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TEM Protocol for Pore Imaging

> This protocol is meant to serve as a reminder of how to image a solid state nanopore with the Technia F-20 TEM. ***Do not attempt to image a pore without proper training from a CCMR staff member***. Improper use of the TEM may result in damage to the instrument, significant material cost, and banishment from the CCMR. Pore imaging should be done immediately after the pore has been drilled via STEM mode. Please refer to the pore drilling protocol for information on how to drill a pore.

Initial Settings: This protocol assumes that the reader has just completed the drilling of a nanopore. This scope will be in STEM mode. The HADAFT Detector will be inserted. The Monochromator will be at a lowered setting and the Spot Size will be 3. The magnification will be 1.3Mx

Protocol:
After you believe that a pore has been drilled (Ronchigram is clear and a dark spot is visible in the STEM scan) you should confirm the pore via a TEM image. This image will also serve to give an accurate measurement of the pore's diameter.

1) In the STEM Tab, select the PREVIEW button. This provides an image of the pore on the computer screen. The pore will appear as a black dot.

2) Use the Right Trackball to place the pore in the center of the view screen.

3) Go to the STAGE tab. Select ADD POSITION under the Stage tab. This insures that when you move to the TEM image mode, the stage will be in the same place because if the pore is centered in STEM mode it should be centered in TEM mode as well.

4) Go to the MONOCHROMATOR tab. Select the FOCUS button and use the INTENSITY knob on the left control panel to bring the Monochromator setting to 60. When done, deselect the FOCUS button.

5) Return to the STEM tab. Remove the HADAFT detector by deselecting the HADAFT button.

6) Use the R3 function key on the right control panel to bring the spot size to 9. The spot size is displayed at the bottom of the computer screen.

7) Exit STEM mode by pressing the STEM button under the STEM tab.

TEM mode:

A large green spot should appear on the phosphorous screen. If this does not occur, adjust the intensity knob. If still absent, make sure the objective aperture is open (C2 aperture is located on the body of the TEM scope). If problem persists seek assistance.
1) Use the magnification knob on the right control panel to bring the magnification to 63,000x.

2) Find the nanopore image on the phosphorous screen. It should appear as a dark spot.

3) Center the pore image in the screen using the trackball and increase the magnification. If the beam changes position while bringing up magnification, use the left trackball to bring it back to center. A final magnification of about 900,000x should work.

4) Switch to the EFTEM tab. This is the only imaging mode that automatically calibrates the camera scale so that the image of the pore is the proper size in the camera.

5) Check the entrance aperture in the pull out tab. It should be 5mm. (If it is not 5mm do not continue. Call John Grazul for assistance.)

6) Click EFTEM button displayed on the computer screen. It will turn yellow.

The center of camera is located at 9 o'clock position on the smaller circle of the phosphorous screen. The beam (observed as a green point of light) should be concentric with this circle. **The beam's diameter should be the same size as that of the small black circle on the phosphorus screen!!!** (If it is smaller or larger the intensity of the beam will be too great and may damage the camera--$200,000)

7) Maneuver the pore image to the 9 o'clock position where the camera should be.

8) There is an INTENSITY ZOOM button in the pull out window of the EFTEM tab. Turn it on and then off. This keeps the zoom intensity reset so it doesn't burn out the camera.

9) On the second computer screen (on the right), there is a small bunny icon under the DIGITAL MICROGRAFT tab. Click this to begin a "rabbit scan" with the camera.

10) Press the R1 button on the right control pad to lift the phosphorous screen and open the camera. An image of the nanopore should now be visible on the second computer screen. Center the pore using the trackball.

11) Open an FFT image by selecting the Process tab. In the pull-down menu select LIVE and then Reduced FFT.

12) Select the "Turtle" icon in the DIGITAL MICROGRAPH window to get a slower scan rate. Use this mode to focus the pore.

13) To focus the pore using the focus knob on the right control panel. Make the FFT image as round as possible. It is difficult to describe how it should look, so be sure to train your eye with a CCMR staff member.

When just out of focus a Fresnel fringe will be apparent as a light area around the pore. Always take one image in focus (no Fresnel fringe/ FFT perfectly round) and then take one with Fresnel fringe for easier size estimate.

14) To take an image, select the icon next to the turtle icon.
Go to the Camera menu at the top of the screen.
Select the Files tab
Select Export as TIFF and export to the proper folder.

To End:

1) Select the window with the animal icons (DIGITAL MICROGRAPH).
2) **Press the spacebar on the keyboard**. This stops the camera from taking images and is very important.
3) Press the R1 button on the right control panel to close the screen.
4) Hit FETEM button (takes the scope out of FETEM mode.)
5) Set magnification to 6200x
6) Close the Column Valves
7) Reset holder
If shutting down see basic TEM protocol for standard shutdown procedure.