FEI TECNAI BIOTWIN

GENERAL USE INSTRUCTIONS

THIS MANUAL IS TO BE USED AS A GUIDELINE FOR GENERAL USE OF THE BIOTWIN. YOU ARE EXPECTED TO TAKE NOTES AND KEEP A NOTEBOOK AS YOU PROGRESS THROUGH USING IT AS A RESEARCH TOOL. PLEASE BRING THESE INSTRUCTIONS AND YOUR NOTEBOOK WITH YOU EACH TIME YOU USE MICROSCOPE.

RULES FOR USING PSB B71

WE NEED TO SET STRICT GUIDELINES FOR USING THIS FACILITY SO THAT WE CAN MAINTAIN A CLEAN AND ORGANIZED RESEARCH ENVIRONMENT. SO THAT YOU GET INTO GOOD HABITS WE WILL BE TREATING ALL SAMPLES UNIFORMLY AND ALL USERS WILL ADHERE TO THESE LAB RULES.

1. **NO FOOD OR DRINK IN THE LAB.**
2. **TO GET 24 HOUR ACCESS TO THE LAB YOU’LL NEED TO COMPLETE TRAINING BY THE TOOL MANAGER.**
3. **NEVER ATTEMPT TO REPAIR ANYTHING IN THE FACILITY, CONTACT MARIENA AND/OR KATIE IMMEDIATELY IF THERE IS A PROBLEM. SOFTWARE AND COMPUTERS ARE INCLUDED.**
4. **NEVER USE YOUR OWN MEDIA (MEMORY STICKS, EXTERNAL HARD DRIVE, ETC) TO ARCHIVE IMAGES. AT THE END OF YOUR SESSION YOU SHOULD UPLOAD YOUR IMAGES TO BOX OR DROPBOX.**
5. **BRING YOUR NOTEBOOK AND INSTRUCTIONS EACH TIME YOU USE THE MICROSCOPE.**
6. **CHECK THE SCHEDULE IN FOM, AND CONFIRM THAT THE MICROSCOPE IS UP AND RUNNING AND AVAILABLE, ESPECIALLY IF YOU PLAN TO USE THE MICROSCOPE AT THE LAST MINUTE.**

PROPER SAMPLE HANDLING PROTOCOL

Don’t worry, you can look at pretty much anything in this microscope, but the samples must be extraordinarily clean before they are loaded. This protocol is not only for the welfare of the microscope, but if your sample contaminates you will have wasted your time. Make sure that when you prepare your samples your tweezers are cleaned with a solvent such as methanol, your grids have been cleaned with a solvent and you must have a clean grid box (you can clean these with ethanol). If you wish, use gloves when you prepare or handle your samples, if not just don’t touch the business end of the tweezers or the grids with your fingers.

Any sample that hits the floor must be cleaned in either the plasma cleaner, under the UV light or just ditch it.

Do not touch the holder from the O-ring on, if you do let the manager know and we will show you how to properly clean the holder.

The cold trap in the microscope must be filled BEFORE you load your samples. Once filled, it will take at least twenty (20) minutes before you can load a sample. The dewar must be checked every 2-3 hours though, and topped off when needed. If you are the last user of the day you MUST put the microscope through a cryo-cycle before you leave.

(1)

GETTING STARTED WITH THE BIOTWIN

When you come into the scope room the RED button on the console will be illuminated indicating that the microscope is on. You should also see two white/yellow lights on, next to the red button, indicating that Vacuum (Vac) and High Tension (HT) of the microscope are enabled/functional. If one of these lights is off, let Mariena and/or Katie know immediately. Do not continue working until you’ve gotten the all-clear by the tool manager. The microscope UI monitor will appear off until you log in enable the instrument in FOM. If there are any issues with the scope during the session please note them in FOM AND send the manager a text or email (text is preferred for faster response).

1. The microscope UI (TEM UI) should be open already, as well as TIA and Gatan’s Digital Micrograph (DM).
2. Under **WORKSET** you will be at **SETUP**. Make note of the **Gun/Col** vacuum value. Note that when the **Col. Valves Closed**, **Filament** and **High Tension** buttons are all **Yellow**, this indicates that the TEM is operational. If you’re the first user of the day, the Filament and High Tension will be off. Turn them on, and keep them on if there are sessions booked after yours.
3. Next to **TECNAI G2** on the lower right of the first monitor open up **Vacuum Overview** in the pull down**.** This diagram shows you a cut away of the vacuum system and all the values.
4. If the dewar is not on the scope, fill the dewar with liquid nitrogen and place the copper braids in the dewar. Place the styrofoam lid on top of the dewar and wait twenty (20) minutes.
5. Look in the lower Right corner of the monitor, you will see **X, Y, Z and A, B.** They should all be at ZERO. If not go to **SEARCH** in **WORKSET.** There will be an arrow at the top of **STAGE**, click it to get the flap out. You can now go to **RESET** and click **HOLDER**; this will zero the goniometer. One or two microns on X, Y, or Z, is tolerable, what you don’t want is for the values to be too far away from 0, 0, 0 (ie. hundreds of microns).
6. Return to **SETUP.**
7. You are now ready to load your sample.

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**LOADING YOUR SAMPLE**

This should be a pretty simple protocol, but over the years I have found that most of my scope problems occur when this procedure is done incorrectly. You have to do this properly each time, so take your time both loading and unloading your sample; think about what you are doing and if anything is out of the ordinary call me immediately.

1. Locate the T-12 holders and choose a holder. We have a single and two cryo holders for the BioTwin.
2. **If you are looking at organic material, or samples that do not need the Alpha/Beta tilt you must use the single tilt holder.**
3. Using the **Clamp opening tool,** place the end into the hole of the sample clamp. Gently lever the clamp up to a **90 degree angle** and remove the tool. **Place your grid in the depression, sample side down, with clean tweezers**, re-insert the clamp tool and lever the clamp onto your grid.
4. Inspect the o-ring for debris. If you find debris, gently sweep the debris away with the tweezers, **never grab at the junk**, this can damage the o-ring.
5. Make sure that the **vacuum overview window** is up, this will show you what the pumping system is doing and when the pump sequence is over.
6. **READ THIS SECTION BEFORE YOU DO ANOTHER THING!** Load the sample in the TEM. Align the pin on the holder to the line at the **five o’clock position on the goniometer**. You have now reached the point of no return, there are light guides in the goniometer that activate the **PVP** (pre vacuum pump); insert the holder carefully until it makes contact with the flange. **You may or may not have hit the slot you need to be in**. Carefully wiggle the holder no more then **1 or 2 degrees** to feel for the slot. The PVP pumps on the goniometer for **60 seconds**. In the highlight area the microscope will ask you which holder you have selected, you must tell the microscope which one (single, cryo, or double-tilt) or you may have to retract the sample and re-insert it. If you choose the wrong holder you will have to remove the holder and reinsert so that the scope knows which holder you are using. When the PVP has stopped pumping you will **carefully rotate the holder counter clockwise until it stops**; the interlock is now open to the high vacuum. **NEVER LET THE HOLDER GO! GUIDE THE HOLDER INTO THE COLUMN CAREFULLY UNTIL IT STOPS.**
7. You must wait until the vacuum in the **GUN/COL** returns to a value of **less than 12** before you open the **COLUMN VALVE**. The lowest value that will be read is 6.

**ALIGNMENT OF THE TEM**

The alignment of the BioTwin is controlled by the computer. It is quite simple as long as you follow the instructions explicitly. What is so useful about this microscope is the HELP menu. It is very detailed and will help you throughout your use of the scope. The HELP automatically pops up when you start the alignment procedure. If you have any questions while using the scope just click on the window of interest and press **F1.**

The alignment is never far off, if you find yourself doing extreme scope gymnastics please call me and I will get you out of your jam. But first we have to set your sample up before alignment.

1. Make sure that the scope is at **2550x on the BIOTWIN**, and that you are in **TEM Bright Field**
2. Open the column valve. **Press the Yellow Col. Valves Closed button**, you will hear the valves open and you should see the beam. If not, move your sample around a bit using the joystick. **IF YOU NEED TO LEAVE THE ROOM FOR ANY REASON YOU MUST MAKE SURE THAT THE COL. VALVES ARE CLOSED.**
3. Find something on your sample that is easy to distinguish. Even though we hammer into you guys that your samples need to be extremely clean, we need schmutz (a piece of junk) sometimes to set the **eucentric height.**
4. Once you have found your schmutz, go to **SEARCH** in the **WORKSET**. Open the flap out under **STAGE2** and activate **WOBBLER**. You will notice that your schmutz is moving. Using the **Z AXIS** controls on the **RHS** control panel move the sample either up or down until the movement has ceased. Increase your magnification to around **20,000x** to fine tune your adjustment.
5. You can now deactivate the **WOBBLER**. Never use the Wobbler button on the RHS panel. It does not activate Z-axis wobbler., and will instead cause your image to wobble and you’ll never get a decent image.
6. Lower the **FOCUS SCREEN** and focus your image. This is very important because all your alignments are going to be based on your focus (aim for the point of lowest contrast on the image).
7. Once you are focused reset your **DEFOCUS** by depressing the **RESET** **DEFOCUS** button labeled **R2** on the **RHS** control panel.
8. Find an area of your grid that is open/off sample (ie. where no support film is present).
9. In **WORKSET** open **TUNE or ALIGN.**
10. Expand the file labeled **GUN.**
11. Open **GUN TILT** and follow the instructions listed, read the **HELP** window that popped up, this will help you do the alignments and explain why you are doing them. There are two pages of instructions, click the arrow to advance to the next page; when you have finished page 2 of 2 click **DONE.**
12. Open **GUN SHIFT** and follow the instructions listed, click **DONE**
13. Expand the file labeled **BEAM HM-TEM.**
14. Open **BEAM TILT PIVOT POINT** and follow the instructions, click **DONE.**
15. Open **ROTATION CENTER** and follow the instructions click **DONE.**
16. Check that our beam is centered by clicking on **Beam Shift** and verifying that the beam stays centered. If not, click **Beam Shift** and center the beam with the Multi Function knobs. Do this for several magnifications, up until the highest one you’ll use.
17. You are now ready to find your sample and acquire images in **TEM.**

**HOW TO TAKE AND ARCHIVE AN IMAGE**

1. Look around your sample until you are satisfied that you have sample and that it is worthy of analysis.
2. You probably need contrast at this point. Find material that is not valuable and press the **DIFFRACTION** button on the **RHS**. Insert the **OBJECTIVE APERTURE** by flipping the silver lever to the left. You should see the image of the **OBJECTIVE APERTURE** around the **DIFFRACTION** spot. There are 4 objective apertures: 30, 50, 70 and 100 microns. Choose the one that best suits your sample by turning the large knob on the assembly. Using the smaller knobs you can move the aperture X and Y making the aperture concentric around the diffraction spot. Again, these are **NEVER TOO FAR OFF**. If you find yourself out of control and sweating profusely call me immediately.
3. Depress the **DIFFRACTION** button to return to **BRIGHTFEILD.**
4. Using the **FOCUSING SCREEN**, focus your image using the binoculars (or insert the camera and use the live image).
5. Spread the beam until it fills the large screen. **YOU MUST MAKE SURE THE INTENSITY KNOB IS AT ITS FINEST SETTING.**
6. Inserting the camera can be done by clicking on the Camera Inserted button on the Digital Micrograph UI. For looking around (Search) you can use 0.25s exposure. For taking images (Acquire), depending on how sturdy the sample is (and whether it’s in ice or carbon), anywhere from 0.5-2s should be enough to collect images. To save images, go to File --> Save Display As --> find or create a folder with your CU ID, and save the images there. This folder is visible (shared) with the Support PC, which you can use to transfer data.

**SHUTTING DOWN**

MAKE SURE THAT YOU SHUT THE SCOPE DOWN IN THIS ORDER EACH TIME.

1. Make sure that your images are saved and transferred.
2. **RETRACT THE CCD CAMERA FROM THE COLUMN**.
3. Lower the magnification to **2500-8000x.**
4. Spread the beam so that it fills out the screen.
5. Remove the **OBJECTIVE APERTURE.**
6. Go to **SEARCH** in **WORKSET** and **RESET** the holder.
7. Close the **COLUMN VALVES**.
8. Bring up **VACUUM OVERVIEW**
9. Remove the sample holder. With your left hand press against the purple faceplate while you pull the holder out of the column. When the holder can be pulled no further rotate the holder **CLOCKWISE** until it stops. You should now gently “POP” the holder out of the goniometer, and remove the holder to the cradle.
10. **YOU MUST LEAVE THE ROOM IN THE ORDER WHICH YOU FOUND IT, THERE IS NO MAID SERVICE.**

**STARTING UP AND SHUTTING DOWN FOR THE NIGHT**

Remember that you are not allowed to use the scope after hours or on the weekend until you have had 20 hours of uneventful microscopy.

**START UP**

1. Log on to FOM and start UI.
2. Put Liquid Nitrogen on the ACD (anti contamination device); wait at least 15 minutes before loading your sample.
3. Make sure the vacuum values are good.
4. Click the HT button.
5. Click the Filament tab and wait approximately 300 seconds for the filament to heat up
6. Load your sample and start breaking down the barriers of science.

**SHUT DOWN**

1. When your last sample is removed from the scope and the scope is at the defaults you may now start shutting the scope down for the night.
2. Make sure there are no other users on after you (check FOM, some users end up booking time at the last minute, so it’s always good practice to double and triple check).
3. Open the flap out in VACUUM under SET UP.
4. Find the CRYO CYCLE tab and click it.
5. Remove the dewar from the copper braids and toss the liquid nitrogen on the foam box.
6. The scope will be in cryo cycle for ~600 minutes or more (10hrs) so make sure that it will be ready for the next user.

Go home