

**Molecular
Devices**
Together through life sciences.

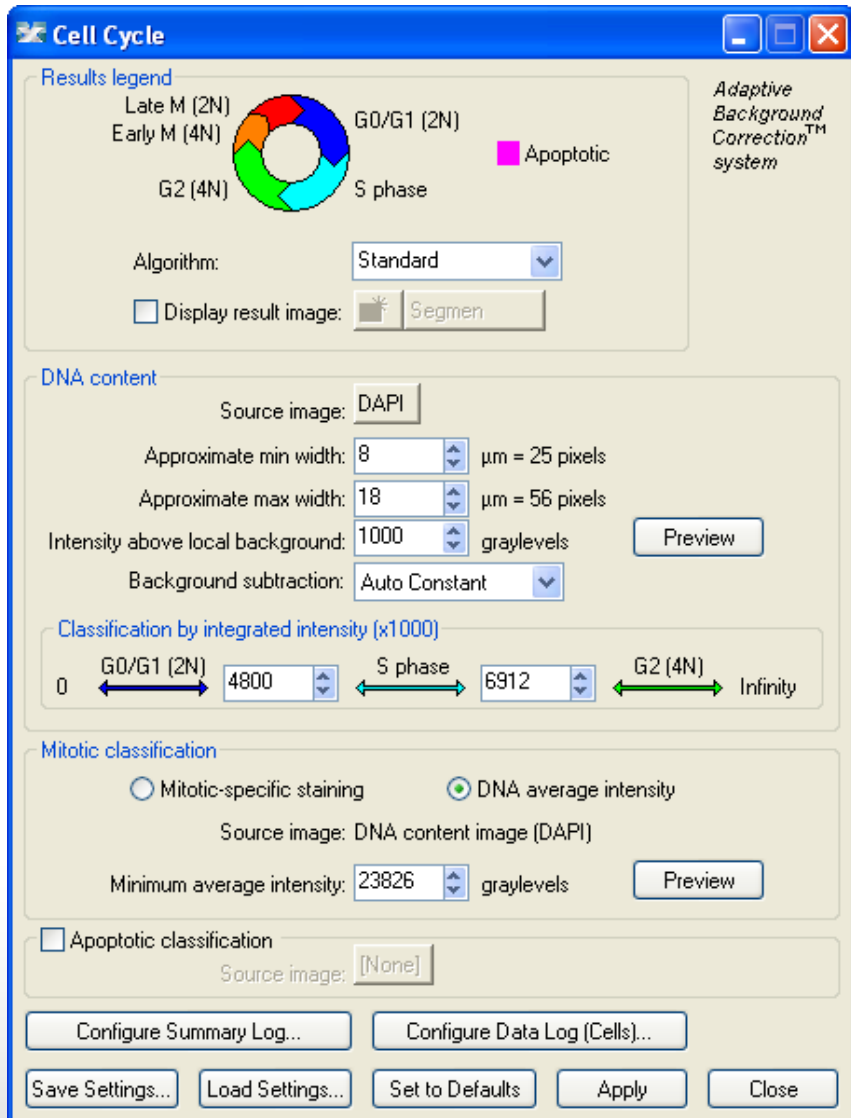
MetaXpress® Software: *Cell Cycle Module*

Together through life sciences.

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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) G0/G1 (2N) S phase 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

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

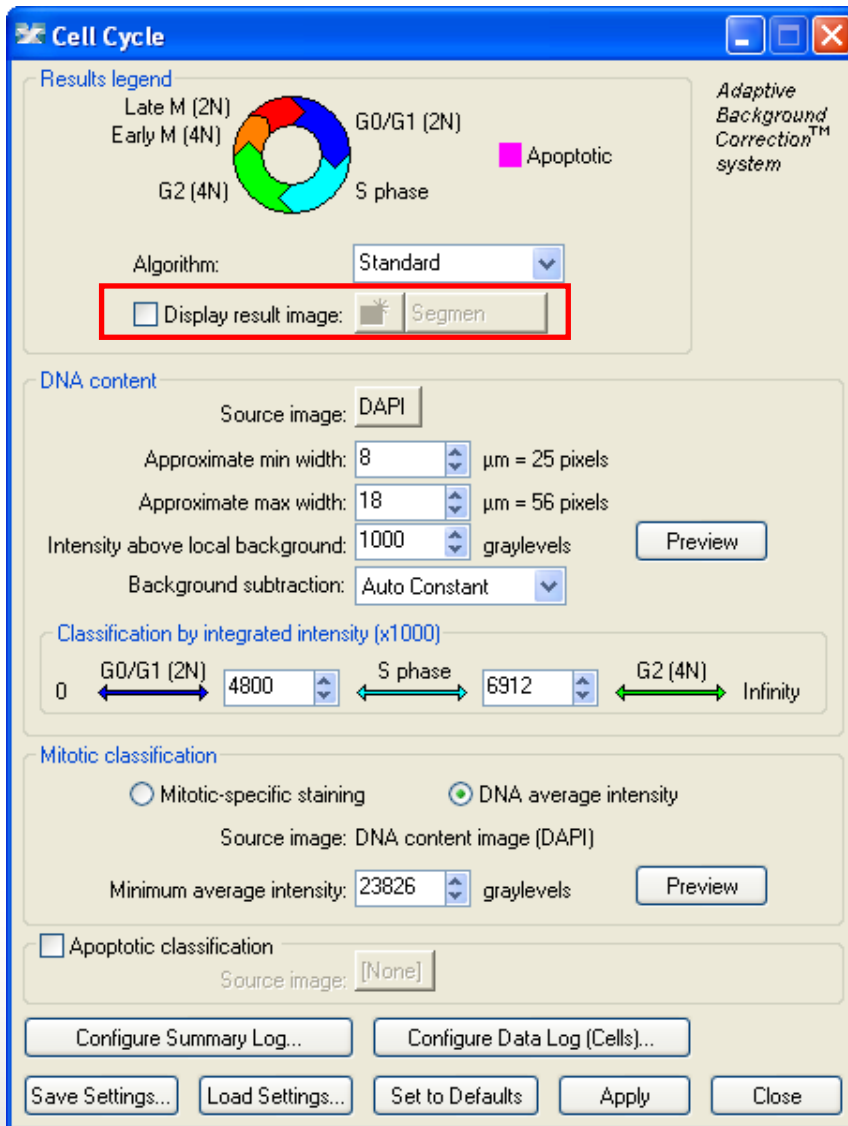
Source image: FITC

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



- 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) G2 (4N) G0/G1 (2N) 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

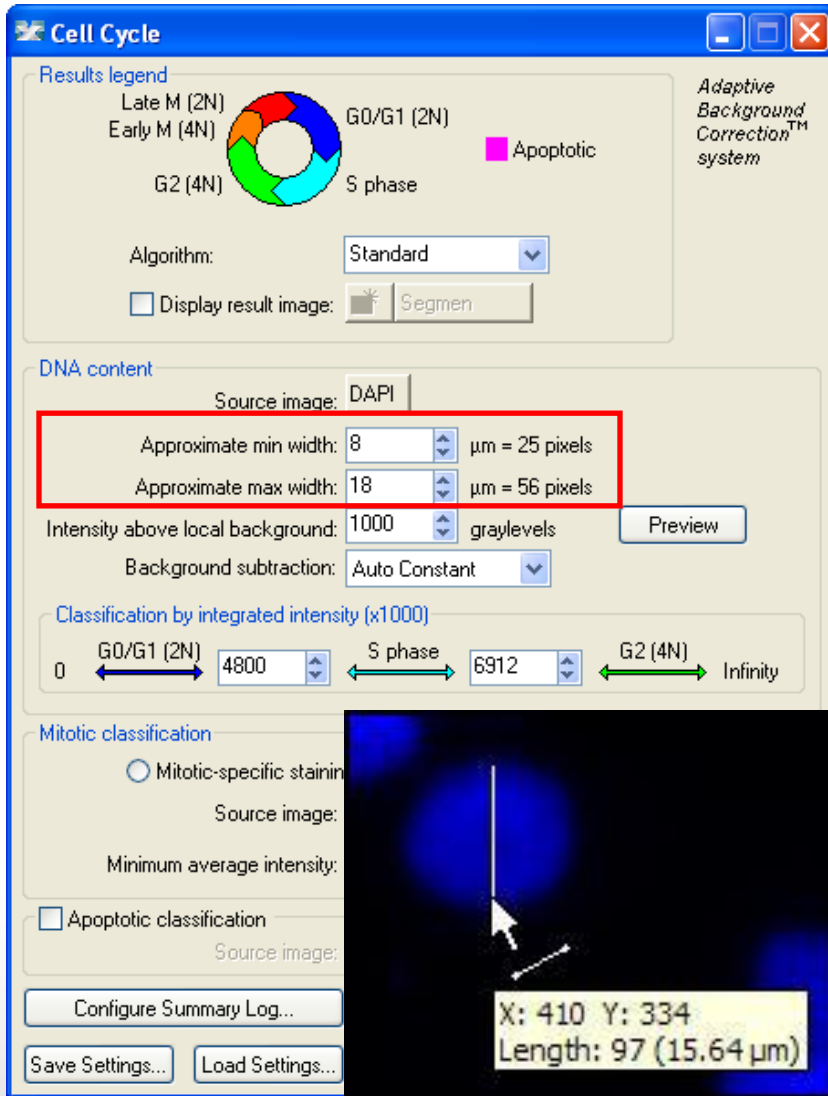
Source image: [None]

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

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

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

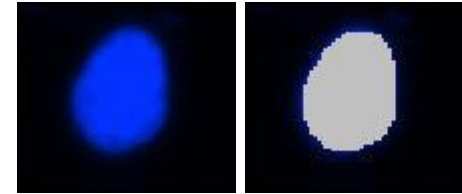
3. Module Settings – width settings



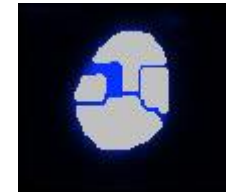
- Set the **Approximate min width** and **Approximate max width** for the range of nuclei that you want to detect
- The width is the short axis of a nucleus (in μm).
- The region tools can be used to measure widths
- Much smaller cells will be ignored
- Much larger cells will be split

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



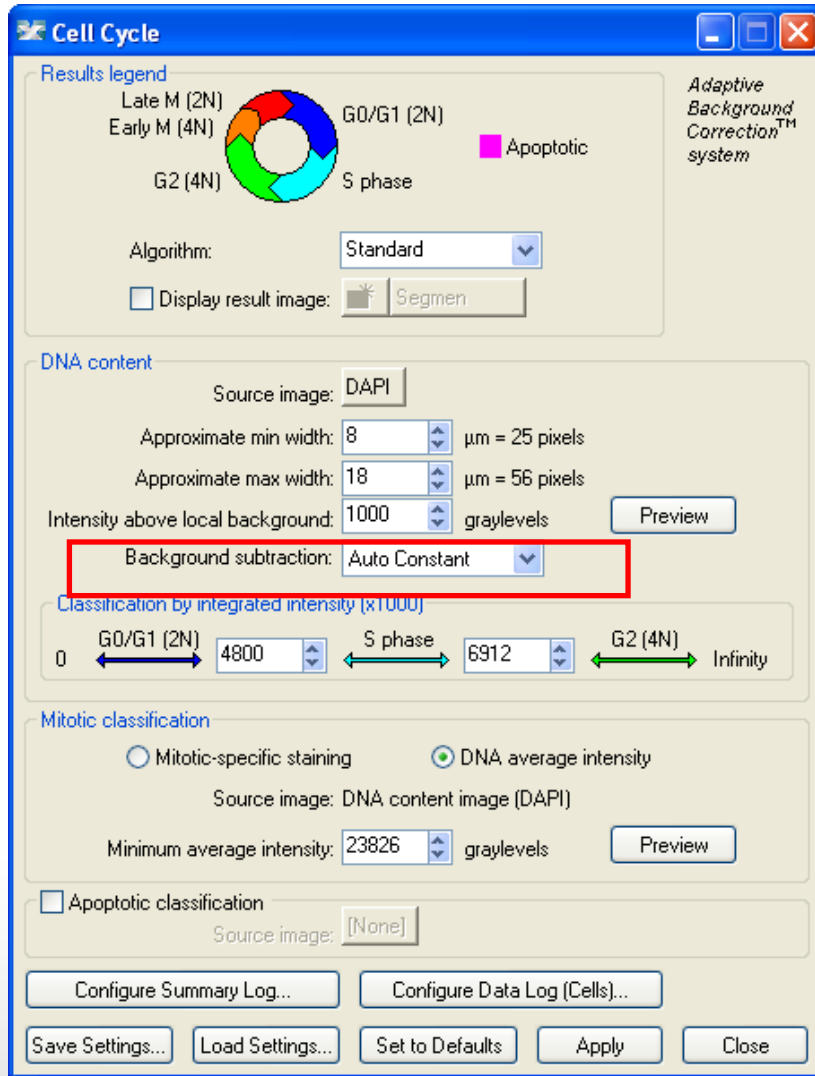
Max width too large: may slightly enlarge nuclear boundaries

4. Module Settings – intensity settings

The screenshot shows the 'Cell Cycle' software interface. The 'Results legend' section includes a circular diagram with segments for Late M (2N), Early M (4N), G2 (4N), S phase, and G0/G1 (2N), along with a pink square for Apoptotic. The 'DNA content' section shows 'Source image: DAPI', 'Approximate min width: 8 μm = 25 pixels', 'Approximate max width: 18 μm = 56 pixels', and 'Intensity above local background: 1000 graylevels' (highlighted with a red box). 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 bottom part of the interface shows a blue-stained cell image and a line scan graph of 'Gray Level (Avg)' vs 'Distance (μm)'.

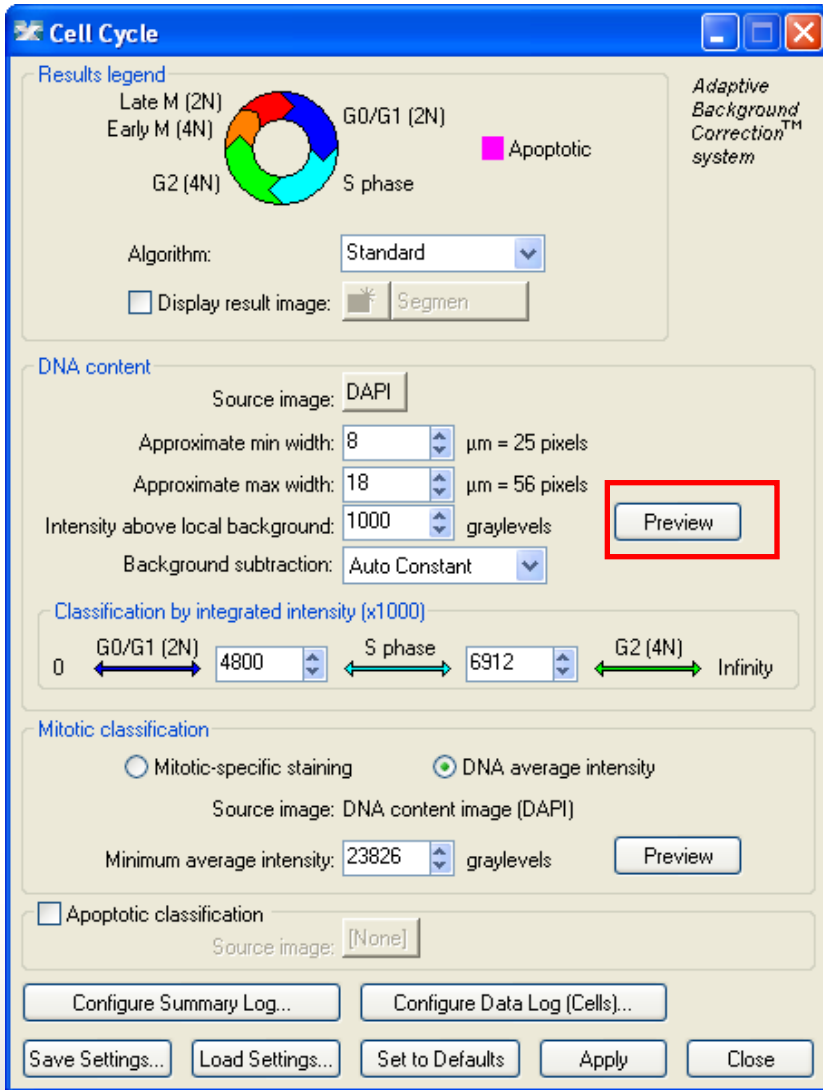
- The **intensity above local background** is used for finding the nuclei
- This value is a minimum and should be set slightly lower than the difference in intensity between a dim cell and its local background. For FAST algorithm, set this value to about half (or less) of the difference in intensity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

Cell Cycle

Results legend

- Late M (2N)
- Early M (4N)
- G2 (4N)
- G0/G1 (2N)
- 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

Classification by integrated intensity (x1000)

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

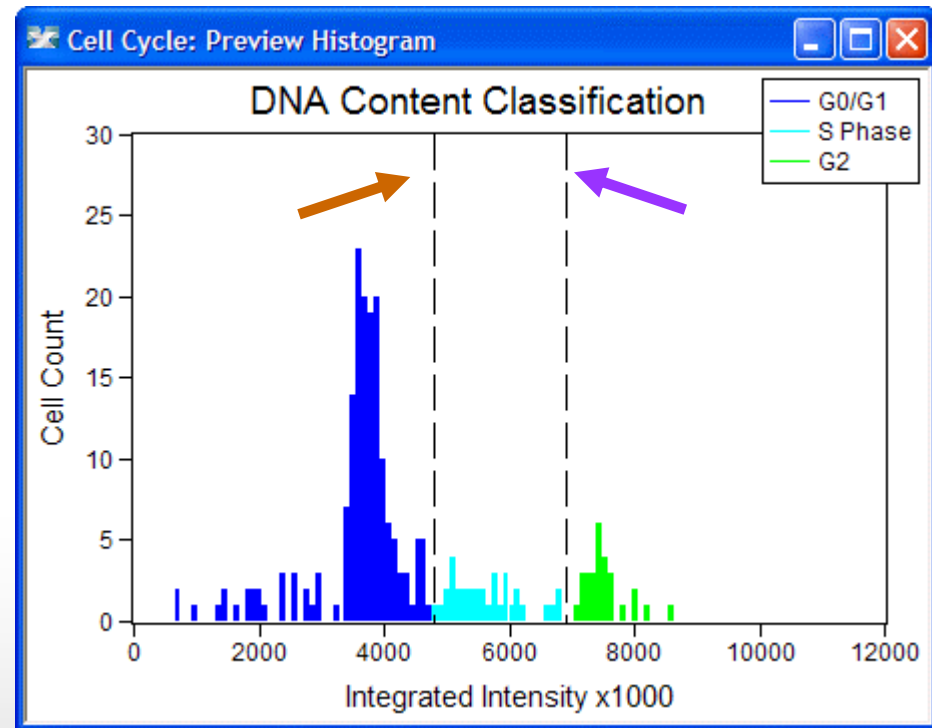
Apoptotic classification

Source image: [None]

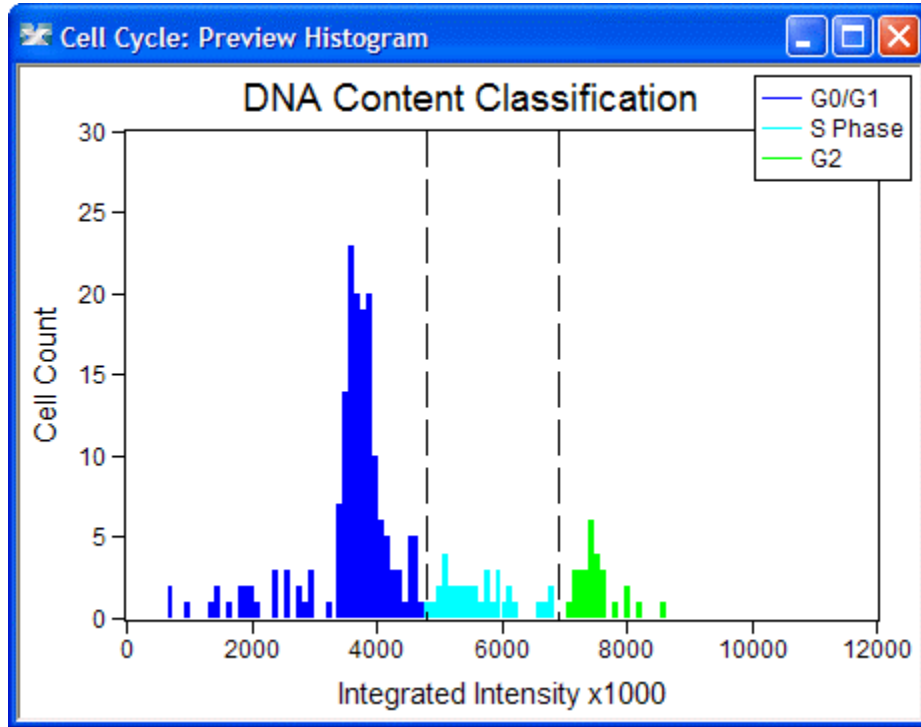
Configure Summary Log... Configure Data Log (Cells)...

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

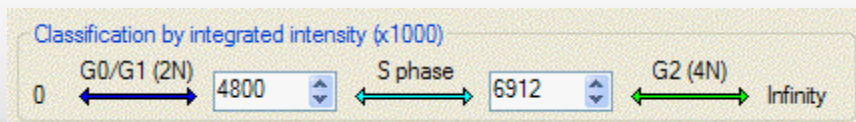
- An interactive graph is shown to adjust the cutoffs for classification.



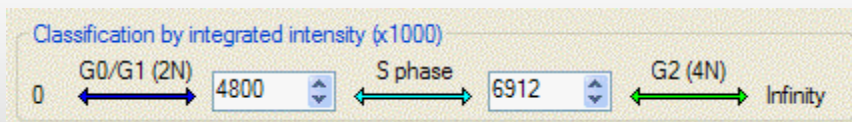
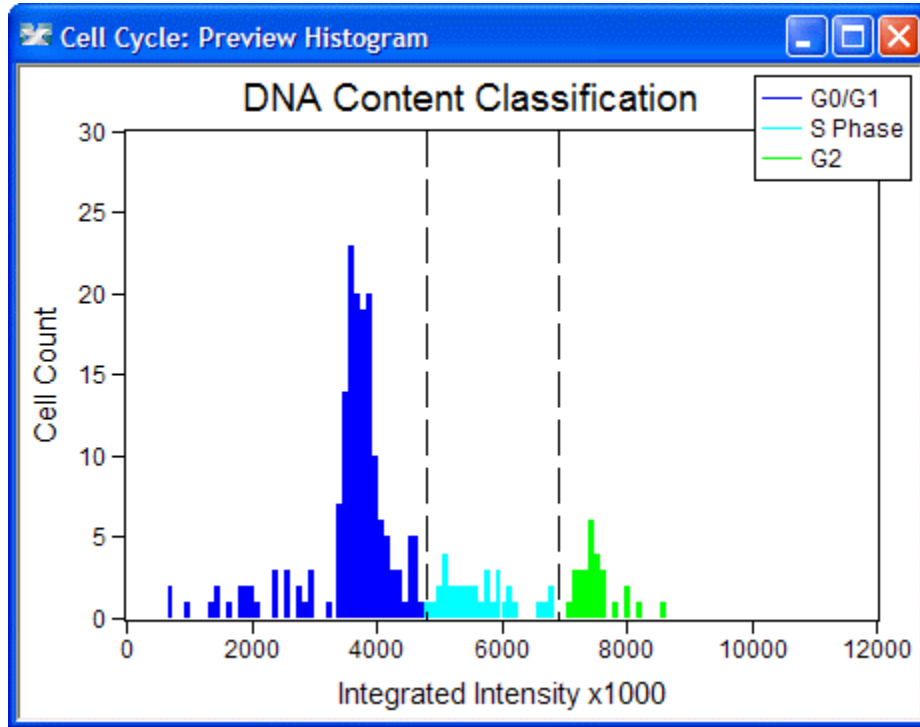
6. Module Settings - DNA content classification



- 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.

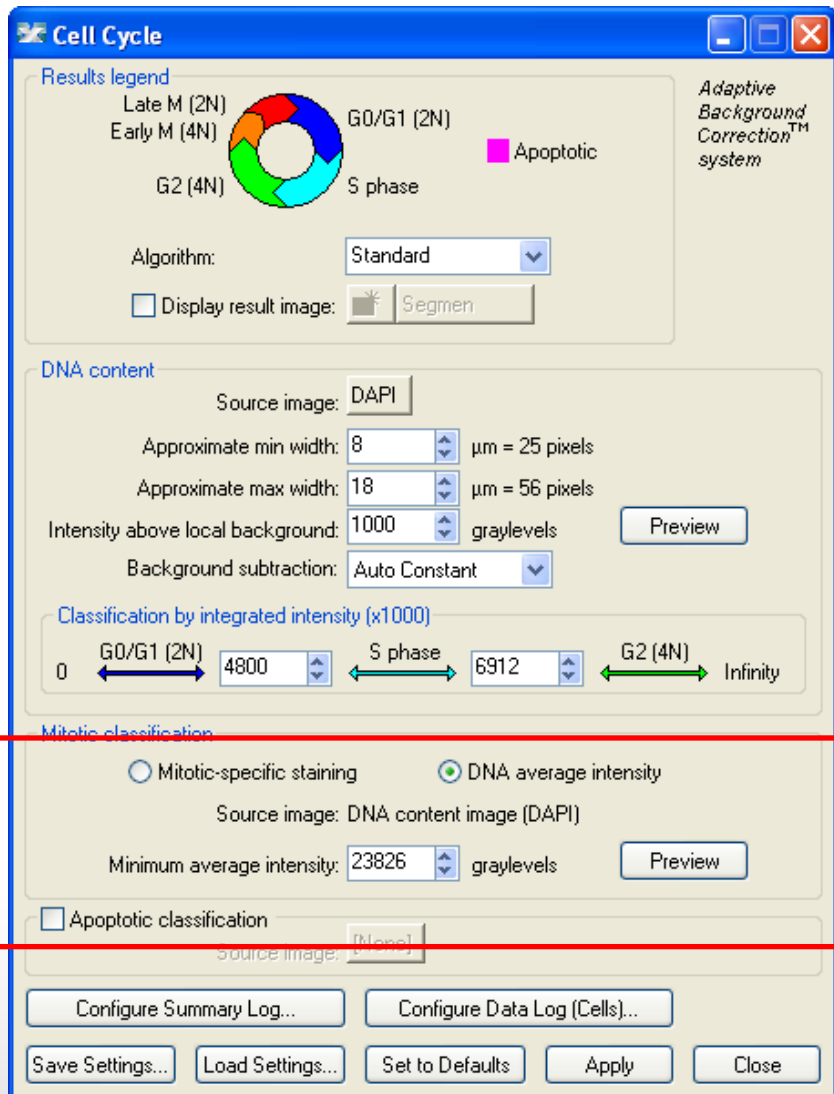


6. Module Settings - DNA content classification



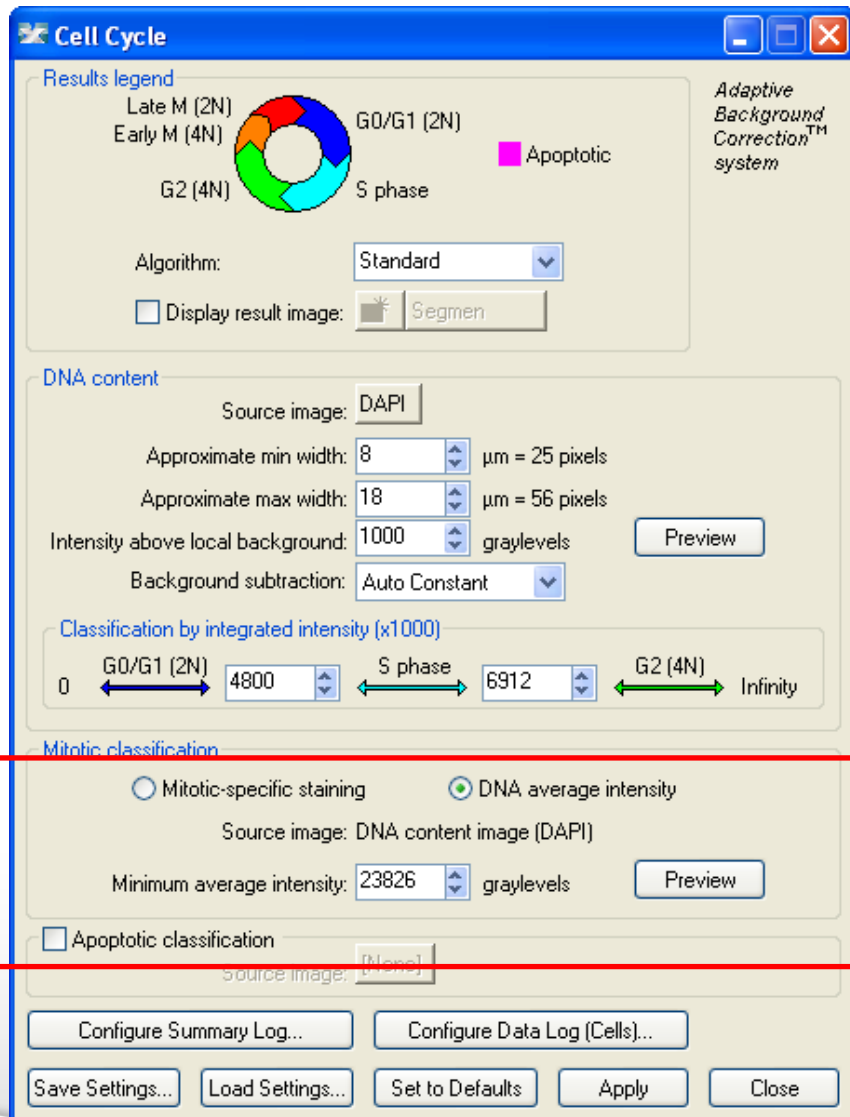
- **Guidelines on setting cutoffs:**
- Drag the sliders to set them, or type in numbers (useful if the sliders are out of scale)
- Usually the first large peak is the 2N peak, and the 4N peak will be smaller and approximately double that
- For more accuracy, do a preliminary analysis on multiple wells,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

7. Module Settings – mitotic classification



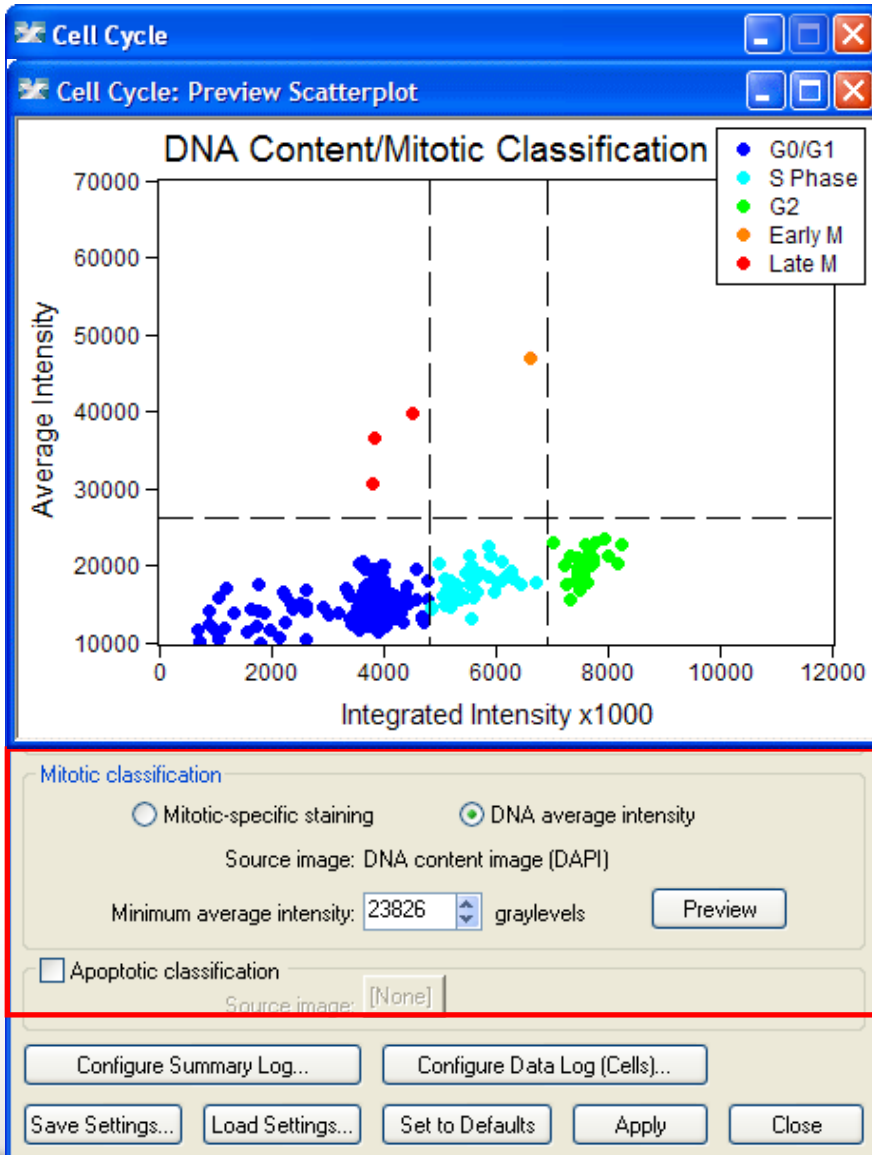
- **Mitotic classification**
- The average intensity (brightness) of the nuclear stain or a separate mitotic-specific marker (e.g. phospho-histone H3) is used to identify mitotic cells.

7. Module Settings – mitotic classification



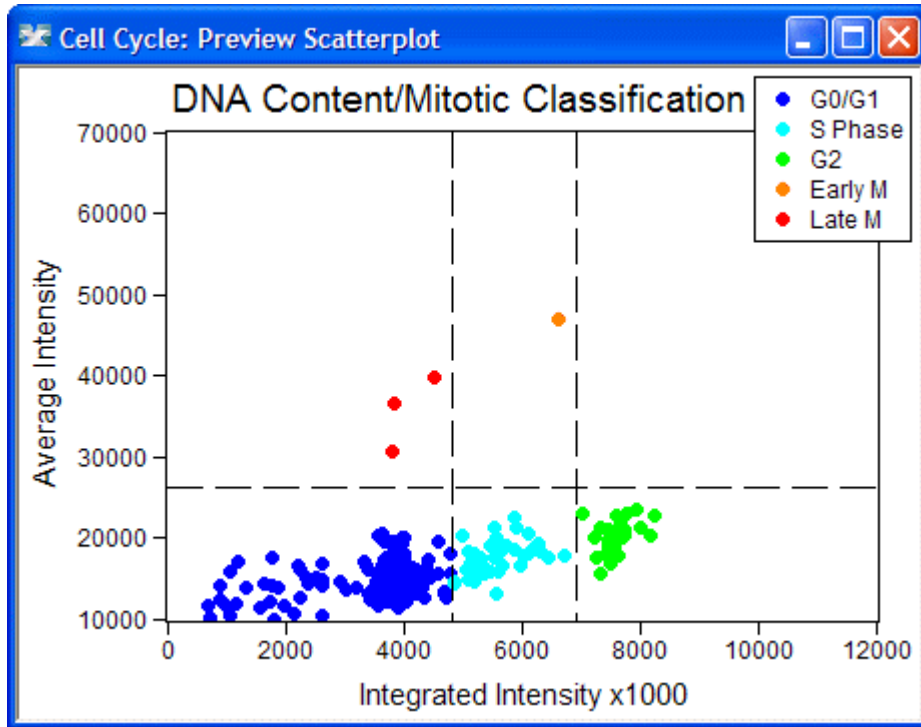
- **DNA average intensity**
- To use the DNA average intensity as a mitotic classifier, simply press Preview.

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”.



Mitotic classification

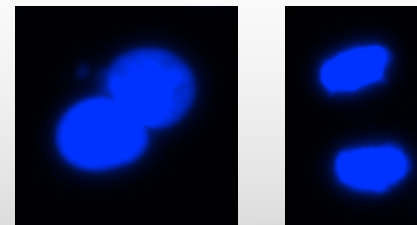
Mitotic-specific staining DNA average intensity

Source image: DNA content image (DAPI)

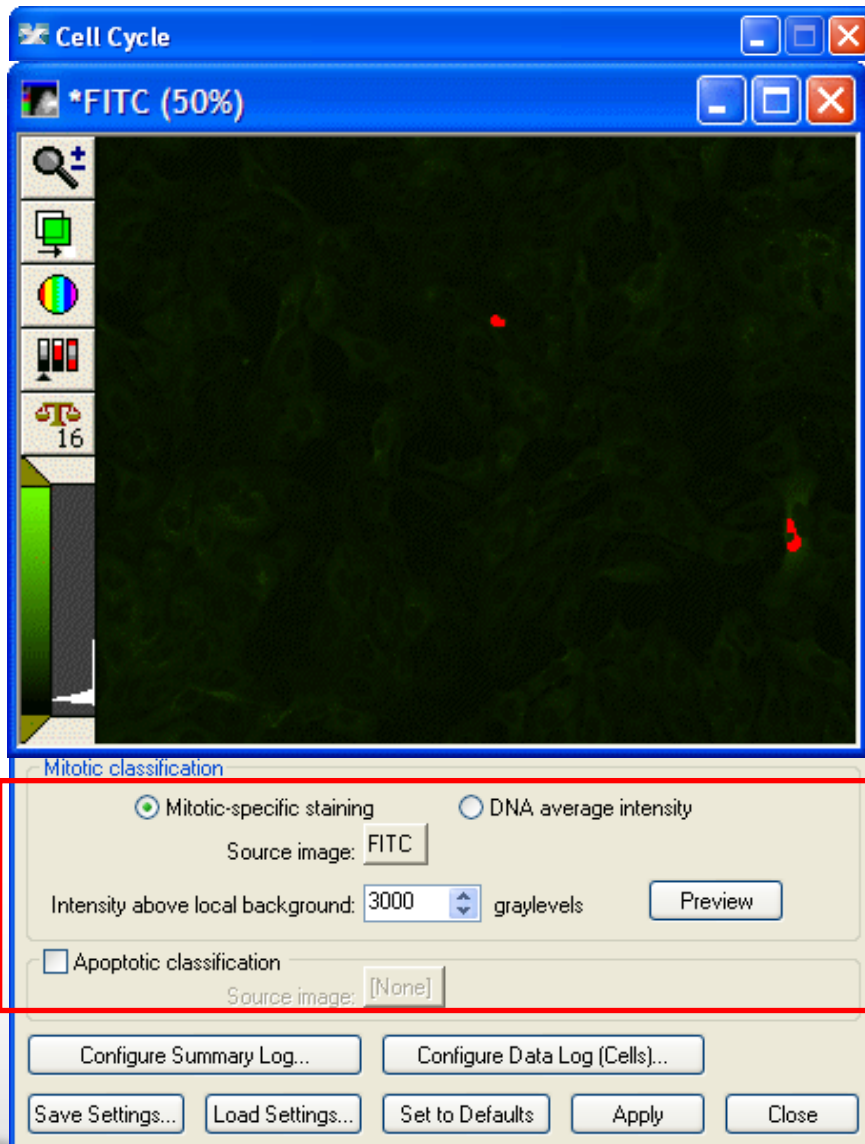
Minimum average intensity: 26296 graylevels

Preview

Early M *Late M*

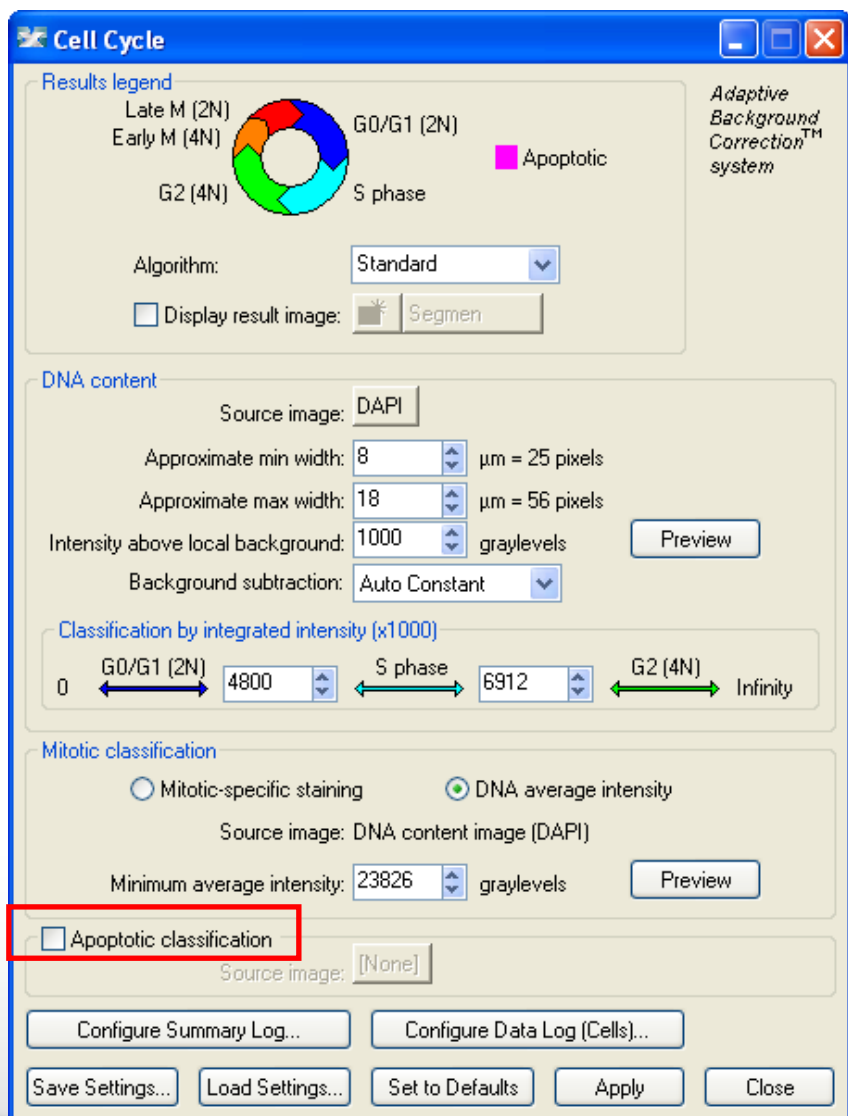


7. Module Settings – mitotic classification



- **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

Cell Cycle

Results legend

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

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: 3000 graylevels

Apoptotic classification

Source image: FITC

Stained area: Nucleus

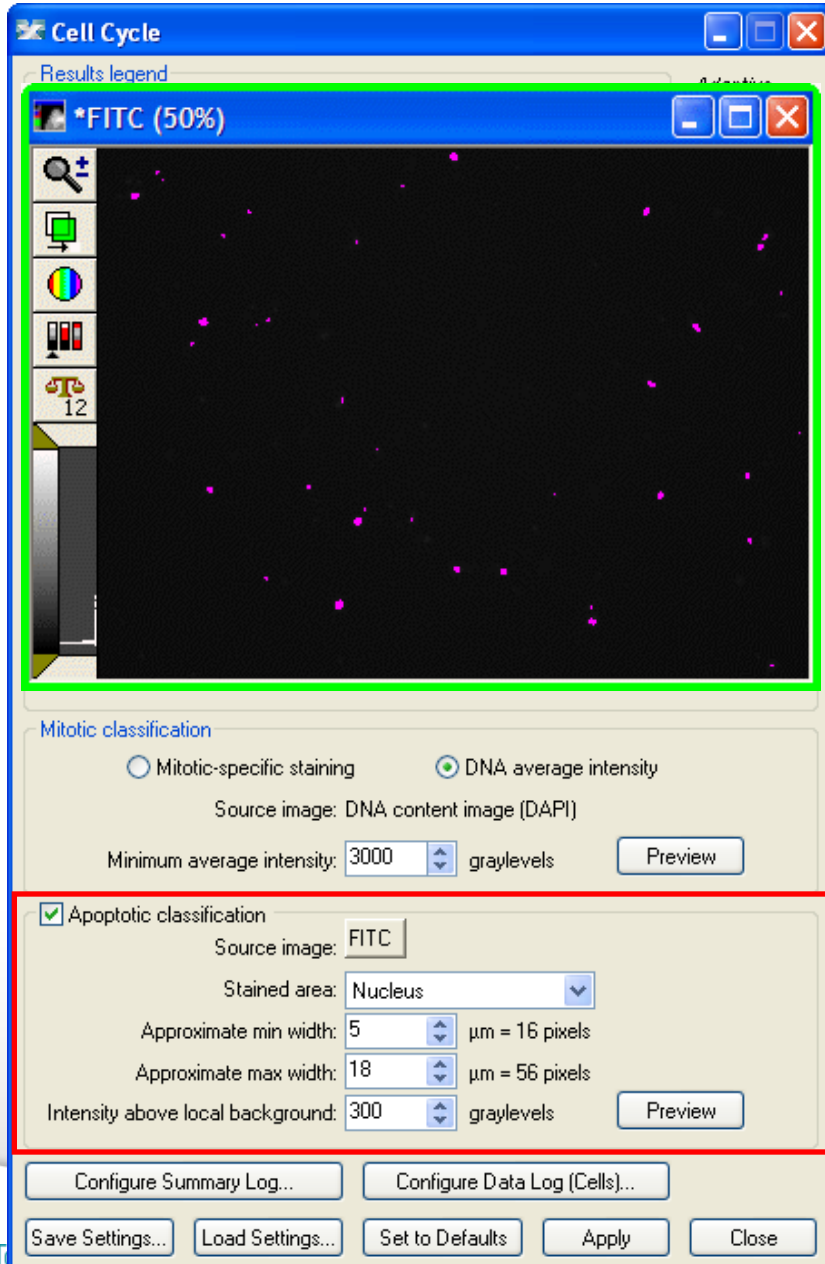
Approximate min width: 5 $\mu\text{m} = 16$ pixels
Approximate max width: 18 $\mu\text{m} = 56$ pixels
Intensity above local background: 3000 graylevels

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

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

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

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

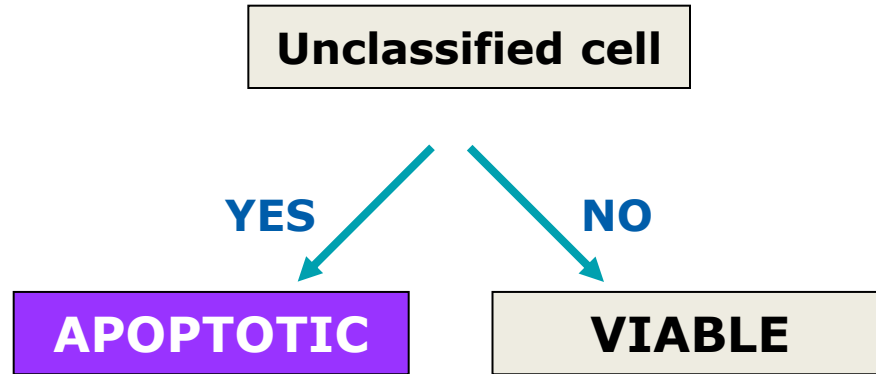
- **Cell: DNA Integrated Intensity** – Total pixel intensity of the nuclear stain in the nucleus
- **Cell: DNA Average Intensity** – Average pixel intensity of the nuclear stain in the nucleus
- **Cell: Mitotic Integrated Intensity** – Total pixel intensity of the mitotic-specific stain overlapping the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

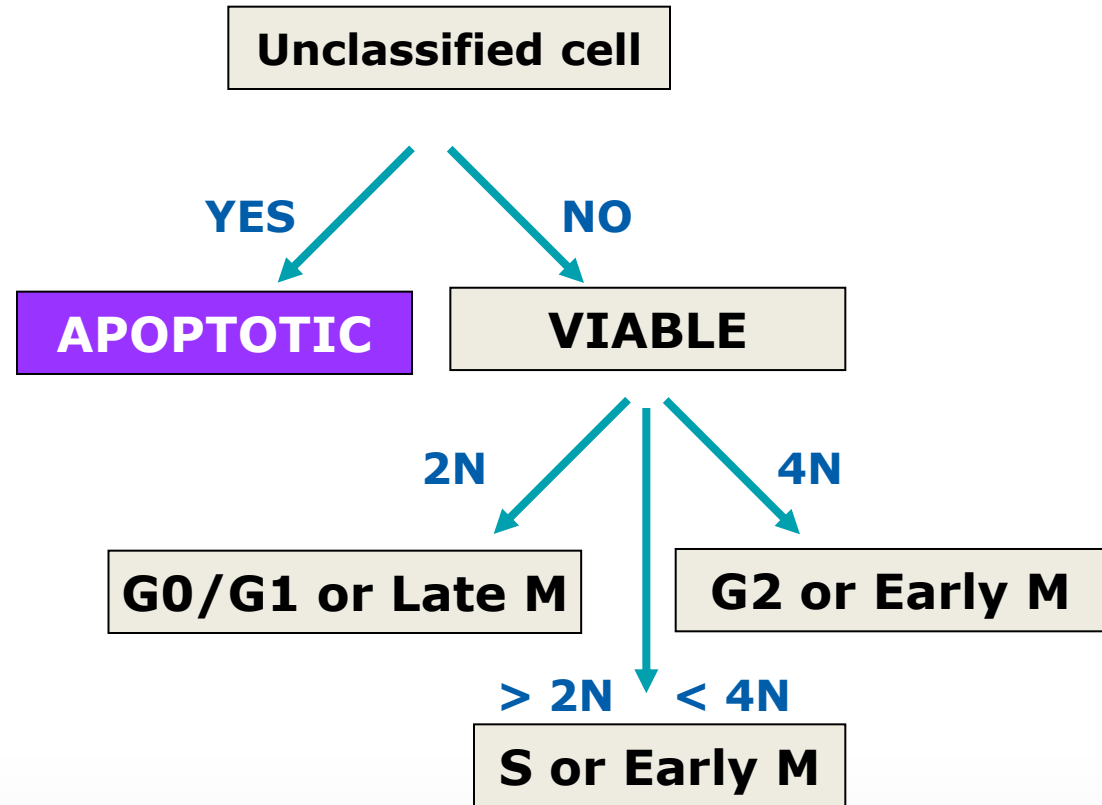
1. (Optional) Is cell positive for apoptotic stain?



Cell cycle module – classification strategy

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

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

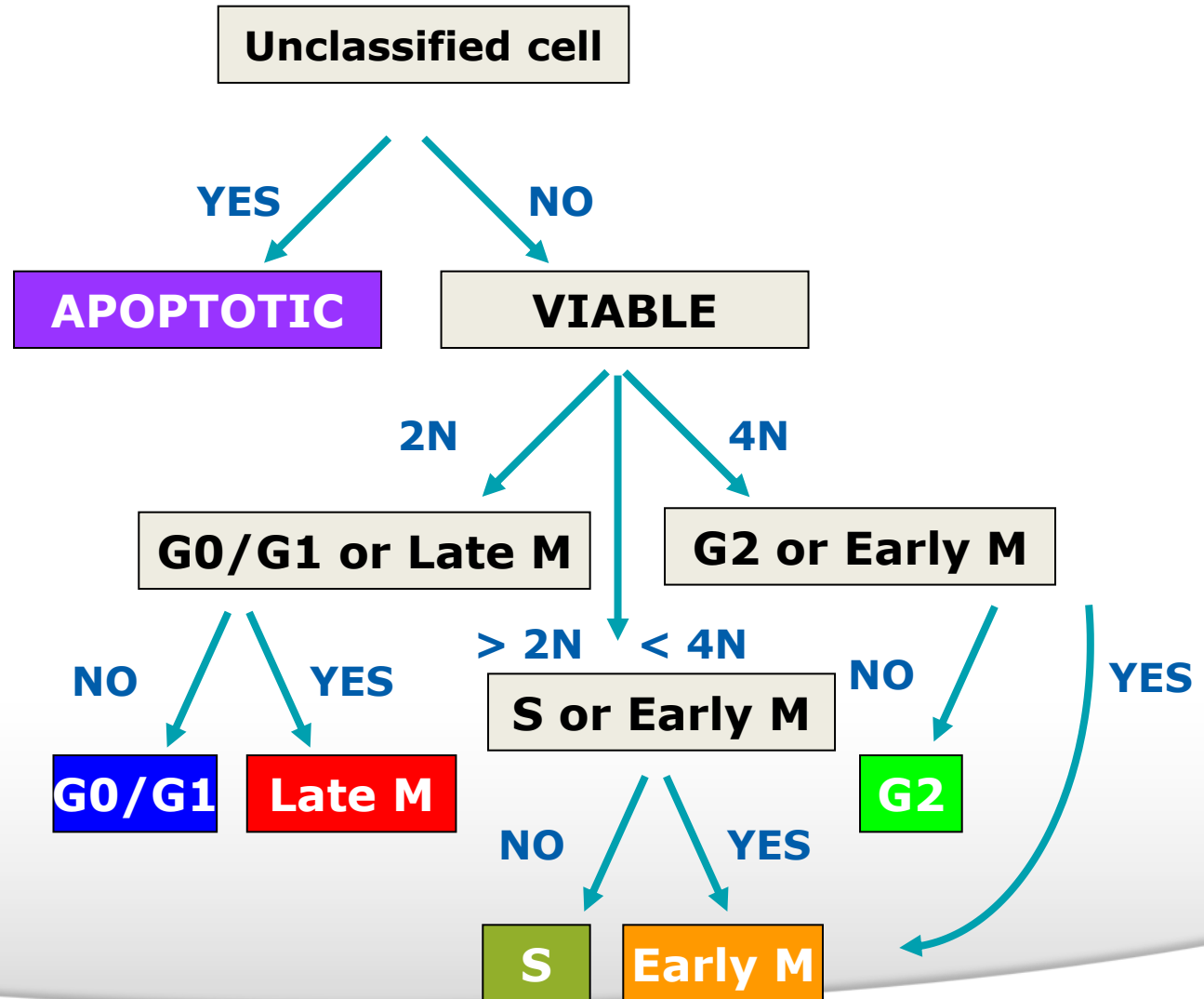


Cell cycle module – classification strategy

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)?





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