Hybrid open-top light-sheet microscopy for high throughput 3D imaging of cleared tissues

Nicholas Reder (nick@alpenglowbiosciences.com), Alpenglow Biosciences, Inc., **Nicholas Reder**, Alpenglow Biosciences, **Steve Pemberton**, Alpenglow Biosciences, Inc., **Cody Cooper**, Alpenglow Biosciences, **Caleb Stoltzfus**, Alpenglow Biosciences, **Madalyn Hardy**, Alpenglow Biosciences, **Marina Bassina**, Alpenglow Biosciences, **Carl Storey**, Alpenglow Biosciences

Hybrid open-top light-sheet microscopy (OTLS) is an emerging microscopy technique well suited to the unique needs for 3D imaging of cleared tissues. The hybrid OTLS uses two collection systems: 1) a dual-axis light-sheet architecture in which the illumination and collection beams intersect at a crossing angle of 90 degrees, and 2) a non-orthogonal dual objective architecture where the collection objective is perpendicular to the sample interface, which allows for high NA objective and maximizes working distance. This geometry enables efficient imaging of the entire 3D volume of a specimen at low resolution and re-imaging of the same specimens at higher resolution without any adjustments to the system, i.e. “scout and zoom.” The unique hybrid open-top approach enables a substantial improvement in throughput and usability over prior technologies.The OTLS system has been used to scan whole mouse brains in less than 2 hours and find a single labeled neuron, image large expanded tissues, image thick multiplex-stained tumors in 3D, and visualize an entire human brain slice measuring 12 cm x 8 cm x 0.7 cm. When paired with cloud-based, parallelized, GPU-accelerated data processing, the system can produce terabytes of high quality data on a daily basis. This technological advance in 3D spatial biology can help unlock more information from intact tissue as part of a multi-assay pipeline in a variety of tissue types.