Introduction

Microendoscopy offers clinicians a powerful tool to image lesions with cellular resolution and create “optical biopsies”. However, subsurface tissue scattering creates undesirable background on the image, reducing the contrast. While there are many systems such as point-scanning confocal and 2-photon microscopy that can reject scattering background, these systems are typically very slow and expensive due to the opto-mechanical parts. We are proposing a structured illumination microscopy that is able to reject scattering while acquiring images in real time using an affordable digital light projector and a color camera. We test background rejection ability of our system both theoretically and experimentally and show that it provides out-of-focus background rejection while costing a fraction of the price of a traditional confocal system.

Axial Response in Microscopy

A microscopic system’s ability to reject background can be measured by axial response. A generic equation for calculating the axial resolution is:

\[ I(u) = \int g^2(u, \delta) \delta \, d \delta \]

This equation states that the axial resolution of a microscope is determined by an average of the behavior of all the spatial frequencies with defocus. Thus a single frequency structured illumination can enhance axial resolution as shown below.

Step 1. One-time Calibration

The system requires a one time calibration to correct crosstalk between color channels. This brightness contribution can be measured for each illumination-detection combinations. Color corrected images are obtained by solving a system of linear equations.

\[ \begin{align*}
I_{R1} &= \alpha_1 I_{R1}^0 + \alpha_2 I_{R2}^0 + \alpha_3 I_{G1}^0 + \alpha_4 I_{B1}^0 + \delta_1 I_{G1}^0 + \delta_2 I_{B1}^0 \\
I_{G1} &= \alpha_1 I_{R1}^0 + \alpha_2 I_{R2}^0 + \alpha_3 I_{G1}^0 + \alpha_4 I_{B1}^0 + \delta_1 I_{R1}^0 + \delta_2 I_{B1}^0 \\
I_{B1} &= \alpha_1 I_{R1}^0 + \alpha_2 I_{R2}^0 + \alpha_3 I_{G1}^0 + \alpha_4 I_{B1}^0 + \delta_1 I_{R1}^0 + \delta_2 I_{G1}^0
\end{align*} \]

Step 2. Image Acquisition

Three phase shifted column pattern is created in each of the channel, and the 3 channels combine to create the illumination pattern. We project the pattern, obtain the image, and correct the color crosstalk.

Step 3. Image Reconstruction

The color corrected images are mean-corrected using a center patch, and then demodulated to obtain the low background image.

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References