

Pipeline

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Analyze Images

Module notes

To begin creating your project, use the Images module to compile a list of files and/or folders that you want to analyze. You can also specify a set of rules to include only the desired files in your selected folders.

File list

D:\20190613 Cinzia manuscript - METHOD

G1_8667final4.tif

Module settings (Images #01)

Filter images?

No filtering

Found 4 image sets

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Module notes

The Metadata module optionally allows you to extract information describing your images (i.e, metadata) which will be stored along with your measurements. This information can be contained in the file name and/or location, or in an external file.

Module settings (Metadata #02)

Extract metadata?

Yes

No

Metadata extraction method

Extract from image file headers

Extract metadata from

All images

Update metadata

Add another extraction method

Metadata data type

Text

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Start Test Mode

Analyze Images

Found 4 image sets

Update	Path / URL	Series	Frame	C	ChannelName	ColorFormat	FileLocation	SizeC	SizeT	SizeX	SizeY	SizeZ	T	Z
1	D:\20190613 C...667final4.tif	0	0	0		monochrome	file:///Dr/20...667final4.tif	3	1	2048	2048	1	0	0
2	D:\20190613 C...667final4.tif	0	1	1		monochrome	file:///Dr/20...667final4.tif	3	1	2048	2048	1	0	0
3	D:\20190613 C...667final4.tif	0	2	2	Leica/FITC	monochrome	file:///Dr/20...667final4.tif	3	1	2048	2048	1	0	0
4	D:\20190613 C...667final4.tif	1	0	0		monochrome	file:///Dr/20...667final4.tif	3	1	2048	2048	1	0	0
5	D:\20190613 C...667final4.tif	1	1	1		monochrome	file:///Dr/20...667final4.tif	3	1	2048	2048	1	0	0
6	D:\20190613 C...667final4.tif	1	2	2	Leica/FITC	monochrome	file:///Dr/20...667final4.tif	3	1	2048	2048	1	0	0
7	D:\20190613 C...667final4.tif	2	0	0		monochrome	file:///Dr/20...667final4.tif	3	1	2048	2048	1	0	0
8	D:\20190613 C...667final4.tif	2	1	1		monochrome	file:///Dr/20...667final4.tif	3	1	2048	2048	1	0	0
9	D:\20190613 C...667final4.tif	2	2	2	Leica/FITC	monochrome	file:///Dr/20...667final4.tif	3	1	2048	2048	1	0	0
10	D:\20190613 C...667final4.tif	3	0	0		monochrome	file:///Dr/20...667final4.tif	3	1	2048	2048	1	0	0
11	D:\20190613 C...667final4.tif	3	1	1		monochrome	file:///Dr/20...667final4.tif	3	1	2048	2048	1	0	0
12	D:\20190613 C...667final4.tif	3	2	2	Leica/FITC	monochrome	file:///Dr/20...667final4.tif	3	1	2048	2048	1	0	0

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Module notes

The NamesAndTypes module allows you to assign a meaningful name to each image by which other modules will refer to it.

Module settings (NamesAndTypes #03)

Assign a name to Images matching rules

Select the rule criteria Match All of the following rules

Metadata Does Have C matching 0

Name to assign these images c1

Select the image type Grayscale image

Set intensity range from Image bit-depth

Duplicate this image

Select the rule criteria Match All of the following rules

Metadata Does Have C matching 1

Name to assign these images c2

Select the image type Grayscale image

Set intensity range from Image bit-depth

Duplicate this image

Remove this image

Select the rule criteria Match All of the following rules

Metadata Does Have C matching 2

Name to assign these images c3

Select the image type Grayscale image

Set intensity range from Image metadata

Duplicate this image

Remove this image

Update	c1	c2	c3
1	G1_8667final4.tif	G1_8667final4.tif	G1_8667final4.tif
2	G1_8667final4.tif	G1_8667final4.tif	G1_8667final4.tif
3	G1_8667final4.tif	G1_8667final4.tif	G1_8667final4.tif
4	G1_8667final4.tif	G1_8667final4.tif	G1_8667final4.tif

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Module notes

The Groups module optionally allows you to split your list of images into image subsets (groups) which will be processed independently of each other. Examples of groupings include screening batches, microtiter plates, time-lapse movies, etc.

Module settings (Groups #04)

Do you want to group your images? ☐ Yes ☒ No

Found 4 image sets

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Input modules

✓ Images

✓ Metadata

✓ NamesAndTypes

✓ Groups

Analysis modules

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✓ ApplyThreshold

✓ ApplyThreshold

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✓ RescaleIntensity

✓ ImageMath

✓ ImageMath

✓ MaskImage

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✓ CorrectIlluminationCalculate

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✓ ApplyThreshold

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✓ IdentifyPrimaryObjects

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✓ GrayToColor

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✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

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Adjust modules: + - < >

Start Test Mode Analyze Images

Module notes

Threshold nuclei for illumination function masking

Module settings (ApplyThreshold #05)

Select the input image c2 (from NamesAndTypes)

Name the output image Thresh_Nuclei_for_mask

Select the output image type Grayscale

Set pixels below or above the threshold to zero? Below threshold

Subtract the threshold value from the remaining pixel intensities? ☐ Yes ☒ No

Threshold strategy Manual

Manual threshold 0.05

Found 4 image sets

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✓ ApplyThreshold

✓ ApplyThreshold

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Analyze Images

Module notes

Threshold nuclei for illumination function masking

Module settings (ApplyThreshold #06)

Select the input image

c2 (from NamesAndTypes)

Name the output image

Thresh_Nuclei_binary

Select the output image type

Binary (black and white)

Threshold strategy

Manual

Manual threshold

0.05

Found 4 image sets

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Module notes

Threshold CD3 staining. Should be based on 2o staining and should perhaps be using intensity normalised CD3

Module settings (ApplyThreshold #07)

Select the input image: c1 (from NamesAndTypes)

Name the output image: Thresh_CD3

Select the output image type: Grayscale

Set pixels below or above the threshold to zero? Below threshold

Subtract the threshold value from the remaining pixel intensities? ☐ Yes ☒ No

Threshold strategy: Global

Thresholding method: Otsu

Two-class or three-class thresholding? Two classes

Minimize the weighted variance or the entropy? Weighted variance

Select the smoothing method for thresholding: No smoothing

Threshold correction factor: 1.0

Lower and upper bounds on threshold: 0.05 1.0

Found 4 image sets

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Module notes

Module settings (ApplyThreshold #08)

Select the input image

c1

(from NamesAndTypes)

?

Name the output image

Thresh_CD3_binary

?

Select the output image type

Binary (black and white)

?

Threshold strategy

Global

?

Thresholding method

Otsu

?

Two-class or three-class thresholding?

Two classes

?

Minimize the weighted variance or the entropy?

Weighted variance

?

Select the smoothing method for thresholding

No smoothing

?

Threshold correction factor

1.0

?

Lower and upper bounds on threshold

0.05

1.0

?

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Analyze Images

Module notes

Module settings (RescaleIntensity #00)

Select the input image

Thresh_CD3

(from ApplyThreshold #07)

Name the output image

ThreshCD3_rescaled

Rescaling method

Stretch each image to use the full intensity range

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Module notes

Module settings (RescaleIntensity #10)

Select the input image

c1

(from NamesAndTypes)

Name the output image

c1_rescaled

Rescaling method

Choose specific values to be reset to the full intensity range

Method to calculate the minimum intensity

Custom

Method to calculate the maximum intensity

Custom

Intensity range for the input image

0.0

0.07

Method to rescale pixels below the lower limit

Mask pixels

Method to rescale pixels above the upper limit

Mask pixels

Found 4 image sets

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Analyze Images

Module notes

Module settings (ImageMath #11)

Operation

Invert

?

Name the output image

Mask

?

Select the first image

Thresh_Nuclei_binary

(from ApplyThreshold #06)

?

Multiply the first image by

1.0

?

Raise the power of the result by

1.0

?

Multiply the result by

1.0

?

Add to result

0.0

?

Set values less than 0 equal to 0?

Yes

No

?

Set values greater than 1 equal to 1?

Yes

No

?

Ignore the image masks?

Yes

No

?

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Analyze Images

Module notes

Module settings (ImageMath #12)

Operation

Invert

?

Name the output image

Mask_CD3

?

Select the first image

Thresh_CD3_binary

(from ApplyThreshold #08)

?

Multiply the first image by

1.0

?

Raise the power of the result by

1.0

?

Multiply the result by

1.0

?

Add to result

0.0

?

Set values less than 0 equal to 0?

Yes

No

?

Set values greater than 1 equal to 1?

Yes

No

?

Ignore the image masks?

Yes

No

?

Found 4 image sets

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Module notes

Module settings (MaskImage #13)

Select the input image: Thresh_Nuclei_for_mask (from ApplyThreshold #05)

Name the output image: Mask_nuclei

Use objects or an image as a mask: image

Select image for mask: Mask (from ImageMath #11)

Invert the mask? ☒ Yes ☐ No

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Module notes

Module settings (MaskImage #14)

Select the input image Thresh_CD3 (from ApplyThreshold #07)

Name the output image Mask_CD3

Use objects or an image as a mask? Image

Select image for mask Mask_CD3 (from ImageMath #12)

Invert the mask? Yes No

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Module notes

Module settings (CorrectilluminationCalculate #15)

Select the input image

Mask_nuclei

(from MaskImage #13)

Name the output image

Thresh_nuc_correction

Select how the illumination function is calculated

Regular

Dilate objects in the final averaged image?

No

Rescale the illumination function?

Yes

Calculate function for each image individually, or based on all images?

Each

Smoothing method

Fit Polynomial

Retain the averaged image?

No

Retain the dilated image?

No

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Module notes

Module settings (CorrectIlluminationCalculate #16)

Select the input imageMask_CD3 (from MaskImage #14)

Name the output imageThresh_CD3_correction

Select how the illumination function is calculatedRegular

Dilate objects in the final averaged image☐ Yes ☒ No

Rescale the illumination function?Yes

Calculate function for each image individually, or based on all images?Each

Smoothing methodFit Polynomial

Retain the averaged image☐ Yes ☒ No

Retain the dilated image☐ Yes ☒ No

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✓ ApplyThreshold

✓ ApplyThreshold

✓ IdentifyPrimaryObjects

✓ ExpandOrShrinkObjects

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ OverlayOutlines

✓ OverlayOutlines

✓ OverlayOutlines

✓ Title

✓ Title

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✓ SaveImages

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Module notes

Module settings (CorrectIlluminationApply #17)

Select the input image: c2 (from NamesAndTypes)

Name the output image: Nuclei_illumCor

Select the illumination function: Thresh_nuc_correction (from CorrectIlluminationCalculate #15)

Select how the illumination function is applied: Divide

Add another image

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✓ MaskImage

✓ MaskImage

✓ CorrectIlluminationCalculate

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✓ ApplyThreshold

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✓ IdentifyPrimaryObjects

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✓ ExportToSpreadsheet

Output

View output settings

Adjust modules:

+

-

^

v

Start Test Mode

Analyze Images

Module notes

Module settings (CorrectIlluminationApply #18)

Select the input image

CD3

(from NamesAndTypes)

Name the output image

CD3_illumCor

Select the illumination function

Thresh_CD3_correction

(from CorrectIlluminationCalculate #16)

Select how the illumination function is applied

Divide

Add another image

Found 4 image sets

Pipeline

Input modules

✓ Images

✓ Metadata

✓ NamesAndTypes

✓ Groups

Analysis modules

✓ ApplyThreshold

✓ ApplyThreshold

✓ ApplyThreshold

✓ ApplyThreshold

✓ RescaleIntensity

✓ RescaleIntensity

✓ ImageMath

✓ ImageMath

✓ MaskImage

✓ MaskImage

✓ CorrectIlluminationCalculate

✓ CorrectIlluminationCalculate

✓ CorrectIlluminationApply

✓ CorrectIlluminationApply

✓ RescaleIntensity

✓ RescaleIntensity

✓ ApplyThreshold

✓ ApplyThreshold

✓ IdentifyPrimaryObjects

✓ ExpandOrShrinkObjects

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ OverlayOutlines

✓ OverlayOutlines

✓ OverlayOutlines

✓ Tile

✓ SaveImages

✓ SaveImages

✓ MeasureObjectSizeShape

✓ MeasureObjectIntensity

✓ ExportToSpreadsheet

Output

View output settings

Adjust modules: + - ^ v

Start Test Mode Analyze Images

Module notes

Module settings (RescaleIntensity #19)

Select the input image Nuclei_illumCor (from CorrectIlluminationApply #17)

Name the output image Nuclei_illumCor_rescaled

Rescaling method Stretch each image to use the full intensity range

Found 4 image sets

Pipeline

Input modules

Images

Metadata

NamesAndTypes

Groups

Analysis modules

ApplyThreshold

ApplyThreshold

ApplyThreshold

ApplyThreshold

RescaleIntensity

RescaleIntensity

ImageMath

ImageMath

MaskImage

MaskImage

CorrectIlluminationCalculate

CorrectIlluminationCalculate

CorrectIlluminationApply

CorrectIlluminationApply

RescaleIntensity

RescaleIntensity

ApplyThreshold

ApplyThreshold

IdentifyPrimaryObjects

ExpandOrShrinkObjects

GrayToColor

GrayToColor

GrayToColor

GrayToColor

GrayToColor

GrayToColor

OverlayOutlines

OverlayOutlines

OverlayOutlines

Tile

Tile

SaveImages

SaveImages

MeasureObjectSizeShape

MeasureObjectIntensity

ExportToSpreadsheet

Output

View output settings

Adjust modules: + - ^ v

Start Test Mode Analyze Images

Module notes

Module settings (RescaleIntensity #20)

Select the input image

CD3_illumCor

(from CorrectIlluminationApply #18)

Name the output image

CD3_illumCor_rescaled

Rescaling method

Stretch each image to use the full intensity range

Found 4 image sets

Pipeline

Input modules

✓ Images

✓ Metadata

✓ NamesAndTypes

✓ Groups

Analysis modules

✓ ApplyThreshold

✓ ApplyThreshold

✓ ApplyThreshold

✓ ApplyThreshold

✓ RescaleIntensity

✓ RescaleIntensity

✓ ImageMath

✓ ImageMath

✓ MaskImage

✓ MaskImage

✓ CorrectIlluminationCalculate

✓ CorrectIlluminationCalculate

✓ CorrectIlluminationApply

✓ CorrectIlluminationApply

✓ RescaleIntensity

✓ RescaleIntensity

✓ ApplyThreshold

✓ ApplyThreshold

✓ IdentifyPrimaryObjects

✓ ExpandOrShrinkObjects

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ OverlayOutlines

✓ OverlayOutlines

✓ OverlayOutlines

✓ Tile

✓ Tile

✓ SaveImages

✓ SaveImages

✓ MeasureObjectSizeShape

✓ MeasureObjectIntensity

✓ ExportToSpreadsheet

Output

View output settings

Adjust modules: + - ^ v

Module notes

Module settings (ApplyThreshold #21)

Select the input image

Nuclei_illumCor_rescaled

(from RescaleIntensity #19)

?

Name the output image

Thresh_nuclei_for_segM

?

Select the output image type

Grayscale

?

Set pixels below or above the threshold to zero?

Below threshold

?

Subtract the threshold value from the remaining pixel intensities?

Yes

No

?

Threshold strategy

Manual

?

Manual threshold

0.1

?

Pipeline

Input modules

✓ Images

✓ Metadata

✓ NamesAndTypes

✓ Groups

Analysis modules

✓ ApplyThreshold

✓ ApplyThreshold

✓ ApplyThreshold

✓ ApplyThreshold

✓ RescaleIntensity

✓ RescaleIntensity

✓ ImageMath

✓ ImageMath

✓ MaskImage

✓ MaskImage

✓ CorrectIlluminationCalculate

✓ CorrectIlluminationCalculate

✓ CorrectIlluminationApply

✓ CorrectIlluminationApply

✓ RescaleIntensity

✓ RescaleIntensity

✓ ApplyThreshold

✓ ApplyThreshold

✓ IdentifyPrimaryObjects

✓ ExpandOrShrinkObjects

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ OverlayOutlines

✓ OverlayOutlines

✓ OverlayOutlines

✓ Tile

✓ Tile

✓ SaveImages

✓ SaveImages

✓ MeasureObjectSizeShape

✓ MeasureObjectIntensity

✓ ExportToSpreadsheet

Output

View output settings

Adjust modules: + - ^ v

Start Test Mode Analyze Images

Module notes

Module settings (ApplyThreshold #22)

Select the input imageCD3_illumCor_rescaled (from RescaleIntensity #20)?

Name the output imageCD3_illumCor_rescaled_thresholded?

Select the output image typeBinary (black and white)?

Threshold strategyGlobal?

Thresholding methodOtsu?

Two-class or three-class thresholding?Two classes?

Minimize the weighted variance or the entropy?Weighted variance?

Select the smoothing method for thresholdingNo smoothing?

Threshold correction factor1.0

Lower and upper bounds on threshold0.1551.0?

Found 4 image sets

Pipeline

Input modules

✓ Images

✓ Metadata

✓ NamesAndTypes

✓ Groups

Analysis modules

✓ ApplyThreshold

✓ ApplyThreshold

✓ ApplyThreshold

✓ ApplyThreshold

✓ RescaleIntensity

✓ RescaleIntensity

✓ ImageMath

✓ ImageMath

✓ MaskImage

✓ MaskImage

✓ CorrectIlluminationCalculate

✓ CorrectIlluminationCalculate

✓ CorrectIlluminationApply

✓ CorrectIlluminationApply

✓ RescaleIntensity

✓ RescaleIntensity

✓ ApplyThreshold

✓ ApplyThreshold

✓ IdentifyPrimaryObjects

✓ ExpandOrShrinkObjects

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ OverlayOutlines

✓ OverlayOutlines

✓ OverlayOutlines

✓ Tile

✓ SaveImages

✓ SaveImages

✓ MeasureObjectSizeShape

✓ MeasureObjectIntensity

✓ ExportToSpreadsheet

Output

View output settings

Adjust modules: + - ^ v

Start Test Mode Analyze Images

Module notes

Module settings (IdentifyPrimaryObjects #23)

Select the input image

Thresh_nuclei_for_segM

(from ApplyThreshold #21)

?

Name the primary objects to be identified

Nuclei

?

Typical diameter of objects, in pixel units (Min,Max)

15

25

?

Discard objects outside the diameter range?

Yes

No

?

Discard objects touching the border of the image?

Yes

No

?

Threshold strategy

Global

?

Thresholding method

RidlerCalvard

?

Select the smoothing method for thresholding

Automatic

?

Threshold correction factor

1.0

?

Lower and upper bounds on threshold

0.1

1.0

?

Method to distinguish clumped objects

Intensity

?

Method to draw dividing lines between clumped objects

Intensity

?

Automatically calculate size of smoothing filter for declumping?

Yes

No

?

Automatically calculate minimum allowed distance between local maxima?

Yes

No

?

Suppress local maxima that are closer than this minimum allowed distance

7.0

?

Speed up by using lower-resolution image to find local maxima?

Yes

No

?

Retain outlines of the identified objects?

Yes

No

?

Fill holes in identified objects?

After declumping only

?

Handling of objects if excessive number of objects identified

Continue

?

Found 4 image sets

Pipeline

- Input modules
- Images
 - Metadata
 - NamesAndTypes
 - Groups

Analysis modules

- ApplyThreshold
- ApplyThreshold
- ApplyThreshold
- ApplyThreshold
- RescaleIntensity
- RescaleIntensity
- ImageMath
- ImageMath
- MaskImage
- MaskImage
- CorrectIlluminationCalculate
- CorrectIlluminationCalculate
- CorrectIlluminationApply
- CorrectIlluminationApply
- RescaleIntensity
- RescaleIntensity
- ApplyThreshold
- ApplyThreshold
- IdentifyPrimaryObjects
- ExpandOrShrinkObjects
- GrayToColor
- GrayToColor
- GrayToColor
- GrayToColor
- GrayToColor
- GrayToColor
- OverlayOutlines
- OverlayOutlines
- OverlayOutlines
- Tile
- Tile
- SaveImages
- SaveImages
- MeasureObjectSizeShape
- MeasureObjectIntensity
- ExportToSpreadsheet

Output

View output settings

Adjust modules: + - ^ v

Start Test Mode Analyze Images

Module notes

Module settings (ExpandOrShrinkObjects #24)

Select the input objects: Nuclei (from IdentifyPrimaryObjects #23)

Name the output objects: integration_contour

Select the operation: Expand objects by a specified number of pixels

Number of pixels by which to expand or shrink: 5

Retain the outlines of the identified objects? Yes No

Found 4 image sets

Pipeline

Input modules

Images

Metadata

NamesAndTypes

Groups

Analysis modules

ApplyThreshold

ApplyThreshold

ApplyThreshold

ApplyThreshold

RescaleIntensity

RescaleIntensity

ImageMath

ImageMath

MaskImage

MaskImage

CorrectIlluminationCalculate

CorrectIlluminationCalculate

CorrectIlluminationApply

CorrectIlluminationApply

RescaleIntensity

RescaleIntensity

ApplyThreshold

ApplyThreshold

IdentifyPrimaryObjects

ExpandOrShrinkObjects

GrayToColor

GrayToColor

GrayToColor

GrayToColor

GrayToColor

GrayToColor

OverlayOutlines

OverlayOutlines

OverlayOutlines

Tile

Tile

SaveImages

SaveImages

MeasureObjectSizeShape

MeasureObjectIntensity

ExportToSpreadsheet

Output

View output settings

Adjust modules

+

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v

Start Test Mode

Analyze Images

Module notes

Module settings (GrayToColor #25)

Select a color scheme

Composite

?

Name the output image

reconstructed_nuclear_staining

?

Image name

Thresh_nuclei_for_segM

(from ApplyThreshold #21)

?

Color

?

Weight

1.0

?

Add another channel

Pipeline

Input modules

✓ Images

✓ Metadata

✓ NamesAndTypes

✓ Groups

Analysis modules

✓ ApplyThreshold

✓ ApplyThreshold

✓ ApplyThreshold

✓ ApplyThreshold

✓ RescaleIntensity

✓ RescaleIntensity

✓ ImageMath

✓ ImageMath

✓ MaskImage

✓ MaskImage

✓ CorrectIlluminationCalculate

✓ CorrectIlluminationCalculate

✓ CorrectIlluminationApply

✓ CorrectIlluminationApply

✓ RescaleIntensity

✓ RescaleIntensity

✓ ApplyThreshold

✓ ApplyThreshold

✓ IdentifyPrimaryObjects

✓ ExpandOrShrinkObjects

✓ GrayToColor

✓ **GrayToColor**

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ OverlayOutlines

✓ OverlayOutlines

✓ OverlayOutlines

✓ Title

✓ Title

✓ SaveImages

✓ SaveImages

✓ MeasureObjectSizeShape

✓ MeasureObjectIntensity

✓ ExportToSpreadsheet

Output

View output settings

Adjust modules: + - ^ v

Start Test Mode Analyze Images

Module notes

Module settings (GrayToColor #26)

Select a color scheme Composite

Name the output image reconstructed_threshCD3_staining

Image name Thresh_CD3 (from ApplyThreshold #07)

Color

Weight 3

Add another channel

Found 4 image sets

Pipeline

Input modules

✓ Images

✓ Metadata

✓ NamesAndTypes

✓ Groups

Analysis modules

✓ ApplyThreshold

✓ ApplyThreshold

✓ ApplyThreshold

✓ ApplyThreshold

✓ RescaleIntensity

✓ RescaleIntensity

✓ ImageMath

✓ ImageMath

✓ MaskImage

✓ MaskImage

✓ CorrectIlluminationCalculate

✓ CorrectIlluminationCalculate

✓ CorrectIlluminationApply

✓ CorrectIlluminationApply

✓ RescaleIntensity

✓ RescaleIntensity

✓ ApplyThreshold

✓ ApplyThreshold

✓ IdentifyPrimaryObjects

✓ ExpandOrShrinkObjects

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ OverlayOutlines

✓ OverlayOutlines

✓ OverlayOutlines

✓ Title

✓ Tile

✓ SaveImages

✓ SaveImages

✓ MeasureObjectSizeShape

✓ MeasureObjectIntensity

✓ ExportToSpreadsheet

Output

View output settings

Adjust modules: + - ^ v

Module notes

Module settings (GrayToColor #27)

Select a color scheme Composite ?

Name the output image reconstructed_threshCD3_RESCALED

Image name ThreshCD3_rescaled (from RescaleIntensity #09) ?

Color ?

Weight 3 ?

Add another channel

Found 4 image sets

Pipeline

Input modules

✓ Images

✓ Metadata

✓ NamesAndTypes

✓ Groups

Analysis modules

✓ ApplyThreshold

✓ ApplyThreshold

✓ ApplyThreshold

✓ ApplyThreshold

✓ RescaleIntensity

✓ RescaleIntensity

✓ ImageMath

✓ ImageMath

✓ MaskImage

✓ MaskImage

✓ CorrectIlluminationCalculate

✓ CorrectIlluminationCalculate

✓ CorrectIlluminationApply

✓ CorrectIlluminationApply

✓ RescaleIntensity

✓ RescaleIntensity

✓ ApplyThreshold

✓ ApplyThreshold

✓ IdentifyPrimaryObjects

✓ ExpandOrShrinkObjects

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ OverlayOutlines

✓ OverlayOutlines

✓ OverlayOutlines

✓ Tile

✓ Tile

✓ SaveImages

✓ SaveImages

✓ MeasureObjectSizeShape

✓ MeasureObjectIntensity

✓ ExportToSpreadsheet

Output

View output settings

Adjust modules: + - ^ v

Start Test Mode Analyze Images

Module notes

Module settings (GrayToColor #28)

Select a color scheme Composite ?

Name the output image reconstructed_threshCD3_ILLUMCOR_RESCALED

Image name CD3_illumCor_rescaled_threshholded (from ApplyThreshold #22) ?

Color ?

Weight 1 ?

Add another channel

Found 4 image sets

Pipeline

Input modules

Images

Metadata

NamesAndTypes

Groups

Analysis modules

ApplyThreshold

ApplyThreshold

ApplyThreshold

RescaleIntensity

RescaleIntensity

ImageMath

ImageMath

MaskImage

MaskImage

CorrectIlluminationCalculate

CorrectIlluminationCalculate

CorrectIlluminationApply

CorrectIlluminationApply

RescaleIntensity

RescaleIntensity

ApplyThreshold

ApplyThreshold

IdentifyPrimaryObjects

ExpandOrShrinkObjects

GrayToColor

GrayToColor

GrayToColor

GrayToColor

GrayToColor

OverlayOutlines

OverlayOutlines

OverlayOutlines

OverlayOutlines

Tile

Tile

SaveImages

SaveImages

MeasureObjectSizeShape

MeasureObjectIntensity

ExportToSpreadsheet

Output

View output settings

Adjust modules: + - ^ v

Start Test Mode

Analyze Images

Module notes

Module settings (GrayToColor #29)

Select a color scheme Composite ?

Name the output image reconstructed_Nuclear_ThreshCD3_staining

Image name Thresh_nuclei_for_segM (from ApplyThreshold #21) ?

Color ?

Weight 1.0 ?

Image name ThreshCD3_rescaled (from RescaleIntensity #09) ?

Color ?

Weight 1.0 ?

Remove this image

Add another channel

Found 4 image sets

Pipeline

Input modules

Images

Metadata

NamesAndTypes

Groups

Analysis modules

ApplyThreshold

ApplyThreshold

ApplyThreshold

ApplyThreshold

RescaleIntensity

RescaleIntensity

ImageMath

ImageMath

MaskImage

MaskImage

CorrectIlluminationCalculate

CorrectIlluminationCalculate

CorrectIlluminationApply

CorrectIlluminationApply

RescaleIntensity

RescaleIntensity

ApplyThreshold

ApplyThreshold

IdentifyPrimaryObjects

ExpandOrShrinkObjects

GrayToColor

GrayToColor

GrayToColor

GrayToColor

GrayToColor

GrayToColor

OverlayOutlines

OverlayOutlines

OverlayOutlines

Tile

Tile

SaveImages

SaveImages

MeasureObjectSizeShape

MeasureObjectIntensity

ExportToSpreadsheet

Output

View output settings

Adjust modules: + - ^ v

Module notes

Module settings (GrayToColor #30)

Select a color scheme Composite

Name the output image reconstructed_raw_staining

Image name c2 (from NamesAndTypes)

Color

Weight 1.0

Image name c1 (from NamesAndTypes)

Color

Weight 1.0

Remove this image

Add another channel

Pipeline

Input modules

✓ Images

✓ Metadata

✓ NamesAndTypes

✓ Groups

Analysis modules

✓ ApplyThreshold

✓ ApplyThreshold

✓ ApplyThreshold

✓ ApplyThreshold

✓ RescaleIntensity

✓ RescaleIntensity

✓ ImageMath

✓ ImageMath

✓ MaskImage

✓ MaskImage

✓ CorrectIlluminationCalculate

✓ CorrectIlluminationCalculate

✓ CorrectIlluminationApply

✓ CorrectIlluminationApply

✓ RescaleIntensity

✓ RescaleIntensity

✓ ApplyThreshold

✓ ApplyThreshold

✓ IdentifyPrimaryObjects

✓ ExpandOrShrinkObjects

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ OverlayOutlines

✓ OverlayOutlines

✓ OverlayOutlines

✓ OverlayOutlines

✓ Title

✓ SaveImages

✓ SaveImages

✓ MeasureObjectSizeShape

✓ MeasureObjectIntensity

✓ ExportToSpreadsheet

Output

View output settings

Adjust modules: + - ^ v

Start Test Mode Analyze Images

Module notes

Module settings (OverlayOutlines #31)

Display outlines on a blank image? ☐ Yes ☒ No

Select image on which to display outlines reconstructed_nuclear_staining (from GrayToColor #25)

Name the output image overlay_nuclei_segM

Outline display mode Color

Width of outlines 1

Load outlines from an image or objects? Objects

Select outline color

Select objects to display Nuclei (from IdentifyPrimaryObjects #23)

Add another outline

Found 4 image sets

Pipeline

Input modules

✓ Images

✓ Metadata

✓ NamesAndTypes

✓ Groups

Analysis modules

✓ ApplyThreshold

✓ ApplyThreshold

✓ ApplyThreshold

✓ ApplyThreshold

✓ RescaleIntensity

✓ RescaleIntensity

✓ ImageMath

✓ ImageMath

✓ MaskImage

✓ MaskImage

✓ CorrectIlluminationCalculate

✓ CorrectIlluminationCalculate

✓ CorrectIlluminationApply

✓ CorrectIlluminationApply

✓ RescaleIntensity

✓ RescaleIntensity

✓ ApplyThreshold

✓ ApplyThreshold

✓ IdentifyPrimaryObjects

✓ ExpandOrShrinkObjects

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ OverlayOutlines

✓ OverlayOutlines

✓ OverlayOutlines

✓ Tile

✓ Tile

✓ SaveImages

✓ SaveImages

✓ MeasureObjectSizeShape

✓ MeasureObjectIntensity

✓ ExportToSpreadsheet

Output

View output settings

Adjust modules: + - ^ v

Start Test Mode Analyze Images

Module notes

Module settings: (OverlayOutlines #32)

Display outlines on a blank image? ☐ Yes ☒ No

Select image on which to display outlines reconstructed_threshCD3_staining (from GrayToColor #26)

Name the output image overlay_threshCD3_SegM

Outline display mode Color

Width of outlines 1

Load outlines from an image or objects? Objects

Select outline color

Select objects to display integration_contour (from ExpandOrShrinkObjects #24)

Add another outline

Found 4 image sets

Pipeline

Input modules

Images

Metadata

NamesAndTypes

Groups

Analysis modules

ApplyThreshold

ApplyThreshold

ApplyThreshold

ApplyThreshold

RescaleIntensity

RescaleIntensity

ImageMath

ImageMath

MaskImage

MaskImage

CorrectIlluminationCalculate

CorrectIlluminationCalculate

CorrectIlluminationApply

CorrectIlluminationApply

RescaleIntensity

RescaleIntensity

ApplyThreshold

ApplyThreshold

IdentifyPrimaryObjects

ExpandOrShrinkObjects

GrayToColor

GrayToColor

GrayToColor

GrayToColor

GrayToColor

GrayToColor

OverlayOutlines

OverlayOutlines

Title

Title

SaveImages

SaveImages

MeasureObjectSizeShape

MeasureObjectIntensity

ExportToSpreadsheet

Output

View output settings

Adjust modules: + - ^ v

Start Test Mode

Analyze Images

Module notes

Module settings (OverlayOutlines #33)

Display outlines on a blank image? ☐ Yes ☒ No

Select image on which to display outlines reconstructed_Nuclear_ThreshCD3_staining (from GrayToColor #29)

Name the output image overlay_threshstaining_SegM

Outline display mode Color

Width of outlines 1

Load outlines from an image or objects? Objects

Select outline color

Select objects to display integration_contour (from ExpandOrShrinkObjects #24)

Add another outline

Found 4 image sets

Pipeline

Input modules

Images

Metadata

NamesAndTypes

Groups

Analysis modules

ApplyThreshold

ApplyThreshold

ApplyThreshold

ApplyThreshold

RescaleIntensity

RescaleIntensity

ImageMath

ImageMath

MaskImage

MaskImage

CorrectIlluminationCalculate

CorrectIlluminationCalculate

CorrectIlluminationApply

RescaleIntensity

RescaleIntensity

ApplyThreshold

ApplyThreshold

IdentifyPrimaryObjects

ExpandOrShrinkObjects

GrayToColor

GrayToColor

GrayToColor

GrayToColor

GrayToColor

OverlayOutlines

OverlayOutlines

OverlayOutlines

Tile

SaveImages

SaveImages

MeasureObjectSizeShape

MeasureObjectIntensity

ExportToSpreadsheet

Output

View output settings

Adjust modules: + - ^ v

Start Test Mode

Analyze Images

Module notes

Module settings (Tile #34)

Select an input image

reconstructed_Nuclear_ThreshCD3_staining

(from GrayToColor #29)

?

Name the output image

TiledImage

?

Tile assembly method

Within cycles

?

Automatically calculate number of rows?

Yes

No

?

Final number of rows

1

?

Automatically calculate number of columns?

Yes

No

?

Final number of columns

3

?

Image corner to begin tiling

top left

?

Direction to begin tiling

row

?

Use meander mode?

Yes

No

?

Select an additional image to tile

overlay_threshstaining_SegM

(from OverlayOutlines #33)

?

Remove above image

Select an additional image to tile

overlay_threshCD3_SegM

(from OverlayOutlines #32)

?

Remove above image

Add another image

Found 4 image sets

Pipeline

Input modules

Images

Metadata

NamesAndTypes

Groups

Analysis modules

ApplyThreshold

ApplyThreshold

ApplyThreshold

ApplyThreshold

RescaleIntensity

RescaleIntensity

ImageMath

ImageMath

MaskImage

MaskImage

CorrectIlluminationCalculate

CorrectIlluminationCalculate

CorrectIlluminationApply

CorrectIlluminationApply

RescaleIntensity

RescaleIntensity

ApplyThreshold

ApplyThreshold

IdentifyPrimaryObjects

ExpandOrShrinkObjects

GrayToColor

GrayToColor

GrayToColor

GrayToColor

GrayToColor

OverlayOutlines

OverlayOutlines

OverlayOutlines

Tile

SaveImages

SaveImages

MeasureObjectSizeShape

MeasureObjectIntensity

ExportToSpreadsheet

Output

View output settings

Adjust modules: + - ^ v

Start Test Mode

Analyze Images

Module notes

Module settings (Tile #35)

Select an input image

c1_rescaled

(from RescaleIntensity #10)

Name the output image

CD3_rescaling_tile

Tile assembly method

Within cycles

Automatically calculate number of rows?

Yes

No

Final number of rows

2

Automatically calculate number of columns?

Yes

No

Final number of columns

2

Image corner to begin tiling

top left

Direction to begin tiling

row

Use meander mode?

Yes

No

Select an additional image to tile

reconstructed_threshCD3_staining

(from GrayToColor #26)

Remove above image

Select an additional image to tile

reconstructed_threshCD3_RESCALED

(from GrayToColor #27)

Remove above image

Select an additional image to tile

CD3_illumCor_rescaled_thresholded

(from ApplyThreshold #22)

Tile

Remove above image

Add another image

Found 4 image sets

Pipeline

Input modules

Images

Metadata

NamesAndTypes

Groups

Analysis modules

ApplyThreshold

ApplyThreshold

ApplyThreshold

ApplyThreshold

RescaleIntensity

RescaleIntensity

ImageMath

ImageMath

MaskImage

MaskImage

CorrectIlluminationCalculate

CorrectIlluminationCalculate

CorrectIlluminationApply

CorrectIlluminationApply

RescaleIntensity

ApplyThreshold

ApplyThreshold

IdentifyPrimaryObjects

ExpandOrShrinkObjects

GrayToColor

GrayToColor

GrayToColor

GrayToColor

GrayToColor

GrayToColor

OverlayOutlines

OverlayOutlines

OverlayOutlines

OverlayOutlines

Tile

Tile

SaveImages

SaveImages

MeasureObjectSizeShape

MeasureObjectIntensity

ExportToSpreadsheet

Output

View output settings

Adjust modules: + - ^ v

Start Test Mode

Analyze Images

Module notes

Module settings (SaveImages #36)

Select the type of image to save

image

?

Select the image to save

TiledImage

(from Tile #34)

?

Select method for constructing file names

Sequential numbers

?

Enter file prefix

analysis_tile_

?

Number of digits

3

?

Saved file format

jpg

?

Output file location

Default Output Folder sub-folder

(d:\cellprofiler data)

?

Sub-folder: Analysis_Tile

?

Overwrite existing files without warning?

Yes

No

?

When to save

Every cycle

?

Rescale the images?

Yes

No

?

Record the file and path information to the saved image?

Yes

No

?

Found 4 image sets

Pipeline

Input modules

- Images
- Metadata
- NamesAndTypes
- Groups

Analysis modules

- ApplyThreshold
- ApplyThreshold
- ApplyThreshold
- ApplyThreshold
- RescaleIntensity
- RescaleIntensity
- ImageMath
- ImageMath
- MaskImage
- MaskImage
- CorrectIlluminationCalculate
- CorrectIlluminationCalculate
- CorrectIlluminationApply
- CorrectIlluminationApply
- RescaleIntensity
- RescaleIntensity
- ApplyThreshold
- ApplyThreshold
- IdentifyPrimaryObjects
- ExpandOrShrinkObjects
- GrayToColor
- GrayToColor
- GrayToColor
- GrayToColor
- GrayToColor
- GrayToColor
- OverlayOutlines
- OverlayOutlines
- OverlayOutlines
- OverlayOutlines
- Tile
- Tile
- SaveImages
- SaveImages
- MeasureObjectSizeShape
- MeasureObjectIntensity
- ExportToSpreadsheet

Output

View output settings

Adjust modules: + - ^ v

Start Test Mode Analyze Images

Module notes

Module settings (SaveImages #37)

Select the type of image to save Image

Select the image to save CD3_rescaling_tile (from Tile #35)

Select method for constructing file names Sequential numbers

Enter file prefix C1_CD3_KCD3rs_KCD3lcrs_tile_

Number of digits 3

Saved file format jpg

Output file location Default Output Folder sub-folder (d:\cellprofiler data)

Sub-folder CD3_Rescaling_Tile

Overwrite existing files without warning? Yes No

When to save Every cycle

Rescale the images? Yes No

Record the file and path information to the saved image? Yes No

Pipeline

Input modules

✓ Images

✓ Metadata

✓ NamesAndTypes

✓ Groups

Analysis modules

✓ ApplyThreshold

✓ ApplyThreshold

✓ ApplyThreshold

✓ ApplyThreshold

✓ RescaleIntensity

✓ RescaleIntensity

✓ ImageMath

✓ ImageMath

✓ MaskImage

✓ MaskImage

✓ CorrectIlluminationCalculate

✓ CorrectIlluminationCalculate

✓ CorrectIlluminationApply

✓ CorrectIlluminationApply

✓ RescaleIntensity

✓ RescaleIntensity

✓ ApplyThreshold

✓ ApplyThreshold

✓ IdentifyPrimaryObjects

✓ ExpandOrShrinkObjects

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ OverlayOutlines

✓ OverlayOutlines

✓ OverlayOutlines

✓ Title

✓ Title

✓ SaveImages

✓ SaveImages

✓ MeasureObjectSizeShape

✓ MeasureObjectIntensity

✓ ExportToSpreadsheet

Output

View output settings

Adjust modules: + - ^ v

Start Test Mode Analyze Images

Module notes

Module settings (MeasureObjectSizeShape #38)

Select objects to measure

Nuclei

(from IdentifyPrimaryObjects #23)

?

Select objects to measure

integration_contour

(from ExpandOrShrinkObjects #24)

Remove this object

Add another object

?

Calculate the Zernike features?

☐ Yes

☒ No

?

Found 4 image sets

Pipeline

Input modules

✓ Images

✓ Metadata

✓ NamesAndTypes

✓ Groups

Analysis modules

✓ ApplyThreshold

✓ ApplyThreshold

✓ ApplyThreshold

✓ ApplyThreshold

✓ RescaleIntensity

✓ RescaleIntensity

✓ ImageMath

✓ ImageMath

✓ MaskImage

✓ MaskImage

✓ CorrectIlluminationCalculate

✓ CorrectIlluminationCalculate

✓ CorrectIlluminationApply

✓ CorrectIlluminationApply

✓ RescaleIntensity

✓ RescaleIntensity

✓ ApplyThreshold

✓ ApplyThreshold

✓ IdentifyPrimaryObjects

✓ ExpandOrShrinkObjects

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ GrayToColor

✓ OverlayOutlines

✓ OverlayOutlines

✓ OverlayOutlines

✓ Title

✓ Title

✓ SaveImages

✓ SaveImages

✓ MeasureObjectSizeShape

✓ MeasureObjectIntensity

✓ ExportToSpreadsheet

Output

View output settings

?

Adjust modules: + - ^ v

▶ Start Test Mode

▶ Analyze Images

Module notes

Module settings (MeasureObjectIntensity #39)

Select an image to measure

c1

(from NamesAndTypes)

?

Select an image to measure

Thresh_CD3

(from ApplyThreshold #07)

?

Remove this image

Select an image to measure

ThreshCD3_rescaled

(from RescaleIntensity #09)

?

Remove this image

Select an image to measure

CD3_illumCor_rescaled_thresholded

(from ApplyThreshold #22)

?

Remove this image

Add another image

Select objects to measure

integration_contour

(from ExpandOrShrinkObjects #24)

?

Add another object

Found 4 image sets

Pipeline

- Input modules
- Images
 - Metadata
 - NamesAndTypes
 - Groups

Analysis modules

- ApplyThreshold
- ApplyThreshold
- ApplyThreshold
- ApplyThreshold
- RescaleIntensity
- RescaleIntensity
- ImageMath
- ImageMath
- MaskImage
- MaskImage
- CorrectIlluminationCalculate
- CorrectIlluminationCalculate
- CorrectIlluminationApply
- CorrectIlluminationApply
- RescaleIntensity
- RescaleIntensity
- ApplyThreshold
- ApplyThreshold
- IdentifyPrimaryObjects
- ExpandOrShrinkObjects
- GrayToColor
- GrayToColor
- GrayToColor
- GrayToColor
- GrayToColor
- OverlayOutlines
- OverlayOutlines
- OverlayOutlines
- Tile
- Tile
- SaveImages
- SaveImages
- MeasureObjectSizeShape
- MeasureObjectIntensity
- ExportToSpreadsheet

Output

View output settings

Adjust modules: + - A V

Start Test Mode Analyze Images

Module notes

Module settings (ExportToSpreadsheet #40)

Select the column delimiter: Tab

Output file location: Default Output Folder (d:\cellprofiler data)

Add a prefix to file names? ☒ Yes ☐ No

Filename prefix: REH_T_Sheep_

Overwrite existing files without warning? ☐ Yes ☒ No

Add image metadata columns to your object data file? ☐ Yes ☒ No

Limit output to a size that is allowed in Excel? ☐ Yes ☒ No

Representation of Nan/Inf: NaN

Select the measurements to export: ☐ Yes ☒ No

Calculate the per-image mean values for object measurements? ☐ Yes ☒ No

Calculate the per-image median values for object measurements? ☐ Yes ☒ No

Calculate the per-image standard deviation values for object measurements? ☐ Yes ☒ No

Create a GenePattern GCT file? ☐ Yes ☒ No

Export all measurement types? ☒ Yes ☐ No

Found 4 image sets