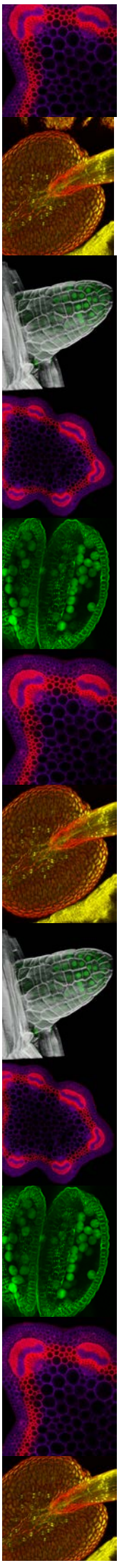

CLEARSEE PROTOCOL

ClearSee solution:

XYLITOL [final 10% (w/v)],

SODIUM DEOXYCHOLATE [final 15% (w/v)]

UREA [final 25% (w/v)]

WATER to the final volume

Mix the solution well on the magnetic stirrer until you get everything completely dissolved!

- Fix the Arabidopsis seedlings with 4% **PFA (paraformaldehyde)** for 30–120 min (seedlings, 30 min; leaves, 120 min; pistil, 60 min; gametophores, 60 min) in **1 x PBS** at room temperature. At least for roots it works very well without vacuum.
- Wash twice the fixed tissues for **1 min in 1 x PBS.**
- Transfer the seedlings to **ClearSee** solution and clear them at room temperature. For young roots (4-6 days) 1-2 days is sufficient. Otherwise clear longer for older roots, shoots and leaves.

According to the publication ClearSee-treated samples could be stored at room temperature for at least 5 months (can confirm at least after 1 week look good).

For **fuchsin** staining after clearing, prepare the fuchsin solution in ClearSee, stain the roots for 12-24 hours and wash in ClearSee for 30min -1 hour. Also longer washing might reduce the background noise.

HOW TO MAKE 4% PFA:

Easy protocol for 4% **PFA (PARAFORMALDEHYDE)**

1. Take 4g of paraformaldehyde powder and add 1X PBS to 100ml to 4% final concentration.
2. Transfer to the stirrer. Heat while stirring to approximately 60 °C. **Take care that the solution does not boil! Don't go over 70**°C!
3. The powder will not immediately dissolve. Slowly raise the pH by adding 1 N NaOH dropwise from a pipette until the solution clears.
4. Once the paraformaldehyde is dissolved, recheck the pH, and adjust it with small amounts of HCl to approximately 6.9 pH;
5. Cool down the solution before use.

Note: the solution can be aliquoted and frozen. Try to use always fresh PFA and store it in -20 no longer that for a week.