APPLICATION NOTE

Multiphoton confocal microscope A1R MP+

The Secret Inside Flowers - Imaging Inside Plants Using ClearSee Clearing Reagent

Observation of the structure is very important in understanding how each organ or tissue of living beings functions, but it is difficult to see deep inside them because the bodies of most living beings, especially plants are not transparent. Dr. Daisuke Kurihara and Dr. Yoko Mizuta of the Institute for Transformative Bio-Molecules, Nagoya University, have developed a reagent named ClearSee which can make a whole plant transparent without diminishing fluorescent proteins. This reagent is capable of making various plants transparent and allows observation deep inside plants, maintaining their structure and fluorescence.
In this Application Note, we introduce examples of clearing the flowers of *Arabidopsis thaliana* with ClearSee and observing them with a multiphoton excitation confocal microscope suited for observation of deep parts of organisms. This enables us to observe in detail how each of the pollen tubes elongate in the pistil for fertilization, using the color-coding of the pollen tubes.

Plant structure and autofluorescence

The pollen tube elongates in the innermost part of flowers, but since plants consist of various cells with different refractive indices, it has been difficult to see the inside of them without section preparation or dissection. However, dissection results in the breaking of tissue structure, making analysis with fluorescent protein difficult. Also, since chlorophyll in chloroplasts has strong autofluorescence, it obstructs observation.

ClearSee plant-clearing reagent

Dr. Daisuke Kurihara and Dr. Yoko Mizuta explored the possibility of a plant-clearing reagent which diminishes chlorophyll from plants but retains fluorescent protein, resulting in the development of ClearSee, consisting of urea, surfactant, and xylitol.
Plants become transparent by simply being immersed in ClearSee after formalin fixation (Fig. 1). Four days later, autofluorescence disappears and plants can be cleared while retaining GFP (green fluorescent protein) (Fig. 2).

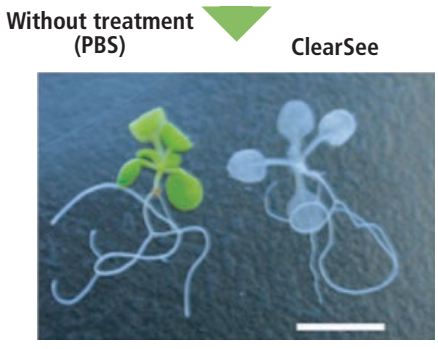

Fig. 1:
The plant can be made transparent simply by immersing it in ClearSee after fixing it with formalin.
Scale bar: 5mm.

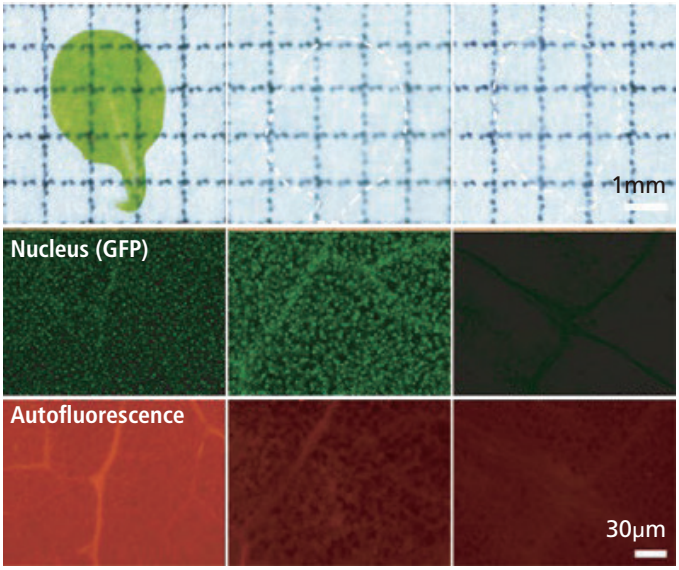

Fig. 2:
Comparison of day four plant leaves after respectively performing no treatment, ClearSee treatment and treatment with a chloral hydrate-based clearing solution.
It can be seen that autofluorescence in leaves treated with ClearSee disappeared and the leaves were cleared while retaining GFP.

Without treatment

ClearSee

Fig. 3:
Multiphoton excitation confocal microscopy images of an untreated pollen tube and a pollen tube after treatment with ClearSee.
Sample: Fluorescent protein (mApple, Venus, mTFP1)-labeled pollen tubes of a pistil of *Arabidopsis thaliana* cleared with ClearSee.
Objective: CFI75 Apochromat 25XC W 1300, NA: 1.10, W.D.: 2.00 mm
Images courtesy of: Drs. Daisuke Kurihara and Yoko Mizuta, Institute for Transformative Bio-Molecules, Nagoya University

NIKON JOICO AWARD
(Japanese only)

Inside of flower observed with multiphoton excitation microscopy

The flowers of *Arabidopsis thaliana* were pollinated with pollens labelled with blue, green, and red fluorescent protein and cleared using ClearSee after formalin fixation, then observed using the A1R MP+ multiphoton confocal microscope with an excitation wavelength of 990 nm (Fig. 3).
As a result, the pistils in the whole flower could be observed without other treatment such as dissection. In addition, spatial studies became possible while retaining the tissue morphology, such as how and where pollen tubes elongate.

Reference:

Daisuke Kurihara, Yoko Mizuta, Yoshikatsu Sato, Tetsuya Higashiyama (2015)
ClearSee: a rapid optical clearing reagent for whole-plant fluorescence imaging.
Development. 142(23): 4168–4179.

Yoko Mizuta, Daisuke Kurihara and Tetsuya Higashiyama (2016)
Visualization of plant sexual reproduction in the whole-mount pistil by ClearSee.
Cytologia 81(1): 1-2.

Product Information

A1R MP+ multiphoton confocal microscope

This microscope achieves high-speed and high-resolution imaging because it is equipped with both resonant and Galvano scanners. It can support simultaneous excitation imaging using a 1300 nm-compatible dual beam IR laser. It can be mounted on both upright and inverted microscopes, and supports single-photon confocal imaging.

- High speed: up to 720 fps (512 x 16 pixels)
- High resolution: up to 4096 x 4096 pixels