

Tethered confocal endomicroscopy capsule for diagnosis and monitoring of eosinophilic esophagitis

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Abstract: Eosinophilic esophagitis (EoE) is an allergic condition that is characterized by eosinophils infiltrating the esophageal wall. The treatment of the disease may require multiple follow up sedated endoscopies and biopsies to confirm elimination of eosinophils. These procedures are expensive, time consuming, and may be difficult for patients to tolerate. Here we report on the development of a confocal microscopy capsule for diagnosis and monitoring of EoE. The swallowable capsule implements a high-speed fiber-based reflectance confocal microscopy technique termed Spectrally Encoded Confocal Microscopy (SECM). SECM scans the sample in one dimension without moving parts by using wavelength swept source illumination and a diffraction grating at the back plane of the objective lens. As the wavelength of the source is tuned, the SECM optics within the 7 x 30 mm capsule are rotated using a driveshaft enclosed in a 0.8 mm flexible tether. A single rotation of the optics covered a field of view of 22 mm x 223 μ m. The lateral and axial resolutions of the device were measured to be 2.1 and 14 μ m, respectively. Images of Acetic Acid stained swine esophagus obtained with the capsule *ex vivo* and *in vivo* clearly showed squamous epithelial nuclei, which are smaller and less reflective than eosinophils. Imaging of esophageal biopsies from EoE patients *ex vivo* demonstrated the capability of this technology to visualize individual eosinophils. Based on the results of this study, we believe that this capsule will be a simpler and more effective device for diagnosing EoE and monitoring the therapeutic response of this disease.

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OCIS codes: (170.1790) Confocal microscopy; (170.2150) Endoscopic imaging; (170.2680) Gastrointestinal; (170.4730) Optical pathology.

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1. Introduction

Eosinophilic esophagitis (EoE) is a prevalent food allergy disorder that manifests by eosinophilic infiltration within the esophageal wall. The cytokines released by eosinophils inflame the surrounding esophageal tissue and result in symptoms such as dysphagia, food impaction, nausea, vomiting, and heart burn [1]. The keys to treating of the disease are identification of the triggering food allergen and attenuation of local inflammation. Therefore, the treatments include an iterative process of eliminating potential allergenic foods from a person's diet and/or corticosteroids. Both treatment methods require that patients be followed up by sedated endoscopies with biopsy to confirm that the eosinophils have been eliminated. These endoscopic procedures are expensive, time consuming, and frequently difficult for patients, many of whom are children, to tolerate. Moreover, large numbers of biopsies are required to achieve acceptable diagnostic yield due to the patchy nature of the disease [2]. By providing a less invasive and potentially less expensive method for evaluating the esophagus on the microscopic level, swallowable capsule endomicroscopy devices capable of resolving eosinophils have the potential to improve the standard of care in EoE.

Confocal microscopy is a popular optical imaging technique in the life sciences that can provide high contrast depth-resolved cellular information from tissue. Recently, confocal microscopy has been introduced in the gastrointestinal endoscopy field, termed confocal laser endomicroscopy (CLE). There are two main configurations for CLE, eCLE integrates a miniature confocal microscope into the tip of an endoscope (Pentax, Japan), while pCLE is a stand-alone fiber-bundle probe delivered through the accessory channel of endoscopes (Mauna Kea Technologies, France) [3]. Both configurations use fluorescent dyes, applied either systemically or topically, to visualize cellular features. Investigators have used CLE to study several gastrointestinal tract disorders such as esophageal dysplasia and carcinoma in Barrett's esophagus [4], colon aberrant crypt foci [5], colorectal polyps [6], and colonic mucosa in patients with ulcerative colitis [7]. Recently [8,9], pCLE has been used to identify eosinophils in patients with EoE disease. Neumann et al. have indicated that pCLE can identify eosinophils as small pale gray cells within the intercellular spaces after injection of 5 ml of 10% fluorescein sodium intravenously [8,9]. Another method for identifying eosinophils is two-photon microscopy. It is well known that eosinophils are highly fluorescent [10]. A recent study conducted by Safdarian et al. [11] has shown that two-photon autofluorescence microscopy can identify eosinophils in biopsy samples. This technology could become useful when multiphoton microscopy probes are available for clinical use.

Alternatively, we have shown in prior work [12] that eosinophils are highly scattering relative to surrounding esophageal tissue and therefore are easily seen with high contrast using Spectrally Encoded Confocal Microscopy (SECM), a fiber-based reflectance confocal microscopy technique [13]. High-speed form of SECM was introduced by Boudoux et al. [14] where a rapid wavelength-swept source is utilized in conjunction with a single-element photodetector for imaging, as opposed to the previous implementation of broadband illumination and spectrometric detection. With SECM, light from a rapid wavelength swept laser is coupled into an optical fiber. At its distal end, light is collimated on to a diffraction grating; once diffracted, the light is focused into the tissue using an objective lens. As the laser wavelength is swept, the focused beam is scanned across the sample. Light reflected from the tissue at each point is transmitted back through the system into the fiber, which serves as a spatial filter that rejects multiply scattered and out of focus light (i.e., confocal microscopy). This configuration enables one to rapidly acquire one line of a confocal microscopy image without high speed moving parts and therefore facilitates miniaturization of confocal microscopy into a small probe. In recent years, SECM has been successfully incorporated into bench top imaging systems [14,15] and probes [16–19] to image cellular features of a variety of disorders.

Here we describe the development of a tethered SECM capsule for the diagnosis and monitoring of EoE. The SECM capsule is designed to be swallowed without sedation and, because eosinophils scatter light much more strongly than surrounding esophageal tissue,

does not require the use of exogenous contrast agents. The SECM system and capsule design are detailed and then the capsule's optical and mechanical performance tests are reported. *Ex vivo* images of swine esophageal tissue and biopsies from EoE patients and swine *in vivo* images are presented to demonstrate that the SECM capsule endomicroscopy device has the potential to evaluate the eosinophil load for this prevalent disease.

2. Methods

2.1 SECM system

Figure 1(a) shows an overview of the developed SECM clinical system. The light source is a 100-kHz wavelength-swept source [20] developed based on an approach previously used for high-speed optical coherence tomography swept sources [21]. The initial swept source spectrum is generated using a polygon mirror tunable filter and a fiber-based laser ring cavity with a sweep rate of 25 kHz and a duty cycle of 25%. Then, a “cut and paste” scheme [21] using four fiber-optic delay lines is incorporated to achieve a wavelength swept source with a tuning rate of 100 kHz and a duty cycle of 100%. The average output power is 80 mW and the FWHM of the spectrum is 91 nm centered at 1296.5 nm.

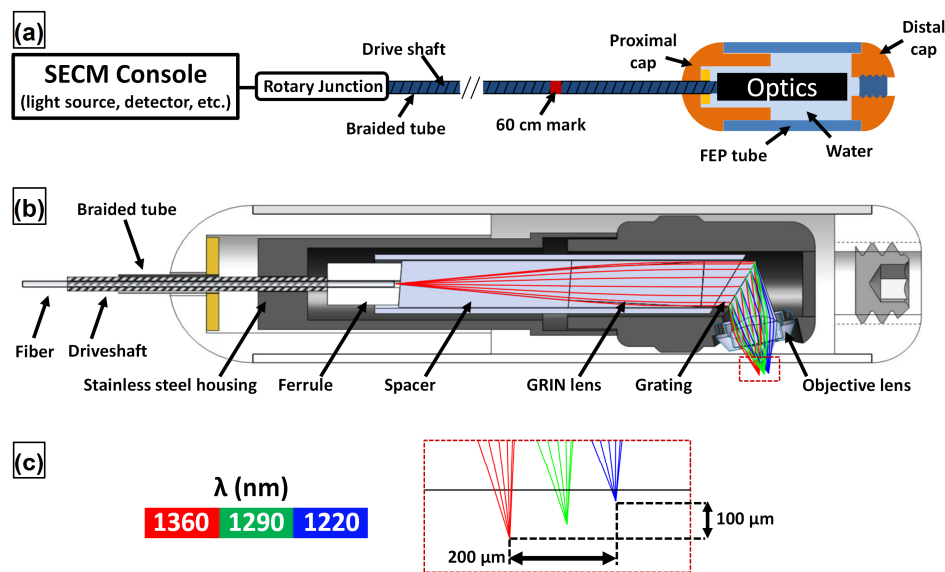


Fig. 1. (a) Schematic view of the SECM clinical system and SECM tethered capsule. (b) ZEMAX optical design integrated to the mechanical design of the capsule. (c) Close up view of the tilted focal line based on the ZEMAX simulation.

The detection unit consists of InGaAs avalanche photo diode (Princeton Lightwave, USA) and a custom-designed post amplifier with bandwidth of 100 MHz. A high-speed DAQ unit, with a bit depth of 16 bit/pixel and sampling rate of 120 MSamples/sec, digitizes the photodiode signal and stores it on a hard drive array in a RAID 0 configuration. Based on this acquisition rate, each A-line (one SECM line) is Nyquist sampled with 256 points. Given the diameter of the capsule of 7 mm, an entire circumferential image is 22 mm. A total of 16,500 A-lines form a single circumferential image, corresponding to a spatial sampling of 1.3 μm in the angular direction, and a frame rate of 6 Hz.

The opto-mechanical connection between the SECM clinical system and the capsule system is achieved by an optical rotary junction made in-house [22]. The swept source and back-reflected light from tissue are coupled at the interface between the stationary and rotary parts of the rotary junction using custom-made fiber collimators. The rotational torque is provided by a DC motor (Micro Motion Solutions, USA), rotating the capsule's optics at a speed of 6 revolutions/s.

2.2 Capsule mechanical design

The SECM capsule device is composed of tether and capsule sections. The innermost component of the tether is a single-mode fiber threaded inside three-layer stainless steel driveshaft (Asahi Intecc, Japan; 0.54mm OD/0.26mm ID). This assembly is covered by medical grade braided tube (MicroLumen, USA). At the proximal end, the driveshaft assembly is locked to the rotary junction by E2000 diamond fiber connector and the braided tube is held stationary by a fixture. At the distal end, the drive shaft is epoxied to the optics assembly and the braided tube is epoxied to the capsule body with a brass ring. This configuration allows for the rotary motion of the fiber/driveshaft/optics assembly while the braided tube/capsule body assembly is stationary and in contact with the luminal organ. The optics assembly is carefully aligned and fixed by epoxy inside the stainless steel housing and the distal end of the housing is sealed, Fig. 1(b).

The capsule body is comprised of three parts. Proximal and distal caps are machined out of medical grade Polymethyl Methacrylate (PMMA) to give mechanical integrity to the capsule and the optical window is made out of medical grade Fluorinated ethylene propylene (FEP) tubing. The inner surface of the FEP tube is chemically etched (Acton Technologies, USA) at both ends to achieve optimal epoxy bonding to the PMMA caps. The capsule is filled with sterile water and sealed at the most distal end by stainless steel set screw. The SECM capsule is 7 mm in diameter and 30 mm in length.

The optics of this SECM probe share similarities to that previously reported [19] with some important differences that reduce the size of the device for easy swallowing. Figure 1(b) shows the ZEMAX design of the SECM capsule optics integrated into the mechanical drawing. Light from the swept-source laser is delivered to the optics by the lensed single-mode fiber (OZ Optics, Canada). The $NA(1/e^2)$ of the lensed single-mode fiber is 0.18 which is twice that of the conventional SMF28 fiber. Use of the high NA fiber allowed us to reduce the length of collimation optics from 23.5 mm to 14.5 mm, which ultimately results in a smaller SECM capsule size. Index-matching epoxies as well as angle-polished surfaces are used to minimize the amount of back-reflected light. The light beam is expanded within the glass spacer (GRIN Tech, Germany) and then collimated by the GRIN lens (GRIN Tech, Germany), filling the full aperture of the 1.8 mm optics. Using a second glass spacer (GRIN Tech, Germany) a high-density polarization independent diffraction grating (Ibsen technologies, Denmark; groove density = 1144 line/mm) is mounted at 60° with respect to the optical axis. This configuration results in optimal diffraction efficiency and a 12.5° propagation angle of the first order diffracted beam relative to the normal axis of the tissue surface. Finally, the swept-source light is focused by a custom-designed water-immersion aspheric singlet objective lens (Japan; NA = 0.5) to an oblique focal line inside the tissue. Using water as the objective immersion medium further minimizes the spherical aberration and diminishes the specular reflection from the tissue surface.

Figure 1(c) is the close up view of the tilted focal line based on the ZEMAX design. The focal line is 200 μm wide and centered 100 μm below the tissue surface, imaging a tilted area of 0.491 mm^2 (0.223mm x 22mm) in a single optics rotation. The oblique focal line design allows inspection of several depths within one sweep of the laser source. This is particularly beneficial in detecting heterogeneously scattered epithelial eosinophils in EoE patients [2]. While the consensus diagnostic recommendations for EoE require histopathologic detection of at least 15 eosinophils per high-power field (approximately 0.16 mm^2), no specific orientation (e.g. iso-depth) is suggested for the inspection. As a result, the SECM capsule is designed to acquire isolated circumferential microscopic image strips from the entire esophagus rather than to perform volumetric imaging through stitching of adjacent image strips. In this scenario, 3 high-power fields worth of microscopic information (0.491 mm^2 / 0.16 mm^2) is acquired in each optics rotation (i.e., image strip). Therefore, a manual pull back of the capsule in 30 seconds provides hundreds of high-power fields microscopic information along the whole esophagus, which covers significantly more tissue than the current 2 to 6 biopsy specimens taken from the proximal, mid, and distal esophagus [1].

2.3 Performance tests

Non-uniform rotational distortion (NURD) is a documented problem with helical scanning systems such as intravascular ultrasound and OCT catheters [23]. NURD is caused by non-uniform transmission of torque from the external motor to the distal end of the driveshaft and results in distortion of the acquired lumen structure. To estimate NURD in the SECM capsule, the distal PMMA cap was removed and a thin radial mark was placed at the distal end of the stainless steel housing. The tether was placed under a mild curvature of 90° and high-resolution optical photography was carried out with a CMOS camera (iDS, Germany) at 100 fps while spinning the optics at 7.25 rev/s. The angular displacement of the radial mark was then quantified in Matlab[®] platform by fitting a circle along the contour of the housing and identifying the location of the mark at each frame. The absolute value of the angle difference between the measured and expected values was calculated and termed the “angle error”. The average angle error over a course of 3 seconds was used as a measure of NURD.

In order to evaluate the optical performance of the capsule several standard tests were carried out. The lateral resolution was quantified by imaging a high-resolution 1951 US Air Force (USAF) resolution target as well as by measuring the full width at half maximum (FWHM) of the line spread function. The field of view was also quantified using this target. The axial resolution was measured as the FWHM of the intensity profile acquired while scanning a mirror through the focus.

To ensure safety of the capsule in clinical practice, the capsule was tested for mechanical integrity, leakage, and dissociation under repeated cycles of acid exposure, mechanical load, and high-level disinfection (Cidex-Opa). It was found that each SECM capsule can be reliably sterilized and reused for at least 5 imaging procedures.

2.4 Tissue imaging studies

In vivo and *ex vivo* experiments were designed to evaluate the performance of the SECM capsule in imaging tissue. All the imaging studies carried out on swine were performed in accordance with a protocol (Protocol# 2013N000143) approved by the Massachusetts General Hospital’s subcommittee on research animal care. For *ex vivo* imaging, excised swine esophagus tissues were collected and treated with 6% acetic acid (AA) prior to imaging to enhance the nuclear contrast. The thinking behind using an exogenous contrast agent to stain epithelial cells was to create features in swine esophageal mucosa that are smaller and less reflective than the target features in EoE patients (i.e. eosinophils). This design of experiment allowed us to indirectly evaluate the imaging capability of the capsule for imaging structures that approximately have the size of eosinophils *in vivo*. The capability of the capsule for directly visualizing eosinophils was studied using human biopsy samples. That is, de-identified pinch biopsies from EoE patients were obtained in collaboration with researchers in food and allergy department at Massachusetts General Hospital (MGH) and in accordance with an IRB-approved protocol (Protocol# 201P001159). AA treatment was not carried out on human biopsy samples. Tissues were placed in imaging chambers and were covered with a 250 μ m thick FEP sheet prior to imaging. In order to perform the imaging, capsule optics assembly was fixed on a 3D raster scanning system and was aligned to place the imaging focal line parallel to the tissue surface. For *ex vivo* studies, raster scanning was preferred over the helical scanning scheme because it allowed for well-controlled positioning of the focal line within the tissue. Water was added between the objective lens and the FEP cover sheet to simulate the SECM capsule *in vivo* conditions. The 3D data was collected and stitched together to form the large-area SECM image stack.

For testing the device *in vivo*, Yorkshire swine (35-42 kgs) were anesthetized, intubated, and ventilated using standard methods. To facilitate the delivery and removal of the SECM capsule to the esophagus, an endoscopy over tube (US Endoscopy, USA) was placed in the proximal esophagus using an endoscope (Pentax Corp, Japan). Then, the esophagus was sprayed with 20ml of 6% AA with a spray catheter and the endoscope was removed. There was no attempt to wash away the excess acetic acid. The SECM capsule was gently pushed to

the mid esophagus by a semi-rigid tube placed over the tether. Once positioned in the desired location, the rigid tube was removed and the imaging commenced with the capsule stationary in esophagus. Upon completion of the study, the capsule was withdrawn and the animal was immediately sacrificed.

2.5 Histology

For histology, tissues were fixed in formalin for 48 hours and were subsequently embedded in paraffin. Sections with $5\mu\text{m}$ thickness were cut *en face* to the SECM imaging plane. Hematoxylin and eosin (H&E) histopathology was conducted per the standard of care at the MGH Gastrointestinal Pathology Service. Slides were digitized by using a full-slide scanner (Aperio Technologies Inc, USA).

3. Results and discussion

3.1 Performance tests

Figure 2 presents the NURD study performed on the SECM capsule. Panels (a) – (c) show the image processing carried out on three representative frames. The image processing program first identifies the center and the boundary of the housing at each frame and then places a radial line at the center of the radial mark. Panel (d) is the plot of the radial mark angular position as a function of time over a course of 10 revolutions. The solid line is the expected trend based on the set rotational speed (7.25 rev/s) and open circles denote the measured angular positions. The plot shows that the measured position closely follow the expected values. Interestingly, it can be seen that the angular errors do not accumulate over time. The average absolute angle error over a course of 3 seconds was found to be $1.86 \pm 1.3^\circ$. This value is significantly smaller than those reported for certain intravascular ultrasound and OCT devices ($3.2 \pm 1^\circ$ and $6.9 \pm 2.1^\circ$, respectively [23]).

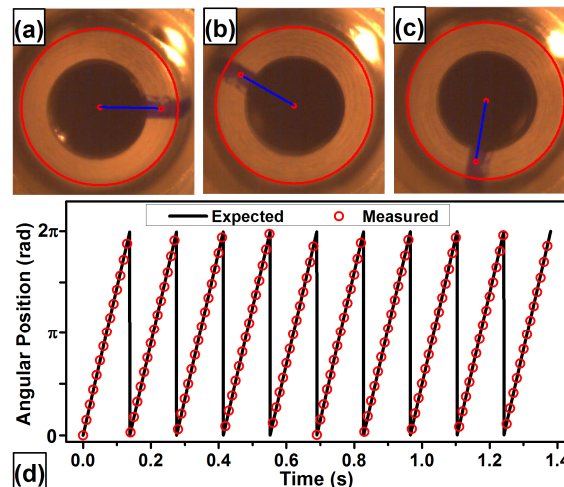


Fig. 2. (a-c) Representative frames captured from the optics housing while rotating, showing the principles of the NURD assessment image processing. (d) Plot of the radial mark's angular position as a function of time over a course of 10 revolutions.

Figure 3 shows the SECM capsule images of the USAF resolution target. The magnified views, panels (b) and (c), indicate that the element 6 of group 8 with a line width of $1.10\mu\text{m}$ is the finest pattern that can be visually recognized. This line width corresponds to spatial frequency of 456.0 lp/mm . The lateral resolution measured from the FWHM of the line spread function was found to be $2.1\mu\text{m}$, which is consistent with that of the USAF resolution target images. Based on the known dimensions of the target patterns, the field of view was estimated to be $200\mu\text{m}$. Figure 3(d) is a plot of the axial response obtained by scanning a

mirror through the focus of the SECM optics. The FWHM of the main lobe (i.e., axial resolution) was measured to be 14 μm .

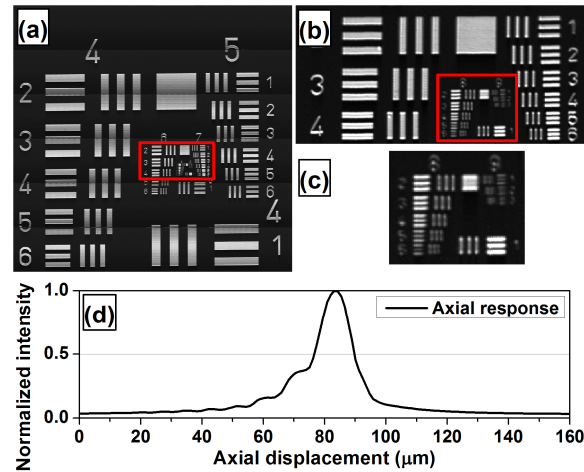


Fig. 3. (a) SECM image of a USAF resolution target constructed by raster scanning the capsule optics over the target. Panels (b) and (c) are the magnified view of regions of interest delineated in panels (a) and (b), respectively. (d) Intensity axial plot obtained by scanning a mirror through the focus. The FWHM is 14 μm .

3.2 *Ex vivo* imaging

Figure 4 shows the SECM images acquired by raster scanning the capsule optics over the swine esophageal tissue. Images were taken at depth of 180 μm from tissue surface.

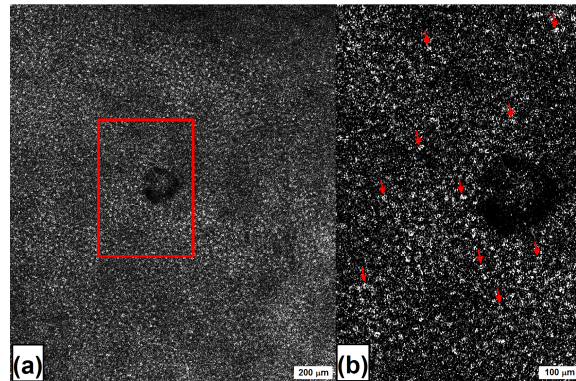


Fig. 4. (a) Large area SECM image of swine esophageal tissue *ex vivo* stained with 6% AA. The image was formed by raster scanning the capsule optics over the tissue. (b) Magnified view of the region of interest delineated in (a) demonstrates brightly scattering structures consistent with epithelial nuclei (arrows).

Panel (a) is a large area view constructed by stitching 11 longitudinal scans together. This image clearly shows enhancement of nuclear contrast due to the 6% AA staining. It has been hypothesized that AA induces spatial fluctuations in the nuclear index of refraction, possibly arising from the coagulation of nuclear proteins. This increases backscattering from the nuclear region and results in clearer delineation of nuclear structure [24]. To compensate for the structural differences between the swine and human tissues, however, the concentration of AA used in this study (6%) was higher than that commonly used in the clinical practice (1.5-3%) [25]. The dense population of nuclei in Fig. 4 suggests the presence of imaging plane at the basal zone.

Figure 4(b) shows a magnified view of the area highlighted in panel (a). The stained cell nuclei appear as highly reflective round features. Similar findings have been reported elsewhere [14,15]. The dark round feature in Fig. 4 is possibly squamous papilla which is expected to be observed in the basal layer. Although key structural features of the esophageal tissue are observed in Fig. 4, high level of speckle noise is evident. However, speckle noise is not expected to jeopardize the performance of the SECM capsule in detecting eosinophils because our prior bench top study [12] indicates that the average intensity of the back reflected light from eosinophils is 4 times higher than those of the surrounding tissue. Eosinophils are of the similar size as epithelial cells; therefore, this study indirectly verifies the performance of the capsule optics for visualizing eosinophils.

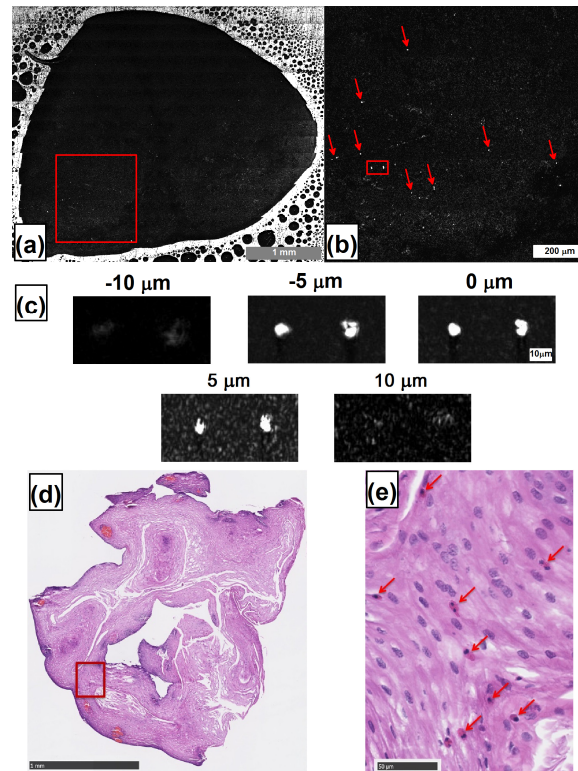


Fig. 5. (a) Large area SECM image of a de-identified biopsy sample obtained from an EoE patient. The SECM image was formed by acquiring frames as the capsule optics were raster scanned over the tissue. The spongy feature enclosing the tissue is water condensation. (b) Magnified view of a region of interest, showing brightly reflecting cells (arrows) with size and distribution that are consistent with the eosinophils seen in corresponding histology (e, arrows). (c) Z-stack of a magnified portion of (b). (d) Histology of biopsy sample (H&E, magnification 3x). (e) Magnified view of a region within (d). Red rectangles show the regions of interest in (a), (b), and (d) that are shown magnified in (b), (c), and (e), respectively.

Figure 5(a) shows the SECM image of a de-identified biopsy sample from an EoE patient. The biopsy was taken from proximal esophagus for research purpose only. The large-area image of panel (a) show several highly reflective features, magnified in panel (b). Panel (c) is a z-stack of the area highlighted in panel (b). The confocal image stack shows the highly reflective features over a depth range of 20 μm . The image taken at the relative depth zero, same depth as panels (a) and (b), shows a bi-lobed structure, which is the characteristic shape of eosinophil nucleus. The significant intensity difference between the feature and the background is also suggestive of eosinophils based on our previous findings [12]. Furthermore, images of panel (c) indicate that speckle noise is not a limiting factor for SECM capsule when visualizing eosinophils. Panel (d) depicts the histology images of the biopsy

sample. The high magnification image confirms the presence of eosinophils in the sample. The bi-lobed structure of the nucleus is also evident for some of the eosinophils. The comparison of the features in Fig. 5 with those previously reported in our bench top study [12] suggests that the capsule optics do not have as high optical performance (e.g. contrast, speckle noise, etc.) as our SECM bench top system. However, it should be noted that a single-mode illumination and multimode detection imaging configuration was used in the bench top system to reduce laser speckle noise and improve the signal-to-noise ratio. Moreover, the bench top system incorporated chromatic and wave front aberration corrected microscope objective lens with an effective NA of 0.7 in water to produce images with axial resolution of 10 μ m. While miniaturization of the bench top system into a swallowable tethered capsule comes at the price of compromises in optical performance, it does not change the physics of the light interaction with eosinophils that provides intrinsic contrast. The *ex vivo* studies of Figs. 4 and 5 suggest that the SECM capsule optics provides images with sufficient resolution and contrast to image the native eosinophils in humans *in vivo*.

3.3 *In vivo* imaging

Figure 6(a) is a photograph of the SECM capsule next to a penny. The SECM capsule was delivered to swine esophagus to study the overall performance of the system *in vivo*. AA staining was performed prior to imaging and then the capsule was placed stationary in mid-esophagus. The image in panel (b) was constructed by stitching images from 188 revolutions of the optics, at the same location, below each other. A close look at this image reveals that the amount of NURD is reasonably low and structural features are of the same size at different revolutions. The black vertical strip is probably a fold in tissue where the focal line was outside the tissue. The puckered nature of swine esophageal mucosa makes esophagus distensible to permit the swallowing of a fairly large bolus of food. The relatively uniform width of this fold along the image is also suggestive of low NURD of the optics. Moreover, it can be seen that the contact between the capsule and tissue was not uniform during optics revolution. For instance, the focal line was placed deeper in the epithelial layer in areas toward the edges of the image, yielding lower average intensity from deep unstained regions. The partial dropouts highlighted by arrows in Fig. 6(b) are probably caused by secondary peristaltic waves placing the focal line out of the tissue. We do not anticipate that these problems will jeopardize the capability of the SECM tethered capsule in diagnosis and screening of EoE in humans; because, our recent *in vivo* studies with OCT tethered capsules [26] had revealed that loss of contact is minimal in unsedated humans due to the contraction of esophageal muscles that grab to capsule during the peristaltic wave.

Figure 6(c) is a magnified view of the area highlighted in panel (b). Nuclei of epithelial cells are clearly visualized by the SECM capsule *in vivo*. Careful examination of Figs. 6(b) and 6(c) indicates presence of a variation in background signal level along the width of each image strip (i.e. spectrally encoded line). The variation is larger towards the edges of each image strip, producing a periodic pattern that can be seen when the strips are stitched together. This variation is due to the larger attenuation of light at the deeper edge of the focal line as well as the spectral variation of source intensity. Source spectrum compensation has already been applied to all the images in this paper but a more optimized processing algorithm can eliminate this artifact more effectively. Nevertheless, as mentioned in the mechanical design section, the SECM tethered capsule is designed to acquire isolated circumferential microscopic images and therefore the image strips do not need to be stitched together for diagnosis of EoE in patients.

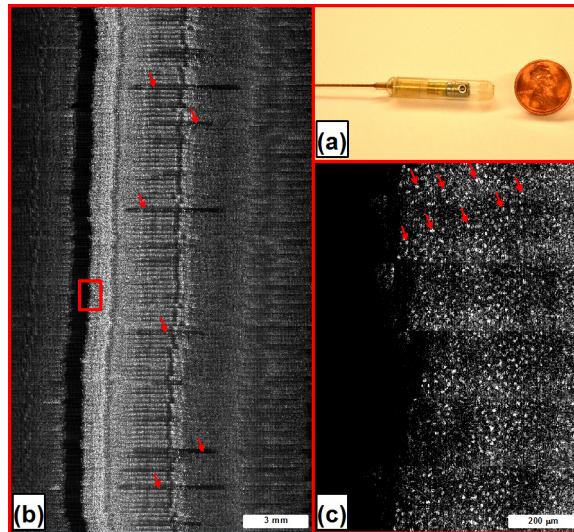


Fig. 6. (a) Photograph of the SECM tethered capsule. (b) SECM image of swine esophagus mucosa, obtained *in vivo*. The image was captured while the capsule was stationary within the esophagus. SECM scans obtained from a total of 188 revolutions are seen from top to bottom. Arrows indicate partial drop outs of focal line due to peristaltic waves. (c) Magnified image of the area highlighted in panel (b) showing structures consistent with nuclei of epithelial cells (arrows).

4. Conclusions

We have miniaturized the SECM technology into a 7x30 mm tethered capsule. The capsule is designed to perform near infrared reflectance confocal microscopy of luminal organs, especially for the diagnosis and monitoring eosinophilic esophagitis disease in unsedated patients. Optical performance tests demonstrate that this capsule has lateral and axial resolutions of 2.1 and 14 μm , which is sufficient for identifying individual eosinophils [12]. NURD was evaluated and the average angular error was found to be $1.86 \pm 1.3^\circ$, significantly lower than those previously reported for certain intravascular ultrasound and OCT catheters [17]. Low NURD is essential for good optical performance of high resolution helical scanning endomicroscopy devices such as the SECM capsule. *Ex vivo* imaging of excised swine esophageal tissue enabled the visualization of epithelial cell nuclei after staining with AA. *Ex vivo* imaging of de-identified biopsy sample from EoE patients suggests that this device enables identification of individual eosinophils. Performance of the SECM capsule was verified *in vivo* in swine, showing the capability of the device to clearly image individual epithelial cell nuclei with no significant NURD generated artifacts. Our research continues towards optimizing the performance of the SECM capsule and performing *in vivo* imaging in patients with EoE disease.

Disclosure statement

Dr. Tearney consults for and receives sponsored research from Ninepoint Medical. Massachusetts General Hospital has a licensing relationship with Ninepoint Medical. Drs. Kang, Tearney, and Nishioka have rights to receive royalties from this licensing relationship.

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