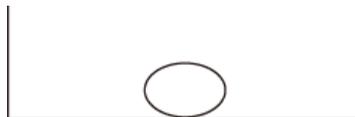


## Immobilizing Scaled samples

**Melted agarose-water solution:** 0.35% (w/v) agarose dissolved in Milli-Q water (Melt in a microwave oven and cool down to 37–40°C).

### Step 1

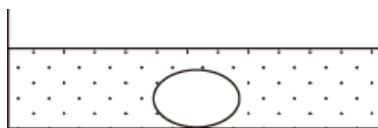
Transfer a Scaled sample from Scale solution onto a plastic dish measuring 60–100 mm diameter. Air-dry for 10 min at RT. To dry the surface of the sample lightly, wick away excess Scale solution carefully with filter paper.



Scaled samples are difficult to cut due to their softness. If the sample needs to be trimmed for observation, follow the procedures in Steps 2–6. If not, go to Step 7.

### Step 2

Fill the dish with a melted agarose-water solution to embed the sample completely. Avoid introducing air bubbles into the solution.



### Step 4

Trim the sample embedded in the agarose gel with a scalpel.



### Step 5

Remove the agarose gel from the trimmed sample carefully.

### Step 6

Place the (trimmed) *Scaled* sample onto a plastic dish measuring 60–100 mm diameter for observation using an upright confocal or two-photon excitation microscope. Position the sample with the observation part on top.



### Step 7

Pour a melted agarose-water solution gently over the top of the sample. Let the viscous solution run radially down the sides. After hardening, the mountain-shaped agarose gel covers most of the sample deeply, but the summit should be covered with only a thin film of agarose gel. In this setup, the observation part should be accessible to an objective lens.



### Step 8

Air-dry the surface of the entire agarose gel for approximately 30 min at RT. Then immobilize the gel edge onto the surface of the plastic dish with fast-drying adhesive (Aron Alpha or Crazy Glue).



### Step 9

Pour *ScaleA2* solution into the dish until the mountain is submerged. Gently shake the plastic dish using an orbital shaker for 3 hours at RT. The *ScaleA2* solution is being diluted with the remaining water. Repeat this step with new *ScaleA2* solution.

**Step 10**

When *ScaleA2* is fully substituted as an immersion medium, the sample is ready for observation.

