

# Appendix A

## ImageJ Macros

The ImageJ macros and Python files described in this appendix are available at [https://github.com/nwespe/ImageJ\\_functions](https://github.com/nwespe/ImageJ_functions). To install the macros, copy the files to the ImageJ or Fiji application plugins folder and restart the application.

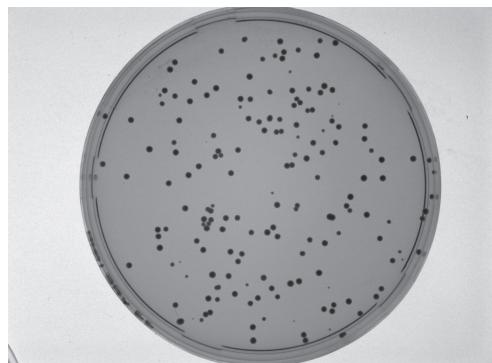
### A.1 Colony counting

1. Take images of plates facing up with trans-illuminating white light. Settings used on Gel-Doc: aperture = 8.00, zoom = 25, focus = 2.0, exposure = 75 ms.
2. In ImageJ/Fiji, run “auto count” from Plugins folder. Select folder containing plate images.
3. Run “manual count” from Plugins folder, selecting same folder as before. Click on unmarked colonies, typically around the edge of the plate. Colony counts (auto, manual, and total) are saved in a text file in the folder selected before.

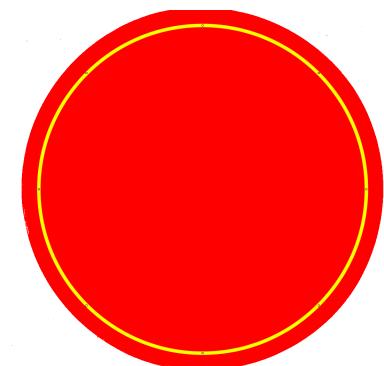
## Colony counting: “auto count” processing steps

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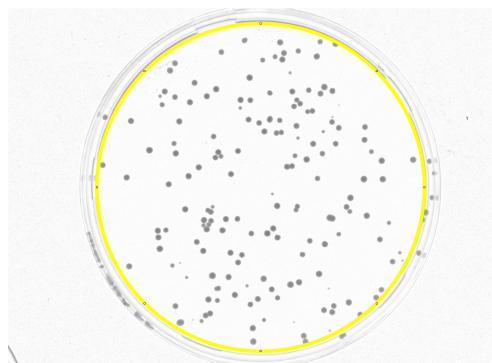
Input image: “somecolonies.tif”



Convert to 8-bit, duplicate as mask and auto-threshold to find plate. Fit circle and shrink to define area for auto particle analysis.



Subtract background of original image and add circle selection defined above.



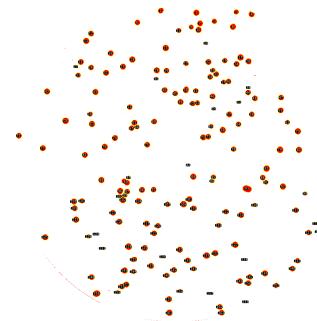
Clear outside and convert to binary. Run Watershed algorithm.



## Colony counting: “auto count” processing steps (continued)

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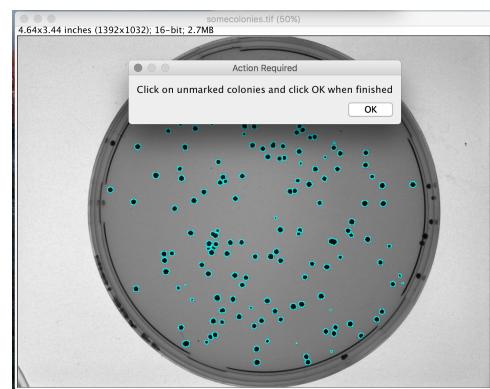
Run “Analyze particles.”



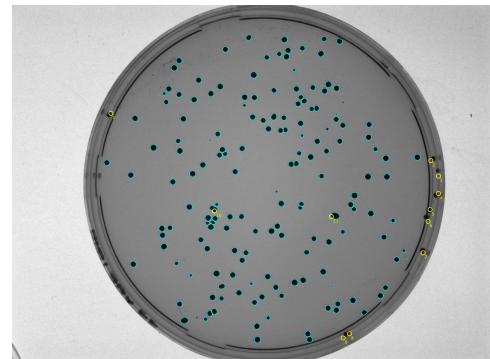
## Colony counting: “manual count” processing steps

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Add outlines of auto-counted colonies to original image and ask user to click on uncounted colonies.



Save original image with overlay of auto-counted and manually counted colonies.



Save text file with auto, manual and total counts for all images: “Colony counts.txt”

	Image	Auto count	Manual count	Total
1	lotsofcolonies.tif	352	11	363
2	somecolonies.tif	162	11	173

## A.2 Frogger sample splitting

1. Take images of plates facing up with trans-illuminating white light. Settings used on Gel-Doc: aperture = 8.00, zoom = 25, focus = 2.0, exposure = 75 ms. The next three steps are run directly from ImageJ/Fiji.
2. Place plate images in a folder. Select “batch plate cropper” from Plugins folder. Select folder with images. Move yellow box to be centered over samples.  
Subroutine information: batch\_plate\_cropper.py calls plate\_cropper.py, which calls save\_-roi.ijm.
3. Select “batch montage” from Plugins folder. Select “Individual” folder created by “batch plate cropper” in first step. Select destination folder.  
Subroutine information: batch\_montage.py calls create\_montage.ijm.
4. Select “combine experiment” from Plugins folder. Select “Montages” folder created by “batch montage” in second step. Select destination folder.  
Subroutine information: combine\_experiment.py calls place\_image.ijm.

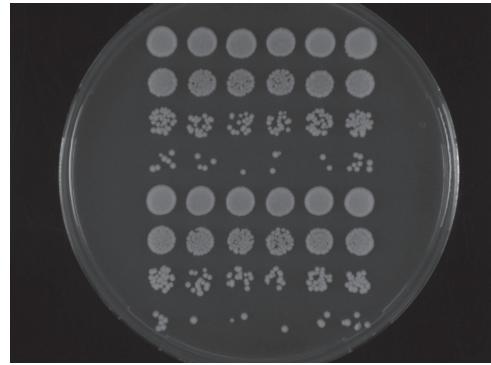
The strain sorting functions below are run from the command line or an ipython notebook and require a CSV file containing information about each sample.

5. strain\_sorter.py: The montage files are copied to a folder created for each strain and named with the information provided. This program could be modified to sort by and include any information, not just strain and medium.
6. strain\_report.py: All montage image files in a folder created by strain\_sorter.py are compiled into a single PDF file.

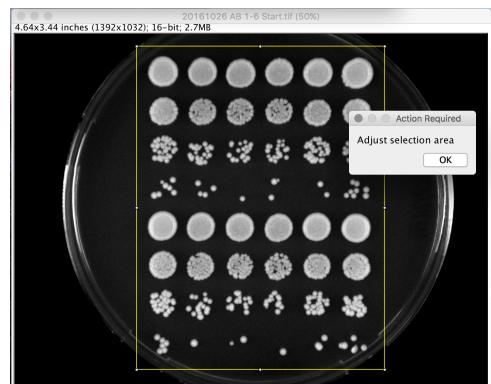
## Frogger sample splitting: “batch plate cropper” processing steps

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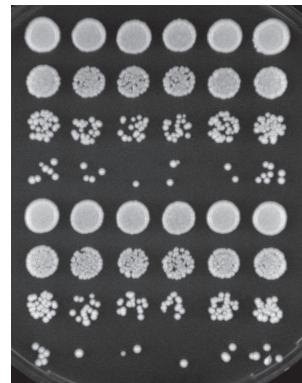
Input image: “20161026 AB 1-6 Start.tif”



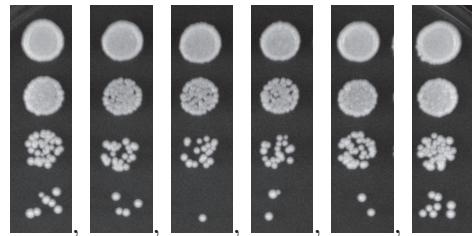
Adjust brightness and contrast.  
Ask user to move selection box to be  
centered over samples.



Crop image and save in “Adjusted” folder.



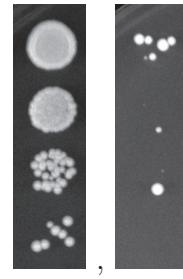
Select regions of individual dilution series  
and save as separate files in “Individual”  
folder.



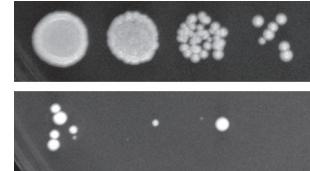
## Frogger sample splitting: “batch montage” processing steps

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Input images: “20161026 A1 Start.tif” and  
“20161026 A1 End.tif.” Program matches each start  
image with its corresponding end image.



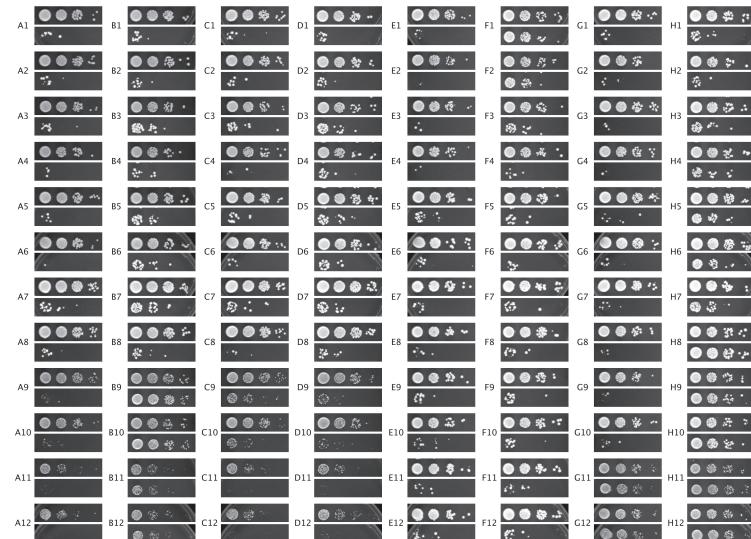
Rotate each image, combine into one image  
file, and save in “Montages” folder.



## Frogger sample splitting: “combine experiment” processing steps

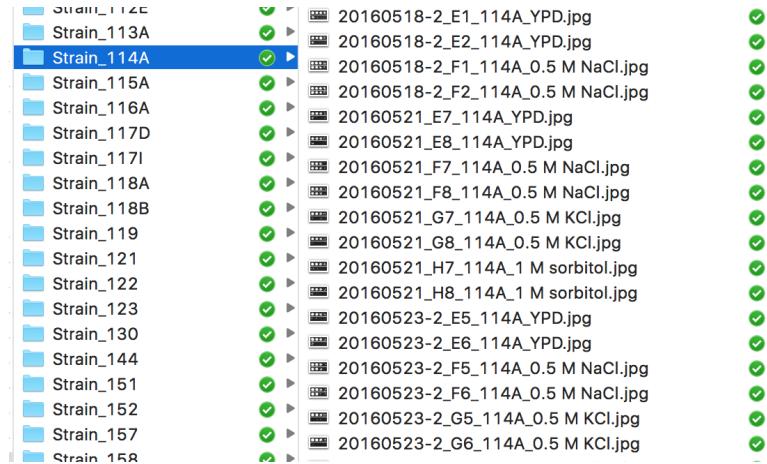
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All montage images from  
one experiment are  
arranged in a single PDF  
document.

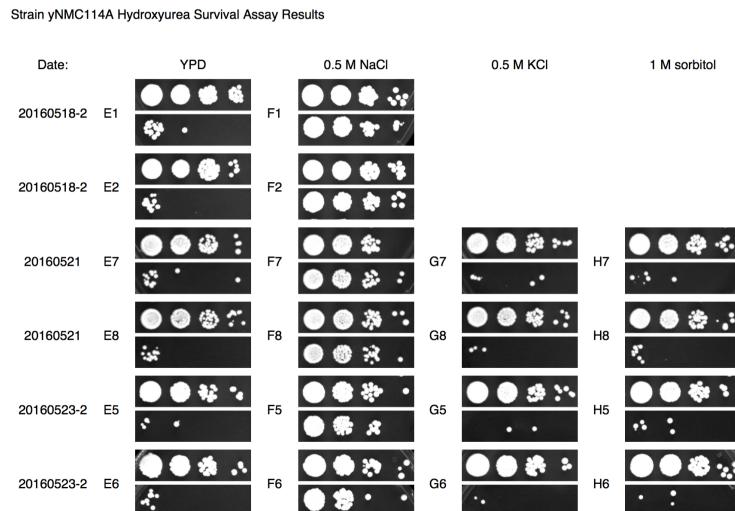


## Frogger sample splitting: sorting and compiling results

Sort montage image files into folders by strain or other characteristic.



Compile report of samples for each strain.



## A.3 Evaluating hydroxyurea survival

The first step is run directly from ImageJ/Fiji and operates on the montage images created by the frogger splitting functions described above.

1.     a. Select “batch survival strain” from Plugins folder. Select folder containing subfolders with montage images for each strain.

Subroutine information: batch\_survival\_strain.py calls analyze\_pixels.ijm.

Output: strainid\_pixel\_analysis.csv file for each strain; “Regions” folder in each strain folder containing copy of each montage image with regions marked.

- b. Alternatively, select “batch survival expt” to run analysis on a single experiment instead of on images grouped by strain.

Output: one pixel\_analysis.csv file; “Regions” folder containing copy of each montage image with regions marked.

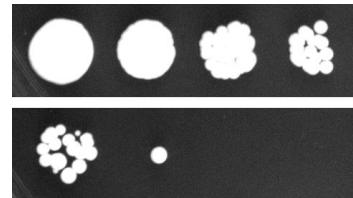
2. HU\_strain\_analysis.py is run from the command line. This uses the pixel analysis files generated in step 1. This program computes end to start area ratios and determine the maximum dilution at which the pixel ratio is at least 0.5. This program also creates graphs displaying the maximum dilution results grouped by media condition.

Output: one summary file plus three files for each strain: strainid\_ratios.csv, strainid\_max\_dilutions.csv, and strainid\_HU\_survival.png.

## Hydroxyurea survival: “batch survival strain” processing steps

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Input image:  
“20160518 E1 114 YPD.jpg”



Select first dilution series in montage,  
duplicate, threshold, and convert to mask.



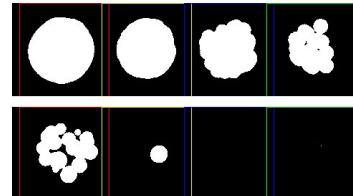
Select region containing first spot,  
duplicate, invert, and measure pixel area.



Repeat for each spot.



Repeat process for second dilution series in  
montage. Mark spot selection regions on  
dilution series image and save in “Regions”  
folder.



Save CSV file containing area  
measurements.

Area	Dilution	Date	Well	Strain	Condition	Time
6682	1	20160518	E1	114	YPD	start
5501	2	20160518	E1	114	YPD	start
5053	3	20160518	E1	114	YPD	start
3565	4	20160518	E1	114	YPD	start
4202	1	20160518	E1	114	YPD	end
472	2	20160518	E1	114	YPD	end
0	3	20160518	E1	114	YPD	end
1	4	20160518	E1	114	YPD	end

## Hydroxyurea survival: “HU strain analysis” example output

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The start and end area measurements for each sample are matched, and the end/start ratio is calculated.

Dilution	Area_start	Area_end	Ratio
1	6682	4202	0.629
2	5501	472	0.086
3	5053	0	0.000
4	3565	1	0.000

The maximum dilution level at which the ratio is still at least 0.5 is determined.

Max Dilution	Date	Well	Strain	Condition
1	20160518	E1	114A	YPD
1	20160518	E2	114A	YPD
3	20160518	F1	114A	0.5 M NaCl
4	20160518	F2	114A	0.5 M NaCl

Maximum dilutions are compiled by strain and condition, and a survival level is assigned based on the mean value.

Condition	N	Strain	mean	median	std	Survival
YPD	14	113A	0.14285714	0	0.53452248	-
0.5 M NaCl	13	113A	3.61538462	4	0.76794765	+++
0.5 M KCl	8	113A	0.5	0.5	0.53452248	-
1 M sorbitol	8	113A	0.25	0	0.46291005	-
YPD	12	114A	0.58333333	0.5	0.66855792	-
0.5 M NaCl	12	114A	3.25	3	0.62158156	+++
0.5 M KCl	8	114A	0.375	0	0.74402381	-
1 M sorbitol	8	114A	0.5	0	0.9258201	-

Maximum dilutions are also graphed by condition for each strain.

