NUFFIELD DEPARTMENT OF SURGICAL SCIENCES
UNIVERSITY OF OXFORD

SOP Number: JWVSU_03_V2.1
SOP Title: OxAAA JWVSU FMD and NMD analysis protocol.

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1. PURPOSE

The purpose of this Standard Operating procedure is to outline a consistent and reproducible method of analysis for Flow Mediated Dilatation and Nitro-glycerine Mediated Dilatation studies.

2. INTRODUCTION

This document outlines the correct procedures for organising study files, and the steps that should be taken to ensure accurate analysis of the flow mediated dilation (FMD) and Nitroglycerin mediated dilatation (NMD) images captured within the Jackie Walton, Vascular Studies Unit (JWVSU) for the Oxford Abdominal Aortic Aneurysm Study (OxAAA).

3. SCOPE

This SOP applies to the clinical research for which the Nuffield Department of Surgical Sciences, University of Oxford has accepted the role of 'Sponsor', in the Jackie Walton Vascular Studies Unit. This SOP may or may not apply to commercially sponsored research or research sponsored by an external non-commercial organisation.

4. RESPONSIBILITIES

a. Investigator or delegate

- Ensure the subject understands the procedure as described below.
- Ensure that the subject will be continuously observed during the study.

5. SPECIFIC PROCEDURE

a. Equipment

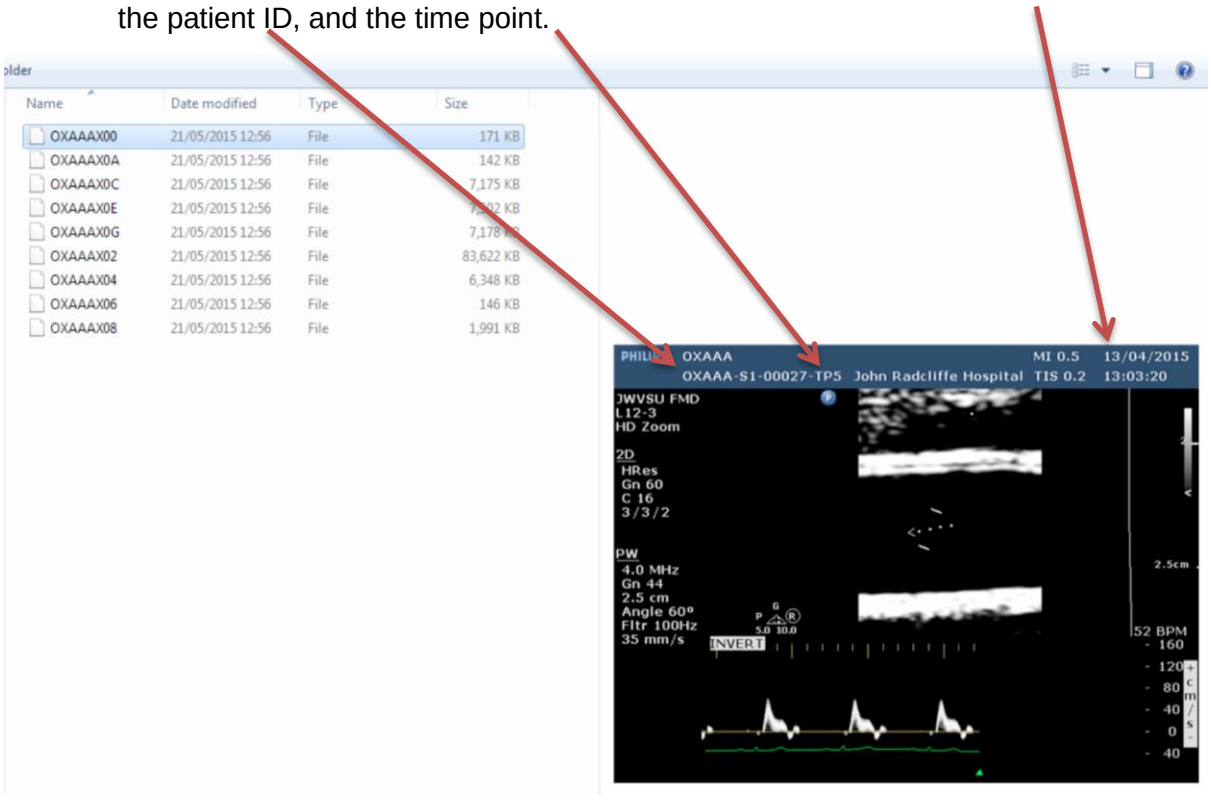
1. The research computer with the Brachial Analyzer for Research software installed
2. A designated, secure USB drive for transport of files
3. The OxAAA hard drive and backup hard drives
4. An extra Windows based PC
5. DICOM preview application (http://www.microdicom.com/dicom-shell-extension.html)
6. The blank folder structure available in the OXAAANDS1TB hard drive within the study image folder.
7. Access to an up-to-date version of the OxAAA FMD data mastersheet, or other excel document in which data is to be entered.
8. Access to OxAAA study appointment calendar and paper files
9. OxAAA backup hard drives

b. Retrieval of images for analysis

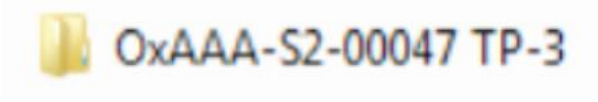
1. Initiate CX50 and allow 5 minutes for machine to fully start up. Although probe may capture images sooner, additional time is required for file retrieval functions to initiate.
2. Insert USB stick in port on the right hand side of machine.
3. In the top row of buttons on the keyboard, locate and press the “review” button.
4. Once in the review screen, hover the cursor over the top row of icons to locate the “export files” button, and press the left mouse button.
5. Select the “files created/modified since last export” option, and check the size of the files for export does not exceed the available space on your USB storage device. DO NOT directly download files to the main hard drive.
6. If not already selected, in the drop down menu, select “USB device”.
7. Click “export” and allow for media to be downloaded. This process typically takes an hour for several scans.
8. Once export has completed, remove USB stick from CX50, power down the machine, and close the cover.

c. Sorting of images

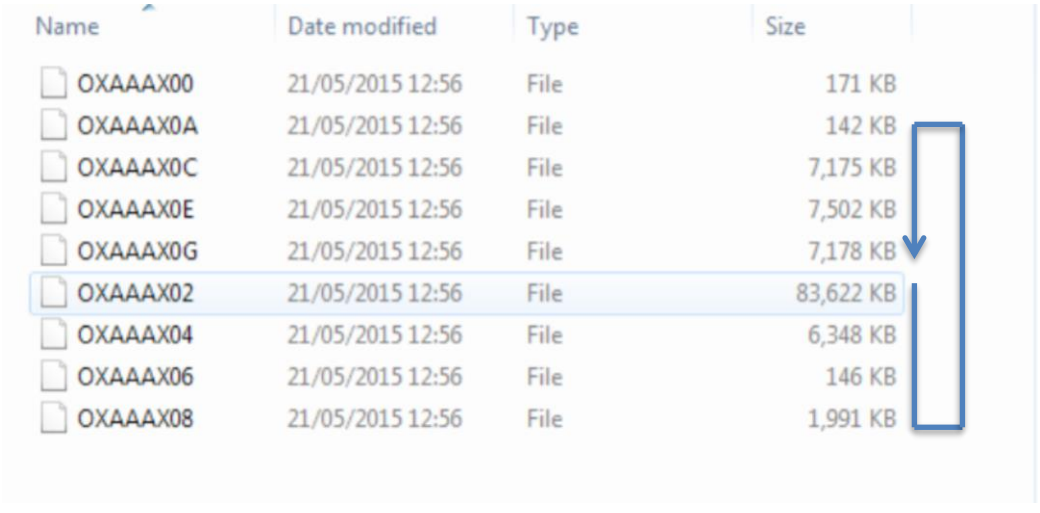
1. On a Windows PC with the DICOM preview software, insert the USB stick and the study hard drive.
2. Unlock the study hard drive by opening the unlocking application and entering the password.
3. Open an explorer window with the USB stick. You will see folders beginning with the extension “OXAA” followed by a letter and number string. Each folder contains all of the images from a particular scan.
4. Open the first folder and click once on the first image. Note the date of the scan, the patient ID, and the time point.



5. Refer to the appointment calendar for that day and verify that the correct patient ID and time point are present on the scan.
6. Relabel the folder in the form "OxAAA-S1-00123 TP-X," using the correct patient stream, patient number and time point, especially if the information on the DICOM file is not fully accurate. See image below for example.



7. Repeat with all folders until each has a unique identifier in the correct form.
8. Open the OxAAA Hard drive and locate the FMD images file. Inside will be the Blank Folder Structure file. Open that, and copy the two folders – "FMD" and "AAA loops" into the newly labelled folders in the USB stick.
9. Click on each image, check the label on the preview window, and drop it into the appropriate folder in either the FMD or AAA loop folder.
    - **i.** Files will be labelled with hexadecimal identification and will not be in order when sorted by name in the filing system.
    - **ii.** However, examining the size of the file can be invaluable in speeding the sorting process. Typically, files will have up to 10 static images resultant from the AAA scan and 2 loops, one AP and one transverse.
    - **iii.** In order of size the files will be as follows:
        1. ~80,000-100,000 kB : Ungated baseline loop
        2. ~20,000-30,000 kB: AAA loops
        3. ~6,000-11,000 kB: FMB, FMD and NMD 60s loops
        4. ~2,000-3,000 kB: FMC and NMD 30s loops
        5. ~100-300 kB: Static images
    - **iv.** Below is an FMD scan without NMD once the AAA images have been sorted away. The first image of the scan is OXAAAX00, which, according to its size, is a static image. This will be BFM-Velocity. The scan then jumps to OXAAA02, selected, which is the largest file and is the ungated baseline loop. From there, the images continue in order; next is the FMB gated loop, the static image for FMC-Velocity, the FMC loop. Having reached the end, the images start again at the top, after the BFM-velocity file, at the PHFM-Velocity image, and then the three FMD 60s loops in order.



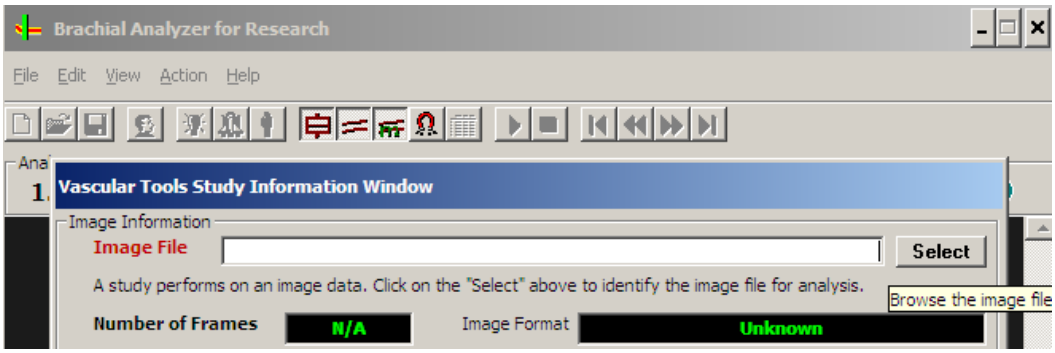
Name	Date modified	Type	Size
OXAAAX00	21/05/2015 12:56	File	171 KB
OXAAAX0A	21/05/2015 12:56	File	142 KB
OXAAAX0C	21/05/2015 12:56	File	7,175 KB
OXAAAX0E	21/05/2015 12:56	File	7,502 KB
OXAAAX0G	21/05/2015 12:56	File	7,178 KB
OXAAAX02	21/05/2015 12:56	File	83,622 KB
OXAAAX04	21/05/2015 12:56	File	6,348 KB
OXAAAX06	21/05/2015 12:56	File	146 KB
OXAAAX08	21/05/2015 12:56	File	1,991 KB

v. Examining several unsorted scans will quickly provide an intuitive understanding of this system and greatly speed sorting. In addition, if loops were mislabelled on the system, this pattern can help identify loops that are not labelled correctly based on their position in the order.

10. Copy a fresh version of the empty folder structure into the next folder and continue repeating this process until all images in all folders are sorted on the USB stick.
11. Open the USB stick and the Hard drive in two separate windows side by side.
12. Drag and drop each scan folder on the USB stick into the matching patient folder in the hard drive.
13. Once it is certain that all scans have been copied, clear the USB stick of all scans.
14. Maintain regular backups of the images folder on the OxAAA hard drive using the two designated backup drives.
15. In addition to sorting scans into the file system and generating backups; it is also advisable to maintain a database of the full scan inventory.

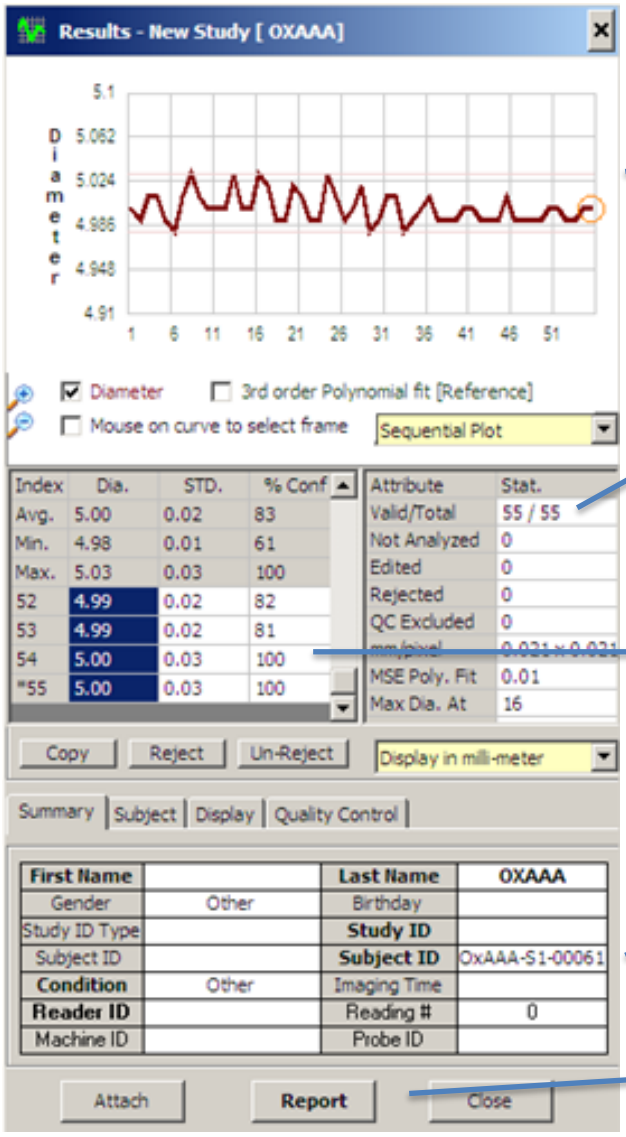
d. Running a basic analysis on an image loop

1. Plug hard drive in analysis laptop and navigate to “My Computer.”
2. Open the external drive labelled “Application” and select the drive unlocking program.
3. Enter password to access hard drive.
4. Open the Brachial Analyzer Program.
5. Select the leftmost button representing a blank sheet of paper to create a new study file.
6. In the dialog box that opens, press the select button to open the file explorer.



7. Navigate to the file you wish to choose, and double click to select the file.
8. Press “okay” in the open dialog box to proceed with analysis of image.

9. Play file from start to end to examine for any shifts in the vessel. Note the region of the image where the vessel is for the most frames.
10. Click Action in the upper menu, and then choose “Initialize.” Alternatively, select the fifth button from the left, depicting the light bulb.
11. The red dotted box that appears is the region of interest, or the ROI. It is within these boundaries that the width of the vessel is measured. The circles can be used to extend the borders of the box, and the central line should always remain within the lumen of the vessel, as one border will be defined on either side of the center.
12. During Step 1, the ROI will jump around as adjustment is attempted, as the software will attempt to offer its own suggestions. Move as necessary, and if required press proceed. During Step 2, the software will interfere less with the placement of the ROI, and will also show yellow lines at the borders of the vessel. If these lines do not correspond to the edges of the vessel, adjust the ROI as required.
13. Once satisfied with the placement of the ROI in Step 2, press process. The analysis process will start automatically. Ensure that the speed is set on 20 fps for speedy analysis, as “FAST” will not proceed as quickly. On the right hand side of the screen, a new window will open will open with analysis statistics, showing a graph at the top of the window and the numerical data in the middle of the screen.



Graph of sizes: frame number on x-axis and diameter on the y-axis.

Number of frames in which software identified walls

Raw numbers and basic stats

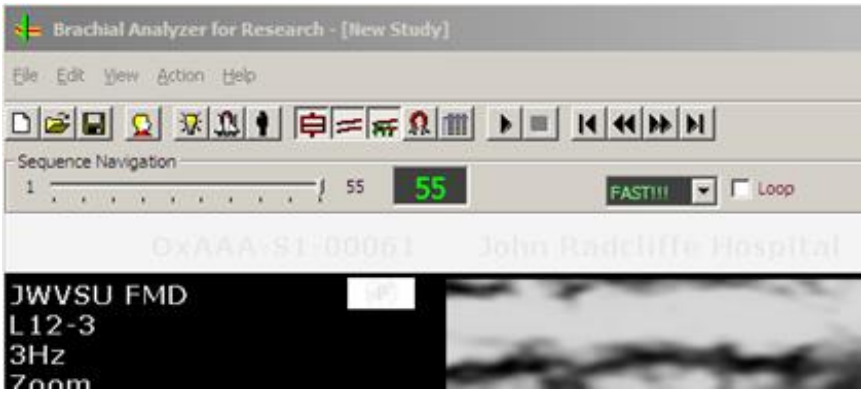
DICOM metadata, reflects only ID in image, not actual study ID if there was a mismatch during filing

Allows export to Excel

14. Once the graph has been acquired and all frame have analysed, frames that should be rejected can be rejected in two ways. Refer to the guidelines in the next section to decide which frames to reject.

    i. Select the frame on the graph or in the raw data table and press the reject button below the raw data table.

    ii. Navigate to the frame by clicking in the sequence navigation bar (shown below) and using the arrow keys or mouse to find the right image find the frame you wish to delete. Press “ctrl-X” on the keyboard and the frame will be rejected. This is best for loops in which many frames need to be deleted, as all frames can be examined by starting at the first frame and navigating through with the arrow key, deleting frames with the keyboard shortcut as required.



15. Once satisfied with the image, take note of data in appropriate location. Procedures for noting official data during analysis of an FMD scan will be detailed in the data management section below.

16. Save the study file by pressing the save button or choosing the option from the File menu.

e. Analysing an FMD scan

1. With the study hard drive plugged in and unlocked, navigate to the scan to be analysed.

2. Select the baseline, gated scan, in the FMB-1 folder. There should only be one baseline scan, but if there is more than one, select the later one. Ensure that if there are multiple, the chosen scan is of full length, or approximately 50-70

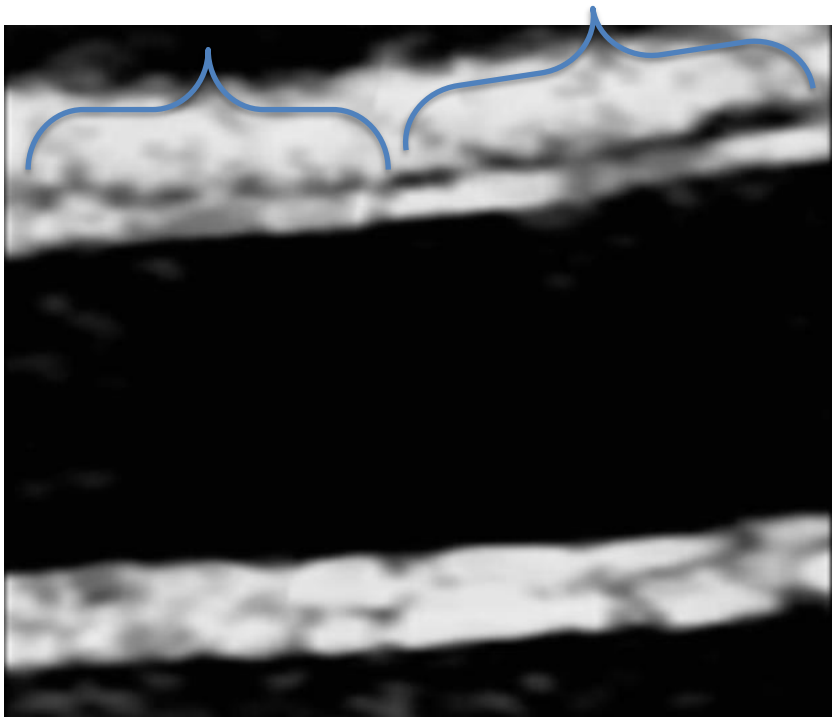
frames. The number of frames will be displayed when first selecting an image for a new scan, but before scan is opened.

3. Examine the baseline scan to examine for vessel drifting.

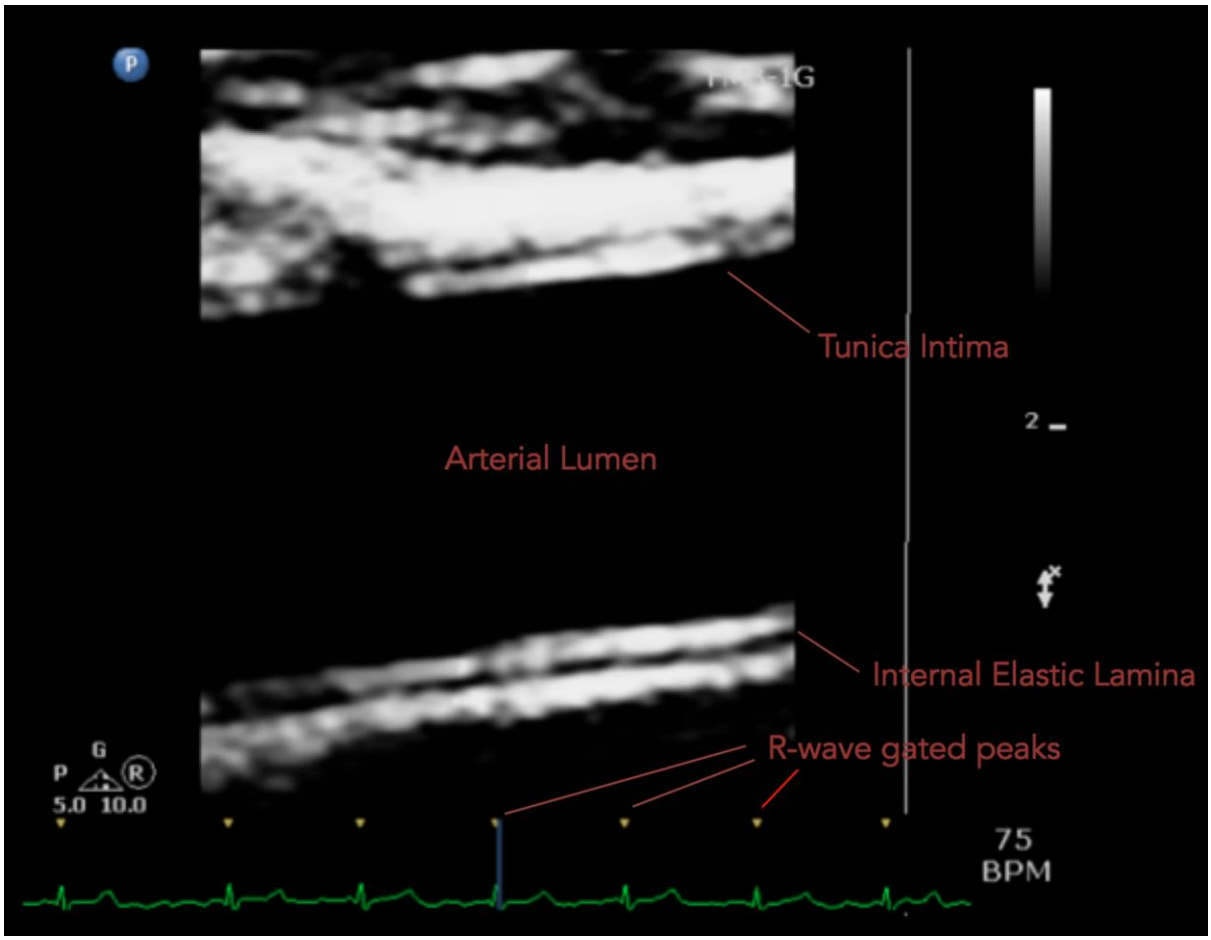
4. If the vessel does drift slightly vertically, expand the length but not width of the ROI to encompass all the regions the vessel occupies over the course of the scan.

5. If the vessel shifts dramatically, find the section of consecutive frames during which the vessel is most stable. Click on the first frame of this section, and select the lightbulb or initialize option on this frame. This will allow for definition of ROI to occur in this frame, as opposed to on the first frame. All frames will still be analysed, so those not in the consecutive set of stable frames should be deleted according to the frame rejection protocol detailed.

6. Avoid excessively lumpy or unusually bright areas of the wall, as this could be caused by calcifications of the vessel or artifacting during imaging, causing aberrantly small readings. During the course of the loop, a section of previously stable wall may become excessively bright or lumpy; these frames should be deleted to avoid skewing the average. See below for an example of a vessel with a region of consistent brightness (left) and region of inconsistent brightness (right).



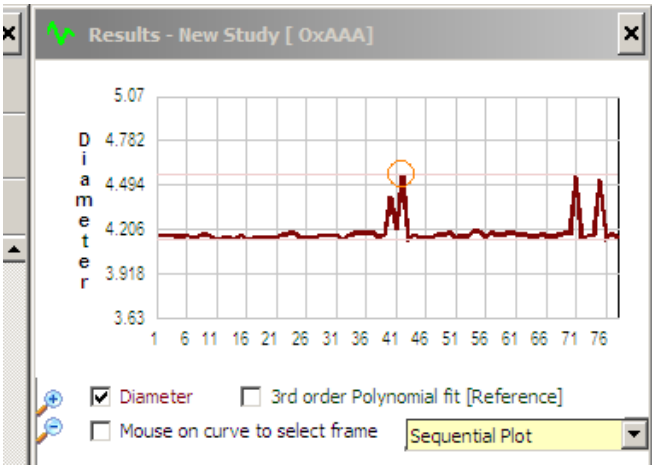
7. Select an ROI of minimal width in a clean and consistent region of the image, and proceed with analysis as detailed in section d. Ensure that the yellow lines of the ROI are capturing the intima of the vessel wall, as labelled in the image below.



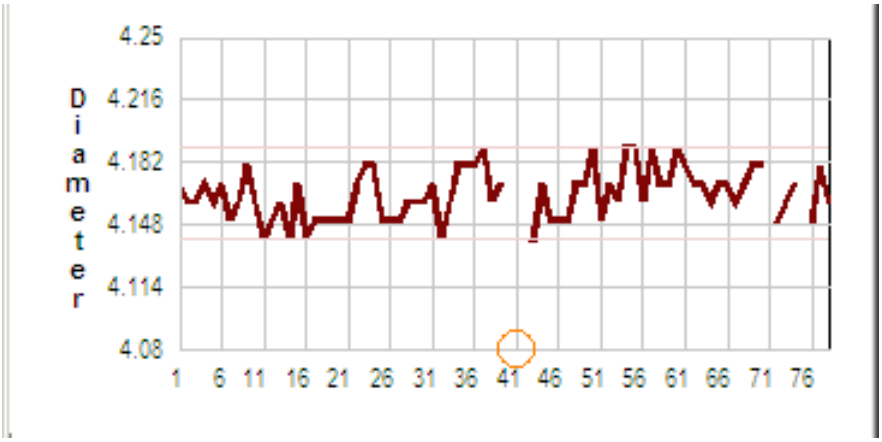
8. Once the analysis is complete, examine the graph.

   i. The orange lines on the graph denote the maximum and minimum brachial diameter values. If they are within 1/10th of a millimetre, there are likely no major aberrations in analysis.

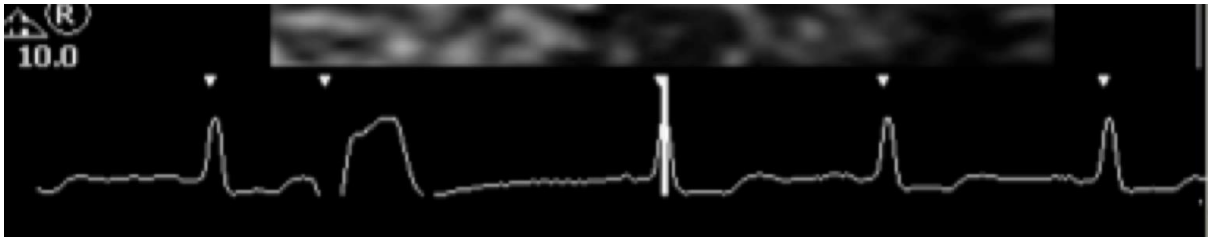
   ii. If the graph looks mostly like a straight line with one or a few peaks (below), those are the frames in which the analyser has failed to properly capture the walls of the intima. The orange lines will also have a much larger distance between them. Select those peaks on the graph and use the reject button to remove them.



iii. The graph dynamically adjusts the scale of the axis, so as the outlying aberrant points are all removed, the graph will readjust and the regions that previously looked straight will look far more jagged. A jagged graph is not a concern so long as the range is not very large; the physiological and imaging noise results in each frame having a slightly different diameter, which will average out during analysis of FMB-1. Below is an example, with gaps where frames have been rejected.



9. Check the ECG trace at the bottom of the screen to ensure that gating is functional and there are no ectopic beats. If there are, start at the beginning of the sequence navigation bar, and using the right arrow, scroll through each frame and delete those that are not gated at the peak of an R-Wave. Below is an example of an ECG in which the frame corresponding to the second beat much be removed.



10. If the entire scan is ungated, as ones taken on EPIQ, allow analysis to run, and reject every single frame by selecting frame one in the raw data table, scrolling to the bottom, and pressing shift-click on the last frame. This will select all frames, and then the reject button or ctrl-X can be used to reject them all. There will be ~300 frames, so find the frames in which R-wave peaks occur by clicking along the navigation bar and pressing ctrl-Z or the un-reject button to restore accurate frames.

11. Once frames from regions in which vessel is not in the ROI, which are obviously aberrant on the graph, are on an ectopic heartbeat or not at the peak of an r-wave have been removed, flip through each frame starting from the beginning to ensure that that all remaining frames are acceptable; that is, they have caught the walls of the intima accurately on a frame that is clear and free of artefacts around the vessel walls in the ROI.

12. However it is generally accepted that for a sample to be useable it requires a minimum of 10 accepted frames.

13. Save the file to a new folder in the appropriate analysis folder, labelled in the form“OxAAAS100081 TP-S1,” with the stream, time point, and patient ID as appropriate the scan that has just been analysed. Label the scan “FMB1.”

14. Do not yet report the scan in the official spreadsheet or export it to excel, until FMD has been analysed and confirmed.

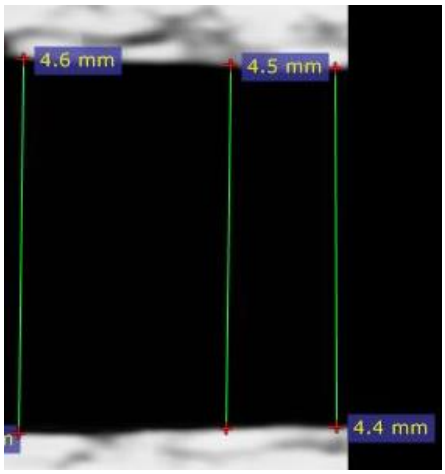
15. Open the first FMD image of the **SAME SCAN** in the brachial analyser tool.

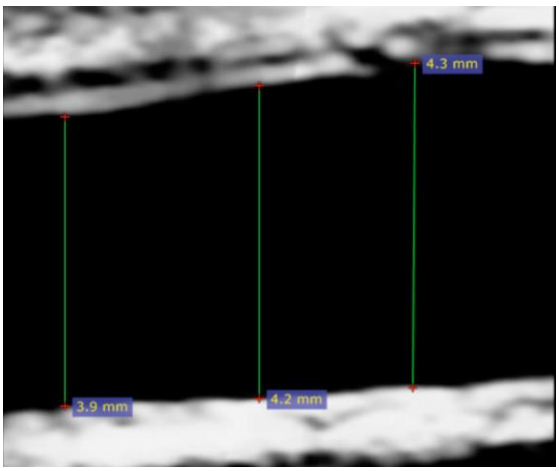
16. Confirm that the vessel has not shifted between FMB and FMD. This can be done by opening the two scans side by side, examining the FMC scan for shifts, and looking for landmarks in the scan such as a characteristic bend in the vessel.

17. Once satisfied that the vessel has not shifted, select the lightbulb icon or “initialize” from the drop down menu.

18. The ROI will appear in the exact same place it was in the previously analysed FMB-1 scan. Adjust it as slightly as possible while still capturing the vessel walls.

19. **It is VERY important that the ROI is as close as possible between the FMB and FMD scan. If the same region of intima from the baseline scan is not visible in the FMD, then return to the baseline scan, select a region which is also visible on the FMD, and re-analyse the baseline. It may be helpful to examine the FMD scan prior to analysing FMB-1, in order to find regions which are clean in both scans. As shown below, the same vessel can have very different diameters depending on region.**





20. As with FMB-1, avoid regions of the intima which are lumpy. Once you are confident that the same region is being analysed in both scans and that the region has stable intima and is of consistent brightness and smooth, proceed with analysis of scan.

21. As before, examine the graph resulting from the analysis. While FMB-1 is based on an average and requires as many frames to be reasonably accurate as possible, FMD measurement is dependant ONLY on the maximum frame, and is therefore highly sensitive.

22. Start from the maximum frame – the point on the graph touching the orange line, and follow the checklist below:

    i. Are the yellow lines touching the intima?

    ii. Is the vessel wall free of imaging artefacts?

    iii. Is this frame gated on a non-ectopic beat at the top of an R-wave?

    iv. Is the measurement in this frame consistent with the frames preceding and following? i.e. no drastic or sudden jumps.

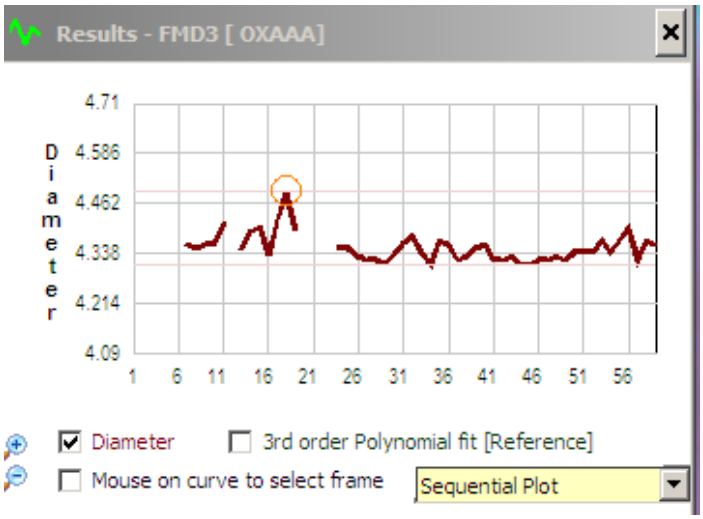
23. If all of these conditions have not been satisfied in the maximum frame, reject the frame and wait for the graph to readjust the scale and orange lines. This will happen quickly.

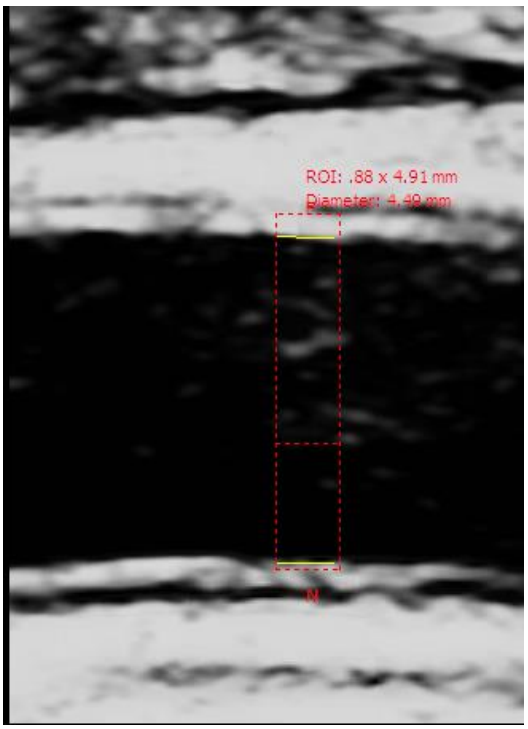
24. Select the new maximum frame, and apply the same checklist as above.

25. Repeat this process until you are satisfied that the maximum frame is acceptable.

26. Examine the resulting graph. Does the value of the maximum frame occur multiple times? If yes, value is likely to be accurate. If the next highest frame/s are very close to the orange line, the maximum is also likely to be accurate.

27. If the clean maximum frame is far higher than the next highest clean frame (>3/100 mm) consult with another analyser to discuss whether frame should be discarded or kept. Shown below, this scan shows a peak 12/100ths of a millimetre above the next highest, which would cause a 2-3% difference in FMD. The decision will depend on the quality of the scan and the smoothness of the analysis graph.





28. Save the study in the previously created folder, named “FMD” and note the maximum diameter of the brachial artery, but do not yet report it in the excel spreadsheet.

29. Open the next FMD image file and repeat steps 13-23. If the resulting maximum diameter is higher than the first image of the FMD analysis, then save this analysis over the previous one. If the maximum diameter found in this scan is lower, do not save it.

30. Repeat step 25 with the final FMD scan of the study. Again, is the maximum diameter in this scan exceeds the previously record one, save this study file as

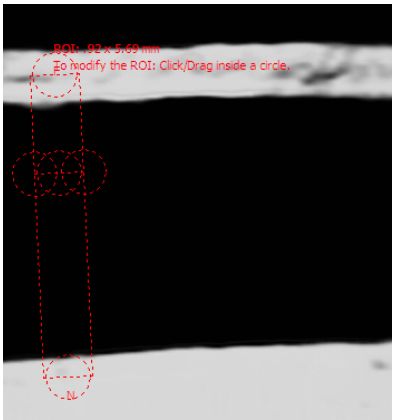
FMD, deleting the previous file. Note the maximum of the final file saved, which should be the maximum FMD brachial artery diameter between all FMD loops.

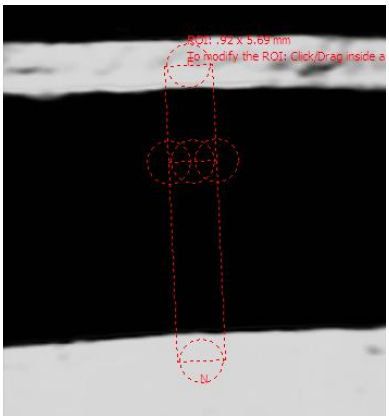
31. Compare the maximum FMD diameter found to the average baseline diameter noted. If FMD max diameter is lower, open the FMD study files saved and re-calibrate the file as described below. This frequently fixes the problem. If, upon opening FMB1, calibration was requested, you **MUST** also calibrate each FMD file. If calibration is not explicitly requested, do not calibrate unless the FMD max is lower than FMB min.

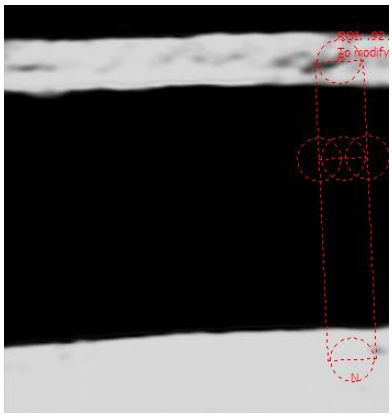
    i. To Calibrate, press the action menu and select calibrate.
    ii. Ensure that vertical calibration is selected.
    iii. Position the crosshair callipers on the top and bottom left-hand corners of the zoom box.
    iv. At the top of the screen, enter 10 mm in the entry box, and press calibrate.
    v. Repeat analysis process.

32. The two study files saved –FMB1 and FMD- should now both have the same ROI, and the FMD should be the file in which the largest diameter was found.

33. It is advisable to obtain measurements of FMB1 and FMD in several areas of the sample to provide an average due to irregularities in vessel lumen diameters. We recommend repeating steps 1-31 for regions within the sample to the left (L), centre (C) and right (R) of an analysable region within the frame. Save each file carefully. Ensure that the ROI analysed each time is consistent between the FMB and FMD scans. If there is not enough visible intima overlapping between both scans, only one or two analyses may be possible.







34. There should now be three scans labelled FMB1, and three scans labelled FMD. When saving, add an indicator of “L,” “C,” or “R,” to denote which of the three analyses is enclosed.

35. Open the first FMB1 file, record the minimum, maximum, number of valid frames, and average in a blank spreadsheet. Press the report button, choose Excel, and save the Excel report in the EXCEL REPORTS folder, under the appropriate filing. The filename should be the patient ID number, and L, C, or R, depending on which of the analyses is enclosed.

36. Repeat for each FMB1 scan with a new row, and use a formula to obtain the average minimum, maximum, frame number, and average.

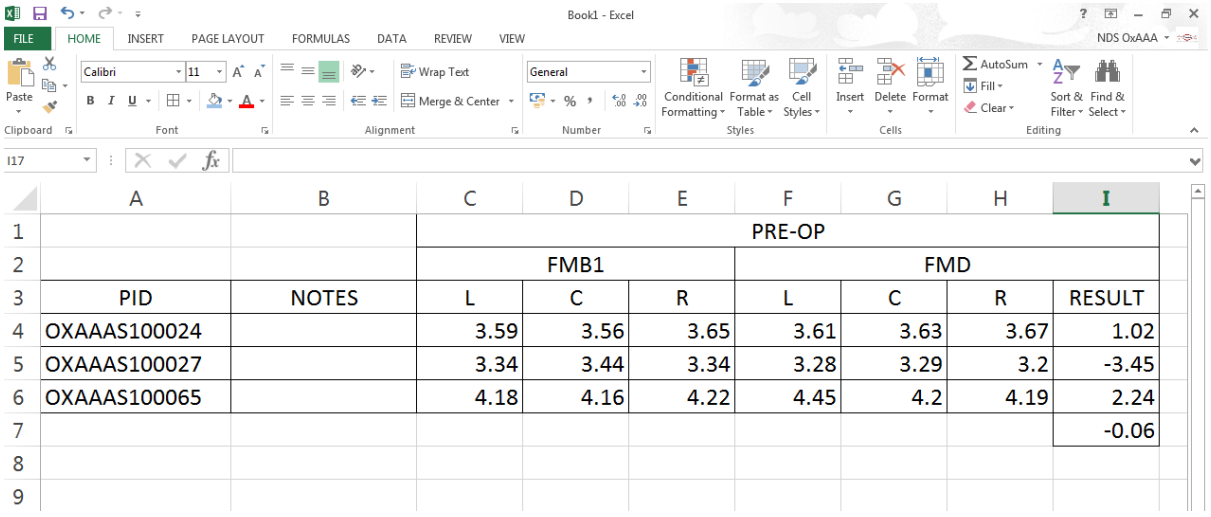
37. Repeat this process for FMD.

38. In the OxAAA master spreadsheet under the tab for the correct study time-point, create a new row for the scan and place the short form of the study ID in the third column. Copy the DICOM tag from the bottom right hand side of the analysis window into the first column, labelled PID. If there were any notes during analysis, such as a gating issue or ambiguous frames, note them in column 2.

39. In the appropriate column, note the calculated values for minimum, maximum, frame number, and average.

40. FMD should first be calculated for each of the different vessel regions (L, C and R) as a percentage increase using the average FMB-1 value and the corresponding FMD max value. Then the 3 FMD percentages can be averaged to provide a measure of overall vascular function independent of irregular lumen diameter across the sample.

41. Step 39 can be completed concisely using the formula:
=IFERROR(100*((F4-C4)/C4 + (G4-D4)/D4 + (H4-E4)/E4)/3,"")
As used in the first row of data in the screenshot below.



	A	B	C	D	E	F	G	H	I
1			PRE-OP						
2			FMB1			FMD			
3	PID	NOTES	L	C	R	L	C	R	RESULT
4	OXAAAS100024		3.59	3.56	3.65	3.61	3.63	3.67	1.02
5	OXAAAS100027		3.34	3.44	3.34	3.28	3.29	3.2	-3.45
6	OXAAAS100065		4.18	4.16	4.22	4.45	4.2	4.19	2.24
7									-0.06
8									
9									

42. If NMD was completed on this patient, the same procedure should be repeated, treating FMB2 as the FMB1 and both NMD25 and NMD400 as the FMD scan. Unlike FMD, only one ROI/analysis needs to be completed for these scans.

43. Report these results in the same row as the FMD results were reported.

f. Tips on analysis

1. Position the ROI as close to the true intima as possible to avoid drifting to the internal elastic lamina or other artefacts – this will skew averages obtained for FMB1 measurements.

2. Use a standard ROI width – the OXAAA study defined this as 0.84mm (the point at which the ROI adjustment circles intersect).

3. Upon obtaining an anomalous result first check for ROI drifting. If this looks normal then check calibration as described in step 31 of section e. Note that the figure of 10mm listed in this SOP may be specific to the OXAAA CX50 ultrasound machine. It is advisable to obtain your own calibrating value by using the digital callipers available on most ultrasound machines.

4. If at any point you are unable to open a saved analysis through the Brachial Analyser, first check that the original sample file still exists. Either has it been deleted or was it stored on a removal storage device? The brachial analyser adopts a fixed file path utilisation mechanism and hence requires access to the initial loop in its original location. Therefore this issue can be resolved either through simply running a new scan or copying the sample file to its original location at the time of first analysing.

5. If the sample falls out of view when using the zoom feature press one of the ‘centre image’ or ‘centre on ROI’ buttons.

6. If the intima is faint or the contrast of the image is unfavourable for other reasons, this can be adjusted by using the ‘window/level’ slider bar in the bottom left of the screen.

7. Consider that the ‘Brachial Analyser’ occupies a large proportion of the CPU usage and it is therefore wise to run as few programs as possible in the background when conducting analysis.