

3D mapping of whole organs with iDISCO⁺ and light-sheet microscopy

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“A picture is worth a thousand words” is a particularly appropriate principle in histology, where micrographies provide the basis for describing and interpreting tissue organization. For more than a century, the obtention of these images required tissue sectioning to generate thin specimens that could be penetrated by light. Although this approach remains fundamental to the study of tissue microarchitecture, sectioning necessarily involves tissue loss, restricts quantitative analyses to sampled subregions, and limits the mesoscopic interpretation of complex three-dimensional (3D) structures.

Tissue-clearing and 3D imaging techniques provide a complementary approach, allowing intact specimens to be stained, optically clarified, and imaged at cellular resolution across large volumes. Moreover, semi-automated image-segmentation methods enable the quantitative and standardized analysis of these datasets. Together, these advances make 3D histology particularly well suited to studying phenomena at the mesoscale, bridging the gap between imaging modalities that operate at different spatial scales. While, MRI enables non-invasive imaging of large samples at low spatial resolution, conventional section-based histology provides high-resolution information but is limited to thin, sampled sections. By combining cellular resolution with a large three-dimensional field of view, 3D histology can capture the organization and connectivity of structures that extend beyond individual tissue sections. Critical applications include tracing long-range axonal trajectories (Figure 1) or characterizing vascular networks down to the capillary level. Looking ahead, the development of reference atlases for different organs, organisms, and developmental stages, together with the implementation of AI-based interfaces that facilitate the implementation of complex image-analysis pipelines, is expected to further expand the applications and accessibility of 3D histology.

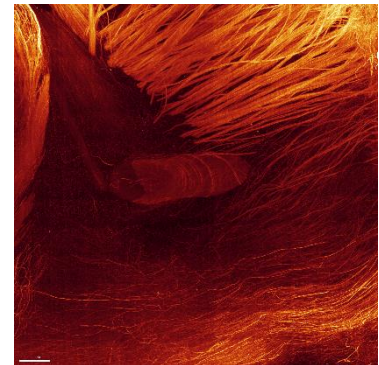


Figure 1. Mouse brain stained for pNFH with iDISCO⁺. Volumetric imaging enables the observation of potentially misguided axons wrapping around the anterior commissure.

In our laboratory, we combine tissue-clearing approaches, including iDISCO⁺ and TRISCO, with light-sheet microscopy and computational image-analysis tools such as ClearMap, to investigate the cellular mechanisms underlying long-range injury spread and circuit remodeling in the adult brain. At the same time, we are working to expand the applications of 3D histology by developing and optimizing new markers and segmentation tools.

In this technical talk, I will first provide an overview of the iDISCO⁺ and TRISCO tissue-clearing methodologies and introduce the ClearMap computational workflow. I will then present AndromeMap, a new ClearMap-compatible tool developed for astrocyte quantification at the whole-brain scale. Finally, I will showcase examples of how these approaches can be used to investigate tissue organization and cellular responses in the brain and other tissues.