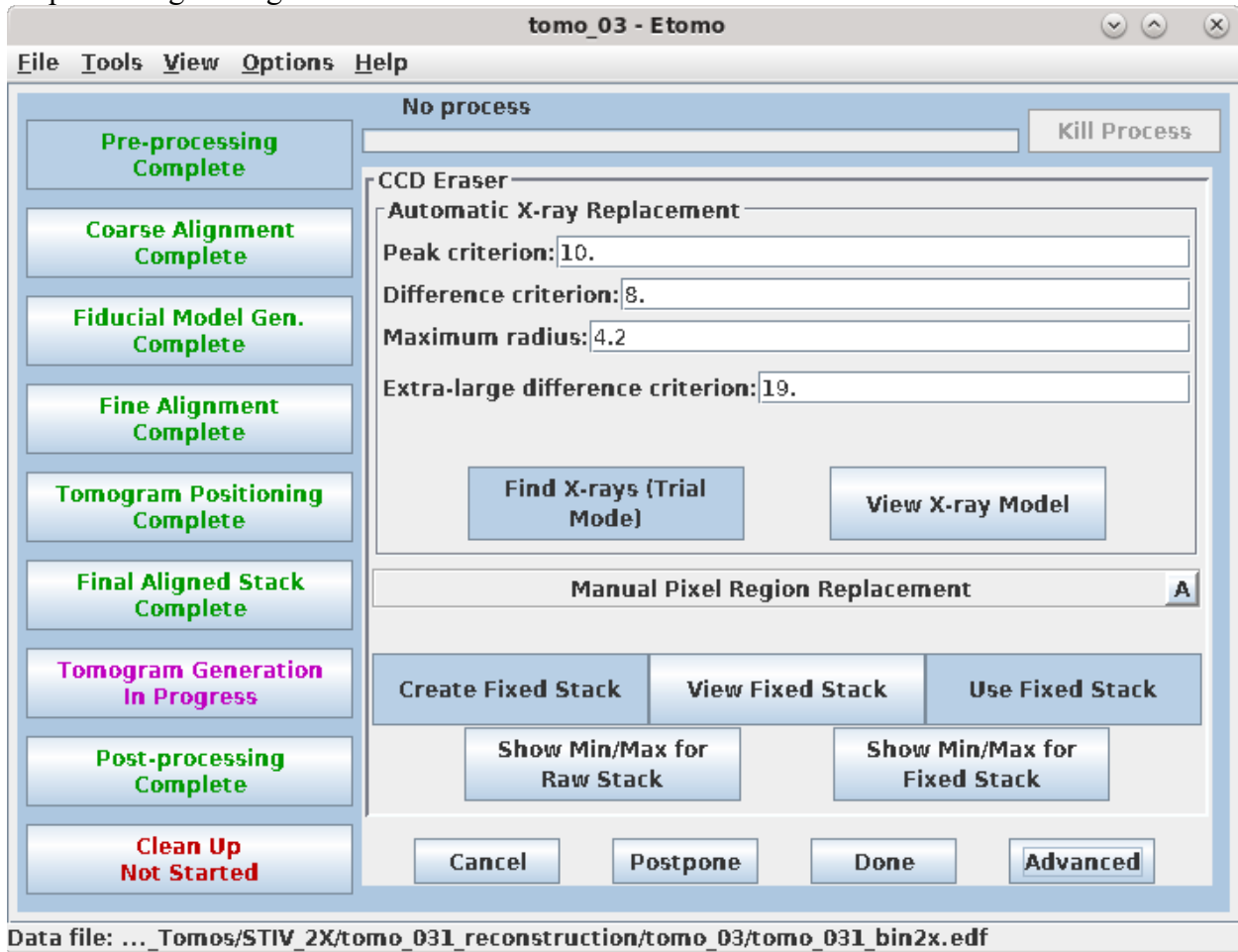


SUPPLEMENTAL FILE ONE

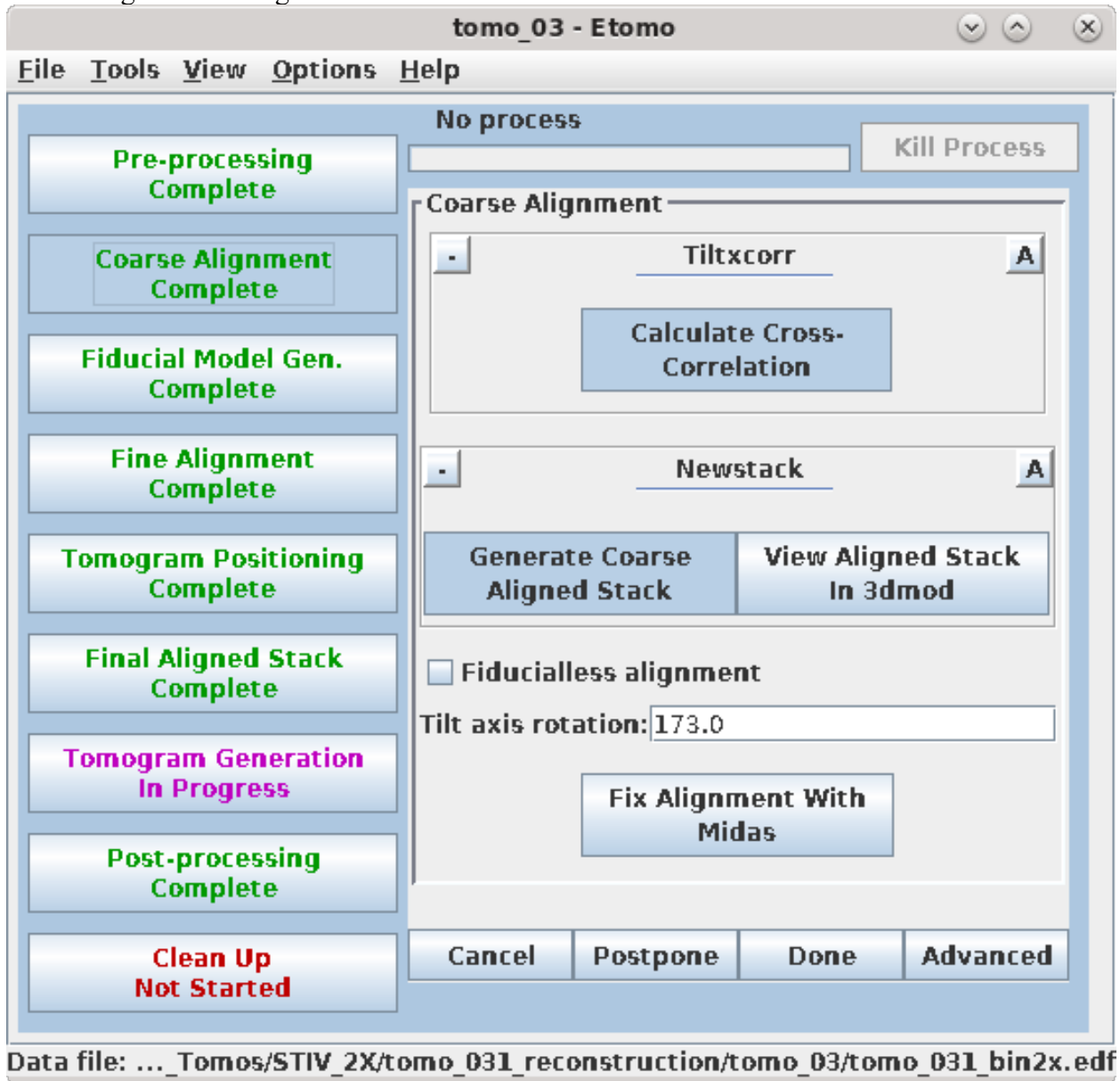
IMOD AND PEET STANDARD SETTINGS

The following images show standard settings for use with IMOD and PEET during tomogram reconstruction and sub- tomogram averaging respectively.

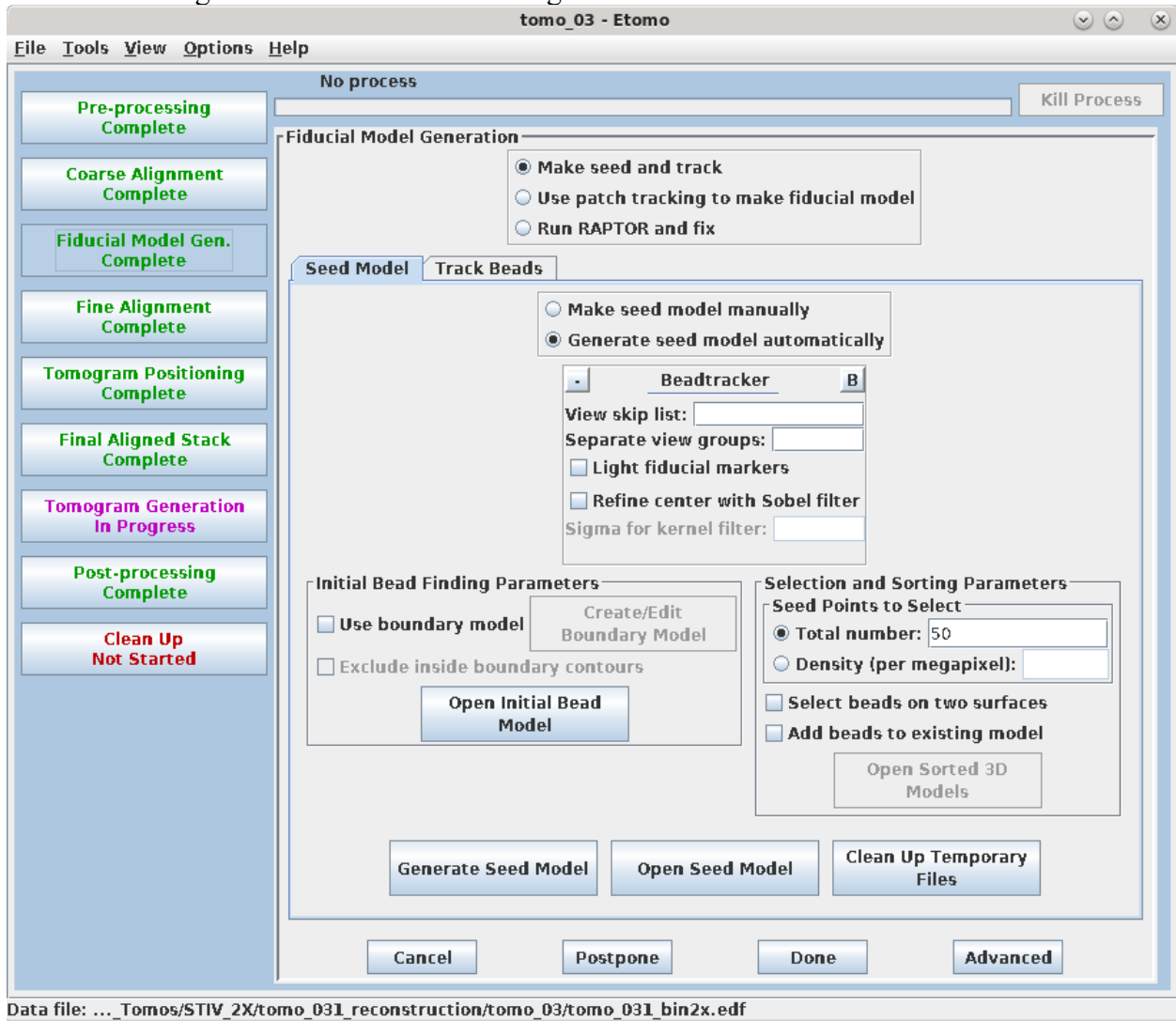
### Preprocessing Settings



Coarse alignment settings



# Fiducial model generation: seed model settings



Fiducial model generation: track beads settings

tomo\_03 - Etomo

File Tools View Options Help

No process Kill Process

**Pre-processing Complete**

**Coarse Alignment Complete**

**Fiducial Model Gen. Complete**

**Fine Alignment Complete**

**Tomogram Positioning Complete**

**Final Aligned Stack Complete**

**Tomogram Generation In Progress**

**Post-processing Complete**

**Clean Up Not Started**

**Fiducial Model Generation**

Make seed and track  
 Use patch tracking to make fiducial model  
 Run RAPTOR and fix

Seed Model **Track Beads**

**Beadtracker** B

View skip list:

Separate view groups:

Tilt angle group size:

Non-default tilt angle groups:

Magnification group size:

Non-default magnification groups:

Minimum # of views for tilt alignment:

Unbinned bead diameter:

Light fiducial markers

Refine center with Sobel filter

Sigma for kernel filter:

Fill seed model gaps

Maximum gap size:

Local tracking

Local area size:

Minimum beads in area:

Minimum beads overlapping:

Max. # views to include in align:

Rounds of tracking:

Minimum tilt range for finding axis:

Minimum tilt range for finding angles:

Search box size (pixels):

**Expert Parameters**

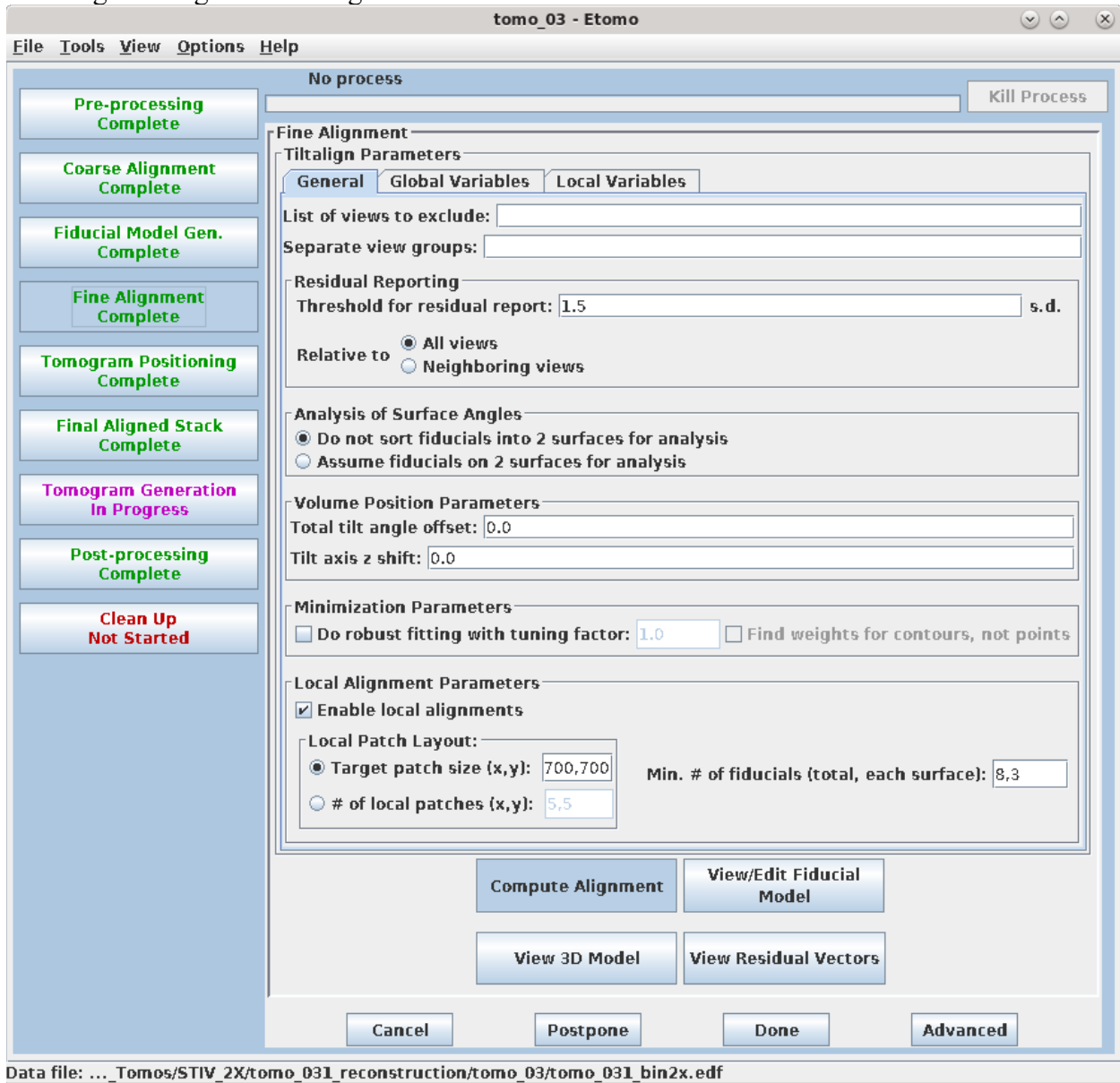
+ Track Seed Model

Fix Fiducial Model Track with Fiducial Model as Seed

Cancel Postpone Done Advanced

Data file: ...\_Tomos/STIV\_2X/tomo\_031\_reconstruction/tomo\_03/tomo\_031\_bin2x.edf

# Fine alignment: general settings



# Fine alignment: global variables

The screenshot shows the 'tomo\_03 - Etomo' software window. The title bar includes 'tomo\_03 - Etomo' and window control buttons. The menu bar contains 'File', 'Tools', 'View', 'Options', and 'Help'. The main window is titled 'No process' and has a 'Kill Process' button. On the left, a vertical sidebar shows progress indicators for various steps: 'Pre-processing Complete', 'Coarse Alignment Complete', 'Fiducial Model Gen. Complete', 'Fine Alignment Complete', 'Tomogram Positioning Complete', 'Final Aligned Stack Complete', 'Tomogram Generation In Progress', 'Post-processing Complete', and 'Clean Up Not Started'. The main area is the 'Fine Alignment' dialog, which has three tabs: 'General', 'Global Variables', and 'Local Variables'. The 'Global Variables' tab is active. It contains several sections: 'Rotation Solution Type' with radio buttons for 'No rotation', 'One rotation', 'Group rotations', and 'Solve for all rotations' (selected), and a 'Rotation angle' field set to '173.0'; 'Magnification Solution Type' with radio buttons for 'Fixed magnification at 1.0', 'Group magnifications' (selected), and 'Solve for all magnifications', and a 'Group size' field set to '4'; 'Tilt Angle Solution Type' with radio buttons for 'Fixed tilt angles', 'Group tilt angles' (selected), and 'Solve for all except minimum ...', and a 'Group size' field set to '5'; 'Distortion Solution Type' with radio buttons for 'Disabled', 'Full solution' (selected), and 'Skew only', and fields for 'X stretch group size' (7) and 'Skew group size' (11); 'Beam Tilt' with radio buttons for 'No beam tilt' (selected), 'Fixed beam tilt (degrees):', and 'Solve for beam tilt'; and 'Restrict Alignment Variables' with 'Ratio of measurements to unknowns to achieve: Target' (3.6) and 'Minimum' (3.2), and a 'Restrict Variables' button. At the bottom of the dialog are buttons for 'Compute Alignment', 'View/Edit Fiducial Model', 'View 3D Model', 'View Residual Vectors', 'Cancel', 'Postpone', 'Done', and 'Advanced'.

File Tools View Options Help

tomo\_03 - Etomo

No process Kill Process

Fine Alignment

Tiltalign Parameters

General Global Variables Local Variables

Rotation Solution Type

No rotation Rotation angle: 173.0

One rotation

Group rotations Group size: 5

Solve for all rotations

Magnification Solution Type

Fixed magnification at 1.0

Group magnifications Group size: 4

Solve for all magnifications

Tilt Angle Solution Type

Fixed tilt angles

Group tilt angles Group size: 5

Solve for all except minimum ...

Distortion Solution Type

Disabled X stretch group size: 7

Full solution Skew group size: 11

Skew only

Beam Tilt B

No beam tilt

Fixed beam tilt (degrees):

Solve for beam tilt

Restrict Alignment Variables

Ratio of measurements to unknowns to achieve: Target 3.6 Minimum 3.2

Restrict Variables

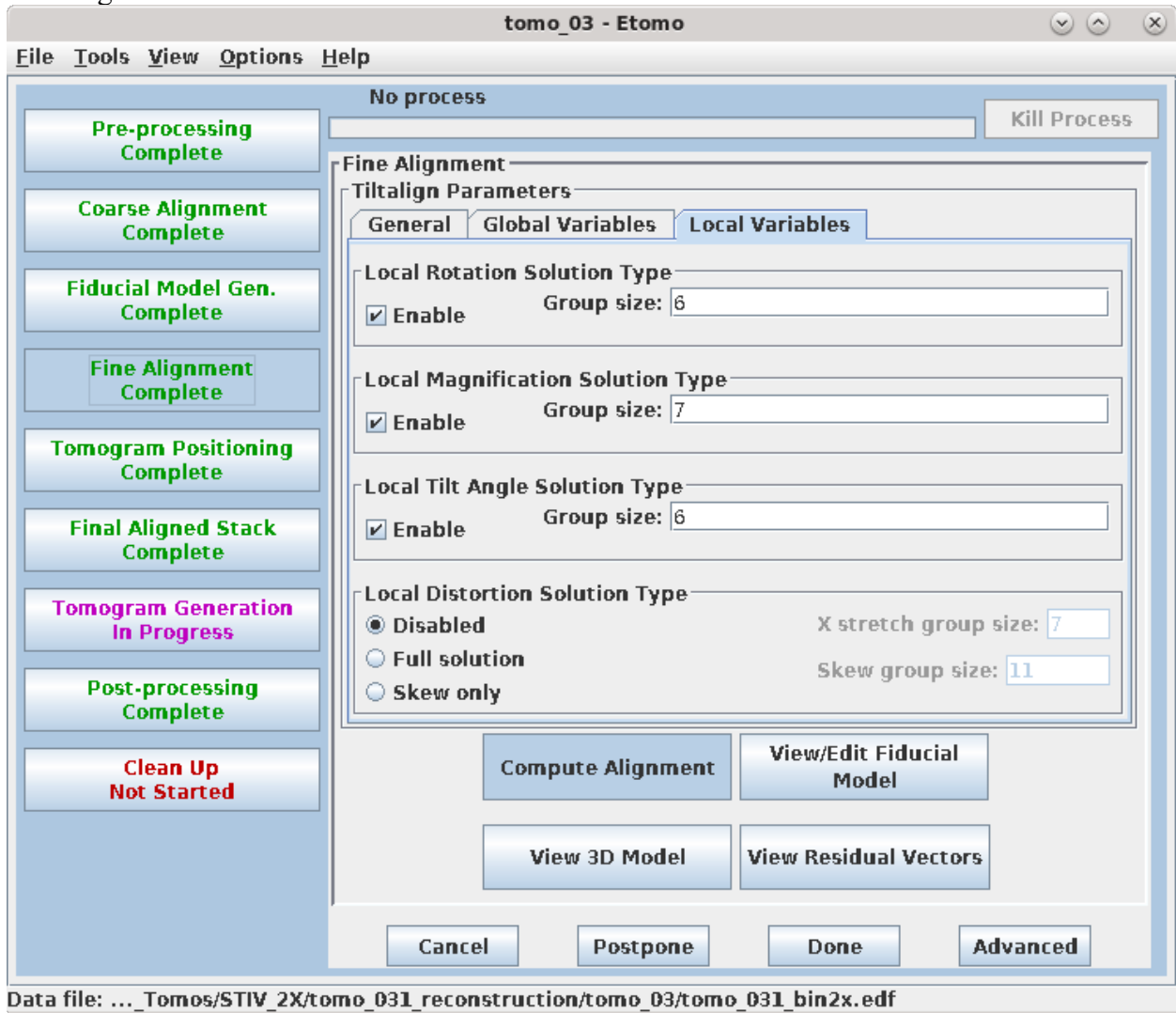
Compute Alignment View/Edit Fiducial Model

View 3D Model View Residual Vectors

Cancel Postpone Done Advanced

Data file: ...\_Tomos/STIV\_2X/tomo\_031\_reconstruction/tomo\_03/tomo\_031\_bin2x.edf

Fine alignment: local variables





# Tomogram positioning

**tomo\_03 - Etomo**

File Tools View Options Help

No process Kill Process

**Pre-processing Complete**

**Coarse Alignment Complete**

**Fiducial Model Gen. Complete**

**Fine Alignment Complete**

**Tomogram Positioning Complete**

**Final Aligned Stack Complete**

**Tomogram Generation In Progress**

**Post-processing Complete**

**Clean Up Not Started**

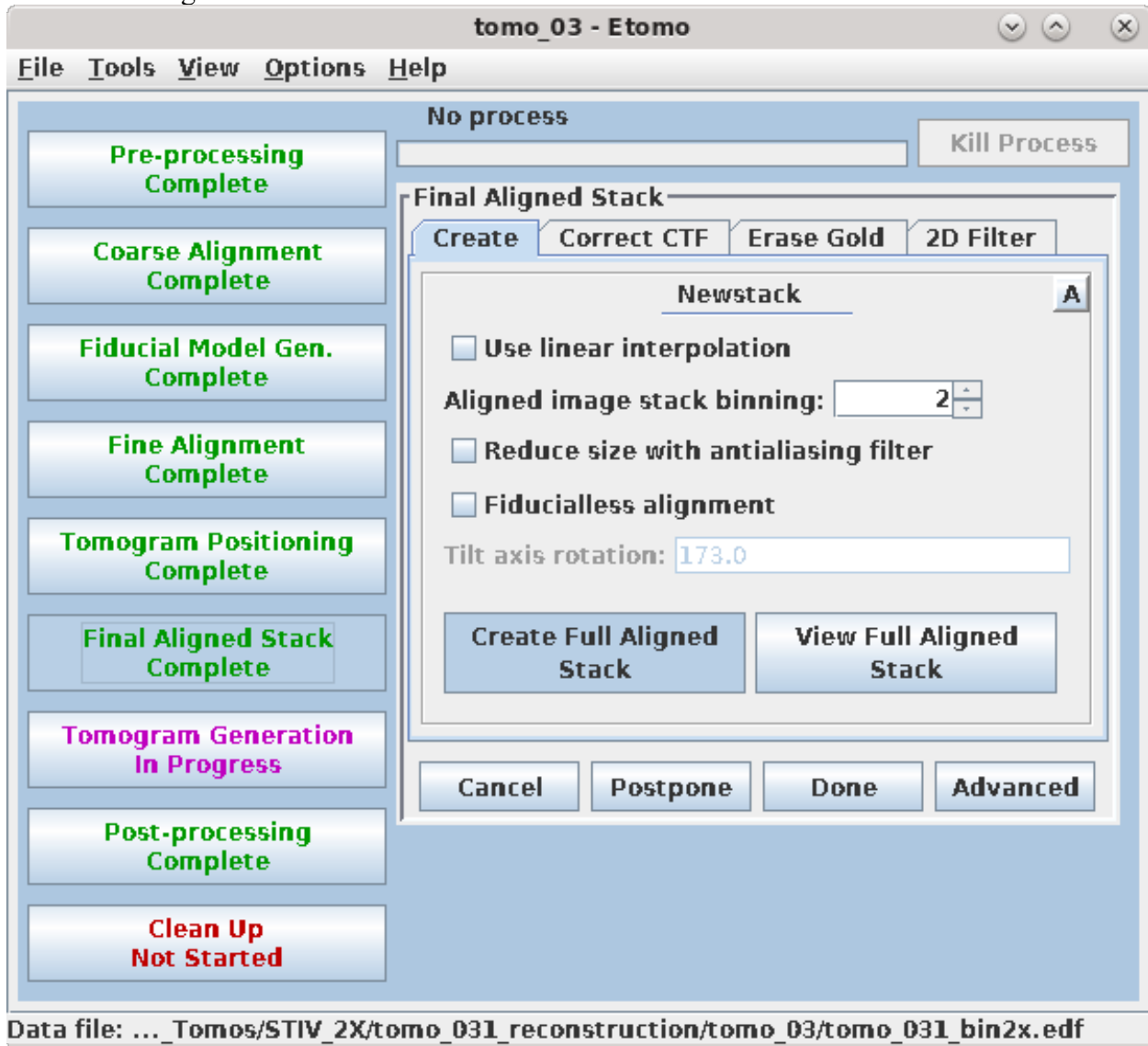
**Tomogram Positioning**

- Use the GPU
- Find boundary model automatically
- Do positioning for cryo sample
- Sample has gold beads of size:  pixels
- Positioning tomogram thickness:
- Fiducialless alignment
- Tilt axis rotation:
- Use whole tomogram Binning
- Create Whole Tomogram
- Create Boundary Model
- Added border thickness (unbinned):
- Compute Z Shift & Pitch Angles
- Final Alignment**
  - Angle offset: Total:
  - Z shift: Total:
  - Create Final Alignment
- Tilt Parameters**
  - X axis tilt: Total:
  - Final Tomogram Thickness:

Cancel Postpone Done Advanced

Data file: ...\_Tomos/STIV\_2X/tomo\_031\_reconstruction/tomo\_03/tomo\_031\_bin2x.edf

Create final aligned stack



# Erase gold beads/fiducials

The screenshot shows the Etomo software interface with the 'Final Aligned Stack' dialog box open. The 'Erase Gold' tab is selected, and the 'Bead Eraser' section is active. The 'Model Creation Method' is set to 'Use the existing fiducial model'. The 'Erase Beads' section has 'Diameter to erase (pixels)' set to 22.2 and 'Polynomial Order' set to 'Use mean of surrounding points'. The 'Iterations to grow circular areas' checkbox is unchecked and set to 2. The 'Clean Up' button is highlighted in red, indicating it has not started.

**Final Aligned Stack**

Create Correct CTF Erase Gold 2D Filter

**Bead Eraser**

**Model Creation Method**

- Use the existing fiducial model
- Use findbeads3d

Transform Fiducial Model View Transformed Model

**Erase Beads**

Diameter to erase (pixels): 22.2 Polynomial Order

- Use mean of surrounding points
- Fit a plane to surrounding points

Iterations to grow circular areas:  2

Erase Beads View Erased Stack Use Erased Stack

Cancel Postpone Done Advanced

**Final Aligned Stack Complete**

**Clean Up Not Started**

Data file: ...\_Tomos/STIV\_2X/tomo\_031\_reconstruction/tomo\_03/tomo\_031\_bin2x.edf

# Tomogram reconstruction

tomo\_03 - Etomo

File Tools View Options Help

No process Kill Process

Parallel Processing

| Computer                                      | # CPUs | Load Average |        | Restarts | Finished | Failure |
|---|--------|--------------|--------|----------|----------|---------|
|   | Used   | 1 Min.       | 5 Min. |          | Chunks   | Reason  |
| <input checked="" type="checkbox"/> localhost | 1      | 0.0          | 0.04   |          |          |         |

CPUs: 1 Restart Load Nice: 15 Pause Resume Save As Defaults

Tomogram Generation

Back Projection  SIRT

Tilt

Use the GPU: Maximum number of GPUs recommended is 3

Take logarithm of densities with offset: 0.0

Tomogram thickness in Z: 3000 Z shift: 0.0

X axis tilt: 0.0

Use local alignments

Use Z factors

Reconstruct subarea

Size in X and Y: 900,900 Offset in Y: 250

SIRT

Radial Filtering

Standard Gaussian cutoff: 0.4 Falloff (1.4 \* sigma): 0.05

Iteration #'s to retain: 5,10,15,20

Scale retained volumes to integers

Do not make vertical slice output files used for resuming

Delete existing reconstructions after starting point

Start from beginning

Resume from last iteration: 10

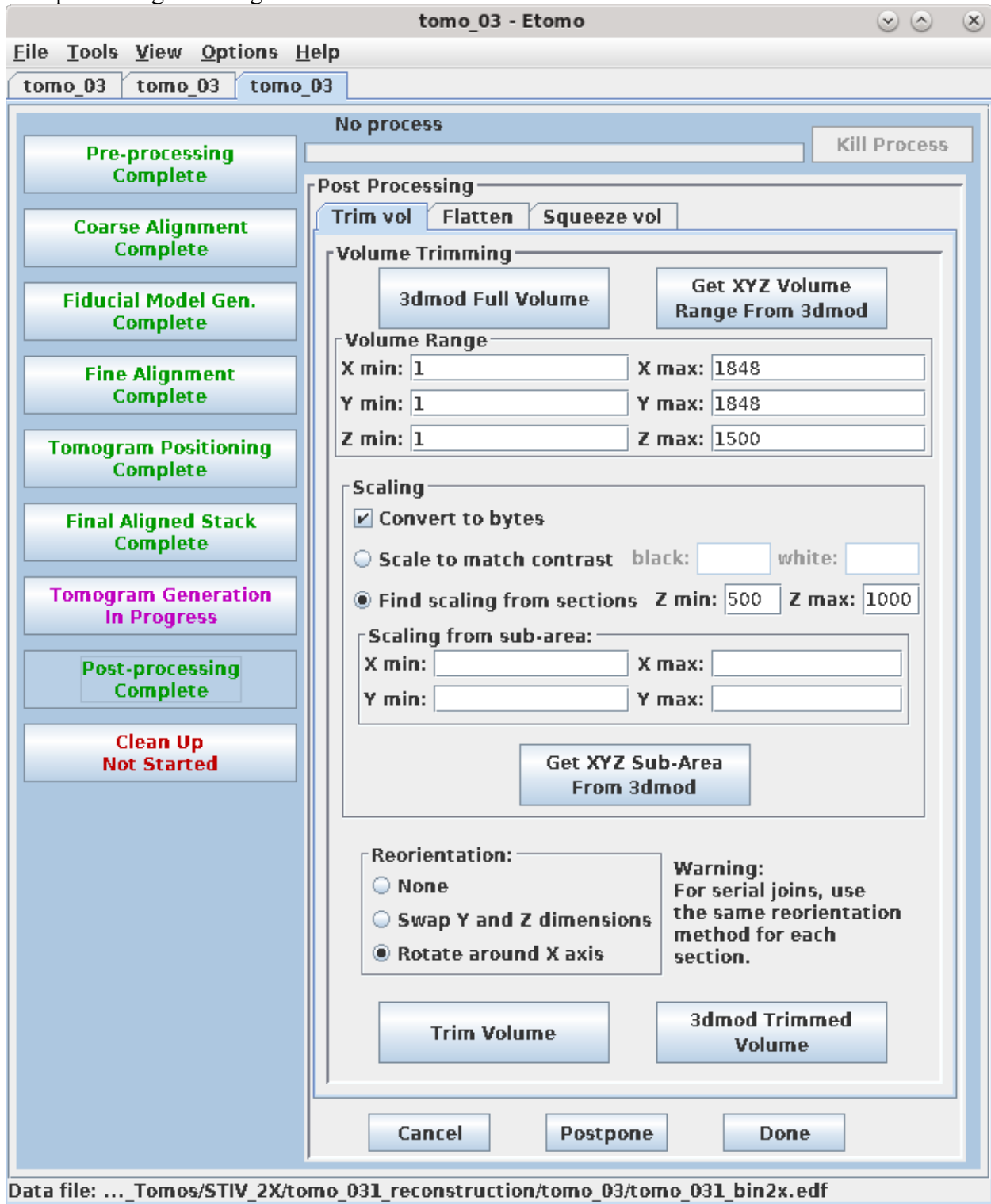
Go back, resume from iteration: 10

Run SIRT View Tomogram(s) In 3dmod Use SIRT Output File

Cancel Postpone Done Advanced

Data file: ...\_Tomos/STIV\_2X/tomo\_031\_reconstruction/tomo\_03/tomo\_031\_bin2x.edf

# Post processing of tomogram



# PEET: Setup settings

align\_tomo03\_3virus - Etomo

File Tools View Options Help

tomo\_03 tomo\_03 tomo\_03 tomo\_041 align\_tomo03\_3virus 2\_3\_4\_all\_A 2\_3\_4\_rotations

No process Kill Process

---

PEET

Setup Run

Directory: /mnt/ffd173af-e748-452d-a079-49af2cdca521/Hartman/STIV\_Tomos/STIV\_2X/tomo\_041\_reconstruction/align\_tomo\_03fxali Root name for output: align\_tomo03\_3virus

**Fix File Paths**

Files may be in separate directories

| Vol # | Volume                | Model                      | Initial | Tilt Range |       |
|-------|-----------------------|----------------------------|---------|------------|-------|
|       |                       |                            | MOTL    | Min        | Max   |
| 1     | tomo_03_S10_fxali.rec | tomo_031fxali_3viruses.mod |         | -59.95     | 59.83 |

File names are templates

Reference

Particle  In Volume:

User supplied file:

Multiparticle reference with  particles

Volume Size (Voxels)

X:  Y:  Z:

Missing Wedge Compensation

Enabled Edge shift:  Weight groups:

Number of Tilt Axes:  1  2 or more

Masking

None

Sphere

Cylinder Height:

User supplied binary file:

Inner radius:  Outer radius:

Blur mask by:

Manual Cylinder Orientation

Z Rotation:  Y Rotation:

Particle Y Axis

Tomogram Y axis

Particle model points

End points of contour

User supplied csv files

Initial Motive List

Set all angles to 0

Align particle Y axes

User supplied csv files

Uniform random rotations

Random axial (Y) rotations

Data file: ...omo\_041\_reconstruction/align\_tomo\_03fxali/align\_tomo03\_3virus.epe

PEET: Run settings

align\_tomo03\_3virus - Etomo

File Tools View Options Help

tomo\_03 tomo\_03 tomo\_03 tomo\_041 align\_tomo03\_3virus 2\_3\_4\_all\_A 2\_3\_4\_rotations

No process Kill Process

Parallel Processing

| Computer                                      | # CPUs | Load Average |               | Restarts | Finished | Failure |
|---|--------|--------------|---------------|----------|----------|---------|
|   |        | Used         | 1 Min. 5 Min. |          |          |         |
| <input checked="" type="checkbox"/> localhost | 1      | 0.2          | 0.11          |          |          |         |

CPUs: 1 Restart Load Nice: 18 Pause Resume Save As Defaults

---

PEET

Setup **Run**

Iteration Table

| Run # | Angular Search Range |      |       |      |      |      | Search Distance | Hi Freq |       | Ref Threshold | Duplicate |       |  |
|-------|----------------------|------|-------|------|------|------|-----------------|---------|-------|---------------|-----------|-------|--|
|       | Phi                  |      | Theta |      | Psi  |      |                 | Filter  |       |               | Shift     | Angle |  |
|       | Max                  | Step | Max   | Step | Max  | Step |                 | Cutoff  | Sigma |               |           |       |  |
| 1     | =>                   | 0.0  | 1.0   | 0.0  | 1.0  | 0.0  | 1.0             | 10      | 0.1   | 0.05          | 3.0       |       |  |
| 2     | =>                   | 36.0 | 10.0  | 36.0 | 10.0 | 36.0 | 10.0            | 2       | 0.25  | 0.05          | 3.0       |       |  |
| 3     | =>                   | 15.0 | 5.0   | 15.0 | 5.0  | 15.0 | 5.0             | 2       | 0.35  | 0.05          | 3.0       |       |  |
| 4     | =>                   | 6.0  | 2.0   | 6.0  | 2.0  | 6.0  | 2.0             | 2       | 0.4   | 0.05          | 3.0       |       |  |
| 5     | =>                   | 3.0  | 1.0   | 3.0  | 1.0  | 3.0  | 1.0             | 2       | 0.5   | 0.05          | 3.0       |       |  |

Remove duplicate particles after each iteration

Spherical Sampling for Theta and Psi

None  Full sphere  Half sphere Sample interval (degrees): 36.0

Number of Particles to Average

Start: 1 Incr.: 1 End: 4 Additional numbers:

Use Equal Numbers of Particles from All Tomograms

For average volumes  For new references

Optional / Advanced Features

Align averages to have their Y axes vertical Particles per CPU: 1

Use absolute value of cross-correlation Debug level: 3

Save individual aligned particles Low frequency cutoff: 0.0 Sigma: 0.05

Strict search limit checking Average only members of classes:

No reference refinement

Randomized particle selection

Run Open averages in 3dmod Open references in 3dmod Remake Averages

Data file: ...omo\_041\_reconstruction/align\_tomo\_03fxali/align\_tomo03\_3virus.epe