FAQs: Protocol

General remark:

The volume of the smaller set is around 12ml (in total), which is usually sufficient for 2-3 mice. Although it depends on what exactly is your organ of interest. There are also bigger sets with around 50-60 ml of total volume. It would eventually be more suitable for you; in case you have many animals in the group.

Here is the description of how we do it (on example of a 25g mouse):

1. I would even call it step zero: you prepare the contrast agent according to the illustrated instruction enclosed in every kit (besides the contrast agent kit and accessories kit you may need a vortex). Prepare the two syringes and let them stand for a while (between 15 min and 2 hours) before using (the idea is for the microbubbles to leave the fluid or at least get close to the surface so that they will not enter the outflow from the syringe). After the syringes are prepared, I start with the mouse.
2. Mouse: we usually inject the heparine a few minutes before or simultaneously with the injection of the mixture for deep anaesthesia. The dose should be enough to prevent coagulation (better too much than not enough). I usually inject around 50ul of heparine solution (Liquemin, concentration 5000 IU/ml) intraperitoneally. Once heparinized, the mouse can wait for some minutes (you do not have the stress to do it as quick as possible).
3. I open the abdominal cavity and then thorax in order to have a practically unrestricted access to the descending aorta of the mouse.
4. The major supplying artery (in case of mouse – it is usually aorta) should then be carefully cannulated. For the hindlimbs or kidneys – in antegrade direction, for brain/eye – in retrograde direction. For the in situ fixation of the cannula I put two ligations (sure is sure). To reduce the volume of the contrast agent needed (it is expensive) and to improve the quality of the perfusion, it is advisable to clamp away the non-needed branches (e.g., abdominal aorta below renal arteries in case of kidney perfusion or mesenteric vessels in case of perfusion of the hind limbs; or the left ventricle in case of brain). Remark: it is possible to make the perfusion “through the heart” – but from my experience, the perfusion is worse and the outcome is not that reproducible (let alone the fact that you will need much more of the contrast agent)
5. The blood should be washed out using the warm (approx. 40 degrees) PBS with heparine. The perfusion with PBS (or NaCl) and heparine should continue till the tissue of interest becomes completely pale. One of the ways is to visually control the outflow) or the color of the muscle tissue. If you perfuse tumors, especially within the bone tissue, or bones, the PBS perfusion should be continued for some time more, even if the outflow and tissues seem to be completely pale (that is our experience). To allow the outflow I usually make a few cuts of the liver edge (3-4 of them, up to 4-5mm deep). Cutting the right atrium makes the outflow too fast.
6. Perfusion with the previously prepared µAngiofil (there should be enough time for the microbubbles to “come out”) through the aforementioned cannula. For that we use the specially modified perfusion pump with the constant volume speed (you can do it manually or use the two separate syringe pumps). Since the contrast agent is blue – the visual control of perfusion success is rather straightforward. The perfusion should be continued till the outflow contains only blue contrast agent (not “interrupted” by some PBS or even blood inclusions) – it is critical for the proper/continuous filling of the venous tree. In case of the

bones – one should continue the perfusion somewhat longer since we cannot visually control the perfusion success within the bone!

7. Once the perfusion with µAngiofil is finished, the major vessels should be clamped to keep the pressure/volume. The sample should be left for some 30 minutes for the contrast agent to polymerise (we cover it with wet paper or gauze to prevent drying).
8. The material can be harvested and fixed via immersion in 2-4% PFA solution (at 4°C). Once fixed it can be transferred to PBS solution and kept for longer time (in the cold room or fridge). We often leave the samples in the PFA solution.
9. The sample can be scanned next day or months after perfusion (tested).

Further remarks:

- it is advisable to have not more than 20-30 minutes between Heparine injection and step 5 (not keeping to that may lead to crucial problems during the perfusion).
- Please avoid having elements like Ca or of even higher atomic number in your solutions – having them could negatively influence the signal-to noise ratio (at least in case of our microCT-systems).
- It is important for the sample/animal to be warm (35-40 degrees) during the perfusion. If you wash out the blood with the cold PBS/saline it will cool down the contrast agent once it is in the vessels and will have a negative influence on its viscosity. That may lead to problems with the perfusion of the capillary bed.
- We usually do not use the vasodilating cocktails, but their usage will be most probably beneficial for the perfusion and the study.
- In the studies with mice we mainly use the volume speed between 0.5 and 1.5ml/min.
- Please pay EXTRA attention for the microbubbles not to get into your vascular system of interest (you will have discontinuous filling!)
- The protocol how to prepare the contrast agent is provided in every set with the contrast agent and is nothing special (having a vortex is advisable).