RESEARCH HIGHLIGHT

TESOS: an integrated approach for uniform mesoscale imaging

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The Transparent Embedding Solvent System, an innovative imaging method, integrates tissue clearing and section-reconstruction imaging, ensuring consistent sub-micron resolution. This technique is adaptable and compatible with various imaging platforms and specimens.

Recently, there has been a profound interest in gaining deeper insights into large biological samples to unravel structural and functional intricacies. 3D large-volume optical imaging, in particular, has received considerable attention due to its high resolution and potential to register molecular and cellular identities via various fluorescent labeling methodologies, such as transgenic mouse models, viral vector expression, and immunolabeling. Large-volume optical imaging of mammalian tissues has historically been difficult due to heterogeneous tissue properties and light scattering. Imaging an intact nervous system is even more challenging due to the long-range projections and complex connections. In recent years, a number of tissue processing and imaging techniques have enabled large-volume optical imaging, which has begun to reveal the 3D cellular profiles and neural circuits in mammals.

There are two main types of large-volume imaging workflows: tissue clearing and block-face imaging. Tissue-clearing techniques have evolved significantly in recent years and been extensively reviewed.[1–3] It removes lipids from the tissues with different detergents or solvents to render the tissue optically transparent. Together with the advancement of lightsheet microscopy, it has been applied to image whole organs, such as brains from rodents and non-human primates, as well as some entire rodents.[4–6] These methods enable detailed examination of cellular and sub-cellular structures in their native 3D environments. Even though tissue clearing can be time-consuming, imaging acquisition is generally quick and can be completed in minutes to a few hours with contemporary lightsheet microscopy. However, the lightsheet also tends to compromise axial resolution. Moreover, a common concern with tissue clearing is diminished optical resolution in the deeper parts of the samples due to inadequate clearing and improper refractive index (RI) matching. Lastly, samples could be distorted during clearing and mounting steps, making quantitative comparisons between samples or registration to established atlases challenging.

Block-face imaging, on the other hand, uses various mechanical and chemical methods to achieve thin sections for continuous imaging and reconstruction. Several automated block-face imaging methods have been developed, such as the micro-optical sectioning tomography series.[7,8] They feature simple tissue preparation, rigid sample properties, and high imaging resolution, making quantitation and registration easier. However, they are less compatible with immunolabeling and can be time-consuming due to the need for precise slicing.

In a new study published in *Cell Research*, Yi and colleagues introduce the Transparent Embedding Solvent System (TESOS), an innovative method combining tissue clearing with section-reconstruction imaging.[9] TESOS has three main steps: pretreatment, clearing, and transparent embedding. In brief, after pretreatment and organic solvent-based delipidation, samples are immersed in a high RI medium supplied with a UV crosslinking initiator. Post UV crosslinking, this embedding can turn the samples into a rigid organo-gel block while maintaining tissue transparency. Subsequently, the sample block can be imaged, similar to a block-face platform, by light microscopy combined with either a microtome for smaller samples or a milling platform for larger tissues. During imaging, planes are captured with a thickness aligned to the objective's working distance. After capturing each plane, it is physically removed to allow the examination of the next layer. The acquired images can then be reconstructed into a cohesive volume, ensuring consistent optical resolution.

The team showcased the adaptability of TESOS across various samples, from individual organs to an entire 6-week-old mouse. They also demonstrated its compatibility with different fluorophores (YFP, GFP) and fluorescent labeling techniques (transgenic mouse lines, viral injection, and antibody staining) as well as with various optical imaging techniques such as confocal and light-sheet microscopies. The study highlighted TESOS's ability to achieve uniform sub-micron resolution, as evidenced by the detailed nerve structures with single-axon resolution visualized in both the brain and periphery.

In summary, TESOS creatively combines tissue clearing and section-reconstruction imaging principles to achieve uniform sub-micron resolution in large biological samples. Its conceptual simplicity and modular design could make TESOS readily adaptable to conventional biology labs. With the strength of fluorescent labeling in pinpointing specific proteins or other biomolecules, TESOS stands as a promising optical counterpart to electron microscopy reconstruction, heralding new opportunities in neuroscience and beyond.

It should be noted that choosing the appropriate approach for large-volume 3D imaging requires careful consideration to balance a variety of factors, such as targeted structures, tissue types, and labeling methods. The users need to find a compromise between optical resolution, processing speed, and data size, among other parameters. TESOS adds a novel bridging technique to the conventional tissue clearing and block-face imaging approaches, with flexible resolution and the potential to apply to a variety of tissue samples. It may be especially useful for applications requiring high-resolution interrogation in relatively large volumes, such as the long-range projection of the peripheral nervous system, as demonstrated by the authors.

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Finally, managing and analyzing large imaging datasets remains a general challenge for the field. A single mouse brain dataset, e.g., may range from 100 GB to 30 TB, depending on the methodologies used.[2] With its sub-micron resolution, TESOS would generate substantially large datasets, and the scale of the data size could further increase when applied to the peripheral nervous system or entire mammalian bodies, making data registration and quantitative analysis more challenging. Thus, additional efforts would be needed from the community to develop effective approaches for visualizing and analyzing huge datasets from these newer imaging methods.

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ADDITIONAL INFORMATION

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