

# High-Resolution Microendoscope Images of Middle Ear Cholesteatoma and Surrounding Tissue: Evaluation of Interobserver Concordance

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## Abstract

**Objective.** Investigate how accurately otolaryngologists could differentiate between images obtained with high-resolution microendoscopy (HRME) of ex vivo cholesteatoma specimens and surrounding middle ear epithelium.

**Study Design.** HRME images of surgically resected cholesteatoma and middle ear epithelium were obtained and otolaryngologists classified these images.

**Setting.** Tertiary medical center.

**Subjects and Methods.** Resected cholesteatoma and middle ear epithelium were stained with a contrast agent, proflavine, and HRME images were captured. Specimens were sent for standard histopathology and compared with HRME images. Quality-controlled images were used to assemble a training set. After viewing training images, otolaryngologists without prior cholesteatoma HRME experience reviewed and classified test images.

**Results.** Ten cholesteatoma and 9 middle ear specimens were collected, of which 17 representative cholesteatoma and 19 middle ear epithelium images were extracted for a testing set. Qualitative analysis for concordance between HRME images and histological images yielded a strong correlation between modalities. The mean accuracy of all reviewers in correctly identifying images was 95% (95% confidence interval [CI], 92%–98%). The sensitivity to correctly detect cholesteatoma images was 98% (95% CI, 93%–100%), and the specificity was 92% (95% CI, 87%–97%). The Fleiss kappa interrater reliability score was 0.83, (95% CI, 0.77–0.89).

**Conclusions.** Medical professionals can quickly be trained to accurately distinguish between HRME images of cholesteatoma and normal middle ear epithelium, both of which have distinct imaging characteristics. Real-time HRME optical imaging can potentially improve the results of otologic surgery

by allowing for extirpation of cholesteatomas while eliminating residual disease.

## Keywords

cholesteatoma, keratoma, high-resolution microendoscope, HRME, proflavine

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## Introduction

Cholesteatoma is the common name for keratoma, a sac of keratinizing squamous epithelium that forms in the middle ear and mastoid. Although the disease is benign, it can grow, become infected, and destroy bone.<sup>1</sup> Complications include hearing loss and spread of disease to the labyrinth, facial nerve, petrous apex, or intracranial structures.<sup>2</sup> The only available treatment is complete surgical removal of the cholesteatoma.<sup>3</sup> However, the disease can re-form after surgery, and recurrence rates have been reported between 5% and 70% in published series.<sup>4–6,7</sup> The mechanism for cholesteatoma regrowth is either the re-formation of a new epithelial sac or residual disease left behind at the initial surgery, and revision surgery is necessary to treat the recurrence. Therefore, completely removing the cholesteatoma at the

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initial surgery can help reduce the need for additional surgery and prevent morbidity. The only current method for detection of cholesteatoma intraoperatively is to visually differentiate its pearlescent appearance from the surrounding mucosa through the operating microscope, an imperfect technique. An objective method to help the surgeon reliably identify residual disease at surgery would help eliminate residual disease and decrease the need for additional surgery.

Optical imaging technologies can provide noninvasive visualization of tissue epithelium in real time.<sup>8</sup> High-resolution microendoscopy (HRME), which has previously been described,<sup>9</sup> is a compact, robust, and inexpensive fiber-optic microendoscopy system based around wide-field LED illumination, a flexible 1 mm diameter fiber-optic bundle, and a color CCD camera. After staining tissues with the contrast agent proflavine, this unique imaging device allows for the spatial resolution of individual nuclei and cellular structures.<sup>9-11</sup> The intrinsic properties of proflavine, a member of the acriflavine family, allow it to reversibly bind to DNA and stain cell nuclei.<sup>12</sup> In previous studies from our lab using HRME to image squamous cell carcinomas in the head and neck, we found that keratinized tissue has a high affinity for proflavine, thus making it difficult to image the cellular structure that differentiates squamous cell carcinoma from surrounding normal mucosa in the oral cavity and esophagus. However, we postulated that this affinity of proflavine for keratin could provide a means to identify and differentiate cholesteatoma from surrounding middle ear epithelium.<sup>10,11</sup>

Prior studies at our institution have shown that cholesteatoma has distinct imaging characteristics from surrounding normal middle ear epithelium and that HRME is a useful imaging modality for differentiating cholesteatoma from uninvolved middle ear mucosa.<sup>13</sup> The purpose of this study is to determine the accuracy and interrater reliability of otolaryngologists without prior experience in distinguishing cholesteatoma from normal middle ear epithelium on HRME images.

## Methods

### Imaging System

The HRME has been previously described.<sup>9</sup> It is a high-resolution fiber-optic fluorescence imaging system for visualizing subcellular detail in living tissue, which is based on wide-field imaging through a coherent fiber bundle. This imaging platform is designed for use on tissue stained with proflavine (Sigma-Aldrich, St. Louis, Missouri), which is buffered with saline to 0.01% solution. A small amount is applied topically to tissue specimens with a cotton-tipped applicator. Proflavine is safe: it has been used in *in vivo* studies of the gastrointestinal tract in Europe and Australia without any reported adverse events and is a component of the dye used to prevent infection of the umbilical stump of newborns.<sup>14-16</sup>

### Specimen Acquisition and Imaging

The study protocol was approved by the Mount Sinai School of Medicine Institutional Review Board (GCO# 12-0707), and informed consent was obtained from patients undergoing tympanomastoidectomy surgery for cholesteatoma. Resected surgical

specimens were labeled by the surgeon as either cholesteatoma or surrounding middle ear epithelium following standard surgical resection. A fragment of the fresh specimen was immediately stained with proflavine for HRME analysis; the remainder of the specimen was submitted to pathology for standard histopathology. After topical application of the dye, the fiberoptic probe was used to view these areas and capture movie clips of 3 seconds duration. Movie clips were converted to still images using Windows Movie Maker (Microsoft, Redmond, Washington). Following resection, staining, and HRME imaging, specimens were stored in 10% buffered formalin and sent for histological processing and preparation of hematoxylin and eosin (H&E) slides correlating with the area imaged.

### Image Database Assembly

Two representative movies were randomly selected from the 4 to 12 movies available from each tissue specimen. Selected movies were then reviewed for quality and were included in further analysis only if the frame contained at least 50% of nuclei or keratin for over 1.5 seconds of the 3 second captured video and the image did not contain oversaturation from residual proflavine or significant motion artifact. A representative still frame was subsequently extracted from each video. For each specimen imaged, the highest quality image was selected.

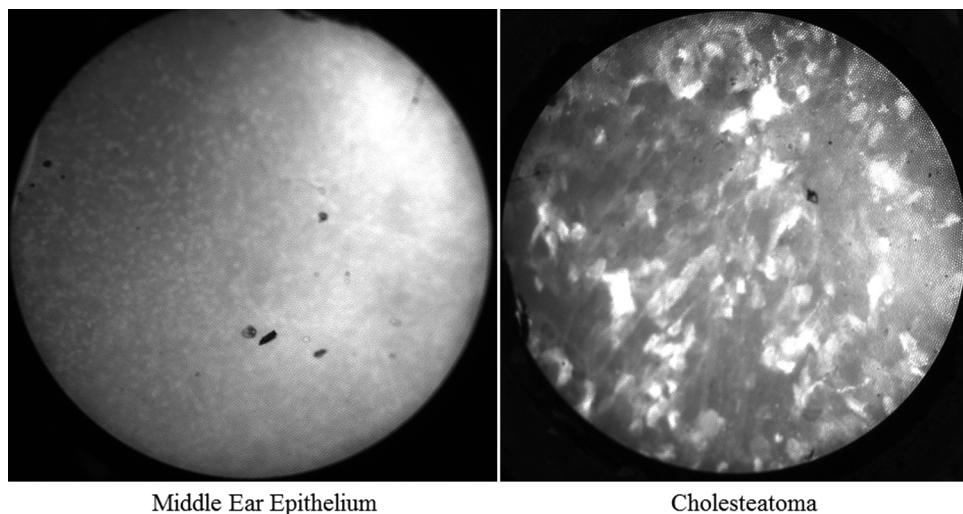
### Interrater Reliability Testing

A training set of images was created that included 3 representative images of normal middle ear epithelium and 2 representative images of cholesteatoma and a written description of imaging characteristics of each tissue type (**Figure 1**). After reviewing training images, otolaryngologists with varying levels of training and without prior cholesteatoma HRME experience reviewed and classified test images from the assembled database as either normal middle ear epithelium or cholesteatoma. No time limit was placed on viewing training or test images. These results were compared against the gold standard histopathology of each specimen. Statistical analysis included accuracy, sensitivity, and specificity values calculated with Microsoft Excel. SAS version 9.2 (SAS Institute, Cary, North Carolina) was used to calculate the Fleiss kappa interrater reliability score between multiple raters, using the MAGREE function. Two-tailed *P* values of .05 were considered to be statistically significant.

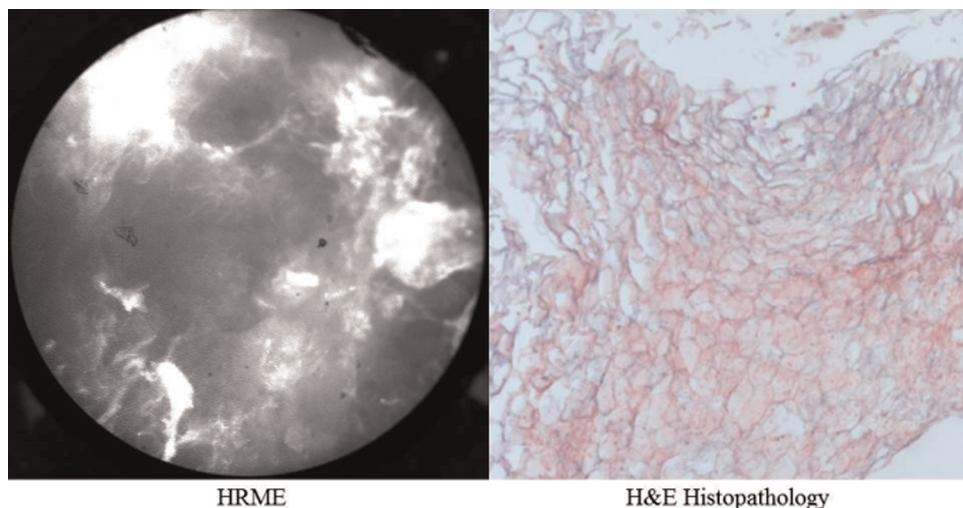
## Results

Ten cholesteatoma specimens and 9 specimens of adjacent normal middle ear mucosa were collected from 10 patients and immediately labeled by the surgeon at the time of resection. By HRME, with proflavine staining, cholesteatoma specimens appeared as areas of confluent hyperfluorescence with a loss of cellular architecture (**Figure 2**), whereas middle ear epithelium displayed normal-appearing nuclei, which correlated with corresponding histology (**Figure 3**).

The image database assembly process yielded 17 representative images from cholesteatoma specimens and 19 representative images from middle ear epithelium specimens to



**Figure 1.** Representative high-resolution microendoscopy (HRME) images used in the training set with middle ear epithelium on the left and cholesteatoma on the right.



**Figure 2.** Representative contrast-enhanced high-resolution microendoscopy (HRME) (left) and corresponding H&E histopathology images of a specimen labeled as cholesteatoma at surgical resection.

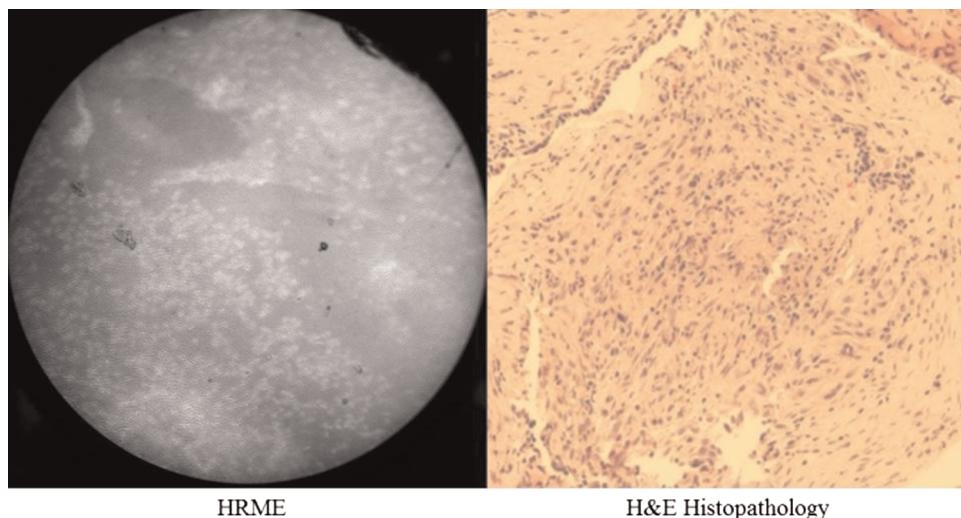
create a testing set containing 36 total images. Eight otolaryngologists viewed the image training set to familiarize themselves with the process and then rated the test set of HRME images as either showing cholesteatoma or normal middle ear mucosa.

The mean accuracy of all reviewers in correctly identifying images as either cholesteatoma or normal middle ear epithelium was 95% (95% confidence interval [CI], 92%-98%). The sensitivity of correctly identifying histologically confirmed images of cholesteatoma as a cholesteatoma was 98% (95% CI, 93%-100%), and the specificity was 92% (95% CI, 87%-97%). The Fleiss kappa interrater reliability score is a measure of the reliability of multiple raters who are assigning variables into fixed categories. In this study, for assigning the 2 categories of normal middle ear epithelium or cholesteatoma, the Fleiss kappa interrater reliability score was 0.83 (95% CI, 0.77-0.89).

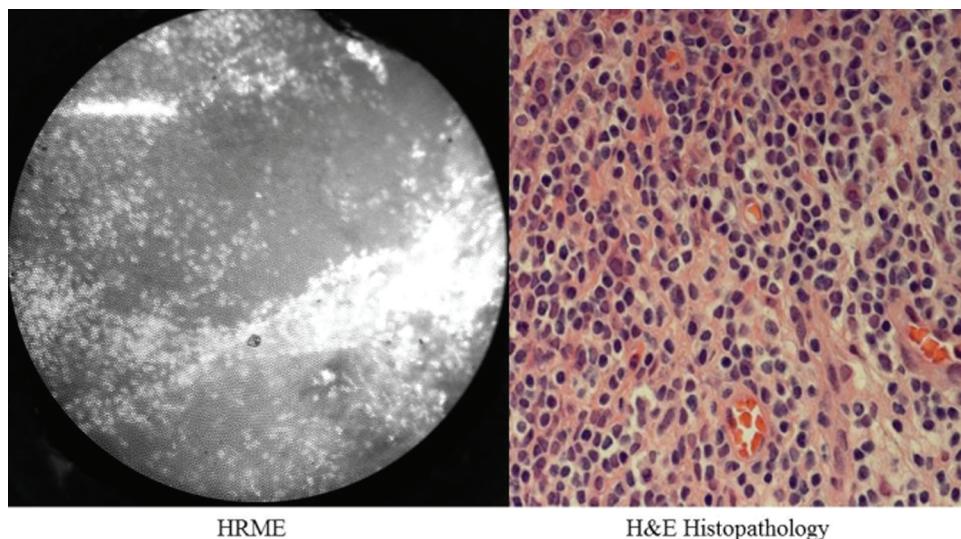
## Discussion

This investigation builds on the previous work done at our institution that established the excellent concordance between HRME and histopathology for differentiating cholesteatoma from middle ear epithelium. In that in vitro study, we compared HRME and histopathology of fresh frozen tissue from cholesteatoma and normal epithelium. In the current study, we immediately captured HRME images of freshly resected specimens and compared those to histopathology. Cholesteatoma was consistently found to show a complete loss of cellular architecture with confluent areas of hyperfluorescence that correlated well with histologically confirmed keratin.

The goal of this study was to examine the reliability and accuracy of multiple observers to correctly label HRME images of each tissue type. After viewing a short training



**Figure 3.** Representative contrast-enhanced high-resolution microendoscopy (HRME) (left) and corresponding H&E histopathology images of a specimen labeled as middle ear epithelium at surgical resection.



**Figure 4.** Representative contrast-enhanced high-resolution microendoscopy (HRME) (left) and corresponding H&E histopathology images of a specimen labeled as cholesteatoma at surgical resection are identified as middle ear epithelium by HRME and confirmed by histopathology.

set of 5 images with descriptions of imaging characteristics for either cholesteatoma or middle ear epithelium, 8 otolaryngologist reviewers were asked to identify 36 different images captured with the HRME. Results following this short training set were quite strong as the group correctly marked images of cholesteatoma 98% of the time and correctly marked normal middle ear epithelium 92% of the time. In addition, the raters demonstrated a high level of reliability with a Fleiss kappa score of 0.83. These results suggest that HRME can successfully differentiate normal from abnormal tissue. Furthermore, otolaryngologists could be quickly and easily trained to correctly and accurately identify specific tissue types on proflavine-enhanced HRME. We therefore believe that HRME is a potentially valuable method to ensure eradication of all diseased tissue during surgery.

Cholesteatoma surgery has not changed in many years, and the surgical results are imperfect. The introduction of the operating microscope in the 1950s, high-speed drills in the 1960s, and endoscopes in the 1990s have been incremental advances. However, the problem of incomplete surgical resection persists, and the rate of residual disease remains high. The cholesteatoma sac is amorphous, and the anatomy of the middle ear is complex, so this disease defies en bloc, *in toto* resection. Under the surgical microscope, cholesteatoma is pearlescent and appears different than the surrounding middle ear mucosa, however, the gross appearance cannot be relied upon to ensure complete excision. As seen in one of our specimens, tissue that was thought to be cholesteatoma at surgery proved to be normal mucosa histologically and by HRME (**Figure 4**).

This study provides evidence that HRME is superior to the microscope for detecting disease, and this method should improve visualization and reduce the rate of residual cholesteatoma. HRME currently has a wide range of potential applications. It has already been studied as a tool to detect neoplastic changes in the mucosa of the head and neck, cervix, and esophagus.<sup>10,11,17</sup> This study demonstrates the utility of contrast-enhanced HRME for detecting cholesteatoma in the middle ear and mastoid.

The previous work from our institution using HRME to differentiate cholesteatoma from normal middle ear epithelium provided the basis for the current study.<sup>13</sup> By demonstrating the ability of easily trained surgeons to reliably differentiate cholesteatoma from normal middle ear epithelium, we have shown the clinical feasibility of this modality and built a foundation for the *in vivo* application of contrast-enhanced HRME imaging to aid in identification of keratin-containing tissue and elimination of residual disease at surgery. The use of proflavine *in vivo* in the human ear is a novel application, and we are seeking a treatment IND as we move forward with this concept. However, we expect it to be safe as the drug has shown low toxicity elsewhere in the GI tract.<sup>10,11,17</sup> Overall, the results from our current study support the future *in vivo* intraoperative application of HRME to provide real-time imaging in cholesteatoma surgery.

## Author Contributions

**James Bradley**, contributions to design, data acquisition, analysis, drafting and revising the article, final approval; **Nancy Jiang**, contributions to design, data analysis, drafting and revising the article, final approval; **Lauren Levy**, contributions to design, data acquisition and analysis, revising the article critically, final approval; **Rebecca Richards-Kortum**, contributions to conception, revising the article critically, final approval; **Andrew Sikora**, contributions to design, data acquisition, analysis, drafting and revising the article, final approval; **Eric Smouha**, contributions to design, data acquisition, analysis, drafting and revising the article, final approval;

## Disclosures

**Competing interests:** Rebecca Richards-Kortum holds patents related to the optical imaging modality used in this study in addition to ownership stake in the company for which these technologies have been licensed.

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