MOUNTING AND MULTIVIEW IMAGING STRATEGY TO EXTEND LIGHSHEET IMAGING DEEPER INTO LARGE WHOLE MOUNT CLEARED ORGANS

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The combination of Lightsheet Microscopy and sample optical clearing has become a natural and powerful technique in Biomedical research to study diseases in model organisms. However, several aspects surrounding how the sample is brought to the lightsheet instrument are key to enable full 3D imaging a large samples and several pitfalls still systematically hamper the versatility of full organ imaging.

On one hand, optimizing optical clearing is sometimes very experimental and does not always yield the expected transparency, though samples (e.g. rodent organs) often have a value that does not enable extensive try and error. On the other hand, one may aim at different clearing solutions according to the targeted labelling strategy which inevitably involves working in different refractive index solutions. In an imaging facility, disposing of a versatile lightsheet instrument that tackles these diverse experimental conditions is key.

We show that a simple sample mounting strategy enables to design a simpler, yet versatile instrument family, and that double-sided detection enables to extend the volume of imaging in a straightforward way to reach sample size of over 2cm. We image very large samples with two macroscopes laid horizontally, first to avoid gravity-related issues of heavy equipment when refocusing, and second to avoid contact of objective lenses with clearing medium (i.e. dipping). We enable sample rotation without physical contact with the sample. The layout enables instant mounting and unmounting of the sample cuvette and simplifies the whole experimental procedure of lightsheet imaging.

We will discuss how partially cleared and multiple-refractive-index samples can be imaged from multiple angles. We show how double-sided detection macroscopy yields a factor two increase in imaging depth and enables to circumvent the need of post-acquisition multiview image fusion, which results extremely challenging computationally with very large tiled datasets (>1TB). Finally, we also show how such principle can be implemented in a cost-efficient way using LEGO hardware and a minimal amount of optical components available off-the-shelf. We show that cleared samples of up to 5 centimeters can be efficiently imaged for a price tag about 100-200 times inferior to custom/commercial available solutions, respectively, thereby providing a first entry solution for any lab to start evaluating optical clearing and collect scientific results, at a cost inferior to that of, for example, whole mount sample immunostaining.