Methanol-based combined Fluorol Yellow and Calcofluor White staining to visualise Arabidopsis root suberin and cell walls

1. Fix your seedling in methanol for at least three days. If you plan to keep them longer, be aware of evaporating methanol and seal you plates with parafilm. Exchange with fresh methanol at least twice;
2. Transfer your seedlings to Fluorol Yellow 088 (0.01% in methanol) for at least 1hour. Stain in dark with gentle agitation;
3. Rinse the seedling shortly in methanol and counter-stain the samples in aniline blue (0.5% in methanol) at room temperature for 1hour in darkness with gentle agitation;
4. Shortly rinse the seedlings in water;
5. Mount the seedlings in water and use the GFP settings to vizualize the suberin. Use low laser power to avoid fast bleaching. Parafilm-based spacers can be used on both sites of the slide to avoid squeezing the root. Optimal images can be achieved by using the chambered coverglass for confocal imaging where a slice of agar is placed on the top of the stained seedlings.

Fluorol Yellow staining combined with Calcofluor White:

- After fixing and clearing you seedlings in methanol for 3 days, transfer them to Calcofluor White (0.1% in methanol) and stain for at least one day (Calcofluor White is very stable, the samples can stay for long time);
- Proceed to the Fluorol Yellow staining as described above;
- To separate the two dyes, use sequential scan mode with 405 laser to visualise Calcofluor White and 488 laser for Fluorol Yellow.