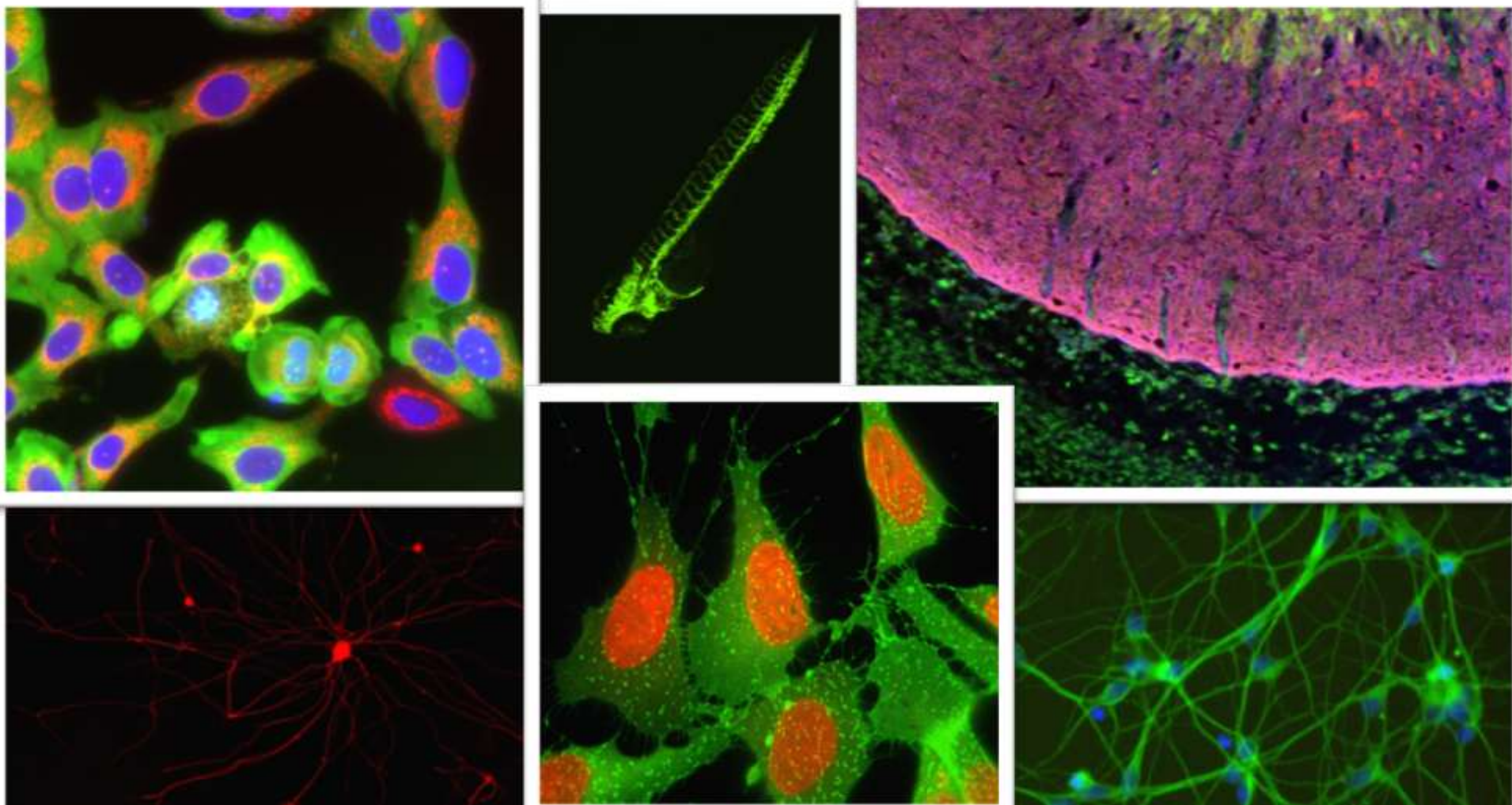




**Molecular
Devices**

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MetaXpress® Software: *Granularity Module*

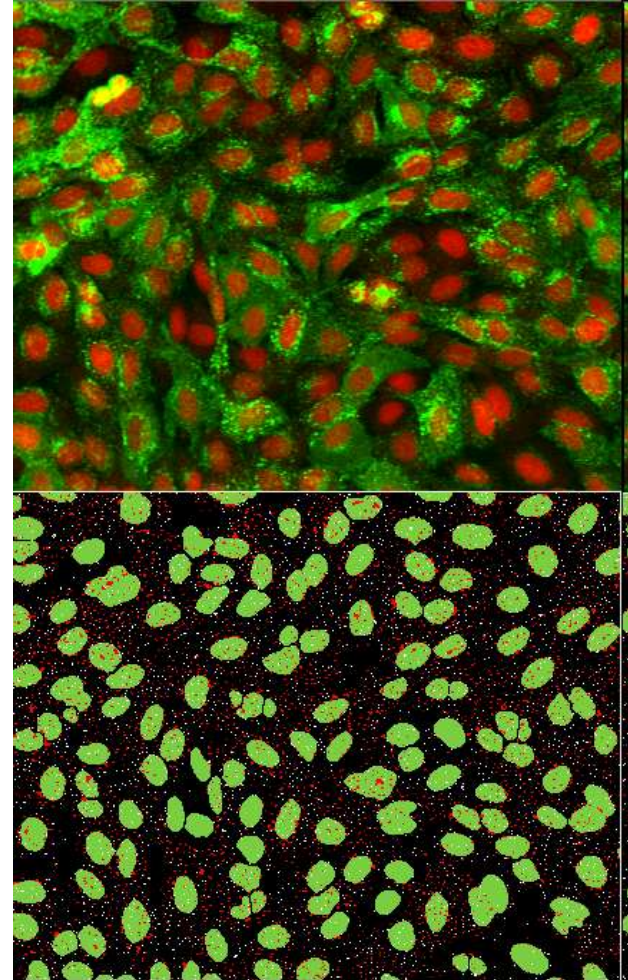
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Granularity Module Overview

- The Granularity module can be used to analyze the number and intensity of granules (spots) per image and per cell
- This module does not require a nuclear wavelength.
- A nuclear stain (e.g. DAPI, Hoechst, or DRAQ5) is required to determine the number of granules per cell.



Module Settings

Configure Settings for Granularity

Granules

Granule image: FITC

☒ Display result image: Granules

Adaptive Background Correction™ system

Algorithm: Fast

Approximate min width: 1 $\mu\text{m} = 3$ pixels

Approximate max width: 6 $\mu\text{m} = 19$ pixels

Intensity above local background: 1000 graylevels

☒ Nuclear stain (optional)

Nuclear image: Cy5

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

Approximate max width: 30 $\mu\text{m} = 93$ pixels

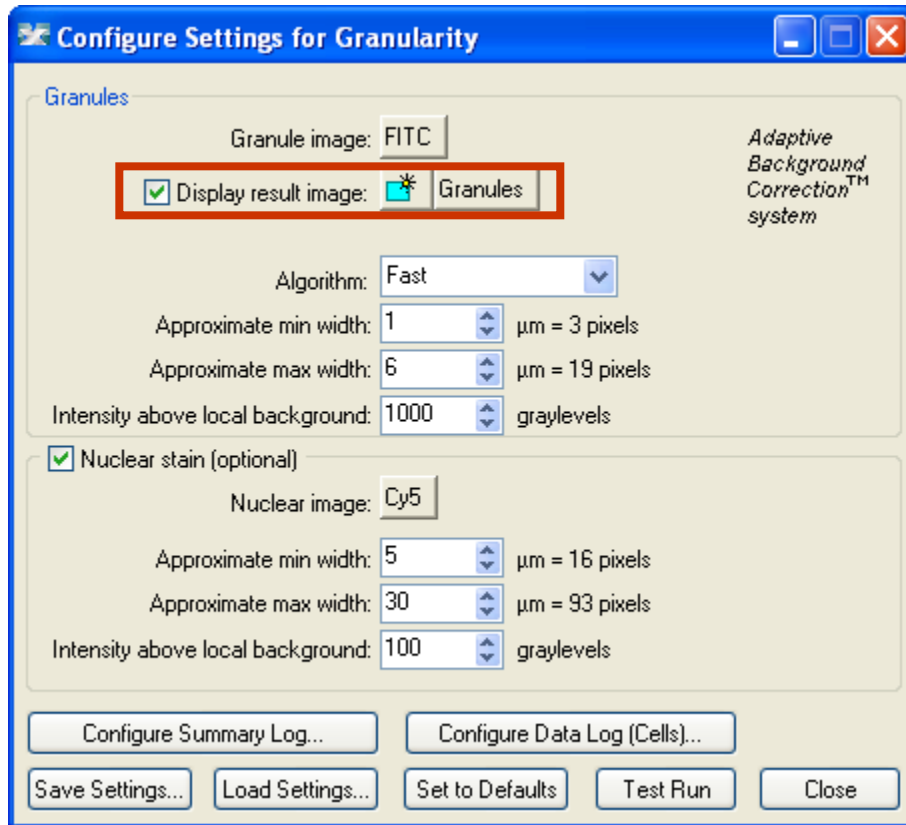
Intensity above local background: 100 graylevels

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

Save Settings... Load Settings... Set to Defaults Test Run Close

- **Granule Image**
- Select the image with granules here

Module Settings



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

Module Settings

Configure Settings for Granularity

Granules

Granule image: FITC

☐ Display result image: Granules

Algorithm: **Fast**

Approximate min width: Standard

Approximate max width: 5 $\mu\text{m} = 19$ pixels

Intensity above local background: 1000 graylevels

☒ Nuclear stain (optional)

Nuclear image: Cy5

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

Approximate max width: 30 $\mu\text{m} = 93$ pixels

Intensity above local background: 100 graylevels

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

Save Settings... Load Settings... Set to Defaults Test Run 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.

Module Settings

Configure Settings for Granularity

Granules

Granule image: FITC

☒ Display result image: Granules

[Adaptive Background Correction™ system](#)

Algorithm: Fast

Approximate min width: 1 μm = 3 pixels

Approximate max width: 6 μm = 19 pixels

Intensity above local background: 1000 graylevels

☒ Nuclear stain (optional)

Nuclear image: Cy5

Approximate min width: 5 μm = 16 pixels

Approximate max width: 30 μm = 93 pixels

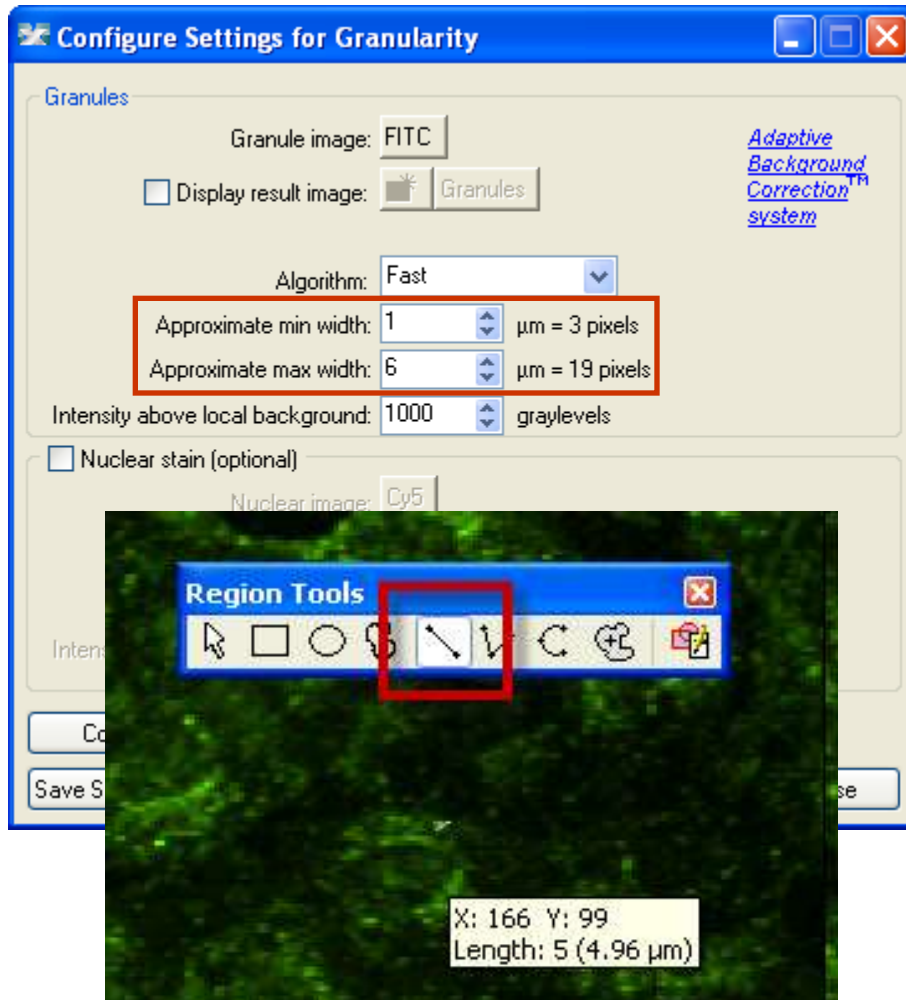
Intensity above local background: 100 graylevels

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

Save Settings... Load Settings... Set to Defaults Test Run Close

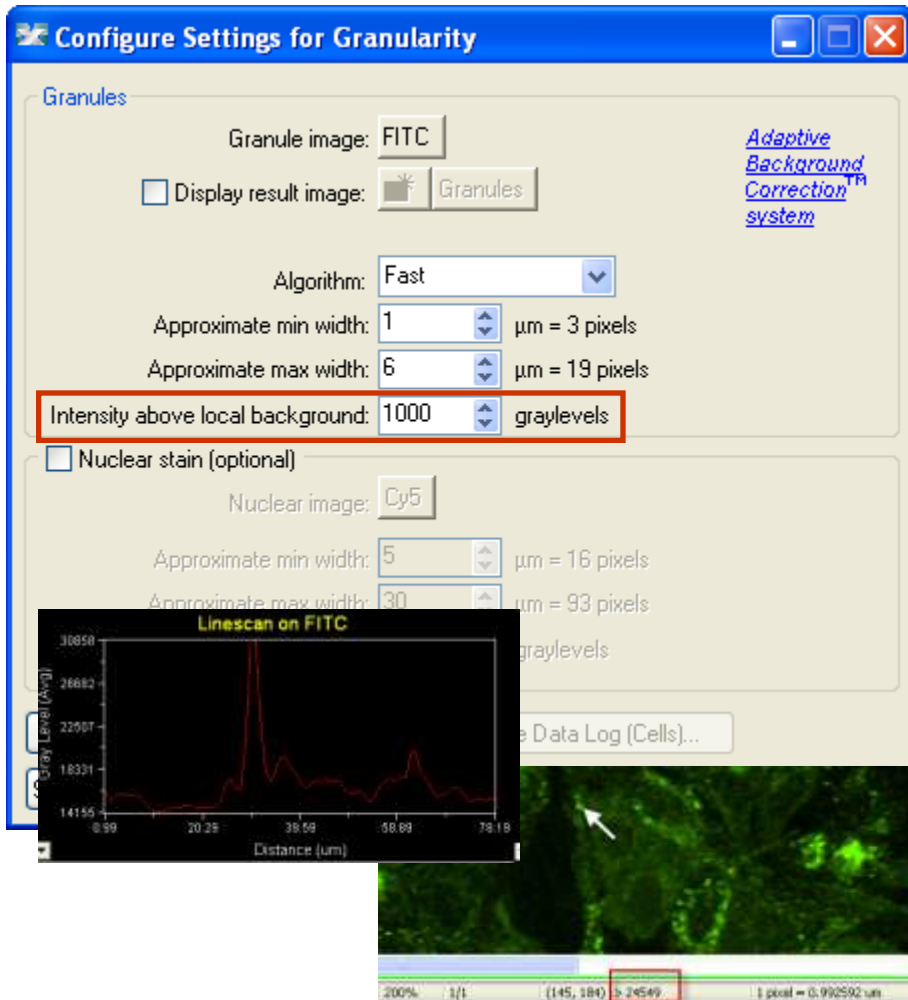
- **Granules**
- Deselect Nuclear stain

Module Settings



- **Granules**
- Using the region tools measure the appropriate min (minimum) and max (maximum) width of qualifying granules.
- Much smaller granules will be ignored
- Much larger granules will be split

Module Settings



- **Granules**
- The **intensity above local background** is used for finding the granules
- This value is a minimum and should be set slightly lower than the difference in intensity between a dim granules and its local background
- Draw a line across a cell into the background and use Measure > Linescan to determine intensity values; or simply mouse over the granules and the background and view the intensity values

Module Settings

Configure Settings for Granularity

Granules

Granule image: FITC

☐ Display result image: Granules

Algorithm: Fast

Approximate min width: 1 $\mu\text{m} = 3$ pixels

Approximate max width: 6 $\mu\text{m} = 19$ pixels

Intensity above local background: 1000 graylevels

☒ Nuclear stain (optional)

Nuclear image: Cy5

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

Approximate max width: 30 $\mu\text{m} = 93$ pixels

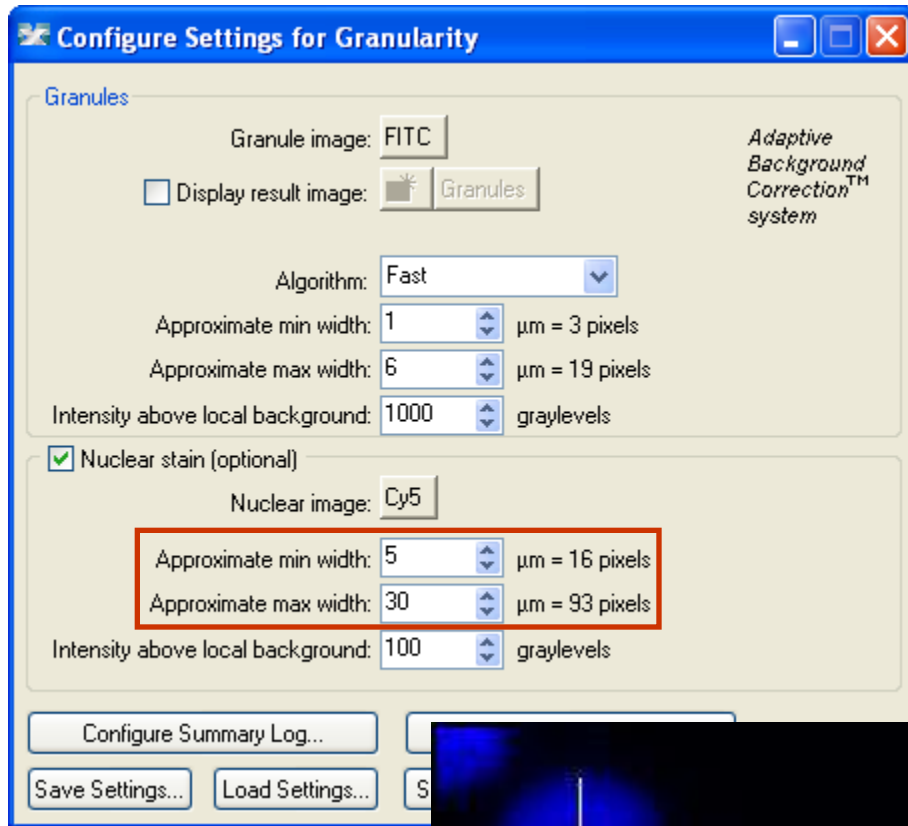
Intensity above local background: 100 graylevels

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

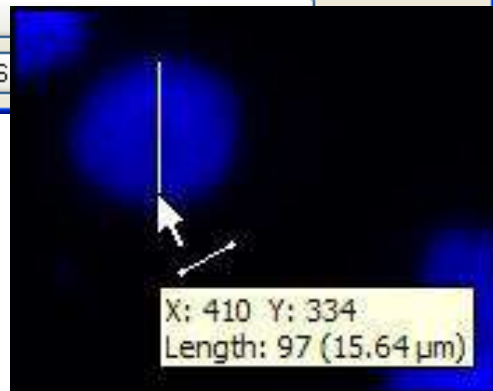
Save Settings... Load Settings... Set to Defaults Test Run Close

- **Nuclear Stain** (not required)
- Select tick mark
- Select the wavelength for the **nuclear image**

Module Settings

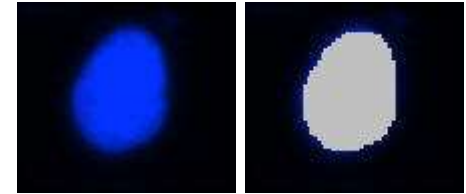


- **Nuclear Stain**
- **Set the Approximate min width and Approximate max width** for the range of nuclei that you want to detect
- The width is the short axis of a nucleus (in μm)
- The region tools can be used to measure widths
- Much smaller cells will be ignored
- Much larger cells will be split



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 Granularity

Granules

Granule image: FITC

☐ Display result image:  Granules

Adaptive Background Correction™ system

Algorithm: Fast

Approximate min width: 1 μm = 3 pixels

Approximate max width: 6 μm = 19 pixels

Intensity above local background: 1000 graylevels

☒ Nuclear stain (optional)

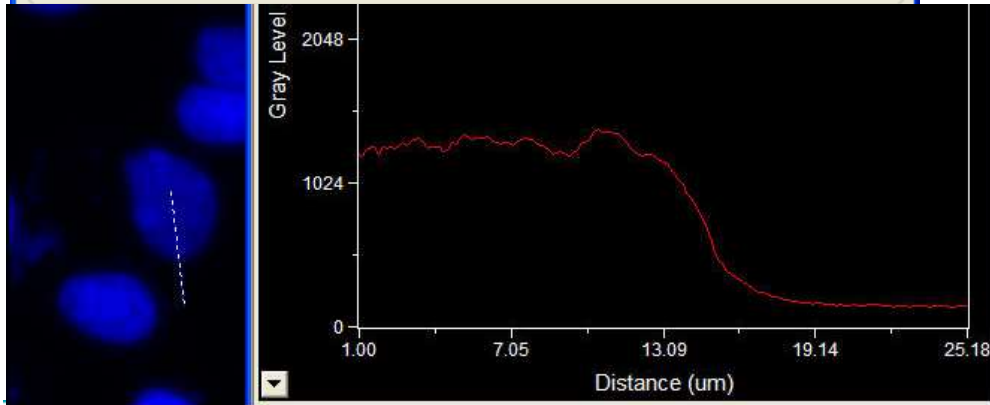
Nuclear image: Cy5

Approximate min width: 5 μm = 16 pixels

Approximate max width: 30 μm = 93 pixels

Intensity above local background: 100 graylevels

- **Nuclear Stain**
- 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
- 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 Granularity

Granules

Granule image: FITC

☐ Display result image: Granules

Algorithm: Fast

Approximate min width: 1 $\mu\text{m} = 3$ pixels

Approximate max width: 6 $\mu\text{m} = 19$ pixels

Intensity above local background: 1000 graylevels

☒ Nuclear stain (optional)

Nuclear image: Cy5

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

Approximate max width: 30 $\mu\text{m} = 93$ pixels

Intensity above local background: 100 graylevels

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

Save Settings... Load Settings... Set to Defaults **Test Run** Close

Adaptive Background Correction™ system

- Select Test Run to view the cell segmentation
- Change settings if needed
- Save the settings

Module Settings – General Settings

Configure Settings for Granularity

Granules

Granule image: FITC

☐ Display result image: Granules

Algorithm: Fast

Approximate min width: 1 $\mu\text{m} = 3$ pixels

Approximate max width: 6 $\mu\text{m} = 19$ pixels

Intensity above local background: 1000 graylevels

☒ Nuclear stain (optional)

Nuclear image: Cy5

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

Approximate max width: 30 $\mu\text{m} = 93$ pixels

Intensity above local background: 100 graylevels

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

Save Settings... Load Settings... Set to Defaults Test Run 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)

- ✓ Granules
- ✓ Granules Per Cell
- ✓ Total Granule Area
- ✓ Mean Granule Area
- ✓ Integrated Granule Intensity
- ✓ Average Granule Intensity
- ✓ Nuclei
- ✓ Total Nuclear Area
- ✓ Mean Nuclear Area
- ✓ Integrated Nuclear Intensity
- ✓ Average Nuclear Intensity
- ✓ Texture Index
- ✓ Cellular Texture Index
- ✓ Gradient Index
- ✓ Cellular Gradient Index
- ✓ Laplacian Index
- ✓ Cellular Laplacian Index

- **Granules:** Total number of granules
- **Granules Per Cell:** Total number of granules divided by the total number of nucleus
- **Total Granule Area:** The total area of the granules found in the image (in μm^2)
- **Mean Granule Area:** The total area of granules for all cells divided by the total number of nucleus (in μm^2)
- **Integrated Granules Intensity:** The total pixel intensity of the granules area
- **Average Granules Intensity:** The total pixel intensity of the granules area divided by the total number of nucleus

Summary Data (site-by-site measurements)

- ✓ Granules
- ✓ Granules Per Cell
- ✓ Total Granule Area
- ✓ Mean Granule Area
- ✓ Integrated Granule Intensity
- ✓ Average Granule Intensity
- ✓ Nuclei
- ✓ Total Nuclear Area
- ✓ Mean Nuclear Area
- ✓ Integrated Nuclear Intensity
- ✓ Average Nuclear Intensity
- ✓ Texture Index
- ✓ Cellular Texture Index
- ✓ Gradient Index
- ✓ Cellular Gradient Index
- ✓ Laplacian Index
- ✓ Cellular Laplacian Index

- **Nuclei:** Total number of nuclei (cell count)
- **Total Nuclear Area:** The total area of the nucleus for all cells found in the image (in μm^2)
- **Mean Nuclear Area:** The average area of nucleus for all cells found in the image (in μm^2)
- **Integrated Nuclear Intensity:** The total pixel intensity of the nuclear stain over the nuclear area
- **Average Nuclear Intensity:** The total pixel intensity of the nuclear stain over the nuclear area, divided by the total number of cells

Summary Data (site-by-site measurements)

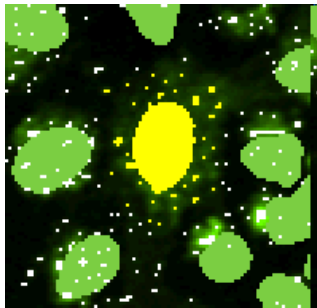
- ✓ Granules
- ✓ Granules Per Cell
- ✓ Total Granule Area
- ✓ Mean Granule Area
- ✓ Integrated Granule Intensity
- ✓ Average Granule Intensity
- ✓ Nuclei
- ✓ Total Nuclear Area
- ✓ Mean Nuclear Area
- ✓ Integrated Nuclear Intensity
- ✓ Average Nuclear Intensity

- ✓ Texture Index
- ✓ Cellular Texture Index
- ✓ Gradient Index
- ✓ Cellular Gradient Index
- ✓ Laplacian Index
- ✓ Cellular Laplacian Index

- **Texture Index:** Standard deviation of intensity values in the image.
- **Cellular Texture Index:** Cell-by-cell standard deviation of intensity values near the nuclei. (Requires use of nuclear stain).
- **Gradient Index:** A texture-dependent measurement that reflects the amount of local intensity contrast. Measures the difference between the maximum and minimum intensity within a local neighborhood.
- **Cellular Gradient Index:** Cell-by-cell Gradient Index measured near the nuclei. (Requires use of nuclear stain).
- **Laplacian Index:** Similar to the morphological gradient, however this morphological measurement reflects fluctuations in the gradient.
- **Cellular Laplacian Index:** Cell-by-cell Laplacian Index measured near the nuclei. (Requires use of nuclear stain).

Cell Data (cell-by-cell measurements)

- ✓ Cell: Assigned Label #
- ✓ Cell: Granule Count
- ✓ Cell: Granule Total Area
- ✓ Cell: Granule Integrated Intensity
- ✓ Cell: Granule Average Intensity
- ✓ Cell: Nuclear Total Area
- ✓ Cell: Nuclear Integrated Intensity
- ✓ Cell: Nuclear Average Intensity
- ✓ Cell: Texture Index
- ✓ Cell: Gradient Index
- ✓ Cell: Laplacian Index



Highlighted cells shows
assigned granules to cell

- **Cell: Assigned Label #** – Cell label number (1 through total cell number)
- **Cell: Granules Count:** Number of granules detected for a specific cell. (Note: a granules is assigned to its nearest nucleus)
- **Cell: Granules Total Area:** Area covered by all the granules assigned to a specific cell in μm^2
- **Cell: Granules Integrated Intensity:** The total pixel intensity of the granules assigned to a specific cell
- **Cell: Granules Average Intensity:** The total pixel intensity of the granules assigned to a specific cell divided by the number of granules assigned to a specific cell

Cell Data (cell-by-cell measurements)

- ✓ Cell: Assigned Label #
- ✓ Cell: Granule Count
- ✓ Cell: Granule Total Area
- ✓ Cell: Granule Integrated Intensity
- ✓ Cell: Granule Average Intensity
- ✓ Cell: Nuclear Total Area
- ✓ Cell: Nuclear Integrated Intensity
- ✓ Cell: Nuclear Average Intensity
- ✓ Cell: Texture Index
- ✓ Cell: Gradient Index
- ✓ Cell: Laplacian Index

- **Cell: Nuclear Total Area** – Total square microns of the nucleus
- **Cell: Nuclear Integrated Intensity** – Total pixel intensity of the nuclear stain in the nucleus
- **Cell: Nuclear Average Intensity** – Average pixel intensity of the nuclear stain in the nucleus

Cell Data (cell-by-cell measurements)

- ✓ Cell: Assigned Label #
- ✓ Cell: Granule Count
- ✓ Cell: Granule Total Area
- ✓ Cell: Granule Integrated Intensity
- ✓ Cell: Granule Average Intensity
- ✓ Cell: Nuclear Total Area
- ✓ Cell: Nuclear Integrated Intensity
- ✓ Cell: Nuclear Average Intensity
- ✓ Cell: Texture Index
- ✓ Cell: Gradient Index
- ✓ Cell: Laplacian Index

- **Cell: Texture Index:** Standard deviation of intensity values of a cell
- **Cell: Gradient Index:** A texture-dependent measurement that reflects the amount of local intensity contrast. Measures the difference between the maximum and minimum intensity within a local neighborhood of a cell
- **Cell: Laplacian Index:** Similar to the morphological gradient, however this morphological measurement reflects fluctuations in the gradient of a cell



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