Motion compensation in structured illumination fluorescence endomicroscopy

Andrew Thrapp, Michael R. Hughes

Andrew Thrapp, Michael R. Hughes, "Motion compensation in structured illumination fluorescence endomicroscopy," Proc. SPIE 10854, Endoscopic Microscopy XIV, 108541D (26 February 2019); doi: 10.1117/12.2509590

SPIE.

Event: SPIE BiOS, 2019, San Francisco, California, United States

Motion compensation in structured illumination fluorescence endomicroscopy

Andrew Thrapp*, Michael R. Hughes
Applied Optics Group, School of Physical Sciences, University of Kent, Canterbury, Kent. CT2 7NH, UK

*at600@kent.ac.uk

ABSTRACT

Endomicroscopy is a technique for obtaining real-time images in vivo, eliminating the need to biopsy a tissue sample. A simple fluorescence endomicroscope can be constructed using a fiber bundle, camera, LED and filters, and individual images can be mosaicked as the probe is moved across the tissue to increase the image size. However, to improve image contrast optical sectioning is required for the removal of returning out-of-focus light. Commonly, this is done using the confocal technique, requiring more expensive laser sources and mechanical scanning mirrors which limits the frame rate. Structured illumination microscopy (SIM) instead uses line patterns projected onto the sample to allow for computational optical sectioning. This eliminates the need for point scanning and allows an incoherent light source, such as an LED, to be used, at the cost of some loss of signal-to-noise ratio. However, as SIM requires multiple images to be combined, motion of the probe results in severe image artefacts, preventing the use of mosaicking techniques. We report a SIM endomicroscope using a digital micro-mirror device (DMD) to generate line patterns at high speed, and with the ability to change the patterns on the fly. Combined with a high-speed camera, this reduces motion artefacts significantly, but not sufficiently to allow for video mosaicking techniques. We therefore demonstrate further reduction of artefacts by orienting the illumination patterns parallel to the direction of motion and performing inter-frame registration and correction. This offers potential for low cost, versatile, optically-sectioned endomicroscopy.

Keywords: Endomicroscopy, digital micro-mirror device, structured illumination, optical sectioning

INTRODUCTION

Clinical demand is motivating research into optical biopsy as an alternative to excisional biopsy and histology. A conventional biopsy requires the removal of a tissue sample from the patient, followed by staining and examination under a microscope by a qualified clinician[1,2]. In contrast, an optical biopsy with an endoscope can be used to provide clinicians with the means to make an on-the-spot diagnosis and in some cases provide immediate treatment[3]. Endoscopic optical biopsy can be used in accessible organs such as skin and oropharynx, hollow organs such as oesophagus, stomach, and rectum, and surgically accessible organs such as the prostate, and ovaries[1].

One form of optical biopsy, endomicroscopy, is essentially endoscopy with microscopic resolution. Endomicroscopy is useful to obtain cellular information such as elevated nuclear to cytoplasmic area ratio, nuclear crowding, and pleomorphic nuclei[3,4]. Endomicroscopy is commonly performed using a multicore fiber imaging bundle (~30,000 cores) with an inter-core spacing of around ~2-4 μm[5]. The imaging bundle, which has a pseudo-hexagonal core layout, allows image transmission in pixelated form[5]. Fluorescent dyes such as acriflavine can be topically or intravenously applied to improve contrast[6]. The distal end of the fiber can be placed in direct contact with the tissue, or imaged onto the tissue using a miniaturized lens assembly. In both cases, for a thick sample, light returns from both in and out-of-focus axial planes, and so optical sectioning is required for high resolution imaging[7].

While confocal endomicroscopy has been widely implemented, it is a relatively complex and expensive approach. Structured illumination microscopy (SIM) is an alternative technique for optical sectioning[8]. SIM works by modulating features in a thin slice around the axial position of the focal plane[8], allowing information from the focal plane to be distinguished from information from out-of-focus planes via numerical post-processing. The theory of SIM requires the focal plane to be modulated with sine waves of a single spatial frequency[8]. However, a well reported approximation uses square lines instead, relying on blurring as they propagate through the optical system due to the point-spread function. To

Endoscopic Microscopy XIV, edited by Guillermo J. Tearney, Thomas D. Wang, Melissa J. Suter, Proc. of SPIE Vol. 10854, 108541D · © 2019 SPIE
CCC code: 1605-7422/19/$18 · doi: 10.1117/12.2509590

remove the overlapping line pattern from images, the line patterns are spatially phase shifted three times, and three-phase demodulation is then used to remove the unwanted background light[8].

The intensity of the reconstructed image is determined by the amplitude of the modulation[9]. This decays with defocus at a rate determined by the spatial frequency[9]. A well-known problem occurs when imperfect modulation at the focal plane leads to a decrease in the signal-to-noise-ratio (SNR)[10]. Nevertheless, SIM has previously been successfully incorporated into endomicroscopy[11]**.** However, a further factor in fibre bundle endomicroscopy is that, due to the constraint arising from the pixel size in the fibre bundle, the width of the line pairs must be optimized between the intensity of the reconstructed image, the axial sectioning strength, and the SNR.

Several well-established methods have been used to provide the non-uniform illumination. These include the interference of coherent lasers, a mask placed immediately after the illumination source and imaged onto the focal plane, or programmable devices such as a spatial light modulator, or a digital micro mirror device[12]. There are advantages and limitations to each approach. Lasers are expensive compared to LED's and the interference of a coherent source introduces speckle-noise[12]. Masks have non-changeable patterns and need to be moved mechanically, approaches which translate the slide at a constant velocity can lead to drift and hysteresis[12]. Programmable sources such as a digital micro-mirror devices (DMDs) or spatial light modulators (SLMs) have an array like structure where pixels can be turned on and off. This allows the use of an inexpensive incoherent source such as an LED, eliminating speckle noise, and dwell time can be customized. However, the line pattern size is limited by the DMD or SLM array, and pattern switching speed rates are then limited by the hardware[12].

A conventional biopsy has an image size of >2.5 mm^2 [13]. In contrast, a fiber bundle endomicroscope, designed to fit in the working channel of a commercial endoscope, has an image diameter of less than 1 mm and an area of less than 0.75 mm^2 [6]. The smaller image size makes it difficult to obtain a broad sense of tissue morphology, and introduces sampling errors when imaging heterogeneous biological tissue[4]. Video mosaicking can be used to stitch together overlapping frames to increase the usable image size of endomicroscopic images[4]. Successful and robust mosaicking requires sufficient signal generating features for template matching, spatial overlap between consecutive images, minimal tissue deformation, no probe rotation, and minimal moving debris[4]. Both online and offline mosaicking can be used. Offline video mosaicking, with no fixed processing-time requirements, can more accurately mosaic images by correcting for non-rigid tissue deformation. Online video mosaicking techniques use simple template matching assuming rigid deformation to provide clinicians real time access to images[4]. The fiber bundle pattern structure interferes with template matching due to the fixed spatial frequencies, and so must be removed before attempting mosaicking. This can be accomplished using spectral filtering in the frequency domain, median filtering in the spatial domain, spatial interpolation, or a nearest-neighbor lookup method[4].

A significant problem with SIM-based endomicroscopy is that three phase-shifted images are required to produce each optically-sectioned image. Any motion of the probe therefore introduces artefacts, making mosaicking impossible. Higher frame rates would limit motion artefacts, and so below we describe a DMD-based implementation of a SIM-endomicroscope which can provide frame rates limited only by the camera. We show that if the illumination pattern is aligned with the motion, simple registration of the three patterns allows for reconstruction with minimal artefacts, and we propose that this may form the basis of an adaptive DMD-SIM endomicroscope.

METHODS

To demonstrate DMD-SIM endomicroscopy, we modify the illumination optics in a widefield fluorescence endomicroscopy system similar to that described by Pierce et al.[1] , as shown in Fig 1. The DMD is a Texas Instruments Lightcrafter DLP 3000, a diamond array of mirrors which can each be individually switched on or off. The DMD is illuminated with the built-in blue LED in the Lightcrafter. A demagnified image of the DMD is formed by a 4x objective. A short pass filter selects light below 450 nm and a dichroic reflects this to onto x10 finite conjugate objective, which images the DMD onto the proximal face of the fiber bundle (Fujikura FIGH-30-650-S). Returning light from the fiber bundle is imaged onto a camera (FLIR Flea3) via a 500 nm long-pass filter.

In order to generate SIM patterns on a rectangular grid, the minimum pitch of the line pattern is six DMD pixels, as it must include prime factors of two and three; two to ensure an equal amount of white and dark space, and three for the three spatial phase positions. The DMD has a diamond pixel structure, and with line pairs around the size of the array the lines are not uniform. To adapt for this, patterns were generated at rotation of 45 degrees, eliminating the jagged edges, as illustrated in Fig. 2.

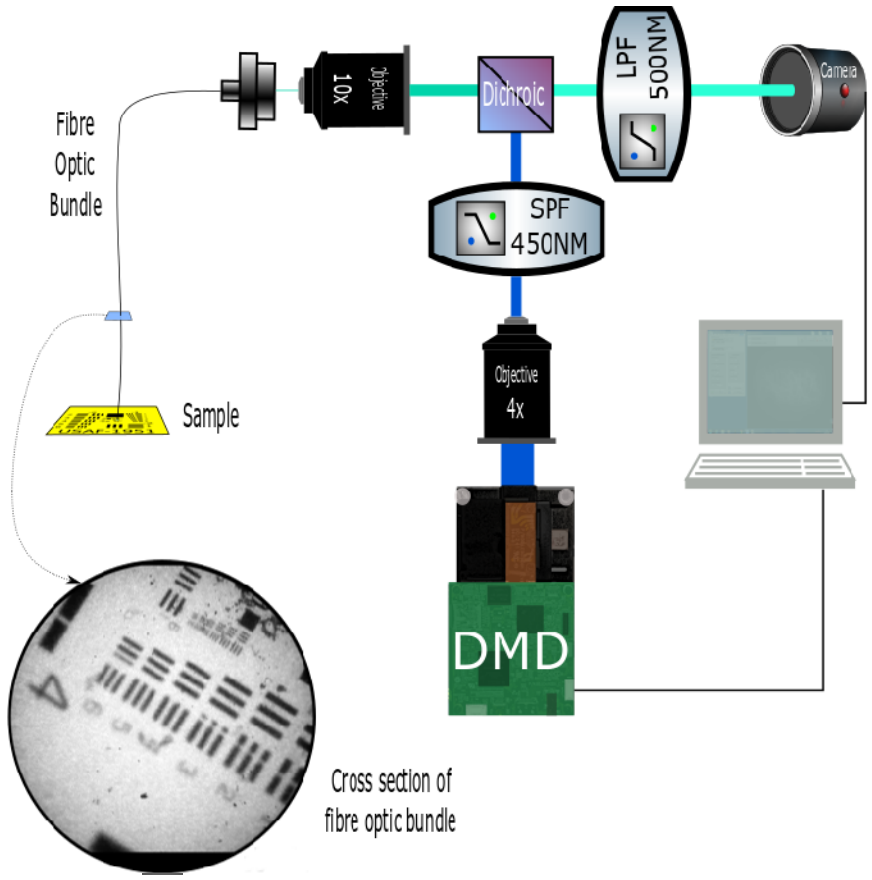

Figure 1. Schematic of endomicroscopy setup. SPF: short-pass filter, LPF: long-pass filter.

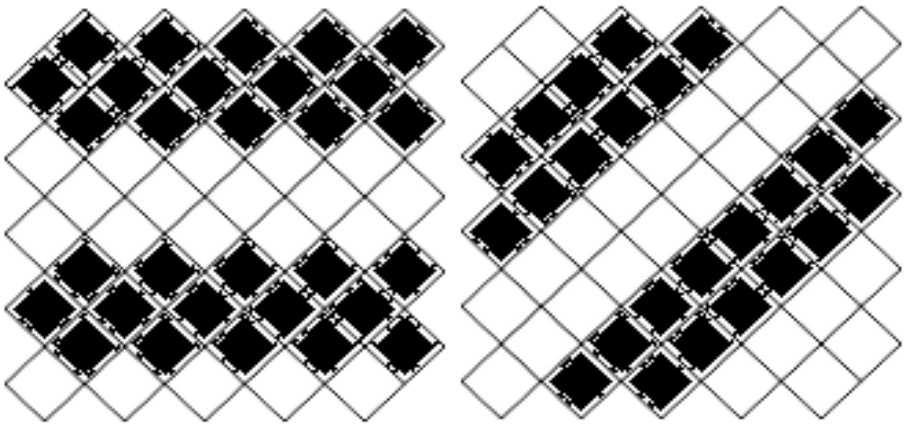

Figure 2. Diamond array of the DLP 3000 Lightcrafter and SIM patterns generated for a rectangular array (left), and optimized for the diamond structure (right).

As discussed above, acquiring multiple images for SIM will lead to motion artefacts when the probe is in motion. We report an extension to the frame sequence required for SIM in which inter-frame registration is used to correct for the motion and reduce artefacts. The SIM scheme requires projecting three line pairs onto a sample. To compensate motion, fully illuminated frames (i.e. without structured illumination) are introduced at the beginning and end of a sequence. Using interframe registration between the two fully illuminated frames, the shift between the SIM frames can be estimated as ¼ the total movement. The three images can then be correctly aligned prior to SIM demodulation.

RESULTS

In order to optimize the line pairs for optical sectioning strength, the axial response was determined by translating a metal plate axially from the focal plane (Fig. 3). This was done for both a fiber bundle system and with the bundle removed. Due to the low NA of the objective, the bundle exhibits superior optical sectioning. The modulation depth in the ideal case was measured and found to be 0.6 for the fiber bundle with line pair widths greater than 45 µm.

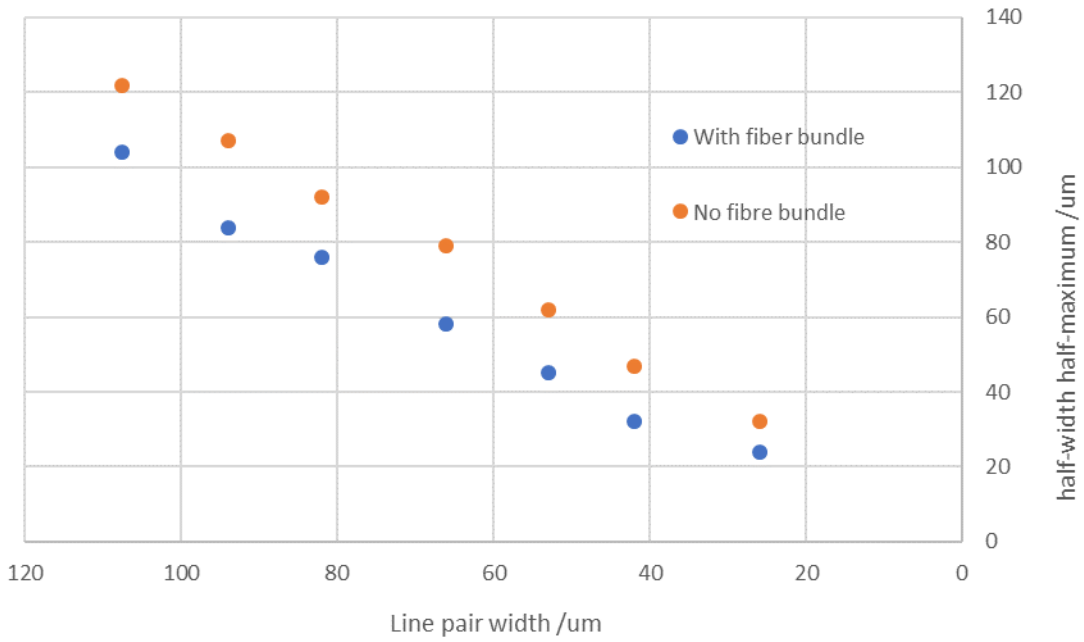

Figure 3. A comparison of relative intensity drop-off distance (half-width, half-maximum) for a FIGH-30-650-S fiber optic bundle, and with the bundle removed. Line pair width measured at the tip of the bundle.

Motion artefacts exhibit different properties depending on the movement of the probe relative to the line pair orientation. We demonstrate these artefacts by translating a stage at a fixed speed parallel and perpendicular to the orientation (Fig. 4). The perpendicular case shows artefacts at a fixed spatial frequency, while the parallel suffers mainly from blurring.

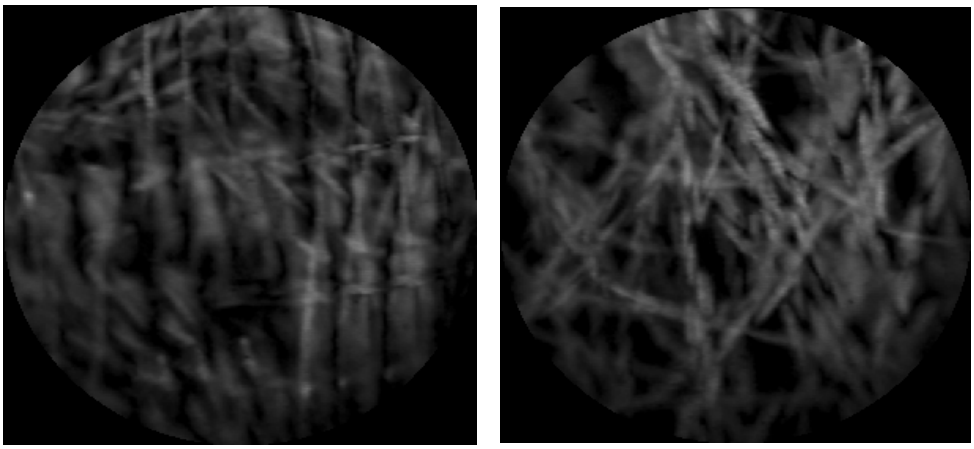

Figure 4. Two images taken showing motion artefacts introduced from a translated probe. Left image translated perpendicular to line patterns with a translation stage speed of 0.5 mm/s, right image translated parallel to line pattern orientation with a stage speed of 0.25 mm/s. Exposure time for both images fixed at of 50 μs, with a delay between images of 50 μs. Probe diameter for scale 720 +/- 35 μm.

To show the reduction of artefacts, line pairs with a width of 88 μm were projected onto the tip of a 568 μm bundle (Fig. 5). The probe was translated 14.5 μm between raw frames and images were acquired. Images were reconstructed for both SIM and WF without inter-frame registration (a,b) and with inter-frame registration (c,e), and comparison images were generated from frames acquired with no movement (d,f). A normalized cross correlation (NCC) comparison was then performed. The peak NCC value between the motion compensated SIM image and the static reference image was 0.917, and for the SIM image without motion compensation it was 0.551. The WF reconstructed image had a NCC value of 0.963 while the one without motion compensated had an NCC value of 0.843. The measured contrast for a SIM reconstruction with no movement between frames was 1, compared to a contrast of 0.679 for the static WF image.

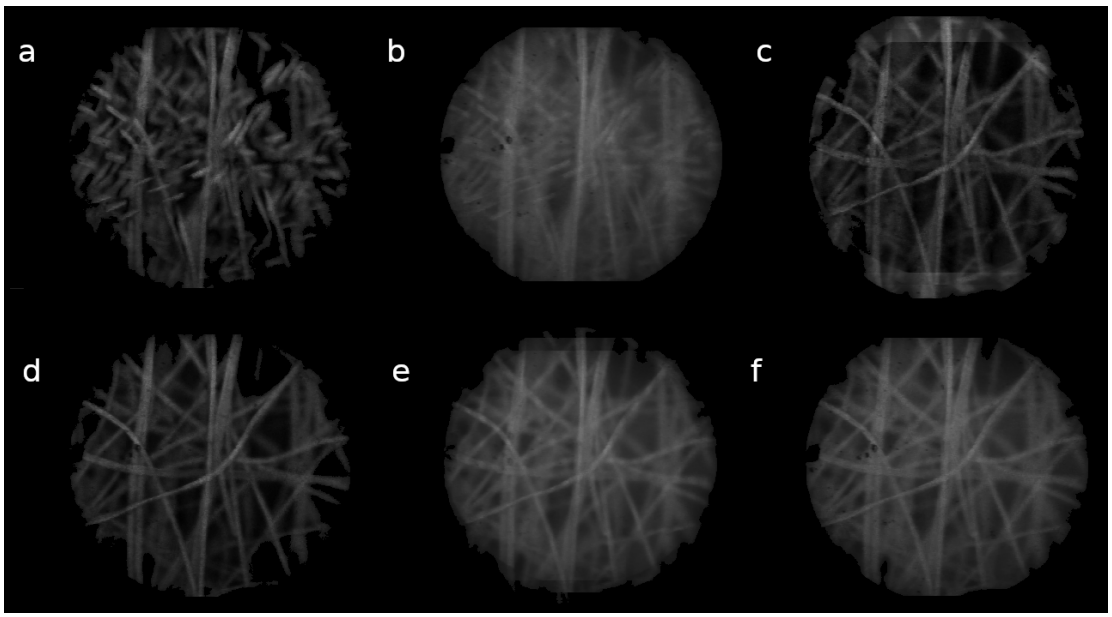

Figure 5. Comparison of SIM and widefield (WF) reconstructions for tissue paper stained with yellow fluorescent highlighter. Image a) SIM reconstruction without motion compensation, b) WF reconstruction without motion compensation (by summing the three SIM acquisitions), c) SIM reconstruction with motion compensation, d) SIM with static frames - contrast measured as 1, e) WF reconstruction with motion compensation, f) Static WF image - contrast measured as 0.679. Probe diameter 568 μm for scale. Line pairs with a width of 88 μm were projected onto the tip of the bundle for SIM and WF reconstruction images. Probe translated 14.5 μm between raw frames in images a,b,c and e. No probe translation in frames d and f. Fiber bundle core pixilation effects removed with a median filter.

DISCUSSION AND FUTURE WORK

A DMD-SIM endomicroscope has been demonstrated, for which registration of frames can result in a reduction of motion artefacts when the line pairs are aligned with direction of travel of the probe. The greater improvement in the normalized cross correlation value for the SIM case compared to the WF case can be explained by the differences in contrast; NCC uses differences in intensity values to determine quality of fit, and greater contrast will produce larger differences between matched and unmatched regions. The elimination of fixed spatial frequency artefacts and reduction of blurring allows for video mosaicking which relies on template matching. We intend to develop an approach which will allow for determining the direction of travel of the probe and aligning the patterns parallel to that direction. This will lead to optically sectioning endomicroscopy which is robust to motion.

ACKNOWLEDGMENTS

This work was partially support by EPSRC Grant EP/R019274/1, *Ultrathin Fluorescence Microscope in a Needle*. Andrew Thrapp is supported by a University of Kent Vice Chancellor's PhD Scholarship.

REFERENCE LIST

[1] Liu, J.T., et al., "Point-of-care pathology with miniature microscopes," Anal Cell Pathol (Amst), 2011. 34(3): p. 81-98.
[2] Muldoon, T.J., et al., "Subcellular-resolution molecular imaging within living tissue by fiber microendoscopy." Optics Express, (2007). 15(25): p. 16413-16423.
[3] Grant, B.D., et al., "High-resolution microendoscopy: a point-of-care diagnostic for cervical dysplasia in low-resource settings." Eur J Cancer Prev, (2017). 26(1): p. 63-70.
[4] Bedard, N., et al., "Real-time video mosaicing with a high-resolution microendoscope." Biomed Opt Express, (2012). 3(10): p. 2428-35.
[5] Oh, G., E. Chung, and S.H. Yun, "Optical fibers for high-resolution in vivo microendoscopic fluorescence imaging." Optical Fiber Technology, (2013). 19(6): p. 760-771.
[6] Wang, T.D. and J. Van Dam, "Optical biopsy: a new frontier in endoscopic detection and diagnosis". Clin Gastroenterol Hepatol, (2004). 2(9): p. 744-53.
[7] Jabbour, J.M., et al., "Confocal endomicroscopy: instrumentation and medical applications." Ann Biomed Eng, (2012). 40(2): p. 378-97.
[8] Neil, M.A.A., R. Juskaitis, and T. Wilson, "Method of obtaining optical sectioning by using structured light in a conventional microscope." Optics Letters, (1997). 22(24): p. 1905-1907.
[9] Wilson, T., "Optical sectioning in fluorescence microscopy." J Microsc, (2011). 242(2): p. 111-6.
[10] Hagen, N., L. Gao, and T.S. Tkaczyk, "Quantitative sectioning and noise analysis for structured illumination microscopy." Opt Express, (2012). 20(1): p. 403-13.
[11] Bozinovic, N., et al., "Fluorescence endomicroscopy with structured illumination." Optics Express, (2008). 16(11).
[12] Karadaglic, D. and T. Wilson, "Image formation in structured illumination wide-field fluorescence microscopy." Micron, (2008). 39(7): p. 808-18.
[13] Gonzalez, S., et al., "Randomized comparison of 3 different-sized biopsy forceps for quality of sampling in Barrett's esophagus." Gastrointest Endosc, (2010). 72(5): p. 935-40.
[14] Pierce, M., D. Yu, and R. Richards-Kortum, "High-resolution fiber-optic microendoscopy for in situ cellular imaging." J Vis Exp, 2011(47), (2011).