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## ***In vivo* cytological observation of liver and spleen by using high-resolution microendoscopy system under endoscopic ultrasound guidance: A preliminary study using a swine model**

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### **Abstract**

Endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) is useful to obtain specimens from lesions underlying deep parts of the liver and spleen. However, the development of novel ancillary techniques must be explored to reduce the number of needle passes and potential adverse effects during this procedure. We conducted an animal study using a swine to demonstrate technical feasibility of *in vivo* cytological observation of liver and spleen using the high-resolution microendoscopy (HRME) system under EUS guidance. We successfully performed *the study*. No significant acute adverse events occurred during the procedure. The HRME system could obtain clear images representing cytology-level morphology of spleen and liver. Hence, it is found out that *in vivo* cytological observation of liver and spleen using the HRME system under EUS guidance is technically feasible.

**Keywords:** High-resolution microendoscopy (HRME), liver, spleen

### **INTRODUCTION**

Mass lesions and focal abnormalities affecting the liver and spleen are found relatively frequently. Because a wide variety of benign and malignant etiologies can cause these structural abnormalities, exact pathological diagnosis is important to facilitate appropriate patient management. Tissue acquisition from a liver or spleen mass is usually performed by computed tomography (CT)-guided or ultrasound (US)-guided aspiration or biopsy.[1,2,3] However, in a certain number of cases, this external puncture can be risky, especially when the lesions are small, located in the deep parts of organs (e.g., hepatic or splenic hilum). In those difficult cases, endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) has been utilized since EUS-FNA enables the lesions to be approached from another side of the body via the stomach or

duodenum. In these circumstances, EUS-FNA has shown high diagnostic ability.[4,5,6] However, procedure-related adverse effects (e.g., bleeding) are still a concern of EUS-FNA since both liver and spleen have rich vasculature. The development of novel ancillary techniques must be explored to reduce the number of needle passes and potential adverse effects during this procedure.

The high-resolution microendoscopy (HRME) system has been developed recently to provide cellular-level resolution images of gastrointestinal tissue. Its potential to aid in the diagnosis of gastrointestinal neoplasms has been explored in a previous study.[7]

In this study, we aimed to evaluate technical feasibility of *in vivo* cytological observation of liver and spleen using the HRME system under EUS guidance in a swine model. Our results demonstrated that it is technically feasible to obtain clear images representing cytology-level morphology.

## MATERIALS AND METHODS

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The study was conducted at the animal facility of The University of Texas MD Anderson Cancer Center after an approval was obtained from the Animal Care and Use Committee (IACUC protocol No. 07-05-06923). One male domestic pig weighing 40 kg was used. The pig was allowed no food by mouth for 24 hours before the procedure. Preanesthesia medications consisted of intramuscular injection of ketamine (22-33 mg/kg) and acepromazine (0.22-1.1 mg/kg). General anesthesia was achieved with isoflurane (1%-3% to up to effective dose) and propofol (12 mg/kg/h). All the procedure was performed with swine in the left lateral position. The findings of pulse oximetry and electrocardiography were continuously monitored during the experimental procedures. Tissues from liver and spleen were collected from the swine.

**High-resolution microendoscopy system** A recently developed prototype HRME system was utilized to obtain images of cellular-level morphology and tissue architecture *in situ* and in real time.[8] Detailed information regarding the system assembly and techniques of image acquisition have been described previously.[9,10] Briefly, a fluorescent contrast agent (Proflavine, Sigma-Aldrich, St. Louis, MO) was applied topically to the targeted tissue to stain nuclei and then a fiber-optic probe was introduced through the needle to contact the tissue. The HRME system is a fiber-optic fluorescence microscope controlled by a laptop. Illumination is provided by a blue light-emitting diode (LED) light. Remitted fluorescence is collected by the bundle, passed through a dichroic mirror and long-pass filter, and is directed to a Charge-Coupled Device (CCD) camera. The HRME system has a spatial resolution of 4.4  $\mu\text{m}$  and it displays images at 12 frames per second in real time. Use of a probe with a 600- $\mu\text{m}$  field of view allows passage through a 19-gauge aspiration needle [Figure 1a and b].

**Endoscopic devices** All procedures were performed with a commercially available upper endoscope (GIF-160, Olympus, Center Valley, PA) and EUS (GF-UC140P-AL5; Olympus). The entire stomach contents were removed with the upper endoscope during the observation. In the next step, EUS was utilized to visualize adjacent organs as well as blood vessels to avoid damage during the procedure. Subsequently, the stomach wall puncture was performed to create access to the spleen or liver with the same method of using EUS-FNA with a 19-gauge EUS-FNA needle. After the removal of a stylet, Proflavine was administered through the needle into the tissue and the HRME probe was advanced through the needle under EUS guidance.

## RESULTS

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We successfully performed *in vivo* cytological observation in a swine using the HRME system under EUS guidance. No significant acute adverse events occurred during the procedure.

We found that delivery of the contrast agent was straightforward, and manipulation of the HRME probe was essentially comparable to working with the EUS-FNA device alone.

[Figure 2](#) shows the HRME images and corresponding histology from the spleen and liver. Normal spleen showed clear nuclei as discrete bright dots, but distribution of cells were scattered throughout the HRME field of view [[Figure 2a](#)]. Normal liver also showed clear nuclei as discrete bright dots throughout the HRME field of view, but they were larger and more crowded in comparison to the spleen [[Figure 2b](#)]. In the corresponding hematoxylin and eosin (H&E) stained section, normal liver and spleen cells have small, regularly spaced, and centrally located round nuclei [[Figure 2c](#) and [d](#)].

## DISCUSSION

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In the current *in vivo* study, we successfully observed hepatic and splenic parenchyma in real time and obtained images representing cellular-level morphology by using the HRME system. To the best of our knowledge, this is the first animal study evaluating the technical feasibility of *in vivo* cytological observation of liver and spleen by using the HRME system under EUS guidance. We believe this procedure could potentially be applied to humans to support diagnostic techniques for spleen and liver diseases.

The ability of the HRME system to visualize cellular-level morphology and tissue architecture in real time may help us to diagnose target lesions in cases in which biopsy sampling is difficult to obtain or hard to interpret, while histopathological diagnosis is the standard method for a definite diagnosis.[\[9,10\]](#) This low-cost portable system (less than \$3,500 to build) can be a reasonable choice for this purpose. Furthermore, compared to other complimentary imaging techniques (e.g., confocal microendoscopy) that require intravenous injection of fluorescein contrast agent, the HRME system can obtain clear cellular-level morphology with topical fluorescent dyes on demand.

The limitations of our study include the small number of samples and the lack of a pathological control. Therefore, further studies utilizing a larger number of animal and human samples including normal and abnormal findings are needed to validate the potential of this procedure.

## CONCLUSION

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In conclusion, we demonstrated the technical feasibility of *in vivo* cytological observation of liver and spleen by using the HRME system under EUS guidance. This technique has the potential to improve the diagnostic ability of EUS-FNA for spleen and liver lesions.

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**Conflicts of interest** Other authors have no conflict of interest or financial ties to disclose relevant to this study.

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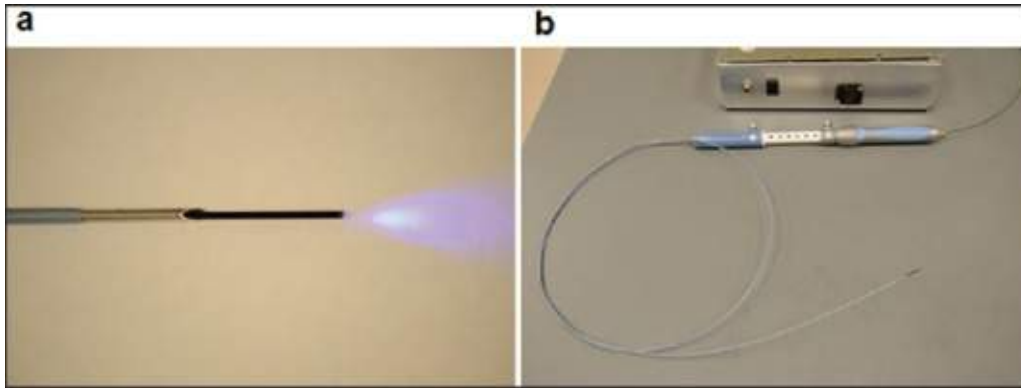
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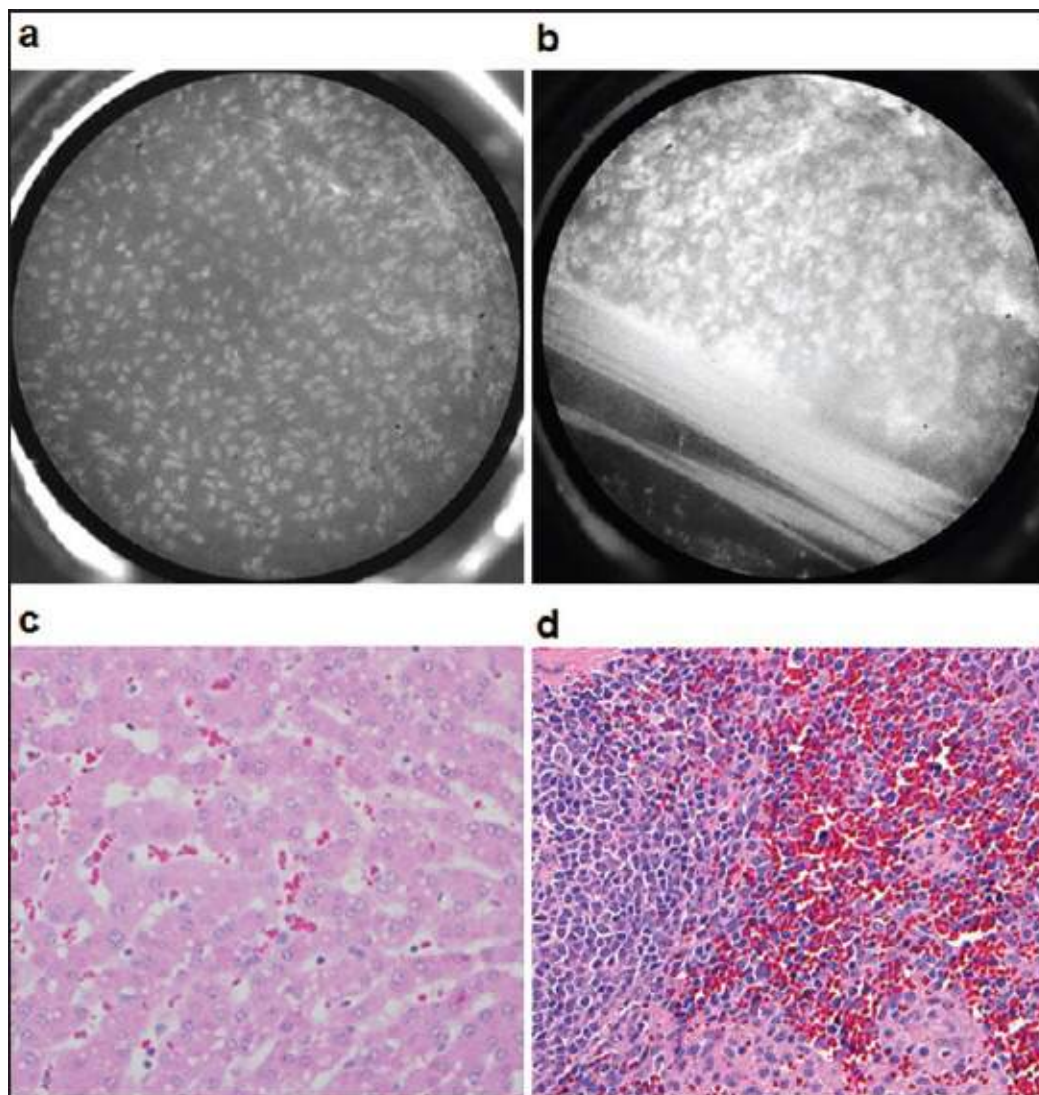
## Figures and Tables

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**Figure 1**



(a and b) Photographs of high-resolution microendoscope fiber-optic probe with 0.45-mm diameter passed through an EUS-guided FNA needle (Echotip ultra 19-gauge; Cook)

**Figure 2**

Representative high-resolution microendoscope images of the hepatic parenchyma (a) and splenic parenchyma (b) in an *in vivo* swine model. Images were acquired with the fiber-optic probe advanced within the lumen of a 19-gauge EUS-guided FNA needle. The nuclei appear as small, discrete dots within the field of view. In the corresponding hematoxylin and eosin (H&E) stained section, normal liver (c) and spleen cells (d) have small, regularly spaced, and centrally located round nuclei

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