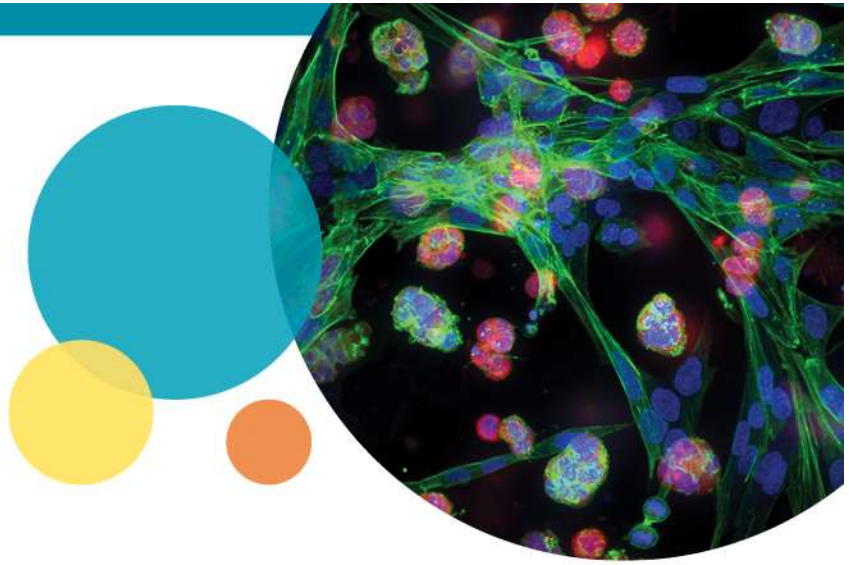
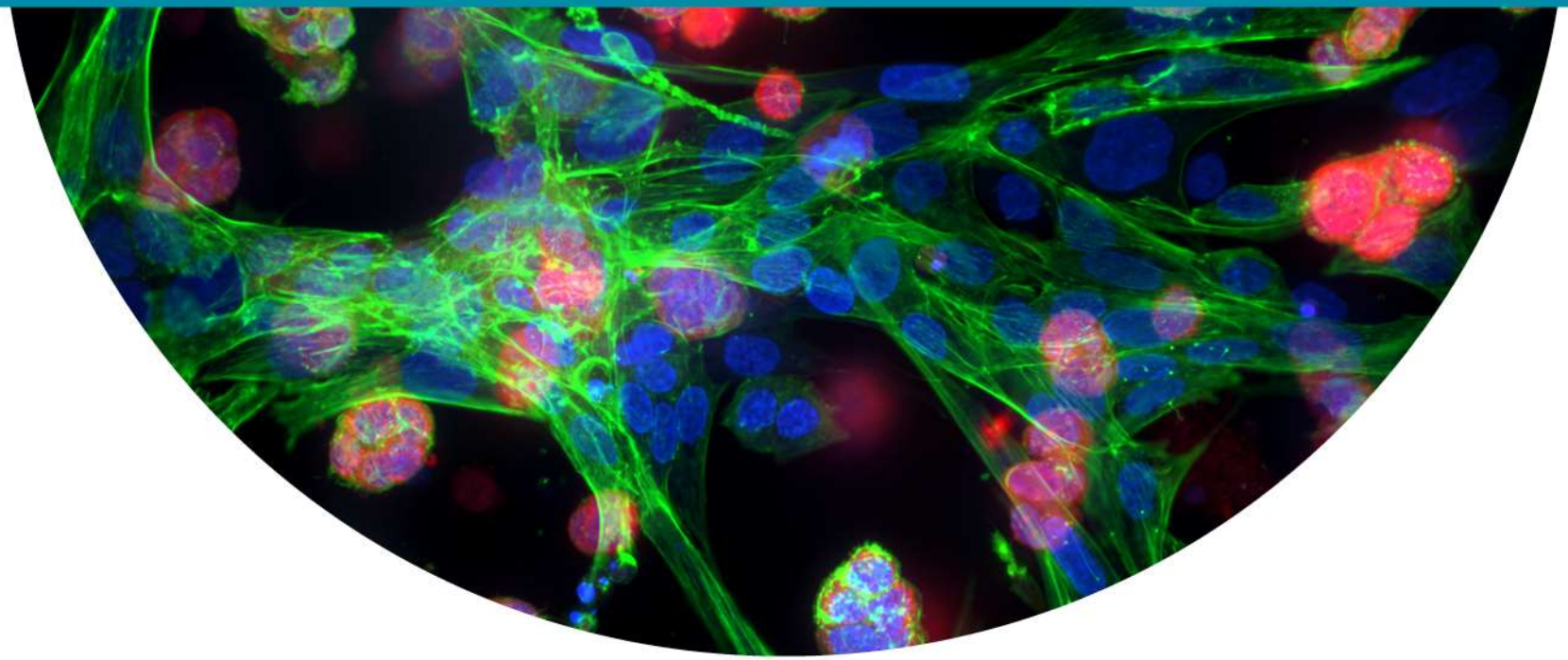


Actin Filament analysis in MetaXpress Custom Module Editor

August 2018

MOLECULAR DEVICES
ADVANCING DISCOVERY

For research use only. Not for use in diagnostic procedures.
© 2012-2018. Trademarks are the property of Molecular Devices, LLC or their respective owners.

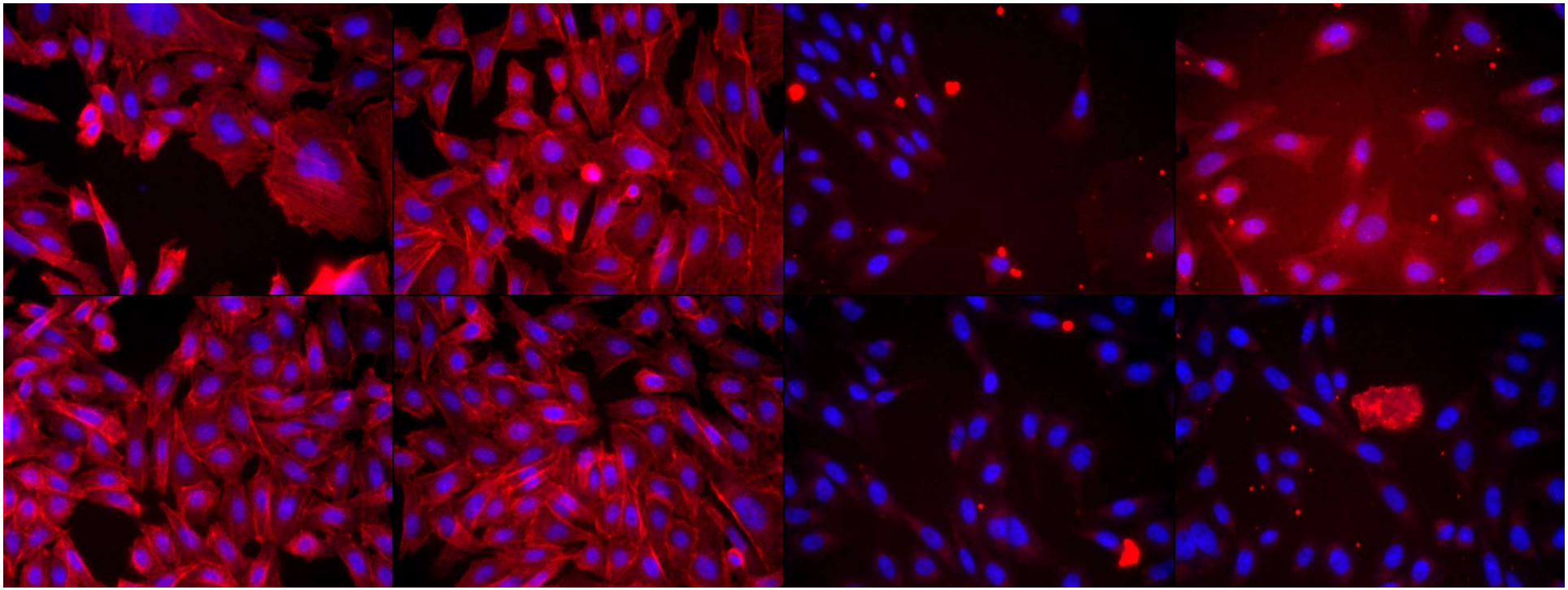


Version A: Transfluor or Granularity module

MOLECULAR DEVICES

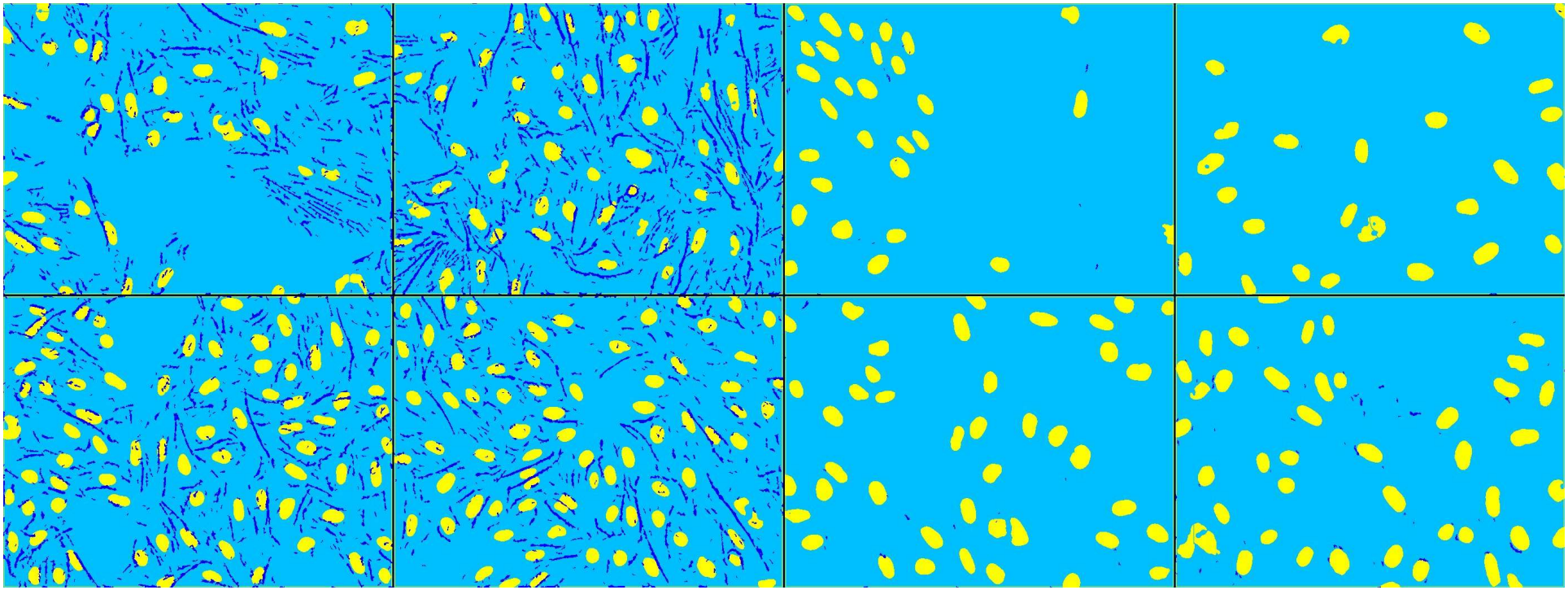
For research use only. Not for use in diagnostic procedures.

Example Plate (8 wells)



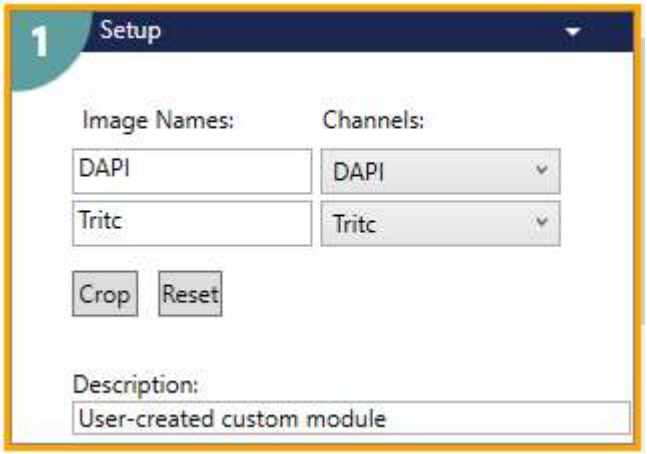
For research use only. Not for use in diagnostic procedures.

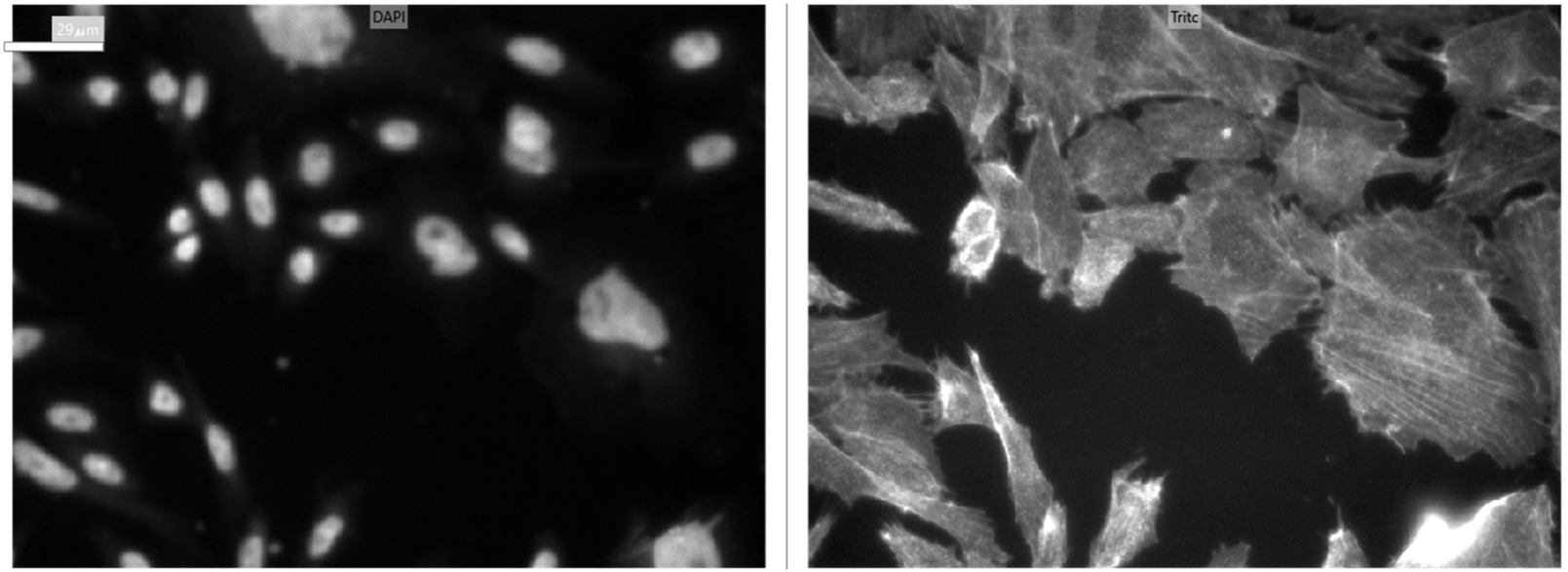
Segmentation Overlay



For research use only. Not for use in diagnostic procedures.

Step 1: Setup

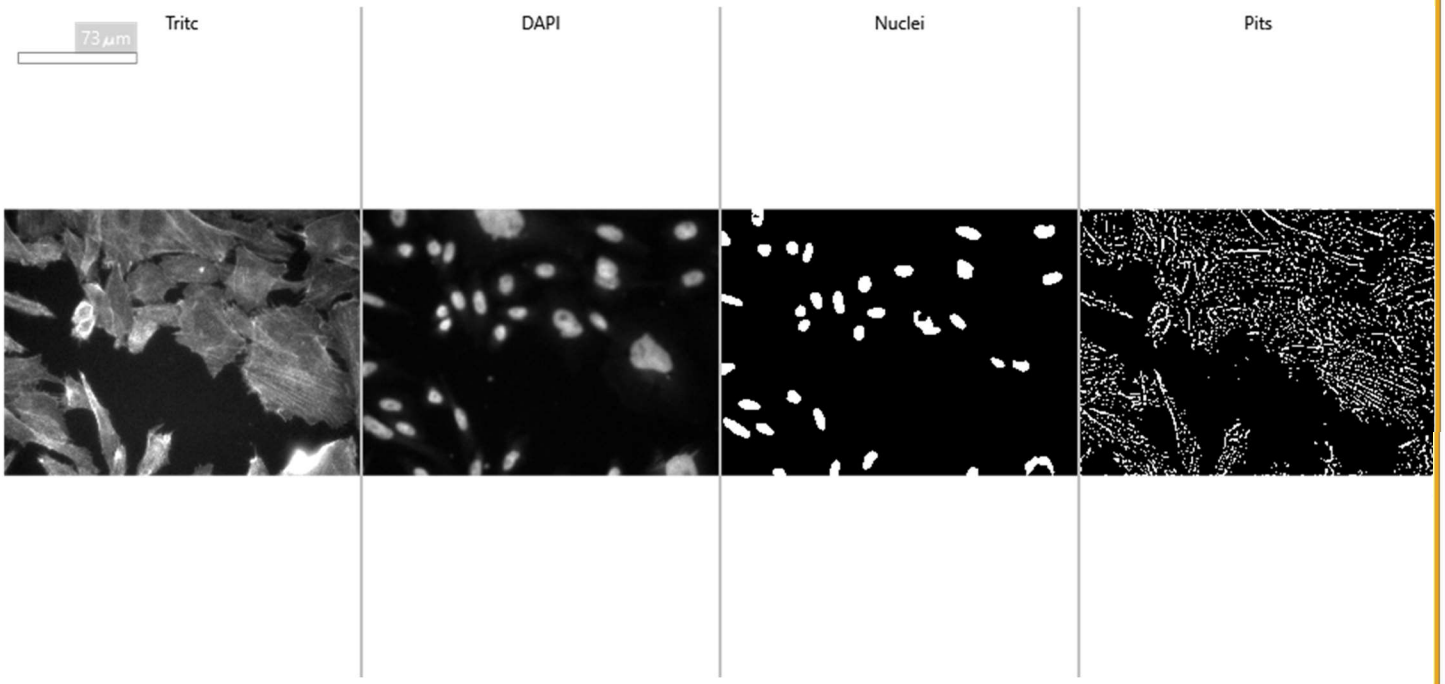


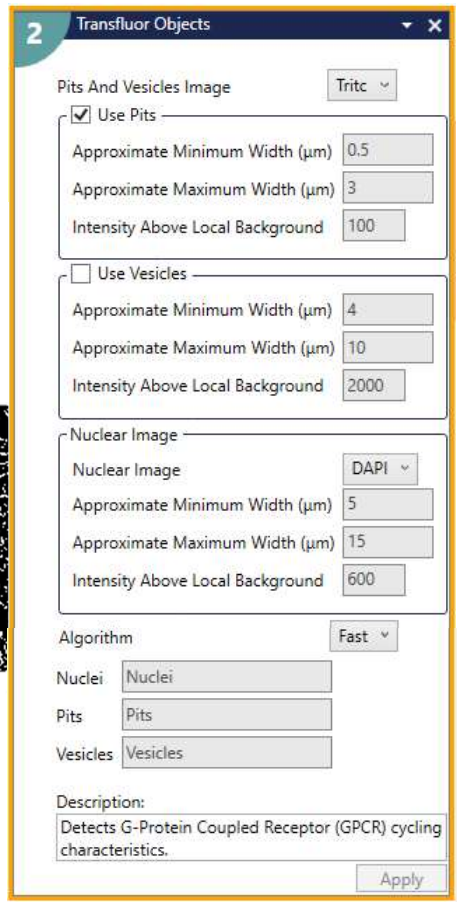


In the Setup step, define the wavelengths for analysis.

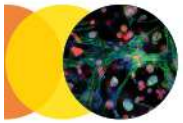
For research use only. Not for use in diagnostic procedures.

Step 2: Transfluor (or Granularity)



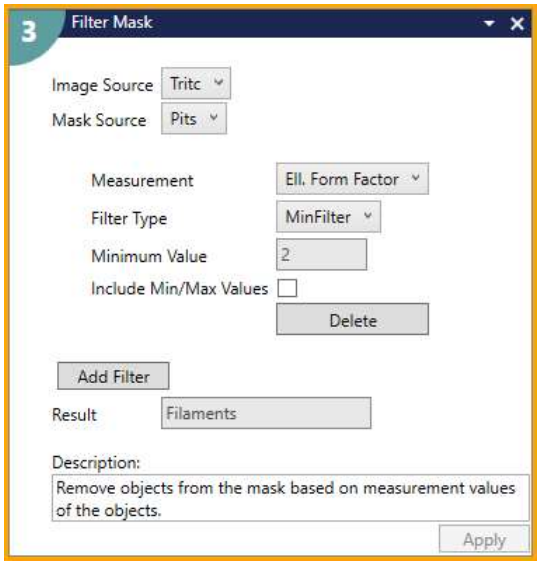


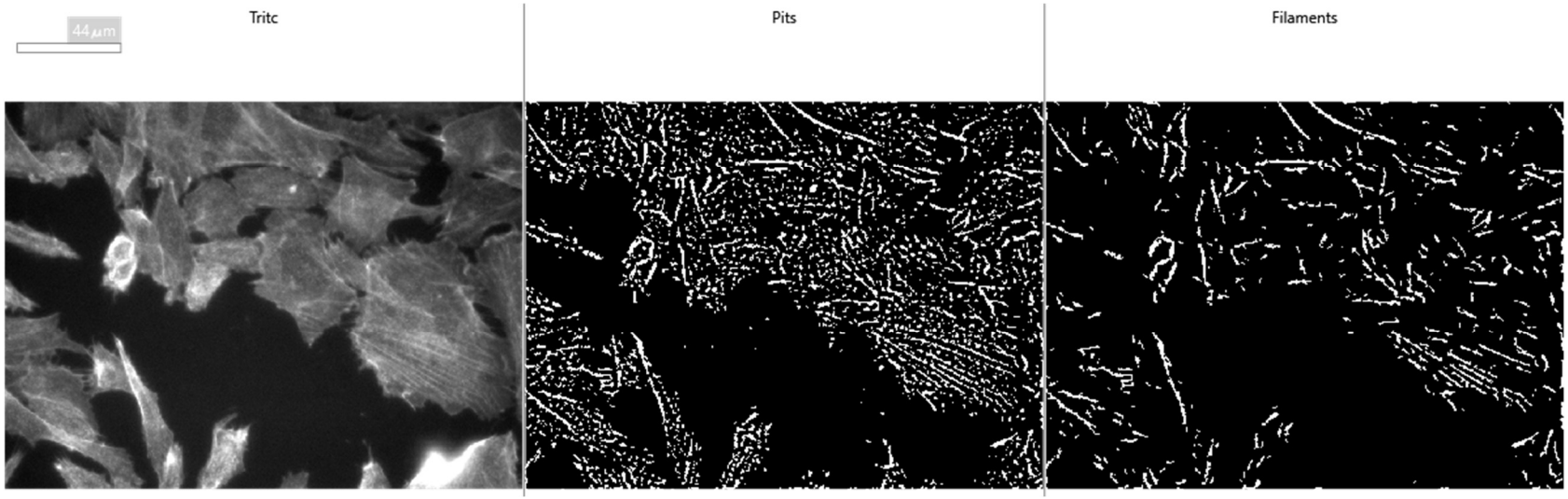
Use the Transfluor or Granularity Application Module to identify nuclei and small bright objects. If using Transfluor, only the Pits option is used. Note: Either the Transfluor or Granularity module must be enabled on your MetaXpress license key.



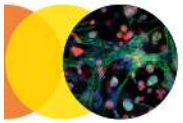
For research use only. Not for use in diagnostic procedures.

Step 3: Filter Mask



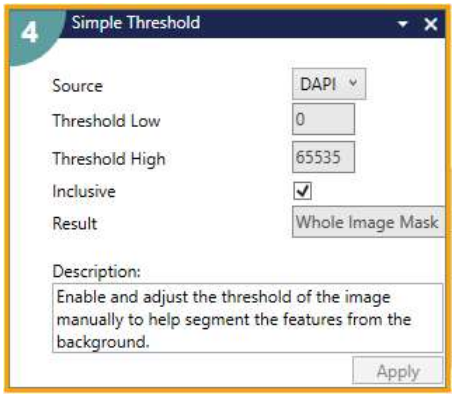


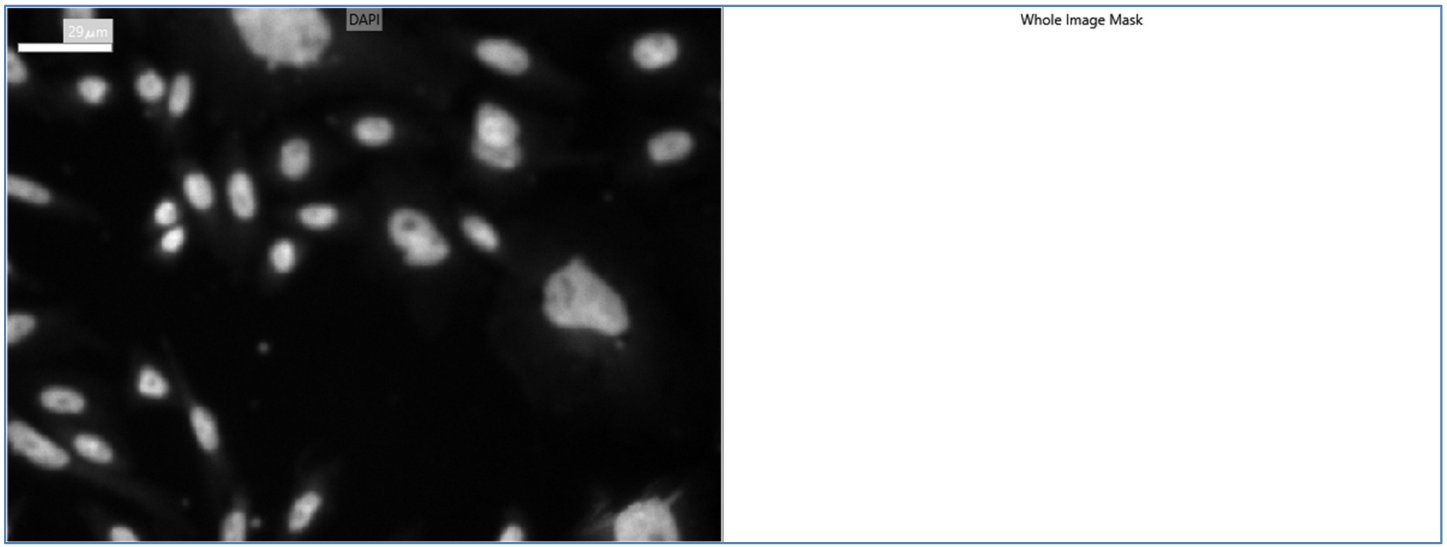
Use a Filter Mask to select objects by size, shape, and/or intensity from the Pits or Granules mask. In this example, Elliptical Form Factor (ratio of length/breadth) is used to select elongated objects.



For research use only. Not for use in diagnostic procedures.

Step 4: Simple Threshold

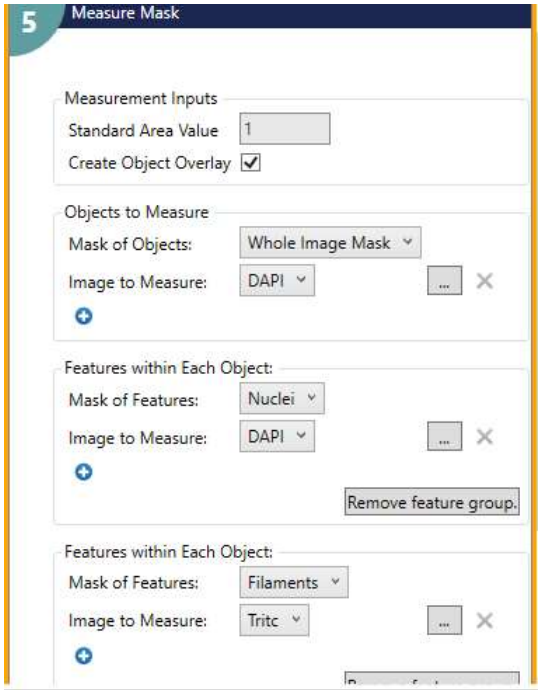


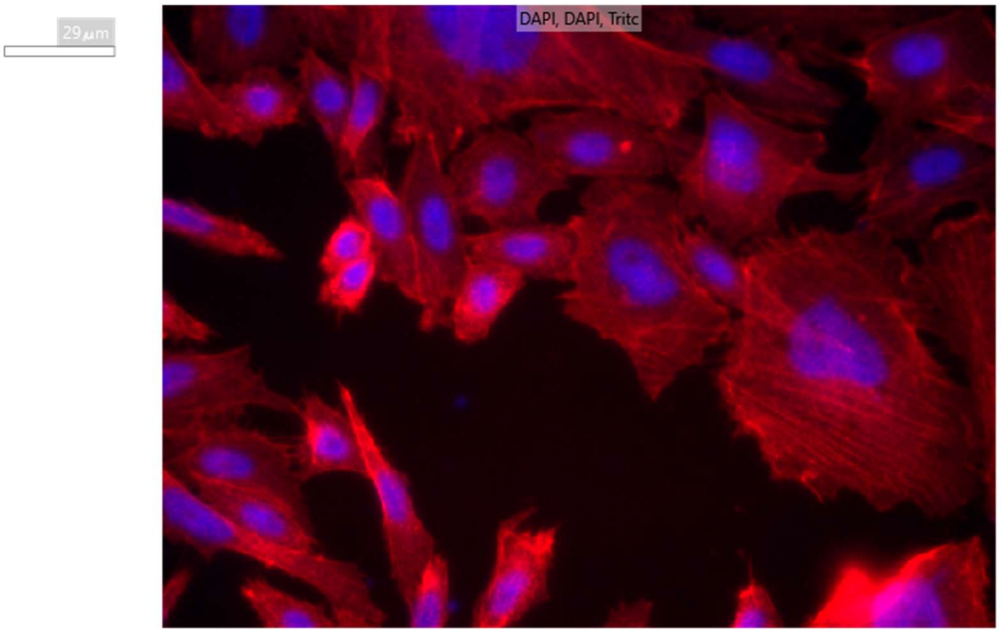


To simplify the output, use Simple Threshold inclusive from 0-65535 to create a mask that represents the entire image area. Any image may be used as the Source for this step.

For research use only. Not for use in diagnostic procedures.

Step 5: Measure Mask

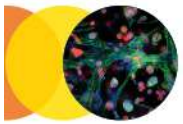




Mask Legend

Layer	Color	Mask Name
1		Whole Image Mask
2		Nuclei
3		Filaments

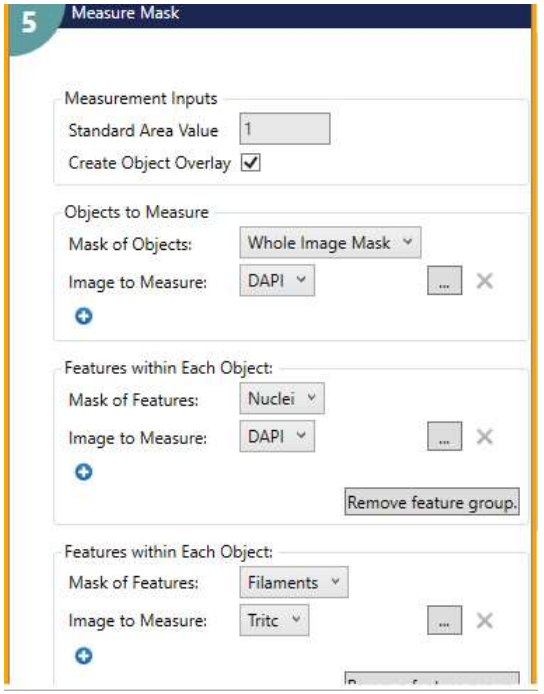
The ‘Objects to Measure’ mask is set to the Simple Threshold result mask, with no measurements selected. This generates 1 row of data per site. The Nuclei and Filaments masks are added as feature groups.

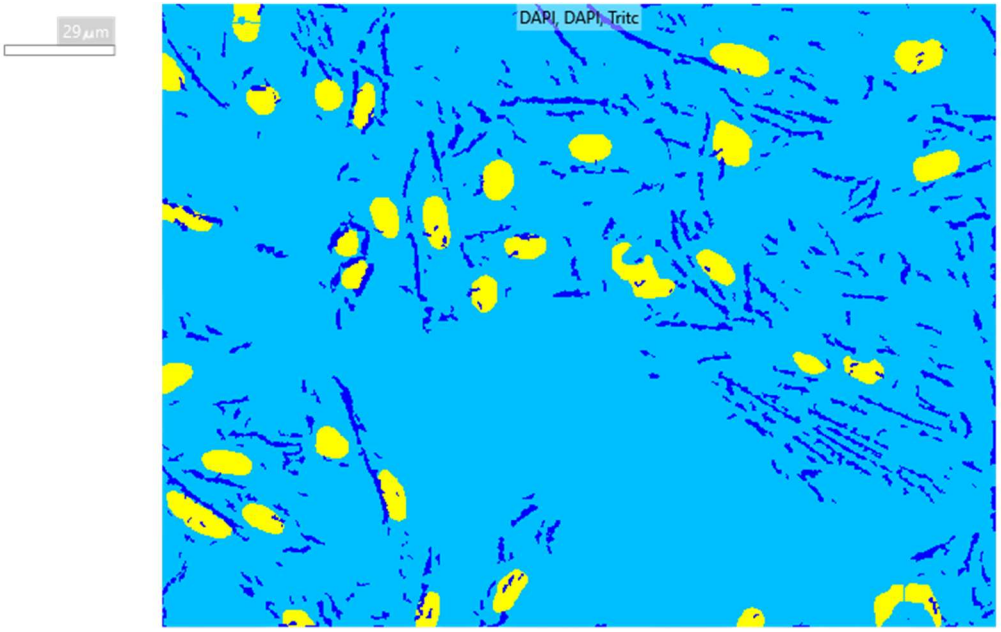


MOLECULAR DEVICES

For research use only. Not for use in diagnostic procedures.

Step 5: Measure Mask

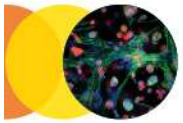




Mask Legend

Layer	Color	Mask Name
1		Whole Image Mask
2		Nuclei
3		Filaments

The ‘Objects to Measure’ mask is set to the Simple Threshold result mask, with no measurements selected. This generates 1 row of data per site. The Nuclei and Filaments masks are added as feature groups.



MOLECULAR DEVICES

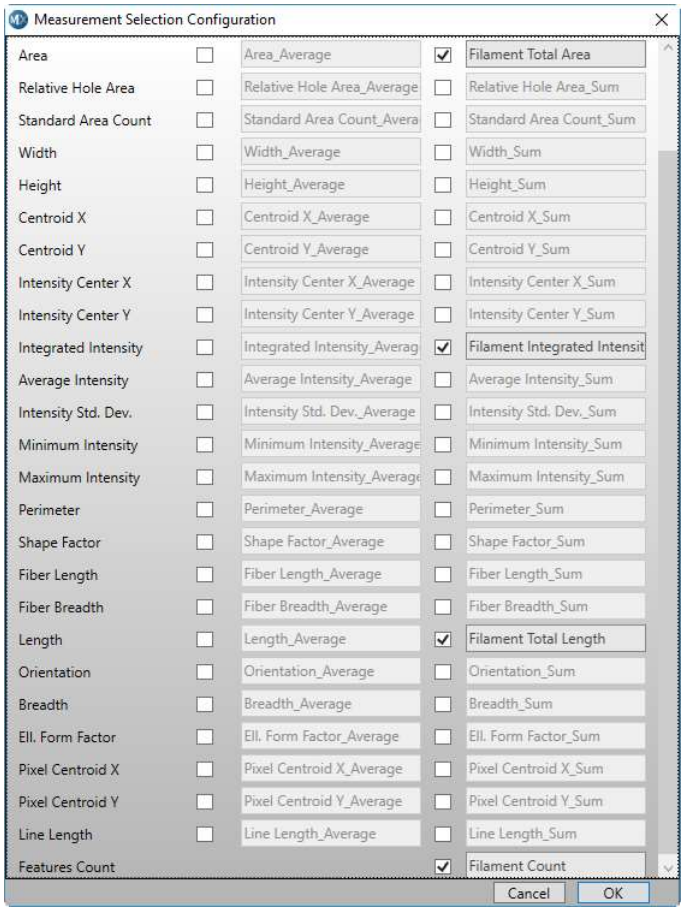
For research use only. Not for use in diagnostic procedures.

Measurement Selections

Whole Image Mask:
No measurements selected

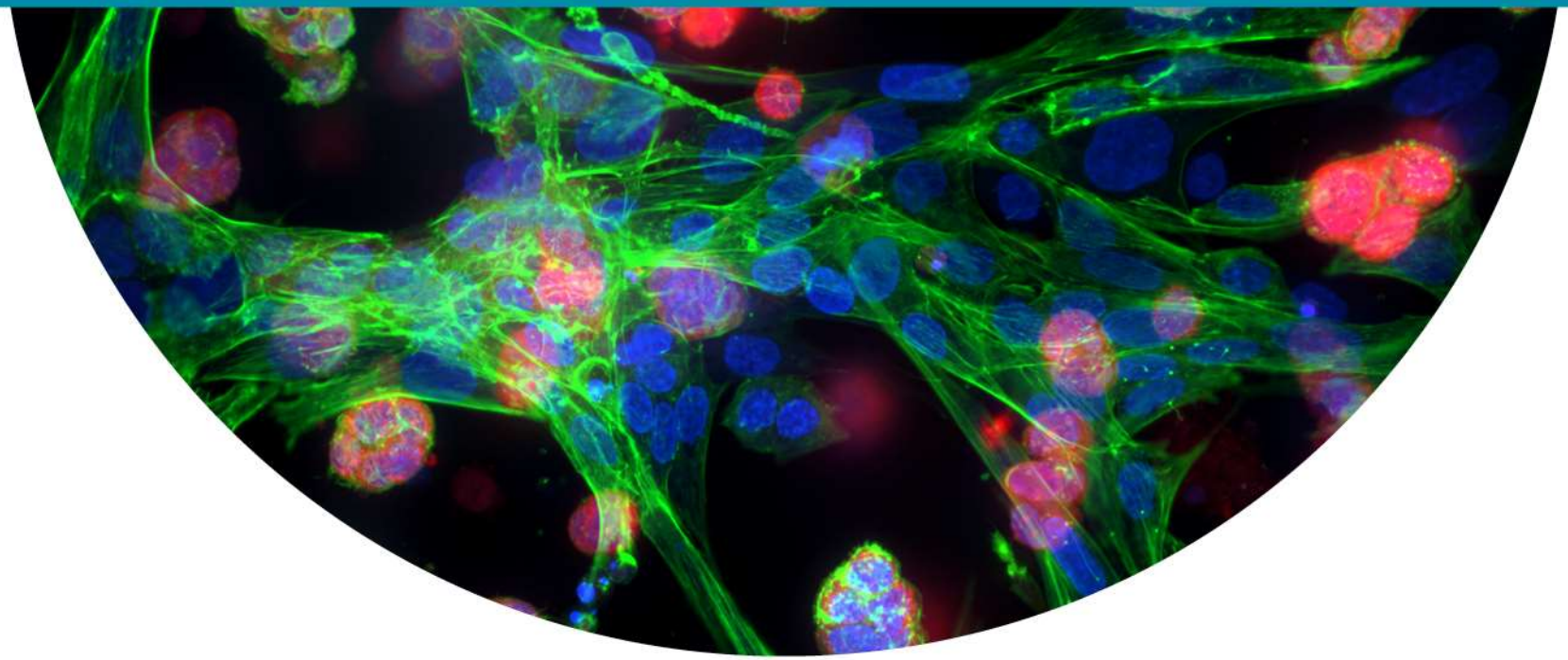
Nuclei Mask:

Filaments Mask:



Selected measurements are calculated for the Nuclei and Filaments masks.

For research use only. Not for use in diagnostic procedures.

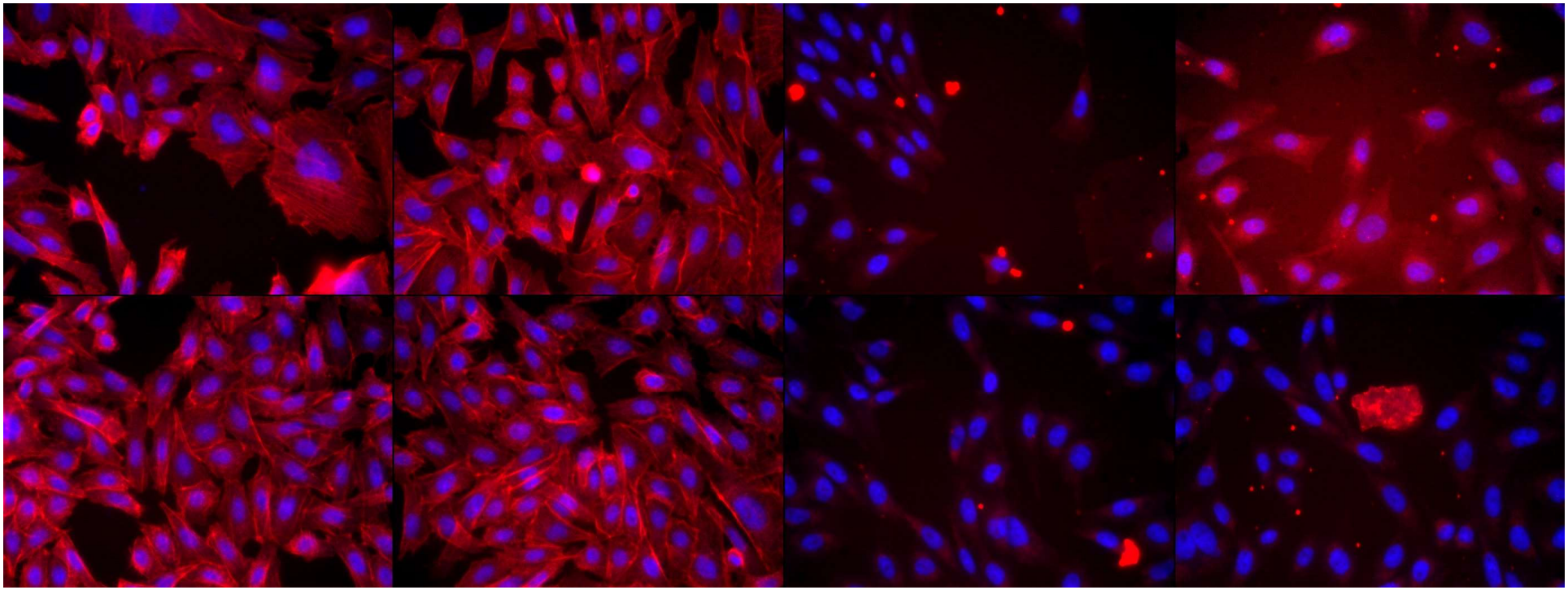


Version B: Top Hat and Find Fibers

MOLECULAR DEVICES

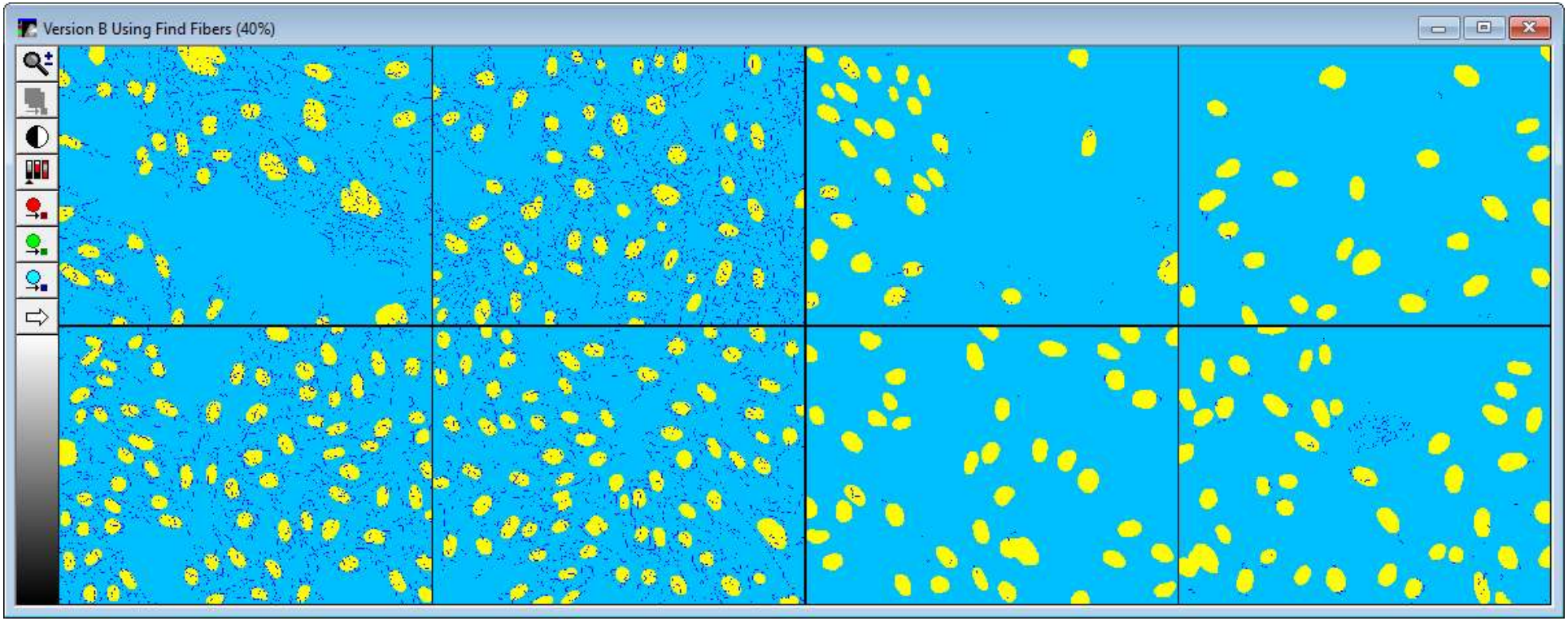
For research use only. Not for use in diagnostic procedures.

Example Plate (8 wells)



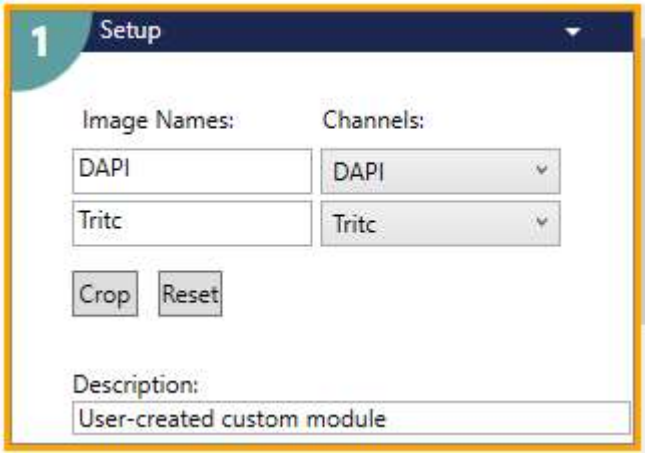
For research use only. Not for use in diagnostic procedures.

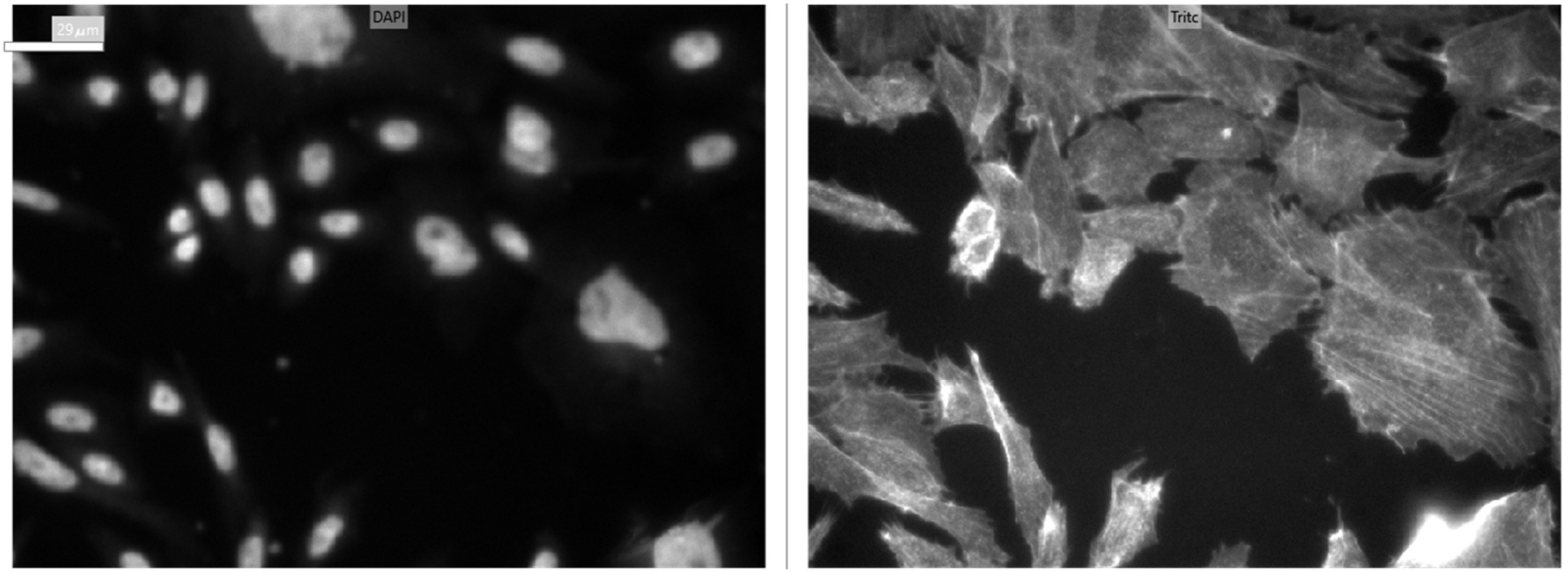
Segmentation Overlay



For research use only. Not for use in diagnostic procedures.

Step 1: Setup

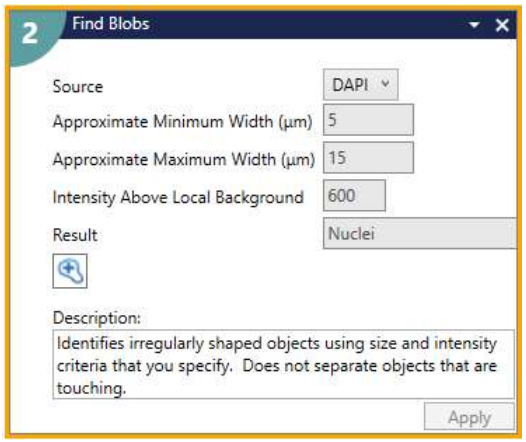


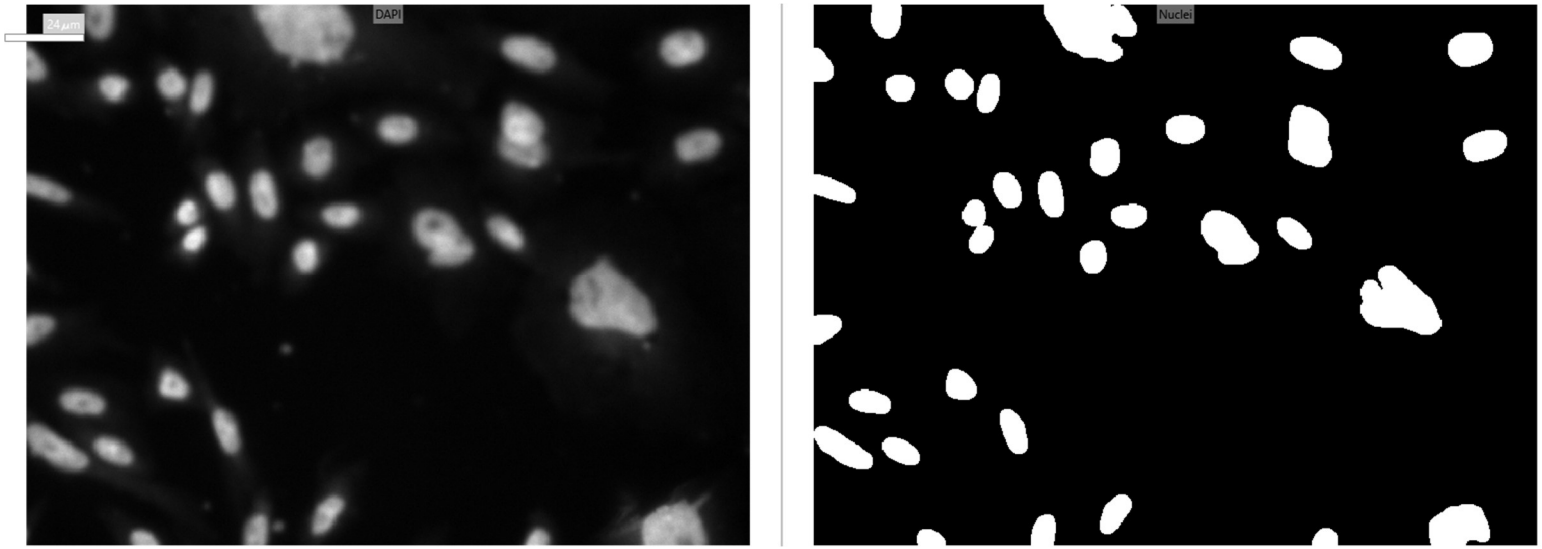


In the Setup step, define the wavelengths for analysis.

For research use only. Not for use in diagnostic procedures.

Step 2: Find Blobs

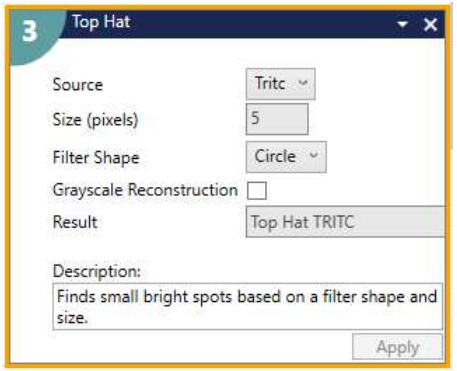


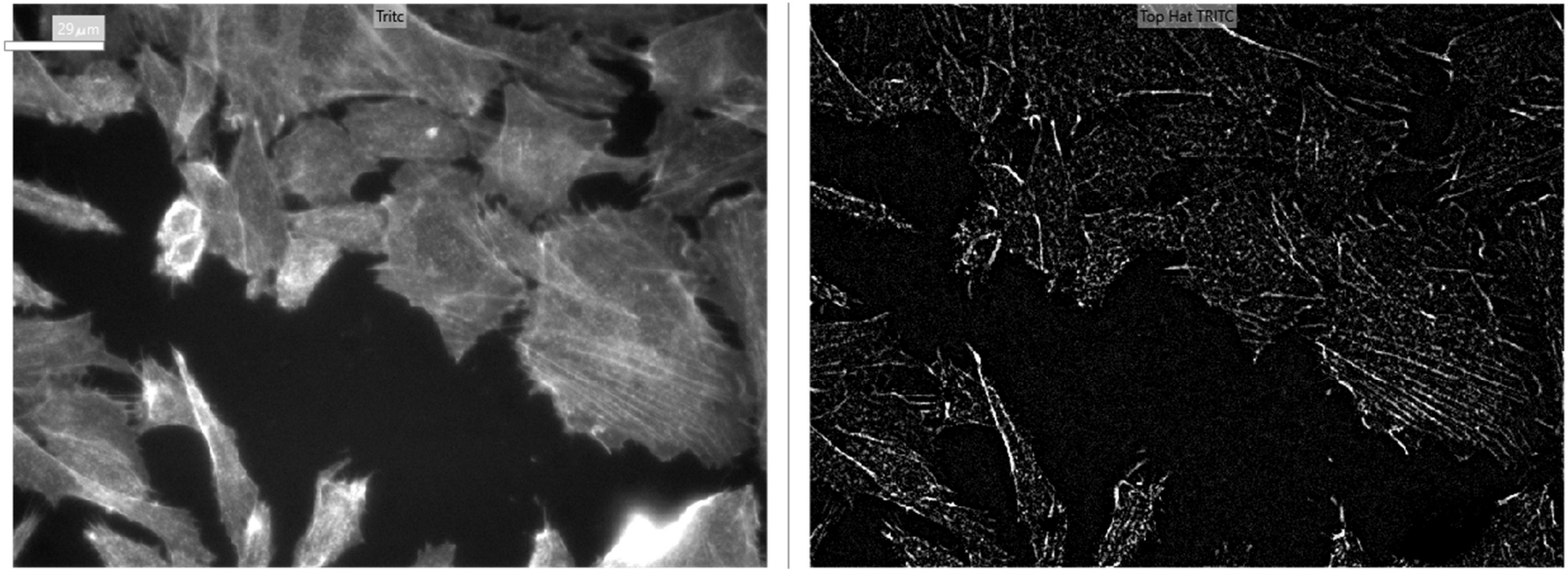


Use the Find Blobs step card to identify nuclei.

For research use only. Not for use in diagnostic procedures.

Step 3: Top Hat

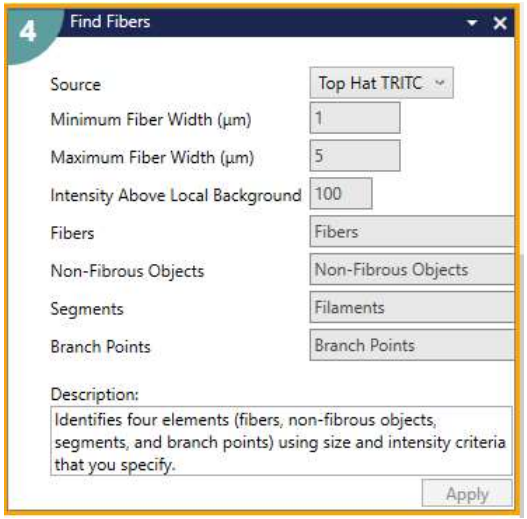


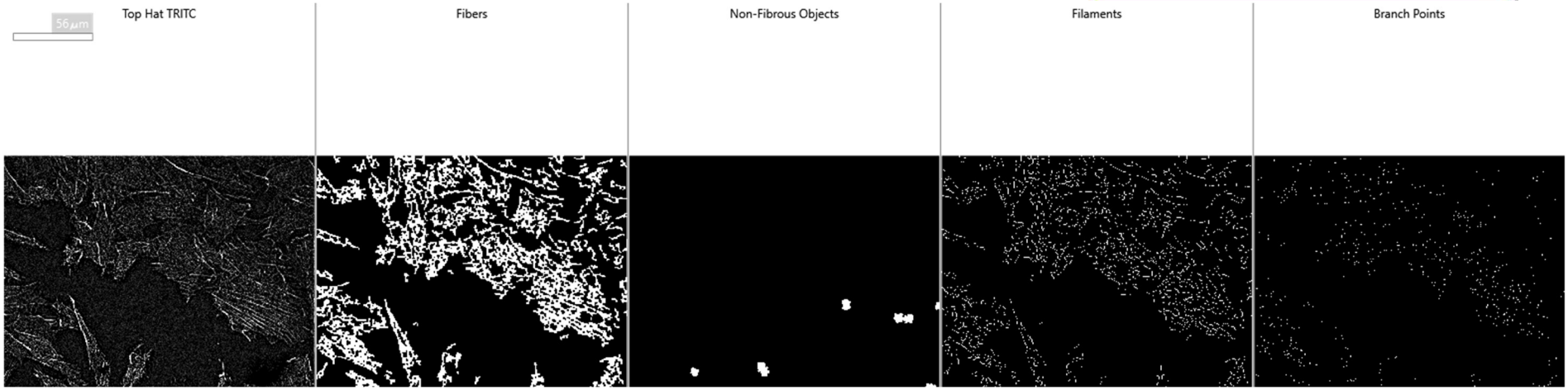


Use the Image Processing step card Top Hat to enhance small bright objects in the image.

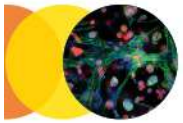
For research use only. Not for use in diagnostic procedures.

Step 4: Find Fibers



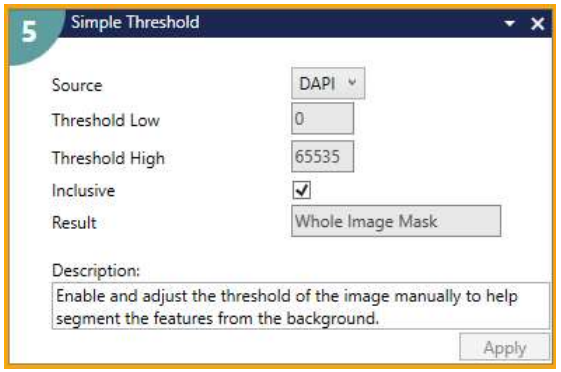


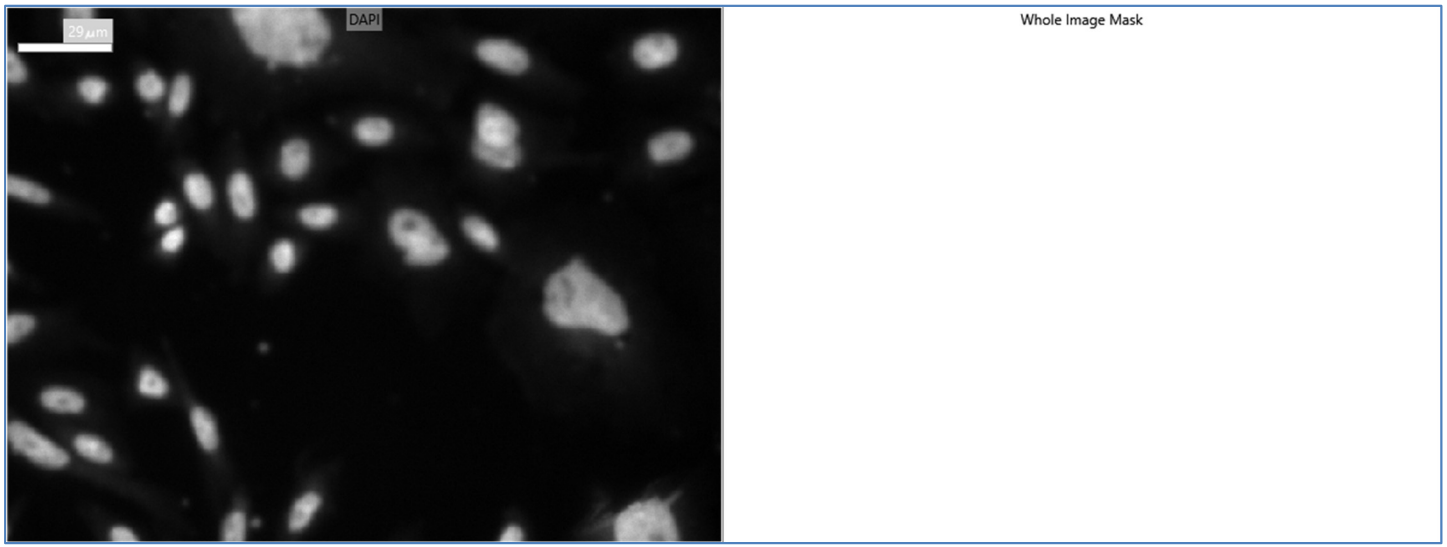
Use the Find Fibers step card to identify fibrous objects. Subsequent steps use the Segments mask which is a skeletonized version of the Fibers mask.



For research use only. Not for use in diagnostic procedures.

Step 5: Simple Threshold

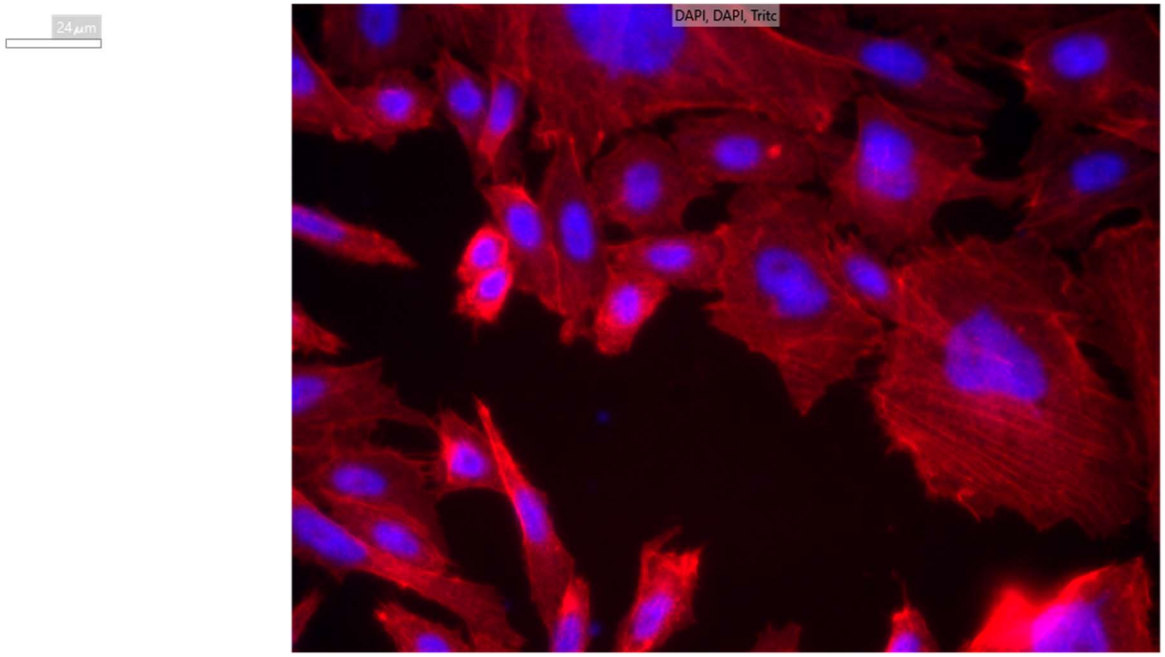


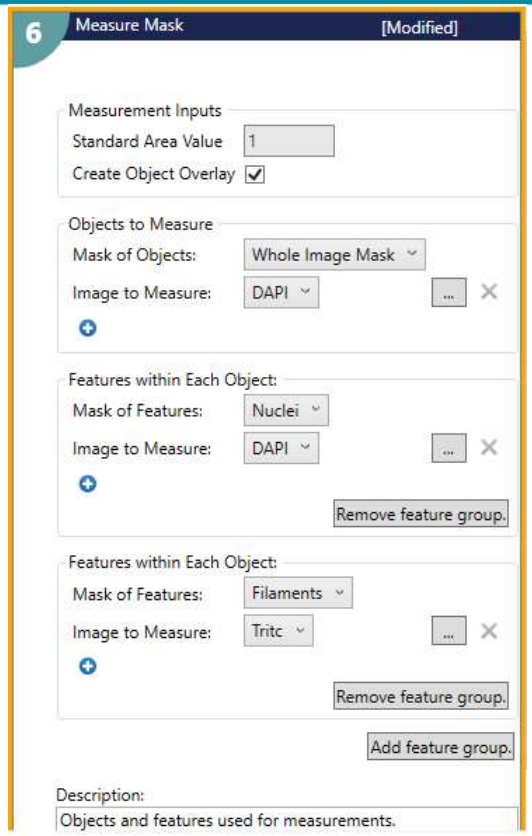


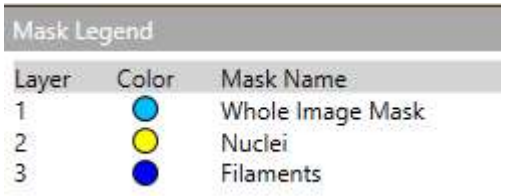
To simplify the output, use Simple Threshold inclusive from 0-65535 to create a mask that represents the entire image area. Any image may be used as the Source for this step.

For research use only. Not for use in diagnostic procedures.

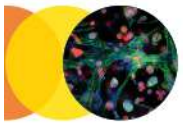
Step 6: Measure Mask





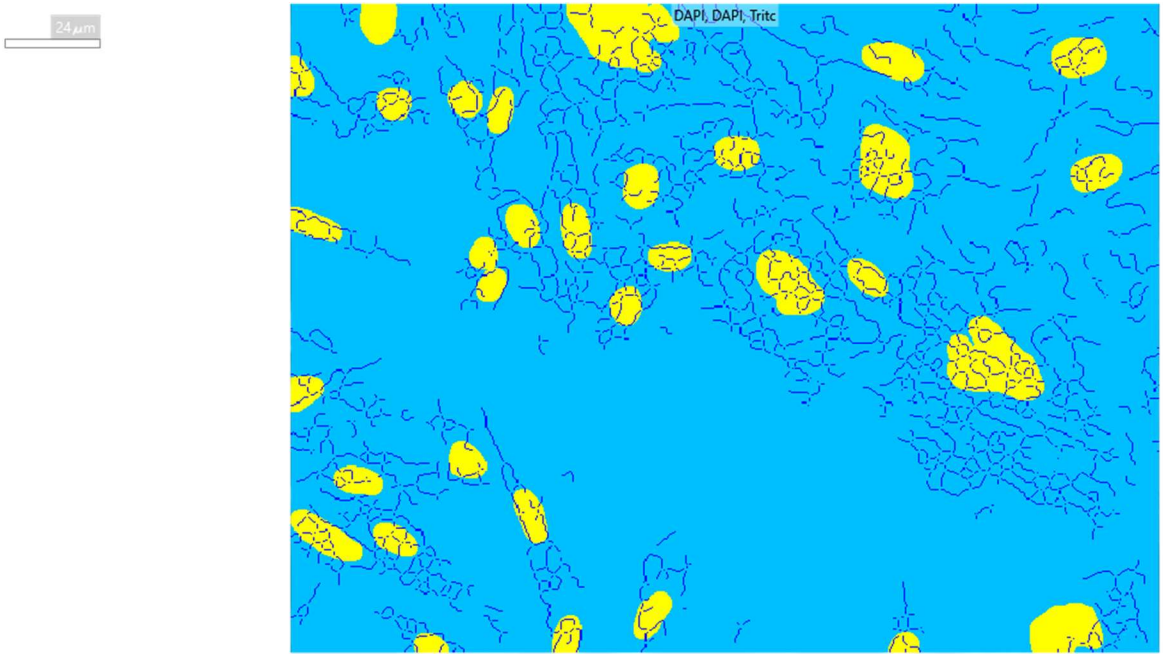


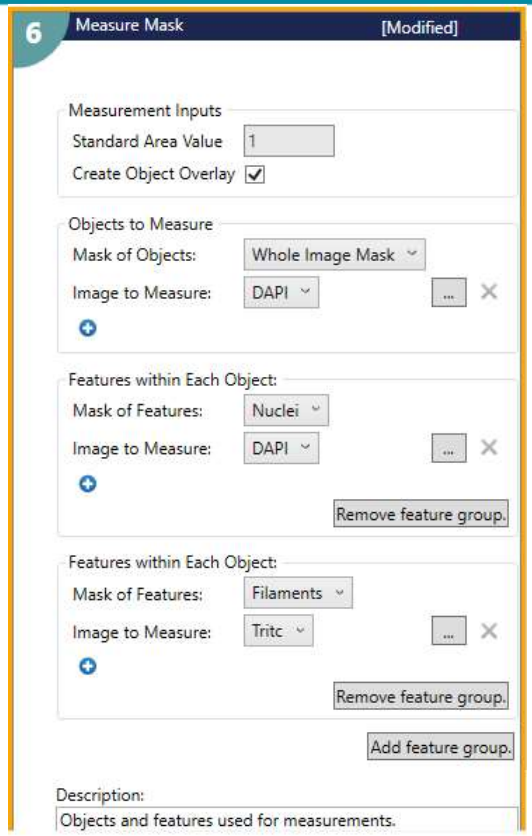
The ‘Objects to Measure’ mask is set to the Simple Threshold result mask, with no measurements selected. This generates 1 row of data per site. The Nuclei and Filaments masks are added as feature groups.

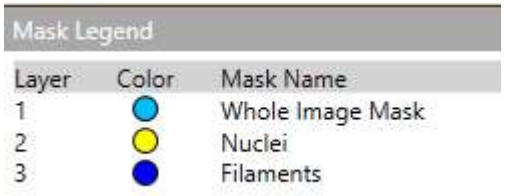


For research use only. Not for use in diagnostic procedures.

Step 6: Measure Mask







The ‘Objects to Measure’ mask is set to the Simple Threshold result mask, with no measurements selected. This generates 1 row of data per site. The Nuclei and Filaments masks are added as feature groups.

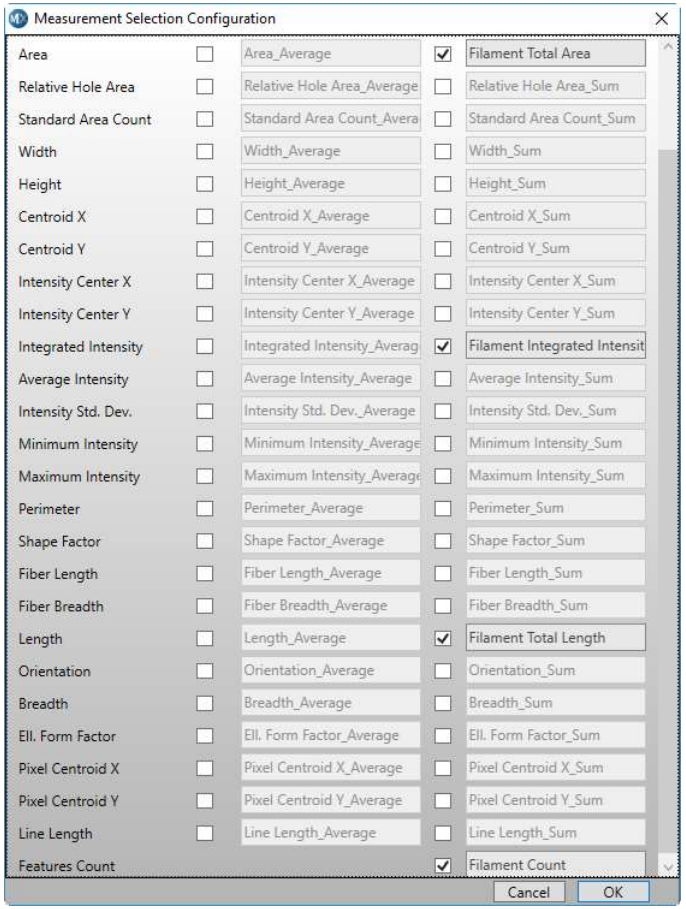
For research use only. Not for use in diagnostic procedures.

Measurement Selections

Whole Image Mask:
No measurements selected

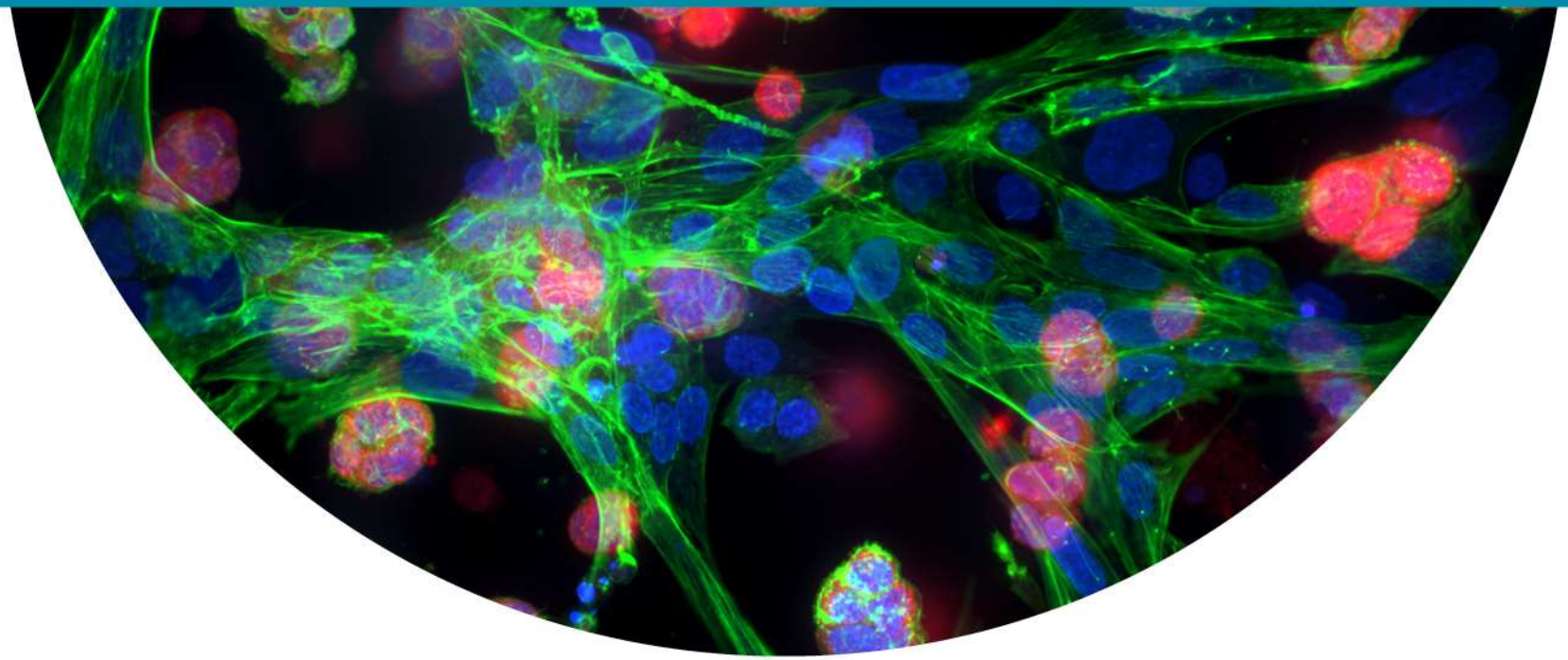
Nuclei Mask:

Filaments Mask:



Selected measurements are calculated for the Nuclei and Filaments masks.

For research use only. Not for use in diagnostic procedures.

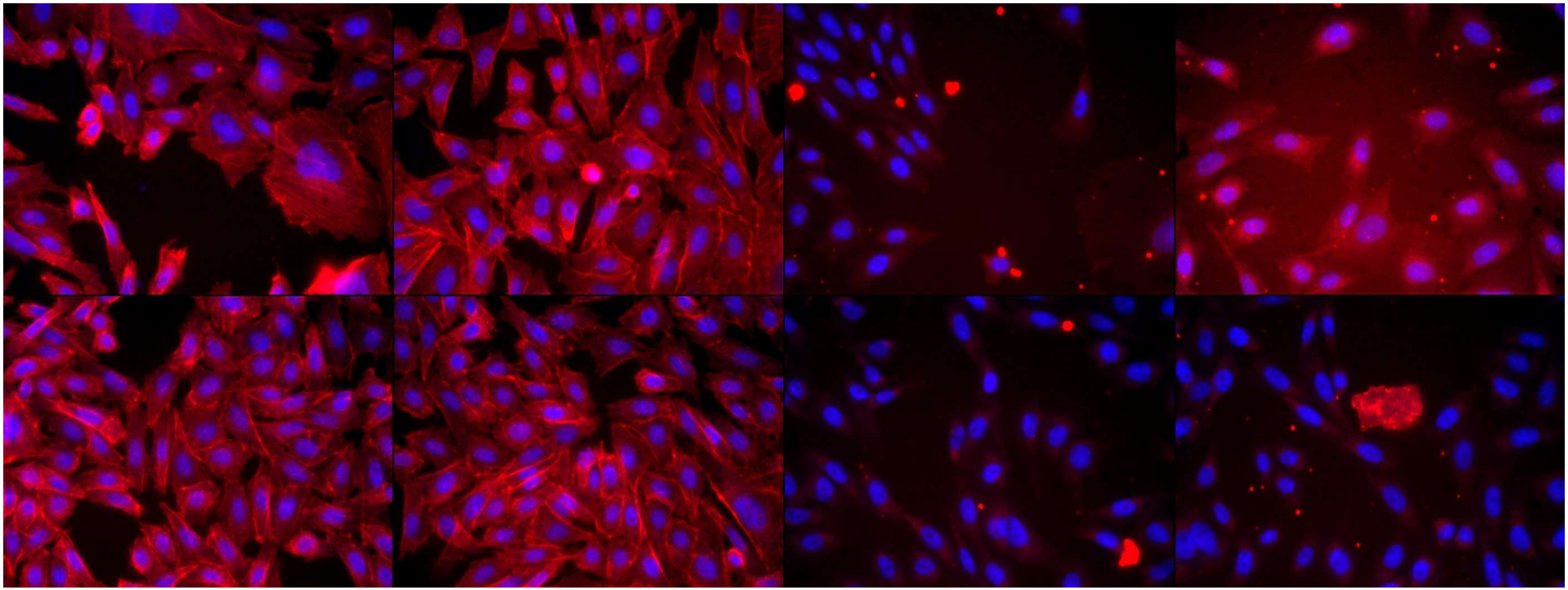


Version C: Top Hat and Adaptive Threshold

MOLECULAR DEVICES

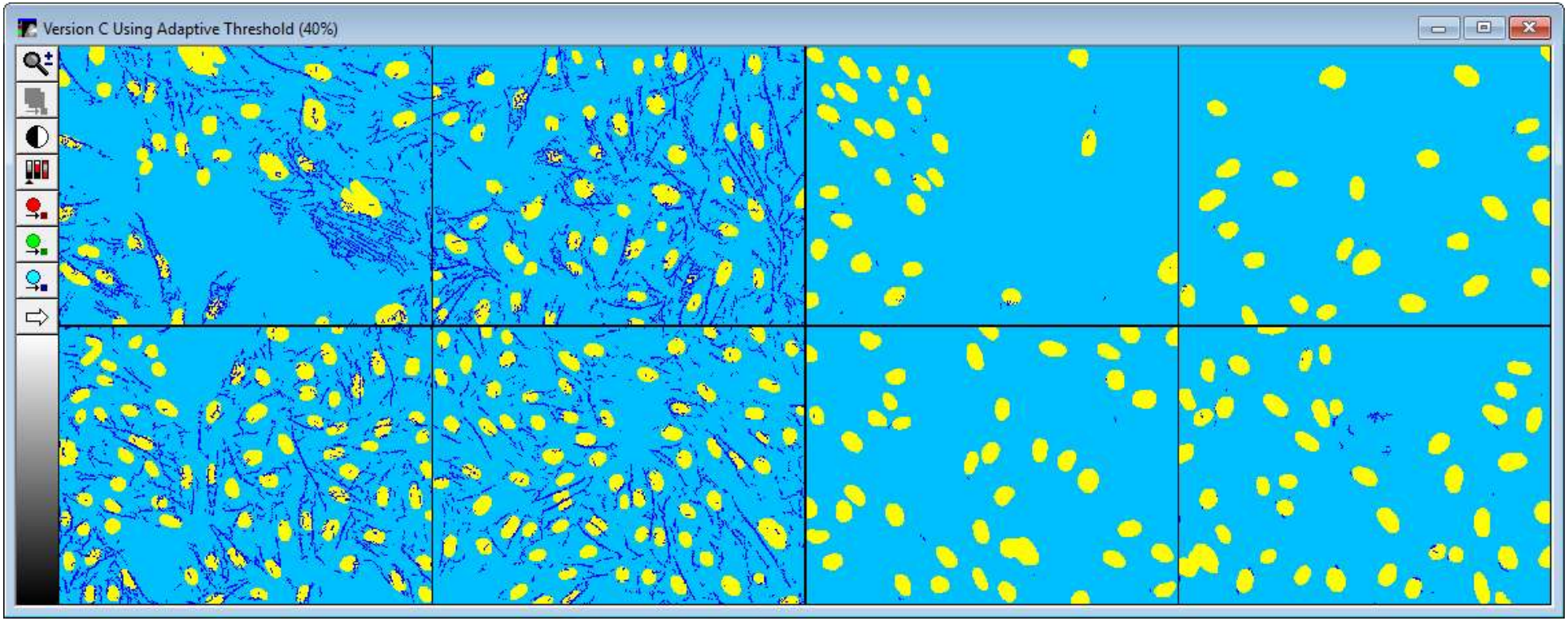
For research use only. Not for use in diagnostic procedures.

Example Plate (8 wells)



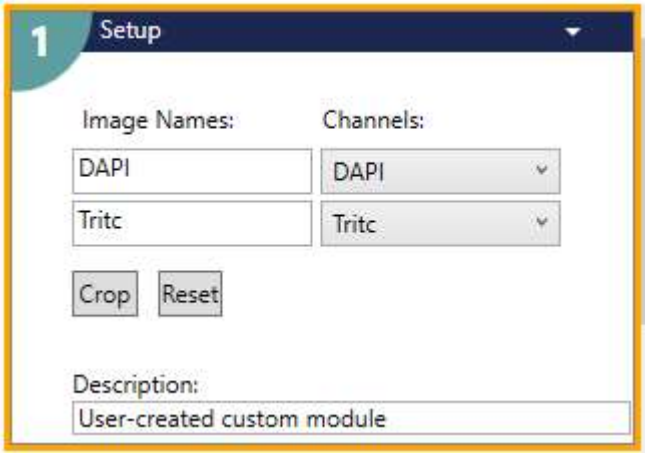
For research use only. Not for use in diagnostic procedures.

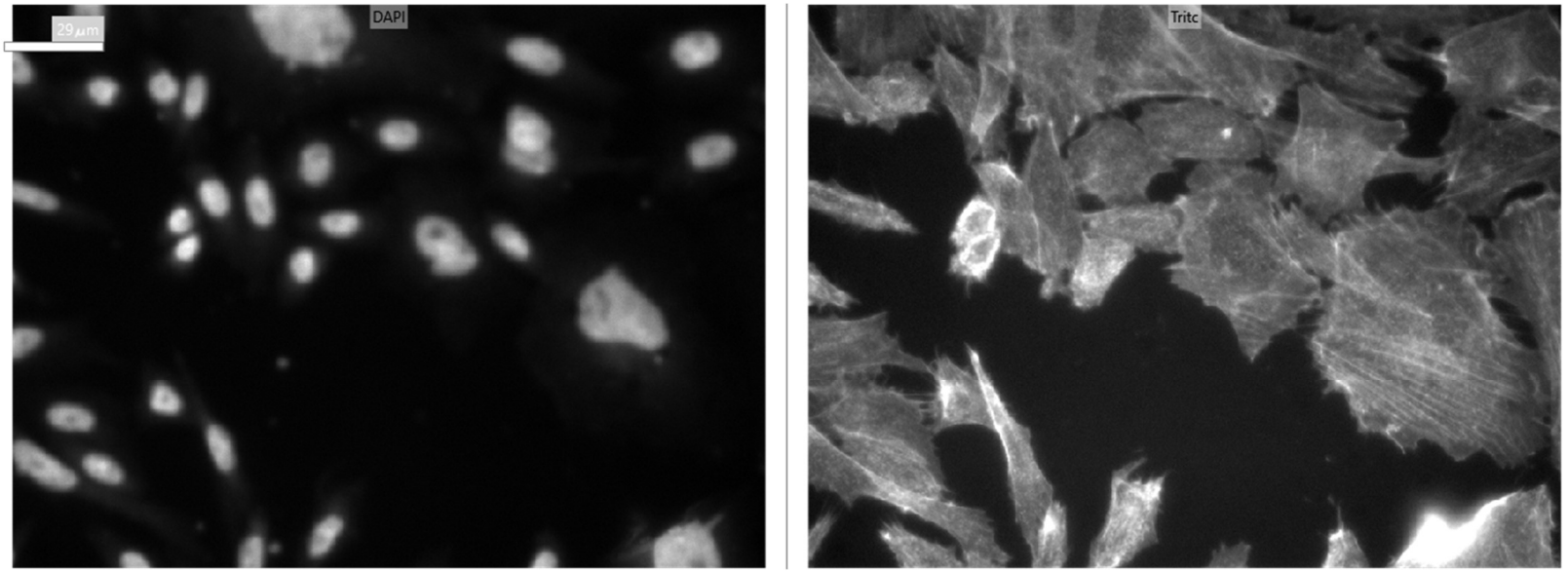
Segmentation Overlay



For research use only. Not for use in diagnostic procedures.

Step 1: Setup

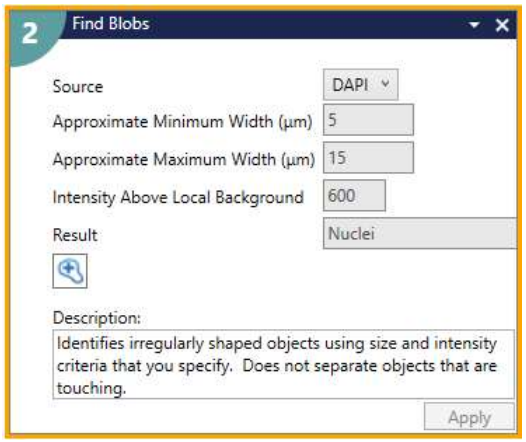


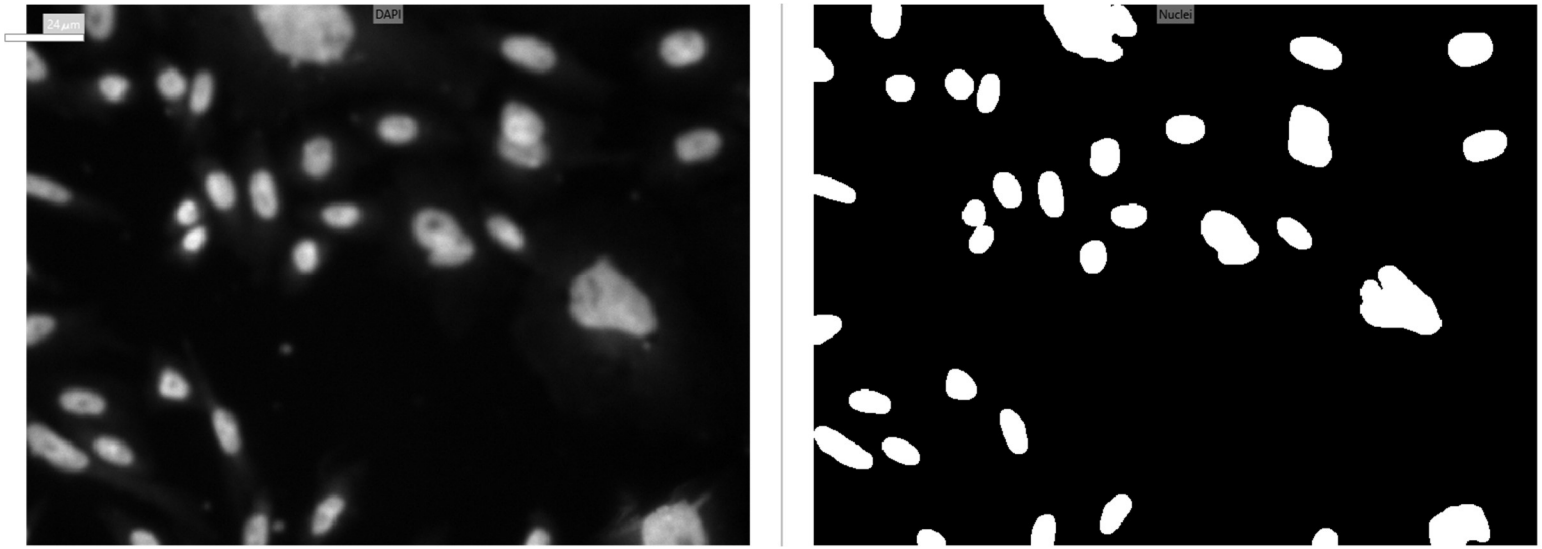


In the Setup step, define the wavelengths for analysis.

For research use only. Not for use in diagnostic procedures.

Step 2: Find Blobs

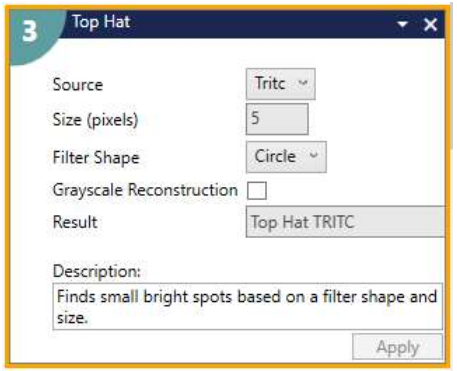


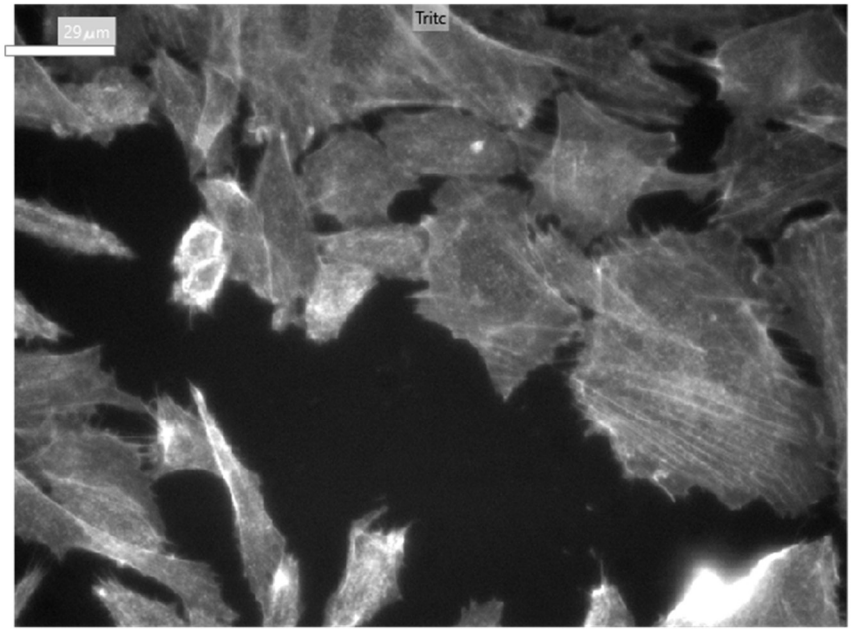


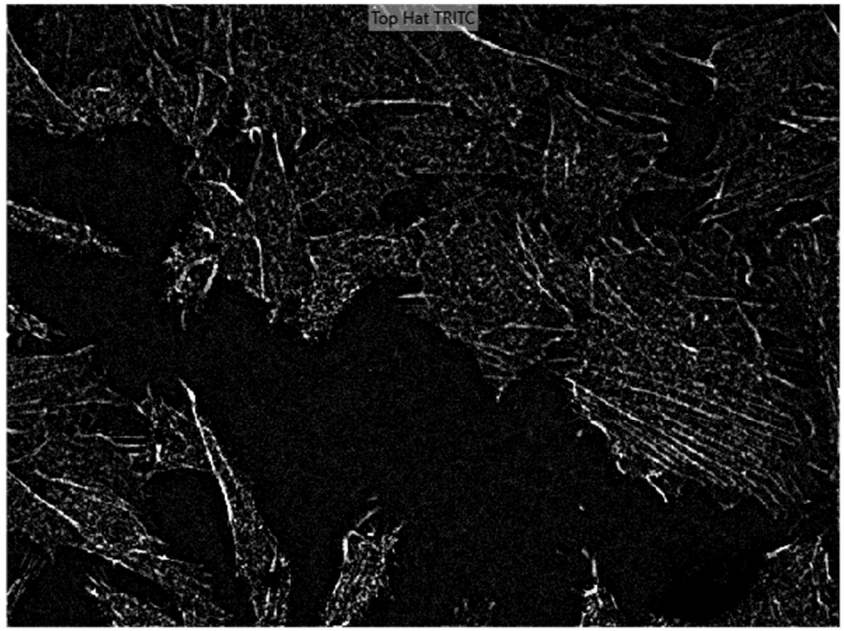
Use the Find Blobs step card to identify nuclei.

For research use only. Not for use in diagnostic procedures.

Step 3: Top Hat



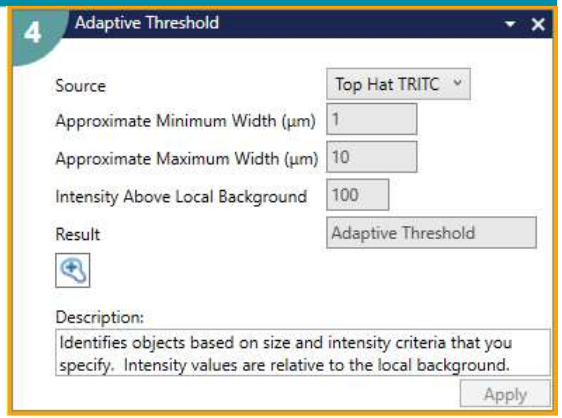


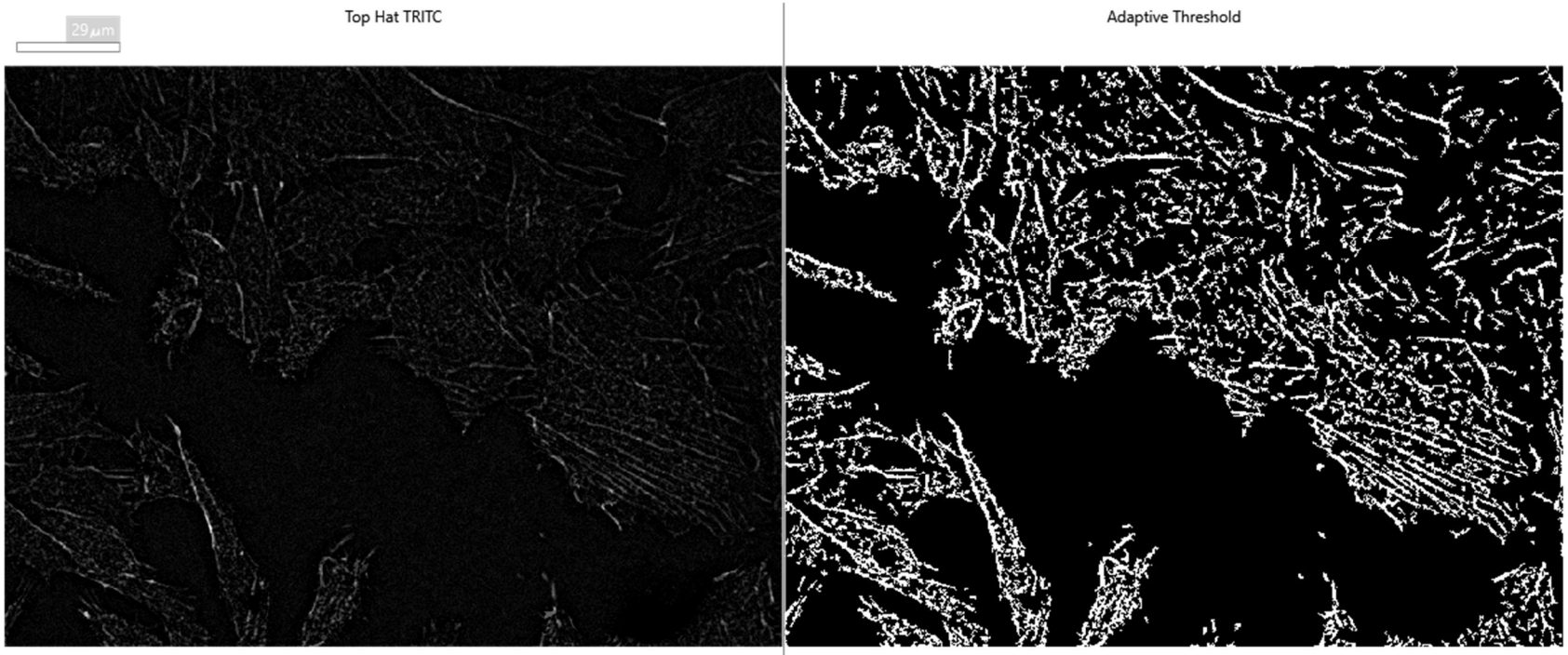


Use the Image Processing step card Top Hat to enhance small bright objects in the image.

For research use only. Not for use in diagnostic procedures.

Step 4: Adaptive Threshold

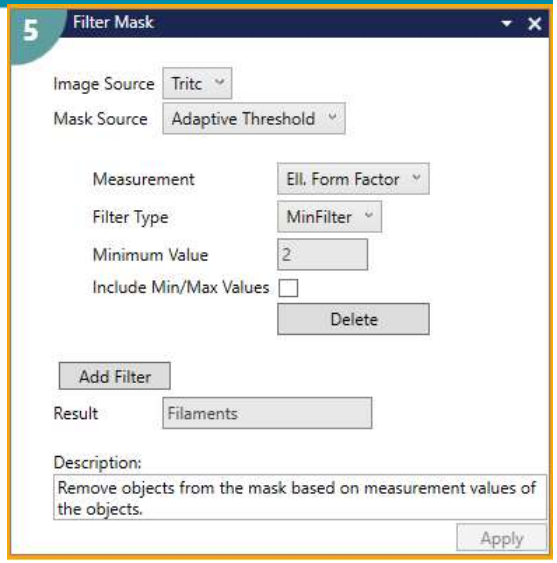


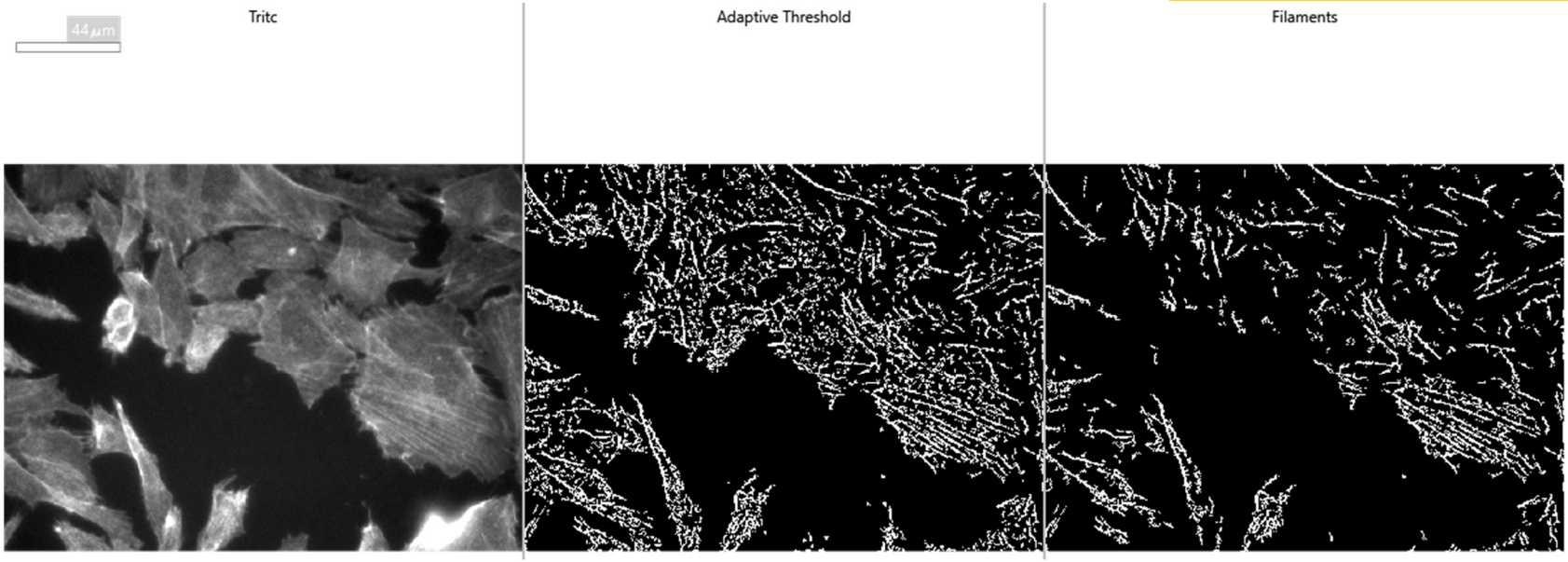


Use the Adaptive Threshold step card to identify irregularly shaped bright objects.

For research use only. Not for use in diagnostic procedures.

Step 5: Filter Mask

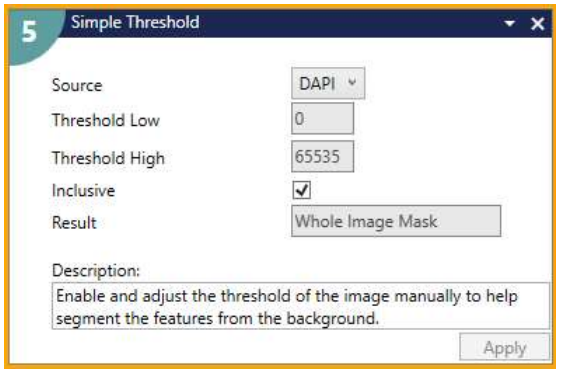


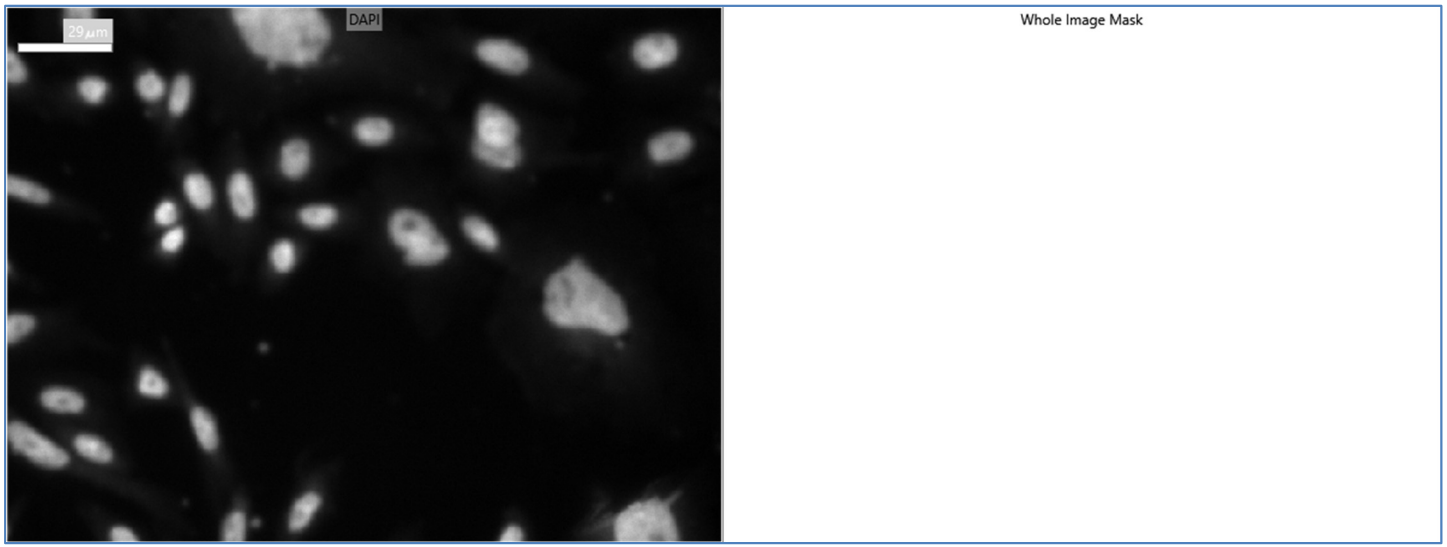


Use a Filter Mask to select objects by size, shape, and/or intensity from the Adaptive Threshold mask. In this example, Elliptical Form Factor (ratio of length/breadth) is used to select elongated objects.

For research use only. Not for use in diagnostic procedures.

Step 6: Simple Threshold

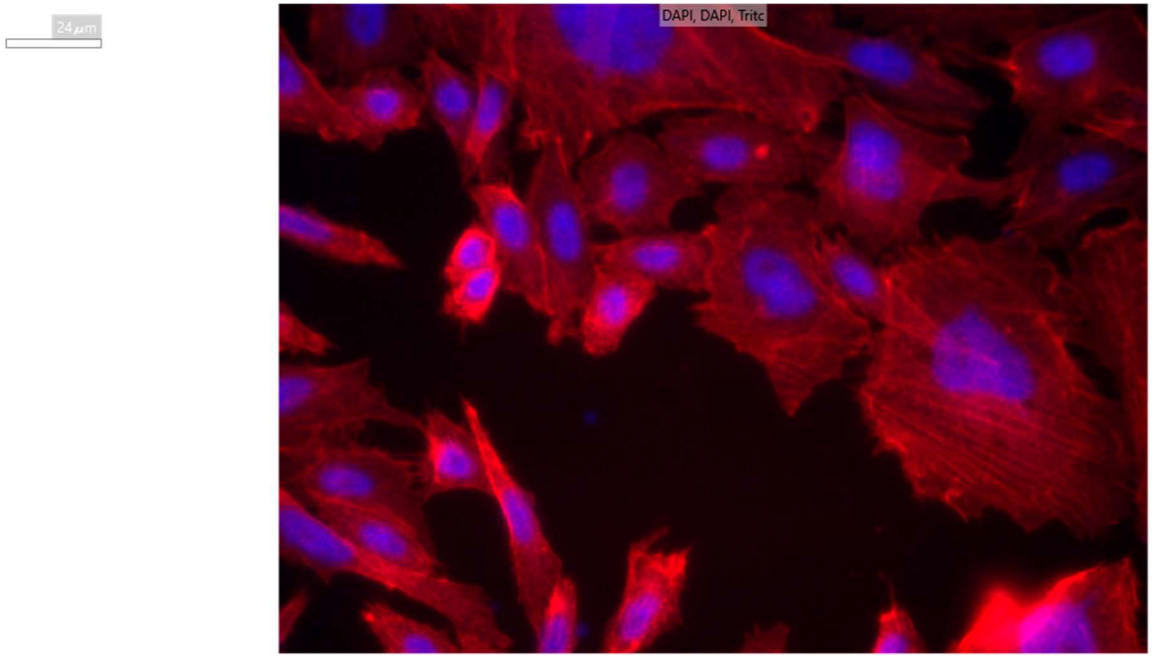


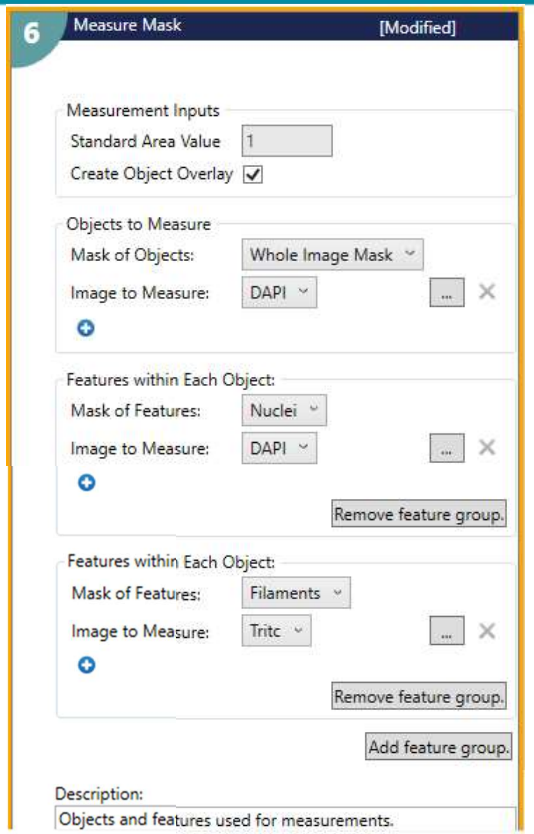


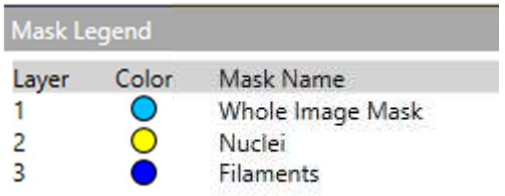
To simplify the output, use Simple Threshold inclusive from 0-65535 to create a mask that represents the entire image area. Any image may be used as the Source for this step.

For research use only. Not for use in diagnostic procedures.

Step 7: Measure Mask



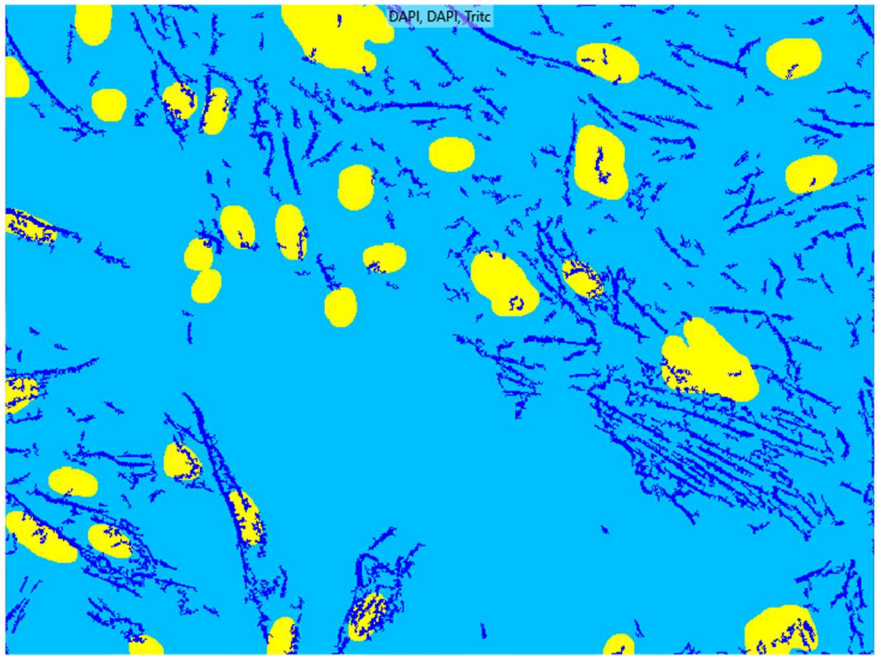


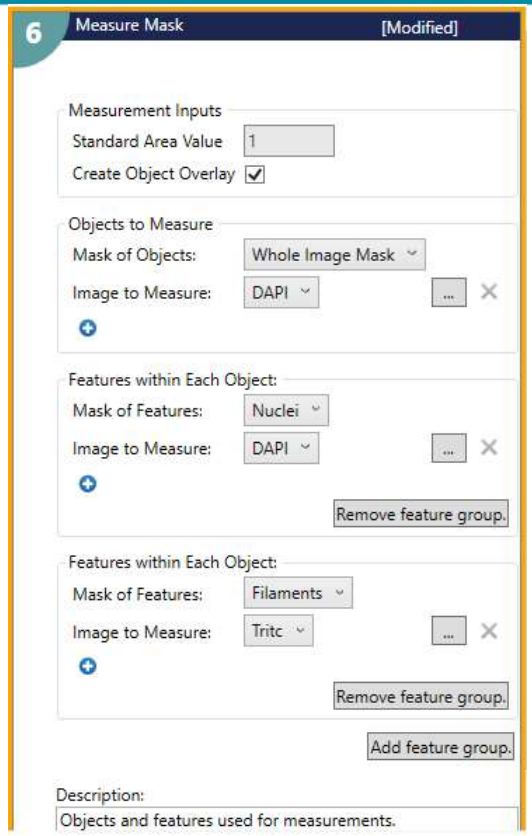


The ‘Objects to Measure’ mask is set to the Simple Threshold result mask, with no measurements selected. This generates 1 row of data per site. The Nuclei and Filaments masks are added as feature groups.

For research use only. Not for use in diagnostic procedures.

Step 7: Measure Mask

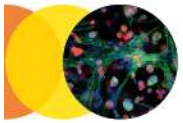




Mask Legend

Layer	Color	Mask Name
1		Whole Image Mask
2		Nuclei
3		Filaments

The ‘Objects to Measure’ mask is set to the Simple Threshold result mask, with no measurements selected. This generates 1 row of data per site. The Nuclei and Filaments masks are added as feature groups.



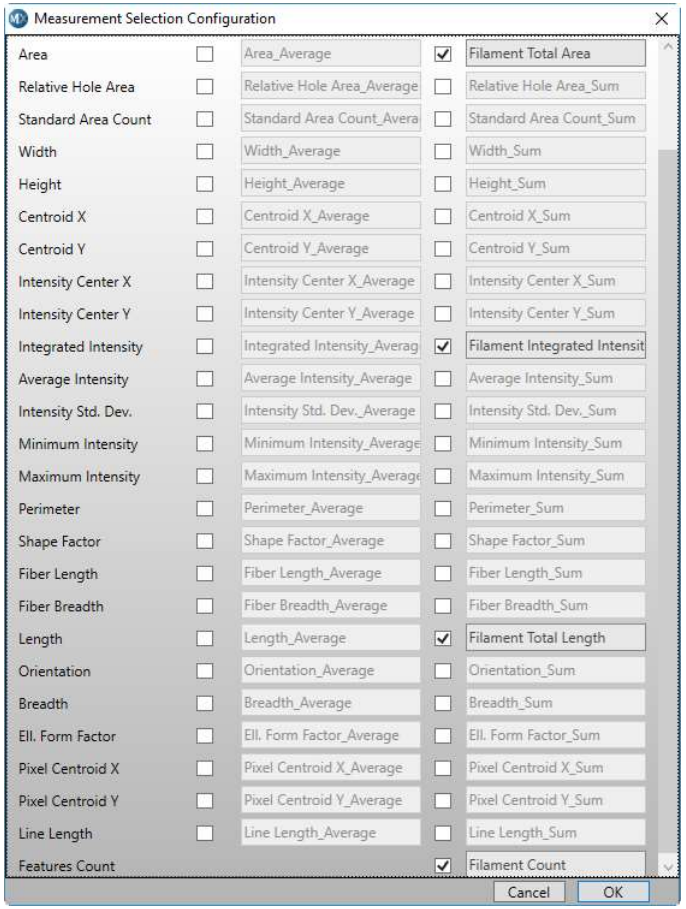
For research use only. Not for use in diagnostic procedures.

Measurement Selections

Whole Image Mask:
No measurements selected

Nuclei Mask:

Filaments Mask:

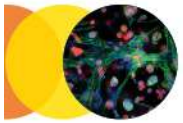


Selected measurements are calculated for the Nuclei and Filaments masks.

For research use only. Not for use in diagnostic procedures.

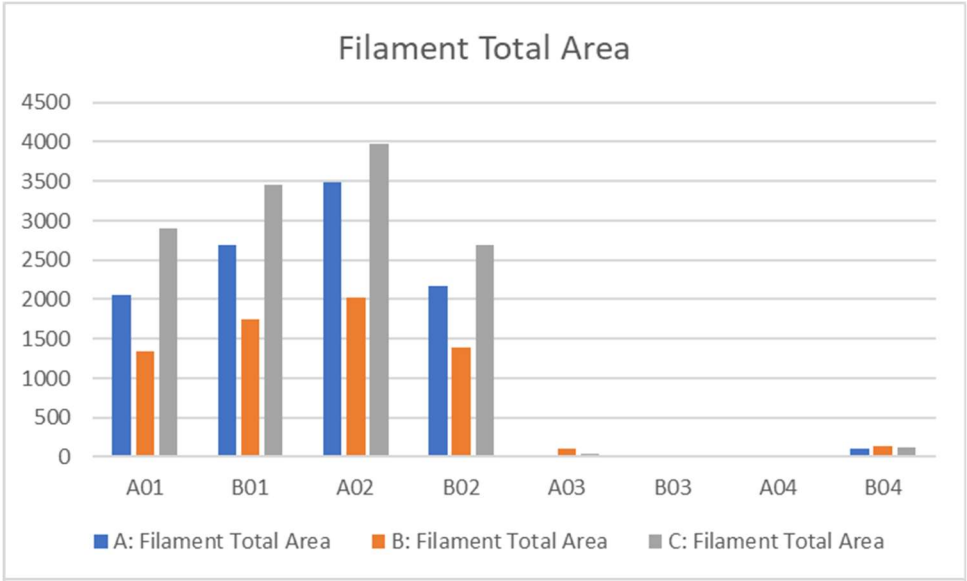
Results Comparison

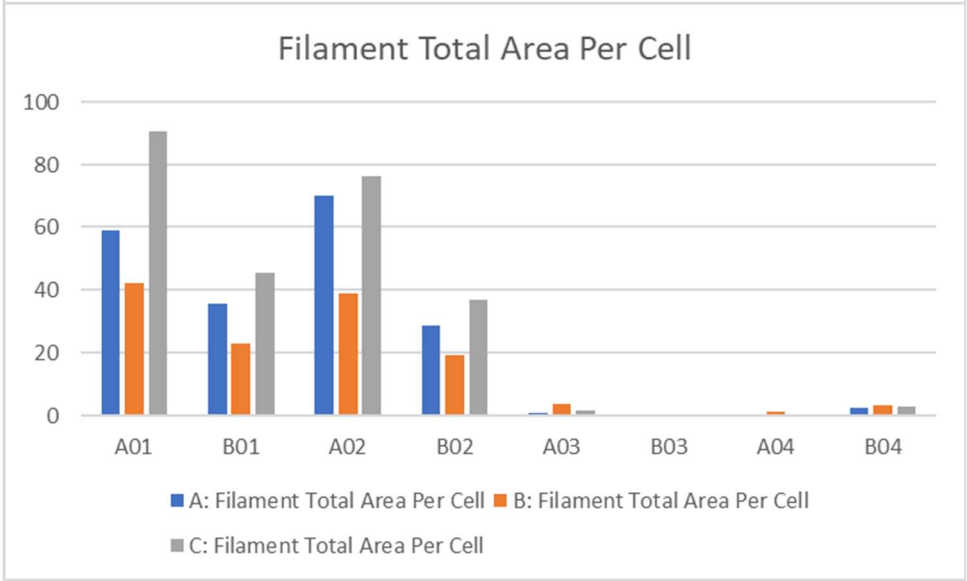
Measurement	Version A	Version B	Version C
Nuclear Count			
Filament Total Area			
Filament Integrated Intensity			
Filament Total Length			
Filament Count			



For research use only. Not for use in diagnostic procedures.

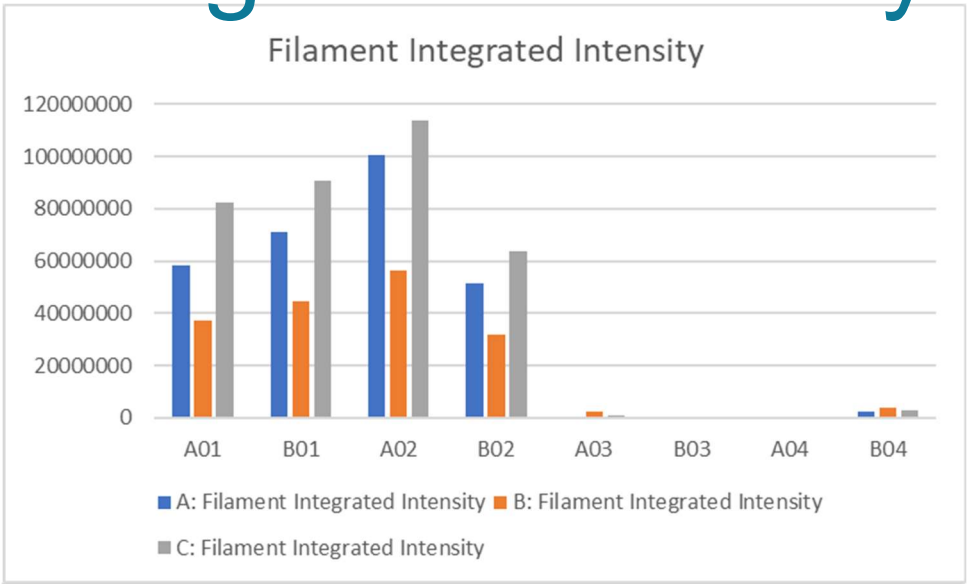
Filament Total Area

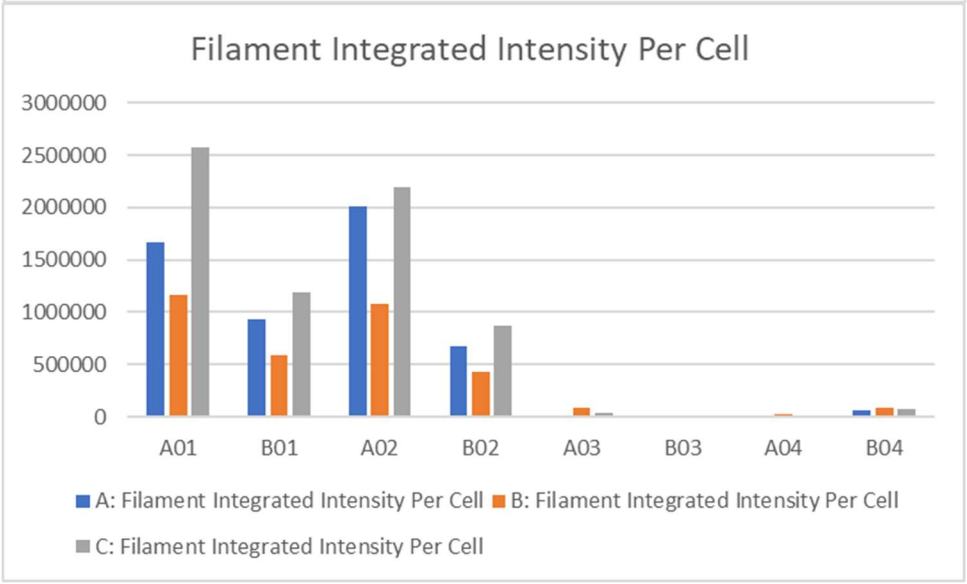


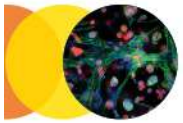


For research use only. Not for use in diagnostic procedures.

Filament Integrated Intensity



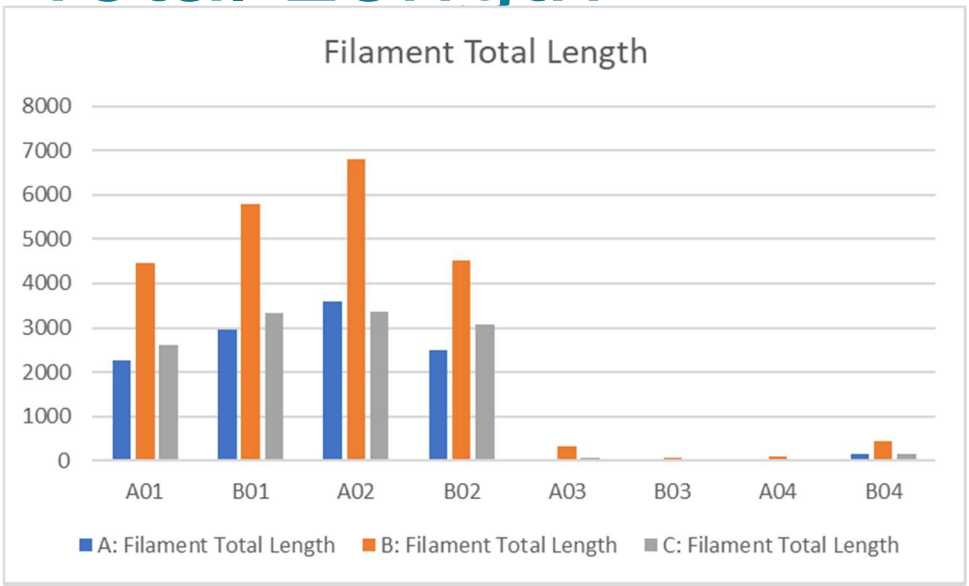


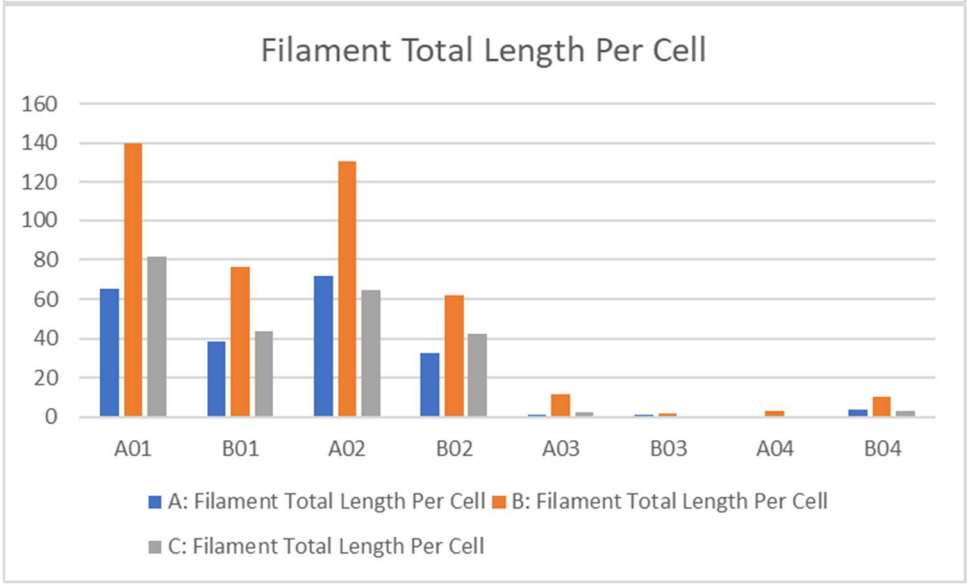


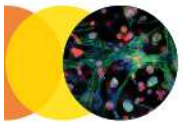
For research use only. Not for use in diagnostic procedures.

MOLECULAR DEVICES

Filament Total Length



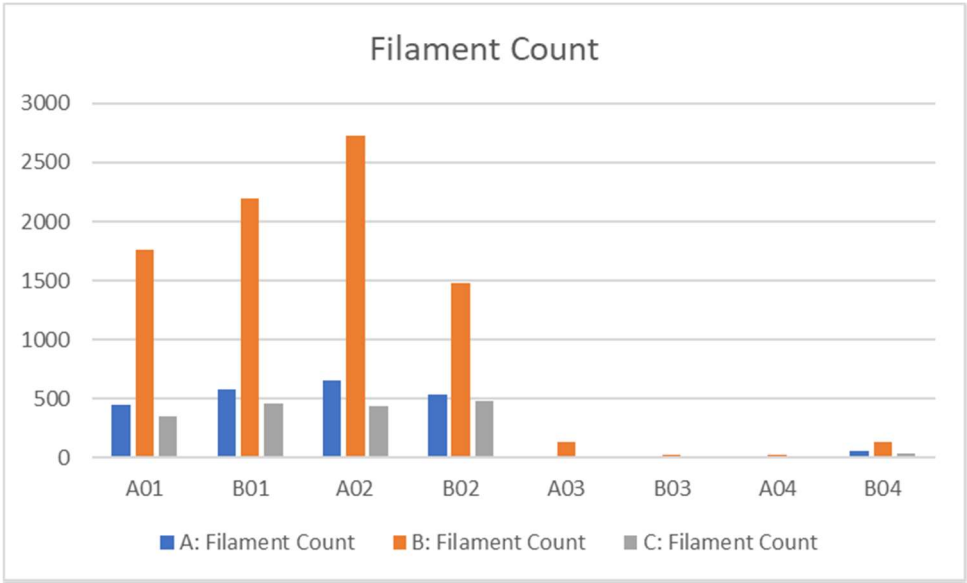


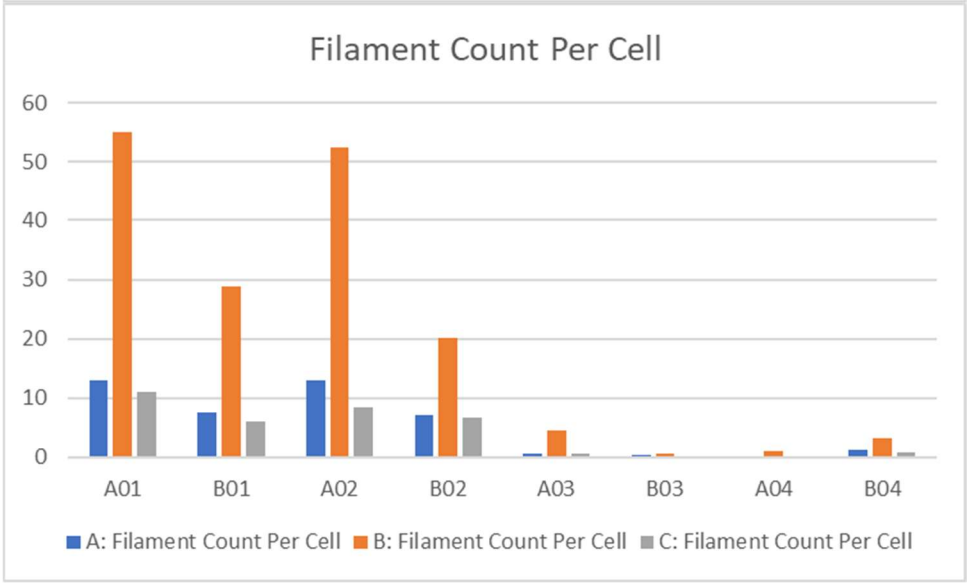


MOLECULAR DEVICES

For research use only. Not for use in diagnostic procedures.

Filament Count

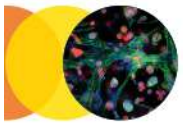




For research use only. Not for use in diagnostic procedures.

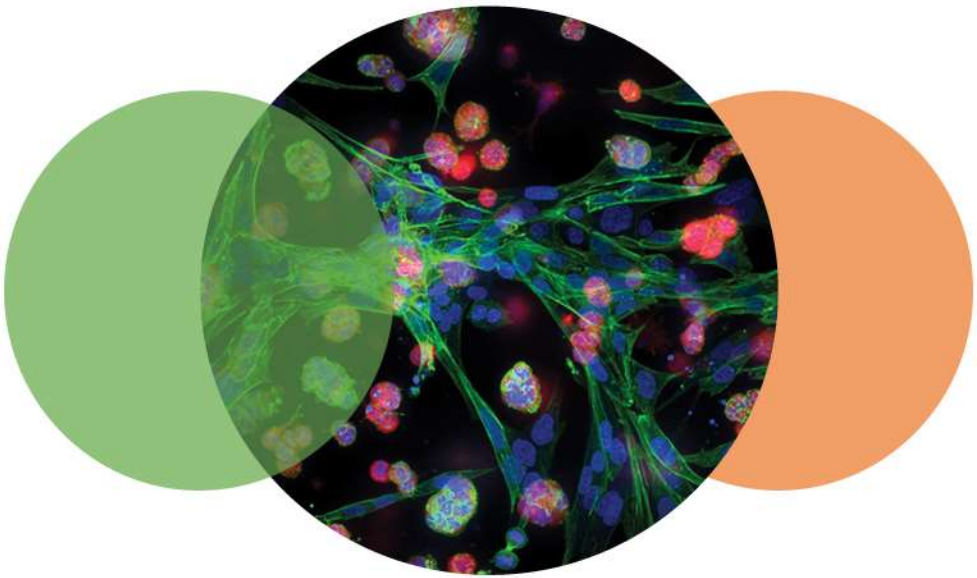
Summary

- Multiple approaches can be used to measure filamentous structure in cell images.
- The best approach depends on the specific assay and the desired output.



For research use only. Not for use in diagnostic procedures.

MOLECULAR DEVICES



MOLECULAR DEVICES

ADVANCING DISCOVERY

For research use only. Not for use in diagnostic procedures.
© 2012-2018. Trademarks are the property of Molecular Devices, LLC or their respective owners.