

Together through life sciences.

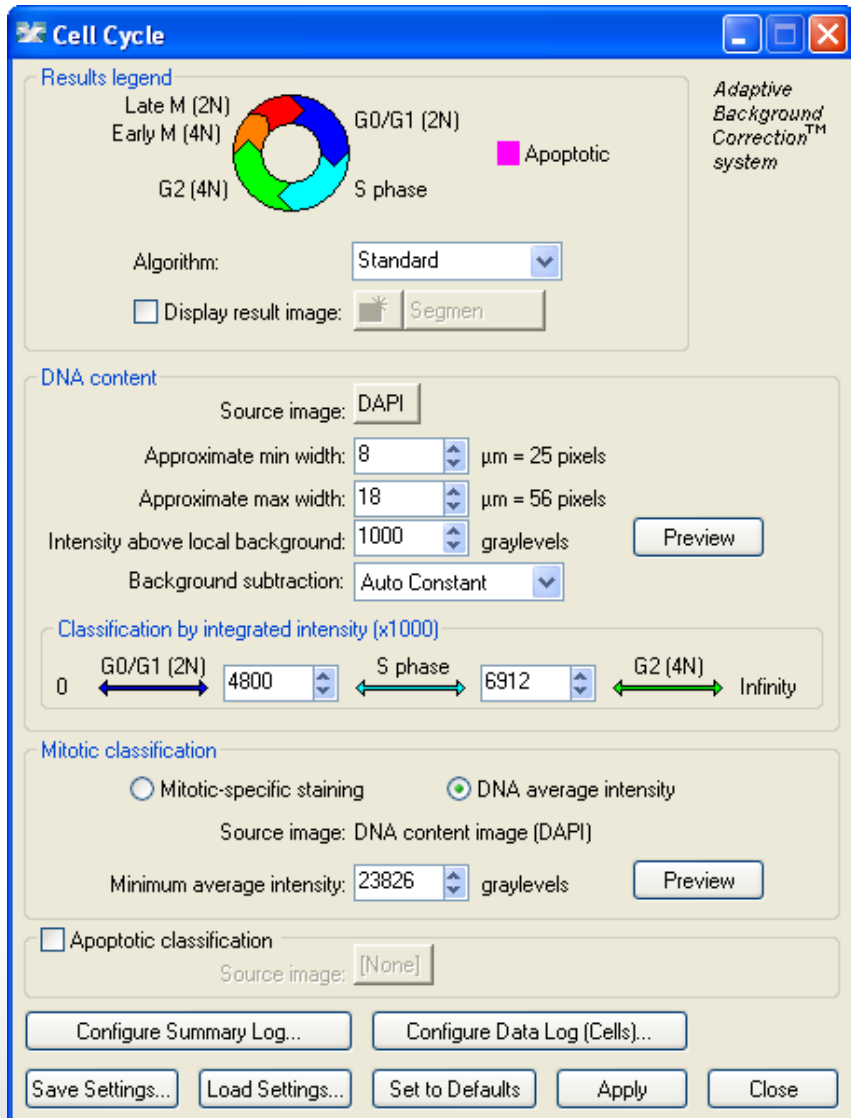
## MetaXpress® Software: *Cell Cycle Module*

Together through life sciences.

©2014 For research use only. Not for use in diagnostic procedures. Trademarks mentioned herein are property of Molecular Devices, LLC or their respective owners.



# 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

**Cell Cycle**

Results legend

Late M (2N)  
Early M (4N)  
G2 (4N)  
S phase  
G0/G1 (2N)  
Apoptotic

Adaptive Background Correction™ system

Algorithm: Standard  
☐ Display result image: Standard  
Fast

DNA content

Source image: DAPI

Approximate min width: 8  $\mu\text{m}$  = 25 pixels  
Approximate max width: 18  $\mu\text{m}$  = 56 pixels  
Intensity above local background: 1000 graylevels  
Background subtraction: Auto Constant

Classification by integrated intensity (x1000)

0  $\longleftrightarrow$  4800  $\longleftrightarrow$  6912  $\longleftrightarrow$  Infinity  
G0/G1 (2N) S phase G2 (4N)

Mitotic classification

☐ Mitotic-specific staining ☒ DNA average intensity

Source image: DNA content image (DAPI)

Minimum average intensity: 3000 graylevels

☐ Apoptotic classification

Source image: FITC

Configure Summary Log... Configure Data Log (Cells)...  
Save Settings... Load Settings... Set to Defaults Apply Close

- **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

**Cell Cycle**

**Results legend**

Late M (2N) Early M (4N) G2 (4N) S phase G0/G1 (2N) Apoptotic

Algorithm: Standard

☒ Display result image: Segmen

**DNA content**

Source image: DAPI

Approximate min width: 8  $\mu\text{m}$  = 25 pixels

Approximate max width: 18  $\mu\text{m}$  = 56 pixels

Intensity above local background: 1000 graylevels

Background subtraction: Auto Constant

**Classification by integrated intensity (x1000)**

0 G0/G1 (2N) 4800 S phase 6912 G2 (4N) Infinity

**Mitotic classification**

☐ Mitotic-specific staining ☒ DNA average intensity

Source image: DNA content image (DAPI)

Minimum average intensity: 23826 graylevels

**Apoptotic classification**

☐ Apoptotic classification

Source image: [None]

Configure Summary Log... Configure Data Log (Cells)...

Save Settings... Load Settings... Set to Defaults Apply Close

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

## 2. Module Settings – DNA content

**Cell Cycle**

**Results legend**

Late M (2N) Early M (4N) G0/G1 (2N) G2 (4N) S phase Apoptotic

Adaptive Background Correction™ system

Algorithm: Standard

☐ Display result image: Segmen

**DNA content**

Source image: DAPI

Approximate min width: 8  $\mu\text{m}$  = 25 pixels

Approximate max width: 18  $\mu\text{m}$  = 56 pixels

Intensity above local background: 1000 graylevels

Background subtraction: Auto Constant

Preview

**Classification by integrated intensity (x1000)**

0 G0/G1 (2N) 4800 S phase 6912 G2 (4N) Infinity

**Mitotic classification**

☐ Mitotic-specific staining ☒ DNA average intensity

Source image: DNA content image (DAPI)

Minimum average intensity: 23826 graylevels

Preview

☐ Apoptotic classification

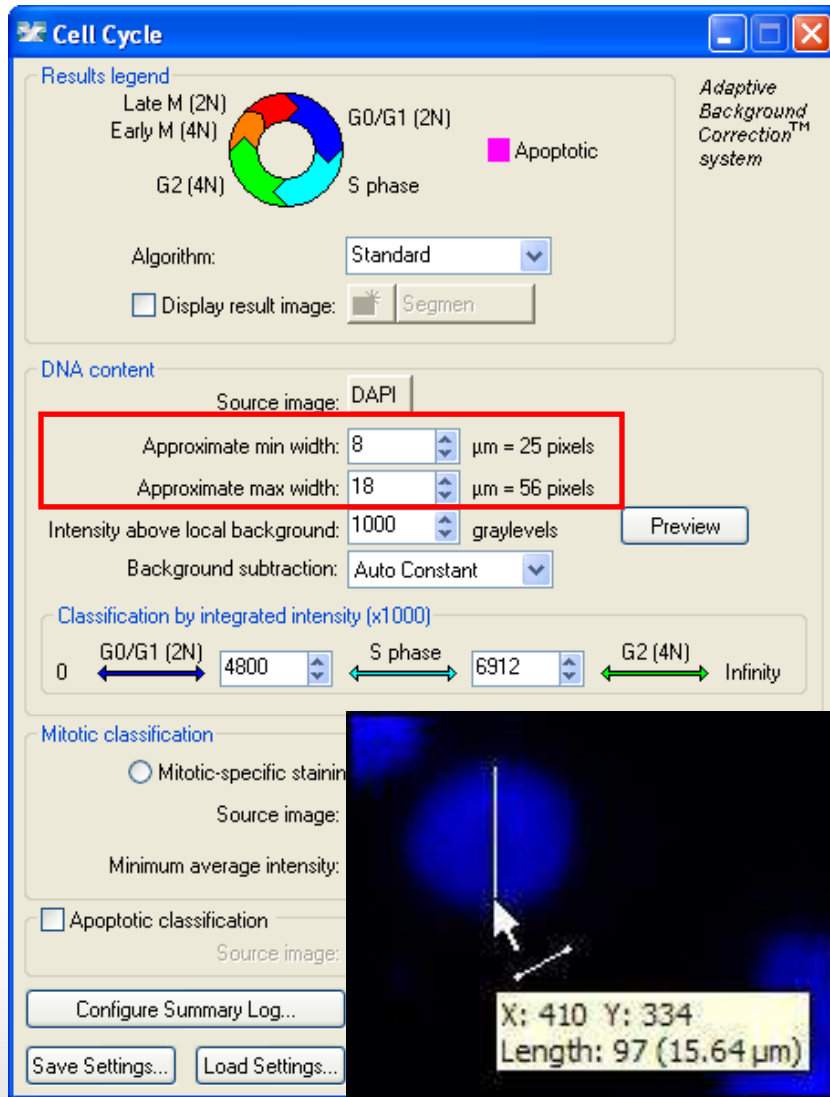
Source image: [None]

Configure Summary Log... Configure Data Log (Cells)...

Save Settings... Load Settings... Set to Defaults Apply Close

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

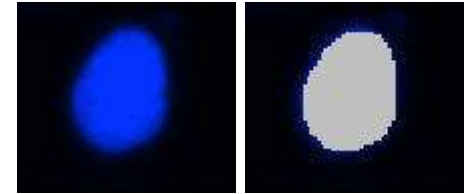
# 3. Module Settings – width settings



- 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  $\mu\text{m}$ ).
- 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

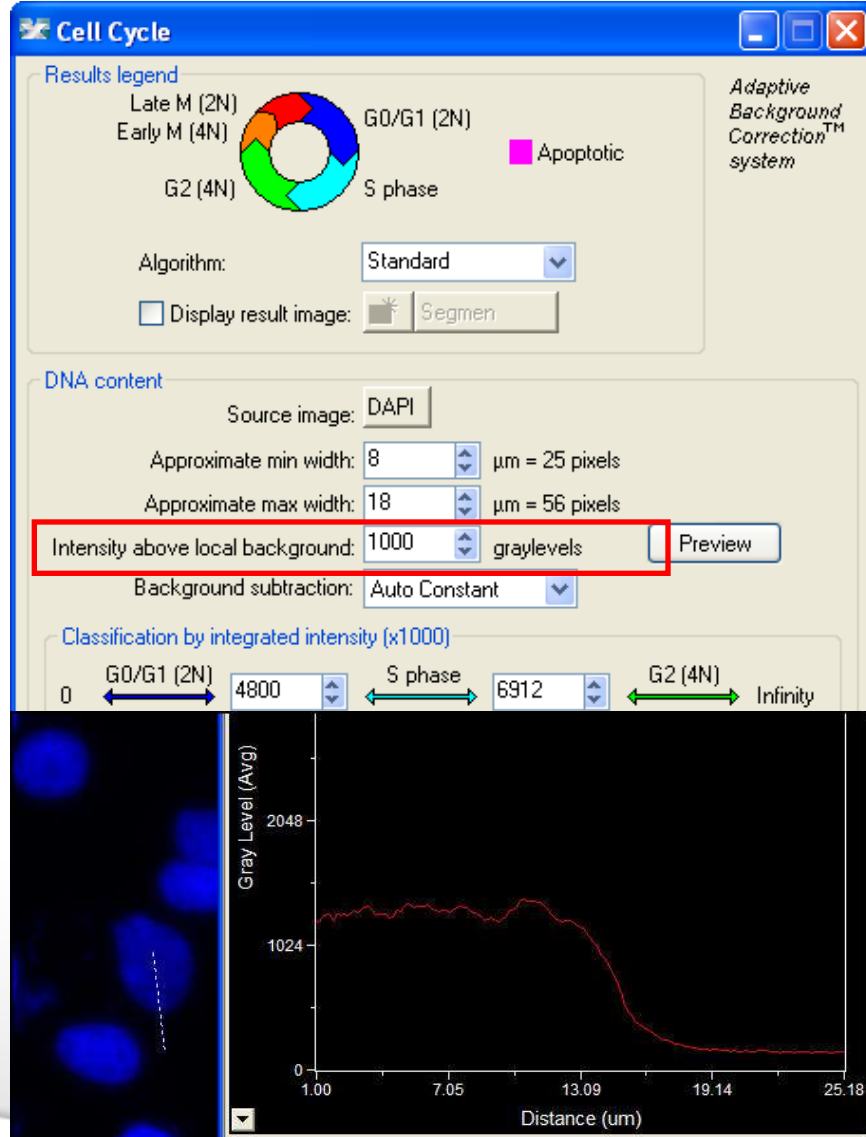


Max width too small: may shrink nuclear boundaries



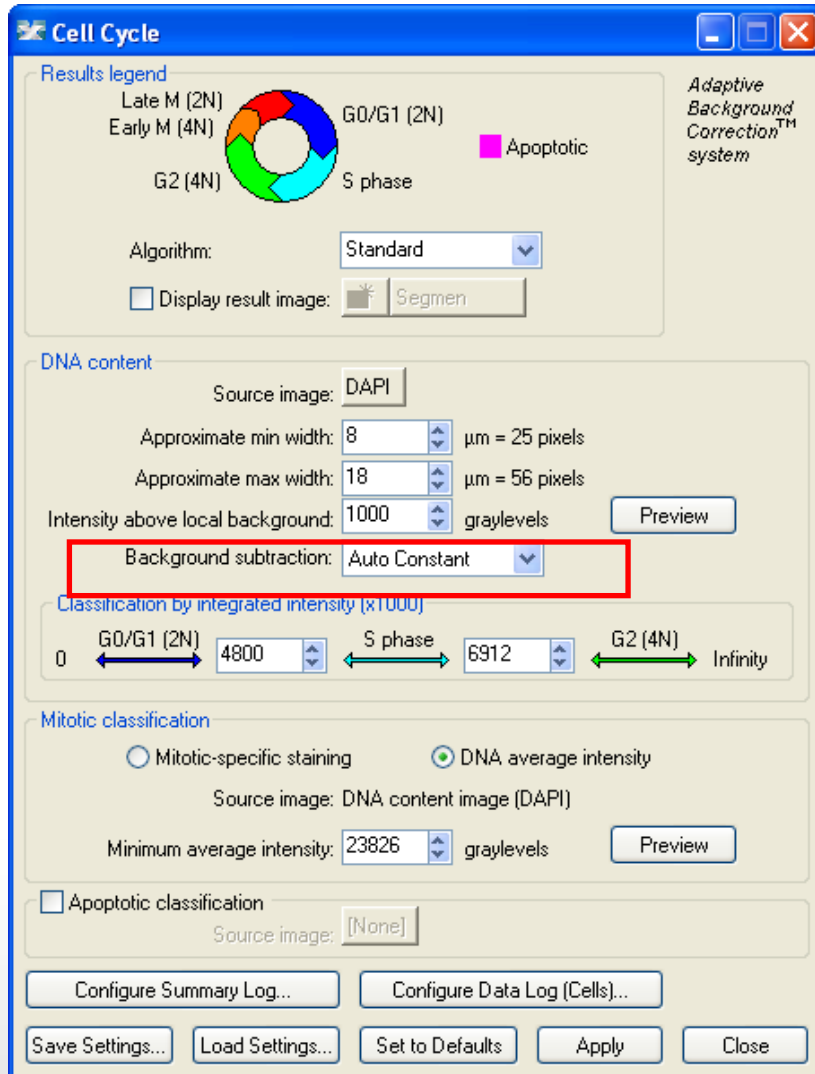
Max width too large: may slightly enlarge nuclear boundaries

# 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

**Cell Cycle**

**Results legend**

Late M (2N) Early M (4N) G2 (4N) S phase G0/G1 (2N) Apoptotic

Algorithm: Standard

☐ Display result image: Segmen

*Adaptive Background Correction™ system*

**DNA content**

Source image: DAPI

Approximate min width: 8  $\mu\text{m} = 25$  pixels

Approximate max width: 18  $\mu\text{m} = 56$  pixels

Intensity above local background: 1000 graylevels

Background subtraction: Auto Constant

**Classification by integrated intensity (x1000)**

0 G0/G1 (2N) 4800 S phase 6912 G2 (4N) Infinity

**Mitotic classification**

☐ Mitotic-specific staining ☒ DNA average intensity

Source image: DNA content image (DAPI)

Minimum average intensity: 23826 graylevels

☐ Apoptotic classification

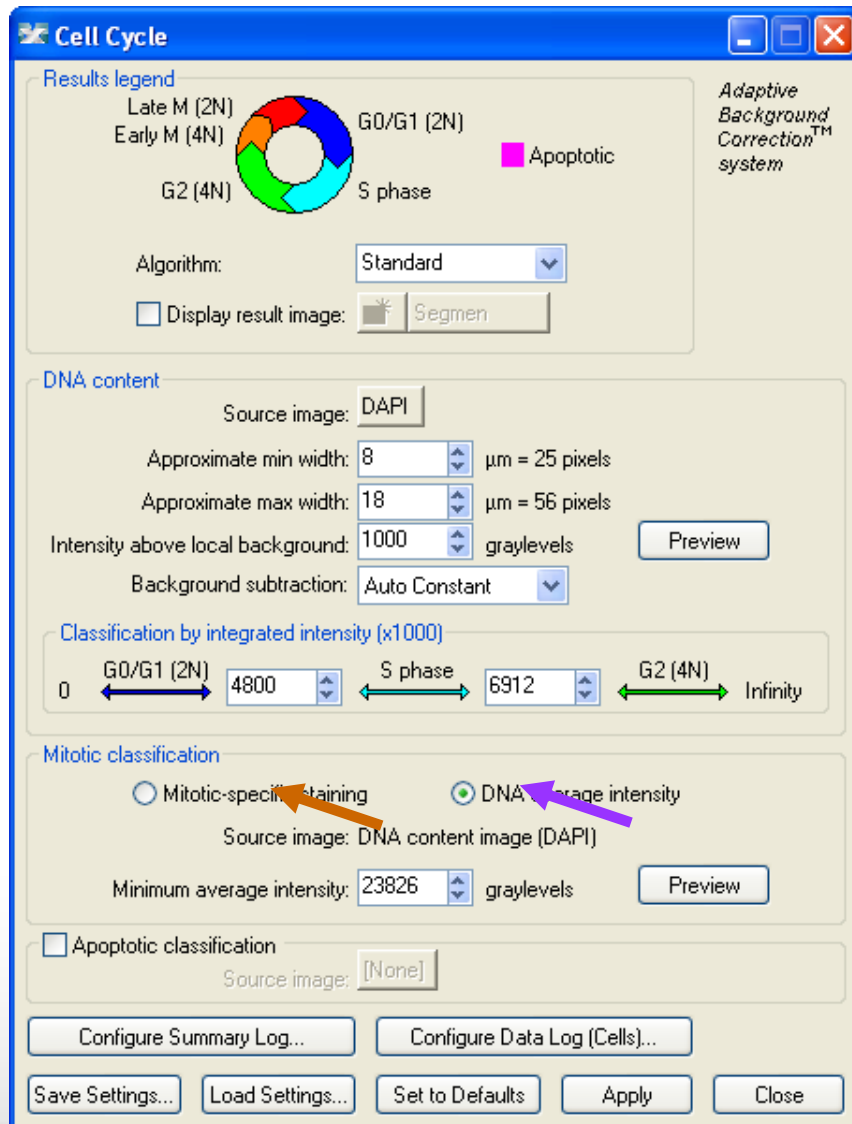
Source image: [None]

Configure Summary Log... Configure Data Log (Cells)...

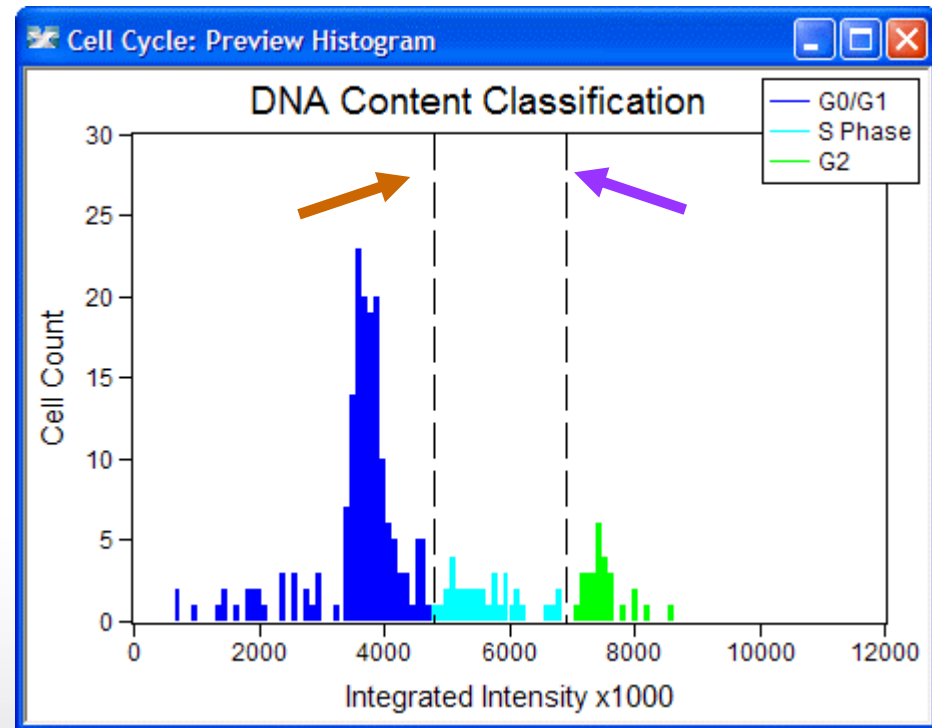
Save Settings... Load Settings... Set to Defaults Apply Close

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

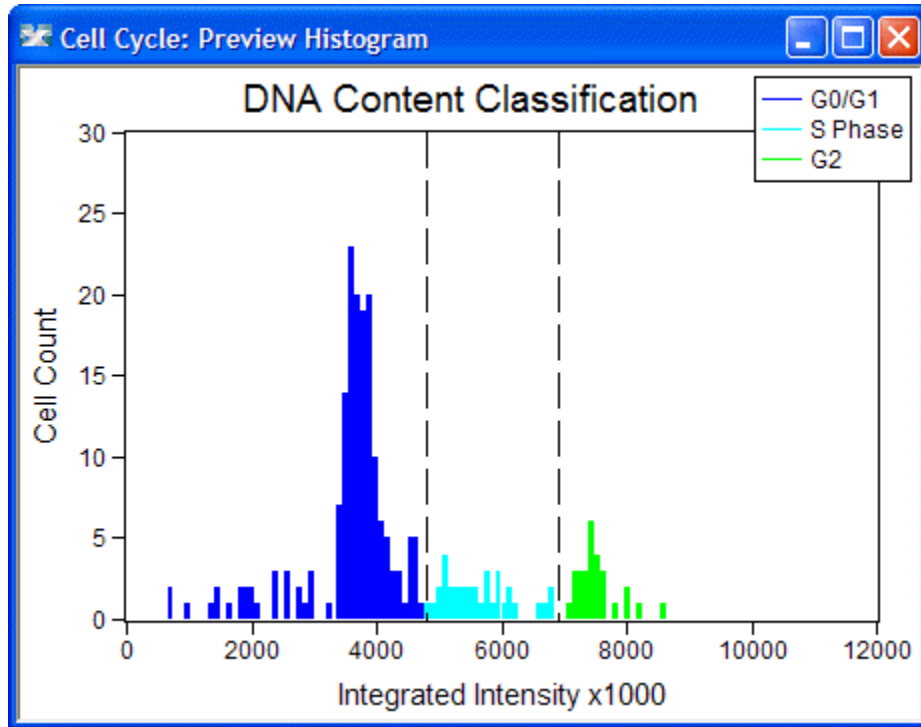
# 6. Module Settings - DNA content classification



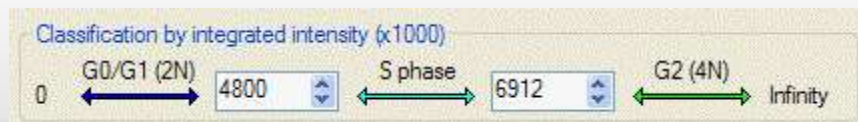
- An interactive graph is shown to adjust the cutoffs for classification.



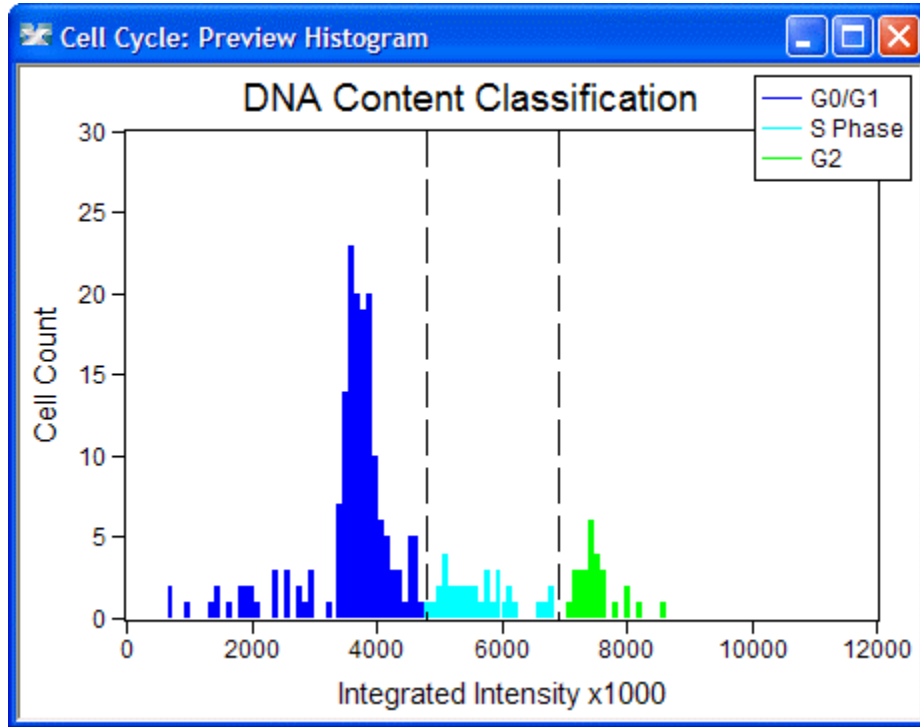
# 6. Module Settings - DNA content classification



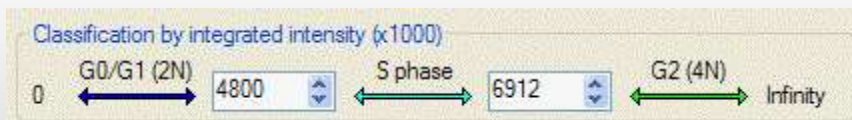
- The **integrated intensity** of the nuclear stain is used to quantify DNA content (similar to flow cytometry cell cycle analysis).
- Cells in G0 or G1 or late mitosis have 2N DNA content.
- Cells in G2 or early mitosis have 4N DNA content.
- Cells in S phase have DNA content in between 2N and 4N.



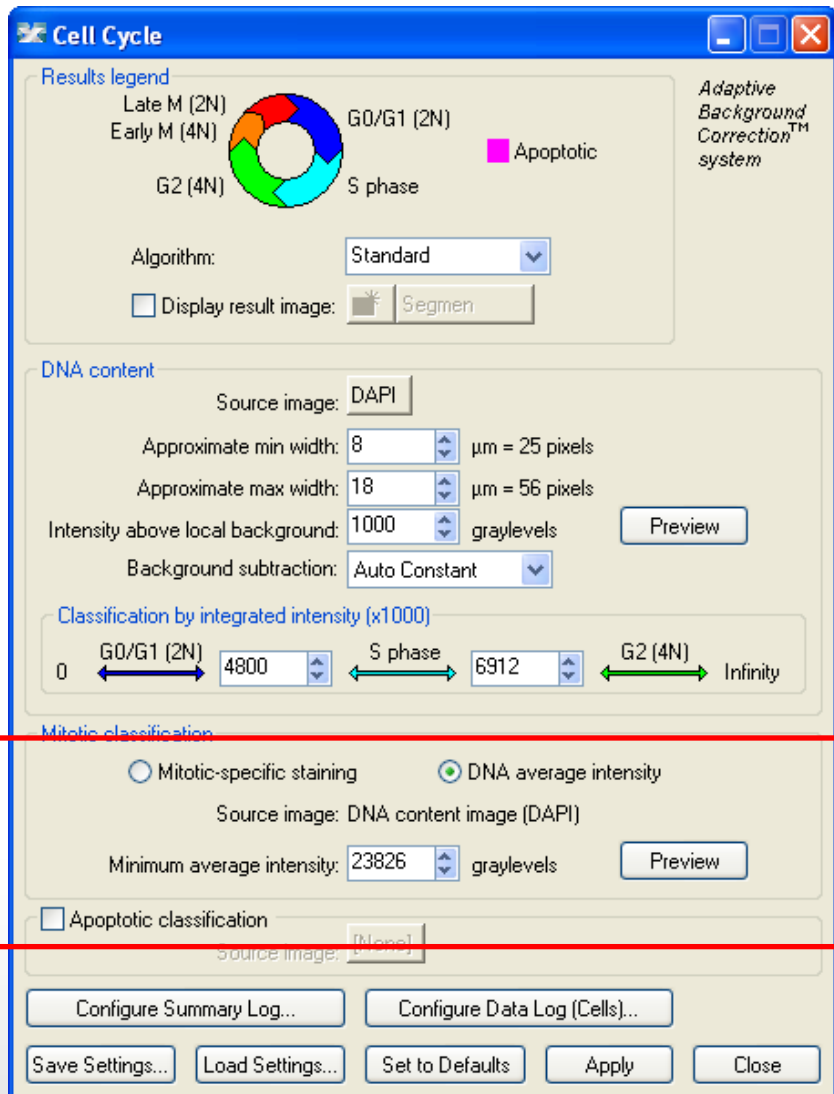
# 6. Module Settings - DNA content classification



- **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

**Cell Cycle**

**Results legend**

Late M (2N)  
Early M (4N)  
G2 (4N)  
S phase  
G0/G1 (2N)  
Apoptotic

Adaptive Background Correction™ system

Algorithm: Standard

☐ Display result image: Segmen

**DNA content**

Source image: DAPI

Approximate min width: 8  $\mu\text{m}$  = 25 pixels

Approximate max width: 18  $\mu\text{m}$  = 56 pixels

Intensity above local background: 1000 graylevels

Background subtraction: Auto Constant

**Classification by integrated intensity (x1000)**

0 G0/G1 (2N) 4800 S phase 6912 G2 (4N) Infinity

**Mitotic classification**

☐ Mitotic-specific staining ☒ DNA average intensity

Source image: DNA content image (DAPI)

Minimum average intensity: 23826 graylevels

☐ Apoptotic classification

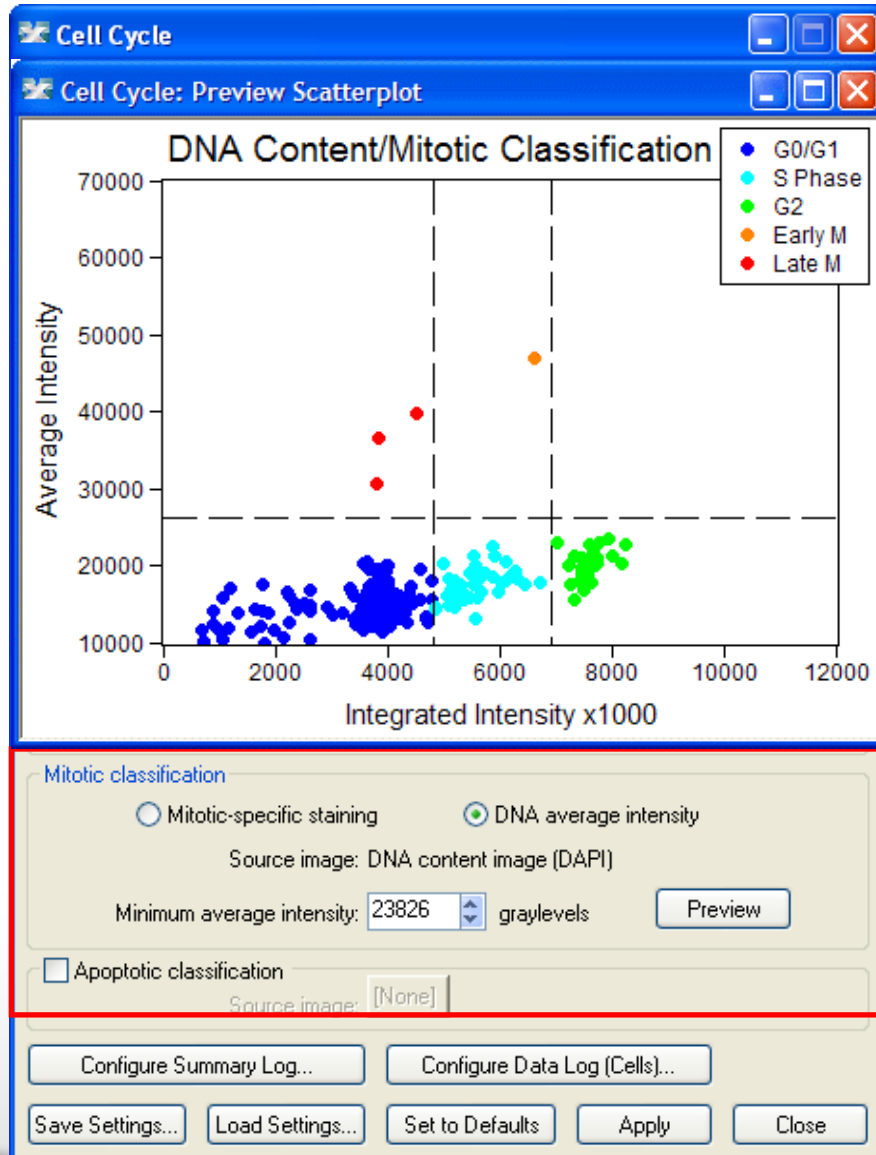
Source image: [None]

Configure Summary Log... Configure Data Log (Cells)...

Save Settings... Load Settings... Set to Defaults Apply Close

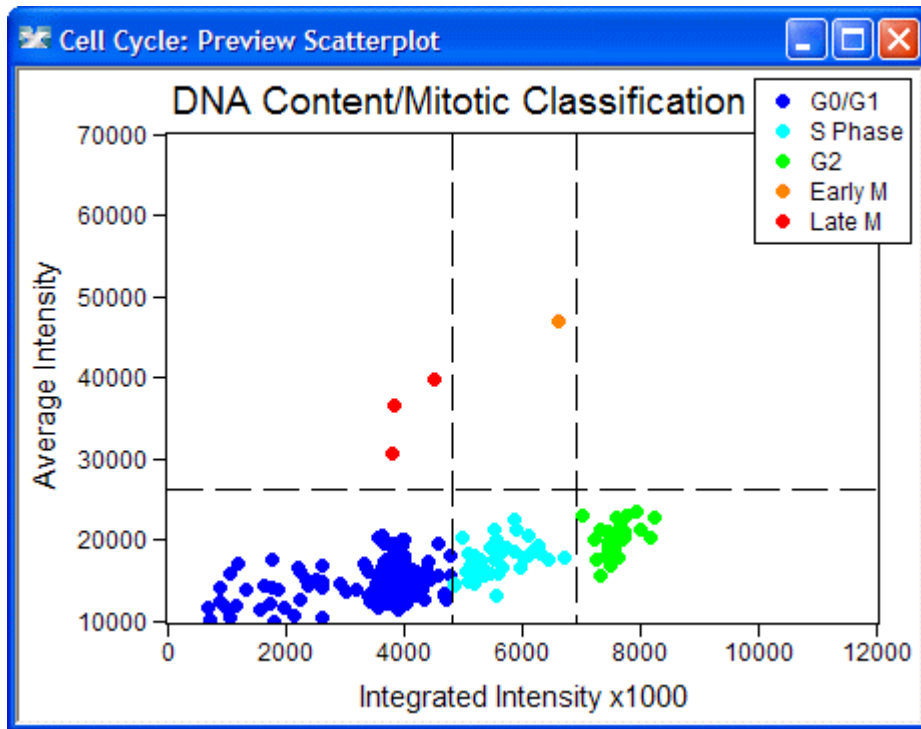
- **DNA average intensity**
- To use the DNA average intensity as a mitotic classifier, simply press Preview.

# 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



Mitotic classification

☐ Mitotic-specific staining ☒ DNA average intensity

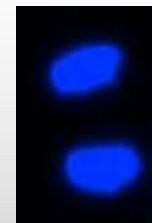
Source image: DNA content image (DAPI)

Minimum average intensity: 26296 graylevels

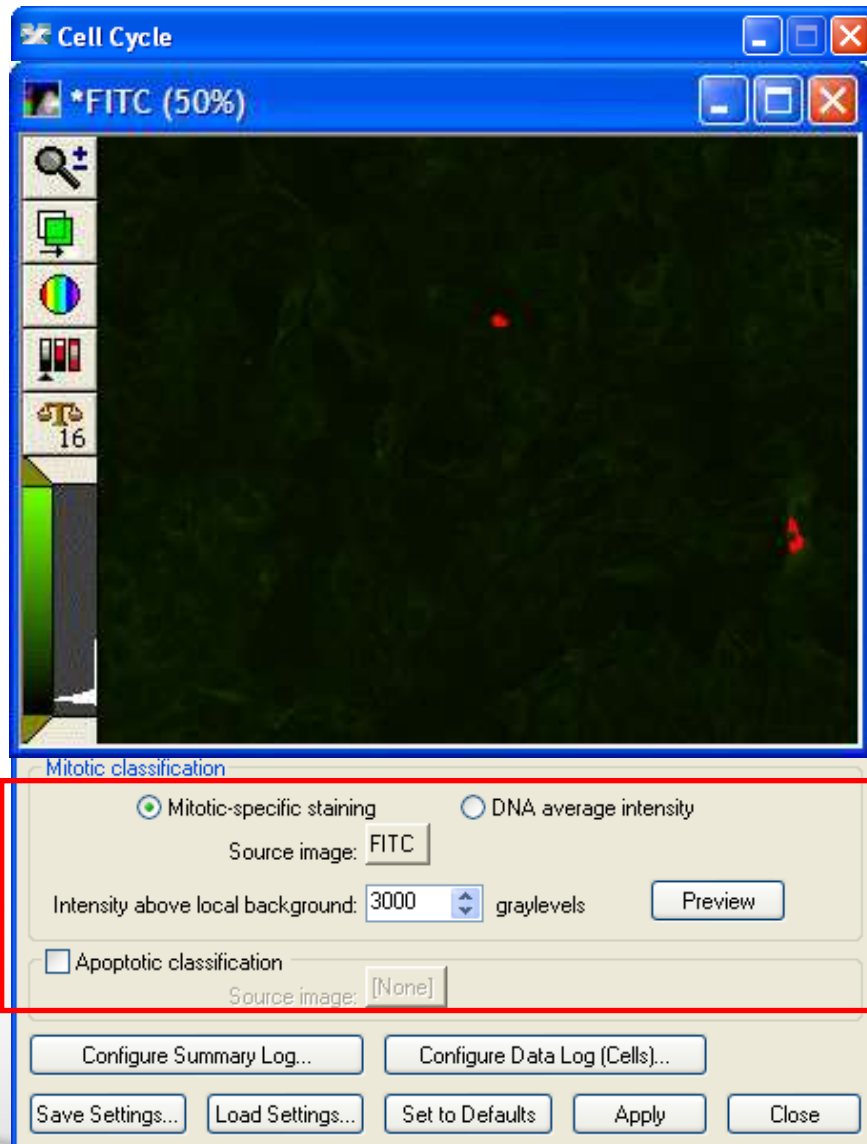
Preview

- **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

**Cell Cycle**

**Results legend**

Late M (2N) Early M (4N) G0/G1 (2N) G2 (4N) S phase Apoptotic

Algorithm: Standard

☐ Display result image: Segmen

**DNA content**

Source image: DAPI

Approximate min width: 8  $\mu\text{m}$  = 25 pixels

Approximate max width: 18  $\mu\text{m}$  = 56 pixels

Intensity above local background: 1000 graylevels

Background subtraction: Auto Constant

**Classification by integrated intensity (x1000)**

0 G0/G1 (2N) 4800 S phase 6912 G2 (4N) Infinity

**Mitotic classification**

☐ Mitotic-specific staining ☒ DNA average intensity

Source image: DNA content image (DAPI)

Minimum average intensity: 23826 graylevels

☐ Apoptotic classification

Source image: [None]

Configure Summary Log... Configure Data Log (Cells)...

Save Settings... Load Settings... Set to Defaults Apply Close

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

# 8. Module Settings – apoptotic classification

The screenshot shows the 'Cell Cycle' software window. The 'Results legend' at the top left displays a circular diagram with segments for Late M (2N), Early M (4N), G2 (4N), S phase, and G0/G1 (2N). A pink square indicates 'Apoptotic'. The 'Algorithm' is set to 'Standard'. The 'Display result image' checkbox is unchecked, and the 'Segment' button is visible. The 'DNA content' section includes settings for 'Source image' (DAPI), 'Approximate min width' (8  $\mu\text{m}$  = 25 pixels), 'Approximate max width' (18  $\mu\text{m}$  = 56 pixels), 'Intensity above local background' (1000 graylevels), and 'Background subtraction' (Auto Constant). The 'Classification by integrated intensity (x1000)' section shows a scale from 0 to Infinity with markers for G0/G1 (2N) at 4800, S phase at 6912, and G2 (4N). The 'Mitotic classification' section has two radio buttons: 'Mitotic-specific staining' (unselected) and 'DNA average intensity' (selected). The 'Source image' is 'DNA content image (DAPI)' and the 'Minimum average intensity' is 3000 graylevels. The 'Apoptotic classification' section is checked, with 'Source image' set to 'FITC'. The 'Stained area' is 'Nucleus'. The 'Approximate min width' is 5  $\mu\text{m}$  = 16 pixels, and the 'Approximate max width' is 18  $\mu\text{m}$  = 56 pixels. The 'Intensity above local background' is 3000 graylevels. A red rectangle highlights the 'Apoptotic classification' section. At the bottom, there are buttons for 'Configure Summary Log...', 'Configure Data Log (Cells)', 'Save Settings...', 'Load Settings...', 'Set to Defaults', 'Apply', and 'Close'.

**Cell Cycle**

Results legend

Late M (2N)  
Early M (4N)  
G2 (4N)  
S phase  
G0/G1 (2N)  
Apoptotic

Algorithm: Standard

☐ Display result image: Segment

DNA content

Source image: DAPI

Approximate min width: 8  $\mu\text{m}$  = 25 pixels

Approximate max width: 18  $\mu\text{m}$  = 56 pixels

Intensity above local background: 1000 graylevels

Background subtraction: Auto Constant

Preview

Classification by integrated intensity (x1000)

0 G0/G1 (2N) 4800 S phase 6912 G2 (4N) Infinity

Mitotic classification

☐ Mitotic-specific staining ☒ DNA average intensity

Source image: DNA content image (DAPI)

Minimum average intensity: 3000 graylevels

Preview

☒ Apoptotic classification

Source image: FITC

Stained area: Nucleus

Approximate min width: 5  $\mu\text{m}$  = 16 pixels

Approximate max width: 18  $\mu\text{m}$  = 56 pixels

Intensity above local background: 3000 graylevels

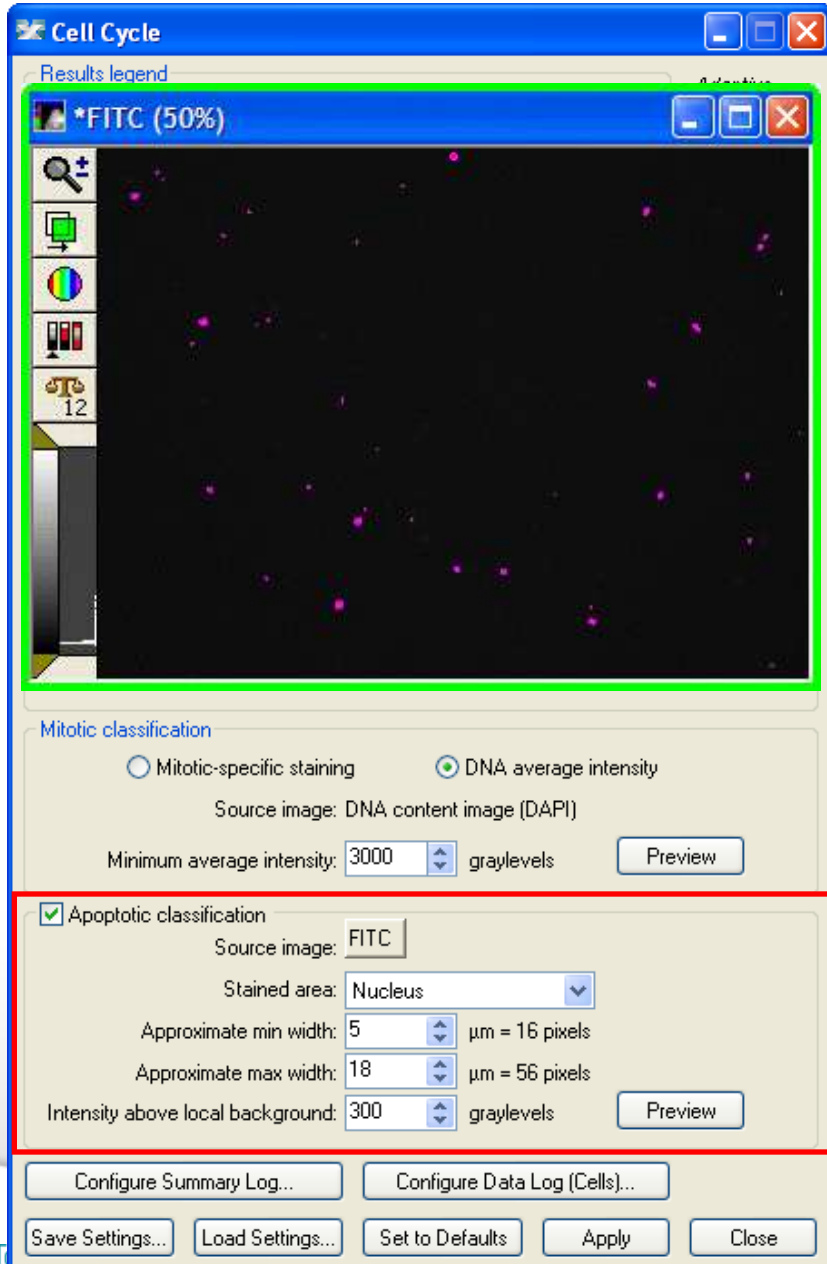
Preview

Configure Summary Log... Configure Data Log (Cells)

Save Settings... Load Settings... Set to Defaults Apply Close

- 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.

# 9. Module Settings – final settings

**Cell Cycle**

**Results legend**

Late M (2N) Early M (4N) G0/G1 (2N) S phase G2 (4N) Apoptotic

Adaptive Background Correction™

Algorithm: Standard

☐ Display result image: Segmen

**DNA content**

Source image: DAPI

Approximate min width: 8  $\mu\text{m}$  = 25 pixels

Approximate max width: 18  $\mu\text{m}$  = 56 pixels

Intensity above local background: 1000 graylevels

Background subtraction: Auto Constant

Preview

**Classification by integrated intensity (x1000)**

0 G0/G1 (2N) 4800 S phase 6912 G2 (4N) Infinity

**Mitotic classification**

☐ Mitotic-specific staining ☒ DNA average intensity

Source image: DNA content image (DAPI)

Minimum average intensity: 3000 graylevels

Preview

☒ Apoptotic classification

Source image: FITC

Stained area: Nucleus

Approximate min width: 5  $\mu\text{m}$  = 16 pixels

Approximate max width: 18  $\mu\text{m}$  = 56 pixels

Intensity above local background: 300 graylevels

Preview

Configure Summary Log... Configure Data Log (Cells)...

Save Settings... Load Settings... Set to Defaults Apply Close

- **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
- ✓ DNA Background Value
- ✓ G0/G1 Cells
- ✓ % G0/G1
- ✓ S Phase Cells
- ✓ % S Phase
- ✓ G2 Cells
- ✓ % G2
- ✓ Early M Cells
- ✓ % Early M
- ✓ Late M Cells
- ✓ % Late M
- ✓ Apoptotic Cells
- ✓ % Apoptotic

- **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: Classification
- ✓ Cell: G0/G1
- ✓ Cell: S Phase
- ✓ Cell: G2
- ✓ Cell: Early M
- ✓ Cell: Late M
- ✓ Cell: Apoptotic
- ✓ Cell: DNA Area
- ✓ Cell: DNA Integrated Intensity
- ✓ Cell: DNA Average Intensity
- ✓ Cell: Mitotic Integrated Intensity
- ✓ Cell: Mitotic Average Intensity
- ✓ Cell: Apoptotic Integrated Intensity
- ✓ Cell: Apoptotic Average Intensity

- **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: Assigned Label #
- ✓ Cell: Classification
- ✓ Cell: G0/G1
- ✓ Cell: S Phase
- ✓ Cell: G2
- ✓ Cell: Early M
- ✓ Cell: Late M
- ✓ Cell: Apoptotic
- ✓ Cell: DNA Area
- ✓ Cell: DNA Integrated Intensity
- ✓ Cell: DNA Average Intensity
- ✓ Cell: Mitotic Integrated Intensity
- ✓ Cell: Mitotic Average Intensity
- ✓ Cell: Apoptotic Integrated Intensity
- ✓ Cell: Apoptotic Average Intensity

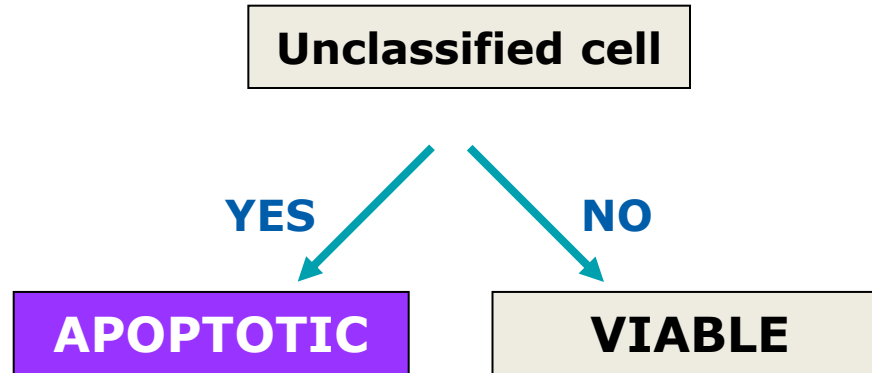
- **Cell: DNA Integrated Intensity** – Total pixel intensity of the nuclear stain in the nucleus
- **Cell: DNA Average Intensity** – Average pixel intensity of the nuclear stain in the nucleus
- **Cell: Mitotic Integrated Intensity** – Total pixel intensity of the mitotic-specific stain overlapping the 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

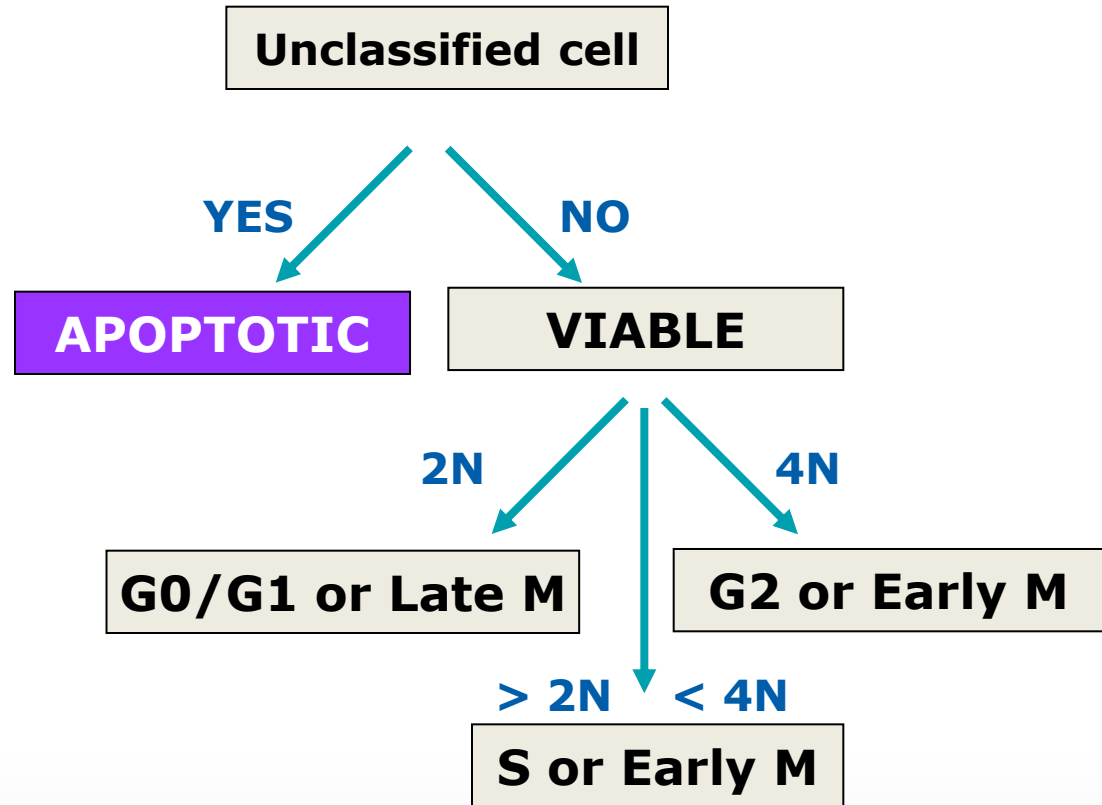
1. (Optional) Is cell positive for apoptotic stain?



# Cell cycle module – classification strategy

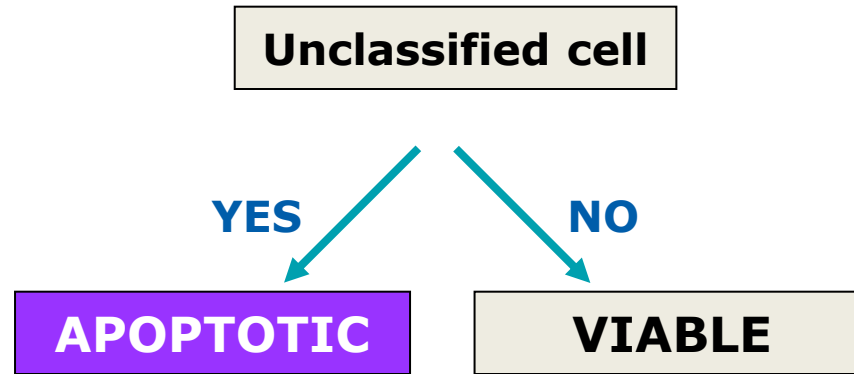
1. (Optional) Is cell positive for apoptotic stain?

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

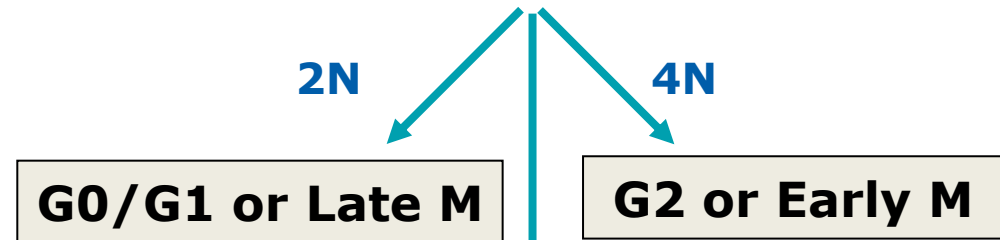


# Cell cycle module – classification strategy

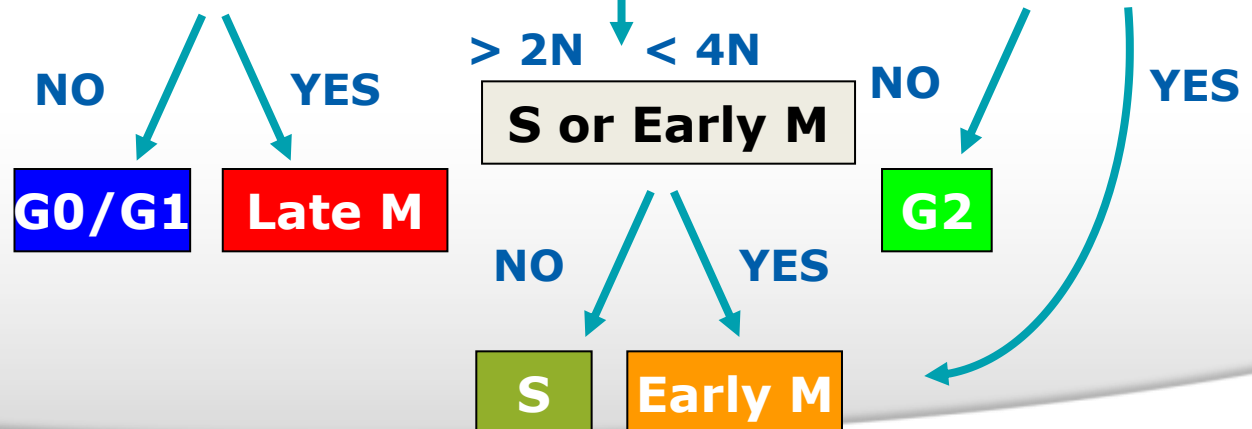
1. (Optional) Is cell positive for apoptotic stain?



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



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





Together through life sciences.

[www.moleculardevices.com](http://www.moleculardevices.com)