

WIDEFIELD MICRO-CAMERA INTEGRATED INTO THE OBJECTIVE LENS OF A
REFLECTANCE CONFOCAL MICROSCOPE FOR CONCURRENT IMAGE REGISTRATION

by

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DEDICATION

I would like to dedicate this work to my parents, Corey and Jennifer Aist.

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ABSTRACT

With millions of new skin disease cases reported annually, non-invasive imaging methods have been developed to diagnose skin disease accurately. Reflectance confocal microscopes (RCM) have led these new technologies with high sensitivity and specificity. However, current methods use multiple devices: a digital camera, a dermoscope, and an RCM, which are not co-registered. Therefore, locating small, microscopic RCM fields-of-view (0.5×0.5 mm) at specific suspicion sites within the larger dermoscopic field-of-view (10×10 mm) is extremely difficult. This “blind” RCM imaging results in lower and more variable diagnostic accuracy, particularly sensitivity, where positive and negative predictive values can drop by up to 30%. Our team has designed a new objective lens with an integrated micro-camera to deliver a concurrent widefield image of the skin surface surrounding the location of microscopic RCM imaging. The widefield image can be used directly to provide context for RCM or can be registered to a previously stored high-resolution clinical image to show where RCM imaging is occurring. In this thesis, the micro-camera is characterized and tested in laboratory and clinical settings. In addition, this thesis investigates a co- and cross-polarized micro-camera and LED system. It compares them to the non-polarized system to explore whether the cross-polarized version enhances feature contrast and enables better dermoscopic imaging. Non-polarized, co-polarized, and cross-polarized mock-up probes of the objective lens with a micro-camera were designed and built for testing. Images of resolution targets, color charts, and skin were taken to obtain modulation transfer function (MTF) measurements, color analysis data, and representative skin images. The results showed improvement in the MTF for the cross-polarized probe when compared to the co- and non-polarized probes. It was also found that the polarization of the imaging system did not significantly affect the color quality of the images. When tested by scientists at Memorial Sloan Kettering Cancer Center, sub-surface features not seen with the co- and non-polarized probes were observed with the cross-polarized probe. The cross-polarized probe suppressed the surface reflections, allowing for sub-surface information to be captured.

CHAPTER 1

INTRODUCTION

State of Skin Cancer Care

Studies show millions of cases of skin cancer being diagnosed each year in countries such as the United States of America (USA), the United Kingdom, European Countries, and Australia [3-6]. The highest rates are reported in Australia, with over 1,000 per 100,000 persons. The lowest rates are reported in areas like London, with 24 for every 100,000 persons [3]. Note that only limited data are available from countries in Africa, meaning that these reports are primarily from predominately white populations. However, the number of skin cancer cases is increasing year to year. To identify malignant versus benign with noninvasive methods, many dermatologists rely on visual inspection and dermoscopes (devices showing the surface of the skin with low-level magnification). This method can achieve high sensitivities (few false negatives), but at the cost of poor and highly variable specificity leading to a considerable amount of false positives, especially in the hands of novice clinicians [7-10]. This means these clinicians diagnose many benign lesions as potentially malignant, leading to unnecessary biopsies [11-16]. A biopsy is a commonly used diagnostic tool for skin diseases because it can definitively identify whether a suspicious lesion is cancerous. Increased awareness of melanoma over the last 30 years in the public and with clinicians has also put pressure to conduct more biopsies, especially in younger demographics where the likelihood of malignant lesions is lower [16].

While misdiagnosis that leads to unnecessary biopsies is a problem, skin disease is still deadly. Proper and early diagnosis is critical for treating skin diseases like melanoma, basal cell carcinoma, and squamous cell carcinoma before they become fatal. With over 3000 per 100,000 reported cases of skin disease and 23,000 deaths a year in the USA [14, 16], the increase in public awareness is warranted. Statistically, over 20% of Americans across all age groups have an average of more than one skin disease, with about 49% of the population of adults over 65 having 2.2 skin diseases and almost 25% of children under 17 with 1.4 skin diseases [14, 16]. While most skin diseases are not life-threatening, carcinomas can metastasize, eventually becoming fatal. Treating these diseases can cost millions of dollars, and it is estimated that billions are spent each year on the direct costs of treating skin diseases [5]. Skin cancer care aims to enhance the patient's standard of living by minimizing any discomfort, morbidity, and negative health impacts resulting from biopsies and excisions while conserving as much tissue anatomy and function as possible. This is achieved by maintaining normal skin, reducing the need for additional biopsies, utilizing preventative methods for proper diagnosis, and implementing treatments that preserve tissue and anatomy, improve appearance and functionality, and minimize unintended harm.

To help meet the goals of skin cancer care, giving dermatologists the tools to better detect and diagnose skin cancer is paramount. Methods to accomplish this noninvasively using optical imaging and spectroscopy are being developed [6, 15, 17-101]. Reflectance confocal microscopy (RCM) imaging is currently one of the best noninvasive imaging techniques for diagnosing and monitoring skin conditions. RCM uses laser light that can penetrate multiple layers of skin and produce images of the underlying structures. The images produced with RCM imaging can be

obtained in real-time and display cellular-level detail in high resolution of subsurface skin structures that are not visible with traditional dermoscopy. RCM images can provide information about the skin layers' size, shape, thickness (up to 200 μm), and the presence of pigmented or non-pigmented structures. This information is crucial for diagnosing and managing various skin conditions. From clinical testing, it was found that RCM imaging helped provide high sensitivity and specificity when used by dermatologists for the discrimination of benign and malignant lesions [38, 42, 43, 46, 102]. Computer vision and machine-learning algorithms have also been applied to diagnose skin conditions. These machine-learning systems proved to have higher sensitivity and specificity than dermatologists when observing smaller pigmented skin lesions [67, 68, 79, 89, 103]. Spectrophotometric Intracutaneous Analysis (SIAscopy) is another skin disease diagnosis tool recently developed and tested [57, 66, 82, 104]. However, both latter two methods showed lower specificity than RCM imaging. Other methods have been demonstrated with higher sensitivities and specificities but have limited clinical studies to back their results or are not tested on all types of lesions [40, 42, 52, 86]. Therefore, more studies must be performed before a valid comparison of these methods can be made. Thus, RCM imaging is currently a front-runner in improving the noninvasive diagnosis of skin diseases and is becoming more widely accepted by medical professionals.

RCM imaging has shown high sensitivity and specificity, yet wide-probe RCM imaging (WP-RCM) can only be done on areas of the skin that do not have many contours, so the large gantry-mounted imaging system can reach them. Hand-held RCM (HH-RCM) allows RCM imaging on curved and harder-to-reach surfaces [30, 53]. For a direct comparison of 54 basal cell carcinoma (BCC) lesions, the WP-RCM showed a sensitivity of 100%, specificity of 78%,

positive predictive value of 96%, and negative predictive value of 100%. The HH-RCM showed a sensitivity of 93%, specificity of 78% (same as the WP-RCM), positive predictive value of 95%, and negative predictive value of 70%. Likely due to the wider field of view, the WP-RCM performed better, yet the ability to reach more parts of the body and faster setup times are still advantages of the HH-RCM [30, 53]. The HH-RCM can image more areas, such as the face and genitals, where WP-RCM cannot be used due to size limited access of the scope to these areas of concern.



Figure 1.1. Image of the VivaScope 3000 HH-RCM by Caliber ID (left). Image of the VivaScope 1500 WP-RCM by Caliber ID (right).

Prior to imaging with RCM, clinicians identify suspicious sites with a dermoscope or widefield CCD camera. Then, using those images for reference, RCM images are captured. The WP-RCM and HH-RCM are guided by a clinical widefield image taken with a dermoscope. A sticky ring attached to the skin is used to mechanically register the location of the dermoscope's image to the objective lens of the WP-RCM. This sticky ring approach is not applicable for the

HH-RCM because it is freely moving, guided by a clinician. For the HH-RCM, the widefield CCD camera or the dermoscope is used to get an overall picture of the lesions of concern. Then, the clinician attempts to center HH-RCM over suspicious parts of the lesion. However, the objective of the freely moving HH-RCM obstructs the view of the lesion. Without simultaneous guidance from a registered and oriented clinical widefield or dermoscope image, the clinician must take blind images, hoping they are covering the most diagnostically relevant parts of the lesion. This process for the HH-RCM, using multiple, non-registered devices, is a slow and tentative procedure that results in significantly lower sensitivity and specificity than the controlled trials that initially validated RCM imaging [30, 53].

To address the problem of “blind” HH-RCM imaging using the handheld probe, this project aims to incorporate simultaneous widefield imaging of the skin surface during HH-RCM microscopy by adding a micro-camera to the HH-RCM instrument. Integrating a widefield micro-camera into the objective of an HH-RCM could significantly improve sampling accuracy and repeatability, and thus diagnostic accuracy compared to the current practice of separate devices and blind HH-RCM imaging. An integrated widefield micro-camera would provide real-time registration and orientation of the three fields-of-view (dermoscopy, widefield, and RCM), leading to quicker and more precise imaging. Furthermore, our team hypothesizes that the more precise imaging using the integrated widefield micro-camera could demonstrably reduce the number of healthy skin biopsies, significantly reducing the burden on patients and healthcare systems.

However, integrating a widefield micro-camera into the objective of an HH-RCM presents multiple challenges that must be addressed for wider adoption. A critical constraint was

how to include the micro-camera and illumination into the objective lens without negatively impacting RCM performance. The second challenge is to provide a widefield image that is of sufficiently high quality to be useful. This must account for the resolution of the image and the quality of the illumination scheme.

To help overcome the obstacle of improving diagnostic accuracy, this thesis will investigate the micro-camera used for widefield imaging, how to integrate the micro-camera and light emitting diodes (LEDs) used for illumination into the objective lens, image quality, and the quality of the illumination scheme. Additionally, the effects of the cross-polarization of the micro-camera and the LEDs on the image quality are investigated. Cross-polarized dermoscopic imaging has been proven to image subsurface structures of the skin that non-polarized imaging cannot, as well as increase the contrast between features [23, 48, 55, 61, 65, 78, 92, 97, 105]. Cross-polarized widefield imaging as guidance could provide clinicians with more information about the lesions and allow for better placement of the HH-RCM over suspicious locations in a lesion.

This project is a collaboration between our group at Montana State University (MSU) and engineers and scientists from Caliber ID, Memorial Sloan Kettering Cancer Center (MSKCC), and Photon Gear Inc. to provide a working HH-RCM imaging device with concurrent widefield registration and orientation through an integrated micro-camera within the objective lens of the HH-RCM. The HH-RCM design is based on the VivaScope 3000 line developed by Caliber ID. Photon Gear Inc. designed the objective lens assemblies for the system. MSKCC is responsible for clinical testing and computer-aided feature recognition to track the movement of the HH-

RCM during imaging. MSU works with all three organizations to design and test the micro-camera system and its illumination scheme.

Reflectance Confocal Microscopy

In RCM, a laser beam is focused beneath the sample surface, such as a patient's skin. The backscattered light is collected by the objective lens and directed toward a pinhole in front of a photodetector. The pinhole is optically conjugate to the focal plane, confocal, in the sample and filters the out-of-focus light from the image, allowing subsurface imaging. This spatial filtering, called optical sectioning, is a key property of RCM. The objective lens is designed to focus the laser beam onto a small point within the skin and to collect the reflected light with high sensitivity. This small point being imaged in the skin is a three-dimensional point spread function, or voxel, capable of resolutions between $0.5\ \mu\text{m}$ and $1\ \mu\text{m}$. RCM can image to depths between $100\ \mu\text{m}$ and $200\ \mu\text{m}$, and usually with the intent of imaging the basal cell layer and dermal-epidermal junction in the skin located between $50\ \mu\text{m}$ and $150\ \mu\text{m}$ [20, 106]. Being able to image at a range of depths in addition to across the skin allows RCM to do three-dimensional imaging, differentiating RCM imaging from dermoscopy or other microscopy of the *surface* of the skin. Generally, the laser beam is scanned across the skin in a raster pattern, and the reflected light is collected at each voxel. The collected light is then used to build up an image of the skin, with each pixel corresponding to a voxel within the skin. The image is created by plotting the intensity of the backscattered light as a function of the position of the laser beam in the skin.

The fundamental requirement for the objective lens in RCM is that it must have a high-numerical aperture (NA), typically greater than 0.7 [106], to allow for the collection of light from a large solid angle and to achieve both thin optical sectioning and high-resolution sub-surface

imaging. The NA is a measure of the ability of the lens to gather light and is proportional to the lens's ability to resolve small features in the sample. It is a dimensionless value related to angles that the objective lens can capture or emit light. A consequence of requiring a high-NA is that the working distance (the distance between the lens and the skin surface) is usually small.

Additionally, working in contact is beneficial for RCM in that it renders the skin stationary with respect to the lens, minimizing motion artifacts. The high-NA and small working distance are important to ensure that the laser beam can be focused onto a small point in the skin, which is necessary for thin optical sectioning and high-resolution imaging. The RCM system with which the micro-camera will be integrated uses an immersion lens for imaging into the skin. Ultrasound gel and mineral oil index-match the skin, slide window, and objective lens. The RCM system is imaging with about a $500 \times 500 \mu\text{m}$ field-of-view, which is limited by its NA [20, 106]. The high-NA objective lens will vignette anything outside of the $500 \mu\text{m}$ field-of-view.

A suggested method of integrating a widefield camera is inserting a beam splitter between the objective lens and tissue. However, the immersion lens system used by Caliber ID does not allow enough space between the objective lens and tissue because it requires such a high NA and short working distance. A second potential method is to put the camera behind the objective lens (again, with a beam splitter). But the camera doesn't see a big enough field-of-view if placed behind the objective lens. Hence, the solution is to put the camera *within* the objective lens, where it can see the desired wide field-of-view without the vignetting caused by the full optical train.

Integrating the micro-camera into the objective lens of the RCM means that the micro-camera will obstruct the RCM beam. The front element in the objective lens is a nearly aplanatic

hyper-hemisphere lens. The hyper-hemisphere was chosen to minimize spherical aberrations in the RCM image when focusing inside the skin tissue. In addition to achieving a high NA, the objective lens is also responsible for reducing spherical aberrations that will blur the image. The micro-camera is placed directly behind this hyper-hemisphere, but before the back lens group, as shown in figure 1.1. With the board used to mount the LEDs and micro-camera (details described in the Flex Board section, the obstruction takes the form of a 2.5 mm thick line in the pupil of the objective lens. This obstruction has been shown to not significantly degrade the optical sectioning and resolution of the RCM, even with the redesign of the objective lens by Photon Gear Inc to accommodate the flex board and camera. The obstruction by the flex board's bridge creates a divided pupil or two D-pupils instead of a full pupil. Divided pupil designs have been shown to increase image contrast because of the angular separation of the detection path from the illumination path compared to the full pupil configuration [85, 91]. The optical sectioning from the divided pupil and full pupil configurations were also comparable. However, the RCM system used for this widefield micro-camera integration has no angular separation of the detection and illumination paths. So, this result with our system may not be comparable and warrants further investigation.

The LEDs used for the micro-camera's illumination do not interfere with the RCM system, provided they are located outside the RCM beam cone. With the capacity to integrate a micro-camera into the objective lens of the RCM, the system delivers a real-time video feed simultaneous to the RCM imaging. This micro-camera feed can potentially even be registered to a high-resolution clinical widefield image (e.g., dermoscope image) to provide better clinical guidance to the user. In this thesis, the micro-camera is characterized and tested in laboratory and

clinical settings. In addition, this thesis investigates a co- and cross-polarized micro-camera and LED system and compares them to the non-polarized system to verify if the cross-polarized enhances feature contrast and enables better dermoscopic diagnosis.

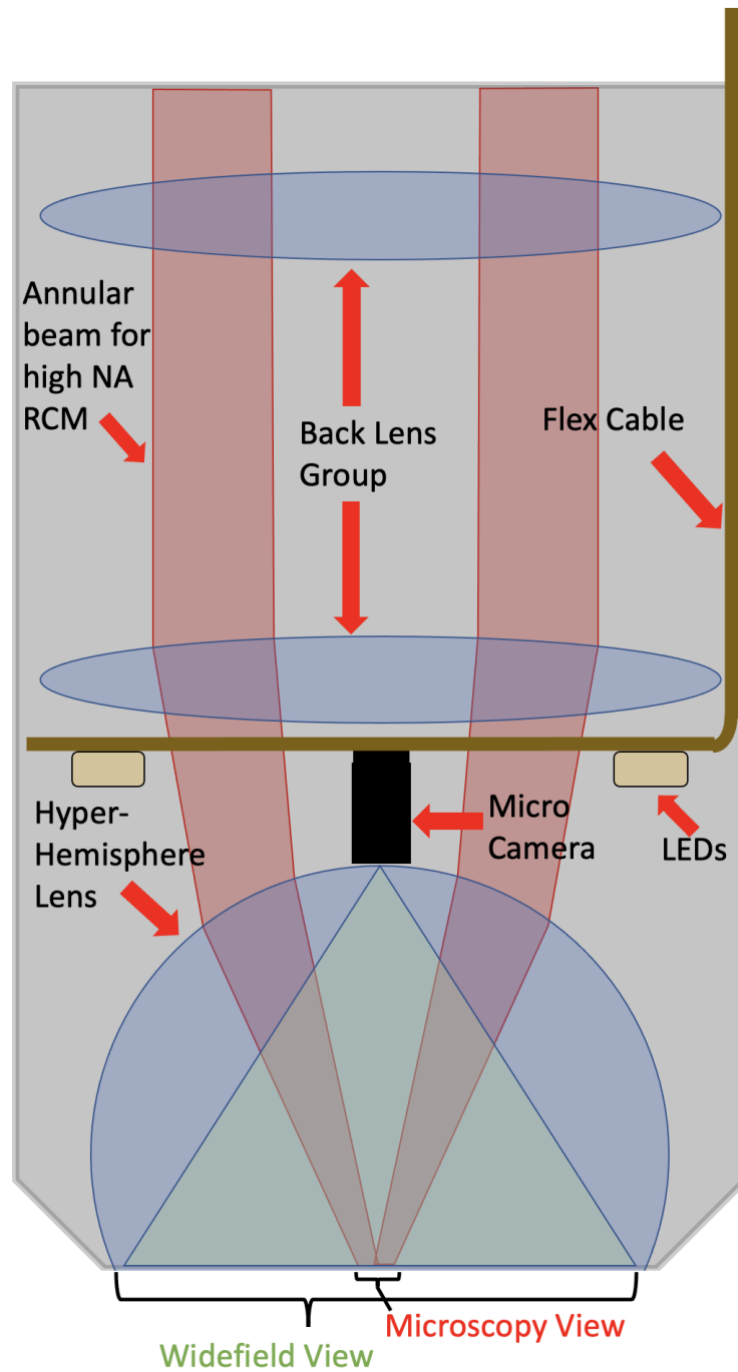


Figure 1.2. Side view of RCM objective lens with integrated micro-camera, not to scale.



Figure 1.3. Objective lens housing partially assembled with flex board and hyper-hemisphere from Photon Gear Inc.

CHAPTER 2

ENGINEERING AND DESIGN

Toward the development of a micro-camera integrated into the objective lens of an RCM imager for concurrent widefield registration, this chapter focuses on the engineering and design decisions regarding the camera and its illumination sources. The engineering design process has been employed to produce a system capable of providing real-time widefield images of the skin surface, allowing for precise analysis and diagnosis of skin lesions. The design section begins with the choice of camera and its characterization, illumination solution, flex board design, and prototyping of imaging probes. Next, an investigation of the effective number of bits to justify the need for 8-bit or 10-bit images. Then, the image acquisition process is described, and the image processing steps are detailed. Zemax simulations of the LEDs' non-uniform illumination are explained after the image processing. Finally, the potential of cross-polarized imaging is presented, and the polarization of the probes is described.

Micro-Camera

In the process of choosing a micro-camera for this project, some constraints were put in place. First was the size of the package. A smaller package size would mean a smaller obstruction in the RCM beam. Another constraint was the location of the best focus and its tolerance, which is constrained by the size of the hyper-hemisphere lens and path length to the skin. Since the hyper-hemisphere lens design by Photon Gear was not completed at this time, the hyper-hemisphere was estimated to have a radius of 3.5 mm and index of refraction of 1.5. This would mean, for a canonical hyper-hemisphere lens, the thickness would be approximately

$r \left(1 + \frac{1}{n}\right) = 5.8333 \text{ mm}$, so the depth of focus of the camera must account for this thickness, as illustrated in figure 2.1 below. A paraxial analysis of the camera's best focus is shown later in the Camera Characterization section. The number and size of the pixels to maximize the resolution was also a constraint. Early prototypes investigated the use of a custom lens to design for the ideal focus. The team elected to not pursue a custom lens. They also investigated a Bayer color mask versus a monochrome sensor with sequential red, green, and blue (RGB) illumination to create a color image. The goal being to increase the resolution with the monochrome sensor. Early work done by Jordan Anspach helped the team chose to not pursue the monochrome sensor with RGB illumination.

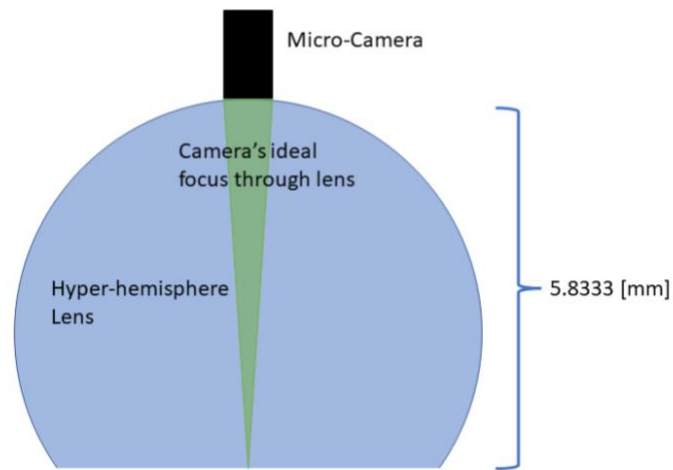


Figure 2.1. Illustration of the ideal focus of the camera through the hyper-hemisphere lens.

The choice of micro-camera was between the AMS NanEyeC Miniature Camera Module and the Omni Vision OV6946-RAJA Camera Cube Chip. These cameras had very similar physical dimensions, with the OV6946-RAJA being $1100 \times 1100 \times 2266 \mu\text{m}$ and the NanEyeC being $1040 \times 1040 \times 2162 \mu\text{m}$. Both cameras used a 4-pin interface with solder balls placed already in place. Both cameras could operate up to 10-bit images and video. However, the

OV6946-RAJA contained 400×400 , $1.75 \times 1.75 \mu\text{m}$ pixels, whereas the NanEyeC contained 320×320 , $2.4 \times 2.4 \mu\text{m}$ pixels. The NanEyeC had a higher frame rate of up to 58 fps at 320×320 resolution, whereas the OV6946-RAJA had a frame rate of 30 fps at 400×400 resolution. Each camera's sensor has a Bayer mask layered on top of it. This allows for interpolation to achieve a color image. A RAW output was desired for this camera, and since both cameras could output RAW images, neither had an advantage here. Both cameras have user interfaces that give the user control of basic parameters and can switch between capture modes. Both cameras require specific drivers from the manufacturer that must be analyzed and replicated by Caliber ID for integration into the HH-RCM system. The depth of focus of the OmniVision camera ranged from 5 [mm] to 50 [mm] in air, this range contains the estimated thickness of the hyper-hemisphere lens. After testing, the main difference that affected the camera's potential was the pixel count and size, which allowed for a higher-resolution image. Therefore, the Omni Vision OV6946-RAJA camera was chosen for this project.

Camera Characterization

The Omni Vision OVM6946-RAJA, color CMOS analog 160 Kpixel (400×400) Camera Cube Chip was chosen to be integrated into the HH-RCM. However, only a limited number of optical parameters for the camera were given, see table 1.1. Therefore, a more thorough characterization was required. For paraxial analysis, equations 1, 2, and 3 can be used. Equation 1 is the paraxial imaging equation that solves for the focal length given parameters S_1 and S_2 , and the indices of refraction of the mediums, n_1 and n_2 . An illustration of these parameters is shown in figure 2.2. Equations 2 and 3 are adaptations of equation 1 using the maximum and minimum depth of focus instead of the S_2 variable, for these equations the indices of refraction

are both equal to 1. From OmniVision, the focal length is known to be 0.418 mm, the maximum depth of focus is 50 mm, and the minimum depth of field is 5 mm, all values for in air. Equation 3 can be manipulated to solve for ΔS_1 , yielding ΔS_1 equal to 0.0173 mm. Then, solving equation 2 for S_1 and entering the calculated value for ΔS_1 calculated before, we get S_1 equal to 0.4388. Now, equation 1 can be solved for S_2 , entering the value calculated for S_1 and the given values, S_2 , the theoretical best focus in air was calculated to be 8.8068 mm.

$$\frac{1}{f} = \frac{n_1}{S_1} + \frac{n_2}{S_2} \quad (1)$$

$$\frac{1}{f} = \frac{1}{S_1 - \Delta S_1} + \frac{1}{\text{Depth of Focus}_{Max}} \quad (2)$$

$$\frac{1}{f} = \frac{1}{S_1 + \Delta S_1} + \frac{1}{\text{Depth of Focus}_{Min}} \quad (3)$$

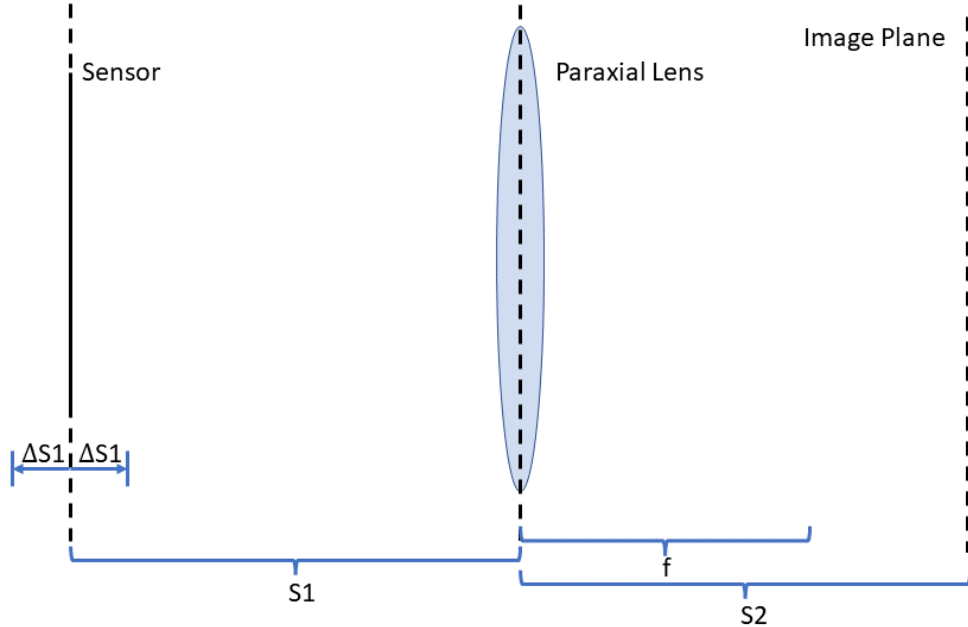


Figure 2.2. Visualization of paraxial parameters used in equations 1, 2 and 3.

Lens Parameter		Specification
Field-of-view (FOV)	Horizontal	85.7°
	Diagonal	120°
F-Number		5.0
Focal Length		0.418 mm
Optical Layout		3-Surface Lens
Maximum Image Circle		1.06 mm diameter
TV Distortion		< 11% @ 95% Field
Relative Illumination (Optical)		60.7% @ y = 0.53 mm
Focus	Mechanism	Fixed Focus
	Depth of Field	5 mm ~ 50 mm
IR Band Pass Filter 50% Cut-Off		665±10 nm

Table 1.1. Optical parameters for the OV6946-RAJA provided by Omni Vision.

Assuming the interface between the camera and the hyper-hemisphere is negligible, therefore ignoring the refraction from the curved surface of the hyper-hemisphere, equations 4, 5, and 6 can be used to calculate the best focus through the hyper-hemisphere, as well as the adjusted depth of focus. The value of n_2 is equal to 1.5 to approximate glass, S_2 is the calculated best focus in air, 8.8068 mm, and the depth of focus values are the same as before. The prime symbol refers to the values calculated through the glass. The new, best focus through the glass will become 5.850 mm, showing good agreement with the desired focus of the camera through the approximated hyper-hemisphere lens. The depth of focus imaging through the lens is 4.375 mm to 9.211 mm.

$$\frac{1}{S_2'} = \frac{1}{n_2} \left(\frac{1}{S_2} - \frac{1}{R} \right) + \frac{1}{R} \quad (4)$$

$$\frac{1}{\text{Depth of Focus}_{Max}'} = \frac{1}{n_2} \left(\frac{1}{\text{Depth of Focus}_{Max}} - \frac{1}{R} \right) + \frac{1}{R} \quad (5)$$

$$\frac{1}{\text{Depth of Focus}_{Min}'} = \frac{1}{n_2} \left(\frac{1}{\text{Depth of Focus}_{Min}} - \frac{1}{R} \right) + \frac{1}{R} \quad (6)$$

Several images were taken with the cameras at varying heights to verify the theoretical best focus calculated above. For this experiment we used the slanted edge method of calculating the modulation transfer function (MTF). MTF is a measure of the contrast of an image as a function of spatial frequency, and it quantifies how well the imaging system can distinguish and resolve fine details in an image. The slanted edge technique is a way to generate an oversampled edge response, which allows computation of the modulus of the optical transfer function (OTF) [107]. A more detailed explanation the MTF is described in a later section. A white Teflon sheet was used as a diffuser, and a microscope LED ring was placed behind the Teflon sheet as a backlight. The microscope LED ring was set to a brightness that did not overexpose the camera. A razor blade was used for the slanted edge. Other than the lens pre-built into the OVM6946-RAJA camera, no additional lens was used. Starting with the camera in contact with the razor blade, a micrometer incrementally adjusted the distance between the camera and the razor blade. Since the depth of field of the camera was given to be 5 mm to 50 mm by OmniVision, the first images were taken at a distance of 5 mm. Smaller increments were used between 7 mm and 10 mm, the distances close to the theoretical best focus. Fewer images were taken for values above 14 mm.

The captured images were then sent through the `sfrmat4.m` function from [108] to generate MTF plots. The calculated MTF curves are normalized to 1 at a frequency of 0 cycles/pixel, and decay as the spatial frequency is increased. A 50% loss of contrast was reached in the images when the frequency at which the spatial frequency response (SFR) reaches 0.5. This is generally a good value to distinguish between the various MTF curves. If the 50% SFR frequency is higher, then the image is more in focus, containing higher contrast. The 50% SFR

frequencies were obtained at each distance and compared from these plots. These plots did not significantly improve the MTF at the theoretical best focus; see figure 2.4. The highest 50% SFR frequencies ranged from 5 mm to 13 mm. The images taken at distances between 20 mm and 50 mm had consistently lower 50% SFR frequencies. However, the lowest 50% SFR frequency was only 2/3 of the maximum and occurred at a distance of 50 mm. Images captured at distances lower than 5 mm showed significant blurring and poor MTF curves. While these results do not accurately confirm the theoretical best focus, they prove that the depth of focus range provided by Omni Vision is accurate.

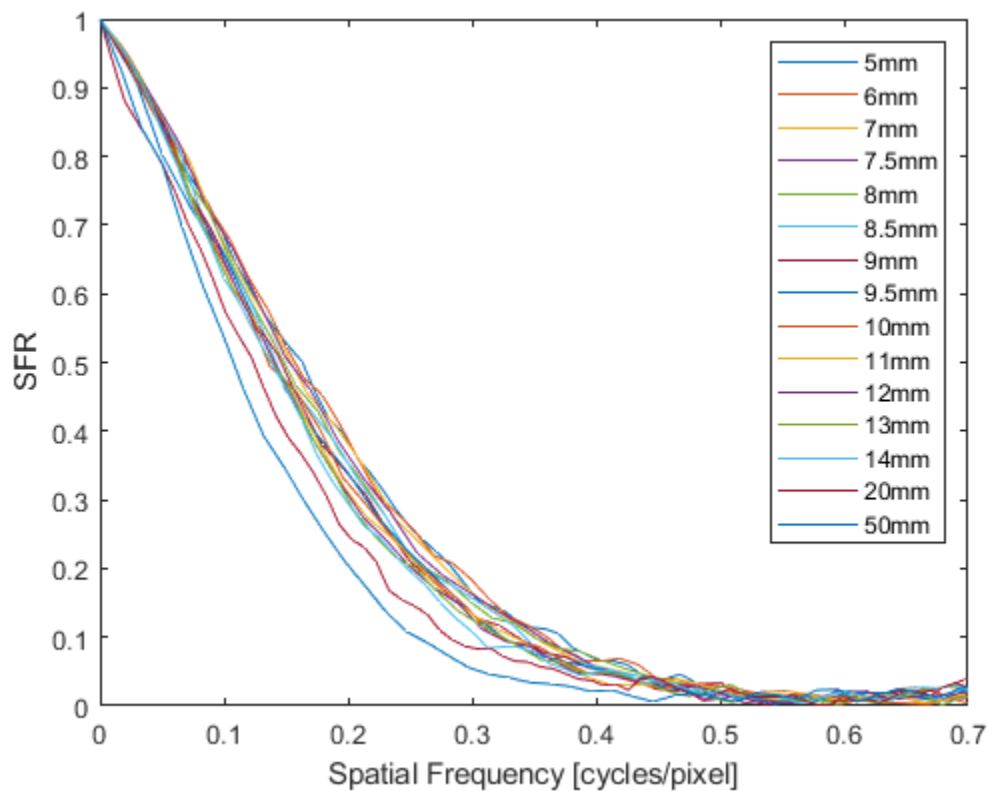


Figure 2.3. MTF curves for the OVM6946-RAJA camera with no external lens at varying heights from the target.

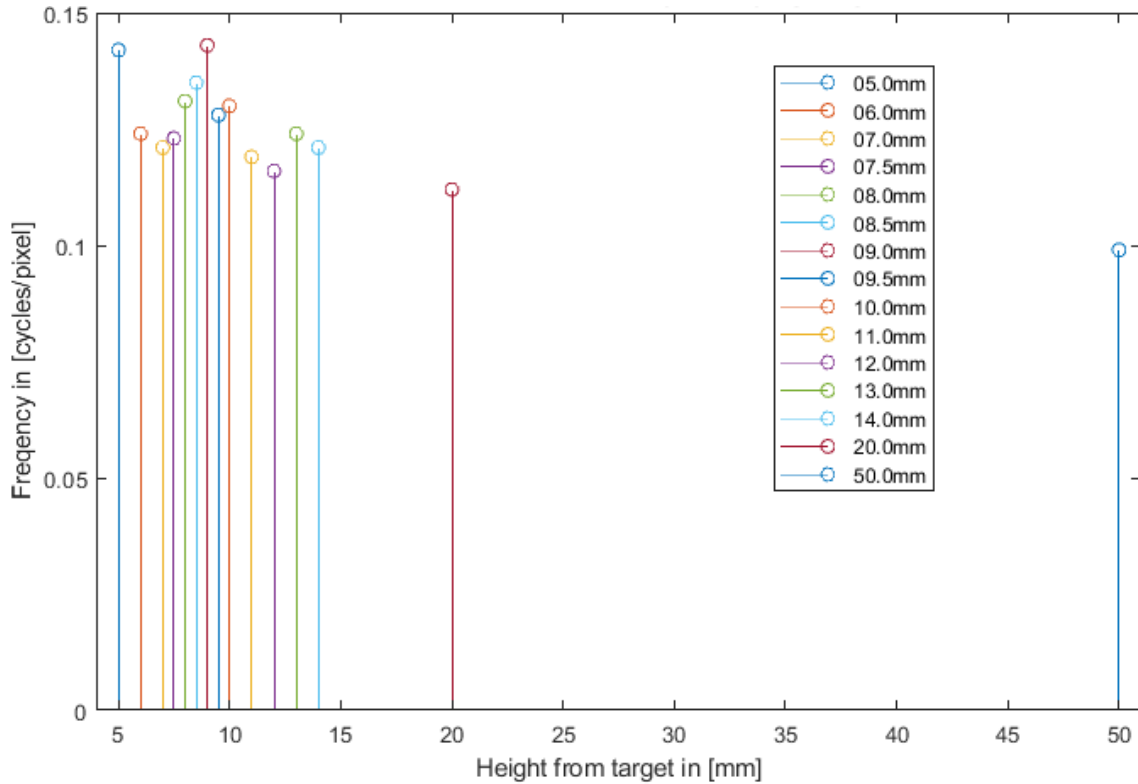


Figure 2.4. 50% MTF Frequency at varying heights from the target.

Illumination Solution

Multiple methods for illuminating the skin for the camera were investigated in early stages of the project. One of the initial methods was using four LEDs pressed to hyper-hemisphere lens. Having the LEDs pressed to the hyper-hemisphere lens produced hot spots where the image was saturated, and this design would likely obstruct the RCM's beam too much. The main constraint was that the LEDs must not impede the RCM imaging beam cone. Within this constraint, it was found that placing the LEDs on a ring, located on the inner edge of the objective housing allowed for some flexibility when considering the height above the hyper-hemisphere and number of LEDs as variables. The ring design helped to remove the obstruction from the RCM's beam's path. Placing the LED ring near the plane of the back of the camera

produced good illumination and removed direct reflections from the top surface of the hyper-hemisphere. After testing different numbers of LEDs, ranging from four to sixteen, a ring of eight LEDs was chosen, and illustrated in figure 2.5. Below is an image taken on a white background of the illumination pattern from eight Inolux IN-S42AT white LEDs on a ring, imaged through a hyper-hemisphere approximation. This pattern shown has the highest intensity in the center of the image and decreasing intensity moving out. Zemax simulations of the LED illumination profile and high-level schematic are included below. The Zemax simulations showed good agreement with the images captured in the lab.

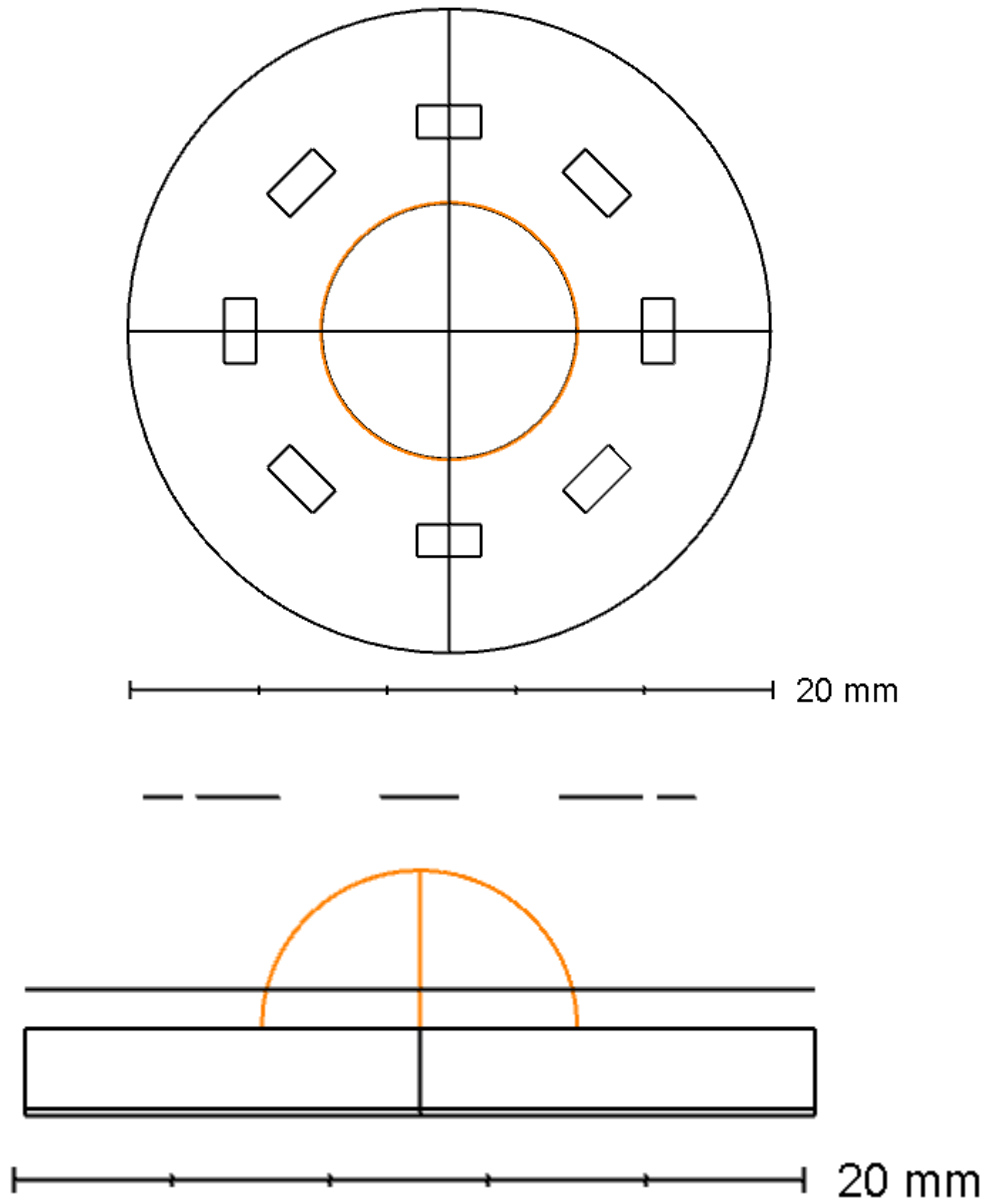


Figure 2.5. Top down and side view of the LED positioning (eight rectangles forming a ring), relative to the mock hyper-hemisphere lens (highlighted orange). The camera is not shown in these illustrations.

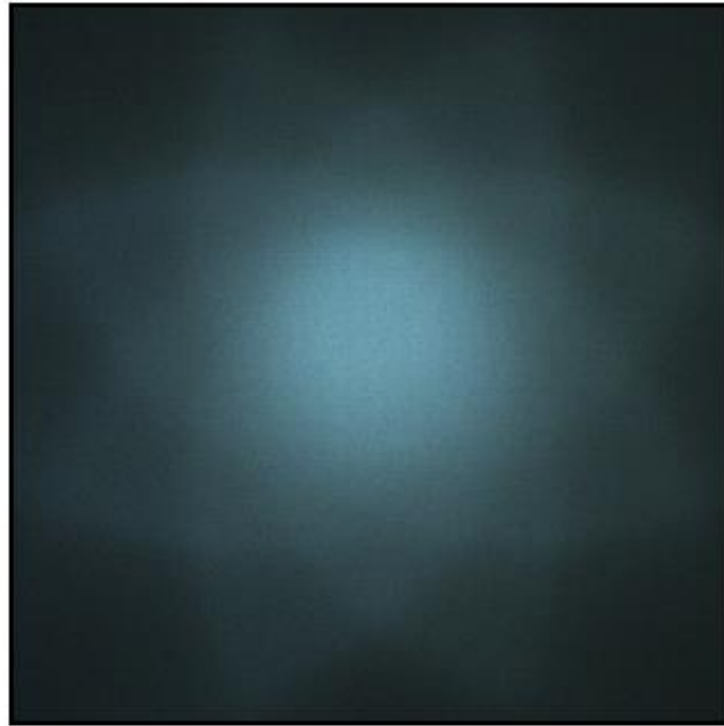


Figure 2.6. Image of the illumination scheme based on eight LEDs on a ring.

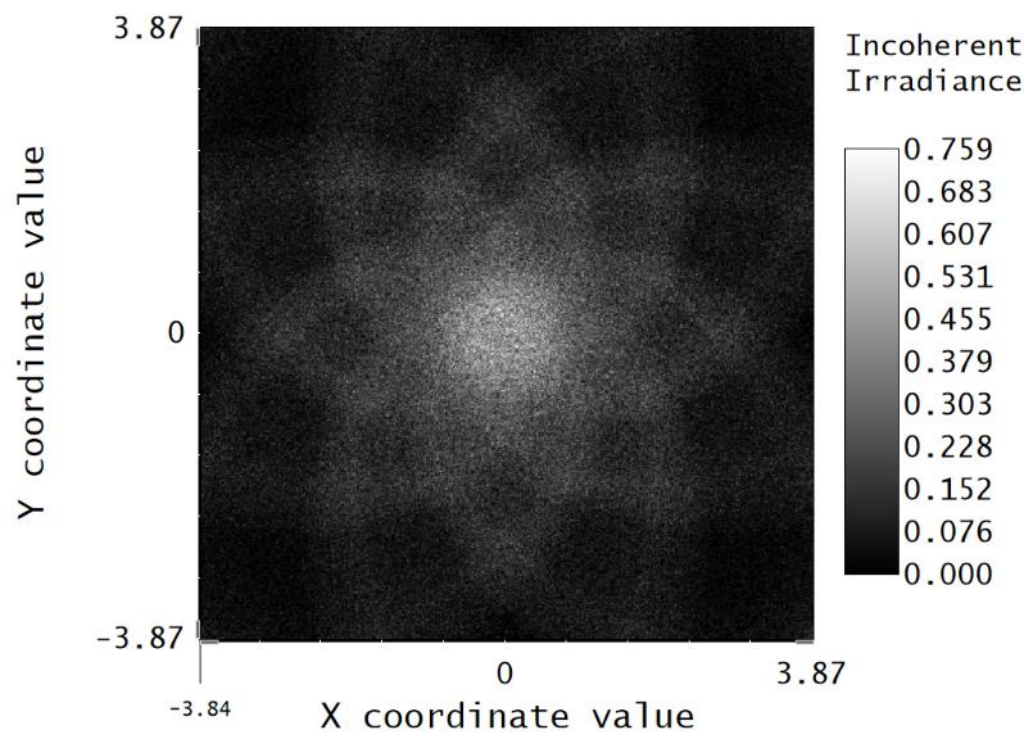


Figure 2.7. Zemax simulation of LED illumination pattern.

From the image captured with a prototype probe and the Zemax simulation, a distinct illumination pattern is observed. The overlap between illumination spots from neighboring LEDs on the ring cause the “flower petals” to form in the image. The non-uniformity of this pattern is shown to be reduced in the Black-Background Subtraction and White-Balance Normalization section of this thesis.

Flex Board

With the OV6946-RAJA camera and LEDs chosen for imaging and illumination, a method to electrically connect and secure them within the objective lens was needed. Initial prototypes glued the camera directly to the hyper-hemisphere with a micro ribbon cable used for the electrical connection. However, the LED illumination solution indicates that a ring of LEDs location approximately at the plane where the back of the camera should sit is near optimal. This suggests that a single flex printed circuit board (PCB) could be used to hold both the camera and LEDs. Furthermore, this flex circuit could be supported at its perimeter by the objective housing, so that no extra assembly step of gluing and aligning of the camera to the hyper-hemisphere is required.

A flex board purchased from PCBWay was chosen for mounting the LEDs and camera. It is a flexible printed circuit board made up of a ring with a bridge across the middle and a 42×3.5 mm flexible tail where the electronic interface is located. The ring’s outer diameter is 13 mm, and the inner diameter is 9.76 mm. The diameter of the flex board in the objective lens assembly developed by Photon gear is larger than this prototype’s (15 mm outer diameter and 13.5 mm inner diameter), consequences of this are discussed in a later chapter. The width of the ring is 1.62 mm and bridge is 1.52 mm thick to accommodate the width of the LEDs and camera

module. Eight LEDs are mounted at equidistant spacing around the flex ring for illumination. At the center of the bridge, centered in the ring, are four pads where the camera is assembled. This will align the camera with the center of the objective lens of the RCM imager. The flexible bridge helps to take pressure off the camera if it is pressed to the lens too hard. The ring with a bridge design was chosen because it allows most of the RCM's scanned laser beam to pass through the objective and still have the camera centered in the objective lens. Photon Gear Inc. and Caliber ID worked closely to redesign the objective lens to compensate for the obstruction that changes the full pupil into a divided pupil. Thus, the bridge does not significantly degrade the RCM optical sectioning.

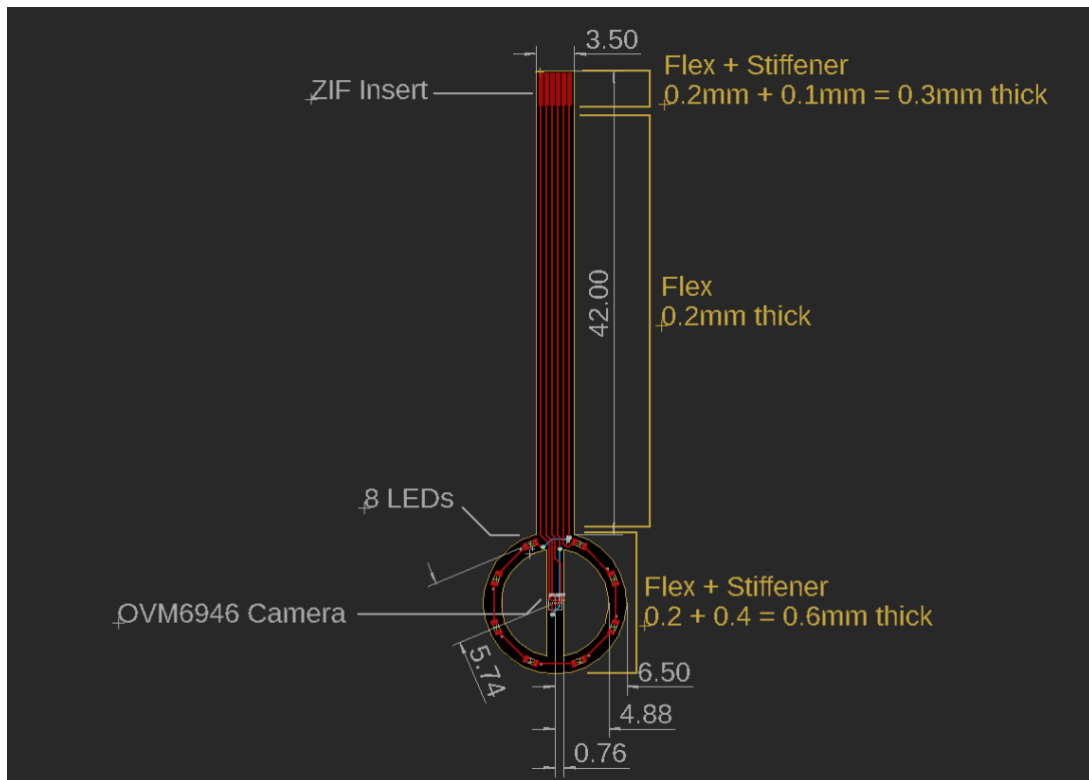


Figure 2.8. Mechanical drawing of flex board

Probe Prototype and Board Assembly

Prior to receiving an objective lens assembly from Caliber ID and Photon Gear Inc, A prototype probe with the camera, LED illumination, and hyper-hemisphere lens needed to be built. The four primary components of the imaging system being integrated into the objective of the RCM are the: OV6946-RAJA camera from Omni Vision, a hyper-hemisphere lens, eight Inolux IN-S42AT white LEDs for illumination, and the flex board to mount the camera and LEDs. The same camera and LEDs were adopted for inclusion in the Photon Gear objective lens assembly. The first step in populating the prototype flex board was cleaning and drying a flex board using isopropanol and air drying. A stencil purchased with the flex board was used to apply solder paste for eight LEDs. Then, using a benchtop microscope, if necessary, the eight LEDs were set and aligned on the flex board, using the solder paste to hold them to the pads before reflowing. Next, a small amount of tacky solder flux was placed on the four pads at the center of the bridge for the OV6946-RAJA on the flex board. The OV6946-RAJA was then placed onto the flex board, and the flux helped to keep it in place. The OV6946-RAJA comes with solder balls on the camera's pads for self-assembly. When the solder reflows, the OV6946-RAJA self-assembles, aligning to the pad on the flex board. A toaster oven with an external digital thermometer was set to 245° C, and the flex board was placed inside the toaster oven. Once the temperature reached 245° C, the solder reflowed, and the oven was turned off and allowed to cool slowly. The door to the toaster oven was left shut to allow for a more gradual cooling to help avoid temperature shock on the components. Once the flex board cooled to room temperature, the flex board was removed from the toaster oven and was tested. A Ziff connector connects to the end of the flex board's cable and the OmniVision OH0093 ISP board, which

connects to the computer. Once connected, using the OVTMedical desktop application, if the camera was assembled correctly, the computer will deliver an indicator signal, and a live video stream can be seen. The LEDs are connected to the LED driver and tested simultaneously with the camera. Epoxy was added around the camera's base to reinforce the camera onto the flex board. Adding the epoxy was a preventative step to protect the solder connections from breaking if the camera was touched too aggressively.

The hyper-hemisphere lens was approximated with an 8 mm diameter half-ball N-BK7 lens and 10×10×2 mm square N-BK7 window from Edmund Optics. The half-ball lens and square window were glued together using the Norland Products Inc. NOA 61 optical adhesive. A 3D-printed objective lens mount was created to hold the mock hyper-hemisphere lens. A 3D-printed post was made to mount the flex ring and allow for camera height adjustment relative to the hyper-hemisphere lens when assembled. The post with the flex ring was inserted into a brass tube that protects the cabling and holds the assembly together. The objective fits over the brass tube and was held in place using set screws. Once the objective lens was set and the post with the flex ring was placed inside the brass tube, a micrometer moved the post, so the camera came into contact with the hyper-hemisphere lens. A live video feed was used for this process since the brass tube blocks a direct view of the camera and lens. In the live video stream, as the camera was brought into contact with the hyper-hemisphere lens, bright reflections of the LEDs off the surface of the hyper-hemisphere were moved out from the center of the camera's field-of-view and eventually completely out of the field-of-view. When the LED surface reflections were pushed out of the frame, the camera and lens could be assumed to be in contact.

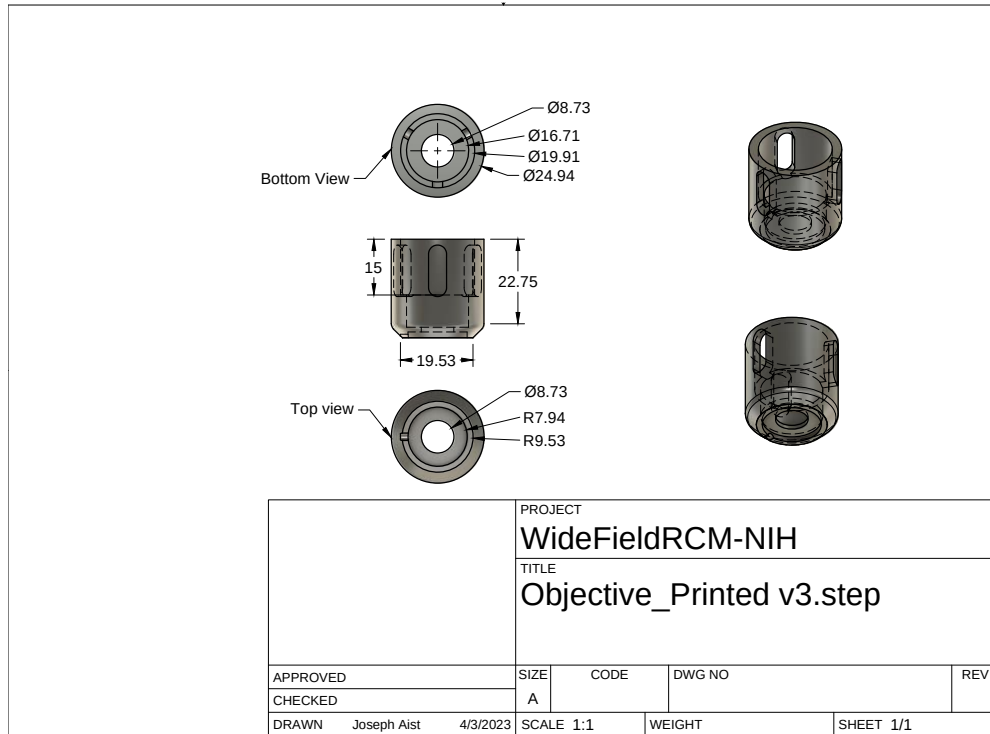


Figure 2.9. Mechanical drawing of prototype objective lens housing for the prototype probes.

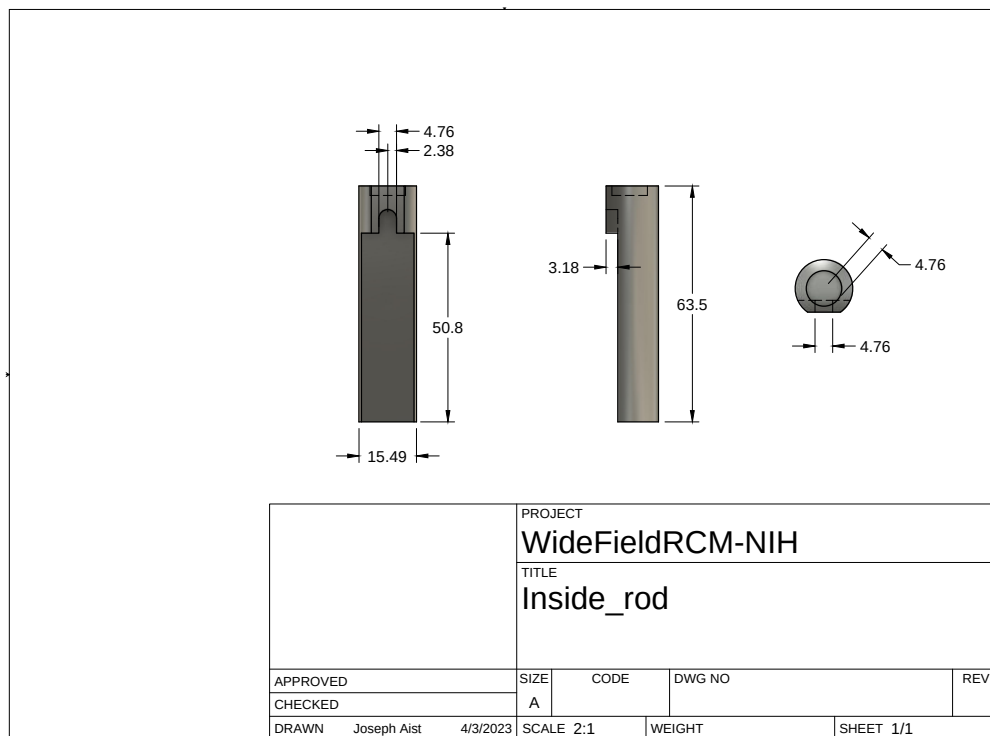


Figure 2.10. Mechanical drawing of the inside rod of the prototype probes.

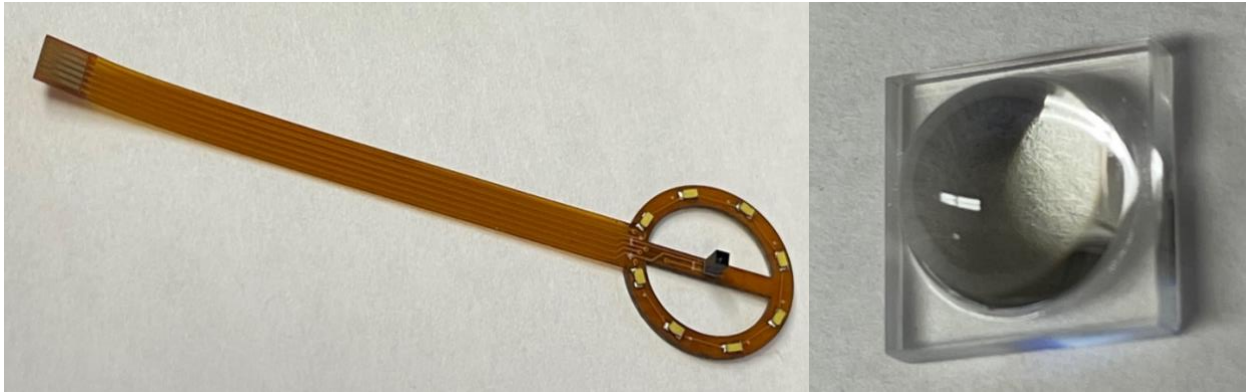


Figure 2.11. Prototype Flex Ring Board with eight IN-S42AT LEDs and OV6946-RAJA camera soldered to it (left). Half-ball and window approximating a hyper-hemisphere lens (right).



Figure 2.12. Populated flex board mounted to the adjustable 3D printed post inside a brass protective enclosure (left). Hyper-hemisphere approximation mounted to 3D printed objective and attached to the brass protective enclosure for imaging (right).

Effective Number of Bits

When using Omni Vision's OV6946 camera with the OH0093 image signal processing (ISP) board through the OvtMedical application, RAW images can be captured and saved as an 8-bit or 10-bit image. This section investigates the potential for additional information present in

the addition of 2-bits of data. Every additional bit doubles the number of values per pixel. So, a single-bit image is represented by ones or zeros, two distinct values. With 2-bits, there are four distinct values, and so on. The 8-bit data was saved as a bitmap (BMP) file. The 10-bit data can be saved in a least-significant-bit (LSB) or most-significant-bit (MSB) form with the respective file extensions ‘.lsb_raw’ and ‘.msb_raw.’ The LSB and MSB files independently contain all 10-bits of data. The data for each pixel in the LSB and MSB images are 2-bytes, following the convention shown below. The analysis of these images was conducted in MATLAB.

$$\text{MSB: } b_9 \ b_8 \ b_7 \ b_6 \ b_5 \ b_4 \ b_3 \ b_2 \ b_1 \quad b_1 \ b_0 \ 0 \ 0 \ 0 \ 0 \ 0 \ 0 \quad (7)$$

$$\text{LSB: } b_7 \ b_6 \ b_5 \ b_4 \ b_3 \ b_2 \ b_1 \ b_0 \quad 0 \ 0 \ 0 \ 0 \ 0 \ 0 \ b_9 \ b_8 \quad (8)$$

The first step is to import the images into MATLAB correctly. The BMP image could be imported using the ‘fread’ or ‘imread’ commands. The image will be loaded as a $400 \times 400 \times 3$ Uint8 image. The image type is RAW and, therefore, grayscale, with each of the three 400×400 arrays identical. Two 400×400 arrays could then be removed, leaving only a single 400×400 Uint8 array representing the image. The LSB image could be imported using the ‘fread’ command with the following preferences: ‘fread(fid, [400×400], ‘uint16’, ‘ieee-le’).’ This command loaded a 400×400 image with values ranging from 0 to $2^{10} - 1$, 1023, read using the least-significant-bit convention. MATLAB does not support a Uint10 image type, so it must be displayed as a double or a Uint16 image. The MSB image can be imported using the ‘fread’ command with the following preferences: ‘fread(fid, [400×400], ‘uint16’, ‘ieee-be’).’ The only difference from the LSB’s command is the most-significant-bit convention.

For the analysis, images containing a repeated pattern, such as the repeating dots of a distortion target shown in figure 2.13, were used. Note, for all the images used in these tests, the maximum pixel values kept around 95% of the maximum pixel value to avoid saturation but still give a full dynamic range. Once the images were loaded, a 2D Fourier transform of the image could be performed to observe any prominent patterns in the Fourier domain. Then, in an iterative process, a bit-shift was applied to the images, replacing the shifted bit with a 0, effectively removing a single bit of data. As the bits were shifted, the images started to lose information, and eventually, only noise remained, or the images would have no information remaining. For example, with an 8-bit image, after 8 bit-shifts, the image would contain no information about the original scene, and every pixel value will be 0. Or, if there was a noise floor at some bit value, once the data for that pixel was shifted by that value, only noise would remain. Another way to think about this is if noise dominates before all the bits are switched to 0, the effective number of bits would be less than the actual number of bits. It should be noted that the MSB images ranged to 2^{16} because of how MATLAB reads in the MSB, but they still only have 10-bits worth of data because the 0 values fill the last six placeholders.

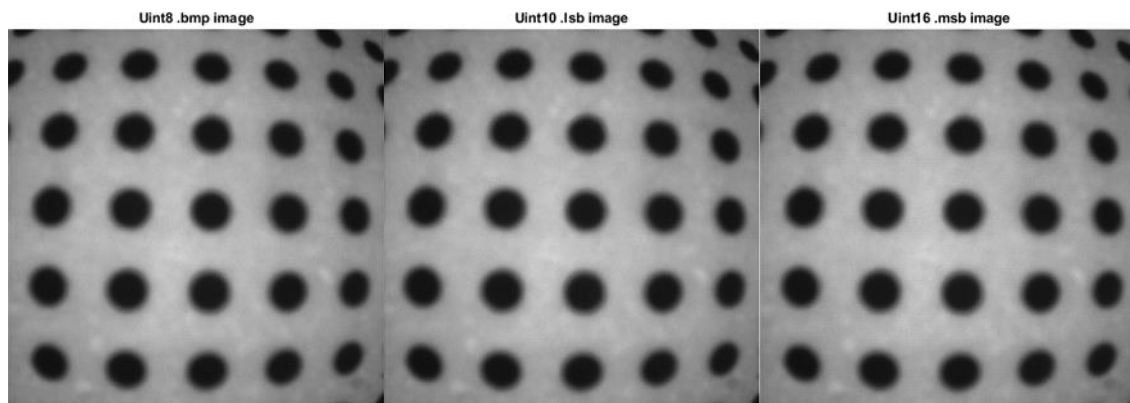


Figure 2.13. Image of repeating pattern on a distortion target. Uint8 means the pixel values range from $0-2^8$. Uint10 pixels range from $0-2^{10}$. Uint16 pixels range from $0-2^{16}$.

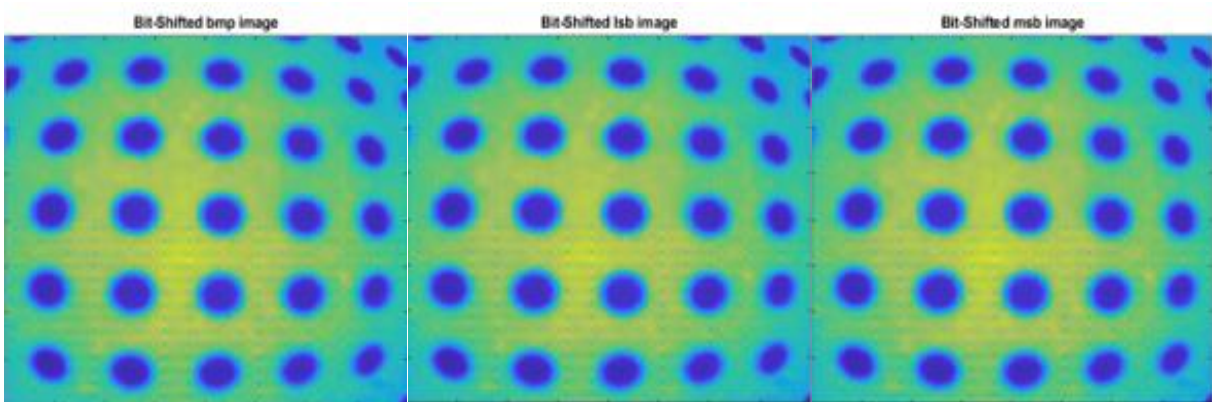


Figure 2.14 Using the `imagesc` command in MATLAB shows the scaled images prior to a bit-shift.

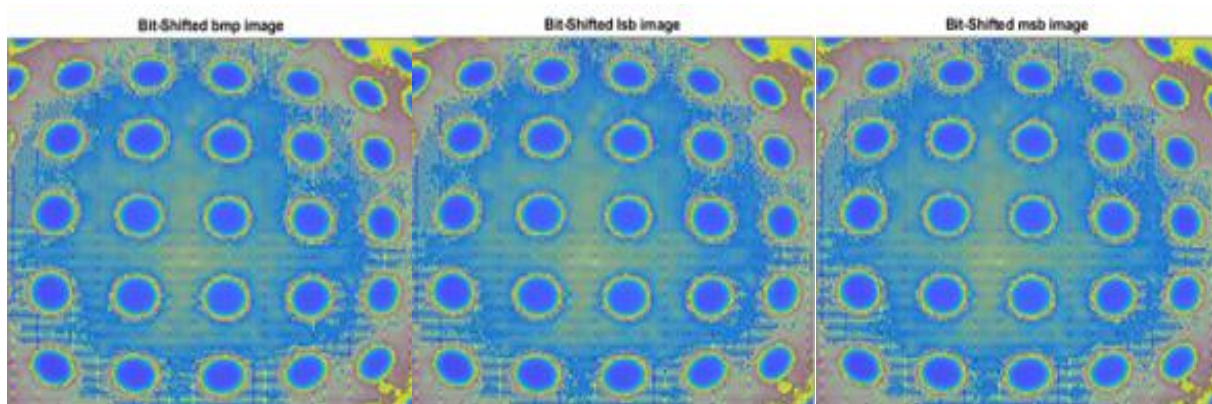


Figure 2.15. Scaled images after a bit-shift of 1.

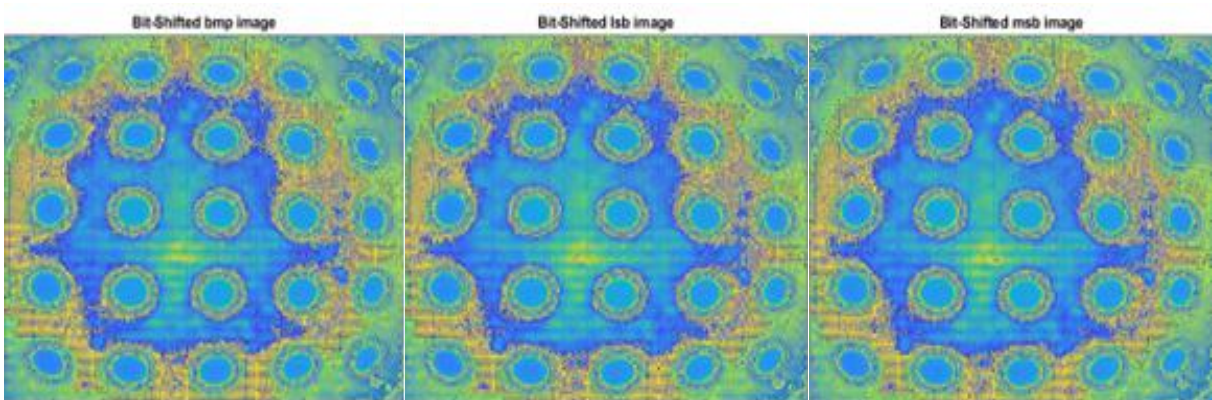


Figure 2.16. Scaled images after a bit-shift of 2.

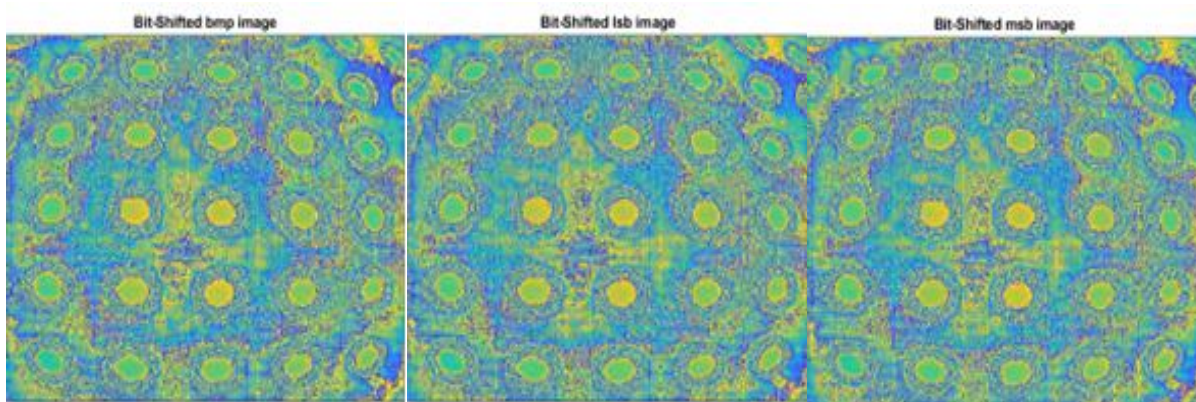


Figure 2.17. Scaled images after a bit-shift of 3.

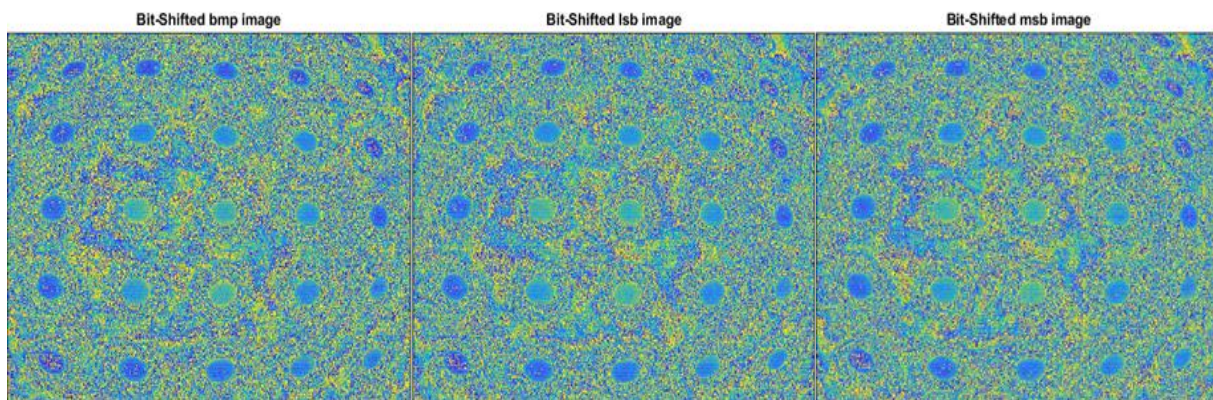


Figure 2.18. Scaled images after a bit-shift of 4.

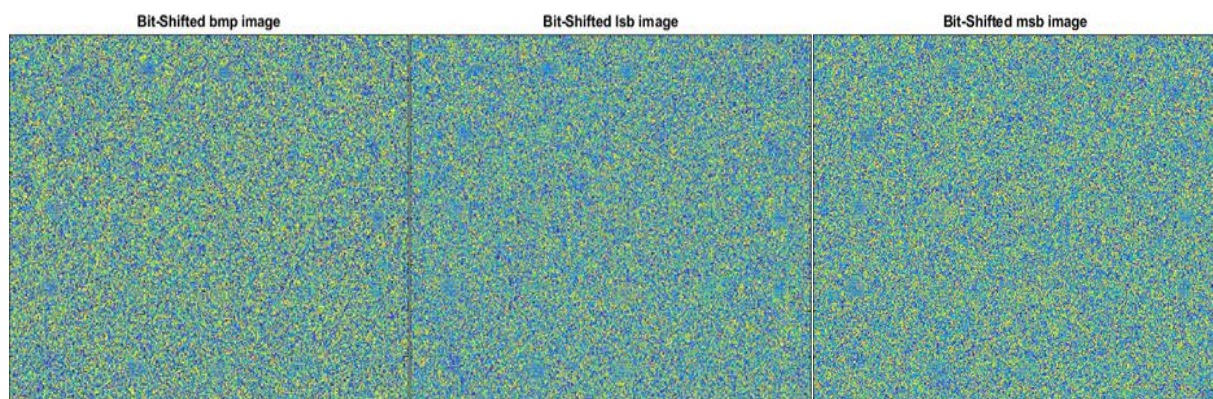


Figure 2.19. Scaled images after a bit-shift of 5.

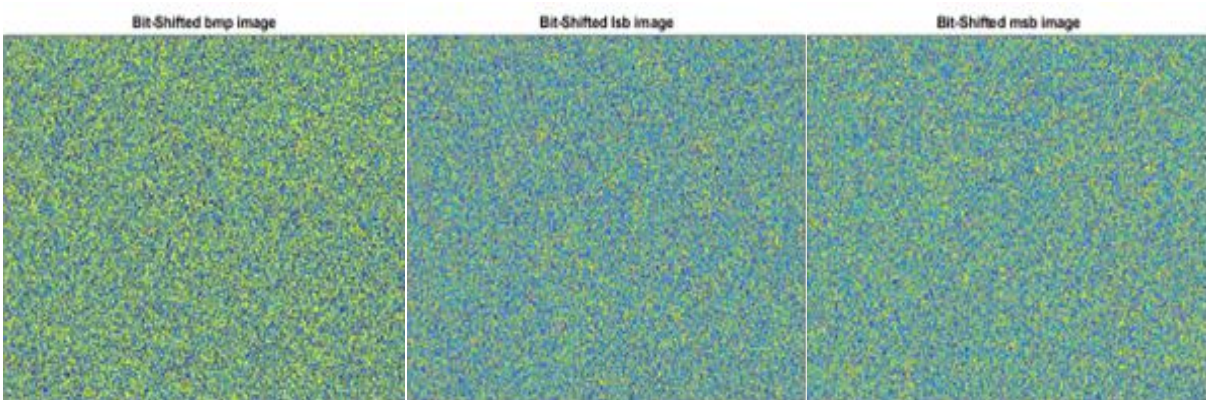


Figure 2.20. Scaled images after a bit-shift of 6.

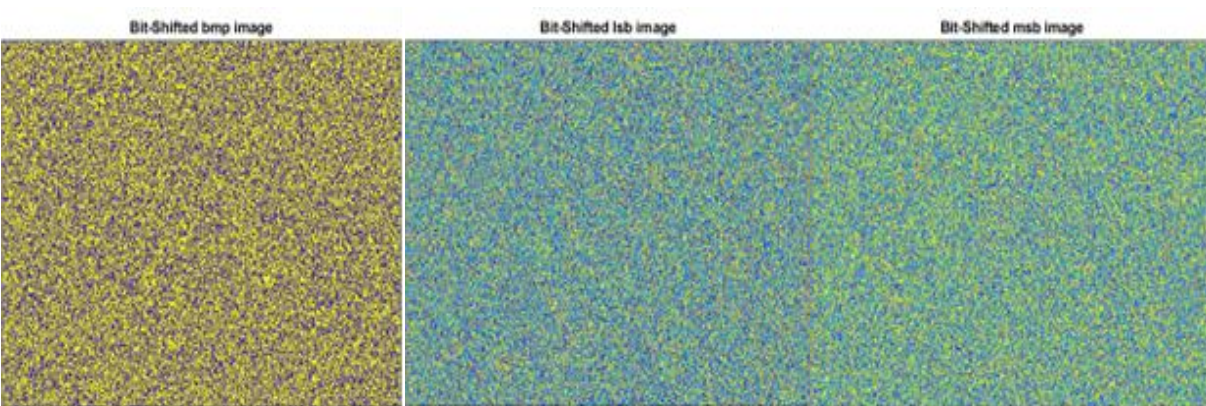


Figure 2.21. Scaled images after a bit-shift of 7.

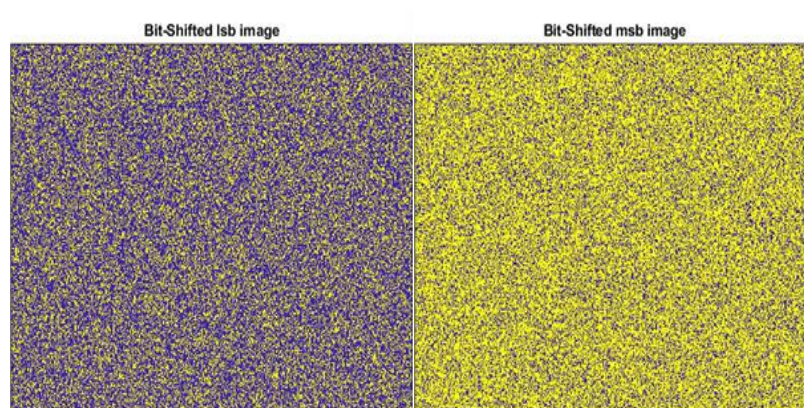


Figure 2.22. Scaled images after a bit-shift of 9.

Figures 2.14 through 2.22 show the degradation of the images as bits of information were removed. As each bit was removed, the range of values represented in the image is reduced.

Once a bit-shift of 8 was completed, the BMP image contained no information. From a cursory observation of figures 2.19 and 2.20, while the BMP image contained less information, the section of the pattern that could be observed with the human eye was the same as the LSB and MSB images. After figure 2.19, corresponding to a bit-shift of 5, it was difficult to see the dots of the distortion target in all three images. Figures 2.20 through 2.22 are essentially just noise. To confirm this, the Fourier transform of these images with the DC component removed could be observed.

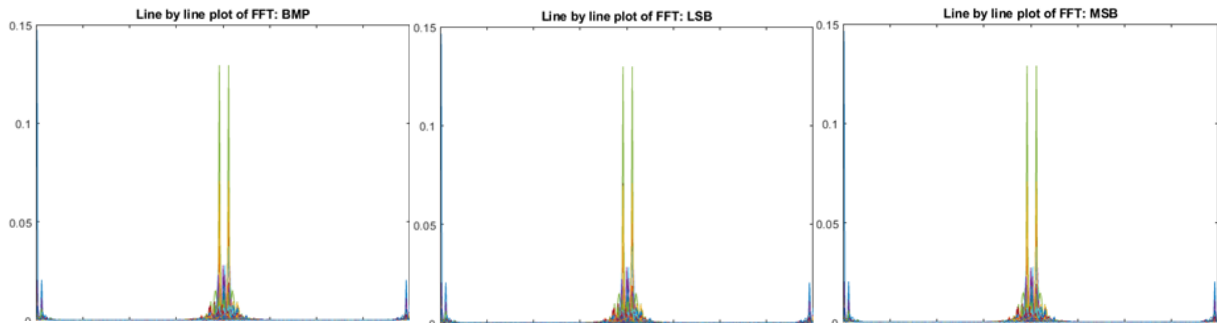


Figure 2.23. Column-by-column plot of the 2-dimensional Fourier transform of the original image with the zero-frequency component removed.

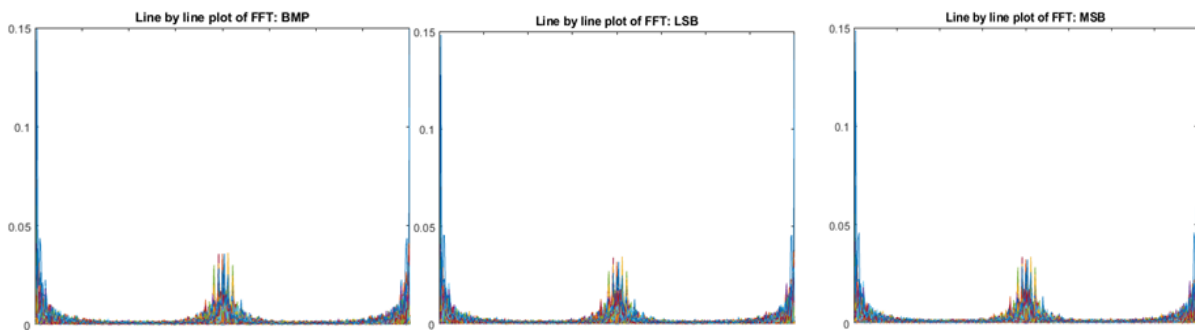


Figure 2.24. Column-by-column plot of the 2-dimensional Fourier transform of the image after a bit-shift of 1.

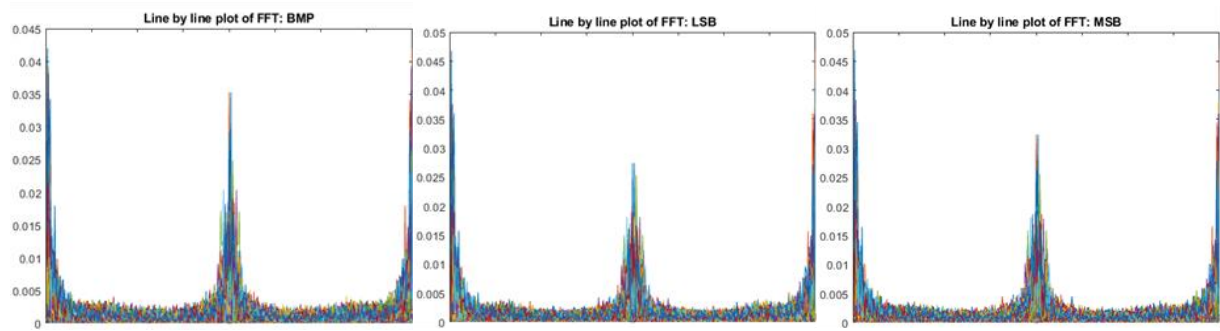


Figure 2.25. Column-by-column plot of the 2-dimensional Fourier transform of the image after a bit-shift of 2.

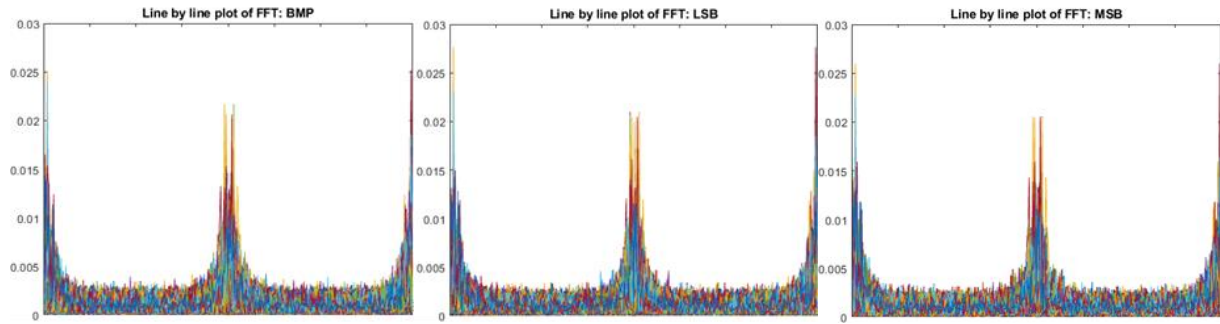


Figure 2.26. Column-by-column plot of the 2-dimensional Fourier transform of the image after a bit-shift of 3.

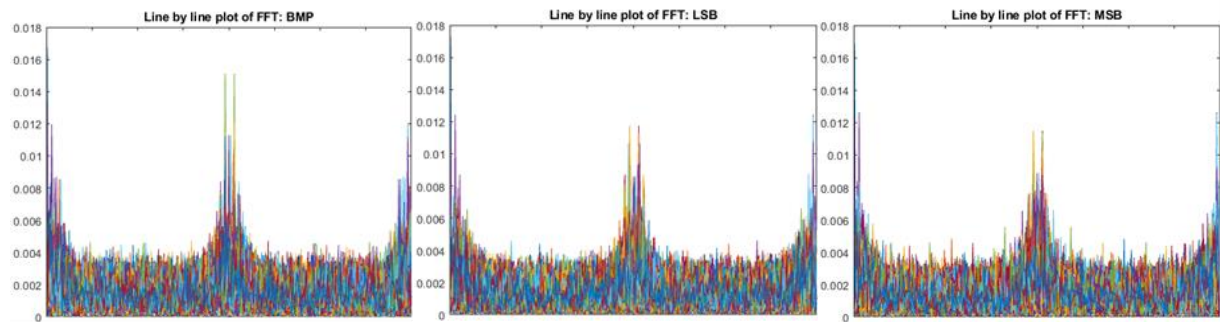


Figure 2.27. Column-by-column plot of the 2-dimensional Fourier transform of the image after a bit-shift of 4.

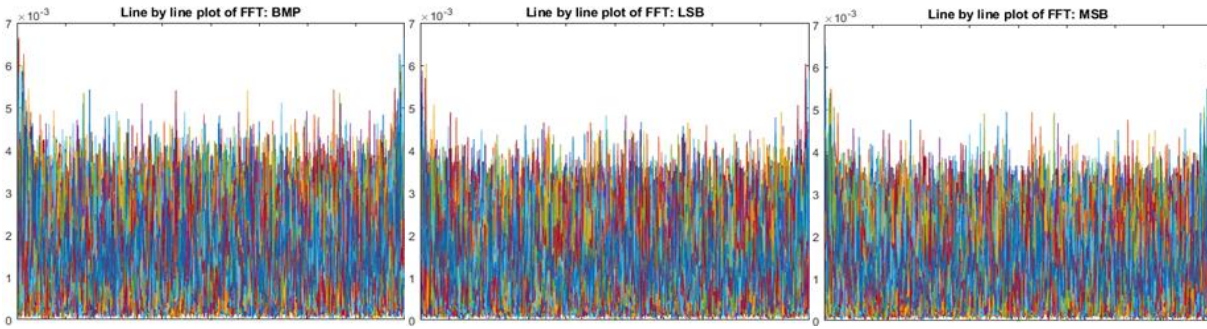


Figure 2.28. Column-by-column plot of the 2-dimensional Fourier transform of the image after a bit-shift of 5.

Figures 2.23 through 2.28 show column-by-column plots of the 2-dimensional Fourier transform of the images as bits were removed in each new figure. Once again, there was no significant difference in the apparent features in each plot. The loss in information from the fourth to fifth bit removed confirmed what was observed in the scaled images above. As information was lost, the frequency components corresponding to the dots pattern on the distortion target disappeared, and noise dominated. Note that the DC component of these Fourier transforms was removed to better see the frequency components of the dot pattern. The DC component persists through all the bit-shifts. The images used for the scaled images and Fourier transforms thus far were without the objective lens, index-matching medium, or LED illumination. To better mimic the environment this camera would be used in, a lower illumination scene using LEDs with the objective lens in contact with the target using an index-matching oil was used.

Figures A.1 through A.5 found in appendix A represent a potentially more accurate scene to the images that the camera will be captured in a clinical setting with lower and non-uniform illumination from LEDs that produce a star-like artifact. Also, these images had a higher light intensity in the center of the image with a low light intensity perimeter. And in these images,

after the fifth bit-shift, all the images seem almost entirely dominated by noise, yet the distortion target's pattern is still distinguishable. The dot pattern can barely be made out in the images with 6 bit-shifts, and the Fourier transform loses the nodes their pattern should introduce, meaning that the noise is dominating the image. No more relevant data was left in the image. Therefore, even with 4-bits remaining for the LSB and MSB images, the features were approximately as visible in the remaining 2-bits of the BMP image.

From the analysis of the images presented in the report and others, the additional 2-bits of information in the LSB and MSB images did not contain more information and detail than the 8-bits of data per pixel in the BMP images but rather were dominated by noise. A dark current likely caused this noise. When images were taken of a black background with no illumination, values up between 6 and 9, 3- to 4-bits of data for the 8-bits images, and values between 30 and 33, 5- to 6-bits of data for the 10-bit images were measured. This means that when the camera operates in its most efficient state, about 5-bits of data were useful. The agreement of the number of useful bits from both analysis methods provided the evidence to claim the effective number of bits for the camera, regardless of if it was 8-bits or 10-bits, was between 4- and 5-bits. Therefore, the additional byte required for 10-bit images was unnecessary for this project. A single byte containing 8-bits of data per pixel should be adequate.

Image Processing

Image Acquisition for Testing

Images were captured from the Omni Vision 6946-RAJA camera using OmniVision's OvtMedical desktop application. The application used the Omni Vision 0093 Image Signal Processing (ISP) board to interface with the computer and process the images. The OVTMedical

application was operated with the camera in a 'RAW Video Mode' to capture RAW $400 \times 400 \times 3$ pixel images in a uint-8 bitmap (.bmp) file type. The 0093 ISP board allowed for 10-bit image and video acquisition with an LSB or MSB format. However, testing found a noise floor, leaving about 5-bits of data containing useful information about the images; see the Effective Number of Bits section above. 10-bit data also required storing two bytes, whereas 8-bit data only required a single byte. Therefore, only using the 8-bit data helped save memory space filled by unusable noise. Using the RAW mode eliminated any non-linear processing steps computed on the 0093 ISP board, allowing for a more accurate characterization of the camera and its image performance. The image file is saved from the OvtMedical application as a $400 \times 400 \times 3$ uint-8 bitmap file, where each of the three-color channels contained identical values ranging from 0 to 255, yielding a RAW, grayscale image. Since the three channels were identical, two of them could be removed without a loss of information. A RAW image at this stage of development gives maximum flexibility to test algorithms for removing artifacts from non-uniform illumination, demosaicing of the Bayer mask pattern, and color correction methods. If Caliber ID were to create its own interface board for the integrated widefield camera, then the hardware would likely be digitizing RAW data before applying algorithms to perform demosaicing, image normalization, and color correction.

Black-Background Subtraction and White-Balance Normalization

The first step in post-processing the images is a black-background subtraction and white-balancing to normalize the illumination profile across the image. The images used for the black-background subtraction and white-balancing were acquired before the images used for dermoscopy. This is because the intensity of the LED ring must not saturate the white-balance

images. Saturation is when the light's intensity exceeds the sensor's dynamic range. For the most efficient normalization, the white-balance image should have pixel values greater than or equal to the brightest pixel value on the dermoscopic image. Often, the skin dermoscopy images were darker due to the melanin in the skin, and if the brightness is adjusted for these, it would likely saturate the white-balance images. The white balance images were taken by placing the probe in contact with a white material such as Teflon or Spectralon (materials with similar scattering patterns to human skin, examples of various materials in figure 2.30), with mineral oil (or another substance with similar properties) as an index-matching medium. The LEDs' brightness was adjusted so that no part of the image was saturated, limiting the values to 254 in the image. The target range for maximum pixel values is around 95% of the max possible value, 255. The black-background image was taken without illumination from the environment or the probe. There are non-zero pixel values when an image was taken with no illumination; these values were attributed to the dark current noise of the camera, which is related to the effective bit depth described earlier, shown in figure 2.29. The black-background subtraction removed the dark current noise from the images. Once a dermoscopy image was captured, the following equation was applied pixel-by-pixel to the captured dermoscopy image using a MATLAB algorithm.

$$\begin{aligned} \text{Normalized Image} \\ = \frac{(\text{Dermoscopy Image}) - (\text{BlackBackground})}{(\text{WhiteBalance}) - (\text{BlackBackground})} (255) \end{aligned} \quad (9)$$

Multiplying by 255 after the normalization converts the image to a uint8 scale. The black-background and white-balance normalization was the first stage of image correction, for it helped make the illumination profile across the image more consistent and removed artifacts

from the LED and lens system. Figure 2.31 shows the correction when applied to the same white-balance image. Figure 2.32 shows the correction when applied to a skin image.

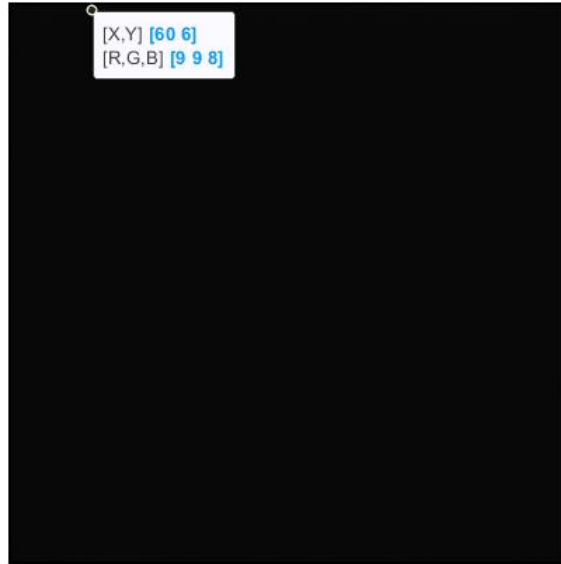


Figure 2.29. Interpolated black-background image showing non-zero dark current values corresponding to 3- and 4- bits of data.

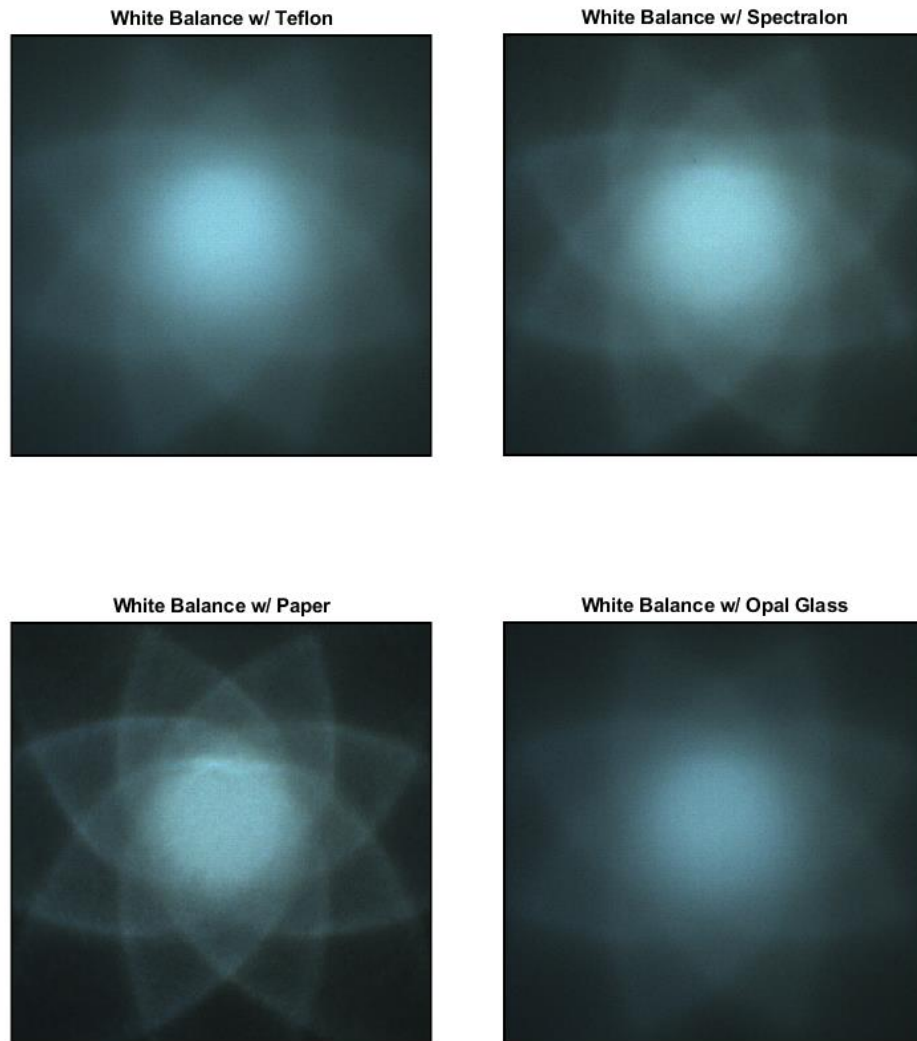


Figure 2.30. Interpolated white-balance images using various white materials with the same illumination. Top left: Teflon. Top right: Spectralon. Bottom left: USAF Resolution Paper Target. Bottom right: Opal Glass.

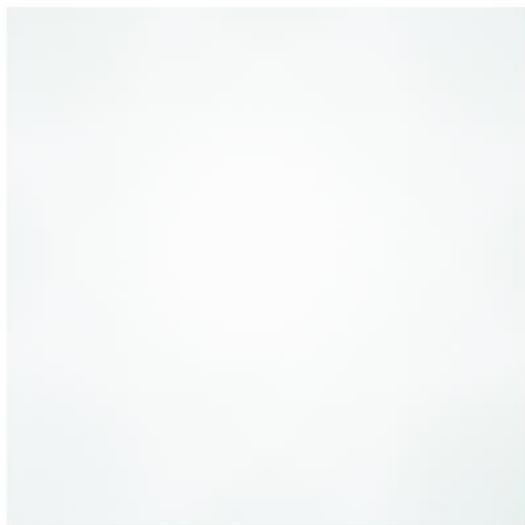


Figure 2.31. Image of the Opal Glass white balance image (bottom right in figure 2.30) after going through the black-background and white-balance normalization.

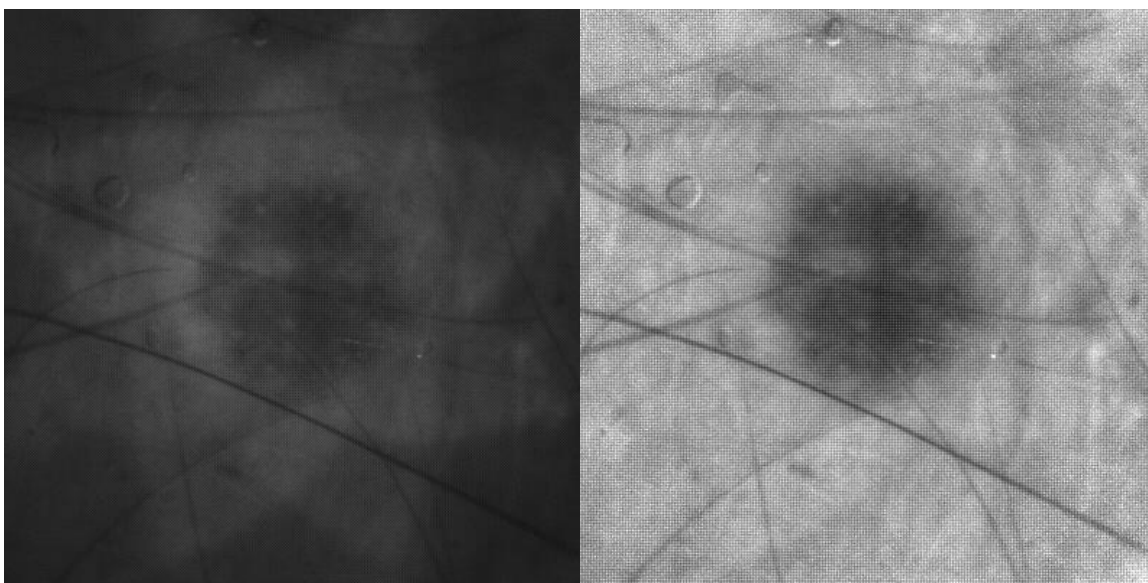


Figure 2.32 Image of a freckle on the skin before and after black-background and white-balance normalization and interpolation (which is explain in the following section).

Gradient-Corrected Bilinear Bayer Mask Interpolation

The Omni Vision 6946-RAJA camera has a Bayer Mask color filter on the sensor array. The Bayer Mask was a repeating pattern of a blue-green-blue-green row followed by a green-red-green-red row, with the top left corner of the sensor (the 1,1 position) containing the blue

filter (this corresponds to a bggr Bayer pattern), a section of the filter pattern can be seen in figure 2.33. Using MATLAB, the RAW image was sent through a gradient-corrected bilinear Bayer Mask interpolation algorithm created for this project. Non-linear interpolation algorithms have been found to perform better than linear ones; however, non-linear interpolations algorithms accomplish this at the cost of computational complexity and speed [1, 109]. This micro-camera will be used simultaneously with RCM imaging, and the performance increase in the micro-camera using non-linear interpolation is insignificant. Therefore, the bilinear interpolation with the gradient-correction algorithm was chosen for this application. Since the RAW image file from the image acquisition contained three identical color channels, the interpolation algorithm only used one of the channels for the interpolation. Thus, the input to the interpolation algorithm was a 400×400 uint-8 array. The output was a $400 \times 400 \times 3$ uint-8 color image with the first channel corresponding to red, the second to green, and the third to blue.

B	G	B	G	B
G	R	G	R	G
B	G	¹ B	³ G	B
G	R	² G	⁴ R	G
B	G	B	G	B

Figure 2.33. Bayer pattern with pixel types denoted by superscript and red, green, or blue filters represented by R, G, and B respectively following the bggr Bayer filter pattern.

The bilinear portion of the demosaicing process estimates a pixel's RGB value based on the nearest neighbors corresponding to that color. Each color channel had four possible pixel types, as seen in figure 2.33 above. Once the pixel types are defined, weight functions (shown with representative matrices, seen below) could be created to interpolate the RGB values for each pixel type. These matrices show how the RGB values for each pixel were estimated since,

for any given pixel, the filter covering that pixel corresponded to only one of the three colors. The sum of the entries is equal to 1. At the edges and corners of the images, RGB values must be estimated using the nearest existing neighbors. While this degraded the accuracy of the color estimation for the edge pixel values, the edge of the image was where the highest aberrations and illumination imbalance were present. Therefore, the object of interest in the image was placed as much in the center as possible, meaning that the edge values could be ignored in general analysis. With a monochrome camera without a Bayer mask, there would be a pixel-limited contribution to the MTF curve, but the bilinear interpolation scheme has its own MTF contribution that contributes to the system MTF. This worsens the MTF, but the gradient-correction method helped improve upon the bilinear interpolation's contribution.

The gradient-correction method in [1] works on the principle that areas of high spatial frequency (sharp transitions such as the edge of a mole) would weight the luminance components more than the chrominance components. Instead of simply discarding the colors you are not trying to estimate as in a purely bilinear interpolation, the color value of that pixel's Bayer filter could be compared to its nearest neighbors. For example, if you estimated a red value at a pixel with a blue Bayer filter, then the nearest neighbor blue pixel values were also used to estimate the red value. If there was a significant difference between the pixel's value and its nearest neighbors', one could assume a significant change in the luminance. The luminance change could then be added to the values estimated using the bilinear interpolation approach with a predetermined weight and gain value calculated in [1]. In figure 2.34, the sum of the pixels containing the color being estimated sums to 8. Summing to 8 is the convention used in [1], which could easily be scaled to 1 to match the methods in [109]. The pixel color that matches the

Bayer mask was used for the gradient-correction and summed to zero. Therefore, if the luminance difference of the nearest neighbors was negligible, then the value added to the bilinear interpolation estimate was also negligible.

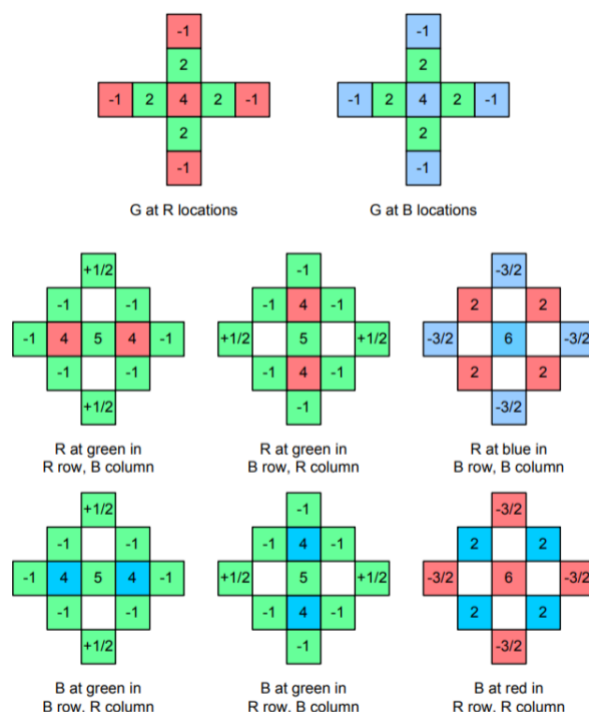


Figure 2.34. Filter coefficients corresponding to pixel location relative to the pixel whose RGB values are estimated for the bilinear gradient-correction method [1].

In order to observe the MTF effects of the gradient-correction in addition to the bilinear interpolation, the kernels shown in figure 2.34 were used to derive the theoretical MTF equations using the same method as in the bilinear case. The gradient-correction uses information from pixels that were two pixels away from the pixels whose RGB values were being estimated, requiring a 5×5 matrix instead of a 3×3 as in the bilinear case. Using the additional pixels would cause the derived equation to contain a second harmonic term. Below are the derivations for the bilinear interpolation MTF which were then applied to calculate the gradient-corrected bilinear interpolation MTF.

Below are the matrices for each pixel type representing the interpolation weight functions used for the bilinear interpolation [109]. The weight functions f_i^j contain superscripts j referring to pixel type, subscripts i refer to the color channel. i equaling one means the red channel, i equaling two means the green channel, i equaling three means the blue channel.

Pixels of type 1:

$$f_1^1 = \begin{bmatrix} 0.25 & 0 & 0.25 \\ 0 & 0 & 0 \\ 0.25 & 0 & 0.25 \end{bmatrix}$$

$$f_2^1 = \begin{bmatrix} 0 & 0.25 & 0 \\ 0.25 & 0 & 0.25 \\ 0 & 0.25 & 0 \end{bmatrix}$$

$$f_3^1 = \begin{bmatrix} 0 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 0 \end{bmatrix}$$

Pixels of type 3:

$$f_1^3 = \begin{bmatrix} 0 & 0.5 & 0 \\ 0 & 0 & 0 \\ 0 & 0.5 & 0 \end{bmatrix}$$

$$f_2^3 = \begin{bmatrix} 0 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 0 \end{bmatrix}$$

$$f_3^3 = \begin{bmatrix} 0 & 0 & 0 \\ 0.5 & 0 & 0.5 \\ 0 & 0 & 0 \end{bmatrix}$$

Pixels of type 2:

$$f_1^2 = \begin{bmatrix} 0 & 0 & 0 \\ 0.5 & 0 & 0.5 \\ 0 & 0 & 0 \end{bmatrix}$$

$$f_2^2 = \begin{bmatrix} 0 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 0 \end{bmatrix}$$

$$f_3^2 = \begin{bmatrix} 0 & 0.5 & 0 \\ 0 & 0 & 0 \\ 0 & 0.5 & 0 \end{bmatrix}$$

(10)

Pixels of type 4:

$$f_1^4 = \begin{bmatrix} 0 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 0 \end{bmatrix}$$

$$f_2^4 = \begin{bmatrix} 0 & 0.25 & 0 \\ 0.25 & 0 & 0.25 \\ 0 & 0.25 & 0 \end{bmatrix}$$

$$f_3^4 = \begin{bmatrix} 0.25 & 0 & 0.25 \\ 0 & 0 & 0 \\ 0.25 & 0 & 0.25 \end{bmatrix}$$

Below are the matrices for each pixel type representing the interpolation weight functions used for the bilinear gradient-corrected interpolation [1]. It should be noted that these weight functions were normalized from zero to one by dividing by eight for the actual calculations, they are easier to read in this form.

Pixels of type 1:

$$f_1^1 = \begin{bmatrix} 0 & 0 & -1.5 & 0 & 0 \\ 0 & 2 & 0 & 2 & 0 \\ -1.5 & 0 & 6 & 0 & -1.5 \\ 0 & 2 & 0 & 2 & 0 \\ 0 & 0 & -1.5 & 0 & 0 \end{bmatrix}$$

$$f_2^1 = \begin{bmatrix} 0 & 0 & -1 & 0 & 0 \\ 0 & 0 & 2 & 0 & 0 \\ -1 & 2 & 4 & 2 & -1 \\ 0 & 0 & 2 & 0 & 0 \\ 0 & 0 & -1 & 0 & 0 \end{bmatrix}$$

$$f_3^1 = \begin{bmatrix} 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 8 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \end{bmatrix}$$

Pixels of type 2:

$$f_1^2 = \begin{bmatrix} 0 & 0 & 0.5 & 0 & 0 \\ 0 & -1 & 0 & -1 & 0 \\ -1 & 4 & 5 & 4 & -1 \\ 0 & -1 & 0 & -1 & 0 \\ 0 & 0 & -1.5 & 0 & 0 \end{bmatrix}$$

$$f_2^2 = \begin{bmatrix} 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 8 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \end{bmatrix}$$

$$f_3^2 = \begin{bmatrix} 0 & 0 & -1 & 0 & 0 \\ 0 & -1 & 4 & -1 & 0 \\ 0.5 & 0 & 5 & 0 & 0.5 \\ 0 & -1 & 4 & -1 & 0 \\ 0 & 0 & -1 & 0 & 0 \end{bmatrix}$$

Pixels of type 3:

$$f_1^3 = \begin{bmatrix} 0 & 0 & -1 & 0 & 0 \\ 0 & -1 & 4 & -1 & 0 \\ 0.5 & 0 & 5 & 0 & 0.5 \\ 0 & -1 & 4 & -1 & 0 \\ 0 & 0 & -1 & 0 & 0 \end{bmatrix}$$

$$f_2^3 = \begin{bmatrix} 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 8 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \end{bmatrix}$$

$$f_3^3 = \begin{bmatrix} 0 & 0 & 0.5 & 0 & 0 \\ 0 & -1 & 0 & -1 & 0 \\ -1 & 4 & 5 & 4 & -1 \\ 0 & -1 & 0 & -1 & 0 \\ 0 & 0 & -1.5 & 0 & 0 \end{bmatrix}$$

Pixels of type 4:

$$f_1^4 = \begin{bmatrix} 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 8 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 \end{bmatrix}$$

$$f_2^4 = \begin{bmatrix} 0 & 0 & -1 & 0 & 0 \\ 0 & 0 & 2 & 0 & 0 \\ -1 & 2 & 4 & 2 & -1 \\ 0 & 0 & 2 & 0 & 0 \\ 0 & 0 & -1 & 0 & 0 \end{bmatrix}$$

$$f_3^4 = \begin{bmatrix} 0 & 0 & -1.5 & 0 & 0 \\ 0 & 2 & 0 & 2 & 0 \\ -1.5 & 0 & 6 & 0 & -1.5 \\ 0 & 2 & 0 & 2 & 0 \\ 0 & 0 & -1.5 & 0 & 0 \end{bmatrix}$$

(11)

Once the interpolation weight functions were defined, the following mathematical steps were computed. Note, ‘*’ referred to a single-dimension convolution and ‘**’ referred to a two-dimensional convolution. Following mathematical steps were from the methods used in [109],

and were applied to only the Bayer interpolation as written here. The method for the gradient-corrected bilinear interpolation is adjusted, changing equations 12 through 36 such that the column-wise convolution accounts for the extra terms in the interpolation weight functions. This meant that when applied for the gradient-corrected bilinear interpolation, a third term representing a second harmonic appeared in the solution.

$$s_i^{int} = \sum_{x'} \sum_{y'} s(x', y') f_i(x, y, x', y') \quad (12)$$

Where S_i was the signal input for a specific color channel. And where S_i^{int} was the interpolated signal below.

$$s_i^{int} = \begin{cases} s ** f_i^1 & xOdd, yOdd \\ s ** f_i^2 & xOdd, yEven \\ s ** f_i^3 & xEven, yOdd \\ s ** f_i^4 & xEven, yEven \end{cases} \quad (13)$$

Because of the Bayer mask's alternating pattern, this system was not shift-invariant (SI), nor separable. To overcome this, [109] uses "a scene that varies at one axis and was invariant on the other," such as a vertical edge. Thus, accounting for spatial frequencies in the x-direction and only the constant DC term in the y-direction. A consequence of treating this not SI system this way was a spurious response from spatial frequencies not included in the input. This spurious response is calculated below but was ignored for further MTF analysis. Using an averaged scene in the y-direction, a column-wise convolution, equation 13 could be written as follows.

$$\begin{aligned}
s_i^{int}(x) &= \frac{1}{N} \sum_{y=0}^N s_i^{int}(x, y) = \frac{1}{N} \sum_{y=0}^N \begin{cases} s ** f_i^1 & xOdd, yOdd \\ s ** f_i^2 & xOdd, yEven \\ s ** f_i^3 & xEven, yOdd \\ s ** f_i^4 & xEven, yEven \end{cases} \\
&= \begin{cases} s * \sum_y \frac{f_i^1 + f_i^2}{2} & xOdd \\ s * \sum_y \frac{f_i^3 + f_i^4}{2} & xEven \end{cases}
\end{aligned} \tag{14}$$

Simplifying equation 14 by reducing the second term.

$$s_i^{int}(x) = \begin{cases} s * h_i^1 & xOdd \\ s * h_i^2 & xEven \end{cases} \tag{15}$$

$$h_i^1 = \frac{1}{2} \sum_y [f_i^1(x, y) + f_i^2(x, y)] \tag{16}$$

$$h_i^2 = \frac{1}{2} \sum_y [f_i^3(x, y) + f_i^4(x, y)] \tag{17}$$

Now, a weighting could be applied to represent the MTF as a luminosity rather than three MTF equations for the RGB channels. Representing the MTF as a luminosity allows for comparison to a grayscale image.

$$L = [K_1 \quad K_2 \quad K_3] \cdot \begin{bmatrix} s_1^{int} \\ s_2^{int} \\ s_3^{int} \end{bmatrix} = K \cdot s^{int} \tag{18}$$

Where, $K = [0.3 \ 0.6 \ 0.1]$ as used in [109].

For this theoretical calculation of the MTF for the interpolation process, the signal input values were irrelevant, so only the terms, including the interpolation weight functions, were kept, reducing equation 18.

$$h^j = K \cdot \begin{bmatrix} h_1^j \\ h_2^j \\ h_3^j \end{bmatrix} \quad j = 1, 2 \quad (19)$$

Writing out the resulting matrices for h^1 and h^2 for the bilinear interpolation, we got the following.

$$h^1 = [0.3 \ 0.6 \ 0.1] \cdot \begin{bmatrix} 0.5 & 0 & 0.5 \\ 0.125 & 0.75 & 0.125 \\ 0 & 1 & 0 \end{bmatrix} = [0.225 \ 0.55 \ 0.225] \quad (20)$$

$$h^2 = [0.3 \ 0.6 \ 0.1] \cdot \begin{bmatrix} 0 & 1 & 0 \\ 0.125 & 0.75 & 0.125 \\ 0.5 & 0 & 0.5 \end{bmatrix} = [0.125 \ 0.75 \ 0.125] \quad (21)$$

And for the gradient-corrected bilinear interpolation, h^1 and h^2 were as follows.

$$\begin{aligned} h^1 &= \left(\frac{1}{8}\right) [0.3 \ 0.6 \ 0.1] \cdot \begin{bmatrix} -1.25 & -1 & 4.5 & -1 & -1.25 \\ -0.5 & 0 & 1 & 0 & -0.5 \\ 0.25 & -1 & 1.5 & -1 & 0.25 \end{bmatrix} \\ &= \left(\frac{1}{8}\right) [-0.65 \ 1.4 \ 6.5 \ 1.4 \ -0.65] \end{aligned} \quad (22)$$

$$\begin{aligned} h^2 &= \left(\frac{1}{8}\right) [0.3 \ 0.6 \ 0.1] \cdot \begin{bmatrix} 0.25 & -1 & 1.5 & -1 & 0.25 \\ -0.5 & 0 & 1 & 0 & -0.5 \\ -1.25 & -1 & 4.5 & -1 & -1.25 \end{bmatrix} \\ &= \left(\frac{1}{8}\right) [-0.35 \ 0.6 \ 7.5 \ 0.6 \ -0.35] \end{aligned} \quad (23)$$

Next, the discrete Fourier transform of the h^j terms were calculated. $H^1(\theta)$ and $H^2(\theta)$ for the bilinear interpolation were as follows.

$$H^1(\theta) = 0.55 + 0.45\cos(2\pi f) \quad (24)$$

$$H^2(\theta) = 0.75 + 0.25\cos(2\pi f) \quad (25)$$

And $H^1(\theta)$ and $H^2(\theta)$ for the gradient-corrected bilinear interpolation were as follows.

$$H^1(\theta) = 0.8125 + 0.35\cos(2\pi f) - 0.1625\cos(2 \cdot 2\pi f) \quad (26)$$

$$H^2(\theta) = 0.9375 + 0.15\cos(2\pi f) - 0.0875\cos(2 \cdot 2\pi f) \quad (27)$$

The MTF could be calculated by dividing the absolute value of the sum of the discrete Fourier transform of the h^j terms by 2. And the spurious response could be calculated by the shifted difference of the H^j terms as seen in equation 36. However, to get the MTF equations, effects of the non-shift invariant system from the even and odd terms needed to be understood. From equation 16, the h^1 term was the convolution kernel for odd column pixels and the h^2 term was the convolution kernel for even column pixels. In order to properly select the even and odd terms for the output, vectors of alternating zeros and ones were created, as seen in equations 28 and 29. The first position was the zeroth position.

$$\text{Odd Vector} = m_{\text{odd}} = [0 \ 1 \ 0 \ 1 \ 0 \ 1 \ 0 \ \dots \ 1] \quad (28)$$

$$\text{Even Vector} = m_{\text{even}} = [1 \ 0 \ 1 \ 0 \ 1 \ 0 \ 1 \ \dots \ 0] \quad (29)$$

The image vector S could be convolved with the interpolation kernel for odd pixels h^1 , for which the result would be correct only for odd values of x . Similarly, S could be convolved with the interpolation kernel for even pixels h^2 , for which the result would be correct only for even values of x . The mask vectors m_{odd} and m_{even} were used to select the proper output from these two convolution operations, with the result summed for the full interpolated output signal $s_i^{int}(x)$. Equation 30 shows the interpolated signal with the even and odd filters as they were Fourier transformed.

$$\begin{aligned} s_i^{int}(x) &= (s * h^1)m_{odd} + (s * h^2)m_{even} \\ &\xrightarrow{\mathcal{F}} S_i^{int}(\theta) = (S(\theta)H^1(\theta)) * M_{odd} + (S(\theta)H^2(\theta)) * M_{even} \end{aligned} \quad (30)$$

The discrete Fourier transforms of m_{odd} and m_{even} are as follows. The second non-zero terms were located at π radians.

$$M_{odd} = \begin{bmatrix} \frac{1}{2} & 0 & 0 & 0 & \dots & \frac{-1}{2} & 0 & 0 & 0 & \dots \end{bmatrix} \quad (31)$$

$$M_{even} = \begin{bmatrix} \frac{1}{2} & 0 & 0 & 0 & \dots & \frac{1}{2} & 0 & 0 & 0 & \dots \end{bmatrix} \quad (32)$$

So,

$$\begin{aligned} S_i^{int}(\theta) &= \frac{1}{2}(S(\theta)H^1(\theta) - S(\theta - \pi)H^1(\theta - \pi)) \\ &\quad + \frac{1}{2}(S(\theta)H^2(\theta) + S(\theta - \pi)H^2(\theta - \pi)) \end{aligned} \quad (33)$$

$$S_i^{int}(\theta) = \frac{S(\theta)(H^2(\theta) + H^1(\theta))}{2} + \frac{S(\theta - \pi)(H^2(\theta - \pi) - H^1(\theta - \pi))}{2} \quad (34)$$

Where the first term was MTF response (where $S_i^{int}(\theta)$ depends only on $S(\theta)$), and the second term was the spurious response (where $S_i^{int}(\theta)$ depends on $S(\theta - \pi)$). Equations 35 and 36 are found by separating equation 34 and dividing out the input signal components.

$$MTF^{Interpolation} = \left| \frac{H^2(\theta) + H^1(\theta)}{2} \right| \quad (35)$$

$$MTF^{Spurious} = \left| \frac{H^2(\theta - \pi) - H^1(\theta - \pi)}{2} \right| \quad (36)$$

Where $H^j(\theta)$ is the Fourier transform of h^j , and $\theta = \pi \frac{f}{f_{nyquist}}$. The MTF calculated for the bilinear interpolation matched the MTF calculated in [109].

$$MTF^{Bilinear\ Interpolation} = 0.65 + 0.35 \cos(2\pi f) \quad (37)$$

$$MTF^{Bilinear\ Spurious\ Response} = 0.1 - 0.1 \cos(2\pi f) \quad (38)$$

$$\begin{aligned} MTF^{Gradient-Corrected\ Bilinear\ Interpolation} \\ = 0.875 + 0.25 \cos(2\pi f) - 0.125 \cos(2 \cdot 2\pi f) \end{aligned} \quad (39)$$

$$\begin{aligned} MTF^{Gradient-Corrected\ Bilinear\ Spurious\ Response} \\ = 0.0625 - 0.1 \cos(2\pi f) + 0.0375 \cos(2 \cdot 2\pi f) \end{aligned} \quad (40)$$

Below, both calculated MTF curves were multiplied by the pixel-limited MTF curve and plotted on the same axis as the pixel-limited MTF curve. The pixel-limited MTF shows how the system was limited by the size and shape of the pixels on an image sensor. This means that the imaging system cannot resolve features that are smaller than the pixel size. The spatial frequency of the MTF curves plotted below was in cycles per pixel, so the pixel-limited MTF curve extends

to 1 cycle/pixel. If it was plotted on cycles per millimeter, the cutoff would be at a different value. The pixels were squares, and the MTF of a pixel can be found by taking the absolute value of the one-dimensional Fourier transform of a square, yielding a sinc pattern. Since the Bayer mask was layered on top of the pixels of the sensor, the MTF curves calculated above must be multiplied by the pixel-limited MTF curve to accurately represent the system. The Nyquist frequency was shown in the below figure to be at 0.5 cycles/pixel. Any input signal frequencies above the Nyquist frequency can be aliased and cause errors when interpreting the MTF.

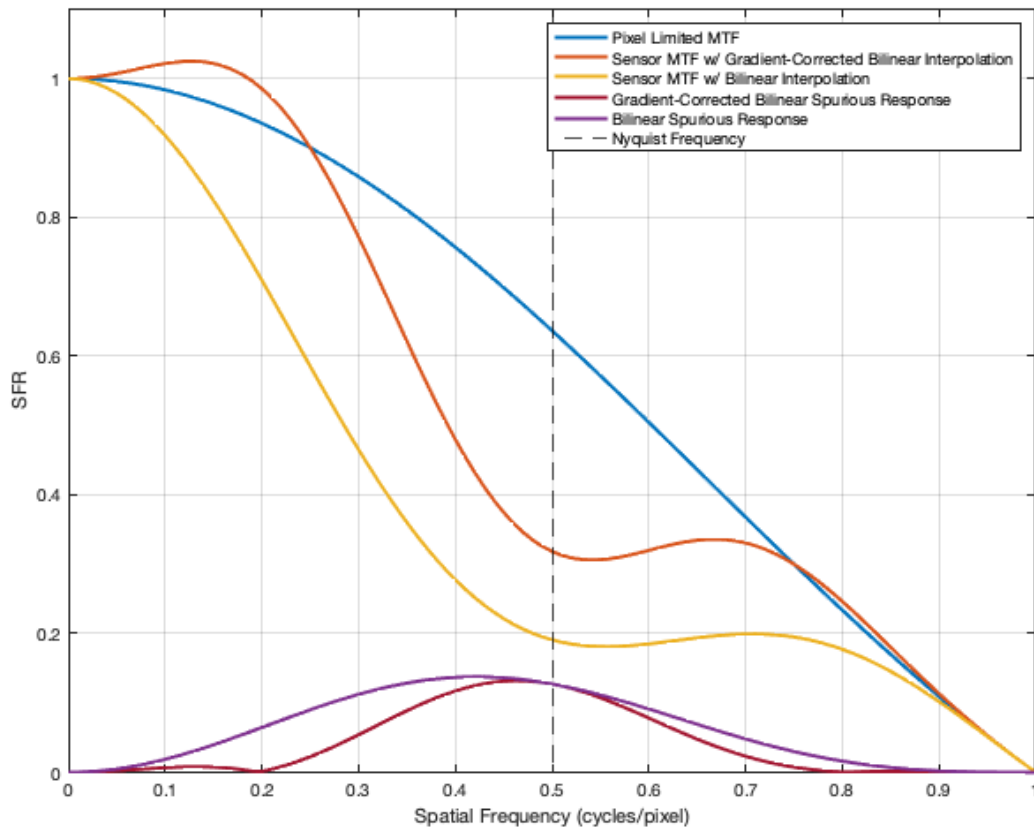


Figure 2.35. The MTF curve for a pixel-limited B/W detector compared to the MTF curve for the gradient-corrected bilinear interpolation, bilinear interpolation, and their spurious responses for the Bayer mask filter. The MTF curves for the bilinear interpolation and gradient-corrected bilinear interpolation are individually multiplied by the MTF curve for the pixel-limited sensor, a sinc, for the above plot.

From the theoretical data, a function was created in MATLAB to conduct both interpolation schemes on full images. Then, a slanted edge image was generated using MATLAB to verify the theoretically calculated curves. The slanted edge was generated by making an image with binary values, one half of a 400×400 pixel image equal to the uint8 value of 20 and the other half equal to the uint8 value of 220. An angle of 5° was assigned for the edge. This simulated slanted edge image was then sent through the gradient-corrected bilinear interpolation function. And the MTF curves from each image were calculated using the `sfrmat4.m` MATLAB function [108], based on the theory from [107], which calculates the MTF curve for an image using a slanted edge in the image. The MTF curves generated from the slanted edge MTF function are shown in figure 2.36 with the theoretical MTF curves for reference.

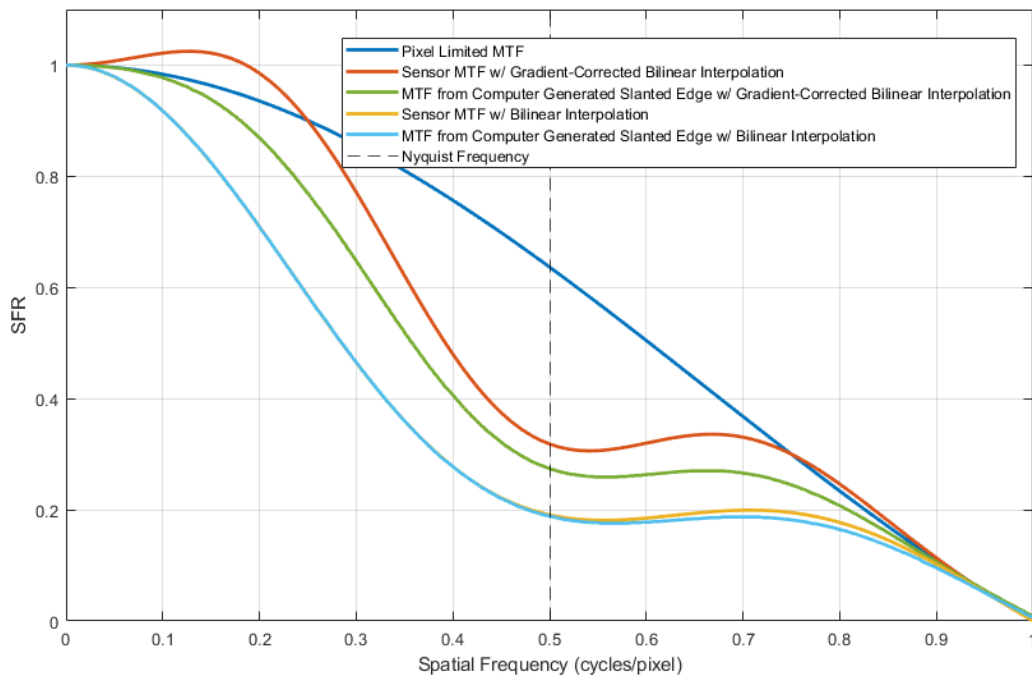


Figure 2.36. Computer generated slanted edge MTF and theoretical MTF for the gradient-corrected bilinear interpolation, with the pixel-limited MTF for reference.

As seen in figure 2.36, the MTF curve for the gradient-corrected bilinear interpolations calculated from the generated slanted edge had good agreement with the theoretically calculated MTF curves. A notable discrepancy was the initial positive slope of the theoretically calculated gradient-corrected bilinear interpolation MTF curve, which gave an offset throughout the frequency spectrum. This positive slope brings the MTF curve above an SFR of 1, where the MTF curve calculated from the generated slanted edge started with a negative slope, not exceeding an SFR of 1, but maintained the relative shape of the theoretical curve.

Images taken with the mockup probes of each polarization variation of a slanted edge on an Opal glass Airforce resolution target were then sent through the black-background and white-balance function to help balance the illumination profile followed by bilinear and gradient-corrected bilinear interpolation functions. These newly processed images were sent through the `sfrmat4.m` function. The calculated MTF curves showed improvement in the MTF for the gradient-corrected bilinear interpolation over the solely bilinear interpolation, as seen in figure 2.37. However, the shape seen in the above simulations could not be made out in these curves. This was likely heavily influenced by the diffraction effects of the probe's lens. The agreement that the gradient-corrected bilinear interpolation performs better when working with theoretical, computer-generated, and real images gave enough evidence to use it as the primary interpolation method moving forward with experimentation and testing.

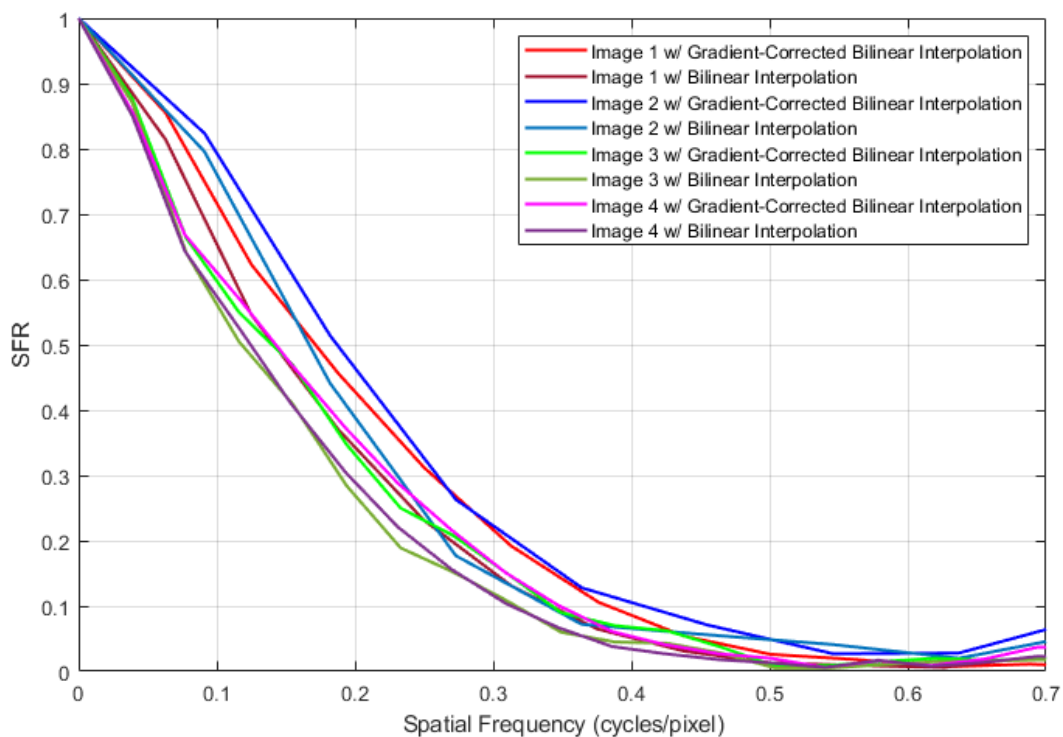


Figure 2.37. Comparison of the MTF curves from slanted edge images with the gradient-corrected bilinear interpolation and bilinear interpolation.

Figure 2.38 shows the gradient-corrected bilinear interpolation applied to the normalized image corrected in figure 2.32. This figure shows a RAW image transformed into an RGB color image.

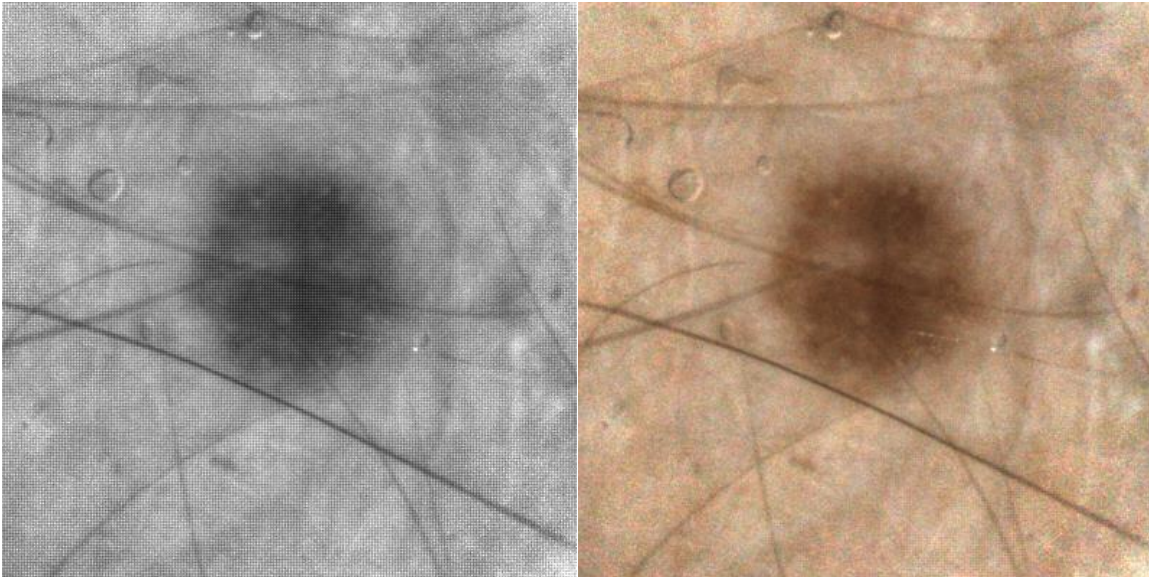


Figure 2.38. Transformation from a RAW image (left) to an RGB color image (right) using the gradient-corrected bilinear interpolation.

Color Spaces and Color Correction

When an image was captured with a color camera, the RGB values it detected were unique for the specific detector because of variables such as the Bayer mask filter, chromatic aberrations from the lens, illumination spectra, and so on. Therefore, calibration or post-processing was required for images to exist in the same color space. However, multiple color spaces exist that were defined for various visual systems (monitors, printers, projectors, televisions, etc.) that aim to approximate the colors in human visual spectra. The root of their differences came from the initial choice of primary colors used to define the tristimulus coordinates in color-matching experiments. The color matching experiments were tests where subjects are shown two colors, one composed of a single wavelength generated through optical manipulation, and the other was the combination of three primary colors, red, green, and blue. The subject was supposed to determine when the three mixing primary colors' weights match the actual color. The tristimulus coordinates were the weights of the three primaries that create the

actual color. Color spaces refer to “... a representation derived with respect to a particular choice of primaries,” [110, 111]. And the color space is the collection of tristimulus coordinates. The choice of color space affects how the colors are interpreted when viewed by a human or clinician for this purpose. But the selection of color space could be irrelevant if the colors captured by the camera were distorted due to the numerous variables listed above.

Images captured with the micro-camera must accurately represent the colors of a lesion for accurate diagnosis. To this end, interpolation and color correction of RAW images was conducted. However, to better understand the corrections needed, baseline assessments of color had to be made for the cameras. From there, multiple methods of color correction were explored. Figure 2.41. compares the measured RGB values from a non-polarized probe imaging the Image Science Associates (ISA) ColorGauge Pico Target, to the Adobe color space values provided by ISA. The measured RGB values were captured as a RAW image, sent through the black-background and white-balance function to help balance the illumination profile, then the bilinear and gradient-corrected bilinear interpolation function to make them a color image. The RGB values of the patches were averaged by drawing a rectangle over the patch in MATLAB. Figure 2.39 shows an example of an image used to measure the RGB values of one patch. The measured values are interpolated from a RAW image using the gradient-corrected bilinear interpolation described in the previous section. These results show that almost every cell (except for the white cell) had lower RGB values, resulting in a darker color. The error from the measured to the reference RGB values for each color patch was unique, implying that a simple linear correction would not make the colors match. Polynomial fitting, least squares approximation, and gamma correction would be investigated as possible color correction schemes for this system. The goal

was to find a function that can be integrated into image processing to reduce the computational power necessary during common use of the concurrent widefield camera and RCM device.

Other, more accurate methods of color correction exist but require more computational power to implement.

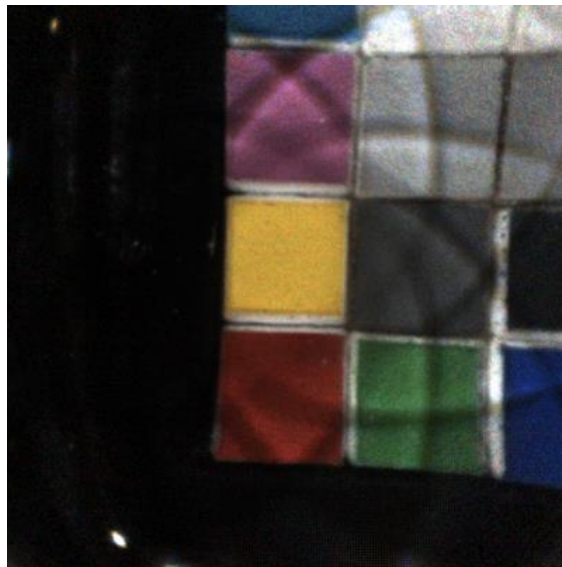


Figure 2.39. Image of the 19th patch on the ISA ColorGauge Pico Target that is used to measure the RGB values and compare them to the reference values for the 19th patch. Image taken with a non-polarized probe built in the MSU lab.



Figure 2.40. Image of the entire ISA ColorGauge Pico Target. Image taken with an iPhone.

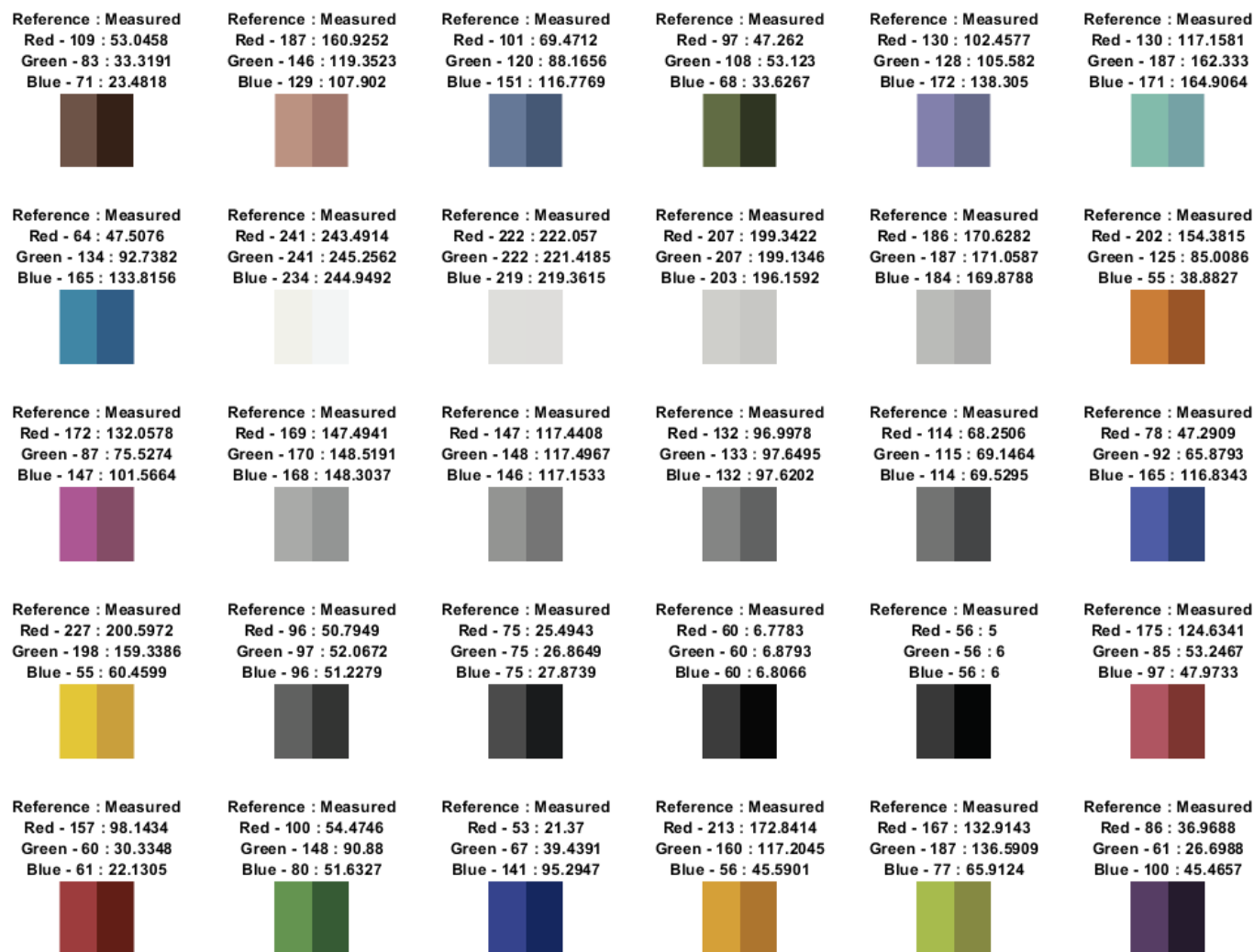


Figure 2.41. Comparison of the measured RGB values for each color on the ISA ColorGauge Pico Target before correction to the Adobe RGB Color Space reference values provided by ISA.

Linear Least Squares Approximation. A linear least squares approximation is a commonly used method in statistical modeling and regression analysis to determine the best fit for a given set of data points. It is used to approximate solutions to overdetermined systems. The goal is to find the parameters of a function that minimize the sum of the squared differences between the function and the data points. Applying the solved function parameters reduces the difference between the measured and model values. The function is typically represented as a mathematical model with unknown parameters that must be estimated. The optimization algorithm iteratively adjusts these parameters to minimize the objective function, the sum of the squared differences between the reference values and actual data. With established color spaces, a conversion between the established color spaces using a least squares approximation has been proven [110, 111]. For these conversions, there exists specific 3×3 matrices specific for each color space and the color space being converted to, calculated from a least squares approximation. Since least squares approximations had been shown to work for conversions between color spaces, it was believed that it could be used to correct the measured values from the micro-camera to the reference values provided by ISA. MATLAB contains a function that, given two matrices of the same size, $\mathbf{X}$ and $\mathbf{Y}$, as seen in the equation below, could conduct the least squares approximation to generate b . $\mathbf{C}$ was a constant offset matrix. For this use, $\mathbf{X}$ would be the RGB values for the reference color space values from ISA in the form of a 3×30 matrix, and $\mathbf{Y}$ would be the RGB values from the images captured with the micro-camera in the form of a second 3×30 matrix. The $\mathbf{C}$ matrix was implemented by calculating the means for every color of the reference patches, resulting in a 3×30 matrix with equal RGB values, and subtracting those values from the reference 3×30 matrix resulting in $\mathbf{X}'$. A $\mathbf{Y}'$ was found using the same

mean subtraction for the measured RGB values. Then, using the MATLAB matrix divide function, *mrdivide.m*, with the command ‘/’ for **X** and **Y**, MATLAB calculated a linear least squares mapping via *b*, a 3×3 matrix. The *b* matrix was the linear least squares best approximation for converting the values in **Y** to equal the values in **X**. When applying the equation below, **C** was a 3×30 matrix of mean values from the reference matrix **X**.

$$\mathbf{X} = \mathbf{bY} + \mathbf{C} \quad (41)$$

Polynomial Curve Fitting. Another method investigated was polynomial curve fitting, where a polynomial function is found that best fits a given set of data points. On a fundamental level, it works by finding the coefficients of a polynomial function that best fits the given data set. One application of polynomial curve fitting is to correct data sets to a reference data set. This is done by comparing the two data sets and identifying their differences. Once the differences are identified, a polynomial function is fit to the differences and used to correct the data set by adding or subtracting the fitted polynomial from the measured data. This method calculates *n* coefficients for a polynomial of degree *n* to map **Y** to **X**, using the same convention as before. The MATLAB function that conducts this process is the *Polyfit* function, and the function that applies the transform is *Polyval*. MATLAB calculates the polynomial coefficients by minimizing the sum of squares of the differences between the polynomial and the data points, a least squares regression. MATLAB constructs a Vandermonde matrix from **Y** and then uses the Vandermonde matrix solved with **X** to generate a coefficient vector. This coefficient matrix can then be applied to the *Polyval* function to convert **Y** to approximate **X**. Polynomials up to the third-order were tested. The degree of the polynomial used for curve fitting depends on the complexity of the data set and the level of accuracy required. Higher degree polynomials can better fit complex data sets

but may overfit data, resulting in poor performance for new, unseen data. Thus, it was chosen to work with polynomials of third-degree or lower to help reduce the risk of overfitting. For each order of polynomial fitting tested, the *Polyfit* function created an array to map a 3×30 **Y** matrix to a 3×30 **X** matrix. In addition to this, the *Polyfit* function was used to map each color channel of **Y** to **X** separately. So, the input was two 1×30 arrays, and the process was repeated three times to map each color channel. In the results section, this method is referred to as ‘color dependent 1st order polynomial fitting’.

Gamma Correction. Gamma correction is a commonly used technique for color correction in digital imaging, which adjusts the brightness and contrast of an image by non-linearly modifying the relationship between pixel value and displayed intensity. It helps compensate for the nonlinear relationship between an image's luminance values and the display's brightness. Gamma correction is used to make images look more natural on a display by adjusting the brightness curve modeled by mathematical functions. A gamma correction function maps measured intensity to displayed intensity using a nonlinear curve that applies higher gain (steeper slope) to weaker signals and lower gain (lower slope) to strong signals. The choice of non-linear function is chosen such that the displayed pixel intensities for the three color channels in the image better approximate the human visual perception of brightness in the image. This means darker color values are increased more than the already bright ones. When viewing figure 2.41, the most measured values from the prototype probe appear to be darker than the reference colors, except for the white values, or the brightest values. Because of this trend, it was hypothesized that a non-linear correction, such as a gamma correction, could help correct the colors. Since it is a non-linear function, gamma correction was applied as the final step in image

processing. Since gamma correction is often applied for converting colors between different devices, each color space has specific gamma values applied to a nonlinear function used for the gamma correction. Note the pixel values must range from 0 to 1 when applying this correction method. The gamma correction functions for the various color spaces represented on the ISA ColorGauge Pico Target are shown below [110]. Since the reference values provided by ISA are for specific color spaces, this method adjusted the intensity of the RGB values for each pixel in an image with the convention for that color space in the hopes of better matching the corrected values to the reference values. In the following equations, u refers to the input signal values, the image before correction, and v is the output signal values, the corrected image. Gamma correction using the Adobe RGB (1998) Standard is shown in equation 42. Gamma correction using the eciv2 RGB and ProPhoto Standards is shown in equation 43. Gamma correction using the sRGB Standard is shown in equation 44.

$$\begin{aligned} v &= u^\gamma, & u &\geq 0 \\ v &= -(-u)^\gamma, & u &< 0 \\ \gamma &= \frac{1}{2.19921875} \end{aligned} \quad (42)$$

$$\begin{aligned} v &= u^\gamma, & u &\geq 0 \\ v &= -(-u)^\gamma, & u &< 0 \\ \gamma &= \frac{1}{1.8} \end{aligned} \quad (43)$$

$$\begin{aligned} v &= -(-u)^\gamma, & u &< 0 \\ v &= c * u, & 0 \leq u &< d \\ v &= a * u^\gamma + b, & u &\geq d \end{aligned} \quad (44)$$

Where: $a = 1.055$, $b = -0.055$, $c = 12.92$, $d = 0.0031308$, $\gamma = \frac{1}{2.4}$

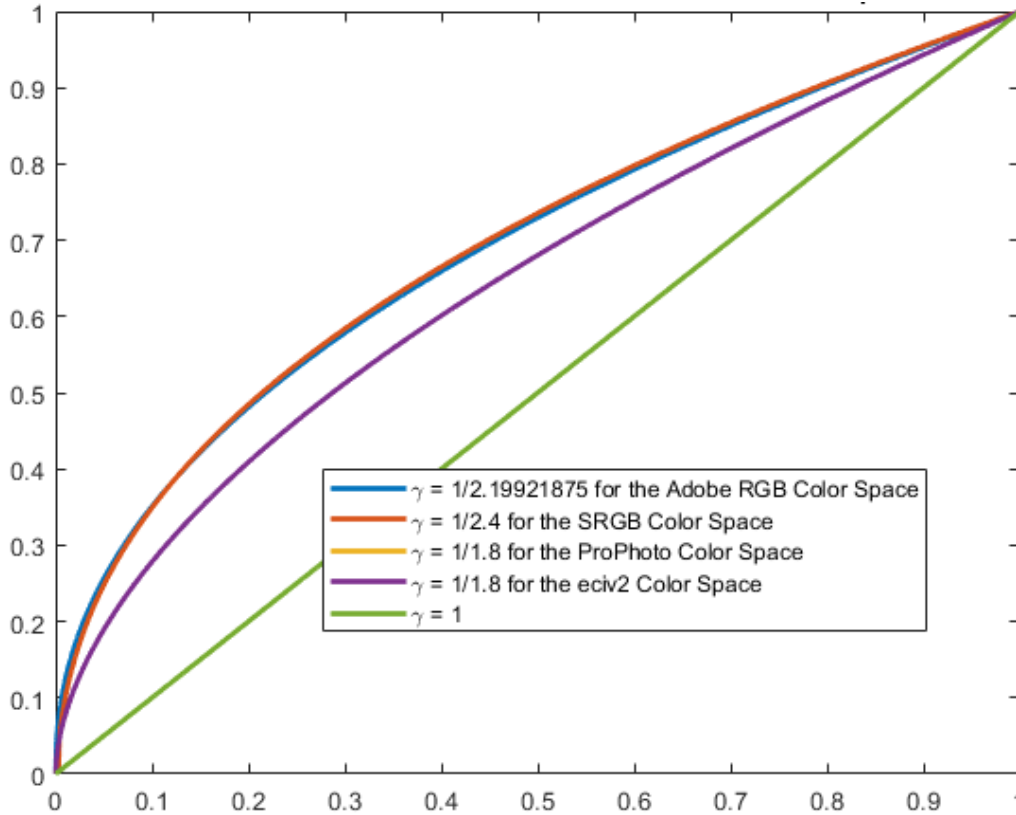


Figure 2.42. Visualization of the normalized gamma curves for each color space of the ISA references.

Color Correction Analysis. The above methods for color correction were tested in order to map measured RGB values for the ColorGauge Pico Target to reference data using the following color spaces: Adobe RGB, eciv2, Pro Photo, and sRGB. The results shown in this analysis are only from the Adobe RGB color space reference data. The results from all the color spaces showed very similar results. The plot below shows the reference RGB values for all thirty patches versus the uncorrected RGB values for all thirty patches, with the black line being the reference versus the reference, showing perfect alignment. From this plot, it was observed that the error from the reference to the measured values is non-linear and unique for RGB values for each color. The red circles correspond to the red values for each patch, the green circles

correspond to the green values for each patch, and the blue circles correspond to the blue values for each patch. From here, the various color correction methods were applied, tables 2.1 through 2.5 show the calculated coefficients for the linear least squares approximation and the polynomial fittings.

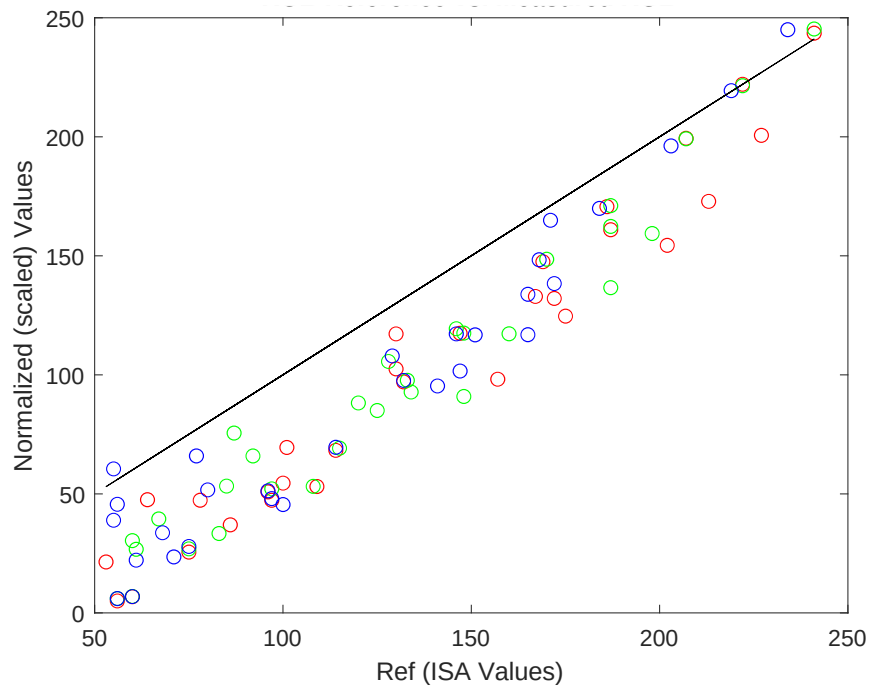


Figure 2.43. Plot of the non-corrected measured RGB values versus the Adobe RGB color space reference values, with the Adobe RGB versus Adobe RGB line shown for reference.

1.0629	-0.2535	0.0161
-0.1645	1.3719	-0.4446
-0.1537	-0.3590	1.1772

Table 2.1 3×3 b matrix values calculated for the linear least squares approximation converting the measures values to the reference values for the Adobe RGB color space.

	0 th Order Term	1 st Order Term	2 nd Order Term	3 rd Order Term
1 st Order Polynomial Fitting	0.1923	0.8276	NA	NA
2 nd Order Polynomial Fitting	0.1631	1.0209	-0.2148	NA
3 rd Order Polynomial Fitting	0.1945	0.6405	0.7934	-0.7081

Table 2.2. Polynomial coefficients calculated for the polynomial fitting methods converting the measures values to the reference values for the Adobe RGB color space.

Red	0 th Order Term	1 st Order Term	2 nd Order Term	3 rd Order Term
Color Dependent 1 st Order Polynomial Fitting	0.2024	0.8328	NA	NA
Color Dependent 2 nd Order Polynomial Fitting	0.1710	1.0403	-0.2294	NA
Color Dependent 3 rd Order Polynomial Fitting	0.1983	0.7105	0.6344	-0.6051

Table 2.3. Color dependent polynomial coefficients for the red channel calculated for the polynomial fitting methods converting the measures values to the reference values for the Adobe RGB color space.

Green	0 th Order Term	1 st Order Term	2 nd Order Term	3 rd Order Term
Color Dependent 1 st Order Polynomial Fitting	0.1961	0.8240	NA	NA
Color Dependent 2 nd Order Polynomial Fitting	0.1655	1.0200	-0.2163	NA
Color Dependent 3 rd Order Polynomial Fitting	0.1916	0.7037	0.6306	-0.5969

Table 2.4. Color dependent polynomial coefficients for the green channel calculated for the polynomial fitting methods converting the measures values to the reference values for the Adobe RGB color space.

Blue	0 th Order Term	1 st Order Term	2 nd Order Term	3 rd Order Term
Color Dependent 1 st Order Polynomial Fitting	0.1822	0.8151	NA	NA
Color Dependent 2 nd Order Polynomial Fitting	0.1565	0.9904	-0.1973	NA
Color Dependent 3 rd Order Polynomial Fitting	0.1935	0.5408	0.9980	-0.8378

Table 2.5. Color dependent polynomial coefficients for the blue channel calculated for the polynomial fitting methods converting the measures values to the reference values for the Adobe RGB color space.

Error from corrected values to the reference values	RMS Error in Red Channel	RMS Error in Green Channel	RMS Error in Blue Channel
Least Squares Approximation	7.5673	6.117	8.3277
1 st Order Polynomial Fitting	12.6012	11.3971	14.8821
Color Dependent 1 st Order Polynomial Fitting	12.2053	11.378	14.3842
2 nd Order Polynomial Fitting	12.0276	10.7798	14.4959
Color Dependent 2 nd Order Polynomial Fitting	11.592	10.769	13.9985
3 rd Order Polynomial Fitting	11.6661	10.4976	14.0523
Color Dependent 3 rd Order Polynomial Fitting	11.2935	10.4658	13.5619
Gamma Correction	26.1737	28.9731	33.7536

Table 2.6. RMS error for each color channel for the corrected RGB values to the reference ISA ColorGauge Pico Target Adobe RGB color space values for each method of color correction.

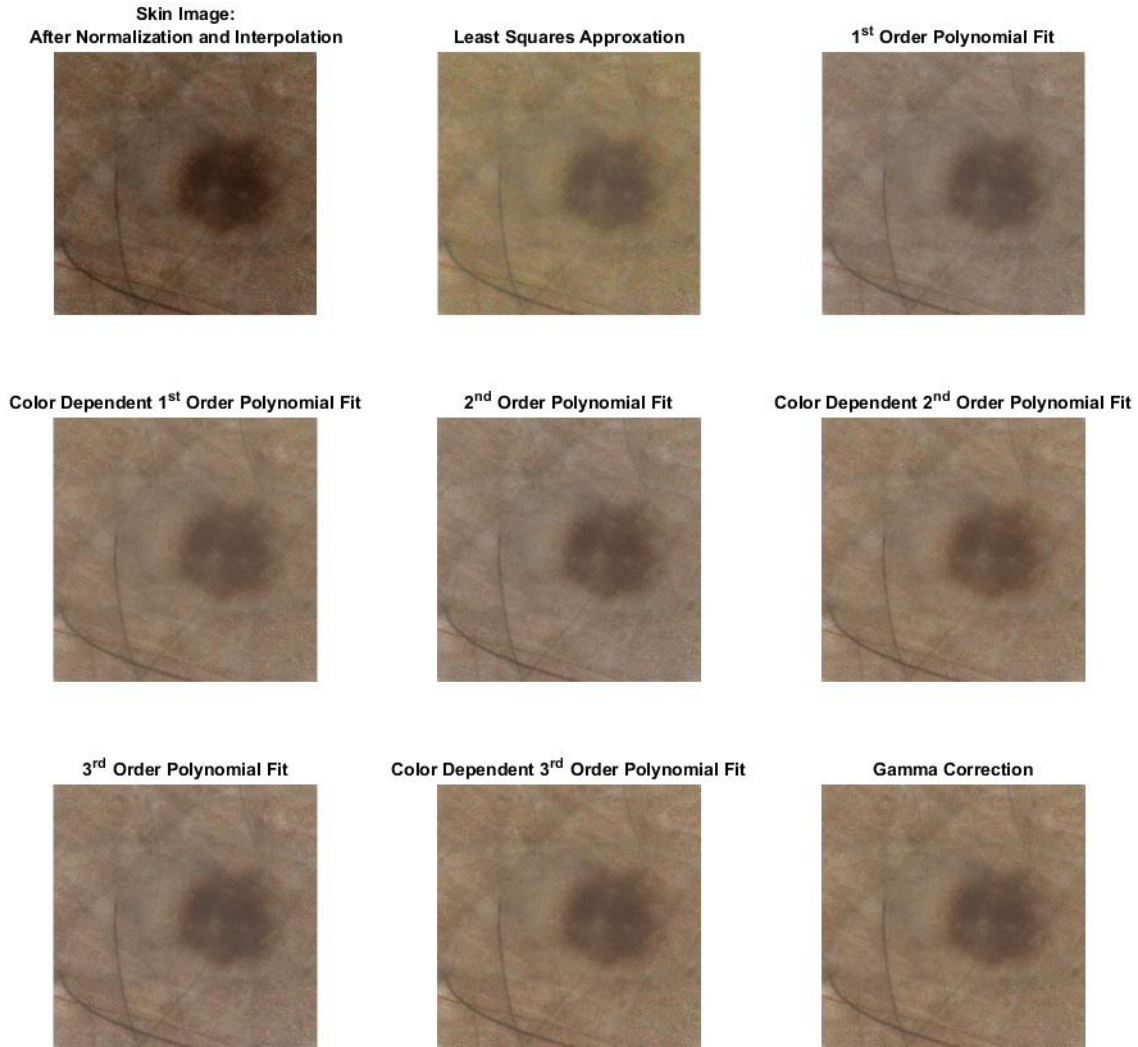


Figure 2.44. Skin image after normalization and interpolation (top left). The other eight images show the results of the tested color correction methods.

When the color correction methods were applied to the ISA ColorGauge Pico Target measured patch values, the least squares approximation method showed the best correction to the reference data set. The six methods using polynomial fitting performed similarly with the third-order color dependents polynomial curve fitting showing the lowest RMS error compared to the reference set, see table 2.6. However, when the generated algorithms were applied to a skin image, the results were harder to quantify, see figure 2.45. Visually, the skin image after the least

squares approximation appears to have the red channel dominant in the image, causing a tint relative to the other corrected images. The images corrected with the various polynomial curve fitting techniques appear more natural than the least squares ones. And while the results are similar between the six polynomial fitting methods, each image has slight variation after correction. However, the best method could not be chosen confidently without a verified method to quantify the color corrections' ability to correct skin color accurately. Each one was able to increase the brightness of the image, making it easier to identify features, but did not brighten the image to the extent that the gamma correction did. The predefined gamma correction functions are more likely to increase all the colors too much, making the overall image seem more washed out. It was observed that the color-dependent polynomial curve fitting methods resulted in images that did not appear as washed out as when the polynomial curve fitting was applied to all three color channels at once. The third-order color-dependent polynomial curve fitting did appear to provide the best increase in brightness while not washing out the image as much as the other methods. More figures of the color correction results can be found in Appendix C.

Another important parameter to consider was the image's contrast after color correction. A correction method that made the images have higher contrast would be desired for the AI-guided feature recognition, which is one project goal. A sharp transition from light to dark in an image could indicate a lesion or other skin artifact that a computer could identify in an automated algorithm. The table below shows the brightness difference for each color channel and correction method of the 200th row, from columns 160 to 220. The correction method that stood out for having two of the most significant changes was the gamma correction. However, the red channel of the gamma correction method shows the lowest change, while the green and blue channels

show the highest. Because of the low contrast in the red, the gamma correction cannot be considered the best method for high contrast. The other methods all show similar results.

	Red Channel	Green Channel	Blue Channel
Least Squares Approximation	15	22	13
1 st Order Polynomial Fitting	16	21	17
Color Dependent 1 st Order Polynomial Fitting	16	20	17
2 nd Order Polynomial Fitting	17	23	20
Color Dependent 2 nd Order Polynomial Fitting	17	23	20
3 rd Order Polynomial Fitting	18	22	17
Color Dependent 3 rd Order Polynomial Fitting	18	22	16
Gamma Correction	14	26	28

Table 2.7. Change in pixel values from columns 160 to 220 in row 200 on the skin image to show contrast between correction methods.

Stanford's Robust AWB Color Correction. After these color correction methods were tested, Caliber ID suggested testing an automatic white balance (AWB) algorithm developed at Stanford, [112]. The white balance developed in [112] uses gray points in the image to estimate color temperature. A gray point is when all RGB components of a pixel are equal, and this algorithm uses the deviation between them to estimate the color temperature. From there, the algorithm iteratively corrects the RGB values of the image until the white balancing is complete. This algorithm does not work on the color patch images and values because there was no gray point, the images captured using the probes have too narrow of a field-of-view to contain the

grayscale patches when imaging the eighteen colored patches. So, this algorithm was not applied to the 3×30 matrices like the other method were. However, it was easily applied to skin images. Below is a comparison between the non-corrected skin image, the robust AWB corrected skin image, and the third-order color dependent polynomial corrected skin image. The result of robust AWB algorithm is a darker image, with a more neutral hue than the other images. This neutral color can be better understood by viewing figures 2.45 and 2.46 below, which shows the RGB values for row 200 from the skin images. The RGB values are much more closely aligned for the robust AWB corrected image than the non-corrected and third-order color dependent polynomial corrected images. Table 2.8 inspects the contrast across row 200 around the freckle region on the image. This does not show a significant contrast difference between any images. Therefore, the neutral hue obtained with the robust AWB was its only advantage over the other correction methods. And a clinician's input would still be needed to choose between the correction methods.

	Red Channel	Green Channel	Blue Channel
Non-Corrected	19	25	21
Robust AWB	14	26	28
Color Dependent 3 rd Order Polynomial Fitting	18	22	16

Table 2.8. Change in pixel values from columns 160 to 220 in row 200 on the skin image to show contrast between correction methods.

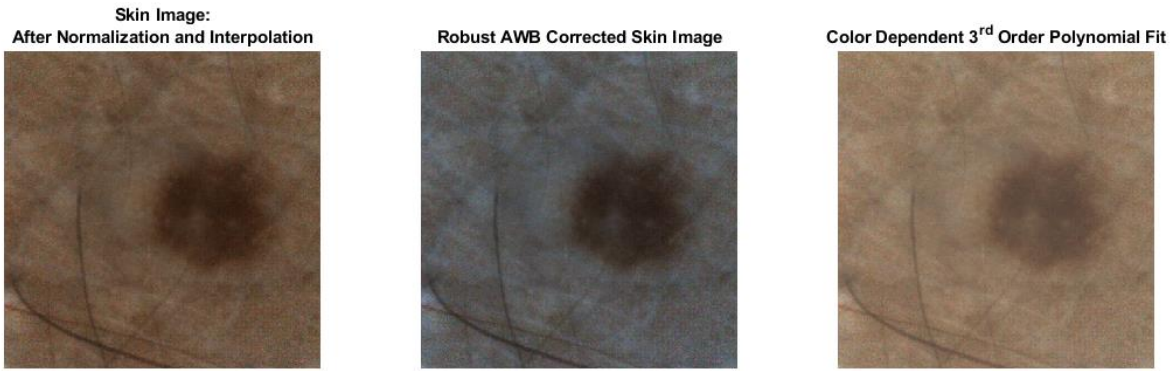


Figure 2.45. Comparison of the non-corrected skin image (left), the Robust AWB corrected skin image (middle) and the 3rd order color dependent polynomial corrected skin image (right).

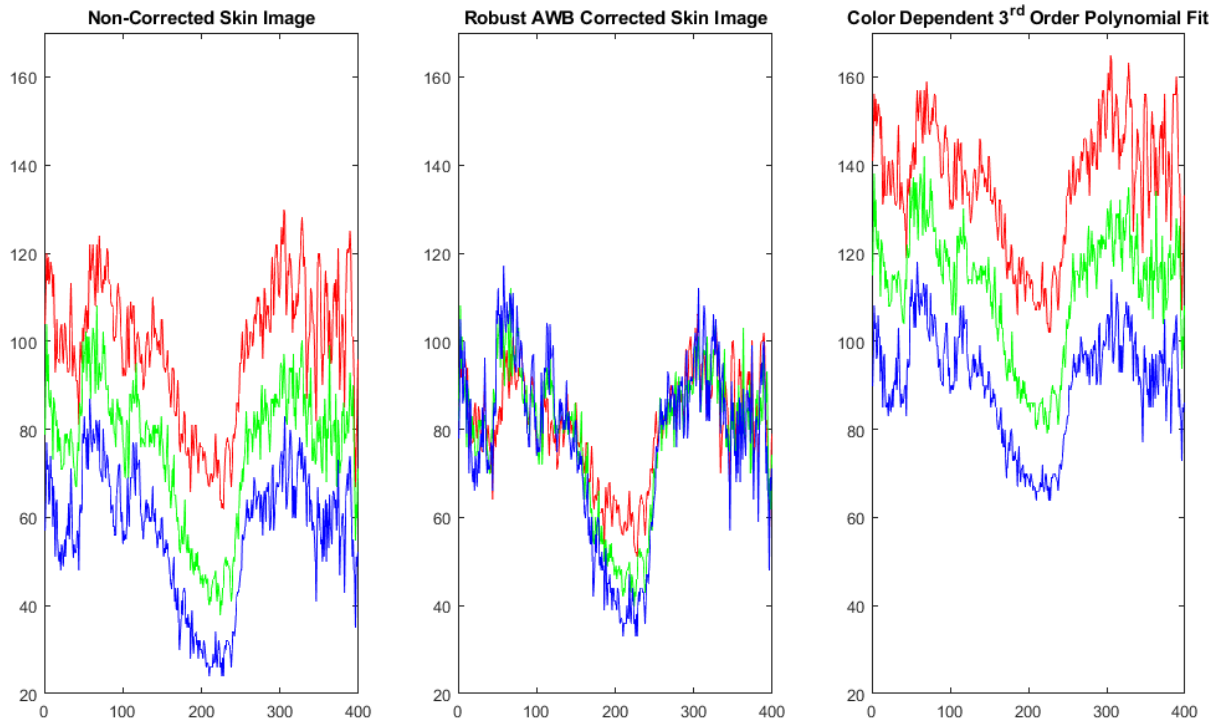


Figure 2.46. RGB values for all of row 200 from the non-corrected skin image (left), the Robust AWB corrected skin image (middle) and the 3rd order color dependent polynomial corrected skin image (right).

Further Illumination Modeling in Zemax

Zemax was used to model the hyper-hemisphere and the illumination profile of the LEDs. The prototype system built at MSU and the objective lens assembly from Photon Gear have different dimensions and glass types, so separate models were created in Zemax for each. The array of eight LEDs attached to the flex board ring created the illumination for the micro-camera inside the objective of the RCM device. However, the illumination profile was non-uniform and requires post-processing correction see figure 2.6 in the Illumination Solution section. A better understanding of the cause of the non-uniform illumination was critical for hardware changes to be made to improve it in future iterations. More importantly, the imaging process used by Caliber ID for the RCM system requires an additional 1 mm window after the end of the objective lens, with ultrasound gel used as a compressible medium for changing the focus depth of the RCM system. The addition of this window caused a dark spot, a section of the image without illumination, to be formed in the center of the images taken with the MSU prototype probe and the objective lens assembly from Photon Gear Inc., seen in figure 2.47. This can be compared to figure 2.6, which is the MSU prototype probe without the extra window. In addition to the dark spot in the center of the image, another notable change was that the images without the window have the lobes of the illumination pattern centered with the LEDs. With the window, the lobes of the illumination pattern are centered in between the LEDs. While the changing lobes could be accounted for with a new white-balance image, the dark spot was a problem since the center of the image was where the highest quality, least distortion, would be found. Losing light in this region made it much harder to use for feature registration. While the size of the dark spot is not the same, the presence in each assembly proves that it results from the overall design.

Another goal of the Zemax simulations is to help explain the cause of this dark spot and help identify a way to remove it from the system.

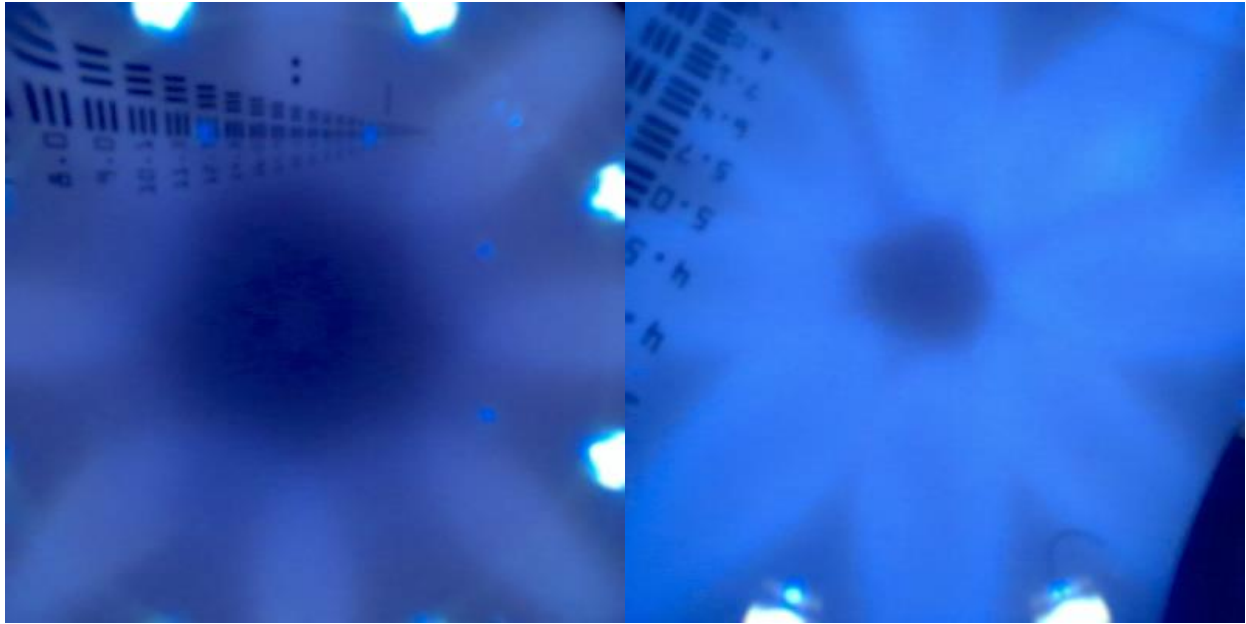


Figure 2.47. Image of dark spot with the Photon Gear objective lens (left), and with the MSU prototype probe (right).

The MSU prototype probes had the LEDs mounted on a ring of radius 6.5 mm, as seen in figure 2.5. The micro-camera had a height of 2.15 mm and was brought into contact with the hyper-hemisphere lens making the LEDs 1.65 mm above the hyper-hemisphere lens. The mock hyper-hemisphere lens created in the MSU lab was made up of a 4 mm radius half-ball BK7 lens and a 10×10×2 mm Square BK7 window from Edmund Optics. Early experiments applied mineral oil to a target or skin and imaged through only the mock hyper-hemisphere lens. A 2 mm BK7 window was added in between the mock hyper-hemisphere and the subject to better match the methods of Caliber ID. Ultrasound gel was used between the mock hyper-hemisphere and the window, and mineral oil was used between the window and the subject. This window and ultrasound gel addition significantly changed the laboratory testing illumination pattern.

The assembly from Photon Gear had the LEDs on a flex board ring of radius 7.225 mm. The other primary difference is that the hyper-hemisphere used in the Photon Gear assembly was a hyper-hemisphere with a radius of curvature 3.6 mm and a thickness of 4.478 mm made from silica. The layer of ultrasound gel was variable but was around 0.4 mm thick and inserted into the cavity of the window shown below. Then the window was an additional 1 mm thick. Below is a figure of the window used by Caliber ID.



Figure 2.48. Window used by Caliber ID for imaging in clinical settings.

Using Zemax's non-sequential modeling, both the prototype probe and Photon Gear assembly were made. The non-sequential modeling mode was used to account for polarization-dependent Fresnel losses for oblique rays, to obtain a more accurate illumination distribution simulation. The LEDs were modeled as rectangle sources that emit in a Gaussian pattern. The following illumination profiles were obtained using a power of 0.1 W per LED. Figure 2.49 shows the illumination pattern for a single LED and figure 2.50 shows the illumination pattern for all eight LEDs. Both simulations include the additional window and ultrasound gel layer.

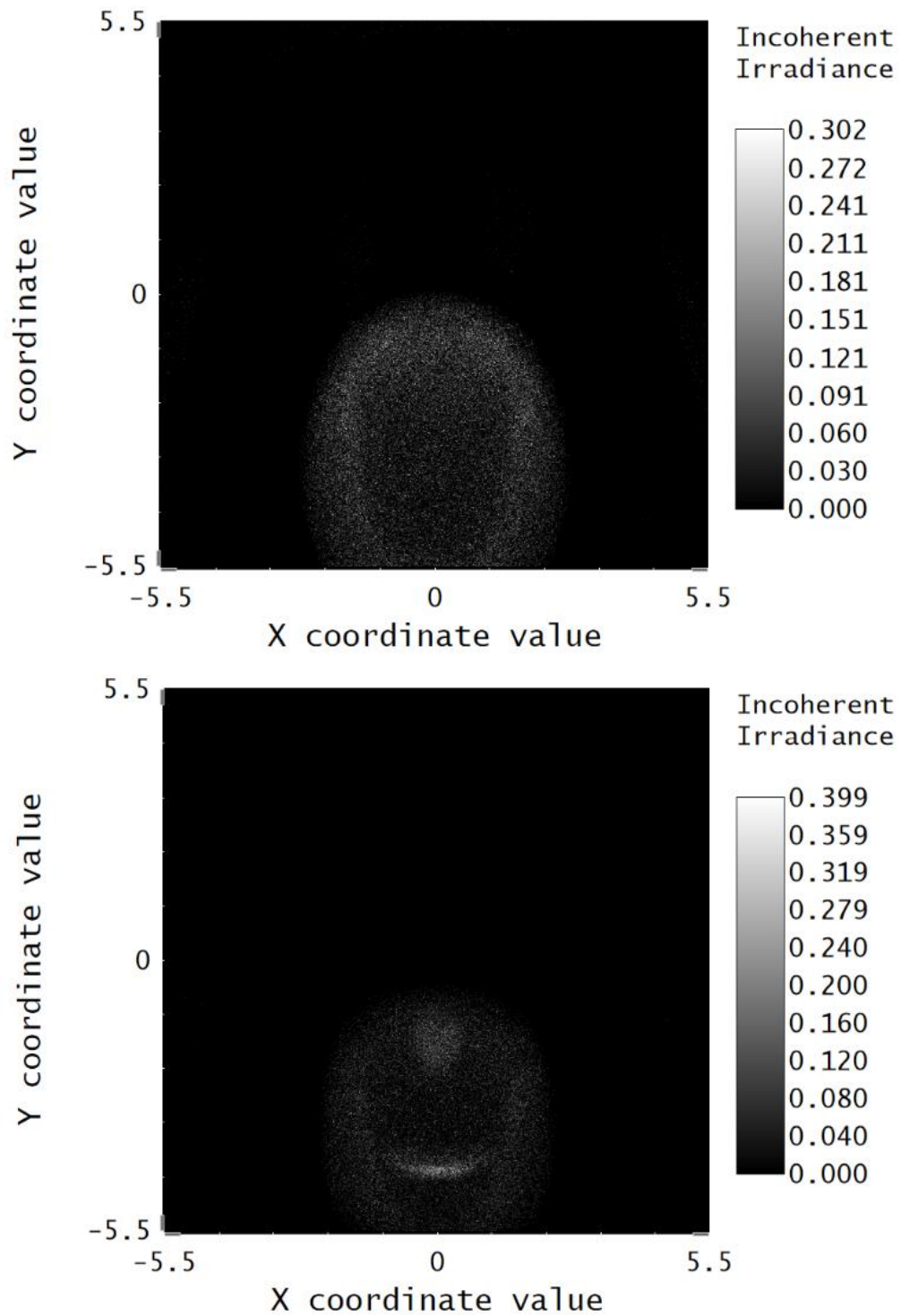


Figure 2.49 Zemax simulations of the illumination pattern from a single LED with a power of 0.1 W, and the additional window for the MSU prototype probe (top) and the Photon Gear objective lens (bottom).

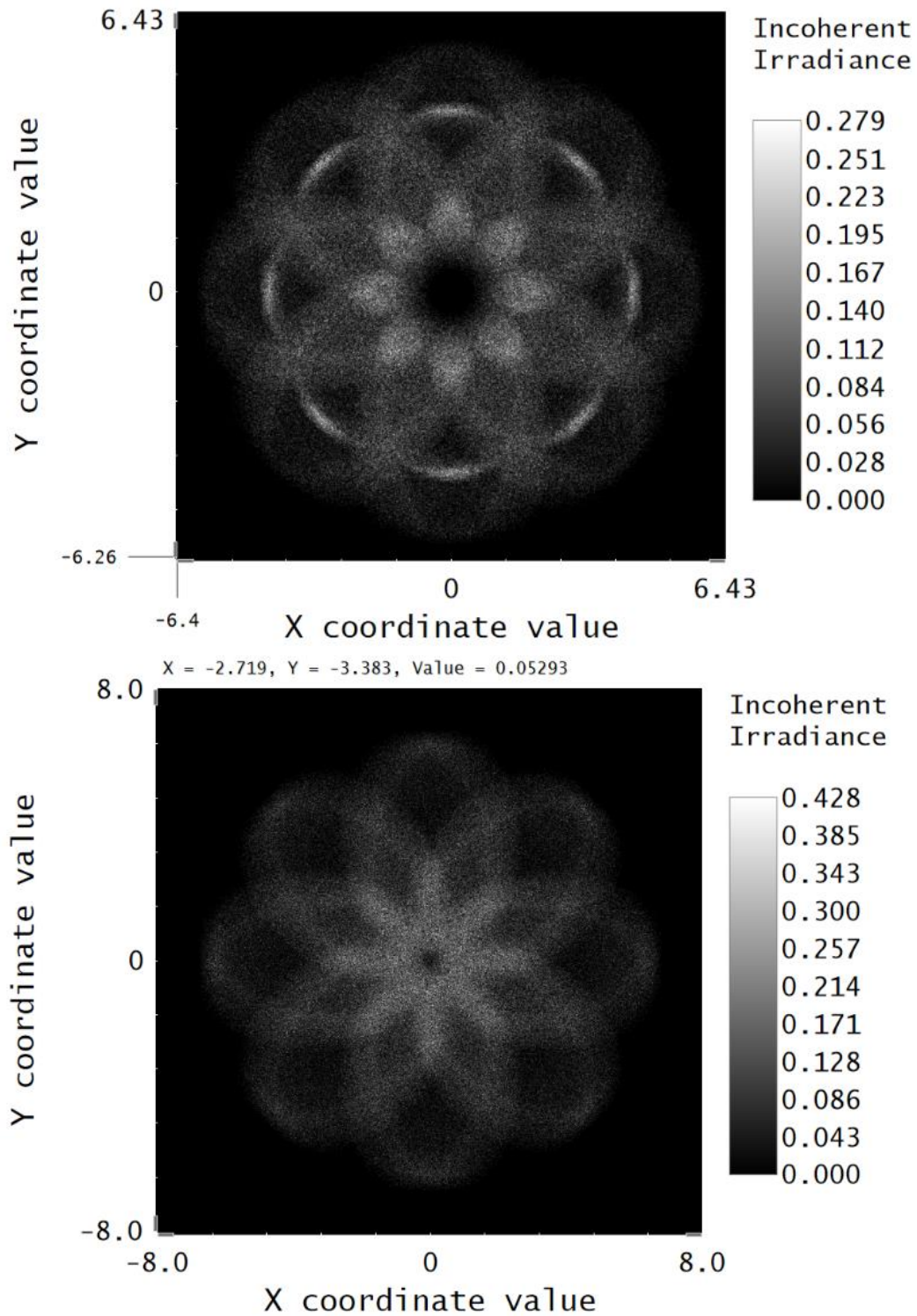


Figure 2.50 Zemax simulations of the illumination pattern for the MSU prototype probe (top) and the Photon Gear objective lens (bottom).

These simulated illumination patterns in Zemax show good agreement with those captured in the lab. The main difference encountered was that the size and severity of the dark spots were not as extreme as was imaged in the lab. However, it was found that part of the cause of the dark spot is an obstruction caused by the mounting of the hyper-hemisphere lens. The mounting point blocks some of light rays from entering the lens and illuminating the skin below. This obstruction was modeled in the above Zemax simulations and showed an increase in the size of the dark spot. The other cause of the dark spot was that the LEDs are located at a radius greater than the hyper-hemisphere lens. So, when the rays from the LEDs pass through the hyper-hemisphere, the angle at which they propagate relative to the optical axis increases. Therefore, when the distance the rays must propagate increases, and an obstruction is placed such that the farthest out rays are vignetted, the rays are entirely missing the center of the image. One proposed solution was to include two LEDs on the bridge of the flex board. A simulation of this is shown in figure 2.49 below. One concern with this solution was that it could introduce hot spots from reflections off the flat surface of the hyper-hemisphere and the ultrasound gel. The index-matching of the gel could minimize this, or it could be removed with a white-balance image if the hot spots did not saturate those sections of the image. A cross-polarized probe could also suppress the direct reflections. Physical prototyping would be required to test this.

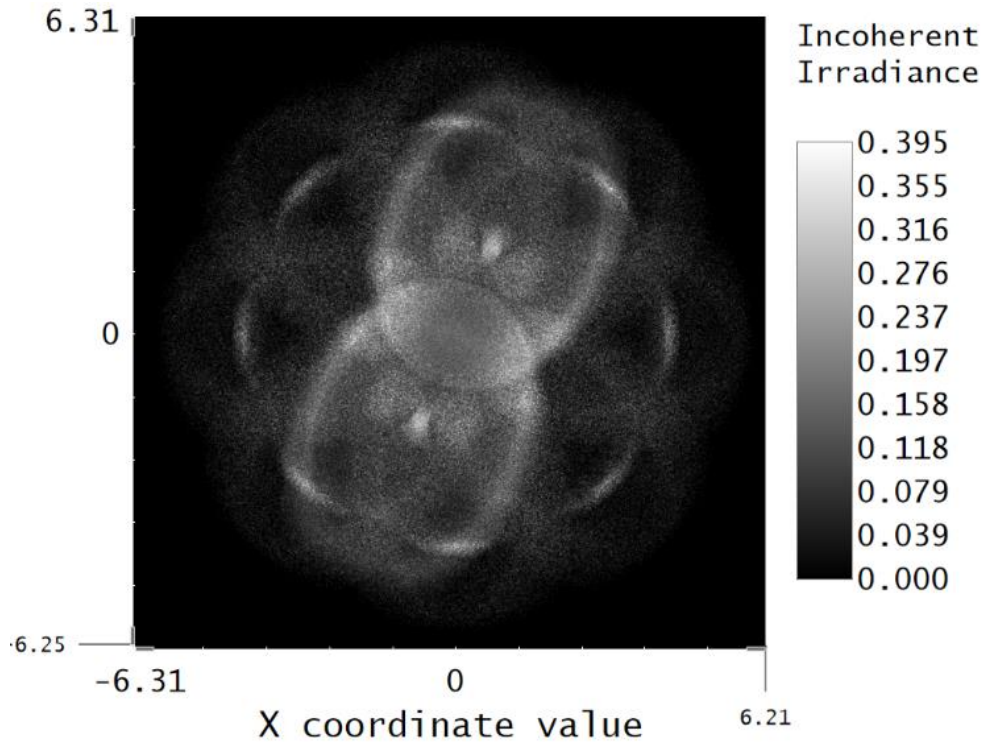


Figure 2.51. Zemax simulation of the addition of 2 LEDs on the bridge of the flex board.

Polarization

Human skin is comprised of multiple layers: stratum corneum, epidermis, dermis, and hypodermis [78, 113, 114]; the stratum corneum is the outermost, followed by the epidermis and then the dermis. Observing each layer of skin may be necessary to diagnose various lesions or cancers accurately. However, observing dermis features can be more difficult due to the other layers' reflections and absorptions. The stratum corneum generally has a normal reflection of 4% - 7% [113], and because the stratum corneum is non-planar nor smooth, the normal reflections are non-specular. Also, when light is reflected out of the skin, internal reflections will occur at the stratum corneum layer, reducing the amount of light that could reach the detector of a camera even more. Within the layers of the skin are inhomogeneities that contribute to the complication of imaging into the skin through various scattering effects. Molecules and particles in the skin

range from a scale of one-tenth of the wavelength of light to much larger than the wavelength of light. The scattering from the small molecules and particles can be modeled as Rayleigh scattering, which is generally weak, nearly isotropic, polarized, and varies inversely with the fourth power of the wavelength. Those particles and molecules whose sizes are of the same order as the wavelength scatter more strongly in the forward direction, modeled as Mie scattering. And the particles and molecules much larger than the wavelength follow the geometric scattering model. Of these multiple scattering types, [113] claims that particles and molecules on the order of wavelength to much larger than the wavelength dominate. A major contributor in the dermis is the collagen fibers. In addition to scattering within the layers, reflections and back reflections at the layer boundaries, and absorption with each layer 93% - 96% of the light that passes through the layers is either scattered or absorbed [113].

Normal reflections from the air to stratum corneum layer account for a much larger percentage of the light reflected toward a detector than the light reflected from the epidermis and dermis. It also increases glare and potential hotspots from the illumination source on the stratum corneum. Thus, making imaging the epidermis and dermis in vivo more challenging. However, normal reflections off the stratum corneum tend to maintain the polarization of the incident light. Therefore, removing the normal reflections off the stratum corneum is possible by polarizing the light source and the detector in perpendicular orientations. The light scattered in the epidermis and dermis layers can experience depolarization as it is scattered. And when the scattered light reaches the detector, it is no longer in the crossed-state [23, 48, 65, 78, 92, 97, 105]. This means that where before, the imaging was mainly of the stratum corneum, now a more significant percentage of the light being imaged is from lower layers. Removing the surface reflections can

also help to increase the contrast. Since the micro-camera in this work was being integrated into the objective of an RCM, this camera was imaging in contact with the skin and with an index-matching medium (mineral oil is the preferred choice from Caliber ID). The index-matching medium works to reduce the normal reflections off the stratum corneum for in contact dermoscopy [48, 92]. Different features can still be observed or made more or less prominent when contact dermoscopy is used based on the system's polarization. The act of putting a lens in contact with the lesion can also change how the appearance for a clinician, for example, flattening the skin and lesion. [92] reports differences in the color sharpness of features within a lesion depending on whether it was polarized. It should be noted that features on the surface and in the deeper layers can both be important for diagnosis depending on the type of lesion [48, 65, 78, 92, 105]. Testing was conducted to characterize cross-polarized, co-polarized, and non-polarized imaging with the micro-camera system for contact dermoscopy with an index-matching medium.

Three separate probes were created to capture images to characterize the cross-polarized, co-polarized, and non-polarized systems. To create co- and cross-polarized probes, a 0.1 mm thin film linear polarizer from American Polarizers Inc. was used. These polarizers had a relatively flat transmission spectrum across the wavelengths associated with the RGB pixel values, as seen in the below figure. Tracking the polarizer's orientation, a ring was cut out of the linear polarizer to cover the LEDs. Then a small square was cut out to be placed over the camera. Then, the ring and square were glued in place using the Norland Products Inc. NOA 68H optical adhesive. The NOA 68H optical adhesive was chosen because it could be cured thermally and with a UV lamp. The polarizers blocked too much of the ultraviolet spectrum for the adhesive to cure with a UV

lamp alone. For one probe, the ring's polarized orientation was set to be 90° from the square polarizer's orientation glued to the camera, thus making a cross-polarized system. The co-polarized system had the polarizer orientation for the LEDs and the camera to be the same. The non-polarized system copied the initial probe design with no polarizers used. Once assembled, the polarization orientations were verified using a mirror to provide a normal reflection with the LEDs on and a live camera feed. The cross-polarized system showed LEDs illumination captured by the camera to be minimized, and the co-polarized system showed LEDs maximized, which was the expected result, see figure 2.54. Figure 2.55 shows the cross-polarized probe imaging through a linear polarizer verifying the polarization axis relative to the image orientation.

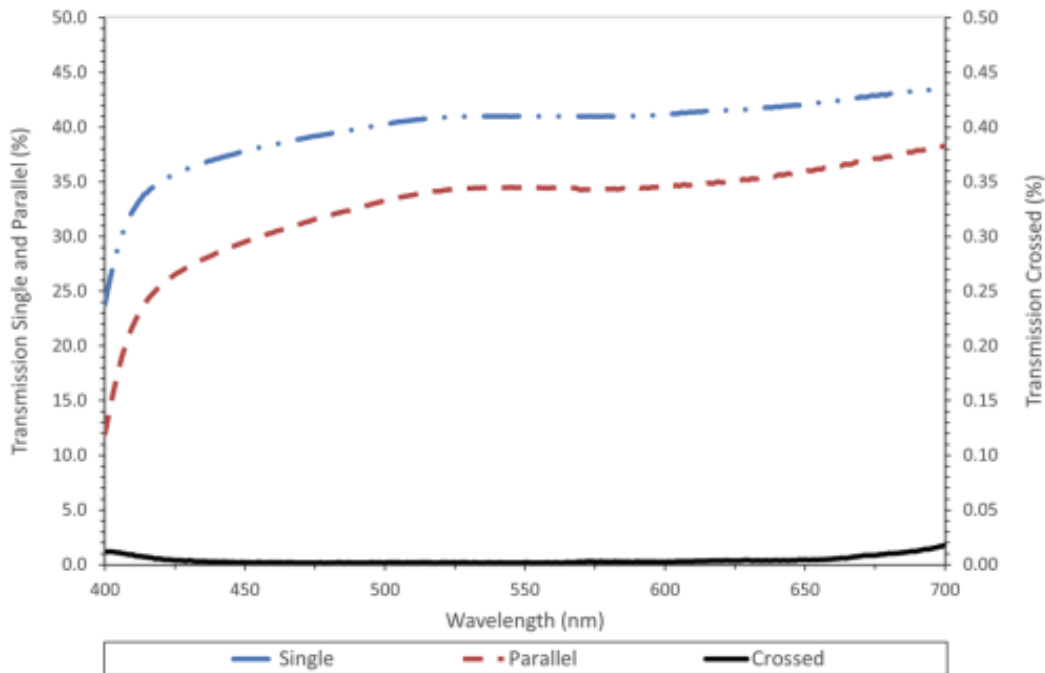


Figure 2.52. Transmission through a single polarizer, polarizers in a parallel state, and polarizers in a crossed state, from the manufacturers datasheet [2].



Figure 2.53. Image of a polarized probe prototype built at MSU.

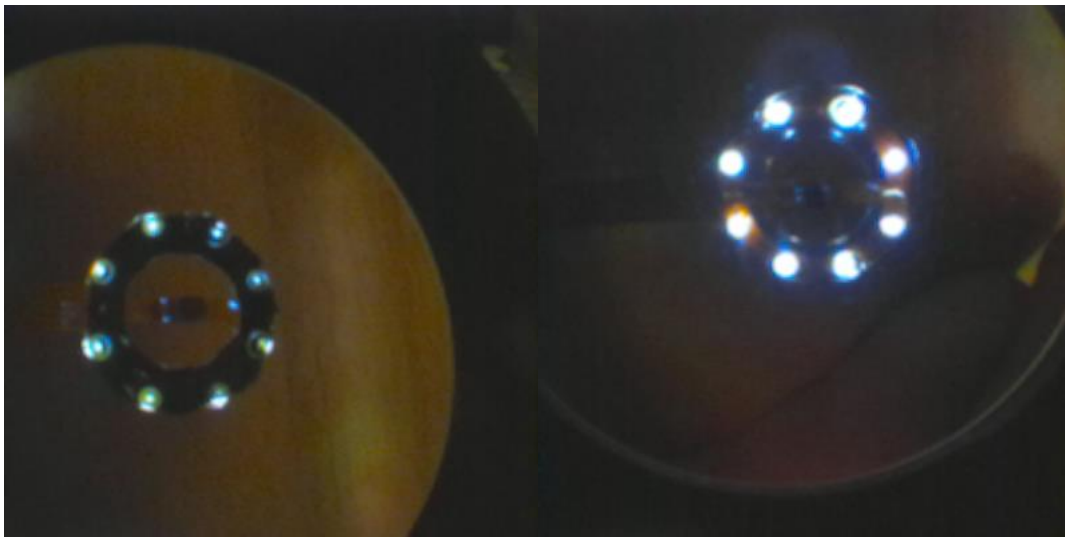


Figure 2.54. Imaging the reflection of the polarized cameras and LEDs off a mirror. Cross-polarized system on the left. Co-polarized system on the right.



Figure 2.55. Linear polarizer in front of sticky note with the polarization axis labeled on the polarizer. The camera's polarizer is oriented perpendicular to the polarizer's polarization axis on the left. The camera's polarizer is oriented parallel to the polarizer's polarization axis on the right.

CHAPTER 3

LABORATORY PERFORMANCE

MTF Characterization

The Modulation transfer function (MTF) is a widely used analysis method for scientific imaging performance. It describes the ability of an imaging system to transfer spatial frequencies from the object to the image. MTF is a measure of the contrast of an image as a function of spatial frequency, and it quantifies how well the imaging system can distinguish and resolve fine details in an image. MTF analysis helps engineers optimize imaging systems for specific applications, not just standard digital color CMOS camera imaging. For example, in microscopy, MTF can be used to optimize the system for high-resolution imaging of low-contrast samples. In astronomy, MTF can be used to optimize telescopes for imaging distant objects with fine details, such as stars and galaxies. The MTF can also be helpful in image processing to measure improvements to image quality. The MTF is typically measured using test targets such as the United States Air Force-1951 Standard Resolution Target (T-20) from Applied Image Inc, used for measurements in this thesis. Test targets like this one have high-precision series of lines of other patterns of known spatial frequency, typically printed black on white. And the MTF can be calculated by measuring the contrast of the black and white lines when imaged onto the camera's sensor. MTF can also be measured from an edge response, which for a digital camera will be accomplished using the slanted edge technique [107, 108].

The primary use of MTF in this work was to evaluate the quality of the micro-camera and how its performance changed when integrated into the RCM objective lens. For this work, the

MTF was calculated by analyzing a slanted edge using the `sfrmat4.m` MATLAB function [107, 108]. This method is common in digital image analysis, providing a precise and objective measurement of the camera or lens's spatial frequency response. The basic principle behind the slanted edge method is to analyze the edge spread function (ESF) of the system. The edge is tilted slightly relative to the pixel grid in order to improve sampling density across the edge. The ESF describes how the image intensity varies across the edge and is obtained by measuring the brightness values along a line that is perpendicular to the edge. The slanted edge target is used to create an edge with a sharp transition from black to white, with a known angle between the edge and the horizontal or vertical axis of the image. The algorithm in `sfrmat4.m` applies a polynomial edge fitting for images where the imaging system distorts the slanted edge [107, 108]. A curve is fitted to the ESF to obtain a smooth function describing the brightness or intensity variation across the edge. Then, the derivative of the fitted ESF curve is calculated to obtain the line spread function (LSF). The LSF describes the system response to an infinitesimally wide line parallel to the edge. The MTF curve is obtained by taking the Fourier transform of the LSF. In these experiments, a “vertical” edge typically used to compute the horizontal MTF, the MATLAB function `sfrmat4.m` rotates the image if the image is taken vertically.

When imaging with the micro-camera through the hyper-hemisphere with the ring of eight LEDs as the illumination source, a non-uniform illumination pattern became present in the image. As described in the Black-Background Subtraction and White-Balance Normalization section, a black-background subtraction and white-balance function is used to help reduce the severity of the non-uniform patterns. While this function did a good job of evening out the illumination, on a target that was black and white, the effects were still noticeable. And since, as

described above, calculating the slanted edge uses the intensity of the image line by line, changes in the intensity of the image that do not correspond to the slanted edge will degrade the calculated MTF. Therefore, generating several radically different MTF curves was possible by sending different sized sections of a slanted edge image through the function. This showed the necessity for an accurate reference MTF curve for verification. A purely theoretical system MTF curve was created using mathematical models to obtain this reference. This reference MTF curve needed to account for the Bayer mask demosaicing, and defocus.

The theoretical system MTF was calculated by multiplying the individual MTF contributions from the finite sized pixels, Bayer mask and interpolation, and the defocus through the hyper-hemisphere. For these calculations, the MTF would be the luminance MTF, weighting the red, green, and blue channels by 0.3, 0.6, and 0.1, respectively, following the convention used in [109]. The MTF contribution from the Bayer mask and gradient-corrected interpolation is derived in the Gradient-Corrected Bilinear Bayer Mask Interpolation section. The contribution from the pixel size is known as the pixel-limited MTF and comes from the Fourier transform of the pixel shape, a square, which transforms in one dimension to a sinc function in the frequency domain. In equation 45, f is in units of cycles per pixel.

$$\text{Pixel Limited MTF} = |\text{sinc}(f)| \quad (45)$$

The diffraction limit is the fundamental limit to the resolution of an incoherent imaging system. Meaning that the diffraction limit sets the lower bound on the size of the smallest resolvable details in an image. The diffraction limit is determined by the wavelength of light and the NA of the system. The smaller wavelength of light and the larger the NA, the better the resolution of the system [115, 116]. Below are the equations describing the diffraction limited

MTF for an incoherent system. For this work, it was assumed that the pupils of this system were aligned to the principal planes of the system. In these equations, λ is the wavelength of the light, z_2 is the distance from the exit pupil to the sensor plane, w is the diameter of the exit pupil, ρ is radial distance in the frequency plane, ρ_o is the cutoff frequency for coherent imaging, the cutoff frequency for incoherent imaging occurs at $2\rho_o$ [115]. Multiplying by the pixel size in the cutoff frequency equation made our MTF in terms of cycles per pixel.

$$\rho_o = \frac{\frac{w}{2}}{\lambda z_2} (\text{pixel size}) = \frac{NA}{\lambda} (\text{pixel size}) \quad (46)$$

$$\text{Diffraction Limited MTF} = \left| \frac{2}{\pi} \left(\cos^{-1} \left(\frac{\rho}{2\rho_o} \right) - \left(\frac{\rho}{2\rho_o} \right) \sqrt{1 - \left(\frac{\rho}{2\rho_o} \right)^2} \right) \right|, \quad (47)$$

$$\rho \leq 2\rho_o$$

The aberration defocus is an optical aberration that occurs when an optical system is imaging at a location other than its focus. In an ideal system, with no defocus, light from a point source will converge to a point corresponding to the diffraction limit in the image plane, but in a system with defocus, the light will converge to a blurred circle. The wavefront coefficient for defocus describes how the wavefront of light changes as it passes through a defocused optical system [116, 117]. For pupil plane aberrations such as defocus, the diffraction effects are included in the evaluation of the Fourier transform over the finite exit pupil size. The defocus will degrade the MTF relative to the aberration-free pupil, which has a diffraction limited MTF. The first step in deriving the MTF for a defocused system is to define the Seidel polynomial function and its components [117]. The Seidel polynomial is described in equation 48 where W_{ijk}

represents the wavefront aberration coefficients. The factor H is the fractional image height from 0 to 1. The ρ term is the fractional pupil radius from 0 to 1. The rotational pupil coordinate is given by the θ term. The Seidel polynomial is made from the sum of wavefront aberration coefficients, where the “ijk” subscripts for the wavefront aberration coefficients are an index, with each wavefront aberration coefficient represented by the numbers. The order of the term in the polynomial is the sum of the i and j subscripts. For example, the defocus wavefront aberration W_{020} is a second-order term, and the spherical wavefront aberration coefficient W_{040} is a fourth-order term. The defocus wavefront aberration W_{020} term is dependent of the difference between the distance from the exit pupil to the object and the distance from the exit pupil to best focus. Wavefront aberration for defocus, W_{020} , is shown in equation 49, where z_1 is the distance from the entrance pupil to the calculated best focus of the system. The object distance is measured from the object to the entrance pupil. w is the radius of the exit pupil. and λ_n is the wavelength of light normalized for the index of refraction of the medium. If z_1 and the object distance are equal, then the system is in focus and the calculated MTF would match the diffraction limited MTF. Using the equation 4 from the Camera Characterization section and using the MSU mock hyper-hemisphere with a radius of curvature of 4 mm, z_1 equals 6.288 mm and the object distance is 6 mm. Using those values, W_{020} was calculated to be -0.0195 waves for the blue wavelength 467 nm, -0.0173 waves for the green wavelength 527 nm, and -0.0146 waves for the red wavelength 624 nm. This was a small amount of defocus, so the generated MTF was expected to look similar to the diffraction limited curve. Using the Seidel polynomial, the pupil function describes the phase in the system's exit pupil. The pupil function is shown in

equation 50, where A is the proportional strength to the electromagnetic field in the exit pupil and is typically set 1, and W is the Seidel polynomial.

$$\text{Seidel Polynomial} = W = \sum_{i,j,k} W_{ijk} H^i \rho^j \cos^k \phi \quad (48)$$

$$W_{020} = -\frac{1}{2} \left(\frac{1}{\text{object distance}} - \frac{1}{z_1} \right) \frac{w^2}{\lambda_n} \quad (49)$$

$$\text{Pupil Function } P = A e^{i(2\pi)W} \quad (50)$$

For this incoherent system, the square magnitude of the Fourier transform of the pupil function yields the point spread function (PSF). And the MTF was calculated by taking the normalized Fourier transform of the PSF, which is represented by equation 51 from [115], which is the autocorrelation of the pupil function. Since these equations are wavelength dependent, the MTF must be calculated separately for the RGB channels, then weighted to calculate the luminance, as disclosed above. The equations from [115] are applied to calculate the defocused MTF in a MATLAB code written by Dr. David Dickensheets, seen in Appendix B.

$$MTF^{defocus} = \left| \frac{\iint_{A(f_x f_y)} e^{jk \left[w \left(x + \frac{\lambda z_1 f_x}{2}, y + \frac{\lambda z_1 f_y}{2} \right) - w \left(x - \frac{\lambda z_1 f_x}{2}, y - \frac{\lambda z_1 f_y}{2} \right) \right]} dx dy}{\iint_{A(0,0)} dx dy} \right|^2 \quad (51)$$

The complete system MTF was found by multiplying each individual MTF component together. The theoretical MTF for the system is shown in figure 3.1. In this figure, each MTF component shown with a dashed line is its individual contribution. This curve followed the

trends of the initial characterization of the camera without the hyper-hemisphere lens and was verified with simulated results.

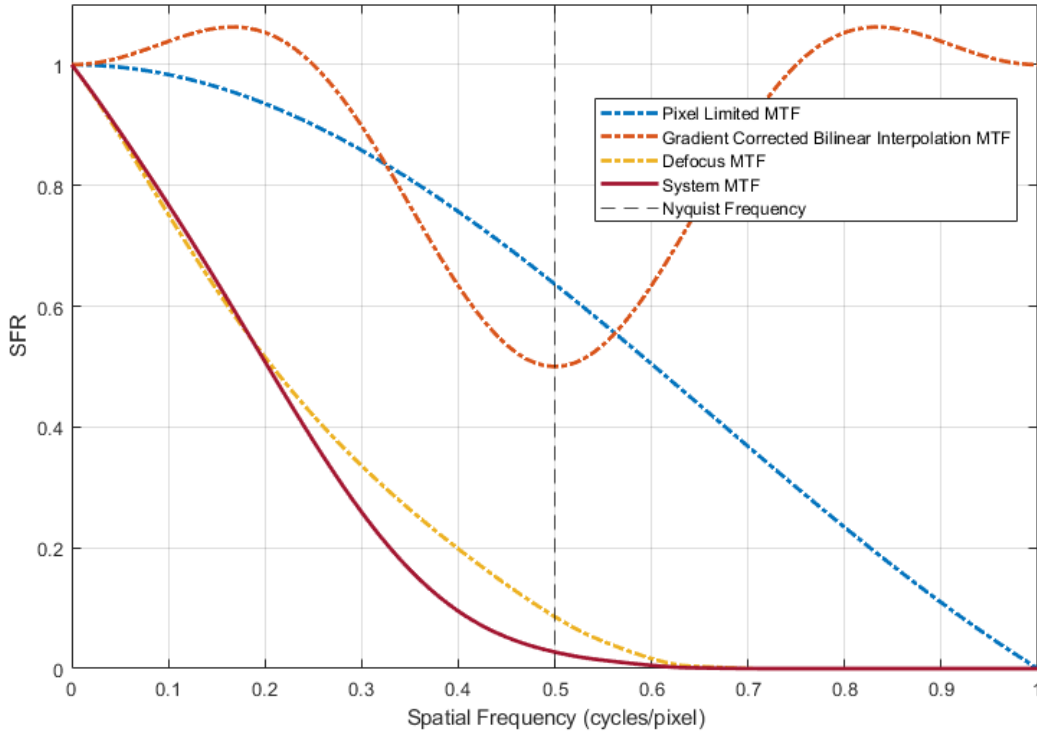


Figure 3.1. Complete theoretical system MTF with individual MTF components shown.

Polarized Probe MTF Testing

MTF and color analysis were performed to characterize the three probes. MTF testing was conducted using slanted edge analysis through the `sfrmat4.m` from [108]. This MATLAB function took in an image of a slanted edge and generated an MTF curve. The slanted edge function `sfrmat4` could work with a grayscale image or a color image where it generates an MTF curve for each RGB channel and the luminance, which is a customizable weighting of the three channels. To best match the theoretically calculated and simulated MTF curves, the luminance weighting for all the `sfrmat4` generated MTF curves was 0.3 for the red channel, 0.6 for the green

channel, and 0.1 for the blue channel, same as was used for the theoretical calculations. Another setting in sfrmat4 that was manipulated was the line fitting. Sfrmat4 can conduct a polynomial line fit for images with distortion to better characterize the image quality. After analyzing the images captured with the probes, a quadratic fit was chosen. The slanted edge images were taken on a chrome on opal glass Airforce resolution target with the LEDs set to a brightness that did not cause saturation and with mineral oil in-between the camera lens and the target. The opal glass target was used to better approximate the scattering and depolarization of skin. Specular reflections from the chrome regions are localized and outside of the region used for edge response measurements, so the chrome areas are the dark part of the edge. The opal glass scatters the light, generating the bright part of the edge. The images were taken in a RAW uint8 (0-255) setting so that no external processing was done to the images. Then, the images were sent through the black-background subtraction and white-balance normalization and a gradient-corrected bilinear interpolation function described in previous sections, respectively. After some initial image analysis, it was noted that even after the black-background subtraction and white-balance normalization, the non-uniform illumination field caused by LEDs was still present to some degree. Because of this, only a small region near the center of each image was used for the sfrmat4 MTF generation. If the whole image was used, the non-uniform illumination would cause extreme degradation of the MTF curve.

Data was collected for each probe on several days with slightly varying illumination intensity, how centered the slanted edge was, and the angle of the slanted edge. The lenses were also interchanged between the probes throughout the testing in case of a defect in one lens. The settings and relative size of the region of interest in the image were held constant throughout the

testing. Once the data was collected, all the MTF curves were imported to MATLAB, and ten curves with the highest 50% SFR frequency were used to calculate the average best 50% SFR frequency for each probe. Only the highest ten frequencies from each data set were used because some of the curves suffered more than others from the non-uniform illumination and caused MTF curves to have significant discrepancies from the theoretical MTF curve described in the MTF Characterization section. The only source of these discrepancies found through simulated images and MTFs was the non-uniform illumination changes throughout the region of interest. From the best ten MTF curves for each probe, the average 50% SFR, and their 50% SFR range are shown in table 3.1.

	Average 50% SFR	Minimum 50% SFR	Maximum 50% SFR
Non-polarized probe	0.1155 cycles/pixel	0.099 cycles/pixel	0.143 cycles/pixel
Cross-polarized probe	0.1663 cycles/pixel	0.144 cycles/pixel	0.188 cycles/pixel
Co-polarized probe	0.1417 cycles/pixel	0.13 cycles/pixel	0.154 cycles/pixel

Table 3.1. Average 50% SFR and the range of 50% SFR values for the ten best MTF curves for each probe.

The ten MTF curves for each probe can be seen in figure 3.2. The cross-polarized probe shows an improvement over the co-polarized and non-polarized probes. However, there was overlap of many of the MTF curves in figure 3.2, and inconsistencies within the curves for each probe. It was believed that the non-uniform illuminations contributed to the variance in the results. Figure 3.2 also shows the theoretical MTF curve calculated above superimposed on the plot. This theoretical MTF shows a higher SFR over the spatial frequencies than the measured data. This was to be expected. The theoretical data did not account for non-uniform illumination

or other aberrations that could affect the system. However, the overall shape and decay of the curves showed good agreement, suggesting that they were meaningful and useful for this analysis. The similarity in the probes' performance using MTF as a metric was not surprising because of the index-matching medium and being in contact with the skin. The index-matching already helps to reduce the normal reflectance off the stratum corneum, so the cross-polarized system only removes light scattered in the same polarization as the LEDs polarizer. And due to numerous epidermal and dermal inhomogeneities, a large portion of the light scattered back out of the skin did not maintain the LED's initial polarization. Due to the variance of these results, further tests of the MTF with the polarized probes should be conducted to confirm its improvement. From these results it could be concluded that the polarization of the probes did not negatively affect the probes performance, but suggested an improvement. The next test was to check any color affects and then send the devices to clinicians for patient trials to see if the probes image the features of lesions differently.

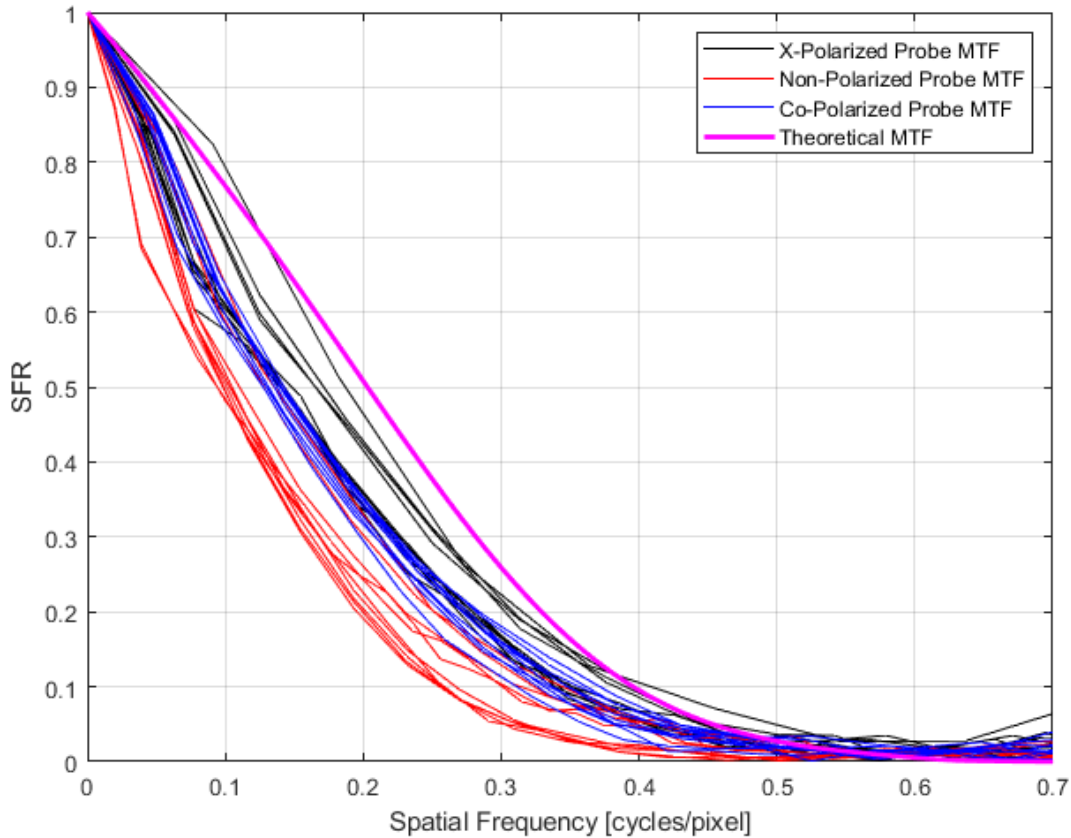


Figure 3.2. Ten highest cross-polarized MTF curves (black). Ten highest co-polarized MTF curves (blue). Ten highest non-polarized MTF curves (red). Theoretical System MTF (purple).

Polarized Probe Color Analysis

The color analysis was performed to identify any colorimetry changes caused by adding polarizers to the probes. Since the video and images captured by these probes would be used to diagnose skin lesions, the accuracy of the color of the images was essential. Understanding how or if the color of an image changed with the polarizers allowed for color correction in post-processing. To find changes in the color, a baseline color test of the OVM6946 camera with no polarizer and the non-polarized probe was needed for reference. The ISA ColorGauge Pico Target was used for these tests. This target contains 18 color patches surrounding 12 grayscale patches. ISA provided an Excel spreadsheet with the RGB values of each patch calibrated for

multiple color spaces. RAW images of the 30 patches are taken, adjusting the LEDs prior to the image acquisition so that no patch was saturated. Then the RAW images are sent through the black-background and white-balance normalization followed by the gradient-corrected bilinear interpolation MATLAB functions. Then, the color image was sent through another MATLAB function that allows the user to draw a box over a single patch. The function then calculates the RGB values for that patch by averaging the separate RGB values for each pixel contained in the drawn box. This function was repeated for all 30 patches. The process was then repeated for images taken with polarized probes.

This stage of color analysis was primarily looking at how the polarizers on the LEDs and camera change the color information. Adjustments of the colors to a usable color space are explained in the Color Spaces and Color Correction section. To this end, the non-polarized probe's color information could be used as a reference since no changes were made to the camera or LEDs. Observing all thirty patches, the maximum difference between the RGB values from the non-polarized probe and the cross-polarized probe were: 8.547, 8.2093, and 8.76757. The average difference for the RGB channels were: 2.1174, 2.4476, and 2.5761. For the non-polarized probe to the co-polarized probe, the maximum differences in the RGB channels were: 7.197, 8.3368, and 9.0335. With the average differences being: -2.8846, -2.9703, and -3.1012. The actual data takes the form of integers from 0-255. As explained above, the non-integer values seen here result from averaging the RGB values from multiple pixels. From these results, the most significant difference equates to about 4-bits of data, and the average was about 3-bits of data. This difference is significantly less than the error between the measured RGB values and the RGB values provided as a reference from ISA for each patch. Figure 3.3 and 3.4 show side-

by-side comparisons of the non-polarized color patches to the co- and x-polarized color patches. Therefore, since color correction was already going to be applied as one of the post-processing steps, the difference in color caused by the addition of the polarizers does not provide a reason not to pursue a cross-polarized probe.

Further verifying that the polarization of the probes did not significantly affect the color of the images, the color patch data collected from the probes were sent through the color correction algorithms and compared to the reference color spaces as before. The below tables and figures show the results of this experiment. These results were similar to those with the non-polarized probe; see the Color Spaces and Color Correction Section. Thus, it can be concluded that the polarization of the probes does not affect the color of the images in a significant way.

Error from corrected values to the reference values	RMS Error in Red Channel	RMS Error in Green Channel	RMS Error in Blue Channel
Least Squares Approximation	8.88636	6.24679	11.9836
1 st Order Polynomial Fitting	13.8325	12.6634	13.7102
Color Dependent 1 st Order Polynomial Fitting	13.4578	12.6457	13.1491
2 nd Order Polynomial Fitting	12.9471	11.9251	13.0782
Color Dependent 2 nd Order Polynomial Fitting	12.5227	11.9153	12.5232
3 rd Order Polynomial Fitting	12.7143	11.7329	12.3138
Color Dependent 3 rd Order Polynomial Fitting	12.3349	11.7171	12.3138
Gamma Correction	24.5403	27.5661	30.3561

Table 3.2. RMS error for each color channel for the corrected cross-polarized RGB values to the reference Adobe RGB color space values for each method of color correction.

Error from corrected values to the reference values	RMS Error in Red Channel	RMS Error in Green Channel	RMS Error in Blue Channel
Least Squares Approximation	7.93254	6.30097	12.8475
1 st Order Polynomial Fitting	13.2952	12.4755	13.1869
Color Dependent 1 st Order Polynomial Fitting	12.8892	12.4573	12.5706
2 nd Order Polynomial Fitting	12.9385	12.1141	12.8946
Color Dependent 2 nd Order Polynomial Fitting	12.5139	12.1036	12.926
3 rd Order Polynomial Fitting	12.2674	11.5548	12.3089
Color Dependent 3 rd Order Polynomial Fitting	11.919	11.5284	11.7037
Gamma Correction	29.1845	32.1694	35.4648

Table 3.3. RMS error for each color channel for the corrected co-polarized RGB values to the reference Adobe RGB color space values for each method of color correction.

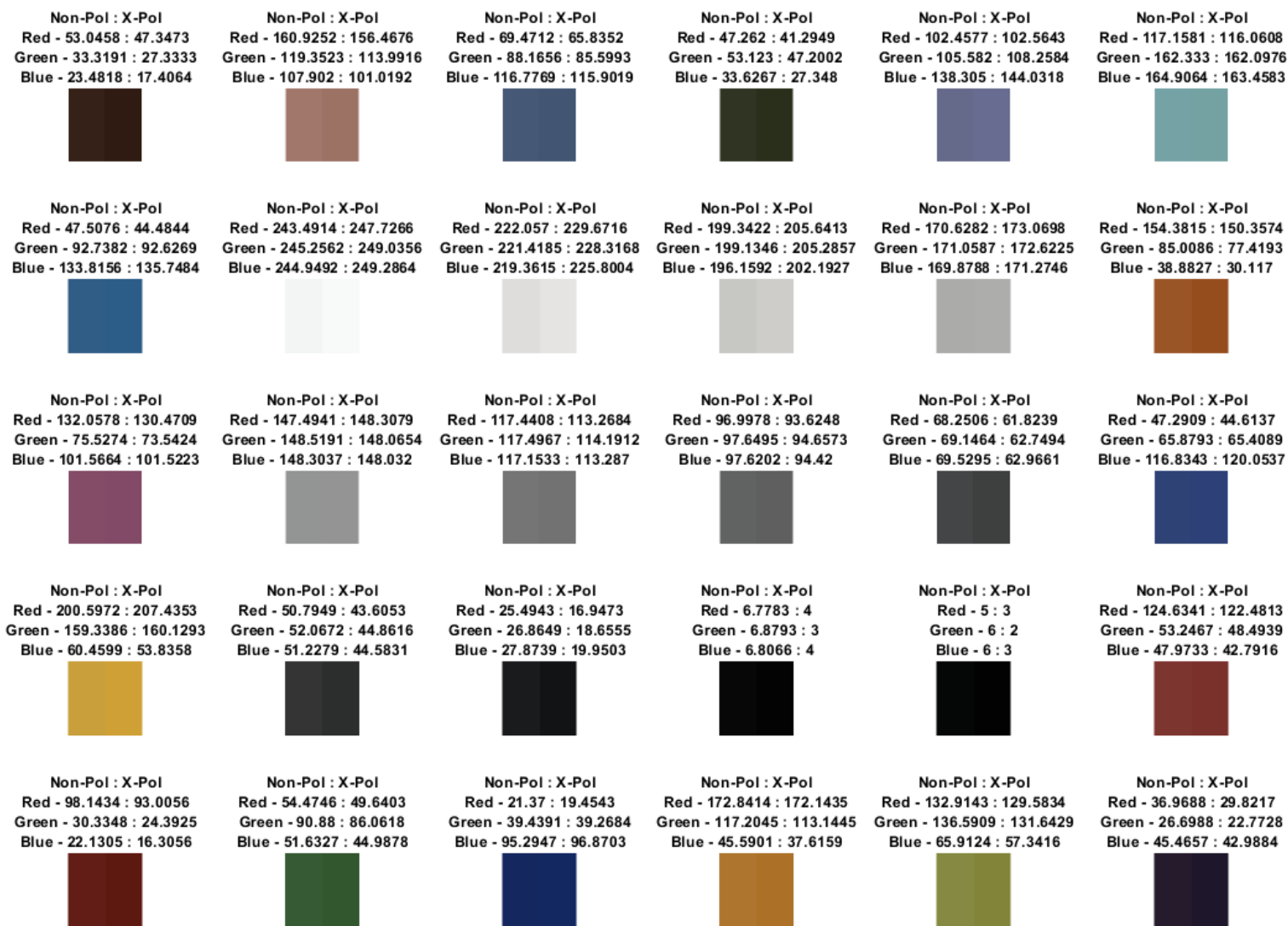


Figure 3.3. Non-polarized to cross-polarized ISA ColorGauge Pico Target values.

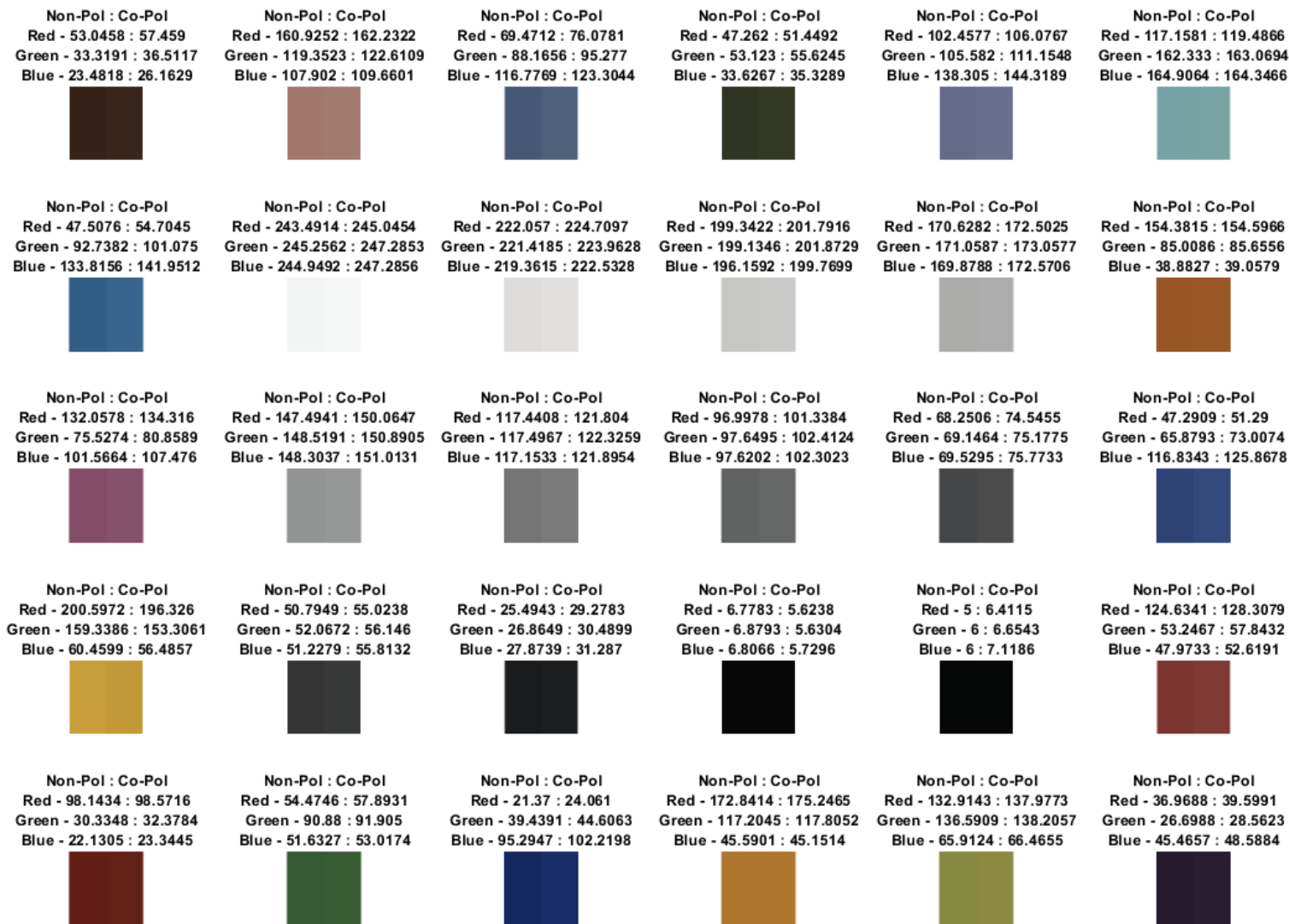


Figure 3.4. Non-polarized to co-polarized ISA ColorGauge Pico Target values.

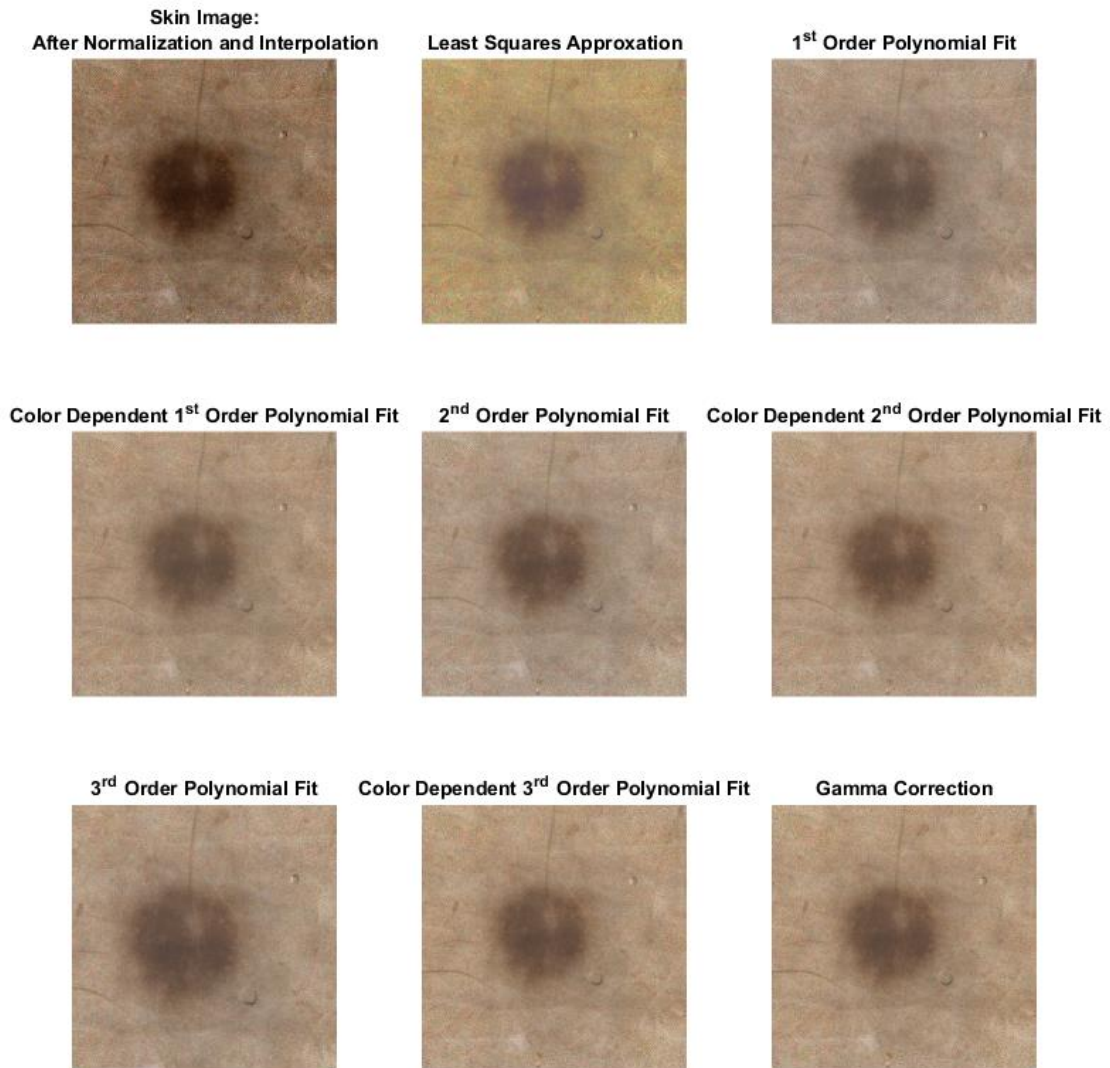


Figure 3.5. Cross-polarized skin image after normalization and interpolation (top left). The other eight images show the results of the eight color correction methods tested.

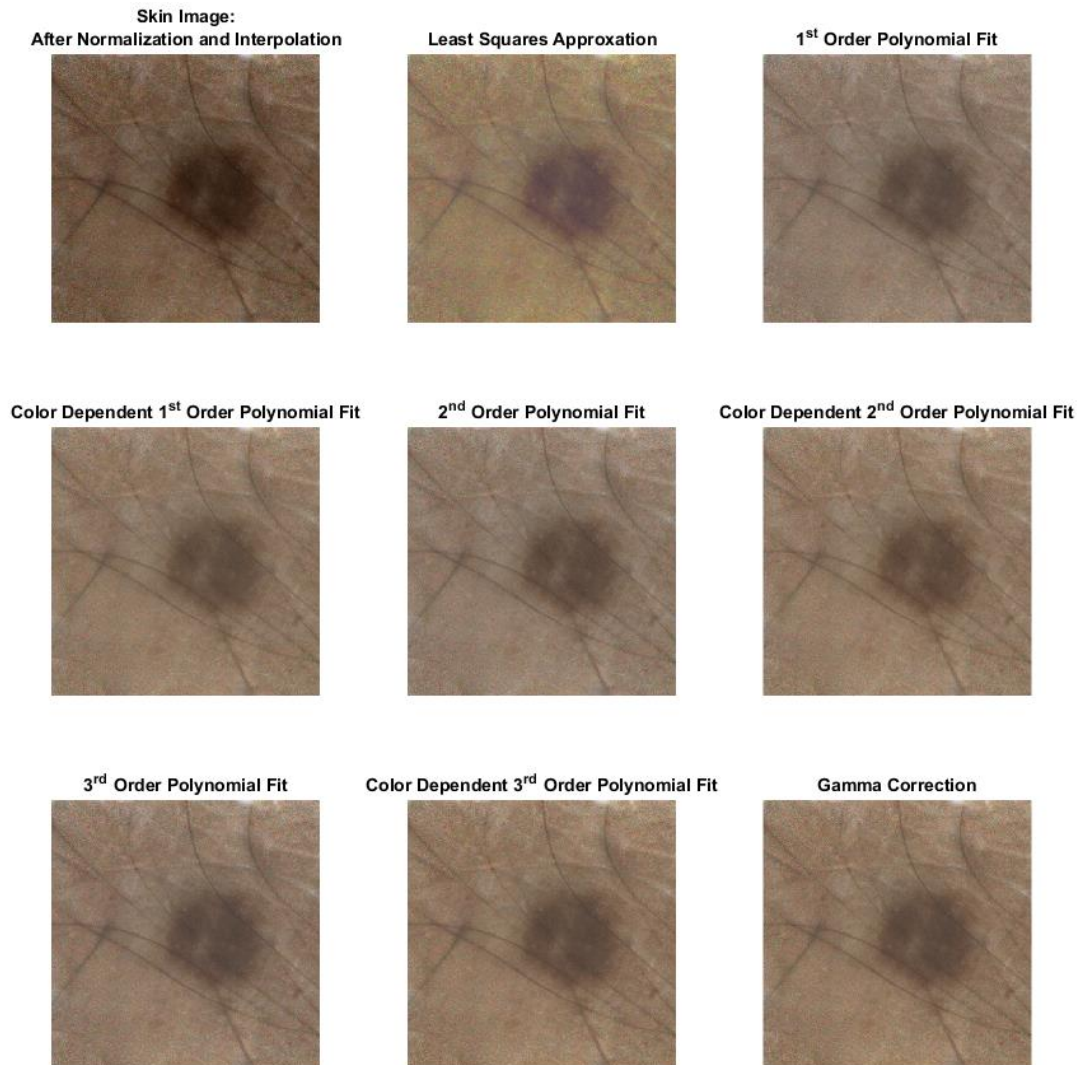


Figure 3.6. Co-polarized skin image after normalization and interpolation (top left). The other eight images show the results of the eight color correction methods tested.

Clinical Testing at MSKCC

The co-, non-, and cross-polarized probes were sent to MSKCC for testing on lesions by their scientists. The goal of these initial tests was to get assessment of image appearance and quality from collaborating researchers with experience in observing skin lesions. These three probes did not include the additional window used by Caliber ID that caused the dark spot in the illumination field. Therefore, the images acquired by MSKCC were only representative images.

Figures 3.7-3.9 are composed of frames exported from videos captured at MSKCC of three different lesions. The cross-polarized probe captured images (rightmost image in all the figures) show reduced surface reflected glare and enhanced subsurface detail of morphology and pattern of pigmentation and blood capillaries that the co- and non-polarized probes did not. One of the features identified was the red from the blood capillaries under the surface of the skin are more prominent in the cross-polarized images such as in figures 3.8 and 3.9. The ability of the cross-polarized probe to image sub-surface structures and reduce the surface glare off the skin provides evidence to pursue integration of cross-polarized illumination in the fully integrated widefield and RCM system.



Figure 3.7. Image of a lesion captured by MSKCC. Non-polarized image on the left. Co-polarized image in the center. Cross-polarized image on the right.



Figure 3.8. Image of a lesion captured by MSKCC. Non-polarized image on the left. Co-polarized image in the center. Cross-polarized image on the right.

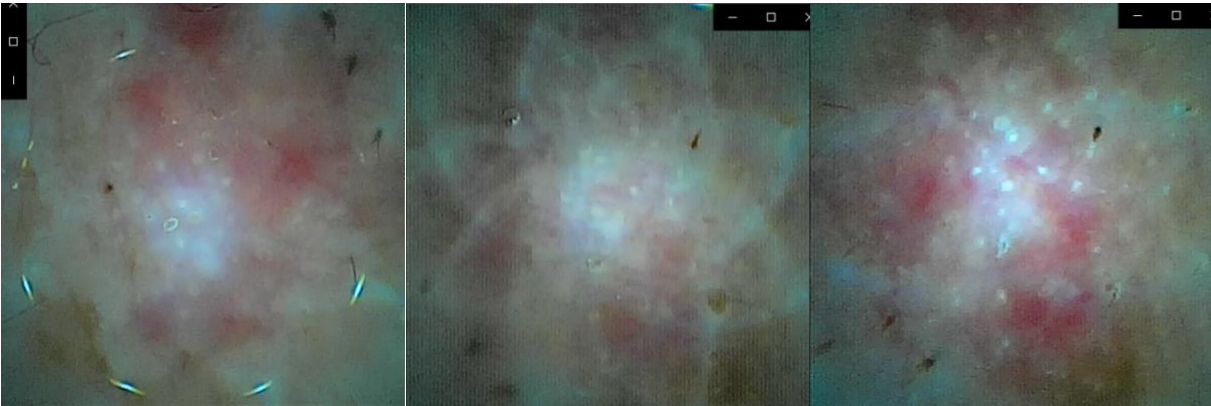


Figure 3.9. Image of a lesion captured by MSKCC. Non-polarized image on the left. Co-polarized image in the center. Cross-polarized image on the right.

CHAPTER 4

CONCLUSIONS

This work has demonstrated how a widefield micro-camera and illumination system can be integrated into the objective lens of an RCM for concurrent image registration. Within the constraints of the RCM system, the micro-camera and illumination profile were characterized and tested. Image processing methods were employed to enhance the images and correct artifacts of the system. The use of a cross-polarized micro-camera and ring LED system was investigated. The cross-polarized probe showed an improvement in the measured MTF. And scientists at MSKCC reported that the cross-polarized probe images sub-surface features in the skin. Integrating these two imaging modalities opens new avenues for non-invasive, high-resolution imaging of skin disease, progressing towards the aims of skin cancer care; to enhance the patient's standard of living by minimizing any discomfort and negative health impacts resulting from biopsies and excisions. A brief summary of the work follows, as well as suggestions for the direction of future research.

Summary of Significant Work

Physically constrained by the RCM's objective lens and the requirement to not significantly impede the RCM's beam, a micro-camera and flex board design was chosen. The OV6946-RAJA with dimensions of $1100 \times 1100 \times 2266 \mu\text{m}$ and a 400×400 pixel array was chosen. Of the choices of micro-cameras presented, this one had the highest pixel count and, therefore, resolution. This micro-camera was mounted onto the bridge spanning across a flex board ring. This bridge was an obstruction in the RCM beam within the objective lens, changing

the pupil to a split annular pupil, which was shown to not degrade the optical sectioning of the RCM. A series of eight white LEDs were mounted around the ring of the flex board to provide illumination for the micro-camera. The flex board fits into the objective lens of the RCM such that the camera was in contact with a hyper-hemisphere lens, the first lens in objective housing. The back lens group was behind the flex board.

The only external lens the micro-camera images through was a hyper-hemisphere lens, the last element of the RCM's objective lens. This lens was custom-made for the system by Photon Gear Inc. and was expensive and hard to acquire in mass quantity for laboratory testing at MSU. Therefore, an approximation of the hyper-hemisphere was made using a half-ball lens and window from Edmund optics. This allowed for rapid prototyping to obtain representative images to characterize the micro-camera system.

The OV6946-RAJA micro-camera that was chosen could output 10-bit or 8-bit RAW images and video. 10-bit data requires 2-bytes to be stored, whereas 8-bit data only requires a single-byte to be stored. The information contained in each bit was investigated with several images under various conditions. With visual inspection and an analysis of the Fourier transform of a high-contrast distortion target, it was found that, at best, only 5-bits of the data contained useful information. It was speculated that dark current noise was the primary contributor to the loss of information. From this result, capturing and saving 10-bit data was deemed unnecessary, and 8-bit images and video were used.

Techniques for processing RAW images were tested to show the potential for improving image quality. Two methods of demosaicing the Bayer mask to create a color image were tested, bilinear interpolation and a gradient-corrected bilinear interpolation. While the bilinear

interpolation only accounted for the nearest neighbors of the color being interpolated, the gradient-corrected method accounts for the change in the luminance of pixels with the same mask filter. This made the interpolation account for sharp transitions better. And an analysis of the MTF contribution for each method showed that the gradient-corrected bilinear interpolation was an improvement to the solely bilinear interpolation. Another processing method demonstrated was a black-background and white-balance normalization. This reduced the star-like pattern from the LEDs illumination propagating through the hyper-hemisphere and mineral oil, giving the image a more uniform brightness. Uniform illumination is critical for computer-aided feature identification. Then, color correction methods were investigated. These methods aimed to make the colors captured by the camera better match a set of reference colors. Accurately representing colors in the images is essential for a clinician's diagnosis. The tested methods showed an improvement in the colors compared to the reference, but when applied to skin images, the best method was not identified. Without a quantitative process to compare the various methods with skin images, clinicians' input would be required to choose best color correction method.

As the project progressed, Photon Gear Inc. and Caliber ID were able to provide an objective lens assembly with the micro-camera and hyper-hemisphere assembly for testing. However, it was discovered that when imaging through a window between the objective lens and the skin, necessary for RCM imaging in a clinical setting, a dark spot develops in the center of the image. Zemax simulations were created to model this dark spot and identify the sources of the dark spot. The simulations could not perfectly match the images taken in the laboratory, but they were close enough to gain insight into the source of the problem. It was found that part of

the dark spot was caused by a slight obstruction of the LEDs' illumination by the mounting ring in the objective lens. The other cause of the dark spot was the angle at which the LEDs' light entered hyper-hemisphere due to their position at the edge of the objective lens. This was not noticed when only imaging through the hyper-hemisphere, but with the additional glass, the rays pass beyond the center of the image, causing the dark spot. One solution tested in Zemax was to place two LEDs on the bridge of the flex board to fill the dark spot. However, this solution must be tested with a physical prototype to prove its validity.

A simple method of polarizing the camera probes was demonstrated to test a co- and cross-polarized micro-camera system compared to a non-polarized one. This was done by cutting a linear polarizer into a ring and a small square with an X-acto blade, then using an UV and thermally cured optical adhesive to glue the cut polarizer pieces to the LEDs and micro-camera. Then, MTF characterization and color analysis was conducted. MTF measurements were made by imaging a slanted edge and using the `sfrmat4.m` MATLAB function to calculate the MTF. To verify that the generated MTF curves were accurate, theoretical MTF curves for the system were derived. The results of the MTF characterization for the three probes showed that the cross-polarized system may improve the MTF. The results were variable, but the difference in average 50% MTF spatial frequency of the cross-polarized to non-polarized probe was an increase of 0.0508 cycles/pixel. The color analysis showed that the polarizers did not significantly affect the images' color, and the color correction methods worked similarly to the non-polarized images. Thus it was concluded that polarizing the probes, did not negatively affect their performance.

The three probe assemblies were sent to MSKCC to receive feedback from their scientists. From initial imaging of multiple lesions, the scientists at MSKCC reported that the

cross-polarized probe was able to capture images and video showing enhanced sub-surface features. A reduction in glare from LED reflections off the skin surface was also observed with the cross-polarized probe. These results warrant further investigation.

With the results of the experiments explained in this thesis, the cross-polarized system showed potential to improve the contrast of the image and enhance subsurface features while reducing surface glare. The polarization of the probes was found to not degrade the any measured elements of the imaging system. From these results, it is my opinion that there should be further pursuit and testing of the cross-polarized imaging system toward the goal of integrating into the HH-RCM system.

Suggestions for Future Work

To fix the illumination dark spot, a prototype of a flex board with eight LEDs around the ring and two LEDs on the bridge should be made. As was shown through Zemax simulations, placing two LEDs on the bridge can fill the dark center. However, proving this with a physical prototype is necessary. Once the initial prototype has verified that this is a valid solution, optimization can occur. Variables such as the radius at which the LEDs are mounted, and the brightness relative to the other LEDs could significantly affect the system. There is also concern that moving the LEDs closer to the camera, more directly over the hyper-hemisphere lens, could introduce direct reflections off the interfaces of glass and index-matching mediums. However, a cross-polarized probe could remove the direct reflections. Filling the center dark spot is critical for this product's success and should be investigated.

Caliber ID has almost completed a fully integrated widefield micro-camera with the redesigned objective lens into one of their existing RCM systems. Once completed, it will be

given to MSKCC for clinical trials. The system will be testing on patient volunteers to capture concurrent widefield and RCM images. This will be a milestone in the project and help define actions for the next generation of prototypes. A cross-polarized version of this fully integrated system should also be created. Along with patient trials, research will be conducted on computer-aided feature identification. The primary goal of feature recognition is for the registration of limited-quality clinical images from the integrated micro-camera to high-quality clinical images taken before the procedure starts.

Another area that must be explored is how the micro-camera system performs on skin with higher melanin content. Currently, the images taken have primarily been on skin with relatively low melanin content. The amount of melanin will affect how much of the LED illumination is absorbed and scattered.

The effects of an increased bridge obstruction on the RCM's optical sectioning should be investigated. It was shown in [85, 91] that a divided pupil configuration, similar to the imposed divided pupil caused by the flex board's bridge, could increase the RCM image contrast when compared to the full pupil configuration while maintaining comparable yet worse optical sectioning. This increased contrast was caused by the rejection of background light from the angular separation of the detection path from the illumination path. A key difference between the experimental setups in these experiments compared to our system is that for these setups, one half-pupil is used for the illumination path while the other path is used for the detection path, whereas both pupil halves are used for the detection and illumination paths for the Caliber ID HH-RCM used. This difference may be significant enough to make the two RCM setups not comparable. Also, these studies used a line-scanning system rather than a point-scanning system.

Due to these differences, we considered other RCM systems with different obstructions. An annular pupil could be created by having a full pupil objective lens with an obscuration in the middle of the pupil, similar to how the flex board's bridge is an obscuration in this RCM system. Annular pupils have been shown to improve the optical sectioning of RCM systems depending on the size of the pinhole and detector, with larger detectors yielding a better cross-sectioning [39, 101, 118, 119]. The divided pupil with full illumination beam RCM system from Caliber ID that the micro-camera is being integrated into may see some of the trends from each of the two methods mentioned thus far. So, optical sectioning of this RCM may likely be comparable yet slightly degraded when compared to the optical sectioning from a fully open pupil. However, these studies have not investigated the effects of varying the width of the division between the pupils; but [120] did investigate varying the size of an annular pupil. In these results, the degradation of the cross-sectioning power was minimal until the obstruction grew to over forty percent of the pupil, and from there, the cross-sectioning power decreased significantly. This study used an infinitesimally small pinhole, which partially explains the different results from the studies referenced above.

It could be theorized that the optical sectioning with the bridge obstruction will behave similarly. Therefore, it may be expected that as the width of the bridge is increased, especially to take up more than forty percent of the pupil, the optical sectioning will degrade, but the contrast may increase because more stray light will be blocked. This prediction is somewhat verified by experimental results after integrating a flex board with a micro camera into the objective lens of an RCM and capturing images with it. It should be noted that increasing the bridge width will also further reduce the number of photons collected by the high NA objective lens. Theoretical

and experimental results for this divided pupil with full illumination RCM system should be investigated. In addition, the effects of increasing the bridge width on the RCM's performance should be understood.

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APPENDICES

APPENDIX A

A MORE ACCURATE SCENE FOR THE EFFECTIVE NUMBER OF BITS ANALYSIS

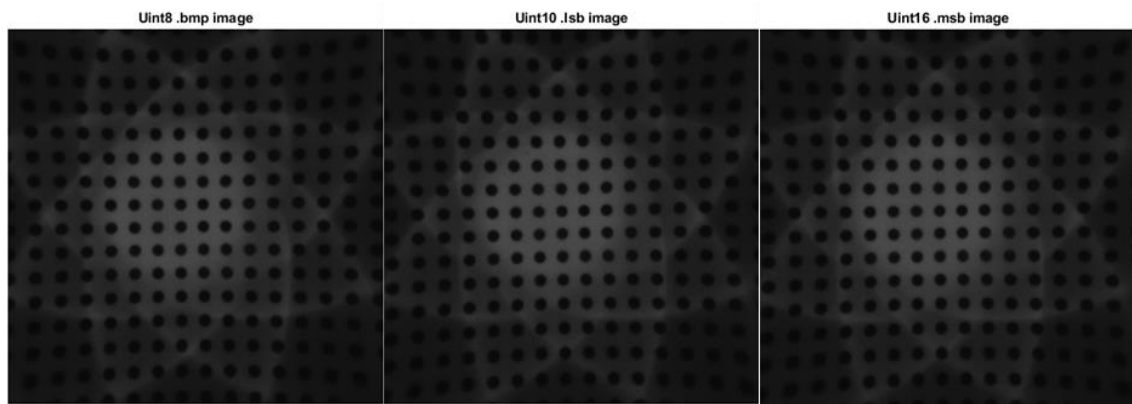


Figure A1. Image of repeating pattern on a distortion target using LED's and an index-matching medium.

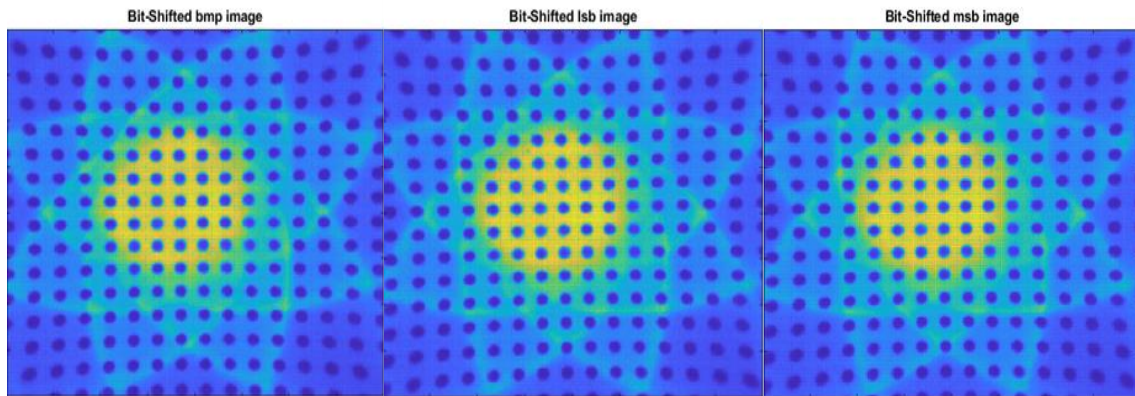


Figure A2. Using the imagesc command in MATLAB shows the scaled images prior to a bit-shift.

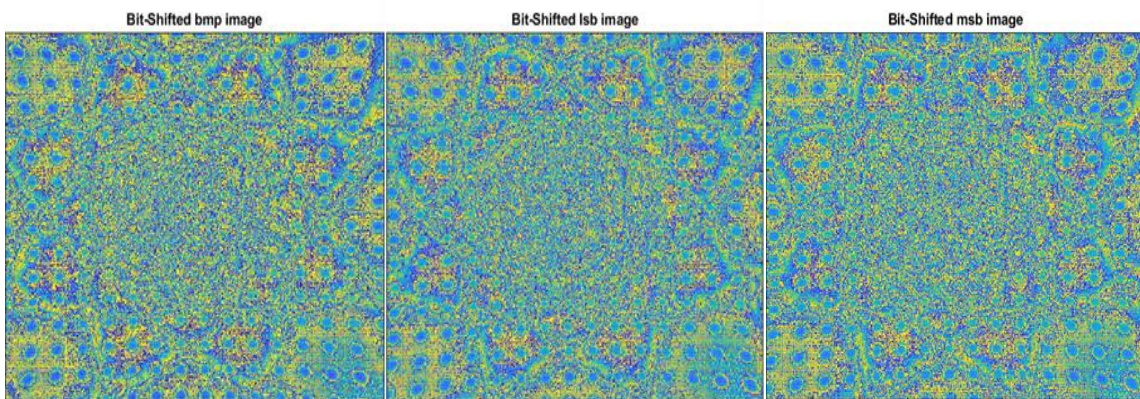


Figure A3. Scaled images after a bit-shift of 5.

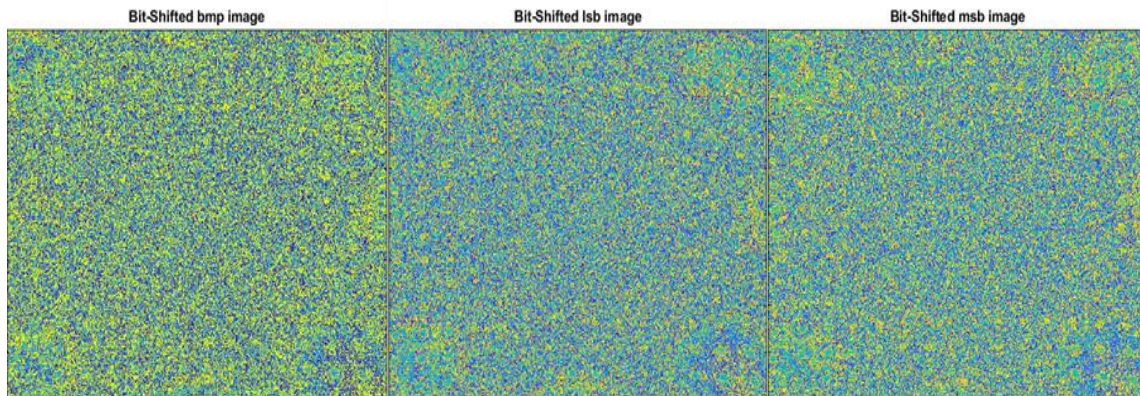


Figure A4: Scaled images after a bit-shift of 6.

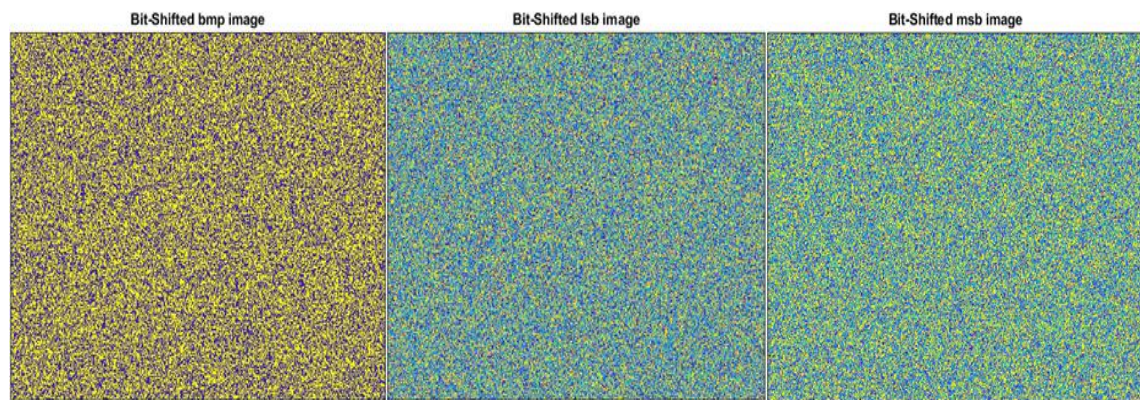


Figure A5: Scaled images after a bit-shift of 7.

From the above figures, after a bit-shift of 6, the dot pattern is almost completely unrecognizable. This result agrees with the results seen in the ‘Effective Number of Bits’ section.

APPENDIX B

MATLAB CODE FOR CALCULATING THE THEORETICAL DEFOCUSED MTF

```

function defocusedMTF =
defocus_mtf(w,z1,nominal_object_distance,spatialFreq,NA,pixelSize,index_of_medium)
% This function computes the MTF curve caused by a defocused lens. The user
% needs to input the calculated radius of aperture 'w'. distance 'z1'.
% Distance from lens to object 'nominal_object_distance'. Spatial Frequency
% vector 'spatialFreq'. Numerical Aperture 'NA'. Pixel Size 'pixelSize'.
% And the index of refraction of the medium (lens) 'index_of_medium'. See
% 'C:\Users\r64g338\OneDrive - Montana State University\Documents -
% Widefield-RCM NIH Project\Matlab Codes\MTF_Simulation.m' for an example
% of this function in use.

% Code by Dr. David Dickensheets

clear x y
N=200;
x=linspace(-1,3,2*N); %pupil coordinates for x
y=linspace(-1,1,N);   %pupil coordinates for y
y=y';
X=(1+y*0)*x;
Y=y*(1+x*0);
R=sqrt(X.^2+Y.^2);
A=0*R;
A(find(R<=1))=1;

```

Weighted color channels

```

rhoNaughtBlue = (NA/(467*10^-6))*pixelSize; % for cycles/pixel
rhoNaughtGreen = (NA/(527*10^-6))*pixelSize; % for cycles/pixel
rhoNaughtRed = (NA/(624*10^-6))*pixelSize; % for cycles/pixel

%for blue...
norm_spatial_freq=spatialFreq./(2*rhoNaughtBlue); %normalized spatial frequency rho/(2*rho_0)
w_def=-1/2*(1/nominal_object_distance-1/z1)*(w^2)/((467*10^-6)/index_of_medium); %blue defocus
coeff on r^2 in "waves"
P=A.*exp(j*2*pi*w_def*R.^2);
OTF=zeros(1,N);
for q=0:N-1
    OTF(q+1)=sum(sum(P.*conj(wshift('2D',P,[0 q]))));
end
MTF_d=abs(OTF);
MTF_d=MTF_d./(max(max(MTF_d)));
nonzero_mtf_range=find(norm_spatial_freq<1);
defocusedMTFBlue=0*norm_spatial_freq;
defocusedMTFBlue(nonzero_mtf_range)=spline(linspace(0,1,N),MTF_d,norm_spatial_freq(nonzero_mtf_range));

%for green...
norm_spatial_freq=spatialFreq./(2*rhoNaughtGreen); %normalized spatial frequency rho/(2*rho_0)
w_def=-1/2*(1/nominal_object_distance-1/z1)*(w^2)/((527*10^-6)/index_of_medium); %green

```

```

defocus coeff on r^2 in "waves"
P=A.*exp(j*2*pi*w_def*R.^2);
OTF=zeros(1,N);
for q=0:N-1
    OTF(q+1)=sum(sum(P.*conj(wshift('2D',P,[0 q]))));
end
MTF_d=abs(OTF);
MTF_d=MTF_d./(max(max(MTF_d)));
nonzero_mtf_range=find(norm_spatial_freq<1);
defocusedMTFGreen=0*norm_spatial_freq;
defocusedMTFGreen(nonzero_mtf_range)=spline(linspace(0,1,N),MTF_d,norm_spatial_freq(nonzero_mtf_range));

%for red...
norm_spatial_freq=spatialFreq./(2*rhoNaughtRed); %normalized spatial frequency rho/(2*rho_0)
w_def=-1/2*(1/nominal_object_distance-1/z1)*(w^2)/((624*10^-6)/index_of_medium); %red defocus
coeff on r^2 in "waves"
P=A.*exp(j*2*pi*w_def*R.^2);
OTF=zeros(1,N);
for q=0:N-1
    OTF(q+1)=sum(sum(P.*conj(wshift('2D',P,[0 q]))));
end
MTF_d=abs(OTF);
MTF_d=MTF_d./(max(max(MTF_d)));
nonzero_mtf_range=find(norm_spatial_freq<1);
defocusedMTFRed=0*norm_spatial_freq;
defocusedMTFRed(nonzero_mtf_range)=spline(linspace(0,1,N),MTF_d,norm_spatial_freq(nonzero_mtf_range));

% This calculates the luminance MTF by weighting the MTF for the RGB
% channels. Weighting follows covention from paper.
defocusedMTF = 0.6*defocusedMTFGreen + 0.3*defocusedMTFRed + 0.1*defocusedMTFBlue;

end

```

APPENDIX C

MORE COLOR CORRECTION FIGURES AND RESULTS

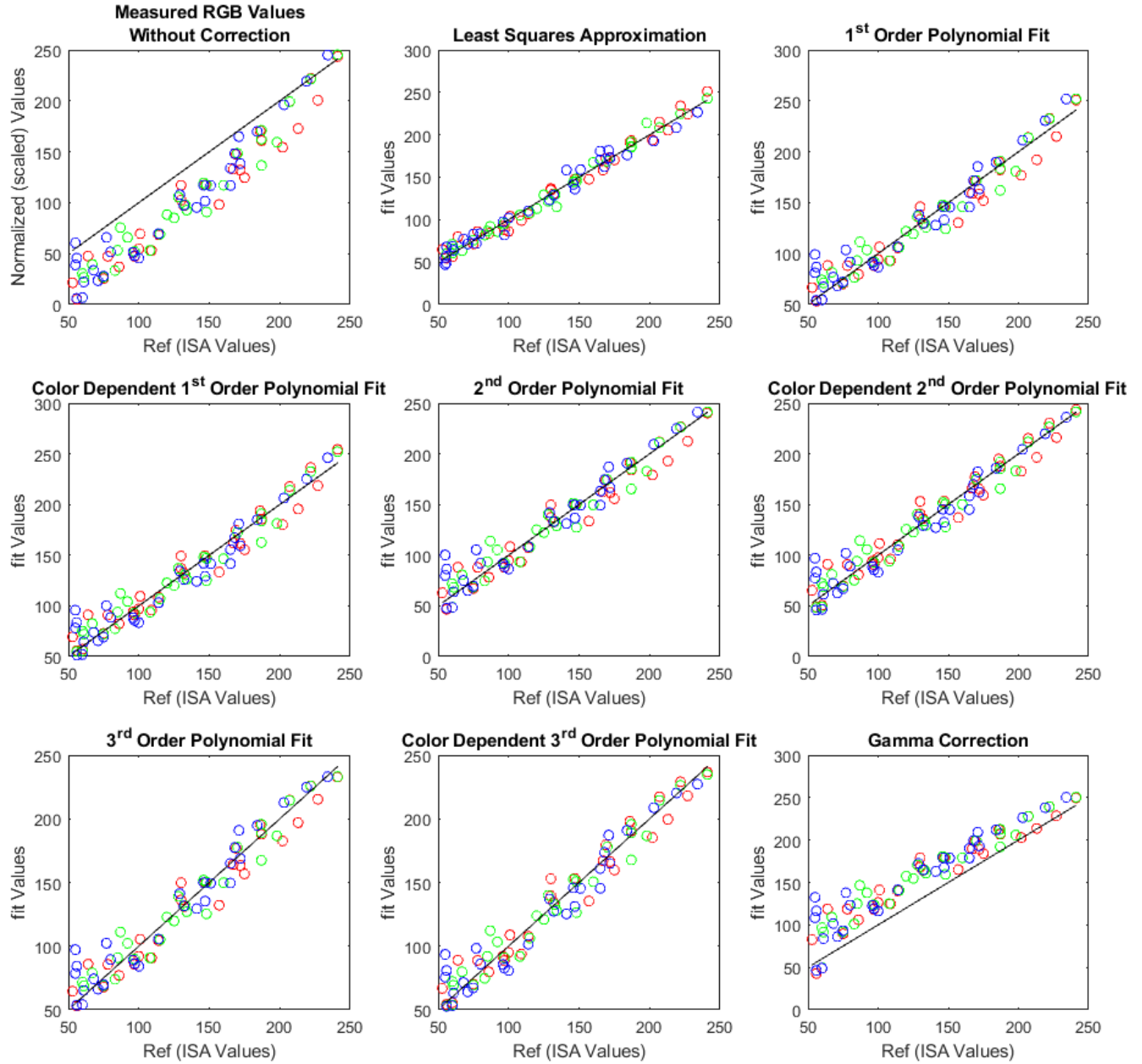


Figure C1. RGB values versus the reference fit line. The top left is the non-corrected measured RGB values, and the rest are the corrected values. The red circles correspond to the red channel, green circles to the green channel, and the blue circles to the blue channel.

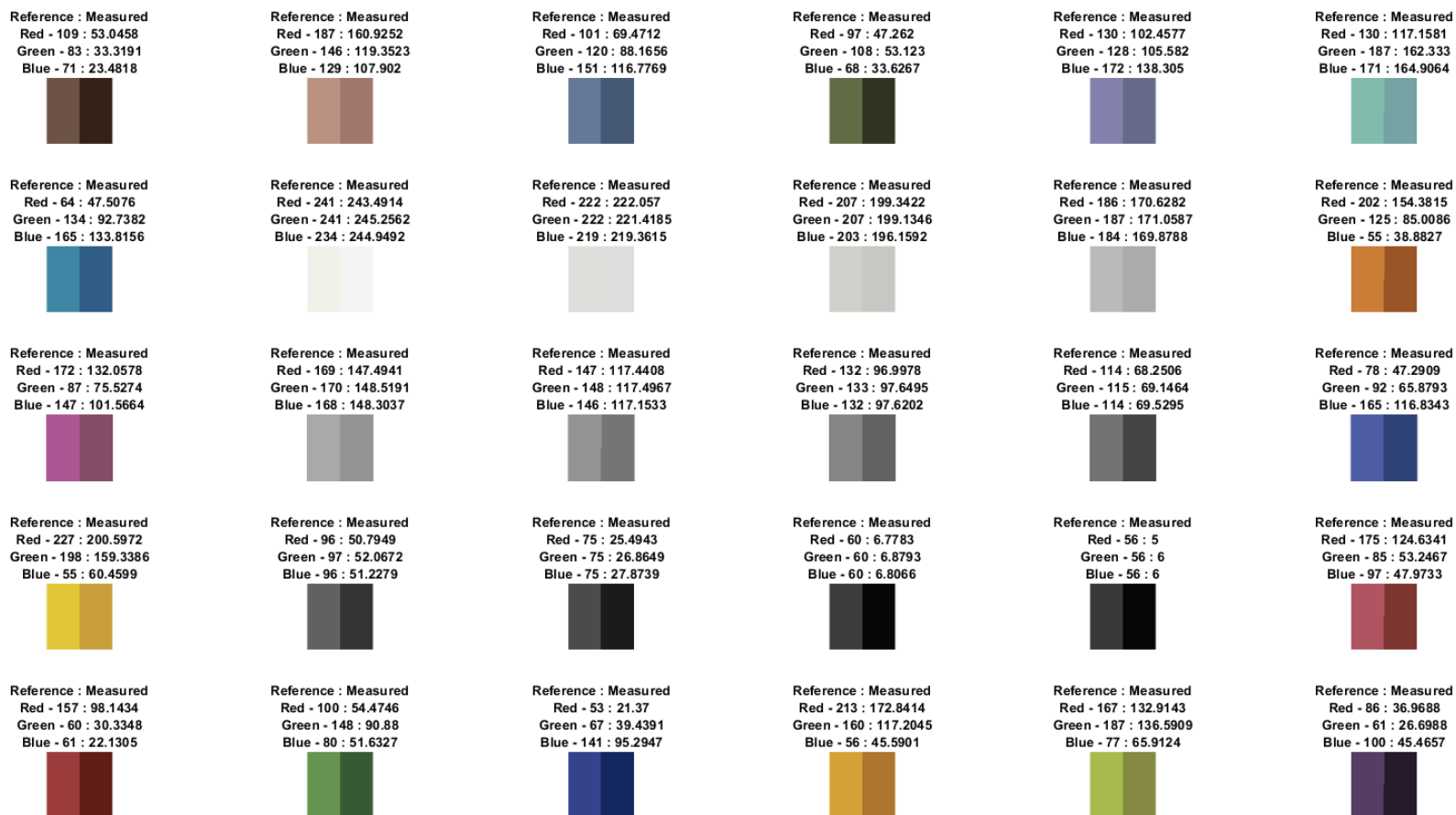


Figure C2. Comparison of the uncorrected RGB values for each color on the ISA ColorGauge Pico Target to the Adobe RGB Color Space reference values provided by ISA.

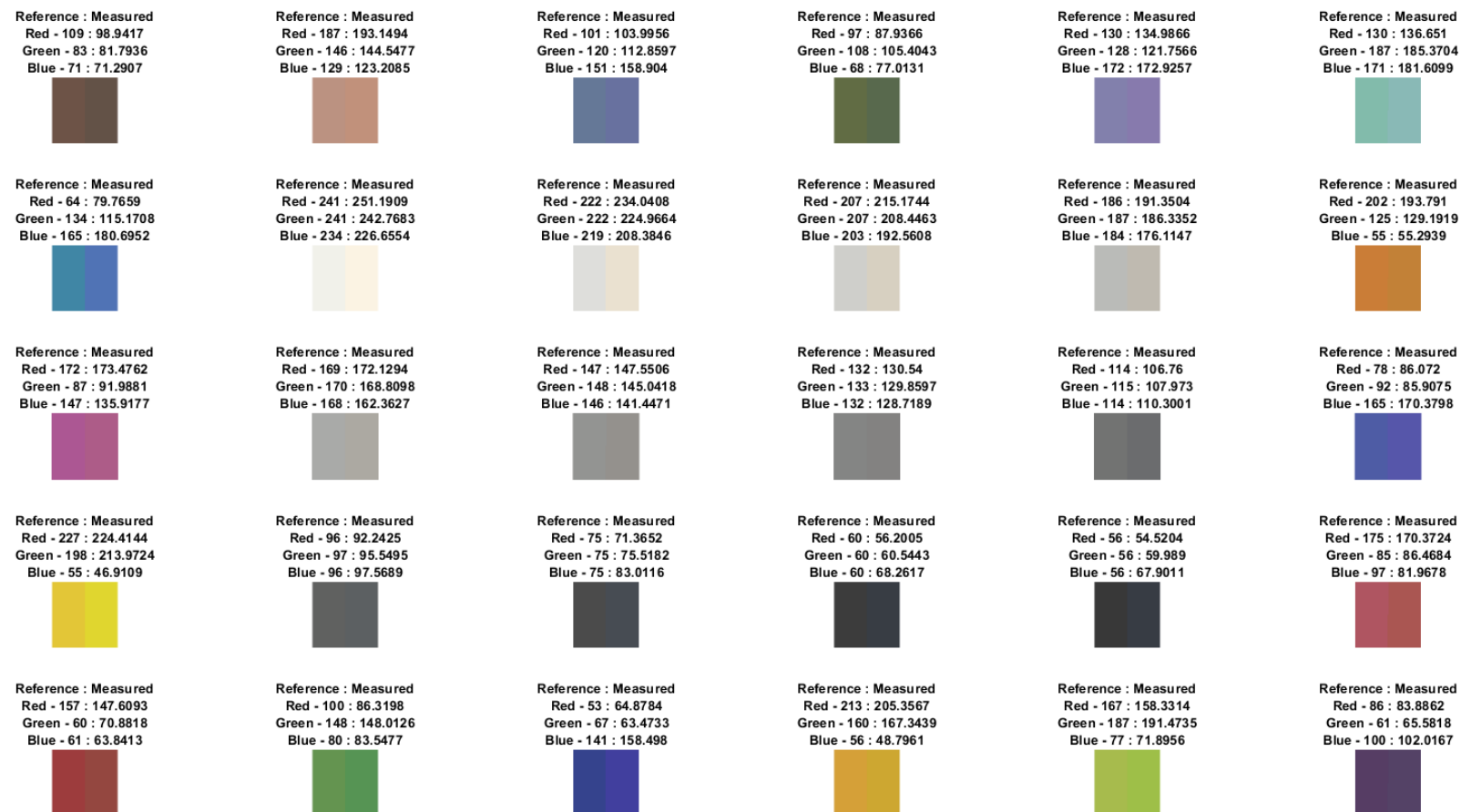


Figure C3. Comparison of the Least Squares Approximation corrected RGB values for each color on the ISA ColorGauge Pico Target to the Adobe RGB Color Space reference values provided by ISA.

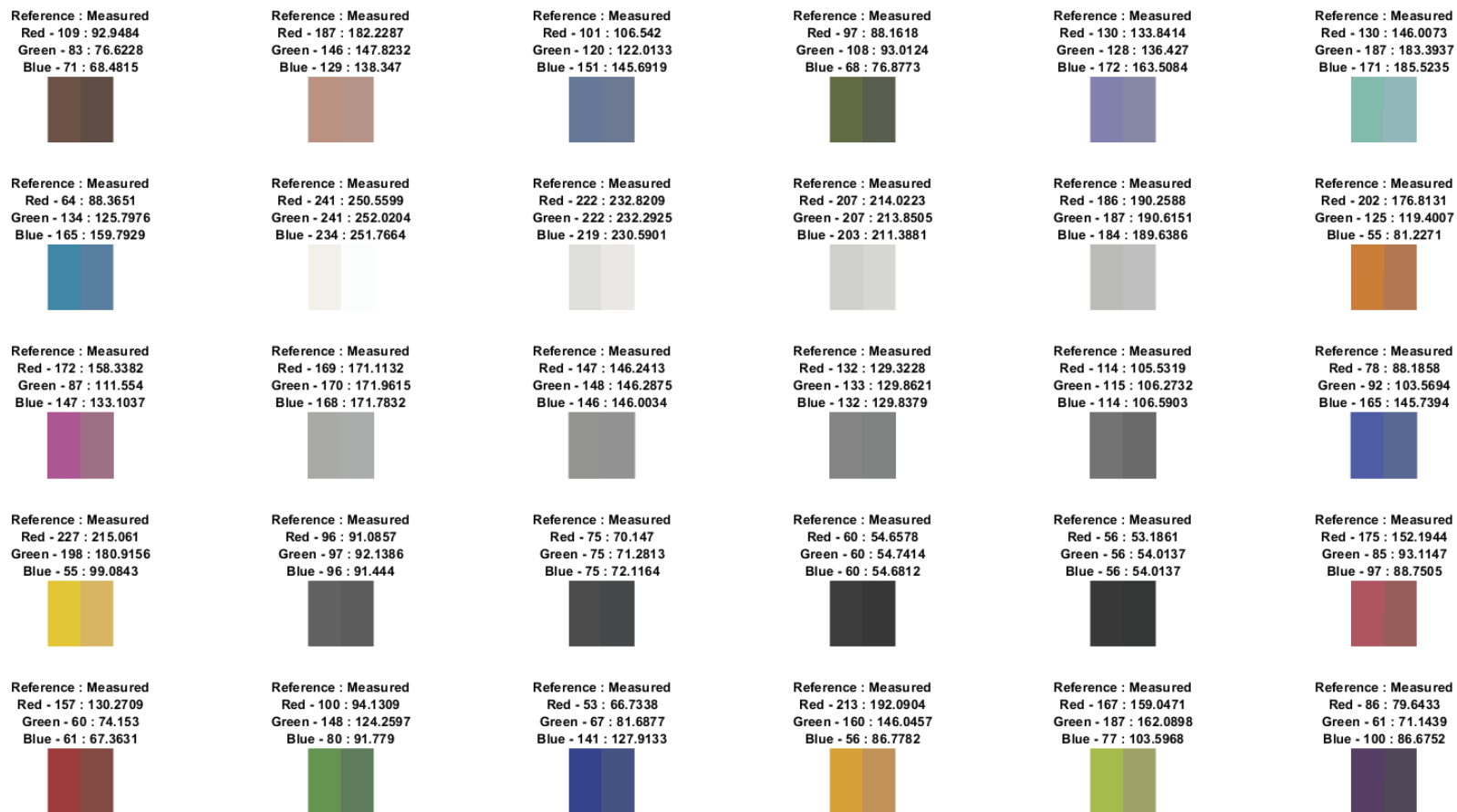


Figure C4. Comparison of the 1st Order Polynomial Fitted RGB values for each color on the ISA ColorGauge Pico Target to the Adobe RGB Color Space reference values provided by ISA.

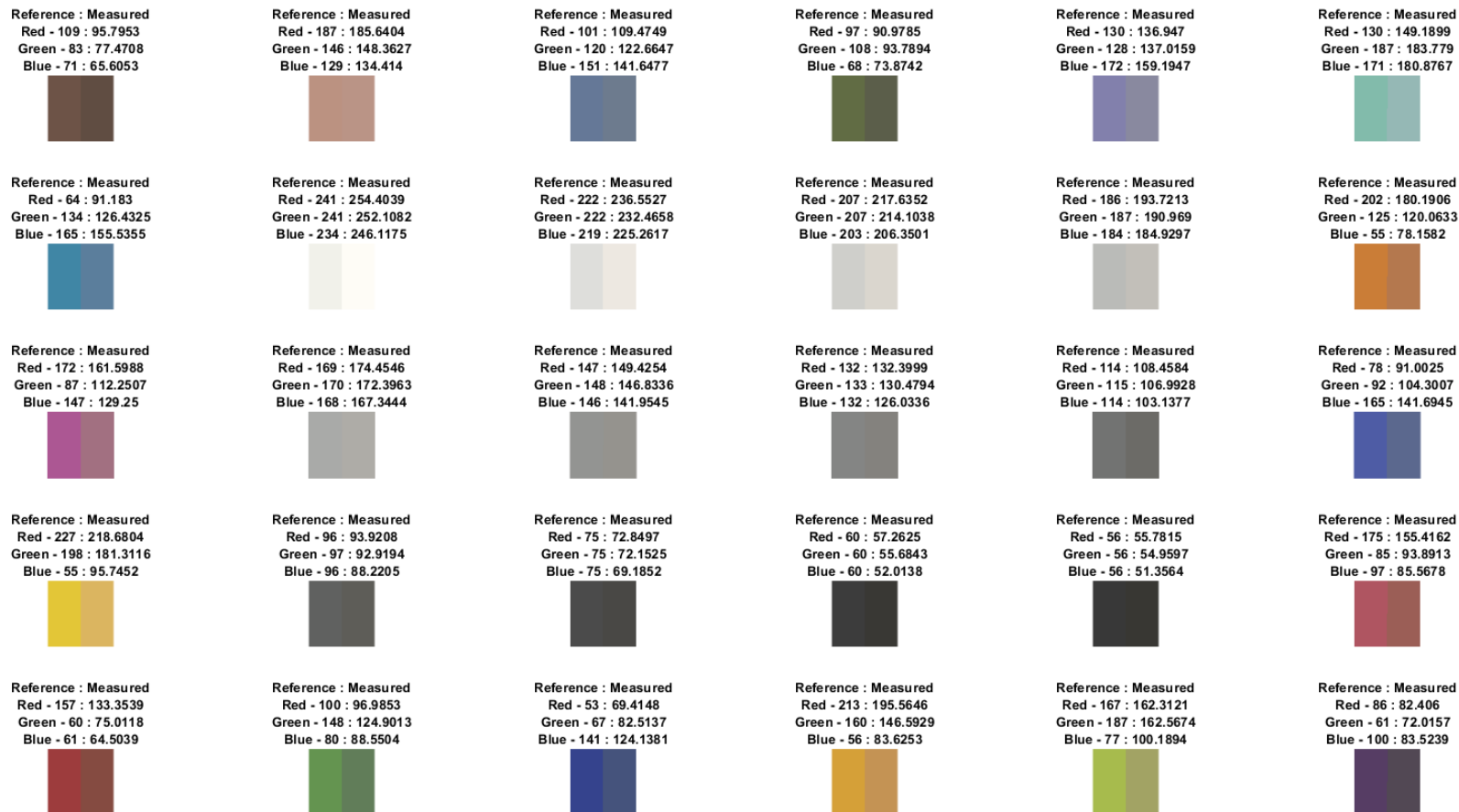


Figure C5. Comparison of the Color Dependent 1st Order Polynomial Fitted RGB values for each color on the ISA ColorGauge Pico Target to the Adobe RGB Color Space reference values provided by ISA.

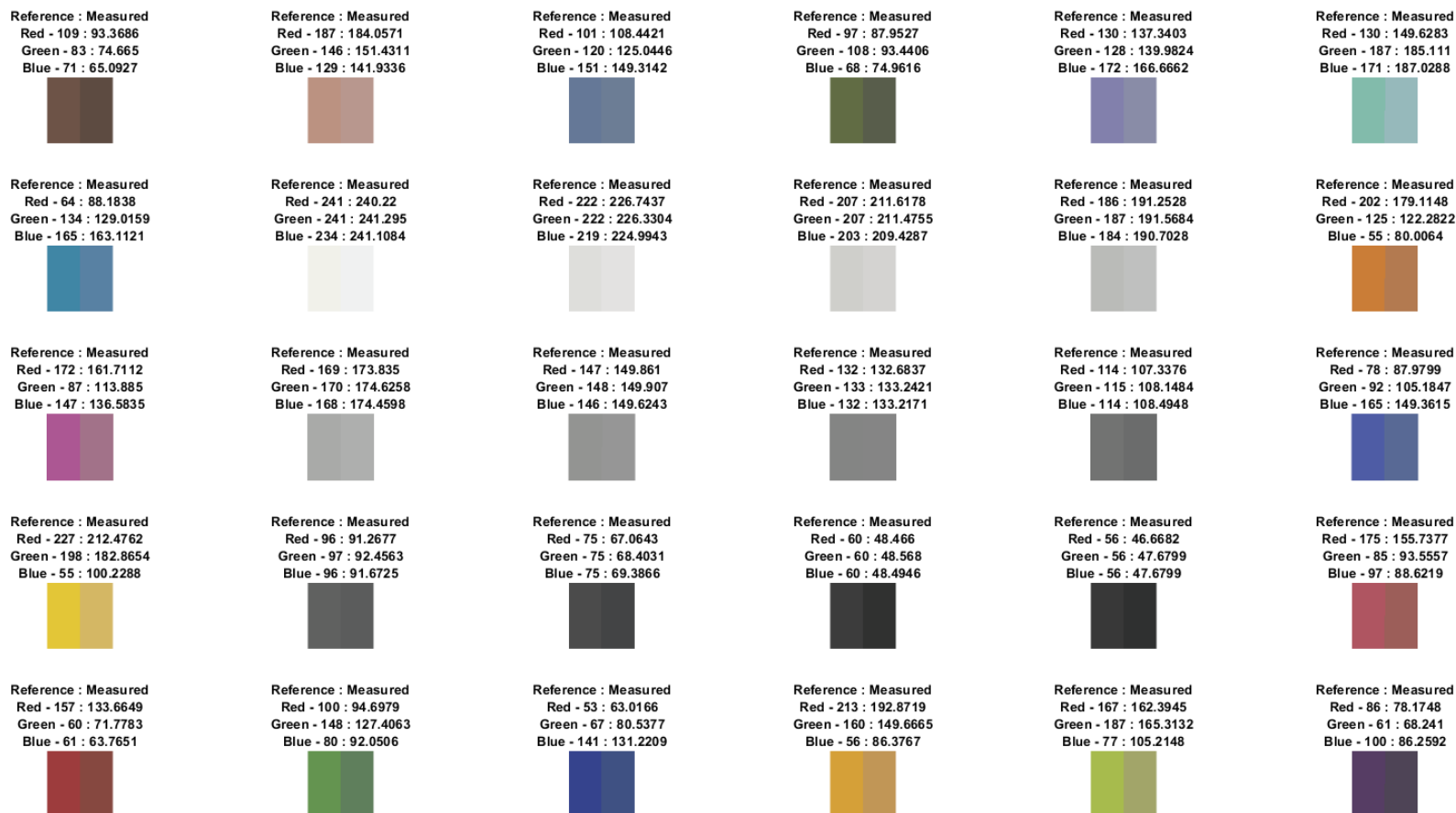


Figure C6. Comparison of the 2nd Order Polynomial Fitted RGB values for each color on the ISA ColorGauge Pico Target to the Adobe RGB Color Space reference values provided by ISA.

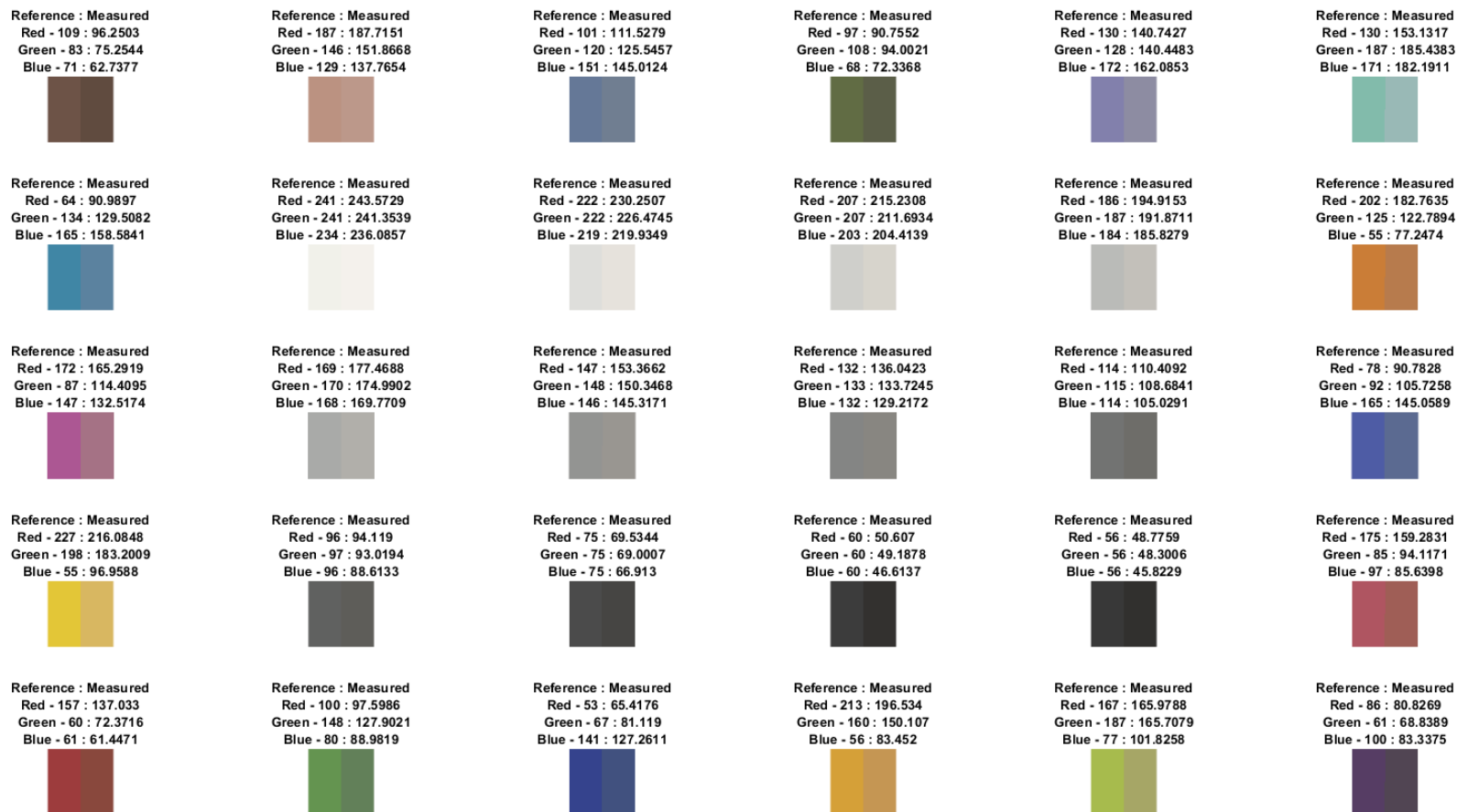


Figure C7. Comparison of the Color Dependent 2nd Order Polynomial Fitted RGB values for each color on the ISA ColorGauge Pico Target to the Adobe RGB Color Space reference values provided by ISA.

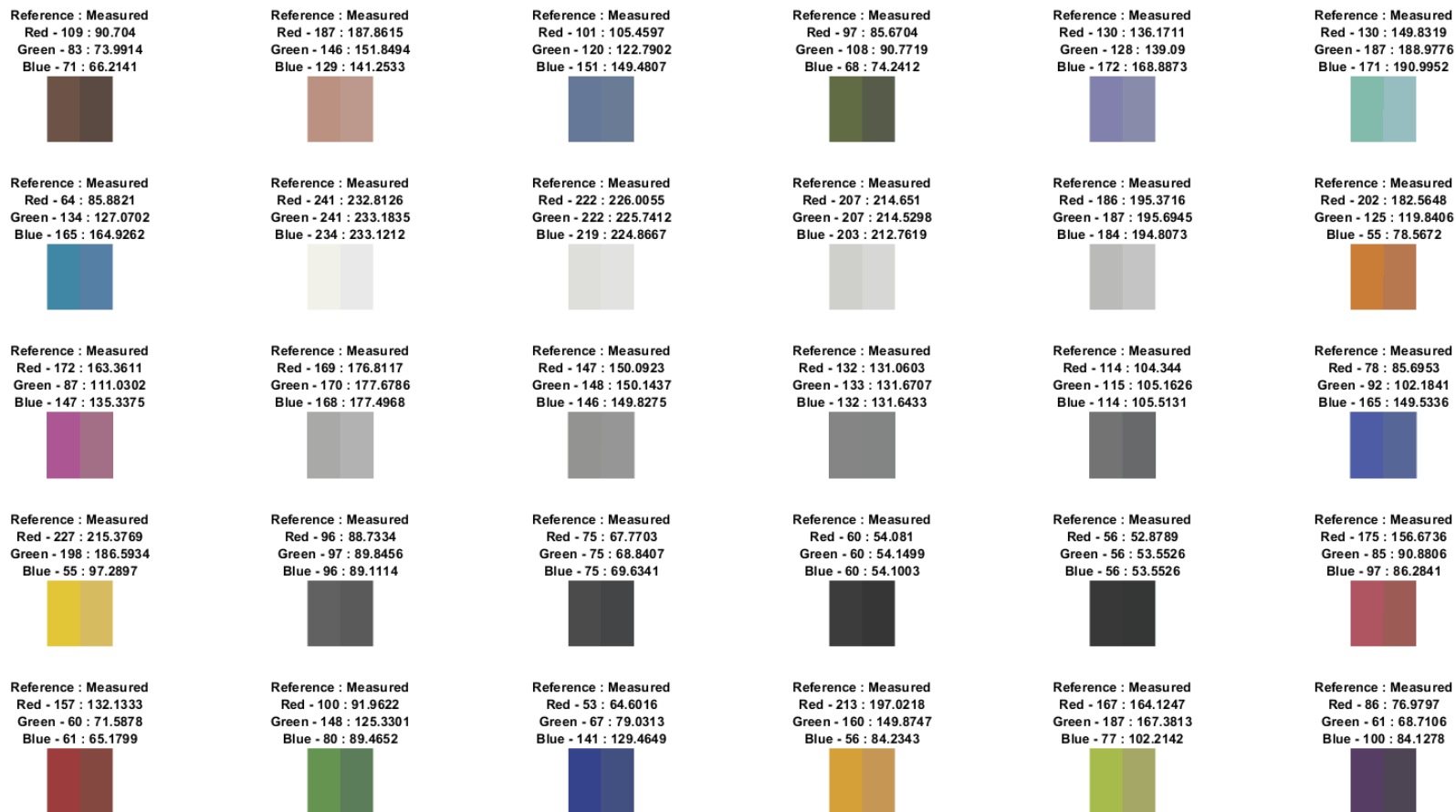


Figure C8. Comparison of the 3rd Order Polynomial Fitted RGB values for each color on the ISA ColorGauge Pico Target to the Adobe RGB Color Space reference values provided by ISA.

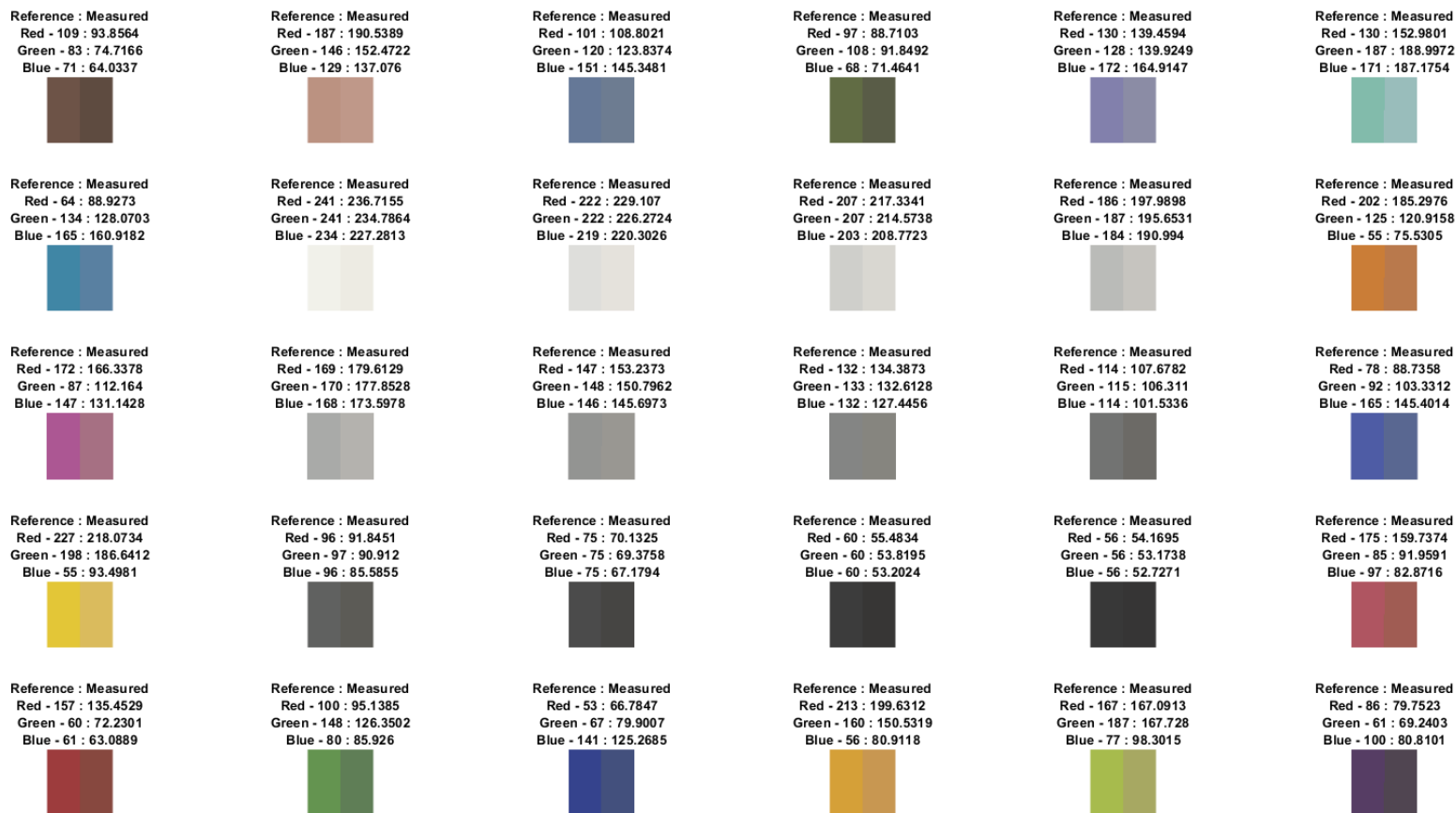


Figure C9. Comparison of the Color Dependent 3rd Order Polynomial Fitted RGB values for each color on the ISA ColorGauge Pico Target to the Adobe RGB Color Space reference values provided by ISA.

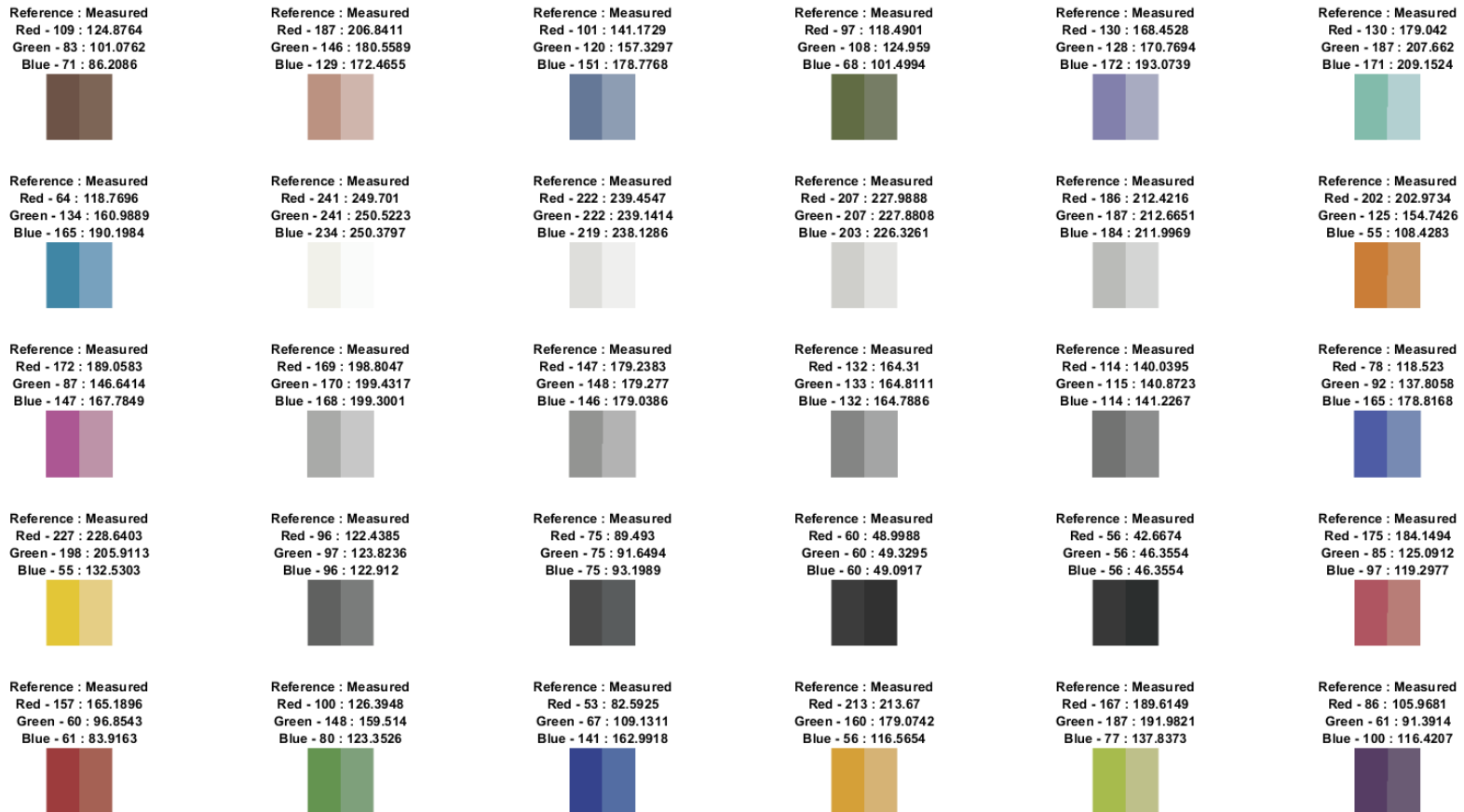


Figure C10. Comparison of the Gamma Corrected RGB values for each color on the ISA ColorGauge Pico Target to the Adobe RGB Color Space reference values provided by ISA.