

Quick Start Guide – ImageXpress Micro XLS and MetaXpress 5.3

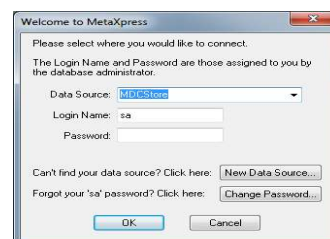
Revision A

1. Turn on the system:
 - a. Light source (if not already on)
 - b. IXM power supply
 - c. IXM options controller (if options installed)
 - d. Computer/monitor (if not already on)
2. Go to the MetaXpress folder and double-click the appropriate shortcut.

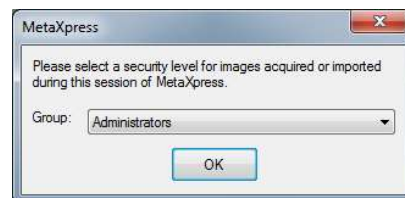


3. Login to MDCStore database with username and password:

Username	<i>moldev</i>
Password	<i>moldev</i>

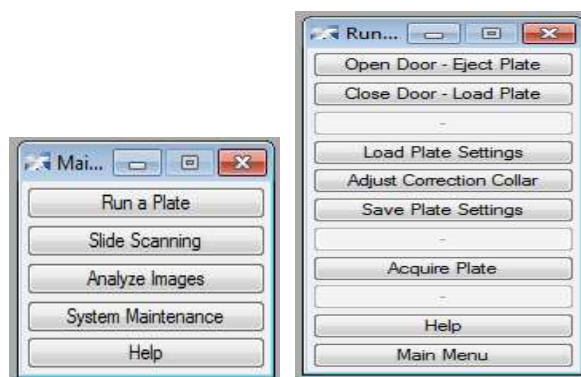


4. If you log in as system administrator (sa), the next window warns you about security risks. Click **OK**.

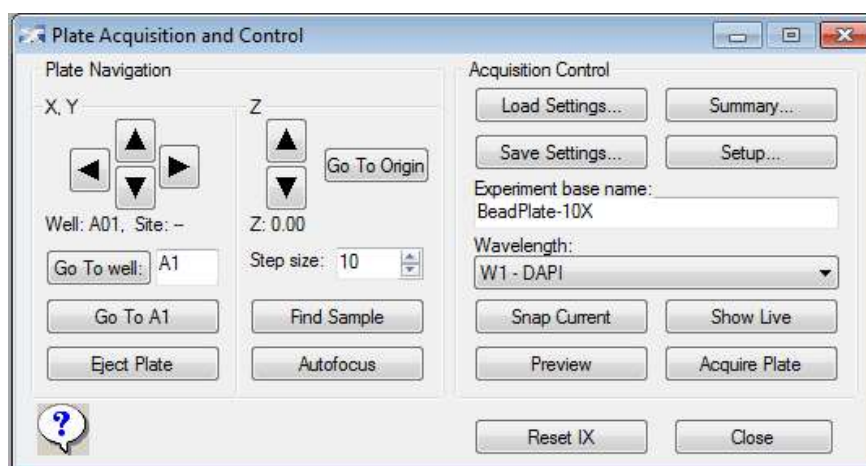


5. Select Group (security level) and click **OK**.

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 Setup**.
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, 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 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.



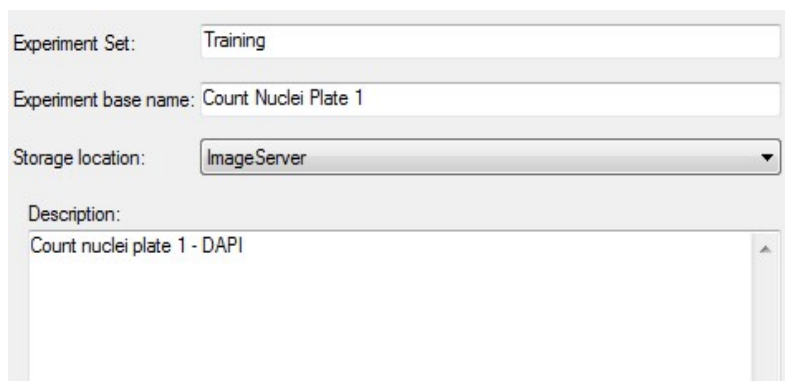
Set up a MetaXpress screening experiment.

☐ Create new settings

☒ Load existing settings file

Load Settings...

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)



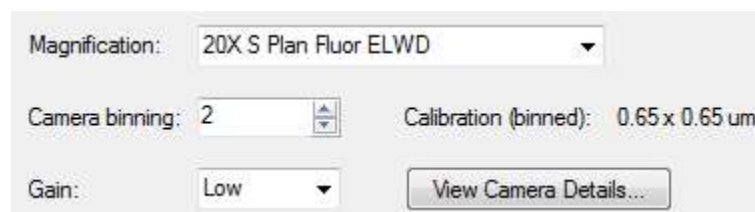
Experiment Set: Training

Experiment base name: Count Nuclei Plate 1

Storage location: ImageServer

Description:
Count nuclei plate 1 - DAPI

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)



Magnification: 20X S Plan Fluor ELWD

Camera binning: 2

Calibration (binned): 0.65 x 0.65 um

Gain: Low

View Camera Details...

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

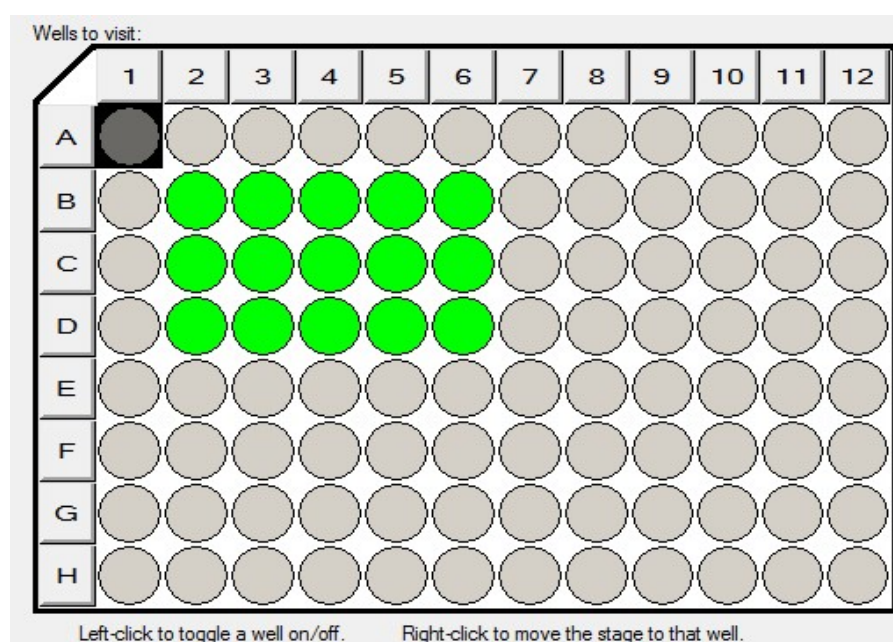
6. **Plate** tab

- a. Select the appropriate plate type from the drop-down list.

Plate name: 96 Wells (8x12)

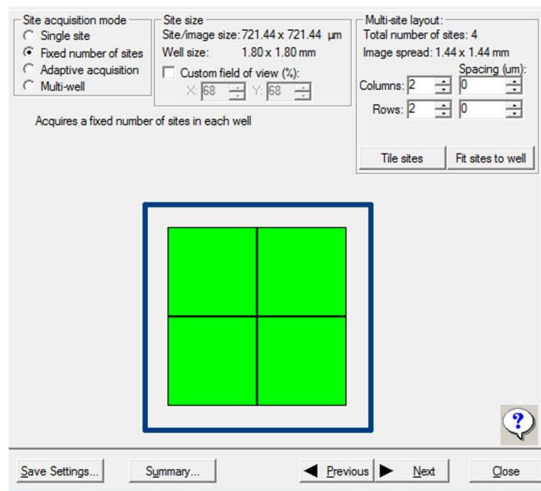
7. **Wells to Visit** tab

- 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.



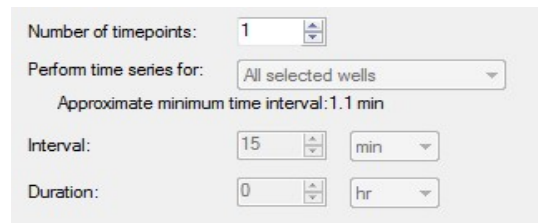
8. **Sites to Visit** tab

- 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.



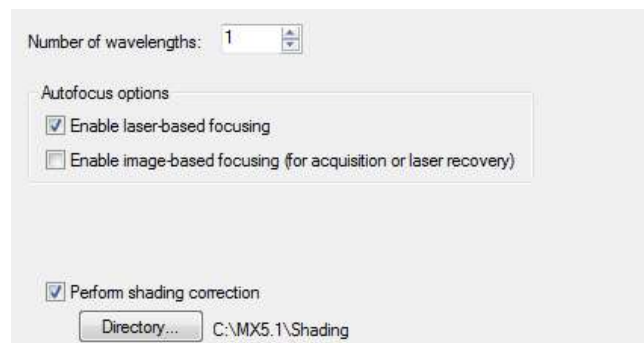
9. Timelapse tab

- Leave this set to 1 time point for any fixed cell assay.



10. Acquisition Loop tab

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



11. Autofocus tab

- Usually set **Well to well autofocus** to **Focus on well bottom**.
- Set **Well to well autofocus** to **Focus on plate bottom...** for the following scenarios:
 - Slide/coverlip 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**.

The screenshot shows a software interface for focusing. It has two main sections: 'Laser-based Focusing' and 'Image-based Focusing'. In the 'Laser-based Focusing' section, there is a 'Configure Laser Settings...' button and a 'Well to well autofocus' dropdown menu set to 'Focus on well bottom'. The 'Image-based Focusing' section includes an 'Algorithm' dropdown set to 'Standard', a 'Binning' spinner set to '2', a 'Custom exposure times' checkbox (unchecked), and an 'Allow image-based focusing for recovery from laser-based well bottom failures' checkbox (unchecked). Below these sections, there are settings for 'Initial well for finding sample' (dropdown set to 'First well acquired'), a column selector 'A', a row selector '1', 'Number of wells to attempt initial find sample' (spinner set to '1'), and 'Site Autofocus' (dropdown set 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 **45000**.
- h. Repeat for each subsequent wavelength.

The screenshot shows a software interface for exposure and autofocus settings. The 'Exposure' section has an 'Illumination setting' dropdown set to 'DAPI', an 'Exposure (ms)' spinner set to '2', an 'Auto Expose' button, and a 'Target max intensity' spinner set to '45000'. The 'Autofocus options' section includes a 'Test Settings' button, a 'Post-laser offset (um)' spinner set to '10', and a 'Laser with z-offset' dropdown. At the bottom, there is a 'Calculate Offset' button, a '<' button, checkboxes for 'Use Z stack' and 'Custom Range' (both unchecked), and two spinners for 'Range (um)' (set to '61.73') and 'Step (um)' (set to '2.47').

13. Journal tab

- a. Usually leave everything deselected (turned off) here.

Acquisition Step	Journal
<input type="checkbox"/> Before each image	[None]
<input type="checkbox"/> After each image	[None]
<input type="checkbox"/> Before focusing	[None]
<input type="checkbox"/> Start of site	[None]
<input type="checkbox"/> End of site	[None]
<input type="checkbox"/> Start of well	[None]
<input type="checkbox"/> End of well	[None]
<input type="checkbox"/> Start of time point	[None]
<input type="checkbox"/> End of time point	[None]
<input type="checkbox"/> Start of plate	[None]
<input type="checkbox"/> End of plate	[None]
<input type="checkbox"/> Prevent asynchronous hardware moves (recommended if any journals are dependent on hardware positioning).	

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**.

Display Setup allows setting image positions and display properties to use during acquisition.

☐ Use default display settings

☒ Manually set image display properties Display Images...

☒ Display images during autofocus

☒ Display images during acquisition

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

Select an analysis and setting from the lists below, and a base folder for the measurement results. Once acquisition is complete, the analysis will start running on a computer connected to the database that is in Auto Run mode.

☒ Auto Run analysis

Analysis: Count Nuclei

Setting: CountNuclei_DAPI_4sites

Timelapse:

☒ All time points

☐ Time point range 1 1

☐ Stack of all time points

Count nuclei on 4 sites - DAPI

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 acquisition.

Save Acquisition Setting

☒ Save to file rather than database

Setting Name:

Stored Settings:

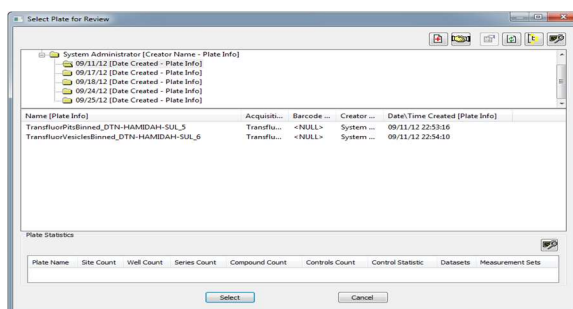
Save... Cancel

Reviewing Images and Setting Up Image Analysis

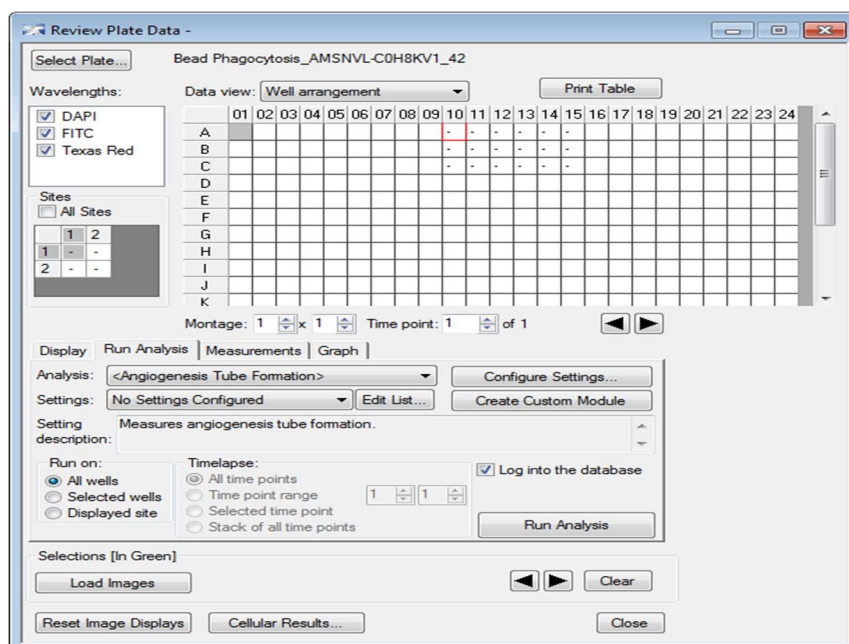
1. Go to **Review Plate Data** under the screening menu and click on **Select Plate**.



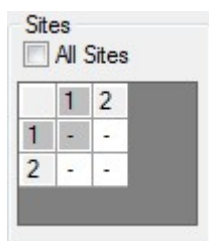
2. Navigate through the folders to find the plate of interest and double click it, or highlight it and click **Select**.



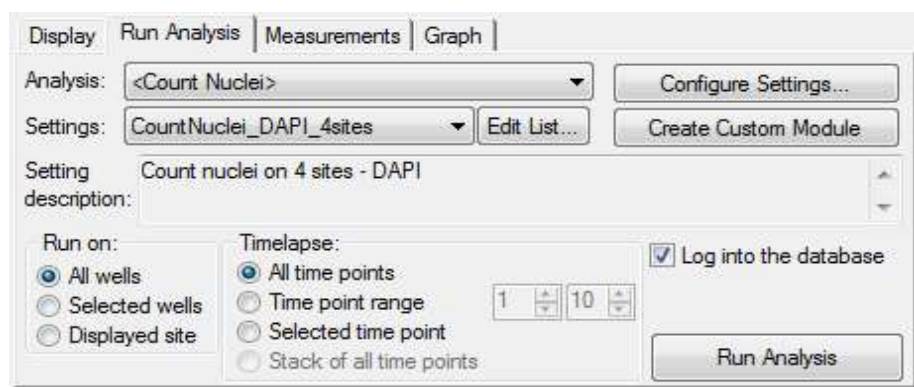
3. In the plate view, you will see a “-” in each well that was imaged. Left-click and drag across the wells you want to view. A thumbnail montage of these wells will open for each wavelength.



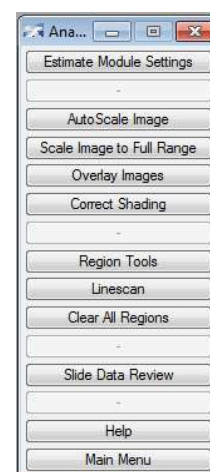
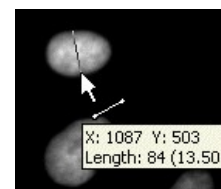
4. If there are multiple sites per well, select an appropriate site to view, or select **All sites**.



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:

The screenshot shows the 'Run Analysis' dialog box with the following settings:

- Analysis:** <Count Nuclei>
- Settings:** CountNuclei_DAPI_4sites
- Setting description:** Count nuclei on 4 sites - DAPI
- Run on:**
 - ☒ All wells
 - ☐ Selected wells
 - ☐ Displayed site
- Timelapse:**
 - ☒ All time points
 - ☐ Time point range (1 to 10)
 - ☐ Selected time point
 - ☐ Stack of all time points
- ☒ Log into the database
- Run Analysis** (button highlighted with a red box)

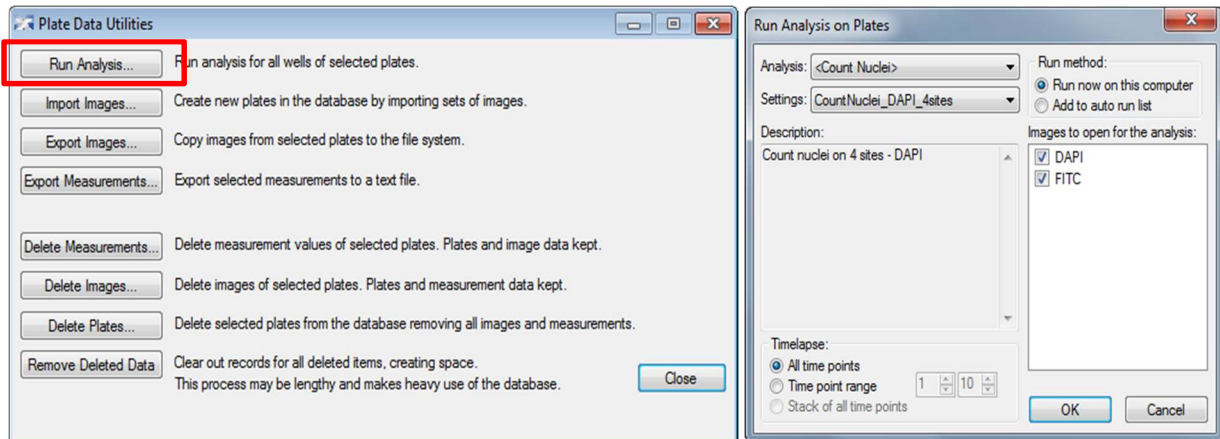
- a. Under the **Run on** section, select the appropriate radio button on which well selection the analysis should run.
 - i. **All wells:** analysis will be run on all wells
 - ii. **Select wells:** select specific wells by right-click on them (select wells are highlighted in green)
 - iii. **Displayed 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

Montage: 1 x 1 Time point: 8 of 10
 - 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 range** 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:

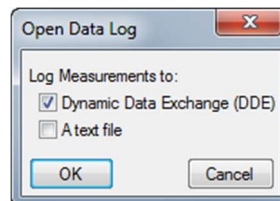
- a. Go to "Review Plate Data" and select plate.
- b. Go to **Measurements** tab.
- c. Select analysis (module and settings names) from drop down menu.
- d. Select the appropriate measurement from drop down.

12. To export data to Excel:

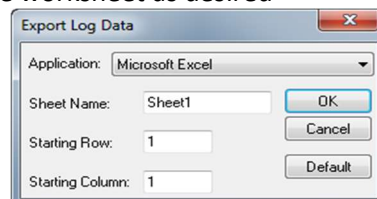
- a. On the **Measurements** tab, click on **Open Log**.



- 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.

Data Log: DDE App

Configure Log...

Log Data

- 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:

- Go to **Graph** tab
- Select Histogram, Scatter Plot, or Measurement vs Well Row/Column/Number
- Select measurements
- Click on **Show Graph**

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