Design of an in-vivo multi-spectral confocal microendoscope for clinical trials

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ABSTRACT

We previously reported on the development and testing of a multi-spectral confocal microendoscope. Here we present a new system that will be used during an early stage clinical trial. The new microendoscope is significantly smaller, uses fewer optical elements, and is structurally more robust. The slit-scanning confocal system employs two synchronized single-axes scan mirrors and an externally coupled imaging catheter with automated focus control and dye delivery systems. In grayscale collection mode the confocal microendoscope operates at 30 frames-per-second with 3μm lateral resolution and 25μm axial resolution. The multi-spectral collection mode operates at 0.5 frames-per-second when acquiring 32 spectral channels with an average minimum resolvable wavelength difference of 12nm. The system will be used, in grayscale mode, to image ovaries during a small scale clinical trial on women undergoing oophorectomy. Recent grayscale and multi-spectral imaging results from ex-vivo human tissues are presented.

Keywords: Fiber, optics, microscopy, confocal, endoscope, fluorescence, spectral, spectroscopy, tissue

1 INTRODUCTION

Optical biopsy strives to transform the practice of traditional biopsy to a minimally invasive procedure that provides instant visualization of pathology. This emerging technology has significant potential in areas where disease screening is not adequately developed and/or in areas where pathology is sparse enough that the inherent sampling nature of traditional biopsy is a problem. We have developed and previously reported[1-7] on a 2nd generation multi-spectral confocal microendoscope (MCME) that has shown significant promise in the field of optical biopsy. The MCME is a custom built full-spectrum slit-scanning confocal microscope coupled to a small and flexible fiber-optic catheter. Previous work has shown that the MCME can provide a high resolution microscopic view of tissue. The MCME has the potential to provide physicians with increased diagnostic capabilities and ultimately improve diagnosis.

Applications for the MCME include the diagnosis of diseases of the gastro-intestinal tract and female reproductive system. More specifically, the system may have significant potential as a screening tool for the early detection of ovarian cancer. Ovarian cancer is the second most lethal gynecologic condition with a 5-year survival rate of only 44%[8]. The disease is routinely diagnosis late in its progression, which usually leads to poor and unsuccessful treatment. With early detection, the 5-year survival rate increases to 94% but only 19% of cancers are caught in this early stage[8]. Unfortunately, an accurate ovarian cancer screening test in not avialable[8]. As a result, many high-risk patients choose prophylactic oophorectomy, which causes premature onset of menopause and has significant risks associated with hormone replacement therapy.

The MCME, with its high resolution grayscale and multi-spectral imaging capability, may enable accurate screening of women at high risk of ovarian cancer. Most ovary-related gynecologic surgeries use laparoscopic techniques to gain intaperitoneal access to the ovaries. The MCME could be inserted through a trocar during laparoscopy, thus enabling microscopic imaging of the epithelial surface of the ovary. This approach should provide an effective view since most ovarian cancers are epithelial in nature.

With this as our primary goal, a new clinical prototype 3rd generation MCME has been built that incorporates a modified catheter and a completely redesigned optical system. The details of the application specific catheter are describe in

another publication[9]. In this paper, we present the MCME's proximal optical assembly as well as preliminary grayscale and multi-spectral images acquired with the new system.

2 SYSTEM DESIGN

The specific details of the 2nd generation MCME have been previously reported[4,5]. Figure 1 shows the layout of the optical system along with photos of the catheter. The bench-top optical system was constructed on a 2' x 4' optical table that could be placed on a mobile cart. However, the size and weight of the system made such transport extremely difficult. The complex optical path consisted of 13 lenses and 6 mirrors, which caused a significant reduction in optical performance and made the system difficult to align. In addition, the complexity of the system made the 2nd generation MCME exceptionally sensitive to slight changes in mechanical layout. As a result, the system was prone to misalignment, particularly during transport. The system used two scientific grade CCD cameras, one for grayscale and one for multi-spectral collection, which added size, weight, and cost to the MCME. The primary excitation sources for the system were laboratory grade water cooled argon-ion and krypton-ion lasers that operate on 208V electrical power. Overall, the 2nd generation MCME was not practical as a portable device. A clinical system would have to be smaller, lighter, more robust, and more reliable. In addition, the catheter of the MCME would need to be sterilizable and incorporate a dye delivery channel. For specifics on the changes made to the catheter please refer to our other publication[9] in this proceeding.

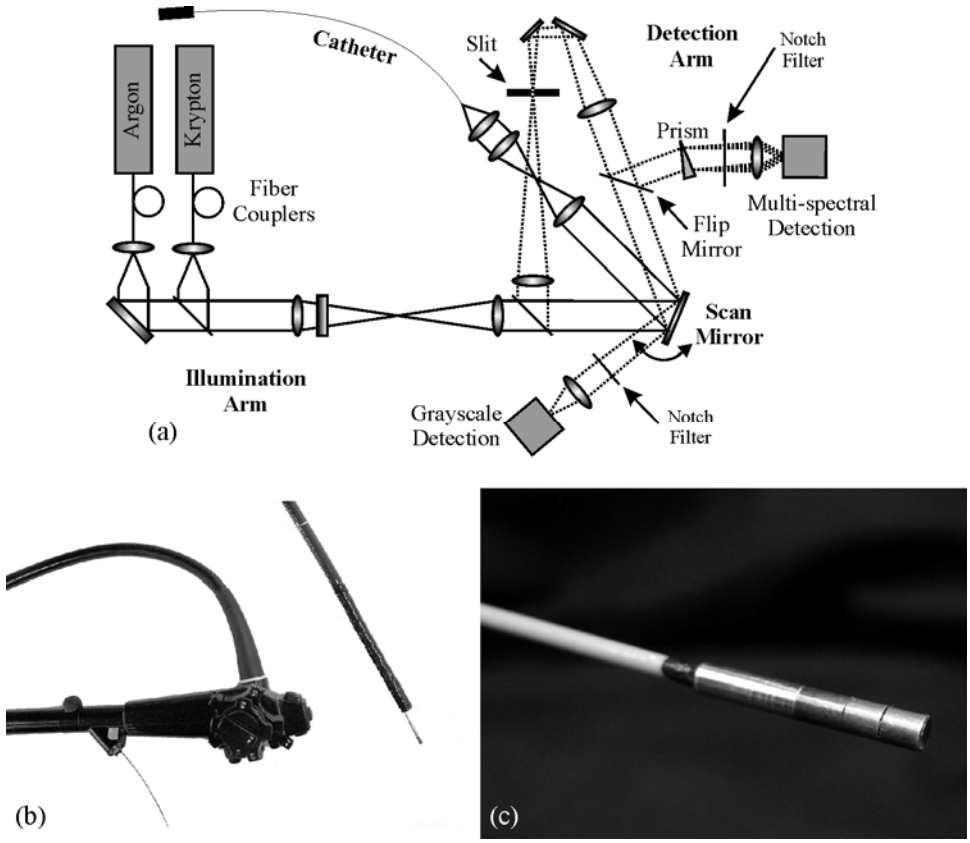

Figure 1 (a) Bench-top layout of the 2nd generation MCME, (b) catheter routed through a colonoscope, and (c) catheter on its own. The same catheter is used in the clinical prototype with a disposable protective sheath.

Several designs were considered for the 3rd generation optical system. Ultimately, the design depicted in Fig. 2 had the correct balance of size and functionality. A 488nm Coherent[10] solid-state laser is used in the system with an Oz Optics[11] fiber coupler. A spherical lens, cylindrical lens, and Olympus 10x microscope objective form an anamorphic optical system that provides a line of illumination on the proximal face of the catheter. The orthogonal view in Fig. 2 shows the illumination subassembly from the orientation that has power in the cylindrical lens. The catheter contains a Sumitomo Electric[12] coherent imaging bundle, which relays the illumination line to the distal tip of the catheter. A miniature

objective images the distal end of the fiber into the tissue and a miniature focusing mechanism enables depth selection to 200μm below the surface of the tissue. Induced sample fluorescence is collected by the catheter and separated from the reflected illumination by a dichroic beamsplitter. The fluorescence signal is focused onto the confocal slit aperture, which rejects the majority of light collected from out-of-focus planes. A pair of lenses and a second scan mirror relay the energy passed by the slit aperture onto a Photometrics[13] Cascade II CCD camera.

In grayscale collection mode the scan mirrors are driven at identical rates so that there is a one-to-one correspondence between the illumination profile on the tissue and the detection profile on the CCD. The frame transfer CCD collects full frames of data at roughly 30fps. The spatial resolution in grayscale collection mode is approximately 3μm lateral and 25μm axial. In multi-spectral collection mode a prism is introduced into the space between the second scan mirror and the camera lens. The second scan mirror is fixed at a constant angle while the first scan mirror sweeps the illumination profile across the tissue. For a fixed position of the first scan mirror, the two-dimensional light distribution on the CCD represents one dimension (along the slit direction) of spatial information and one dimension (perpendicular to the slit) of spectral information. A full three-dimensional image (2 spatial and 1 spectral) is read out sequentially as the first scan mirror sweeps across the tissue. The spectral resolution in multi-spectral collection mode is approximately 12nm using the current configuration. Figure 3 shows a photo of the clinical prototype in its current form. The total optical assembly, without the camera, will fit on a 1' x 2' optical breadboard.

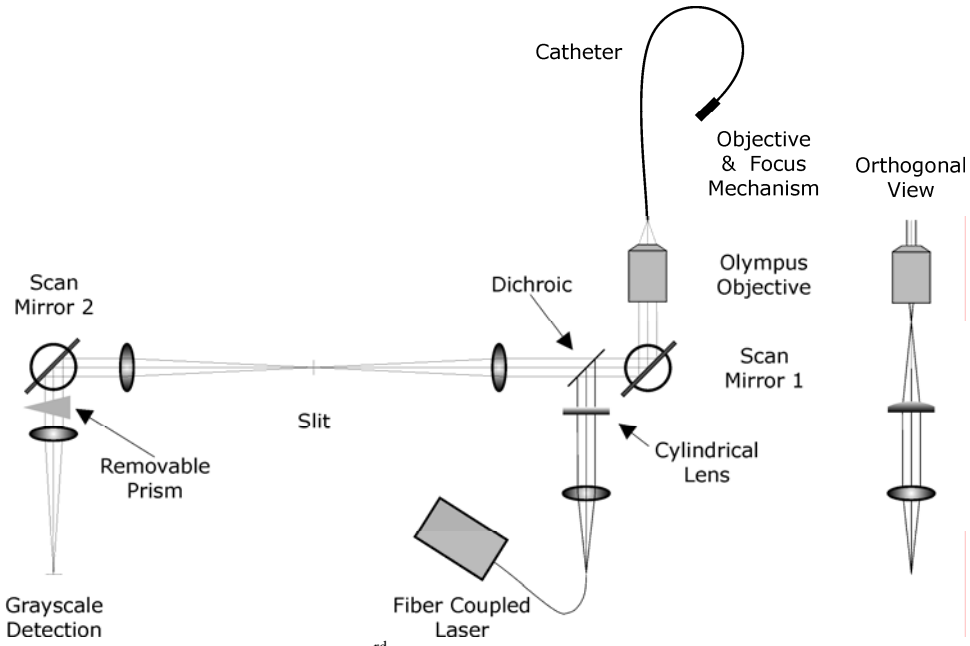

Figure 2 Layout of the clinical prototype 3rd generation MCME. The orthogonal view shows the illumination path from the cylindrical lens power direction.

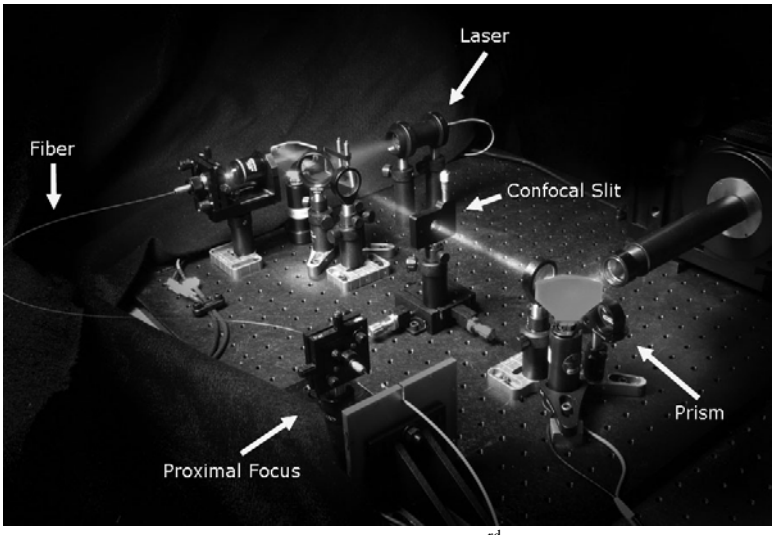

Figure 3 Photo of the clinical prototype 3rd generation MCME.

There are several major advantages to the new clinical prototype design. The most noticeable is the reduction in complexity. The optical path of the new system has one half the elements of the previous design. This yields dramatically higher throughput as well as a qualitatively noticeable increase in optical performance. The mounting hardware is more mechanically stable, which combined with the smaller design creates a very robust, low maintenance, and easy to align system. Overall, the footprint of the clinical prototype is one third the size of the previous system. The air-cooled solid state laser is very stable and easily coupled to the system through a FC terminated fiber. A three-way fiber coupler may be added in the future to introduce green and red laser excitation options. The proximal end of the fiber-optic catheter is now SMA terminated, which enables interchangeable application-specific catheters. The two scan mirrors further simplify the design and enable rapid transition between grayscale and multi-spectral collection on a single camera. With the addition of a low cost motorized prism mount the MCME could easily collect a full spectral image, by switching from grayscale to multi-spectral and back to grayscale collection modes, with less than a one second delay in the real time grayscale video display.

3 GRAYSCALE IMAGING RESULTS

Initial testing of the clinical prototype MCME was performed on a mouse that was sacrificed immediately prior to the experiment. An incision was made to give the MCME's catheter access to the peritoneal cavity of the animal. Figure 4 shows three images obtained from this experiment. The data were acquired in-vivo after topical application of 330μM acridine orange[14] (AO). Acridine orange is a vital nucleic acid fluorescent dye that intercalates with DNA and RNA. AO is efficiently excited by the MCME's 488nm excitation source and has dual emission spectra peaks at 525nm and 650nm. These images reveal significant differences between tissue types and show the 3rd generation MCME's ability to distinguish different tissues based on morphology.

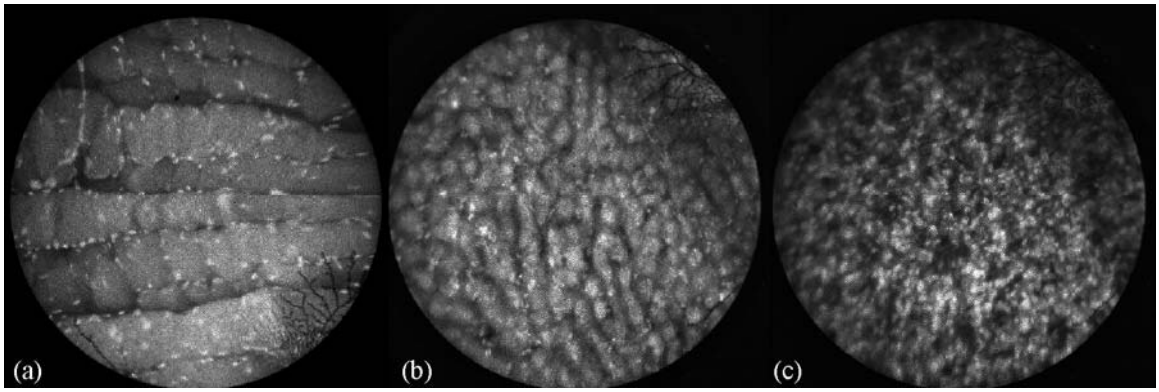

Figure 4 In-vivo grayscale images of mouse (a) peritoneal wall, (b) liver, and (c) lung.

Figure 5 shows a montage of preliminary grayscale results obtained from excised human ovary. For these experiments, a small sample of the resected ovary was imaged with the MCME immediately following prophylactic oophorectomy. The samples were stained with 330μM AO and imaged within one hour of surgical resection. The images were collected from several patients and provide excellent examples of the powerful imaging capabilities of the new system. The images are uniform and of high quality out to the edge of the field of view. The resolution and field-of-view of the MCME enable visualization of individual cells as well as local tissue morphology. Unfortunately histological results are not yet available for these tissues; however, a qualitative impression may be given based on our experience. Figures 5(a)-(c) show typical healthy ovarian epithelium, which is commonly manifested by a fairly uniform layer of cells. The irregular columnar patterns in Fig. 5(d) suggest the possibility of disease. Figure 5(e) shows normal undulations in the epithelium and Fig. 5(f) shows the slightly more sparse and elongated cells of the underlying stromal layer.

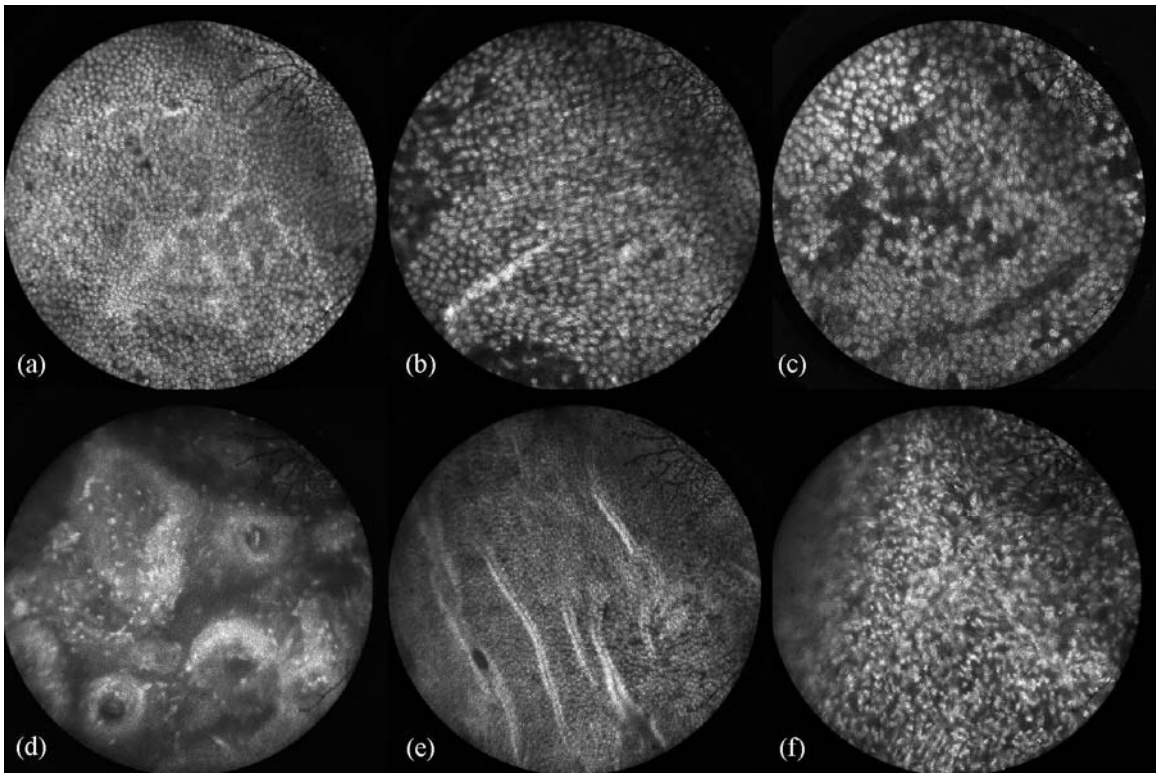

Figure 5 Grayscale imaging result from excised human ovary. (a)-(c) normal appearing epithelium, (d) unusual area of unknown diagnosis, (e) normal appearing epithelium showing slight undulations on the surface of the ovary and, (f) normal appearing stroma. Histological results unknown.

The immediate intended application for the new clinical prototype MCME is the study of ovarian cancer. However, there are many promising application for the system. Figure 6 shows three images collected from a small biopsy of human esophagus. In this project the MCME is being tested as a means to track the progression of Barrett's esophagus to fully invasive cancer. Barrett's esophagus is a premalignant condition that commonly develops into invasive cancer through a series of steps including low-grade and high-grade dysplasia. The MCME may be able to provide the necessary imaging capabilities to enable real-time minimally invasive investigation into the progression of this disease, ultimately providing a low cost screening tool for those at high risk of esophageal cancer. The MCME may also be useful as a means to guide endoscopic treatment of dysplasia and cancer of the esophagus by helping to define the margins of disease. The images in Fig. 6 were collected from a sample of human esophagus of unknown diagnosis. As the work progresses, we will develop a database of MCME images, which when matched to their corresponding histological results should provided insight into the systems ability to detect the early cellular and morphologic changes of esophageal cancer.

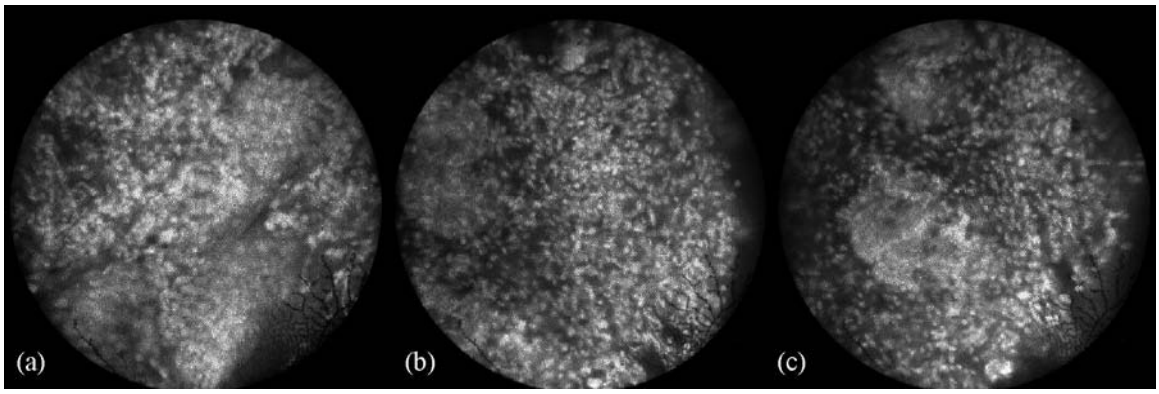

Figure 6 Grayscale imaging result from excised human esophagus. Histological results unknown.

4 MULTI-SPECTRAL IMAGING RESULTS

The data presented in the previous section clearly demonstrate that the clinical prototype MCME provides high quality grayscale images of cells and tissue morphology. The MCME may also act as an imaging spectrometer and collect spatially localized spectral information across the entire grayscale field-of-view. Figure 7 shows results obtained from a mixture of 15µm fluorescent beads with various spectral emission profiles. The image in Fig. 7 shows a grayscale projection of the multi-spectral data cube (i.e. the raw data summed over the spectral dimensions). The grayscale projection is approximately equivalent to an image that would be collected by the MCME in grayscale imaging mode. The plot in Fig. 7 shows the spectrum of two microspheres in the sample. The vertical scale in this plot is simply a relative digital camera unit and the horizontal scale is pixel position. Ultimately, the MCME will be properly calibrated, providing a direct correlation between wavelength and pixel position. The plot reveals significant overlap between the two emission spectra. One might envision that spectral information collected by the MCME could be used to deconvolve the emission profiles of multiple dyes with significant spectral overlap, thus enabling the use of a wider variety of fluorophors.

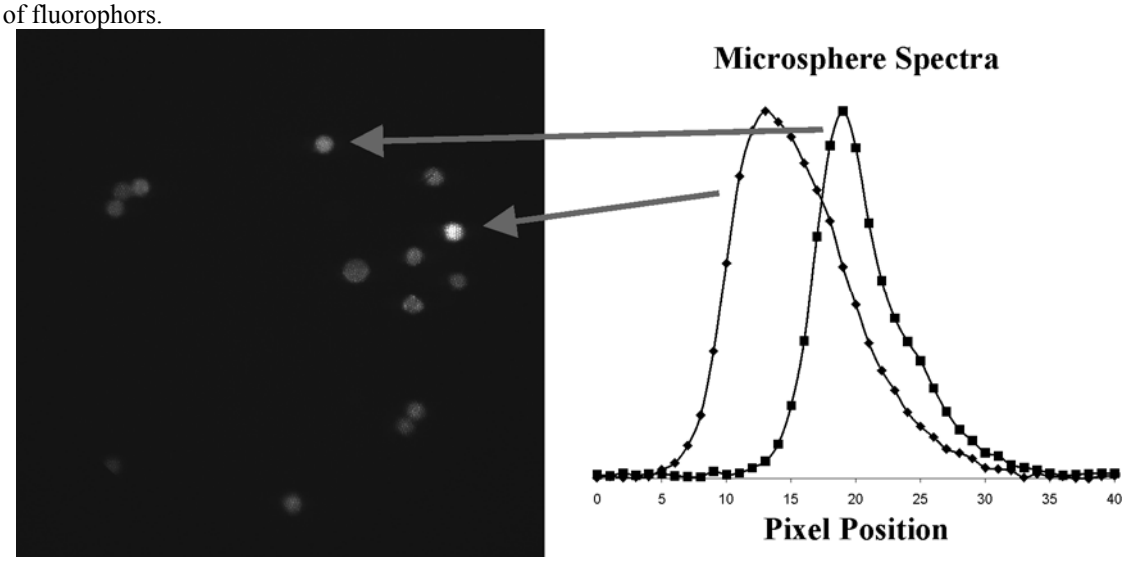

Figure 7 Multi-spectral data collected from a phantom of 15µm fluorescent microspheres with excitation/emission maxima of 505/512, 515/534, 540/560, 565/580, and 580/605. Spectra is show for two microspheres. Specific wavelength data will be available once a spectral calibration is performed.

Figure 8 shows multi-spectral data acquired from the same sample of human esophagus discussed in the previous section. The sample was topically stained with a 330µM AO. At proper concentration, acridine orange exhibits dual emission characteristics, staining cell nuclei with a peak emission of 525nm and staining cytoplasm with a peak emission of 650nm. The plots in Fig. 8 were extracted from a specific cell and clearly show a red shifted emission in the cytoplasm. Pathologist often look for enlarged nuclei as one of the morphologic indicators of cancer. With the proper

fluorescent dye or dyes, it may be possible for the MCME to aid in the diagnosis and detection of disease by providing an accurate measurement of nuclear to cytoplasmic ratio. This could be automated to provide an audible alert or visual overlay when the ratio becomes abnormal for the tissue type under observation.

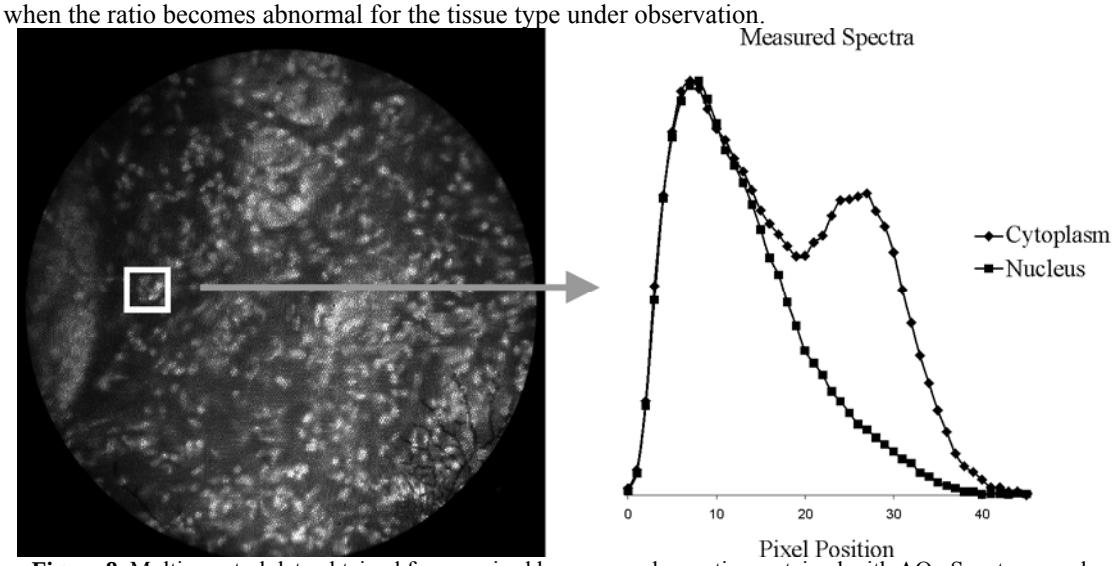

Figure 8 Multi-spectral data obtained from excised human esophagus tissue stained with AO. Spectra reveals that AO provides red and green staining of cytoplasm and nucleus, respectively. Specific wavelength data will be available once a spectral calibration is performed.

5 CONCLUSION

We have presented a new 3rd generation multi-spectral confocal microendoscope. The system is a clinical prototype that will be used during an early stage clinical trial in the study of ovarian cancer. The portable device is based on a simple and robust dual scan mirror design that maintains alignment when transported. The system is capable of rapidly switching between grayscale and multi-spectral collection modes. The MCME provides video-rate high resolution grayscale images with 3μm lateral resolution and 25μm axial resolution. In multi-spectral mode the system can collect 32 spectral channels with full spatial resolution in less than a one second. With the current configuration the average minimum resolvable wavelength difference is 12nm. Preliminary grayscale and multi-spectral imaging results show an overall improvement in optical performance when compared to the previous system. Overall, the MCME provides real-time grayscale and multi-spectral cellular imaging to the field of medical endoscopy. Upcoming ovarian clinical trials should prove the MCME's efficacy as a diagnostic clinical instrument.

ACKNOWLEDGMENTS

Human tissues were provided through clinical collaborations with Dr. Molly Brewer at the Arizona Health Sciences Center and Dr. Richard Sampliner at the Veterans Administration Hospital in Tucson. This work was supported by NIH grants CA73095 and CA115780, and ADCRC grant 9711.

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