



Maximum intensity:255.0

Groups:[module\_num:4|svn\_version:\'Unknown\'|variable\_revision\_number:2|show\_window:False|notes:\x5B\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.\x5D|batch\_state:array(\x5B\x5D, dtype=uint8)|enabled:True|wants\_pause:False]

Do you want to group your images?:No

grouping metadata count:1

Metadata category:None

EnhanceOrSuppressFeatures:[module\_num:5|svn\_version:\'Unknown\'|variable\_revision\_number:5|show\_window:True|notes:\x5B\x5D|batch\_state:array(\x5B\x5D, dtype=uint8)|enabled:True|wants\_pause:False]

Select the input image:RPE

Name the output image:Enhanced

Select the operation:Enhance

Feature size:10

Feature type:Neurites

Range of hole sizes:1,10

Smoothing scale:5

Shear angle:0.0

Decay:0.95

Enhancement method:Tubeness

Speed and accuracy:Slow / circular

IdentifyPrimaryObjects:[module\_num:6|svn\_version:\'Unknown\'|variable\_revision\_number:10|show\_window:True|notes:\x5B\x5D|batch\_state:array(\x5B\x5D, dtype=uint8)|enabled:True|wants\_pause:False]

Select the input image:RPE

Name the primary objects to be identified:cells

Typical diameter of objects, in pixel units (Min,Max):3,150

Discard objects outside the diameter range?:Yes

Try to merge too small objects with nearby larger objects?:No

Discard objects touching the border of the image?:Yes

Method to distinguish clumped objects:Intensity

Method to draw dividing lines between clumped objects:Intensity

Size of smoothing filter:0

Suppress local maxima that are closer than this minimum allowed distance:8

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

Name the outline image:CellOutlines

Fill holes in identified objects?:After both thresholding and declumping

Automatically calculate size of smoothing filter for declumping?:No

Automatically calculate minimum allowed distance between local maxima?:No

Retain outlines of the identified objects?:Yes

Automatically calculate the threshold using the Otsu method?:Yes

Enter Laplacian of Gaussian threshold:0.5

Automatically calculate the size of objects for the Laplacian of Gaussian filter?:Yes

Enter LoG filter diameter:5.0

Handling of objects if excessive number of objects identified:Continue

Maximum number of objects:500

Threshold setting version:2

Threshold strategy:Global

Thresholding method:RidlerCalvard

Select the smoothing method for thresholding:No smoothing

Threshold smoothing scale:1

Threshold correction factor:0

Lower and upper bounds on threshold:0.4,1  
Approximate fraction of image covered by objects?:0.01  
Manual threshold:0.0  
Select the measurement to threshold with:None  
Select binary image:ImageAfterMath  
Masking objects:None  
Two-class or three-class thresholding?:Two classes  
Minimize the weighted variance or the entropy?:Weighted variance  
Assign pixels in the middle intensity class to the foreground or the background?:Foreground  
Method to calculate adaptive window size:Image size  
Size of adaptive window:10  
Use default parameters?:Default  
Lower outlier fraction:0.05  
Upper outlier fraction:0.05  
Averaging method:Mean  
Variance method:Standard deviation  
# of deviations:2.0

OverlayOutlines:[module\_num:7|svn\_version:\'Unknown\'|variable\_revision\_number:3|show\_window:True|notes:\x5B\x5D|batch\_state:array(\x5B\x5D, dtype=uint8)|enabled:True|wants\_pause:False]  
Display outlines on a blank image?:No  
Select image on which to display outlines:RPE  
Name the output image:RPEOutlines  
Outline display mode:Color  
Select method to determine brightness of outlines:Max possible  
Width of outlines:2  
Select outlines to display:CellOutlines  
Select outline color:magenta  
Load outlines from an image or objects?:Image  
Select objects to display:cells

MeasureImageAreaOccupied:[module\_num:8|svn\_version:\'Unknown\'|variable\_revision\_number:3|show\_window:True|notes:\x5B\x5D|batch\_state:array(\x5B\x5D, dtype=uint8)|enabled:True|wants\_pause:False]  
Hidden:1  
Measure the area occupied in a binary image, or in objects?:Objects  
Select objects to measure:cells  
Retain a binary image of the object regions?:Yes  
Name the output binary image:Stain  
Select a binary image to measure:RPE

MeasureObjectNeighbors:[module\_num:9|svn\_version:\'Unknown\'|variable\_revision\_number:2|show\_window:True|notes:\x5B\x5D|batch\_state:array(\x5B\x5D, dtype=uint8)|enabled:True|wants\_pause:False]  
Select objects to measure:cells  
Select neighboring objects to measure:cells  
Method to determine neighbors:Adjacent  
Neighbor distance:5  
Retain the image of objects colored by numbers of neighbors?:Yes  
Name the output image:ObjectNeighborCount  
Select colormap:Default  
Retain the image of objects colored by percent of touching pixels?:Yes  
Name the output image:PercentTouching  
Select a colormap:Default

MeasureObjectSizeShape:[module\_num:10|svn\_version:\'Unknown\'|variable\_revision\_number:1|show\_window:True|n

otes:\x5B\x5D|batch\_state:array(\x5B\x5D, dtype=uint8)|enabled:True|wants\_pause:False]

Select objects to measure:cells

Calculate the Zernike features?:Yes

SaveImages:[module\_num:11|svn\_version:\'Unknown\'|variable\_revision\_number:11|show\_window:True|notes:\x5B\x5D|batch\_state:array(\x5B\x5D, dtype=uint8)|enabled:True|wants\_pause:False]

Select the type of image to save:Image

Select the image to save:RPE

Select the objects to save:None

Select the module display window to save:None

Select method for constructing file names:From image filename

Select image name for file prefix:RPE

Enter single file name:OrigBlue

Number of digits:4

Append a suffix to the image file name?:Yes

Text to append to the image name:\_cellOutlines

Saved file format:tif

Output file location:Default Output Folder\x7C

Image bit depth:8-bit integer

Overwrite existing files without warning?:No

When to save:Every cycle

Rescale the images? :No

Save as grayscale or color image?:Grayscale

Select colormap:gray

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

Create subfolders in the output folder?:Yes

Base image folder:Default Input Folder sub-folder\x7CDesktop\\\\\\\\\\\\\\\\LightInjury RPE-FM\\\\\\\\\\\\\\\\ManuallyTraced\\\\\\\\\\\\\\\\ManuallyTraced\_smoothed

Saved movie format:avi

SaveImages:[module\_num:12|svn\_version:\'Unknown\'|variable\_revision\_number:11|show\_window:True|notes:\x5B\x5D|batch\_state:array(\x5B\x5D, dtype=uint8)|enabled:True|wants\_pause:False]

Select the type of image to save:Image

Select the image to save:RPEOutlines

Select the objects to save:cells

Select the module display window to save:None

Select method for constructing file names:From image filename

Select image name for file prefix:RPE

Enter single file name:OrigBlue

Number of digits:4

Append a suffix to the image file name?:Yes

Text to append to the image name:\_Overlay

Saved file format:tif

Output file location:Default Output Folder\x7C

Image bit depth:8-bit integer

Overwrite existing files without warning?:No

When to save:Every cycle

Rescale the images? :No

Save as grayscale or color image?:Grayscale

Select colormap:gray

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

Create subfolders in the output folder?:Yes

Base image folder:Default Input Folder sub-folder\x7CDesktop\\\\\\\\\\\\\\\\LightInjury RPE-FM\\\\\\\\\\\\\\\\ManuallyTraced\\\\\\\\\\\\\\\\ManuallyTraced\_smoothed

Saved movie format:avi

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SaveImages:[module_num:13|svn_version:\'Unknown\'|variable_revision_number:11|show_window:True|notes:\x5B\x5D|batch_state:array(\x5B\x5D, dtype=uint8)|enabled:True|wants_pause:False]
  Select the type of image to save:Image
  Select the image to save:CellOutlines
  Select the objects to save:None
  Select the module display window to save:None
  Select method for constructing file names:From image filename
  Select image name for file prefix:RPE
  Enter single file name:OrigBlue
  Number of digits:4
  Append a suffix to the image file name?:Yes
  Text to append to the image name: _Cells
  Saved file format:tif
  Output file location:Default Output Folder\x7C
  Image bit depth:8-bit integer
  Overwrite existing files without warning?:No
  When to save:Every cycle
  Rescale the images? :No
  Save as grayscale or color image?:Grayscale
  Select colormap:gray
  Record the file and path information to the saved image?:No
  Create subfolders in the output folder?:Yes
  Base image folder:Default Input Folder sub-folder\x7CDesktop\LightInjury RPE-
  FM\ManuallyTraced\ManuallyTraced_smoothed
  Saved movie format:avi
```

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SaveImages:[module_num:14|svn_version:\'Unknown\'|variable_revision_number:11|show_window:True|notes:\x5B\x5D|batch_state:array(\x5B\x5D, dtype=uint8)|enabled:True|wants_pause:False]
  Select the type of image to save:Image
  Select the image to save:Stain
  Select the objects to save:None
  Select the module display window to save:None
  Select method for constructing file names:From image filename
  Select image name for file prefix:RPE
  Enter single file name:OrigBlue
  Number of digits:4
  Append a suffix to the image file name?:Yes
  Text to append to the image name: _AreaOccupied
  Saved file format:tif
  Output file location:Default Output Folder\x7C
  Image bit depth:8-bit integer
  Overwrite existing files without warning?:No
  When to save:Every cycle
  Rescale the images? :No
  Save as grayscale or color image?:Grayscale
  Select colormap:gray
  Record the file and path information to the saved image?:No
  Create subfolders in the output folder?:Yes
  Base image folder:Default Input Folder sub-folder\x7CDesktop\LightInjury RPE-
  FM\ManuallyTraced\ManuallyTraced_smoothed
  Saved movie format:avi
```

SaveImages:[module\_num:15|svn\_version:\'Unknown\'|variable\_revision\_number:11|show\_window:True|notes:\x5B\x5D|batch\_state:array(\x5B\x5D, dtype=uint8)|enabled:True|wants\_pause:False]  
Select the type of image to save:Image  
Select the image to save:ObjectNeighborCount  
Select the objects to save:None  
Select the module display window to save:None  
Select method for constructing file names:From image filename  
Select image name for file prefix:RPE  
Enter single file name:OrigBlue  
Number of digits:4  
Append a suffix to the image file name?:Yes  
Text to append to the image name:\_ColorNeighbors  
Saved file format:tif  
Output file location:Default Output Folder\x7C  
Image bit depth:8-bit integer  
Overwrite existing files without warning?:No  
When to save:Every cycle  
Rescale the images? :No  
Save as grayscale or color image?:Grayscale  
Select colormap:gray  
Record the file and path information to the saved image?:No  
Create subfolders in the output folder?:Yes  
Base image folder:Default Input Folder sub-folder\x7CDesktop\\\\\\\\\\\\\\\\LightInjury RPE-  
FM\\\\\\\\\\\\\\\\ManuallyTraced\\\\\\\\\\\\\\\\ManuallyTraced\_smoothed  
Saved movie format:avi

ExportToSpreadsheet:[module\_num:16|svn\_version:\'Unknown\'|variable\_revision\_number:11|show\_window:True|note  
s:\x5B\x5D|batch\_state:array(\x5B\x5D, dtype=uint8)|enabled:True|wants\_pause:False]  
Select the column delimiter:Comma (",")  
Add image metadata columns to your object data file?:Yes  
Limit output to a size that is allowed in Excel?:No  
Select the measurements to export:Yes  
Calculate the per-image mean values for object measurements?:Yes  
Calculate the per-image median values for object measurements?:Yes  
Calculate the per-image standard deviation values for object measurements?:Yes  
Output file location:Default Output Folder\x7C  
Create a GenePattern GCT file?:No  
Select source of sample row name:Metadata  
Select the image to use as the identifier:None  
Select the metadata to use as the identifier:None  
Export all measurement types?:Yes

:cells\x7CNeighbors\_SecondClosestDistance\_Adjacent,cells\x7CNeighbors\_FirstClosestDistance\_Adjacent,cells\x7CNeighbors\_FirstClosestObjectNumber\_Adjacent,cells\x7CNeighbors\_SecondClosestObjectNumber\_Adjacent,cells\x7CNeighbors\_PercentTouching\_Adjacent,cells\x7CNeighbors\_NumberOfNeighbors\_Adjacent,cells\x7CNeighbors\_AngleBetweenNeighbors\_Adjacent,cells\x7CNumber\_Object\_Number,cells\x7CLocation\_Center\_Y,cells\x7CLocation\_Center\_X,cells\x7CAreaShape\_Perimeter,cells\x7CAreaShape\_FormFactor,cells\x7CAreaShape\_MinorAxisLength,cells\x7CAreaShape\_Orientation,cells\x7CAreaShape\_Area,cells\x7CAreaShape\_MinFerretDiameter,cells\x7CAreaShape\_Solidity,cells\x7CAreaShape\_MaxFerretDiameter,cells\x7CAreaShape\_EulerNumber,cells\x7CAreaShape\_MedianRadius,cells\x7CAreaShape\_Compactness,cells\x7CAreaShape\_Extent,cells\x7CAreaShape\_Eccentricity,cells\x7CAreaShape\_MaximumRadius,cells\x7CAreaShape\_MeanRadius,cells\x7CAreaShape\_MajorAxisLength,cells\x7CAreaShape\_Center\_Y,cells\x7CAreaShape\_Center\_X,Image\x7CCount\_cells,Image\x7CAreaOccupied\_Perimeter\_cells,Image\x7CAreaOccupied\_AreaOccupied\_cells,Image\x7CAreaOccupied\_TotalArea\_cells,Image\x7CFileName\_RPE,Experiment\x7CPipeline\_Pipeline

Representation of Nan/Inf:NaN  
Add a prefix to file names?:Yes  
Filename prefix:ManualTraced\_  
Overwrite existing files without warning?:Yes  
Data to export:Image  
Combine these object measurements with those of the previous object?:No  
File name:DATA.csv  
Use the object name for the file name?:Yes  
Data to export:cells  
Combine these object measurements with those of the previous object?:No  
File name:DATA.csv  
Use the object name for the file name?:Yes

ExportToDatabase:[module\_num:17|svn\_version:\'Unknown\'|variable\_revision\_number:27|show\_window:True|notes:\x5B\x5D|batch\_state:array(\x5B\x5D, dtype=uint8)|enabled:True|wants\_pause:False]

Database type:MySQL / CSV

Database name:DefaultDB

Add a prefix to table names?:Yes

Table prefix:MyExpt\_

SQL file prefix:SQL\_

Output file location:Default Input Folder sub-folder\x7CDesktop\LightInjury RPE-FM\Fig.RPE cells\copies for figure 5

Create a CellProfiler Analyst properties file?:Yes

Database host:

Username:

Password:

Name the SQLite database file:DefaultDB.db

Calculate the per-image mean values of object measurements?:Yes

Calculate the per-image median values of object measurements?:Yes

Calculate the per-image standard deviation values of object measurements?:Yes

Calculate the per-well mean values of object measurements?:No

Calculate the per-well median values of object measurements?:No

Calculate the per-well standard deviation values of object measurements?:No

Export measurements for all objects to the database?:All

Select the objects:

Maximum # of characters in a column name:64

Create one table per object, a single object table or a single object view?:Single object table

Enter an image url prepend if you plan to access your files via http:

Write image thumbnails directly to the database?:No

Select the images for which you want to save thumbnails:

Auto-scale thumbnail pixel intensities?:Yes

Select the plate type:None

Select the plate metadata:None

Select the well metadata:None

Include information for all images, using default values?:Yes

Properties image group count:1

Properties group field count:1

Properties filter field count:0

Workspace measurement count:1

Experiment name:MyExpt

Which objects should be used for locations?:cells

Enter a phenotype class table name if using the classifier tool:

Export object relationships?:Yes

Overwrite without warning?:Never

Access CPA images via URL?:No  
Select the classification type:Object  
Select an image to include:None  
Use the image name for the display?:Yes  
Image name:Channel1  
Channel color:red  
Do you want to add group fields?:No  
Enter the name of the group:  
Enter the per-image columns which define the group, separated by commas:ImageNumber, Image\_Metadata\_Plate,  
Image\_Metadata\_Well  
Do you want to add filter fields?:No  
Automatically create a filter for each plate?:No  
Create a CellProfiler Analyst workspace file?:No  
Select the measurement display tool:ScatterPlot  
Type of measurement to plot on the X-axis:Image  
Enter the object name:None  
Select the X-axis measurement:None  
Select the X-axis index:ImageNumber  
Type of measurement to plot on the Y-axis:Image  
Enter the object name:None  
Select the Y-axis measurement:None  
Select the Y-axis index:ImageNumber