

Numerical Spectral Demultiplexing Microscopy of Measurements from an Anatomical Specimen

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Abstract

Multispectral microscopy is a method of capturing spectral bands using a microscope, and is used to observe specimens on a micron or nano scale. However, these systems are limited because they cannot capture transient phenomena since they cannot capture simultaneous spectral information. We propose a new method called numerical spectral demultiplexing microscopy (NSDM) which utilizes a Raspberry Pi camera to capture RGB measurements and then infer narrow-band multispectral spectra. This is accomplished by training a non-linear regression random forest model based on the spectral sensitivity of the camera which allows for a low-cost, portable, and simultaneous capture multispectral microscopy system. We use the NSDM system as a bright-field multispectral microscope and a dark-field fluorescence multispectral microscope on an anatomical specimen and show that additional information can be gathered by combining a bright-field and dark-field fluorescence microscope.

1 Background

Microscopes are optical instruments that allow the study and observations of objects on the micron and nano scale. Most microscopy systems capture spectral data in a limited number of wavelength bands which results in limited spectral information about the specimen. Moreover, when a multispectral microscope captures multiple spectral bands using a variety of light sources or optical filters, it is not done in a simultaneous manner, which prevents capturing transient phenomena when imaging samples *in-vivo*. These systems quickly become very large and expensive which limits their mobility and accessibility.

To combat the issues that arise when sequentially capturing multispectral data, one approach is to capture RGB signals simultaneously by using a consumer three-band RGB camera, and then inferring additional spectral information during the post-processing of the imaging data. We propose to use a new method to predict narrow-band spectra from RGB measurements and use this method for microscopy applications. We call this method Numerical Spectral Demultiplexing Microscopy (NSDM) which allows for a low-cost, portable multispectral microscope, which can be used for point-of-care application.

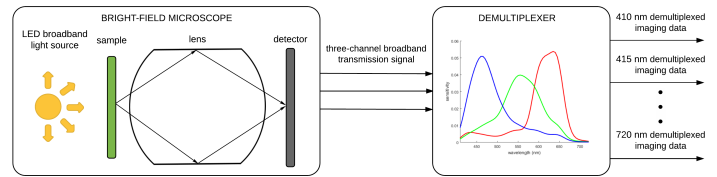
2 Methodology

The NSDM system is an extension of previous work by Deglint *et al.* [1] and is broken into two components, as illustrated in Fig. 1. As the first component, we built a microscopy instrument using the Raspberry Pi camera system. To turn this camera into a digital microscope a cemented achromatic doublet lens with a focal length of 10 mm and a diameter of 8 mm was placed in the optical path of the Raspberry Pi camera.

The second component is the numerical spectral demultiplexer, which is used to demultiplex the multiplexed RGB signals into a series of demultiplexed narrow-band imaging signals at different wavelengths using a non-linear regression model created from the prior knowledge of the spectral characterization of the detector.

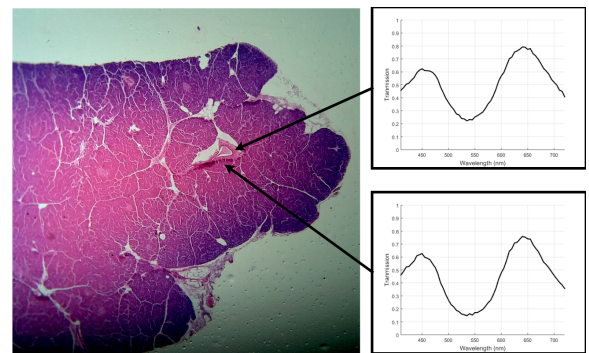
The spectral sensitivity of the camera was measured using a monochromator from 420 nm - 720 nm at a resolution of 5 nm, resulting in 63 measurements. Using this spectral sensitivity, a forward model that mathematically describes the relationship between 63 narrow-band wavelength signals and three multiplexed RGB imaging signals, can be written as $M_{3 \times 1} = O_{3 \times 63} \Lambda_{63 \times 1}$ where M denotes the broadband multiplexed imaging measurements for the three different RGB channels and Λ represents the narrow-band spectral signals at 63 different wavelengths. The spectral characterization of the detector can be described by the observation matrix, O .

Since the inverse mapping $\Lambda = O^{-1}M$ is non-linear, we employ a non-linear regression model to model this mapping. The non-linear modeling approach used in this paper is a decision-tree strategy known as random forest modeling (RFM) [2].

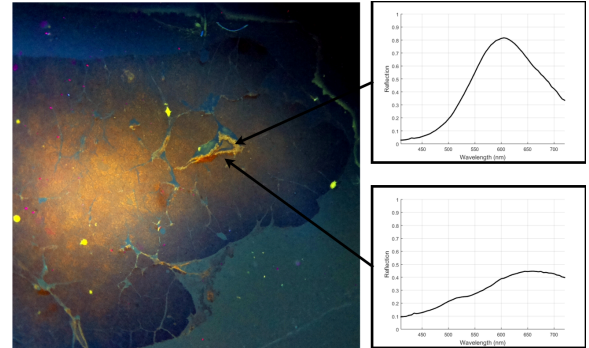


(a) Bright-field Numerical Spectral Demultiplexing Microscope (NSDM)

Fig. 1: A digital microscope was built using the Raspberry Pi camera and a simple lens. As seen, this microscope can operate as a bright-field microscope, or as a dark-field fluorescence microscope by placing a UV light source between the sample and the lens. The three channel broadband measurements of the bright-field or dark-field fluorescence microscope system are fed into the demultiplexer to predict narrow-band spectral signals.



(a) Islet of Langerhans of the Pancreas Bright-field Image



(b) Islet of Langerhans of the Pancreas Dark-field Fluorescence Image

Fig. 2: The NSDM was used to capture images of the islet of Langerhans region of the pancreas using (a) a bright-field microscope and (b) a dark-field fluorescence microscope. In (a) different parts of the anatomical specimen have similar predicted spectral signatures. In (b) the same pixel locations have much different predicted spectral signatures and therefore can be distinguished.

3 Results and Discussion

The transmission spectra at two different pixels of the islet of Langerhans region can be seen in Fig. 2a. Comparing these two spectral signatures demonstrates that they are very similar in nature. However, if we inspect the same pixels in the reflection spectra of the dark-field fluorescence image (Fig. 2b), we can see that the pixels predict significantly different signatures. This illustrates that additional information can be gathered by combining a bright-field NSDM system with a dark-field fluorescence NSDM system.

References

- [1] Deglint, J., Kazemzadeh, F., Shafiee, M. J., Li, E., Khodadad, I., Saini, S. S., Wong, A., and Clausi, D. A., "Virtual spectral multiplexing for applications in in-situ imaging microscopy of transient phenomena," in [SPIE Optics and Photonics], (August 2015).
- [2] Breiman, L., "Random Forests," *Machine Learning* (2001).