

Low Dose Mode, Navigator, and Energy Filtering in SerialEM

Low Dose Mode

- Low dose mode lets you define 4 “areas” with independent mag and beam settings
 - View: a low mag overview ($\sim 5000\times$)
 - Focus: for focusing, displaced from center along tilt axis
 - Trial: for tracking, also displaced, usually same as Focus
 - Record: centered area of interest, for final acquisition
- An area is activated by taking a picture of the corresponding type or by dropping the screen and selecting the “Area to show when screen down”
 - “Preview” also takes a picture of the Record area
- Tasks generally use View area for low mag images
 - Rough and fine eucentricity, reverse tilt, image shift reset
 - Image shift reset has an option to use Trial area
 - Walk up uses Trial area, regardless of mag
 - User is responsible for setting mag low enough to run tasks reliably
- There are two autoalign buffers, and Record/Preview and Trial are automatically aligned to first or second one (usually D, E)

Setting Area Properties and Location

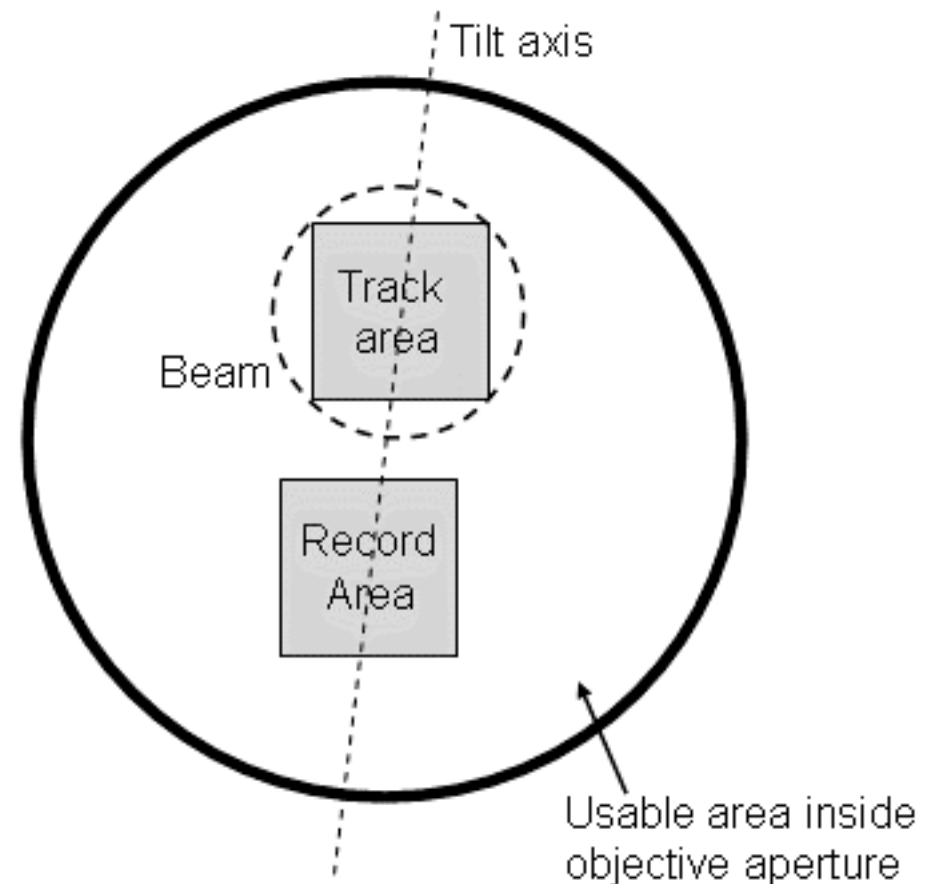
- Properties of an area are set up by turning on “Continuous update” and adjusting them – mag, beam, filter settings
 - “Set Intensity” will also change stored intensity of current area without Continuous update being on
 - But otherwise, changes will go away when you take a new picture
 - The property line is grayed out except when Continuous update is on
- “Copy current area mag and beam to” is useful for areas with the same mag, although you will still want to adjust the beam
- To set the trial/focus area offset, select one under “Define position of area” then set/adjust position by any method:
 - Typing in a position
 - Taking a view image and clicking in it
 - Taking a trial/focus and clicking in it or shifting it
- “Maximum area separation” is distance from circle circumscribing the Trial area to edge of Record area
 - Size of each area is defined by camera parameters
 - So watch out if you have a separate, smaller Focus area

Dealing with Beam Centering Problems

- Condenser lens hysteresis can make beam centering vary between areas and depend on last area visited before going to an area
- “Normalize beam through View” passes intensity through View setting when switching between other areas: makes position reproducible
- “Additional beam shift” can then be set to center beam for particular area
- These features are needed at very high mag or when there is high lens hysteresis (e.g., Berkeley JEOL 3100)
- Additional beam shift is also good for providing extra safety factor in separation from Record area. To use it:
 - Turn on “Set”, move beam, turn off “Set”: the beam movement will be recorded as an incremental shift for that area
 - Get the beam centered on one area with no shift (i.e., Record) then set shift in other areas

Dealing with Limited Image Shift

- Use “Balance Shifts” to shift the Record and Track/Focus areas by equal and opposite amounts instead of applying all the shift to the Track/Focus area
- Image shift away from center is thus half as much, which is useful when image shift is limited (e.g., by objective aperture on F30)
- To use, center objective aperture with low dose off or before pressing “Balance Shifts” (and with image shift at zero!)
- When either button is pressed, there is a (non-intuitive) shift of image and of viewed specimen position, dictated by the logic of the situation



Assessing Dose

- When dose is calibrated, the Low Dose panel shows a readout of the dose from taking a picture at current area
- Dose is also shown in Camera Setup dialog – this is the only way to see Preview dose
- In Tilt Series Setup, there is an estimate of total dose as a multiple of the dose at 0 degrees and at the current angle
 - Requires constant or $1/\cosine$ (to power) intensity control
 - Takes into account the current Record dose and the designated number of tilts
 - Takes into account whether you say intensity is already set up for high tilt or for zero tilt

Low Dose Tilt Series Setup

- No low mag tracking – you need a Trial area with low enough mag to track reliably
- Use 1/cosine intensity; adjust power to balance dose distribution versus unevenness in SNR through series
 - 1/cosine will give twice the dose at 60° as at 0°: this uses a lot of dose at high tilt if you want a certain minimum at 0°
 - $(1/\cosine)^{1/2}$ will give 1.41 x the dose at 60° as at 0°: this gives a more even distribution of dose with modest loss in SNR at high tilt
- For cryo, at least with Gatan holder, it is recommended to go to high tilt before starting tilt series
 - You can set up the intensity there that will give the right exposure at zero tilt (if you can do the arithmetic)
 - Or you can leave intensity at zero tilt value and select “Use current intensity at zero tilt”

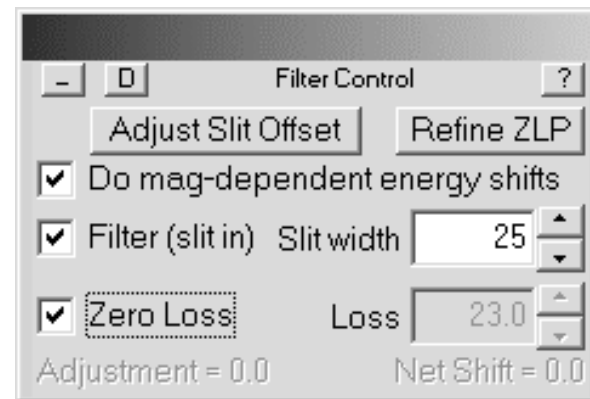
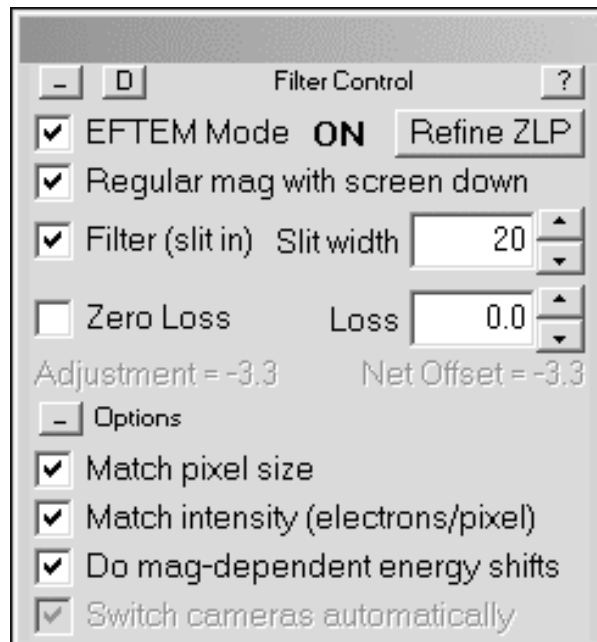
Tracking Control in Low Dose

- You can make the “Repeat Record” percentage higher or uncheck it
 - In Low Dose mode, it will ask before retaking Record images
- If drift is a problem, you can track after instead of before autofocusing, or even before and after (until things settle down)
- In Low Dose there are two references (Trial and Record) which can conflict
 - Small conflicts are resolved by shifting the tracking reference by the same amount that a Record is shifted to align with its reference
 - If conflict is bigger than amount set in “Get new track reference if...”, a new track reference is taken
 - If camera is slow and there is drift, this reference won’t be in the right place any more since the Record was taken > 15 sec before
 - Thus the option to “Align with Preview before getting new track reference”: the track reference is taken very soon after this Preview
 - Preview must have enough counts to give a good correlation
 - If many new references are taken to reduce conflicts, raise the percentage criterion or give up and “Align only with tracking images”

Using an Energy Filter

- With a filter, the basic modes of image acquisition are:
 - Unfiltered: slit open or retracted
 - Zero-loss filtered; typically with 20-25 eV slit
 - Filtered at a particular loss for elemental detection or most probable loss imaging
- Filter Control Panel has controls for these modes of imaging:
 - Slit in
 - Slit width, usually limited to 5- 50 eV
 - Energy loss, and a “Zero-loss” checkbox for convenience

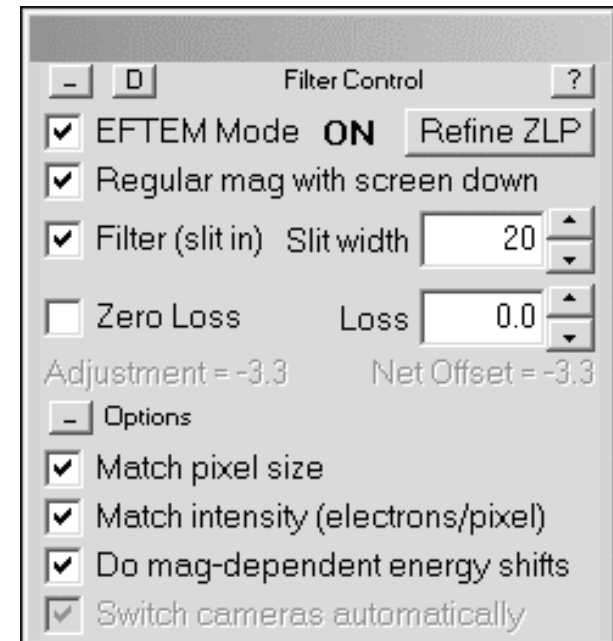
GIF



Omega filter

Problems in Energy Filtering and Features to Help

- Huge post-column mag in GIF requires EFTEM lens mode
 - Option to go out of mode with screen down for viewing specimen and beam
 - Options to make the switching more useful (Match pixel size)
- Need to recenter the zero loss peak periodically
 - Refine ZLP routine that assumes the alignment is close, uses minimal dose, and can be run periodically in tilt series
- Shift in zero-loss alignment with mag
 - Has a calibration routine
 - Option in panel to use the calibrated shift
- Adjustment is computed from:
 - Shift due to mag change
 - Refinement in ZLP position
 - Half change in GIF slit width
- Net offset should match TUI value



Navigator Concepts

- Positions are all kept track of as stage coordinates
 - Current stage position added directly with “Add Stage Pos”
 - In an image with known stage position and mag, each point added with “Add Points” is converted to a stage position
- Items can be points, polygons, or maps
 - A map is an image in a file whose position and other features the Navigator keeps track of
- “Go To” moves the stage to the center position of an item
 - You can click in an image and use “Go To Marker” to move there without making it an item
- All items overlapping an image will be drawn on the image

Maps

- Most maps are montages. The Navigator lets you set up montage parameters for making a map in three ways:
 - Defining points as corners
 - Drawing a polygon
 - Taking the whole grid (limits can be set in property file)
- These montages can skip unneeded pieces
- “New map” makes an image a map after it has been saved
- “Load map” or double clicking table entry loads map into read buffer

Automatic Map Acquisition

- To acquire maps at a series of locations, first add a point at each location
- Mark each added point as “Acquire”
- Prepare an image file to receive the maps:
 - For single frame images, the current file must be able to store Record images
 - For montage maps, draw a polygon of desired area and use “Setup Polygon Montage”
- Select “Acquire Areas” and go for coffee/lunch
- Each new image will become a map and the original point at that location will be turned off

Registrations and Transforming Positions

- A “registration” is a fixed relationship between specimen and stage positions
- If this relationship changes, specimen points will not be at the correct stage positions – the grid is are in a new registration
- If you mark well-defined points in the original relationship as registration points, then mark the corresponding points in the new situation, all the stage coordinates can be corrected
- Rotation and even stretch between the two sets of registration points can be determined
- When you move the grid in the holder, it is intuitively obvious that you are in a new registration – so increase the registration number and find corresponding points
 - But what about when you haven’t moved the grid, but the map doesn’t take you where it should?

Fixing Local Map Positions with a Single Point

- After large stage movements, (our) stages don't seem to come back to defined points very well
- Thus, after taking a series of medium mag maps, and going to one, the features do not appear at the expected locations
- To correct this with a single pair of registration points:
 - Pick a well-defined feature in the map that is recognizable in a new image
 - Add a point and set it as a registration point
 - Increment the current registration
 - Take a new image, add a point at the feature, and mark it as a registration point with the same number
 - Select “Transform Items” from the Navigator menu
- Now when you click in the map, “Go To Marker”, and take an image, the marked position should appear
- If you change the current registration back to 1, you can transform items back to where they were (which might be better for a map in some other area...)