1 **A miniaturized, tethered, spectrally-encoded confocal endomicroscopy capsule**

2 **(Running title: A miniaturized confocal endoscopic capsule)**

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18 **Research funding:** American Partnership For Eosinophilic Disorders (APFED), Tummala Family Foundation,
19 NIH/NIDDK R01 (R01DK091923).

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30 **Acknowledgements**

31 This research has been supported by National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK
32 Grant # R01DK091923), American Partnership For Eosinophilic Disorders (APFED), and the Tummala Family
33 Foundation. Authors thank Valerie J. Madden for her help taking photographs of the SECM capsule.

34

35 **Disclosures**

36 Dr. Tearney received a research sponsorship from Nine Point Medical to develop SECM technology.

Abstract

Background and Objective: The tethered spectrally-encoded confocal endomicroscopy (SECM) capsule is an imaging device that once swallowed by an unsedated patient can visualize cellular morphologic changes associated with gastrointestinal tract diseases *in vivo*. Recently, we demonstrated a tethered SECM capsule for counting esophageal eosinophils in patients with eosinophilic esophagitis (EoE) *in vivo*. Yet, the current tethered SECM capsule is far too long to be widely utilized for imaging pediatric patients, who constitute a major portion of the EoE patient population. In this paper, we present a new tethered SECM capsule that is 33% shorter, has an easier and repeatable fabrication process, and produces images with reduced speckle noise.

Materials and Methods: The smaller SECM capsule utilized a miniature condenser to increase the fiber numerical aperture and reduce the capsule length. A custom 3D-printed holder was developed to enable easy and repeatable device fabrication. A dual-clad fiber was used to reduce speckle noise.

Results: The fabricated SECM capsule (length = 20 mm; diameter = 7 mm) had a similar size and shape to a pediatric dietary supplement pill. The new capsule achieved optical sectioning thickness of 13.2 μm with a small performance variation between devices of 1.7 μm . Confocal images of human esophagus obtained *in vivo* showed the capability of this new device to clearly resolve microstructural epithelial details with reduced speckle noise.

Conclusions: We expect that the smaller size and better image performance of this new SECM capsule will greatly facilitate the clinical adaptation of this technology in pediatric patients and will enable more accurate assessment of EoE-suspected tissues.

Keywords: confocal endomicroscopy; spectrally encoded confocal microscopy; reflectance confocal microscopy; tethered endoscopic capsule; eosinophilic esophagitis; esophageal imaging

1 Introduction

2 Confocal endomicroscopy is an imaging technology that can visualize cellular details of internal organs *in vivo*.
3 Previous studies showed that confocal endomicroscopy has the potential to be used for guiding biopsy and
4 monitoring the treatment of gastrointestinal (GI) tract diseases.(1-5) Conventional confocal endomicroscopy,
5 however, has a relatively small field of view (FOV), less than 0.5 mm (6,7), which makes it prone to sampling
6 error. Video mosaicking of multiple confocal endomicroscopy can increase the FOV but the resulting FOV still
7 is limited to a few mm (7,8), which falls short for imaging the large tissue areas that need comprehensive
8 microscopic examinations.

9 We previously developed a high-speed confocal endomicroscopy technology, termed spectrally encoded
10 confocal microscopy (SECM).(9) In SECM, a diffraction grating is used in conjunction with a wavelength-
11 swept source to conduct line confocal imaging without using mechanical beam scanning devices. Since readily
12 available high-speed wavelength-swept light sources scan much faster than mechanical beam scanners, SECM
13 can achieve a significantly higher imaging speed than conventional confocal endomicroscopy devices. The high
14 imaging speed can be combined with a helically-scanned confocal optics to image over very large FOV,
15 possibly even the entire organ that is at risk of containing disease.(10) Through previous studies imaging
16 gastroesophageal biopsies with SECM *ex vivo*, we have demonstrated that SECM can visualize characteristic
17 cellular features associated with various gastroesophageal diseases. (11,12) Using these unique properties of
18 SECM, confocal imaging of the entire human distal esophagus has been conducted using an SECM endoscopic
19 probe *in vivo*.(13) We have also developed a tethered SECM endoscopic capsule, which can be used to image
20 unsedated patients in outpatient settings.(14) A preliminary study imaging eosinophilic esophagitis (EoE)
21 patients showed that the SECM capsule could image very long segments of the esophagus and visualize
22 eosinophils, the density of which plays a key role in diagnosing EoE. (15) In the same study, however, we also
23 found several technological challenges with the SECM endoscopic capsule: 1) while our previous SECM
24 capsule (diameter = 7 mm; length = 30 mm) was successfully swallowed by 92% of the adult EoE patients, the
25 relatively long length may hamper use of the capsule in pediatric patients, who constitute a major portion of the
26 EoE patient population; 2) the device fabrication was time-consuming and achieving consistent performance for
27 different devices was often difficult; and 3) the speckle noise caused by the single-mode (SM) illumination and
28 detection posed challenges during image analysis. In this paper, we present and demonstrate clinically a new
29 tethered SECM capsule that is significantly smaller for imaging pediatric patients, is easier to fabricate, and also
30 achieves improved performance.

31 Materials and Methods

32 Figure 1 shows the schematic of the SECM imaging system (16) and endoscopic capsule. Light from a custom
33 wavelength-swept source (repetition rate = 100 kHz; central wavelength = 1296.5 nm; bandwidth = 91 nm) was
34 delivered to a dual-clad fiber (DCF) coupling optics, where the source light was coupled mostly to the core of
35 the custom DCF (core diameter = 7.18 μm ; inner clad diameter = 29.1 μm). The DCF was then connected to a
36 custom-built fiber-optic rotary junction. Light from the rotary junction was delivered to the SECM endoscopic
37 capsule through a 1.6-m-long DCF and illuminated the tissue. Reflected light from the tissue was coupled to the
38 DCF and delivered back to the DCF coupling optics. The reflected light was then coupled to a multi-mode
39 (MM) fiber (GIF625, Corning; core diameter = 62.5 μm ; clad diameter = 125 μm) and finally detected by a
40 high-speed InGaAs photodetector (PLA641, Princeton Lightwave; bandwidth = 150 MHz) and digitized at 120
41 MSamples/sec by a high-speed DAQ card (PX14400, Signatec).

42 While the new SECM capsule in this paper uses the same objective lens as our previous SECM capsule (17),
43 there are several changes in the optics design that enabled significant reduction of the capsule length. Inside the
44 SECM capsule (inset of Fig. 1), light exiting from the DCF was coupled to a miniature condenser. A detailed
45 view of the miniature condenser is shown in Fig. 2a. A coreless fiber (FG125LA, Thorlabs; diameter = 125 μm ;
46 length = 450 μm), gradient index (GRIN) fiber (GIF625, Corning; length = 192 μm), and another coreless fiber
47 (FG125LA, Thorlabs; length = 300 μm) were sequentially fusion-spliced and cleaved to construct the miniature
48 condenser. The condenser changed the illumination NA from 0.09 (Gaussian NA of the DCF core) to 0.20,

which significantly reduced the optics length. The end surface of the condenser was angled at 8° to reduce the back reflection from the end surface.

Light from the condenser was collimated by an aspheric singlet (focal length = 4.5 mm). The aspheric singlet was used in place of a GRIN lens of the previous SECM capsule. Use of the aspheric singlet also helped reduce the length of the optics since the light from the condenser propagated in the air instead of inside the GRIN lens and thus maintained a large divergence angle. As a result, the length between the fiber and grating was reduced by approximately three times compared to our previous SECM capsule. The collimated light was diffracted by a transmission grating (PING-Sample-106, Ibsen Photonics; groove density = 1,144 lp/mm) and focused on the tissue by a custom objective lens (water-immersion aspheric singlet; focal length = 2.13 mm; NA = 0.55). The focal plane was located 100 μm below the tissue surface. Optical axis of the objective lens was tilted by 5.7° relative to the normal axis of the tissue surface, which reduced the specular reflection from the tissue surface. The tilt of the objective lens generated an imaging depth variation of $\pm 3 \mu\text{m}$, which was smaller than the axial resolution. Optics performance simulation (ZEMAX) was used to calculate the RMS wavefront error over the source spectral band. Diffraction-limited performance (RMS wavefront error < 0.072) was achieved over 86% of the spectrum (Fig. 2b). This result corresponded to a diffraction-limited FOV of 247 μm .

The DCF was used to increase the detection aperture diameter and subsequently reduce the speckle noise.(18) Effects of the detection aperture diameter on speckle noise and confocal axial resolution were thoroughly analyzed and experimentally validated in Glazowski and et. al.'s work.(19) In the new SECM capsule, the inner clad and core of the DCF were used as the detection aperture. With the DCF NA of 0.09 and central wavelength of 1296.5 nm, the number of resels for the detection aperture diameter of 29.1 μm was calculated as 4.0. For this number of resels, the theoretical speckle noise was 0.27 and axial resolution 9.5 μm .

The small SECM capsule optics design created challenges in device fabrication since many of the optical components were not co-axial to the capsule's mechanical housing (inset of Fig. 1). In order to assemble these optical elements at precise positions and angles, we developed a custom optics holder, which later was fabricated by 3D printing (Form 2, Formlabs). The 3D printer had a laser spot diameter of 140 μm and a printing step size of 50 μm . Dental SG resin (Formlabs) was used due to its superior water tightness to other 3D printing resins. The optics holder (Fig. 3) had holes and slots for retaining the collimation lens, grating, and objective lens. The shapes and dimensions of the holes and slots were optimized through an iterative print-and-test process. Use of the 3D-printed optics holder greatly reduced the complexity and time for the device fabrication.

Lateral resolution of the SECM capsule was measured by imaging a USAF resolution target and calculating the full-width-half-maximum (FWHM) of the line spread function (LSF) along each of the spectrally-encoded and capsule-rotation directions. The USAF resolution target was placed on a three-axis manual translation stage. The SECM capsule was held by a separate, stationary holder and placed over the resolution target. The resolution target was then translated so that the smallest pattern was shown at the center of the SECM image. The laser power was reduced to avoid the detector saturation. Axial resolution was measured by z-scanning a mirror with a motorized stage and calculating the FWHM of the axial response curve. Axial resolutions were measured for nine SECM capsules to test the repeatability of the fabrication process.

Tissue imaging performance was evaluated by imaging the human esophagus *in vivo*. The study protocol was reviewed and approved by the Partners Healthcare IRB (protocol # 2013-0863). Details of the study protocol were described in our previous work. (15) Briefly, the tethered SECM capsule was swallowed by a volunteer and translated over the entire length of the esophagus by peristalsis of the esophagus (downward translation) and gentle pull back on the tether by the operator (upward translation). During the capsule translation, the SECM optics rotated at 360 rpm and confocal images were continuously acquired. Upon completion of SECM imaging, the capsule was retrieved by gently pulling the capsule out. Raw data were processed for background subtraction and spectral variation compensation. A two-dimensional map of minimum intensity over multiple rotational images was calculated and used as the background noise map. An average of multiple rotational images ($n = 50$) was calculated and used during the spectral variation compensation. The processed data were

saved as multiple TIFF images. SECM capsules were examined for presence of any air bubbles in front of the lens prior to imaging. Only the SECM capsules without air bubbles were used for imaging patients.

Speckle noise was measured at 10 different tissue regions that exhibited uniform tissue reflectance without any visible cellular features. The tissue image instead of an image of a standard specimen was used to calculate the speckle noise since the speckle noise from the tissue image directly affects the analysis. Speckle noise was calculated by dividing the standard deviation of the intensity values by the average. In order to evaluate the image quality improvement, we compared the MM-detection SECM images with those obtained with SM detection, where the core of the DCF was mainly used to collect the reflected light. The tissue contact area was estimated by first binarizing the SECM image with a threshold intensity that was above the background noise level and by calculating the proportion of the non-zero intensity areas from the binarized SECM image.

Results

A photo of the tethered SECM capsule is shown in Fig. 4. The SECM capsule had a length of 20 mm and diameter of 7 mm, similar to that of a pediatric dietary supplement pill. A SECM image of the USAF resolution target is shown Fig. 5a. The pattern in the group 9, element 1 is visible along the spectrally-encoded direction and group 8, element 6 for the rotational direction. Lateral resolutions were measured as $2.0 \pm 0.1 \mu\text{m}$ and $2.2 \pm 0.2 \mu\text{m}$ along the spectrally-encoded and rotational directions, respectively. A representative axial response curve is shown in Fig. 5b (FWHM of $13.4 \mu\text{m}$). The average axial resolutions for nine SECM capsules fabricated per the current design was $13.2 \mu\text{m}$ and standard deviation was $1.7 \mu\text{m}$. The small standard deviation indicated that the new design and fabrication process produced capsules with consistently good performance. Fabrication time was reduced from over a week to a few days.

A representative SECM image of a human esophagus is shown in Fig. 6. The subject was 18 years old and previously diagnosed with EoE. The measured signal level from the *in vivo* SECM image was similar to that from the *ex vivo* SECM image of esophageal tissue (16), which had a good agreement with the theoretical signal level. The low-magnification SECM image (Fig. 6a) covers a 20-cm length of the esophagus and stomach. 61.4 % of the SECM imaging area had contact with the tissue, which was similar to the tissue contact proportion measured with the longer SECM capsule, 66.5%. (15) Imaging time was 30 seconds. While most regions of the image contained tissue data, there were areas where the contact between the capsule and tissue was not maintained (asterisks in Fig. 6a). A magnified image (Fig. 6b) reveals characteristic features of the gastroesophageal junction (GEJ), squamous epithelium of the esophagus and gastric cardia mucosa of the stomach.

A comparison of images obtained with the MM detection (Fig. 7a) and SM detection (Fig. 7b) is also shown. Each image was normalized by four times its average intensity. In both images, squamous epithelial cells are visualized with bright cell nuclei (arrows) and a dark cytoplasm background. A closer look at the images (insets in Fig. 7) reveals that cell nuclei are distinguished better from cytoplasm in the MM detection image due to the reduced speckle noise. The speckle noise for the MM-detection images was measured as 0.31 ± 0.01 , similar to the theoretical expectation of 0.27. The signal level in the MM detection was higher by 4.0 ± 0.2 times than that of the SM detection. Line profiles along the yellow dotted lines in Fig. 7a and b also show approximately 4 times signal enhancement in the MM detection (black line in Fig. 7c). Normalized intensity line profiles show that the MM detection (black line in Fig. 7d) provides reduced signal fluctuation compared with that of the SM detection (red line in Fig. 7d).

In vivo SECM images revealed different cell types. Fig. 8a shows a group of bright cell nuclei, while Fig. 8b shows an isolated cell nucleus. Both images were normalized with the same intensity threshold. A magnified view of the Fig. 8a (inset) clearly shows bright cell nuclei (arrows) surrounded by dark cytoplasm and bright cell borders (arrow heads), indicative of squamous epithelial cells. A magnified view of Fig. 8b shows a bi-lobed cell nucleus (arrow) without a surrounding dark background, suggestive of an eosinophil. (15)

Discussion

1 In this paper, we have presented a new tethered SECM capsule that is tailored for imaging pediatric patients.
2 The new SECM capsule had a significantly shorter length and provided reduced speckle noise than our previous
3 capsule. The mechanical design of the new capsule allowed for easy and repeatable device fabrication. *In vivo*
4 SECM images of human esophagus obtained with the new capsule clearly visualized cellular details relevant to
5 the diagnosis of EoE and other esophageal diseases. While we did not qualitatively notice a significant
6 difference in non-uniform rotational distortion (NURD) artifacts between the small SECM capsule and longer
7 SECM capsule, we will investigate this potential issue further as we image more patients with the small SECM
8 capsule. In future, we will validate this smaller, tethered SECM capsule for diagnosing EoE in pediatric
9 patients. As this clinical study progresses, we will measure swallowability in pediatric patients and evaluate
10 diagnostic sensitivity and specificity, compared to endoscopy with biopsy, the current gold standard. We
11 anticipate that the smaller size and better image performance provided by the new SECM capsule will greatly
12 facilitate the clinical testing and adoption of the device in both adult and pediatric patients.

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Figure legends

Figure 1 Schematic of the SECM imaging system and capsule. DCF – dual-clad fiber.

Figure 2 Schematic of the miniature condenser (A) and simulated RMS wavefront error of the confocal capsule optics inclusive of the collimator and objective lens (B).

Figure 3 Schematic of the 3D-printed optics holder and assembly procedure.

Figure 4 Photo of the SECM capsule in comparison with a pediatric dietary supplement pill.

Figure 5 SECM image of the USAF resolution target (A) and axial response curve (B).

Figure 6 Large-area SECM image of human esophagus *in vivo* (A) and magnified portion of image (A) at the gastroesophageal junction (GEJ) marked by the yellow box in (A). asterisks – lack of contact between the tissue and capsule.

Figure 7 Comparison of SECM images obtained with MM detection (A) and SM detection (B) and line profiles along the yellow dotted lines in (A) and (B) with the measured intensity (C) and normalized intensity (D). Arrows – cell nuclei.

Figure 8 SECM images of squamous epithelial cells (A) and an eosinophil (B). Arrows – cell nuclei; arrowheads – cell border.

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Fig. 7. Comparison of SECM images obtained with MM detection (A) and SM detection (B) and line profiles along the yellow dotted lines in (A and B) with the measured intensity (C) and normalized intensity (D). Arrows—cell nuclei.

Fig. 8. SECM images of squamous epithelial cells (A) and an eosinophil (B). Arrows—cell nuclei; arrowheads—cell border.