MINIATURE MICROSCOPE DESIGN AND CONSTRUCTION
BASED ON TILTED ROTATIONALLY ASYMMETRIC PRINTED
LENSES

by

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SIGNED: ____________________________

Jeremy David Rogers
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For Kara
TABLE OF CONTENTS

LIST OF FIGURES ................................................................. 9

LIST OF TABLES ................................................................. 11

ABSTRACT ................................................................. 12

CHAPTER 1 INTRODUCTION AND BACKGROUND .................. 14
1.1 Enabling Fabrication Technologies ................................. 14
   1.1.1 Deep Reactive Ion Etching and the Bosch Process .......... 15
   1.1.2 Deep X-Ray Lithography or LIGA .............................. 16
   1.1.3 Grayscale Lithography .......................................... 17
1.2 Idea: Zero Adjustment Precision Assembly .................... 19
1.3 Optical Sectioning ..................................................... 20
1.4 Goal: Build a Miniature Microscope for \textit{in vivo} Cellular Imaging .... 22

CHAPTER 2 OPTICAL DESIGN ................................................. 24
2.1 Introduction ............................................................... 24
2.2 Miniaturization and scaling considerations ....................... 25
2.3 Basic Design Constraints ............................................. 26
2.4 First Order Design ...................................................... 29
2.5 Tilted Element System Design ....................................... 34
2.6 Nonsequential Ray Trace Analysis ................................... 40

CHAPTER 3 GRAYSCALE MASK DESIGN ............................... 42
3.1 Introduction ............................................................... 42
3.2 Mask Properties ......................................................... 42
3.3 Grayscale Encoding Methods ......................................... 46
3.4 Positioning Features and Lens Constraint ......................... 51
3.5 Mask Layout ............................................................. 53

CHAPTER 4 LENS FABRICATION ............................................. 55
4.1 Introduction ............................................................... 55
4.2 Hybrid Sol-gel Glass Material ........................................ 56
4.3 Lithographic Patterning ............................................... 61
   4.3.1 Sample cleaning and preparation ................................ 61
   4.3.2 Spin coating ...................................................... 62
### TABLE OF CONTENTS – Continued

4.3.3 Exposure ........................................... 65
4.3.4 Development Process ................................. 66
4.4 Summary of lens fabrication ......................... 67

**CHAPTER 5 SYSTEM ASSEMBLY** ................................ 69
5.1 Introduction ............................................. 69
5.2 MOT Substrates ........................................ 70
5.3 Alignment Features and Insertion .................. 73
5.4 Mounting of the spherical glass lens ............... 77
5.5 Cementing .............................................. 81

**CHAPTER 6 TESTING AND PERFORMANCE** ......................... 84
6.1 Introduction ............................................. 84
6.2 Lens surface metrology ............................... 85
   6.2.1 Lens surface roughness ............................ 85
   6.2.2 Lens shape ........................................ 91
6.3 MTF and Tilted Edge Measurements ............... 93
6.4 Ghost images and Stray Light ...................... 99

**CHAPTER 7 IMAGING RESULTS** ................................ 103
7.1 Introduction - challenges of *in vivo* imaging .... 103
7.2 Transmission Illumination Imaging ................. 106
7.3 Fluorescence Imaging ................................ 108
7.4 Reflectance Imaging .................................. 110
7.5 Optical Sectioning using Structured Illumination . 111

**CHAPTER 8 CONCLUSIONS AND FUTURE DIRECTIONS** ............ 118
8.1 Technological successes .............................. 118
   8.1.1 LIGA and DXRL for Mechanical Alignment Systems . 119
   8.1.2 Printed lenses .................................... 119
8.2 Technological weaknesses and pitfalls ............. 120
   8.2.1 Inconsistent and variable lens patterning .......... 120
   8.2.2 Assembly is labor intensive ...................... 121
8.3 Future design considerations ...................... 122
   8.3.1 Limitations imposed by CMOS camera ............ 122
   8.3.2 Limitations imposed by the material sag .......... 122
8.4 Final Words ........................................... 123
## TABLE OF CONTENTS – Continued

APPENDIX A  Mask Design Scripts ........................................... 124
  A.1  Calibration Features .................................................. 124
  A.2  Pixelated Aspheric Lens ............................................. 126
  A.3  Aspheric Lens with Uniform Step Height ......................... 127
  A.4  Polynomial Lens with Regular OD Increments .................... 128
  A.5  Polynomial Lens ..................................................... 130
  A.6  Alvarez Phase Plate .................................................. 132

REFERENCES ................................................................. 133
## LIST OF FIGURES

1.1 Grayscale lithography process ........................................... 18
1.2 Confocal microscope schematic ....................................... 21
1.3 Structured illumination schematic .................................... 22
2.1 Design constraints ......................................................... 31
2.2 Untilted design ............................................................. 32
2.3 Reflections from tilted elements ......................................... 36
2.4 Spot diagram for tilted element system .............................. 39
2.5 Comparison of ghost reflections .......................................... 41
3.1 Development dependence on shape ..................................... 44
3.2 Undercutting during development ....................................... 45
3.3 Grayscale encoding methods ............................................. 48
3.4 Spoke artifact in lenses encoded as annuli ............................ 49
3.5 Comparison of annular and pixelated grayscale masks ............. 50
4.1 Transmission of hybrid glass ............................................. 59
5.1 MOT alignment ............................................................. 71
5.2 Alignment features ......................................................... 75
5.3 Glass lens embedded in MOT ............................................. 78
5.4 Fold mirror geometry ...................................................... 79
5.5 Glass lens mount ........................................................... 80
5.6 Assembled 4M system ....................................................... 82
6.1 Roughness measurement .................................................. 87
6.2 Roughness varies across the lens aperture ............................ 88
6.3 Correlation of roughness across substrates ........................... 90
6.4 Stitched profile map of lens B ........................................... 92
6.5 Profiles of patterned lens B .............................................. 94
6.6 MTF of the relay optics .................................................. 97
6.7 MTF of the 4M device ..................................................... 98
6.8 Ghost reflections in tilted and untilted design ........................ 101
7.1 Transilluminated cells ..................................................... 107
7.2 Transilluminated tissue slice ............................................. 107
7.3 Fluorescence image of cells on a slide ............................... 109
LIST OF FIGURES – Continued

7.4 Reflectance image of cells ........................................ 111
7.5 Reflectance image with optical sectioning ...................... 113
7.6 Fluorescence image with optical sectioning ................... 115
7.7 Fluorescence image of pig tissue ................................. 116
<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Optical systems scaling</td>
<td>26</td>
</tr>
<tr>
<td>5.1</td>
<td>Cantilever spring forces</td>
<td>77</td>
</tr>
</tbody>
</table>
Successful treatment of many types of cancer is improved when early detection is possible. One method of early detection is microscopic inspection of the tissue at risk. Microscopic inspection can be performed by extracting a biopsy and using traditional microscopes, but biopsy is painful and inconvenient which limits its use. An alternative is \textit{in vivo} microscopy using an endoscope or microendoscopy. This dissertation describes the design, construction, and evaluation of a miniature microscope or microendoscope based on new microfabrication technologies.

All components typically found in a traditional bench-top microscope were designed and built on a miniature scale. The objective was comprised of one planoconvex spherical glass lens and three printed microlenses. The printed lenses were patterned using grayscale lithography in a custom engineered photosensitive hybrid sol-gel glass material. Illumination was delivered by high-brightness Light Emitting Diodes (LEDs) via multimode fiber. The design incorporated a custom imaging detector and a Micro-Electrical-Mechanical-Systems (MEMS) actuator for optical sectioning using structured illumination. The opto-mechanical system is designed using a new concept called “zero alignment assembly” in which the lens elements snap into place and are constrained to a precise position with tolerances tighter than the optical tolerances. This scheme requires no post-assembly alignment or adjustment and simplifies system assembly. The miniature microscope was designed to image in several modes including reflectance, fluorescence, and using structured illumination for optical sectioning. A unique optical design incorporated tilted elements to remove ghost images and internal reflections from the image plane. This design enabled microscopic imaging of extremely low reflectance samples like tissue where the “in-focus” component of the object reflects only 0.04% of the illumination. The miniature microscope was built and tested by imaging a variety of test
objects including cancer tissue phantoms and pig tissue. The results demonstrated the successful implementation of many new microfabrication technologies and design concepts to build a working prototype miniature microscope measuring only $3 \times 4 \times 15$ mm capable of imaging cellular structure of tissue in reflectance or fluorescence.
The ultimate goal of the work described in this dissertation was to explore new opportunities to create miniature optical imaging systems that result from new fabrication methods. Specifically, a miniature microscope was designed, constructed, and used to image tissues and other biological samples. During the research, unforeseen challenges were addressed using some of the unique advantages of these fabrication methods. This chapter introduces the technology and ideas that were assimilated to produce a miniature microscope for \textit{in vivo} imaging of cellular tissue.

1.1 Enabling Fabrication Technologies

In the past few decades, technologies have emerged that enable fabrication of very small structures with micrometer scale precision. Many of these technologies are descendant of the microlithography industry’s technology for manufacturing electronics and integrated circuits. These extremely accurate patterning techniques can be applied to create mechanical and optical structures that take advantage of the sub-micron precision and resolution of microlithography. This section describes the technologies used to fabricate the components of a miniature microscope.
1.1.1 Deep Reactive Ion Etching and the Bosch Process

Deep Reactive Ion Etching (DRIE) is one method of etching a pattern formed on the surface of a silicon wafer. The pattern is first formed in photoresist using a standard lithographic process. Once the pattern is formed, the photoresist acts as a barrier to protect some regions while other regions are exposed to the etching chemistry. DRIE is a combination of mechanical and chemical etching where ions of reactive molecules are accelerated through an electric field towards the patterned wafer. The chemical reaction that takes place when these ions bombard the exposed silicon is what enables fast material removal. The acceleration of the ions towards the surface produces an anisotropic etch since the rate of interaction is much higher for surfaces facing the bombardment than side walls. A wet etch process can etch isotropically or anisotropically along material crystal planes, but can not produce arbitrary prismatic structures (2-dimensional shapes extruded through a third dimension). The DRIE process etches primarily in the direction of bombardment and is key to propagating an arbitrary pattern deep into a silicon surface. Unfortunately, the process is not purely directional and the sidewalls of the etched pattern erode slightly by the reactive ions during the etch. This produces some degradation in the shape as it propagates downward into the silicon wafer.

A solution to this problem was developed by Bosch and the resulting Bosch process produces much higher quality prismatic etches[1]. The Bosch process uses alternating cycles of etching and deposition. During the deposition cycle, a pro-
tective polymer coating is applied to the part. This protective layer coats the side
walls and the bottom of the etch feature uniformly. Once the etch cycle begins, the
coating on the bottom is eroded away much more quickly by the mechanical bom-
bardment of particles and soon leaves the silicon exposed to the chemical process.
The coating on the sidewalls of the feature is preserved for some time, and the etch
continues in the downward direction. Eventually, the sidewalls become exposed, but
before this happens, the cycle is repeated and a new protective coating is applied.
The Bosch process allows a pattern to be propagated through the entire depth of a
silicon wafer up to 500 $\mu$m thick.

1.1.2 Deep X-Ray Lithography or LIGA

LIGA or Deep X-Ray Lithography (DXRL) is another printing technology that pro-
duces precise prismatic shapes deep in a material[1]. LIGA is a German acronym
($LIthographie$, $Galvanoformung$ und $Abformung$) that means ‘lithography, electro-
plating and molding’. The process uses high energy X-rays to expose PolyMethyl-
MethAcrylate (PMMA), a plastic. The X-rays are the byproduct of synchrotron
particle accelerators and have wavelengths from 1-5 Å depending on the facility.
This short wavelength reduces the effect of diffraction when patterning thick resists.
The collimation of the beam is better than 0.1 mrad. The short wavelength and high
degree of collimation allows the patterning of the PMMA to large thicknesses up to
millimeters. Even thicker components can be fabricated by stacking multiple parts.
The stacking of parts also provides a means for producing non-prismatic structures. The exposure of PMMA to X-rays results in a breakdown of chemical bonds and the exposed PMMA becomes soluble and is washed away. In this sense, the PMMA acts as a negative tone photoresist. The developed PMMA can be used directly if the plastic can be used as the part, or the PMMA can be used as a mold. Metal is electroplated into the mold so that it overfills the plastic mold and the excess can be removed by planarization techniques such as lapping. Once the metal is planarized, the plastic is removed and the metal parts are released. This method is capable of producing components similar in shape to the Bosch process, but in a wider variety of materials such as metals and plastic. Another advantage of the LIGA process is the improvement in side wall straightness and smoothness.

1.1.3 Grayscale Lithography

Grayscale lithography is a process very similar to traditional lithography, but uses variations in irradiance of the resist to produce 3-dimensional variations in resist height. Figure 1.1 shows how the exposure and development produces a surface profile with only 3 levels. In practice, many gray levels are used to create a virtually continuous surface. Using an irradiance pattern that varies with radius can, for example, produce a lens shape in the photo resist. This technology requires a method of controlling the irradiance or exposure of the resist. Methods of modulating the irradiance include halftone masks, phase masks or holograms, grayscale masks, and
Figure 1.1: The process of grayscale lithography begins with a spatially varied exposure of a photosensitive material. In this case, the exposure is modulated by a grayscale mask with a variation in optical density (indicated in the diagram as line thickness of the mask). Regions of the material that are exposed to more light become more polymerized than regions less light. The component is then placed in a developer bath where the material is removed at a rate that depends on the degree of polymerization.

direct writing of material with a laser or e-beam[2]. Grayscale masks can be written using High Energy Beam Sensitive (HEBS) glass. A HEBS glass mask contains a thin layer that darkens upon exposure to high energy beams such as an electron beam (e-beam). Whatever the technology, the purpose of the mask is to spatially control the irradiance at the resist such that after development, the resist height varies according to the desired pattern.

Choosing the resist to use for grayscale lithography is not trivial. The goal of the majority of the microlithography industry is to produce binary patterns (circuits) of very small scale. To achieve this, most resists have a virtually binary response such that variations in exposure still result in binary features. This binary response
reduces defects due to small, unintentional variations in exposure of the circuit pattern. It also enables thresholding methods to produce line widths much smaller than the resolution limit of the lithographic lens. However, when a grayscale feature is the goal, these resists pose a challenge. Some resists offer a better grayscale response, and additional materials are under development.

Grayscale lithography enables the printing of refractive lenses. Binary lenses or diffractive elements such as Fresnel zone plates may be printed with more traditional lithography methods, but the efficiency of diffractive optics is not as high as refractive optics. Refractive microlenses can also be produced using wet etch techniques, reflow, diamond turning, and other similar techniques, but grayscale printing provides a larger degree of control over the surface shape.

1.2 Idea: Zero Adjustment Precision Assembly

Using the new technologies described in the previous section, a highly accurate method of miniature system assembly can be implemented. The use of microlithography technology to print optical and mechanical components leverages a mature and highly accurate method of patterning. Lithography photomasks of even economical quality are capable of reproducing features accurate to sub-micron dimensions. This sub-micron accuracy of the pattern can be extended to the opto-mechanical system by using various lithographically printed features in the assembly of an optical system. For example, if positioning features are printed alongside optical surfaces using
grayscale lithography, the positioning features can be used to constrain the lenses relative to some base substrate. This approach allows for the design and fabrication of an entire optical system in which all components are constrained. The resulting assembly requires no adjustment or tuning to achieve optical alignment[3]. In this scheme, the substrate is analogous to an optical table in which lens elements and other optical components are plugged into place. The substrate was hence given the name Micro-Optical Table (MOT) and is discussed in greater detail in chapter 5.

1.3 Optical Sectioning

Optical sectioning is a general term that describes various methods of obtaining an image of a slice from some depth within a nebulous object. Most methods work by filtering light out of an image that originates from out-of-focus object planes. The problem arises in nebulous or diffuse scattering objects such as biological tissue viewed microscopically. In the case of imaging a thick tissue sample, the semi-transparent nature of the tissue allows light scattered or reflected from defocused planes to reduce the contrast of the image. This undesired background light is removed using optical sectioning.

The most common method of optical sectioning is confocal microscopy. A confocal microscope eliminates defocused light by spatially filtering the light using a pinhole or slit that is scanned around the image. The schematic in figure 1.2 shows how rays originating from out-of-focus are blocked by the pinhole.
Figure 1.2: Optical sectioning in a confocal microscope is accomplished by spatial filtering. Rays originating from an in-focus spot (solid) within the object are focused onto a pinhole and reach the detector. Rays originating from other points (dashed) come to focus in front or beyond the pinhole and are mostly blocked. The point in object space conjugate to the pinhole is scanned around the object plane to form a full image.

A new method of optical sectioning depicted in figure 1.3 uses structured illumination to sample the object plane[4]. Instead of uniformly illuminating the object plane, a low frequency grating or Ronchi ruling is projected into the object. The grating is scanned laterally and several images are taken for different positions of the grating. At depths in the object where the grating is in sharp focus, image points are modulated as the grating is scanned. Object depths where the grating is out of focus are not modulated. Using the collected images, a single image is reconstructed that consists of only the modulated signal. This reconstructed image exhibits none of the out-of-focus background light.

Micro-Electro-Mechanical Systems (MEMS) technology is capable of making the small actuators and gratings needed to implement structured illumination in a minia-
Figure 1.3: Optical sectioning via structured illumination works by projecting a grating or Ronchi ruling into the object. The contrast of the grating drops rapidly for out-of-focus planes. The grating is scanned laterally and several images are captured for different lateral grating positions. The in-focus object points are thereby modulated in time as the grating is scanned, while out-of-focus areas have steady uniform illumination. The multiple images are used to reconstruct a single image based only on the in-focus light. The method is analogous to a lock-in amplifier.

turized device. The actuator and grating can be produced using LIGA processing. A few changes to the mode of operation and reconstruction algorithm are useful in such a miniaturized system[5].

1.4 Goal: Build a Miniature Microscope for \textit{in vivo} Cellular Imaging

The technologies and ideas discussed so far can be tied together to produce a miniature microscope[6]. Such a microscope has potential to be extremely useful in healthcare fields to aid inspection and diagnosis in human tissues. If the device is small enough, it could be used like an endoscope to inspect accessible tissues without surgery. Since the image is not sent through a fiber bundle, it has the potential for
higher resolution and than optical fiber based endoscopes. Such a device has many requirements: it must be small enough to be used as an endoscope; the device must have resolution better than that of conventional fiber bundle endoscopes; and the device should be capable of optical sectioning to reduce or eliminate background light from defocused tissue. The goal was to design and build a miniature microscope capable of imaging cells \textit{in vivo} using either fluorescence or reflectance with optical sectioning. The challenges inherent in such a goal provides many opportunities for developing new ideas. The remainder of this dissertation details these ideas and demonstrates a working prototype of a miniature microscope capable of imaging biological tissue.
CHAPTER 2

OPTICAL DESIGN

2.1 Introduction

There are many unique considerations to attend to in the optical design of miniaturization imaging systems. The results presented here represent the most recent in a number of iterations incorporating ever more details discovered during a previous design. First Chen Liang and later Junwon Lee contributed excellent designs that are presented in their dissertations[7, 8]. This chapter will first discuss some advantages and challenges of miniaturization and then detail the specific design of a miniature multimodal microscope device (4M device). Section 2.2 discusses the general aspects of miniaturization. Section 2.3 provides an overview of the general design requirements of \textit{in vivo} cellular imaging as well as specific design constraints imposed on the 4M device project. Section 2.4 outlines a first order design of the 4M system. Section 2.5 describes the details of a design solution that reduces ghost images and stray reflection by tilting lens elements. Finally, section 2.6 describes a non-sequential ray trace model of the stray light and ghost images of the system design.
2.2 Miniaturization and scaling considerations

The trend to miniaturize imaging systems such as cameras and microscopes is driven by a number of factors. The most obvious factor is that smaller devices are more portable. This is important in consumer devices such as mobile phone cameras where small and light devices are preferable. Smaller is also important in the field of biomedical imaging. The small size of imaging devices results in easier access to tissues and less invasive screening or diagnosis. Another advantage is the potential for reduction of manufacturing costs. By printing lenses rather than traditional grinding and polishing, lens fabrication of even aspheric and complex surfaces can become very economical.

In addition to these basic factors, some quantifiable technical reasons also point towards advantages of miniaturization for certain applications. To understand the finer details of scaling an optical system, it is important to examine how scale affects resolution, field of view, working distance, etc. These properties are affected differently depending on how the system is scaled. A discussion of optical system scaling and different modes of scaling appears in Microoptics [2]. The discussion found there examines several modes of scaling that are specific to diffractive optical systems. For a system based on refractive lenses, it is most appropriate to consider constant $F/\#$ scaling where all diameters and focal lengths are scaled by the same factor and ray angles remain constant. Since the $F/\#$ or Numerical Aperture (NA) is not changed, the resolution does not change. This is crucial because it means
that it is possible to make a miniature microscope that has the same resolution as a conventional microscope. On the other hand, the working distance and Field of View (FOV) are reduced. Interestingly, all lengths are scaled by the same factor except wavelength which obviously remains constant. This scaling of length includes the length of ray errors and wavefront errors and thus aberrations tend to be reduced when the scale of an optical system is reduced. Table 2.1 summarizes some of the effects of scale.

### 2.3 Basic Design Constraints

Design of any optical system should begin with a careful examination of the requirements of the system. These requirements will define the design space available and allow one to develop a good first order design. For the 4M system, these constraints arise from the nature of the object, the limits on the detector geometry, limits on the sag of the printed lenses, and the desire to make the system as small as possible.

---

**Table 2.1: Advantages and disadvantages of scaling optical systems.**

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<td><strong>Size</strong></td>
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<tr>
<td>Diffraction Limited Resolution</td>
<td>No change</td>
<td>No change</td>
</tr>
<tr>
<td>Aberrations / Geom. Spot Size</td>
<td>Worse</td>
<td>Better</td>
</tr>
<tr>
<td>Strehl Ratio</td>
<td>Worse</td>
<td>Better</td>
</tr>
<tr>
<td>Field Diameter (finite object)</td>
<td>Increases</td>
<td>Decreases</td>
</tr>
<tr>
<td>Field of View (angular)</td>
<td>No change</td>
<td>No change</td>
</tr>
<tr>
<td>Image size (detector)</td>
<td>Increases</td>
<td>Decreases</td>
</tr>
<tr>
<td>Working Distance</td>
<td>Longer</td>
<td>Shorter</td>
</tr>
<tr>
<td>Weight</td>
<td>Increases as $scale^3$</td>
<td>Decreases as $scale^3$</td>
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On a microscopic or cellular scale, biological tissue has a nebulous, semi-transparent nature that allows for imaging cells below the surface. It is desirable to image below the surface since many tissues are covered in a thin epithelial layer, and the types of cells of the underlying tissue may not exist on the surface. Longer wavelengths from 650 nm to 850 nm scatter less and allow for imaging up to several hundred microns below the surface. To take full advantage of this, the working distance should therefore be at least 300 \( \mu m \). In addition, the cells of interest are typically on the order of 10-30 \( \mu m \) in diameter, so a sampling of around 1 \( \mu m \) is desirable to provide good sampling of each cell. Also, the use of the device in vivo requires that the microscope work in immersion. The first surface of the device is placed in contact with the tissue or fluid surrounding the tissue, so the immersion fluid can be assumed to be saline solution with 1.34 refractive index.

On the opposite side of the system, some limits are imposed by the detector. In keeping the size of the system as small as possible, the detector also needs to be small in area. The detector designed for the 4M system is a custom engineered CMOS detector array. To retain good sampling resolution, the pixels should also be as small as possible, and yet the size of the collection area and readout electronics imposes some practical limits. In CMOS detector technology, readout and address lines are run throughout the detector array. These lines have a minimum size and as pixel size is reduced, the address lines become a significant portion of the area of the detector array. The detector array designed for the 4M device has four micron
center-to-center spacing of the pixels in both directions. This spacing allowed for 50% fill factor of the pixels, which was deemed acceptable. For one micron sampling of the object, this requires a transverse magnification of -4.

The sampling of one micron also sets a limit on the Numerical Aperture (NA) of the system. A sampling of 1 \( \mu m \) of the object leads to a diffraction limited resolution of 2 \( \mu m \) so that the detector elements sample two diffraction spots separated by the Raleigh criterion (this corresponds to the Nyquist frequency limit). Equation 2.1 shows that for the longer wavelength of interest (850 nm), the system should have an NA of at least 0.26 to retain two micron diffraction limited resolution. An NA of 0.4 was used in the initial designs to improve light gathering ability of the system. At this NA, the system is detector limited rather than diffraction limited.

\[
d = 1.22 \frac{\lambda}{2NA}
\]  

Equation 2.1

Another limit imposed on the system is due to the fabrication technology. The use of grayscale printed lenses provides many advantages such as easily fabricated aspheric surfaces. However, these advantages come at a cost. The patterned lenses have an upper limit on their sag. The hybrid glass used as the material for these lenses is continuing to improve, but the the upper limit on sag at the time of the design was 80 \( \mu m \).

To summarize, the following list of limitations can be used to constrain a first order optical design:

- Imaging wavelength: 650 nm
• Numerical Aperture: $NA \geq 0.4$

• Object in immersion: $n = 1.34$

• Magnification: $m = -4.0$

• 1 micron sampling (at object plane)

• Lens sag: $s \leq 80 \mu m$

• Working distance: $WD \geq 300 \mu m$

2.4 First Order Design

The first order design should leave some degrees of freedom to allow for optimization of the final design. However, many constraints can be outlined which determine limits and are sure to aid the designer in arriving at an optimal system. This section works through the various constraints to construct a first order design.

A good starting point is to set some boundaries on the scale of the system. To provide a practical amount of information in the image, the detector should have at least $256 \times 256$ pixels. The detector has $4 \mu m$ pixels, so the size of the detector must be at least $1 \text{ mm}$ across. This sets the scale of the diameter of the optical system and also sets the FOV at the object to around 250 microns. This FOV would allow imaging of around $20 \times 20$ cells which is also reasonable.

Next, consider the first lens by examining the geometry restrictions of the NA, working distance, and FOV. The NA and index of refraction set the half angle of the
marginal ray. The working distance then sets the height of 94 µm for the marginal ray at the first surface. Since the microscope is used in immersion, it is advantageous to have a planar first surface. If the first element is a grayscale printed lens, the substrate of the printed lens is a 150 µm thick piece of borosilicate float glass. The index of 1.5 puts the marginal ray at a height of 135 µm requiring the first lens to have a diameter of at least 270 µm.

Next, consider the Field Of View (FOV). Microscopes should be telecentric so that the magnification or apparent size of an object does not change as it comes into focus. Requiring the system to be telecentric in object space means that the diameter of the first lens must actually be 270 µm plus the 250 µm FOV or 520 µm. This diameter along with the 80 µm sag limit of the hybrid glass requires the radius of curvature of the surface to be greater than 316 µm. This radius of curvature is too weak and is unable to keep the rays from diverging rapidly and ultimately increasing the size of the system. The conclusion is that a more powerful first lens is needed, so a commercially available ground glass lens is used as the first element.

It should be noted that while this argument for using a commercial glass lens as the first element is valid, its use is partially due to the legacy of earlier designs where the sag limit of the hybrid glass was not to exceed 50 µm. It is plain to see that this reduced sag made the situation even worse. The current sag limit of 80 µm is close to allowing an all hybrid glass lens design. Any further increase in the material sag limit, index of refraction or decrease in the acceptable working distance would make
Figure 2.1: Working Distance (WD) and Numerical Aperture (NA) constrain the marginal ray height (h). For a telecentric system, the Field Of View (FOV) adds to the ray height constraining the lens diameter. For a printed lens, the additional limits on the substrate thickness and sag of the printed lens material impose too long a radius and consequently a weak lens. This weak lens allows the rays to diverge too much and leads to an unacceptably large system diameter.
The object is immersed in water with a 0.4 numerical aperture. The printed lenses are spaced 0.8 mm apart to allow space for mounting features in the substrate. The working distance is 0.38 mm and the transverse magnification is -4. The object height is 125 microns allowing for a 250 micron full field of view. The largest diameter is 1.4 mm and the maximum printed lens sag is 60 microns. The spherical glass lens provides most of the power, but introduces significant spherical aberration. The printed lenses are conic sections with a conic constant of -2.84 which corrects the spherical aberration.

such an all-printed-lens design attainable.

The number of commercially available glass lenses with diameters of 1 mm is quite limited. The Edmund Optics lens chosen for the design is a plano-convex high index glass lens. The short radius of curvature and high power result in severe spherical aberration, but fortunately this can be corrected by aspheric surfaces on the printed lenses of the system. Once the first lens is constrained, the rest of the first order optical design falls into place rather simply. A few additional constraints arise from the lens mounting mechanism of the system substrate, which requires that the lenses be spaced by at least 600 µm from one another. Figure 2.2 shows the optical design produced by these constraints.
Already this design incorporates some unconventional attributes. The system has a small amount of field curvature, but the nature of the object allows this. The nebulous aspect of the tissue means that the object plane is embedded within the tissue. By allowing the object plane to have some small amount of curvature, the performance is increased. Since structured illumination is used to section the object, the system’s field curvature simply makes the optical section a curved surface within the object. This is convenient because the curvature introduced by the system in imaging the grating into the tissue is exactly removed in imaging the curved object plane back onto the detector. Since the grating and detector are both planar, allowing this slight field curvature in the system design is not problematic. The field curvature is kept small enough so that the sag of the object plane is less than one cell depth (around 10 µm) so that the optical section is always approximately the same cell layer.

The system is also not perfectly telecentric. In a microscope system, it is desirable to have a telecentric object space. This means that the chief ray from each object point is parallel to the axis, or that the entrance pupil is at infinity. The effect of this constraint is that as the object is defocused it does not change in magnification. This is enormously useful in a microscope since a change in image size while focusing is confusing to the observer and makes focusing difficult. In the 4M system, however, the optical sectioning cuts out the light of any object that is not in focus. Therefore, the requirement of telecentricity can be relaxed. In practice,
it is still useful to be close to telecentric since the device is also used in wide-field imaging without optical sectioning. The image space of a microscope is not generally telecentric, but due to the detector design, this may be a requirement for future designs of the 4m system. This is discussed in detail in chapter 8.

2.5 Tilted Element System Design

The design described in the previous section was built and tested and an important discovery concerning the reflectance of tissue was made. Despite the use of Anti-Reflection (AR) coating on the lens surfaces, the small residual reflections were still as strong or stronger than the reflection from the cells of the tissue. The result was reflections or ghost images that completely dominated the image.

To estimate the reflectance of the tissue under investigation, a simple comparison was performed. Two samples were imaged, a gold-labelled tissue phantom[9] in immersion and an uncoated glass surface in immersion. The Fresnel reflection from a glass surface in water is calculated to be less than 0.4% and the image plane irradiance for the tissue was measured to be almost identical to that of the glass slide. However, the reflected signal from the in-focus cells must be even less since the tissue reflects light from several hundred microns of depth, but only a thin layer of a few microns is in focus at a given time. While using the structured illumination method[4, 5], the maximum contrast of the grating projected into the tissue phantom is measured to be 12% of the reflected light. It is therefore estimated
that the maximum reflectance from the in-focus cells to be 12% of 0.4% or 0.05% of the incident light. Clearly, simple AR coatings alone cannot reduce the surface reflections enough to allow imaging of such an object without further reduction of ghost reflections.

Interestingly, Mohammed Rahman while working to image the tissue with a conventional benchtop microscope, was having the same difficulty and noticed that by slightly tilting the objective (by rotating the turret), the reflections could be reduced and a clearer image could be seen. This led to the idea that each lens element in the 4M device could be intentionally tilted to send the reflected light out of the image plane. The concept of tilting or decentering lens elements to remove stray light was first formally discussed by Buchroeder[10]. The technique is difficult to employ since it requires very careful design to control the aberrations that are inevitably introduced by the tilted or decentered lenses. Such a design requires more elements than a symmetric design and aberrations are controlled by balancing third order aberrations with higher orders. The 4M system, however, is not constrained to use spherical or even rotationally symmetric lenses. Grayscale printing of the lens elements allows the freedom to print almost any functional surface. The aberrations introduced by a tilted lens can be easily compensated by asymmetric lens surfaces. The aberrations are directly corrected at each lens surface, and no additional elements are needed.

As mentioned earlier, the commercial objective also suffered from ghost reflec-
Figure 2.3: The optical design shows illumination rays (originating on-axis from the left) and the reflected rays (shaded) that are sent out of the system into baffles. For simplicity, only the reflected rays from the (a) first surface and (b) third surface are shown. The device is designed for immersion, and the medium on the far right is water.

tions, but the printed lenses compounded the problem by having flat surfaces on each lens element. Since the lens elements are printed on one side of a flat substrate of glass, each lens must inherently be planoconvex or planoconcave. The flat surface of these lenses caused the most concentrated ghost reflections. Figure 2.3 shows how the reflections from the first two flat surfaces are removed from the image plane by tilting the lenses.

The restriction of this scheme is that the grayscale patterning technique imposes a limit on the sag of the lens surface. In a rotationally symmetric design, the sag limit constrains the F/# or the optical power of each lens for a given diameter. In a tilted component design, some of the sag must also be used in the correction of aberrations. The challenge is then to minimize the tilt of each element while still removing the reflections from the image plane. This method will maximize the sag
available for power in each element.

The tilted component system is designed in stages of increasing complexity. The first step is to design the optimal rotationally symmetric system. Required tilts can be estimated by sequentially treating each surface as a mirror and examining the beam footprint back at the starting plane. Next, the estimated tilts are introduced and a polynomial function is added to each surface. The polynomial coefficients are varied during optimization with care taken to ensure the surface function does not dip below the level of the substrate or exceed the maximum allowed sag for the component. Finally, a non-sequential ray trace is used to determine the actual size, strength, and location of reflections. The simulated overlap of reflections with the image plane can be used to iteratively fine tune the tilts needed to completely remove the reflection from a given surface.

Some reflections cannot be removed from the image plane without excessive tilts. This case occurs when the reflection from a curved surface results in a rapidly diverging beam. A tilt approaching $90^\circ$ could be required to completely remove such a reflection. Note however, that such a rapidly diverging beam will have a very large footprint at the image plane and will therefore have a very low energy density. It follows that the brightest reflections will tend to be the ones with the smallest spatial extent near the image plane. This is fortunate because these reflections require the smallest tilts to completely remove them from the image plane.

Once the tilts have been introduced, the surfaces can be modified to correct
aberrations. A biconic may be enough to correct the aberrations in some systems, but a 3\textsuperscript{rd} order polynomial was used in our design which provides more degrees of freedom. Equation 2.2 represents the surface function of the lenses where \( c \) is the curvature, \( k \) is the conic constant, and coefficients \( a_1 - a_2 \) are the polynomial coefficients to be varied during optimization of tilted design.

\[
z(x, y) = \frac{c(x^2 + y^2)}{1 + \sqrt{1 - (1 + k)c^2(x^2 + y^2)}} + a_1 x + a_2 y + a_3 x^2 + a_4 xy + a_5 y^2 + a_6 x^3 + a_7 x^2 y + a_8 xy^2 + a_9 y^3
\]

In our case, the result of this design process was a diffraction limited system with Strehl ratio above 0.8 over the entire field of view. Figure 2.4 shows the spot diagram for a selection of field points.

Such an unconventional optical design deserves a brief discussion of tolerances. Since the lens surfaces contain polynomial functions, it is reasonable to expect that the positional tolerance on these surfaces could be much tighter than for rotationally symmetric equivalent designs. Due to the unique assembly method afforded by the MOT concept (discussed in chapter 5), the dominant assembly error is decenteration. Investigation shows that decentering the lenses by up to 5 \( \mu \text{m} \) does not significantly degrade performance. This displacement is greater than the error in assembly using our current technology\[6\]. A more significant error in the lens system is due to scaling of the patterned lenses. This effect is discussed in detail in Chapter 4.
Figure 2.4: The spot diagram for the tilted component system. The circles represent the size of the Airy disk with diameter 7.8 µm. The field points range from ±0.5 mm in both X and Y directions at the image plane.
2.6 Nonsequential Ray Trace Analysis

The non-sequential ray trace analysis of the tilted design predicted a 75% reduction of stray light in the image plane. Measured reflections from the actual device were actually better than predicted and were reduced by 85%. The difference from the predicted reduction and measured results were most likely due to limitations of the model. Figure 2.5 shows a comparison of the reflections from a system with untilted rotationally symmetric lenses and a tilted component system.
Figure 2.5: Ghost images formed by (a) untitled conventional system and (b) tilted component system. The circles depict the designed FOV (radius 0.5 mm at the image) for the system. In the tilted system, a faint ghost is visible on the right, just overlapping with the FOV.
CHAPTER 3

GRAYSCALE MASK DESIGN

3.1 Introduction

At first, it may seem that design and layout of the grayscale mask is a simple task. The various regions of the mask should simply have an Optical Density (OD) that corresponds to the desired surface height of the part at each relative location. However, it turns out that many subtle aspects of grayscale mask design require detailed consideration. The most obvious item is that a calibration curve or mapping between OD and surface height must be determined. This calibration is important, but is only a first step. Over the course of designing and testing many masks, a number of important issues and design methods have been learned. This chapter discusses the subtle but important aspects of grayscale mask design.

3.2 Mask Properties

A number of methods exist to produce grayscale masks. Each type of mask has advantages and disadvantages, and other methods may work well for some applications. The mask type chosen for this research was based on High Energy Beam Sensitive (HEBS) glass[11]. This glass is available as blanks that can be written
with an electron beam (e-beam) writer, or as already rendered grayscale masks from Canyon Materials, Inc.[12] The HEBS glass works as a mask because it darkens locally when exposed to high energy beams. The glass contains a thin layer of around 3 µm thick in which a silver compound is doped[13]. This doped layer accounts for the darkening effect. Once the layer is darkened by exposure to e-beam, the glass can be used as a grayscale photomask.

Since the layer is around 3 µm thick, the lateral resolution of the HEBS glass mask is not as fine as a chrome mask, but features around one micron can be patterned. In practice, the resolution of the hybrid glass material is the limiting factor and not the HEBS mask. In principle, the active layer of the HEBS glass can be made thinner for high resolution, but the OD range will be consequently reduced. The OD range of 0.126 to 1.530 is standard for a HEBS mask, and OD of up to 2.0 can be produced (using a proportionally thicker doped layer). The OD of a particular region is determined by the dwell time or dose of the e-beam in that region. The OD resolution is somewhat dependant on the writer used, but masks can be ordered with up to 500 distinct gray levels.

Choosing the optical density to write for each corresponding region of the lens depends on the relationship between OD and material height after development. Initially, determining this relationship was done by exposing the hybrid glass with a calibration mask that contained a number of separated boxes of specified OD. The height of each box was measured after the hybrid glass was developed, and the
Figure 3.1: During grayscale exposure, material is polymerized to a degree proportional to the exposure. Each region $dx$ has a degree of polymerization that sets the rate of development of that region. As the lens surface evolves out of the initial material plane, the material removal occurs normal to the surface at a rate $p(x)$ that depends on the local degree of polymerization. The vertical rate of material removal $dz/dt$ is then greater than $p(x)$. This leads to a dependence of surface height on the adjacent geometry.

data curve was plotted and used as a mapping between the height and OD. Further testing revealed that this relationship is dependent on variations of exposure and development processes of the hybrid glass material. Additionally, it was found that the relationship depends on the geometry of adjacent regions.

The dependence on adjacent features can be explained by the three dimensional nature of the development process. The situation shown in figure 3.1 should be readily corrected for, but an additional complication exists. In the figure, the entire region represented by $dx$ is assumed to have the same exposure and therefore the
Figure 3.2: This feature, intended to be a cylindrical pillar, exhibits undercutting due to vertical variation of development rate. The rounding of the top is due to the development of the corners from both side and top.

same development rate. In reality, the exposure drops off in the material due to absorption of the exposing beam. This effect is the cause of undercutting of some features that occurs under certain process conditions. Furthermore, the development rate is influenced by some complex oxygen induced quenching effects. The combined effect is shown in figure 3.2 which shows a feature that should have been a cylindrical pillar. The study of these influences is beyond the scope of this research. The solution to these problems is to either iteratively calibrate or use calibration features that closely resemble the desired features.
3.3 Grayscale Encoding Methods

Once a relationship between mask OD and material surface height is attained, it should be straightforward to draw up a grayscale mask. However, there are a range of choices in how to encode the grayscale information in a photomask containing discrete zones of constant OD. Once again, these choices have subtle influences in the final lens surface and must be made with care.

Although in principle the HEBS glass mask could contain continuous variations in OD, the mask is written using an e-beam writer that is controlled digitally. The writer must be provided with something like an image or map that is comprised of discrete zones or patterns of a given OD. The choice is in how to encode the continuous lens surface as discrete zones of OD and two such methods are shown in Figure 3.3. If a constant step height is used, the pattern looks something like a Fresnel Zone Plate, and it might be thought that by using appropriate geometry, the phase difference between steps could be used advantageously. However in patterning experiments, the material response is somewhat blurred and sharp corners tend to get rounded off during development. Provided the steps are smaller than about half a micron, the surface is smooth and continuous. On the other hand, if the constant step height method is used, the central zone can be as large as tens of microns in diameter. This large central zone of constant OD results in a large flat central region of the lens that has no focusing power that could lead to large spot sizes or halos. Based on these arguments, the better encoding method is one in which each annulus
of constant OD is the same width and the OD for each zone is assigned based on the desired surface height.

Although initial lenses fabricated using the bull’s-eye style pattern looked good, they exhibited a subtle but consistent artifact. Examining Figure 3.4 closely, one will notice a radial spoke pattern superimposed on top of the lens surface. This pattern can also be seen in the mask itself. During file preparation for the e-beam writer, each zone or shape in the file must first be fractured into polygon zones. This spoke artifact is due to slight variations in fracturing of the annular rings as a function of angle. As the local angle of the annulus edge changes with respect to the square fracturing grid of the e-beam address space, a slight difference arises in the local fracturing. This difference manifests itself as a very slight variation in OD around the annulus. Due to the highly sensitive nature of the grayscale photoresist, this difference which would normally not be noticeable becomes a major error in the lens surface. To eliminate this artifact, the shapes of the mask must first be broken up into sub-shapes of equal shape and size so that file fracturing will treat every region identically. The lens pattern is first broken up into pixels, and each pixel is assigned an OD based on its position in the lens. Figure 3.5 shows the schematic comparison of the two methods and the resulting masks and lenses.

Close examination of both masks or lenses depicted in Figure 3.5 shows a second artifact present in both encoding schemes. The rings or discontinuous steps in radius are due to an slight error in the calibration of the e-beam writer. The exposure dose
Figure 3.3: A spherical lens must be sampled discretely into layers that represent the grayscale levels of the mask. Two methods are shown. On the left, the sphere is sampled into layers of constant thickness like a contour map producing a grayscale pattern that resembles a Fresnel zone plate. On the right, the sphere is sampled into annuli of constant change in radius producing a bull’s-eye pattern. The OD for each zone is chosen to match the intended lens profile.
Figure 3.4: SEM micrograph of a lens exhibiting the radial spoke artifact. Masks encoded using annular rings as zones of constant OD contain a subtle artifact that produces a spoke-like pattern on the lens surface. The artifact is due to aliasing of the square grid of address space in the e-beam writer with the varying local angle of the annulus edge.
Figure 3.5: A comparison of mask encoding techniques. The lens comprised of annular rings (a) leads to a spoke-like radial artifact in the grayscale mask (b) that prints through into the lenses (c). If the mask is encoded by first pixelating the features into small squares of uniform size (d), the resulting mask (e) and printed lenses (f) do not contain the spoke artifact. The mask images were taken with a 10x microscope objective and the lenses were imaged with a Scanning Electron Microscope (SEM).
that produces variations in OD is controlled by changing the dwell time of the beam in various locations. If this dwell time is slightly off, the wrong OD will be written for a particular intended OD. This artifact will be removed by better calibration of the writer in future masks.

The pixelation of the lens features could potentially lead to a diffractive artifact in the images similar to the spiders of a telescope, but as mentioned before, the tendency of the development to smooth out sharp corners leaves no detectable trace of the mask pixelation in the fabricated lenses. Masks designed for this research have typically used 128 gray levels to represent the lenses with 50-80 µm of sag. The step height is not constant, but the steps are typically less than a micron. As the sag of the material increases, the encoding may need to use a larger number of gray levels to keep the step size small and indiscernible after developing.

3.4 Positioning Features and Lens Constraint

A fundamental technology of the 4M and MOT technology is the precise positioning of lens elements relative to some base substrate. The design of positioning features should promote precise and accurate position of each lens. This requires that all degrees of freedom are accounted for, but that the element is not overconstrained which would lead to instability or stress and deformation. A good analogy for positional constraint is a stool built with different numbers of legs. A two legged stool is under-constrained, and does not stand up on its own. A four legged stool
is over-constrained and will often rock when placed on an uneven surface. Three legs is the ideal for constraining the position of a stool, but the positioning of a lens element is slightly more complex.

Many types of positioning features were explored, but the final working design was driven by both constraints discussed above and by the material properties of the hybrid glass. Ideally, the lens element should have one constraint per degree of freedom. There are six degrees of freedom for a lens element: \( x \), \( y \), \( z \), tip, tilt, and rotation. An ideal mounting mechanism should contact the lens element in six places with no redundant constraints. However, the fabrication technology for the substrate requires that the shapes machined out of the substrate are prismatic. This tends to lead to some redundancy of the mounting features, but the precision of the substrate and the lens elements is so great that over constraint is not observed to be problematic. Furthermore, the use of points of contacts in not practical in the case of the hybrid glass because the material is not strong enough to withstand high pressures or shearing forces. Therefore, rather than using small knobs or bumps of hybrid glass, longer ridges and blocks are used that improve the adhesion and strength of the hybrid glass positioning features.

Figure 3.4 shows the lens surrounded by 4 separate positioning feature schemes. The same lens in the mask can be tested with different positioning features by dicing out the lens along with the desired scheme. The small bumps seen in the figure should in theory provide better kinematic mounting of the lens. In practice,
however, the bumps failed under shear during insertion of the element. The longer ridges worked well and did not exhibit stress or instability due to over-constraint. The ridges sit into two complimentary v-shaped grooves in the sidewall of the slots in the base substrate. These ridges constrain tip, tilt, rotation, $z$, and lateral position $x$. To constrain the height of the lens above the substrate, the large blocks seen below each lens are used. These blocks are thick and overhang the base substrate. As the lens element is inserted, the blocks come to rest on the top surface of the base substrate constraining the lens height or position along $y$. In this way, all degrees of freedom are constrained by precise lithographically printed features.

3.5 Mask Layout

The layout of the lens elements on the mask is somewhat arbitrary, but by using some clever arrangements, costs can be minimized while the number of lenses and alignment schemes present can be maximized. Since the cost of generating the grayscale photomask is dominated by the $e$-beam write time, dark or high OD regions are more expensive to write.

The lens areas are one of the largest consumers of beam time in the mask writer, so it is advantageous to maximize the use of the lenses in the mask layout. In initial masks, several styles of alignment feature were to be tested, but the lenses were symmetric. This allowed four sets of various positioning features to be printed on each of four sides of the lenses. After printing the lenses on a particular substrate,
the desired positioning features can be selected and cut out of the glass substrate along with the lens.

Another consideration is the spacing of the lenses. The lens elements which consist of the lens and the surrounding positioning features must have enough space between them to allow for the breadth of the dicing saw blade. Since the dicing saw blade width varies from machine to machine and even over time as a blade wears, it is wise to use two separate cuts between each lens element and allow a few hundred microns extra space between each lens element.

Most of the generation of the mask file can be scripted. The scripts written to generate the mask file can be found in appendix A. The masks were designed using Design Workshops DW2000 and the scripts are written in that software’s GPE scripting language. Many subtle but important details regarding the specific encoding and mask layout may be found in the comments of the scripts.
4.1 Introduction

Early concepts of the miniature microscope and Micro-Optical Table (MOT) utilized diffractive optics printed with binary lithography. At the time, printing Fresnel zone plates on a small substrate alongside positioning features seemed to be a promising fabrication method. Printing these diffractive elements in photoresist is simple, but the resist is not an ideal optical material. When photoresist is used, an etch process is typically employed to transfer the pattern into the substrate material[14]. The substrate, often glass or silicon, is a better optical material and is also much more mechanically robust.

An alternative to photoresist was developed by Juha Rantala and Ari Kärkäinen using a sol-gel process to create a photosensitive hybrid glass[15]. This material could be used in place of photoresist, but after processing, had glasslike properties without requiring a pattern transfer into the substrate. It was discovered that by adjusting the chemistry of the hybrid glass, thicknesses in excess of ten microns could be patterned. This exciting breakthrough made possible the patterning of refractive lenses using grayscale lithography. Refractive optical components are advantageous
over diffractive elements because no light is lost to diffraction orders, so they are more “efficient” and so offer better image contrast. Refractive lenses also exhibit significantly less dispersion than diffractive lenses making operation over a broader spectral range possible.

The chemistry and synthesis of the hybrid sol-gel glass material was entirely the work of others, but much of the process of using the material was developed and contributed to through the course of this research and so will be reported here. For details regarding the chemistry and properties of this material, please refer to papers by Ari Kärkäinen [15, 16, 17, 18]. A more general overview of the sol-gel process and materials can be found in Hench [19]. Section 4.2 reviews some of the most relevant material properties.

4.2 Hybrid Sol-gel Glass Material

The hybrid sol-gel glass was engineered to have many properties desirable for printing refractive lenses. Since the material was to be used directly as the optical surface, the most critical properties are the bulk optical properties of the material. Just as with optical glass, the chemistry of the hybrid glass can be altered to produce a desired index of refraction or variations in dispersion. However, adjusting the chemistry of the hybrid glass also affects the fabrication process as discussed later so the variations have not yet been studied exhaustively. The material used in this work exhibits a refractive index of $n_d = 1.53$ with a dispersion of $\nu = 45$. 
Another important property for an optical material is its compatibility with optical thin film coatings. Early experiments showed that the material is quite stable in conditions required for Anti-Reflection (AR) coating processes. The lenses produced are routinely sent to a commercial coating house and given AR coatings.

Although the material has a large inorganic component, the molecules are linked by organic bonds. The post processing bake of the lenses promotes crosslinking within the material and improves the hardness and stability of the lenses, but the surfaces remain softer than glass. The surfaces can be easily scratched and would not be suitable as an exposed surface for a long use instrument. This is not expected to pose a problem for the intended application where all surfaces are protected and the lifetime of each unit is short as with a disposable one-use endoscope.

Another concern is the transmission of the material. Figure 4.1 shows the transmission of the material across the spectral range for which it is intended to be used. The measurement was taken with a Cary 500 8.01 using quartz (SiO$_2$) substrates. One substrate was coated with hybrid glass and the other is left uncoated for a baseline measurement. The difference of transmission between the two substrates is what is measured. The thickness of the hybrid glass was about 7 µm which caused interference that shows up as the oscillations observed in the plot. The slight dip at 800 nm corresponds to a detector switch in the instrument. Over the visible spectrum there is an overall slope indicating slightly more absorption in the blue. This is confirmed visually, as centimeter thick quantities of the material take on a
slight yellowish tint. This color is only observed for volumes of material hundreds of times thicker than those used in the printed lenses. A Gaussian filter was applied ($\sigma = 15$) to average out the oscillations and more accurately represent the material transmission. It should also be noted that the difference in index of the quartz substrate and the hybrid glass material leads to a slight increase in the reflection from the hybrid glass. Equation 4.1 gives the reflection for normal incidence from one medium to another.

$$R = \left(\frac{n_2 - n_1}{n_2 + n_1}\right)^2$$ (4.1)

Calculations at 587 nm give 3.48% reflection from quartz ($n = 1.46$), and 4.38% reflection from hybrid glass ($n = 1.53$). With a difference of 0.9% reflection, the bulk of the loss shown in figure 4.1 is likely due to this difference in reflection. The thickness used in patterning lenses is up to ten times thicker than the film measured here, but the transmission remains over 99% over the wavelengths of interest. It follows that absorption is not a significant issue for lenses made from hybrid sol-gel glass.

Finally, the property that really enabled fabrication of refractive elements is the ability to produce a thick layer. Most photoresists are designed to work at a thickness of less than a few microns, but refractive lenses need a sag or thickness much greater. The hybrid glass can be spin coated to over 200 $\mu$m in thickness. The process for coating photoresist typically uses a spinner to evenly spread the material.
Figure 4.1: The transmission of hybrid sol-gel glass remains better than 99% over the intended spectral range. The oscillations are due to thin film interference from the 7 µm layer of material. A gaussian filter was applied to show a more realistic transmission curve. The inset shows the transmission over the full range of the instrument.
across a substrate. Sheats and Smith [20] provide a good basic introduction to spin coating of photoresist. The thickness of the coating is determined primarily by the photoresist viscosity and the spinner’s acceleration and speed. The result is a very uniform and well controlled thickness. A very convenient feature of photoresist is that it comprised of dissolved solids in a volatile solvent. Once the material spreads out on a substrate, the increase in surface area to volume ratio allows the solvent to quickly evaporate leaving behind the solids. The solid film is then easy to handle and process. This process works well for the thin films of photoresist, but the hybrid sol-gel glass must work differently. With the hybrid glass, the thickness is so great that solvent can not easily escape the film. The material is therefore not a solvent based liquid, but actually a liquid soup of precursors.

The small precursor molecules form a liquid with a high viscosity. The high viscosity allows the spin coating process to create films of hundreds of microns thick. However once the spinning has stopped, the material is still liquid and will flow. This flow can easily lead to uneven films and hard to control thicknesses. Additionally, hybrid glass continues to get thinner so the time the substrate is spun is as important as the speed, unlike photoresist which stops thinning once the solvent has evaporated. Fortunately, the viscosity of the material is comparable to honey, so the material flow is quite slow. The uniformity is maintained by exposing the substrate quickly after spin coating. If processed fast enough, the edge bead (material that builds up on the edge of the substrate during spinning) does not
have time to flow back to the central region of the substrate. This central region of
the substrate is then still a uniform thickness of material that can be patterned to
produce consistent lenses.

4.3 Lithographic Patterning

A critical step in producing high quality lenses is the lithographic exposure of the
photosensitive hybrid glass. This required an extremely uniform exposure beam, a
reduction in reflections during exposure, and a high quality grayscale photomask.
The details of grayscale photomask design are found in chapter 3. This section will
describe in detail the steps used to pattern the lenses.

4.3.1 Sample cleaning and preparation

Before any lens patterning can begin, substrates must be cleaned and prepared.
The cleaning process can have a surprisingly dramatic affect on the quality of the
lenses produced. The substrates used to back the printed lenses are typically #1
cover slips. These coverslips are 140–170 µm thick borosilicate glass. This material
has an index of refraction of 1.524 at 546 nm. The glass uniformity is not great
over many samples, but over the small feature size of the lenses, the thickness
variations are negligible. Any coverslip material could be used, but a convenient
50 mm diameter circular size is available from ProSciTech [21]. The cover slips
are often dusty and dirty and require cleaning. The cleaning process begins by
submerging the substrates in an ultrasonic solvent bath such as isopropanol. The ultrasonic agitation helps to remove dust and particles while the solvent attacks any soluble particles and displaces adsorbed moisture. The substrates are then rinsed in deionized water and blow dried with a nitrogen gun.

To promote adhesion of the material to the glass substrate, the substrate must be free of water. At a microscopic level, water vapor from the air can adsorb onto the glass surface and dramatically change the surface chemistry weakening the bonds between the hybrid glass and glass substrate. The effect is a peeling or delamination of the lenses after post-bake, or sometimes even weeks later. To remove the surface water, several methods have been used. A plasma cleaning in which substrates are placed in partial vacuum and microwaves ignite a plasma along the glass surface was predominately used. The combination of microwaves, heat and vacuum were very effective in removing water and producing a clean substrate surface. Another method to dehydrate the substrates is a simple hotplate bake at 150\degree C for 20 minutes. This method worked well enough once the material chemistry had been modified to increase adhesion. An additional increase in the adhesion was attained once the hot deposition method described below was employed.

4.3.2 Spin coating

In earlier procedures, the substrate was placed on the spinner and 0.25–0.5 mL of hybrid glass would be deposited on the substrate. The spinning action was used to
spread the material and achieve film uniformity. This method proved inconsistent and required much more material to completely coat the substrate than was actually used. This is typical for spin coating since the final film thickness is a function of viscosity, spin speed and other material properties. The material flows outward from the center and excess material piles up on the edge forming an ‘edge bead’ or thicker zone near the edge. Excess material is thrown off if the edge bead gets too large, but the film thickness away from the edge remains constant and consistent. However, the hybrid glass remains liquid throughout the spinning process, so material continues to pile up as an edge bead and is eventually thrown off. The material thickness in the central region is as much a function of spin time as speed and viscosity. It is easy to see that the process of throwing off excess material is not constant and as the material thins, the rate of thinning will decrease. That means the final thickness is somewhat dependent on the initial thickness. For this reason, the spin coating process was modified as described next.

The clean dry substrates were placed on a hotplate set to 80°C and a precise volume (typically 0.1 mL) of hybrid glass was deposited onto the center of the substrate. As the material warms, a decrease in viscosity is observed and the material becomes easy to spread around the substrate by tilting in a circular motion. If the liquid glass is deposited on a cold substrate and then heated, a change in the material at the glass is observed that allows the material to be more easily spread. This observed change appears to be a “wetting” of the glass surface and is hypothesized
to be bonds forming between the material and glass. The coverslips will sometimes even warp from stress, but relax again once the material is spread evenly. Once the material is spread around on the substrate so that the entire surface is ‘wetted’, the substrate is placed on a second hotplate at 120° C for 5 minutes. The substrate is then placed on a heat sink and cooled to room temperature for 1 minute. This ensures a constant temperature (and so a constant viscosity) during spinning. At this stage, the substrate has a thick, uniform and consistent volume of viscous liquid material on its surface.

Next, the substrate is placed on the spinner with care to keep the substrate level to avoid flow that leads to wedge in the material layer. The spinning parameters are tuned to provide material thickness appropriate for the desired lens sag. Typical values are 30 seconds at 600 rpm. After spinning, the edge bead is clearly visible around the outer 1–5 mm of the substrate. The edge bead will immediately begin to flow like a slow wave back towards the center of the substrate. After one minute, the front can progress as much as a centimeter, so the substrate must be moved quickly to the mask aligner for exposure. After exposure, the material is polymerized and the edge bead is frozen. It is useful to examine the edge bead to ensure that the front did not have time to flow back to the region where the lenses and features were patterned.
4.3.3 Exposure

The exposure of the material is easy to control with a mask aligner, but there are a few differences from a typical photoresist process. The material is one of extremely large dynamic range and sensitivity. This is advantageous for grayscale patterning, because it permits precise patterning of a very large range of heights. The disadvantage is that the material is sensitive to any variation in exposure due to variation in the exposure beam or reflections. For a 100 μm thick lens, a 1% variation in exposing beam could lead to as much as a 1 μm change in surface height. Early experiments used an Carl Suss MJB3 mask aligner, but the exposure uniformity caused variations in lens thickness. Recently a Carl Suss MA6 was used that provides better uniformity and more consistent exposure. Since the substrate material is transparent, the exposing beam passes through and can reflect off the chuck. Even using blackened low reflection chucks, the reflections were enough to cause a print through of the chuck or interference fringes superimposed over the lens pattern. To eliminate this problem, the substrates were placed on a wafer coated with camera black. A surface blackened with candle soot also worked well, but was easily damaged. Since the hybrid glass material is still liquid during exposure, it cannot come into contact with the mask like photoresist. The mask is held in carefully controlled proximity to the material using spacers. The MA6 has a mask chuck with special features to control the spacing. Another option is to use additional coverslips as surrounding shims to control the mask height above the edge.
bead. Typical exposures on the MA6 were 15–20 s using the I-line filter. During exposure, the material polymerizes and crosslinks. The degree of polymerization is proportional to the exposure.

4.3.4 Development Process

After the exposure, the sample is developed in a solvent bath of isopropanol (IPA) and Methyl-isobutenyl-ketone (MIBK) or acetone. Developer solutions were varied to test linearity, but a typical solution was five parts IPA to one part MIBK. During development, the material is slowly dissolved into the solvent at a rate proportional to the degree of polymerization. The sample is held with tweezers and slow gentle agitation using a slow waving motion is used to disrupt convective flow. If no agitation is used, the material dissolving into solution at the surface will sink and sets up a laminar flow across the surface of the hybrid glass. This flow produces a directional preference in development and skews the lens shapes. Depending on the exposure and thickness of the material, the time needed to develop away the unexposed regions surrounding the lenses is from one to three minutes. After the material clears in the unexposed regions, the sample is removed and dipped in ethanol for a few seconds to rinse away any developer and stop development. The substrate is then laid flat and blown off with a nitrogen gun.

The dry sample can then be baked at 110°C for a few hours to complete crosslinking of any available bonds. This post-bake procedure results in a hard stable mate-
rial. Measurement of samples before and after post-bake show that the lenses will shrink in thickness by up to 10%. This is probably due to densification as solvent that soaks into the polymer matrix is expelled.

4.4 Summary of lens fabrication

The following is a list summarizing the steps involved in lens fabrication. Some example parameters are given, but these parameters are only a starting point and may need tuning to produce the desired lens shapes. Example process steps:

1. Heat substrate on a hot plate at 80° C

2. Add 0.1 mL (3 drops) hybrid glass to center of substrate with a syringe

3. While hot, tilt to flow material around to 'wet' entire substrate

4. Bake on hotplate at 105–120° C for 5 minutes

5. Cool on heat sink to room temperature for 1 minute

6. Spin at 600 rpm for 30 seconds

7. Move to mask aligner immediately, keeping flat to avoid flow

8. Use spacers or gap setting to keep mask above edge bead

9. Expose for 16 seconds using I-line filter on an MA-6 mask aligner

10. Develop in 5:1 IPA: MIBK for 3 minutes
11. Hold substrate with tweezers and gently swish during development

12. Dip/rinse in ethanol or IPA bath to quench developer

13. Gently blow dry with dry nitrogen gun or filtered compressed air

14. Post bake at 110° C for 2 hours to complete crosslinking
CHAPTER 5

SYSTEM ASSEMBLY

5.1 Introduction

The inspiration leading to the development of a miniature microscope system was
the ability to assemble microoptics and other components with high precision[6].
Recent advances in micromachining techniques could produce slots in silicon with
submicron precision and the idea was to use these slots as a mechanical alignment
base on which lenses could be precisely affixed to produce a miniature optical system.
This high-precision mechanical substrate was named the Micro-Optical Table or
MOT.

The MOT was initially micro-machined using a lithography and etch process
known as the Bosch process and later using the LIGA process. This use of lithog-
raphy to define the mechanical base of the optical system provided slot features
with sub-micron alignment precision between components. It was conceived that
an optical system could be assembled using the MOT in which the assembly errors
were less than the optical tolerances. The optical components could then be assem-
bled without requiring the labor intensive and costly adjustments and tuning that
is normally required during assembly of precision optical systems.
Much of the knowledge learned over the course of this work is not of a quantifiable nature. Nevertheless, the lessons learned during years of trial and error accumulate to an invaluable wealth of understanding essential in any attempt to reproduce such efforts. This chapter is primarily a description of the art of assembling the 4M prototype systems.

5.2 MOT Substrates

The MOT design began with very simple rectangular slots etched in silicon wafers. Optical elements were designed with simple tabs on one side that were to be inserted into the slots and constrain the position of the optical element. It was quickly demonstrated that something more sophisticated would be required.

By adding a cantilever spring to the back side of the slot and small alignment grooves to the front side, the optical elements could be slipped into place and held by the force provided by the spring. Complimentary ridges were patterned on the elements that sit into the grooves providing constraint over lateral displacement and tip/tilt. This scheme is illustrated in figure 5.1.

The springs to be machined in silicon were designed by Dr. Chris Tigges of Sandia National Laboratories. A finite element analysis of the spring design was carried out by Bahattin Kilic and Prof. Erdogan Madenci of the University of Arizona. The spring shape was etched with the Bosch process at Sandia National Laboratories by Christi Willison and Dr. Randy Shul.
Figure 5.1: The MOT substrate holds the lens elements in place by mating high precision lithographically defined surfaces while less precise component edges do not make contact. The slots in the MOT (brown) have grooves patterned into the front edge of each slot. The lens elements (blue) have ridges that fit into the MOT grooves. The element is lowered into place until the alignment block comes to rest on the top surface of the MOT substrate. Only the front edge of the MOT slot is shown. The back of the slot contains a cantilever spring that provides force to hold the lens element against the front of the MOT slot.
Initial attempts at fabricating the MOT substrate revealed a problem with lens insertion and alignment. As the etch progresses down through the silicon wafer, the rate of etch depends on the shape and area of the pattern. Smaller holes tend to etch more slowly than larger holes. The result was that certain regions of each slot etch through to the back of the silicon wafer before other regions. Additionally, as the etch propagates, a rounded bottom develops in the feature being etched. These two physical processes result in an artifact referred to as a foot at the bottom of each slot or spring features. The foot is a small rough sloped buttress that intrudes on the slot area. The roughness of this remaining material caused excessive friction with optical element during insertion and cantilever springs would often break off. If elements were successfully inserted, the foot would cause a misalignment of the optical element which relied on the straightness of the slot’s sidewall to constrain the vertical angle of the lens. Careful adjustment of the Bosch process by Dr. Shul eliminated the foot, but silicon remained a problematic material due to its brittle nature. Nevertheless, many working 4M optical systems were assembled using the silicon MOT substrates.

In 2004, an alternative process for fabricating the MOT was pursued. A process known as LIGA used Deep X-Ray Lithography (DXRL) to produce the prismatic parts of the MOT substrate in metal materials. The process uses high energy X-rays from synchrotron radiation sources to expose PolyMethylMethacrylate (PMMA) in thicknesses of hundreds of microns. The exposed PMMA is developed and then
used as a mold in which metal is electroplated. The X-ray beamline is collimated to better than 0.1 milliradians and the wavelength of single digit Ångstroms eliminates diffraction effects for features on the scale of microns. The resulting metal parts are less rough and the vertical walls in the slots are more parallel and precise than the silicon counterparts. In addition, the metal is much more forgiving than silicon MOT substrates. Although Young’s modulus for silicon is nearly identical to that of the metal used in electroplating, the catastrophic nature of the failure in silicon results in frequent breakage. The metal parts do exhibit more deviation from a plane than silicon parts due to buckling under internal stress of the electroplated metal. The result is warping of the part, but this warping was observed at less than a few microns over the 15 mm of length of the MOT substrate. This keeps the lens positions within the optical tolerances of the system.

5.3 Alignment Features and Insertion

The features of both the lens elements and the MOT substrate were designed to constrain all degrees of freedom. Ideally, mounting designs should be kinematic providing constraint over all degrees of freedom without over constraint. Overconstraint leads to uncertainty in alignment (potential for rocking) or stress and warping of parts. Consider the difference between a three-legged stool versus a four legged chair on uneven ground.

The fabrication technology used in the 4M device does impose some limita-
tions on the mounting design. The prismatic nature of the MOT substrate and the mechanical strength of the hybrid glass require that some deviation from perfect kinematic mounting be allowed. First, the V-shaped grooves in the front of the slot are matched to ridges on the front of each lens element. The force provided by the cantilever spring pushes the ridges to the center of the grooves. This centering provides alignment laterally. The length of the groove/ridge contact provides constraint from rotation of the lens. The contact between the ridge and the vertical wall of the groove constrains tilt of the lens. The last degree of freedom is the vertical position which is constrained by a block feature that protrudes out from the lens element and overhangs the top surface of the MOT. The lens element is tapped down until this block makes contact with the MOT surface. These features act together to constrain all degrees of freedom of each lens element. Figure 5.2 shows how the lens element with alignment features is held in place by the spring and grooves of the MOT substrate.

Any deviation of each element from its exact intended position are due to errors in fabrication or patterning of the features. These errors depend on the feature. The LIGA fabricated parts are typically accurate to better than 0.1 microns which is far more precise than the grayscale patterned lens element features. The errors can be considered to be completely due to errors in the lens element fabrication with the one exception of stress induced warping mentioned above.

The actual assembly process was performed using a combination of micrometers,
Figure 5.2: The vertical ridges printed on the lens element fit into complementary grooves in the front wall of the MOT slots. The cantilever spring provides a small force to hold the element in place. Once the ridges/grooves are mated, the element is pressed down until the alignment blocks contact the top surface of the MOT substrate. The top view at the right shows the rectangular ridges seated in the V-shaped grooves of the MOT slot.

stages, stereoscope and steady hands. When using the silicon MOT substrates, breakage was common, and use of a specially designed tweezers stage developed by Robert Bedford was necessary. LIGA based metal MOT substrates are much more forgiving, and assembly by hand using tweezers is possible and usually much quicker. Early thoughts on the assembly assumed that the lens element would be inserted in a wide slot to the side of the cantilever spring. The element would then be slid into place between the spring and slot wall. When the ridges aligned with the grooves, the part would snap into place. In practice this worked, but led to chipping of the ridges due to high roughness of the silicon sidewalls and the resultant binding and slipping of the part often caused breakage of the springs.

A better method was to simply use the bottom edge of the lens element to pull the
spring back and then lower the element directly into the slot. This method required some practice, but worked reliably. The greatest advantage of this small change is that the slots no longer needed the extra width to accommodate the insertion. Almost half the the width of each slot could be removed, reducing the overall width of the 4M device.

The force provided by the current spring design is enough to hold the lens elements in place temporarily, but shocks from dropping or bumping the system can be enough to dislodge the lens elements. Several cantilever spring designs were tested using different spring thicknesses. The spring designs are listed in table 5.1. The springs provide from 20 to 120 mN of force and the higher force springs required less deflection. The lens elements are printed on coverslips with a very loose thickness tolerance of 150±20 μm. In practice the lens elements are very close to 150 μm thick, but a variation of only 5 μm would double the deflection of the thicker springs and would usually result in catastrophic failure. The thinner, low force springs were less prone to breakage and still provided enough force to hold the lens element in place during assembly. The 40 μm thick spring worked best and was used in all later designs. The spring contacts the lens element in the center between the two outer points of contact of the ridge/groove features on the front of the element. The result is a bridging of the lens element between the two outer points of contact. This bridging results in a 1-2 μm deflection in the center of the tab of the lens element. Clearly, the force provided by the spring should not be increased too much. The
Table 5.1: Several designs for the silicon spring were tested. This table shows the spring thickness, intended deflection, and resulting force on the lens element.

<table>
<thead>
<tr>
<th>Spring thickness (µm)</th>
<th>Deflection (µm)</th>
<th>Force (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>29</td>
<td>0.017</td>
</tr>
<tr>
<td>40</td>
<td>19</td>
<td>0.074</td>
</tr>
<tr>
<td>50</td>
<td>7.7</td>
<td>0.034</td>
</tr>
<tr>
<td>60</td>
<td>7.5</td>
<td>0.110</td>
</tr>
<tr>
<td>80</td>
<td>6.6</td>
<td>0.120</td>
</tr>
</tbody>
</table>

springs should be regarded as merely an aid in assembly alignment. Once the lens elements are inserted, a small amount of optical epoxy is used to cement them in place.

5.4 Mounting of the spherical glass lens

One problem with the assembly of the 4M system is the mounting of the first element. As discussed in chapter 2, the first element is a conventional plano-convex glass lens. This lens can not be mounted using alignment features like the printed lenses.

One solution was to insert the glass lens in a precise hole in the MOT substrate as shown in figure 5.3. This hole had a diameter only a few microns larger than the outer diameter of the lens. The lens would be placed in the hole and cemented in place with UV-curing epoxy. The z-axis position of the lens was controlled by placing the lens on a glass slide and placing the MOT over the lens, lowering it down around the lens. Depending on the thickness of the MOT substrate, the bottom surface of the MOT and lens would be flush, or precision shims or spacers
could be used to keep the MOT a fixed distance above the lower surface of the lens during cementing.

By embedding the first lens in the MOT substrate, the optical axis needed to be folded 90°. This was easily accomplished by a fold mirror, but careful consideration was needed to ensure precise alignment of the fold mirror. Since the mirror has no power, the lateral position is of no importance, but the mirror surface must be constrained to the correct plane. This was accomplished by again making use of the precision of the lithographically printed features of both the printed lens elements and the MOT substrate. On the substrate, a thin slot is made that the lower edge of the mirror rests in. The front edge of the slot is in contact with the front surface of the mirror providing a pivot or line intersection with the mirror plane. At the
Figure 5.4: The fold mirror position is constrained by the edges of a 75 µm wide slot in the MOT substrate and the top edge of alignment blocks above the first printed lens element. The position of the surface of the mirror is thereby constrained only by lithographically defined features. The lens elements and mirror are cut to the proper size using a dicing saw which has limited accuracy of tens of microns. If the alignment were dependent on the edges cut by the saw, the positional accuracy would be quite poor. This geometry affords a great deal of tolerance. The lens element can be cut to any dimension and the fold mirror has a 70 µm tolerance (for 50 µm tall alignment blocks).

top of the mirror, the front surface rests on an alignment block on the printed lens element above the lens as shown in figure 5.4. This provides a completely constrained position for the mirror ensuring 45° angle and correct position for the fold mirror. Since the mirror leans against the printed lens, it was dubbed a 'lean-to' mirror.

The lean-to mirror design successfully positioned the fold mirror, but the embedded glass lens was not constrained as precisely as the other elements. To improve the overall system performance, a better glass lens mounting mechanism was desirable. The LIGA fabrication technology made possible a much needed solution. The lens holder was made from three layers of LIGA parts. On one side, a plate with a round aperture just smaller than the lens diameter provided a backing that constrained one surface of the lens in the z-axis. This layer also comprised a tab that could
Figure 5.5: The glass lens is mounted in this miniature self-centering mount. On the left is the back of the mount with the circular hole that centers the spherical surface. Right is the spiral spring-loaded front of the lens holder.

be inserted into a precision MOT slot to position the lens mount. On the opposite side of the holder, the plate had a round aperture suspended by spiral springs. This aperture was able to float to accommodate variation in the lens thickness and to provide some force to hold the lens against the fixed aperture. Between these two plates was a spacer layer that contained spiral springs intended to center the lens by providing a center force around the cylindrical edge of the lens. Testing of the lens holder showed that the centering springs were not as effective at centering the lens about its cylindrical edge, but the fixed aperture was centering the lens about the spherical surface. This proved to be an advantage, since from an optical perspective, the alignment of the spherical surface is more important than the alignment of the lens edge allowing for looser tolerance on the fabrication of the spherical glass lens.

The lens mount design allowed for the optical system to be unfolded providing a
straight system design capable of looking forward rather than to the side. By implementing designs similar to the lens holder, other components such as a fiber holder and condenser lens mount were designed and constructed by Todd Christenson at HTmicro[22].

5.5 Cementing

Once the elements was inserted, a small amount of epoxy was used to permanently cement the elements in place. This epoxy can easily wick into the space between the lenses if too much is used. Only small fractions of a picoliter should be applied at a time. This tiny volume is not easily dispensed, so instead, best results were obtained by dipping or scraping the tip of a hypodermic needle into a small drop of epoxy. The bead of epoxy collected on the tip of the needle should be no more than 30-50 microns in diameter. This bead can then be safely applied to the MOT substrate near the slot edge and teased into the slot.

Once some epoxy contacts the interface of the lens element and MOT substrate, it quickly wicks into the narrow spaces around the element. To facilitate this wicking process, some slots were designed with 10 micron wide trenches leading away from the slot. A small pool of epoxy could be applied over the trench and would wick into the slot around the lens element. The epoxy was then cured with an Ultra Violet (UV) lamp.

The epoxy also acts as a sealant against fluids that come in contact with the
Figure 5.6: A complete 4M system assembled with all components shown on a US penny for scale. A large core optical fiber delivers the illumination. The grating position is controlled by applying voltage to the electrostatic comb-drive actuator. The three printed lenses are held in place by alignment features in the MOT substrate. The condenser lens and first glass lens are assembled lens holders and mounted in bistable spring latched slots. The beam splitter or dichroic folds the image out to the side of the device where the CMOS chip will eventually be mounted to the side wall of the enclosure (not shown).
MOT substrate during imaging. Since the device works in immersion, the fluid surrounding tissue samples could easily contaminate the optical surfaces. After the 4M system was assembled and the components were cemented in place, all holes and spring slots in the MOT substrate were filled with epoxy to seal the lower surface of the system. Likewise, the lens mount for the first glass lens is sealed with care to avoid contaminating the clear aperture with epoxy. This is accomplished by introducing very small amounts of epoxy at a time and curing the epoxy before there is time for it to spread into the clear aperture. The viscosity of the epoxy tends to help by keeping the epoxy contained in the small cracks and vias surrounding the lens. The prototype 4M systems have only been used to image tissue samples and biopsies on slides, but ultimately, the entire device will be sealed to avoid contamination of the optical surfaces and electronics when the device is used inside a body cavity. Several methods for encasing the 4M device include diffusion bonded LIGA components or coating the outer walls of the 4M device with an encapsulant.
6.1 Introduction

The success of the 4M project relies on the synergy of several new technologies and ideas. To evaluate the merit of these technologies, several methods of testing and metrology were utilized for different aspects of the system. Metrology of individual lens elements is required to validate the grayscale lens printing technology. Verifying alignment of elements relative to the substrate validates the MOT concept. Tilted or slanted edge measurements were used to test the imaging performance of the system as a whole. Imaging tests of an artificial object were used to demonstrate the effectiveness of tilted component design to remove background and ghost images. Finally, the ultimate test is the successful imaging of tissue and other biological samples. The results of surface metrology, MTF measurements, and ghost image removal are described in this chapter. The imaging results for biological samples are presented in chapter 7.
6.2 Lens surface metrology

Deviations of an optical surface from the ideal surface occur over a range of different length scales [23]. The term roughness is used to describe very high spatial frequencies with length scales on the order of microns. One the other side of the range, low spatial frequencies with length scales comparable to the size of the lens are referred to as the optical figure. The deviations from an ideal surface occur at all length scales in between these two extremes.

The effect of these deviations on the optical performance also varies continuously with these length scales. At the high spatial frequency end of the spatial-frequency spectrum, roughness corresponds to scattered light that results in reduced image contrast. The light is scattered into all angles and becomes evenly distributed across the image. One the other side of the scale, optical figure introduces very small angle deviations or small wavefront errors. These errors lead to an increased spot size, or what looks like image blur. Deviations also occur on a length scale between these two extremes resulting in small angle scattering. This small angle scattering causes a halo surrounding bright points in the image [24].

6.2.1 Lens surface roughness

The printed hybrid sol-gel glass lenses do exhibit surface deviations at all these length scales to varying degree, and it is useful to measure the surface to ensure that these deviations do not limit imaging performance. In most conventional lenses, the
measurement of deviations across this range of scales would require several metrology methods. However, the small extent of the printed lenses allows measurement of the entire range from roughness to optical figure using an optical profilometer. An optical profilometer uses white-light interference to probe the surface height of a sample through a microscope objective. The lateral sampling depends on the magnification or objective used, but can be as small as 0.09 µm. The field of view of the instrument is limited, but newer instruments can step and repeat and then stitch the fields to create one surface profile of a large component. The measurements of the printed lenses were taken with a Veeco NT2000 or a Veeco NT9800 prototype [25].

Roughness was measured using the 50× objective producing a lateral sampling of 0.165 µm and a FOV of 120×92 µm. Over this region, the lens curvature is apparent and can be seen in the left of figure 6.1. At the right of the figure, the best fit spherical term is subtracted leaving a view of the deviation from a sphere.

To measure the high spatial-frequency roughness, the optical profilometer was used with a 50× objective and a 1× field of view lens. This setup corresponds to a lateral sampling of 0.09 µm on the lens surface. The profile map is recorded and a 25×25 µm square sub-region is selected for the calculation of roughness. Choosing this small region ensures that the lower spatial frequency errors are not considered. The roughness increases from the center of a convex lens (4.4 nm RMS) to the edge (13.5 nm RMS), probably due to magnification of variations as the surface is developed away during lens fabrication. Since the center of the convex lens is not
Figure 6.1: Lens surface measurement using a Vecco NT2000. At the left is the raw measurement with a \(120 \times 92\ \mu m\) FOV and 0.165 \(\mu m\) sampling. At the right is the same measurement with the spherical term removed showing the departure from sphere. The RMS surface roughness (Rq) for this measurement is 26 nm after the curvature is subtracted.

Developed very deep, the roughness remains small. Near the edge of the lens the full initial thickness of material is developed away and variations in the material cause a greater magnitude of roughness near the edge. Figure 6.2 shows the comparisons for three lenses measured at the center and edge of the lens. In the figure, a larger field of view is used and deviations of a slightly larger length scale are most prominent. At the larger FOV, the deviations on a scale of tens of micrometers are the dominant error. The corresponding increase in FOV results in a slight increase in the RMS roughness to around 20-35 nm.

The mid-range spatial frequencies with periods on the order of tens or hundreds of microns exhibit a roughness of 30-50 nm RMS. This range of roughness is measured with the optical profilometer using a 50\(\times\) objective and 0.5\(\times\) FOV lens providing a field of view of 243\(\times\)185 \(\mu m\) and a pixel sampling of 0.331 \(\mu m\). This type of
Figure 6.2: These measurements show the departure from sphere at the lens center and departure from cylinder at 80% of the lens aperture for three lenses. The RMS roughness increases slightly from the center to the lens edge, but remains below 40 nm at this 0.165 μm lateral sampling over the 121×92 μm field of view. Cylinder is removed from the edge measurements because the aspheric nature of the lenses means cylinder is the dominant term in the measurement.
mid-spatial frequency roughness will tend to contribute to a halo around images of point-like objects.

While measuring printed lenses from several different substrates, it was noticed that the pattern of deviation was similar for the same lens on different substrates. Further analysis demonstrates that there is a strong correlation of the apparent random pattern between substrates. Figure 6.3 shows measurements for “LensD” from three different substrates (i.e. different printings). The figure also shows difference measurements obtained by subtracting one surface from the other. The difference measurement shows that the RMS roughness over hundreds of microns is only around 20 nm. There is no observed correlation between the same lens within a single substrate. This strongly suggests that the surface deviations are mostly caused by some systematic error in the exposure process. Such systematic error could be due to irregularities in the illumination or imperfections in the grayscale mask. In either case, it is promising to note that the roughness is not a limitation of the material. If the roughness is introduced by imperfections in the mask, then improvements could be expected by improving the mask technology or by using a new method of varying exposure. If variations in the illumination is the culprit, a possible solution would be to vary the illumination during the exposure to average out any fluctuations. This could be accomplished by creating a mount that fixes the relative position between the mask and substrate while allowing motion relative to the exposure beam.
Figure 6.3: The measurements on the left are of the same lens (row 1, lensD) from three different substrates with the spherical term subtracted. Several dust particles were masked out (black circles) to allow uniform scaling of the color map. On the right are shown the result of subtracting these surface maps from each other. The correlation strongly suggests a systematic error in the exposure process exists. This error is likely due to imperfections in the grayscale mask or non-uniformities in the illumination. The systematic nature of these errors indicates that it is possible to eliminate these deviations. The circular ripple patterns are measurement artifacts due to vibration in the interferometer during measurement. The subtracted maps show that the surface deviation due to random errors are around 20 nm RMS even over hundreds of microns.
6.2.2 Lens shape

So far, the deviations of lens surface have been examined on the small and middle length scales. Next, variations of the surface that influence the lens shape are examined. Deviations from the ideal lens shape are on the length scale of a few cycles per aperture and are referred to as the optical figure. This is the realm of the Raleigh criterion, where the system can be considered diffraction limited if the wavefront error is less than one quarter of the wavelength.

This scale of measurement is usually done using a Fizeau interferometer or other more traditional methods of optical testing. The aspheric and asymmetric nature of the printed lenses limits the utility of these techniques. Fortunately, the optical profilometer has the ability to measure the whole lens element when used in stitching mode. For the 4M system lenses, the largest lenses are less than 2 mm in diameter, and the profilometer can be used to measure the entire surface. By using a low magnification, the entire lens will be within the field of view of the profilometer, but the entire surface cannot be measured. For lenses, the issue is the slope of the lens surface. Two problems arise as the slope of a surface increases. The first is that the fringe spacing decreases and for a given magnification, at some point the detector is unable to sample the fringes resulting in no signal. The second problem is the result of the reflection being tilted off-axis for a sloped surface which reduces the amount of light returned to the interferometer and so reduces the signal. Fortunately, the 4M system lenses have a limited slope and can be measured using the 50× objective.
Surface Statistics:
Ra: 26.16 um
Rq: 28.43 um
Rz: 93.21 um

Set-up Parameters:
Size: 1317 X 1279
Sampling: 1.41 um

Processed Options:
Terms Removed:
Tilt
Filtering:
None

Figure 6.4: A stitched surface map of lens B and the alignment blocks above and below the lens surface. The total lens sag is 75 \( \mu \)m. The black areas are regions of the lens element where the slope exceeded that which could be measured with the 50\( \times \) objective.

The NT9800 has the ability to step and measure and stitch measurements together to produce large maps of the surface from multiple measurements. The result can be seen in figure 6.4 which depicts a surface map of lens B and the alignment blocks above and below the lens. The data from the stitched measurement can be used to evaluate the lens shape or optical figure.

The surface fabrication error is highly process dependent, but when process parameters are tuned properly, the primary fabrication error is a linear scaling of the surface function. This error is most likely due to an over or under development of the exposed hybrid sol-gel glass. Since the fabrication process can be tuned
to produce surface heights proportional to mask optical density, an over or under development will simply expand or shrink the surface function. This effect is shown in Figure 6.5(c) where a scaled version of the designed surface function closely matches the fabricated surface. The effect of this scaling error was investigated by scaling the lens function in the optical design. The printed lenses were scaled by ±5% corresponding to lens sags of 76-84 μm and the most significant effect was an overall change optical power. By compensating for this change with some defocus or change in the image plane location, the system remained diffraction limited for this scaling error. The conclusion is that the component of fabrication error that is due to a linear scaling of the lens surface function results in a change in system power and can be compensated for by refocusing. The system will have a slight change in magnification, but remains diffraction limited.

6.3 MTF and Tilted Edge Measurements

In addition to measuring the individual lenses, it is also useful to make some quantitative measurement of the system as a whole. One very useful method to characterize an optical system is to measure the Modulation Transfer Function (MTF) of the system. The MTF is a function that describes how the contrast of frequencies is transferred through and optical system. The MTF is the modulus of the Optical Transfer Function (OTF) and cannot be negative. For a perfect diffraction limited system illuminated incoherently, the OTF is the autocorrelation of the pupil func-
Figure 6.5: A lens surface profile along the x-axis showing (a) both the designed profile (solid gray) and the fabricated surface (black points) as measured using a Wyko optical profilometer, (b) the lens figure error, (c) the lens surface compared to the designed profile linearly scaled by 80% to match the fabricated lens surface, and (d) the error of the lens surface and the scaled function. This shows that the error in lens shape is primarily a linear scaling error.
tion. Equation 6.1 shows the relationship where $P$ is the pupil function and $a$ is a normalization constant.

$$MTF = |OTF| = a|P \ast P|$$  

(6.1)

For optical systems that are not diffraction limited, the MTF is reduced. A plot of the MTF for an optical system can provide a good idea of the optical performance of that system. Measurement of the MTF by successively measuring the contrast for different grating pitches would be tedious, but a fast method was described by Tzannes[26]. The idea is that the Edge Spread Function (ESF) can be used to calculate the MTF, but in most optical systems, the detector sampling is so coarse that the ESF can not be obtained. However, by tilting and edge slightly relative to the detector grid, many ESF profiles can be measured with slight displacement of the sampling for each row. By averaging the rows in the image, an accurate ESF can be obtained. The MTF is then calculated as the Fourier Transform of the derivative of the ESF as shown in equation 6.2. A program was written by Chidley that calculates MTF curves for images of tilted edges and is described in his dissertation[27].

$$MTF = \mathcal{F}\{\frac{d}{dx} ESF\}$$  

(6.2)

The 4M device is designed to work with a custom CMOS imaging detector with $4 \times 4 \mu m$ pixels. This detector was still under development and so all imaging tests
were performed by relaying the image to a larger format CCD camera. The relay system used two achromatic lenses with focal lengths of 75 mm and 15 mm to provide 5× magnification. The large format CCD has 6.45×6.45 µm pixels and a magnification of only 1.5× would provide sampling comparable to the CMOS detector, but some over sampling during image testing was useful. The relay system is arranged to relay the intermediate image formed by the 4M system to the CCD with a total magnification of 20×. There exists the potential that the relay system could degrade the image formed by the 4M device. The MTF of the relay system was measured independently from the 4M system to establish that the relay optics were not limiting the performance. The measured MTF is shown in figure 6.6. The object was a chrome grating transilluminated with a diffuser.

Once the relay optics were aligned and tested, the 4M device was placed in front of the relay optics so that the image is relayed to the CDD. The object was again transilluminated, but in this instance the grating was placed in water immersion to simulate the designed imaging conditions. The MTF measurement of the 4M device combined with relay system is shown in figure 6.7. The MTF of this 4M device drops to zero at around 500 line pairs per mm (lppm). This corresponds to an imaging resolution of just less than 2 microns which matches the Nyquist sampling limit for the CMOS detector. The Strehl Ratio is calculated to be 0.41 assuming a 0.4 NA. Clearly there is room for improvement, but even with this performance, the system performance is well matched to the detector.
Figure 6.6: The MTF of the relay optics measured by the tilted edge method. The relay system has a diffraction limited performance with a calculated 0.97 Strehl Ratio. The red dashed curve represents the ideal MTF for a wavelength of 650 nm and 0.1 NA. The green curve is the measured MTF used to calculate Strehl Ratio and the blue segment (raw data) is assumed to be noise and is truncated by a threshold level.
Figure 6.7: The MTF of the 4M device and relay system combined measured by the tilted edge method. The red dashed line represents the ideal MTF. The green curve is the measured MTF and the blue segment (raw data) is assumed to be noise and is blocked by a threshold level. The calculated Strehl Ratio is 0.41 for the device with 0.4 NA.
6.4 Ghost images and Stray Light

Stray light and ghost images were the single most inhibiting factor during first reflectance imaging tests of the 4M system. The extremely low reflectivity and contrast of cells while imaging tissue in reflectance requires very high illumination irradiance in comparison to the reflected light that forms the image. This large dynamic range in energy results in strong ghost reflections that are returned to the image plane from the optical elements. As discussed in chapter 2, Anti-Reflection (AR) coatings alone are not sufficient to allow microscopic reflectance imaging of thick tissue. The tilted component design, however, was able to reduce the ghost reflections and background light to allow imaging.

To quantify the reduction in reflected light, a simple comparison was made between the tilted component system and the previously constructed untilted 4M device. First, the untilted system was used to image a black absorbing object in immersion (black electrical tape). The experiment must be done in immersion so that the reflections from the first lens are not increased by an air-glass interface that would not exist during normal operation. Reflections from the black object are further reduced by defocusing the object extremely far from the designed object plane. This extreme defocus allows most of the light reflected from the object to miss the aperture of the 4M device. With the untilted system viewing a black object, the illumination and CCD integration time are set to levels that result in pixel counts just under the CCD saturation level. This illumination and integration
time is then held constant and the untilted 4M system is replaced with the tilted component system. Images from these two configurations can be seen in figure 6.8. The plots below the images show a cross section of the pixel counts. The pixel counts in the center of the image are reduced from around 950 to less than 50. One ghost reflection can be seen at the left edge of the FOV on the tilted system with pixel counts around 200. This reflection was not completely removed from the FOV because of limits on the amount of tilt due to lens sag. The reflection was expected to be weak, but this reflection is visible as the brightest part of the field in images of biological samples shown in chapter 7.

The reduction in background light afforded by the tilted component 4M system enabled quantification of the reflectance of the tissue. As a measurement of the reflected signal from cell samples, a comparison was made by imaging a gold-labeled tissue phantom [9] in immersion and an uncoated glass surface in immersion. The back side of the glass was coated with an absorbing film to minimize the reflection from the second surface. The thickness of the glass sample also removed the second surface far from the object plane so that reflected light from this surface is mostly reflected away from the 4M device aperture. The reflected signal is measured by examining the average pixel values on the CCD camera at the image plane. The Fresnel reflection from a glass surface in water is calculated to be less than 0.4%, and the image plane irradiance for the tissue was measured to be almost identical to that of the glass slide. However, the reflected signal from the in-focus cells
Figure 6.8: Ghost images formed by (a) untilted conventional system and (b) tilted component system. The circles depict the designed FOV (radius 0.5 mm at the image) for the system. In the tilted system, a faint ghost is visible on the right, just overlapping with the FOV.
must be even less, since the tissue reflects light from several hundred micrometers of depth, but only a thin layer of a few micrometers is in focus at a given time. With the structured illumination method[4, 5] the maximum contrast of the grating projected into the tissue phantom is measured to be 12% of the reflected light. The maximum reflectance from in-focus cells is therefore estimated to be 12% of 0.4%, or 0.05% of the incident light. Simple AR coatings alone cannot reduce the surface reflections enough to allow imaging of such an object without further reduction of ghost reflections.
CHAPTER 7

IMAGING RESULTS

7.1 Introduction - challenges of in vivo imaging

In the previous chapter a number of tests were performed to provide quantitative 4M device performance. Those data are well suited to show the strengths and weaknesses of the 4M system, but the true measure of success for the 4M device is the ability to take meaningful images. This chapter illustrates the imaging capability of the 4M device. The device is intended to work in some very unusual scenarios, so some understanding of the challenges of in vivo tissue imaging is required. In addition, comparisons between images taken with the 4M device and with commercial microscopes are useful to demonstrate the capability of the 4M device. These comparisons also underline the challenges of imaging scenarios for which the 4M device is designed.

The case of in vivo imaging at the microscopic level is an excellent example of a task rich in challenges. Cells that make up the tissue have very low reflectance and the semi-transparent or nebulous nature of tissue at the microscopic scale leads to out-of-focus light being scattered or reflected back into the image. The result is that microscopic images of thick tissue are always accompanied by large amounts of
background light. This background light reduce image contrast and often makes it impossible to discern the cellular structure.

When cells and tissue are to be imaged microscopically, a number of techniques are used to overcome these challenges. Phase contrast, dark-field, polarization, modulation contrast, and differential interference contrast (DIC) are all methods of microscopy that enhance the contrast and hence visibility of individual cells[28]. Confocal and two-photon microscopy methods are used on thick tissue samples to optically section the tissue sample and eliminate background light from out-of-focus layers. Fluorescence has an additional benefit of illuminating and imaging at different wavelengths so that the illumination light can be filtered out to reduce background light in the image. Scanning Electron Microscopes (SEM) and Transmission Electron Microscopes (TEM) are used to give the highest resolution images of cells, but require extensive sample preparation.

All of the methods used to microscopically image tissue use some method of reducing background light from out-of-focus tissue. However, some of these methods are clearly inappropriate for in vivo imaging. TEM requires thin slices of tissue and so would require tissue to be first removed from the body. Phase contrast microscopy requires transmission illumination, and so would require tissue to be illuminated from behind and like TEM, requires thin slices of tissue. Fluorescent microscopy has limitations on dyes that can be used since many dyes are toxic. Some dyes may allow for in vivo microscopy, but this does not eliminate out of focus background
from reducing the contrast in the image. It can also be difficult to get dyes uniformly distributed below the tissue surface. The use of autofluorescence is one possible way to eliminate the need for a dye, but illumination must be kept to a level that does not cause damage to the tissue. The fluorescence is then often too weak to image without excessive integration time thus eliminating the possibility of video rate imaging. Two photon fluorescence is one other promising possibility and provides an optical sectioning effect, but this method relies on scanning and again is limited by scan speeds. Finally, confocal microscopy certainly has excellent potential for imaging tissue \textit{in vivo} and is used in commercial instruments. The primary limitation is the size of the objective which constrains its use in endoscopic applications. Work on fiber coupled confocal microscopes is under active development by a number of groups, but ultimate resolution and throughput is always limited by the tradeoff between fiber spacing and numerical aperture.

The idea to miniaturize a microscope design to the point that the entire device could fit in an endoscopic probe is exciting, but does not by itself address the challenges described above. The following sections discuss how these challenges are met for specific imaging modalities, and show images taken with the 4M device. When possible, images taken with conventional microscope systems are shown for comparison.
7.2 Transmission Illumination Imaging

Transmission illumination imaging is a configuration where the object is illuminated from one side and the image is collected from the other side. Transmission imaging requires that the object be thin enough so that the light penetrates the object. Though transmission illumination is not useful for \textit{in vivo} imaging, it provides an excellent baseline for examining the imaging quality of the optical system. By separating the illumination path from the imaging system, all reflections and ghost images become insignificant, providing a great sense of the 4M device’s usefulness as a miniature microscope. Figure 7.1 shows an image of a thin layer of cells deposited on collagen. The slide is transilluminated by a red LED with a peak wavelength at 650 nm.

Optical sectioning is not practical when using transmission illumination, so thin samples were used that have very little out-of-focus structure and as a consequence have very little scattered background. The cells are grown in culture, released into a solution and then deposited on a layer of collagen on a microscope slide to form a thin layer of live cells.

Another example of transmission illumination is shown in figure 7.2 in which a slice of tissue was stained to provide higher contrast. The figure shows the same sample imaged with a traditional benchtop microscope and with the 4M microscope optics for comparison. This imaging modality demonstrates the potential of the 4M device for use as a cheap and ultra-portable microscope.
Figure 7.1: A thin layer of cells deposited on a collagen layer transilluminated by a red LED with a peak wavelength at 650 nm. The FOV is 250×250 µm.

Figure 7.2: A slice of tissue imaged with both a traditional benchtop microscope (left) and the 4M device objective (right). Both images are courtesy of Mohammed S. Rahman, Rice University.
From the transmission illumination images it can be seen that the quality of the 4M device optics is good enough to allow high resolution imaging of cellular structure. Although the resolution is not as high as commercial objectives, the images certainly have the resolution required to make qualitative assessments about the tissue structure.

7.3 Fluorescence Imaging

The first fluorescence imaging was also performed on a thin layer of cells deposited on a slide to reduce background. The cells were labeled with Quantum-Dots (QDs) used as the fluorescent agent. QDs provide a strong fluorescent signal and have a broad absorption making them very useful in this application. The illumination source was a blue LED (peak wavelength at 450 nm) and the peak emission of the quantum dots is centered at 650 nm. In this imaging experiment, the illumination optics, dichroic beam splitter, and relay optics are all macroscopic components. A lens-only 4M device was used that consisted of the glass lens and 3 printed lenses. In this sense, the 4M device acted as a miniature microscope objective and the image formed was relayed to the camera sensor.

Figure 7.3 shows the fluorescent monolayer of cells imaged with the 4M device objective and a conventional benchtop microscope. The 4M device is used in an epillumination configuration where the illumination is delivered to the object through the microscope optics. A dichroic beamsplitter is used to transmit the blue illumi-
nation into the sample and pass the red imaging wavelength to the camera. The relay lens system discussed in chapter 6 was used to couple an intermediate image formed by the 4M device onto the large format CCD camera. An additional band-pass filter was used at the camera to pass the fluorescent light while blocking any stray or scattered light from the blue illumination.

The fluorescent image capability provides confirmation that the 4M device can be used in epi-fluorescence and produce images of cell membranes labelled with quantum dots. The use of fluorescence allows the separation in wavelength of the illumination and imaging light allowing filters to clearly separate stray light and internal reflections.
7.4 Reflectance Imaging

Reflectance imaging is the most challenging of the imaging modalities for a number of reasons. The fact that the illumination and image share the same wavelength requires that a beamsplitter be used which immediately drops a significant amount of light in both directions. Also, the common wavelength eliminates the possibility of filtering the stray light and ghost reflections from the image. The extremely low reflectance of cells provides a very limited contrast in the image and the large scattered light signal from out-of-focus cells further reduces the contrast. In early 4M device prototypes, the reflections from the untilted lens surfaces made any imaging of cells in reflectance impossible.

The introduction of the tilted element design allowed imaging of cells in reflectance and the results are shown in figure 7.4. The cells are labelled with gold nanoparticles to boost reflectance and scattering from the cell membranes. The gold nanoparticles are useful as a contrast agent that can selectively attach to cancer cells and not to normal cells providing a means to differentiate between the two.

One very interesting feature of this image is the saturated bright region at the right of the 4M image. This bright region is actually due to the reflection from one of the lens surfaces. For comparison, this is the same reflection that shows up as a dim region in figure 6.8. In that figure, the reflection was a barely significant 200 counts on the CCD compared to the 1000 counts from back reflections of the untilted system. Now in the reflectance imaging of cells, this weak reflection is by
Figure 7.4: A thin layer of A431 human carcinoma cells labeled with gold nanoparticles imaged by a Zeiss Axiovert 100M (left) and by the 4M device optics (right). The FOV is 250×250 µm and is again highlighted by the ring.

far the brightest region of the image confirming that internal reflections from lens surfaces severely limit reflectance imaging of cells.

7.5 Optical Sectioning using Structured Illumination

Images shown thus far have all been of thin layers of cells or slices of tissue to reduce the effects of out-of-focus background light. In this section the optical sectioning capability is demonstrated using structured illumination. This method works by projecting a grating or Ronchi ruling into the tissue. The grating frequency is chosen such that the modulation of the grating drops off quickly with defocus. The result is that regions of the object that are out of focus are illuminated uniformly while regions of object in the focal plane are strongly modulated. The grating is scanned and multiple images are taken for different grating positions. The images
are then used to reconstruct a single image that contains only the modulated signal.

To demonstrate structured illumination, two configurations were used. In the first configuration, an external grating was controlled by a large motorized stage and motion controller. The grating was back-illuminated and imaged into the 4M device using one set of relay optics and a beamsplitter. A second set of relay optics in the other path of the beamsplitter then relayed the 4M device image to the CCD. This configuration provided fine control of the grating for testing purposes. In a second configuration, the integrated MEMS grating was used and controlled by an electrostatic comb-drive actuator. The integrated grating was designed to work as a mass-spring system in resonance to provide large grating displacement with minimal power consumption. The use of the grating in resonance requires an imaging sensor capable of capturing 500 frames per second. The custom CMOS sensor designed for the 4M device is still under development and was not yet available for integrated testing, so the electrostatic actuator was of limited use.

By using the first configuration with an externally controlled grating, the structured illumination technique was tested and shown to work for the 4M device. In figure 7.5 two images of a monolayer of cells are shown. One image shows the wide-field image with no optical sectioning. The second image shows the effect of optical sectioning with structured illumination.

Structured illumination was also demonstrated using fluorescence. In figure 7.6 a comparison is shown between widefield images and optical sectioning for the 4M
Figure 7.5: A comparison of reflectance imaging with and without optical sectioning by structured illumination. On the left is the wide-field view of gold labeled cells on collagen. On the right is the reconstruction of the image using structured illumination to remove the background light. The FOV is $250 \times 250 \, \mu m$ in each image. Note the strong reflection on the left of the image saturated the CCD and resulted in a black region on the reconstructed image. This strong reflection is the same reflection shown at the end of chapter 6.
device and for commercial benchtop microscopes. The quantum dot labeled cells were deposited on a glass slide and imaged in widefield using the 4M device optics and the Zeiss Axiovert 100M. For optical sectioning, the 4M device was using with structured illumination from an externally controlled grating just as in the reflectance image. The optically sectioned image for a commercial microscope was taken using a Leica confocal microscope.

Finally in figure 7.7 a 4M device with integrated illumination and MEMS grating is shown to work in fluorescence. The 4M system used to take this image was a complete system with the exception of the imaging detector which remains in testing at the time of this writing. The system uses a 450 nm high brightness LED coupled to a fiber as illumination. On the 4M device, the fiber is held by a high precision fiber holder designed to fit the MOT substrate. A condenser lens illuminated the 1.3×1.3 mm grating which is mounted on the MEMS actuator. The grating is scanned electrostatically by providing up to 300 V to the comb drive. This high voltage repositions the grating statically so that long integration times of up to one second can be used to capture images. The figure shows the widefield image taken with a Zeiss AxioImage Z1 using a 20× EC Epiplan Apochromat objective and the widefield image taken with the 4M device. Structured illumination was possible using the integrated MEMS grating, but did not significantly improve the contrast in the pig tissue sample since the fluorescent quantum dot agents labeled only near the surface in this sample.
Figure 7.6: A comparison of fluorescence imaging with and without optical sectioning. The top row shows widefield images obtained using the Zeiss Axiovert 100M to the left and the 4M device optics to the right. Below shows the effect of optical sectioning with a commercial confocal microscope (Leica SPS2) on the left and the 4M device using structured illumination. In this case the external grating was used and projected into the 4M device object plane. The $250 \times 250 \mu$m FOV is depicted by the circle in each 4M device image.
Figure 7.7: Widefield images of pig tissue labeled with fluorescent quantum dots. The image on the left was taken using a Zeiss AxioImage Z1 while the image on the right was taken with the 4M device. The sample is a thick slice of epithelial pig tissue labeled with anti-EGFR conjugated quantum dots. The FOV of each image is roughly $200 \times 200 \, \mu m$. 
This image is extremely important as it demonstrates imaging of real tissue as opposed to cultured cells grown in media. In both the commercial system and the 4M device, the cell membranes of epithelial cells are clearly visible.
118

CHAPTER 8

CONCLUSIONS AND FUTURE DIRECTIONS

Through the course of this work, a tremendous wealth of information was learned. As with many endeavors, this work included many successes as well as some failures. In either case it is important to learn from these experiences and recognize where the strengths and weaknesses are so that future work can improve upon the strengths and avoid the weaknesses. In this final chapter, a summary is provided of the good and the bad points surrounding this work. Finally, some discussion of the future directions and recommendations are made in the earnest hope that this does not conclude the work that this dissertation has begun.

8.1 Technological successes

The greatest accomplishment of this research has been the successful integration of a large number of novel technologies to create a working miniature microscope. Throughout this research, the project depended on breakthroughs and improvements in technologies on all fronts. Cutting edge technologies included the design and construction of a custom high speed CMOS imaging sensor, the implementation of MEMS and electrostatic comb-drive actuators, the use of structured illumination with a mechanical resonator grating, the development of new optical materials for
printing refractive microlenses, the MOT zero alignment concept, and the use of tilted polynomial surfaces to reduce ghost images. The technologies were all quite progressive, but two technologies stand out as truly successful ideas with broad potential for use in future projects: printed lens technology and LIGA/DXRL micromachining for mechanical alignment.

8.1.1 LIGA and DXRL for Mechanical Alignment Systems

LIGA and DXRL are accurate and economical micromachining methods that have a bright future. The precision with which the parts are produced is impressive and the alignment accuracy that these components can lend to the optical design is inspiring. When viewed under even high magnification, the components produced with these technologies seem flawless leading one to imagine the parts were fabricated at a large scale and somehow zapped with a shrink-ray gun. Through clever design, the precision of these components leads directly to incredibly precise mechanical assembly of entire systems of optical and mechanical components.

8.1.2 Printed lenses

The grayscale lithographic patterning of refractive lenses was very successful, but what is really impressive about this technology is the flexibility. The use of hybrid sol-gel glass and grayscale patterning opens up enormous opportunity in optical design. In the lens design described in this work, the polynomial surfaces enabled
the correction or compensation of aberrations of tilted lens elements that reduced ghost images of the bright illumination source. This unique design is only the tip of the iceberg. Many exciting optical designs could be enabled by lifting the restrictions typically assumed in optical design such as spherical surfaces and rotational symmetry.

8.2 Technological weaknesses and pitfalls

In addition to some tremendous strengths demonstrated by these new fabrication technologies, a number of weaknesses have been uncovered. Some of these weaknesses are the consequence of working with limited equipment and resources and some are the result of working with very new materials that have not been well characterized. Many of the weaknesses may be easily avoided or eliminated by future work.

8.2.1 Inconsistent and variable lens patterning

One of the most frustrating aspects of the work was the variability of the lens fabrication process. The number of adjustable parameters that influence lens fabrication is astounding, and the influence and interplay of these parameters is not yet well characterized. The hybrid glass material is a very new material and much work is needed to produce a reliable process and consistent lenses. There is no doubt that the process can be made more reliable, but one proposed intermediate solution is to
use the grayscale patterning to produce a master part that could be replicated via molding. The use of molding could improve the uniformity across many substrates, but the master would still need to be made with grayscale printing. Although the printing might be inconsistent, only one master would be needed to create many replicas.

8.2.2 Assembly is labor intensive

Another weakness that became evident during system assembly is the labor required in assembling the systems. Initial conception of the 4M device anticipated that the mass production of individual components could produce extremely low cost systems. This is certainly true of individual components. For example, the CMOS detector could be produced for as little as $0.20 per chip and the lenses and other optical components are equally economical to fabricate. The weakness is the labor required in assembly. In the current system, the lens elements can be assembled in a few hours, but the entire system takes close to a day to put together. This labor intensive assembly would clearly dominate the cost of mass producing the device. Again, this is likely not a fundamental problem, as much of the assembly could likely be automated. Another solution may be to radically change the mechanical design to emphasize ease in assembly.
8.3 Future design considerations

8.3.1 Limitations imposed by CMOS camera

One interesting consequence of using CMOS technology is due to the geometry of the readout lines of the CMOS detector which are printed on top of the pixels. These circuits are as tall as 6 \( \mu \text{m} \) even for a pixel size of only 4 \( \mu \text{m} \). These tall readout wires cast a shadow on the pixel active area for light incident at an angle. This is normally not a problem, but the geometry of these wires is not symmetric and each line is used to readout rows of pixels on either side. The wires are only present in between every other row of pixels. The effect of this asymmetry is that if the detector array is illuminated from off-axis, every other line is shadowed causing a striping artifact in the image. The only way to avoid the effect is to ensure that all illuminating beams are incident normal to the detector. This is only true in a telecentric system. The microscope should already be telecentric in object space to ensure that defocus does not affect magnification. This new requirement means that the system must be doubly telecentric in future designs.

8.3.2 Limitations imposed by the material sag

Another important design consideration for future work is the limit imposed by material sag. The current material process works well to produce lenses with sag in excess of 80 \( \mu \text{m} \), and lenses have been patterned with sag of almost 200 \( \mu \text{m} \). In principle, the sag is limited only by the thickness with which a uniform layer can
be spin coated. In practice, however, the linearity and consistency of the process degrades as the thickness increases. Another problem is that the exposure process may be hard to control as thickness increases. Diffraction from the mask or errors in collimation of the illumination beam will be magnified as the thickness increases since the lower region of material will need to be further removed from the mask. Lastly, the increasing thickness will have an impact on the development process requiring very careful analysis of the absorption during exposure and the development dynamics. It may no longer be enough to empirically calibrate the lens features, but thorough modelling of the development process may be required. One potential method for modeling the development is level set methods[29]. This method is well suited to modeling the development process in three dimensions.

8.4 Final Words

In the end, the marriage of technologies used in this research provides a solid foundation for future work. A continued evolution of materials and technologies will no doubt enable extremely exciting developments in optical imaging and instrumentation. This work will hopefully provide some guidance for others pursuing the future development of these technologies. It has been a thrill to be a contributor in this exiting time for technology and optics research.
APPENDIX A

Mask Design Scripts

This appendix includes a listing of scripts used to generate the grayscale mask features. They are provided for reference since many subtle aspects of mask design are easily missed, and the scripts may be useful to someone reproducing this work. To format the scripts for printing, line wraps were added using the " " character.

The scripts are provided with the hope that they may save someone some time. They are free to use and modify as needed, but it is requested that credit be given to the author by including the following in any scripts that are reproduced:

\A collection of scripts for producing grayscale lenses
\and calibration ramps or various phase objects using
\DW2000 and the GPE scripting language
\Scripts originally written by:
\  Jeremy Rogers
\  College of Optical Sciences
\  University of Arizona
\  Originally written between Aug. 2000 - Nov. 2006
\  Modified by: <insert names and dates as appropriate>

A.1 Calibration Features

These functions generate various features for calibration or verification of the process linearity. There are functions to create arrays of squares or rectangles. The individual boxes are placed on different layer numbers so that each layer can be assigned an Optical Density to produce ramps, staircases, or an array of separated boxes.

\********************************************************************
\ Generate calibration features for grayscale masks.
\ Written by Jeremy Rogers on 9/20/04
\ Last modified 9/20/04
\********************************************************************
\This makes a calibration curve in the form of a ramp.
Menu "calramp" endmenu
niladic procedure calramp
    local n; heigth; wid; numlayers; skip; width1; origin; box; ^
hieght := ScalarInput "Enter hieght in microns:"
width1 := ScalarInput "Enter width in microns:"
numlayers := ScalarInput
    "Enter the number of Grey levels:"
IF (ostruct) = "" THEN error "Structure is closed" ENDIF
origin := coordinput "Enter the lower lefthand position:"
IF origin = "" THEN goto endsub ENDIF

wid := ( width1 / numlayers )
xpos1 := origin[1]
ypos1 := origin[2]
ypos2 := origin[2] + hieght
FOR n RANGE iota (numlayers) DO
    layer (n - 1)
    xpos2 := origin[1] + n*wid
    box := PolyClose(4 2 Reshape(xpos1, ypos1, xpos2, ypos1, ^
        xpos2, ypos2, xpos1, ypos2))
    putel (3,0,-2); (layer); (datatype);;; box
    xpos1 := xpos2
ENDDO
endsub

\********************************************************************
\This makes a calibration spot array.
Menu "calspots" endmenu
niladic procedure calspots
    local n; hieght; wid; numlayers; skip; width1; origin; box; ^
        xpos1; ypos1; xpos2; ypos2
    hieght := ScalarInput "Enter hieght in microns:"
    width1 := ScalarInput "Enter width in microns:"
    numlayers := ScalarInput
        "Enter the number of Grey levels:"
    IF (ostruct) = "" THEN error "Structure is closed" ENDIF
    origin := coordinput "Enter the lower lefthand position:"
    IF origin = "" THEN goto endsub ENDIF

    wid := 50
    xpos1 := origin[1]
ypos1 := origin[2]
ypos2 := origin[2] + hieght
    FOR n RANGE iota (numlayers) DO
layer (n - 1)
xpos2 := xpos1 + wid
box := PolyClose(4 2 Reshape(xpos1, ypos1, xpos2, ypos1, ^
xpos2, ypos2, xpos1, ypos2))
putel (3,0,-2); (layer); (datatype);;; box
xpos1 := xpos2 + width1
ENDDO
endsub

A.2 Pixelated Aspheric Lens

These functions generate lens features with polynomial surface functions by breaking the lens region up into “pixels” or squares of a given dimension. Each pixel is then assigned a layer number proportional to the OD needed to produce the required height at that location of the lens.

\********************************************************************
\ Generate various structures using a pixel grid of specified size.
\ Written by Jeremy Rogers on 6/27/02
\ Last modified 9/1/04
\********************************************************************
\pixellens generates a grid of pixels in a circular
\ aperture with layer number increasing with radius.
\ There are an odd number of pixels, so there is a central pixel.
Menu "pixellens" endmenu
niladic procedure pixellens
  local n; m; rad; pix; numlayers; origin; box; x; y; xpos1; ^
ypos1; xpos2; ypos2; nmax; mmax
  rad := ScalarInput "Enter lens semi-diameter in microns:";250
  numlayers := ScalarInput ^
    "Enter the number of Grey levels:";64;2;255
  IF (ostruct) = "" THEN error "Structure is closed" ENDIF
  origin := coordinput "Enter the center position:"
  IF origin = "" THEN goto endsub ENDIF

  pix := 4.
  nmax := 2*(floor(rad / pix)) + 1
  FOR n RANGE iota nmax DO
    FOR m RANGE iota nmax DO
      x := ((origin[1] - rad) + pix*(m - 1))
      y := ((origin[2] - rad) + pix*(n - 1))
IF \( (x^2+y^2) < ((\text{rad}+\text{pix}/2.)*(\text{rad}+\text{pix}/2.)) \) THEN
xpos1 := x - pix/2.
ypos1 := y - pix/2.
xpos2 := x + pix/2.
ypos2 := y + pix/2.
box := PolyClose(4 2 Reshape(xpos1, ypos1, xpos2, ypos1, ypos2, xpos2, ypos2))
layer round ((\text{numlayers} - 1) * ((\sqrt{x^2+y^2})/\text{rad}); 0)
putel (3,0,-2); (layer); (datatype);;; box
ENDIF
ENDDO
ENDDO
endsub

A.3 Aspheric Lens with Uniform Step Height

\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*
\text{asphere lens surface generator}
Menu "aspherelens" endmenu
niladic procedure aspherelens
  local \( n; m; \text{pix}; \text{numlayers}; \text{origin}; \text{box}; x; y; \text{xpos1}; \text{ypos1}; \text{xpos2}; \text{ypos2}; \text{nmax}; \text{mmax}; \text{lens}; \text{maxsag} \)
  local \( \text{sd}; \text{rad}; k; a1; a2; a3; a4; a5; a6; a7; a8; a9 \)
  \text{lens} := ScalarInput\
     "Enter lens number to generate [B=1, C=2, or D=3]";1;1;3
  \text{numlayers} := ScalarInput\
     "Enter the number of Grey levels..";128;2;256
  IF (\text{ostruct}) = "" THEN error "Structure is closed" ENDIF
  \text{origin} := coordinput "Enter the center position.."
  IF \text{origin} = "" THEN goto endsub ENDIF
  \text{SWITCH} \text{lens} \text{OF}
  \text{CASE} 1: "Prescription B" ! \text{sd}:=.60 ! \\
  rad:=4.568778 ! \text{k}:-4.786044
  \text{CASE} 2: "Prescription C" ! \text{sd}:=.72 ! \\
  rad:=3.472021 ! \text{k}:=1.699308
  \text{CASE} 3: "Prescription D" ! \text{sd}:=.77 ! \\
  rad:=3.501300 ! \text{k}:-9.665478
  \text{OUT:} "Not a valid lens prescription" ! GOTO endsub
ENDSWITCH
sd := sd * 1000
maxsag := 80
pix := 1.6
nmax := 2*(floor(sd / pix))
FOR n RANGE iota nmax DO
  FOR m RANGE iota nmax DO
    x := ((origin[1] - sd) + pix*(m - .5))
    y := ((origin[2] - sd) + pix*(n - .5))
    IF (x*x+y*y) < ((sd + .5 * pix) * (sd + .5 * pix)) THEN
      xpos1 := x - pix/2.
      ypos1 := y - pix/2.
      xpos2 := x + pix/2.
      ypos2 := y + pix/2.
      box := PolyClose(4 2 Reshape(xpos1, ypos1, xpos2, ypos1, xpos2, ypos2))
      \scaling to mm so the layer function below works
      \scale x := x/1000 ! y := y/1000
      \scale layer round ((numlayers - 1)*((1000/maxsag*(((x*x+y*y)/(rad*(1 + SQRT (1-(1+k)*(x*x+y*y)/(rad*rad)))) )^ Power 0.5)); 0)
      \scale putel (3,0,-2); (layer); (datatype);; box
    ENDIF
  ENDDO
ENDDO
endsub
endDO
endDDO
endsub

A.4 Polynomial Lens with Regular OD Increments

\*********** polynomial lens surface generator using even increments of OD
Menu "polynomiallenslinearOD" endmenu
niladic procedure polynomiallenslinearOD
  local n; m; pix; numlayers; origin; box; x; y; xpos1; ypos1;
  \scale xpos2; ypos2; nmax; mmax; lens; maxsag
  local sd; rad; k; a1; a2; a3; a4; a5; a6; a7; a8; a9
  \scale lens := ScalarInput
  "Enter which lens to generate [B=1, C=2, or D=3]";1;1;3
  numlayers := ScalarInput
  "Enter the number of Grey levels..";128;2;256
  IF (ostruct) = "" THEN error "Structure is closed" ENDIF
origin := coodinput "Enter the center position.."
IF origin = "" THEN goto endsub ENDIF

SWITCH lens OF
  CASE 1: "Prescription B" ! sd:=.60 ! rad:=4.300652 ! ^
    k:=1.689004 ! a1:=-.005030 ! a2:=-.003595 ! ^
    a3:=0.028415 ! a4:=0 ! a5:=0.008694 ! ^
    a6:=-.002469 ! a7:=-.000670 ! a8:=0.009871 ! ^
    a9:=0.004579
  CASE 2: "Prescription C" ! sd:=.72 ! rad:=3.595048 ! ^
    k:=-2.464507 ! a1:=0.003506 ! a2:=0.002514 ! ^
    a3:=-.001620 ! a4:=0 ! a5:=0.009462 ! ^
    a6:=0.009734 ! a7:=0.001779 ! a8:=-.006074 ! ^
    a9:=-.006521
  CASE 3: "Prescription D" ! sd:=.77 ! rad:=3.909325 ! ^
    k:=-8.30917 ! a1:=-.004818 ! a2:=-.003873 ! ^
    a3:=-.009397 ! a4:=0 ! a5:=0.007001 ! ^
    a6:=-.015386 ! a7:=-.001130 ! a8:=-.004978 ! ^
    a9:=0.003949
  OUT: "Not a valid lens prescription" ! GOTO endsub
ENDSWITCH

sd := sd * 1000
maxsag := 80
pix := 5
nmax := 2*(floor(sd / pix))
FOR n RANGE iota nmax DO
  FOR m RANGE iota nmax DO
    x := ((origin[1] - sd) + pix*(m - .5))
    y := ((origin[2] - sd) + pix*(n - .5))
    IF (x*x+y*y) < ((sd + .5 * pix) * (sd + .5 * pix)) THEN
      xpos1 := x - pix/2.
      ypos1 := y - pix/2.
      xpos2 := x + pix/2.
      ypos2 := y + pix/2.
      box := PolyClose(4 2 Reshape(xpos1, ypos1, xpos2, ^
                                 ypos1, xpos2, ypos2, xpos1, ypos2))
      \scale to mm so the layer function below works
      x := -x/1000 ! y := y/1000
      layer round ((numlayers - ^
                    1)*(1000/maxsag*((x*x+y*y)/(rad*(1 + ^
                                    SQRT (1-(1+k)*(x*x+y*y)/(rad*rad)))) + ^
                                    1))
    ENDIF
  ENDFOR
ENDFOR
A.5 Polynomial Lens

This function produces a pixelated lens based on polynomial functions.

\******************************************************************************
\Polynomial lens surface generator
Menu "polynomiallens" endmenu
niladic procedure polynomiallens

local n; m; pix; numlayers; origin; box; x; y; xpos1; ypos1;^n
  xpos2; ypos2; nmax; mmax; lens; maxsag
local sd; rad; k; a1; a2; a3; a4; a5; a6; a7; a8; a9
lens := ScalarInput "Lens prescription[B=1, C=2, or D=3]:" ^\n  ;1;1;3
numlayers := ScalarInput "Enter the number of Grey levels:" ^\n  ;128;2;256
IF (ostruct) = "" THEN error "Structure is closed" ENDIF
origin := coordinput "Enter the center position.."
IF origin = "" THEN goto endsub ENDIF

SWITCH lens OF
  CASE 1: "Prescription B" ! sd:=.60 ! ^\n      rad:=4.300652 ! k:=1.689004 ! ^\n      a1:=-.005030 ! a2:=-.003595 ! ^\n      a3:=0.028415 ! a4:=0 ! ^\n      a5:=0.008694 ! a6:=-.002469 ! ^\n      a7:=-.000670 ! a8:=0.009871 ! ^\n      a9:=0.004579
  CASE 2: "Prescription C" ! sd:=.72 ! ^\n      rad:=3.595048 ! k:=-2.464507 ! ^\n      a1:=0.003506 ! a2:=0.002514 ! ^\n      a3:=-.008415 ! a4:=0 ! ^\n      a5:=0.008694 ! a6:=-.002469 ! ^\n      a7:=-.000670 ! a8:=0.009871 ! ^\n      a9:=0.004579
CASE 3:  "Prescription D"  ! sd:=.77  ! 
       rad:=3.909325  ! k:=-8.30917  !
       a1:=-.004818  ! a2:=-.003873  !
       a3:=-.009397  ! a4:=0  !
       a5:=0.007001  ! a6:=-.015386  !
       a7:=-.001130  ! a8:=-.004978  !
       a9:=0.003949

OUT:  "Not a valid lens prescription"  !

GOTO endsub

ENDSWITCH

sd := sd * 1000
maxsag := 80
pix := 1.6
nmax := 2*(floor(sd / pix))
FOR n RANGE iota nmax DO
  FOR m RANGE iota nmax DO
    x := ((origin[1] - sd) + pix*(m - .5))
    y := ((origin[2] - sd) + pix*(n - .5))
    IF (x*x+y*y) < ((sd + .5 * pix) * (sd + .5 * pix)) THEN
      xpos1 := x - pix/2.
      ypos1 := y - pix/2.
      xpos2 := x + pix/2.
      ypos2 := y + pix/2.
      box := PolyClose(4 2 Reshape(xpos1, ypos1, xpos2, ^
          ypos1, xpos2, ypos2))
      \scale to mm so the layer function below works:
      x := x/1000  ! y := y/1000
      layer round (numlayers - 1)*((1000/maxsag*^
          ((x*x+y*y)/(rad*(1 + SQRT (1-(1+k)*(x*x+y*y)/^
             (rad*rad)))) + a1*x + a2*y +a3*x*x + a4*x*y + ^
             a5*y*y + a6*x*x*x + a7*x*x*y + a8*x*y*y + ^
             a9*y*y*y + .0001)) Power 0.5); 0)
      putel (3,0,-2); (layer); (datatype);;; box
  ENDIF
ENDDO
ENDDO
endsub
A.6 Alvarez Phase Plate

This function uses a method very similar to the pixelated lens script, but generates a phase plate for use as an Alvarez plate. Two such components can then be translated relative to each other laterally to produce a variable power lens.

\************************************************************
\phase plate code
Menu "phaseplate" endmenu
niladic procedure phaseplate
  local n; m; sd; rad; pix; numlayers; origin; box; x; y; ^
    xpos1; ypos1; xpos2; ypos2; nmax; mmax
  sd := ScalarInput "Phase plate semi-diameter in microns:";250
  numlayers := ScalarInput ^
    "Enter the number of Grey levels:";64;2;255
  IF (ostruct) = "" THEN error "Structure is closed" ENDIF
  origin := coordinput "Enter the center position.."
  IF origin = "" THEN goto endsub ENDIF
  pix := 2.5
  nmax := 2*(floor(sd / pix))
  rad := rad * sqrt (2)
  FOR n RANGE iota nmax DO
    FOR m RANGE iota nmax DO
      x := ((origin[1] - sd) + pix*(m - .5))
      y := ((origin[2] - sd) + pix*(n - .5))
      IF (x*x+y*y) < ((sd + .5 * pix) * (sd + .5 * pix)) THEN
        xpos1 := x - pix/2.
        ypos1 := y - pix/2.
        xpos2 := x + pix/2.
        ypos2 := y + pix/2.
        box := PolyClose(4 2 Reshape(xpos1, ypos1, xpos2, ^
          ypos1, xpos2, ypos2, xpos1, ypos2))
        layer round ((numlayers - 1)*
          (((x-origin[1]) power 3) +
            ((y-origin[2]) power 3)) /
          (2*((sd - .5 * pix) power 3)) +.5); 0)
        putel (3,0,-2); (layer); (datatype);;; box
      ENDIF
    ENDDO
  ENDDO
endsub
REFERENCES


