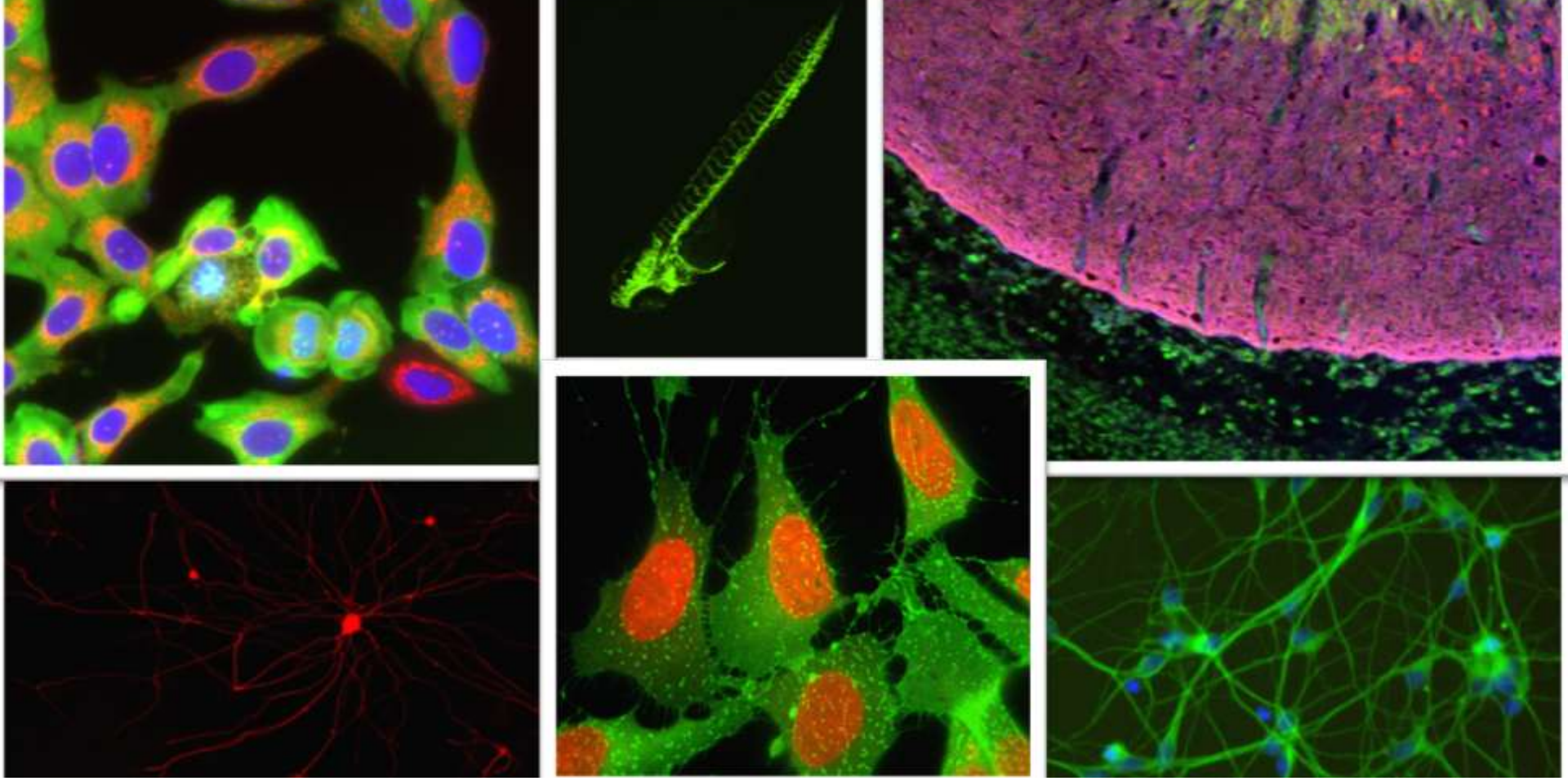

Molecular Devices

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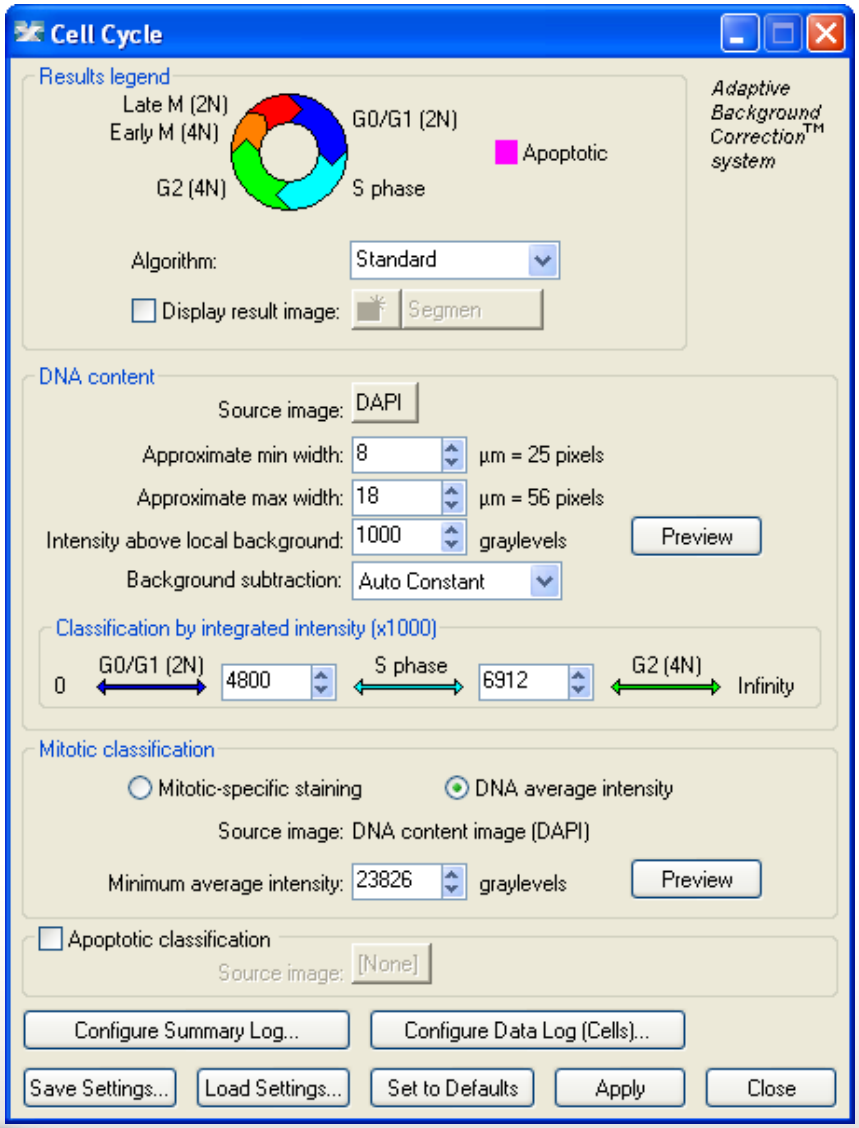
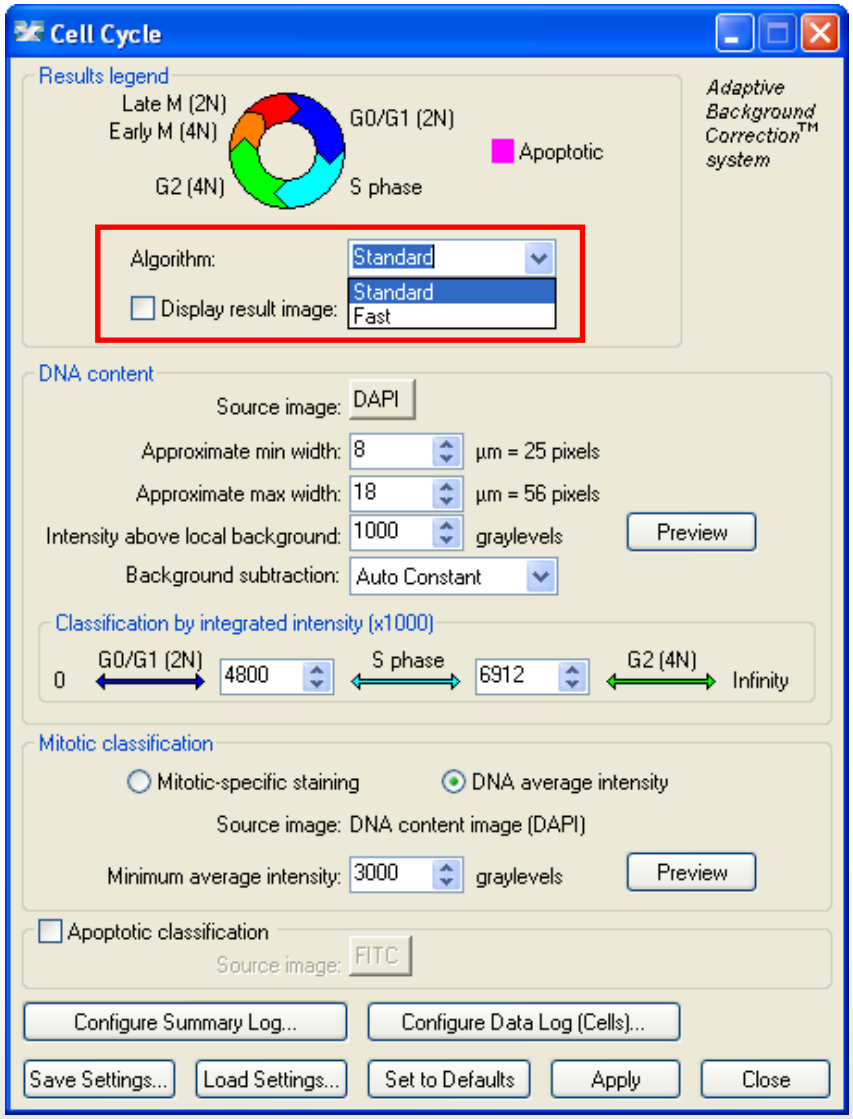
MetaXpress® Software:
Cell Cycle Module

Cell Cycle Module Overview



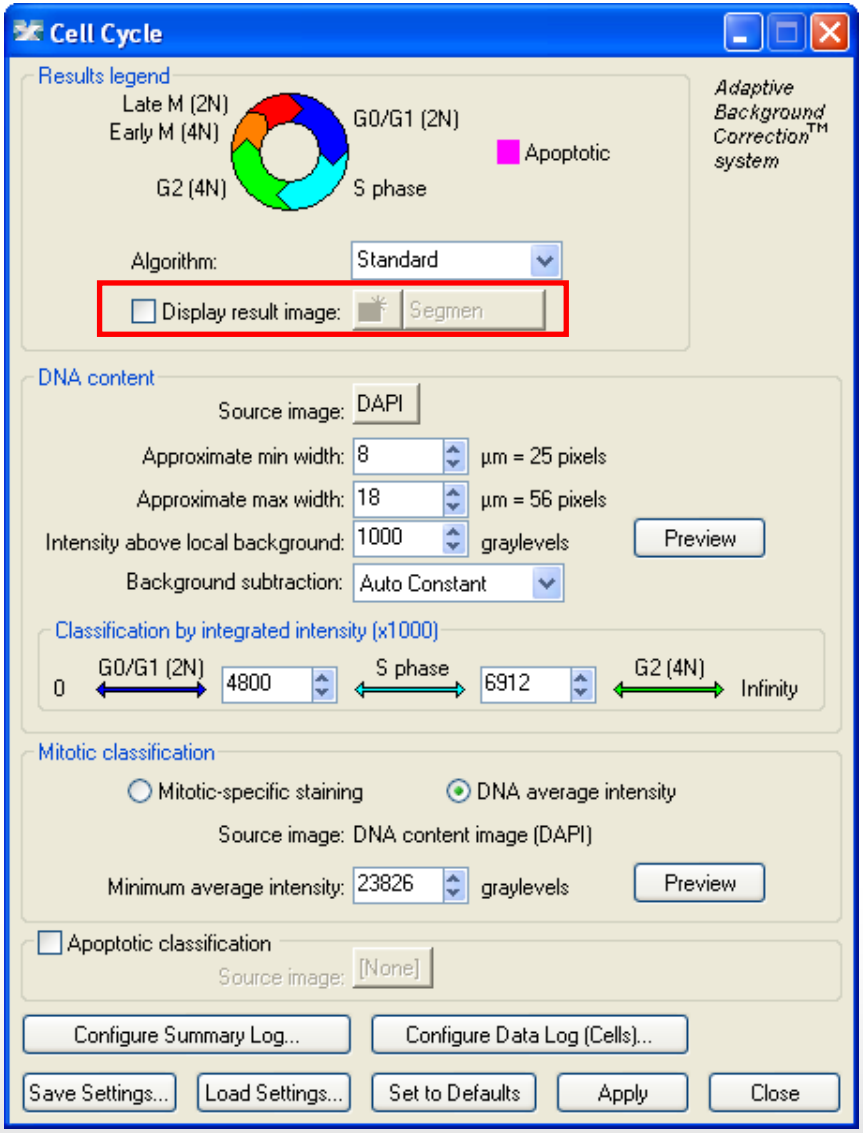
- Cell-by-cell cell cycle classification into G0/G1, S phase, G2, early mitosis, late mitosis, and (optional) apoptosis using 1-3 wavelengths:
- DNA stain (required)
- Optional mitotic stain (such as phospho-histone H3)
- Optional apoptotic stain

Module Settings



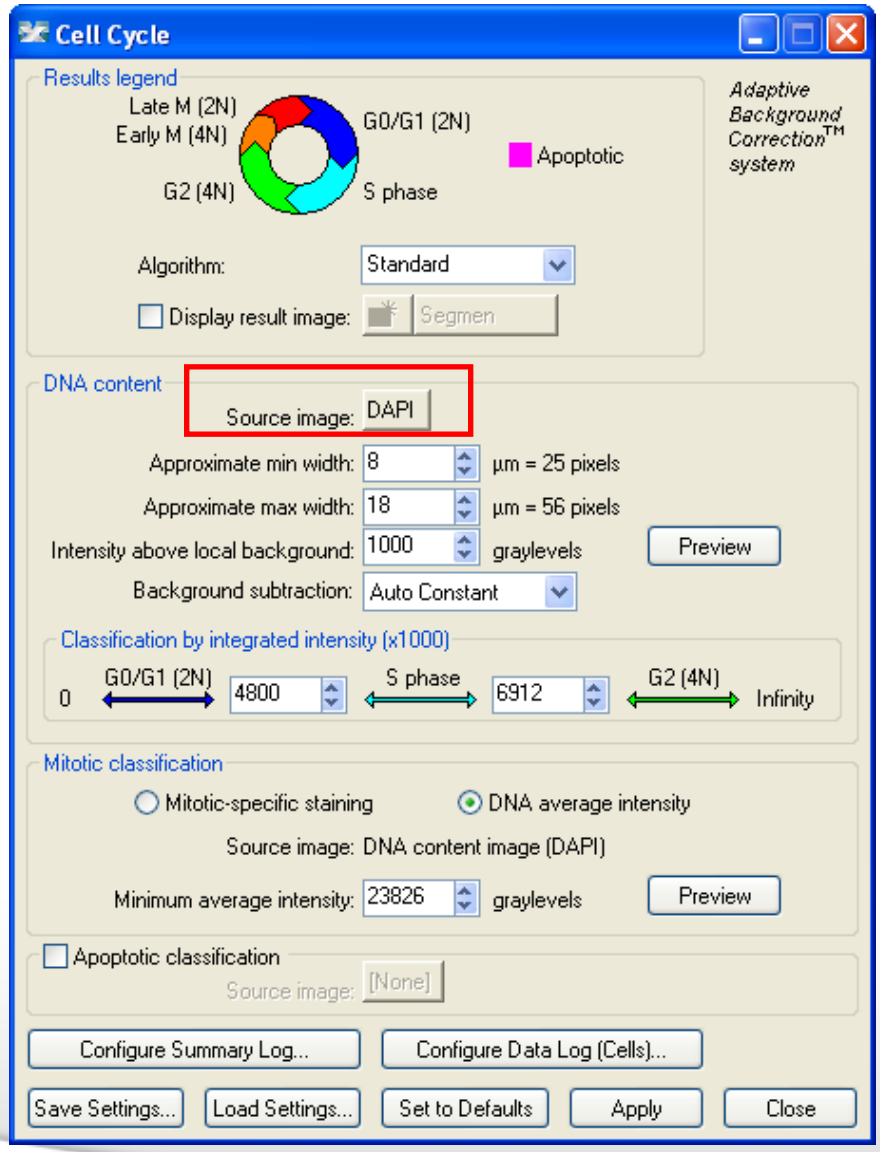
- **Algorithm**
- This option is only available in MetaXpress software version 4.0 and higher and determines how quickly the analysis is performed.
- **Fast** algorithm can perform analysis up to twice as fast as **Standard**.
- Both algorithms produce similar but not identical results.

1. Module Settings – result image



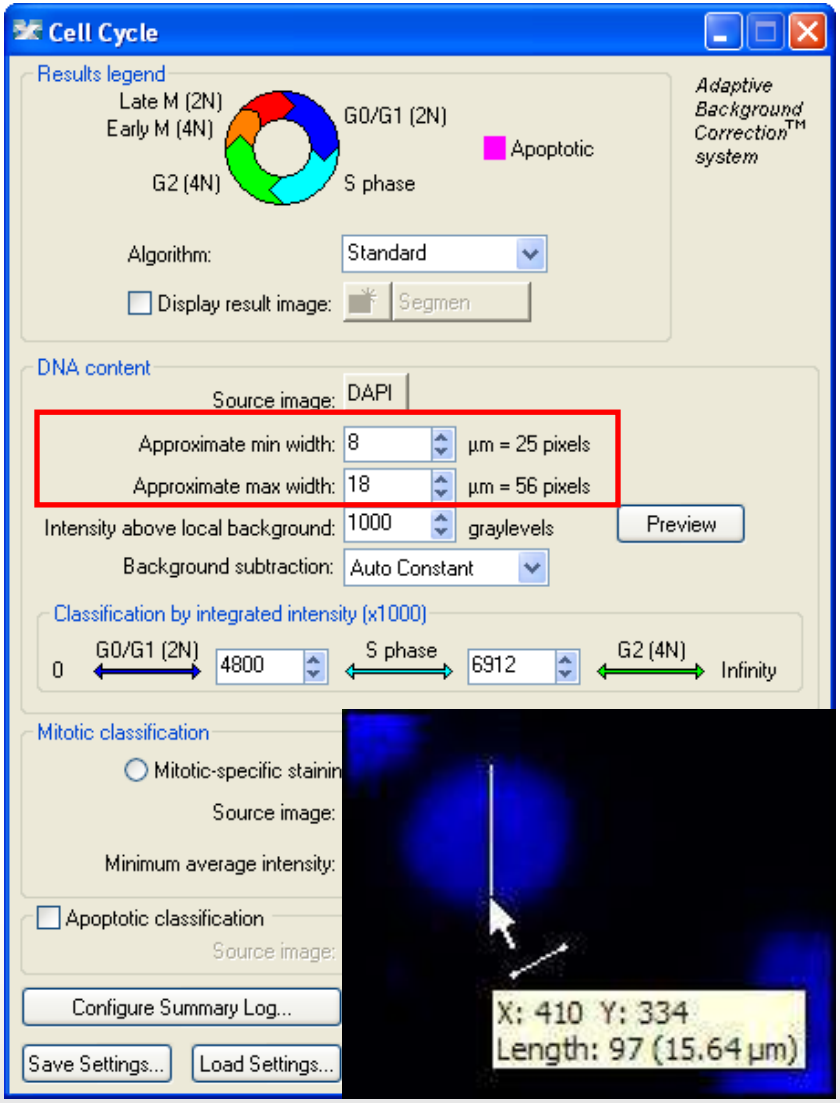
- Leave "**Display result image**" deselected (this is generally only used when journaling)

2. Module Settings – DNA content



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

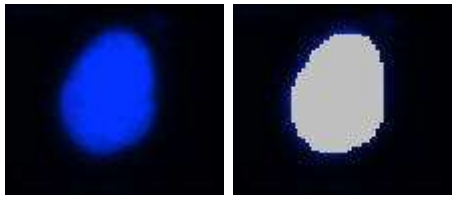
3. Module Settings – width settings



- **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



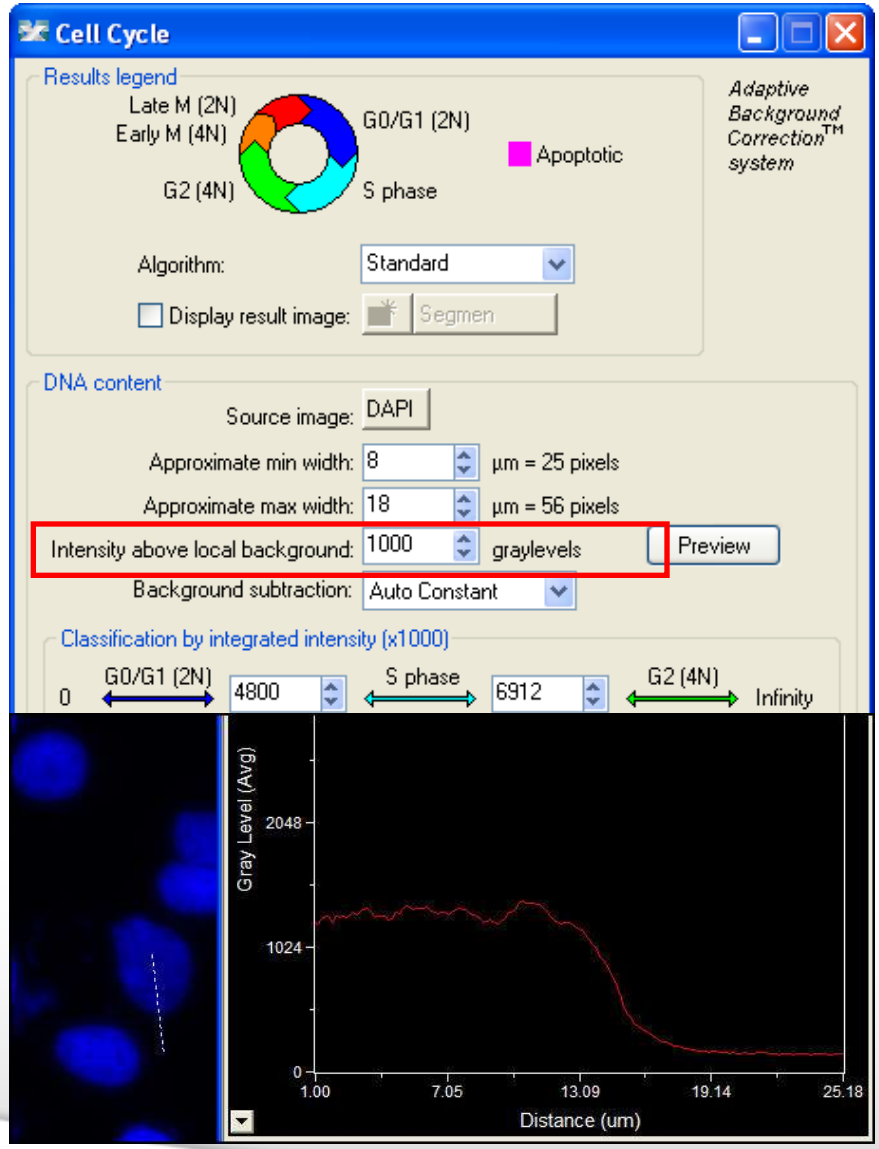
Min width too small: splits nuclei

Min width too large: omits smaller nuclei

Max width too small: may shrink nuclear boundaries

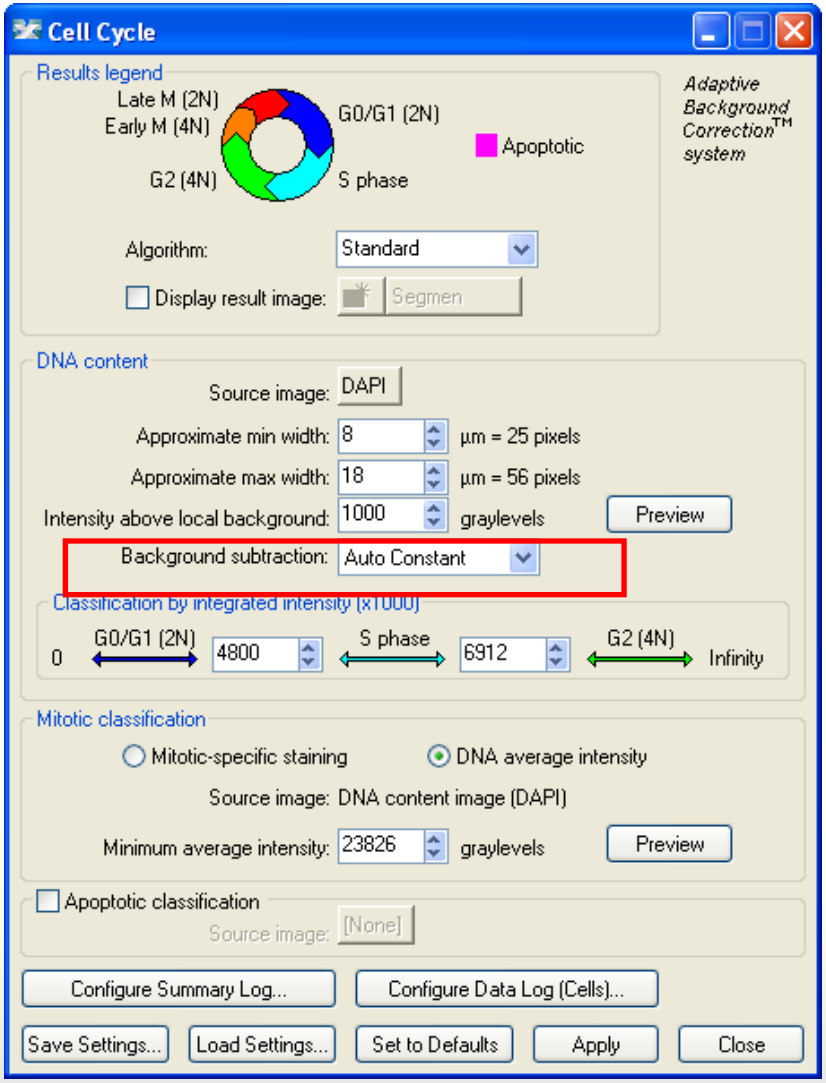
Max width too large: may slightly enlarge nuclear boundaries

4. Module Settings – intensity settings



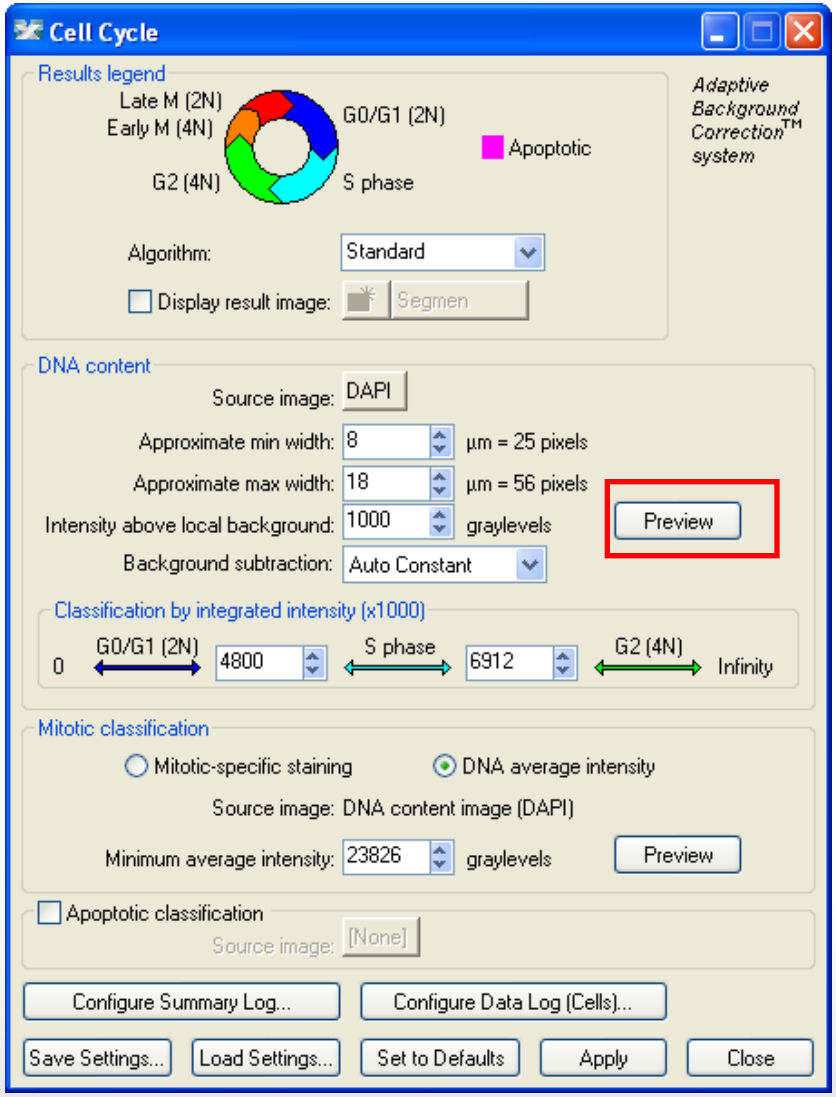
- The **intensity above local background** is used for finding the nuclei
- This value is a minimum and should be set slightly lower than the difference in intensity between a dim cell and its local background. For FAST algorithm, set this value to about half (or less) of the difference in intensity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

5. Module Settings – background subtraction



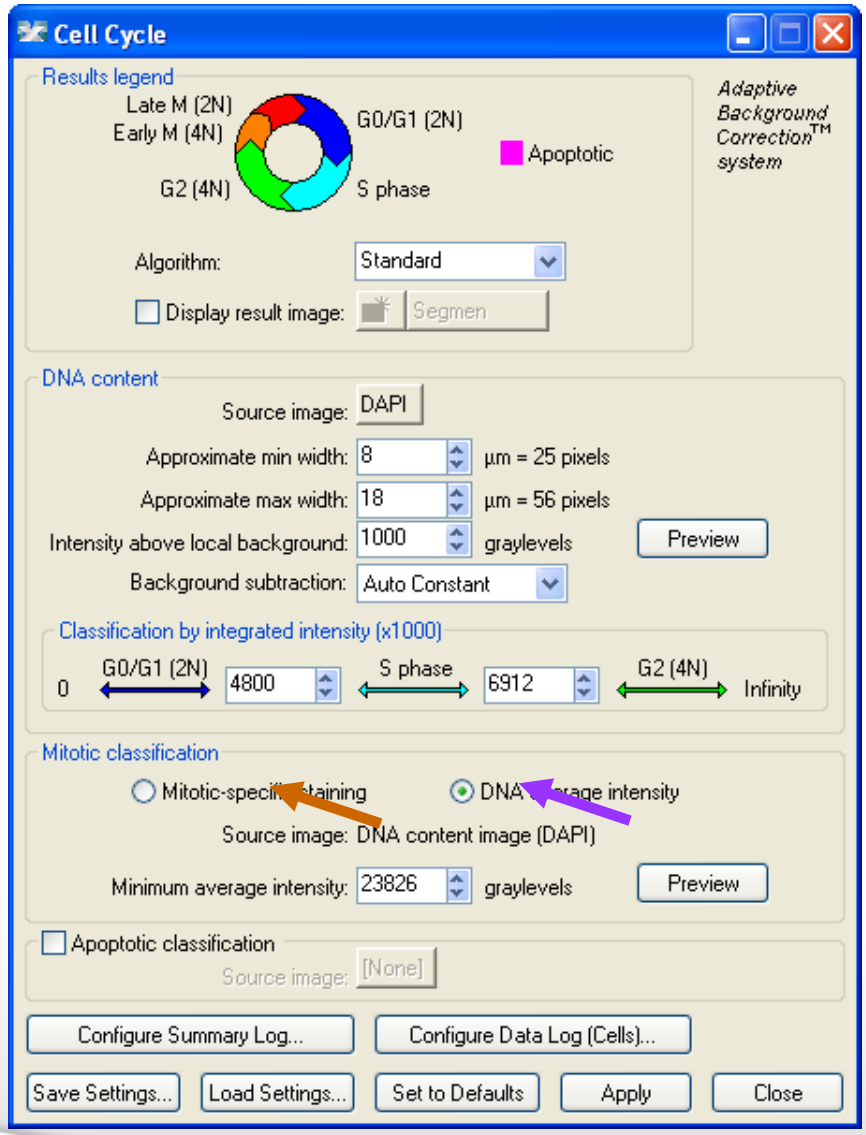
- The background intensity is subtracted from the probe intensities before measurements are performed and recorded.
- **None:** no background subtraction is performed
- **Auto Constant:** an average background value is calculated for each image and subtracted
- **Constant:** input a fixed background intensity to be subtracted

6. Module Settings – DNA content classification

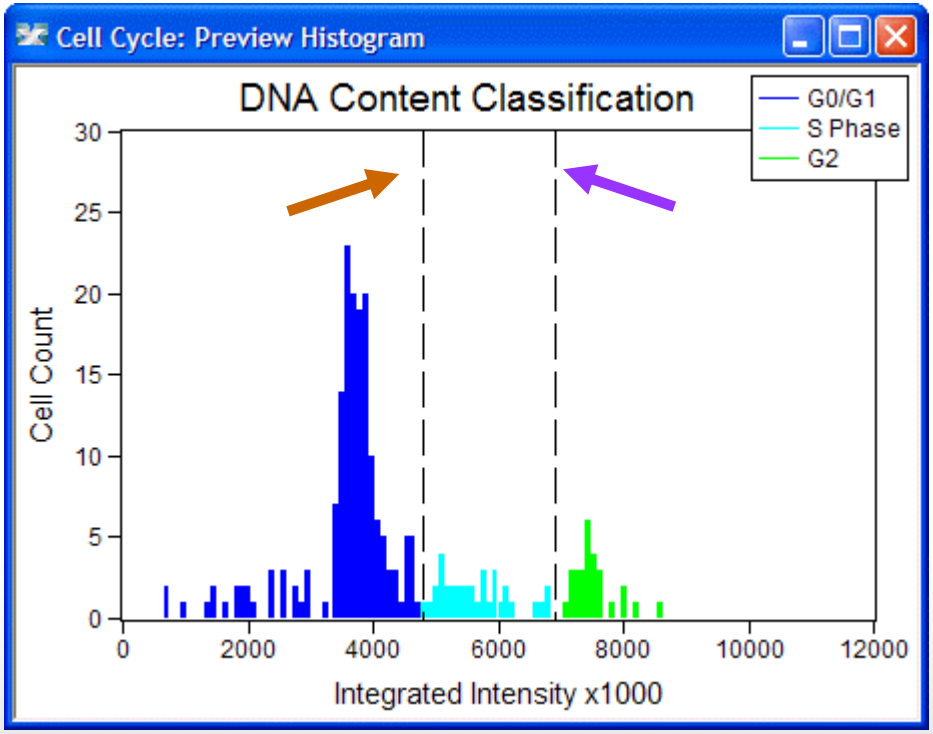


- Click on **Preview** to test settings and set the classification parameters

6. Module Settings - DNA content classification

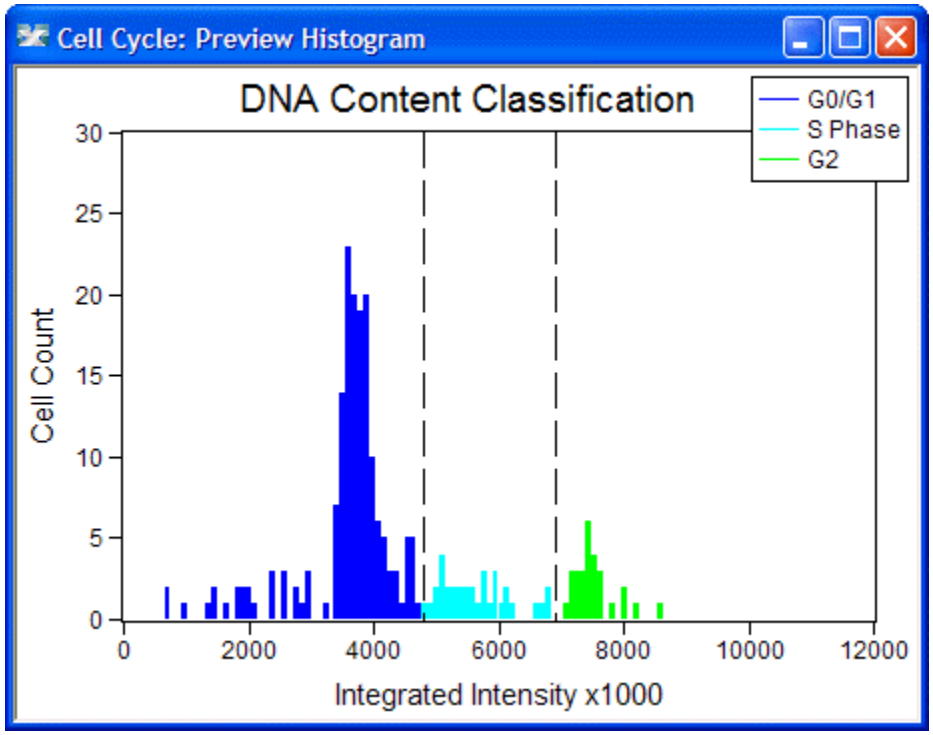


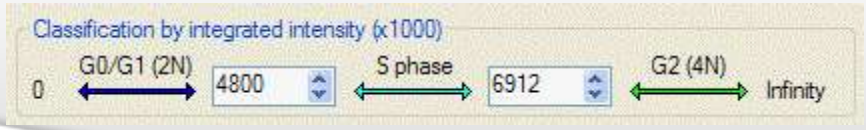
- An interactive graph is shown to adjust the cutoffs for classification.



Molecular Devices

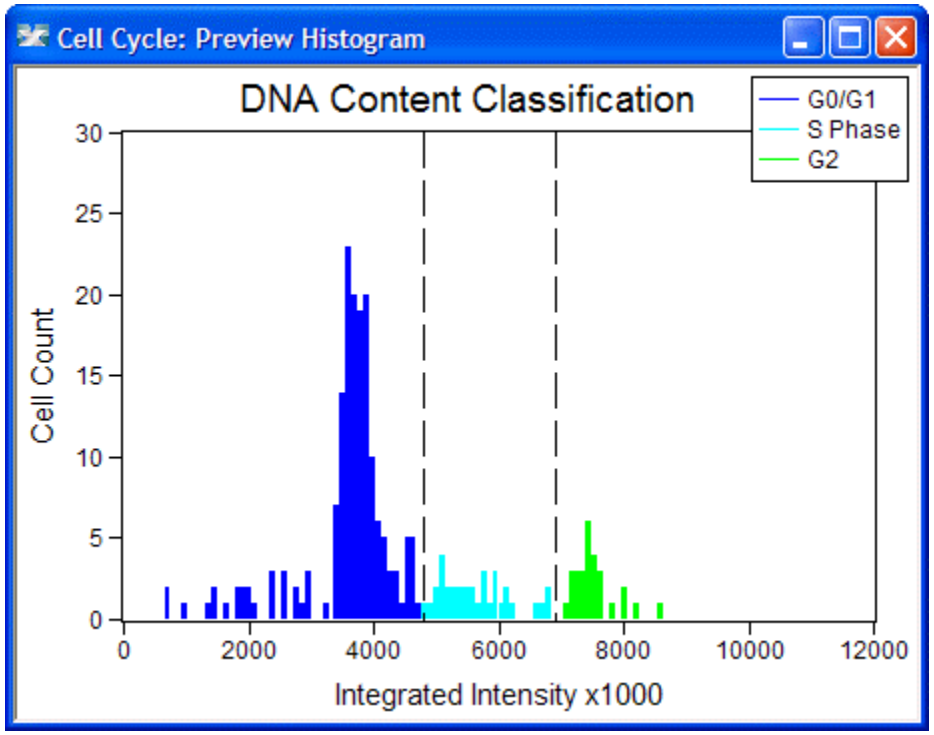
6. Module Settings - DNA content classification

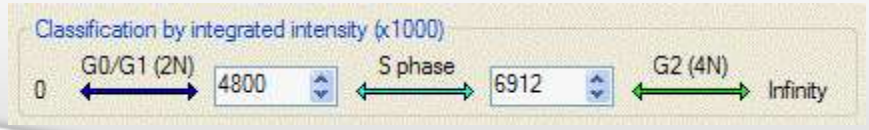




- The **integrated intensity** of the nuclear stain is used to quantify DNA content (similar to flow cytometry cell cycle analysis).
- Cells in G0 or G1 or late mitosis have 2N DNA content.
- Cells in G2 or early mitosis have 4N DNA content.
- Cells in S phase have DNA content in between 2N and 4N.

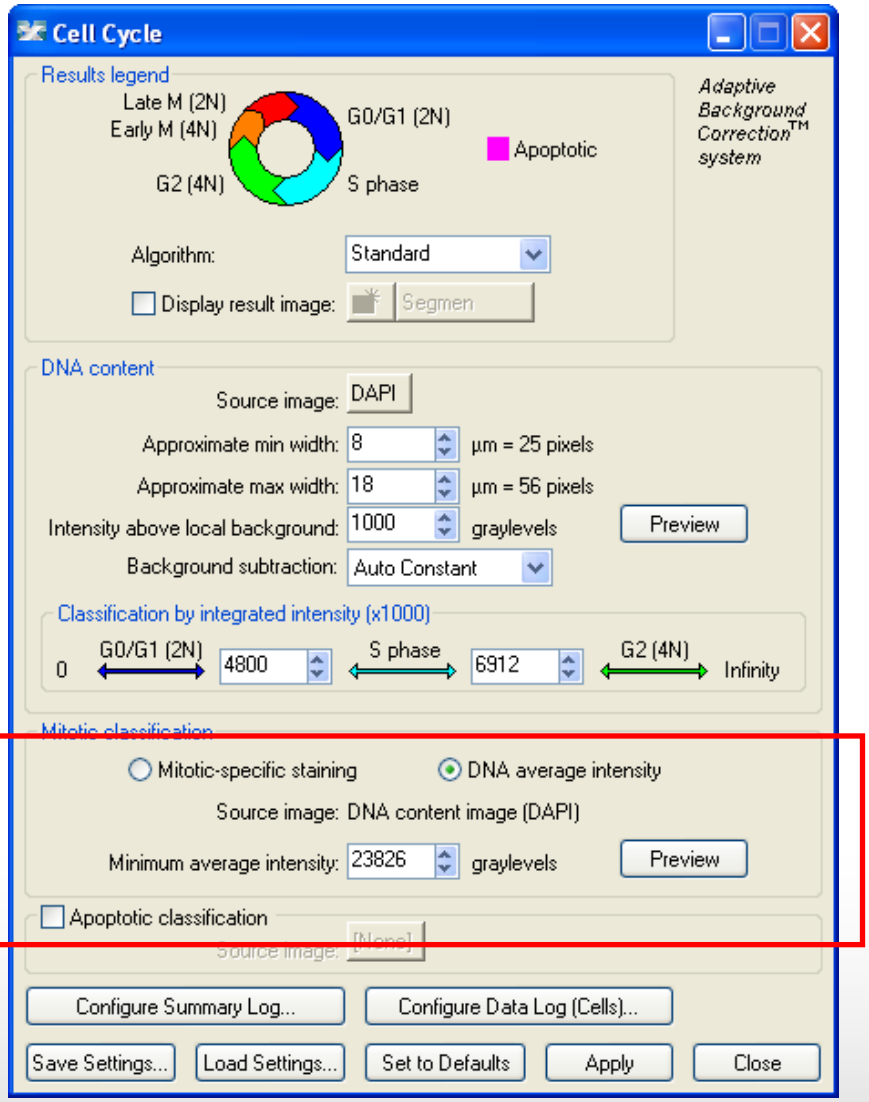
6. Module Settings - DNA content classification





- **Guidelines on setting cutoffs:**
- Drag the sliders to set them, or type in numbers (useful if the sliders are out of scale)
- Usually the first large peak is the 2N peak, and the 4N peak will be smaller and approximately double that
- For more accuracy, do a preliminary analysis on multiple wells, then use AcuityXpress to view a histogram of DNA content across those wells and determine cutoffs

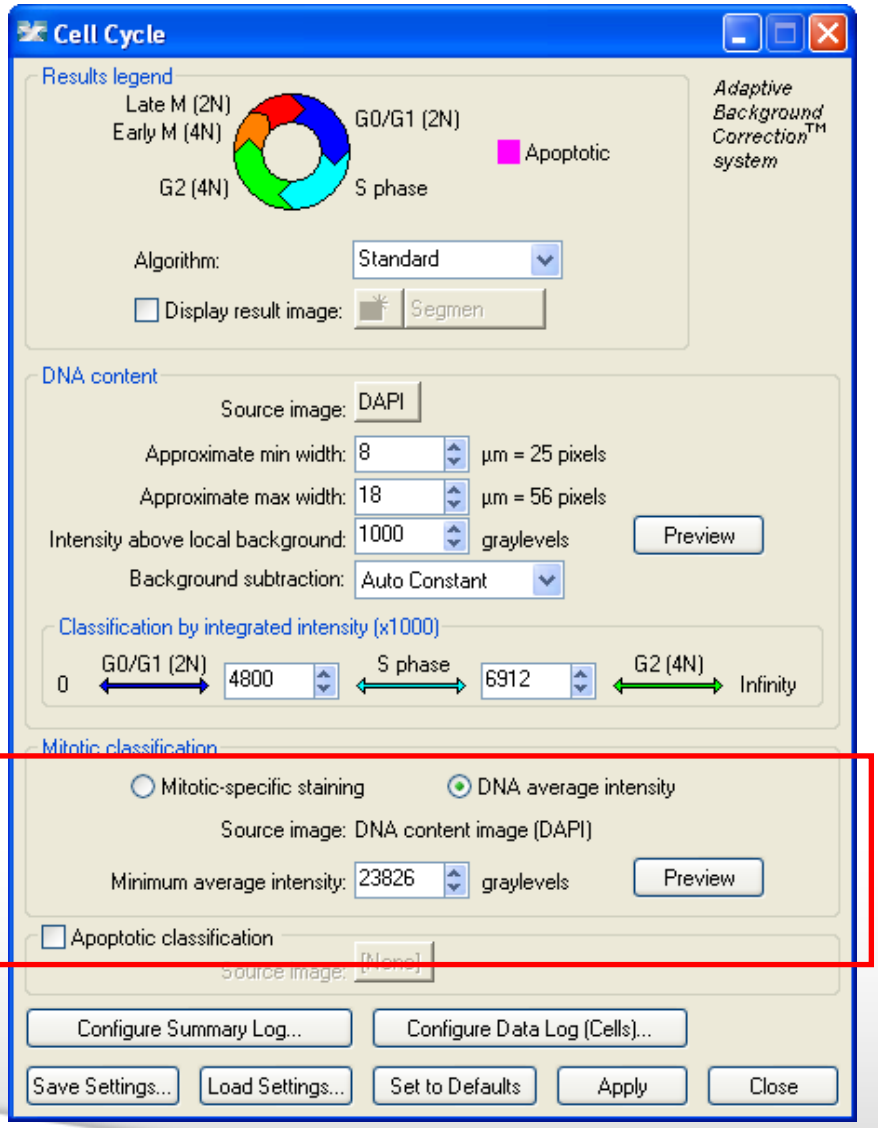
7. Module Settings – mitotic classification



- **Mitotic classification**

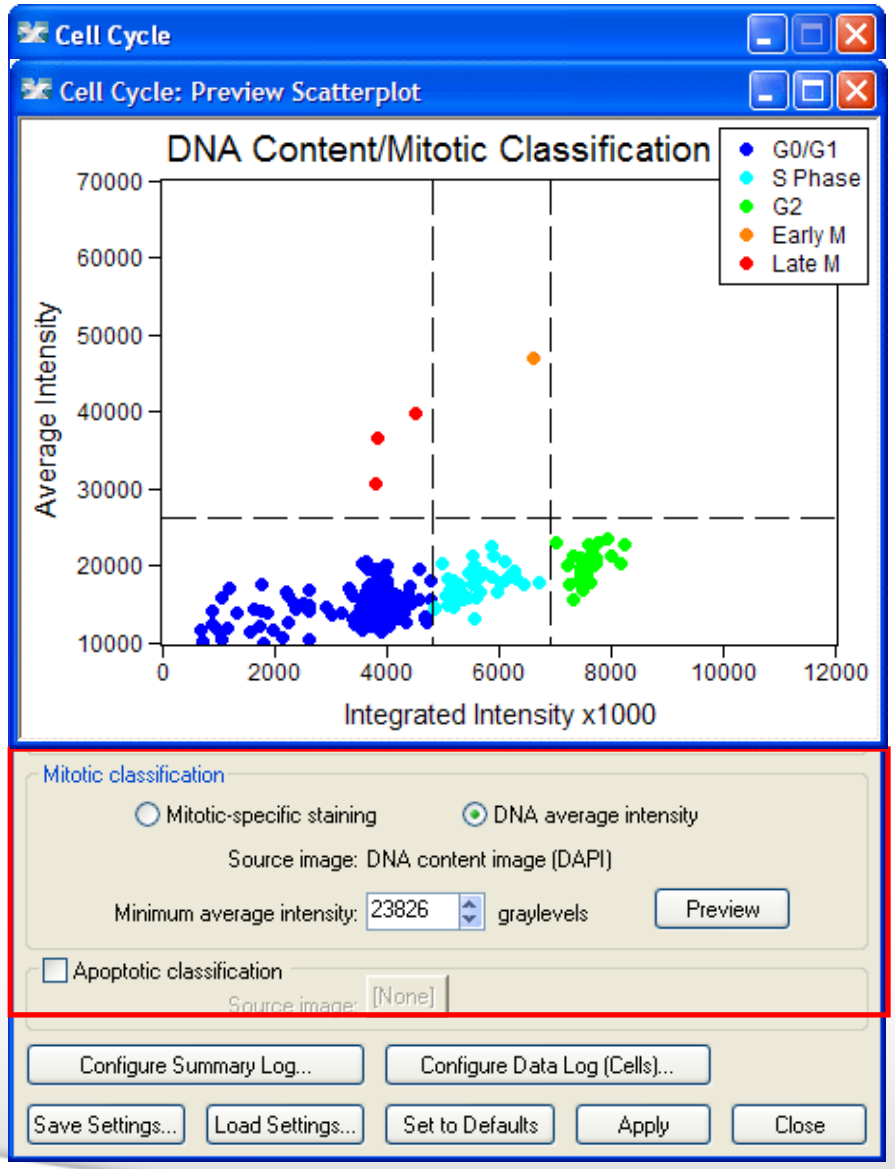
- The average intensity (brightness) of the nuclear stain or a separate mitotic-specific marker (e.g. phospho-histone H3) is used to identify mitotic cells.

7. Module Settings – mitotic classification



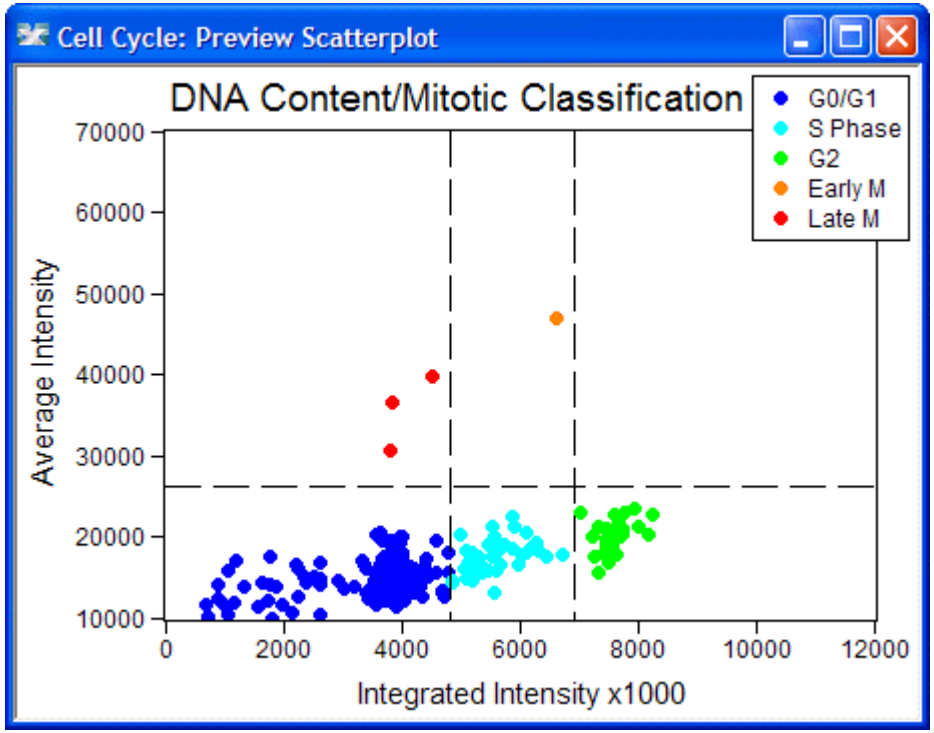
- **DNA average intensity**
- To use the DNA average intensity as a mitotic classifier, simply press Preview.

7. Module Settings – mitotic classification



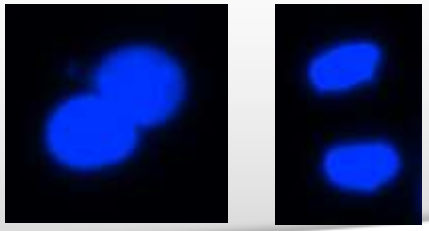
- **DNA average intensity**
- To use the DNA average intensity as a mitotic classifier, simply press Preview.
- A scatter plot will appear with an interactive horizontal slider for setting the intensity cutoff.

7. Module Settings – mitotic classification

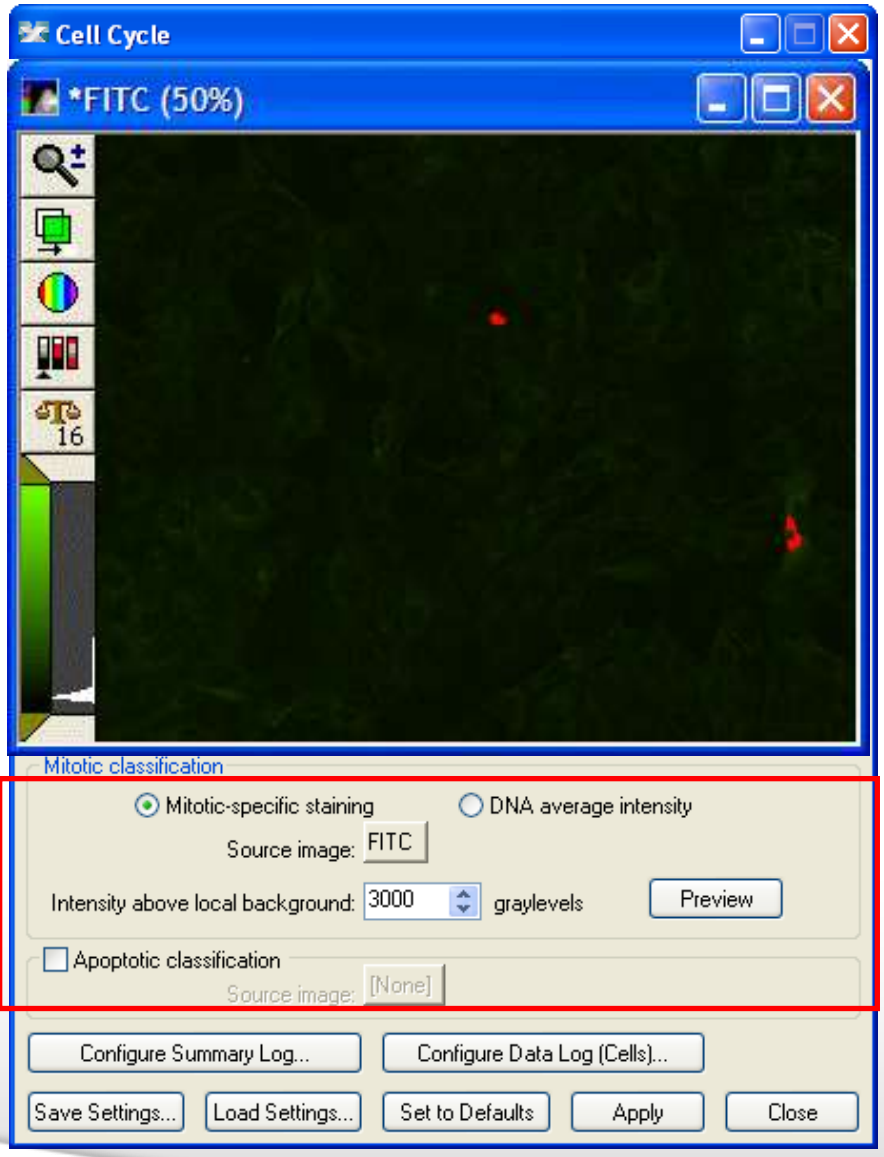


- **DNA average intensity**
- In the scatter plot, cells above the horizontal line are mitotic; cells below it are not mitotic.
- Mitotic cells with 2N content are classified as “Late M” and those with >2N content are classified as “Early M”.

Early M ***Late M***

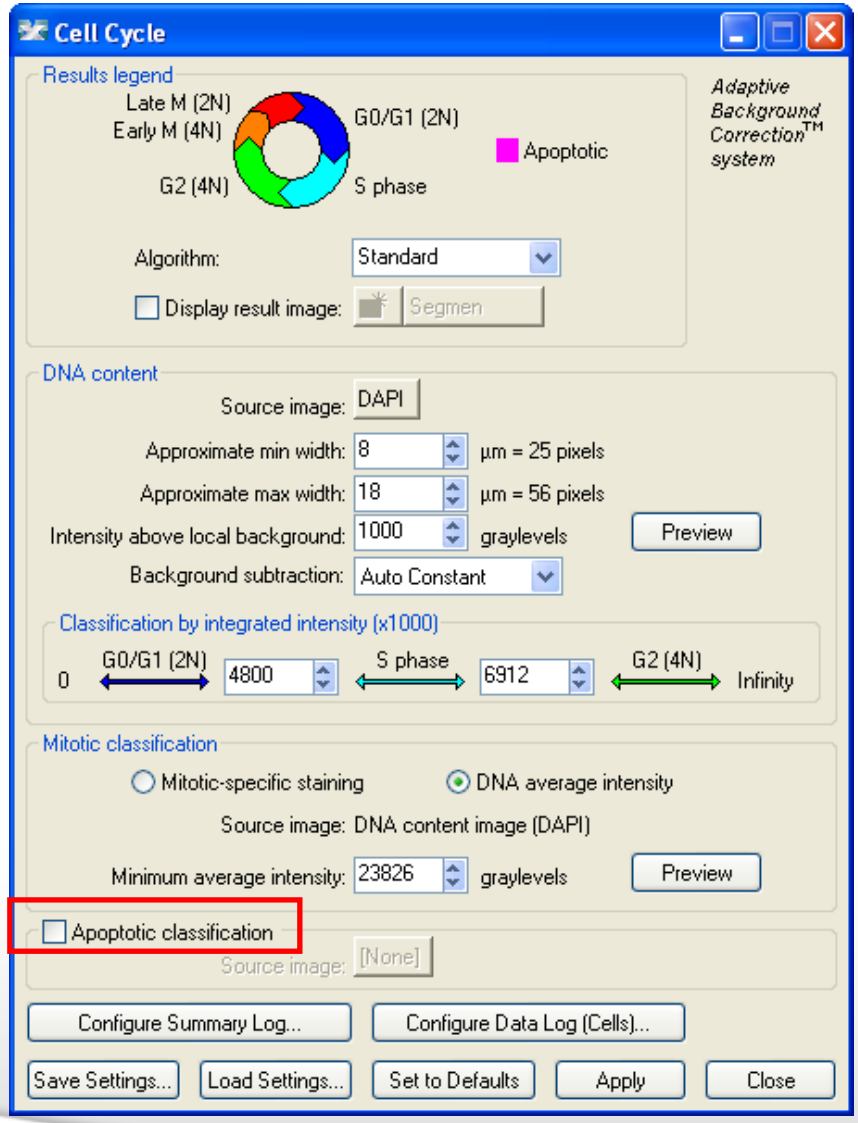


7. Module Settings – mitotic classification



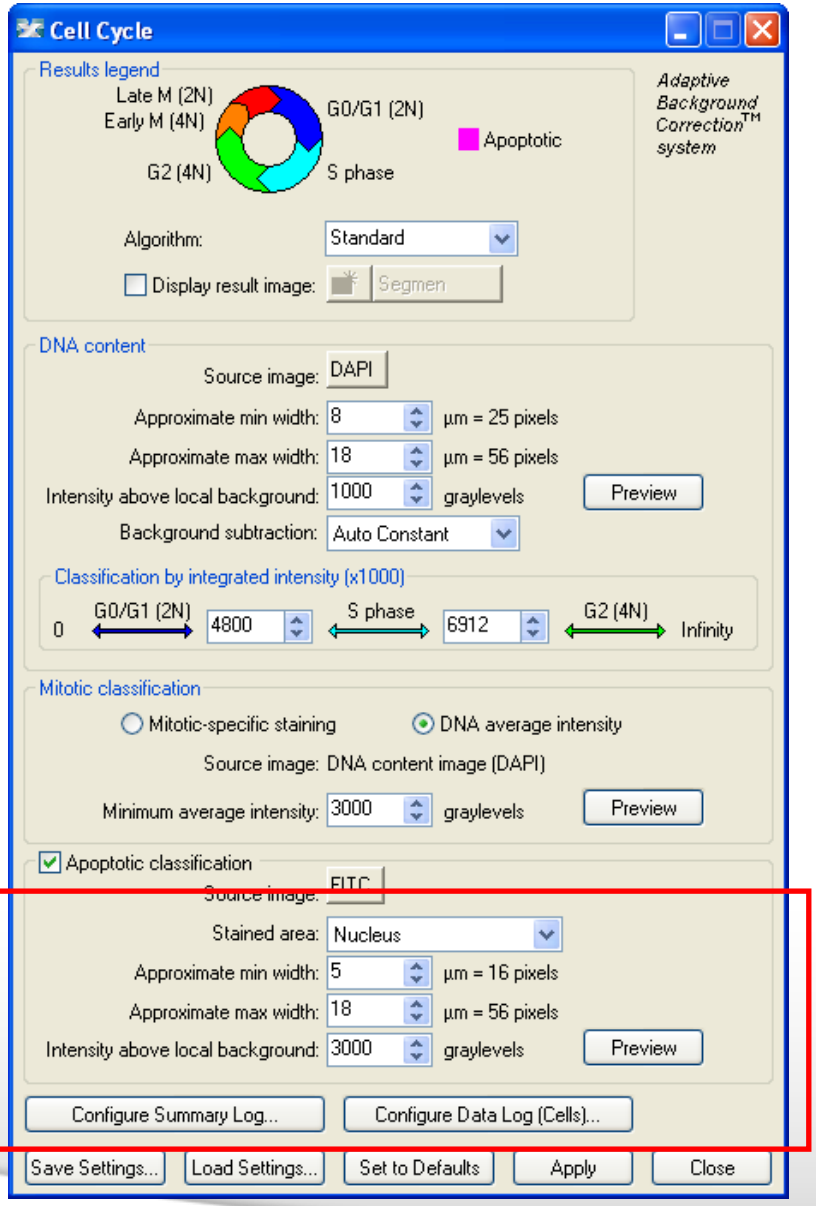
- **Mitotic-specific stain**
- Select the wavelength for the mitotic marker, enter a cutoff intensity value, and press Preview to see cells with that level of staining highlighted in the image.
- Mitotic cells with 2N content are classified as “Late M” and those with >2N content are classified as “Early M”.
- Using a mitotic-specific marker, if available, is typically more accurate than using the DNA average intensity.

8. Module Settings – apoptotic classification



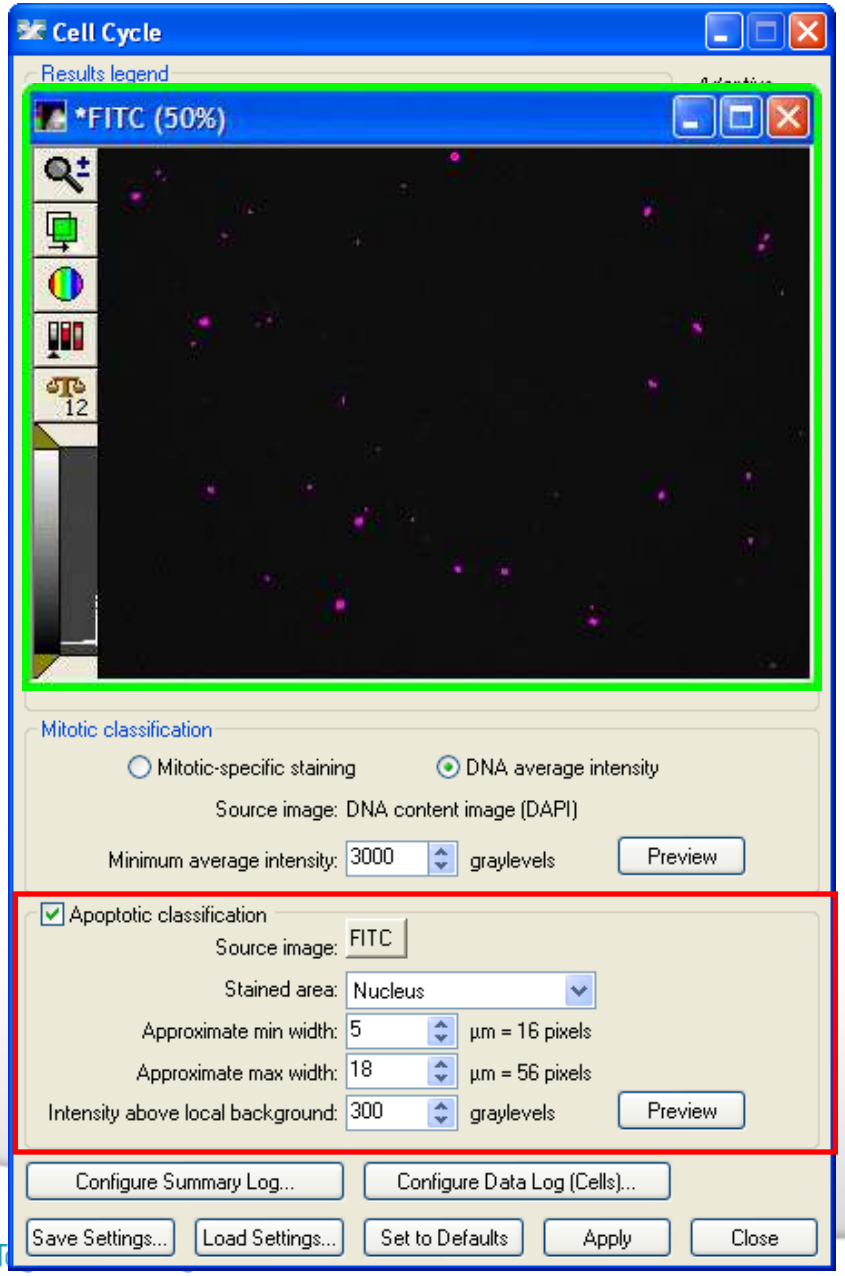
- If the assay has an apoptotic-specific marker, select the "Apoptotic classification" checkbox.

8. Module Settings – apoptotic classification



- Select the wavelength of the **apoptotic** marker.
- Define the stained area as nuclear, cytoplasmic, or both.
- Set the width settings (if the stain is nuclear, it should match the prior width settings).
- Set an intensity cutoff.

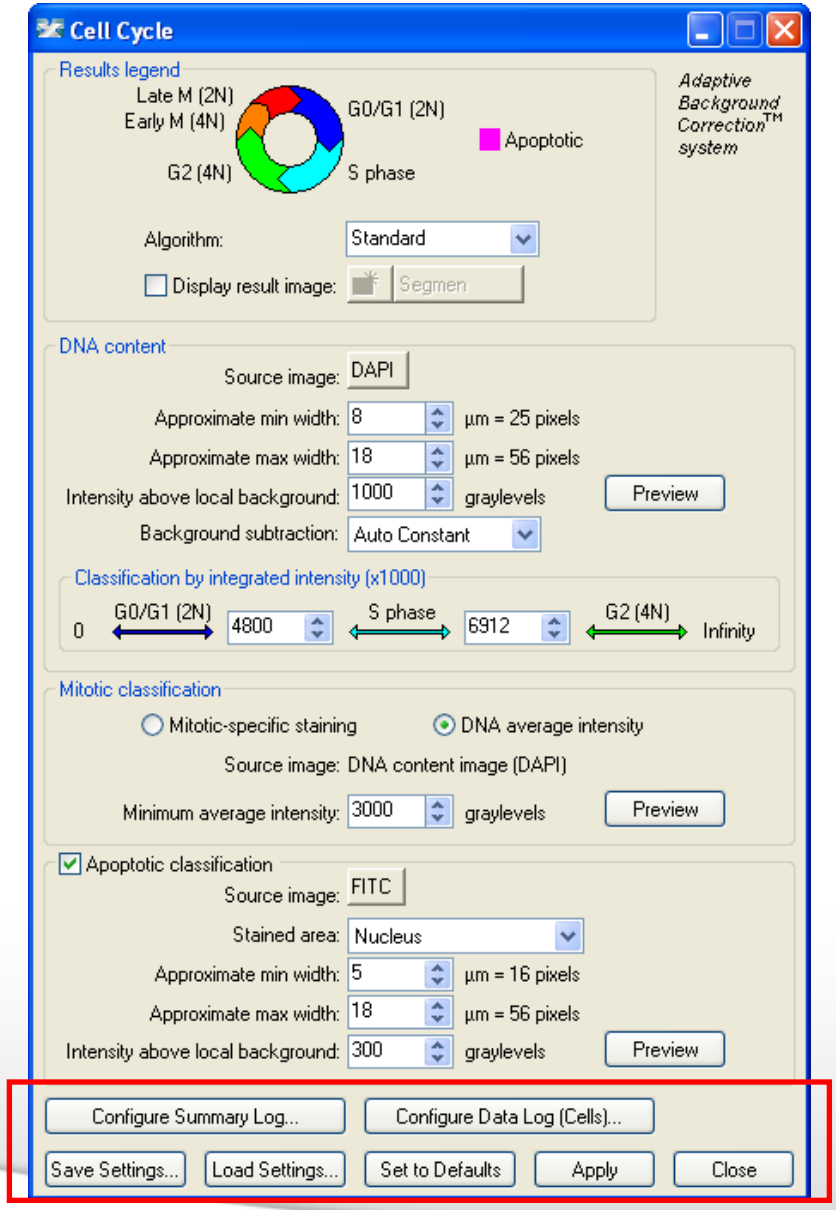
8. Module Settings – apoptotic classification



- Select the wavelength of the apoptotic marker.
- Define the stained area as nuclear, cytoplasmic, or both.
- Set the width settings (if the stain is nuclear, it should match the prior width settings).
- Set an intensity cutoff.
- Press Preview to test settings.

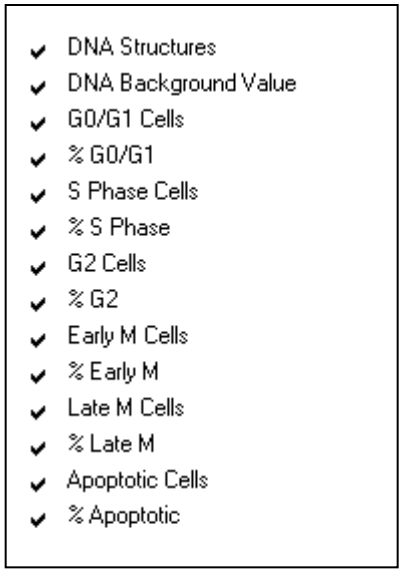
Molecular Devices

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)



- **DNA Structures:** Total number of nuclei

- **DNA Background Value:** The average background pixel intensity of the DNA image. This is the value that has been subtracted from intensity measurements if the "Auto Constant" option was chosen

- **G0/G1 Cells:** Number of nuclei classified as G0/G1
- **% G0/G1:** Percentage of nuclei classified as G0/G1

- **S Phase Cells:** Number of nuclei classified as S phase
- **% S Phase:** Percentage of nuclei classified as S phase

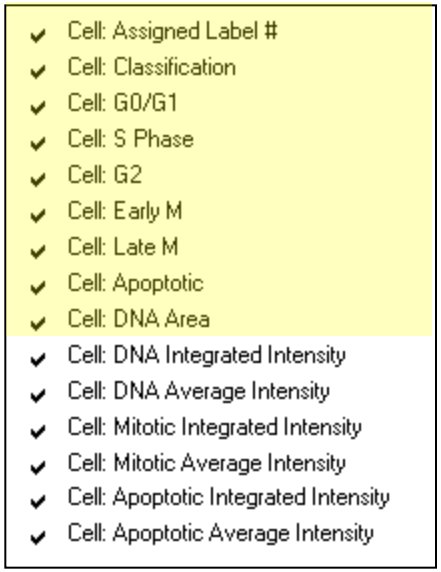
- **G2 Cells:** Number of nuclei classified as G2
- **% G2:** Percentage of nuclei classified as G2

- **Early M Cells:** Number of nuclei classified as early mitotic
- **% Early M:** Percentage of nuclei classified as early mitotic

- **Late M Cells:** Number of nuclei classified as late mitotic
- **% Late M:** Percentage of nuclei classified as late mitotic

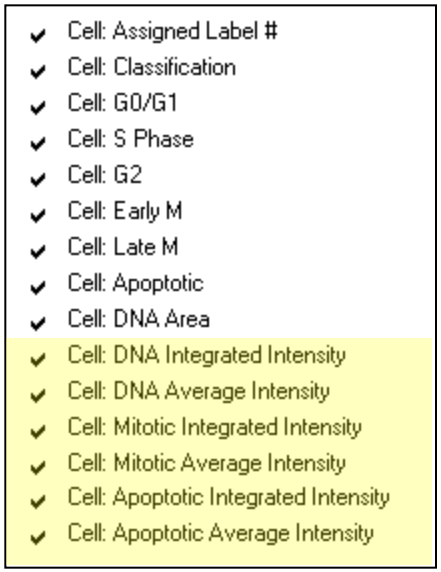
- **Apoptotic Cells:** Number of nuclei classified as apoptotic
- **% Apoptotic:** Percentage of nuclei classified as apoptotic

Cell Data (cell-by-cell measurements)



- **Cell: Assigned Label #** – Cell label number (1 through total cell number)
- **Cell: Classification** – The classification for this cell, i.e. “G0/G1” or “Apoptotic” or “S Phase”
- **Cell: G0/G1** – 1 if the cell is in G0/G1, 0 if it is not
- **Cell: S Phase** – 1 if the cell is in S Phase, 0 if it is not
- **Cell: G2** – 1 if the cell is in G2, 0 if it is not
- **Cell: Early M** – 1 if the cell is early mitotic, 0 if it is not
- **Cell: Late M** – 1 if the cell is late mitotic, 0 if it is not
- **Cell: Apoptotic** – 1 if the cell is apoptotic, 0 if it is not
- **Cell: DNA Area** – Total square microns of the nucleus

Cell Data (cell-by-cell measurements)



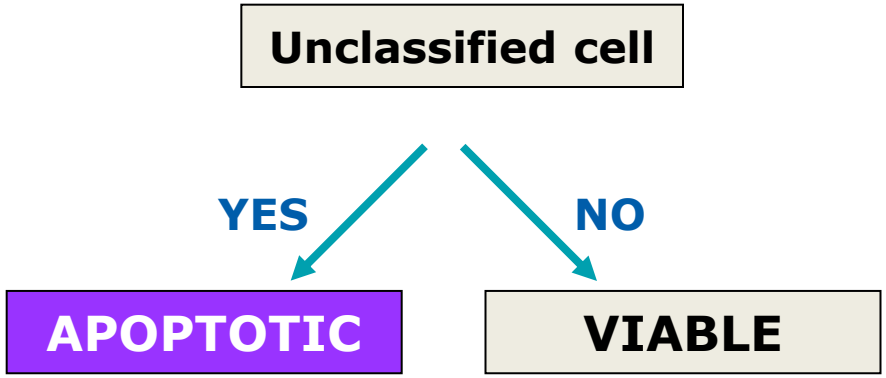
- **Cell: DNA Integrated Intensity** – Total pixel intensity of the nuclear stain in the nucleus
- **Cell: DNA Average Intensity** – Average pixel intensity of the nuclear stain in the nucleus
- **Cell: Mitotic Integrated Intensity** – Total pixel intensity of the mitotic-specific stain overlapping the nucleus
- *Note: appears only if Mitotic-specific staining used*
- **Cell: Mitotic Average Intensity** – Average pixel intensity of the mitotic-specific stain overlapping the nucleus
- *Note: appears only if Mitotic-specific staining used*
- **Cell: Apoptotic Integrated Intensity** – Total pixel intensity of the apoptotic-specific stain overlapping the nucleus
- *Note: appears only if Apoptotic classification used*
- **Cell: Apoptotic Average Intensity** – Average pixel intensity of the apoptotic-specific stain overlapping the nucleus
- *Note: appears only if Apoptotic classification used.*

Cell cycle module – classification strategy

Unclassified cell

Cell cycle module – classification strategy

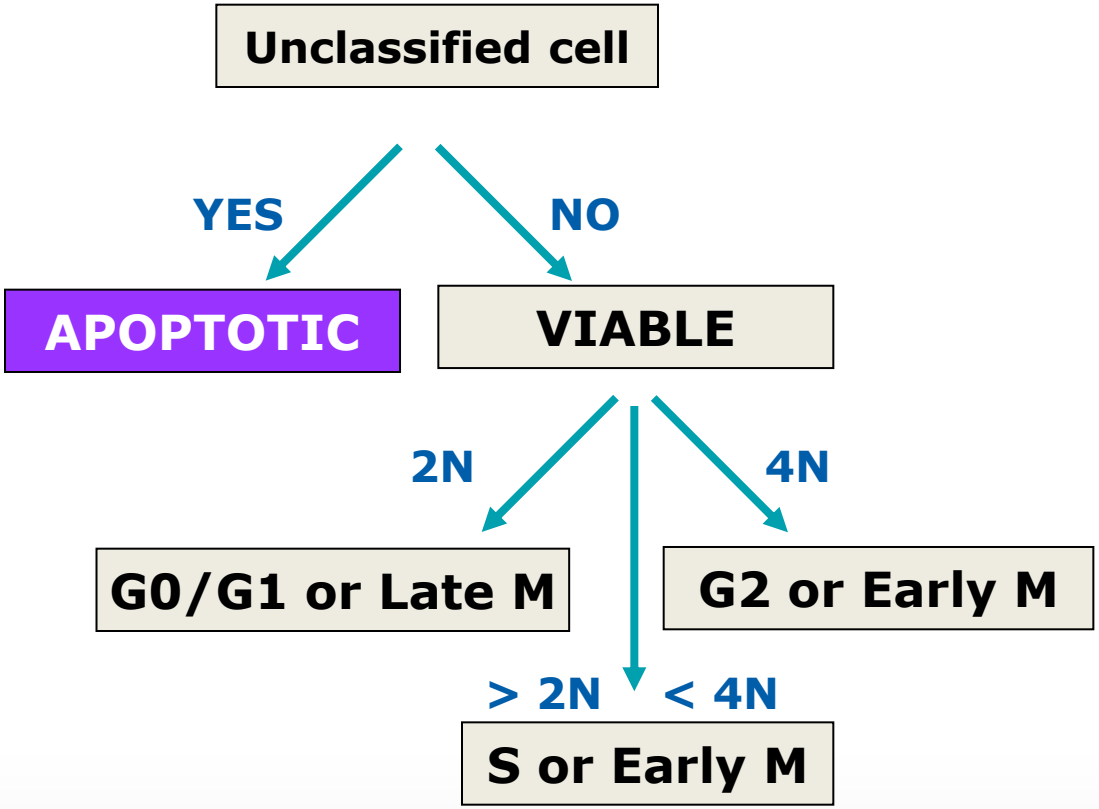
1. (Optional) Is cell positive for apoptotic stain?



Cell cycle module – classification strategy

1. (Optional) Is cell positive for apoptotic stain?

2. What is DNA content (integrated nuclear intensity)?

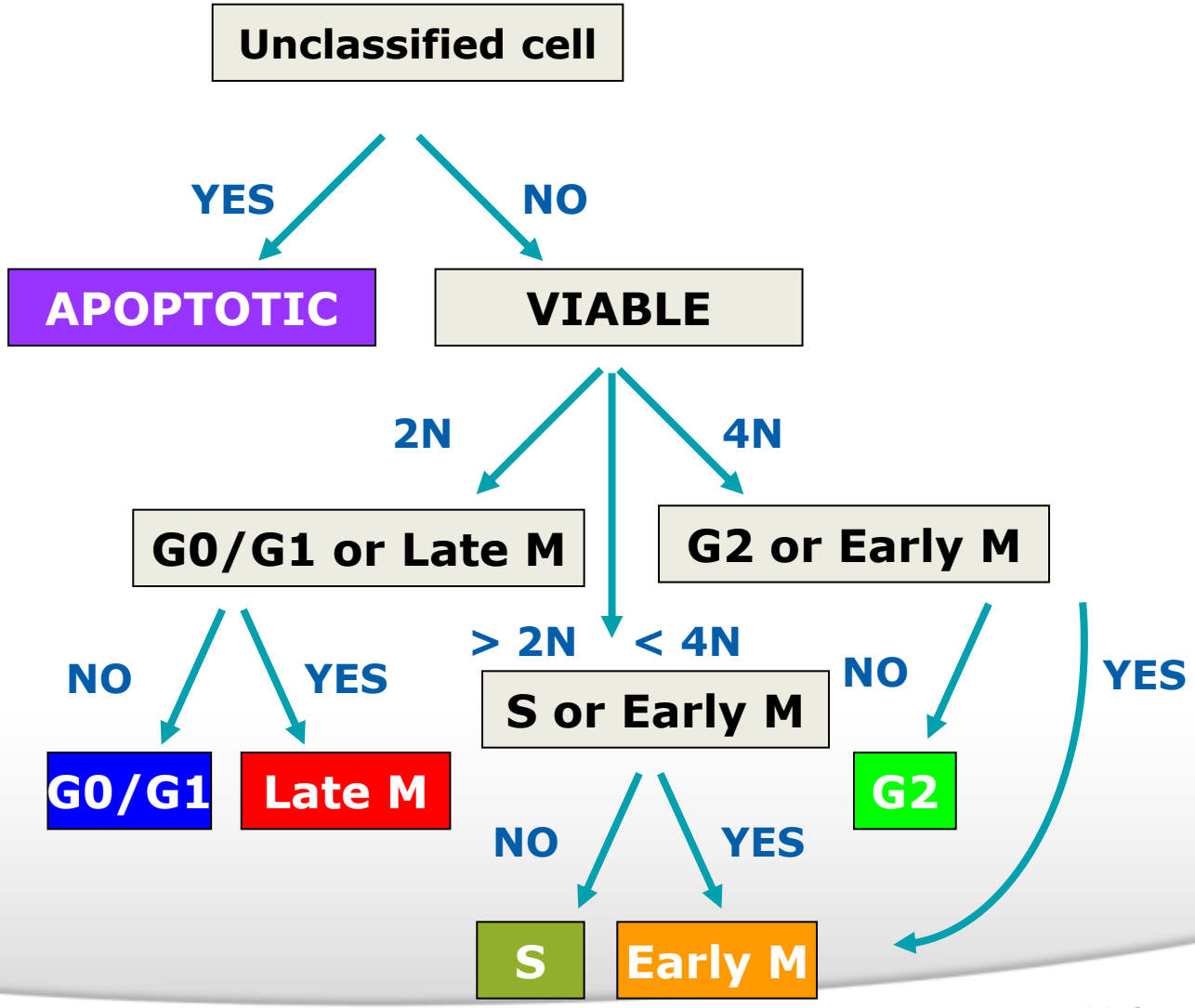


Cell cycle module – classification strategy

1. (Optional) Is cell positive for apoptotic stain?

2. What is DNA content (integrated nuclear intensity)?

3. Is cell mitotic (mitotic stain or average nuclear intensity)?



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