

Actin Filament analysis in MetaXpress Custom Module Editor

August 2018



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Version A: Transfluor or Granularity module



Example Plate (8 wells)







Segmentation Overlay







Step 1: Setup

Image Names:	Channels:	
DAPI	DAPI	~
Tritc	Tritc	*



In the Setup step, define the wavelengths for analysis.





Step 2: Transfluor (or Granularity)

Tritc	DAPI	Nuclei	Pits

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Vesicles Image	Tritc 🖌
ximate Minimum Width (μm) ximate Maximum Width (μm) ity Above Local Background	0.5 3 100
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m	Fast v
Nuclei	
Pits	
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ion: G-Protein Coupled Receptor	(GPCR) cyclin
	Apply
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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.







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

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Measurement Inputs	
Standard Area Value	1
Create Object Overlag	у 🔽
Objects to Measure -	
Mask of Objects:	Whole Image Mask 👻
Image to Measure:	DAPI ~
0	22
Features within Each (Object:
Mask of Features:	Nuclei Y
Image to Measure:	DAPI ~
0	
	Remove feature
Features within Each (Object:
Mask of Features:	Filaments Y
Image to Measure:	Tritc Y
0	

Mask Le	egend	
Layer	Color	Mask Name
1	0	Whole Image Mask
2	0	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 5: Measure Mask

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Measurement Inputs	
Standard Area Value	1
Create Object Overla	y 🔽
Objects to Measure	
Mask of Objects:	Whole Image Mask 💙
Image to Measure:	DAPI ~
0	10
Features within Each	Object:
Mask of Features:	Nuclei Y
Image to Measure:	DAPI ~
0	
C.	Remove feature
Features within Each (Object:
Mask of Features:	Filaments Y

Mask Le	egend		
Layer	Color	Mask Name	
1	0	Whole Image Mask	
2	0	Nuclei	
3	0	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:

Features Count

✓ Nuclear Count

Selected measurements are calculated for the Nuclei and Filaments masks.





Filaments Mask:

Measurement Selection	on Contig	Juration			~
Area		Area_Average	\checkmark	Filament Total Area	1
Relative Hole Area		Relative Hole Area_Average		Relative Hole Area_Sum	
Standard Area Count		Standard Area Count_Avera		Standard Area Count_Sum	1
Width		Width_Average		Width_Sum	1
Height		Height_Average		Height_Sum	
Centroid X		Centroid X_Average		Centroid X_Sum	
Centroid Y		Centroid Y_Average		Centroid Y_Sum	
Intensity Center X		Intensity Center X_Average		Intensity Center X_Sum	
Intensity Center Y		Intensity Center Y_Average		Intensity Center Y_Sum	
Integrated Intensity		Integrated Intensity_Averag	\checkmark	Filament Integrated Intensit	1
Average Intensity		Average Intensity_Average		Average Intensity_Sum	
Intensity Std. Dev.		Intensity Std. Dev. Average		Intensity Std. DevSum	
Minimum Intensity		Minimum Intensity_Average		Minimum Intensity_Sum	
Maximum Intensity		Maximum Intensity_Average		Maximum Intensity_Sum	
Perimeter		Perimeter_Average		Perimeter_Sum	
Shape Factor		Shape Factor_Average		Shape Factor_Sum	
Fiber Length		Fiber Length_Average		Fiber Length_Sum	
Fiber Breadth		Fiber Breadth_Average		Fiber Breadth_Sum	
Length		Length_Average		Filament Total Length	1
Orientation		Orientation_Average		Orientation_Sum	<u>\$</u>
Breadth		Breadth_Average		Breadth_Sum	
Ell. Form Factor		Ell. Form Factor_Average		Ell. Form Factor_Sum	1
Pixel Centroid X		Pixel Centroid X_Average		Pixel Centroid X_Sum	1
Pixel Centroid Y		Pixel Centroid Y_Average		Pixel Centroid Y_Sum	1
Line Length		Line Length_Average		Line Length_Sum	1
Features Count				Filament Count	1



Version B: Top Hat and Find Fibers



Example Plate (8 wells)







Segmentation Overlay







Step 1: Setup

Image Names:	Channels:	
DAPI	DAPI	~
Tritc	Tritc	¥



In the Setup step, define the wavelengths for analysis.







Use the Find Blobs step card to identify nuclei.

Step 2: Find Blobs









Step 3: Top Hat



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







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



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 ~ >
0	
Features within Each O	bject:
Mask of Features:	Nuclei 👻
Image to Measure:	DAPI ~
0	
	Remove feature grou
Features within Each O	bject:
Mask of Features:	Filaments *
Image to Measure:	Tritc ~
0	
	Remove feature grou
	Add feature gro
Description:	
Objects and features us	ed for measurements.

Mask Legend		
Layer	Color	Mask Name
1	0	Whole Image Mask
2	0	Nuclei
3	0	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

DAPI, DAPI, Trite



Mask Legend			
Layer	Color	Mask Name	
1	0	Whole Image Mask	
2	0	Nuclei	
3	0	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:

Features Count

✓ Nuclear Count

Selected measurements are calculated for the Nuclei and Filaments masks.





Filaments Mask:

Area	Area_Average	-	Filament Total Area	
Relative Hole Area	Relative Hole Area_Average		Relative Hole Area_Sum	
Standard Area Count	Standard Area Count_Avera		Standard Area Count_Sum	
Width	Width_Average		Width_Sum	
Height	Height_Average		Height_Sum	
Centroid X	Centroid X_Average		Centroid X_Sum	
Centroid Y	Centroid Y_Average		Centroid Y_Sum	
ntensity Center X	Intensity Center X_Average		Intensity Center X_Sum	
ntensity Center Y	Intensity Center Y_Average		Intensity Center Y_Sum	
ntegrated Intensity	Integrated Intensity_Averag		Filament Integrated Intensi	
Average Intensity	Average Intensity_Average		Average Intensity_Sum	
ntensity Std. Dev.	Intensity Std. Dev. Average		Intensity Std. DevSum	
Vinimum Intensity	Minimum Intensity_Average		Minimum Intensity_Sum	
Maximum Intensity	Maximum Intensity_Average		Maximum Intensity_Sum	
Perimeter	Perimeter_Average		Perimeter_Sum	
Shape Factor	Shape Factor_Average] Shape Factor_Sum	
iber Length	Fiber Length_Average		Fiber Length_Sum	
iber Breadth	Fiber Breadth_Average		Fiber Breadth_Sum	
Length	Length_Average	\checkmark	Filament Total Length	
Orientation	Orientation_Average		Orientation_Sum	
Breadth	Breadth_Average		Breadth_Sum	
Ell. Form Factor	Ell. Form Factor_Average		Ell. Form Factor_Sum	
Pixel Centroid X	Pixel Centroid X_Average		Pixel Centroid X_Sum	
Pixel Centroid Y	Pixel Centroid Y_Average		Pixel Centroid Y_Sum	
Line Length	Line Length_Average		Line Length_Sum	
Features Count		~	Filament Count	



Version C: Top Hat and Adaptive Threshold



Example Plate (8 wells)







Segmentation Overlay







Step 1: Setup

Image Names:	Channels:	
DAPI	DAPI	~
Tritc	Tritc	*



In the Setup step, define the wavelengths for analysis.







Use the Find Blobs step card to identify nuclei.

Step 2: Find Blobs







Step 3: Top Hat



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







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







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



Measure Mask		[Modified]
Measurement Inputs		
Standard Area Value	1	
Create Object Overlay		
Objects to Measure		
Mask of Objects:	Whole Image N	lask 🛩
Image to Measure:	DAPI ~	
0		
Features within Each (Object:	
Mask of Features:	Nuclei ~	
Image to Measure:	DAPI ~	
0	La resta de la compañía de	
	Rem	iove feature gr
Features within Each (Object:	
Mask of Features:	Filaments 👻	
Image to Measure:	Tritc v	
0		
	Rem	iove feature gr
		Add feature g
escription:		14 - 14 A
bjects and features us	sed for measureme	ents.

Mask Legend					
Layer	Color	Mask Name			
1	0	Whole Image Mask			
2	0	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



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 ~
0	
Features within Each (Dbject:
Mask of Features:	Nuclei 👻
Image to Measure:	DAPI ~
0	
	Remove feature gro
Features within Each (Dbject:
Mask of Features:	Filaments 👻
Image to Measure:	Tritc v
0	
	Remove feature gro
	Add feature on
	nao rebuie gr
escription:	

Mask Legend					
Layer	Color	Mask Name			
1	0	Whole Image Mask			
2	0	Nuclei			
3	0	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:

Features Count

✓ Nuclear Count

Selected measurements are calculated for the Nuclei and Filaments masks.





Filaments Mask:

Measurement Selection	on Config	juration			1
Area		Area_Average	\checkmark	Filament Total Area]
Relative Hole Area		Relative Hole Area_Average		Relative Hole Area_Sum	
Standard Area Count		Standard Area Count_Avera		Standard Area Count_Sum	1
Width		Width_Average		Width_Sum	
Height		Height_Average		Height_Sum	
Centroid X		Centroid X_Average		Centroid X_Sum	
Centroid Y		Centroid Y_Average		Centroid Y_Sum	
Intensity Center X		Intensity Center X_Average		Intensity Center X_Sum	
Intensity Center Y		Intensity Center Y_Average		Intensity Center Y_Sum	
Integrated Intensity		Integrated Intensity_Averag	\checkmark	Filament Integrated Intensi	
Average Intensity		Average Intensity_Average		Average Intensity_Sum	
Intensity Std. Dev.		Intensity Std. Dev. Average		Intensity Std. DevSum	
Minimum Intensity		Minimum Intensity_Average		Minimum Intensity_Sum	
Maximum Intensity		Maximum Intensity_Average		Maximum Intensity_Sum	
Perimeter		Perimeter_Average		Perimeter_Sum	
Shape Factor		Shape Factor_Average		Shape Factor_Sum	
Fiber Length		Fiber Length_Average		Fiber Length_Sum	
Fiber Breadth		Fiber Breadth_Average		Fiber Breadth_Sum	
Length		Length_Average		Filament Total Length	1
Orientation		Orientation_Average		Orientation_Sum	
Breadth		Breadth_Average		Breadth_Sum	
Ell. Form Factor		Ell. Form Factor_Average] Ell. Form Factor_Sum	
Pixel Centroid X		Pixel Centroid X_Average		Pixel Centroid X_Sum	
Pixel Centroid Y		Pixel Centroid Y_Average		Pixel Centroid Y_Sum	
Line Length		Line Length_Average		Line Length_Sum	1
Features Count				Filament Count	1

Results Comparison

Measurement	Version A	Version B	Version C
Nuclear Count	01 02 03 04 05 06 A 95 00 50.00 29.00 26.00 4 4 B 76 00 76.00 42.00 44.00 4 4 C	01 02 03 04 05 06 A 32.00 52.00 29.00 25.00 2 2 B 76.00 73.00 41.00 42.00 2 2 C	01 02 03 04 05 06 A 32.00 52.00 29.00 25.00 9 9 B 76.00 73.00 41.00 42.00 9 9 9 D Image: Constraint of the second s
Filament Total Area	01 02 03 04 05 06 A 2059 18 3490.33 19.04 6.15 6 6 B 2697.95 2163.57 25.88 106.25 6 6 C	01 02 03 04 05 06 A 1345.21 2020.80 107.32 27.73 5 5 B 1742.77 1330.92 24.12 141.89 5 5 C	01 02 03 04 05 06 A 2900.33 3976.45 47.95 8.89 B 3461.72 2684.18 17.38 114.26 C
Filament Integrated Intensity	01 02 03 04 05 06 A 59208760.00 100672888.00 441331.00 131208.00 I B 71086312.00 51301424.00 562869.00 2605125.00 I C D I I I I	01 02 03 04 05 06 A 37128264.00 56388296.00 2400957.00 577784.00 6 B 44589580.00 31627472.00 509481.00 3725097.00 6 C 6 D	01 02 03 04 05 04 A 92334224.00 113821944.00 1130019.00 191341.00 1 B 90640448.00 63486460.00 386055.00 3023410.00 1 C D D D D D D D
Filament Total Length	01 02 03 04 05 06 A 2276.56 3595.42 37.03 13.23 5 B 2946.42 2495.46 39.63 156.64 5 C	01 02 03 04 05 06 A 4464.31 6806.75 332.94 82.73 6 6 B 5782.34 4528.87 76.65 430.04 6 6 C 0 0 0 0 0 0 0 D 0 0 0 0 0 0 0	01 02 03 04 05 06 A 2605.66 3370.33 71.39 16.55 5 5 B 3325.03 3075.49 26.37 145.68 5 5 C
Filament Count	01 02 03 04 05 06 A 452.00 651.00 19.00 6.00 6 6 B 576.00 540.00 17.00 58.00 6 6 C D	01 02 03 04 0' A 1761.00 2726.00 130.00 28.00 B 2195.00 1476.00 24.00 140.00 C D	01 02 03 04 05 06 A 354.00 435.00 20.00 6.00 6 B 465.00 486.00 9.00 37.00 6 6 C Image: Constraint of the state of the





Filament Total Area









Filament Integrated Intensity



Filament Integrated Intensity Per Cell







Filament Total Length





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Filament Count





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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.









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