

ct-dSPIM imaging protocol

Citation for using this protocol:

Schueth, A., Hildebrand, S., Samarska, I., Sengupta, S., Kiessling, A., Herrler, A., ... & Roebroek, A. (2023). Efficient 3D light-sheet imaging of very large-scale optically cleared human brain and prostate tissue samples. *Communications Biology*, 6(1), 170.

Notes:

The ct-dSPIM microscope is aimed at 3D imaging of very large, cleared tissue samples with a thickness of up to 5 mm and a lateral size limited only by XY-stage travel and imaging time limits. It was derived from the diSPIM (dual view inverted Selective Plane Illumination Microscopy) system³¹ and co-developed together with Applied Scientific Instrumentation Inc. (ASI, Eugene, US). Any optically cleared human or animal tissue can be used for ct-dSPIM imaging.

Hardware and software specification:

Hardware

- Coherent obis, laser line 552nm LS 40mW LASER SYSTEM: FIBER PIGTAIL: FC
- electronically tunable (ETL) lens (C 60-TUNELENS-100, ASI)
- light-sheet “scanner” (MM-SCAN-1.2, ASI)
- 2D micro-electro-mechanical mirror (MEMS)
- multi-immersion illumination objective (#54-10-12, Special Optics/ Applied Scientific Instrumentation (ASI) with a refractive index (RI) range from 1.33 to 1.56 and a working distance (WD) of 12 mm
- polychroic mirror (ZT488/543/635rpc-UF2, Chroma) and a motorized filter wheel (FW) (FW-1000-8, ASI) with three emission filters (ET519/26m; ET576/31m; ET655lp, Chroma)
- sCMOS camera (ORCA-Flash4.0 V3, Hamamatsu)
- C60-TUBE-B, ASI; f=200 mm
- motorized XY stage (MS-8000, scan-optimized)
- ASI tiger controller (TG-1000)
- For additional info see <https://www.asiimaging.com/products/light-sheet-microscopy/dual-selective-plane-illumination-microscopy-for-cleared-tissue/#tab-id-7>

Software

- microscope is controlled by μ Manager (<https://micro-manager.org/>, here you can download new nightly builds), a free open-source microscope control software
- The ASI diSPIM plug-in in μ Manager is used to both align the microscope and to setup and perform acquisitions

Image acquisition:

	Isotropic Resolution	Speed	Slice step
Mosaic 16	16.4 μm	1.7hrs/1cm ³	11.60 μm
Mosaic 4	4.1 μm	5 hrs/1cm ³	2.90 μm
Mosaic 0.5*	0.5 μm * (0.725 μm x 0.5127 μm x 0.5127 μm)	15.8 hrs/1cm ³	0.363 μm

Table 1. Resolution, imaging speed and slice step for Mosaic 16, 4 and 0.5 ct-dSPIM scans, respectively

Other useful resources:

<http://dispim.org/> Wiki page for all things dispim

<https://www.asiimaging.com/support/> for ASI hardware and software specific questions and downloads

<https://www.asiimaging.com/products/light-sheet-microscopy/dual-selective-plane-illumination-microscopy-for-cleared-tissue/> very specific information and specs on the ct-dSPIM

References:

Schuetz and Hildebrand et al, *Communications Biology* (2023), <https://doi.org/10.1038/s42003-023-04536-4>