F20 User Manual at the Simons Electron Microscopy Center

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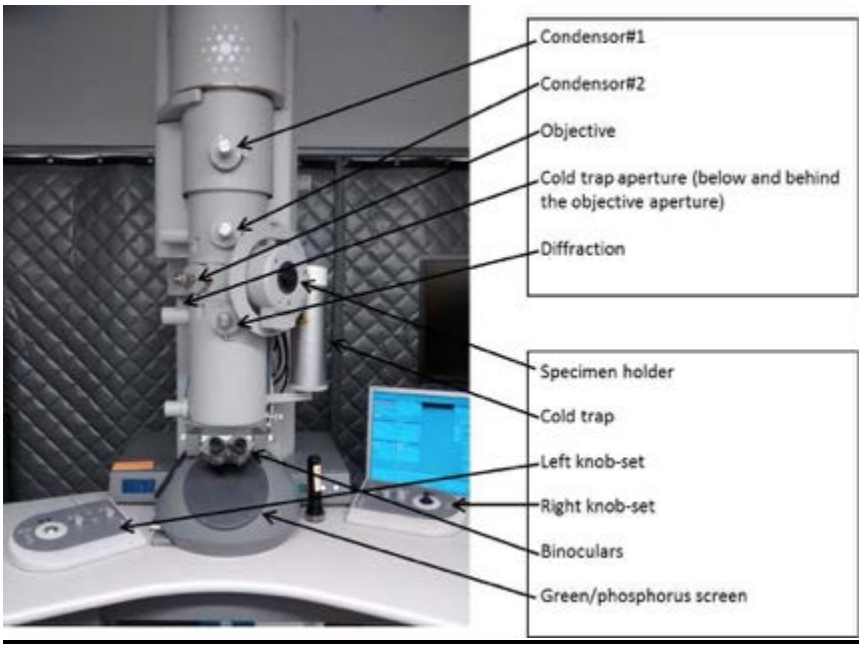
F20 Information Sheet



- Wavelengths
  - 80 kV = 0.04176 Å
  - 120 kV = 0.03349 Å
  - 200 kV = 0.02508 Å
  - 300 kV = 0.01969 Å

- Lens & magnification range
  - LM = 21X → 2100X
  - M = 1700X → 3500X
  - SA = 65,000X → 280,000X
  - Mh = 390,000X → 700,000X

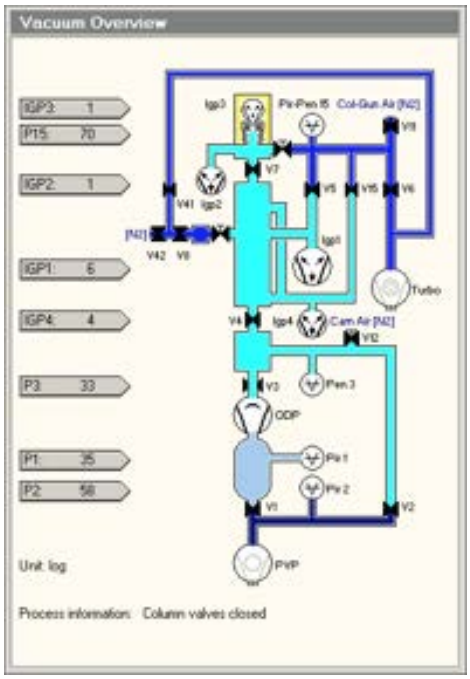
- Objective lens Cs = 2.1mm

- Aperture sizes

Aperture	**#1**	**#2**	**#3**	**#4**
Condenser	50 um	70 um	100 um	150 um
Objective	40 um	70 um	70 um	100 um
Selected area	40 um	70 um	100 um	200 um

F20 User Guide (starting your session)

1. Make sure microscope is in stable condition:
    a. Make sure HT and FEG are on
        i. Under “Setup” tap, “High Tension” and “Operate” are highlighted yellow
    b. Make sure that column valves are closed and turbo pump is on
        i. Under “Setup” tab, “Col. Valves Closed” and “Turbo on” are highlighted yellow
    c. Make sure that EM column vacuum is stable (IGP1 = 6)
    d. Stage neutral
        i. Under “Search” tab, click “Holder” button to zero out the X, Y, Z & α coordinates of the stage
    e. Fill cold trap with LN2 (can last for 16 hours); refill every 1 - 2 hours



2. Login to leginon workstation with your LDAP account (ex: bcarragher)
    a. Given to you on a slip of white paper during new user orientation
3. Transfer cryo holder from the pumping station; cool down holder and insert your sample
    a. See “Dry Pumping Station User Guide (for cryo holders)” for detailed instructions (page 15)
4. Insert cryo holder into EM
    a. See “Cryo Holder Insertion/Retraction Guide” for detailed instructions (page 16)
    b. CHECKLIST:
        1) Reset = Holder
        2) Alpha = -60°
        3) flu screen of microscope is down (R1)
        4) objective aperture is out
        5) turbo is on and ready (yellow)
        6) shield of cryo holder is closed
        7) airlock pump time is = 120s
        8) pre-pump airlock
5. After insertion, wait for IGP1 to reach <10 before opening the column valves and starting your session
6. Select the camera/acquisition type needed for your session:
    a. If you are screening multiple cryo grids today, use the TVIPS camera (“Cryo Screening with TVIPS” guide)
        i. This camera has a faster readout and yields higher contrast images in a single exposure
        ii. If your grid looks good, you can switch to the DE20 for data collection
    b. If you plan to collect data for a reconstruction, use the DE20 camera (“Cryo Data Collection with the DE20” guide)

i. This camera has a slower readout, but can give you higher resolution details within multiple frames of a movie

F20 User Guide (ending your session)

1. After your screening and/or data collection is done, remove the cryo holder from the microscope
    a. See “Cryo Holder Insertion/Retraction Guide” for detailed instructions
2. If you used the DE20, make sure the camera is “retracted” and “warmed”
3. Cryo cycle the microscope
    a. reset = holder
    b. column valves closed
    c. flu screen is down
    d. objective aperture is out
    e. high mag lenses on (send scope to EN/FA/FC preset)
    f. click “Cryo Cycle” on microscope PC (600 minutes before midnight)
4. Remove cold trap from the stand when IGP1 and IGP4 read 99
    a. You can keep the remaining LN2 inside the cold trap (off the coils) for the next user
5. Bake out the cryo holder
    a. See “Dry Pumping Station User Guide (for cryo holders)” for detailed instructions
6. Log off leginon workstation
7. Close Leginon client on both “camera” and “microscope” PC
8. Cleanup the bench space for the next user
9. Log any issues into the EM notebook or notify SEMC staff directly

Cryo Screening with Tietz TVIPS F416

Introduction:

The TVIPS is a CMOS (complementary metal-oxide-semiconductor) 16 megapixel camera with a 50 frame/sec readout (4k x 4k). It sits below the column of the microscope, below the DE20 camera (thus, the DE20 must be retracted to be able to use the TVIPS). It is a robust camera that has good SNR (good contrast) that is used primarily for screening grids. It is housed in a vacuum housing separate from the EM vacuum and is not retractable and always cooled. Dark and gain references are taken infrequently (once per month). It is controlled using “EM-Menu” software, which is found on the “camera” monitor in monitor input “DVI-2”. Suggested mags for EN/FA/FC = 62,000x; HL = 5000x; SQ = 1700x. We recommend the use of condensor2 (C2) aperture = 50um (#2) with beam spot size 5.

MCL server (on microscope computer) must be on in order to turn on EMMENU4 (software for F416) (on camera computer). If F416 computer restarted, login as supervisor.

1. After step 6 of “F20 User Guide”....
2. Open Leginon client on “camera” & “microscope” computer
    a. TVIPS camera control on monitor input DVI-2
3. Open Leginon program on leginon PC
    a. In terminal, type [betaleginon]
4. Setup Leginon session settings:
    a. Select “create new session” → “next”
    b. Select holder (TF20-cryo-holder_AMI-2)
    c. In session description, type information about sample → “next”
    d. Select project → “next”
    e. Image directory (use default… “/gpfs/leginon/username”)
    f. Connect to clients= tf20-f416 & tf20leginon → “next”
    g. C2 size = 70 um → “finish”
5. Once Leginon has started up, open the “application”
    a. Run → Application → MSI-T2 (3.3)
        i. Scope = tf20-d394
        ii. Camera = tf20-f416
        iii. Main = TF20Leginon
    b. “Run”
6. Upload your presets
    a. Preset manager → “import presets from another session” (* icon)
        i. TEM = tecnai; Digital Camera = Tietz F416 (if you do not see a camera available, restart both clients on EM and camera computers)
    b. “Find” presets from past 20 days; select desired preset (usually from the most recent user)

c. Highlight all the presets (GR, SQ, HL, FA, FC, EN) and “import” → “done”
    i. Suggested mags for EN/FA/FC = 62,000x; HL = 5000x; SQ = 1700x
d. Close window

7. “Cycle” presets to minimize hysteresis in the beam
    a. Under “preset manager” node, select “settings” button and check the “cycle presets” option (see image)

8. Wait until IGP1 = 6
    a. Open shield of cryo holder when IGP1 <15; you may see a slight increase in pressure, but should settle within a few seconds
9. Center preset beams
    a. Send stage to square of non-interest (square with carbon that you don’t mind trashing)
        i. Under “square_targeting”, select “acquisition” mouse and select square of non-interest; submit target (click green “play” button)
    b. Once the stage stops moving, check that beam is centered at all presets
        i. Center “square” preset beam
            1. Send scope to “square” preset
            2. Lower green screen
            3. Center beam using left trackball
            4. Open the “Edit” box and click “apply beam shift from scope”
        ii. Center “hole” preset beam
            1. Send scope to “hole” preset
            2. Lower green screen
            3. Center beam using left trackball
            4. Open the “Edit” box and click “apply beam shift from scope”
        iii. Center and adjust intensity of FA/FC/EN preset beam
            1. Send scope to “EN” preset
            2. Lower green screen
            3. For the EN preset, adjust beam intensity to ~0.5 sec (little screen on flu screen), with a beam diameter that is about 1.5X the size of the second circle
                a. For the FA/FC preset you can make this beam a bit brighter and smaller
            4. Open the “Edit” box and apply intensity “from scope” and beam shift “from scope”
            5. Repeat for FA and FC preset
10. Get eucentric height of your grid
    a. Send scope to sq preset
    b. Open column valves + lower green screen (R1 on knobset)
    c. Center beam using Left Knobset trackball

   d. Using Right Knobset trackball, move stage until you see a grid square with unique fiducial; center that square on the green screen
   e. "Z_focus" node→ simulate target (+ icon)
   f. Recommended- record the eucentric height value; it is useful to have an idea of what the eucentric height of your grid is, especially as you navigate around during data collection; (the eucentric height should stay relatively similar, +/- 20 um; this means your grid is fairly flat; if your eucentric height varies much more than that, your grid is probably bent/wrinkled)
11. Collect atlas of your grid (this is a series of low mag images that are stitched together to give you a whole view of your grid; ~10 minutes)
   a. Under the "Grid_targeting" node, click "settings" button (upper left hand side) and input your grid description
      i. Radius = 0.0009m = 0.9mm
   b. Click "calculate atlas"
   c. Click "play" to collect atlas
12. Insert and center objective aperture
   a. In "square targeting" node and using green "acquisition" mouse, go to a junk square
   b. Once at the junk square location, send scope to EN mag
   c. Lower green screen and send scope to "DIFFRACTION" mode (right knobset)
      i. Should see bright green small beam on the green screen with diffuse carbon diffraction ring around it
   d. Insert objective aperture and center
   e. Exit "DIFFRACTION" mode
   f. If you plan to take a break, send microscope to high mag (FA/FC/EN) to turn on the high mag lenses and close the column valves
13. You are now ready to collect some images!
14. In "Square_Targeting" select 1 square of interest
   a. Using "acquisition" mouse, select the center of square of interest
   b. Click "Submit target"
   c. Click "Submit queued targets"
15. In "Hole_Targeting" select 1 - 2 regions of holes of interest
   a. Using "acquisition" mouse, select holes of interests (on the carbon, every 2x2 holes away from each other)
   b. Using "focus" mouse, select area of carbon towards the center of the hole areas you selected in the step above (this is where leginon will acquire the eucentric height of the square)
   c. Click "Submit target"
   d. Click "Submit queued targets" <u>when you are done with every hole target</u>
16. In "Exposure_Targeting" select exposure targets
   a. Using "acquisition" mouse, select the center of the holes of interests, on the ice. Select up to 4 holes that are closest to the center of the screen (use "toggle center crosshair" tool to find where center is). This ensures that you do not collect images with a lot of image shift.

   b. Using “focus” mouse, select area of carbon towards the center of the stage (use “toggle center crosshair” tool to find where center is). This is where leginon will acquire auto-focusing for the exposure targets selected in the step above.
   c. Click “Submit target”
   d. Click “Submit queued targets”
      i. Note- at this point you have queued up a number of exposure targets for leginon to acquire. Refer to “Leginon/Appion User Guide” to view micrographs and for further instructions.
17. Under “exposure” node, select “toggle queue timeout” so that column valves will close once exposure queue is done collecting
18. To collect a new square, repeat steps 14 - 16 until you are done collecting data.
19. If you are done for the day… read “**F20 User Guide (ending your session)”**

Cryo Data Collection with the DE20

Introduction:

The DE-20 camera system is a direct detection device (DDD) manufactured by Direct Electron. It has high signal-to-noise ratio (SNR) with consistent performance across all spatial frequencies and TEM magnifications. “Movie mode” proves high-speed acquisition of a continuous stream of frames (32 frames per sec max, unbinned full-frame) with nearly 100% duty cycle (no dead time between frames). Increased data quality compared to TVIPS thru drift correction, per-particle motion correction, radiation damage compensation, post-acquisition exposure setting, etc. It has a 5k x 4k field of view (5120 x 3840) with a 6.4um pixel pitch. It is fully retractable and needs to be manually retracted and warmed when not in use, and inserted and cooled when in use. The temperature changes make it so that the camera needs a new dark and gain reference with each new session. The DE20 is sensitive to stray light (operate with window covered and lights off).

Dose should be 2.1 - 2.2 electrons/pixel/frame (no more than 2.5 otherwise clipping will occur). At 29,000X this corresponds to ~45 electrons/$Å^2$ for a 2 second exposure (1.54 Å/pixel).

1. After step 6 of “F20 User Guide”....
2. Open DE20 software to insert and cool down camera (NOTE- can only open MicroManager or Leginon client/server at one time); DE-20 camera control on monitor input DVI-1
    a. Close Leginon client on “camera” computer
    b. Open DE server GUI on “camera” computer (this program is laggy; wait 30 sec)
    c. Open MicroManager on “camera” computer
    d. Using pull-down flaps, “cool down” camera and “extend” camera (when not in use, DE20 should be retracted and warmed); it takes ~ 5 min to cool down camera
    e. Make sure that you “do not autosave”
    f. Close MicroManager
3. Open Leginon client on “camera” computer
4. Open Leginon client on “microscope” computer
5. Open Leginon program on main computer (TF20Leginon)
    a. In terminal, type [betaleginon]
6. Setup Leginon session settings:
    a. Select “create new session” → “next”
    b. Select holder (TF20-cryo-holder_AMI-2)
    c. In session description, type information about sample → “next”
    d. Select project → “next”
    e. Image directory (use default… “/gpfs/leginon/username”)
    f. Connect to clients= tf20-f416 & tf20leginon → “next”
    g. C2 size = 70 um → “finish”
7. Once Leginon has started up, open the “application”

   a. Run → Application → MSI-T2 (3.3)
      i. Scope = tf20-d394
      ii. Camera = tf20-de20
      iii. Main = TF20Leginon → “run”
8. “Cycle” presets to minimize hysteresis in the beam
   a. Under “preset manager” node, select “settings” button and check the “cycle presets” AND “optimize cycling” option
9. Wait until IGP1 = 6
   a. Open shield of cryo holder when IGP1 <15; you may see a slight increase in pressure, but should settle within a few seconds
10. Center preset beams
   a. Send stage to square of non-interest (square with carbon that you don’t mind trashing)
      i. Under “square_targeting”, select “acquisition” mouse and select square of non-interest; submit target (click green “play” button)
   b. Once the stage stops moving, check that beam is centered at all presets
      i. Center “square” preset beam
         1. Send scope to “square” preset
         2. Lower green screen
         3. Center beam using left trackball
         4. Open the “Edit” box and click “apply beam shift from scope”
      ii. Center “hole” preset beam
         1. Send scope to “hole” preset
         2. Lower green screen
         3. Center beam using left trackball
         4. Open the “Edit” box and click “apply beam shift from scope”
      iii. Center and adjust intensity of FA/FC/EN preset beam
         1. Send scope to “EN” preset
         2. Lower green screen
         3. For the EN preset, adjust beam intensity to ~0.5 sec (little screen on flu screen), with a beam diameter that is about 1.5X the size of the second circle
            a. For the FA/FC preset you can make this beam a bit brighter and smaller
         4. Open the “Edit” box and apply intensity “from scope” and beam shift “from scope”
         5. Repeat for FA and FC preset
11. Get eucentric height of your grid
   a. Send scope to sq preset
   b. Open column valves + lower green screen (R1 on knobset)
   c. Center beam using Left Knobset trackball
   d. Using Right Knobset trackball, move stage until you see a grid square with unique fiducial; center that square on the green screen
   e. “Z_focus” node→ simulate target (+ icon)

   f. Recommended- record the eucentric height value; it is useful to have an idea of what the eucentric height of your grid is, especially as you navigate around during data collection; (the eucentric height should stay relatively similar, +/- 20 um; this means your grid is fairly flat; if your eucentric height varies much more than that, your grid is probably bent/wrinkled)

12. Collect atlas of your grid (this is a series of low mag images that are stitched together to give you a whole view of your grid; ~10 minutes)
    a. Under the “Grid_targeting” node, click “settings” button (upper left hand side) and input your grid description
       i. Radius = 0.0009m = 0.9mm
    b. Click “calculate atlas”
    c. Click “play” to collect atlas
13. Insert and center objective aperture
    a. In “square targeting” node and using green “acquisition” mouse, go to a junk square
    b. Once at the junk square location, send scope to EN mag
    c. Lower green screen and send scope to “DIFFRACTION” mode (right knobset)
       i. Should see bright green small beam on the green screen with diffuse carbon diffraction ring around it
    d. Insert objective aperture and center
    e. Exit “DIFFRACTION” mode
    f. If you plan to take a break, send microscope to high mag (FA/FC/EN) to turn on the high mag lenses and close the column valves
14. Obtain dark and gain reference (needs to be done every time since the camera is warmed up after every use; use same settings as EN preset)
    a. For dark - close column valves → “correction” node → “settings” → tecnai & de-20 → 5120 x 3840 bin1, exposure = 1000ms, frame = 40ms, do not save frames, image to combine = 10, average → “dark” and “both channels” → acquire (you should see black image)
    b. For bright - open column valves → find empty square → send scope to EN preset → adjust brightness to 0.5sec on small screen → “correction” node → “settings” → tecnai & de-20 → 5120 x 3840 bin1, exposure = 2000ms, frame = 40ms, image to combine = 10, average → “bright” and “both channels” → acquire (you should see image of the camera sensor)
    c. Confirm that correction is done → “correction” and “both channels” → acquire; you should see a blank image
15. You are now ready to collect some images!
16. In “Square_Targeting” select 1 square of interest
    a. Using “acquisition” mouse, select the center of squareof interest
    b. Click “Submit target”
    c. Click “Submit queued targets”

17. In “Hole_Targeting” select 1 - 2 regions of holes of interest
    a. Using “acquisition” mouse, select holes of interests (on the carbon, every 2x2 holes away from each other)
    b. Using “focus” mouse, select area of carbon towards the center of the hole areas you selected in the step above (this is where leginon will acquire the eucentric height of the square)
    c. Click “Submit target”
    d. Click “Submit queued targets” when you are done with every hole target
18. In “Exposure_Targeting” select exposure targets
    a. Using “acquisition” mouse, select the center of the holes of interests, on the ice. Select up to 4 holes that are closest to the center of the screen (use “toggle center crosshair” tool to find where center is). This ensures that you do not collect images with a lot of image shift.
    b. Using “focus” mouse, select area of carbon towards the center of the stage (use “toggle center crosshair” tool to find where center is). This is where leginon will acquire auto-focusing for the exposure targets selected in the step above.
    c. Click “Submit target”
    d. Click “Submit queued targets”
        i. Note- at this point you have queued up a number of exposure targets for leginon to acquire. Refer to “Leginon/Appion User Guide” to view micrographs and for further instructions.
19. Under “exposure” node, select “toggle queue timeout” so that column valves will close once exposure queue is done collecting
20. To collect a new square, repeat steps 16 - 18 until you are done collecting data.
21. Start frame alignments
    a. In Appion/Leginon, select your session
    b. Login with LDAP info (upper right hand side)
    c. Select “processing”
    d. In “Direct Detector Tools” → “Select Frame Alignment” → “Full Frame alignment for Direct Electron Co. Camera”
    e. Input the following parameters:
        i. Default everything except…
        ii. Turn off dose-weighting (radiation damage compensation switch = 0”
        iii. 5 N frames to average for rolling average (odd)
        iv. Link file handling
        v. /gpfs/tmp/username queue scratch
        vi. 8 number of jobs
        vii. 8 queue memory
    f. Select “Just Show Command”
    g. Copy the command line
    h. Open new terminal and type “ssh de20framealignment”
    i. Paste the command line from step f
22. Start CTF correction (after first movie is done aligning)

a. CTF → estimate the CTF → CTFFIND v4
b. Default all settings except preset = en
c. Run on semc-head

23. If you are done for the day…refer to "**F20 User Guide (ending your session)**"

Dry Pumping Station User Guide (for cryo holders)

Normal state:
V1 – closed
V2 – open
V3 – open
V4 – open

Warm up holder
~30min cycle

Removing holder from dry pumping station:

1. Unplug holder jacket from temperature controller
2. Close V4,V3, V1, then 2 of holder
3. Unhook tygon tubing from holder and re-connect to silver stopping plug (near V1 & V2)
4. Turn pumping station off
    a. Wait for green “MDP ready” to turn off
5. Using one hand to stabilize V1/V2 vacuum area, gently apply force to pull out cryo holder
    a. Immediately upon removing cryo holder, store in cryo transfer station
6. Place blanking plug (black) into opening where cryo holder was

Inserting holder into dry pumping station (warm up):

1. Connect cold holder to the temperature controller and run the “warm up cycle”
2. Turn on pumping station and wait for green “MDP ready” state (~1 minute).
3. Make sure all valves are closed.
4. Remove the blanking plug and insert cryo holder and invert it to facilitate condensation removal.
5. Connect the plastic tygon tubing to V4 of the cold cryo holder
6. Open V2, then V1 and wait until vacuum stabilizes
7. Once warm up is done, open V3 and V4
8. Start the “zeolite cycle” for 2 hours

Cryo Holder Insertion/Retraction Guide

***Cryo holder insertions on the F20 may only be performed by SEMC staff. NO EXCEPTIONS. *** Refill holder dewar every 2-3 hours.

Cryo Holder insertion:

1. Before insertion confirm that….
    a. IGP1 = 6
    b. column valves closed
    c. holder = reset
    d. objective aperture out
    e. flu screen down
    f. airlock default pump time = 120 sec
    g. turbo on and ready (button will be orange while spinning up and yellow when ready)
2. Set holder alpha value at -60 degrees. Goniometer will rotate.
3. Position Styrofoam box below goniometer to catch LN2
4. “Pre-pump airlock.” Takes 4 seconds
5. Insert holder to first stop with holder top at 3 o’clock position. Wait 2 minutes while airlock pumps down
6. Select ‘ST Cryo Holder’ as specimen holder
7. With hand on the holder dewar, reset alpha value to 0
8. Once stage is set to 0 degrees, turn holder counter-clockwise from the 3 o’clock to 12 o’clock position. Slowly let the vacuum pull the holder in.
9. Ensure that “High Tension” and “Operate” are still yellow (did you crash the vacuum? Will be grey if vacuum crashed.)
10. Top off holder dewar with LN2 once inserted
11. Turn off turbo pump
12. Do not open the column valves until IGP1/Column < 10. If you are ever in doubt, close the column valves and contact an EMG staff member immediately

Cryo Holder retraction:

1. Close column valves, reset holder, objective out, flu screen down, cryo shield of holder closed.
2. Gently brace against the column and pull holder back till it stops, then turn clockwise to 5 o’clock and pull straight out of the column.
3. Watch IGP1/Column vacuum for spike. If vacuum spikes and column pressure crashes, contact an EMG staff member immediately.
4. Turn turbo on to prepare for next insertion.

Notes on Leginon

- Due to the top-down workflow of Leginon, if you decide that you want to abort a target or a queue of targets….
    - Click “abort” and “abort queue” at the node (square_targeting, hole_targeting, or exposure_targeting)
    - Wait for Leginon to “park” (no more moving green circles at any of the nodes)
- If you want to skip a square that you do not like….
    - Submit zero targets for that square
    - Go to “Square_Targeting” node
    - Select new targets with acquisition mouse
- If you want to do a small movement or recenter your image
    - Under “Navigation” node, select the preset (mag) that you want to adjust in (pull down window); “send to scope”
    - Acquire image (click camera icon)
    - Select “stage position”
    - Using “toggle crosshair tool” and “click tool”, click on the image where you’d like to center the image
- If you do not like the square you are in and want to abort the submitted queue of targets…
    - Go to the “exposure” node, and click “Abort” and then “Abort Queue”.
    - Wait until active nodes are static (no more green circles rotating at any node).
    - Repeat steps 14 - 16 to pick a new square (and new holes, and new exposure targets)
- If you have selected too many square or hole targets…
    - Go to the node above, and press PAUSE; this will direct leginon to stop queueing more square or hole targets, and will begin collecting hole or exposure targets
- If you would like hole targets to continuously collect as you select exposure targets…
    - Hole node settings → wait for a node to process image = false

F20 Alignments

F20 alignments should be done by EMG staff ONLY!!

Alignments can be done with room temperature (RT) holder or cryo holder.

*Note- if you're confident that the alignments on the FEG are good, skip gun alignments (steps 1 & 2)

1. Gun tilt
    a. Remove objective aperture (silver tab pointed right)
    b. Center the stage (with stage shift trackball/joystick on right knobset) over a square with intact carbon and some large features that are visible at low mag.
    c. With microscope computer, under "Tune" tab → "Gun" folder → "Gun Tilt"
    d. Focus beam (with intensity knob)
    e. Center beam (beam shift trackball on left knobset)
    f. Open beam (intensity knob) CW past crossover (past second circle) and use MF X and Y to get the brightest beam (lowest exposure time)
        i. Stay away from edge of FEG
    g. Condense and center the beam (trackball)
    h. Open beam up to the RIGHT so that it's just past second circle
    i. Center using condenser 2 aperture
    j. Click next, repeat steps b-e
2. Gun Shift ("Tune" → "Gun" → "Gun Shift")
    a. Condense beam, then center beam using MF x and Y
    b. Repeat for next step
3. Eucentric Height and Focus (want to be at slightly higher mag 5-10K)
    a. Find a piece of dirt that is large (easily visible at low mag, ~5k)
    b. "Stage/beam" tab → "Stage2" flapout → "Wobbler".
    c. Use "Z axis" +/- (right knob-set) to adjust stage z-height until feature on screen stops moving
    d. Rough focus by pressing "Eucentric focus" on right knob-set
    e. Focus beam using "Focus" knob on right knob-set and camera/FFT. Reset defocus to 0 (press R3 on right knobset)
4. Condensor Astigmatism (high mag, ~135k)
    a. "STIG/FEG" tab → "Condensor"

This is a side view of the objective aperture, but adjustments to condensor aperture 2 is the same

   b. Use MF X and Y to make sure beam opens and closes circularly and concentrically over both sides of crossover
   c. Click “None” when done
5. Objective Aperture Centering (~25 – 50K)
   a. Focus and center beam to a point
   b. Click “Diffraction” on right knob-set
   c. Center beam using MF X and Y
   d. Insert objective aperture (silver tab pointed left) and center aperture
6. Objective Astigmatism (~25 – 50k)
   a. At slightly underfocus (2-3 thon rings) use Live FFT and get rings to be a circle (elliptical = objective astigmatism). Use MF X and Y to correct the astigmatism.
7. Coma Free Pivot Point X and Y (“Tune” → “Direct Alignments” → “Coma free pivot point X”)
   a. Go to zero focus using camera and live fft.
   b. Green screen down. Focus beam to the size of an pencil eraser
   c. Click “Coma free pivot point x”
   d. Use MF X and Y to minimize beam movement between the 2 pivot points
   e. Repeat for pivot point Y
8. Coma Free Alignment X and Y (“Tune” → “Direct Alignments” → “Coma free alignment X”)
   a. Using Live FFT, set the beam to be a little under focused (-250 - 500nm)
   b. Set the camera so that you can get a quick readout from the camera during the coma free alignment (set exposure to ~0.5s and condense beam to just inside the inner circle)
   c. Use MF X and Y so that rings to stay the same size (It’s OK if it looks like a “rotation”, but need to correct if the rings are changing in diameter)

Troubleshooting

HT should be brought up by EMG staff ONLY!!

1. If the column pressure crashes and HT and FEG turn off… (This can happen for a number of reasons; during cryo holder insertion/retraction)
    a. Notify EM staff
    b. Wait for TMP rollout to recover (~7 minutes)
    c. Wait for column pressure to recover (IGP < 0) (30 - 60 minutes)
    d. Manually set HT to 80 kV
    e. Click “HT” to turn on
        i. Wait till measured HT is 80 kV (~ 2 minutes)
    f. Click “Operate” to turn FEG on (~ 2 minutes)
        i. Emission should be < 10 uA
        ii. After any increase in HT, wait a few minutes for emission to recover and stabilize
    g. 80 → 120 kV, 10 kV steps
        i. Free high tension = true
        ii. 10,000 eV steps
        iii. Select >
    h. 120 → 160 kV, 3 kV steps
        i. Free high tension = true
        ii. 3,000 eV steps
        iii. Select >
    i. 160 → 200 kV, 1 kV steps
        i. At 200 kV, should read ~ 6 uA emission
    j. Make sure FEG is operating at 4150V (may need to select the ↲ button to refresh it)
2. Align presets to one another (only necessary if targeting between different presets are inaccurate)
    a. Find feature that is visible at low mag (using “navigation” tab, send microscope to “square” and take picture; using “stage position”, “crosshair tool” and the “clicking tool” center a distinguishable edge of a feature in the center of the stage
    b. Send scope to EN mag, and find that feature with the flu screen
    c. Take a picture under EN; center the feature (using stage position)
    d. Send scope to “HL” preset and take a picture; using “image shift”, center the feature using the click tool
    e. Go to “preset manager” and apply the image shift to the HL preset “from scope”
    f. Under “navigation” node, send scope to “SQ” preset and take a picture; using “image shift”, center the feature using the click tool
    g. Go to “preset manager” and apply the image shift to the SQ preset “from scope”

h. Under “navigation” node, send scope to “GR” preset and take a picture; using “image shift”, center the feature using the click tool (you may need to take out the objective aperture) (from SQ to GR, there is a flip/translation in the image)
i. Go to “preset manager” and apply the image shift to the GR preset “from scope”
j. You are done!
    i. Go back to navigation node and reset back to “stage position” for movements