Lab #3: Basic microscopy, histology and cortical neuroanatomy

The goal of today’s lab is to do some simple investigations involving tissue slices from real human brains. In the process, you’ll learn how some histological research is done, and you should become familiar with both the appearance of neurons under magnification, and the basic stratified structure of cerebral cortex.

The department only has 5 microscopes, so split into four evenly distributed groups (given class size, there should be about 3 per group), and turn in one lab assignment per group. Some of the questions require sketching tissue slices, so you may want to identify any particularly talented artists in your group (though artistic skill or expression will not be a grading criterion). Also, even though one person will most likely do the actual drawing on those items, make sure everyone participates, by discussing what features to emphasize, etc.

The microscopes in the lab have four objectives, providing 4x, 10x, 40x and 100x magnification. When combined with the magnification of the eyepieces (10x magnification), these result in objects being 40, 100, 400 or 1000 times. Note that the 100x objective is an oil immersion lens and doesn’t work the same as the others (I’ll demonstrate for you how to use it).

General Microscopy advice:

When you look at each slide, start by looking at it first without magnification – identify where the surface of the cross section is, and note any distinguishing features so that you know what you’re looking at when they’re under the microscope. Then begin under the lowest magnification level and work your way toward higher levels of magnification, in order. Keep in mind that under higher levels of magnification you may not be able to see as much area as under lower levels. If a layer or feature disappears, that may be why. Move the slide around a little bit and make sure you’re not missing anything.

In order to see individual cells and draw a nice representation of layering, I recommend using the 40x objective (400x magnification). Lower levels may not reveal a satisfying level of detail, and higher levels may make it difficult to distinguish layers. However, try different levels and use what is most comfortable for you.

Once you’ve put oil on the slide in order to use the oil immersion lens, it can be messy to go back to lower magnification, since you want to avoid getting oil on the dry-use objectives. For this reason, I recommend leaving the highest level of magnification for last. If you decide to go back to a lower magnification level, please clean off the slide before you do so.
Slide Preparations

Individual neurons are difficult to see unless the tissue has been prepared so that they stand out from other types of tissue. This process, for obvious reasons, is called staining. The slides we’ll be looking at today use one of three common types of stains, each of which is a different color, and each of which targets different kinds of tissues. Hence, none of the colors you see through the microscope are naturally occurring ones – they’re created by the stain. You’ll see the usefulness of this as you begin to identify structural patterns.

The stain types are:

1) *Hematoxylin and Eosin*. These slides will appear mostly pink (for example, slides 1 – 4, 8, 9, …)
2) *Luxol Fast Blue*. These will be (you guessed it) blue or purple in color (for example, slides 7, 10, 14…)
3) *Silver stain*. These slides will be a bit darker. Strangely not silver - more of a brown color (for example, slides 5, 6, 11, 12…)

Each type of stain has its own advantages and disadvantages, so some things that are obvious when prepared with one kind of stain will be imperceptible with another stain. I recommend looking at all three before committing to any answers or beginning to sketch tissue. Don’t become frustrated if you can’t identify all the layers you think should be there right away. Be patient – try another area on your slide, or another slide altogether. A different stain may give you some good clues about what to look for.

Each group will have a similar set of slides, but obviously won’t be identical. You may also want to swap slides with another group to see some variation (and some sets lack slides that are present in other sets due to differential attrition). If you borrow from another group, make sure to keep the slide sets’ contents intact, so that one set doesn’t have two of one slide type and none of another. The handy colored labels will hopefully make this easy. Also, feel free to look at any of the other slides not mentioned directly in the handout. They’re just as interesting. I also have some slides of brain tumors that you can look at that aren’t in with the rest.

When you’re finished, make sure all the slides in your set are there in their plastic case, with slides in their proper numerical order, and return it to me. After you’ve cleaned off any immersion oil and the microscope is clean, make sure the microscope is turned off and replace its dust cover.
1. Types of stains

You already know a little about the structure of cortex and spinal cord sections (also see
the diagrams on the end of this handout). Compare the appearance of the different slides of
cortex and spinal cord sections provided. Then use your knowledge of structure to infer what
each stain targets, and specify what color it turns those structures (some stains may color
multiple structures different colors).

I think it’s helpful to try to answer the following questions for each stain type:

What does grey matter in cortex look like? (and what does grey matter contain?)
What does white matter in cortex look like? (and what does white matter contain?)
What does the central (“grey”, H-shaped) part of the spinal cord look like?
What does the outer (“white”) part of the spinal cord look like?

Based on the answers to the above questions, infer what the stain is coloring – possibilities
include cell bodies, nuclei, glial cells, myelin, everything, etc… State your conclusions below,
and summarize what you saw in the slides that led you to those conclusions.

Note: if you’re a biology aficionado and already know what these do, don’t just write them
down. You still need to justify your answers with observations from today’s slides. Some of
you may find it helpful to sketch out the appearance of each slide under magnification (you’ll
need to do this for #2 below anyway).

Fast Blue:

Silver:

Hematoxylin and Eosin:
1b. Application of staining techniques
Based on the above, what type of stain would be best suited for the following purposes? Explain your logic, based on the answers given above. Discuss these with your group and come to a rational, defensible conclusion. In addition to the stain, indicate why you made the choice you did.

a) Searching for the presence of a potential tumor? (remember that brain tumors consist of glial cells, not neurons)

b) Tracing the connections of axons?

c) Counting the density of cell bodies (somas) in a brain structure?

d) Determining the progression of a demyelinating disease such as ALS (Lou Gehrig’s** disease)?

**A bonus point if you can tell me which now retired major league baseball player broke the Iron Horse’s consecutive games streak (albeit without having to battle a progressive neurodegenerative disease).
2. **Stratification of cerebral cortex**

Sketch out the layered structure of cortex, under one type of stain (indicate which one at the top of your sketch). Refer to the diagram of cortical layering on the back of this handout to get your bearings. Mark on your drawing your best estimate of where the borders between layers are located, and the contents of each layer. Are any layers left indistinguishable? Which ones, and why? (hint: might this have to do with the stain that was used to prepare the slide?)
3. **Layering in a different brain structure: the cerebellum**

We haven’t discussed the cellular structure of the cerebellum yet. However, by now you should be able to make some accurate guesses by comparing the cerebellum to cerebral cortex. Draw a rough sketch of a cross-section through cerebellar cortex. Draw lines where you would propose making a distinction between layers. For each of your layers, indicate your best estimate of what that layer contains (ie, mostly axons, cell bodies, glial cells, etc…) and explain how you arrived at your estimate.
4. Neural Diversity: Structuring of non-cortical tissue

Finally, investigate one more type of slide. In your slide sets are the following (among others):

- Nerve
- Pacinian Corpuscle (a touch receptor cell)
- Taste buds (these are very cool looking if you ask me)
- Pons
- Medulla
- Basal Ganglia (be sure to look at different areas of the same slide for this one)

Select one of the above, and sketch out the structure, as it appears under your microscope. Indicate the magnification level and the type of stain used in your drawing. Finally, label in your diagram any distinguishing features or details, and indicate the contents (axons, glia, soma, etc…) of the slice where there are differences in distribution.
SPINAL CORD ORGANIZATION.

LANDMARKS
- POST. MEDIAN SULCUS
- POST. MEDIAN SEPTUM
- POST. INTERMED. SEPTUM
- POST. LATERAL SULCUS
- ANT. LATERAL SULCUS
- ANT. MEDIAN FISSURE

WHITE MATTER
- POST. FUNICULUS
- LISSAUER'S FASCIC
- LAT. FUNICULUS
- ANT. FUNICULUS

GRAY MATTER
- POST. COLUMN/HORN
- LAMINA I/PST. MARG.
- NUCLEUS
- LAMINA II/SUBST. GELAT.
- LAMINAE III, IV/NUC. PROP.
- LAMINA V
- LAMINA VI

LAMINA VII/INTER. ZONE

ANT. COLUMN/HORN
- LAMINA VIII
- LAMINA IX

GRAY COMMISSURE
- LAMINA X

POSTERIOR ROOT FIBERS

ANTERIOR ROOT FIBERS
You should be able to see the existence of layers in your slides, but it will probably be tough to differentiate and identify all of them. The more important contrast for answering question #1 will be between white matter and grey matter (ie, all 6 layers collectively).