MICROSCOPY

Imaging cleared tissues made easy

A flexible open-top light-sheet microscope has been developed that can perform deep three-dimensional imaging on all clearing protocols with low and high optical resolution.

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An obstacle in the field of three-dimensional (3D) imaging of cleared tissues is that there are few flexible imaging systems that can be used to effortlessly visualize all clearing protocols and different-sized specimens with various optical resolutions. This issue confines researchers to a narrow set of clearing methods and experimental approaches that match their choice of light-sheet microscope. In this issue of *Nature Methods*, Glaser and colleagues[1] present a hybrid open-top light-sheet microscope, which uses a unique configuration of multiple objective lenses mounted in an open-top architecture that allows for multipurpose 3D imaging.

Microscopic imaging of biological tissues predominantly occurs in two dimensions because visual light has difficulties penetrating through biological material. However, biological life occurs in 3D; therefore, it is desirable to visualize biological samples in 3D. In the past decade, tissue clearing has received increasing attention, which has resulted in the development of a plethora of clearing protocols that have different optical properties and biological applications[2]. This rapid development of tissue clearing protocols has fueled the need for 3D imaging, an area in which light-sheet microscopy has gained the most attention. The advances in light-sheet microscopy are due to the diligent work of many independent researchers who have custom-designed their own instruments for their specific needs, resulting in a variety of light-sheet microscopes with different strengths and weaknesses.

Now, Glaser and co-workers have succeeded in merging several optical techniques into one instrument that has enormous flexibility. This hybrid open-top light-sheet microscope: (1) is able to image single or multiple specimens with standard holders, (2) is compatible with all clearing protocols, (3) has no limits on lateral specimen size, and offers (4) deep imaging and (5) tunable multi-scale imaging (Fig. 1). Central to the design is the open-top configuration in which the objective lenses

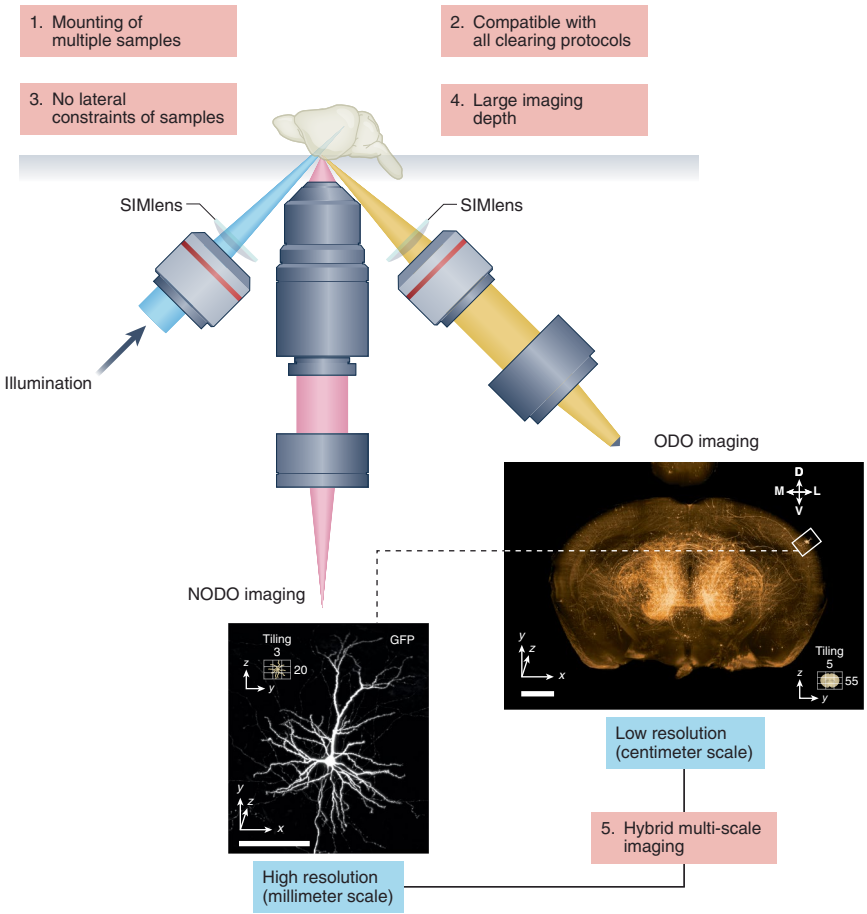

Fig. 1 | Simplified schematic of the optical layout of the hybrid open-top light-sheet microscope. The illumination beam (blue beam) passes through the illumination objective and the solid immersion meniscus lens (SIMlens), creating the light-sheet that penetrates the cleared tissue and excites the fluorophore. Emission is then collected by either the non-orthogonal dual objective (NODO) configuration (red beam) or the orthogonal dual objective (ODO) configuration (yellow beam) to produce either a high-resolution millimeter-scale image or a low-resolution centimeter-scale image, respectively. D, dorsal; L, lateral; M, medial; V, ventral. Image adapted with permission from ref. [1], Springer Nature America, Ltd.

are placed underneath the microscope stage, offering user-friendly accessibility from above[3]. However, this leaves open the question of how to avoid introducing aberrations when oblique light beams pass through a horizontal sample plate. Glaser et al.[1] overcame this obstacle by developing a solid immersion meniscus lens (SIMlens) that suppressed aberrations occurring when the oblique illumination and detection beam transition between air and immersion media with a high refractive index range, covering all current clearing protocols[4]. Additionally, using the SIMlens allows for rapid

transitions between air-based objectives for high- and low-resolution 3D imaging[5]. This is desirable because spatial resolution trades off with imaging and analysis times. For the open-top light-sheet microscope, switching between high- and low-resolution imaging is solved by a hybrid optical architecture that combines a non-orthogonal dual objective (NODO) beam path with an orthogonal dual objective (ODO) beam path, all in the same instrument.

The NODO configuration, which has recently gained popularity (especially for live-cell imaging), can be designed with both single- and double-sided illumination. Here, Glaser et al.[1] chose a single-objective light-sheet architecture that minimizes the aberration caused by refractive index mismatch. For fluorescence collection, a vertically oriented multi-immersion objective with a long 1-cm working distance is used, which provides excellent imaging depth and great tolerance to differences in refractive index. The ODO configuration is used to achieve low-magnification imaging, providing fast mesoscale screening of the entire sample and location of regions of particular interest for high-resolution NODO imaging. In this way, large amounts of high-resolution data that are difficult to process can be avoided, offering the advantage of a substantial time reduction.

The flexibility of the hybrid open-top light-sheet microscope was validated by performing 3D imaging on various tissues cleared with a large variety of protocols ranging from aqueous CUBIC, ClearSee/DEEP-Clear, Ce3D and SHIELD to hydrophobic iDISCO, ECi and PEGASOS. Their results are remarkable and show both centimeter-scale low-resolution overviews and millimeter-scale high-resolution zooms (Fig. 1). It is impressive how well the hybrid instrument can visualize intricately detailed objects such as spines on individual neuronal dendrites and the spatial 3D distribution of breast-cancer metastasis deep inside mouse brains. What is most amazing is that all these images have been recorded with the same light-sheet microscope.

Although the hybrid light-sheet microscope delivers astonishing results, there are features of the system that can be further improved (for example, the resolution). The ODO resolutions are (x) 4.1 µm, (y) 4.4 µm, and (z) 5.5 µm, and the NODO resolutions are (x,y) 0.5 µm and (z) 2.9 µm. Incorporating a Bessel beam or a Gaussian beam with a higher numerical aperture for light-sheet illumination would enhance the axial resolution. Another detail that can be improved is the tolerance for semi-transparent samples. The hybrid light-sheet microscope uses single-sided illumination, which has limitations because the light-sheet loses intensity when it penetrates thick tissues. In other configurations, this issue is commonly solved by dual-sided illumination[6]. However, recently developed clearing protocols render the sample highly transparent, which reduces the negative effect of single-sided illumination.

In summary, an obvious strength of the hybrid open-top light-sheet microscope is that we can more efficiently perform 3D imaging of intricate details, such as molecules and single cells, and of biological structures (for example, tumor vasculature) in intact and undamaged tissues. However, this technique also enables high-throughput, cell-by-cell analysis of spatial location and expression profile of each cell in the entire specimen[7], which can be up to tens of millions of cells. The achievement of Glaser et al.[1] will be a powerful tool in researchers' quests to uncover information about complex neuronal circuits and structures of the nervous system[8], and to obtain results that answer how diseases occur and can be treated[9]. ❐

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Competing interests

The authors declare no competing interests.