

Protocol for generating training data in ImageJ (or Fiji)



1. Make sure that ImageJ or Fiji is updated and the necessary macros are uploaded
 - a. *RGB_measure* may come standard, if it did not you can download the java file and drag and drop it into the GUI.
 - b. Depending on the version of macro the output may be different from what is presented here.
 - c. This is not a problem except the portion of the R script that reads the ImageJ csv output will need to be modified.
 - d. All of the examples on the GitHub page come with pre-made metadata so you can see what the ImageJ files look like for it to work with the current system



2. Change the settings in the *Set Measurement* tab so the columns work with the R script

<input checked="" type="checkbox"/> Area	<input checked="" type="checkbox"/> Mean gray value
<input type="checkbox"/> Standard deviation	<input type="checkbox"/> Modal gray value
<input type="checkbox"/> Min & max gray value	<input type="checkbox"/> Centroid
<input type="checkbox"/> Center of mass	<input type="checkbox"/> Perimeter
<input type="checkbox"/> Bounding rectangle	<input type="checkbox"/> Fit ellipse
<input type="checkbox"/> Shape descriptors	<input type="checkbox"/> Feret's diameter
<input type="checkbox"/> Integrated density	<input type="checkbox"/> Median
<input type="checkbox"/> Skewness	<input type="checkbox"/> Kurtosis
<input type="checkbox"/> Area fraction	<input type="checkbox"/> Stack position
<input checked="" type="checkbox"/> Limit to threshold	<input checked="" type="checkbox"/> Display label
<input type="checkbox"/> Invert Y coordinates	<input type="checkbox"/> Scientific notation
<input type="checkbox"/> Add to overlay	
Redirect to: <input type="text" value="None"/>	
Decimal places (0-9): <input type="text" value="3"/>	
<input type="button" value="Help"/> <input type="button" value="Cancel"/> <input type="button" value="OK"/>	

3. Download your image or use an image from your hard drive
 - a. The image shown here is a perennial ryegrass spaced plant
 - b. Several features are present: background (imaging mat, rocks, soil...), healthy plant tissue, and diseased plant tissue.



4. Drag and drop your image into ImageJ
 - a. Zoom in on a portion of the image that you want to select
 - b. Use the multi-point selection tool to click on pixels that you want to classify
 - c. In the following image the background is being classified



5. Use the RGB measure function to measure your selections
 - a. Save this as a csv file
 - b. Name the file something meaningful, such as image0001_background.01
 - c. Keep all of the files in a training data folder specific to a project

	Label	Area	Mean	X	Y
1	PRG_7102.jpg	0	162	952.750	724.500
2	PRG_7102.jpg	0	154	956.750	723.500
3	Red	0	157	956.500	726.750
4	PRG_7102.jpg	0	140	952.750	724.500
5	PRG_7102.jpg	0	138	956.750	723.500
6	Green	0	133	956.500	726.750
7	PRG_7102.jpg	0	65	952.750	724.500
8	PRG_7102.jpg	0	61	956.750	723.500
9	Blue	0	59	956.500	726.750
10	PRG_7102.jpg	0	122	952.750	724.500
11	PRG_7102.jpg	0	118	956.750	723.500
12	(R+G+B)/3	0	116	956.500	726.750
13	PRG_7102.jpg	0	138	952.750	724.500
14	PRG_7102.jpg	0	134	956.750	723.500
15	0.299R+0.587G+0.114B	0	132	956.500	726.750

6. Next classify the foreground
 - a. Here the foreground is everything but the soil, red imaging mat and rocks
 - b. So the foreground will contain both healthy and diseased tissue
 - c. Measure these as well using the RGB Measure function and save using another name such as image0001_foreground.01



7. Now measure the pixels that look like diseased or in this case rust pustules and save this csv file with a name like image0001_disease.01



8. This process should be repeated across several images so that there are around 1200 total observations in the training data folder
9. Most images and features can be characterized with around this many thoughtful observations, but doing more will not hurt
10. Use the R script provided in each example in lines ~101-131 under the training data code chunk to make models for whatever is being classified