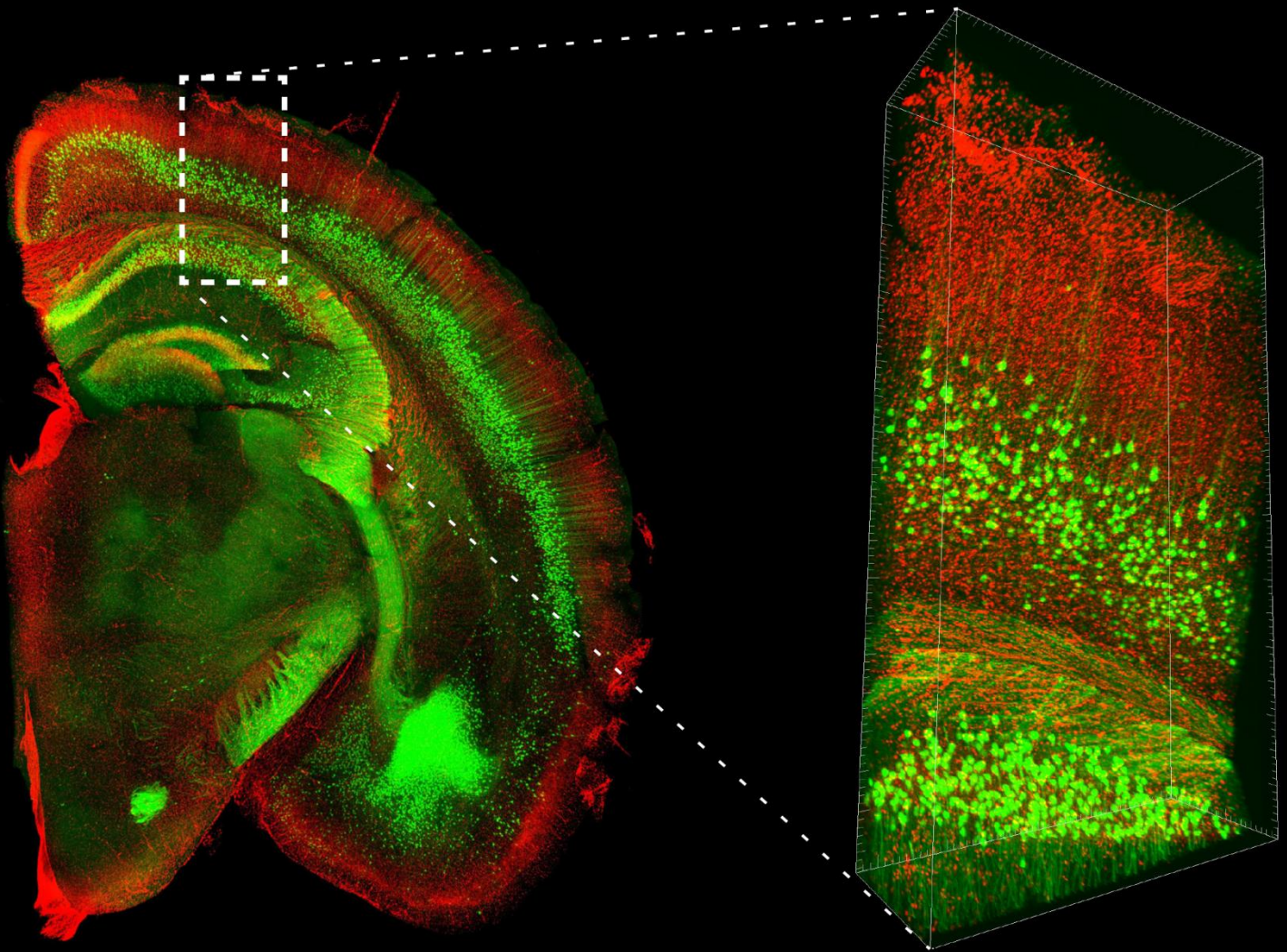


Tissue Optical Clearing Reagent

Take a Revolutionary Approach to Deep Image

SCALEVIEW-S



Data provided by Dr. Hiroshi Hama, Tetsushi Hoshida and Dr. Atsushi Miyawaki,
Laboratory for Cell Function Dynamics, Brain Science Institute, RIKEN
Biotechnological Optics Research Team, Center for Advanced Photonics, RIKEN
Cooperation with Olympus

SCALEVIEW-S Summary

The original recipe reported by the Miyawaki team in 2011 termed *Scale* was an aqueous solution based on urea that limited because the transparency process itself can damage the structures under study..

The research team spent 5 years improving the effectiveness of the original recipe to overcome this critical challenge, and the result is *ScaleS*, (we called SCALEVIEW-S) a new technique with many practical applications.

SCALEVIEW-S creates transparent brains with minimal tissue damage, that can handle both florescent and immunohistochemical labeling techniques, and is even effective in older animals.

The new technique creates transparent brain samples that can be stored in SCALEVIEW-S solution for more than a year without damage. Internal structures maintain their original shape and brains are firm enough to permit the micron-thick slicing necessary for more detailed analyses

Benefits of SCALEVIEW-S

- Easy-to-use
- Less damage to sample
- No special equipment required
- Compatible with IF, FP and other fluorescent labels

SCALEVIEW-S Applications

| | Fixation | Clearing | | | | | | | Imaging |
|------------------------|---|--------------------------|--------------------------|--------------------------|--------------------------|-----------------------------|--------------------------|---------------------------|-----------------------------|
| | Perfusion fixation + post fixation 4%PFA/PBS(-) [pH7.6-7.8] | Step 1. SCALEVIEW@-S0 | Step 2. SCALEVIEW@-S1 | Step 3. SCALEVIEW@-S2 | Step 4. SCALEVIEW@-S3 | Step 5. deScale Solution | Step 6. SCALEVIEW@-S4 | Step 7. SCALEVIEW@-SMT | Mounting: SCALEVIEW@-SMT |
| Processing temperature | 4 °C | 37 °C | 37 °C | 37 °C | 37 °C | 4 °C | 37 °C | 37 °C | RT |
| Processing time | 3days | 30 min | 30 min | 30 min | 30 min | 3 hrsx2 | 12 ~ 24 hrs | 1 hr | |

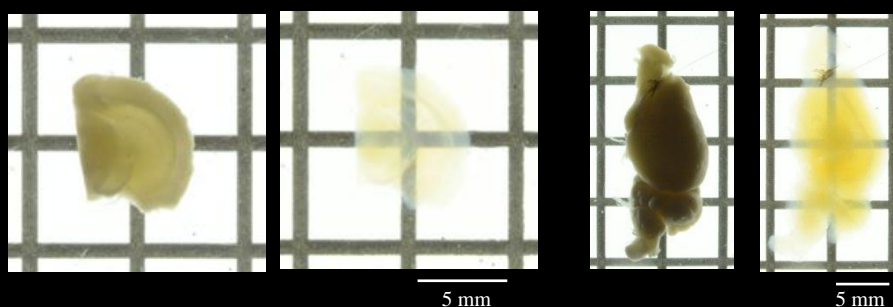


Figure 1. Transmittance images of mouse brain before and after clearing with SCALEVIEW-S Solutions.

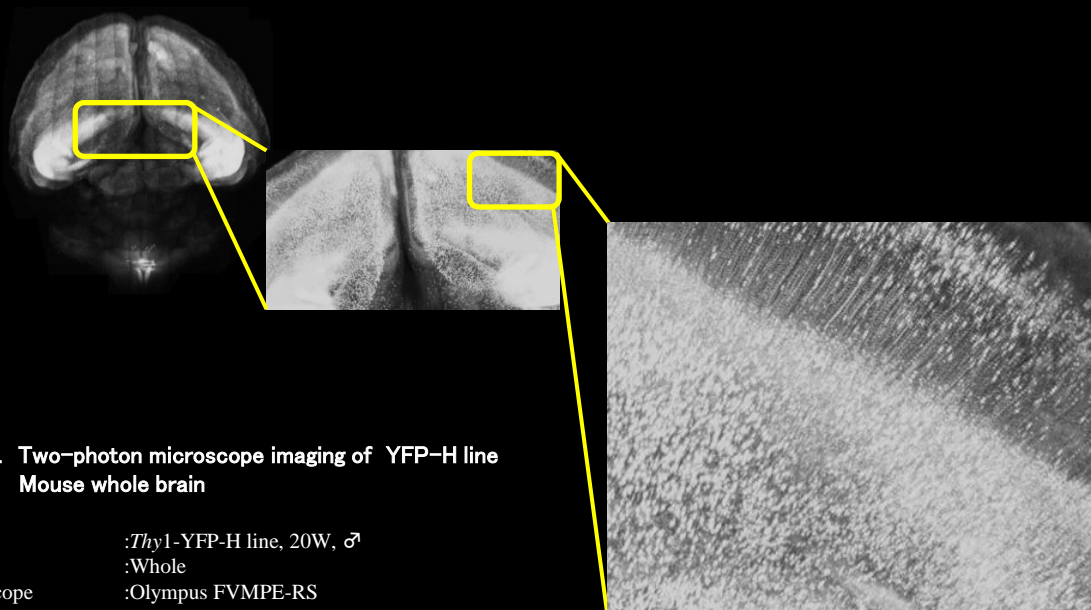


Figure 2. Two-photon microscope imaging of YFP-H line Mouse whole brain

| | |
|----------------|--|
| Mouse | : <i>Thy1</i> -YFP-H line, 20W, ♂ |
| Size | :Whole |
| Microscope | :Olympus FVMPE-RS |
| Objective lens | :XLPLN10XSVM (NA 0.6) |
| Laser | :960 nm (for YFP), |
| Image size | :512 x 512, 170 tiles, Z=8000 μ m, Z Step 16 μ m |

SCALEVIEW-S Applications: IHC(AbScale)

| | Fixation | Preprocessing | | | | | IHC | | |
|------------------------|--|----------------------------------|--------------------------|-----------------------------|--------------------------|-----------------------------|--|-------------------------------------|----------------------------------|
| | Perfusion fixation + post fixation 4%PFA/PBS(-) [pH7.6-7.8] | Step 1. SCALEVIEW@-S0 | Step 2. SCALEVIEW@-A2 | Step 3. 8M Urea Solution | Step 4. SCALEVIEW@-A2 | Step 5. deScale Solution | Step 1. Blocking 1% Blocking Reagent(Roche)/PBS(-) | Step 2. Staining Antibody stains | Step 3. Wash AbScale Solution |
| Processing temperature | 4 °C | 37 °C | 37 °C | 37 °C | 37 °C | 4 °C | RT | 37 °C | RT |
| Processing time | 3days | 4 hrs | 4 hrs | 12 hrs | 4 hrs | 6 hrs | 2 hrs | > 1 day | 2 hrsx1, 1 hrx1 |
| IHC | | Clearing | | Imaging | | | | | |
| | Step 4. Re-fixation 4%PFA/PBS(-) [pH7.6-7.8] | Step 5. Wash deScale Solution | | Mounting: SCALEVIEW@-S4 | | | | | |
| | 4 °C | 4 °C | | 37 °C | | RT | | | |
| | 1 hr | 6 hrs | | 6 - 8hrs | | | | | |

*AbScale Solution:0.33 M Urea and 0.1 %(wt/vol) Triton X-100 in PBS(-) Solution

Iba1 (RF635: Green) Amyloid-β (Alexa Fluor 488: Red) Tomato lectin (Texas Red: Blue)

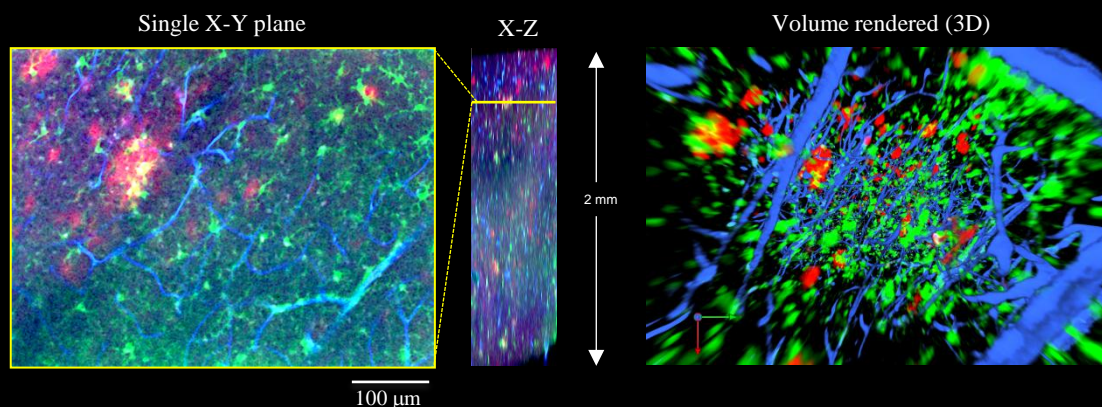


Figure 3. 3D visualization of A β plaques (red), microglia (green) and blood vessels (blue) from a 17-month-old AD model mouse.

Microscope (CLSM) :Olympus FV1200
Objective lens :XLPLN10XSVM (NA 0.60)

SCALEVIEW-S Applications: Fluorescent labels (ChemScale)

| | Fixation | Preprocessing | | | | | Fluorescent labels | | |
|------------------------|--|----------------------------|--------------------------|-----------------------------|--------------------------|-----------------------------|---|-------------------------------|----------------------------------|
| | Perfusion fixation + post fixation 4%PFA/PBS(-) [pH7.6-7.8] | Step 1. SCALEVIEW@-S0 | Step 2. SCALEVIEW@-A2 | Step 3. 8M Urea Solution | Step 4. SCALEVIEW@-A2 | Step 5. deScale Solution | Step 1. FL labels ex:DAPI(500nM), PI(1μg/ml)/SCALEVIEW@-A2 | Step 2. Wash SCALEVIEW@-A2 | Step 3. Wash deScale Solution |
| Processing temperature | 4 °C | 37 °C | 37 °C | 37 °C | 37 °C | 4 °C | 37 °C | 37 °C | 4 °C |
| Processing time | 3days | 4 hrs | 4 hrs | 12 hrs | 4 hrs | 6 hrs | 6 - 8 hrs | 2 hrsx1, 1 hrx1 | 3 hrs |
| Clearing | | Imaging | | | | | | | |
| | SCALEVIEW@-S4 | Mounting: SCALEVIEW@-S4 | | | | | | | |
| | 37 °C | RT | | | | | | | |
| | 6 - 8hrs | | | | | | | | |

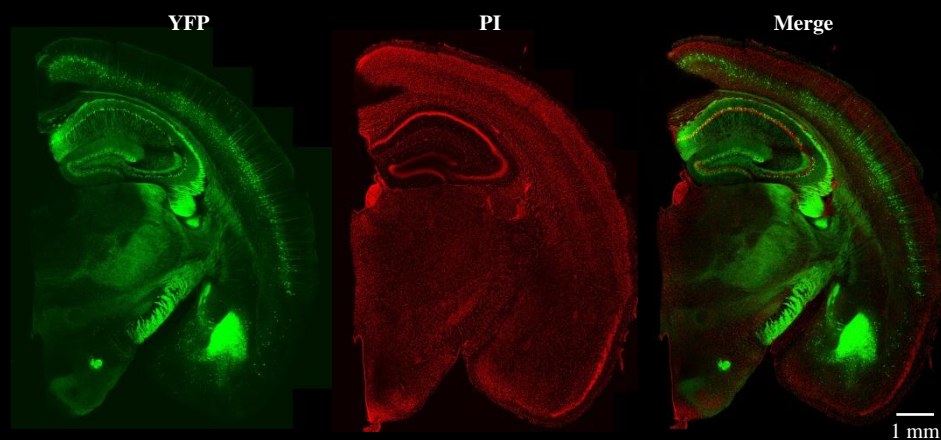


Figure 4. Confocal laser scanning microscope imaging of fluorescent labeled (PI) YFP-H line mouse brain slice (2 mm thick).

Mouse :*Thy1*-YFP-H line, 42W, ♂
Size :Coronal Slice (2 mm)
Microscope (CLSM) :Olympus FV3000 (Inverted)
Objective lens :UPLSAPO10x2 (NA 0.40)
Laser :488 nm (for YFP), 561 nm (for PI)

SCALEVIEW-S Applications: Neurosphere

| | Fixation | Preprocessing | | | | | IHC | | |
|------------------------|--|----------------------------------|--------------------------|-----------------------------|----------------------------|-----------------------------|--|-------------------------------------|----------------------------------|
| | Perfusion fixation + post fixation 4%PFA/PBS(-) [pH7.6-7.8] | Step 1. SCALEVIEW®-S0 | Step 2. SCALEVIEW®-A2 | Step 3. 8M Urea Solution | Step 4. SCALEVIEW®-A2 | Step 5. deScale Solution | Step 1. Blocking 1% Blocking Reagent(Roche)/PBS(-) | Step 2. Staining Antibody stains | Step 3. Wash AbScale Solution |
| Processing temperature | RT | 37 °C | 37 °C | 37 °C | 37 °C | 4 °C | RT | 37 °C | RT |
| Processing time | 1 hr | 4 hrs | 4 hrs | 12 hrs | 4 hrs | 6 hrs | 2 hrs | > 1 day | 2 hrs×1, 1 hr×1 |
| | | IHC | | Clearing | Imaging | | | | |
| | Step 4. Re-fixation 4%PFA/PBS(-) [pH7.6-7.8] | Step 5. Wash deScale Solution | | SCALEVIEW®-S4 | Mounting: SCALEVIEW®-S4 | | | | |
| | RT | 4 °C | | 37 °C | RT | | | | |
| | 1 hr | 3 hrs | | 4 hrs | | | | | |

Primary Neurosphere from Adult Rat Hippocampus (5 days *in vitro*)

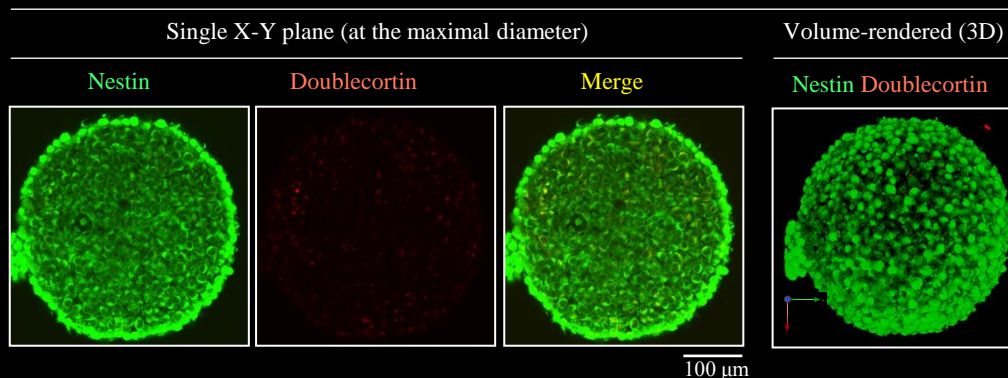


Figure 5. 3D visualization of Neurosphere

【References】

- 1)Hama,H.et al. : *Nature Neuroscience* **14**, 1481(2011).
- 2)Hama,H.et al. : *Nature Neuroscience* **18**, 1518(2015).
- 3)Hama H, et al. : *Protocol Exchange* (2016), doi:10.1038/protex.2016.019
- 4)Molly E, Boutin, et al : *Scientific Reports*, **8**, 11135 (2018).

Microscope (CLSM) :Olympus FV1000
Objective lens :UMPLFLN10XW (NA 0.3)

| Product name | Wako cat. No. | Package Size | Storage Condition | Grade |
|-----------------------|---------------|--------------|-------------------|---------------------------------|
| SCALEVIEW-S0 | 196-18521 | 250ml | Keep at 2 – 10°C | Tissue Optical Clearing Reagent |
| SCALEVIEW-S1 | 193-18531 | 250ml | Keep at 2 – 10°C | Tissue Optical Clearing Reagent |
| SCALEVIEW-S2 | 190-18541 | 250ml | Keep at 2 – 10°C | Tissue Optical Clearing Reagent |
| SCALEVIEW-S3 | 197-18551 | 250ml | Keep at 2 – 10°C | Tissue Optical Clearing Reagent |
| SCALEVIEW-S4 | 194-18561 | 250ml | Keep at 2 – 10°C | Tissue Optical Clearing Reagent |
| SCALEVIEW-SMt | 191-18571 | 250ml | Keep at 2 – 10°C | Tissue Optical Clearing Reagent |
| deScale Solution | 041-34425 | 500ml | Keep at 2 – 10°C | Tissue Optical Clearing Reagent |
| SCALEVIEW-S Trial Kit | 299-79901 | 1kit | Keep at 2 – 10°C | Tissue Optical Clearing Reagent |
| SCALEVIEW-A2 | 193-18455 | 500ml | Keep at RT | Tissue Optical Clearing Reagent |

- Listed products are intended for laboratory research use only, and not to be used for drug, food or human use.
- This brochure may contain products that cannot be exported to your country due to regulations.
- Bulk quote requests for some products are welcome. Please contact us.

FUJIFILM Wako Pure Chemical Corporation

1-2, Doshomachi 3-Chome
Chuo-Ku, Osaka 540-8605, Japan
Tel: 81-6-6203-3741
Fax: 81-6-6203-1999

FUJIFILM Wako Chemicals U.S.A. Corporation

Toll-Free (U.S. only): 1-877-714-1920
Head Office (Richmond, VA)
Tel: 1-804-714-1920
Fax: 1-804-271-7791

FUJIFILM Wako Chemicals Europe GmbH

European Office:
Fuggerstraße 12, D-41468, Neuss, Germany
Tel: 49-2131-311-0
Fax: 49-2131-311100