

Wako

Tissue Optical Clearing Reagent

Sugar solution makes tissues see-through

SeeDB

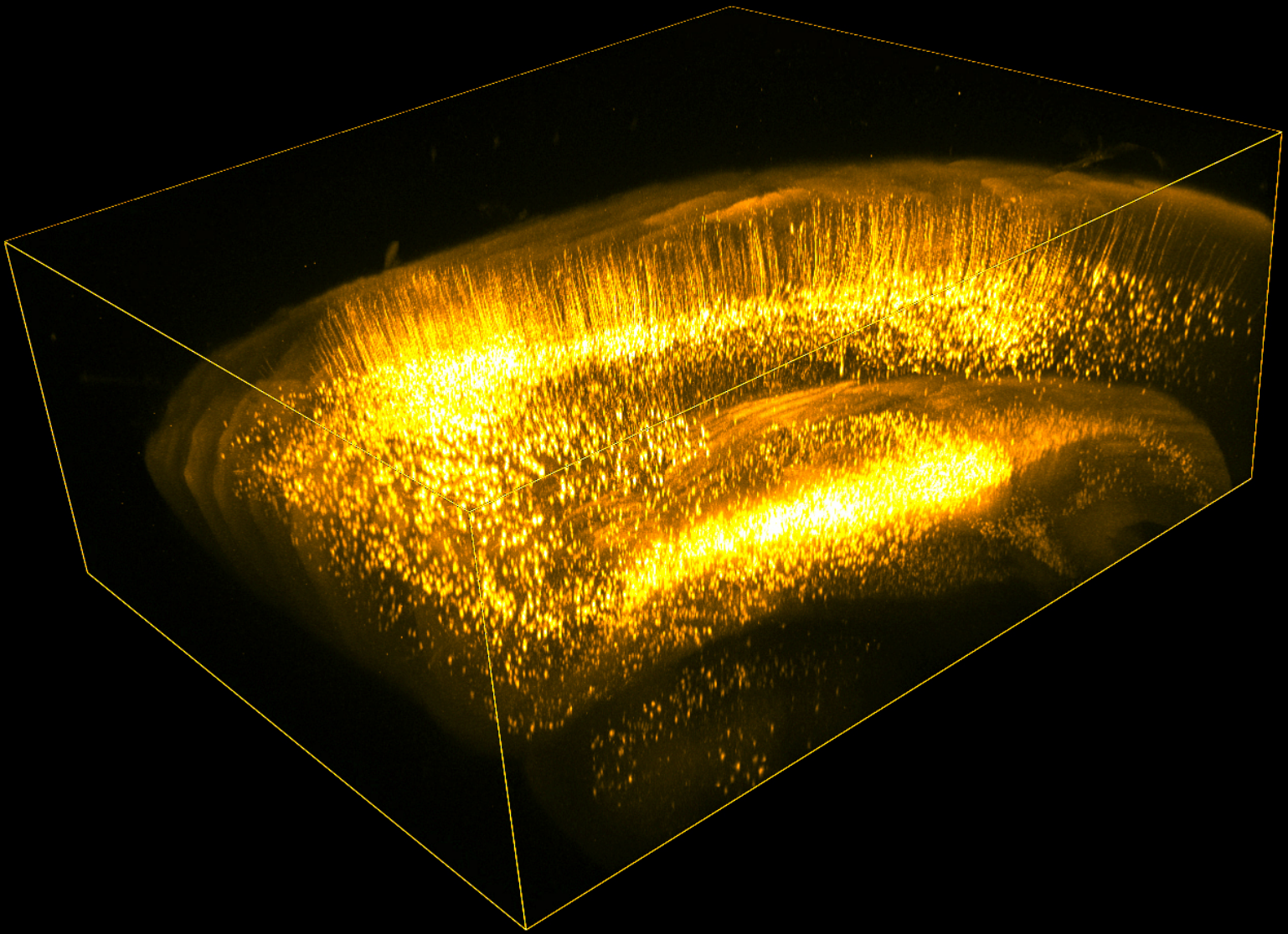


Image data courtesy of: Takeshi Imai, Meng-Tsen Ke,
Laboratory for Sensory Circuit Formation RIKEN Center for Developmental Biology

SeeDB protocol

Dr. Takeshi Imai, et al. developed a water-based optical clearing reagent, SeeDB (See Deep Brain), which clears fixed brain samples in a few days without quenching many types of fluorescent dyes, including fluorescent proteins and lipophilic neuronal tracers. SeeDB is a saturated solution of fructose in water with thioglycerol. This method facilitates comprehensive and quantitative analyses for understanding neuronal circuitry, both in the adult and developing mouse brain.

【Procedure】

(Fixation)

- 1) Fix the sample in 4% PFA at 4 ° C with gentle shaking overnight.
- 2) Wash the sample in PBS three times (10 min each).

(Clearing)

- 3) Incubate the sample in ~20 ml of SeeDB:20w/v% Fructose Solution in 50 ml conical tube, and then place the conical tube on a tube rotator (~4 rpm) or a seesaw shaker (~17 rpm) for 4-8 h, respectively.
A small piece of sample (e.g., slices) requires less time for optical clearing. Incubation should be performed at 25-37 ° C.
- 4) Incubate the sample in ~20 ml SeeDB:40w/v% Fructose Solution for 4-8 h as above.
- 5) Incubate the sample in ~20 ml SeeDB:60w/v% Fructose Solution for 4-8 h.
(Samples may no longer sink in 60% or higher concentrations of fructose.)
- 6) Incubate the sample in ~20 ml SeeDB:80w/v% Fructose Solution for 12 h.
- 7) Incubate the sample in ~20 ml SeeDB:100w/v% Fructose Solution for 12 h.
- 8) Incubate the sample in ~20 ml SeeDB for 24 h. The incubation time can be extended up to 48 h.
- 9) The transparency can be evaluated by eye at this stage . If the sample is successfully cleared, the adult brain sample should look amber under a light source.

(Observation)

- 10) Observe the SeeDB-treated samples under a fluorescence microscope. Samples should be mounted in SeeDB solution.

1. Image of a mouse brain after SeeDB process

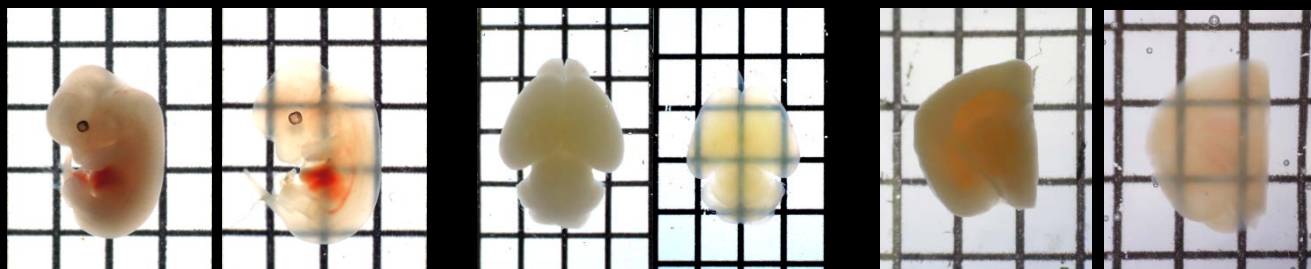


Figure.1 Mouse embryo and mouse brain before and after treatment with SeeDB

2. Imaging data using SeeDB

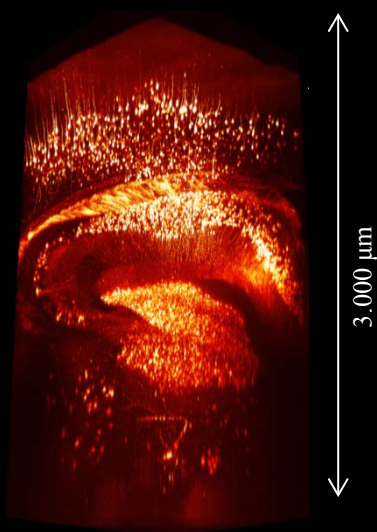


Figure2. Imaging of Thy1-YFP(H Line) mouse brain after SeeDB process using Multiphoton microscope and Multiphoton dedicated objective :OLYMPUS, model:XLPLN10XSVM.

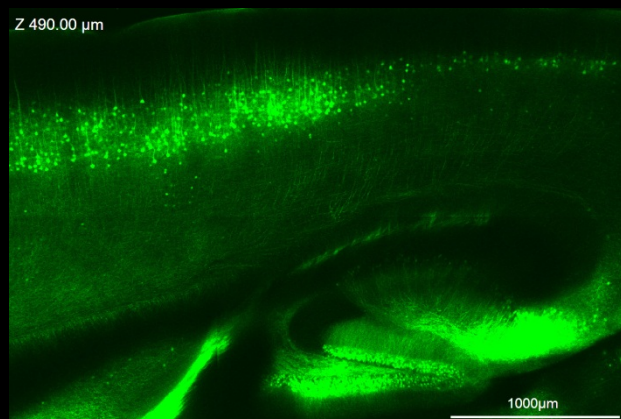


Figure 3. Imaging of Thy1-YFP(H Line) mouse brain after SeeDB process using Confocal microscope and objective :OLYMPUS, UPLSAPO 10X2.

Imaging data using SeeDB

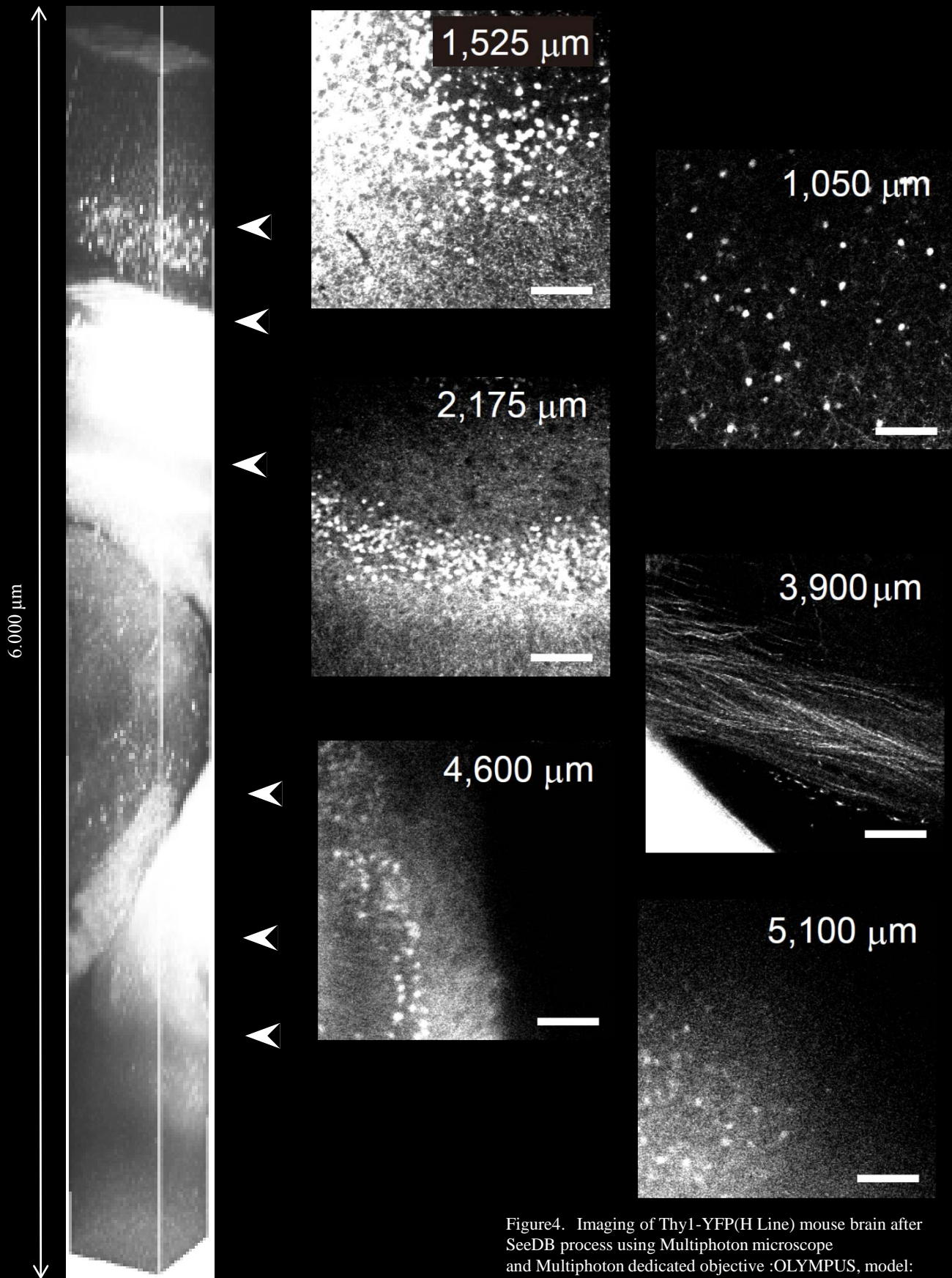


Figure4. Imaging of Thy1-YFP(H Line) mouse brain after SeeDB process using Multiphoton microscope and Multiphoton dedicated objective :OLYMPUS, model: XLSLPLN25XGMP. Bars, 50 μm .

Imaging data using SeeDB

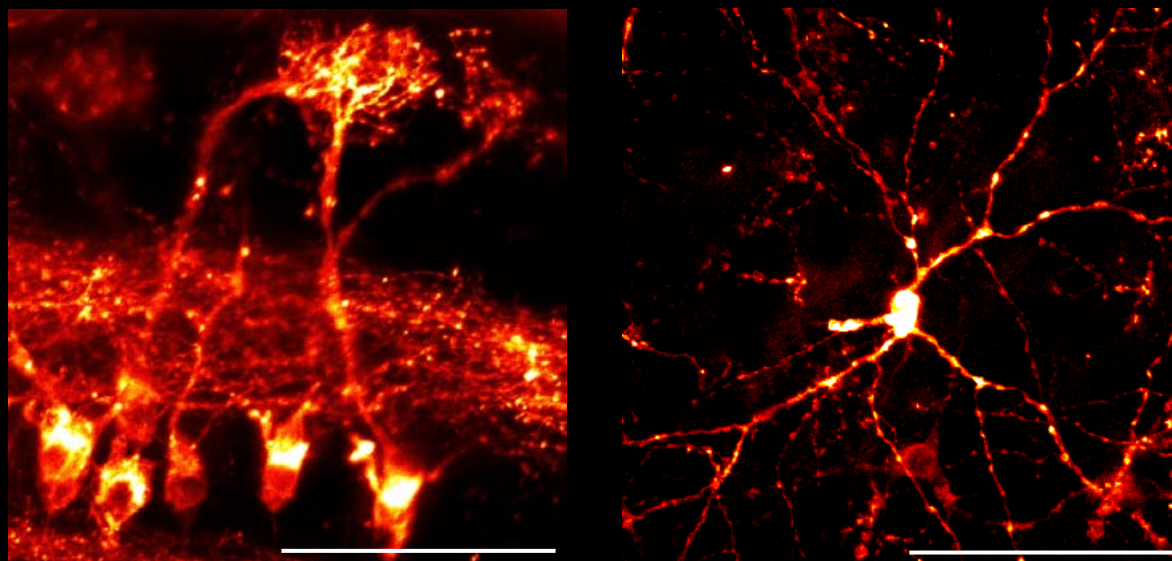


Figure.5 Imaging of DiI-labeled mitral cells in the olfactory bulb after SeeDB process using Confocal microscope and objective :OLYMPUS, UPLSAPO 20X. Bars, 100 μ m.

References

- 1) Ke, M. T., Fujimoto, S. and Imai, T. : *Nat Neurosci* ,**16** (8),1154 (2013).
- 2) Ke, M. T., Fujimoto, S. and Imai, T.: *Bio-protocol* **4**(3), e1042 (2014).
- 3) Ke, M. T., and Imai, T. : *Curr Protoc Neurosci* ,**66**,2.22.1-2.22.19 (2014).

Wako cat. No.	Product name	Grade	Package Size	Storage
291-79601	SeeDB Trial Kit	Tissue Optical Clearing Reagent	1kit	Keep at RT
193-18391	SeeDB:20w/v% Fructose Solution	Tissue Optical Clearing Reagent	250ml	Keep at RT
196-18401	SeeDB:40w/v% Fructose Solution	Tissue Optical Clearing Reagent	250ml	Keep at RT
193-18411	SeeDB:60w/v% Fructose Solution	Tissue Optical Clearing Reagent	250ml	Keep at RT
190-18421	SeeDB:80w/v% Fructose Solution	Tissue Optical Clearing Reagent	250ml	Keep at RT
197-18431	SeeDB:100w/v% Fructose Solution	Tissue Optical Clearing Reagent	250ml	Keep at RT
194-18441	SeeDB	Tissue Optical Clearing Reagent	250ml	Keep at RT

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