j-1} - y_j c_j (n_j - n_{j-1}) , \]

where \( c_j \) is the surface curvature \( c = 1/r \).

The lateral magnification is:

\[ m = \frac{y'}{y} = \frac{nu}{n'u'} . \]

OSLO computes several paraxial constants that describe the overall system such as focal length \((EFL)\), the Gaussian image height \((GIH)\), the numerical aperture in the image space \((NA)\) and the f/number \((FNB)\) as:

\[ EFL = f' = - \frac{eh}{u' b e + u' a h} \]

\[ GIH = h' = \frac{nu_a}{n'u_a} h \]

\[ NA = n' \sin u' \]

\[ FNB = \frac{1}{2NA} \]

where \( e \) is the entrance pupil radius, the axial ray data in object and image space are \( y, u \) and \( y', u' \), and the refractive indices are \( n \) and \( n' \).

The paraxial limit, while informative, does not portray the real optical performance over large apertures and therefore more detail is required.

### 2.2.3 Real Ray Tracing

Real ray tracing provides exact information about how a ray propagates and gives real insight into the behaviour of an optical system with the limitation that the data is drawn from rays that are traced, and not those over of the whole optical aperture.

Real ray tracing involves two stages: the development of accurate algorithms to compute a ray translating through a complex optical system and determining the specification of the rays to be traced.

The approach in real ray tracing is to describe the ray as a vector in the direction of propagation with amplitude equal to the refractive index of the propagation medium.
Between two refracting media, the tangential component of the ray vector continuously changes until it intersects perpendicular to the next surface. This is done by differentiation over the next surface which is defined by a \( z = F(x, y) \) function. Once the intersection point of the ray and the next surface is established, the emergent ray is determined. The ray translation stage can be difficult if there is a complex surface definition and may include iterative procedures to find the intersection point.

Specifying the ray to be traced can be complicated, for example, by the need to specify the region on the surface where a ray strikes or the aperture of the surface.

Real ray tracing obeys Snell’s law exactly. To evaluate the system, OSLO uses two types of rays. The first type is an ordinary or Lagrangian ray which is traced from a given object point in a prescribed direction. The second is a reference Hamiltonian ray, which starts from a given object point in a direction determined by the requirement that the ray passes through some prescribed point in the system. An example is the chief ray which starts from a field edge and passes through the centre of the aperture stop. To compute the trajectory of a Hamiltonian ray, OSLO repetitively calculates and corrects the ray direction until the ray passes close to the prescribed point. Once, the exact ray has been traced, the rays which are slightly displaced in the aperture field are traced and used to compute the tangential and sagittal rays around a real chief ray and the paraxial constants of the system.

### 2.2.4 Specification of Ray Data

An important aspect of ray tracing is to specify the correct ray and this requires an understanding of the coordinate system used to describe an optical system and the quantities for describing the ray in it.

#### 2.2.4.1 Coordinate System

OSLO describes an optical system using a surface-based model which, for a lens, can include several surfaces. Each surface is described by a radius of curvature, thickness, the glass material employed and special parameters including aspheric constants, multilayer coating etc.

The position of each surface is described in a local coordinate system which has its origin defined in a base coordinate system for this surface. The base coordinate system
is located in the z-axis of the previous local coordinate system or at some point (x,y,z) of the global coordinate system. Each coordinate system is right-handed (x, y, z), where the z-axis is the direction of beam propagation, see Figure 2-2. Each surface is separated by the thickness of the previous surface. The surfaces are numbered sequentially, starting from the 0 for the object surface.

Figure 2-2. Optical system with four surfaces. The local coordinate system of each surface is centred on the optical axis.

The coordinate system of each surface can be tilted and/or decentred relative to the coordinate system of the previous surface. The new local coordinate system is described respectively to the previous coordinate system by specifying the decentre in the X, Y and Z direction, and tilting in degrees about the x, y and z axis by DCX, DCY, DCZ, TLA, TLB and TLC parameters respectively. Figure 2-3 [178] shows the tilting of the coordinate system about X, Y and Z axis and decentres the vertex of the local coordinate system.

2.2.4.2 Fractional Coordinates

The input ray in OSLO is specified by its fractional coordinates on two separate surfaces. Normally, it is the ratio of the ray height in the object surface to the overall object height (FBY, FBX), and the ratio of the ray height in the pupil surface to the overall pupil size (FY, FX) as shown in Figure 2-4.
The fractional coordinates are important in tracing a single ray through a system. This feature was used in chapter 6 to evaluate the optical path difference between peripheral and on-axis rays in the propagated pulsed beam in order to calculate the pulse time distortion across the field.

The definition of the fractional coordinates can be complicated for an off-axis object point, a decentred pupil, a large angle illuminating system and/or the positions of object or image surfaces. There are different methods, including central reference ray-aiming, rim reference ray-aiming, extended aperture ray-aiming and telecentric ray-aiming to
address these complexities. In these cases a reference ray can be used iteratively with feedback from an error function to determine the rays and performance.

### 2.2.5 Lens Surfaces

The majority of lens surfaces are spherical and these are considered as a special case of conic surfaces. OSLO uses various forms of surfaces: conic, aspheric and polynomial and defines them in the coordinate system.

The conic surface with the origin at the centre of symmetry is defined by the equation:

\[
z = \frac{c(x^2 + y^2)}{1 + \sqrt{1 - c(1 + cc)(x^2 + y^2)}}
\]

where \( c = 1/r \) is the curvature of the surface at the vertex, \((x^2 + y^2)\) is the surface equation for rotation about the \( z \) axis and \( cc \) is the conic parameter. The radius of curvature \( r \) is determined by the radius of the sphere in the vertex of the surface. The conic constant is determined by the geometry of the conic surface and the calculation code may be very complicated, consisting in the case of aspherical surfaces of several iterative loops to trace rays.

The standard aspheric surface with rotational symmetry has a set of deformation coefficients that can be of the 4\(^{th}\) (\( ad \)), 6\(^{th}\) (\( ae \)), 8\(^{th}\) (\( af \)) and 10\(^{th}\) (\( ag \)) order:

\[
z = \frac{c(x^2 + y^2)}{1 + \sqrt{1 - c(1 + cc)(x^2 + y^2)}} + ad(x^2 + y^2)^4 + ae(x^2 + y^2)^6 + af(x^2 + y^2)^8 + ag(x^2 + y^2)^{10}
\]

To trace a ray through a polynomial aspheric surface, sophisticated iterative procedures are employed to find the ray tracing solution.

### 2.2.6 Interpretation of Ray Data

The ray tracing routine includes setting up an object point according to its fractional coordinates (FBY, FBX, FBZ) and its direction. The object point will automatically trace a reference ray from this point, usually through the centre of aperture stop. OSLO can trace a single ray or the ray fan from the chosen object point.

The single ray tracing shows the trajectory of a single Lagrangian ray through the optical system. One of the output characteristics is the total optical path length (OPL) from the object sphere, a sphere with its vertex at surface 1, and its centre on the object
point. The main single rays are a chief (principal) ray and an axial ray. The chief ray is traced from the top of the object through the centre of the aperture stop to the edge of the field stop. A marginal (axial) ray is traced from the axial object point to the edge of the aperture stop and through the centre of the field stop. These are illustrated in Figure 2-5.

![Figure 2-5](image1.png)

**Figure 2-5.** Schematic diagram of a system and its entrance and exit pupils. Also shown are the axial (marginal) rays from on-axis object point \( P_0 \) and the chief ray from the off-axis object point \( P \).

OSLO traces an uniformly distributed fan of rays through the entrance aperture stop from a pre-defined single object point to the image surface as illustrated in Figure 2-6.

![Figure 2-6](image2.png)

**Figure 2-6.** Ray tracing concept in OSLO: fans of rays from three object points pass uniformly through an aperture stop, propagate in a lens system and intersect at the image surface.

An aperture stop or an entrance pupil limits the area over which light is collected. The exit pupil is the image of the aperture stop through the lens and this limits the light passing through the system to the image, effectively the NA.
2.2.6.1 Image Evaluation Technique

Ray fan tracing provides excellent characterization of an optical system’s performance. The ray tracing information is used for spot diagrams. The spot diagrams data are then used for calculating quantities such as radial energy distribution or modulation transfer functions which will be described in the sections 2.2.6.6 and 2.2.6.7. The basic concept used in ray tracing is that the light source energy flows along rays and by determining the trajectory of a ray through an optical system one can find the path of the energy flow through the system.

Ray tracing is also used as the basis for calculation of wavefront propagation through the lens. For this, OSLO traces several rays from a single object point through different aperture points and defines the wavefront surface as a surface with equal optical path lengths along each ray. As a result, the effect of diffraction on the image can be evaluated. The wavefront analysis tools include peak-to-valley optical path difference (P-V OPD) and RMS OPD.

2.2.6.2 Ray Intercept Curve Analysis

Ray intercept curves are graphical presentations of ray fan data and provide the aberration data for the optical system.

Ray analysis curves present the transverse ray aberrations DY for a defined object point in the meridional YZ plane and the DX aberration in the orthogonal XZ plane for different fractional aperture coordinates FY or FX respectively. For an afocal system, the aberrations are calculated as angular values DYA and DXA (directional tangents).

The astigmatism curves show the variation of the focal point position across the field for the meridional section (YZ plane, transverse aberration) and the sagittal section (the XZ plane, sagittal aberration).

In the plot of the axial image point FY versus DZ, the longitudinal spherical aberration curves are shown as the error in the focal point position (DZ) along the propagation axis for different radii in the pupil.

The distortion curve shows the error in the paraxial magnification for different object points.
These curves can be used as a diagnostic tool to analyse the different types of aberration in the two orthogonal directions. These graphs however, do not show the aberrations between the two orthogonal planes.

### 2.2.6.3 Spot Diagram

The spot diagram is a pattern created at the image surface by intersection of ray fans traced from a single object point through the aperture stop points. Usually the aperture stop is divided into equal square grid elements, shown in Figure 2-7, and rays are traced evenly through. The number of rays needed to make a representative spot diagram depends on the quality of the system and the intended application. The ray fan propagation is computed by geometrical ray propagation and does not include the wave nature of light. Therefore, the spot diagrams can only show the approximate light distribution at the image plane of a point source but they are a useful geometrical representation of optical performance.

![Spot Diagram Illustration](image)

Figure 2-7. (a) Grid pattern in the system aperture to provide equal ray distribution through the aperture. The grid illustrated has 34 cells across the aperture stop in each axis. Increasing the number of cells will increase the accuracy of calculation. (b) is an example of a spot diagram of the focal spherical scan engine evaluated in this thesis showing the Airy disk, and the ray spread across a 400 square micron panel. The grid size is in mm, the Airy disk radius is equal to 0.006 mm.

The data on the spot diagram contains the image coordinates xyz, the geometrical spot size, the calculated root-mean-square (RMS) spot size and the diffraction-limited spot size. The spot diagram also shows the Airy disk in order to evaluate diffraction-limited performance of the actual system, see Figure 2-7. The size of the Airy disk is:
\[ r = 0.61 \frac{\lambda f}{EBR}, \] where \( \lambda \) is the wavelength, \( f \) is the focal length and EBR is the entrance beam radius.

The spot diagram does not show the exact intensity distribution in the image as the diagram does not show any weighting of the rays.

### 2.2.6.4 Wavefront Analysis

The geometrical wavefront can be constructed from the rays used in the spot diagram. For a geometrically perfect point image, the corresponding incoming wavefront is hemispherical with its centre on the image point located on the image surface. This is the reference sphere. Any departure of the actual wavefront from the reference sphere, which is calculated as the optical path difference (OPD), is the wavefront aberration. The amount of wavefront aberration depends on the reference sphere location and radius. The point with minimal RMS wavefront error is the peak of the point image diffraction pattern.

### 2.2.6.5 Point Spread Function

The true intensity distribution of light in the image of a point monochromatic source with diffraction due to the wave nature of light is conveyed by the point spread function (PSF). The PSF is calculated in OSLO by using the geometrical wavefront, available from the ray tracing for the spot diagram. A detailed derivation of the equation can be found in the literature [179-181] and is repeated in the OSLO manual. The main steps summarised here include defining the pupil function \( P(x, y) \) which has a complex form expressed by the amplitude distribution in the exit pupil \( A(x, y) \) and wavefront aberration \( W(x, y) \) in the form:

\[
P(x, y) = A(x, y) \exp[\imath k W(x, y)],
\]

where \( k = 2\pi / \lambda \) and \( \lambda \) is the wavelength. Since the pupil function is zero outside the pupil, the Kirchhoff approximation may be used to show the diffracted amplitude \( U(x', y') \) is given by:

\[
U(x', y') = \frac{i}{\lambda} \iint_D P(x, y) \frac{\exp(-\imath k R')}{R'} dA,
\]
where $A$ is the area of the pupil and $R'$ is the distance from the pupil sphere point $(x,y)$ to the observation point $(x', y')$.

For most cases the equation is approximated to:

$$U(x', y') = i \exp\left\{ -\frac{ik[R + \varepsilon(x', y')]}{\lambda M_R R} \right\} \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} P(x, y) \exp\left[ i \frac{2\pi}{\lambda R} (xx' + yy') \right] dxdy,$$

where $R$ is the radius of the reference sphere, $\varepsilon(x', y')$ is a quadratic phase factor, and $M_R$ is the $z$-axis direction cosine of the reference ray. The double integral is a two-dimensional Fourier transform with frequency variables $\nu_x = x'/\lambda R$ and $\nu_y = y'/\lambda R$.

The point spread function at the image point in terms of irradiance is:

$$PSF(x', y') = |U(x', y')|^2$$

OSLO uses two techniques to numerically calculate the integral: direct integration and the Fast Fourier Transform (FFT). Both of methods have advantages and disadvantages. The direct method only allows calculation of PSF one point at a time. The FFT method is much faster but has a fixed sampling interval in the image plane and a fixed number of points. The sampling interval in the object $\Delta y$ and the sampling interval in the image $\Delta y'$ are related by:

$$\Delta y' = \frac{\lambda R}{N \Delta y},$$

where $N$ is the number of points in the array for FFT calculation. For the M rays traced across the pupil of diameter $D$, $\Delta y = D / M$ so:

$$\Delta y' = \frac{\lambda R M}{N \Delta y} = \frac{\lambda}{2NA} \frac{M}{N},$$

where $NA$ is the numerical aperture.

The PSF is reported in OSLO as a normalised value to the diffraction–limited peak value of a perfect system. The figure of merit in OSLO is the so called Strehl ratio which is the normalised intensity at the central peak of the PSF and this is used to characterise the diffraction-limited performance of the real system. A system with Strehl ratio of more than 0.8 produces a diffraction-limited performance according the Maréchal criterion.
2.2.6.6 Energy Distribution

The energy distribution in the image of a point can be calculated by geometrical methods using the spot diagram or by diffraction using the PSF. The energy distribution is centred at the centre of PSF for the diffraction method or the spot diagram for the geometrical method. It is possible to define the radius of a circle with a particular amount of the total energy or to calculate the amount of energy within the Airy disk. The energy distribution is compared to the performance of a perfect lens.

2.2.6.7 MTF

The optical transfer function (OTF) shows how different frequency components in the object surface are reproduced in the image. OTF is the normalised Fourier transform of the PSF and such is the function of the spatial frequencies $\nu_x$ and $\nu_y$:

$$OTF(\nu_x, \nu_y) = \frac{\int \int_{-\infty}^{\infty} PSF(x', y') \exp\{2\pi (\nu_x x' + \nu_y y')\} dx' dy'}{\int \int_{-\infty}^{\infty} PSF(x', y') dx' dy'}$$

The maximum spatial frequency $\nu_0$ for OTF which can be presented in the image is given by:

$$\nu_0 = \frac{2NA}{\lambda}$$

The OTF is a complex function. The modulus of the OTF is called the modulation transfer function (MTF) and the phase of the OTF is the phase transfer function (PTF). The MTF shows the ratio of the modulation in the image to the modulation in the object. The PTF measures the shift of the spatial frequency component from the ideal position.

The OTF is independent of the precise nature of the object. It can be computed from the wavefront of the lens and used to calculate the image quality. OTF can be calculated using geometrical optics by replacing the PSF in the equation by the spot diagram. MTF is calculated using a Fast Fourier Transform.
2.2.6.8 Radiometric Concept of Ray Tracing

OSLO uses the basic laws and geometrical relationships to calculate the amount of light that passes through an optical system. The light flux is calculated as a light per unit of solid angle coming from a small source. To compute irradiance, OSLO determines the solid angle subtended by the apparent source in the image plane multiplied by the radius of the source. Irradiance is calculated relative to the on-axis value. The model for calculation uses a grid which is set up so that each ray propagated subtends an equal amount of the solid angle in image space, and is weighted according to the solid angle in object space.

There are different sources: Lambertian source, non-uniform, and off-axis source.

2.2.7 Tolerance

The tolerance data describes the allowable variation in the parameters of the optical system. They include all surface data parameters: radius of curvature, thickness, tilt and etc. and specify the system sensitivity information in order to evaluate performance. There are many different methods used by designers.

The default tolerance data which is established in OSLO according to the ISO 10110 standard and shown in Table 2-1[182] was used in this work. The tolerances depend on the clear aperture of the surface.

Table 2-1. Some of Default tolerance data according ISO 10110 standard. Ø is the lens diameter.

<table>
<thead>
<tr>
<th>Property</th>
<th>Clear aperture diameter, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ø&lt;10</td>
</tr>
<tr>
<td></td>
<td>10&lt;Ø&lt;30</td>
</tr>
<tr>
<td></td>
<td>30&lt;Ø&lt;100)</td>
</tr>
<tr>
<td>Edge length, Ø mm</td>
<td>±0.2</td>
</tr>
<tr>
<td>Thickness, mm</td>
<td>±0.1</td>
</tr>
<tr>
<td>Stress birefringence, nm/cm</td>
<td>0/20</td>
</tr>
<tr>
<td>Surface form tolerances, 3/A(B), where A is the max sagitta error in fringes, B is the maximum irregularity in fringes</td>
<td>3/5(1)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Centering tolerances, 4/A, where A is the tilt in minutes (')</td>
<td>4/30’</td>
</tr>
<tr>
<td>Surface imperfection tolerances, 5/AxB, where A is scratches and digs</td>
<td>5/3 x 0.16</td>
</tr>
</tbody>
</table>
The tolerance data in our simulated optical systems is as follows. Fringe power shows the surface form tolerance and is specified in units of fringe spacing where one fringe space is equal to \( \frac{1}{2} \) of the default 0.546 µm light wavelength. Fringe irregularity shows the irregularity in the surface form. For spherical and parabolic reflectors used for modelling in this thesis, the fringe power is given over a 30 mm diameter test area, per ISO 10110, and is 10 with the irregularity of 2. The tilt tolerance is 0.1667°. For small diameter flat surfaces in scan engine simulations the thickness tolerance is 0.1 mm, for 10 mm diameter surfaces it is 0.2 mm, for the parabolic reflectors it is 0.8 mm and for the spherical reflectors it is 0.4 mm.

For tilted surfaces, scan mirrors and spherical and parabolic reflectors, the tilting tolerance depends on the clear aperture. For 5 mm diameter flat reflector surfaces, it is 0.5°, for large spherical and parabolic reflectors, it is 0.1167°.

### 2.3 Modelling Scan Engines Used in This Thesis

In this section the concept and the simulation setup of a laser beam source and scan engines for deflecting a beam in two perpendicular directions, such as X and Y is presented. The scan engines include a single mirror reference scan engine (RS), close-coupled engine (CCE), spherical (SSE) and parabolic (PSE) four mirror afocal relays. The PSE is a new scan engine described in chapter 3. Other scan engines are used to compare and highlight the performance of the new PSE.

Modelling the scan engines involves several steps. The first is to model the two separate scan surfaces to deflect the incoming beam in only X or Y direction. The different approaches are dictated by the goal to achieve a straight scan line at the image surface in the required direction. Every scan mirror setup includes several surfaces: the mirror surface itself and additional auxiliary coordinate break surfaces. Coordinate break surfaces are introduced to optimise the lens drawing.

The simulation of two separate scan mirrors for the X and Y scan, were then adopted for modelling two-dimensional CCE, SSE and PSE. A single mirror scanning in two dimensions was also simulated.
2.3.1 Modelling the Laser Source and the Image Surface

The idealized beam parameters are chosen from the typical laser output of the Spectra-Physics Tsunami and Coherent Chameleon commercial ultra-short pulsed Titanium doped Sapphire(Ti:S) lasers. The laser wavelength is 790 nm with a $1/e^2$ beam waist radius of 0.4 mm, half angle divergence of 0.629 mRad and an M-squared factor of 1. For all scan engines, the object, i.e., the beam waist, is positioned 2 m away from the first scanning mirror and the aperture stop is on the first scanning mirror surface.

A uniformly illuminated source in OSLO is modelled by tracing a fan of rays with equal weight. An unobstructed Gaussian laser source is formed by introducing a Gaussian apodized aperture stop, at surface 1, which gives different weights to the propagated rays, as shown in Figure 2-8. For our model, the $1/e^2$ apodized aperture stop radius can be calculated to match the laser beam 0.4 mm radius beam waist at 2 m distance:

$$w(z) = w_0 \left[ 1 + \left( \frac{z\lambda}{\pi w_0^2} \right)^2 \right]^{0.5} = 0.4\text{mm} \left[ 1 + \left( \frac{2000\text{mm} \times 0.00079\text{mm}}{3.14 \times 0.4\text{mm} \times 0.4\text{mm}} \right)^2 \right]^{0.5} \approx 1.32\text{mm}$$

The overall size of the ray bundle evaluated is determined by the entrance beam radius at surface 0. Hence, to simulate an untruncated Gaussian beam, the entrance beam radius should be about twice the 1.32 mm apodized Gaussian aperture stop radius [182] which in this study is 2.5 mm.

Figure 2-8. (a) Uniformly illuminated entrance pupil and (b) Gaussian apodized pupil. OSLO computes the spot diagram with a Gaussian apodized pupil. The aperture stop diameter is $d_2$ and $d_1$ is the diameter at which irradiance drops to $1/e^2$ of its axial value at surface 1.
In this work, the scan engines were analysed as afocal or focal systems. The position and size of an image surface is the same in all scan engines. For analysing afocal engine performance, the image aperture radius is 10 mm at a distance of 15 mm from the last scanning mirror specified in the scan engine optical arrangement. The distance from the scan engine to the scan lens is chosen to fill the aperture of the scan lens. For analysing focal scan engine performance (scan engine and the scan lens), the scan lens is positioned 15 mm away from the last scanning surface, with an image aperture radius of 14.5 mm. The distance from the last scan lens surface to the image surface is set to be the distance to produce a minimal RMS spot size for the zero-deflected beam for all systems modelled. This is the usual focal plane of the scan lens however there is a small difference between modelled scan engines due to the aberrations introduced by the engines.

2.3.2 Calculating the Pulse Time Distortion Using Optical Path Difference

In this thesis, the method of calculating the pulse time distortion across the scan field induced by a scan engine is introduced. A beam interacting with some surface will have a wavefront distortion (or time delay) as a result of the optical path length (OPL) for peripheral rays being different. At any scan position the spot intersection with the surface is elliptical with a major axis, as shown in Figure 2-9.

![Figure 2-9. Calculating optical path difference between peripheral rays for any scan point.](image)
For the beam deflected at the point O, the highest optical path difference (OPD) is created by the peripheral beam rays OC and OA which generate the longest major radius CA. Hence, OPD is equal to BC. For known beam incident angle $\alpha$ to a surface, the OPD can be geometrically calculated. OSLO provides the elliptical Gaussian spots major axis values and the value of the incident angle. Hence, the OPD can be calculated from:

$$OPD = BC = AC \cdot \sin \alpha$$

The time delay induced is: $\Delta t = \frac{OPD}{c}$, where $c$ is the speed of the light.

### 2.3.3 Mirror Scanning in the Vertical Direction

The scan mirror for deflecting the beam in vertical direction is shown in Figure 2-10 (a). The mirror surface M1 is initially tilted 45° relative to an incident beam and then deflects the beam along one axis as shown in Figure 2-10 (b). The scan line created is shown in Figure 2-10 (c). The surface data spreadsheet description is attached in Appendix B.

![Figure 2-10](image)

Figure 2-10. Scanning mirror (a) 2D and (b) 3D drawing, and (c) the footprint on the image surface.

### 2.3.4 Mirror Scanning in the Horizontal Direction

The mirror M1 scanning in the X direction and perpendicular to the axis previously described, is shown in Figure 2-11. The resultant scan line is a straight line as shown in Figure 2-11(c). The surface data spreadsheet description is attached in Appendix B.
2.3.5 Two Close Coupled Mirror Scanning System

The close coupled scanning mirror system CCE has been modelled by combination of the X and Y scanning mirror designs described previously. The lens drawing in OSLO is shown in Figure 2-12. The separation between the two scanning surfaces M1 and M2 is 10 mm. The system produces straight scan lines which are elongated due to the separation between the two mirrors. The surface data spreadsheet and the special data are attached in Appendix B.

2.3.6 One Mirror Scanning System

A single scan mirror RS system is schematically presented in Figure 2-13(a). The OSLO performance is shown in Figure 2-13(b) and (c). The scan pattern created at the image surface, Figure 2-13 (c), is symmetrical in both directions. Performance of this
engine has been used in this work as a reference scan system. The surface data spreadsheet and special data are attached in Appendix B.

![Figure 2-13. One mirror scanning in both directions: (a) design, (b) scanning rays and (b) scan pattern for 25 beam positions.](image)

### 2.3.7 Scanning Systems with Two Flat Mirrors and Two Concave Reflectors as Relay Optics

The modelling of two scanning systems with relay reflectors between two flat scan mirrors is presented in this section. The systems parameters and performance analyses are described and evaluated later in this work.

The first simulation uses two spherical reflectors and presents the well known optical scan engine patented by Amos, described in chapter 1. The second design uses two off-axis parabolic reflectors and is the new optical design proposed in chapter 3.

#### 2.3.6.1 Two Spherical Reflectors Afocal Relay Optics

The schematic diagram of the scan engine with two scan mirrors, 1 and 2, and two spherical reflectors, 3 and 4, is illustrated in Figure 2-14 (a). The ray propagation is shown in Figure 2-14 (b). The data spreadsheet is attached in Appendix B.
2.3.6.2 Scanning System with Two Flat Mirrors and Two Parabolic Reflectors as a Relay Optics

The scan system with two flat scan mirrors, 1 and 2, and two parabolic reflectors, 3 and 4, acting as relay optics is schematically illustrated in Figure 2-15 (a). The ray propagation across the created scan pattern is shown in Figure 2-15 (b). The surface data is attached in Appendix B.

2.3.8 Scan Lens

Within a typical laser scanning microscope, the scan lens is placed so as to create a flat intermediate image plane as was shown in chapter 1. Usually, the $F - \theta$ scan lenses are corrected for field distortion creating linearity between the scan angle and the position of the flying spot in the field of view. There are several evaluations of the scan lens in
the literature [2, 175, 176]. For evaluation of the scan engines in this study, a well optimised four element scan lens was taken from the OSLO database as shown in Figure 2-16. The incident rays with different angle to the lens’ optical axis produce a sharp focus within the diffraction-limited spot as shown in Figure 2-17.

Figure 2-16. Scan lens design: (a) 2D lens drawing and (b) 3D image.

Figure 2-17. Diffraction-limited performance of the scan lens used in this work: (a) Spot diagram and (b) point spread function (PSF) which describes the intensity distribution at the image of the point object. The black circle in (a) represents the Airy disk.

In the performance evaluation of focal scan engines, the scan lens is positioned 15 mm from the last scan surface and the distance from the scan lens to the image plane is chosen to focus an on-axis object point to a minimal RMS spot size. With this choice and in this manner the aberrations introduced and evaluated will be primarily due to the scan engine performance. This choice of scan lens exceeds the optical performance of those commonly used. The parameter, group velocity dispersion, was not considered in this choice but will be a factor of primary concern in the application, dictating singlet or doublet scan lens.
2.3.9 Scan Field Evaluation Points

In this thesis, the performance of the scan engines has been evaluated at 25 scan points (5 x 5 point scan pattern) created by rotating one or both flat scan mirrors by ±10° about its rotational axis and corresponding to ±20° optical beam deflection in both directions and shown in Figure 2-18.

![Scan Field Evaluation Points Diagram](image)

Figure 2-18. Twenty five point scan pattern.

2.3.10 Programming in OSLO

OSLO functions as advanced optical design software by incorporating an integrated programming environment which uses a Compiled Command Language (CCL) to allow enhanced user commands speeding up operations, extending provided toolbox commands and functions, manipulating data and performing calculations.

Several CCL routines were developed during this work and used to generate results reported in chapters 2 to 5. The CCL files used in Chapter 5 are attached in Appendix D.

2.4 Summary

Optical software generates a virtual optical system which can be evaluated for performance and optimised prior to manufacturing. Traditional methods of system evaluation through spot diagram, PSF, MTS and aberration are used and have been defined and detailed. A new method to evaluate the laser beam pulse front distortion by using the optical path difference between peripheral and on-axis rays has been introduced.
The optical arrangements of all scan engines modelled have been defined and the processes and procedures to evaluate the optical performance across the scan field both with and without an optimised scan lens in the beam path have been stated.
Chapter 3  An Improved Field Scan Engine Design Incorporating Parabolic Optics

An unobstructed afocal scan engine design employing two off-axis parabolic reflectors as relay optics between two flat scan mirrors is introduced in this chapter. Its performance is investigated using OSLO optical software as described in chapter 2. Due to the geometry of this design, the symmetric arrangement of parabolic reflectors with appropriate selection of the first scan mirror rotational axis allows this system to produce linear scan lines at the image surface. The point spread function results in all scan positions investigated are excellent. Comparison of its performance to known scan engines has shown that the new design is functionally equivalent to a single mirror scan engine and superior in every metric to a comparable spherical mirror arrangement. The design is suited to two-dimensional laser scan engines and for fast confocal and two-photon microscopy in particular.

This chapter is based on our paper published in Applied Optics, 2009 [183].

3.1 Introduction

The whole microscope system specification may be determined by the performance of the two-dimensional laser scanning system used prior to the microscope proper and there are numerous approaches to laser scanning of the beam across the sample [3, 20, 40, 44, 46, 54] as reviewed in chapter 1. Linearity of scan is important for many applications and the design of scan engines continues to be an active area of research [8, 44, 46, 55].

The simplest approach is to use a single mirror, gimballing about a point on its surface, to scan the beam across a target area. However, this remains impractical as the fast and precise control of an appropriate mount is not commercially available. The next simplest scanning system of two close-coupled scan mirrors is limited in speed, defined by the size, or strictly, the moment of inertia of the second scan mirror. Relay optics may be used between the two deflecting mirrors to keep the size and mass of the both scan mirrors small so that imaging speed can be increased. As was shown in chapter 1, lens relay optics introduces limits to optical performance due to chromatic aberrations as well as temporal and spatial distortions in the ultra-short pulses used in multi-photon
microscopy. The best approach therefore, is to use reflecting optics as achromatic relay optics between the two scanning mirrors.

A scan engine including four reflecting elements, comprising two flat scanning mirrors and two spherical reflectors, was designed by Amos [54] and employed in the BioRAD confocal microscopy scanning systems as the ‘gold standard’ in scanning microscopy. Denk patented a similar optical system as a scan engine in a two-photon microscopy system [8] well prior to the ultra-short pulse based systems currently available. The two spherical mirrors within these systems are in a confocal arrangement and are necessarily tilted to avoid obstructing the beam, as detailed in Figure 1-17, and hence the degree of aberrations affecting a beam propagating through the scan engine is increased. The aberrations in the focusing reflecting elements can be reduced by using the off-axis section of a parabolic surface instead of spherical mirror surfaces and we have proposed a new design of an afocal scan engine based on two parabolic optical elements placed in the mirror train as relay optics.

The scope of this chapter is to model and examine the performance of afocal scan engines prior to any microscope. The coupling of these engines into the microscope objective conjugate plane reveals the extent of focused beam distortion. However, as this involves other microscope-specific optical elements, this research is limited to discussion of the scan engine design itself. OSLO optical software (Lambda Research Corporation) has been used to design and simulate the performance of the two comparable four-mirror scan engines and a reference system of a single gimballing mirror. The scan engines are compared to each other in terms of the point spread function, the spot size of a Gaussian beam and the flatness of the scan lines in the image plane.

The chapter begins with a review of the aberrations in the optical system and comparative performance of a spherical and parabolic reflector.

### 3.2 Aberrations in the Optical System

In any optical system, there are image defects or optical aberrations which are caused by deviation of the geometrical rays from ideal propagation [177, 184]. Aberrations prevent diffraction-limited performance being achieved, increase the focal spot size, change the spot shape and redistribute the intensity across the spot resulting in a decrease of the peak intensity. Laser imaging systems require highly corrected optics to
minimise geometric aberration and a microscope objective lens is an example of a well corrected lens. However, aberrations can also be introduced by other optical elements in the optical train before the objectives.

The common optical aberrations in microscopes are spherical aberration, coma, astigmatism, field curvature and distortion. The general expression for the five aberration components of the lowest (third) order or Seidel aberrations which describes relationships between amount of aberration and the aperture size and field of view can be found in [85] and will be discussed in this section with reference to a spherical mirror as the performance of these is widely discussed in this thesis.

Spherical aberration results from paraxial and peripheral light rays focusing in different planes to form concentric circles with centres at the Gaussian image point (Figure 3-1(a) and (b)) and has the effect of reducing the peak intensity at the focal point. Spherical aberration varies with the third power of the aperture size and is independent of the position in the field of view. It may be calculated from the equations below and is prescribed by the a non-zero value for $B$:

$$\Delta^{(3)}_x = B \rho^3 \sin \theta$$
$$\Delta^{(3)}_y = B \rho^3 \cos \theta,$$

where $\Delta^{(3)}_x$ and $\Delta^{(3)}_y$ measure the image distortion in two orthogonal axes $x$ and $y$, $B$ is a constant and $\rho$ and $\theta$ are the polar coordinates of the object point.

![Figure 3-1. Spherical aberration and coma induced by a reflective optical element.](image-url)

- (a) The ray tracing through the spherical mirror aperture. The inset shows that the rays from the mirror edges intersect the optical axis closer to the mirror than peripheral rays.
- (b) The spot diagram at the focal point.
- (c) The ray tracing through the tilted spherical mirror. The inset shows the asymmetric rays propagating through the focal point.
- (d) The effect of coma at the focal point.
Coma is primarily an off-axis spherical aberration and has significance in laser scanning systems. It affects point-like sources away from the optical axis as the rays passing through different zones of the aperture experience different magnification. Coma results in a streak-like radial distortion of image points (Figure 3-1 (c) and (d)). The blur image spot size varies with the square of the aperture size multiplied by the field of view:

\[
\Delta^{(3)}x = - F y_0 \rho^2 \sin 2\theta \\
\Delta^{(3)}y = - F y_0 \rho^2 (2 + \cos 2\theta)
\]

where \( F \) is the constant, \( y_0 \) is the position of the object in the field of view. Coma forms circles of radius \( |F y_0 \rho^2| \) with its centre at a distance \(- 2F y_0 \rho^2 y_0\) from the Gaussian focus in the negative y-direction. Coma and spherical aberration can be corrected by an appropriate combination of lens elements and objectives.

In an astigmatic system, the image of a perfectly symmetrical light source has two different orthogonal focal planes (sagittal and tangential) in which the images of off-axis source points are radially or tangentially elongated (Figure 3-2 (a) and (b)). The image of a point object has an oval shape and is blurred. Uncorrected astigmatism reduces image intensity, sharpness and contrast with increasing effects at greater distance from the optical axis. When the best focus is chosen at a compromise position between the two extremes (the circle of least confusion) the resulting Airy disk is asymmetrical resulting in image degradation. Astigmatism is increased by other misalignments in the optical path of the microscope.

Figure 3-2. Astigmatism in an optical element caused by the rays from an off-axis object point. (a) The ray tracing from an off-axis object point. (b) The magnified image of the focal volume. The rays form the sagittal and tangential focal planes with the circle of least confusion in between.
A field curvature or flatness of field of a lens is important and a critical characteristic in high-resolution scanning applications. Simple spherical lenses focus flat object points from different regions onto a curved image surface that reflects the shape of the lens surface. This results in the central and peripheral areas of the field not being simultaneously brought into sharp focus (Figure 3-3 (a)). Complex multiple lens elements are necessary to correct this filed curvature, e.g. scan lenses (Figure 3-3 (b)) and objectives. Objectives designed as flat field correct the flatness of the field in the final image plane. The image flatness also depends on the intermediate optical elements prior to the objective.

![Figure 3-3. (a) Field curvature in an optical element. The rays from different object points form a curved focal image plane. (b) Performance of the scan lens used in this thesis for simulations. Its performance is corrected for field curvature.](image)

Astigmatism and field of curvature are studied together and described by two constants $C$ and $D$ as:

\[
\Delta^{(3)} x = D\rho y_0^2 \sin \theta \\
\Delta^{(3)} y = (2C + D)\rho y_0^2 \cos \theta 
\]

Non-linearity of magnification from the centre to the edges of the image field produces geometric distortions of the specimen features, causing their true dimensional profiles to be skewed in the image. Distortion described by the constant $E$:

\[
\Delta^{(3)} x = 0 \\
\Delta^{(3)} y = -Ey_0^3 
\]

These are two types of distortions which are commonly referred to as barrel ($E>0$) and pincushion ($E<0$) distortion (Figure 3-4 (a) and (b)).
Figure 3-4. Image distortion effects; (a) barrel distortion, and (b) pincushion distortion.

3.3 Advantage of Off-Axis Parabolic Reflector Compared to Spherical

Spherical and paraboloid mirrors find many applications in image forming optical systems as reflective focusing elements and are especially used in astronomical telescopes. These mirrors can reflect parallel light coming from infinity to a focal point which is situated on the mirrors’ optical axis and at a distance R/2 from the vertex point where R is the curvature radius as shown in Figure 3-5(a) and (b). Spherical reflectors however, have disadvantages. Firstly, due to spherical aberration, they focus a beam to a caustic surface as the rays reflected from reflector extremities intersect the optical axis closer to the mirror surface compared to the paraxial rays. The second disadvantage arises from the necessary tilt of spherical surfaces to avoid beam vignetting which induces coma and astigmatism as shown in Figure 3-5 (c) and detailed using OSLO in Figure 3-6.

A parabolic reflector was defined by the ancient Greeks and reflects light from infinity to a single point focus as shown in Figure 3-5 (b). The optical performance advantages have been periodically rediscovered [5, 185] and the off-axis part of a parabolic reflector can be used to focus the light to an unobstructed point with minimal aberration as shown in Figure 3-5 (d) and detailed using OSLO in Figure 3-7. The use of off-axis parabolic reflectors “backwards”at the 90° reflection angle is detailed in Figure 3-8 and shows the coma increasing with an increase in the entrance beam radius. Note that this is for a single parabolic surface and not representative of the optical performance of a four mirror relay.
Figure 3-5. Spherical and parabolic reflectors. (a) The spherical reflector focuses a beam from infinity to a caustic surface. (b) The parabolic reflector focuses to one focal point. (c) Beam reflection from a tilted spherical surface introduces astigmatism and coma at the focal point highlighted in the inset. (d) An off-axis parabolic surface focuses the beam to one unobstructed focal point which lies on its optical axis. A magnified image is shown in inset.
Figure 3-6. Aberrations introduced to a narrow and wide parallel incident beam reflecting from a spherical reflector. (a) Spot diagram and MTF of a normal incidence axis showing focus within the Airy disk (black circle). (b) Spherical surface tilted 10°. The incident beam induces astigmatic focus and loss of diffraction limited performance in the spot diagram and MTF. (c) The caustic surface from a wider incident beam degrades the spot diagram and MTF further.
Figure 3-7. A parabolic reflector focuses a beam of any size, incoming parallel to its optical axis, from any off-set part to a geometrical point within the diffraction limit. The MTF function is coincident with the ideal. (a) Incident beam is on-axis. (b) Beam is incident parallel to the optical axis and reflected from 90° off-axis segment. (c) Beam is as (b) but with increased incident beam radius.

Scale changes in the spot diagram and MTF graphs are consistent with diffraction limit theory aperture dependence.
Figure 3-8. An off-axis parabola focuses light emerging from the focal point to a parallel beam. When a low-divergence beam [(a) 1 mm beam radius and (b) 2 mm beam radius] is incident as shown, the aberrations at the focal point increase with the beam size. Intensity PSFs show the distorted energy distribution at the focus.

3.4 Method and Modelling Design

Three scan engines including a single flat reflector reference engine (RS) and two different four reflector afocal optical relays comprising two flat scanning mirrors and two concave surfaces were simulated using OSLO optical software as specified in chapter 2 with their performances compared across the scan field.

3.4.1 Spherical Scan Engine

Parameters for the spherical scan engine (SSE) simulation are largely drawn from the Amos patent[54] and data in [54]. The SSE engine is illustrated in Figure 3-9. The two plane mirrors 1 and 5 are driven by galvanometers deflecting the beam in two orthogonal directions. Mirror 1 rotates around the axis 2, reflecting the incident beam to a scan line on the spherical surface 3. Mirror 4 gathers the light reflected from the mirror 3 to one point on the surface 5. Mirror 5 is rotated in the direction illustrated by
About an axis which lies on its surface and is perpendicular to the page plane to create the two-dimensional scanning pattern.

![Figure 3-9. A spherical mirror based achromatic scanning engine after Amos [54]](image)

The patent quotes a 75 mm spherical reflector focal length, however, to facilitate a better comparison with the 101.6 mm focal length parabolic reflectors, the spherical reflector focal length dimension chosen for the simulation is also 101.6 mm. This has the effect of slightly reducing the spherical aberrations of the spherical reflector engine. To minimize the scan line distortion, Amos [43] found that the two plane mirrors 1 and 5 need to be oriented at an angle to each other. In this simulation, mirror 1 is tilted at the angle $\alpha = 10$ degrees, as distinct from its rotation of $\pm 10^\circ$. The spherical surfaces 3 and 4 are tilted at an angle $\beta = 10$ degrees to ensure the focal point of reflector 3 is not vignetted. In general, the value of this angle depends on the geometrical size of the reflectors chosen and the physical separation between the scanner mirrors. A smaller angle will introduce less spherical aberration in the system and less curvature of the scan lines as discussed later in this chapter.

The light reflected from mirror 3 is focused at an intermediate point, the position of which is found in OSLO by solving for distance $d$ from mirror surface 3 to focus the diverging beam to the smallest spot. The spherical reflector 4 is positioned at distance $d$ from the intermediate point to minimize the aberration in the symmetrical optical system.
3.4.2 Parabolic Scan Engine

The new parabolic scan engine (PSE) incorporates two static off-axis parabolic mirrors and its design is illustrated in Figure 3-10. The parabolic reflectors 4 and 6 are the off-axis circular segment from the parabolic surfaces 3 and 5 respectively with a radius of curvature $R$ and a focal length $R/2$. The parabolic reflectors 4 and 6 are modeled as identical Newport Corporation off-axis replicated parabolic mirrors 50338Al with the radius of curvature of 101.6 mm and 50.8 mm focal length. As it is the 90° off-axis paraboloid section, the effective focal length is 101.6 mm. Use of identical parabolic reflectors is not strictly necessary but allows a symmetrical optical system to be constructed and therefore aberrations introduced by the first concave surface 4 to be minimized and a uniform beam diameter to be maintained. The deflection axes of the scanning mirrors 1 and 7 are positioned at the focal points of the parabolic reflectors 4 and 6 respectively. Mirrors 1 and 7 rotate respectively around axis 2, and at an axis lying on the surface of the mirror 7 perpendicular to both the rotation axis 2 and to the plane of this page. Line 8 demonstrates the direction of movement of mirror 7. Mirror 1 is angled at 45° to the incident beam and rotational axis 2 is coaxial with the incident beam.

![Figure 3-10. Optical layout of the improved PSE scan engine design. Two dashed arcs 3 and 5 illustrate two separate parabolic reflective surfaces from which sections 4 and 6, representing the 90° reflective off-axis sections, are employed. Surfaces 3 and 5 have a common optical axis. The scan mirrors 1 and 7 are positioned at the focal points of parabolic reflectors 4 and 6 respectively.](image)

This arrangement, and particularly the alignment and rotation of scan mirror 1 about axis 2, ensures that the beam traces a geometrical circle on the surface of parabolic reflectors 4 and 6 and results in the beam impinging on the centre of mirror 7 independent of the scan deflection angle, within the limit of the aperture stop defined by the physical size (38 mm) of the surface 4.
The distance from parabolic reflector 4 to reflector 6 is determined by the parabola’s parameters and was held at 2R as shown in Figure 3-10 due to the choice of a symmetric design. Parabolic reflector 4 focuses the beam at a mid point due to the incident laser beam diameter. The exact position of the mid-point focus is determined by the beam divergence and will vary slightly from the distance R to give a slight magnification of the beam size at mirror 7. Mirror 7 was modelled as a conventional galvanometer scanner rotating about an axis in the plane of the mirror surface.

3.4.3 Other Modelled Specifications

The close coupled (CCE) engine was simulated as rotational mirrors separated by distance equal to 10 mm, and specified in chapter 2. For all scan engines the illuminating Gaussian laser beam source was simulated as described in Chapter 2. In short, it is the circular 790 nm wavelength Gaussian beam with a $1/e^2$ waist radius of 0.4 mm, a half-angle divergence of 0.629 mRad and an M-squared factor of 1, and which is positioned 2 m away from the first scanning mirror. The aperture stop is on the first scanning mirror surface. The image aperture radius is 10 mm at a distance of 15 mm away from the last scanning mirror. The aperture radius and distance were chosen to mimic the entrance aperture of a scan lens which usually follows a scan engine and is not included in this investigation. The engines are analyzed as afocal systems. In all engine designs the scanning mirror surfaces move ± 10° in each single axis, or both axes for RS, producing a ± 20° line scan about the original zero position. With the four mirror systems, the first mirror scans in the horizontal direction and the second mirror scans the vertical axis.

Twenty five scan field points were defined as described in chapter 2 and shown in Figure 3-11, and these have been used as the basis for calculations. The 25 points include zero and maximum deflection in each and both axes and are representative of the complete frame in a scan pattern. In further discussion, these 25 points define separate optical configurations within OSLO and will be referred to as configurations.
3.5 Results and Discussion

3.5.1 The Shape of the Scan Lines

The beam outlines in the image surface plane for the RS, SSE and PSE scan engines are shown in Figure 3-12 for each of the configurations. The spherical reflector SSE engine exhibits curved scan lines at the final image surface whereas the scan lines created by the RS have a slight pincushion distortion and the horizontal scan lines within the PSE engine are straight and free of field distortion for any scan deflection angle though there is some pincushion distortion in the vertical scan lines.

The PSE engine gives a straight scan line due to geometry of the design. The deflection by the first scanning mirror, mirror 1 in Figure 3-10, traces a circular arc on the surface of the first off-axis parabolic mirror with a radius equal to the curvature radius of the parabolic reflector at that line. Points on this line are subsequently reflected to the second parabolic reflector and form a similar radius circle segment. Finally, all these points are focused by the second parabolic reflector to its focal point where the deflection point of the second scanning mirror is situated. Hence, the scan lines are straight.

Figure 3-13 highlights the difference in the beam position across the scan field for the four scan engines. The CCE engine produces straight horizontal scan lines and the scan pattern is elongated due to the separation distance between scan mirrors. Three other engines for the same deflection angles scan across very similar sized areas. The ideal RS engine produces symmetrical pincushion scan pattern. The PSE engine is similar to
the RS engine in the vertical direction but produces straight lines in the horizontal. The SSE system produces curved lines with different curvature in each axis.

The scan line curvature in the SSE engine was found by Amos to rely on the orientation of the first scanning mirror to the incident beam. Analysis of the scan line field curvature for the SSE engine during this investigation and as it can be seen from Figure 3-13 showed that, for a fixed radius of curvature of the spherical reflectors used, the field curvature in the image plane depends most strongly on the tilt angle of the second spherical reflector, surface 4 in Figure 3-9. In reality, this angle is defined by the distance between the two scanning mirror mounts and will be a factor defined by engineering choices.

Figure 3-12 The image surface scanned beam footprint and the scan field distortion for (a) the single mirror reference engine, (b) the spherical mirror SSE engine and, (c) the parabolic mirror PSE engine.
The scanning laser beam propagation parameters and the beam spot sizes are calculated for the twenty five configurations by the astigmatic Gaussian beam analysis option within OSLO. The calculated results for the RS, PSE and SSE are displayed graphically in Figure 3-14. This data shows that the spot sizes for the PSE engine are almost the same as for the RS engine and, in the central scan area, the beam is almost circular and non-astigmatic. In the SSE system in contrast, the beam spot is elliptical and suffers from aberrations.

A beam tracking along a scan line becomes azimuthally turned, as evidenced in the footprints in Figure 3-12, and this can be seen in the difference between the x and y spot radii for all systems and the increase or decrease in the beam size from the centre position in a scan line towards the edges.

The deviation from a circular scanning beam spot is larger and increases to a greater extent along the scan lines for the SSE than for the PSE engine. In the SSE engine there is a lack of symmetry between the four extreme configurations and the different circularity is due to the coma arising from the tilt of the spherical mirrors. It is also caused by the different curvature of the scan lines created (see Figure 3-12). The magnitude of the deviation from circularity is approaching a factor of two worse for the SSE engine than the PSE system while the reference RS engine and the parabolic PSE engine are almost identical.

The aberrations in the SSE engine are predominantly caused by tilting the spherical surfaces. The spherical aberration engendered is detailed in the spot diagram images for different tilting angle in Figure 3-15. The series displayed changes the tilt angle from 0°
through 10º, with 5º degrees being the first physically reasonable angle. An angle of 10º was chosen as the tilt angle for the modelling and experiments in this thesis.

Figure 3-14. Beam spot dimensions R1 and R2 in two orthogonal axes for three scan engines: (a) RS, (b) PSE and (c) SSE. There is almost complete overlap of the PSE and RS results.
Figure 3-15. Aberration in the spherical SSE engine caused by the tilt angle of the spherical surfaces: (a) 0°, (b) 2.5°, (c) 5°, (d) 7.5° and (e) 10°. The size of individual squares is 0.1 mm. The white circle within all individual squares indicates the Airy disk. In figure (f), the spot diagrams at position 13 from (a) to (e) are collected and show the rapid increase of the aberrated geometrical RMS radius spot size.

### 3.5.3 Spot Diagrams and Point Spread Functions

The spot diagrams at infinity with an outline of the Airy disk for all twenty-five configurations in the afocal RS, PSE and SSE systems are shown in Figure 3-16 (a), (b) and (c) respectively highlighting the differences between the scan engines. The point spread functions (PSFs) at infinity for all systems are illustrated in Figure 3-17 (a) to (c). The spot diagrams and the PSFs are computed in OSLO as images of a single on-axis object point via geometrical optics for spot diagrams and as a diffracted image in the case of the PSF.
From the spot diagrams in Figure 3-16 (c), it is evident that for the SSE system, the image of the beam for the central zero scanning deflection, configuration 13, is distorted due to coma and astigmatism in the vertical axis and this also extends to configurations 3, 8, 16 and 19. As the scan moves horizontally from the centre in the five scan lines, the spot size increases in the vertical direction and gradually increases in the horizontal direction. These results are in stark contrast to the PSE system with parabolic reflectors illustrated in Figure 3-16 (b) and the reference system of Figure 3-16 (a), where the spot size as well as the shape does not change substantially with deviation from the central position.

The point spread functions of each configuration and system in Figure 3-17 (a) to (c) highlight the stark difference in performance between the spherical and parabolic surfaces. These results are closely related to the spot diagram image of each configuration, and graphically emphasize the difference, or lack of, between the two curved optic scan engines PSE and SSE compared to the ideal reference RS engine. The normalized peak amplitude of the point spread functions are analyzed graphically in Figure 3-18. The point spread functions are almost identical for the parabolic mirror PSE engine (Figure 3-18(b)) and RS engines (Figure 3-18 (a)) only varying effectively in the fifth decimal place with the range of values falling within ± 0.00009. The spherical mirror equivalent in contrast varies from 0.013 to 0.073 within a range of ± 0.03 and a factor of 5.

### 3.5.4 Positioning Error

The beam positioning error on the surface of the second scan mirror for 25 beam positions for the PSE and SSE engines is shown by the beam outlines (Figure 3-19). The beam walk across the second scan mirror surface is due to the design geometry thus inherent in the model and is the choice of real beam divergence. The “node” in Figure 3-19 (b) is narrower than the corresponding “node” in Figure 3-19 (a) and represents the positioning error arising from different angles of incidence on this mirror. This error evaluates as 9.3 % for the SSE and 4.1 % for the PSE, and is representative of relative resolution loss in the final image.
Figure 3-16. Spot diagram array for the twenty-five scan engines configurations examined: (a) the RS engine; (b) is for the PSE engine and (c) is for the SSE engine. The black circle represents the Airy disk.
Figure 3-17 Point spread function data for the twenty-five system configurations examined: (a) RS engine; (b) is for PSE engine and (c) is for SSE engine.
Figure 3-18 The variation in point spread function maxima for the twenty-five system configurations examined in each of the three scan engines: (a) the RS engine, (b) the PSE engine and (c) the SSE engine. Plates (a) and (b) vary by 0.1% across the field while (c) has a wide range of values.

Figure 3-19. Beam outlines on the second scan mirror surface for 5 mm aperture radius: (a) in the SSE engine and (b) in the PSE engine.

3.5.5 Comparison of Modelling Results with Specific Literature Results

Howard and Stone [186] proved mathematically that for a plane-symmetrical optical system with two spherical reflectors, the image of a point object can be achieved
without blur when the object point, the centres of curvature of both mirrors and the image point are all aligned on the axis of symmetry for the system as shown in Figure 3-20. The models presented here, the SSE and PSE designs, confirm their conclusions. This condition is not true for the SSE engine and is true for the PSE engine.

The new PSE engine can be compared with the scan design ascribed to Seel [57], reviewed in chapter 1, which uses a parabolic torus surface as a relay between scan mirrors. The parabolic torus surface is circular in the x-y plane and is a line parabola in the y-z plane as detailed in Figure 1-21 and as in Figure 3-21 (a) below. Optically, the beam reflects from a parabolic surface only in the plane of reflection and the real beam width interacts with cylindrical surfaces above and below the plane of incidence.

Nonetheless, this design does transfer the beam in afocal modelling with a smaller amount of aberration than the SSE design.
Figure 3-22. Comparative performance of off-axis parabolic toroid (as in Seel’s design) and 90° off-axis parabolic reflector (as in the PSE) at the focal point. Spot diagrams match the scan angle of the first scan mirror in the range +10°, +5°, 0°, -5°, and -10°. The optical performance for both systems are aberrated at the focal plane between the first and the second curved surface reflections.

As shown in Figure 3-22 the two designs are different in the aberrations induced by the reflection of a diverging Gaussian laser beam off the different surfaces cause non-diffraction limited focussing at a hypothetical surface between the two curved surfaces. The differences may be ascribed to coma, being the off-axis component, and astigmatism. Strictly, the PSE does not have 1st and 3rd order spherical aberration for input parallel light. The PSE design uses the parabola “backwards” and only one on-axis ray of the laser passes through the focal point of the parabola. The pattern in panel (d) shows the on-axis ray well centred while the peripheral rays form each half of the comatic images. The Seel design shows an aberrated image, with a better centre of mass of the rays than PSE, but with distortion arising from the circularly curved toroidal surface. Both designs have a second reflection that alters the aberration performance due to the symmetry in both optical arrangements.

Figure 3-23 shows the correction to the afocal field that result from the second reflection. Row (a) in Figure 3-23 shows the scan field footprint pattern for both, row (b) in Figure 3-23 has the spot diagrams showing slight differences in the shape and centre of mass of the spot diagrams while row (c) has the PSF images showing the diffraction of the on-axis rays and as expected these are identical. The on-axis rays for both systems pass through both focal points in the PSE design and the focal point in the Seel design while reflecting at 45 degrees from both curved surfaces and, as such, are functionally identical parabolic optical paths.
Figure 3-23. Comparative performance for 25 configurations across the two dimensional ±10° scan field for the Seel design and the PSE. Row (a) illustrates the simulated lens drawing and the image of 25 spots at the image surface. Rows (b) and (c) show the spot diagrams and the PSFs for the 25 scan points.

3.6 Summary

A new design of an achromatic, afocal optical relay incorporating two off-axis parabolic reflectors and an appropriate rotational axis for the scan mirrors, is presented. This eliminates line curvature in the scan pattern created and exhibits good Gaussian spot
size performance and excellent spot diagram and point spread function parameters for the range of scan angles evaluated. This system was compared to a long-established standard optical relay design using spherical mirrors. The bulk of the modelling was done at 10° and at this angle the PSE was found to be demonstrably superior in every metric. However, different tilt angles of the two spherical optics will change the performance of this design and this work did not comprehensively pursue optimising the SSE design. The differences between the new PSE design and an ideal single scan mirror are only very slight. Finally, the spot diagrams and point spread functions show in stark detail an impressive performance advantage associated with the parabolic mirror design. The comparison with the Seel design does not detail a large optical performance difference however, the cost of a diamond machined parabolic torus is prohibitive against the cost of two replicated off-axis parabolas. The PSE design should find immediate application in scanning microscopy and in the wider field of two-dimensional laser scanning.
Chapter 4 Experimental Verification of New Scan Engine Performance

4.1 Introduction

The scan engine is a critically important part of a laser scanning microscope as was shown in chapter 1. An afocal scan relay deflects a coherent laser beam in two-directions before a scan lens focuses the deflected beam to its focal plane. In a microscope, this location is called the intermediate image plane and is conjugated to the objective focal plane. Any beam aberrations induced by the afocal scan relay and seen at the intermediate image plane will be further multiplied by the optics that follows, including the objective lens and, as a result, will distort the system diffraction-limited performance. In two-photon microscopy particularly, the aberrations induced by the scan engine also cause pulse time distortion and therefore, will severely decrease the two-photon excitation efficiency.

This chapter presents and compares experimental parabolic scan engine (PSE) performance evaluating it as an afocal relay and with measurements made at an intermediate image plane after a scan lens. The other engines evaluated are a spherical scan engine (SSE) formed by a four mirror (flat, spherical concave, spherical concave, flat) afocal relay and an ideal reference scan engine (RS) formed by a single mirror gimballing about a point on its reflective mirror surface. Evaluating the scan engines as afocal relays shows wavefront distortion and aberrations introduced to the relayed laser beam across the scan field. Evaluation made at the focal plane of a scan lens shows how the aberrations induced by the scan engine affect diffraction limited performance and directly matches the coupling to a microscope through an intermediate image plane. Experimental evaluation of the scan engines required a Gaussian laser beam profile and a He-Ne laser was used for this reason.

The paper with the results of this study was recently submitted to Applied Optics [187]

4.2 Method

The experimental investigations involved comparing the performance of the new PSE scan engine with the known spherical scanning engine (SSE) and an ideal single scan
mirror engine (RS) one. Measured parameters include beam spot profile images, spot radii and ellipsity across the scan field.

In the first part of experiment, in order to evaluate experimental results for the SSE and PSE afocal engines, both scan engines were analysed by Gaussian beam simulation using the optical modelling software OSLO. The performances of the SSE and PSE afocal engines were then contrasted with the geometrically calculated performance of an ideal single mirror RS scan engine.

In the second part, the effect of the scan engines on beam propagation was evaluated.

In the third part, three focal engines, RS, PSE and SSE were compared at the intermediate image plane, including the induced field curvature for the PSE and RS engines, and astigmatism in the SSE engine.

The beam spot intensity profile images measured by the BeamScan detector present the normalised intensity distribution across the beam spot and they were compared with the OSLO calculated point spread function (PSF) images across the scan field for three afocal scan engines shown in the chapter 3. This comparison is possible as a PSF defined by OSLO is the intensity distribution in the image of a point object.

### 4.3 Experimental setup

Prototypes of three scan engines; the new parabolic scanning engine (PSE), spherical scanning engine (SSE), and an ideal single scan mirror engine (RS) were assembled according the designs described in chapter 3.

The PSE afocal scan engine includes two 90° off-axis parabolic reflectors as relay optics between two flat scan mirrors and its setup is shown in Figure 4-1. Two Newport off-axis replicated parabolic mirrors 50338AL, with a radius of curvature equal to 101.6 mm, were placed opposite each other in a custom-built adjustable slide mount to ensure correct positioning of the parabolic reflectors. The specifications of the parabolic mirror surfaces are typically: roughness is 2.5 nm, the wavefront distortion, ≤ 2λ @633 nm and reflectance ≥ 85%.
The experimental setup of the SSE afocal scan engine compromising two spherical reflectors as relay optics between two flat scan mirrors is shown in Figure 4-2. This was constructed according to the Amos patent [54] which was discussed in chapter 1 and 3. Two identical 100 mm focal length spherical reflectors, Thorlabs CM508-050-G01, were positioned opposite each other and tilted at an angle $\beta = 10^\circ$. The angle between the first flat mirror 1 and the first spherical reflector 3 is $\alpha = 10^\circ$. The mirror quality is typically: front surface is 40-20 scratch-dig and flatness is $\lambda/4$ at 633 nm.
For both the SSE and PSE, two small flat silver mirrors were used as an alternative to scanner driven mirrors. The mirrors were separately positioned in rotational mounts which simulate the movements of the scanning reflector in appropriate directions and provide the ability to deflect a beam at a particular angle. This arrangement makes it possible to fix the beam at selected scanning field points in order to measure its profile. In both PSE and SSE engines, the first flat mirror executes the line scanning and the second scan mirror does the vertical scanning. The first and the second flat mirrors are able to rotate $\pm 10^\circ$ respectively and deflect the incoming beam up to $\pm 20^\circ$.

The second part of the investigation aimed to evaluate the performance of the focal scan engines at the intermediate image plane. For this, an achromatic doublet lens of 100 mm focal length was used at a distance of 10 mm from the second scan mirror for both PSE and SSE engines and 10 mm after the single scan mirror in the RS engine as shown in Figure 4-3, Figure 4-4 and Figure 4-5. In the RS engine, only one flat mirror is used to provide one pivot point on its surface to reflect the beam in both directions as shown in Figure 4-5.

![Figure 4-3. The focal parabolic scanning engine (PSE).](image)
Figure 4-4. The focal spherical scanning engine (SSE).

Figure 4-5. A single mirror reference scan engine RS. The two dotted lines mark the two orthogonal rotational axes of this mirror with the intersection of these in the mirror plane of reflection forming the pivot point in the RS engine.
The scan engines performance was investigated at 25 scan field points, shown in Figure 4-6, and chapter 2. In the SSE and RS engines, the beam profile measurements were completed for all 25 positions. In the PSE engine, due to the physical size of the off-axis parabolic reflectors, the rotation amplitude of the first flat mirror was restricted to 5° or 10° optical.

Illumination came from a Melles-Griot He-Ne laser at wavelength 632.8 nm with the beam waist diameter less than 0.59 mm and full beam divergence of 1.35 mrad. The Rayleigh range for this laser beam was 0.45 m and the laser positioned at 0.71 m from the first scan mirror for all engines.

The beam profile images and their cross-sectional measurements at different scan field points were taken by a Photon Inc. BeamScan detector. This device measures the beam profile by scanning two orthogonal slits across the profile while recording the transmitted intensity. The beam profile is then reconstructed in software. The real beam profile can be different due to the effects of diffraction from the scanning slits, as can be seen in Figure 4-8 below, however the BeamScan serves as a good qualitative guide to spot size and shape. The spot profile images do not show the beam spot azimuthal rotation because the BeamScan detector presents the image along main axes of the elliptical spot. The measured parameters include: the elliptical beam spot width in two
main axes; ellipsity and Gaussian fit along two main axes. The Gaussian fit shows the
deviation in the spot distribution from the Gaussian (TEM00 mode). The results are the
average of 400 scans across the incoming beam and the root mean square deviation\(^1\) for
each parameter. Beam width is measured at \(1/e^2\) FWHM. The BeamScan was mounted
rigidly on an optical dovetail rail. This construction enables the detector head to be
shifted horizontally and vertically across the simulated scanning field and to be kept
perpendicular to the non-deflected position.

In the second part, the BeamScan head was mounted on a mechanical translation stage
which provided fine position adjustment of the BeamScan head along the beam
propagation axis. It provided also the ability to find a fine focal position and perform
the beam profile measurements in the appropriate plane and to measure effective field
curvature in this plane.

In further discussions, the term “ratio of the spot radius” is used to define the relative
beam spot radii at a specified scan field point over the spot radius at the central position.

4.4 Results and Discussions

4.4.1 The Investigation of Afocal Scan Engines

4.4.1.1 Beam Spot Measurements

The beam cross-sectional images created by the PSE and SSE afocal scan engines at
respective scan field positions are shown in Figure 4-7 and Figure 4-8. The results are	
tabulated in Table 4-1 and Table 4-2 respectively. At the zero-deflected position, the
PSE afocal engine produces a circular non-astigmatic spot with ellipsity of \((1.0 \pm 0.01)\) while the SSE engine shows an elongated spot with ellipsity of \((1.39 \pm 0.003)\). For both
engines, spot sizes increased from the central position 13 towards the scan edges.

From Figure 4-7, for the PSE afocal scan engine, the beam spots across the field did not
change markedly in shape, and spot only exhibited a small increase ellipsity from the
centre to the periphery. For the SSE afocal engine from Figure 4-8, results indicate that
the spot ellipsity decreased from the marked ellipsity in the central position to the
periphery. The spot ellipsity decrease in the SSE afocal engine is due to rapidly
increased spot size caused by spherical aberrations induced by the two tilted spherical
reflectors. The spherical aberrations also caused deformation of the Gaussian beam

\(^1\) The root mean square deviation is the square root of the mean square error.
profile. It can be seen from results in Table 4-1 and Table 4-2, where Gaussian fit for the PSE afocal engine across the scan field are from 0.55 to 0.76 and for the SSE afocal engine are from 0.23 to 0.56.

Line curvatures created by the SSE engine were visually detected while translating the detector head vertically and horizontally during measurements.
Figure 4-7. Experimental cross-sectional images of the laser beam propagated through PSE engine at fifteen scan field points. The spots correspond to the red labelled region of Figure 4-6. The images in rows represent beam spots deflected by the first scan mirror and the images in columns are for five tilted angles of the second scan mirror.
Table 4-1. Experimental measurements of the laser beam spot parameters across the scan field after the PSE afocal scan engine. Data presented for the spots corresponding to the red labelled region of Figure 4-6. All results are the average of 400 scans across the incoming beam and root mean square deviation for each parameter.

<table>
<thead>
<tr>
<th>Spot number</th>
<th>22</th>
<th>23</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1, mm</td>
<td>1.38±0.02</td>
<td>1.36±0.01</td>
<td>1.38±0.02</td>
</tr>
<tr>
<td>D2, mm</td>
<td>1.32±0.01</td>
<td>1.28±0.01</td>
<td>1.30±0.01</td>
</tr>
<tr>
<td>Ellipsity b)</td>
<td>1.04±0.01</td>
<td>1.06±0.01</td>
<td>1.06±0.01</td>
</tr>
<tr>
<td>Gaussian Fit 1 c)</td>
<td>0.57±0.05</td>
<td>0.72±0.03</td>
<td>0.63±0.04</td>
</tr>
<tr>
<td>Gaussian Fit 2 c)</td>
<td>0.66±0.05</td>
<td>0.69±0.02</td>
<td>0.65±0.03</td>
</tr>
<tr>
<td>Spot number</td>
<td>17</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td>D1, mm</td>
<td>1.31±0.01</td>
<td>1.31±0.01</td>
<td>1.30±0.01</td>
</tr>
<tr>
<td>D2, mm</td>
<td>1.28±0.01</td>
<td>1.28±0.01</td>
<td>1.28±0.01</td>
</tr>
<tr>
<td>Ellipsity</td>
<td>1.02±0.01</td>
<td>1.02±0.01</td>
<td>1.02±0.01</td>
</tr>
<tr>
<td>Gaussian Fit 1</td>
<td>0.73±0.04</td>
<td>0.72±0.03</td>
<td>0.71±0.05</td>
</tr>
<tr>
<td>Gaussian Fit 2</td>
<td>0.68±0.05</td>
<td>0.74±0.03</td>
<td>0.67±0.02</td>
</tr>
<tr>
<td>Spot number</td>
<td>12</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>D1, mm</td>
<td>1.28±0.01</td>
<td>1.28±0.01</td>
<td>1.29±0.01</td>
</tr>
<tr>
<td>D2, mm</td>
<td>1.29±0.01</td>
<td>1.27±0.01</td>
<td>1.28±0.01</td>
</tr>
<tr>
<td>Ellipsity</td>
<td>0.99±0.01</td>
<td>1.00±0.01</td>
<td>1.01±0.01</td>
</tr>
<tr>
<td>Gaussian Fit 1</td>
<td>0.75±0.02</td>
<td>0.76±0.03</td>
<td>0.74±0.04</td>
</tr>
<tr>
<td>Gaussian Fit 2</td>
<td>0.69±0.03</td>
<td>0.68±0.02</td>
<td>0.67±0.02</td>
</tr>
<tr>
<td>Spot number</td>
<td>7</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>D1, mm</td>
<td>1.30±0.01</td>
<td>1.30±0.01</td>
<td>1.30±0.01</td>
</tr>
<tr>
<td>D2, mm</td>
<td>1.28±0.01</td>
<td>1.29±0.01</td>
<td>1.28±0.01</td>
</tr>
<tr>
<td>Ellipsity</td>
<td>1.01±0.01</td>
<td>1.01±0.01</td>
<td>1.02±0.01</td>
</tr>
<tr>
<td>Gaussian Fit 1</td>
<td>0.67±0.04</td>
<td>0.72±0.02</td>
<td>0.74±0.02</td>
</tr>
<tr>
<td>Gaussian Fit 2</td>
<td>0.71±0.03</td>
<td>0.70±0.02</td>
<td>0.68±0.03</td>
</tr>
<tr>
<td>Spot number</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>D1, mm</td>
<td>1.38±0.02</td>
<td>1.36±0.01</td>
<td>1.38±0.01</td>
</tr>
<tr>
<td>D2, mm</td>
<td>1.31±0.01</td>
<td>1.29±0.01</td>
<td>1.30±0.01</td>
</tr>
<tr>
<td>Ellipsity</td>
<td>1.06±0.02</td>
<td>1.05±0.01</td>
<td>1.06±0.01</td>
</tr>
<tr>
<td>Gaussian Fit 1</td>
<td>0.55±0.05</td>
<td>0.73±0.03</td>
<td>0.66±0.03</td>
</tr>
<tr>
<td>Gaussian Fit 2</td>
<td>0.70±0.03</td>
<td>0.66±0.03</td>
<td>0.66±0.02</td>
</tr>
</tbody>
</table>

a D1 and D2 are beam widths along the spot major and minor axis and are measured at 1/e² FWHM.

b Ellipsity is the ratio D1/D2.

c Gaussian Fit 1 and 2 show how close the beam profile distribution to the Gaussian distribution for TEM00 mode.
Figure 4-8. Experimental cross-sectional images of the laser beam at twenty scan field points after the SSE engine. The spots correspond to the blue labelled region of Figure 4-6. The images in rows represent beam spots deflected by the first scan mirror and the images in columns are for five tilt angles of the second scan mirror.
Table 4-2. Experimental measurements of the laser beam spot parameters across the scan field after the SSE afocal scan engine. Data presented for the spots corresponding to the blue labelled region of Figure 4-6. All results are the average of 400 scans across the incoming beam and root mean square deviation for each parameter.

<table>
<thead>
<tr>
<th>Spot number</th>
<th>21</th>
<th>22</th>
<th>23</th>
<th>24</th>
<th>25</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1, mm&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.27±0.02</td>
<td>1.15±0.03</td>
<td>1.03±0.02</td>
<td>1.11±0.02</td>
<td>1.27±0.02</td>
</tr>
<tr>
<td>D2, mm&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.66±0.06</td>
<td>0.95±0.02</td>
<td>0.78±0.02</td>
<td>0.94±0.02</td>
<td>1.67±0.07</td>
</tr>
<tr>
<td>Ellipsity&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.77±0.03</td>
<td>1.21±0.04</td>
<td>1.33±0.04</td>
<td>1.18±0.04</td>
<td>0.76±0.03</td>
</tr>
<tr>
<td>Gaussian Fit 1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.56±0.06</td>
<td>0.36±0.09</td>
<td>0.46±0.08</td>
<td>0.38±0.09</td>
<td>0.56±0.06</td>
</tr>
<tr>
<td>Gaussian Fit 2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.24±0.08</td>
<td>0.43±0.11</td>
<td>0.39±0.11</td>
<td>0.39±0.10</td>
<td>0.23±0.08</td>
</tr>
<tr>
<td>Spot number</td>
<td>16</td>
<td>17</td>
<td>18</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>D1, mm</td>
<td>1.15±0.04</td>
<td>1.09±0.02</td>
<td>1.06±0.02</td>
<td>1.15±0.03</td>
<td>1.33±0.05</td>
</tr>
<tr>
<td>D2, mm</td>
<td>1.45±0.04</td>
<td>0.9±0.02</td>
<td>0.76±0.02</td>
<td>0.94±0.02</td>
<td>1.43±0.04</td>
</tr>
<tr>
<td>Ellipsity</td>
<td>0.94±0.04</td>
<td>1.22±0.04</td>
<td>1.38±0.04</td>
<td>1.22±0.04</td>
<td>0.94±0.04</td>
</tr>
<tr>
<td>Gaussian Fit 1</td>
<td>0.24±0.1</td>
<td>0.45±0.09</td>
<td>0.46±0.08</td>
<td>0.36±0.09</td>
<td>0.25±0.12</td>
</tr>
<tr>
<td>Gaussian Fit 2</td>
<td>0.29±0.09</td>
<td>0.42±0.11</td>
<td>0.44±0.11</td>
<td>0.42±0.09</td>
<td>0.31±0.09</td>
</tr>
<tr>
<td>Spot number</td>
<td>11</td>
<td>12</td>
<td>13</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>D1, mm</td>
<td>1.33±0.04</td>
<td>1.1±0.02</td>
<td>1.06±0.02</td>
<td>1.1±0.03</td>
<td>1.33±0.04</td>
</tr>
<tr>
<td>D2, mm</td>
<td>1.45±0.04</td>
<td>0.91±0.02</td>
<td>0.76±0.01</td>
<td>0.91±0.02</td>
<td>1.46±0.04</td>
</tr>
<tr>
<td>Ellipsity</td>
<td>0.94±0.04</td>
<td>1.21±0.03</td>
<td>1.39±0.03</td>
<td>1.22±0.04</td>
<td>0.91±0.04</td>
</tr>
<tr>
<td>Gaussian Fit 1</td>
<td>0.26±0.09</td>
<td>0.42±0.1</td>
<td>0.52±0.12</td>
<td>0.37±0.1</td>
<td>0.25±0.09</td>
</tr>
<tr>
<td>Gaussian Fit 2</td>
<td>0.29±0.08</td>
<td>0.48±0.08</td>
<td>0.54±0.12</td>
<td>0.46±0.08</td>
<td>0.28±0.08</td>
</tr>
<tr>
<td>Spot number</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>D1, mm</td>
<td>1.31±0.06</td>
<td>1.11±0.02</td>
<td>1.06±0.02</td>
<td>1.1±0.02</td>
<td>1.32±0.05</td>
</tr>
<tr>
<td>D2, mm</td>
<td>1.41±0.04</td>
<td>0.93±0.02</td>
<td>0.76±0.01</td>
<td>0.92±0.02</td>
<td>1.41±0.04</td>
</tr>
<tr>
<td>Ellipsity</td>
<td>0.93±0.05</td>
<td>1.19±0.03</td>
<td>1.39±0.04</td>
<td>1.2±0.04</td>
<td>0.93±0.05</td>
</tr>
<tr>
<td>Gaussian Fit 1</td>
<td>0.26±0.11</td>
<td>0.39±0.08</td>
<td>0.47±0.09</td>
<td>0.38±0.08</td>
<td>0.25±0.12</td>
</tr>
<tr>
<td>Gaussian Fit 2</td>
<td>0.3±0.09</td>
<td>0.43±0.09</td>
<td>0.5±0.1</td>
<td>0.4±0.08</td>
<td>0.29±0.09</td>
</tr>
<tr>
<td>Spot number</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>D1, mm</td>
<td>1.37±0.04</td>
<td>1.17±0.03</td>
<td>1.04±0.02</td>
<td>1.15±0.03</td>
<td>1.34±0.04</td>
</tr>
<tr>
<td>D2, mm</td>
<td>1.31±0.06</td>
<td>0.97±0.03</td>
<td>0.79±0.02</td>
<td>0.96±0.03</td>
<td>1.39±0.06</td>
</tr>
<tr>
<td>Ellipsity</td>
<td>1.05±0.06</td>
<td>1.21±0.05</td>
<td>1.31±0.04</td>
<td>1.2±0.04</td>
<td>0.97±0.05</td>
</tr>
<tr>
<td>Gaussian Fit 1</td>
<td>0.23±0.09</td>
<td>0.33±0.11</td>
<td>0.45±0.08</td>
<td>0.33±0.1</td>
<td>0.27±0.1</td>
</tr>
<tr>
<td>Gaussian Fit 2</td>
<td>0.26±0.12</td>
<td>0.37±0.11</td>
<td>0.38±0.1</td>
<td>0.37±0.11</td>
<td>0.26±0.12</td>
</tr>
</tbody>
</table>

<sup>a</sup> D1 and D2 are beam widths along the spot major and minor axis and are measured at 1/e<sup>2</sup> FWHM.

<sup>b</sup> Ellipsity is the ratio D1/D2.

<sup>c</sup> Gaussian Fit 1 and 2 show how close the beam profile distribution to the Gaussian distribution for TEM00 mode.
4.4.1.2 Comparison of the experimental and simulated results

The OSLO simulations were used to calculate the beam sizes and ellipsity for the SSE and PSE afocal engines at the same 25 scan field positions and then the results compared with experimentally measured data. The results are shown in Figure 4-9 and Figure 4-10 for the PSE and SSE afocal engines respectively, and tabulated in Table 4-3.

For the PSE afocal engine, Figure 4-9, the beam becomes slightly elliptical towards the edges. The measured beam spot sizes at all positions are larger than those calculated using OSLO by a maximum of 4.5 %. The difference is a result from the quality of the off-axis parabolic reflector surface and 2 % accuracy of the BeamScan scanning slit profiler [188]. The spot size ratio across the scan field confirms the experimentally measured statistics.

For the SSE engine, the experimentally measured and OSLO calculated beam spot sizes are illustrated in Figure 4-10. In the central part of the scan field, the results are very similar and at extremities, experimental data exceed modelling results by up to 25 %.

This difference is ascribed to the quality of the optical surface employed especially towards the edge of the specified clear aperture. Minor misalignment in five axes (x, y, z, 0, φ) of the curved elements relative to the optical axis and each other will compound the enhanced spherical aberration expected at the scan periphery.

4.4.1.3 The Spot Size Ratio across the Scan Field Compare to an Ideal Single Mirror Scan Engine (RS)

A single mirror RS scan engine is the best possible scan engine as it does not introduce any wavefront distortion to a Gaussian laser beam and creates a symmetrical scan field about the central position. The performance of the RS engine is used as the ideal system and the performance of other engines is evaluated relative to RS performance. The method to calculate the elliptical beam spot sizes along two main axes for the RS engine is described in Appendix B. Based on these calculations, the RS engine spot size ratios (ratio of the elliptical spot sizes along two main axes at any scan position relative to the spot size at undeflected position 13) for a quarter of the scan field are shown in Table 4-4. The spot size ratio based on the experimentally measured spot sizes for the PSE and SSE engines are tabulated in Table 4-5. The results confirm the similarity of the PSE performance to RS within 0.1 % while the SSE spot size ratio is different.
Experimental Verification of New Scan Engine Performance

Figure 4-9. (a) Experimental and (b) OSLO calculated beam spot radii $R_1$ and $R_2$ across the scan field in the PSE afocal engine.

Figure 4-10. (a) Experimental and (b) OSLO calculated spot radii $R_1$ and $R_2$ along the scanning field in the SSE afocal engine.
Table 4-3. Beam spot diameters calculated by OSLO for the PSE and SSE engines across the scan field. Data presented respectively for the spots corresponding to the blue and red labelled region of Figure 4-6.

<table>
<thead>
<tr>
<th>Spot number</th>
<th>PSE</th>
<th>SSE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spot number</td>
<td></td>
</tr>
<tr>
<td></td>
<td>22 23 24</td>
<td></td>
</tr>
<tr>
<td>D1, mm&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.37 1.34 1.37</td>
<td>D1, mm</td>
</tr>
<tr>
<td>D2, mm&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.26 1.26 1.26</td>
<td>D2, mm</td>
</tr>
<tr>
<td>Ellipsity</td>
<td>1.08 1.06 1.08</td>
<td>Ellipsity</td>
</tr>
<tr>
<td></td>
<td>17 18 19</td>
<td></td>
</tr>
<tr>
<td>D1, mm</td>
<td>1.26 1.28 1.26</td>
<td>D1, mm</td>
</tr>
<tr>
<td>D2, mm</td>
<td>1.30 1.26 1.30</td>
<td>D2, mm</td>
</tr>
<tr>
<td>Ellipsity</td>
<td>0.97 1.02 0.97</td>
<td>Ellipsity</td>
</tr>
<tr>
<td></td>
<td>12 13 14</td>
<td></td>
</tr>
<tr>
<td>D1, mm</td>
<td>1.26 1.25 1.26</td>
<td>D1, mm</td>
</tr>
<tr>
<td>D2, mm</td>
<td>1.28 1.25 1.28</td>
<td>D2, mm</td>
</tr>
<tr>
<td>Ellipsity</td>
<td>0.98 1.00 0.98</td>
<td>Ellipsity</td>
</tr>
<tr>
<td></td>
<td>7 8 9</td>
<td></td>
</tr>
<tr>
<td>D1, mm</td>
<td>1.26 1.28 1.26</td>
<td>D1, mm</td>
</tr>
<tr>
<td>D2, mm</td>
<td>1.30 1.26 1.30</td>
<td>D2, mm</td>
</tr>
<tr>
<td>Ellipsity</td>
<td>0.97 1.02 0.97</td>
<td>Ellipsity</td>
</tr>
<tr>
<td></td>
<td>2 3 4</td>
<td></td>
</tr>
<tr>
<td>D1, mm</td>
<td>1.37 1.34 1.37</td>
<td>D1, mm</td>
</tr>
<tr>
<td>D2, mm</td>
<td>1.26 1.26 1.26</td>
<td>D2, mm</td>
</tr>
<tr>
<td>Ellipsity</td>
<td>1.08 1.06 1.08</td>
<td>Ellipsity</td>
</tr>
</tbody>
</table>

<sup>a</sup> D1 and D2 are beam widths along the spot major and minor axis and are measured at 1/e<sup>2</sup> FWHM.

<sup>b</sup> Ellipsity is the ratio D1/D2.
Table 4-4. Single mirror scan engine spot size ratio over one quarter of the scan area. The central position is 13. Data presented respectively for the spots shown in Figure 4-6.

<table>
<thead>
<tr>
<th>Spot number</th>
<th>23</th>
<th>24</th>
<th>25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.07</td>
<td>1.09</td>
<td>114</td>
</tr>
<tr>
<td>Ratio 2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.01</td>
<td>1.01</td>
<td>1.01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Spot number</th>
<th>18</th>
<th>19</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio 1</td>
<td>1.02</td>
<td>1.03</td>
<td>1.09</td>
</tr>
<tr>
<td>Ratio 2</td>
<td>1</td>
<td>1</td>
<td>1.01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Spot number</th>
<th>13</th>
<th>14</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio 1</td>
<td>1</td>
<td>1.02</td>
<td>1.01</td>
</tr>
<tr>
<td>Ratio 2</td>
<td>1</td>
<td>1</td>
<td>1.07</td>
</tr>
</tbody>
</table>

<sup>a</sup> Ratio 1 and Ratio 2 are calculated elliptical spot size along two main axes relative to the spot size in the position 13.

Table 4-5. The spot size ratio in PSE and SSE scan engines. Data presented are for the spots corresponding to the blue and red labeled regions of Figure 4-6 respectively.

<table>
<thead>
<tr>
<th>Spot</th>
<th>22</th>
<th>23</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.09</td>
<td>1.07</td>
<td>1.09</td>
</tr>
<tr>
<td>Ratio 2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.04</td>
<td>1.01</td>
<td>1.02</td>
</tr>
<tr>
<td>Spot</td>
<td>17</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td>Ratio 1</td>
<td>1.03</td>
<td>1.03</td>
<td>1.02</td>
</tr>
<tr>
<td>Ratio 2</td>
<td>1.01</td>
<td>1.01</td>
<td>1.01</td>
</tr>
<tr>
<td>Spot</td>
<td>12</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>Ratio 1</td>
<td>1.02</td>
<td>1.01</td>
<td>1.02</td>
</tr>
<tr>
<td>Ratio 2</td>
<td>1.01</td>
<td>1</td>
<td>1.01</td>
</tr>
<tr>
<td>Spot</td>
<td>7</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Ratio 1</td>
<td>1.02</td>
<td>1.02</td>
<td>1.02</td>
</tr>
<tr>
<td>Ratio 2</td>
<td>1.01</td>
<td>1.02</td>
<td>1.01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Spot</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio 1</td>
<td>1.09</td>
<td>1.07</td>
<td>1.09</td>
</tr>
<tr>
<td>Ratio 2</td>
<td>1.03</td>
<td>1.01</td>
<td>1.02</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Spot</th>
<th>21</th>
<th>22</th>
<th>23</th>
<th>24</th>
<th>25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.67</td>
<td>1.25</td>
<td>1.03</td>
<td>1.24</td>
<td>1.67</td>
</tr>
<tr>
<td>Ratio 2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.18</td>
<td>1.51</td>
<td>1.36</td>
<td>1.46</td>
<td>2.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Spot</th>
<th>16</th>
<th>17</th>
<th>18</th>
<th>19</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio 1</td>
<td>1.78</td>
<td>1.18</td>
<td>1.00</td>
<td>1.24</td>
<td>1.75</td>
</tr>
<tr>
<td>Ratio 2</td>
<td>1.91</td>
<td>1.43</td>
<td>1.39</td>
<td>1.51</td>
<td>1.88</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Spot</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio 1</td>
<td>1.75</td>
<td>1.20</td>
<td>1.00</td>
<td>1.20</td>
<td>1.75</td>
</tr>
<tr>
<td>Ratio 2</td>
<td>1.91</td>
<td>1.45</td>
<td>1.39</td>
<td>1.45</td>
<td>1.92</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Spot</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio 1</td>
<td>1.72</td>
<td>1.22</td>
<td>1.00</td>
<td>1.21</td>
<td>1.74</td>
</tr>
<tr>
<td>Ratio 2</td>
<td>1.86</td>
<td>1.46</td>
<td>1.39</td>
<td>1.45</td>
<td>1.86</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Spot</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio 1</td>
<td>1.72</td>
<td>1.28</td>
<td>1.04</td>
<td>1.26</td>
<td>1.76</td>
</tr>
<tr>
<td>Ratio 2</td>
<td>1.80</td>
<td>1.54</td>
<td>1.37</td>
<td>1.51</td>
<td>1.83</td>
</tr>
</tbody>
</table>

<sup>a</sup> Ratio 1 and Ratio 2 are calculated elliptical spot size along two main axes relative to the spot size in the position 13.
4.4.2 The Effect of the Scan Engines on Beam

4.4.2.1 Evaluation of the Beam Collimation

To explore the impact of the PSE and SSE four mirror reflective optical relays on the propagated laser beam, the He-Ne laser beam properties with and without the optical relays in the zero-deflected position (position 13) were measured and also compared with the calculated beam propagation size:

\[ w(z) = w_0 \left[ 1 + \left( \frac{z}{z_R} \right)^2 \right]^{1/2} \]

where \( w_0 \) is the beam waist, \( w(z) \) is the beam radius at the distance \( z \) from the beam waist and \( z_R \approx \frac{\pi w_0^2}{\lambda} \) is the Rayleigh distance.

The results of calculated and experimentally measured propagated beam radii without any obstruction are shown in Figure 4-11. The real beam is elliptical, to within 2.3 %, and its far-field half-angle divergence is higher than the specifications would indicate.

A comparison of the beam size measured with the PSE and SSE relays on the beam path is illustrated in Figure 4-12 (a). The beam propagating after the PSE relay (lines 2 and 3) is within 1 % of circular, while the beam propagating after the SSE relay (lines 4 and 5) is elliptical, producing a line image of the circular Gaussian beam at infinity, due to spherical aberration, astigmatism and coma.
The PSE afocal engine beam radii, lines 2 and 3, are almost parallel to the free propagating beam and geometrical translation of these lines on the graph by a distance of 406.4 mm, which is equal to the path created by the PSE engine, shows that the size and divergence of the beam after the second scan mirror in PSE engine is similar to the beam incident upon its first scan mirror (Figure 4-12 (b)). This result indicates that the PSE afocal relay can also be used to control and correct beam divergence which was investigated further below.

Figure 4-12. (a) Beam propagation after the PSE and SSE relays compared to theoretical beam propagation size. Line 1 is the theoretical beam radius, lines 2 and 3 are the two beam radii after the PSE engine, lines 4 and 5 are the two beam radii for the elliptical spot after the SSE engine. (b) Line 6 is the experimental results for the PSE engine (line 2) shifted left by the optical path length in the PSE engine.
4.4.2.2 *OSLO simulation of the PSE optical relay*

OSLO simulation confirmed that there is no dependence on beam waist position from the first scan mirror and for a fixed \(2f = 203.2\) mm distance between parabolas, thus the output beam parameters (size and divergence) are almost equal to the incident beam parameters on the first mirror independent of how far away the laser is (see Figure 4-13).

![Figure 4-13. The beam radius propagated through the PSE relay for different positions of the input beam waist relative to the first scan mirror. Position of the first and second scan mirrors, and the first and second parabola, are indicated by vertical lines with the distance from the first scan mirror indicated in mm above.](image)

The distance between two parabolic reflectors also affects the output beam parameters. Figure 4-14 (a) details the results and Figure 4-14 (b) identifies that the collimated output beam with smallest divergence is achieved for a separation of 208.2 mm.

4.4.2.3 *Focal plane shift dependence on inter-parabola distance*

The beam divergence after the PSE relay depends on the distance between parabolic reflectors, which will cause a change in the position of the focal plane of a scan lens after the PSE engine. The axial spot positions after the PSE relay and scan lens were experimentally measured for different distances between the two off-axis parabolic
Figure 4-14. The output beam divergence after the PSE engine for the beam waist positioned 2 m away from the first scan mirror surface. The radius of curvature of two parabolic reflectors is 101.8 mm. Zero at the X axis represents position of the first scan mirror. (a) Variation of the propagated laser beam radius depending on the distance between two parabolic reflectors. The vertical line shows the position of the first parabola and the number above it shows its distance from the first scan mirror. Line labels represent the distance between parabolic reflectors. (b) Collimated output is achieved for 208.2 mm separation between parabolic reflectors.

reflectors. Results are shown in Figure 4-15. The focal spot position moves further away from the focusing lens with decreasing parabolic mirror separation. This result can be used to investigate volume scanning in a microscope system employing a PSE, where the two dimensions in a given lateral plane have the third dimension, along the optical axis of the microscope objective, probed by a focal plane shift within a sample arising from a change in mirror separation. The slope of Figure 4-15 shows a 4 mm shift in focus position for a 3 mm change in mirror separation placing this effect well within the capability of piezo-electric actuators to dynamically scan the third dimension over the hundred micrometer order of multiple cell layers.
4.4.3 Investigation at the intermediate image plane

In a microscope, a scan lens positioned after the scan engine produces an intermediate image plane that is conjugated to the objective focal plane (Figure 1-3). We can simulate the intermediate image plane by using a focusing lens after a scan engine and evaluate the performance in this plane. This next section investigates the scan engine performance at the intermediate image plane.

4.4.3.1 RS beam spot results

The numerical results of the beam spot sizes measured for the RS engine at the intermediate image plane are tabulated in Table 4-6 and plotted in Figure 4-16. Figure 4-17 shows the beam profiles across the scan field. Measurements show that the spots across the scan field are nearly circular with ellipsity from 1.01 at the central position 13 to 1.1 at the periphery positions. The spot size increases from the central position rapidly rising up to 2.6 times at the four extreme positions as a result of a field curvature created by the scan lens. The field curvature was measured and is presented in the next section.

4.4.3.2 PSE beam spot results

The numerical results of the beam spot sizes measured for the PSE engine at the intermediate image plane are tabulated in Table 4-7 and plotted in Figure 4-18. Figure 4-19 shows the beam profiles across the scan field. The results across the scan field show that the spots are nearly circular with small ellipsity from 1.01 at the central position 13 to 1.19 at the periphery. The spot sizes increase by up to 45% from the central position towards extremities. This is caused by the field curvature as described later in this chapter. Measurements show an absence of astigmatism in the beam after the focusing lens.
Experimental Verification of New Scan Engine Performance

Figure 4-15. Measured axial focal shift dependence on parabola separation in PSE engine.

Figure 4-16. The RS engine: elliptical spot radii R1 and R2 along two main axes at the intermediate image plane. The radii increase evenly in both directions from the central spot.
Figure 4-17. RS engine: spots profiles along created scan field at the intermediate image plane. The images in rows represent beam spots deflected by the first scan mirror and the images in columns are for five tilt angles of the second scan mirror. The spots correspond to the blue labelled region of Figure 4-6.
Table 4-6. RS engine: spot profile data at the intermediate image plane. Data presented for the spots corresponding to the blue labelled region of Figure 4-6. All results are the average of 400 scans across the incoming beam and root mean square deviation for each parameter.

<table>
<thead>
<tr>
<th>Spot number</th>
<th>21</th>
<th>22</th>
<th>23</th>
<th>24</th>
<th>25</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1, µm</td>
<td>257.9±1.6</td>
<td>138.7±1.2</td>
<td>93.2±0.5</td>
<td>137.6±1.4</td>
<td>257.4±1.4</td>
</tr>
<tr>
<td>D2, µm</td>
<td>274.1±2.3</td>
<td>150.7±1.4</td>
<td>99.8±0.7</td>
<td>150.6±1.4</td>
<td>272.8±2.4</td>
</tr>
<tr>
<td>Ellipsity</td>
<td>1.06±0.01</td>
<td>1.09±0.01</td>
<td>1.07±0.01</td>
<td>1.1±0.02</td>
<td>1.06±0.01</td>
</tr>
<tr>
<td>Gaussian Fit 1</td>
<td>0.72±0.04</td>
<td>0.66±0.05</td>
<td>0.66±0.02</td>
<td>0.68±0.04</td>
<td>0.7±0.05</td>
</tr>
<tr>
<td>Gaussian Fit 2</td>
<td>0.73±0.05</td>
<td>0.54±0.05</td>
<td>0.76±0.03</td>
<td>0.56±0.07</td>
<td>0.73±0.05</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Spot number</th>
<th>16</th>
<th>17</th>
<th>18</th>
<th>19</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1, µm</td>
<td>130.1±1.8</td>
<td>94.1±0.9</td>
<td>73.1±0.4</td>
<td>92.3±0.9</td>
<td>133.9±1.2</td>
</tr>
<tr>
<td>D2, µm</td>
<td>139.5±1.9</td>
<td>97.1±1</td>
<td>74.2±0.4</td>
<td>96.8±1</td>
<td>145.9±1.3</td>
</tr>
<tr>
<td>Ellipsity</td>
<td>1.07±0.02</td>
<td>1.03±0.01</td>
<td>1.02±0.01</td>
<td>1.05±0.01</td>
<td>1.09±0.01</td>
</tr>
<tr>
<td>Gaussian Fit 1</td>
<td>0.68±0.08</td>
<td>0.7±0.04</td>
<td>0.81±0.02</td>
<td>0.69±0.05</td>
<td>0.69±0.04</td>
</tr>
<tr>
<td>Gaussian Fit 2</td>
<td>0.58±0.06</td>
<td>0.67±0.07</td>
<td>0.72±0.02</td>
<td>0.67±0.05</td>
<td>0.57±0.05</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Spot number</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1, µm</td>
<td>88.6±0.5</td>
<td>72.2±0.4</td>
<td>70.2±0.5</td>
<td>72.1±0.4</td>
<td>88.4±0.9</td>
</tr>
<tr>
<td>D2, µm</td>
<td>93.5±0.5</td>
<td>72.9±0.4</td>
<td>71±0.4</td>
<td>72.4±0.5</td>
<td>93.9±0.8</td>
</tr>
<tr>
<td>Ellipsity</td>
<td>1.06±0.01</td>
<td>1.01±0.01</td>
<td>1.01±0.01</td>
<td>1±0.01</td>
<td>1.06±0.01</td>
</tr>
<tr>
<td>Gaussian Fit 1</td>
<td>0.68±0.03</td>
<td>0.81±0.02</td>
<td>0.85±0.03</td>
<td>0.83±0.03</td>
<td>0.69±0.04</td>
</tr>
<tr>
<td>Gaussian Fit 2</td>
<td>0.72±0.03</td>
<td>0.79±0.03</td>
<td>0.83±0.03</td>
<td>0.78±0.04</td>
<td>0.67±0.02</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Spot number</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1, µm</td>
<td>129.6±1.7</td>
<td>92.6±0.9</td>
<td>77.4±0.6</td>
<td>92.5±0.9</td>
<td>130.2±1.7</td>
</tr>
<tr>
<td>D2, µm</td>
<td>140±2</td>
<td>96±1</td>
<td>80.2±0.8</td>
<td>96.1±1</td>
<td>140.9±1.9</td>
</tr>
<tr>
<td>Ellipsity</td>
<td>1.08±0.02</td>
<td>1.04±0.01</td>
<td>1.04±0.01</td>
<td>1.04±0.01</td>
<td>1.08±0.02</td>
</tr>
<tr>
<td>Gaussian Fit 1</td>
<td>0.69±0.08</td>
<td>0.68±0.04</td>
<td>0.76±0.03</td>
<td>0.69±0.04</td>
<td>0.67±0.06</td>
</tr>
<tr>
<td>Gaussian Fit 2</td>
<td>0.57±0.08</td>
<td>0.66±0.04</td>
<td>0.64±0.04</td>
<td>0.67±0.06</td>
<td>0.57±0.07</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Spot number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1, µm</td>
<td>249.8±3.3</td>
<td>134.5±1.2</td>
<td>85.3±0.4</td>
<td>134.8±1.2</td>
<td>246.9±2.8</td>
</tr>
<tr>
<td>D2, µm</td>
<td>258.3±4.3</td>
<td>147.8±1.4</td>
<td>91.5±0.5</td>
<td>147.9±1.4</td>
<td>257.6±4.1</td>
</tr>
<tr>
<td>Ellipsity</td>
<td>1.03±0.02</td>
<td>1.1±0.01</td>
<td>1.07±0.01</td>
<td>1.1±0.01</td>
<td>1.04±0.02</td>
</tr>
<tr>
<td>Gaussian Fit 1</td>
<td>0.65±0.09</td>
<td>0.69±0.04</td>
<td>0.69±0.02</td>
<td>0.68±0.04</td>
<td>0.65±0.09</td>
</tr>
<tr>
<td>Gaussian Fit 2</td>
<td>0.63±0.08</td>
<td>0.56±0.05</td>
<td>0.68±0.03</td>
<td>0.56±0.05</td>
<td>0.63±0.09</td>
</tr>
</tbody>
</table>

a D1 and D2 are beam widths along the spot major and minor axis and are measured at 1/e^2 FWHM.
b Ellipsity is the ratio D1/D2.
c Gaussian Fit 1 and 2 show how close the beam profile distribution to the Gaussian distribution for TEM00 mode.
Figure 4-18. PSE engine: spot radii for 15 positions (correspond to the red labelled region of Figure 4-6) at the intermediate image plane. Horizontal scan angle represents the tilting angle of the first scan mirror and vertical scan angle represents the tilting angle of the second scan mirror. The spot size increases from the central spot in both directions. The greater increase is for the deflection by the second mirror.
Figure 4-19. PSE engine: spots profiles at chosen positions (the red labelled region of Figure 4-6) at the intermediate image plane. The images in rows represent beam spots deflected by the first scan mirror and the images in columns are for five tilted angles of the second scan mirror.
Table 4-7. PSE engine: spot profile data at the intermediate image plane. Data presented for the spots corresponding to the red labelled region of Figure 4-6. All results are the average of 400 scans across the incoming beam and root mean square deviation for each parameter.

<table>
<thead>
<tr>
<th>Spot number</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1, µm</td>
<td>95.7±0.8</td>
<td>93.6±1.1</td>
<td>98±0.8</td>
</tr>
<tr>
<td>D2, µm</td>
<td>106.2±1.2</td>
<td>99.7±1.2</td>
<td>108.8±1.1</td>
</tr>
<tr>
<td>Ellipsity</td>
<td>0.9±0.01</td>
<td>0.9±0</td>
<td>0.9±0</td>
</tr>
<tr>
<td>Gaussian Fit 1</td>
<td>0.7±0</td>
<td>0.6±0.1</td>
<td>0.7±0</td>
</tr>
<tr>
<td>Gaussian Fit 2</td>
<td>0.7±0.1</td>
<td>0.6±0.7</td>
<td>0.7±0.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Spot number</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1, µm</td>
<td>76.4±0.6</td>
<td>76.5±1.1</td>
<td>81.6±0.7</td>
</tr>
<tr>
<td>D2, µm</td>
<td>88.2±0.7</td>
<td>77±1</td>
<td>89.9±0.8</td>
</tr>
<tr>
<td>Ellipsity</td>
<td>0.9±0</td>
<td>1±0</td>
<td>0.9±0</td>
</tr>
<tr>
<td>Gaussian Fit 1</td>
<td>0.7±0</td>
<td>0.7±0.1</td>
<td>0.7±0</td>
</tr>
<tr>
<td>Gaussian Fit 2</td>
<td>0.7±0</td>
<td>0.8±0.1</td>
<td>0.7±0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Spot number</th>
<th>12</th>
<th>13</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1, µm</td>
<td>75.7±0.8</td>
<td>73.1±1</td>
<td>76.4±0.8</td>
</tr>
<tr>
<td>D2, µm</td>
<td>80.4±1.2</td>
<td>73.5±0.8</td>
<td>80.6±1.2</td>
</tr>
<tr>
<td>Ellipsity</td>
<td>0.9±0</td>
<td>1±0</td>
<td>0.9±0</td>
</tr>
<tr>
<td>Gaussian Fit 1</td>
<td>0.8±0.1</td>
<td>0.8±0.1</td>
<td>0.8±0.1</td>
</tr>
<tr>
<td>Gaussian Fit 2</td>
<td>0.7±0.1</td>
<td>0.7±0.1</td>
<td>0.7±0.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Spot number</th>
<th>17</th>
<th>18</th>
<th>19</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1, µm</td>
<td>82.2±1</td>
<td>77.9±0.8</td>
<td>82.1±1</td>
</tr>
<tr>
<td>D2, µm</td>
<td>86.9±1.3</td>
<td>77.9±0.9</td>
<td>86.8±1.3</td>
</tr>
<tr>
<td>Ellipsity</td>
<td>0.9±0</td>
<td>1±0</td>
<td>0.9±0</td>
</tr>
<tr>
<td>Gaussian Fit 1</td>
<td>0.1±0.1</td>
<td>0.1±0.1</td>
<td>0.7±0</td>
</tr>
<tr>
<td>Gaussian Fit 2</td>
<td>0.1±0.1</td>
<td>0.7±0.1</td>
<td>0.7±0.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Spot number</th>
<th>22</th>
<th>23</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1, µm</td>
<td>106.1±1.2</td>
<td>97.8±0.8</td>
<td>106.2±0.8</td>
</tr>
<tr>
<td>D2, µm</td>
<td>108.3±2.6</td>
<td>104.6±0.7</td>
<td>107.2±1.2</td>
</tr>
<tr>
<td>Ellipsity</td>
<td>1±0</td>
<td>0.9±0</td>
<td>1±0</td>
</tr>
<tr>
<td>Gaussian Fit 1</td>
<td>0.6±0.1</td>
<td>0.7±0</td>
<td>0.7±0</td>
</tr>
<tr>
<td>Gaussian Fit 2</td>
<td>0.6±0.1</td>
<td>0.7±0</td>
<td>0.7±0</td>
</tr>
</tbody>
</table>

a D1 and D2 are beam widths along the spot major and minor axis and are measured at 1/e² FWHM.
b Ellipsity is the ratio D1/D2.
c Gaussian Fit 1 and 2 show how close the beam profile distribution to the Gaussian distribution for TEM00 mode.
4.4.3.3 SSE beam spot results

The initial experimental measuring the beam spot for the SSE engine revealed that the propagated beam was subject to astigmatism and coma, with the beam profile changing from elliptical along the X axis to elliptical along the Y axis. Therefore, the beam has sagittal and tangential foci. To measure the spot size across a scan field, the position of the intermediate image plane has to be defined. It was chosen as the distance from the focusing lens to the point where the undeflected beam, position 13, has tangential focus. The beam profiles across the scan field for all spots were measured at three distances from the lens: namely at two positions representing the beam foci in two perpendicular directions, and at the distance equal to the chosen intermediate image plane. The resultant data was also used for calculating the astigmatism in the focal SSE engine.

The beam profiles across the scan field in the intermediate image plane are shown in Figure 4-20, numerical beam radii results are plotted in Figure 4-21 and tabulated in Table 4-8. The astigmatism is shown Figure 4-22. From Figure 4-20, the spot pattern along horizontal scan lines are similar. Within a single horizontal line the beam profiles are nearly symmetrical about the central spot. Along vertical columns, the profiles are similar. The spot pattern indicates that beam aberrations are introduced by the first scan mirror followed by reflection from the two tilted spherical reflectors. The central vertical line, which represents the beam spots created by beam deflection with only the second scan mirror, as the first scan mirror is in zero scan, has the most elliptical spots, with ellipsity from (2.82±0.04) to (3.23±0.05) as shown in Table 4-8. This is due to the chosen position of the intermediate plane with the middle points close to their tangential plane and the peripheral points beyond the focal plane.

The changes in astigmatism along scan lines are similar and are in the range from 5.2 to 17.5 mm (see Figure 4-22).
Figure 4-20. Beam profile across the chosen scan field at 25 positions. The images in rows represent beam spots deflected by the first scan mirror and the images in columns are for five tilt angles of the second scan mirror. Spots are more elliptical in the central column which represents zero deflection of the first scan mirror. Beam circularity for other positions is due to astigmatism created by the two tilted spherical reflector surfaces.
Table 4-8. SSE: spot profile data at the intermediate image plane. Data presented for the spots corresponding to the blue labelled region of Figure 4-6. All results are the average of 400 scans across the incoming beam and root mean square deviation for each parameter.

<table>
<thead>
<tr>
<th>Spot number</th>
<th>21</th>
<th>22</th>
<th>23</th>
<th>24</th>
<th>25</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1, µm</td>
<td>68.9±1.2</td>
<td>188.6±4.1</td>
<td>265.6±16.7</td>
<td>186.2±4.1</td>
<td>78.8±0.9</td>
</tr>
<tr>
<td>D2, µm</td>
<td>76.9±1.55</td>
<td>87.4±1.03</td>
<td>92.8±1.01</td>
<td>88±1.04</td>
<td>80.4±0.94</td>
</tr>
<tr>
<td>Ellipsity</td>
<td>0.9±0.02</td>
<td>2.16±0.05</td>
<td>2.86±0.18</td>
<td>2.12±0.05</td>
<td>0.98±0.02</td>
</tr>
<tr>
<td>Gaussian Fit 1</td>
<td>0.67±0.15</td>
<td>0.6±0.12</td>
<td>0.22±0.07</td>
<td>0.6±0.11</td>
<td>0.57±0.1</td>
</tr>
<tr>
<td>Gaussian Fit 2</td>
<td>0.57±0.2</td>
<td>0.67±0</td>
<td>0.67±0</td>
<td>0.65±0</td>
<td>0.63±0.1</td>
</tr>
<tr>
<td>Spot number</td>
<td>16</td>
<td>17</td>
<td>18</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>D1, µm</td>
<td>93±2</td>
<td>130±2.1</td>
<td>205.3±2.8</td>
<td>132.5±2.1</td>
<td>88.5±1.7</td>
</tr>
<tr>
<td>D2, µm</td>
<td>72.3±1.98</td>
<td>68±0.69</td>
<td>69.6±0.47</td>
<td>68.4±0.65</td>
<td>73±2.51</td>
</tr>
<tr>
<td>Ellipsity</td>
<td>1.29±0.04</td>
<td>1.91±0.04</td>
<td>2.95±0.05</td>
<td>1.94±0.04</td>
<td>1.22±0.04</td>
</tr>
<tr>
<td>Gaussian Fit 1</td>
<td>0.58±0.2</td>
<td>0.57±0.09</td>
<td>0.55±0.11</td>
<td>0.55±0.08</td>
<td>0.63±0.22</td>
</tr>
<tr>
<td>Gaussian Fit 2</td>
<td>0.56±0.2</td>
<td>0.73±0</td>
<td>0.75±0</td>
<td>0.79±0</td>
<td>0.52±0.2</td>
</tr>
<tr>
<td>Spot number</td>
<td>11</td>
<td>12</td>
<td>13</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>D1, µm</td>
<td>86.2±1.2</td>
<td>102.8±1.2</td>
<td>182.2±2.1</td>
<td>103.5±1.3</td>
<td>88.5±1.3</td>
</tr>
<tr>
<td>D2, µm</td>
<td>73.6±1.27</td>
<td>65.9±0.71</td>
<td>64.7±0.42</td>
<td>65.4±0.6</td>
<td>73.2±1.14</td>
</tr>
<tr>
<td>Ellipsity</td>
<td>1.17±0.03</td>
<td>1.56±0.02</td>
<td>2.82±0.04</td>
<td>1.58±0.03</td>
<td>1.19±0.03</td>
</tr>
<tr>
<td>Gaussian Fit 1</td>
<td>0.75±0.08</td>
<td>0.75±0.09</td>
<td>0.67±0.07</td>
<td>0.77±0.1</td>
<td>0.73±0.08</td>
</tr>
<tr>
<td>Gaussian Fit 2</td>
<td>0.7±0.1</td>
<td>0.81±0</td>
<td>0.8±0</td>
<td>0.83±0</td>
<td>0.73±0.1</td>
</tr>
<tr>
<td>Spot number</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>D1, µm</td>
<td>72±0.7</td>
<td>106.8±1.6</td>
<td>216.6±2.6</td>
<td>109.3±0.7</td>
<td>70.2±1</td>
</tr>
<tr>
<td>D2, µm</td>
<td>69.3±0.81</td>
<td>68.8±0.79</td>
<td>69.7±0.31</td>
<td>71.7±0.03</td>
<td>67.1±1.14</td>
</tr>
<tr>
<td>Ellipsity</td>
<td>1.04±0.02</td>
<td>1.55±0.03</td>
<td>3.11±0.04</td>
<td>1.52±0.11</td>
<td>1.04±0.02</td>
</tr>
<tr>
<td>Gaussian Fit 1</td>
<td>0.79±0.05</td>
<td>0.75±0.11</td>
<td>0.63±0.1</td>
<td>0.69±0.03</td>
<td>0.79±0.09</td>
</tr>
<tr>
<td>Gaussian Fit 2</td>
<td>0.8±0.1</td>
<td>0.78±0.1</td>
<td>0.81±0</td>
<td>0.73±0</td>
<td>0.72±0.1</td>
</tr>
<tr>
<td>Spot number</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>D1, µm</td>
<td>92.3±1.6</td>
<td>140.1±2.3</td>
<td>297±4.8</td>
<td>143.4±3.4</td>
<td>90.9±1.9</td>
</tr>
<tr>
<td>D2, µm</td>
<td>79.5±1.66</td>
<td>94.2±0.56</td>
<td>91.9±0.57</td>
<td>93.2±1.06</td>
<td>78.1±1.28</td>
</tr>
<tr>
<td>Ellipsity</td>
<td>1.16±0.03</td>
<td>1.49±0.03</td>
<td>3.23±0.05</td>
<td>1.54±0.04</td>
<td>1.16±0.03</td>
</tr>
<tr>
<td>Gaussian Fit 1</td>
<td>0.51±0.16</td>
<td>0.6±0.08</td>
<td>0.63±0.07</td>
<td>0.55±0.16</td>
<td>0.52±0.14</td>
</tr>
<tr>
<td>Gaussian Fit 2</td>
<td>0.58±0.1</td>
<td>0.71±0</td>
<td>0.72±0</td>
<td>0.64±0.1</td>
<td>0.66±0.1</td>
</tr>
</tbody>
</table>

a D1 and D2 are beam widths along the spot major and minor axis and are measured at 1/e² FWHM.

b Ellipsity is the ratio D1/D2.

c Gaussian Fit 1 and 2 show how close the beam profile distribution to the Gaussian distribution for TEM00 mode.
4.4.3.4 Comparison RS and PSE Engines for Field Curvature

The field curvatures at the intermediate image plane across the scan field for the RS and the PSE scan engines were calculated from the experimental data by evaluating the difference between the focal locations of the beams at all scan points and the focal position for undeflected beam, position 13. The field curvature for the SSE engine was difficult to estimate as the beam is astigmatic. Results are illustrated in Figure 4-23 and tabulated in Table 4-9. The field curvature created by the PSE engine along the horizontal and vertical is smaller than that created by the RS engine. Comparison of the differential field curvature between the RS and PSE engines is shown in Figure 4-23 (c).
Figure 4-23. Comparison of the field curvature created by the RS and PSE engines. (a) and (b) are the field curvatures in RS and PSE engines respectively, and (c) presents the difference in field curvature between RS and PSE engines.

Table 4-9. The values of the calculated field curvatures in RS and PSE engines along the scan lines.

<table>
<thead>
<tr>
<th>Scan Mirror Angle</th>
<th>PSE (mm)</th>
<th>RS (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+5°</td>
<td>0°</td>
</tr>
<tr>
<td>Horiz.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vertical</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+10°</td>
<td>6.88</td>
<td>4.29</td>
</tr>
<tr>
<td>+5°</td>
<td>2.75</td>
<td>1.59</td>
</tr>
<tr>
<td>0°</td>
<td>1.86</td>
<td>0</td>
</tr>
<tr>
<td>-5°</td>
<td>4.35</td>
<td>2.32</td>
</tr>
<tr>
<td>-10°</td>
<td>7.84</td>
<td>4.22</td>
</tr>
</tbody>
</table>
It shows that, for nearly all the scan field points, the field curvature created by the PSE engine is smaller than that created by the RS engine. The field curvature compensation in the PSE, we believe, is due to collimation properties of the PSE afocal relay described above and the slight differences in the beam diameter due to the additional optical path length through the PSE optical relay. Asymmetry in the PSE values arises due to the aberrated focus between the two parabolic elements and reflects the centre of mass distribution.

4.5 Summary

The experimental evaluation of the performance of three scan engines, the PSE, SSE and RS, as afocal and focused scan engines has been presented. Comparison with a freely propagated beam affirms that, for the PSE afocal relay, no astigmatism is introduced on axis and the dependence on axial parabolic mirror separation reveals the PSE relay works as a collimator with potential for volume scanning.

Afocal analysis results show that the performance of the PSE afocal engine is very similar to an ideal RS engine with the central spot being circular and beam spot becoming slightly elliptical towards the scan edges in both cases. The PSE afocal engine spot size ratio is identical, within measurement error, to that of the RS engine and superior to an SSE design.

The focused scan engine performance evaluated at the intermediate image plane showed the new PSE design again closely paralleled an ideal single mirror scan engine. In contrast, the SSE engine produced a highly astigmatic beam where the wavefront distortion will significantly decrease the improvements in resolution offered by two-photon excitation in scanning microscopy. The spot profiles measured in this experiment by a beam profiler reproduce the calculated point spread function of the beam in both the afocal and focused measurement series. The profile images at the intermediate plane (Figure 4-17, Figure 4-19, Figure 4-20) correspond to the published OSLO calculated images at infinity [183].

Measurements of the field curvature were made for the focused PSE and RS engines revealing that the PSE field curvature is smaller than the RS engine while the SSE engine produced large astigmatism.

In summary, the experimental measurements have matched the results predicted by modeling and the scan engine produced by the four (flat, off-axis parabola, off-axis
parabola, flat) mirror afocal relay has significant advantages in optical performance and potential as compared to alternative relays.
Chapter 5 Calculated Two-Photon Fluorescence Correction Factors for Reflective Scan Engines

The excitation laser’s spatial and temporal characteristics at the objective focal point are critical to the performance of two-photon scanning microscopes as reviewed in chapter 1. Optical aberrations in scanning systems increase the microscope objective focal spot area and introduce pulse time broadening in the deflected beam resulting in degradation of two-photon induced fluorescence across the scan field. The geometrical pulse broadening is investigated for the first time and then combined with the focused spot area, to provide a normalized two-photon fluorescence intensity correction factor. This factor, calculated using OSLO optical software, is compared to four reflective scan engines and it allows compensation of the detected signal with position across the scan field. This new metric highlights that a parabolic mirror afocal relay exhibits superior performance as a reflective scan engine for two-photon scanning microscopy.

This work was published in Applied Optics [189]

5.1 Introduction

Multiphoton fluorescence scanning microscopy [8] is employed in biomedical research because of its ability to produce images of biological activities and structure deep within live tissues [3-7] and was reviewed in chapter 1. When focused by a high numerical aperture objective, near-infrared ultra-short laser pulses create the very high photon flux at the focal point required for the fluorescent molecules in the sample to absorb two photons simultaneously. The average two-photon induced fluorescence emission per fluorophore per incident photon per second is given by Diaspro and Sheppard [1] as:

\[
\langle I_{fl}(1) \rangle = \frac{\delta_2 P_{ave}^2}{\tau_p f_p \frac{\pi NA^2 \gamma^2}{hc\lambda}},
\]

where \(\delta_2\) is the two-photon absorption cross section, \(P_{ave}\) is the laser average power, \(\tau_p\) is the pulse duration, \(f_p\) is the laser pulse repetition rate and \(NA\) is the objective lens numerical aperture, while \(h\) is the Planck constant and \(c\) is the speed of light of wavelength \(\lambda\). When diffraction limited performance is convolved with this expression, the two-photon induced fluorescence intensity from the excited volume element
emerges as being inversely proportional to the pulse duration of the exciting laser and
the square of the focused spot area for a particular fluorophore:

\[ I_p \propto \frac{1}{\text{Area}^2 \tau_p} = F, \]

where \( F \), the fluorescence intensity factor, is introduced within this research as a
parameter of the optical system. The evaluation of \( F \) across the scan field is a useful
metric, as for any chosen wavelength, average laser beam power and pulse repetition
rate, any increase in focal spot size and any temporal broadening degrades the
multiphoton fluorescence emission.

The minimum focal spot size, ideally the diffraction limit, can be achieved in optical
systems when a planar wavefront uniformly illuminates the back aperture of an
objective. However, in scanning microscopy the beam must be deflected in order to
build the scanned image of the sample and, as a result, the beam wavefront is tilted with
respect to the objective back entrance at some angle for all scan pattern positions except
on-axis. Moreover, the beam wavefront can be distorted prior to the objective by other
optical elements including the scan engine. The combination leads to the actual optical
performance at the raster in the sample falling below the benchmark diffraction-limit, as
is evident in Figure 5-1 which shows lens performance for on-axis and skew incident
beams.

The temporal pulse broadening in a microscope system is dominated by, and is usually
ascribed completely to, the effect of group velocity dispersion (GVD) by lens materials
(see chapter 1). This effect has been well investigated [97] and arises from the different
wavelengths within an ultra short pulse propagating with different velocities through
each of the different glass elements in the optical train resulting in different chromatic
elements of the pulse arriving at different times in the focal plane. The net effect is to
decrease the peak intensity at the focal point. The modern multi-photon microscope can
employ a pulse compressor system [92, 190] or a negative chirp GVD compensator
[191] to effectively pre-correct for the on-axis temporal pulse broadening associated
with the microscope objective and other refractive or coated optical elements. The
GVD associated with off-axis beam propagation through the optical train while scanning
is impossible to compensate for dynamically and difficult to measure experimentally.
Using only reflective surfaces in the scan system limits the GVD induced pulse
broadening to that associated with the atmospheric optical path distance for any system.
The contribution of the scan engine itself to pulse time broadening has, until now, been overlooked. For fast scanning by any scan engine, the mirrors used must have a low moment of rotational inertia arising from mirror mass and size [3] and effectively proportional to the cube of the aperture [26]. However, even with small mirrors, the optical path difference between the peripheral rays creates a distorted pulse front and an effective temporal broadening at the sample.

This Chapter investigates the optimum scan engine design to achieve the best possible two-photon fluorescent signal by comparing and contrasting four scan engines. These are evaluated by spot size within and across the scan field as well as, for the first time, the wavefront distortion associated with the scan engine, allowing the calculation of the effective temporal broadening induced by the scan engine itself. We introduce the new fluorescence intensity factor as a system metric and calculate these values across the scan field for three scan engines normalized to an ideal reference system of a single mirror. The analysis further highlights the advantage of a new parabolic reflective
scanning engine design introduced in chapter 3 and published [183] which employs two parabolic reflectors as an afocal relay between two scan mirrors.

5.2 Method

In this chapter, the OSLO simulation, reviewed in the chapter 2, was used to calculate the spot diagrams, point spread functions and wavefront distortion of input Gaussian beams based on the propagation of a grid of rays launched from an on-axis object point and evenly spaced through the system entrance pupil with an evaluation of the rays at the intermediate image plane. The beam spot areas at the different scan field points evaluated were calculated using the astigmatic Gaussian beam analysis option within OSLO. The input beam source is simulated as described in chapter 2 and represents the TEM00 Titanium-doped Sapphire near-infrared ultrashort pulsed laser used in two photon microscopy. Pulse time broadening arising from the difference in optical path length between peripheral rays was calculated based on analysis of OSLO results for spot sizes and angle of incidence as described in chapter 2. The fluorescence intensity factors were calculated then from the spot area and the pulse time broadening.

The performance advantages of particular scan engines are shown through comparison with other known and commonly used systems. There are many scan engine designs in optical scanning microscopy from single mirror deflectors to very complex multiple element arrangements designed to reduce beam aberration and field distortion as reviewed in the chapter 1 and in literature [3, 28, 30]. Not all of them can be applied in fast imaging microscopy due to mass of moving elements and, particularly in the two-photon fluorescence microscopy case, the engines with only achromatic metallic-coated reflective elements are optimal as they do not introduce additional GVD to the propagated beam.

Three established reflective designs and the recent parabolic scan system have been simulated as described in chapter 2. All systems deflect the incoming beam in two perpendicular directions, as shown in Figure 5-2 panels (a)-(d). They represent: (a) single flat scan mirror RS; (b) two flat scan mirrors as a close coupled engine CCE; (c) the spherical mirror based SSE system, with two flat scan mirrors each with its rotational axis in an orthogonal direction and two spherical mirrors between them as reflective relay optics; and (d) the PSE system, including two flat scan mirrors with rotational axes in two orthogonal directions and two parabolic reflectors between them.
as reflective relay optics. The 25 points of the scan pattern used to analyse performance of the scan engines at the intermediate image plane are shown in Figure 5-2 (e).

![Figure 5-2. Four simulated scanning designs: (a) single mirror engine used as the reference design RS, (b) close coupled engine CCE, (c) spherical reflector engine SSE, and (d) parabolic reflector engine PSE. Panel (e) describes the scan pattern points analysed.](image)

Three macros which were created during this work to retrieve the data, calculate parameters and plot graphs used in this chapter are presented in Appendix D.

### 5.3 Evaluation Limit

The illumination path in a typical scanning microscope was reviewed in chapter 1 and for a two-photon microscope is schematically illustrated in Figure 5-3. The Ti: S laser beam is deflected by a scan engine, and then focused by a scan lens to the intermediate image plane which is then conjugated to the microscope objective plane, and then enters the microscope body.

As microscope objectives, and any tube lens for an infinity-corrected objective, are well corrected optics located inside the microscope body, the scan engine performance analysis presented here is calculated at the intermediate image plane, in essence a magnified replica of the scanned beam in the objective focal plane.
An optimized scan lens design from the OSLO database, as detailed within Figure 5-3 and described in chapter 2, was used in this work. This choice is optimal for optical aberrations but it should be noted would that it would be a poorer choice for GVD due to the number and thickness of glass elements. In practice a two-photon system may well use an eyepiece with reduced wide field performance or even a singlet lens for better GVD performance. The distance from the last surface of the scan lens to the intermediate image plane was determined on the basis of minimal RMS spot size for the on-axis beam in position 13 from Figure 5-2(e).

5.4 Results and Discussion

Results are shown through comparison of spot diagrams, point spread functions (PSF), maximum PSF values, wavefront errors, calculated spot areas, pulse front aberrations, related pulse time dispersion and the relative fluorescence intensity factors. The relative scan geometry for the various engines are shown in Figure 5-4 which are similar to the simulation results in chapter 3, Figure 3-12.
The RS system scan pattern is symmetrical and shows symmetrical pincushion distortion. The PSE engine creates straight scan lines with pincushion distortion in both sides similar to the RS system. The CCE system produced elongated scan lines with pincushion distortions on the sides. The SSE created scan lines have a different curvature which increases down the scan area. Created scan lines are straight only for the PSE system.

5.4.1 Spot Diagrams and PSF

The spot diagrams for an on-axis object point for four examined scan engines at 25 scan field points are shown in Figure 5-5 (a). It can be seen that diffraction-limited performance is achieved only for the RS, CCE and PSE designs at the central nine configurations of the examined scan area and performance degrades at the extreme angles. These results are reflected on the appropriate normalized intensity PSF map graphs for the same points, Figure 5-5 (b). Note the bell-shaped spots distributed around the peak for the central nine configurations with the maximum peak close to 1 and distorted spots with reduced maximum value at the sides. The intensity PSFs for the CCE along scan lines are distorted faster compared to the RS and PSE designs as the CCE produces elongated scan lines for the same deflecting angle compared to both the RS and PSE designs as shown in Figure 5-5 (b). The SSE design produces the worst performance with aberrated spot footprints due to astigmatism and coma arising from the tilted dual spherical reflectors. The maximum PSF value is only 0.12 in the off-centre configurations 12 and 14 arising from extreme astigmatism in these scan positions.

Figure 5-5 (c) and (d) show the PSF maximum across the whole scan field at a resolution of 0.1 degrees. Diffraction-limited performance, as determined from PSF
max values (Strehl ratio) between 0.75 and 1, is produced by RS and PSE designs across the central area while for the CCE this ideal is reached only in the middle of scan lines. The SSE design does not approach diffraction limited performance across the whole area with PSF maximum values less than 0.16. The 2-D panels in Figure 5-5 (d) are instructive in highlighting the symmetry of the PSF variation. The panel for the PSE shows a very slight performance decrease in the upper half due to the optical magnification experienced by the expanding spherical wavefront interacting with identical parabolic surfaces spaced symmetrically, while the RS is ideal and the CCE reflects scan elongation.

Figure 5-5. (a) Spot diagrams and b) intensity PSFs for four scan engines in 25 scan configurations. Scan angle range in both directions is from -10 to +10 degrees. (c) and (d) show PSF maximum values as contour and 2D maps across whole scanning field at a resolution of 0.1 degrees. The color scale bar is common for (d) and (c) of each column.
5.4.2 Wavefront Contours and Wavefront Error

The resultant geometrical wavefront contours for an on-axis object point for the 25 scan field points are shown in Figure 5-6 (a). The wavefront error, defined as the peak-to-valley optical path difference in wavelength (P-V OPD), across the whole scanning field is shown in Figure 5-6 (b). These results confirm the diffraction-limited performance of RS, CCE and PSE for the nine central points of the scanning pattern where the wavefront error for RS and PSE systems is less than one quarter wave. For CCE the wavefront distortion is approaching a half-wave with increasing distortion towards the extremities, and for the SSE, distortion is more than three wavelengths for all configurations.

![Figure 5-6. Wavefront aberrations for four scan engines. (a) wavefront contours, (b) ln(peak-to-valley optical path difference), (c) Scaled 2-D maps of central (± 5°) scan field.](image)

The wavefront aberration in the close-coupled mirror design arises from the distance separating the two flat mirrors and the deflection angle of both mirrors. The CCE result shows similarity with the reference system for the central vertical axis where the first scan mirror is at 0° deflection and the beam position is defined only by the deflection of the second scan mirror. The P-V OPD distortion increases rapidly with deviation from the centre along horizontal zones and is only comparable to the RS result when the second scan mirror is within ± 5° of the central position.

The worst wavefront distortion results are observed in the SSE system where even in the central position, the P-V OPD error reaches the value of 3.37λ. The P-V OPD is
higher at the top two corners in comparison to the lower two and this arises from the SSE system not being completely symmetrical compared to the other systems as the two scanning mirrors are angled differently to obtain an unobstructed two dimensional scan engine. When those scan mirror tilt angles are close in value to the static mirror tilt as at configurations 21 to 25, the wavefront error decreases.

Panels in Figure 5-6 (c) are of the central field within \( \pm 5^\circ \) of the central position and show the wavefront error to be lowest for the RS and PSE designs.

### 5.4.3 Fluorescence Intensity Factor

The fluorescence intensity factor \( F \), as described in the introduction to this chapter, has been calculated and the performance of the scan engines normalized with respect to the RS system evaluated. The necessary data are the spot area and broadened pulse time i.e. the initial pulse time plus the additional time distortion caused by the scan engine.

A spot area has been calculated using astigmatic beam analysis data for a propagated Gaussian beam which presents the beam radii at the image surface in two perpendicular directions, X and Y. Where the beam spot is an ellipse, the spot size is calculated as

\[
\text{Area} = \pi ab, \quad \text{where} \quad a \text{ and } b \text{ are the two beam radii.}
\]

The spot area plots for four systems are shown in Figure 5-7.

The spot area for three systems, RS, PSE and CCE, are nearly the same in the \( \pm 5^\circ \) scanning range. With the CCE design, the spot area increases up to 25 times toward the scan extremities.

The SSE result is from 5 to 15 times larger at the central configurations along all scan lines and sharply increases at the extremities.

![Figure 5-7. Spot areas at the intermediate image plane for (a) RS, (b) PSE, (c) CCE and (d) SSE systems.](image-url)
The concept of pulse time broadening calculation at any scan field point was presented in chapter 2.

The resultant time delays induced by the scan engines are shown in Figure 5-8.

Figure 5-8. Time delay introduced by scan engines for (a) RS, (b) PSE, (c) CCE and (d) SSE.

The PSE system showed a very similar result to the RS system with both below 14 fs maximum at scan extremities and below 0.3 fs within ± 5° of the central position. These engines show a difference of less than 0.07 fs across the central area.

The CCE induces a 1.1 fs broadening which is 5.5 times larger in the central area compared to RS, and increases towards the scan extremities. The SSE system produces up to 1.84 fs pulse broadening for the central region, and sharply increases at the scan extremities.

Calculated relative fluorescence intensity factors are plotted in Figure 5-9 and value ranges are shown in Table 5-1. These results assume an initial pulse duration of 80 fs and the additional time arising from the scan engine path length detailed in Figure 5.8 is added to this period. Results confirm that the performance of the new parabolic scan engine design is similar to the RS and if employed in two-photon scanning microscopy will produce higher intensity two-photon fluorescence signals. The evaluation of F across the whole scan area reveals more uniform and even values for the PSE design compared to the CCE and SSE designs. Within the ± 5° scan range, the calculated two-photon fluorescence signal is up to 1.74 times higher for the PSE design compared to a single mirror scanning system, which is 10 % higher than for a CCE system and 32 times higher than in an SSE system which shows a maximum of 0.06 in all configurations indicating that it suffers a 94 % reduction in signal compared to RS.
Calculated TP fluorescence Correction Factors for Reflective Scan Engines

Figure 5-9. The relative fluorescence intensity factor for (a) PSE, (b) CCE and (c) SSE across the investigated area.

Table 5-1. The minimal and maximum relative fluorescence intensity factors for PSE, CCE and SSE compare to RS range of values for the central section of the investigation area.

<table>
<thead>
<tr>
<th></th>
<th>PSE/RS</th>
<th>CCE/RS</th>
<th>SSE/RS</th>
</tr>
</thead>
<tbody>
<tr>
<td>min</td>
<td>1.2</td>
<td>1.14</td>
<td>0.005</td>
</tr>
<tr>
<td>max</td>
<td>1.74</td>
<td>1.64</td>
<td>0.055</td>
</tr>
</tbody>
</table>

5.5 Summary

In this chapter a new metric for scan engine performance evaluation, the fluorescence intensity factor, was introduced and used to evaluate the performance of four scan engines. The fluorescence intensity factor is determined by spot area, as determined by spot diagrams and point spread functions, wavefront distortion and the associated temporal broadening. This temporal broadening, attributable to the scan engine, was calculated for the first time and found to be small (< 2 fs) in the central regions of the scan field but much higher in the extremities. The central field wavefront distortion as a contributing proportion of overall pulse temporal characteristics will become more significant as shorter incident laser pulses are employed in two-photon microscopy.

It was found from the study above that the scan engine design employing two off-axis parabolic reflectors, appropriately positioned relatively to the rotational axis of the scan mirrors, is the best choice of scan system to apply in two- and multi-photon fast
imaging microscopy at least for illumination of a plane. The wavefront aberrations introduced to the pulsed laser beam over the range of the scan angles are minimal compared to existing systems and hence minimize pulse distortion. The geometrical spot diagrams and point spread functions of the on-axis object point are equal to the reference system confirming that optically, the PSE design is very similar to an ideal single mirror scan engine. The PSE is a practical realizable design with the unique ability to employ small-sized scan mirrors and hence scan rapidly, and represents an ideal choice for multi-photon scanning microscopy.
Chapter 6  Summary of Thesis and Future Work

The data presented and discussed in the previous chapters introduced a new optical scan engine design and investigated its performance. In this final chapter, the study is summarised and directions for future research and applications are highlighted.

6.1 Summary of Thesis

The design of optical scan engines continues to be an active research area with scan speed, width and linearity key target parameters. There is an ongoing demand for efficient scan engines in fields ranging from military, through commerce (bar code scan engines), atmospheric science, to high-performance microscopes applied in biomedical science [192, 193]. The performance of the 2D laser scan system in a biomedical microscope is fundamental to imaging system and many approaches have been tried.

This research introduced a new afocal scan engine design based on two parabolic optical elements as reflective relay optics. Using OLSO optical software the system was modelled and its performance was examined in comparison to other known afocal scan engines over a scan amplitude of ±10° deflecting angle for both scan mirrors. Modelling results showed that the new parabolic reflector design and an appropriate scan mirror rotational axis, eliminated line curvature in the scan pattern and exhibited good Gaussian spot size performance and excellent spot diagram and point spread function parameters for the range of scan angles evaluated. The new system performance was very similar to a single mirror scanning engine as it effectively created one optical pivot point and the fast imaging speed can be achieved by employing a fast resonant mirror.

Prototypes of the new scan engine and known engines were assembled and their performance was measured.

Results confirmed the OSLO modelling predictions for the performance of the PSE and SSE engines with the PSE producing better spot size results across the scan field, compared to the SSE, and showing an absence of astigmatism. It was also shown that the PSE engine produces less field curvature compare to the single mirror engine and it can be used as an afocal relay.

In this study, the time pulse distortion induced by a scan engine across the scan field has been investigated for the first time. The optical path difference between the peripheral
rays in an ultra-short pulsed laser excitation beam creates a distorted pulse front and an effective temporal broadening at the sample. The new evaluation metric introduced for optical performance across the scan field, showed that the new design introduced less pulse time distortion across the field and the relative factor showed that the new scan engine design will produce better TPE signal from the sample compared to a close coupled scan engine and SSE.

This study takes the scan engine to the next logical step of the development.

### 6.2 Future Research

The plan for future studies is outlined as a flowchart in Figure 6-1. It includes further exploration of the new afocal reflective scan engine performance in terms of scan field flatness, linearity of scan lines, wide-angle scan approaching hemispherical, optical zoom ability and miniaturising the design.

These selected areas are ones in which the new scan engine offers significant innate performance advantage when compared to existing scan engine designs. The goal of multi-beam multi-focal two-photon microscopy is particularly attractive and the area of ultra-wide field scanning matches the enhanced capability of this design.

Further comparative optimization of the PSE, CCE and SSE are possible as improved modelling software allows for parameter values to be set based on iterative aberration minimization. The outcome of this process would be the most advantageous scan engine for single and multi-photon microscopy. For example, the PSE and SSE designs would merge in the limit of a spherical surface approximating the parabolic, however an unobstructed beam path is required.

#### 6.1.1 Wide Angle Scan

The geometry of the new parabolic scan engine and its scan mirror rotational axis orientations allows for wide angle scanning (Figure 6-2 (a)). The highlighted section of the parabolic surface, as shown in Figure 6-2 (b), can be illuminated by the first scan mirror providing of 360° rotation.
Figure 6-1. Flowchart for future research.
In practice this would be limited to $\sim 170^\circ$ in order to illuminate the front surface of scan mirror 2 at other than grazing incidence. Scan mirror 2 can be rotated over an angle range of $\sim 85^\circ$ with the limits being grazing incidence at $90^\circ$ and vignetting from the second parabolic surface close to $0^\circ$ angle of incidence.

The parabolic elements reduce to diamond machined strip mirrors just wide enough to take the beam ($\sim 5$ mm) in the $z$-axis and semicircular in the $x$-$y$ plane as shown in Figure 6-2 (c). This allows for a rugged design.

The potential for extremely wide field scanning is attractive for military and other scientific scanning applications.

![Figure 6-2. PSE design proposal for a wide scan: (a) the PSE design; (b) strip of parabolic surface needed for the wide angle scan; and (c) the side view of the strip from parabolic surface. Its width could be only 5 mm.](image)

### 6.1.2 Linear Scan and Scan Field Flatness

In microscopy, the flat field scanning of the PSE across the objective field is very beneficial in minimizing image distortion for both confocal and two-photon microscopy. The degree to which this affects image performance remains to be quantified.
6.1.3 Advantage in Two-Photon Microscopy

The inherent features of this scan engine including minimal temporal and spatial wavefront distortion (as shown in chapter 5) may substantially improve the image quality in two-photon microscopy and this needs to be quantified.

In any two-photon excitation system, the image contrast can be increased by increasing excitation beam power and/or decreasing the pulse duration. Increased beam power opens the possibility of harm to the live tissue and photodamage to the fluorophore properties. The shorter pulse duration will undergo the greatest time broadening due to GVD. However, this is the preferred and best approach in two-photon imaging.

The new scan engine provides the best possible and most uniform image contrast across the scan field due to the excitation spot dimensions and minimal pulse time distortion, which is important for shorter pulses.

6.1.4 Miniaturisation

The PSE physical size can be described by the radius of curvature of the parabolic surfaces employed as shown in Figure 6-3. Therefore, the PSE has potential for miniaturisation with limits to size anticipated to emerge as the discrete and finite size of the beam diameter becomes an effective extended source at the system entrance.

![Figure 6-3](image_url)

Figure 6-3. The size of the PSE scan engine is a function of the radius of curvature R of parabolic reflectors.

6.1.5 Optical Zoom Ability

The zooming capability arises from two very different approaches. The first is to produce digital zoom by acquiring the same pixel information (e.g. 512 x 512 pixel) from a smaller area of interest as the mirrors scan a smaller angle. This is an imaging software controlled zoom with the limit determined by scan mirror performance. The
second method is to vary the distance between the two parabolic elements as was shown in chapter 4. This will distort the afocal nature of the scan engine and result in a shift of the objective focal plane deeper into or less deep into the tissue. In this way, one can build a 3D volume scan system independent of moving the objective lens or the microscope stage.

### 6.1.6 Multi-beam Scan

During this research, the investigation of the PSE as multi-beam scan engine was initiated. The three beam generator which included three mirrors M1 to M3 and two beamsplitters is shown in Figure 6-4. The laser beam is reflected at a small angle from the mirror M1, incident on the 30/70 T/R beamsplitter. 30% of the beam is transmitted by the beamsplitter B1 and forms the first beamlet. 70% of the beam is reflected to the mirror M3. The beam then reflects from the 50/50 beamsplitter B2 which produces the second beamlet with an intensity of 35% of the initial beam and reflects the rest to the mirror M3. The mirror M3 produces the third beamlet with the remaining 35% of the laser beam intensity.

The 1.2 m distance between the mirrors and beamsplitters is calculated to produce a 4 ns time delay between beams propagated in air: \( d = 3 \times 10^8 \text{m/s} \times 4\text{ns} = 1.2\text{m} \).

The beamlets can be positioned close to each other so that cross-talk between beamlets due to the time separation can be avoided. Additionally, the beamsplitters will create a small time delay. The 4 ns time difference is chosen to match the 4.1 ns fluorescence lifetime of Oregon Green Bapta-1 dye. Therefore, during one excitation pulse (duration 12 ns), the fluorescence molecules can be excited three times (Figure 6-5 (a) and (b)) and produce three emission signals which can be recorded by separate detectors. Hence, this multi-beam setup increases the imaging speed three times.

### 6.3 Conclusion

This novel scan engine has great potential in areas where its innate and particular optical characteristics match with the application requirements. The flatness of the scan field and the ease of wide field scanning are particular attributes that should attract interest in this engine. By employing the new scan engine in a two-photon microscope, the image resolution should be significantly enhanced. Ability to control the stepping of the focal
plane with the new scan engine can be beneficial for continuous three dimensional imaging when stability of the tissue/objective interface needs to be maintained.

Figure 6-4. Three beam generator. M1 is a small 100% reflectance mirror. M2 and M3 are 100% D-shaped mirrors. B1 and B2 are beam-splitters. B1 is 30/70 T/R and B2 is 50/50 T/R. Resultant three beams have a power of 30%, 35% and 35% of incident beam.

Figure 6-5. Spatio-temporal decoupling for the 3 beam multi-photon multi-beam multiplexing (see chapter 1.3). (a) Three beams (1, 2 and 3) separated in time during one pulse. (b) Three fluorescence excitations during one pulse.
Appendix A. Pulsed Laser Beam Parameters

The energy delivered in one pulse in the laser pulse train $E_p$ is the energy of all the photons in this pulse:

$$E_p = \sum_n n h\nu_n = \sum_n \frac{hc}{\lambda_n},$$

where $n$ is the number of photons, $h$ is Planck’s constant, $\lambda$ is the peak wavelength and $c$ is the speed of light in vacuum.

Due to the spread of wavelengths in a mode locked pulse it is better to approach this statistically with the time averaged energy:

$$\langle E_p \rangle_t = \frac{\langle P_{ave} \rangle_t}{f_p},$$

where $P_{ave}$ is the average power of the laser and $f_p$ is the pulse repetition rate.

Therefore, the averaged number of photons per pulse is:

$$n = \frac{P_{ave} \lambda_{Peak}}{f_p hc}.$$

For a Ti:S laser operating at 1W average power and emitting at a central wavelength of 800 nm, with 100 fs pulse duration at an 80 MHz repetition rate, the average energy per pulse is:

$$E_p = \frac{1W}{80MHz} = 12.5nJ.$$

The average number of photons per pulse is:

$$n = \frac{1W \times 800nm}{80MHz \times 6.6 \times 10^{-14} J/s \times 3 \times 10^5 m/s} = 5 \times 10^{10}.$$

For this number of photons per pulse, the peak power of the 100 fs pulse will be on average:

$$P_p = \frac{E_p}{t_p} = \frac{12.5nJ}{100 \times 10^{-15} s} = 12.5 \times 10^4 J/s = 125kW.$$

A 1.4 NA objective can focus 800 nm laser beam to a diffraction-limited Airy spot with diameter at $1/e^2$ calculated as:

$$d = 2\frac{\lambda f}{\pi w} = \frac{2\lambda}{\pi (NA)} = 0.64 \times \frac{800nm}{1.4} = 0.37\mu m.$$

The total radiance per pulse is:
Here are some additional spatio-temporal pulse characteristics which can be useful. For 100 fs pulse with repetition rate of 80 MHz the time between pulses is:

\[ t = \frac{1}{80 \text{MHz}} = 12.5 \text{ns}. \]

The pulse length is:

\[ l_p = c \times \tau_p = 3 \times 10^8 \text{ m/s} \times 100 \times 10^{-15} \text{ s} = 3 \times 10^{-5} \text{ m} = 30 \mu \text{m}. \]

The distance between two sequential pulses is:

\[ d_p = c \times t = 3 \times 10^8 \text{ m/s} \times 12.5 \times 10^{-9} \text{ s} = 37.5 \times 10^{-1} \text{ m} = 3.75 \text{ m}. \]

The number of 800 nm wavelengths per pulse is:

\[ 30 \mu \text{m} / 800 \text{nm} \approx 37.5. \]

Performing a line scanning with a resonant scanner at 8 kHz, the pulse and time characteristics per pixel are shown in Figure A-1 indicating a single pixel recorded represents a voxel illuminated by 20 pulses.
Appendix B. Database for Scan Engines

B.1 Mirror scanning in the Y direction

The scan mirror M1 deflecting the beam in the vertical direction, is tilted 45° relative to an incident beam and rotates about point O in the direction shown in Figure B-1 (a). The initial tilting angle of the mirror is dependent on the scanning amplitude and can be changed depending on the system requirements.

Figure B-1. (a) Scan mirror in the Y direction. (b) The changes in local coordinate systems in OSLO. The X axis is perpendicular to the page surface.

Table B-1 Scan mirror in the Y direction: the lens spreadsheet, special data and configuration data.

| Lens: Y scan mirror | Ent beam radius | Field angle | 10.000000 Primary wave
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>OBJ</td>
<td>0.000000</td>
<td>1.000000e+20</td>
<td>1.7633e+19</td>
</tr>
<tr>
<td>AST</td>
<td>0.000000</td>
<td>0.000000</td>
<td>1.000000  AS</td>
</tr>
<tr>
<td>S</td>
<td>0.000000</td>
<td>0.000000</td>
<td>1.000000  S</td>
</tr>
<tr>
<td>3</td>
<td>0.000000</td>
<td>0.000000</td>
<td>1.000000  S</td>
</tr>
<tr>
<td>4</td>
<td>0.000000</td>
<td>0.000000</td>
<td>5.000000  REFLECT</td>
</tr>
<tr>
<td>5</td>
<td>0.000000</td>
<td>0.000000</td>
<td>1.000000  S</td>
</tr>
<tr>
<td>6</td>
<td>0.000000</td>
<td>-50.000000</td>
<td>1.000000  S</td>
</tr>
<tr>
<td>IMS</td>
<td>0.000000</td>
<td>0.000000</td>
<td>10.000000</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TILT/DECENTER DATA</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>DT 1</td>
<td>DCX 45.000000</td>
<td>TLA -- TBB --</td>
</tr>
<tr>
<td>3</td>
<td>DT 1</td>
<td>DCX -- DCY -- DCZ --</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>DT 1</td>
<td>TLA DCX -- DCY DCZ --</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>DT -1</td>
<td>DCX -- DCY -- DCZ --</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>DT 1</td>
<td>TLA 45.000000</td>
<td>TLA DCB --</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NBR</th>
<th>ITEM</th>
<th>SURFACE</th>
<th>ZOOM</th>
<th>QUALIFIER</th>
<th>VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TLA</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>10.000000</td>
</tr>
<tr>
<td>2</td>
<td>TLA</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>5.000000</td>
</tr>
<tr>
<td>3</td>
<td>TLA</td>
<td>3</td>
<td>4</td>
<td>0</td>
<td>-5.000000</td>
</tr>
<tr>
<td>4</td>
<td>TLA</td>
<td>3</td>
<td>5</td>
<td>0</td>
<td>-10.000000</td>
</tr>
</tbody>
</table>
The design includes five surfaces, 2 to 6, shown in Table B-1. Each surface is described in the local coordinate system as it was discussed in chapter 2. The local coordinate systems for incident beam, for mirror M1 and for reflected beam are shown in Figure B-1 (b).

Surface 2 tilts the coordinate system at 45° about the X axis. Surface 3 creates the deflection of the scan mirror, surface 4, to the desired scanning angle by changing TLA in the coordinate data. The tilting of a scanner is organized by using configurations for the angle TLA on surface 3, shown in Table B-1.

Surface 4 is the flat mirror surface itself. The “Tilt and Bend” option is on. The local coordinate system 5 is tilted at 90° to the previous coordinate system. Surface 5 is the coordinate break surface and it tilts the local coordinate system for the subsequent surfaces in the opposite direction to the scanning deflection created by the surface 3. The coordinate break surface 6 further tilts the coordinate system at the same angle as the surface 2. As a result, the scan mirror surface in the lens drawing remains at the same position for all configurations.

**B.2 Scan Mirror in the X direction.**

The scan mirror M1 deflecting the beam in the horizontal direction, is tilted 45° relative to an incident beam and rotates about point O in the direction shown in Figure B-2 (a) and (b). The initial tilting angle of the mirror is dependent on the scanning amplitude and can be changed depending of the system requirements.

![Figure B-2. (a) Scan mirror in the Y direction. The X axis is perpendicular to the page surface. (b) The incident beam is perpendicular to the page.](image)

There were two approaches to model a scan system in the X direction. The first approach was to model a setup similar to the previously described design: firstly tilt the mirror surface about the X axis by TLA=45° and then perform the scanning around Y
axis by changing TLB (sets the angle in degrees about the Y axis). However, this scan setup produced the curved scanning “line” at the image surface as shown in Figure B-3.

![Figure B-3. Ray diagram and curved scan line created on the image surface by the first version of a X scan system.](image)

The second approach was to first scan the mirror about the Y axis by tilting TLB $\pm 10^\circ$ and then to tilt it about the X axis TLA $\pm 90^\circ$. The resultant scan pattern has straight lines as shown in Figure 2-11. The data for the X scan system is shown in Table B-2.

The X scan system includes four surfaces. Surface 2 allows scanning of the flat reflected mirror, surface 3, along the X axis of the local coordinate systems by specifying the angle TLB in configurations. The coordinate break surface 4 picks up the negative value of the tilting angle TLB on surface 2. Surface 5 provides the initial tilting angle on the scan mirror relative to the incident beam at $45^\circ$ about X axis and reflects the scan line at $90^\circ$ to the incident beam. The initial tilting angle of the scan mirror is dependent on the scanning amplitude and can be changed depending on the optical system requirements.
**B.3 Two Close Coupled Mirrors**

A two close coupled scanning mirror system has been modelled according to the schematic diagram shown in Figure B-4. It combines the X scan mirror design to model the galvo scanner M1 rotating about axis 1 and the Y scan mirror design to model the scan mirror M2 rotating about axis 2. The separation distance $d$ is 10 mm and this has been determined as the most suitable to achieve a minimum scan line and field curvature. The lens setup is shown in Table B-3.

![Figure B-4. Two closed coupled scan engine.](image-url)
**Table B-3. Two close coupled mirror modelling setup.**

<table>
<thead>
<tr>
<th>SRF</th>
<th>RADIUS</th>
<th>THICKNESS</th>
<th>APERTURE RADIUS</th>
<th>GLASS</th>
<th>SPECIAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>OBJ</td>
<td>0.000000</td>
<td>2.000000e+03</td>
<td>2.500000</td>
<td>AIR</td>
<td></td>
</tr>
<tr>
<td>AST</td>
<td>0.000000</td>
<td>25.000000</td>
<td>2.500000</td>
<td>A</td>
<td>F</td>
</tr>
<tr>
<td>2</td>
<td>0.000000</td>
<td>0.000000</td>
<td>2.562500</td>
<td>S</td>
<td>AIR</td>
</tr>
<tr>
<td>3</td>
<td>0.000000</td>
<td>0.000000</td>
<td>2.000000</td>
<td>REFLECT</td>
<td>FC</td>
</tr>
<tr>
<td>4</td>
<td>0.000000</td>
<td>0.000000</td>
<td>1.500000</td>
<td>AIR</td>
<td>C</td>
</tr>
<tr>
<td>5</td>
<td>0.000000</td>
<td>-10.000000</td>
<td>5.000000</td>
<td>REFLECT</td>
<td>C</td>
</tr>
<tr>
<td>6</td>
<td>0.000000</td>
<td>0.000000</td>
<td>2.537500</td>
<td>S</td>
<td>AIR</td>
</tr>
<tr>
<td>7</td>
<td>0.000000</td>
<td>0.000000</td>
<td>2.537500</td>
<td>S</td>
<td>AIR</td>
</tr>
<tr>
<td>8</td>
<td>0.000000</td>
<td>0.000000</td>
<td>5.000000</td>
<td>REFLECT</td>
<td>C</td>
</tr>
<tr>
<td>9</td>
<td>0.000000</td>
<td>0.000000</td>
<td>2.537500</td>
<td>S</td>
<td>AIR</td>
</tr>
<tr>
<td>10</td>
<td>0.000000</td>
<td>15.000000</td>
<td>2.537500</td>
<td>S</td>
<td>AIR</td>
</tr>
<tr>
<td>IMS</td>
<td>0.000000</td>
<td>0.000000</td>
<td>5.000000</td>
<td>F</td>
<td></td>
</tr>
</tbody>
</table>

**B.4 A single mirror scan engine**

A single scan mirror system (RS) is schematically presented in Figure 2-13 (a) as a flat mirror M1 rotating simultaneously about axes 1 and 2. The simulated optical design of this mirror has shown that the scanning image pattern in the image plane produces a curved line, Figure 2-13 (b), which is caused by the shifting of the pivot point as a result of tilting.

![Figure B-5. One mirror scanning in both directions: (a) design and (b) scanning pattern for 25 configurations.](image)

To make a propagated beam deflect at one pivot point in two directions, the simulation has been realised through the setup shown in Table B-4 which is a true gimbal about the reflective surface.
Table B-4. A single scan mirror modelling setup.

<table>
<thead>
<tr>
<th>Surface data</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRF</td>
</tr>
<tr>
<td>OBJ</td>
</tr>
<tr>
<td>AST</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>IMS</td>
</tr>
</tbody>
</table>

Surface 3 is the mirror surface itself. Scanning is performed by surface 2 which tilts the beam about X and/or Y axis by changing angles TLA and/or TLB respectively. Surface 4 is the coordinate break surface which picks up a negative value for TLA and TLB on scanning surface 2.

The scan pattern created at the image surface is symmetrical in both directions and for this reason performance of RS engine has been used as a reference scan system.

**B.5 Scan engine with spherical reflectors**

The scan engine with spherical reflectors was introduced by Amos and the parameters for the model are mostly from the Amos patent [54]. Schematic diagram is drawn in Figure 3-9.

Lens data for the two spherical reflectors is shown in Table B-5. The first spherical reflector surface, surface 2, has a radius of curvature of 101.6 mm, an aperture radius of 25 mm, tilted 10° about the X axis and the coordinate data spreadsheet has the “tilt and bend” option as enabled. Hence, the reflected rays propagate 20° relative to the incident and the next surface coordinate system is tilted appropriately on a reflective surface. The direction of propagation Z-axis is in the opposite direction.

Surface 3 is the auxiliary surface and has been used to specify the position of the intermediate focal plane between the two spherical reflectors. The thickness of this surface is calculated by solving it for the axial ray height =0 (focal point).
Surface 4 is the intermediate surface which is the defined position of the second spherical reflector. Its thickness is chosen from the thickness of surface 3 to create a symmetrical arrangement.

Surface 5 is the second spherical reflector and has the same radius of curvature and aperture radius as the first spherical reflector but is tilted about X axis at $-10^\circ$.

Table B-5. The spherical scan engine setup.

<table>
<thead>
<tr>
<th>SRF</th>
<th>RADIUS</th>
<th>THICKNESS</th>
<th>APERTURE RADIUS</th>
<th>GLASS</th>
<th>SPECIAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>OBJ</td>
<td>0.000000</td>
<td>1.000000e+06</td>
<td>8.74890e+06</td>
<td>AIR</td>
<td></td>
</tr>
<tr>
<td>AST</td>
<td>0.000000</td>
<td>50.000000</td>
<td>5.000000</td>
<td>A</td>
<td>F</td>
</tr>
<tr>
<td>2</td>
<td>-101.600000</td>
<td>0.000000</td>
<td>25.000000</td>
<td>REFLECT</td>
<td>C</td>
</tr>
<tr>
<td>3</td>
<td>0.000000</td>
<td>-50.800000e+06</td>
<td>5.374000e+04</td>
<td>S</td>
<td>AIR</td>
</tr>
<tr>
<td>4</td>
<td>0.000000</td>
<td>-50.800000e+06</td>
<td>5.000000</td>
<td>F</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.000000</td>
<td>50.000000</td>
<td>25.000000</td>
<td>REFLECT</td>
<td>C</td>
</tr>
<tr>
<td>INS</td>
<td>0.000000</td>
<td>0.000000</td>
<td>5.000000</td>
<td>F</td>
<td></td>
</tr>
</tbody>
</table>

B.6 Scan engine with parabolic reflectors

The function and parameters of the PSE engine, shown in Figure 3-10, are described here. Parabolic reflectors 4 and 6 are off-axis circular segment from parabolic surfaces 3 and 5, respectively, with a radius of curvature $R$ and a focal length of $R/2$. Parabolic reflectors 4 and 6 are identical Newport Corporation off-axis replicated parabolic mirrors (50338 Al) and have an effective focal length of 101.6 mm, which is also the radius of curvature of the surface, and a true focal length of 50.8 mm. Use of identical parabolic reflectors is not strictly necessary, but allows a symmetrical optical system to be constructed and, therefore, aberrations introduced by the first concave surface, 4, to be minimized and a uniform beam diameter to be maintained. The deflection axes of scanning mirrors 1 and 7 are positioned at the focal points of parabolic reflectors 4 and 6, respectively. Mirrors 1 and 7 rotate around axis 2 and an axis lying on the surface of mirror 7, perpendicular to both rotation axis 2 and to the plane of this page. Line 8 demonstrates the direction of the mirror 7 movement. Mirror 1 is angled at 45° to the incident beam and rotational axis 2 is coaxial with the incident beam. This arrangement, and particularly the alignment and rotation of scan mirror 1 about axis 2, ensures that
the beam traces a geometric circle on the surface of parabolic reflectors 4 and 6 and results in the beam impinging on the centre of mirror 7, independent of the scan deflection angle, within the limit of the aperture stop defined by the physical size (38 ) of surface 4. The distance from parabolic reflector 4 to reflector 6 is determined by the parabola’s parameters and was held at 2R, as shown in Fig. 2, due to the choice of a symmetric design. Parabolic reflector 4 focuses the beam at a midpoint due to the incident laser beam diameter. The exact position of the midpoint focus is determined by the beam divergence and will vary slightly from the distance R to give a slight magnification of the beam size at mirror 7. Mirror 7 was modelled as a conventional galvanometer scanner rotating about an axis in the plane of the mirror surface.

The lens data is shown in Table B-6.
### Table B-6. The parabolic scan engine setup.

<table>
<thead>
<tr>
<th>SRF</th>
<th>RADIUS</th>
<th>THICKNESS</th>
<th>APERTURE RADIUS</th>
<th>GLASS</th>
<th>SPECIAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>OBJ</td>
<td>0.000000</td>
<td>1.0000E+03</td>
<td>2.5000E00</td>
<td>AIR</td>
<td></td>
</tr>
<tr>
<td>AST</td>
<td>0.000000</td>
<td>20.000000</td>
<td>2.5000E00</td>
<td>AS</td>
<td>AIR</td>
</tr>
<tr>
<td>2</td>
<td>0.000000</td>
<td>0.000000</td>
<td>4.0000E00</td>
<td>AIR</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.000000</td>
<td>0.000000</td>
<td>5.0000E00</td>
<td></td>
<td>REFLECT</td>
</tr>
<tr>
<td>4</td>
<td>0.000000</td>
<td>0.000000</td>
<td>4.0000E00</td>
<td>AIR</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.000000</td>
<td>-101.600000</td>
<td>10.0000E00</td>
<td></td>
<td>REFLECT</td>
</tr>
<tr>
<td>6</td>
<td>101.600000</td>
<td>0.000000</td>
<td>110.0000E00</td>
<td></td>
<td>REFLECT</td>
</tr>
<tr>
<td>7</td>
<td>0.000000</td>
<td>101.600000</td>
<td>0.127000</td>
<td>S</td>
<td>AIR</td>
</tr>
<tr>
<td>8</td>
<td>0.000000</td>
<td>101.600000</td>
<td>10.0000E00</td>
<td></td>
<td>AIR</td>
</tr>
<tr>
<td>9</td>
<td>-101.600000</td>
<td>0.000000</td>
<td>110.0000E00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.000000</td>
<td>0.000000</td>
<td>5.0000E00</td>
<td>AIR</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>0.000000</td>
<td>0.000000</td>
<td>5.0000E00</td>
<td>AIR</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>0.000000</td>
<td>0.000000</td>
<td>5.0000E00</td>
<td>AIR</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>0.000000</td>
<td>0.000000</td>
<td>10.0000E00</td>
<td></td>
<td>REFLECT</td>
</tr>
<tr>
<td>14</td>
<td>0.000000</td>
<td>0.000000</td>
<td>5.0000E00</td>
<td>AIR</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>0.000000</td>
<td>15.000000</td>
<td>5.0000E00</td>
<td>AIR</td>
<td></td>
</tr>
<tr>
<td>IMS</td>
<td>0.000000</td>
<td>0.000000</td>
<td>10.0000E00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### CONIC AND POLYNOMIAL ASPHERIC DATA

<table>
<thead>
<tr>
<th>SRF</th>
<th>CC</th>
<th>AD</th>
<th>AE</th>
<th>AF</th>
<th>AG</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>-1.0000E+00</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>9</td>
<td>-1.0000E+00</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

#### TILT/DECEMENT DATA

<table>
<thead>
<tr>
<th>CONFIGURATION 13</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRF</td>
<td>DT</td>
<td>DT</td>
<td>DT</td>
<td>DT</td>
<td>DT</td>
<td>DT</td>
<td>DT</td>
<td>DT</td>
<td>DT</td>
<td>DT</td>
<td>DT</td>
<td>DT</td>
<td>DT</td>
<td>DT</td>
</tr>
<tr>
<td>RADIUS</td>
<td>10</td>
<td>20</td>
<td>25</td>
<td>30</td>
<td>35</td>
<td>40</td>
<td>45</td>
<td>50</td>
<td>55</td>
<td>60</td>
<td>70</td>
<td>80</td>
<td>90</td>
<td>100</td>
</tr>
<tr>
<td>THICKNESS</td>
<td>3.50</td>
<td>5.00</td>
<td>6.50</td>
<td>8.00</td>
<td>9.50</td>
<td>11.00</td>
<td>12.50</td>
<td>14.00</td>
<td>15.50</td>
<td>17.00</td>
<td>18.50</td>
<td>20.00</td>
<td>21.50</td>
<td>23.00</td>
</tr>
<tr>
<td>APERTURE RADIUS</td>
<td>1.50</td>
<td>2.00</td>
<td>2.50</td>
<td>3.00</td>
<td>3.50</td>
<td>4.00</td>
<td>4.50</td>
<td>5.00</td>
<td>5.50</td>
<td>6.00</td>
<td>6.50</td>
<td>7.00</td>
<td>7.50</td>
<td>8.00</td>
</tr>
<tr>
<td>GLASS</td>
<td>AIR</td>
<td>AIR</td>
<td>AIR</td>
<td>AIR</td>
<td>AIR</td>
<td>AIR</td>
<td>AIR</td>
<td>AIR</td>
<td>AIR</td>
<td>AIR</td>
<td>AIR</td>
<td>AIR</td>
<td>AIR</td>
<td>AIR</td>
</tr>
<tr>
<td>SPECIAL</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>
Appendix C. Calculations of the Beam Spot Ratio across the Scan Field Created by an Ideal Single Mirror Scan Engine

We defined the spot size ratio as the spot size relative to the central non-deflected spot size.

The beam size at any position in an ideal single mirror scanning engine (RS) can be calculated based on illustration, Figure C-1. Mirror M, tilted 45º to an incoming beam, reflects it at the angle 90º for the zero-scan position. There are no wavefront distortions. The mirror can be rotated at the point O about two orthogonal axes X and Y in the coordinate system shown. The pivot point is in the point O and the beam waist position is located at the distance \( z_o \) prior the point O. Created scan field pattern will be symmetrical about the X and Y axes.

![Figure C-1. Gaussian beam propagation deflected at point O.](image)

At the non-deflected beam position, point D, the beam radii in both directions, \( w_x \) and \( w_y \), at the plane perpendicular to the beam, are identical and defined by the beam propagation formula:

\[
w(z) = w_0 \left[ 1 + \left( \frac{z}{z_R} \right)^2 \right]^{1/2},
\]  
4-1
where $w_0$ is the beam waist, $w(z)$ is the beam radius at the distance $z$ from the beam waist and $z_R = \pi w_0^2 / \lambda$ is the Rayleigh distance.

Therefore, to define the beam size at any scan field point, we have to define the beam propagation distance to this point and the angle the beam cross-section forms with the plane. Two cases can be considered: the mirror rotated about a single axis and the mirror rotated simultaneously about both $X$ and $Y$ axes.

a). For the mirror rotated about a single axis at the angle $\alpha$, let it be the $X$ axis, the reflected beam propagates at the angle $2\alpha$ to the non-deflected beam and intersects the DD1 plane at the point D1 as it is shown in Figure C-2. At the point D1, beam radii in two directions, $X$ and $Y$, are different.

The beam size along the mirror rotation axis, the axis $X$, can be calculated by equation 4-1 with a corrected value of $z$ for the new beam travel distance to the point D1.

![Figure C-2. Beam propagation in a single mirror scanning engine deflected about one axis.](image)

From Figure C-2, the factor that relates the increase the beam propagation distance between beam propagation from the point O to the point D1 compared to the point D is:

$$k_1 = \frac{OD_1}{OD} = \frac{OD}{\cos 2\alpha} \times \frac{1}{OD} = \frac{1}{\cos 2\alpha},$$

where $k_1$ is the beam propagation path enlargement factor.

Therefore, the $X$ beam radius at the point D1 for any mirror rotation angle $\alpha$ about a single axis is:
Calculations of the Beam Spot Ratio

\[ w_x(D_1) = w_1 = w_0 \left[ 1 + \left( \frac{z_O + OD_1}{z_R} \right)^2 \right]^{1/2} = w_0 \left[ 1 + \left( \frac{z_O + k_1 \cdot OD}{z_R} \right)^2 \right]^{1/2}, \quad 4-2 \]

The Y beam radius at the point D₁ is larger compared to the X beam radius at the same point due to the beam interaction angle with the detection surface DD₁ (see Figure C-2). The beam size at the DD₁ surface for any scan position will be \( BC \) rather than \( AB \). The angle \( \angle ABC \) depends on the mirror surface rotation at the point O and, from geometrical analysis, is equal to \( 2\alpha \). Therefore the beam radius \( w_y(D_1) \) can be calculated by:

\[ w_y(D_1) = w_2 = BC = \frac{AB}{\cos 2\alpha} = \frac{w_x(D_1)}{\cos 2\alpha} \quad 4-3 \]

The beam size in the Y direction for any mirror deflection about a single axis is larger than the beam size in the X direction by \( 1/\cos 2\alpha \) times.

For the mirror rotated only about the Y axis, the beam radii can be calculated in the same way as above.

b). The second case for calculating a beam spot size created by a single mirror scan engine, is when the mirror is simultaneously rotated about the X and Y axes at pivot point O at angles \( \alpha \) and \( \beta \) respectively, as shown in Figure C-3. The reflected beam travels to point D₃ which makes an angle \( \angle ODD_3 = \gamma \) with the non-deflected beam.

Figure C-3. A single mirror rotated simultaneously about two axes.
The factor that relates the increase in the beam optical path from a scan mirror at point O to the point D3 compared to the path to point D can be expressed as:

\[ k_2 = \frac{OD_3}{OD} = \frac{(OD^2 + DD_3^2)^{1/2}}{OD} \]

where:

\[ DD_3^2 = DD_1^2 + DD_2^2 = (OD \times \tan 2\alpha)^2 + (OD \times \tan 2\beta)^2 = OD^2 \times \left(\tan 2\alpha + \tan 2\beta\right)^2 \]

Therefore:

\[ k_2 = \left[ \frac{OD^2 + OD^2 \times \left(\tan 2\alpha + \tan 2\beta\right)^2}{OD} \right]^{1/2} = \left[ 1 + \tan 2\alpha + \tan 2\beta \right]^{1/2} \]

The resultant optical path from the mirror to the surface created by a mirror simultaneously rotated about two axes increases in \( k_2 = \left(1 + \tan 2\alpha + \tan 2\beta\right)^{1/2} \) times compared to the non-deflected beam.

The first beam radius can be calculated using equation 4-2 as:

\[ w_1(D_3) = w_0 \left[ 1 + \left( \frac{z_0 + OD_3}{z_R} \right)^2 \right]^{1/2} \]

Equation 4-3 can be used to calculate the longest beam radius. The beam deflection angle at point D3 from the non-deflected beam OD is \( \gamma \) and can be calculated from its tangent:

\[ \tan \gamma = \frac{DD_3}{OD} = \frac{OD \times \left[ \left(\tan 2\alpha + \tan 2\beta\right)^2 \right]^{1/2}}{OD} = \left(\tan 2\alpha + \tan 2\beta\right)^{1/2} \]

Then the beam radius \( w_2 \) at the point D3 is:

\[ w_2(D_3) = w_1(D_3) / \cos \gamma. \]

In our experiment, the He-Ne laser 0.3 mm beam waist radius, is positioned at a distance of 0.71 m from the first scanning mirror and the distance from the last scanning surface to a detector is 0.11 m in the PSE engine. The beam radii at the non-deflected position and positions created by the mirror rotation either about a single axis or simultaneously about both axes at 5\(^\circ\) and 10\(^\circ\) degrees are shown in Table C-1. The asymmetry of the plotted spot sizes for SSE reflects the scan angle correction to spherical aberration in the engine.
Table C-1. The beam radii ratio in a single point scan system.

<table>
<thead>
<tr>
<th>Scan mirror deflection angles in one and/or two directions</th>
<th>Beam deflected about single axis</th>
<th>Beam deflected about two axes simultaneously</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0°</td>
<td>5°</td>
</tr>
<tr>
<td>Pathlength Factor ( k )^a)</td>
<td>1</td>
<td>1.0154</td>
</tr>
<tr>
<td>Calculated beam radius , ( w_1 ) ( \text{mm} )^b)</td>
<td>0.627</td>
<td>0.628</td>
</tr>
<tr>
<td>Calculated beam radius , ( w_2 ) ( \text{mm} )^b)</td>
<td>0.627</td>
<td>0.638</td>
</tr>
<tr>
<td>Spot ellipcity for a quarter of scan field , ( w_1 / w_2 )</td>
<td>1</td>
<td>1.0154</td>
</tr>
</tbody>
</table>

^a) Pathlength factor (k) was determined from the equation \( k_1 = 1/\cos2\alpha \) and \( k_2 = \left(1 + \tan2\alpha^2 + \tan2\beta^2\right)^{1/2} \) for single and dual axes respectively.

^b) Both radii, \( w_1 \) and \( w_2 \), of the elliptical spot along main axes were calculated from \( w_2(D_1) = w_1(D_1)/\cos2\alpha \) and \( w_2(D_3) = w_1(D_3)/\cos2\gamma \).

Appendix D. CCL Macro

The following CCL routines were developed and used to generate results reported in chapter 5. There include calculations of: PSF maximum, P-V OPD and the relative fluorescence intensity factor.

The main steps in those CCL routines are: open required lens file, define the scan surface number, insert the maximum scan angle, organise two loops to change the scan angles for both scan mirrors with the step of 0.1°, execute required code, retrieve the data, perform calculation, collect the results into the 2D matrix and plot the graph. The PSF max and wavefront error calculations were performed using geometrical ray tracing for on-axis object point. The spot area, time delay and the relative fluorescence intensity factor calculations were computed using an astigmatic Gaussian beam tracing option.
D.1 PSF Maximum Across the Scan Field

cmd collectingpsf(int Scan_System_RS1_PSE2_CCE3_SSE4, real Insert_max_scan_angle)
{
close;
int a, b, a1,b1;
real i,j, g,d,c;

int scansurf;
textwin_reset;

c=Insert_max_scan_angle;
scansurf=Scan_System_RS1_PSE2_CCE3_SSE4;

int nmb;
nmb=10*c;

double value_2D[2*nmb+1][2*nmb+1];// declare matrix

if(scansurf>=4)
{
    open "C:/Program Files/OSLO/Prm64/private/len/Galiya/Scan systems with scan lens/SSE two spher refl and scan lens .len";
    a=2;
b=10;}
else if (scansurf>=3)
{
    open "C:/Program Files/OSLO/Prm64/private/len/Galiya/Scan systems with scan lens/CCE two close-coupled mirrors and scan lens.len";
    a=2;
b=7;}
else if (scansurf>=2)
{
    open "C:/Program Files/OSLO/Prm64/private/len/Galiya/Scan systems with scan lens/PSE two parab refl and scan lens .len";
    a=2;
b=12;}
else if(scansurf>=1)
{
    open "C:/Program Files/OSLO/Prm64/private/len/Galiya/Scan systems with scan lens/RS one scan mir in two dir and scan 1.len";
    a=2;
b=2;}
else ;

a1=tlb[a];
b1=tlb[b];
//scanning and printing PSF max
for (i=-nmb; i<=nmb; i++) //loop for the second scan mirror
{d=i/10;
tla b d;

  for (j=-nmb; j<=nmb; j++) //loop for the first scan mirror, creates scan line
  {g=j/10;
tlb a g;
  psf;
  value_2D[j+nmb][i+nmb]=ssb(1,4);
  textwin_reset;
  }
}

set_preference Graphics_autoclear off;
set_preference Graphics_labels off;

//plot Gplot 2D contour max=1
  graphwin_open 1 400 300 0 100;
gwt "PSF max contour, max=1";
gvp(0.0, 1.0, 0.0, 1.0, 1.0);
gplot2D(2*nmb+1, value_2D,"PSF max", "(first, second)",
  "con");

set_preference Graphics_autoclear on;
set_preference Graphics_labels on;

//plot Gplot 2D map
  graphwin_open 2 400 300 0 400;
gwt "PSF max map";
gvp(0.0, 1.0, 0.0, 1.0, 1.0);
gplot2D(2*nmb+1, value_2D,"PSF max", "(first, second)",
  "map");

//restore initial values
tlb a a1;
tla b b1;
}

D.2 Wavefront Error (P-V OPD) Across the Scan Field
cmd collectingwavefront(int ScanSystem_to_open_PSE1_CCE_2_SSE_3_RS_4, real Insert_max_scan_angle)
{
close;
int a, b, a1, b1;
real i, j, g, d, c, wvf;;
c=Insert_max_scan_angle;
int scansystem;

scansystem=ScanSystem_to_open_PSE1_CCE_2_SSE_3_RS_4;
int nmb;// number of points
nmb=10*c;

double value_2Dln[2*nmb+1][2*nmb+1];// declare matrix
double value_2D[2*nmb+1][2*nmb+1];// declare matrix

if(scansystem<=1)
    {open "C:/Program Files/OSLO/Prm64/private/len/Galiya/Scan systems with scan lens/PSE two parab refl and scan lens .len";
        a=2;
        b=12;}
else if(scansystem<=2)
    {open "C:/Program Files/OSLO/Prm64/private/len/Galiya/Scan systems with scan lens/CCE two close-coupled mirrors and scan lens.len";
        a=2;
        b=7;}
else if(scansystem<=3)
    {open "C:/Program Files/OSLO/Prm64/private/len/Galiya/Scan systems with scan lens/SSE two spher refl and scan lens .len";
        a=2;
        b=10;}
else if(scansystem<=4)
    {open "C:/Program Files/OSLO/Prm64/private/len/Galiya/Scan systems with scan lens/RS one scan mir in two dir and scan 1.len";
        a=2;
        b=2;}
else message "Enter Scan system PSE=1, CCE=2, SSE=3 Rs=4";

a1=tlb[a];
b1=tla[b];

trr usr 0 0;//set object point
textwin_reset;

a1=tlb[a];
b1=tla[b];
//collect wavefront error data across the scan field. Calculate ln and plot the graph

for (i=-nmb; i<=nmb; i++) //loop for the second scan mirror
{d=i/10;
tla b d;

for (j=-nmb; j<=nmb; j++) //loop for the first scan mirror, creates scan line
{g=j/10;
tlb a g;

textwin_reset;
wvf;// print data
value_2D[j+nmb][i+nmb]=ssb(1,1); //P-V OPD in wavelength

wvf=log(ssb(1,1)); //log means natural log
value_2Dln[j+nmb][i+nmb]=wvf;
}

//plot Gplot 2D contour
graphwin_open 1 400 300 0 100;
gwt "ln(P-V OPD) contour";
gvp(0.0, 1.0, 0.0, 1.0, 1.0);
gplot2D(2*nmb+1, value_2Dln,"ln(P-V OPD) ", "(first, second)",
"con", -1.0, 2.0);

//plot Gplot 2D map
graphwin_open 2 400 300 0 400;
gwt "ln(P-V OPD) map";
gvp(0.0, 1.0, 0.0, 1.0, 1.0);
gplot2D(2*nmb+1, value_2Dln,"ln(P-V OPD) ", "(first, second)",
"map", -1.0, 2.0);

set_preference Graphics_autoclear off;
set_preference Graphics_labels off;

//plot Gplot 2D contour maximum value =5
graphwin_open 3 400 300 0 700;
gwt "ln(P-V OPD) contour, max=5";
gvp(0.0, 1.0, 0.0, 1.0, 1.0);
gplot2D(2*nmb+1, value_2Dln,"ln(P-V OPD) ", "(first, second)",
"con", -1.0, 2.0);

set_preference Graphics_autoclear on;
D.3 Relative Fluorescence Intensity Factor

```c
int cmd relativefactorusingIANG1 (int ScanSystem_to_open_PSE_1_CCE_2_SSE_3_RS_4, real Insert_max_scan_angle, real Initial_pulse_duration)
{
    int a, b, a1,b1;
    real i,j, g,d,s, c,s1;
    real xrad, yrad, relfact;
    real majoraxis, SquaredSpotArea, spotarea;
    real angle, PositionAngle, OptPathDif, MaxTimeDelay, sinPositangle;

    int scansystem;
```
scansystem = ScanSystem_to_open_PSE_1_CCE_2_SSE_3_RS_4;

real PulseDuration;
PulseDuration = Initial_pulse_duration;

c = Insert_max_scan_angle;

int nmb; // number of points
nmb = 10 * c;

double value_2DRS[2*nmb+1][2*nmb+1]; // declare matrix
double value_2D[2*nmb+1][2*nmb+1]; // declare matrix
double value_2D2[2*nmb+1][2*nmb+1]; // declare matrix

// Part 1. Calculate factor for RS

open "C:/Program Files/OSLO/Prm64/private/len/Galiya/Scan systems with scan lens/RS one scan mir in two dir and scan.len";
a = 2; // for RS, scan surface in both directions is surface number 2
b = 2;

// save the initial value for the tilt angle to reconstruct it at the end of calculations
a1 = tlb[a];
b1 = tla[b];

trr usr 0 0; // set the object point = on-axis (0,0)
textwin_reset;

// at any scan positions

for (i = -nmb; i <= nmb; i++) // loop for the second scan mirror
{d = i/10; // angle in degrees
tla b d;

for (j = -nmb; j <= nmb; j++) // loop for the first scan mirror (scan lines)
{g = j/10; // angle in degrees
tlb a g;

// for every scan position

textwin_reset;
tgb std srf 0.4 0.4 0 0 0 ims; // trace Gaussian beam at image (ims) surface; the X and Y beam radii = 0.4 mm; the wavefront radii of curvature (Y and X) = 0.

// from data spreadsheet, retrieve the value for Y and X spot size
Xrad = ssb(2, 2);
yrad = ssb(2, 1);
// calculate the spot area
SpotArea=(pi*xrad*yrad);
SquaredSpotArea=(pi*xrad*yrad)**2;

//the time delay
//at the image surface, find the largest spot radius (Y or X)
majoraxis=max(yrad, xrad);
textwin_reset;

tra ful loc srf usr 0 0;//trace a single ray from the current object point

PositionAngle=fabs(ssb(2,4));//retrieve absolute value of the incident angle to surface, in degrees

sinPositangle=sin(PositionAngle*pi/180);

//calculate timedelay
maxtimedelay=majoraxis*sinPositangle/0.0003;

//Create matrix for 1/F(F=fluorescence intensity factor for RS)=(pulse time)*(square of spot area)
value_2DRS[j+nmb][i+nmb]=(maxtimedelay+PulseDuration)*SquaredSpotArea;

} }

} 

tlb a a1;//restore initial values
tlb b b1;


//Part2. Calculate Factor for chosen scan system

//open required lens
if(scansystem<=1)
  {open "C:/Program Files/OSLO/Prm64/private/len/Galiya/Scan systems with scan lens/PSE two parab refl and scan lens .len";
a=2;
b=12;}
else if(scansystem<=2)
  {open "C:/Program Files/OSLO/Prm64/private/len/Galiya/Scan systems with scan lens/CCE two close-coupled mirrors and scan lens.len";
a=2;
b=7;
}

else if(scansystem<=3)
{
open "C:/Program Files/OSLO/Prm64/private/len/Galiya/Scan systems with scan lens/SSE two sphere refl and scan lens .len";
  a=2;
  b=10;
}

else if(scansystem<=4)
{
open "C:/Program Files/OSLO/Prm64/private/len/Galiya/Scan systems with scan lens/RS one scan mir in two dir and scan 1.len";
  a=2;
  b=2;
}

else message "Enter Scan system PSE=1, CCE=2, SSE=3 RS=4";

// save the initial value of the tilt angle to reconstruct it at the end of calculations
a1=tlb[a];
b1=tla[b];

trr usr 0 0; // set object point
textwin_reset;

// for spot at any scan positions
for (i=-nmb; i<=nmb; i++)  // loop for the second scan mirror
  {d=i/10;
   tla b d;
   
   for (j=-nmb; j<=nmb; j++)  // loop for the first scan mirror, creates scan line
     {g=j/10;  // angle in degrees
      tlb a g;
      textwin_reset;
      tgb std srf 0.4 0.4 0 0 0 0 ims;
      
      // find longest major axis
      Xrad=ssb(2,2);
      yrad=ssb(2,1);
      
      majoraxis=max(yrad, xrad);  // establish the major axis of elliptical spot
      SpotArea=(pi*xrad*yrad);  // calculate the Spot Area
      SquaredSpotArea=(pi*xrad*yrad)**2;
      
      // calculate the time delay
      textwin_reset;
tra ful loc srf usr 0 0; //trace a single ray. Output-full data, 0 and the last surface is 0, which means the results will be presented only for the last surface.

PositionAngle=fabs(ssb(2,4)); //retrieve the elliptical spot angle

sinPositangle=sin(PositionAngle*pi/180);
maxtimedelay=majoraxis*sinPositangle/0.0003;

//Create matrix for 1/F (F=fluorescence intensity factor for chosen engine)=(pulse time)*(square of spot area)

value_2D[j+nmb][i+nmb]=(maxtimedelay+PulseDuration)*SquaredSpotArea;

} }

//Part 3. Calculate relative factor=(Factor for chosen engine)/(Factor for RS engine)

for (i=-nmb; i<=nmb; i++)
{ for (j=-nmb; j<=nmb; j++)

    if(value_2D[j+nmb][i+nmb]<=0)
value_2D2[j+nmb][i+nmb]=value_2DRS[j+nmb][i+nmb]/1; //to avoid divide by zero
else
value_2D2[j+nmb][i+nmb]=value_2DRS[j+nmb][i+nmb]/value_2D[j+nmb][i+nmb];

}

//plot Gplot 2D

graphwin_open 5 400 300 0 100;
graphwin_reset;
gwt "Relative Factor";
gvp(0.0, 1.0, 0.0, 1.0, 1.0);
gplot2D(2*nmb+1, value_2D,"Relative Factor", "(first, second)", "con");

//plot Gplot 2D mpa

graphwin_open 6 400 300 0 400;
graphwin_reset;
gwt "Relative Factor";
gvp(0.0, 1.0, 0.0, 1.0, 1.0);
gplot2D(2*nmb+1, value_2D,"Relative Factor", "(first, second)", "map");
}
References


[33] LEICA Microsystems, in Application Letter 12 Heidelberg.
[38] I. Parker, *Build Your Own Video-Rate 2-Photon Microscope*, 2006.


[165] B. Qi et al., Dynamic Focus Control in High-Speed Optical Coherence Tomography Based on a Microelectromechanical Mirror, Optics Communications 232, 123, 2004.


List of Figures

Figure 1-1. Galvo and resonant scanner oscillating waveform detailing rotational angle $\Phi$ versus time. $T$ is the duty cycle. The galvo scanner provides a linear scan over the 70 % duty cycle while the resonant has an approximate linear section of 33 % for scan in one direction. $\tau$ is the retrace time (adapted from [20]). .................................................7

Figure 1-2. A high-performance scanning mirrors: (a) the mirrors for galvo and resonant scanners (adapted from [25]); (b) the optimal mirror design in beryllium with nickel plating support for 2 kHz application [26]). ..............................................................8

Figure 1-3. Simplified diagram of the optical components of a laser scanning microscope. Intermediate image plane is conjugated to the objective focal plane. The centre of the scanner movements (pivot point) must be positioned at the plane conjugated to the objective telecentric plane.................................................................10

Figure 1-4. (a) Single mirror scanning in two directions [3]. (b) Projection of the scan engine pivot point to the objective telecentric plane and forming the scan pattern in the focal plane [33]. 11

Figure 1-5. The close coupled mirror scan engine. (a) The performance and image distortions in the created scan pattern [34], (b) The field curvature in the intermediate plane and two telecentric planes due to separation distance between two scanning mirrors [33]. ........................................12

Figure 1-6. Paddle mirror scan engine: (a) scan engine configuration and (b) scanner motion [3]. 15

Figure 1-7. Golf club scan engine configuration diagram (a) and scanning motion(b) [3]........16

Figure 1-8. Schematic depiction of the (a) mutually independent rotary drive unit and (b) scan engine for variable oscillating frequency (adapted from [39]). ............................................................17

Figure 1-9. Improved scanning mechanism for providing a linear motion by resonant driver. 6 and 7 are galvanometric drivers, and 4 and 5 are resonant drivers. 6 provides the scanning in the Y direction and the assembly of 4, 5 and 7 provides deflection in the X direction (adapted from [40]). ...........................................................................................................18

Figure 1-10. Three moving optical element scan engine setup (adapted from [41]). ................18

Figure 1-11. Rotating polygon mirror..................................................................................19

Figure 1-12. Schematic layout of the scan system for a confocal microscope redrawn from White’s UK patent application field in 1986 (adapted from [43]). ..........................................................20

Figure 1-13. Optical arrangement for deflecting a light beam in two perpendicular directions [44]. ...........................................................................................................................................21

Figure 1-14. Schematic diagram of compact arrangement of optical scan engine [45]. ........22

Figure 1-15. Optical scanning microscope apparatus assigned by Olympus Corporation [46]. .........................................................23

Figure 1-16. Schematic diagram of the single-fibre confocal microscope system [53]: PBS is a polarising beamsplitter, QWP is a quarter-wave plate, and APD is an avalanche photodiode. ..............................................................................................................................24

Figure 1-17. Schematic presentation of the achronatic scanning system [54]. .........................25

Figure 1-18. Imaging system. The angle $\alpha$ of the lens 1 tilting is 45.6° [56]. ........................25

Figure 1-19. System for collimation and imaging [56]...........................................................26

Figure 1-20. (a) Beam deflecting rotational device including two scanning mirrors 2 and 3, and symmetrical mirror arrangement 5. (b) Mirror surface 4 images the rotational point P on surface 2 into point P’ on surface 3 9 (adapted from [57]). .................................................................26

Figure 1-21. (a) Parabolic torus surface. The blue sections show the section used for reflecting the light. (b) and (c) Optical design of scan engine by Seel along x and y axes. For every angle of the first scanner rotation, the beam rays in the YZ plane are reflected from a 90° off-axis parabolic surface and the rays in the XY plane are reflected from a 45° tilted spherical surface.................................................................27

Figure 1-22. Cosine fourth off-axis image distortion [34]. ......................................................28

Figure 1-23. Compensation for the mirror reflective surface being off-axis [34]. .................29

Figure 1-24. Jablonski diagram for (a) one-photon, (b) two-photon and (c) three-photon excitation: $S_0$ is the ground state, $S_1$ is the excited state and $V$ is the virtual state which exists only for a very short time.................................................................32

Figure 1-25 Spatial and temporal compression of photons in (a) continuous and (b) 100 fs pulsed laser beam. If this beam has the same average power (i.e. mean number of photons per second) then the pulsed laser will cause a $10^7$ temporal compression of photons. Hence nonlinear TPE fluorescence is enhanced by $10^{10}$ (adapted from [75]) .........36
Figure 1-26. Timescale for TPE shown relation in the pulse duration, pulse repetition rate and fluorescence lifetime. ................................................................. 37
Figure 1-27. Measured TPE cross-section for common fluorescent molecules measured with commonly utilized tunable laser sources [1]. ................................................. 40
Figure 1-28. One- and two-photon excitation volume [14]. .............................................................. 41
Figure 1-29. The comparison of fluorescence excitation area at the objective focal plane. The blue region together with the red region shows the area by one-photon excitation and the red area only illustrates two-photon excitation area. ................................................................................. 42
Figure 1-30. Illumination and square of illumination PSFs: (a) cross-section in lateral and axial planes, and (b) intensity profiles for OPE and TPE (adapted from [14]). ............................................. 43
Figure 1-31. Two-photon excitation resolution for different excitation wavelengths. The vertical axis is the values for lateral and axial spot sizes ($\omega_x$ and $\omega_z$) in $\mu$m and for the volume in $\mu$m$^3$. .................................................................................................................................................. 44
Figure 1-32. Ti:Sapphire energy level diagram with absorption and emission spectra. Interaction of the titanium electron with the Al$_2$O$_3$ produces a wide spreading of the energy levels and results in broad tuning (adapted from [101]). ................................................................. 45
Figure 1-33. The output power tuning curve for Coherent Chameleon mode-locked Ti:Sapphire laser (adapted from [102]). .............................................................................. 46
Figure 1-34. Pulse broadening due to GVD (after Frosz [105]). .......................................................... 47
Figure 1-35. Broadening of a femtosecond pulse at 800 nm after propagation through 20 mm of BK7 glass [107]. ........................................................................................................... 48
Figure 1-36. Schematic diagram illustrating the two-photon excitation microscope with the resonant mirror scanning. The red coloured path represents the excitation beam path and the yellow is the fluorescence pathway [153]. ........................................................................................................... 52
Figure 1-37. Multi-focal imaging system: three cameras Cam 1, Cam 2 and Cam 3 detect the two-photon fluorescence signal from three focal planes. The insert (a) shows the axial x-y point spread functions for the 3 cameras obtained by focusing through a sub-resolution (100 nm) fluorescent bead [155]. .................................................................................................................. 53
Figure 1-38 Schematic presentation of multi-beam microscope: L1 and L2 form a beam expander, L3-L5 are intermediate optics to image the array of foci at the virtual prefocusing PFP plane into the focal plane of the objective; ST stop the outer part of Gaussian laser beam profile; ML- microlens disk; M- optional mirror; F- short-pass filter; DM-dichroic mirror. Inset shows the rotating microlens array. Each lens is 460 $\mu$m diameter and 6.0 mm focal length and are positioned in the rotating disk to form a 10 row spiral of ~6 $\mu$m beam waist spots in the prefocusing plane [142]. ........................................................................................................... 54
Figure 1-39 The images of fluorescence spots in a uniform sea of Rhodamine 6G dissolved in immersion oil of a (b) resting and (c) rotating microlens disk. A laser wavelength of 765 nm was used with a 100x NA 1.4 oil immersion objective and 1.6x tube lens [115]. ......................... 55
Figure 1-40. Setup of Time Multiplexing Multifocal Multiphoton Microscope [156]. ............................. 56
Figure 1-41. Disk (left) and photograph (right) of a section of the microlens array. A, B and C represent the different classes of temporally delay beamlets. The amplitude transmission is nearly the same for all classes [156]. ........................................................................................................... 57
Figure 1-42. (a) and (b) x-y images of pollen grain acquired at the same axial position by MMM and with TMX respectively. (d) and (e) are 3-D reconstructed images of the same pollen grain with MMM and with TMX respectively [156]. ........................................................................................................... 58
Figure 1-43. Beam generator including a 50 % p-polarisation beam splitter and four high reflective mirrors, to generate 8 beams in the same plane. The distance between beams depends on the distance between mirrors and the beamsplitter, and can be changed from 3 $\mu$m to 6 $\mu$m by the moving mirrors M1-M4 respectively to the beamsplitter [158]. ...................................................... 59
Figure 1-44. Autofluorescence images of an urothelial cell: (a) by one-photon excitation and (b) by multifocal two-photon excitation [158]. ................................. 59
Figure 1-45. Setup of MMM with 64 beamlets generator. In the right top corner – two-photon excited fluorescence of Rhodamine 6G dissolved in water [159]. ................................................. 60
Figure 1-46 Fast scan imaging in the X-Z plane and X-Y plane [159]. .................................................. 60
Figure 1-47. Image of the fluorescence of all beams in a homogeneous fluorescence solution [160]. 61
Figure 1-48. Experimental MMM setup, employed 4 x 4 fan-out diffractive element and two galvo mirrors (VMS00, GSI Lumonics). L1, L1-telescope lens pair with 2x magnification; GX and GY –galvo scanning mirrors; L3 and L4 –telescope lens pair; L5 and tube lens TB-telescope lens pair with 4x magnification; DM-dichroic mirror; BF –IR blocking filter. Mode-locked Ti:Sa laser at 800 nm with 100 fs pulse duration at 80 MHz repetition rate [162]. ................. 62
Figure 1-49. (a) Theoretical diffraction orders from the 4 x 4 fan-out DOE, where + in the centre represents the location of an unwanted zero-order beam. (b) Theoretical spatial spread of a 100-fs pulse diffracted by a DOE (3,3 order). (c) Three-dimensional intensity plot of a fluorescence image obtained with a Rhodamine solution excited by a 4 x 4 DOE multi-beam. The average laser power was 1 mW per spot at excitation wavelength of 800 nm and the distance between spots in the focal plane was 25 μm [162].

Figure 1-50. (a) SS-MMM setup. DOE-binary diffractive optical element; L1(f=20cm) and L2 (f=35cm) are NIR achromate lenses; MB- mechanical block to stop the residual undiffracted beam at the focus of the telescope L1-L2; L3- NIR achromate scan lens(f=15cm); L4-tube lens (f=20 cm). (b) two-photon fluorescence image of the multifocal array in a solution of Rhodamine 6G [163].

Figure 1-51. (a) single frame two-photon fluorescence multifocal raster scan image of a packed monolayer of 500 nm diameter microspheres at 1 frame/s. (b) single frame SS-MMM two-photon fluorescence image over the same area [163].

Figure 1-52. Histograms distribution of simulated fast axis residency times at 1 frames/s for (a) raster and (b) stochastic scanning [163].

Figure 1-53. Three dimensional continuous line scanning microscope: (a) schematic construction of a microscope, and (b) reconstruction of the 3D scan trajectory, and (c) direct visualisation of 3D scan pattern [167].

Figure 1-54(a) Reference image stack of 10 μm fluorescence beads embedded in agar. Single beads are shown in isosurface view, yellow. Three-dimensional scan lines(b) spiral, (c) square-spiral, (e) Lissajous, and (f) user-defined. Scale bars, 50 μm. (d) Close view of a single bead and segments of scan trajectory [167].

Figure 2-1. (a) Paraxial ray tracing. Optical surfaces are replaced by their tangent planes. Notations: y is a ray height, u and u' - angles with the optical axis, and t, thickness, the distance between surfaces. (b) Ray refraction on a spherical surface.

Figure 2-2. Optical system with four surfaces. The local coordinate system of each surface is centred on the optical axis.

Figure 2-3. Tilting and decentring surfaces in OSLO [178].

Figure 2-4. Single ray tracing concept: the ray traced from a specific object point through a specified pupil point. The object and the pupil point are both defined by their fractional coordinates.

Figure 2-5. Schematic diagram of a system and its entrance and exit pupils. Also shown are the axial (marginal) rays from on-axis object point P₀ and the chief ray from the off-axis object point P.

Figure 2-6. Ray tracing concept in OSLO: fans of rays from three object points pass uniformly through an aperture stop, propagate in a lens system and intersect at the image surface.

Figure 2-7. (a) Grid pattern in the system aperture to provide equal ray distribution through the aperture. The grid illustrated has 34 cells across the aperture stop in each axis. Increasing the number of cells will increase the accuracy of calculation. (b) is an example of a spot diagram of the focal spherical scan engine evaluated in this thesis showing the Airy disk, and the ray spread across a 400 square micron panel. The grid size is in mm, the Airy disk radius is equal to 0.006 mm.

Figure 2-8. (a) Uniformly illuminated entrance pupil and (b) Gaussian apodized pupil. OSLO computes the spot diagram with a Gaussian apodized pupil. The aperture stop diameter is d2 and d1 is the diameter at which irradiance drops to 1/e² of its axial value at surface 1.

Figure 2-9. Calculating optical path difference between peripheral rays for any scan point.

Figure 2-10. Scanning mirror (a) 2D and (b) 3D drawing, and (c) the footprint on the image surface.

Figure 2-11. The X scan system performance: (a) 3D lens drawing, (b) 2D lens drawing, and (c) scan beam footprint on the image surface.

Figure 2-12. Two close coupled scan mirrors.

Figure 2-13. One mirror scanning in both directions: (a) design, (b) scanning rays and (b) scan pattern for 25 beam positions.

Figure 2-14. Two spherical mirrors design: (a) schematic, and (b) 3D performance.

Figure 2-15. Scan system with two flat scan mirrors and two parabolic reflectors as a relay optics: (a) schematic diagram and (b) scan rays.

Figure 2-16. Scan lens design: (a) 2D lens drawing and (b) 3D image.
Figure 3-1. Spherical aberration and coma induced by a reflective optical element. (a) The ray tracing through the spherical mirror aperture. The inset shows that the rays from the mirror edges intersect the optical axis closer to the mirror than peripheral rays. (b) The spot diagram at the focal point. (c) The ray tracing through the tilted spherical mirror. The inset shows the asymmetric rays propagating through the focal point. (d) The effect of coma at the focal point. ................................................................. 96
Figure 3-2. Astigmatism in an optical element caused by the rays from an off-axis object point. (a) The ray tracing from an off-axis object point. (b) The magnified image of the focal volume. The rays form the sagittal and tangential focal planes with the circle of least confusion in between. ................................................................................................................... 97
Figure 3-3. (a) Field curvature in an optical element. The rays from different object points form a curved focal image plane. (b) Performance of the scan lens used in this thesis for simulations. Its performance is corrected for field curvature. ........................................................................ 98
Figure 3-4. Image distortion effects; (a) barrel distortion, and (b) pincushion distortion. ........ 99
Figure 3-5. Spherical and parabolic reflectors. (a) The spherical reflector focuses a beam from infinity to a caustic surface. (b) The parabolic reflector focuses to one focal point. (c) Beam reflection from a tilted spherical surface introduces astigmatism and coma at the focal point highlighted in the inset. (d) An off-axis parabolic surface focuses the beam to one unobstructed focal point which lies on its optical axis. A magnified image is shown in inset. 100
Figure 3-6. Aberrations introduced to a narrow and wide parallel incident beam reflecting from a spherical reflector. (a) Spot diagram and MTF of a normal incidence axis showing focus within the Airy disk (black circle). (b) Spherical surface tilted 10°. The incident beam induces astigmatic focus and loss of diffraction limited performance in the spot diagram and MTF. (c) The caustic surface from a wider incident beam degrades the spot diagram and MTF further. .......................................................................................................................... 101
Figure 3-7. A parabolic reflector focuses a beam of any size, incoming parallel to its optical axis, from any off-set part to a geometrical point within the diffraction limit. The MTF function is coincident with the ideal. (a) Incident beam is on-axis. (b) Beam is incident parallel to the optical axis and reflected from 90° off-axis segment. (c) Beam is as (b) but with increased incident beam radius. ........................................................................................................ 102
Figure 3-8. An off-axis parabola focuses light emerging from the focal point to a parallel beam. When a low-divergence beam [(a) 1 mm beam radius and (b) 2 mm beam radius] is incident as shown, the aberrations at the focal point increase with the beam size. Intensity PSFs show the distorted energy distribution at the focus. ........................................................................ 103
Figure 3-9. A spherical mirror based achromatic scanning engine after Amos [54]................. 104
Figure 3-10. Optical layout of the improved PSE scan engine design. Two dashed arcs 3 and 5 illustrate two separate parabolic reflective surfaces from which sections 4 and 6, representing the 90° reflective off-axis sections, are employed. Surfaces 3 and 5 have a common optical axis. The scan mirrors 1 and 7 are positioned at the focal points of parabolic reflectors 4 and 6 respectively.......................................................... 105
Figure 3-11 Symmetrical raster scanning pattern illustrated by the black lines with 25 defined analysis points. .................................................................................................................. 107
Figure 3-12 The image surface scanned beam footprint and the scan field distortion for (a) the single mirror reference engine, (b) the spherical mirror SSE engine and (c) the parabolic mirror PSE engine. ............................................................................................................... 108
Figure 3-13. Comparison of the created scan pattern for the same 25 scan field configurations... 109
Figure 3-14. Beam spot dimensions R1 and R2 in two orthogonal axes for three scan engines: (a) RS, (b) PSE and (c) SSE. There is almost complete overlap of the PSE and RS results. .... 110
Figure 3-15. Aberration in the spherical SSE scan engine caused by the tilt angle of the spherical surfaces: (a) 0°, (b) 2.5°, (c) 5°, (d) 7.5° and (e) 10°. The size of individual squares is 0.1 mm. The white circle within all individual squares indicates the Airy disk. In figure (f), the spot diagrams at position 13 from (a) to (e) are collected and show the rapid increase of the aberrated geometrical RMS radius spot size........................................................................ 111
Figure 3-16. Spot diagram array for the twenty-five scan engines configurations examined: (a) the RS engine; (b) is for the PSE engine and (c) is for the SSE engine. The black circle represents the Airy disk. ................................................................................................. 113
Figure 3-17 Point spread function data for the twenty-five system configurations examined: (a) RS engine; (b) is for PSE engine and (c) is for SSE engine .................................................................114
Figure 3-18 The variation in point spread function maxima for the twenty-five system configurations examined in each of the three scan engines: (a) the RS engine, (b) the PSE engine and (c) the SSE engine. Plates (a) and (b) vary by 0.1% across the field while (c) has a wide range of values ...........................................................................................................115
Figure 3-19. Beam outlines on the second scan mirror surface for 5 mm aperture radius: (a) in the SSE engine and (b) in the PSE engine. .................................................................115
Figure 3-20. Examples of the arrangement for the optical system with two spherical reflectors to get the image of the point object without blur [186] .............................................................................116
Figure 3-21. Simulated scan engines: (a) as in Seel patent and (b) the PSE design .......................116
Figure 3-22. Comparative performance of off-axis parabolic toroid (as in Seel’s design) and 90° off-axis parabolic reflector (as in the PSE) at the focal point. Spot diagrams match the scan angle of the first scan mirror in the range +10°, +5°, 0°, -5°, and -10°. The optical performance for both systems are aberrated at the focal plane and the second curved surface reflections .................................................................117
Figure 3-23. Comparative performance for 25 configurations across the two dimensional ±10° scan field for the Seel design and the PSE. Row (a) illustrates the simulated lens drawing and the image of 25 spots at the image surface. Rows (b) and (c) show the spot diagrams and the PSFs for the 25 scan points .................................................................118
Figure 4-1. The experimental setup of the parabolic scanning engine (PSE) which includes four reflective surfaces: two off-axis parabolic reflectors and two flat mirrors ........................................122
Figure 4-2. A spherical mirror based four mirror afocal scan engine SSE .................................122
Figure 4-3. The focal parabolic scanning engine (PSE) ...............................................................123
Figure 4-4. The focal spherical scanning engine (SSE) .............................................................124
Figure 4-5. A single mirror reference scan engine RS. The two dotted lines mark the two orthogonal rotational axes of this mirror with the intersection of these in the mirror plane of reflection forming the pivot point in the RS engine .............................................................................124
Figure 4-6. The twenty five points of the scanning field to measure the beam size in an afocal scanning engine. The investigation points for PSE and SSE engines are surrounded by the red and blue rectangle respectively .........................................................................................125
Figure 4-7. Experimental cross-sectional images of the laser beam propagated through PSE engine at fifteen scan field points. The spots correspond to the red labelled region of Figure 4-6. The images in rows represent beam spots deflected by the first scan mirror and the images in columns are for five tilted angles of the second scan mirror .........................................................128
Figure 4-8. Experimental cross-sectional images of the laser beam at twenty scan field points after the SSE engine. The spots correspond to the blue labelled region of Figure 4-6. The images in rows represent beam spots deflected by the first scan mirror and the images in columns are for five tilt angles of the second scan mirror .........................................................................................130
Figure 4-9. (a) Experimental and (b) OSLO calculated beam spot radii R1 and R2 across the scan field in the PSE afocal engine .........................................................................................133
Figure 4-10. (a) Experimental and (b) OSLO calculated spot radii R1 and R2 across the scan field in the SSE afocal engine .........................................................................................133
Figure 4-11 The experimental and calculated beam size along the propagation axis ........................136
Figure 4-12. (a) Beam propagation after the PSE and SSE relays compared to theoretical beam propagation size. Line 1 is the theoretical beam radius, lines 2 and 3 are the two beam radii after the PSE engine, lines 4 and 5 are the two beam radii for the elliptical spot after the SSE engine. (b) Line 6 is the experimental results for the PSE engine (line 2) shifted left by the optical path length in the PSE engine .........................................................................................137
Figure 4-13. The beam radius propagated through the PSE relay for different positions of the input beam waist relative to the first scan mirror. Position of the first and second scan mirrors, and the first and second parabola, are indicated by vertical lines with the distance from the first scan mirror indicated in mm above .........................................................................................138
Figure 4-14. The output beam divergence after the PSE engine for the beam waist positioned 2 m away from the first scan mirror surface. The radius of curvature of two parabolic reflectors is 101.8 mm. Zero at the X axis represents position of the first scan mirror. (a) Variation of the propagated laser beam radius depending on the distance between two parabolic reflectors. The vertical line shows the position of the first parabola and the number above it shows its distance from the first scan mirror. Line labels represent the distance between parabolic
Figure 4-15. Measured axial focal shift dependence on parabola separation in PSE engine........141
Figure 4-16. The RS engine: elliptical spot radii R1 and R2 along two main axes at the intermediate
image plane. The radii increase evenly in both directions from the central spot.................141
Figure 4-17. RS engine: spots profiles along created scan field at the intermediate image plane. The
images in rows represent beam spots deflected by the first scan mirror and the images in
columns are for five tilt angles of the second scan mirror. The spots correspond to the blue
labelled region of Figure 4-6. ..................................................................................................142
Figure 4-18. PSE engine: spot radii for 15 positions (correspond to the red labelled region of Figure
4-6) at the intermediate image plane. Horizontal scan angle represents the tilting angle of the
first scan mirror and vertical scan angle represents the tilting angle of the second scan mirror.
The spot size increases from the central spot in both directions. The greater increase is for the
deflection by the second mirror.................................................................144
Figure 4-19. PSE engine: spots profiles at chosen positions (the red labelled region of Figure 4-6) at
the intermediate image plane. The images in rows represent beam spots deflected by the first
scan mirror and the images in columns are for five tilt angles of the second scan mirror. 145
Figure 4-20. Beam profile across the chosen scan field at 25 positions. The images in rows represent
beam spots deflected by the first scan mirror and the images in columns are for five tilt angles
of the second scan mirror. Spots are more elliptical in the central column which represents
zero deflection of the first scan mirror. Beam circularity for other positions is due to
astigmatism created by the two tilted spherical reflector surfaces.................................148
Figure 4-21. The beam spot radii in two directions in the SSE engine for 25 positions corresponding
to the blue labelled region of Figure 4-6. ................................................................................150
Figure 4-22. Astigmatism in the SSE engine at the intermediate image plane. The largest
astigmatism is for the zero deflection by the first scan mirror.................................150
Figure 4-23. Comparison of the field curvature created by the RS and PSE engines. (a) and (b) are
the field curvatures in RS and PSE engines respectively, and (c) presents the difference in
field curvature between RS and PSE engines................................................................151
Figure 5-1. (a) Performance of a simple lens for tilted and on-axis incident beam. (b) Four spot
diagrams and (c) four intensity point spread functions (PSF) represent incident beam angles
at the lens of (1) 0°, (2) 10° in the horizontal direction , (3) 10° in the vertical direction and (4)
10° simultaneously in both directions relative to the optical axis respectively. The scale on the
spot diagrams is different and relative size may be gauged by the black Airy disk circle on the
spot diagrams.................................................................156
Figure 5-2. Four simulated scanning designs: (a) single mirror engine used as the reference design
RS, (b) close coupled engine CCE, (c) spherical reflector engine SSE, and (d) parabolic
reflector engine PSE. Panel (e) describes the scan pattern points analysed. ......................158
Figure 5-3. Scan engine position in a typical two-photon scanning microscope arrangement. The
scan lens used in this work was an optimized scan lens design from the OSLO database as
shown in the expanded detail. .................................................................159
Figure 5-4. Centre of mass points for 25 configurations of the four scan engines...............160
Figure 5-5. (a) Spot diagrams and b) intensity PSFs for four scan engines in 25 scan configurations.
Scan angle range in both directions is from -10 to +10 degrees. (c) and (d) show PSF
maximum values as contour and 2D maps across whole scanning field at a resolution of 0.1
degrees. The color scale bar is common for (d) and (c) of each column..........................161
Figure 5-6. Wavefront aberrations for four scan engines. (a) wavefront contours, (b) ln(peak-to-valley optical path difference), (c) 2D maps of central (± 5º)scan field..........................162
Figure 5-7. Spot areas at the intermediate image plane for (a) RS, (b) PSE, (c) CCE and (d) SSE
systems.................................................................................................................163
Figure 5-8. Time delay introduced by scan engines for (a) RS, (b) PSE, (c) CCE and (d) SSE.....164
Figure 5-9. The relative fluorescence intensity factor for (a) PSE, (b) CCE and (c) SSE across the
investigated area..............................................................................165
Figure 6-1. Flowchart for future research. .............................................................................169
Figure 6-2. PSE design proposal for a wide scan: (a) the PSE design; (b) strip of parabolic surface
needed for the wide angle scan; and (c) the side view of the strip from parabolic surface. Its
width could be only 5 mm.................................................................170
Figure 6-3. The size of the PSE scan engine is a function of the radius of curvature R of parabolic
reflectors...............................................................................................171
Figure 6-4. Three beam generator. M1 is a small 100% reflectance mirror. M2 and M3 are 100% D-shaped mirrors. B1 and B2 are beam-splitters. B1 is 30/70 T/R and B2 is 50/50 T/R. Resultant three beams have a power of 30%, 35% and 35% of incident beam. .......................... 173

Figure 6-5. Spatio-temporal decoupling for the 3 beam multi-photon multi-beam multiplexing (see chapter 1.3). (a) Three beams (1, 2 and 3) separated in time during one pulse. (b) Three fluorescence excitations during one pulse. .................................................................................. 173

Figure A-1. Relation between pulsed beam characteristics for scanning with resonant scanner...175

Figure B-1. (a) Scan mirror in the Y direction. (b) The changes in local coordinate systems in OSLO. The X axis is perpendicular to the page surface...............................................................176

Figure B-2. (a) Scan mirror in the Y direction. The X axis is perpendicular to the page surface. (b) The incident beam is perpendicular to the page.................................................................177

Figure B-3. Ray diagram and curved scan line created on the image surface by the first version of a X scan system.................................................................................................................178

Figure B-4. Two closed coupled scan engine. ..................................................................................179

Figure B-5. One mirror scanning in both directions: (a) design and (b) scanning pattern for 25 configurations.................................................................180

Figure C-1. Gaussian beam propagation deflected at point O..............................................................185

Figure C-2. Beam propagation in a single mirror scanning engine deflected about one axis ........186

Figure C-3. A single mirror rotated simultaneously about two axes..............................................187

