ZEISS Xradia Versa 620 3D X-ray Microscope

Complete Standard Operating Procedure

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CONTENTS

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INTRODUCTION

The Zeiss Xradia Versa 620 3D X-ray Microscope (AKA: nanoCT, microCT, x-ray CT) is a characterization tool capable of creating 3D reconstructions of specimens with contrast based on a convolution of material density and atomic Z, up to a resolution of about 900 nm. This system is distinct from conventional microCT due to the objective lenses placed in front of the detector, enabling a variety of fields of view and resolutions of specimens without a loss of scan quality.

Specimens are scanned at ambient conditions (atmospheric pressure, approx. 28 °C.) Specimens **MUST** be stable inside the chamber (unchanging shape, etc.)

The system is equipped with a mechanical testing stage for in situ experiments.

Most specimen types are permitted in this microscope, with few exceptions:

- **NO MAGNETS.**
- **NO LIVING ANIMAL SPECIMENS** (See Penn's Center for Musculoskeletal Disorders MicroCT Imaging Core for assistance.)
- Hazardous specimens **MUST** be properly contained.

TRAINING AND QUALIFICATIONS

Users **MUST** complete training with MCC staff to independently operate this equipment.

- Training starts with a hands-on demo of setup with a user-provided specimen, with the goal of achieving a successful reconstruction of the specimen at the desired field of view OR resolution.
- Training requirements are considered fulfilled after successful completion of a "driver's test" training session, during which the trainee sets up a scan of their specimen following procedures outlined in this document.
- Users **MUST** also complete x-ray safety training.
- Users **MUST** consult with the Equipment Manager before using new modes (i.e. flat panel imaging, mechanical testing, any unconventional use cases.)

USER RESPONSIBILITIES AND SAFETY

- This tool uses x-rays! Complete any requisite x-ray safety training courses.
  - Ensure the source is off before opening the doors.
  - Ensure the door interlock is enabled before turning on the source.
- The doors are very heavy! Be careful not to pinch fingers.
- Watch the opening of the chamber for head collisions.
- Be very careful not to touch the source aperture, filters, or objectives with specimens or your hands.
- Clamp large or heavy specimens to the stage.
- Transfer **and delete** data from the EquipmentData drive in a timely manner.
- When in doubt, contact the equipment manager for help.
- Log scan times in iLab.
- Fill out the logbook for each use.

SAMPLE PREP

GOALS

- See the sample size table (in Appendix B and on the benchtop) for sample size recommendations.
- Specimens should be self-supporting and stable. If a sample is flexible and likely to fall over, use available mounting supplies or custom-make a holder (e.g. 3D printing) to support the sample so it does not move during the scan.
    - If a sample moves during a scan, it is highly unlikely that you will be able to recover a quality reconstruction.
- Position the scan center location as close to the center of the holder as possible.
- Use the tip of a toothpick, a small piece of graphite, or another small part to "label" the scan center on curved or large samples (this helps with alignment.)
    - Use the Scout and Zoom procedure (notes in Appendix D) for defining scan centers of difficult-to-identify tomography locations (e.g. specific cracks, interior features of interest.)
- Samples can be held within containers during a scan. The container material will affect scan quality, acquisition time, and achievable resolution/field of view. Plastic or Kapton (polyimide) tubes are typically acceptable containers. Aluminum, stainless steel, and other thin-walled, lightweight metals may be acceptable for certain scans (e.g. batteries.) Aim for low atomic Z, thin-walled containers as close to the size of the specimen as possible.
    - Specimens that are susceptible to temperature changes may be kept inside the chamber for a few hours to overnight before a scan to acclimate to the slightly elevated temperature (approx. 28 °C.) Place acclimating specimens in the front tray of the chamber to avoid interference with any scans.
    - Specimens that must be in a certain environment (e.g. hydrated) may be prepped in that environment. Contact the equipment manager for ideas.
- The selected specimen holder should ideally be smaller than or equal to the specimen diameter for high resolution scans, if possible. Use provided materials to hold extra small samples high enough that the base of the holder will fit underneath the source and filter wheel.
    - UV glue and graphite rods (mechanical pencil lead) work great for holding specimens in the smallest pin vise.
    - Pins are not recommended for low-Z specimens due to the likelihood of metal artifacts.
- The flat edge of each sample holder faces the chamber door (runs between source and detector) at stage rotation of 0°.
- Multiple specimens may be scanned in sequence if they can be appropriately arranged on a holder (e.g. mounting multiple vertically stacked, alternating sides, etc. Keep in mind that different arrangements may affect how close the source and detector may be placed relative to the specimen.

SAMPLE CONSTRAINTS

- Specimens do not need to be conductive.
- No magnets! No live animals!
- Stage maximum weight is 25 kg.
- Maximum scannable area varies by objective. Maximum dimensions may depend on source-detector positions. If it fits and can rotate, we can scan it (although not always with ideal results)

AVAILABLE MATERIALS

- UV glue + UV light: dab small amount of UV glue onto joint between specimen and e.g. graphite rod. Cure for about 30 s with UV light. May take multiple applications from multiple directions for optimal security. Dab small amounts at a time – too large of a dab may not cure internally. Cured glue removes easily (snaps off – may require razor blade or other fine edge.) Glue may wick into porous materials.
- Blue Loctite putty (poster tack): blue putty that bonds temporarily to most materials with reasonably strong hold. Removes easily by rubbing/rolling/dabbing excess blue putty onto any residue.
- Graphite rods (mechanical pencil lead): 0.7 mm graphite fits into the smallest pin vise holder. Ideal for holding small specimens above the specimen holder for optimal placement of source and detector for high resolution scans.
- Pins: fit into smallest pin vise. May result in metal artifacts. Not recommended for low-Z materials.
- Kapton (polyimide) tubing: thin walled plastic tubing great for containing small, air-sensitive specimens. May be combined with e.g. small amount of water or hydrated cotton in one end of the tube to ensure specimens remain hydrated during a scan. Both ends of the tube should be sealed (e.g. with UV glue.)
- Kapton (polyimide) tape: thin low attenuation tape great for securing larger samples to larger holders.
- A variety of sample holders with interchangeable bases:
  - Pin vises: great for holding graphite rods and pins. SEM pin stubs fit in the larger vise.
  - Clamps: large and small options. Good for relatively flat samples, or when a flat support surface is needed. Avoid scanning below the top of the clamp for best results.
  - Flat-topped holders: for mounting most self-supporting specimens, or when a flat base is needed.
    - Modular plates and risers: for larger specimens requiring a flat bottom.
  - All sample holders *may* be used with the stage clamp. However: Do not use the stage clamp unless necessary (i.e. for large specimens likely to topple.) Use of this clamp may damage the system if the source or detector collide with the specimen and there is no range of motion for the specimen to "lean" away.
- You are welcome to use other supplies to prepare specimens. A little creativity goes a long way!

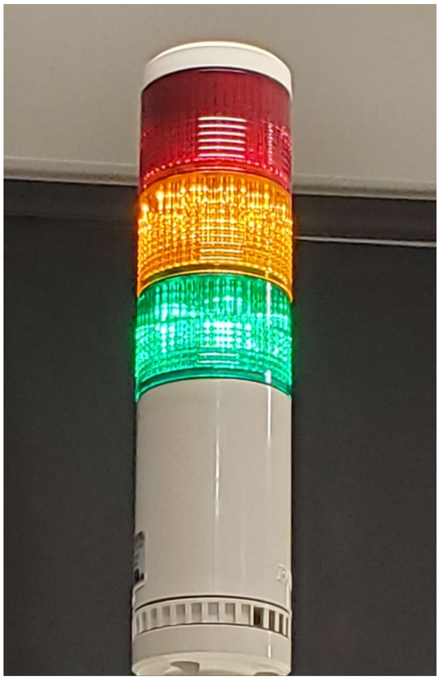
Available supplies (back to front, left to right):
- Parafilm, Kapton tubes, light metal tubes
- Lab tape, modular holders, tweezers, etc.
- UV flashlight and UV glue, more modular stage parts, dress pins.
- Scissors, poster tack, large flat holders, adjustable clamps, toothpicks.
- Tweezers, spatula, small allen key, small scissors, tweezers, graphite rods.

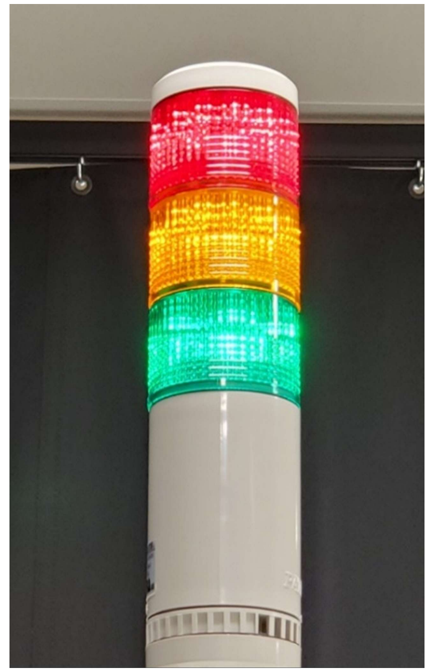
Ask the equipment manager for assistance with sample preparation if you are having issues.

SAFETY

INTERLOCKS

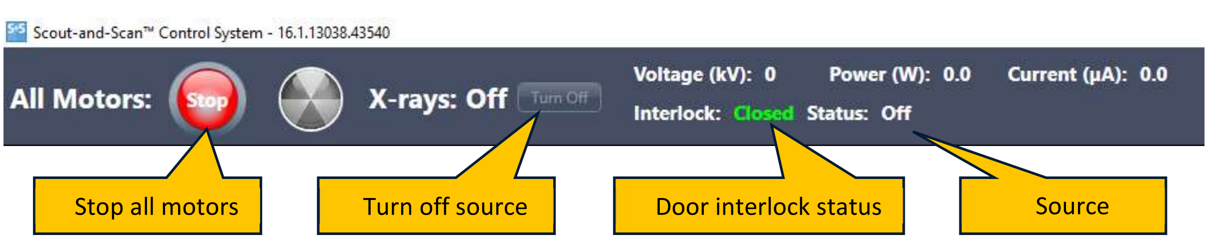
The system is interlocked so that IF the door is open, the source will not turn on, and IF the doors *are opened* while the source is on, a shutter drops and the beam is shut off immediately.





Light	Status	Meaning
Red	Off	X-ray source is off. Safe to open doors.
Red	**On**	X-ray source is **on**. **Turn off source before opening doors.**
Yellow	**Off**	Doors are **open**. **Close doors before turning on x-ray source**.
Yellow	On	Doors are closed. Safe to turn on source.

- ALWAYS check that the source is **off** before opening the doors (tower and monitor.)
- ALWAYS check that the doors are closed before turning on the source (tower and monitor.)
- If you need to turn off the source, click **Turn Off** (or set the voltage and power to 0 and click Apply.)
- If it looks like the source, sample, and/or detector are about to collide, IMMEDIATELY click **Stop All Motors** (multiple locations, and a physical button above the mouse.)



STARTUP (SAMPLE)

GOAL: LOG IN, OPEN THE SOFTWARE, SELECT THE FILE PATH, AND ADD RECIPE POINTS

LOGGING IN

1. Log in to the paper logbook.
    a. Note any recently reported problems. If there are recurring problems, contact the equipment manager before proceeding.
    b. Throughout the session, track problems in the logbook.
2. Log in to iLab to unlock the control monitor.

SOFTWARE

1. If Scout and Scan is not open, click the icon below from the taskbar.

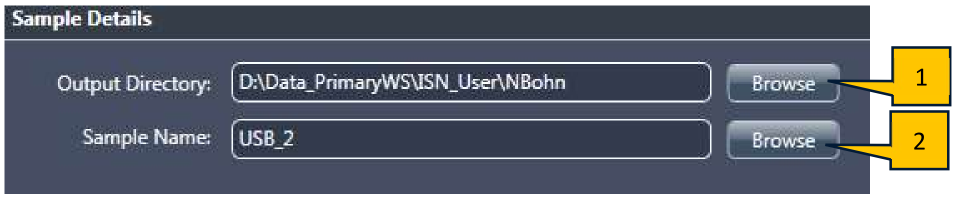
2. If Scout and Scan is open and on the Run screen, ensure that there are no scans in progress, then click "New Sample" from the bottom right to return to the "Sample" page.
    a. If a scan is in progress and you have reserved the microscope, *you have a right to cancel the scan and proceed with your own*. You may also contact the equipment manager for assistance.

Scout-and-Scan Control System View Tabs[a]	Step	Operation
Sample	Step 1 – Sample	Set the **Sample**'s data folder. Initiate the creation of a new recipe.
Load	Step 2 – Load	Use the Visual Light Camera to **Load** and roughly position the sample.
Scout	Step 3 – Scout	Use X-ray images to **Scout** the sample to locate the sample's region of interest (ROI) and field of view (FOV), and set imaging parameter values.
Scan	Step 4 – Scan	Set up the recipe's 3D **Scan** acquisition and auto reconstruction parameter values.
Run	Step 5 – Run	**Run** the recipe and acquire the tomographies.

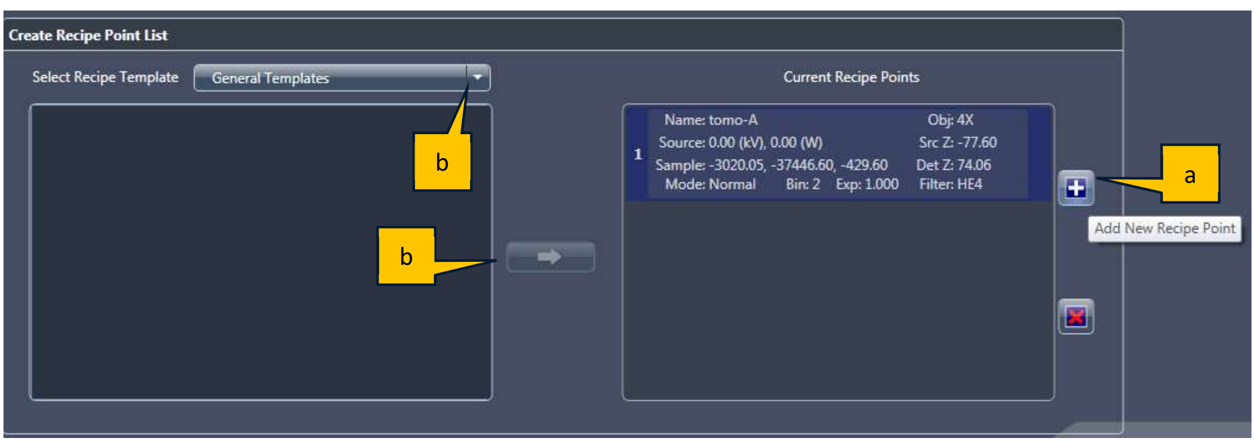
A summary of Scout and Scan's setup procedure. Tabs will be grayed out until proceeding to them the first time, then any tab may be returned to by clicking on the tab itself. Otherwise, use the large arrows in the bottom left and right corners to navigate.

SAMPLE

1. Set the file path for the Output directory as the folder with your name.
2. Select or type in a Sample Name; this will be a subfolder within your main folder.



3. Add recipe points (one or multiple from the following options)
   a. Click the plus to add a new recipe point.
   b. Browse for an existing recipe* (.rcp file) from a previously successful scan of a similar specimen, then click the arrow to transfer all recipe points, or click the dropdown arrow then select individual recipe points to transfer with the arrow.
      i. Specimens should be similar in material, size, density, and field of view.
      ii. Changes in specimen characteristics may mean that one or more parameters must be updated. (e.g. different filter if the specimen is more dense or larger)
      iii. All existing recipes should be verified for stage, source, and detector coordinates.



4. Click the arrow in the bottom right to proceed to LOAD.

** Recipes are a list of recipe points that each contain the scan parameters and coordinate information for an individual tomography location. Recipes may be composed of many recipe points with different parameters. All recipes should contain at minimum a warmup scan and a sample scan.*

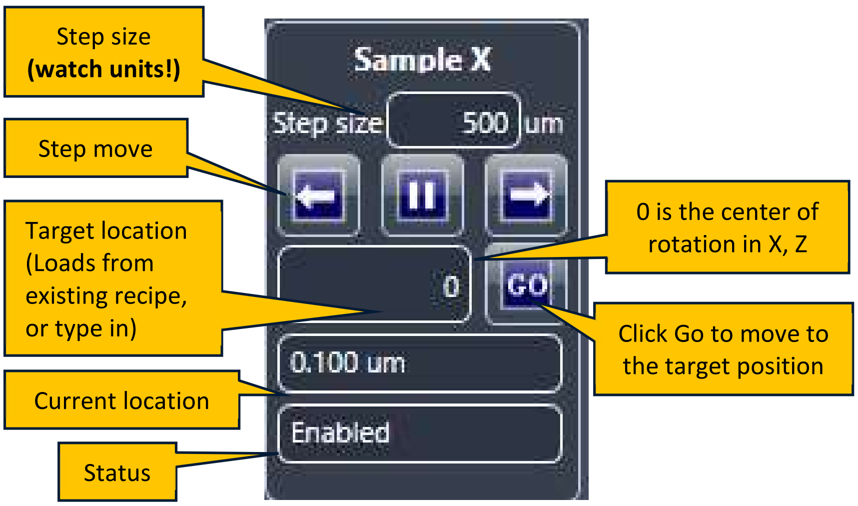
SAMPLE LOADING (LOAD)

THIS PAGE IS INFORMATIONAL

STAGE SAFETY

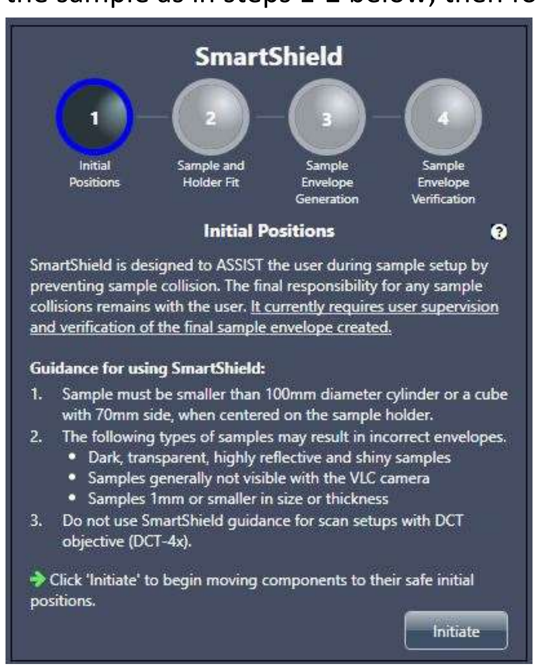
For the following sections, you will need to use the motion controllers

- Motion controllers have the same basic setup. Use them as necessary throughout the setup process to adjust sample, source, and detector positions.
- Press STOP (or Stop All Motors) at any time if it looks like the sample/source/detector may collide. Press the physical Stop All Motors button to unlock the motion controllers.



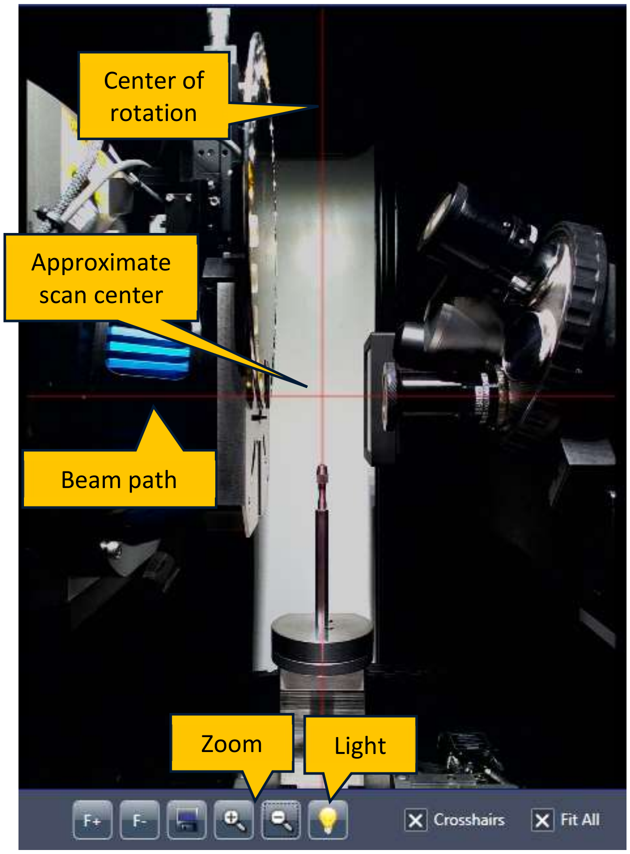
This system is equipped with SmartShield: an optional, automated way to reduce the risk of collisions.

- Not using SmartShield may save scan time or result in less noisy data, but means the user must be certain that the sample will not collide with the source or detector at any point in the 360° rotation or while fine aligning the scan center in Scout. These steps follow.
- If using SmartShield, load the sample as in steps 1-2 below, then follow the prompts.



LOAD

GOAL: LOAD SAMPLE, ROUGHLY ALIGN SCAN CENTER, FIND SOURCE AND DETECTOR LIMITS



1. Move the source and detector away from the sample and remove any specimens in the chamber.
2. Type in 0 for stage X, Z, and R and click Go for each.
3. Ensure the source is off, then load your prepared sample onto the stage. Ensure the flat edges align, and the three raised bearings fit in the 3 grooves on the underside of the specimen holder.
    a. If your specimen is very large or heavy, or otherwise seems like it might tip over, use the mounting bracket to secure your specimen to the stage.
4. Roughly align the scan center:
    a. Ensure the source and detector are far enough that they will not collide with the widest part of the specimen.
    b. Use the Y motion controller to center the sample/region of interest along the beam path.
    c. At 0°, use the Z motion controller to center the sample along the center of rotation.
    d. At -90°, use the X motion controller to center the sample along the center of rotation.
    e. Rotate the sample to -180°, and then to 180°. Watch for collisions and make a note of where your sample is closest to the source and to the detector. Remember these angles. (Alternatively: use SmartShield.)
5. Select the objective (whichever you will use for the final scan: see Appendix B for the objective selection table,) the filter (if known, otherwise use Air,) set the voltage and power to 0, and click Apply.
    a. SAFETY NOTE: If the voltage and power are NOT 0, the source WILL turn on when you click Apply.

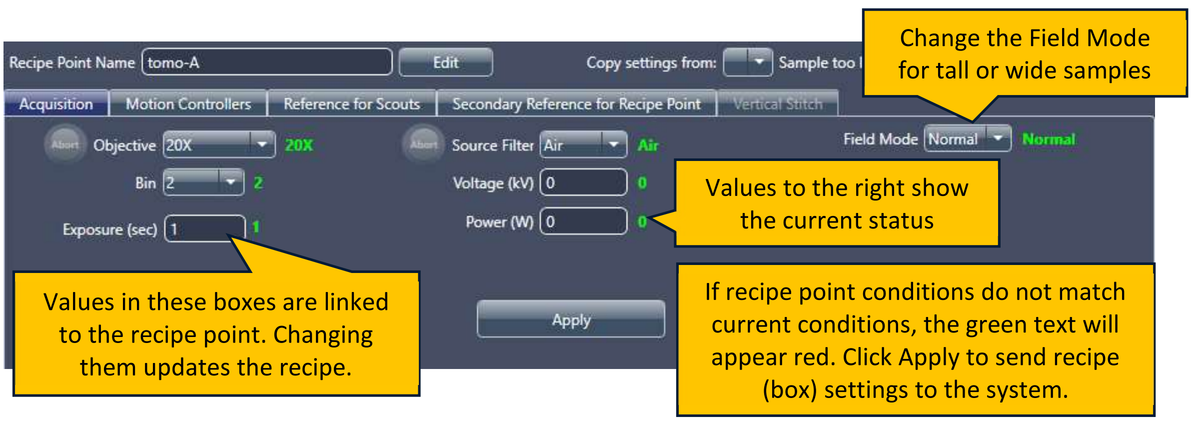
6. Position the source and detector as close as possible to your sample using the following procedure (except for 0.4X, which should be moved to at least 50 mm.)
    a. Rotate to where the sample is closest to the source.
    b. Slowly move the source in as close as possible.
    c. Rotate to where the sample is closest to the detector.
    d. Slowly move the detector in as close as possible.
    e. Rotate first to -180°, then to 180° and watch for collisions. Hit STOP if necessary.
    f. For very close positions, you may complete this process with the chamber doors open. Ensure the source is off before opening the doors should you choose to do so.
7. Press the arrow to advance to SCOUT

SCAN SETUP (SCOUT)

INITIAL CONDITIONS

GOAL: SET STARTING CONDITIONS WHICH WILL BE REFINED LATER IN THIS SECTION.

IF YOU ARE USING AN EXISTING RECIPE FROM A SIMILAR SAMPLE (SIZE, MATERIAL, DENSITY, FOV,) CLICK APPLY AND SKIP AHEAD TO "ALIGN SCAN CENTER"

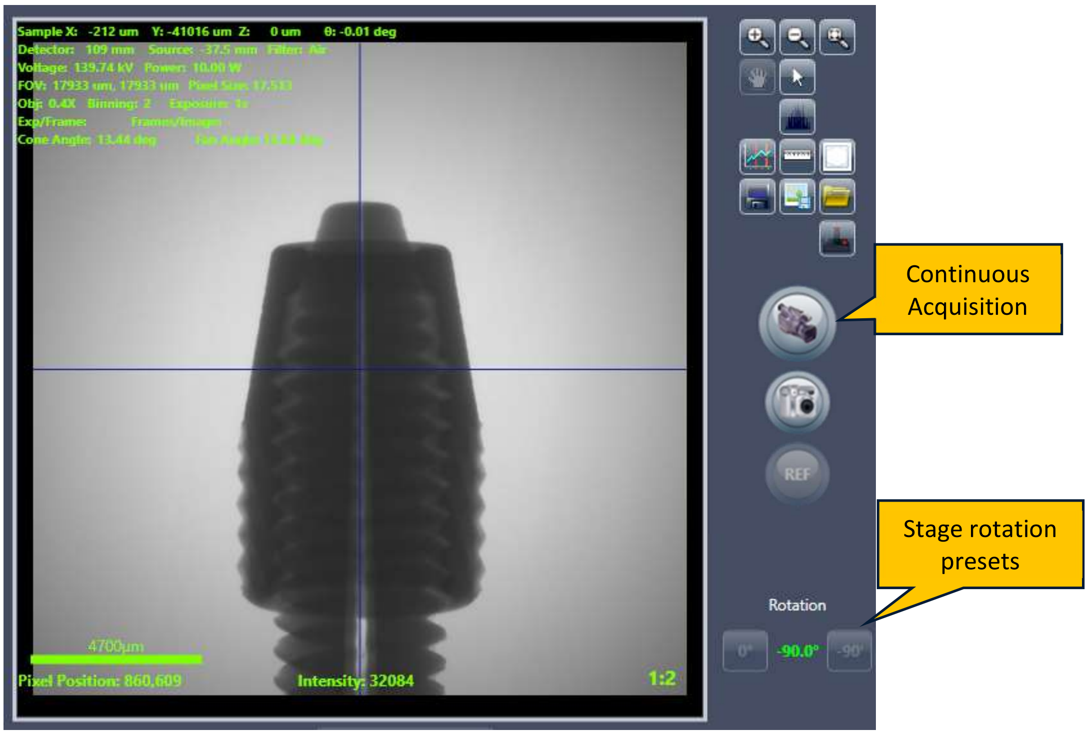


1. For new samples, use the following conditions:
    - Objective: 4X for small samples (under ~5 mm diameter,) or 0.4X for large samples (for rough alignment only – you will change to the target objective later.)
    - Bin: 1 for high resolution, 2 for faster scans.
    - Exposure: 1 s
    - Source Filter: Air
    - Voltage: 80 kV for
    - Power: maximum available (updates after typing in Voltage and clicking elsewhere.)

2. Click Apply to update the active system settings.
    a. Not clicking Apply will change only the recipe point.

ALIGN SCAN CENTER

GOAL: FIND AND CENTER THE REGION OF INTEREST AT 0° AND -90°



1. When the source is ready, the Status in the top bar will say "On."

2. Use the stage rotation preset to rotate to 0°.
    - Move the detector to change the field of view.
    - If you cannot move the detector closer but need to, move the source away instead.
    - If needed, select a different objective and click Apply.
    - The scan field of view will be set next – we only need to center the region of interest for now.

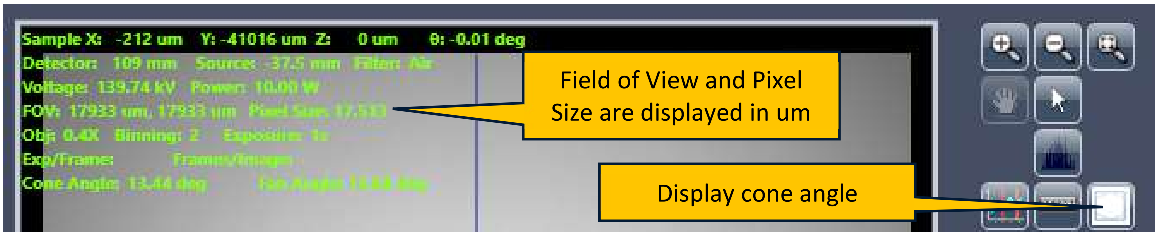
3. Click Continuous Acquisitions to begin collecting projections.
4. Double click on the projection to center your sample or an identifiable feature at the center of your desired region of interest. The stage will move to center wherever was clicked.
    a. Double clicking only works at the rotation presets. Otherwise, use the motion controllers. See Appendix H for directionality of axes.

5. Stop the acquisition, click the preset to rotate to -90°, and center the same feature or region.
6. If the region of interest is internal and not clearly visible in projections, you may need to use the Scout and Zoom procedure (see Appendix D for more information.)

SET FIELD OF VIEW AND RESOLUTION

GOAL: POSITION THE SOURCE AND DETECTOR, SET THE FIELD OF VIEW, AND SELECT THE BINNING FACTOR

1. If using a different objective than was used for rough alignment, select it now and click Apply.



2. See the text at the top of the projections for the current field of view (FOV) and pixel size.
    - Reducing the pixel size below the limit in the Objective Selection Table results in blurred features.

3. If your features of interest are near the top or bottom edge of the projection, click the cone angle icon.
    - Any features outside the displayed red lines will not reconstruct well.

4. Select the binning factor for your resolution and FOV requirements, then click Apply.
    - Bin 1 has 2048x2048 pixels per projection (larger file, higher resolution, longer scan.)
    - Bin 2 has 1024x1024 pixels per projection (¼ file size, half resolution, ¼ scan time vs bin 1.)
    - Select the highest binning factor that will give you your target resolution in the FOV.

5. Move the detector and/or source to change the FOV. Watch for collisions!
    - Remember your source/detector limits from the Load step!

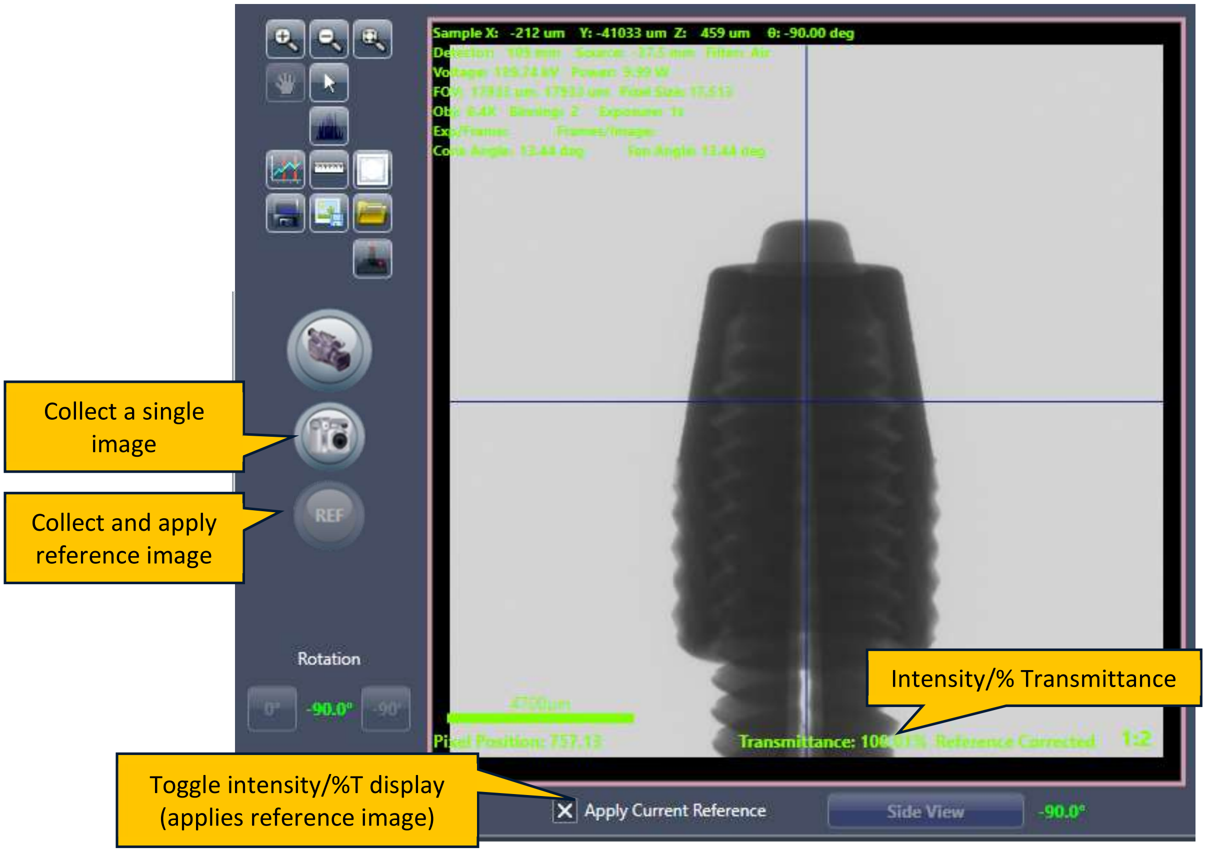
6. Rotate the stage to 180° and then to -180° while watching the camera for collisions.
    - If you used SmartShield, it will prevent you from moving too close. You may skip the 180°-180° check if using SmartShield or the source and detector are *very* far from the specimen.

SELECT FILTER

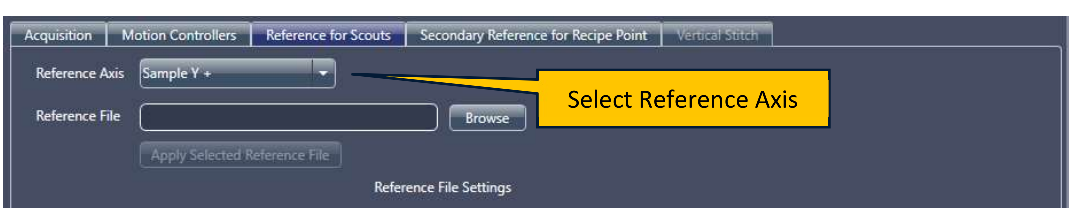
GOAL: FIND THE %TRANSMITTANCE AND SELECT THE APPROPRIATE FILTER

FOR HIGH ASPECT RATIO SAMPLES (E.G. >1X4 LXW,) SEE APPENDIX D FOR HART AND VARIABLE EXPOSURE

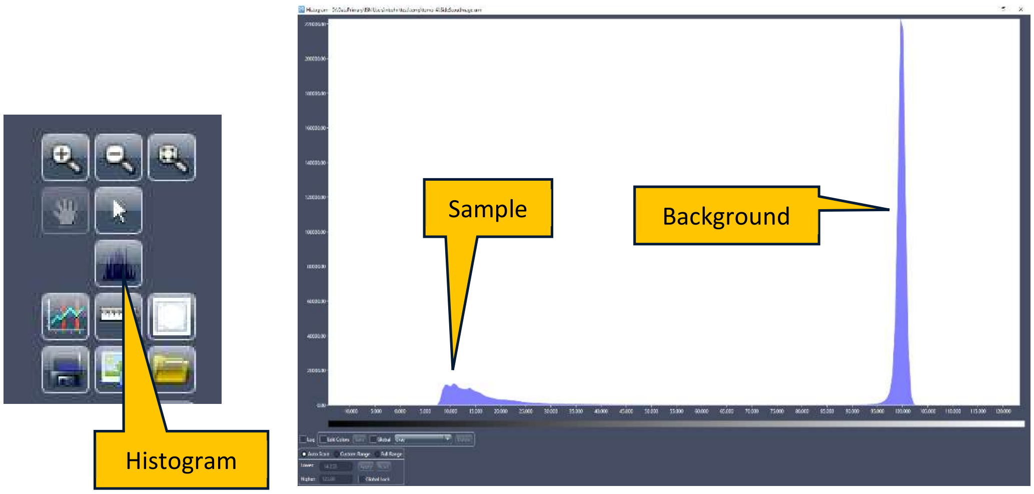
1. For samples of roughly equal dimensions in X and Z, use 0° or -90°.
   - For samples of moderate to high aspect ratios, the intensity is noticeably different at 0° vs -90°. Rotate the sample to an intermediate angle instead for the next steps, i.e. between 30° - 60°.



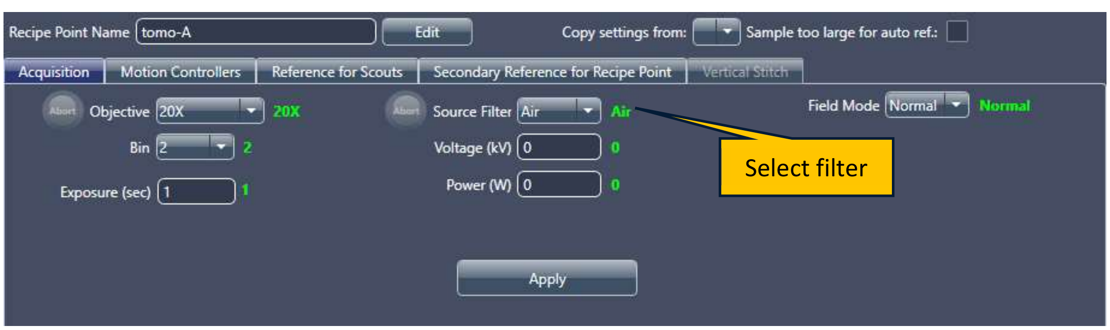
2. Collect a single image.
3. Hover the mouse over all regions of interest in the sample and note the intensity at the bottom of the image. It should be at least 600-1000. If needed, increase the exposure.
   - Intensity scales linearly with exposure, so if intensity = 500 counts at 1 s, use 2 s or more.
   - Remember to click Apply to update the exposure time in the system.
4. Click REF to capture and apply a reference image.
   - References are images of the beam itself, without your specimen. If you see a bright "shadow" of your sample, change the reference axis to ±X or ±Z so the sample is out of the beam path.
   - Very large samples may need to be removed completely. Turn off the source, remove the sample, turn it back on, and click REF.



5. Estimate the overall % transmittance by hovering over the specimen just as you did for estimating the exposure.
    - Or, click the histogram icon and estimate the range for your sample.
    - The histogram should ideally *not* look like a simple bell-curve if air is in the image or if there are multiple distinct regions in the sample. This indicates high noise.



6. Check the filter table in Appendix C
7. Select the most appropriate filter and click Apply.



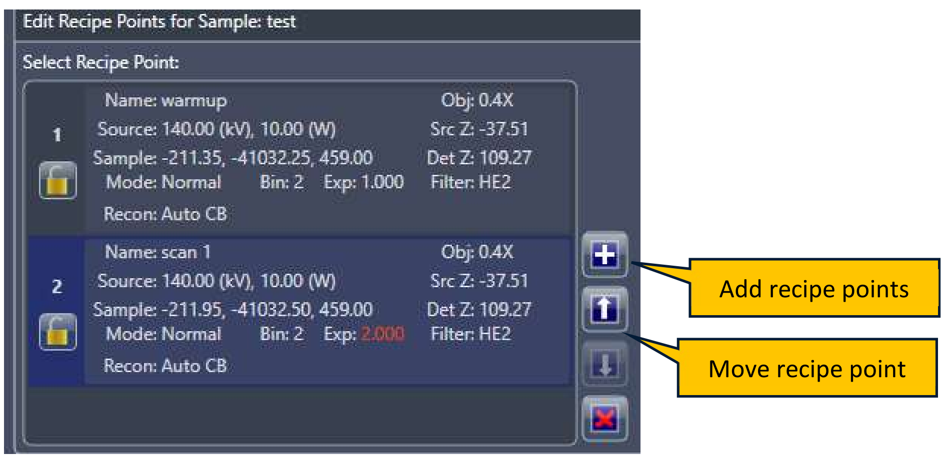
8. Collect another single image and reference image (repeat steps 2-5 of this section.)
    - The ideal range is 15-35% through the region of interest. If the %T is too high, decrease the voltage and repeat steps 2-5. If %T is too low, increase the voltage and repeat steps 2-5.
        - Change voltage in steps of 10kV, adjusting the power to its maximum each time.
    - Anything between 2-95% will reconstruct, but may be noisy or faint.
    - Air should appear as 100% ±5%
    - More details about filter selection, recommended ranges, and edge cases (too low or too high transmittance) are in Appendix C

SET EXPOSURE AND ADD WARMUP POINT

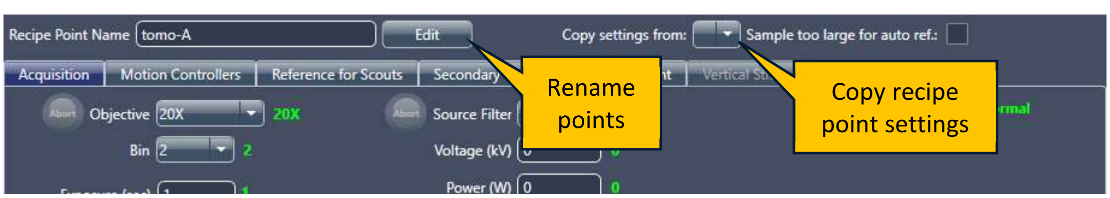
GOAL: INCREASE EXPOSURE TO REDUCE NOISE, ADD A WARMUP POINT

1. Uncheck the reference application to view the intensity, or collect a new single image.
2. Adjust the exposure so the intensity is at least 5000 counts through the regions of interest.
   - Intensity scales linearly with time. If 1 s exposure = 1000 counts, set exposure to ≥ 5 s
   - If 5000 counts is unreasonable, aim for at least 2000 counts.
   - Do not exceed 50,000 counts (64,000 is detector saturation.)
   - You can refine the exposure on the next page before starting the scan.

3. Rotate the sample from -180° to 180° and watch for collisions.
4. Click + to add a warmup point and any additional scan points.
   - *Warmups are not required if the source has been on and running at the desired voltage and power for at least 30 minutes.*
   - For additional scan points, repeat the scan setup steps as necessary (e.g. align scan center, set field of view and resolution, select filter.) You do not need to select a new filter unless imaging with a different objective, a different sample, or a significantly different part of the same sample.



5. Move the warmup point to the first position.
   - Click Edit to rename points. Each point will have its own folder for data within the Sample folder.
   - All warmup point settings (except binning, exposure) should match at least the first recipe point.
   - If values do not match: type them into the appropriate boxes, select another point under "Copy settings from," *or* capture a single image with the correct values applied (best for stage positions.)

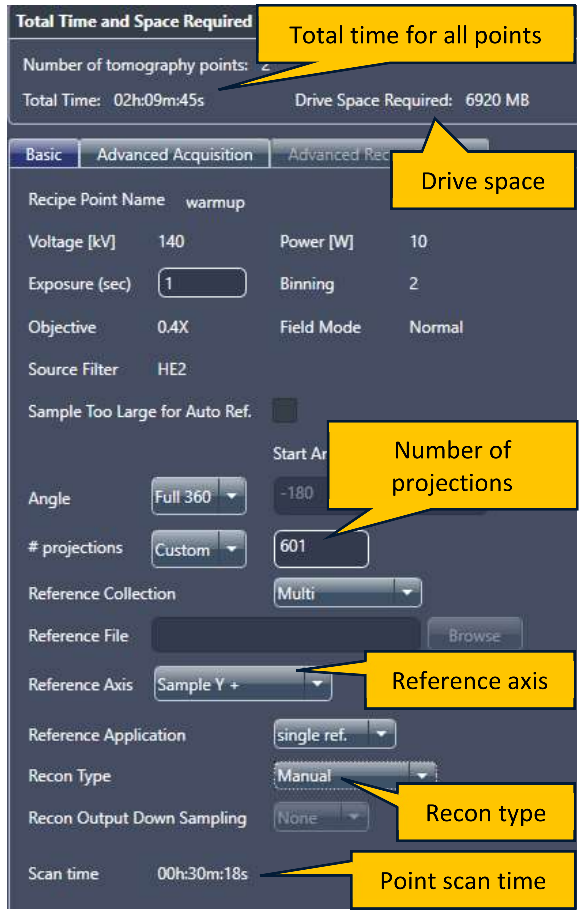


6. Click the arrow to advance to SCAN.

SCANNING (SCAN AND RUN)

ACQUISITION PARAMETERS

GOAL: SET FINAL SCAN CONDITIONS FOR THE WARMUP POINT AND ALL SCAN POINTS



1. Select the number of projections.
   - >1601 if you can see all edges (less than 1201 loses fine detail)
   - >3201 if you cannot see all edges (2401 may be sufficient if features are large relative to pixels.)
   - 201-801 for the warmup
     - Total warmup time (including beam on before this) should be 30 minutes for < 7 hr scans, or 60 minutes for > 20 hr scans.
   - Custom numbers of projections may be input in multiples of 20 and end in 1 (i.e. 20N + 1)

2. Confirm the reference axis, if not using Y.

3. Select Auto under "recon type" for scan points.
   - Select Manual for the warmup (it will not reconstruct.)

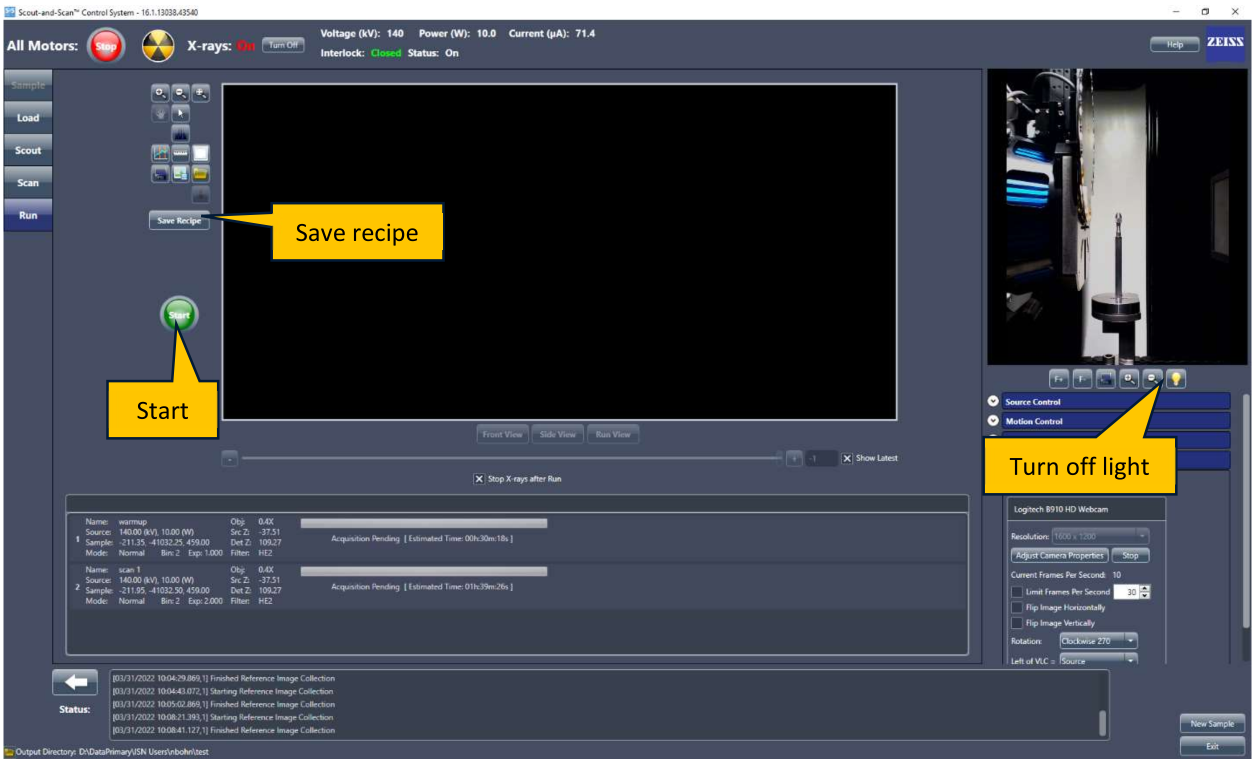
4. Confirm the drive ("EquipmentData") has enough space for all data.

5. Note the total time and check the schedule.
   - Contact the next user, alter your run time, or save the recipe on the next page to run at a later date if it will run into the next user's scheduled time.

6. Click the arrow to advance to RUN.

START THE SCAN

GOAL: START THE SCAN. YOU MADE IT!



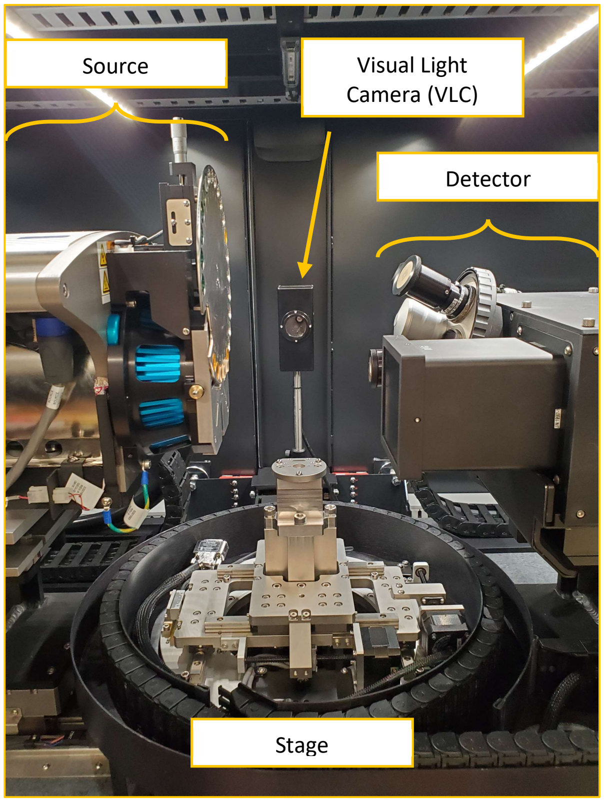
1. If using SmartShield, the system will run a pre-check for collisions.
2. Press Start.
3. Turn off the chamber light.
4. Wait for the first few projections to collect before leaving the equipment.
5. Update your reservation in iLab:
   a. Edit your reservation so the end time is a half hour before the scheduled end. iLab will automatically log you out a half hour *after* the end of your reservation.
   b. Mark in the log book the end time and the total scan time.

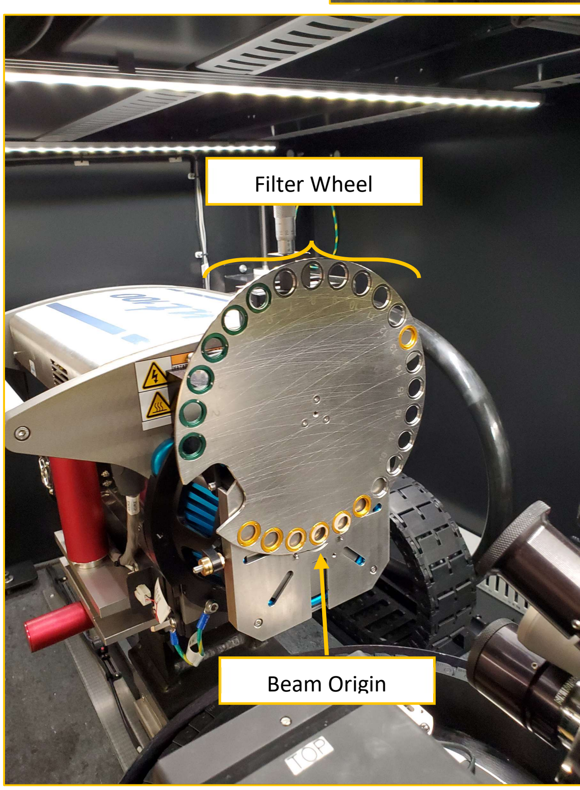
AFTER SCANNING

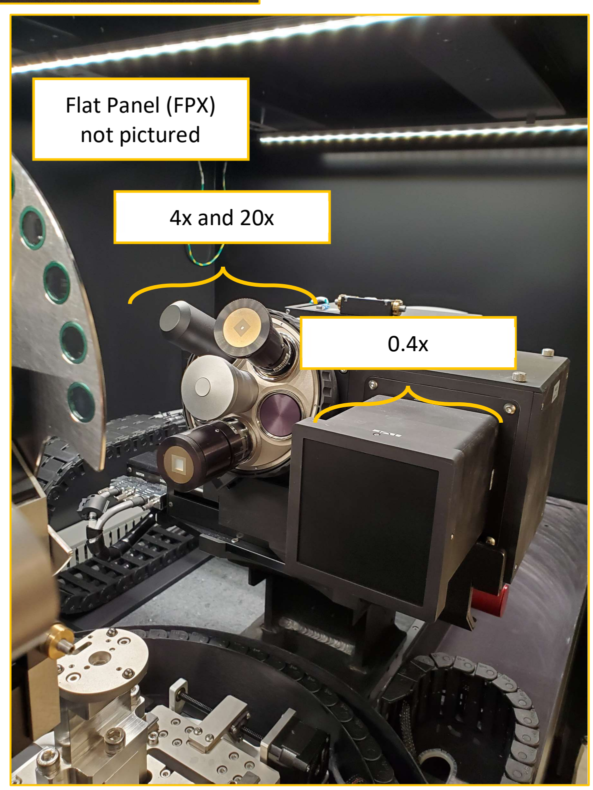
1. Pick up your sample from the table or stage (move the source and detector away if needed.)
2. View the reconstruction on the data processing computer (i.e. in Dragonfly Pro.)
3. Convert .txm files to tiff stacks in the Reconstructor if not using Dragonfly Pro.
4. See *CZXRM_QuickGuide_Scout-and-Scan_Reconstructor* in the CZXRM folder for basic reconstruction processes if you need to manually correct a reconstruction or reconstruct a failed dataset.
5. Tips for Dragonfly are available online. Contact the Equipment Manager for more assistance.
6. Copy and **delete** your data after processing.
   - .txrm files are your raw data. Keep this if you want the possibility of reconstructing again.
   - .txm are proprietary reconstruction datasets. They work in Dragonfly Pro, local software, and there may be python or matlab packages for these files.
   - .rcp are your recipe files. These may stay on the computer.

APPENDICES

A: INSIDE THE CHAMBER







B: OBJECTIVE/FIELD OF VIEW/RESOLUTION/SAMPLE SIZE TABLE

Table 3: Objective recommendations for typical user scan conditions

Objective	Sample Size (Maximum*) (mm)	Maximum 3D FOV (WFM[7]) (mm)	Voxel Size Bin1 (µm)	Voxel Size Bin2 (µm)
FPX	15 - 140 (200*)	140	6 - 57	12 - 115
0.4X	6 - 50 (100*)	50 (90)	3 - 30	6 - 60
4X	2 - 20 (50*)	6 (10)	0.7 - 3	1.4 - 6
20X	0.5 - 4.0 (10*)	1.1	0.3 - 0.6	0.5 - 1.2

How to read this table:

- **<u>Sample Size</u>**:
  - Recommended range allows optimal placement of source/detector
  - Larger samples may be scanned but will take more time, depending on density, atomic Z, and amount of solid material

* Recommended maximum is in parentheses.

- **<u>Maximum 3D FOV</u>**:
  - Field of View – volume visible in the reconstruction (diameter of final cylinder)

  (7) WFM: Wide Field Mode – an alternate scanning mode yielding a wider scan area (about 1.7:1 instead of 1:1,) but takes nearly twice as long.

- **<u>Voxel Size</u>**:
  - Edge length of 3D pixel (pixel size when viewing raw projections)
    - A scale factor appears in the bottom of projections when pixels are not actual size.
  - Aim for 3 pixels to a feature if you want to measure it, 2 pixels per feature to see it.
  - **At Bin 1, voxel:FOV = 1:2048 (i.e. FOV (mm) / 0.682 = resolution to measure (um))**
  - **At Bin 2, voxel:FOV = 1:1024 (i.e. FOV (mm) / 0.341 = resolution to measure (um))**

E.g. a reconstruction with 0.3um voxels at bin 1 has a ~600um FOV and can theoretically measure 900nm features this is the resolution limit of a setup with a 20X objective.

C: FILTER SELECTION TABLE AND NOTES

Table 1: Macro and 4X Filter Selection

Transmission (%) @ 80 kV For Thin samples and/or Low Z Materials[c]	**Transmission (%) @ 140kV** For Thick samples and/or High Z Materials[d]	**Filter**
>74	Re-Check at 80 kV	No Filter
74 – 58		*LE #1*
58 – 46		*LE #2*
46 – 36		*LE #3*
36 – 28		*LE #4*
28 – 20		*LE #5*
20 – 12		*LE #6*
Re-Check at 140 kV	32 – 20	**HE #1**
	20 - 12	**HE #2**
	12 - 8	**HE #3**
	8 - 5	**HE #4**
	5 - 3	**HE #5**
	<3	**HE #6**

Table 2: 20X and 40X[be] Filter Selection

Transmission (%) @ 80 kV For Thin samples and/or Low Z Materials[c]	**Transmission (%) @ 140kV** For Thick samples and/or High Z Materials[d]	**Filter**
> 63	Re-Check at 80 kV	No Filter
63 – 44		*LE #1*
44 – 34		*LE #2*
34 – 28		*LE #3*
28 – 21		*LE #4*[d]
21 – 14		*LE #5*
14 – 8		*LE #6*
Re-Check at 140 kV	30 - 18	**HE #1**
	18 - 8	**HE #2**
	8 - 6	**HE #3**
	6 - 4	**HE #4**
	4 - 3	**HE #5**
	< 3	**HE #6**

Filter Selection Quick Guide

(Detailed instructions can be found in the user's manual)

1. Take an image of the sample by clicking button.
2. Click on the reference button. Make sure that the reference is collected on air. If the sample is too tall go to the "Reference for Scouts" tab and change the reference axis to +X and try again.
3. Select a filter based on the transmission values from the newly created image with the help of the correct filter table.
4. With the filter in place, adjust kV to obtain an ideal transmission in the range of 20% - 35%. If no filter is used, let the transmission value be at least 15% – 20%.

Depending on the sample material and size, ideal transmission may not be obtainable. If this is the case, please image using a transmission value closest to the ideal range.

- Use the histogram or hover over features to estimate the %T in regions of interest.
- Compare feature detail to background (if visible.) These may display as two or more distinct peaks in the histogram (most samples should not display as a typical bell curve)
- Find the appropriate row in the tables for the average %T for the objective used.
- Most samples will not achieve the 15-35%T recommendation. Try to stay between 2-95%

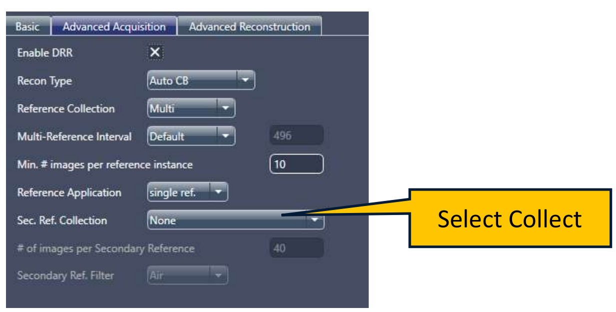
Continued on next page for high and low transmittance specimens…

FOR HIGH TRANSMITTANCE SAMPLES:

With no filter, %T should generally be less than 95% (e.g. end of the bell curve)
Background where there is no sample may be 100% or greater, as will certain voids or features.

- **Reduce the voltage:** lowest available is 30kV. 60kV may be sufficient
  - Lower voltages use lower powers, which require longer exposures.
- **Move the source and detector further away**
  - To keep the current resolution and FOV:
    - Note the current positions of the source and detector.
    - Find the ratio between these (e.g. source -20: detector 30 has a 2:3 ratio)
    - Maintain this ratio when moving the source and detector.
- **Use a higher magnification objective**, if available.
- If using no filter, consider adding a **filtered secondary reference**:
  - To select a secondary reference filter:
    - Image your sample with no filter and note the intensity near the center.
    - Image your sample with a filter (e.g. LE1 or LE2) and note the intensity around the same area.
    - Under the Secondary Reference for Recipe Point tab, select the filter that yields an intensity roughly 80% of the unfiltered image

    - In the Advanced Acquisition tab of the SCAN page, select Collect from the Sec. Ref. Collection menu and confirm the filter.



FOR LOW TRANSMITTANCE SAMPLES:

With HE6 filter, %T should generally be greater than 2%

- Carefully **move source and detector closer**, if possible
- **Increase voltage** (maximum 160kV)
- Use a **lower magnification objective** or the flat panel detector, if possible
- Use the **HE18 filter** (especially with the flat panel detector.)
- Use HART or variable exposure if your sample is wider in one dimension (ask the Equipment Manager for more details.)

D: ADVANCED OPERATING MODES

For more advanced or specialized methods, see the **CZXRM** folder on the desktop. There you will find Zeiss provided documents for techniques including:

CT Scaling: use to scale reconstructions for e.g. air vs water or two known materials. Will scale reconstructions to a standard for interpolation of composition.

- Requires a phantom samples (e.g. water, or custom material)
- *CZXRM_TechnicalProcedure_CT_Scaling_Ver08*

Dual Energy and DSCoVer: collect two scans at different voltages to enhance contrast between similar looking features.

- DSCoVer: Dual Scan Contrast Visualizer: program to view dual energy contrast
- Requires binning 2
- See documentation for parameter selection.
- *CZXRM_TechnicalProcedure_Dual_Energy_Ver02*
- *CZXRM_TechnicalProcedure_DSCoVer*

High Aspect Ratio Tomography (HART): increases concentration of projections around the long axis of a sample to reduce certain artifacts and provide higher quality scans in less time. Use for flat samples with >1:4 %transmission difference between the 0° and -90° views. May be used with variable exposure.

- Samples must be oriented so the long path is parallel to the beam at -90° (long axis perpendicular to the flat edge of the sample holder base) or 0°
- Use 180°+fan projection mode instead of 360° rotation (set under Advanced Acquisition)
- *ZEISS XRM Technical Procedure – High Aspect Ratio Tomography…*

Filtered Secondary Referencing: use with high transmission samples with no filter to reduce the appearance of beam hardening artifacts.

- *ZEISS XRM Technical Procedure – Filtered Secondary Referencing…*

Phase Contrast: use to enhance edges and contrast of high transmission samples

- Set up the scan as normal, then move the source and detector away, maintaining the ratio of their locations.
- *CZXRM_Technical Procedure_PhaseContrast-Ver05*

Scout and Zoom: use to center a difficult to find region of interest.

- begin with a short (~20 minute) low mag scan and reconstruction, then select a new scan center from the reconstruction for a high mag scan. Samples cannot be moved from the sample holder between scans.
- *CZXRM_TechnicalProcedure_Scout-and_Zoom_Ver6*

Variable Exposure: use with high aspect ratio samples/samples with significantly different intensities through the long vs short axis of the sample.

- Ensure as best as possible that the long axis of the sample is parallel or perpendicular to the beam path (use the flat edge of the sample holder for reference)
- Use a 30-60° orientation for filter selection as an average of the high vs low transmission orientations.
- Set up scan otherwise as normal. Determine the scaling factor by comparing single images at 0° and -90°
- *CZXRM_TechnicalProcedure_Variable_Exposure_Ver05*

E: COMMON SAMPLES: (SAMPLE PREP NOTES, TYPICAL SCANNING CONDITIONS)

Aerogels: If structure is stable in air, secure with no container to a holder – UV glue not recommended on sample directly due to wicking. Tape or mechanical clamping recommended. If a holder is needed: use polyimide tubing or other thin-walled plastic container close to sample size.

Scan at low voltage (30-40kV,) with no filter. Filtered Secondary Reference recommended. Phase Contrast may be used with 20X (not recommended for 40X due to scan times.) 40X recommended to enhance contrast for extra small features.

Hydrogels (Hydrated): Polyimide tubing or other thin-walled container close in size to the sample recommended, with a small "sponge" of material in the bottom to keep the sample hydrated during the scan. Samples may be left in the chamber for a few hours to overnight to stabilize at system temperature.

Scan at low voltage (30-40kV,) with no filter. Filtered Secondary Reference recommended. Phase Contrast may be used with 20X (not recommended for 40X due to scan times.) 40X recommended to enhance contrast for extra small features. Some larger hydrogels (or hydrogels with salts, etc. embedded) may need a weak low energy filter.

Porous Metals: Typically easy to scan – avoid metals with higher Z than tungsten. Prepare sample as small as possible (especially for small pores.) May use UV glue and graphite to suspend sample in smallest sample holder, or use any holder suitable to sample geometry.

Start with 140kV. May need 160kV. Heavier metals and/or larger samples may need HE18 filter.

Coin Cell/Pouch Batteries: If these need to be protected from air: seal in thin pouch (lighter materials typically better.) Smaller clamp holder recommended. If sealed in pouch, pinch corner and scan near top of sample to reduce overall beam path through the sample.

Conditions depend on battery contents and goals: Start with 140kV

Large Cell Batteries: Ensure these are secure in the holder before scanning. Depending on height and objective used, may need axis other than Y for reference images.

Most likely conditions: 160kV, HE18 filter. Depends on individual samples and goals. FPX detector may be faster, depending on voxel size/field of view goals.

Biological (animal): Stain, if possible, to improve contrast (depending on goals.) Ensure samples are well sealed and unlikely to move in a plastic container close to the sample size. **Contact EHS before scanning to discuss safety**.

Typically use intermediate voltage and filter. Start with 80kV and adjust based on sample (slightly higher or lower.) May need Scout and Zoom to identify a specific region within the sample.

Biological (vegetable): Ensure sample will not leak. If hydrated (like fruit,) encapsulate sample in thin walled plastic – polyimide tubing or another plastic container works fine – with a small amount of water/fluid in a sponge/spongy material to maintain humidity. Sample may need to stabilize at chamber temperature prior to scan.
Samples likely to shrink, dry, or otherwise change during the scan may benefit from faster scans: If possible, use lowest magnification lens (or flat panel,) with bin 2. Most samples will start at 80kV, and may need slightly lower or higher voltage, depending on size and composition. May need Scout and Zoom to identify specific internal structures for higher magnification scans.

Carbon-based 3D Prints: Ensure sample is stable. If sample is on a substrate, the lowest density substrate available is recommended. Si may yield Feldkamp artifacts for very short samples.

Typical scan conditions (depending on size) use low energy filter and 60kV. Check and adjust for individual samples for the first scan.

Si Chip Devices: If attempting to reconstruct generally higher density/higher atomic Z materials on Si, prepare sample mounted vertically in a clamp holder (for larger samples,) or with UV glue bonded to graphite (for small samples.)

Scan with High Aspect Ratio Tomography Mode (typically. Not necessary for chips similar in depth and width.) Choose starting voltage based on surface layers (80kV for lower Z, 140kV for higher Z, such as metals.)

Small/Light Structures on Si: Trim Si as close to the region of interest as possible. Use a different substrate (lower Z/density) and/or a lattice structure on top of the substrate, if possible. Mount as flat as possible on the smallest sample holder. This may mean the cylindrical column or custom printing a holder than can interface with either pin vise holder (0.7mm or 1-3mm diameter pin.) Any amount of tilt may yield Feldkamp artifacts that distort or hide features of very short samples/features.

Scans will likely use low voltage (40-60kV,) no filter, maybe phase contrast (4X or 20X.) If features are not visible in projections, it is likely not worth scanning.

F: ARTIFACTS

Beam Hardening (Low Voltage): Appears as concentric rings originating from the center of a reconstructed slice. Use of a filter is recommended to reduce the appearance of these rings. For samples that should not be scanned with a primary filter (e.g. aerogels, anything in Phase Contrast mode,) use a Filtered Secondary Reference.

Beam Hardening (High Voltage): Appears as a gradient through a high density region of a sample. Use the beam hardening correction in the Reconstructor to attempt to correct, and/or higher voltage, stronger filter.

Metal (Photon Starvation): Especially apparent in samples with multiple distinct regions of high density/low transmittance materials (e.g. a metal lattice, Si devices.) These appear as dark shadows between low transmittance materials where the beam is significantly attenuated. Attempt to scan with highest voltage, strongest filter (up to HE18,) lowest magnification (0.4X of FPX,) and position sample so a row is NOT at the vertical center of the projection. The latter takes advantage of the cone shaped beam to reduce feature overlap.

Metal (Scatter): Bright lines radiating from metal features or corners. Attempt higher voltage, stronger filter, but these may be unavoidable (and sometimes appear on corners of non-metal samples.)

Feldkamp Artifacts: Likely to be problematic when scanning a low density sample on Si or substrate with higher density than sample. Appear as lines emanating from the substrate (or higher density material) and may hide or distort lower density materials. Attempt to scan with lowest possible cone angle (higher mag or greater source-detector separation) and lighter substrate, if possible.

Speckle: Single pixel-wide streaks across slices of a reconstruction. This is NOT noise. These may result from stray cosmic rays or scattering impacting a single pixel of the detector. Use the despeckle filter in the Reconstructor to attempt to remove these artifacts.

Center Shift: Appears as doubled edges, star-shaped artifacts from points, or donut-shapes for interior shapes/voids. Load the .txrm dataset in the Reconstructor and adjust center shift values until the slice appears focused (note: this is not actual focus, it just looks sharp.) If center shift does not resolve the artifacts, you may have Sample Drift or a very large sample (see below.)

Sample Drift: Small amounts of drift with the high magnification detectors can be automatically compensated for. Datasets may fail to reconstruct, or reconstruct with doubled-edge artifacts that appear similar to center shift artifacts at first, but cannot be resolved with a center shift correction. This indicates the sample moved or changed shape during the scan. **Solution**: better preparation to stabilize sample (e.g. hydrated environment, custom holder)

Sample too Large: The dataset may fail to reconstruct, or reconstruct with doubled-edge artifacts that appear similar to center shift artifacts at first, but are approximately equal in magnitude and opposite in direction from one side of the sample to the other. **Solution:** reduce cone angle by moving source and detector farther apart.

G: BASIC TROUBLESHOOTING

Noise: Easiest fix is longer exposures, however for very low or very high transmittance samples, longer exposures alone may not resolve the problem (too little signal.) Increase binning and/or use a lower magnification objective, if possible. The general goal is to increase intensity through the sample. Post-processing and image filtering may help with a given reconstruction.

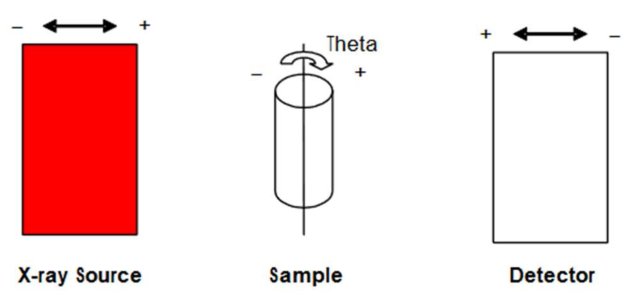
Low Contrast: Use a different filter (stronger for low transmittance samples, weaker for high transmittance), voltage (higher for low transmittance samples, lower for high transmittance), or Phase Contrast mode (low transmittance samples, no filter, 30-40kV, 20X.) The typical goal is to keep transmittance as close to 15-35% as possible.

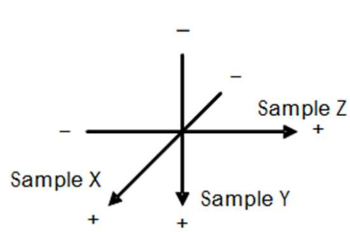
Radial Streaks: If radial streaks emanate from the center (and are NOT metal/scatter artifacts,) this typically indicates too few projections. Scan again with more projections.

Low Resolution: Use a lower binning factor (if possible) for the largest FOV with a smaller resolution, or use a higher magnification lens (will yield a smaller FOV.) Recommend: Scout and Zoom to identify region for higher mag scan.

H: ORIENTATION OF AXES

Direction (Axis)	Sample Theta Position	Movement	Movement From User's Perspective
Sample X	0°	Toward and away orthogonal to the beam line from the X-ray source to the detector	IN and OUT
	-90°	Toward and away on the beam line from the X-ray source to the detector	
Sample Y	0°, -90°	From top to bottom of the enclosure	UP and DOWN
Sample Z	0°	Beam line from the X-ray source to the detector	LEFT and RIGHT
	-90°	Orthogonal to the beam line from the X-ray source to the detector	
Sample Theta	–	Circular angle	CLOCKWISE and COUNTERCLOCKWISE





Directions when the Sample Theta stage is at 0°. The Sample X and Sample Z axes rotate with the stage. When the Sample Theta stage is at -90°, Sample Z moves orthogonal to the beam line and Sample X moves on the beam line.