Amsterdam UMC
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Standard Operation Procedure:

Mouse cardiac MRI with mouse body coil

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1. Scan Preparation

- Check level of isoflurane in evaporator, and fill if necessary (instructions are on evaporator).
- Preparation:
    o Turn on the heater of the animal cradle (2 buttons, controller and pump), and set the temperature at 37 degrees (using the 1/2/3/4 button, the settings are for number 3).
    o Attach the mouse (or rat) bed to the connector in the cradle.
    o Make tape, forceps, body coil, ECG electrodes and respiration balloon available on the preparation table.
    o Connect the ECG and respiration box to the battery and the fiber-optic cable that runs through the magnet bore. Make sure the battery is charged.
    o Put the temperature probe through the front cable-opening of the cradle. Take care not to bend the fiber-optic cable too much as it is fragile.
    o Turn on the OpSens temperature measurement device, which is situated in control room next to the pc.

2. PC-SAM software for monitoring and triggered MRI

- Open the PC-SAM program on the PC to monitor the ECG and respiration (detailed settings are shown later).
- A quick set-up window appears. Click on 'use default settings' to start monitoring.
- Quickly move the program to the left computer screen to keep the PC-SAM window visible during scanning. Note: Moving the window may cause the PC-SAM program to freeze. Moving the window fast seems to help. If the program freezes, quit via the Windows task manager and reopen.

3. Animal preparation

- Weigh the mouse and put the animal in the anesthesia induction box and turn on the isoflurane between 3.0 and 4.0. Use a mixture O_2 (~0.25 L/min) and medical air (~0.15 L/min) as carrier gas.

- When the animal is fully anesthetized, turn the three-way valve to redirect the anesthesia gas flow, either to the animal cradle or, if desired, to the anesthesia mask of the heating platform on the table for further animal preparation (such as tail vein cannulation).

- Transfer the animal to the animal cradle of the scanner.

- The nose cone in the animal cradle of the MRI scanner can be moved back and forward to ensure that the nose is tightly positioned in the outflow track.

- Adjust the isoflurane to around 2.0. The effect on the animal will be noticed with some delay.

- Apply eye cream to prevent dehydration of the eyes.

- If a catheter was inserted in the tail vein, tape the tail with catheter to the cradle to avoid movement. You might want to check whether the catheter is still functional by injecting a small amount of saline. Tape the syringe to the scanner table to keep it stationary and prevent accidental tension on the tail-vein catheter during animal preparation.

- Apply the needle ECG electrodes under the skin near the fore legs (**Figure 1**) and attach the respiration balloon on the belly of the animal. Insert the temperature probe rectally with the help of some Vaseline.
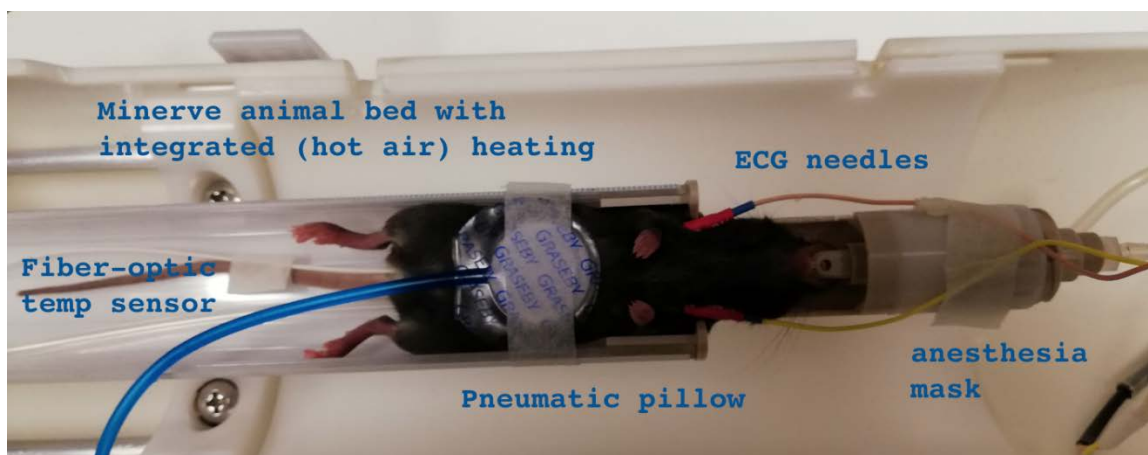

Figure 1: Mouse positioned on top of the Minerve animal bed.

- Continuously check the ECG and respiration rates with the PC-SAM software. Typically for a mouse, the respiration rate should be between 50 and 100 bpm and the heart rate between 400 and 600 bpm.
- ECG and respiratory signal quality will deteriorate in the scanner bore during the course of a long experiment. Therefore, make sure that the ECG and respiratory readings are sufficiently high at the start of the experiment. If ECG quality is insufficient, re-insert the ECG needles at slightly different position.
- Move the Minerve animal bed to put the heart in the iso-center of RF coil and magnet.
- Briefly disconnect the ECG electrode connectors from the PC-SAM interface box. Place the mouse body RF coil around the animal. Re-connect the ECG connectors and connect the 0- and 90-degree RF coils connectors.
- Tape the cable of the respiratory balloon to the top of the coil.
- Transfer the syringe for contrast injection through the magnet bore.
- Put the cradle in the MRI scanner. Make sure that the PC-SAM fiber-optic cable and catheter tubing do not get stuck or entangled.

4. Cardiac and respiratory triggering

- For cardiac and respiratory triggering click 'Gating' (Error! Reference source not found.) and the 'Gating Setup Form' will pop up. Click the 'Gates' boxes for both 'ECG' and 'Respiration'.
- *Important: confirm every entry with 'Enter'. The box will change from yellow to white.*
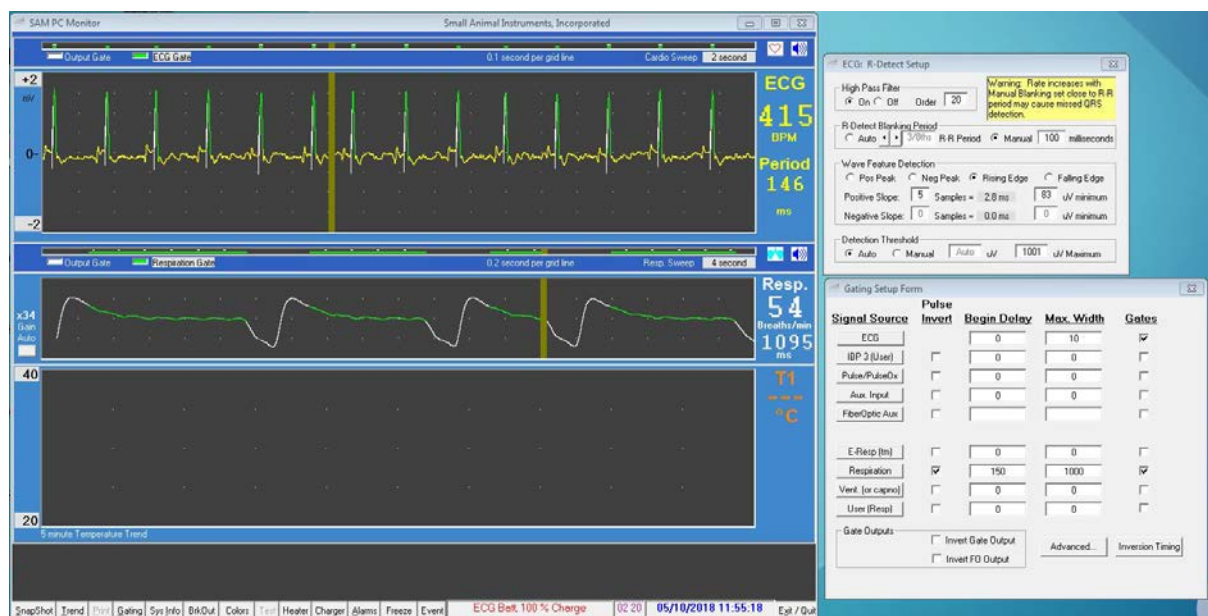
Figure 2: PC-SAM software after setup showing correct triggering of the ECG and respiratory readings.

- Click on 'Respiration'. In this window you can adjust the following:
    o A trigger delay can be set using the 'Begin Delay' fields. For normal measurements these should be set to 150 ms, max width 1000 ms, which can be adjusted if necessary.
- For ECG peak triggering, click on 'ECG' in the 'Gating Setup Form'. A window as shown in **Figure 2 on the left** will pop up. Adapt the following things:
    o 'High Pass Filter' is always on and has order 16-20.
    o The 'R-Detect Blanking Period' should be set manually. This is a fixed period after which no new trigger is detected. Set this at ~80% of the duration of the cardiac period. Adjust this value when the heart rate changes throughout the experiment.
    o In the 'Wave Feature Detection' part, the method for peak detection can be chosen. Adjust the values to get the right detection of the peaks. Sometimes 'Rising Edge' or 'Positive Peak' detection is used. Adjust the values depending on how the ECG looks like. Remember to keep checking this throughout the experiment.
    o Make sure the correct cardiac cycles are green by clicking on resp/ECG gate in the monitor window.
- For correct triggering of the ECG and respiration triggering, adjust the parameters until all peaks are detected correctly.
- Once the triggering is correct, it will look like **Figure 2**.
    o Every detected peak is shown with a green bar above the ECG signal and a white bar depicts every trigger. In this example, not all detected peaks result in valid triggers, since some R-peaks occur during respiration.
    o Above the respiratory signal the detected respiratory plateaus are shown in green and the valid triggers are shown in white.

5. MRI preparation and planning

During all MR measurements, regularly check breathing rate, heart rate and temperature. All parameters should be stable and the heart rate should be between 400 and 600 bpm, the breathing rate between 50 and 100 breaths per minute and the temperature between 36 and 37°C. If necessary, adjust the temperature to control heart rate and level of anesthesia to control the respiration rate. If the respiratory rate fluctuates or heart rate becomes low,

increase the flow of oxygen. In case you used oxygen, note this in your logbook, since this can influence the cardiac parameters.

- Start MR Solutions Preclinical Scan 1.2.
  Log in using user = manager and password = manager.
- Choose 'New subject' in the 'subject tab' window and add project and mouse details if necessary. Click 'scan' to accept.
- In protocol window, make sure the mouse-body RF coil is selected.

- The orientation of short- and long-axis views of the heart have to be determined first. Use the preset MRI scanning protocols for this. They can be found under 'Mouse cardiac', and are given in the supplement
- Run a scout, and check if animal is position in the middle of the coil. If not so, adjust the mouse cradle.
- Turn on the cardiac and respiratory triggering (top left button) to synchronize subsequent acquisitions with cardiac and respiratory readings. This will improve image quality substantially.
- Click on the "Central Frequency" and perform a GRE scout scan.
- After the initial scouts, plan the first apparent short axis measurement; this is a first guess of the short axis of the heart using the protocol *1. Scout_multislice SA (FLASH)*. (**Figure 3**)

Figure 3: Planning of apparent short-axis slice

  - o Open the GRE scout in the coronal orientation with the heart in middle of the field of view.
  - o Position the slices in the heart in a mid-left ventricular position. Make sure that the slices are roughly perpendicular to the longitudinal cardiac axis, and run the scan.
  - o Note: in case the gradient echo sequence disturbs the ECG signal such that triggering becomes irregular and unreliable, it is advised to take out the animal now and adjust the leads for stronger ECG readings.
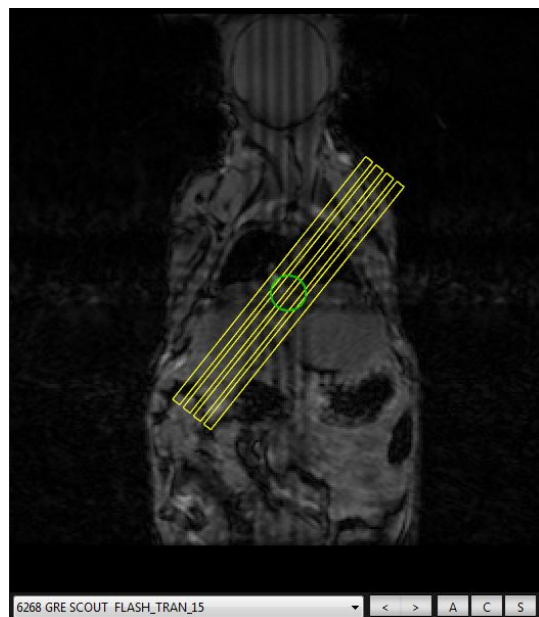
Before continuing, now you need to calibrate the 90 degrees pulse power. Due to cardiac and respiratory movement, the automatic calibration can be unreliable. It is therefore advised to adjust the power manually with the following procedure:

- Open MRS power scan.
- Click on the triangle with the magnet (next to the magnet sign).
- Open smis/calb/PPR/RF_cal_manual.
- Click on the "setup button" (two arrows) to run the program.
- Open scaling under Tx/ attenuation (under view).
- Make sure that both echo-signals have a similar height by changing the Tx attenuation. Due to cardiac and respiratory movement, the two echo signals might fluctuate and it can therefore be sometimes difficult to find the optimal value.
- If both peaks have the same height, fill in the Tx attenuation value (dB) under 'coil configuration' in Preclinical.
- Stop the acquisition by clicking on the "hand button". This is important as the scanner will not run otherwise.

- Planning initial 2-chamber long axis view
- Open the protocol: *2. Scout_2CH*:
    - o Open the GRE scout in the frontal plane and the short axis scan.
    - o Move and rotate the measurement slice such that it is aligned with the right ventricular insertion points (green spots in **Figure 4**).
    - o Next, move the measurement slice to the middle of the left ventricle.
    - o Adjust the slice orientation such that it goes through the apex in the coronal view of the GRE scout.
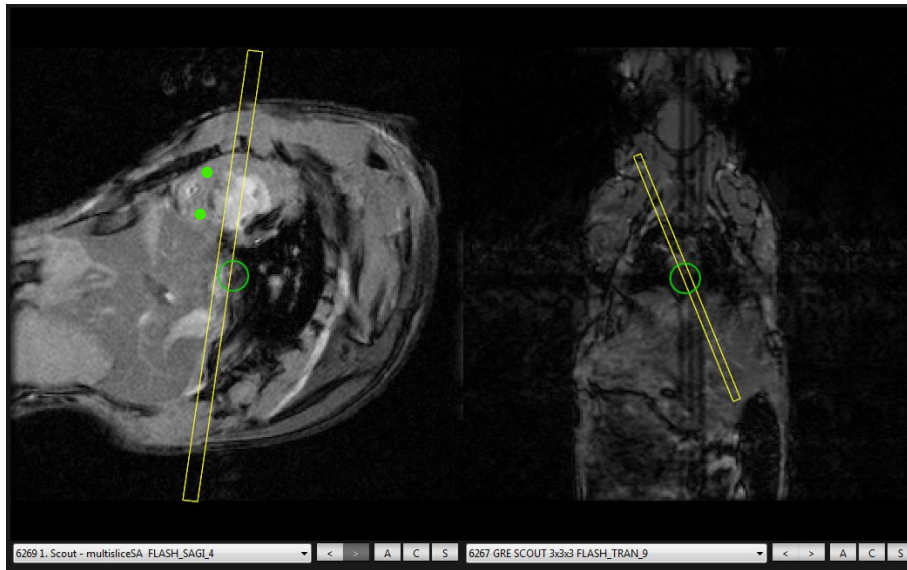
Figure 4: Planning of an apparent long-axis view (left) on the first cine in the apparent short-axis view and (right) on the coronal GRE scout.

- Planning 4-chamber long axis view
    - o Open the protocol: *3. Scout_4CH*:
    - o Load the apparent long-axis view (from the *2. Scout_2CH* acquisition) and plan as shown in **Figure 5**.
    - o Create a perpendicular slice by pressing the C button.
    - o Move and rotate the slice so that it goes through the apex and in between the valves (green dots in **Figure 5**).
    - o Inspect the position of the slice throughout the cardiac cycle. Adjust the slice position on the 'best' average position.
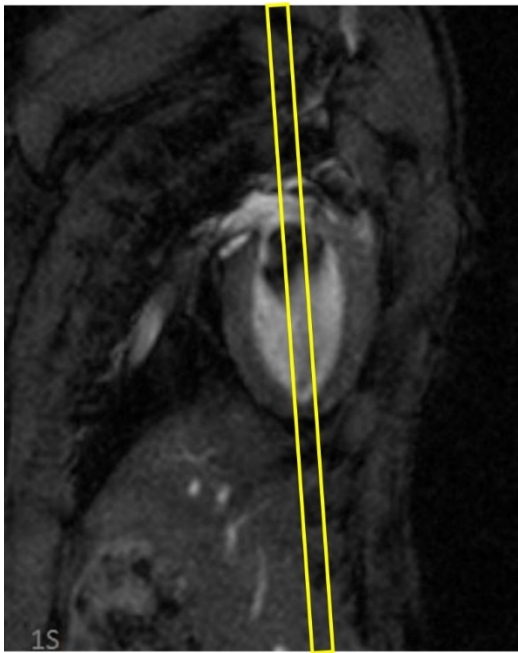
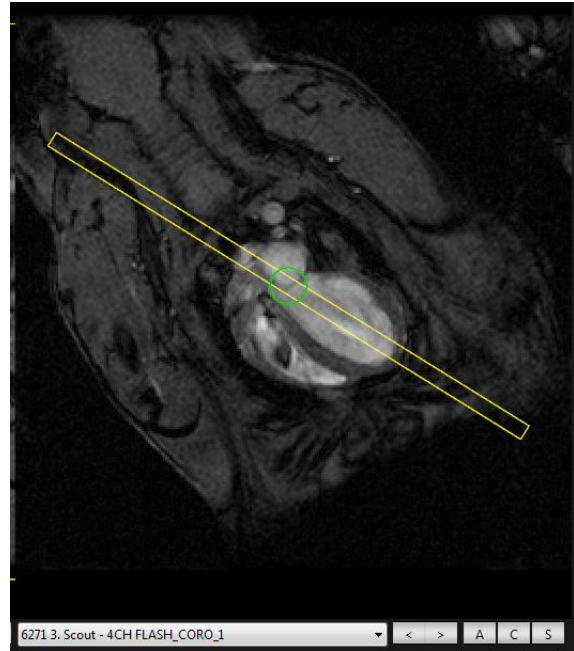**Figure 5: Planning of the four-chamber long-axis measurement.**

Figure 6: Planning of two-chamber long-axis measurement.

- Planning final 2-chamber long axis view
    - Open the protocol: *4. 2CH_final*
    - Load "3. Scout_4CH", and plan as shown in **Figure 6**.
    - Create a perpendicular slice by pressing the C button.
    - Move and rotate the measurement slice such that it is going through the apex and in between the valves.
    - Inspect the position of the slice throughout the cardiac cycle. Adjust the slice position on the 'best' average position.
    - *If required, record a good quality scan of this view.*
    - *If a final 4-chamber view is required, repeat the 4-chamber protocol above.*

- Planning short-axis view
    - Open the protocol: *5. SA final test*
    - Load "3. Scout_4CH" and "4. 2CH_final", and plan as shown in **Figure 7**.
    - Create a perpendicular slice by pressing the **S** button.
    - Move and rotate the measurement slice in an orientation perpendicular to the mid-left ventricular wall in both the two- and four-chamber scans.
    - Make sure that the slice heart is positioned approximately in the middle of the slice and the field-of-view fits the mouse to prevent aliasing.

- o Change the number of slices to 5 or 7 to inspect whether you can cover the whole heart. This sometimes also helps to adjust the slice orientation. When satisfied with the slice orientation, click "copy slices".
- o Set the number of slice back to 1. Run the scan with only one slice in the middle of the heart.
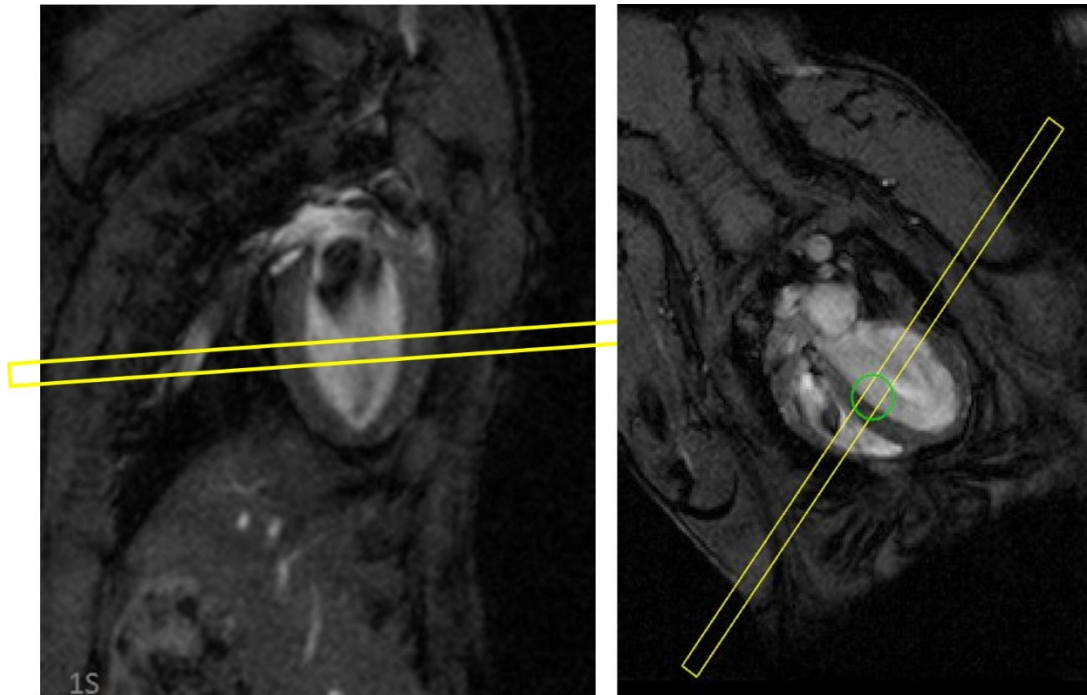- o If required, record a good quality scan of this view.

Figure 7: Planning of short-axis slice orientation.

Now you have a short axis scan of the heart that is needed for various functional measurements. Some of them are described below.

This following chapters describe the various applications that can be used after determining the correct mid-cardiac slice selection. They do not need to be run in this order.

6. Application: CINE-MRI for systolic function measurement

- Open the protocol: *6. Systolic function multislice SA*
- Copy the slices from the short axis scan
- Adjust the number of slices to cover the whole heart. Typically, 7 or 9, 1-mm-thick slices are needed. An odd number is advised because this preserves the position of the middle slice.
- Determine and set the number of desired cardiac time frames:
  - The measurement of one frame (TR) takes 7 ms. Therefore, change the echos/frame (under protocol) so that the number of frames covers about 60-70% of the R-R interval. For example, an R-R interval = 120 ms allows for 14 to 15 cardiac time frames.
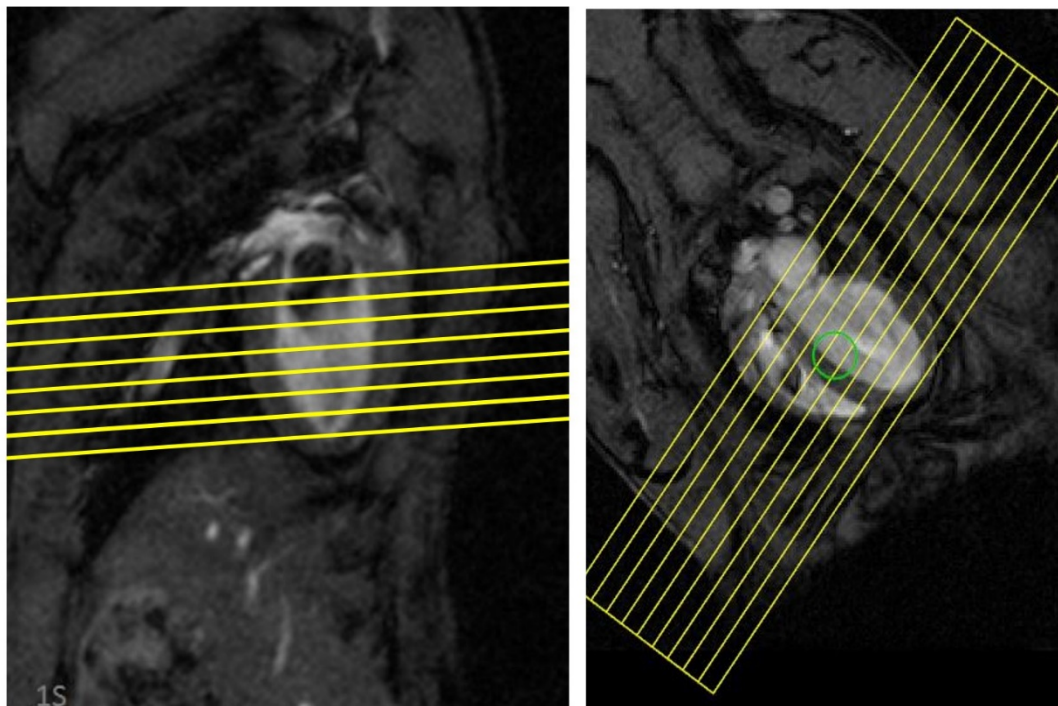

Figure 8: Systolic function measurement with 9 slices.

7. Application: CINE-MRI for diastolic function by retrospective gating

- Open the protocol: *7. Diastolic Function*.
- Copy the slice orientation from the middle short axis CINE scan.
- Turn off the cardiac and respiratory gating.
- Run the protocol.

8. Application: Late gadolinium enhancement for infarct size

- A tail vail cannulation is required for this technique, with the syringe filled with gadolium.
- Inject the gadolium into the tain vein
- Open the protocol: 8. *LGE*.
- Copy the slice orientation from the middle short axis CINE scan.
- Run the protocol multiple times for a duration of about 20 minutes.

9. After the measurements

- Remove the RF coil from the scanner and take the mouse out of the cradle.
- Remove ECG leads and respiratory balloon.
- Shut down the PC-SAM software by clicking 'Exit/Quit' and choose 'Fast exit: Save all measurements and exit'.
- Do NOT shut down the measurement computer.
- Clean the table in front of the scanner.
- Properly clean and store all the items you used.