Johns Hopkins Engineering

Methods in Neurobiology

Advancements in Imaging to Increase Brain Resolution

JOHNS HOPKINS
WHITING SCHOOL
of ENGINEERING

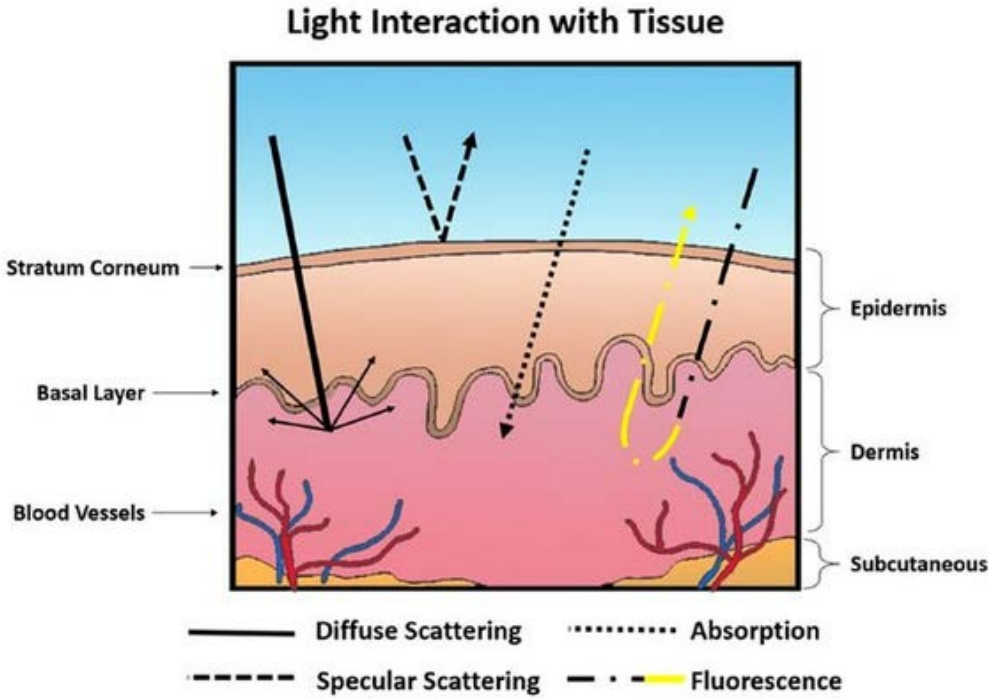
Imaging Depth

Issues:

- Physical limitations: objective working distance
- Tissue penetration: absorption and scattering.

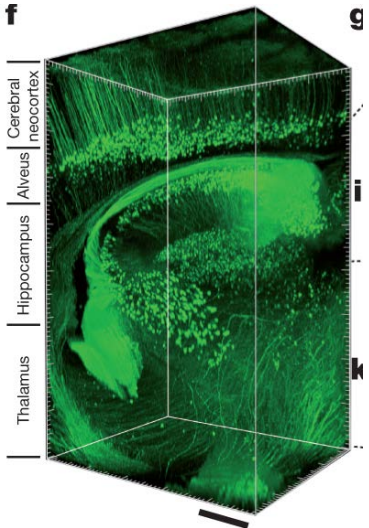
Solutions:

- Clarity
- Multiphoton microscopy

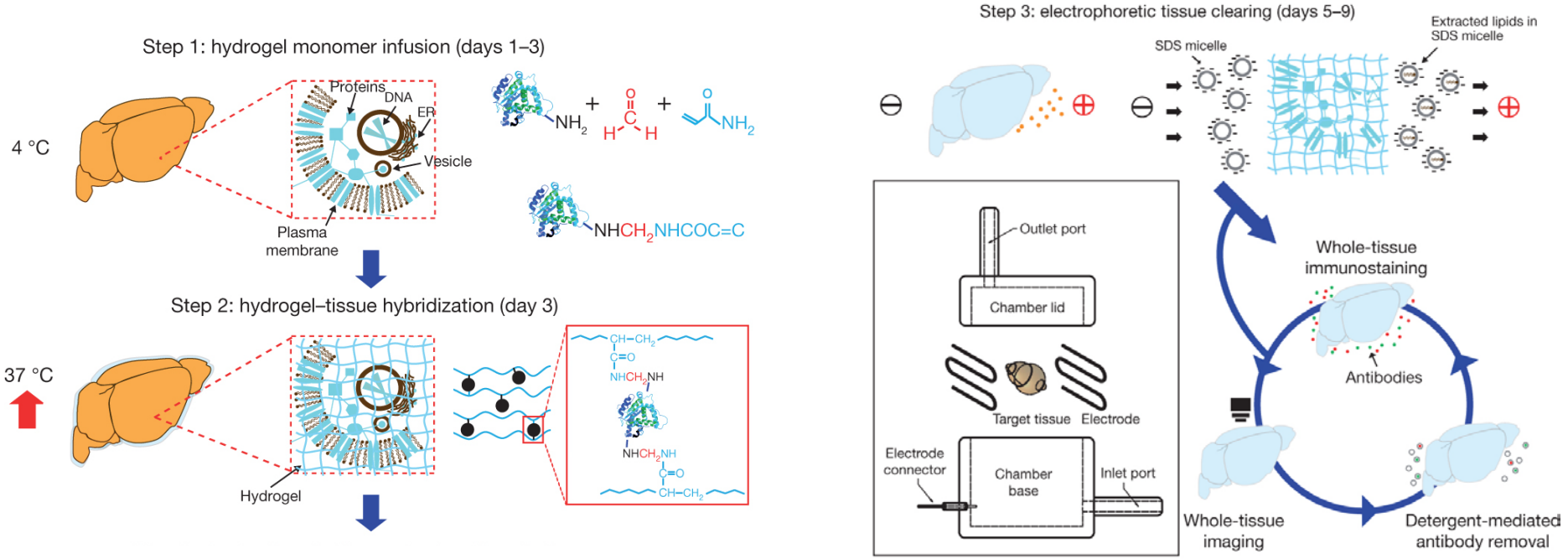


Clarity

- A chemical treatment that turns whole organs transparent.
- GFP family maintains fluorescence during the treatment;
- Possible to use regular immunohistochemistry techniques.
- No live cell imaging – Fixed samples



Clarity: Tissue Clearing Steps



Clarity Advantages

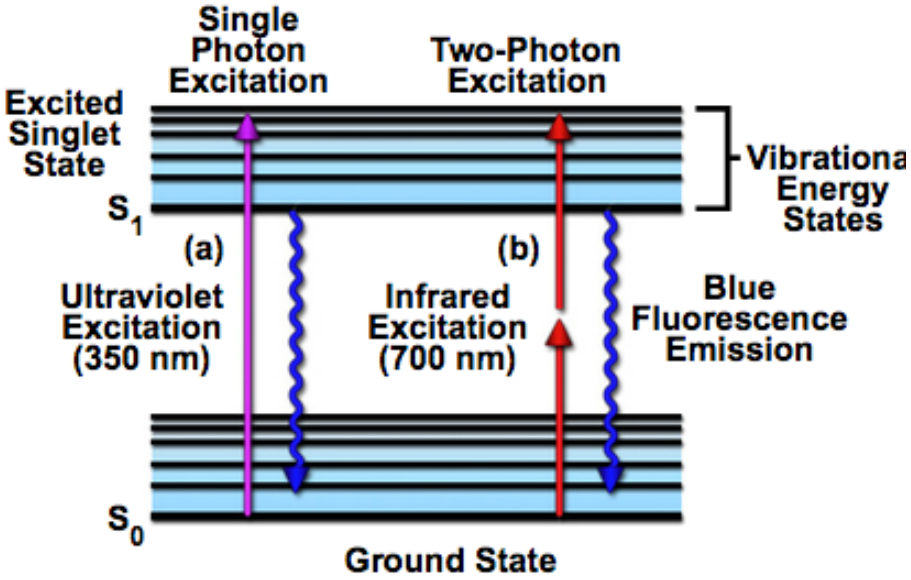
Advantages
1) Preserve native antigens structure
2) Rapid diffusion of molecular probes deep into intact tissue
3) Enable multi-round molecular phenotyping
4) Removal of lipid membranes allows high-resolution imaging.
5) Applicable to any tissue or animal model, including human postmortem brain stored for long time.
6) Applicable to any type of microscopy including electron microscopy

Multiphoton Microscopy

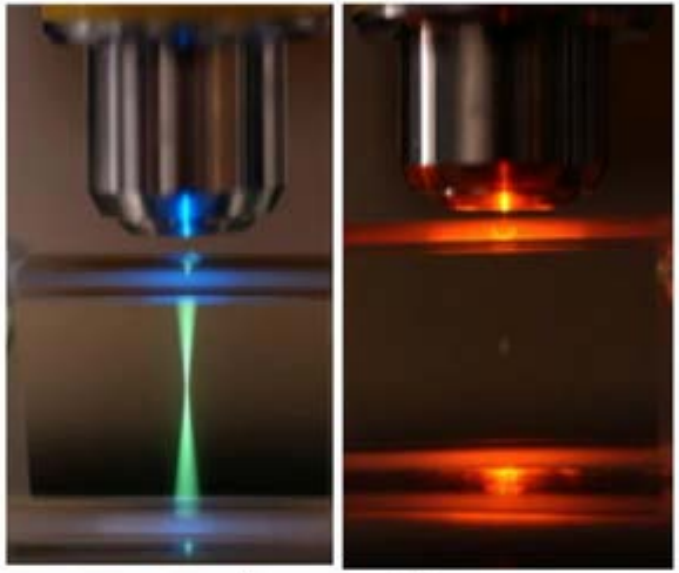
- Imaging of living, intact biological tissues on length scales.
- Minimal sample invasion over long periods of time.
- Employing near infrared (NIR) femtosecond lasers.
- Reduction of light scattering, photodamage of the sample and photobleaching.

Multiphoton Microscopy

Figure 1 - Two-Photon Jablonski Energy Diagram



1-photon vs. 2-photon



Photos by Steve Ruzin

Fluorescence from out of focus planes

Fluorescence from focal spot only

Probes for Multiphoton Microscopy

- Conventional fluorescent probes (Hoechst, AlexaFluor488, fluorescent proteins, genetic optical indicators);
- New NIR indicators: small molecules

  - pH sensitive NIR cyanine, H-ICG;
  - ROS cyanine;
  - Receptor conjugated cyanine dyes;
  - ….
- via IV delivery (fluorescein, rhodamine…)

References

Slide	Reference
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3,4	Chung, K., Wallace, J., Kim, S. *et al.* 2013 Structural and molecular interrogation of intact biological systems. *Nature* 497, 332–337.
7	Piston, D.V., Fellers, T.J., Davidson, M.J. (n.d.) Multiphoton Microscopy. Nikon Microscopy U. https://www.microscopyu.com/techniques/multi-photon/multiphoton-microscopy Ruzin, S., Aaron, H. (n.d.) Biological Imaging Facility. UC Berkeley http://microscopy.berkeley.edu/courses/tlm/2P/index.html