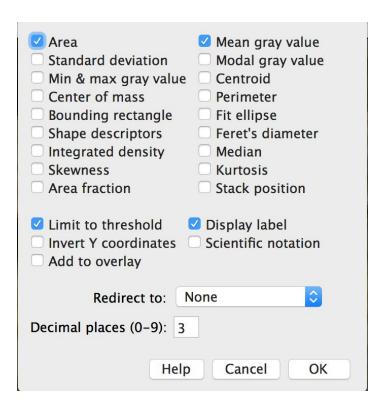
## Protocol for generating training data in ImageJ (or Fiji)



- 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



- 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       | Υ       |
|----|----------------------|------|------|---------|---------|
| 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