

Technical Procedure: Vertical Stitching

1 Abstract

This document describes the procedure to stitch multiple reconstructed volumes, offset in the vertical direction using version 11. The resulting volume will have a larger field of view while maintaining the desired higher resolution.

2 Overview

Vertical stitching is a technique which allows users to combine multiple datasets which have an offset in the Y-direction (vertical direction) only. This procedure will guide users in obtaining and stitching datasets from multiple regions collected over the length of a sample.

The field of view (FOV) in a 3D tomography is determined by the pixel size and number of pixels used. For a single scan, a larger FOV results in larger pixel size thus lower resolution.

E.g.: In Figure 1, the complete 70mm long tube is too large to be accommodated in one FOV. Without stitching, the best pixel resolution that can be achieved is 30 μm (binning 1) while imaging a 62mm portion of the length of the sample. However, by collecting and stitching 3 datasets, the user can obtain a much higher resolution of 40 μm (20 μm by using binning 1) over the full length of the sample.

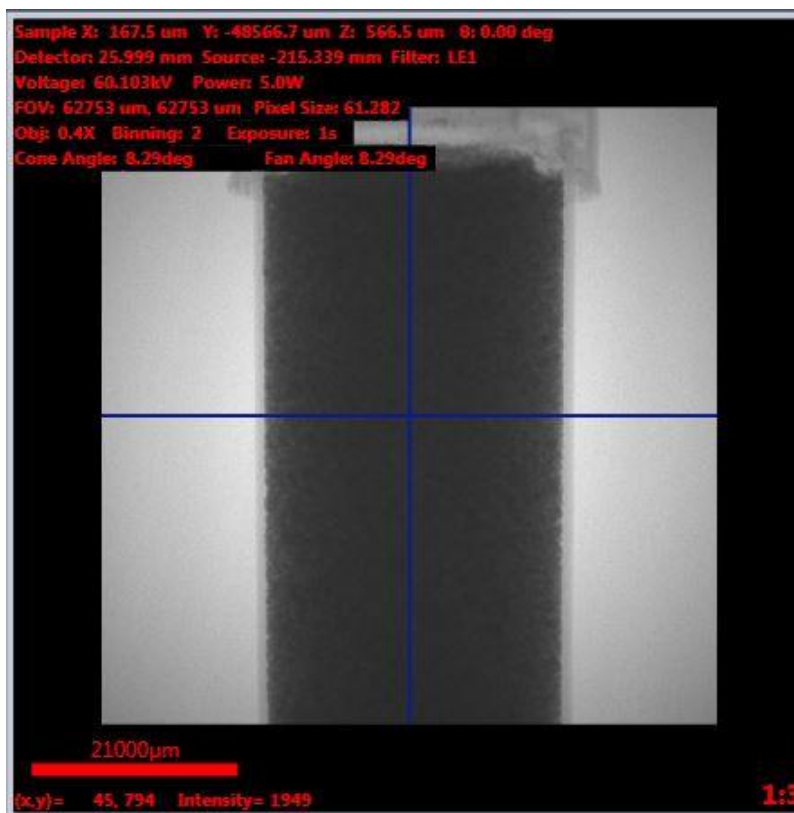


Figure 1: Unless vertical stitching is used, the maximum FOV possible to image of a 70mm tube is a 60mm portion, with the highest resolution achievable being 20 μm (binning 1).

3 Procedure

Vertical stitching can be performed in two ways

I. Auto stitching

Auto stitching is enabled by default for every stitch tomography. The user defines the end points of the stitch and the software calculated the number of segments required to cover the vertical FOV. Once a tomography is completed, the reconstruction starts automatically, while the next scan is in progress. When two tomographies are reconstructed, a stitch begins in parallel to the next scan and/or reconstruction.

II. Manual stitching

Auto stitching involves auto reconstruction of the acquired tomography. Sometimes, the software may not be able to complete an auto reconstruction of a particular tomography due to incorrect center shift correction or the inability to find a center shift.

This requires manual reconstruction of the individual scans followed by manually stitching them. The stitch set-up would be same as for the auto-stitch.

For use cases where the BH constant or the global scaling is not to the users liking, or the user decides simply not have the auto stitching enabled, the manual stitching procedure has to be followed.

I. Auto Stitching

1. Follow the instrument user guide to establish standard scanning parameters, such as FOV, kV, acquisition time, physical filter, etc. using the Scout and Scan Interface for your sample. Make sure your sample is mounted as vertical as possible, and centered in both 0 and 90 degree planes.

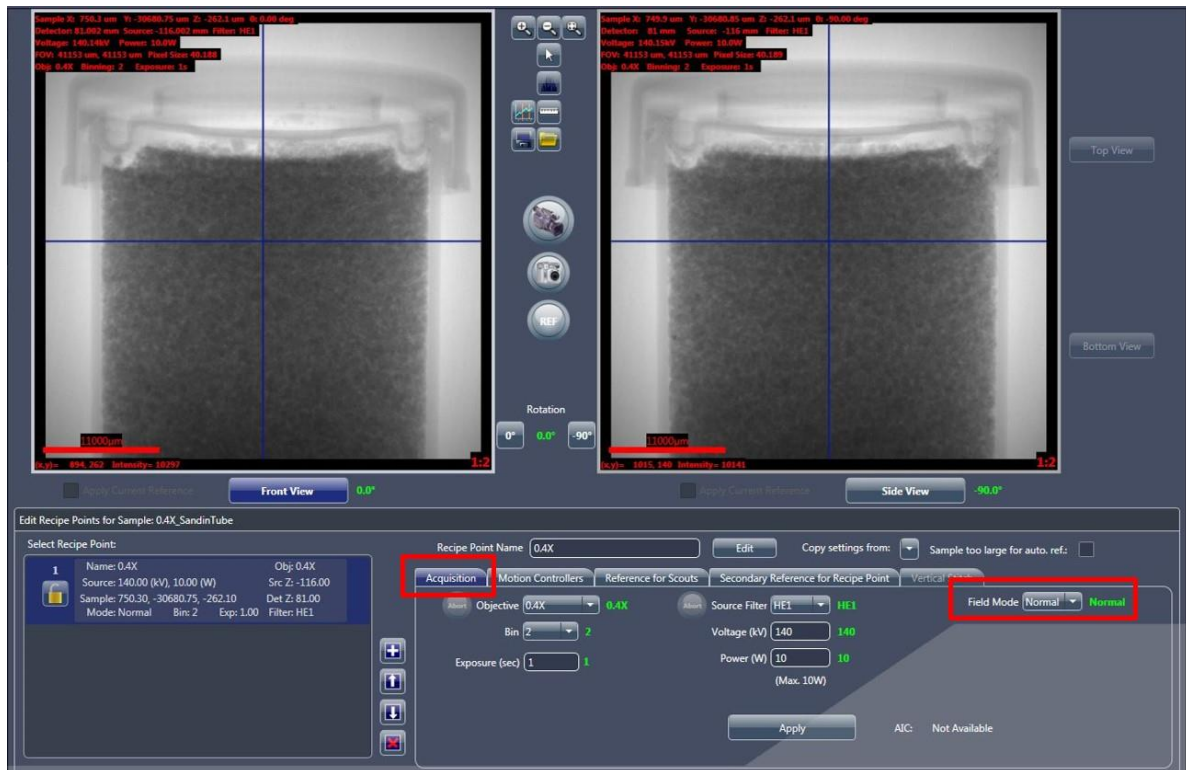


Figure 2 : Setup your scan as usual, using the acquisition and motion controller tabs and the Normal field mode.

NOTE: For the most efficient use of stitching it is recommended to have the sample mounted as straight as possible. Each individual tomography will have the same X and Z position for accurate stitching, so any sample that is not aligned vertically may not be fully covered.

2. Change the Field mode to "Stitch" and click on "Apply" to enable vertical stitching.



Figure 3: To enable Vertical Stitch, change the Field Mode to Stitch

3. Switch to the "Vertical Stitch" tab where the setup for the stitch will be carried out.

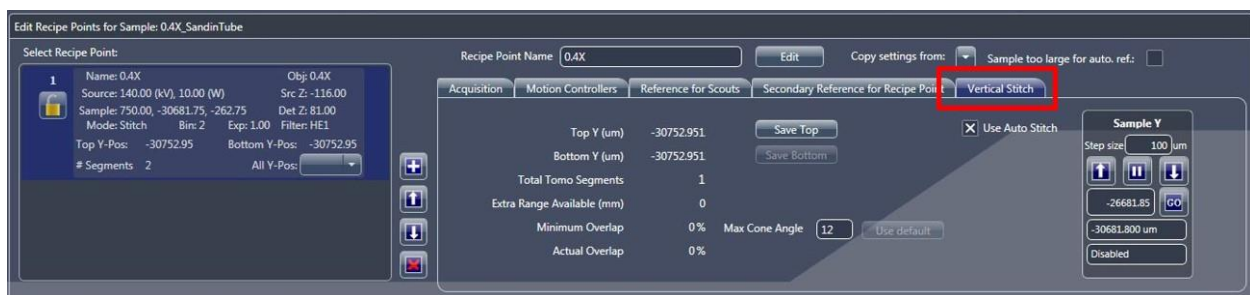


Figure 4: Setting up a stitch from the Vertical Stitch Tab

4. Saving top view

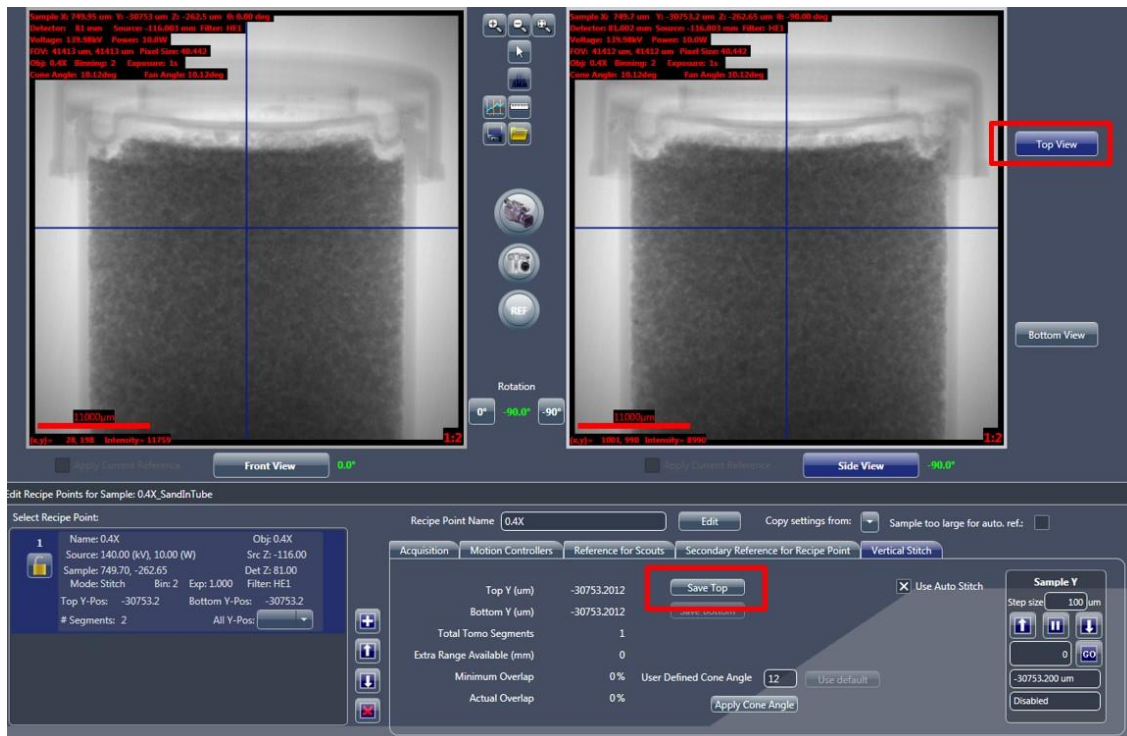


Figure 5: Saving the TOP View of the stitch

- 4.1. Click on the Top View button.
- 4.2. Start a continuous scan and use the Sample Y motion controller to move the sample till the TOP of the sample or the desired ROI is in the FOV.
- 4.3. Click the "Save Top" button to "lock – in" the Top Y position.

NOTE: An image either single or continuous must be taken if any changes are made to the scan parameters (such as the objective, sample X or Z positions, source or detector positions) or the recipe will not be updated. However taking an image does not update the saved Top Y position in the recipe. If the saved 'top of the ROI' needs to be changed in the recipe, then the 'Save Top' button needs to be clicked at that desired position.

5. Saving bottom view



Figure 6: Saving the BOTTOM view of the stitch

- 5.1. Click on the Bottom View button.
- 5.2. Start continuous scan and use the Sample Y motion controller to move the sample till the BOTTOM of the sample or the desired ROI is in the FOV.

NOTE: With the continuous acquisition on, as the user moves the sample, the "Total Tomo Segments" parameter changes, to inform the user of the total number of stitch points needed to cover the range.

- 5.3. Click the "Save Bottom" button to "lock-in" the Bottom Y position.

NOTE: Taking an image either single or continuous does not update the saved bottom Y- position in the recipe. If the saved 'bottom of the ROI' needs to be changed in the recipe, then the 'Save Bottom' button needs to be clicked at that desired position.

- The number of segments needed to cover the desired vertical range is shown under # Segments in the recipe point. The Y positions of the individual segments are also displayed in the recipe point.

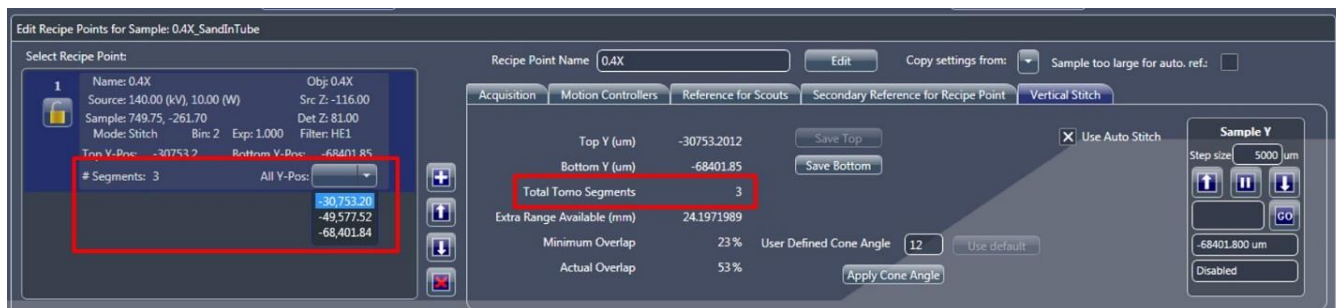


Figure 7: Total number of segments needed for the stitch

- If an acquisition was taken at the Y position different from the "locked -in" Top/ Bottom Y positions (in the recipe), the parameters under the Vertical Stitch tab will show different numbers from those seen in the Recipe point. Care needs to be taken, to acquire and "Save" the desired Y positions.

E.g.: In Figure 8, after saving the Top (-30753.20) and Bottom (-68401.84) Y -positions as seen in the recipe point, the user moved the top sample- Y position and acquired an image at -34401.4, as seen under the VS tab. This changed the rest of the parameters under the VS tab. These new values, under the Vertical Stitch tab, will be used, ONLY if the user SAVES the new Top-Y position by clicking the "Save Top" button.

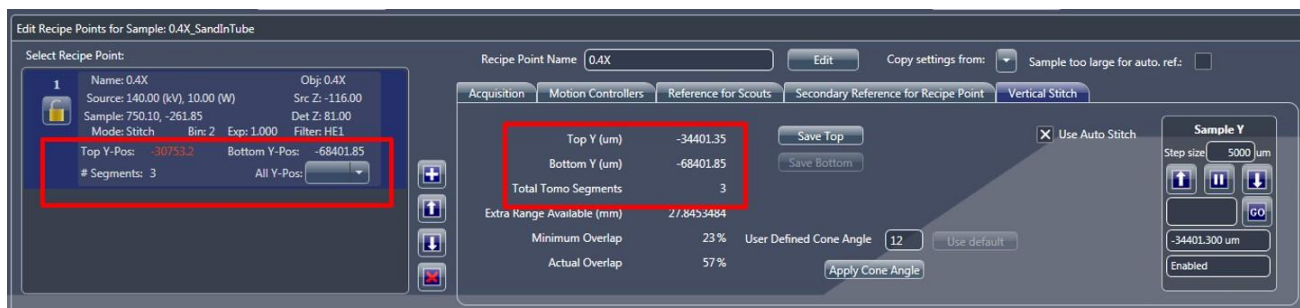


Figure 8: Mis-matched parameters in the VS tab and the saved recipe point.

Essentially, after saving the top and bottom Y positions, the stitch is set up, and the user can choose to move to the Scan Page to set up the rest of the parameters. However, if desired, there are a few more options available.

8. Extra Range Available

Depending on the geometric configuration (objective, cone angle, etc.), the software calculates the ideal "overlapping region" required for an accurate stitch. This is displayed as the "Minimum Overlap" parameter under the VS tab.

The “Actual Overlap” parameter is calculated from the current Top and Bottom Y positions (in the VS tab) and provides the user information on the overlapped region. The “Extra range available” parameter gives the user information on how much extra range in mm is available on the Y axis.



Figure 9: For a more efficient stitch, the user can make changes in the stitch recipe to reduce the excess overlapping between segments

Depending on how much excess blending is performed, the user can decide to do one of the 3 things (use Figure 9 as an example)

- Make no changes: This will lead to the use of the “extra overlap” as the total blending region enabling a higher quality of blend in terms of signal to noise. E.g.: In Figure 9, the actual overlap is much more that the minimal required overlap.
- Change the Y positions to increase the range: As in Figure 10, out of the extra 24mm of space (shown initially in Figure 9), 16mm were used to increase the vertical range of the stitch, 8mm being added to each end of the range. The user can choose to add the extra range however they please.

Alternatively, the saved Y positions can be altered (if actual overlap is too large), which decreases the vertical range and reduces the number of segments.



Figure 10: To reduce the extra blending region, the user may move the Y positions to get a larger vertical stitched range

NOTE: It is advised to leave at least 0.5mm under the “Extra Range Available” to ensure any slight motor movements do not increase the number of segments by 1.

- Change the max cone angle:

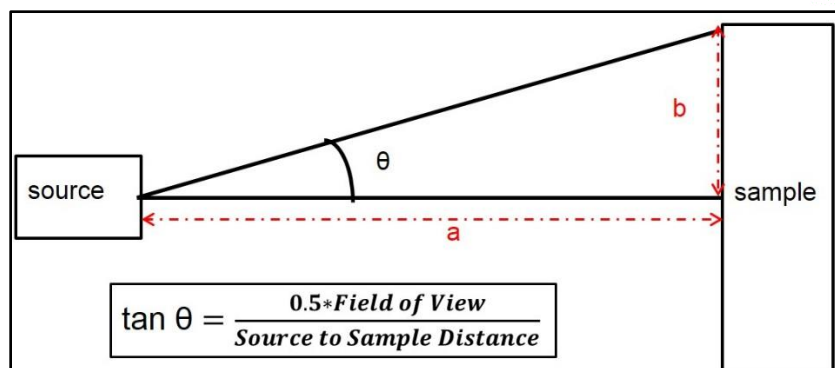


Figure 11 : Cone angle definition

The cone angle (CA) θ can be calculated as shown in Figure 11. If an advanced user desires to perform stitching at a particular angle, they can do so by changing the parameter “User Defined Cone Angle” to the desired value, and clicking on “Apply Cone Angle” to ensure the changes are saved in the recipe.

NOTE: The user-defined cone angle defines the cone angle at which the segments get STITCHED. Changing this parameter, does not change the physical CA of the scan, which is defined by the objective, source – RA and detector – RA distances.

E.g.: As seen in Figure 12, the CA was changed from default of 12 (Figure 9) to 8. This led to the reduction of the blending region, because slices at cone angles higher than 8 degrees were excluded from the stitch.



Figure 12 : Changing the User defined Cone angle, changes the Cone angle at which the stitch is performed.

9. Unless auto stitch is not desired, please leave the “Auto Stitch” option enabled.

NOTE: Only when the Auto stitch option is unchecked, the Recon Type can be changed to “manual”, in the run page.

- Once the stitch is set up, go to the Scan page and setup the scan parameters, just like in any normal scan setup. The parameters are defined for one section, and are applied to the remaining stitch sections. The auto- recon is by default enabled for all the sections if the Auto Stitch option under the VS tab is selected.

The Scan time per segment estimates the time to acquire one segment. The Overall scan time estimates the total time for all the segments in the stitch tomo point to finish acquisition. The total time on the top is the total time for the acquisition of all the tomo points in the recipe.



Figure 13: The timings for individual sections and total stitched tomography point are listed in the Scan page.

11. Once the desired parameters are selected, go to the Run page and click on start. If the option of Auto Stitch is enabled, the Auto reconstruction and Auto stitch progress bars will appear. Auto –recon and stitching will be executed in parallel to acquiring the tomographies.

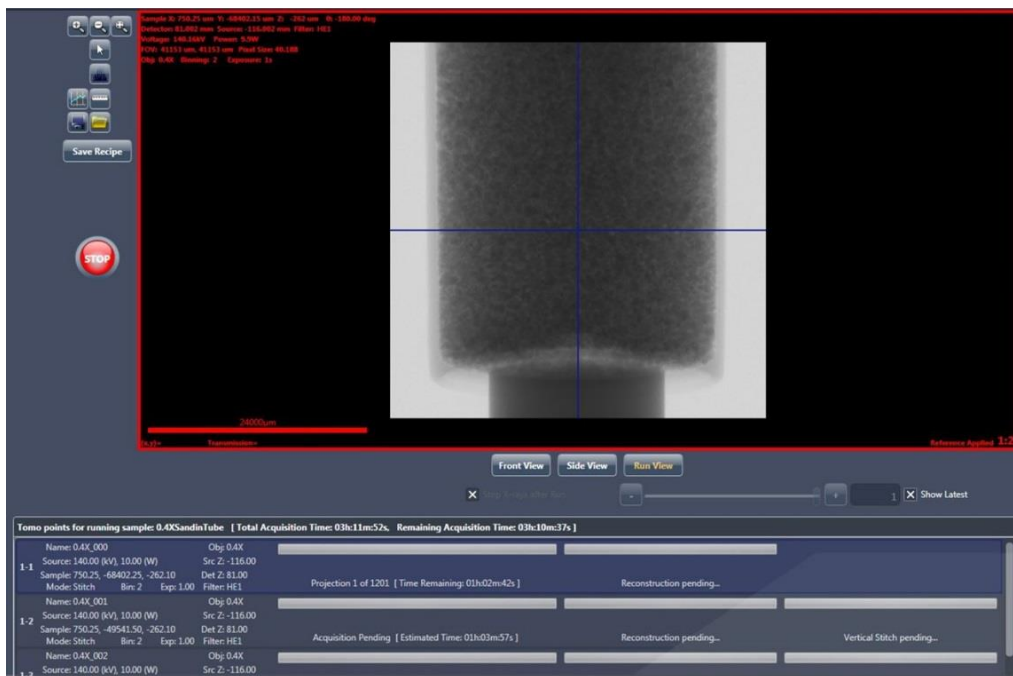


Figure 14: Individual progress bars for auto- recon and stitching appear (if enabled) on the run page.

12. Once the scans and the stitches are all successfully complete, the progress bars should all be green.

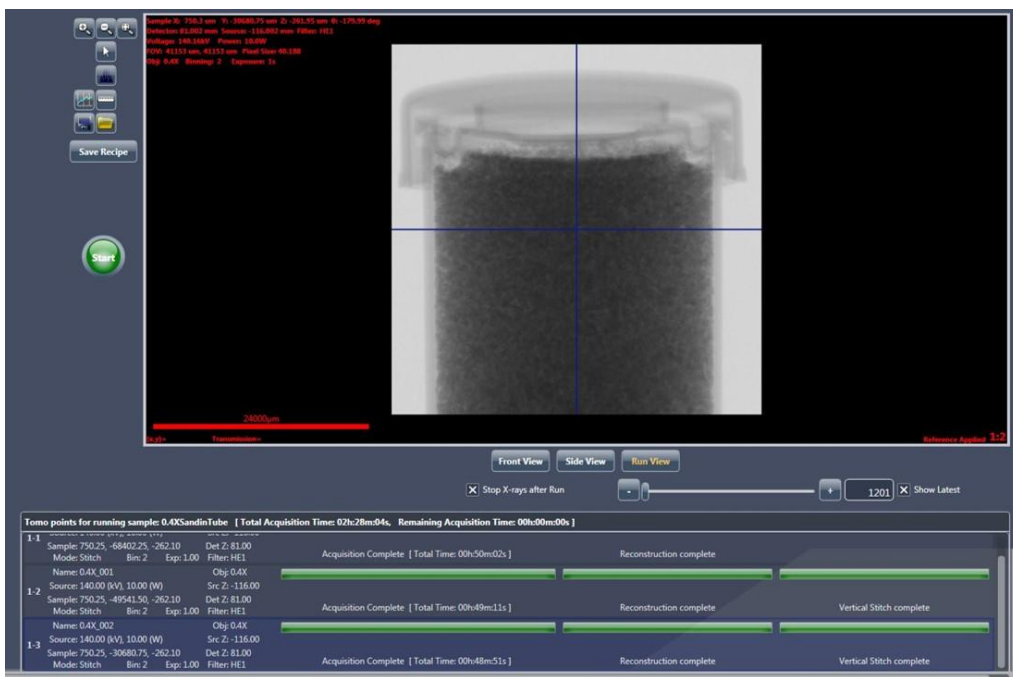


Figure 15: Green progress bars denote successful completion of the task.

13. The individual sections are going to be auto-labeled using numbers; _000 for the first scan, _001 for the second scan, and so on. At the successful completion of data collection, reconstruction, and stitching, a file with a name like "xxx_Stitch.txm" will be present in the data folder; a file containing the full stitched 3D volume image for the entire sample (**Error! Reference source not found.**). This stitched data set can be opened directly into XM3DViewer or using Visual SI Advanced.

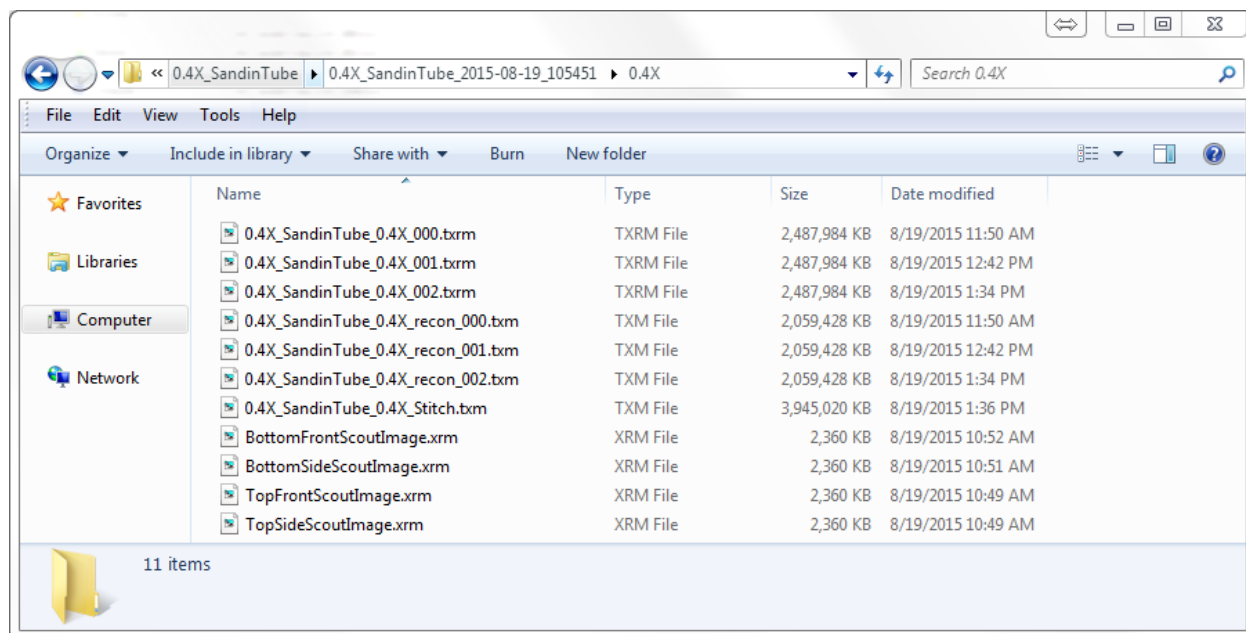


Figure 16: List of files seen after a completed stitched tomography point

Note: Since several tomographies are being collected the overall amount of data may be quite large, especially if collecting data using binning1 or Wide Field Mode. The fully stitched data set will be much larger than any individual section. In this example for binning 2 datasets, the fully stitched file is ~4 GB.

II. Manual Stitching

In the case of unsatisfactory center shift or beam hardening values used for auto-stitch, manual stitching should be performed. This requires reconstructing each tomography with the same byte scaling, beam hardening, and filtering. These values are typically based on the first section reconstructed. Do not rotate or resize the ROI in the XMReconstructor. The following steps demonstrate this case.

1. Open the "xxx_000.txrm" in the Scout-and-Scan Control System Reconstructor - 11.x. Determine the center shift and beam hardening using the standard protocol. Reconstruct the data allowing for global min/max calculation of the byte scaling. (Figure 17) You may wish to use a new file name such as "xxx_000_manualrecon.txm" or you may choose to write over the auto-reconstructed file.

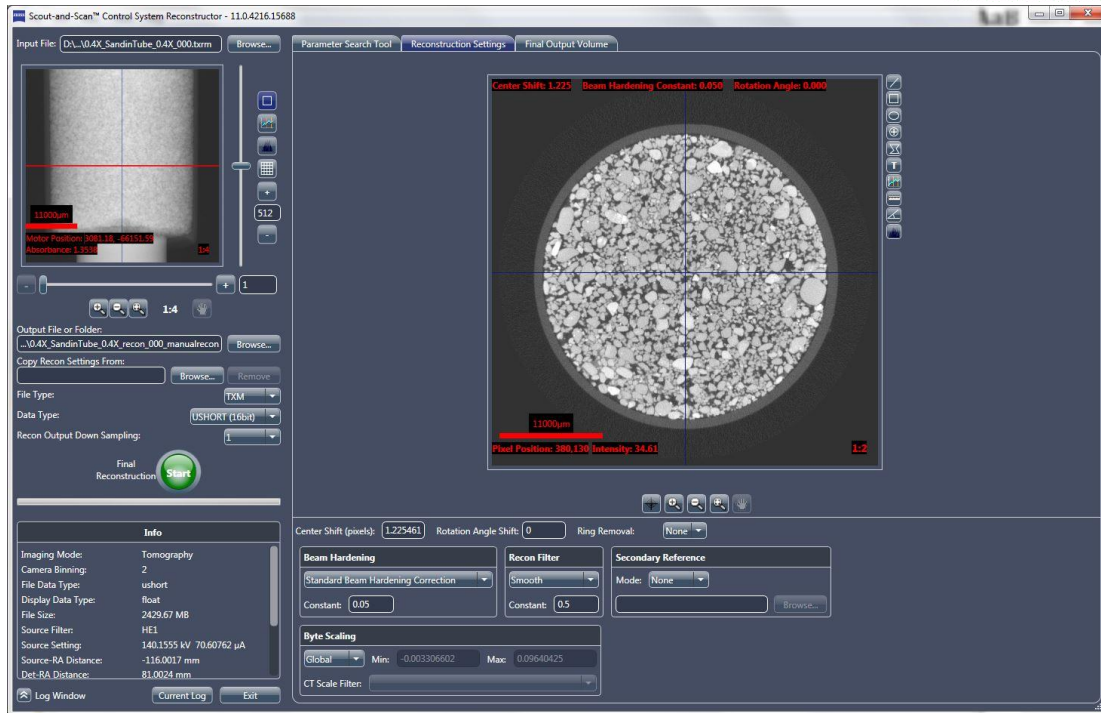


Figure 17: Manual reconstruction of the 1st tomography point.

2. After reconstruction is complete, open the next segment "xxx_001.txrm" in the Reconstructor Scout-and-Scan 11.xx.xxx. Under the "Copy Recon Settings From" parameter, select the "xxx_000.txrm" or the reconstructed "xxx_000.txm". The setting (center shift, beam hardening correction, global scaling, recon filter) used to reconstruct the new file will be the same.
3. The center shift between the sections may be slightly different. The user should check for center shift for each individual segment. All other parameters (beam hardening correction, global scaling, recon filter) must be identical between the to-be stitched sections.

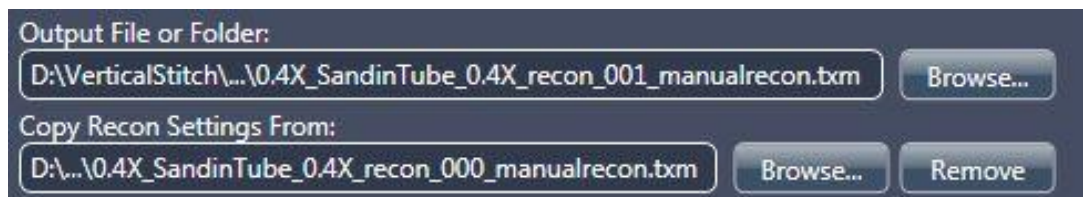


Figure 18: Recon settings are copied over from the selected dataset using "Copy Recon Settings From" tab

4. Now that all the reconstructed files (.txm) are ready, the Manual Stitcher Scout-and-Scan 11.0.x interface will be used to stitch them together.
5. Open the manual stitcher from Programs> Carl Zeiss X-Ray Microscopy>Xradia Versa 11.0.xxxx> Manual Stitcher Scout-and-Scan 11.0.xxxx.
6. Select all the reconstructed files (.txms) using the  button. User can use Shift Select to choose multiple files. Also provide an output file name.

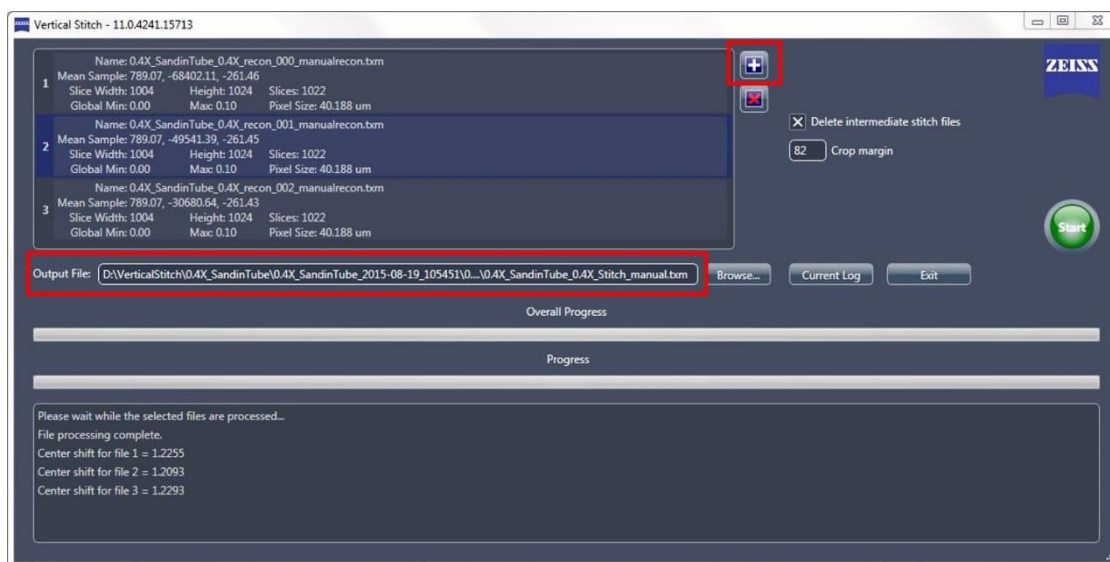


Figure 19: Select the reconstructed files to be stitched, and provide an output file name

7. If desired, the user can uncheck the "Delete intermediate stitch files" option to keep the intermediate files. Click on the START button to initiate the stitch.
8. The "Progress" bar gives the information to the individual stitches. The "Overall Progress" bar gives the user the information regarding the time to complete the whole stitch.

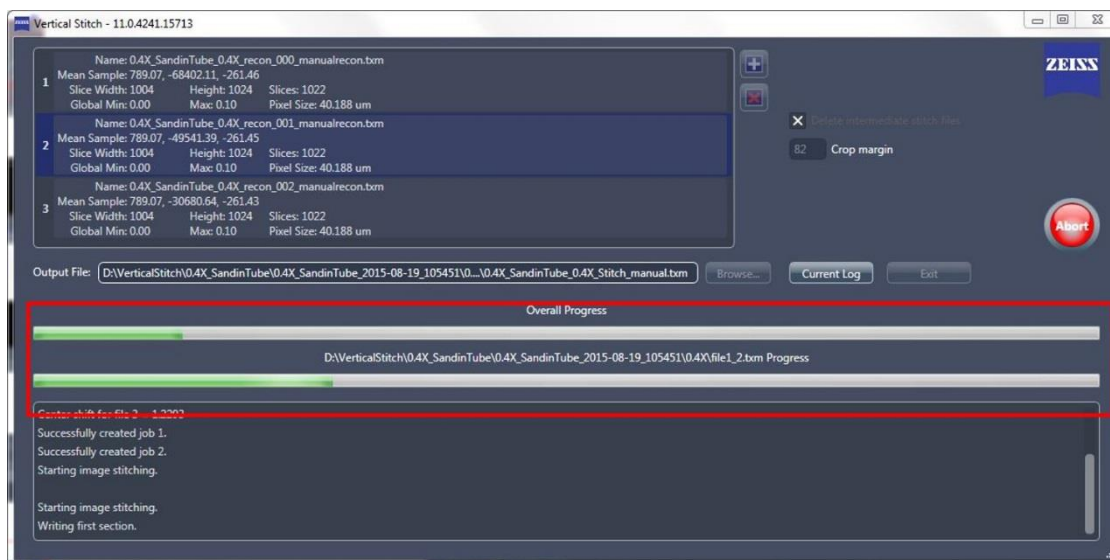


Figure 20: The overall progress of the stitch is shown as a progress bar.

9. Once the stitch is complete, the progress bars are completely green.

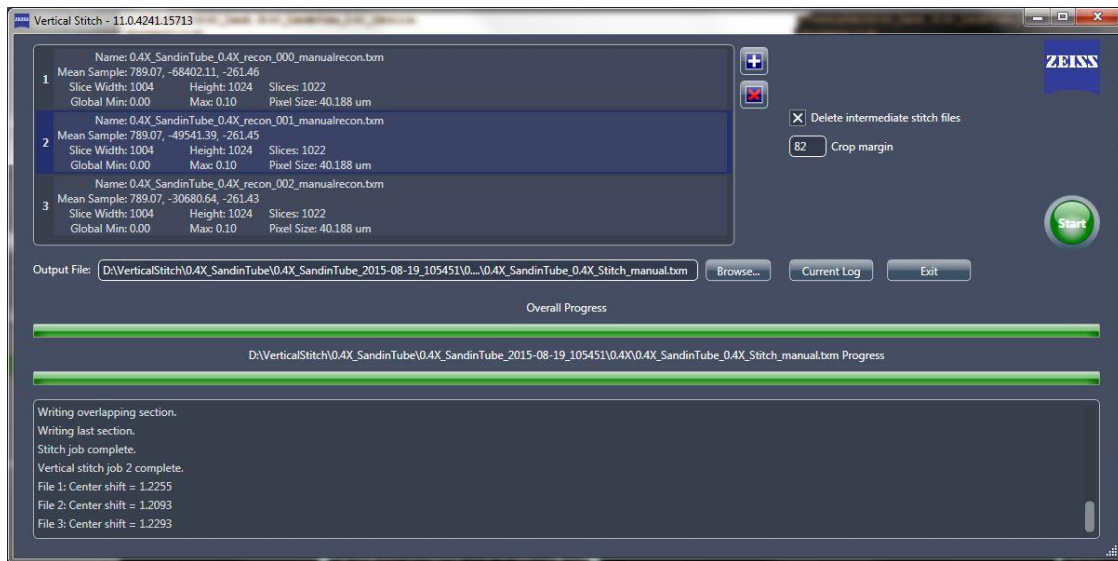


Figure 21: Green progress bars denote a successfully completed stitch.

10. The user can now load the stitched file in Visual SI Advanced or the XM3DViewer

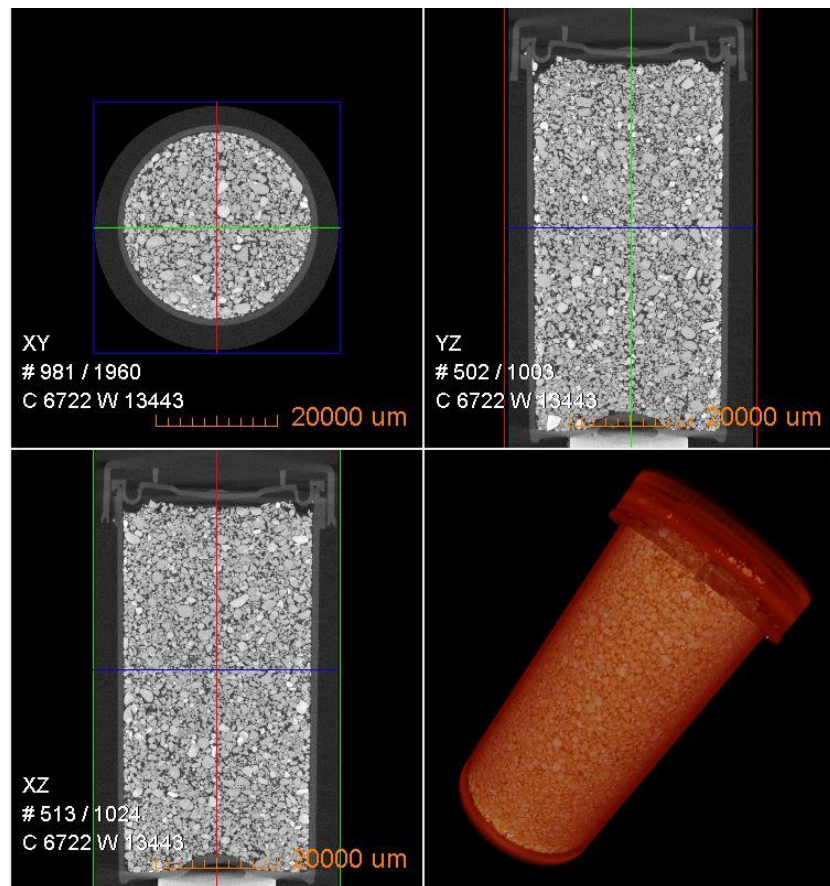


Figure 22: Section A2E is the vertically stitched dataset of 5 consecutive sections



4 Summary

This document has provided detailed instruction for performing vertical stitching using ZEISS X-ray Microscopy (XRM) instruments. This technique allows users to obtain a large volume reconstruction while maintaining the desired high resolution.