MetaXpress® 6 Software Guide

Application Modules: Count Nuclei

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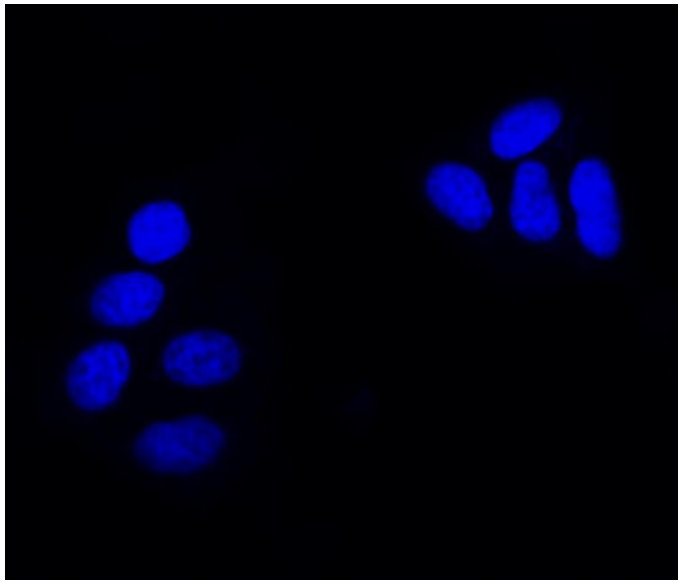
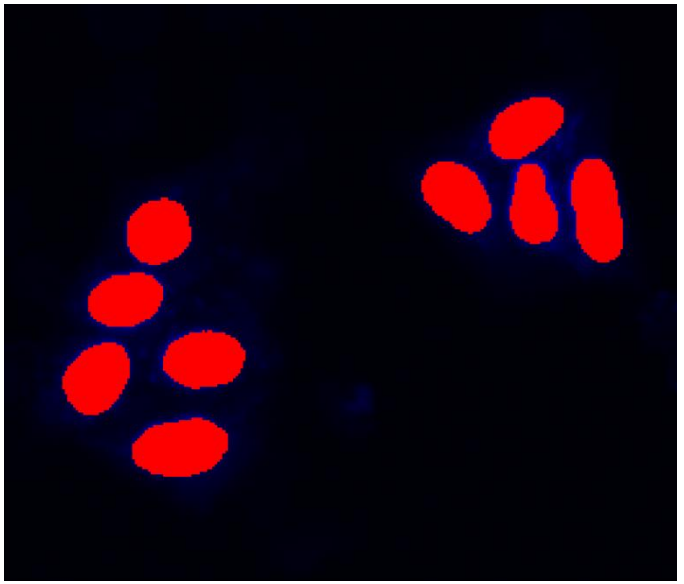
Count Nuclei Application Module Overview

The Count Nuclei application module can be used to identify nuclei.

- A fluorescent nuclear stain (i.e. DAPI, Hoechst, DRAQ5, etc.) is required.

NOTE Application modules can be used to measure different biological processes. Nuclei can be any structure with a roundish shape. For example, bacteria, whole cells, beads, etc.





Module Settings: Selecting an Image & Algorithm

Source Image

- Select the image with nuclei

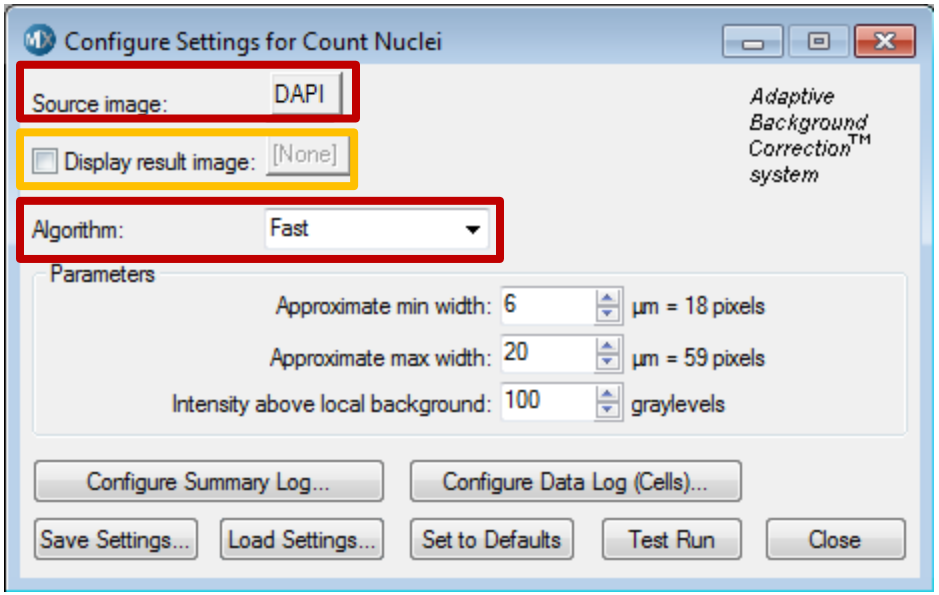
NOTE Do not choose images with “HTS” in the name

Display result image:

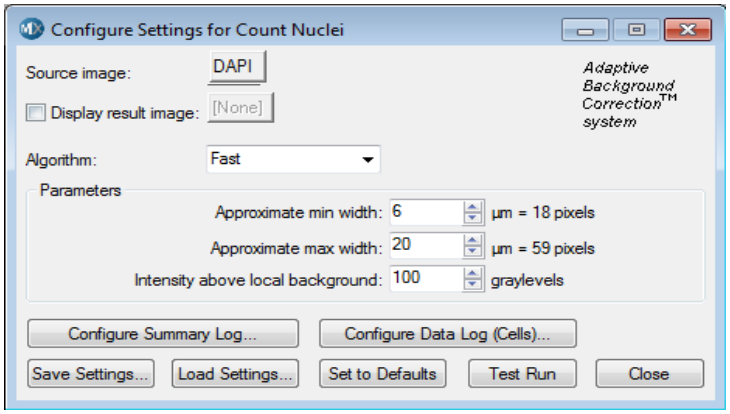
- Leave **Display result image** deselected (this is generally only used when creating a journal)

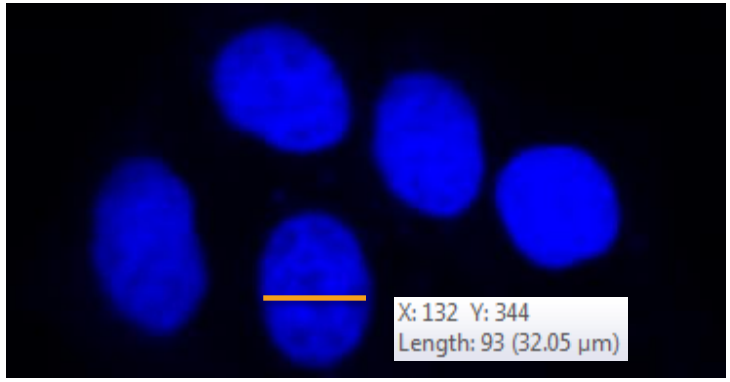
Algorithm

- This option is only available in MetaXpress software version 4.0 and higher and determines how quickly the analysis is performed
- **Fast** algorithm can perform analysis up to twice as fast as **Standard**
- Molecular Devices recommends starting with the **Standard** algorithm for Transfluor and Granularity



Module Settings: Defining the Size of Objects



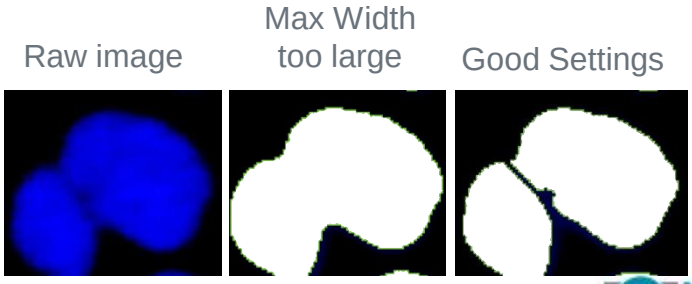


Approximating min and max widths

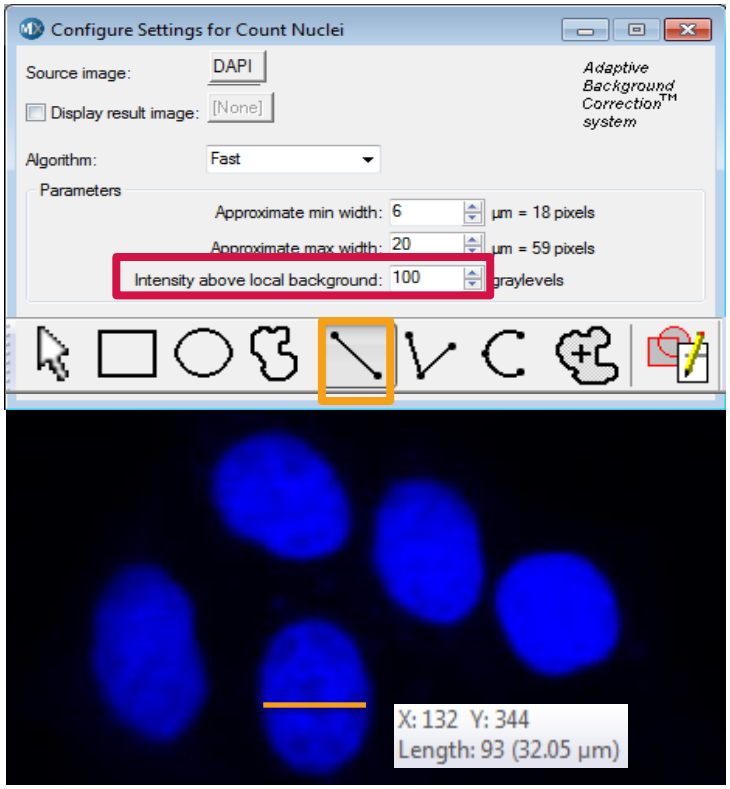
- Select the single line region from **Regions Tools**
- Click and drag across a representative small and large object; a tooltip will show the length of the line

NOTE Do not click the image again. This will cause the tooltip to disappear. If the tooltip disappears, repeat the drawing procedure.

- The width is the short axis of a object (in µm)
- Much smaller or much larger cells will be ignored
- Click **Test Run** and adjust values as necessary
- Repeat for all desired objects (pits, vesicles, and nuclei)

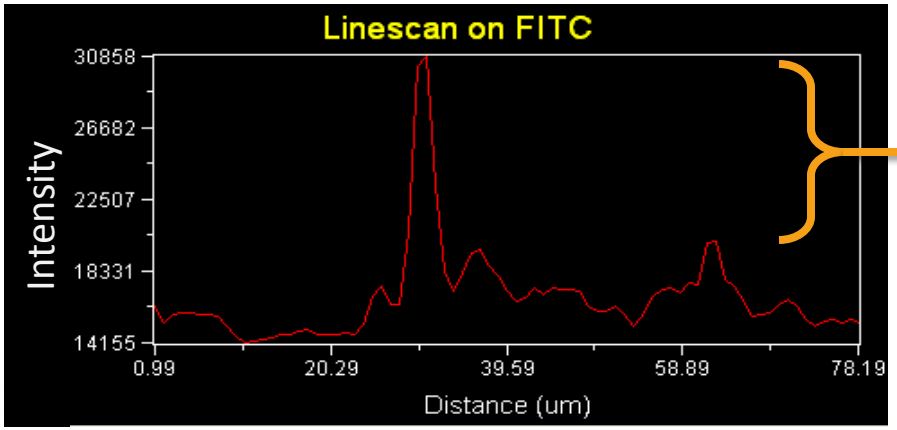


Module Settings: Defining Object Intensities



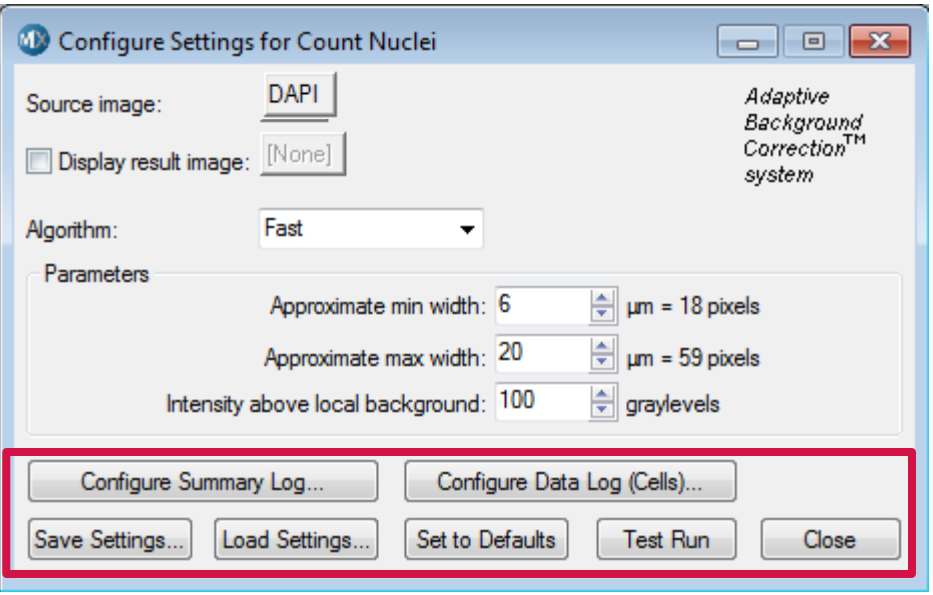
Intensity above local background

- Draw a line over the dimmest object of interest that covers both the object and background using the Single line tool
- In the main menu, select **Measure > Intensities > Linescan**. This will display a histogram of intensity values.
- For **Fast** algorithms, set **Intensity above local background** to half (or less) of the difference in intensity between an object and background
- For **Standard** algorithms, set this value slightly lower than this difference
- Click **Test Run** to preview the measurement mask and adjust values as necessary



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

Module Buttons



Configure Summary Log: Select image-by-image measurements.

Configure Data Log: Select cell-by-cell measurements.

Save Settings: Save application module settings.

Load Settings: Load saved application module settings

Set to Defaults: Restore default application module settings.

Test Run: Test all settings together and display cell-by-cell results for the displayed image (if **Show Cellular Results** is enabled under the **Measure** menu)

Configuring Summary or Data (Cell) Logs

Double click on parameter to select or deselect it for logging into the database.

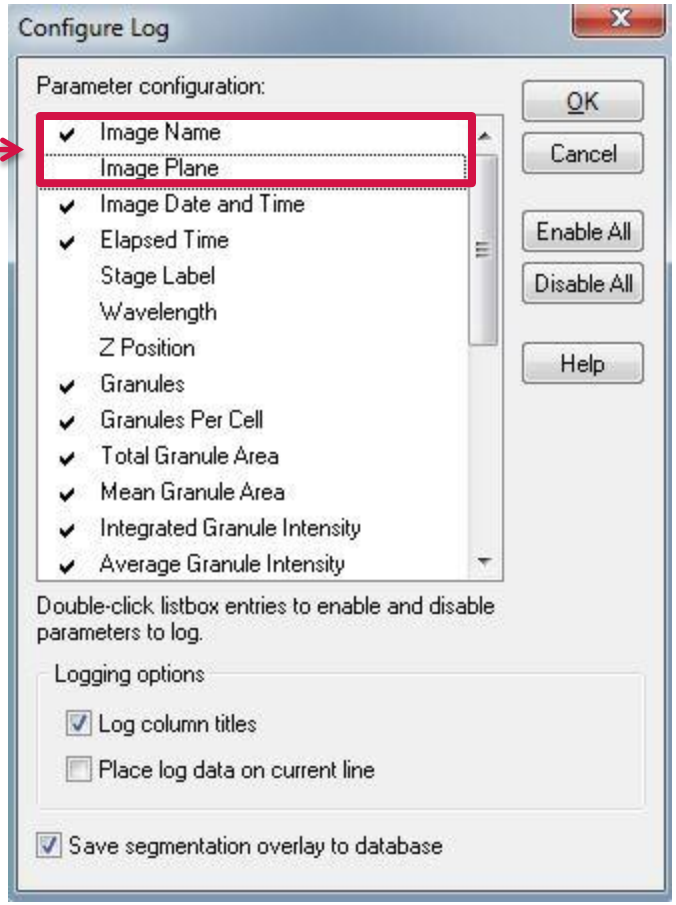
- ✓ Indicates a parameter that will be logged into the database (or Excel/text file log if open)

Log column titles: Does not affect database logging. If you have an Excel or text file log open, this records the parameter name as the column header for easy review. Recommend enabling this option.

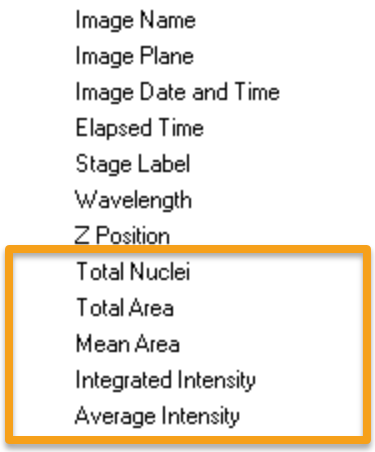
Place log data on current line: Does not affect database logging. If you have an Excel or text file log open, this records the data into the last row used, to the right of the previous data. Recommend disabling this option to ensure that new data is recorded into a new row.

Save segmentation overlay to database: A mask (or binary image) is temporarily created for every single raw image analyzed on a plate (see Transfluor overview for an example of a mask). This option allows you to save these masks to the database. Recommend enabling this option for assay development and disabling it for screening.

- Pro: Allows you to quickly review your segmentation results after analysis has been run across the entire plate
- Con: These masks take up a significant amount of space in a database, which may be limited in size. Saving the masks may also slow down analysis.

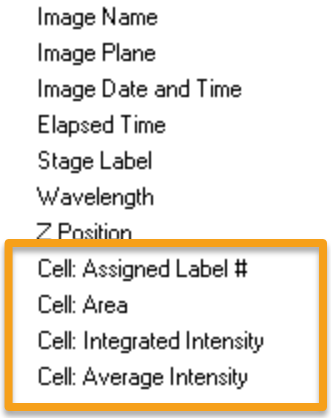


Configure Summary Log (Image Measurements)



- **Total Nuclei:** Total number of nuclei (cell count) in the image.
- **Total Area:** Total area of the nuclei for all cells found in the image (in μm^2).
- **Mean Area:** Mean area of nuclei for all cells found in the image (in μm^2).
- **Integrated Intensity:** Total pixel intensity of the nuclear stain over all of the nuclear areas.
- **Average Intensity**: Average pixel intensity of the nuclear stain over all of the nuclear areas.

Configure Data Log (Cell-by-Cell Measurements)



- **Cell: Assigned Label #**: Cell label number for the current image (1 through total cell number).
- **Cell: Area:** Total area in μm^2 of a given nucleus.
- **Cell: Integrated Intensity:** Total pixel intensity of a given nucleus.
- **Cell: Average Intensity:** Average pixel intensity of a given nucleus.

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

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