Three-Dimensional Virtual Histology in Unprocessed Resected Tissues with Photoacoustic Remote Sensing (PARS) Microscopy and Optical Coherence Tomography

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Abstract: A combined Photoacoustic Remote Sensing (PARSTM) microscope and Optical Coherence Tomography (OCT) system is used to capture label-free wide-field high-resolution three-dimensional visualizations of nuclear morphology in unprocessed resected tissues.

Histopathological visualization of tissues plays a crucial role in biological research, cancer screening, and surgical oncology. However, the preparation of such visualizations requires laborious multi-step processing [1]. First, bulk resected tissues are fixed in formalin. A macroscopically representative subset of the bulk sample is selected for further analysis. Selected tissues are dehydrated with alcohol and xylene, then embedded into paraffin wax. Sections (4- to 10-microns thick) are then cut from the formalin fixed paraffin embedded tissues using a microtome. The resulting thin sections are fixed to glass slides, resulting in a translucent fixed tissue preparation. At this point, the sample may be stained with immunohistochemical dyes to highlight relevant subcellular structures. The most common dye combination being hematoxylin and eosin (H&E). Hematoxylin highlights nuclei in purple, while eosin colors filaments in muscle cells, intracellular membranes, and extracellular fibers pink [2]. Once stained, the slide is finally complete and may be interpreted by a pathologist using a transmission light microscope. This demanding workflow requires multiple days, sometimes delaying diagnostic reports over a week. For complex specimens, processing may take longer than ten days [3]. Moreover, visualizing subsurface morphology necessitates the preparation of multiple thin sections. As an alternative, faster techniques such as frozen section analysis are sometimes employed. However, this technique can introduce artifacts hindering clinical interpretation [4]. These challenges are especially prevalent in fatty samples, such as breast tissues. Moreover, the preparation of frozen sections requires processing which is incompatible with long term storage of samples. Once a tissue undergoes frozen sectioning, the bulk sample cannot be reused.

Therefore, there remains an unmet need for a rapid clinically compatible histological imaging system. Ideally, H&E-like diagnostic images could be captured label-free directly on unprocessed freshly resected tissue. A technique capable of such imaging would permit rapid intraoperative assessment of surgical margins, directly on the excised tissues. This would not only save valuable time during surgery but would guide total resection of tumors and reduce re-operation rates. Moreover, unlike frozen sectioning, such a technique would preserve tissue for paraffin preparation. In recent years, several modalities such as light sheet microscopy (LSM) [5], non-linear microscopy [6], optical resolution photoacoustic microscopy (OR-PAM) [7], stimulated Raman scattering microscopy [8] and microscopy with ultraviolet surface excitation (MUSE) microscopy [9], have aimed to provide intraoperative histopathology capabilities like this. However, most have fallen short of the ideal solution. Of these modalities only OR-PAM has shown reflection mode label-free H&E-like imaging in thick tissue samples. OR-PAM techniques have demonstrated efficacious visualization of nuclear structures by targeting the unique ultraviolet (UV) absorption contrast attributed to DNA. [7]. However, OR-PAM is a hybrid opto-acoustic imaging modality. Absorption contrast is detected with an acoustically coupled ultrasound transducer, requiring physical contact with the sample [7]. In the popular work of T. Wong et al., tissues were submerged in a water tank during imaging [7].

Recently, a novel non-contact technique Photoacoustic Remote Sensing (PARS) microscopy has emerged as an alternative to traditional OR-PAM. PARS replaces the acoustic transducer of conventional OR-PAM with a co-focused detection beam [10]. The detection beam observes photoacoustic signals as elasto-optic induced back-reflected intensity modulations. Here, we present a conjoined PARS microscope and swept source optical coherence
tomography (OCT) system for rapid virtual histology in bulk unprocessed resected tissue specimens. This novel multimodal system provides co-registered OCT and PARS visualizations. While the PARS microscope provides histology-like imaging of nuclear structures, the OCT system provides visualizations of the tissues surface, and subsurface morphology. Previously, PARS has demonstrated recovery of nuclear contrast in resected tissues with limited resolution, field of view and imaging speed [11]. In contrast, our optimized PARS and OCT system captures wide-area, high-resolution diagnostic quality histological visualizations in bulk unprocessed tissue specimens. Moreover, by leveraging the optical sectioning capabilities of the PARS system we capture the first three-dimensional volumetric imaging of subsurface nuclear morphology in unprocessed tissues. The resulting PARS and OCT images provide qualitatively identical diagnostic features to conventional H&E preparations.

To capture nuclear contrast, the PARS system targets the optical absorption peak of DNA using a 266 nm excitation. PARS pressure and temperature signals are then detected with a co-focused 1310 nm continuous-wave super-luminescent diode. To reduce chromatic effects a 0.5 NA reflective objective lens is used to co-focus the PARS excitation and detection onto the sample. With the proposed architecture, the PARS resolution is measured on 200 nm gold nanoparticles as ~300 nm laterally and ~1.1 μm axially. Concurrently, the OCT system uses a MEMS-VSCEL source centered at ~1060 nm with ~100 nm full width sweep bandwidth. The OCT axial resolution and SNR were measured experimentally using a mirror. The FWHM of the axial PSF was 10.1 μm in free space, corresponding to 7.3 μm in biological tissue, assuming an average refractive index of n = 1.38. Depending on the desired field of view the NA of the OCT objective is switched between 0.14 and 0.4 corresponding to ~15.6 μm and ~3.9 μm lateral resolution, respectively.

This system was then applied directly in unprocessed resected human, rattus and murine tissues. While samples were stored in formalin solution to prevent degradation during transport, no further preparation or processing was performed prior to imaging. To prevent movement during mechanical scanning, tissues were first placed into a sample immobilization jig. Tissues were first scanned with the PARS microscope, then shifted to the OCT imaging head. OCT scans were captured with the sample in the same orientation generating co-registered OCT images. One such combined PARS and OCT dataset is presented in Fig. 1 (a). The PARS image of the nuclear structures (Purple) is projected onto the 3-dimensional OCT (0.14 NA) representation of the bulk tissue morphology (Gray). Enlarged subsections of the large field PARS image can be observed in Fig. 1 (b) and (c). Within the PARS images, we may assess both dense nuclear structures and individual sparse nuclear morphology. Concurrently, a small field OCT (0.4 NA) frame may be observed in Fig. 1 (d) and (e). While Fig. 1 (d) shows a vertical slice into the tissue, Fig. 1 (e) shows a horizontal subsurface slice from the tissue sample. These images reveal subsurface tissue structures, including adipocytes (circular structures), and a possible ductal structure (black void).

![Fig. 1](image)

Fig. 1. Combined PARS and OCT (0.14 NA) imaging of unprocessed excised rattus mammary tumor tissue. (a) Shows co-registered OCT and PARS images projected directly onto the excised tissue specimen. (b and c) Subsections of the wide field PARS image presented in (a). (d) Vertical profiled (at location shown (e)) of a small field OCT (0.4 NA). (e) Horizontal slice (at depth shown in (d)) of a small field OCT (0.4 NA).

While OCT captures subsurface morphology of the bulk tissue, it does not provide nuclear visualizations. To explore and visualize the subsurface nuclear structures within the resected tissues, we leverage the optical sectioning capabilities of the PARS system. We capture a depth resolved series of UV-PARS images, which are reconstructed.
to form a depth resolved 3-dimensional representation of the subsurface nuclear morphology within the resected tissue sample. One such result is shown in Fig. 2. Images are captured from the sample surface through to ~30 μm in depth (Fig. 2 (a)), then reconstructed into a 3-dimensional representation (Fig. 2 (c)). In contrast to the ~1.5 mm penetration of the OCT system, the PARS imaging depth is limited to approximately the transport mean free path of the UV excitation (~50 μm). As the UV excitation is highly absorbed and scattered within tissues, the UV-PARS system begins to rapidly lose contrast beyond the transport mean free path of the excitation. However, within this bound we can observe multiple layers of sparse nuclei, adjacent to regions of dense nuclear structures.

This talk will focus on the development, implementation, and future advancements of this revolutionary multimodal histology system. We will explore salient results from the proposed microscope to investigate the techniques full capabilities. This work represents; (1) the first report of a combined PARS and OCT system for label-free visualizations of nuclear and extranuclear morphology in resected tissue specimens; (2) the first report of 3-dimensional imaging of subsurface nuclear morphology in resected tissue specimen with a non-contact photoacoustic system. This microscope provides co-registered sub-micron depth-resolved nuclear imaging, and micron scale depth resolved tissue morphology in an all-optical reflection mode format. Moreover, these visualizations may be recovered label-free directly from thick unprocessed tissues. These clinically relevant features are not accessible through any other means. The unparalleled clinical diagnostic capabilities of this system could revolutionize the diagnostics, treatment, and resection of cancers and other neoplasms.

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