# MetaXpress® 6 Software Guide

## An Overview of Application Modules

MOLECULAR DEVICES
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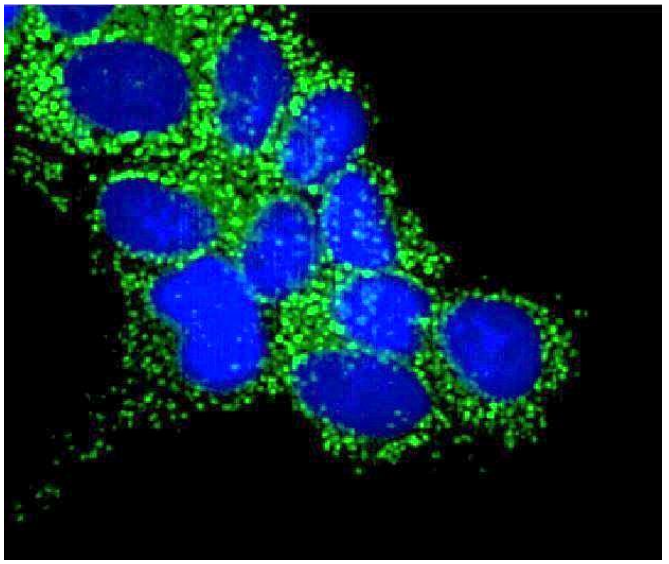
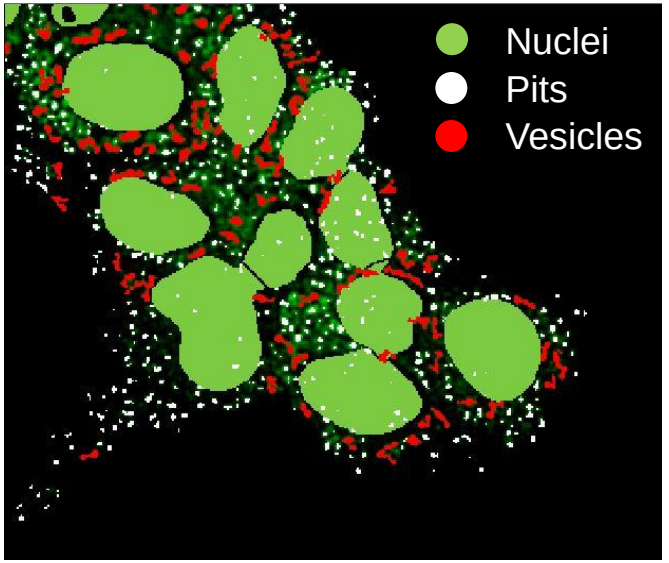
# Application Module Overview

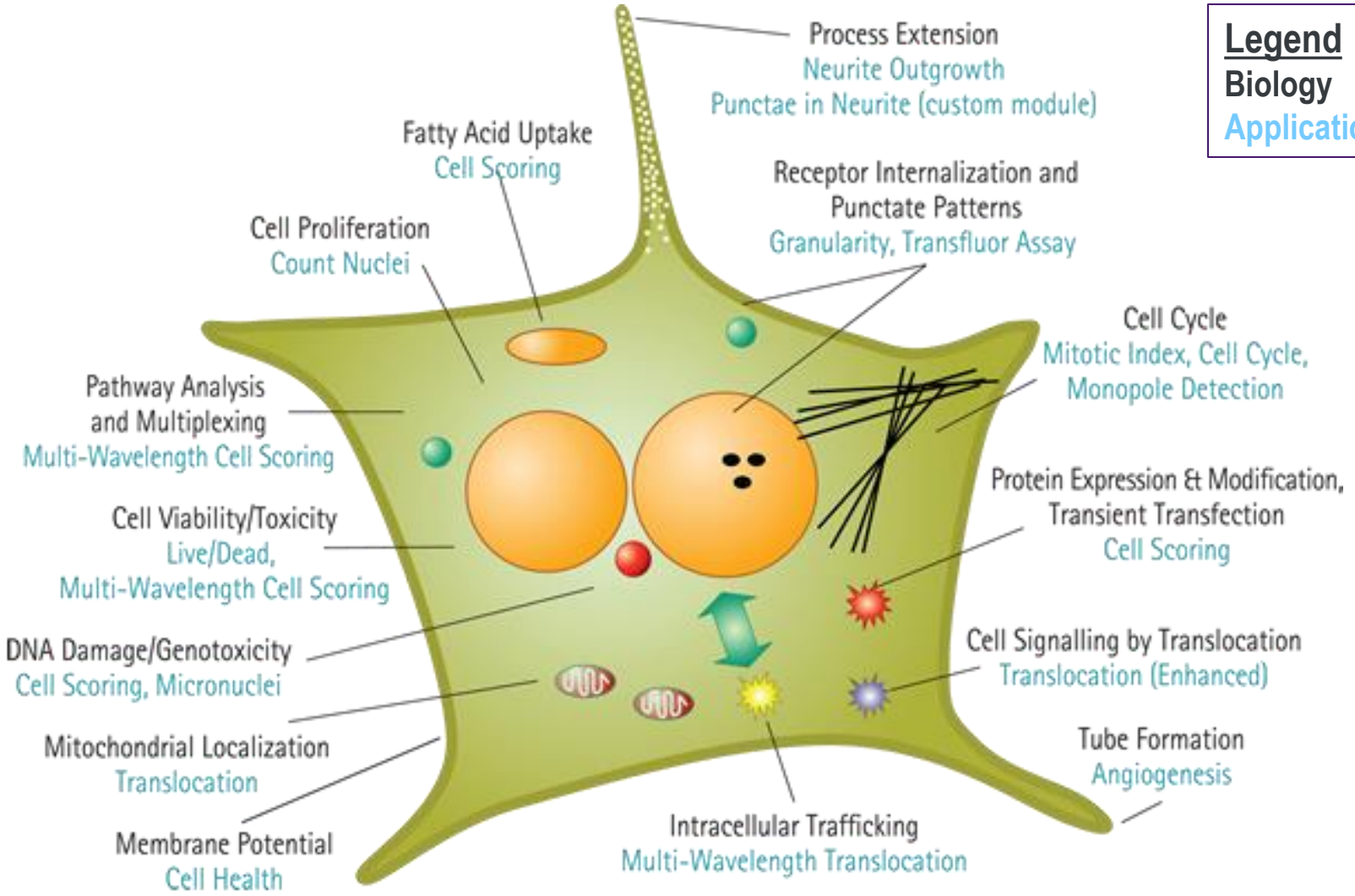
MetaXpress Application Modules are turnkey analysis solutions for measuring the most common biologies. Application modules feature:

- Advanced segmentation, feature detection and measurement capabilities.
- Image-by-image and cell-by-cell data.
- Validated results.
- Can be incorporated into Custom Modules and journals for increased customization.





# Applications and Relevant Modules




**Legend**
**Biology**
**Application Module**

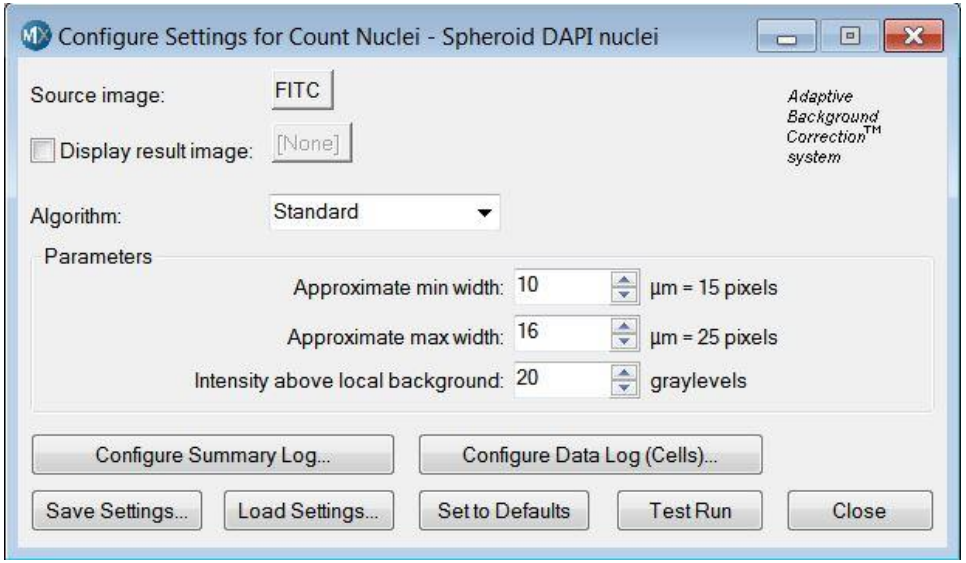
# Application Modules Overview

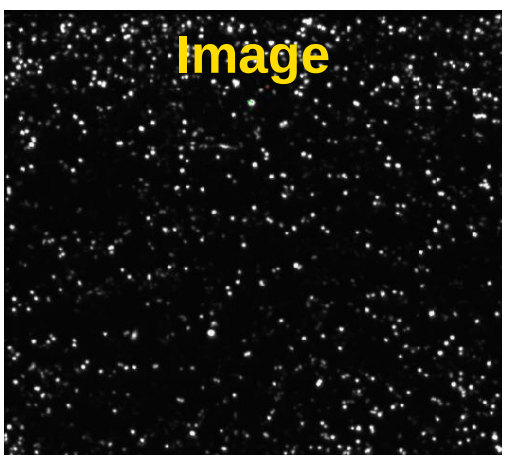
MetaXpress offers 19 Application Modules that share the same basic inputs for finding objects of interest.

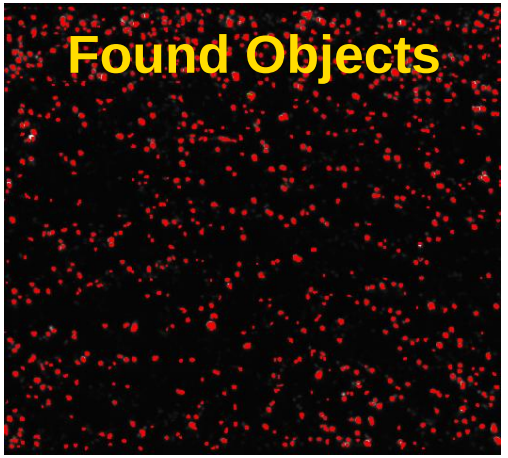
Each module has a simple configuration:

- Select wavelength(s) (Source image).
- Set size range of objects and intensity threshold.
- Select measurements.
- Test and save settings.

All Application Modules utilize the Adaptive Background Configuration™ System and automatically split touching cells.

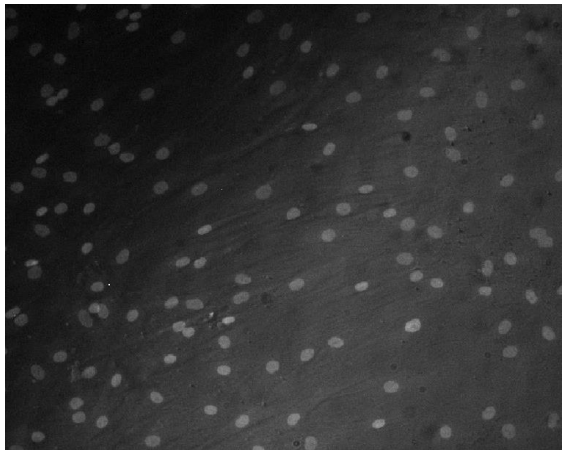


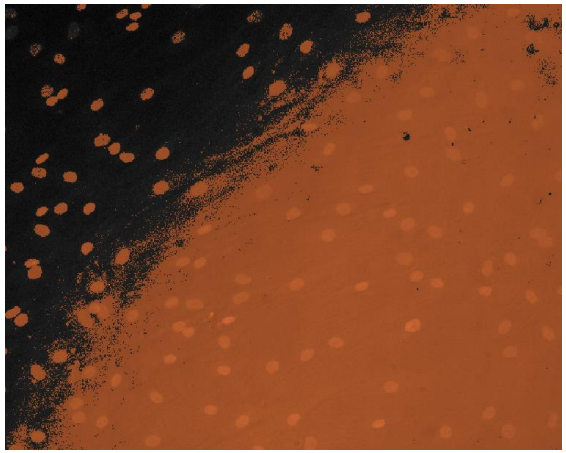



# Adaptive Background Correction™ System

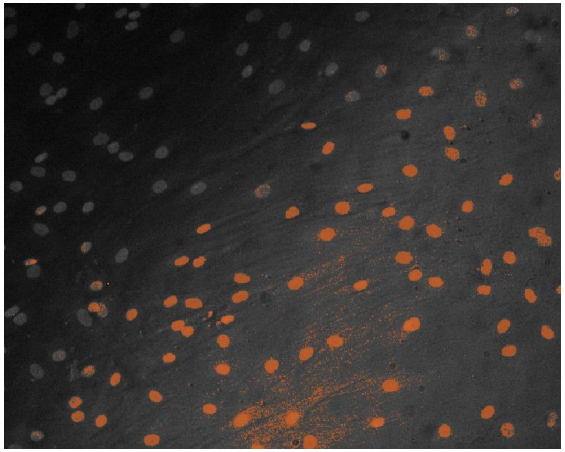
**Raw Image**



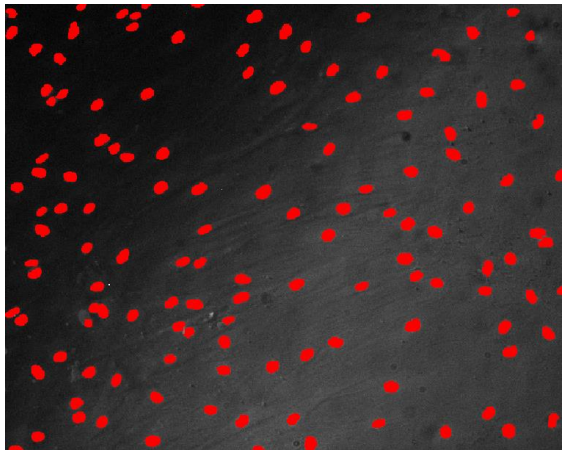
**Detection with low intnesity threshold**



**Detection with high intensity threshold**



**Adaptive Background Correction applied**



The **Adaptive Background Correction** system corrects uneven image backgrounds throughout the image by adapting to local content. This allows for more robust segmentation and analysis repeatability.

- Performed by each application module.
- Allows detection even in noisy and poorly stained images.
- Splits touching cells.
- Consistent performance across multiple images.

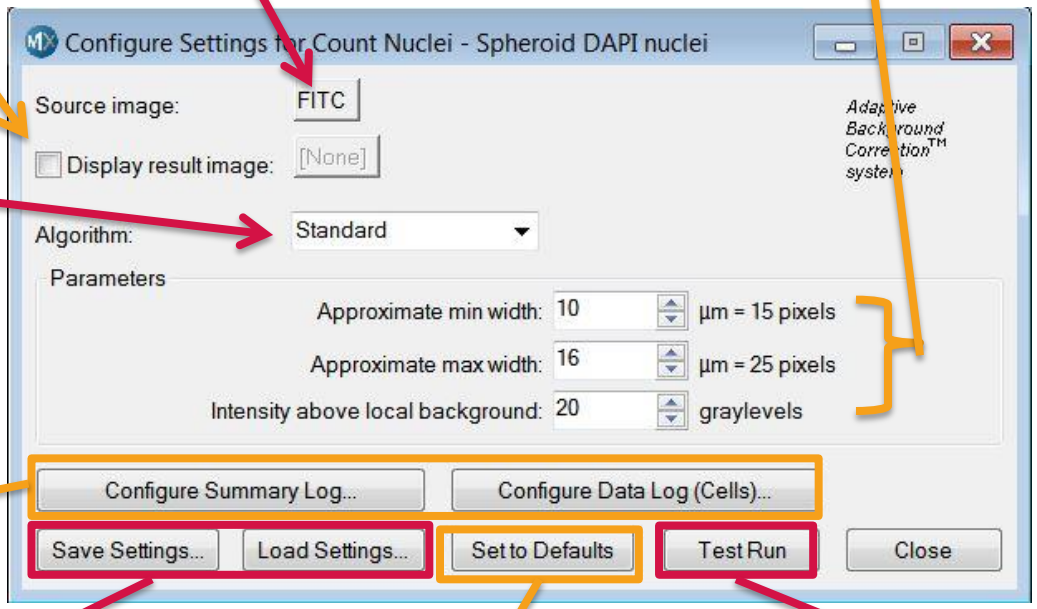
# Application Module Interface

Deselect the **Display result image** check box. This is generally only used when creating a journal.

Click the **Source image** button to select wavelength (image) to find objects. *DO NOT select images that are preceded with "HTS".*

**Width** and **Intensity** parameters for finding objects

Most application modules feature two **algorithms** (**Standard and Fast**). The **Fast** algorithm typically runs twice as fast and is available in MetaXpress version 4.0 and higher.



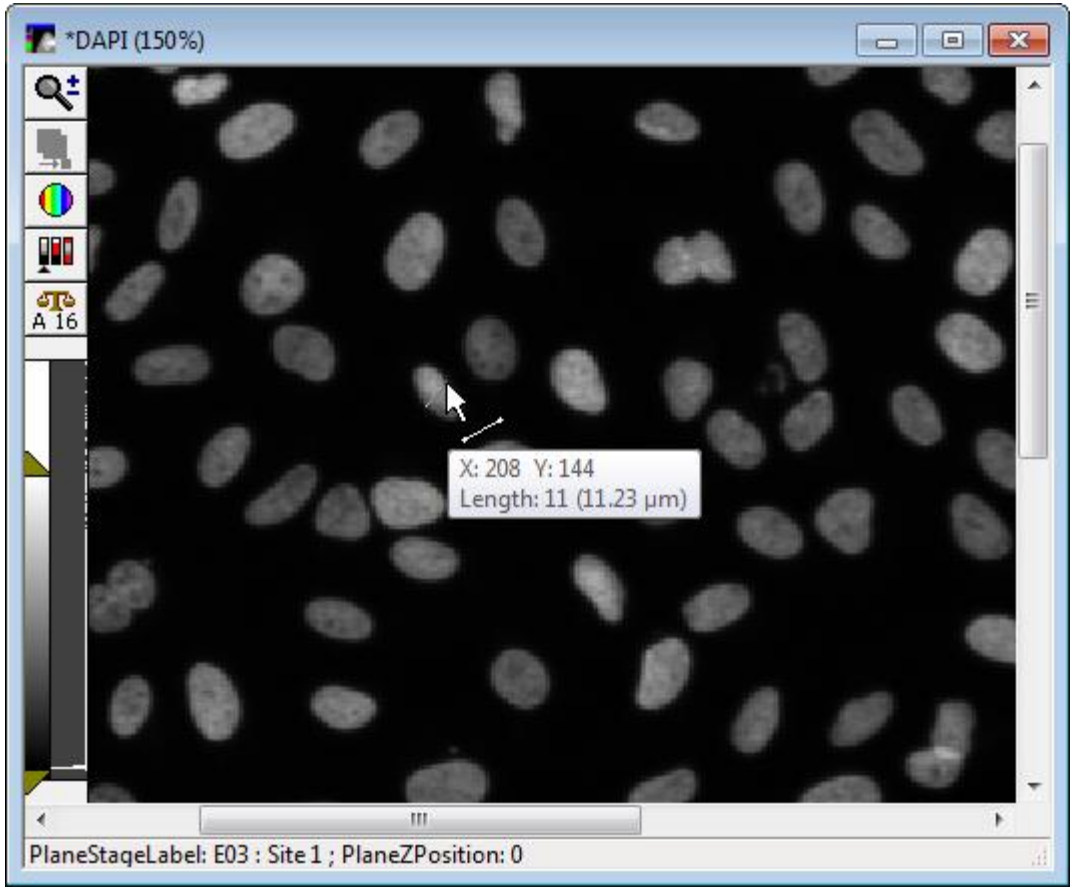

Use the **Configure Summary Log** and **Configure Data Log** buttons to enable measurements made for both image and cell data

Use the **Load Settings** and **Save Settings** buttons to load and save settings

Use the **Set to Defaults** button to reset all options to default

Click the **Test Run** button to apply settings and view segmentation results

# Configuring Modules: Measuring Object Width




## Method 1: Single Line Tool

- In the main toolbar, select the **Single line** tool in Region tools.
- Single-Click and drag across the *shortest* axis of a small object to determine its **Approximate min width.**
- The software will display a tool tip of the line length in µm

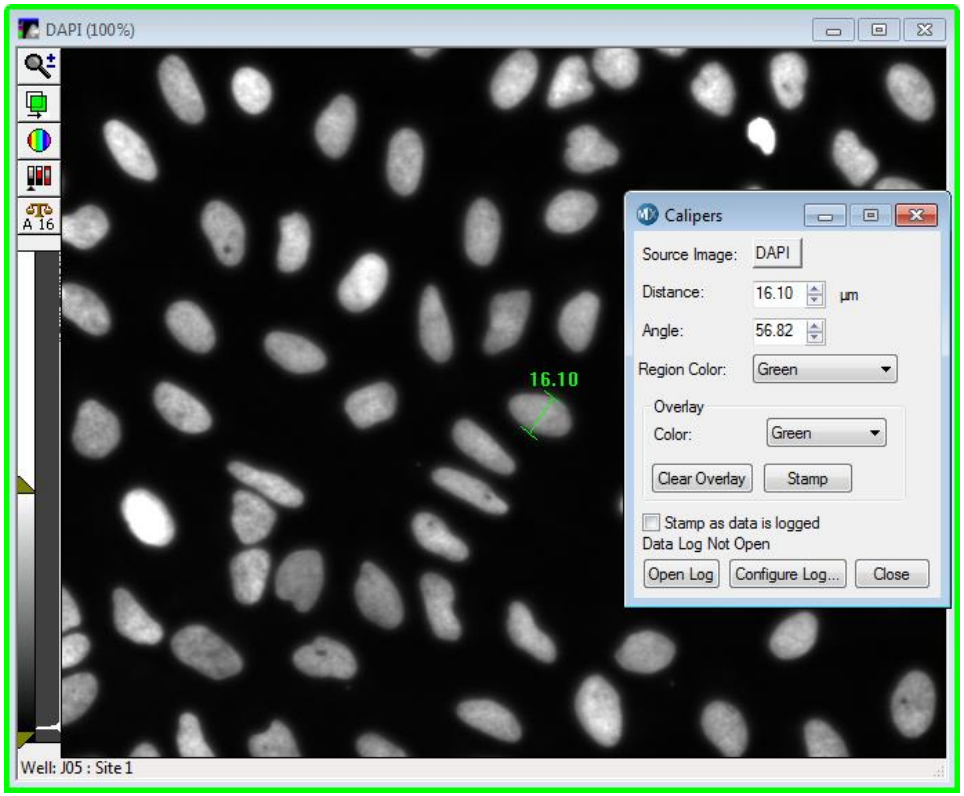
***NOTE* Do not click the image again as this will cause the tooltip to disappear. If the tooltip disappears, repeat the drawing procedure as the lines will not affect any image settings.**

- Repeat the above process with a large object to determine **Approximate max width**

# Configuring Modules: Measuring Object Width

## Method 2: Using Calipers

- In the main menu, select **Measure > Distances > Calipers**
- Click and drag to resize and rotate calipers across the shortest axis of a small object to determine **Approximate min width**
- Repeat the above process with a large object to determine **Approximate max width**



# Effects of Varying Width Settings

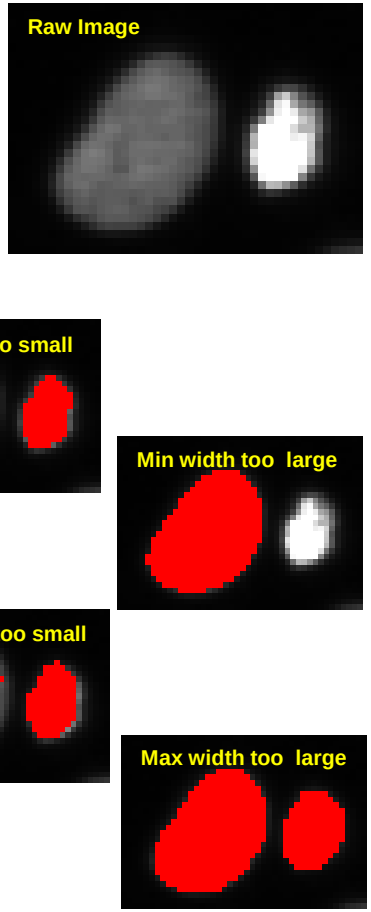
Molecular Devices recommends only changing one parameter at a time in order to determine optimal settings.

**Min width Too Small:** splits single objects into multiple smaller objects.

**Min width Too Large**: omits smaller objects.

**Max width Too Small:** may shrink object boundaries to within the actual edge.

**Max width Too Large:** may slightly enlarge object boundaries to beyond the actual edge.



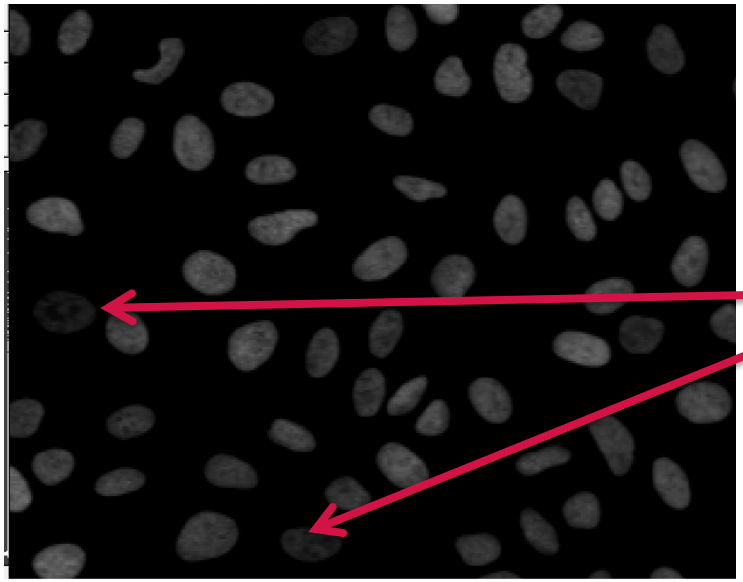

**NOTE* The above suggestions are simply meant to be guidelines for determining values. You will need to test and optimize these values for each application module/sample type. In most cases, if multiple application modules are being used parameters can be transferred between modules (i.e. for identifying nuclei).*

# Intensity Above Local Background

The **Intensity above local background** is a parameter used to determine if an object should be identified based on its intensity level compared to its local background.

- This value should be set to the difference in intensity between a dim object and its local background
  - For Fast algorithms, set the value to half (or less) of the difference in intensity between an object and background
  - For Standard algorithms, set this value to slightly lower than the difference

**NOTE* The above suggestions are only meant to be guidelines for determining values. You will need to test and optimize these values for the application module. In most cases, if multiple application modules are being used parameters can be transferred (i.e. nuclei objects).*

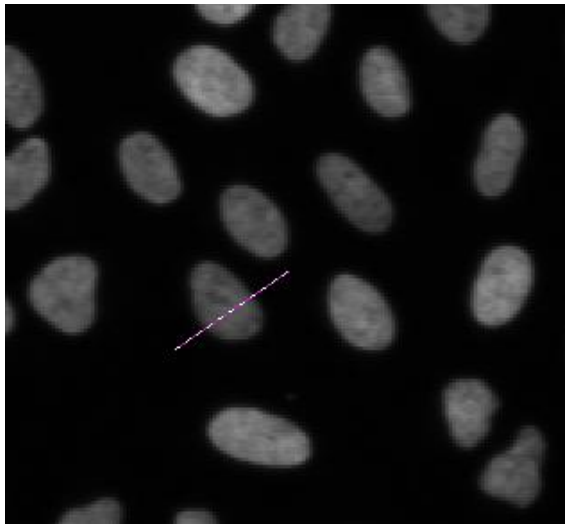


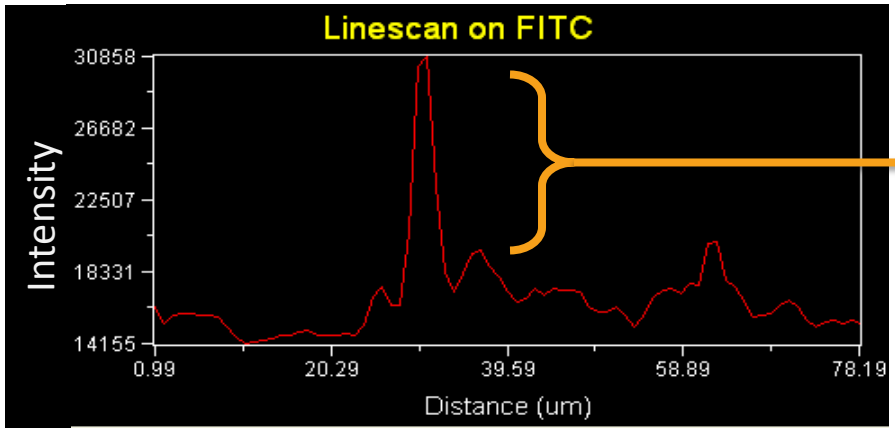
These nuclei appear to be the dimmest objects in the image and are ideal for determining **Intensity above local background**

# Configuring Modules: Intensity Above Local Background

## Method 1: Using Linescan

- Using the Single line tools, draw a line across an object and its local background as shown below.
- In the main menu, select **Measure > Intensities > Linescan.**
- Determine the intensity change on the y-axis.
- Enter a number slightly lower than this result in the **Intensity above local background** field in the application module settings. For this example:
  - Standard: (30858 - 18331) = 12527 (enter 12,000)
  - Fast: ([30858 - 18331] / 2) = 6263.5 (enter 6,000)





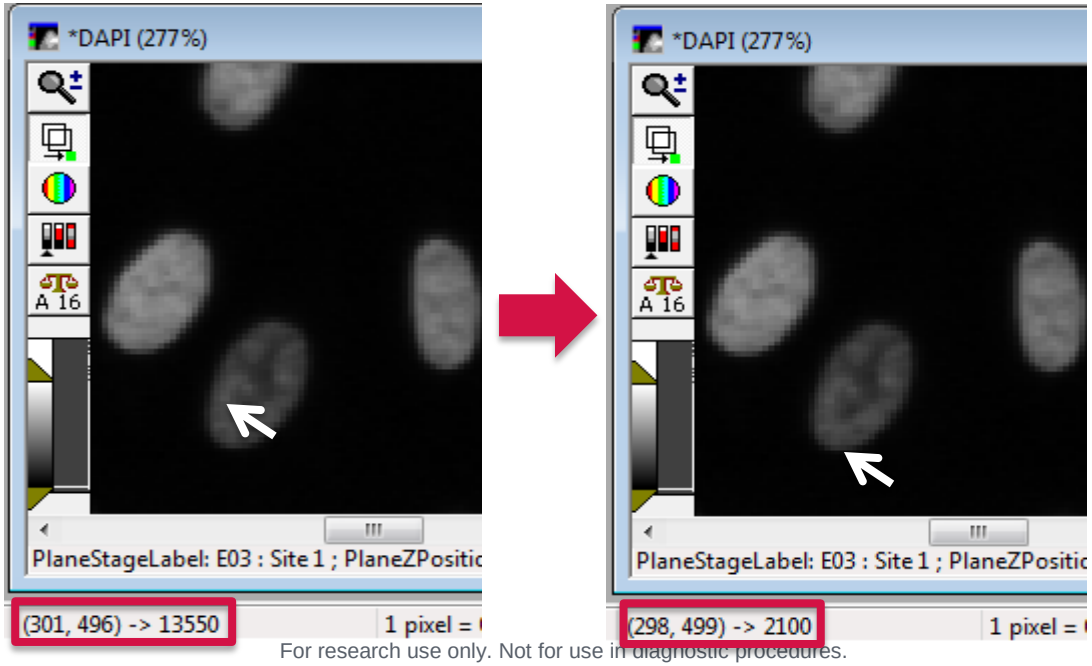

Use the difference in intensity values to set **Intensity above local background**

# Configuring Modules: Intensity Above Local Background

## Method 2: Using your mouse

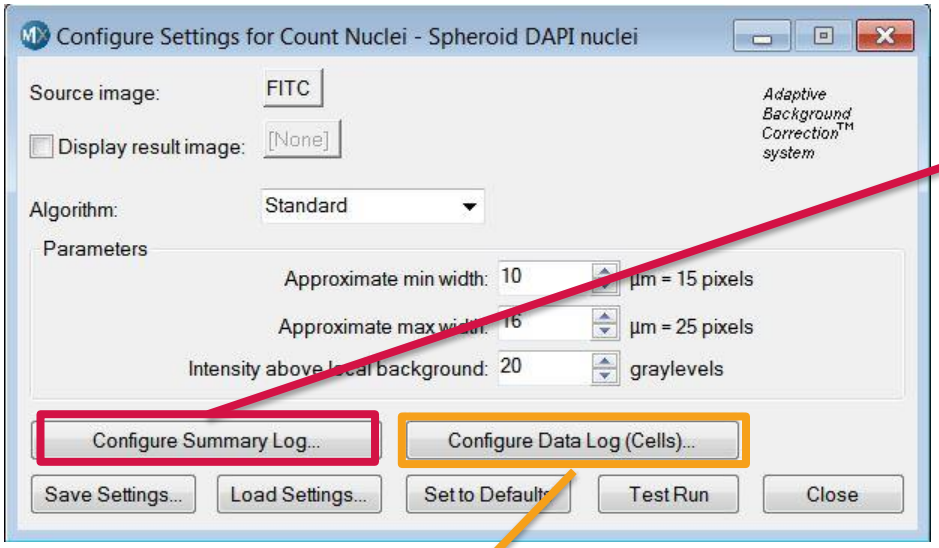
- Point your mouse inside and just outside of the object of interest and note the pixel intensity.
  *NOTE* Pixel (x,y) coordinates and intensity information is located at the bottom of the MetaXpress window
- Subtract the background intensity from the object intensity.
- Enter a slightly lower value in the **Intensity above local background** field within the application module settings. For this example:
  - Standard: (13550 - 2100) = 11450 (enter 10,000)
  - Fast: ([13550 - 2100] / 2) = 5275 (enter 5,000)

**Pointer Inside of Cell**
X coordinate: 301
Y coordinate: 496
Intensity: 13550



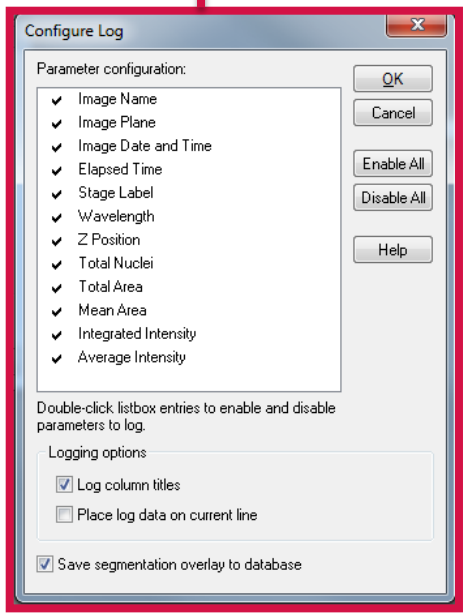

**Pointer Outside of Cell**
X coordinate: 298
Y coordinate: 499
Intensity: 2100

# Selecting Measurements



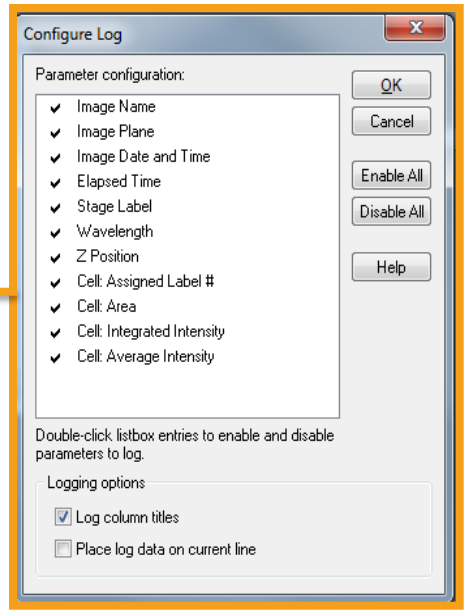

Click on the **Configure Summary Log** button to:

- Enable/disable image measurements (i.e. total number of nuclei, average intensity of all nuclei, average area of all nuclei).
- Enable/disable saving cell segmentation to the database.



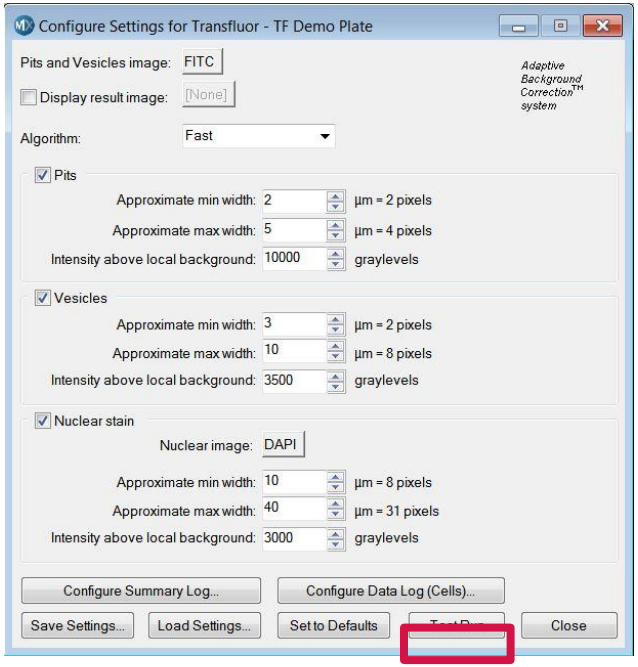

Click on the **Configure Data Log** button to:

- Enable/disable cell measurements (i.e. area of each nucleus, total intensity of each nucleus).



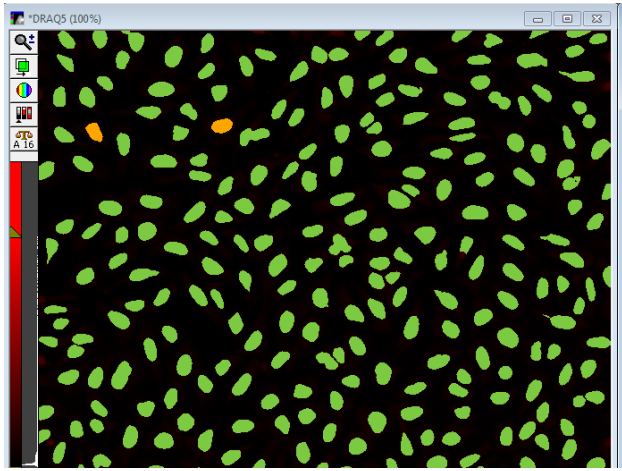
# Interactive Feedback

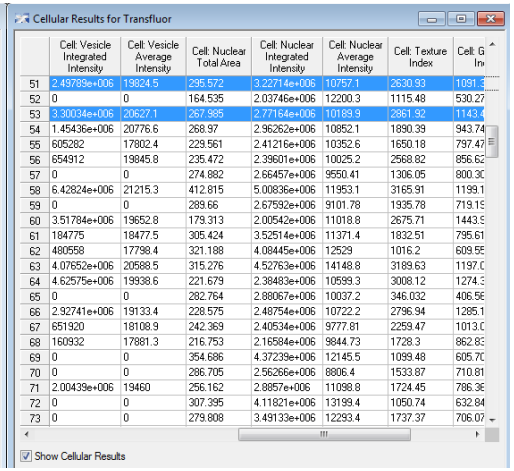
Interactive optimization of analysis parameters




- Changing parameters in application modules automatically updates segmentation on the image and object (cell-by-cell data) after clicking **Test Run** or **Apply.**
- Clicking on an object of interest highlights the corresponding data in the table and vice versa
- To enable the Cellular Results table, from the main menu select **Measure** > **Show Cellular Results.**

Immediate graphic feedback on detection results

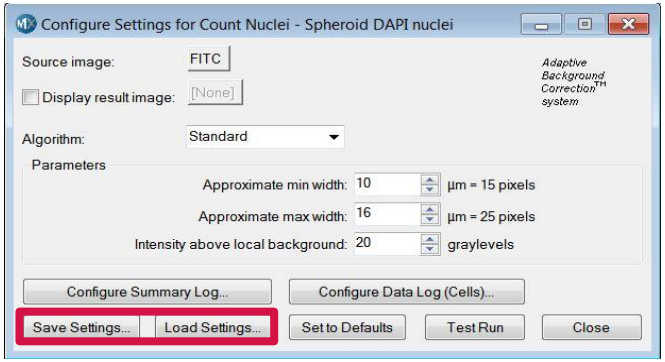


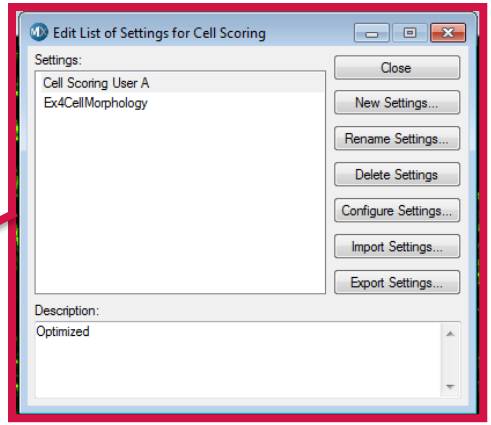

Links numerical results to image overlays

# Loading and Saving Settings

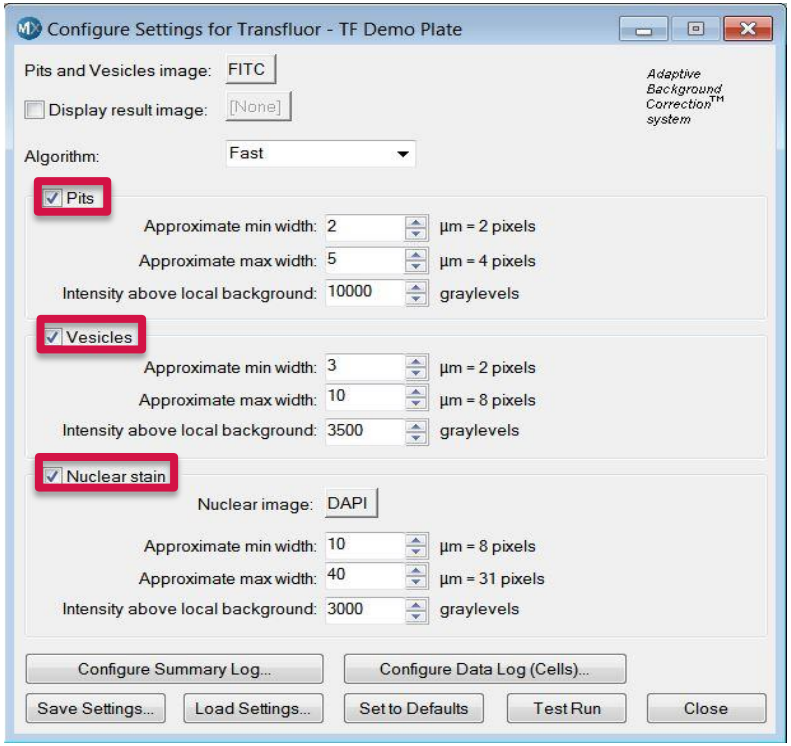
Application modules can be accessed either through the **Review Plate Data** dialog or in the main menu (**Measure > Objects and Cells**)




- Each application module has its own file extension.
- If the application module is opened through the main menu, settings are loaded / saved as files.
- If the application module is opened through **Review Plate Data**, settings are loaded / saved directly to the database. To import / export / delete settings:
  - In **Review Plate Data**, select the **Run Analysis** tab and choose the desired **Application module** from the drop-down menu.
  - Click on the **Edit List** button.
  - This dialog will allow you to import / export settings saved to the hard drive as well as delete settings in the database.



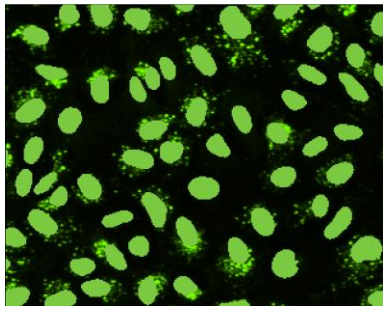
# Tips On Working with Application Modules



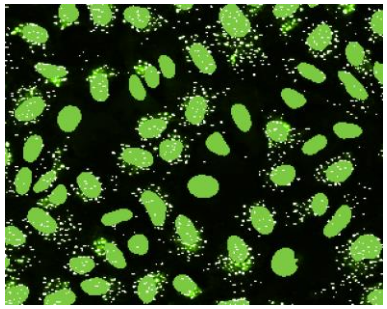

Some application modules enable measurement of multiple objects types. For example, in the Transfluor application module you can select and measure any combination of Pits, Vesicles, and Nuclei object types.

- When all objects are enabled, the cell segmentation will display all object types.
- Select only the object type(s) of interest to display in cell segmentation

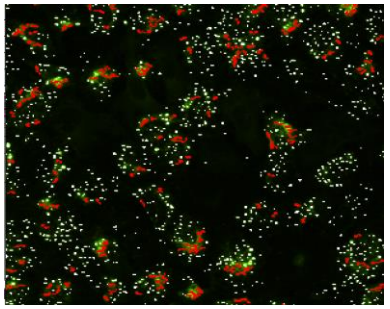
***NOTE* Be sure to enable all desired object types before saving and exiting the application module.**



Nuclei only

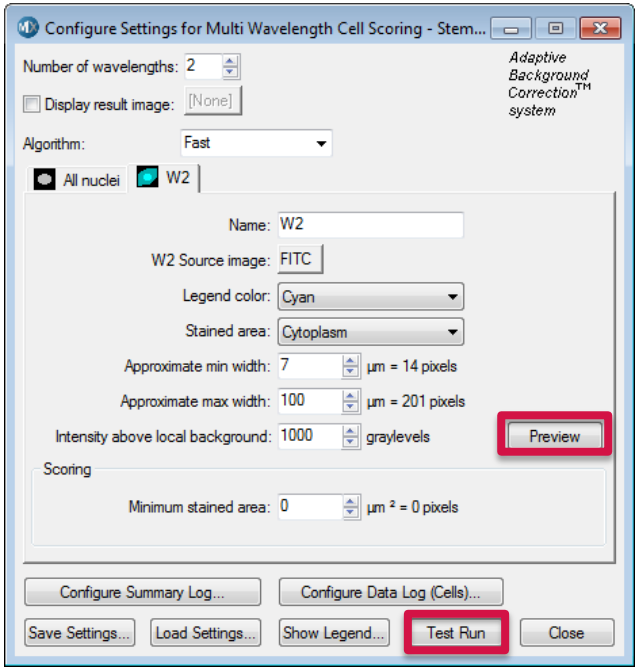


Nuclei and Pits

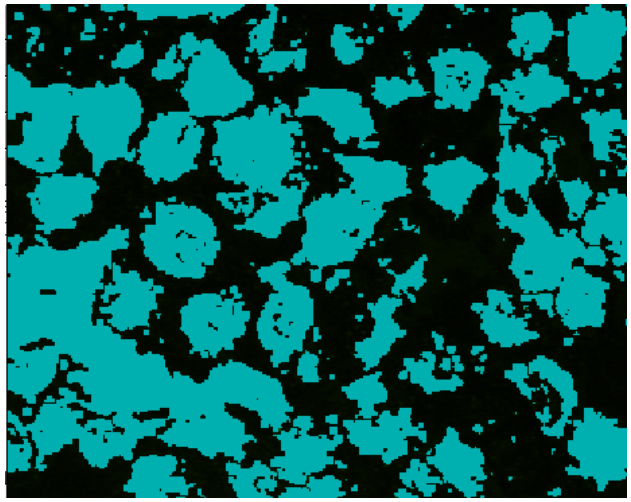


Pits and Vesicles

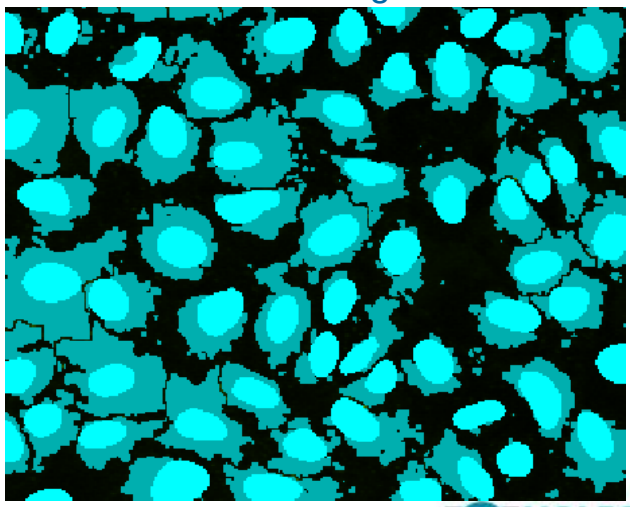
# Tips On Working with Application Modules




**Preview:** Only W2 settings



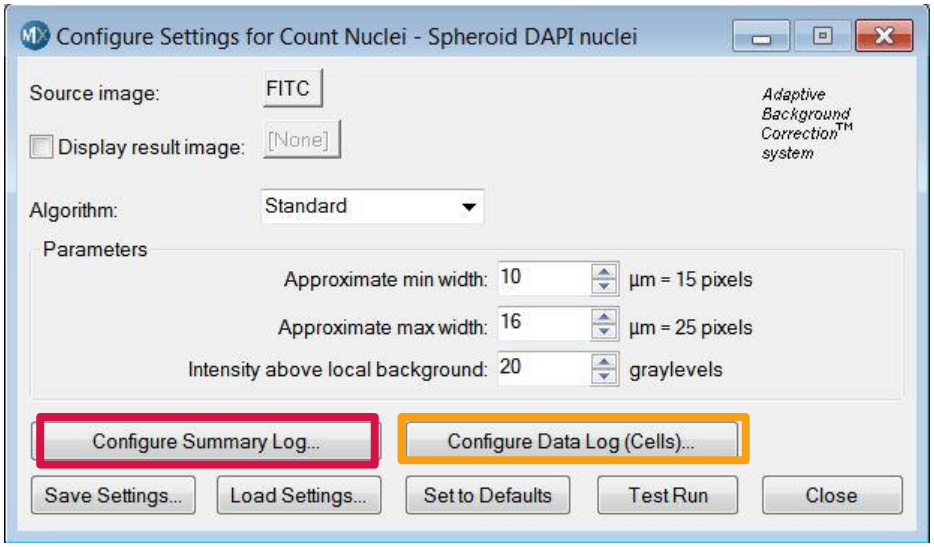
**Test Run/Apply**: All nuclei and W2 settings



Some application modules may have a **Preview** button:

- The **Preview** button will display all objects found based *solely* on the settings for that selected wavelength
- Click the **Test Run** (or **Apply**) button to display cell segmentation based on the settings entered for *all* wavelengths

# Tips On Working with Application Modules




While optimizing settings in application modules, it is possible to log both image (Summary) and/or cell-by-cell data directly to Excel or to a text file:

- **Image (Summary Log)**: in the main menu, select **Measure > Log > Open Summary Log**
- **Cell-by-Cell Data (Data Log)**: in the main menu, select **Measure > Log > Open Data Log.**
- Both logs can be open at the same time.

**NOTE* Refer to corresponding module on exporting data for details on logging to an Excel or text file*

# Support Resources

- F1 / HELP within MetaXpress® Software
- Support and Knowledge Base: http://mdc.custhelp.com/
- User Forum: http://metamorph.moleculardevices.com/forum/
- Request Support: http://mdc.custhelp.com/app/ask
- Technical Support can also be reached by telephone:
  - 1 (800) 635-5577
  - Select options for Tech Support → Cellular Imaging Products → ImageXpress Instruments

MOLECULAR DEVICES

ADVANCING PROTEIN AND CELL BIOLOGY