QUICK-START GUIDE:
POWER DOPPLER-3D IMAGING WORKFLOW

This document’s objective is to describe the sequence of steps used to optimize the settings when imaging in Power Doppler and Power Doppler-3D modes for best results in relative vasculature quantification.

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1. Imaging in Power Doppler 3D

1. Enter the Vevo application and initialize the 3D motor;
2. Adjust the imaging plane in B-Mode, and then initiate Power Doppler Mode;
3. Deselect the Auto Histogram check box;
4. Adjust the Color Box for the size of the region of interest;
5. Adjust the range on the Histogram so all the color spectrum fills the color box (as in Fig.1)

![Fig.1 – Histogram](image)
6. The default settings allow for the detection of some flow in the region of interest; the color changes from orange to yellow when a Doppler signal is detected.

### 1.1 Optimizing the acquisition parameters in Power Doppler

The purpose of optimizing the parameters when imaging in Power Doppler is to maximize the sensitivity of the system; this will allow for detection of flow which cannot be detected by Pulse-Wave Doppler, and for minimization of artifacts mainly caused by respiration.

The parameters optimization will be covered for three most common imaging areas: subcutaneous tumor (MeWo hind limb), testicle, and kidney.

#### 1.1.1 Subcutaneous Tumor Imaging:

With a subcutaneous tumor located on the hind limb, respiration is not a major factor when setting the imaging parameters, so the focus will be on increasing the sensitivity for lower velocity blood flow.

1. With the **Color box** and **Histogram** set as described in steps 3 and 4 above the image should look similar to **Fig. 2** bellow:

![Fig. 2 – MeWo Tumor – default acquisition parameters](image)

2. The yellow speckles are a clear indication of flow in the tumor;
3. Scan for a couple of seconds and verify that the yellow spots are continuously present in the image, and that there are no yellow parallel lines caused by respiratory movement;
4. To increase sensitivity for low velocity flow decrease the **Scan Speed and Wall Filter** until you observe artifacts caused by respiration (this could be as low as 0.8 mm/s). Set the Scan Speed and Wall Filter to the same value in subsequent steps (from now on they will be referred to as only Scan Speed) but make sure that both Scan Speed and Wall Filter are being adjusted.
5. To remove the respiration artifacts the **Scan Speed** needs to be increased step by step;

**Note:** Increasing the **Scan Speed** reduces the ability to visualize low velocity flow so the next steps are meant to increase the sensitivity of the system without detecting the respiration artifact. All of these changes may enhance the...
sensitivity of the system, however they may not enhance the resolution and are included here only guidelines to try when optimizing the Power Doppler settings.

6. Increase Power Doppler Gain in small increments, this should enhance the intensity of the Power Doppler signal.

7. Change the Velocity (Pulse Repetition Frequency) setting to “Slow” (this may allow for the detection of lower velocity blood flow)

8. Increase the number of RF Cycles
   a. Increasing the number of RF Cycles increases the sensitivity, however it broadens the range in the color spectrum making the signal appear more bloomed, this will make adjusting the min Range more difficult (separating the noise from the true Power Doppler signal).

9. Decrease the Grayscale Priority in order to reduce the amount of signal displayed from the skin line, be sure that you are not eliminating signal displayed within the tumor while adjusting this parameter;

10. After making these adjustments the image should look similar to Fig.3:

![Fig.3 - MeWo Tumor - optimized parameters](image)

**Observations:** From a close comparison in between Fig.2 and Fig.3 it is obvious that minor adjustments of the acquisition parameters help in obtaining a better picture of tumor vascularization.

11. Once the parameters are adjusted for increased sensitivity, adjust the Histogram on the right side of the image to only keep the color for the flow areas, removing the dark red color from the image. This is done by sliding the min Range bar up until the red background is removed from the image.

12. After adjusting the color Histogram the image will look similar to Fig.4:

![Fig.4](image)
13. Save the parameters file for future references for MeWo tumor imaging.

1.1.2 Testis Imaging:

Power Doppler imaging of the testis is very similar to the tumor imaging; respiration artifact should still be minimized only by adjusting the Scan Speed even if the testis are in the abdominal area due to changes in the animal's body temperature.

Briefly, adjusting the parameters in Power Doppler is a similar process as described above for tumor imaging:

1. Set the imaging plane in B-Mode and then initiate the Power Doppler Mode;
2. Un-check the Auto Histogram;
3. Adjust the size of the color box for the area of interest;
4. Adjust the Histogram so the full spectrum of color is shown in the color box (this is a way to identify the color coming from flow and color coming from respiration or cardiac movement);
5. Increase the amount of lower velocity blood flow by decreasing the Scan Speed and Wall Filter. Again the Scan Speed and Wall Filter should be set to the same value, and will be referred to as only Scan Speed in subsequent steps. (in the case of testis the Scan Speed could be 1.5mm/s, but this will depend on the physiological state of the animal)
6. When decreasing the Scan Speed continue to lower the value until artifacts from respiration are seen, then return the speed to the previous value;
7. Try to, step by step, increase the Gain and RF Cycles (first Gain and then RF Cycles if needed), (these adjustments may or may not improve the sensitivity of the Power Doppler imaging, as stated before they are just guidelines);
8. Change Velocity to “Slow” (this is not imperative but again it may increase the amount of low velocity flow which is detected);
9. After setting these parameters the image should be optimized for 3D imaging.

Imaging using Respiration Gating:

6’. In case respiration brings strong respiratory artifacts at the default Scan Speed, check “Enable Gating”;
7’. Increase the Scan Speed step by step, one unit at a time until the respiratory artifact is removed.

!Note: Please note that when increasing the Scan Speed the blood flow with a lower velocity will not be observed in the image unless the Gain and RF Cycles are adjusted.

For 3D acquisition and data processing follow the steps described in the 1.2 Adjusting the 3D parameters before starting the Power Doppler-3D imaging and 1.3 Processing the Power-3D image

Examples of Power Doppler images in the testicle:
Image saved with default parameters:

![Image](image1.png)

Image saved with Scan Speed too high – the smaller flow is not observed in the image:

![Image](image2.png)

Image saved with optimum Scan Speed – before starting 3D acquisition:

![Image](image3.png)
1.1.3 Kidney Imaging:

Power Doppler imaging in the kidney must account for respiration artifacts, due to the location of the organ in the abdominal cavity.

The parameters optimization is done in the same succession as described for the tumor and testis; the only difference is that the adjustments of the Scan Speed have to be combined with Respiration gating.

**Note:** Checking the “Enable Gating” check box does not automatically minimize the respiration artifact in the image, it has to be combined with further Scan Speed adjustments.

1. Adjust the imaging plane in B-Mode and then initiate the Power Doppler Mode;
2. Un-check the Auto Histogram;
3. Adjust the size of the color box for the area of interest;
4. Adjust the Histogram so the full spectrum of color is shown in the color box (this is a way to identify the color coming from flow and color coming for respiration or cardiac movement);
5. Check “**Enable Gating**”;
6. Increase the **Scan Speed** and **Wall Filter** step by step, one unit at the time until the respiration artifact is removed from the image.

**Note:** Please note that when increasing the **Scan Speed** the blood flow which has a lower velocity than the set value will not be detected unless the **Gain** and **RF Cycles** are adjusted to higher values. The **Scan Speed** and **Wall Filter** should be set to the same value, and will be referred to as only **Scan Speed** in subsequent steps

**Note:** For optimum imaging conditions and low respiration artifacts, it is recommended that the respiration rate be in the range of 40-65 BPM which will allow for values of the Scan Speed to be between 3.5-5.5 mm/s. This should allow for a good response from a full range of vasculature.

7. Increase the **Gain** and **RF cycles** (first Gain and then RF Cycles if needed). These adjustments may or may not improve the sensitivity of the Power Doppler imaging;
8. Change **Velocity** to **Slow** (this is not imperative but again it may increase the amount of low velocity flow which is detected);
9. After setting these parameters the image should be optimized for 3D imaging.

For 3D acquisition and data processing follow the steps described in the **1.2 Adjusting the 3D parameters before starting the Power Doppler-3D imaging** and **1.3 Processing the Power-3D image**
Examples of images for Power Doppler of the kidney:

Image saved with default parameters:

Image saved with adjusted parameters before starting 3D acquisition:
1.2 Adjusting the 3D parameters before starting the Power Doppler-3D imaging

1. Initialize the 3D motor - the max Range is ~ 40 mm, depending on the motor, it could go at least 37.95 mm with the default setting for Step Size (0.1 mm);
   - **Range**: max value = between 37.95 to 40 mm  
     - min value = 0.5 mm;
   - **Step Size**: min value = 0.03175mm  
     - max value = 0.5mm;

   The maximum number of 3D slices is 500 and can be calculated as:
   \[ \text{nr. of slices} = \frac{\text{Range}}{\text{Step Size}} \]

   Given the Range and Step Size limits and the formula above, the only limitation is in the number slices for a given volume. The application adjusts the max Range for a given Step Size automatically and min Step Size for a given Range.

2. Select the **Range** and **Step Size**.
   Minimum for the Step Size should be close to the RMV resolution (ex: 706 (50-60 microns), 707B (60 microns), etc.)

3. From Power Doppler, with the parameters set as described in section 1.1 and 1.1.1/1.1.2/1.1.3, start 3D acquisition;
4. Obtain the Power-3D data set.

1.3 Processing the Power Doppler 3D image

To obtain a Power Doppler 3D image of the vasculature

- Display only the Cube View on the screen;
- Adjust the cube so only the tumor is in view;
- Move through the cube to the desired plane;
- Select **Render** from the 3D panel and then choose “Render” from the list of display options,
  - this will render the image and display the vasculature in a 3D volume
- Select the greyscale tool button on the bottom right of the toolbar buttons to remove the B-Mode image data, leaving only the Power Doppler spectrum highlighting the flow in the area of interest;
- Use the rotation tool to rotate the image for optimal view.
This is an example of a MeWo tumor image acquired in Power Doppler-3D:

Power Doppler-3D of the testis – Fig.6:
To record the 3D file
- To record the 3D rendered volume in an .avi format select Record;
- The current window is the one that is being recorded, so every rotation, to show the vascular network, or slide in the volume is going to be part of the .avi file;
- When you are finished click Stop Recording.

To save the 3D images
- Click Cine Store to save the initial file.
- Click on Control and Cine Store simultaneously to save subsequent copies of the initial 3D volume with the latest updates.

1.4 Using the 3D Measurements Tools

Creating a Volume

Once you are in the 3D Mode, adjust the size of the image in the cube view, to the size of the area of interest (i.e. tumor). Then choose the volume option from the Mode Setup.

Volume measurements come with 2 options:
1. Parallel: for awkwardly shaped tumors or organs
2. Rotational: for spherical shapes or uniformly shaped tumors or organs

Within both these options you can choose to have the volume done in an Auto mode or a Semi automated mode.

For most of the tumors it is recommended to use the Rotational, Semi-Automated tool because you can refine right away and end up with a tumor overlay.
a) When in Auto mode:
With the Angle set to 18 degrees the rotation of the 3D volume will be every 18 degrees and thus 10 volume contours will be generated for the entire volume. If the angle of rotation is decreased more contours will be generated.

- First adjust the plane in the cube that is close to the middle of the tumor. Hit start: the software will prompt you to set the rotational axis. Left click once to set the start of the axis and then again at the end of the rotational line. Trace the axis of rotation along the long axis of the image acquired (i.e. most of the tumors have an elliptic shape so trace the rotational axis along the longest axis of the tumor).
- Draw the contour of that volume such that it intersects the rotational axis. Select the rotational direction, Clockwise or Counterclockwise, click Refine (for the contour) if needed, and then Proceed.
- The software will automatically generate the subsequent contours.
- Once the volume has been created, the volume measurement and percent vascularization (PV) will be displayed in the left lower corner of the Cube view. The measurements values are only visible in the Cube view or Measurements Browser.
- You can refine any of the contours once the volume has been generated, to match the actual contour of the tumor.
- After you have changed one or more contours in the volume, click on “PV Recalc” to get the percent vascularization (PV) recalculated for the updated volume.

b) When in Semi mode:
When choosing the Semi rotational option, set the angle to 18 and step to 3, this way you get the tumor overlay in 10 volume contours in steps of 3.
- The software will copy the initial contour 3 times. All the contours can be refined before proceeding for another series of 3 contours.
- After running the 10 volume contours click Finish to have the volume measurement and the PV value displayed.

Selecting a volume for Parallel segmentation:
Setting the parameters for Parallel segmentation:

- Start in the middle of the tumor.
- Select Both from the Direction dropdown
- Select the appropriate step number for the size of the volume ;
- Trace and refine the contour and click Proceed.