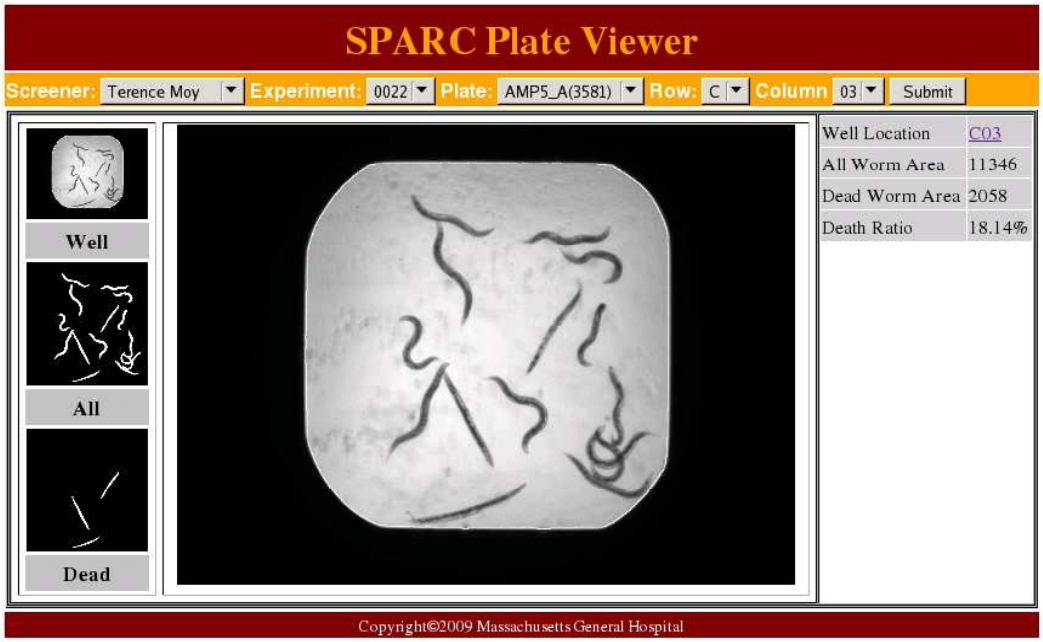
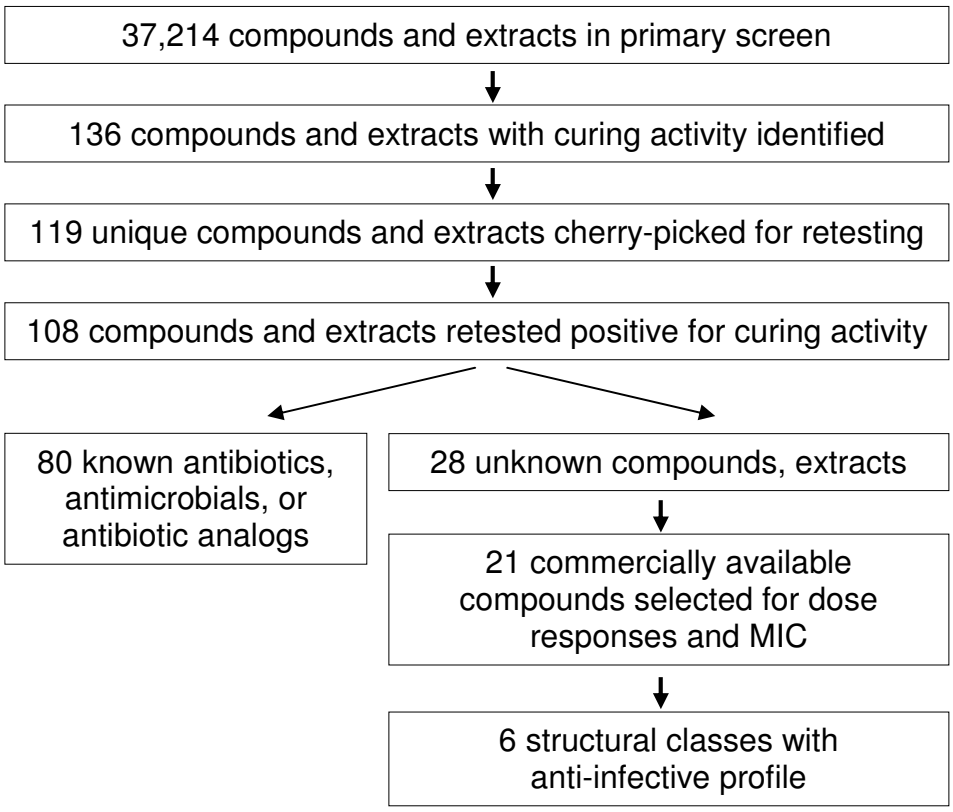
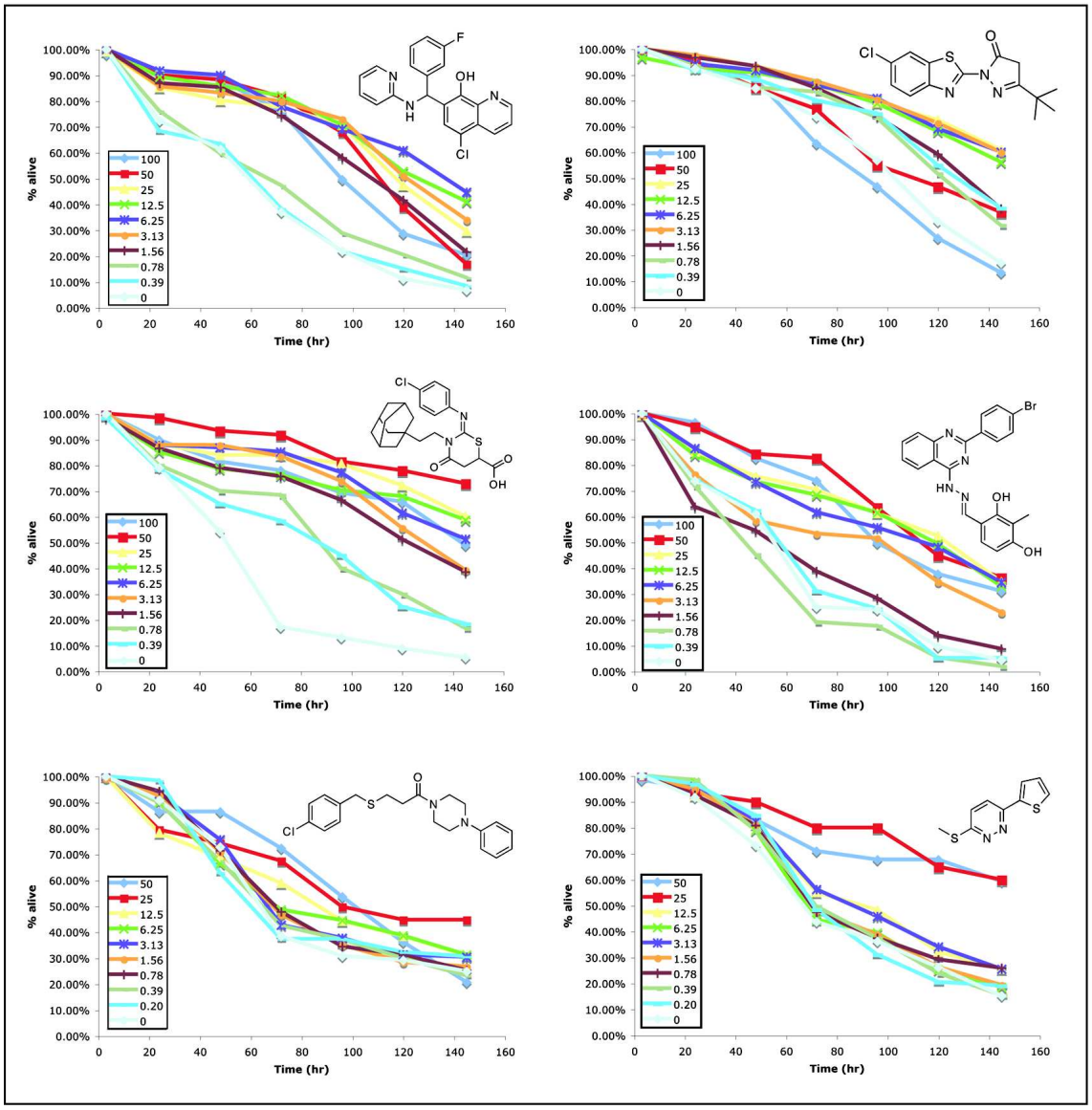


Supplementary Figure 1. Data viewer web interface. The data viewer is connected to the Oracle database of images acquired in a screen, and output images and data from the automated CellProfiler analysis.

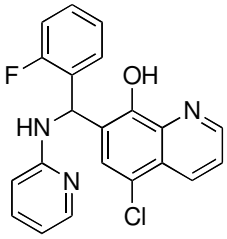


Supplementary Figure 2. Flow chart of the categorization of hit compounds.

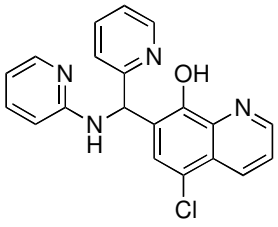


Supplementary Figure 3. Dose response curves of representative anti-infective compounds from each of the 6 structural groups. The compound structures are shown in the top right hand corner of the graphs. Each colored curve represents a survival curve at a different concentration (µg/ml).

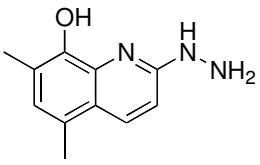
1



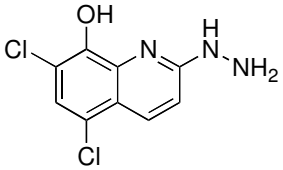
MIC=12.5
EC=0.78



MIC=12.5
EC=3.13

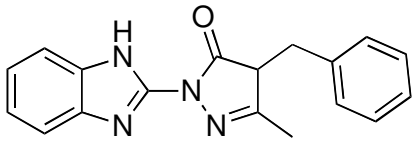


MIC=n.d.
EC=n.d.



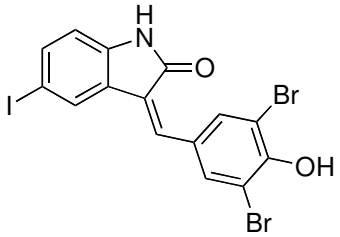
MIC=6.25
EC=0.20

2



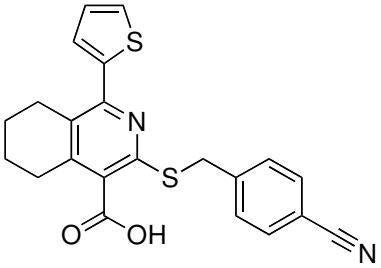
MIC=n.d.
EC=n.d.

11



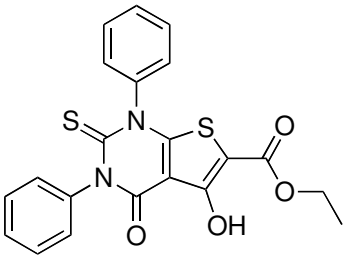
MIC=6.25
EC=6.25

3



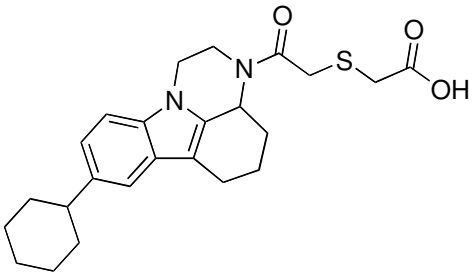
MIC=n.d.
EC=n.d.

12



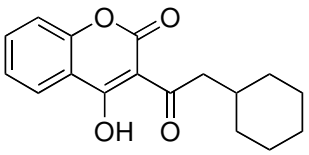
MIC<0.78
EC=0.39

4

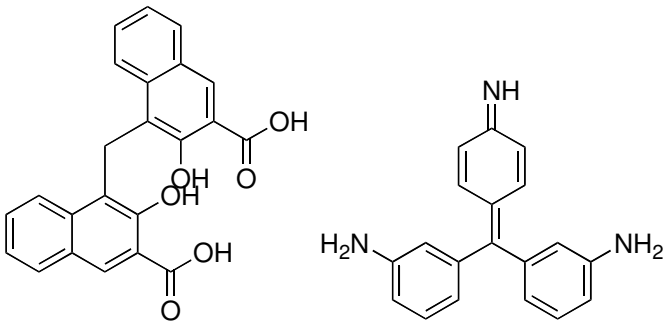
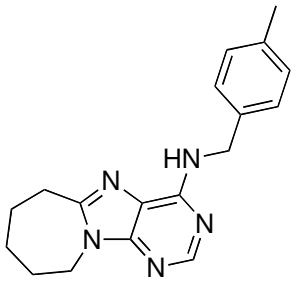
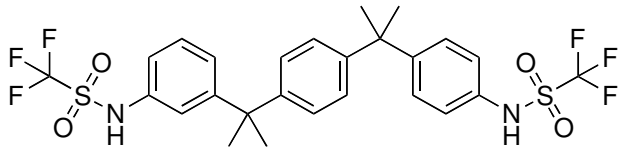
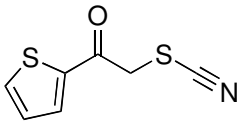
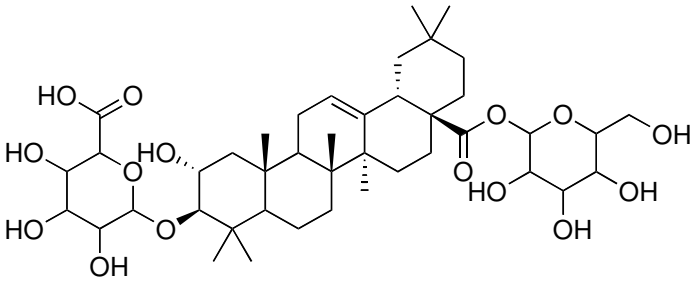
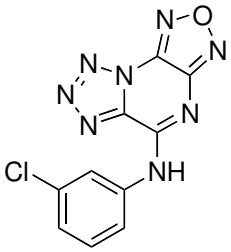
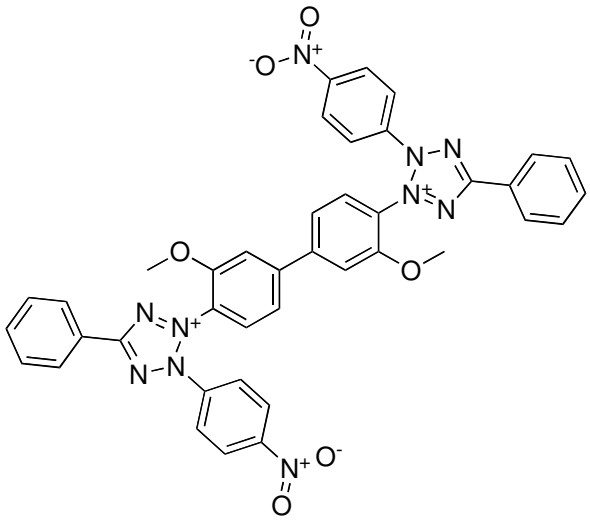
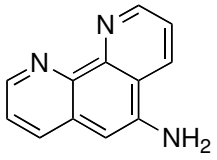


MIC=n.d.
EC=n.d.

13



MIC<0.78
EC=1.56

7  MIC=25 EC=6.25	14  MIC=6.25 EC=4.17
8  MIC<0.78 EC=6.25	15  MIC=n.d. EC=n.d.
9  MIC=n.d. EC=n.d.	16  MIC=n.d. EC=n.d.
10  MIC<0.78, EC=0.78	17  MIC=n.d. EC=n.d.

Supplementary Figure 4. Compound structures, MIC and minimum EC values of all non-antimicrobial hits excluding those shown in Table 2. The structures are presented in structural groups.

Supplementary Method 1. CellProfiler Pipeline

Module #1: LoadImages
How do you want to load these files? Text-Regular expressions
Type the text that one type of image has in common (for TEXT options), or their position in each group (for ORDER option): _w2[^_][^t]
What do you want to call these images within CellProfiler? RawBrightfield
Type the text that one type of image has in common (for TEXT options), or their position in each group (for ORDER option): _w1[6_][^t]
What do you want to call these images within CellProfiler? RawSYTOX
If using ORDER, how many images are there in each group? 3
Are you loading image or movie files? Image
Analyze all subfolders within the selected folder? Yes
Enter the path name to the folder where the images to be loaded are located. Type period (.) for default image folder.

Module #2: RescaleIntensity
What did you call the image to be rescaled? RawBrightfield
What do you want to call the rescaled image? RescaledBrightfield
Rescaling method. (S) Stretch 0 to 1

Module #3: IdentifyPrimAutomatic
What did you call the images you want to process? RescaledBrightfield
What do you want to call the objects identified by this module? WellArea
Typical diameter of objects, in pixel units (Min,Max): 100,1000
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? No
Select an automatic thresholding method or enter an absolute threshold in the range [0,1]. Otsu Global
Threshold correction factor 1
Lower and upper bounds on threshold, in the range [0,1] 0,1
Speed up by using lower-resolution image to find local maxima? (if you are distinguishing between clumped objects) Yes
Do you want to fill holes in identified objects? Yes
Do you want to run in test mode where each method for distinguishing clumped objects is compared? No

Module #4: ExpandOrShrink
What did you call the objects that you want to expand or shrink? WellArea
What do you want to call the expanded or shrunken objects? ShrunkenWell
Were the objects identified using an Identify Primary or Identify Secondary module (note: shrinking results are not perfect with Secondary objects)? Primary
Do you want to expand or shrink the objects? Shrink
Enter the number of pixels by which to expand or shrink the objects, or "Inf" to either shrink to a point or expand until almost touching, or 0 (the number zero) to simply add partial dividing lines between objects that are touching (experimental feature). 5
What do you want to call the outlines of the identified objects (optional)? WellOutline

Module #5: CorrectIllumination_Calculate
What did you call the images to be used to calculate the illumination function? RescaledBrightfield
What do you want to call the illumination function? w2illum2
Do you want to calculate using regular intensities or background intensities? Regular
For REGULAR INTENSITY: If the incoming images are binary and you want to dilate each object in the final averaged image, enter the radius (roughly equal to the original radius of the objects). Otherwise, enter 0. 0
For BACKGROUND INTENSITY: Enter the block size, which should be large enough that every square block of pixels is likely to contain some background pixels, where no objects are located. 45
Do you want to rescale the illumination function so that the pixel intensities are all equal to or greater than one (Y or N)? This is recommended if you plan to use the division option in CorrectIllumination_Apply so that the resulting images will be in the range 0 to 1. Yes
Enter Each to calculate an illumination function for Each image individually (in which case, choose Pipeline mode in the next box) or All to calculate an illumination function based on All the specified images to be corrected. See the help for details. Each

Are the images you want to use to calculate the illumination function to be loaded straight from a Load Images module, or are they being produced by the pipeline? See the help for details. Pipeline

Enter the smoothing method you would like to use, if any. Median Filtering

For MEDIAN FILTERING, SUM OF SQUARES, or SQUARE OF SUMS, specify the approximate width of the artifacts to be smoothed (in pixels), or leave the word "Automatic". 200

If you want override the above width of artifacts and set your own filter size (in pixels), please specify it here. Otherwise leave "/". /

(For "All" mode only) What do you want to call the averaged image (prior to dilation or smoothing)? (This is an image produced during the calculations - it is typically not needed for downstream modules) Do not save

What do you want to call the image after dilation but prior to smoothing? (This is an image produced during the calculations - it is typically not needed for downstream modules) Do not save

Module #6: CorrectIllumination

What did you call the image to be corrected? RescaledBrightfield

What do you want to call the corrected image? CorrBrightfield

What did you call the illumination correction function image to be used to carry out the correction (produced by another module or loaded as a .mat format image using Load Single Image)? w2illum2

How do you want to apply the illumination correction function? Divide

If you chose division, Choose rescaling method. Stretch 0 to 1

Module #7: InvertIntensity

What did you call the image to be inverted (made negative)? CorrBrightfield

What do you want to call the inverted image? InvertedBrightfield

Module #8: Crop

What did you call the image to be cropped? InvertedBrightfield

What do you want to call the cropped image? Brightfield

For RECTANGLE + ELLIPSE, into which shape would you like to crop? See the help for several other options. ShrunkenWell

Would you like to crop by typing in pixel coordinates or clicking with the mouse? Coordinates

Should the cropping pattern in the first image cycle be applied to all subsequent image cycles (First option) or should each image cycle be cropped individually? Individually

For COORDINATES + FIRST + RECTANGLE, specify the (Left, Right) pixel positions (the word "end" can be substituted for right pixel if you do not want to crop the right edge) 1,100

For COORDINATES + FIRST + RECTANGLE, specify the (Top, Bottom) pixel positions (the word "end" can be substituted for bottom pixel if you do not want to crop the bottom edge) 1,100

For COORDINATES + FIRST + ELLIPSE, what is the center pixel position of the ellipse in form X,Y? 500,500

For COORDINATES + FIRST + ELLIPSE, what is the radius of the ellipse in the X direction? 400

For COORDINATES + FIRST + ELLIPSE, what is the radius of the ellipse in the Y direction? 200

Do you want to use Plate Fix? (see Help, only used when cropping based on previously identified objects) No

Module #9: IdentifyPrimAutomatic

What did you call the images you want to process? Brightfield

What do you want to call the objects identified by this module? Worms

Typical diameter of objects, in pixel units (Min,Max): 13,200

Discard objects outside the diameter range? Yes

Try to merge too small objects with nearby larger objects? Yes

Discard objects touching the border of the image? No

Select an automatic thresholding method or enter an absolute threshold in the range [0,1]. To choose a binary image, select "Other" and type its name. Choosing "All" will use the Otsu Global method to calculate a single threshold for the entire image group. The other methods calculate a threshold for each image individually. "Set interactively" will allow you to manually adjust the threshold during the first cycle to determine what will work well. RobustBackground Global

Threshold correction factor 1

Lower and upper bounds on threshold, in the range [0,1] 0,1

For MoG thresholding, what is the approximate percentage of image covered by objects? 10 Method to distinguish clumped objects (see help for details): None

Method to draw dividing lines between clumped objects (see help for details): None

Size of smoothing filter, in pixel units (if you are distinguishing between clumped objects). Enter 0 for low resolution images with small objects (~< 5 pixel diameter) to prevent any image smoothing. Automatic

Suppress local maxima within this distance, (a positive integer, in pixel units) (if you are distinguishing between clumped objects) Automatic

Speed up by using lower-resolution image to find local maxima? (if you are distinguishing between clumped objects) Yes

Enter the following information, separated by commas, if you would like to use the Laplacian of Gaussian method for identifying objects instead of using the above settings: Size of neighborhood(height,width),Sigma,Minimum Area,Size for Weiner Filter(height,width),Threshold /

What do you want to call the outlines of the identified objects (optional)? Do not save

Do you want to fill holes in identified objects? No

Do you want to run in test mode where each method for distinguishing clumped objects is compared? No

Module #10: ConvertToImage
What did you call the objects you want to convert to an image? Worms
What do you want to call the resulting image? WormImage
What colors should the resulting image use? Binary (black & white)
For COLOR, what do you want the colormap to be? Default

Module #11: MeasureImageAreaOccupied
What did you call the images you want to process? WormImage
What do you want to call the staining measured by this module? WormArea
Select an automatic thresholding method or enter an absolute threshold in the range [0,1]. Choosing "All" will use the Otsu Global method to calculate a single threshold for the entire image group. The other methods calculate a threshold for each image individually. Set interactively will allow you to manually adjust the threshold to determine what will work well. .5
Threshold correction factor 1
Lower and upper bounds on threshold, in the range [0,1] 0,1
For MoG thresholding, what is the approximate percentage of image covered by objects? 10
Module #12: SaveImages revision - 12
What did you call the images you want to save? If you would like to save an entire figure, enter the module number here WormImage
Which images" original filenames do you want use as a base for these new images" filenames? Your choice MUST be images loaded directly with a Load module. Alternately, type N to use sequential numbers for the file names, or type =DesiredFilename to use the single file name you specify (replace DesiredFilename with the name you actually want) for all files (this is *required* when saving an avi movie). RawBrightfield
Enter text to append to the image name, type N to use sequential numbers, or leave "" to not append anything. ALL
In what file format do you want to save images (figures must be saved as fig, which is only openable in Matlab)? jpg
Enter the pathname to the directory where you want to save the images. Type period (.) for default output directory. .
Enter the bit depth at which to save the images (Note: some image formats do not support saving at a bit depth of 12 or 16; see Matlab"s imwrite function for more details.) 8
Do you want to always check whether you will be overwriting a file when saving images? No
At what point in the pipeline do you want to save the image? When saving in avi (movie) format, choose Every cycle. Every cycle
If you are saving in avi (movie) format, do you want to save the movie only after the last cycle is processed (enter "L"), or after every Nth cycle (1,2,3...)? Saving movies is time-consuming. See the help for this module for more details. L
Do you want to rescale the images to use a full 8 bit (256 graylevel) dynamic range (Y or N)? Use the RescaleIntensity module for other rescaling options. No
For grayscale images, specify the colormap to use (see help). This is critical for movie (avi) files. Choosing anything other than gray may degrade image quality or result in image stretching. gray
Enter any optional parameters here ("Quality",1 or "Quality",100 etc.) or leave / for no optional parameters. /
Update file names within CellProfiler? See help for details. No
Warning! It is possible to overwrite existing files using this module! n/a

Module #13: RescaleIntensity
What did you call the image to be rescaled? RawSYTOX
What do you want to call the rescaled image? ScaledSYTOX
Rescaling method. (S) Stretch the image (0 to 1). (E) Enter the minimum and maximum values in the boxes below. (G) rescale so all pixels are equal to or Greater than one. (M) Match the maximum of one image to the maximum of another. (C) Convert to 8 bit. See the help for details. Enter min/max below
(Method E only): Enter the intensity from the original image that should be set to the lowest value in the rescaled image, or type AA to calculate the lowest intensity automatically from all of the images to be analyzed and AE to calculate the lowest intensity from each image independently. 0
(Method E only): Enter the intensity from the original image that should be set to the highest value in the rescaled image, or type AA to calculate the highest intensity automatically from all of the images to be analyzed and AE to calculate the highest intensity from each image independently. .0625
(Method E only): What should the lowest intensity of the rescaled image be (range [0,1])? 0
(Method E only): What should the highest intensity of the rescaled image be (range [0,1])? 1
(Method M only): What did you call the image whose maximum you want the rescaled image to match? RawBrightfield

Module #14: Crop revision
What did you call the image to be cropped? ScaledSYTOX
What do you want to call the cropped image? SYTOX
For RECTANGLE + ELLIPSE, into which shape would you like to crop? See the help for several other options. ShrunkenWell
Would you like to crop by typing in pixel coordinates or clicking with the mouse? Coordinates
Should the cropping pattern in the first image cycle be applied to all subsequent image cycles (First option) or should each image cycle be cropped individually? Individually
For COORDINATES + FIRST + RECTANGLE, specify the (Left, Right) pixel positions (the word "end" can be substituted for right pixel if you do not want to crop the right edge) 1,100
For COORDINATES + FIRST + RECTANGLE, specify the (Top, Bottom) pixel positions (the word "end" can be substituted for bottom pixel if you do not want to crop the bottom edge) 1,100

For COORDINATES + FIRST + ELLIPSE, what is the center pixel position of the ellipse in form X,Y? 500,500
For COORDINATES + FIRST + ELLIPSE, what is the radius of the ellipse in the X direction? 400
For COORDINATES + FIRST + ELLIPSE, what is the radius of the ellipse in the Y direction? 200
Do you want to use Plate Fix? (see Help, only used when cropping based on previously identified objects) No

Module #15: IdentifyPrimAutomatic
What did you call the images you want to process? SYTOX
What do you want to call the objects identified by this module? DeadWorms
Typical diameter of objects, in pixel units (Min,Max): 13,200
Discard objects outside the diameter range? Yes
Try to merge too small objects with nearby larger objects? Yes
Discard objects touching the border of the image? No
Select an automatic thresholding method or enter an absolute threshold in the range [0,1]. To choose a binary image, select "Other" and type its name. Choosing "All" will use the Otsu Global method to calculate a single threshold for the entire image group. The other methods calculate a threshold for each image individually. "Set interactively" will allow you to manually adjust the threshold during the first cycle to determine what will work well. Otsu Global
Threshold correction factor .8
Lower and upper bounds on threshold, in the range [0,1] 0.03,1
For MoG thresholding, what is the approximate percentage of image covered by objects? 10 Method to distinguish clumped objects (see help for details): None
Method to draw dividing lines between clumped objects (see help for details): None
Size of smoothing filter, in pixel units (if you are distinguishing between clumped objects). Enter 0 for low resolution images with small objects (~< 5 pixel diameter) to prevent any image smoothing. Automatic
Suppress local maxima within this distance, (a positive integer, in pixel units) (if you are distinguishing between clumped objects) Automatic
Speed up by using lower-resolution image to find local maxima? (if you are distinguishing between clumped objects) Yes
Enter the following information, separated by commas, if you would like to use the Laplacian of Gaussian method for identifying objects instead of using the above settings: Size of neighborhood(height,width),Sigma,Minimum Area,Size for Weiner Filter(height,width),Threshold /
What do you want to call the outlines of the identified objects (optional)? Do not save
Do you want to fill holes in identified objects? No
Do you want to run in test mode where each method for distinguishing clumped objects is compared? No

Module #16: MeasureObjectAreaShape
What did you call the objects that you want to measure? DeadWorms
Would you like to calculate the Zernike features for each object (with lots of objects, this can be very slow)? No

Module #17: MeasureObjectIntensity
What did you call the greyscale images you want to measure? SYTOX
What did you call the objects that you want to measure? DeadWorms

Module #18: ConvertToImage
What did you call the objects you want to convert to an image? DeadWorms
What do you want to call the resulting image? DeadWormImage
What colors should the resulting image use? Binary (black & white)
For COLOR, what do you want the colormap to be? Default

Module #19: MeasureImageAreaOccupied
What did you call the images you want to process? DeadWormImage
What do you want to call the staining measured by this module? DeadWormArea
Select an automatic thresholding method or enter an absolute threshold in the range [0,1]. Choosing "All" will use the Otsu Global method to calculate a single threshold for the entire image group. The other methods calculate a threshold for each image individually. Set interactively will allow you to manually adjust the threshold to determine what will work well. .5
Threshold correction factor 1
Lower and upper bounds on threshold, in the range [0,1] 0,1
For MoG thresholding, what is the approximate percentage of image covered by objects? 10
Module #20: SaveImages revision - 12
What did you call the images you want to save? If you would like to save an entire figure, enter the module number here DeadWormImage
Which images" original filenames do you want use as a base for these new images" filenames? Your choice MUST be images loaded directly with a Load module. Alternately, type N to use sequential numbers for the file names, or type =DesiredFilename to use the single file name you specify (replace DesiredFilename with the name you actually want) for all files (this is *required* when saving an avi movie). RawSYTOX
Enter text to append to the image name, type N to use sequential numbers, or leave "" to not append anything. DEAD
In what file format do you want to save images (figures must be saved as fig, which is only openable in Matlab)? jpg
Enter the pathname to the directory where you want to save the images. Type period (.) for default output directory. .

Enter the bit depth at which to save the images (Note: some image formats do not support saving at a bit depth of 12 or 16; see Matlab"s imwrite function for more details.) 8
Do you want to always check whether you will be overwriting a file when saving images? No
At what point in the pipeline do you want to save the image? When saving in avi (movie) format, choose Every cycle. Every cycle
If you are saving in avi (movie) format, do you want to save the movie only after the last cycle is processed (enter "L"), or after every Nth cycle (1,2,3...)? Saving movies is time-consuming. See the help for this module for more details. L
Do you want to rescale the images to use a full 8 bit (256 graylevel) dynamic range (Y or N)? Use the RescaleIntensity module for other rescaling options. No
For grayscale images, specify the colormap to use (see help). This is critical for movie (avi) files. Choosing anything other than gray may degrade image quality or result in image stretching. gray
Enter any optional parameters here ("Quality",1 or "Quality",100 etc.) or leave / for no optional parameters. /
Update file names within CellProfiler? See help for details. No
Warning! It is possible to overwrite existing files using this module! n/a

Module #21: Subtract
Subtract this image: WormImage
From this image: DeadWormImage
What do you want to call the resulting image? SubtractTotalfrDead
Enter the factor to multiply the first image by before subtracting: 1
Enter the factor to multiply the second image by before subtracting: 1
Do you want negative values in the image to be set to zero? Yes

Module #22: Subtract
Subtract this image: SubtractTotalfrDead
From this image: DeadWormImage
What do you want to call the resulting image? SubtractedDeadFinal
Enter the factor to multiply the first image by before subtracting: 1
Enter the factor to multiply the second image by before subtracting: 1
Do you want negative values in the image to be set to zero? Yes

Module #23: MeasureImageAreaOccupied
What did you call the images you want to process? SubtractedDeadFinal
What do you want to call the staining measured by this module? SubtrDeadWorms
Select an automatic thresholding method or enter an absolute threshold in the range [0,1]. Choosing "All" will use the Otsu Global method to calculate a single threshold for the entire image group. The other methods calculate a threshold for each image individually. Set interactively will allow you to manually adjust the threshold to determine what will work well. 0.5
Threshold correction factor 1
Lower and upper bounds on threshold, in the range [0,1] 0,1
For MoG thresholding, what is the approximate percentage of image covered by objects? 10
Module #24: SaveImages revision - 12
What did you call the images you want to save? If you would like to save an entire figure, enter the module number here SubtractedDeadFinal
Which images" original filenames do you want use as a base for these new images" filenames? Your choice MUST be images loaded directly with a Load module. Alternately, type N to use sequential numbers for the file names, or type =DesiredFilename to use the single file name you specify (replace DesiredFilename with the name you actually want) for all files (this is *required* when saving an avi movie). RawSYTOX
Enter text to append to the image name, type N to use sequential numbers, or leave "" to not append anything. SUBTRD
In what file format do you want to save images (figures must be saved as fig, which is only openable in Matlab)? jpg
Enter the pathname to the directory where you want to save the images. Type period (.) for default output directory. .
Enter the bit depth at which to save the images (Note: some image formats do not support saving at a bit depth of 12 or 16; see Matlab"s imwrite function for more details.) 8
Do you want to always check whether you will be overwriting a file when saving images? Yes
At what point in the pipeline do you want to save the image? When saving in avi (movie) format, choose Every cycle. Every cycle
If you are saving in avi (movie) format, do you want to save the movie only after the last cycle is processed (enter "L"), or after every Nth cycle (1,2,3...)? Saving movies is time-consuming. See the help for this module for more details. L
Do you want to rescale the images to use a full 8 bit (256 graylevel) dynamic range (Y or N)? Use the RescaleIntensity module for other rescaling options. No
For grayscale images, specify the colormap to use (see help). This is critical for movie (avi) files. Choosing anything other than gray may degrade image quality or result in image stretching. gray
Enter any optional parameters here ("Quality",1 or "Quality",100 etc.) or leave / for no optional parameters. /
Update file names within CellProfiler? See help for details. No
Warning! It is possible to overwrite existing files using this module! n/a

Module #25: OverlayOutlines
On which image would you like to display the outlines? RescaledBrightfield
What did you call the outlines that you would like to display? WellOutline

Would you like to set the intensity (brightness) of the outlines to be the same as the brightest point in the image, or the maximum possible value for this image format? Max of image
What do you want to call the image with the outlines displayed? WellBrightfield
For color images, what do you want the color of the outlines to be? White

Module #26: OverlayOutlines
On which image would you like to display the outlines? ScaledSYTOX
What did you call the outlines that you would like to display? WellOutline
Would you like to set the intensity (brightness) of the outlines to be the same as the brightest point in the image, or the maximum possible value for this image format? Max of image
What do you want to call the image with the outlines displayed? WellSYTOX
For color images, what do you want the color of the outlines to be? White

Module #27: SaveImages
What did you call the images you want to save? If you would like to save an entire figure, enter the module number here WellBrightfield
Which images'' original filenames do you want use as a base for these new images'' filenames? Your choice MUST be images loaded directly with a Load module. Alternately, type N to use sequential numbers for the file names, or type =DesiredFilename to use the single file name you specify (replace DesiredFilename with the name you actually want) for all files (this is *required* when saving an avi movie). RawBrightfield
Enter text to append to the image name, type N to use sequential numbers, or leave "" to not append anything. WELL
In what file format do you want to save images (figures must be saved as fig, which is only openable in Matlab)? jpg
Enter the pathname to the directory where you want to save the images. Type period (.) for default output directory. .
Enter the bit depth at which to save the images (Note: some image formats do not support saving at a bit depth of 12 or 16; see Matlab''s imwrite function for more details.) 8
Do you want to always check whether you will be overwriting a file when saving images? No
At what point in the pipeline do you want to save the image? When saving in avi (movie) format, choose Every cycle. Every cycle
If you are saving in avi (movie) format, do you want to save the movie only after the last cycle is processed (enter "L"), or after every Nth cycle (1,2,3...)? Saving movies is time-consuming. See the help for this module for more details. L
Do you want to rescale the images to use a full 8 bit (256 graylevel) dynamic range (Y or N)? Use the RescaleIntensity module for other rescaling options. No
For grayscale images, specify the colormap to use (see help). This is critical for movie (avi) files. Choosing anything other than gray may degrade image quality or result in image stretching. gray
Enter any optional parameters here (''Quality'',1 or "Quality",100 etc.) or leave / for no optional parameters. /
Update file names within CellProfiler? See help for details. No
Warning! It is possible to overwrite existing files using this module! n/a

Module #28: SaveImages
What did you call the images you want to save? If you would like to save an entire figure, enter the module number here WellSYTOX
Which images'' original filenames do you want use as a base for these new images'' filenames? Your choice MUST be images loaded directly with a Load module. Alternately, type N to use sequential numbers for the file names, or type =DesiredFilename to use the single file name you specify (replace DesiredFilename with the name you actually want) for all files (this is *required* when saving an avi movie). RawSYTOX
Enter text to append to the image name, type N to use sequential numbers, or leave "" to not append anything. WELL1
In what file format do you want to save images (figures must be saved as fig, which is only openable in Matlab)? jpg
Enter the pathname to the directory where you want to save the images. Type period (.) for default output directory. .
Enter the bit depth at which to save the images (Note: some image formats do not support saving at a bit depth of 12 or 16; see Matlab''s imwrite function for more details.) 8
Do you want to always check whether you will be overwriting a file when saving images? No
At what point in the pipeline do you want to save the image? When saving in avi (movie) format, choose Every cycle. Every cycle
If you are saving in avi (movie) format, do you want to save the movie only after the last cycle is processed (enter "L"), or after every Nth cycle (1,2,3...)? Saving movies is time-consuming. See the help for this module for more details. L
Do you want to rescale the images to use a full 8 bit (256 graylevel) dynamic range (Y or N)? Use the RescaleIntensity module for other rescaling options. No
For grayscale images, specify the colormap to use (see help). This is critical for movie (avi) files. Choosing anything other than gray may degrade image quality or result in image stretching. gray
Enter any optional parameters here (''Quality'',1 or "Quality",100 etc.) or leave / for no optional parameters. /
Update file names within CellProfiler? See help for details. No
Warning! It is possible to overwrite existing files using this module! n/a

Module #29: CalculateRatios
What do you want to call the ratio calculated by this module? RatioDeadtoTotal
Which object would you like to use for the numerator? Image
Which category of measurements would you like to use? AreaOccupied_SubtrDeadWorms
Which feature do you want to use? (Enter the feature number - see help for details) 1

For INTENSITY or TEXTURE features, which image"s measurements would you like to use? RawBrightfield
Which object would you like to use for the denominator? Image
Which category of measurements would you like to use? AreaOccupied_WormArea
Which feature do you want to use? (Enter the feature number - see help for details) 1
For INTENSITY or TEXTURE features, which image"s measurements would you like to use? RawBrightfield
Do you want the log (base 10) of the ratio? No

Module #30: ExportToExcel
Which objects do you want to export? Image

Supplementary Method 2. Liquid Chromatography Mass Spectrometry

Tandem high performance liquid chromatography/mass spectral (LC/MS) analyses were performed on a Waters Corporation 3100 Mass Detector in positive and negative electrospray ionization (ESI) mode after separation on a Waters separation module (2545 Binary Gradient Module). The actual separations were performed on a Waters Xterra column C18 (5μm, 4.6 x 50 mm) with a flow rate of 5 mL/min and a 2 min gradient of 5-100% CH3CN in H2O, with a constant 0.1% formic acid buffer. Elution chromatograms were acquired using a Waters 2998 photodiode array (PDA) detector monitoring 200-400nm. Purity was determined by integration at 214nm.