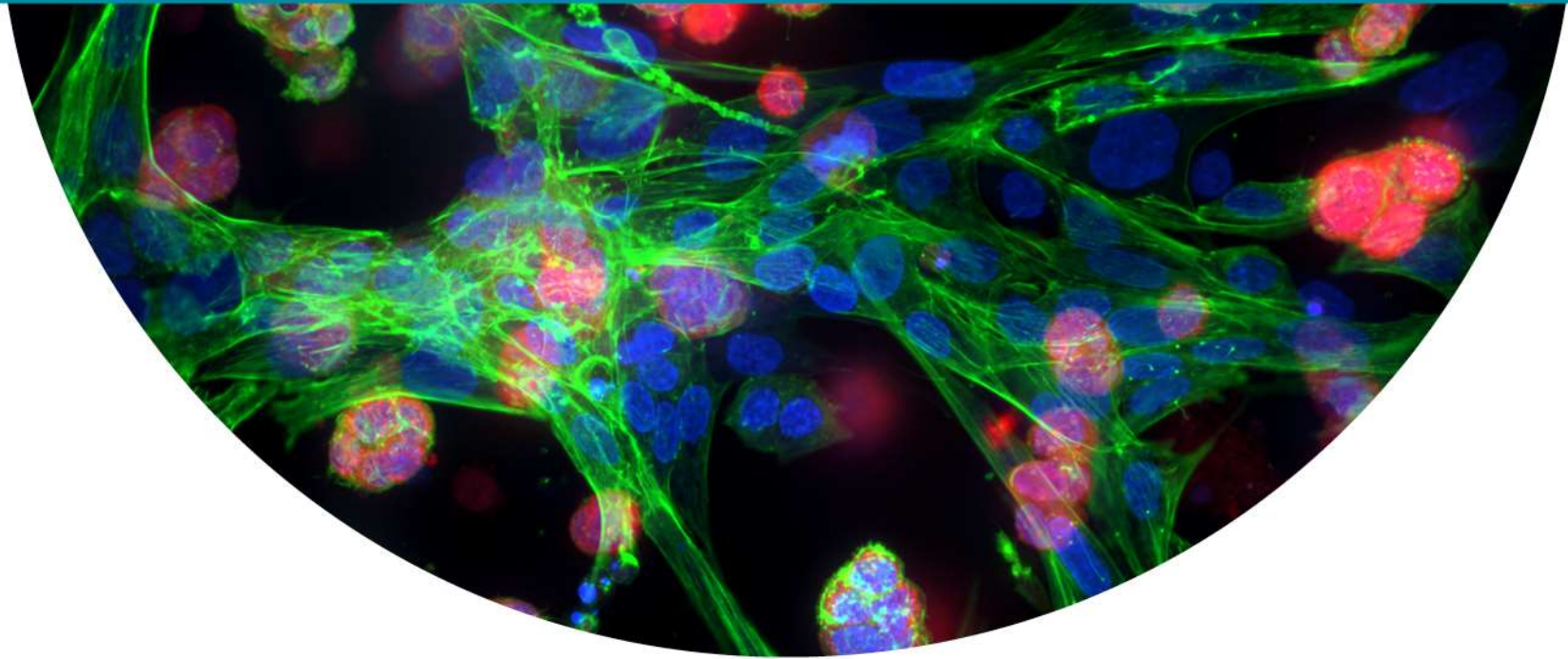


Actin Filament analysis in MetaXpress Custom Module Editor

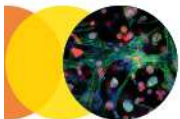
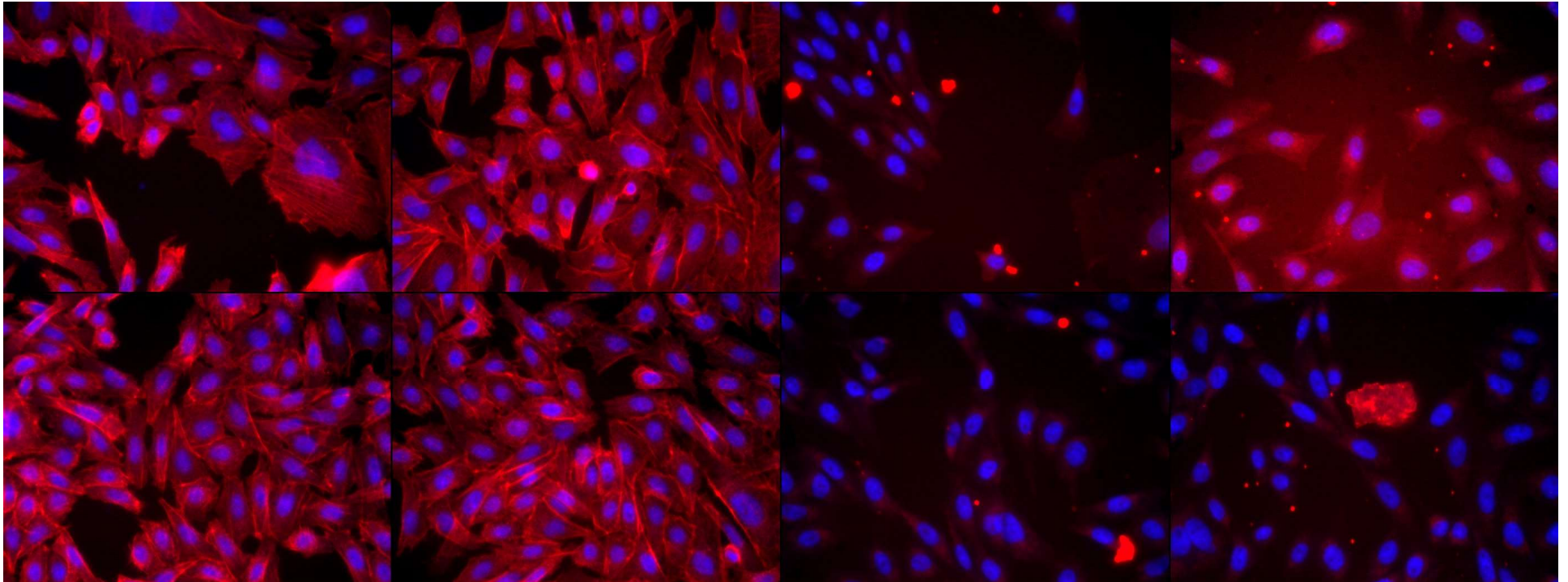
August 2018



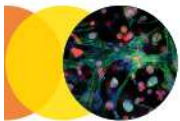
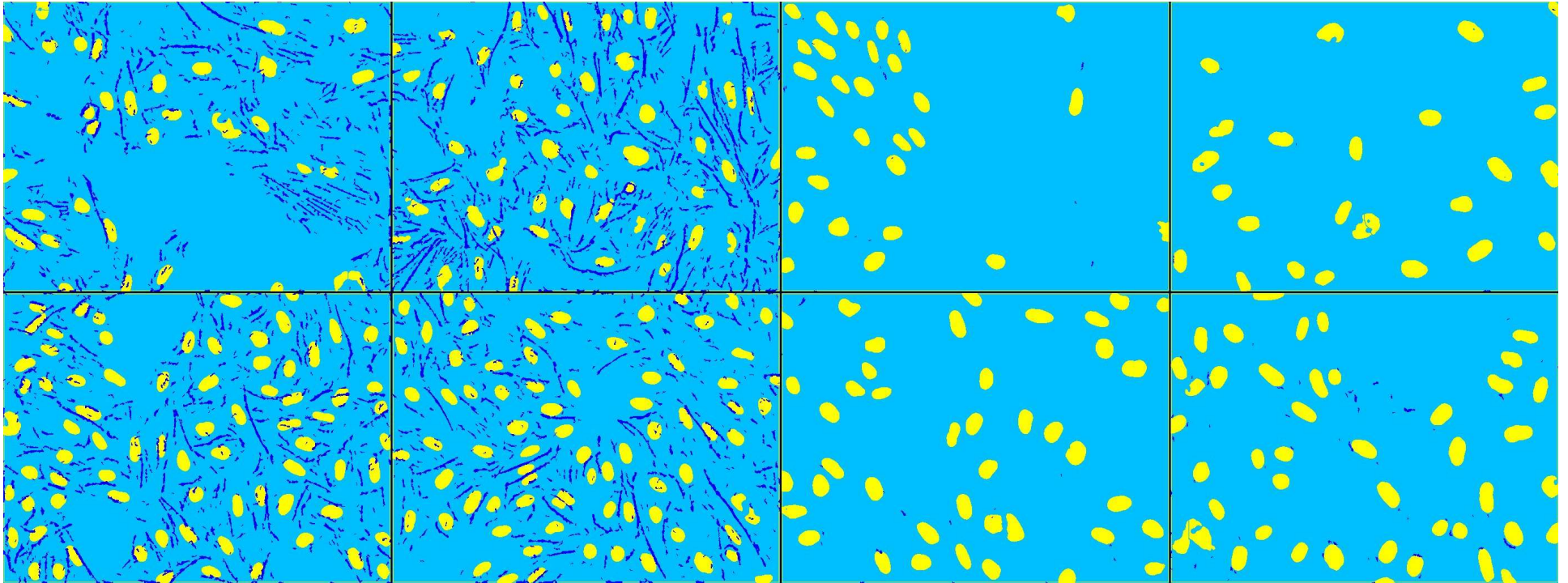


Version A: Transfluor or Granularity module

Example Plate (8 wells)



Segmentation Overlay



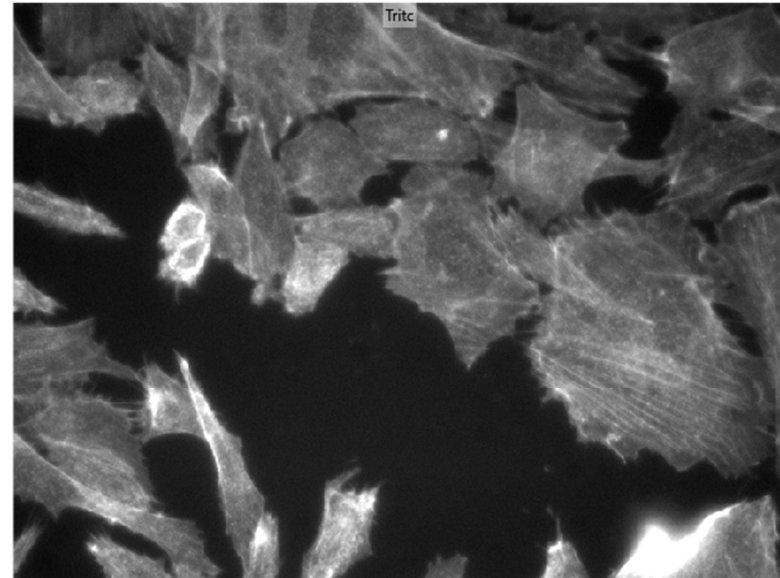
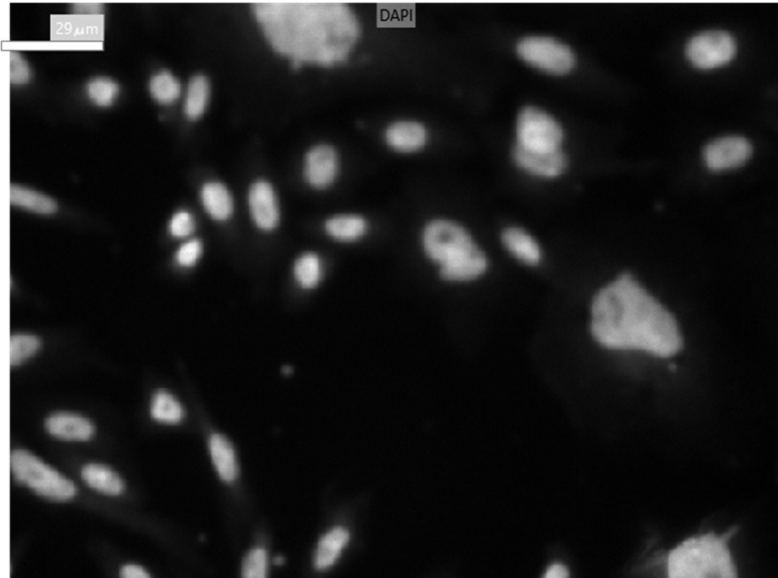
Step 1: Setup

1 Setup

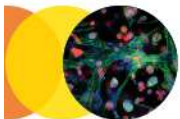
Image Names:	Channels:
DAPI	DAPI ▾
Tritc	Tritc ▾

Crop Reset

Description:
User-created custom module



In the Setup step, define the wavelengths for analysis.



Step 2: Transfluor (or Granularity)

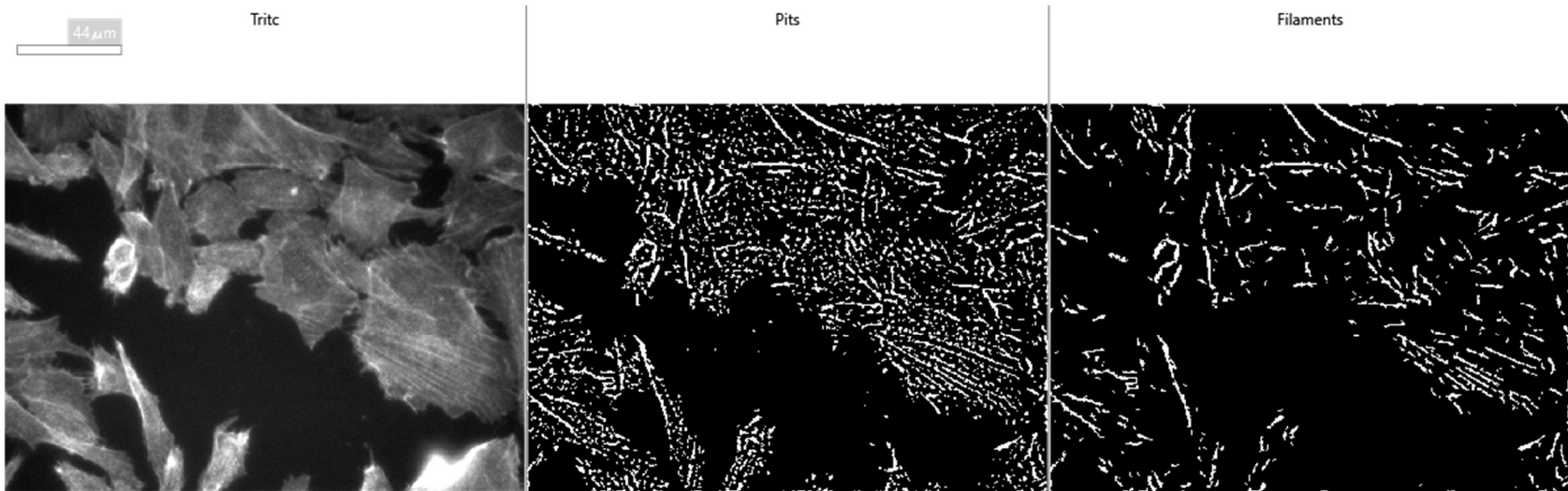
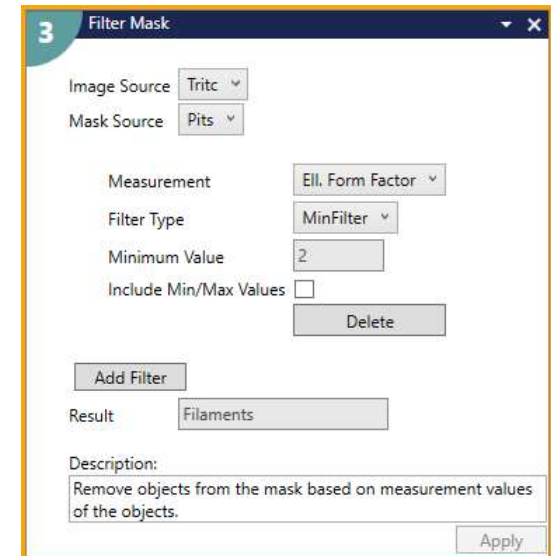
The figure illustrates the workflow for identifying nuclei and small bright objects using the Transfluor or Granularity Application Module. It shows four stages of image processing: Tritc, DAPI, Nuclei, and Pits. A scale bar indicates 73 μm. The software interface on the right shows the following settings:

- Transfluor Objects** (Window title)
- Pits And Vesicles Image:** Tritc
- Use Pits**
 - Approximate Minimum Width (μm): 0.5
 - Approximate Maximum Width (μm): 3
 - Intensity Above Local Background: 100
- Use Vesicles**
 - Approximate Minimum Width (μm): 4
 - Approximate Maximum Width (μm): 10
 - Intensity Above Local Background: 2000
- Nuclear Image**
 - Nuclear Image: DAPI
 - Approximate Minimum Width (μm): 5
 - Approximate Maximum Width (μm): 15
 - Intensity Above Local Background: 600
- Algorithm:** Fast
- Nuclei:** Nuclei
- Pits:** Pits
- Vesicles:** Vesicles
- Description:** Detects G-Protein Coupled Receptor (GPCR) cycling characteristics.
- Apply** button

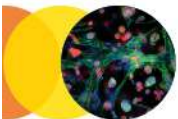
Use the Transfluor or Granularity Application Module to identify nuclei and small bright objects. If using Transfluor, only the Pits option is used. Note: Either the Transfluor or Granularity module must be enabled on your MetaXpress license key.



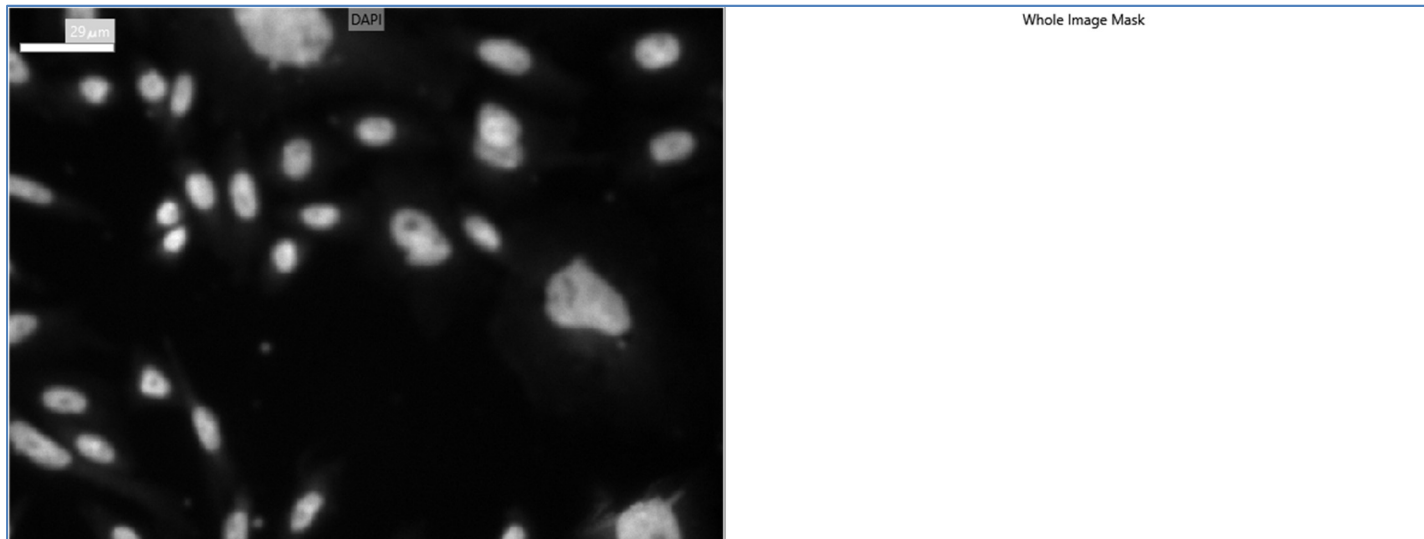
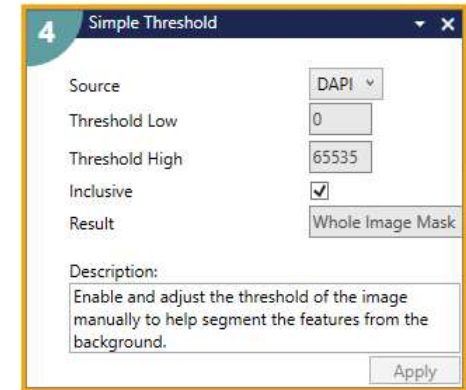
Step 3: Filter Mask



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.



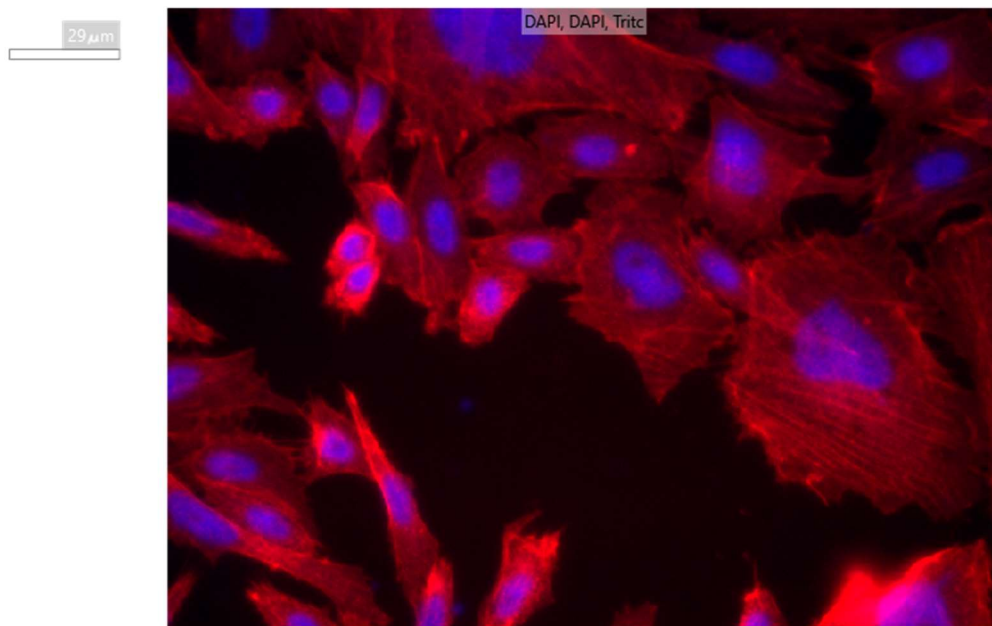
Step 4: Simple Threshold



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.



Step 5: Measure Mask



5 Measure Mask

Measurement Inputs
Standard Area Value: 1
Create Object Overlay:

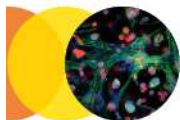
Objects to Measure
Mask of Objects: Whole Image Mask
Image to Measure: DAPI

Features within Each Object
Mask of Features: Nuclei
Image to Measure: DAPI
Remove feature group.

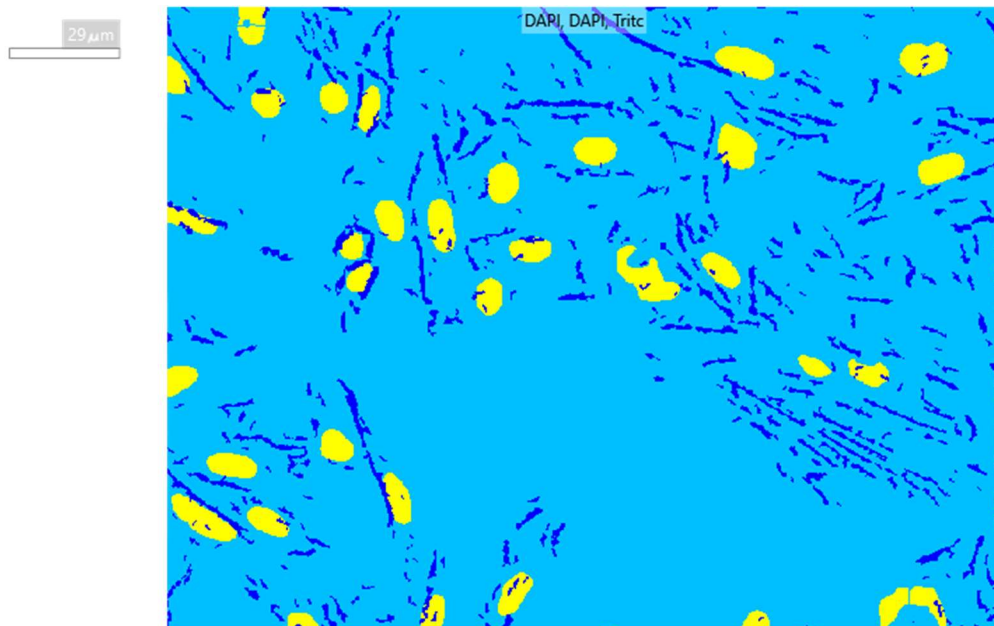
Features within Each Object
Mask of Features: Filaments
Image to Measure: Tritc

Mask Legend		
Layer	Color	Mask Name
1	Yellow	Whole Image Mask
2	Blue	Nuclei
3	Red	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.



Step 5: Measure Mask



5 Measure Mask

Measurement Inputs

Standard Area Value: 1

Create Object Overlay:

Objects to Measure

Mask of Objects: Whole Image Mask

Image to Measure: DAPI

Features within Each Object

Mask of Features: Nuclei

Image to Measure: DAPI

Remove feature group.

Features within Each Object

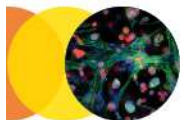
Mask of Features: Filaments

Image to Measure: Tritc

Mask Legend

Layer	Color	Mask Name
1	Light Blue	Whole Image Mask
2	Yellow	Nuclei
3	Dark Blue	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.



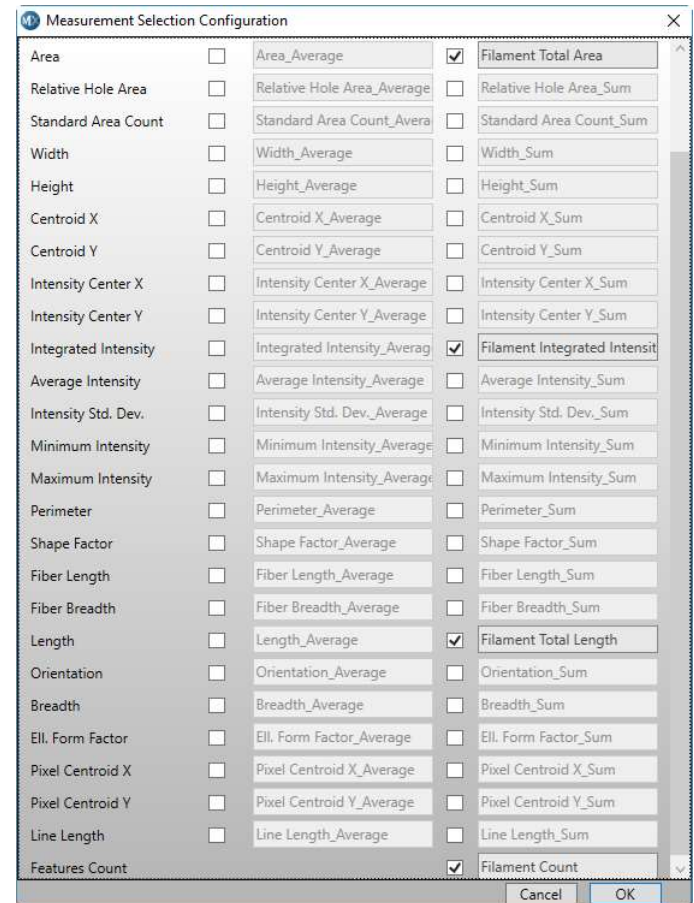
Measurement Selections

Whole Image Mask:
No measurements selected

Nuclei Mask:

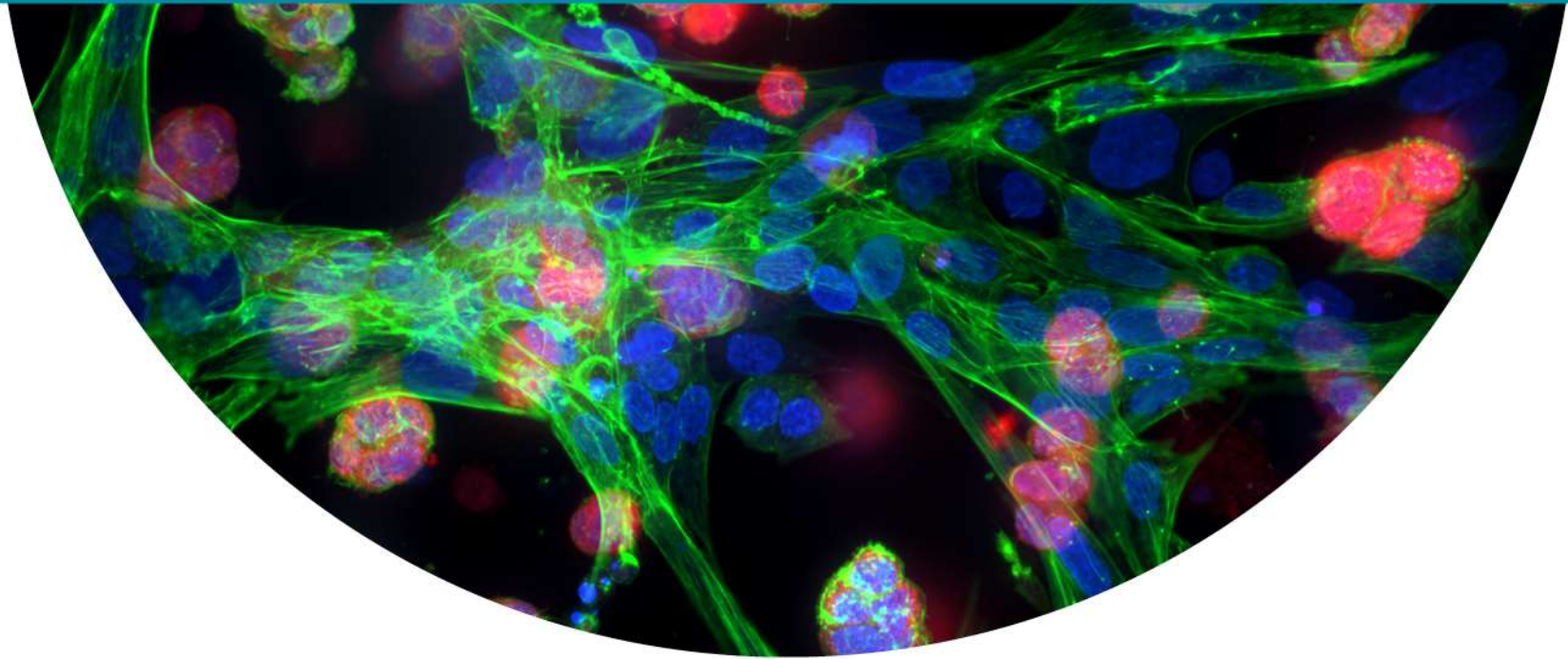


Filaments Mask:



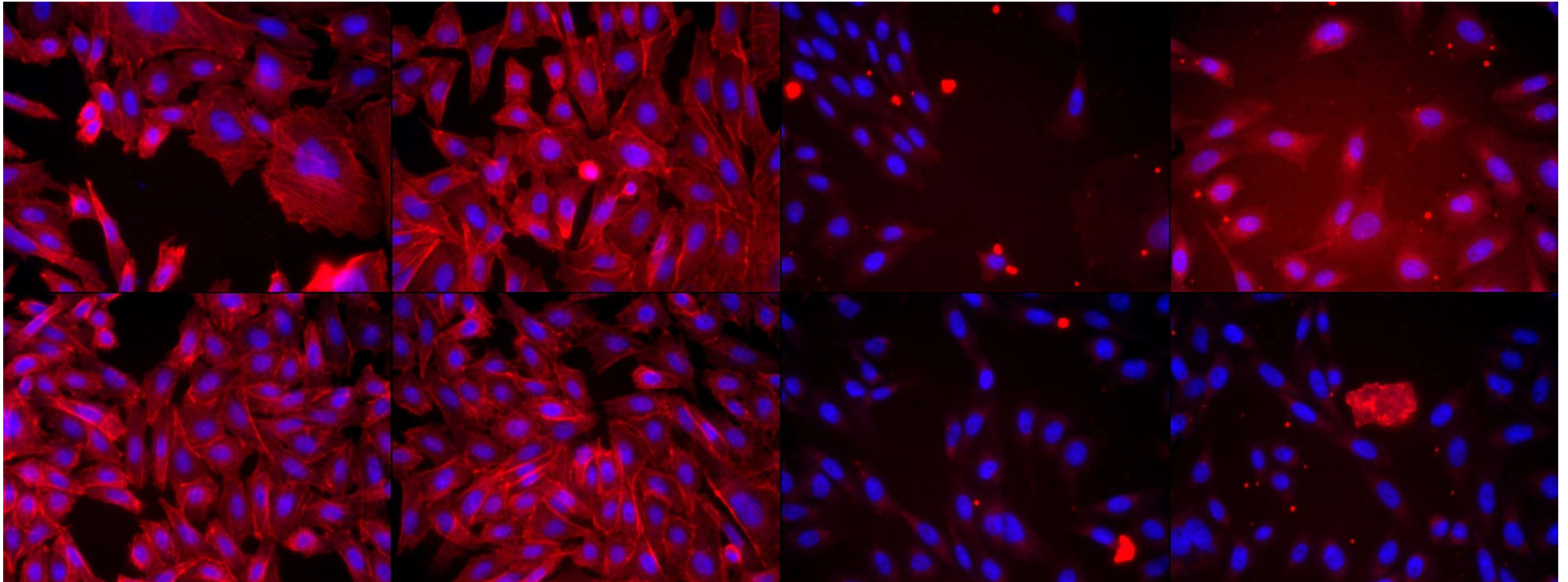
Selected measurements are calculated for the Nuclei and Filaments masks.



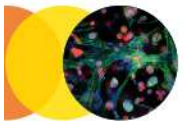
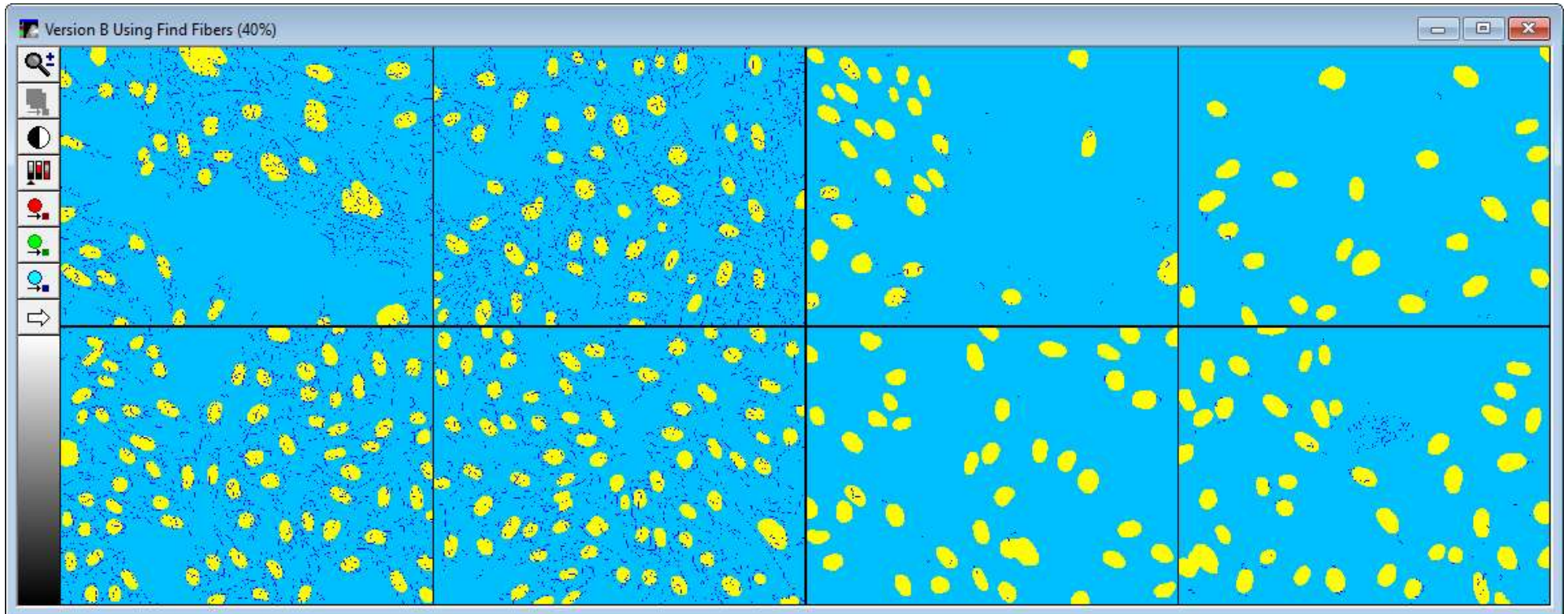


Version B: Top Hat and Find Fibers

Example Plate (8 wells)



Segmentation Overlay

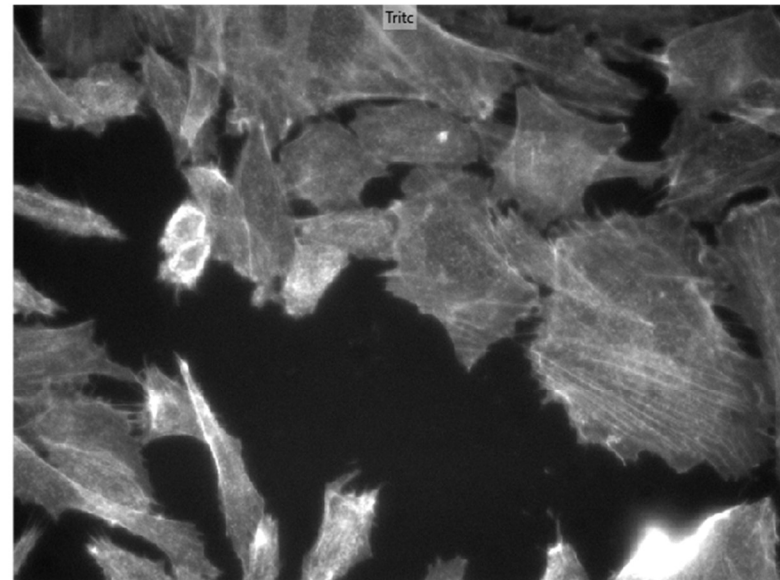
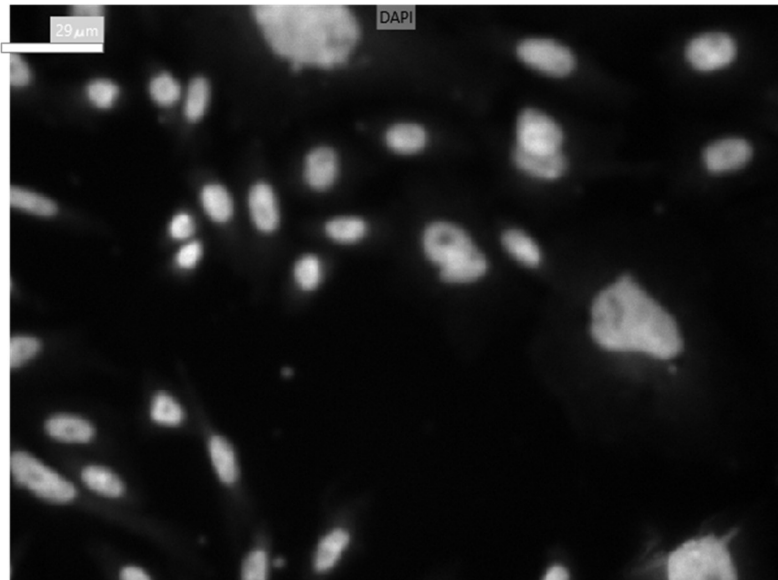


Step 1: Setup

1 Setup

Image Names:	Channels:
DAPI	DAPI ▾
Tritc	Tritc ▾

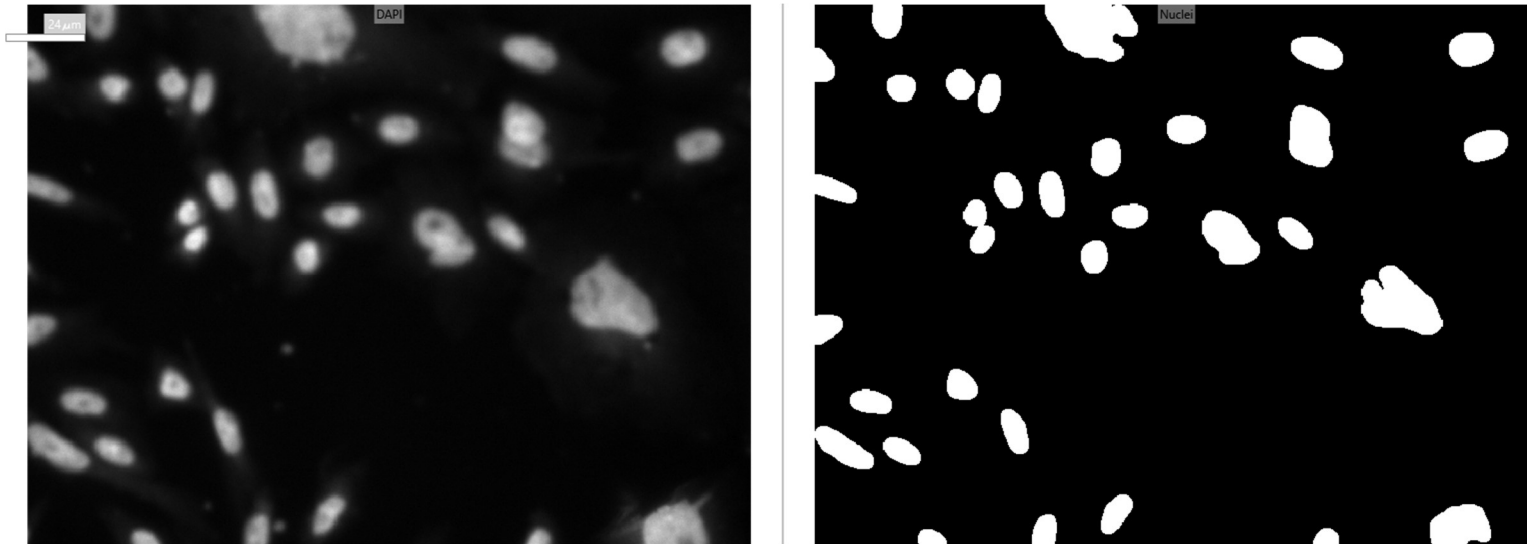
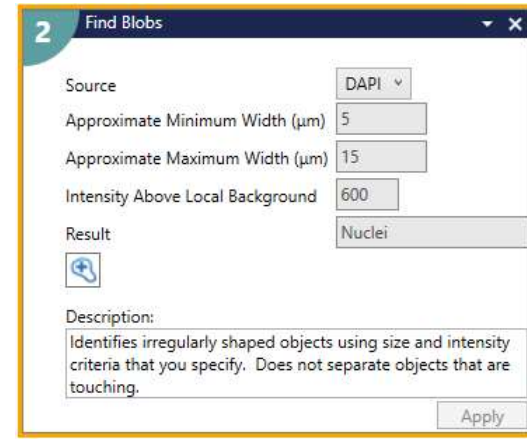
Description:
User-created custom module



In the Setup step, define the wavelengths for analysis.



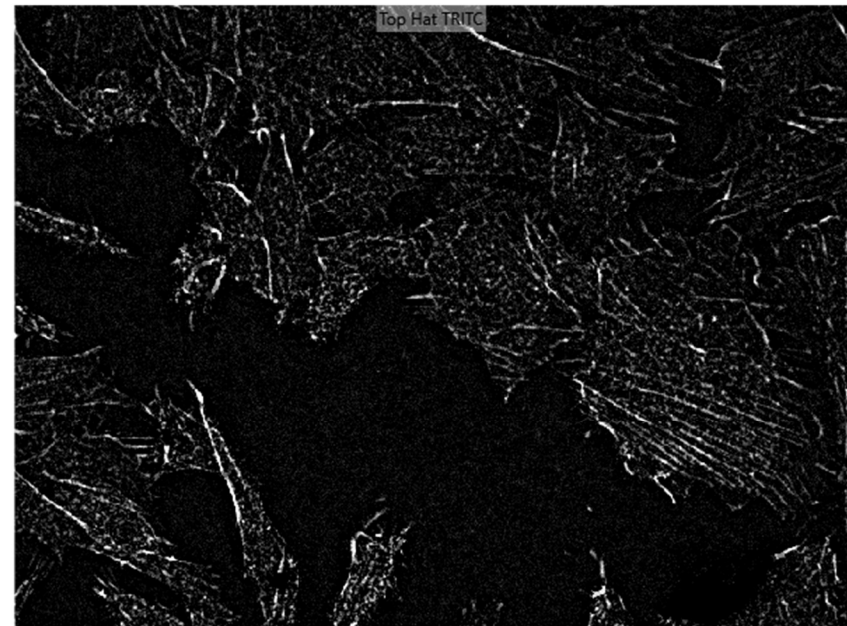
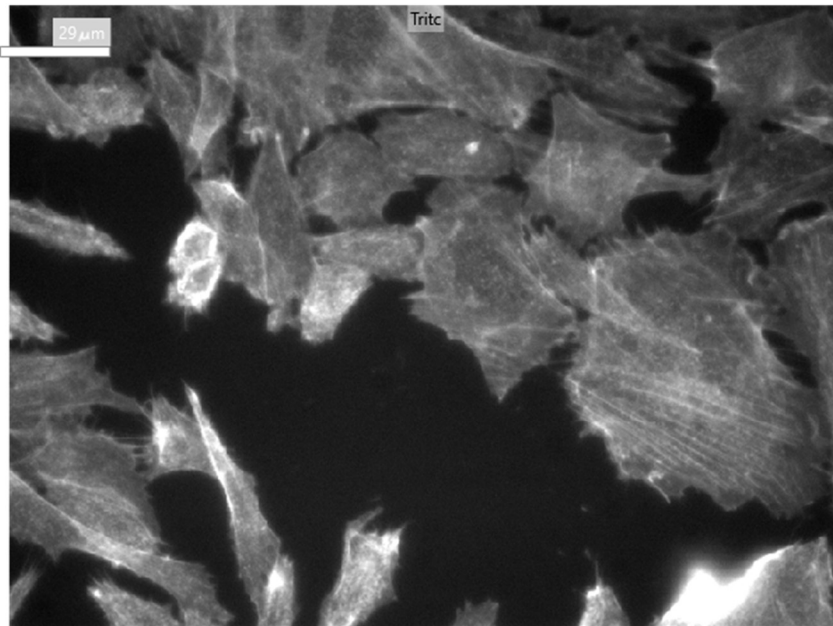
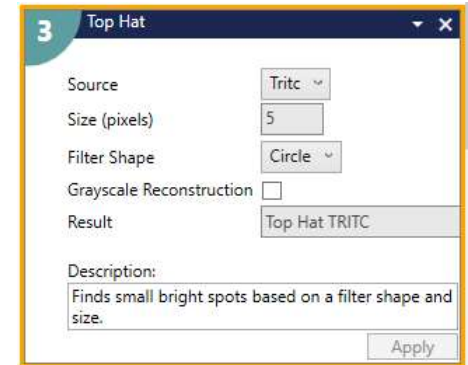
Step 2: Find Blobs



Use the Find Blobs step card to identify nuclei.



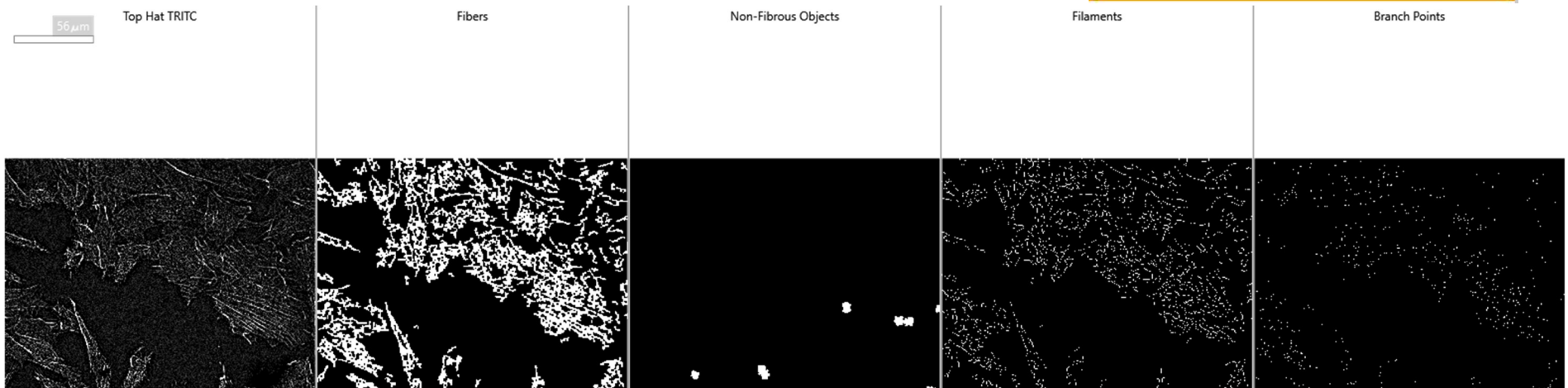
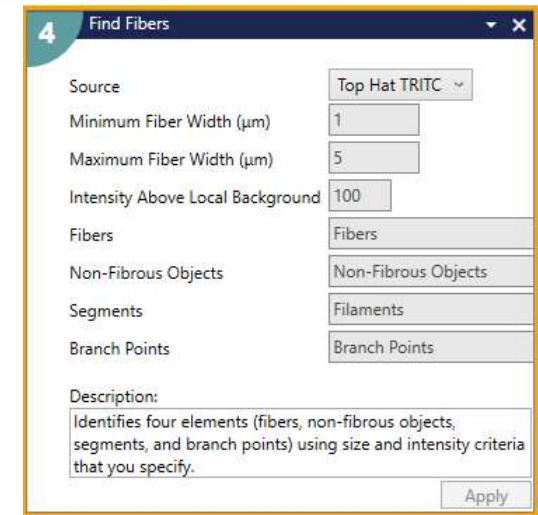
Step 3: Top Hat



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



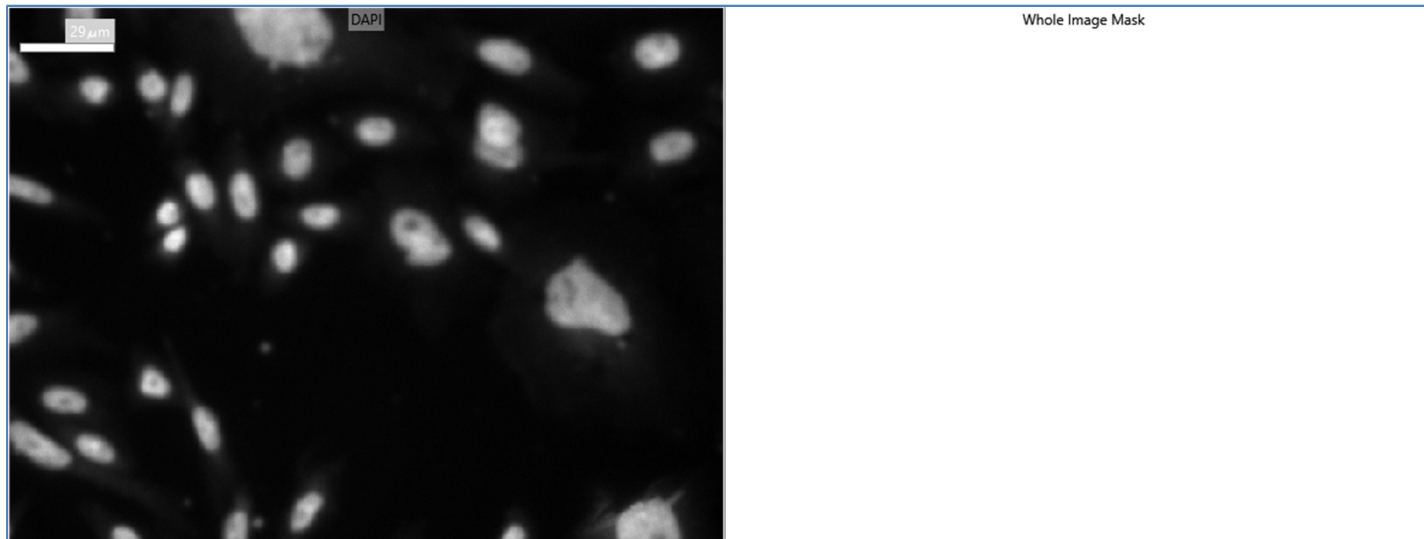
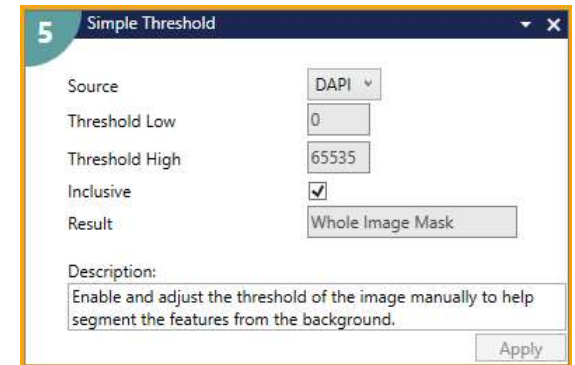
Step 4: Find Fibers



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.



Step 5: Simple Threshold

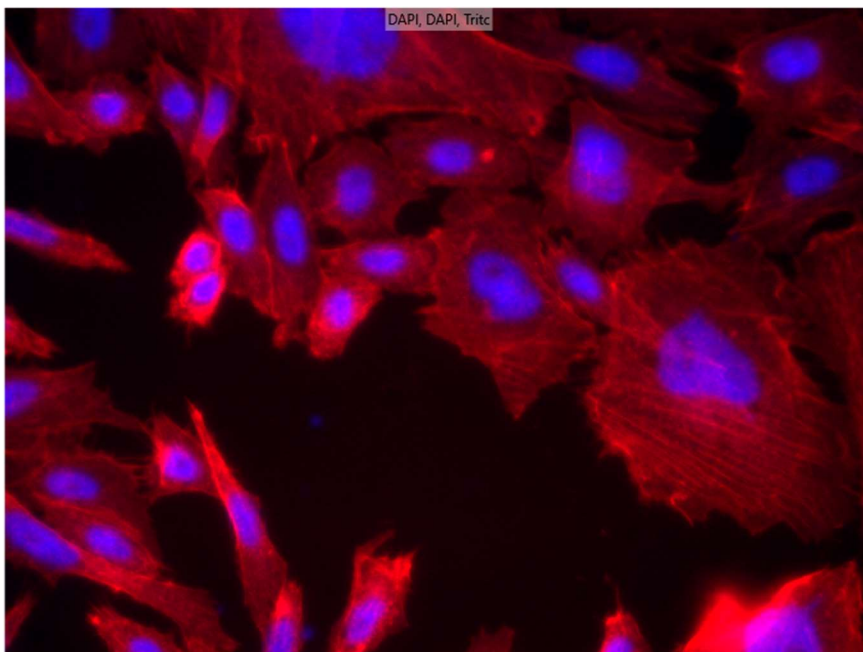


To simplify the output, use Simple Threshold inclusive from 0-65535 to create a mask that represents the entire image area. Any image may be used as the Source for this step.



Step 6: Measure Mask

24 μm



6 Measure Mask [Modified]

Measurement Inputs
Standard Area Value: 1
Create Object Overlay:

Objects to Measure
Mask of Objects: Whole Image Mask
Image to Measure: DAPI

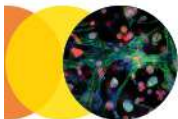
Features within Each Object
Mask of Features: Nuclei
Image to Measure: DAPI

Features within Each Object
Mask of Features: Filaments
Image to Measure: Tritc

Description:
Objects and features used for measurements.

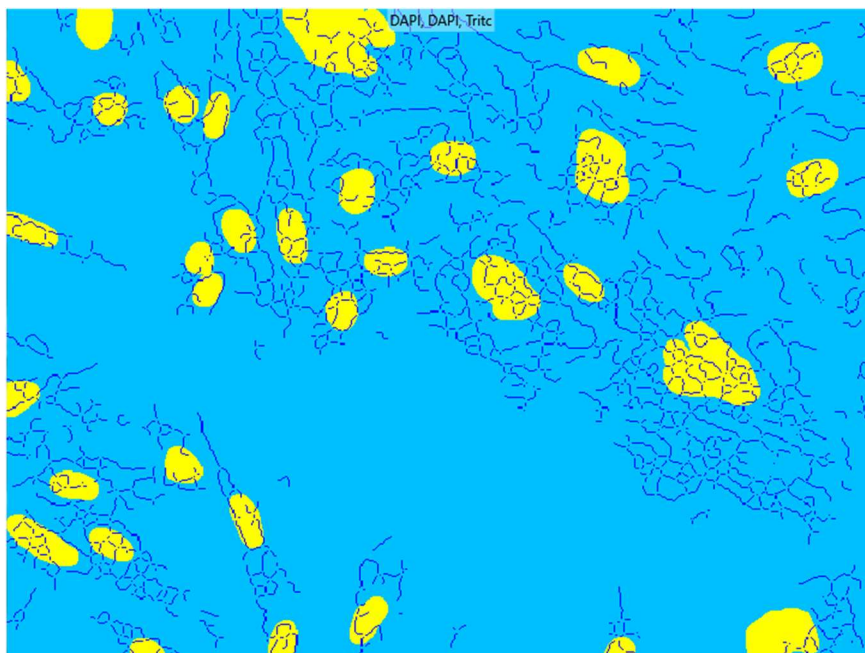
Layer	Color	Mask Name
1	Light Blue	Whole Image Mask
2	Yellow	Nuclei
3	Dark Blue	Filaments

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.



Step 6: Measure Mask

24 μm



6 Measure Mask [Modified]

Measurement Inputs
Standard Area Value: 1
Create Object Overlay:

Objects to Measure
Mask of Objects: Whole Image Mask
Image to Measure: DAPI

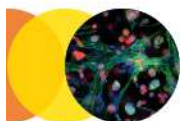
Features within Each Object
Mask of Features: Nuclei
Image to Measure: DAPI

Features within Each Object
Mask of Features: Filaments
Image to Measure: Tritic

Description:
Objects and features used for measurements.

Layer	Color	Mask Name
1	Light Blue	Whole Image Mask
2	Yellow	Nuclei
3	Dark Blue	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.



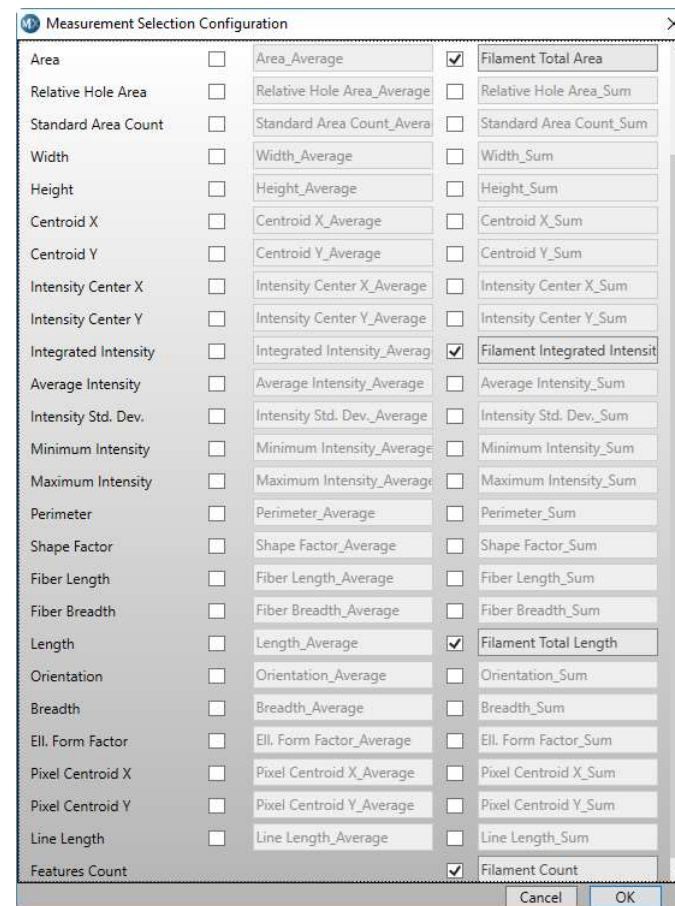
Measurement Selections

Whole Image Mask:
No measurements selected

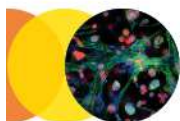
Nuclei Mask:

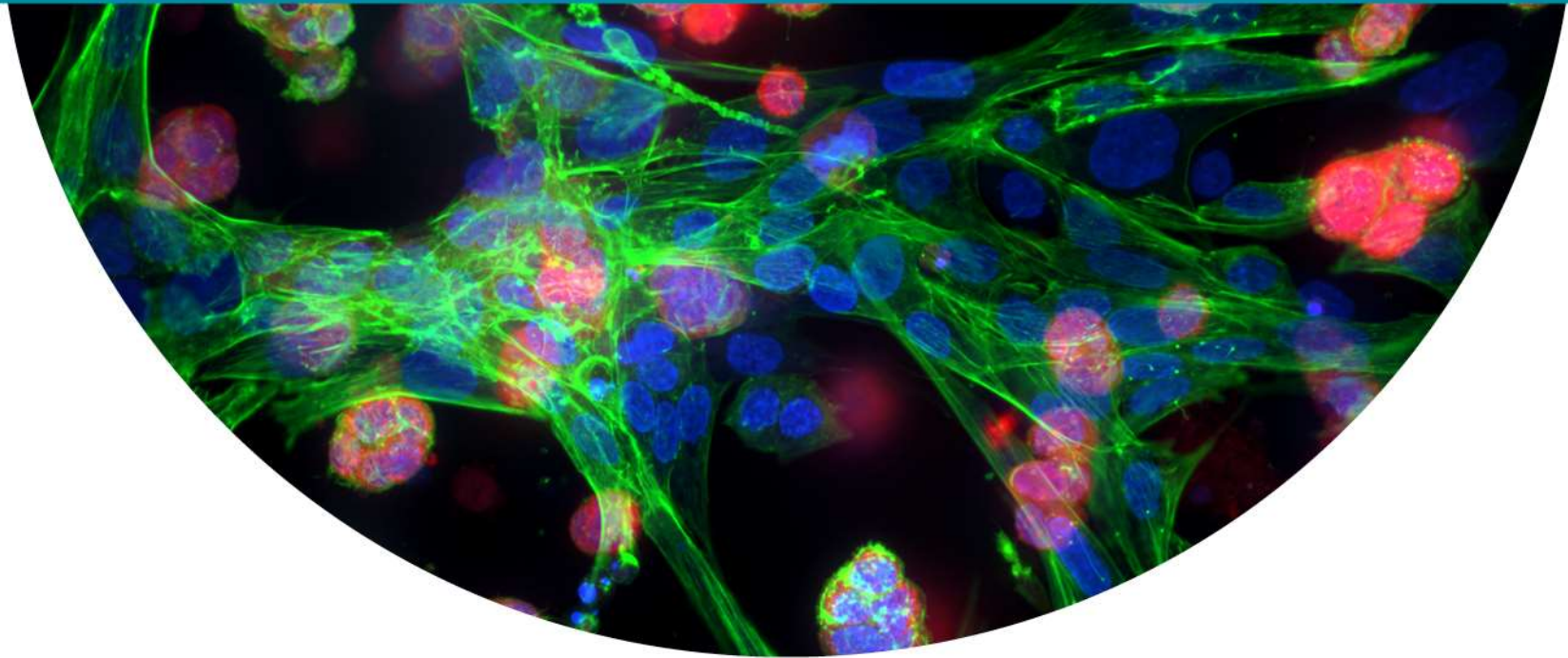


Filaments Mask:



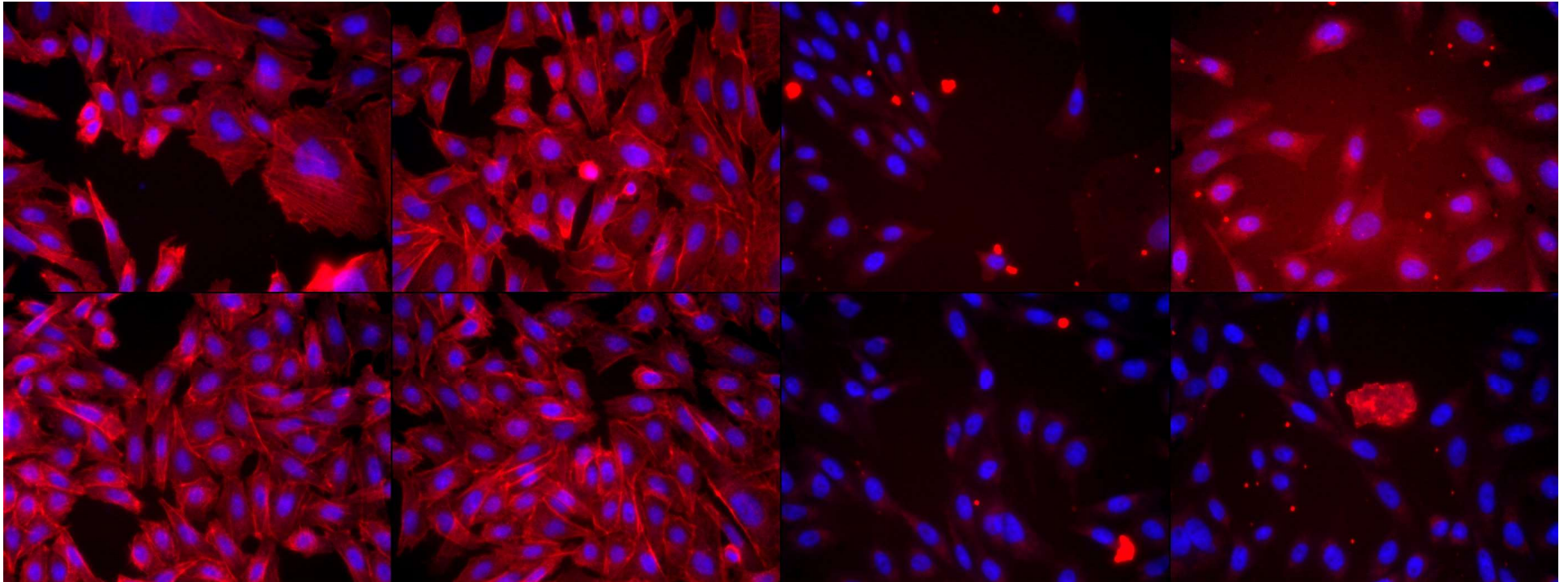
Selected measurements are calculated for the Nuclei and Filaments masks.



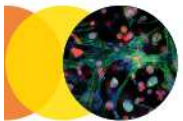
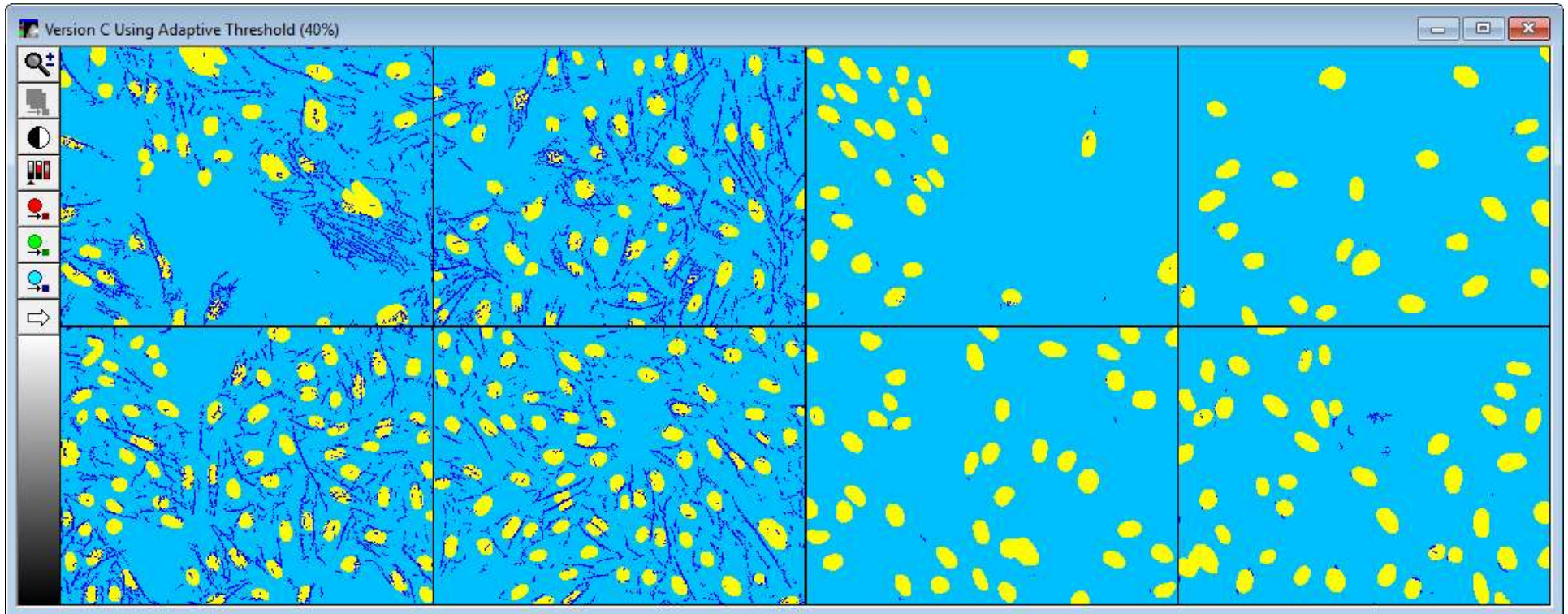


Version C: Top Hat and Adaptive Threshold

Example Plate (8 wells)



Segmentation Overlay



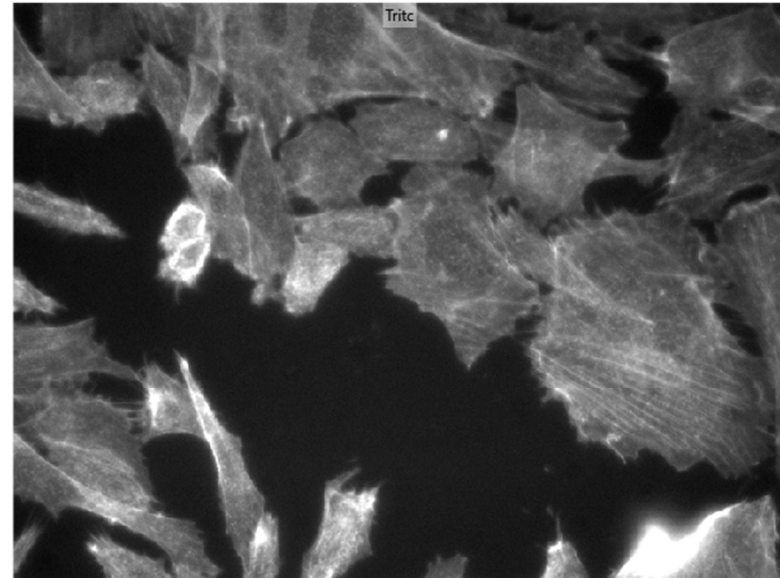
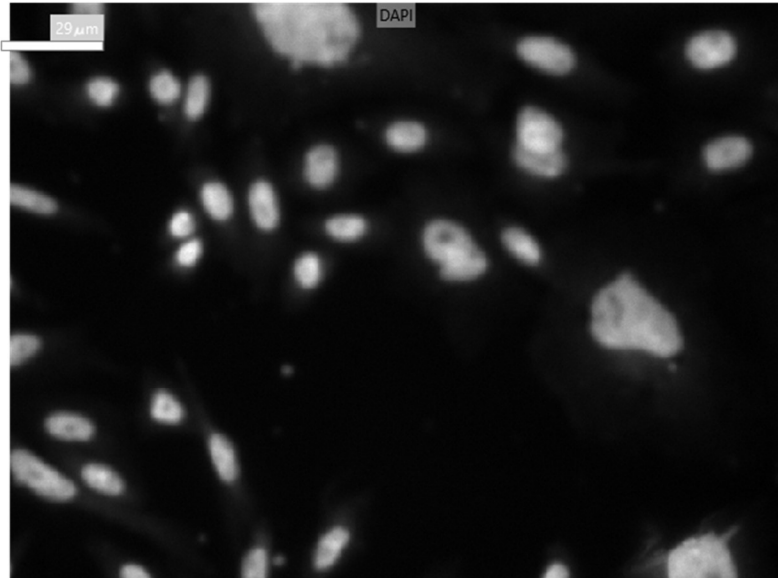
Step 1: Setup

1 Setup

Image Names:	Channels:
DAPI	DAPI
Tritc	Tritc

Crop Reset

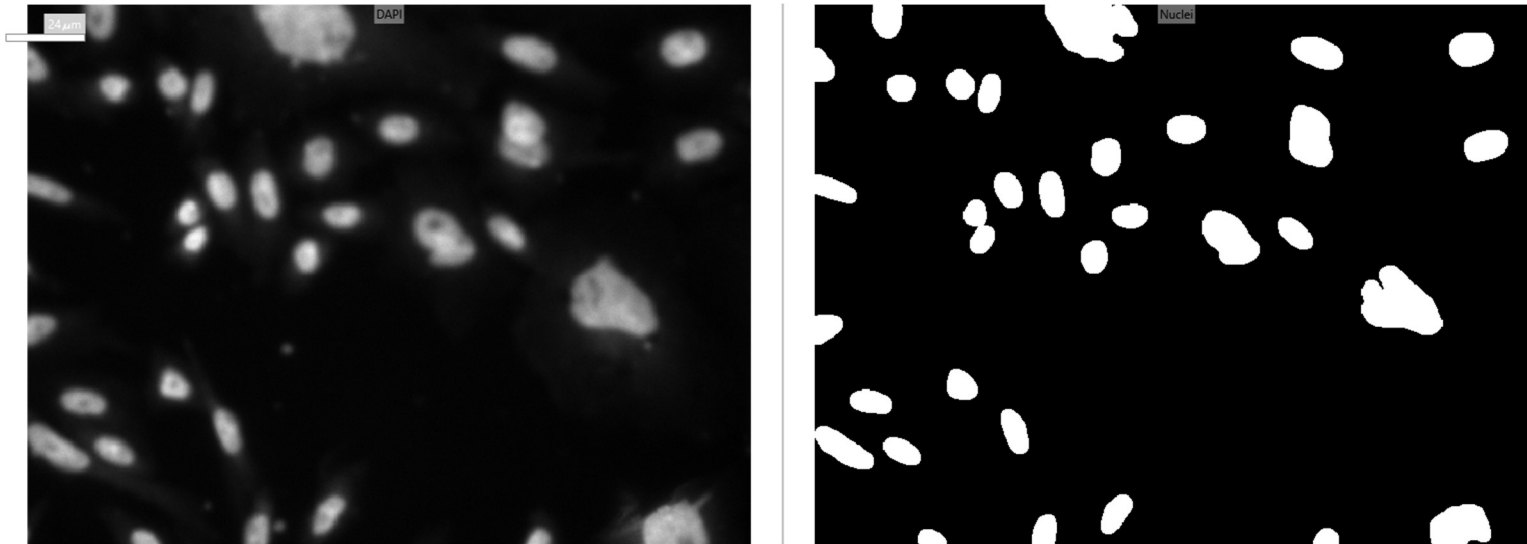
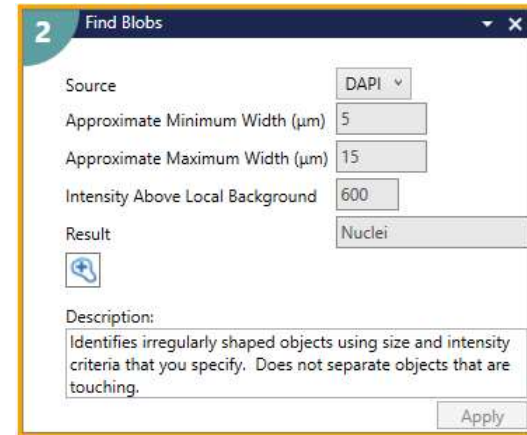
Description:
User-created custom module



In the Setup step, define the wavelengths for analysis.



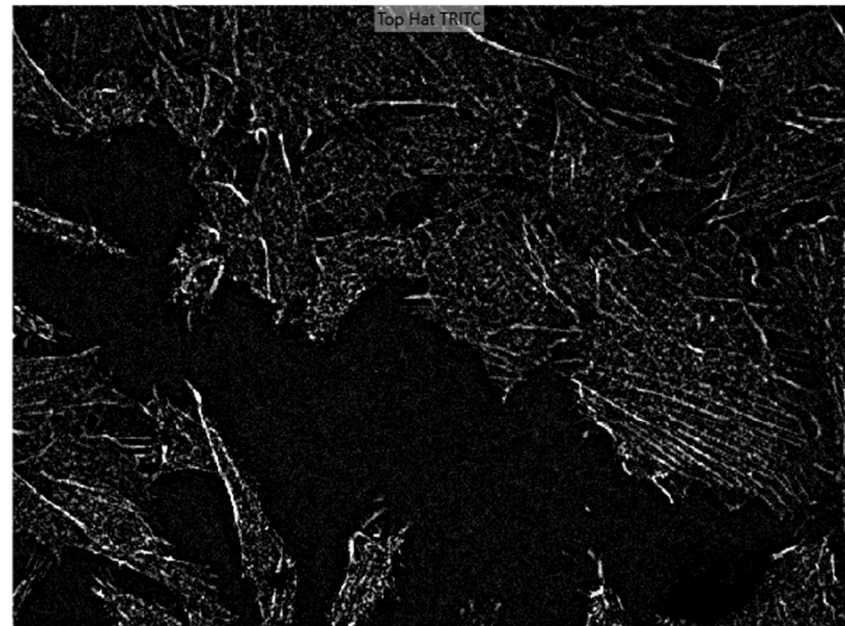
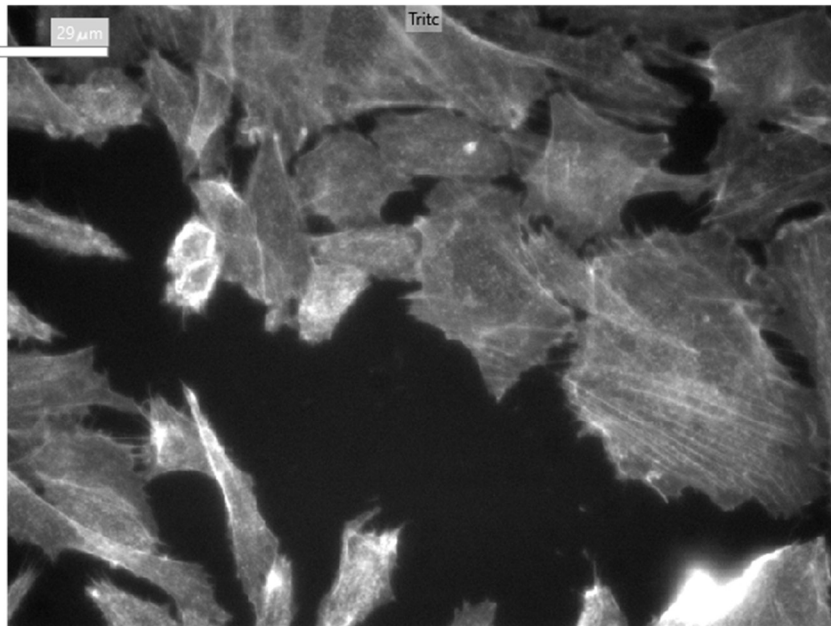
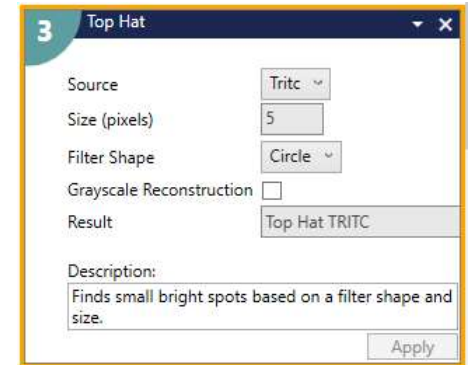
Step 2: Find Blobs



Use the Find Blobs step card to identify nuclei.



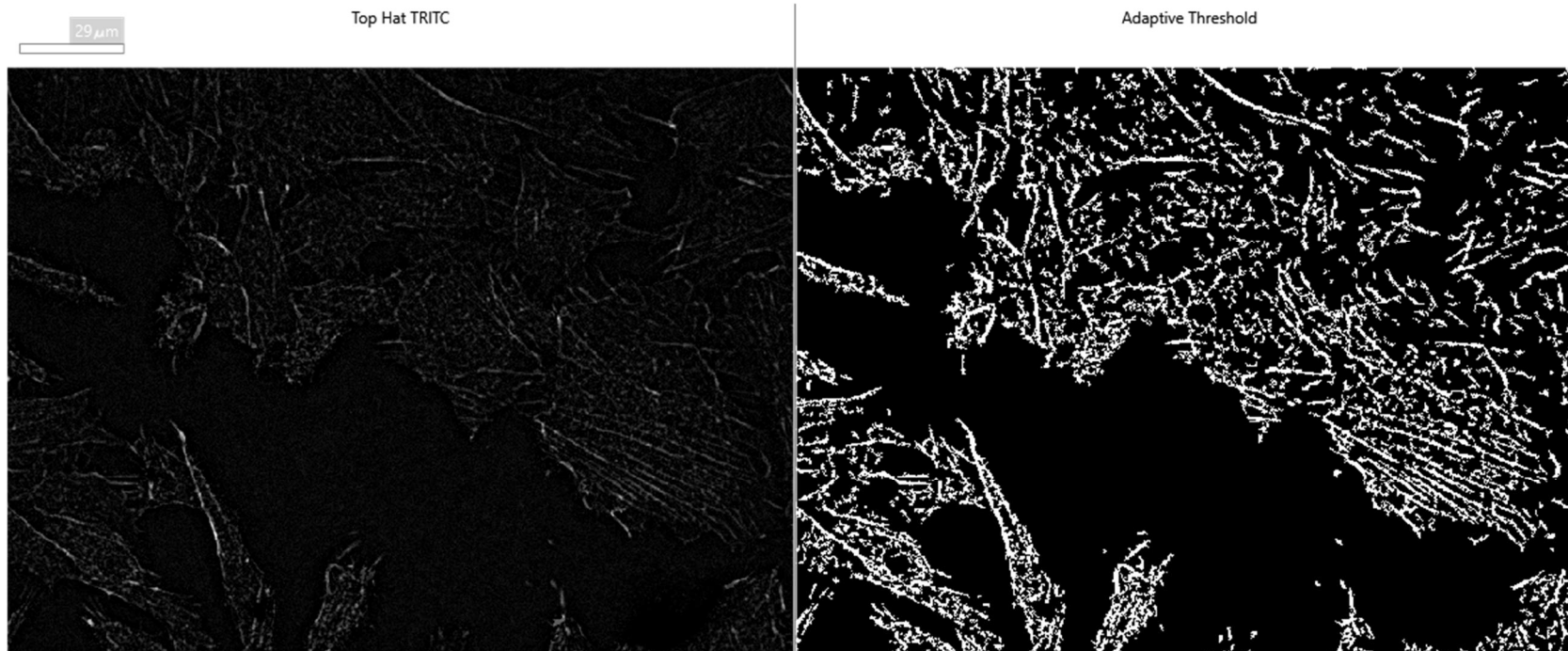
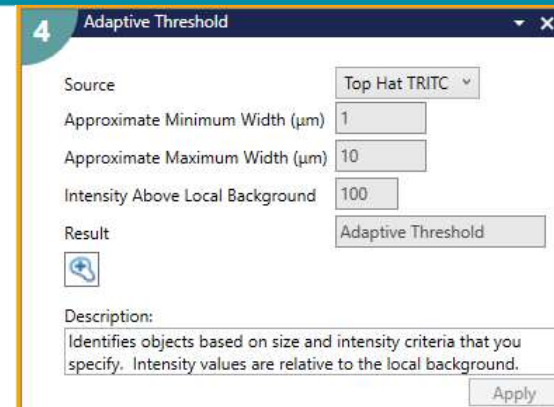
Step 3: Top Hat



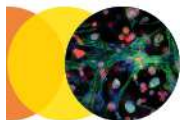
Use the Image Processing step card Top Hat to enhance small bright objects in the image.



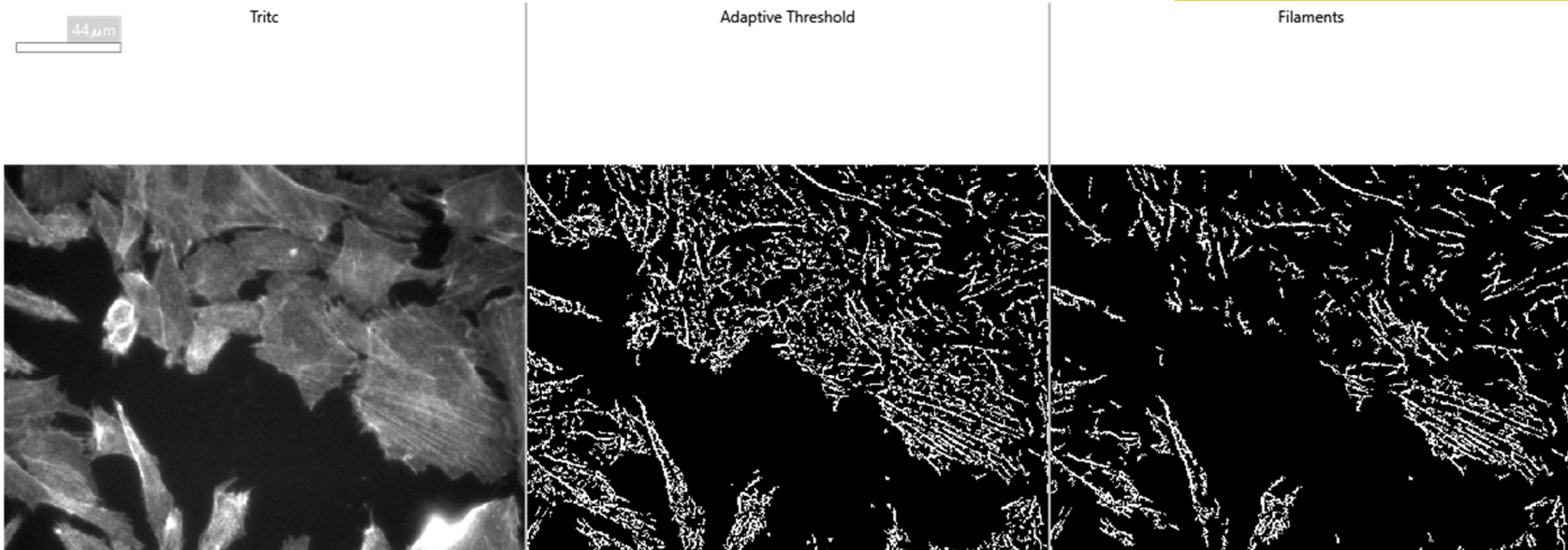
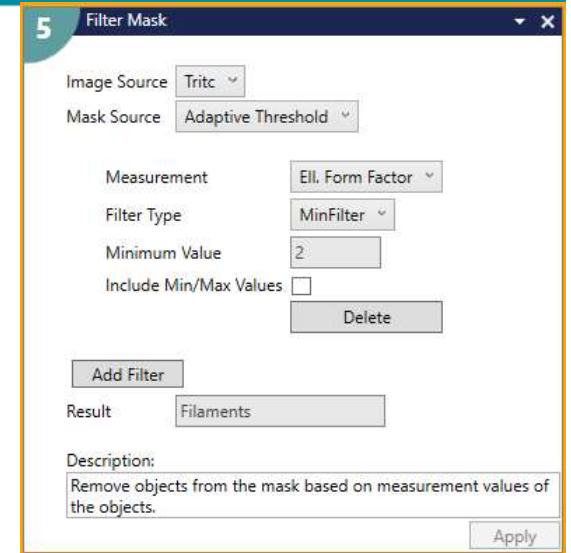
Step 4: Adaptive Threshold



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



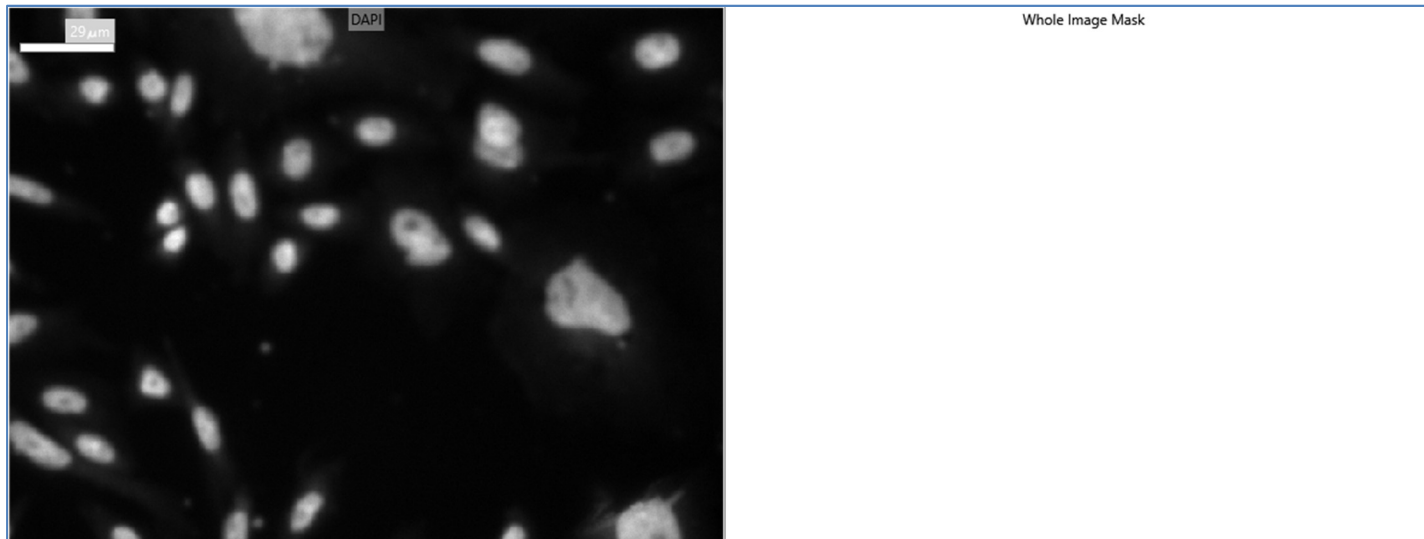
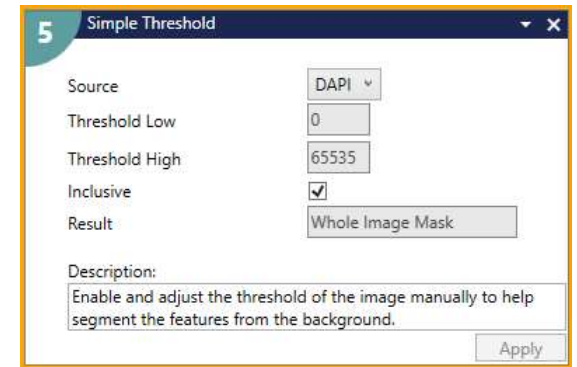
Step 5: Filter Mask



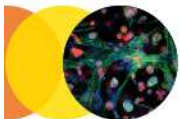
Use a Filter Mask to select objects by size, shape, and/or intensity from the Adaptive Threshold mask. In this example, Elliptical Form Factor (ratio of length/breadth) is used to select elongated objects.



Step 6: Simple Threshold

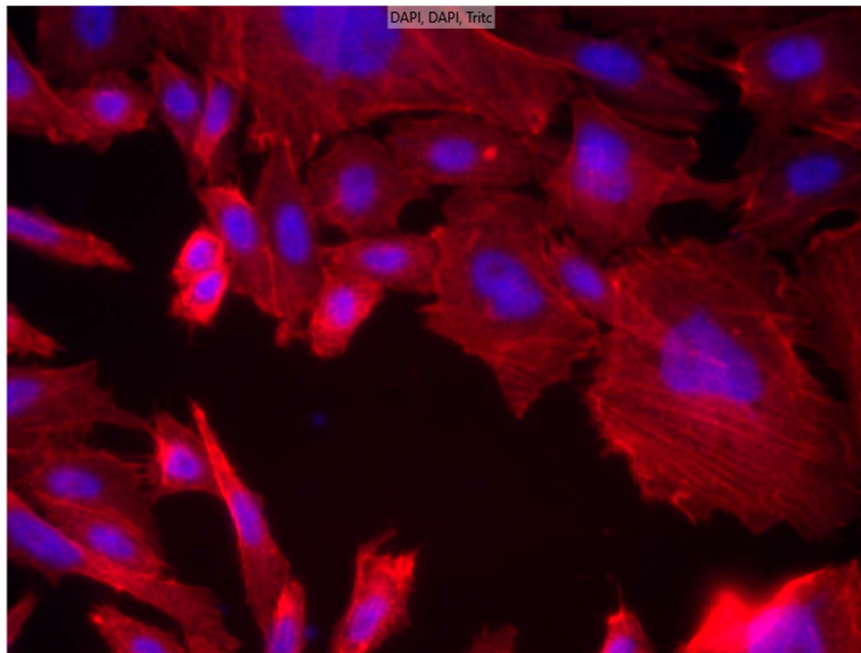


To simplify the output, use Simple Threshold inclusive from 0-65535 to create a mask that represents the entire image area. Any image may be used as the Source for this step.



Step 7: Measure Mask

24 μm



6 Measure Mask [Modified]

Measurement Inputs
Standard Area Value: 1
Create Object Overlay:

Objects to Measure
Mask of Objects: Whole Image Mask
Image to Measure: DAPI

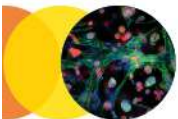
Features within Each Object
Mask of Features: Nuclei
Image to Measure: DAPI

Features within Each Object
Mask of Features: Filaments
Image to Measure: Tritc

Description:
Objects and features used for measurements.

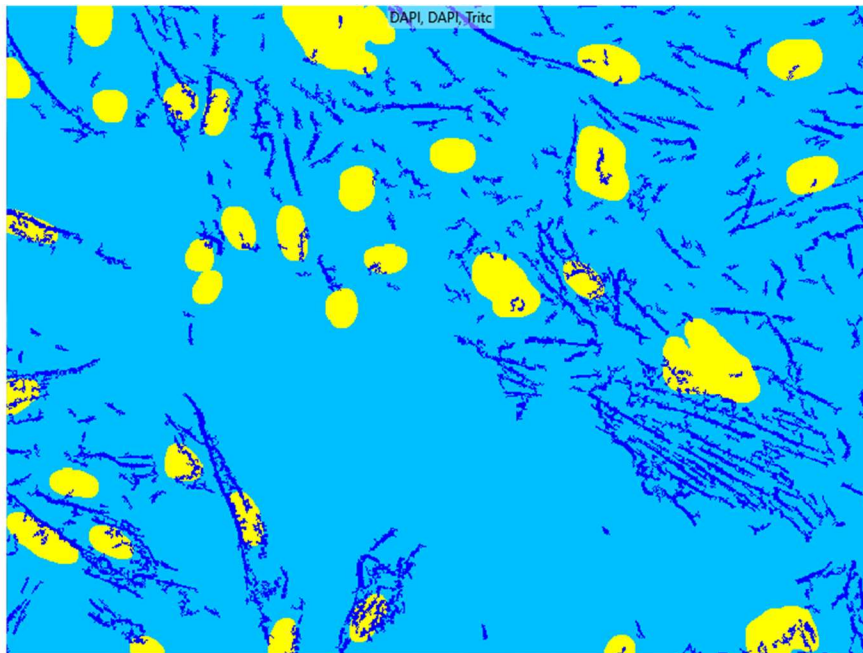
Mask Legend		
Layer	Color	Mask Name
1		Whole Image Mask
2		Nuclei
3		Filaments

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.



Step 7: Measure Mask

24 μm



6 Measure Mask [Modified]

Measurement Inputs
Standard Area Value: 1
Create Object Overlay:

Objects to Measure
Mask of Objects: Whole Image Mask
Image to Measure: DAPI

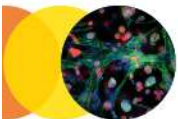
Features within Each Object
Mask of Features: Nuclei
Image to Measure: DAPI

Features within Each Object
Mask of Features: Filaments
Image to Measure: Tritc

Description:
Objects and features used for measurements.

Mask Legend		
Layer	Color	Mask Name
1	Light Blue	Whole Image Mask
2	Yellow	Nuclei
3	Dark Blue	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.



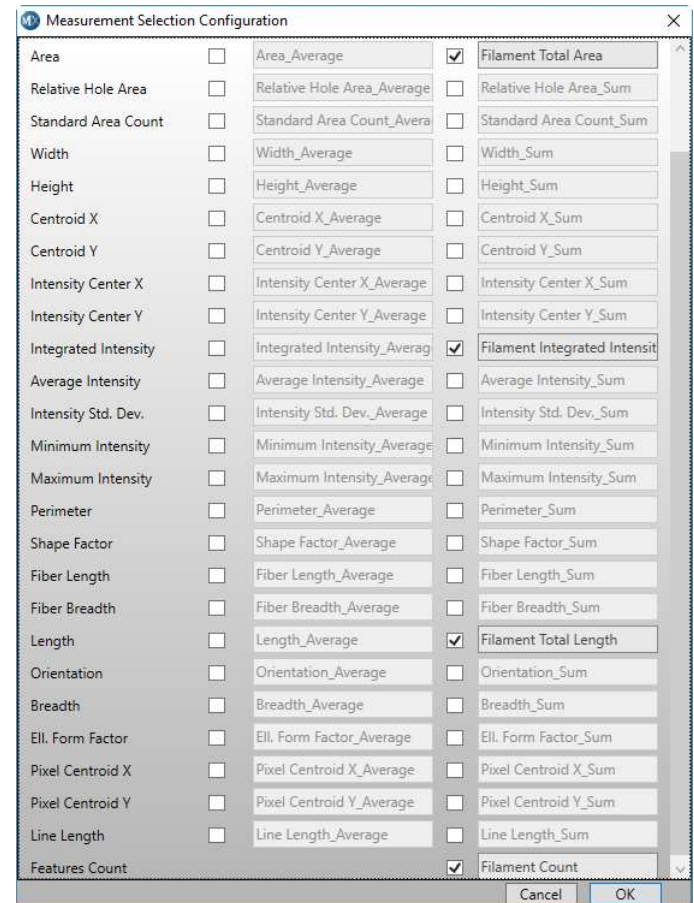
Measurement Selections

Whole Image Mask:
No measurements selected

Nuclei Mask:



Filaments Mask:



Selected measurements are calculated for the Nuclei and Filaments masks.

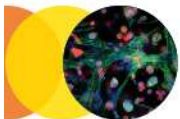
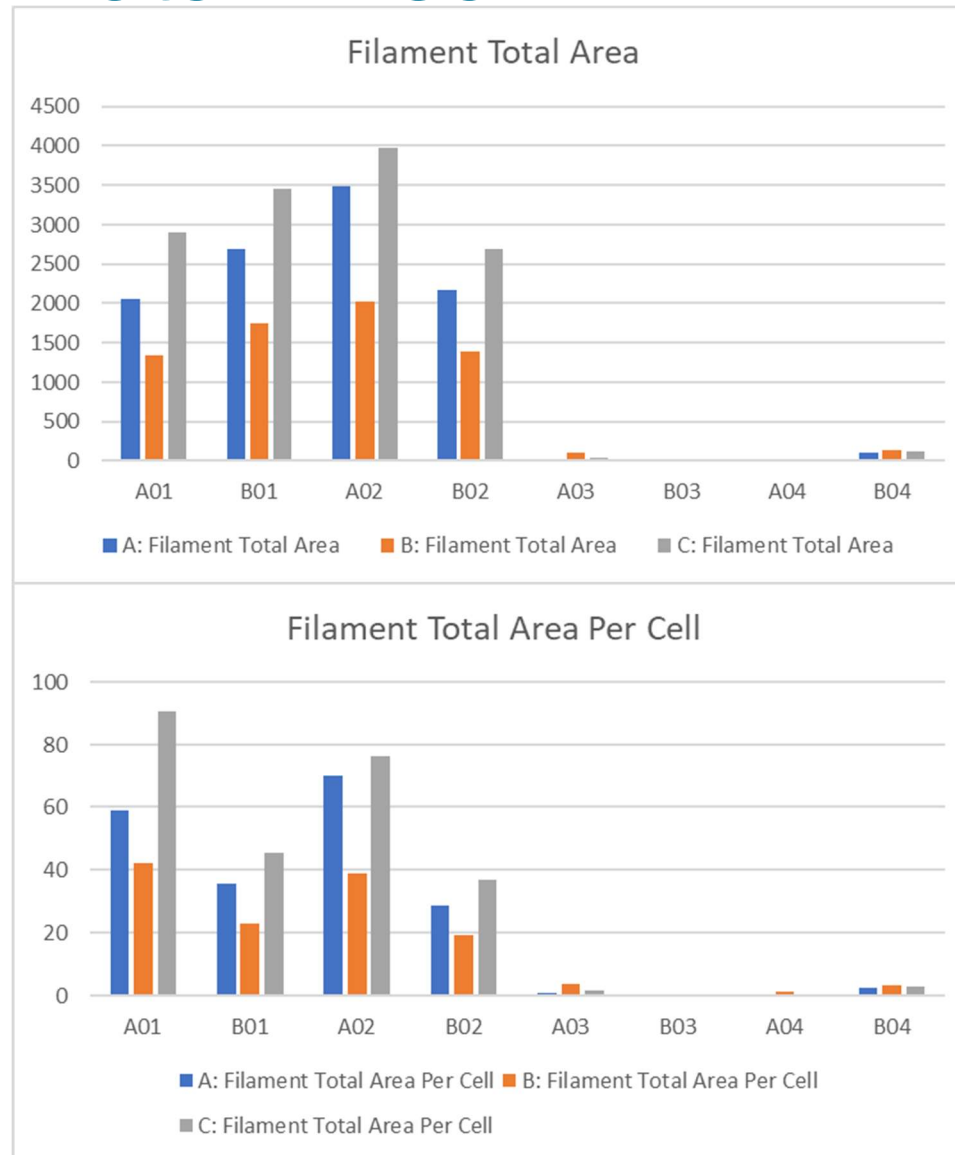


Results Comparison

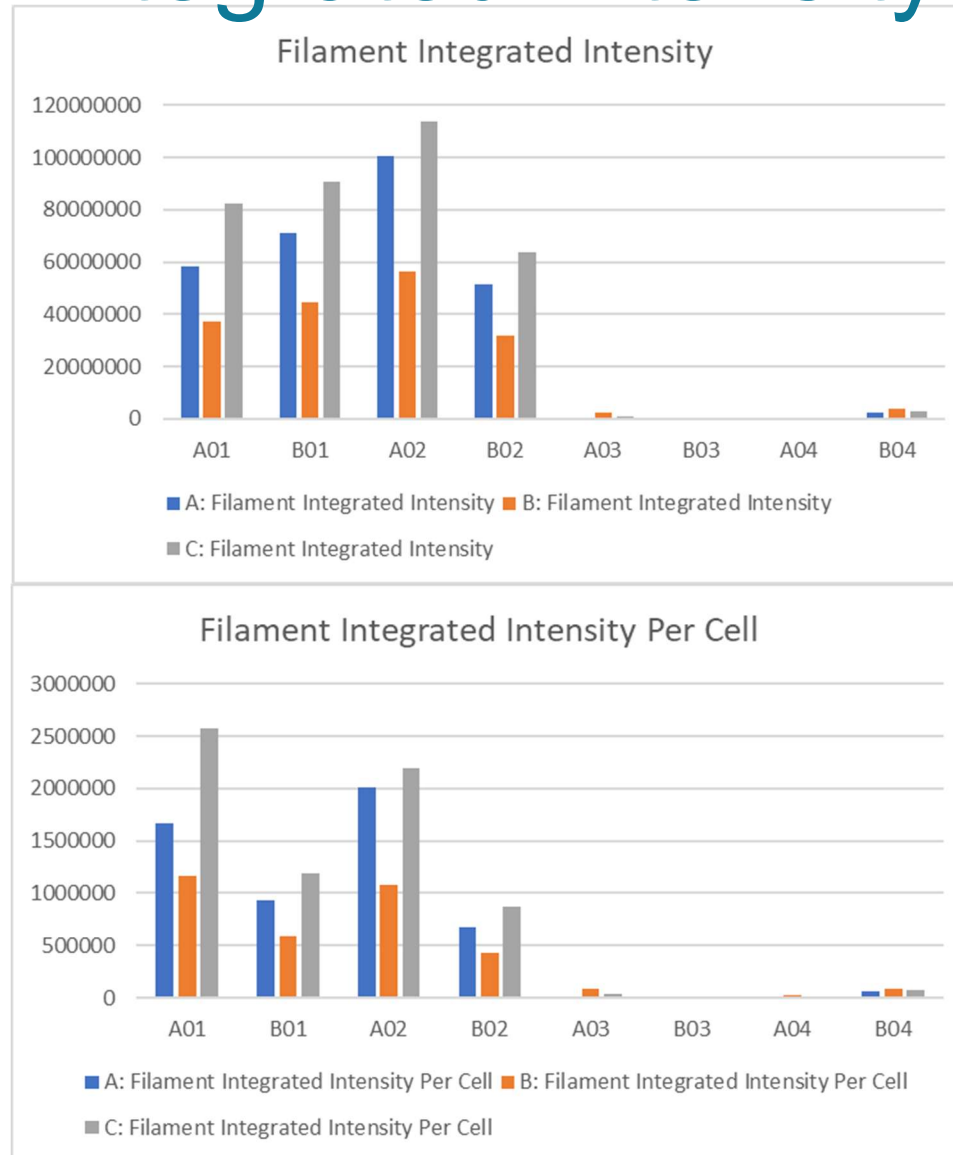
Measurement	Version A	Version B	Version C	
Nuclear Count				
	A	35.00 50.00 29.00 26.00	A 32.00 52.00 29.00 25.00	A 32.00 52.00 29.00 25.00
	B	76.00 76.00 42.00 44.00	B 76.00 73.00 41.00 42.00	B 76.00 73.00 41.00 42.00
	C		C	C
Filament Total Area				
	A	2059.18 3490.33 19.04 6.15	A 1345.21 2020.80 107.32 27.73	A 2900.39 3976.46 47.95 8.89
	B	2697.95 2163.57 25.88 106.25	B 1742.77 1390.92 24.12 141.89	B 3461.72 2684.18 17.38 114.26
	C		C	C
Filament Integrated Intensity				
	A	58208760.00 100672898.00 441331.00 131208.00	A 37128264.00 56388296.00 2400957.00 577784.00	A 82334224.00 113821944.00 1130019.00 191341.00
	B	71086312.00 51301424.00 562869.00 2605125.00	B 44589580.00 31627472.00 509481.00 3725037.00	B 90640448.00 63486460.00 386055.00 3023410.00
	C		C	C
Filament Total Length				
	A	2276.56 3595.42 37.03 13.23	A 4464.31 6806.75 332.94 82.73	A 2605.66 3370.33 71.39 16.55
	B	2946.42 2495.46 39.63 156.64	B 5782.34 4528.87 76.65 430.04	B 3325.03 3075.49 26.37 145.68
	C		C	C
Filament Count				
	A	452.00 651.00 19.00 6.00	A 1761.00 2726.00 130.00 28.00	A 354.00 435.00 20.00 6.00
	B	576.00 540.00 17.00 58.00	B 2195.00 1476.00 24.00 140.00	B 465.00 486.00 9.00 37.00
	C		C	C



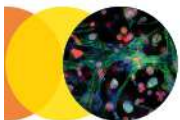
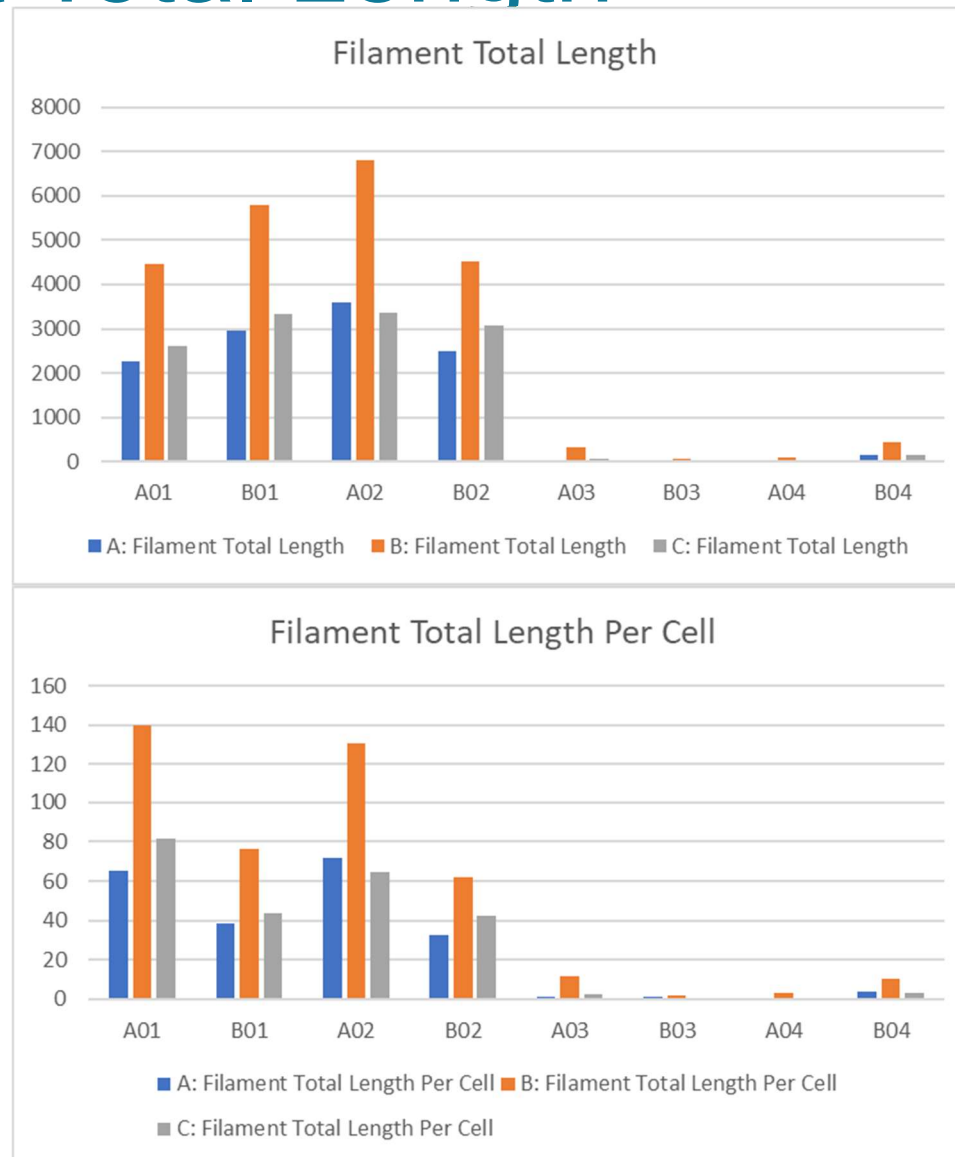
Filament Total Area



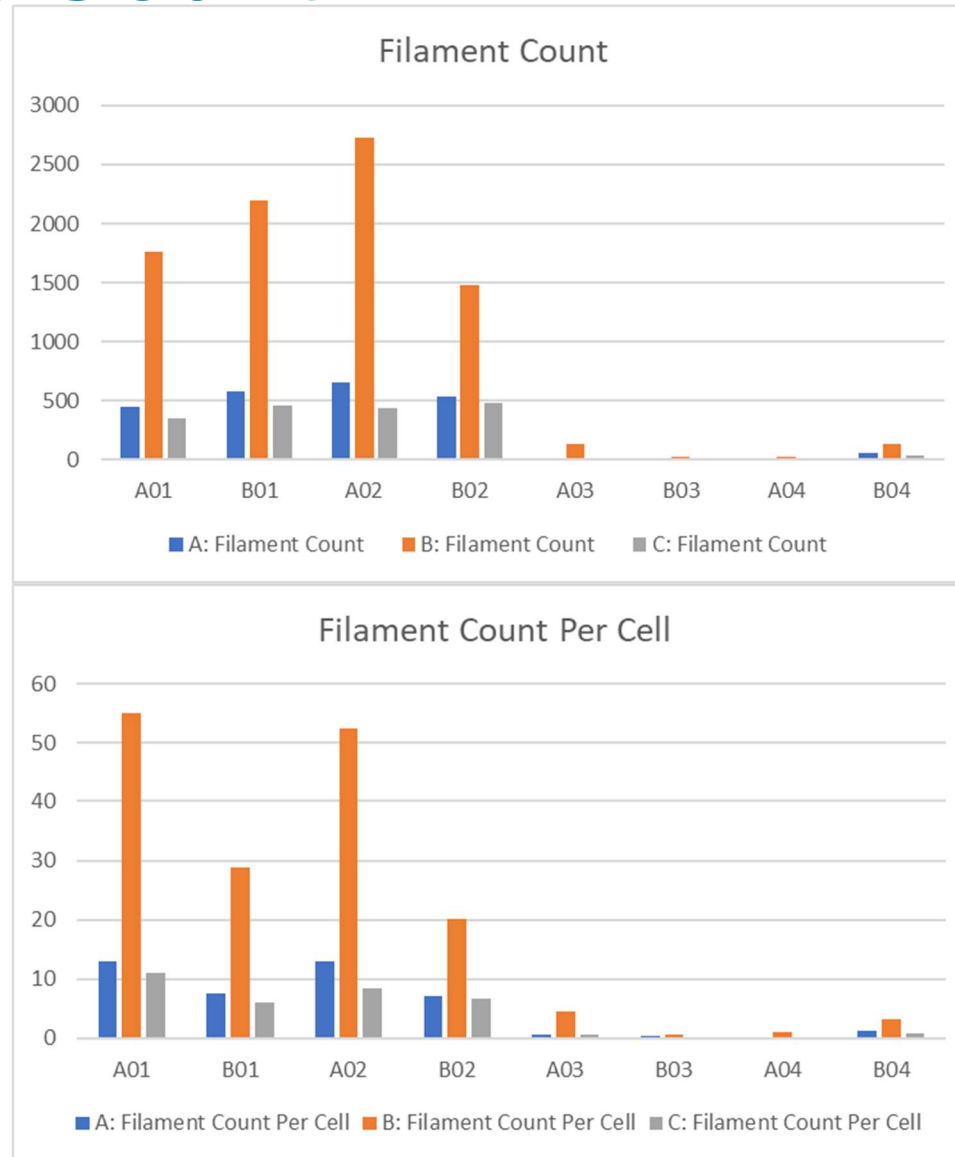
Filament Integrated Intensity



Filament Total Length



Filament Count



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.



