Optical clearing of biological tissue with Sca*l*eA2 (20110927)

The following is a typical protocol for mouse brain.

Step 1

Fix a mouse via transcardial perfusion with 4% PFA (paraformaldehyde) /PBS (w/v) (pH 7.5–8.0) at room temperature (RT).

Acid fixatives (pH < 7.0) may quench FPs irreversibly. On the other hand, use of alkaline fixatives (pH > 8.0) may results in damage to samples later at Step 8.

Step 2

Remove the brain and postfix it with 4% PFA/PBS (pH 7.5–8.0) at 4 ºC for 8–12 hrs.

It may be hard to fix the brain of older mice (> 3 weeks old) across the pia mater. For older mice, it is recommended to split the brain into two pieces. For example, unless commissural connections are examined, cut the brain at mid-plane into two cerebral hemispheres. The following procedure assumes that a cerebral hemisphere is processed as a sample to be cleared. Alternatively, if you prefer to keep the entire brain intact, making a few incisions will facilitate fixation throughout the whole brain.

Step 3

After washing with PBS, incubate the sample in 20% sucrose /PBS (w/v) (pH 7.4–7.8) at 4 ºC (or RT) for 1–2 days.

Step 4

Embed the sample in OCT compound (Sakura) and freeze with liquid N_2.

Step 5

Thaw the sample in PBS (25 ml/ 0.5 g tissue) at RT for 20 min with gentle shaking.

Step 6

Rinse the sample in PBS (25 ml/ 0.5 g tissue) at RT for 20 min with gentle shaking.

Step 7

Fix the sample again with 4% PFA/PBS (pH 7.5–8.0) for 30 min at RT.
It is highly recommended that sample fluorescence is checked at every step prior to Scaling (Step 8). It is somewhat common that the fluorescence can easily be lost during the fixation process. For example, incomplete fixation may result in wash out of soluble forms of fluorescent proteins (FPs).

Step 8

Transfer the sample into Sca*l*eA2 solution (20 ml/ 0.5 g tissue) in a see-through vial.
Since ScaleA2 is free of salt, PBS-derived salt remaining in the sample is gradually washed out. In the process of tissue clearing, salts cause white precipitates and thus should be avoided.
It is important to use a container where you can easily assess sample transparency.

Step 9

Incubate the sample in Sca*l*eA2 at 4 ºC (or RT) for 2–14 days or longer with gentle shaking. Exchange Sca*l*eA2 if necessary.
Clearing larger or harder (from older animals) samples requires longer incubation times. Check the transparency of the sample intermittently. Scale makes samples soft and fragile, like jelly, so care should be taken not to damage or destroy the sample.
It may be hard to clear the brain of old mice (> 3 weeks old) across the pia mater. It is thus recommended the brain be split into two pieces or introduced with a few slits (see Step 2).

Step 10

Observe the sample under an upright microscope. Fresh Sca*l*eA2 solution is used as the immersion medium.
If the sample needs to be stabilized over an extended time period for observation, please see "the procedure for immobilizing cleared sample."

Step 11

Store the cleared sample in fresh Sca*l*eA2 solution at 4 ºC (or RT). Fluorescence is not lost over time during long-term storage. There is no need to add preservatives such as sodium azide.