The Microscope and Neurolucida

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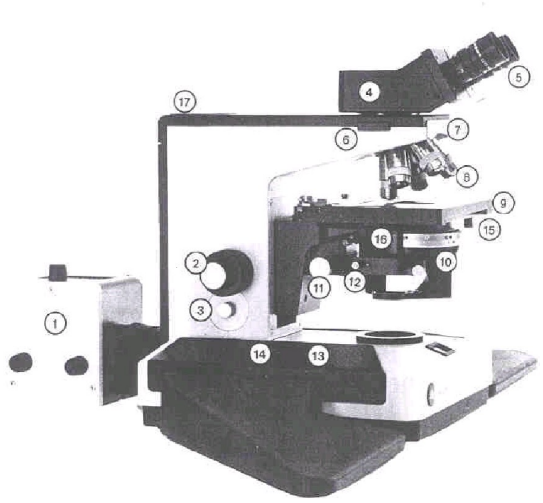

1. Lamphousing 103Z
2. Coarse and Find focus Controls
3. Focus Stop Screw
4. Binocular observation tube S
5. Periplan 2.5x/20 M eyepieces
6. Filter Slot
7. Quintuple Objective nosepiece
8. PL FLUOTAR objectives
9. No. 87 Mechanical Stage
10. UKO universal condenser
11. Condenser Height Control

I.Set up and Callibration

II.Connection and Operation

III.Tracing

Before any tracing can be done, the microscope must first be setup and calibrated correctly. Without this the results of any traces will be very skewed. This lab uses a Leitz Diaplan with Lamphousing 103Z, UKO Universal Condenser, No. 87 mechanical Stage, and binocular observation tube S. The Leitz objectives are oil immersion. They're magnification range from 4x - 100x and numerical aperture increases from 0.12 -1.32.

In order to setup the lamp housing, first remove the light trap and any filters from the filter mount. Locate the centering in the light path. Focus the image of the lamp filament on the centering aid's screen using the collector knob. Use the centering knob to move the image into the upper half of the illuminated area, and then use the centering knob to move the image horizontally until it completely fills the upper half of the illuminated area. Now use the centering knobs to move the mirror image into the lower half of the illuminated areas with the image. Finely adjust the knobs until the two images just touch in the center. Finally, remove the centering aid and replace the filter and/or light trap.

In order to correctly view a specimen, the condenser, field diaphragm and aperture diaphragm must all be set correctly for each individual specimen and magnification. First the condenser must be centered. Close the field diaphragm and raise the condenser as high as possible. Now looking through the eyepiece, lower the condenser till edge of the field diaphragm is in focus. Use the two screws on the condenser to move the

image of the field diaphragm to the center of the field of view. Finally, open the field diaphragm until its image is just larger than the field of view. Centering of the condenser may have to be done for each different objective used.

The field diaphragm keeps the specimen from receiving unnecessary light and heat. As the objective get larger, The aperture diaphragm determines the contrast and resolution of the image. The best optical performance is obtained when the objective aperture and aperture diaphragm are the same size. If the aperture diaphragm is close to a size less than that of the objective aperture, the resolution will be reduced, but the contrast increased. This technique can be used successfully; to map out dendritic trees especially when the dye used to stain the cell is not sufficient enough to provide enough contrast against the surrounding medium. However, if the aperture diaphragm closes to less than one-third of the aperture of the objective, the eye will notice a significant decrease in resolution. To set the aperture diaphragm correctly, remove an eyepiece from its mount, and close the aperture diaphragm until the image is just visible on the rear objective element. This is the normal setting. Replace the eyepiece. In addition, the aperture diaphragm should not be used to adjust the image brightness. Only the rotary control should be used for this purpose. When using objectives less than 0.25, slide the condenser top out of the light path.

Light microscopes are limited by the optical resolution that can be obtained. For a microscope that limit is approximately 0.6*l/NA where l is the wavelength of light and NA is the numerical aperture of the objective. For our microscope, the maximum resolution at 100x magnification is 0.23 mm - meaning that any dendrite for instance whose width is less than 0.23 mm will appear to be 0.23 mm due to light diffraction. This can cause some error when mapping out the dendrite branches. Another problem is caused by orientation. If a dendrite runs vertically through the slice especially underneath or above the soma, the reconstruction may suffer in accuracy (De Shutter). However, this is not a problem for the cell done so far. Pyramidal Hippocampal cell do not exhibit such geometry and seem to stay planar for the most part.

Microbrightfield, Inc., makes Neurolucida. It seems to be the only software available, which provides the ability to map 3D neurons. Before tracing can begin, Neurolucida must be calibrated with the xyz-motorized stage. First, in order to use the software, the Prior OptiScan machine must be turned on. Then Neurolucida can be started. Once running, the video feed from the microscope automatically comes up. After the desired slide is placed under the microscope, progressively greater units of magnification should be used to locate and view the cell. The joystick should be used to move the slide around. Z-control however must be done manually. When the highest-powered magnification is in use, one must use caution against cracking or damaging the cell while using the Z-control. One should notice that on that setting, the Z-control can make the lens physically touch the slide. After adjusting the z-control, adjust the condenser to view the cell more clearly. Condensers work by collecting the light and concentrating it uniformly over the specimen. It can not be over emphasized how important it is to obtain the right angle and intensity of light over the specimen. For lower powered magnifications, the diameter of illumination must be much greater than for higher-powered magnifications. Therefore, as magnification increases, the height of the condenser must increase to decrease the size of the field of illumination. The second important aspect is the aperture diaphragm. The aperture diaphragm controls the angle of light hitting the specimen. If it is opened too much, light will come at oblique angels and will cause large amounts of glare. If it is closed too much, there will not be enough light for proper contrast of the specimen. Generally, as the power of magnification increases, the aperture must be opened more and more.

Once a suitable image is obtained, the cell must be mapped. In Neurolucida, you must specify your point of reference. It is usually helpful to click on a point somewhere on the cell that is easily recognizable. Once this is done, there are a few things to check. Make sure that the magnification selected matches the objective magnification. Once this is done, on the cell part selector, click "cell body". Now adjust the radius of the circle that appears at the crosshairs. This is done with the mouse wheel. You use this circle to indicate the thickness of the cell body and dendrites. Begin clicking points along the cell body. The more points you get, the greater the resolution of the image. It's important in when plotting the cell body to finish it by clicking at your last point, your first point. You don't have to get exactly the same coordinates, just a good

approximation. Then, right click and select "Finish Cell Body". Once the cell body is done, change to cell parts selector to dendrites and begin mapping out the dendrite. Remember to keep the circle on the cross hairs equivalent to the diameter of the dendrite. When you get to the first branch, click on the branch node itself, then right click and select what kind of node you have come to i.e. a bifurcating node or a trifurcating node. Once you have selected your type of node, click on the node point again before continuing down one of the branches. Repeat this step as you move through the cell. When you come to an ending, simply right click again and click on "ending". Neurolucida will then automatically take you to the last node you passed and you can start down the other branch. Once all the branches are done, save the file as an ASCII file.