CM120 Operation Instruction

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Abstract

This document tries to list steps and procedures for a typical daily operation on CM120. You can use it as guidebook to help you when you are sitting with CM120, especially if you are a new user. I here assume you are already familiar with scope interface, what the knobs and buttons do etc.. You might use the section titles as quick bullet p ints, but the explanation inside each sections are supposed to be useful and informative. A pdf version of this document is also available.

If you have suggestion how to improve this document to make it more useful, please feel free to let me know. Thank you!

NOTE: all the turning knobs on the panel and push buttons are marked in Bold.

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1. Check log book to see if there have been any problems

This is the

It is always a good idea to check the LOG book. You can find useful information listed there, such as:

- If there have been any recent problems with the scope.
- The conditions that were used last, especially the filament saturation.
- Any special note that last user wants you to know.

2. Check vacuum status

The status must be *Ready* before you can operate the scope. If not, you should ask for help and report it to the manager. Usually, if the vacuum is not ready, it is due to one of the following reasons:

- The scope is malfunctioning.
- The air pressure is not within a good range(the building compressed air may be down?).
- The cooling water is off.

Vacuum being *Ready* means:

- Top line of vacuum page shows "ready".
- **P3** < 50, **IGP** < 26 (normally, they are shown 0, 5).
- LEDs for UVAC and HiVAC are lit.

3. Add Liquid Nitrogen to the BIG anti-contaminator dewar

LN2 in the cold trap dewar is necessary for fast vacuum recovery. It is very useful, especially when you need to change grids and/or do a cryo session. However, if you are working with a negative stain or plastic section - a dry grid, the scope can still run without LN2 cold trap. Unless you are running automatic, long overnight session, you should always use it.

Tip

Generally, when you first put LN2 dewar to its stand, you want to be SLOW, or the strong evaporation will make LN2 spilling.

4. Apply High Tension

Turn High Tension (H.T.) on if it is off by pressing the H.T. button on the panel. From Parameter page, set it to 120kV or the voltage you want.

5. Turn filament on

It is recommended to turn the filament on while on the configuration page where the actual filament current number is shown and the limit can be checked(highlighted).

6. Check saturation and gun tilt, and then saturate the filament.

The procedure is as following:.

- Desaturate the filament about 2-3 clicks.
- Press Align button, highlight Gun Tilt.
- Adjust the multi-function X, Y to get the best shape for the tip and maximum intensity as well.

- Bring the filament current to saturation.

7. Check C2 aperture mechanical position and C2 stigmatism

Steps to adjust the C2 aperture mechanical position are:

- Make sure the C2 aperture is in.
- At around 5kX, cross the beam first and center beam using deflectors (Beam Shift X,Y), then spread the beam until its diameter is close to 5cm ring on the screen. Adjust screws X & Y on C2 aperture to center the beam. Repeat this process 2-3 times.
- At high mag ~30kX or higher, change the intensity of beam(C2) through the crossover and adjust the screws X,Y to make the beam symmetric when spreading out.

There are two ways to check and adjust the **C2** stigmatism:

- When the filament is desaturated, press Stig button and select Cond, then adjust Multi-function knobs so that the details in the filament shadowing image can be clearest and sharpest.
- When changing the beam intensity, use the Multi-function knobs to adjust the beam into symmetric and "round" shape, i.e. not elliptical.

Note

For this purpose, don't pay attention to the beam shape when beam is exactly at cross point, as that more reflects the shape of the crystal tip, rather than stigmatism. You want to make beam "round" when spreat out.

8. Check specimen holder & load grid

Important

This is important. If you see any problem with any of the holders, report it to the manager *immediately*. Otherwise, you could be the one held accountable for the damage. Several details about the holder must be checked carefully before use:

- Overall shape is good, and there is not obvious damage.
- Make sure there is no crack or any other damage on the O-ring. If you do see a damage, like a cut etc., ask manager to replace it for you. Check if there any dirt or fibril on the O-ring. You might want to clean it gently with alcohol and slightly re-grease it. Do not over-grease. The main function of the grease is to lubricate.

Gently secure the specimen grid on its position. Use the tool pin to open and close the clamping device.

9. Insert specimen holder into column

Important

Be careful! Only at this stage, you might damage the scope or specimen holder *mechanically*. Be sure that you understand what you are doing. Should you feel any confusion about this procedure, please stop and ask for help.

Note

For the sake of filament crystal, it is REQUIRED to turn down the filament to *10* before inserting the specimen rod. That way, in case **`IGP`** shoots high, there will be no subtancial damage to the LaB6 tip crystal. In general, filament should be kept at *10* or completely off until **`IGP`** recovers to below *26*.

The procedure to insert a room temperature specimen rod is below.

- define airlock pumping time as 60 seconds, from Vacuum - Cryo page.
- Reset stage tilt angle to 0 if it is not.
- Insert rod in, with the Pin at 3'oclock position.
- As soon as it reach the end, rotate rod CLOCKWISE with some pushing force so that the pin slides into the locking groove at 5'oclock position. You should feel the rod goes "in" about 8mm.
- Wait until the red LED on the stage disappears. Dismiss the "non-standard" flushing message on the screen by pressing "Reset" button at lower left corner of the screen display.
- Turn rod Count-Clockwise until pin is at 12'oclock position, while watching **`IGP`** reading. You should adjust your rotating speed to keep **`IGP`** < *40*.

10. Adjust the specimen height to the eucentric height

Eucentricity is a fixed reference point in a scope. It is the intercross point of stage axis and column axis. We want to observe our specimen grid at this height level so that the actual magnification doesn't differ much from day to day. And scope is designed to perform better when specimen is at such height. It is good to have the habbit to always adjust specimen to eucentric height after rod insertion. On CM120, the procedure is as following:

- Have beam seen on large screen, at ~3000X, and find a feature on the grid.
- rotate stage back and forth by pressing CompuStage - A-wobbler.
- Adjust **`joystick Z`** to minimize the shifting of the feature.

11. Check Beam Tilt Pivot Point X, Y

Note

The prerequisites for Pivot-Point is specimen being at eucentric height and objective being preceisly at focus level.

- Make sure the specimen is at eucentric height.
- Take out Obj. aperture.
- Press button Align - Direct Alignment - Beam Tilt Pivot X.
- Merge image feature by adjusting Focus knob. This is to *precisely* focus the image.
- Merge beam using Multi-fcuntion knobs.
- Repeat the last two steps for Beam Tilt Pivot Y.
- Press Align button again to exit.

12. Check voltage and current rotation centers

This step is to align the beam to make it parallel to the axis of the column. The purpose of this step is to make beam to hit specimen perpendicularly. A coma is not a good thing, as it generates some phase error to the data.

The procedure is below.

- Press Align button and select Rotation Center.
- Select Voltage or Current from the same page.
- Adjust the Multi-function Knobs to let the wobbling be symmetrical around the center of the beam. The feature at very center of the large screen has minimum shift.
- If you perform this with Obj aperture in, then re-check the certering of objective aperture.

Note

The step size button on Focus is used to control the amplitude of the beam wobbling.

The steps used here only give "roughly" parallel beam to the axis. If you need very acurate "0" tilt beam, a different alignment procedure - Coma-Free is needed.

13. Put in objective aperture and center it

It is important to know what size of the obj. aperture you are using. You don't want to use too small size to actual cut off useful high resolution signal. Meantime, you don't want to use the aperture size too large, as the non-usable high resoltion beam becomes noise to your image. This reduces signal to noise ration unneccesarily.

The position of the aperture could affect the obj. lens stigmatism. Therefore, you want to do this step before you finally check Obj lens stigmatism.

Here are the steps to insert and center Objective lens aperture:

- Make sure the large screen is down, to prevent CCD from damage.
- Switch to diffraction mode by pressing the diffraction "D" button.
- Adjust camera length to ~1m using magnification knob.
- Adjust the Intensity (C2) and Defocus knobs to see the shape edge of the obj. aperture.
- Adjust the related mechanical screws on aperture holder to choose the proper size of the aperture and center it to the central beam on diffraction pattern.
- Switch back to image mode by pressing D button again.

14. Check Objective Lens stigmatism

The obj. stigmatism should be corrected as much as possible, and it should be checked for *every* netative stain low-dose image that you are taking, as staining material might change field in local area. This is a bit hard by hand. Even with lastest version of SerialEW, this can be done by software, it is still not easy and time efficient. However, slight stigmated image is not critically bad, as it can be corrected as part of CTF correction computationally.

Here are steps to correct Obj lens stigmatism, manually:

- Go to a relatively high mag., such as 100,000X, and focus the image.
- If possible, acquire continous CCD image with live FFT so Thon rings can be seen.
- Press the Stig button, highlight Obj, and select proper stepsize.
- Adjust the stigmatism using Multi-function knobs until it becomes minimum at all defocus levels. (It shows up more at close to focus.)

15. Typical Low-Dose setup parameters

Here are some typical setup for Low-Dose condition.

- Search: Mag=2650X(3000X with screen up), Spotsize=3-5, image mode

  Alternatively, at 2650-3400x, switch to Diff. mode by pressing "Diff" button. Adjust camera length to 680mm using "Mag" knob. Focus the diffraction spot using Focus knob, and then adjust "defocus" until the image inside central spot expands to proper size. Personally, I like to "defocus" to clockwise side. What you got is a shadowing image inside of defocused diffraction central beam. The advantage of this is high contrast compared to a normal image mode.

- Focus: Mag=175kX, Spotsize=6. When work with tilting stage, make sure S1 or S2 sits on tilting axis of the geonimeter, the angles will posted on the panel.
- Record: Mag=53000X(60kX), spotsize=3-5

16. Align an identified area under Exposure and Search

This step is to insure that what you see under low mag. (Search mode) will be the same area you get under imaging mag. Here is how I do it:

- At Exposure mode, MECHANICALLY drive an identified spot to the centber of the screen.

- At Search mode(and usually in Diffraction mode also), using the Multi-function knobs to backtrack the identified spot at the center of the screen (electronically). This uses Image Shift or Diffaction Shift (when Search mode is set up in Diff mode) to "shift" image without actually moving the stage position.

Note

You can use a corner of a mesh as the identified spot for a negative stain specimet or to use an ice burn mark in the cryo case.

17. Check S1 and S2 parameters

In Focus mode, S1 and S2 are linked to the positions of the beam which is deflected away from the center of the imaging area on the specimen. When highlighted, their parameters can be changed by the Multi-function keys.

Normally, we use 175kX as the magnification. The radius of the spot is about 1.5 microns. Depending on the specimen, you can change those parameters to fit your needs.

18. Finishing Up

When you are done with your session, perform finishing up procedure.

- Specimen rod out.
- Reset Stage Position, X, Y, Z and A.
- Filament 0.
- H.T. OFF.
- Cryo-cycle, normally for 2-3 hours.
- Data display OFF.
- Display OFF.
- Log your session on logbook.