FUJIFILM
Value from Innovation

Wako

Plant Optical Clearing Reagent

ClearSee™

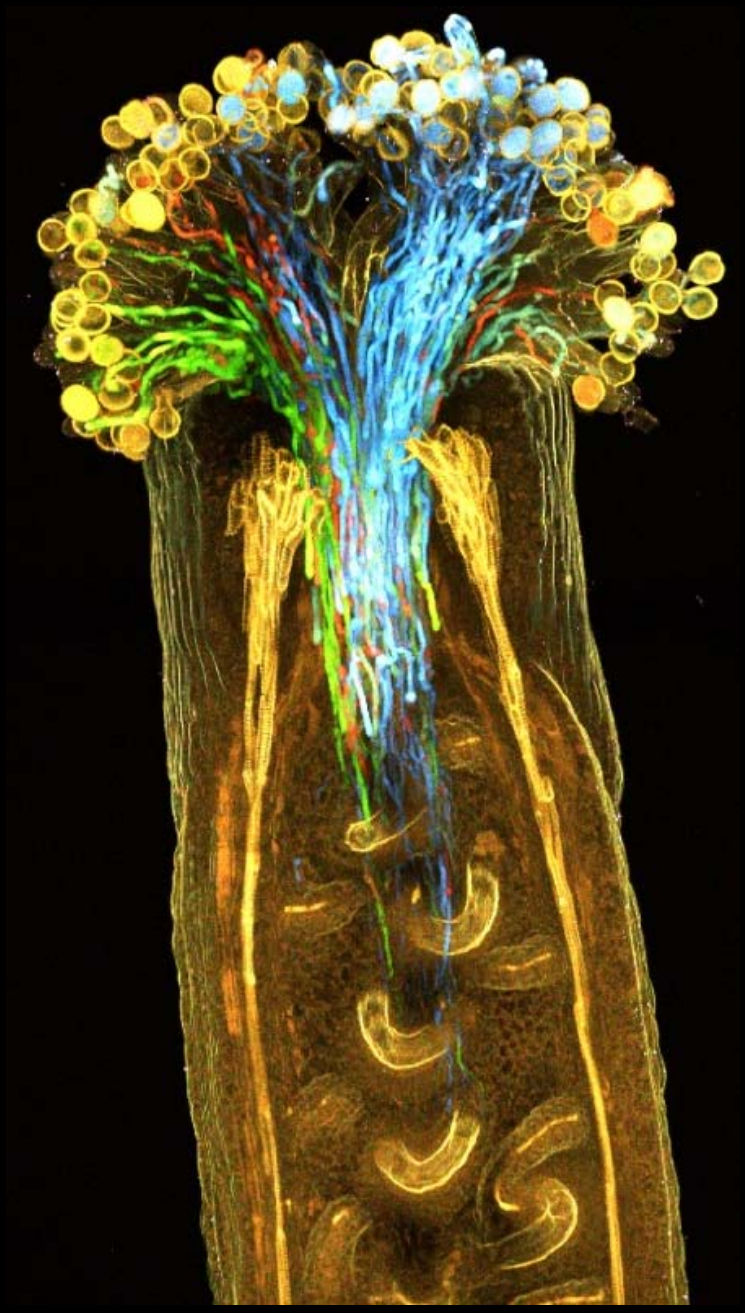


Image data courtesy of: Daisuke Kurihara and Yoko Mizuta
from Graduate School of Science, Nagoya University, Nagoya, Japan

Protocol of ClearSee™ treated sample

Dr. Daisuke Kurihara (Nagoya University) developed the clearing solution, termed ClearSee™, to diminish chlorophyll autofluorescence while maintaining fluorescent protein stability and tissue structures of plant samples. ClearSee™ is applicable to multicolor deep imaging of various plant samples without sectioning.

【Features】
- Procedures are only 3 steps.
- Every fluorescence microscopes can be used.
- ClearSee™-treated samples can be store for long term (at least 6 months).
- The ClearSee™-treated samples can be observed for many times.

1. Fixation

1.Put plant samples in a microtube and add 1.3 ml of fixative solution

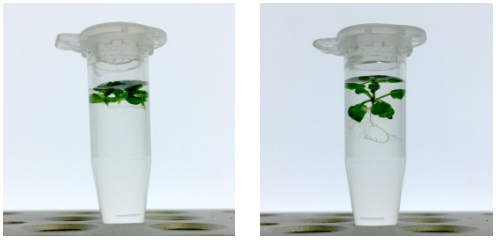


Fig.1 *Arabidopsis* leaves and seedling in fixative solution

2. Put the samples in the desiccator and turn on the vacuum pump. After closing a desiccator and turn off the vacuum pump, fix for 30 minutes at room temperature.

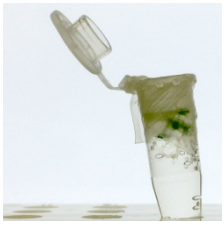


Fig.2 Bubbles from the samples in a microtube

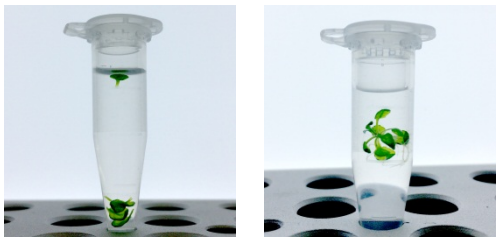


Fig.3 Arabidopsis leaves and seedling after fixative treatment

2.. Wash

3. Slowly open the desiccator. After removing the fixative solution, add 1 ml of 1x PBS and store for 1 minute (in the draft chamber).
4 After removing 1x PBS, add new 1 ml of 1x PBS and store for 1 minute.

3. Clearing

5. After removing 1x PBS, add 1.3 ml of ClearSee™ .
6. Seal the microtube with parafilm and open holes by a needle. Put the samples in the desiccator and turn on the vacuum pump. After closing a desiccator and turn off the vacuum pump, store for 1 hour at room temperature.

4. Clearing process

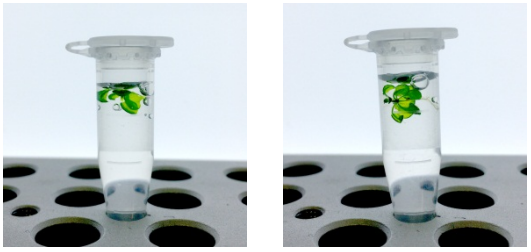


Fig. 4 *Arabidopsis* leaves and seedling in ClearSee™ solution

Fig. 5 Chlorophyll are observed around the samples (1~2 days incubation)

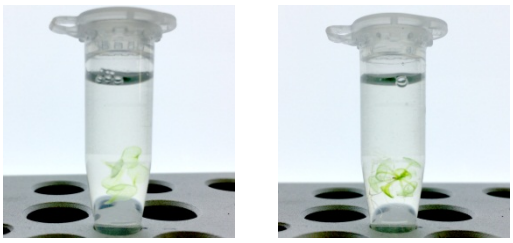


Fig. 6 Substitute new ClearSee™

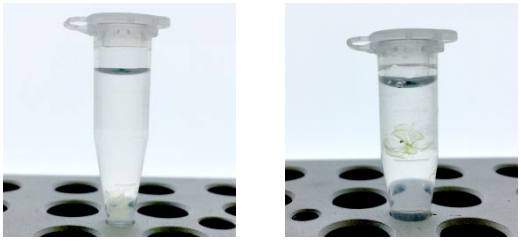


Fig. 7 Cleared Arabidopsis leaves and seedling (3~5 days incubation)

Data of ClearSee™ treated samples

5. Observation

7. Put vaseline on a slide glass.
8. Transfer the ClearSee™ -treated samples into ClearSee™ on a slide glass.
9. Cover with a cover glass and seal with the cover glass with vaseline.
10. Observe the ClearSee™ -treated samples under a fluorescent microscope.

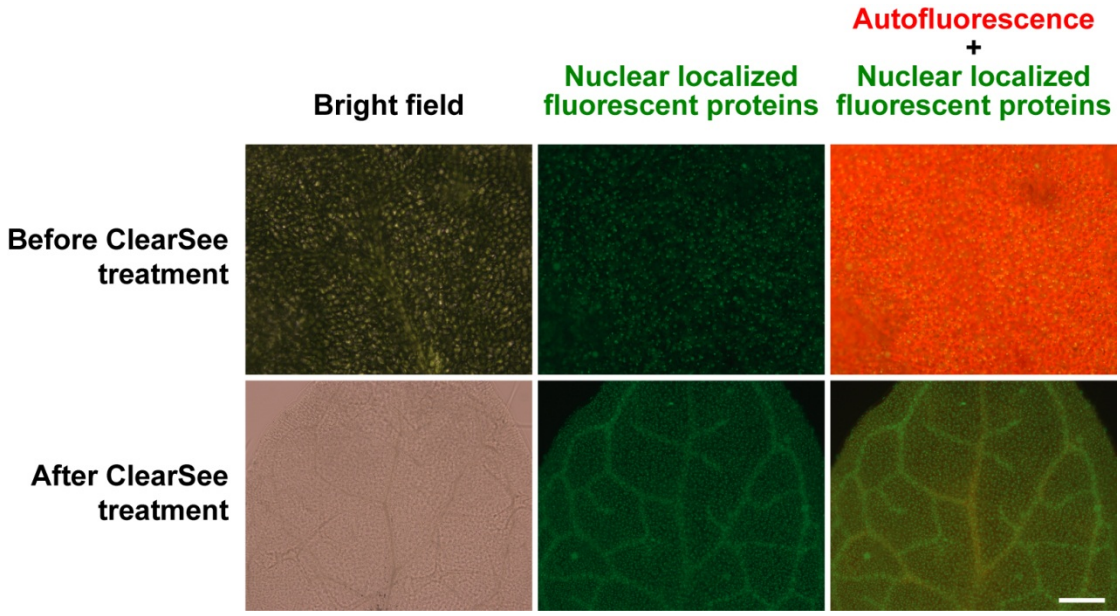


Fig. 8 The ClearSee™-treated samples are cleared under a bright field. Autofluorescence of chlorophyll are diminished. The fluorescent proteins are detected even in the vascular bundles. Bar : 200 μm.

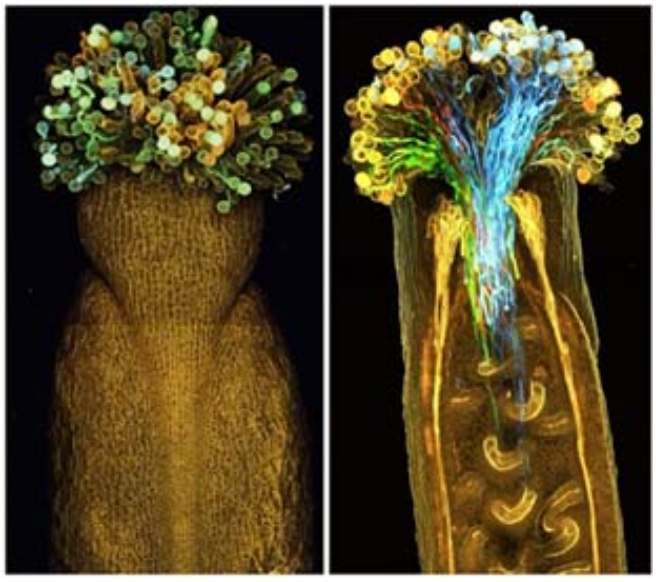
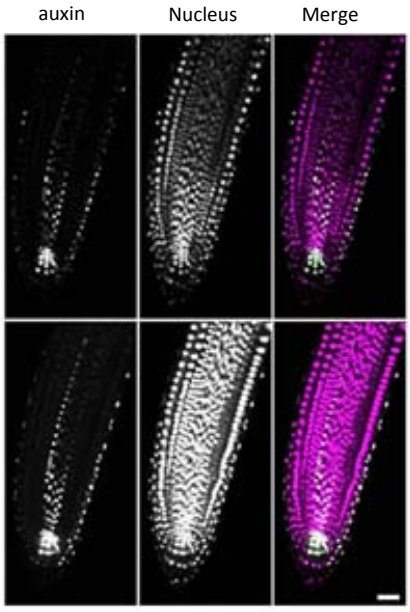


Fig 9 Image of ClearSee™ for multi color imgin g of the whole pistil

Left: a whole pistil before ClearSee™ process Right: a whole pistil after ClearSee™ process



Bar:30μm

Fig.10 Image of ClearSee™ treated *Arabidopsis* root

Data of ClearSee™ treated samples

ClearSee™ is applicable to various plant species other than Arabidopsis. Fig. 11 shows ClearSee™ -treated leaves of torenia, petunia, tobacco, tomato, cucumber, and rice after 6-days incubation. In the case of rice, removal of cuticular wax are required for penetration of solution. Immerse the rice samples into organic solvents such as chloroform for 10~30 seconds and then fix with a fixative solution.

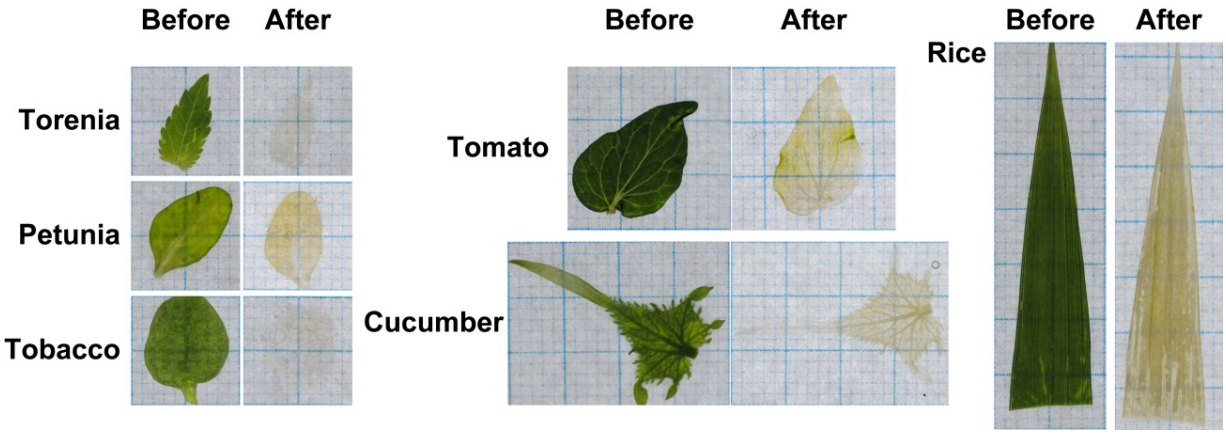


Fig. 11 ClearSee™-treated leaves of various plant species (6-days incubation).

References

1) Kurihara, D. *et al.* : *Development*, **142**, 4168-4179（2015）.
2) Nagaki ,K. *et al.* : *Scientific Reports* **7**, 42203(2017).
3) Ohtsu,M. *et al.* : *Protoplasma* (2017).
4) Kalmbach,L. *et al.*:*Nature plants*, **3**,17058(2017)..

Reagent List

Wako cat. No.	Product name	Grade	Package Size	Storage Condition
031-25151	ClearSee	Plant Optical Clearing Reagent	50ml	Keep at room temperature

■ 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:
Fuggerstraβe 12, D-41468, Neuss, Germany
Tel: 49-2131-311-0
Fax: 49-2131-311100