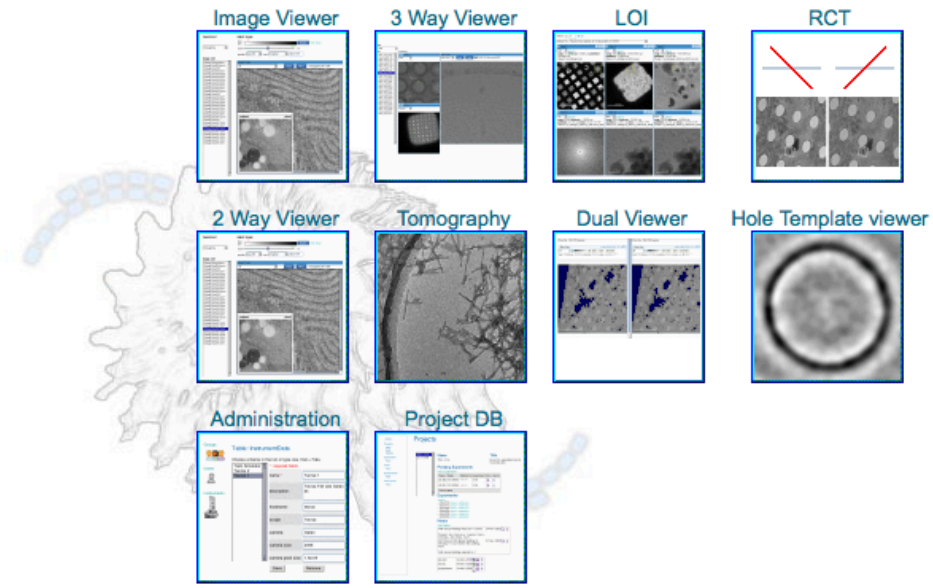
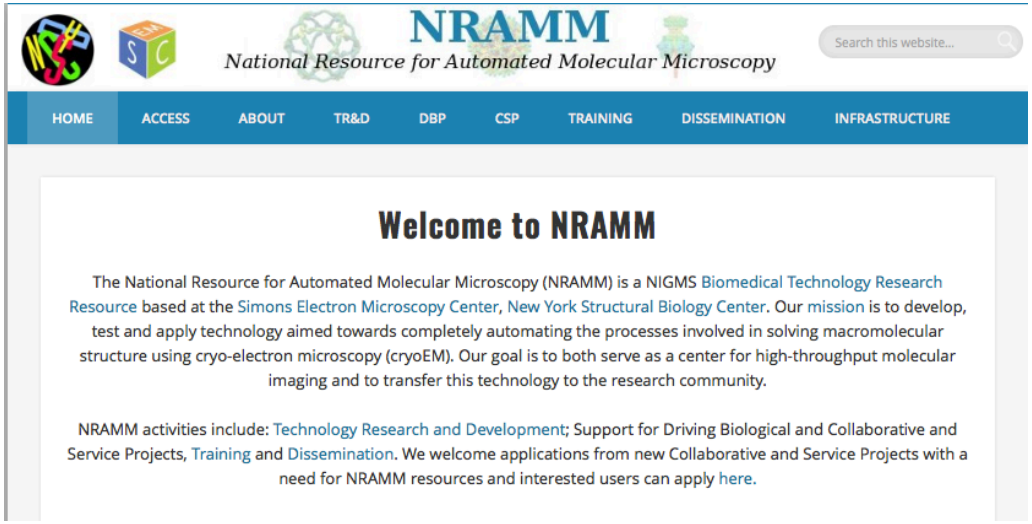
Appion and Leginon DB Tools

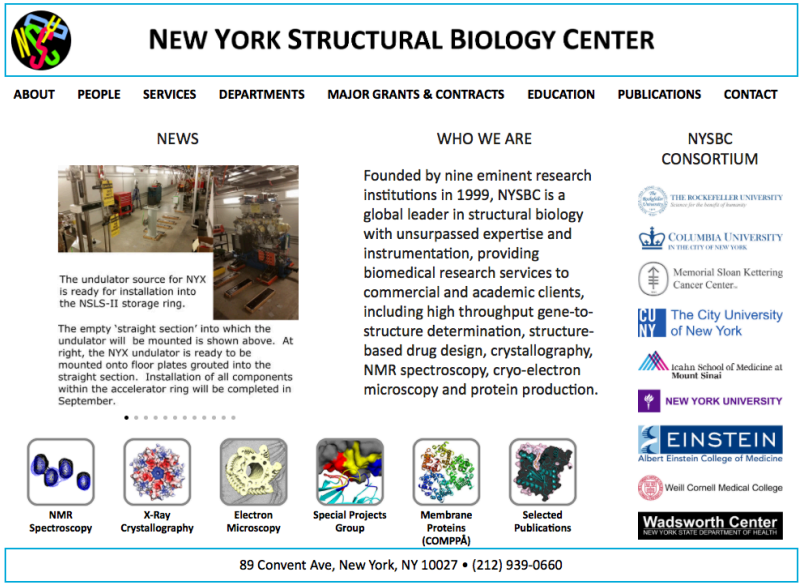


Introduction to Appion and Leginon tools

Chi-yu Fu (ICOB)

NRAMM/AMI at New York Structural Biology Center

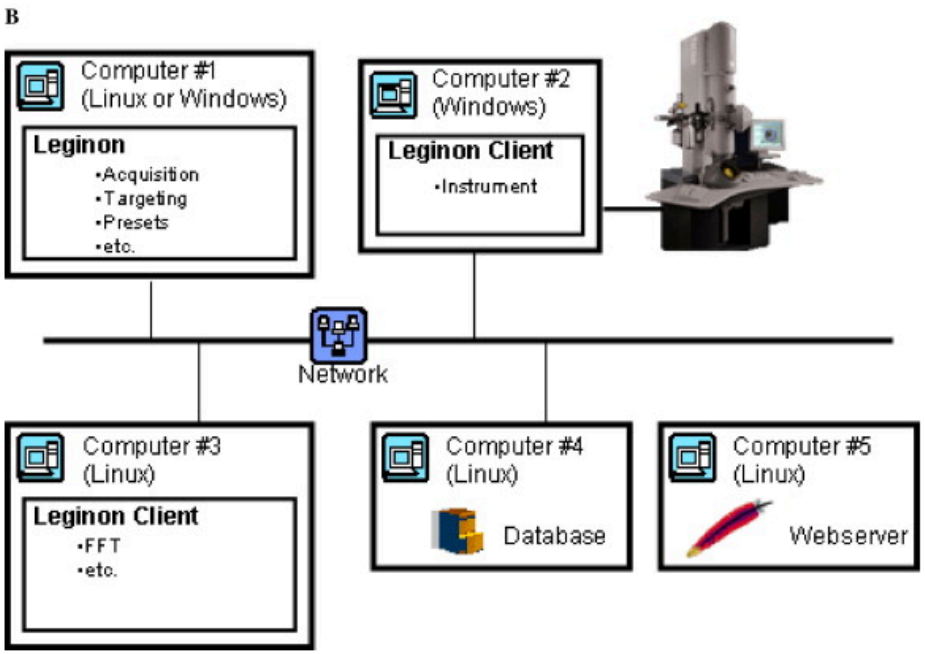


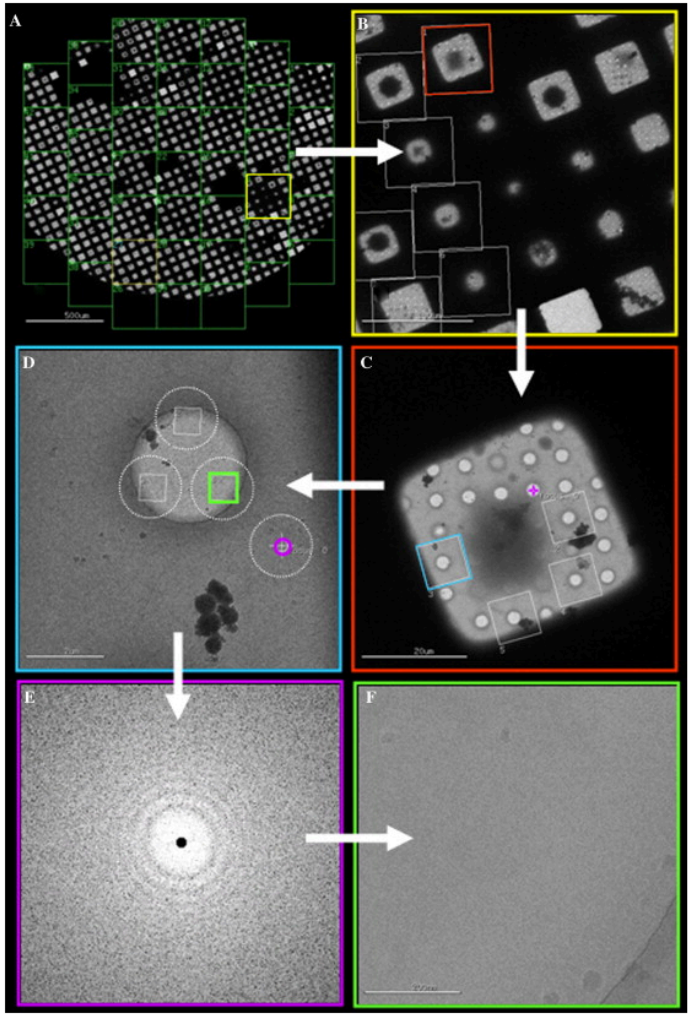


http://emg.nysbc.org/redmine/projects/leginon/wiki/Leginon_Homepage

http://emg.nysbc.org/redmine/projects/appion/wiki/Appion_Home

Leginon/Appion pipeline





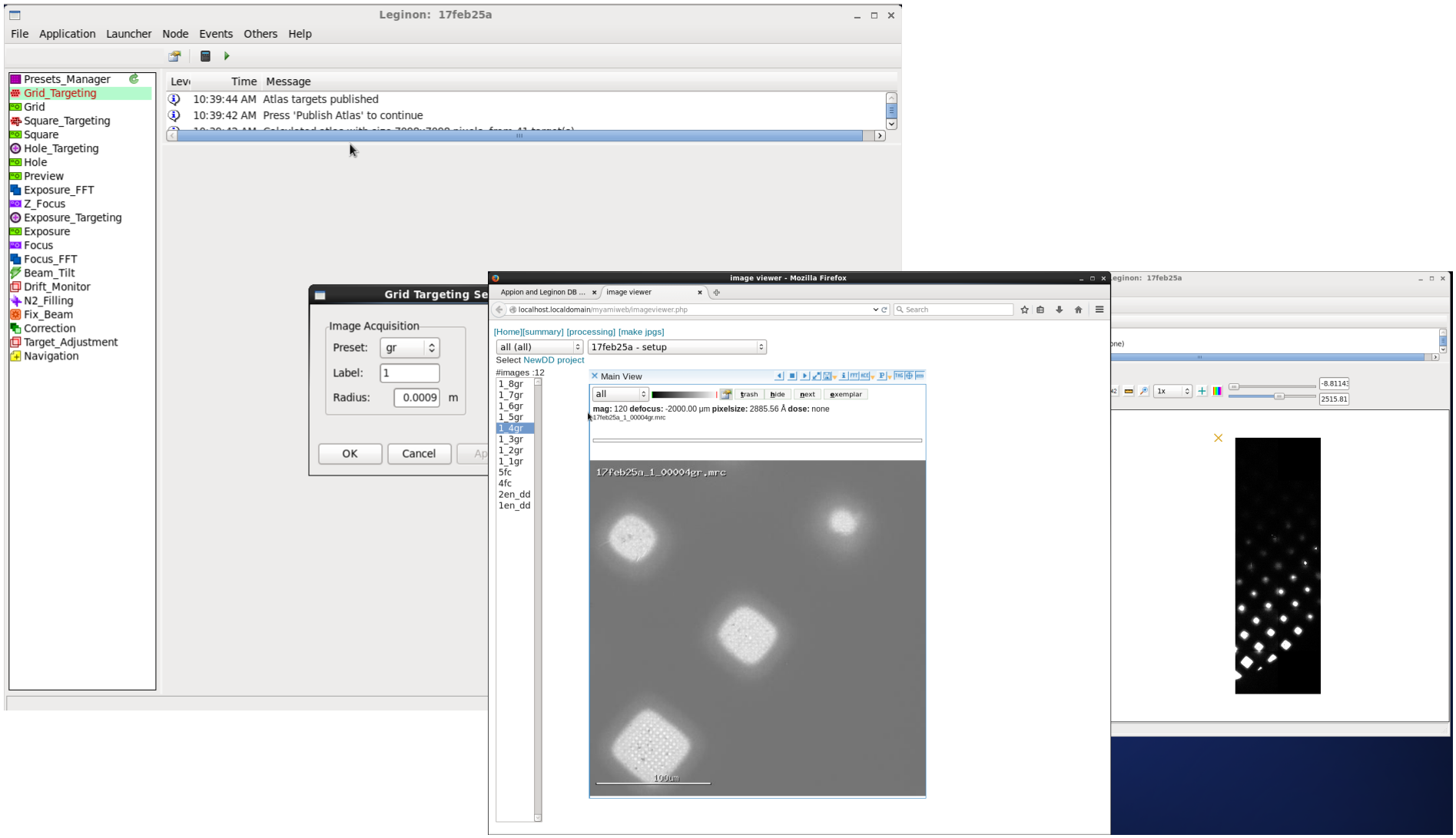
Installed in cryoEM core

Special thanks to

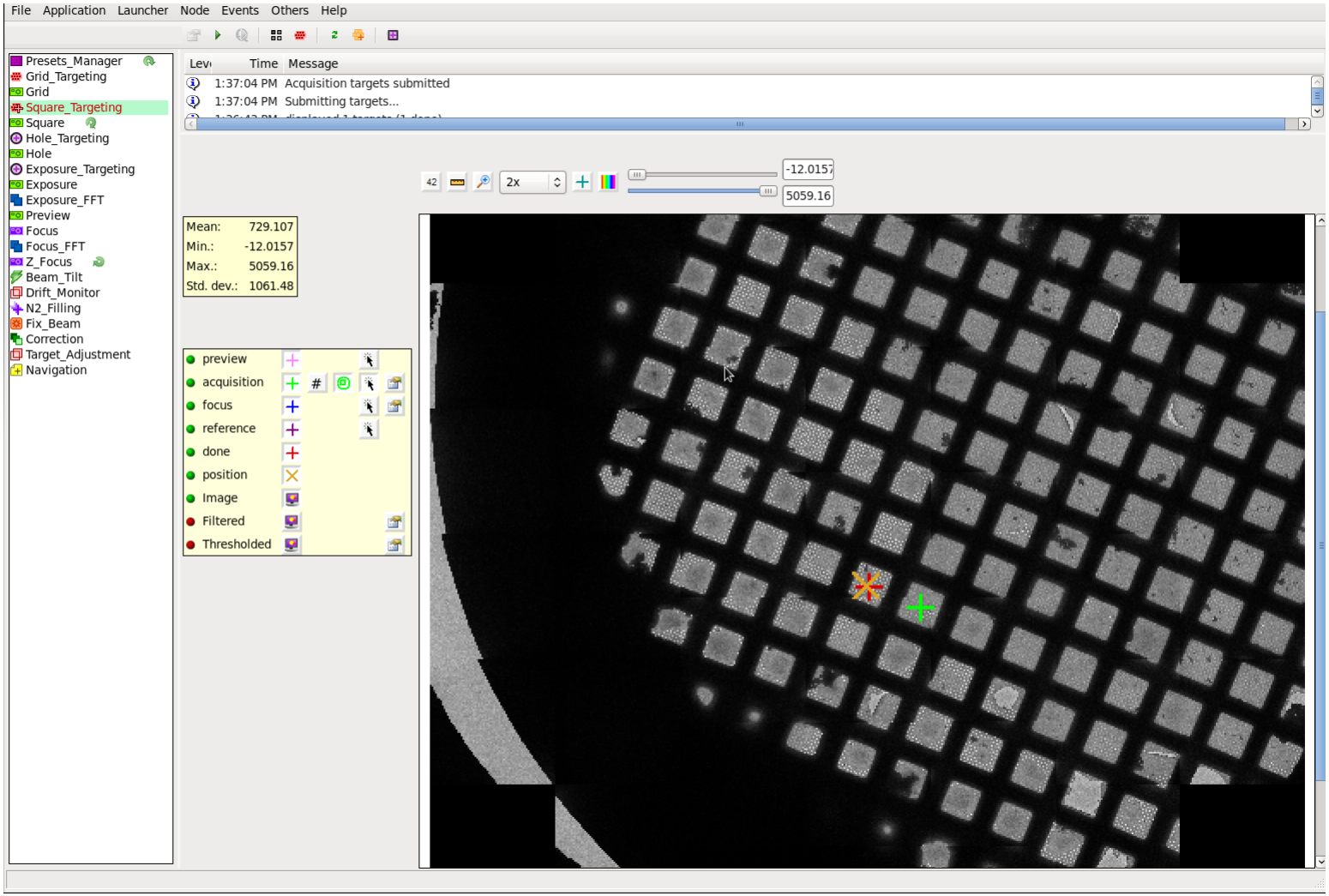
Anchi Cheng (AMI)

Shin-bo Cheng (ICOB computer center)

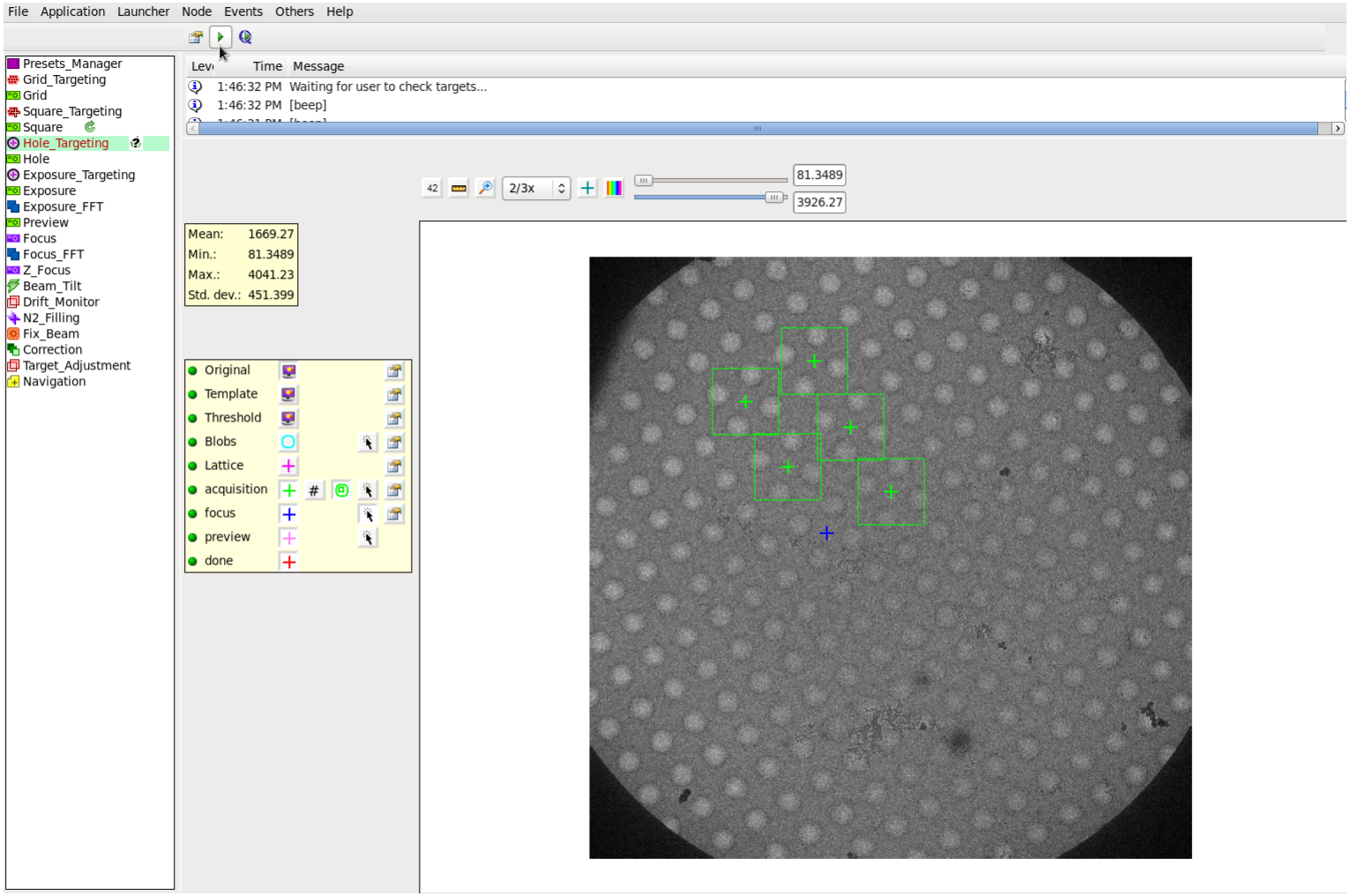
Atlas collection



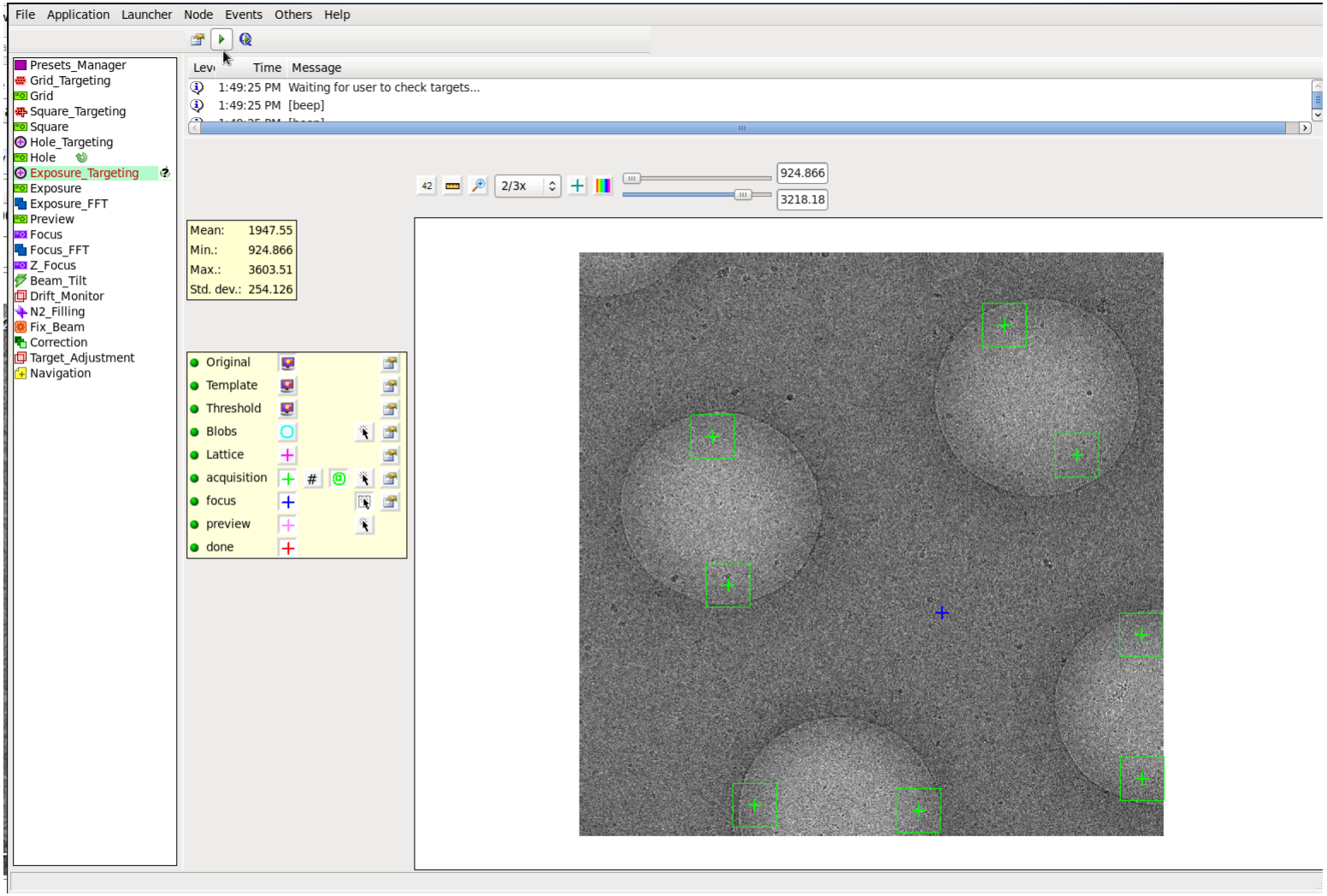
Square targeting



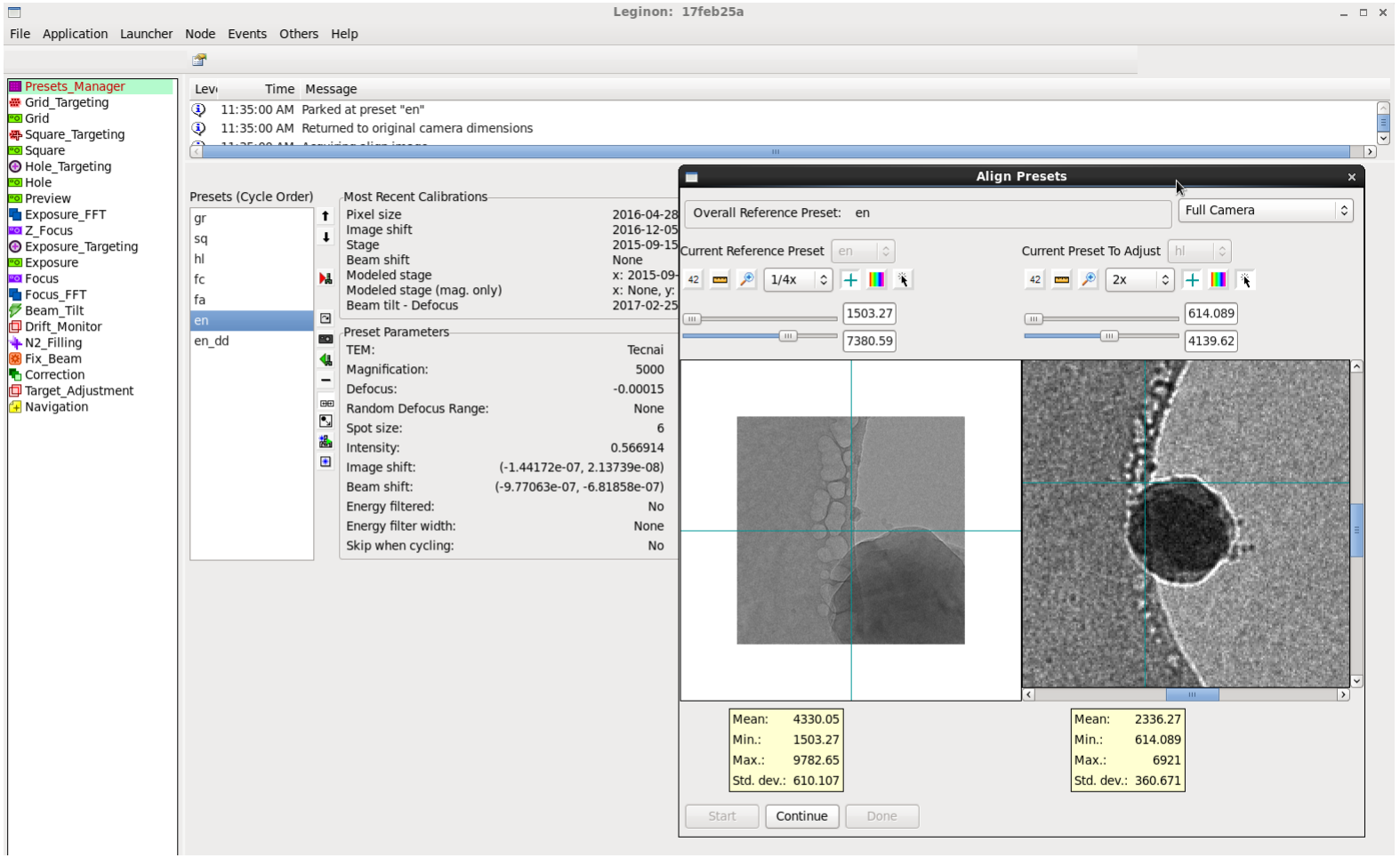
Hole targeting



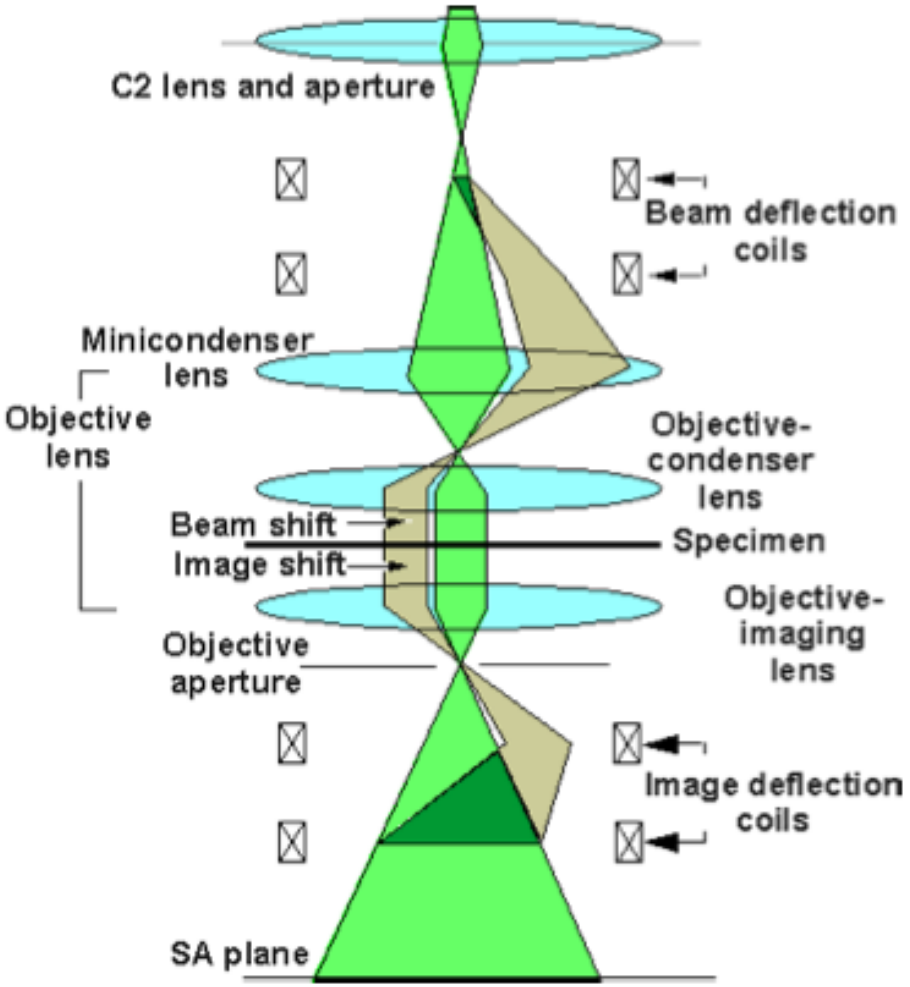
Exposure targeting



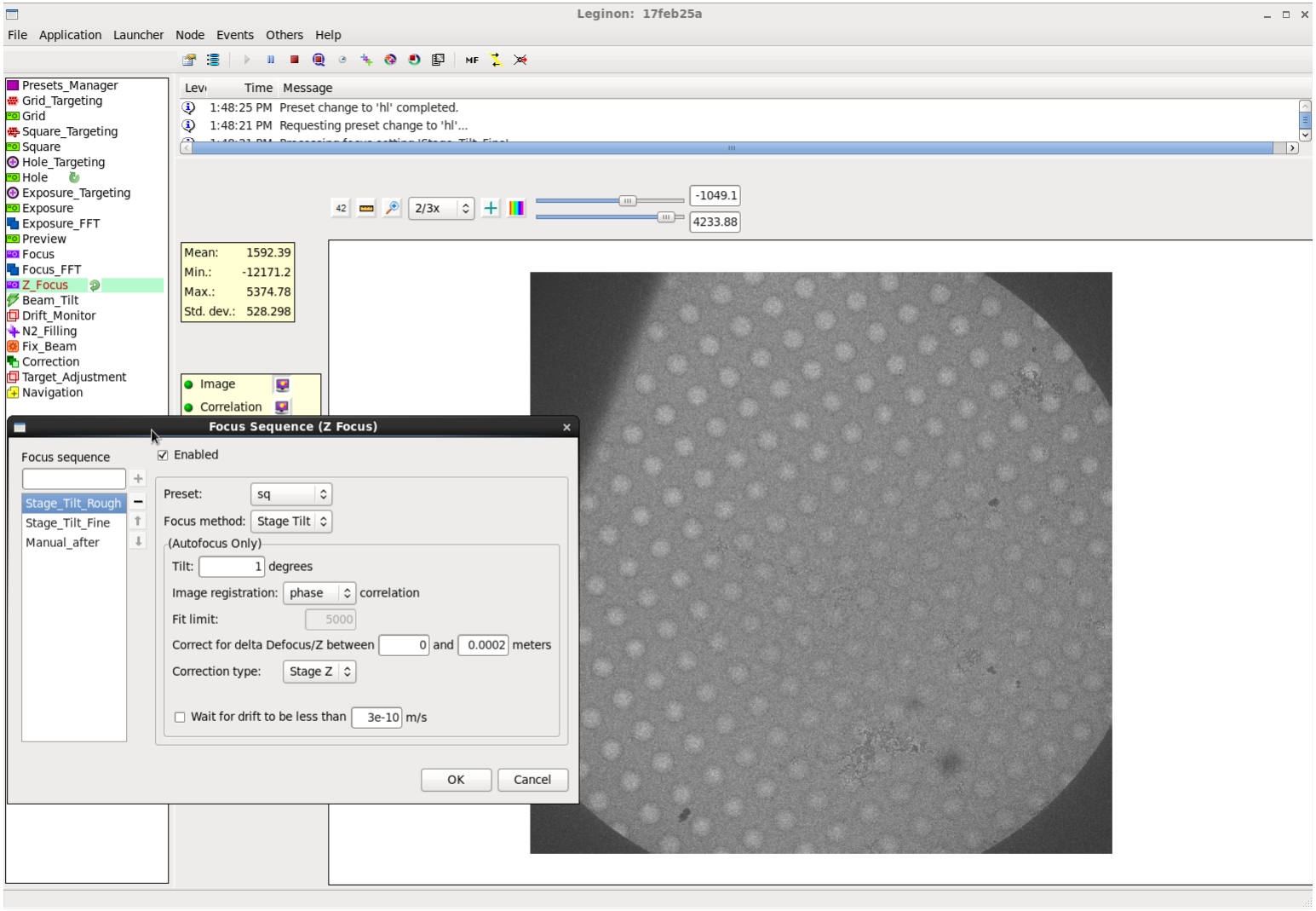
Alignment of different resets



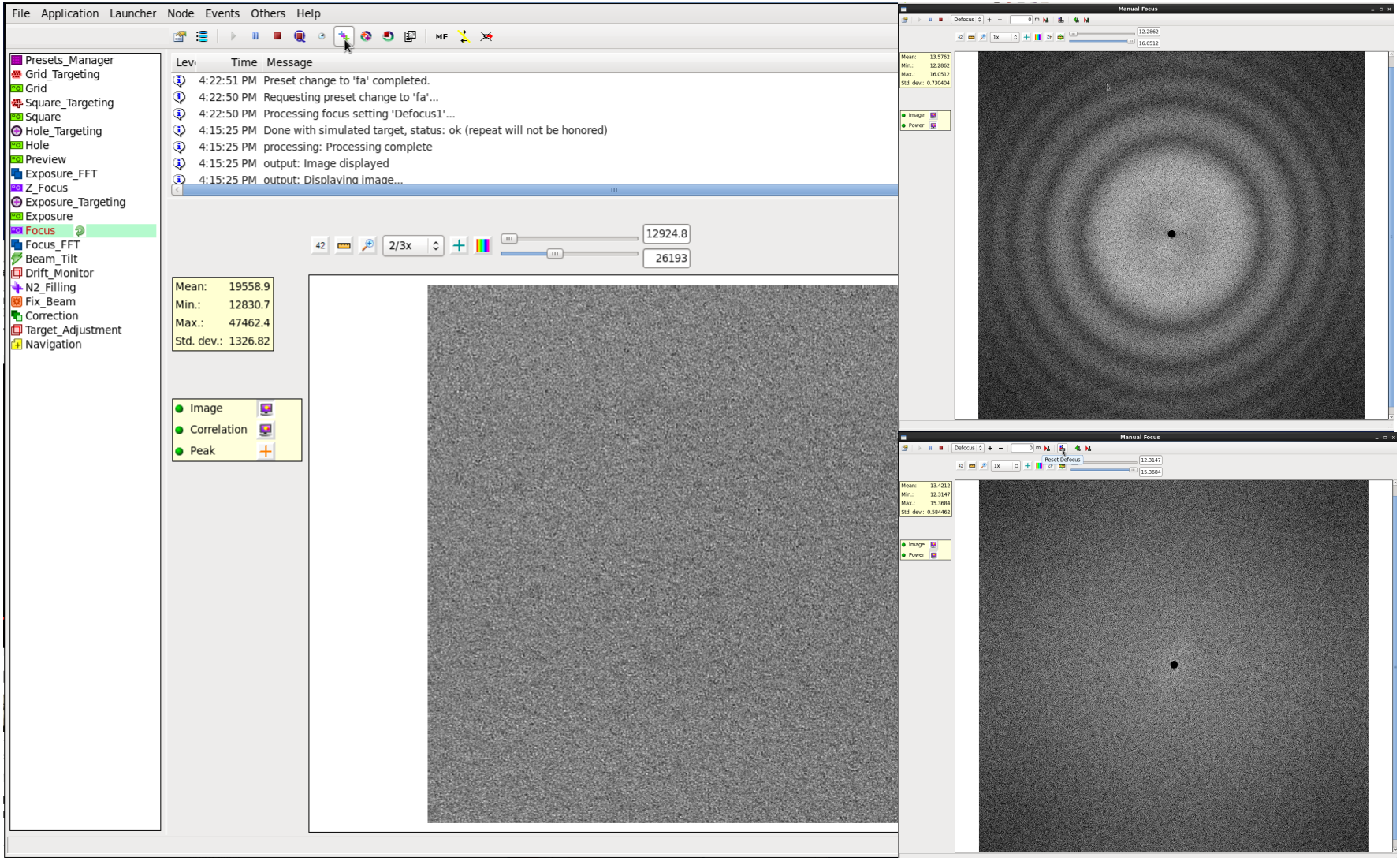
Beam shift and image shift in preset alignment



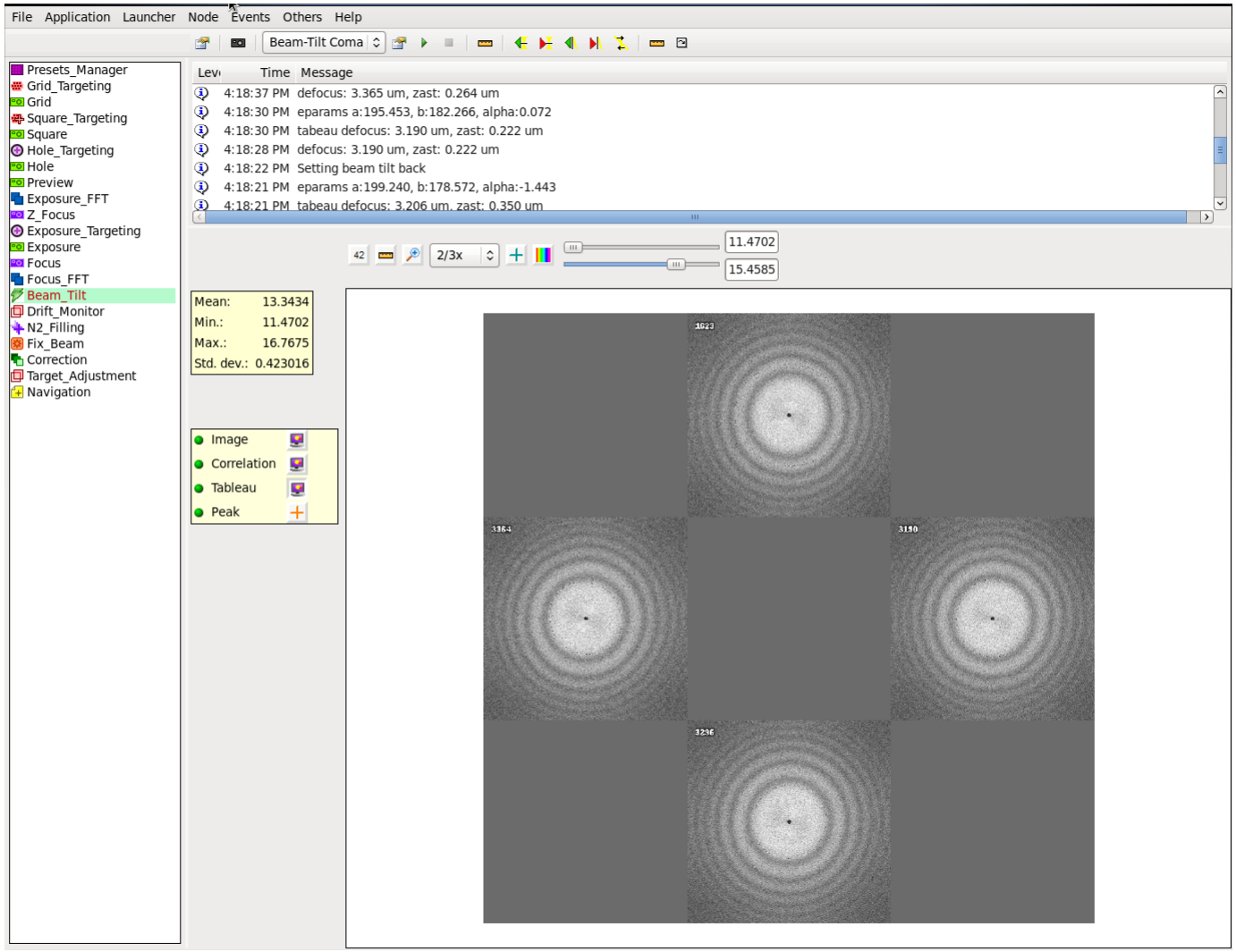
Z-focusing


Leginon: 17feb25a
File Application Launcher Node Events Others Help
Presets_Manager
Grid_Targeting
Grid
Square_Targeting
Square
Hole_Targeting
Hole
Exposure_Targeting
Exposure
Exposure_FFT
Preview
Focus
Focus_FFT
Z_Focus
Beam_Tilt
Drift_Monitor
N2_Filling
Fix_Beam
Correction
Target_Adjustment
Navigation
Time Message
1:48:25 PM Preset change to 'hl' completed.
1:48:21 PM Requesting preset change to 'hl'...
2/3x
-1049.1
4233.88
Mean: 1592.39
Min.: -12171.2
Max.: 5374.78
Std. dev.: 528.298
Image
Correlation
Focus Sequence (Z Focus)
Focus sequence
Enabled
Stage_Tilt_Rough
Stage_Tilt_Fine
Manual_after
Preset: sq
Focus method: Stage Tilt
(Autofocus Only)
Tilt: 1 degrees
Image registration: phase correlation
Fit limit: 5000
Correct for delta Defocus/Z between 0 and 0.0002 meters
Correction type: Stage Z
Wait for drift to be less than 3e-10 m/s
OK
Cancel

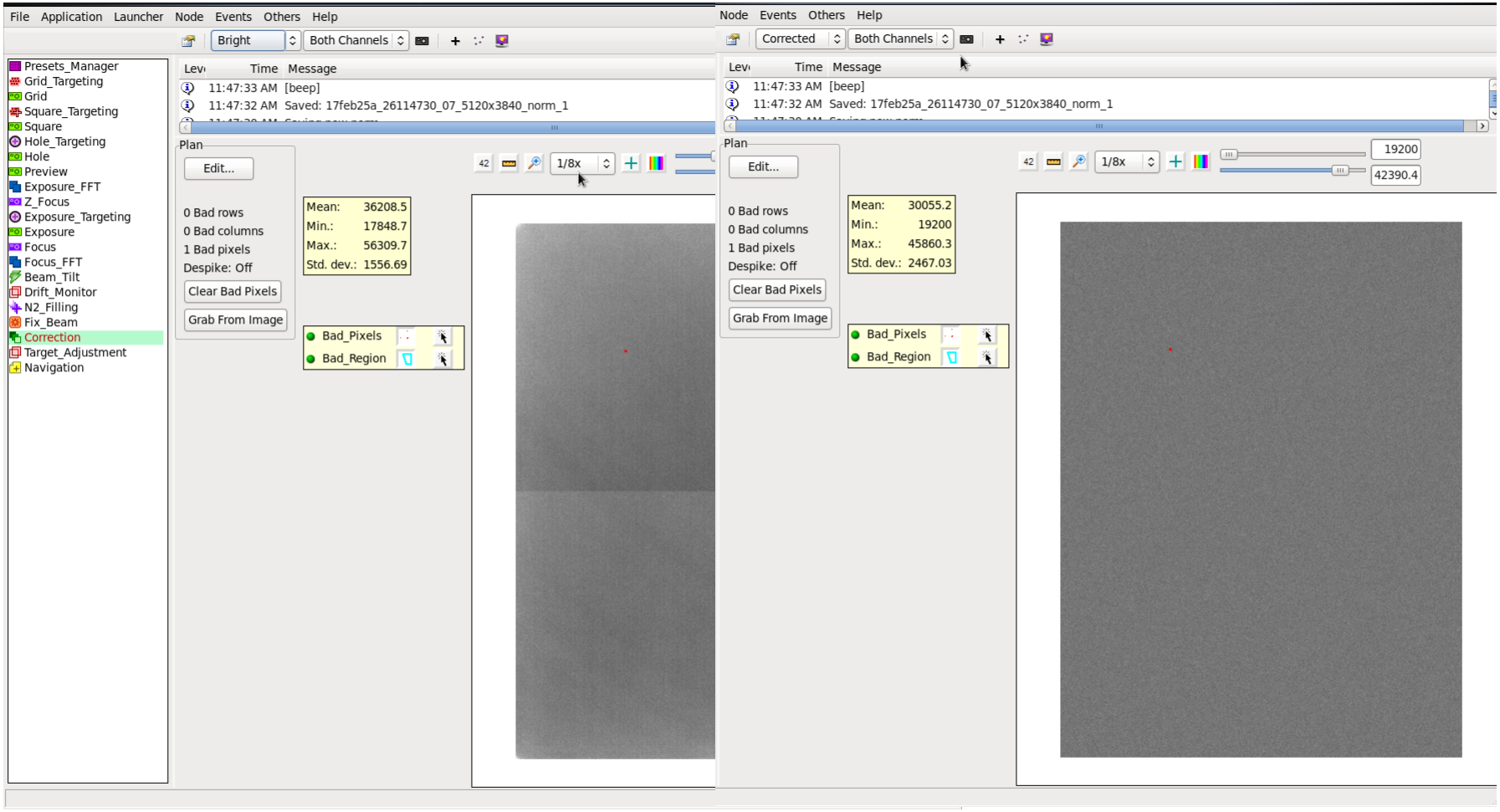
Focusing


File Application Launcher Node Events Others Help
Presets_Manager
Grid_Targeting
Grid
Square_Targeting
Square
Hole_Targeting
Hole
Preview
Exposure_FFT
Z_Focus
Exposure_Targeting
Exposure
Focus
Focus_FFT
Beam_Tilt
Drift_Monitor
N2_Filling
Fix_Beam
Correction
Target_Adjustment
Navigation
Time Message
4:22:51 PM Preset change to 'fa' completed.
4:22:50 PM Requesting preset change to 'fa'...
4:22:50 PM Processing focus setting 'Defocus1'...
4:15:25 PM Done with simulated target, status: ok (repeat will not be honored)
4:15:25 PM processing: Processing complete
4:15:25 PM output: Image displayed
4:15:25 PM output: Displaying image...
2/3x
12924.8
26193
Mean: 19558.9
Min.: 12830.7
Max.: 47462.4
Std. dev.: 1326.82
Image
Correlation
Peak
Manual Focus
Manual Focus

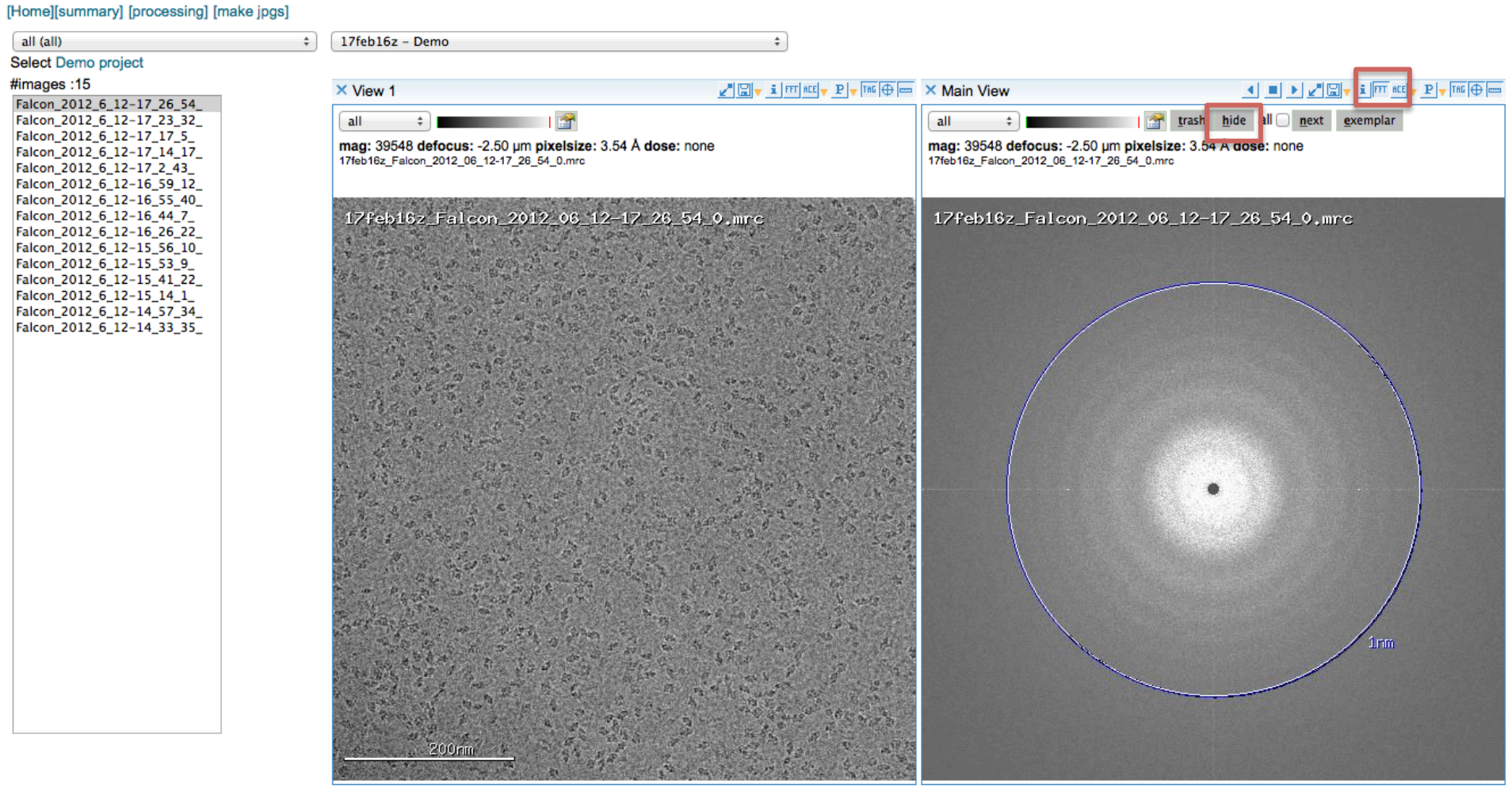
Coma-free alignment



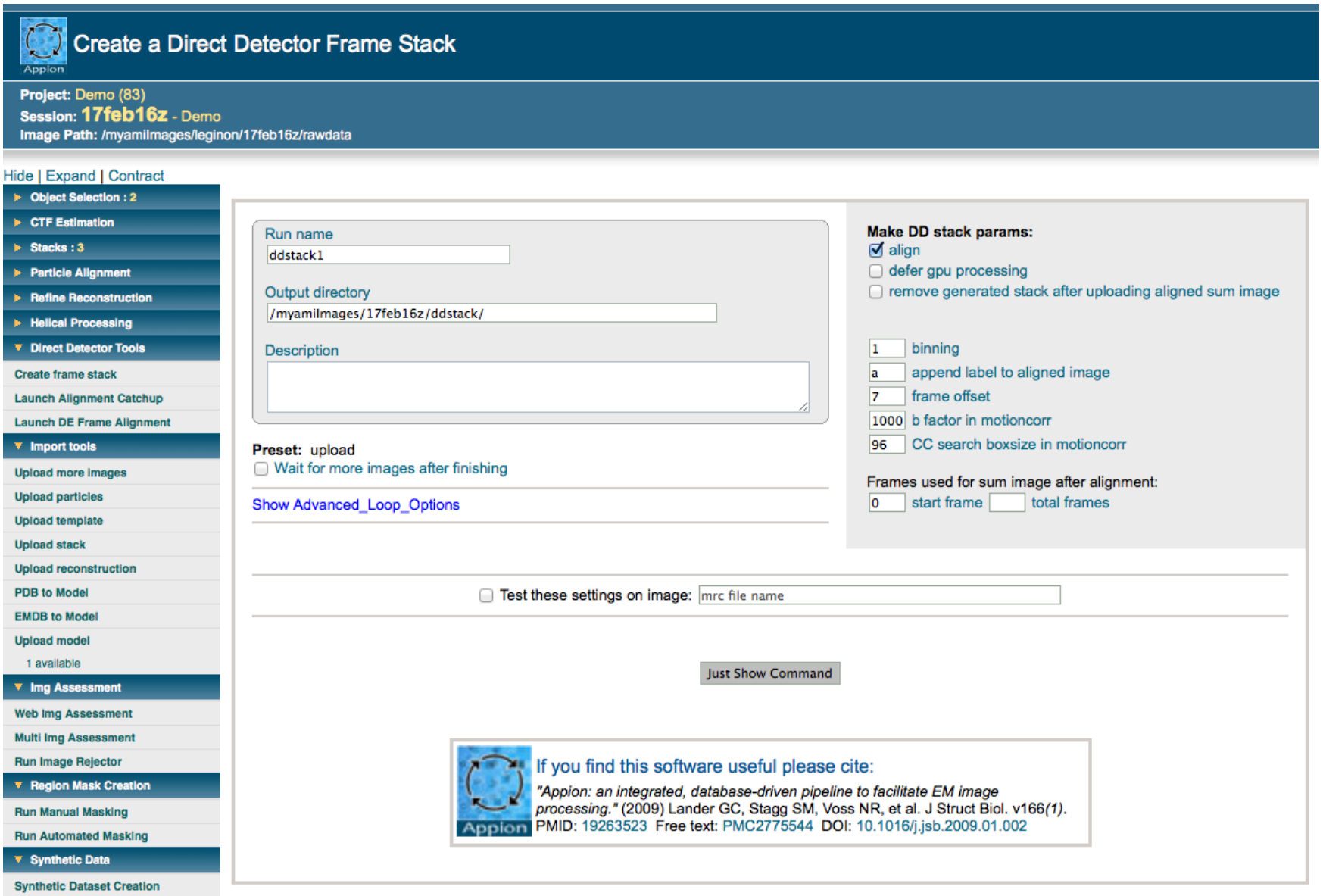
Camera gain correction



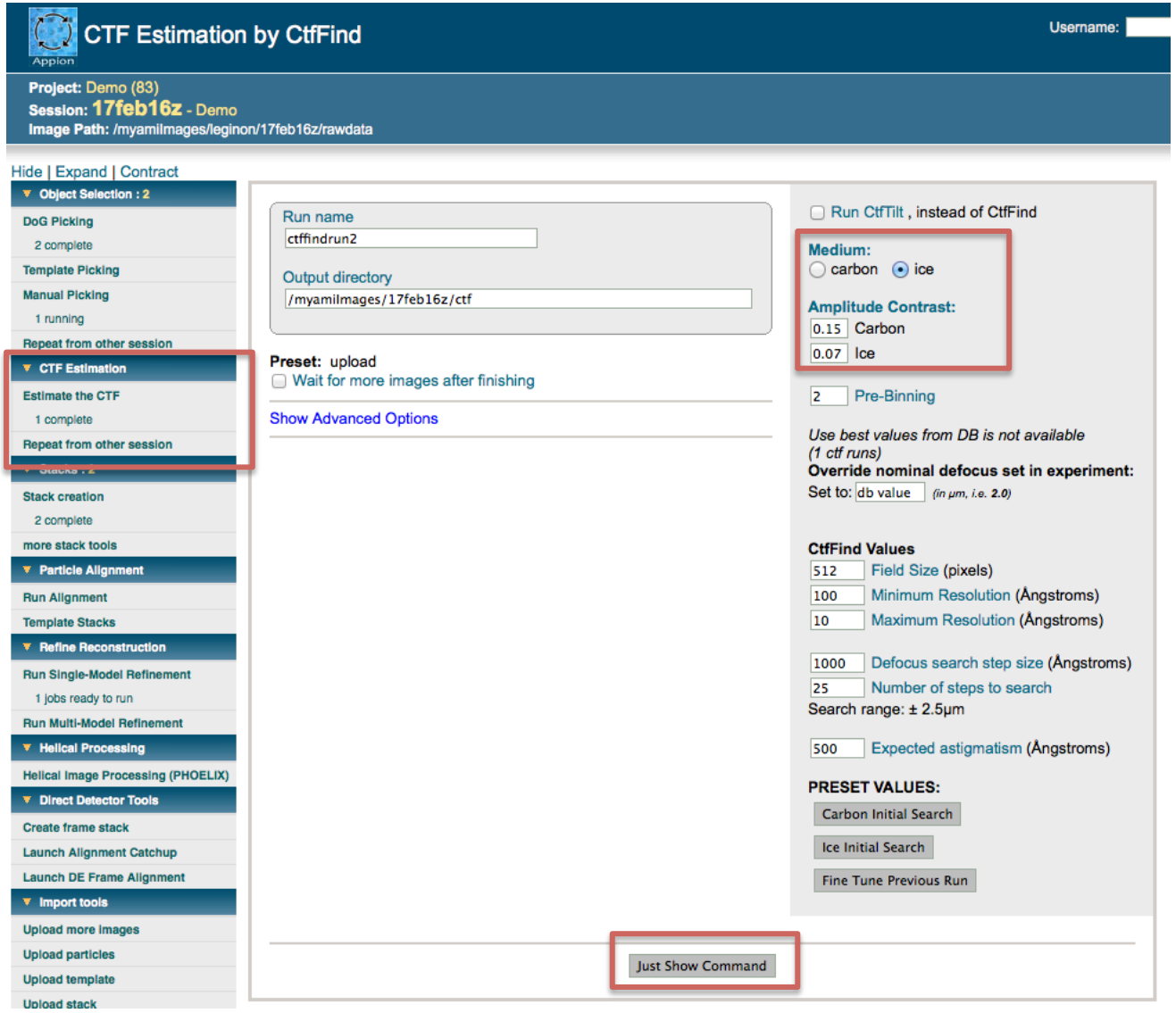
Appion
Image assessment



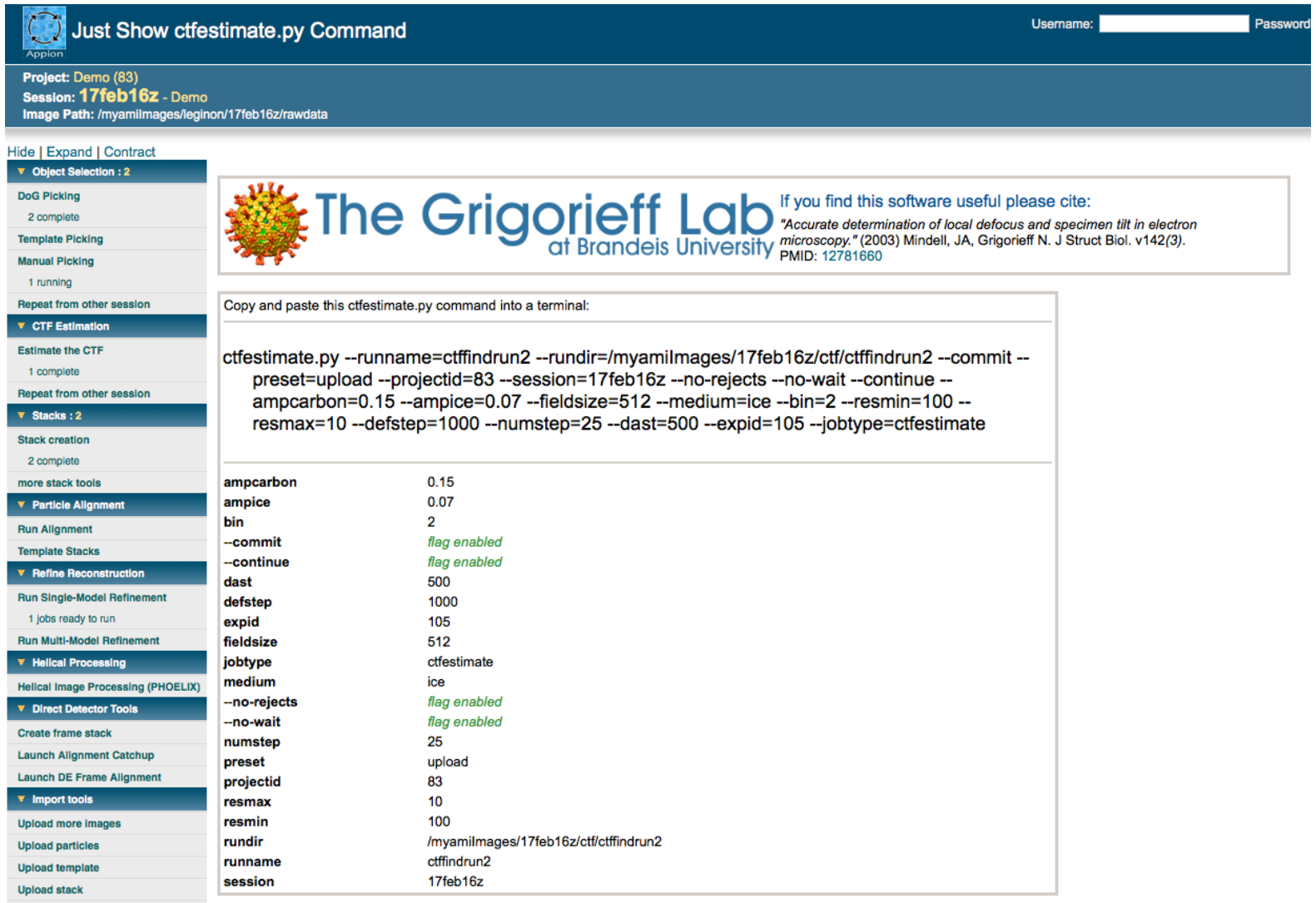
DD frame alignment



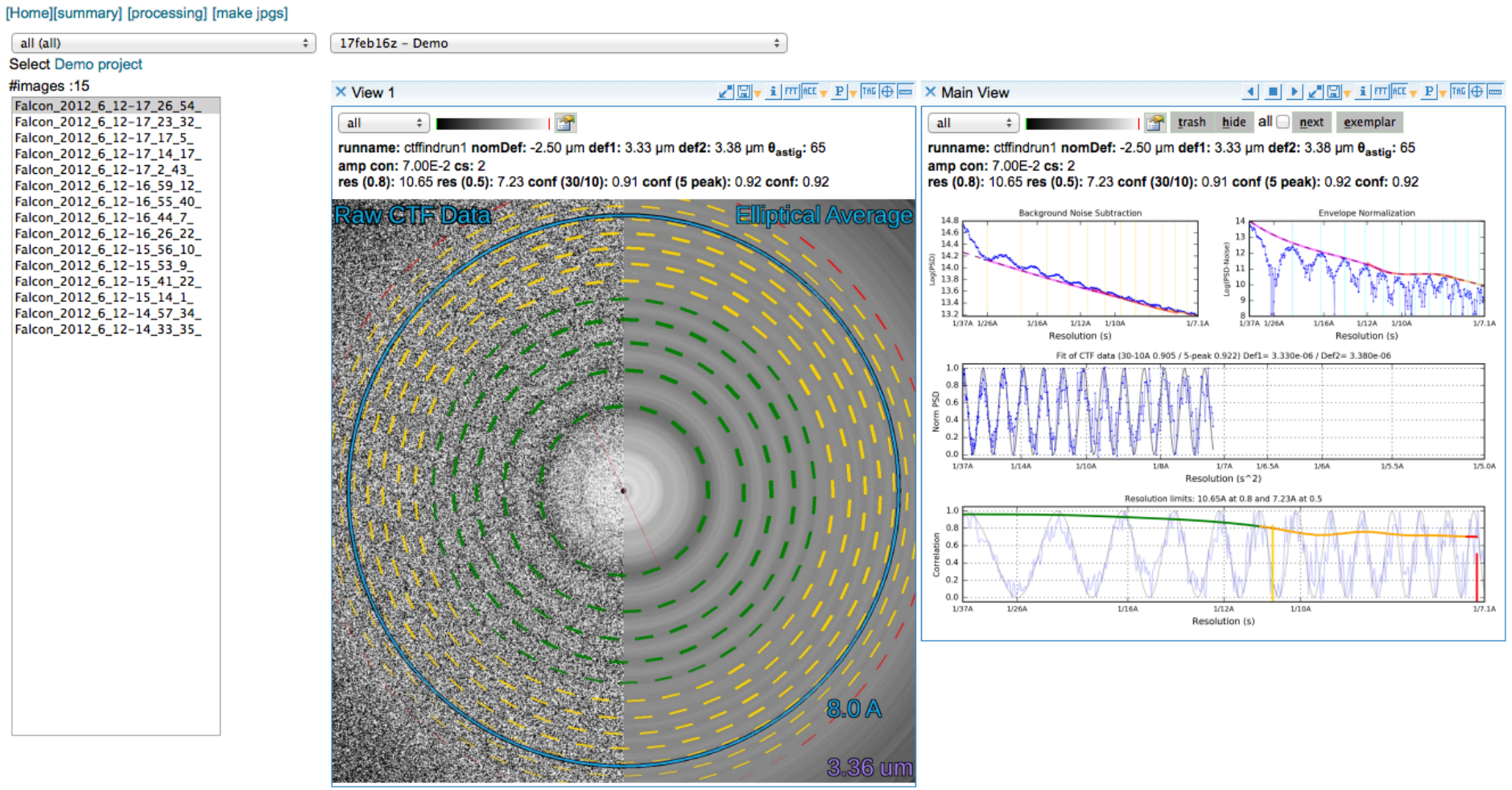
CTF correction



CTF correction



CTF correction



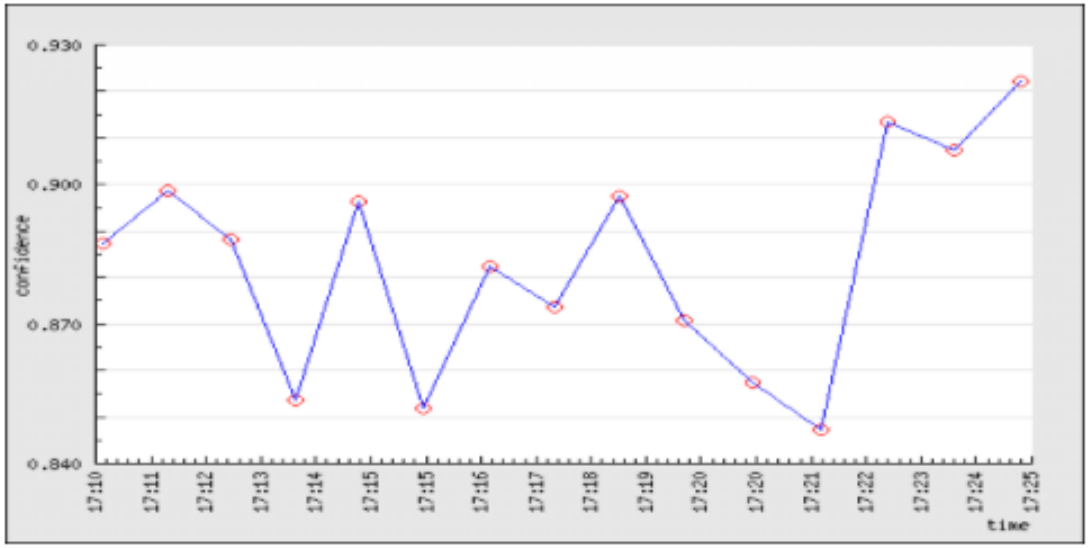
Ctf Run: 1 ctffindrun1 hide delete (download ctf data)

Time: *2017-02-16 17:52:10*

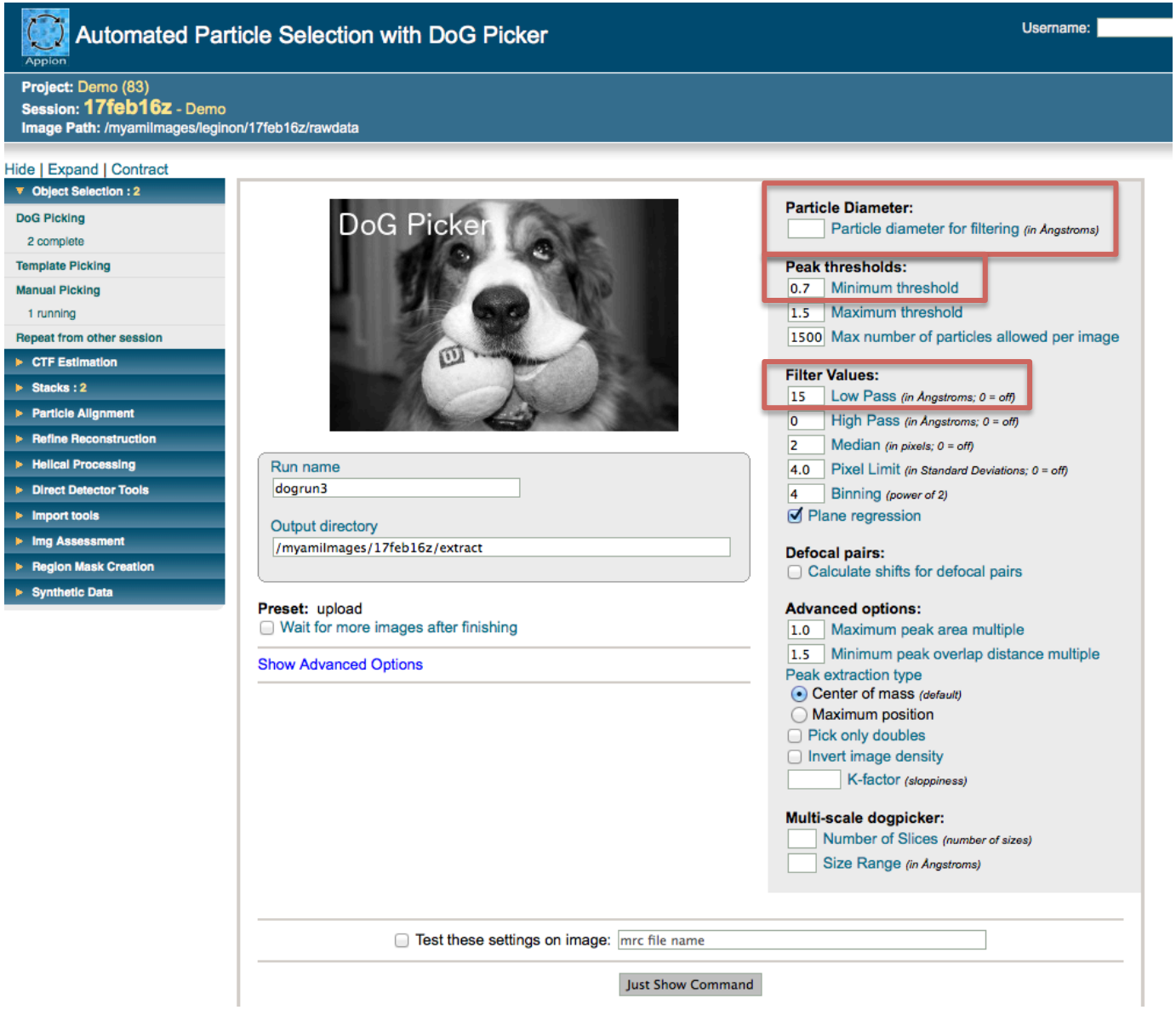
Path: */myamiImages/appion/17feb16z/ctf/ctffindrun1*

	nb	min	max	avg	stddev
defocus1	15	2.02 µm	3.41 µm	2.73 µm	0.43 µm
defocus2	15	2.03 µm	3.44 µm	2.77 µm	0.44 µm
angle_astigmatism	15	-87.290	85.000	27.654	43.560
amplitude_contrast	15	7.000×10^{-2}	7.000×10^{-2}	7.000×10^{-2}	0
confidence_30_10	15	0.823	0.913	0.872	2.626×10^{-2}
confidence_5_peak	15	0.767	0.922	0.872	3.774×10^{-2}
resolution_80_percent	15	8.674	13.737	11.261	1.393
resolution_50_percent	15	7.198	9.457	8.024	0.784

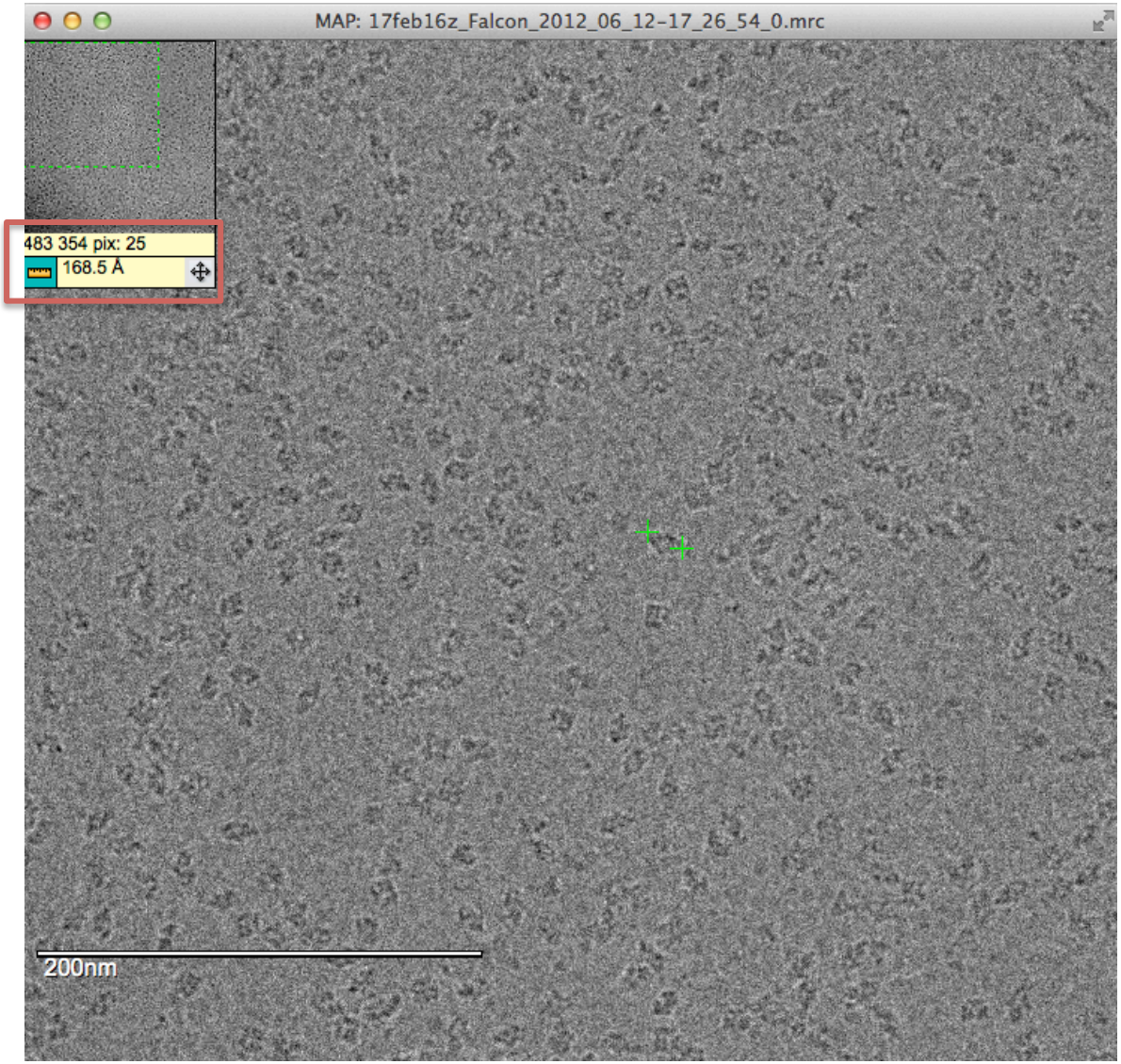
Confidence values during run



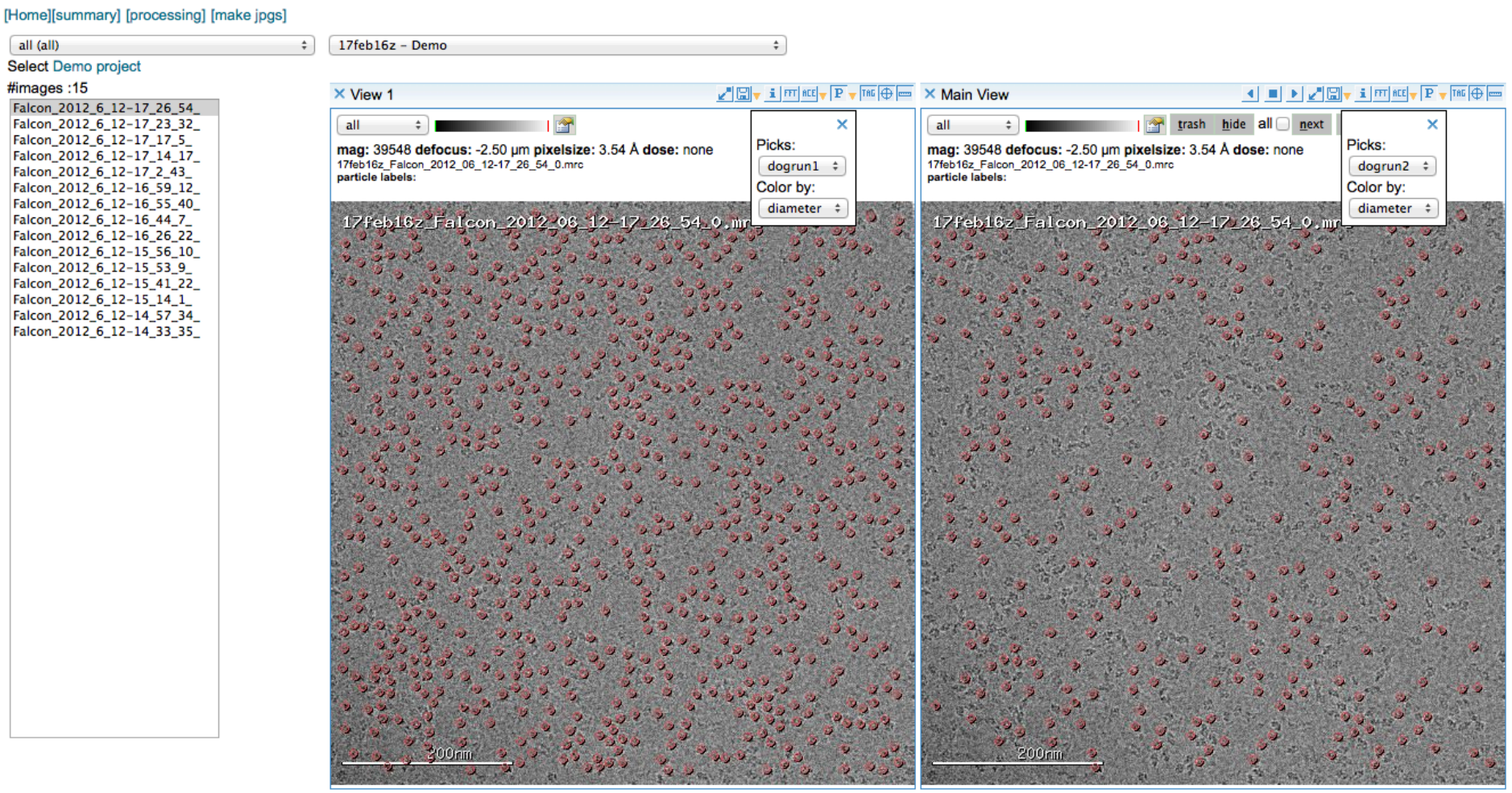
Particle picking



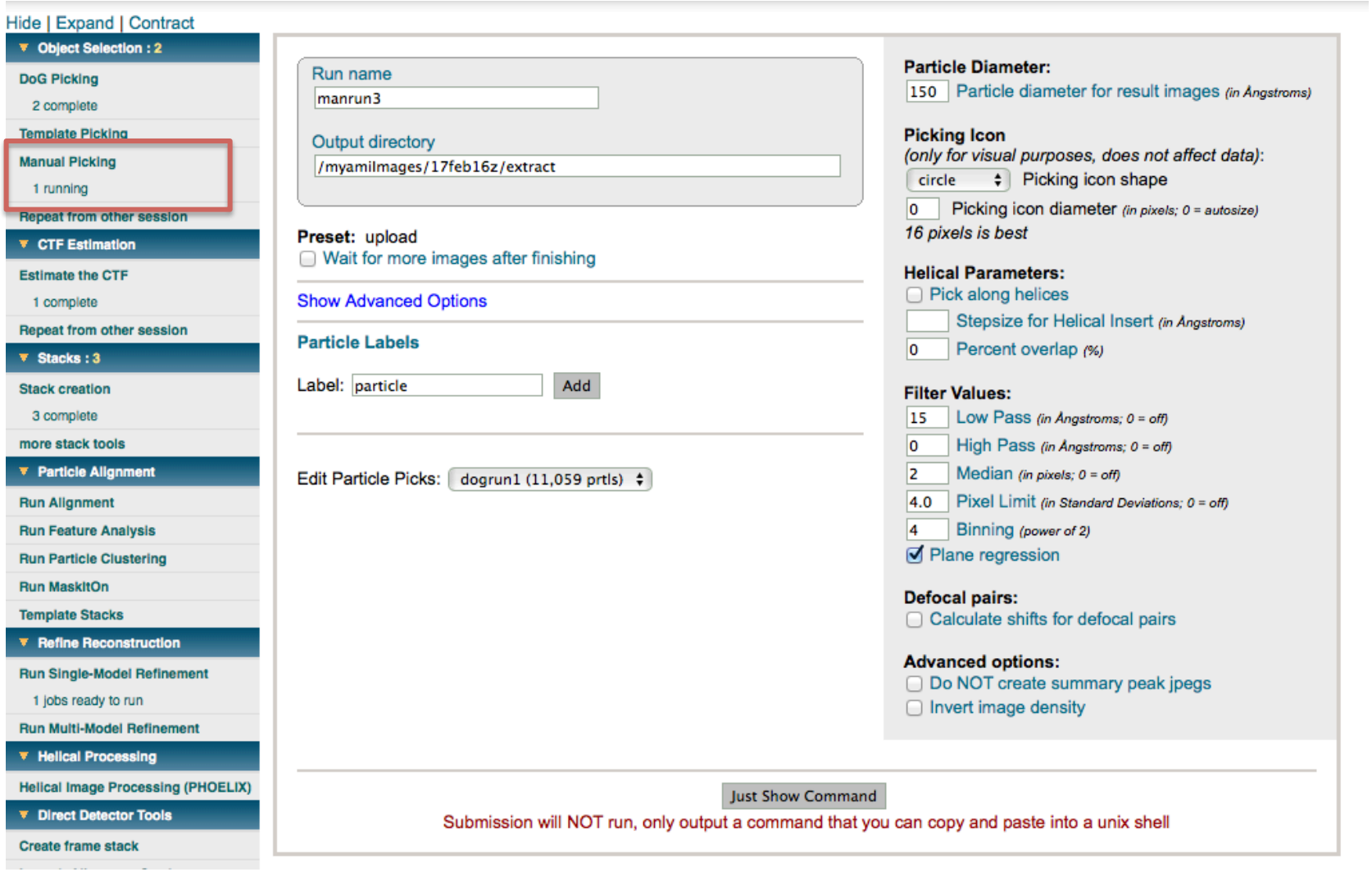
Assess particle sizes



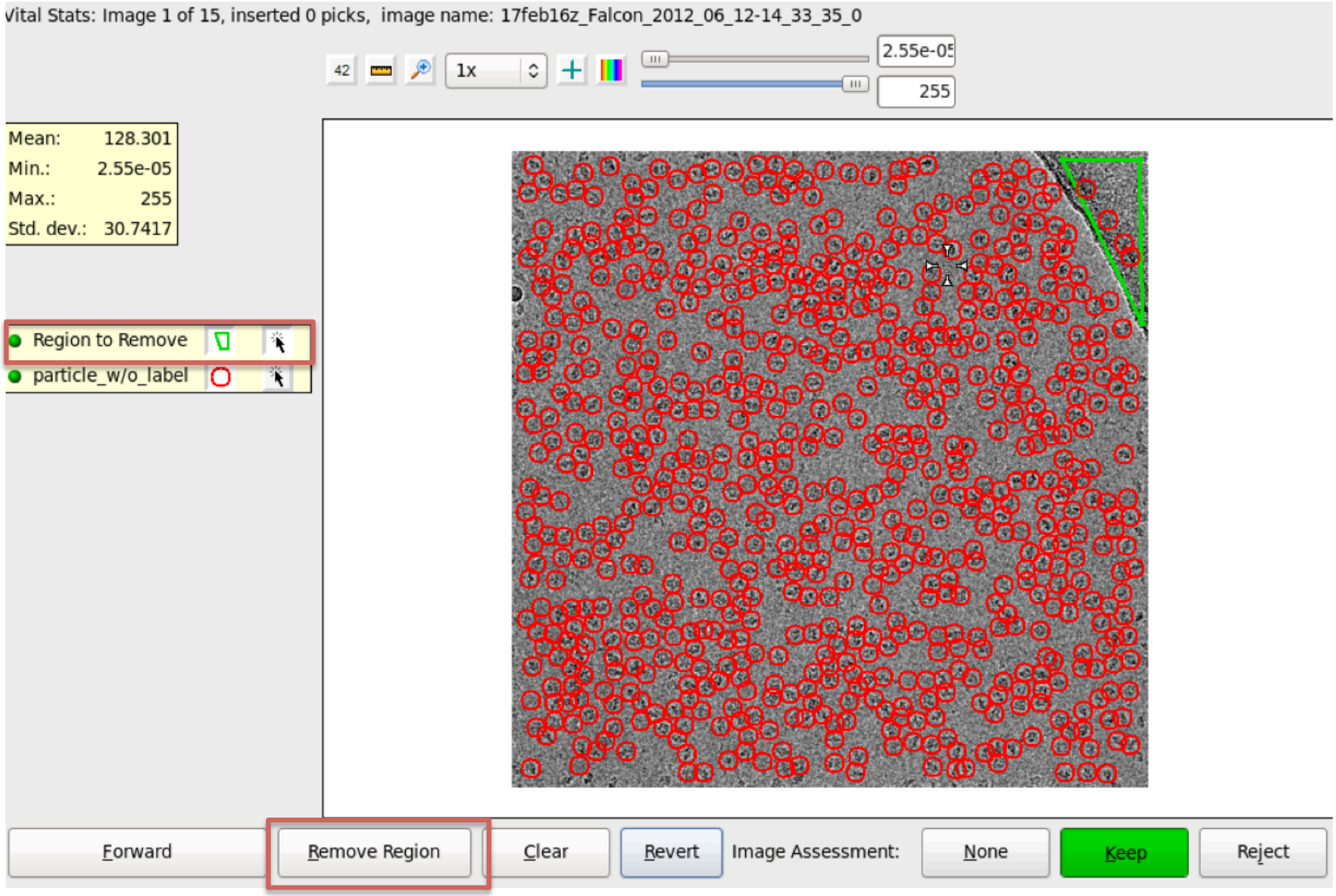
Particle picking



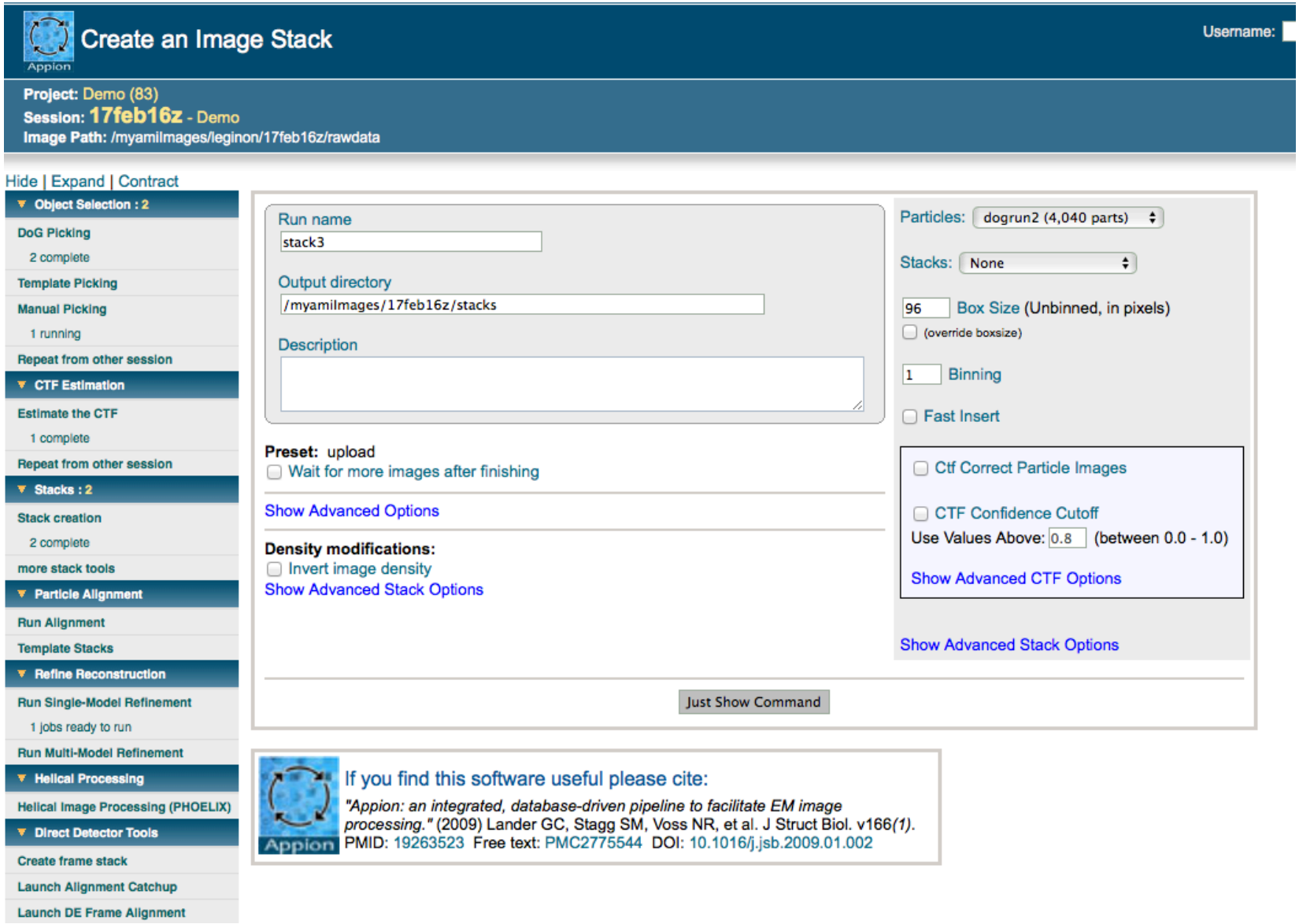
Manual picking



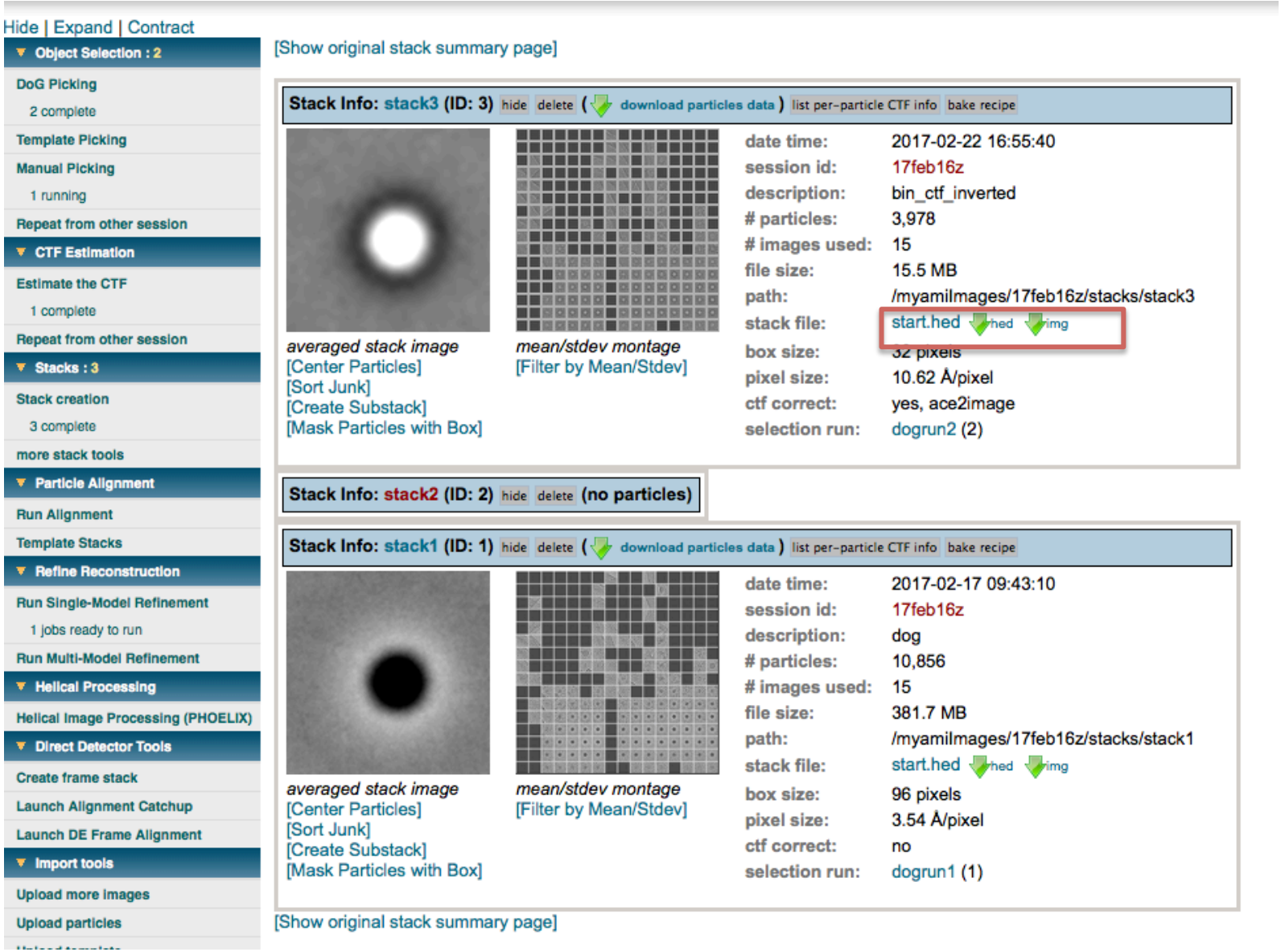
Clean up particle picks



Stack creation



Stack creation



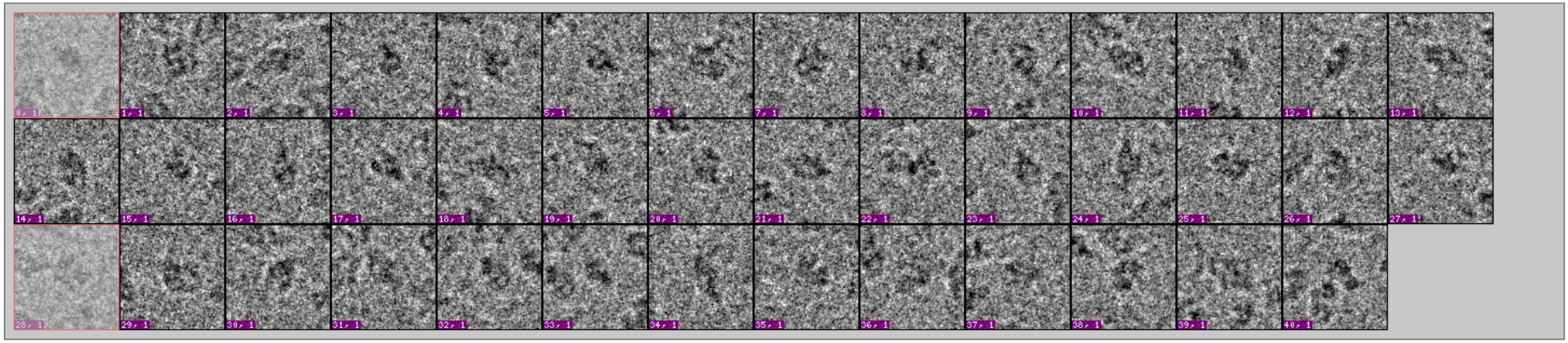
Assessment of particle picks

stack: /myamiImages/17feb16z/stacks/stack1/start.hed
#images: 10856
from: 0 to: 40 binning: 1 quality: jpeg 50 info: scale bar: Load
Selection mode: exclude

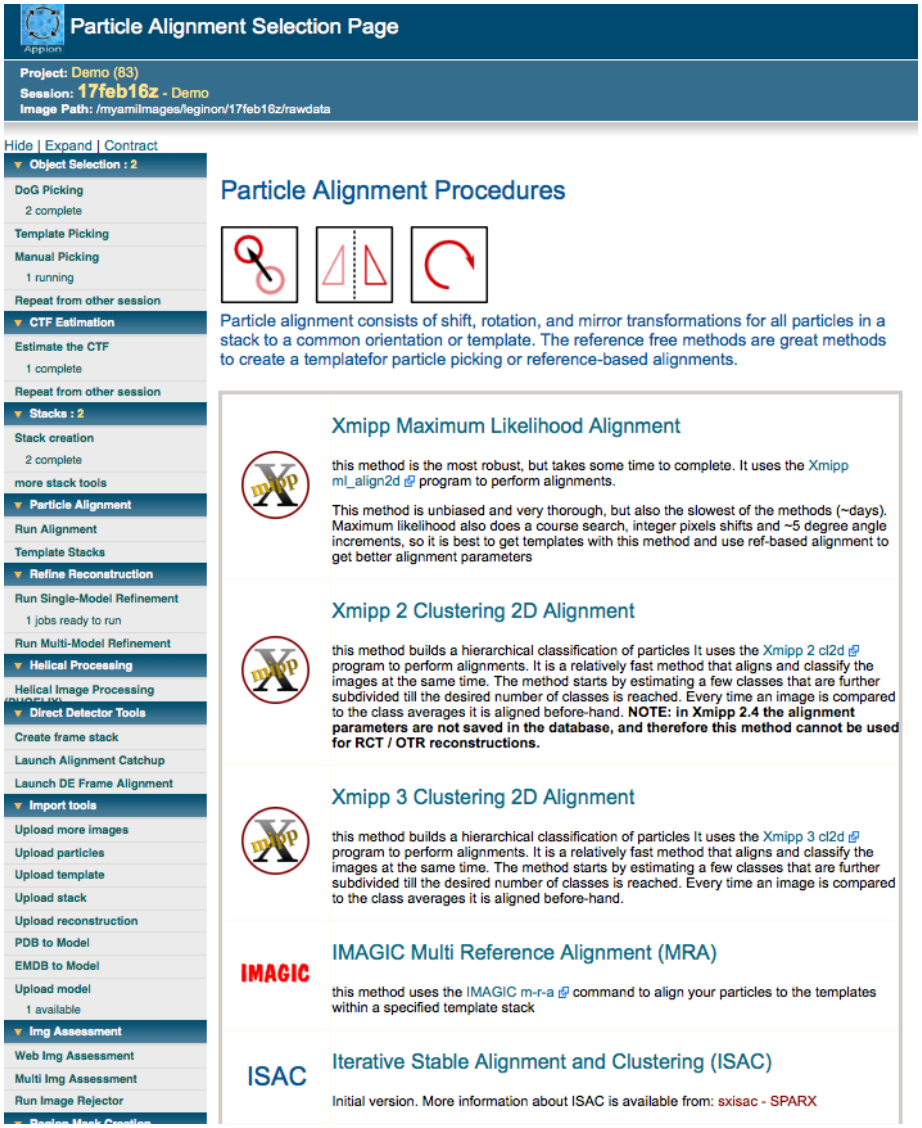
Selected images:
Select: Create Templates Include Particles

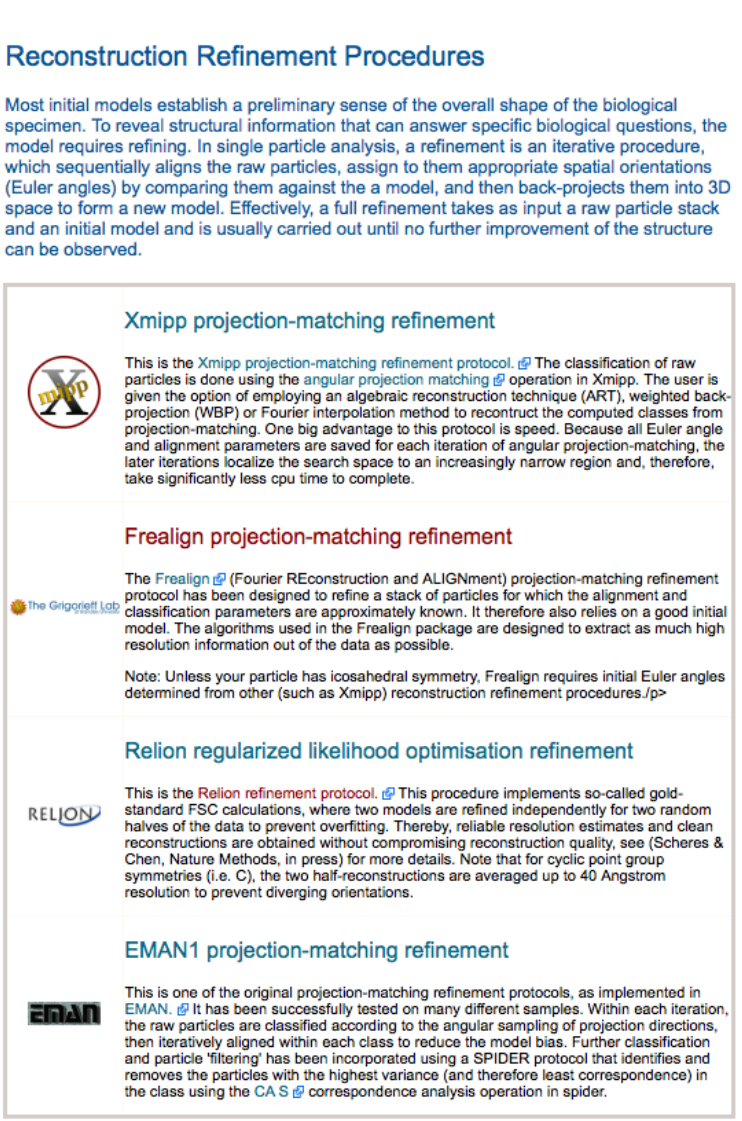
Excluded images: 28,0
Exclude: Remove Particles

Average images as template

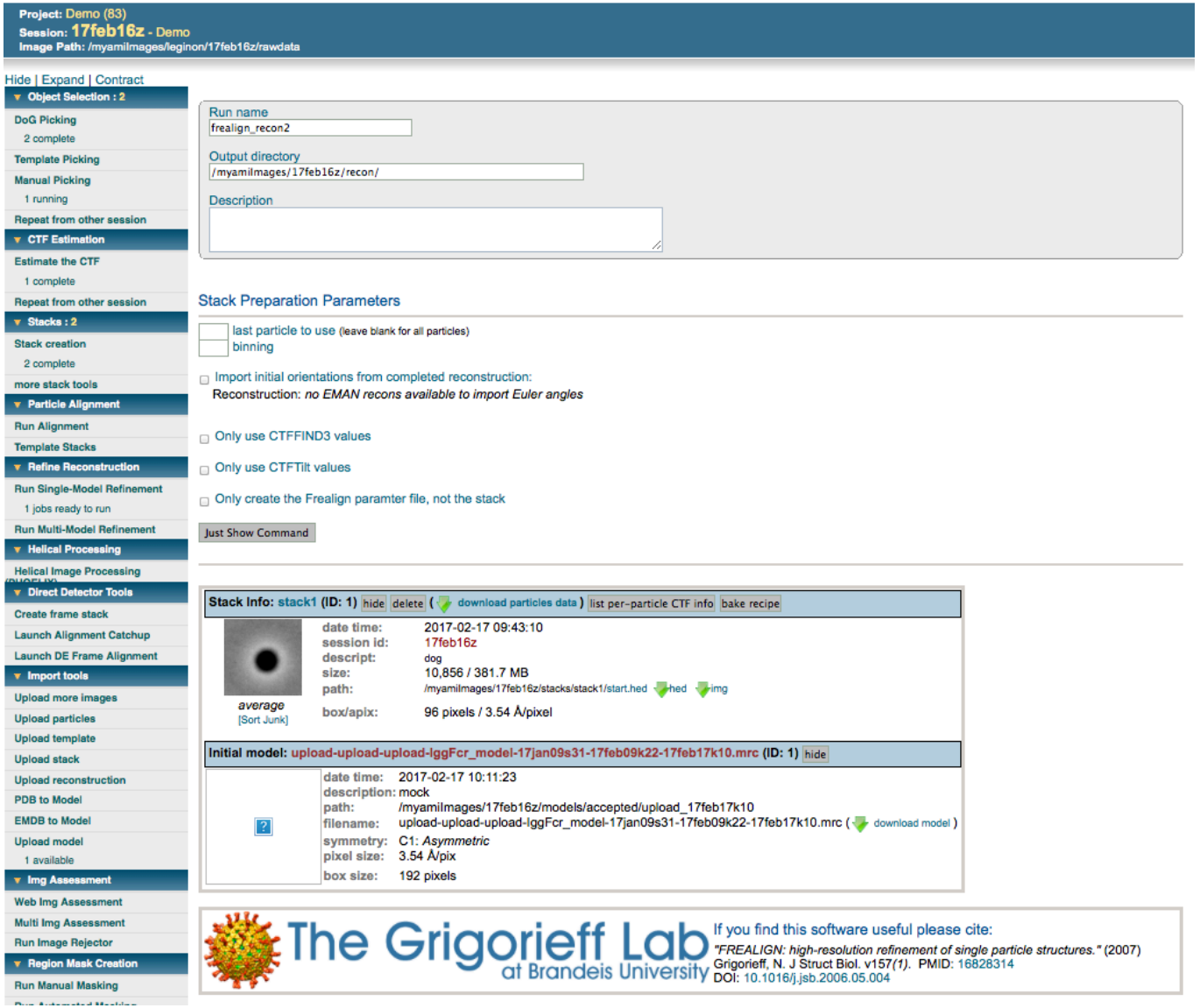


2D classification and 3D refinement





Import particle stack to Frealign



[user1@fulab frealign_recon1]$ ls
files_to_remote_host initmodel0001.mrc note params.000.par
prepRefineFrealign.log start.mrc thread000.log thread001.log

[cyfu@pcf00243 refinement]$ ls
frealign.log mparameters pdh_2_r1.mrc pdh_2_r1.par pdh_stack.mrc scratch

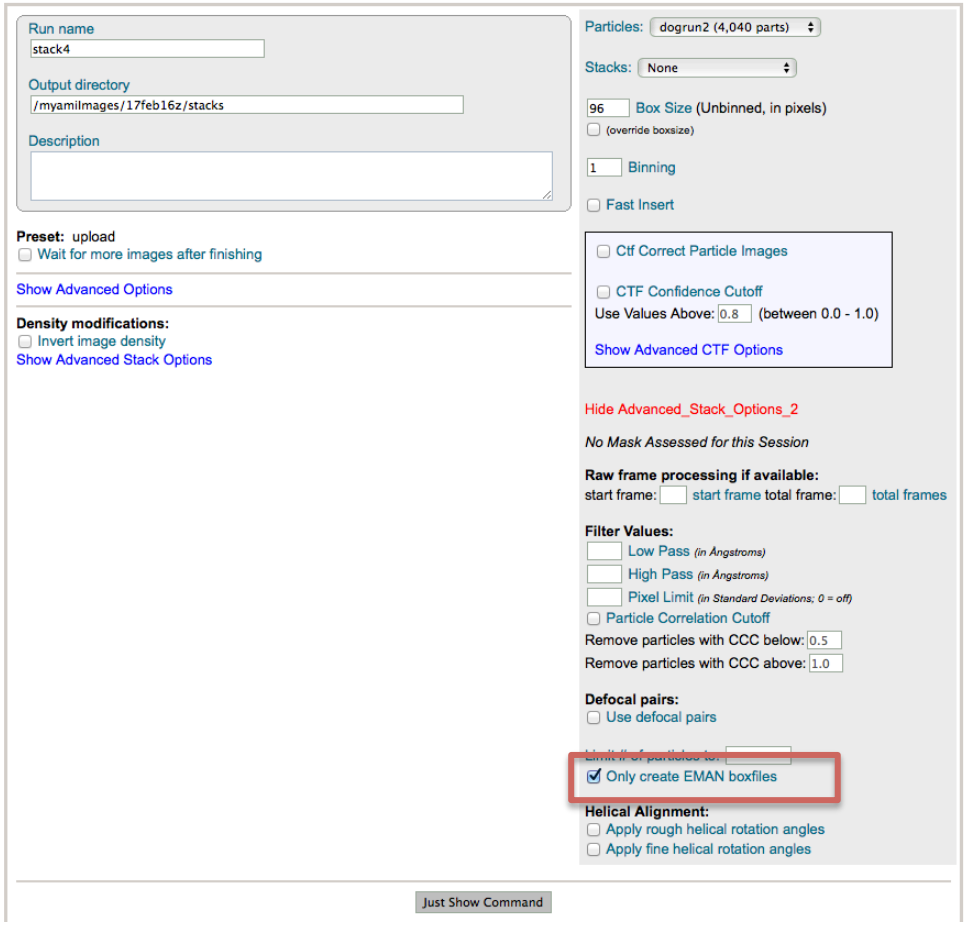
```
1     0.00     0.00     0.00     0.00     0.00  10000.      1  34109.6  34384.8   29.41   0.00     0.00       1
2     0.00     0.00     0.00     0.00     0.00  10000.      1  34109.6  34384.8   29.41   0.00     0.00       2
3     0.00     0.00     0.00     0.00     0.00  10000.      1  34109.6  34384.8   29.41   0.00     0.00       3
4     0.00     0.00     0.00     0.00     0.00  10000.      1  34109.6  34384.8   29.41   0.00     0.00       4
5     0.00     0.00     0.00     0.00     0.00  10000.      1  34109.6  34384.8   29.41   0.00     0.00       5
6     0.00     0.00     0.00     0.00     0.00  10000.      1  34109.6  34384.8   29.41   0.00     0.00       6

Control parameter file to run Frealign
======================================

This file must me kept in the project working directory from which the refinement scripts are launched.

Note: Please make sure that project and scratch directories are accessible by all sub-processes that are run on cluster nodes.

# Computer-specific setting
cluster_type         none       ! Set to "sge", "lsf", "slurm" or "pbs" when running on an SGE, LSF, SLURM or PBS cluster, otherwise set to "none".
nprocessor_ref        4         ! Number of CPUs to use during refinement.
nprocessor_rec        2         ! Number of CPUs to use during reconstruction.
mem_per_cpu        2048         ! Memory available per CPU (in MB).

# Refinement-specific parameters
MODE                  1         ! 1, 2, 3 or 4. Refinement mode, normally 1. Set to 2 for additional search.
start_process         3         ! First cycle to execute. Output files from previous cycle (n-1) required.
end_process          10         ! Last cycle to execute.
res_high_refinement  35.0       ! High-resolution limit for particle alignment.
res_high_class        8.0       ! High-resolution limit to calculate class membership (OCC).
thresh_reconst        0.0       ! Particles with scores below this value will not be included in the reconstruction.
nclasses              1         ! Number of classes to use.

# Search-specific parameters
res_search           30.0       ! High-resolution limit for orientational search.
thresh_refine        70.0       ! Score threshold above which search will not be performed.
DANG                200.0       ! Angular step for orientational search.
ITMAX               200         ! Number of repetitions of grid search with random starting angles.
Bsearch            2000.0       ! B-factor filtering (when > 0) applied during search.

# Dataset-specific parameters
data_input           pdh        ! Root name for parameter and map files.
raw_images           /usr1/cyfu/frealign/examples_for_frealign_v9/refinement/pdh_stack
image_contrast        N         ! N or P. Set to N if particles are dark on bright background, otherwise set to P.
outer_radius        146.0       ! Outer radius of spehrical particle mask in Angstrom.
inner_radius          0.0       ! Inner radius of spehrical particle mask in Angstrom.
mol_mass           1500.0       ! Molecular mass in kDa of particle or helical segment.
Symmetry              I         ! Symmetry of particle.
pix_size              3.11      ! Pixel size of particle in Angstrom.
dstep                14.0       ! Pixel size of detector in micrometer.
Aberration            2.0       ! Sherical aberration coefficient in millimeter.
Voltage             120.0       ! Beam accelleration voltage in kilovolt.
Amp_contrast          0.07      ! Amplitude contrast.
```

Import particle stack to Relion

```
[user1@fulab frealign_recon1]$ ls
all_particles.star  initmodel0001.mrc  params.000.par       start.mrc   thread000.log
files_to_remote_host  note             prepRefineFrealign.log  start.mrcs  thread001.log
[user1@fulab frealign_recon1]$
```

```
data_
loop_
_rlnImageName
_rlnMicrographName
_rlnDefocusU
_rlnDefocusV
_rlnDefocusAngle
_rlnVoltage
_rlnSphericalAberration
_rlnAmplitudeContrast
1@/usr1/cyfu/demo/start.mrcs 1 34109.6 34384.8 29.41  300 2 0.07
2@/usr1/cyfu/demo/start.mrcs 1 34109.6 34384.8 29.41  300 2 0.07
3@/usr1/cyfu/demo/start.mrcs 1 34109.6 34384.8 29.41  300 2 0.07
4@/usr1/cyfu/demo/start.mrcs 1 34109.6 34384.8 29.41  300 2 0.07
5@/usr1/cyfu/demo/start.mrcs 1 34109.6 34384.8 29.41  300 2 0.07
6@/usr1/cyfu/demo/start.mrcs 1 34109.6 34384.8 29.41  300 2 0.07
7@/usr1/cyfu/demo/start.mrcs 1 34109.6 34384.8 29.41  300 2 0.07
8@/usr1/cyfu/demo/start.mrcs 1 34109.6 34384.8 29.41  300 2 0.07
9@/usr1/cyfu/demo/start.mrcs 1 34109.6 34384.8 29.41  300 2 0.07
10@/usr1/cyfu/demo/start.mrcs 1 34109.6 34384.8 29.41  300 2 0.07
11@/usr1/cyfu/demo/start.mrcs 1 34109.6 34384.8 29.41  300 2 0.07
12@/usr1/cyfu/demo/start.mrcs 1 34109.6 34384.8 29.41  300 2 0.07
```

Import particle coordinates to Relion

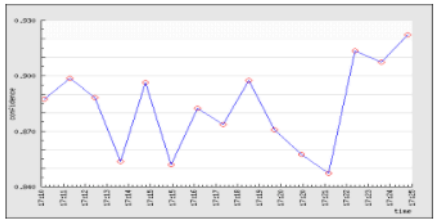



```
[user1@fulab stack2]$ pwd
/myamiImages/appion/17feb16z/stacks/stack2
[user1@fulab stack2]$ ls
17feb16z_Falcon_2012_06_12-14_33_35_0.box
17feb16z_Falcon_2012_06_12-14_57_34_0.box
17feb16z_Falcon_2012_06_12-15_14_01_0.box
17feb16z_Falcon_2012_06_12-15_41_22_0.box
17feb16z_Falcon_2012_06_12-15_53_09_0.box
17feb16z_Falcon_2012_06_12-15_56_10_0.box
17feb16z_Falcon_2012_06_12-16_26_22_0.box
17feb16z_Falcon_2012_06_12-16_44_07_0.box
17feb16z_Falcon_2012_06_12-16_55_40_0.box
17feb16z_Falcon_2012_06_12-16_59_12_0.box
```

```
1803    672     96      96      -3
868     1639    96      96      -3
1570    1550    96      96      -3
1050    26      96      96      -3
1633    315     96      96      -3
1742    722     96      96      -3
1412    439     96      96      -3
397     671     96      96      -3
1380    220     96      96      -3
1248    768     96      96      -3
1089    814     96      96      -3
1530    516     96      96      -3
1584    1092    96      96      -3
412     724     96      96      -3
169     930     96      96      -3
888     179     96      96      -3
```

Import CTF star file to Relion

Confidence values during run Difference fr



Generate defocus histogram with applied cutoff

Cutoff method: confidence_30_10

Cutoff value (0-1):

Generate Histogram

download star file for RELION

download best ctf data

download best ctf EMX file

Ctf Run: 1 ctffindrun1 hide delete (download ctf data)

Time: *2017-02-16 17:52:10*

Path: */myamiImages/appion/17feb16z/ctf/ctffindrun1*

	nb	min	max	avg	stddev
defocus1	15	2.02 µm	3.41 µm	2.73 µm	0.43 µm
defocus2	15	2.03 µm	3.44 µm	2.77 µm	0.44 µm
angle_astigmatism	15	-87.290	85.000	27.654	43.560
amplitude_contrast	15	7.000×10^{-2}	7.000×10^{-2}	7.000×10^{-2}	0
confidence_30_10	15	0.823	0.913	0.872	2.626×10^{-2}
confidence_5_peak	15	0.767	0.922	0.872	3.774×10^{-2}
resolution_80_percent	15	8.674	13.737	11.261	1.393
resolution_50_percent	15	7.198	9.457	8.024	0.784

```
 1
 2 data_
 3
 4 loop_
 5 _rlnMicrographName #1
 6 _rlnCtfImage #2
 7 _rlnDefocusU #3
 8 _rlnDefocusV #4
 9 _rlnDefocusAngle #5
10 _rlnVoltage #6
11 _rlnSphericalAberration #7
12 _rlnAmplitudeContrast #8
13 _rlnMagnification #9
14 _rlnDetectorPixelSize #10
15 _rlnCtfFigureOfMerit #11
16 micrographs/17jan09y_20170107b_1_00023gr_00004sq_v02_00004hl_v01_00002en.mrc micrograph
   s/17jan09y_20170107b_1_00023gr_00004sq_v02_00004hl_v01_00002en.ctf:mrc 19969.210000 209
   25.760000 86.640000 200.000000 2.000000 0.070000 10000.000000 1.080000 0.716469
17 micrographs/17jan09y_20170107b_1_00023gr_00004sq_v02_00004hl_v01_00003en.mrc micrograph
   s/17jan09y_20170107b_1_00023gr_00004sq_v02_00004hl_v01_00003en.ctf:mrc 16739.590000 175
   16.950000 -85.340000 200.000000 2.000000 0.070000 10000.000000 1.080000 0.695531
18 micrographs/17jan09y_20170107b_1_00023gr_00004sq_v02_00005hl_v02_00003en.mrc micrograph
   s/17jan09y_20170107b_1_00023gr_00004sq_v02_00005hl_v02_00003en.ctf:mrc 19290.750000 202
   95.960000 -79.540000 200.000000 2.000000 0.070000 10000.000000 1.080000 0.515900
19 micrographs/17jan09y_20170107b_1_00023gr_00004sq_v02_00007hl_v01_00004en.mrc micrograph
   s/17jan09y_20170107b_1_00023gr_00004sq_v02_00007hl_v01_00004en.ctf:mrc 17319.640000 195
   09.240000 -74.580000 200.000000 2.000000 0.070000 10000.000000 1.080000 0.574156
20 micrographs/17jan09y_20170107b_1_00023gr_00004sq_v02_00008hl_v01_00002en.mrc micrograph
   s/17jan09y_20170107b_1_00023gr_00004sq_v02_00008hl_v01_00002en.ctf:mrc 22308.230000 239
   53.470000 -60.000000 200.000000 2.000000 0.070000 10000.000000 1.080000 0.691933
```

Appion and Leginon DB Tools