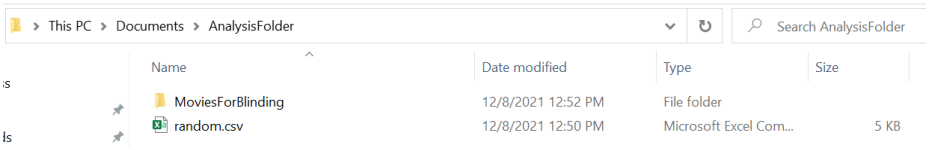
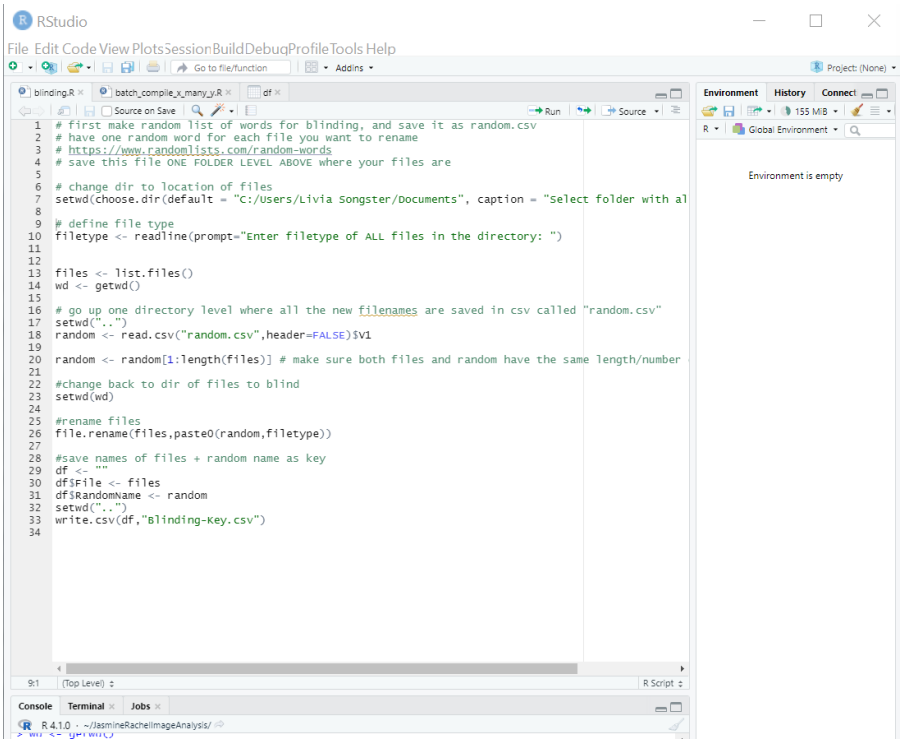
Blinding your data

1. Move all the files you want blinded into a new folder (aka directory). You can name this folder whatever you like, just make sure it is intuitive for you so you know/remember what it is.
    a. Everything in this folder MUST be the same file type (typically .nd2 or .tif).
    b. Make sure you have these files saved in another location in case you need to go back to the original files.
2. One level above where your files are, save a copy of random.csv. This contains all the random new names that will be assigned to the files you wish to blind. The default random.csv file I provide has 600 file names. Feel welcome to change or add to this list as you wish. Example directory layout for analysis is below.

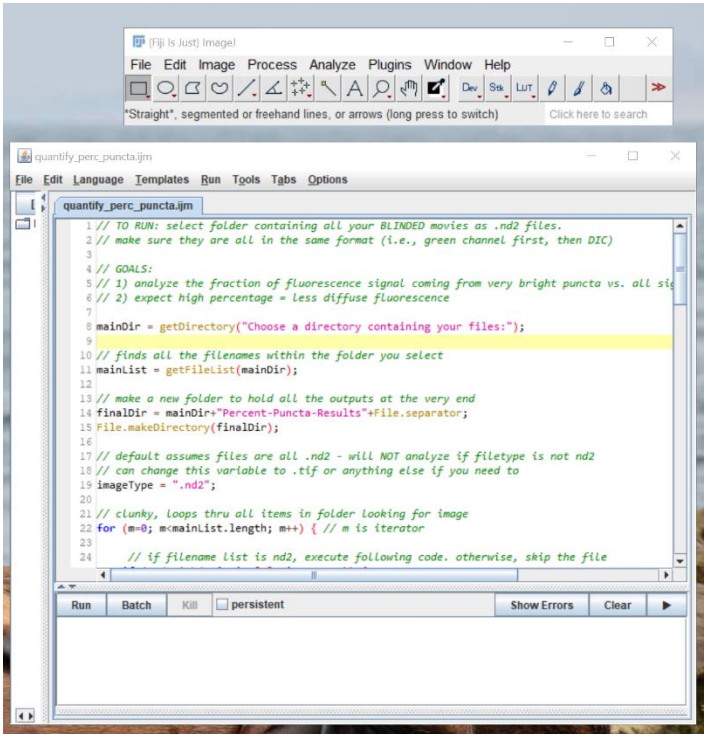


3. Open blinding.R in Rstudio. This is how it should look.

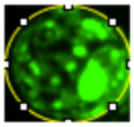


4. Run through the lines of code one at a time. Click on the first line and then type CTRL+Enter, or use your mouse to click “Run” at the top of the code. When prompted on line 7, select the directory containing the files you want blinded. In the screenshot above, this would be the folder labeled MoviesForBlinding.
5. In line 10, the code will ask you to define the file type. After running this line, click on the console below (next to the > at the bottom) and type the image type (like .nd2 or .tif)
6. Continue running through the code until you reach the end
    a. IF the code ever gives you an error, try googling it or message Livia!
    b. Remember, this will change the name of EVERY file in the folder you select, so be careful and make sure if you input files are .nd2, you are outputting them in the same format, otherwise you risk loss of data.

Percent Puncta Analysis

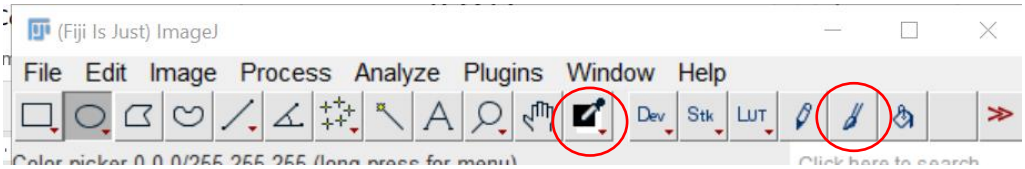


1. Open FIJI. Update if it asks. (always close and restart after updating)
2. Open quantify_perc_puncta.ijm in FIJI so you can edit and see the code – there are two ways to do this
    a. Drag and drop quantify_perc_puncta.ijm into FIJI
    b. In FIJI, go to Plugins > Macros > Edit… and select quantify_perc_puncta.ijm
3. This is how it should look (right). Next you can click “Run” and the program will prompt you to select the directory containing your images for analysis – AKA the directory that contains blinded files.
    a. As you can see in line 19, the default input is .nd2 files. If you want to run this on a .tif, you will need to MANUALLY go into the code and change .nd2 to .tif on line 19 prior to clicking Run.
4. When prompted, trace the cells and press “t” to save them as a region of interest/ROI. Try to trace as accurately as you can (see right). Be consistent in which types of cells you choose to include in the analysis. You can click and drag the little white dots to adjust the oval to better fit before you press t to save.

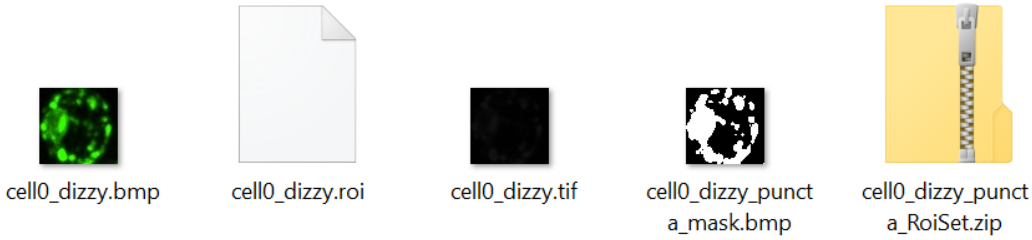


    a. Tip: check the “Show all” box on the ROI manager if you want to check / see where all your ovals currently are

5. Once you are done, click OK on the popup. Now the program will ask you to manually correct the puncta masks. White = an area that the program will detect as "puncta". You will need to erase any white areas that are NOT within the main cell that is entirely in view – for example, any extra cells or background that is in the crop but not your cell of interest.
    a. click the paintbrush tool to change its pixel width (circled below).
    b. Make sure you are drawing with 100% black – you can check this using the eyedropper tool (also circled below).
    c. Use Ctrl+z to undo your most recent change to the image. Note you can only ctrl-z to undo actions once; there is not a long memory log of all the changes you make so be careful!



6. Once you are done with the mask, click OK and continue through the rest of the script until all your movies are analyzed.
7. DOUBLE CHECK THE OUTPUT FILES as needed. Each movie that was analyzed will produce a new analysis folder that contains the following files for each cell



    a. cell crop (cellX_filename.bmp for easy checking in windows/mac photo viewer and cellX_filename.tif to reanalyze)
    b. the roi for the cell crop (cellX_filename.roi). you can open the original movie in imagej and then drag-drop this .roi to check where the cell was from on the original movie
    c. the puncta mask (cellX_filename_puncta_mask.bmp)
    d. the roi for all the puncta (cellX_filename_punctaROIset.zip). same deal as the .roi above; open the tif for the cell you want to check and drag-drop the punctaROIset to see all the highlighted regions