CellProfiler Pipeline: http://www.cellprofiler.org

Version:5

DateRevision:426

GitHash:

ModuleCount:33

HasImagePlaneDetails:False

Images:[module\_num:1|svn\_version:'Unknown'|variable\_revision\_number:2|show\_window:False|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.']|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

:

Filter images?:Images only

Select the rule criteria:and (extension does isimage) (directory doesnot containregexp "[\\\\/]\\.")

Metadata:[module\_num:2|svn\_version:'Unknown'|variable\_revision\_number:6|show\_window:False|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.']|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Extract metadata?:Yes

Metadata data type:Text

Metadata types:{}

Extraction method count:2

Metadata extraction method:Extract from file/folder names

Metadata source:File name

Regular expression to extract from file name:^(?P<SampleID>N[0-9]+)\_(?P<ImageType>[A-Za-z]+)\.(?P<FileExtension>[a-z]+)$

Regular expression to extract from folder name:(?P<Date>[0-9]{4}\_[0-9]{2}\_[0-9]{2})$

Extract metadata from:All images

Select the filtering criteria:and (file does contain "")

Metadata file location:Elsewhere...|

Match file and image metadata:[]

Use case insensitive matching?:No

Metadata file name:None

Does cached metadata exist?:Yes

Metadata extraction method:Extract from image file headers

Metadata source:File name

Regular expression to extract from file name:^(?P<Plate>.\*)\_(?P<Well>[A-P][0-9]{2})\_s(?P<Site>[0-9])\_w(?P<ChannelNumber>[0-9])

Regular expression to extract from folder name:(?P<Date>[0-9]{4}\_[0-9]{2}\_[0-9]{2})$

Extract metadata from:All images

Select the filtering criteria:and (file does contain "")

Metadata file location:Elsewhere...|

Match file and image metadata:[]

Use case insensitive matching?:No

Metadata file name:None

Does cached metadata exist?:Yes

NamesAndTypes:[module\_num:3|svn\_version:'Unknown'|variable\_revision\_number:8|show\_window:False|notes:['The NamesAndTypes module allows you to assign a meaningful name to each image by which other modules will refer to it.']|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Assign a name to:All images

Select the image type:Color image

Name to assign these images:AP\_liver\_unmasked

Match metadata:[]

Image set matching method:Order

Set intensity range from:Image metadata

Assignments count:1

Single images count:0

Maximum intensity:255.0

Process as 3D?:No

Relative pixel spacing in X:1.0

Relative pixel spacing in Y:1.0

Relative pixel spacing in Z:1.0

Select the rule criteria:and (metadata does Tissue "Liver")

Name to assign these images:AP\_liver

Name to assign these objects:Cell

Select the image type:Color image

Set intensity range from:Image metadata

Maximum intensity:255.0

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

Do you want to group your images?:No

grouping metadata count:1

Metadata category:sample\_id

UnmixColors:[module\_num:5|svn\_version:'Unknown'|variable\_revision\_number:2|show\_window:True|notes:[]|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Stain count:1

Select the input color image:AP\_liver\_unmasked

Name the output image:BKG

Stain:Custom

Red absorbance:0.431515

Green absorbance:0.609995

Blue absorbance:0.664605

Threshold:[module\_num:6|svn\_version:'Unknown'|variable\_revision\_number:12|show\_window:False|notes:[]|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Select the input image:BKG

Name the output image:BKGThreshold

Threshold strategy:Global

Thresholding method:Minimum Cross-Entropy

Threshold smoothing scale:0.0

Threshold correction factor:1.0

Lower and upper bounds on threshold:0.0,1.0

Manual threshold:0.0

Select the measurement to threshold with:None

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

Log transform before thresholding?:No

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

Size of adaptive window:50

Lower outlier fraction:0.05

Upper outlier fraction:0.05

Averaging method:Mean

Variance method:Standard deviation

# of deviations:2.0

Thresholding method:Minimum Cross-Entropy

RemoveHoles:[module\_num:7|svn\_version:'Unknown'|variable\_revision\_number:1|show\_window:False|notes:[]|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Select the input image:BKGThreshold

Name the output image:BKGremoveholes

Size of holes to fill:30

ReduceNoise:[module\_num:8|svn\_version:'Unknown'|variable\_revision\_number:1|show\_window:False|notes:[]|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Select the input image:BKGremoveholes

Name the output image:BKGReduceNoise

Size:50

Distance:25

Cut-off distance:0.3

Threshold:[module\_num:9|svn\_version:'Unknown'|variable\_revision\_number:12|show\_window:False|notes:[]|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Select the input image:BKGReduceNoise

Name the output image:BKGBinary

Threshold strategy:Global

Thresholding method:Otsu

Threshold smoothing scale:0.1

Threshold correction factor:1

Lower and upper bounds on threshold:0.0,1.0

Manual threshold:0.0

Select the measurement to threshold with:None

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

Log transform before thresholding?:No

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

Size of adaptive window:50

Lower outlier fraction:0.05

Upper outlier fraction:0.05

Averaging method:Mean

Variance method:Standard deviation

# of deviations:2.0

Thresholding method:Minimum Cross-Entropy

ConvertImageToObjects:[module\_num:10|svn\_version:'Unknown'|variable\_revision\_number:1|show\_window:False|notes:[]|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Select the input image:BKGBinary

Name the output object:TissueObjects

Convert to boolean image:Yes

Preserve original labels:No

Background label:0

Connectivity:0

MeasureObjectSizeShape:[module\_num:11|svn\_version:'Unknown'|variable\_revision\_number:3|show\_window:False|notes:[]|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Select object sets to measure:TissueObjects

Calculate the Zernike features?:Yes

Calculate the advanced features?:No

MeasureImageAreaOccupied:[module\_num:12|svn\_version:'Unknown'|variable\_revision\_number:5|show\_window:False|notes:[]|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Measure the area occupied by:Objects

Select binary images to measure:

Select object sets to measure:TissueObjects

CalculateMath:[module\_num:13|svn\_version:'Unknown'|variable\_revision\_number:3|show\_window:False|notes:[]|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Name the output measurement:TissueObjects\_PercentArea

Operation:Divide

Select the numerator measurement type:Object

Select the numerator objects:TissueObjects

Select the numerator measurement:AreaShape\_Area

Multiply the above operand by:1.0

Raise the power of above operand by:1.0

Select the denominator measurement type:Image

Select the denominator objects:None

Select the denominator measurement:AreaOccupied\_TotalArea\_TissueObjects

Multiply the above operand by:1.0

Raise the power of above operand by:1.0

Take log10 of result?:No

Multiply the result by:1.0

Raise the power of result by:1.0

Add to the result:0.0

How should the output value be rounded?:Not rounded

Enter how many decimal places the value should be rounded to:0

Constrain the result to a lower bound?:No

Enter the lower bound:0.0

Constrain the result to an upper bound?:No

Enter the upper bound:1.0

FilterObjects:[module\_num:14|svn\_version:'Unknown'|variable\_revision\_number:10|show\_window:False|notes:[]|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Select the objects to filter:TissueObjects

Name the output objects:AP\_liverobject

Select the filtering mode:Measurements

Select the filtering method:Limits

Select the objects that contain the filtered objects:None

Select the location of the rules or classifier file:Elsewhere...|

Rules or classifier file name:rules.txt

Class number:1

Measurement count:1

Additional object count:0

Assign overlapping child to:Both parents

Keep removed objects as a separate set?:No

Name the objects removed by the filter:RemovedObjects

Select the measurement to filter by:Math\_TissueObjects\_PercentArea

Filter using a minimum measurement value?:Yes

Minimum value:0.25

Filter using a maximum measurement value?:No

Maximum value:1.0

Allow fuzzy feature matching?:No

MaskImage:[module\_num:15|svn\_version:'Unknown'|variable\_revision\_number:3|show\_window:False|notes:[]|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Select the input image:AP\_liver\_unmasked

Name the output image:AP\_liver

Use objects or an image as a mask?:Objects

Select object for mask:AP\_liverobject

Select image for mask:None

Invert the mask?:No

UnmixColors:[module\_num:16|svn\_version:'Unknown'|variable\_revision\_number:2|show\_window:False|notes:[]|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Stain count:2

Select the input color image:AP\_liver

Name the output image:AP\_binary

Stain:Custom

Red absorbance:0.4374

Green absorbance:0.683165

Blue absorbance:0.584779

Name the output image:AP\_bkg

Stain:Custom

Red absorbance:0.434541

Green absorbance:0.640396

Blue absorbance:0.633299

Threshold:[module\_num:17|svn\_version:'Unknown'|variable\_revision\_number:12|show\_window:False|notes:[]|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Select the input image:AP\_bkg

Name the output image:Threshold

Threshold strategy:Global

Thresholding method:Otsu

Threshold smoothing scale:0.0

Threshold correction factor:1.0

Lower and upper bounds on threshold:0.0,1.0

Manual threshold:0.0

Select the measurement to threshold with:None

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

Log transform before thresholding?:No

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

Size of adaptive window:50

Lower outlier fraction:0.05

Upper outlier fraction:0.05

Averaging method:Mean

Variance method:Standard deviation

# of deviations:2.0

Thresholding method:Minimum Cross-Entropy

RescaleIntensity:[module\_num:18|svn\_version:'Unknown'|variable\_revision\_number:3|show\_window:False|notes:[]|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Select the input image:AP\_binary

Name the output image:AP\_binary\_rescale

Rescaling method:Stretch each image to use the full intensity range

Method to calculate the minimum intensity:Custom

Method to calculate the maximum intensity:Custom

Lower intensity limit for the input image:0.0

Upper intensity limit for the input image:1.0

Intensity range for the input image:0.0,1.0

Intensity range for the output image:0.0,1.0

Select image to match in maximum intensity:None

Divisor value:1.0

Divisor measurement:None

GaussianFilter:[module\_num:19|svn\_version:'Unknown'|variable\_revision\_number:1|show\_window:False|notes:[]|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Select the input image:AP\_binary\_rescale

Name the output image:AP\_binary\_GaussianFilter

Sigma:1

ReduceNoise:[module\_num:20|svn\_version:'Unknown'|variable\_revision\_number:1|show\_window:False|notes:[]|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Select the input image:AP\_binary\_GaussianFilter

Name the output image:AP\_ReduceNoise

Size:10

Distance:15

Cut-off distance:0.2

EnhanceOrSuppressFeatures:[module\_num:21|svn\_version:'Unknown'|variable\_revision\_number:7|show\_window:False|notes:[]|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Select the input image:AP\_ReduceNoise

Name the output image:AP\_SuppressFeatures

Select the operation:Suppress

Feature size:5

Feature type:Speckles

Range of hole sizes:1,10

Smoothing scale:2.0

Shear angle:0.0

Decay:0.95

Enhancement method:Tubeness

Speed and accuracy:Fast

Rescale result image:No

Threshold:[module\_num:22|svn\_version:'Unknown'|variable\_revision\_number:12|show\_window:False|notes:[]|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Select the input image:AP\_SuppressFeatures

Name the output image:AP\_Threshold\_Adaptive

Threshold strategy:Adaptive

Thresholding method:Otsu

Threshold smoothing scale:0.075

Threshold correction factor:1.25

Lower and upper bounds on threshold:0.0,1.0

Manual threshold:0.0

Select the measurement to threshold with:None

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

Log transform before thresholding?:No

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

Size of adaptive window:50

Lower outlier fraction:0.05

Upper outlier fraction:0.05

Averaging method:Mean

Variance method:Standard deviation

# of deviations:2.0

Thresholding method:Otsu

Threshold:[module\_num:23|svn\_version:'Unknown'|variable\_revision\_number:12|show\_window:False|notes:[]|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Select the input image:AP\_SuppressFeatures

Name the output image:AP\_Threshold\_Global

Threshold strategy:Global

Thresholding method:Otsu

Threshold smoothing scale:0.075

Threshold correction factor:1.25

Lower and upper bounds on threshold:0.0,1.0

Manual threshold:0.0

Select the measurement to threshold with:None

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

Log transform before thresholding?:No

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

Size of adaptive window:50

Lower outlier fraction:0.05

Upper outlier fraction:0.05

Averaging method:Mean

Variance method:Standard deviation

# of deviations:2.0

Thresholding method:Otsu

Threshold:[module\_num:24|svn\_version:'Unknown'|variable\_revision\_number:12|show\_window:False|notes:[]|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Select the input image:AP\_SuppressFeatures

Name the output image:AP\_Threshold\_Robust

Threshold strategy:Global

Thresholding method:Robust Background

Threshold smoothing scale:0.075

Threshold correction factor:1.25

Lower and upper bounds on threshold:0.0,1.0

Manual threshold:0.0

Select the measurement to threshold with:None

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

Log transform before thresholding?:No

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

Size of adaptive window:50

Lower outlier fraction:0.05

Upper outlier fraction:0.05

Averaging method:Mean

Variance method:Standard deviation

# of deviations:2.5

Thresholding method:Otsu

SaveImages:[module\_num:25|svn\_version:'Unknown'|variable\_revision\_number:16|show\_window:False|notes:[]|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Select the type of image to save:Image

Select the image to save:AP\_Threshold\_Adaptive

Select method for constructing file names:From image filename

Select image name for file prefix:AP\_liver\_unmasked

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:\_Threshold\_adaptive

Saved file format:tiff

Output file location:Default Output Folder|

Image bit depth:8-bit integer

Overwrite existing files without warning?:Yes

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:Elsewhere...|

How to save the series:T (Time)

Save with lossless compression?:Yes

SaveImages:[module\_num:26|svn\_version:'Unknown'|variable\_revision\_number:16|show\_window:False|notes:[]|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Select the type of image to save:Image

Select the image to save:AP\_Threshold\_Global

Select method for constructing file names:From image filename

Select image name for file prefix:AP\_liver\_unmasked

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:\_Threshold\_global

Saved file format:tiff

Output file location:Default Output Folder|

Image bit depth:8-bit integer

Overwrite existing files without warning?:Yes

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:Elsewhere...|

How to save the series:T (Time)

Save with lossless compression?:Yes

SaveImages:[module\_num:27|svn\_version:'Unknown'|variable\_revision\_number:16|show\_window:False|notes:[]|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Select the type of image to save:Image

Select the image to save:AP\_Threshold\_Robust

Select method for constructing file names:From image filename

Select image name for file prefix:AP\_liver\_unmasked

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:\_Threshold\_robust

Saved file format:tiff

Output file location:Default Output Folder|

Image bit depth:8-bit integer

Overwrite existing files without warning?:Yes

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:Elsewhere...|

How to save the series:T (Time)

Save with lossless compression?:Yes

MeasureObjectSizeShape:[module\_num:28|svn\_version:'Unknown'|variable\_revision\_number:3|show\_window:False|notes:[]|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Select object sets to measure:AP\_liverobject

Calculate the Zernike features?:Yes

Calculate the advanced features?:No

MeasureImageAreaOccupied:[module\_num:29|svn\_version:'Unknown'|variable\_revision\_number:5|show\_window:False|notes:[]|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Measure the area occupied by:Binary Image

Select binary images to measure:AP\_Threshold\_Adaptive, AP\_Threshold\_Global, AP\_Threshold\_Robust

Select object sets to measure:AP\_liver

CalculateMath:[module\_num:30|svn\_version:'Unknown'|variable\_revision\_number:3|show\_window:True|notes:[]|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Name the output measurement:PercentArea\_AP\_Adaptive

Operation:Divide

Select the numerator measurement type:Image

Select the numerator objects:None

Select the numerator measurement:AreaOccupied\_AreaOccupied\_AP\_Threshold\_Adaptive

Multiply the above operand by:1.0

Raise the power of above operand by:1.0

Select the denominator measurement type:Object

Select the denominator objects:AP\_liverobject

Select the denominator measurement:AreaShape\_Area

Multiply the above operand by:1.0

Raise the power of above operand by:1.0

Take log10 of result?:No

Multiply the result by:1.0

Raise the power of result by:1.0

Add to the result:0.0

How should the output value be rounded?:Not rounded

Enter how many decimal places the value should be rounded to:0

Constrain the result to a lower bound?:No

Enter the lower bound:0.0

Constrain the result to an upper bound?:No

Enter the upper bound:1.0

CalculateMath:[module\_num:31|svn\_version:'Unknown'|variable\_revision\_number:3|show\_window:True|notes:[]|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Name the output measurement:PercentArea\_AP\_Global

Operation:Divide

Select the numerator measurement type:Image

Select the numerator objects:None

Select the numerator measurement:AreaOccupied\_AreaOccupied\_AP\_Threshold\_Global

Multiply the above operand by:1.0

Raise the power of above operand by:1.0

Select the denominator measurement type:Object

Select the denominator objects:AP\_liverobject

Select the denominator measurement:AreaShape\_Area

Multiply the above operand by:1.0

Raise the power of above operand by:1.0

Take log10 of result?:No

Multiply the result by:1.0

Raise the power of result by:1.0

Add to the result:0.0

How should the output value be rounded?:Not rounded

Enter how many decimal places the value should be rounded to:0

Constrain the result to a lower bound?:No

Enter the lower bound:0.0

Constrain the result to an upper bound?:No

Enter the upper bound:1.0

CalculateMath:[module\_num:32|svn\_version:'Unknown'|variable\_revision\_number:3|show\_window:True|notes:[]|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Name the output measurement:PercentArea\_AP\_Robust

Operation:Divide

Select the numerator measurement type:Image

Select the numerator objects:None

Select the numerator measurement:AreaOccupied\_AreaOccupied\_AP\_Threshold\_Robust

Multiply the above operand by:1.0

Raise the power of above operand by:1.0

Select the denominator measurement type:Object

Select the denominator objects:AP\_liverobject

Select the denominator measurement:AreaShape\_Area

Multiply the above operand by:1.0

Raise the power of above operand by:1.0

Take log10 of result?:No

Multiply the result by:1.0

Raise the power of result by:1.0

Add to the result:0.0

How should the output value be rounded?:Not rounded

Enter how many decimal places the value should be rounded to:0

Constrain the result to a lower bound?:No

Enter the lower bound:0.0

Constrain the result to an upper bound?:No

Enter the upper bound:1.0

ExportToSpreadsheet:[module\_num:33|svn\_version:'Unknown'|variable\_revision\_number:13|show\_window:True|notes:[]|batch\_state:array([], dtype=uint8)|enabled:True|wants\_pause:False]

Select the column delimiter:Comma (",")

Add image metadata columns to your object data file?:No

Add image file and folder names to your object data file?:No

Select the measurements to export:Yes

Calculate the per-image mean values for object measurements?:No

Calculate the per-image median values for object measurements?:No

Calculate the per-image standard deviation values for object measurements?:No

Output file location:Default Output Folder|

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

Press button to select measurements:Image|Metadata\_ImageType,Image|Metadata\_SampleID,Image|AreaOccupied\_AreaOccupied\_AP\_Threshold\_Global,Image|AreaOccupied\_AreaOccupied\_AP\_Threshold\_Robust,Image|AreaOccupied\_AreaOccupied\_AP\_Threshold\_Adaptive,Image|AreaOccupied\_TotalArea\_AP\_Threshold\_Robust,Image|AreaOccupied\_TotalArea\_AP\_Threshold\_Global,Image|AreaOccupied\_TotalArea\_AP\_Threshold\_Adaptive,Image|FileName\_AP\_liver\_unmasked,Experiment|Pipeline\_Pipeline,AP\_liverobject|AreaShape\_Area,AP\_liverobject|Math\_PercentArea\_AP\_Robust,AP\_liverobject|Math\_PercentArea\_AP\_Global,AP\_liverobject|Math\_PercentArea\_AP\_Adaptive

Representation of Nan/Inf:NaN

Add a prefix to file names?:Yes

Filename prefix:Results\_

Overwrite existing files without warning?:No

Data to export:Do not use

Combine these object measurements with those of the previous object?:No

File name:DATA.csv

Use the object name for the file name?:Yes