Endoscopic Full-Field Swept-Source Optical Coherence Tomography Neuroimaging System

by

Ilan Felts Almog

A thesis submitted in conformity with the requirements
for the degree of Master of Applied Science
The Edward S. Rogers Sr. Department of Electrical & Computer Engineering
University of Toronto

© Copyright 2018 by Ilan Felts Almog
Abstract

Endoscopic Full-Field Swept-Source Optical Coherence Tomography Neuroimaging System

Ilan Felts Almog
Master of Applied Science
The Edward S. Rogers Sr. Department of Electrical & Computer Engineering
University of Toronto
2018

Optical Coherence Tomography (OCT) has the capability to differentiate brain elements with intrinsic contrast and at a resolution an order-of-magnitude higher than other imaging modalities. This thesis investigates the feasibility of OCT for neuroimaging applied to neurosurgical guidance. We present, to our knowledge, the first Full-Field Swept-Source OCT system operating near a wavelength of 1310 nm, achieving a transverse imaging resolution of 6.5 µm, an axial resolution of 14 µm in tissue and a field of view of 270 µm × 180 µm × 400 µm. Imaging experiments were performed on rat brain tissues ex vivo, human cortical tissue ex vivo, and rats in vivo. A multi-level threshold metric applied on the intensity of the images led to a plausible correlation between the observed density and location in the brain. The proof-of-concept OCT system can be improved and miniaturized for clinical use.
To my beautiful family and to my spiritual teacher, with all my love and gratitude.

You continuously bring light and joy into my life.
Acknowledgements

I would like to extend my sincere gratitude and appreciation to my academic supervisor, Professor Joyce Poon, first for granting me the opportunity of conducting this very interesting piece of research, and then for the immense support and guidance throughout the project and this thesis.

We had the honour of collaborating with Dr. Andres Lozano, who provided a direction and resources for this project to be viable. I thank Dr. Lozano for being part of my thesis committee as well.

I also thank Professor Peter Herman and Professor George Eleftheriades for serving in my thesis committee and providing valuable feedback.

Thank you to my colleagues in the Micro/Nano Photonics Group for keeping things interesting when the work was tough. In particular, I would like to thank Fu Der Chen (Fred) for the countless hours he invested in collaborating with me on this project, even when he was only volunteering.

Thanks to all of Dr. Lozano’s group members at the Krembil Research Institute who also took part in this project by providing guidance on surgical procedures and needs, carrying out experiments involving animals and evaluating our results according to the required specifications for usefulness in a clinical setting. From Dr. Lozano’s group, Dr. Yann-Suhan Senova was the biggest contributor to this project, regularly evaluating our developments and carrying out the first in vivo experiment. Dr. Anton Fomenko executed the second in vivo procedure, helped us understand the structural elements in the imaged regions, and participated in the analysis of histology images for comparison with our OCT-derived results. Dr. Elise Gondard was instrumental in preparing the histology sections for comparison with OCT images. Dr. Francesco Sammartino participated at the beginning of the project and provided the starting specifications for the system.

Lastly, I thank my wife Juliana for her patience, support and care, and my son Theo for keeping me grounded and brightening my days.
# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acknowledgements</td>
<td>iv</td>
</tr>
<tr>
<td>Table of Contents</td>
<td>v</td>
</tr>
<tr>
<td>List of Tables</td>
<td>viii</td>
</tr>
<tr>
<td>List of Figures</td>
<td>ix</td>
</tr>
<tr>
<td>List of Abbreviations</td>
<td>xv</td>
</tr>
<tr>
<td><strong>1 Introduction</strong></td>
<td>1</td>
</tr>
<tr>
<td>1.1 Motivation: Neurosurgical Intraoperative Guidance</td>
<td>1</td>
</tr>
<tr>
<td>1.1.1 Deep Brain Stimulation</td>
<td>2</td>
</tr>
<tr>
<td>1.1.2 DBS Electrode Insertion Procedure</td>
<td>3</td>
</tr>
<tr>
<td>1.2 Background</td>
<td>4</td>
</tr>
<tr>
<td>1.2.1 Optical Coherence Tomography</td>
<td>4</td>
</tr>
<tr>
<td>1.2.2 Types of OCT</td>
<td>6</td>
</tr>
<tr>
<td>1.3 Thesis Objectives and Organization</td>
<td>11</td>
</tr>
<tr>
<td>1.3.1 Objectives</td>
<td>11</td>
</tr>
<tr>
<td>1.3.2 Thesis Organization</td>
<td>11</td>
</tr>
<tr>
<td><strong>2 Imaging Metrics and Optical Properties of Tissue</strong></td>
<td>13</td>
</tr>
<tr>
<td>2.1 Imaging Metrics</td>
<td>13</td>
</tr>
<tr>
<td>2.1.1 Resolution and DOF</td>
<td>13</td>
</tr>
<tr>
<td>2.1.2 Gaussian Beams &amp; Depth-of-Field</td>
<td>16</td>
</tr>
<tr>
<td>2.1.3 Field-of-View</td>
<td>18</td>
</tr>
<tr>
<td>2.2 GRIN Lenses</td>
<td>19</td>
</tr>
</tbody>
</table>
2.3 Optical Properties of Tissue ........................................... 21
2.4 Summary ................................................................. 24

3 Specifications and Design .................................................. 25
3.1 Required Specifications .................................................. 25
  3.1.1 Physical Requirements ............................................. 25
  3.1.2 Imaging Requirements ............................................. 26
  3.1.3 Acquisition & Processing Requirements ......................... 26
3.2 Optical System Design ................................................... 27
  3.2.1 Endoscope Design .................................................. 28
  3.2.2 Bulk Optical Components ........................................ 30
3.3 Summary ................................................................. 36

4 Implementation ............................................................. 37
4.1 System Integration ....................................................... 37
  4.1.1 Optical System ..................................................... 37
  4.1.2 Light Sources ...................................................... 41
  4.1.3 Camera ............................................................. 42
4.2 Acquisition Control ...................................................... 42
4.3 OCT-Resolving Algorithm .............................................. 43
4.4 Displaying the Tomogram ............................................... 46
4.5 Summary ................................................................. 47

5 System Characterization .................................................... 48
5.1 Optical Power and Sensitivity .......................................... 48
  5.1.1 Power ............................................................... 48
  5.1.2 Sensitivity ........................................................ 48
5.2 Transverse Imaging Properties: Resolution and FOV .............. 49
  5.2.1 Transverse Resolution ............................................. 49
  5.2.2 FOV ............................................................... 49
5.3 Axial Imaging Properties: Resolution and DOF ..................... 50
  5.3.1 Axial Resolution .................................................. 50
  5.3.2 DOF ............................................................. 51
5.4 Summary ................................................................. 53
6 Ex Vivo and In Vivo Experiments

6.1 General Experimental Procedure ........................................... 54
6.2 Rat Brain Tissue ................................................................. 55
  6.2.1 Tissue Sections ............................................................. 55
  6.2.2 Ex Vivo Pieces ............................................................... 59
6.3 Human Cortical Tissue .......................................................... 61
6.4 In Vivo Experiment ............................................................... 63
  6.4.1 Experimental Procedure .................................................... 64
  6.4.2 First Tract .................................................................. 65
  6.4.3 Second Tract ................................................................ 69
  6.4.4 Threshold Metric ............................................................. 73
6.5 Summary ........................................................................... 74

7 Discussion, Conclusion and Outlook ......................................... 77

7.1 Discussion ........................................................................ 77
7.2 Conclusion ......................................................................... 79
7.3 Outlook, Extensions and Future Work ..................................... 80

Appendix A OCT Algorithm ......................................................... 82
Bibliography ........................................................................... 84
List of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Parameter comparison for different types of OCT systems</td>
<td>6</td>
</tr>
<tr>
<td>1.2</td>
<td>Comparison between fiber-based and FF-OCT systems</td>
<td>10</td>
</tr>
<tr>
<td>3.1</td>
<td>Summary of refractive index values in the GRIN lens</td>
<td>29</td>
</tr>
<tr>
<td>3.2</td>
<td>Simulated results for the placement of optics</td>
<td>33</td>
</tr>
<tr>
<td>3.3</td>
<td>Beam diameter simulated with Gaussian beam propagation at different positions of the illumination path</td>
<td>33</td>
</tr>
<tr>
<td>3.4</td>
<td>Summary of simulated results for the complete optical system</td>
<td>34</td>
</tr>
</tbody>
</table>
# List of Figures


1.2 Illustration of a Michelson interferometer for OCT. Image by the author.

1.3 Overview of the different types of OCT systems. Extracted from Ref. [38].

2.1 A simulated and a real Airy disk. Images in the public domain.

2.2 Numerical aperture of a thin lens. Image in the public domain.

2.3 Illustration of the Rayleigh criterion for resolution. Image from Leica, Ref. [45].

2.4 (a) A Modulation Transfer Function generated for a modeled optical system and (b) an illustration of contrast versus spatial frequency. Images from Ref. [46].

2.5 Cross-section of a focused z-propagating Gaussian beam showing its waist $w_0$, its Rayleigh range $z_R$ and the confocal parameter $b$. Image by Rodolfo Hermans [47].

2.6 Transverse cross-section of a Gaussian beam. Image in the public domain.

2.7 Effect of the DOF in an image. Image extracted from Ref. [49].

2.8 Illustration of the trade-off between Numerical Aperture and Depth-of-Field. Image extracted from Ref. [50].

2.9 Rays from an object at the input surface of a relay GRIN lens showing their periodic trajectories and an intermediate image. The object has its image at the output surface of the GRIN lens. Image extracted from Ref. [51].

2.10 Focal distance $f$ and working distance $s$ as defined for a GRIN lens of length $z_l$ and diameter $d$. Image extracted from Ref. [51].
2.11 Wavelength-dependent attenuation in biological tissue (log scale). Adapted from an image in Ref. [55].

2.12 Water absorption in the visible and near-infrared ranges. Image by the author based on data from Ref. [58].

3.1 A diagram of the entire FF-SS-OCT system. $L_{\text{coll}}$: collimation lens with focal length $f_{\text{coll}}$. $L_{\text{inj}}$: injection lens with focal length $f_{\text{inj}}$. BS: beamsplitter. $L_{\text{obj}}$: objective lens, which can be translated for dynamic focusing. $L_{\text{cam}}$: camera lens with focal length $f_{\text{cam}}$.

3.2 Radial refractive index profile of the GRIN lens for 1310 nm light.

3.3 Ray-tracing simulation of the endoscope showing image formation of two points from the sample. Rod diameter: 1 mm. The glass spacer at the distal end is 7.1 mm long and the imaging GRIN is 3.18 mm long. The relay GRIN lens shown here has 0.5 pitch and is approximately 11 mm long; our endoscope uses a relay which is 5 times longer.

3.4 (a) Cross-section of the simulated PSF from the modeled endoscope. The first zero from the center is the Airy radius, with approximately 13.45 µm after a magnification of 1.5, equivalent to an Airy radius of 8.97 µm in object space. (b) Simulated MTF for the same components. The MTF has 20% contrast at 61 cycles/mm (equivalent to 8.2 µm). (c) Chromatic focal shift in the endoscope over our wavelength range.

3.5 (a) Simulation of the illumination path from fiber to sample with 75 mm lenses and the designed endoscope including the relay GRIN, delivering collimated illumination to the sample. (b) Simulation of the imaging path from the sample to the camera, with 75 mm and 1000 mm focal length lenses. Simulations obtained by sequential paraxial ray-tracing in Zemax OpticStudio. Calculated total magnification: 19.84x. Separation distances are listed in Table 3.2.

3.6 Simulated illumination spot size at the sample for the different wavelengths in the range. The diameter is approximately 560 µm, close to the value in Table 3.3.

3.7 (a) Simulated FOV of the complete system, showing a cutoff at approximately 250 µm, not accounting for the dimensions of the CCD sensor. (b) Resulting PSF from the entire imaging path on the CCD, with an Airy radius of 185 µm covering approximately 6 camera pixels.

3.8 Simulated image of a 500 µm × 500 µm square grid. The different wavelengths, size of the detector and pixel pitch were accounted for in the simulation.
4.1 A picture of the system set up in the animal experiment room at the Krembil Research Institute. Circles label the different components. The schematic is presented in Fig. 3.1.

- Red: \(L_{\text{coll}}\)
- Green: \(L_{\text{inj}}\)
- Blue: beamsplitter
- Purple: endoscopic probe
- Yellow: \(L_{\text{obj}}\)
- Grey: \(L_{\text{cam}}\)
- Orange: InGaAs CCD
- Pink: fiber collimator for trans-illumination

The fiber collimator and the two mirrors in the back are not depicted in the diagram of Fig. 3.1.

4.2 Detailed image of the assembled endoscope attached to the cage system.

4.3 Side view of the imaging path showing the connection and alignment of the endoscope to the rest of the optical system.

4.4 A top view of the complete system. The spatial constraint imposed by the beamsplitter cube and the long imaging path required to compensate for it can be clearly seen in this image.

4.5 Flowchart for the OCT-resolving algorithm.

4.6 A screenshot of the Matlab plugin IMAGINE 2.0 with four sweeps of an OCT C-scan loaded. The software features interactive contrast adjustment for several colormaps, the option to work simultaneously with multiple volumes, parallel line profiling and easy manipulation of 3D data.

5.1 Resolved amplitude in dB for a single reflector positioned 295 \(\mu\)m away from the reference. This was obtained from the pixel exhibiting the maximum sensitivity.

5.2 Smallest resolvable features from a USAF-1951 resolution target, illuminated with a broadband NIR source. Each line in Element 5-5 is 49 \(\mu\)m long and 9.84 \(\mu\)m wide.

5.3 (a) Resolved depth profile along a cross-section through the center of the phantom. Scale bar (red): 25 \(\mu\)m. (b) Trans-illuminated image of the phantom in microscope mode. Scale bar (green): 85 \(\mu\)m. Axes are shown in red for the depth map and in green for the BOA image.

5.4 Resolved depth slices from a phantom made of glass beads in agarose gel at depths (a) 40 \(\mu\)m, (b) 50 \(\mu\)m, (c) 90 \(\mu\)m and (d) 177 \(\mu\)m. Scale bar (red): 40 \(\mu\)m. The bottom image shows a maximum intensity projection along the \(x\) dimension and the positions from where the slices were taken. Axes are shown in black for the slices and in red for the projection depth map.
5.5 (a) Depth profile of an OCT-resolved bead showing a FWHM of 19 µm, with the refractive index $n$ of the medium equal to approximately 1.34. (b) Transverse profile of the bead with a measured diameter of 50 µm. (c) The individual bead in the OCT-resolved volume from which the profiles in (a) and (b) were taken.

5.6 Resolution target showing Element 5-5, positioned at: (a) best focus, (b) 50 µm away, (c) 150 µm away, (d) 250 µm away, (e) 300 µm away and (f) 400 µm away. Line separation: 9.84 µm; line length: 49 µm. Cutoff for the half-DOF set at 200 µm.

6.1 A region of a Nissl-stained rat brain cortex section imaged using (a) our system, illuminated from behind the sample with a broadband source and (b) a bench-top optical microscope with a 50x-magnification objective. Scale bars: 50 µm.

6.2 Image of a CA1 neuronal band in the hippocampus of a rat brain section (a) via OCT and (b) in microscope mode, epi-illuminated with the BOA. General features are strongly correlated. Scale bar (red): 50 µm. (c) Pixel depth profile showing the resolved thickness of 70 µm for the section (approximately 50 µm when correcting for refractive index).

6.3 Comparison of a neuronal band of the hippocampus, near the dentate gyrus, of a rat brain section, imaged by OCT and with the BOA. The OCT-resolved frames in (a), (b) and (c) are taken 15 µm apart. The same region was imaged with the BOA with (d) trans-illumination and (e) epi-illumination. The OCT frames appear to have features which appear exclusively in the epi-illuminated image (yellow arrow) and in the trans-illuminated image (green arrows). Scale bars: 50 µm.

6.4 Depth profile for an arbitrary spot in the thalamus of a thawed rat brain (maximum intensity projection along the $y$ dimension). Bottom image obtained from a sweep two minutes after the top image. Auto-correlation and endoscope artifacts appear near zero. The scale is the same in all dimensions.

6.5 Experimental setup for imaging frozen ex vivo pieces of tissue to verify the repeatability of sweeps over long depths.

6.6 OCT-resolved frames from sequential sweeps 20 seconds apart showing a region of the thalamus in a frozen rat brain at depths (a) 280 µm, (b) 485 µm and (c) 670 µm. Scale bar: 50 µm.

6.7 The setup for imaging human cortical tissue.

6.8 Images of a preserved human brain section in microscope mode, with (a) epi-illumination and (b) trans-illumination. Scale bar: 50 µm.
6.9 The same region of a preserved human brain section imaged (a) by OCT and (b) with the BOA, trans-illuminated. Scale bar: 50 µm.

6.10 Cut brain from the in vivo experiment showing the penetration location of a failed run. A lacerated blood vessel distorted the data, and the tract cannot be properly identified.

Ruler markings: 500 µm.

6.11 The setup for the in vivo experiment, with the vertical positioning of the stereotactic frame in front of the endoscope.

6.12 Page from the Rat Brain Atlas (ML+5 mm, AP+0.5 mm, DV+7.8 mm) showing the likely final position of the endoscope in the first penetration. The region is the piriform nucleus.

6.13 Cut brain from the in vivo experiment showing the first tract, mostly through cortical regions. There appears to be a kink in the tract. Ruler markings: 0.5 mm.

6.14 Histology comparison with OCT from the first penetration: (a) Nissl-stained section from the piriform cortex 150 µm after the deepest position of the endoscope and (b) an OCT-resolved frame of the same approximate location and depth. Scale bars (red): 50 µm. (c) Larger scale image of the piriform cortex showing (with red arrow) residual blood from the endoscope tract. Scale bar (green): 1 mm.

6.15 Selected frames showing OCT-resolved depth from the first tract, nominally at (a) 1 mm, (b) 3 mm, (c) 5 mm and (d) 7 mm into the brain. Yellow arrows indicate what appear to be large continuous darker structures, possibly vessels. Images cover from 100 µm to 590 µm. Scale bar: 50 µm.

6.16 Cut brain from the in vivo experiment showing the second tract, through white matter and into the putamen. Ruler markings: 0.5 mm.

6.17 Page from the Rat Brain Atlas (ML−3 mm, AP−1.2 mm, DV+3.8 mm) showing the likely final position of the endoscope in the second penetration, located in the putamen.

6.18 Histology comparison with OCT from the second penetration: (a) Nissl-stained section from the putamen 157 µm after the deepest position of the endoscope and (b) an OCT-resolved frame of the same approximate location and depth showing some similarity to the histology section. Scale bars (red): 50 µm. (c) Larger scale image of the putamen showing (red arrow) residual blood from the endoscope tract. Scale bar (green) 500 µm.
6.19 Selected frames showing OCT-resolved depth from the second tract, nominally at (a) 1 mm, (b) 3 mm, (c) 4 mm and (d) 5 mm into the brain. Images cover from 100 µm to 590 µm. Yellow arrows indicate distinct large, darker features which may represent structures or vessels. Scale bar: 50 µm.

6.20 Results from applying the threshold metric on data from all positions and sweeps of the first tract. The standard deviations over positions and sweeps is also shown along the axes.

6.21 Results from applying the threshold metric on data from all positions and sweeps of the second tract. The standard deviations over positions and sweeps is also shown along the axes.

6.22 Average metric result overlapped with the endoscope tract indicating the approximate imaged positions in the first penetration. Scale bar (black): 1 mm. Color bar shows values for the metric.

6.23 Average metric result overlapped with the endoscope tract indicating the approximate imaged positions in the second penetration. Scale bar (black): 1 mm. Color bar shows values for the metric.
List of Abbreviations

1D  one-dimensional
2D  two-dimensional
3D  three-dimensional
AR  anti-reflection
BOA  Booster Optical Amplifier
CCD  Charge-Coupled Device
CMOS  Complementary Metal-Oxide Semiconductor
CT  Computed Tomography
DBS  Deep Brain Stimulation
DFT  Discrete Fourier Transform
DOF  Depth-of-Field
FD-OCT  Fourier-Domain OCT
FF  Full-Field
FF-OCT  Full-Field OCT
FFT  Fast Fourier Transform
FOV  Field-of-View
FT  Fourier Transform
FWHM  Full-Width Half-Maximum

GP  globus pallidus

GRIN  GRaded INdex

MER  microelectrode recording

MRI  Magnetic Resonance Imaging

MTF  Modulation Transfer Function

NA  Numerical Aperture

OCT  Optical Coherence Tomography

PD  Parkinson’s disease

PSF  Point Spread Function

SD-OCT  Spectral-Domain OCT

SNR  Signal-to-Noise Ratio

SS-OCT  Swept-Source OCT

STN  subthalamic nucleus

SWIR  short-wave infrared

TD-OCT  Time-Domain OCT
Chapter 1

Introduction

1.1 Motivation: Neurosurgical Intraoperative Guidance

Over the last century, neurosurgical interventions have evolved to provide effective treatment for a variety of disabling conditions including cancer [1], epilepsy [2] and Parkinson’s Disease [3–5]. Experimental treatments for depression and Alzheimer’s disease are also being studied [6, 7]. Given the small scale and intricate complexity of brain structures, accurate localization of structures and careful guidance of instruments is of utmost importance and has a significant impact on clinical outcomes. However, current guidance methods are limited by an absence of real-time visual feedback of the region ahead of surgical instruments inserted in brain tissue. Although auxiliary imaging technologies have been developed (e.g., ventriculoscopy, intraoperative Magnetic Resonance Imaging (MRI), X-ray or Computer Tomography-assisted angiography), they fail to provide the spatial resolution required to precisely determine the specific regions and structures targeted by the neurosurgical procedure. Fig. 1.1 shows an overview of different imaging modalities, with the spatial resolution they provide and the depth they are able to image.

While neuron bodies and other identifying features are a few micrometers to tens of micrometers large, even state-of-the-art MRI systems only provide spatial resolution in the order of hundreds of micrometers [13]. One problem that may arise from such limitation is the misguidance and misplacement of stimulating electrodes in Deep Brain Stimulation (DBS), which could lead to inferior therapeutic performance, no beneficial effect or serious detrimental collateral effects [17, 18]. Another potential issue is the laceration of blood vessels, not detectable by the previously-mentioned imaging technologies, causing surgical complications such as intracerebral hemorrhage (morbidity ≈ 2%) or death (mortality ≈
Chapter 1. Introduction

Figure 1.1: Overview comparison of the resolution and penetration depth of different biomedical imaging technologies. CM: Confocal Microscopy [8, 9], OCT: Optical Coherence Tomography, OCM: Optical Coherence Microscopy [10], US: Ultrasound [11], CT: Computed Tomography [12], MRI: Magnetic Resonance Imaging [13, 14], PET: Positron Emission Tomography [15, 16].

2%) [19, 20]. Additionally, MRI or Computed Tomography (CT) devices may be very large and require the patient to be confined to a tight area, which is often unsuitable due to the size of the stereotactic frame required for surgery. Finally, most metallic objects are incompatible with MRI, which also makes imaging electrodes challenging.

1.1.1 Deep Brain Stimulation

(DBS) is a well-established neurosurgical treatment for medication-refractory Parkinson’s disease (PD). PD is caused by insufficient availability of the neurotransmitter dopamine in the brain, leading to irregular neuronal activity in specific areas. This manifests as debilitating symptoms such as tremor, rigidity, stiffness, slowed movement, and walking problems. While the history of DBS and its application to treating Parkinson’s extend back to at least the 1950s [21], it was in the last 20 years that its usage has expanded, nowadays bringing about significant beneficial results and improving patient quality of life. DBS works by delivering current pulses to specific nuclei within the brain. Such stimulation acts as a pacemaker and leads to the regulation of neuronal activity, mitigating or even suppressing the symptoms of PD, such as tremor. To achieve this goal, neurosurgeons insert an electrode in the target region to be stimulated, most often the subthalamic nucleus (STN) and/or the globus pallidus (GP). The choice of target region is tailored to the patient’s specific clinical presentation and anatomy [22, 23]. The electrode is attached to a pulse generator with a battery, which is implanted under the skin of the patient’s chest. The frequency and amplitude of the delivered pulses are calibrated after implantation.
of the electrode in order to maximize benefit to the patient.

1.1.2 DBS Electrode Insertion Procedure

For the insertion of DBS electrodes, a preoperative MRI or CT scan is obtained to identify and locate the exact target for electrode implantation. A suitable path through the brain reaching the target region is calculated by a specialized software, and coordinates for that path are obtained. A Leksell stereotactic frame is attached to the patient’s head, who must refrain from taking their symptom-alleviating medications the day of the procedure and a couple of days prior to it. The neurosurgeon drills a small hole in the patient’s skull and inserts an 18 cm-long metal cannula along the traced path, with the aid of the coordinates in the Leksell frame, usually while the patient is awake.

Microelectrode recording (MER) is the current standard for guiding DBS electrode implantation [24]. MER provides the neurosurgeon recordings of neuronal activity along the insertion path so it can be determined whether they are passing through the intended regions. MER is often noisy and accurate localization may be challenging. The neurosurgeon must average several results, sometimes along different tracts, in order to determine from which brain region the recordings originate. This procedure may take several hours. Moreover, MER usage carries additional risk of lesion to the brain [25, 26]. Once the target region is located with sufficient confidence, the microelectrodes are removed and the surgeon inserts the DBS electrode. If at any point there is doubt that the proper path/target region is reached, the neurosurgeon removes the metal cannula from the brain and restarts the entire procedure from the beginning. In fact, even if the target is reached as intended, the patient is surveyed after the placement for undesirable effects (for example, visual light flashes when the stimulation is on) and the electrode may require repositioning [27].

The electrode insertion can be lengthy and demanding on both the neurosurgeon and the patient. It is also costly in terms of time and usage of hospital resources. Therefore, all stakeholders would gain from having a faster, more precise and more efficient way of identifying the different brain regions and structures as the cannula is inserted into the patient’s brain. From consultations with Dr. Andres Lozano and neurosurgeons in his group it became clear that the practice would benefit from having an endoscopic imaging system which provide sufficient guidance during surgery, complementing or replacing the current method. The most important features of such a system would be: 1) the ability to distinguish in real-time between different brain regions, 2) dimensions compatible with neurosurgical insertion, 3) compatibility with existing equipment and technologies, and 4) significant speed improvement over electrophysiological recording.
In order to address the challenges of safety, accuracy, comfort and efficiency of stereotactic neurosurgery, Optical Coherence Tomography (OCT) has emerged as a potential guidance technology [28]. While OCT methods are limited in the depth they can image (1-2 mm in tissue), they achieve higher resolution and are simpler, cheaper, faster and more portable than imaging technologies such as MRI and CT. Fig. 1.1 illustrates how OCT compares to other imaging modalities. Another advantage of OCT is the ability to differentiate structures with intrinsic contrast (i.e., without the injection of a contrast agent) [29] and without ionizing radiation. In particular, it has been shown to discriminate grey versus white matter, tumorous versus non-tumorous cells, and blood vessels [10] [30-33].

1.2 Background

1.2.1 Optical Coherence Tomography

OCT is a three-dimensional (3D)-imaging technique based on interferometry [34]. Initially derived from low-coherence interferometry with broadband white light in the early 1990s [35], several different variations of OCT have been developed since then. OCT is now one of the most valuable technologies available for retinal imaging and clinical diagnosis [36]. Both free-space and fiber-based systems have been developed [37]. By varying one or more parameters in the system, such as the length of the reference arm or the wavelength of the source, changes in optical intensity due to constructive or destructive interference between light from the two arms can be resolved to yield a depth profile of the sample.

Referring to Fig. 1.2 for clarification of the OCT mechanism, we start with a simple Michelson

Figure 1.2: Illustration of a Michelson interferometer for OCT. Image by the author.
interferometer and monochromatic light with free-space wavelength $\lambda_0$. Consider an $(x, y, z)$ coordinate system for a cubic sample and let the plane $x$-$y$ be the optical plane of incidence at the sample, and $x'$-$y'$ the plane of incidence at the photodetector. The two planes are related by the spatial transfer function, known as the Point Spread Function (explained in Section 2.1.1). A mirror in the reference arm is positioned $z_{\text{ref}}$ away from the common arm length $L$, as indicated in Fig. 1.2. Light propagating in the reference arm is reflected by the mirror and reaches the detector after traveling an optical path length $2(L + z_{\text{ref}})$. Let its intensity at the detector be $I_{\text{ref}}$ for all $(x'_0, y'_0)$. Light propagating along the sample arm is backscattered by discrete reflectors enumerated by $i$, whose depths in the sample are $z_i$. Backscattered light reaches the detector with intensity $I_{\text{samp}}(x_0, y_0, z_i)$ after traveling an optical path length of of $2(L + z_i)$. Due to the interference of light from both arms at the detector, for each point $(x_0, y_0)$ of the sample the light intensity observed at the corresponding photodetector point $(x'_0, y'_0)$ varies according to the wavelength and location of the reflector in the sample. The optical intensity at the photodetector point $I_{\text{det}}(x'_0, y'_0)$ is

$$I_{\text{det}}(x'_0, y'_0) \propto I_{\text{ref}} + \sum_{i} \max_{z_i} I_{\text{samp}}(x_0, y_0, z_i) + 2\sqrt{I_{\text{ref}}I_{\text{samp}}(x_0, y_0, z_i)} \cos \left[ \frac{2\pi n}{\lambda_0}(z_i - z_{\text{ref}}) \right]$$

$$+ \sum_{m, n; m \neq n} 2\sqrt{I_{\text{samp}}(x_0, y_0, z_m)I_{\text{samp}}(x_0, y_0, z_n)} \cos \left[ \frac{2\pi n}{\lambda_0}(z_m - z_n) \right]. \quad (1.1)$$

In Eq. 1.1 above, $z_{\text{ref}}$ is the depth of the reference reflector, $z_{\text{max}}$ is the maximum distance from which backscattered light is observable, $n$ is the refractive index of the sample, $I_{\text{samp}}(x_0, y_0, z_i)$ is the intensity of backscattered light from each reflector $(x_0, y_0, z_i)$ in the sample. $z_{\text{max}}$ depends on the optical penetration depth in the sample at each wavelength, as well as other factors, such as the depth of field and scattering anisotropy. In Eq. 1.1 the refractive index of the sample has been assumed to be uniform, and $I_{\text{samp}}(x_0, y_0, z_i)$ is the unknown to be solved for. The cosine terms represent the interference effect due to different optical path lengths; the factor of 2 accounts for the round-trip length. The second summation term in Eq. 1.1 corresponds to auto-correlation terms, caused by interference between backscattered light from different points within the sample itself (represented by $z_m, z_n$). These terms have a small magnitude and appear near $z = 0$ after the signal is resolved. Their effect is discussed in Section 4.3.

The model above assumes a single path, single scattering and a single polarization. For a continuous surface of reflectors the summations would be replaced with integrals, but the end result is the same.

Eq. 1.1 shows that varying either $z_{\text{ref}}$ or the wavelength changes the detected intensity due to the cosine term. By sweeping either parameter and applying the appropriate signal processing techniques to the collected signal Eq. 1.1 can be solved to find $I_{\text{samp}}(x_0, y_0, z_i)$ for each reflector $i$. The exact
processing algorithm depends on the modality of OCT, as detailed in the next section. Repeating the procedure over all points \((x_0, y_0)\) yields a 3D representation of the relative reflectance, penetration depth and scattering properties of the various points of the sample.

### 1.2.2 Types of OCT

Fig. [1.3](#) shows an overview of the components in different types of OCT system. The three main types of OCT are Time-Domain OCT (TD-OCT), Fourier-Domain OCT (FD-OCT), both implemented with a broadband source, and Swept-Source OCT (SS-OCT), which is mathematically equivalent to FD-OCT. The following subsections will provide a brief overview of these main types of OCT. Table [1.1](#) compares these main types of OCT.

#### Table 1.1: Parameter comparison for different types of OCT systems.

<table>
<thead>
<tr>
<th>OCT type</th>
<th>Source</th>
<th>Axial Resolution</th>
<th>Maximum Depth</th>
<th>Speed</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>TD</td>
<td>broadband</td>
<td>coherence length</td>
<td>reference translation</td>
<td>slow</td>
<td>low</td>
</tr>
<tr>
<td>FD</td>
<td>broadband</td>
<td>source bandwidth</td>
<td>pixel pitch</td>
<td>fast</td>
<td>higher, w/roll-off</td>
</tr>
<tr>
<td>SS</td>
<td>tunable</td>
<td>sweep range</td>
<td>(\lambda) steps</td>
<td>fastest</td>
<td>highest</td>
</tr>
</tbody>
</table>

---
**TD-OCT**

The first and simplest type of OCT developed is based on broadband light, a Michelson interferometer and a movable mirror at the reference arm of the interferometer. This modality is now known as TD-OCT. As the light source for TD-OCT is broadband (i.e., composed of a wide range of wavelengths), it exhibits low temporal coherence and a short coherence length. One estimate for the coherence length based on $\Delta \lambda_{FWHM}$, the Full-Width Half-Maximum (FWHM) bandwidth of a Gaussian source, is

$$L_c = \frac{2 \ln 2}{n \pi} \frac{\lambda_c^2}{\Delta \lambda_{FWHM}}$$  \hspace{1cm} (1.2)

In general, interference effects between two electromagnetic waves are only observed when the optical path length difference between the two waves falls within the coherence length. For a source with a Gaussian-like spectrum, for example, an optical path length difference equal to the coherence length $L_c$ results in a 50% reduction in fringe visibility \[38\] (see the concept of “contrast” in Eq. 2.5). For TD-OCT, the coherence length is equivalent to the axial (i.e., $z$) resolution of the system, thus a low coherence source is preferred. For each position of the translated mirror the detected intensity varies proportionally to the reflected intensity from structures in the sample that are at most one coherence length away from the reference path length. The coherence length of a broadband source for TD-OCT is of the order of at most several micrometers. The peak detected intensity is observed when $z_i = z_{ref}$ due to constructive interference. Translating the mirror over a longer range (millimeters, for example) yields several of these oscillations, with relative peak intensity values determining the relative reflectance of the sample at different depth points. Acquisition speed is limited by the reference-translating mechanism. As in all OCT systems, the maximum resolvable depth here depends on the sensitivity and dynamic range of the photodetector, as well as on the intensity of backscattered light.

**FD-OCT**

Another modality is Frequency-Domain OCT or Spectral-Domain OCT (SD-OCT), in which the length of the reference arm ($z_{ref}$) is kept fixed while the intensity of interfered light is measured for each wavelength. Taking the Fourier transform of the acquired spectrogram leads to the immediate calculation of the reflected intensity at each depth point. The spectra can be obtained by either a dispersive detector, which spatially separates the intensity corresponding to each wavelength via a diffraction grating, for example, or by varying temporally the wavelength of the light source by using a tunable laser source. FD-OCT usually refers to the former type, and the latter is called SS-OCT.

In FD-OCT, employing a broadband source, the spatially-separated intensities are projected onto a
Chapter 1. Introduction

multi-pixel sensor. Thus, its spectral resolution is limited by the pixel size of the detector. Intensity variations caused by reflections at a larger distance from the reference exhibit higher frequency. The ability of the detector to resolve oscillations drops as the frequency increases. That behavior is called the “high-frequency sensitivity roll-off” of FD-OCT systems, and limits the maximum depth that can be resolved by the system. The FWHM axial resolution $\delta z_{FWHM}$ here depends on the bandwidth of the source as well as on the sensitivity bandwidth of the detector, given for a Gaussian spectrum as

$$n\delta z_{FWHM} = \frac{2 \ln 2}{\pi} \frac{\lambda_c^2}{\Delta \lambda_{FWHM}}.$$  \hspace{1cm} (1.3)

In the equation above, $n$ is the refractive index of the medium, $\lambda_c$ is the central wavelength of the source (assuming a Gaussian spectral profile) and $\Delta \lambda_{FWHM}$ is the FWHM bandwidth of either the source or the detector (whichever is narrower). For a detector with spectral resolution $\delta \lambda$, the maximum one-sided resolvable depth according to the Nyquist limit is [34]

$$n\Delta z_{max} = \frac{\lambda_c^2}{4\delta \lambda}.$$  \hspace{1cm} (1.4)

SS-OCT

In SS-OCT, the mathematics involved is almost identical to FD-OCT. Two major differences are that SS-OCT requires a tunable high-coherence source, and that a single pixel can be used per spectra because the wavelengths are sampled temporally as the laser wavelength is swept. For that reason, the spatial configuration of the detector no longer dictates the maximum depth that can be resolved. While SS-OCT does not suffer from high-frequency roll-off due to the detector itself, it does have similar limitations which are further elaborated below.

As fast-sweeping tunable lasers with a broad wavelength range have become available, SS-OCT has become a common choice for the development of OCT systems, although these laser-based systems have not yet matched the resolution, reliability and maturity of broadband source-based systems [35].
Chapter 1. Introduction

Nonetheless, SS-OCT is superior to FD-OCT due to higher scanning speeds, reduced sensitivity roll-off at longer depths, reduced fringe washout from motion due to transverse scanning or from the sample itself, higher wavelength resolution and increased Signal-to-Noise Ratio (SNR) in balanced detection schemes [39].

In SS-OCT, the axial resolution $\delta z$ and the maximum resolvable depth $\Delta z_{\text{max}}$ are similar to the FD-OCT case. For a tunable laser source sweeping wavelengths from $\lambda_{\text{start}}$ to $\lambda_{\text{end}}$ with constant power and wavelength steps $\delta\lambda$,

$$n\delta z_{\text{FWHM}} = \pi \left( \frac{2\pi}{\lambda_{\text{start}}} - \frac{2\pi}{\lambda_{\text{end}}} \right)^{-1}, \text{ and}$$

$$n\Delta z_{\text{max}} = \frac{\lambda_{\text{start}} \lambda_{\text{end}}}{4\delta\lambda}.$$  

(1.5)  

(1.6)

Scan types

Finally, the signal for all points $(x_0, y_0)$ can be obtained individually (scanning the points sequentially) or by parallel acquisition using a Charge-Coupled Device (CCD) or Complementary Metal-Oxide Semiconductor (CMOS) camera. In OCT terminology, obtaining the entire signal for a single pixel is called an “A-scan”, running A-scans over a line of points is a “B-scan” and scanning over a two-dimensional (2D) surface is a “C-scan”. One A-scan yields a one-dimensional (1D) depth image of the sample; likewise, a B-scan yields a 2D image and a C-scan a 3D image. The acquisition speed of OCT systems is often given in “A-scans per second”. Parallel acquisition of a C-scan is known as a Full-Field (FF) or “En face” acquisition scheme.

Fiber-based & FF schemes

OCT can be implemented via fiber-based or FF schemes. The vast majority of OCT systems reported are fiber-based. That is, they acquire the OCT signal by scanning in either a 1D or a 2D pattern. This acquisition scheme has several advantages, the most significant of which are:

- Uses a single photodetector.
- Allows for easy implementation of balanced detection, which greatly increases the overall signal-to-noise ratio by removing the strong interference-independent background.
- Allows for coherent detection.
- Concentrates all laser power on each A-scan
- Is mostly flexible and compact, up to the scanning mechanism/optics.
Table 1.2: Comparison between fiber-based and FF-OCT systems.

<table>
<thead>
<tr>
<th>Acq. scheme</th>
<th>Power</th>
<th>Sensor</th>
<th>Speed limitation</th>
<th>Balanced detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fiber-based</td>
<td>concentrated 1-4 photodetectors; lower cost</td>
<td>tranverse scanning</td>
<td>simple</td>
<td></td>
</tr>
<tr>
<td>FF</td>
<td>distributed 10^3 to 10^6 pixels; higher cost</td>
<td>frame rate of detector</td>
<td>challenging</td>
<td></td>
</tr>
</tbody>
</table>

- Can easily be used for polarization-sensitive OCT with polarization-maintaining fibers.

However, it has three main disadvantages compared to Full-Field OCT (FF-OCT). The first is that scanning requires moving parts. The second is that the acquisition speed is limited by the scanning mechanism and the repeated wavelength sweep of the laser at each pixel. The third is that the user is only able to obtain an image of the sample after the acquisition and processing is complete. Table 1.2 summarizes the comparison between the fiber-based and FF-OCT.

In comparison, for each spectrogram acquisition in FF-OCT the wavelength range of the laser only has to be swept once. Moreover, while the number of points that can be simultaneously “scanned” is limited by the number of pixels and the frame rate of the camera, this type of system has no moving parts as in the fiber-based case. Another advantage of a FF acquisition scheme is that the system also functions as an epi-illumination microscope, simply by switching the coherent light source with a broadband one. This alternative mode of operation is capable of providing information not captured by the OCT mode, or in preparation for the OCT scan. For example, imaging can be used to check the position of an electrode, or to verify the illumination alignment without interference effects.

On the other hand, the disadvantages of FF-OCT are:

- They may be bulkier using fully packaged cameras, but they can be miniaturized with original equipment manufacturer (OEM) parts.

- The laser power is distributed over all of the pixels, and there is more loss in the system due to aperture mismatches.

- Current short-wave infrared (SWIR) cameras are more expensive, have fewer and larger pixels and inferior performance compared to CMOS cameras for visible and near-infrared light.

- Challenging to implement balanced detection.

- More optical components to align.
Chapter 1. Introduction

To date, FF-OCT systems which have been demonstrated \[40-42\] use low-coherence light sources, and thus do not benefit from the advantages of a swept-source system. Moreover, their optical systems tend to have higher numerical aperture, which limits the imaging depth.

Common-path OCT

Most OCT systems, especially fiber-based ones, have a reference arm separate from the sample arm as shown in Fig. 1.2. Thus, the reference intensity can be adjusted according to the reflectance of the sample, which allows for maximal usage of the dynamic range of the photodetector without saturation. However, for resolving the OCT signal the dispersion in both arms must be balanced. Common-path OCT refers to a system where the reference is positioned along the sample arm. Common-Path OCT systems are more stable, especially in highly-dispersive systems such as those that use long GRaded INdex (GRIN) lenses \[43\].

1.3 Thesis Objectives and Organization

1.3.1 Objectives

This thesis presents a proof-of-concept endoscopic full-field swept-source OCT (FF-SS-OCT) system that aims to satisfy the imaging requirements for neuroimaging and stereotactic neurosurgery guidance. The designed and demonstrated system is unique compared to existing OCT systems in terms of the form factor, wavelength used, and modality. Although SS-OCT systems are now the standard in terms of high-SNR OCT, FF-SS-OCT implementations are rare. To our knowledge, this is the first report of FF-SS-OCT using a coherent source at wavelengths $>1\mu m$. It is also the first endoscopic version of such an OCT system.

1.3.2 Thesis Organization

This thesis is structured as follows: Chapter 2 presents additional background relating to imaging metrics, the mechanism of GRIN lenses and the interaction of light with biological tissue. Chapter 3 discusses the required technical specifications, design trade-offs and presents ray-tracing simulations for the design of the optical system. Chapter 4 deals with the physical implementation of the optical system and its integration with the hardware, including the acquisition control software and the OCT-processing algorithm. Chapter 5 addresses the characterization of the power and imaging properties of the system. The obtained results validate the design metrics. Chapter 6 covers the experimental
procedures undertaken for \textit{ex vivo} and \textit{in vivo} tissue experiments, and presents the results obtained from those experiments including a comparison with histology-stained tissue. Results were reproducible containing features which seem to correlate with the properties of different brain regions. Chapter 7 covers the analysis of these results in light of the objectives and offers a perspective of possible directions for continuation of the research.
Chapter 2

Imaging Metrics and Optical Properties of Tissue

The design of the OCT system must achieve the required specifications on the transverse and axial resolution, field of view, and imaging depth. In this chapter, we review the relevant metrics for optical imaging, as well as the optical properties of tissues. This information will be used in Chapter 3 in the design of the OCT system.

2.1 Imaging Metrics

2.1.1 Resolution and DOF

In OCT systems, the transverse and axial resolutions are specified independently from one another. The transverse resolution is determined exclusively by the optics of the system, identically to most optical microscope systems. Thus, a transverse resolution analysis follows the same conditions as in standard microscopy, namely diffraction limits and wavelength considerations, as is further elaborated next.

Point Spread Function, Airy disk, Rayleigh criterion

As all optical systems have a finite aperture, they are unable to reproduce an object with infinite accuracy. The limit to that accuracy due to diffraction is the Point Spread Function (PSF) and it determines the resolution of the optical system. The PSF is the impulse response of an imaging system and its output when the object is a point. The PSF may be experimentally determined by imaging very small objects, such as quantum dots or fluorescent beads. For the sake of a design metric, the PSF can be estimated...
from the diffraction pattern resulting from a circular aperture, which is known as the Airy disk (Fig. 2.1).

According to Fraunhofer diffraction theory, the intensity profile of the diffraction pattern on a plane a large distance $d_{\text{image}}$ away from the aperture is given in [44] as

\[ I(r) = I(0) \left[ \frac{2J_1(\frac{ka}{d_{\text{image}}}}}{ka} \right]^2, \]

where $k$ is the propagation wavenumber, $J_1$ refers to the first-order Bessel function of the first kind, $a$ is the radius of the aperture, and $r$ is the radial distance from the optical axis on the image plane. That intensity pattern is shown in Fig. 2.1. The Airy radius is the distance from the center to the first zero of the central lobe, which corresponds to the first zero of the Bessel function. Taking a lens with diameter $D_{\text{lens}} = 2a$ as the aperture, and an image formed from a collimated monochromatic beam with a wavenumber $k = \frac{2n\pi}{\lambda_0}$ at the focal length $f$ of the lens, Eq. 2.2 leads to

\[ r_{\text{Airy}} \simeq \frac{1.22}{\lambda_0} \frac{f}{D_{\text{lens}}}. \]

The quantity $\frac{f}{D_{\text{lens}}}$ is the f-number or $f/\#$ of the imaging system. The Numerical Aperture (NA) is related to the acceptance angle of the lens, $\theta$, and is defined as

\[ NA = n \sin \theta \approx n\theta, \]

for small angles. From Fig. 2.2 it can be seen that $\theta \approx \tan(\theta) = \frac{D}{f} = \frac{1}{f/\#}$, from which we obtain the relation $\frac{f}{D} = f/\# = \frac{\lambda_0}{2nNA}$. Therefore, Eq. 2.2 simplifies to:

\[ r_{\text{Airy}} \approx 0.61 \frac{\lambda_0}{NA}. \]
This form makes it more intuitive to understand that a system with a higher NA has a smaller $r_{\text{Airy}}$ and thus a greater ability to resolve smaller features.

Since the PSF is the impulse response of the imaging system, the input (i.e., the object) can be convolved with this function to yield the output (i.e., the image). Since every point in the object is expanded to the size of the Airy disk at the output of the imaging system, if features in the object are smaller than the size of the Airy disk they will be blurred and will not be considered resolvable. Therefore, the PSF sets the limiting resolution of the system. In particular, the Rayleigh criterion for resolution states that for two nearby points to be deemed resolvable, the centers of their Airy disks must be at least one Airy radius apart. Thus, the smallest features we consider resolvable are the ones with size greater or equal to $r_{\text{Airy}}$. That condition is illustrated in Fig. 2.3.

The resolution of an optical imaging system can be further described using the concept of Modulation Transfer Function (MTF). The MTF is the amplitude part of the complex spatial Fourier Transform of the PSF. While the Rayleigh criterion imposes a sharp cutoff for the resolution, the MTF allows for a continuous metric, relating spatial frequencies to contrast. For example, a fringe width of 25\,\mu m is equivalent to 20\,lp/mm, and a fringe width of 8\,\mu m is equivalent to 62.5\,lp/mm. Since the resolution of an imaging system is limited at smaller features (i.e., higher frequencies), the MTF of the system is a
low-pass filter of the spatial frequencies of its object. In the case of a digitized image, frequencies can also be normalized to the spatial Nyquist sampling limit, namely 0.5 cycles/pixel at the image sensor. For a line pair consisting of a brighter line and a darker line, the contrast is defined as

$$100 \times \left( \frac{I_{\text{bright}} - I_{\text{dark}}}{I_{\text{bright}} + I_{\text{dark}}} \right),$$

where $I_{\text{bright}}$ and $I_{\text{dark}}$ are the highest and lowest intensities, respectively, of a fringe pattern.

Thus, the MTF is the ratio of the image contrast to the object contrast, at a specific spatial frequency. It is illustrated in Fig. 2.4. The MTF of a diffraction-limited system can be well-approximated by two lines. The MTF is 1 at low frequencies and is linear with an asymptote intercepting the frequency axis at $\frac{NA}{\sin(\theta)}$ (the Rayleigh limit). For higher frequencies near the Rayleigh limit the MTF diverges from that asymptote and more slowly decreases towards 0 at the cutoff frequency, which is given by $\frac{2NA}{\lambda R}$.

These approximations are for ideal, in-focus, aberration-free optical systems.

### 2.1.2 Gaussian Beams & Depth-of-Field

The resolvable depth of OCT, without a moving focus, is limited by the Depth-of-Field (DOF) of the imaging system. The DOF can be approximated using Gaussian beam optics. The electric field of an x-polarized z-propagating Gaussian beam is given by the vector

$$\vec{E}(r, z) = \hat{x} E_0 \frac{w_0}{w(z)} e^{-\frac{r^2}{w(z)^2}} e^{-j(kz + k \frac{r^2}{2w^2} - \arctan \frac{z}{z_R})} e^{j\omega t},$$

where $w(z)$ is the half-diameter of the beam, $w_0$ is the beam waist, and $z_R$ is Rayleigh range. As depicted in Fig. 2.5, the Rayleigh range is defined as the point at which the half-diameter of the beam...
increases by a factor of $\sqrt{2}$ with respect to its minimum value, $w_0$. The increase in half-diameter is equivalent to a doubling of the transverse ($x$-$y$) area of the beam ($\frac{\pi(\sqrt{2}w_0)^2}{\pi w_0^2} = 2$). Since the beam has a Gaussian intensity profile as depicted in Fig. 2.6, the half-diameter is defined as the radial distance from the center at which the intensity drops by a factor of $\frac{1}{e^2}$. From Eq. 2.6 the half-diameter of the beam is:

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

with:

$$z_R = \frac{n\pi w_0^2}{\lambda_0}.$$  

From Eq. 2.7 it can be shown that the divergence angle of the Gaussian beam, $\Theta$, which is defined as the angle between the center of the beam at the focal spot and the $\frac{1}{e^2}$ intensity diameter at the Rayleigh range, is:

$$\Theta \approx \frac{2\lambda_0}{n\pi w_0}.$$  

\[\text{Figure 2.5:} \ \text{Cross-section of a focused } z\text{-propagating Gaussian beam showing its waist } w_0, \text{ its Rayleigh range } z_R \text{ and the confocal parameter } b. \ \text{Image by Rodolfo Hermans [47].}\]

\[\text{Figure 2.6:} \ \text{Transverse cross-section of a Gaussian beam. Image in the public domain.}\]
Chapter 2. Imaging Metrics and Optical Properties of Tissue

Figure 2.7: Effect of the DOF in an image. Image extracted from Ref. [49].

If we take the Gaussian beam as being focused by a lens, comparing Fig. 2.5 with Fig. 2.2, we see that
\[ \Theta \approx 2\theta. \]
Since \( \theta \approx \frac{D}{f} \), Eq. 2.9 becomes:
\[ w_0 \approx \frac{2\lambda_0 f}{n\pi D}. \] (2.10)

The DOF is considered to be the longitudinal range in object space for which the image of the object
is “in focus”, as illustrated in Fig. 2.7. The DOF corresponds to the Rayleigh range, \( z_R \), before and
after the focal spot, and is thus equivalent to the confocal parameter, \( b = 2z_R \), of the Gaussian beam.
Therefore, from Eq. 2.8 the DOF of an imaging system is
\[ \text{DOF} = 2z_R = \frac{2\pi n}{\lambda_0} \left( \frac{2\lambda_0}{n\pi} \right)^2 \left( \frac{f}{D} \right)^2 = \frac{8\lambda_0}{n\pi} \left( \frac{f}{D} \right)^2 = \frac{2n\lambda_0}{\pi NA^2}. \] (2.11)

The relation \( NA = \frac{1}{2\pi f} \) was used in deriving the last part of the equation above. Eqs. 2.11 and
2.4 show the trade-off between DOF and resolution due to NA, which is also illustrated in Fig. 2.8. A
higher NA leads to improved transverse resolution, but reduced DOF. Additionally, since DOF decreases
as the square of the NA, a small improvement in resolution leads to a more significant reduction of the
DOF. Note that a higher refractive index does not increase the DOF because the NA also includes the
refractive index; a higher refractive index reduces the DOF.

2.1.3 Field-of-View

The transverse Field-of-View (FOV) is a function of the total magnification of the system and the camera
sensor, with an additional restriction imposed by the endoscope diameter. A linear magnification \( M \)
and detector dimensions \( d_x, d_y \) limit the FOV to at most \( \frac{d_x}{M}, \frac{d_y}{M} \). Thus, the higher the magnification,
the smaller the FOV. At the same time, the magnification must be sufficiently high to resolve the
desired features. The optically-limited resolution previously described should match the pixel size of the
camera. In practice, the PSF should cover at least a few pixels. Thus, an upper and a lower bound for the magnification are derived from the aforementioned parameters.

### 2.2 GRIN Lenses

To fit the form factor of the endoscope, we use GRIN lenses rather than standard lenses. GRIN lenses are designed to achieve the same wavefront shaping effects as standard lenses by varying the refractive index in the lens instead of varying the shape. Their main advantage is greater control over the form factor, allowing for thin and/or long lenses with flat surfaces on the edges. They can be designed to function in contact with various media of different refractive indices (for example, fluids), since they do not rely on the medium-to-lens index to shape the wavefront as with standard lenses. The refractive index profile or gradient determines the properties of the GRIN lens. A radial GRIN lens has an index profile that varies radially from the center. A typical index profile that can be manufactured is

\[ n(r) = n_0 \text{sech}(\Gamma r), \]  

(2.12)

where \( \Gamma \) is the gradient constant and \( r \) is the radial distance. The refractive index is highest at \( r = 0 \), where it is equal to \( n_0 \). A high value of \( \Gamma \) is similar to a standard lens with a large radius of curvature and a short focal length. As light propagates in a GRIN lens rod, its rays trace periodic trajectories that repeatedly converge and diverge as illustrated in Fig. 2.9.
The pitch of a GRIN lens, \( P \), is the number of trajectory periods spanned by rays over the lens length. The periodic trajectories make possible relay and focusing/collimating types of GRIN lenses. A relay GRIN replicates the incident wavefront at its output. The pitch of a non-inverting relay GRIN is an integer multiple of 1. The pitch of an inverting relay GRIN is an odd multiple of 0.5. For collimation, GRIN lenses have pitch roughly equal to 0.25. We can associate a focal length to the focusing/collimating type of GRIN lens, which is

\[
f = \frac{1}{n_0 g \sin(\Gamma z_l)},
\]  

(2.13)

where \( z_l \) is the length of the rod as depicted in Fig. 2.10. As a GRIN lens has to be treated as a thick lens, the separation, \( s \), between the focal plane of the GRIN and its input facet is given by

\[
s = \frac{1}{n_0 g \tan(\Gamma z_l)}.
\]

(2.14)

Eqs. 2.13 and 2.14 show that from a GRIN rod with specified \( n_0 \) and \( \Gamma \) lenses of different pitches, focal lengths and working distances can be produced by cutting the rod at different lengths \( z_l \). For a GRIN lens with diameter \( d \), the NA is defined in the same way as optical fibers, such that

\[
\sin \theta = \sqrt{n_{\text{max}}^2 - n_{\text{min}}^2} = \sqrt{n_0^2 - n_0^2 \sech \left( \frac{\Gamma d}{2} \right)} = n_0 \sqrt{1 - \sech \left( \frac{\Gamma d}{2} \right)}
\]

(2.15)

Finally, the analysis of image formation by a GRIN lens can be carried out analytically using ray optics and ABCD matrices, or computationally using ray-tracing software such as Zemax OpticStudio or Synopsys CODE V. In Chapter 4, we use these principles to design the GRIN lens for our OCT system.
2.3 Optical Properties of Tissue

We will present here a brief overview of the practical analysis of light-tissue interaction. For a more detailed approach, the reader can refer to [52], from which a substantial portion of the content that follows was extracted. Generally speaking, two aspects have to be considered with regards to the interaction of light and biological tissue: absorption and scattering. Absorption refers to attenuation due to energy that is transferred to the tissue, while scattering refers to how and by how much light is reflected or deflected through the sample. The absorption and scattering per length are quantified by the wavelength-dependent coefficients $\mu_a(\lambda)$ and $\mu'_s(\lambda)$, respectively. Both processes depend on the type of particles in the medium and their concentration, while scattering also depends on the relative size of the particles to the wavelength of light. The intensity attenuation is described by the Beer-Lambert law; the intensity, $I$, of a light beam traced along a path $r$ is

$$I(r) = I_0 e^{-\int \mu_t(r) dr}, \quad (2.16)$$

where $I_0$ is the initial intensity, and $\mu_t = \mu_a + \mu'_s$ [53].

Absorption

Absorption depends on the optical excitation of the electronic and phononic transition in a medium. The value for $\mu_a(\lambda)$ is usually obtained from a more formal quantum analysis of the material, dispersion models (e.g., the Lorentz oscillator model) and/or experimentally.
Scattering

The most pertinent scattering processes for OCT are Mie and Rayleigh scattering. Mie theory explains the general behavior of scattering, for particles of any size, while the Rayleigh regime is the limit of Mie theory for scatterers which are much smaller than the wavelength of light \[54\]. Within the Mie regime, there is less dependence on the wavelength, but stronger dependence on the indices of refraction of the scatterer and the surrounding medium, and the size of the scatterer. Since in the Rayleigh scattering regime both the anisotropy and the effect of scattering decrease proportionally to \(\frac{1}{\lambda^4}\), longer wavelengths are less affected by it. As neurological elements such as neurons and glial cells are 8 to 40 µm in diameter, much larger than the optical wavelengths for OCT, the Mie regime more accurately describes the scattering of light from these structures. For structures with size of the order of hundreds of nanometers, Rayleigh scattering is the appropriate model. The dimensionless anisotropy coefficient \(g\) characterizes the average directionality of the scattering. The anisotropy parameter is bounded in \([-1, 1]\). A medium with \(g = 0\) is isotropic, with uniform distribution of scattering over all angles. Negative values of \(g\) mean the medium is predominantly backward-scattering and positive values mean the medium is predominantly forward-scattering. Most types of biological tissue have \(g\) in \([0.8, 0.98]\), meaning they are strongly forward-scattering \[54\]. Due to the cumulative contribution of several scattering events, after a certain distance the trajectories of photons become random and backscattered signal can no longer be acquired. That distance is called the scattering depth and can be estimated from \(\frac{1}{\mu_s'}\). A formulation for \(\mu_s'\) which accounts for both Mie and Rayleigh contributions is presented in Ref. \[54\].

The case for choosing 1310 nm light

The optical or therapeutic window refers to the ranges of wavelengths, mostly within the near-infrared (NIR) range, at which attenuation in biological tissue is minimal. As such, optical penetration depth reaches a maximum for those wavelengths. The NIR range is divided into four wavelength windows: NIR-I corresponds to 650-950 nm; NIR-II corresponds to 1100-1350 nm; NIR-III corresponds to 1600-1870 nm; and NIR-IV corresponds to 2100-2300 nm \[56\]. Fig. 2.11 illustrates the light attenuation spectrum for a few select biological materials, with the NIR-I and NIR-II windows highlighted. The NIR-II region was traditionally ignored in OCT due to stronger absorption by water relative to the NIR-I, as shown in Fig. 2.12. However, comparing Figs. 2.11 and 2.12 the attenuation from tissue dominates over water absorption, motivating recent interest in using NIR-II for in vivo studies \[55, 57\].

Additional advantages of a longer wavelength range are a longer scattering depth and a longer DOF. Moreover, since the O-band (1260 to 1360 nm) is a standard wavelength range for optical communica-
Figure 2.11: Wavelength-dependent attenuation in biological tissue (log scale). Adapted from an image in Ref. [55].

Figure 2.12: Water absorption in the visible and near-infrared ranges. Image by the author based on data from Ref. [58].
tions, there are many commercially-available tunable light sources and optical components optimized for that range. One downside is that FF-OCT at this wavelength range necessitates the use of InGaAs CCD cameras, which, today, are more expensive, have larger pixels, and have lower frame rates than CMOS cameras. Considering all factors, we decided it would still be advantageous to use the O-band as our design wavelength range.

**Optical parameters for brain tissue**

For reference, we include here a few optical parameters for brain tissue at 1310 nm. The refractive index of artificial cerebral spinal fluid (aCSF) was measured to be $1.342 \pm 0.007$. The refractive index in the corpus callosum (white matter) was measured to be $1.407 \pm 0.015$, while in grey matter regions such as the putamen and the cortex the index varied from 1.361 to 1.369, with no statistically significant variation between different grey matter regions. The average refractive index over several measured brain regions is $1.380$ [59]. An estimated $\mu_s(1310\text{nm}) = 7.624\text{cm}^{-1}$ suggests a scattering depth of approximately 1.3 mm for 1310 nm light [54].

### 2.4 Summary

In this chapter, we have reviewed the concepts of resolution, contrast, DOF and FOV in imaging. The NA is the parameter at the core of the trade-off between the transverse resolution and DOF. We have motivated the use of 1310 nm wavelength light for imaging due to its deeper scattering depth in brain tissue.
Chapter 3

Specifications and Design

This chapter introduces and analyzes the required specifications for the imaging system, and explains the design trade-offs that were made throughout the development process. Simulation results are included for all design parameters.

3.1 Required Specifications

3.1.1 Physical Requirements

The system needs to be physically compatible with existing instruments and operating environments. The form factor and dimensions of a viable endoscope are the strictest requirement. Neurosurgical procedures almost always employ rigid stainless steel cannulas, which facilitate the insertion of instruments for execution of different tasks (e.g., recording electrophysiology signals or removing a piece of tissue for biopsy). Cannulas used in neurosurgery have a range of internal diameters, from 1 mm to 1.8 mm. The design goal was an endoscope with less than 1.3 mm outer diameter, so that it would fit inside most cannulas. The endoscope also needs to be at least 18 cm long. Cannulas are inserted deep into the brain, with a portion remaining outside of the head. That external portion is attached to the stereotactic frame for precise positioning and control. Since there is usually sufficient space for large devices in operating rooms, components other than the endoscope need not be as compact. In a clinical implementation, however, optical components must be packaged so that they can be handled by the operating personnel and can be attached to the stereotactic frame.
3.1.2 Imaging Requirements

The purpose of the system is to allow the neurosurgeon to identify the brain region located immediately next to the endoscope tip. This can be achieved by the ability to observe features which are decidedly distinguishable from features in nearby regions. It has to be faster and provide equal or better accuracy than existing methods. A few candidates for such features are the diameter of neuron bodies, neuronal densities or neuronal organization (for example, in columnar arrangements), and the presence or absence of specific elements. Two sets of parameters determine the ability to identify these structures: resolution and FOV. A high SNR is also required. Additional image properties such as contrast and brightness can be adjusted a posteriori and are usually application-specific.

Resolution

There are several brain elements at the micrometer scale, some of which could serve as distinguishing features for region identification: human soma vary from 10\(\mu\)m to 25\(\mu\)m depending on their location in the brain [60]; capillaries that vascularize the brain are 5\(\mu\)m to 10\(\mu\)m wide [61]; neuroglia are of the order of 6\(\mu\)m to 10\(\mu\)m [62]; myelinated axons have approximately 2\(\mu\)m diameter [63]. These structures must be resolvable in order to be quantified, which sets the required resolution. Our design goal was a resolution equal to or better than 8\(\mu\)m, a range which encompasses several of the key structures listed above.

FOV

In the cerebral cortex, neurons are grouped into layers of different density. In other gray matter regions a high density of neuron bodies is expected, while in white matter regions, composed primarily of myelin and myelinated organelles, there should be few of no neuron bodies. Thus, the FOV should encompass at least several neuron bodies for identification of the region of interest. For example, neuron bodies in the STN and in the GP, regions commonly targeted by DBS procedures, have an average diameter of 15\(\mu\)m to 20\(\mu\)m. An estimate of soma density could be obtained from the observation of tens to a few hundred of these neurons in a volume. That translates to an average of at least 10 to 15 neurons in all directions (x, y and z), and sets a minimum FOV of approximately 200\(\mu\)m along each dimension.

3.1.3 Acquisition & Processing Requirements

Current methods for neurosurgical navigation take several hours, mainly due to repeated electrophysiology measurements for improved reliability. A method providing equal or better information in a shorter
time would make the procedure more comfortable for the patient and less taxing on the neurosurgeon. A short signal acquisition time is also important because structures in the brain of live subjects exhibit constant motion due to fluid drift, blood flow in vessels and the subject’s breathing. From initial estimates of our acquisition and processing capabilities, our goal was to acquire and process C-scans in less than a couple of minutes, with an acquisition time of less than 10 seconds. Displaying the processed tomogram could also be automated, although we believe it would be advantageous to the neurosurgeon to be able to manipulate and investigate the tomogram (i.e., rotate it, slice it along different axes, adjust the contrast). In a more advanced system, the classification of features such as neuronal population and organization patterns could be automated through machine vision algorithms, possibly with inferred identification of the region being inspected.

3.2 Optical System Design

This section discusses the design of the optical system, from its individual components to complete integration. The integrated system is shown in Fig. 3.1. Its parts are described in detail in the following sections.
3.2.1 Endoscope Design

In order to satisfy the cannula form factor requirement, we chose to use GRIN lenses in our endoscope design. There are some advantages to that choice:

- there are options of GRIN lenses with diameters less than 1 mm.
- GRIN lenses can be manufactured to custom focal lengths.
- to a certain extent, GRIN lenses can be arbitrarily long.
- the edges of GRIN lenses connect flatly with glass spacers and windows, leaving no air gaps to create spurious reflections or be filled with leaked epoxy in the assembly process.
- the edges of a GRIN lens can be angle-polished to reduce reflections.

We start from the resolution and DOF requirements: a resolution $\leq 8\,\mu\text{m}$, and a DOF $\geq 200\,\mu\text{m}$. The longest wavelength in our range, 1345 nm, yields the worst resolution. Therefore, taking $r_{\text{Airy}} \leq 8\,\mu\text{m}$ at 1345 nm in Eq. 2.4 we obtain $\text{NA} \geq 0.1026$. The shortest wavelength in the range, 1260 nm, yields the shortest DOF. Using $\text{NA} = 0.1026$ at a wavelength of 1260 nm in Eq. 2.11 we find the lower bound for the DOF to be 76 $\mu\text{m}$, which does not satisfy the axial imaging requirement. The trade-off requires the a reduction in NA for a longer DOF.

Imposing a minimum DOF of 200 $\mu\text{m}$ in Eq. 2.11 we obtain $\text{NA} \leq 0.0633$, which yields a resolution $\geq 12.96\,\mu\text{m}$ in air. Assuming a refractive index of 1.35, those metrics convert to a 9.6 $\mu\text{m}$ resolution and a 148 $\mu\text{m}$ DOF in tissue. We use those values as guidelines for the design. It is not possible to obtain a higher NA for a GRIN lens than the one determined by its refractive index gradient, but the NA can be reduced. One way to do so is to add an opaque ring aperture to the front edge of the endoscope. Another way is to add a glass spacer in front of the GRIN lens, which effectively reduces the span of angles of light from the sample that can couple onto the endoscope. However, the latter restricts the range of possible magnification levels, as it imposes a minimum object distance from the lens.

The OCT axial resolution is calculated from Eq. 1.5. The usable wavelength range [1260 nm, 1345 nm] yields $\nu \Delta z = 9.97\,\mu\text{m}$. In tissue with 1.35 refractive index, it is further improved to 7.38 $\mu\text{m}$. The maximum Nyquist-resolvable depth $\nu \Delta z_{\text{max}}$, calculated from Eq. 1.6 for a wavelength step $\delta \lambda$ of 0.042 nm, is approximately 1 cm, reduced to 7.5 mm in tissue. Both parameters satisfy the imaging requirements, and there is flexibility to increase $\delta \lambda$ for lower triggering rates.

The next aspect to be analyzed is the endoscope working distance. Having a working distance of at least DOF/2 from the edge of the endoscope assures that the entire range of the DOF is being used
Table 3.1: Summary of refractive index values in the GRIN lens

<table>
<thead>
<tr>
<th>λ [nm]</th>
<th>r [mm]</th>
<th>0.25</th>
<th>0.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1260</td>
<td>1.5166</td>
<td>1.5134</td>
<td>1.5041</td>
</tr>
<tr>
<td>1345</td>
<td>1.5162</td>
<td>1.5131</td>
<td>1.5038</td>
</tr>
</tbody>
</table>

to image the sample, and not the glass. Moreover, the working distance should be extended by an
additional 20 to 40 µm to avoid corruption of the image by a residual DC component and low-frequency
auto-correlation artifacts. Thus, we consider a working distance of 150 µm.

In order to address all factors simultaneously we modeled the endoscope in the ray-tracing software
OpticStudio (Zemax LLC.; WA, USA). The software was also used to model the other optical components
in the illumination and imaging paths.

Ray-tracing simulations

The GRIN lens manufacturer GRINTech (Jena, Germany) provided modeling parameters for the refrac-
tive index gradient and the dispersion of the glass in their lenses. GRINTech also supplies custom glass
spacers. Our endoscope was modeled from a 1 mm-diameter GRIN rod with NA 0.2 (GT-LFRL-100),
with radial gradient profile

\[ n_{\text{ref}}(r, \lambda_{\text{ref}}) = 1.524 - 0.05r^2 + 0.00149r^4, \] (3.1)

for \( \lambda_{\text{ref}} = 0.67 \) µm. The dispersive refractive index is determined with a method based on the Sellmeier
formula, with two auxiliary quantities \( K(r) \) and \( L(r) \) calculated at each radial position by

\[ K(r) = 0.7989765 - 1.6362388n_{\text{ref}}(r) + 1.1367279n_{\text{ref}}^2(r) - 0.2761276n_{\text{ref}}^3(r), \] (3.2)

\[ L(r) = 0.0471047 + 0.2227973n_{\text{ref}}(r) - 0.1407245n_{\text{ref}}^2(r) - 0.0168879n_{\text{ref}}^3(r). \] (3.3)

The values obtained from Eqs. 3.2 and 3.3 are then used to calculate the radial refractive index at each
wavelength \( \lambda \) as

\[ n(\lambda, r) = \sqrt{n_{\text{ref}}^2(r) + \frac{K(r)(\lambda^2 - \lambda_{\text{ref}}^2)}{(\lambda^2 - L(r))}}. \] (3.4)

Table 3.1 summarizes the refractive index values in the GRIN lens over our wavelength range, and
Fig. 3.2 shows the radial gradient profile for \( \lambda = 1310 \) nm.

The initial design idea was a GRIN endoscope head connected to a flexible imaging fiber bundle.
Thus, the endoscope was designed to project an image at a fixed distance, with a glass spacer on each side of the imaging GRIN lens. A magnification greater than unity was also required, as the “pixels” of the fiber bundle were approximately 8 µm wide and demagnification would further deteriorate the transverse resolution.

Setting the length of the GRIN lens and of the glass spacers as variables, we defined a merit function which included the NA, FOV, working distance, magnification, chromatic focal shift and diffraction-limited focus. The parameters calculated above for the working distance and NA were inserted in the merit function. The variables were optimized via routines available in OpticStudio (“Optimize!” and “Hammer Current”).

The final endoscope design is shown in Fig. 3.3 with a GRIN lens of length 3.18 mm and a glass spacer 7.1 mm long. The effective focal length of the endoscope is 3.56 mm and it provides a magnification of -1.5x for a working distance of 150 µm. Fig. 3.4 shows plots of the PSF, MTF and chromatic focal shift for the endoscope, with an Airy radius of approximately 8.97 µm after adjusting for magnification, an MTF with 20% contrast at 61 cycles/mm and 3.6 µm chromatic focal shift over the wavelength range.

### 3.2.2 Bulk Optical Components

For the other optical components in the system, collimation, beam diameter and image magnification were analyzed. Physical constraints were also considered (e.g., the size of the beamsplitter cage). The lenses were chosen to achieve three conditions:
Figure 3.3: Ray-tracing simulation of the endoscope showing image formation of two points from the sample. Rod diameter: 1 mm. The glass spacer at the distal end is 7.1 mm long and the imaging GRIN is 3.18 mm long. The relay GRIN lens shown here has 0.5 pitch and is approximately 11 mm long; our endoscope uses a relay which is 5 times longer.

1. Light reaching the sample is collimated to maximize specular backscattering.

2. The diameter of the beam reaching the sample is maximized for a wider FOV.

3. The magnification from the endoscope to the camera assures that the PSF encompasses at least a few pixels in the sensor.

We refer to Fig. 3.1 for an overview of the components. The detailed mechanism and implementation are presented in Chapter 4. Different combinations of commercially-available achromatic doublets were tried and their optimal focal lengths and placement positions were determined for both the illumination and imaging paths, shown separately in Fig. 3.5. The chosen focal lengths were 75 mm for the collimation lens $L_{\text{coll}}$, the injection lens $L_{\text{inj}}$, and the objective lens $L_{\text{obj}}$, and 1000 mm for the camera lens $L_{\text{cam}}$. Table 3.2 shows the simulated results for the placement of lenses. Simulations show the system is insensitive to the separation between $L_{\text{coll}}$ and $L_{\text{inj}}$ and to the separation between $L_{\text{obj}}$ and $L_{\text{cam}}$.

The collimated illumination spot size at the sample was estimated to be 560 µm from ray-tracing, shown in Fig. 3.6. Table 3.3 shows the diameter of the beam along the illumination path, simulated with Gaussian beam propagation starting from a standard single-mode fiber. The diameter at the sample is 645.76 µm, similar to the ray-tracing value.
Figure 3.4: (a) Cross-section of the simulated PSF from the modeled endoscope. The first zero from the center is the Airy radius, with approximately 13.45 µm after a magnification of 1.5, equivalent to an Airy radius of 8.97 µm in object space. (b) Simulated MTF for the same components. The MTF has 20% contrast at 61 cycles/mm (equivalent to 8.2 µm). (c) Chromatic focal shift in the endoscope over our wavelength range.
Figure 3.5: (a) Simulation of the illumination path from fiber to sample with 75 mm lenses and the designed endoscope including the relay GRIN, delivering collimated illumination to the sample. (b) Simulation of the imaging path from the sample to the camera, with 75 mm and 1000 mm focal length lenses. Simulations obtained by sequential paraxial ray-tracing in Zemax OpticStudio. Calculated total magnification: 19.84x. Separation distances are listed in Table 3.2.

Table 3.2: Simulated results for the placement of optics

<table>
<thead>
<tr>
<th>Components</th>
<th>Distance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fiber to $L_{coll}$</td>
<td>65.00 mm</td>
</tr>
<tr>
<td>$L_{inj}$ to endoscope</td>
<td>84.92 mm</td>
</tr>
<tr>
<td>Endoscope to $L_{obj}$</td>
<td>73.04 mm</td>
</tr>
<tr>
<td>$L_{cam}$ to CCD</td>
<td>957.90 mm</td>
</tr>
</tbody>
</table>

Table 3.3: Beam diameter simulated with Gaussian beam propagation at different positions of the illumination path

<table>
<thead>
<tr>
<th>Fiber output</th>
<th>After $L_{coll}$</th>
<th>After endoscope</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.6 µm</td>
<td>13.06 mm</td>
<td>645.76 µm</td>
</tr>
</tbody>
</table>
Figure 3.6: Simulated illumination spot size at the sample for the different wavelengths in the range. The diameter is approximately 560 µm, close to the value in Table 3.3.

Table 3.4: Summary of simulated results for the complete optical system

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total magnification</td>
<td>19.84x</td>
</tr>
<tr>
<td>NA</td>
<td>0.102</td>
</tr>
<tr>
<td>f#</td>
<td>8.35</td>
</tr>
<tr>
<td>Transverse resolution</td>
<td>8.97 µm (in air)</td>
</tr>
<tr>
<td>FOV</td>
<td>500 µm (circular)</td>
</tr>
<tr>
<td>DOF</td>
<td>150 µm</td>
</tr>
<tr>
<td>Working distance</td>
<td>150 µm</td>
</tr>
<tr>
<td>Strehl ratio</td>
<td>0.993</td>
</tr>
<tr>
<td>Chromatic focal shift (endoscope only)</td>
<td>3.6 µm</td>
</tr>
<tr>
<td>Airy radius on CCD</td>
<td>185 µm</td>
</tr>
</tbody>
</table>

The simulated transverse FOV is shown in Fig. 3.7. The FOV is radially symmetric, so it encompasses a circle of approximate radius 500 µm, not accounting for the size of the CCD sensor. Thus, the size of the incident beam is appropriate for illuminating the FOV. Fig. 3.7 also shows the PSF at the camera sensor after a magnification of 19.84x, with an Airy radius of 185 µm. The Airy radius covers approximately 6 pixels in the sensor. Finally, a simulated image of a 500 µm × 500 µm square grid with 50 µm-spaced dots is shown in Fig. 3.8. Diffraction, magnification, wavelength shifts, the dimensions of the sensor and of the individual pixels were considered for that simulation. OpticStudio calculated a 0.993 Strehl ratio, which is a quality metric for imaging systems that is equal to unity for a perfectly unaberrated system.
Figure 3.7: (a) Simulated FOV of the complete system, showing a cutoff at approximately 250 µm, not accounting for the dimensions of the CCD sensor. (b) Resulting PSF from the entire imaging path on the CCD, with an Airy radius of 185 µm covering approximately 6 camera pixels.

Figure 3.8: Simulated image of a 500 µm × 500 µm square grid. The different wavelengths, size of the detector and pixel pitch were accounted for in the simulation.
3.3 Summary

In this chapter, we presented the design of the endoscopic FF-SS-OCT optical system. A key component is the GRIN lens, which must be specified to meet the resolution and DOF requirements. The FOV is set by the magnification of the system, which can be adjusted through the combination of the GRIN and choice of other lenses in the system. The FOV also depends on the diameter of the GRIN and the dimensions of the sensor. The endoscope GRIN lens has been designed to be compatible with a flexible fiber bundle, although that is not a mandatory requirement and has not been implemented. Ray tracing simulations of the optical system show that it meets the specifications for surgical guidance.
Chapter 4

Implementation

This chapter describes the implementation of the complete proof-of-concept OCT system, starting from the design presented in Chapter 3. We describe the components involved, the assembly process, hardware control for signal acquisition and the OCT-resolving algorithm.

4.1 System Integration

4.1.1 Optical System

Following from the design in Chapter 3, we proceeded to physically implement the proof-of-concept system. GRINTech manufactured the imaging GRIN lens and the glass spacer with the requested lengths, namely 3.18 mm for the GRIN lens and 7.1 mm for the glass spacer. From another manufacturer, GoFoton (NJ, USA), we obtained a 5 cm and a 18 cm relay GRIN lenses designed to work in our wavelength range. Unfortunately, the longer relay GRIN broke during the course of the project. The final endoscope with the shorter relay was approximately 6 cm long. With the aid of custom 3D-printed holders mounted on translation stages, the facets of the GRIN rods and glass spacer were coated with index-matched optical UV-curable epoxy (NOA 76, Norland Products; NJ, USA) and the components were inserted in hypodermic stainless steel tubing (304H18TW, Microgroup Inc.; MA, USA). The measured outer diameter of the tube was 1.5 mm. A custom tube with thinner walls can be requested for a clinical implementation. After testing the image quality of the endoscope, the epoxy was cured by axial UV irradiation.

The system shown in Fig. 4.1 was put together from commercially-available parts. Due to the novelty of this type of OCT as well as the uncertainty regarding the functionality of the designed endoscope,
Figure 4.1: A picture of the system set up in the animal experiment room at the Krembil Research Institute. Circles label the different components. The schematic is presented in Fig. 3.1 Red: $L_{coll}$. Green: $L_{inj}$. Blue: beamsplitter. Purple: endoscopic probe. Yellow: $L_{obj}$. Grey: $L_{cam}$. Orange: InGaAs CCD. Pink: fiber collimator for trans-illumination. The fiber collimator and the two mirrors in the back are not depicted in the diagram of Fig. 3.1.
Figure 4.2: Detailed image of the assembled endoscope attached to the cage system.

A few variations were tried before reaching this final setup. With the same endoscope design a fiber-bundle-based iteration was attempted. Under ideal conditions, we measured the roundtrip loss from the bundle alone to be -18 dB. Moreover, cross-core coupling worsened the resolution by an order of magnitude. We were unable to obtain OCT images with the flexible endoscope.

In order to facilitate the alignment of components, a 30 mm cage system was used. All of the commercial optics used are anti-reflection (AR)-coated and achromatic over our wavelength range. All of the component models in this section are from Thorlabs Inc. (NJ, USA) unless otherwise noted.

The assembled endoscope was attached to a 3D-printed plastic adapter and inserted in a ferrule adapter (SM1LCM). The ferrule adapter was connected to a cage-system compatible kinematic mount (KC1-S), shown in Figs. 4.2 and 4.3.

AC254-75-C and AC508-1000-C lenses were used for $L_{\text{coll}}$, $L_{\text{inj}}$, $L_{\text{obj}}$ and $L_{\text{cam}}$ in Fig. 3.1. Lenses were positioned according to the values in Table 3.2.

A standard FC/PC SMF-28 fiber is connected to the cage system via an adapter (SM1FC) placed at the focal length of $L_{\text{coll}}$ for collimation. The collimated $\frac{1}{\pi}$ diameter $d_{\text{coll}}$ is estimated from

$$d_{\text{coll}} = 4\lambda \frac{f_{\text{coll}}}{\pi(MFD)}, \quad (4.1)$$
where $f_{\text{coll}}$ is the focal length of $L_{\text{coll}}$, and MFD is the Mode-Field diameter for the fiber at wavelength $\lambda$, given by the manufacturer as $9.2 \, \mu m$ for $\lambda = 1310 \, nm$. Inserting those yields a $13.6 \, mm$ collimated beam diameter after $L_{\text{coll}}$, matching the simulations, and was verified experimentally. The injection lens $L_{\text{inj}}$ forms a $4f$ system with the endoscope through the beamsplitter (BS015), and the illumination beam diameter at the sample $d_{\text{sample}}$ is given by

$$d_{\text{sample}} = d_{\text{coll}} \frac{f_{\text{GRIN}}}{f_{\text{inj}}}.$$ 

From OpticStudio we know the focal length of the imaging GRIN lens $f_{\text{GRIN}}$ is $3.56 \, mm$, thus $d_{\text{sample}} = 645 \, \mu m$ as previously described. The endoscope forms an image of the sample at approximately $7.75 \, mm$ away from the endoscope, reflected by the beamsplitter towards $L_{\text{obj}}$. The position of $L_{\text{obj}}$ is adjusted via a translation stage (SM1Z) for the endoscope image to be in the focal plane of the lens. $L_{\text{obj}}$ collimates the rays which are focused by the camera lens $L_{\text{cam}}$ and form an image at the camera. The total magnification $M_{\text{total}}$ of the system is given by $M_{\text{total}} = M_{\text{GRIN}} \left( \frac{f_{\text{cam}}}{f_{\text{obj}}} \right)$, where $M_{\text{GRIN}}$ is the magnification provided by the endoscope, equal to 1.5 by design, and $f_{\text{cam}}$, $f_{\text{obj}}$ are the focal lengths of $L_{\text{cam}}$ and $L_{\text{obj}}$, respectively. Thus, $M_{\text{total}}$ is equal to 20x as simulated and each $30 \, \mu m$ pixel of the camera is equivalent to approximately $1.5 \, \mu m$ in the sample. The maximum FOV is $480 \, \mu m \times 380 \, \mu m$ at this magnification level due to cropping at the camera.

It is relevant to note that the magnification and beam diameter depend mostly on the ratios of the focal lengths of the lenses. The physical separation imposed by the beamsplitter cage restricted the choice of $L_{\text{inj}}$, $L_{\text{obj}}$, which in turn required $L_{\text{cam}}$ to have a long focal length for the required magnification to be achieved. A custom packaged system could have the same parameters with a significantly smaller
footprint as long as the focal length ratios were maintained. Fig. 4.4 shows a top view of the system, in which the physical constraints described above can be seen.

This system also allows for dynamic focusing as illustrated in Fig. 3.1. Translating \( L_{\text{obj}} \) focuses at different depths of the sample. Thus, the DOF can be extended by several times. With a precise computer-controlled translation stage, acquisitions could be carried out at different focal planes separated by approximately 100 \( \mu \text{m} \) and then stitched together through standard algorithms. Note that the translated objective distance and the imaged sample depth are related non-linearly through the lens equation.

### 4.1.2 Light Sources

For the SS-OCT, we used the Keysight 81600B-130 (Keysight Technologies; CA, USA) tunable laser. The laser is a telecom tunable laser and provides a wavelength sweep range from 1260 nm to 1378 nm, -1 dBm maximum output optical power during sweeps, and a maximum sweep speed of 80 nm s\(^{-1}\). The broadband infrared source was a S9FC1132P Booster Optical Amplifier (BOA) (Thorlabs Inc.; NJ, USA) with a maximum output power of 15 dBm, center wavelength 1305 nm and a FWHM bandwidth of 87
nm. The BOA is designed to amplify an optical signal, but when used without an input it functions as a broadband source. Having a broadband source was imperative when adjusting the focus or otherwise trying to determine the region to be imaged in ex vivo samples, since images obtained with the laser exhibited pervasive interference effects which prevented the recognition of virtually any features in the object. Optical alignment was carried out with a 625nm visual fault detector and then actively refined with the BOA and the camera.

4.1.3 Camera

For the FF imaging, we used an InGaAs charge-coupled device (CCD) camera with a single-stage thermoelectric cooler (Xeva 1.7-320 T1, Xenics nv; Leuven, Belgium). Its sensor has 320 × 256 pixels, with a 30µm pixel pitch. It is T1-cooled for reducing dark current and noise and captures full frames at a maximum of 350 Hz when using a CameraLink framegrabber, or 100 Hz over USB. That is equivalent to a maximum of 28.67 megapixels per second over CameraLink. While the camera is specified to operate in the 950 nm to 1700 nm range, it displayed a large sensitivity oscillation at specific wavelengths within the 1345 nm to 1378 nm. This defect limited our usable wavelength range from 118 nm to 85 nm, proportionally reducing the achievable axial resolution by 26%.

4.2 Acquisition Control

Integrating the camera was the most challenging aspect for control. The standard software development kit (SDK) for the camera control was slow for processing, since images were being saved to disk. With the help of Fu Der Chen, the C++ code of the SDK was ported to MEX (Matlab executable). Control of the camera and the laser was done from within Matlab and all acquired data was kept in RAM, transferred directly to the Matlab workspace. Signal processing algorithms and visualization were also done in Matlab. Camera acquisition worked via USB and via CameraLink Framegrabber transport. The control code provides the following options for sweeping:

- Starting and ending wavelengths, $\lambda_{\text{start}}$ and $\lambda_{\text{end}}$. These were always set to the maximum usable range, namely 1260 to 1345 nm.

- Approximate wavelength step $\delta \lambda$ for triggering frame acquisition at the camera. An optimal choice for $\delta \lambda$ was determined to be 0.042 nm, which yields approximately 2020 frames per sweep.

- Dimensions of the camera acquisition window. Reducing the acquisition window was necessary for faster acquisition rate.
• Sweep speed, from 0.5, 2, 5, 10, 20, 40 or 80 nm/s. The maximum sweep speed we could use due to the frame rate limitation of the camera was 10 nm/s, and each sweep took 8.5 seconds.

• Integration time. The maximum integration time compatible with the aforementioned parameters was 2800 µs. That maximum value was used for most tissue experiments due to weak backscattering. A lower integration time was used for more strongly reflecting samples.

• Number of sequential sweeps, for averaging or comparison. Limited by the available RAM in the machine. Each additional sweep requires approximately 350 MB of RAM for 320 × 256 pixel windows. After sequential sweeps, a 4-dimensional array of data and a 2D array of measured wavelength data points were loaded onto the Matlab workspace for processing.

The camera frame rate scaled nonlinearly with windowing. The window was set to 192 × 124 pixels (29% of full frame). With a 2800 µs integration time, the maximum frame rate of the camera over USB was 250 Hz. A framegrabber would triple that frame rate. At 10 nm/s and 420 pm steps, we triggered the camera at approximately 238 Hz. The acquisition speed was approximately 2800 A-scans per second.

A shorter integration time would have been preferred to avoid motion artifacts. However, a shorter integration time leads to lower observed optical power and lower SNR. The SNR limitation was significant in the tissue experiments and so the long integration time was kept.

The 420 pm step was carefully chosen to control aliased artifacts. As the proximal edge of the GRIN endoscope was not AR-coated, reflections from that surface introduced noise over a long range. The originating reflection was beyond our Nyquist range and thus the blur was aliased. By adjusting δλ we were able to shift that aliased artifact out of our usable image range. This choice of wavelength step can be further investigated and eventually increased, leading to faster acquisition and shorter processing time due to fewer data points.

We chose values for the 14-bit analog-to-digital converter in the camera to capture small variations in the signal (i.e., finer intensity resolution) without cropping low intensity values.

4.3 OCT-Resolving Algorithm

The acquired signal was processed with Matlab code implemented purely with vector-based operations. The code is included in Appendix A. The sequence of processing steps is illustrated in Fig. 4.5. Up to three sequential sweeps can be acquired, processed and displayed in less than a minute.
Figure 4.5: Flowchart for the OCT-resolving algorithm.

Background removal

The background, which includes the illumination ring artifact and the line pattern of the sensor, is removed. The calibration function packaged with the camera was incompatible with triggered acquisition in the C++-based SDK.

Linear wavenumber interpolation

A linear wavenumber space is required for the OCT algorithm. The laser triggers the camera at approximately linear wavelength steps, but not at linear wavenumber steps ($\frac{2\pi}{\lambda}$). Also, triggered data points do not have the exact same separation, but the laser provides the logged wavelengths. The data is interpolated in Matlab. Interpolation can be avoided either by manually setting the individual wavelengths to be swept (pre-calculated to yield a linearly-spaced $k$-space), or by introducing a Mach-Zender interferometer-based triggering clock. The former approach is very slow, leading to sweeps that are longer than 20 minutes. The latter approach is standard in current OCT systems [64][65][66]. As interpolation was not the bottleneck for speed in our system, we did not pursue that approach.

Optional averaging

Multiple sweeps were averaged to enhance the SNR. This step was optional. Sometimes there were individual outliers in sequential sweeps that differed significantly from the others, probably due to abrupt motion; those were discarded when averaging.
**Pixel-level low-frequency removal**

Auto-correlation artifacts in OCT arise from interference from multiple scattering within the sample, separated by small distances. Most of them resolve to the first spatial bin or first few bins. In order to reduce these, the mean over all wavelengths for each pixel is subtracted. It is also possible to compensate for the low-frequency wavelength-dependent intensity variation arising from multiple components in the system. We observed negligible improvement in the overall result after compensating for those low-frequency variations, which is likely due to that variation being resolved to the first few spatial bins and lumped with the other artifacts.

**Windowing**

An important part of the algorithm is applying a window function. The mathematics behind the Fast Fourier Transform (FFT) have an underlying assumption that the signal being transformed is infinitely-periodic and abrupt discontinuities at the ends translate into spurious high-frequency components. Any data array has “by default” a rectangular window applied to it. The width of the central lobe of the Fourier Transform (FT) determines the frequency resolution, similarly to the Airy disk. A rectangular window yields best resolution. However, its high-amplitude side lobes introduce a distribution of frequency information over the signal, known as spectral leakage. Other window functions yield reduced spectral leakage by trading off resolution. There are several well-known windows and choosing one depends on the application [67]. It is commonly accepted that the Hann window is appropriate for a large number of scenarios, as it provides a 19 dB reduction in side lobe amplitude with respect to the rectangular window, but at the cost of 63% worse axial resolution. We employed the Hann window in our algorithm, implemented in Matlab as the function `hann`.

**Zero-padding**

Acquiring data over a wider wavelength range leads to higher frequency resolution, similar to a higher NA in optics. In FF-SS-OCT the range is limited by both the laser source and the camera. Zero-padding is a processing technique that simulates a longer acquisition range by appending zeros at the extremes of the signal. Although it does not improve the *de facto* ability to distinguish closely-spaced frequencies, it reduces the size of the frequency bins resulting from the FT. Effectively, it interpolates the data over smaller bins, which has two benefits: a smoother-looking resulting curve, and reduced scalloping loss. Scalloping loss happens when a resolvable frequency falls exactly at the midpoint between two frequency bins, and as such is split (i.e., scalloped) between those two bins. Smaller frequency bins make it is less
likely that such previously-split frequencies will fall at a midpoint, and thus those frequencies are more accurately represented. The length of the array after zero-padding is usually made a power of 2 in order to maximize FFT efficiency.

**FFT**

The FFT is applied along the wavenumber $k$ dimension, independently for each pixel independently. Continuous lines in the resolved image reflect identical frequency components in the raw data of independent pixels.

**Axis adjustments**

From Eq. [1.1] we apply the appropriate factor correction to obtain the conversion from signal frequency to depth ($z$, in microns). We also discard half of the resulting array, which is redundant due to the complex conjugate ambiguity: since the acquired data in a non-coherent scheme is purely real, its Fourier Transform will have conjugate components in the positive and negative frequencies. There are coherent detection techniques to suppress the complex conjugate ambiguity [68].

**Post-processing**

The literature shows different types of post-processing algorithms applied to brain OCT images [33, 69]. We applied 2D Gaussian filtering to our images in order to reduce the granularity resulting from the low SNR. All OCT images presented in this thesis have been filtered.

### 4.4 Displaying the Tomogram

The final step is the adjustment of contrast and brightness for displaying the data. A common way of doing so is $\gamma$-correction, with

$$I_{\text{corrected}} = \alpha (I_{\text{original}})^\gamma.$$  

(4.2)

However, that requires knowledge of $\alpha$ and $\gamma$, which could vary between images. We used a free software plugin for Matlab, IMAGINE 2.0 [70], which offers interactive contrast adjustment. A snapshot of the software being used is shown in Fig. [4.6]. Other software packages offer similar features, especially DICOM-based packages for medical image visualization. An alternative way to present the C-scan is to generate a video from depth-frames. It was suggested by our collaborators that the ability to rotate, slice or adjust the contrast of the tomogram may be preferable for clinical purposes.
Figure 4.6: A screenshot of the Matlab plugin IMAGINE 2.0 with four sweeps of an OCT C-scan loaded. The software features interactive contrast adjustment for several colormaps, the option to work simultaneously with multiple volumes, parallel line profiling and easy manipulation of 3D data.

4.5 Summary

This chapter explained the physical implementation of the optical system, including assembly of the endoscope, and the integration with light sources and the camera. It also addressed acquisition control and the algorithm for processing OCT signals. Our software design enabled automatic sequential sweeps entirely in RAM, then loaded directly into the Matlab workspace for processing with the fully vector-based OCT-resolving algorithm. This expedited the data acquisition and processing time to about 40 s per C-scan. Although the implemented OCT system (Fig. 4.1) consists of cage parts and cannot yet be directly applied to a clinical setting, it is suitable for a first investigations of brain imaging using OCT and the designed GRIN lens. The GRIN lens would function as the endoscope tip in a clinically deployed system.
Chapter 5

System Characterization

This chapter addresses the characterization of the OCT system with regards to optical power, sensitivity, transverse and axial resolution, DOF and FOV.

5.1 Optical Power and Sensitivity

5.1.1 Power

The optical loss in the illumination path was measured using the InGaAs camera to be -5 dB. 3 dB of the optical loss was due to the beamsplitter. Applying an AR-coating to the proximal end of the endoscope would reduce the loss by 0.18 dB. Since the laser input power is -1 dBm throughout the experiments, the expected power delivered to the sample is about -6 dBm or 248 µW.

5.1.2 Sensitivity

We measured a maximum per-pixel sensitivity of 58 dB per sweep, in air. Sensitivity was taken to be

\[ 20 \log_{10} \left( \frac{A_{\text{max}}}{\sigma_{\text{noise}}} \right), \]

where \( A_{\text{max}} \) is the resolved peak amplitude for a single mirror reflector at the pixel and \( \sigma_{\text{noise}} \) is the standard deviation of the noise floor for the same pixel in the region surrounding the peak. The resolved amplitude for a single reflector 295 µm away from the reference is shown in Fig. 5.1. From approximately \( 1.18 \times 10^4 \) pixels, the minimum measured sensitivity was 38 dB and the average was 47 dB. Higher SNR correlated with points of higher backscattered power from the reflector as the illumination
was not uniform. Since the power is distributed over the window pixels, a correction factor of 40 dB could be added, bringing the sensitivity on par with other OCT systems. Averaging $N$ sequential sweeps is expected to improve the sensitivity by a factor of $N$.

5.2 Transverse Imaging Properties: Resolution and FOV

5.2.1 Transverse Resolution

For adjusting the focus and measuring the transverse resolution, a negative USAF-1951 resolution target (R3L3S1N, Thorlabs Inc.; NJ, USA) was used. From the OpticStudio simulations, the system was expected to resolve a minimum feature size of approximately $8.5 \, \mu m$ in air. This corresponds to Elements 5-6 of the resolution target, which has a line separation of $8.77 \, \mu m$. Results matched this expectation as shown in Fig. 5.2: lines in Element 5-6 are resolvable with contrast between 10 and 20%. Assuming the refractive index of tissue equal to 1.35, that is equivalent to about $6.5 \, \mu m$ resolution in tissue.

All OCT images presented in this thesis were Gaussian-filtered with a kernel of 4.0, which deteriorates the transverse resolution by approximately 12%. After filtering, the smallest resolvable lines are in Element 5-4 (11 $\mu m$ line separation, 8.15 $\mu m$ in tissue). BOA images were not filtered.

5.2.2 FOV

Fig. 5.2 also shows the FOV, which covers 94% or more of the area of the sensor (corners subtracted). Calculations show 1.42 $\mu m$ per pixel in this image, matching the expected total magnification of 20x.
Chapter 5. System Characterization

5.3 Axial Imaging Properties: Resolution and DOF

5.3.1 Axial Resolution

The expected FWHM axial resolution in air was 10 µm; 16 µm with the application of the Hann window, which is equal to approximately 12 µm in tissue.

To quantify the axial resolution and the sensitivity of the imaging system to backscattered light, we prepared a phantom sample of high refractive index glass beads in 1% agarose gel to mimic brain tissue. An OCT-resolved B-scan and a trans-illuminated BOA image of the phantom are shown in Fig. 5.3.
Chapter 5. System Characterization

Figure 5.3: (a) Resolved depth profile along a cross-section through the center of the phantom. Scale bar (red): 25 µm. (b) Trans-illuminated image of the phantom in microscope mode. Scale bar (green): 85 µm. Axes are shown in red for the depth map and in green for the BOA image.

with detailed OCT-resolved frames shown in Fig. 5.4.

The glass beads (Corbeads4-55, Corpuscular Inc.; NY, USA) have a diameter of 55+/-1 µm, and a refractive index of 1.95. The agarose gel has a refractive index of 1.34, and is commonly used to mimic brain tissue [71]. However, typically only a limited portion of one surface of the spherical beads reflected enough light to be resolved, so it was challenging to quantify the axial resolution from two adjacent reflections. The depth profile of a single bead shown in Fig. 5.5 exhibits a FWHM of 19 µm, which is equivalent to 14 µm in tissue after adjusting for the refractive index. That value may be taken as a metric of axial resolution, as it closely matches the expected value of 16 µm. The extracted diameter of 45 to 50 µm from the transverse profile in Fig. 5.5 is also in agreement with the specified diameter of the bead, assuming reflection from a limited portion of its surface.

5.3.2 DOF

OpticStudio estimated the diffraction-limited DOF to be 150 µm. We measured the DOF by stepped translation of the resolution target. The experimental metric for the DOF was chosen as the point at which the dark lines in the pattern are no longer separable with at least 5% contrast. We used Element 5-5 equivalent for that distinction, which represents 7.4 µm in tissue. As seen in Fig. 5.6, the cutoff happens between 150 µm and 250 µm, where lines start to merge. This represents a unilateral translation from the focal plane, and the same blur distance is expect along the opposite direction. Thus, from this metric the DOF is estimated to be 400 µm, better than the estimate obtained from OpticStudio. Moreover, the beads shown in Fig. 5.3 appear to be uniform in size over a range of at least 300 µm.
Figure 5.4: Resolved depth slices from a phantom made of glass beads in agarose gel at depths (a) 40 µm, (b) 50 µm, (c) 90 µm and (d) 177 µm. Scale bar (red): 40 µm. The bottom image shows a maximum intensity projection along the x dimension and the positions from where the slices were taken. Axes are shown in black for the slices and in red for the projection depth map.

Figure 5.5: (a) $n \times$ Depth profile of an OCT-resolved bead showing a FWHM of 19 µm, with the refractive index $n$ of the medium equal to approximately 1.34. (b) Transverse profile of the bead with a measured diameter of 50 µm. (c) The individual bead in the OCT-resolved volume from which the profiles in (a) and (b) were taken.
Chapter 5. System Characterization

Figure 5.6: Resolution target showing Element 5-5, positioned at: (a) best focus, (b) 50 µm away, (c) 150 µm away, (d) 250 µm away, (e) 300 µm away and (f) 400 µm away. Line separation: 9.84 µm; line length: 49 µm. Cutoff for the half-DOF set at 200 µm.

5.4 Summary

The system characterization confirmed the estimated parameters in the design. The one-way optical loss was measured to be -5 dBm, and the maximum per-pixel sensitivity 58 dB per sweep in air. The measured transverse resolution is 6.5 µm in tissue with a maximum FOV of 450 µm × 360 µm. The axial resolution was determined to be 14 µm in tissue with a DOF of approximately 400 µm. Extending the DOF of OCT is a topic of substantial interest in the field and has been demonstrated with the use of micro-optic axicons and phase-masks [72, 73]. The long DOF observed here is unique given the simplicity of the probe and validates the design strategy. Other imaging requirements were almost entirely satisfied, with the exception of axial resolution which could not be more precisely determined. The common-path feature of our setup lead to resolvable tomographies even in the presence of vibrations due to a relatively unstable setup (i.e., mounted on long posts and placed on a non-floating table). These results confirm the viability of a GRIN-based probe for FF-SS-OCT and suggest the technology can be employed for the guidance of cannula-based procedures.
Chapter 6

**Ex Vivo and In Vivo Experiments**

This chapter presents the results from experiments on brain tissue. Images of thin sections (thicknesses of 20-60 µm) of tissue, cm-thick pieces of tissue and from an *in vivo* experiment are included. Human cortical tissue sections were also imaged. Both OCT and BOA images are presented.

### 6.1 General Experimental Procedure

All data acquisition, including the characterization of the system, followed the process described here. The BOA is first set as the light source and the USAF-1951 resolution target is positioned for imaging. Using the software package supplied with the camera (Xeneth Advanced 64-bit, Xenics nv; Leuven, Belgium), the system is optically aligned and the objective lens is translated for focusing of the smallest resolvable features in the resolution target. The resolution target is replaced with the sample and the region of interest is located. For some samples, custom 3D-printed holders mounted on translation stages were used. Pictures are taken for reference. Depending on the sample, trans-illuminated images are also acquired for comparison, with a pre-aligned fiber collimator (PAFA-X-4-C, Thorlabs Inc.; NJ, USA) positioned behind the sample. Once the position in the sample is defined, the laser is set as the laser source at its maximum power, -1 dBm. The histogram and acquisition window in the software are configured to previously-determined settings. A test sweep is executed to determine if the pixels saturate. The integration time is adjusted to maximize acquired intensity without saturation. Images obtained with the laser are dominated by speckle and interference effects and it is practically impossible to obtain information from them prior to OCT processing; thus, snapshots were rarely taken at this stage. We switch to Matlab (R2017a, The MathWorks Inc.; MA, USA), configure the sweep parameters and run the control script.
6.2 Rat Brain Tissue

Imaging experiments of rat brain tissue, both *in vivo* and *ex vivo*, were designed and executed. All tissue samples in these studies were obtained from animals euthanized following ethical protocols and carried out by Dr. Yann-Suhan Senova and Dr. Anton Fomenko. This project was listed under the Animal Utilization Protocol # 4987.4, experiment 2. The animals were sacrificed by decapitation under deep anesthesia. The brain was excised from the skull immediately after euthanasia. In some cases, the brains were frozen directly, placed on aluminum foil on top of dry ice; in others, they were cut in large pieces before freezing. The thin sections of brain tissue were used to verify the transverse imaging capability of the OCT, and the thicker tissue samples enabled testing of the axial imaging capabilities.

6.2.1 Tissue Sections

For the section-on-slide experiments, the brain was sectioned according to standard histology preparation procedures, embedded in Optimal Cutting Temperature and cut in a cryomicrotome. Sections were 20 to 60 micrometers thick depending on the experiment. The sections were then placed over standard microscope slides and dried while exposed to air. Some of the sections were Nissl-stained for histological analysis under a standard optical microscope. For imaging, the slides were inserted into a translational target holder (XYFM1, Thorlabs Inc.; NJ, USA) mounted on an axial translation stage (PT1, Thorlabs). The system was aligned and focused. As the sections were relatively thin, there were strong reflections from the glass slide on which they were mounted. To mitigate that, double distilled water was added as an interface between the endoscope and the tissue. We imaged sections that were pre-stained and freshly-dried (without a coverslip).

**Stained tissue**

A simple experiment was performed to verify the system was capable of displaying features in standard histology sections. A Nissl-stained section of rat brain cortex on a glass slide was imaged with a standard bench-top optical microscope. The same slide was imaged with our system, with BOA trans-illumination. Fig. 6.1 shows the result of that test, with a pattern of eight neurons recognizable in both images. The tissue section was approximately 20$\mu$m thick, covered with resin and placed between two glass slides. The strong reflection from the coverslip-resin interface, co-located with the tissue, overwhelmed the tissue signal and prevented resolution via OCT.
Chapter 6. Ex Vivo and In Vivo Experiments

Figure 6.1: A region of a Nissl-stained rat brain cortex section imaged using (a) our system, illuminated from behind the sample with a broadband source and (b) a bench-top optical microscope with a 50x-magnification objective. Scale bars: 50 µm.

Freshly-dried sections

From a brain which had been previously excised for another experiment, 60 µm sections were prepared by Dr. Elise Gondard. The sections were placed on a glass slide and dried exposed to air. For imaging, the endoscope was placed in direct contact with the tissue (no glass coverslip), with the addition of distilled water as an interface. With the expectation of observing higher contrast in the features, we chose neuronal bands in the hippocampus for imaging. The imaged location in Fig. 6.2 was in the CA1 region of the hippocampus.

The OCT-resolved image has strong qualitative correlation with the BOA image, but the features are clearer and more distinguishable in the OCT frame. That suggests the phase contrast resolved by OCT yields higher-quality images of fresh tissue. For the section shown in Fig. 6.3, the dentate gyrus was targeted for imaging.

The OCT-resolved frames in the figure show a distribution of features at sequential depths 15 µm apart, which appear to be a combination of features observed exclusively in the trans-illuminated and epi-illuminated BOA images.

The sections were stained with cresyl violet after imaging. The goal was to create a reference of tissue features for OCT, for comparison with histology-stained sections. However, the endoscope damaged the tissue in imaged locations, so it was not possible to compare the images after staining. This experiment should be reproduced on a floating optical table, eliminating the need for contact between the tissue and the endoscope, which deforms and damages the tissue. The resolved thickness was around 50 µm per section after dividing by the refractive index of 1.35, as shown in Fig. 6.2. The thickness is slightly less than the 50 µm of the preparation, which can be explained by deformation caused by the endoscope pressed against the section. Pressing was necessary for the sample and the
Figure 6.2: Image of a CA1 neuronal band in the hippocampus of a rat brain section (a) via OCT and (b) in microscope mode, epi-illuminated with the BOA. General features are strongly correlated. Scale bar (red): 50 µm. (c) Pixel depth profile showing the resolved thickness of 70 µm for the section (approximately 50 µm when correcting for refractive index.)
Figure 6.3: Comparison of a neuronal band of the hippocampus, near the dentate gyrus, of a rat brain section, imaged by OCT and with the BOA. The OCT-resolved frames in (a), (b) and (c) are taken 15 µm apart. The same region was imaged with the BOA with (d) trans-illumination and (e) epi-illumination. The OCT frames appear to have features which appear exclusively in the epi-illuminated image (yellow arrow) and in the trans-illuminated image (green arrows). Scale bars: 50 µm.
Chapter 6. *Ex Vivo* and *In Vivo* Experiments

59

Figure 6.4: Depth profile for an arbitrary spot in the thalamus of a thawed rat brain (maximum intensity projection along the y dimension). Bottom image obtained from a sweep two minutes after the top image. Auto-correlation and endoscope artifacts appear near zero. The scale is the same in all dimensions.

endoscope to form a coupled mechanical system, reducing vibrations which would otherwise render the interference fringes unresolvable. This was feasible due to the common-path feature of our setup.

Variations may also be due to contraction of the tissue after drying and the uncertainty of the refractive index of the imaged tissue.

6.2.2 *Ex Vivo* Pieces

Although the sections allow for comparison with microscopy, they were too thin to test the axial imaging capability of the OCT system. Therefore, we prepared cubic-centimeter sized pieces of brain tissue for *ex vivo* OCT measurements. The brain pieces were kept frozen and cut while still frozen immediately prior to the experiments, exposing the region of interest for imaging. We then inserted the endoscope in different regions of the tissue. The tissue thawed at room temperature as imaging experiments progressed. Usually the tissue would degrade within 1 hour. A depth-resolved image from an undetermined location in the thalamus of a thawed rat brain is shown in Fig. 6.4.

Specific cellular structures are difficult to delineate, but it is possible to identify distinct regions in
terms of general brightness and density of features. These features may not necessarily be cell bodies, but rather blood vessels or myelinated axons. Given the scale of these distinct regions, it is more likely they are vessels. They may also represent brain areas with a high concentration of cell bodies, whose individual structure was not resolved. Vessels and cell bodies have been reported to appear darker myelinated structures when imaged by OCT [10]. The different setup of an endoscope inserted directly into the tissue and the lower resolution or our system may alter the way the various structures appear in the images. Alternatively, it is possible that the long integration time in our sweeps leads to a mixing of different small scale elements in the image, due to vibration or drift in the sample.

We also carried out imaging experiments on frozen pieces of tissue, which were attached to the dry-ice cooled copper frame using Optimal Cutting Temperature compound. The purpose of these experiments was to verify the repeatability of OCT sweeps. The setup is shown in Fig. 6.5. A few results from these tests are shown in Fig. 6.6, which verified the repeatability of several sweeps over a long range.

![Experimental setup for imaging frozen ex vivo pieces of tissue to verify the repeatability of sweeps over long depths.](image)

These images show a certain variability of the smallest features, which could be due to ill-resolved scattering at our resolution levels. Nonetheless, even most small features are reproduced repeatably. Matlab’s Structural Similarity evaluation function (SSIM) applied to sequential sweeps returned a similarity value greater than 90%. Higher intensity levels may be correlated not with specific structures (i.e., cell bodies or axons) but with the local density of structures in general, as transitions through matter of different refractive indices would increase the overall scattering.
Chapter 6. Ex Vivo and In Vivo Experiments

Figure 6.6: OCT-resolved frames from sequential sweeps 20 seconds apart showing a region of the thalamus in a frozen rat brain at depths (a) 280 µm, (b) 485 µm and (c) 670 µm. Scale bar: 50 µm.

6.3 Human Cortical Tissue

We also had the opportunity of experimenting on a section of preserved human cortical tissue, kindly provided by Sara Mahallati and Dr. Taufik Valiante. The use of the tissue was authorized under REB: 08-0617-TE.

These sections were 4 mm x 2 mm x 500 µm, extracted from the temporal cortex, containing cortical layers starting from the pia mater down to white matter. They were kept in a paraformaldehyde solution. For imaging these, the tissue samples were removed from the solution and placed on standard microscope slides. The setup is shown in Fig. 6.7. Figs. 6.8 and 6.9 show the imaging results for the human brain section.

The section was originally 500 µm thick and had been preserved in paraformaldehyde for over 2 years. The sections were transferred to glass slides and imaged by direct contact with the endoscope. A certain amount of pressure was required to stabilize vibrations in the system, which deformed the tissue. Thus, thickness was not maintained, and it was undetermined in the OCT scans. Nonetheless, Fig. 6.9a was resolved from an OCT sweep and exhibits particular features not seen in the BOA-illuminated image of
Chapter 6. Ex Vivo and In Vivo Experiments

Figure 6.7: The setup for imaging human cortical tissue.

Figure 6.8: Images of a preserved human brain section in microscope mode, with (a) epi-illumination and (b) trans-illumination. Scale bar: 50 µm.
Chapter 6. Ex Vivo and In Vivo Experiments

6.3 Ex Vivo Experiment

Figure 6.9: The same region of a preserved human brain section imaged (a) by OCT and (b) with the BOA, trans-illuminated. Scale bar: 50µm.

the same region.

Human and rat tissue images were quite distinct. Rat brain OCT images appeared to have significantly more local variability, while structure contrast in the human brain was greater. The BOA images of human tissue also contained larger and more distinct features compared to the ones we observed in the rat brain sections. Apart from anatomical differences, this may also be due to the difference in preparation of the sections. Human tissue sections were 10x thicker than rat tissue sections and preserved in paraformaldehyde for a long time, which could have altered the structure and composition of different elements.

6.4 In Vivo Experiment

In the in vivo experiment, three different penetrations into the brain were executed independently. Of the three, one did not yield usable data: blood from a lacerated vessel distorted the images, and a distinct straight tract was not observed in the post mortem dissection, given that a nominal penetration of 6mm was expected for that tract. The cut brain exposing the tract is shown in Fig. 6.10. The following sections present data from the other two tracts.

The biggest issue in these experiments was position and depth inaccuracy. Faster acquisition is also necessary for mitigating motion artifacts.
6.4.1 Experimental Procedure

The following description of the in vivo surgical procedure was kindly provided by Dr. Anton Fomenko, who executed the surgery.

**Subject**: Male adult rat, weight 300g. Species: Wistar. White fur. Housed at the KDT animal facility in paired cages, exposed to standard day/night cycles, standard diet.

**Induction**: Rats were injected with urethane (1.25 g/kg, IP). Induction of stage 3 anesthesia was confirmed by observation of regular respiration, absence of spontaneous movement, and absence of blink reflex. After trimming the fur of the cranial region artificial tear lubricant was applied. The animal skull was rigidly pinned into a stereotactic head frame (Kopf instruments). The animal’s body was gently secured to the stereotactic frame using adhesive tape. Maintenance of regular respirations and mucosal membrane colour was ensured to ensure breathing was not compromised.

**Surgery**: The scalp was lightly disinfected with povidone/iodine solution. Lidocaine was applied topically in the midline of the scalp (2%, 0.04cc of lidocaine) as an analgesic. The skin was incised longitudinally by about 4-5 cm at midline with a #15-blade.

Bilateral craniectomies over the frontal and parietal regions were then made, using a Dremel drill bit followed by a Rongeur bone cutter. The meninges were peeled away in sterile fashion. The surface of the brain was irrigated to wash away any debris.

The dura mater at the planned penetration location was punctured. The stereotactic frame was rotated vertically and mounted to a custom rigid frame holder on an xyz-translation stage. A similar arrangement for the frame is shown in Fig. 6.11.

The stage was translated to align the OCT probe to the punctured spot, then advanced until the surface of the endoscope was covered in fluid, verified with the camera and the BOA as light source.
The stage was slowly advanced to several depths, measured by the micrometer on the translation stage (PT1, Thorlabs Inc.; NJ, USA). At least five sequential acquisitions were carried out at each depth. The endoscope was then gently retracted, and the tract was irrigated to control any bleeding. The process procedure was repeated for a different tract.

**Sacrifice:** The animal was sacrificed under deep anesthesia. The induction of stage 3 anesthesia was re-confirmed by observing regular respiration, absence of spontaneous movement, absence of blink reflex, and absence of withdrawal reflex. Transcardiac perfusion with 250 cc of 0.9% saline and 250 cc of 4% paraformaldehyde fixative solution was used to euthanize the animals, followed by decapitation. The brains were removed and sectioned for histological staining. Nissl-stained sections were then compared to the OCT images.

### 6.4.2 First Tract

The first tract was in the right hemisphere. Entry point was expected to be located approximately 3 mm from the center (ML $-3$ mm), but *post mortem* examination indicated it was closer to ML $-5.5$ mm. The tract was expected to be mostly vertical. The weight of the stereotactic frame on the custom holder...
caused an angular deviation of approximately $2^\circ$ measured with a level. Post mortem observation confirms a small angular deviation, but there appears to be a kink in the trajectory. Entry AP position was marked to be Bregma $-1.3$ mm, but tract appears to be closer to Bregma $-0.3$ mm. Moreover, the entire length of the tract was measured to be about 6.8 mm, but the stage was translated by a total of 8.5 mm starting from our reference position. There is likely uncertainty in the reference position, chosen at the point the endoscope was in full contact with fluid, but additional deformation or shifting of the tissue may have taken place. This variability in position happened in all in vivo experiments performed even when great care was taken.

Sequential scans were acquired at nominal depths of 1, 2, 3, 4, 5, 6, 7, 8 and 8.5 mm, carefully measured with the translation stage micrometer.

After slicing the brain for histology, the final endoscope position is estimated to be at ML+5 mm, AP+0.5 mm and DV+7.8 mm. The atlas location is shown in Fig. 6.12 and the tract in Fig. 6.13.

The region was identified as the piriform cortex after examination of the cut brain and of the histology slides. The imaged positions were almost entirely in the cortex, with possible imaging of a part of the external capsule, inferred from a higher reflectance region in the OCT scans. As the OCT endoscope destroys the tissue along its tract, only the final depth can be used for an attempt at histology comparison.

The blood in the tracts is likely due to laceration from the sharp edges of the cut metal tube surrounding the glass endoscope. This can be addressed by the introduction of a rounded/blunt-tip
window to the endoscope. A thinner endoscope would also be favourable. Thinner GRIN lenses are commercially-available, but the resulting FOV would be reduced. Moreover, as these experiments were carried out via the direct insertion of the endoscope into the tissue, they do not represent the surgical scenario. The proper surgical procedure would be to insert the cannula first and then the endoscope through its lumen. Finally, it is possible to acquire OCT images as the endoscope is gradually inserted, identifying at-risk vessels along the path. Fig. 6.14 shows side by side a stained histology section for that last position and an OCT scan of the approximate region and depth.

It also shows a larger scale image of the same spot, with a blood stain signaling the endoscope tract. Since the tissue undergoes demyelination in preparation for staining, it is possible that the features observed in OCT were removed in the process if they were primarily myelin-based. Moreover, the tissue may undergo deformation in the process of staining, due to slicing and dehydration. Thus, finding a match with histology is not a trivial task. A more controlled process for co-localization would be ideal.

Fig. 6.15 shows selected OCT-resolved frames from the first tract data.

The nominal values for depths are unreliable, for the reasons previously explained. Some large features (100 µm or more) are recognizable. These features are likely to be vessels given their dimension.
Figure 6.14: Histology comparison with OCT from the first penetration: (a) Nissl-stained section from the piriform cortex 150µm after the deepest position of the endoscope and (b) an OCT-resolved frame of the same approximate location and depth. Scale bars (red): 50µm. (c) Larger scale image of the piriform cortex showing (with red arrow) residual blood from the endoscope tract. Scale bar (green): 1 mm.
Figure 6.15: Selected frames showing OCT-resolved depth from the first tract, nominally at (a) 1 mm, (b) 3 mm, (c) 5 mm and (d) 7 mm into the brain. Yellow arrows indicate what appear to be large continuous darker structures, possibly vessels. Images cover from 100 $\mu$m to 590 $\mu$m. Scale bar: 50 $\mu$m.

Blood is more absorbing than other types of tissue, which would explain these regular features appearing as darker regions. The same structures appear in sequential sweeps. Overall, the images obtained from the second tract, presented in the next section, seem to contain more distinctive features than these. A possible explanation is that the endoscope in the second tract certainly passed through white matter regions (i.e., with stronger scattering), whereas the endoscope in the first tract was confined to the cortex. Categorization of the regions without a priori knowledge is addressed in Subsection 6.4.4.

6.4.3 Second Tract

We again sought to penetrate the brain vertically, starting at Bregma -2.3, left hemisphere. Here, too, the penetration ended up not being vertical as seen in the post mortem, shown in Fig. 6.16. Moreover, while we moved the translation stage by a total of 5.5 mm starting from the surface of the brain, the entire length of the tract was only 3.7 mm. The total depth discrepancy of 1.8 mm was approximately the same as in the previous tract. We chose as starting point for translation the point where the endoscope was covered in fluid (seen at the camera), and so it may have been 1 mm or more away from the actual pia. The final endoscope position appears to be ML−3 mm, AP−1.2 mm and DV+3.8 mm, shown in Fig. 6.17. The region is the putamen. The histology sections in this case exhibit a more distinct pattern than the previous ones, a network of high density of soma in a background of low density. The OCT frames also exhibit a distinct pattern of connected high-intensity elements, suggesting a similarity to
Figure 6.16: Cut brain from the in vivo experiment showing the second tract, through white matter and into the putamen. Ruler markings: 0.5 mm.

Figure 6.17: Page from the Rat Brain Atlas (ML−3 mm, AP−1.2 mm, DV+3.8 mm) showing the likely final position of the endoscope in the second penetration, located in the putamen. [74]
Chapter 6. *Ex Vivo and In Vivo Experiments*

Figure 6.18: Histology comparison with OCT from the second penetration: (a) Nissl-stained section from the putamen 157 µm after the deepest position of the endoscope and (b) an OCT-resolved frame of the same approximate location and depth showing some similarity to the histology section. Scale bars (red): 50 µm. (c) Larger scale image of the putamen showing (red arrow) residual blood from the endoscope tract. Scale bar (green) 500 µm.

Figure 6.18 shows those patterns, along with a larger-scale image of the region indicating the position of the tract.

The OCT depth profiles for this tract, a few of which are shown in Fig. 6.19, also display more intricate features compared to the ones obtained in the first penetration. In particular Fig. 6.19d, which represents a nominal penetration depth of 5 mm, displays a distinct oval shape at around $z = 250$ µm. The feature also appears in sequential sweeps. Considering the smooth and continuous borders of this feature, it is more likely to be a contained structure (i.e., a vessel, a vesicle or a membrane of some sort) than neuronal bands. It also seems to have a wrapped cord-like structure at the bottom.

Generally speaking, the OCT images from the *in vivo* experiments were noisier than the images from thick *ex vivo* pieces, which can be seen by comparing Figs. 6.15 and 6.19 to Fig. 6.4. That is expected, due to the dynamic nature of a living sample. However, it indicates the need for a more robust system.
Chapter 6. *Ex Vivo and In Vivo Experiments*  

Figure 6.19: Selected frames showing OCT-resolved depth from the second tract, nominally at (a) 1 mm, (b) 3 mm, (c) 4 mm and (d) 5 mm into the brain. Images cover from 100 µm to 590 µm. Yellow arrows indicate distinct large, darker features which may represent structures or vessels. Scale bar: 50 µm.

with respect to motion artifacts. Integration time can be reduced with the use of a framegrabber and a higher-powered laser source, and the optical system can be packaged in a more compact manner to reduce vibrations. An increase of the wavelength step would reduce the number of data points, yielding faster acquisition and processing. Nevertheless, the common-path feature of the setup led to resolvable tomographies even in the presence of motion.

For accurate identification of the features we have observed, better accuracy in the placement of the endoscope is required. Location uncertainty affects many aspects of the experiment: the initial choice of tract for identifying specific regions, the control of penetration depths for acquisition and the strategy for perfusing the animal, sectioning and staining the excised brain.

Another path would be to employ injected contrasts for the purpose of mapping how different elements appear in OCT, leading towards the application of OCT for neurosurgery guidance. For example, if these features represent blood vessels and the subject is injected with an infrared-absorbing compound, the distinction between them and the surrounding medium would be enhanced.

A more complex approach would be to have two endoscopic probes designed for the same OCT system, one with high NA and another one with low NA. Since high-NA OCT has been shown to enable visualization of brain structures with intrinsic contrast but with a short DOF, alternating between probes with the aid of a cannula would yield complementary images, possibly leading to the proper identification
of structures. Also, since a higher-NA objective would collect more backscattered light, the described strategy would address, to a certain extent, the SNR limitation of the system.

6.4.4 Threshold Metric

As there was no direct reference for comparison of features, we sought to derive a metric by applying different signal processing techniques. Fu Der Chen was instrumental in this process. We present one that generated consistent results.

Due to the low SNR, the vast dark regions in the resolved volumes contributed to counting or averaging schemes. Thus, there was not much variance among results from applying those to different regions. The solution was applying a multi-level thresholding algorithm based on Otsu’s method. The function is implemented as multi thresh in Matlab.

Before applying the function, the resolved volumes along the penetration tract were cropped to remove the first few bins (where the artifacts accumulate) and up to a certain depth into the volume (usually 750 µm). The cropped volumes were then concatenated into one large array. The thresholding function segments intensities into distinct values, normalized to the entire histogram of the array. In our case, 8 levels were chosen. After thresholding, the volumes are split again and the average value for each is calculated.

The metric was consistent over sweeps and variable over penetration position, as shown in Figs. 6.20 and 6.21. Simply put, it represents the backscattered intensity from the structures in each sweep. It appears to yield higher values for regions with higher neuron density and near white matter.

For the first penetration, the metric increases as the brain is penetrated, but then appears to suddenly decrease. This may be related to the apparent kink or shift in the tract at around that depth. It then proceeds to increase again. A better-controlled experiment would be required to determine a correlation with the neuronal density of cortical layers.

In the second tract, the metric seems to correlate more with the structure. There was no data for a nominal penetration of 2 mm, which was intentionally skipped during the in vivo. The metric value for the nominal 1 mm was copied over for the purpose of matching the penetration length to the metric bar. The metric value seems to increase steeply as the probe approaches the white matter region, then decreases gradually as the probe passes through it. The value in the deeper regions is still higher than in cortex, which is what would be expected if the intensity is correlated with a higher density of neurons.

Figs. 6.22 and 6.23 show the metric next to the approximate positions from where data was obtained. The size of the metric was scaled to the total penetration length, as the nominal values were off. More
Figure 6.20: Results from applying the threshold metric on data from all positions and sweeps of the first tract. The standard deviations over positions and sweeps is also shown along the axes.

data is necessary for a global reference or normalization of values, as the metrics are calculated here on a per-tract basis. Notwithstanding, if the defined metric indirectly evaluates structural density and scattering properties of tissue, it may be applied to the categorization of the different brain regions without the need for identifying individual elements. That is, just as MER provides an ensemble metric of electrical activity, this method would serve as an ensemble metric of optical interactions, providing guidance to neurosurgical procedures much quicker than MER.

6.5 Summary

Ex vivo and in vivo experiments were presented in this chapter. Several different samples were studied and select images were included. OCT experiments on slides, while yielding higher-quality images than their microscope counterparts, had limited depth information. A potential match with histology should be further investigated, in particular by the repetition of sectioned tissue experiments on an optical table. The in vivo images have high granularity but seem to contain distinct features. Cell-level
Chapter 6. *Ex Vivo* and *In Vivo* Experiments

**Figure 6.21:** Results from applying the threshold metric on data from all positions and sweeps of the second tract. The standard deviations over positions and sweeps is also shown along the axes.

Identification was not possible, but the larger structures observed may lead to a better understanding of how different elements are represented in OCT images. A metric was also defined, based on thresholding and counting. The initial results from the metric indicate it could be a possible method for identification of brain regions without *a priori* knowledge. More data is required for proper evaluation of that proposed potential. However, if such a method is shown to satisfy the surgical guidance requirements it may lead to the relaxation of OCT system specifications, making FF-SS-OCT a more accessible and interesting alternative to MER or other technologies.
Figure 6.22: Average metric result overlapped with the endoscope tract indicating the approximate imaged positions in the first penetration. Scale bar (black): 1 mm. Color bar shows values for the metric.

Figure 6.23: Average metric result overlapped with the endoscope tract indicating the approximate imaged positions in the second penetration. Scale bar (black): 1 mm. Color bar shows values for the metric.
Chapter 7

Discussion, Conclusion and Outlook

This chapter provides an analysis of the results obtained in light of the thesis objectives, and addresses potential solutions to some of the issues encountered. It then proceeds to state the key findings and contributions of this work. The chapter ends by offering a perspective of potential paths for continuation of the research.

7.1 Discussion

The purpose of this thesis is to investigate whether FF-SS-OCT can be used as a fast, real-time imaging modality for neurosurgical guidance. Although OCT has been used to image neural tissues before, the previous demonstrations typically only imaged ex vivo samples [29, 32], contained optical components that are too large to be compatible with the surgical cannula [10, 30, 42], had rotating components in direct contact with the tissue [28] and produced 2D images with insufficient resolution for identifying cell-level features [75, 76].

At the outset of the thesis research, it was not known whether the modality of FF-SS-OCT near a wavelength of 1310 nm and with a small GRIN lens imaging objective can be used to image neuronal features. More specifically, there was uncertainty with regards to the dispersion in GRIN lenses, the apparent inability to modify the NA of a GRIN lens, the reduced collection of backscattered light for a small-diameter low-NA lens, and the achievable resolution, DOF and contrast for the purpose of identifying different brain structures. It was also undetermined if an imaging fiber bundle could be used with FF-SS-OCT with infrared light.

The research has shown that a simple but carefully-designed GRIN-lens-based endoscope is not a limitation for FF-SS-OCT and can meet the required physical and imaging specifications for in vivo
neuroimaging. Most interestingly, in our *ex vivo* experiments without a floating optical table we learned that the direct contact of the small GRIN lens with the sample stabilizes vibrations, forming a coupled system between the sample and the endoscope. Without this stabilization, OCT was not possible even for imaging a static mirror, which suggests that large systems with standard lens objectives cannot work well *in vivo*.

Unfortunately, imaging fiber bundles seem to be incompatible with the technology due to the large pixel size required for avoiding cross-core coupling, which leads to multi-mode coupling and reduction of resolution. The high optical losses and wavelength-dependent coupling in bundles are also contributing factors to their incompatibility with FF-SS-OCT. Custom multi-core fibers would need to be developed for fiber-bundle imaging using telecommunication band wavelengths. Therefore, in the short term, future iterations of this OCT system should retain the use of relay lenses.

Other improvements to the system include boosting the SNR, increasing the image acquisition rate, and increasing the axial imaging range. A low SNR was the main obstacle for obtaining better-quality images in the results shown in Ch. 6, and this can be resolved by employing a higher-powered source, a higher-sensitivity sensor, and increasing the reflected power of the reference beam in the endoscope. Additional image processing techniques may be considered for the removal of artifacts to improve image quality. The image acquisition rate can be increased using a framegrabber. A faster rate would reduce motion artifacts and enable an expansion of the FOV without sacrificing the resolution. The FOV can also be increased by reducing the total magnification of the system. The Airy radius of the current system spanned approximately 6 camera pixels, the magnification can be reduced by a factor of 2 to 3 by reducing the focal length of the camera lens. This would also increase the collected power.

Lastly, the dynamic focusing feature can be implemented in the future to achieve axial imaging over a longer, continuous range. Dynamic focusing would enable the use of a higher NA GRIN lens, increasing the transverse resolution as well as the collected backscattered power. While the setup can be improved, the results in this thesis shows FF-SS-OCT with the desired form factor and resolution is feasible.

However, a much more fundamental and interesting question that has emerged in this work is the correlation between the OCT and cellular structures, and how light interacts with structures in the brain. Microscopy and histology images are easier to correlate with expected features in the brain, but our results in Ch. 6 show that tomography does not necessarily capture the cellular structure in the same way as visible light microscopy due to the complexity in the shape, materials and sizes of the features in the brain. As shown by our experiment with beads presented in Ch. 5, even when imaging a relatively large object of known shape and optical properties one surface may be visible (i.e., the front), but not the other (i.e., the back), depending on the shape and details of the optical refraction and scattering. The
brain is much more complex. There are multiple cell types (glial and neurons) and vascular structures, and even a single cell, like a neuron, has multiple parts (e.g., cell body, axons, dendrites). Many of these different elements have similar refractive index and overlapping size scales. For example, in the 8 to 10 µm scale there may be neuron cell bodies, glial cells and capillaries. Furthermore, he combined optical scattering and absorption properties are not known. Both optically bright and dark regions can represent physical structures. In our attempts to match OCT images with histology (Section 6.4), even if they originate from precisely the same region, one may be looking at completely different objects due to the treatment of tissue in preparation for staining (e.g., demyelination). Repeating the ex vivo experiments with thicker sections, on an optical table and with better control over the location of imaging spots may lead to the much-needed reference images for the one-to-one correspondence of features. With a proper reference to relate histological features to the ones observed in OCT, better metrics for brain region identification can be implemented.

Nonetheless, distinguishable features were observed in tissue experiments, which reinforces the case for the viability of FF-SS-OCT and indicates a potential for it to be used in neurosurgery guidance. Our experiments also show that pattern density may be a viable way to discern general locations in the brain without the need for identification of individual elements. With a mm-range penetration depth, a 400 µm DOF, 6.5 µm transverse and 14 µm axial resolutions, the OCT system presented offers imaging capabilities and a form factor that is unique compared to other imaging modalities, while the implementation itself is unique compared to other OCT systems.

7.2 Conclusion

Summarizing, this thesis has described the requirements, design, implementation, characterization and application of a novel endoscopic FF-SS-OCT system. We have presented the first demonstration of FF-SS-OCT with >1 µm light as well as the first demonstration of such a system with an endoscopic probe tip. The presented processes for design and implementation serve as a basis for the development of other FF-SS-OCT systems. This system was tested for imaging brain tissue ex vivo and in vivo in rats, and in ex vivo human tissue. The images obtained still need to be improved, but currently have no direct counterpart in the literature. They serve as a guide for the research and implementation of other imaging systems. FF-SS-OCT presents itself as a promising and novel modality for in vivo brain imaging.

The main contributions of this thesis are

1. The confirmation of the viability of FF-SS-OCT with NIR light near a wavelength of 1310 nm.
2. The validation of the compatibility of a stereotactic-surgery compatible endoscopic probe for such a system.

3. A complete basis for the design and implementation of endoscopic FF-SS-OCT systems.

4. A comprehensive set of imaged samples taken ex vivo and in vivo in two species (rats and human), which may serve as a guideline for future endoscopic OCT brain research.

5. The definition of a metric for identifying different brain regions without a priori knowledge and without the ability to distinguish cell-level structures.

7.3 Outlook, Extensions and Future Work

Technologies for brain imaging and surgery guidance are a topic which continues to attract interest. Due to the simplicity of its optical system design and compatible form factor, endoscopic FF-SS-OCT emerged as a candidate for replacement of current neurosurgery guidance methods. Faster and higher resolution InGaAs cameras, along with higher-power, wider-range tunable laser sources will lead to better, faster systems with higher resolution and image quality. A real-time FF-SS-OCT system will soon be possible. Nonetheless, modifications of the system presented can be implemented with current technologies, potentially addressing key limitations of this implementation and advancing the state of the technology towards clinical deployment. For example, given the commercial availability of GRIN lenses with less than 1 mm diameter, thinner and longer iterations of the endoscopic probe can be designed following the process described in this thesis. The other components in the optical system can be miniaturized and packaged so the device can be used for clinical testing. Packaging would also facilitate the implementation of automated dynamic focusing, relaxing DOF constraints. Another possibility for improvement is the addition of polarization-sensitivity and Doppler capabilities. With regards to the specific application of OCT to tissue imaging, studies of the correlation between the density of elements in brain regions and the observed OCT, and studies for matching OCT with known histological features could aid in the identification of OCT features. In parallel, better signal processing techniques and the development of metrics for the automated identification of brain structures or regions would pave the way for clinical use.

From a more fundamental perspective, there are several questions which remain open for exploration. The development of models for the collective interaction of light and tissue could explain how different elements in the brain yield different results in the OCT images. Relatedly, the ability to distinguish between structures of similar size and scattering properties through the use of structured illumination
could be investigated. An additional path would be the exploration of longer wavelengths for tissue FF-SS-OCT, or even the simultaneous employment of two distinct wavelength ranges for combined effects.
Appendix A

OCT Algorithm

```matlab
%% RESAMPLING (INTERPOLATION AT LINEARIZED WAVEVECTOR POINTS)
% Resample the signal using cubic spline interpolation after creating the wavenumber vector from the triggered wavelength array

k_array = flip(2*pi./(lambda_array*10^(-9)));
k_lin = linspace(k_array(1), k_array(end), length(lambda_array));
flipped_signal = flip(signal,3);
resampled_signal = spline(k_array, flipped_signal, k_lin);

%% Removing DC component (mean or detrending)
% subtract the mean value of the signal along different wavelength to remove DC component

meansarray = mean(resampled_signal,3);
meanscube = repmat(meansarray, [1 1 size(resampled_signal,3)]);
descaled_signal = resampled_signal - meanscube;

%% WINDOWING FOR SPECTRAL LEAKAGE REDUCTION
```
% Hanning window is applied to reduce spectral leakage caused by sharp transitions at the ends of the signal

```matlab
hannwindow = transpose(hann(size(descaled_signal,3),'periodic'));
windowcube = repmat(reshape(hannwindow, [1 1 size(hannwindow, 2)]), [size(descaled_signal,1) size(descaled_signal,2)]);
refinedsignal = windowcube .* descaled_signal;
```

%% ZERO-PADDING
% zero-padding is applied to improve FFT performance, reduce scalloping loss and generate a smoother looking profile

```matlab
padding = zeros(size(refinedsignal,1), size(refinedsignal,2), 2^(2+nextpow2(size(refinedsignal,3))-size(refinedsignal,3));
padded_signal = cat(3, refinedsignal, padding);
```

%% EXTRACT DEPTH INFORMATION WITH FOURIER TRANSFORM
% FFT is applied with respect to the linear wavenumber. Magnitude of the resolved signal is preserved, phase and complex conjugate are discarded

```matlab
tempft = fft(padded_signal,[],3);
finalsize = round(.5*size(tempft,3));
final_result = abs(tempft(:, :, 1:finalsize));
```

%% CALCULATING FREQUENCY BIN SIZE AND RESOLUTION
% Convert wavenumber indices into depth information using frequency to radian relation
delta_k = k_lin(3) - k_lin(2);

k_range = k_lin(end) - k_lin(1);

f_res = 2*pi/k_range;

f_sampling = 1/delta_k;

df = f_sampling / size(tempft,3);

zstep = pi*df;
Bibliography


[65] J. Xi, L. Huo, J. Li, and X. Li, “Generic real-time uniform K-space sampling method for high-speed swept-source optical coherence tomography.” *Optics express*, vol. 18, no. 9, pp. 9511–7,


[73] K.-S. Lee and J. P. Rolland, “Bessel beam spectral-domain high-resolution optical coherence tomography with micro-optic axicon providing extended focusing range,” *Optics Letters*, vol. 33,

