Quick Start Guide – ImageXpress Micro and MetaXpress 5.3

Revision A

1. Turn on the system:
   a. Light source (Xenon lamp should be turned on at least 20 minutes before first use)
   b. IXM power supply
   c. Camera power supply
   d. IXM options controller (if options installed)
   e. Computer/monitor (if not already on)

2. Go to the MetaXpress folder and double-click the appropriate shortcut.

3. Login to MDCStore database with username and password:

Username	***moldev***
Password	***moldev***

4. If you log in as system administrator (sa), the next window warns you about security risks. Click **OK**.

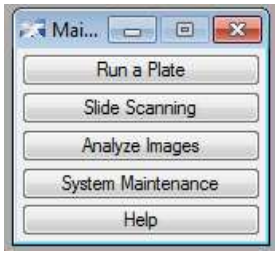
5. Select Group (security level) and click **OK**.

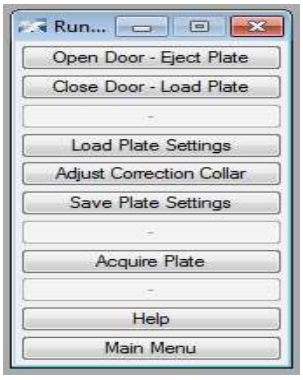
6. On the taskbar, select **Run a Plate**.

7. Click on **Open Door - Eject Plate**.

8. Place plate into system with A1 facing the labeled corner (towards the plate clamp).

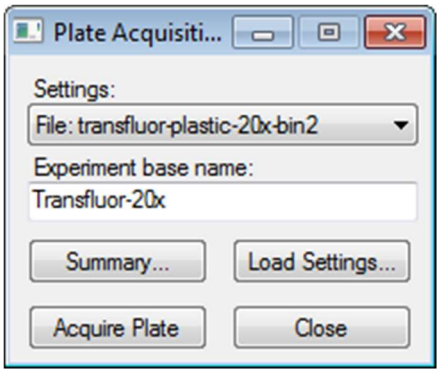
9. Click on **Close Door - Load Plate**.





10. In the menus, go to **Screening → Plate Acquisition**.

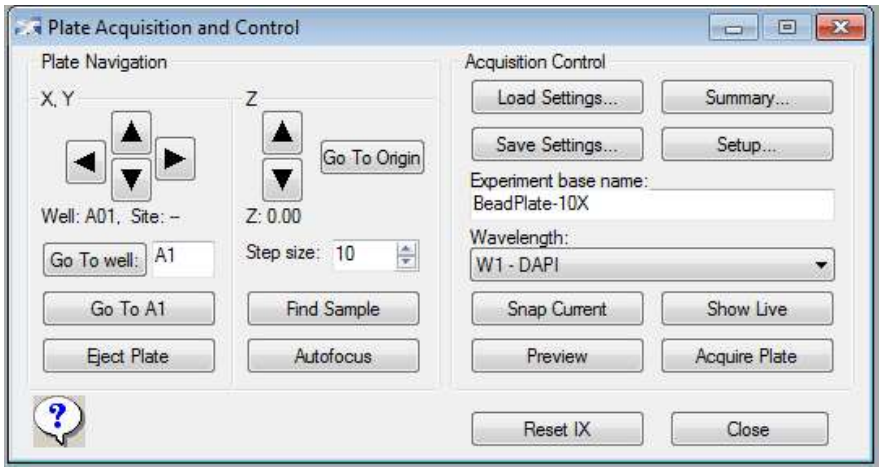
11. Select **Load settings**, then select ***-From File-*** option from dropdown list.



12. Browse for the saved settings file.

13. Adjust the objective correction collar for your plate thickness, if necessary.

14. If desired, test experiment settings:
    a. Open up the **Plate Acquisition and Control** window.
    b. Move to a well of interest.
    c. Select a channel to look at from the **Wavelength** drop down.
    d. Click on **Find Sample** to focus and snap an image.
    e. See section below to adjust acquisition settings, if necessary.



15. Make sure experiment base name is appropriate.

16. In the **Plate Acquisition** dialog, click on **Acquire Plate**.

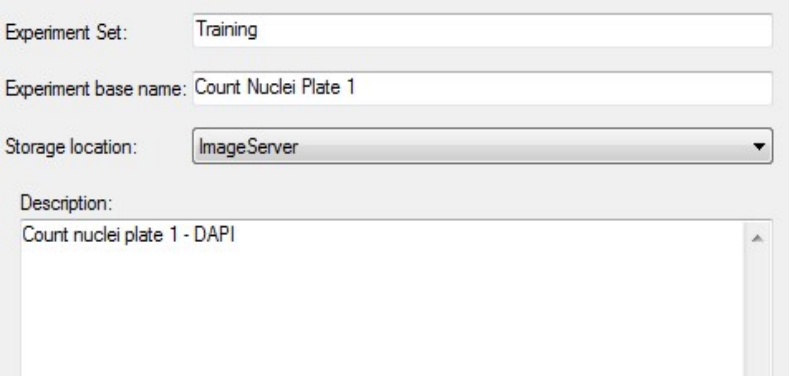
17. Once plate is acquired, go to **Review Plate Data** (under the Screening menu) and **Select Plate** to view images and set up analysis.

Defining and Optimizing Settings for Plate Acquisition

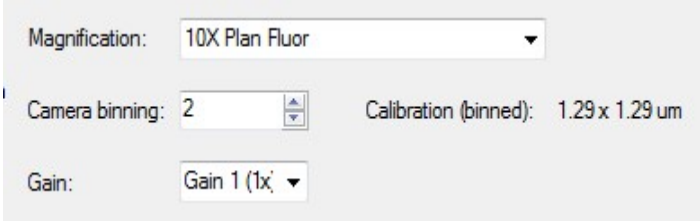
1. Go to **Plate Acquisition Setup** under the screening menu.

2. Load existing settings or select new settings, then click **Next** to advance to the next tab.

3. **Names and Description** tab
    a. Experiment Set = folder (your project, PI name, etc.)
    b. Experiment Base Name = plate name (this experiment)
    c. Storage Location (select appropriate server for image storage)



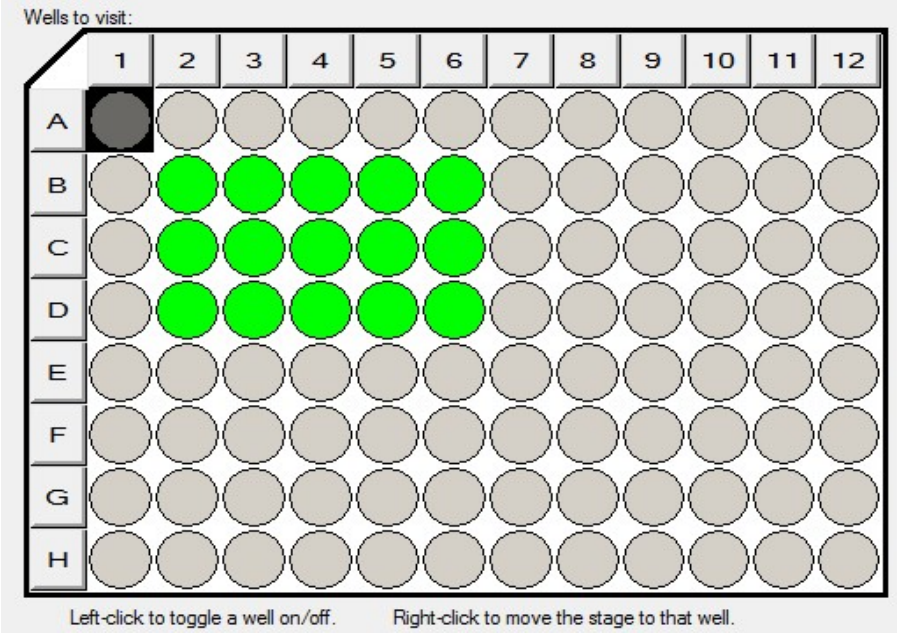
4. **Objective and Camera** tab
    a. Select the appropriate magnification.
    b. Set binning (1 or 2 are most common).
    c. Set gain (gain of 2 for binning of 1, 1 for binning of 2 or higher)



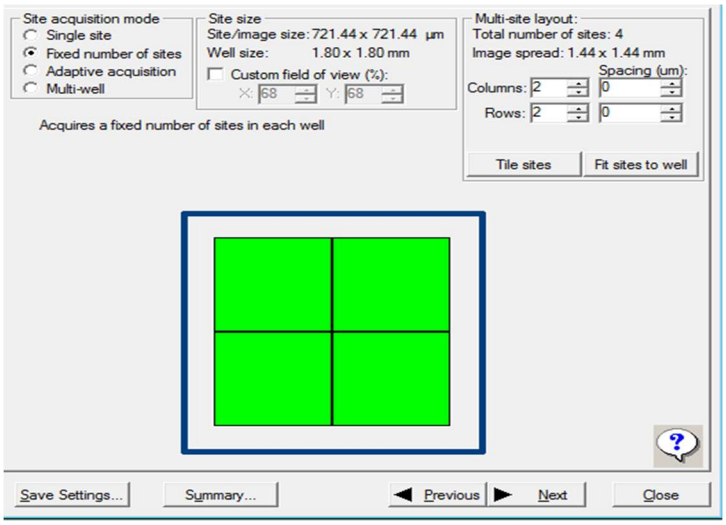
5. Adjust the objective correction collar if necessary (needs to match plate bottom thickness).

6. **Plate** tab
    a. Select the appropriate plate type from the drop-down list.

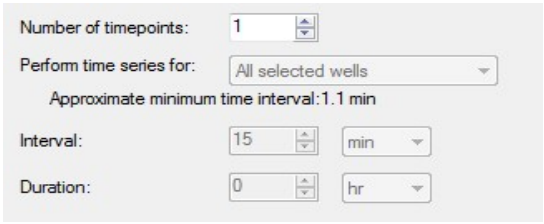
7. **Wells to Visit** tab
    a. Left-click on rows, columns, or individual wells to select them for imaging (green wells are selected, grey wells are not selected).
    b. Click and drag to select or deselect a block of wells.
    c. Right-click on any well (selected or not) to physically move the plate to that position.



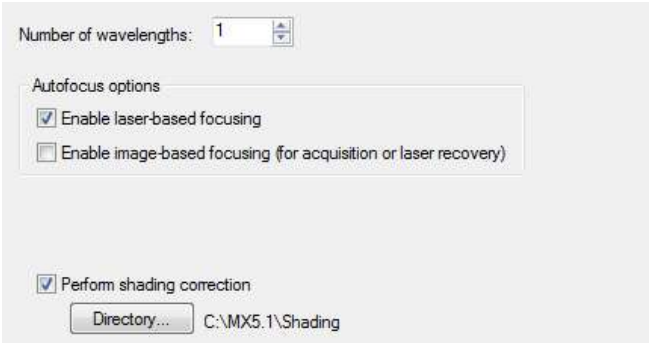
8. **Sites to Visit** tab
    a. **Single site** will image once per well in the center.
    b. **Fixed number of sites** will always image the selected sites for every well. Adjust number and spacing of sites. Left-click on sites to select them for imaging. Green sites are selected, grey sites are not selected. Right-click on any site to physically move the plate to that position within the current well (site will turn dark green).
    c. **Adaptive acquisition** means that the system will collect a variable number of sites depending on the number of cells present in the well.
    d. **Multi-well** means that the system will collect multiple wells in a single field of view and then crop out the images to single well images automatically. If the field of view is too small to accommodate this, the option will be greyed out.



9. **Timelapse** tab
    a. Leave this set to 1 time point for any fixed cell assay.



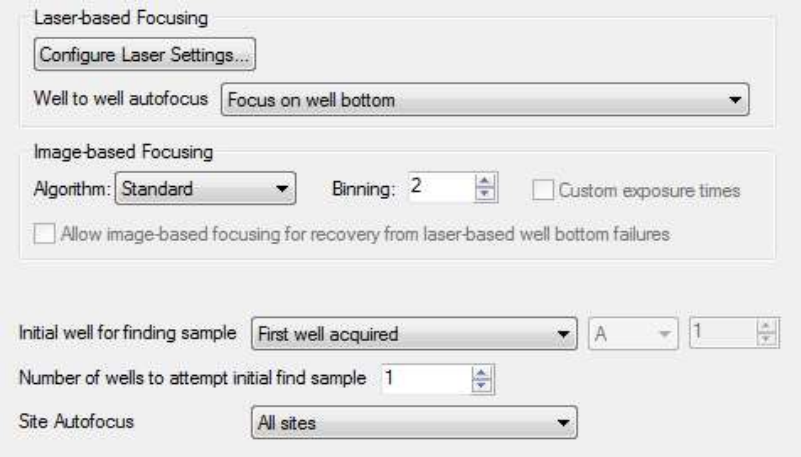
10. **Acquisition Loop** tab
    a. Set the number of wavelengths.
    b. Make sure laser-based focusing is enabled.
    c. For most assays, image-based focusing can be disabled.
    d. Turn shading correction on (optional). If used, select the directory for reference images.



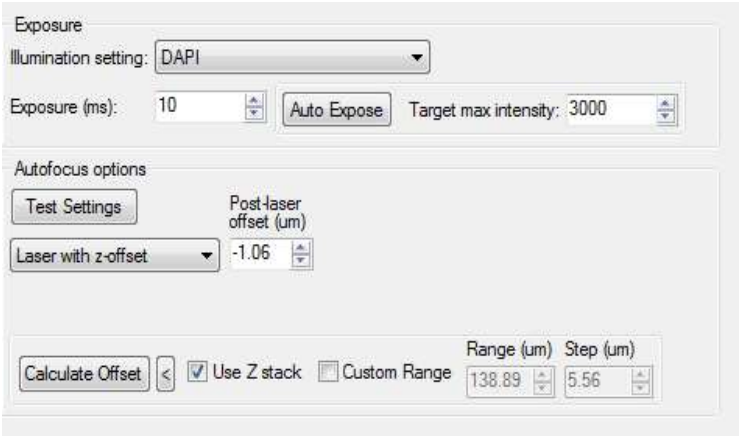
11. **Autofocus** tab
    a. Usually set **Well to well autofocus** to ***Focus on well bottom***.
    b. Set **Well to well autofocus** to ***Focus on plate bottom…*** for the following scenarios:

i. Slide/coverslip imaging
ii. Thin-bottom plates with a low-magnification objective (4x and below)

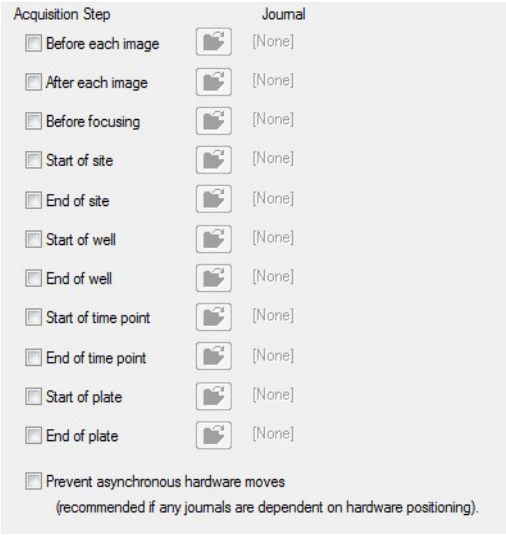
c. Set **Initial well for finding sample** to ***First well acquired***.
d. If you are imaging multiple sites, usually set **Site Autofocus** to ***All sites***.



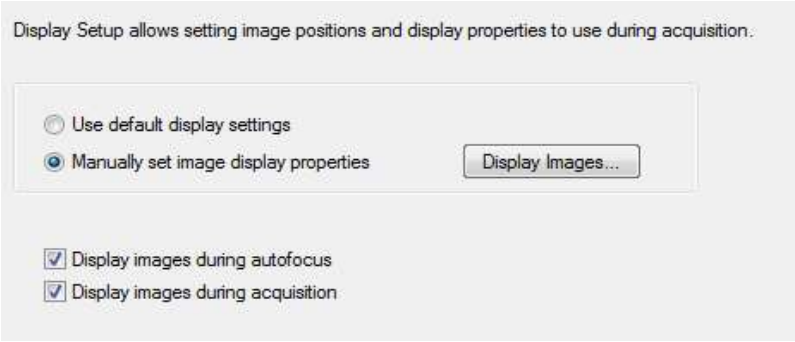
12. **Wavelength** tabs
    a. Select appropriate filter set under **Illumination Setting**.
    b. Move to an appropriate well/site, then click on **Test Settings**.
    c. Look at image (if you don't see an image, this is because autofocus settings are not optimized for this plate). If it is saturating, reduce exposure time for this wavelength.
    d. Click on **Calculate Offset**.
        i. For more control, turn on ***Use Z Stack*** and follow the prompts
        ii. If necessary set a custom range
    e. Click on **Test Settings** again to test Z offset.
    f. If image is too dim, increase exposure time. If image is too bright, decrease exposure time. Click on **Test Settings** to test.
    g. You may also try using the **Auto Expose** button with the ***Target max intensity*** set to **3000**.
    h. Repeat for each subsequent wavelength.



13. **Journal** tab
    a. Usually leave everything deselected (turned off) here.

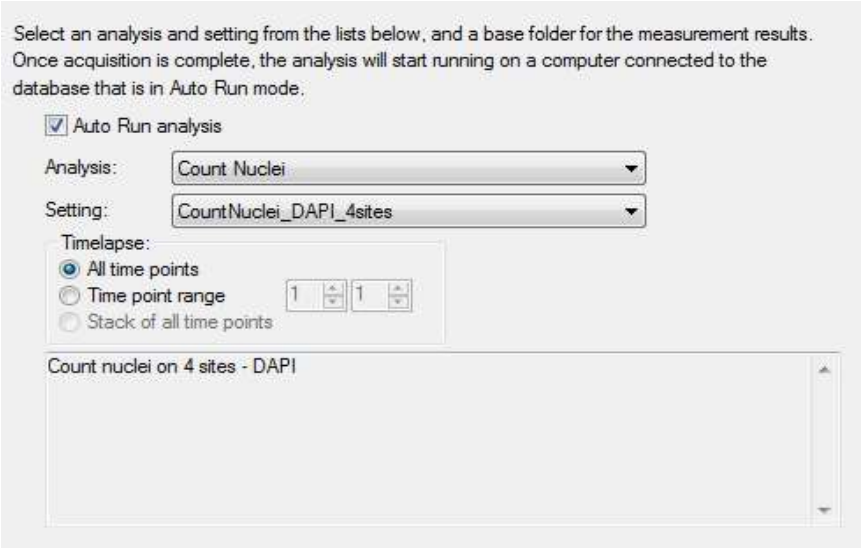


14. **Display Settings** tab
    a. You can just use default display settings.
    b. ***Display images during acquisition*** is usually on.
    c. Optional: to customize display (only affects display during acquisition, does not affect raw data), select the ***Manually set image display properties*** option and click the ***Display Images*** button. Resize and position windows as desired, then click ***OK***.



15. **Post Acquisition** tab
    a. Only turn on Auto Run Analysis if you already have optimized analysis settings for the assay.
    b. If you are not sure of analysis settings to use, make sure this option is turned off.
    c. **Timelapse** (available if you specified a time series on the *Timelapse* tab)**:**
        i. Select **All time points** to run the analysis on all time points in the data set
        ii. Select **Time point range** to run the analysis on a range of time points or on a single time point

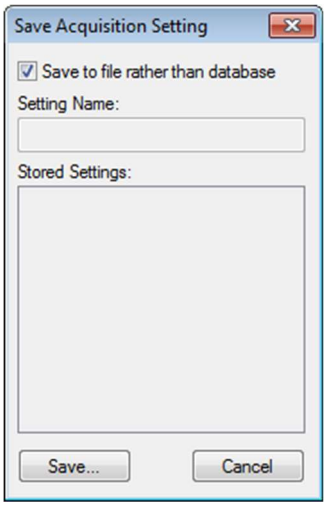
iii. Select **Stack of all time points** if in the *Analysis* field, you selected a legacy timelapse journal which analyzes the planes in a stack as separate time points



16. **Summary** tab
    a. Review summary of settings and print if desired.

17. Click on **Save Settings** and select ***Save to file***. Click on **Save** and it will prompt you for a location and filename.

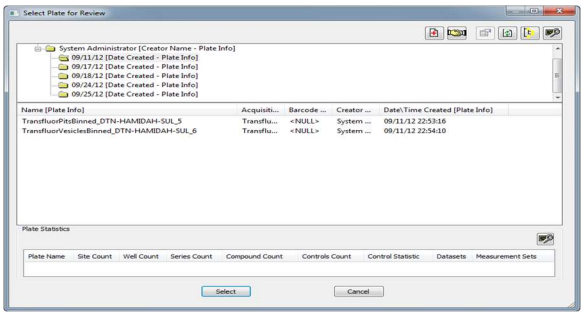
18. Click **Acquire Plate** to begin image acquisition.



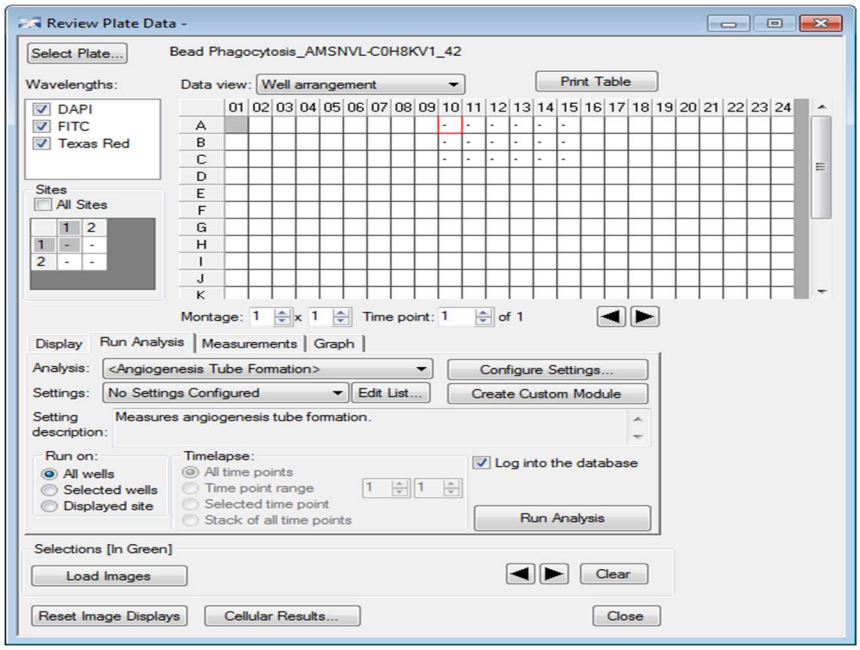
Reviewing Images and Setting Up Image Analysis

1. Go to ***Review Plate Data*** under the screening menu and click on **Select Plate**.

2. Navigate through the folders to find the plate of interest and double click it, or highlight it and click **Select**.



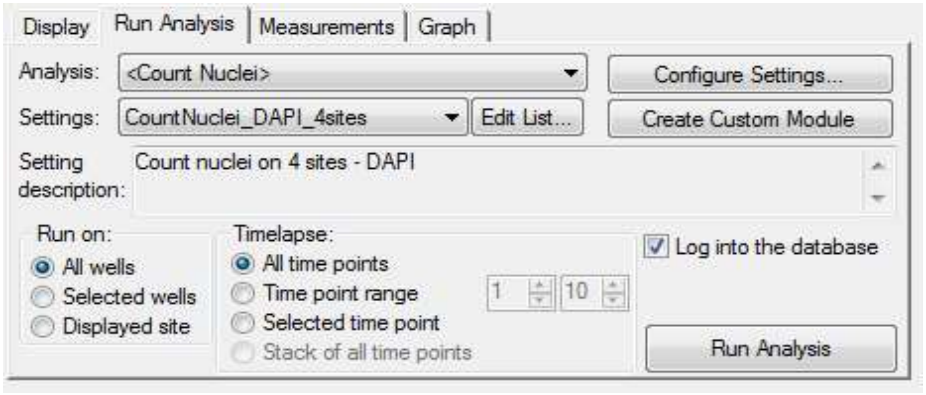
3. In the plate view, you will see a "-" in each well that was imaged. Left-click and drag across the wells you want to view. A thumbnail montage of these wells will open for each wavelength.



4. If there are multiple sites per well, select an individual site to view, or select ***All sites***.

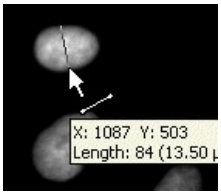
5. Left-click on a single thumbnail to view full resolution images (all wavelengths).

6. Go to the ***Run Analysis*** tab in Review Plate Data.



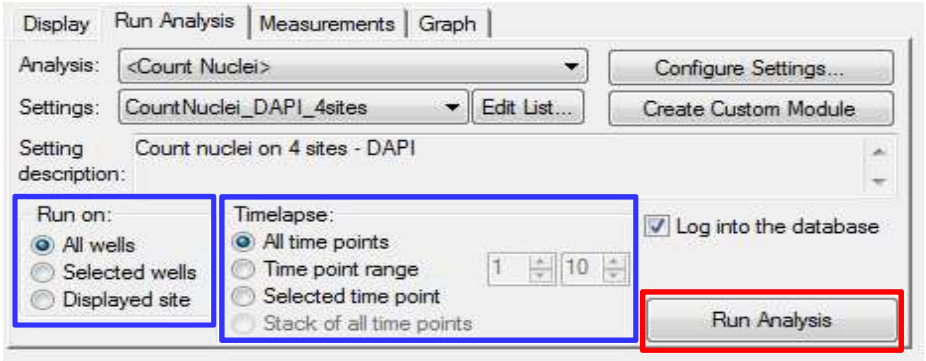
7. Select the appropriate application module from the drop-down list or click on ***Create Custom Module*** to open the Custom Module Editor (please refer to the Custom Module Editor Quick Start Guide for instructions on creating a custom module).

8. For application modules, click on ***Configure Settings***.
    a. Select the appropriate wavelengths for each part of the analysis.
    b. Turn off ***Display result image***.
    c. Measure size ranges using the line tool in the toolbar. Cells should be measured across their short axis. Enter the minimum and maximum width (in um) or approximate width (in um), depending on the module.
    d. Measure intensity inside cells and outside of cells by moving the mouse arrow across the image and reading the intensity value displayed at the bottom of the window, or by drawing a line across the background and the cell and viewing **Linescan**. Make this measurement on a dim cell, reduce it slightly and use it as the ***Intensity above local background*** value.
    e. Alternatively, use the **Estimate Module Settings** button on the **Analyze Images** taskbar, select interactive mode and follow prompts.
    f. Enter other appropriate settings for the module.
    g. Click on Preview to test individual channels (not available for all modules), or Test Run to test entire analysis on the selected images.
    h. Modify settings as necessary and re-test.

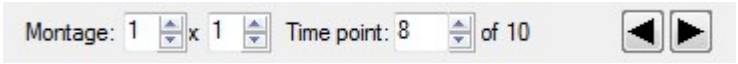


i. Go to both positive and negative control wells by left-clicking on the appropriate thumbnail images and test analysis settings.
j. Click on **Configure Summary Log** and enable the measurements you want.
k. Click on **Configure Data Log** and enable the measurements you want.
l. Click on **Save Settings** and name and save analysis settings.
m. Close **Configure Settings** dialog.

9. Run analysis on current plate:



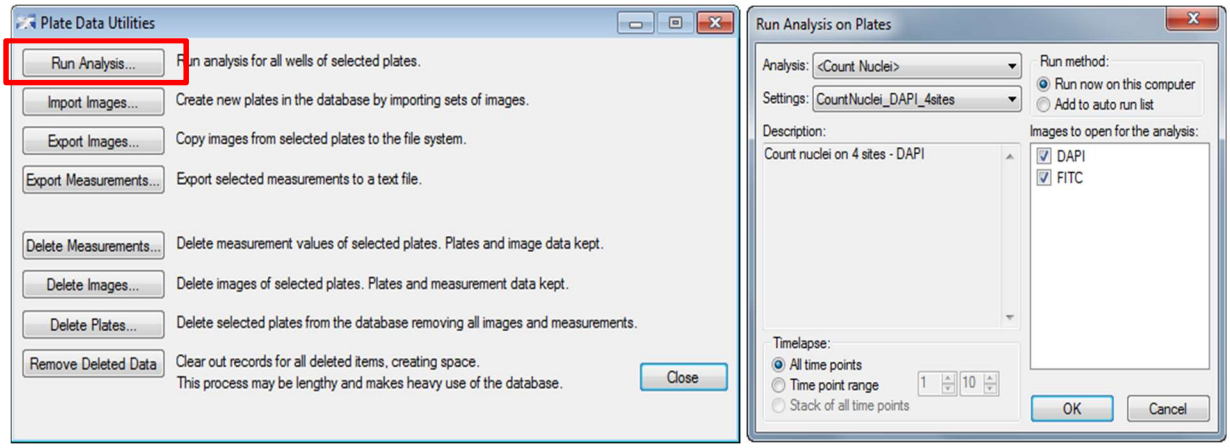
a. Under the **Run on** section, select the appropriate radio button on which well selection the analysis should run.
    i. **All wells:** analysis will be run on all wells
    ii. **Select wells:** select specific wells by right-click on them (select wells are highlighted in green)
    iii. **Displayed site:** only the select site displayed in the montage will be analyzed
b. If the data set is a Timelapse data set, select the appropriate radio button on which time points the analysis be run under the **Timelapse** section.
    i. Select **All time points** to run the analysis on all time points in the data set
    ii. Select **Time point range** to run the analysis on a range of time points and enter a consecutive time range
    iii. Select **Selected time point** to run the analysis on only one time point. Select the appropriate time point in the **Time point** section next to the montage box



    iv. Select **Stack of all time points** if in the *Analysis* field, you selected a legacy timelapse journal which analyzes the planes in a stack as separate time points
c. Click on **Run Analysis** to begin the analysis.
d. If you have already analyzed this plate with these settings, you will be asked whether you want to overwrite your data. If you are not sure, save the settings with a new name before analyzing your plate.

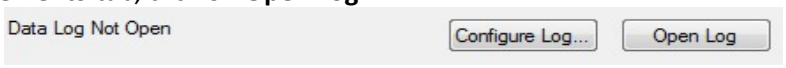
10. Run analysis on multiple plates:
    a. Go to ***Plate Data Utilities*** and click on **Run Analysis**.
    b. Select plate and/or plates to be analyzed.

c. Select analysis module and saved settings from drop down lists.
d. If plates contain time lapse data,
    i. select **All time points** to run analysis on all time points
    ii. select **Time point rage** and analyze a range of time points
    iii. select **Stack of all time points** if in the *Analysis* field, you selected a legacy timelapse journal which analyzes the planes in a stack as separate time points
e. Select ***Add to auto run list*** to flag it for offline computers to analyze and click **OK**.

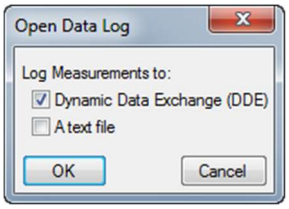


11. View analysis results:
    a. Go to "Review Plate Data" and select plate.
    b. Go to ***Measurements*** tab.
    c. Select analysis (module and settings names) from drop down menu.
    d. Select the appropriate measurement from drop down.

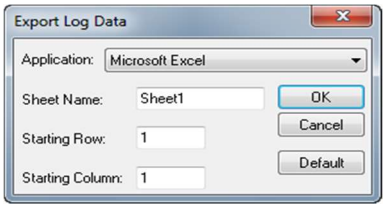
12. To export data to Excel:
    a. On the ***Measurements*** tab, click on **Open Log**.



    b. Select ***Dynamic Data Exchange.***



    c. Select ***Microsoft Excel*** and name worksheet as desired



d. Click on **Log Data**. Currently viewed data will be logged into the Excel sheet.

e. To view all measurements in a list view, set Data View in Review Plate Data to ***Measurement vs. Well***.
f. To view a single measurement in plate view, set Data View to ***Well arrangement***

13. To create simple graphs in MetaXpress:
    a. Go to ***Graph*** tab
    b. Select Histogram, Scatter Plot, or Measurement vs Well Row/Column/Number
    c. Select measurements
    d. Click on **Show Graph**

14. AcuityXpress can be used for more sophisticated analyses & visualizations.