Leica MZ6 and DMLB Instructions

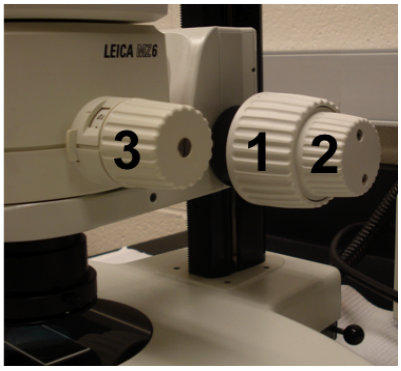
Sample preparation

Place a small amount of sample on a glass slide and spread it out. If the sample contains a significant amount of solvent, put a cover glass on it. Note that a dense sample may lead to a low quality image.

Using the LEICA MZ6

1. Turn on the power of MZ6 (between 70 and 80).

2. Place the sample under the object piece, in the center of the light field.
3. Start with 0.63X, tune the coarse focus **(knob 1** below**)** until the sample is in sight (might need to move the sample to bring it in sight). Then fine tune **(knob 2** below**)** to get a clear image.
4. Turn the magnification knob **(knob 3** below**)** for higher magnifications. Fine tune **(knob 2** below**)** to re-focus once the magnification is changed.

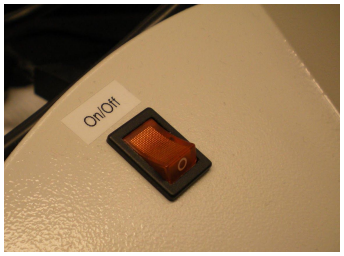


5. To obtain polarized images, turn around the polarized plate until a dark field image is obtained.

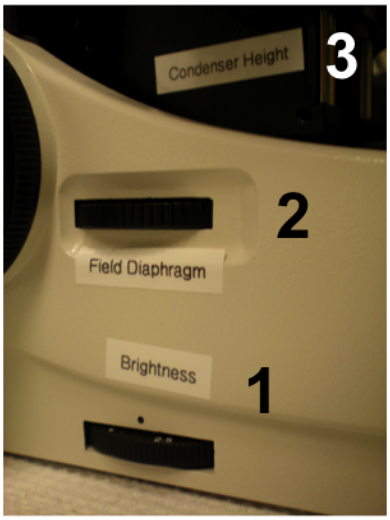
6. Turn off the light source when finished. Clean the eyepieces or object piece with lens paper. Clean with iPrOH if necessary.

Using the LEICA DMLB

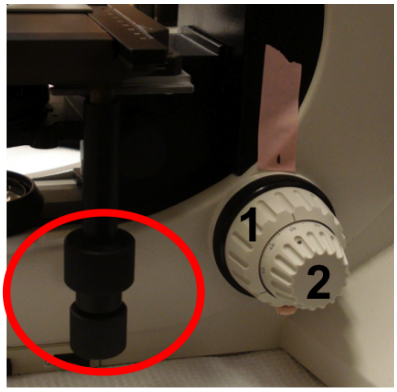
1. Turn on the microscope.



2. Place the sample underneath the 10X objective. Adjust brightness (**knob 1**, default is 5~6).



3. Tune the coarse focus **(knob 1** from #4 above**)** until the sample is in sight. Then fine-tune **(knob 2 from** #4 above**)** the image until it is in focus. Move the sample via the knobs underneath the stage (circle).



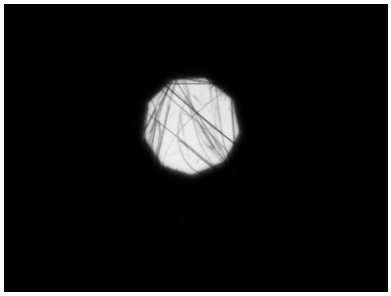
4. To get the optimal illumination of microscope, you need to align the condenser lens properly. The condenser should be close to the sample stage before doing the following steps.

   (a) First, focus the sample in brightfield.

   (b) Close the field diaphragm (**knob 2** in point 2), so it looks something like this.

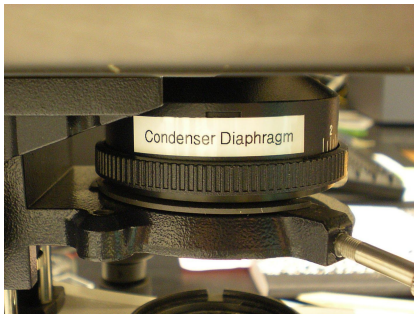
   (c) Focus the edge of the diaphragm by adjusting the condenser height (**knob 3** in point 2), so it looks like this:

   (d) Center the image using the centering screws.



(e) Open the field diaphragm (**knob 2** in point 2) until it is at the edge of the field of view.

(f) Close the condenser diaphragm just to the point where the image begins to get dark and no further.



5. Select different magnifications by switching the objectives (2.5, 5, 10, 20, 40, 100X). Fine tune **(knob 2** in point #4 above**)** to refocus once the magnification is changed.

6. After using the microscope, turn off the power. Switch back to the 10X objective and remove the sample.

7. Clean the objectives with lens paper. Use iPrOH if necessary.

Image capture via LEICA DMLB

1. Once the sample is in-focus under DMLB, turn on the microscope camera.

2. Double click the **Q-Capture Pro** icon 

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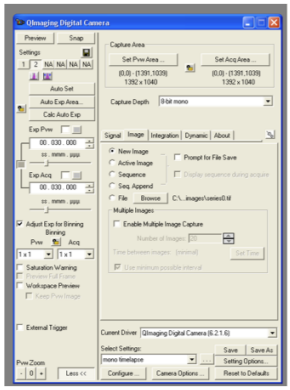
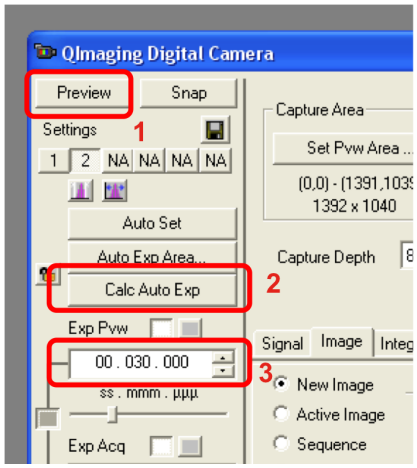
3. Click the digital camera icon, a dialogue box as below should show up.

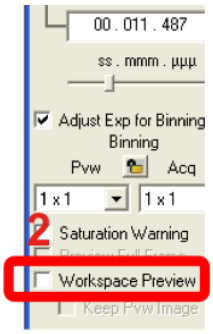


4. Pull the side pore all the way out.

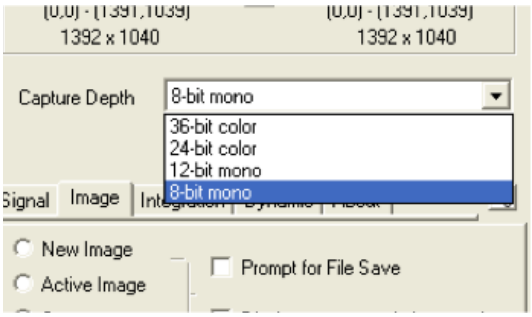
5. Click **Preview (1)** to see the image (at low resolution). If the image is too dark or too bright, click **Calc Auto Exp (2)** to adjust the exposure time. To fine-tune the exposure time, double click the time **(3)** and type in a value.



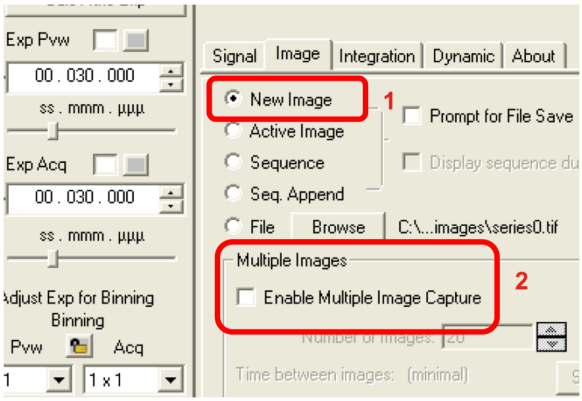
6. To get a higher resolution of the preview, check **Workspace Preview**. Then fine-tune the focus and brightness to get the optimal image.



7. Select **8-bit mono** in *Capture Depth* for black-and-white images; **24-bit color** for colored images.

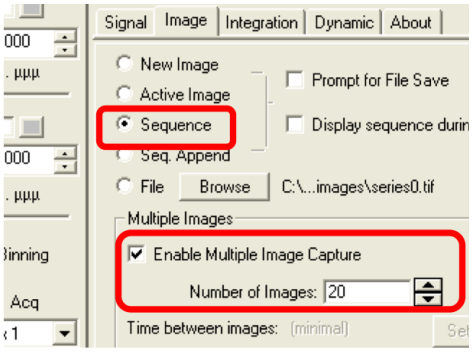


8. To take a picture of the sample, click **New Image**. Make sure uncheck the **Multiple Images** box. Then click **Snap** to capture the image.

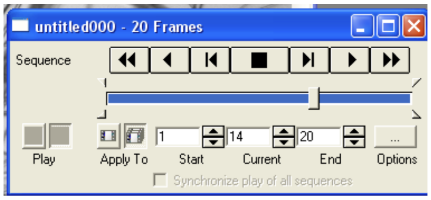


9. Click **File__ Save File as**, name the file and save it as a TIFF file.

10. To make a movie for the sample, click **Sequence**, check **Multiple Images** and type in the number of images you want (< 1000). Click **Snap** to start collecting the images.



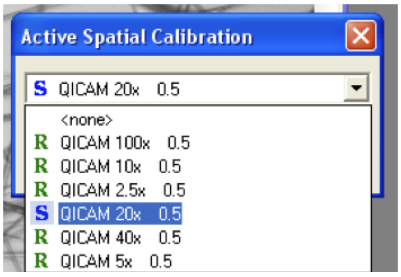
11. Once the capture is finished, the sequence can be played forward or backward, or by frames.



12. Click **File__ Save File as**, name the sequence and save it as a TIFF file (recommended), or an AVI file.

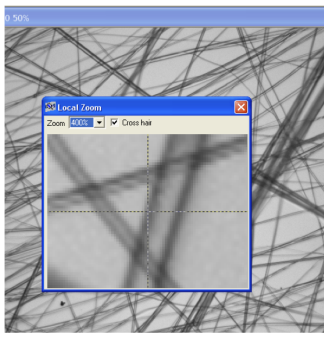
Distance measurements

1. Click  , and set the right magnification.

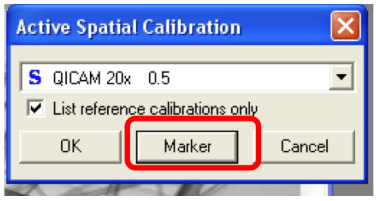


2. Click  to open the Measure Distance box. Left click and drag a line across the distance you want to measure.

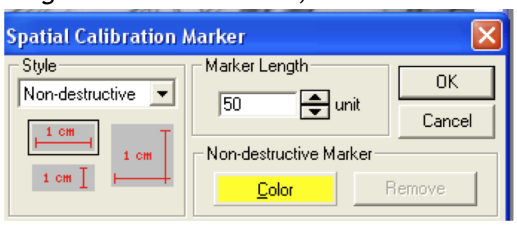
3. For higher accuracy, right click and select **Local Zoom.** Use the cross-hairs to more accurately measure by left-clicking and dragging.



4. To place a calibration bar, click  , and click **Marker**.



Select the calibration *Style* (Non-destructive means the marker is not saved together with the image; Destructive means the marker is permanently saved with the original image), *Marker Length* and *Marker Color*, click **OK**.



5. To save the measurements and calibration bar together with the picture, click  and save the new image.

Hot-Stage Instructions

Initial Setup

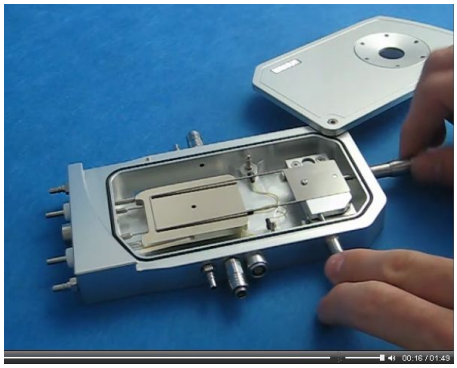
1. Switch on the microscope and the camera.

2. Switch on the cooling pump (the top box) followed by the temperature controller (the bottom box). This order is important!

3. Open the heating stage by slowly turning the top cover, and load your sample. Make sure that the sample is close to the small cavity in the center of the stage.

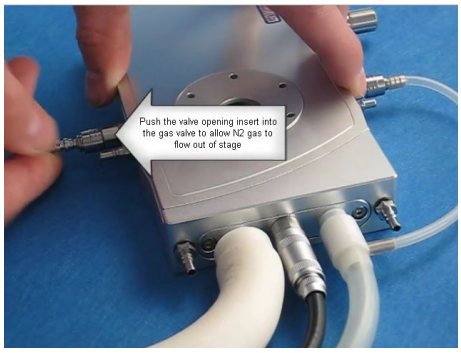
Close the stage gently.

4. You can use the X-Y screws to move your sample. (NOTE: The screws can come out if they are turned too far).



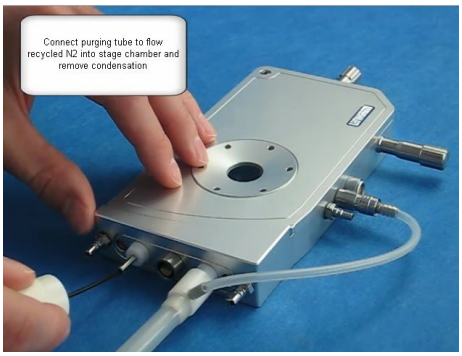
5. Turn the bino/phot knob on the microscope in and focus the sample using the coarse and fine focusing knobs. You can use the polarizer by pushing the polarizer knob in.

6. Connect the tubing as shown in the figure and push the valve insert into the gas valve as shown. This is to allow purging the stage with N_2.



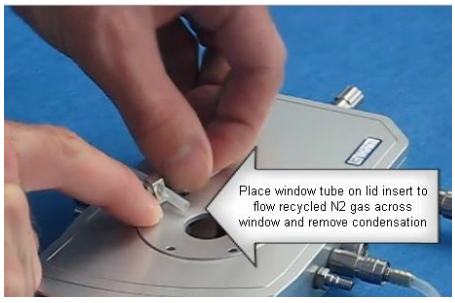
7. Fill liquid nitrogen in the dewar to approximately 3 cm from the top of the vessel. Cover the dewar with the lid but do not fasten catches until the boiling off subsides.

8. Connect the dewar to the stage by slowly inserting the black tubing into the appropriate place as shown.



Push it all the way till the end.

9. Connect the window tube with the clamps.

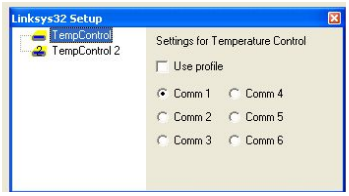


10. Click on the Linksys32 icon on the desktop. 

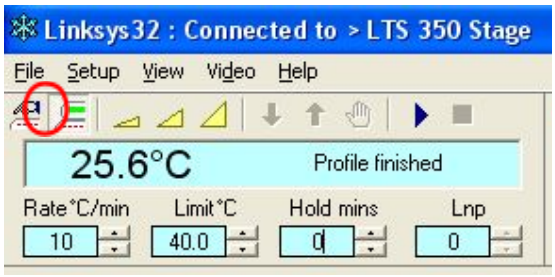
11. Click on **File** -> **Connect** and click on OK.

NOTE: Steps 12-20 required only for experiments involving temperatures below RT.

12. Click on **Setup** -> **Temp Control**. Make sure the "Use profile" box is **UNCHECKED**.

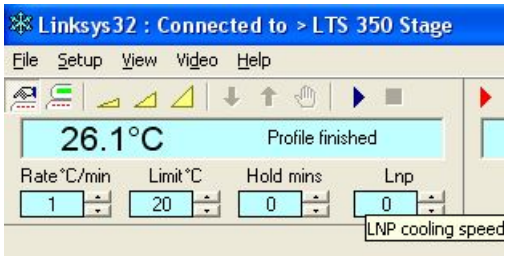


13. Set the LNP control to manual mode. Set the limit (40 °C) which indicates the temperature you want the set the stage to, the rate (10 °C/min) and hold time (1 min). You need to click outside the dialog boxes to make sure the instrument receives the command.



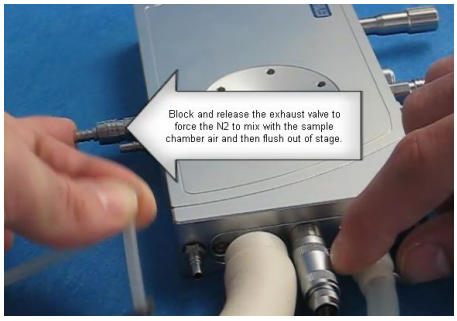
Hit the play button. 

14. Once temperature reaches the setting, increase the LNP speed to maximum using the nudge buttons on the side of where LNP is displayed.

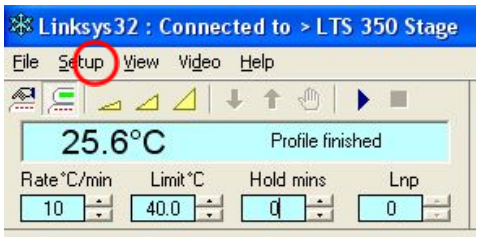


15. Fold and pinch the window tube with your one hand and block the exhaust hole in LNP tube in order to redirect flow of dry nitrogen into the purging tube.

16. Block and release the exhaust valve to force the nitrogen to mix with the sample chamber and flush out of stage. Do this for 60 seconds.

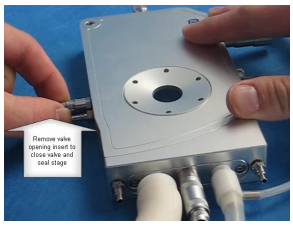


17. Set the LNP back to auto.

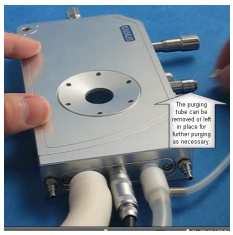


18. Set the limit to 20°C and hit play again. This will bring the stage back to the original temperature.

19. Once the temperature is reached, remove the valve opening insert, and place it back in the drawer.



20. The purging tube can be removed or left in place for further purging.

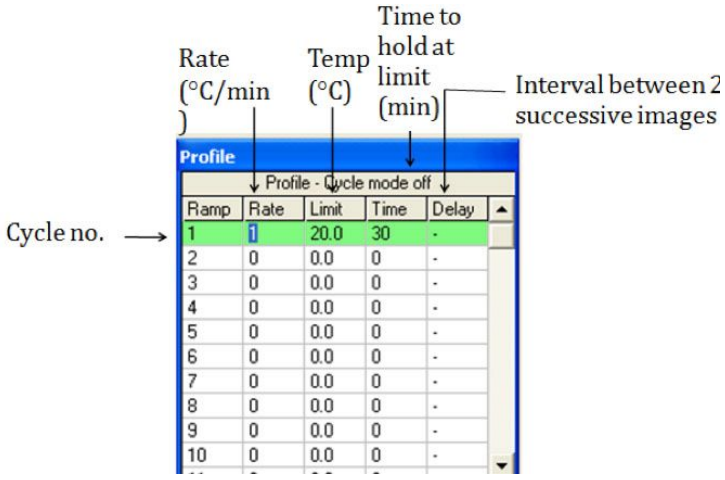


21. Clamp the window tube back to its original position.

SETTING UP A PROFILE

22. Click **View** -> **Temperature Profile**

23. You can enter the rate of heat/cool (˚C/min)-column "Rate", the start/stop temperature (˚C)-colum "Limit", the time to hold at a particular temperature (min)-column "Time" and the delay time between 2 subsequent images (in seconds)-column "delay". After you have entered all the values, make sure to click on a cell on the same spreadsheet apart from the ones you have entered your values, to save your values.

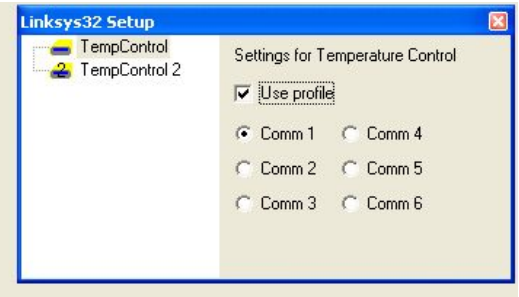


24. Click on **File** -> **Save** -> **Temperature Profile** to save your profiles.

NOTE: The last step of your profile should set limit as 20 ˚C at 10 ˚C/min to bring back the stage to RT at the end of the experiment.

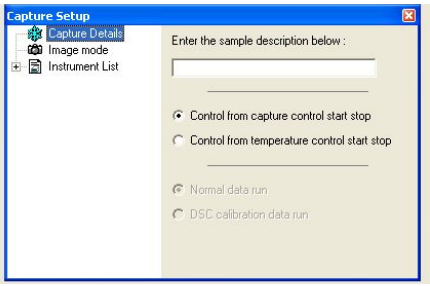
RECORDING DATA

25. Click on **Setup** -> **Temperature Controller** and **"CHECK"** the "Use Profile" box.



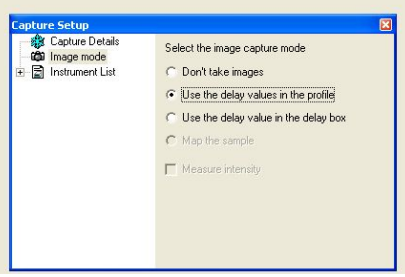
Close the window.

26. Right click on the camera button toolbar. You can select either option.



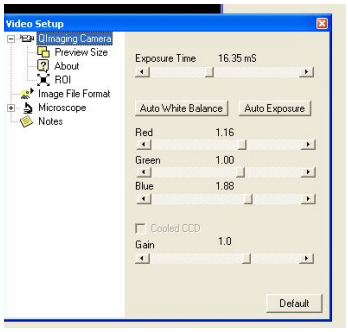
The "control from capture control start stop" will start recording the images only after the red play button is clicked. The "control from temperature control start stop" option will start capturing images the moment the profile is started by hitting the blue play button.

27. Click on image mode. If you want to capture images with a delay interval as mentioned in the profile then check the "use the delay values in the profile" option.



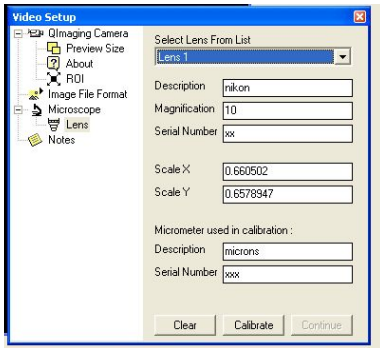
28. Click on the **Video** -> **Show Window**.

29. Right click anywhere in the new window and click on auto exposure. Make sure that the bino/photo knob on the microscope is pulled out.



Focus the image using the "Fine focus" knob.

30. Click on the Lens button on the side bar of the same dialog box and choose the correct lens.



Close the dialog box.

31. Hit the play button  to start recording the data.

32. The moment the profile is finished, or the stop button is clicked, a dialog box will prompt asking you to save your file.

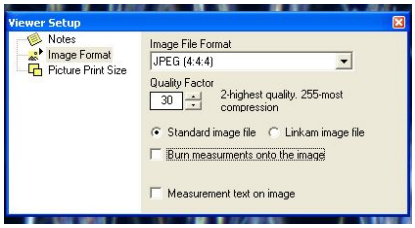
DATA PROCESSING

33. Click on **File** -> **Disconnect** to disconnect communication between controller and computer as data processing is memory intensive.

34. Click on **File** -> **Open** -> **Data File**. Click on the gallery  button to open your images. Click on the image to open it.

35. Click on the ruler tool to perform distance measurements.

36. Right click on the image toolbar and select "Image format". Select the standard image format to view the image on all computers.



37. Export the data by clicking on the camera  button on the image toolbar. You can create your own sub-folder for each experiment but **DO NOT change the location of the sub-folder from "Exports"**.

CAUTION: EXPORTING IMAGES IS A VERY MEMORY INTENSIVE PROCESS AND CAN TAKE UP TO 15-20 MIN DEPENDING ON THE NUMBER OF IMAGES. DO NOT PERFORM ANY OTHER ACTION ON THE COMPUTER UNTIL THE IMAGES HAVE BEEN EXPORTED.