

Microscopy Guidelines
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1/15/18

Before you begin:

1. Before using the microscopes, you must attend training with a Peyton Lab member.
2. After training, you will be granted access to the shared google calendar to schedule microscope time.
 - a. If you need the scope for 24+ hr, schedule over the weekend.
 - b. If you would like the microscope left on when the person before you is finished, there is a contact sheet located at each microscope.
3. Before entering the microscope room, check that the sign above the door is not illuminated before turning on the light (if you need to turn on the light, the switch is located behind the confocal microscope)
4. If sign is illuminated, do not turn on the light as you may disrupt the experiments that are currently running.
5. Do not attempt to transfer data from computers while experiment is running.

General notes:

1. When using CO₂, make sure that you only turn on/off the main tank valve.
2. With tissue imaging, avoid using green stains because there is some natural green fluorescence.
3. The channel(s) that is checked will be included in experiment.
4. The channel that is highlighted is what you will see in live view.
5. If the person before you didn't use incubation (CO₂ or heat), you must reboot the microscope.

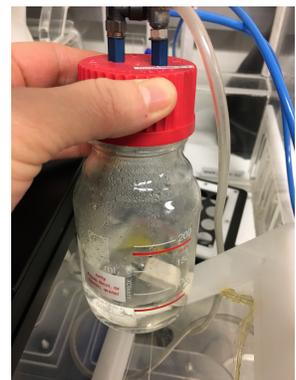
Wide Field (Vivatome) Microscope

Steps to turn on microscope:

1. Sign in with name, lab, date, and time that you are starting.
2. Turn on heater/CO₂ switch in back (if using). If heat is necessary, turn on the microscopy 20 minutes prior to using.
3. Turn on basic scope switch on bottom right on scope (labeled basic scope).
4. Turn on x-cite box only if you'll be using fluorescence.
5. Turn on scope body on left, rear side of scope.
6. Open Zen program.
7. Calibrate the microscope when prompted by Zen.
8. Check that water level is between two red lines in glass humidifier inside incubator.

Using touchscreen:

1. Choose objective through the Control option.
2. Confirm whether you want heat and CO₂ on during imaging through the Incubation option. If you have turned on the CO₂ via the touchscreen and the CO₂ levels are not rising, check to make sure the tank is open.
3. If using 63x, make sure you are using the oil immersion medium, 518F.



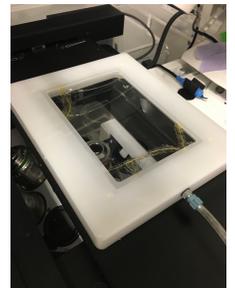
Setting up experiment:

1. At top left side of screen, choose Acquisition tab.
2. From dropdown menu, choose saved experiment or create a new experiment.
3. Choose which camera to use, IC (color camera) or MR R3 (black/white camera), in dropdown menu under Acquisition tab.
4. Determine whether you want to include z-stack, tile, or time series experiment and choose channels to be included in experiment under Acquisition tab left menu options.
5. For z-stack experiment:
 - a. Determine whether to set a center point and image a distance above and below that point or whether you want to set a first and last point and image between. You can either set the distance between images or how many slices to image over a specific distance.
 - b. Imaging a z-series:
 - i. Gallery - to see all slices
 - ii. Ortho - gives you a cross section at any location
 - iii. 3D - allows live rotation
6. For tile experiment:
 - a. You can either determine an area with tiles (regular or irregular shapes) or choose specific points as tiles.
7. For time experiment:
 - a. Choose how often you want to image tile over what amount of time (make sure you can finish experiment in time that microscope is reserved).
8. Make sure that you know where the experiment will be saved using the AutoSave option on the left menu options, preferably to an external hard drive so you do not have to take computer space and microscope time to retrieve experiments. Do not attempt to retrieve data while someone has an experiment running as it can cause Zen to crash.
9. Before running experiment make sure that channels you want to be included have a check next to the channel.



Loading and Finding Sample:

1. Make sure either microscopic slide or well plate insert is secure and does not wobble.
2. If using CO₂, place plate cover over sample. Make sure you don't lower condenser too far which cracks the CO₂ plate.
3. Find sample using either the live view or through the eyepiece.
4. For live view:
 - a. Use joystick to move sample under lightsource and under Acquisition tab click on Live view.
 - b. It is helpful to click on min/max under the display option at the bottom of the screen to ensure that the
 - c. Use the focus knobs on the touchscreen to focus your sample.
5. For eyepiece view:
 - a. Touch the Make it Visible button on the touchscreen to view sample with brightfield.
 - b. Click on the Locate tab and choose a channel at the top to view.
 - c. Make sure the light pathway leads to the eyepiece. The amount of light can be adjusted by the turning knob at the bottom of the microscope.
6. If you are using 40x or higher objective, locate what will be imaged in a lower objective before switching. If you switch to a higher objective that requires an immersion medium, there will be a popup to remind you to apply the medium before continuing.



- If you want to take an image, make sure the channels you want included in the image have a check next to them and click Snap button. You can change the brightness of individual channels using the histogram.
- To add a scale bar, click on the ruler on the top menu bar, drag to set length or right click to change properties.
- To export czi files as images (jpeg or tiff), open files and choose either image export or batch export if there are many files under the Processing Tab.

Confocal Microscope

Steps to turn on microscope:

- Sign in with name, lab, date, and time that you are starting.
- Turn on heat/CO₂ if necessary on the powerstrip to right of microscope. If heat is necessary, turn on the microscopy 20 minutes prior to using.
- Turn on 2 powerstrips under the scope on right and leg sides.
- Turn on HXP 120 C box if you'll need fluorescence during imaging
- Open Zen program
- Calibrate the microscope when prompted by Zen.
- Check that water level is between two red lines in glass humidifier inside incubator.



Using touchscreen:

- Choose objective through the Control option.
- Confirm whether you want heat and CO₂ on during imaging through the Inkubation option. If you have turned on the CO₂ via the touchscreen and the CO₂ levels are not rising, check to make sure the tank is open.
- If using 25x, 40x, or 63x make sure you are using water immersion medium, W2010.

Setting up experiment:

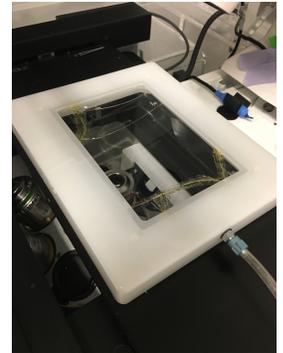
- At top left side of screen, choose Acquisition tab.
- From dropdown menu, choose saved experiment or create a new experiment.
- Choose which camera to use, IC (color camera) or MR R3 (fluorescent camera), in dropdown menu under Acquisition tab.
- Determine whether you want to include z-stack, tile, or time series experiment and choose channels to be included in experiment under Acquisition tab left menu options.
- For z-stack experiment:
 - Determine whether to set a center point and image a distance above and below that point or whether you want to set a first and last point and image between. You can either set the distance between images or how many slices to image over a specific distance.
 - Imaging a z-series:
 - Gallery - to see all slices
 - Ortho - gives you a cross section at any location
 - 3D - allows live rotation
- For tile experiment:
 - You can either determine an area with tiles (regular or irregular shapes) or choose specific points as tiles.
- For time experiment:



- a. Choose how often you want to image tile over what amount of time (make sure you can finish experiment in time that microscope is reserved).
8. Make sure that you know where the experiment will be saved using the AutoSave option on the left menu options, preferably to an external hard drive so you do not have to take computer space and microscope time to retrieve experiments. Do not attempt to retrieve data while someone has an experiment running as it can cause Zen to crash.
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Troubleshooting:

Cannot see sample through eyepiece

1. Make sure sample is directly above light source
2. Make sure switch under eyepiece is turned to open eye
3. Try turning knob at very bottom front of scope to allow more transmitted light through
4. Try touching the "Make it Visible" button on touchscreen
5. Make sure light path under locate tab has the eyepiece illuminated at the end of path
6. If trying to see fluorescence, make sure X-cite box or HXP 120 C is turned on

Cannot get lasers to fire

1. When the confocal microscope is first turned on, sometimes it takes some time for the lasers to warm up
2. Try turning the microscope off and back on again. Wait up to 15 minutes to allow the microscope to cool down.
3. Try switching experiments to see if a setting has been changed in the experiment you are using