SPATIAL FREQUENCY MODULATION
FOR IMAGING WITH INCOHERENT LIGHT SOURCES

by
Gina M. Eldridge
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Golden, Colorado
Date ______________________

Signed: ______________________
Gina M. Eldridge

Signed: ______________________
Dr. Jeffrey A. Squier
Thesis Advisor

Signed: ______________________
Dr. Charles G. Durfee
Thesis Advisor

Golden, Colorado
Date ______________________

Signed: ______________________
Dr. Jeffrey A. Squier
Professor and Head
Department of Physics
This thesis discusses the initial execution of a microscope that achieves imaging by implementing the technique SPatIal Frequency modulation for Imaging (SPIFI). SPIFI is a robust imaging technique even in the presence of scattering because a single element detector is used in place of a camera. A line geometry is used to illuminate the sample and each place along the line is modulated at a specific temporal frequency. In this way the location information is encoded in the illumination light. After passing through the sample the light reaches the single element detector, which produces a time varying signal. The Fast Fourier Transform (FFT) of the signal gives the spectrum of modulation frequencies and their respective amplitudes. Since each of those frequencies corresponds to a position on the sample, the spectrum of the signal can be used to produce an image. Scanning the line across the sample and stitching the data together generates an entire image. Prior work has demonstrated a coherent version of SPIFI using a spinning disk to modulate the laser line-focus with linearly varying frequencies across its spatial extent. In this work, light emitting diodes (LEDs) are used as the light source and are electronically modulated, eliminating the need for a spinning reticle. There are a number of advantages to using LEDs over a laser. They are inexpensive compared to pump lasers that cost thousands of dollars before they are even implemented in an expensive femtosecond laser oscillator. The cost of an LED array with supporting electronics costs only around hundred dollars. Also, laser light is coherent and typically collimated making it easily focused by the lens of the eye to an intense spot on the retina, which can cause permanent damage depending on the energy of the beam. LED light is incoherent and highly divergent, making it much safer and the implementation of these microscopes in K-12 schools more realistic. Other imaging techniques, such as confocal microscopy, have been able to image through scattering media by physically rejecting out of focus photons. While video-rate (30 frames/s) confocal microscopy has been demonstrated, the pathway to
video-rate SPIFI in terms of complexity, is quite straightforward and imminently achievable.
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LIST OF ABBREVIATIONS

SPatIal Frequency modulation for Imaging ........................................ SPIFI
Light Emitting Diode ................................................................. LED
Fast Fourier Transform ............................................................... FFT
Surface Mounted Device ............................................................. SMD
Field-Programmable Gate Array .................................................... FPGA
Modulation Transfer Function ...................................................... MTF
Organic Light Emitting Diode ......................................................... OLED
PhotoMultiplier Tube ................................................................. PMT
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I look forward to each new fiasco with the utmost relish.

Evelyn Waugh, Decline and Fall
CHAPTER 1
INTRODUCTION

Advances in microscopy have guided shifts in biological paradigms and accordingly, the biological sciences have driven the demand for advancements in microscopy [1]. Traditional wide-field imaging methods illuminate the sample over an entire field of view and use a camera (i.e. a two-dimensional multi-pixel detector) to capture an image. In the presence of scattering, this method can yield blurry images because photons are redirected and registered by pixels that do not necessarily correspond to the correct location in the sample. In order to get clear images, specimens need to be thinly sliced, or flattened between slides, making imaging inside intact live samples difficult [2]. In 1957 Marvin Minsky patented the confocal microscope [3], which circumvented poor optical sectioning and contrast due to scattered light posed by thick specimens [4]. Instead of requiring the mechanical slicing of samples, confocal microscopy achieves sufficient axial resolution (<1 µm) by rejecting out-of-focus light [5, 6]. The basic mechanism of confocal microscopy is illustrated in Figure 1.1.

Figure 1.1: A basic illustration of the mechanism of confocal microscopy. The black rays represent light from the desired sample plane and the dashed red and blue lines represent light from outside that plane [4].

A point source is produced by placing a pinhole (a) in front of an illumination source. The point then is imaged onto the sample (b) by a condenser lens. An objective lens collects
the light from the sample and focuses it to an optically conjugate image plane where a second pinhole (c) acts as a spatial filter ensuring that only light that originated from the desired sample plane reaches the detector. Light coming from above or below that plane is out of focus at the second pinhole and is blocked. The image is built up one pixel at a time by scanning the point across the sample and using a single element detector to capture all the light that comes from that location. Figure 1.2 compares two images. The top image (A) was taken using a wide-field fluorescent technique in which out-of-focus light significantly degrades the quality of the image. The bottom image was taken using a single photon confocal laser scanning microscope. The contrast and resolution of the image improve dramatically, which is evident in the visible striated pattern of the myocyte sarcomeres and the definition of the distinct cells.

Figure 1.2: Myocyte sarcomeres imaged with wide field florescence (A), and single photon laser scanning confocal microscopy (B) [4].

Despite the advantages of confocal microscopy, its single-photon imaging nature has some drawbacks [5]. Limited imaging depth (∼150 µm at 514 nm [5]), photo bleaching, and photo toxicity are the result of samples being exposed to intense (∼ 5 × 10^5 W/cm²) high energy (∼488 nm) light [5]. These issues are mitigated by multiphoton microscopy which uses
light in the near infrared (800-1000 nm) instead of the standard short wavelength (400-600 nm) excitation beam [7]. Figure 1.3 (a) shows two low energy photons (1.58 - 0.886 eV) undergoing simultaneous excitation in place of one high energy photon (2.38 - 2.19 eV).

Like confocal microscopy, multiphoton microscopy is able to image through scattering media by using a single element detector; however it does not require the use of a pinhole. Superior optical sectioning is achieved by an inherent rejection of out-of-focus light due to nonlinear optical processes such as two- and three-photon fluorescence, harmonic generation, sum-frequency generation, and coherent Raman scattering [8]. These nonlinear effects require an enormous flux of photons ($\sim 5 \times 10^{24}$ photons cm$^{-2}$ s$^{-1}$ [9]) such that the probability of simultaneous absorption ($\sim 10^{-16}$ s) of two or more photons in the sample is high [6]. Pulsed lasers concentrate the energy from what would be continuous wave emission into pulses that are hundred(s) of femtoseconds long. Intensities on the order of tens of GW/cm$^2$ can be reached by focusing the beam of a pulsed laser with a modest NA objective ($\geq 0.65$) [10, 11]. Two photon absorption depends quadratically on excitation intensity which means two-photon events only have a high probability of occurring at focus [6]. This gives multiphoton microscopy several advantages. The near infrared wavelengths can penetrate deep (hundreds of micrometers) and have been shown to be less damaging [11]. Because the photons that produce the signal have half the wavelength of the illumination beam, nearby single photon fluorescence, which produces photons at a wavelength closer to the excitation light than the
signal light, does not hurt the resolution (Figure 1.3c vs. Figure 1.3b).

While multiphoton microscopy has overcome some of the drawbacks of confocal microscopy, both techniques are limited in their ability to produce images quickly as single point methods. Images can be produced in real-time at video rates (30 frames/s [12]) when an extended source, such as a line, is used [13]. This poses a problem, however, because if multiple locations are illuminated on the sample at one time, a single element detector cannot be used, and the issue of scattering media resurfaces. Spatial Frequency modulated Imaging, or SPIFI, is an imaging method that allows for the illumination of the sample by an extended source while still permitting the use of a single element detector. The technique was first demonstrated by Sanders, et al. [14] in 1991, but Futia, et al. [15] developed the imaging theory and its limitations in 2011.

Figure 1.4 illustrates a set up that helps explain how SPIFI works. A cylindrical lens focuses an incoming beam to a line, and then a spinning reticle modulates the line across its spatial extent with a spatial frequency that changes linearly with time. Imaging optics relay the modulated pattern onto the sample, and then collection optics direct the light to the detector. Previous demonstrations of SPIFI have used a laser as the illumination source.

![Figure 1.4: Illustration of SPIFI set-up [15].](image)

[12, 15, 16]. The modulation on the sample can be viewed as a consequence of the coherent nature of the light. The pattern on the reticle acts as a grating, which causes the beam to
diffract. The bandwidth of spatial frequencies on the reticle vary the angle of the diffracted orders with time. The diffracted orders are focused onto the sample where they interfere to set up fringes that modulate each place along the line at a different frequency.

In order for spatial information to be encoded onto the beam, the modulator maps local space to temporal frequency. Equation 1.1 shows the modulation function of the line as a function of the space \((x)\) and time \((t)\), where the cosine is truncated by the window, \(w(t) = \text{rect}(\frac{t}{T_m})\), where \(T_m\) is the modulation period [17].

\[
m(x, t) = \frac{w(t)}{2}[1 + \cos(2\pi\kappa xt)]
\]  
(1.1)

By mapping a frequency to a specific location on the line, the information about the sample at each location is multiplexed in the time domain, and can be recovered with a Fourier transform. Single-photon SPIFI is sensitive to scattering that occurs before the illumination light reaches the focus, but not after. This extends the imaging depth of SPIFI two times over wide-field imaging.

In this thesis, we present an imaging method that employs the concept of SPIFI with incoherent sources: light emitting diodes (LEDs). The greatest advantage of using LEDs over laser light is in their comparative cost. Commercial LED arrays can be purchased for under $100, and the cost of a custom LED array would not exceed $1000 [18]. The price of a commercial femtosecond laser oscillator ranges between $50,000 - $150,000. Homebuilt systems can bring the cost down to $10,000 - $30,000, but this is still two orders of magnitude more expensive than LED arrays.

LEDs are currently being used in academic research as light sources for imaging. In the implementation of an imaging method called Fourier ptychography, Tian, et al. and Dong, et al. use an array of LEDs to illuminate the sample from varying angles [19, 20]. While this technique produces enhanced resolution images, it uses wide-field imaging and is not robust in the presence of scattering. The microscope presented in this thesis uses single element detection, which means it has the ability to image through scattering media while using an
inexpensive LED array as a light source.
CHAPTER 2
THEORY OF SPATIAL FREQUENCY MODULATION FOR IMAGING

The theory of coherent SPIFI is developed by Futia, et al. where the modulation of the illumination light at different locations on the sample can be viewed as a consequence of the coherent nature of the light [15, 17]. It differs from incoherent SPIFI in that the modulations are imparted to the field instead of the direct modulation of the intensity of the light. The transverse extent of the illumination field along the modulation direction (x) can be written as

$$E_{\text{IllumCoh}}(x, t) = E_0 u(x) e^{i\omega_0 t} + \text{c.c.}$$

where c.c. is the complex conjugate and $u(x)$ is the normalized spatial profile of the field. The mask used to modulate the line cursor has a pattern produced by Equation 1.1. Shifting the field with respect to the modulator mask by a distance $x_c$ has the effect of moving the field so that it does not straddle the DC frequency in the mask, which would lead to the same frequencies being imparted to different locations along the beam. The light at a distance $x_c$ across the line cursor is modulated at the center frequency, $f_c = \kappa x_c$.

$$m_{\text{shifted}}(x, t) = \frac{w(t)}{2} [1 + \cos(2\pi \kappa (x - x_c) t)]$$

After the beam passes through the modulation reticle and the object to be imaged, the field becomes

$$E_{\text{mod}}(x, t) = E_0 u(x) g(x) m(x - x_c, t) e^{i\omega_0 t} + \text{c.c.}$$

where $g(x)$ represents the transmission function of the microscope subject, which is in general complex. Squaring Equation 2.3 gives the intensity of the field

$$I(x, t) = I_0 |u(x)g(x)m(x - x_c, t)|^2$$

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where \( \gamma \) represents detector efficiency, and \( I_0 = \frac{n_\text{rep}}{2} |E_0|^2 \) is the intensity coefficient. The field is relayed to the detector, which integrates over its entire area to generate a time-varying signal according to

\[
s(t) = \gamma \int I(x, t) dx \tag{2.5}
\]

Substituting Equation 2.4 and Equation 1.1 into Equation 2.5 gives the signal

\[
s(t) = \frac{\gamma I_0}{4} \int |u(x)g(x)|^2 |w(t)|^2 [1 + \cos(2\pi \kappa (x - x_c) t)]^2 dx \tag{2.6}
\]

The absolute value of the square of the time window, \( |w(t)|^2 \), can be replaced by \( w(t) \) since it is a rectangle function. Expanding Equation 2.6 and using the identity \( \cos^2(u) = \frac{1}{2} + \frac{\cos(2u)}{2} \), the signal can be written as

\[
s(t) = \frac{\gamma I_0}{4} \int |u(x)g(x)|^2 w(t) \left[ \frac{3}{2} + 2 \cos(2\pi \kappa (x - x_c) t) + \frac{1}{2} \cos(4\pi \kappa (x - x_c) t) \right] dx \tag{2.7}
\]

From this we can write the signal in terms of the harmonics, \( s(t) = \frac{\gamma I_0}{4} [s_0(t) + s_1(t) + s_2(t)] \)

where the D.C., first harmonic, and second harmonic terms are

\[
s_0(t) = \frac{3}{2} w(t) \int |u(x)g(x)|^2 dx \tag{2.8}
\]

\[
s_1(t) = 2w(t) \int |u(x)g(x)|^2 \cos(2\pi \kappa (x - x_c) t) dx \tag{2.9}
\]

\[
s_2(t) = \frac{1}{2} w(t) \int |u(x)g(x)|^2 \cos(4\pi \kappa (x - x_c) t) dx \tag{2.10}
\]

respectively. Considering the first harmonic, the cosine in Equation 2.9 can be rewritten as a sum of exponentials to give

\[
s_1(t) = w(t)e^{-i2\pi f_x x_c} \int |u(x)g(x)|^2 e^{i2\pi f_x x} dx + c.c. \tag{2.11}
\]

where Equation 2.11 makes it clear Equation 2.12 has the form of a spatial Fourier transform with \( f_x \to \kappa t \)

\[
G(f_x) \equiv \int |u(x)g(x)|^2 e^{i2\pi f_x x} dx = \mathcal{F}\{|u(x)g(x)|^2\} \tag{2.12}
\]
Rewriting Equation 2.11 in terms of the phase and amplitude of Equation 2.12, the signal on the photodiode is found to be

\[ s_{\text{SigCoh}}(t) = 2w(t)|G(\kappa t)| \cos(2\pi f_c t + \angle G(\kappa t)) \] (2.13)

which shows in coherent SPIFI the signal is the product of one oscillating function and the amplitudes of the spatial frequencies. This method of modulating the beam along a continuous line would work the same way with incoherent light except there would not be a second harmonic signal. In this thesis, however, a spinning disk is not used to modulate the light.

The illumination light is electronically modulated, which uncouples the modulation frequencies from their location in the line cursor so the positions of the frequencies with respect to each other are arbitrary. The signal of each LED can be represented by \( \sin^2(\omega t) \). However, for these experiments, the real signals resemble a saw-tooth or triangle wave (Figure 2.1). The net intensity of the LED array can be represented as the sum of individual intensities

\[ I_{\text{IllumInco}}(t) = I_1 \sin^2(\omega_1 t) + I_2 \sin^2(\omega_2 t) + ... + I_N \sin^2(\omega_N t) \] (2.14)

where \( N \) is the number of LEDs. The light from each LED is focused to a different place on the sample, and is attenuated according to the features at that location. The strength of the attenuation of the incoherent illumination light at a given location in the sample can be modeled by multiplying each term in Equation 2.14 by a number between 0 − 1 (\( a_n \) in Equation 2.15).

\[ s_{\text{SigInco}}(t) = a_1 I_1 \sin^2(\omega_1 t) + a_2 I_2 \sin^2(\omega_2 t) + ... + a_N I_N \sin^2(\omega_N t) \] (2.15)

This intensity produced by all eight LEDs is collected on a detector, which produces a signal that is the sum of \( N \) oscillating functions. A Fast Fourier Transform (FFT) is used to separate the contribution from each LED that reaches the detector, which is the information needed to render an image. It is in this way SPIFI exploits a kind of temporal multiplexing that allows for the use of a single element detector.
To simulate the incoherent imaging process, a gray-scale $16 \times 16$ pixel picture is used as the sample to be imaged (Figure 2.2(a)). Eight LED signals were used simultaneously so multiple “scans” were taken to image more than eight pixels. Again, the LEDs are made spatially distinct by modulation at a unique carrier frequency. Features in the sample attenuate the light from certain LEDs, which varies the frequency content of the total signal. To demonstrate this, two coherence spikes and the absolute value of their FFTs (from here simply referred to as FFTs) are compared from different places in the sample. The two highlighted strips in Figure 2.2(b) show which locations are used.

The attenuated signals are added together in the time domain. Figure 2.3(a) shows the two total signals from these locations. They have distinctive shapes due to their different spectral content. The different frequency components that make up the two signals are shown in their respective FFTs in Figure 2.3(b). The magnitudes are used as intensities to construct an image of the “sample” (Figure 2.2(b)).

The actual time signals from the individual LEDs were used in the image simulation in Figure 2.2, as opposed to simulating them with a sine or triangle wave. Figure 2.1 shows the fastest (1.65 kHz) and slowest (975 Hz) of the eight signals. The blue graph in Figure 2.4(a) shows the coherence spike that is produced by adding all eight signals together. The red graph in Figure 2.4(a) is the signal recorded by an oscilloscope. The two graphs align almost
Figure 2.2: (a) The original $16 \times 16$ pixel image used as a “sample” to simulate incoherent SPIFI. (b) The image rendered by the simulation of incoherent SPIFI. The coherence spikes and FFTs of the pixels highlighted in green and orange are shown in Figure 2.3.

Figure 2.3: (a) Plots of coherence spikes from the signals recorded for the locations highlighted in Figure 2.2. (b) The FFT of the two signals in (a) demonstrating the difference in their frequency components.
exactly with small deviations in the extreme intensities and several missed undulations near the noise floor. Figure 2.4(b) shows the FFT of the calculated total (blue) and experimental total (red) signal together where its no surprise they are nearly identical.

In each LED trace, the number of data points was adjusted to synchronize the phases of all eight signals. Without this step, the coherence spike produced by adding the signals together (Figure 2.5(a)(green)) is less similar to the one seen on the oscilloscope (Figure 2.5(a)(red)) than the calculated signal in Figure 2.4(a)(blue). As expected, the FFT gives the same frequency components (Figure 2.5(b)). This shows that if two clocks are used to modulate separate LED arrays, as long as the LEDs have distinct modulation frequencies, the individual signals can be recovered. Experimentally, all frequencies modulating the individual LEDs are derived from the same clock.

Figure 2.3(a) shows the two total signals generated from highlighted locations in Figure 2.2(a). They have distinctive shapes due to the varying magnitudes of certain frequencies. The different frequency components that make up the two signals are shown in their respective FFTs in Figure 2.3(b). Figure 2.2(a) shows the original “sample” image, and the image rendered by the SPIFI simulation is shown in Figure 2.2(b). The code used for this simulation is included in the Appendix. This demonstration shows it is reasonable to believe SPIFI will work with incoherent sources and illustrates the mechanism.
Figure 2.4: (a) A plot of the calculated (blue) and experimental (red) coherence pattern from the sum of all eight LEDs signals. (b) The FFT of the calculated (blue) and experimental (red) signal.

Figure 2.5: (a) Plot calculated by summing the signals of the LEDs without synchronizing their phases (green) (in contrast to figure Figure 2.4) and the experimental coherence pattern (maroon). (b) The FFT of the calculated (dashed green) and experimental (maroon) signal.
CHAPTER 3
DESIGN AND MODELING OF THE MICROSCOPE

The basic architecture of the microscope can be broken down into three domains: light source, optics, and detection/image rendering. The following sections discuss the design and execution of each.

3.1 Light Source

The light source is a Surface Mounted Device (SMD) LED array on the DE0-Nano Field-Programmable Gate Array (FPGA) development platform which costs $79. Figure 3.1 shows the eight rectangular diffusers, \( \sim 1 \times 2 \text{ mm}^2 \), over the green LEDs (\( \sim 532 \text{ nm} \)) that span approximately 9 mm. All the LEDs will be imaged onto the sample at the same time which means each of these must be modulated at a different frequency in order for a single-element detector to be used. The simplest (and fastest) way to do this is to attach each LED to a different bit output of an 8-bit counter. Figure 3.2(b) illustrates how this modulates all the LEDs at different rates at the maximum speed possible for the FPGA, which has a 50MHz
clock oscillator. The problem is the modulations are not spaced linearly in the frequency domain, which causes the lower frequencies to bunch up as shown in Figure 3.2(a). This makes it difficult to know what sampling rates to use on the detection end: too low and the high frequencies will be cut out, too high and resolution is lost in the frequency domain.

In order to modulate the LEDs so their frequency distribution is linear, a more sophisticated design was developed. The idea behind how this circuit works is discussed here, but the actual Quartus II code is shown in the Appendix. Instead of using each bit of one counter to dictate the frequencies, eight separate counters are used to control the periods of the LED modulations. All eight counters are clocked synchronously at the same rate and each counter counts to a different number that, when reached, tells the LED to turn on or off. “These numbers” are determined by back-calculating them from pre-selected linearly spaced frequencies. The next paragraph gives an example of how this calculation is done (Mathematica notebook included in the Appendix), and then how these numbers are used in a circuit to modulated the LEDs is discussed.

The clock oscillator on the FPGA is 50 MHz so the frequency produced by the eighth bit of a counter is $50 \text{ MHz}/2^8 = 195.31 \text{ kHz}$, which will be referred to as the “base frequency”. To get a frequency spacing of 10 kHz, 10,000 is added to the base frequency to give eight target frequencies: 195 kHz, 205 kHz, 215 kHz, 225 kHz, 235 kHz, 245 kHz, 255 kHz, and 265 kHz. We find the period of these frequencies ($1/f$) to be: $5.12 \mu s$, $4.87 \mu s$, $4.64 \mu s$, 

4.43 µs, 4.24 µs, 4.07 µs, 3.91 µs, and 3.76 µs. The fraction these periods are of the longest period, or “base period”, determines what number on the eight bit counter will turn the LED on or off: 1.000000, 0.951294, 0.907112, 0.866852, 0.830013, 0.796178, 0.764994, 0.736160. We determine this number by multiplying this fraction by the base period in bit counts \(2^8 = 256\) and then rounding: 255, 243, 231, 221, 211, 203, 195, 187. Figure 3.3 shows a graph of these periods (in counter units, not seconds) inverted and graphed with respect to LED number. This confirms that this method does give linearly spaced frequencies.

Figure 3.3: Vertical axis is the inverse of the period in counts (not seconds) and the horizontal axis is number of LED. Because the trend is a straight sloped line, the numbers determined for the counters in the circuit will give us linearly spaced frequencies.

Converting these numbers into binary is crucial to the circuit design: 11111111, 11110011, 11100111, 11011101, 11010011, 11001011, 11000011, 10111011. Figure 3.4 shows how each of these numbers is used in the circuit to turn the LEDs on and off. All bits on the counter are fed to a NAND gate which either passes a 1 or 0 to the LED depending on the number reached by the counter which is illustrated in Figure 3.4. Since these numbers were picked because they were the periods of linearly spaced frequencies, the modulation of the LEDs in the time domain now yields evenly spaced peaks in the frequency domain. This is illustrated in Figure 3.5, which shows a trace taken directly off the oscilloscope.

Mild vignetting from the aperture of the optics causes the LEDs on the ends to be slightly dimmer. Spacing the modulation frequencies linearly in time has the advantage of leaving
Figure 3.4: Circuit layout to be programmed to the FPGA to modulate the LEDs at evenly spaced frequencies.

Figure 3.5: Trace taken off the oscilloscope showing the signal of the eight modulated LEDs in time (yellow, and the FFT of that signal in pink. The evenly spaced peaks confirm the spacing of the modulation frequencies is linear). Vignetting from optics causes intensity variation between the peaks in the middle and the peaks on the ends.
smaller gaps in the frequency space than the previously mentioned nonlinear modulation scheme. In the future when a larger array of LEDs is used, packing more frequencies into a smaller range in the Fourier domain will raise the upper bound on the maximum number of LEDs that can be used simultaneously.

## 3.2 Optics

A schematic to illustrate the general layout of the optics is illustrated in Figure 3.6.

![Schematic of Optics](image.png)

Figure 3.6: A birds eye illustration of the microscope (except the computer and oscilloscope) not to scale.

The LEDs are “collimated” with a $f = 100$ mm lens and two ThorLabs Galvo scan mirrors arranged to form a periscope redirect the “beam” through the 4F image relay system where a $f = 100$ mm and a $f = 75$ mm lens focus the image of the collimated LEDs to the back of the objective. The setup is designed so the LEDs can be scanned and each still fully fills the back aperture of the condensing objective. This objective ($\times 40$, NA= 0.65) creates a demagnified image of the LEDs which can be scanned over the sample with the scan mirrors. The sample is placed between the condenser objective and the collection asphere. The scan mirrors are controlled using two DC voltage supplies, one for each axis. When taking an image, in order to fill the gap between LEDs, five steps must be taken in the dimension of
the LED array for a total voltage change of 1 V (and a trace from the oscilloscope taken at each step). This was determined by placing a screen where the LEDs focus after the first focusing lens (Figure 3.6), and seeing the voltage change required to move the image of one LED until it was against the place the neighboring LED had started. This was done to the accuracy of the power supply which was 0.1 V. A single “step” taken by the scanners is equivalent to a change in the voltage of ±0.2 V, which was determined by measuring the width of the LED light on the screen.

Before construction of the microscope, a model of the system from light source to image formation was created in the optical ray tracing program ZEMAX. The LED array was modeled as three point sources with one point source centered on the optical axis and the other two ±5 mm on either side to simulate the extent of the LED array on the FPGA. In order for an objective to focus incoming light to the smallest spot possible, the entire back aperture must be filled. Figure 3.7 shows the three point sources depicted in red, blue, and green.

![Figure 3.7](image)

Figure 3.7: (a) Paraxial model of the system with a scan angle of 0 degrees and relative distances indicated. An image of the LEDs at the sample plane is magnified in the box. (b) Paraxial model of the system with a scan angle of 5 degrees. While the system is no longer centered, the back aperture of the objective is still filled by all three sources.
The first lens is placed a focal distance (10 cm) away from the sources in order to collimate them. The collimated light overlaps on the scan mirror and is relayed to the back of the objective, which is depicted as a red line at the bottom of Figure 3.7. All three light sources fill the back aperture of the objective at a scanning angle of zero degrees (Figure 3.7(a)). Figure 3.7(b) shows the paraxial system with a scan angle of five degrees. The light is no longer centered on the optics, but all three sources still fully fill the back aperture. This is expected since we imaged a plane where the light from each source overlapped (on the scan mirror) onto the back of the objective.

After modeling the system with paraxial lenses, real lenses were inserted in the lens design editor. The lens separation was optimized so the design could act as a blueprint for construction of the microscope. Expected sizes of the demagnified LEDs could also be calculated, since these play a part in determining the resolution of the system. By tracing two point sources separated by the measured width of the LED diffuser (2 mm) and seeing the distance they are separated after being focused by the objective, the size of the LEDs at the sample plane (\(\sim 100 \, \mu m\)) could be determined. Figure 3.8 illustrates this process with the scan mirror removed.

The collection optics were not modeled in ZEMAX, but are shown in Figure 3.6. An asphere \((f = 2.8 \, \text{mm})\) on the transmission side of the sample “re-collimates” the focused image of the LEDs and a \(f = 100 \, \text{mm}\) lens focuses the light onto the photodiode. A picture of the actual optics setup is shown in Figure 3.9. The camera was used to get wide-field images of the sample. This allowed for the desired field of view to be easily selected with the translation stages on which the sample was mounted.

### 3.3 Detection and Image Rendering

The detector is a ThorLabs DET100A biased silicon photodiode sensitive in a wavelength range of (400-1100 nm). The rise time is 43 ns and the area of detection is relatively large, 75.4 mm\(^2\) [22]. It is connected to a LeCroy WaveAce 234 300 MHz oscilloscope with a maximum sampling rate of 2 GS/s. With the LEDs at one location on the sample, a trace is
Figure 3.8: Scan system designed with real lenses from ThorLabs catalog. The left box shows the point source separation (2 mm). The right box shows the objective focus. The bottom box shows the ZEMAX Full Field Spot Diagram and the distance between the two focused sources (∼ 100 µm).

Figure 3.9: A photograph of the actual microscope setup.
recorded on a USB drive plugged into the oscilloscope. The LEDs are then scanned one step in the $x$-direction ($\pm 0.2$ V) and another trace is recorded. After the LEDs have been scanned far enough in the $x$-direction to fill in the gaps between them (1 V), they are scanned one step in the $y$-direction ($\pm 0.2$ V), and the process for filling the spaces between them starts again. In order to record what traces have been taken where, spreadsheets were used to correlate the trace number with its position in voltage. A sample of one of these spreadsheets is shown in Figure 3.10.

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Figure 3.10: Spreadsheet used to record the position of traces taken for imaging. The bold numbers indicate the voltage reading on the controllers for each axis when a trace was taken and the consecutive integers indicate the number of the trace taken.

The trace data was processed in Mathematica. Each trace contains a signal in time with information for eight pixels of an image, corresponding to the eight LEDs. An FFT extracts the frequency components of each signal. The peaks that correspond to the modulation frequencies of the LEDs represent the intensities of the pixels. A normalization trace is taken at the scan location (0.6 V, 1.6 V) and the sample position is adjusted so none of the LEDs are attenuated by features on the sample. It is an option to take a normalization trace for every scan location, but this practice is time intensive and only slightly improves the contrast of the image near the edges. After the normalized intensities of each pixel have been collected into lists and appropriately ordered, Mathematica’s Image[] function converts these numbers into an image. The code that rendered the images can be found in the Appendix.
CHAPTER 4
RESULTS

The first iteration of the microscope used an aspheric lens with a focal length of 2.8 mm as the condensing optic. While Zemax predicts the imaging system will produce a spot size of $\sim 80 \, \mu m$, features spaced closer than this were resolved, which is evident in Figure 4.1. The ZEMAX calculation assumes the widest dimension of the diffuser on the LED, 2 mm, is demagnified at the sample plane when in practice, a much smaller area reaches the sample plane with adequate intensity. The Zemax calculation was done using sequential ray-tracing, and does not model the nature of the source.

![Figure 4.1](image)

Figure 4.1: Images taken with an aspheric condenser lens of a 1951 Air Force resolution test target: (a) Horizontal line sets 5 and 6 from group 3 with respective line widths 39.37 $\mu m$, and 35.08 $\mu m$. (b) Line sets 2, 3, and 4 from group 4 with respective line widths 27.84 $\mu m$, and 24.80 $\mu m$, and 22.10 $\mu m$.

Images taken of a 1951 Air Force resolution test target were used to determine the resolution limit of the microscope. Figure 4.1(a) shows the horizontal lines of elements 5 and 6, from group 3 of the test target. The width of one line in each element are 39.37 $\mu m$, and 35.08 $\mu m$, respectively. Figure 4.1(b) shows elements 2, 3, and 4, from group 4 of the test target. The line widths are 27.84 $\mu m$, and 24.80 $\mu m$, and 22.10 $\mu m$, respectively. The unresolved element in Figure 4.1(b) does not necessarily mean the resolution limit for the
asphere is at 22.10 µm. That element is not centered in the field of view, which is quite limited due to the use of an aspheric condensing lens. If the position of the element had been more centered, it might have been resolved.

Before imaging finer elements, the asphere was replaced by an objective with magnification 40× and an NA of 0.65. The resolution predicted by sequential ray-tracing in ZEMAX is \( \sim 100 \) µm (Figure 3.8), but we were able to resolve elements with line widths finer than this. Figure 4.2(a) shows an image of the elements 5 and 6 in the third group on the test target and Figure 4.2(b) shows an image taken with a camera of the same area of the target. Element 5 has a line width of 39.37 µm and is fully resolved. It is not obvious why element 6 is not clearly resolved because Figure 4.3(a) and Figure 4.4(a) show elements with thinner line widths resolved, namely, element 1 and 2 from group 4 with line widths 31.25 µm and 27.84 µm, respectively. We speculate that this effect is due to the reliability of the power supply to accurately be at the voltage it reads, which directly effects the position of the LEDs at the sample. Also since scanning the LEDs creates a grid of points, if the sample is not oriented favorably with respect to this grid, certain elements of the target may get smeared out.

![Figure 4.2](image)

Figure 4.2: Line sets 5 and 6 from group 3 of a 1951 Air Force resolution test target with respective line widths 39.37µm, and 35.08 µm. (a) Image taken with SPIFI. (b) Image taken with camera. (c) Intensity profiles corresponding to the red and blue lines in (a).

The most widely accepted standard to measure the resolution limit of an imaging system is the modulation transfer function (MTF) [23]. The MTF can be thought of as the spatial
Figure 4.3: Line set 1 from group 4 of a 1951 Air Force resolution test target with line width 31.25 µm. (a) Image taken with SPIFI. (b) Image taken with camera. (c) Intensity profiles corresponding to the red and blue lines in (a).

Figure 4.4: Line sets 2, 3, and 4 from group 4 of a 1951 Air Force resolution test target with respective line widths 27.84 µm, and 24.80 µm, and 22.10 µm. (a) Image taken with SPIFI. (b) Image taken with camera. Intensity profiles corresponding to the (c) red and blue lines, (d) green and orange lines, and (e) Purple and yellow lines.
frequency response of the system. In this case, the spatial frequencies are the evenly spaced lines on the target. In the 1951 test target, these are not pure frequencies since they have hard edges as opposed to being sinusoidally apodized, but they give a good idea of the resolution limit of the system. The modulation or contrast $M(\nu)$ is given by

$$M(\nu) = \frac{I_{\text{max}} - I_{\text{min}}}{I_{\text{max}} + I_{\text{min}}}$$

(4.1)

where $I_{\text{max}}$ is the peak value and $I_{\text{min}}$ is the minimum value of a line intensity profile perpendicular to the frequencies (Figure 4.2(c) is an example of this profile). The contrast is calculated for each element in a given direction and those points are normalized by the contrast of the object to give the MTF:

$$\text{MTF}(\nu) = \frac{M_{\text{image}}}{M_{\text{object}}}$$

(4.2)

Figure 4.5 shows the MTF of the microscope with the characteristic shape of a low-pass frequency response curve. It suggests a resolution limit between 0.020 $\mu$m$^{-1}$ and 0.023 $\mu$m$^{-1}$ spatial frequencies, or the microscope can resolve features as small as $\sim 25$ $\mu$m. Each point was calculated from the intensity profiles for the images shown in Figure 4.2, Figure 4.3, and Figure 4.4. The resolution of this microscope is far above the diffraction limit of the objective, $7.69 \times 10^{-7}$ m, which is due to the fact that the LEDs used are extended sources. Because the resolution is worse than the diffraction limit of the objective, this calculation was presented to show how the resolution of imaging systems is quantified, not to serve as an argument for these exact results.
Figure 4.5: Plot of the MTF of the microscope.
CHAPTER 5
LIMITATIONS AND FUTURE WORK

The ultimate limitations of future versions of the microscope reside in one of two regimes: optical limitations or electronic limitations. Each is discussed to give insight to the potential of this microscope.

5.1 Optical Limitations

The resolution of the images produced by the microscope is determined by the size of the image of one LED at the sample. The smallest focused point achievable by an objective of a given NA and a light source of a given wavelength, $\lambda$, is

$$d = \frac{\lambda}{2\text{NA}}$$  \hspace{1cm} (5.1)

which specifies the diameter of the diffraction limited spot size. The diffraction limited diameter for the objective in this thesis is 409 nm. The spot size achieved was $\sim 25 \, \mu\text{m}$ due to the fact that the individual LEDs are extended sources. This is a smaller spot size than the ZEMAX model predicted (Figure 3.8) according to the dimensions of the diffuser over the LED. ??(c) shows the LEDs after several orders of magnitude of attenuation. The circular shape of the LEDs after attenuation accounts for the discrepancy in predicted vs. measured resolution. By taking the spot size of the LED at the sample plane and the magnification of the imaging system to be

$$\text{mag} = \frac{-f_2}{f_1}$$  \hspace{1cm} (5.2)

the portion of the LED that contributes to the size of the spot at the sample plane can be back-calculated to be $\sim 0.5 \, \text{mm}$. To arrive at this number, $f_2$ is the focal length of the objective, 3.84 mm, and $f_1$ is the focal length of the second collimating lens, 75 mm (as shown in Figure 3.6). The focal length of the objective was calculated using
NA = \frac{nD}{2f} \tag{5.3}

where \( D \) is the diameter of the objective and \( n \) is the index of refraction between the objective and the subject, which is approximated to be 1 in this calculation. In order to get a diffraction limited spot, the size of the LED must be small enough that, after being demagnified and imaged at the sample, it matches the diameter calculated according to Equation 5.1. The maximum diameter of the source that would give a diffraction limited spot can be calculated using

\[ D_{\text{FocusedSpot}} = \text{mag} \times D_{\text{Source}} \tag{5.4} \]

Plugging in the diffraction limited spot size, the focal lengths of the optics, and solving for \( D_{\text{Source}} \) gives the maximum source size to get diffraction limited imaging.

\[ D_{\text{Source}} = \frac{75 \times 10^{-3}}{3.84 \times 10^{-3}} \times 4 \times 10^{-7} = 8 \times 10^{-6} \tag{5.5} \]

The illumination source would need to be \( \sim \) 8 \( \mu \)m in diameter to get diffraction limited imaging. Even the smallest LED emitters are 40 \( \mu \)m across.

Future versions of this microscope will use a mask with eight precisely drilled pinholes to cut the extent of each LED so the images of the LEDs at the sample meet the diffraction limit. This will have to be weighed against the attenuation of the intensity of the light and effects from diffraction.

The luminous intensity of an LED in the array is \( \sim \) 250 mcd. Taking the area of a rectangular LED diffuser to be 2 mm\(^2\) and the radius of a pinhole to be 4\( \mu \)m, the luminous intensity scaled by the mask is 250 mcd\( \times \frac{4^2 \pi}{2000 \times 1000} = 6.3 \mu \)cd. In this estimation, effects due to diffraction are not considered. The power per steradian (sr) is \( 9.2 \times 10^{-9} \frac{W}{\text{sr}} \) given that 1 cd = \( \frac{1}{683} \) \( \frac{W}{\text{sr}} \) at a wavelength of 555 nm. It is assumed that the light collimated by the first 2 inch collimating lens makes it to the detector. The area subtended by 1 steradian at the distance of the collimating lens 10 cm away is 100 cm\(^2\) implying the intensity is \( 9.2 \times 10^{-11} \frac{W}{\text{cm}^2} \). Because the area of the collimating lens is relatively small compared to
the area subtended by 1 steradian, the power captured by the lens is approximated as

\[ P = \text{Area of Lens} \times \text{Intensity at Lens} = 2.54^2 \pi \text{ cm}^2 \times 9.2 \times 10^{-11} \frac{W}{\text{cm}^2} = 1.9 \times 10^{-9} \text{ W}. \]

This number is converted into photon counts per second with

\[ n = \frac{P \lambda}{hc} = \frac{1.9 \times 10^{-9} \times 532 \times 10^{-9}}{6.626 \times 10^{-34} \times 3 \times 10^8} = 5.2 \times 10^9 \] (5.6)

The photon counting regime is in the MHz range or several million photons per second. This calculation suggests that the reduction in intensity that would result from a mask will not require a more sensitive, photon counting detection scheme [24].

Organic LED (OLED) displays are not as bright (10^2-10^4 cd m^{-2} compared to LEDs at 10^6-10^7 cd m^{-2} [25]) but might work well for this application. OLED pixels can be as small as 3.3 \times 9.9 \mu m [26] and come in small screens with thousands of pixels. Economical solid-state photo-multiplier tubes (PMTs) are becoming available which could make these screens a practical illumination source [27]. If a custom board is made, another possible alternative illumination source is surface mounted laser diodes. These sources would be bright enough and it would not be necessary to have a pinhole to get diffraction limited imaging.

As mentioned in the introduction, SPIFI is robust to scattering on the way from the focus to the detector. One way to further decrease the sensitivity to scattering would be to re-image the image plane in the sample before the detector, and put an array of pinholes there. As in confocal microscopy, this would have the effect of physically blocking out-of-focus light.

5.2 Electronic Limitations

The electronic limitations of the data collection and detection are related to the electronic limitations of the source modulation. The clock oscillator on the FPGA, 50 MHz, limits the maximum modulation frequency of the fastest LED to half this frequency, 25 MHz (see Section 3.1).

\[ f_{\text{max}} = \frac{f_{\text{FPGA}}}{2} \] (5.7)
To avoid aliasing, the minimum rate the oscilloscope and photodiode can sample the signal is the Nyquist rate: twice the rate of the highest frequency (50 MHz)

\[ r_{\text{Nyquist}} = 2f_{\text{max}} \] (5.8)

The oscilloscope used in this thesis is a Lecroy WaveAce with a bandwidth of 300 MHz and sampling capabilities of 2 GS/s. While it is possible to detect signals at or higher than the bandwidth frequency, their amplitudes will be attenuated according to Figure 5.1. The strength of the signal compared to the noise will determine whether or not those frequencies are accessible [28]). The required sampling rate of at least 50 MHz is met by the oscilloscope,

![Figure 5.1: Oscilloscope frequency response [29].](image)

but that is not sufficient. The detector must also be able to sample at this frequency. The photodiode has a rise time of 43 ns, which implies the highest frequency it can detect is 8 MHz according to \( t_{\text{rise}} = \frac{0.35}{f_{\text{bandwidth}}} \) [22]. While the oscilloscope far exceeds the bandwidth needs of the FPGA, the rise time of the photodiode means none of the LEDs can be modulated above 8 MHz. The highest modulation frequency used for imaging in this thesis is 1.65 kHz: far below the sampling limitations of either the oscilloscope or the photodiode.

The bandwidth and rise time of the photodiode dictate the highest modulation frequency of the LEDs, which sets an upper bound on the number of LEDs that can be used simultaneously. The slowest frequency (produced by an 8-bit counter run by a 50 MHz clock, 195 kHz) also influences the number of LEDs that can be used because it is the “base frequency” from which all other frequencies can be calculated. These other frequencies are calculated
based on the “frequency step size” (mentioned in Section 3.1 to be 10 kHz). The smaller this step size, the more potential modulation frequencies there are, and the more potential LEDs that can be used to image at the same time. The theoretical limit to the number of light sources used together is

\[ N_{\text{LED}} = \frac{f_{\text{max}} - f_{\text{min}}}{\Delta f} \]  

(5.9)

where \( \Delta f \) is the step size in the frequency domain, \( f_{\text{max}} \) is the maximum frequency and, \( f_{\text{min}} \) is the “base frequency”. The step size cannot be less than the two times the separation of the points in the frequency domain of the FFT

\[ \delta f_{\text{min}} = \frac{2}{N_{\text{points}} \delta t} \]  

(5.10)

where \( \delta t \) is the inverse of the sampling rate.

In future versions of this microscope, \( f_{\text{min}} \) could be lowered by using two counters per LED in the FPGA code (Section 3.1) to increase the number of possible modulation frequencies available. This will allow for larger arrays of LEDs to be used, which will improve data acquisition speed, and extend the field of view.

The following calculation shows how LED SPIFI extends to higher numbers of LEDs. Given the bandwidth of the photodiode is 8 MHz, the fastest frequency that can be reliably detected is 4 MHz. Assuming an acquisition time of 10 milliseconds, the number of points in one data set will be \( 0.01 \text{ s} \times 4 \text{ MHz} = 80,000 \) points, and the lowest possible frequency that could be detected is \( 0.01^{-1} \text{ s} = 100 \text{ Hz} \). The amount of time between sample points is \( \delta t = \frac{1}{8 \text{ MHz}} = 125 \text{ ns} \). In the Fourier domain, the minimum spacing between frequency points is \( \delta f = \frac{2}{80000 \text{ points} \times 1.25 \times 10^{-7} \text{ s}} = 200 \text{ Hz} \). Solving Equation 5.9 for \( N_{\text{LED}} \) gives \( N_{\text{LED}} = 19,999 \).

If an LED array is 100 \( \times \) 100 pixels, only the first 10,000 frequencies need to be used, namely 4 MHz - 2 MHz. Keeping the lowest frequency greater than half the highest frequency also helps ensure the harmonics from the lower frequencies are not contributing to the signal of the higher frequencies. If a custom board is made to include analog control of LED brightness, they may be taken from off to on in a precisely sinusoidal pattern, which would
avoid the issue of higher harmonics.

Other improvements that will drastically improve the microscope include automating the scanning and data acquisition process, and refining the Mathematica code to render images more quickly. As mentioned above, using a screen with sub–10 × 10 µm emitters is a possibility to eliminate the need for scanning and to achieve diffraction limited imaging.
REFERENCES CITED


Many of the calculations and data processing done for this thesis was done in Mathematica. The Mathematica notebooks and Quartus II code previously referenced in this thesis are included as supplemental electronic files. The files that are included are listed and described in Table A.1.

Table A.1: A table of tabular goodness.

<table>
<thead>
<tr>
<th>File Name</th>
<th>Description of File</th>
</tr>
</thead>
<tbody>
<tr>
<td>ImageRenderingCode.nb</td>
<td>This code can be used to generate images from data taken off the oscilloscope.</td>
</tr>
<tr>
<td>8-bit LED Frequencies Generation.nb</td>
<td>This code can be used to generate linearly spaced frequencies using 8-bit counters.</td>
</tr>
<tr>
<td>quartusIIrr.pdf</td>
<td>This is an image of the Quartus II code that modulates the 8 LEDs on the FPGA at linearly spaced frequencies.</td>
</tr>
<tr>
<td>ImageSimulation.nb</td>
<td>This code simulates incoherent SPIFI.</td>
</tr>
</tbody>
</table>