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3D Imaging of BABB Cleared Whole-Mount Organs Using Light Sheet Microscopy

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There are significant advantages to imaging thick 3D specimens with light sheet microscopy, but it is still challenging due to the imaging geometry, sample mounting, sample size, and incompatibility with non-aqueous clearing agents such as benzyl alcohol-benzyl benzoate (BABB). Here, we present an approach to expand the range of compatible specimens for Lightsheet Z.1 and other related light sheet instruments where we introduce a protocol that combines BABB clearing and Periodic Acid Schiff (PAS) staining of whole-mount intact tissues to achieve imaging depths of more than 3 mm in intact organs. With BAAB clearing and PAS staining we are able to observe 50µm glomeruli structures (50µm in diameter) in whole kidney specimens and vasculatures (50µm in diameter) in whole heart specimens.

Factors prohibiting the use of organic solvents in the sample chamber include: the abrasiveness of such chemicals and changes to the index of refraction. The harshness of these chemicals prohibits the use of dipping objectives, restricting the imaging objective to a 5x air objective. To accommodate harsh organic solvents we manufactured an aluminum sample chamber (Fig. 1). The Z.1 has a fixed objective design, thus any change to the refractive index of the chamber media results in a misalignment of the image plane and a shift in the light sheet waist. To account for a change in the imaging plane, we made a ring adapter to allow realignment of the imaging objective. Repositioning the objective aligns the objective focal plane, making focusing possible even without the shift in the light sheet waist correction for different clearing solutions of variable refractive index. Our custom designed chamber enabled LSFM imaging in RIMS and BABB solutions. The point spread function (PSF) in figure 1 was taken with the 5x objective with fluorescent bead in agarose while the chamber was filled with water. The PSF demonstrates a 3D Gaussian shape with a typical lateral width of 1.3µm and an axial width of 7µm at 561nm; this supports an expected 1.5µm lateral resolution with the 5x 0.16NA objective. This PSF provides a lower limit to the resolution available from our Lightsheet Z.1 using our customized chamber.

There are two main factors that limit the specimen size – the chamber size and signal to noise ratio. The chamber size places a mechanical limit on the size of the specimen that can be imaged. The chamber window is 1.7 cm in diameter and fixes the lateral field of view accordingly. Stitching methods may be able to improve this, however, commercial software options are limited. Imaging through thick tissue, even with clearing, can result in scattered light decreasing the contrast of the image. To improve the contrast of imaging through thick tissue we followed the procedure of BABB clearing and PAS labeling as outlined by Sivaguru et al[1]. The PAS label creates a strong fluorescent signal which enables high contrast imaging through several millimeters of cleared tissue (Fig 2).

Recently, Torres et al[2], demonstrated the benefits of combining multiphoton microscopy and BABB clearing to image human biopsied whole-mount kidneys in clinical settings, without the need for the laborious task of embedding and sectioning. In this study, the BABB cleared tissues were stained with

SYTOX Green (Thermo Fisher, Eugene, Oregon) to improve the contrast. While good morphological detail was achievable, they obtained imaging depths up to about 0.5 mm. Similarly, we evaluated the depth of penetration and contrast performance of the Lightsheet Z.1 using whole-mount BABB cleared and PAS stained heart[1]. We obtained depths of penetration of ~3.5mm. We note that our approach of staining the BABB cleared heart tissues with PAS instead of SYTO green and imaging with LSFM produced an improvement in depth of penetration and contrast compared to recent reports[3,4]. This increase in imaging depth, made possible by the BABB clearing and PAS staining, enables imaging of specimens that would ordinarily require a system with lightsheet capability for larger samples such as an ultramicroscope. The other reports listed above[3,4] make use of CLSM imaging to obtain morphological detail of their specimens. We sought to compliment this effort by using our approach of BABB clearing and PAS staining and followed by imaging with lightsheet. We obtained high contrast images such that it was possible to compare the glomeruli structures of the kidney specimen as well as the vasculature of the heart specimen. These results validate the approach of combining BABB clearing and PAS staining for LSFM imaging of whole-intact tissues. Obviously we are not limited to organic clearing methods. By isolating the imaging objective and sample chamber, any imaging medium could be used. The versatility afforded by this chamber greatly increases the range of specimens and protocols compatible with the Lighsheet Z.1 and can serve as a template for others trying to increase the functionality of a commercial LSFM system.

References:

[1] M Sivaguru *et al*, Biotechniques **59** (2015) p. 259.
[2] R Torres, S Versuna and M Levene, Arch Pathol Lab Med **138** (2014), p. 395.
[3] CE Miller *et al*, Microsc Microanal **11** (2005) p. 216
[4] RM Smith, A Matiukas, CW Zemlin, and AM Pertsov, Microsc Res Tech **71** (2008) p. 510

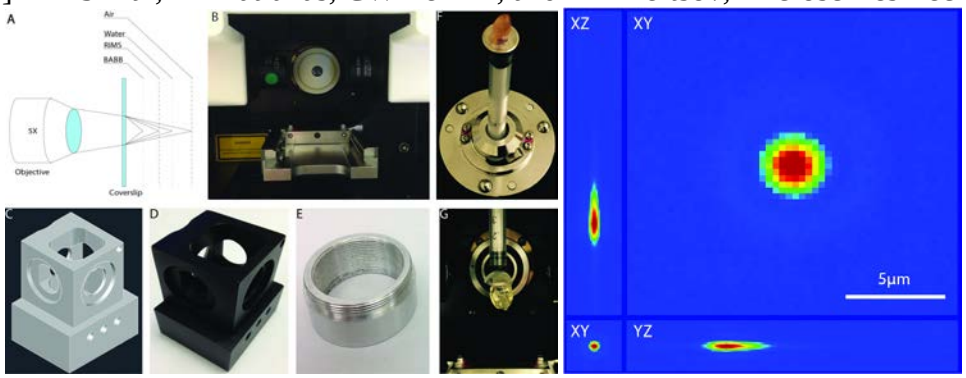

Figure 1. The Custom Lightsheet chamber with illustration of the change in focal plane of the objective based on the index of refraction of the sample media. Shown is the PSF for 5x objective with fluor-beads.

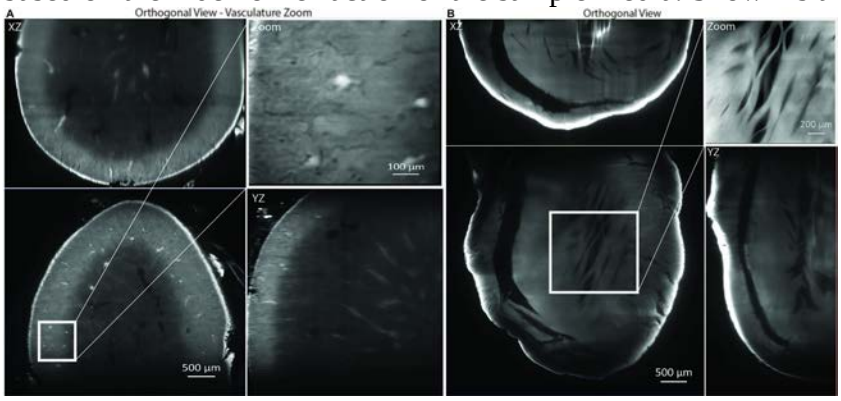

Figure 2. Whole mount imaging of BABB cleared and PAS labelled mouse kidney (A) and heart (B)

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