

MosaicExplorerJ: Interactive Stitching of Terabyte-size Tiled Datasets from Lightsheet Microscopy

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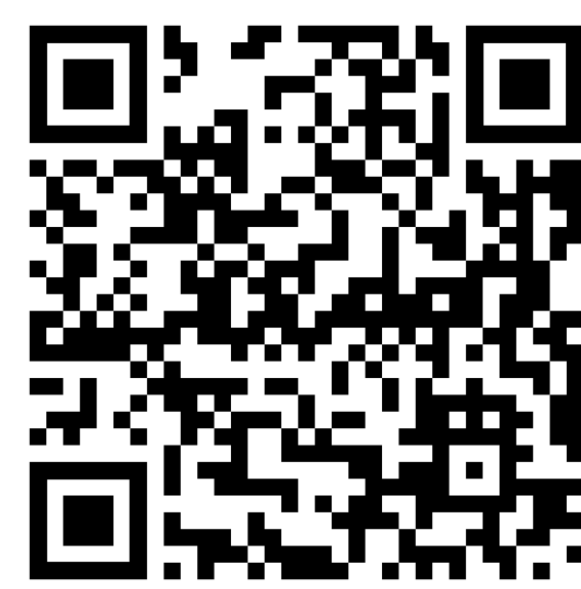
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Software, Documentation & Datasets:

<https://github.com/SebastienTs/MosaicExplorerJ>

Article:

<https://doi.org/10.12688/f1000research.27112.1>



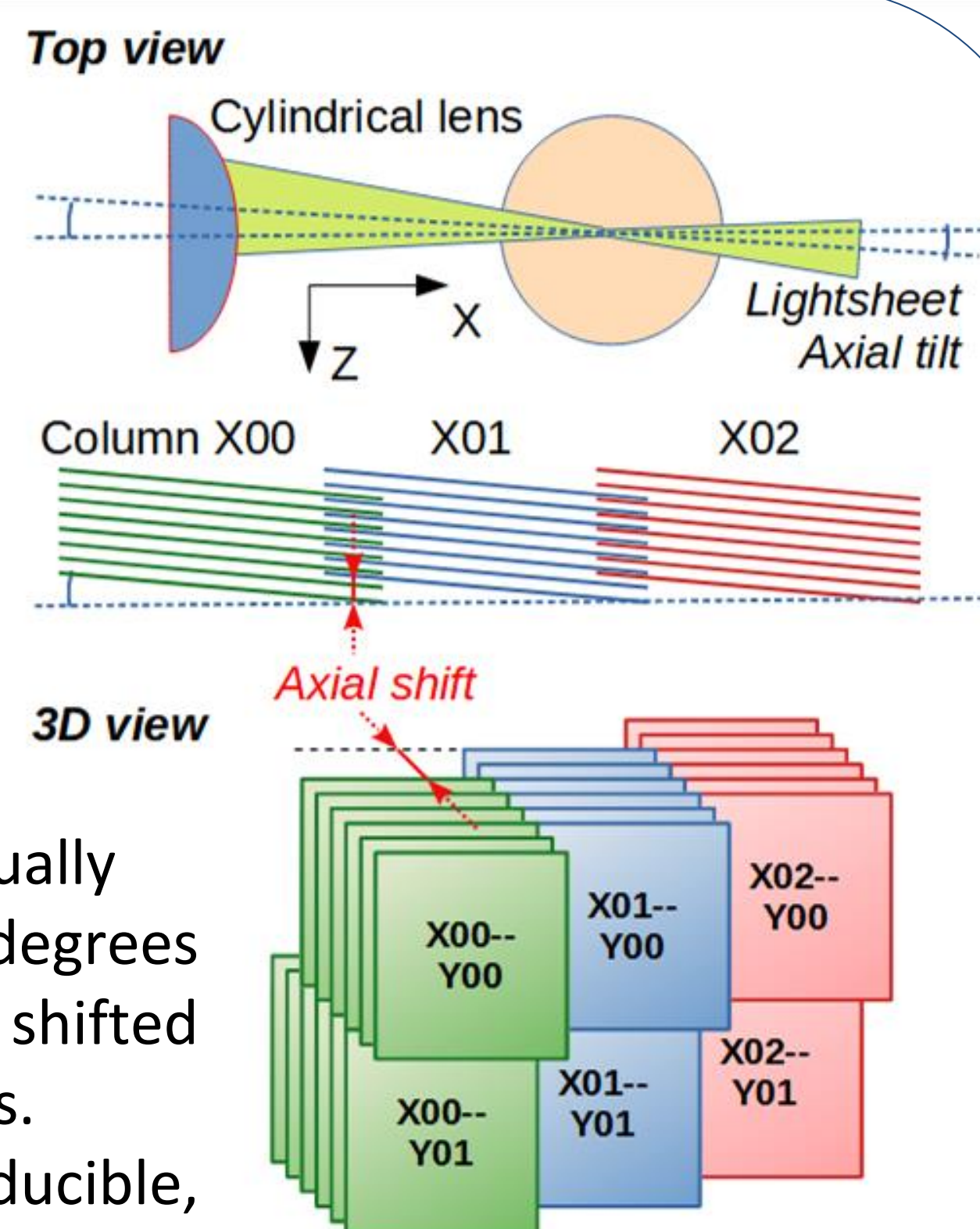
ABSTRACT

MosaicExplorerJ is an ImageJ script to align and stitch three-dimensional tiles from terabyte-size microscopy datasets. As opposed to existing solutions, aligning the tiles does not require any prior information on their actual physical positions, sample fiducials, or data conversion. MosaicExplorerJ was specifically designed to process lightsheet microscopy datasets from optically cleared samples [1]. It can handle multiple fluorescence channels, dual-side lightsheet illumination and dual-side camera detection. Sparse datasets are also supported, for instance to monitor data acquisition, or if specific sample regions have not been imaged.

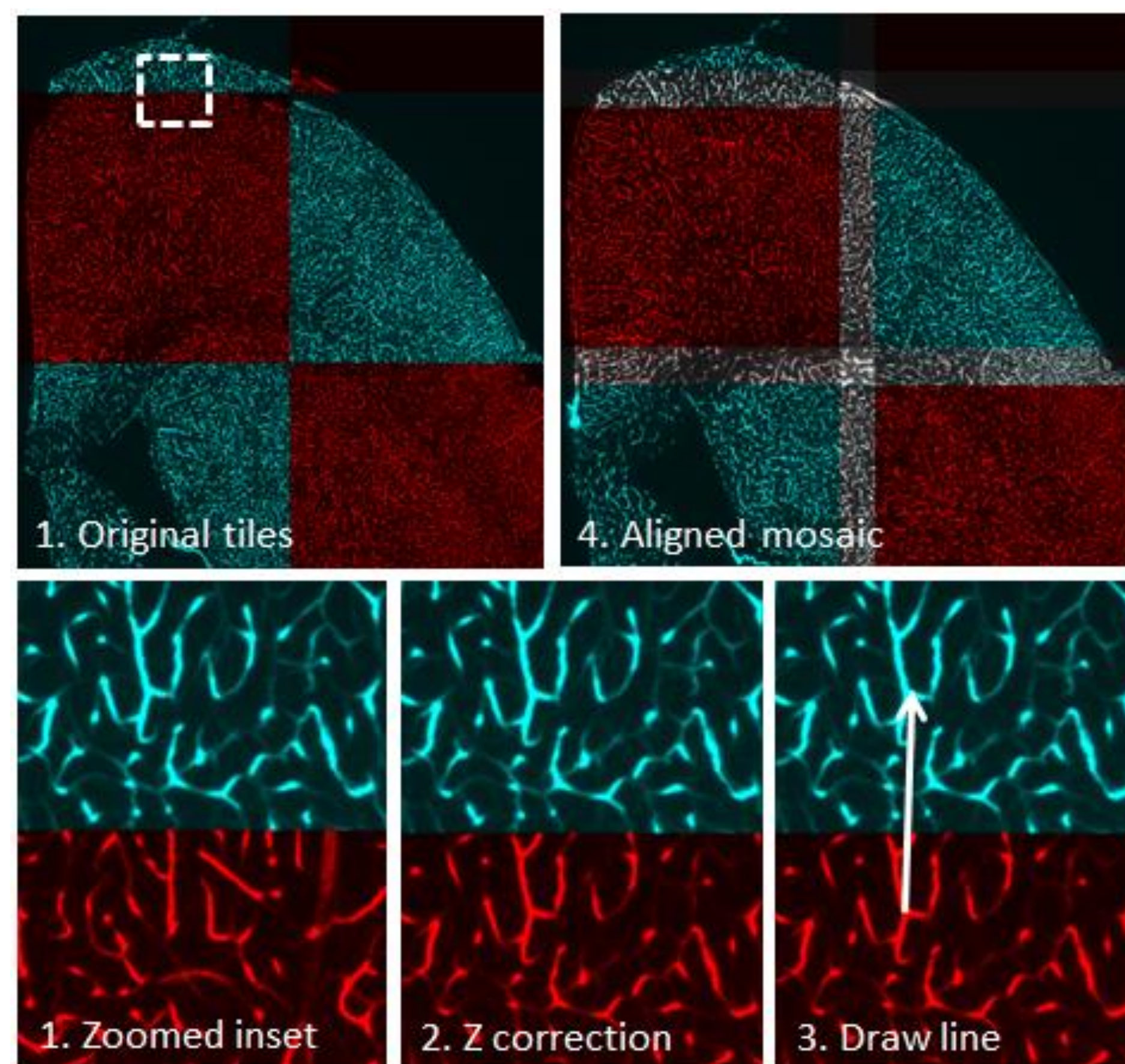
Challenges when aligning lightsheet mosaics

- Lightsheets can be tilted / focal plane (and sample movement axes)
- Motor axes can suffer axial wobbling
- Lightsheet non-uniformity (or lateral misalignment) → differences in regions of tile overlap.

MosaicExplorerJ assists the user in visually aligning the tiles along their possible degrees of freedom. First, the tiles are axially shifted to compensate for the first two effects. Since motor defects are usually reproducible, a XY separable correction is typically applied (shifts adjusted across first column / row).



User Interface and alignment workflow



Dataset exploration

ZPos

CPos

MaxInt

CAM

Side

Stitch Mode Tiles Fusion

OvlX (%)

OvlY (%) Tiles XY alignment

XCor

YCor

ZxyCor (Camera Common)

ZxCor Tiles Z correction

ZyCor

BigSteps

Color mode

Free Zxy Correction

Grid+CAM+LR Panel CAM/LR

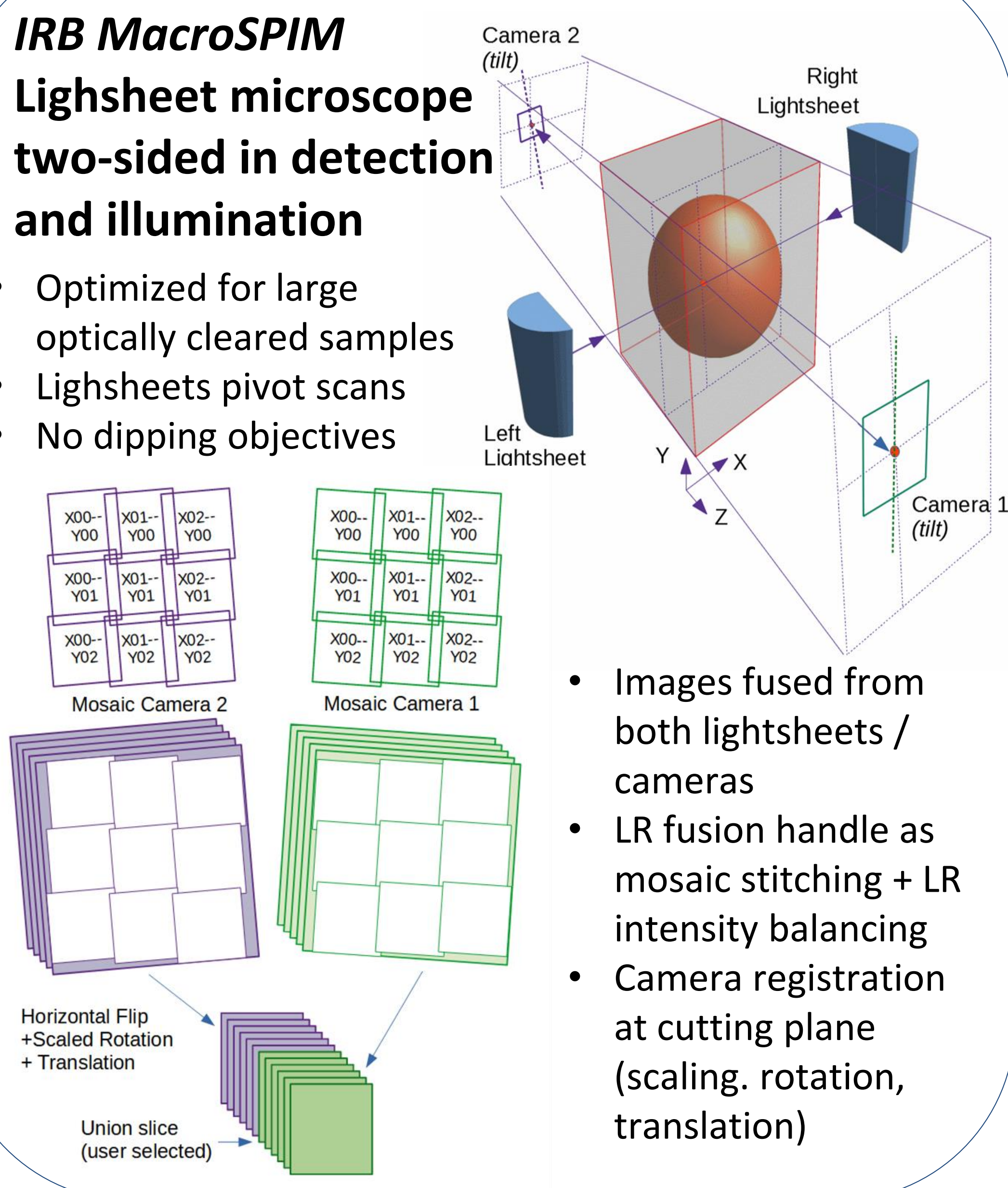
Export stack

Exit

IRB MacroSPIM

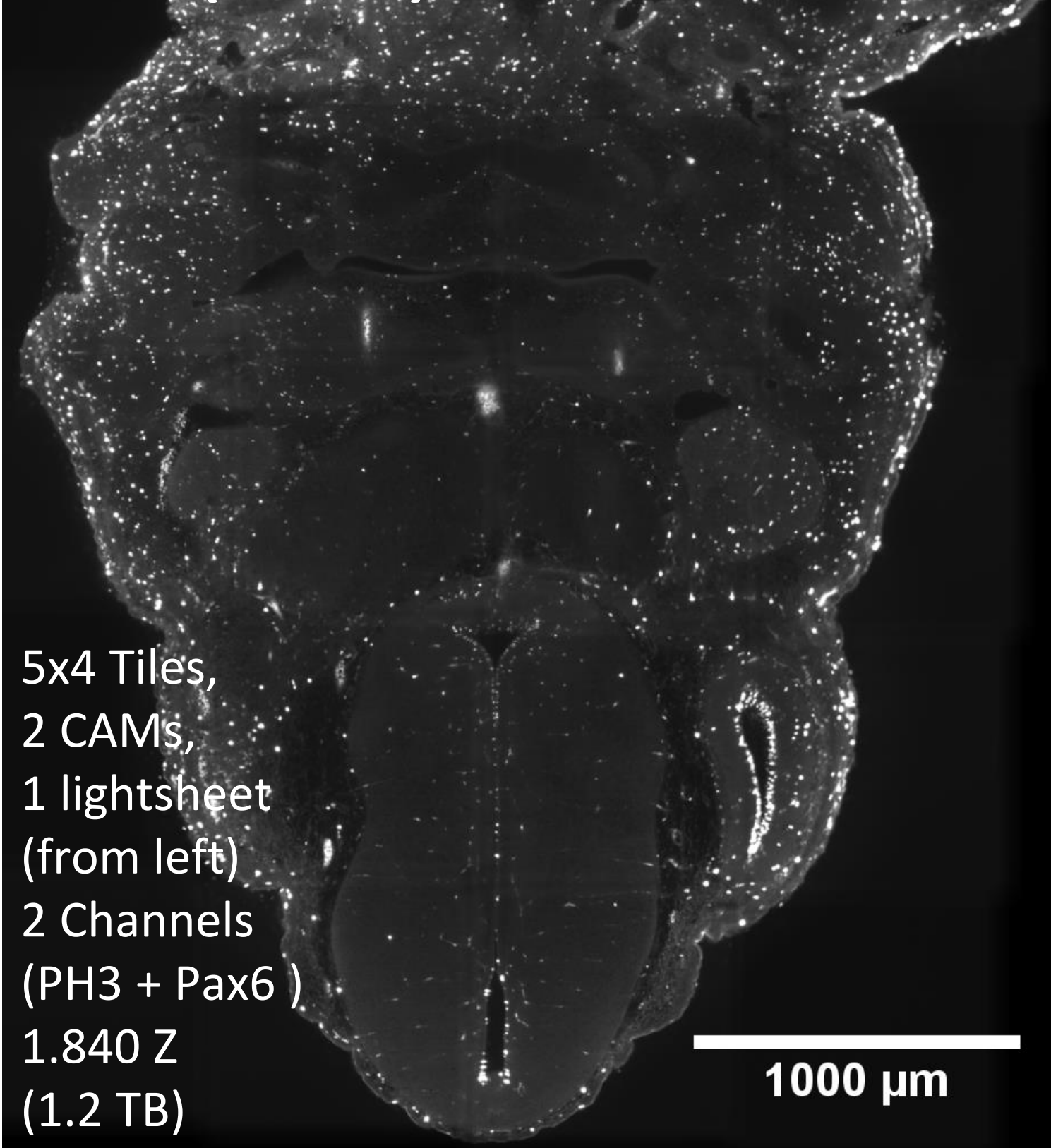
Lightsheet microscope two-sided in detection and illumination

- Optimized for large optically cleared samples
- Lightsheets pivot scans
- No dipping objectives



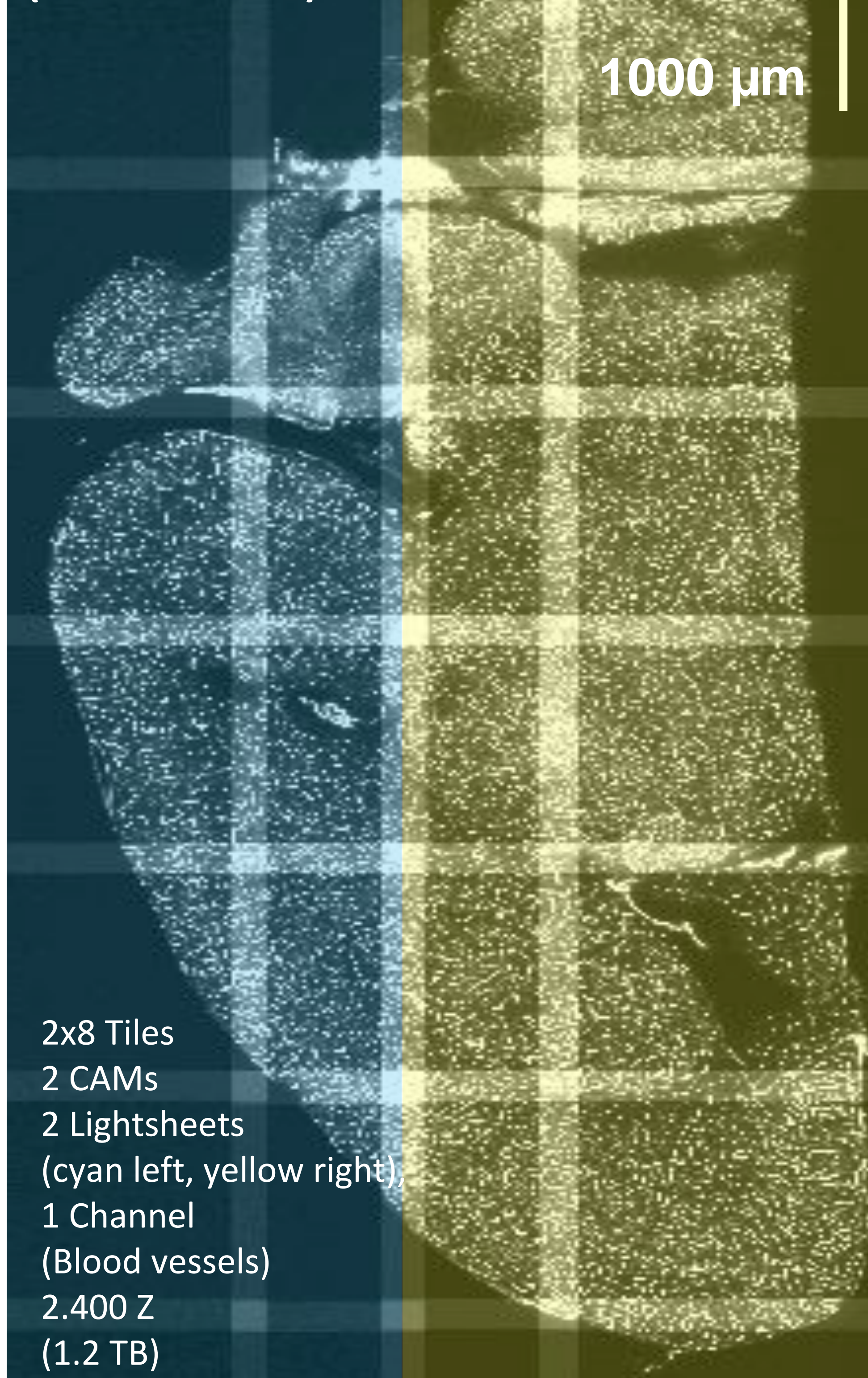
- Images fused from both lightsheets / cameras
- LR fusion handle as mosaic stitching + LR intensity balancing
- Camera registration at cutting plane (scaling, rotation, translation)

Mice embryonic head (nuclei)



5x4 Tiles,
2 CAMs,
1 lightsheet
(from left)
2 Channels
(PH3 + Pax6)
1.840 Z
(1.2 TB)

Mice brain (blood vessels)



2x8 Tiles
2 CAMs
2 Lightsheets
(cyan left, yellow right),
1 Channel
(Blood vessels)
2.400 Z
(1.2 TB)

Comparison with IJ BigStitcher [2]

Features	Mosaic ExplorerJ	Big Stitcher
2D TIFF series / 3D TIFF tiles	YES	NO
Dual-camera	YES	NO
Multi-angle views	NO	YES
Model constrained alignment	YES	NO
Automated alignment	NO	YES
Automatic tile coloring	YES	NO
Tile correlation metrics	NO	YES
Intensity correction	Scaling	Flat field

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REFERENCES

- [1] Dodt, HU., Leischner, U., Schierloh, A. *et al.* Ultramicroscopy: three-dimensional visualization of neuronal networks in the whole mouse brain. *Nat Methods* **4**, 331–336 (2007).
- [2] Hörl, D., Rojas Rusak, F., Preusser, F. *et al.* BigStitcher: reconstructing high-resolution image datasets of cleared and expanded samples. *Nat Methods* **16**, 870–874, 2019.