

Hippocampus Tracing Using Nikon Model Eclipse E400

Protocol:

1. Turn on Nikon Model Eclipse E400 microscope.
2. Turn on the desk lamp.
3. Use Kimwipes to clean lens and mirrors.
4. Place the slide under the microscope upside down so that the label is to the right and is upside down.
5. Set the resolution to its lowest (4x/0.10, red).
6. Make sure the magnification scale is at 7 (green tape holds the knob in place).
7. Make sure the "Draw vs. Observe" knob is pushed in.
8. In order to see the camera view necessary for tracing, the lighting knob must be turned counterclockwise to decrease the lighting. The knob can be turned clockwise to increase the light when needed.
9. Adjust the clarity of the image using the finer control.
10. Do not move the clipboard or the paper until the entire hemisphere is completely traced. If necessary, tape down the paper and clipboard.
11. Include your name or initials, date of work completed, magnification scale, and the bird I.D. number somewhere on your paper. Always use a sharp pencil to produce tracings that are clear and detailed.
12. Beware of tissue overlapping. In your tracing, be sure to include the section of the tissue that overlaps with the other hemisphere.
13. Edges of the hippocampus may be torn.
 - a. If the tear smooth like a semicircle hole, then the tear is most likely a ventricle. In such cases, you must include the hole in your tracing.
 - b. If the tear is sharp, has ridges, and looks unnatural, then the tear is most likely a tear that occurred when the slide was mounted. In such cases, assume that the hippocampus is there and trace over the tear.
14. The middle of the hippocampus may have tears. If the tear is minor, then it is okay to disregard the tear in your tracing. However, if the tear is significant enough, you can change the volume of the hippocampus by using a dashed line to subtract the added volume in your tracing.
15. Often, the boundaries of the hippocampus are ambiguous.
 - a. Look for color differences. The color of the hippocampus is a lighter purple than the surrounding tissue.
 - b. Look for changes in cell density. Neuron cells in the hippocampus are further apart, thus the hippocampus region looks like it has a smaller cell density than the surrounding tissue.