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Development of low-cost optical detection systems for point-of-care infectious disease detection and monitoring in low-resource settings

by

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ABSTRACT

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Nearly half the world's population lives below the global poverty line of $2.50 per day. This population is disproportionately affected by infectious diseases due to a lack of regular screenings and early detection and intervention. Optical diagnostics provide a highly sensitive and specific, low-cost method by which to improve access to disease screening and monitoring at the point of care in low-resource settings. Point of care settings range from a patient’s bedside to a hospital, and there is a need for instrumentation that may be used across the point of care setting spectrum. This work describes a platform of miniature fluorescence microscopes designed for use in resource-limited point of care settings ranging from rural communities to hospital laboratories. The prototype units of the platform described here are three miniature microscopes:

1) A monochromatic infinity-corrected 0.25 NA microscope that was used for optical readout of multiplexed bead-based bioassays. This prototype was validated with a commercially available assay reader (Luminex's MAGPIX);
2) An achromatic 0.35 NA finite conjugate objective designed for three-part white blood cell differential counts in rural areas. This unit was validated against a commercially available benchtop hematology analyzer (Beckman Coulter Ac T diff2) and is currently in use in clinical trials at Lyndon B. Johnson General Hospital; and

3) A tunable 0.25 NA infinity-corrected fluorescence microscope designed for use with a variety multiplexed biological samples. The tunable system does not require manual adjustment by a trained user between emission wavelengths due to the addition of two low-cost, commercially available electrowetted lenses. It was validated by isolating green and red emission signal of blood stained with acridine orange.

Each microscope prototype costs at least one order of magnitude less than its commercially available instrumentation counterpart. Additionally, the per-test cost for all devices is <$1.00 since the sample platform used for all three modules is a standard microscope slide with low cost reagents, not a specialized cartridge or cuvette. With the platform of low-cost, high-performance microscopes described in this work, it is possible for diagnostic tests to be performed without expensive equipment or highly-trained clinicians, thereby delivering testing access to low-resources settings.
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# Contents

Acknowledgments .................................................................................................................. iii

Contents ................................................................................................................................. v

List of Figures ......................................................................................................................... viii

List of Tables ............................................................................................................................ xiv

List of Equations ...................................................................................................................... xvi

Nomenclature .......................................................................................................................... xvii

Introduction .............................................................................................................................. 1

1.1. Summary ......................................................................................................................... 1

1.2. Objective and Specific Aims .......................................................................................... 2

1.3. Outline of Text ................................................................................................................ 3

Challenges in medicine at the point of care in low-resource settings ......................... 7

2.1. Introduction ..................................................................................................................... 7

2.2. Addressing challenges in medicine in low-resource settings ................................... 9

Background: Current Optical Screening Techniques at the Point-of-Care, from Benchtop to Bedside ...................................................................................................................... 13

3.1. Commercially available benchtop instrumentation ...................................................... 15

3.1.1. Commercially available instrumentation and techniques for multiplex assay readout ............................................................................................................................ 15

3.1.2. Clinical importance and benchtop instrumentation for white blood cell differential counting .................................................................................................................. 17

3.2. Miniature optical diagnostic devices designed for point-of-care use ....................... 18

Development of a miniature monochromatic fluorescence microscope for molecular imaging .................................................................................................................. 28

4.1. Introduction ..................................................................................................................... 29

4.2. Experimental Methods ................................................................................................. 35

4.2.1. Luminex's MagPlex Beads ...................................................................................... 35

4.2.2. Optical Design of the 0.25 NA Miniature Fluorescence Microscope .............. 36

4.2.3. Optical Design of Illumination System ................................................................. 48

4.2.4. Optomechanical System ........................................................................................ 51
4.2.4.1. Miniature Microscope Optomechanics .............................................. 53
4.2.4.2. Illumination Optomechanics ......................................................... 56
4.2.5. Sample Preparation .......................................................................... 57
4.2.6. Image Acquisition ............................................................................. 59
4.2.7. Miniature Microscope Image Analysis ............................................... 61
4.2.8. Luminex MAGPIX Experiments ......................................................... 65
4.3. Results and Discussion ........................................................................ 65
  4.3.1. Limit of Detection: Miniature 0.25 NA Microscope and Luminex MAGPIX 65
  4.3.2. Analyte Classification ..................................................................... 67
4.4. Conclusions ............................................................................................ 68
4.5. Acknowledgements ................................................................................ 69

Development of an achromatic miniature objective for three part white blood cell differential measurements ......................................................... 70

5.1. Introduction ............................................................................................ 71
5.2. Experimental Methods ........................................................................... 74
  5.2.1. Optical Design of Miniature Objective .............................................. 74
  5.2.2. Sample Cartridge Design ................................................................. 85
  5.2.3. Optomechanical Design .................................................................. 85
  5.2.4. Cost analysis .................................................................................... 90
  5.2.5. Image Acquisition .......................................................................... 92
5.3. Conclusions ............................................................................................ 94
5.4. Acknowledgements ................................................................................ 95

Challenges in fabrication and testing of static monochromatic and achromatic systems ........................................................................................................ 96

6.1. Monochromatic objective image acquisition challenges ....................... 96
6.2. Achromatic objective design and fabrication challenges ...................... 98
6.3. Benefits of infinity-corrected objectives ................................................. 102
6.4. Conclusions ............................................................................................ 105

Development of a tunable monochromatic infinity-corrected objective ...... 106

7.1. Introduction ............................................................................................ 106
7.2. Experimental Methods ......................................................................... 110
7.2.1. Optical Design of a miniature tunable infinity-corrected objective........ 110
7.2.2. Optomechanical Design ................................................................. 120
7.2.3. Image Acquisition ........................................................................ 123
7.3. Conclusions and Future Work ............................................................ 124

Conclusions and Suggestions for Future work ........................................... 126
List of Figures

Figure 2.1 Image from the World Health Organization’s (WHO) report on Global Health Risks: Mortality and burden of disease attributable to selected major risks, 2009. A world map illustrating population, gross national income per capita, life expectancy at birth, for low and middle income countries and high income countries [2]. ................................................................. 8

Figure 2.2 Spectrum of point of care settings and common tests performed at each [5]. ............................................................................................................................................... 10

Figure 3.1 An image of the Luminex’s MAGPIX assay platform (estimated cost $35000 USD) [19]. ........................................................................................................................................ 16

Figure 3.2 (a) The mobile phone microscope layout schematic. (b) Image of the prototype. (c) brightfield images of fluorescent beads (6 um diameter) (d) Fluorescent images of beads. The field of view of is outlined in the dashed white line. [28] ........................................................................................................................................ 19

Figure 3.3 (a) Schematic of the Cellscope Loa. (b) Simplified optical diagram of microscope [30] ........................................................................................................................................ 20

Figure 3.4 Image from Steingart et al. 2006. The Global Focus microscope is shown above. The spatial resolution of the system is 0.8 um and can achieve magnification of 1000x. (a) The trans-illumination light path. (b) Left image is an M. tuberculosis bacilli stained with auramine orange dye. The image was obtained with the Global Focus microscope at a magnification of 400x. Digital magnification detail is shown on the right [33]. ........................................................................................................... 23

Figure 4.1 The figure above illustrates the MagPlex bead hybridization. B is biotin and PE is streptavidin phycoerythrin. The Classifier (CL1/CL2) channels determine the analyte being detected while the magnitude of the PE-derived signal is measured in the Reporter channel. ...................................................................................... 36

Figure 4.2: The optical schematic of the 0.25 NA miniature fluorescence microscope. All dimensions given are in millimeters (mm) ........................................................................................................ 37

Figure 4.3 Performance metrics of the miniature tunable fluorescence microscope for nominal working conditions: a) modulation transfer function plots for two tangential field points located on optical axis and -0.6mm away from it. MTF plots for tangential and sagittal field points are drawn in blue and
for field point located -0.6mm away from the optical axis in green. Tangential and sagittal MTF plots for diffraction limited system are drawn in black. All MTF plots are for the design wavelength of 590nm; b)Spot diagrams for all designed, tangential field points of: 0.0mm, -0.1mm, -0.2mm, -0.3mm, -0.4mm, -0.45mm, -0.5mm, -0.6mm, for design wavelength of 590nm. Horizontal extent and vertical extent of sub-field rectangles: 20nm. The airy disk is depicted in spot-diagrams sub-field images as black circle. The theoretically calculated Airy disk radius is 5.9µm.

Figure 4.44 Cross-section through 3D model of the opto-mechanical assembly of the microscope objective (a) and the tube lens (b)

Figure 4.5 Images of 1951 USAF resolution target taken using Zeiss Axio Imager Z1 through: a) miniature all plastic microscope objective, b) miniature plastic tube lens, c) and reference Zeiss 10x/0.25 microscope objective. Please note that due to difference in focal lengths of tested systems, images of resolution target were recorded at different magnifications. Magnified, red marked regions of 1951 USAF resolution target, close to assessed limit of resolution are zoomed and presented in inlays in respective images.

Figure 4.6 Optical layout of the dual wavelength illumination system.

Figure 4.7 Spectral bandwidths of excitation (ex.) and emission (em.) filters together with spectral densities of illumination sources for Classifier (a) and Reporter (b) channels.

Figure 4.8 a) A photo of the complete miniature system prototype. The system contains (from top to bottom): a camera, a tube lens, a sliding emission filter holder, an objective lens a sample tray that is designed to fit a standard size microscope slide, and an illumination module that houses optics and light sources for two different illumination pathways. b) A Solidworks rendering of the cross section of the complete system. A cross section of the camera mount, adjustable tube and objective lens holders, emission filter holder, sample tray, and illumination module is shown. In the illumination module, the Classifier channel pathway is highlighted in red and the Reporter channel illumination pathway is highlighted in blue. Both channels intersect at a dichroic beamsplitter and then share a subsequent common optical pathway, highlighted in purple.

Figure 4.9 A cross section of a Solidworks rendering of the miniature microscope is shown above. The rendering illustrates an exploded view of the
system cross section highlighting the threaded objective and tube lens holders. The inset image shows an enlarged view of the threaded objective holder.

Figure 4.10 The disassembled infinity-corrected miniature microscope components (camera cap, camera mount, objective mount, tube lens mount, and emission filter holder). Examples of the 3/10 dowel pins (Briksen, Germany) used to connect the modules are shown in the top right corner. Dowel pin holes are visible on the tube and objective lens modules. Extra unmounted tube lens and objective lens modules are shown to the right of the microscope.

Figure 4.11 A magnified view of the illumination system and its components.

Figure 4.12 Images taken with the miniature microscope from the Classifier 1 channel (a) and Classifier 2 channel (b) at an exposure time of 300 milliseconds. Note that due to filter spectral characteristic, Classifier 1 channel exhibits stronger background signal.

Figure 4.13 Image processing algorithm; flow of operation diagram.

Figure 4.14 An example of a post-processed HDR image from the Reporter channel of bead 39 tagged with $10^{11}$ copies of DNA (detailed description of image processing operations in the main text). Please note beads intensity variations, caused by difference in concentration of target analyte.

Figure 4.15 The two figures above illustrate limits of detection of the miniature microscope from a singleplexed experiment that used bead type #39. (a) and Luminex MAGPIX (b). Normalized intensity is plotted on the y-axis and concentration in 10x copies of target is plotted on the x-axis. The limit of detection is calculated from the intersection of the baseline (red squares) with the measured median intensities at each concentration (black dots). The limit of detection of the miniature microscope was $10^{9.3}$ copies of target (synthetic DNA). For Luminex’s MAGPIX, the limit of detection was found to be $10^{8.0}$ copies of target in one experiment. MAGPIX’s limit of detection is reported to be between $10^{9.8}$ and $10^{10.6}$ copies of target in the literature.

Figure 4.16 A bead map from a multiplexed bead sample imaged on the miniature microscope. The sample contains 137 total beads comprised of
three distinct bead types, which can be resolved into three distinct groups (outlined with red ellipses). Each group is spaced approximately 0.6 units apart in the y-direction and 0.8 units apart in the x-direction.

Figure 5.1 Optical schematic of the miniature achromatic objective.

Figure 5.2 Performance metrics of the miniature tunable fluorescent microscope for nominal working conditions: a) modulation transfer function for expected performance at 525 nm b) modulation transfer function for expected performance at 650 nm. (c) spot diagram for expected performance at 525 nm (shown in blue) and 625 nm (shown in green). All plots are for image surface for design wavelength of 590nm, for following object points: 0.0mm, 0.25mm, 0.35mm, 0.45 mm, 0.50 mm, 0.55 mm, 0.6 mm. Airy disk radii for 525 nm and 650 nm: 4.179 and 5.126µm, respectively.

Figure 5.3 Assembled plastic objective inside brass tubing (left). 3D printed threaded objective holder (right).

Figure 5.4 Images of high resolution USAF target taken with the miniature 0.35 NA achromatic objective. a) Image acquired at 525 nm b) Image acquired at 650 nm. Magnified inlays of the smallest resolvable elements are shown outlined in each image. Contrast has been enhanced for presentation purposes.

Figure 5.5 Optomechanical schematic of the miniature WBC microscope (a), cross-section through sample chamber with 3D printed microscope slide positioning springs (b), details of the optomechanical assembly of the prototype of the WBC objective (c), photo of the assembled WBC microscope, (d) photograph of assembled 3D printed system is shown on an optical bench. The spacing between the holes is 1 inch for reference.

Figure 5.6 (a) Schematic of measurement system used to evaluate sample insertion repeatability. (b) Slide insertion repeatability test: blue line – measured distance, black line – mean distance, red lines – ±1σ standard deviation boarder lines.

Figure 5.7(a) Image of acridine orange taken at 400 ms exposure stained white blood cells in whole blood. Image acquired with the miniature all-plastic objective. (b) Magnified image of granulocyte. (c) Magnified image of a lymphocyte. (d) Magnified image of a monocyte. Contrast has been enhanced.
for presentation purposes. Red-to-green ratios were calculated using raw data ................................................................................................................................. 93

Figure 6.1 Binarized, coregistered beads from Classifier channels and Reporter channel. Red represents signal from Classifier 1 channel, green represents signal from Classifier 2 channel, blue represents the binarized bead as it appears in the reporter channel, and white is the overall of all three channels. White indicates that the images are coregistered.............................. 98

Figure 6.2 Optimum 3CC syringe barrel schematic.................................................. 100

Figure 6.3 Image of Lens 2 (left). Image of lens 2 and 3 affixed with NOA61 optical glue. .......................................................................................................................... 101

Figure 6.4 (a) Zemax optical layout of the 0.5 NA/-8x infinity corrected monochromatic objective (b) optical layout for transmission illumination (c) optical layout for epi-illumination............................................................. 103

Figure 7.1 Example of axial chromatic aberation of white light focused through a positive lens. F represents blue light (486 nm), the dashed line C represents red light (656 nm) and the distance d is the axial difference between the two focal planes [78]. ............................................................................................................. 108

Figure 7.2 Electrowetting lens with applied voltages between 0 and 120V. Voltage applied between conductive substrate and liquid results in change in curvature of insulating hydrophobic layer (oil drop) [79]. ........................................ 109

Figure 7.3 Optical schematic of the infinity-corrected tunable objective. The red arrows indicate the tunable Arctic 316 lenses. ....................................................... 111

Figure 7.4 Relationship between Voltage (V) and Optical Power (D) of the Arctic 316 lens [79]. The lowest power of the tunable lens (P₀) is -5 diopters and the highest power of the tunable lens is +12 diopters. ............................................ 114

Figure 7.5 Performance metrics of the miniature tunable fluorescent microscope for nominal working conditions: (a) modulation transfer function for expected performance at 590 nm, (b) modulation transfer function for expected performance at 660 nm, (c) modulation transfer function for expected performance at 720 nm, (d) spot diagram for expected performance at 590 nm, (e) spot diagram for expected performance at 660 nm, and (f) spot diagram for expected performance at 720 nm. All plots are for image surface for design wavelength of 590nm, for following object points: 0.0mm, 0.10 mm,
0.20 mm, 0.30 mm, 0.40 mm, 0.45 mm, 0.50, 0.60 mm. Airy disk radii for
Configuration 1 (c), 590 nm, is 7.34 µm, Configuration 2 (d), 660 nm, is 8.042
µm, and Configuration 3 (e), 720 nm is 7.758 µm. .......................................................... 115

Figure 7.6 (a) Arctic 316-P tunable lens [source: arctic manual]. (b) 3D printed
holder for tunable objective. (c) 3D printed tube lens holder with C mount
threading for Flea3 image detector .................................................................................. 119

Figure 7.7 (a) Images of high resolution USAF target taken with miniature
tunable microscope. (a) an image acquired at 525 nm, (b) an image acquired
at 650 nm with no adjustment of the tunable lenses. ...................................................... 120

Figure 7.8 (a) Solidworks cross section of microscope optomechanics. (b)
Photograph of illumination module, slide holder with slide inside, optical
detection element chamber, tube lens holder, and camera............................................ 122

Figure 7.9 Raw image of AO-stained blood with objective focused on 525 nm
wavelength emission. ........................................................................................................ 123

Figure 7.10 (a) Greyscale image of red channel of RGB camera for raw image
shown in 7.9. (b) Greyscale image of green channel of RGB camera for Figure
7.9. Contrast has been enhanced for display purposes. .................................................... 124
List of Tables

Table 2.1 Target product profile for fluorescence microscope subunits of the platform...............................................................................................................................................11

Table 3.1 TPP of the Cellscope based on minimally acceptable profile defined in Table 2.1 ........................................................................................................................................... 21

Table 3.2 TPP of the Global Focus Microscope based on Minimally acceptable profile defined in Table 2.1 ........................................................................................................................................... 24

Table 3.3 Summary of attributes of lensless imaging system (Ozcan group) ... 25

Table 3.4 Summary of attributes of DNA imaging and sizing platform (Ozcan group).................................................................................................................................................. 25

Table 3.5 Summary of attributes of Foldscope .......................................................................................................................................................... 26

Table 4.1: Summary of optical parameters of 0.25 NA miniature fluorescent microscope. ...........................................................................................................................................37

Table 4.2 Optical prescription data of miniature fluorescent microscope. ..... 38

Table 4.3 Tolerance parameters of the miniature fluorescent microscope. .... 42

Table 5.1 Summary of optical parameters of miniature fluorescent microscope ...........................................................................................................................................75

Table 5.2 Optical prescription data of miniature microscope (Radii, thicknesses and semi-diameters are in units of [mm]) ................................................................. 76

Table 5.3 Measured vs. reported refractive index values for polystyrene and PMMA..................................................................................................................................................80

Table 5.4 Calculated vs. reported (in Zemax) Vd for polystyrene and PMMA..81

Table 5.5 Tolerance parameters of the miniature fluorescent microscope .... 81

Table 5.6 Cost of complete optical detection hardware for prototype device and conservative cost estimate for mass production .................................................... 92

Table 7.1 Summary of optical parameters of tunable objective .................... 111
Table 7.2 Optical prescription data of the tunable microscope (Radii, thicknesses, semi-diameters (SD), and conic values are given in units of [mm])

Table 7.3 Tolerance parameters of the miniature tunable microscope

Table 8.1 Target product profile for fluorescence microscope subunits of the platform

Table 8.2 Target product profile and validation method

Table 8.3 Design considerations, economic and data import properties of three miniature systems
List of Equations

Equation 1: Depth of Field .............................................................................................................. 54

Equation 2: Abbe number equation ............................................................................................ 80

Equation 3: Equation of the airy disk radius .............................................................................. 104

Equation 4: Optical Power (D) vs Voltage (V) of the Arctic 316 lens between $V_L$ and $V_H$, and $P_L$ and $P_H$ .......................................................................................................................... 113
## Nomenclature

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>AO</td>
<td>Acridine orange</td>
</tr>
<tr>
<td>DALY</td>
<td>Disability-adjusted life years</td>
</tr>
<tr>
<td>DoF</td>
<td>Depth of Field</td>
</tr>
<tr>
<td>FOV</td>
<td>Field of view</td>
</tr>
<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
</tr>
<tr>
<td>ID</td>
<td>Inner Diameter</td>
</tr>
<tr>
<td>MTF</td>
<td>Modulation transfer function</td>
</tr>
<tr>
<td>mV</td>
<td>millivolts</td>
</tr>
<tr>
<td>NA</td>
<td>Numerical aperture</td>
</tr>
<tr>
<td>NOA</td>
<td>Norland optical adhesive</td>
</tr>
<tr>
<td>OD</td>
<td>Outer Diameter</td>
</tr>
<tr>
<td>PMMA</td>
<td>Poly(methyl methacrylate)</td>
</tr>
<tr>
<td>POC</td>
<td>Point of care</td>
</tr>
<tr>
<td>PS</td>
<td>Polystyrene</td>
</tr>
<tr>
<td>RMS</td>
<td>Root mean squared</td>
</tr>
<tr>
<td>SD</td>
<td>Semi-diameter</td>
</tr>
<tr>
<td>TPP</td>
<td>Target product profile</td>
</tr>
<tr>
<td>WBC</td>
<td>White blood cell</td>
</tr>
<tr>
<td>WD</td>
<td>Working Distance</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
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</table>
YLL  Years of life lost to premature mortality

YLD  Years of life lost to disease
1.1. Summary

The work presented in this thesis describes the design, fabrication, and validation of a platform of low-cost, high-performance miniature optical detection systems for the monitoring and detection of infectious diseases at the point of care. Optical detection is a common method for screening and monitoring of many infectious diseases that disproportionately affect the world’s population living in developing countries. Instruments such as high-performance assay readers or high-end fluorescence microscopes are not cost-effective for implementation in developing countries and other low-resource settings. Components such as expensive objectives, arc lamps, and excitation and emission filters contribute to a high price tag in these devices. The platform described in this work consists of three sub-units, each consisting of a miniature fluorescence microscope that contains an
illumination module, a sample module, and a detection module. The miniature plastic fluorescence microscopes sub-units were validated against commercial instrumentation and designed to provide optical readout for a variety of biological samples tagged with single or multiple fluorophores. The prototype target applications were: (1) the optical readout of bead based bioassays, and (2) white blood cell counting and three-part differentials. Both of these tests are commonly used and have a variety of applications in the context of infectious disease diagnosis and monitoring.

The modular nature of the microscopes described in this work means that each device is customizable depending on the target application. In addition to an illumination, detection, and sample module, each microscope contains cost-effective electronics comprised of off-the components such as low-cost light emitting diodes (LEDs) and single board computers. The shift to mass-production of these standardized elements will result in lower cost-per-unit. In order to reduce the demand for clinical expertise in rural settings, each miniature system produces a digital output which may be used for remote automated image processing and analysis to classify and quantify the biological target.

1.2. Objective and Specific Aims

The goal of the research presented in this thesis is to develop a family of low-cost, high-performance microscopes that can be used at the point of care in low-resource settings to aid in the detection and diagnosis of infectious diseases. The
three generations of microscopes presented retain the desired functionality of their commercially available counterparts (assay reader and white blood cell differential) but cost at least one order of magnitude less for each prototype.

The specific aims are:

**Specific Aim 1:** Design and manufacture a family of miniature fluorescence microscope objectives (microscope units #1 and #2 in platform) to screen for infectious disease via molecular and morphological detection at the point of care in low-resource settings.

**Specific Aim 2:** Integrate microscope objectives from Aim 1 into modular optical interrogation units and validate with biological samples.

**Specific Aim 3:** Based on benefits and limitations of microscope units #1 and #2, design and fabricate a custom low-cost, high-performance all-plastic microscope (third unit in platform) that does not require manual readjustment between fluorescent channels.

**Specific Aim 4:** Validation of miniature fluorescence microscope from Aim 3 with a multiplexed biological sample.

### 1.3. Outline of Text

This thesis highlights the regions of the world where robust, low-cost diagnostic testing is needed and the challenges of designing instrumentation for
these areas (Chapter 2). Commercially available benchtop diagnostic instruments are discussed as well as the current landscape of devices designed for global health use at the point of care (Chapter 3). The design and manufacturing process for two microscopes that meet the required design characteristics needed for global health instrumentation are described: (1) a miniature microscope objectives for optical readout of bead based bioassays (Chapter 4) and (2) three-part white blood cell differential counts (Chapter 5). The integration of these custom optical systems into modular interrogation units, and the validation of the systems with biological samples are also discussed in these chapters (Chapters 4 and 5). These sections cover the material corresponding to Specific Aims 1 and 2 from Section 1.2.

Challenges of the design and fabrication process are discussed for these microscopes (Chapter 6) and the design, fabrication, and validation of a third microscope that was designed based on previous challenges is described (Chapter 7). This section corresponds to material from Specific Aims 3 and 4 described in Section 1.2. Specifically, the dissertation is organized in the following manner:

Chapter 1 provides an overview of the thesis and the list of specific aims.

Chapter 2 provides an overview of the challenges in screening, detection, and diagnosis of infectious disease at the point of care in low-resource settings, including analysis of geographic regions of interest and the most prevalent infectious diseases in these regions.
Chapter 3 is a literature review of common commercially available benchtop techniques for detecting infectious disease at the point of care as well as currently available low-cost alternatives and their limitations.

Chapter 4 details the fabrication and validation of a miniature 0.25 NA monochromatic, infinity-corrected fluorescence microscope designed for low-cost optical readout of bead-based bioassays. The results obtained with this 0.25 NA microscope were validated against results from a commercially available assay reader.

Chapter 5 describes the design, fabrication and validation of a 0.35 achromatic objective for three-part white blood cell differential counts at the point of care. The results from this objective were validated against a commercially available hematology analyzer.

Chapter 6 highlights the benefits and limitations of the optical systems described in Chapters 4 and 5. This chapter gives an in-depth description of the benefits of infinity-corrected system but the mechanical challenges of static monochromatic objectives for multiplexed samples (the interchange between multiple emission filters and the manual readjustment of the objective). Additionally I will highlight the benefits of achromatic systems but describe fabrication challenges of making achromatic doublets and the disadvantages of a finite objective. These factors were considered prior to design and fabrication of tunable system described in Chapter 7.
Chapter 7 presents a system that combines the benefits of monochromatic and achromatic optical systems described in Chapters 4 and 5 while eliminating the mechanical and fabrication challenges described in Chapter 6. The system described is a 0.25 tunable infinity-corrected fluorescence microscope designed to image multiplexed samples. The system was validated using whole blood stained with acridine orange (AO).

Chapter 8 summarizes the work described in Chapters 3-7, its impact, and possible future work.
Chapter 2

Challenges in medicine at the point of care in low-resource settings

2.1. Introduction

2.4 billion people live in low-income countries and lack resources to undergo routine health screenings or obtain treatment for diseases [1]. Figure 2.1 depicts low and middle income countries as defined by the World Health Organization [2].

These low-income countries account for more than half of total disability-adjusted life years (DALYs) [3]. One DALY is defined as one year lost of a traditionally healthy life. This metric provides a method for the burden of disease to be measured since burden of disease may be thought of as the gap between the ideal health status of an individual and the actual health status. A DALY is defined as

\[ \text{DALY} = \text{YLL} + \text{YLD} \]
Where YLL for a disease is years of life lost due to premature mortality and YLD is years lost to disability for the incident cases of the disease for a particular disease. YLL are defined as:

\[
YLL = \text{number of deaths at each age due to particular disease} \\
\times \text{global standard life expectancy of the age at which death occurs}
\]

and YLD are defined as:

\[
YLD = \left[\text{number of disease cases in that period}\right] \\
\times \left[\text{average duration of disease}\right] \times \left[\text{disability weight}\right]
\]

where “disability weight” is a factor dependent on a specific disease.

Figure 2.1 Image from the World Health Organization’s (WHO) report on Global Health Risks: Mortality and burden of disease attributable to selected
major risks, 2009. A world map illustrating population, gross national income per capita, life expectancy at birth, for low and middle income countries and high income countries [2].

Premature mortality from certain diseases disproportionately affects low-income areas. For example, worldwide, Africa accounts for roughly 90% of all child deaths due to malaria, 90% of child deaths due to AIDS and 50% of child deaths due to diarrheal disease and pneumonia [4]. In low-income regions, such as the WHO African Region, the risk of a child dying before reaching five years of age is 13 times higher than in high-income countries (6 deaths per 1000 births) [4].

2.2. Addressing challenges in medicine in low-resource settings

Widely available screening tools could significantly impact health outcomes in low and middle income countries and reduce the number of DALY cases. Point of care settings, or target product profiles (TPPs), range from tests used in the home (TPP1) to hospital (TPP5) tests and are outlined in Figure 2.2.

With the low-cost platform described in this work, the user may perform tests ranging from white blood cell differential counts to multiplexed bead based bioassays. These tests must meet requirements for a TPP that can be used across the spectrum of POC settings.
Figure 2.2 Spectrum of point of care settings and common tests performed at each [5].

The target product profile settings of the devices presented in this thesis range from TPP2 (a standalone white blood cell differential microscope) to TPP5 (automated optical readout of multiplexed bead based bioassays and testing of patients in hospitals) as shown in Figure 2.2. Table 2.1 displays the TPP minimum requirements for all miniature fluorescence microscope units of the platform that will be described in this thesis. Devices meeting the listed requirements in the categories of test performance, design considerations, economic and data transfer attributes will be useful in multiple low-resource point of care settings, ranging from rural clinics to hospital laboratories.
### Table 2.1 Target product profile for fluorescence microscope subunits of the platform

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test performance</td>
<td>• Comparable to established gold standard product</td>
</tr>
<tr>
<td>Design Considerations</td>
<td>• Portable (50 cm³ or smaller)</td>
</tr>
<tr>
<td></td>
<td>• Runs off local 110-220 AC power with rechargeable battery backup</td>
</tr>
<tr>
<td></td>
<td>• Must work with fluorescent samples (containing single or multiple fluorophores)</td>
</tr>
<tr>
<td></td>
<td>• Does not require complex redesign between sample targets</td>
</tr>
<tr>
<td></td>
<td>• Must interface with standard microscope slide (no custom sample cartridge or cuvette required)</td>
</tr>
<tr>
<td>Cost</td>
<td>• Must be at least 1 order of magnitude cheaper than established gold standard counterpart</td>
</tr>
<tr>
<td></td>
<td>• Per-test cost less than $1.00</td>
</tr>
<tr>
<td>Data transfer</td>
<td>• USB or wireless</td>
</tr>
</tbody>
</table>

The application for the first device target profile was optical readout of multiplexed assays because of the versatile applications for this diagnostic test. Multiplexed assays can simultaneously detect a panel of pathogens rather than single pathogens, and can be used to reduce the cost of test per pathogen [6]. Tests to detect RNA, DNA, and antibodies or antigens from bacteria, viruses or parasites may be conducted in a single assay rather than multiple and multiplexed assays may even be able to help identify drug resistance for treatments to widespread diseases such as malaria [6], [7].

The application for the second device TPP was three-part white blood cell differential counts. The white blood cell differential is one component of a complete
blood count (CBC), an evaluation performed for standard blood work-ups. Three-part white blood cell differentials measure the percentage of lymphocytes, monocytes, and granulocytes present in a sample of whole blood. This differential count can give community health care workers an indication if a patient has certain conditions such as an acute infection, anemia, chronic infection, or sepsis.

Each fluorescence microscope unit of the platform was validated against its commercial counterpart (Luminex’s MAGPIX assay platform or Beckman Coulter AcT diff2 hematology analyzer) to validate that the limits of detection and classification abilities of each device were comparable to their commercially available benchtop counterparts. All microscopes in the modular platform presented in this work met the power requirements in Table 2.1, were portable (<50 cm³), were at least one order of magnitude less expensive than their commercial counterpart, used a USB to transfer data to a laptop, and were designed to perform optical readout of samples with single or multiple fluorophores.

The next chapter will discuss the commercially available bench-top diagnostic instruments for white blood cell differential counts and optical readout of multiplexed bead based bioassays that were used as the standard to validate the miniature devices against. Chapter 3 will also discuss alternative miniature optical point of care diagnostic devices and why they do not meet the requirements of the specific TPP profile given in Table 2.1.
Background: Current Optical Screening Techniques at the Point-of-Care, from Benchtop to Bedside

In a high-income country (Figure 2.1), a patient’s diagnosis typically relies on trained clinicians, analyzing diagnostic test results in a hospital or external laboratory setting. Many instruments used to screen for disease or make diagnoses in hospitals and laboratories are expensive, costing on the order of tens of thousands of dollars or more. These expensive readers and microscopes also typically require trained staff to use them and are not portable or power-efficient. They also often have a high per-test cost. In low and middle income countries, widespread patient access to such devices is unrealistic and for the 2.4 billion patients living on less than $2.50 per day, a per-test cost of anything more than $1 is
considered unsustainable. Therefore there is a need for diagnostic instruments that retain high performance yet do not impose a high financial burden.

Point-of-care (POC) diagnostics designed for low resource areas can be used to provide diagnostic results to patients at a lower cost than conventional laboratory analysis and can be used in a spectrum of settings, from a rural community clinics to hospital laboratories. POC diagnostics that use optical detection techniques will be primarily discussed since they are more versatile than other methods of detection, which typically perform only a single or few tests and inherently lower cost as they do not require expensive reagents. POC devices must be as sensitive and specific as their benchtop counterparts, while being more durable, cost-effective, power-efficient, and portable and meeting all the requirements described in Table 2.1.

Section 3.1 describes the commercially available benchtop methods and instrumentation used for readout of bioassays and white blood cell differentials. The Luminex MAGPIX and Beckman Coulter Ac T diff2 hematology analyzer instrumentation mentioned in this section are used as the gold standards of validation (Table 2.1). Section 3.2 describes the current market for low-cost POC devices designed for use in low-resource settings. Section 3.2 focuses on optical diagnostics rather than other technologies such as flow cytometry due to the inherent low cost of microscopy compared to other detection methods as well as its versatility. The ability of these optical diagnostic instruments to meet the minimum requirements listed in Table 2.1 will be discussed.
3.1. Commercially available benchtop instrumentation

3.1.1. Commercially available instrumentation and techniques for multiplex assay readout

Multiplexed assays refer to assays that measure multiple analytes simultaneously [8]. These assays can be used for diagnostic purposes ranging from determining the cause of a patient’s fever, to screening patients for HIV [9]. There has been ongoing development of bench-top instruments with high specificity and sensitivity, which have the potential to screen for a large number of targets simultaneously [10]. These multiplexed assays are typically either planar or bead-based. Planar arrays consist of either an antibody array used for antigen profiling or membrane-based arrays [11]. Planar arrays may be used to run multiplexed ELISA assays [12]–[16]. In the bead-based assay format, immunodetection occurs on spheres that are single microns in diameter rather than on a flat surface. Beads contain a unique blend of specific fluorophores that act as a “signature” for a single analyte [11]. Bead-based assays require a flow-cytometer or a special reader, such as Luminex’s MAGPIX. Luminex’s technology is open and large amounts of different analyte combinations are available, however, making it useful for many different disease applications [17].

The MAGPIX fluorescent detection system utilizes magnetic MagPlex microspheres and Luminex’s XMAP technology to measure multiple proteins or nucleic acids in a single reaction [18]. The beads are illuminated by two LEDs and
their multi-channel fluorescence emission intensities are analyzed to classify the
analyte being detected and to determine its concentration [7]. Although the system
reduces the cost of traditional assays and reduces required sample volume, the
MAGPIX reader, shown in Figure 3.1, is not suited for point-of-care applications. It is
bulky and expensive, measuring 16.5 x 60 x 43 cm with a weight of 38.5 pounds and
has retail cost of approximately $35,000 USD [19].

![MAGPIX Reader](image)

**Figure 3.1 An image of the Luminex’s MAGPIX assay platform (estimated cost $35000 USD) [19].**

The ability to have an inexpensive, mass-produced platform to read spatially
multiplexed samples may have the potential to enable assays that could, for
example, allow multiple antibiotic resistance determinations at once. Detecting
multiple analytes in a single test is an emerging trend in point-of-care tests that can
provide identification for 80-90% of serious infections [20], possibly replacing
laboratory culture-based analysis for infection-causing pathogens in blood samples.
For this reason, optical readout of bead-based multiplexed assays was chosen as a preliminary target sample for the miniature microscope described in Chapter 4.

3.1.2. Clinical importance and benchtop instrumentation for white blood cell differential counting

The white blood cell (WBC) differential count is one of the most commonly performed tests at the point of care. Each white blood cell type is counted by determining its percentage of the total WBC population. The five types of white blood cells counted are granulocytes (neutrophils), eosinophils, basophils, lymphocytes and monocytes. For diagnostic purposes discussed throughout this thesis, I will be focusing only on the three-part differential WBC count, which determines the population of granulocytes, monocytes, and lymphocytes since it has been shown to still be a reliable clinical benchmark [21]–[24]. The WBC differential is a useful diagnostic tool at the point of care, and can help clinicians determine information such as if a patient is suffering from a viral or bacterial infection [25]. In high resource areas, commercially available benchtop hematology analyzers are used to perform these tests, but require trained staff and are expensive [26]. In low resource settings, these tests are often performed using a light microscope and a special microscope (hemocytometer) slide with counting chambers, where multiple fields of view must be counted by a technician. This process is time-intensive and prone to errors. Hematology analyzers designed for point of care applications, such as the HemoCue WBC, have been developed, although they are not yet available in the U.S. They also have a per-test cost that is
unsustainable in many low-resource settings. Commercially available points of care devices for clinical use impedance cell counting and spectrophotometric measurement of hemoglobin concentration in a small sample (20 microliters) of blood [23]. The Beckman Coulter Ac T diff2 hematology analyzer is used as the gold standard in this work and retails for approximately $30,000 [27]. There are also commercially available instruments designed for portable use such as the HemoCue WBC, which is not currently available for purchase in the United States. It performs a five-part WBC differential, but retails for more than $1000 and has a per test cost of $3.52, making it unsustainable for people living below the poverty line in low-resource areas. Chapters 5 and 7 discuss the development of miniature microscopes that perform a three-part WBC differential on Acridine Orange-stained whole blood for a per-test cost of less than $0.10.

3.2. Miniature optical diagnostic devices designed for point-of-care use

Section 3.1 described the clinical standard techniques to which the low-cost microscope platforms will be validated against (Table 2.1). A selection of currently available optical diagnostic instruments designed for global health are discussed in the following section. Several groups have developed low-cost platforms that aim to reduce instrumentation expenses of fluorescence readout for use in low-resource settings. These devices will be assessed based on design criteria given in Table 2.1.
The CellScope, by the Fletcher group [28], is shown in Figure 3.2. This portable microscope uses an off-the-shelf objective (0.85 NA 60X Achromat objective) and a 20x wide field eyepiece, resulting in a system field of view of 180 um in diameter, a magnification of 28X, and a resolution of 1.2 um, which is 3x larger than the theoretical Rayleigh resolution limit =0.61λ/NA=0.4 um [28]. The CellScope, while portable and lower in cost than traditional bench-top microscopes, is less versatile and not designed for multiplexing applications. The microscope performs in brightfield and fluorescence modes, but only with a single illumination and filter set. The emission filter is not located in an infinity space so the system would require redesign or manual adjustment if the excitation or emission wavelengths were changed.

Figure 3.2 (a) The mobile phone microscope layout schematic. (b) Image of the prototype. (c) brightfield images of fluorescent beads (6 um diameter) (d) Fluorescent images of beads. The field of view of is outlined in the dashed white line. [28]
This platform also relies on a mobile phone camera for a detector. Devices that use mobile phone platform microscopes inherently restrict the adjustment of capture parameters such as exposure time, master gain, and relative color gain [29]. Additionally, with a mobile phone camera, users are unable to set processing parameters such as compression, custom corrections, edge enhancement and noise reduction [29]. Ultimately the user interface is simplified through use of mobile phone cameras but control of capture and image processing parameters are sacrificed [30]. The current commercially available Cellscope retails for $79 [31] but is only compatible with the iPhone 5, 5s, 6, and 6s, increasing the total device cost by several hundred dollars.

![Diagram of Cellscope](image)

**Figure 3.3 (a) Schematic of the Cellscope Loa. (b)Simplified optical diagram of microscope [30].**

Additionally, the Cellscope does not work with multiplexed samples and requires redesign depending on application. A newer model of the device by the
group, uses multiple mobile phone camera lenses [30], [32]. The simplified optical schematic of one of the device models, the Cellscope Loa, is shown in Figure 3.3. This device also requires the use of an iPhone (5s) and requires a specialized capillary tube in a holder for the sample platform. It also requires an array of LEDs and an additional iPhone lens used in reverse and has not yet been tested in fluorescence mode [30].

The profile of the Cellscope based on the criteria from Table 2.1 is shown in Table 3.1.

**Table 3.1 TPP of the Cellscope based on minimally acceptable profile defined in Table 2.1**

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Minimally acceptable profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test performance</td>
<td>• Yes. [Uses commercially available lenses]</td>
</tr>
<tr>
<td>Design Considerations</td>
<td>• Portable</td>
</tr>
<tr>
<td></td>
<td>• Runs off local 110-220 AC power with rechargeable battery backup</td>
</tr>
<tr>
<td></td>
<td>• Does not work with multiplexed fluorescent samples</td>
</tr>
<tr>
<td></td>
<td>• Newer models only work in brightfield modality</td>
</tr>
<tr>
<td></td>
<td>• Requires complex redesign between sample targets and illumination modality (trans to epi)</td>
</tr>
<tr>
<td></td>
<td>• Requires manual readjustment</td>
</tr>
<tr>
<td></td>
<td>• Requires specialized sample holder</td>
</tr>
<tr>
<td>Cost</td>
<td>• Optics and holder meet cost requirements</td>
</tr>
<tr>
<td></td>
<td>• Costly detector required</td>
</tr>
<tr>
<td></td>
<td>• Per-test cost unknown</td>
</tr>
<tr>
<td>Data transfer</td>
<td>• Uses USB or wireless connection</td>
</tr>
</tbody>
</table>
The Cellscope meets some of the profile requirements, but does not have the versatility to be used with multiplexed samples. Its optical layout needs modification between sample targets in addition to exchanging filters and light sources.

Another portable microscope designed for use at the point of care in low-resource settings is the Global Focus Microscope (Figure 3.4). The Global Focus Microscope uses an LED flashlight with a white LED as a brightfield imaging light source and a blue LED with narrow band excitation filter for fluorescence imaging of *M. tuberculosis* samples [33]. The estimated cost of the prototype was $240 USD. Although the low price point and imaging performance of the microscope are sufficient to image *M. tuberculosis* bacilli stained with fluorescent dye, the microscope is not designed for a wide array of sample applications since it possesses only one alternative illumination wavelength to white light. It also requires manual adjustment by the user and skill to locate the bacteria and is not intended for use with multiplexed samples.
Figure 3.4 Image from Steingart et al. 2006. The Global Focus microscope is shown above. The spatial resolution of the system is 0.8 um and can achieve magnification of 1000x. (a) The trans-illumination light path. (b) Left image is an M. tuberculosis bacilli stained with auramine orange dye. The image was obtained with the Global Focus microscope at a magnification of 400x. Digital magnification detail is shown on the right [33].

The profile of the Global Focus microscope is shown in Table 3.2. The global focus microscope meets performance standards for one sample type (fluorescent dye stained sputum smears). It is portable and low-power but only works with samples that contain a single fluorophore (auramine orange). Other sample types would require a redesign of the system.
Table 3.2 TPP of the Global Focus Microscope based on Minimally acceptable profile defined in Table 2.1

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Minimally acceptable profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test performance</td>
<td>• Comparable results to established gold standard (Nikon E400)</td>
</tr>
</tbody>
</table>
| Design Considerations      | • Portable  
• Runs off local 110-220 AC power with rechargeable battery backup  
• Works with single fluorophore (Auramine o.)  
• Requires complex redesign between sample targets and imaging modalities (trans- to epi- illumination)  
• Interfaces with standard microscope slide (no custom sample cartridge or cuvette required) |
| Cost                       | • Cost estimated $240 not including detector (Canon 69)  
• Per-test cost less than $1.00                                                                                                                              |
| Data transfer              | • USB or wireless (requires external detector, used Canon G9 camera)                                                                                         |

The Ozcan group has created a lens-free cell phone microscope that uses in-line digital holography to image biological samples such as blood smears with a large field of view (~20 mm²) [34], [35]. Lens free microscopy still faces several challenges, however, such as difficulty in imaging fluorescent and thick specimens [34]. More details of its design specifications are shown in Table 3.3.

The Ozcan group has also developed a mobile phone based systems designed for imaging single DNA molecules, described in Table 3.4 [36]. This device is designed to image in dark field mode and fluorescence mode. The authors were able to measure lengths of DNA molecules. Although the FOV of the device is large (2 mm²), the integration time required for imaging the DNA in fluorescence mode is
roughly 4 seconds per frame, and leads to photobleaching of the sample. The device is intended for field-use, but has only been used in a laboratory setting at present.

Table 3.3 Summary of attributes of lensless imaging system (Ozcan group)

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Minimally acceptable profile</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Test performance</strong></td>
<td>• Requires digital reconstruction, reconstructed images compared to benchtop imaging results</td>
</tr>
<tr>
<td><strong>Design Considerations</strong></td>
<td>• Portable</td>
</tr>
<tr>
<td></td>
<td>• Power requirement unknown</td>
</tr>
<tr>
<td></td>
<td>• Cannot image high density samples in fluorescence mode</td>
</tr>
<tr>
<td></td>
<td>• Only designed for bright-field or single wavelength fluorescence</td>
</tr>
<tr>
<td><strong>Economic</strong></td>
<td>• Detailed cost analysis not performed</td>
</tr>
<tr>
<td><strong>Data import</strong></td>
<td>• USB or wireless</td>
</tr>
</tbody>
</table>

Table 3.4 Summary of attributes of DNA imaging and sizing platform (Ozcan group)

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Minimally acceptable profile</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Test performance</strong></td>
<td>• Validated against benchtop fluorescence microscope (1.3/100x oil-immersion objective)</td>
</tr>
<tr>
<td><strong>Design Considerations</strong></td>
<td>• Portable</td>
</tr>
<tr>
<td></td>
<td>• Meets power requirement</td>
</tr>
<tr>
<td></td>
<td>• Only works at a single excitation and emission wavelength</td>
</tr>
<tr>
<td></td>
<td>• Requires specialized sample holder</td>
</tr>
<tr>
<td><strong>Economic</strong></td>
<td>• $400 estimated cost (not including mobile phone)</td>
</tr>
<tr>
<td><strong>Data import</strong></td>
<td>• USB or wireless</td>
</tr>
</tbody>
</table>
Other microscopes that have been developed for point of care use include the Foldscope (Table 3.5) [37], which is an origami inspired microscope containing an LED and microlenses as well as the ability for manual adjustment to focus on samples. These devices are summarized in Table 3.3 based on the minimum requirements for TPP profile described in Table 2.1.

Although the platforms described in this section have advanced the field of miniature optical diagnostics for global-health applications, they do not meet all the requirements of the product specifications listed in Table 2.1. There is still a need a platform that is comparable to an established gold standard product, runs off low-power, and is portable. Any platform should interface with a standard sample, such as a microscope slide, so that expensive (>$1.00 per use) disposable sample containers such as cuvettes do not need to be purchased.

**Table 3.5 Summary of attributes of Foldscope**

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Minimally acceptable profile</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Foldscope</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Test performance</strong></td>
<td>• Reliability not measured for fluorescence imaging of biological samples</td>
</tr>
<tr>
<td><strong>Design considerations</strong></td>
<td>• Interfaces with microscope slide</td>
</tr>
<tr>
<td></td>
<td>• Requires manual adjustment for focusing</td>
</tr>
<tr>
<td></td>
<td>• Different microscopes are required for each modality (brightfield, fluorescence, darkfield)</td>
</tr>
<tr>
<td></td>
<td>• Cannot image multiplexed fluorescent samples</td>
</tr>
<tr>
<td><strong>Cost</strong></td>
<td>• Estimated cost &lt;$1.00 per microscope (not including camera cost)</td>
</tr>
<tr>
<td><strong>Data transfer</strong></td>
<td>• USB or wireless</td>
</tr>
</tbody>
</table>
Ideally diagnostic imaging devices will have the capability to image samples containing single or multiple fluorophores without requiring complex redesign or the purchase of new devices. Additionally, all of the current microscope platforms described interface only interface with cell-phone cameras, which are not included in the cost analysis or the discussion of sustainability of such platforms. Chapters 4-8 will discuss the development of a family of modular all-plastic microscopes designed for use at the point of care in low resource settings that meet the product requirements listed in Table 2.1. These microscopes contain low-cost electronics and detectors, were designed to image a wide spectrum of sample types, and provide automated digital readout, which may also lead to potential networking of patient results and a strengthening of patient monitoring and health systems [38].
Chapter 4

Development of a miniature monochromatic fluorescence microscope for molecular imaging

*The contents of this chapter were published with in the following journal article: Alessandra Forucci, Michal Pawlowski, Zachary Crannell, Catherine Majors, Ina Pavlova, and Rebecca Richards-Kortum, and Tomasz S. Tkaczyk, 3D printed miniature fluorescence microscope for point-of-care readout of bead-based bioassays, J. Biomed. Opt. 20 (10), 105010 (October 21, 2015). All figures have been published with permission from SPIE.

As discussed in Chapters 2 and 3, there is a need for low cost optical devices to analyze biological samples at the point of care in settings that are considered to be low-resource. The following chapter describes a low-cost 3D printed fluorescence microscope that can be used as a detector for multiplexed, bead-based assays to support point-of-care applications. Images obtained with the microscope were analyzed to differentiate multiple analytes in a single sample with a
comparable limit of detection to commercially available macroscopic assay platforms.

4.1. Introduction

The World Health Organization (WHO) has called for an increase in access to reliable diagnostic tests in low-resource areas [39]. Access to diagnostic devices is integral to reducing long term complications that may result from diseases such as HIV/AIDS, malaria, respiratory infections, perinatal conditions, and diarrhoeal diseases [9]. Point-of-care (POC) tests often require an optical system for signal detection as well as imaging, and in low-resource environments, size, cost, and infrastructure requirements of these optical systems is critical [40].

POC diagnostics that do not require operation by highly trained laboratory staff are used to rapidly provide results to patients at a lower cost than conventional laboratory analysis would entail. Often these diagnostics eliminate the need for samples to travel to a lab, and because they are more specialized than conventional laboratory equipment, are designed to perform only a single or few tests. These POC devices are typically designed for targets of proteins, metabolites and other small molecules, nucleic acids, human cells, and microbes or pathogens. Fluorescence signal detection is a commonly used technique to detect and diagnose infectious and hematological diseases due to its high sensitivity and specificity, and bench-top detection systems, especially those that perform fluorescence signal detection, often
require expensive illumination sources and multiple optical elements, such as lenses and emission and excitation filters.

To reduce instrumentation costs that are typical for commercial fluorescence microscopy systems, several groups have used 3D printing to develop custom optical instrumentation. The CellScope, a 3D-printed microscope designed to attach to a cellular phone, developed by the Fletcher group, is an example of one portable device that uses commercially available optics. It consists of a commercially available objective (0.85 NA, 60X Achromat) and a commercially available 20x wide-field eyepiece, resulting in a system field of view 180 μm in diameter, a magnification of 28x, and a resolution of 1.2 microns [28]. The CellScope Oto, a digital otoscope sold commercially by the group, currently retails for $299 USD [31]. The CellScope Oto is designed to perform ear exams at the point of care via digital brightfield imaging. The device does not operate in fluorescence mode; therefore the listed price does not include components found in these typically more expensive systems, such as excitation and emission filters. The price also does not take into account cost of the detector (iPhone). When compared to scientific cameras, cell phone cameras restrict the adjustment of capture parameters such as exposure time, master gain, and relative color gain [29]. Additionally, with a mobile phone camera, users are unable to set processing parameters such as compression, custom corrections, edge enhancement, and noise reduction [29]. Although the user interface is simplified through use of a mobile phone camera, control of image capture and processing is sacrificed.
The Ozcan group also created a cost-effective microscope cell-phone attachment [41]. This attachment combines commercially available optics and 3D printed optomechanics to create a transmission microscope that interfaces with a modified flow cytometer to quantify white blood cells in blood samples and can resolve features on the order of two to four microns [41]. This attachment is low-cost (estimated at <10 USD in mass production) but can only perform tests using two LEDs of the same color. Also, the plastic filter used is an absorption filter, which reduces overall device cost but does not permit narrowband excitation of a sample.

Other 3D-printed microscopes designed for global health applications, such as the Global Focus Microscope, use low-cost components such as LED flashlights containing a white LED for brightfield imaging and a blue LED with narrow band excitation filter for fluorescence. The Global Focus Microscope is designed specifically for a single application, fluorescence and brightfield imaging of M. tuberculosis samples [33]. The Global Focus Microscope uses multiple commercially available microscope objectives for brightfield and fluorescence imaging, however it is limited in the versatility of its fluorescence applications since the filters and illumination source are specifically chosen for auramine orange fluorescent dye. The estimated cost of the prototype was $240 USD.

Many portable fluorescence systems designed for the point of care, including the CellScope and Global Focus microscope, use high-power LEDs as light sources due to their long lifetime (~50,000 hours), ambient operating temperature, and low power requirements in addition to their low cost [42]. Traditional fluorescence
microscopy illumination setups typically use high-energy, expensive light sources such as Xenon or mercury arc lamps. These bulky illumination sources are unsuitable for work in the field since the bulbs are delicate and provide illumination for only \( \sim 200 \) hours \([43]\). Our prototype device uses two high power LEDs to provide dual excitation for multiplexed imaging. Instrumentation costs will typically increase if quantification of signal in samples with multiple fluorescence emission channels is needed to make more informed diagnoses at the point-of-care setting.

The Ligler group has developed a microflow cytometer platform for performing multiplexed bead-based assays. Multiplexed microsphere immunoassays using the system were successfully performed for six discrete bacteria and toxins and the microflow cytometer produced results comparable to that of commercial systems \([44]\). Recently this group fabricated a spinning magnetic trap platform. This platform, the MagTrap, interfaces with magnetic core polystyrene microbeads \([45]\). The MagTrap system was integrated with the existing microflow cytometer to automate sample preparation and detect multiple pathogens simultaneously\([45]\).

There has also been ongoing development of bench-top readers with high specificity and sensitivity, which have the potential to screen for a large number of targets simultaneously \([8], [46]\). Currently, a number of such bench-top instruments for molecular assays are available on the market \([47], [48]\). Luminex’s MAGPLEX system of labeled beads is one such technology that exemplifies spatial multiplexing. The MAGPIX fluorescent detection system utilizes magnetic MagPlex microspheres and Luminex’s XMAP technology to measure multiple proteins or nucleic acids in a
single reaction [49]. The Luminex system utilizes pre-set ratios of two dyes dissolved in their polymer beads, which exhibit peak emission at two discrete wavelengths [18]. The antibody or capture oligomer for a given target is immobilized on beads with a particular dye ratio, and readout in a cytometry-like fashion includes identifying the bead type according to the ratio of the two dyes (excited by a common wavelength) and quantifying the bound target using a different excitation wavelength for a fluorescent label [20]. The beads are illuminated by two LEDs and multi-color fluorescence images are analyzed to classify the analyte being detected and to determine its concentration[7]. Although the system reduces the cost of traditional assays and reduces required sample volume, the MAGPIX reader is not suited for point-of-care applications. It is bulky and expensive, measuring 16.5 x 60 x 43 cm with a weight of 38.5 pounds and retail cost of approximately 35,000 USD [19].

The sections that follow describe a low-cost prototype of a miniature optical detection system designed for point-of-care optical readout of bead-based bioassays. The system was fabricated using the rapid prototyping techniques of diamond turning and high resolution 3D printing. The system is modular and contains two main subunits: a miniature microscope and an illumination system designed for two fluorescent excitation pathways. Single point diamond turning was used to fabricate all custom lenses in the prototype miniature system. Single point diamond turning is primarily used to fabricate inserts for lenses for injection
molding, and has been well documented as a method by which to fabricate high performance, custom miniature plastic lenses [50]–[54].

Unlike the low-cost prototypes discussed previously [3]–[8], our modular prototype can independently excite two fluorophores. It may be used in brightfield or fluorescence modes, and has three fluorescent emission channels. Unlike many other low-cost microscopes [31], [33], our prototype was designed with a specific application in mind (multiplexed bead-based bioassays). The illumination system contains two high-power LEDs to provide dual excitation for multiplexed imaging. The initial sample for demonstrating successful optical readout was the MagPlex bead platform due to its commercial availability and simple sample preparation, though the modular nature of the assembled prototype provides versatility for future applications. The stackable, modular optomechanics units were made via 3D printing, an additive rapid fabrication process that is used to make components of custom geometries and sizes, such as the tunable objective lens holder and tube lens holder used in the miniature microscope subunit. Unlike the Cellscope and other cell phone based platforms [28], [29], [31], [41], our device uses low-cost board-level camera. The use of a board-level camera ensures that the user can, if necessary, modify image acquisition settings, which is difficult or impossible to do on many cellular phones. The diffraction-limited optical design is monochromatic, which relaxes manufacturing tolerances and simplifies overall design and assembly. Single point diamond turning of prototype plastic lenses is used to create the custom spherical and aspheric lenses. The all-plastic composition of the prototype, including
the diamond-turned PMMA objective and tube lenses, is critical for demonstrating the potential for the device to be mass produced via injection molding. Our resulting point-of-care prototype is 1.5 orders of magnitude cheaper than its commercially available counterpart, Luminex’s MAGPIX, and can perform comparable analyte classification and quantification.

### 4.2. Experimental Methods

For the prototype system, optical readout of bead-based bioassays was validated using Luminex’s system of MagPlex beads due to their simple experimental preparation and commercial availability. The spectral properties of the MagPlex beads are described in Section 4.2.1. Sections 4.2.2 and 4.2.3 describe the optical design of the miniature microscope and illumination system, respectively. Sections 4.2.4.1 and 4.2.4.2 describe the design and fabrication process for the microscope and illumination optomechanics, respectively.

#### 4.2.1. Luminex’s MagPlex Beads

The MagPlex microspheres are magnetic core-containing polystyrene beads 6.5 microns in diameter. There are two fluorophores present in the MagPlex beads, referred to as Classifier 1 (CL1) and Classifier 2 (CL2), which allow the beads to be identified by an optical reader. The Classifier 1 dye has excitation and emission peaks at 615 nm and 661 nm, respectively, and the Classifier 2 dye has excitation and emission peaks at 615 nm and 720 nm, respectively. When irradiated by a
common excitation channel, the bead type can be identified by the ratio of the intensity of the two classifier dyes. Each bead type is functionalized with a different type of oligonucleotide capture sequence complementary to a target sequence of interest. Extended PCR products are biotinylated, hybridized to the beads, and labeled with a streptavidin coupled “Reporter” fluorescent dye (Figure 4.1). The Reporter dye has an excitation peak at 510 nm and an emission peak at 590 nm.

![Figure 4.1](image.png)

**Figure 4.1** The figure above illustrates the MagPlex bead hybridization. B is biotin and PE is streptavidin phycoerythrin. The Classifier (CL1/CL2) channels determine the analyte being detected while the magnitude of the PE-derived signal is measured in the Reporter channel.

### 4.2.2. Optical Design of the 0.25 NA Miniature Fluorescence Microscope

An optical schematic of the miniature fluorescence microscope designed for the preliminary application of optical readout of bead-based bioassays is presented in Figure 4.2.
Figure 4.2: The optical schematic of the 0.25 NA miniature fluorescence microscope. All dimensions given are in millimeters (mm).

The microscope objective and tube lens were custom designed using Zemax® (Radiant Zemax, Redmont, WA, USA). A summary of the basic optical parameters of the microscope is presented in Table 4.1. Note that the optical system presented in Figure 4.2 has flat object and image surfaces.

Table 4.1: Summary of optical parameters of 0.25 NA miniature fluorescent microscope.

<table>
<thead>
<tr>
<th>Optical Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA image side</td>
<td>0.25</td>
</tr>
<tr>
<td>Magnification</td>
<td>4x</td>
</tr>
<tr>
<td>Telecentric object space</td>
<td>Yes</td>
</tr>
<tr>
<td>Object side Field Of View diameter</td>
<td>1.2 mm</td>
</tr>
<tr>
<td>Objective working distance</td>
<td>1.2 mm</td>
</tr>
<tr>
<td>Design wavelength</td>
<td>590 nm</td>
</tr>
<tr>
<td>Largest Clear Aperture (1st surface of tube lens)</td>
<td>8.07 mm</td>
</tr>
<tr>
<td>'Infinity space' length</td>
<td>15 mm</td>
</tr>
<tr>
<td>Total length of optical system</td>
<td>43.02 mm</td>
</tr>
</tbody>
</table>

The optical prescription data of the microscope objective and the tube lens are given in Table 4.2. The microscope objective has a numerical aperture (NA) of
0.25, focal length of 4 mm and a 1.2 mm diameter Field of View (FOV). The tube lens has a focal length of 16mm, and together with the objective, forms an infinity-corrected microscope.

Table 4.2 Optical prescription data of miniature fluorescent microscope. Radii, thicknesses and semi-diameters are in units of [mm].

<table>
<thead>
<tr>
<th>Surface</th>
<th>Radii</th>
<th>Thickness</th>
<th>Glass</th>
<th>Semi-Diameter</th>
<th>Conic</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>∞</td>
<td>1.200</td>
<td></td>
<td>0.600</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-2.565</td>
<td>3.125</td>
<td>PMMA</td>
<td>0.868</td>
<td>1.839</td>
</tr>
<tr>
<td>2</td>
<td>-3.032</td>
<td>0.732</td>
<td>PMMA</td>
<td>1.738</td>
<td>0.062</td>
</tr>
<tr>
<td>3</td>
<td>9.389</td>
<td>3.200</td>
<td>PMMA</td>
<td>1.930</td>
<td>-2.937</td>
</tr>
<tr>
<td>4</td>
<td>-7.061</td>
<td>1.001</td>
<td>PMMA</td>
<td>1.981</td>
<td>-0.746</td>
</tr>
<tr>
<td>5</td>
<td>4.749</td>
<td>3.200</td>
<td>PMMA</td>
<td>1.793</td>
<td>0.194</td>
</tr>
<tr>
<td>6</td>
<td>3.817</td>
<td>0.500</td>
<td>PMMA</td>
<td>1.152</td>
<td>0.955</td>
</tr>
<tr>
<td>7</td>
<td>∞</td>
<td>14.500</td>
<td></td>
<td>1.014</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>7.380</td>
<td>3.108</td>
<td>PMMA</td>
<td>4.035</td>
<td>-0.954</td>
</tr>
<tr>
<td>9</td>
<td>-7.859</td>
<td>1.345</td>
<td>PMMA</td>
<td>3.962</td>
<td>-2.875</td>
</tr>
<tr>
<td>10</td>
<td>-12.868</td>
<td>3.000</td>
<td>PMMA</td>
<td>3.403</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>7.775</td>
<td>9.215</td>
<td></td>
<td>2.793</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>∞</td>
<td></td>
<td></td>
<td>2.501</td>
<td></td>
</tr>
</tbody>
</table>

The microscope objective and tube lens were designed to be infinity-corrected to increase system flexibility. This flexibility enables switching of components auxiliary to the imaging system, such as filters, prisms and polarizers, without affecting the image-object plane location and resolution. The combined magnification of the microscope is -4x. The microscope objective is built from three aspheric lenses, is 11.34 mm long, and has a maximum clear aperture diameter of 4 mm. The tube lens, due to the ‘infinity space’ length of 15 mm, has the largest clear aperture, measuring 8 mm. The tube lens is 7.5mm long and consists of two elements, one spherical and one aspheric. The total length of the optical system is 43 mm, which is a length comparable to many commercial microscope objectives. For
example, the Zeiss Primo Plan-Achromat 10x/0.25 is 40mm long, as measured from front lens to stop face.

To simplify the optical design and relax the manufacturing tolerances, the system was optimized for a single wavelength. The objective and the tube lens are made exclusively from Poly(methy methacrylate) (PMMA) due to its excellent machinability on diamond turning machines [55], [56] and low autofluorescence [52]. The modulation Transfer Function (MTF) that was calculated using the Fast Fourier Algorithm (FFF) for the design wavelength in nominal working conditions is presented in Figure 4.3a, together with corresponding spot diagram plot depicted in Figure 4.3b.

The design was optimized for 8 field points, evenly distributed along the Y-field object axis from 0 mm to 0.6mm. The MTF and the spot diagram plots shown in Figure 4.3 indicate diffraction limited performance for all field points for nominal working conditions. Small deviation of sagittal MTF plot for marginal field point indicates presence of uncorrected astigmatism, but at a level that is not detrimental to overall system performance. Diffraction limited performance of the miniature fluorescence microscope was achieved due to extensive use of aspheric components throughout the design.
Figure 4.3 Performance metrics of the miniature tunable fluorescence microscope for nominal working conditions: a) modulation transfer function
plots for two tangential field points located on optical axis and -0.6mm away from it. MTF plots for tangential and sagittal field points are drawn in blue and for field point located -0.6mm away from the optical axis in green. Tangential and sagittal MTF plots for diffraction limited system are drawn in black. All MTF plots are for the design wavelength of 590nm; b)Spot diagrams for all designed, tangential field points of: 0.0mm, -0.1mm, -0.2mm, -0.3mm, -0.4mm, -0.45mm, -0.5mm, -0.6mm, for design wavelength of 590nm. Horizontal extent and vertical extent of sub-field rectangles: 20nm. The airy disk is depicted in spot-diagrams sub-field images as black circle. The theoretically calculated Airy disk radius is 5.9µm.

The optical system of the microscope was designed in a two-stage process. In stage one the microscope objective was designed, and in stage two the tube lens was designed. We corrected aberrations of the microscope objective and the tube lens individually, i.e. aberrations of the microscope objective were not corrected by the tube lens and vice versa. This complicated the design, but resulted in compatibility of the microscope objective and tube lens with a large selection of commercially available infinity-corrected systems. The expected system performance was evaluated using the Root Mean Square (RMS) wavefront error during the design process. The RMS wave front error of the microscope optical train was 0.054 for nominal working conditions. The fabrication tolerance parameters used during optimization stage are presented in Table 4.3.

Based on 10,000 runs of Monte-Carlo simulations, it was estimated that the miniature fluorescence microscope would achieve diffraction limited performance on axis with 80% probability and there would be a 20% of chance of reaching diffraction limited performance within the field of view enclosed by a disk of radii of
0.42mm (70% of object height). The estimated change in performance due to manufacturing tolerances calculated using the Root Sum Square (RSS) algorithm was estimated to be 0.058 and the total estimated system performance (nominal + change) was estimated to be 0.112. In nominal working conditions, as defined by the Rayleigh criteria, diffraction effects will limit the resolution of the miniature fluorescent microscope to ~1.4 μm. Taking the size of the target objects (6.5 μm) and the system resolution into account, we accepted the predicted performance of the system because our application did not require a diffraction-limited 0.25NA imaging system for sufficient object sampling.

### Table 4.3 Tolerance parameters of the miniature fluorescent microscope.

Legend for table superscript: *1-* for surface 7 (‘infinity space’)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Radii [%]</th>
<th>Thickness [mm]</th>
<th>Element Decent [mm]</th>
<th>Element Tilt [°]</th>
<th>Surface Decenter Tilt [mm]</th>
<th>Irregularity [Fringes]</th>
<th>Abbe [%]</th>
<th>n [a.u.]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td>±0.4 %</td>
<td>±0.035</td>
<td>±0.02</td>
<td>±0.03</td>
<td>±0.02</td>
<td>±0.2</td>
<td>±0.57</td>
<td>±0.00</td>
</tr>
<tr>
<td>±0.2’1</td>
<td></td>
<td>±0.2’1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The tube lens and microscope objective were manufactured on the Precitech Optimum 2400 (Ametek, Precitech, Keene, NH, USA) diamond turning lathe. Pellets of optical grade plastics were mounted on the diamond turning machine using ER16-UP (Rego-Fix, Tenniken, Switzerland) collets housed in a custom vacuum chuck compatible holder. ER16-UP collets limited the diameter of manufactured parts to 10 mm, which was taken into account during design stage. Diamond turning paths were generated using the Diamond Turning Path Generator (DTPG v2.54,
Precitech, NH, USA) program based on the Zemax lens drawings. In order to simplify assembly of the system, lenses were manufactured with integrated spacing features and were stacked inside brass hypodermic tubes. The outer diameter of all lenses was manufactured to meet tight, interference fit tolerances with the inner diameter of brass tubes. Both brass tubes that held the microscope and tube lens assemblies, had ~1mm wide slit cut throughout their lengths parallel to the mechanical axis of the optical system, which allowed non-invasive insertion of the fragile plastic lenses. Elastic expansion of the brass tubes, due to interference fit between lens OD and tube ID resulted in radial clamping force, with its vector pointing towards mechanical axis of the optical system that held both the microscope objective and tube lens assemblies.

The microscope objective was mounted inside a hypodermic brass tube that had a 7.13 mm outer diameter (OD) and a 6.34 mm internal diameter (ID). The tube lens assembly was inserted into brass tube with a 10.3 2mm OD and a 9.54 mm ID. A drawing of the optical system assembly of the microscope objective is presented in Figure 4.4a. A schematic of the opto-mechanical assembly of the tube lens is depicted in Figure 4.4b.
The assembled prototypes of the microscope objective and tube lens were tested individually on the Zeiss Axio Imager Z1 (Zeiss, Oberkochen, Germany) microscope. We limited stray light attenuating features by internal blackening of the brass hypodermic tubes and coating of lens spacing stop surfaces with permanent marker prior to inserting lenses. Additionally, before testing we masked non-active front and back areas of both systems (tube lens and microscope objective) with laser cut stops, made from thin, volume blackened plastic. The prototypes were mounted sequentially in a custom designed 3D printed holder, which interfaced with the Zeiss microscope’s revolving nosepiece. The resolution of the system components was assessed using positive, high resolution 1951 USAF resolution test (Stock No:58-198, Edmund Optics, NJ, USA). The Zeiss standard halogen illumination unit was used with a narrowband, 656 nm filter with a 10 nm
bandwidth (#65-656, Edmund Optics, NJ, USA) inserted in the illumination path. We tested all components of the system at a wavelength of 656 nm to use the 1951 USAF target for quality assessment, since at the design wavelength of 590 nm the lens will out-resolve the high-resolution 1951 USAF target, making testing with a commercially available resolution target impossible. Images of the 1951 USAF resolution target were acquired through a standard 1x microscope tube lens using a black and white Axiocam MRm camera (Zeiss, Oberkochen, Germany).

Three optical systems were tested to assess the optical performance of each individually: the miniature plastic microscope objective, the miniature plastic tube lens, and a commercially available Zeiss 10x/0.25 objective. Raw images of the resolution target recorded using the miniature fluorescent microscope objective, the miniature tube lens, and the Zeiss objective are presented in Figure 4.5(a-c).

![Images of 1951 USAF resolution target taken using Zeiss Axio Imager Z1 through: a) miniature all plastic microscope objective, b) miniature plastic tube lens, c) and reference Zeiss 10x/0.25 microscope objective. Please note that due to difference in focal lengths of tested systems, images of resolution target were recorded at different magnifications. Magnified, red]
marked regions of 1951 USAF resolution target, close to assessed limit of resolution are zoomed and presented in inlays in respective images.

The miniature microscope objective was tested in nominal, designed working conditions, with the resolution target located in object plane. The tube lens was tested in reverse, with 1951 USAF resolution target located in its designed image plane. The Zeiss microscope objective was tested in nominal working conditions to provide a reference point for the components of the miniature system. All three tested lenses have different focal lengths, which in the infinity-corrected set-up of Zeiss microscope resulted in different magnifications of the 1951 USAF target images.

The spatial resolution of the microscope objective was measured to be $\nu_{ob}@656nm=575lp/mm$. The spatial resolution of the tube lens was measured to be $\nu_{tb}@656nm=203lp/mm$. The Zeiss microscope objective successfully resolved features up to $\nu_{zeiss}@656nm=287lp/mm$. Theoretically a diffraction-limited 0.25NA objective at a wavelength of 656 nm, according to Rayleigh criterion, should resolve $\nu_{theo}@656nm=625lp/mm$, a spatial resolution located in group 9 between elements 2 and 3. Our microscope objective successfully resolved both horizontal and vertical features of element 2 group 9 as well as horizontal features of group 9 element 3, confirming satisfactory, as designed, diffraction-limited performance. The tube lens in nominal ‘as designed’ working conditions, has an image NA of 0.063 and theoretically, according to Rayleigh criterion, illuminated with wavelength of 656nm
should resolve spatial features up to $v_{RTL}=157\text{lp/mm}$ (a spatial frequency located in group 7 between elements 2 and 3). Because the tube lens was designed independently from the microscope objective for compatibility with future components of the microscope, it had a larger than required clear aperture. The tube lens was tested with the microscope Koehler illumination aperture set to a numerical aperture of 0.1 (the minimum NA of the illumination possible on the Zeiss system). The system was designed to work with an illumination beam of 0.063 NA but for experimental settings using an illumination NA of 0.1, the expected spatial resolution limit was met (203 lp/mm). Because the tube lens was designed independently from the objective so that it may interface with future generation custom-designed miniature infinity-corrected objectives of higher numerical apertures (e.g. NA of 0.5), it has a clear aperture larger than that required to interface with the 0.25 NA objective lens. Due to the higher than designed illumination beam NA, we were able to resolve 203 lp/mm features on the 1951 USAF target, but at the cost of an unwanted increase of stray light (clearly visible on the edges of the image of the 1951 USAF target).

The miniature microscope objective as directly compared to commercially available 0.25NA objective exhibited higher resolution but visibly lower contrast. While our system out-resolved the commercial microscope objective, it should be noted that we compared performance of both systems for a single wavelength. Resolution of commercially available achromatic microscope objectives is a result of balance between spectral range, opto-mechanical complexity and cost. We attribute
decreased contrast of the miniature microscope objective and tube lens compared to the reference commercial objective to stray light leaking through them.

4.2.3. Optical Design of Illumination System

The optical schematic for the illumination system is shown in Figure 4.6.

Figure 4.6 Optical layout of the dual wavelength illumination system.

The dual wavelength Abbe illumination system shown in Figure 4.6 was designed using Zemax®(Radiant Zemax, Redmont, WA, USA) based on the peak excitation wavelengths of the MagPlex microspheres described in Section 4.2.1. Abbe illumination is characterized by imaging the source directly onto the sample.
plane [57]. This illumination configuration was designed primarily to reduce the number of optical elements (e.g. lenses). Spectral characteristics of filters and light sources used in the Classifier channels are presented in Figure 4.7a. The excitation filter, emission filter, and LED spectra for the Reporter channel are shown in Figure 4.7b.

![Figure 4.7](image1.png)  
**Figure 4.7** Spectral bandwidths of excitation (ex.) and emission (em.) filters together with spectral densities of illumination sources for Classifier (a) and Reporter (b) channels.
The Reporter illumination pathway, highlighted in blue in Figure 4.7, consists of a 505 nm LED (part no. SR-01-E0070, Luxeon, USA), a commercially available collector lens (ACL1210, Thorlabs, USA), a bandpass filter (FF03-510/20, Semrock, USA), two long pass filters to block UV light from exciting the polystyrene substrate of the MagPlex beads (BLP01-355R and BLP01-442R, Semrock, USA), and a dichroic mirror (DMLP567, Thorlabs, USA). A second commercially available aspheric was used as the condenser lens for both the Classifier and Reporter channels (ACL1512, Thorlabs, USA).

The Classifier excitation optical path, highlighted in red in Figure 4.7, has an analogous layout to the Reporter pathway. The pathway elements include a 617 nm LED (part no. SR-01-H2060, Luxeon, USA), a commercially available aspheric collector lens (ACL1215, Thorlabs, USA) and a bandpass filter centered at 615 nm (FF01-615/20, Semrock, USA). The Classifier illumination pathway shares the dichroic mirror and condenser lens common to the Reporter pathway. The common pathway for the two channels is shown in purple in Figure 4.6.

Uniformity of illumination is contingent on proper alignment of all optical elements. Elements in the illumination channels (lenses, filters, etc.) are press-fit into 3D printed housing. The Projet HD 3000 3D printer has accuracy on the order of 0.025-0.05 mm per inch of part dimension, which may cause slight element misalignment. The lenses are positioned in 3D-printed indented holders and are held in place via printed alignment features once the two mirrored halves of the illumination housing are pressed together. The LED and heat sink are mounted in a
3D printed slot that holds them centered on the optical axis of each illumination pathway. The 3D-printed hardware of the illumination system eliminates the need for expensive commercial optomechanics and allows for rapid iterative prototyping and rebuilding. A potential downside to 3D-printed alignment features is that printer tolerances and plastic shrinkage may compromise alignment of optical elements.

In the classifier and reporter pathways, the illumination field uniformity varied between 11% and 13%, respectively. Experimental validation on multiple images, however, showed that the intensity differences between all observable beads in the three channels was high enough to allow for accurate classification, indicating that these background intensity fluctuations have little influence over system performance. In addition, geometric properties such as bead size are measured to eliminate beads that are barely visible in the sample.

4.2.4. Optomechanical System

All optomechanical components of the miniature system were designed in Solidworks (Solidworks Premium, Dassault Systèmes SolidWorks Corporation, USA) and fabricated on a 3D printer (ProJet HD 3000, 3D Systems, USA). After printing, all system components were airbrushed matte black with enamel paint (Matte Black 33 A0360, Humbrol, UK) over internal and external surfaces. Additional light-blocking masks were laser cut from thin sheets of volume blackened plastic and placed under the sample tray as well as between the objective and tube lens holders. A labeled
photograph and Solidworks design of the complete modular miniature system is shown in Figure 4.8(a) and Figure 4.8(b), respectively.

Figure 4.8 a) A photo of the complete miniature system prototype. The system contains (from top to bottom): a camera, a tube lens, a sliding emission filter holder, an objective lens, a sample tray that is designed to fit a standard size microscope slide, and an illumination module that houses optics and light sources for two different illumination pathways.  b) A Solidworks rendering of the cross section of the complete system. A cross section of the camera mount, adjustable tube and objective lens holders, emission filter holder, sample tray, and illumination module is shown. In the illumination module, the Classifier channel pathway is highlighted in red and the Reporter channel illumination pathway is highlighted in blue. Both channels intersect at a dichroic beamsplitter and then share a subsequent common optical pathway, highlighted in purple.
**4.2.4.1. Miniature Microscope Optomechanics**

A Solidworks rendering of the miniature infinity-corrected microscope is shown in Figure 4.9.

![Solidworks rendering of the miniature microscope](image)

**Figure 4.9** A cross section of a Solidworks rendering of the miniature microscope is shown above. The rendering illustrates an exploded view of the system cross section highlighting the threaded objective and tube lens holders. The inset image shows an enlarged view of the threaded objective holder.

The miniature infinity-corrected microscope sits above the sample tray, which was designed to hold a standard microscope slide. The objective lens is enclosed in a threaded holder, shown in Figure 4.9, which allows for manual focus adjustment using a focusing knob. The Projet HD 3000 3D printer has a lateral resolution of 13 x 13 dots per millimeter in x and y. The axial resolution is 24 dots
per millimeter with accuracy of 0.025-0.05 millimeter depending on the part geometry. The high lateral resolution ensures that the tube lens and objective lens may be aligned on-axis in the 3D printed housing without the need for additional machining or alignment features, since the lateral printer resolution falls within the design tolerances values listed in Table 4.3. The high axial resolution of the printer allows for printing of fine mechanical features, such as the 0.0635 millimeter pitch threading on the objective and tube lens holders, which enable fine manual adjustments of the objective and tube lens. The theoretical depth of field (DoF) of the 0.25 NA objective lens corresponds to:

$$DoF = \frac{\lambda}{2NA^2} = 4.72 \, \mu m$$

**Equation 1:Depth of Field**

at the design wavelength of 590 nm. The monochromatic objective lens requires a focal adjustment of 30 microns between the fluorescent channels, corresponding to a half rotation of the objective holder. The large depth of focus of the tube lens does not require manual adjustment between Classifier and Reporter emission channels.

The removable emission filter holder is inserted into the infinity-space above the microscope objective lens and below the tube lens. It houses three emission filters; one for each Classifier channel and one for the Reporter channel. Classifier Channel 1 uses a bandpass emission filter centered at 661 nm (FF01-661/20,
Semrock, USA), Classifier Channel 2 uses a bandpass emission filter centered at 720 nm (FF01-720/13 nm, Semrock, USA), and the Reporter channel uses a bandpass emission filter centered at 590 nm (FF01-590/20, Semrock, USA). All filters were ordered in custom 1/2” diameter housing to fit the small footprint of the microscope.

To reduce costs, we used an inexpensive CCD, the Pointgrey Chameleon CCD Monochrome Camera (CMLN-13S2M-CS, Point Grey Research Inc, Canada). The camera was dismounted to board level and placed in the custom 3D printed camera mount above the tube lens holder. The camera was connected to a laptop via USB 2.0 cable for image acquisition and transfer. A photograph of the disassembled miniature microscope modules is shown in Figure 4.10. Note that Dowel pins were used to connect the modules.
Figure 4.10 The disassembled infinity-corrected miniature microscope components (camera cap, camera mount, objective mount, tube lens mount, and emission filter holder). Examples of the 3/10 dowel pins (Brikksen, Germany) used to connect the modules are shown in the top right corner. Dowel pin holes are visible on the tube and objective lens modules. Extra unmounted tube lens and objective lens modules are shown to the right of the microscope.

4.2.4.2. Illumination Optomechanics

The dual wavelength Abbe illumination module delivers light to a sample chamber designed to fit a standard microscope slide. The slide is inserted through a slot and can be manually adjusted to acquire images in multiple fields of view. A photograph view of the illumination unit cross section and its components is shown in Figure 4.11. Note that unlike the miniature microscope components, the components of the illumination system (heat sinks, Peltier coolers, LEDs, lenses,
filters, and dichroic beamsplitter), are aligned via press fitting, rather than stacked and aligned with dowel pins in standalone modules.

Figure 4.11 A magnified view of the illumination system and its components.

4.2.5. Sample Preparation

For experiments with data collected using the miniature microscope, DNA hybridization to MagPlex-TAG microsphere beads (MTAG-XXX, Luminex, USA) was performed according to the manufacturer’s instructions with the following modifications: the beads were diluted and filtered through a 3.0 µm filter and resuspended in hybridization buffer prior to hybridization. Biotinylated DNA oligonucleotides complimentary to bead numbers 39 (5’-biotin-ACAAATATCTAATCTATCACA-3’), 73 (5’-biotin-CTTTATCAAATTCTAATTCTCAAC-3’), and 78 (5’-biotin-TTTACAAATCTAATCACACTATAC-3’) were purchased from IDT (USA). For each
hybridization experiment, a dilution series was prepared for each bead such that there were \(10^{11}, 10^{10.5}, 10^{10}, 10^{9.5}, 10^{9}, 10^{8.5}, 10^{8}, \) or 0 complementary DNA copies per 25 µl water. Singleplex samples containing one bead type and its complementary oligonucleotide were prepared in triplicate for each DNA concentration. Multiplex samples containing all three bead types and their complementary oligonucleotides were prepared in triplicate for \(10^{11}\) DNA copies per sample.

Twenty five microliters of DNA at each concentration were then mixed with 25 µl prepared complementary beads in the wells of a 96-well plate. Beads were prepared for miniature microscope imaging by removing debris from the bead solution by diluting seven microliters beads per sample at stock concentration in one milliliter 2x concentrated hybridization buffer (0.2 M Tris-HCl, 0.4 M NaCl, 0.16% Triton X-100). The bead solution was filtered through a 3.0 µm filter (WHA110412, Sigma Aldrich, USA). The filter containing the beads was then washed in 25 µl 2x hybridization buffer per sample and centrifuged at 2,500 RCF to re-suspend the beads.

For experiments with data collected using the Luminex MAGPIX, DNA hybridization to MagPlex-TAG microsphere beads was performed according to the manufacturer's instructions. Briefly, Beads were prepared for MAGPIX processing by diluting 2 µl beads per sample at stock concentration in 23 µl 2x hybridization buffer. Diluted beads were mixed in a 96 well plate with a dilution series of DNA target as described above.
After samples were prepared with DNA and beads in a 96 well plate, the plate was sealed with film (MSA-5001, Biorad, USA) and incubated in a thermal cycler. After an initial 90 seconds at 96°C, the plate was incubated for 30 minutes at 37°C. At the end of the initial incubation period, 25 µl 20 µg per ml streptavidin phycoerythrin (S866, Life Technologies, USA) in 1x hybridization buffer was added to each sample. The plate was incubated for an additional 15 minutes, removed from the thermal cycler, and placed in the Luminex MAGPIX or processed for imaging on the miniature microscope.

Samples were processed for imaging on the miniature microscope by washing twice with 1x hybridization buffer on a magnetic stand, with the supernatant discarded after the final wash. Two point five microliters of the washed beads were then placed on a rinsed slide in the center of a spacer (S24737, Life Technologies, USA) and sealed with a #0 coverslip (260366, Ted Pella, USA) and clear nail polish on the edges.

4.2.6. Image Acquisition

The same sample bead batch used for imaging experiments with the MAGPIX was later used for imaging experiments with the miniature microscope experiments. Samples were imaged on the MAGPIX and on the miniature microscope within two hours of preparation. All images acquired with the miniature microscope were captured using custom software. The illumination system of the miniature microscope interfaced with an LED driver (DC4100, Thorlabs, USA). The driver was
triggered automatically through a software command to illuminate the sample. Once an LED was triggered, an image was captured and saved automatically.

Eleven images were acquired in each fluorescence channel over exposure times ranging from 10 milliseconds to 1000 milliseconds due to the large dynamic range of signal in the Classifier and Reporter channels. One dark frame image was also acquired at each exposure time, for image correction purposes. Typical images acquired in Classifier 1 and Classifier 2 channels at exposure time of 300ms are shown in Figure 4.12.

Figure 4.12 Images taken with the miniature microscope from the Classifier 1 channel (a) and Classifier 2 channel (b) at an exposure time of 300 milliseconds. Note that due to filter spectral characteristic, Classifier 1 channel exhibits stronger background signal.
4.2.7. **Miniature Microscope Image Analysis**

Matlab version R2014a (MathWorks, USA) was used to analyze images acquired using the miniature microscope. The flow chart of image processing operations is illustrated in Figure 4.13.

The dynamic range of signals analyzed by the miniature fluorescence microscope spans four orders of magnitude. Due to the limited dynamic range of the detector (Chameleon USB 2.0, Point Gray, Canada), detection of fluorescent signal is performed on High Dynamic Range (HDR) images, a sequence of numerically merged Low Dynamic Range (LDR) raw images acquired at increasing exposure times (typically eleven images obtained over evenly spaced exposure time range of 10-1000 ms). Each fluorescent LDR image in our system is accompanied by a dark image, acquired at identical exposure time with light source turned off.
In the first step of data processing all LDR images, and their corresponding dark images, are loaded into computer memory. In the next step dark images are subtracted from the corresponding fluorescent images. The Chameleon camera image detector is larger than the designed image circle of the miniature microscope; for this reason, processed raw fluorescent images are multiplied in the next step by a circular binary mask. The mask has a central area filled with ones which encompasses the intended FOV of the microscope. HDR image synthesis from a
series of masked LDR is performed next. The resultant HDR fluorescent images are stored in double floating point format in order to preserve the dynamic range of the signal. Due to spectral and spatial characteristics of the light source and spectral leakage of the excitation/emission filter combination, images acquired in the CL1 fluorescent channel suffer from low spatial frequency background variations. To spatially unify the intensity distribution within CL1 HDR images, we subtract the background obtained by parameterized median filtration of the source CL1 HDR image. Typically we use a median filter with kernel size of 41 pixels in order to obtain a smooth, slowly varying background intensity approximation. A sample filtered HDR image from the Reporter channel is shown in Figure 4.14.

Next in non-linear low pass filtration, we mask all pixels of HDR images with intensity below a pre-set threshold (typically 15000 intensity counts) and leave all other pixels unchanged. This filtering removes random noise and prepares the images for the next step of data processing, which is image alignment. We designed the optical system of the microscope using multiple monochromatic system configurations. As a result, in order to acquire sharp images in each of the mutually separated fluorescent channels it is necessary to manually adjust the working distance of the microscope. Micro movement of the objective assembly within the system chassis is the main reason behind relative lateral shift between HDR images. We spatially align the fluorescent HDR images using parameterized two-dimensional correlation. During two dimensional alignment we minimize the sum of absolute difference between intensity values of overlapping pixels. Aligned
fluorescent HDR images are binarized using global intensity threshold levels adjusted separately for each spectral channel. Binarized fluorescent images are segmented and basic statistic metrics of segments are calculated (area, width, height, mean intensity, median intensity, etc...). In the next step of image processing, we remove segments that do not correspond in fluorescent HDR images to isolated beads. We filter out unwanted segments using morphological criteria (pixel count, height, width, etc.) together with segment intensity conditions to select only single, isolated, non-clustered beads. Segments that correspond to same bead in filtered, binarized and segmented images of sample, recorded through Classifier 1 and 2 filters are identified next. In the final step, a scatter plot, called “bead map” is created. The mean intensity of the bead in the Classifier channels is used to mark bead location in Cartesian coordinate system and the intensity of a bead in the Reporter channel is used to quantify target analyte.

Figure 4.14 An example of a post-processed HDR image from the Reporter channel of bead 39 tagged with $10^{11}$ copies of DNA (detailed description of
image processing operations in the main text). Please note beads intensity variations, caused by difference in concentration of target analyte.

4.2.8. Luminex MAGPIX Experiments

The Luminex MAGPIX was used according to manufacturer recommendations with the stage preheated to 37\(^\circ\)C. Fluorescence intensity for each bead was measured and median fluorescence intensity was reported for each bead type in every sample. All data was saved and exported to Microsoft Excel for analysis.

4.3. Results and Discussion

4.3.1. Limit of Detection: Miniature 0.25 NA Microscope and Luminex MAGPIX

In a dilution series experiment conducted on the miniature microscope and the MAGPIX system, six bead samples were imaged with different levels of target (copies of synthetic DNA). The target concentrations were \(10^{9.0}, 10^{9.5}, 10^{10.0}, 10^{10.5}\), and \(10^{11.0}\) copies of synthetic DNA in addition to a non-target control (shown as 10\(^0\) copies of DNA in all subsequent figures) on the miniature microscope. MAGPIX experiments included \(10^{8.0}, 10^{8.5}, 10^{9.0}, 10^{9.5}, 10^{10.0}, 10^{10.5}\), and \(10^{11.0}\) synthetic DNA copies and a non-target control.
For the same sample bead batch, the miniature system reached a limit of detection of $10^{9.3}$ copies of target (synthetic DNA) while the limit of detection on the MAGPIX was found to be $10^{8.0}$ copies of target. MAGPIX’s limit of detection is reported to be between $10^{9.8}$ and $10^{10.6}$ copies of target in the literature. Limit of detection plots are shown in Figure 4.15. The line of best fit is plotted in black dots; the normalized median intensity at each concentration is plotted in blue triangles. The red squares plotted at a concentration of zero represents the non-target control sample, which was comprised of beads with no DNA. Multiplex experiments demonstrated comparable results to singleplex samples containing the same amount of target (data not shown).

Figure 4.15 The two figures above illustrate limits of detection of the miniature microscope from a singleplexed experiment that used bead type #39. (a) and Luminex MAGPIX (b). Normalized intensity is plotted on the y-axis and concentration in 10x copies of target is plotted on the x-axis. The limit of detection is calculated from the intersection of the baseline (red squares) with the measured median intensities at each concentration (black dots). The limit of detection of the miniature microscope was $10^{9.3}$ copies of target.
target (synthetic DNA). For Luminex’s MAGPIX, the limit of detection was found to be $10^{8.0}$ copies of target in one experiment. MAGPIX’s limit of detection is reported to be between $10^{9.8}$ and $10^{10.6}$ copies of target in the literature [48].

The limit of detection of the miniature microscope was found to be consistent over a series of independent experiments and ranged between $10^{9.2}$ and $10^{9.6}$. Results with Luminex’s MAGPIX had larger distribution and the limit of detection varied between $10^{9.0}$ and $10^{10.8}$. This is consistent with results reported in literature [58] although the limit of detection may be target-dependent. Luminex recommends using concentrations on the order of $10^{11.0}$ or above for consistent measurements.

4.3.2. Analyte Classification

![Bead map from a multiplexed bead sample imaged on the miniature microscope.](image)

Figure 4.16 A bead map from a multiplexed bead sample imaged on the miniature microscope. The sample contains 137 total beads comprised of three distinct bead types, which can be resolved into three distinct groups.
(outlined with red ellipses). Each group is spaced approximately 0.6 units apart in the y-direction and 0.8 units apart in the x-direction.

Figure 4.16 shows a “bead map”, a two dimensional scatter plot of the measured intensity of beads in Classifier channel 2 vs. the measured intensity of beads in Classifier channel 1. This bead map represents a multiplexed sample containing three different bead types, imaged with the miniature microscope. Each blue dot represents the mean intensity of the brightest twenty five pixels in one bead in a sample of 137 beads. The centers of each group are spaced approximately 0.6 units apart in the x direction and approximately 0.8 units apart in the y-direction. The red dashed ellipses were arbitrarily drawn on top of the clusters to highlight the three bead groups. The separation of the tight groups of individual points illustrates the ability of our system to classify different analytes in a multiplexed sample. This method of visualizing the bead distribution also allows us to quantify the number of pathogens that can be analyzed in one sample by our device.

4.4. Conclusions

We developed a 3D printed prototype of a miniature optical detection platform. Our device achieved diffraction limited performance superior to commercially available optics at a single wavelength. For proof-of-concept applications, the miniature microscope successfully differentiated multiple bead types in a single multiplexed sample and exhibited a limit of detection comparable to a commercial bead-based assay system at $10^{9.3}$ copies of synthetic DNA. These
experiments demonstrated the ability of a low-cost, rapid-prototype device with simplified, inexpensive hardware to produce a comparable limit of detection to a commercially available system.

The cost of the prototype device is estimated at 1.5 orders of magnitude less than the commercially available system. In the future, mass production of the lenses and optomechanical components via injection molding, as well as selection of specialized electronic components may reduce manufacturing costs. Additionally, mass production of filters via methods such as deposition on plastic substrates could further reduce cost, resulting in a device with a price tag suitable for point-of-care applications in low-resource areas. Rapid prototyping techniques, which we validated for possible point-of-care use in this preliminary application, will allow relatively rapid development of future generations of miniature point-of-care optical readout devices for different biological applications, such as infectious disease detection or complete blood counts.

4.5. Acknowledgements

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Chapter 5

Development of an achromatic miniature objective for three part white blood cell differential measurements

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The automated miniature fluorescence microscope described in Chapter 4 was monochromatic and required manual adjustment between fluorophores of different emission wavelengths. To image multiple fluorophores simultaneously, there is a need for an achromatic system. The chapter that follows describes a low-cost, miniature achromatic microscope that was fabricated for identification of lymphocytes, monocytes, and granulocytes in samples of whole blood stained with
acridine orange, which fluoresces at two distinct wavelengths simultaneously depending on if it is bound to RNA or DNA. The microscope was manufactured using rapid prototyping techniques of diamond turning and 3D printing and is intended for use at the point-of-care in low-resource settings. The custom-designed microscope requires no manual adjustment between samples and was successfully able to classify three white blood cell types (lymphocytes, granulocytes, and monocytes) using samples of peripheral whole blood stained with acridine orange.

5.1. Introduction

The white blood cell (WBC) differential is one component of a complete blood count (CBC), an evaluation performed for standard blood work-ups. The WBC differential count is essential at the point-of-care when screening for quantitative abnormalities in otherwise morphologically normal white blood cell populations, a condition which may occur with certain infectious diseases, such as Dengue fever [59]–[61]. Three-part WBC differentials identify and quantify lymphocytes, monocytes, and granulocytes. The three part WBC differential has been shown to be a reliable clinical benchmark when compared to conventional differential counts or five part white blood cell counts [21]–[24].

At the point of care, especially in developing countries, having immediate access to WBC differential results aids clinicians in selecting appropriate treatment, such as the proper choice of antibiotics to treat a disease [62]. For example, an increased or decreased total white blood cell count (WBC) may indicate abnormal
bone marrow pathology [63]. Leukocytosis with an associated lymphocyte count may indicate the presence of a microbial or viral infection [64]. Clinicians may also use the WBC biomarker to mitigate risks of improper treatment and identify patients in need of immediate treatment and a closer follow-up [65]. Two commonly used techniques for WBC differentials, flow cytometry and optical methods such as fluorescence imaging and light scattering, have replaced manual cell counting by a laboratory technician with a microscope and hemacytometer [23], [66], [67]. Many flow-based counting devices are impractical for point of care use due to their high cost and infrastructure requirements [68]. Typical image based counting methods, despite shortcomings such as a small field of view, require less complex sample preparation, and have the potential to be miniaturized. Small field of view necessitates a technician to acquire multiple fields of view to get an accurate differential and complete count. For optical evaluation, the readily available, nonspecific fluorescent dye acridine orange may be used to stain samples of whole blood[67],[69]–[71]. Acridine orange (AO) is a stain that may be applied directly to an undiluted wet sample without rinsing and can be used to provide a diagnosis within approximately ten minutes [68], [72]. Acridine orange binds strongly to double stranded DNA, and when bound its excitation maximum is 502 nm and its emission maximum is 525 nm. It also binds strongly to RNA and single stranded DNA, with a shifted excitation maximum of 460 nm and an emission maximum of 650 nm. It is hydrophobic and neutral in pH and therefore can easily diffuse through cell membrane and cell nucleus membrane to bind to RNA and DNA. The
WBC cell nucleus is stained green with small quantities of red because of double stranded DNA and single stranded RNA, while the cell cytoplasm is stained predominantly red because of the presence of RNA and lysosomes [67]. Differentiation is performed by quantifying signal from 525 nm and 650 nm channels.

Commercially available points of care devices for clinical use in the United States include the Chempaq, the HemoCue WBC, and the HemoCue WBC Diff. These devices use impedance cell counting and spectrophotometric measurement of hemoglobin concentration in a small sample (20 microliters) of blood [24], [73]. The HemoCue WBC measures total WBC count but does not provide a differential [59]. The Chempaq provides the user with a differential output and has a coefficient of variation <15% for lymphocyte and granulocyte counts, but has poor monocyte correlation when compared to the Beckman Coulter LH 750, used as a reference standard [74]. The HemoCue WBC performs a five-part WBC differential, but retails for more than $1000 and has a per test cost of $3.52, making it undesirable for low-resource areas. There remains need for a low-cost point of care instrument that has the capability to accurately perform monocyte classification, since this group of cells is typically harder to identify even with commonly used hematology analyzers.

An achromatic miniature microscope, optimized for the peak emission maxima of acridine orange bound to DNA and RNA (525 nm and 650 nm, respectively), was designed for the observation and classification of white blood cells in undiluted whole blood samples stained with acridine orange. The custom
microscope objective was fabricated out of plastic via single point diamond turning for rapid prototyping purposes. Single point diamond turning produces plastic lenses with optical quality surface roughness [52], [53], [56]. Plastic substrates for the custom fabricated lenses were carefully selected to minimize axial chromatic aberration. The plastic objective was integrated into all-plastic, 3D-printed housing. Once the objective was initially adjusted to the proper working distance using the 3D-printed optomechanics, the system required no further manual adjustments to refocus the optics between different samples. Because the sample consists of fluorescently stained undiluted blood, only one field of view (1.2 mm) was necessary to capture statistically significant information (>100 WBCs/FOV) regarding quantities of various white blood cell types. The digital microscope classifies WBC types (lymphocytes, monocytes, granulocytes) based on the ratio of red to green intensity within each cell, rather than morphology. The sections that follow describe the optical design and fabrication of a custom designed, all-plastic prototype of an achromatic miniaturized microscope for identification of white blood cells in whole blood for use at the point-of-care in resource-limited settings.

5.2. Experimental Methods

5.2.1. Optical Design of Miniature Objective

An optical schematic of the miniature objective designed for classification of white blood cells is shown in Figure 5.1.
Figure 5.1 Optical schematic of the miniature achromatic objective.

The objective was custom designed using Zemax® (Radiant Zemax, Redmont, WA, USA). A summary of the basic optical parameters of the microscope is presented in Table 5.1.

Table 5.1 Summary of optical parameters of miniature fluorescent microscope

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA (object side)</td>
<td>0.35</td>
</tr>
<tr>
<td>Magnification</td>
<td>-4.5x</td>
</tr>
<tr>
<td>Telecentric object space</td>
<td>Yes</td>
</tr>
<tr>
<td>Object side Field Of View diameter</td>
<td>1.2 [mm]</td>
</tr>
<tr>
<td>Objective working distance</td>
<td>1.33 [mm]</td>
</tr>
<tr>
<td>Design wavelengths</td>
<td>525,650 [nm]</td>
</tr>
<tr>
<td>Total length of optical system</td>
<td>78.95 [mm]</td>
</tr>
</tbody>
</table>

The optical prescription data of the microscope objective is given in Table 5.2. The microscope objective has a numerical aperture (NA) of 0.35, focal length of 11 mm and a 1.2 mm diameter Field of View (FOV). The field of view was designed
to be greater than 1 mm so that at least 130 cells would be present in each field for statistical significance when quantifying three-part white blood cell differential.

Table 5.2 Optical prescription data of miniature microscope (Radii, thicknesses and semi-diameters are in units of [mm])

<table>
<thead>
<tr>
<th>Surface</th>
<th>Radii</th>
<th>Thickness</th>
<th>Material</th>
<th>Semi-diameter</th>
<th>Conic</th>
<th>6th Order Term</th>
<th>8th Order Term</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Sample)</td>
<td>∞</td>
<td>0.200</td>
<td>Seawater</td>
<td>0.600</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (Slide)</td>
<td>∞</td>
<td>0.100</td>
<td>BK7</td>
<td>0.654</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>∞</td>
<td>1.326</td>
<td></td>
<td>0.678</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (Coverslip)</td>
<td>∞</td>
<td>0.150</td>
<td>BK7</td>
<td>1.164</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>∞</td>
<td>0.300</td>
<td></td>
<td>1.199</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>-3.3219</td>
<td>1.147</td>
<td>Polystyrene melt</td>
<td>2.698</td>
<td>-0.338</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>∞</td>
<td>7.405</td>
<td></td>
<td>2.957</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 (Lens 2)</td>
<td>20.538</td>
<td>6.500</td>
<td></td>
<td>3.777</td>
<td>0.711</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>4.434</td>
<td>0.300</td>
<td>NOA 61</td>
<td>3.678</td>
<td>-1.682</td>
<td>1.697E-005</td>
<td>-6.041E-007</td>
</tr>
<tr>
<td>10 (Lens 3)</td>
<td>4.341</td>
<td>6.500</td>
<td>PMMA</td>
<td>3.678</td>
<td>-1.637</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-10.003</td>
<td>3.816</td>
<td></td>
<td>3.850</td>
<td>-1.199</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11(Em. filter)</td>
<td>∞</td>
<td>4.754</td>
<td>BK7</td>
<td>3.681</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 (IR filter)</td>
<td>∞</td>
<td>10.000</td>
<td></td>
<td>3.610</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>∞</td>
<td>1.000</td>
<td>D263T_BRO</td>
<td>3.382</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>∞</td>
<td>30.736</td>
<td></td>
<td>3.367</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>∞</td>
<td>2.664</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

The magnification of the microscope is -4.5x. The objective has a maximum clear aperture diameter of 7.700 mm. It is 27 mm long as measured from front lens to final lens surface. The elements following the objective in the optical schematic shown in Figure 5.1 include the emission filter (BLP-1-532R-25, Semrock, USA) and the IR filter on the detector (Flea3, Point Grey Research Inc., Canada). The total length of the optical system is 78.95 mm as measured from sample to detector plane.
The design was optimized at seven field points, distributed along the Y-field object axis from 0 mm to 0.6 mm. The modular transfer function (MTF) and spot diagram plots for each wavelength (525 nm and 650 nm) are shown in Figure 5.2. These diagrams indicate that diffraction limited performance was expected for all field points and wavelengths in nominal working conditions, achieved through use of aspheric components and one achromatic doublet. To retain a simple and compact design, the design was corrected for axial chromatism only. Additionally, lateral chromatism may be numerically corrected in post-processing of images.
Figure 5.2 Performance metrics of the miniature tunable fluorescent microscope for nominal working conditions: a) modulation transfer function for expected performance at 525 nm b) modulation transfer function for expected performance at 650 nm. (c) spot diagram for expected performance at 525 nm (shown in blue) and 625 nm (shown in green). All plots are for image surface for design wavelength of 590nm, for following object points: 0.0mm, 0.25mm, 0.35mm, 0.45 mm, 0.50 mm, 0.55 mm, 0.6 mm. Airy disk radii for 525 nm and 650 nm: 4.179 and 5.126µm, respectively.

The achromatic design was optimized for two wavelengths, 525 nm and 650 nm, which correspond to the peak emission wavelengths of acridine orange bonded to double stranded DNA and RNA/single stranded DNA, respectively. The objective is comprised of three aspheric lenses, which are easier to manufacture via diamond turning than with traditional manufacturing methods. Two lenses are made of Poly(methyl methacrylate) (PMMA) and one lens is made of polystyrene. These materials were chosen due to their well-documented machinability on diamond
turning machines and low autofluorescence [52]. To achieve achromatic performance, the design contains only one achromatic doublet, consisting of a polystyrene lens and a PMMA lens. The lenses comprising the doublet are affixed with NOA 61 optical glue (Norland, New Jersey, USA). The glue is set using UV light to permanently bond the lenses.

Proper characterization of the lens substrate materials is critical to accurately model the chromatic performance of the system. Note in Table 5.2, the material of the second lens in the objective is listed as “polystyrene melt” rather than “polystyrene”. The original system prescription was modeled in Zemax using the default “polystyrene” material properties from the software’s glass catalogue. Measurements obtained on an Abbe refractometer (DR-M2, Atago, USA) did not match the refractive index data listed in Zemax. The refractive index of materials varies with batch and between manufacturers. This was observed experimentally since the refractive index measurements of our selected batch of polystyrene fell outside the default refractive index and Abbe number tolerance values used to optimize the design. We also measured the PMMA batch and found that our experimental (PMMA measurements agreed with the values given in the Zemax catalogue). Table 5.3 displays the default refractive index values for polystyrene as defined in the Zemax glass catalogues as well as the refractive index values measured for the selected batches of material on the Abbe refractometer. The default refractive index tolerances used in the group are ± 0.001. The differences in measured and default polystyrene refractive index values for the chosen batch of
polystyrene fell outside of the tolerance boundaries, therefore prompting a modification of the material properties in the Zemax models with a best-fit curve based on the Schott formula using the refractive index measurements taken at four wavelengths shown in Table 5.3.

**Table 5.3 Measured vs. reported refractive index values for polystyrene and PMMA**

<table>
<thead>
<tr>
<th>λ (nm)</th>
<th>Measured</th>
<th>Reported (Zemax)</th>
<th>Difference</th>
<th>Measured</th>
<th>Reported (Zemax)</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>486</td>
<td>1.5964</td>
<td>1.6041</td>
<td>0.0077</td>
<td>n/a*</td>
<td>1.4978</td>
<td>n/a</td>
</tr>
<tr>
<td>546</td>
<td>1.5884</td>
<td>1.5950</td>
<td>0.0066</td>
<td>1.4934</td>
<td>1.4938</td>
<td>0.0004</td>
</tr>
<tr>
<td>589</td>
<td>1.5834</td>
<td>1.5915</td>
<td>0.0079</td>
<td>1.4910</td>
<td>1.4917</td>
<td>0.0007</td>
</tr>
<tr>
<td>656</td>
<td>1.5778</td>
<td>1.5850</td>
<td>0.0072</td>
<td>1.4883</td>
<td>1.4892</td>
<td>0.0009</td>
</tr>
</tbody>
</table>

*value outside measurement range of Abbe refractometer

The Abbe number ($V_d$) was calculated using the equation:

**Equation 2: Abbe number equation**

$$V_d = \frac{n_d - 1}{n_F - n_C}$$

where $n_d$, $n_F$, and $n_C$ are the refractive indices of polystyrene at the Fraunhofer spectral lines (589.3 nm, 486.1, and 656.3 nm, respectively). Abbe numbers are shown in Table 5.4.
Table 5.4 Calculated vs. reported (in Zemax) Vd for polystyrene and PMMA

<table>
<thead>
<tr>
<th></th>
<th>Polystyrene</th>
<th>PMMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_d$ (calculated)</td>
<td>31.366</td>
<td>n/a*</td>
</tr>
<tr>
<td>$V_d$ (reported)</td>
<td>30.867</td>
<td>57.441</td>
</tr>
<tr>
<td>% change</td>
<td>1.16%</td>
<td>n/a</td>
</tr>
</tbody>
</table>

*value of $n_D$ outside measurement range of Abbe refractometer

During the design process, the default refractive index tolerance value used in the group ($\pm$ 0.001) was modified to $\pm$ 0.002 for NOA 61 optical glue and $\pm$ 0.004 for polystyrene and PMMA and the Abbe number tolerance value was loosened from $\pm$ 1% to $\pm$ 2% to allow to match measured values for the selected batch of material and to allow use of materials from other sources if necessary. The revised tolerance parameters are displayed in Table 5.5.

Table 5.5 Tolerance parameters of the miniature fluorescent microscope

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td>±0.5%</td>
<td>±0.02</td>
<td>±0.02</td>
<td>±0.20</td>
<td>±0.02</td>
<td>±0.2</td>
<td>±2</td>
<td>±0.004*</td>
</tr>
</tbody>
</table>

*index tolerances loosened to ±0.004 for PMMA and polystyrene lenses
**index tolerances loosened to ±0.002 for NOA 61 optical glue

Based on 1,000 runs of Monte-Carlo simulations, it was estimated that the miniature objective would achieve diffraction limited performance on-axis with 50% probability and there would be a 50% of chance of reaching diffraction limited performance within the field of view enclosed by a disk of radii of 0.42mm (70% of object height). Using RMS wavefront as the criterion, the estimated change in performance due to manufacturing tolerances calculated using the Root Sum Square
(RSS) algorithm was estimated to be 0.041 and the total estimated system performance (nominal + change) was estimated to be 0.144. The Rayleigh criterion-defined resolution of the system was 0.89 mm at 525 nm and 1.1 mm at 650 nm. Because the sample target (white blood cells) was larger than the theoretical diffraction limit by a factor of approximately ten, the expected system performance was determined to be suitable for the application.

The microscope objective was manufactured on the Precitech Optimum 2400 (Ametek, Precitech, Keene, NH, USA) diamond turning lathe. Pellets of optical grade plastics were mounted on the diamond turning machine using ER16-UP (Rego-Fix, Tenniken, Switzerland) collets housed in a custom vacuum chuck compatible holder. Diamond turning paths were generated using the Diamond Turning Path Generator (DTPG v2.54, Precitech, NH, USA) program based on the Zemax lens drawings. A brass spacing feature was diamond turned to space the singlet lens from the achromatic doublet. The lenses and brass spacing feature were stacked inside a larger brass tube. The outer diameter of all lenses and the brass spacer was manufactured to meet tight tolerances to fit within the inner diameter of the outer brass tube. Figure 5.3 illustrates the assembled objective in the brass tubing (left) and the 3D printed threaded objective holder (right).
The microscope objective was tested at 525 nm and 650 nm using a white light source (MWWHL3, Thorlabs, USA) and two 10 nm bandpass filters (Stock No: #65-639 and #65-656 Edmund Optics, USA) that were manually exchanged. A high resolution, negative 1951 USAF resolution test (Stock No: #55-622, Edmund Optics, NJ, USA) was used to assess the microscope performance. Based on the Rayleigh criterion, the microscope should out resolve the resolution target at both wavelengths. The distance between the objective and the USAF target was stationary between wavelengths. For as designed chromatic performance, the resolution for both wavelengths would be the same. Figure 5.4 shows a magnified image of the target at both wavelengths. The resolution at 525 nm was found to be group 8 element 6, corresponding to 456.1 lp/mm. At 650 nm the resolution was group 8 element 4, corresponding to 362.0 lp/mm. A magnified image of the smallest resolvable element at each wavelength is shown in Figure 5.4(a) and Figure
5.4(b) as insets. The system was accepted because the minimum resolvable line pair was adequate to resolve the target sample.

Figure 5.4 Images of high resolution USAF target taken with the miniature 0.35 NA achromatic objective. a) Image acquired at 525 nm b) Image acquired at 650 nm. Magnified inlays of the smallest resolvable elements are shown outlined in each image. Contrast has been enhanced for presentation purposes.

The use of a 0.35 NA objective allows us to acquire properly exposed images over the period of 400 ms, which falls below the exposure time experimentally found to rapidly photobleach the fluorescent dye (1500 ms). The limited depth of field (6 μm at 525 nm) of the optics may cause some of the fluorescently stained WBC to appear blurred on the camera. This will be discussed further in the section 2.3. However, to correctly classify WBC into three groups (lymphocytes, monocytes, granulocytes) we only need to distinguish between individual WBCs and compare their respective relative ratio of fluorescence in two mutually separated emission
channels centered at 525 nm and 650 nm. Because we do not perform any morphological analysis, we can successfully process blurred images of WBCs up to the point when adjacent WBCs starts to overlap. Since typically, even in case of patients in severe illness, density of WBCs in non-concentrated smears is low, we decided to deliberately leave nominal, as designed NA of the system, in order to avoid photobleaching as well as reduce data acquisition time.

5.2.2. Sample Cartridge Design

Capillary blood was obtained from healthy adult donors and immediately imaged. Blood samples were prepared using a novel cartridge design [75] consisting for layers of tape and a glass cover slip on a glass slide. The cartridge spreads approximately 20 µL of blood into a thin layer of 88 µm directly from a finger prick. Acridine orange is dried in the cartridge and automatically dissolves upon the introduction of the blood sample to a final concentration of [10 µg/mL], staining the white blood cells in the sample.

5.2.3. Optomechanical Design

The optomechanical assembly of the miniature WBC microscope is presented in Figure 5.5. We designed mechanical components of the system in SolidWorks (Dassault Systèmes SolidWorks Corp., USA). All custom parts of the WBC assembly were 3D printed (ProJet HD 3000, 3D Systems, USA) and painted black using matt enamel (Matte Black 33, Humbrol, UK) to attenuate stray light.
Figure 5.5 Optomechanical schematic of the miniature WBC microscope (a), cross-section through sample chamber with 3D printed microscope slide positioning springs (b), details of the optomechanical assembly of the prototype of the WBC objective (c), photo of the assembled WBC microscope, (d) photograph of assembled 3D printed system is shown on an optical bench. The spacing between the holes is 1 inch for reference.

The mount base, located at the bottom of the system, has four ports for mounting bolts that are designed to mount the assembly within the system enclosure (not shown). The main holder (Figure 5.5a) is screwed into the mount base in order to allow for height adjustment of the microscope slide port. The illumination system is mounted beneath the microscope slide port and comprise of an LED located between sample and the catadioptric reflector. A 5mm thick round aluminum disk, placed in contact with bottom part of the LED, serves as a heat sink. The illumination assembly is fixed by threaded 3D printed locking ring marked in Figure 5.5a. Samples are inserted through microscope slide port. The sample chamber has 3D printed springs, depicted in Figure 5.5b, that provide downward
pushing force. The force effectively fixes the distance between the sample and the microscope optics.

The WBC optical system is mounted within the holder depicted in Figure 5.5c. Both objectives that comprise optical assembly are mounted within brass tube that is subsequently inserted into objective enclosure. The aperture stop is made of laser cut black construction paper and is mounted within a diamond cut recess in brass spacer. Front and retaining cups are threaded and when locked provide an axial clamping force that fixes the position of both lenses within the objective assembly. The WBC objective is screwed into the main holder using a custom 3D printed thread (1 mm pitch, 0.87 mm thread tooth height) and its position is adjusted in reference to the sample at the assembly stage. Thread printing dimensions were adjusted to provide tight fit between the objective enclosure and sample holder in order to immobilize optical assembly. The emission filter (BLP-1-532R-25, Semrock, USA) is mounted using a locking ring within custom 3D printed camera interface module, located directly above the optical assembly. The 3D printed camera interface module has an integrated C-mount thread that is used to fix a Flea3 (Point Grey Research Inc., Canada) image detector. The position of the camera is controlled by 3D printed custom threaded connection (1 mm pitch and 0.87 mm thread tooth height) between camera interface and main holder. The position of the camera module is locked in respect to main module using commercially available worm-drive clamp tightened around external surface of the
camera interface module. Figure 5.5d shows a photograph of the assembled 3D printed system.

![Camera Interface Module](image)

**Figure 5.6 (a) Schematic of measurement system used to evaluate sample insertion repeatability. (b) Slide insertion repeatability test: blue line – measured distance, black line – mean distance, red lines – ±1σ standard deviation boarder lines.**

The optical system of the WBC microscope has object side NA=0.35. For the shorter design wavelength of 525 nm the depth of field of the optical system is 6 μm. Consequently, the sample must be placed 1.3 mm (nominal working distance) beneath the microscope objective with ±3 μm tolerance in order to acquire focused images of fluorescently stained white blood cells. Repeatability of the insertion of a sample microscope slide in 3D printed enclosure is tested in an experimental system depicted in Figure 5.6(a). A laser-based displacement meter (IL-030, Keyence Corp, Osaka, Japan) is used to measure distance between micrometer and top/proximal surface of cover slip glass that encapsulates sample chamber. The
laser displacement meter is set to acquire data with relative speed of 2 and a moving average window width to 4096 samples to avoid random fluctuation of indicated distance. A sample microscope slide is inserted into the measurement chamber fifteen times, each time removing it completely from the sample slit. The measured distance between the laser displacement meter and cover slip mounted on top of the microscope slide is graphically presented in Figure 5.6(b). Mean height is measured to be -3.198 mm and standard deviation is \( \sigma=0.007 \) mm. The measured displacement between ten different cartridges has a standard deviation of \( \sigma=0.009 \) mm.

The measured deviation of position of the sample relative to fixed position of the optical system is nearly twice larger than depth of field of the optical system. Optical system depth of field is inversely proportional to \( NA^2 \), and its throughput is directly proportional to \( NA^2 \). Reducing the system’s numerical aperture results in a larger depth of field, but at the expense of lowering the system’s light collection efficiency. Subsequently, optical systems with reduced NA suffer from a lower Signal to Noise Ratio (SNR), due to inherent statistical properties of light acquisition with solid-state detectors, if image acquisition time is not increased. Since fluorescently stained white blood cells fluoresce weakly (due to limited quantum efficiency of the acridine orange dye and inherent low efficiency of fluorescent process), we decided to sacrifice image quality to reduce data acquisition time necessary to record images with sufficient signal to noise ratio.
5.2.4. Cost analysis

Cost burden in relation to point-of-care instrumentation is two-fold, and depends on the one-time cost of the instrumentation as well as cost of the device’s consumables, such as cuvettes. For example, the HemoCue WBC DIFF analyzer, marketed as a point-of-care reader, retails for approximately $1300, but the per-test cost (per-cuvette) is $3.59. An ideal per-test cost for a low resource setting is well below the $1.00 mark [76]. By using a basic slide and coverglass as the sample chamber as well as small quantities (20 microliter) of acridine orange, the per test cost for our device comes to $0.42 for low volume production and ~$0.0004 for high volume quantities (the sum of the cost of a glass slide and acridine orange in microliter quantities).

The cost of fabricating the custom optics can be analyzed based upon the assumption of low, mid and high-volume production [40]. The spacer and housing material for an objective is made from standard hypodermic tubing cut to length using a diamond saw. The total cost of each tube is estimated to be $1.00 for low-volume production. Assembly of the objective is assumed to add another $15 / objective for volumes below one thousand units.

For prototype quantities (<50 units), the use of diamond turning can produce lenses two orders of magnitude more costly than injection molding, but without the initial investment of designing molds for the lenses. At high volume production
quantities (>10,000), the use of injection molding becomes more beneficial, significantly decreasing the costs of the objectives.

The miniature optics interface with a variety of electronic and opto-electronic components. The Luxeon high power LEDs diode (SR-01-B0040, Quadica Developments Inc., Ontario, Canada) are priced in retail at approximately $10 if bought in quantities below 10 units, dropping to $0.50 at quantities greater than 1,000. The system is controlled by a $20 microcontroller (Arduino, Italy), a $200 board-level computer (JNP93-2930, Jetway, USA), and $60 solid state drive (SSD) (SV300S37A/120G, Kingston, USA). A cost breakdown of the optics, electronics, and housing for low-volume production is shown in Table 5.6

Table 5.6 illustrates that in production of volumes on the order of 10,000, the device retails for roughly half the price of commercially available point of care readers such as the HemoCue WBC Diff. Additionally, our per-test cost (~$0.42 in low volume quantities and ~$0.0004 in high volume production) reduces the cost burden compared to the commercially available HemoCue WBC Diff, which has a per test cost of $3.52. The reduction in the per-test cost coupled with the reduction in device cost is important for use in low-resource environments.
Table 5.6 Cost of complete optical detection hardware for prototype device and conservative cost estimate for mass production

<table>
<thead>
<tr>
<th>Component</th>
<th>Cost / part</th>
<th>Estimated cost / part</th>
</tr>
</thead>
<tbody>
<tr>
<td>LED light source</td>
<td>Luxeon Rebel LED $9.00</td>
<td>~ $0.50</td>
</tr>
<tr>
<td>Power supply (5V, 12 A)</td>
<td>5V, 12 A (STDA61A-S05, Mepos) $15</td>
<td>~ $10 (power supply)</td>
</tr>
<tr>
<td>Image Sensor</td>
<td>Pointgrey Flea3 Color CMOS (FL3-U3-88S2C-C) $550</td>
<td>~ $500 (depending on bit depth &amp; sensor size). Cost includes design and manufacturing. If off the shelf, can be significantly lower.</td>
</tr>
<tr>
<td>Housing/Optomechanics</td>
<td>$500 (3D printed)</td>
<td>$2.00</td>
</tr>
<tr>
<td>Optical System</td>
<td>$500 (labor, diamond turning)</td>
<td>~ $10 (injection molding)</td>
</tr>
<tr>
<td></td>
<td>$250/filter (Semrock excitation and emission filters)</td>
<td></td>
</tr>
<tr>
<td>Control Unit</td>
<td>$280 (microcontroller, computer, SSD) Approx $2000</td>
<td>Aprox. $560</td>
</tr>
</tbody>
</table>

5.2.5. Image Acquisition

Preliminary samples of white blood cells in whole blood stained with acridine orange were imaged with the miniature microscope and on the miniature microscope within ten minutes of sample preparation. All images acquired with the miniature microscope were captured using Pointgrey FlyCap Software (Pointgrey Inc., Canada). The high-power LED is driven by a constant current LED driver (Product ID: 03021-D-E-700, BuckPuck, LEDdynamics, USA). Power was measured in an area that matched the optical power meter detector diameter (Model 130C, Newport, USA). The illumination system consists of a 470 nm LED (SR-01-B0040, Quadica Developments Inc., Ontario, Canada) mounted within a catadioptric reflector (Fraen 9°, Quadica Developments Inc., Ontario, Canada) and excitation
filter (FF01-470/28-25, Semrock, USA). The power measured at the sample plane was 23.1 mW/cm² at 470 nm.

A typical image (FOV = 1.13 mm²) acquired at a gain of 1 and an exposure time of 400 ms is shown in Figure 5.7. There must be a minimum of 100 cells in a field of view to get statistically meaningful differential data. The relatively large (1.2 mm) field of view of our microscope is intended to visualize at least 200 cells in a general patient population assuming a Poisson distribution the coefficient of variation will be 200¹/² or ~14 cells [77].

![Figure 5.7](image_url)

**Figure 5.7** (a) Image of acridine orange taken at 400 ms exposure stained white blood cells in whole blood. Image acquired with the miniature all-plastic objective. (b) Magnified image of granulocyte. (c) Magnified image of a lymphocyte. (d) Magnified image of a monocyte. Contrast has been enhanced for presentation purposes. Red-to-green ratios were calculated using raw data.
The out of focus appearance of some cells indicates a tilt in the sample plane. For this reason, we have designed a new sample holder with three points of contact for the slide, which will ensure that future samples are uniformly held in place.

Figure 5.7 (b) depicts a typical image of a granulocyte. The red-to-green ratio of this granulocyte is 0.993. Figure 5.7(c) shows a typical image of a lymphocyte. The red-to-green ratio of this cell is 0.595. By recording each cell’s red-to-green ratio, we see two distinct groups of cells and we are able to classify the white blood cells into lymphocytes (lowest red-to-green ratios) or granulocytes (highest red-to-green ratios). Monocytes (cells with red-to-green ratios that fall in the middle of the other two groups) are the most difficult to identify via visual inspection. A magnified image of a monocyte is shown in Figure 5.7 (d). The red-to-green ratio of this monocyte is 0.741. Future work will entail developing an automated algorithm to identify and quantify these three cell types.

### 5.3. Conclusions

We developed a prototype of a miniature all-plastic achromatic microscope to identify monocytes, lymphocytes, and granulocytes based on the ratio of red to green intensity within each cell. The microscope was successfully able to capture images of white blood cells in whole blood stained with acridine orange. The microscope housing and objective were fabricated using the rapid prototyping techniques of 3D printing and single point diamond turning, respectively. The all plastic microscope is intended for use in the field for point-of-care applications and
requires no manual adjustment of the optics. We were able to reach micrometric level of mechanical repeatability in an optomechanical system that was exclusively 3D printed using UV curable epoxy. We achieved this goal by including 3D printed plastic springs into the sample chamber and incorporating into the mechanical design manufacturing tolerances characteristic for our 3D printer. The use of low cost components such as LEDs, reflectors, and USB detectors, combined with the all-plastic housing and lenses will allow for future versions of the prototype to be mass-produced. Future work will entail developing an automated algorithm for WBC identification and comparing our differential counts to those obtained using commercially available benchtop hematology analyzers.

5.4. Acknowledgements

Funded by a grant from the Bill & Melinda Gates Foundation through the Grand Challenges in Global Health initiative.
Chapter 6

Challenges in fabrication and testing of static monochromatic and achromatic systems

This chapter will review the respective limitations of monochromatic and achromatic objectives based on the design, fabrication, and validation processes discussed in Chapters 4 and 5. I will also highlight the benefits of infinity-corrected objective designs. Finally, I will discuss why, as described in Chapter 7, the final microscope in the contained a tunable, infinity-corrected objective.

6.1. Monochromatic objective image acquisition challenges

Chapter 4 describes a fluorescence microscope that required manual readjustment between emission channels of the three fluorophores present in Luminex's MAGPLEX platform of magnetic beads. This manual readjustment
(exchanging emission filters and refocusing the objective) led to movement of bead positions between images acquired in each of the three emission channels. Because of this movement of a bead's location in the image frame between image acquisition in each channel, it was necessary to include a colocalization MATLAB script in the image processing algorithm. This additional post processing step coregistered images of beads from the three different emission channels. The output from this step of the code is shown in Figure 6.1.

In this Figure 6.1, the beads have been binarized and assigned one of three colors (red, green, or blue). Red represents signal from Classifier 1 channel, green represents signal from Classifier 2 channel, and blue represents the reporter channel, White indicates that the beads from all three emission channels are coregistered, or overlap. A region of coregistered beads is shown in the inset image of Figure 6.1.
Figure 6.1 Binarized, coregistered beads from Classifier channels and Reporter channel. Red represents signal from Classifier 1 channel, green represents signal from Classifier 2 channel, blue represents the binarized bead as it appears in the reporter channel, and white is the overall of all three channels. White indicates that the images are coregistered.

To reduce post-processing time required for each set of acquired images and to reduce the need for the operator to manually focus the objective, an achromatic objective was designed and built for imaging of the next sample containing multiple emission wavelengths (AO-stained blood samples). Achromatic objectives do not require adjustment between wavelengths. The challenges of design and fabrication of achromatic objectives is described in Section 6.2.

6.2. Achromatic objective design and fabrication challenges

An achromatic objective eliminates the need for manual refocus or post-processed coregistration between emission wavelength channels, as described in
Section 6.1. The primary design challenge in fabricating the achromatic objective is described in Section 5.3, particularly Tables 5.3 and 5.4 which describe the challenges in modeling material properties of polystyrene based. Specifically, there was a measured variation in refractive index and Abbe number between batches of the plastic, which differed from the standard Zemax values for the material. Because of these differences in refractive indices and Abbe number from the Zemax models, I established the following new protocol to be used when modeling the performance of an achromatic doublet in Zemax:

1) Characterize pellets of doublet substrate material using Abbe refractometer at (find $n$ and Abbe number). Measure indices of refraction ($n$) at Fraunhofer spectral lines (589.3 nm, 486.1, and 656.3 nm). Calculate Abbe number ($V_d$) using Equation 2: Abbe number equation.

2) The material properties are to be updated in Zemax’s glass catalog using the “Fit Melt Data” function.

3) The material properties are updated and used in the lens editor in place of the default material given by Zemax.

4) In Zemax’s “Tolerance Data Editor” Abbe number tolerance is increased to ±2% (originally ±1%) and refractive index tolerances are multiplied by a factor of at least 2-4x the nominal tolerance value (±0.001) depending on the magnitude of the difference between measured $n$ and the nominal $n$ value given in Zemax.
Once the modeling and material characterization protocol had been established, the main challenges in manufacturing the achromatic objective was during the assembly of the achromatic doublet. To assemble the doublet, the second lens of the objective was placed in an ER16-UP (Rego-Fix, Tenniken, Switzerland) collet housed in a custom vacuum chuck compatible holder. Drops of NOA61 optical glue were administered with Optimum Dispense Tips (Nordson EFD, United States) attached to an Optimum 3CC syringe barrel (Nordson EFD, United States) connected to the air output of a fluid dispenser unit. A schematic of the NOA61 inside the syringe is shown in Figure 6.2.

![Figure 6.2 Optimum 3CC syringe barrel schematic.](image)

The NOA 61 is applied to the collet-mounted lens in single drops by pressing down on the foot pedal of the Ultimus Automatic Fluid dispenser (Nordson EFD, United States). The pressure and time of drop are adjusted via knobs on the front of the device. Five to six 0.2 second length droplets of glue were applied to the surface.
Then, Lens 3 of the objective was applied on the top of the second lens, as shown in Figure 6.3.

![Image of Lens 2 (left). Image of lens 2 and 3 affixed with NOA61 optical glue.](image)

The images in Figure 6.3 were acquired using a Hitachi CCD color camera (model #KP-D20AU, Hitachi, Japan) attached to an Edmund Optics stereomicroscope with magnification 0.75x. The tilt and decentration of lens 2 from lens 3 were then calculated to ensure they fell within the tolerance values given in Table 5.5. Optical glue was cured using the ELC-410 UV Spot Cure System (Electro-Lite, United States) and cured optical glue residue was diamond turned off the sides of the doublet to reach the ID needed to fit inside the brass tubing. On average, to successfully fabricate one doublet within the desired tolerance values for tilt and decentration, two “test copies” (spare plastic lenses with same sag values) had to be cut per doublet lens. Overall this process was time consuming and required spare lenses to be manufactured. This would not have been a large time constraint for a
single objective, but four achromatic objectives were built in parallel, so diamond turning eight test lenses and assembling four doublets was not as time efficient as assembling singlet lenses.

6.3. Benefits of infinity-corrected objectives

The infinity corrected systems, such as the one described in Chapter 4, are useful for fluorescence imaging because of their versatility. The user may interchange components such as filters and beamsplitters inside the “infinity space” without the need to redesign the objective. In addition to the 0.25 NA infinity-corrected system, I designed a 0.5 NA/8x infinity-corrected system to show the versatility of such objectives. The optical layout for the 0.5 NA system is shown in Figure 6.4 9(a).

The 0.5 NA system was initially designed for fluorescence imaging with transmission illumination (Figure 6.4 (b)), but it can also be used in an epi-fluorescence configuration without adjusting the position of the objective or tube lenses. Figure 6.4(b) illustrates the 0.5 NA objective with epi-illumination. A beamsplitter has been added to the infinity space to direct excitation light toward the sample. This beamsplitter coupled with the emission filter blocks the illumination wavelength from the detector.
Figure 6.4 (a) Zemax optical layout of the 0.5 NA/-8x infinity corrected monochromatic objective (b) optical layout for transmission illumination (c) optical layout for epi-illumination.
The infinity-corrected objective has an NA of 0.50, a magnification of -8 and a WD of 1.2 mm. The field of view is smaller than the designs discussed in Chapters 4 and 5 (0.6 mm diameter). Based on the standard fabrication tolerances used in the Tkaczyk lab as listed in Table 4.3, the expected performance based on the sum of nominal RMS wavefront error and RSS change was 0.109 (above the diffraction limit of 0.07). Monte Carlo analysis was performed to estimate the likelihood of achieving diffraction limited performance. One thousand simulations were performed in which each set variable is perturbed a random amount between the minimum and maximum defined values. Two different criterion were used, RMS wavefront error and RMS spot size. The RMS spot size was used as criteria in addition to the RMS wavefront error to more closely estimate the expected system performance. According to the Monte Carlo analysis performed using RMS wavefront error as criterion, the objective’s performance is expected to be diffraction limited for roughly 50% of built systems. For Monte Carlo analysis performed using RMS spot size, the airy disk radius is defined as:

**Equation 3 Equation of the airy disk radius**

\[
    r_{\text{airy}} = \frac{1.22\lambda}{2NA_{\text{objective}}}
\]

At the image plane, Zemax calculated the airy radius to be 5.027 microns. Because the system will have an expected RMS spot radius of 5.084 microns (57 nm greater than calculated airy disk radius) for 50% of systems built, the system performance
was determined to be sufficient for imaging of blood constituents (ranging from platelets on the order of 2-3 microns to white blood cells, on the order of 6 to 10 microns).

The target application for this objective was imaging of blood constituents but because the design is monochromatic, manual adjustment would be required in blood stained with multiple dyes or single dyes with multiple emission wavelengths. The post-acquisition coregistration of images would also be necessary since there would be a manual exchange of filters to image the emission from AO bound to DNA and AO bound to RNA. Taking this limitation into consideration, it was determined that although it is preferable for design and fabrication reasons to design an infinity corrected monochromatic system, the final objective in the platform must have the capability to be adjusted without manual intervention.

6.4. Conclusions

Based on the simplified design and relaxed tolerances required to design infinity-corrected monochromatic systems compared to the finite conjugate achromatic objective, the final microscope in the miniature fluorescence microscope family I will discuss in Chapter 7 was designed to be monochromatic and infinity corrected. It is tunable instead of static, however, and focal adjustment may be performed via electrowetted lenses rather than manual adjustment, thus eliminating the need for image coregistration in post processing.
Chapter 7

Development of a tunable monochromatic infinity-corrected objective

*A portion of the following material is in preparation for submission to Biomedical Optics Express.

This chapter will discuss the design, fabrication, and validation of a tunable infinity-corrected miniature fluorescence microscope. This system has the capability of imaging multiplexed samples without requiring manual refocusing by the user. The system was validated using AO-stained whole blood.

7.1. Introduction

The capability of a point of care diagnostic instrument to resolve multiple signals simultaneously is useful for several clinical purposes. For example, multiplexed bioassays are generally faster and more efficient since allow the user to
analyze multiple analytes simultaneously. Some fluorescent dyes, such as acridine orange, which is used at the point of care because it is relatively inexpensive and shelf stable, emits at two discrete wavelengths depending on if it is bound to DNA or RNA. Because it is clinically relevant for many point of care diagnostic tests to have multiplexing capability, the optical detection system described in this chapter will allow the user to image multiplexed samples without manual adjustment between emission wavelengths.

Because the samples of interest contain multiple fluorophores, a monochromatic objective design would not focus all the wavelengths of light to the same plane, since different wavelengths of light have different indices of refraction [78]. A monochromatic design would result in axial chromatic aberration. Axial chromatic aberration of a positive lens is illustrated in Figure 7.1 where the dotted line F represents blue light (486 nm) and the dashed line C represents red light (656 nm). The distance d is the axial difference between the two focal planes of the different wavelengths.
Figure 7.1 Example of axial chromatic aberration of white light focused through a positive lens. F represents blue light (486 nm), the dashed line C represents red light (656 nm) and the distance d is the axial difference between the two focal planes [78].

In a monochromatic system, manual refocus of the system is required to adjust distance d (Figure 7.1). Because the goal of the design is to eliminate manual adjustments in the microscope, alternative designs including objectives containing achromatic doublets were considered. A compact design intended for point of care use that contained an achromatic doublet assembly was described in Forcucci et al, 2015. Achromatic doublet assembly generally requires fabrication of a high number of lenses since doublet assembly and centering of the elements is non-trivial and has been described previously [54].

One alternative to an achromatic design, which requires a greater number of optical elements in an infinity-corrected design, was to add an electronically tunable component to a monochromatic infinity-corrected system. The optical power of a tunable lens is altered by changing the voltage that is applied to a conductive
material immersing the lens. A conceptual drawing of an electrowetting activated lens, such as the Arctic 316, is illustrated in Figure 7.2.

![Figure 7.2](image_url)

**Figure 7.2 Electrowetting lens with applied voltages between 0 and 120V. Voltage applied between conductive substrate and liquid results in change in curvature of insulating hydrophobic layer (oil drop) [79].**

Changing optical power of one or more elements in a monochromatic objective allows the user to compensate for the difference in the focal plane (distance d in Figure 7.1) between emission wavelengths. Therefore the user can image multiple fluorophores in the same sample without manual adjustment and without a high number of optical elements.

The following chapter describes the third miniature microscope in the platform of all-plastic fluorescence microscopes designed for multiplexed optical detection at the point of care [80], [81]. The microscope uses a combination of custom lenses fabricated via single point diamond turning as well as off-the-shelf
electrowetted lenses. The microscope objective and tube lens were integrated into 3D printed plastic housing. The system requires no manual refocusing, and instead voltage applied to the tunable lenses is altered to adjust the focus. The preliminary sample used was whole blood stained with acridine orange, which has peak emission maxima at 525 nm and 650 depending on if the dye is bound to DNA or RNA, respectively. The microscope has a field of view (1.2 mm in diameter), which is sufficiently large enough to significantly sample the white blood cells without requiring a second field of view. The sections that follow describe the optical design and fabrication of the microscope as well as validation with blood samples.

7.2. Experimental Methods

7.2.1. Optical Design of a miniature tunable infinity-corrected objective

An optical schematic of the tunable miniature infinity-corrected fluorescence microscope is shown in Figure 7.3. The two red arrows indicate the Arctic 316 lenses. The tunable objective lens is 19.6 mm in length and the tube lens is 7.45 mm in lens. The infinity space is 6.95 mm.
The microscope objective and tube lens were custom designed using Zemax (Radiant Zemax, Redmont, Washington). A summary of the optical parameters of the monochromatic microscope is presented in Table 7.1.

**Table 7.1 Summary of optical parameters of tunable objective**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA image side</td>
<td>0.25</td>
</tr>
<tr>
<td>Magnification</td>
<td>-5x</td>
</tr>
<tr>
<td>Telecentric object space</td>
<td>Yes</td>
</tr>
<tr>
<td>Object side Field Of View diameter</td>
<td>1.2 [mm]</td>
</tr>
<tr>
<td>Objective working distance</td>
<td>1.2 [mm]</td>
</tr>
<tr>
<td>Design wavelengths</td>
<td>590, 660, 720 [nm]</td>
</tr>
<tr>
<td>Largest Clear Aperture (1st surface of tube lens)</td>
<td>8.07 [mm]</td>
</tr>
<tr>
<td>‘Infinity space’ length</td>
<td>7 [mm]</td>
</tr>
<tr>
<td>Total length of optical system</td>
<td>45.0 [mm]</td>
</tr>
</tbody>
</table>

The optical prescription data of the microscope objective and tube lens are given in Table 7.2. The microscope objective has a numerical aperture of 0.25, a focal length of 2.4 millimeters and a 1.2 millimeter diameter field of view. The tube

---

**Figure 7.3 Optical schematic of the infinity-corrected tunable objective. The red arrows indicate the tunable Arctic 316 lenses.**
lens has a focal length of 12 millimeters and together, with the objective, forms an infinity-corrected microscope with a combined magnification of -5x.

**Table 7.2** Optical prescription data of the tunable microscope (Radii, thicknesses, semi-diameters (SD), and conic values are given in units of [mm])

<table>
<thead>
<tr>
<th>Surface</th>
<th>Radii</th>
<th>Thickness</th>
<th>Glass</th>
<th>SD</th>
<th>Conic</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>∞</td>
<td>1.200</td>
<td></td>
<td>0.600</td>
<td>0.000</td>
</tr>
<tr>
<td>1</td>
<td>-1.363</td>
<td>3.213</td>
<td>PMMA</td>
<td>0.830</td>
<td>-0.327</td>
</tr>
<tr>
<td>2</td>
<td>-3.138</td>
<td>0.892</td>
<td></td>
<td>1.919</td>
<td>-0.037</td>
</tr>
<tr>
<td>3</td>
<td>9.850</td>
<td>3.200</td>
<td>PMMA</td>
<td>2.324</td>
<td>-1.761</td>
</tr>
<tr>
<td>4</td>
<td>-6.018</td>
<td>1.001</td>
<td></td>
<td>2.488</td>
<td>-1.606</td>
</tr>
<tr>
<td>5</td>
<td>4.277</td>
<td>3.202</td>
<td>PMMA</td>
<td>2.288</td>
<td>-0.377</td>
</tr>
<tr>
<td>6</td>
<td>3.277</td>
<td>2.003</td>
<td></td>
<td>1.559</td>
<td>-0.361</td>
</tr>
<tr>
<td>7</td>
<td>∞</td>
<td>0.550</td>
<td>LQ lens, cover</td>
<td>1.650</td>
<td>0.000</td>
</tr>
<tr>
<td>8</td>
<td>∞</td>
<td>0.320</td>
<td>Tunable Liquid</td>
<td>1.650</td>
<td>0.000</td>
</tr>
<tr>
<td>9</td>
<td>2.700*</td>
<td>0.330</td>
<td>Tunable Liquid</td>
<td>1.350</td>
<td>0.000</td>
</tr>
<tr>
<td>10</td>
<td>∞</td>
<td>0.300</td>
<td>LQ lens, cover</td>
<td>1.350</td>
<td>0.000</td>
</tr>
<tr>
<td>11</td>
<td>∞</td>
<td>3.126</td>
<td></td>
<td>1.216</td>
<td>0.000</td>
</tr>
<tr>
<td>12</td>
<td>∞</td>
<td>0.550</td>
<td>LQ lens, cover</td>
<td>1.650</td>
<td>0.000</td>
</tr>
<tr>
<td>13</td>
<td>∞</td>
<td>0.320</td>
<td>Tunable Liquid</td>
<td>1.650</td>
<td>0.000</td>
</tr>
<tr>
<td>14</td>
<td>-2.183**</td>
<td>0.330</td>
<td>Tunable Liquid</td>
<td>1.350</td>
<td>0.000</td>
</tr>
<tr>
<td>15</td>
<td>∞</td>
<td>0.300</td>
<td>LQ lens, cover</td>
<td>1.350</td>
<td>0.000</td>
</tr>
<tr>
<td>16</td>
<td>∞</td>
<td>0.000</td>
<td></td>
<td>1.350</td>
<td>0.000</td>
</tr>
<tr>
<td>17</td>
<td>∞</td>
<td>6.952</td>
<td></td>
<td>1.350</td>
<td>0.000</td>
</tr>
<tr>
<td>18</td>
<td>7.227</td>
<td>3.108</td>
<td>PMMA</td>
<td>4.035</td>
<td>-0.796</td>
</tr>
<tr>
<td>19</td>
<td>-7.878</td>
<td>1.345</td>
<td></td>
<td>3.962</td>
<td>-3.772</td>
</tr>
<tr>
<td>20</td>
<td>-12.137</td>
<td>3.000</td>
<td>PMMA</td>
<td>3.403</td>
<td>0.000</td>
</tr>
<tr>
<td>21</td>
<td>6.674</td>
<td>11.000</td>
<td></td>
<td>2.793</td>
<td>0.000</td>
</tr>
</tbody>
</table>

*Value for radii of tunable liquid in first Arctic 316 in configuration 1. Radii value for configuration 2= 2.672 mm, configuration 3=10.739 mm

**Value for radii of tunable liquid in second Arctic 316 in configuration 1. Value for radius of tunable liquid in configuration 2= -2.292 mm, configuration 3= -9.973 mm

The microscope objective was designed to operate in three monochromatic configurations, at wavelengths of 590 nm, 660 nm, and 720 nm (corresponding to configurations 1, 2, and 3, respectively). These three design wavelengths were chosen because they correspond to the emission wavelengths of the three fluorophores in the MAGPLEX microspheres and also because these emission
wavelengths are close to that of other common fluorophores, such as acridine orange (emission wavelength at 650 nm). The custom designed objective includes two Arctic 316 (Varioptic, Lyon, France) tunable lenses, which have varying optical power based on applied voltage. The relationship between optical power and voltage for the Arctic 316 is shown in Figure 7.4. The optical power required for the configurations shown in Figure 7.4 require voltages between 35 and 42 Volts, where the relationship is linear. From this plot provided by Varioptic, and knowing that the range of the Arctic 316 spans from -5 diopters to +13 diopters, the calculated relationship between Optical Power (D) and voltage (V) is:

**Equation 4:** Optical Power (D) vs Voltage (V) of the Arctic 316 lens between $V_L$ and $V_H$ and $P_L$ and $P_H$

$$D = 1.64V - 62.3$$

Where $V_L$ is approximately 34.5 V and $V_H$ is approximately 46 V. $P_L$ is -5 diopters and $P_H$ is +13 diopters. For testing, the voltage was controlled with the VPS-3 driver and FocusLab Software (Varioptic, Lyon, France).
Figure 7.4 Relationship between Voltage (V) and Optical Power (D) of the Arctic 316 lens [79]. The lowest power of the tunable lens (P_l) is -5 diopters and the highest power of the tunable lens is +12 diopters.

The complete microscope objective is comprised of three aspheric custom lenses and two Arctic 316 tunable lenses. It is 19.6 mm long (including the two variable lenses) and has a maximum clear aperture of 5 mm. The maximum clear aperture of the tube lens is 8.1 mm due to the 7 mm long “infinity space”. The total length of the optical system is 45 mm, comparable to commercial objectives (insert example here).
Figure 7.5 Performance metrics of the miniature tunable fluorescent microscope for nominal working conditions: (a) modulation transfer function for expected performance at 590 nm, (b) modulation transfer function for expected performance at 660 nm, (c) modulation transfer function for
expected performance at 720 nm, (d) spot diagram for expected performance at 590 nm, (e) spot diagram for expected performance at 660 nm, and (f) spot diagram for expected performance at 720 nm. All plots are for image surface for design wavelength of 590 nm, for following object points: 0.0 mm, 0.10 mm, 0.20 mm, 0.30 mm, 0.40 mm, 0.45 mm, 0.50, 0.60 mm. Airy disk radii for Configuration 1 (c), 590 nm, is 7.34 µm, Configuration 2 (d), 660 nm, is 8.042 µm, and Configuration 3 (e), 720 nm is 7.758 µm.

To simplify the optical design and relax the manufacturing tolerances, the system was optimized for three single wavelengths (590, 660, 720 nm) using the Multi Configuration function in Zemax. The objective and the tube lens are made exclusively from Poly(methy methacrylate) (PMMA) due to its proven machinability on diamond turning machines [ref] and low autofluorescence [ref]. The modulation Transfer Function (MTF) that was calculated at each wavelength using the Fast Fourier Algorithm (FFF) for each design wavelength in nominal working conditions is presented in Figure 7.5(a-c) together with corresponding spot diagram plots depicted in Figure 7.5 (d-e).

The design was optimized for eight field points, evenly distributed along the Y-field object axis from 0 mm to 0.6 mm. The MTF and the spot diagram plots shown in Figure 7.5 indicate nearly diffraction limited performance for all field points for nominal working conditions. Diffraction limited performance of the miniature fluorescence microscope according to the Rayleigh criterion would mean that sample features ~1.4 mm in size would be visible. Because, however, we are not observing morphology and the polystyrene beads are 6.5 microns in diameter, diffraction limited performance was not required.
The aberrations of the microscope objective were corrected by the tube lens in a single design process. The expected system performance was evaluated using the Root Mean Square (RMS) wavefront error during the design process. The RMS wavefront error of the microscope optical train was 0.060 for nominal working conditions. The fabrication tolerance parameters used during optimization stage are presented in Table 7.3. The estimated change in performance due to manufacturing tolerances calculated using the Root Sum Square (RSS) algorithm was estimated to be 0.059 and the total estimated system performance (nominal + change) was estimated to be 0.118.

**Table 7.3 Tolerance parameters of the miniature tunable microscope**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Radii [%]</th>
<th>Thickness [mm]</th>
<th>Element Decenter [mm]</th>
<th>Element Tilt [°]</th>
<th>Surface Decenter Tilt [mm]</th>
<th>Irregularity [Fringes]</th>
<th>Abbe n [a.u.]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Value</td>
<td>±0.4%</td>
<td>±0.02</td>
<td>±0.02</td>
<td>±0.02</td>
<td>±0.02</td>
<td>±0.2</td>
<td>±2</td>
</tr>
</tbody>
</table>

Legend for table superscript {*} for surface 17 ('infinity space')

Based on 1000 runs of Monte-Carlo simulations using the RMS spot radius as the criterion, it was estimated that the miniature fluorescence microscope would achieve diffraction limited performance on axis with 80% probability. Not all field points off-axis would be completely diffraction-limited, but because the sample target is comprised of white blood cells, which are larger than the theoretical diffraction limit by an approximate factor of at least six, the expected system performance was determined to be sufficient for the microscope.
The microscope objective and tube lens were manufactured on the Precitech Optimum 2400 (Ametek, Precitech, Keene, NH, USA), the in-house diamond turning lathe. Pellets of PMMA were mounted on the machine using ER16-UP (Rego-Fix, Tenniken, Switzerland) collets housed in a custom vacuum chuck compatible holder. All diamond turning paths for each surface were generated using the Diamond turning Path Generator program (DTPG v 2.54, Precitech, NH, USA) based on the lens drawings from Zemax. Brass spacing features were diamond turned to space the singlet lenses in the objective and tube lens.

The outer diameter of all the custom objective and tube lenses as well as brass spacers were manufactured on the diamond turning lathe to meet tight tolerances to fit within the inner diameter of the outer brass tube. Figure 7.6(a) illustrates the assembled objective minus the two Arctic 316 lenses in brass tubing. Figure 7.6(b) shows the tube lens in the brass tubing.

A picture of a tunable Arctic 316 lens is shown in Figure 7.6 (a). The Arctic 316-P-A model has a straight FlexCable and comes pre-packaged by Varioptic. It has an external diameter of 9.4 mm and a thickness of 3.5 mm. A holder was 3D printed to hold the custom objective shown in Figure 7.6 (a) as well as the two Arctic lenses. The printed holder is displayed in Figure 7.6(b).
The tunable objective and tube lens were tested at 525nm and 650 nm to assess performance for the prototype sample of acridine orange (AO) stained whole blood. The use of acridine orange stained white blood cells for three-part differential blood counts was described in Forcucci et al 2015. Based on if acridine orange is bound to DNA or RNA, the AO-stained white blood cells will fluoresce at 525 nm (green) or 650 nm (red), respectively. The objective was tested using a green LED (SR-01-M0100, Quadica Developments Inc., Ontario, Canada) and a red LED (SR-01-D2040, Quadica Developments Inc., Ontario, Canada) in a custom illumination holder [81]. The test sample was a high resolution negative 1951 USAF resolution target (Stock No. #55-622, Edmund Optics, USA). The distance between the objective and the USAF target was stationary between each wavelength, only the voltage applied to each Arctic 316 lens was changed. Figure 7.7(a) shows a magnified image of the resolution target at 525 nm and 650 nm. The resolution at
525 nm was group 8 element 6, corresponding to 456.1 lp/mm, consistent with the Zemax model at this wavelength. An image at 650 nm is shown in Figure 7.7(b) when the voltage applied to the Arctic lenses between 525 nm and 650 nm was left constant. The image is no longer in focus, groups 8 and 9 of the target are blurred. The image shown in Figure 7.7(c) corresponds to a voltage change of 20 mV being applied to each Arctic lens at 650 nm. The resolution at 650 was group 8 element 5, corresponding to 406.4 lp/mm.

Figure 7.7 (a) Images of high resolution USAF target taken with miniature tunable microscope. (a) an image acquired at 525 nm, (b) an image acquired at 650 nm with no adjustment of the tunable lenses. (c) an image acquired at 650 nm with adjustment of the tunable lenses. Contrast has been enhanced for presentation purposes.

### 7.2.2. Optomechanical Design

The assembly of the tunable microscope is shown in Figure 7.8. All mechanical components were designed in SolidWorks (Dassault Systèmes SolidWorks Corp.,
USA). All printed parts were printed on the ProJet 3000 3D printer (3D Systems, USA) and painted black using a matte enamel paint (Matte black 33, Humbrol, UK to attenuate stray light). Figure 7.8(a) illustrates a Solidworks cross-section of the optomechanics. Figure 7.8(b) shows a photograph of the assembled miniature tunable microscope. The light source used was a 470 nm LED(SR-01-B0040, Quadica Developments Inc., Ontario, Canada) coupled with an excitation filter (FF01-470/28-25, Semrock, USA).

Two commercially available lenses (ACL1210 and ACL1512, Thorlabs, USA) were used to illuminate the sample [76]. A longpass filter was used to filter emission wavelengths (BLP-1-532R-25, Semrock, USA).

To maintain a similar modularity consistent with past generations of low-cost point of care microscopes developed in the Tkaczyk lab, the illumination system is the same as described in Forcucci et al 2015 since one prototype sample is the MAGPLEX multiplexed beads. For AO-stained blood samples, only the blue channel of the illumination system is used, as highlighted in Figure 7.8(b). The
sample holder is designed to fit a standard sized microscope slide.

Figure 7.8 (a) Solidworks cross section of microscope optomechanics. (b) Photograph of illumination module, slide holder with slide inside, optical detection element chamber, tube lens holder, and camera.

The main holder (optical detection element chamber) of the optomechanical assembly includes a threaded (0.635 pitch, 0.56 mm tooth height) cylinder for the objective and tube lens holder. The objective holder screws in from the bottom of the main holder and the tube lens holder screws in from the top. A slot for a 1” emission filter holder is located in the infinity space between the objective and the tube lens. The main holder also includes a sample tray the size of a standard microscope slide. The main holder sits on top of the illumination module and can be secured with screws. The tube lens holder has an integrated C-mount thread interfaces with the Flea3 (Point Grey Research Inc., Canada) detector as shown in Figure 7.8(b). The position of the camera is controlled by 3D printed custom
threaded connection (1 mm pitch and 0.87 mm thread tooth height) between Flea3 C mount and main holder. The cost of the prototype is estimated to be $2000 and in mass production the cost (~10,000 units) is estimated to be on the order of $500[75], [76].

7.2.3. Image Acquisition

The tunable miniature microscope was tested with AO-stained blood and MAGPLEX beads. Raw images of AO stained blood is shown in Figure 7.9(a) and (b). In Figure 7.9 the microscope has been tuned to have green light (525 nm) in the focal plane.

![Figure 7.9](image)

**Figure 7.9 Raw image of AO-stained blood with objective focused on 525 nm wavelength emission.**

Future work entails the
development of an algorithm to use the signal from the red channel and the green channel to accurately identify WBC types.

Figure 7.10 (a) Greyscale image of red channel of RGB camera for raw image shown in 7.9. (b) Greyscale image of green channel of RGB camera for Figure 7.9. Contrast has been enhanced for display purposes.

7.3. Conclusions and Future Work

A prototype of a tunable miniature fluorescence microscope designed for multiplexed imaging has been designed and validated. The microscope was successfully able to isolate signal at discrete emission wavelengths. The all plastic system is designed for use at the point of care and may be used to image a number of multiplexed or singleplex samples including but not limited to acridine orange or multiplexed assays. The system requires no manual readjustment at each emission wavelength and is instead adjusted by increasing or decreasing the voltage applied to each electrowetted lens. The infinity-corrected design of the system means that components such as emission filters may be changed without requiring a redesign of the optical system. The use of low-cost light sources, detector, and 3D printed
housing allows for future versions of this system to be mass produced. For future work, this system will be used to image multiplexed assays and other multiplexed biological samples stained with multiple dyes or a dye with more than one fluorescent emission wavelength.
Conclusions and Suggestions for Future work

The work presented in this dissertation describes a family of modular low cost fluorescence microscopes that retain high resolution and multiplexing ability, but are one to two orders of magnitudes less expensive than their commercially available counterparts. These microscopes are intended for use at the point of care in low resource settings and address a need for high-performance, low-cost optical readers at such settings shown in Table 8.1.

The first 0.25 NA infinity-corrected microscope was designed for optical readout of bead based bioassays using the MAGPLEX family of multiplexed beads. This system design had relaxed tolerances and allowed for interchange of filters and other optical components in the infinity space without redesign, but because it was monochromatic it required manual focusing between each emission channel.
Additionally, images from each channel had to be coregistered in post processing to perform image analysis. The prototype microscope was 1.5 orders of magnitude less expensive than the commercially available counterpart (Luminex’s MAGPIX reader), and future generations, if produced in mass quantities with technologies such as injection molding (~10,000 units), have the potential to be reduced in cost by one additional order of magnitude.

Table 8.1 Target product profile for fluorescence microscope subunits of the platform

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test performance</td>
<td>• Comparable to established gold standard product</td>
</tr>
<tr>
<td>Design Considerations</td>
<td>• Portable (50 cm$^3$ or smaller)</td>
</tr>
<tr>
<td></td>
<td>• Runs off local 110-220 AC power with rechargeable battery backup</td>
</tr>
<tr>
<td></td>
<td>• Must work with fluorescent samples (containing single or multiple fluorophores)</td>
</tr>
<tr>
<td></td>
<td>• Does not require complex redesign between sample targets</td>
</tr>
<tr>
<td></td>
<td>• Must interface with standard microscope slide (no custom sample cartridge or cuvette required)</td>
</tr>
<tr>
<td>Cost</td>
<td>• Must be at least 1 order of magnitude cheaper than established gold standard counterpart</td>
</tr>
<tr>
<td></td>
<td>• Per-test cost less than $1.00</td>
</tr>
<tr>
<td>Data transfer</td>
<td>• USB or wireless</td>
</tr>
</tbody>
</table>

To address the challenges of imaging multiplexed samples without requiring the user to manually refocus, an achromatic 0.35 NA objective was designed. This objective was not infinity corrected but was designed to perform three part differential counts of white blood cells in AO-stained whole blood. Modeling of the
achromatic doublet in Zemax led to the creation of a new protocol for characterizing and tolerancing material properties in optical designs (Chapter 6.2). The assembled system was designed as a low-cost alternative to benchtop hematology analyzers, such as those produced by Beckman Coulter. The final prototype was one order of magnitude cheaper than these instruments, but in higher volume production quantities (>10,000), it has the potential to become an additional order of magnitude cheaper via injection molding of housing and lenses and mass production of electronics and filters. This device also reduced the per-test cost associated with blood analyzers from $3.59 to well below the $1.00 mark. It also showed promising performance, with over 70% of samples analyzed falling within 15% of the true WBC, therefore meeting the requirements for CLIA-waved devices. It is currently being used in clinical trials at the Lyndon B. Johnson General Hospital in Houston, TX and will be used in further trials in Malawi.

The final generation of low-cost microscope was a tunable infinity-corrected 0.25 NA system. This system was created because it retains the simple geometry and flexibility of an infinity-corrected system but does not require manual adjustment or assembly of achromatic doublets. It contains an objective lens with three custom aspheric lenses produced via diamond turning as well as two commercially available electrowetted lenses. The tube lens contains two custom diamond turned lenses also. The infinity space allows the user to change components such as the emission filter without the need to redesign or manually readjust the system. This microscope also maintains the modular nature of the
previous two microscopes and even interfaces with the illumination system designed for the original 0.25 NA monochromatic microscope designed for assay readout. All adjustments are made by varying the voltage applied to each Arctic 316 lens. This system was successfully used to isolate red and green fluorescent emission signal from AO-stained blood samples. In the future this system will be used to image multiplexed assays using samples such as those described in Chapter 4. A summary of the ability of the three miniature microscopes to meet the profile requirements described in Table 8.1 is presented in Table 8.2 and 8.3.

**Table 8.2 Target product profile and validation method**

<table>
<thead>
<tr>
<th>System</th>
<th>Validation method</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25 NA, Infinity-corrected, Mono-chromatic</td>
<td>Successfully compared to Luminex's MAGPIX classification ability and reporter quantification LOD</td>
</tr>
<tr>
<td>0.35 NA, Finite conjugate, Achromat</td>
<td>Fell within CLIA waived device margin when Validated against benchtop hematology analyzer</td>
</tr>
<tr>
<td>0.25 NA, Infinity-corrected, Tunable</td>
<td>Successfully able to isolate green and red emission signal in acridine orange stained blood samples</td>
</tr>
</tbody>
</table>
Table 8.3 Design considerations, economic and data import properties of three miniature systems

<table>
<thead>
<tr>
<th>System</th>
<th>Low Power?</th>
<th>Multiplexing ability?</th>
<th>Interfaces with standard microscope slide?</th>
<th>Prototype at least 1 order of magnitude cheaper than commercial instrument?</th>
<th>Per-test cost less than $1.00?</th>
<th>USB or Wireless?</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25 NA, Infinity-corrected, Monochromatic</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes ($2000)</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>See Section 4.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.35 NA, Finite conjugate, Achromat</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes ($2000)</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>See Table 5.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25 NA, Infinity-corrected, Tunable</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes ($2000)</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>See Section 7.2.2</td>
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Each miniature fluorescence microscope in the platform met the requirements for the target product profile of low cost high performance optical diagnostic instrumentation. These microscopes can be used at all points of care, from rural clinics to hospitals, to deliver healthcare to those that need it most.
References


