Camera Alignment (Draft v002)

Please note that demonstrations of these calibrations are located in MBF's support tutorials!!

Use the grid slide provided by MBF Bioscience

1. Bring grid into focus
    a. Options→Display preferences → Grid (make sure you drop a reference point first or grid won't show up!)
        i. Check☑ 'Grid enable'
        ii. Bright Grid
        iii. Grid Spacing (250 x 250 (5-20x) or 25 x 25 @ 20-63x)
    b. Find speck of dust
        i. Follow from right to left on the grid using the joystick
        ii. Adjust and retest until perfect
            1. I've found that lining up the grid marks are an excellent way to get started!
            2. Loosen the screws slightly on camera and turn slightly clockwise or counterclockwise until grid lines up! – When you tighten the screws, the camera WILL shift.
        iii. ***most important step b/c all other calibrations will be affected
        iv. Turn off grid enabler
2. Calibrate Objectives
    a. Tools→ grid tune current lens (make sure tool bar notation of objective matches actual objective!!)
            1. 5x -20x obj. calibrate with the 250µm grid
            2. 20x-100x calibrate with the 25µm grid
            3. Check☑ OK
        ii. Drag anchor to line of grid, stay to inside and above gridlines
        iii. Tweak lines until the grid lines are lined up.
        iv. Right click to finish
        v. Repeat for each objective
3. Calibrate z
    a. On MFB grid slide find where the X is drawn. One line of the x is drawn on the bottom and the other is drawn on the top of the slide. (makes sure that your z-piston connects to your stage)
    b. Turn on z-axis measuring pop-up tool (real-time depth measures)
    c. Test fast focus (another pop-up tool) to make sure that the measuring tool is working in 20um increments.
    d. Focus on one side of the x, where the e lines cross, and note z-location.
    e. Move the z-axis until the line on the opposite side of the slide comes into focus. Note z-location.
    f. The slide's width is 1.5mm. The difference should be 1500um.
4. Parcentric Calibration – DO NOT USE OIL!! (Only after objectives calibrated!) **make sure objectives are screwed in tight!
    a. Tools→parcentric/parfocal calibration
        i. Start at highest magnification (Make sure your objective is at 63X or 100x)
            1. Bring grid into focus
            2. Move crosshairs + to top right corner
            3. Click without moving stage
        ii. Change toolbar magnification to 40x
            1. Then the actual objective
            2. Line up and click
            3. Repeat for 20, 10 and 5x → click OK
        iii. Checking that all is well calibrated
            1. Go to 63x, draw contour
            2. Switch through all objectives (physical) and magnifications (on tool bar)
            3. Ensure that the box lines up

Any movement of the actual camera on the Axioskop will require a repeat of these procedures!

FYI's

- Course adjustment on scope
- Fine adjustment on x-y-z controller
- Always drop a reference point first! – Then everything else will work!

Questions or tips for Masha

1. How do we acquire calculations for – Neuroleucida Explorer
    a. branch length,
    b. branch points,
    c. soma area
2. How do we differentiate
    a. spine types
    b. dendritic spine Numbers/designated length (density)
3. Pros and cons of using contour mapping versus manual
4. dendritic density (Sholl)- number of intersections?
5. We need to ID the best cortical layers to measure!

Notes from first training seession:

Watch Neuroleucida Webinars. – they are 40 minutes long and will inform you how to work with the basics of Neuroleucida!

http://www.mbfbioscience.com/webinars

1. In order to acquire calculations for branch points, lengths, soma area
    a. Make a full tracing of neuron in Neuroleucida (you can go between contour and manual tracing). Contour will provide soma volume which might be an interesting DV.
    b. Save the file
    c. Open the file in Neuroleucida Explorer (the icon below the regular neuroleucida icon).
        i. At this point you cannot make any changes, but you can analyse and extract data.
    d. Analysis→Branched Structure Analysis→check dendrites and other parameters you are interested in..
    e. Export to Excel
2. For Soma Volume
    a. Choose contour mapping and make multiple closed contours 5-10 microns apart
    b. Save data file and open in Nerolucida Explorer
    c. Analysis → Markers and Regional Analysis → 3D summary → Enclosed Volume
3. For Spine Types
    a. Go to Neuroleucida tracing file
    b. Drag tool bar down (if you want).
    c. Click on spine type. Right click on head of spine and connect with branch. Repeat for all similar spines.
    d. Use 63x oil or 40x no oil. Best if you use 100x (we do not have).
    e. Deselect spine to go back to tracing mode
4. In order to modify tracings
    a. Select objects→Right click nearby the dendrite you want to add to and select “insert a node”
    b. Right click to splice in new branch point and choose smooth adjustment to make if blend in with the z-axis. N.B. you can only splice to a separated dendrite
5. Options → Display preferences – view colors of each branch or 1^{o}, 2^{o}, 3^{o} branches, change opacity, remove node dots
6. Contours – use multiple closed contours at a variety of depths to reconstruct and calculate soma volume
7. Sholl Analysis
    a. Neurolucida Explorer → Analysis → Sholl Analysis
    b. Check Axon, dendrites , radius , intersections, etc.
    c. Export to Excel
        i. Ecell provides 2 tabs – one with axon parameters and one with dendrite parameters.
8. Helen
    a. Decide which layers to analyze and why?
    b. Which layers are primarily involved with somatosensory circuitry?
    c. barrel field in layer IV of the rat somatosensory cortex.

Hints and Tips

- Try to keep the exposure below 100 to prevent lag between the camera and the computer. You can also reduce the amount of colour by whitening the image through camera settings.
- Pick an easily identifiable reference point e.g. at the edge of the section.
- Display preferences >> adjust transparency of contour lines
- Place markers then select Go to >> click on markers
- It is useful to have Macroview pulled up at the top as this shows you where you are in your contour lines.
- Holding down shift allows you to adjust the thickness of the most recently placed point.