

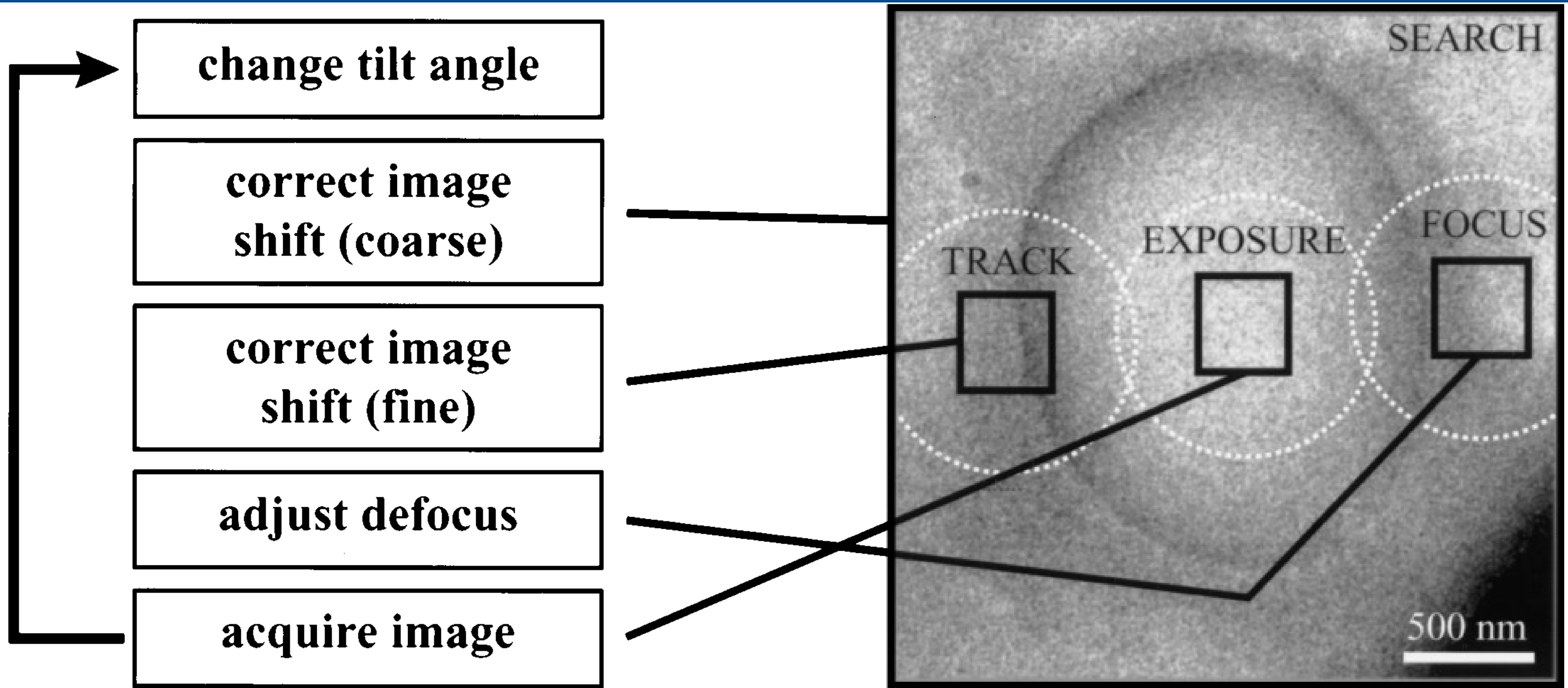


Cryo-ET data collection and reconstruction demonstration

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Cryo-ET data collection



Many cryo-ET data acquisition packages

SerialEM

UCSF Tomo

Leginon

FEL tomography

EM-Manu

SerialEM

Automated electron microscope tomography using robust
prediction of specimen movements

2005

David N. Mastronarde *

*Boulder Laboratory for Three-Dimensional Electron Microscopy of Cells, Department of Molecular, Cellular, and Developmental Biology,
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*SerialEM provides a flexible interface.
The script capability provides a relatively easy
way to add commands requested by users.*

SerialEM (David Mastronarde)

The image shows the SerialEM software interface. The central window displays a grayscale microscopy image of a sample. Overlaid on this image are three red rounded rectangles containing text labels:

- Top Left:** Defocus, Magnification, Stage tilt angle
- Bottom Left:** View, Focus, Trial, Record
- Bottom Right:** Setup, Preview

The interface includes several panels:

- Left Panel:** Buffer Status, Buffer Controls, Image Display Controls, Microscope, Tilt Control, and Camera & Macro Controls.
- Right Panel:** Low Dose Control, K2 Direct Detection, Camera View, and Navigator.

The Microscope panel shows parameters: 0.0355 nA, 2300X, Def -40.00 um, IS 0.00 um, Obj 86.41%, VAC, Spot 6. The Tilt Control panel shows 0.00. The Camera & Macro Controls panel has buttons for Setup, View, Focus, Trial, Record, Preview, End, Resume, and STOP. The Low Dose Control panel has checkboxes for Low Dose Mode, Continuous update of mag & beam, and Define position of area. The K2 Direct Detection panel has a Mode dropdown set to Counted and checkboxes for Background Subtraction and Gain Correction. The Camera View panel has a Setup dropdown set to Search and checkboxes for Auto Exposure, Focus Loupe, Auto Survey, and Camera Inserted. The Navigator panel shows a list of items with columns for Label, Color, X, Y, Z, Type, Reg, Acq, and No.

SerialEM – Navigator

File Settings Camera Calibration Focus Macro Tasks Tilt Series Process Navigator Window Help

Buffer Status
E: Montage Overview
Size: 2116 x 2136 bin 8 Tilt
Stage: -325.25, -232.62 Def: -40.00
A: Montage Center
B: Montage Overview

Buffer Controls
Copy Active Image to Buffer
A B C D E New
SAVE A Save Active To 1
Options Memory = 15 MB
Roll Buffers A -> C Delete
Copy on Save to D
Align to B instead of D/E
Read into Buffer E
Protect unsaved Record images
Align on Save

Image Display Controls
Blk 7
Wht 252
Bri
Con 0.47
Zoom

Microscope
0.0355 nA 2300X
Def -40.00 um IS 0.00 um
Obj 86.41% VAC Spot 6

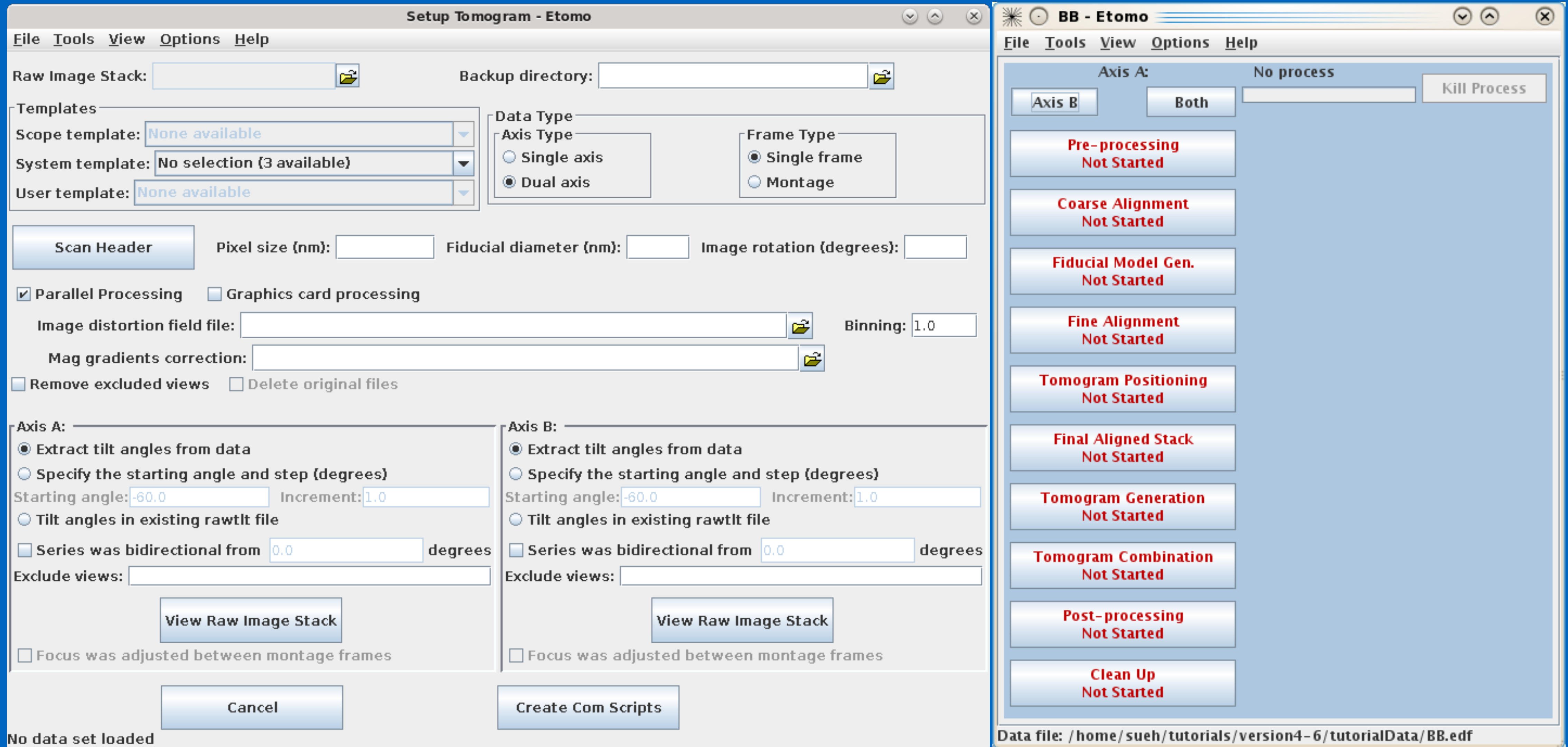
Tilt Control
Tilt
0.00 Up Down To

Camera & Macro Controls
Setup View Focus Trial Record
Preview End Resume STOP
TargetDel TargetDel Macro 3

Navigator
Label: 1 Registration point 1 Corner point (C)
Color Blue Draw Rotate when load For anchor state
#1 Note: Sec 0 - montage01.st
Acquire (A) Tilt series New file at item New file at group
Set File Properties Imaging State TS Parameters Filename
Acquire map or image or run macro at this location automatically
Add Stage Pos Registration 1 Draw all reg. Draw none
Add Points Collapse groups Show Acquire area
Add Polygon Label Color X Y Z Type Reg. Acq. Not
Add Marker 1 Blu -325.2 -232.6 118.7 Map 1
Move Item
Update Z
Go To XY
Go To XYZ
Go To Marker
Load Map
New Map
Anchor Map
Delete Item
Realign to Item

SerialEM Demo

Aligning tilt series (IMOD)



Aligning tilt seires (AreTomo)

AreTomo -InMrc original_tilt.mrc -OutMrc aligned_tilt.mrc

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AreTomo: An integrated software package for automated
marker-free, motion-corrected cryo-electron tomographic
alignment and reconstruction

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Thanks

- **Any questions?**