

Announcing the JEOL 3200FS as a Walk-Up 300 kV (S)TEM for NUANCE

NUANCE January Tech Talk: BioCryo
January 21, 2026

Dr. Christopher Sharpe
Postdoctoral Research Associate
NUANCE BioCryo & EPIC-FIB



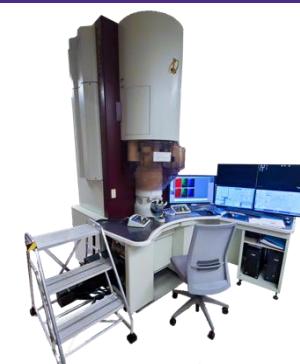
Last week, an information security issue was identified that required immediate maintenance be performed on all NUANCE computer systems, which is interrupting network connectivity between instruments and the Northwestern network (including the Research Data Storage Service). At this time, most NUANCE instruments are working normally, however, all data must be saved locally on the instrument computer, and no systems or computers should be connected to a network port while maintenance proceeds.

In the meantime, please save your data with the format “yyyymmdd_netid” in your individual folder, and NUANCE staff will work to get your data to you. If you urgently need a piece of data, please let us know.

Please also remember that USB devices (flash drives, hard drives) are not permitted on any NUANCE computers.

We are grateful for your patience as we complete this maintenance. We are working diligently to have an updated data transfer solution in place as soon as possible.

TEMs at NUANCE



Instrument	Hitachi 2300A STEM	JEOL 1400 Flash TEM	JEOL 3200FS (S)TEM	JEOL ARM300F (S)TEM	JEOL ARM200CF AC-(S)TEM
Source	Schottky FEG	LaB ₆	Schottky FEG	Cold FEG	Cold FEG
Voltage(s)	80, 120, & 200 kV	120 kV	300 kV	40, 80, & 300 kV	60, 80, & 200 kV
Modes	STEM, Cryo	TEM, Cryo	TEM, STEM, Cryo, Diffraction, <i>in situ</i>	TEM, STEM, Cryo, Diffraction, <i>in situ</i>	STEM, TEM, Diffraction, <i>in situ</i>
Detectors	HAADF, TE-BF or SE Dual EDS	4K OneView with Drift Correction	Rio & K2 Summit, BF & ADF	OneView IS, K3 IS, Stela, BF/ABF/ADF/HAADF, EDS, Dual EELS, 4D-STEM	OneView, K2 Summit, HAADF/BF/ADF/BSE, Dual EDX, Dual EELS, ARINA 4D-STEM
Unique Advantage	Aligned like an SEM; 30-45 min sessions are typical	Intro TEM, Primary Cryo-TEM, Very user friendly	In-column Ω Energy Filter for contrast; SerialEM for ET & Montage Mapping	Most detectors, Wide-gap pole piece for <i>in situ</i> holders	Aberration-Corrected: 0.8 Å Resolution (STEM)

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TEMs at NUANCE: Access Policies



	Instrument	Source Voltage(s)	Modes	Detectors	Unique Advantage
Instrument	Hitachi 2300A STEM	JEOL 1400 Flash TEM	JEOL 3200FS (S)TEM	JEOL ARM300F (S)TEM	JEOL ARM200CF AC-(S)TEM
Source Voltage(s)	Minimum Reservation: 30 minutes	Minimum Reservation: 30 minutes	Minimum Reservation: 30 minutes 1-hour cut-off	Minimum Reservation: 60 minutes	Minimum Reservation: 120 minutes
Modes	Minimum Reservation: 30 minutes	Minimum Reservation: 30 minutes	Pre-Aligned Walk-Up		
Detectors	Aligned like an SEM; 30-45 min sessions are typical	Intro TEM, Primary Cryo-TEM, Very user friendly	In-column Ω Energy Filter for contrast; SerialEM for ET & Montage Mapping	Most detectors, Wide-gap pole piece for <i>in situ</i> holders	Aberration-Corrected: 0.8 Å Resolution (STEM)
Unique Advantage					© Dr. Christopher Sharpe, CC BY-NC.

JEOL 3200FS

Walkup Policy



JEOL 3200FS (S)TEM

Schottky FEG
300 kV

TEM, STEM, Cryo,
Diffraction, *in situ*

Rio & K2 Summit,
BF & ADF

In-column Ω Energy
Filter for contrast;
SerialEM for ET &
Montage Mapping

What does “Pre-Aligned Walk-Up” mean?

- BioCryo staff will fill the ACD cold finger and do a TEM alignment every (weekday) morning
- From 10 am to 5 pm each weekday when the microscope is available, users can simply walk up, make a reservation, and quickly check a sample (<30 min session)
 - For TEM imaging, there should be minimal to no alignment needed – just spot size, apertures, and energy filter (if desired)
 - For diffraction, only spot size, apertures, camera length, and diffraction focus should need to be changed
- For STEM, BioCryo staff will save an alignment file for users to load; from there, only minor alignment with the ronchigram and adjusting detector gain will be needed for imaging
- Users will NOT need to fill the ACD heater between 10 am and 5 pm
- Users with advanced projects (e.g. *in situ*, electron tomography, whole-grid montage mapping) can still schedule their sessions normally
- **HOWEVER**, this access mode will require the microscope to be under a maintenance hold from 5 pm to 8 pm every weekday. **If you wish to use the microscope in that window, email BioCryo staff before 3 pm that day.**

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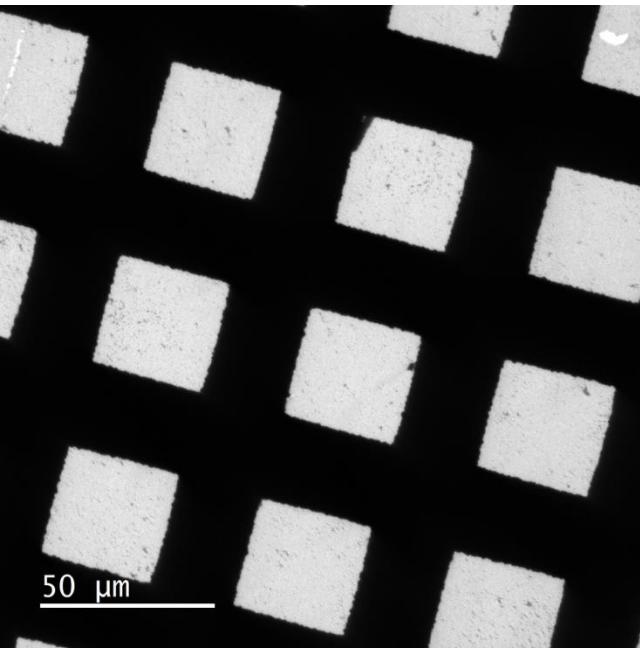
JEOL 3200FS Features



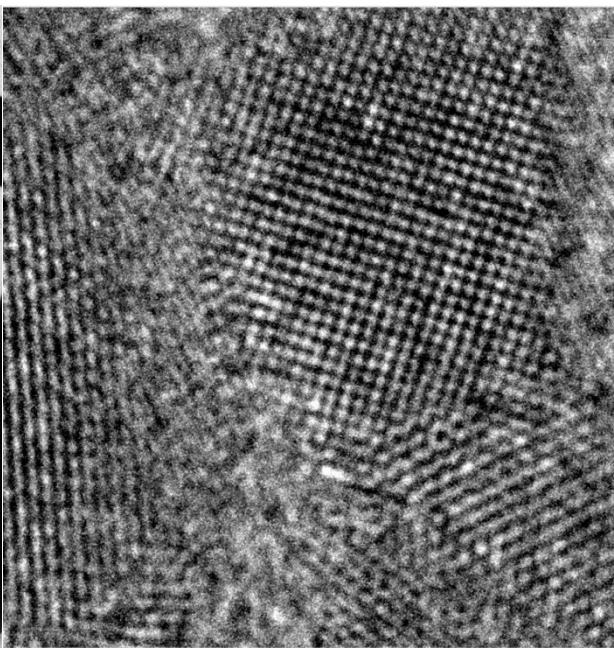
		What makes this microscope a good walk-up instrument for NUANCE?
Instrument	JEOL 3200FS (S)TEM	<ul style="list-style-type: none">➤ TEM, STEM, & Diffraction modes
Source Voltage(s)	Schottky FEG 300 kV	<ul style="list-style-type: none">➤ The anti-contamination device (ACD) dewar is quite large, so it can hold temperature for an entire 8-hour day
Modes	TEM, STEM, Cryo, Diffraction, <i>in situ</i>	<ul style="list-style-type: none">➤ The microscope's mag series cover an uncommonly large range. Low Mag: 100x-3000x Mag: 2500x-6000x 8000x-500,000x 600,000x-1,500,000x
Detectors	Rio & K2 Summit, BF & ADF	<ul style="list-style-type: none">➤ Unique gun valve + in-column energy filter configuration allows for exchanging samples without needing to adjust apertures or discharge the filament
Unique Advantage	In-column Ω Energy Filter for contrast; SerialEM for ET & Montage Mapping	<ul style="list-style-type: none">➤ Stored alignment files for resetting alignment <p>What is this microscope missing?</p> <ul style="list-style-type: none">➤ EDX & EELS

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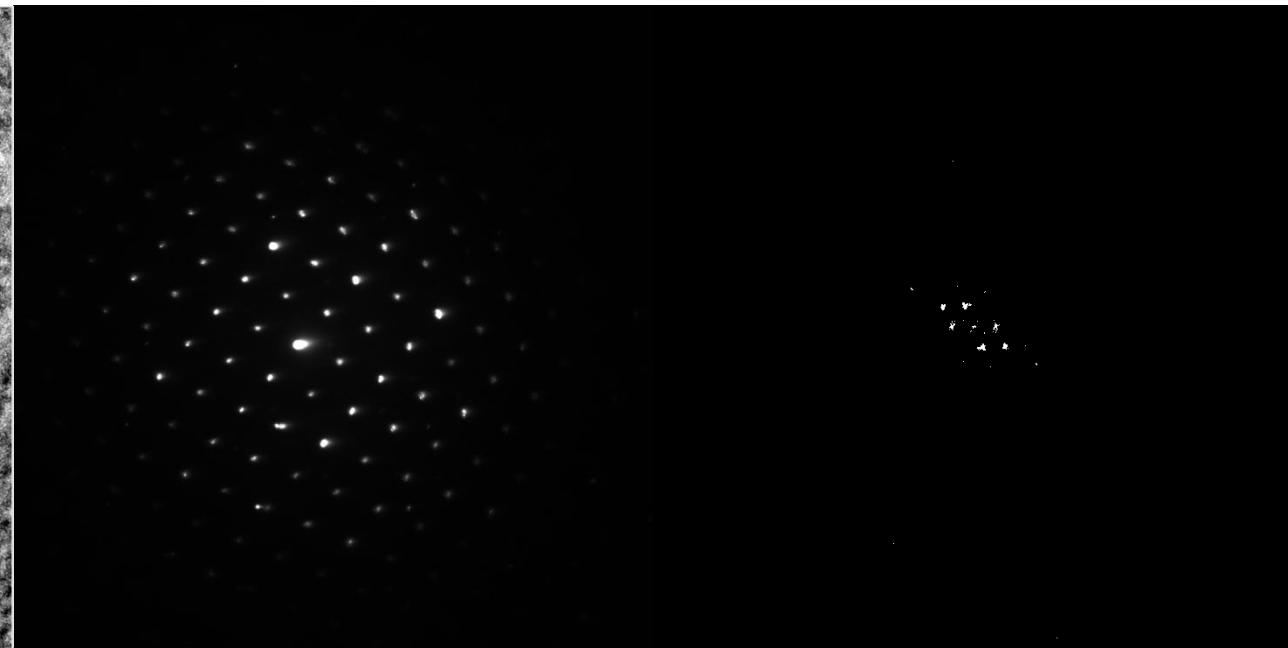
What kind of data can I get with a walk-up session?



Grid Screening w/ Low Mag
Chris Sharpe & Reiner Bleher



AuNP Atomic Lattice Planes
Reiner Bleher



Hard Matter Diffraction
Xiaobing Hu & Tirzah Abbot

Soft Matter Diffraction
Jonathan Lei

Our goal is to streamline access to high resolution TEM and provide an option for pre-screening samples prior to sessions on the ARMs

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JEOL 3200FS

History



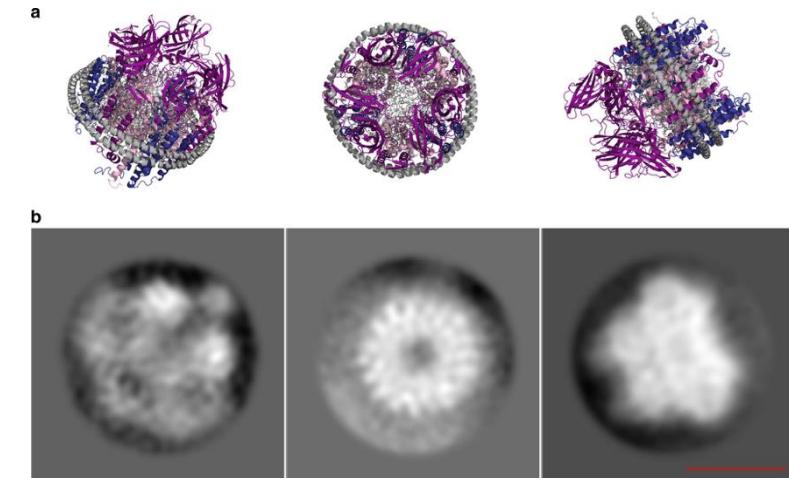
Instrument	JEOL 3200FS (S)TEM
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Unique Advantage	In-column Ω Energy Filter for contrast; SerialEM for ET & Montage Mapping

Microscope was built as a custom cryo-TEM for Northwestern's Center for Structural Biology

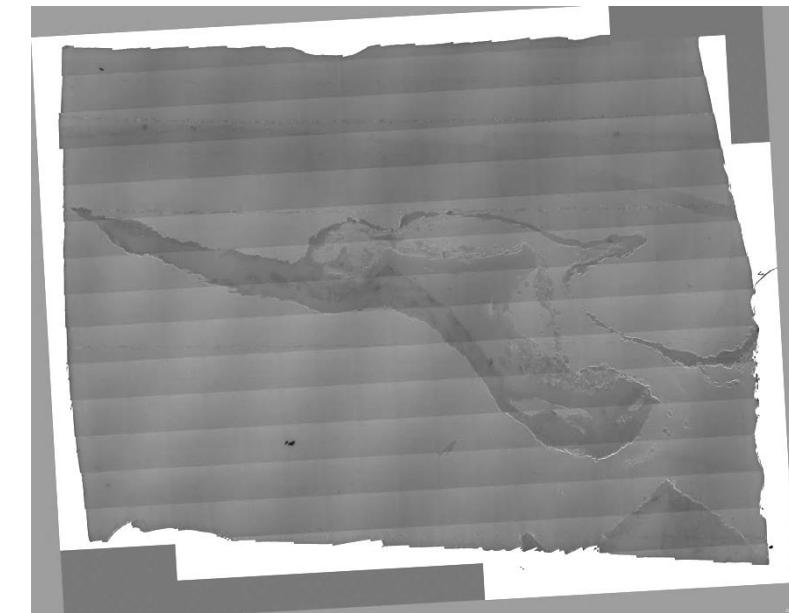
- Most similar to an Ultra-High Resolution (UHR) JEOL 3200FS
- Extensively used for high-resolution Single Particle Analysis (SPA)

Transferred to NUANCE BioCryo in 2022 for soft matter imaging

- Thin section imaging
- Cryo-TEM
- Whole-Grid Montage Mapping
- Tilt Series Electron Tomography
- Energy Filtered Diffraction
- Methods Development



(a) Modeled and
(b) SPA class averages of pMMO nanodisc complexes. *Nat. Comm.*, 2019. NU Structural Biology.



SerialEM
Montage Map of an entire black widow major ampullate silk gland section.
C. Sharpe dissertation.
NUANCE BioCryo.

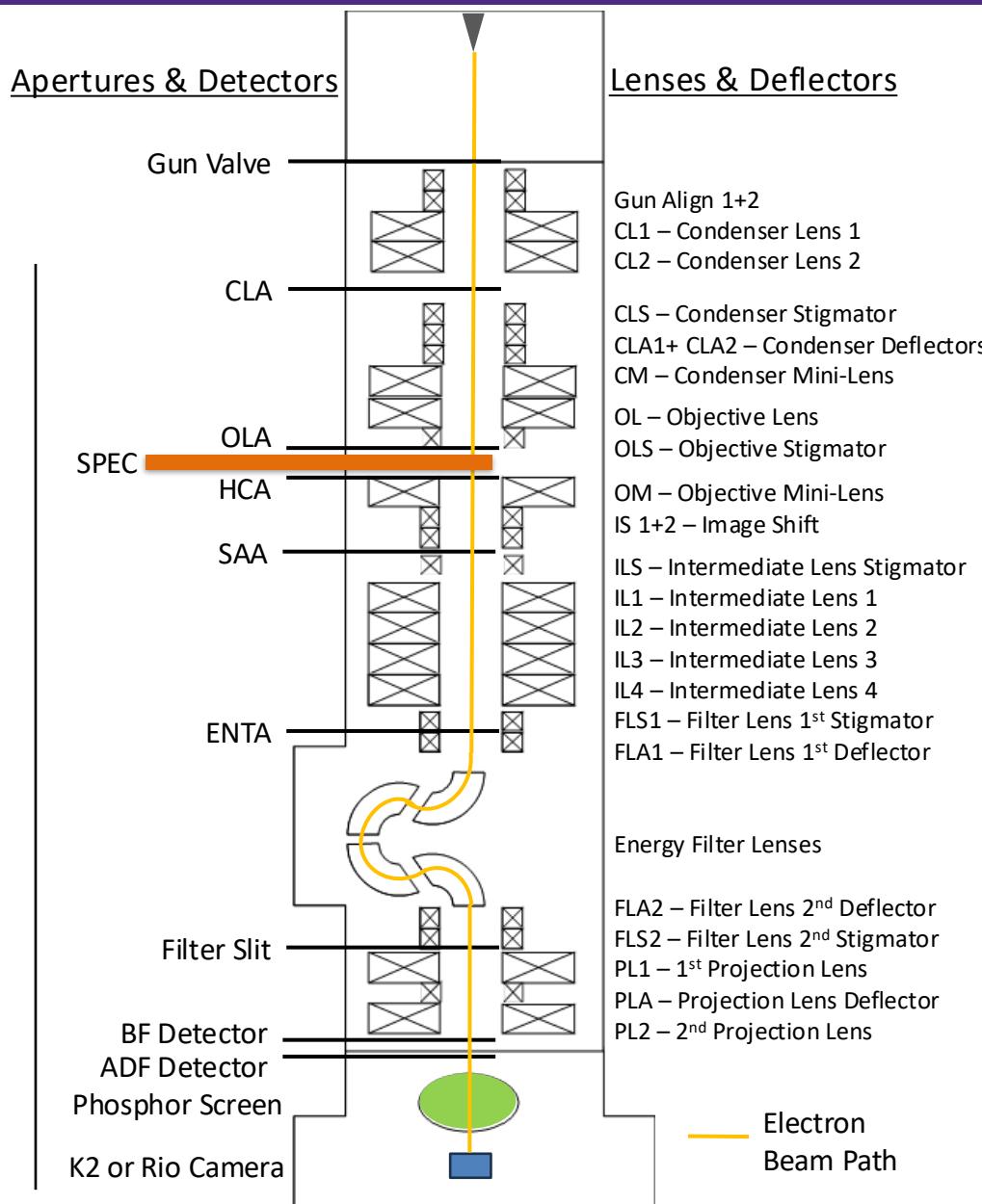
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JEOL 3200FS

Features



Instrument	JEOL 3200FS (S)TEM
Source Voltage(s)	Schottky FEG 300 kV
Modes	TEM, STEM, Cryo, Diffraction, <i>in situ</i>
Detectors	Rio & K2 Summit, BF & ADF
Unique Advantage	In-column Ω Energy Filter for contrast; SerialEM for ET & Montage Mapping



Available Holders

- Single-Tilt
- High-Tilt Tomography (single axis)
- Double Tilt
- Cryo: ELSA & Gatan Holders
- *in situ*: Protochips Liquid Cell (Gianneschi group)

Available Apertures & Energy Filter

- Condenser Lens Apertures (CLA)
 - 3 options (L, M, S)
- Objective Lens Apertures (OLA)
 - 2 phase plates, 1 aperture (OLA3)
- High Contrast Apertures (HCA)
 - 3 options (L, M, S)
- Selected Area Apertures (SAA)
 - 3 options (L, M, S)
- Filter Entrance Apertures (ENTA)
- In-Column Ω Energy Filter
- Filter Slit: 15-30 nm width (typical)

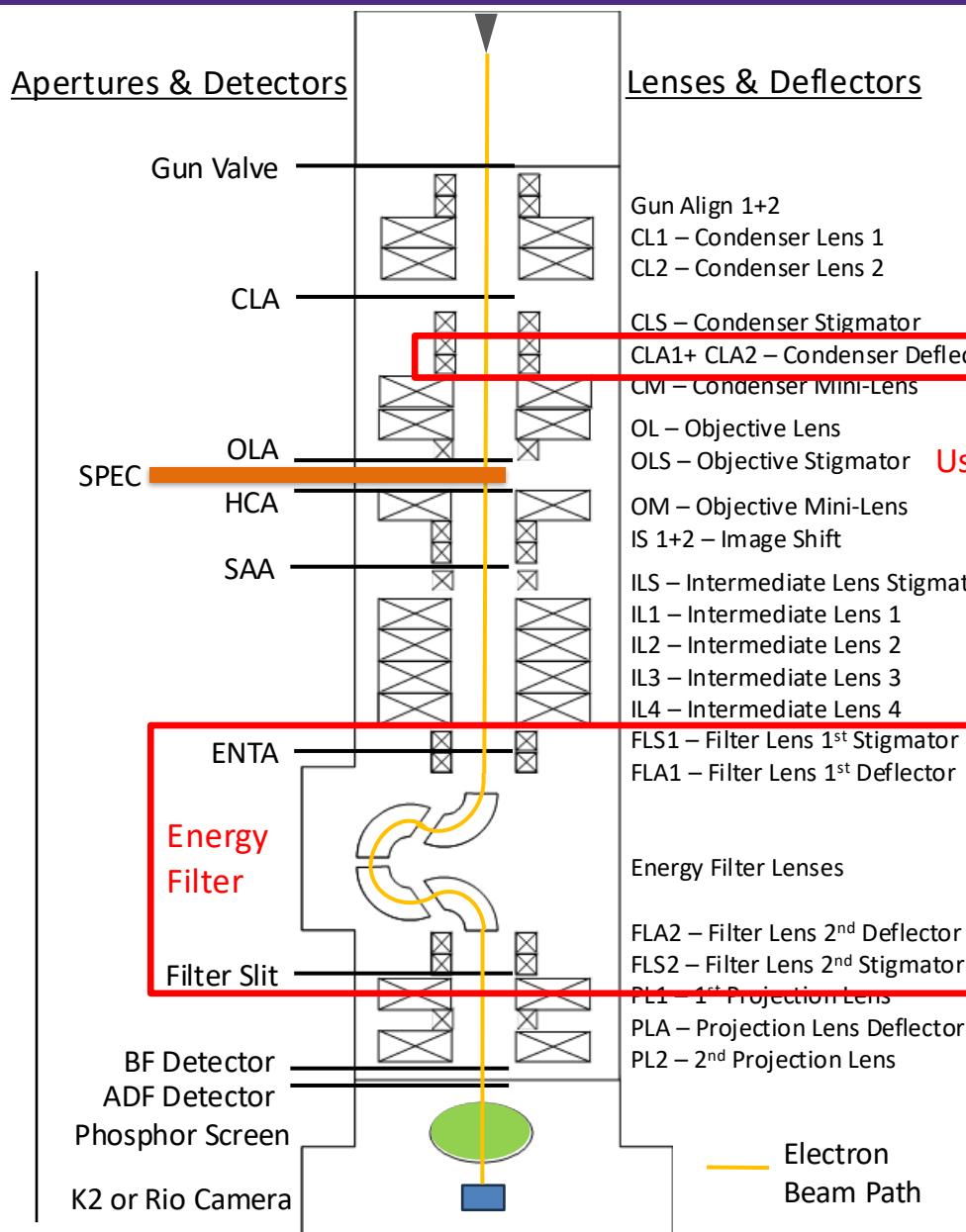
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JEOL 3200FS

Features



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Unique Advantage	In-column Ω Energy Filter for contrast; SerialEM for ET & Montage Mapping



Available Holders

- Single-Tilt
- High-Tilt Tomography (single axis)
- Double Tilt
- Cryo: ELSA & Gatan Holders
- *in situ*: Protocols Liquid Cell (Gianneschi group)

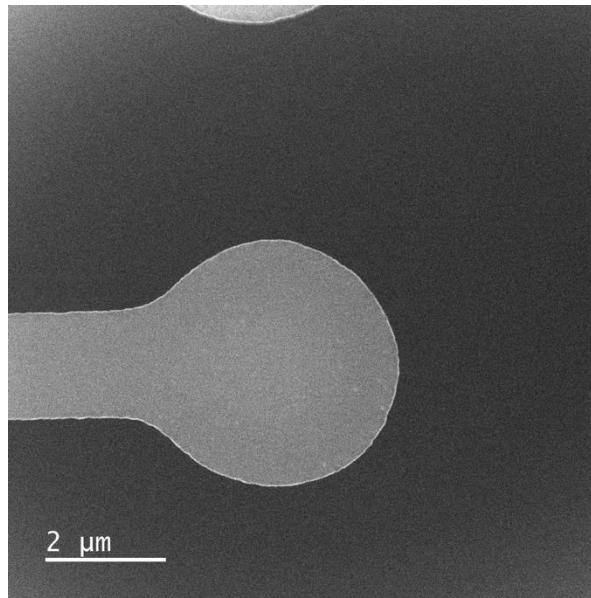
Used to raster beam for STEM

Available Apertures & Energy Filter

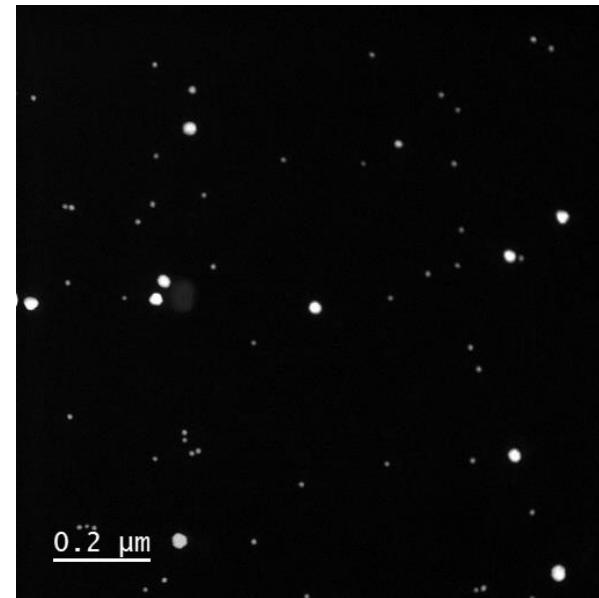
- Condenser Lens Apertures (CLA)
 - 3 options (XL, L, M, S)
- Objective Lens Apertures (OLA)
 - 2 phase plates, 1 aperture (OLA3)
- High Contrast Apertures (HCA)
 - 3 options (L, M, S)
- Selected Area Apertures (SAA)
 - 4 options (XL, L, M, S)
- Filter Entrance Apertures (ENTA)
- In-Column Ω Energy Filter
- Filter Slit: 15-30 nm width (typical)

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STEM on the JEOL 3200FS

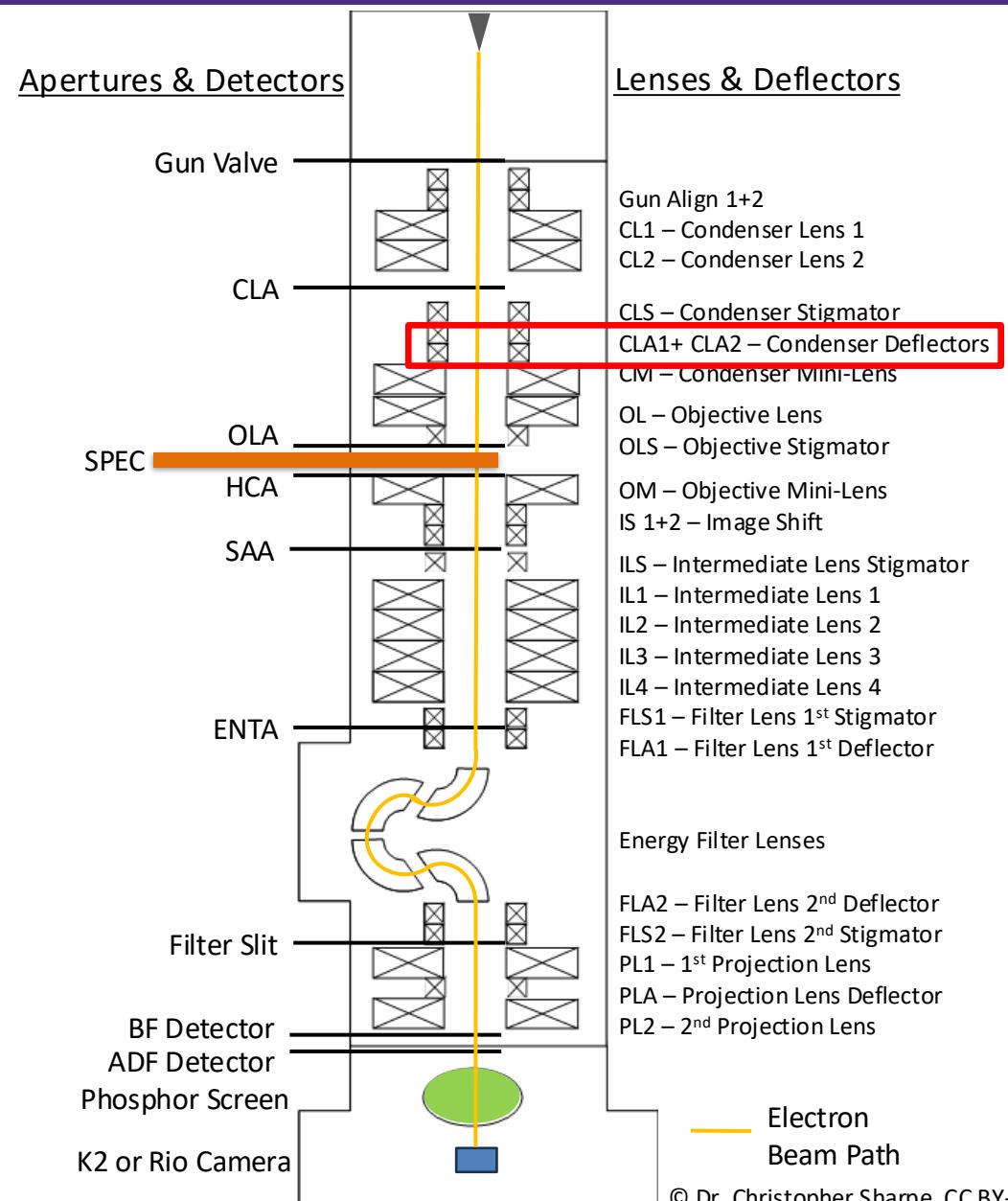


Liquid-filled nanochannel,
30kX, ADF. *Insight Chips*



Gold Nanoparticles,
250kX, ADF. *Chris Sharpe*

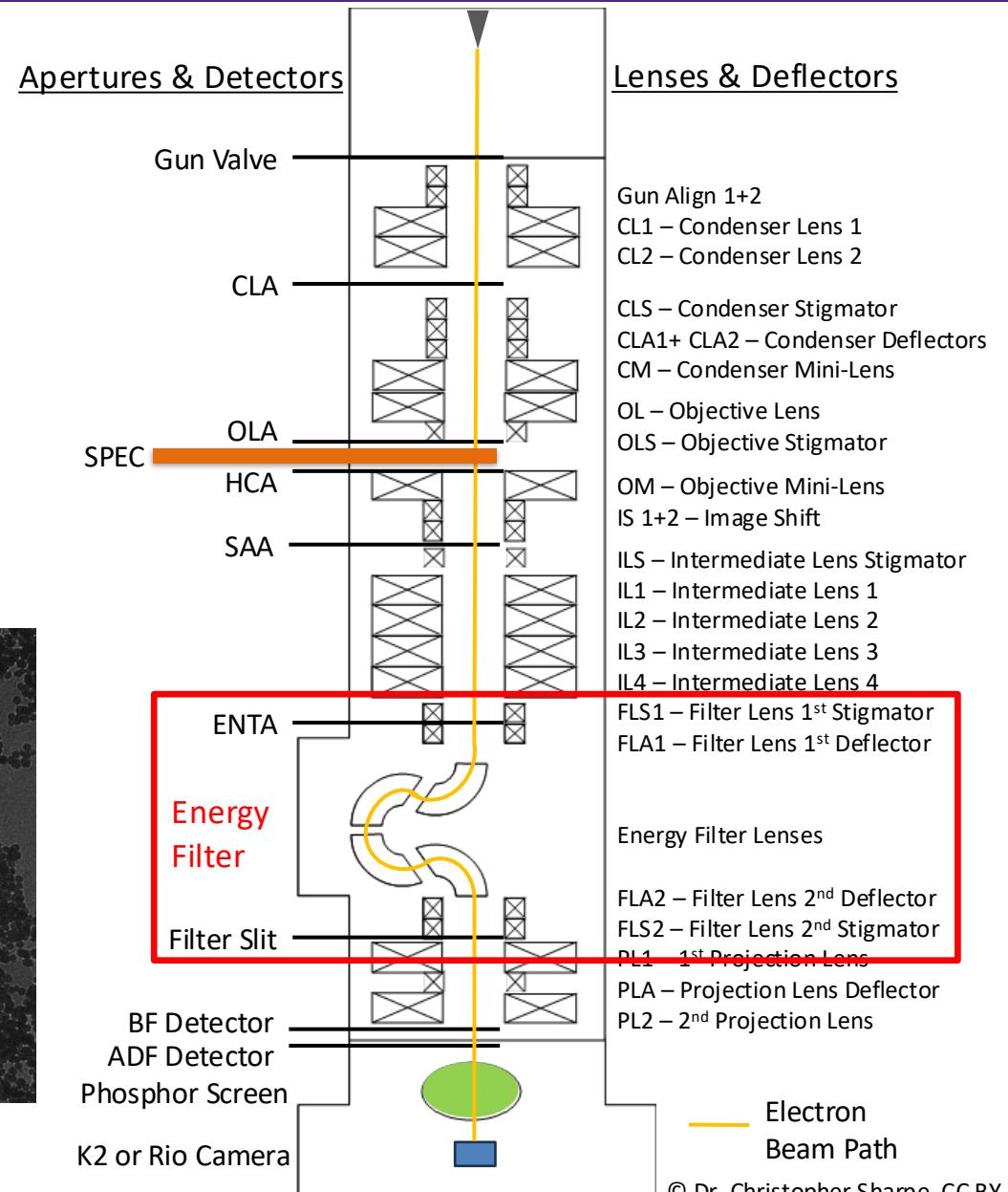
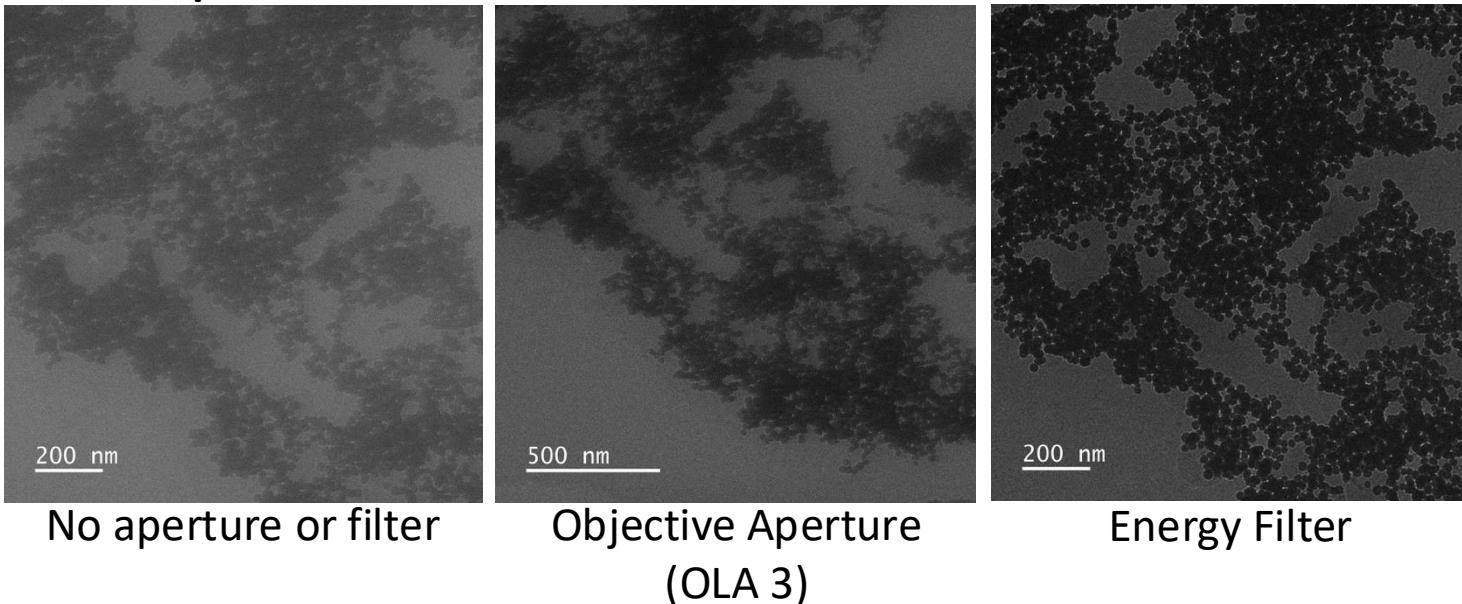
- Re-developed capability for *in situ* liquid cell imaging
- As the microscope lacks dedicated scan coils, the minimum STEM magnification is ~20kX
- Maximum STEM magnification is at least 1.5MX
- BF or ADF available; no HAADF, EDX, EELS, or 4D STEM



Why use the energy filter?

- The energy filter is basically a better objective aperture
- The Ω filter generates an EELS spectrum in the column; the filter slit is then placed to select a specific electron energy, which produces a filtered image from only those electrons
 - Similar in effect to the ARM300's GIF for the K3 & Stela
 - Added benefit of removing chromatic aberrations
 - Usually, we select the ZLP; sometimes the MPL
- Result: higher contrast + higher resolution images

Example: AuNPs in water



What makes this microscope a good walk-up instrument for NUANCE?

- TEM, STEM, & Diffraction modes
- The anti-contamination device (ACD) is quite large, so it can hold temperature for an entire 8-hour day
- The microscope's mag series cover an uncommonly large range.

Low Mag: 100x-3000x

Mag: 2500x-6000x

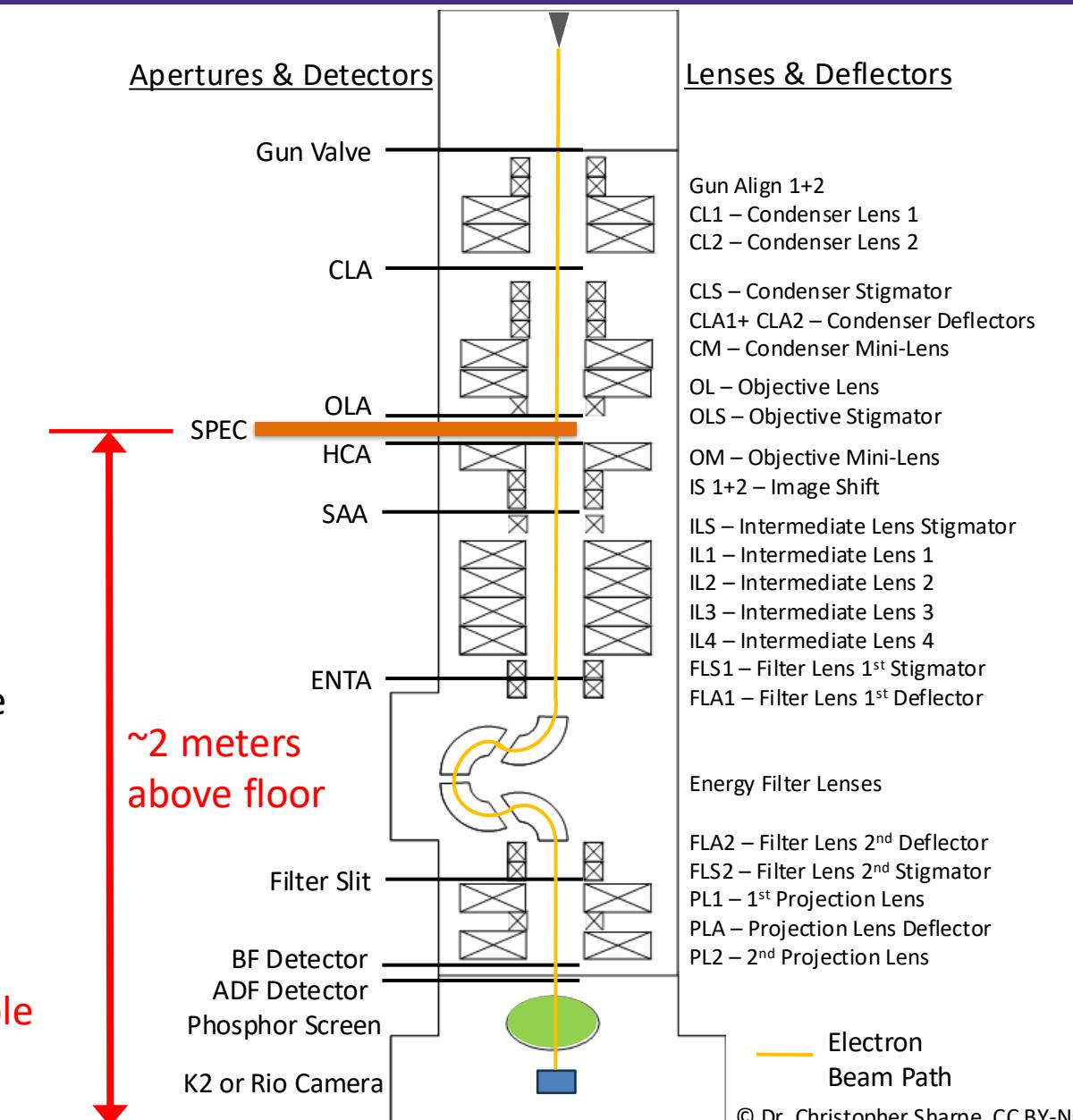
8000x-500,000x

600,000x-1,500,000x

- The combination of gun valve and energy filter allows for rapid screening of multiple grids without needing to change any alignment or aperture settings
- Stored alignment files for resetting alignment

Any downsides?

- No EDX & EELS
- ACD needs 3-4 hours of heating after use to recover
- You will likely need to use the stair-ladder to load your sample
- You'll still need to do minor alignments for excellent images



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What kinds of minor alignments?

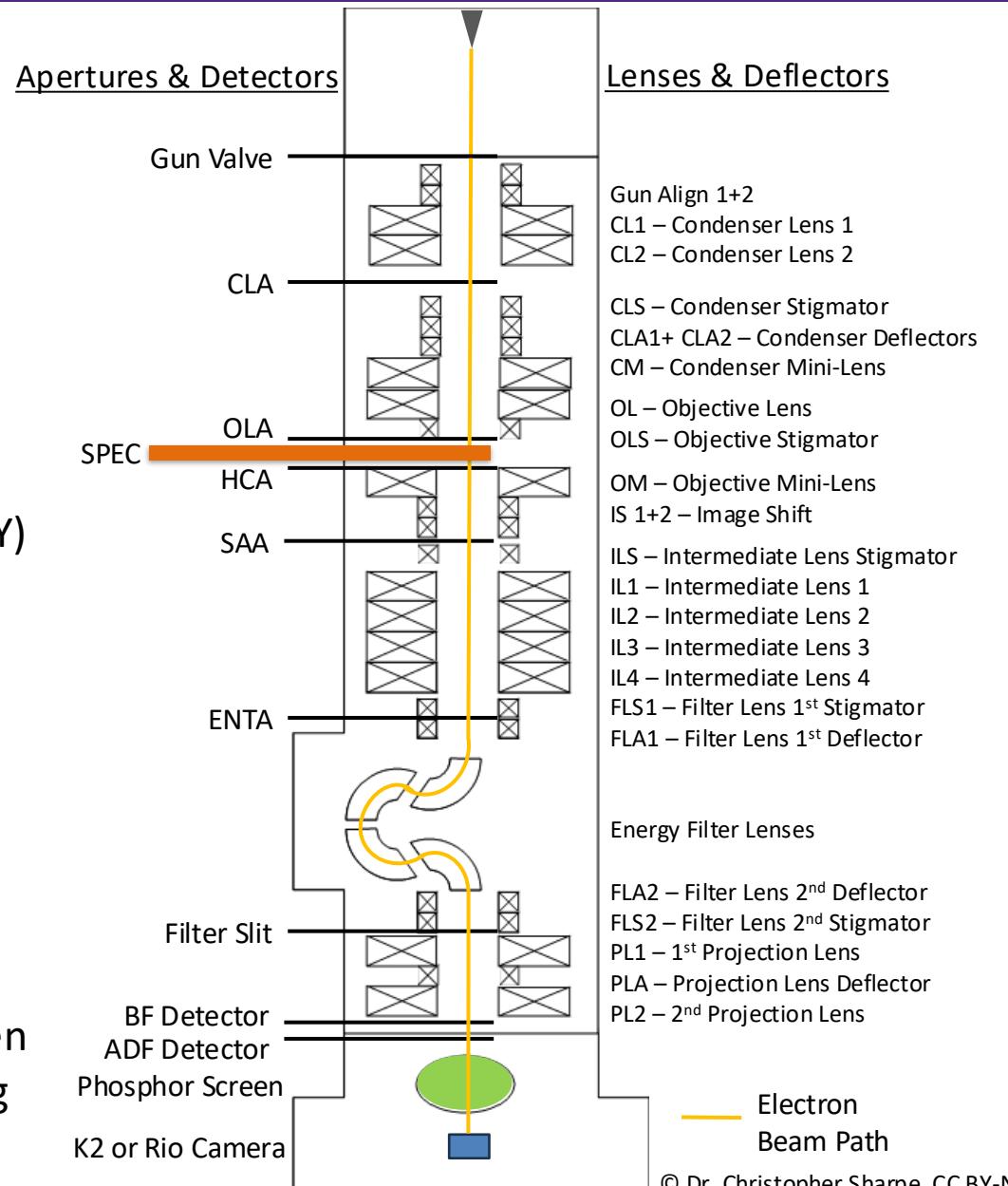
For each mag:

- Condense beam, beam shift to center (Shift-X, Y)
- Spread beam, center CLA (Aperture Arrow Buttons)
- Check eucentric height (IMAGE WOBB-X & $\pm Z$ height)
- Check current centering (OL Wobbler + Bright Tilt & Def/Stig-X, Y)
- Objective Stigmatism (w/ live FFT on camera, OBJ-STIG + Def/Stig-X, Y)
- Energy Filter: SPCTR mode, center beam with FL knob. Insert slit and adjust position with arrows on JEOL computer screen to bracket ZLP

For each sample:

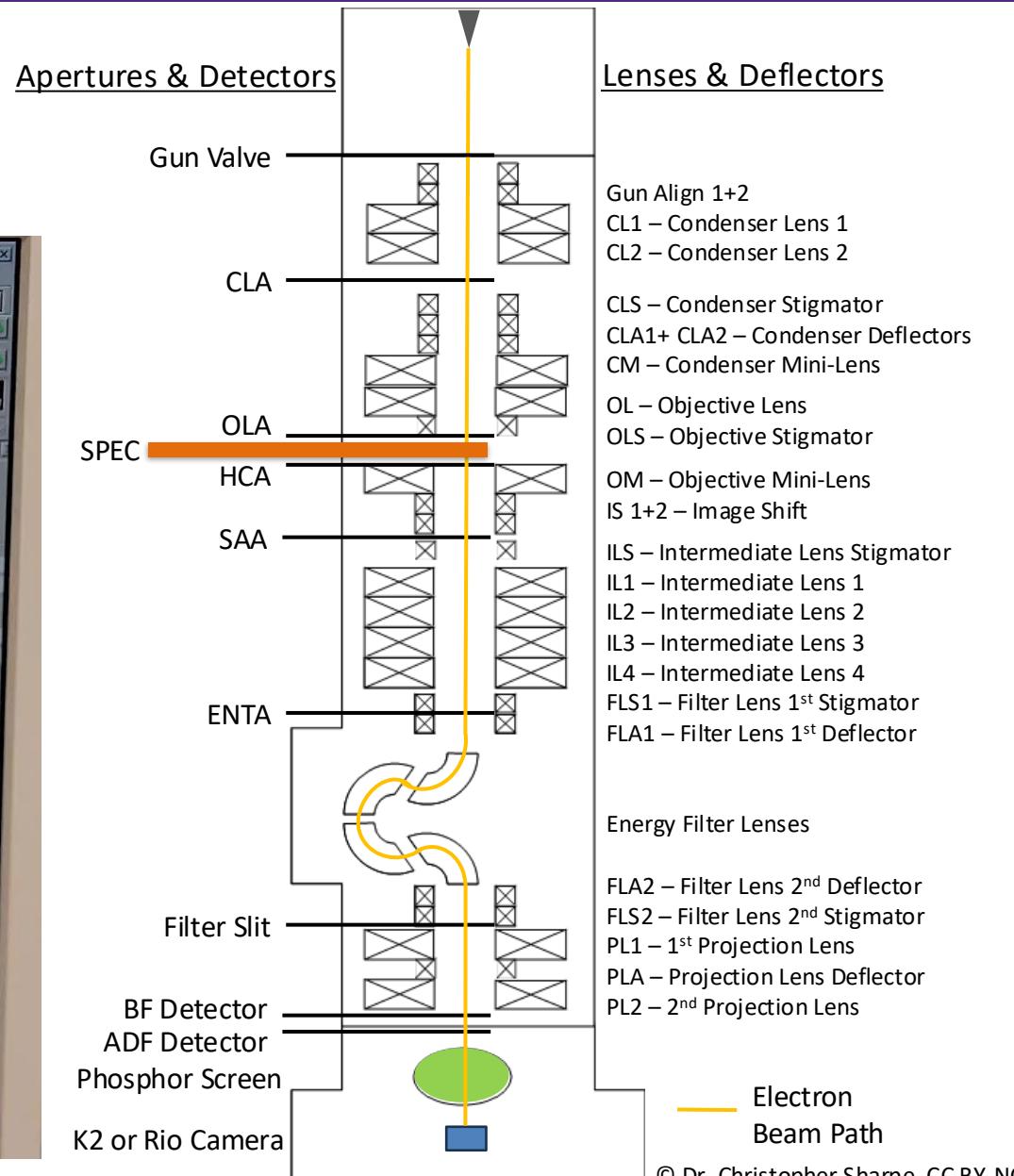
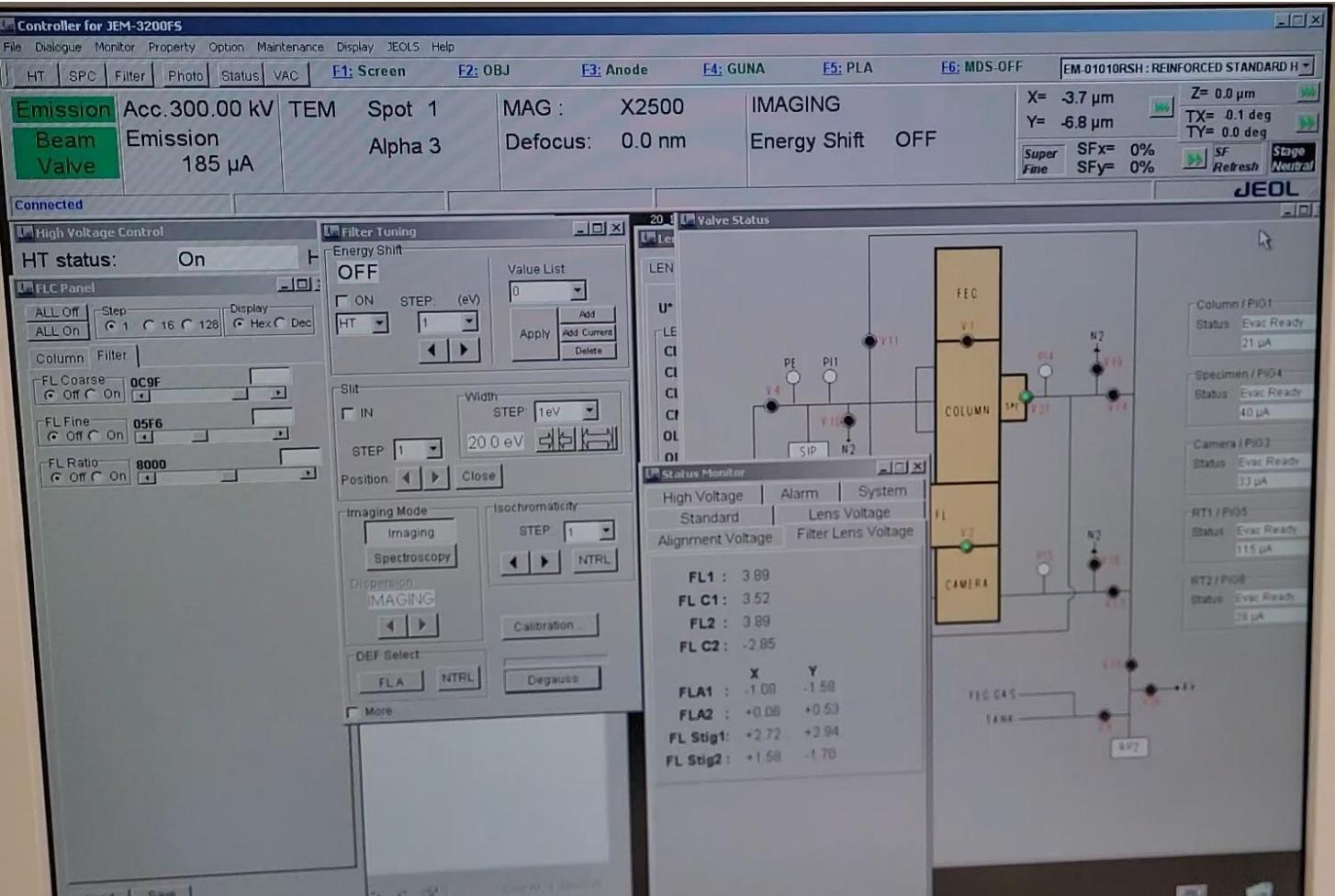
- Eucentric height

If you are looking at multiple samples with the same magnification & energy filter slit (& without OLA or HCA), you ONLY need to zero specimen position before sample removal and adjust eucentric height after loading the next sample. The other alignments will stay correct.

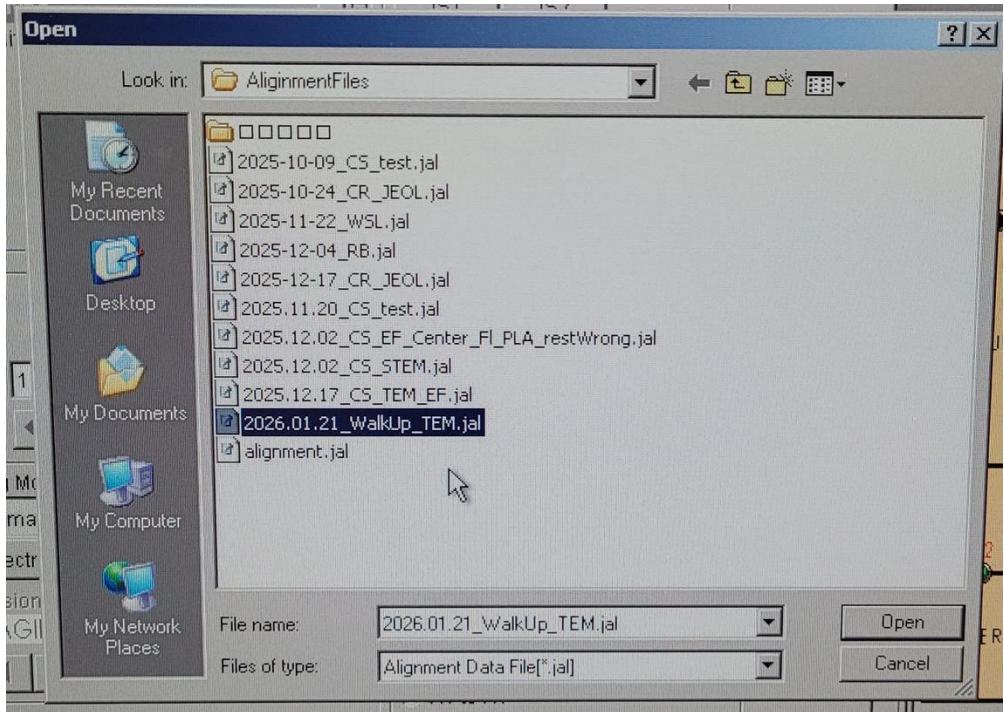


What if I mess up the alignment or it is really bad? So bad that I can't fix it?

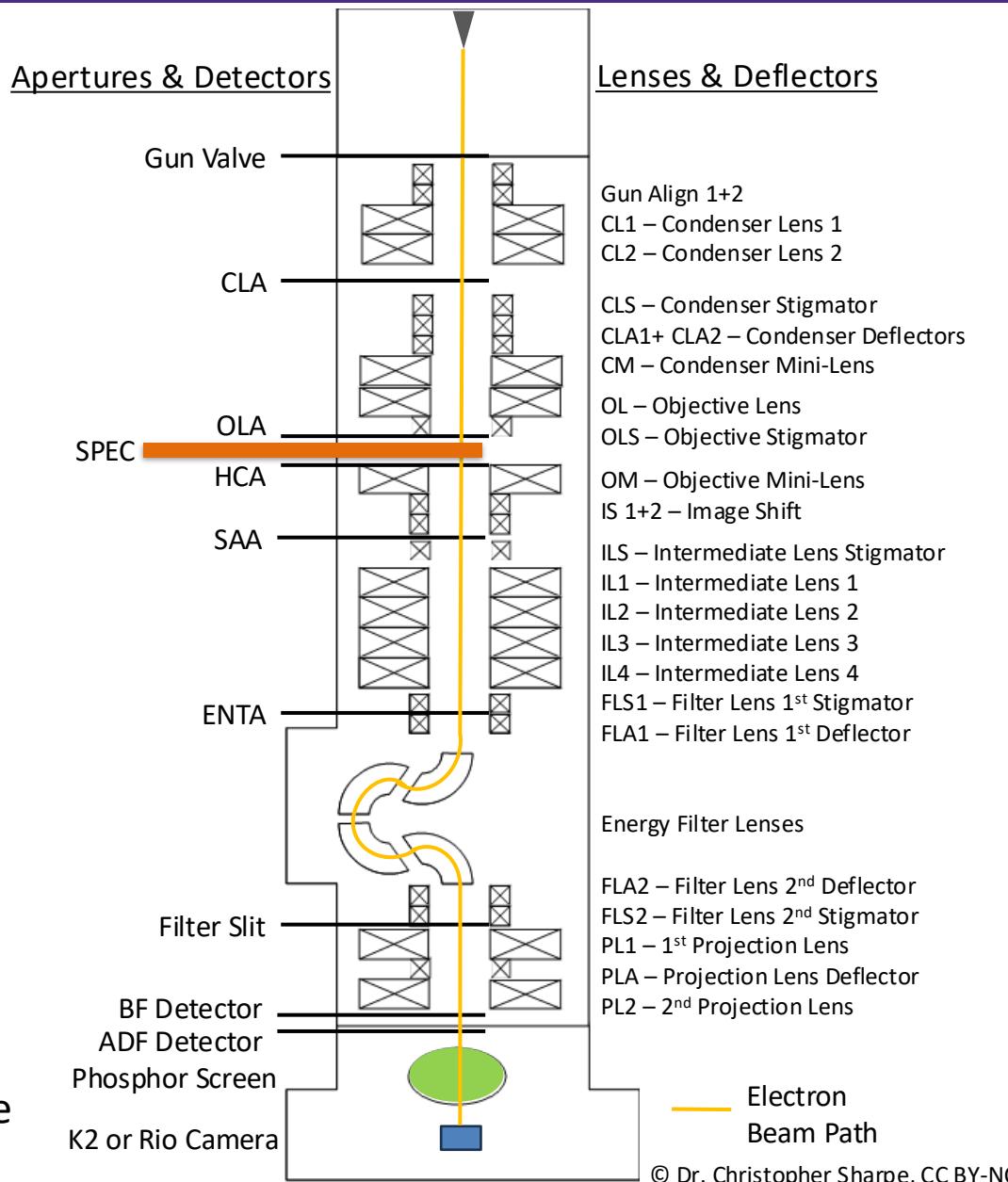
- The 3200 has *alignment files* and they are *fantastic*



What if I mess up the alignment or it is really bad? So bad that I can't fix it?

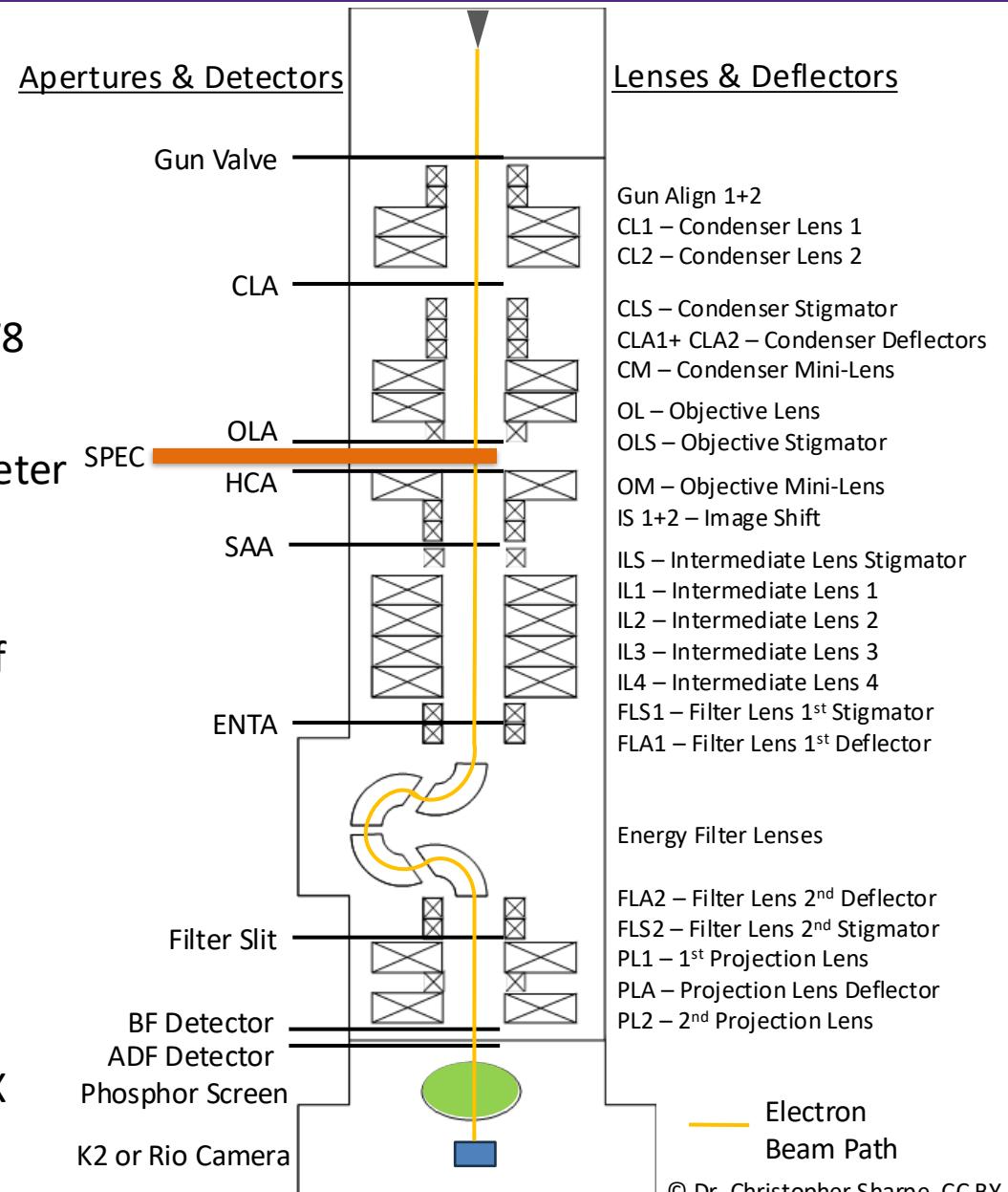


- Each day, BioCryo staff will save the day's TEM alignment to a file
- If the alignment is extremely off, just load the day's alignment file!
- Please do not save over alignment files; if you do, they must be re-created manually
- We will also save a STEM alignment; those are more stable, so just use the most recent one as your starting point



How does a walk-up session work?

1. Have sample(s) on TEM grid(s) that you want to look at
2. Check if the microscope is available
3. If it is, make a reservation for in a few minutes and walk down to AG78
4. Put your grid into the holder. Start your reservation.
5. Insert the holder in the microscope, start pumping down the goniometer
6. Set up Digital Micrograph to save your images into your folder on the local drive while the goniometer pumps down
7. Insert your sample into the column, wait for penny gauge to be left of vertical.
8. Open the gun valve. Set eucentric height, go to the appropriate mag, tweak alignments, and take images/diffraction patterns.
9. Neutralize the stage and remove OLA and HCA apertures. Close gun valve, remove sample holder.
10. Repeat 4-10 for however many grids you have time for
11. When done, remove slit & all apertures and leave microscope at 40kX with the beam spread. End your reservation.



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Frequently Asked Questions

How do I get trained on this microscope? What about STEM?

- For TEM, request training on NUCore. For STEM, email Chris & Reiner.

When will walk-up access become available?

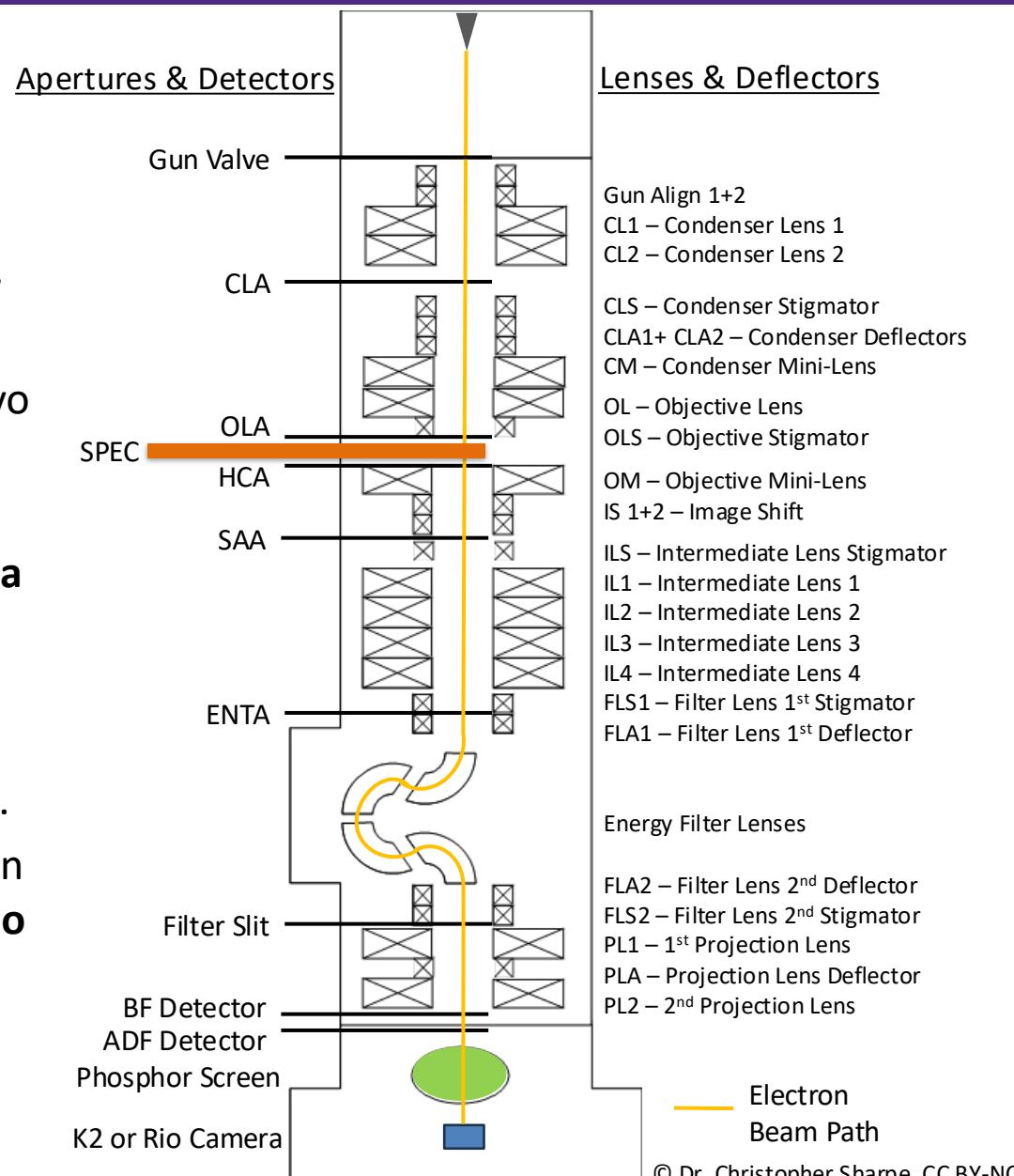
- JEOL is finishing some microscope maintenance this week, and BioCryo is updating our user guides for walk-up use. Hopefully we will roll out walk-up access as early as next week!

Why is there a maintenance hold from 5-8 pm, and what if I want to do a session during that time?

- If we don't bake out the ACD at the end of the day, the microscope vacuum will crash (bad). However, we can refill the ACD dewar if you want to do a late session! But you need to tell us that well in advance.
- Simply email Chris or Reiner before 3 pm, we will input the reservation for you and top up the dewar before we leave. **You will be expected to put in and turn on the ACD heater during your session. Even if you cancel the session, you still need to put in and turn on the heater.**

Why isn't there EDX on this microscope?

- None of our users have needed it yet. Let us know if you want it!



More Frequently Asked Questions

How does this effect longer microscope sessions I'd like to do during the week?

- Users will still be able to make reservations normally. If you have the time reserved, walk-up users should not interrupt your session.

What if I want to start my session before 10 am or on the weekend?

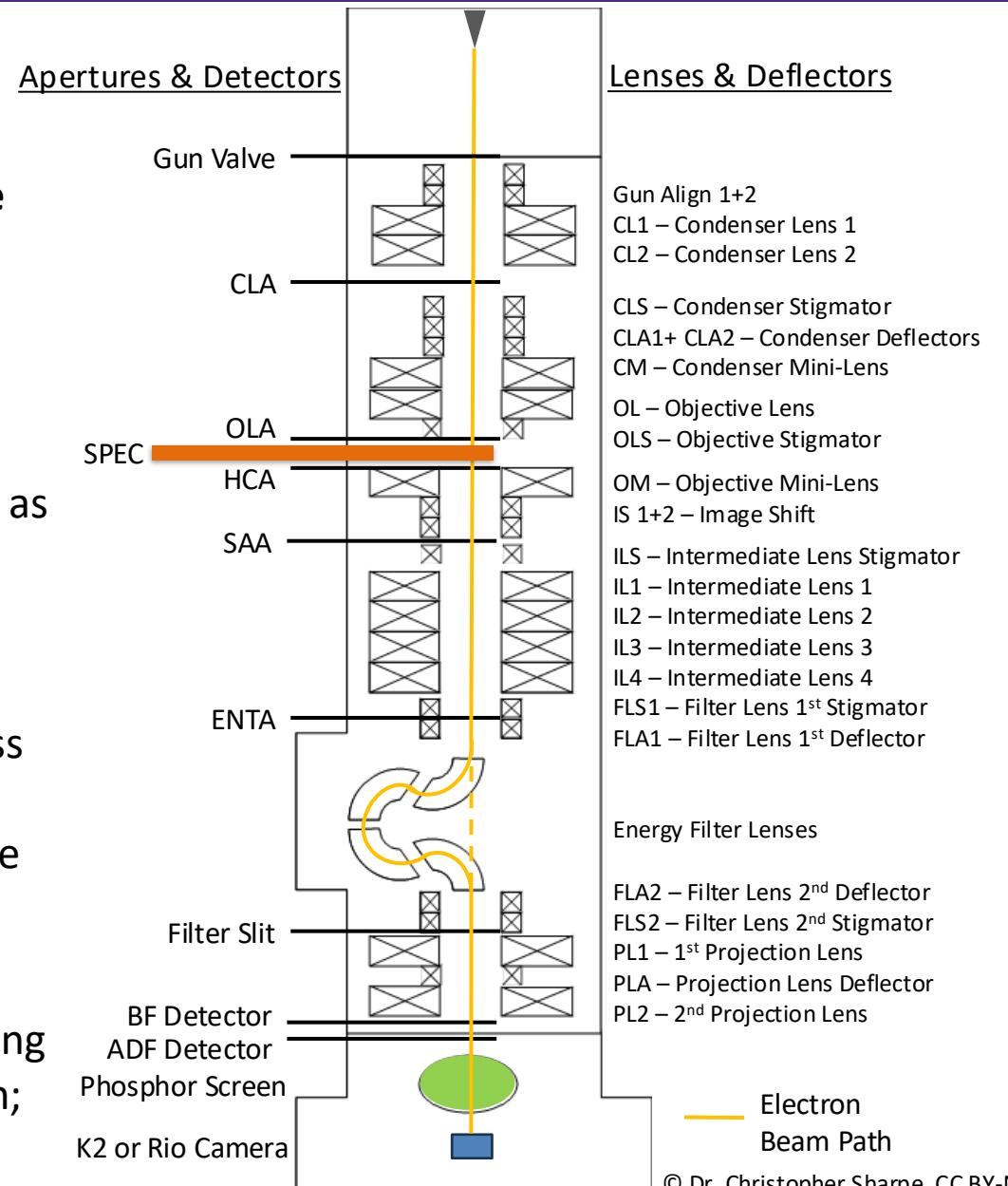
- You will need to fill the ACD dewar and do the normal alignment, just as we taught you in the training. However, staff will be around to help if you need it starting at about 9 am on weekdays.

The energy filter is weird and I'd like to bypass it. Can I?

- No. While it is possible to bypass the filter, it is a non-standard process that requires the assistance of a JEOL technician. Please schedule a meeting with us to discuss your research problem in more detail; if we can't see a workaround, we are happy to reach out to JEOL for you.

Can I do *in situ* work under the walk-up policy?

- No, NUANCE requires a microscope staff member to be available during *in situ* work. Please email Chris & Reiner prior to booking your session; we will let you know if we are available in case of a cell rupture.



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► **BioCryo** ◀
Electron Microscopy

If you have any questions, please do not hesitate to reach out!

BioCryo is happy to schedule a meeting to discuss your samples or any technical questions you have.

chris.sharpe@northwestern.edu

Dr. Reiner Bleher, *r-bleher@northwestern.edu*
Eric Roth, *eric-roth@northwestern.edu*

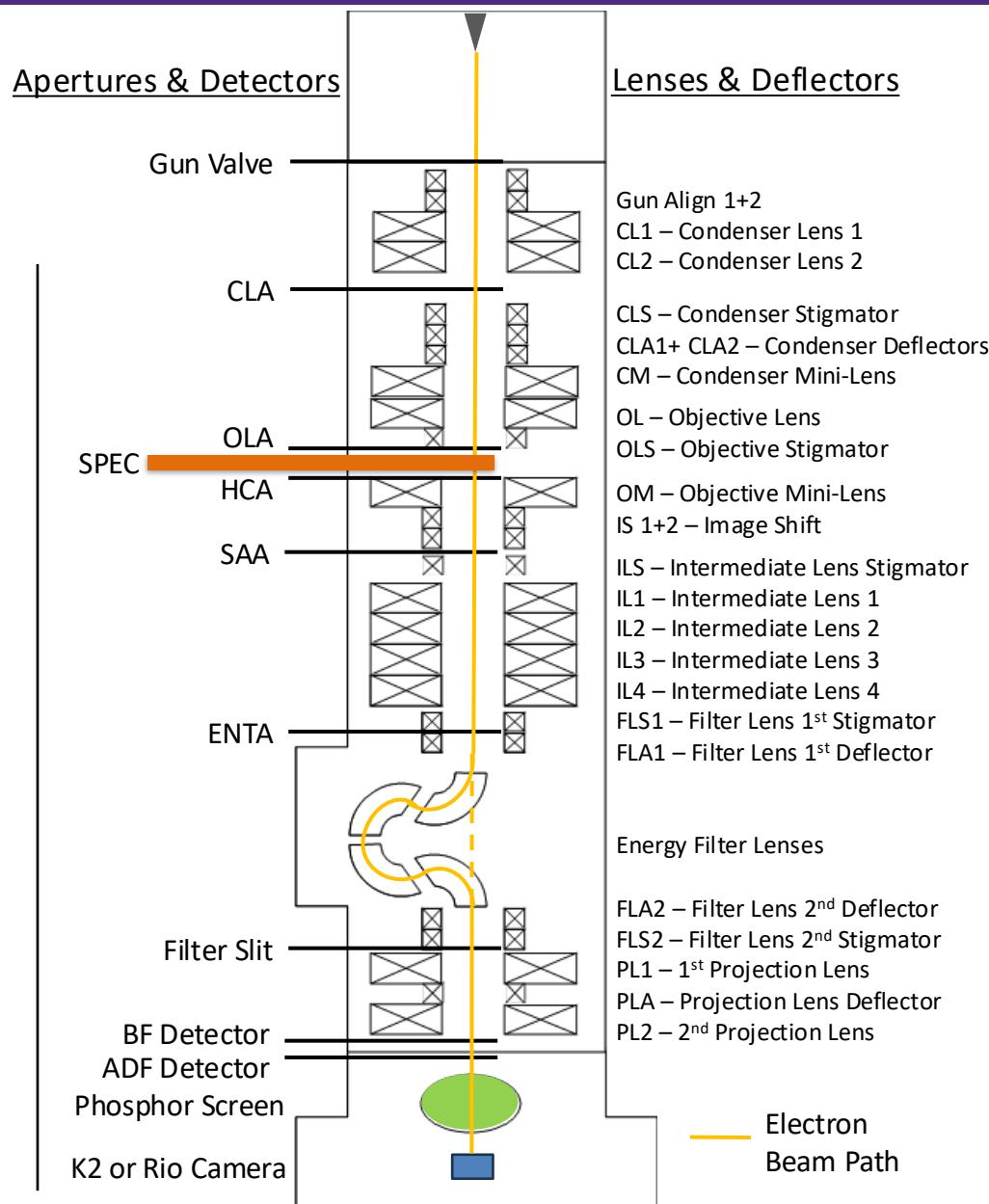


Want to learn more about the instruments and techniques available at NUANCE?
All of our prior Tech Talks are available on the NUANCE Center YouTube page!

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8000x-500,000x

600,000x-1,500,000x

- The combination of gun valve and energy filter allows for rapid screening of multiple grids without needing to change any alignment or aperture settings
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What is this microscope missing?

- EDX & EELS

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