

CellProfiler_Version 4.2.1

Images:

ChannelType_Acti Grayscale

ChannelType_DAPI Grayscale

ChannelType_Tubu Grayscale

Metadata:

Metadata extraction method:Extract from file/folder names

Metadata source:File name

Regular expression to extract from file

name:^(?P<Date>.*)(?P<CellLine>.*)(?P<DiffState>.*)(?P<Substrate>.*)(?P<Site>[0-9]{1,2})_c(?P<Chanel>[0-9])

Modules:

RescaleIntensity:

Select the input image:DAPI

Name the output image:RescaledDAPI

Rescaling method:Choose specific values to be reset to a custom range

Method to calculate the minimum intensity:Minimum for each image

Method to calculate the maximum intensity:Maximum for each image

Lower intensity limit for the input image:0

Upper intensity limit for the input image:1

Intensity range for the input image:0.000000,1.000000

Intensity range for the output image:0.000000,1.000000

Smooth:

Select the input image:RescaledDAPI

Name the output image:rescaledDAPIsmooth

Select smoothing method:Gaussian Filter

Calculate artifact diameter automatically?:No

Typical artifact diameter:3.0

Edge intensity difference:0.1

Clip intensities to 0 and 1?:Yes

RescaleIntensity:

Select the input image:Acti

Name the output image:RescaledActi

Rescaling method:Choose specific values to be reset to a custom range

Method to calculate the minimum intensity:Minimum for each image

Method to calculate the maximum intensity:Maximum for each image

Lower intensity limit for the input image:0

Upper intensity limit for the input image:1

Intensity range for the input image:0.000000,1.000000

Intensity range for the output image:0.000000,1.000000

RescaleIntensity:

Select the input image:Tubu

Name the output image:RescaledTubu

Rescaling method:Choose specific values to be reset to a custom range

Method to calculate the minimum intensity:Minimum for each image

Method to calculate the maximum intensity:Maximum for each image

Lower intensity limit for the input image:0
Upper intensity limit for the input image:1
Intensity range for the input image:0.000000,1.000000
Intensity range for the output image:0.000000,1.000000
Select image to match in maximum intensity:None

GrayToColor:

Select a color scheme:RGB
Rescale intensity:No
Select the image to be colored red:RescaledActi
Select the image to be colored green:RescaledTubu
Select the image to be colored blue:rescaledDAPIsmooth
Name the output image:RGBImage
Relative weight for the red image:1.0
Relative weight for the green image:1.0
Relative weight for the blue image:1.0

SaveImages:

Select the type of image to save:Image
Select the image to save:RGBImage
Saved file format:tiff

IdentifyPrimaryObjects:

Select the input image:rescaledDAPIsmooth
Name the primary objects to be identified:Nuclei
Typical diameter of objects, in pixel units (Min,Max):30,70
Discard objects outside the diameter range?:Yes
Discard objects touching the border of the image?:Yes
Method to distinguish clumped objects:Shape
Method to draw dividing lines between clumped objects:Shape
Size of smoothing filter:10
Suppress local maxima that are closer than this minimum allowed distance:8
Speed up by using lower-resolution image to find local maxima?:Yes
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
Handling of objects if excessive number of objects identified:Continue
Maximum number of objects:500
Use advanced settings?:Yes
Threshold setting version:12
Threshold strategy:Global
Thresholding method:Minimum Cross-Entropy
Threshold smoothing scale:1.3488
Threshold correction factor:2
Lower and upper bounds on threshold:0.000000,1.000000

IdentifySecondaryObjects:

Select the input objects:Nuclei
Name the objects to be identified:Cells
Select the method to identify the secondary objects:Propagation
Select the input image:RescaledActi
Regularization factor:0.05

Discard secondary objects touching the border of the image?:Yes
Discard the associated primary objects?:Yes
Name the new primary objects:NucleiOK
Fill holes in identified objects?:Yes
Threshold setting version:12
Threshold strategy:Global
Thresholding method:Otsu
Threshold smoothing scale:0
Threshold correction factor:0.4
Lower and upper bounds on threshold:0.000000,1.000000
Manual threshold:0.0
Select the measurement to threshold with:None
Two-class or three-class thresholding?:Two classes
Log transform before thresholding?:No

MeasureObjectSizeShape:

Select object sets to measure:Cells
Calculate the Zernike features?:No
Calculate the advanced features?:No

MeasureObjectIntensity:

Select images to measure:Acti, RescaledActi, RescaledTubu, Tubu
Select objects to measure:Cells

OverlayOutlines:

Display outlines on a blank image?:No
Select image on which to display outlines:RGBImage
Name the output image:Overlay
Outline display mode:Color
Select method to determine brightness of outlines:Max of image
How to outline:Inner
Select outline color:yellow
Select objects to display:Cells
Select outline color:red
Select objects to display:Nuclei

SaveImages:

Select the type of image to save:Image
Select the image to save:Overlay
Select method for constructing file names:From image filename
Select image name for file prefix:DAPI
Enter single file name:OrigBlue
Saved file format:tiff
Image bit depth:8-bit integer
When to save:Every cycle
Record the file and path information to the saved image?:No
Create subfolders in the output folder?:No
Base image folder:Default Input Folder
Save with lossless compression?:No

IdentifySecondaryObjects:

Select the input objects:Nuclei

Name the objects to be identified:NucleiBig
Select the method to identify the secondary objects:Distance - N
Select the input image:RescaledActi
Number of pixels by which to expand the primary objects:55
Regularization factor:0.05
Discard secondary objects touching the border of the image?:Yes
Discard the associated primary objects?:Yes
Name the new primary objects:NucleiBigOK
Fill holes in identified objects?:Yes

ConvertObjectsToImage

Select the input objects:Cells
Name the output image:CellBinary
Select the color format:Binary (black & white)
Select the colormap:Default

Morph:

Select the input image:CellBinary
Name the output image:SkelCell
Select the operation to perform:skelpe
Number of times to repeat operation:Custom
Repetition number:10
Rescale values from 0 to 1?:Yes

MeasureObjectSkeleton:

Select the seed objects:NucleiBig
Select the skeletonized image:SkelCell
Retain the branchpoint image?:Yes
Name the branchpoint image:BranchpointImage
Fill small holes?:Yes
Maximum hole size:10
Export the skeleton graph relationships?:No

SaveImages:

Select the type of image to save:Image
Select the image to save:BranchpointImage
Saved file format:tiff
Save with lossless compression?:No

MeasureObjectIntensityDistribution:

Select images to measure:Acti, RescaledActi, RescaledTubu, Tubu
Calculate intensity Zernikes?:Magnitudes and phase
Maximum zernike moment:6
Select objects to measure:Cells
Object to use as center?:Centers of other objects
Select objects to use as centers:Nuclei
Scale the bins?:Yes
Number of bins:4
Maximum radius:100

MeasureTexture:

Select images to measure:Acti, RescaledActi, RescaledTubu, Tubu

Select objects to measure:Cells
Enter how many gray levels to measure the texture at:256
Texture scale to measure:3

ExportToSpreadsheet

ExportToDatabase:

Database type:SQLite
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?:Select...
Select the objects:Cells,NucleiOK
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?:Yes
Select the images for which you want to save thumbnails:RGBImage
Which objects should be used for locations?:NucleiOK
Enter a phenotype class table name if using the Classifier tool in CellProfiler Analyst:
Select the classification type:Object
Select an image to include:None
Use the image name for the display?:Yes
Do you want to add group fields?:Yes
Enter the name of the group:Date
Enter the per-image columns which define the group, separated by commas:ImageNumber,
Image_Metadata_Date
Enter the name of the group:CellLine
Enter the per-image columns which define the group, separated by commas:ImageNumber,
Image_Metadata_CellLine
Enter the name of the group:DiffState
Enter the per-image columns which define the group, separated by commas:ImageNumber,
Image_Metadata_DiffState
Enter the name of the group:Substrate
Enter the per-image columns which define the group, separated by commas:ImageNumber,
Image_Metadata_Substrate
Do you want to add filter fields?:No