Excitation-scan mirror array system advancements to hyperspectral imaging applications

Parker, Marina, Browning, Craig, Mayes, Sam, Deal, Joshua, Gunn-Mayes, Samantha, et al.

Marina Parker, Craig M. Browning, Sam A. Mayes, Joshua Deal, Samantha Gunn-Mayes, Thomas C. Rich, Silas J. Leavesley, "Excitation-scan mirror array system advancements to hyperspectral imaging applications," Proc. SPIE 11245, Three-Dimensional and Multidimensional Microscopy: Image Acquisition and Processing XXVII, 112450Y (17 February 2020); doi: 10.1117/12.2546485

SPIE.

Event: SPIE BiOS, 2020, San Francisco, California, United States

Excitation-scan Mirror Array System Advancements to Hyperspectral Imaging Applications

Marina Parker[1,4], Craig M. Browning[1,4], Sam A. Mayes[1,4], Joshua Deal[2,3], Samantha Gunn-Mayes[1], Thomas C. Rich[2,3], Silas J. Leavesley[1,2,3,4]

[1]Chemical and Biomolecular Engineering, University of South Alabama, AL 36688

[2]Pharmacology, University of South Alabama, AL 36688

[3]Center for Lung Biology, University of South Alabama, AL 36688

[4]Department of Systems Engineering, University of South Alabama, AL 36688

ABSTRACT

Hyperspectral imaging (HSI) technology has been applied in a range of fields for target detection and mixture analysis. While its original applications were in remote sensing, modern uses include agriculture, historical document authentications and medicine. HSI has shown great utility in fluorescence microscopy; however, acquisition speeds have been slow due to light losses associated with spectral filtering. We are currently developing a rapid hyperspectral imaging platform for 5-dimensional imaging (RHIP-5D), a confocal imaging system that will allow users to obtain simultaneous measurements of many fluorescent labels. We have previously reported on optical modeling performance of the system. This previous model investigated geometrical capability of designing a multifaceted mirror imaging system as an initial approach to sample light at many wavelengths. The design utilized light-emitting diodes (LEDs) and a multifaceted mirror array to combine light sources into a liquid light guide (LLG). The computational model was constructed using Monte Carlo optical ray software (TracePro, Lambda Research Corp.). Recent results presented here show transmission has increased up to 9% through parametric optimization of each component. Future work will involve system validation using a prototype engineered based on our optimized model. System requirements will be evaluated to determine if potential design changes are needed to improve the system. We will report on–spectral resolution to demonstrate feasibility of the RHIP-5D as a promising solution for overcoming current HSI acquisition speed and sensitivity limitations.

Keywords: Systems Design, LED, Tissue imaging, HSI, Spectroscopy, Microscopy, Transmission, Spectral, Fluorescence, Molecules, Cells

1. INTRODUCTION

Hyperspectral imaging, also known as imaging spectrometry[1] combines two modalities; imaging and spectroscopy. This combination allows for the creation of three-dimensional data which contains data of the target of interest, measured at different wavelengths[2]. Original hyperspectral imaging applications were in the field of earth remote sensing[3]. However, since then it has been applied to numerous areas including archaeology and art conservation, vegetation and water resource control, food quality and safety control, forensic medicine, crime scene detection, and biomedical applications[1,4–9]. In the medical field, a common goal in hyperspectral imaging applications is tissue discrimination. Hyperspectral imaging delivers assessments of tissue pathophysiology based on the spectral characteristics of different tissue. After showing great promise in fluorescence microscopy applications for separating signatures from many fluorescent molecules, HSI has become popular. Unfortunately, the necessity to acquire signal in many spectral bands and the light losses associated with spectral filtering has resulted in slow acquisition speeds. The purpose of this research is to develop new optical approaches to improve acquisition speeds, specificity and sensitivity limitations of hyperspectral imaging microscopy through fluorescence excitation scanning methods. We have previously reported that to address acquisition speeds, specificity and sensitivity limitations, we have designed RHIP-5D – a system for high-speed microscopy that can detect the entire fluorescence emission in a single channel, while simultaneously providing spectral discrimination by rapidly scanning through a wide range of excitation wavelength bands[10–13]. RHIP-5D is expected to increase sensitivity by 30-100x, in contrast to the currently used emission-filtering based systems[14,15]. Here, we report on the theoretical spectral resolution of the system.

Three-Dimensional and Multidimensional Microscopy: Image Acquisition and Processing XXVII, edited by Thomas G. Brown, Tony Wilson, Laura Waller, Proc. of SPIE Vol. 11245, 112450Y
 · doi: 10.1117/12.2546485

2. SYSTEM MODEL DESIGN

The current model design consists of light-emitting diodes, a multifaceted mirror array, and lenses that are used to focus light sources into a liquid light guide. TracePro – a Monte Carlo based software was used for optical ray tracing. LEDs were simulated by importing irradiance properties using manufacturer data and lenses were simulated by importing manufacturer lens files. Characteristic of each element in the model were specified to reflect each components optical properties and experimental characteristics; this included defining the appropriate surface to emit, absorb, or reflect light. An example of the geometric model is shown in Figure 1, while a simplified version of the model is shown in Figure 2. We have previously reported on the results of our parametric optimization of each element in the model. A summary of these results is shown in Table 1.

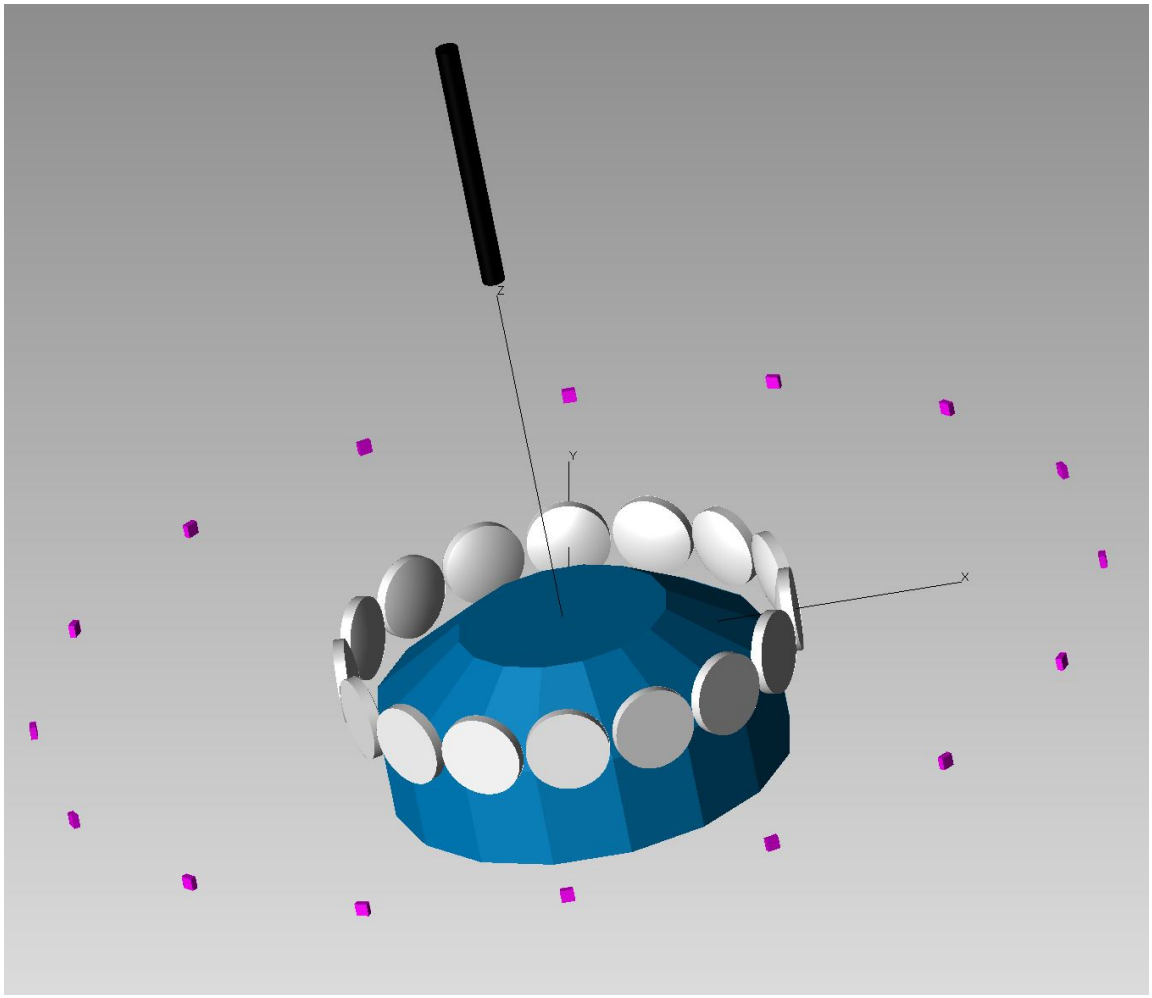

Figure 1: TracePro model created to analyze the light path of each light source (LED) and the transmission collection at the liquid light guide (LLG) entrance aperture. Pink blocks represent each LED, white circular geometry represents the lenses, blue geometry represents the mirror-array and the black cylindrical geometry represents the LLG.

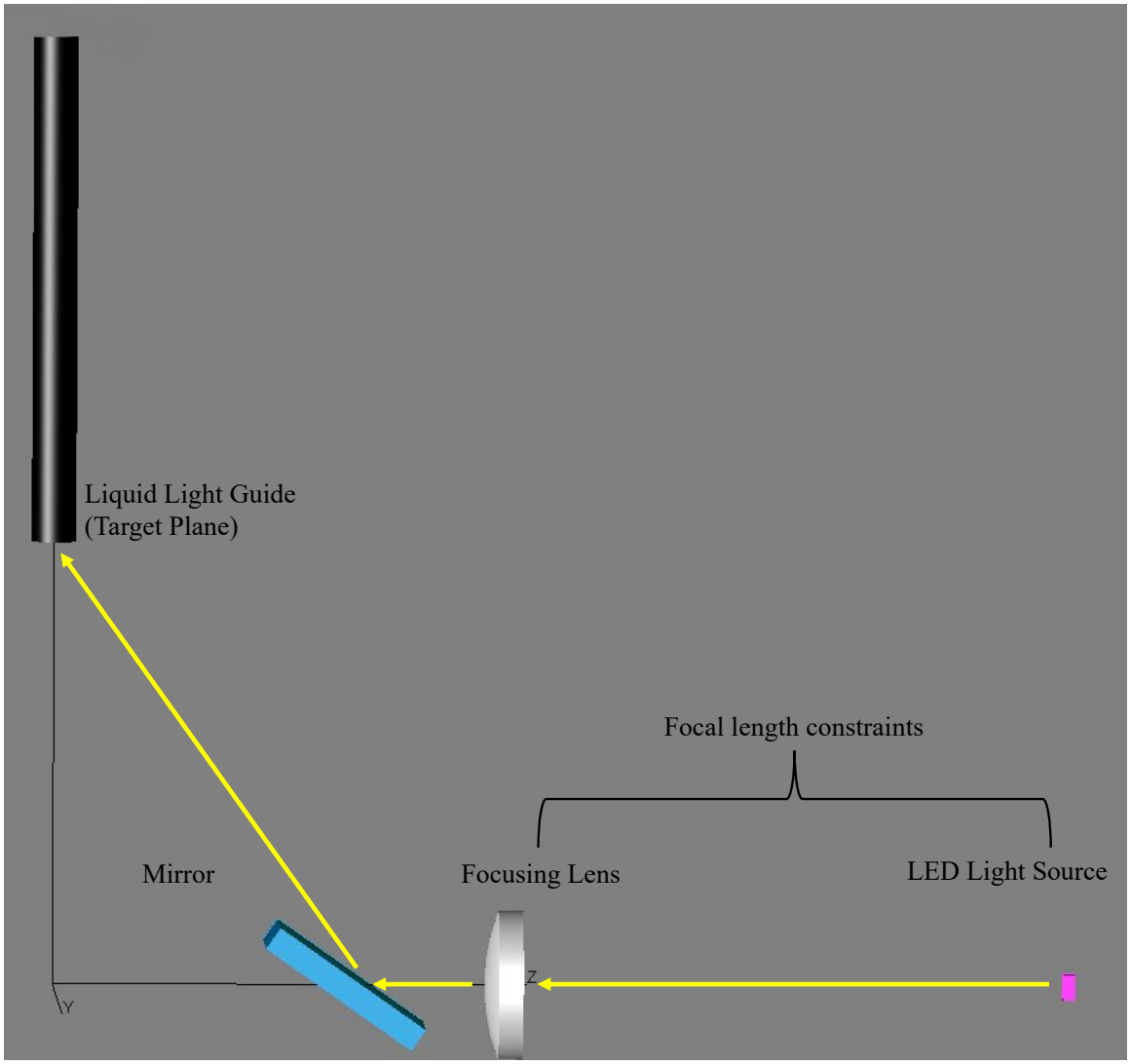

Figure 2: Simplified version of the TracePro geometric model created to analyze the light path and transmission collected at the liquid light guide. Yellow arrows in this figure show that the light path emitted by the LED (light source) is then transmitted through the focusing lenses and finally, reflected by the mirror to the entrance aperture of the LLG. Focal length constraints refer to the minimum distance required between the lens and the LED which can vary based on the wavelength of each LED.

Table 1: Transmission results of optical ray tracing for a range of wavelengths.

LED wavelength (nm)	Incident Rays at LLG (#)	Transmission at LLG (%)
365	94,840	8.77
375	98,401	9.12
395	92,463	8.58
405	92,614	8.59
420	86,482	8.03
450	68,328	6.34
470	69,736	6.46

490	11,579	9.04
515	83,126	7.70
520	65,868	6.09
525	88,004	8.18

3. SYSTEM SPECTRAL RESOLUTION

In microscopy, resolution of a microscope refers to how well we can resolve two points at a given distance from each other. The highest achievable point-to-point resolution in an optical microscope is governed by the objective lens and design of the aperture. Resolution limitations are present due to the diffraction of visible light wave fronts as they pass through the circular aperture at the rear focal plane of the objective making the numerical aperture of the microscope objective directly related to the resolution of the microscope. Limitations in resolutions – also known as diffraction barriers, restrict the ability of optical instruments to distinguish between two objects separated by a lateral distance less than approximately half the wavelength of light used to image the specimen. Due to diffraction of light, the image of a given specimen is never a perfect representative of the real details present in the specimen because there is a lower limit below which the microscope optical system cannot resolve structural details[3,9,1016]. The wavelength spectrum of a light source used to image any sample affects the level of resolution of the microscope. Therefore, a theoretical spectral resolution analysis was performed on the LEDs listed in Table 1 to show the system's ability to distinguish between two fluorescent proteins.

3.1. SPECTRAL RESOLUTION METHODS AND RESULTS

To investigate the model's spectral resolution, a MATLAB code was used to determine if the system is capable of discriminating the spectral signatures between green fluorescent protein (GFP) and yellow fluorescent protein. The typical spectral signature (data obtained from ThermoFisher Scientific Fluorescence Spectral Viewer) of GFP is shown in Figure 3, while the YFP spectral signature can be seen in Figure 4. Spectral signatures of the LEDs listed in Table 1 were obtained from manufacturer data and can be seen in Figure 5.

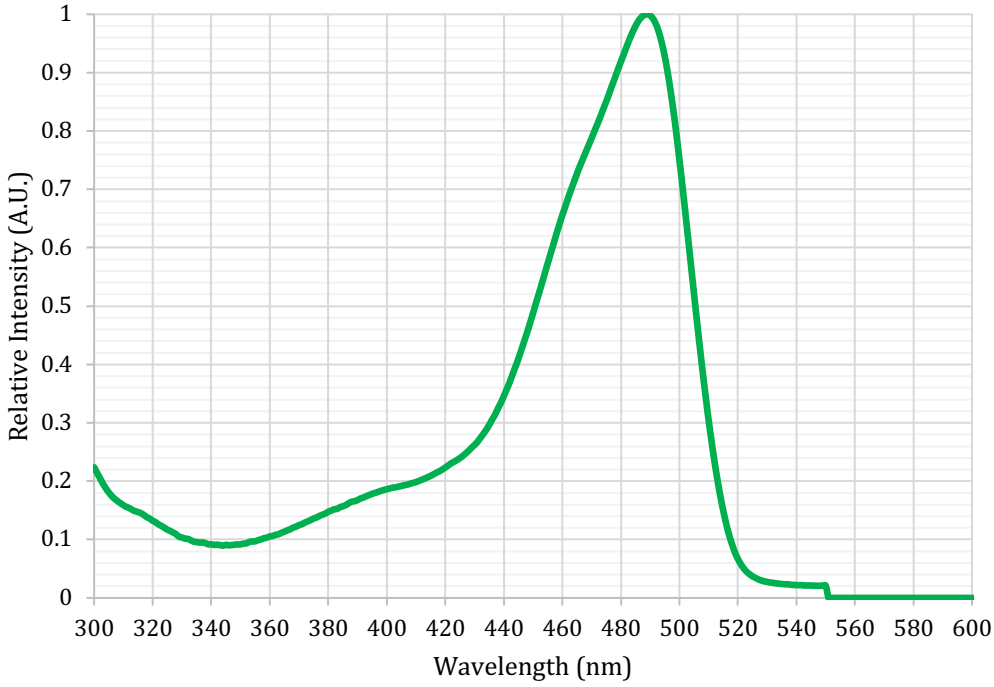

Figure 3: Excitation spectrum of GFP normalized to peak intensity)

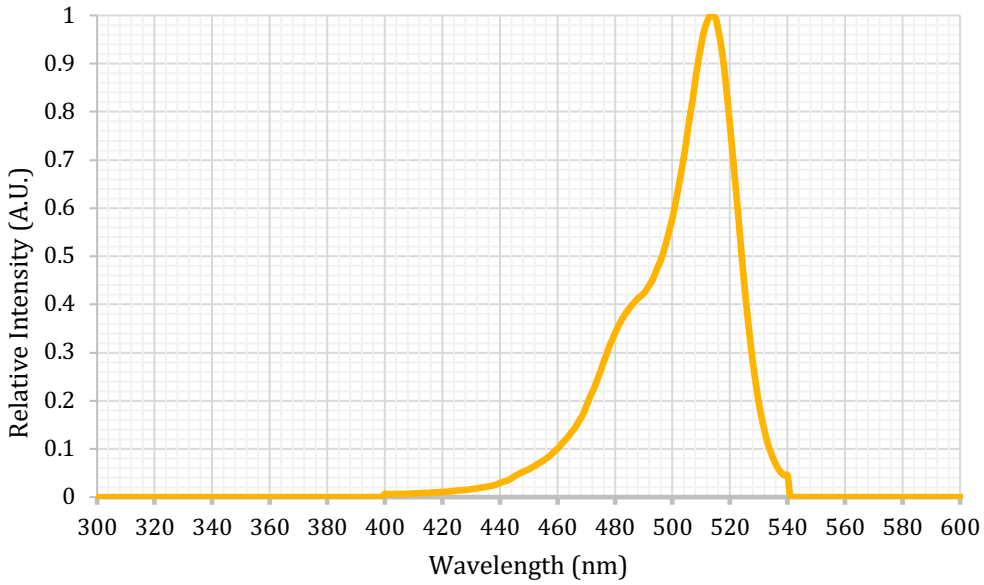

Figure 4: Excitation spectrum of YFP normalized to peak intensity (data obtained from ThermoFisher Spectra Viewer website: https://www.thermofisher.com/order/spectra-viewer)

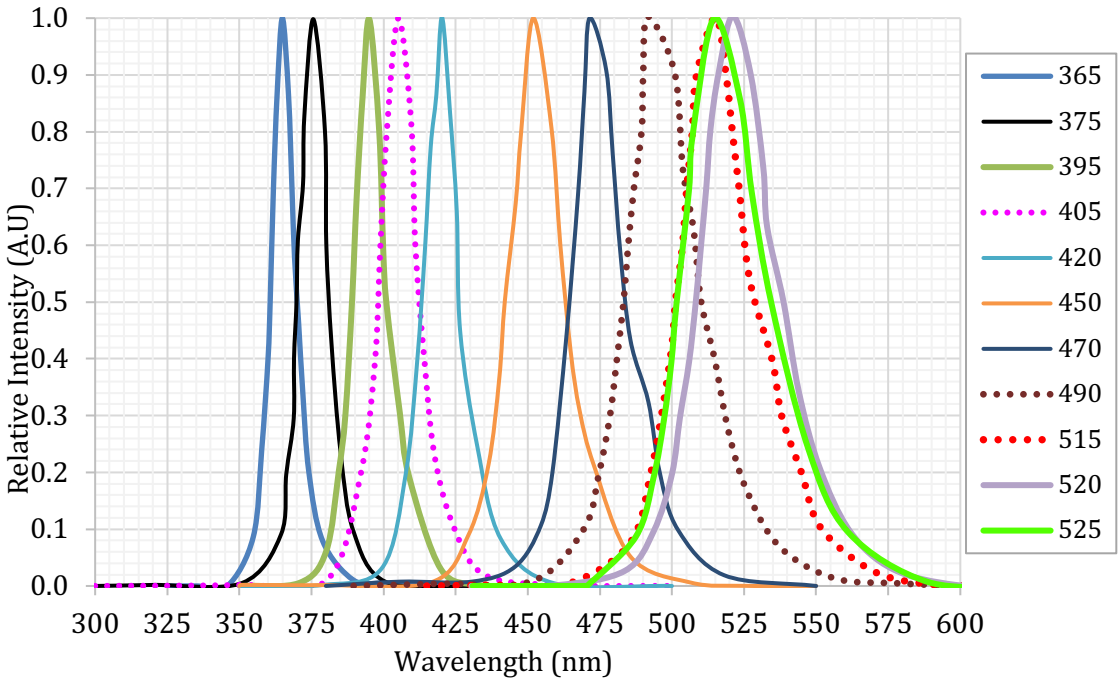

Figure 5: Spectral profiles of LEDs used in our model simulation. Each profile was obtained from the LED manufacturer data. Similar to the GFP and YFP data, the LED data shown here was normalized to peak intensity.

A custom MATLAB script was written that normalized the spectral profile of each LED to the integrated intensity and then convolved each LED spectral profile with both GFP and YFP spectral profiles. The goal of this code was to evaluate whether it was possible to distinguish between GFP and YFP spectral signatures when illuminated with our LED light sources. To successfully discriminate between the two fluorescent proteins, it was expected that result data plotted would clearly show two identifiable peaks, one representing GFP and the other YFP. Results of this analysis are shown in Figure 6.

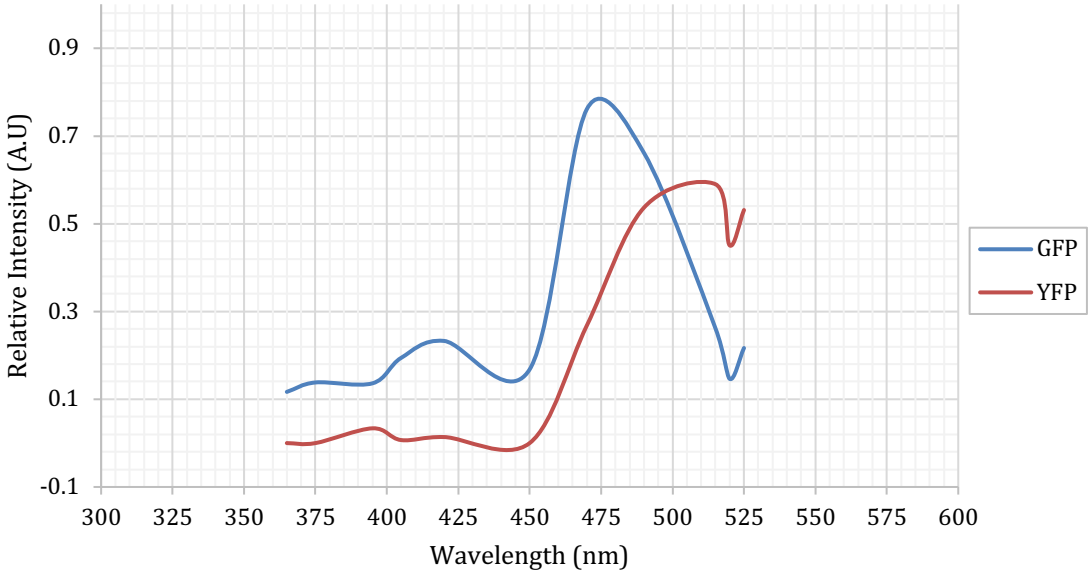

Figure 6: MATLAB analysis results showing two distinguishable peaks for GFP and YFP. The data were normalized to the area under the curve of GFP and YFP.

4. CONCLUSION AND FUTURE WORK

Hyperspectral imaging has become prevalent in the medical field, specifically in microscopy applications due to its ability to assess tissue pathophysiology based on the spectral characteristics of different tissue. It has shown great promise in fluorescence microscopy applications for separating signatures from many fluorescent molecules. In this work, we have shown our first step in analyzing our system's spectral resolution capabilities. Future work will focus on addressing spatial resolution, and signal to noise ratio (SNR) of the system. The preliminary data suggest that the RHIP-5D system has great potential for increasing the sensitivity and speed of HSI techniques in contrast to current approaches. This will be further investigated in future work by characterizing the spectral and spatial resolution as well as the SNR of the system prototype.

5. ACKNOWLEDGEMENTS

The authors would like to acknowledge support from NIH grant P01 HL066299, NSF grant 1725937, and the Abraham Mitchell Cancer Research Fund. Drs. Leavesley and Rich disclose financial interest in a start-up company, SpectraCyte LLC, formed to commercialize spectral imaging technologies.

References

1. Fei, B. Chapter 3.6 - Hyperspectral imaging in medical applications. in *Data Handling in Science and Technology* (ed. Amigo, J. M.) vol. 32 523–565 (Elsevier, 2020).
2. Garini et al. - 2006 - Spectral imaging Principles and applications.pdf.
3. Harris, A. T. Spectral mapping tools from the earth sciences applied to spectral microscopy data. *Cytometry A* **69A**, 872–879 (2006).
4. Padoan, R., Steemers, T. A. G., Klein, M. E., Aalderink, B. J. & Bruin, G. D. *QUANTITATIVE HYPERSPECTRAL IMAGING OF HISTORICAL DOCUMENTS: TECHNIQUE AND APPLICATIONS*.
5. Fink, D. J. Monitorng Earth's Resources from Space. *Technol. Rev.* 10 (1973).
6. Goltz, D., Attas, M., Young, G., Cloutis, E. & Bedynski, M. Assessing stains on historical documents using hyperspectral imaging. *J. Cult. Herit.* **11**, 19–26 (2010).
7. Haboudane, D. Hyperspectral vegetation indices and novel algorithms for predicting green LAI of crop canopies: Modeling and validation in the context of precision agriculture. *Remote Sens. Environ.* **90**, 337–352 (2004).
8. Sun, D.-W. *Hyperspectral Imaging for Food Quality Analysis and Control*. (Elsevier, 2010).
9. Lu, G. & Fei, B. Medical hyperspectral imaging: a review. *J. Biomed. Opt.* **19**, 010901 (2014).
10. Annamdevula, N. S. *et al.* An Approach for Characterizing and Comparing Hyperspectral Microscopy Systems. *Sensors* **13**, 9267–9293 (2013).
11. Leavesley, S. J., L. Britain, A., Cichon, L. K., Nikolaev, V. O. & Rich, T. C. Assessing FRET using spectral techniques: Spectral FRET Measurements. *Cytometry A* n/a-n/a (2013) doi:10.1002/cyto.a.22340.
12. Leavesley, S., Jiang, Y., Patsekin, V., Rajwa, B. & Robinson, J. P. An excitation wavelength–scanning spectral imaging system for preclinical imaging. *Rev. Sci. Instrum.* **79**, 023707 (2008).
13. Mayes, S. A. *et al.* Applications and assessment of an excitation-scanning hyperspectral imaging system. in *Imaging, Manipulation, and Analysis of Biomolecules, Cells, and Tissues XVI* (eds. Farkas, D. L., Nicolau, D. V. & Leif, R. C.) 5 (SPIE, 2018). doi:10.1117/12.2290120.
14. Parker, M., Browning, C., Rich, T. C. & Leavesley, S. J. Optimization of light transmission through an excitation-scan hyperspectral mirror array system. in *Imaging, Manipulation, and Analysis of Biomolecules, Cells, and Tissues XVII* (eds. Farkas, D. L., Leary, J. F. & Tarnok, A.) 23 (SPIE, 2019). doi:10.1117/12.2510555.
15. Leavesley, S. J., Sweat, B., Abbott, C., Favreau, P. & Rich, T. C. A theoretical-experimental methodology for assessing the sensitivity of biomedical spectral imaging platforms, assays, and analysis methods. *J. Biophotonics* **11**, e201600227 (2018).
16. Optical System and Detector Requirements for Live-Cell Imaging. *Nikon's MicroscopyU* https://www.microscopyu.com/techniques/fluorescence/optical-system-and-detector-requirements.