A NOVEL CROSS BEAM LIGHT SHEET MICROSCOPE FOR LARGE CLARIFIED TISSUE

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KEY WORDS: Light Sheet Microscope, Tissue Clearing, Expansion microscopy.

One of most popular application of light sheet microscopy is rapidly getting 3D volume of data from large clarified tissue or whole organ. With orthogonal arranged thin sheet illumination, light sheet microscopy have lowest photobleach and phototoxic compared with other microscopy technology. But the lateral thin sheet illumination also bring stripe artifact in light sheet acquired image. To decrease the stripe artifact in light sheet system, a lot of efforts have been made: using self-reconstructing beam (scanning bessel beam, airy beam, lattice light sheet), using pivot scan, using multiple-view, dual-side illumination or axially swept. All the these efforts lead to more complex light path design and/or more difficulties at hardware synchronization, light path alignment, data processing. Therefore, more complex hardware and customized software are required for these more advance light sheet system.

Here, we created a simple light sheet microscope with multi-beam multi-direction illumination and named it cross beam light sheet microscope. From single light source, we simply separated the laser beam with custom beam splitter that generated 12 rays arranged as a circle. We redirected the rays to sample plane as a large circle then changed the propagating direction to the circle’s center — also the sampling center of detection objective. Array of cylinder lens on sample chamber further shape the rays into thin sheets. Finally, the sample was illuminated from all direction in sample plane and a homogeneous illumination was created.

In detection part, we used the camera lens as objective and tube lens in microscope system. Camera lens with large f number like 50mm f1.2 or 85mm f1.2 were used as objective. Those camera lens provide us both large field of view and long working distance with relative large Numerical Aperture (about 0.5). A 75mm-300mm telescope zoom lens was used as tube lens that can change the zoom factor easily up to 4X difference.

We build a uniform illumination with multi-directions light sheet base on simple planar light sheet. Other advance light sheet strategy can also adapted to our system and instantly duplicated to all direction. Our system is currently controlled by Micromanager but other acquisition software with analog output ability can also be used. With simple optical design and no special controlling software required, the new cross beam light sheet microscope is easy to build, control and maintain. We hope the cross beam light sheet microscope can make light sheet more available and further strengthen the application of light sheet microscope.