

SerialEM for Cryo Tomography

Types of samples

- Very small whole cells (bacteria, some algae)
- FIB milled lamellae
- Vitreous sections
- Isolated organelles (mitochondria, microtubules)
- Viruses and virus-like-particles
- Individual proteins

Setting up Low Dose

- Remember your record image is the most important and all other images should 'revolve' around that image
- When changing exposure times, make sure ALL images have 0 drift settling!!!
- Maximize the Low Dose Control window, it may be helpful to float it and put it on a second monitor
- Check 'Low Dose Mode'

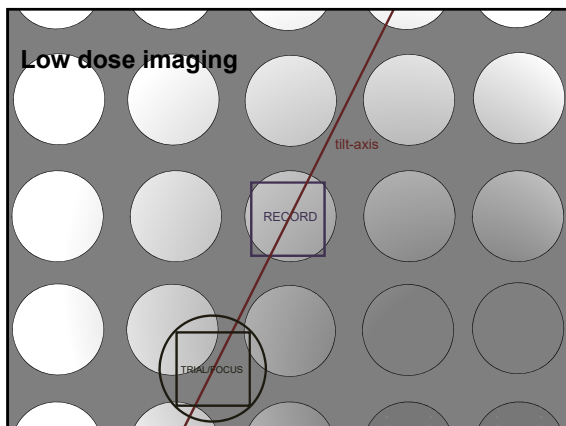


Image types for Cryo

- Record: Final image that goes into tilt-series
 - Try to keep dose around 1 e/A2
- Trial/Focus: Used for tasks and tracking during tilt-series and for autofocus
 - Make beam just bigger than the area of the camera
 - Can be higher dose than record
- View: Lower mag search mode
 - Use a mag that allows you to see several holes at a time
 - Can add some defocus to see particles
 - Make extremely low dose <<<1 e/A2
- Preview: Exact area/mag/C2%/spot size as record, but binned x4 or x8
 - Used to 'see' the record area, but with really low dose
 - Use exposure time and binning to lower dose but have decent image
 - Check dose in Camera Parameters dialogue box

Low Dose Control

Low Dose Mode 2.12 e/A2

Record: 3300x Sp 0 C2 36.72%

Continuous update (see tooltip)

Define position of area

None Focus Trial

Position on tilt axis: 0.00 um

Additional beam shift (and DF tilt)

Set Reset 0.00, -0.00

Go to area / show when screen down

Wa. Foc. Tr. Rec. Sca.

BLANK BEAM when screen down

Blanked Unblank

Offsets for View

Defocus: -100

Normalize beam through View

Keep Focus and Trial identical

Copy current area mag & beam to

Rotate interference rows

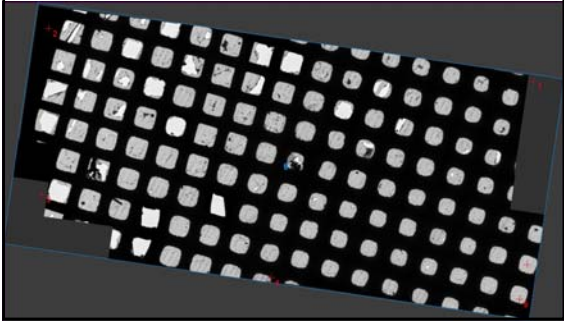
This allows you to change the beam for each image type

Lets you correct beam position if it is not centered the same in different modes (or offset it from the center away from the record area in focus/trial)

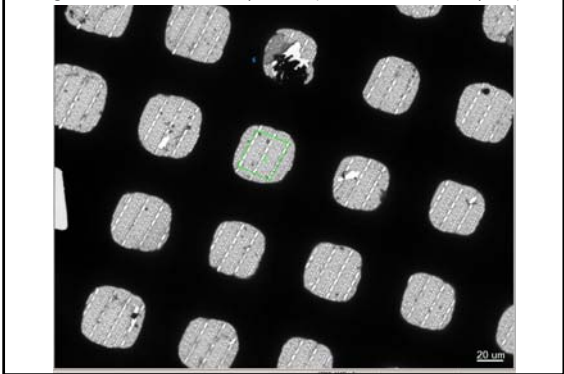
Lets you put a high defocus in view mode so you can see small particles in the holes

Typically focus and trial are identical unless you are at very high mag, where it could be advantageous to have a lower tracking mag

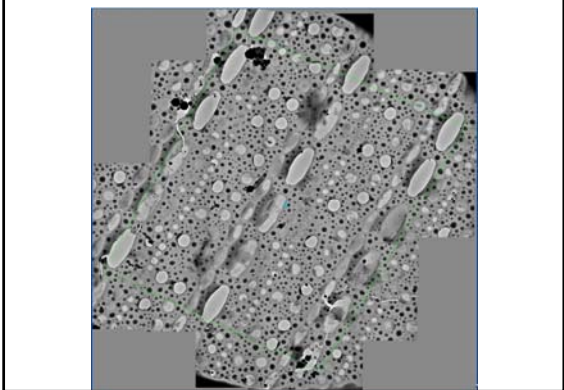
Generate a map of the grid where a tilt-series is possible. Red points are 'corners' of stage positions to tell SerialEM where to collect the map



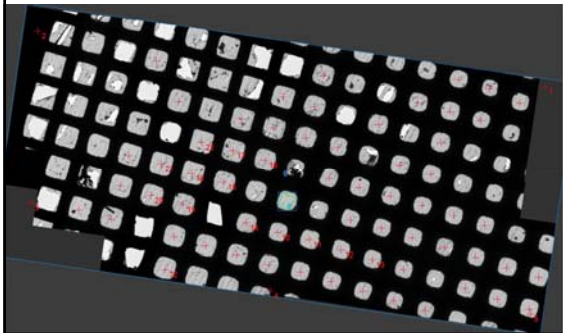
Zoom in and place a polygon within a grid square—best to size it to fit the region where a tilt-series is possible (basic center of the square)



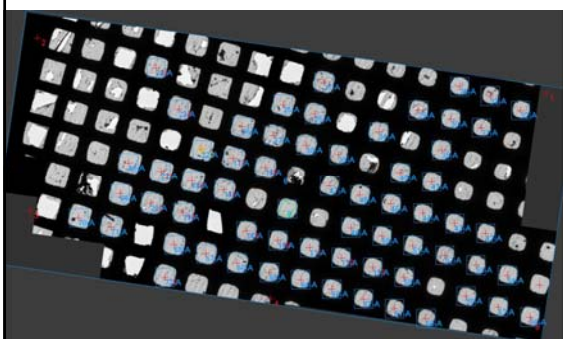
Completed polygon map for the grid square



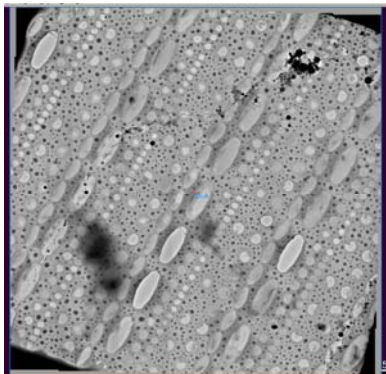
Mark points in the center of each grid square of interest and use Navigator to automatically acquire polygon maps around those points

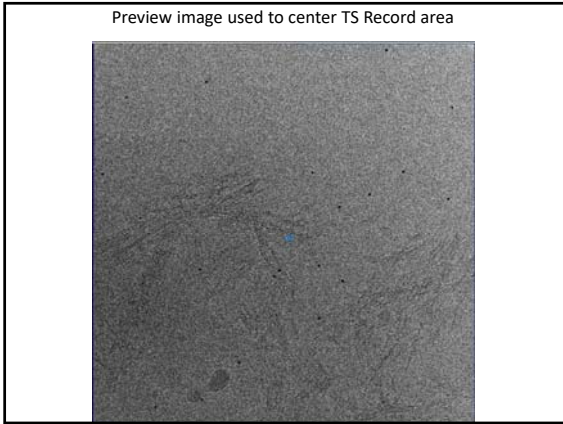


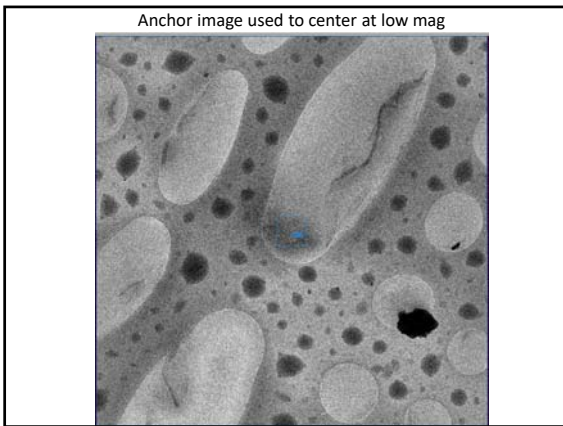
All blue squares are now maps that were acquired automatically with the same settings as the original green polygon

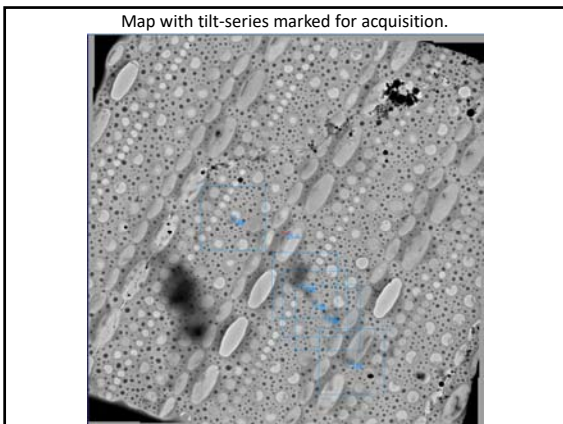


Example of automatically acquired map



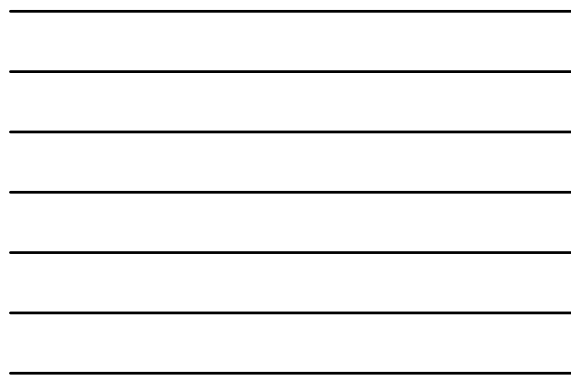






List of tilt-series to acquire overnight

ID	Name	Start Time	End Time	Other Parameters
1	Shu 575.8 -202.4	8.8 Mag 1	Anchor -Sec 3-127-TS mag	
2	Shu 575.8 -202.4	8.8 Mag 1	Sec 4-127-TS mag	
3	Shu 575.8 -202.4	8.8 Mag 1	Anchor -Sec 5-127-TS mag	
4	Shu 575.8 -202.4	8.8 Mag 1	Sec 6-127-TS mag	
5	Shu 583.1 -202.1	8.8 Mag 1	Anchor -Sec 7-127-TS mag	
6	Shu 583.1 -202.1	8.8 Mag 1	Sec 8-127-TS mag	
7	Shu 583.1 -202.1	8.8 Mag 1	Anchor -Sec 9-127-TS mag	
8	Shu 583.1 -202.1	8.8 Mag 1	Sec 10-127-TS mag	
9	Shu 583.1 -202.1	8.8 Mag 1	Anchor -Sec 11-127-TS mag	
10	Shu 583.1 -202.1	8.8 Mag 1	Sec 12-127-TS mag	
11	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 13-127-TS mag	
12	Shu 452.8 -219.8	8.8 Mag 1	Sec 14-127-TS mag	
13	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 15-127-TS mag	
14	Shu 452.8 -219.8	8.8 Mag 1	Sec 16-127-TS mag	
15	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 17-127-TS mag	
16	Shu 452.8 -219.8	8.8 Mag 1	Sec 18-127-TS mag	
17	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 19-127-TS mag	
18	Shu 452.8 -219.8	8.8 Mag 1	Sec 20-127-TS mag	
19	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 21-127-TS mag	
20	Shu 452.8 -219.8	8.8 Mag 1	Sec 22-127-TS mag	
21	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 23-127-TS mag	
22	Shu 452.8 -219.8	8.8 Mag 1	Sec 24-127-TS mag	
23	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 25-127-TS mag	
24	Shu 452.8 -219.8	8.8 Mag 1	Sec 26-127-TS mag	
25	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 27-127-TS mag	
26	Shu 452.8 -219.8	8.8 Mag 1	Sec 28-127-TS mag	
27	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 29-127-TS mag	
28	Shu 452.8 -219.8	8.8 Mag 1	Sec 30-127-TS mag	
29	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 31-127-TS mag	
30	Shu 452.8 -219.8	8.8 Mag 1	Sec 32-127-TS mag	
31	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 33-127-TS mag	
32	Shu 452.8 -219.8	8.8 Mag 1	Sec 34-127-TS mag	
33	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 35-127-TS mag	
34	Shu 452.8 -219.8	8.8 Mag 1	Sec 36-127-TS mag	
35	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 37-127-TS mag	
36	Shu 452.8 -219.8	8.8 Mag 1	Sec 38-127-TS mag	
37	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 39-127-TS mag	
38	Shu 452.8 -219.8	8.8 Mag 1	Sec 40-127-TS mag	
39	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 41-127-TS mag	
40	Shu 452.8 -219.8	8.8 Mag 1	Sec 42-127-TS mag	
41	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 43-127-TS mag	
42	Shu 452.8 -219.8	8.8 Mag 1	Sec 44-127-TS mag	
43	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 45-127-TS mag	
44	Shu 452.8 -219.8	8.8 Mag 1	Sec 46-127-TS mag	
45	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 47-127-TS mag	
46	Shu 452.8 -219.8	8.8 Mag 1	Sec 48-127-TS mag	
47	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 49-127-TS mag	
48	Shu 452.8 -219.8	8.8 Mag 1	Sec 50-127-TS mag	
49	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 51-127-TS mag	
50	Shu 452.8 -219.8	8.8 Mag 1	Sec 52-127-TS mag	
51	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 53-127-TS mag	
52	Shu 452.8 -219.8	8.8 Mag 1	Sec 54-127-TS mag	
53	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 55-127-TS mag	
54	Shu 452.8 -219.8	8.8 Mag 1	Sec 56-127-TS mag	
55	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 57-127-TS mag	
56	Shu 452.8 -219.8	8.8 Mag 1	Sec 58-127-TS mag	
57	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 59-127-TS mag	
58	Shu 452.8 -219.8	8.8 Mag 1	Sec 60-127-TS mag	
59	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 61-127-TS mag	
60	Shu 452.8 -219.8	8.8 Mag 1	Sec 62-127-TS mag	
61	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 63-127-TS mag	
62	Shu 452.8 -219.8	8.8 Mag 1	Sec 64-127-TS mag	
63	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 65-127-TS mag	
64	Shu 452.8 -219.8	8.8 Mag 1	Sec 66-127-TS mag	
65	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 67-127-TS mag	
66	Shu 452.8 -219.8	8.8 Mag 1	Sec 68-127-TS mag	
67	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 69-127-TS mag	
68	Shu 452.8 -219.8	8.8 Mag 1	Sec 70-127-TS mag	
69	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 71-127-TS mag	
70	Shu 452.8 -219.8	8.8 Mag 1	Sec 72-127-TS mag	
71	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 73-127-TS mag	
72	Shu 452.8 -219.8	8.8 Mag 1	Sec 74-127-TS mag	
73	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 75-127-TS mag	
74	Shu 452.8 -219.8	8.8 Mag 1	Sec 76-127-TS mag	
75	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 77-127-TS mag	
76	Shu 452.8 -219.8	8.8 Mag 1	Sec 78-127-TS mag	
77	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 79-127-TS mag	
78	Shu 452.8 -219.8	8.8 Mag 1	Sec 80-127-TS mag	
79	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 81-127-TS mag	
80	Shu 452.8 -219.8	8.8 Mag 1	Sec 82-127-TS mag	
81	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 83-127-TS mag	
82	Shu 452.8 -219.8	8.8 Mag 1	Sec 84-127-TS mag	
83	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 85-127-TS mag	
84	Shu 452.8 -219.8	8.8 Mag 1	Sec 86-127-TS mag	
85	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 87-127-TS mag	
86	Shu 452.8 -219.8	8.8 Mag 1	Sec 88-127-TS mag	
87	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 89-127-TS mag	
88	Shu 452.8 -219.8	8.8 Mag 1	Sec 90-127-TS mag	
89	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 91-127-TS mag	
90	Shu 452.8 -219.8	8.8 Mag 1	Sec 92-127-TS mag	
91	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 93-127-TS mag	
92	Shu 452.8 -219.8	8.8 Mag 1	Sec 94-127-TS mag	
93	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 95-127-TS mag	
94	Shu 452.8 -219.8	8.8 Mag 1	Sec 96-127-TS mag	
95	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 97-127-TS mag	
96	Shu 452.8 -219.8	8.8 Mag 1	Sec 98-127-TS mag	
97	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 99-127-TS mag	
98	Shu 452.8 -219.8	8.8 Mag 1	Sec 100-127-TS mag	
99	Shu 452.8 -219.8	8.8 Mag 1	Anchor -Sec 101-127-TS mag	
100	Shu 452.8 -219.8	8.8 Mag 1	Sec 102-127-TS mag	



Tilt-series dialog

The dialog box is titled "Tilt-series dialog" and is divided into several sections:

- Tilt Angle Specifications:** Includes fields for "Tilt angle" (set to 0.0), "Start time" (set to 0.0), and "End time" (set to 0.0). There are checkboxes for "Placement proportional to cosine of angle" and "Full series when direction less than 1.0 degrees".
- Camera and Acquisition Parameters:** Includes fields for "Average period" (set to 0.0 seconds) and "Subtraction control" (set to "None").
- Tracking Control Parameters:** Includes checkboxes for "Track", "Align with tracking images", and "Align with tracking images when error in N-V prediction is less than 1.0 degrees".

