

OPT Quick Reference Guide
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First and foremost: be mindful of what you are doing at all times. The OPT microscope is delicate and must be treated as such.

SAMPLE CLEANING AND PREPARATION

- Make sure your sample is *scrupulously* clean, dust and fluff will ruin brightfield images.
- Ensure you don't have any air bubbles near your sample as these will appear as bright spots in your final image ruining the reconstruction.
- Ensure that there is enough empty space (atleast the size of your sample) either above or below your sample to perform background and flatfield correction imaging.
- Ensure that the agarose is trimmed to approximate a cylinder with no sharp edges
- Do Not trim the agarose to close to your sample if performing brightfield imaging, or you will image the edge of the agarose, preventing 3D reconstruction.
- Ensure the sample is bonded very strongly to the metal post and aligned to the centre of the post as much as possible.

STARTUP

- Inspect the scope to be sure that all the covers are in place, that there is no liquid where it should not be, no BABB on the scope or table, etc. If there is mess, clean it up before continuing and report it on the feedback sheet.
- First, turn on the computer and log in.
- Then, turn on the Remote Control and allow the system to power up completely.
- Turn on the Exfo Exacte Lightsource if you are going to image fluorescence.
- Start the OPTiscan software
- Question 1: The Hardware will now be Initialised:
 - A. CONTINUE, to Continue and use the Software
 - B. QUIT, to Quit the software
- Question 2: Will you be using the Exfo Lightsource?
 - A. YES, if you have turned on the exfo exacte lightsource for fluorescence imaging.
 - B. No, if you are only imaging in Brightfield
- Question 3: Press Home to Calibrate the Sample Robot
 - A. HOME, if the system power was turned off when you came into the room.
 - B. NOT NOW, if you have just restarted the software due to a crash etc
- In case of a Device Communication Error, a dialog box will appear.
 - A. – if the radio button next to the Zoom Lens is black, cycle the power on the *Stepping Motor Control II*, If this doesn't work, shutdown the software, and cycle the power on the system.
- If a full restart does not work, contact John Griffin, Darren Paul or James Springfield

- **Sample Robot Menu**
 - A. Sample Height – Vertical arrow buttons and vertical sidebar
 - B. Sample Focus – Horizontal arrow buttons and horizontal sidebar
 - C. Sample Rotation – Fixed movement buttons, arrow buttons and rotation dial button
 - D. Abort Motion – Press big red button to immediately stop robot motion in an emergency
 - E. Mark Sample Position – Record Sample Height, focus and rotation positions
 - F. Return to Marked Position – Return to the marked sample position

OBJECTIVES AND FILTERS

- Home Robot, if you didn't perform this step at software startup
- Loosen the two knobs on the robot slider at the bottom of the robot.
- Slide the robot away from the camera
- Mount your objective: 1x, 0.33x or 0.18x magnification.
- Slide the robot to the correct objective position marked on the rail, lightly tighten the knobs.
- Click on System Settings, and select the objective you are using.
- Pixel Size Correction rescales the size of the pixels but is generally fine at the default setting of 1.0
- Ensure you have the correct filter cubes for your experiment, if not change them and update the software.
 1. Excitation and Emission Wavelengths are listed.
 2. Clicking on a filter will bring up a list of available filters. Check with microscopy staff on how to change a filter.
- Device Com Ports, click on this to check all devices are communicating correctly.

SAMPLE MOUNTING AND ALIGNMENT

- Set all of the Coloured Knobs (Yellow, Red, Blue and Green) on the robot to their center positions
- Mount your sample onto the steel plate on the robot, using the magnet on your sample post.
- **IMPORTANT:** Rotate the sample post with your fingers until the front of your sample is facing the camera, then move the post into the center of the metal plate. It's very important that your sample is square with the camera, to help with the alignment procedures we will use later.
- Using the software controls, move the sample down until it is about 1cm above the cuvette, check to make sure it isn't going to hit the sides of the cuvette and is roughly centered on the cuvette.
- White Knob: adjust as necessary to bring your sample into the centre of the cuvette
- Turn on the Brightfield Lamp via "LED Shutter", set to 7 intensity, and adjust the exposure time down until you see the cuvette on the screen (0.001 – 0.100 sec exposure)
- Activate the "Symmetric Cursor" button below the image window
- Use the SAMPLE ROBOT software controls to move the sample down into the cuvette until you can see the metal post on the screen
- Adjust the lamp brightness and exposure until you have a good image.
- Manually adjust the focus, by loosening the two knobs on the robot slider and then sliding the robot, lock the knobs when done.

POST ALIGNMENT

- Place the software cursors on the edge of the post image:
 - If the post isn't vertical, move the Robot to the "0" degrees position, and adjust the YELLOW knob until it is vertical, move the Robot to the "180" degrees position and adjust the YELLOW knob until the post is vertical. Repeat until the post is vertical at both positions.
 - Repeat the steps above at "90" and "270" degrees adjusting the RED knob.
 - Recheck the post is vertical at "0, 90, 180, 270" degrees, adjust if necessary.

SAMPLE ALIGNMENT

- Use the SAMPLE ROBOT controls to lift the sample up into the center of the screen.
- Adjust the lamp brightness and exposure until you have a good image.
- Press the "Align Sample" button at the top of the screen
 - Adjust Coarse Position
 - Tweak the WHITE knob to center your sample horizontally on the screen.
 - Use the software focus controls to bring your sample into focus
 - Aperture controls affect resolution and image brightness
 - A large Aperture gives the highest resolution and brightest image, but only a very thin focal plane in the center of your sample, this is not good for OPT
 - A small Aperture gives lower resolution and a darker image, but all of your sample is in focus at once, this is required for OPT
 - Make sure you have the Aperture set in the green region when scanning.

- Use your fingers to rotate the sample if necessary so that the face of the sample is looking straight into the screen, and repeat the steps above until the sample is centered and facing the camera.
 - Align Axis 1
 - The sample rotates to 0 degrees, use cursors to mark distinct features in your sample that should be visible from the front or back
 - Press the “Rotate” button
 - Use the BLUE knob to move the sample halfway between the old and new position
 - Press “Rotate” and repeat the steps above until the sample stops precessing.
 - Press “Continue”
 - Align Axis 2
 - As per Axis 1, except using the GREEN knob.
 - Repeat the steps above until the sample rotates with no precession in the centre of the screen.
 - Exit
- Adjust the zoom and use the WHITE knob to center the image on the screen if required

IMAGE SETTINGS

- Select the “Excitation Filter” you want to use
 - If the software crashes, type “CTRL + .” to close OPTiscan, wait for the software to close thoroughly and restart OPTiscan; you don’t have to home the robot at Question 3.
- Open the “Xcite Shutter”
- Set intensity on “Fluorescence Lamp” to something low (~20)
- Adjust the Image Capture and lamp settings to achieve a desirable image.
- Ensure that the aperture is between 0-2
- Make sure that the image brightness is below 50,000 at all rotation angles
- **Image Capture Menu**
 - Select “Resolution” - size of your image
 - select “Binning” – resolution and brightness of your image
 - select “Gain” – brightness and Signal to Noise ratio (S/N) of your image
 - select “Avg” (Averaging) – S/N of your image
 - select “Speed” (fast for set-up, slow for image collection)
 - select “Exposure” – adjust brightness of image
 - Set aperture to ~1-2 (or just low enough to get the entire sample into focus)
 - “Live” - update the image onscreen
 - “Snap” - captures a single image and saves to file.
- Repeat steps above for any other required Excitation filters.

ACQUISITION

- **Scan Settings Menu**
 - Select all desired “Excitation Filters” you wish to use for the scan, by ticking the radio buttons on the left of the Filter name.
 - The “padlock” button allows you to unlock/lock the filter settings
 - Set the “# of Images” = 400
 - “Stepsize” = 0.9
 - “Save Settings” – to record the listed Image Capture settings for all excitation filters
 - “Load Settings” – to reuse settings previously saved
 - “Start Scan” – Press to start a new scan
 - “Time Remaining” – indicates an approximate total scan time
 - “Pause Scan” – will pause the scan at the present position
 - “Abort Scan” – will quit the current scan and return to the start position.

- **Start Scan**
 - Press “Start Scan”, choose a location in which to save your data, then create and name a new folder in which to put your data.
 - The “Scan Settings” window will appear
 - “Number of Scans” = 1
 - “Save Corrected Data” to perform flatfield and background subtraction on your images (required for reconstruction)
 - “Save RAW Data” – if you wish to manually adjust the raw data in ImageJ etc
 - “Shutdown lamps” if your scan will finish at night time.
 - “Email details”, if you wish to be notified when your scan finishes.
 - Background Correction
 - Block light to the camera if requested, by using the black plastic sheet and press “Continue”, then
 - Unblock the Camera, when requested and press “Continue”
 - Flatfield Correction
 - Move the sample up or down to image only the agarose with no sample, press continue and wait for the scan to start
 - “Start Scan” becomes “Abort Scan”
 - When the scan is finished the shutter will close; if you wish to run another scan, you must remember to open the shutter again, or you wont get an image on that excitation filter.
 - To remove sample click the “Home Robot/Mount Sample” button at the top of the interface
 - Click “Quit OPTiscan”
 - “Do you wish to quit OPTiscan?”
 - “Yes”
 - “Turn off the lamps?”
 - “Yes”

SHUT DOWN

- **Exit the software** by Pressing the “Quit OPTiscan” button
- **Turn off the lamps**
- Transfer files from local computer to network storage
- **Shut down computer**
- **Cleaning up the scope**
 - Wipe up *any* spills you may have made
- **Turn off the microscope** by switching off the remote control.