ImageXpress protocol

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Configure Imager

1. Turn on the imager (on the left side of the instrument control box above imager).
2. Open MetaXpress on the desktop.
3. Login to MetaXpress (username: moldev, password: moldev)
4. Select “Administrators” as the security level.
5. From the top menu select **Screening > Acquisition Setup**.
6. Within the Acquisition Setup window click on “Load Protocol…” and select the protocol you would like to use then click “Load from DB”.

Imaging plates

1. The best images for CellProfiler require treating worms with sodium azide for 10 min before image acquisition. If live worm images are desired, skip the addition of sodium azide and proceed to step 2.
    a. Pour M9 solution with 50 mM sodium azide into a disposable 100 mL reagent reservoir (cat# 1306-1010, USA scientific).
    b. Using a 12-channel 300 µL pipette, add 334 µL of the 50 mM sodium azide M9 solution to the plate wells. Filling the wells entirely minimizes the curvature of the well meniscus and ensures the most even illumination in images. If you notice a meniscus in the wells after adding the sodium azide solution, adjust the volume such that the surface of the solution in the well is flat with the top of the well edge.
    c. Incubate in sodium azide solution for 10 minutes before imaging.
2. Load your plate into the imager
    a. Click the “eject plate” button in the Acquisition Setup window to open the door to the stage.
        i. NOTE: The stage lock will close 90 seconds after the eject plate button is clicked. If the stage lock triggers before you load the plate, click the “load plate” button then click the “eject plate” button again to load your plate correctly.
    b. Wipe the bottom of the plate with 95% ethanol and a kimwipe to remove any dirt or moisture. Be gentle to avoid scratching the plate bottom.
    c. Remove the plate lid prior to loading the plate.
    d. Make sure A1 is in the top-left corner of the stage and the plate is seated flat in the stage. Make sure the plate is not raised in the A1 corner, which often

happens when the stage lock triggers before the plate is loaded. If the stage lock is triggered, see the note in 2a-i above.

3. Click “load plate” in the Acquisition Setup window once you have properly loaded your plate.
4. Click on the wells of the plate you wish to image within the Acquisition Setup window. The green wells will be imaged, and the grey wells will not be imaged.
5. Review the image protocol, check focus, and check exposure time.
    a. Go into the “Configure” tab in the Acquisition Setup window.
        i. Under “Object and Camera”, make sure your desired objective and magnification are selected.
        ii. Check exposure time
            1. Identify a well that you expect to have typical optical density, *i.e.*, a well that is not too dark or too light.
            2. Move the stage to that well by right clicking the well in the Acquisition setup window.
            3. Click “preview” to see a preview of the image for that well.
            4. Mouse over the preview image to check the intensity values in the center of the well. Ideally, the intensity will be ~3000 in the center of the well. If it is higher than 3500, lower the exposure time. If it is lower than 3000, increase the exposure time.
                a. If needed, adjust “Exposure (ms)” in the transmitted light tab under the wavelengths tab. Typically, you will only need to adjust the exposure by 1 - 5 milliseconds.
            5. Recheck the exposure after adjusting to make sure intensity values are in range.
        iii. Check focus
            1. Click “Calculate Offset” under the wavelength you are trying to focus, *e.g.*, “ W1 Transmitted Light 20”.
            2. The imager will create a Z-stack. Scroll through the images, using the arrows at the top of the image stack, to find the image with the best focus.
                a. NOTE: you may have to compromise the focus of some worms so that the majority of worms are in focus.
            3. Once you have selected the image in the stack with the best focus, click “OK” in the pop-up window.
            4. Click “Yes” to replace the current offset with the calculated offset.
            5. Click “Focus” (the square icon with the glasses).
            6. Select another well and click “Focus” to make sure this offset works with that well. If desired, repeat this step with other wells around the plate to make sure the focus is consistent throughout the plate.
6. Go into the “Run” tab
    a. Create a folder name using the Andersen Lab convention

        i. <date>_<experiment_name>
            1. date = 20200820
            2. experiment_name = anything without “-”
    b. Name the plate with Andersen Lab naming convention
        i. <date>-<experiment_name>-<plate>-<magnification>
            1. date = 20200820
            2. experiment_name = anything without “-”
            3. plate = p001, p002, p003, …, p010, …, p100, ...
            4. magnification = m2X, m4X, m10X, ...
7. Click “Acquire Plate” to image.
8. Once done imaging, click “Eject Plate”.
9. Remove plate from the imager.
10. Load your next plate or leave the imager empty if you have finished imaging.

Review Plates

This section is optional. If you desire to review images before export and analysis the MetaXpress software can help.

1. Click “Review Plate” in the top left-hand corner of the toolbar (next to “Acquisition Setup)
2. Click “Select Plate…”
    a. In the pop-up window, find the folder with your plate and double click on it, then click on the plate you wish to review and click the “Select” button.
3. Choose the wavelengths you want to see and then highlight the wells you want to view.
4. Click “Load Selected Images” in the bottom left-hand corner to view the selected wells.
5. You can left-click on an individual well in the thumbnail images to view a full-sized image of that well.

Exporting Images

1. Plug in the 4TB ECA_image external drive into the ImageXpress PC. The drive is stored on top of the ImageXpress PC when not in use. If you don’t see it there, talk to Erik.
2. Open the MetaXpress software on the ImageXpressPC, the imager does not need to be turned on to export images.
3. In MetaXpress click on Screening -> Plate Utilities
4. In the popup window, click the “Export Images” button.
5. In the new popup window, titled “Select Plate For Image Export”, double click the folder that contains the images you intend to export. Note, these folders are titled by their creation date, so if you want to export images for a particular experiment, you should click on the folder with the date you imaged your plates.
    a. After double clicking the folder you will see rows of data populate in the bottom portion of the split window. Each row represents a set of images for a particular plate.

6. Select all the plates you wish to export images for by clicking on the desired rows.
    a. To quickly select all plates for export, click on the first plate in the list, then hold down shift and click on the last plate in the list.
    b. To remove or add back a single plate to the list, hold down control and click on a plate.
7. Once all the plates are selected, click on the “Select” button at the bottom of the window.
8. In the new popup window titled “Browse for Folder”, click on your folder within the ECA_image external drive. Click the “New” button. Name the new folder with standard convention <date>_<experiment_name>. Click “OK”.
    a. The plates will begin to export after you hit “OK”, you can monitor the export progress with the green status bar at the bottom of the MetaXpress software.
9. After images have been downloaded on an external drive copy the raw images to the `qbiodata.bmbc.northwestern.edu` server.
    a. Once inside `qbiodata.bmbc.northwestern.edu` place your images in the following directory: `qbiodata.bmbc.northwestern.edu/home/projects`.
    b. Create a new project folder for your images in the following format: `YYYYMMDD_projectdescription`.
    c. Within your project folder create the following directory structure:
        i. `raw_images`
        ii. `raw_plate_thumbs`
        iii. `raw_image_thumbs`
        iv. `processed_images_YYYYMMDD`
        v. `design_files`
    d. Upload all RAW images under your `raw_images` folder.
    e. Upload design files, processed images from cell profiler, raw plate thumbs and raw image thumbs from easy express in their respective folders.
    f. These folders serve as a backup storage for image files and respective metadata. These are not working folders.

Preprocessing Exported Images

1. These steps will pre-process images into a standard structure. Once they are preprocessed, they can be uploaded to QUEST for analysis with CellProfiler.
    a. Plug the ECA_image drive into a Mac with the latest version of easyXpress installed.
    b. Open an R studio session and use `easyXpress::tidyProject` function to preprocess the images.
        i. Run `easyXpress::tidyProject(project_dir = <your PATH>)`
        ii. Use the full path to the folder you created in Exporting Images step 8 for `<your PATH>`.
    c. You will be prompted in the Rstudio console with: `Do you want to tidy all .TIF files in <your PATH> (y/n):`

i. If `<your PATH>` is the correct, then enter `y` in the console.
ii. If not, enter `n` in the console and return to step 1ba.
iii. This function will take several minutes to process images from multiple plates.

Analyzing images using CellProfiler

Follow the Analyzing images using CellProfiler protocol to upload the pre-processed images onto QUEST.