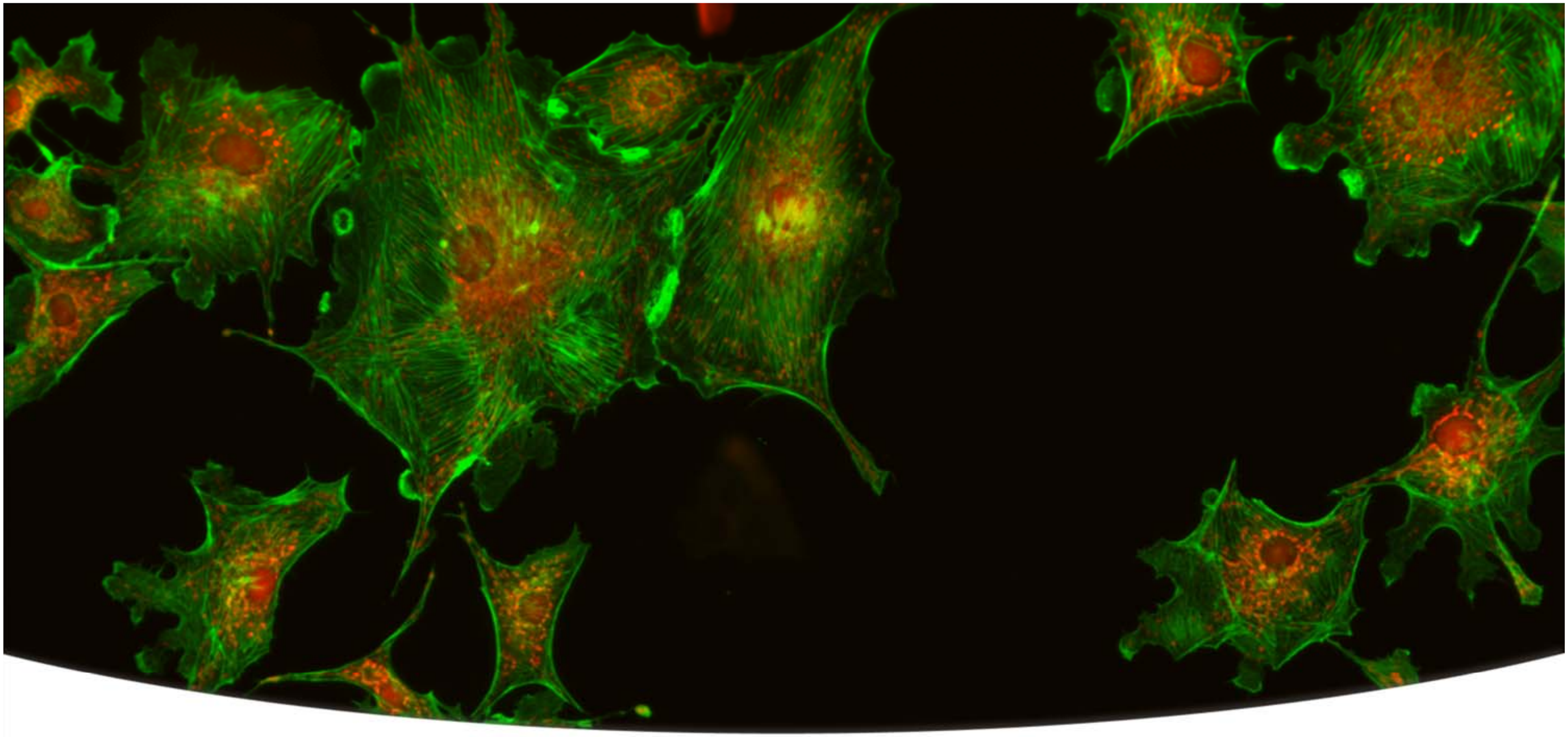


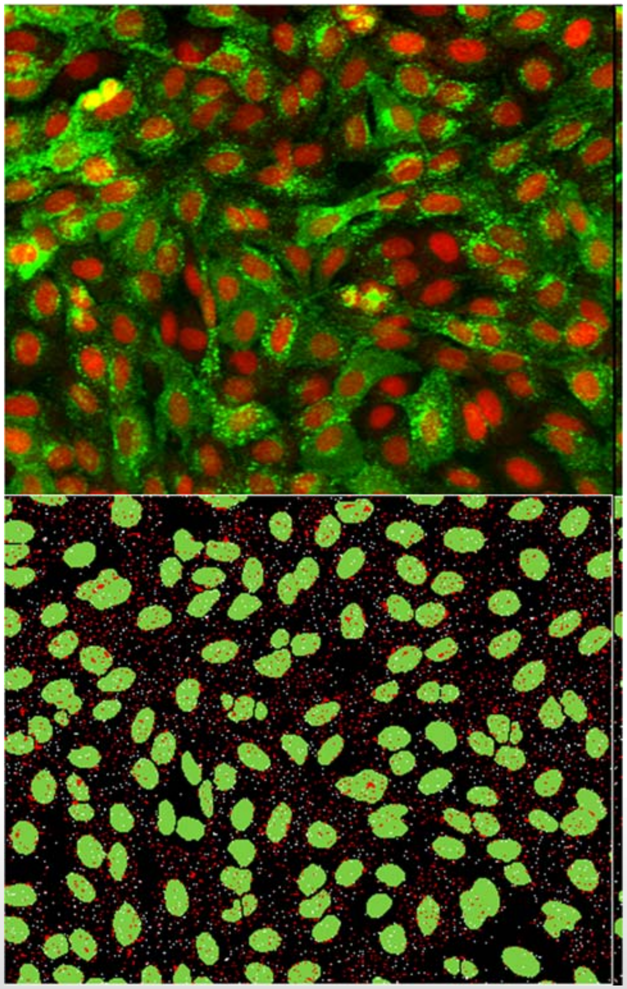
Together through life sciences.

Molecular Devices

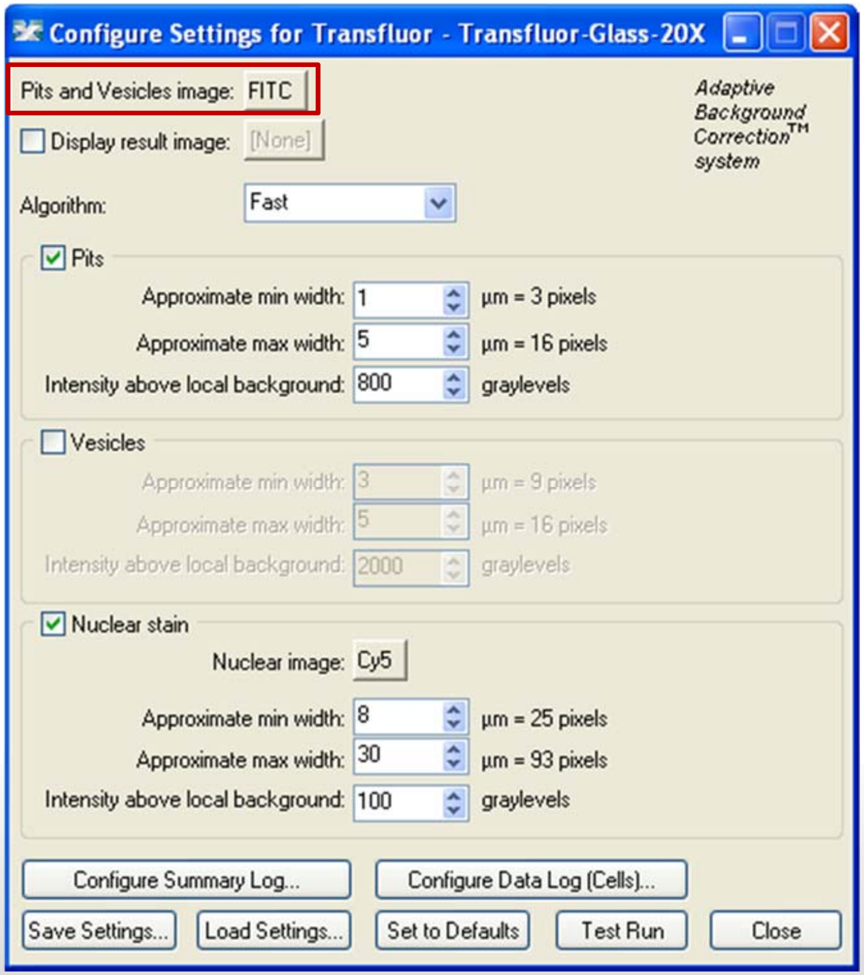
MetaXpress® Software: Transfluor Module

Transfluor Module Overview

- The Transfluor module can be used to analyze the number and intensity of Pits (small) and/or vesicles (larger and brighter) 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 objects (Pits/ vesicles) per cell.



Module Settings



Pits and Vesicles image: FITC

☐ Display result image: [None]

Algorithm: Fast

☑ Pits

Approximate min width: 1 µm = 3 pixels

Approximate max width: 5 µm = 16 pixels

Intensity above local background: 800 graylevels

☐ Vesicles

Approximate min width: 3 µm = 9 pixels

Approximate max width: 5 µm = 16 pixels

Intensity above local background: 2000 graylevels

☑ Nuclear stain

Nuclear image: Cy5

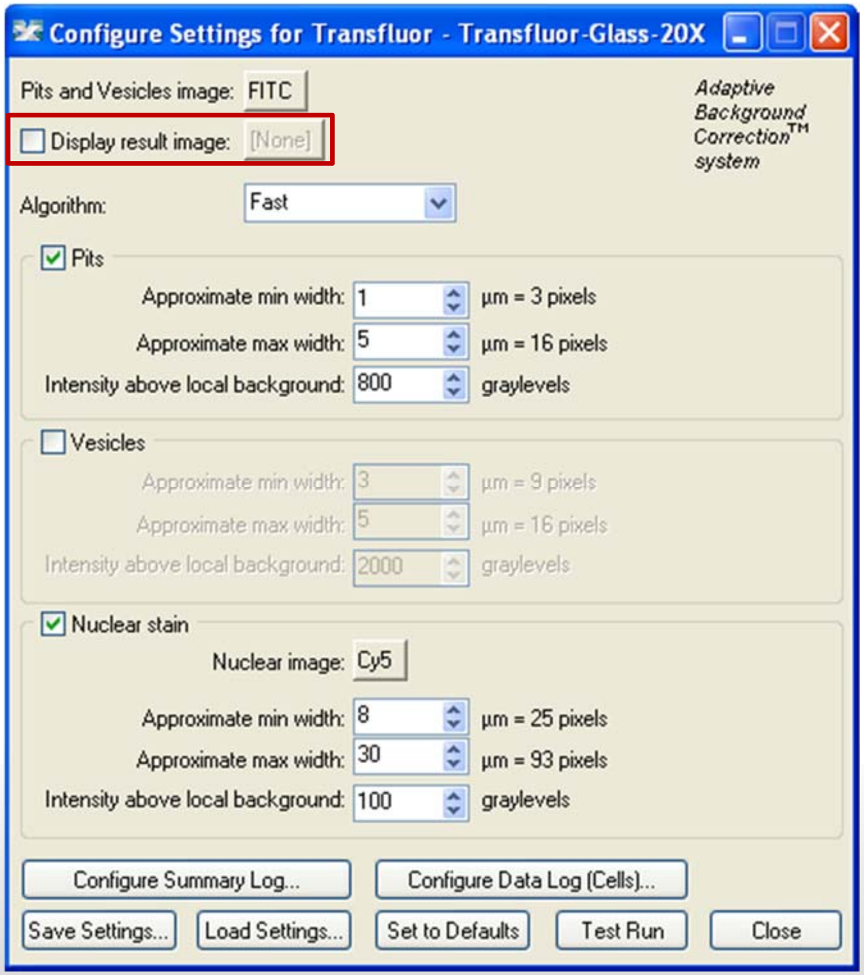
Approximate min width: 8 µm = 25 pixels

Approximate max width: 30 µm = 93 pixels

Intensity above local background: 100 graylevels

- **Pits and Vesicles Image**
- Select the image with pits and vesicles here

Module Settings



Pits and Vesicles image: FITC

☐ Display result image: [None]

Algorithm: Fast

☑ Pits

Approximate min width: 1 µm = 3 pixels

Approximate max width: 5 µm = 16 pixels

Intensity above local background: 800 graylevels

☐ Vesicles

Approximate min width: 3 µm = 9 pixels

Approximate max width: 5 µm = 16 pixels

Intensity above local background: 2000 graylevels

☑ Nuclear stain

Nuclear image: Cy5

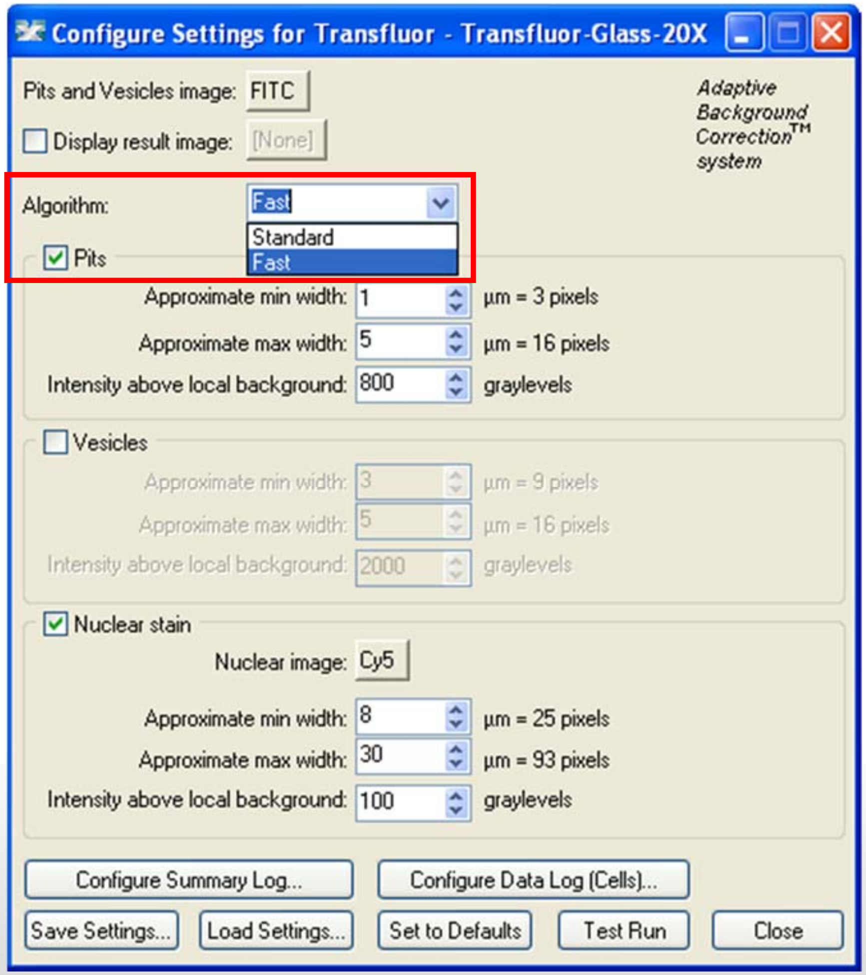
Approximate min width: 8 µm = 25 pixels

Approximate max width: 30 µm = 93 pixels

Intensity above local background: 100 graylevels

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

Module Settings



Configure Settings for Transfluor - Transfluor-Glass-20X

Pits and Vesicles image: FITC

☐ Display result image: [None]

Algorithm: Fast

☑ Pits

Approximate min width: 1 µm = 3 pixels

Approximate max width: 5 µm = 16 pixels

Intensity above local background: 800 graylevels

☐ Vesicles

Approximate min width: 3 µm = 9 pixels

Approximate max width: 5 µm = 16 pixels

Intensity above local background: 2000 graylevels

☑ Nuclear stain

Nuclear image: Cy5

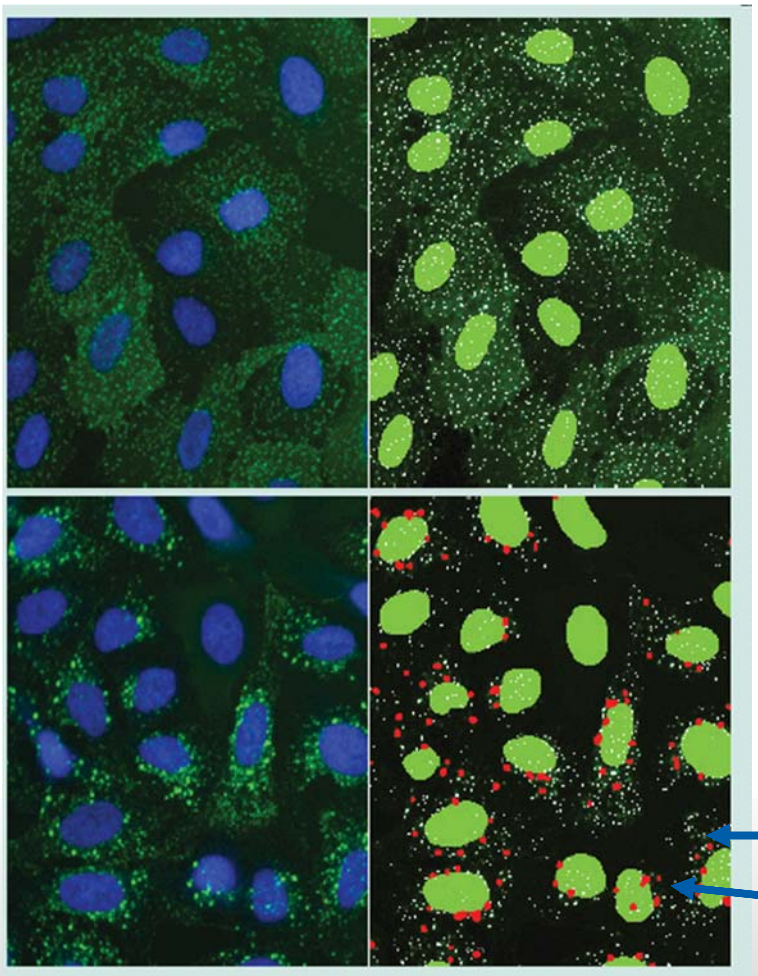
Approximate min width: 8 µm = 25 pixels

Approximate max width: 30 µm = 93 pixels

Intensity above local background: 100 graylevels

- **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.

Pits and Vesicles

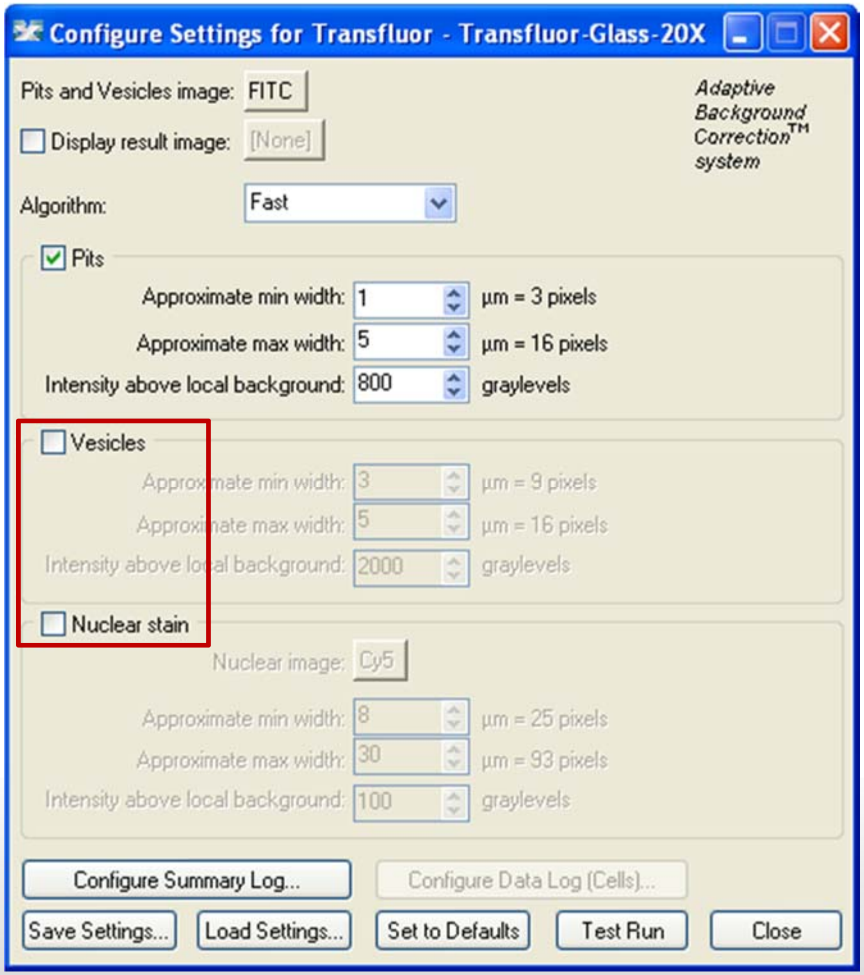


- Pits are smaller and dimmer
- Vesicles is typically an aggregate of multiple Pits
- Vesicles are larger in size and are typically brighter
- Pit detection is indicated in white, Vesicle detection is indicated in red

Pits

Vesicles

Module Settings



Configure Settings for Transfluor - Transfluor-Glass-20X

Pits and Vesicles image: FITC

☐ Display result image: [None]

Adaptive Background Correction™ system

Algorithm: Fast

☑ Pits

Approximate min width: 1 µm = 3 pixels

Approximate max width: 5 µm = 16 pixels

Intensity above local background: 800 graylevels

☐ Vesicles

Approximate min width: 3 µm = 9 pixels

Approximate max width: 5 µm = 16 pixels

Intensity above local background: 2000 graylevels

☐ Nuclear stain

Nuclear image: Cy5

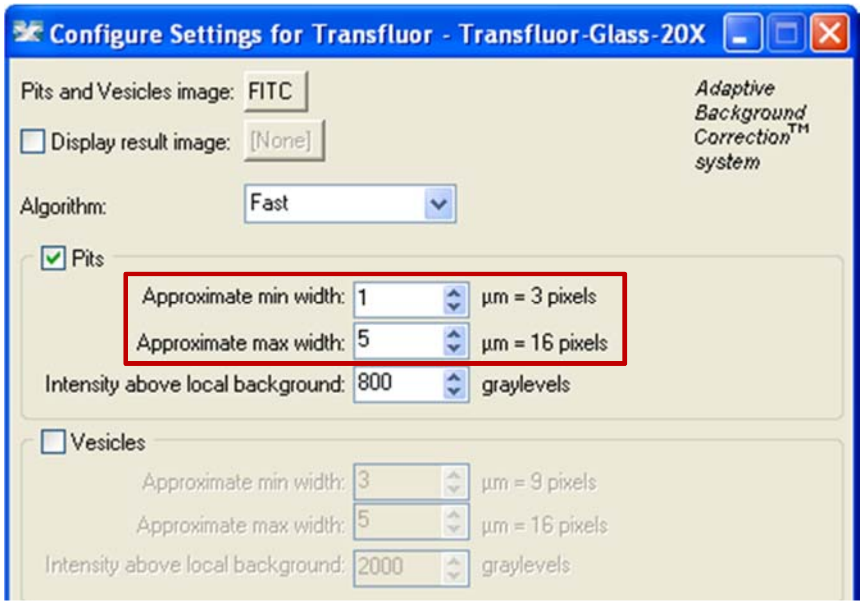
Approximate min width: 8 µm = 25 pixels

Approximate max width: 30 µm = 93 pixels

Intensity above local background: 100 graylevels

- **Pits**
- Deselect Vesicles and Nuclear stain

Module Settings



Configure Settings for Transfluor - Transfluor-Glass-20X

Pits and Vesicles image: FITC

☐ Display result image: [None]

Algorithm: Fast

Adaptive Background Correction™ system

☑ Pits

Approximate min width: 1 µm = 3 pixels

Approximate max width: 5 µm = 16 pixels

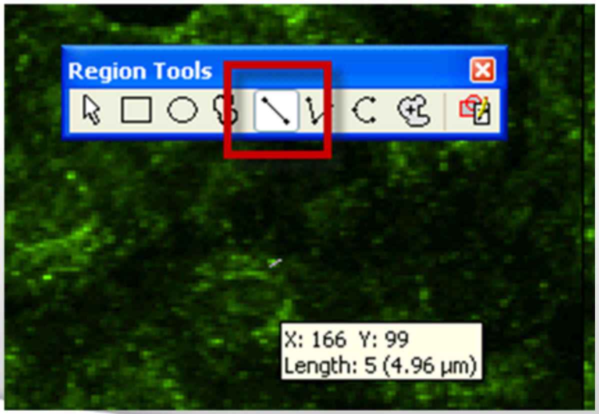
Intensity above local background: 800 graylevels

☐ Vesicles

Approximate min width: 3 µm = 9 pixels

Approximate max width: 5 µm = 16 pixels

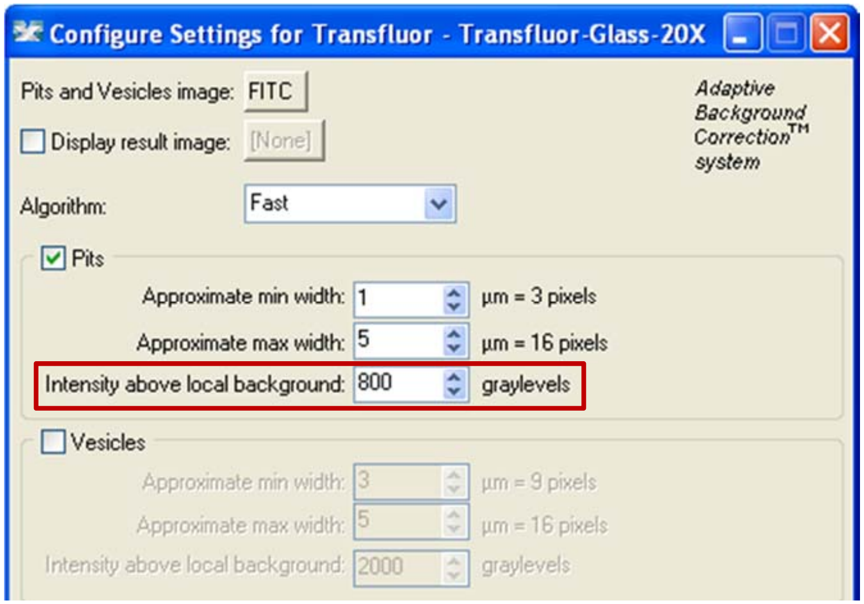
Intensity above local background: 2000 graylevels



X: 166 Y: 99
Length: 5 (4.96 µm)

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

Module Settings



Configure Settings for Transfluor - Transfluor-Glass-20X

Pits and Vesicles image: FITC

☐ Display result image: [None]

Algorithm: Fast

☑ Pits

Approximate min width: 1 µm = 3 pixels

Approximate max width: 5 µm = 16 pixels

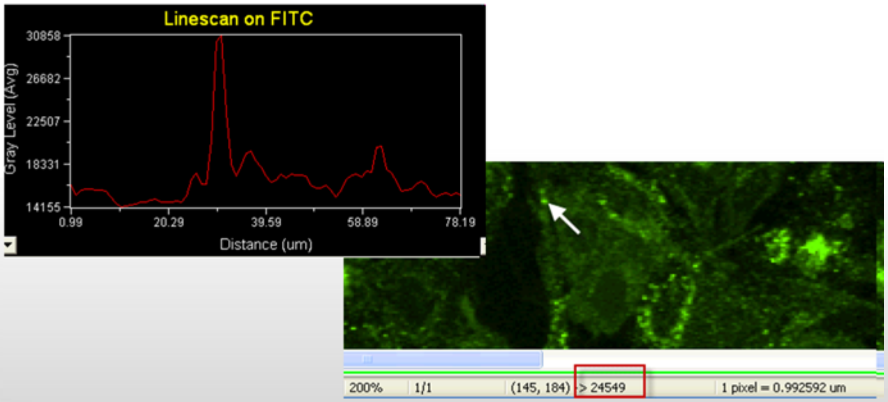
Intensity above local background: 800 graylevels

☐ Vesicles

Approximate min width: 3 µm = 9 pixels

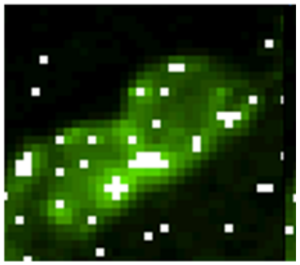
Approximate max width: 5 µm = 16 pixels

Intensity above local background: 2000 graylevels

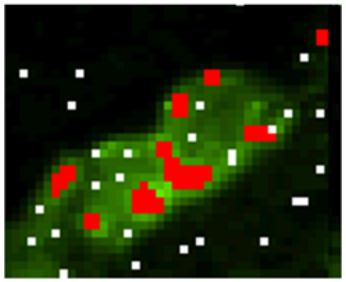


- **Pits**
- The **intensity above local background** is used for finding the Pits
- This value is a minimum and should be set slightly lower than the difference in intensity between a dim Pits and its local background. For FAST algorithm, set this value to about half (or less) of the difference in intensity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

Module Settings

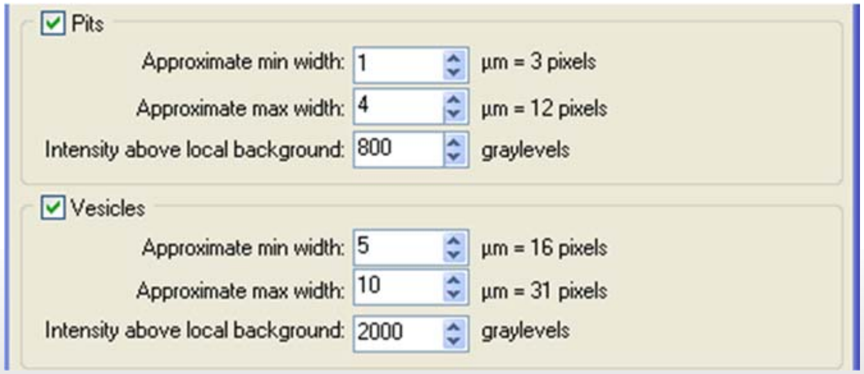


Only pits selected



Pits and vesicles selected

- **Vesicles**
- Repeat the same steps for vesicles
- If an object fulfills the vesicle settings (size and intensity) it will be classified as a vesicle



☑ Pits

Approximate min width: 1 µm = 3 pixels

Approximate max width: 4 µm = 12 pixels

Intensity above local background: 800 graylevels

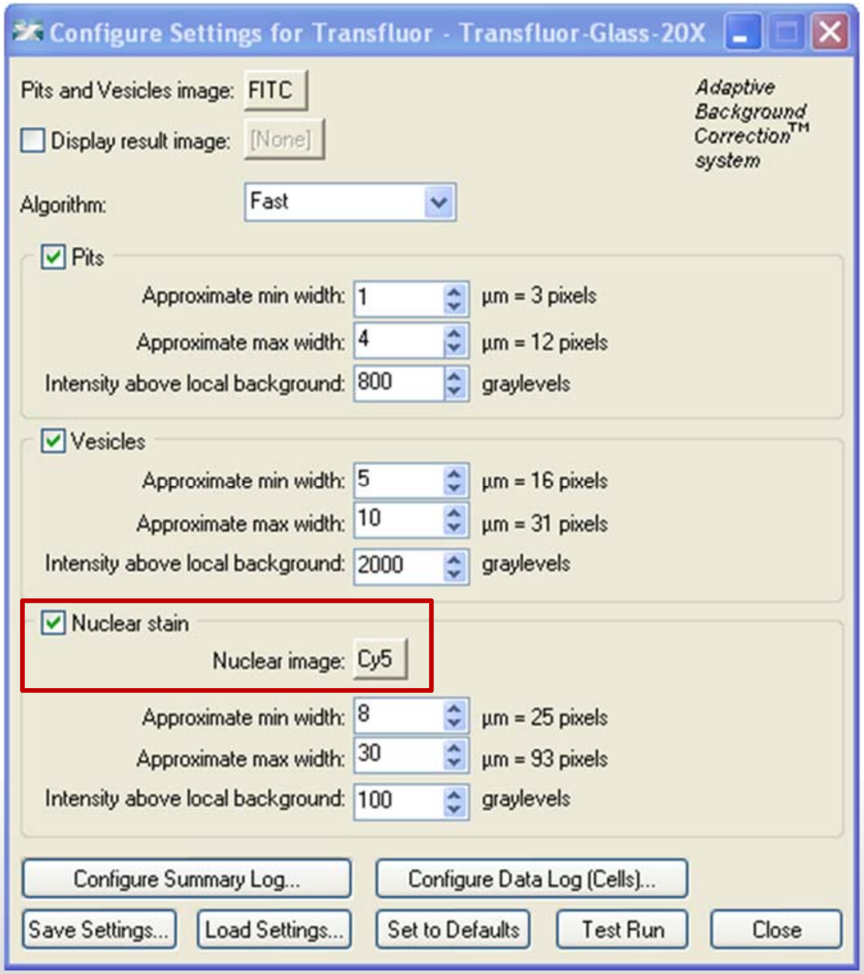
☑ Vesicles

Approximate min width: 5 µm = 16 pixels

Approximate max width: 10 µm = 31 pixels

Intensity above local background: 2000 graylevels

Module Settings



Pits and Vesicles image: FITC

☐ Display result image: [None]

Algorithm: Fast

☑ Pits

Approximate min width: 1 µm = 3 pixels

Approximate max width: 4 µm = 12 pixels

Intensity above local background: 800 graylevels

☑ Vesicles

Approximate min width: 5 µm = 16 pixels

Approximate max width: 10 µm = 31 pixels

Intensity above local background: 2000 graylevels

☑ Nuclear stain

Nuclear image: Cy5

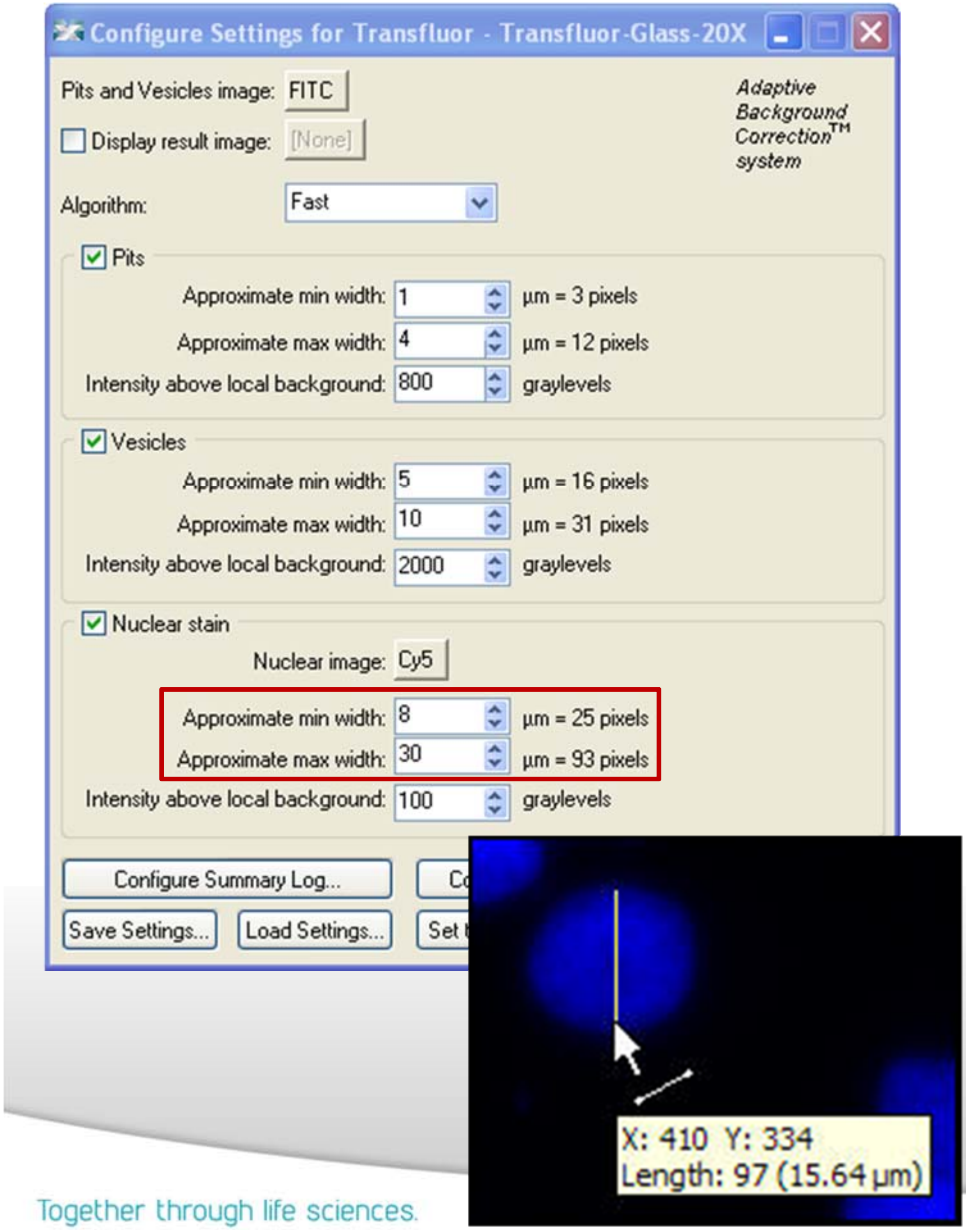
Approximate min width: 8 µm = 25 pixels

Approximate max width: 30 µm = 93 pixels

Intensity above local background: 100 graylevels

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

Module Settings



Configure Settings for Transfluor - Transfluor-Glass-20X

Pits and Vesicles image: FITC

☐ Display result image: [None]

Algorithm: Fast

Adaptive Background Correction™ system

☑ Pits

Approximate min width: 1 µm = 3 pixels

Approximate max width: 4 µm = 12 pixels

Intensity above local background: 800 graylevels

☑ Vesicles

Approximate min width: 5 µm = 16 pixels

Approximate max width: 10 µm = 31 pixels

Intensity above local background: 2000 graylevels

☑ Nuclear stain

Nuclear image: Cy5

Approximate min width: 8 µm = 25 pixels

Approximate max width: 30 µm = 93 pixels

Intensity above local background: 100 graylevels

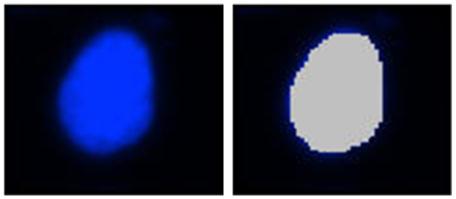
X: 410 Y: 334

Length: 97 (15.64 µm)

- **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 um)
- The region tools can be used to measure widths
- Much smaller cells will be ignored
- Much larger cells will be split

Module Settings

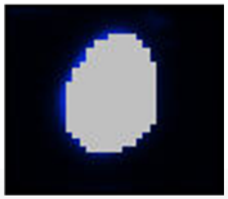
Effects of varying width settings



Min width too small: splits nuclei

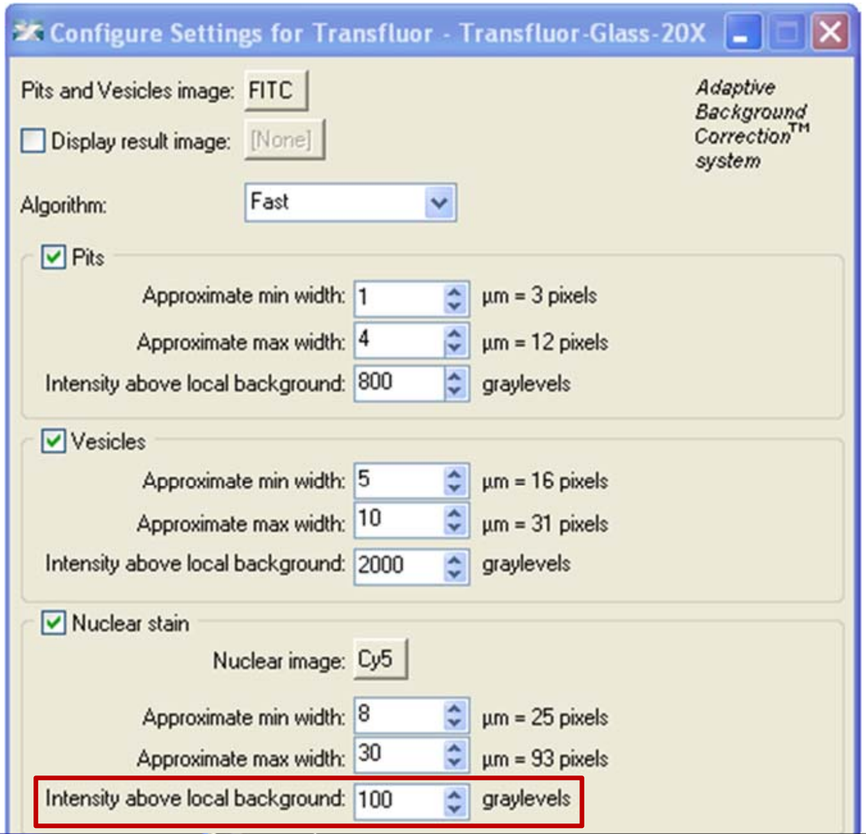
Min width too large: omits smaller nuclei

Max width too small: may shrink nuclear boundaries



Max width too large: may slightly enlarge nuclear boundaries

Module Settings



Configure Settings for Transfluor - Transfluor-Glass-20X

Pits and Vesicles image: FITC

☐ Display result image: [None]

Algorithm: Fast

Adaptive Background Correction™ system

☑ Pits

Approximate min width: 1 µm = 3 pixels

Approximate max width: 4 µm = 12 pixels

Intensity above local background: 800 graylevels

☑ Vesicles

Approximate min width: 5 µm = 16 pixels

Approximate max width: 10 µm = 31 pixels

Intensity above local background: 2000 graylevels

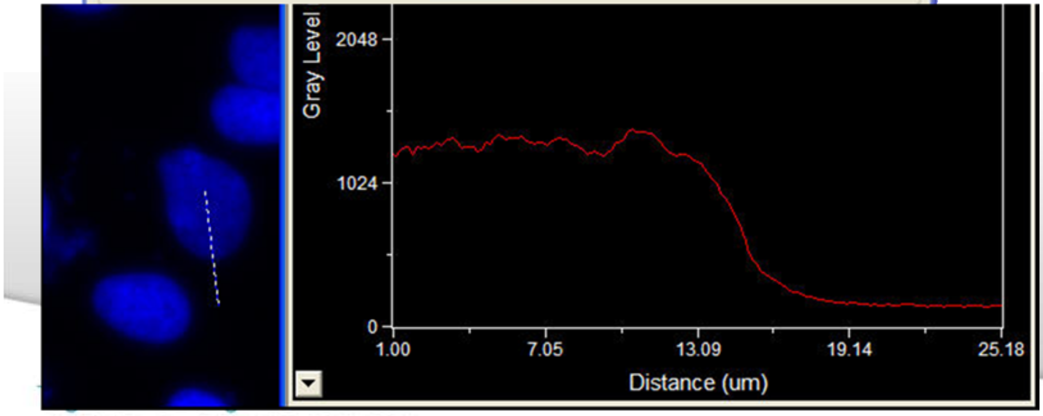
☑ Nuclear stain

Nuclear image: Cy5

Approximate min width: 8 µm = 25 pixels

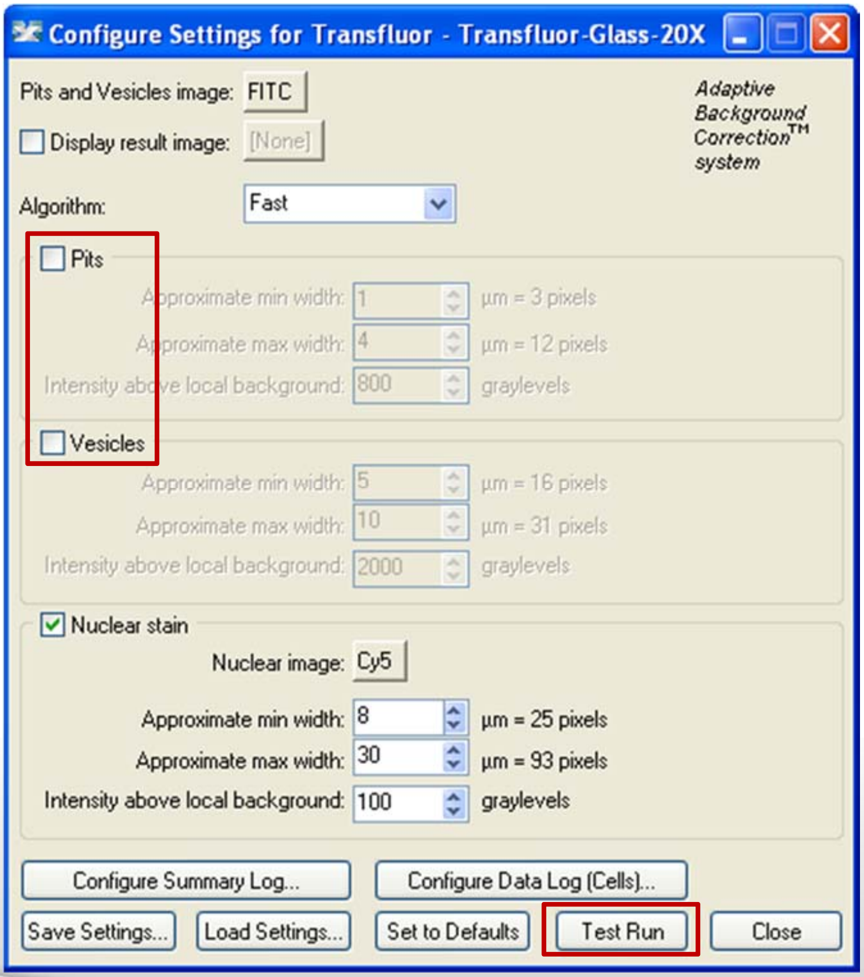
Approximate max width: 30 µm = 93 pixels

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. 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

Module Settings



Pits and Vesicles image: FITC

☐ Display result image: [None]

Algorithm: Fast

☐ Pits

Approximate min width: 1 µm = 3 pixels

Approximate max width: 4 µm = 12 pixels

Intensity above local background: 800 graylevels

☐ Vesicles

Approximate min width: 5 µm = 16 pixels

Approximate max width: 10 µm = 31 pixels

Intensity above local background: 2000 graylevels

☑ Nuclear stain

Nuclear image: Cy5

Approximate min width: 8 µm = 25 pixels

Approximate max width: 30 µm = 93 pixels

Intensity above local background: 100 graylevels

- **Nuclear Stain**
- Deselect the Vesicles and vesicle measurements
- Select Test Run to view the cell segmentation
- Change settings if needed
- Reselect the pit and vesicle options and save the settings

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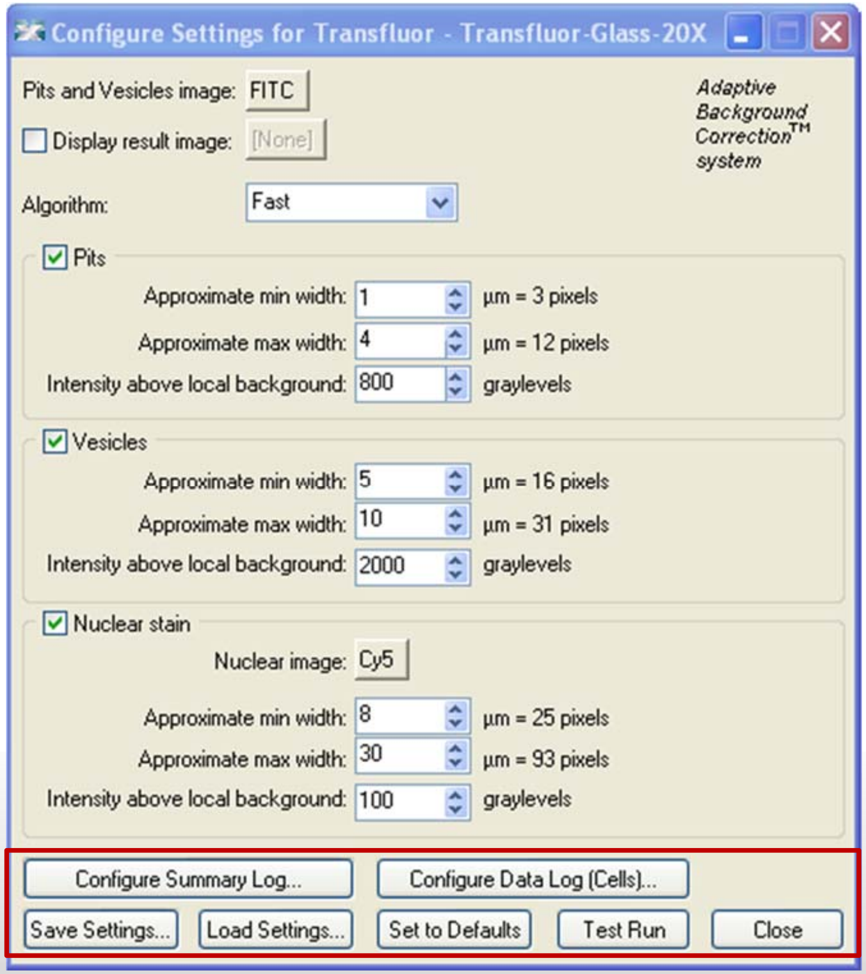
Module Settings – General Settings



Pits and Vesicles image: FITC

☐ Display result image: [None]

Adaptive Background Correction™ system

Algorithm: Fast

☑ Pits

Approximate min width: 1 µm = 3 pixels
Approximate max width: 4 µm = 12 pixels
Intensity above local background: 800 graylevels

☑ Vesicles

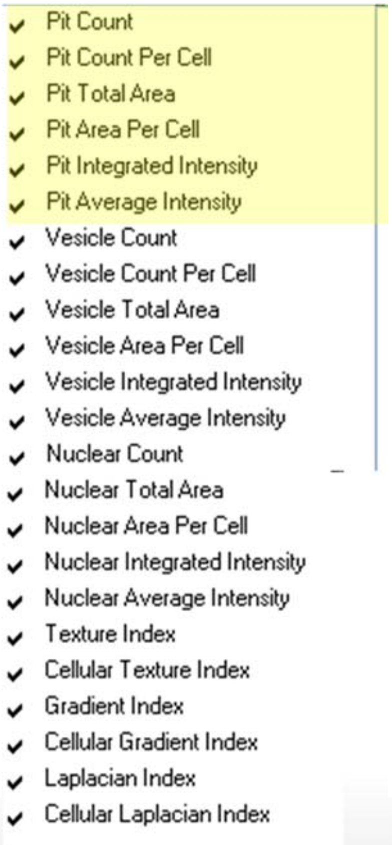
Approximate min width: 5 µm = 16 pixels
Approximate max width: 10 µm = 31 pixels
Intensity above local background: 2000 graylevels

☑ Nuclear stain

Nuclear image: Cy5
Approximate min width: 8 µm = 25 pixels
Approximate max width: 30 µm = 93 pixels
Intensity above local background: 100 graylevels

- **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

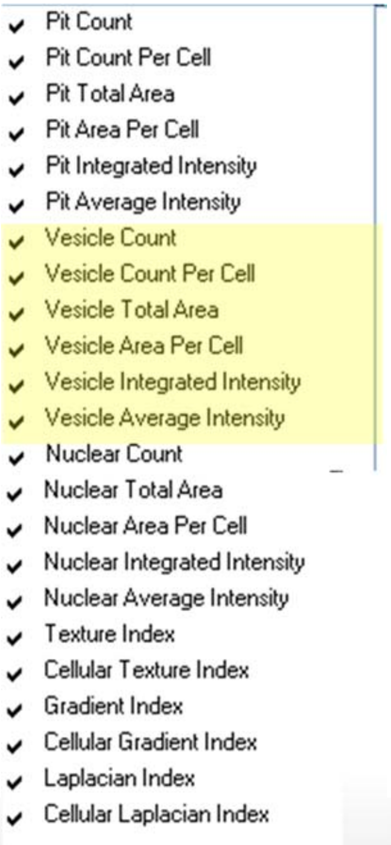
Summary Data (site-by-site measurements)



- ✓ Pit Count
- ✓ Pit Count Per Cell
- ✓ Pit Total Area
- ✓ Pit Area Per Cell
- ✓ Pit Integrated Intensity
- ✓ Pit Average Intensity
- ✓ Vesicle Count
- ✓ Vesicle Count Per Cell
- ✓ Vesicle Total Area
- ✓ Vesicle Area Per Cell
- ✓ Vesicle Integrated Intensity
- ✓ Vesicle Average Intensity
- ✓ Nuclear Count
- ✓ Nuclear Total Area
- ✓ Nuclear Area Per Cell
- ✓ Nuclear Integrated Intensity
- ✓ Nuclear Average Intensity
- ✓ Texture Index
- ✓ Cellular Texture Index
- ✓ Gradient Index
- ✓ Cellular Gradient Index
- ✓ Laplacian Index
- ✓ Cellular Laplacian Index

- **Pit Count:** Total number of pits
- **Pit Count Per Cell:** Total number of pits divided by the total number of nucleus
- **Pit Total Area:** The total area of the pits found in the image (in um^2)
- **Pit Area Per Cell:** The total area of pits for all cells divided by the total number of nucleus (in um^2)
- **Pit Integrated Intensity:** The total pixel intensity of the pit area
- **Pit Average Intensity**: The total pixel intensity of the pit area divided by the total number of nucleus

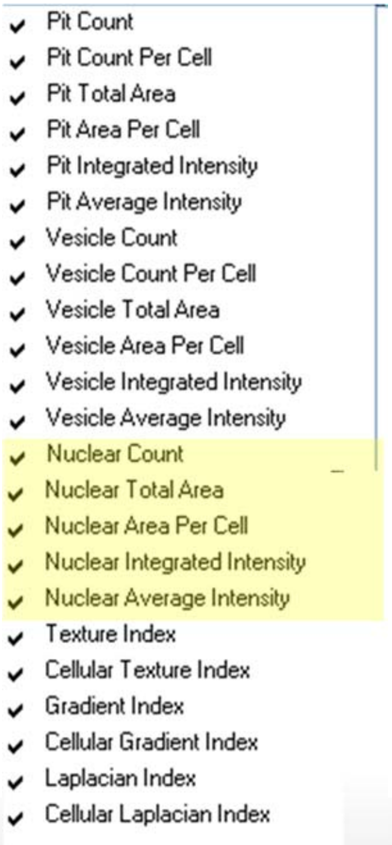
Summary Data (site-by-site measurements)



- ✓ Pit Count
- ✓ Pit Count Per Cell
- ✓ Pit Total Area
- ✓ Pit Area Per Cell
- ✓ Pit Integrated Intensity
- ✓ Pit Average Intensity
- ✓ Vesicle Count
- ✓ Vesicle Count Per Cell
- ✓ Vesicle Total Area
- ✓ Vesicle Area Per Cell
- ✓ Vesicle Integrated Intensity
- ✓ Vesicle Average Intensity
- ✓ Nuclear Count
- ✓ Nuclear Total Area
- ✓ Nuclear Area Per Cell
- ✓ Nuclear Integrated Intensity
- ✓ Nuclear Average Intensity
- ✓ Texture Index
- ✓ Cellular Texture Index
- ✓ Gradient Index
- ✓ Cellular Gradient Index
- ✓ Laplacian Index
- ✓ Cellular Laplacian Index

- **Vesicle Count:** Total number of vesicle
- **Vesicle Count Per Cell:** Total number of vesicle divided by the total number of nucleus
- **Vesicle Total Area:** The total area of the vesicle found in the image (in um^2)
- **Vesicle Area Per Cell:** The total area of vesicle for all cells divided by the total number of nucleus (in um^2)
- **Vesicle Integrated Intensity:** The total pixel intensity of the vesicle area
- **Vesicle Average Intensity**: The total pixel intensity of the vesicle area divided by the total number of nucleus

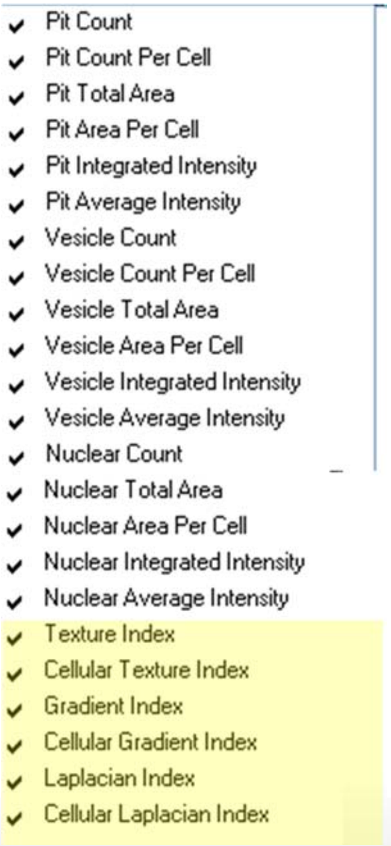
Summary Data (site-by-site measurements)



✓ Pit Count
✓ Pit Count Per Cell
✓ Pit Total Area
✓ Pit Area Per Cell
✓ Pit Integrated Intensity
✓ Pit Average Intensity
✓ Vesicle Count
✓ Vesicle Count Per Cell
✓ Vesicle Total Area
✓ Vesicle Area Per Cell
✓ Vesicle Integrated Intensity
✓ Vesicle Average Intensity
✓ Nuclear Count
✓ Nuclear Total Area
✓ Nuclear Area Per Cell
✓ Nuclear Integrated Intensity
✓ Nuclear Average Intensity
✓ Texture Index
✓ Cellular Texture Index
✓ Gradient Index
✓ Cellular Gradient Index
✓ Laplacian Index
✓ Cellular Laplacian Index

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

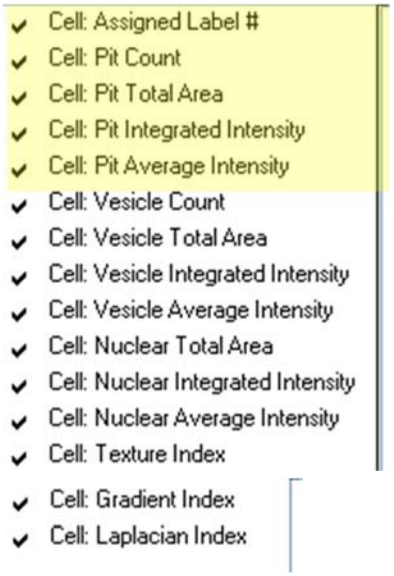
Summary Data (site-by-site measurements)



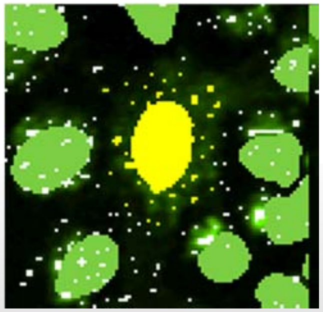
- ✓ Pit Count
- ✓ Pit Count Per Cell
- ✓ Pit Total Area
- ✓ Pit Area Per Cell
- ✓ Pit Integrated Intensity
- ✓ Pit Average Intensity
- ✓ Vesicle Count
- ✓ Vesicle Count Per Cell
- ✓ Vesicle Total Area
- ✓ Vesicle Area Per Cell
- ✓ Vesicle Integrated Intensity
- ✓ Vesicle Average Intensity
- ✓ Nuclear Count
- ✓ Nuclear Total Area
- ✓ Nuclear Area Per Cell
- ✓ Nuclear Integrated Intensity
- ✓ Nuclear Average 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)



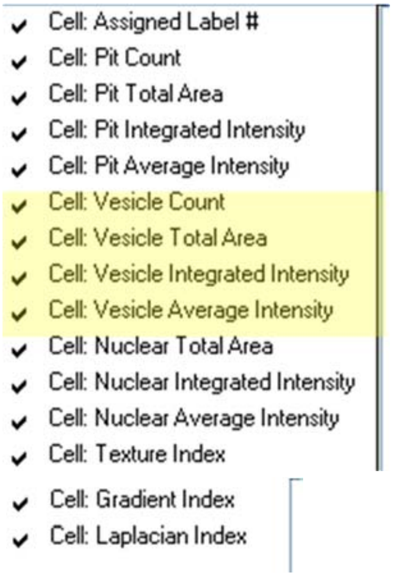
- ✓ Cell: Assigned Label #
- ✓ Cell: Pit Count
- ✓ Cell: Pit Total Area
- ✓ Cell: Pit Integrated Intensity
- ✓ Cell: Pit Average Intensity
- ✓ Cell: Vesicle Count
- ✓ Cell: Vesicle Total Area
- ✓ Cell: Vesicle Integrated Intensity
- ✓ Cell: Vesicle Average Intensity
- ✓ Cell: Nuclear Total Area
- ✓ Cell: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



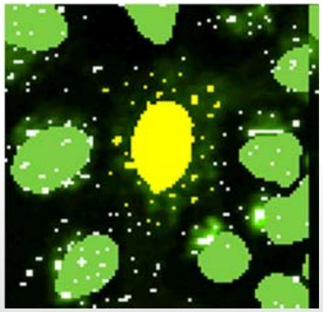
Highlighted cells shows assigned pits to cell

- **Cell: Assigned Label #** – Cell label number (1 through total cell number)
- **Cell: Pit Count:** Number of pits detected for a specific cell. (Note: a pit is assigned to its nearest nucleus)
- **Cell: Pit Total Area:** Area covered by all the pits assigned to a specific cell in um2
- **Cell: Pit Integrated Intensity:** The total pixel intensity of the pits assigned to a specific cell
- **Cell: Pit Average Intensity:** The total pixel intensity of the pits assigned to a specific cell divided by the number of pits assigned to a specific cell

Cell Data (cell-by-cell measurements)



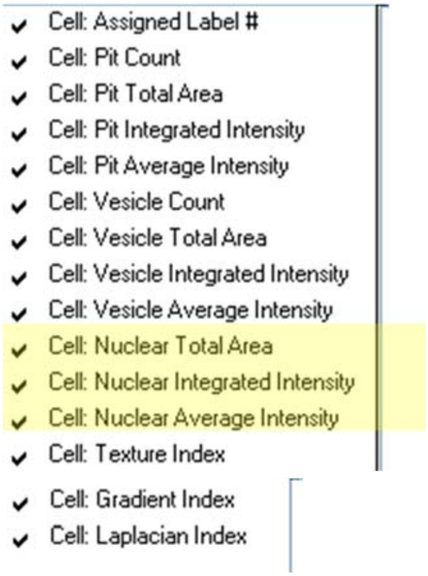
- ✓ Cell: Assigned Label #
- ✓ Cell: Pit Count
- ✓ Cell: Pit Total Area
- ✓ Cell: Pit Integrated Intensity
- ✓ Cell: Pit Average Intensity
- ✓ Cell: Vesicle Count
- ✓ Cell: Vesicle Total Area
- ✓ Cell: Vesicle Integrated Intensity
- ✓ Cell: Vesicle Average Intensity
- ✓ Cell: Nuclear Total Area
- ✓ Cell: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



Highlighted cells shows assigned pits to cell

- **Cell: Vesicle Count:** Number of Vesicles detected for a specific cell. (Note: a Vesicle is assigned to its nearest nucleus)
- **Cell: Vesicle Total Area:** Area covered by all the Vesicles assigned to a specific cell in um2
- **Cell: Vesicle Integrated Intensity:** The total pixel intensity of the Vesicles assigned to a specific cell
- **Cell: Vesicle Average Intensity:** The total pixel intensity of the Vesicles assigned to a specific cell divided by the number of Vesicles assigned to a specific cell

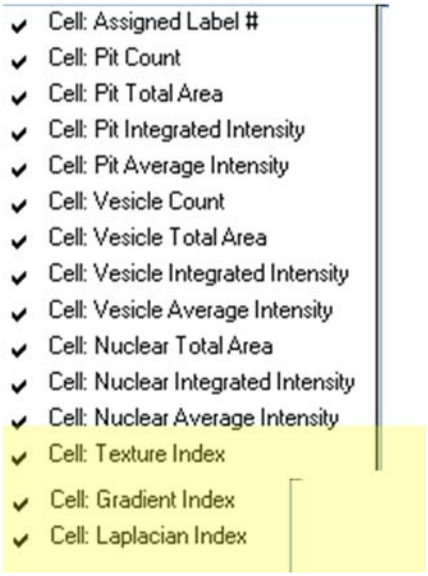
Cell Data (cell-by-cell measurements)



✓ Cell: Assigned Label #
✓ Cell: Pit Count
✓ Cell: Pit Total Area
✓ Cell: Pit Integrated Intensity
✓ Cell: Pit Average Intensity
✓ Cell: Vesicle Count
✓ Cell: Vesicle Total Area
✓ Cell: Vesicle Integrated Intensity
✓ Cell: Vesicle 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: Pit Count
- ✓ Cell: Pit Total Area
- ✓ Cell: Pit Integrated Intensity
- ✓ Cell: Pit Average Intensity
- ✓ Cell: Vesicle Count
- ✓ Cell: Vesicle Total Area
- ✓ Cell: Vesicle Integrated Intensity
- ✓ Cell: Vesicle 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

Molecular Devices

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