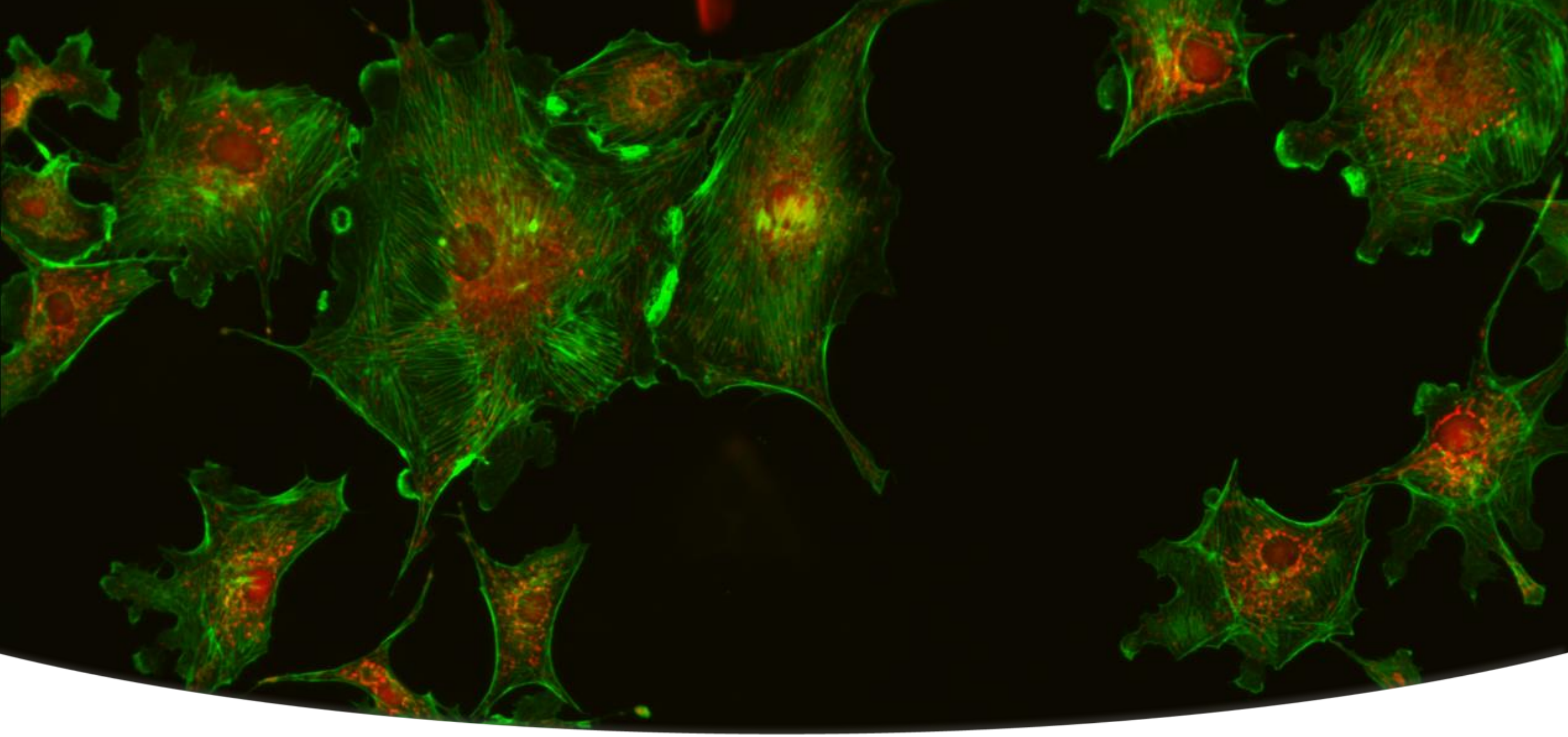

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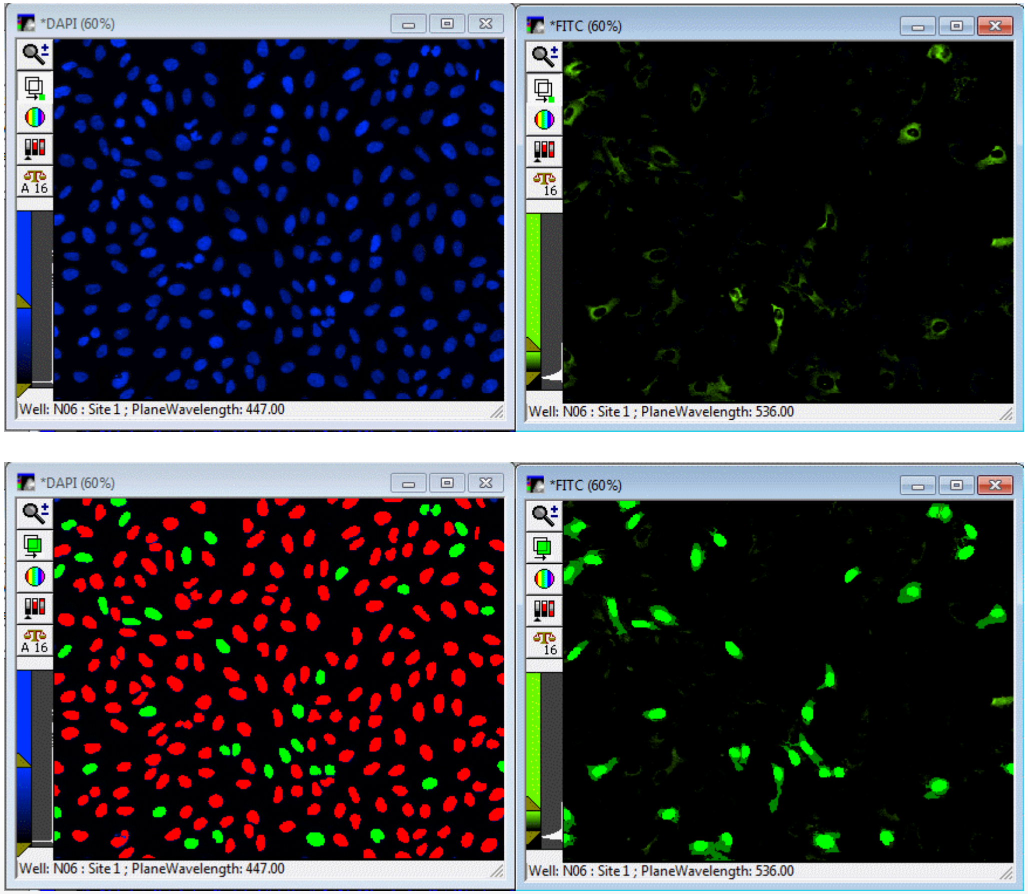
Molecular Devices

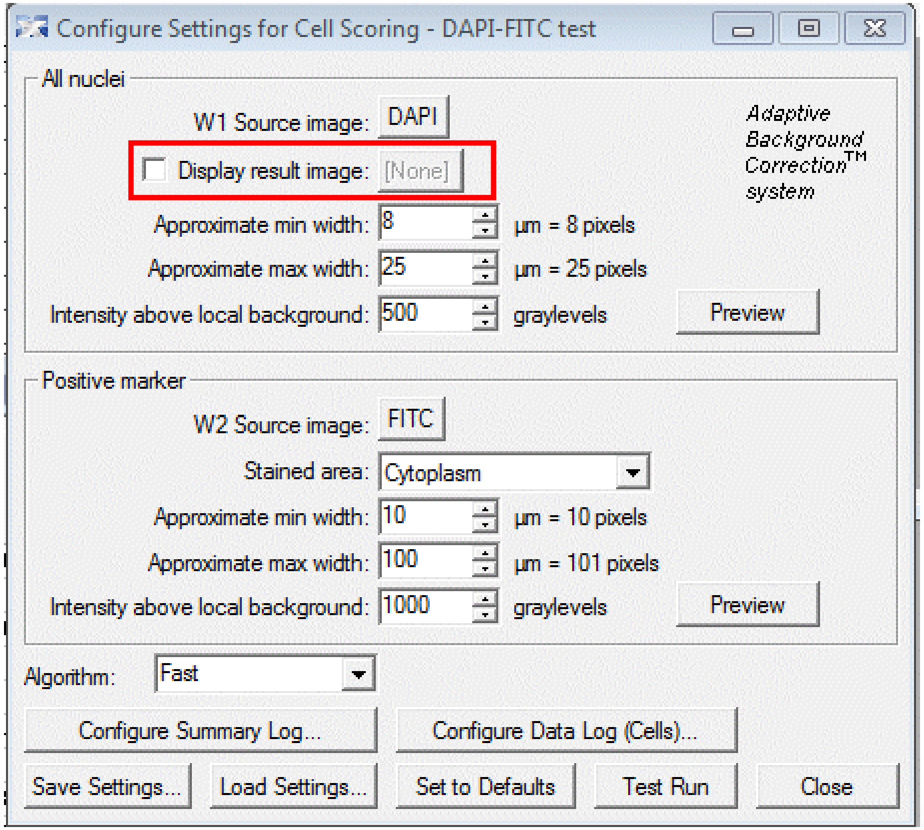
MetaXpress® Software: Cell Scoring Module

Cell Scoring Module Overview

- The Cell Scoring module can be used to analyze cells imaged in 2 wavelengths
- W1 should be a stain for all nuclei (e.g. DAPI, Hoechst, or DRAQ5)
- W2 is the stain of interest for scoring the cells
- The module will score cells as positive (green) or negative (red) for W2



Module Settings


Configure Settings for Cell Scoring - DAPI-FITC test

All nuclei

W1 Source image: DAPI

☐ Display result image: [None]

Approximate min width: 8 µm = 8 pixels

Approximate max width: 25 µm = 25 pixels

Intensity above local background: 500 graylevels

Positive marker

W2 Source image: FITC

Stained area: Cytoplasm

Approximate min width: 10 µm = 10 pixels

Approximate max width: 100 µm = 101 pixels

Intensity above local background: 1000 graylevels

Algorithm: Fast

- **Display result image**
- Leave “**Display result image**” deselected (this is generally only used when journaling)

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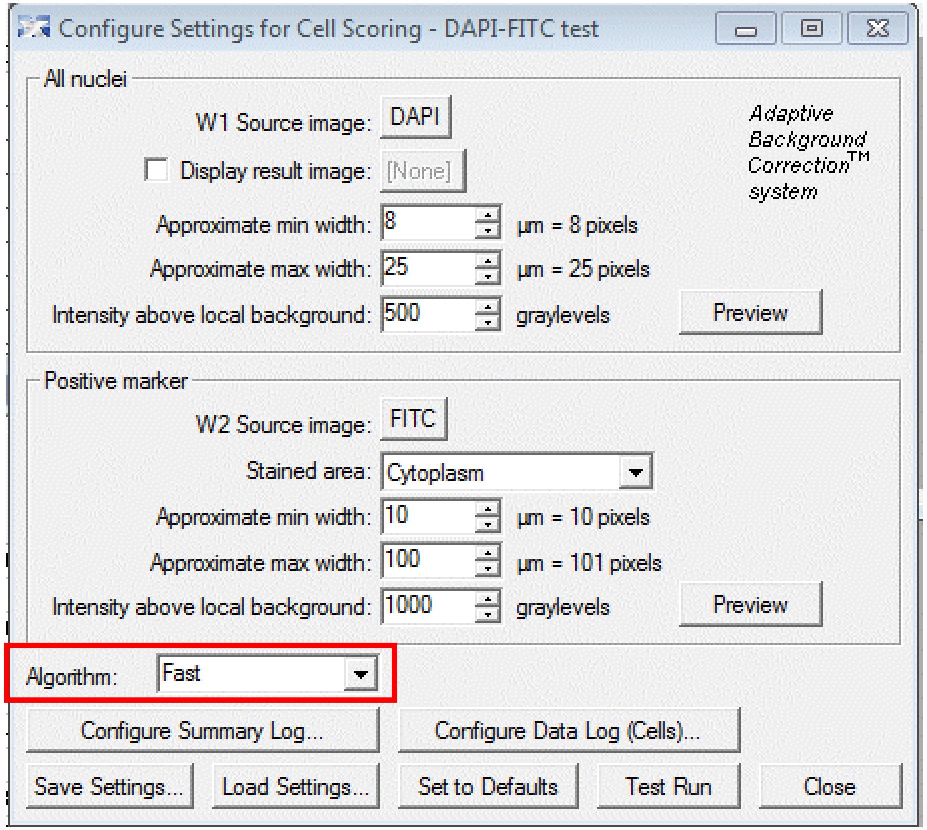
Molecular Devices

Module Settings



Configure Settings for Cell Scoring - DAPI-FITC test

All nuclei

W1 Source image: DAPI

☐ Display result image: [None]

Approximate min width: 8 µm = 8 pixels

Approximate max width: 25 µm = 25 pixels

Intensity above local background: 500 graylevels

Positive marker

W2 Source image: FITC

Stained area: Cytoplasm

Approximate min width: 10 µm = 10 pixels

Approximate max width: 100 µm = 101 pixels

Intensity above local background: 1000 graylevels

Algorithm: Fast

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**.
- Both algorithms produce similar but not identical results.

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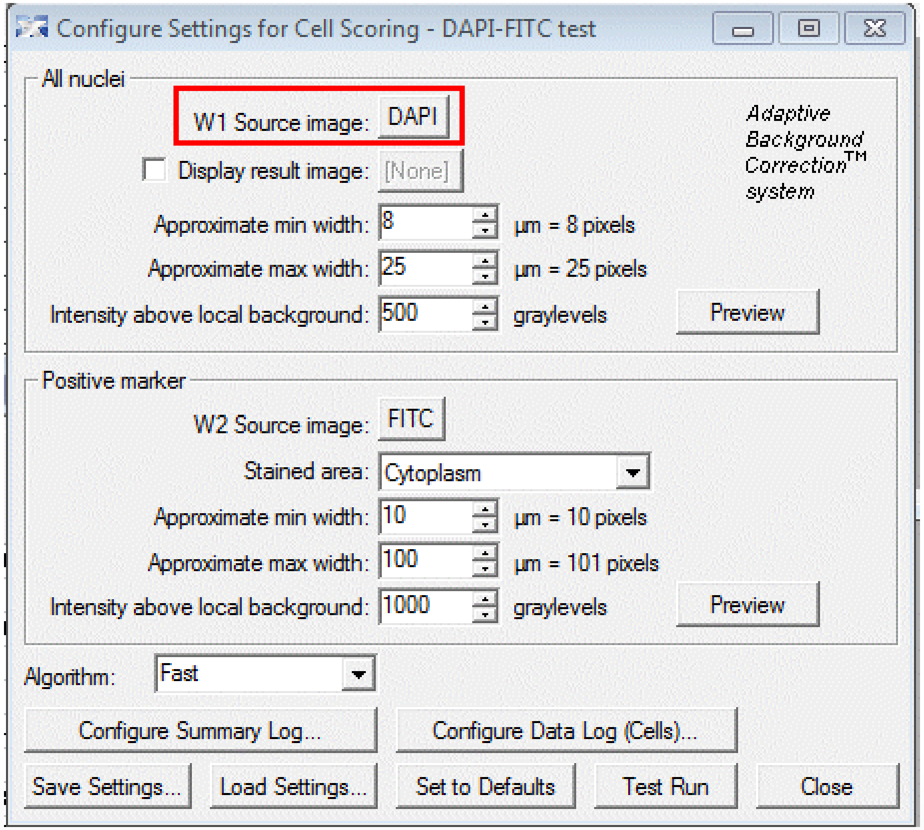
Module Settings



W1 Source image: DAPI

☐ Display result image: [None]

Approximate min width: 8 µm = 8 pixels

Approximate max width: 25 µm = 25 pixels

Intensity above local background: 500 graylevels

W2 Source image: FITC

Stained area: Cytoplasm

Approximate min width: 10 µm = 10 pixels

Approximate max width: 100 µm = 101 pixels

Intensity above local background: 1000 graylevels

Algorithm: Fast

- **Wavelength 1 (W1)**
- Select the wavelength for the **nuclei**
- Do not select the "HTS" image (thumbnail montage)

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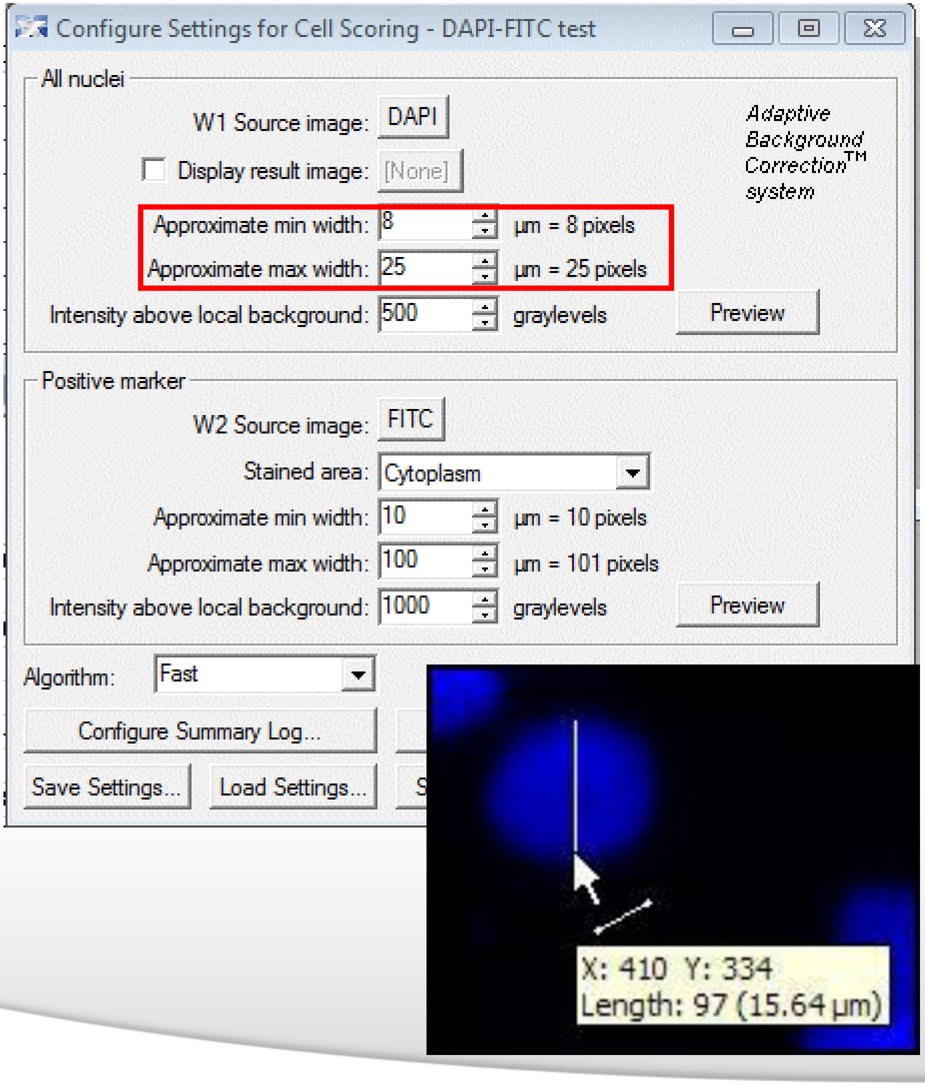
Module Settings



Configure Settings for Cell Scoring - DAPI-FITC test

All nuclei

W1 Source image: DAPI

☐ Display result image: [None]

Approximate min width: 8 µm = 8 pixels

Approximate max width: 25 µm = 25 pixels

Intensity above local background: 500 graylevels

Positive marker

W2 Source image: FITC

Stained area: Cytoplasm

Approximate min width: 10 µm = 10 pixels

Approximate max width: 100 µm = 101 pixels

Intensity above local background: 1000 graylevels

Algorithm: Fast

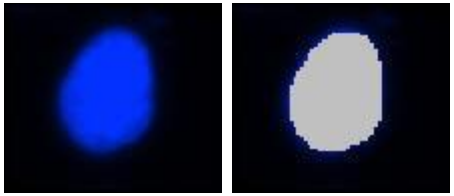
X: 410 Y: 334
Length: 97 (15.64 µm)

Wavelength 1 (W1)

- **Set the Approximate min width** and **Approximate max width** for the range of nuclei that you want to detect
- The width is the short axis of a nucleus (in um)
- The region tools can be used to measure widths
- Much smaller cells will be ignored
- Much larger cells will be split

Module Settings

Effects of varying width settings



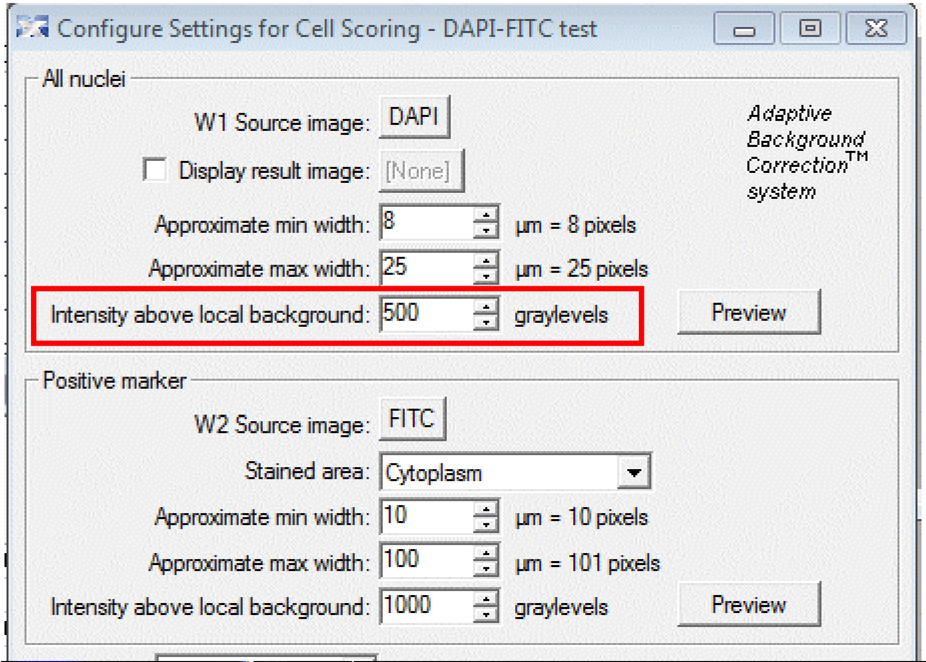
Min width too small: splits nuclei

Min width too large: omits smaller nuclei

Max width too small: may shrink nuclear boundaries

Max width too large: may slightly enlarge nuclear boundaries

Module Settings



Configure Settings for Cell Scoring - DAPI-FITC test

All nuclei

W1 Source image: DAPI

☐ Display result image: [None]

Approximate min width: 8 µm = 8 pixels

Approximate max width: 25 µm = 25 pixels

Intensity above local background: 500 graylevels

Adaptive Background Correction™ system

Positive marker

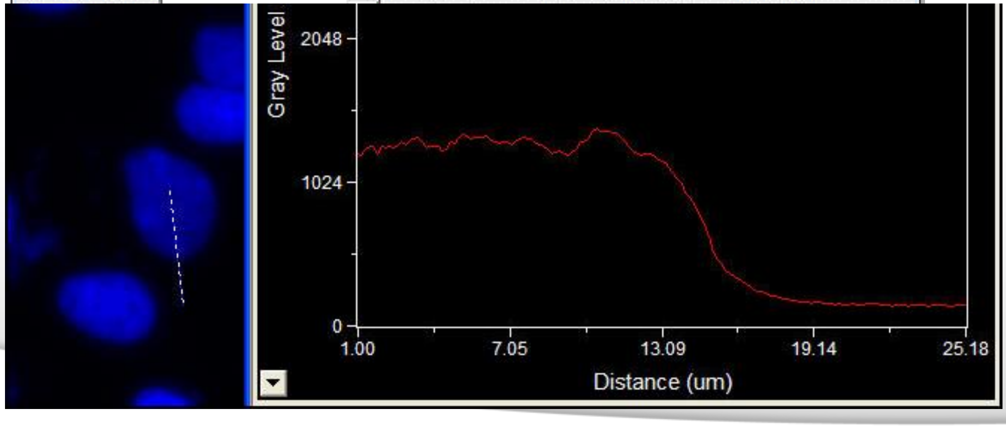
W2 Source image: FITC

Stained area: Cytoplasm

Approximate min width: 10 µm = 10 pixels

Approximate max width: 100 µm = 101 pixels

Intensity above local background: 1000 graylevels

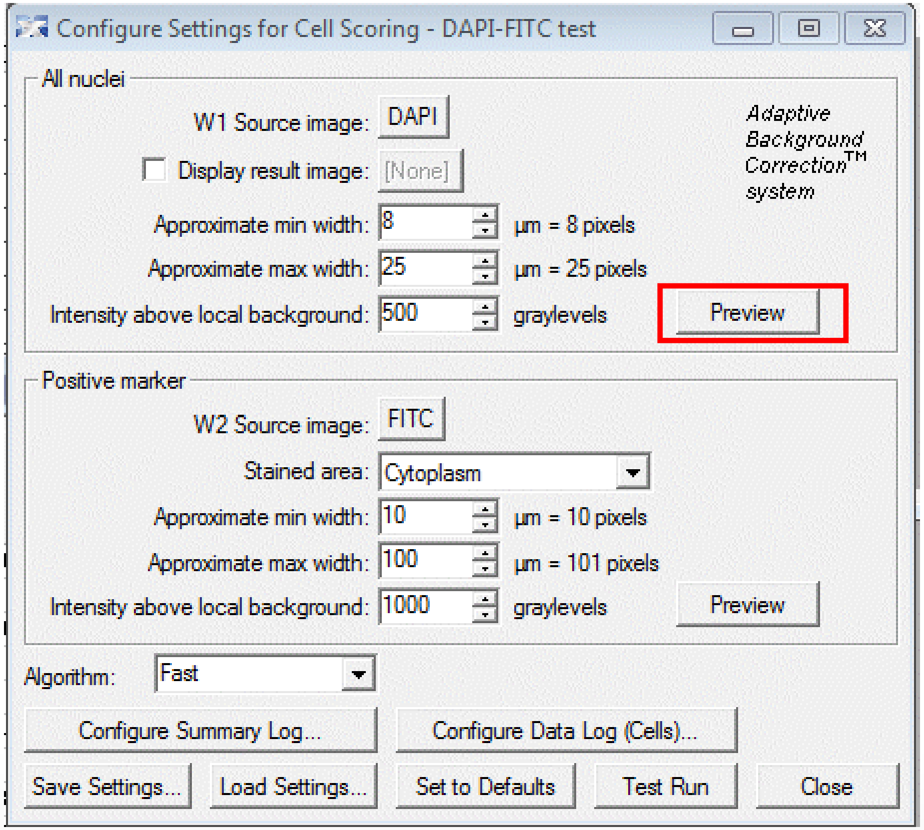


Wavelength 1 (W1)

- The **intensity above local background** is used for finding the nuclei
- This value is a minimum and should be set slightly lower than the difference in intensity between a dim cell and its local background (**Standard** algorithm)
- If using the **Fast** algorithm, set intensity value to about half of this

Draw a line across a cell into the background and use Measure > Linescan to determine intensity values; or simply mouse over the cell and the background and view the intensity values

Module Settings


Configure Settings for Cell Scoring - DAPI-FITC test

All nuclei

W1 Source image: DAPI

☐ Display result image: [None]

Approximate min width: 8 µm = 8 pixels

Approximate max width: 25 µm = 25 pixels

Intensity above local background: 500 graylevels

Adaptive Background Correction™ system

Positive marker

W2 Source image: FITC

Stained area: Cytoplasm

Approximate min width: 10 µm = 10 pixels

Approximate max width: 100 µm = 101 pixels

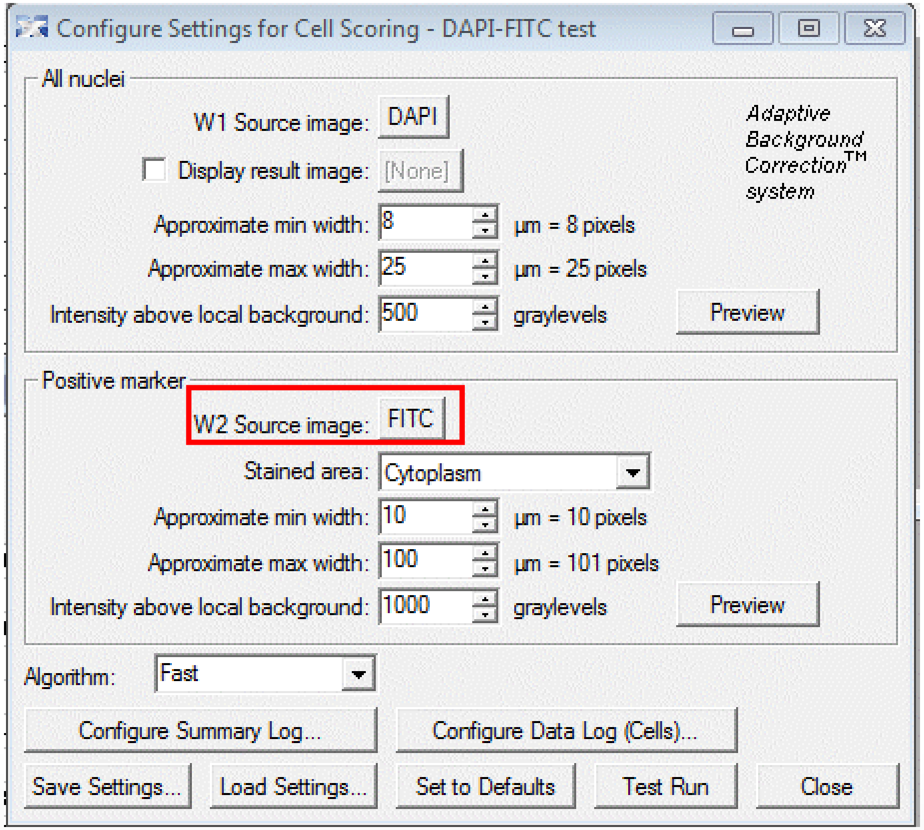
Intensity above local background: 1000 graylevels

Algorithm: Fast

Wavelength 1 (W1)

- Click on **Preview** to test settings for the current wavelength only

Module Settings



Configure Settings for Cell Scoring - DAPI-FITC test

All nuclei

W1 Source image: DAPI

☐ Display result image: [None]

Approximate min width: 8 µm = 8 pixels

Approximate max width: 25 µm = 25 pixels

Intensity above local background: 500 graylevels

Positive marker

W2 Source image: FITC

Stained area: Cytoplasm

Approximate min width: 10 µm = 10 pixels

Approximate max width: 100 µm = 101 pixels

Intensity above local background: 1000 graylevels

Algorithm: Fast

Wavelength 2 (W2)

- Select wavelength for the **marker stain**
- Do not select the "HTS" image (thumbnail montage)

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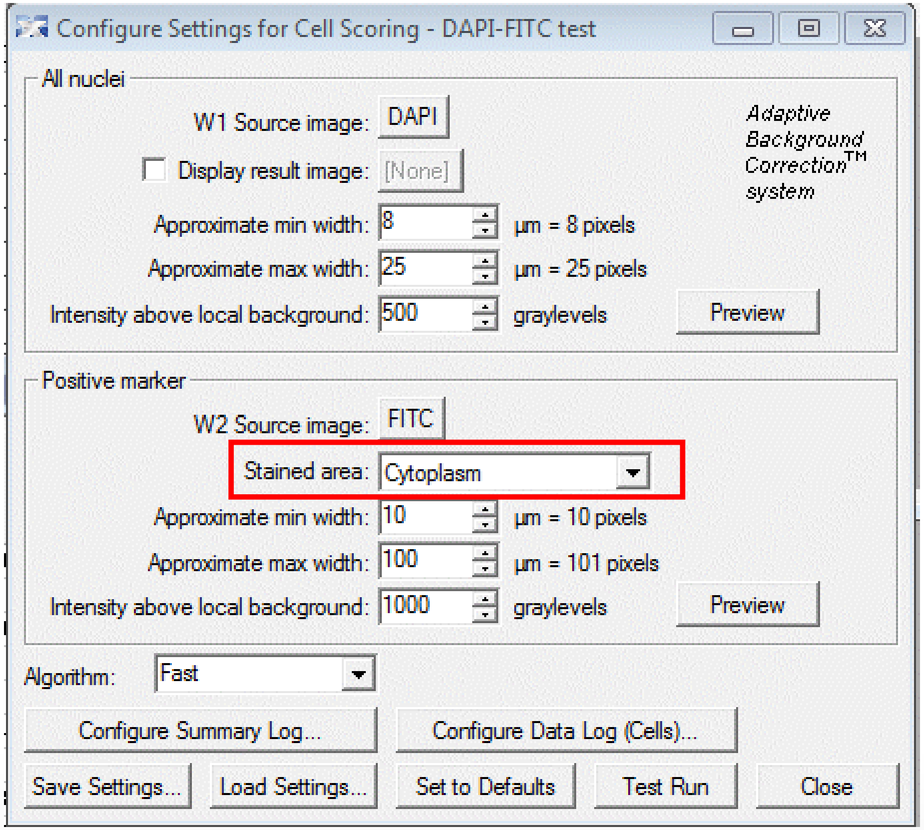
Module Settings



Configure Settings for Cell Scoring - DAPI-FITC test

All nuclei

W1 Source image: DAPI

☐ Display result image: [None]

Approximate min width: 8 µm = 8 pixels

Approximate max width: 25 µm = 25 pixels

Intensity above local background: 500 graylevels

Positive marker

W2 Source image: FITC

Stained area: Cytoplasm

Approximate min width: 10 µm = 10 pixels

Approximate max width: 100 µm = 101 pixels

Intensity above local background: 1000 graylevels

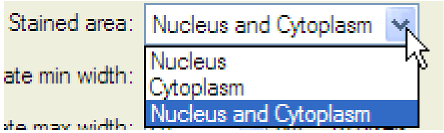
Algorithm: Fast

Wavelength 2 (W2)

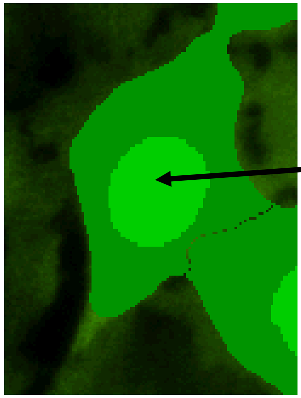
- Define **Stained area**:
  - Nucleus (stain matches W1 area)
  - Cytoplasm (stain is surrounding but not including W1 area)
  - Nucleus and Cytoplasm (stain encompasses W1 and surrounding area)

Stained Area

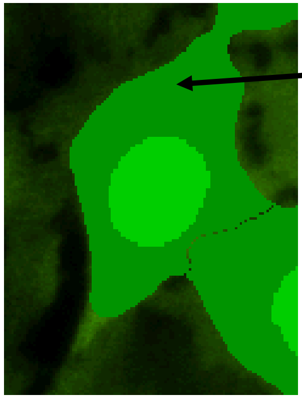
Stained Area (as defined in settings)



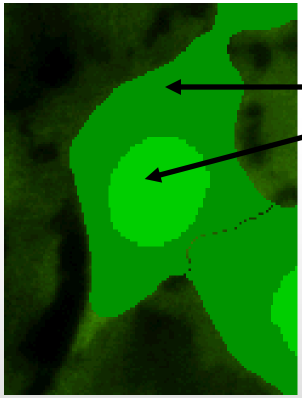
Stained area: Nucleus and Cytoplasm



Nucleus

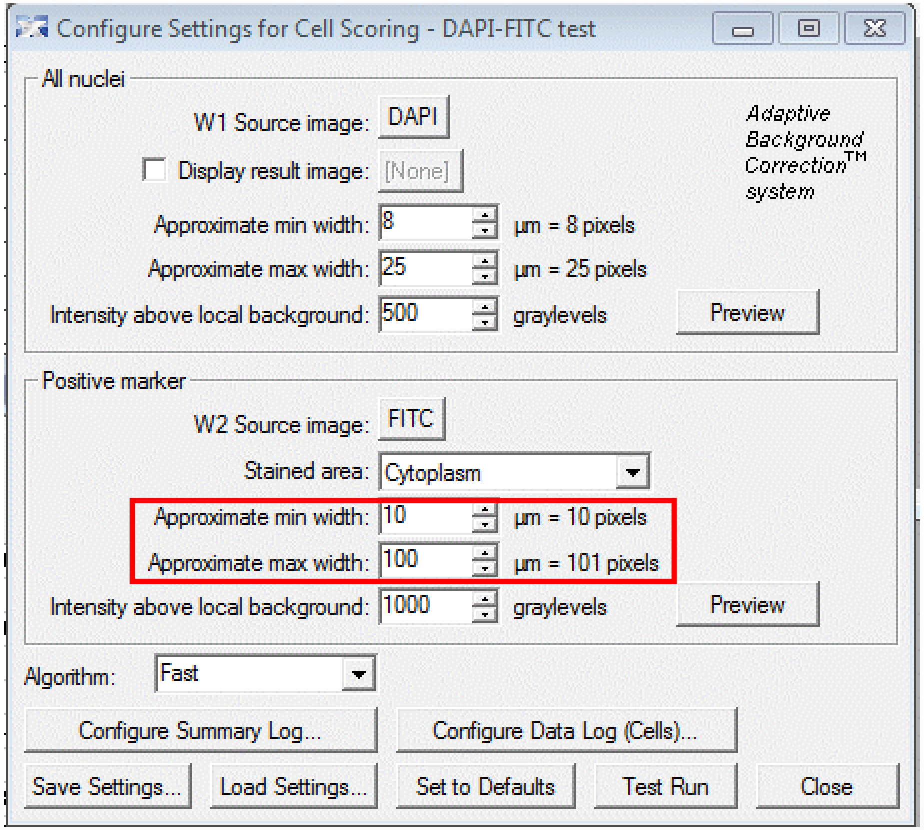


Cytoplasm



Cell (nucleus + cytoplasm)

Module Settings



All nuclei

W1 Source image: DAPI

☐ Display result image: [None]

Approximate min width: 8 µm = 8 pixels

Approximate max width: 25 µm = 25 pixels

Intensity above local background: 500 graylevels

Positive marker

W2 Source image: FITC

Stained area: Cytoplasm

Approximate min width: 10 µm = 10 pixels

Approximate max width: 100 µm = 101 pixels

Intensity above local background: 1000 graylevels

Algorithm: Fast

Wavelength 2 (W2)

- **Set the Approximate min width** and **Approximate max width** for the stain of interest
- For a nuclear stain, use the same settings as W1
- For a cytoplasmic or nuclear + cytoplasmic stain, measure the whole cell the same way you measured the nucleus for W1

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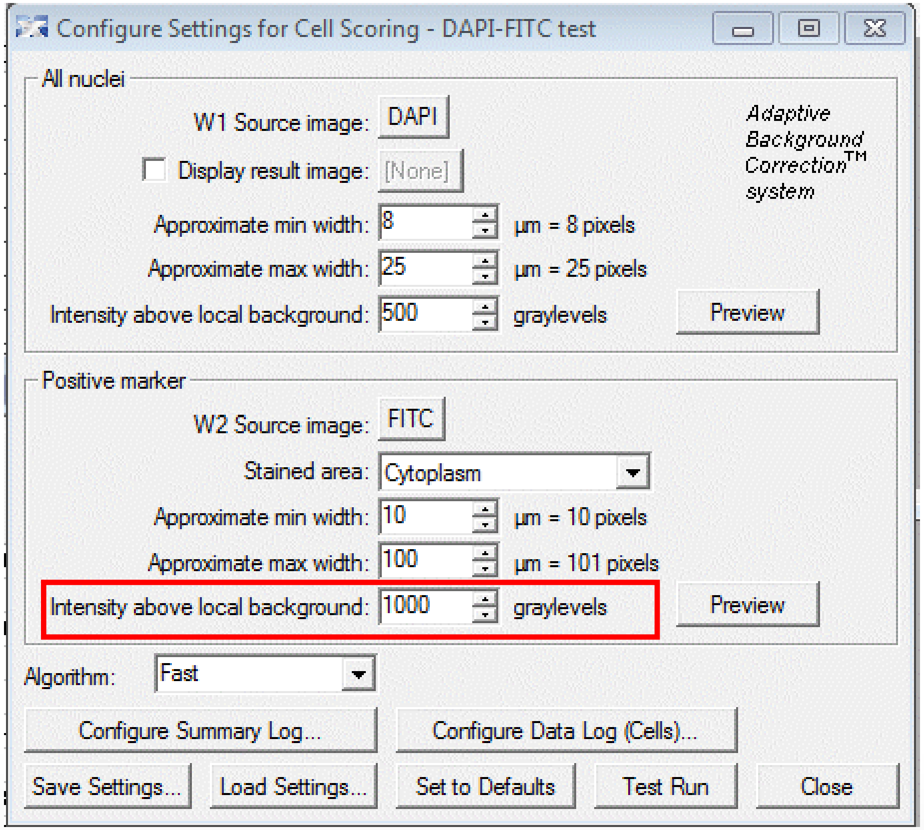
Molecular Devices

Module Settings



Configure Settings for Cell Scoring - DAPI-FITC test

All nuclei

W1 Source image: DAPI

☐ Display result image: [None]

Approximate min width: 8 µm = 8 pixels

Approximate max width: 25 µm = 25 pixels

Intensity above local background: 500 graylevels

Adaptive Background Correction™ system

Positive marker

W2 Source image: FITC

Stained area: Cytoplasm

Approximate min width: 10 µm = 10 pixels

Approximate max width: 100 µm = 101 pixels

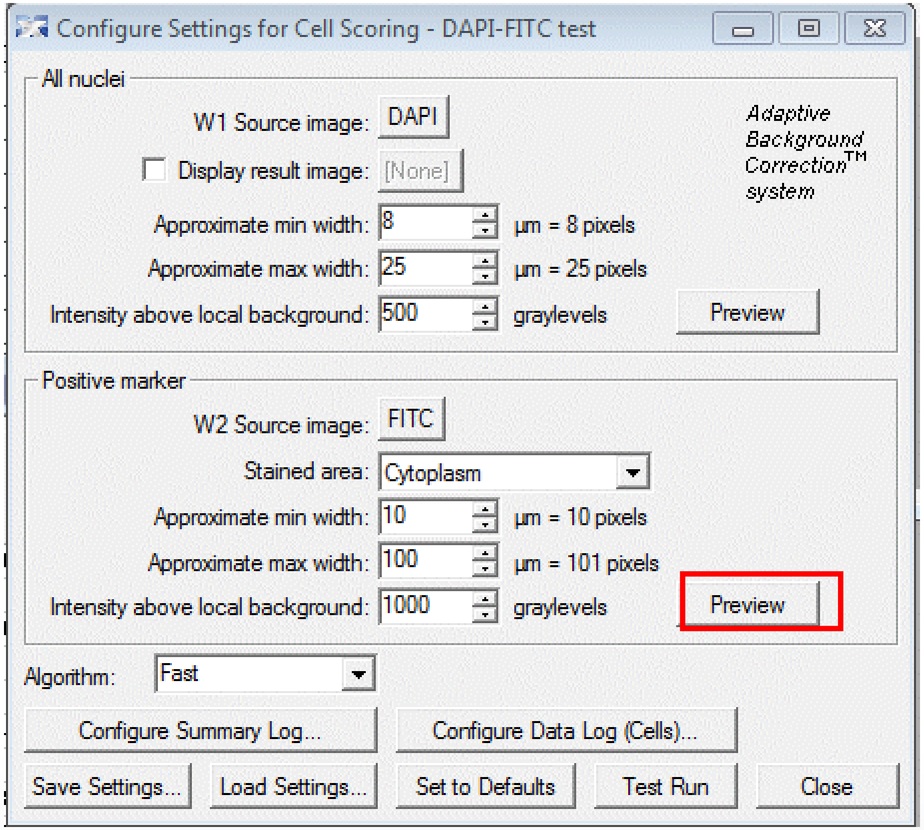
Intensity above local background: 1000 graylevels

Algorithm: Fast

Wavelength 2 (W2)

- **Intensity above local background** for the stain of interest is used to distinguish positive from negative cells
- Measure the stain intensity vs background just as for W1
- Note that the optimal intensity setting for the **Fast** algorithm is about half of the optimal setting for the **Standard** algorithm
- Check both positive and negative control images to help set a suitable cutoff

Module Settings


Configure Settings for Cell Scoring - DAPI-FITC test

All nuclei

W1 Source image: DAPI

☐ Display result image: [None]

Approximate min width: 8 µm = 8 pixels

Approximate max width: 25 µm = 25 pixels

Intensity above local background: 500 graylevels

Positive marker

W2 Source image: FITC

Stained area: Cytoplasm

Approximate min width: 10 µm = 10 pixels

Approximate max width: 100 µm = 101 pixels

Intensity above local background: 1000 graylevels

Algorithm: Fast

Wavelength 2 (W2)

- Click on **Preview** to test settings for the current wavelength only
- Note that this may not accurately segment the cells as this Preview does not use the nuclear staining
- This is most useful for testing the Intensity settings for this wavelength

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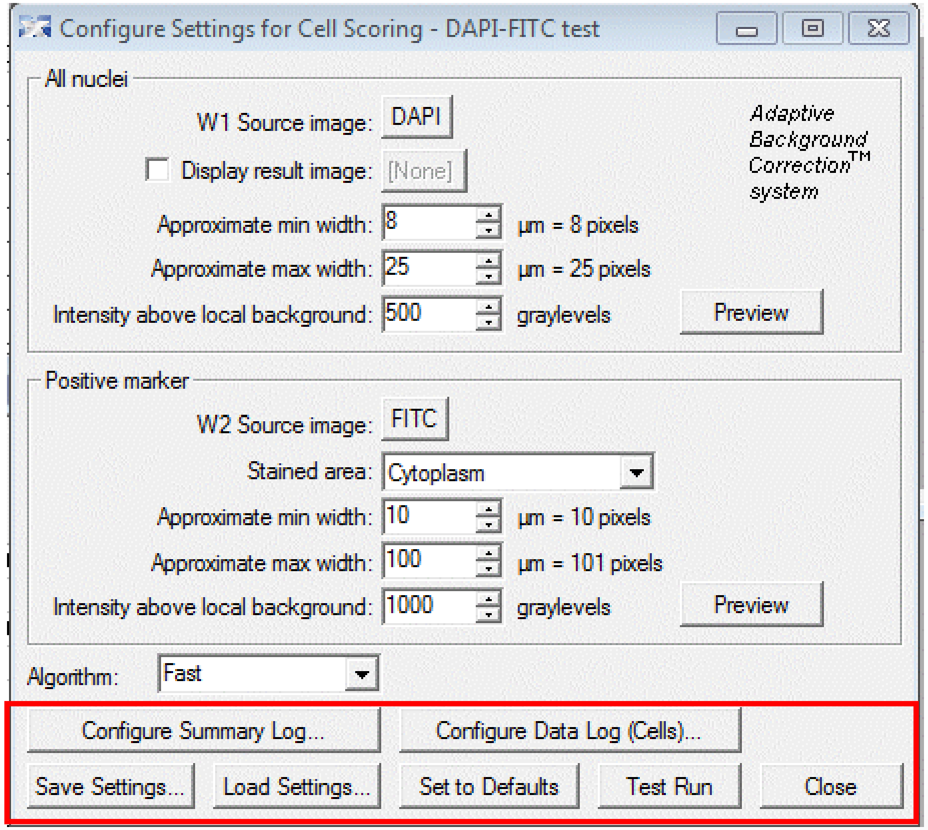
Module Settings – General Settings



Configure Settings for Cell Scoring - DAPI-FITC test

All nuclei

W1 Source image: DAPI

☐ Display result image: [None]

Approximate min width: 8 µm = 8 pixels

Approximate max width: 25 µm = 25 pixels

Intensity above local background: 500 graylevels

Positive marker

W2 Source image: FITC

Stained area: Cytoplasm

Approximate min width: 10 µm = 10 pixels

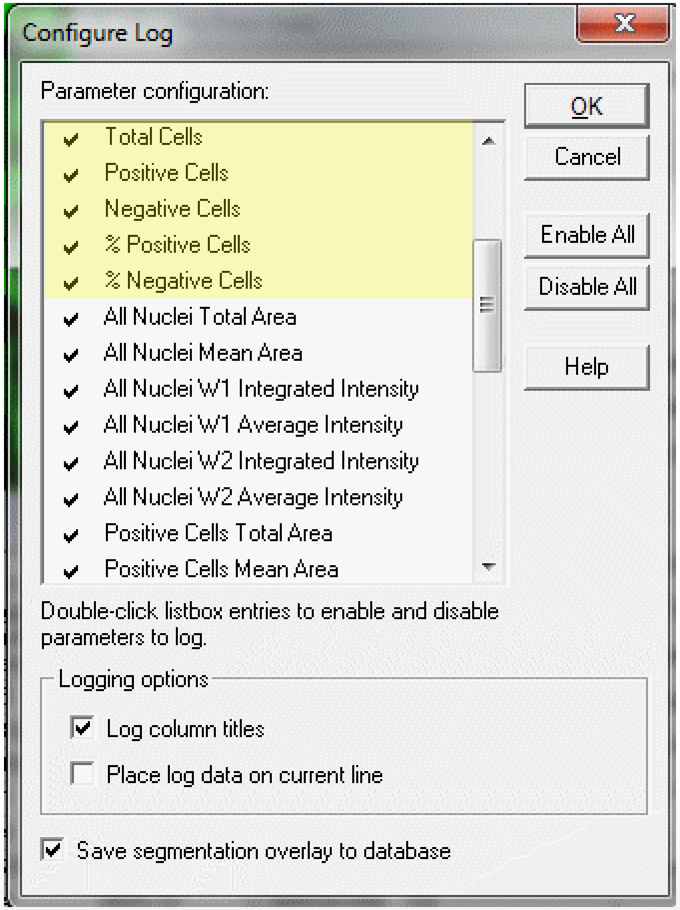
Approximate max width: 100 µm = 101 pixels

Intensity above local background: 1000 graylevels

Algorithm: Fast

- **Configure Summary Log** – select site-by-site measurements
- **Configure Data Log** – select cell-by-cell measurements
- **Save Settings** – save analysis parameters to database
- **Load Settings** – load saved analysis parameters
- **Set to Defaults** – restore default analysis parameters
- **Test Run** – test all settings together and display cell-by-cell results for this site

Summary Data (site-by-site measurements)



Parameter configuration:

- ✓ Total Cells
- ✓ Positive Cells
- ✓ Negative Cells
- ✓ % Positive Cells
- ✓ % Negative Cells
- ✓ All Nuclei Total Area
- ✓ All Nuclei Mean Area
- ✓ All Nuclei W1 Integrated Intensity
- ✓ All Nuclei W1 Average Intensity
- ✓ All Nuclei W2 Integrated Intensity
- ✓ All Nuclei W2 Average Intensity
- ✓ Positive Cells Total Area
- ✓ Positive Cells Mean Area

Double-click listbox entries to enable and disable parameters to log.

Logging options

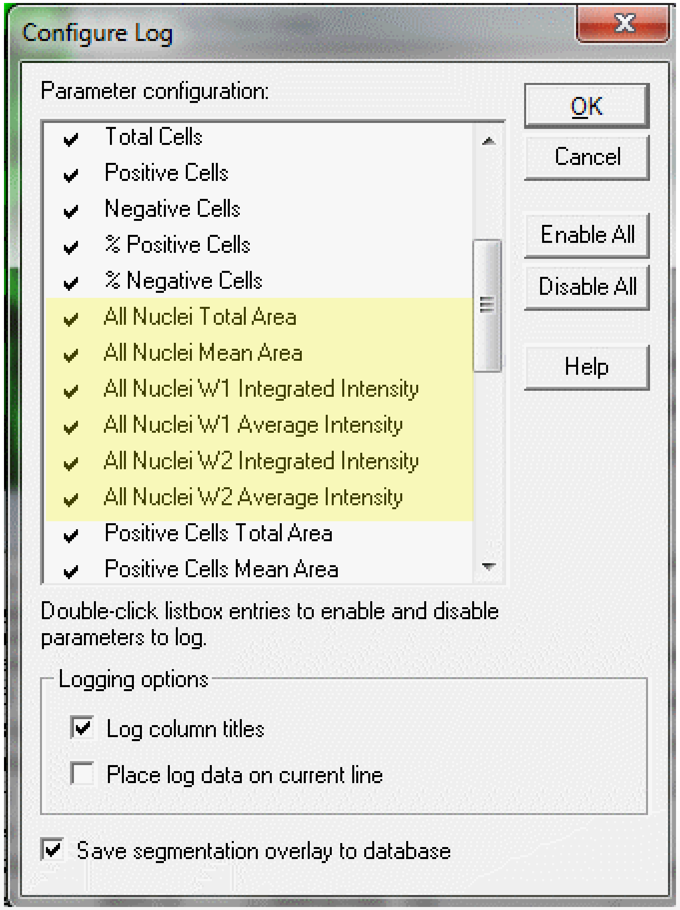
☑ Log column titles

☐ Place log data on current line

☑ Save segmentation overlay to database

- **Total cells:** Total number of nuclei (cell count)
- **Positive Cells:** The total number of cells positive for staining with wavelength 2 as defined in the settings
- **Negative Cells:** The total number of cells negative for staining with wavelength 2 as defined in the settings
- **% Positive Cells:** The number of positive W2 cells divided by the total number of cells, times 100
- **% Negative Cells:** The number of negative W2 cells divided by the total number of cells, times 100

Summary Data (site-by-site measurements)



Configure Log

Parameter configuration:

- ✓ Total Cells
- ✓ Positive Cells
- ✓ Negative Cells
- ✓ % Positive Cells
- ✓ % Negative Cells
- ✓ All Nuclei Total Area
- ✓ All Nuclei Mean Area
- ✓ All Nuclei W1 Integrated Intensity
- ✓ All Nuclei W1 Average Intensity
- ✓ All Nuclei W2 Integrated Intensity
- ✓ All Nuclei W2 Average Intensity
- ✓ Positive Cells Total Area
- ✓ Positive Cells Mean Area

Double-click listbox entries to enable and disable parameters to log.

Logging options

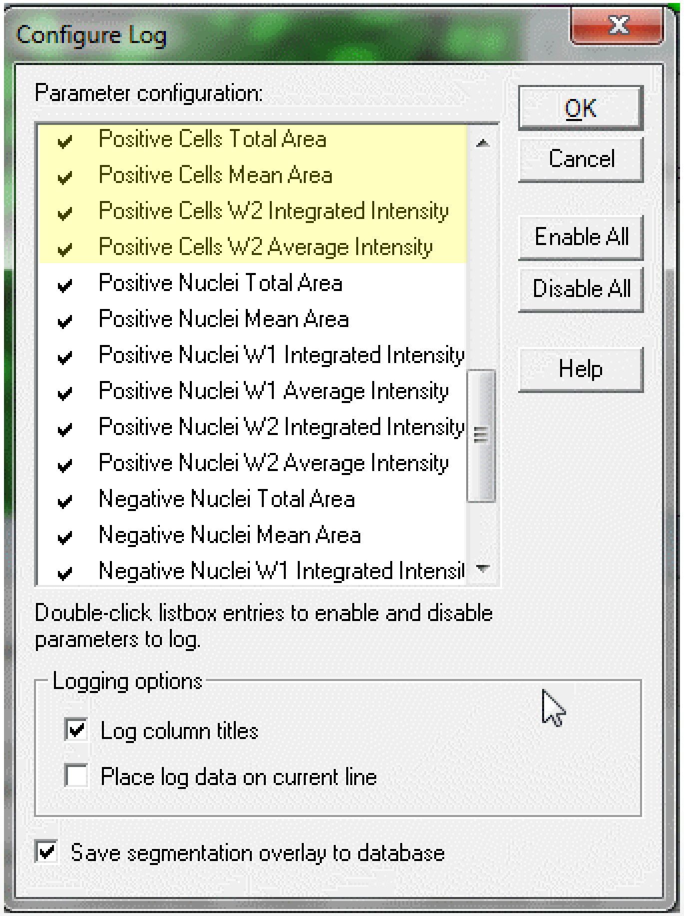
☑ Log column titles

☐ Place log data on current line

☑ Save segmentation overlay to database

- **All Nuclei Total Area:** The total area of nucleus for all cells found in the image (in um^2)
- **All Nuclei Mean Area:** The average area of nucleus for all cells found in the image (in um^2)
- **All Nuclei W1 Integrated Intensity:** The total pixel intensity of the nuclear stain over the nuclear area
- **All Nuclei W1 Average Intensity:** The average pixel intensity of the nuclear stain over all the nuclear areas in the image
- **All Nuclei W2 Integrated Intensity:** The total pixel intensity of the marker stain over the nuclear area
- **All Nuclei W2 Average Intensity:** The average pixel intensity of the marker stain over all the nuclear areas in the image

Summary Data (site-by-site measurements)



Configure Log

Parameter configuration:

- ✓ Positive Cells Total Area
- ✓ Positive Cells Mean Area
- ✓ Positive Cells W2 Integrated Intensity
- ✓ Positive Cells W2 Average Intensity
- ✓ Positive Nuclei Total Area
- ✓ Positive Nuclei Mean Area
- ✓ Positive Nuclei W1 Integrated Intensity
- ✓ Positive Nuclei W1 Average Intensity
- ✓ Positive Nuclei W2 Integrated Intensity
- ✓ Positive Nuclei W2 Average Intensity
- ✓ Negative Nuclei Total Area
- ✓ Negative Nuclei Mean Area
- ✓ Negative Nuclei W1 Integrated Intensit

Double-click listbox entries to enable and disable parameters to log.

Logging options

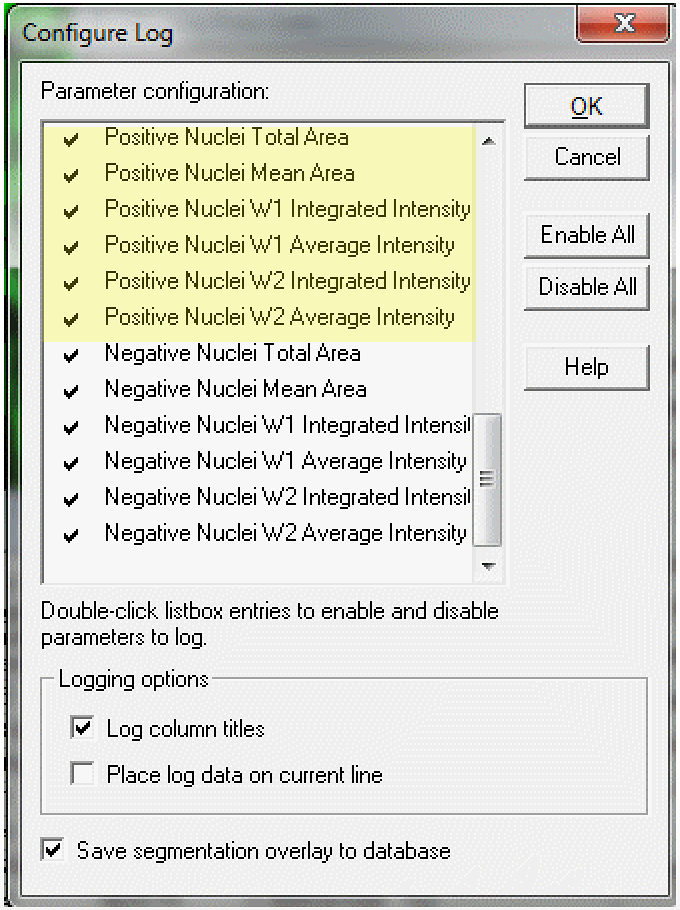
☑ Log column titles

☐ Place log data on current line

☑ Save segmentation overlay to database

- **Positive Cells Total Area:** The total cell area for all positive cells found in the image (in um^2)
- **Positive Cells Mean Area:** The average cell area for all positive cells found in the image (in um^2)
- **Positive Cells W2 Integrated Intensity:** The total pixel intensity of the marker stain over the cell area
- **Positive Cells W2 Average Intensity:** The average pixel intensity of the marker stain over all the cell areas in the image

Summary Data (site-by-site measurements)



Parameter configuration:

- ✓ Positive Nuclei Total Area
- ✓ Positive Nuclei Mean Area
- ✓ Positive Nuclei W1 Integrated Intensity
- ✓ Positive Nuclei W1 Average Intensity
- ✓ Positive Nuclei W2 Integrated Intensity
- ✓ Positive Nuclei W2 Average Intensity
- ✓ Negative Nuclei Total Area
- ✓ Negative Nuclei Mean Area
- ✓ Negative Nuclei W1 Integrated Intensi
- ✓ Negative Nuclei W1 Average Intensity
- ✓ Negative Nuclei W2 Integrated Intensi
- ✓ Negative Nuclei W2 Average Intensity

Double-click listbox entries to enable and disable parameters to log.

Logging options

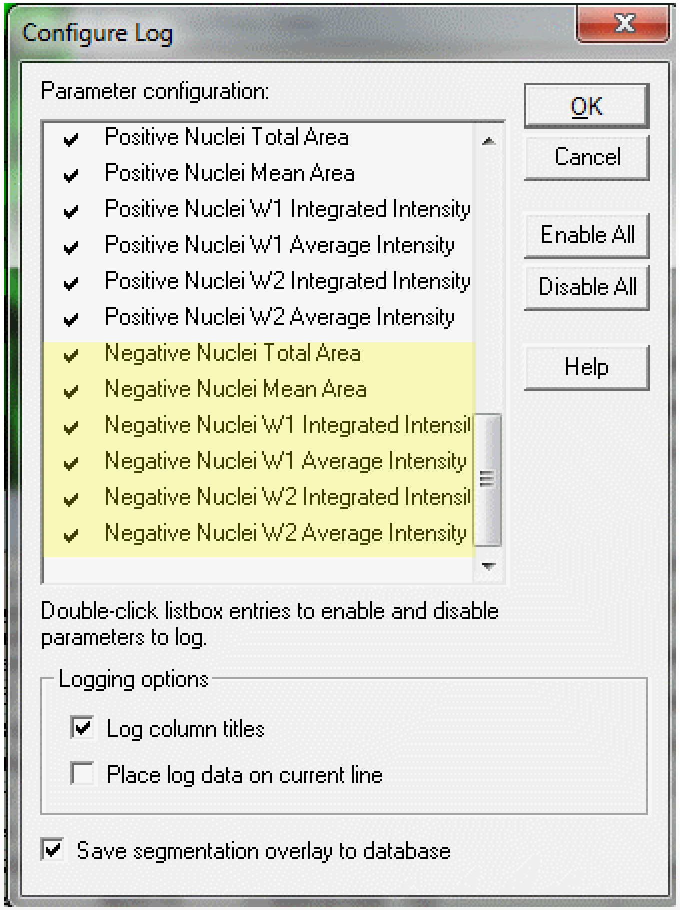
☑ Log column titles

☐ Place log data on current line

☑ Save segmentation overlay to database

- **Positive Nuclei Total Area:** The total area of nucleus for positive cells found in the image (in um^2)
- **Positive Nuclei Mean Area:** The average area of nucleus for positive cells found in the image (in um^2)
- **Positive Nuclei W1 Integrated Intensity:** The total pixel intensity of the nuclear stain over the nuclear area in positive cells
- **Positive Nuclei W1 Average Intensity:** The average pixel intensity of the nuclear stain over all the nuclear areas in positive cells
- **Positive Nuclei W2 Integrated Intensity:** The total pixel intensity of the marker stain over the nuclear area in positive cells
- **Positive Nuclei W2 Average Intensity:** The average pixel intensity of the marker stain over all the nuclear areas in positive cells

Summary Data (site-by-site measurements)



- ✓ Positive Nuclei Total Area
- ✓ Positive Nuclei Mean Area
- ✓ Positive Nuclei W1 Integrated Intensity
- ✓ Positive Nuclei W1 Average Intensity
- ✓ Positive Nuclei W2 Integrated Intensity
- ✓ Positive Nuclei W2 Average Intensity
- ✓ Negative Nuclei Total Area
- ✓ Negative Nuclei Mean Area
- ✓ Negative Nuclei W1 Integrated Intensil
- ✓ Negative Nuclei W1 Average Intensity
- ✓ Negative Nuclei W2 Integrated Intensil
- ✓ Negative Nuclei W2 Average Intensity

Double-click listbox entries to enable and disable parameters to log.

Logging options

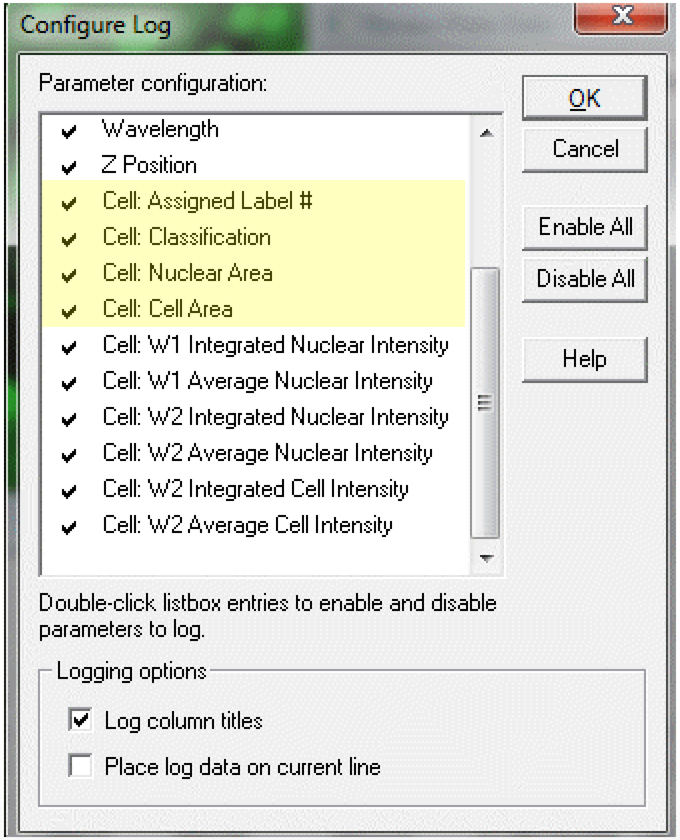
☑ Log column titles

☐ Place log data on current line

☑ Save segmentation overlay to database

- **Negative Nuclei Total Area:** The total area of nucleus for negative cells found in the image (in um^2)
- **Negative Nuclei Mean Area:** The average area of nucleus for negative cells found in the image (in um^2)
- **Negative Nuclei W1 Integrated Intensity:** The total pixel intensity of the nuclear stain over the nuclear area in negative cells
- **Negative Nuclei W1 Average Intensity:** The average pixel intensity of the nuclear stain over all the nuclear areas in negative cells
- **Negative Nuclei W2 Integrated Intensity:** The total pixel intensity of the marker stain over the nuclear area in negative cells
- **Negative Nuclei W2 Average Intensity:** The average pixel intensity of the marker stain over all the nuclear areas in negative cells

Cell Data (cell-by-cell measurements)



Parameter configuration:

- ✓ Wavelength
- ✓ Z Position
- ✓ Cell: Assigned Label #
- ✓ Cell: Classification
- ✓ Cell: Nuclear Area
- ✓ Cell: Cell Area
- ✓ Cell: W1 Integrated Nuclear Intensity
- ✓ Cell: W1 Average Nuclear Intensity
- ✓ Cell: W2 Integrated Nuclear Intensity
- ✓ Cell: W2 Average Nuclear Intensity
- ✓ Cell: W2 Integrated Cell Intensity
- ✓ Cell: W2 Average Cell Intensity

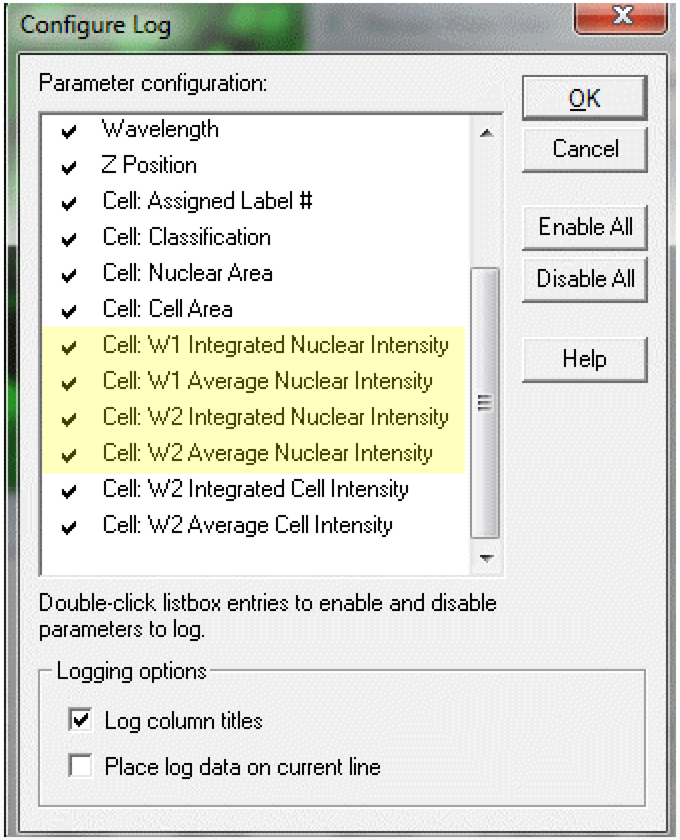
Double-click listbox entries to enable and disable parameters to log.

Logging options

- ☑ Log column titles
- ☐ Place log data on current line

- **Cell: Assigned Label #** – Cell label number (1 through total cell number)
- **Cell: Classification** – “Positive” or “Negative”
- **Cell: Nuclear Area** – Total square microns of the nucleus as defined by the W1 stain
- **Cell: Cell Area** – Total square microns of the cell (nucleus + cytoplasm if cytoplasm selected in the settings)

Cell Data (cell-by-cell measurements)



Parameter configuration:

- ✓ Wavelength
- ✓ Z Position
- ✓ Cell: Assigned Label #
- ✓ Cell: Classification
- ✓ Cell: Nuclear Area
- ✓ Cell: Cell Area
- ✓ Cell: W1 Integrated Nuclear Intensity
- ✓ Cell: W1 Average Nuclear Intensity
- ✓ Cell: W2 Integrated Nuclear Intensity
- ✓ Cell: W2 Average Nuclear Intensity
- ✓ Cell: W2 Integrated Cell Intensity
- ✓ Cell: W2 Average Cell Intensity

Double-click listbox entries to enable and disable parameters to log.

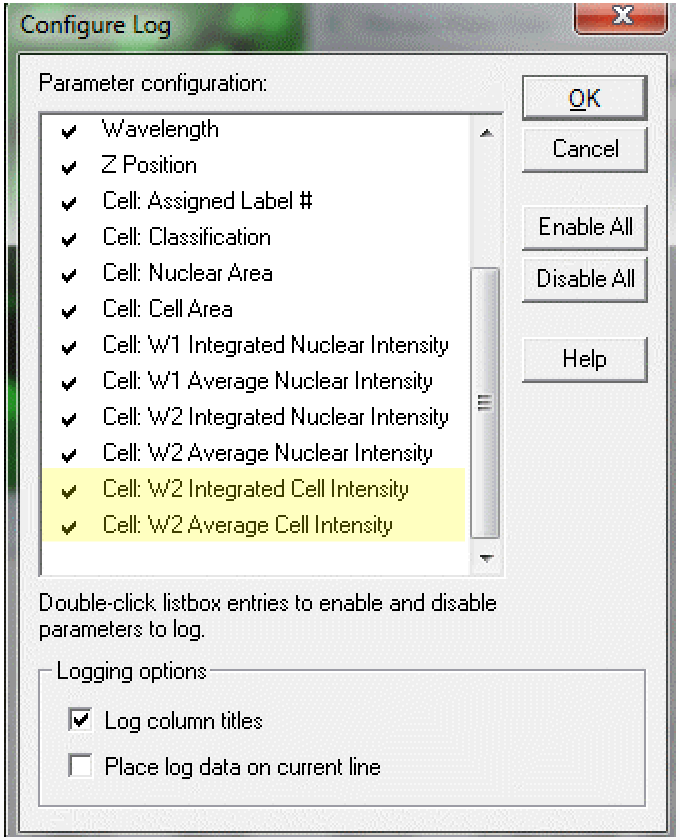
Logging options

☑ Log column titles

☐ Place log data on current line

- **Cell: W1 Integrated Nuclear Intensity** – Total pixel intensity of the nuclear stain in the nucleus
- **Cell: W1 Average Nuclear Intensity** – Average pixel intensity of the nuclear stain in the nucleus
- **Cell: W2 Integrated Nuclear Intensity** – Total pixel intensity of the W2 stain in the nucleus
- **Cell: W2 Average Nuclear Intensity** – Average pixel intensity of the W2 stain in the nucleus

Cell Data (cell-by-cell measurements)



Configure Log

Parameter configuration:

- ✓ Wavelength
- ✓ Z Position
- ✓ Cell: Assigned Label #
- ✓ Cell: Classification
- ✓ Cell: Nuclear Area
- ✓ Cell: Cell Area
- ✓ Cell: W1 Integrated Nuclear Intensity
- ✓ Cell: W1 Average Nuclear Intensity
- ✓ Cell: W2 Integrated Nuclear Intensity
- ✓ Cell: W2 Average Nuclear Intensity
- ✓ Cell: W2 Integrated Cell Intensity
- ✓ Cell: W2 Average Cell Intensity

Double-click listbox entries to enable and disable parameters to log.

Logging options

☑ Log column titles

☐ Place log data on current line

- **Cell: W2 Integrated Cell Intensity** – Total pixel intensity of the W2 stain in the cell (nucleus + cytoplasm if cytoplasm selected in the settings)

- **Cell: W2 Average Cell Intensity** – Average pixel intensity of the W2 stain in the cell (nucleus + cytoplasm if cytoplasm selected in the settings)

Molecular Devices

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www.moleculardevices.com