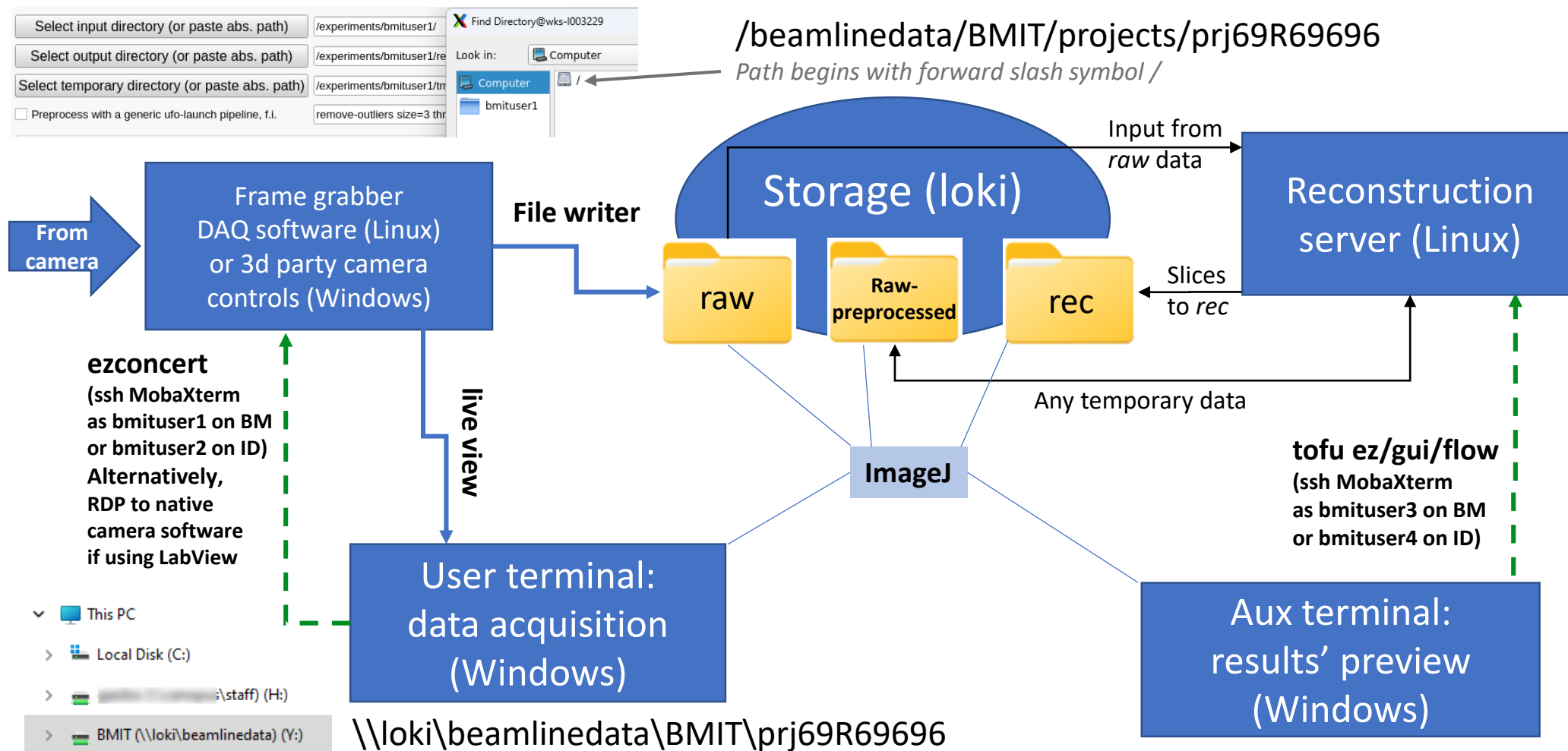


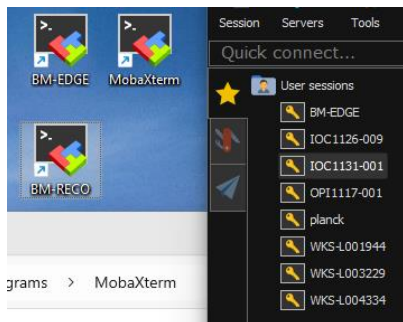
Essential things to know in order to do X-ray microCT at Light exploring life **BMIT**

1. An overview of the data flow between BMIT computers, accounts and the project directory structure



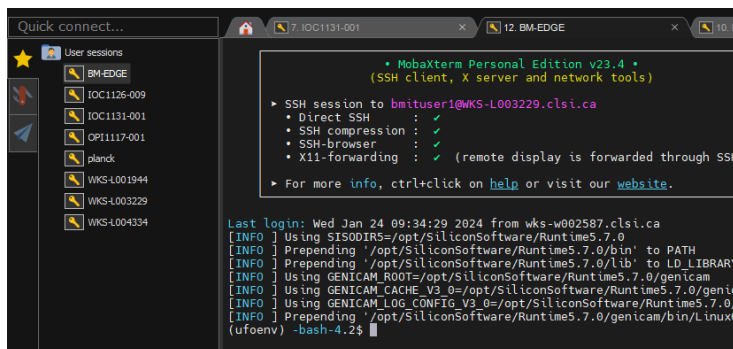
Following pages: (2) Starting applications; (3) Acquiring a CT set; (4) Restarting frozen GUI and resetting a non-responsive CT stage; (5) How to preview slices; (6) Troubleshooting axis of rotation; (7) Retrieving phase and dealing with typical artifacts; (8) Half-acquisition mode (9) Reconstructing half-acquisition mode data and stitching multiple vertical tiles

2. Starting data acquisition and reconstruction applications

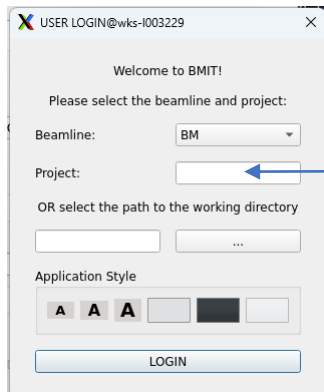


Double click on the shortcut or launch MobaXterm and double click on the session in the right hand side (click on large yellow start if the tab is not visible)

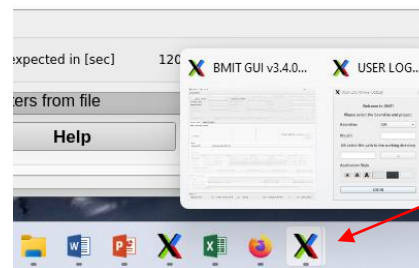
Once connection established, a command line prompt with activated *ufoenv* will be available



To start data acquisition software type *ctgui* and press enter.

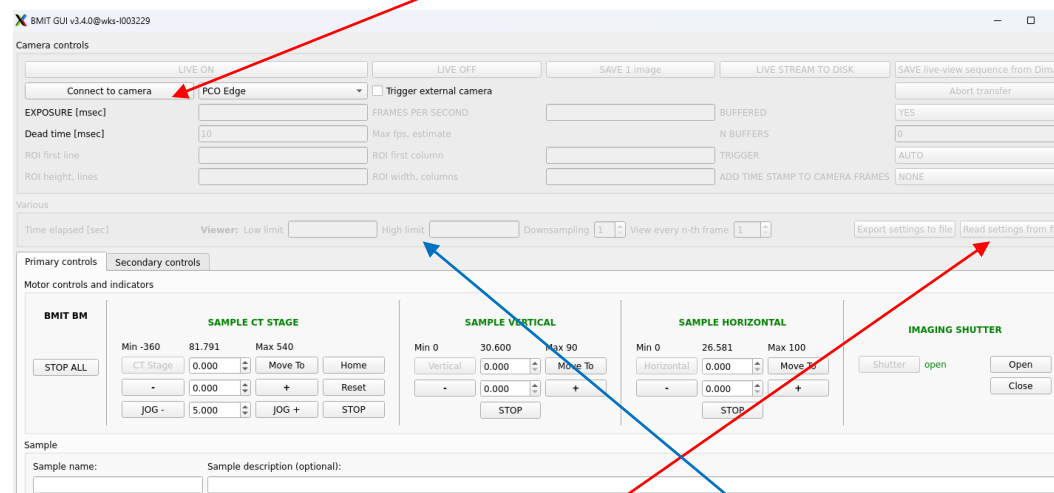


An application will open but it will not be active until you enter your project number in the login prompt



It may happen that the login screen pops in the background. You can switch to it by locating MobaXterm cross symbol in the Windows taskbar at the bottom of the screen

Select the camera from the drop down list and press connect. Beamline scientist will tell you the camera model



Once connection to camera is established, camera control tab and all other boxes will be enabled. To resume experiments you can import the configuration file which was saved automatically together with your last successful CT scan. Note that low and high limits of the viewer are not set

To start **data reconstruction** application, open a new connection to the respective server and execute *tofu ez* in the command line. Note that the reconstruction parameters are saved in the output directory in *parameters.yaml* file and can be imported

```
(ufoenv) -bash-4.2$ tofu ez
```

If *ufoenv* does not appear in the beginning of the prompt: execute *source /opt/ufoenv/bin/activate* and then to open data acquisition application use

```
(ufoenv) -bash-4.2$ python /beamlinedata/BMIT/scripts/ezconcert3-master/gui.py --debug
```

and the same *tofu ez* command to start data reconstruction application

3. Acquiring a CT set

Definition: in parallel-beam geometry a valid X-ray CT set is any number of projections acquired at a constant angular spacing while sample is being rotated by 180 degrees at a constant velocity. This set of radiograms (or *tomo*) has to be accompanied by a set of reference beam intensity images (a.k.a. *flats*) taken when sample is moved out of the beam and a set of detector background noise images (*darks*) taken with the closed shutter.

BMIT GUI v3.4.0@ioc1126-009

Camera controls

Turn on to positioning sample before scan

Select camera and click to begin work

Camera control group

Find the detector counts by hovering the mouse cursor in the live view window and adjust Low/High limits accordingly. Increase exposure time if count are low

Vertical motor and fine XY stages are used to position region of interest in the detector FOV before scan. Horizontal motor must not be moved

Top row: absolute position
Second row: relative moves

CT stage return and jog velocity. Do not change without consulting the beamline scientist first

Must enter "Sample name" if using "Buffer on the local drive" option

Hit to Start scan. Once scan is started only Viewer limits and Abort button will be active. Scan log can be monitored in the MobaXterm window

Must be enabled if data stream exceeds 0.8 GBps (>10 full frames per second). Not used for Dimax and step-and-shoot

Camera controls

LIVE ON (CONNECTED) LIVE OFF SAVE 1 image LIVE STREAM TO DISK SAVE live-view sequence from Dimax Abort transfer

PCO Edge Trigger external camera

EXPOSURE [msec] 300 FRAMES PER SECOND 3.00 BUFFERED NO

Dead time [msec] 10 Max fps, estimate 210 N BUFFERS 0

ROI first line 512 ROI first column 0 TRIGGER AUTO

ROI height, lines 1024 ROI width, columns 2048 ADD TIME STAMP TO CAMERA FRAMES NONE

Various

Time elapsed [sec] Viewer: Low limit 2000 High limit 11000 Downsampling 1 View every n-th frame 1 Export settings to file Read settings from file

Primary controls Secondary controls

Motor controls and indicators

BMIT ID SAMPLE CT STAGE SAMPLE VERTICAL SAMPLE HORIZONTAL IMAGING SHUTTER

Min -360 0.000 Max 540 Min 0 128.000 Max 300 Min 0 312.001 Max 500

STOP ALL CT Stage 0.000 Move To Home Vertical 0.000 Move To Horizontal 0.000 Move To Shutter closed Open

- 0.000 + Reset - 0.000 + - 0.000 +

JOG - 10.000 JOG + STOP STOP STOP STOP

Runout Compensation Runout offset: 0.000 Real position: 312.001

Slope: 0.0

Sample

Sample name: Bambuk007 Sample description (optional): This-is-too-easy-when-hardware-works-well

Scan controls

START ABORT RETURN Delay start by [min] 0 Repeat 1 Readout in the end

Live View MOTOR FLATS START POSITION NUMBER OF POINTS RANGE OR STEP SIZE ENDPOINT FLATS MOTION

Outer loop Vertical [mm] Before 128 0 9 -9.0 Include After On-the-fly

Inner loop CT Stage [deg] Before 0 2000 180 0.09 Include After On-the-fly

Flat-field correction settings

MOTOR Horizont Radio position [mm] 312 Flat position [mm] 325 Num of flats 50 Num of darks 20

File-writer settings

Root dir: /beamlinedata/BMIT/projects/prj36M12564/raw/sergei-tmp

CT scans' name pattern scan_{:>03} Use bigtiff containers Number of images per bigtiff 5000 Filename pattern frame_{:>05}.tif

Local Drive Buffer dir: /data

Scan setting can be imported from *parameters.yaml* file which is automatically generated in the directory with the last successful CT scan

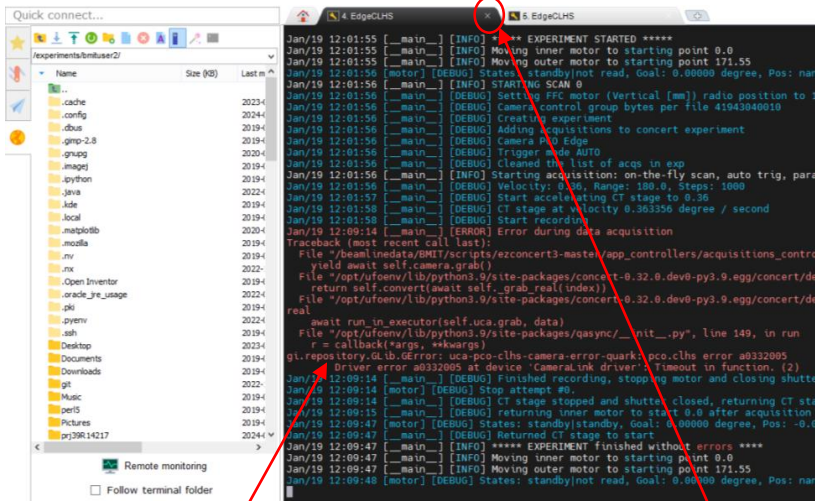
Scan begins at 0 and sample is rotated counter-clockwise. Stage will return to 0 in the clockwise direction in the end. At the end of each CT scan stage should be at 180 (or 360 deg if using half acquisition mode) plus a little amount. Homing always happens counter-cw.

Radio position is found during the alignment stage and, unless lost, it is typically where Sample Horizontal motor is at when the scan is over. Flat field is the position of the motor when sample is moved fully out of the beam. Both are absolute positions.

Select *Root dir* for the new sample in your *project_dir/raw* or use *Sample name*. Data won't be overwritten if the name is the same. DO NOT USE SPACES IN THE FILENAMES

4. Restarting frozen *ezconcert* application and clearing Aerotech CT stage faults

Note that there is a live log of things happening during data acquisition



There is a known issue with PCO Edge cameras which can result in scan failures. If that happened to you or if software has frozen or crashed for other reasons (e.g. scan had to be aborted and controls would not respond):

- 1) Make sure that the application is killed, e.g. that the prompt is back to `(ufoenv) -bash-4.2$`

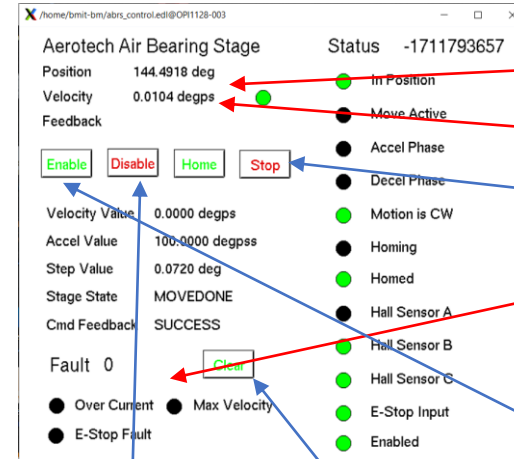
A reliable way of ensuring this is to close the connection tab at the top and then reconnect

- 2) Make sure that CT stage is not moving

3) If you use PCO Edge and saw a CameraLink driver error. In the terminal type `restartpc0` and hit enter. If the command is not recognized execute `sudo systemctl restart pcoclhs`

- 4) Wait 5 seconds and start the PyQT application as explained on the page 2

Aerotech CT stage is free floating or non-responsive or motion has to be urgently stopped? There are two applications opened in the top right monitor which can be used to control and troubleshoot Aerotech CT stage at the low level. Be very careful!



Shows current position. If the number is changing monotonically then the states is moving

Shows approximate current velocity value

Try *Stop* first if you need to stop sample rotation

Shows faults. Note that E-stop fault must be cleared by releasing the emergency stop button inside the hutch

Note: for a successful scan, CT stage must travel at least 180 degrees plus a little extra depending on the exposure time and the number of projections, f.i. 181.6°

To reset the CT stage if it is non-responsive:

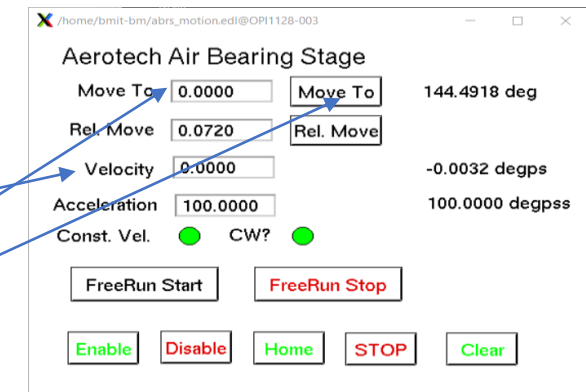
Hit *Disable* button → Click *Clear* in the Fault section → Click *Enable*

Be very careful about how you return stage to 0 after a problem if wires or tubes are attached to sample Firstly, note that the positive direction of rotation is counter-clockwise and the *Home* command will complete a full rotation in this direction and reset the position to 0

You might first need to move stage to f.i. -30 degrees before clicking *Home* button or resuming the scans.

You can do it in this application by

- 1) Set velocity to 5 (type 5 and hit Enter)
- 2) Enter the absolute position in *Move To* entry and clicking on the adjacent button



5. Data preview

To preview the results in tofu ez you would have to set the directories like so

Input/output and misc settings

Select input directory (or paste abs. path)	/beamlinedata/BMIT/projects/prj69R69696/raw/Samp1
Select output directory (or paste abs. path)	/beamlinedata/BMIT/projects/prj69R69696/rec/Samp1/preview
Select temporary directory (or paste abs. path)	/beamlinedata/BMIT/projects/prj69R69696/raw-preprocessed/tmp-ezufo/

And select several detector rows, for which CT slices will be reconstructed

Region of Interest and Histogram Settings

☒ Select rows which will be reconstructed	
First row in projections	100
Number of rows (ROI height)	1000
Step (reconstruct every Nth row)	50

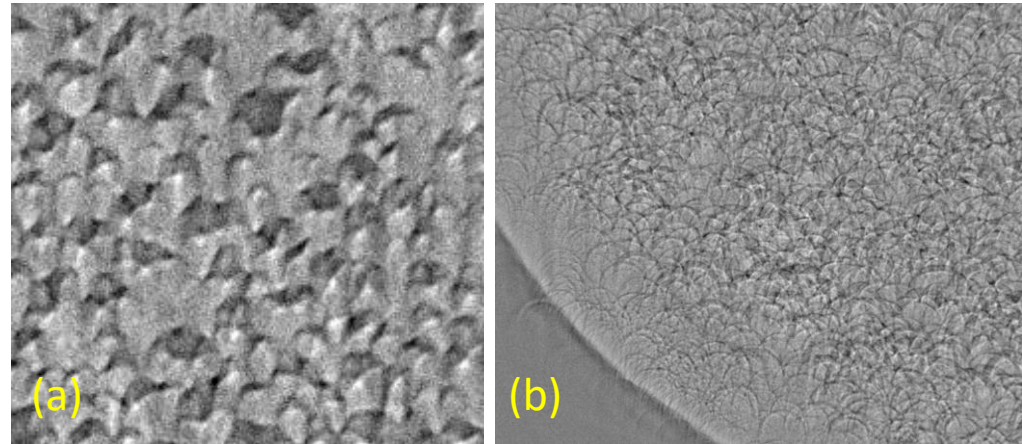
We assume that there is sample between rows 100 and 1100

Estimated value of the *center of rotation* will be printed in the terminal.

The estimator doesn't work correctly in every situation. Below is an example of a slice reconstructed with an imperfect axis

```
***** Analyzing input directory *****
Total folders 1, good folders 1
Path to CT set Typ: 0 bad, 3 no flats2, 4 with flats2
4
***** AXIS INFO *****
Exporting values to: /data/sg-tmp-ezufo/parameters.yaml
Finished exporting
CTset: /data/Test/Tshort/z00
Number of projections: 2250, dimensions: [460, 2048]
***** PROCESSING *****
>>>> PROCESSING
Cleaning temporary directory
- CT with axis 1014.5; ffc:True, PR:False
INFO: X region: (-1024.0, 1024.0, 1.0)
INFO: Y region: (-1024.0, 1024.0, 1.0)
INFO: Parameter region: (-130.0, 70.0, 20.0)
```

On the left, note the broken contours and curly strokes around the features. With the correct *axis of rotation* there must be nice enclosed contours. In the picture on the left pores can still be guessed indicating that the axis estimate is off by small amount (5 pixels) Right picture illustrates strong semi-circles which happen when the axis is 34 pixels off.



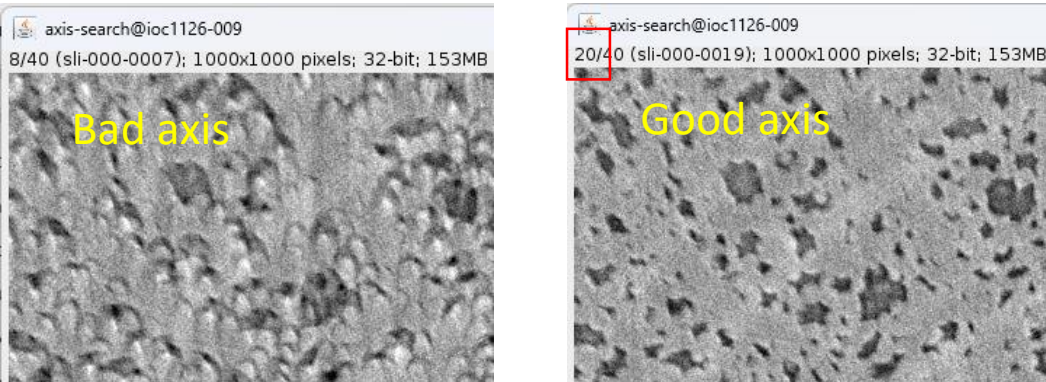
In order to find the correct value of the axis of rotation, select the second option *Minimize std of a slice* Select row which intersects the sample and increase the reconstructed patch size if needed. For this estimator to work well, there must be a good amount of structures in the reconstructed patch

Centre of Rotation

☐ Auto: Correlate first/last projections	
☒ Auto: Minimize STD of a slice	☐ Apply PR while searching
Search rotation axis in [start, stop, step] interval	1004,1024,0.5
Search in slice from row number	100
Size of reconstructed patch [pixel]	1000
☐ Define rotation axis manually	
Axis is in column No [pixel]	980.0

Set the search range based on the inspection of the first reconstructed slice. In example (a) the axis is pretty close to the correct one. The axis estimate was 1014.5 so we set the range to ± 10 pixels with the step 0.5 (note that in the case (b) I would set axis search range to 900,1100,2 and then refine it in the second iteration if needed)

It is possible that both automatic estimators will fail. In this case the sequence of patches generated by the “Minimize std” algorithm can be inspected manually. This sequence can be found in the “axis-search” directory in the temporary directory which you set up in the beginning. Import the sequence to *ImageJ*, find the good slice, note its index (assume *j*) and compute the axis of rotation as $start+(j-1)\times step$. In the example below index of the well-reconstructed slice is 20, hence the correct axis is at 1013.5 which is $1004+19\times 0.5$. You can now select *Define rotation axis manually* in the *Center of rotation* tab and set the value to 1013.5.



If you cannot find good axis of rotation after multiple attempts, one of the following things could have happened: (1) sample has moved during exposure (2) CT stage didn't reach 180 degrees (3) CT stage hugely over-travelled because of data transfer bottlenecks (4) one or more projections in the beginning or the end are actually flat or dark images. Please contact beamline scientist to help with the troubleshooting. You can also open new connection to data reconstruction server and use *tofu gui* application to compare the first and last projections in order to rule out sample motion (next page).

6. Checking integrity of CT sets and recognizing sample motion

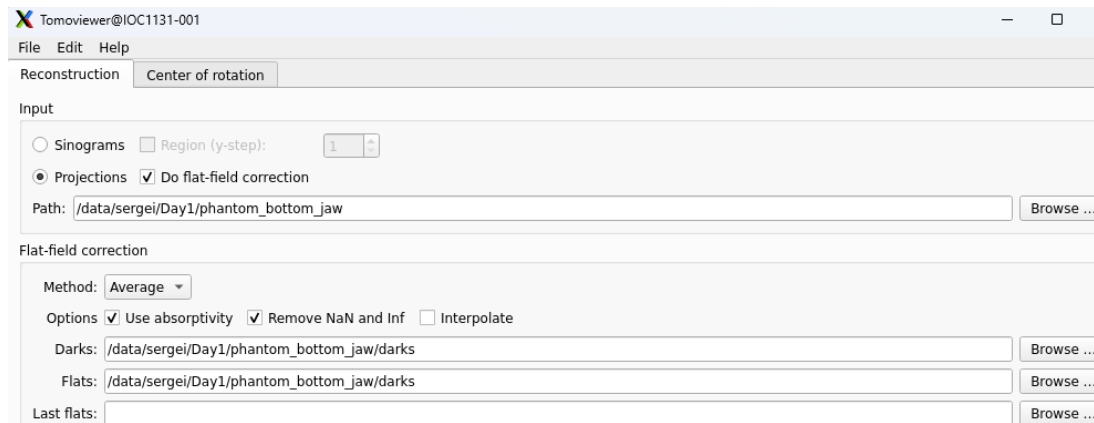
If reconstruction fails then the most likely reasons are:

(1) CT set is incomplete or corrupted (2) Sample has moved during the scan.

To verify integrity of a CT set, navigate to it in the *raw* directory and check the directory structure. A valid CT set is comprised of *flats*, *darks*, and *tomo* directories, which contain only *tif* files (note that Windows can hide *Thumbs.db* file in one of these directories!).

Then inspect files in *Fiji/ImageJ*. The number of images must be consistent with the entries made in the data acquisition interface. Images of different type must not be mixed together, e.g. there are no *flats* in *tomo* directory or bigtiff container. If you spotted a *flat* field in the end of the *tomo* image sequence, delete it or replace it with an adjacent projection.

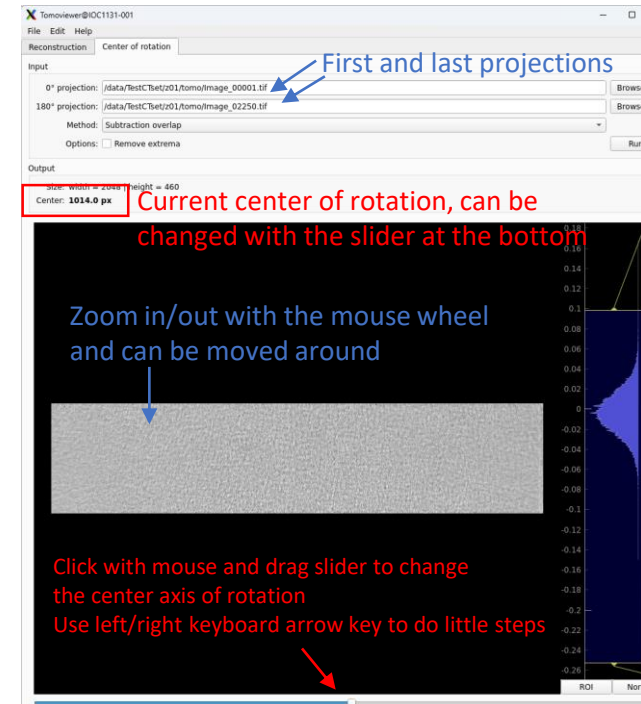
The sample motion can be registered with `(ufoenv) ~>tofu gui` program:



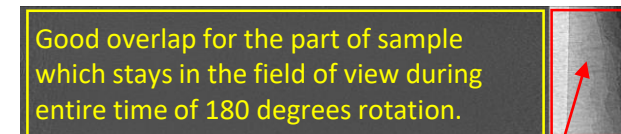
First, change the type of input data to *Projections*. Then select *Do flat-field correction*. Additional entries will appear. Substitute paths to the directories with images of each required type to perform the flat-field correction. Enable *Use absorptivity* and *Remove NaN and inf* to take negative logarithm of transmission projections and replace non-physical values with zeros accordingly.

In the *Center of rotation* tab load two projections separated by 180° from the *tomo* directory (typically first and last files; if you have a bigtiff container then simply point to it twice and program will extract the first and the last pages). Pressing *Run* will trigger three things: selected projections will be flat-filed corrected according to parameters configured in the *Reconstruction* tab, (2) internal algorithm will estimate position of the center of rotation, and the differential image corresponding to this center of rotation will appear on the screen.

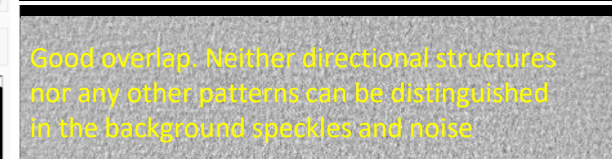
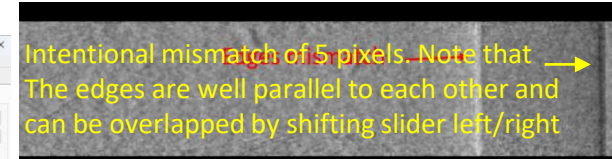
You can now adjust COR using slider in the bottom part of the window until differential image looks as uniform and free of any details as possible. It is useful to note that after the slider is highlighted, keyboard left-right arrows can be used to make fine steps accurately. You can zoom in and out to the features of interest with the mouse wheel



Note, that when doing local CT, an ideal overlap over the entire field of view (FOV) is not necessarily possible to achieve.

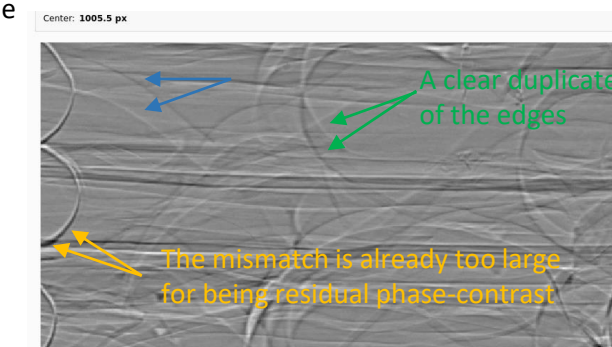


Parts of sample which only were in the detector FOV for a fraction of time during the rotation



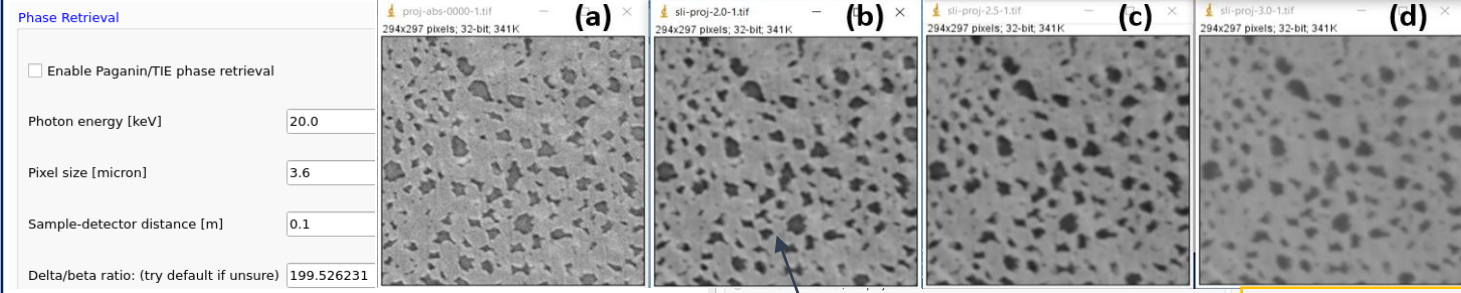
The second tab of the *Tofu gui* interface (on the left hand side) and example of a wrong and correct positions of the axis of rotation (above). Note, that the near-field diffraction fringes (a.k.a. propagation based phase contrast) will not always cancel out perfectly like in the example above. They might still be seen as a pixel thin vertical and horizontal lines.

However, if you can distinctly notice that there are two outlines of the same structure and they are vertically shifted and/or crossing each other then the sample has moved during the scan. An example of a bad CT set is shown at the bottom.



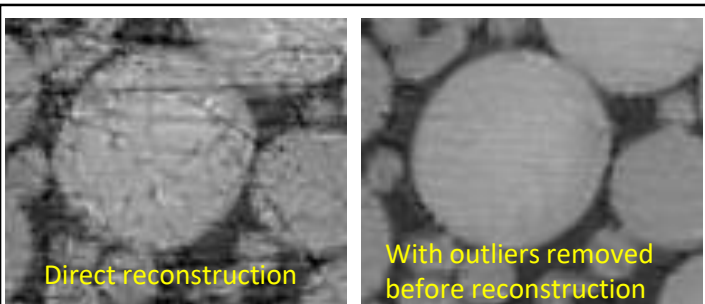
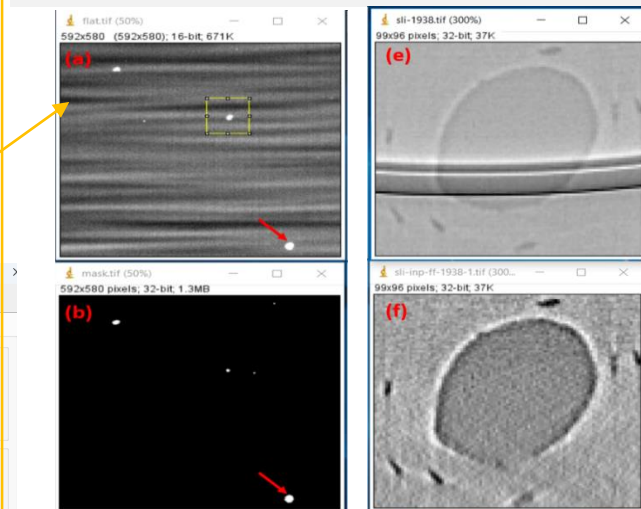
7. Reconstruction with phase retrieval and suppression of typical artifacts

Phase retrieval is not quantitative but it can significantly boost the signal to noise ratio in the reconstructed slices. In the example below *Delta/beta* ratio was varied from 0 (a) to 100, 300, and 1000 (panels b,c,d respectively). If the value is too small, it doesn't boost contrast much and leave residual fringes; if the value is too large, CT slices are very blurry

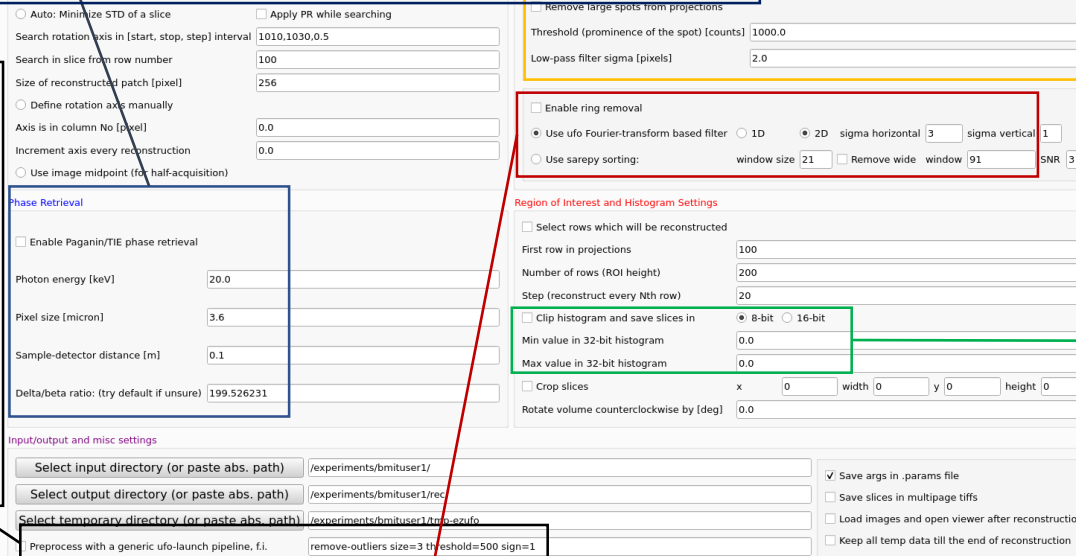


Images generated at each step of the reconstruction pipeline can be viewed in the *temporary directory* if “Keep all temporary data till the end of reco” switch is enabled.

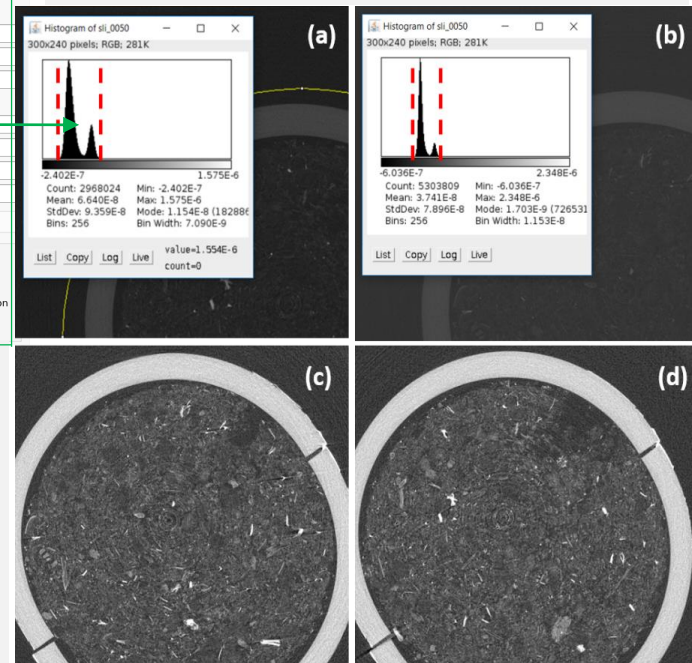
Removal of large spots: artifacts in the flat field due to scintillator defects (a), binary image showing their locations (b); slices reconstructed without (e) and after inpainting of located large spots (f)



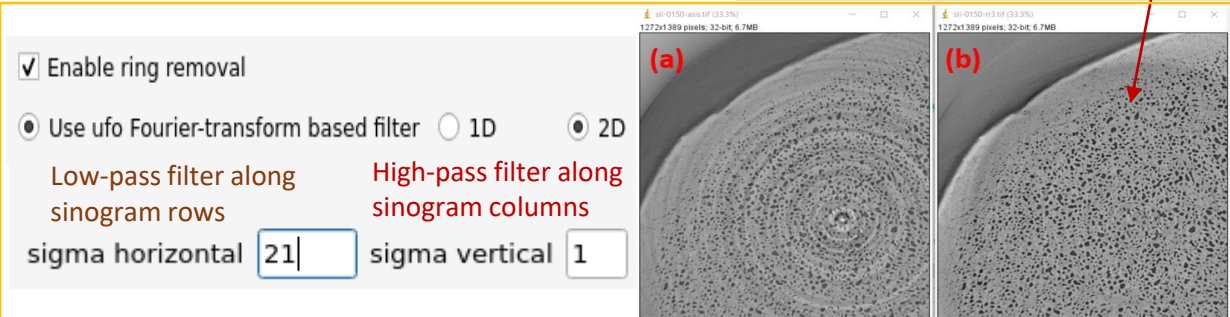
Removal of hot pixels from projections eliminates “Zingers” (strait streaks) artifact. It is basically a mandatory operation for high energy (50+ keV) scans made on ID beamline



Bit-depth reduction with histogram equalization

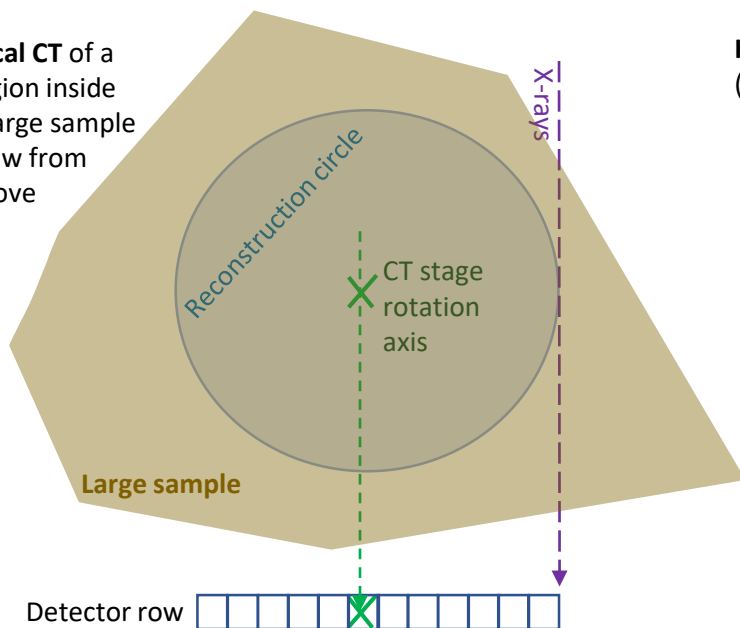


Excluding tails of the histograms and reducing size of the reconstructed data. Slices (a) and (b) belong to two different vertical views of of the same sample. Note that although full range of reconstructed values is different in each histogram, information about the sample is contained in the -1×10^{-7} to 3×10^{-7} interval in both of them. Slices in panels (c) and (d) have their histograms were clipped to $[-1.3] \times 10^{-7}$ range and converted to 8 bit



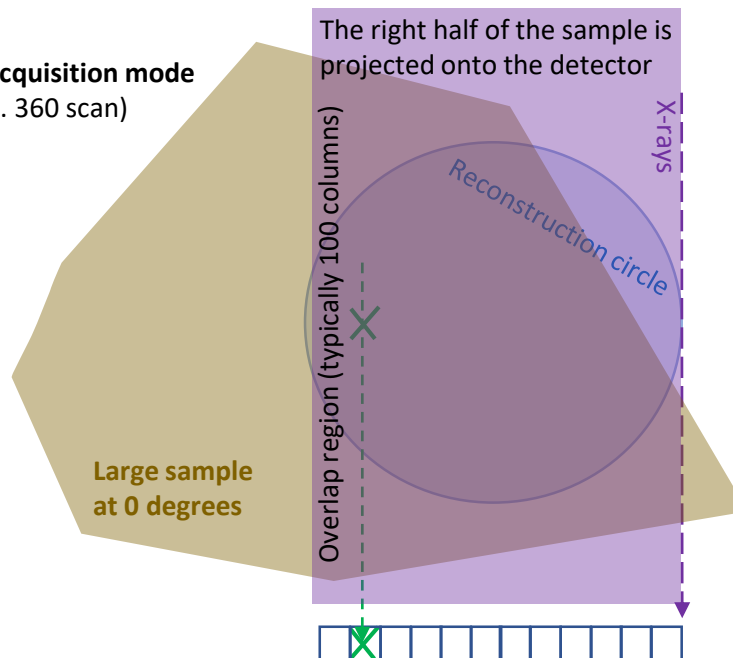
8. Increasing the size of the examined volume with the same detector pixel

Local CT of a region inside a large sample
View from above

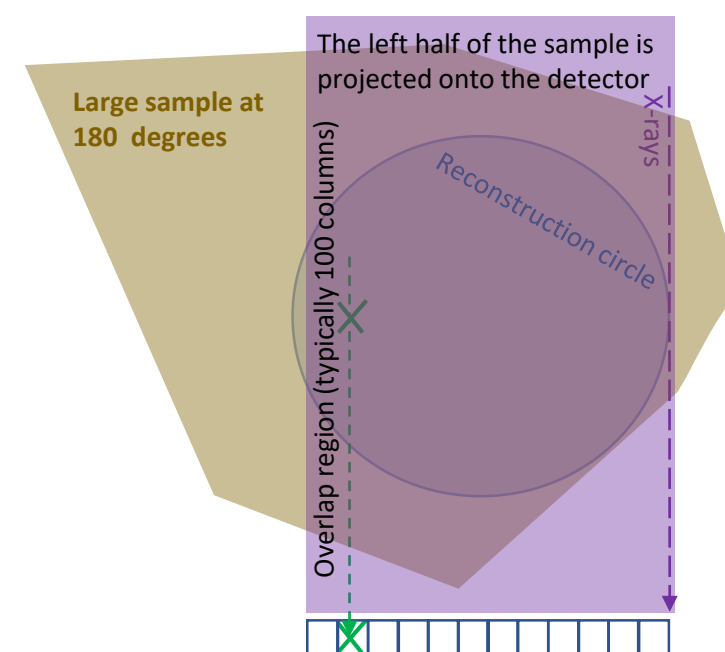


Projection of the axis of rotation projection onto the detector plane. Moving Horizontal motor will change position of the CT rotation axis with respect to the detector columns

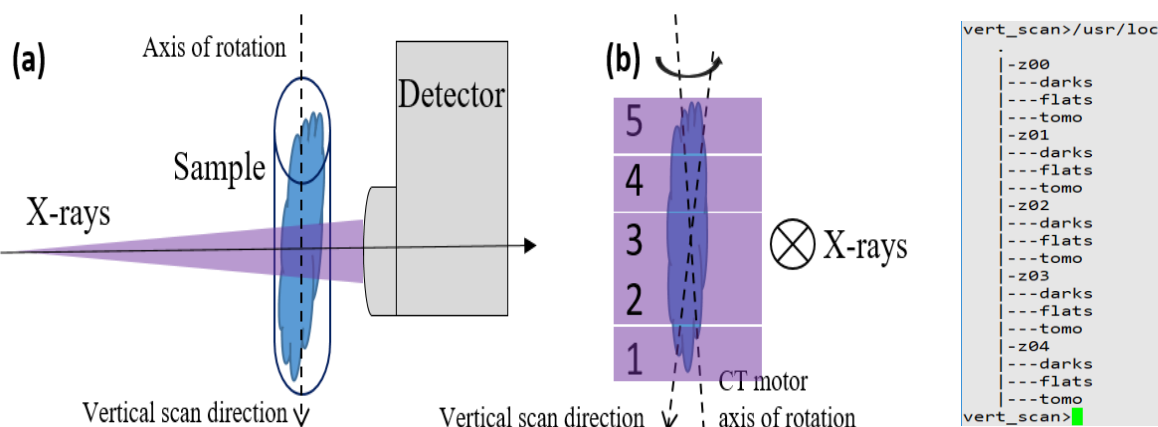
Half acquisition mode
(a.k. a. 360 scan)



We offset the axis of rotation to the edge of the detector and do a 360 degrees rotation. In this way we collect two CT sets for adjacent parts of the sample. They can be stitched together to double the size of the examined volume.

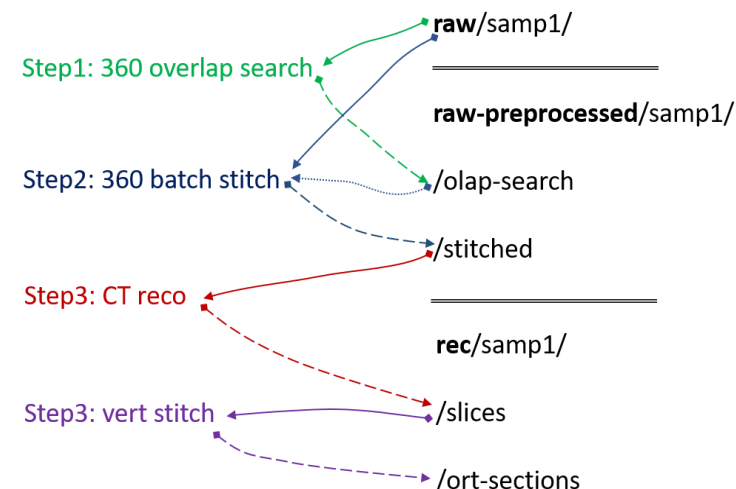


Vertical scan of an extended sample and resulting directory structure



Note that the horizontal position of the axis of rotation changes as samples is moved vertically.
On ID beamline this shift is normally systematic and can be compensated in the data acquisition software.
On BM beamline these shifts are random and can be as large as 5 microns or 8 pixels with 10x objective.

Workflow for generating orthogonal sections through the entire examined volume of a large specimen scanned in half acquisition mode protocol with multiple vertical shifts



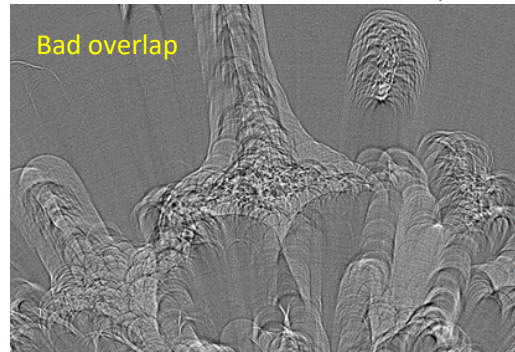
1. Find amount of overlap between projections in half acq. mode scan (in all vertical tiles if applicable)
2. Convert half acq. data to ordinary 180 data set. Crop so that all vertical tiles have the same dimensions
3. Reconstruct all slices (axis of rotation is in the middle)
4. Stitch vertical views and generate orthogonal slices

9. Identifying overlap in the half acquisition mode projections, converting them to ordinary 180° data, and vertical stitching

All stitching operations can be done in the third tab of the *tofu ez* software.



1. The first step is to find the overlap between two halves of the half acquisition mode scan and convert data to an ordinary 180 scan. To do this we reconstruct a bunch of slices with variable overlap and find the best one. Generated slices can be analyzed in the way similar to that which we employed to find axis of rotation with the “STD minimization” procedure. Slice reconstructed with the good overlap will be free of artifacts. Note the index of the slice and compute the overlap as explained on Page 5 or in image below.



5. Reslice a couple of slices first, e.g. 1000, 3000, 100. Once all settings are good, selected the entire volume (step=1)

In this case tiles must be flipped as sample was moved down during scan (top of z01 overlaps with bottom of z02).

Interpolation of the overlapping regions is generally preferable, but finding the equalization factors can be very sensitive to artifacts at the edges of the images.

If interpolation is selected, generated orthogonal sections can be produced in 8-bit with global histogram equalization. Min and max values must be found in ImageJ in advance

Concatenation is very robust as it simply trims the tiles and stack them, but it can result in fluctuating mean values in the adjacent tiles in the composite image

360-AXIS-SEARCH

Select input directory
/beamlinedata/BMIT/projects/prj36M12564/raw/sergei/vert_scan

Select temp directory
/beamlinedata/BMIT/projects/prj36M12564/raw-preprocessed/tmp-olap-search

Select output directory
/beamlinedata/BMIT/projects/prj36M12564/raw-preprocessed/sergei/vert_scan/olap-search

Row to be reconstructed: 1000

Lower limit of stitch/axis search range: 60

Upper limit of stitch/axis search range: 120

Value by which to increment through search range: 1

☒ Apply ring removal

Help Generate slices

2. Script can also process multiple CT sets located at the same input directory. In this way overlaps can be found for all vertical tiles of a long multi-view scan. This information can be used to stitch all tiles in one go

360-MULTI-STITCH

Select input directory
/beamlinedata/BMIT/projects/prj36M12564/raw/sergei/vert_scan

Select temporary directory - default value recommended
/beamlinedata/BMIT/projects/prj36M12564/raw-preprocessed/tmp-sg

Directory to save stitched images
/beamlinedata/BMIT/projects/prj36M12564/raw-preprocessed/sergei/vert_scan_stitched

☒ Crop all projections to match the width of smallest stitched projection

Axis of Rotation (Dir 00): 245 Axis of Rotation (Dir 0N): 245

☒ Enter axis of rotation manually

Axis of Rotation (Dir 00): 66 Axis of Rotation (Dir 06):

Axis of Rotation (Dir 01): 68 Axis of Rotation (Dir 07):

Axis of Rotation (Dir 02): 71 Axis of Rotation (Dir 08):

Input directory must contain at least one subdirectory which is a CT set. Overlaps for each CT set can be entered exactly as shown here or linear interpolation between overlaps in the first and the last set can be applied. Enable switch “Crop all projections to match the width ...” Before pressing *Stitch* button

```
vert_scan>usr
-z00
---darks
---flats
---tomo
-z01
---darks
---flats
---tomo
-z02
---darks
---flats
---tomo
```

3. Once stitching is over, all slices can be in the main tab of *tofu ez* by setting center of rotation to “Use image midpoint” setting. The output slices must be saved to *rec* directory inside your project. Note that *tofu ez* can batch process all vertical tiles if *input* directory contains more than one data set

4. Before generating orthogonal sections though the entire volume, the vertical overlap between adjacent tiles (e.g. z00, z01, etc) must be found. This can be done by finding the same slice in set z00 and z01 and noting respective indices. *Fiji/ImageJ* File Import → Image Sequence. Check “Use virtual stack”. Whether top of z00 overlaps with bottom of z01 or other way round depends on the direction of the vertical scan and whether camera frames were flipped. This can be done again during stitching with the “Flip images...” checkbox.

EZ-STITCH

Select input directory
/beamlinedata/BMIT/projects/prj36M12564/rec/sergei/vert_scan/slices

Select temporary directory
/beamlinedata/BMIT/projects/prj36M12564/raw-preprocessed/tmp-sg

Directory to save stitched images
/beamlinedata/BMIT/projects/prj36M12564/rec/sergei/vert_scan/ort

Name of subdirectory with the same type of images to stitch sli

☒ Stitch orthogonal sections

Which images to be stitched: start,stop,step: 0,4000,1

☒ Flip images upside down before stitching

☒ Interpolate overlapping regions and equalize intensity

Number of overlapping rows: 317

☐ Clip histogram and convert slices to 8-bit before saving

Min value in 32-bit histogram: -0.0003

Max value in 32-bit histogram: 0.0002

☐ Concatenate only

First row: 217 Last row: 900

Proceed to final step

