Guide to Small Animal  
**Adrenal Gland Imaging**  
using the Vevo® 2100 Imaging System
Guide to Small Animal Adrenal Gland Imaging using the Vevo® 2100 Imaging System

Course Objectives

This guide is designed to aid the user in:
- recognizing and imaging the adrenal gland
- optimizing the imaging parameters, probe position and system settings to obtain the desired images
- performing the measurements of interest to get anatomical and physiological data

Overview of the Research Areas

The adrenal glands reside immediately above both kidneys (superomedial to the kidney). It contains a medulla core and an outer cortex shell that can be clearly identified in micro-ultrasound imaging.

Figure 1. Schematic of the adrenal gland.

http://www.informatics.jax.org/cookbook/figures/figure112.shtml
Overview of the Imaging Modes to be used in this Guide

B-Mode:

B-Mode or brightness mode, imaging is used to acquire two dimensional images of an area of interest. The grayscale image allows for identification of anatomical structures and the software allows for various measurements in this mode of imaging. A motor can be attached to the ultrasound probe to translate the probe over a defined range with a defined step-size, the software then recompiles the various 2D slices into a 3D volume. The 3D software allows for various measurements and imaging planes in this type of imaging.

Color Doppler Mode:

Color Doppler Mode allows for quick visualization of blood flow and provides information on the direction of flow, the color is assigned accordingly with blood flowing towards the transducer colored on a scale from red to white and blood flowing away from the transducer colored on a scale from blue to white. BART is an acronym commonly used to remember the colors associated with the direction of flow; Blue Away, Red Towards. Please see our Quick-Start Guide: Imaging in Color Doppler, Power Doppler and Power Doppler 3D Modes for further information on the various Doppler imaging modes.

Contrast Mode:

Contrast Mode is very similar to B-Mode in that it is used to look at 2D anatomical structures. However it includes specific analysis tools to allow a reference subtraction of two images to apply a copper (or green) contrast overlay to the injected contrast agents. Contrast agents are used to enhance the visualization of blood flow in very small blood vessels, as small as capillaries. The software not only allows for enhanced visualization but also provides quantification tools so that useful hemodynamic parameters can be determined.

Overview of the Parameters to be optimized

B-Mode Parameters:

There are numerous parameters which can be set to optimize a B-Mode image. Outlined below are a few key parameters. For a full list of parameters and how they affect B-Mode imaging, please consult our Vevo 2100 Operators Manual.

   Display Maps

Display maps are used to assign grayscale colors to detected ultrasound signal intensities. This allows optimized grayscale differentiation of specific anatomical targets. The Vevo 2100 imaging system comes with numerous factory settings which can be selected, to provide the best quality images.
Dynamic Range

Dynamic range determines the range of grayscale intensities which can be displayed on an image. Together with the display maps, dynamic range can be adjusted to provide the best quality images.

Focal Zones

The Vevo 2100 imaging platform can have one, two or three focal zones, with the spacing of the two and three focal zones being narrow or wide. Using additional focal zones provides an image which is focused over a broader depth, however increasing the focal zones will decrease the maximum frame rate. The number of focal zones and their placement should be selected based on the anatomical structures to be imaged.

Line Density

Line density allows the user to change the space between lines on the ultrasound image. Increasing the line density will increase the resolution, texture and overall will increase the quality of the image. However it will decrease the maximum frame rate at which the system can image.

Color Doppler Mode Parameters:

There are various parameters which can be set to optimize Doppler imaging in PW, Color and Power Doppler imaging modes. Please see our Quick-Start Guide: Imaging in Color Doppler, Power Doppler and Power Doppler 3D Modes for an explanation of these parameters and how they will affect the image quality.

Selecting the Appropriate Probe for Imaging

The selection of the probe for imaging becomes important when considering which images are required and how large the animal is and how large the gland being scanned is. When acquiring B-Mode images on a mouse the ideal probe would be the MS550D. When imaging a rat B-Mode images should be acquired with the MS400 or the MS250 probes depending on the size of the animal.

Software Measurements

The Vevo 2100 imaging software allows the user to make several measurements and calculations. In B-Mode imaging general size measurements can be made, including area and volume measurements. In Doppler and Contrast imaging modes an assessment of vascularization can be completed.
Animal Preparation

The animal should be anaesthetized so as to prevent movement during the imaging session. Typically isofluorane is used, at a concentration of 1.5-2%. Once the animal is anaesthetized in a knock down box it should be positioned on the platform ventral side up. Eye lubricant should be placed on each eye to prevent drying of the area. A small amount of ECG gel should be placed on the copper leads on the platforms and the paws taped to them, this will provide the ECG and respiratory physiology. A rectal probe should be inserted to monitor the temperature of the animal during the imaging session. The hair around the area to be imaged should be removed using a depilatory cream, such as Nair and rinsed with water prior to imaging.

B-Mode Imaging

Place the transducer in the midline in transverse orientation just below the rib cage and liver in the abdominal area. Move transducer left or right to visualize the left or right adrenal gland respectively. If the kidney is in view, simply move up (the adrenal gland resides immediately above and medial to the kidney) until the adrenal gland comes into view.

Figure 2. B-Mode image of adrenal gland showing the cortex and medulla.
Color and Power Doppler Mode Imaging

Color and Power Doppler Mode imaging can be done to visualize flow in the adrenal gland. Flow in the adrenal gland is relatively slow.

Figure 3. Color Doppler Mode image of adrenal gland (L). Power Doppler Mode image of adrenal gland (R).

Contrast Mode Imaging

Untargeted MicroMarker™ Contrast Agent Imaging
To assess flow in the adrenal gland, untargeted MicroMarker contrast agent imaging can be performed. By injection this contrast agent and assessing the wash-in of the contrast agent, one can quantify relative perfusion. Please refer to MicroMarker contrast agent imaging protocols for more details.

Figure 4. Contrast Mode imaging of the adrenal gland showing green contrast signal following an untargeted MicroMarker bolus injection (L). Intensity vs. time curve of the adrenal gland (R).
Targeted MicroMarker Contrast Agent Imaging
Targeted MicroMarker contrast agent imaging can be performed in the adrenal gland to assess intravascular and endovascular markers. Simply mix a biotinylated antibody of interest with the Targeted MicroMarker contrast agent and inject this to quantify the expression levels of the antibody of interest. Please refer to MicroMarker contrast agent imaging protocols for more details.

3D-Mode Imaging

3D-Mode imaging can be done to quantify the volume of the adrenal gland. To perform 3D imaging, using the micromanipulator, scan across the adrenal gland while noting the size by reading the ruler located on the micromanipulator. Return the transducer to the center of the region of interest. The size of the adrenal gland is entered as the “range” of the motor. Enter the desired “step size” (slice thickness) and click on “start 3D” to begin 3D imaging.

Figure 4. 3D mesh view of the adrenal gland; cortex (red) and medulla (yellow).