Tissue and Blood Optical Clearing for Biomedical Applications

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Advanced optical methods pave the way to study the molecular and cellular structure and function of tissues *in vivo* or *in vitro.* However, the high scattering of turbid biological tissues limits the penetration of visible and near-infrared light, and the imaging resolution and contrast decrease as light propagates deeper into the tissue. Tissue optical clearing (TOC) techniques have an increasingly prominent role in biomedical imaging and applications. This special section on TOC includes one review and ten original papers.

Recently, TOC techniques have made major advances in three-dimensional reconstruction of thick tissue sections and intact organs. In the review paper, Silvestri et al. discuss TOC of fixed tissues from the perspective of the end user. They propose a taxonomy of clearing techniques based on matching the various TOC protocols with different optical microscopy techniques. This taxonomy will help researchers identify the protocol best suited to their studies.

A primary application of TOC of intact organs is in the field of neurobiology. Many exciting advances have been made in the visualization of cerebral structures, yet limitations remain with regards to the complexity of implementation of some protocols, and with the long incubation times required especially for imaging of entire organs. Yu et al. describe a rapid and prodium iodide-compatible optical clearing method for brain tissue. Drops of sorbitol, sucrose, and fructose can quickly render mouse brain samples transparent, and result in a threefold enhancement in imaging depth. This rapid optical clearing method is expected to enable optical imaging with sectioning and clearing, and provides a new approach for large-volume reconstruction.

Skin optical clearing continues to be a primary area of research. Feng et al. studied attention to the optical clearing potential of disaccharides with use of molecular dynamics simulations and ex-vivo and in-vivo experiments. Their results show that the two disaccharides exhibit a better optical clearing potential than fructose at the same concentration, and that the use of sucrose with optical coherence tomography (OCT) can achieve a more significant increase in imaging depth and signal intensity. Liopo et al. report on a two-step method for enhancement of light penetration through skin. This method involved pretreatment by hyaluronic acid prior to application of clearing agents (PEG and PPG), and resulted in a ~47-fold increases in transmission of red and near-infrared light and significantly enhanced contrast of optoacoustic images.

OCT is a common method used to evaluate the efficacy of TOC, and seven papers in this section involve use of different OCT approaches. Two papers describe the combination of

TOC and OCT to image the cutaneous microcirculation. Guo et al. report on the use of OCT angiography (Angio-OCT) to perform comprehensive evaluation of skin optical clearing *in vivo.* They performed simultaneous quantification of optical properties and imaging of blood flow and correlated the scattering coefficient with refractive-index matching. In combination with TOC, Angio-OCT demonstrates enhanced performance in imaging cutaneous hemodynamics with satisfactory spatiotemporal resolution and contrast. Enfield et al. applied correlation-mapping OCT to image vascular networks at the capillary level. With *in-vivo* topical application of a high-concentration fructose solution, they measured a 13% increase in OCT penetration depth, thereby enabling the visualization of vessel features at deeper depths within the tissue. Pires et al. used near-infrared OCT to evaluate the effect of a topically-applied optical clearing agent (OCA) to study melanoma *in vivo* and image the microvascular network with speckle variance and depth-encoded algorithms. After treatment with optical clearing for 250 min, they observed improved contrast resolution up to a depth of $\sim$750 μm within the tumor. Based on these findings, optical clearing techniques are a promising adjuvant to clinical optical characterization of melanoma, such as assessing prognosis and treatment responses.

Besides skin, the optical clearing of the gastrointestinal tract, cochlea, and teeth are reported on in this section. Liang et al. applied the mixture of liquid paraffin and glycerol to reduce attenuation of pig and guinea pig esophagus *ex vivo*, and enhance the imaging depth of spectral-domain OCT at 800 nm. With swept-source OCT, Lee et al. studied the effectiveness of decalcification using ethylenediaminetetra-acetic acid (EDTA) as an optical clearing method to enhance depth-resolved visualization of internal soft tissues of cochlea. They showed that mouse and guinea pig cochlear samples required decalcification for 7–14 days to obtain optimal optical clearing results. In addition, Kang et al. imaged extracted teeth with natural occlusal lesions with OCT with and without the addition of a transparent vinyl polysiloxane impression material (VPS) currently used *in vivo.* They showed that OCAs can be used to improve the ability of OCT to visualize subsurface lesions and the dentin—enamel junction under sound and demineralized enamel.

As a more fundamental application of TOC, Mueller matrix polar decomposition (MMPD) is affected by the refractive index matching process, which is one of the major mechanisms of TOC. Chen et al. studied the interaction of TOC with polarization measurements, using Monte Carlo simulations with anisotropic tissue-mimicking models. The depth-resolved polarization features indicate that the refractive index matching process increases the depth of polarization measurements and may lead to higher contrast between tissues of different anisotropies in deeper layers. Furthermore, MMPD-derived polarization parameters can enable qualitative characterization of the refractive index matching process.

In conclusion, we thank all authors for their contribution to this special section. As we look forward, we believe that optical clearing will enhance biomedical optical imaging performance and can make significant contributions in the fields of light-based diagnostic and biomedical imaging techniques.

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