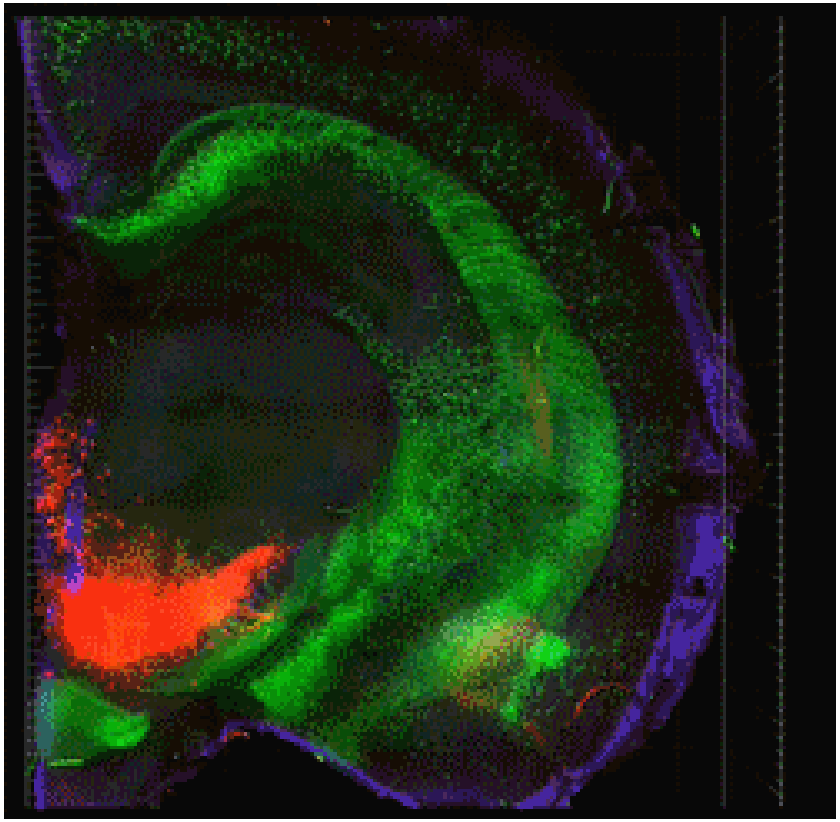
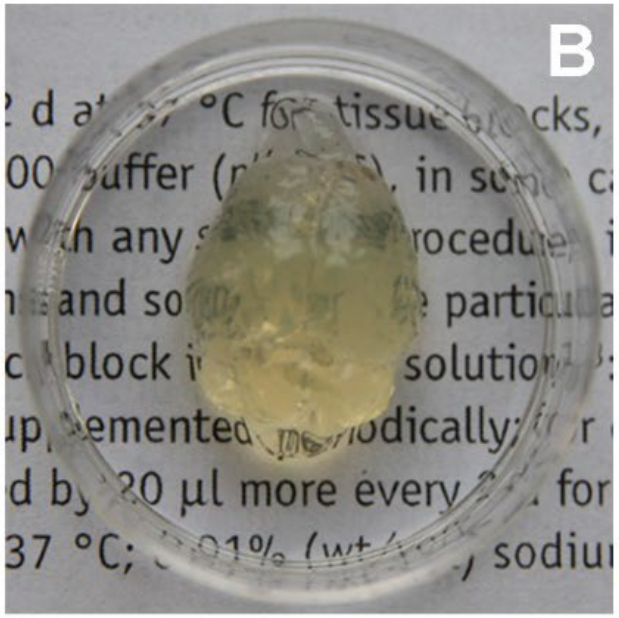
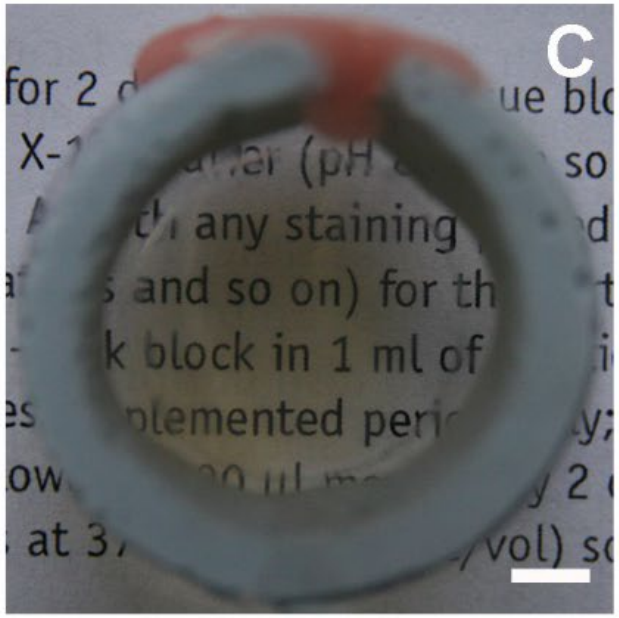
High Throughput Microscopy and Brain Clearing



Lecture 11

Anita Autry, Ph.D.





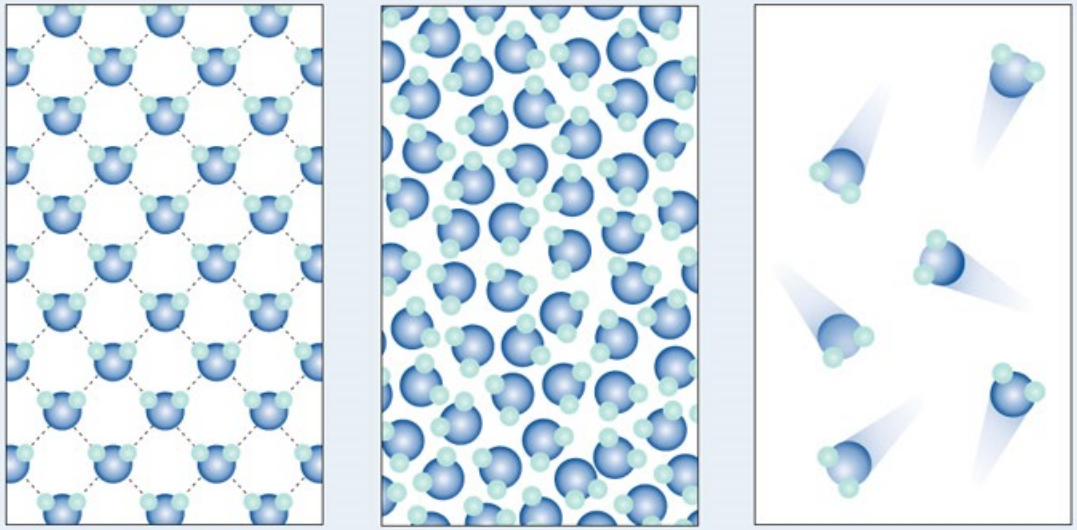
Topics

- Physical Sectioning
- Scanning, stitching, and shading
- Atlas registration
- Serial block face imaging
- Brain Clearing
- Imaging of cleared tissue (optical sectioning)
  - Confocal
  - Lightsheet microscopy

Sectioning brain tissue

Equipment	Ultramicrotome	Cryostat (cryomicrotome)	Freezing/sliding microtome	Vibratome (vibrating microtome)
Tissue preparation	Resin embedding or freezing	Perfused, cryoprotected, OCT embedded; Fresh frozen, OCT embedded	Perfused, cryoprotected	Perfused, agarose embedded (for histology*)
Sectioning size	0.01-2.5 µm	0.5-200 µm (in situ 10-20 µm)	1-60 µm (min 20 best for brain section histology)	10-500 µm (less than 50 no good for brain tissue)
Downstream applications	Electron microscopy	In situ or antibody staining	Antibody staining	Antibody staining

Cryoprotection and snap freezing

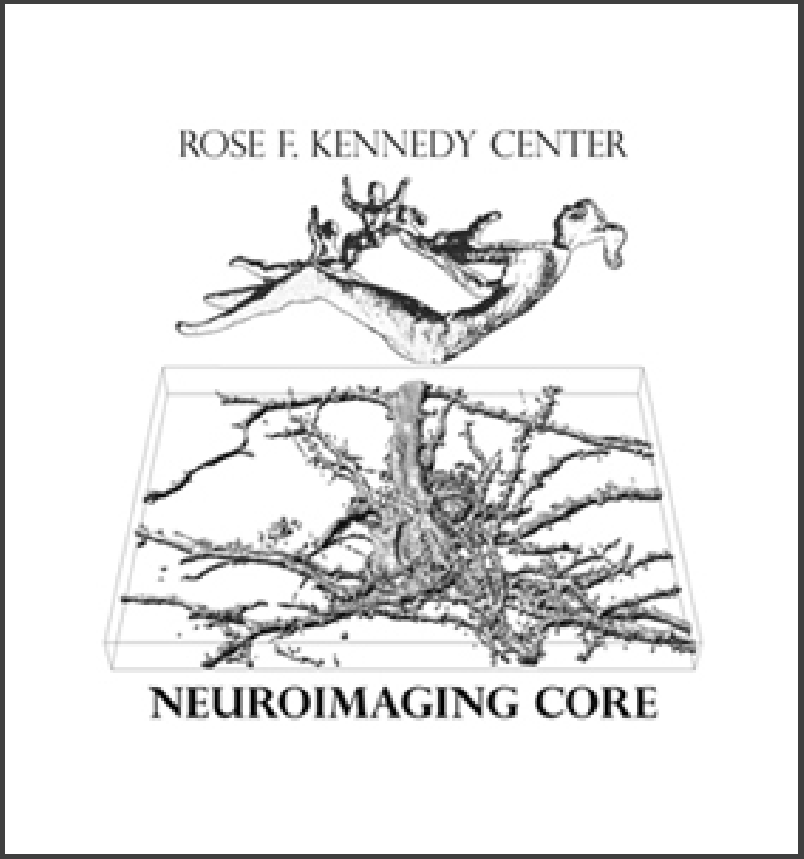


- Snap freezing on dry ice, in isopropanol/dry ice slurry, or in liquid nitrogen will prevent ice crystal formation and stop actions of proteases and nucleases
- After perfusion, tissue may be sunk in hypertonic sucrose to release water from tissue to prevent ice crystal formation

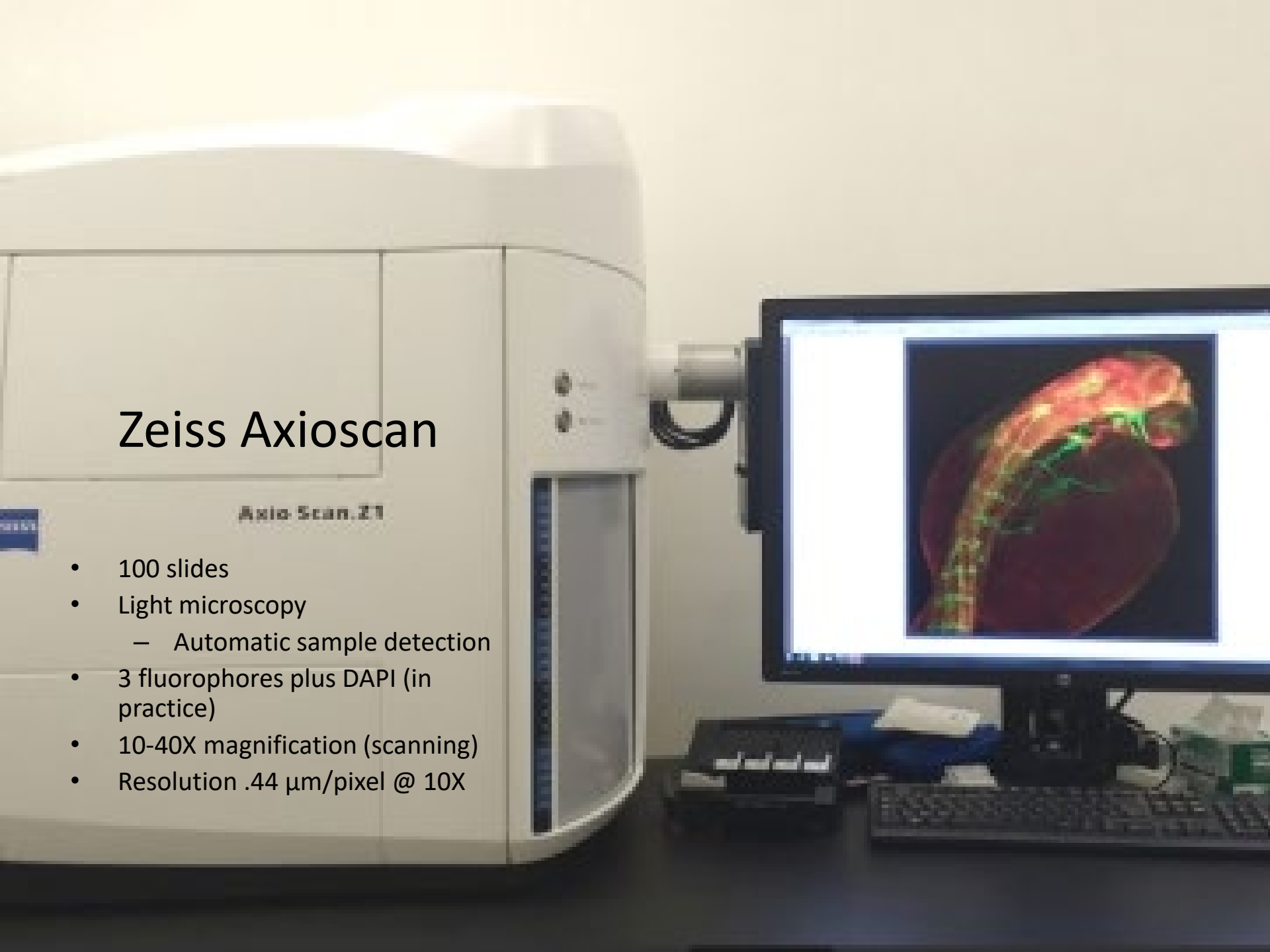
lab ice is not for personal use

Microscopes in Kennedy Core

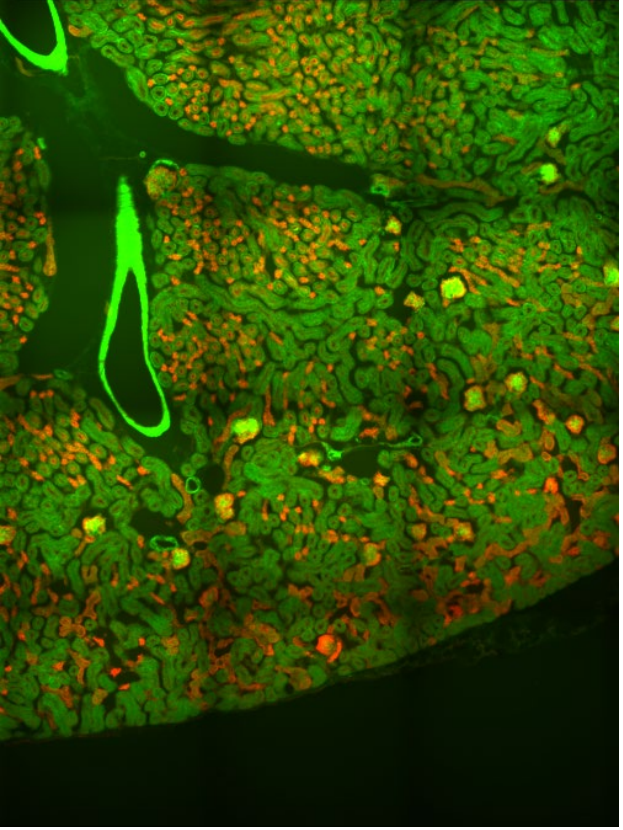
- Olympus Multi-Time Multi-Sample Widefield Epifluorescence
- **Zeiss Axioscan**
- Image Xpress
- Zeiss Airyscan confocal
- Brucker Multiphoton

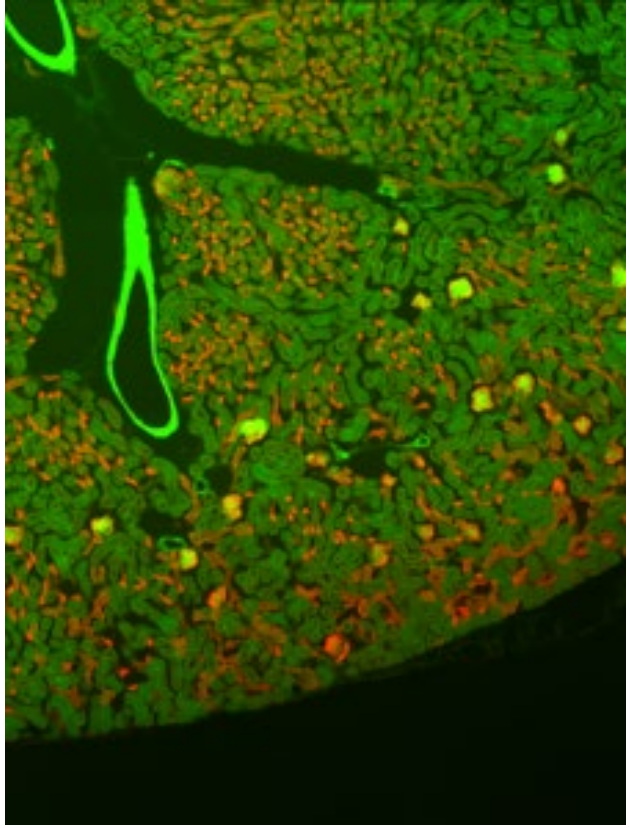


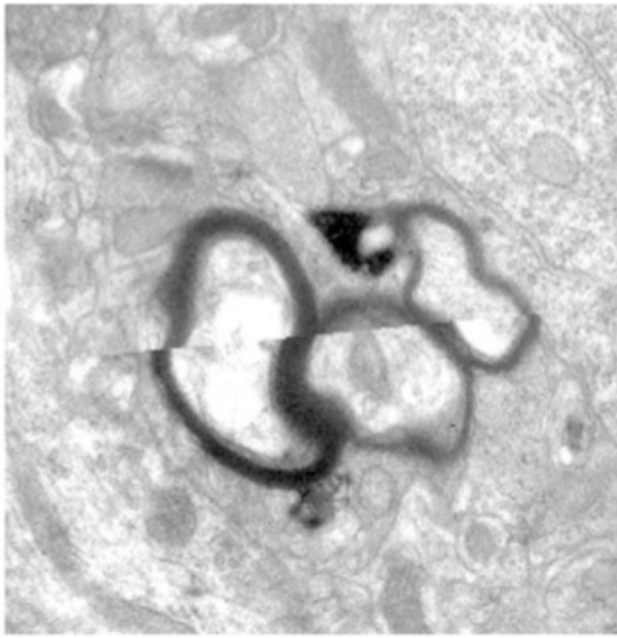
Zeiss Axioscan

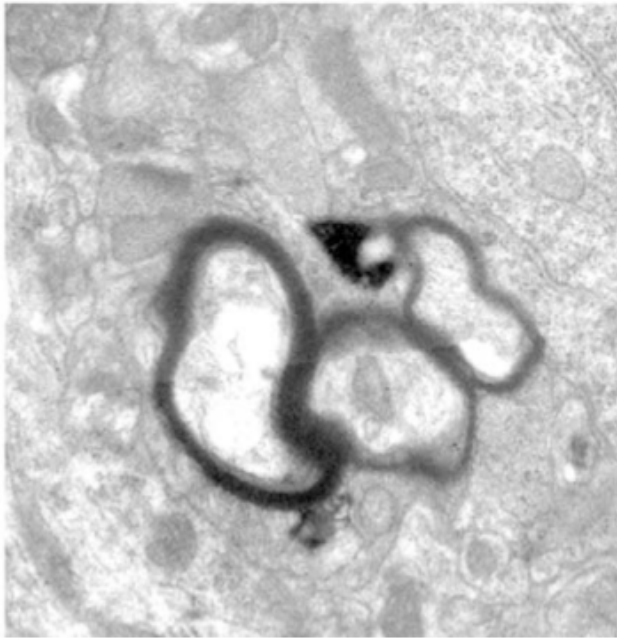


- 100 slides
- Light microscopy
  - Automatic sample detection
- 3 fluorophores plus DAPI (in practice)
- 10-40X magnification (scanning)
- Resolution .44 µm/pixel @ 10X







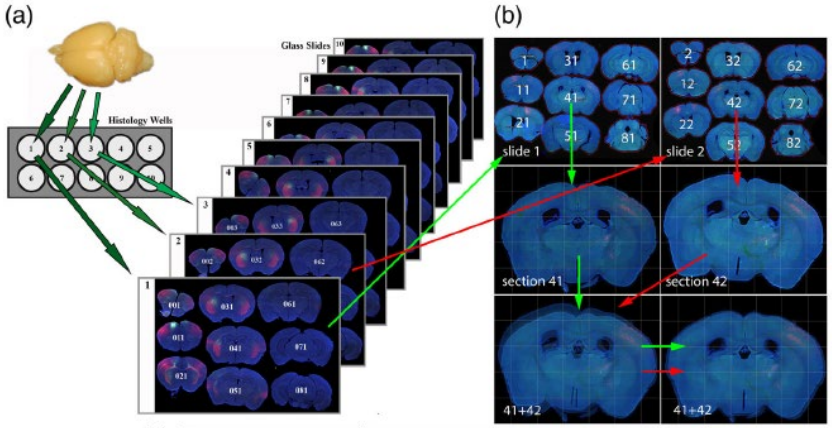


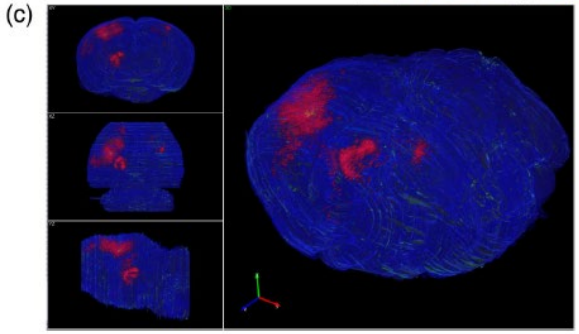
Tiling, stitching and shading

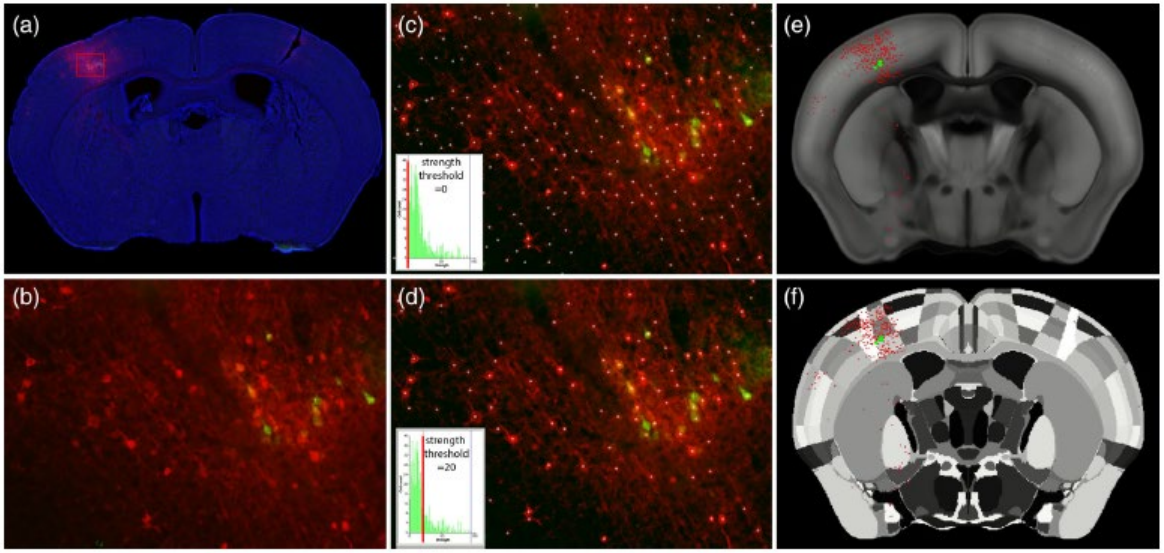
- When acquiring imaged that will be stitched, tiling parameters are key (at least 5% overlap)
- Requires a good reference signal (DAPI)
- Done automatically on the Axioscan
- Can be done in ImageJ using stitching macros

Atlas registration

- Morph 2D (or 3D) samples onto the Allen Mouse Brain Common Coordinate Framework
- Make a 3D representation of 2D data
- Segment and quantify within brain regions automatically



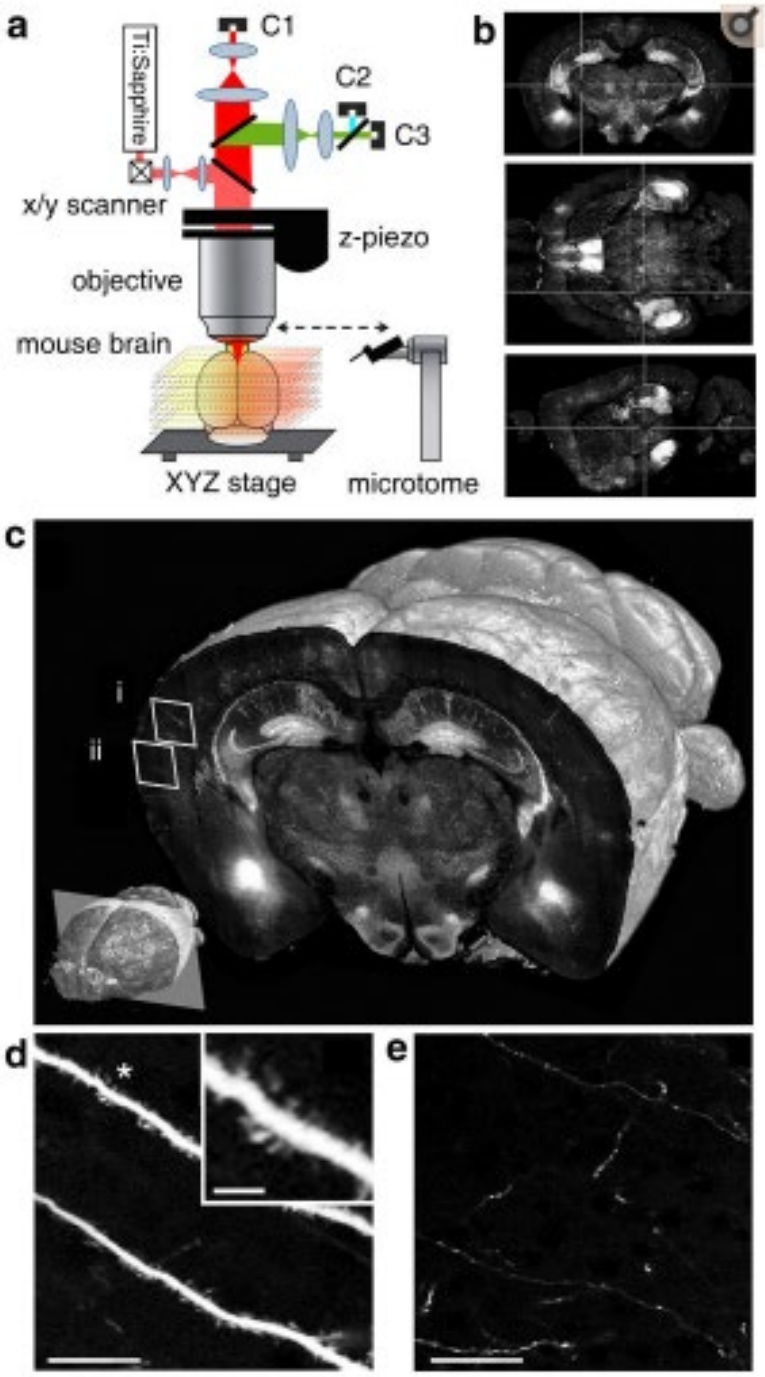




Eastwood et al., J. Comp. Neurol., 2018.

STP tomography

- Serial two-photon tomography
- High resolution
- Incorporates a blade so registration is minimal
- Used for most data on Allen Brain connectivity atlas
- Improvements in 2012 made process faster

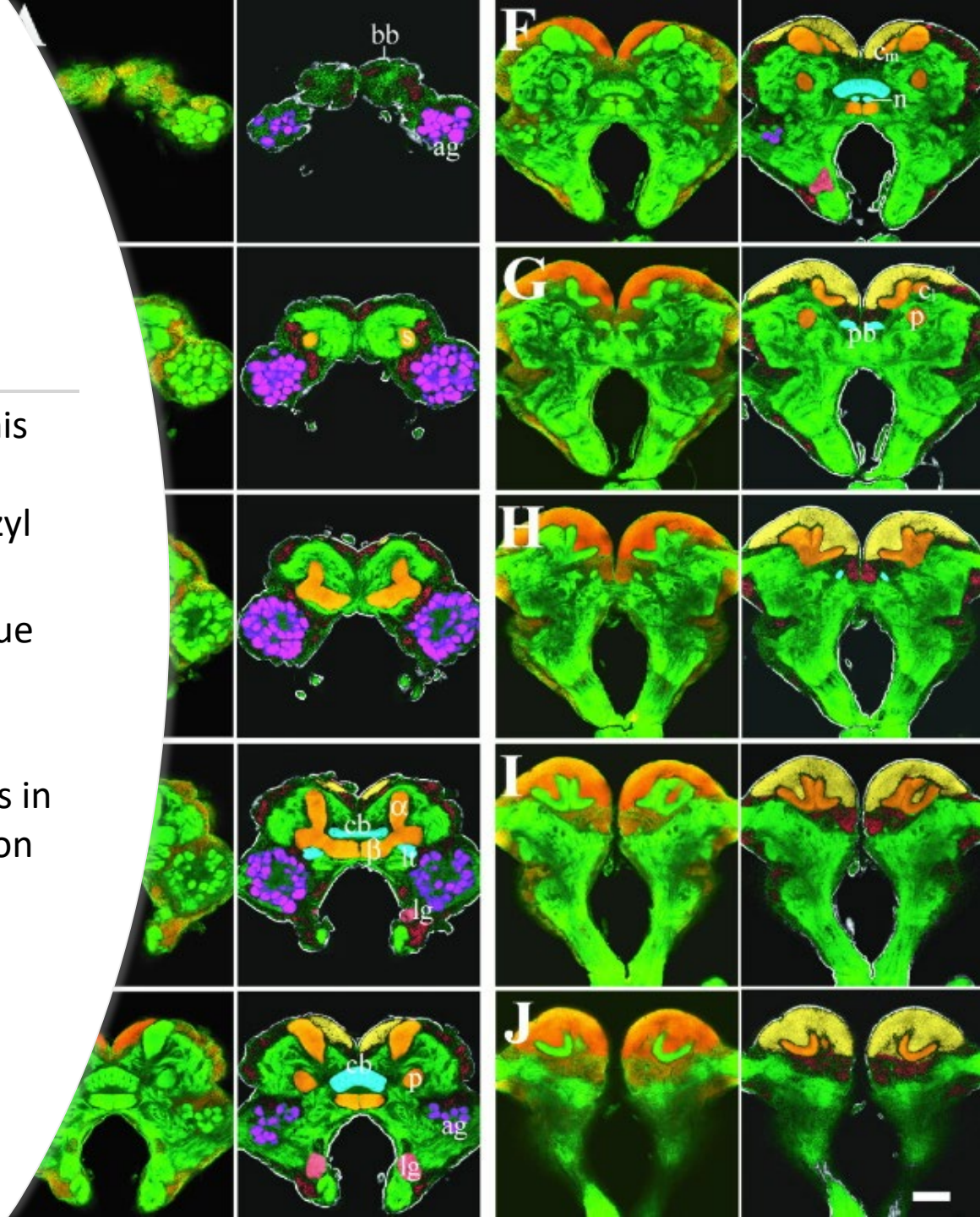


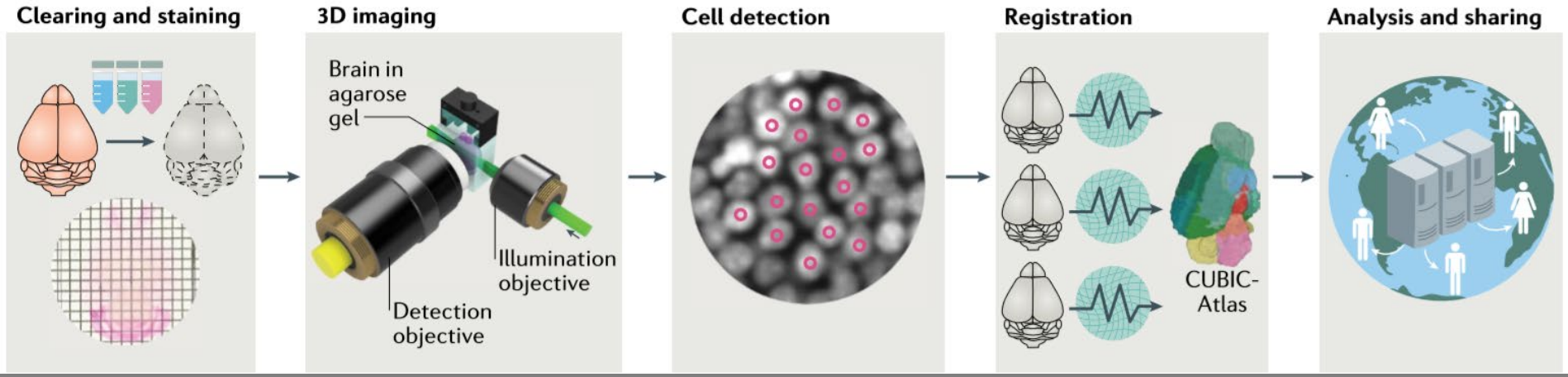
Ragan et al., Nat. Methods, 2012.

Brain clearing

- Spalteholz began working on this problem in 1914 using solvent based on methylsalicylate/benzyl benzoate and wintergreen
- Minimize light scatter from tissue
- Optimize high resolution microscopy
- Renewed interest after progress in aqueous clearing led to invention of FocusClear and imaging of whole cockroach brain in 2001

Chiang et al., J. Comp. Neurol., 2001.



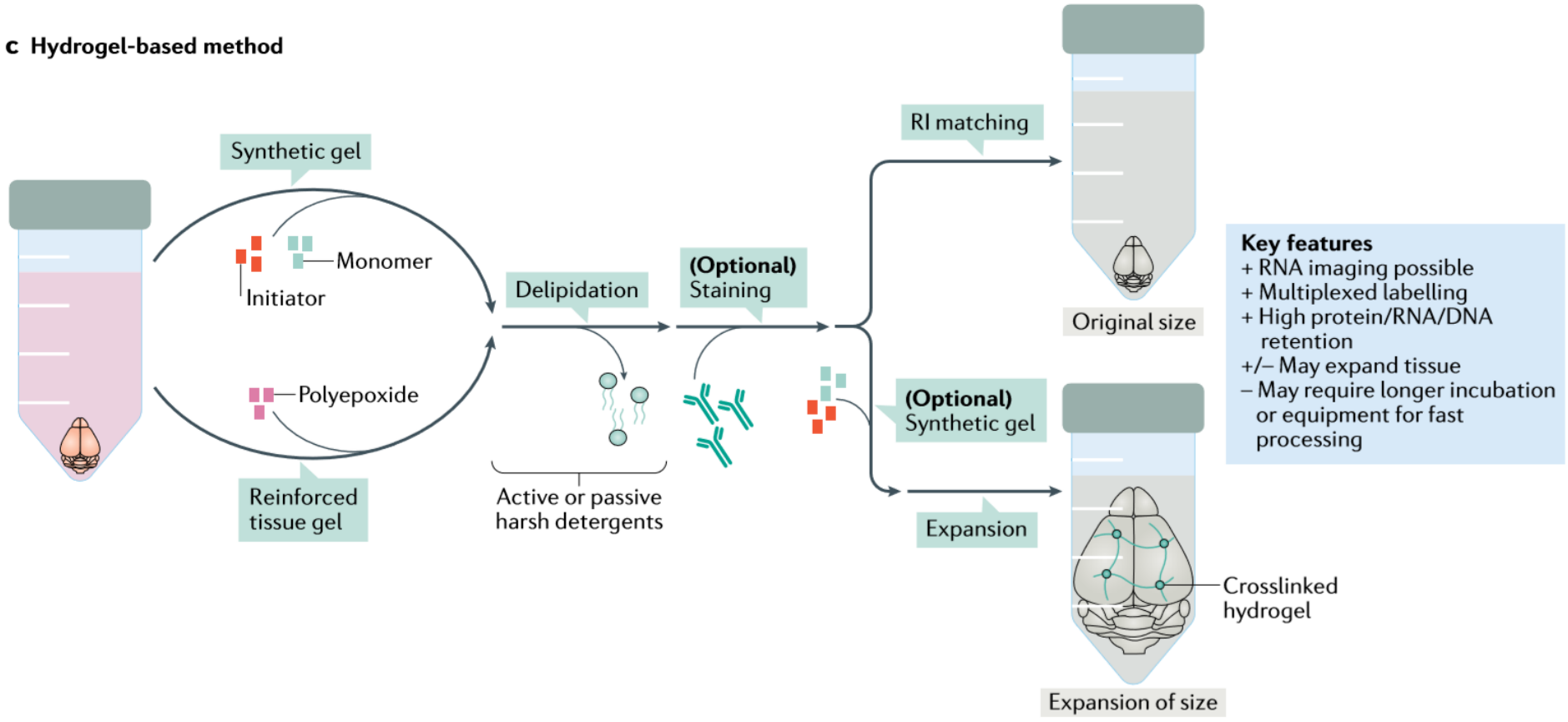


Ueda et al., Nat. Rev. Neurosci., 2020.

Brain clearing principles

- Delipidation
- Decolorization
- Decalcification
- Refractive Index Matching (RI tissue ~1.44-1.56)

Brain clearing: techniques



Ueda et al., Nat. Rev. Neurosci., 2020.

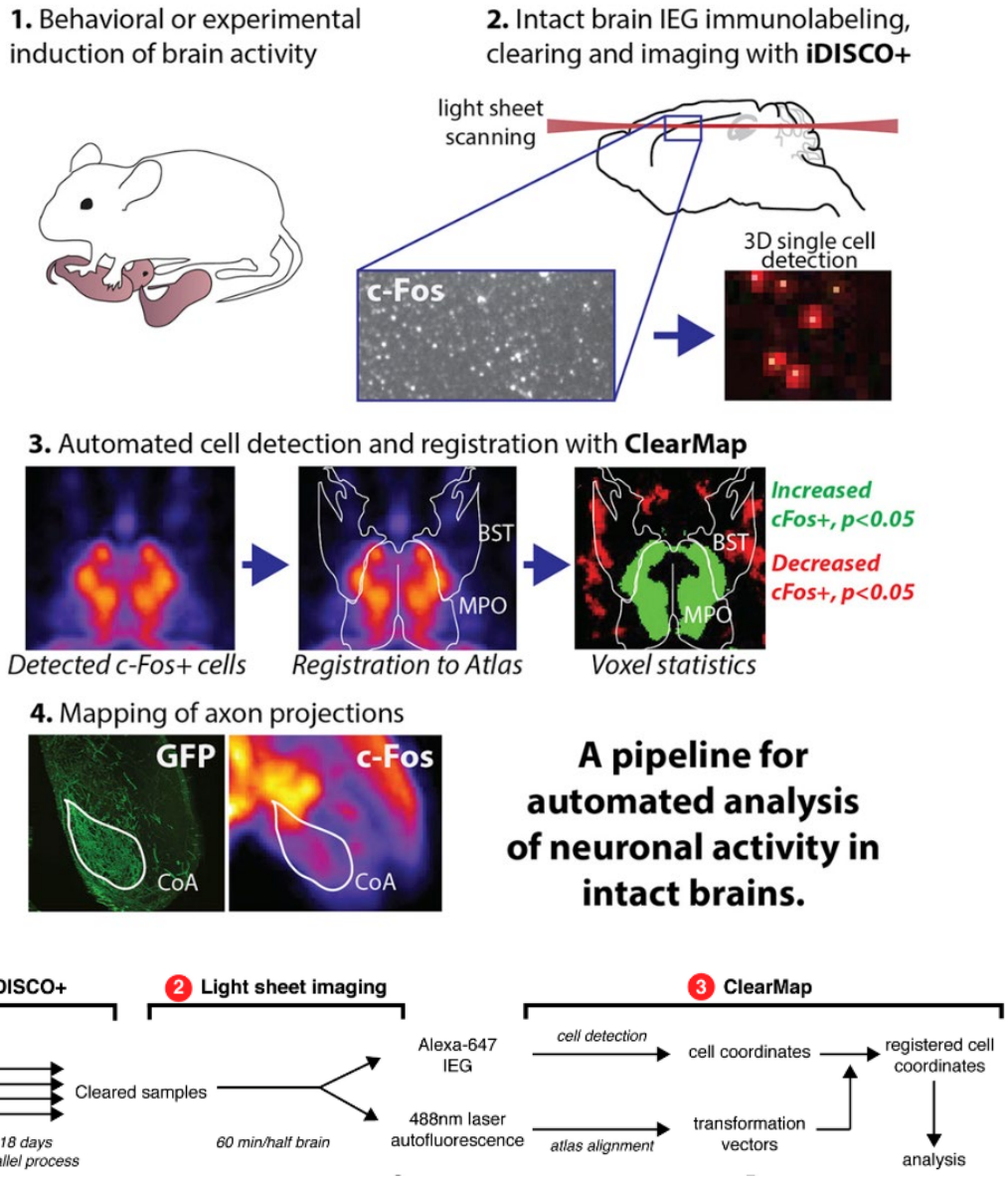
Techniques

- Hydrophobic (solvent)
  - 3DISCO, **iDISCO,** uDISCO*, vDISCO
- Hydrophilic (aqueous)
  - Sca*le,* **Sca*le*S**/AbSca*le*
  - See Deep Brain, SDB2
  - CUBIC, CUBIC-L, CUBIC-R, CUBIC-X*
- Hydrogel (aqueous plus matrix)
  - CLARITY, **PACT/PARS**
  - SWITCH-SHIELD

Ueda et al., Nat. Rev. Neurosci., 2020.

iDISCO+

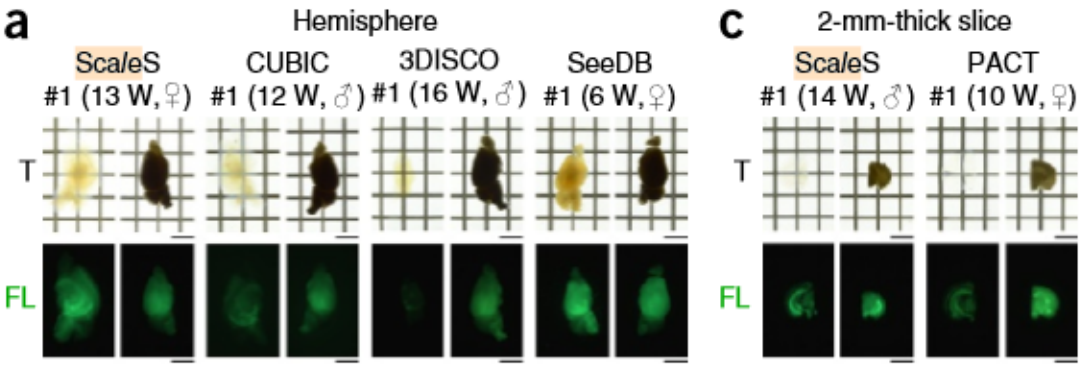
- 3-Dimensional imaging of solvent cleared organs with immunolabeling +
- Simple buffers, methanol etc.
- Only special equipment is solution chamber for imaging
- https://idisco.info/
- Pros: easy, compatible with immunolabeling, limited distortion of tissue, permanent preservation enables re-imaging
- Cons: may affect expressed fluorophores, shrinkage occurs (but is isotropic), not compatible with all antibodies

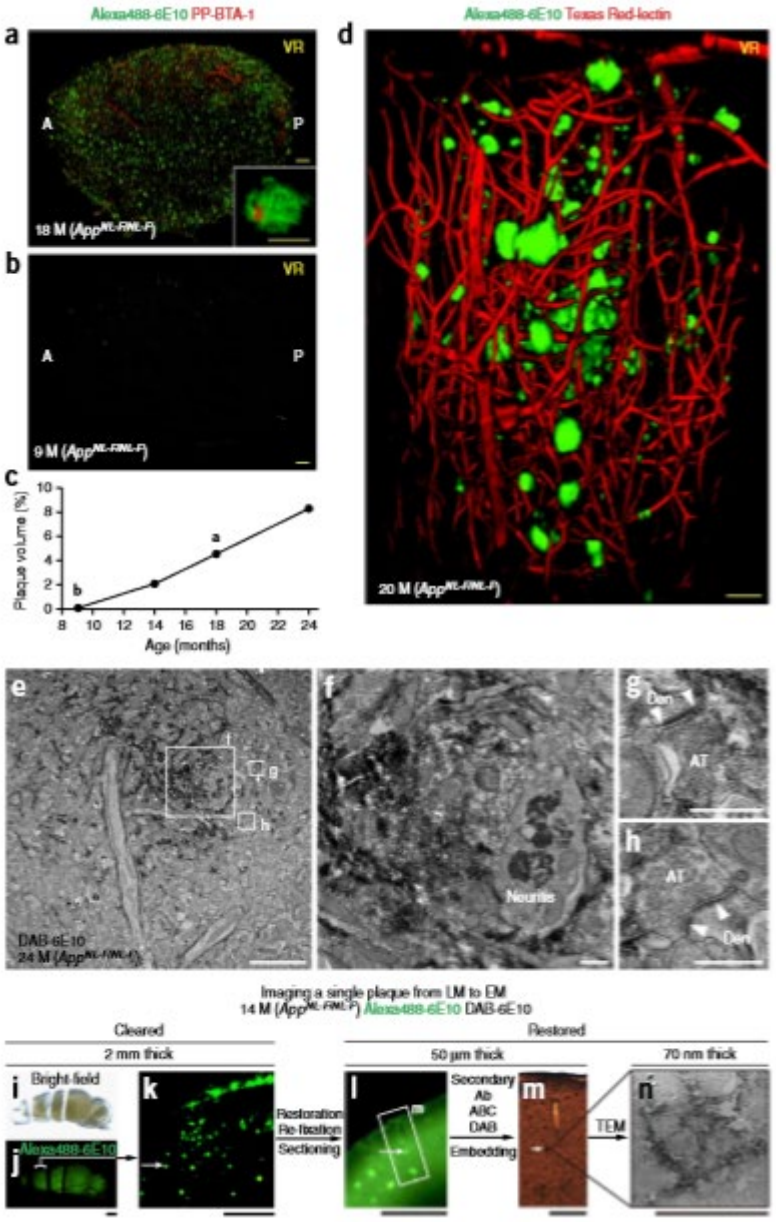


Renier et al., Cell, 2016.

Sca*le*S

- Sca*le* with sorbitol (original solution based on urea)
- Reproducible across samples due to low tissue warping
- Pros: preserves ultrastructure
- Cons: harsh conditions may lead to loss of some proteins

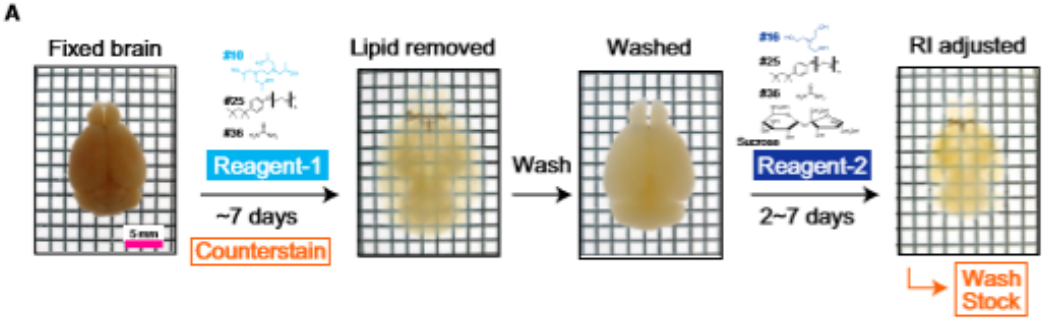




Hama et al., Nat. Neurosci., 2015.

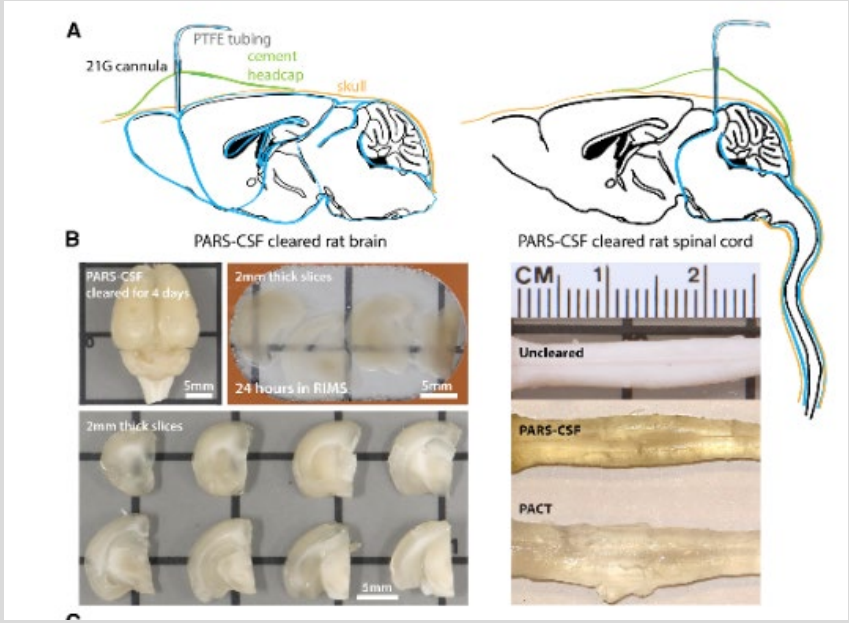
CUBIC

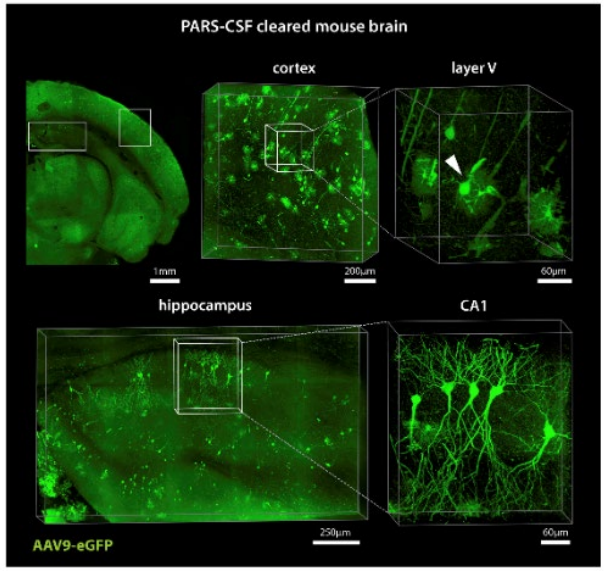
- Clear, unobstructed brain or body imaging cocktails and computational analysis
- Systems level approach to search for chemicals with decoloration/delipidation properties
- L= delipidation; R= Refractive index matching
- Cubic-X adds a hydrogel and allows 10-fold expansion
- Pros: preserves brain structure, compatible with staining procedures
- Cons:

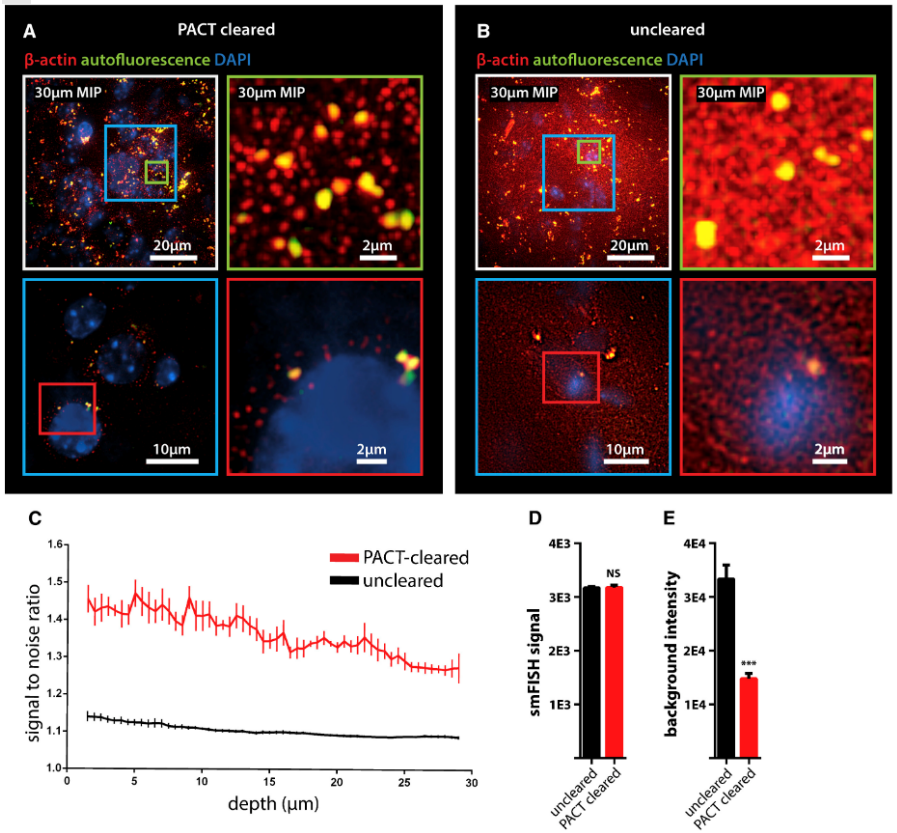


Susaki et al., Cell, 2014.

- **PACT: Passive CLARITY technique**
- "**C**lear, **l**ipid-exchanged, **a**crylamide-hybridized, **r**igid, **i**maging/immunostaining compatible, **t**issue h**y**drogel"
- PARS (perfusion assisted agent release in situ)
- No need for electrophoresis
- Describes RIMS, similar to FocusClear



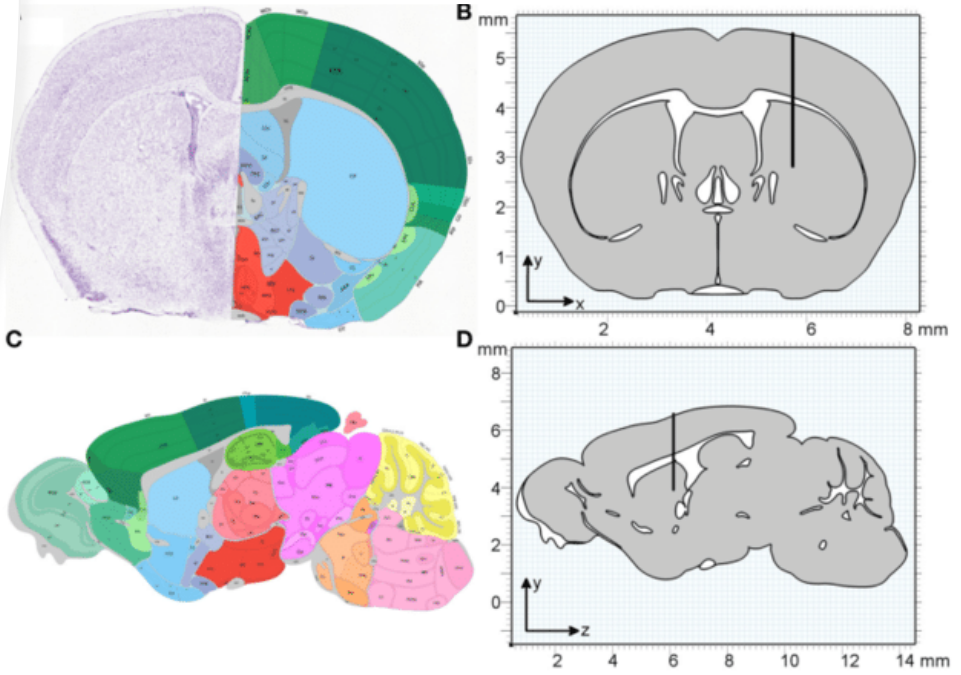




Yang et al., Cell, 2014.

Objective considerations

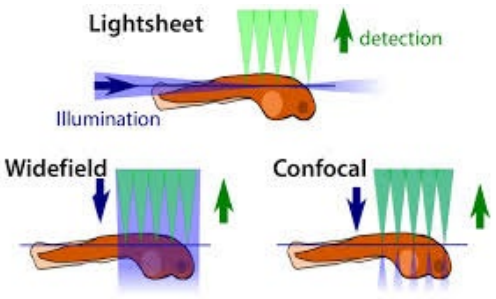
- Working distance
  - Objective (Airyscan ~2mm; Lightsheet up to 5mm)
  - Stage
- Numerical aperture
  - High: above 0.9* (but alternatives possible)
- Refractive index
  - Typically 1.33 (RI of water)
  - Want something closer to 1.44-1.56 which is what the tissue is
  - Paper describes a recipe for OptiView; economical alternative to FocusClear and solution is capable of clearing 1-3 mm sections of tissue without clearing, RI is adjustable
- Computational requirements
- Solution compatibility
  - Cleared tissue may be imaged in air or in RIMS; need to ensure any solution does not damage lenses

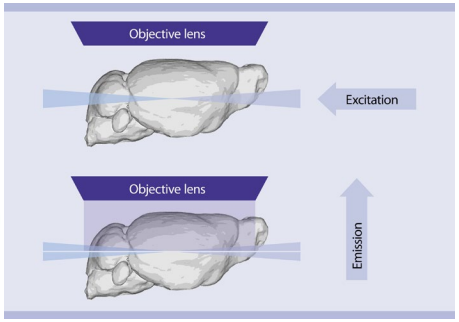


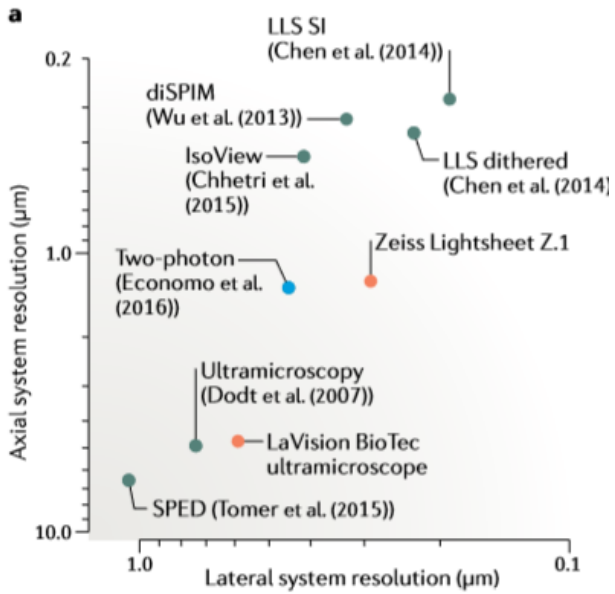
Isogai et al., Synapse Dev., 2016.

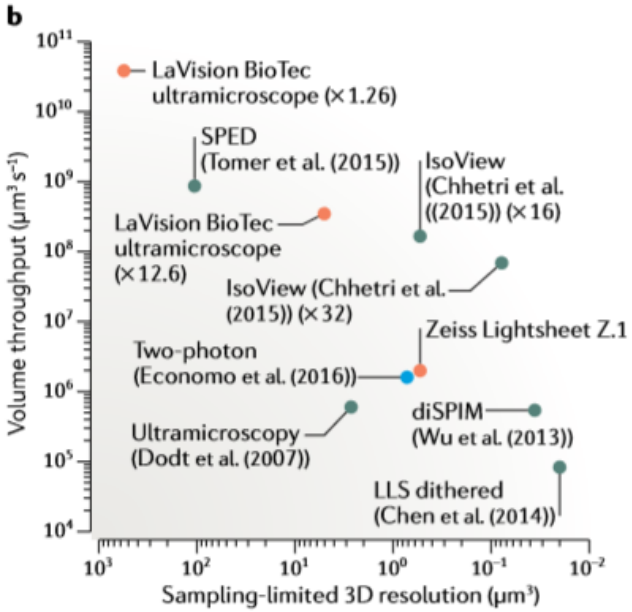
Light sheet microscopy

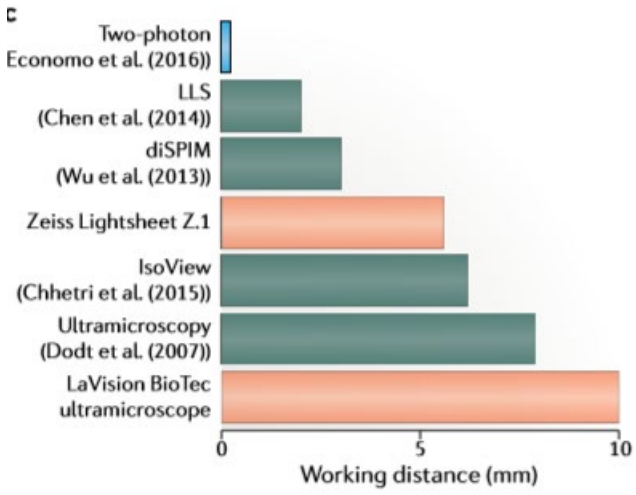
- Single plane illumination microscopy (SPIM)
- High frame rate
- Less photodamage
- Low sample bleaching







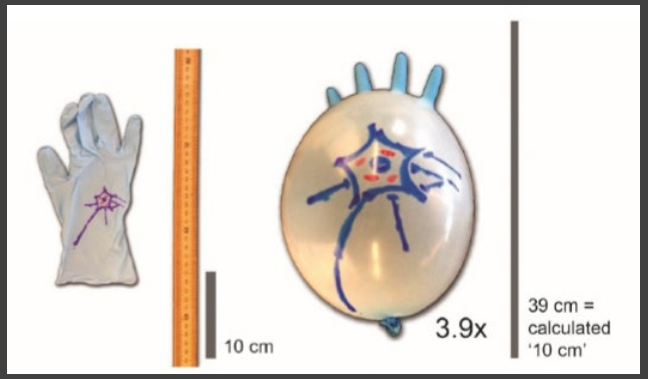


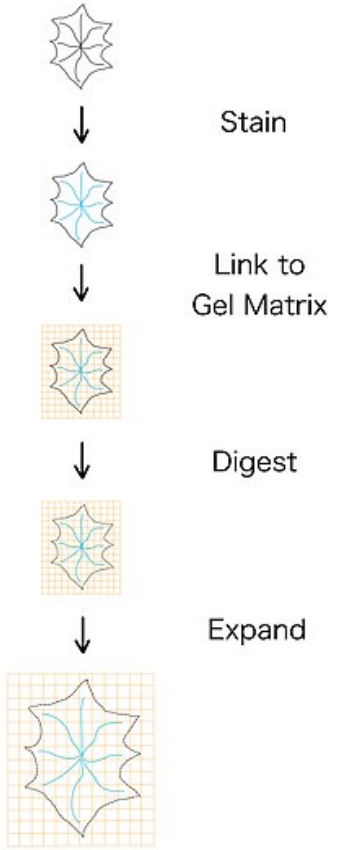


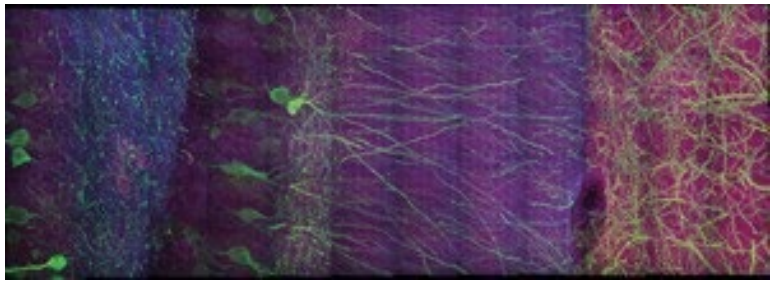
Ueda et al., Nat. Rev. Neurosci., 2020.

Expansion microscopy

- Embed sample in polymer, expand isotropically up to 16x original size

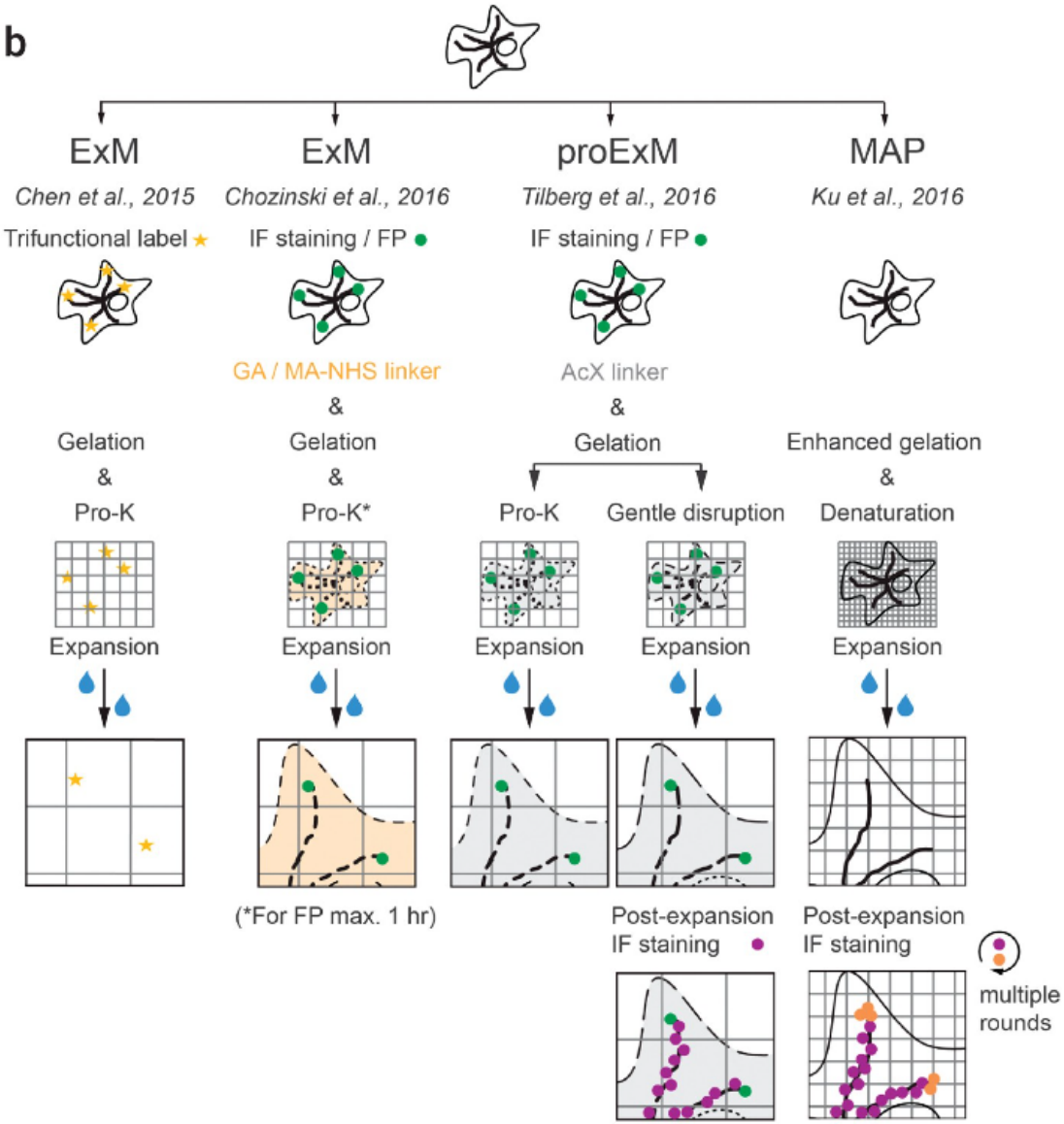






Chen et al., Science, 2015.

Expanded expansion microscopy



Engerer et al., Nat. Biotech., 2016.

Table 1| Comparison of demonstrated expansion microscopy techniques as reported

	ExM[1] Boyden laboratory	ExM[4] Vaughan laboratory	proExM[2]	MAP[3]	ExFISH[9]
Target	Proteins	Proteins and DNA (dye)	Proteins	Proteins and sugar residues	RNA and DNA (dye)
Matrix	2.5% acrylamide sodium acrylate and MBAA	2.5% acrylamide sodium acrylate and MBAA	2.5% acrylamide sodium acrylate and MBAA	30% acrylamide, sodium acrylate and BA	2.5% acrylamide sodium acrylate and MBAA
Linking agent	Trifunctional DNA-oligomer	MA-NHS, GA	AcX	None	LabelX, (AcX plus Label-IT amine)
Sample disruption	Digest proteinase K, >12 h, RT	Digest proteinase K, 30 min to >12 h, 37 °C	Digest proteinase K, >12 h, RT; 4 h, 60 °C	Denaturation with SDS, 37 °C and dissociation at 70/95 °C, 6 h	Digest proteinase K, >12 h, 37 °C
Expansion factor (resolution)	4.5 (~70 nm)	4.0–4.2 (65 nm)	~4.0 (~70 nm)	4.0 (~60 nm)	3.3 (not reported)
Time (brain slice)	~6 d (100 µm)	~4.5 d (100 µm)	~3.0 d (100 µm)	~7 d (100–500 µm)	~4.5 d (50–200 µm)
FP preservation	No	Yes, max. digest 30 min to 1 h	Yes, ~50% intensity	No	Not applicable (combination with proExM[2])
IF staining	No	Yes, modified mAb	Yes	Yes	No
Sample	Cells, tissue: brain	Cells, tissue: brain	Cells, tissue: brain, pancreas, lung, spleen	Cells, tissue: brain, lung, heart, spinal cord, liver, kidney, intestine	Cells, tissue: brain
Comment	First report of the concept, customized reagents needed	Pre-expansion staining	Pre-expansion staining; post-expansion staining possible	Post-expansion staining of preserved epitopes; multiplexing	Post-expansion FISH with multiplexing and HCR amplification

AcX, Acryloyle-X; mAb, antibody; BA, bisacrylamide; FISH, fluorescence *in situ* hybridization; FP, fluorescent protein; GA, glutaraldehyde; HCR, hybridization chain reaction; MA-NHS, methacrylic acid-*N*-hydroxysuccinimidyl ester; MBAA, *N*,*N*′-methylenebisacrylamide; RT, room temperature.

Expansion technique comparison

Engerer et al., Nat. Biotech., 2016.