Data Processing for Serial Sectioning Microscopy of Neural and Vascular Microstructure

MSM WG3 Discussion

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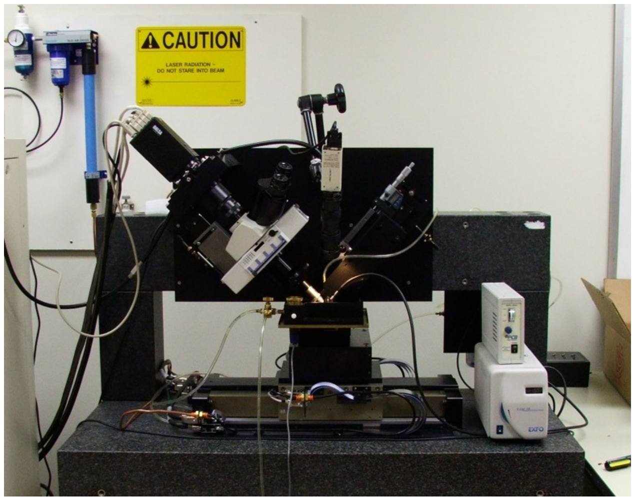
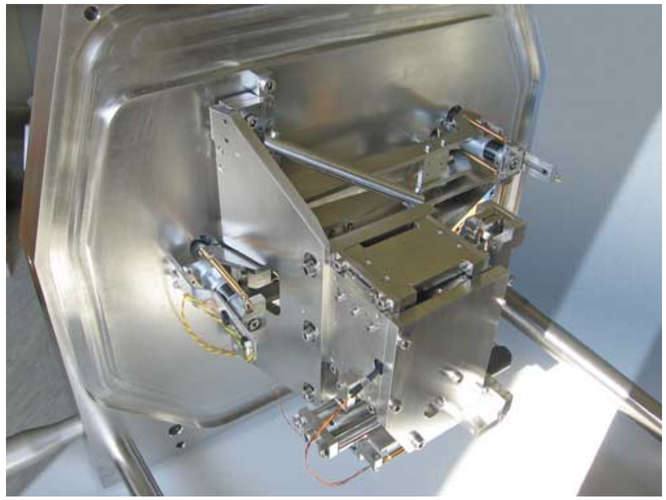
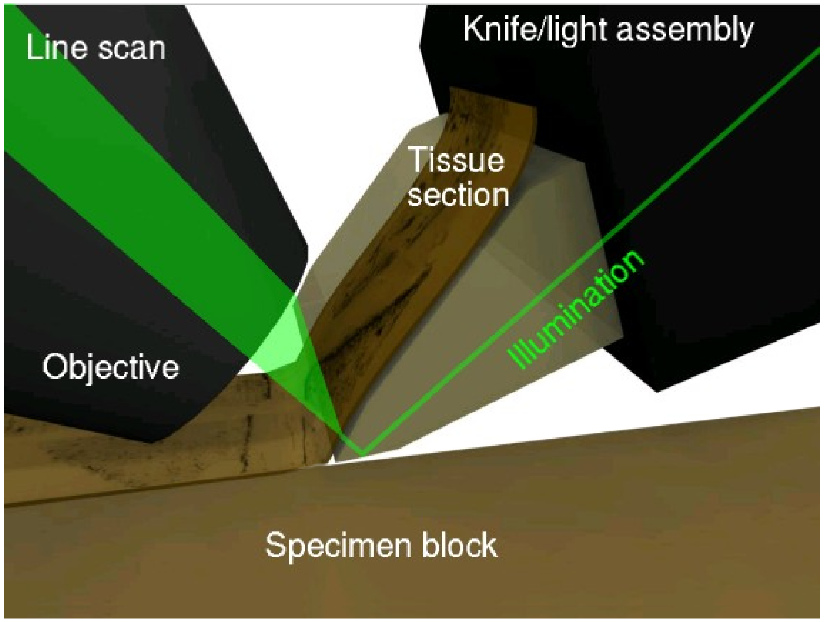
Project Overview: Mouse Brain Microstructure

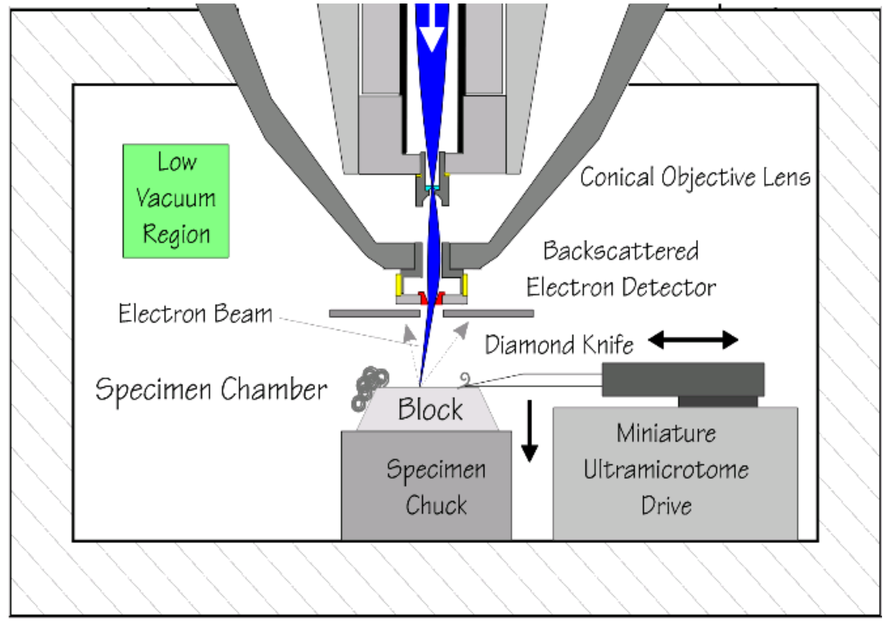
Micro	Nano	Macro
		
KESM, Optical, 0.3–0.6 µm Texas A&M	* SBF-SEM, EM, 20–30 nm Stanford	† MRM, MR, ∼60 µm UCLA

- Surveying mouse brain microstructures
- Three imaging modalities, three scales, one hard problem.
- Imaging effort focused on KESM, and SBF-SEM (+ Array Tomography).

* SBF-SEM image source: Gatan http://www.gatan.com/sem/3view.php

KESM and SBF-SEM: Serial-Sectioning Imaging



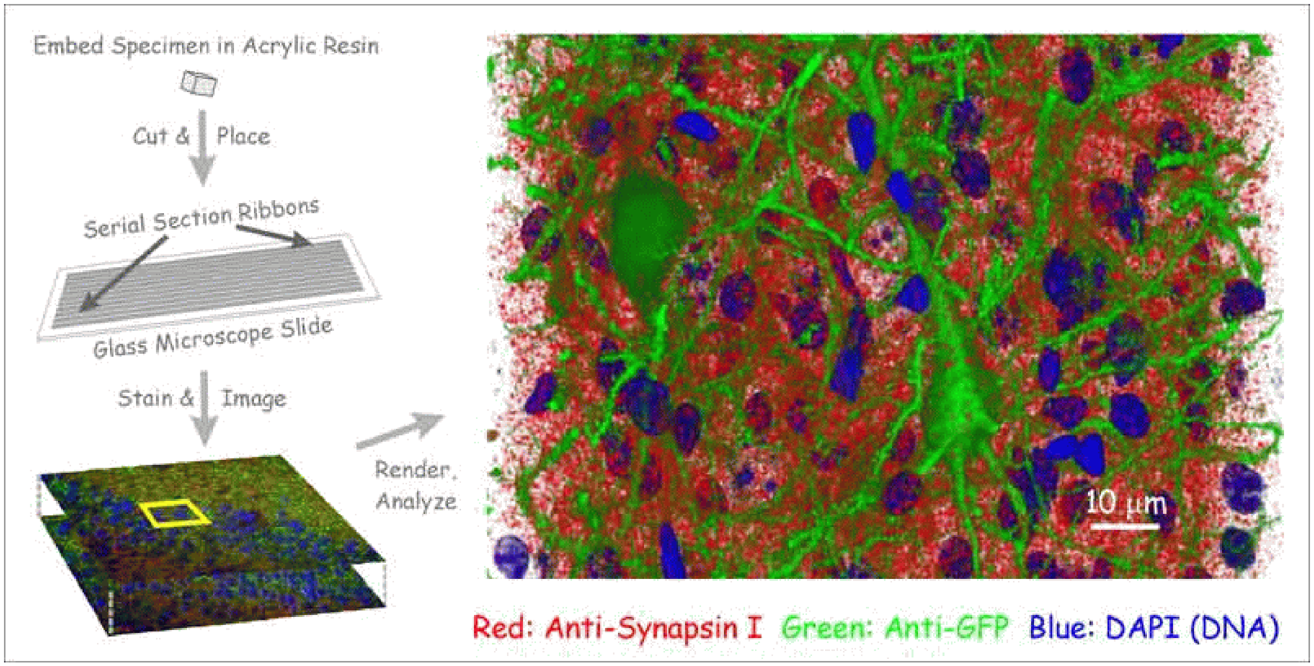


KESM

SBF-SEM

- Serial, physical sectioning for high z-axis resolution.
- Immune to blurring artifacts in optical sectioning such as confocal microscopy or multiphoton microscopy.

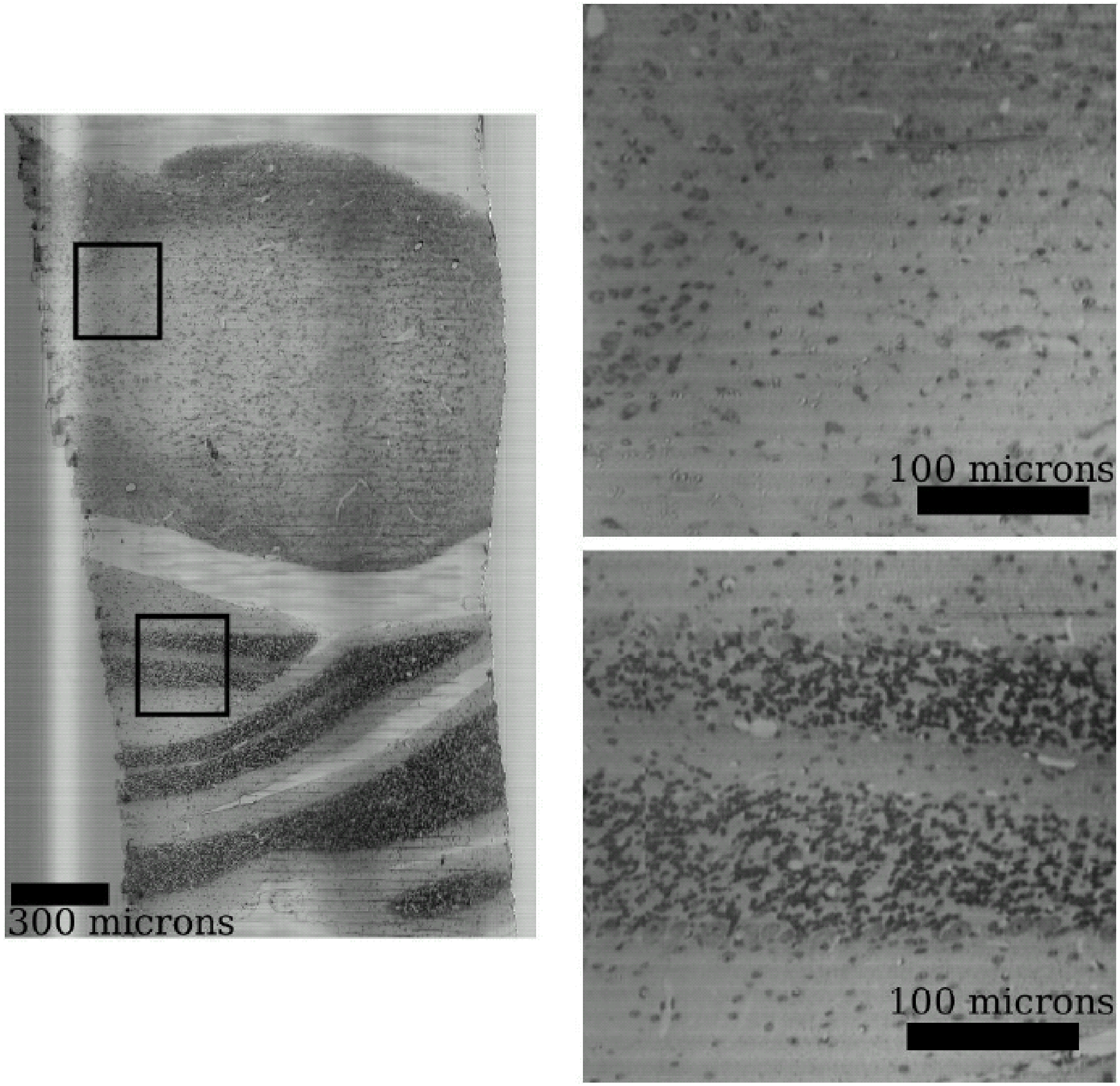
Array Tomography: Multimodal Serial-Sectioning Imaging



Micheva and Smith (2007)

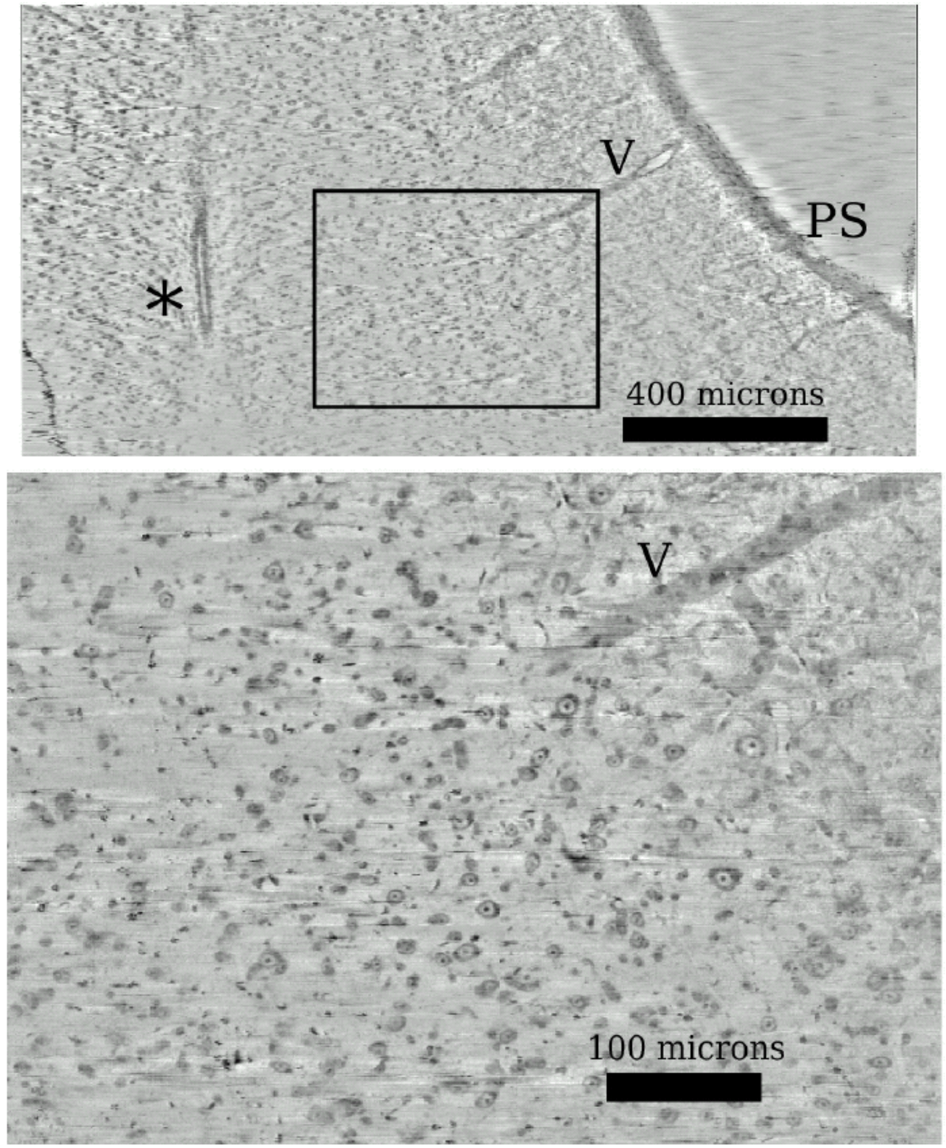
- Serial sections precisely aligned on microscoe slide.
- Multiple fluorescent markers imaged at a time.
- EM as a final step to register ultrastructure to molecular markers.

Typical Data: Nissl Stain, KESM



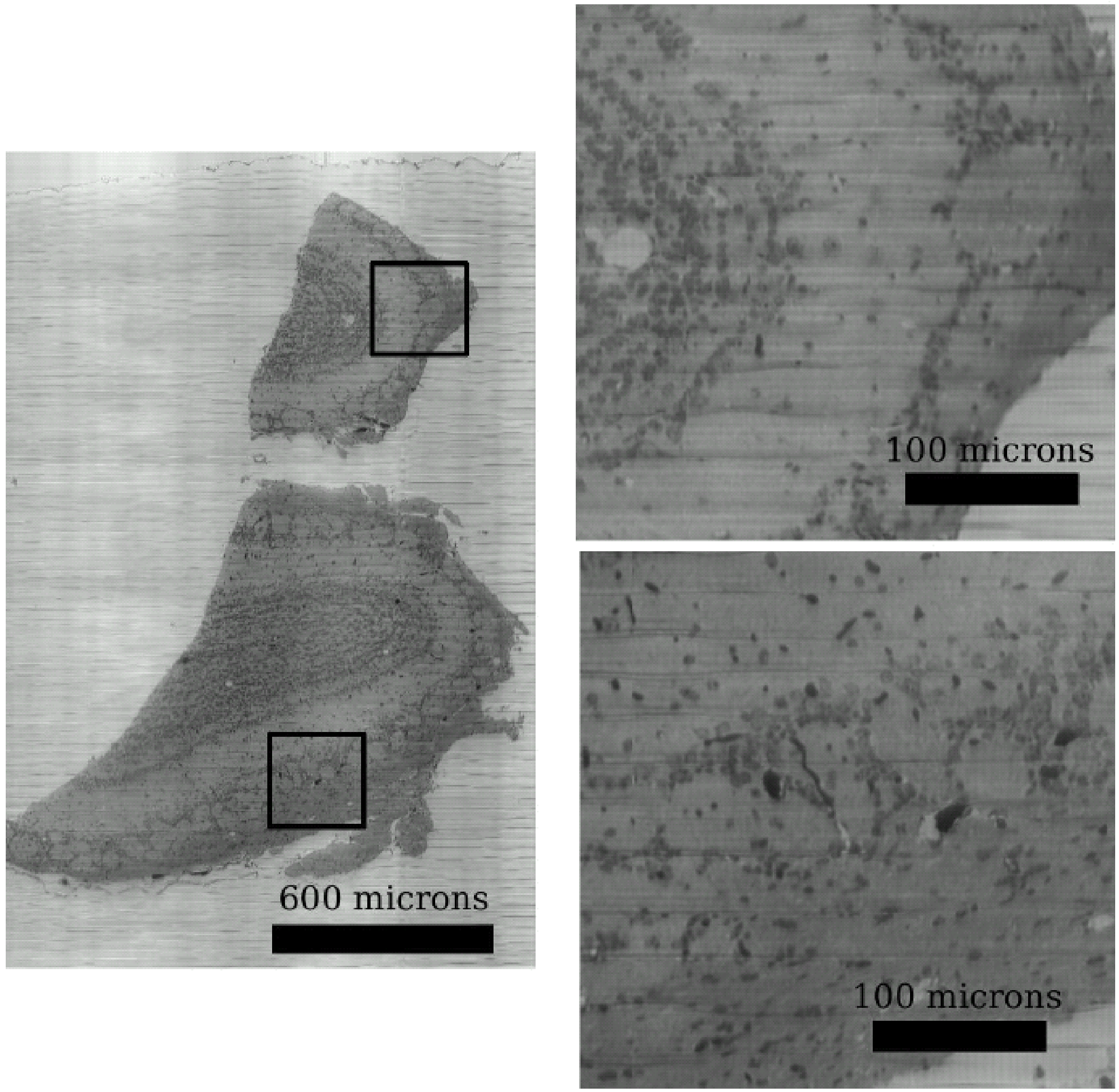
- Mouse brain stem and cerebellum.

Typical Data: Nissl Stain, KESM



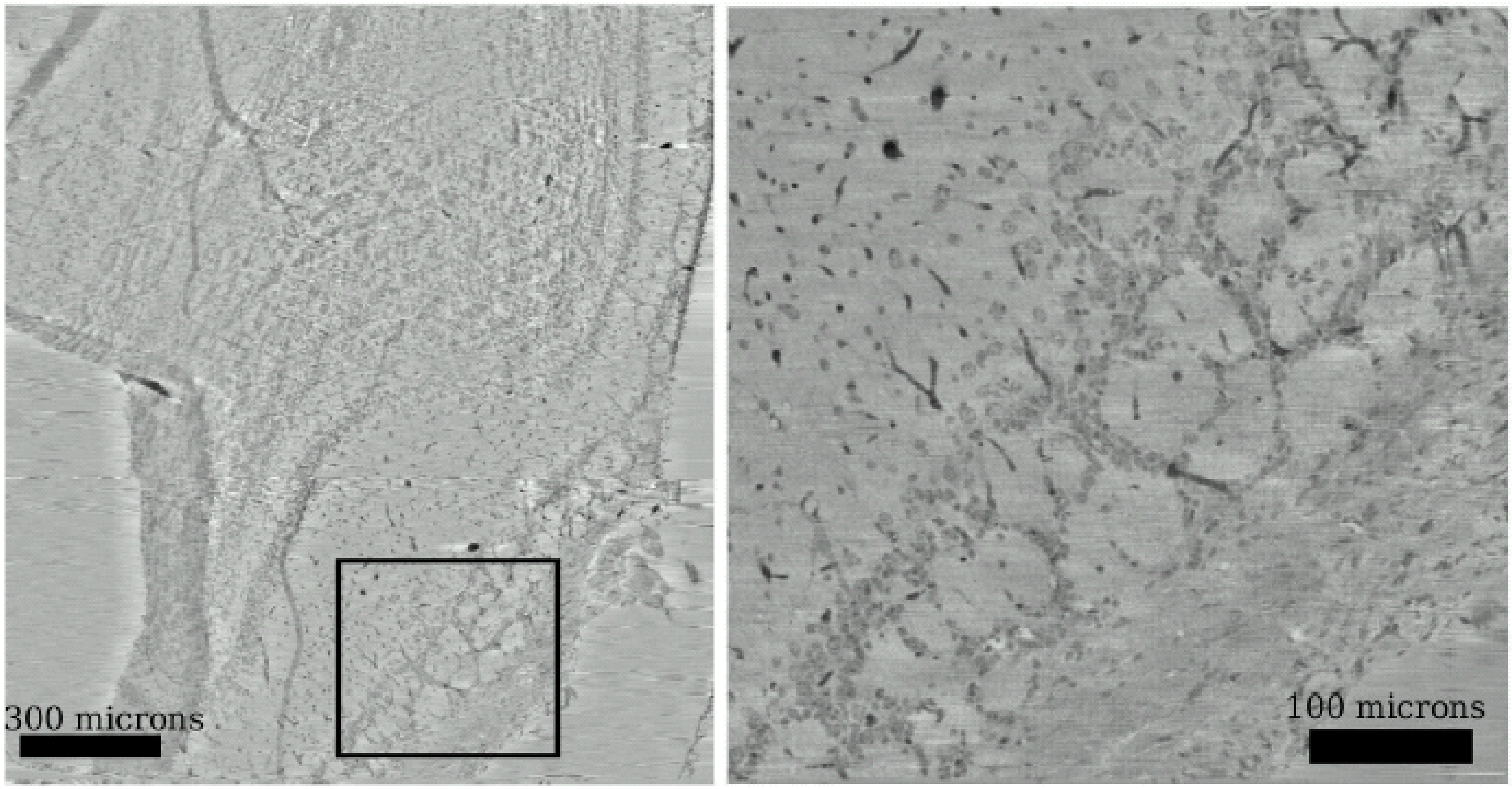
- Cross section of mouse brain stem and spinal cord (PS: pial surface; *: central canal; V: blood vessel).

Typical Data: Nissl Stain, KESM



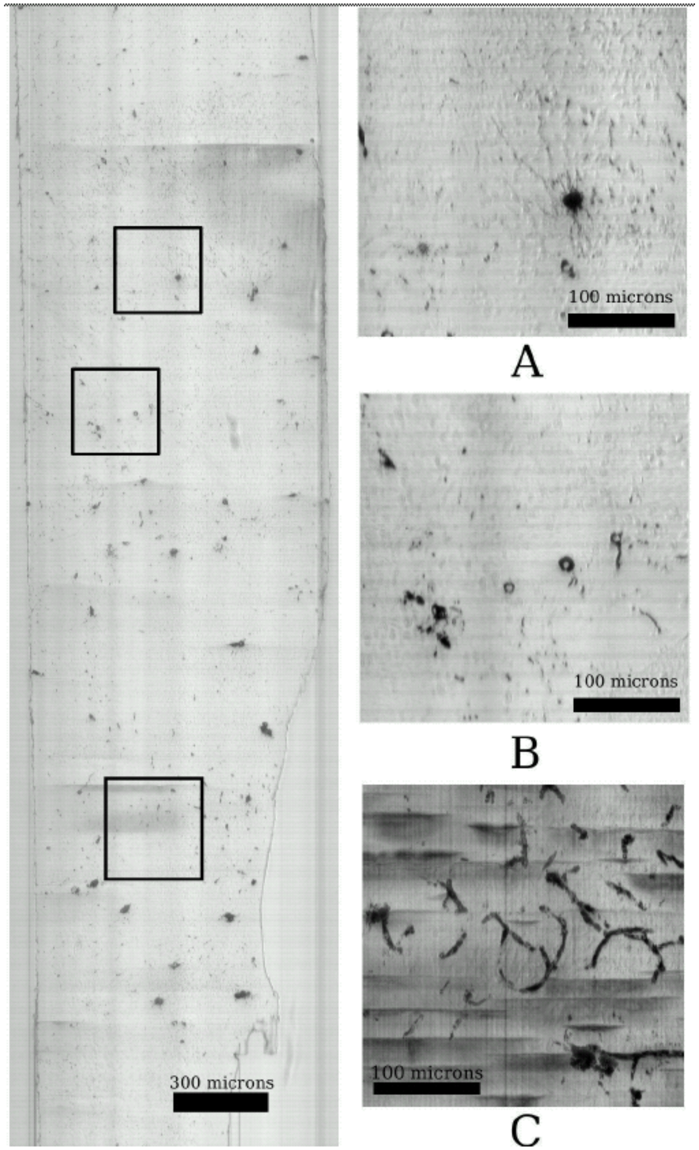
- Mouse olfactory bulb.

Typical Data: Nissl Stain, KESM



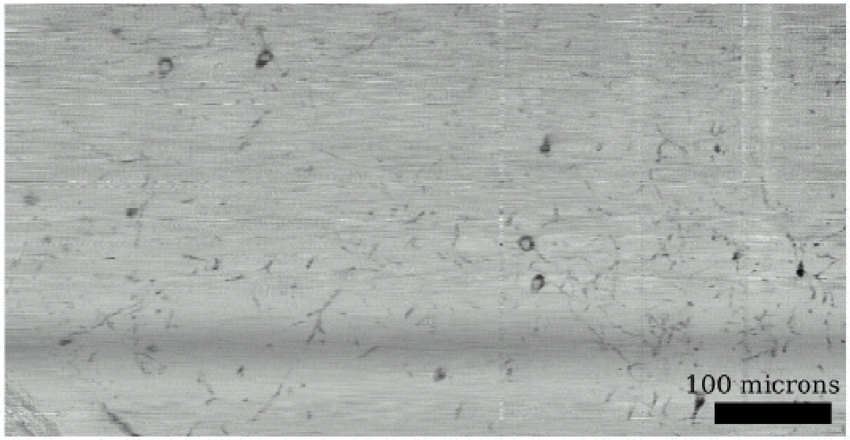
- Mouse olfactory bulb (vasculature stained with india ink).

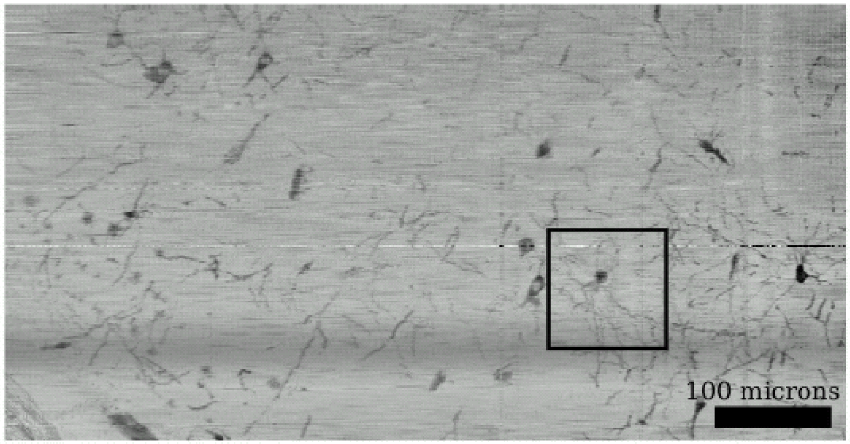
Typical Data: Golgi Stain, KESM

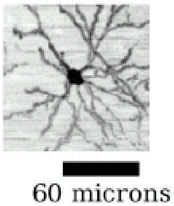


- Mouse cortex.

Typical Data: Golgi Stain, KESM

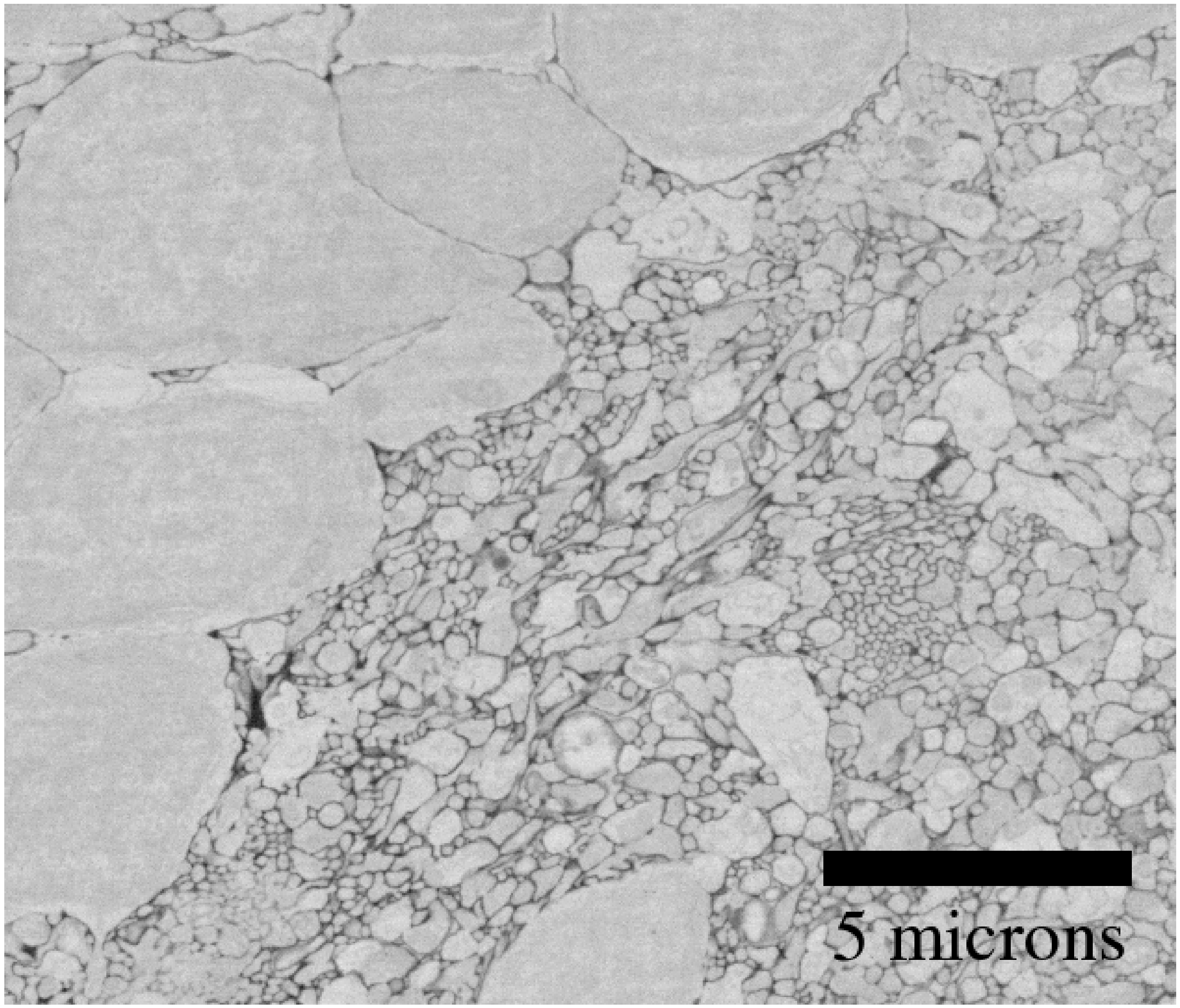






- Mouse cortex (sagital section).

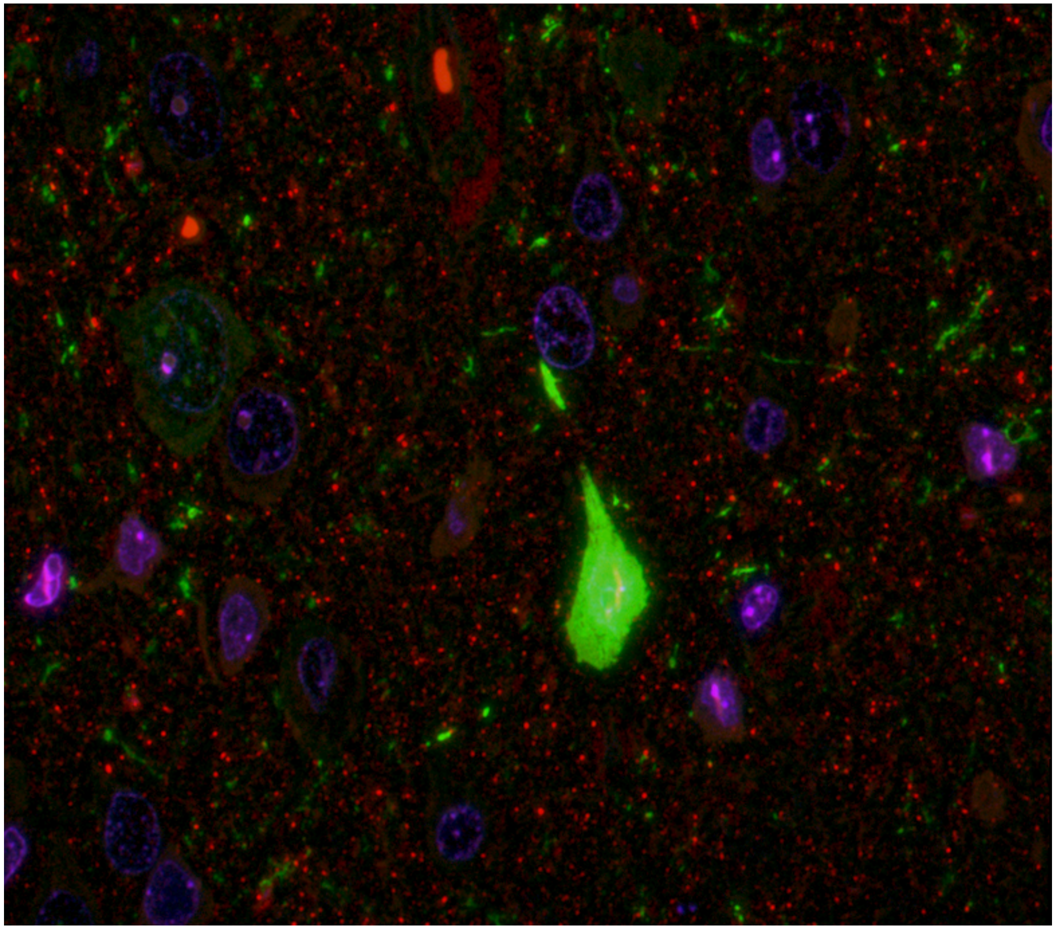
Typical Data: Heavy-metal Stain, SBF-SEM



Zebra fish tectum

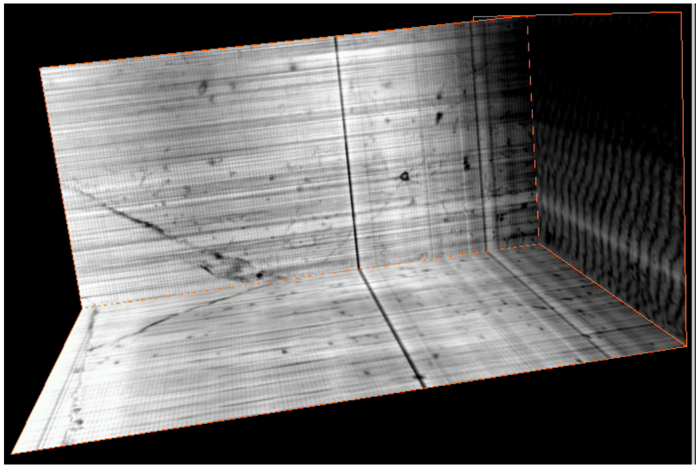
- Heavy-metal staining of extracellular matrix.

Typical Data: Array Tomography

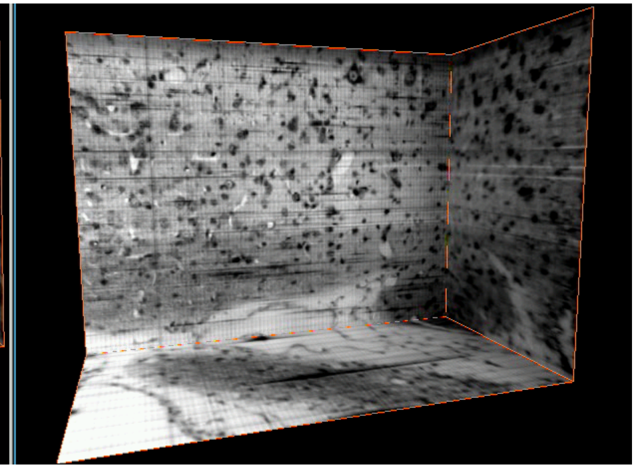


- Blue: DAPI-DNA (cell nuclei); Red: Anti-synapsin I (synapses); Green: FITC anti-GFP (neuron morphology); Scalebar: 10 µm.

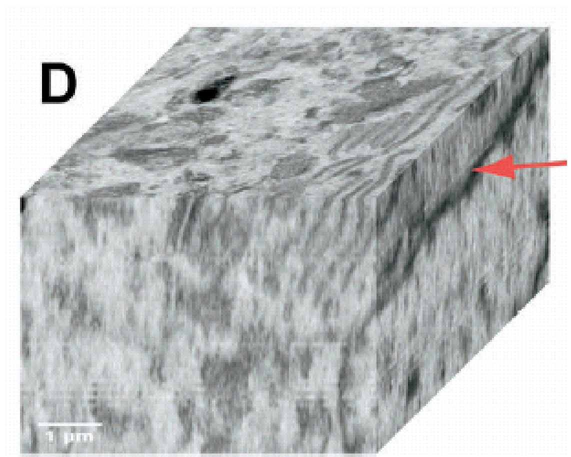
Typical Data Voulmes



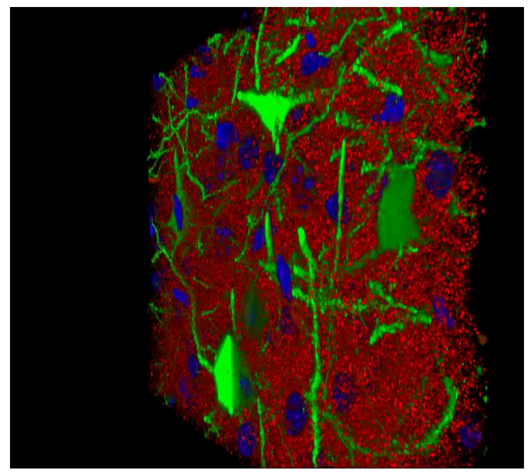
KESM Golgi



KESM Nissl



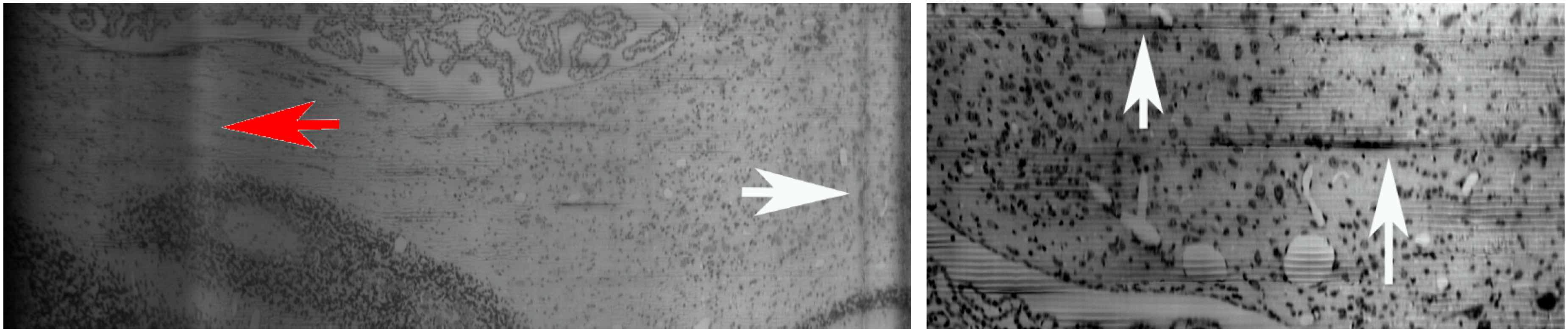
SBF-SEM (Denk and Horstmann 2004)



Array Tomography

- A stack of images are obtained, representing a volume of brain tissue.

Noise in Obtained Image Data

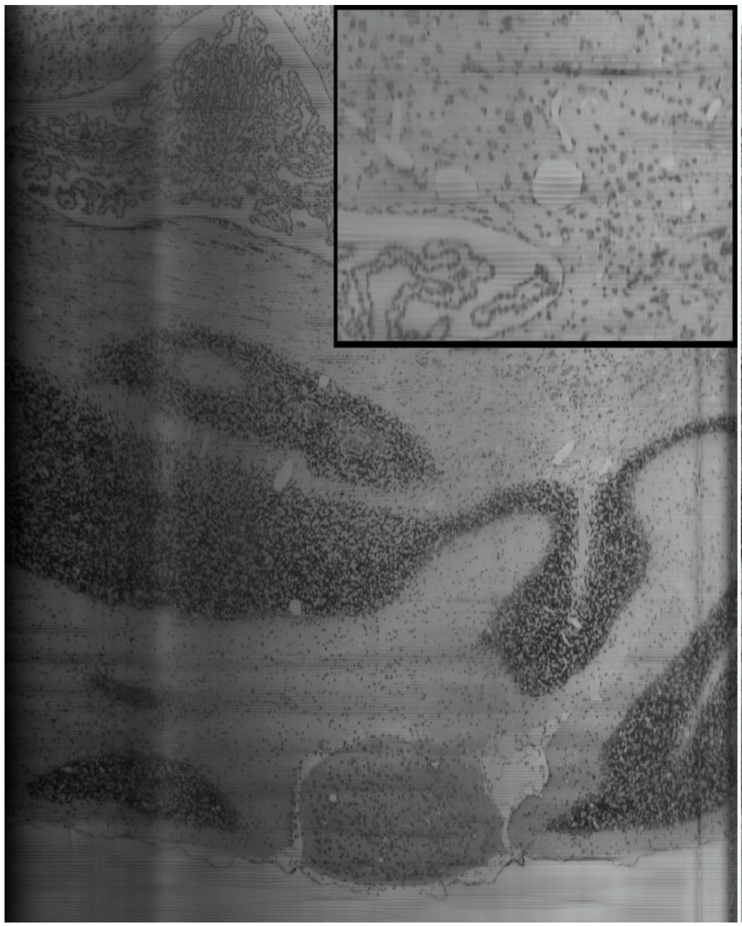


The source of noise are typically:

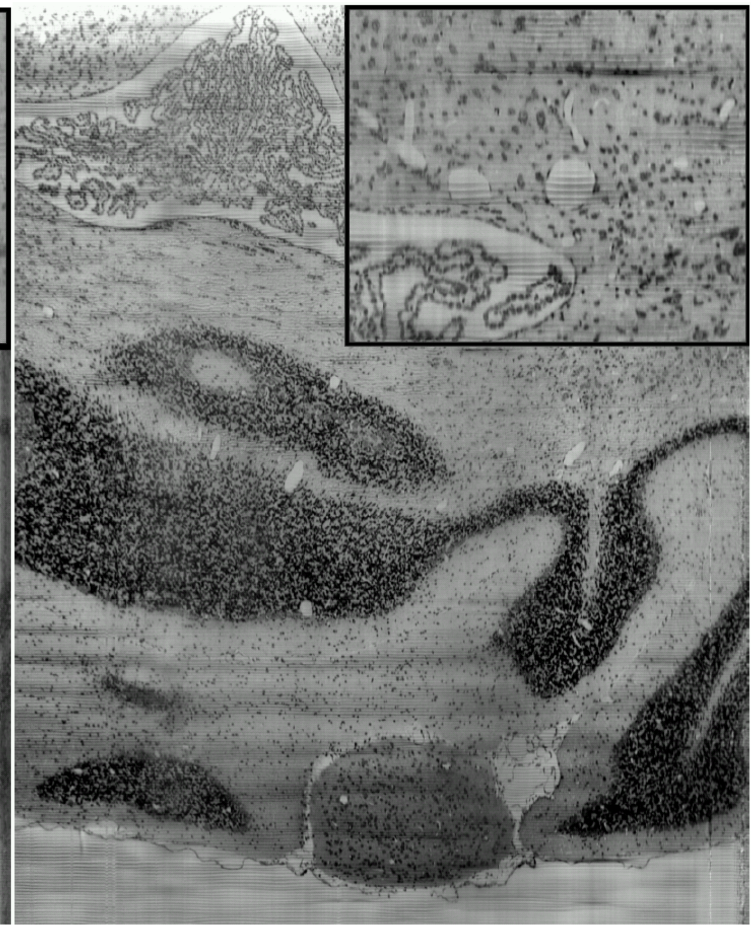
- Optical/illuminance irregularities (left, red arrow)
- Knife defects (left, white arrow)
- Chatter (undesired vibration) in cutting process (right)

No other random noise, optical noise (blurring of background), or registration issues. Some issues with quality of staining.

Fixing Optical/Illuminance Irregularities and Knife Defect



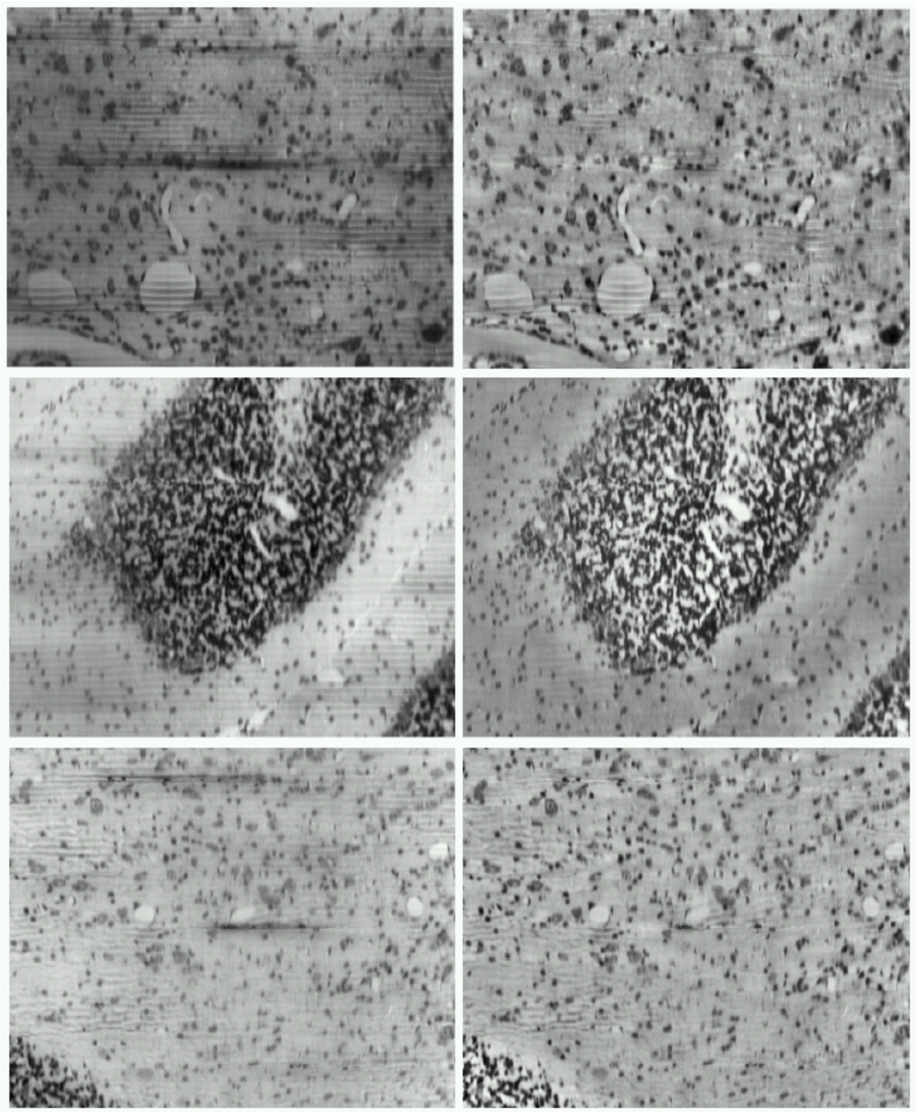
Before



After

- The irregular bands are also present in blank runs, so images obtained from the blank run are used to subtract out these irregularities.

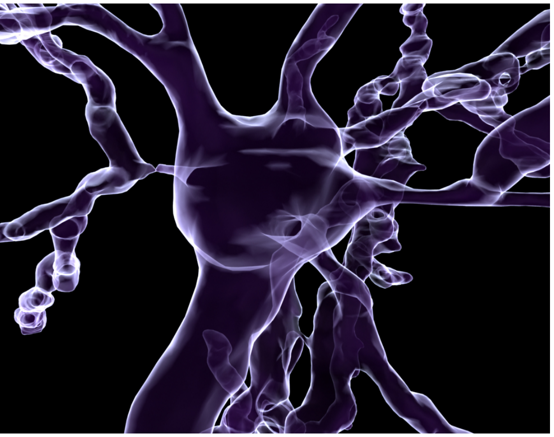
Fixing Knife Chatter Artifacts



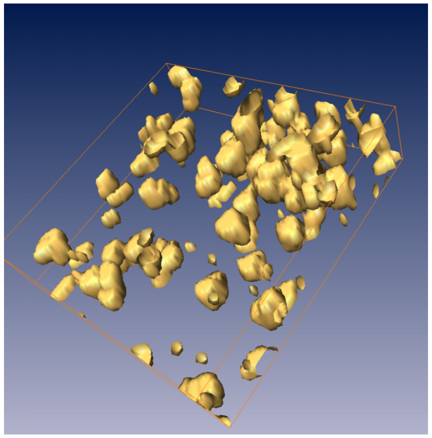
Before After

- A small rectangular sliding window is used to find local mean intensity which is then used to scale the pixel value at the center of the window.

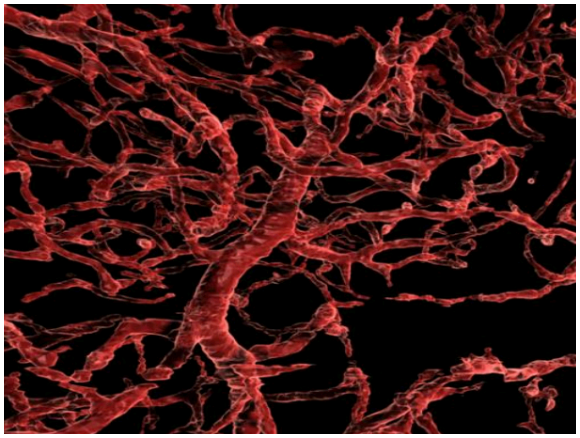
3D Reconstruction



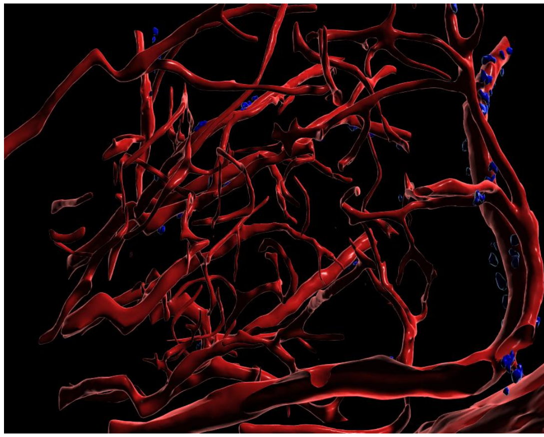
Golgi (Neuron)



Nissl (Soma)



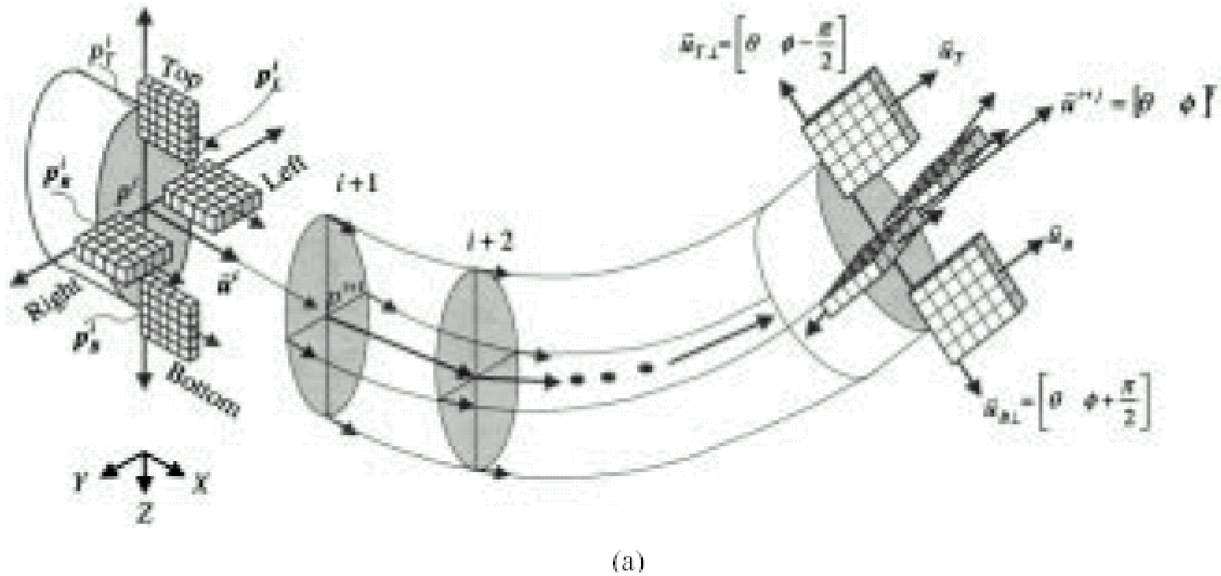
Nissl (Vasculature)



Nissl (Vasculature)

- Thresholding and iso-surface reconstruction can be used for sparse data.
- This approach does not work well for densly packed objects.
- This approach does not result in morphological models.

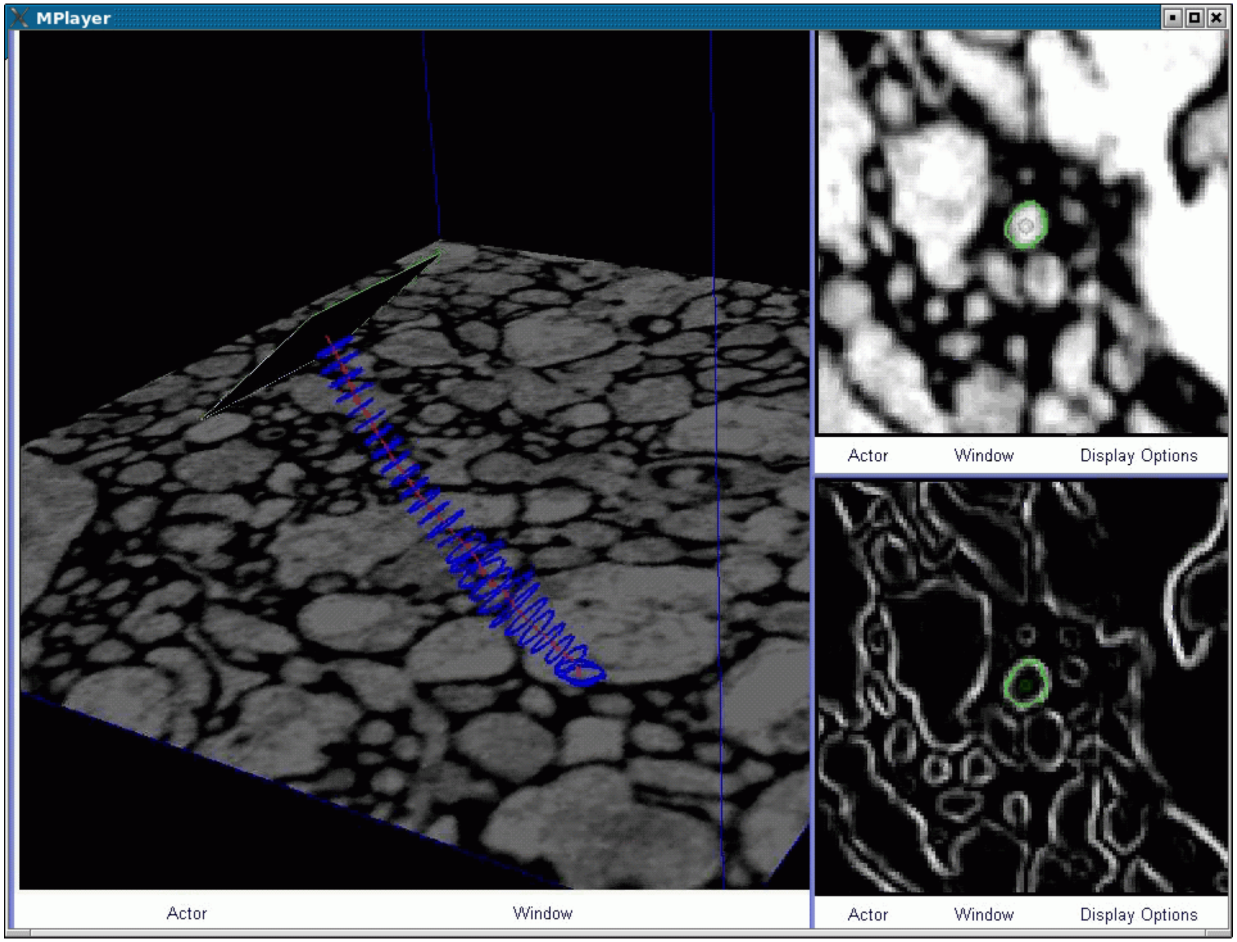
3D Reconstruction: Vector Tracing



(Al-Kofahi et al. 2002)

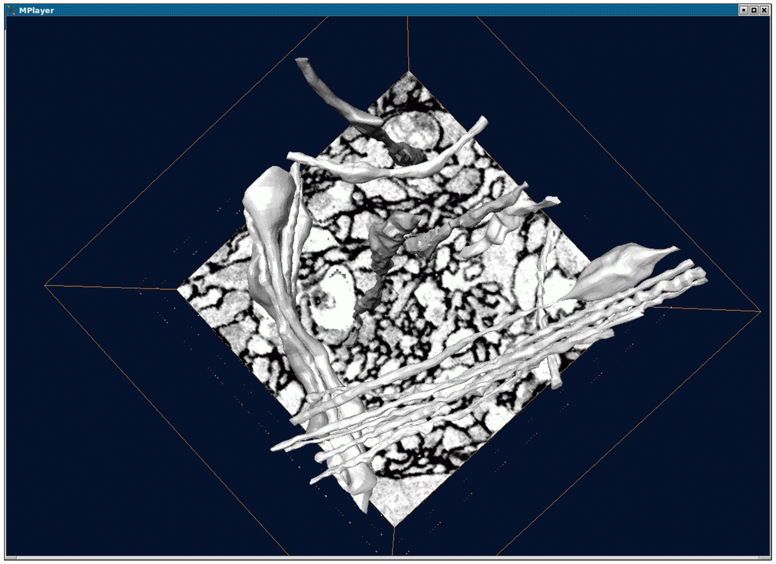
- Template-based vector tracing algorithm: computationally efficient.
- Templates are like orientable paddles (yaw, pitch, roll, translation, and distance from central axis).

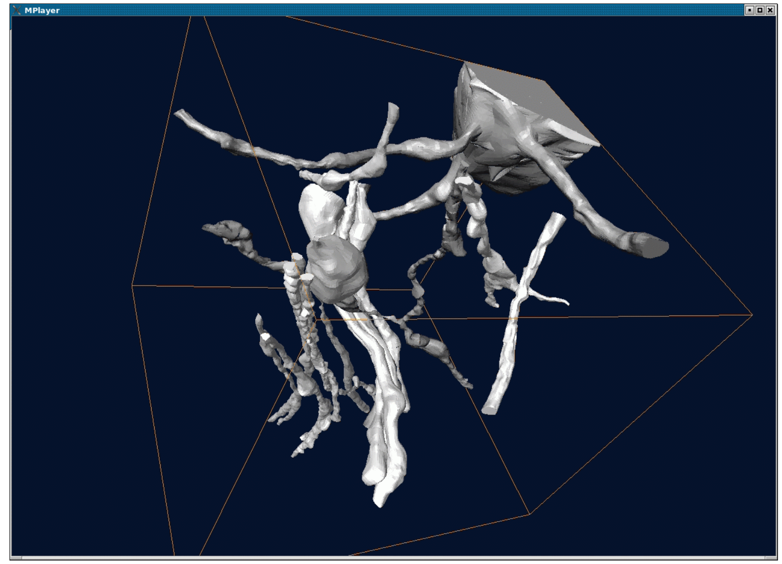
3D Reconstruction: Vector Tracing



- Virtual sectioning along plane perpendicular to fiber path (local coordinate system), used with vector tracing algorithm. Level-set methods for segmentation.

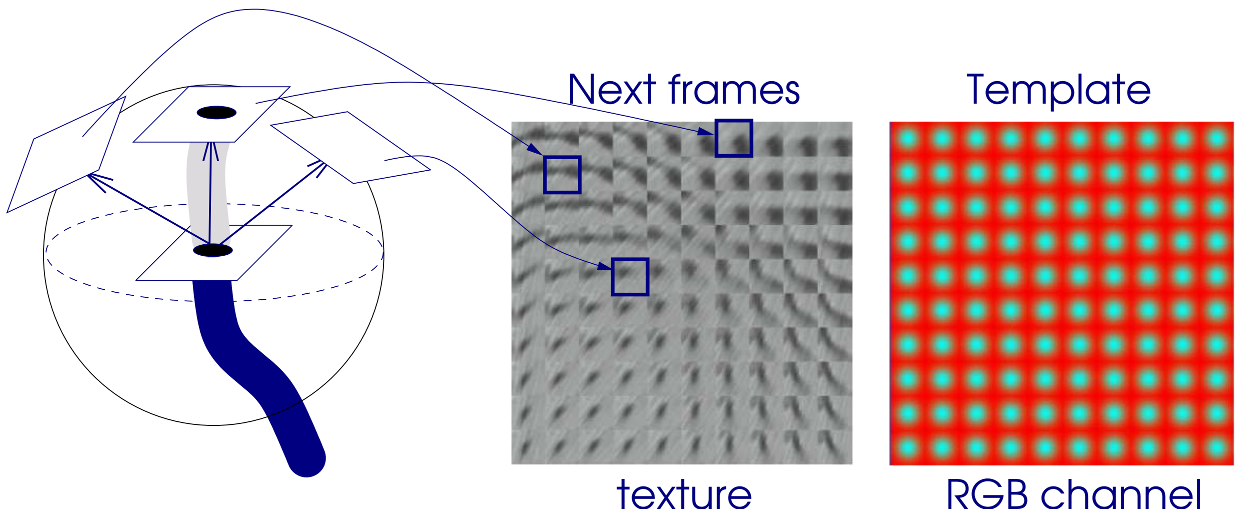
3D Reconstruction: Vector Tracing Results





- Partial reconstruction of zebra fish data is shown.
- The method is equally applicable to Golgi KESM neural morphology data, and Nissl KESM neurovasculature data.

Template-Based Fiber Tracing

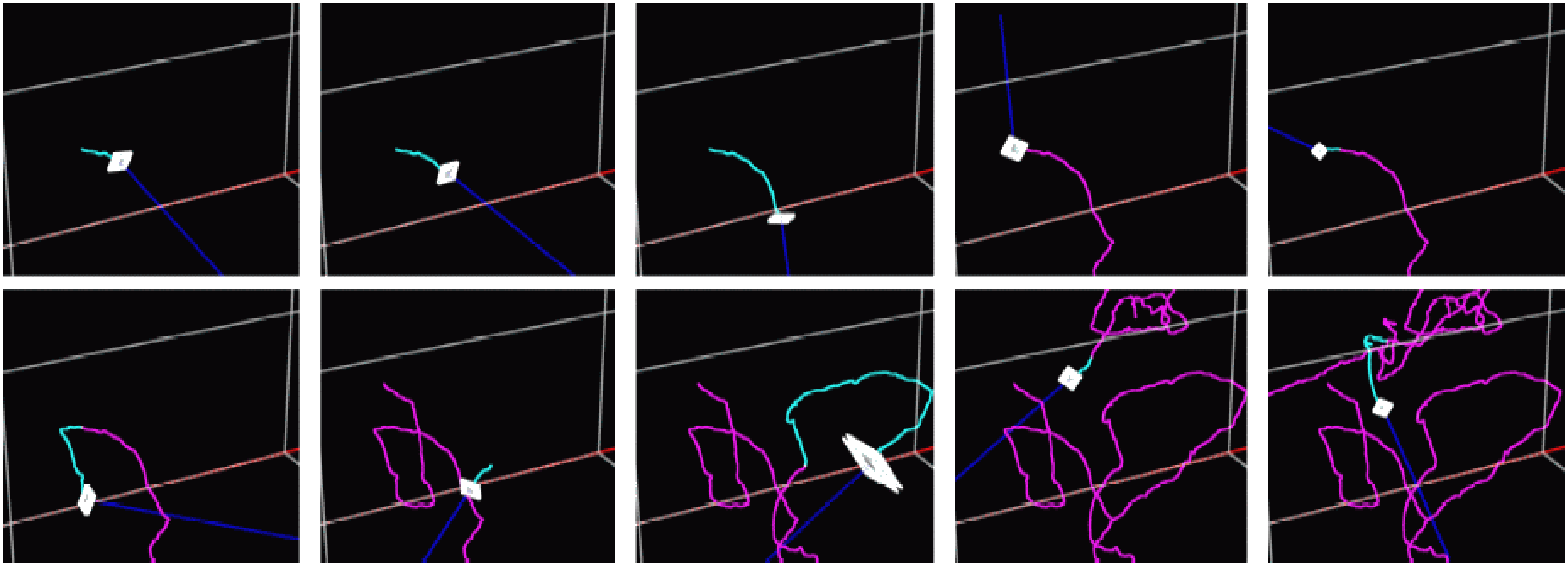


Predictor-corrector algorithm based on fast template matching.

- Predictor: Obtain multiple virtual slices (frames) of planes tangential to the sphere centered at the current tracking point, and pick best match with the Gaussian template.
- Corrector: Correct scale (diameter) and offset using the same template-based approach.

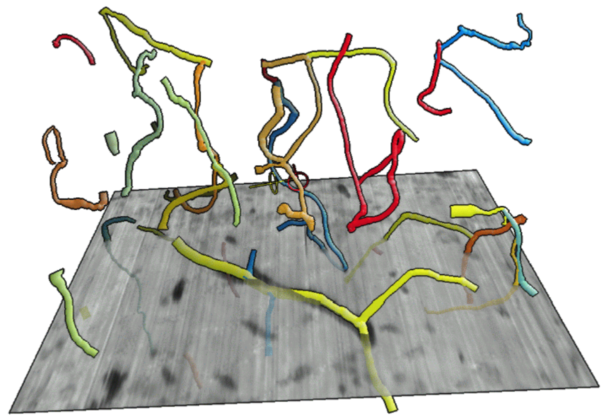
Use GPU (graphical processing unit) to vastly speed up computation.

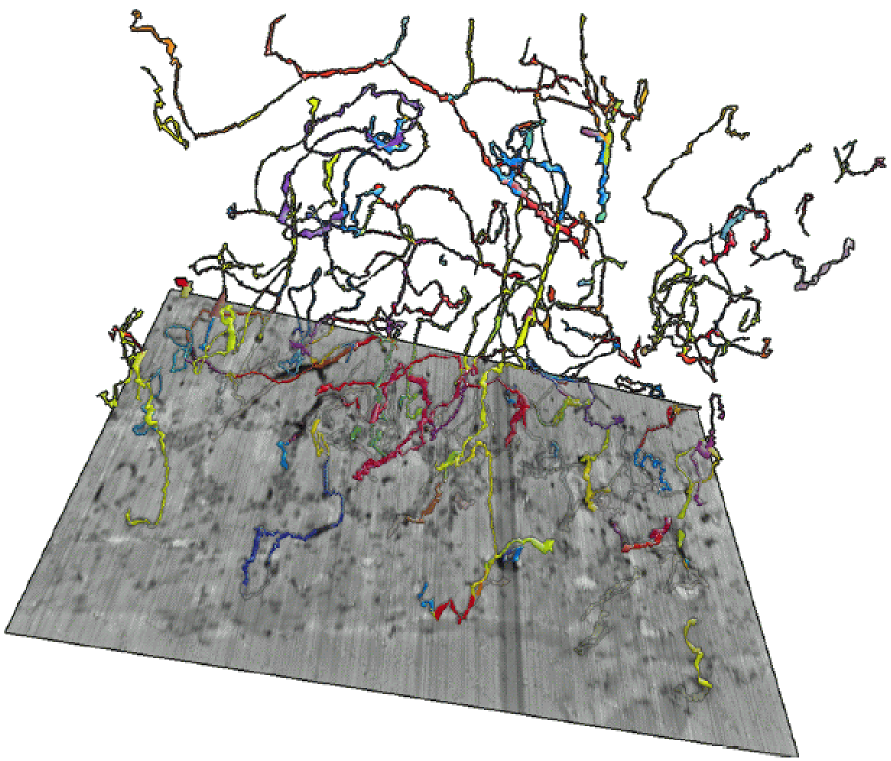
Template-Based Fast Tracing

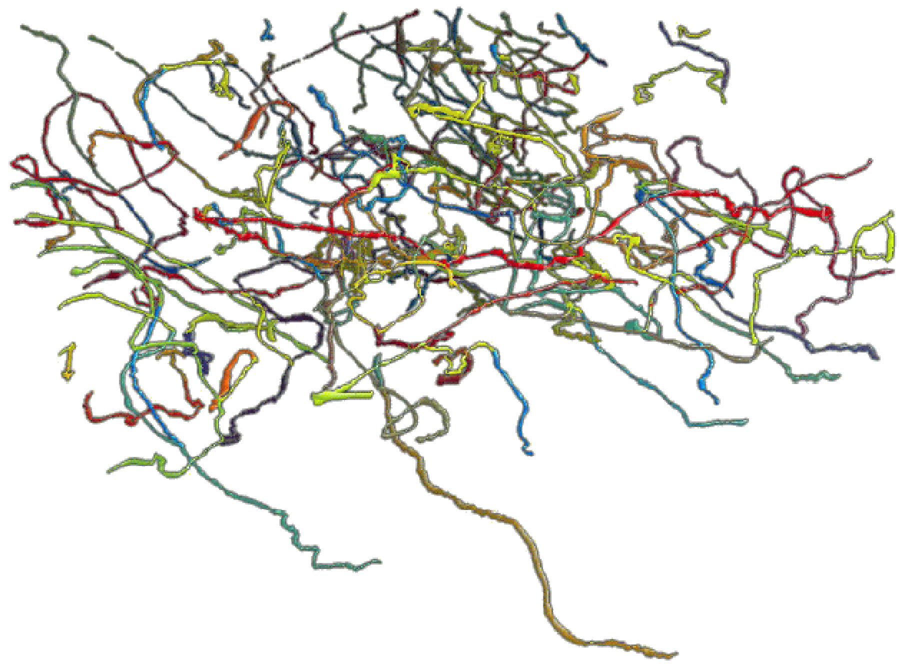


- Tracing microvasculature in the mouse olfactory bulb.

Template-Based Fast Tracing: Results







On-going Work and Future Directions

- Improved *en bloc* staining methods.
- Chatter abatement in KESM sectioning.
- Superresolution methods to link SBF-SEM and KESM.
- Texture segmentation of Nissl KESM data to link KESM and MRM-based mouse brain atlas.
- Morphological model and model fitting.
- 3D reconstruction and validation.
- Databasing reconstructed data and statistical quering.
- Integrated software environment and processing pipeline.
- Interactive, selective visualization using self-orienting surfaces.

Acknowledgments

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  - Stanford: Stephen J. Smith, Kristina Micheva, Brad Busse
- Contributors: Charles A. Taylor, R. Y. Arakaki (Stanford); Arthur Toga (UCLA); Winfried Denk (Max Planck)

Image source: `http://www.mouseatlas.org/data/mouse/stages/t47/view`

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