

Images

Metadata

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MeasureObjectSizeShape

MeasureObjectIntensity

FilterObjects

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SaveImages

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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.

▼ /Users/Proikas-CezanneLab/Desktop/Carmen /E38_JoVe manuscript experiment/E38_JoVe journal experiment

20221124_CJPM_E38_488_Ferritin_546_LAMP2_Set1_F-CO-60_JoVe_40xair_testrun_deffocus-softautofocus.czi

✓ Show files excluded by filters

Filter images? Images only

Apply filters to the file list

Apply filters to the file list

?

?

?

Output SettingsView Workspace

?

Adjust modules:

+

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Start Test Mode

Analyze Images

Found 162 rows

- Images

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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.

Extract metadata?

Yes

No

Metadata extraction method

Extract from image file headers

Extract metadata from

All images

Extract metadata

Metadata extraction method

Import from file

Metadata file location

Default Input Folder

(/Users/heptapus1)

Metadata file name

JoVe_Metadata copy.csv

Extract metadata from

All images

Match file and image metadata

CSV Metadata

Image Metadata

Series

Series

+

Use case insensitive matching?

Yes

No

Remove this extraction method

Add another extraction method

Metadata data type

Text

Update	Path / URL	Series	Frame	C	ChannelName	ColorFormat	FileLocation	SizeC	SizeT	SizeX	SizeY	SizeZ	T	Z
1	/Users/Proikz	0	0	0	AF488-T1	monochrome	file:/Users/P.	3	1	1404	1536	1	0	0
2	/Users/Proikz	0	1	1	DAPI-T2	monochrome	file:/Users/P.	3	1	1404	1536	1	0	0
3	/Users/Proikz	0	2	2	AF546-T3	monochrome	file:/Users/P.	3	1	1404	1536	1	0	0
4	/Users/Proikz	1	0	0	AF488-T1	monochrome	file:/Users/P.	3	1	1404	1536	1	0	0
5	/Users/Proikz	1	1	1	DAPI-T2	monochrome	file:/Users/P.	3	1	1404	1536	1	0	0
6	/Users/Proikz	1	2	2	AF546-T3	monochrome	file:/Users/P.	3	1	1404	1536	1	0	0
7	/Users/Proikz	2	0	0	AF488-T1	monochrome	file:/Users/P.	3	1	1404	1536	1	0	0
8	/Users/Proikz	2	1	1	DAPI-T2	monochrome	file:/Users/P.	3	1	1404	1536	1	0	0
9	/Users/Proikz	2	2	2	AF546-T3	monochrome	file:/Users/P.	3	1	1404	1536	1	0	0
10	/Users/Proikz	3	0	0	AF488-T1	monochrome	file:/Users/P.	3	1	1404	1536	1	0	0
11	/Users/Proikz	3	1	1	DAPI-T2	monochrome	file:/Users/P.	3	1	1404	1536	1	0	0
12	/Users/Proikz	3	2	2	AF546-T3	monochrome	file:/Users/P.	3	1	1404	1536	1	0	0
13	/Users/Proikz	4	0	0	AF488-T1	monochrome	file:/Users/P.	3	1	1404	1536	1	0	0
14	/Users/Proikz	4	1	1	DAPI-T2	monochrome	file:/Users/P.	3	1	1404	1536	1	0	0
15	/Users/Proikz	4	2	2	AF546-T3	monochrome	file:/Users/P.	3	1	1404	1536	1	0	0
16	/Users/Proikz	5	0	0	AF488-T1	monochrome	file:/Users/P.	3	1	1404	1536	1	0	0
17	/Users/Proikz	5	1	1	DAPI-T2	monochrome	file:/Users/P.	3	1	1404	1536	1	0	0
18	/Users/Proikz	5	2	2	AF546-T3	monochrome	file:/Users/P.	3	1	1404	1536	1	0	0
19	/Users/Proikz	6	0	0	AF488-T1	monochrome	file:/Users/P.	3	1	1404	1536	1	0	0
20	/Users/Proikz	6	1	1	DAPI-T2	monochrome	file:/Users/P.	3	1	1404	1536	1	0	0
21	/Users/Proikz	6	2	2	AF546-T3	monochrome	file:/Users/P.	3	1	1404	1536	1	0	0
22	/Users/Proikz	7	0	0	AF488-T1	monochrome	file:/Users/P.	3	1	1404	1536	1	0	0
23	/Users/Proikz	7	1	1	DAPI-T2	monochrome	file:/Users/P.	3	1	1404	1536	1	0	0
24	/Users/Proikz	7	2	2	AF546-T3	monochrome	file:/Users/P.	3	1	1404	1536	1	0	0
25	/Users/Proikz	8	0	0	AF488-T1	monochrome	file:/Users/P.	3	1	1404	1536	1	0	0
26	/Users/Proikz	8	1	1	DAPI-T2	monochrome	file:/Users/P.	3	1	1404	1536	1	0	0

- IdentifyPrimaryObjects
- IdentifySecondaryObjects
- MeasureObjectSizeShape
- MeasureObjectIntensity
- FilterObjects
- IdentifyPrimaryObjects
- IdentifyPrimaryObjects
- RelateObjects
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- SaveImages
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[illegible]

- Images
- Metadata
- NamesAndTypes
- Groups

- IdentifyPrimaryObjects
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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.

Do you want to group your images?

☐ Yes ☒ No

?

Output Settings View Workspace

? Adjust modules: + - ^ v

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Adjust modules:

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▶ Start Test Mode

▶ Analyze Images

Found 162 rows

Use advanced settings?

Yes

No

Select the input image

DAPI

(from NamesAndTypes)

Name the primary objects to be identified

Nuclei

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

100

400

Discard objects outside the diameter range?

Yes

No

Discard objects touching the border of the image?

Yes

No

Threshold strategy

Global

Thresholding method

Otsu

Two-class or three-class thresholding?

Two classes

Threshold smoothing scale

1

Threshold correction factor

0.7

Lower and upper bounds on threshold

0

1.0

Log transform before thresholding?

Yes

No

Method to distinguish clumped objects

None

Fill holes in identified objects?

After both thresholding and declumping

Handling of objects if excessive number of objects identified

Continue

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Start Test Mode

Analyze Images

Select the input image

Ferritin

(from NamesAndTypes)

?

Select the input objects

Nuclei

(from IdentifyPrimaryObjects #05)

?

Name the objects to be identified

Cells

?

Select the method to identify the secondary objects

Watershed - Image

?

Threshold strategy

Global

?

Thresholding method

Otsu

?

Two-class or three-class thresholding?

Three classes

?

Assign pixels in the middle intensity class to the foreground or the background?

Background

?

Threshold smoothing scale

1.5

?

Threshold correction factor

0.7

?

Lower and upper bounds on threshold

0

1.0

?

Log transform before thresholding?

Yes

No

?

Fill holes in identified objects?

Yes

No

?

Discard secondary objects touching the border of the image?

Yes

No

?

Found 162 rows

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Cells

(from IdentifySecondaryObjects #06)

Nuclei

(from IdentifyPrimaryObjects #05)

?

Select object sets to measure

Calculate the Zernike features?

Yes

No

?

Calculate the advanced features?

Yes

No

?

Output Settings

View Workspace

?

Adjust modules:

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Start Test Mode

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Select images to measure

☐

DAPI

(from NamesAndTypes)

☒

Ferritin

(from NamesAndTypes)

☐

LAMP2

(from NamesAndTypes)

?

Select objects to measure

☒

Cells

(from IdentifySecondaryObjects #06)

☐

Nuclei

(from IdentifyPrimaryObjects #05)

?

Output Settings

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?

Adjust modules:

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▶ Start Test Mode

▶ Analyze Images

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Start Test Mode

Analyze Images

This module filters out abnormal cells.

Select the objects to filter

Cells

(from IdentifySecondaryObjects #06)

Name the output objects

NormalCells

Select the filtering mode

Measurements

Select the filtering method

Limits

Select the measurement to filter by

Category: AreaShape

Measurement: Area

Filter using a minimum measurement value?

Yes

No

Minimum value

400

Filter using a maximum measurement value?

Yes

No

Select the measurement to filter by

Category: Intensity

Measurement: UpperQuartileIntensi

Image: Ferritin

Filter using a minimum measurement value?

Yes

No

Filter using a maximum measurement value?

Yes

No

Maximum value

1.6

Remove this measurement

Select the measurement to filter by

Category: Intensity

Measurement: MeanIntensity

Image: Ferritin

Filter using a minimum measurement value?

Yes

No

Minimum value

0.03

Filter using a maximum measurement value?

Yes

No

Maximum value

1

Remove this measurement

Select the measurement to filter by

Category: Intensity

Measurement: LowerQuartileIntensi

Image: Ferritin

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Use advanced settings?

Yes

No

Select the input image

Ferritin

(from NamesAndTypes)

Name the primary objects to be identified

FerritinPuncta

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

5

60

Discard objects outside the diameter range?

Yes

No

Discard objects touching the border of the image?

Yes

No

Threshold strategy

Adaptive

Thresholding method

Otsu

Two-class or three-class thresholding?

Three classes

Assign pixels in the middle intensity class to the foreground or the background?

Background

Threshold smoothing scale

1

Threshold correction factor

1.35

Lower and upper bounds on threshold

0.1

1

Size of adaptive window

25

Log transform before thresholding?

Yes

No

Method to distinguish clumped objects

Intensity

Method to draw dividing lines between clumped objects

Shape

Automatically calculate size of smoothing filter for declumping?

Yes

No

Automatically calculate minimum allowed distance between local maxima?

Yes

No

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

Yes

No

Display accepted local maxima?

Yes

No

Fill holes in identified objects?

Never

Handling of objects if excessive number of objects identified

Continue

Images

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MeasureObjectIntensity

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RelateObjects

RelateObjects

OverlayOutlines

SavelImages

DisplayDataOnImage

SavelImages

ExportToSpreadsheet

Output Settings

View Workspace

?

Adjust modules:

+

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v

Start Test Mode

Analyze Images

Found 162 rows

Use advanced settings?

YesNo

Select the input image

LAMP2

(from NamesAndTypes)

Name the primary objects to be identified

LAMP2Puncta

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

4

50

Discard objects outside the diameter range?

YesNo

Discard objects touching the border of the image?

YesNo

Threshold strategy

Adaptive

Thresholding method

Otsu

Two-class or three-class thresholding?

Three classes

Assign pixels in the middle intensity class to the foreground or the background?

Background

Threshold smoothing scale

1

Threshold correction factor

1.3

Lower and upper bounds on threshold

0

1

Size of adaptive window

40

Log transform before thresholding?

YesNo

Method to distinguish clumped objects

Intensity

Method to draw dividing lines between clumped objects

Propagate

Automatically calculate size of smoothing filter for declumping?

YesNo

Automatically calculate minimum allowed distance between local maxima?

YesNo

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

YesNo

Display accepted local maxima?

YesNo

Fill holes in identified objects?

After both thresholding and declumping

Handling of objects if excessive number of objects identified

Continue

Images

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RelateObjects

OverlayOutlines

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SaveImages

ExportToSpreadsheet



Parent objects

NormalCells

(from FilterObjects #09)

?

Child objects

FerritinPuncta

(from IdentifyPrimaryObjects #10)

?

Calculate per-parent means for all child measurements?

Yes

No

?

Calculate child-parent distances?

None

?

Do you want to save the children with parents as a new object set?

Yes

No

?

Name the output object

CellularFerritinPuncta

?

Images

Metadata

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SaveImages

ExportToSpreadsheet



Parent objects

NormalCells

(from FilterObjects #09)

?

Child objects

LAMP2Puncta

(from IdentifyPrimaryObjects #11)

?

Calculate per-parent means for all child measurements?

Yes

No

?

Calculate child-parent distances?

None

?

Do you want to save the children with parents as a new object set?

Yes

No

?

Name the output object

CellularLAMP2Puncta

?

Images

Metadata

NamesAndTypes

Groups

IdentifyPrimaryObjects

IdentifySecondaryObjects

MeasureObjectSizeShape

MeasureObjectIntensity

FilterObjects

IdentifyPrimaryObjects

IdentifyPrimaryObjects

RelateObjects

RelateObjects

MeasureObjectSizeShape

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RelateObjects

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ExportToSpreadsheet



Select object sets to measure

☐

Cells

(from IdentifySecondaryObjects #06)

☒

CellularFerritinPuncta

(from RelateObjects #12)

☒

CellularLAMP2Puncta

(from RelateObjects #13)

☐

FerritinPuncta

(from IdentifyPrimaryObjects #10)

☐

LAMP2Puncta

(from IdentifyPrimaryObjects #11)

☐

NormalCells

(from FilterObjects #09)

?

Calculate the Zernike features?

☒ Yes

☐ No

?

Calculate the advanced features?

☐ Yes

☒ No

?

Images

Metadata

NamesAndTypes

Groups

IdentifyPrimaryObjects

IdentifySecondaryObjects

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RelateObjects

MeasureObjectSizeShape

RelateObjects

RelateObjects

OverlayOutlines

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SaveImages

ExportToSpreadsheet



Parent objects

CellularFerritinPuncta

(from RelateObjects #12)

?

Child objects

CellularLAMP2Puncta

(from RelateObjects #13)

?

Calculate per-parent means for all child measurements?

Yes

No

?

Calculate child-parent distances?

None

?

Do you want to save the children with parents as a new object set?

Yes

No

?

Name the output object

ColocFerritinLAMPPuncta

?

Images

Metadata

NamesAndTypes

Groups

IdentifyPrimaryObjects

IdentifySecondaryObjects

MeasureObjectSizeShape

MeasureObjectIntensity

FilterObjects

IdentifyPrimaryObjects

IdentifyPrimaryObjects

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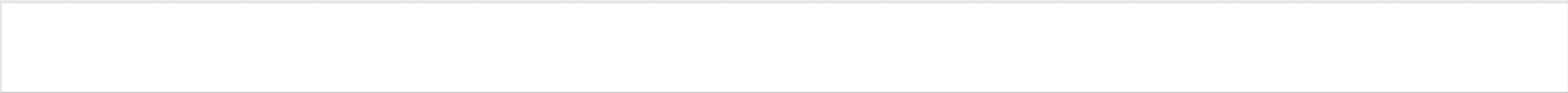
OverlayOutlines

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Parent objects

NormalCells

(from FilterObjects #09)

?

Child objects

ColocFerritinLAMPPuncta

(from RelateObjects #15)

?

Calculate per-parent means for all child measurements?

Yes

No

?

Calculate child-parent distances?

None

?

Do you want to save the children with parents as a new object set?

Yes

No

?

Name the output object

CellularFerritinLAMPPuncta

?

Images

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Adjust modules:

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Start Test Mode

Analyze Images

Found 162 rows

Display outlines on a blank image?

Yes

No

Select image on which to display outlines

Ferritin

(from NamesAndTypes)

Name the output image

PunctaCellsOverlay

Outline display mode

Color

How to outline

Thick

Select objects to display

Nuclei

(from IdentifyPrimaryObjects #05)

Select outline color

Select objects to display

NormalCells

(from FilterObjects #09)

Select outline color

Remove this outline

Select objects to display

CellularFerritinPuncta

(from RelateObjects #12)

Select outline color

Remove this outline

Select objects to display

CellularLAMP2Puncta

(from RelateObjects #13)

Select outline color

Remove this outline

Select objects to display

CellularFerritinLAMP2Puncta

(from RelateObjects #16)

Select outline color

Remove this outline

Add another outline

Images

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▶ Start Test Mode

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Select the type of image to save

Image

?

Select the image to save

PunctaCellsOverlay

(from OverlayOutlines #17)

?

Select method for constructing file names

Sequential numbers

?

Enter file prefix

CJPM_E38_F-CO-60_Ferritinophagy_PunctaCellsOverlay_

?

Number of digits

3

?

Saved file format

tiff

?

Image bit depth

8-bit integer

?

Save with lossless compression?

Yes

No

?

Output file location

Default Input Folder sub-folder

(/Users/heptapus1)

?

Sub-folder:

Desktop\CP_output\PunctaCellsOverlay

Overwrite existing files without warning?

Yes

No

?

When to save

Every cycle

?

Record the file and path information to the saved image?

Yes

No

?

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Display object or image measurements?

Object

?

Select the input objects

NormalCells

(from FilterObjects #09)

?

Measurement to display

Category: Number

?

Measurement: Object_Number

?

Display background image?

Yes

No

?

Select the image on which to display the measurements

PunctaCellsOverlay

(from OverlayOutlines #17)

?

Display mode

Text

?

Text color

?

Font size (points)

40

?

Number of decimals

0

?

Annotation offset (in pixels)

0

?

Name the output image that has the measurements displayed

PunctaCellsOverlayNumbers

?

Image elements to save

Image

?

Images

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?

Adjust modules:

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▶ Analyze Images

Found 162 rows

Select the type of image to save

Image

?

Select the image to save

PunctaCellsOverlayNumbers

(from DisplayDataOnImage #19)

?

Select method for constructing file names

Sequential numbers

?

Enter file prefix

CS_E09_HAP_WIPI4KO_DAPI_PunctaCellsOverlayNumbers_

?

Number of digits

3

?

Saved file format

tiff

?

Image bit depth

8-bit integer

?

Save with lossless compression?

Yes

No

?

Output file location

Default Input Folder sub-folder

(/Users/heptapus1)

?

Sub-folder:

CP_output\PunctaCellsOverlayNumbers

Overwrite existing files without warning?

Yes

No

?

When to save

Every cycle

?

Record the file and path information to the saved image?

Yes

No

?

Images

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Adjust modules:

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Start Test Mode

Analyze Images

Select the column delimiter

Comma (",")

?

Output file location

Default Input Folder sub-folder (/Users/heptapus1)

?

Sub-folder: JoVe_output

?

Add a prefix to file names?

Yes

No

?

Filename prefix

CJPM_E38_F-CO-60_Ferritinophagy_CellProfilerResults_

?

Overwrite existing files without warning?

Yes

No

?

Add image metadata columns to your object data file?

Yes

No

?

Add image file and folder names to your object data file?

Yes

No

?

Representation of Nan/Inf

NaN

?

Select the measurements to export

Yes

No

?

Press button to select measurements

Press button to select measurements

?

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

?

Data to export

Image

?

Use the object name for the file name?

Yes

No

?

File name

Image.csv

?

Remove this data set

?

Data to export

Experiment

?

Use the object name for the file name?

Yes

No

?

Remove this data set

?

Data to export

NormalCells

(from FilterObjects #09)

?

Use the object name for the file name?

Yes

No

?

File name

NormalCells_All.csv

?

Remove this data set

?

Data to export

ColocFerritinLAMPPuncta

(from RelateObjects #15)

?

Found 162 rows

