



VISUALSONICS

Guide to obtaining consistent and reproducible results in the mouse kidney, using Micromarker Contrast Agents

Note: Suggestions below can also be applied to other biological targets

Animal Handling:

1. Temperature regulation is very important; the temperature should be maintained between 37-38°C: once stable the temperature should be maintained within 0.1 to 0.2°C throughout the study, or during subsequent imaging sessions. To accomplish this one must be acutely aware of the animals temperature and be constantly monitoring this parameter.
2. Anesthesia should be maintained at the same level for all animals, by controlling the temperature and the anesthesia the heart rate and respiration rate should be fairly similar within and between imaging sessions in the study.
3. When handling the animals all procedures should be done as quickly as possible, of course maintaining proper procedure and protocol, however the amount of time under anesthesia should be minimized.

Diluting and Administering the Contrast Agent:

1. When a dilution of the stock contrast agent is necessary it should be done using pipettes to be as accurate a possible, and so that each subsequent dilution is the same as the previous ones.
2. A fresh dilution of the stock contrast agent should be completed just prior to each injection; this is crucial as once the contrast agent is removed from the vial it is destabilized. The dilution should be used quickly, and any stock removed from the vial and diluted agent should be discarded.
3. If delivering a small quantity of contrast agent, less than 50ul, than a more accurate method of delivery is advisable, as less than 50ul is very hard to deliver accurately with the provided syringes. Something like a Hamilton syringe is ideal for this type of delivery.
4. If using an infusion pump to deliver the bolus of contrast agent, preloaded into your tubing, the infusion speed should be set to 800ul/min, with an infusion volume large enough to deliver the entire bolus to the animal. This

speed will allow the user to see the wash-in of the bubbles rather than just a slow gradual increase in signal.

5. If not using an infusion pump to deliver the bolus of contrast agent than a constant and consistent hand deliver technique needs to be established before beginning a large study.
6. When possible a tail vein cannulation should be utilized. With practice this is a very quick and stable route of administering the contrast agent, and is less invasive than other cannulation techniques.

Image Acquisition:

1. The imaging plane is very important to getting good results. It is important that the plane be the same in every animal, but also that the image itself is optimized; the user should ensure that there is no gas in the intestines above or to the side of the kidney, this may be improved if the animals are fasted overnight prior to the study or by massaging the abdomen gently with a Q-tip to move the gas away from the kidney. Imaging the kidney from the back of the animal may also allow the user to reduce the interference caused by gas in the intestines.
2. To increase the maximum length of the cine loop the field of view (FOV) can be decreased; this is accomplished either by using the FOV or Sector X or Y buttons, minimizing the amount of surrounding tissue.
3. Increasing the length of the cine loop allows to increase the amount of frames that can be used as reference; this is important for kidney imaging where the organ is moving due to respiration. If the cine loop is 800 frames in length the bolus should be delivered just prior to the half way point, this will allow for roughly 350 frames for reference, and enough remaining frames to establish the baseline and plateau contrast intensity levels.

Processing data:

1. When processing the cine loop, always use the "none" persist option, as using the smoothing option is actually averaging the signal in between frames and so is minimizing the signal from the microbubbles flowing through the image.
2. Placement of the region of interest (ROI) is also important; the ROI should always be placed within the focal zone of the transducer you are working with, and should be placed in the same location from animal to animal.