Neuromuscular Physiology Lab

FloWave.US Ultrasound Blood Flow Analysis Software

Instruction Manual

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Overview

This manual describes the operation of a custom, semi-automated MATLAB program to analyze duplex ultrasound blood flow data. MATLAB® is an interactive program for numerical analysis and data visualization. Basic operations of MATLAB® are not explained in detail, and the user is encouraged to review tutorials available at www.mathworks.com for more information about using MATLAB®.

Manual Sections

- Part 1 – Platform Calibration
  - Setting the scale positions and color profiles for your ultrasound equipment
- Part 2 – *FloWave.US* Operation
  - Executing the automated blood flow analysis code
- Part 3 – *BMode* Operation
  - Detecting vessel diameter in a high resolution B-Mode image sequence
- Part 4 – Acquiring and Editing Analog Video Data
  - Acquiring and editing analog video to use with *FloWave.US* and *BMode* programs

System Requirements

- MATLAB®
  - Version R2011b or newer – requires *VideoReader* function
- Video Converter
  - Use an '.avi' format for a windows operating system.
  - Use a '.mov' format (MPEG4 codec) for a MAC operating system.
- Blood Flow Analog Video Recordings
  - Run the *PlatformCalibration.m* program (see Part 2 of this manual) to create calibration settings for your ultrasound platform. Default settings created for a GE Logiq Book *e* can be loaded if needed.
  - Edit analog videos so the first frame contains a full update of the pulse wave spectrum (see Part 3 of this manual for information about video recording and editing).

MATLAB® M-Files

- *FloWaveUS.m* – primary m-file for automated blood flow analysis
- *PlatformCalibration.m* – primary m-file to calibrate FloWaveUS for a specific ultrasound platform. Platform calibration settings can be saved and imported into FloWaveUS.
- *FiguresLoopingFcn.m* – function loops through the initial frames of the video allowing the operator to define the correct 'start' frame (i.e. a full update of the pulse wave spectrum).
- *FrameCalibrate.m* – convert pixels to units of interest (e.g. velocity, distance, time)
- *GapInterpolate.m* – creates a composite time-series of blood velocity data using a linear interpolation scheme

- *VesselROI.m* – interactively select a rectangular region of interest (ROI) around the imaged vessel in the B-Mode image
- *AutoDiameter.m* – measure the vessel's diameter within the user-defined ROI
- *Imgaussian.m* – applies a Gaussian image file to enhance vessel edges (called by AutoDiameter; written by Dirk-Jan Kroon – see associated third party license)
- *GInputc.m* – adapts the ginput cursor properties (written by Jiro Doke – see associated third party license)
- *BMode.m* – primary m-file to analyze B-Mode vessel images to measure vessel diameter

MATLAB Basics

1. Open MATLAB®.
2. Desktop Overview (Figure 1.1):
    a. Current Folder Menu Bar (Top of Screen) – Lists the folder location MATLAB® is referencing. At startup, the default location is typically "Documents/MATLAB".
        i. To run a function or m-file, the file **must** be in this folder location.
        ii. Click on the arrow or folder icon to change the file path.
    b. Current Folder Contents (Left of Screen) – Lists the contents of the reference folder.
        i. Use this list to check that m-files or functions are in the specified folder.
            1. MATLAB® will report an error if the m-file or function referenced by a program is not in the current folder.
        ii. ***NOTE***: Result files created by the analysis program will be listed in this folder when the program has completed.
    c. Command Window Prompt (Center of Screen) – The main area to enter commands to run functions, programs, or visualize data.
        i. Enter statements after the >> symbol. If this symbol is not visible, the code may be processing (indicated by a 'BUSY' in the lower left corner of the screen).
        ii. After you type a command, press **Enter** or **Return** to execute the instruction.
        iii. Be careful to not enter additional characters in response to an on-screen prompt. For example, values should be entered as integers or decimals. Do not add spaces, quotations, or text.
        iv. When entering filenames, be sure to include the file extension (e.g. ".csv", ".xls", ".jpg")
        v. ***NOTE:*** If you enter an incorrect command, you can exit the program by pressing "ctrl + C". To restart, you will need to change the current folder back to the location with the m-file.
3. To exit MATLAB®, do one of the following:
    a. Click on the close box ('X') in the MATLAB® desktop.
    b. Select **Exit MATLAB** from the **File** menu.
    c. Type quit in the Command Prompt

Figure 1.1 Overview of the MATLAB® desktop. First, change the folder directory to include the location of the automated ultrasound data analysis m-files. To execute the m-file and other commands in the program, enter commands in the MATLAB® prompt command window after the >> symbol. Specific instructions regarding the program will also be printed in the command window.

Error Reporting

MATLAB® reports error messages to the command prompt window in red text. If an error occurs, attempt to rerun the code from the beginning to determine if the error can be repeated. If so, post the error as an "issue" on the github page (https://github.com/ccoolbaugh/FloWave.US). Please include a copy of the error message and a description of what caused the error (i.e. did you type something in the command prompt? Or did you click on a figure?).

PART 1: PLATFORM CALIBRATION

Starting PlatformCalibration.m

1. Open MATLAB®
2. Change the current folder (menu bar at top of Figure 1.1) to the folder location containing the ultrasound analysis m-files.
    a. Check that the m-files are listed in the current folder contents (left panel in Figure 1.1).
3. Type PlatformCalibration in the command prompt and press **Enter**
    a. ***NOTE:*** You will see instructions displayed in the command prompt window to guide you throughout the analysis program. Choose the file directory that contains the video that you want to analyze.
4. Browse for the folder and click **Open** when at the appropriate folder path.
        i. ***NOTE:*** Do not click on a single video file.
5. A list of the folder contents will be displayed in the command prompt.
    a. Type the filename and extension (e.g. Resting1.avi) of the video to be analyzed and press **Enter.**

Setting the Scale Positions

The program will present a screen capture (Figure 1.2) to select a region of interest (ROI) around the velocity, time, and distance calibration scales on the ultrasound screen. The ROIs should include as much of the scale and its markings as possible to improve pixel level calibration in the *FloWave.US* program.

1. Enlarge the figure to view the scale positions.
2. When you move the mouse, a colored cursor will move over the figure. The color of the cursor changes to correspond to each scale position.
    a. Velocity Scale Position – Green Cursor
    b. Time Scale Position – Red Cursor
    c. Distance Scale Position – Yellow Cursor
3. Click on 2 positions on the image to define a ROI around the scale position.
    a. Move the crosshair to the upper left corner of the velocity scale and click the left mouse button.
    b. Move the crosshair to the lower right corner of the scale position and click the left mouse button.
    c. After selecting the ROI the cursor color will change for the next scale.
4. Repeat the ROI selection process for the three scales.
5. A figure will be presented for each scale based on the selected ROI. Press a key to advance through the figures.
6. Type ‘y’ if the scale positions were selected correctly or type ‘n’ to repeat the scale selection process.

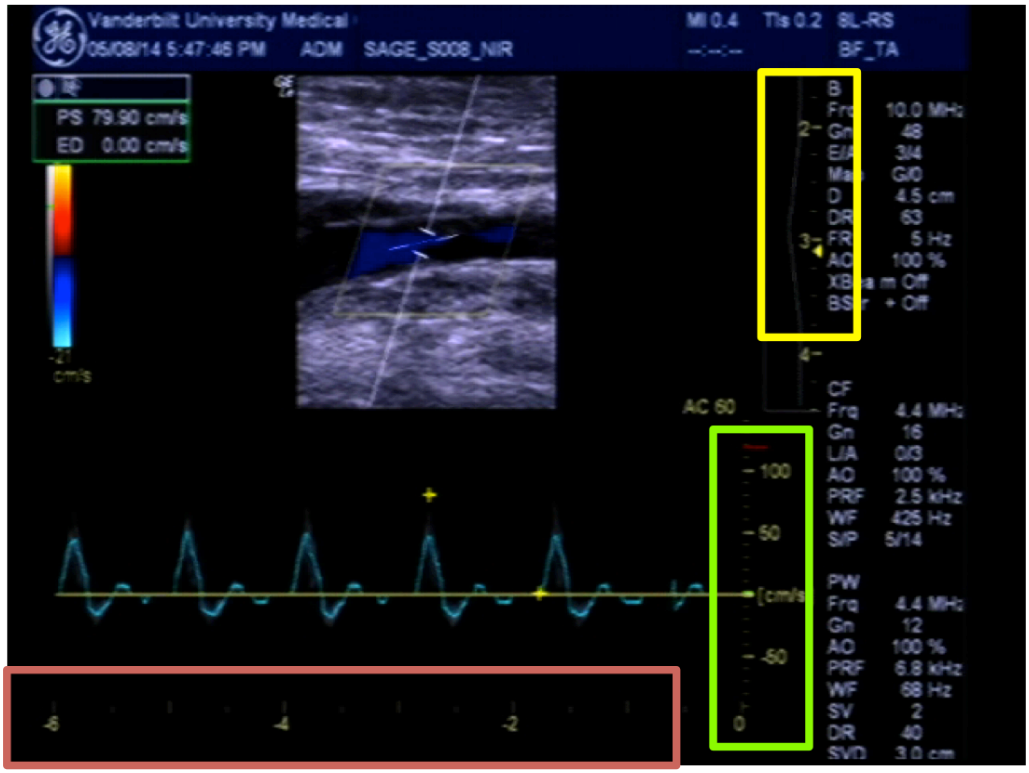

Figure 1.2 First image frame presented for setting the scale positions. Using an interactive cursor, the operator will define ROIs around the velocity (green), time (red), and distance (yellow) scale positions.

Define the Pulse Wave Spectrum ROI

The program will again present a screen capture (Figure 1.3) to select an ROI around the pulse wave spectrum. Care should be taken to select the entire dynamic range of the pulse wave data to avoid missing data.

1. Enlarge the figure to view the pulse wave data.
2. When you move the mouse, a magenta cursor will move over the figure.
3. Click on 2 positions on the image to define a ROI around the pulse wave data.
    a. Move the crosshair to the upper left corner of the velocity scale and click the left mouse button.
    b. Move the crosshair to the lower right corner of the scale position and click the left mouse button.
    c. ***NOTE:*** The complete dynamic range and time scale of the pulse wave data should be selected to avoid missing data during analysis.
4. A figure will be presented of the selected ROI.
5. Type ‘y’ if the pulse wave data were selected correctly or type ‘n’ to repeat the ROI selection process.

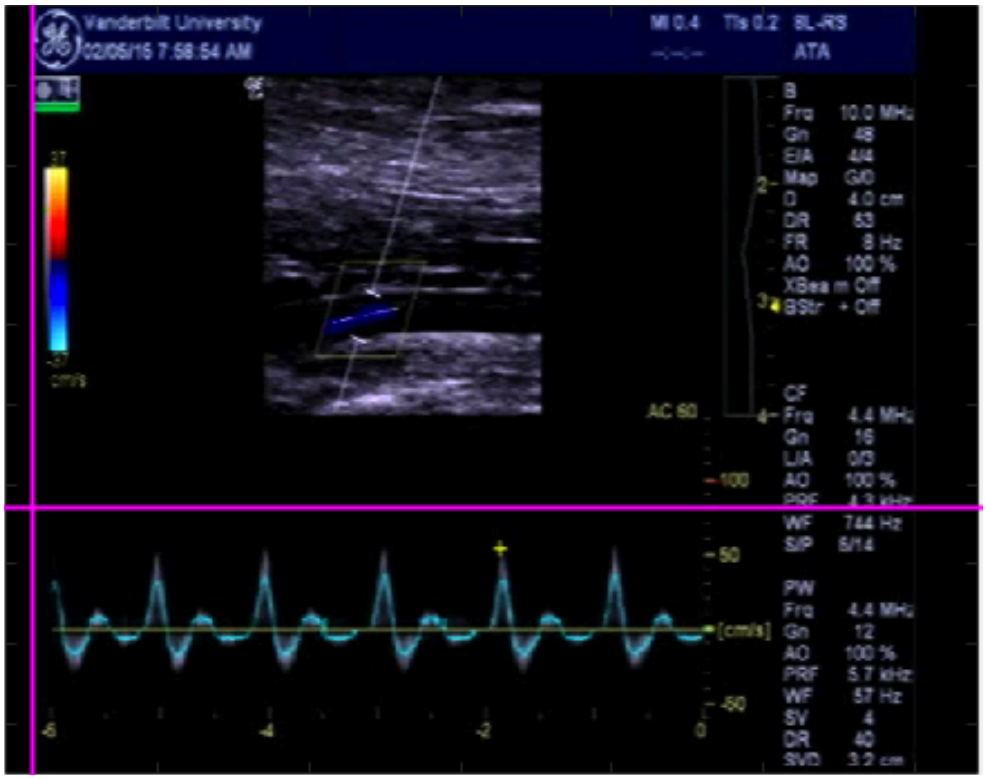

Figure 1.3 First image frame presented for setting the pulse wave spectrum ROI. Using the interactive cursor (magenta), the operator will define an ROI around the pulse wave spectrum.

Set the Zero Velocity (Baseline) Color Profile

The program will present the pulse wave data spectrum (Figure 1.4) to allow the operator to define the zero velocity (baseline) color profile. This selection process will aid in the scaling of velocities values in the *FloWave.US* program.

1. Enlarge the figure to view the pulse wave data.
2. When you move the mouse, a teal cursor will move over the figure (Figure 1.4).
3. Click on 5 positions on the baseline (shown here as a horizontal yellow line) to define the color profile associated with zero velocity.
    a. ***NOTE:*** The positions clicked on the baseline should be varied to allow for a broad color profile range.
    b. ***NOTE:*** Be careful to not deviate the vertical position of the cursor as blurred pixels may distort the selected color. The selected cursor positions are displayed in the upper left corner of the figure in a yellow box.
4. The average red, green, blue pixel values for the 5 selected points are displayed in the command prompt.
5. Type ‘y’ if the color profile is correct or type ‘n’ to repeat the process.

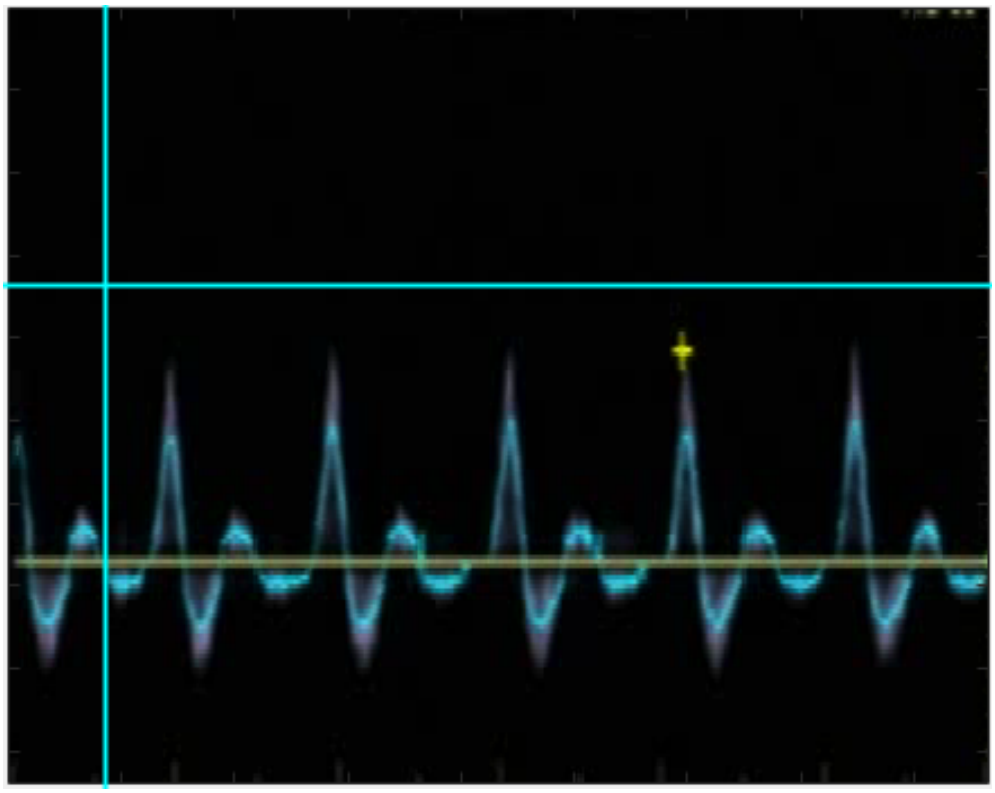

Figure 1.4 Display of the pulse wave data for setting the zero velocity (baseline) color profile. Using the interactive cursor (teal), the operator will select 5 points on the baseline (shown as the yellow horizontal line) to define the average color profile equal to zero velocity.

Set the Time Averaged Mean (TAMean) Velocity Color Profile

The *FloWave.US* program uses the color profile of the calculated TAMean velocity to recreate the velocity time series. In the platform calibration, the operator can redefine the color profile for the TAMean to improve data extraction.

1. A figure of the extracted TAMean data based on the default color ratios is displayed to the operator (Figure 1.5).
    a. ***NOTE:*** The data extraction displays the intensity of the color values not the recreated time series. Increases in gaps in the individual waveforms will result in difficulty recreating the TAMean time series.
2. Type ‘y’ if the default settings are appropriate or type ‘n’ to select a new color profile.
3. Enlarge the figure to view the pulse wave data (Figure 1.6).
4. When you move the mouse, a red cursor will move over the figure.
5. Click on 10 positions on the calculated TAMean velocity (shown as the teal line) to define the color profile.
    a. ***NOTE:*** The positions clicked on the TAMean should be varied to allow for a broad color profile range.
6. A figure of the extracted pixel intensities based on the new TAMean color definition is displayed. The TAMean high ratio is also displayed in the command prompt.

7. In the menu, select to either the “new” or the “default” parameters. The operator can also choose to repeat selection of the color profile.

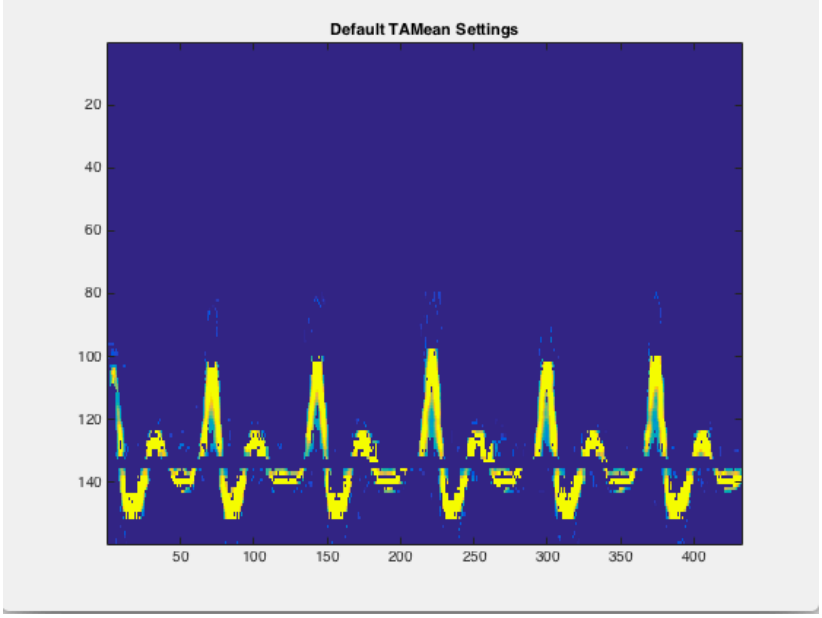

Figure 1.5 Display of the extracted TAMean data using the default settings for the color profile. This example represents an adequate recreation of the data with minimal gaps in the individual waveforms. Note the large horizontal gap represents data excluded by the position of the zero velocity line in the screen.

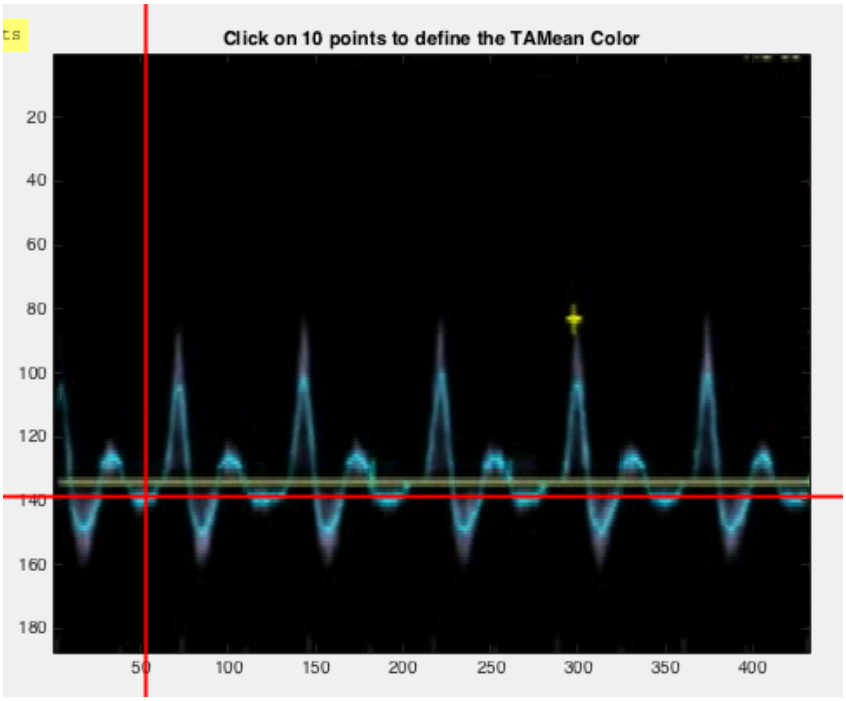

Figure 1.6 Display of the pulse wave data for selecting the TAMean color. Using the interactive cursor (red), the operator will select 10 points to define the TAMean color profile.

Saving the Platform Calibration Parameters

The operator can save the new platform calibration settings to a MATLAB® file (.mat file extension) to load into the *FloWave.US* program.

1. Type the desired filename into the command prompt to save the platform calibration settings.
    a. ***NOTE:*** The filename must include the MATLAB® file extension (*.mat).
2. To create a new set of calibration parameters, type PlatformCalibration into the command prompt to restart the program.

PART 2: FloWave.US OPERATION

Starting FloWaveUS

1. Open MATLAB®
2. Change the current folder (menu bar at top of Figure 1.1) to the folder location containing the ultrasound analysis m-files.
    a. Check that the m-files are listed in the current folder contents (left panel in Figure 1.1).
3. Type FloWaveUS in the command prompt and press **Enter**
    a. ***NOTE:*** You will see instructions displayed in the command prompt window to guide you throughout the analysis program.
4. Load the platform calibration settings file (Figure 2.1).
    a. Default settings for a GE Logiq Book *e* can be selected if needed.

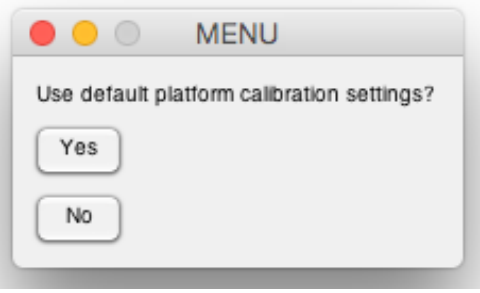

Figure 2.1 Load Platform Calibration Settings Menu. Default parameters for a GE Logiq Book *e* ultrasound are loaded as the default settings.

5. Choose the file directory that contains the video that you want to analyze.
    a. Browse for the folder and click **Open** when at the appropriate folder path.
        i. ***NOTE:*** Do not click on a single video file.
6. A list of the folder contents will be displayed in the command prompt.
    a. Type the filename and extension (e.g. Resting1.avi) of the video to be analyzed and press **Enter.**
7. Inspect the first video frame (Figure 2.2). Note the maximum time range.
    a. **Max Time Range** – the amount of time needed to update the ultrasound video screen (i.e. Sweep Speed).
        i. For example in Figure 2.2, the max time range is 6 seconds.
    b. ***NOTE:*** Any MATLAB figure can be enlarged using the 'maximize' window icons.
8. Type the max time range into the command prompt and press **Enter**.

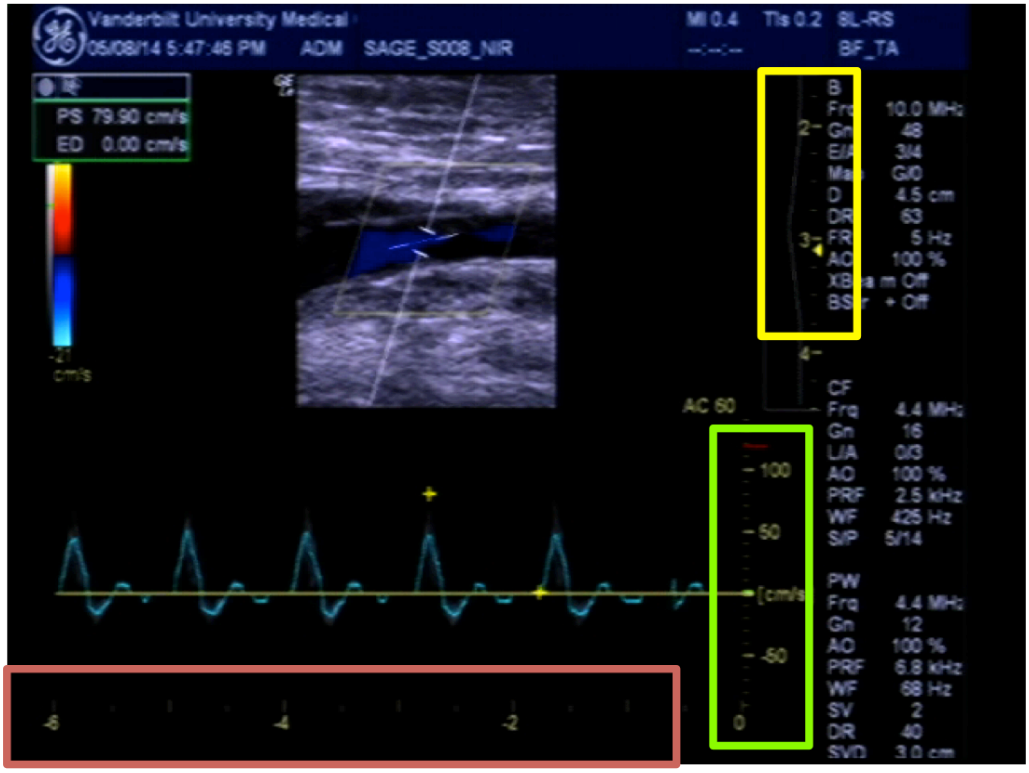

Figure 2.2 First image frame presented for inspection. The user should enlarge the figure and note some key figures to use for future command prompts. The red box shows the time scale for the velocity data. The Max Time Range, 6 seconds in this example, is the amount of time needed to refresh the data on the ultrasound screen. For calibration purposes, the user should also note the major and minor scale values for time (red), velocity (green), and diameter (yellow) values.

Pixel Scale Calibration

The program will present three figures (Figure 2.3): velocity, time, and diameter scales to calibrate the image frame. For each scale, the user will click on 2 points representing the extremes of the scale and enter the scaling factor between these points into the command prompt. The calibration function will calculate the scaling factor to convert the video pixels into the unit of interest (e.g. velocity, time, or distance).

8. Enlarge the figure to view the scale markings.
    a. When you move the mouse, you will see a red cursor + move over the figure.
9. Align the crosshair with a scale marking and click the left mouse button.
10. Move the crosshair to a scale marking at the opposite extreme of the scale and click the left mouse button. A total of 2 points are selected.
    a. ***NOTE:*** Click on scale positions that are extremes but the markings are still visible. For example, velocity scale points could be -20 cm/s and 40 cm/s; time scale points could be 6 s and 1 s; or distance scale points could be 0.5 cm and 2 cm.

11. A menu will appear in the left corner of the screen asking for the direction of the scale ("Vertical" or "Horizontal"). Click on the appropriate option (e.g. Vertical – velocity or distance; Horizontal – time).
12. Minimize the figure.
13. Type the scaling factor into the command prompt and press **Enter**.
    a. ***NOTE:*** The scaling factor is the value between the scale markings. For example, if you selected velocity positions -20 and 40 cm/s, the scaling factor would be 60 cm/s or a value of 60 entered on the command prompt. Enter only the integer value and not the units in the command prompt.
14. Repeat calibration steps for each figure/scale.

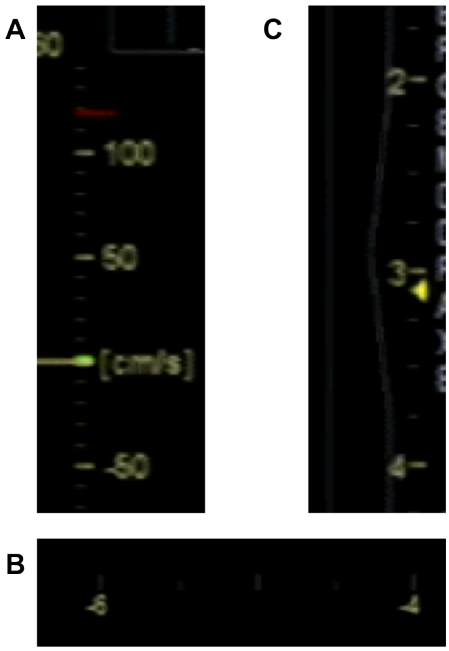

Figure 2.3 Example A) velocity, B) time, and C) diameter scales used to calibrate the pixel positions in the ultrasound image frame. The user will view each scale as a separate figure and select 2 points with the mouse cursor that represent extremes on the scale. The scaling factor can be entered into the MATLAB command prompt.

15. After the third scale, you can repeat the calibrations if needed (e.g. an incorrect scaling factor was entered). Type 'y' or 'n' in the command prompt and press enter to either repeat the calibrations or advance to the next code section, respectively.

First Frame Selection

The objective of portion of the code is to identify the start frame for the analog video data. The first frame should be selected to include a full update of the pulse wave spectrum to prevent repetition in the data analysis. Many video editing programs only enable data to be cropped to a certain time point rather than a specific image frame in the video sequence. To refine the video editing, this code allows the user to visualize individual frames to choose the precise starting frame.

1. The following message is displayed in the command prompt prior to the first frame selection: "An animation of the video frames will be displayed. Press the spacebar to stop the video at the desired start frame (i.e. a full pulse wave update). Paused. Press any key to continue."
2. Press a key on the keyboard to start the frame selection code.
3. An animated figure will be displayed (Figure 2.4). The figure will increment through each frame of the analog video simultaneously updating the frame number in the title.

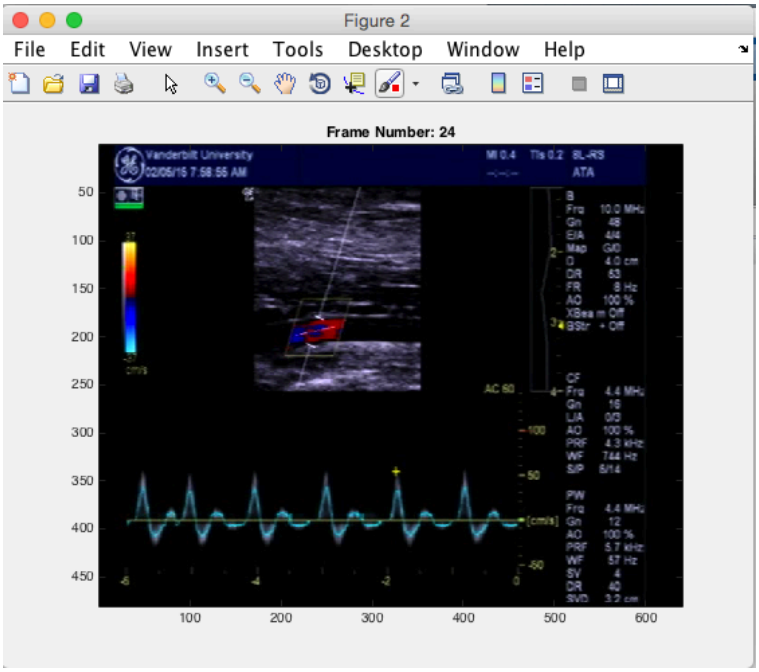

Figure 2.4 Animated figure displaying the initial frames of the analog video. Note the frame number in the title updates to allow the user to identify the start frame for the video. The start frame should be selected to include a complete screen update of the pulse wave spectrum.

4. Press the spacebar to stop the animation.
    a. ***NOTE:*** The selected frame should contain a full update of the pulse wave spectrum.
5. The start frame selected will be displayed in the command prompt.
6. Type 'y' if the correct frame was selected or 'n' to repeat the frame selection process.
    a. ***NOTE:*** The animated frame selection process can be repeated as many times as needed.

Data Epoch Selection

The objective of portion of the code is to divide the velocity data into sections based on changes in either the shape of the waveform or the frequency of the wave content. For example, a muscle contraction protocol can be divided into four sections: 1) baseline, 2) contraction, and 3) post-contraction recovery. Epoch selection also determines the image frame chosen to detect the vessel diameter in the B-mode image. Once epochs are selected, the user will interactively select a ROI around the vessel to automatically detect the vessel diameter. Sections of the data that reflect errors in data collection such as movements of the ultrasound probe can also be removed.

1. A figure of the composite velocity data set will be displayed (Figure 2.5).
2. Enlarge the figure.
3. Use the mouse cursor to click on the data points that designate the **end** of a data section (marked by red dots in Figure 2.5). Press **Enter** when you have selected the final point for the entire data set.
    a. ***NOTE:*** You select the end of the data not the start of the data (time = 0).
    b. ***NOTE:*** The points selected must be within the data set. For example if the total number of data points is 1000, the user should take caution to not select point 1001 on the axis. If a point outside the data range is selected, an error message will be displayed in the prompt. If this occurs, repeat the epoch selection process.
4. The figure will automatically close after you press **Enter**.
5. Check that the number of epochs (displayed on the command prompt) agrees with what was selected in the figure.
    a. ***NOTE:*** Recall that you will mark the **end** of a data section. For example, if you marked 3 points on the figure, this would indicate the selection of 3 data epochs.
6. A series of figures of the velocity data within each selected epoch will be displayed.
    a. Inspect the figure to determine if the epoch was correctly selected.
    b. Press the **Space Bar** to advance to the next figure.
    c. Type Y or N to indicate that the number of epochs was correctly or incorrectly selected and press **Enter**.
        i. **Y or Yes** – The program will advance to the next analysis step.
        ii. **N or No** – Repeat the selection of the data epochs as instructed in steps 1-5 in this section.
        iii. ***NOTE:*** Selection of epochs can occur as many times as needed.

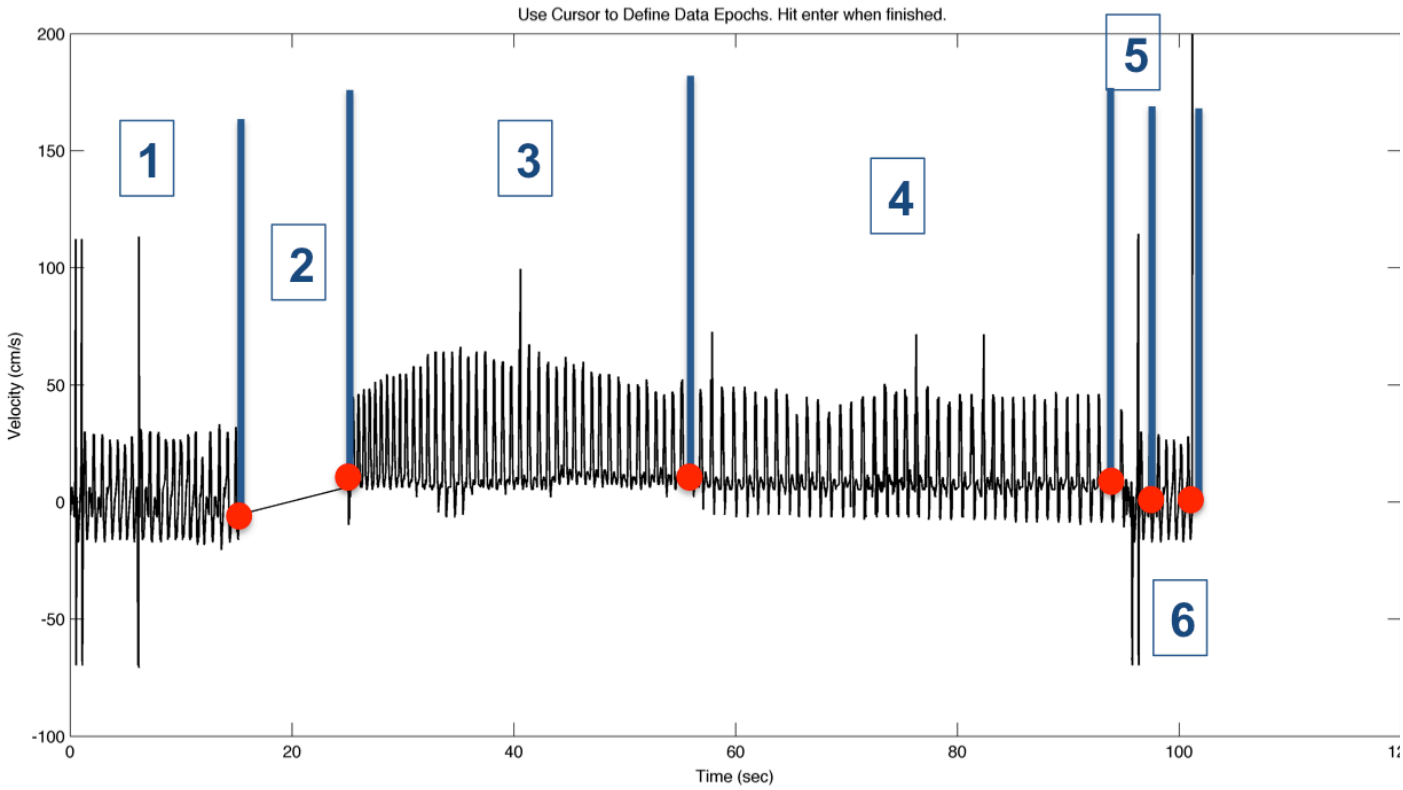

Figure 2.5 Example composite velocity vs. time series presented for data epoch selection. The user will align the mouse cursor with the data and left click on data points that designate the end of an epoch. These epochs can include data related to portions of the data protocol (e.g. epochs 1, 2, 3, and 4 reflect resting, contraction, post-contraction, and recovery data, respectively) or be used to eliminate motion artifacts in the data (e.g. epoch 5 includes a motion artifact resulting in the recovery portion being divided into epochs 4 and 6). The user should be cautious to click on a data point and not click outside of the data range. In this example, a point selected past 105 seconds will result in a program error. Epochs can also be modified to improve vessel diameter detection.

Vessel Diameter Measurements

The objective of this portion of the code is to measure the vessel diameter for each of the selected data epochs. An automated diameter detection code is used to extract the vessel diameter from the B-mode images. If a vessel diameter is not detected, the user can choose to enter a single diameter value extracted from high-resolution B-mode images.

1. Choose to use either a single vessel diameter measurement (e.g. extracted from a high resolution B-mode image) or the automated vessel diameter approach (Figure 2.6).

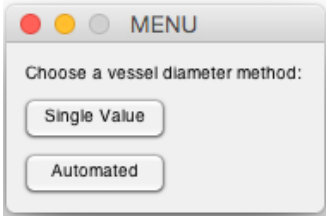

Figure 2.6 Select the vessel diameter detection method. Single values can be typed into the command prompt (units of cm).

2. A single image frame from the beginning of a data epoch will be displayed (Figure 2.7) along with a menu to select an ROI (Figure 2.8).
    a. ***NOTE:*** The image time is displayed in the title of the figure. This time point can be used as a reference for the data epoch (e.g. Time = 0 at resting, Time = 10 s at start of contraction, Time = 12 s at end of contraction, Time = 30 s at end of post-contraction). A vessel ROI should not be selected if the vessel walls are not visible.

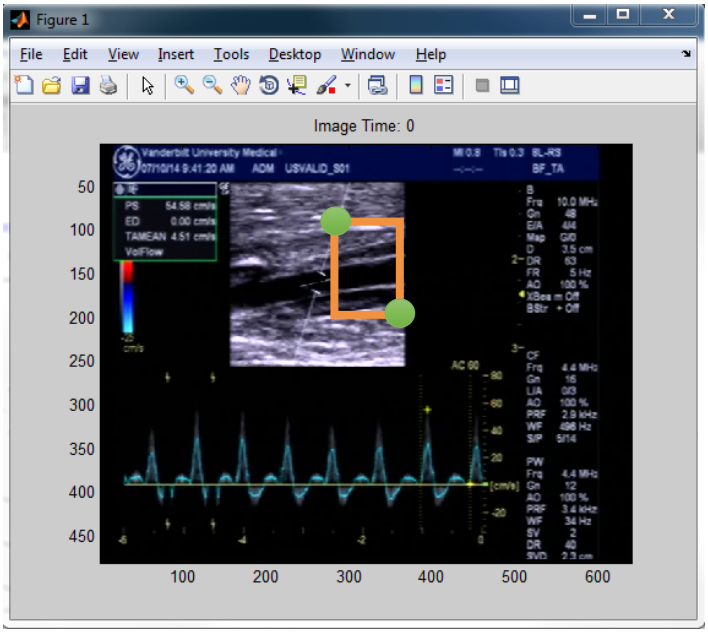

Figure 2.7 Image Frame used for Vessel ROI selection. If the option to define an ROI is selected, the user positions the mouse cursor in the B-mode image to define a rectangular ROI (example shown as the orange box). The ROI should be in close proximity but not include the US cursor gates (shown in the center of the vessel). The user defines the ROI with two mouse clicks (green circles): first to define the top left corner and second to define the lower right corner.

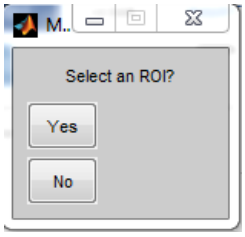

Figure 2.8 Vessel ROI selection menu. The user can proceed with diameter measurement or skip the measurement for the current data epoch.

3. The user can choose to measure the vessel diameter ("Yes") or skip the vessel diameter measurement for the data epoch ("No") in the menu.
    a. ***NOTE:*** A ROI should not be selected if the vessel walls are not visible such as during a muscle contraction.
4. **Yes** – Automated Vessel Diameter Detection for the data epoch.
    a. Enlarge the figure.
    b. Use the mouse cursor to click on two points in the B-mode image of the vessel. The first click defines the upper-left corner of the ROI. The second click defines the lower-right corner of the ROI.
        i. ***NOTE:*** The ROI should include a portion of the vessel where the vessel walls are bright and visible and the inner portion of the vessel is dark. If possible, this ROI should be in close proximity but not include the US cursor gates. See Figure 2.7 for an example of a possible ROI.
        ii. ***NOTE:*** The size of the ROI affects the processing time for the code. A larger ROI will take longer to process.
    c. A series of figures of the vessel ROI that has been rotated 90 degrees will appear (Figure 1.10). Enlarge the figure to define points of interests in the ROI.
        i. First Figure (1 mouse click) – Define the Center of the Vessel.
        ii. Second Figure (2 mouse clicks) – Define the width of the image mask. A red line will appear on the figure to demonstrate the selected mask.
            1. ***NOTE:*** The mask darkens the pixels in the vessel lumen; thus, reducing the amount of noise in the vessel diameter measurement. Caution should be used when selecting the mask to not mask the vessel walls.
        iii. Third Figure (2 mouse clicks) – Define the angle of the vessel wall. A green line will appear on the figure to demonstrate the selected angle.
            1. ***NOTE:*** The vessel wall angle is used to calculate the perpendicular distance between the pixels.
        iv. Type Y or N in the command prompt to choose to repeat the point selection for the ROI or to advance to the next portion of the code.

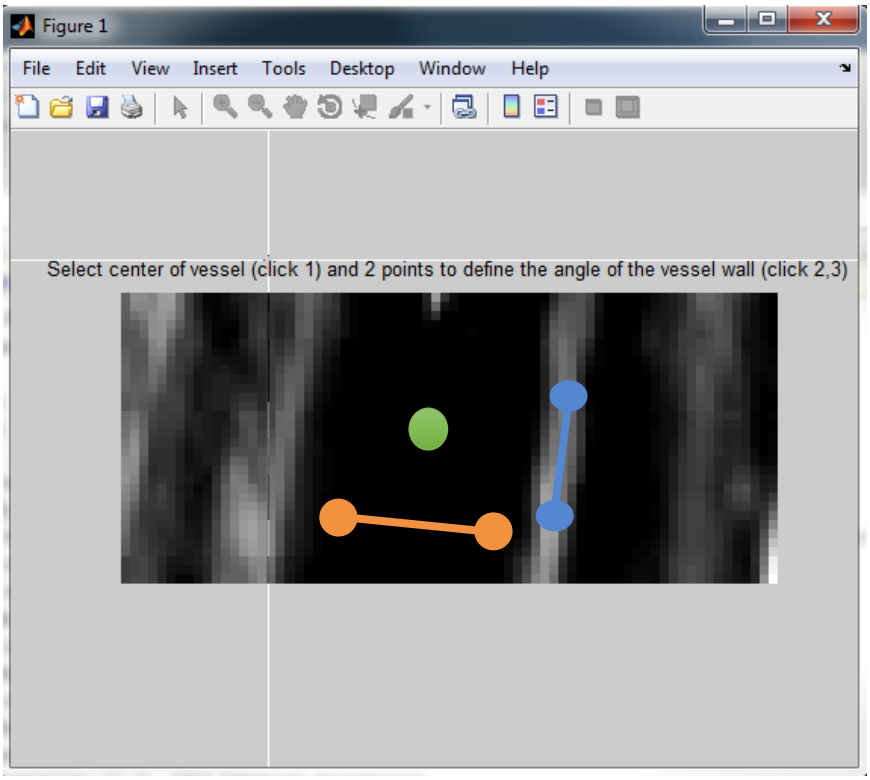

Figure 2.9 Vessel Center and Wall Angle Definition. The user uses the mouse cursor to select five points in the vessel ROI: 1) the center of the vessel (green circle), 2&3) two point in the vessel lumen to define the mask width (the orange line connecting the two points), and 4&5) two points on the vessel wall to define its angle (the blue line connecting the two points).

d. Repeat steps (a-c) for each data epoch.
e. A figure of the measured diameter versus time will be displayed. The user should inspect the time series to assess the quality of the vessel wall detection.
    i. See *Vessel Diameter Quality Control* for tips for ROI selection and examples of "good" and "poor" diameter detection.
f. In the diameter quality control menu (Figure 2.10), the user should select to repeat ("Yes"), keep ("No"), or use a single value.

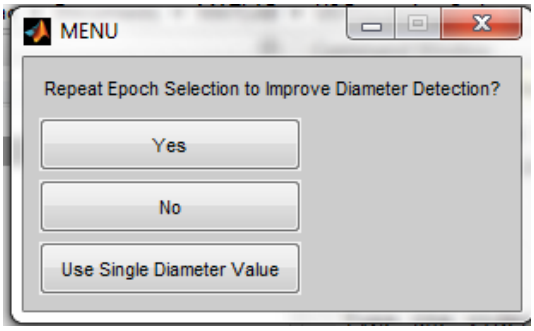

Figure 2.10 Vessel Diameter Quality Control Menu. A plot of the filtered vessel diameter time series is displayed to enable the user to assess the quality of the automated detection.

Vessel Diameter Quality Control

The user must evaluate the vessel diameter values to assess if the measures are accurate. The automated diameter detection code does removes diameter values for single pixels that deviate > 20% from the mean diameter. The diameter time series is also smoothed with a Savitsky Golay filter. However, in some cases the user may need to repeat data epoch selection, for example, it may be difficult to see the vessel walls immediately following a muscle contraction. The following examples represent some possible cases that may occur during the automated detection process and how the user might proceed with the analysis process.

Repeat Data Epoch Selection

1. Possible Scenarios:
    a. Diameter exceeds error limits (+/- 10% of the mean diameter) (Figure 2.11).
    b. The diameter time series is a straight line (Figure 2.12).
        i. ***NOTE:*** You can use the zoom tools in the figure menu bar to inspect the diameter time series in a closer view.
2. How to Repeat Selection to Improve Vessel Detection:
    a. In the vessel diameter time series, note the time that the error occurs. For example in Figure 2.11, the diameter is missing or exceeds the error limits from approximately 11 to 15 seconds.
    b. In the vessel diameter quality control menu (Figure 2.10), select the option “Yes” to repeat the epoch selection process.
    c. Click on the velocity time series (Figure 2.5) so the first epoch ends at the start of the error (e.g. 11 seconds) and the second epoch ends at the end of the error (e.g. 15 seconds). The third epoch in this example could be the end of the velocity time series.
    d. Repeat the vessel diameter measurement steps and evaluate the new diameter time series.
3. What if the Vessel Detection does not improve?
    a. If the repetition of the data epoch selection does not improve the errors, the user can choose to enter a single diameter value on the quality control menu (Figure 2.10).
        i. The BMode.m program can be used to analyze resting BMode images to determine a single diameter value.
    b. Type the diameter value (in centimeters) in the command prompt and press enter.

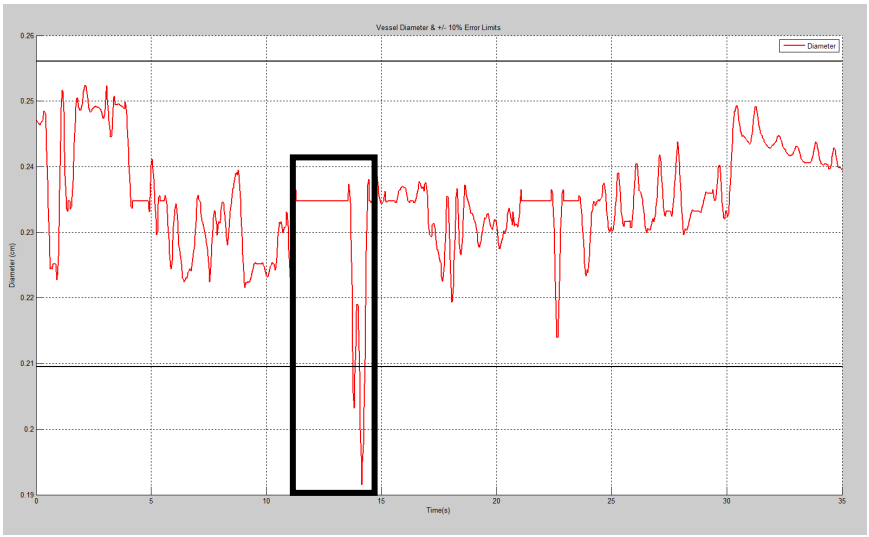

Figure 2.11 Vessel Diameter Time Series Quality Control Assessment. A plot of the filtered diameter time series (red line) and +/- 10% of the mean diameter error limits (solid black horizontal lines) is displayed to the user to evaluate the quality of the automated detection. In this example, the diameter exceeds the error limits (black box). The user should note the time that this error occurs and repeat the data epoch selection. A data epoch selected at the start and end of the times of this error may allow for better registration of the vessel walls.

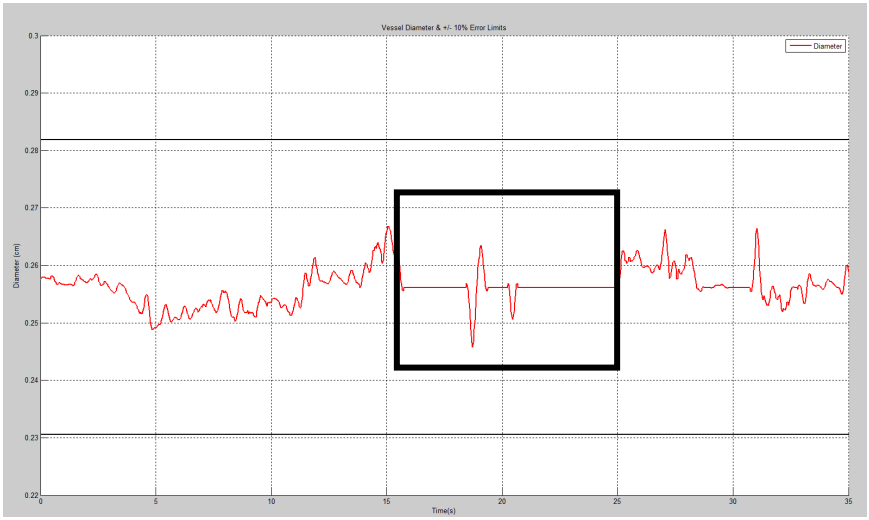

Figure 2.12 Vessel Diameter Time Series Quality Control Assessment. A plot of the filtered diameter time series (red line) and +/- 10% of the mean diameter error limits (solid black horizontal lines) is displayed to the user to evaluate the quality of the automated detection. In this example, the diameter is not detected (black box). The mean diameter is used to correct for the error. If the duration of the missing data is short or not during a critical period of the data collection, the user can choose to ignore this error. Otherwise, the user may want to repeat the data epoch selection to attempt to improve these data.

Cardiac Cycle Analysis

The objective of this portion of the code is to analyze the individual cardiac cycles included in the previously selected data epochs. The user determines if the data epoch should be analyzed, which allows data during a muscle contraction or motion artifact to be excluded. The waveforms are automatically identified based on the frequency content of the signal. Finally, figures of the analyzed data are created.

1. A figure of the velocity data within an epoch will be displayed.
2. Click on a menu option to choose whether the data should be analyzed (Figure 2.13).

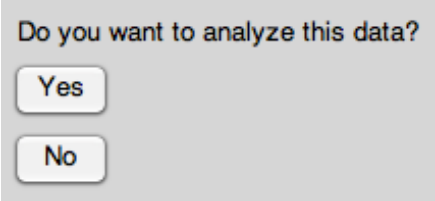

Figure 2.13 Individual Cardiac Cycle Analysis Menu. This menu is displayed with a corresponding figure of the velocity data for a selected epoch. If the user selects "no", the data are not analyzed and zeros are inserted in the variable arrays as placeholders.

3. **Yes –** Analyze the Velocity Data within the Epoch
    a. Peak Identification Part 1: Setting the Peak Height Threshold (Figure 2.14)
        i. A figure will display the velocity-time series for the epoch with the maximum velocity peaks identified for each waveform (red dots in Figure 2.14).
            1. **NOTE:** Default peak find settings = 20 cm/s.
        ii. Inspect the peaks and determine if the vertical peak height threshold is correct.
        iii. Type Y or N if the peaks are correctly or incorrectly identified.
            1. **Y or Yes –** The program will advance to the next step.
            2. **N or No –** Type a different peak height threshold.
                a. ***NOTE:*** The goal is to select a peak height threshold that identifies the majority of the peaks in the data set. The user is encouraged to repeat the threshold selection multiple times until an appropriate setting is chosen.
        iv. The figures will automatically close.

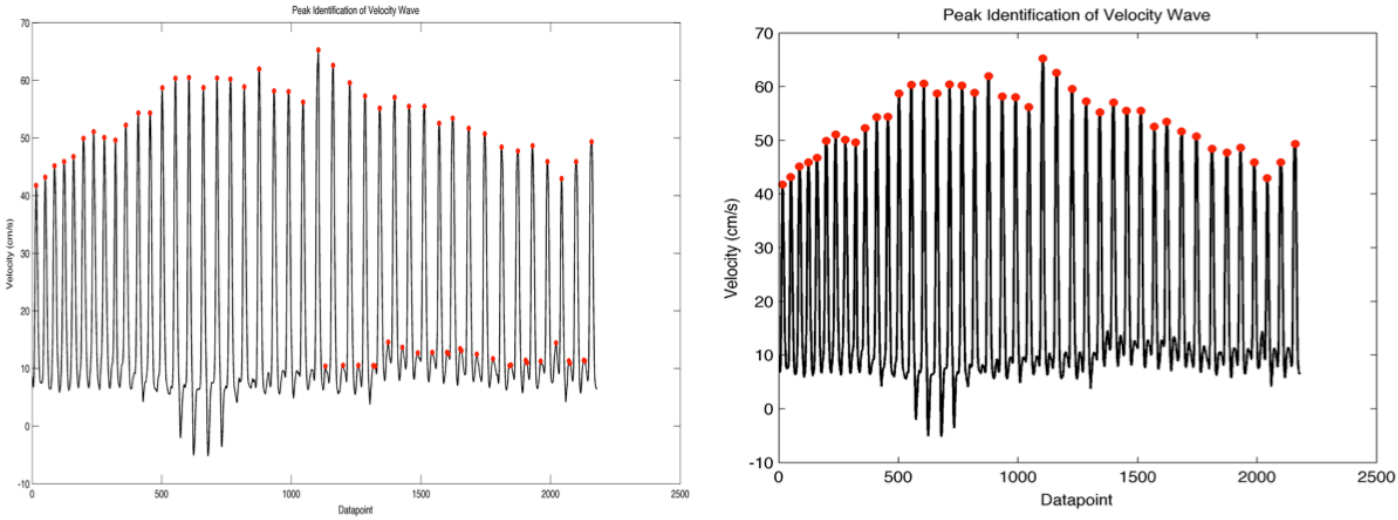

Figure 2.14 Peak Identification Part 1: Setting the Vertical Peak Height Threshold. In this example, the peak height threshold incorrectly identifies peaks (Left). Increasing the peak height threshold identifies the maximum peaks correctly (Right).

b. Peak Identification Part 2: Setting the Distance Between Peaks (Figure 2.15)
    i. A figure will display the velocity-time series for the epoch with the maximum velocity peaks identified for each waveform (red dots in Figure 2.15). Double-peaks, peaks that occur due to noise in the signal, are removed by a filtering step.
        1. ***NOTE:*** Default double-peak settings = 20 data points. For example, if peak 1 occurs at data point 10 and peak 2 occurs at data point 15, then both peaks will be excluded.
    ii. Inspect the peaks and determine if the double-peak width threshold is correct.
    iii. Type Y or N if the peaks are correctly or incorrectly identified.
        1. **Y or Yes** – The program will advance to the next step.
        2. **N or No** – Type a different peak width threshold.
            a. ***NOTE:*** The goal is to select a peak width threshold that rejects noise in the data but identifies the majority of peaks. The user should repeat threshold selection multiple times until an appropriate setting is chosen.
    iv. The figures will automatically close.

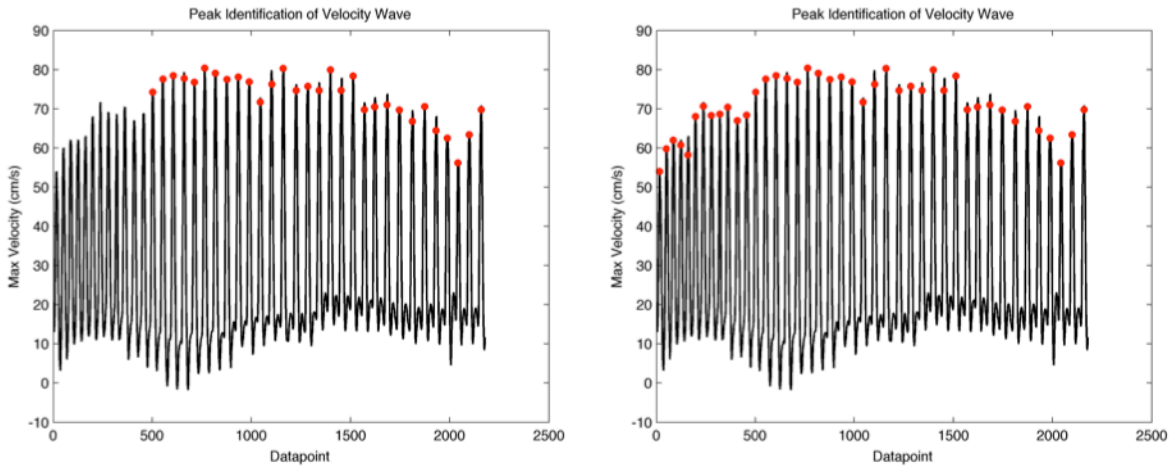

Figure 2.15 Peak Identification Part 2: Setting the Distance between Peaks to Filter Artifacts. In this example, the default distance between peaks (20 data points) filters too many of the waveforms (Left). Reducing the distance between peaks (15 data points) correctly identifies the maximum peaks.

c. Individual Waveform Analysis:
    i. Points of Interest in the blood flow data including: 1) blood flow at start of systole, 2) blood flow at peak systole, 3) blood flow at nadir of diastole, 4) blood flow at end diastole are extracted for each cardiac cycle.
        1. ***NOTE:*** Blood flow at the nadir of diastole are selected using a minimum peak height threshold. Minimum diastolic velocity values are selected using a minimum peak height threshold of 5 ml/min. If the blood flow does not reach this threshold (e.g. post-contraction), a warning message is displayed in the MATLAB command prompt: "Warning: No peaks found" or "Warning: Invalid MinPeakHeight". The user does not need to take action. A value of zero ml/min is used for these cycles.
    ii. A figure of an individual waveform with the key points identified in the velocity data will be displayed. These position indices are used to extract the appropriate data points from the blood flow time series.
    iii. Inspect the figure for the accuracy of the point identification (Figure 2.16).
    iv. Press **Space Bar** to advance to the next figure. The first three waveforms for a data epoch will be displayed.

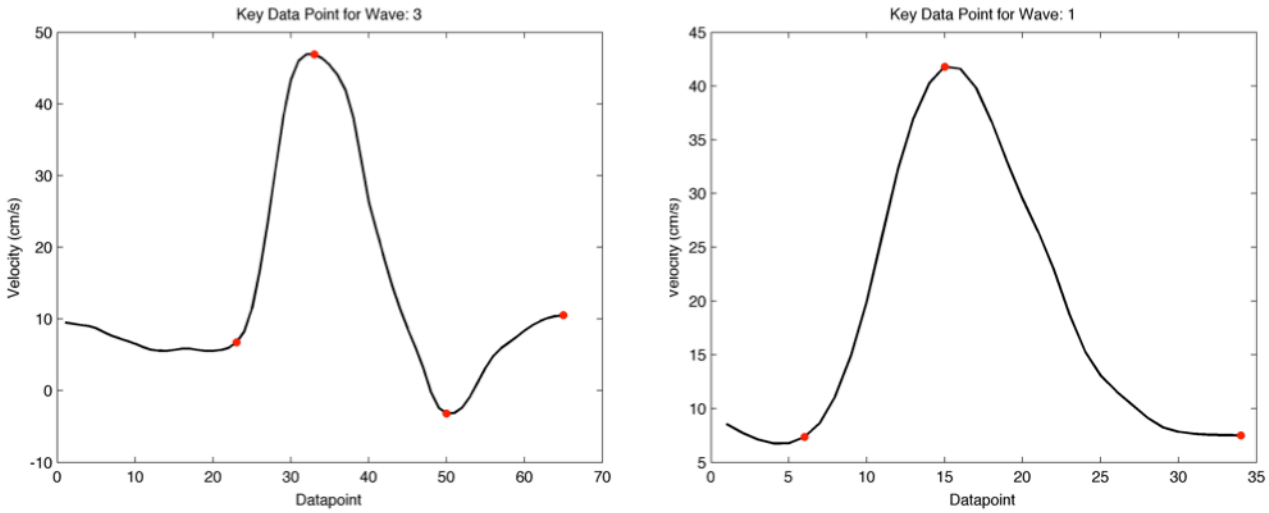

Figure 2.16 Individual Waveform Analysis. Key points for each waveform are automatically extracted. For triphasic waveforms (Left) expected during resting or recovery velocity data epochs, 4 points should be identified. The minimum diastolic velocity will not be identified if the velocity does not reach a -5 cm/s threshold. Only 3 points will be identified for biphasic waveforms (Right). This shape of waveform is common for post-contraction velocity data.

i. Type Y or N in the command prompt if the points on the waveforms are correctly or incorrectly identified.
    1. **Y or Yes** – The program will advance to analysis for the next data epoch. If all the epochs have been analyzed, the program will advance to the data storage stage.
    2. **N or No** – The program will exit. There may be an error in the data or different epochs should be selected.

4. **No** – Do not Analyze the Velocity Data within the selected Epoch
    a. ***NOTE:*** This option should be selected when no waveforms are visible in the data epoch (e.g. during a muscle contraction or during a motion artifact). Zeros will be inserted in the data arrays for the velocity variables.
5. Two figures displaying the blood flow at points in the cardiac cycle and the mean blood flow will be displayed. The user can review these figures to determine if the waveform analysis was accurate for the entire data set. If many errors are present, the user should repeat the analysis from the beginning.
    a. If desired, the user can click on the Save option in the File menu to store the figures for future editing.
        i. Saving figures with the "MATLAB Figure (*.fig)" option will enable editing of the figure settings in MATLAB in the future.

Data Storage

Cardiac cycle analyses, blood flow time series, and data processing settings will be written to a file for future analyses. Saved files can be found in the current folder location in MATLAB®.

1. Write to File
    a. Cardiac Cycle Data:
        i. Type the desired filename including file extension (*.csv) in the command prompt and press **Enter**.
            1. ***NOTE*:** A file extension **must** be included in the filename.
        ii. The format of the data file is displayed in the command prompt. Data are stored in the following format:
            1. Systole Time, Blood Flow @ Systole, Diastole Time, Blood Flow @ Diastole, End Diastole Time, Blood Flow @ End Diastole, Start of Systole Time, Blood Flow @ Start of Systole, Mean Blood Flow (Method 1), Mean Blood Flow (Method 2), Mean Blood Flow (Method 3), TAMean, Diameter @ End Diastole, Systole Time, Diastole Time, Oscillatory Shear Index.
                a. Mean Blood Flow (Method 1): Mean of Blood Flow from Start of Systole to End of Diastole
                b. Mean Blood Flow (Method 2): Mean Blood Flow Weighted for the time spent in Systole and Diastole.
                c. Mean Blood Flow (Method 3): Mean Blood Flow calculated with single diameter value taken from end of diastole
    b. Time Series Data:
        i. Type the desired filename including file extension (*.csv) in the command prompt and press **Enter**.
            1. **NOTE:** A file extension **must** be included in the filename.
        ii. Data are stored in the following format:
            1. Time, Blood Flow, Filtered Blood Flow, Shear, Filtered Shear, Velocity, Diameter
    c. Processing Settings:
        i. Type the desired filename including file extension (*.csv) in the command prompt and press **Enter**.
            1. **NOTE:** A file extension **must** be included in the filename.
        ii. Processing settings are stored for each data epoch in the following format:
            1. Peak height threshold, peak width threshold, average RR interval, number of peaks, vessel ROI time points, scaling factors – velocity, time, distance, and position of the zero velocity baseline.
2. Starting a New Analysis Session
    a. Type FloWaveUS in the command prompt to begin analysis of a new ultrasound image sequence.
        i. All variables will be cleared and figures will close automatically when restarting the analysis.

PART 3: B-MODE VESSEL DIAMETER MEASURES

This section of the manual describes the operation of a custom, semi-automated MATLAB program to analyze B-mode ultrasound images.

System Requirements

- MATLAB®
  - Version R2011a or newer – requires *Video Reader* function
- Video Converter
  - If using a windows operating system, video formats should be an '.avi' type.
  - If using a MAC operating system, video formats should be a '.mov' type using a MPEG4 codec.
- Analog Video Recordings
  - This program was developed for video recorded from the General Electric Logiq Book *e* ultrasound instrument. Certain sections of the code may need to be adapted if a different ultrasound device is used.
  - ***NOTE:*** See Part 3 of this manual for more information about video recording and editing.

MATLAB M-Files

- BMode.m – primary m-file to analyze B-Mode vessel images to measure vessel diameter
- *FrameCalibrate.m* – convert pixels to units of interest (e.g. velocity, distance, time)
- *VesselROI.m* – interactively select a rectangular region of interest (ROI) around the imaged vessel in the B-Mode image
- *AutoDiameter.m* – measure the vessel's diameter within the user-defined ROI
- *Imgaussian.m* – applies a Gaussian image file to enhance vessel edges (called by AutoDiameter; written by Dirk-Jan Kroon – see associated third party license)
- *GInputc.m* – adapts the ginput cursor properties (written by Jiro Doke – see associated third party license)

B-Mode Vessel Diameter Measurements

Many of the features of the BMode.m MATLAB® program function similarly to the FloWave.Us program. For additional details, please refer to PART 2 of this manual.

1. Open MATLAB®
2. Change the current folder to the folder location containing the m-files.
3. Type BMode in the command prompt and press **Enter**
   a. ***NOTE:*** You will see instructions displayed in the command prompt window to guide you throughout the analysis program.
4. Choose the file directory that contains the video that you want to analyze.
   a. Browse for the folder and click **Open** when at the appropriate folder path.
      i. ***NOTE:*** Do not click on a single video file.
5. A list of the folder contents will be displayed in the command prompt.

a. Type the filename and extension (e.g. Resting1.avi) of the video to be analyzed and press **Enter.**

6. Define an ROI around the distance scale (Figure 3.1).
7. Select points on the distance scale to calibrate the pixels (see *Pixel Scale Calibration* in PART 2 for additional steps and details).

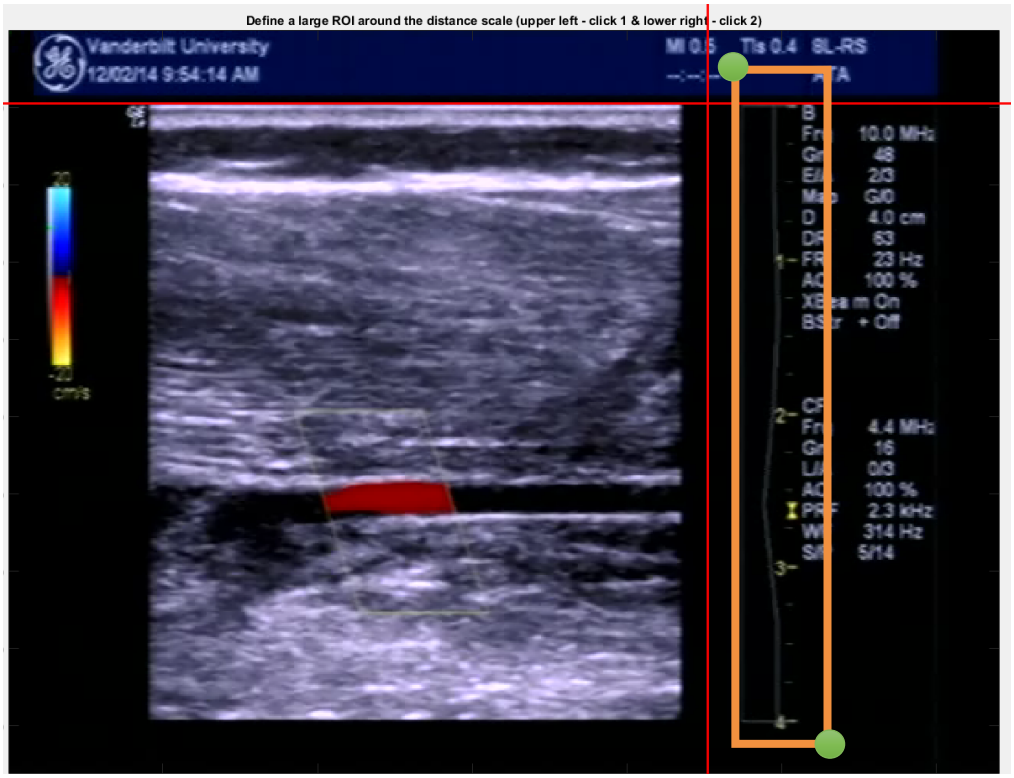

Figure 3.1 Image Frame used for diameter scale ROI selection. Position the mouse cursor (red lines) to define a rectangular ROI (shown as the orange box). The ROI should include as much of the scale as possible. The user defines the ROI with two mouse clicks (green circles): first to define the top left corner and second to define the lower right corner.

8. Select an ROI around the vessel walls to proceed with the vessel diameter measurement (see *Vessel Diameter Measures* in PART 2 for additional steps and details).
9. A plot of the diameter time series will be displayed. The user may also choose to record the mean diameter value (cm) displayed in the command prompt. This value could be used in the *FloWave.US* program if an automated diameter measurement is not viable in the duplex ultrasound data.

PART 4: ANALOG VIDEO CAPTURE

This section of the manual describes the procedures to record analog video data from the GE Logiq Book *e* portable ultrasound to a DVD and to convert these video files to a format compatible with MATLAB.

Equipment

- Portable Ultrasound (GE Logiq Book e)
- Sony Multi-Functional DVD Recorder (VRD-MC6)
- RCA Analog Video Cable
- Blank DVD-R/DVD-RW
- Video Conversion Software (WinX HD Converter Deluxe)
    - Other software can be used; however, it must be able to convert *.VOB files to *.avi or *.mov files.

DVD Recorder

The DVD recorder can be connected to the analog video output on the back of the portable ultrasound via an RCA analog video cable. Please refer to the manufacturer's instruction manual for detailed information regarding formatting a blank DVD, settings for analog video input, and finalizing a DVD.

1. Connect the AC adapter to the DVD recorder and to a power supply.
2. Connect the RCA Analog Video Cable between the portable ultrasound and the DVD recorder.
3. Turn the DVD recorder on.
    a. Press the eject button to insert a blank DVD-R.
    b. Press the Return key to view the Menu.
        i. Select the option Video -> DVD.
        ii. Select OK to format the DVD for recording.
        iii. ***NOTE:*** The ultrasound screen should be visible on the DVD recorder display along with the total recording time.
4. Press the Record button to begin recording the ultrasound video.
    a. To create separate "chapters" on the DVD, press stop to end recording. Pressing the Record button a second time will pause recording and save data to the same video file.
5. At the end of recording, press Return to view the recording menu.
    a. Select the Setup menu.
    b. Select "Finalize Disc". Agree to finalize the disc.
        i. ***NOTE:*** Finalizing the disc allows it to be played back on other devices. You cannot add videos after finalizing the disc.
6. Eject the disc and disconnect the recorder for storage.

Video Conversion for MATLAB

DVD video files are stored on the disc in a *.VOB file format. Video files must be converted to an *.avi file format (Windows) or a *.mov file format (MAC) to be imported in MATLAB® via the *VideoReader* function. The following instructions are specific for the WinX HD Video Converter Deluxe software to create an *.avi file. However, variety of other software options are available to complete the video conversion.

1. Open WinX Video Converter Software (Figure 4.1).

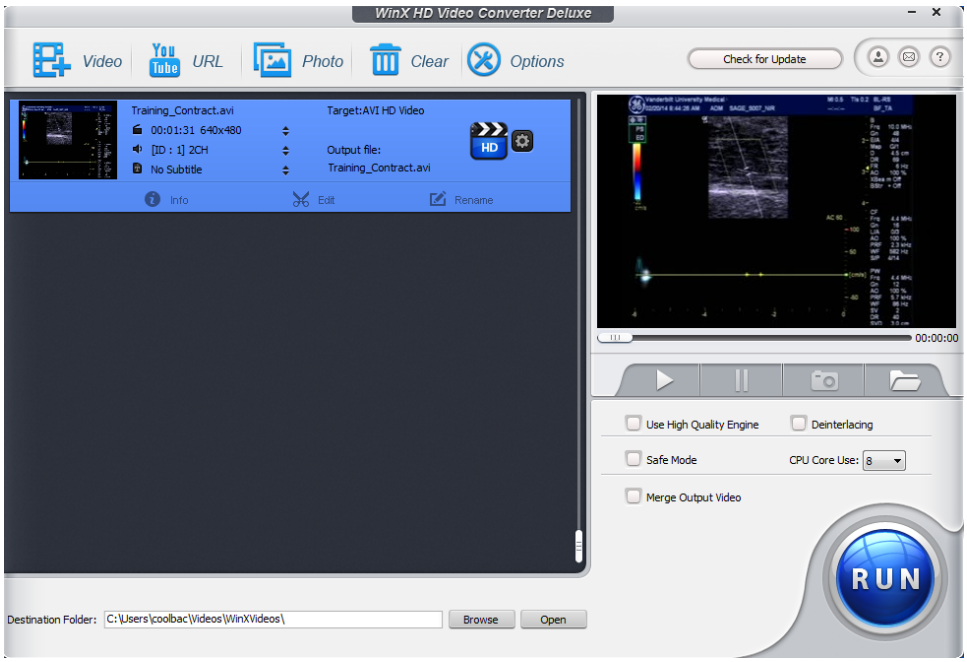

Figure 4.1 WinX HD Video Converter Software. Click on the "Video" icon in the top-left corner to select DVD files for conversion.

2. Click on the "Video" icon in the top-left corner to browse for the DVD files for conversion.
    a. Videos will appear as separate items in a list on the left of the screen. The user can rename the files, edit export settings, and select an output file format for each video.
3. Video output format can be changed by selecting the "HD" button (Figure 4.2).
    a. Select AVI HD Video as the video codec to use for file conversion. The slider bar should be moved to HQ to use a high quality codec.

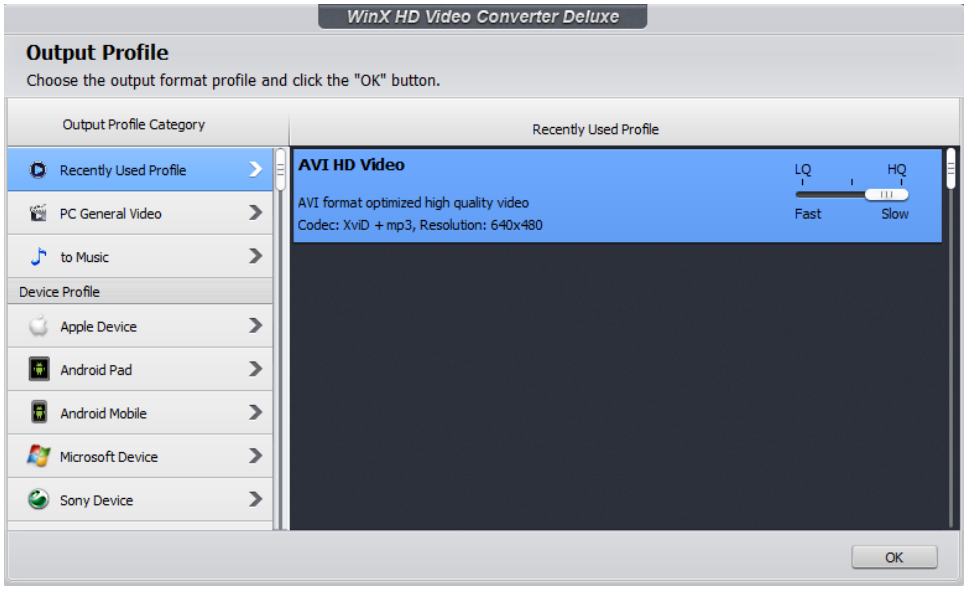

Figure 4.2 Selecting a Video Output File Format. An AVI HQ video codec should be used to convert files to a format compatible with MATLAB.

4. Set the AVI file output settings for import in MATLAB® (Figure 4.3).
    a. Video frame rate and aspect ratio should “keep origin”.
    b. Choose an appropriate resolution for your ultrasound equipment.
    c. No Audio options require editing.

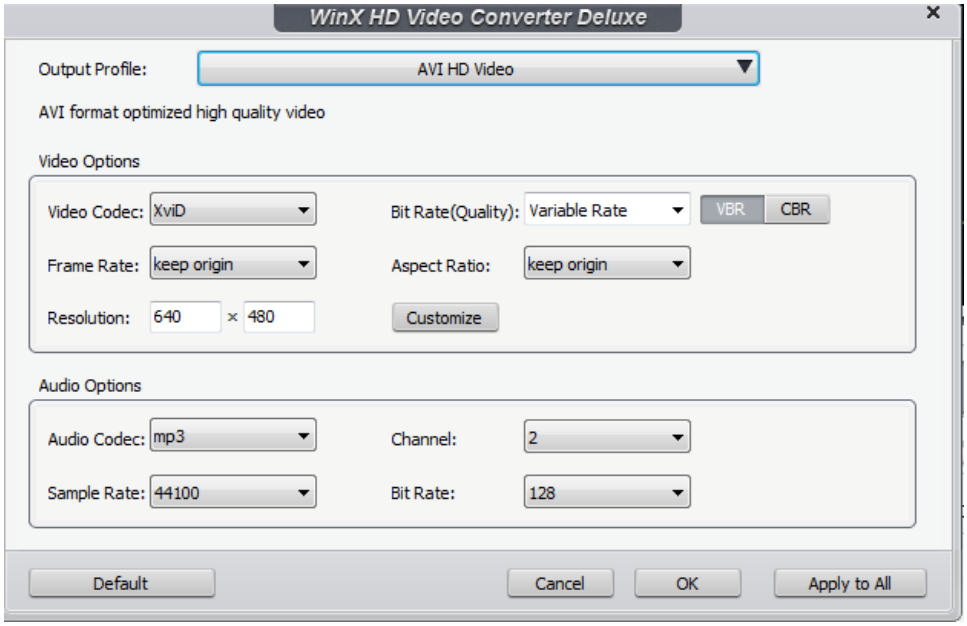

Figure 4.3 Setting AVI Video Output Settings. Here a resolution of 640 x 480 was selected for a GE Logiq Book *e* system.

5. Edit the video to clip extra frames at the beginning or end of the video using the “scissor” button (Figure 4.1).

a. ***NOTE:*** Ultrasound videos should be edited such that the first video frame includes a complete "refresh" of the pulse-wave velocity data (Figure 4.4). Additional editing to select a specific video frame is performed in FloWave.US.

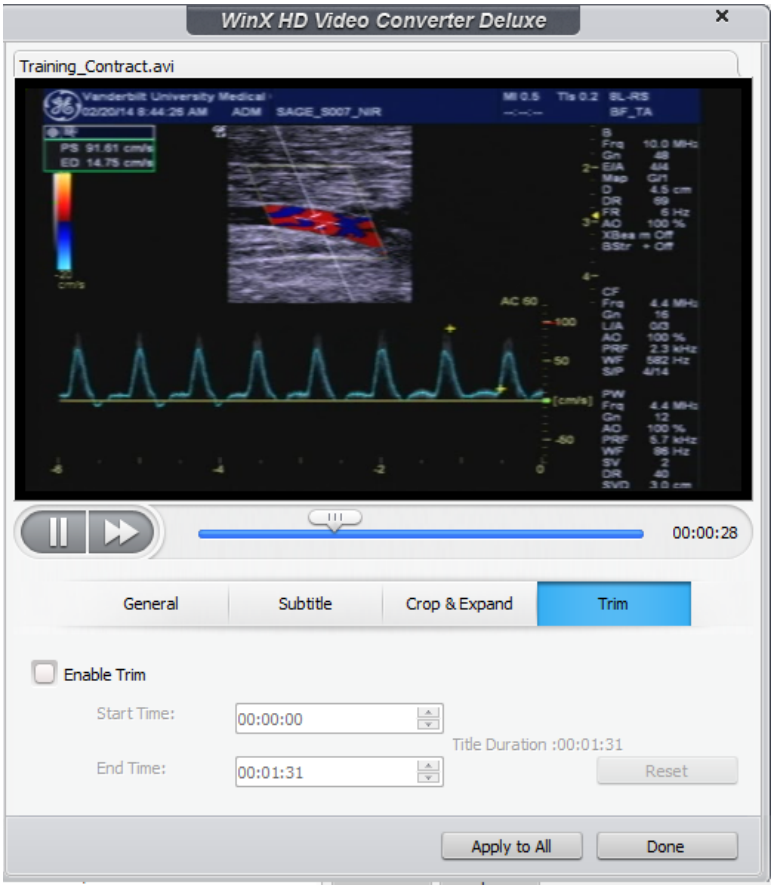

Figure 4.4 Video Editing Display. Select "Enable Trim" to remove frames at the beginning or end of the video. If the video is not visible in the display, close the conversion software and reopen the video.

6. Select a destination folder for the converted video file (Figure 4.1).
7. Click "Run" to convert the video.