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Evaluating micro-optical coherence tomography (μ OCT) as a feasible imaging tool for pancreatic disease diagnosis

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Abstract—Pancreatic cancer is one of the leading causes of cancer mortality worldwide due to the lack of reliable tools for early diagnosis of this cancer. In this study, we evaluated the feasibility of micro-optical coherence tomography (μ OCT) as an imaging tool for the diagnosis of pancreatic cancers. Specifically, we constructed a μ OCT device that achieves a resolution of $1.67 \pm 0.01 \mu\text{m}$ and $1.79 \pm 0.01 \mu\text{m}$ in axial and lateral directions, respectively, and acquires three-dimensional μ OCT images of mouse, rat and human pancreatic specimens *ex vivo*. We compared the results of μ OCT with those of the corresponding histology. In μ OCT images of normal pancreatic specimens, the detailed cellular and subcellular-level pancreatic microstructures, e.g., the islet of Langerhans (IL), IL cell nuclei, blood vessels and serous acini, could be clearly resolved in different cases. To the best of our knowledge, this study is the first to demonstrate that the cellular and subcellular structures of pancreatic tissues were identified using OCT. More importantly, we showed that these normal cellular-level structures were lost in μ OCT images of cancerous specimens, demonstrating the feasibility of differentiating malignant lesions from normal tissues using μ OCT. Moving forward, the development of an intraoperative imaging device may realize optical biopsies *in vivo* or real-time cellular-resolution examination of specimens from needle aspiration biopsies.

Index Terms—optical coherence tomography, pancreatic imaging, high-resolution, morphology.

I. INTRODUCTION

THE pancreas is one of the critical organs in the digestive system with both endocrine and exocrine functions, and it is responsible for human glucose homeostasis along with insulin secreted by islets β cells [1]. In practice, however, the pancreas is prone to various diseases. For example, acute necrotic pancreatitis is one of the most dangerous diseases in gastroenterology with high morbidity and mortality [2], while diabetes affects hundreds of millions of individuals worldwide, initiating whole body complications [3]. Pancreatic cancer, possessing characteristics of insidious onset, high aggressiveness and chemoresistance, is the fourth leading cause of worldwide cancer mortality, and is also called “incurable cancer” in clinical practice [4]. Pancreatic diseases can impose huge personal and social healthcare burdens.

There are two main reasons for the poor prognosis of pancreatic diseases, with one being a strong tendency to form micro-metastases before detectable symptoms arise and, the other being the lack of reliable high-resolution diagnostic tools for observing

the malignant morphological changes at an early stage [5]. To meet the clinical requirements for early pancreatic disease diagnosis, high-resolution imaging tools are necessary to understand tumor growth mechanisms and dissemination patterns at a cellular/subcellular-level. Currently, although various imaging tools have been utilized for such purposes, e.g., computed tomography (CT), magnetic resonance imaging (MRI), endoscopic retrograde pancreatography (ERP), ultrasound (US), radionuclide imaging (RNI), endoscopic ultrasonography (EUS), and positron emission tomography (PET) [6–8], they still suffer from certain limitations, such as low resolution, limited imaging depths, low diagnostic sensitivities, and the destructive nature of intraoperative or needle aspiration biopsies, as well as difficulties in obtaining the specimens. Hence, it is imperative to explore novel devices or strategies to obtain high-resolution images of the pancreatic tissues in a non-invasive or minimally invasive way [9].

Optical coherence tomography (OCT) is an emerging imaging modality based on low coherence interferometry [10]. Through utilizing a broadband laser source and measuring the magnitude and echo time delay of the backscattered light, OCT is capable of providing microstructure images of the biological tissues non-invasively with typical axial resolutions of $\sim 1\text{--}10 \mu\text{m}$ [11]. OCT has attracted extensive research interests since its invention and various efforts have been devoted to improve its performances [12, 13]. Currently, OCT has been applied in various fields, e.g., ophthalmic imaging [14, 15], skin disease diagnosis [16], coronary artery disease diagnosis [17, 18], airway function evaluation [19, 20], and endoscopic GI tract imaging [20, 21], and is widely regarded as a viable tool due to early disease diagnosis for its noninvasive and high-resolution properties. To date, although there have been reports on OCT for pancreatic tissue imaging [22–24], few studies have reported on cellular/subcellular level pancreatic structure imaging, let alone on pancreatic diseases.

In this study, we evaluated the feasibility of OCT as an imaging tool for the diagnosis of pancreatic diseased at cellular/sub-cellular resolution. A lab-built micro-OCT (μ OCT) system that achieves a spatial resolution of $\sim 1.67 \mu\text{m}$ and $1.79 \mu\text{m}$ in axial and lateral directions, respectively was constructed for such purposes, and *ex vivo* images of mouse, rat and human pancreatic specimens were also acquired and compared to their corresponding histology images in different cases. The primary objective of this study was to evaluate the cellular and sub-cellular imaging capability of μ OCT and subsequently to determine its feasibility as a noninvasive (or minimally invasive) potential tool for pancreatic disease diagnosis, which thus could lay a foundation for needle based optical biopsy and intraoperative image guidance [25, 26]. To the best of our knowledge, this report describe the first time that cellular and subcellular structures of pancreatic tissues were visualized using OCT.

The remainder of this paper is organized as follows. Section II describes the constructed μ OCT and its performance characterization, as well as the study protocol. Section III presents the OCT imaging results of mouse, rat and human pancreatic specimens and compares

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Fig. 1. Schematic of the constructed free-spaced μ OCT system. SMF: single-mode-fiber; BS: beam splitter; AI: actuated iris; AO: analog output; L1 - L5: achromatic lens; RM: reference mirror; CL: camera link cable; DAQ: data acquisition card.

them to their corresponding histology in different cases. Section IV discusses the imaging results and the limitations of the system. Section V concludes the paper.

II. STUDY DESIGN AND METHODS

A. μ OCT Imaging System

Previously, we built a compact fiber based μ OCT for rat colon imaging [21]. Due to the limited broadband spectral response of the single mode fiber used, however, the achievable spatial resolution was limited to $\sim 2.0 \mu\text{m}$. To further improve μ OCT spatial resolutions, we constructed a new free-spaced system as shown in Fig. 1. Compared with those in the previous fiber-based system, the components in this new μ OCT were exactly the same except that the fiber coupler was replaced by a 50:50 non-polarizing beam splitter (BS, BS: BS005, Thorlabs Inc.). Specifically, in this system, the collimation lenses L1 and L3 with focal lengths of $f = 15 \text{ mm}$ (AC050-015-B-ML, Thorlabs Inc.) were selected, while for L2 and L4, the infinity corrected, near-infrared radiation range metallurgical bright field plan apochromat $20\times$ objective long working distance lenses (M Plan Apo NIR $20\times$, Mitutoyo Inc.) were chosen, the focal lengths and theoretical apertures of which are $f = 10 \text{ mm}$ and $NA = 0.4$, respectively.

Figure 2 presents the measured spatial resolution of this new μ OCT. As seen in Fig. 2(a), the line patterns of group 7 element 6 with a line spacing of $2.19 \mu\text{m}$ can be clearly resolved; thus, the system lateral resolution should be much finer than $2.19 \mu\text{m}$. To accurately characterize the system lateral resolution, we reasonably assumed that the beam focused by the objective lens is a Gaussian spot, and adopted the method presented in [27] for lateral resolution measurement, wherein the lateral resolution is defined to be the e^{-2} radius of the spot, i.e., 0.78 times the 10-90% width of the rising edge for a line pattern bar. In the experiments, the transverse resolution was measured to be $1.79 \pm 0.01 \mu\text{m}$. With the power ratio of the supercontinuum laser source (SC-5, Yangtze Soton Laser Co., Ltd.) set to be 90%, the system axial resolution was measured to be $\Delta z = 1.67 \pm 0.01 \mu\text{m}$ as shown in Fig. 2(b), wherein the normalized signal amplitude decreases with an increasing path length difference. Such a signal falling-off is due to the fact that in spectral-domain OCT (SD-OCT) [28], larger path length differences result in smaller fringe spacing in spectral interferometry. Since the finite spectrometer resolution reduces the fringe visibility more strongly at higher fringe-frequencies, the signal sensitivity decays with an increasing depth [29]. Therefore, we estimated the μ OCT system spatial resolution to

Fig. 2. Measured spatial resolution of μ OCT. (a) *En face* image ($0.22 \text{ mm} \times 0.22 \text{ mm}$, 1024×1024 pixels) of the 1951 USAF resolution chart. (b) Measured axial PSFs versus path length differences between the sample and the reference arm optics.

be $1.67 \pm 0.01 \mu\text{m}$ in the axial direction and $1.79 \pm 0.01 \mu\text{m}$ in the transverse direction. Such measured results matched well with our predicted respective values of $\Delta z = 1.40 \mu\text{m}$ and $\Delta x = 1.96 \mu\text{m}$. In addition, the illumination power on the sample was set to be 10.0 mW in the experiments, and we measured the system sensitivity to be $103.4 \pm 0.01 \text{ dB}$ at a path length difference of $80.0 \pm 0.1 \mu\text{m}$. The 6-dB sensitivity rolling-off occurred at $0.65 \pm 0.01 \text{ mm}$. In the imaging process, the system data acquisition rate was set to be 30, 720 A-lines/s with each frame consisting of 1, 024 A-lines, i.e., the imaging frame rate was 30 frames per second (fps).

B. Study Design and Imaging Protocol

We established the acute pancreatitis (AP) model using a routine retrograde cholinic bile duct injection of sodium taurocholate ($0.1 \text{ mL}/100 \text{ g}$ body weight with injection rate of $0.1 \text{ mL}/\text{min}$). Five minutes (mins) after the injection, fresh pancreatic tissues were harvested immediately after sacrifice, and *ex vivo* imaging experiments were conducted 3-5 mins after the fresh specimen excisions. Specifically, all excised specimens were flushed with phosphate buffered saline (PBS, $\text{PH}=7.4$) solution to clean the blood on the tissue surface before imaging, while in the imaging process, a small amount of PBS solution was added onto the tissue surface to prevent strong reflections caused by refractive index mismatching at the tissue surface. Three mice and rats were utilized in this study, and each imaging experiment was repeated at least three times. All imaging experiments with mice and rats were finished within 15 mins postmortem, and those regions of interests (ROIs) were marked with tissue marking dye in the organ harvesting and μ OCT imaging processes.

For the human specimens imaging, both pancreatic cancer tissues and cancer adjacent specimens from three patients with pancreatic cancer were acquired, and imaged within 1 hour of tissue resection. Once μ OCT imaging was completed, those ROIs were excised and fixed immediately with 10% neutral buffered formalin solution for histological analysis. Finally, histology images of the ROIs were acquired and utilized to compare with the OCT images. First, a number of consecutive cross-sectional images of the tissue were acquired, and then they were combined together to form three-dimensional (3D) images. Finally, *en face* images could be extracted with the reconstructed 3D images. All μ OCT images utilized in the study were averaged over five consecutive frames to alleviate the influence of speckle noise in different cases. Our studying protocols were reviewed and approved by the institutional animal care and use committee (IACUC) at Nanyang Technological University [ARF-SBS/NIE-A0312], and the institutional review board (IRB) at Renmin Hospital of Wuhan University [2017K-C053].

Fig. 3. Representative cross-sectional images of normal mice pancreatic tissue. (a) A cross-sectional μ OCT image of the mice pancreas ($0.872 \text{ mm (W)} \times 0.44 \text{ mm (H)}$, $1024 \text{ pixel} \times 800 \text{ pixel}$). (b) Histology, hematoxylin and eosin; $200\times$. The cellular-level pancreatic microstructures, e.g., islet of Langerhans (IL), blood vessels (BVs, dash-dotted white arrow) as well as serous acini (SA, solid yellow arrow), together with subcellular-level IL cell nuclei (red arrows) within the IL structure were clearly identified. In histology, the corresponding pancreatic structures could also be resolved. Scale bar: $100 \mu\text{m}$.

III. RESULTS

A. Mouse Pancreas Imaging

Figure 3(a) presents a representative cross-sectional μ OCT image of a normal mouse pancreas *ex vivo*. The image is comprised of $1024 \text{ pixels} \times 800 \text{ pixels}$ covering $0.872 \text{ mm (W)} \times 0.44 \text{ mm (H)}$. The results in Fig. 3(a) show that the main representative pancreatic structures, such as the islet of Langerhans (IL), blood vessels (BVs) as well as the serous acini (SA) that are surrounding the IL could be clearly resolved, and all those structures appear to be high-intensity due to their high scattering properties. Specifically, as the main subcellular-level microstructure, the IL cell nuclei as denoted by the red arrows within the IL could also be identified. Fig. 3(b) presents the corresponding histology of the normal mouse pancreas. It could be observed that both cellular-level structures, e.g., the SA, BVs, IL, and the subcellular-level tissue microstructures, such as IL cell nuclei, could be identified and had good correspondence to those in the μ OCT image in Fig. 3(a).

Figure 4 further shows the corresponding *en face* images of the normal mouse pancreatic tissue at imaging depths of $20 \mu\text{m}$, $120 \mu\text{m}$, $220 \mu\text{m}$, and $270 \mu\text{m}$. Again, it can be seen that the representative detailed pancreatic structures, e.g., BVs, SA, and the IL, could be clearly identified at different imaging depths. Specifically, the SA were brighter at a smaller imaging depth, while the structural color became darker, and the number of micro-structures that could be identified decreased with increasing depth due to the decreased scattering capabilities of the tissues. Both cross-sectional and *en*

Fig. 4. Corresponding μ OCT *en face* images of normal mouse pancreas at imaging depths of (a) $20 \mu\text{m}$, (b) $120 \mu\text{m}$, (c) $220 \mu\text{m}$ and (d) $270 \mu\text{m}$, respectively. All images consist of $1024 \times 1024 \text{ pixels}$ covering an area of $0.872 \text{ mm (W)} \times 0.872 \text{ mm (H)}$. The representative pancreatic structures, e.g., islet of Langerhans (IL), blood vessels (BVs) as well as serous acini (SA) could be identified. Scale bar: $100 \mu\text{m}$.

face images convincingly demonstrated that μ OCT could be applied for cellular/sub-cellular mice pancreatic tissue imaging, and the imaging results obtained with μ OCT were comparable with those from histology.

B. Rat Pancreas Imaging

1) *Normal Rat Pancreatic Imaging*: Figure 5(a) shows a representative cross-sectional image of rat pancreas *ex vivo*. This image consists of $1024 \times 600 \text{ pixels}$ covering an area of $0.872 \text{ mm (W)} \times 0.33 \text{ mm (H)}$. The imaging results show that the main representative cellular-level pancreatic microstructures, e.g., IL, interlobular duct (ID) and blood capillaries (BCs), were clearly resolved as marked. As a key microstructure surrounding the IL, the SA are also resolved due to their high scattering properties. When further enlarged, the subcellular-level microstructure, i.e., the IL cell nuclei as denoted by the red arrows in figure inset, could also be clearly identified. Fig. 5(b) presents the corresponding histology of the pancreas of a rat. As seen, both the detailed cellular structures, e.g., BC, ID and SA, as well as the subcellular-level tissue microstructures, such as IL cell nuclei, could be resolved and had good correspondence to those in the μ OCT image in Fig. 5(a).

The tissue microstructures could be further verified by their *en face* images. Figs. 6(a-d) present the corresponding *en face* images of normal rat pancreas tissue at imaging depths of $50 \mu\text{m}$, $100 \mu\text{m}$, $125 \mu\text{m}$, and $150 \mu\text{m}$, respectively, starting from the tissue surface. These images consist of $342 \times 848 \text{ pixels}$ covering an area of $0.29 \text{ mm (W)} \times 0.72 \text{ mm (H)}$. Again, it can be observed that the cellular-level pancreatic microstructures of IL, ID, BCs as well as SA were clearly identified in Fig. 6(a). When further enlarged, the IL cell nuclei can also be resolved as shown in the figure inset. When penetrating deeper into the tissue, the IL disappears while the ID and the BCs can still be resolved as shown in Figs. 6(b-d). This is because the IL is typically

Fig. 5. Cross-sectional images of normal rat pancreas *ex vivo*. (a) μ OCT cross-sectional image of the rat pancreas (0.872 mm (W) $\times$ 0.33 mm (H), 1024 pixel $\times$ 600 pixel). (b) Histology, hematoxylin and eosin: 200 $\times$. The representative cellular-level pancreatic microstructures, e.g., islet of Langerhans (IL), interlobular duct (ID, dashed green arrow), blood vessel (BV, dash-dotted white arrow) as well as serous acini (SA, solid yellow arrow), together with subcellular-level IL cell nuclei (red arrows) shown in the inset were clearly resolved. In histology, the small artery is also resolved. Scale bar: 100 μ m.

Fig. 7. Representative pancreatic image of AP diseased rats. (a) Representative μ OCT image of a rat pancreas with AP disease (0.872 mm (W) $\times$ 0.425 mm (H), 1024 pixel $\times$ 680 pixel). (b) Histology, hematoxylin and eosin: 200 $\times$. Both cellular and sub-cellular-level micro-structures cannot be identified. Scale bar: 100 μ m.

Fig. 6. Corresponding μ OCT *en face* images of a normal rat pancreas at imaging depths of (a) 50 μ m, (b) 100 μ m, (c) 125 μ m and (d) 150 μ m. All images consist of 342 $\times$ 848 pixels covering an area of 0.29 mm (W) $\times$ 0.72 mm (H). The representative pancreatic structures, e.g., islet of Langerhans (IL, orange dashed line), blood capillary/vessel (BC/BV, dash-dotted yellow arrow), interlobular duct (ID, dashed green arrow) as well as serous acini (SA, solid yellow arrow) were resolved. The IL cell nuclei (denoted by the red arrows) were also clearly resolved as shown in the inset of Fig. 6(a). Scale bar: 100 μ m.

cluster shaped with limited size, while both ID and BCs are reticular structured, and thus, can be visualized at different imaging depths. For detailed pancreatic microstructures, the readers are referred to the supplementary video documents.

2) *AP diseased Rat Pancreas Imaging*: Fig. 7 presents a representative cross-sectional image of a rat pancreas together with its comparison to histology. The results in Fig. 7(a) show that both

cellular and sub-cellular structures are quite different from those in normal tissues, as shown in Fig. 5(a). Specifically, in Fig. 7(a), these tissue micro-structures are gathered together and became mass shaped structures, with those detailed sub-cellular pancreatic structures, as seen in the normal tissues, were not identified. This lack of detail is probably observed because for the AP disease model, the tissue micro-structures, especially the islet of Langerhans and the serous acini around the islets, were destroyed with congestive blood, and the structural distinctions between those cellular/sub-cellular microstructures had interstitial edema; thus, the tissue structures could not be identified.

The feasibility of μ OCT for diseased tissue identification can be further verified by the reconstructed *en face* images of the pancreatic tissues. Fig. 8 presents the reconstructed *en face* images of the diseased pancreatic tissue at imaging depths of 20 μ m, 70 μ m and 120 μ m. Again, it can be observed that the cellular/sub-cellular level microstructures, e.g., the serous acini, IL and BCs/BVs, could not be identified. Instead, these structures turned out to be mass shaped with the micro-structures gathered together. All these structures remained the same at a larger depth. Such imaging results convincingly demonstrated that the constructed μ OCT system was able to differentiate the diseased microstructures from normal ones, and thus could be utilized as a viable tool for pancreatic disease diagnosis.

C. Human Pancreas Imaging

1) *Normal Human Pancreas Imaging*: Fig. 9(a) depicts a representative μ OCT image of normal pancreatic tissue, which consists of 1024 pixels $\times$ 453 pixels covering an area of 0.872 mm (W) $\times$ 0.33 mm (H). As shown in Fig. 9(a), all of the representative cellular-level

Fig. 8. *En face* rat pancreatic images of AP diseased tissue at imaging depths of (a) 20 μm , (b) 70 μm and (c) 120 μm , respectively. All images consist of 1024×1024 pixels covering an area of 0.872 mm (W) $\times$ 0.872 mm (H). The representative pancreatic structures could not be identified as they are destroyed in the AP diseased model. Scale bar: 100 μm .

Fig. 9. Representative images of human pancreatic cancer adjacent tissue. (a) μOCT cross-sectional image of the pancreatic cancer adjacent tissue (0.872 mm (W) $\times$ 0.453 mm (H), 1024 pixel $\times$ 725 pixel). (b) Histology, hematoxylin and eosin: 200 $\times$. The cellular-level structures of the pancreatic tissues, e.g., pancreatic duct (PD), islet of Langerhans (IL) and serous acini (SA), could be clearly identified. Scale bar: 100 μm .

structures, e.g., the IL, pancreatic duct (PD) and SA, could be clearly identified, and are comparable with those from histology, as shown in Fig. 9(b). Specifically, for those layered structures, as shown in Fig. 9(b), they could also be clearly identified in the μOCT image, and they had very good correspondence to those in the μOCT cross-sectional image. Due to the decreased light scattering capability of the tissue, however, the color of the tissue structures became darker with increasing imaging depth in the μOCT image.

The detailed structures of the human pancreatic tissues can be further verified by the reconstructed *en face* images. Figs. 10(a)-10(c) present the corresponding *en face* images of the pancreatic cancer adjacent to the imaging depths of 50 μm , 100 μm , and 150 μm , respectively. Again, it can be observed that the detailed cellular-level pancreatic structures, e.g., the PD, IL and SA, are clearly identified, verifying the pancreatic structures that were observed in the cross-sectional images. Hence, the imaging results of cellular-level pancreatic structures in both cross-sectional and *en face* images convincingly demonstrate the imaging capability of μOCT , and μOCT could be utilized as a viable imaging tool for structure identifications.

Fig. 10. *En face* images of human pancreatic cancer adjacency at imaging depths of (a) 30 μm , (b) 80 μm and (c) 130 μm , respectively. All images consist of 1024×1024 pixels covering an area of 0.872 mm (W) $\times$ 0.872 mm (H). Again, the representative pancreatic structures, such as the pancreatic duct (PD), islet of Langerhans (IL) and serous acini (SA), could be clearly identified. Scale bar: 100 μm .

Fig. 11. Representative images of human pancreatic cancer tissues. (a) A typical μOCT cross-sectional image of human pancreatic cancer tissue (0.872 mm (W) $\times$ 0.425 mm (H), 1024 pixel $\times$ 680 pixel). (b) Histology, hematoxylin and eosin: 200 $\times$. None of these cellular and sub-cellular-level pancreatic micro-structures could be identified, instead, they were largely destroyed by luminal necrosis (LN), infiltrative growth pattern (IGP) and serous acini hyperplasia (SAH). Scale bar: 100 μm .

Fig. 12. *En face* images of human pancreatic cancer at imaging depths of (a) 50 μm , (b) 100 μm . Both images consist of 1024×1024 pixels covering an area of 0.872 mm (W) $\times$ 0.872 mm (H). No representative pancreatic structures were identified, instead, they were destroyed and replaced by infiltrative growth pattern (IGP). Scale bar: 100 μm .

2) *Human Pancreatic Cancer Imaging*: To evaluate the feasibility of μOCT for pancreatic disease diagnosis for human beings, tissues of human pancreatic cancer were also imaged. Fig. 11(a) depicts a typical cross-sectional image of human pancreatic ductal adenocarcinoma, which consists of 1024 pixel $\times$ 680 pixel covering an area of 0.872 mm (W) $\times$ 0.425 mm (H). The results showed that none of these representative cellular-level pancreatic micro-structures, e.g., IL, PD or SA, could be identified in pancreatic cancer tissues. Instead, the images showed that the infiltrating glands of ductal adenocarcinomas grow in a haphazard fashion and luminal necrosis was observed.

The infiltrative growth pattern of tissues in pancreatic cancer could be further verified by μOCT *en face* images. Fig. 12 presents the reconstructed *en face* images at imaging depths of 50 μm and 100 μm . Such imaging results demonstrated that the distinction between cancer tissues and normal tissues was obvious. Specifically, in the cancerous tissues, none of the lobular acini could be identified, and all of the structural boundaries were largely destroyed with only enlarged and distorted ducts could be observed. Such imaging results convincingly demonstrated that the cancerous tissue being easily identified by high-resolution μOCT systems, and thus, a high-resolution μOCT system could be utilized as a viable imaging tool for pancreatic disease diagnosis.

IV. DISCUSSIONS

The prognosis for pancreatic cancer and acute necrotic pancreatitis is extremely poor in clinical practice; therefore, cellular/sub-cellular level pancreatic tissue imaging is highly desired since it could help visualize the subtle tissue morphological changes during the

progression of malignancies, which ultimately helps to significantly reduce morbidity and prevent complications. However, for islet transplantations, it is highly important to both identify minute structures of the pancreas and quantify the number of islets with a non-invasive imaging method, which also helps determine the underlying mechanism of diabetes pathogenesis and β cell loss. Currently, although endoscopic and radiographic technologies, such as ERP, EUS, MRI, CT and US, could be utilized for pancreatic imaging, they are incapable of real-time cellular/sub-cellular level tissue imaging, and thus could not meet clinical needs. In this study, we evaluated the feasibility of μ OCT as a non-invasive, high-resolution imaging tool that provides cellular/sub-cellular level tissue micro-structure images for pancreatic disease diagnosis.

A typical μ OCT system, which achieves a resolution of $\sim 1.67 \mu\text{m}$ in the axial direction and $1.79 \mu\text{m}$ in the lateral direction, was constructed. Images of mouse, rat and human pancreatic tissue specimens were acquired, and compared to their corresponding histology images for both structure confirmations and normal/diseased tissue identifications in different cases. The results showed that for the normal pancreatic tissues, the detailed pancreatic microstructures, e.g., blood vessels, and the parenchyma and adjacent soft tissues, i.e., the islet of Langerhans and the IL cell nuclei, were clearly identified. All of the OCT images were comparable to their histology in different cases. In diseased tissues, such structures are largely destroyed and none could be identified. Such imaging results convincingly demonstrated that μ OCT could be a viable tool for noninvasive or minimal invasive cellular/subcellular level pancreatic imaging, and thus, could be utilized as a candidate tool to perform an intraoperative biopsy or needle aspiration biopsy in the future.

A major factor, however, that limits the clinical applications of μ OCT system is its limited lateral scanning range because there exists a trade-off between the achievable spatial resolutions and the lateral scanning range, which makes it difficult to visualize the tissue morphological changes at a large scanning area for practical use. To address this issue, a possible solution is to develop μ OCT systems with both large-area scanning and high-resolution scanning modes that can be exchanged freely without affecting the imaging process. Such a multi-mode system could help achieve faster diagnosis with a diagnosis-area-locking function.

Another major limitation in the current imaging system is the limited imaging depth. Our results showed that *en face* images with good contrast and resolution can only be obtained at a maximum depth of $\sim 300 \mu\text{m}$ beneath the tissue surface. Such an imaging depth could not meet the requirements for *in vivo* evaluations of microstructure, which typically ranges from several millimeters to centimeters deep inside the tissue in clinical practice. To address this issue, the recent advancements in needle aspiration techniques [30, 31] and high-resolution endoscopic imaging probes [32, 33] have provided a possible solution, i.e., with OCT combined, the former could penetrate into the pancreas with accurate probe placement and minimal invasiveness, while the latter could be utilized for luminal pancreatic duct imaging noninvasively. Since limited depth of focus (DOF) largely impacts on OCT imaging depth, especially for endoscopic OCT systems, extensive efforts have been devoted to DOF extension techniques recently [34–36]. In addition, scanning speed of the μ OCT also needs to be improved for *in vivo* imaging in clinical practice.

V. CONCLUSION

To the best of our knowledge, this study is the first to evaluate the feasibility of μ OCT as a cellular/subcellular resolution imaging tool for pancreatic disease diagnosis. Imaging results with mouse, rat and human pancreatic tissues showed that μ OCT is able to differentiate

between normal and diseased pancreatic tissues in different cases, and the detailed tissue microstructures could also be clearly resolved and comparable to those in histology. Such a study convincingly demonstrated that μ OCT could be a powerful tool for pancreatic disease diagnosis and thus could be potentially utilized in needle-based optical biopsy and intraoperative imaging guidance.

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