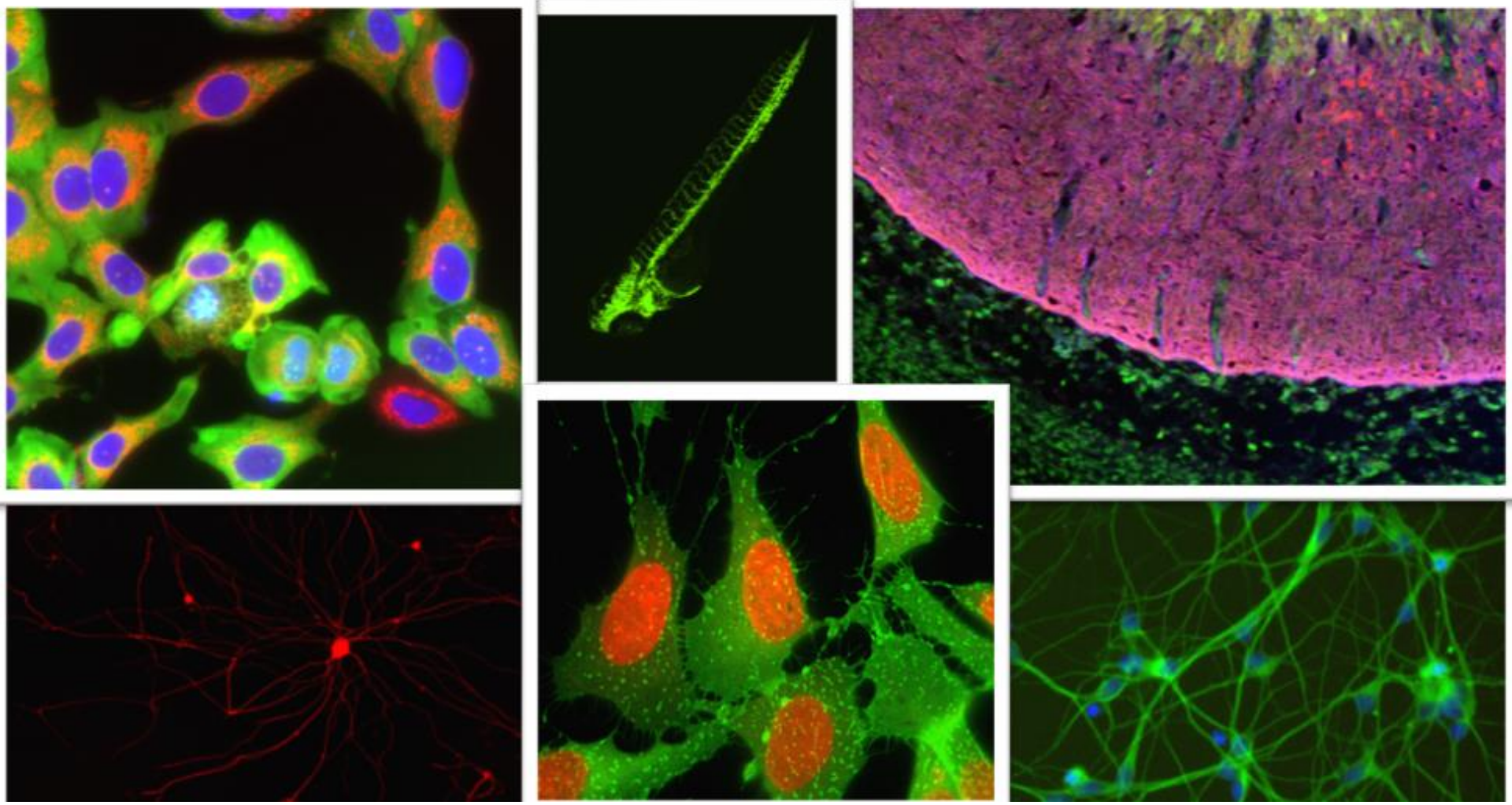

Molecular Devices

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MetaXpress® Software:
Mitotic Index Module

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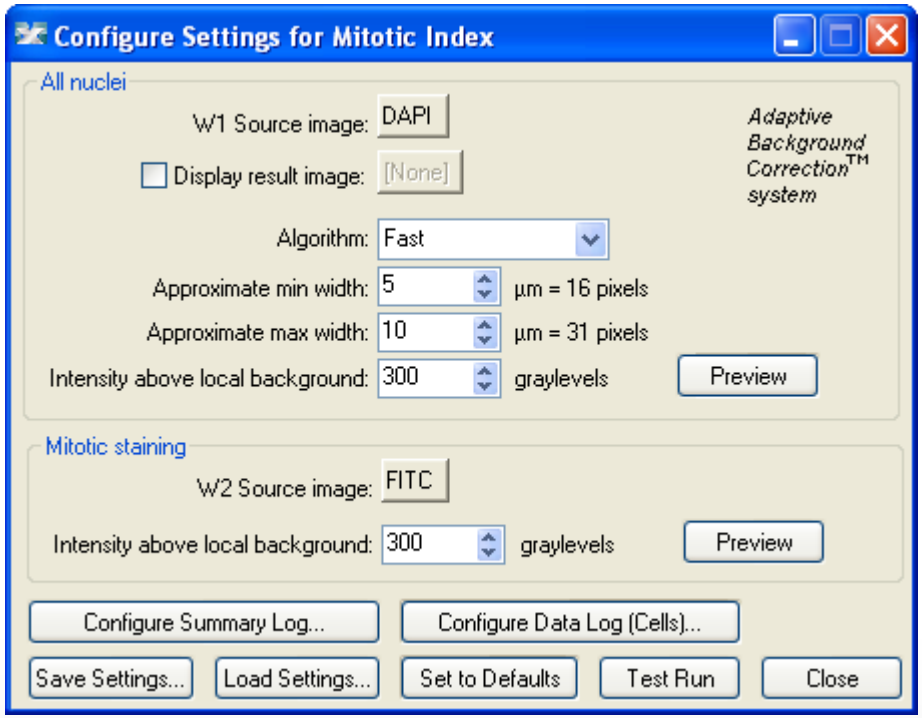
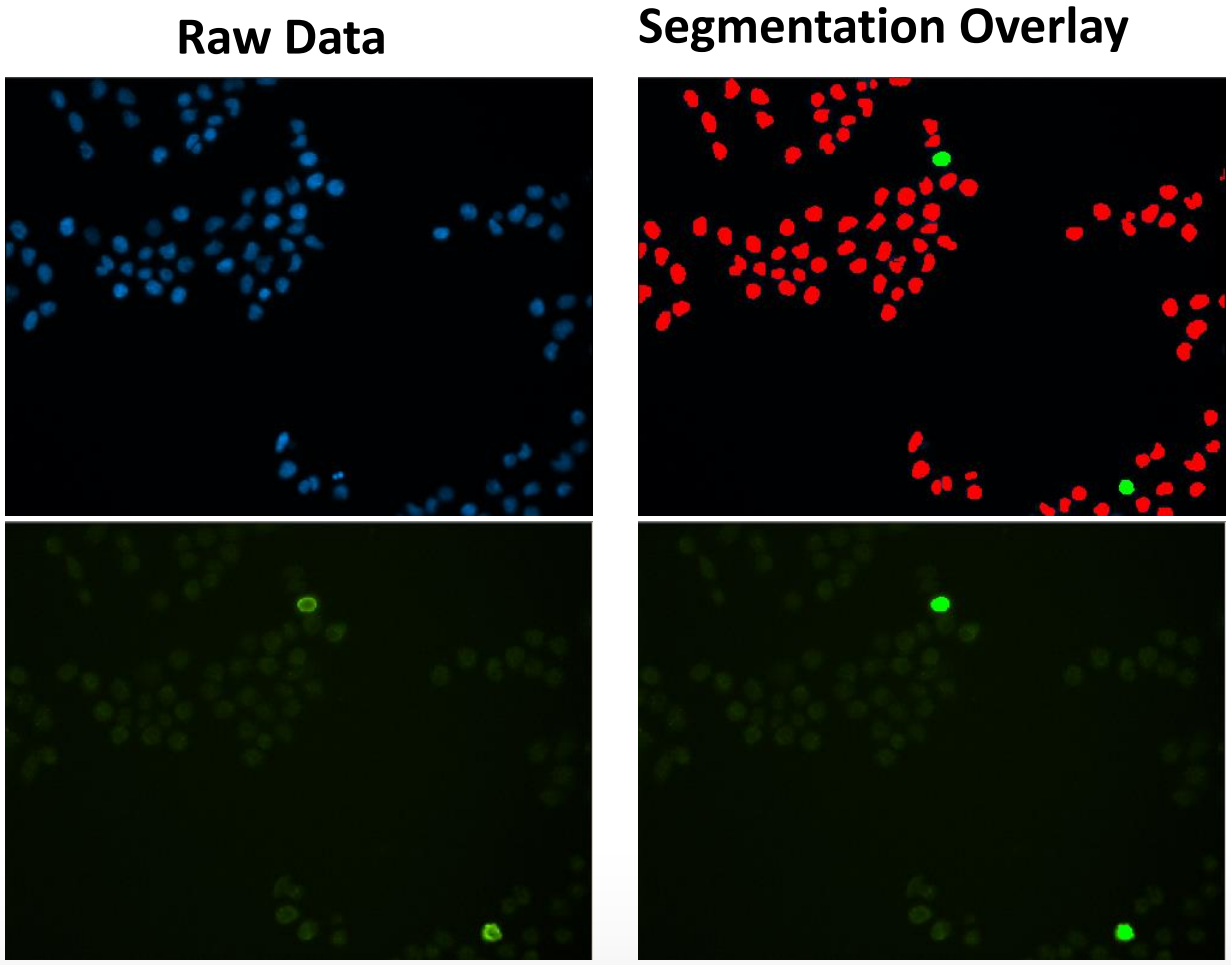
Mitotic Index Module Overview



- The Mitotic Index module can be used to analyze cellular images to differentiate between Mitotic and Interphase cells in a normal cell cycle.
- This module requires a nuclear wavelength and mitotic-specific stain wavelength.
- A typical mitotic-specific stain used for this module is Histone 3 S10 phosphorylation. DNA stain labels all cells and the mitotic cells are labeled only with the mitotic-specific stain.

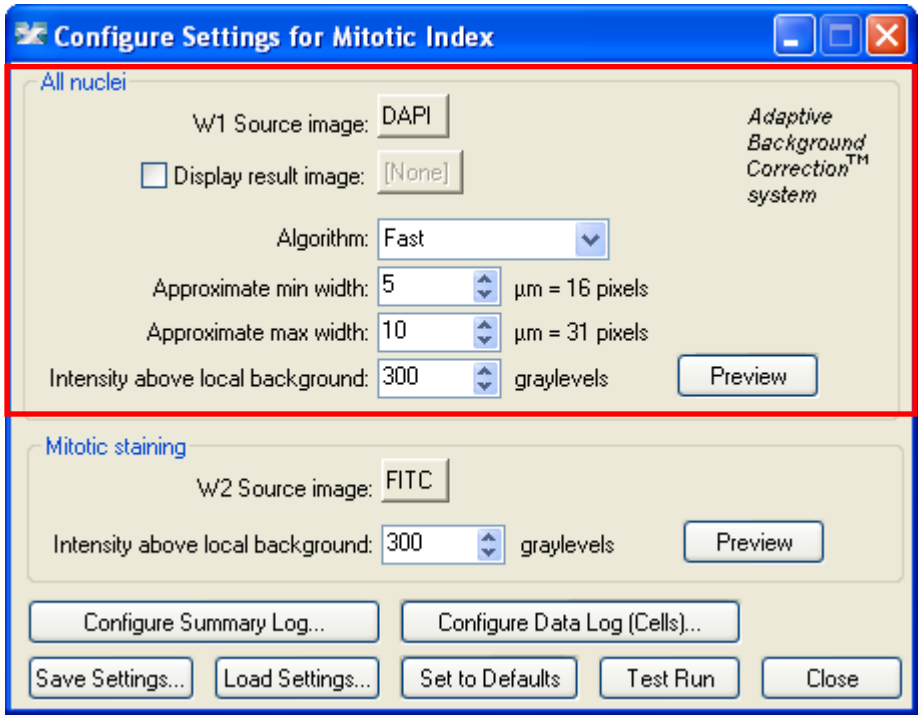
Mitotic Index – Example images

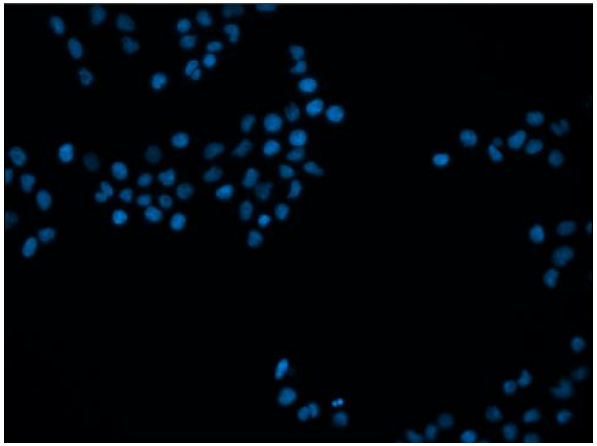


Interphase nuclei are shown with a red overlay and mitotic nuclei with a green overlay.

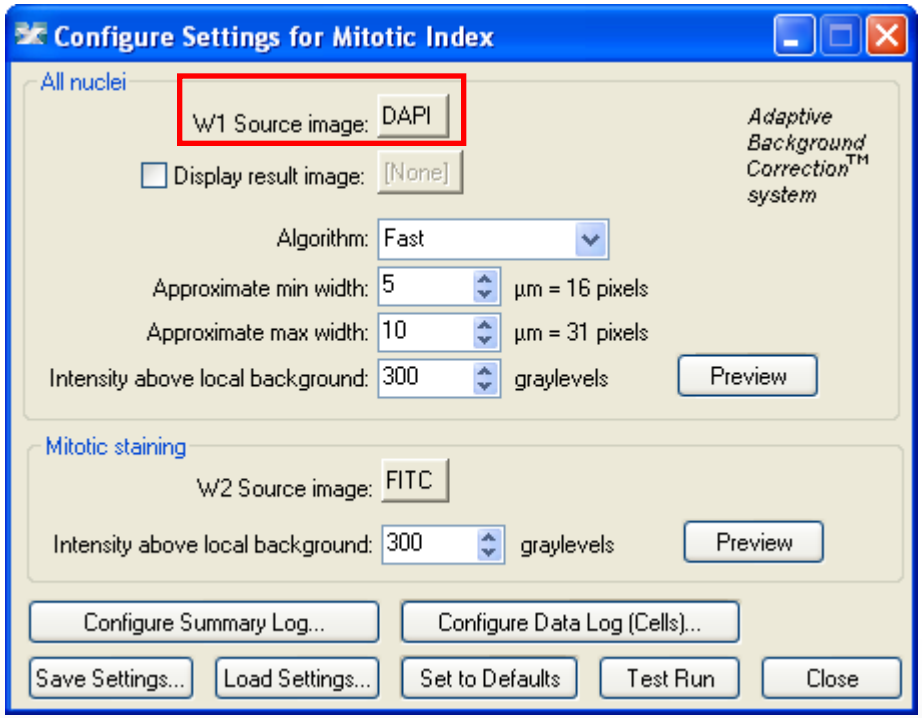
Module Settings – Nuclei classification

- **Nuclei classification**



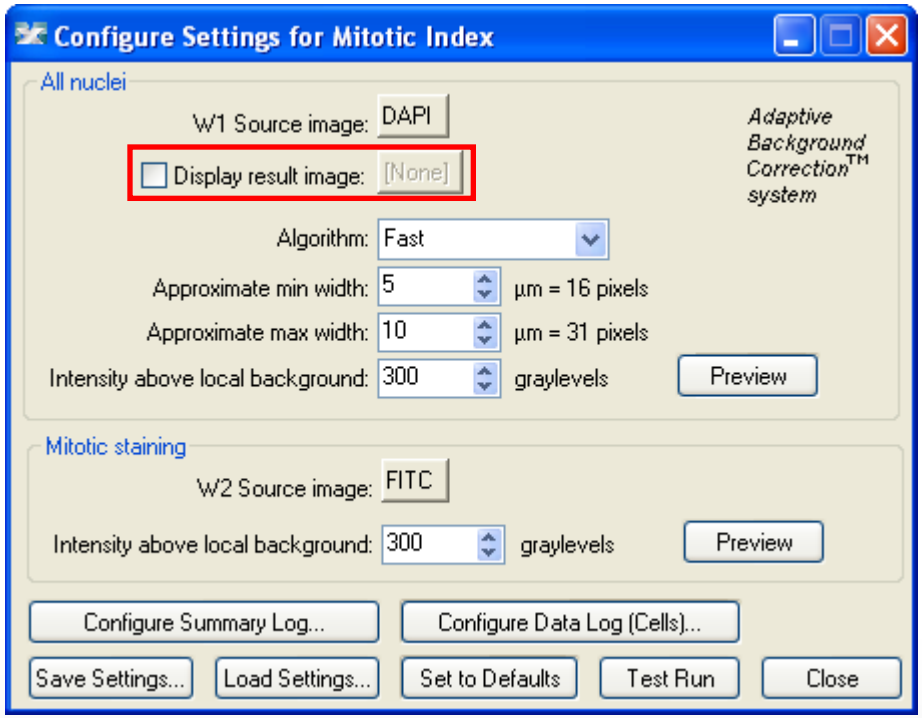


2. Module Settings – DNA content



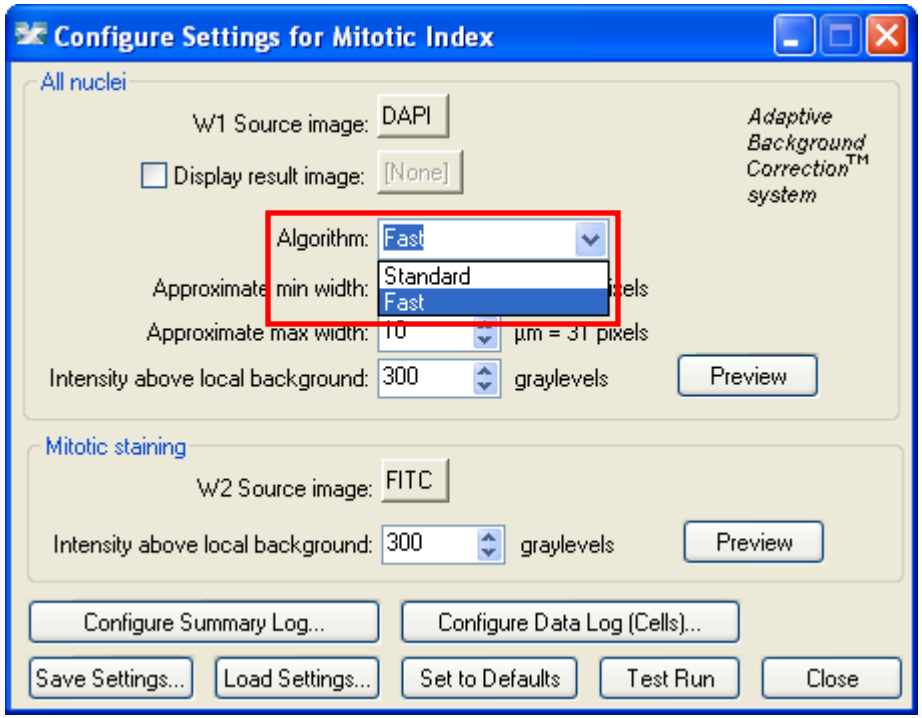
- Select the wavelength for the **DNA content** (nuclear stain)

1. Module Settings – result image



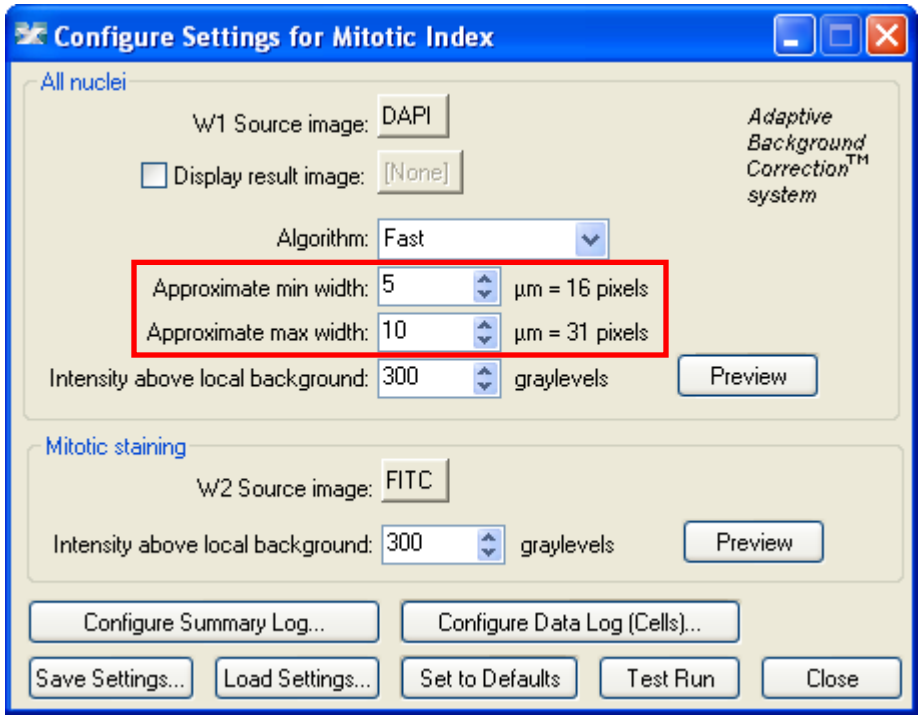
- Leave "**Display result image**" deselected (this is generally only used when journaling)

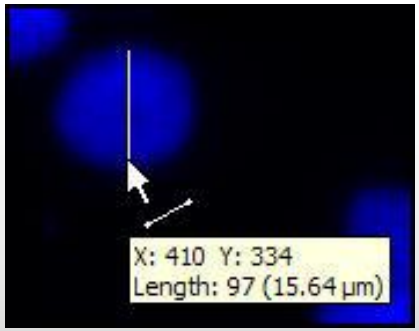
Module Settings



- **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.

3. Module Settings – width settings

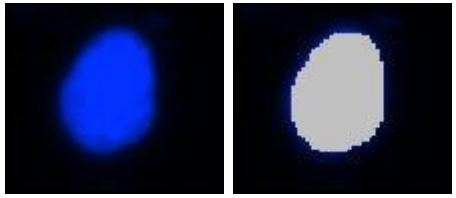




- **Set the Approximate min width** and **Approximate max width** for the range of nuclei that you want to detect
- The width is the short axis of a nucleus (in um).
- The region tools can be used to measure widths
- Much smaller cells will be ignored
- Much larger cells will be split

3. Module Settings – width settings

Effects of varying width settings



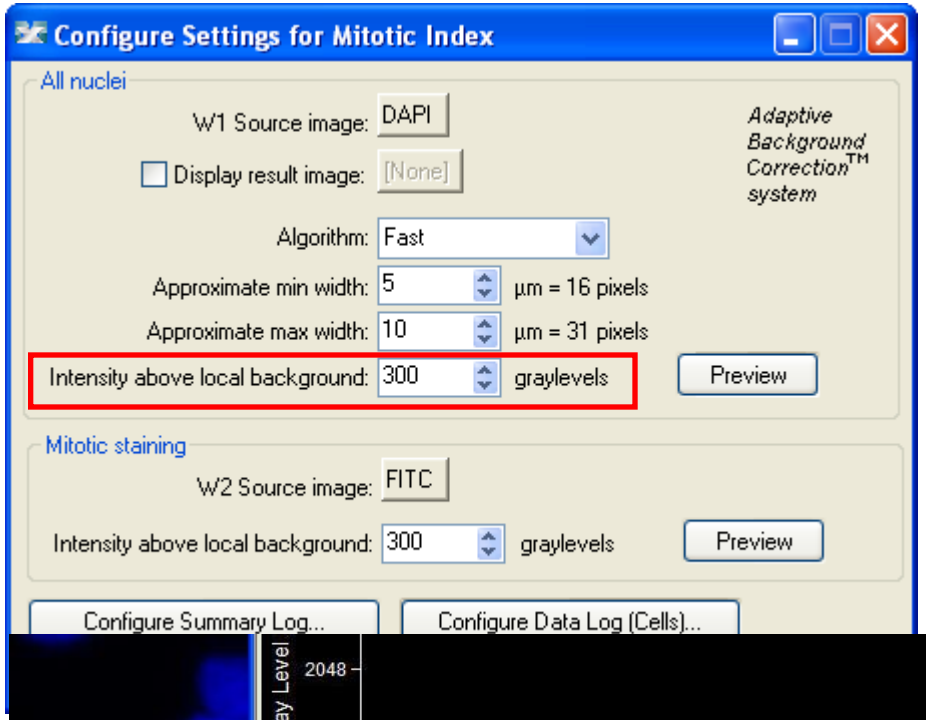
Min width too small: splits nuclei

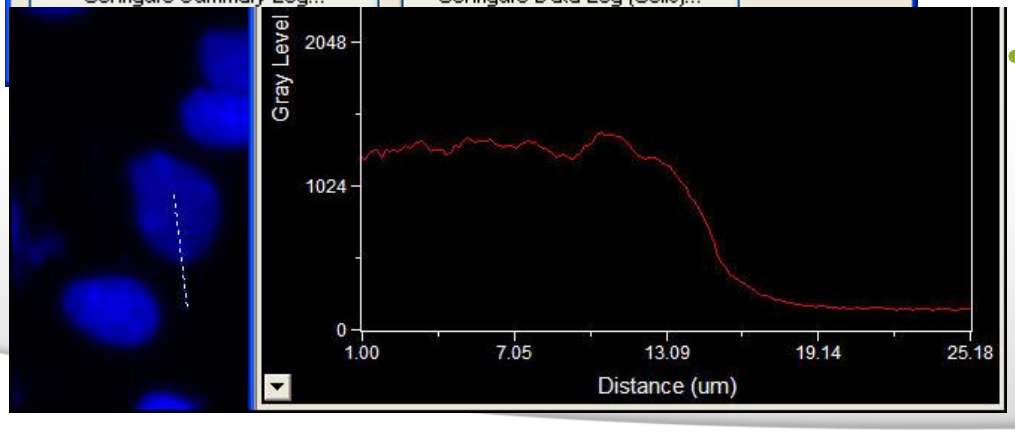
Min width too large: omits smaller nuclei

Max width too small: may shrink nuclear boundaries

Max width too large: may slightly enlarge nuclear boundaries

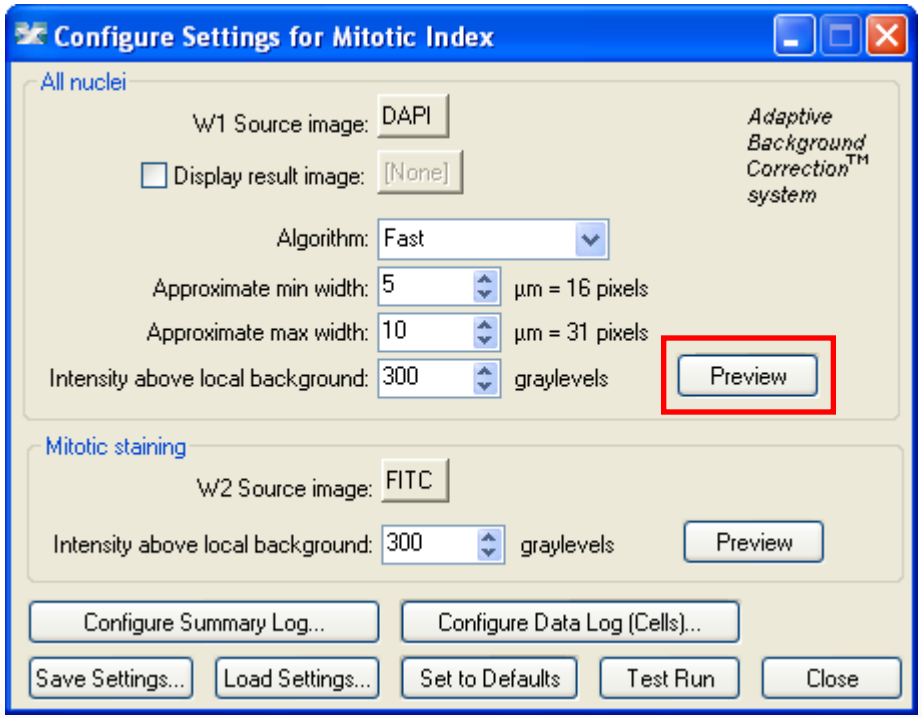
Module Settings – Intensity above background



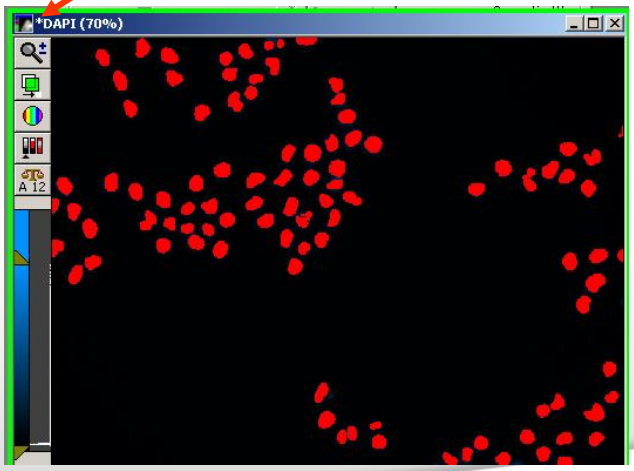


- The **intensity above local background** is used for finding the nuclei
- This value is a minimum and should be set slightly lower than the difference in intensity between a dim cell and its local background. For FAST algorithm, set this value to about half (or less) of the difference in intensity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

4. Module Settings – Nuclei segmentation

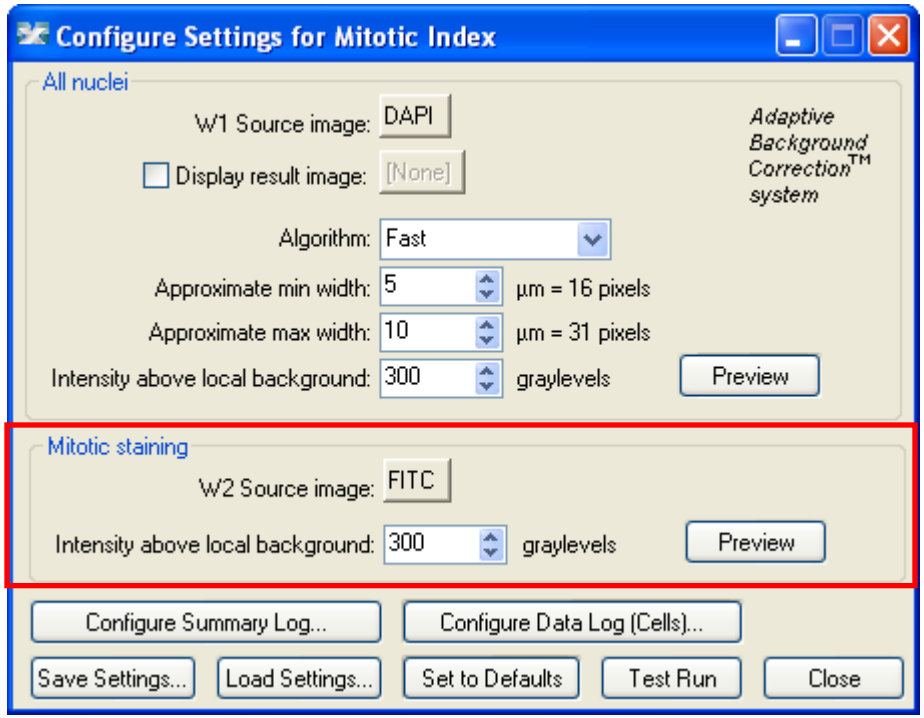


- Click on **Preview** to test the segmentation settings for the nuclei
- The Preview image will show a red overlay over the segmentated nuclei. The overlay can be toggled on and off.

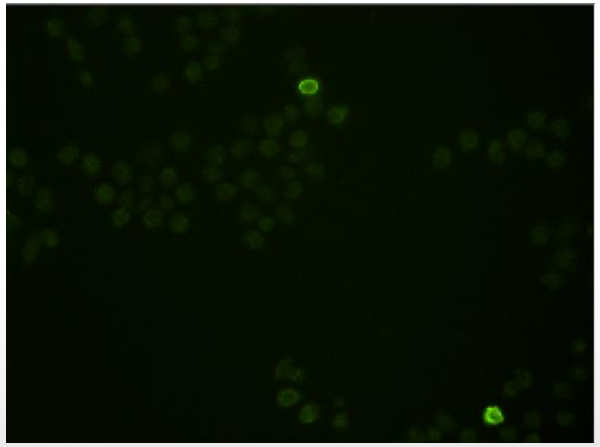


5. Module Settings – Mitotic Cell classification

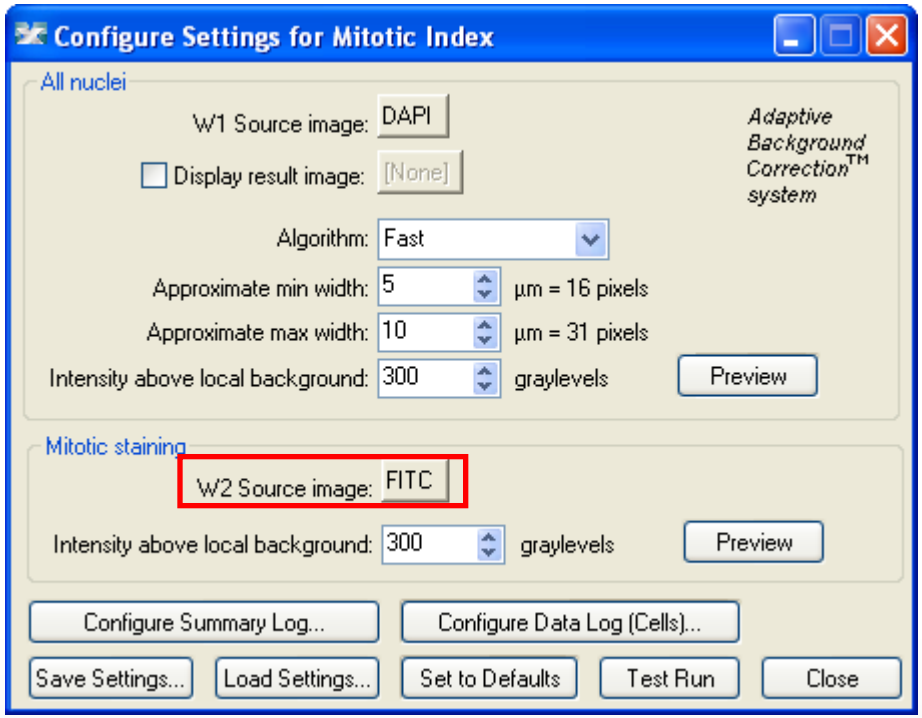
- **Mitotic classification**



- The average intensity (brightness) of the mitotic-specific marker (e.g. phospho-histone H3) is used to identify mitotic cells.

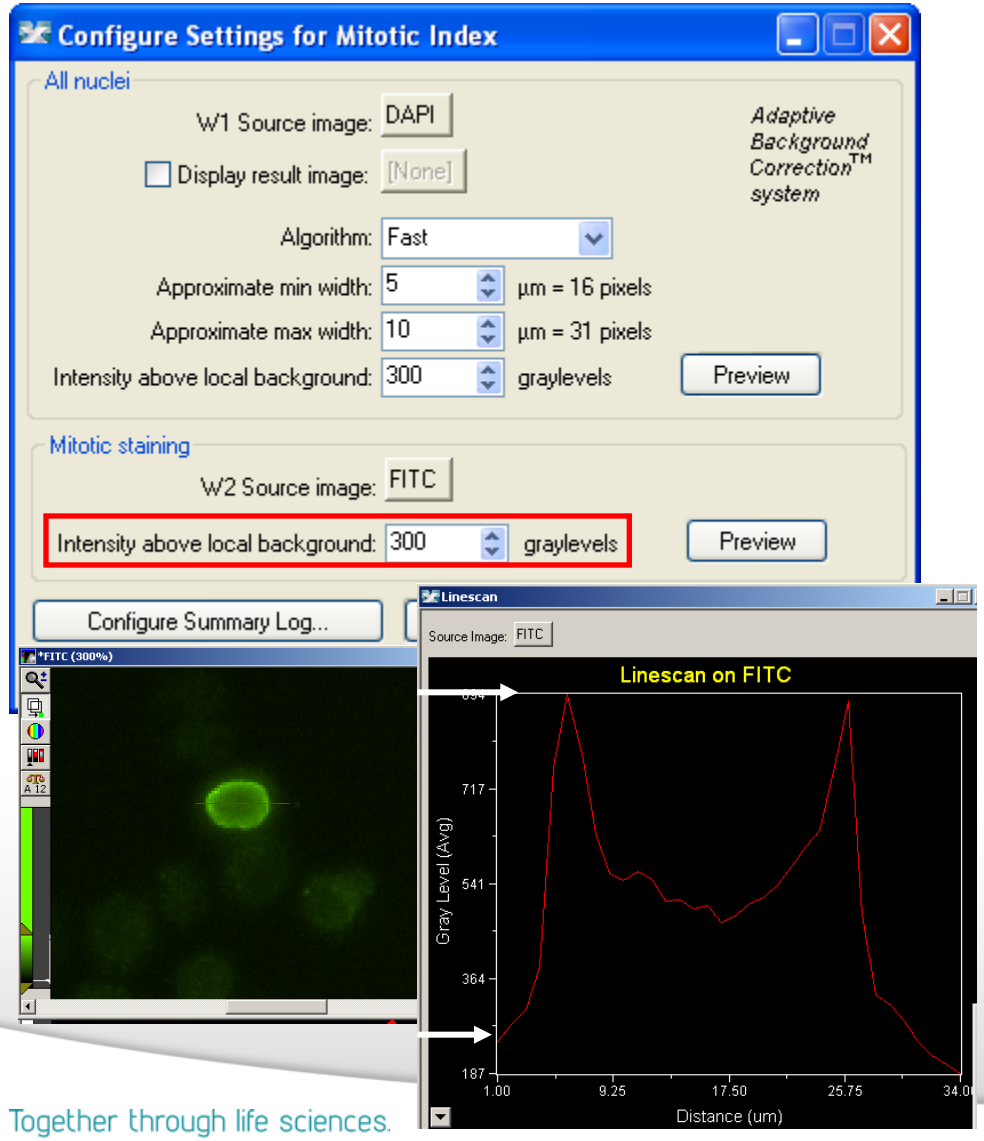


6. Module Settings – mitotic classification



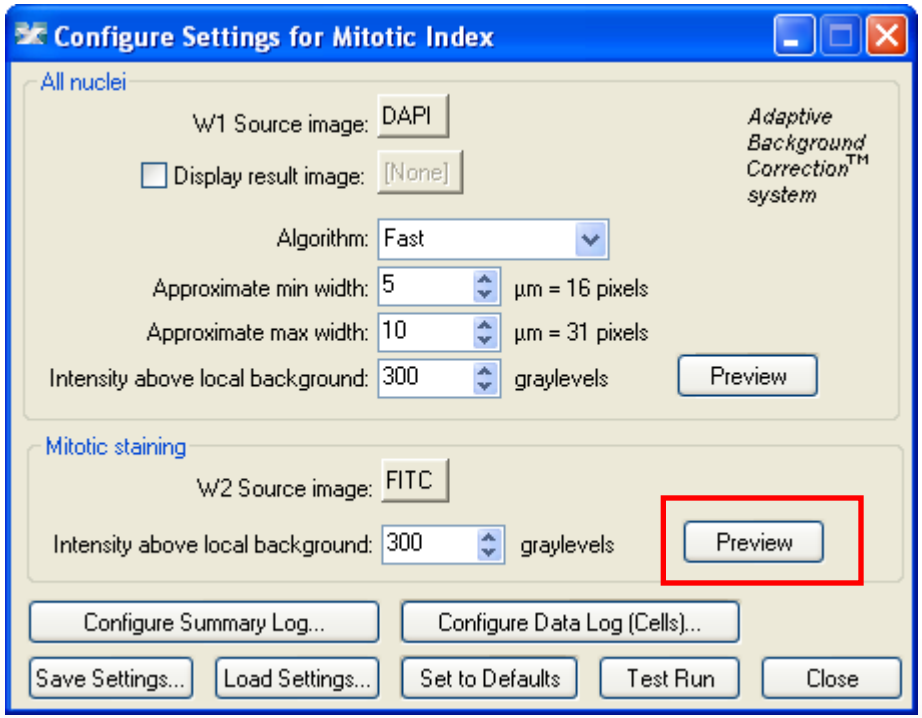
- **Mitotic-specific stain**
- Select the wavelength for the mitotic marker

7. Module Settings – mitotic classification

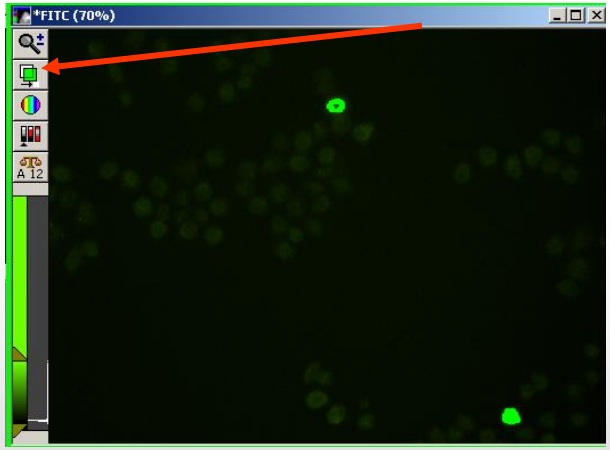


- Enter a cutoff intensity value for the mitotic specific stain.
- This value is a minimum and should be set slightly lower than the difference in intensity between a dim mitotic cell and its local background. For FAST algorithm, set this value to about half (or less) of the difference in intensity between a dim cell and local background.
- 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.

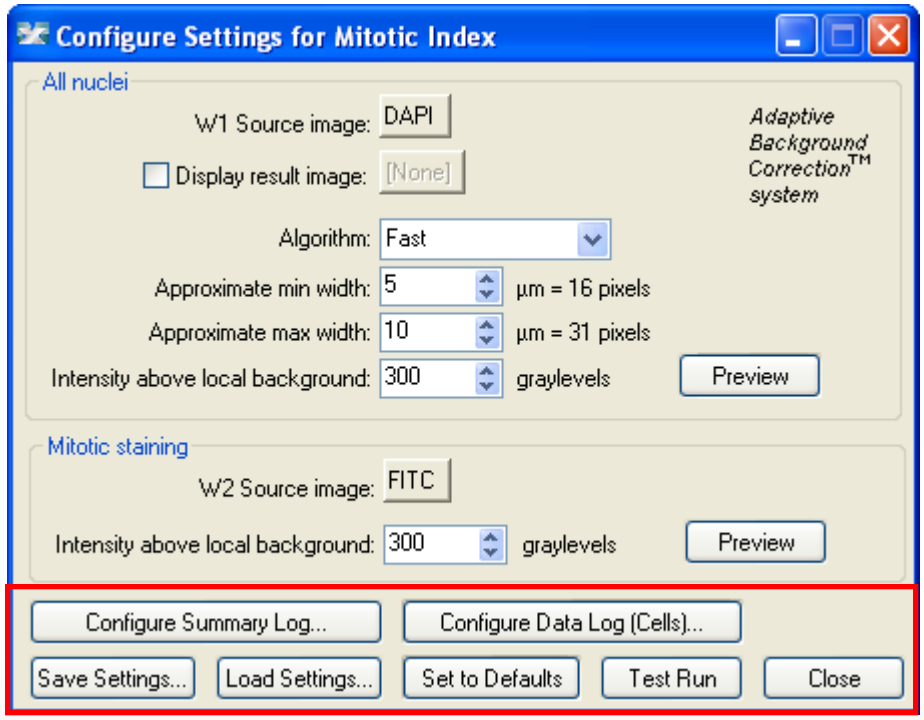
8. Module Settings – mitotic classification



- Press **Preview** to see cells with that level of staining highlighted in the image.
- The Preview image will show a green overlay on the mitotic staining image to show the segmentation derived from the image. The overlay can be toggled on and off.

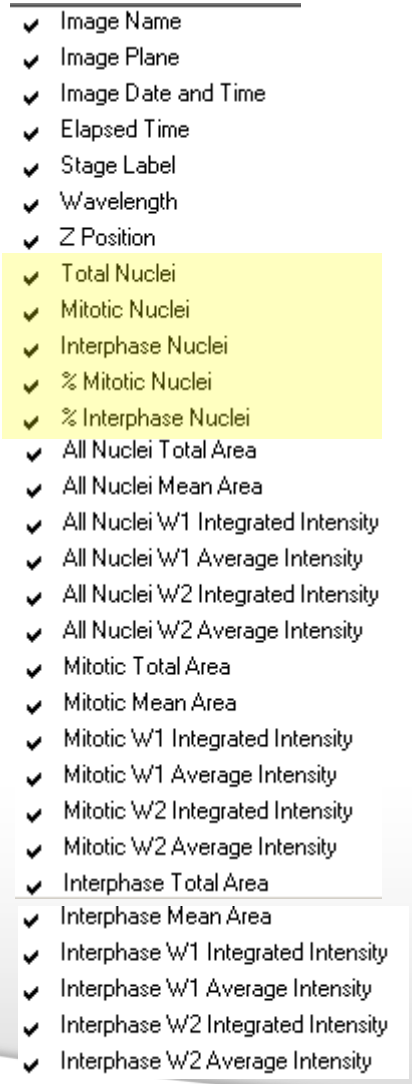


9. Module Settings – final settings



- **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)



- **Summary or site-by-site measurements are for the entire image and include:**

- **Total Nuclei:**

Total number cells determined from wavelength 1.

- **Mitotic Nuclei:**

Total number nuclei determined from wavelength 1 that were positive in wavelength 2.

- **Interphase Nuclei:**

Total number nuclei determined from wavelength 1 that were negative in wavelength 2.

- **% Mitotic Nuclei:**

100 * Mitotic Nuclei/Total Nuclei.

- **% Interphase Nuclei:**

100 * Interphase Nuclei/Total Nuclei.

Summary Data (site-by-site measurements)

- Image Name
- Image Plane
- Image Date and Time
- Elapsed Time
- Stage Label
- Wavelength
- Z Position
- Total Nuclei
- Mitotic Nuclei
- Interphase Nuclei
- % Mitotic Nuclei
- % Interphase Nuclei
- 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
- Mitotic Total Area
- Mitotic Mean Area
- Mitotic W1 Integrated Intensity
- Mitotic W1 Average Intensity
- Mitotic W2 Integrated Intensity
- Mitotic W2 Average Intensity
- Interphase Total Area
- Interphase Mean Area
- Interphase W1 Integrated Intensity
- Interphase W1 Average Intensity
- Interphase W2 Integrated Intensity
- Interphase W2 Average Intensity

- **Summary or site-by-site measurements are for the entire image and include:**

- **All Nuclei Total Area:**

Total µm²s in wavelength 1 in all nuclei.

- **All Nuclei Mean Area:**

All Nuclei Total Area/Total Nuclei.

- **All Nuclei W1 Integrated Intensity:**

Summed grayscale values in wavelength 1 in all nuclei.

- **All Nuclei W1 Average Intensity:**

All Nuclei Integrated Intensity/All Nuclei Total (Pixel) Area.

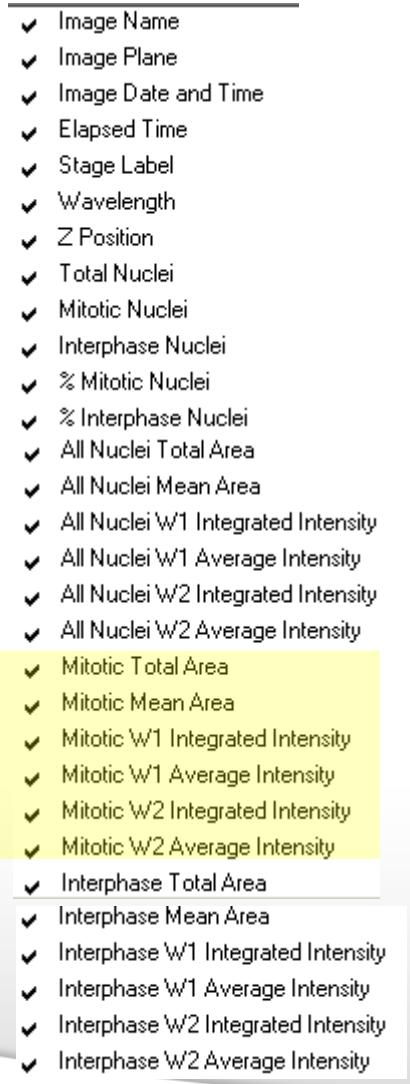
- **All Nuclei W2 Integrated Intensity:**

Summed grayscale values in wavelength 2 in all nuclei.

- **All Nuclei W2 Average Intensity:**

All Nuclei W2 Integrated Intensity/All Nuclei Total (Pixel) Area.

Summary Data (site-by-site measurements)



- **Mitotic Total Area:**

Total µm²s in wavelength 1 in mitotic nuclei.

- **Mitotic Mean Area:**

Mitotic Total Area/Mitotic Nuclei.

- **Mitotic W1 Integrated Intensity:**

Summed grayscale values in wavelength 1 in mitotic nuclei.

- **Mitotic W1 Average Intensity:**

Mitotic Nuclei W1 Integrated Intensity/Mitotic Nuclei Total (Pixel) Area.

- **Mitotic W2 Integrated Intensity:**

Summed grayscale values in wavelength 2 in mitotic nuclei.

- **Mitotic W2 Average Intensity:**

Mitotic W2 Integrated Intensity/Mitotic Total (Pixel) Area.

Summary Data (site-by-site measurements)

- **Interphase Total Area:**

Total µm²s in wavelength 1 in interphase nuclei.

- **Interphase Mean Area:**

Interphase Total Area/Interphase Nuclei.

- **Interphase W1 Integrated Intensity:**

Summed grayscale values in wavelength 1 in interphase nuclei.

- **Interphase W1 Average Intensity:**

Interphase W1 Integrated Intensity/Interphase Total (Pixel) Area.

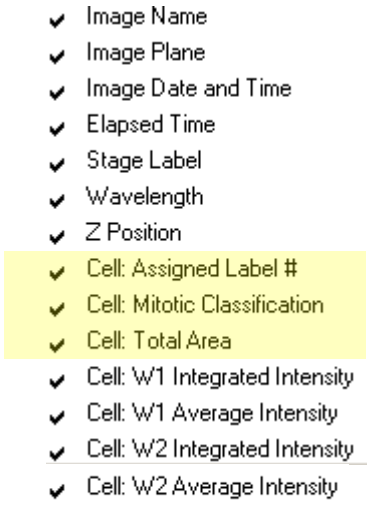
- **Interphase W2 Integrated Intensity:**

Summed grayscale values in wavelength 2 in interphase nuclei.

- **Interphase W2 Average Intensity:**

Interphase W2 Integrated Intensity/Interphase Total (Pixel) Area.

Cell Data (cell-by-cell measurements)



- **Data Log (Cell-by-Cell Measurement) -** For each nucleus, the following measurements are included in the Data Log:

- **Cell: Assigned Label #**:

Cell label number (1 through total cell number)

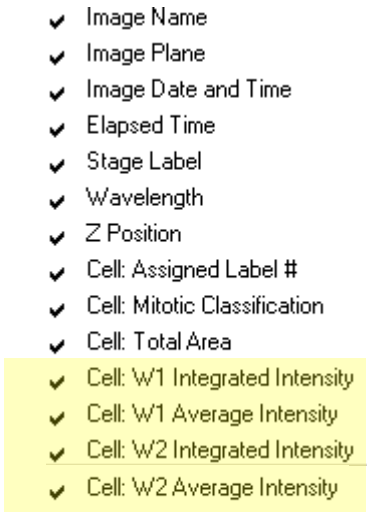
- **Cell: Classification:**

Interphase, Mitotic

- **Cell: Total Area:**

Total µm²s in the nucleus.

Cell Data (cell-by-cell measurements)



- **Data Log (Cell-by-Cell Measurement):**For each nucleus, the following measurements are included in the Data Log:

- **Cell: W1 Integrated Intensity:**

Summed grayscale values in wavelength 1 for this nucleus.

- **Cell: W1 Average Intensity:**

W1 Integrated Intensity/Total (Pixel) Area

- **Cell: W2 Integrated Intensity:**

Summed grayscale values in wavelength 2 for this nucleus.

- **Cell: W2 Average Intensity:**

W2 Integrated Intensity/Total (Pixel) Area

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