

Wako

Tissue Optical Clearing Reagent

Take a Revolutionary Approach to Deep Image

SCALEVIEW-A2

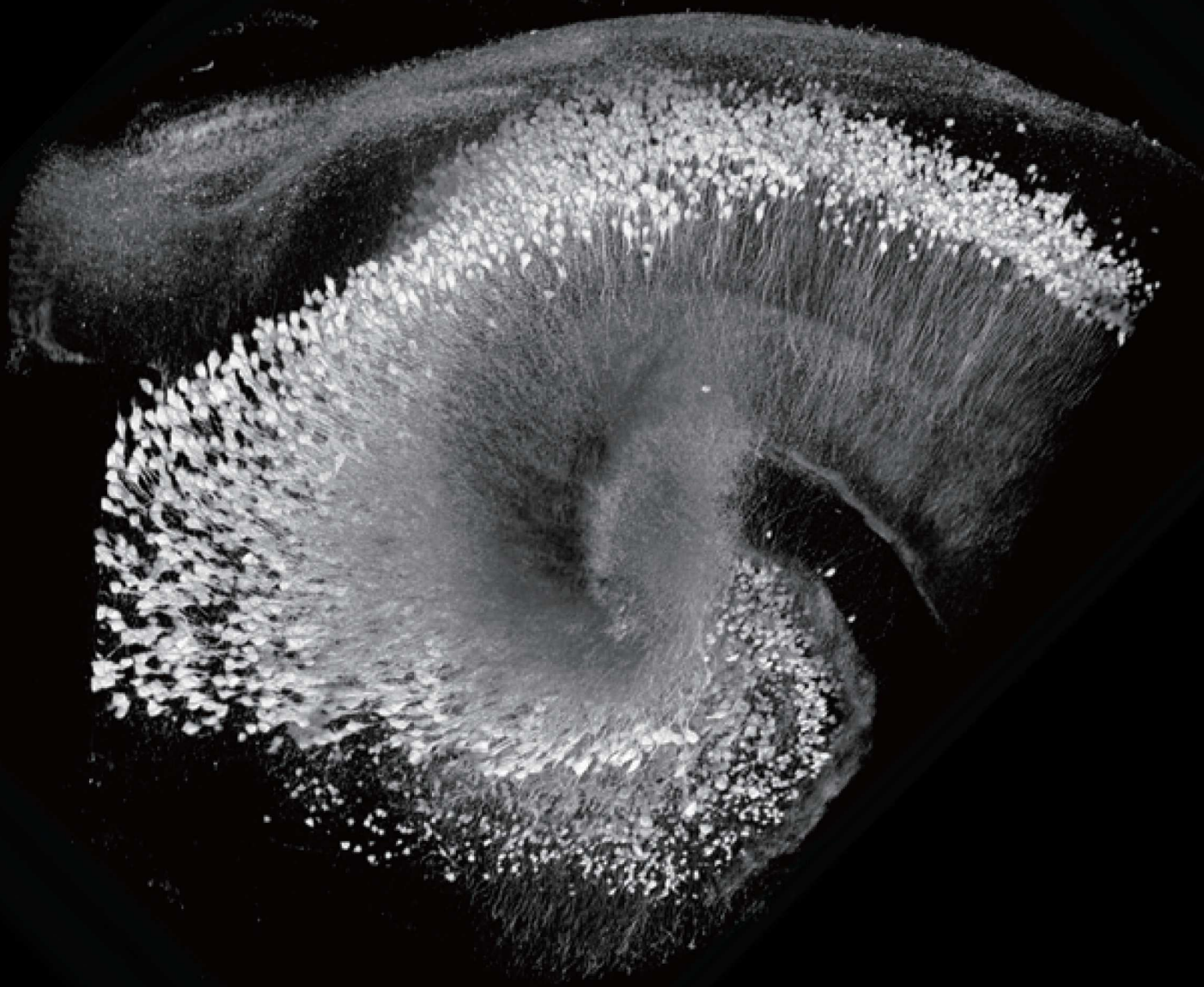


Image data courtesy of: Hiroshi Hama, Atsushi Miyawaki Laboratory for Cell Function Dynamics RIKEN Brain Science Institute and cooperate with Olympus Corporation

SCALEVIEW-A2 Protocol

Dr. Atsushi Miyawaki et al. developed a water-based optical clearing reagent, *Scale*, which clears fixed biological samples without quenching fluorescent protein. Using SCALEVIEW-A2, bodily tissues can be cleared of their opacity to provide a clearly defined image. The SCALEVIEW approach integrates seamlessly with multiphoton microscopes, providing the power to visualise 3-dimensional structures at unprecedented depths in morphologically intact tissue.

Optical characteristic : Refractive index $n_e(20^\circ\text{C})$ of SCALEVIEW-A2 is 1.377 – 1.381.

Example: Rendering mouse brain tissue transparent for deep imaging according to reference ¹⁾.

- 1) Transcardially perfuse an anaesthetised mouse with 4% paraformaldehyde (PFA)/PBS (pH 7.5~8.0).
- 2) Remove the whole brain and subject it to post-fixation in 4% PFA/PBS at 4 ° C for 10 hrs and cryo-protection in 20% sucrose/PBS at 4 ° C for 24 hrs.
- 3) Embed the sample in OCT compound and freeze it with liquid nitrogen.
- 4) Thaw and rinse the sample in PBS, and fix it again with 4% PFA/PBS for 20 min at room temperature.
- 5) Clear the sample by incubation in SCALEVIEW-A2 at room temperature.

More than 30 ml of SCALEVIEW-A2 is required for an adult mouse brain.

A full week of incubation may be necessary for transparency.

During incubation, stir slowly using an orbital shaker.

Exchanging SCALEVIEW-A2 each day accelerates the transparency process.

The sample might be expanded 10-30% in one direction after incubation.

- 6) Perform deep imaging of the transparent brain using an appropriate dipping objective lens. Use SCALEVIEW-A2 as the immersion medium.

If necessary, immobilize the sample to the bottom of the imaging vessel with agarose.

- 7) The transparent brain can be stored for a long time in SCALEVIEW-A2 at 4 ° C.

1. Image of a mouse brain after SCALEVIEW-A2 process



Figure1. A mouse brain after SCALEVIEW-A2 process

Left: a mouse brain before SCALEVIEW-A2 process

Right: a mouse brain after SCALEVIEW-A2 process

2. Imaging data using SCALEVIEW-A2

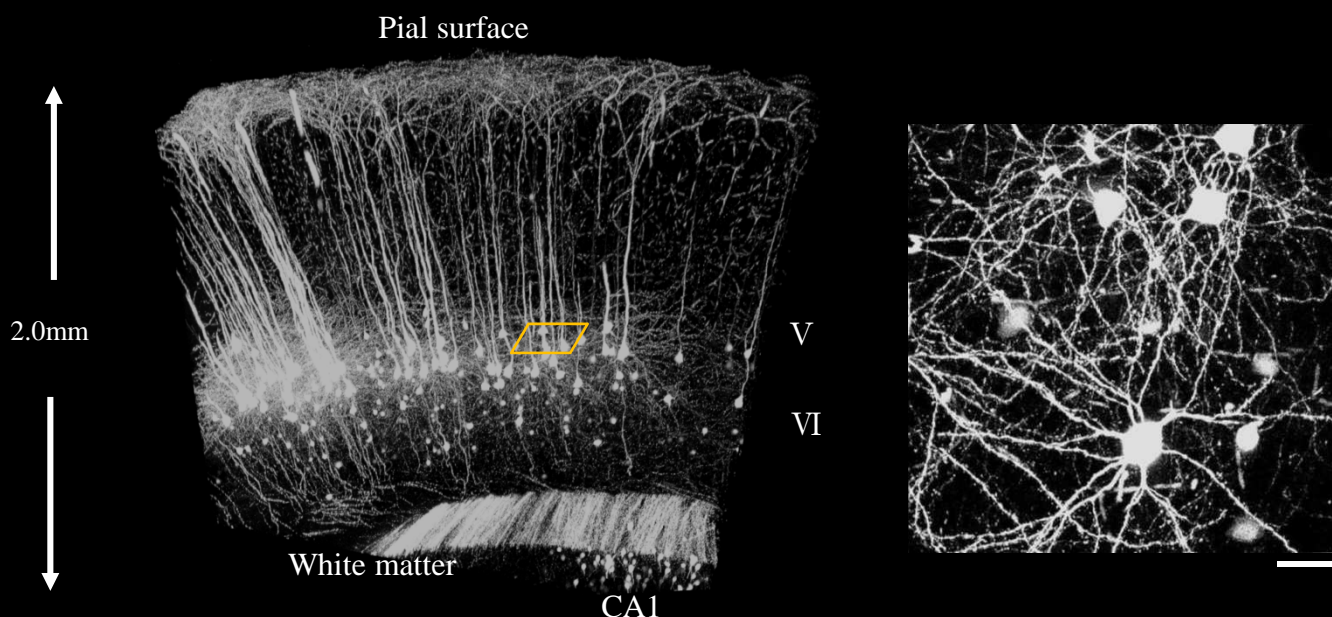


Figure2. Imaging of Thy1-YFP(H Line) mouse brain after SCALEVIEW-A2 process using Multiphoton microscope and Multiphoton dedicated objective :OLYMPUS, model:XLPLN10XSVMP. Bars, 50 μm .

Imaging data using SCALEVIEW-A2

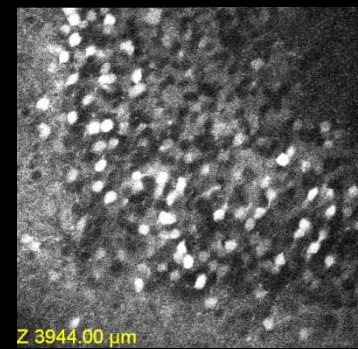
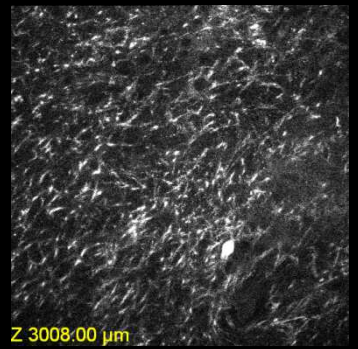
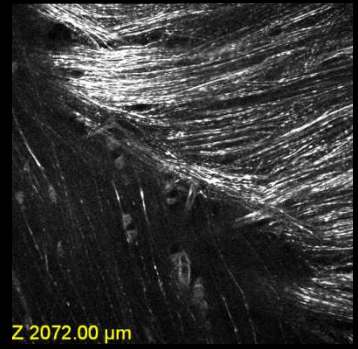
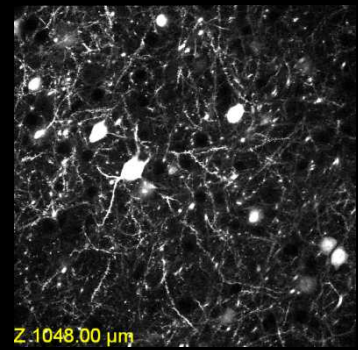
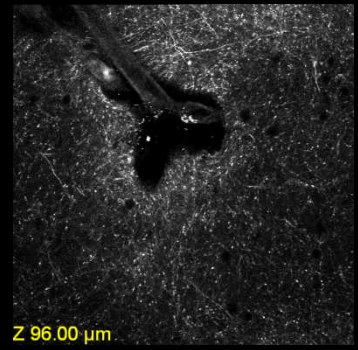
Surface

1mm

2mm

3mm

4mm



IV

V

VI

White Matter

CA1

DG

Figure3. Deep imaging of Thy1-YFP(H Line) mouse brain after SCALEVIEW-A2 process using Multiphoton microscope and Multiphoton dedicated objective :OLYMPUS, model:XLPLN25XSVM(NA1.0 WD4mm).

Imaging data using SCALEVIEW-A2

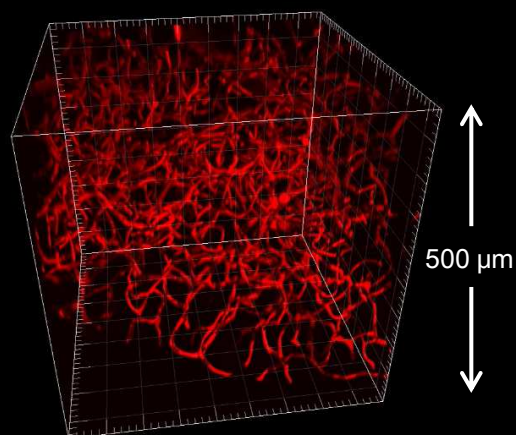


Figure 4. Image of mouse pancreas (labeling process the blood vessel using Lectin Texas Red) after SCALEVIEW-A2 process using Confocal microscope and objective : OLYMPUS, UPLSAPO30XS.

Reference

- 1) Hama, H. et al. : *Nature Neuroscience* **14**, 1481 (2011).

Reagent List

Wako cat. No.	Product name	Grade	Package Size	Storage
193-18455	SCALEVIEW-A2	Tissue Optical Clearing Reagent	500ml	Keep at RT

In cooperation with OLYMPUS.

 **Reagent** www.e-reagent.com

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