ContactAnalysis Instructions:

***** The DipImage Toolbox is required to run this program. http://www.diplib.org/

For a full explanation of this project, please see:

A New Tool to Quantify Receptor Recruitment to Cell Contact Sites during Host-Pathogen Interaction
M. Graus, C. Pehlke, M. Wester, L. Davidson, S. Steinberg, A. Neumann

1. Download ContactAnalysis files and unzip them into a folder in your matlab directory.
2. If the folder is on your matlab path, you can run the program by typing ContactAnalysis at the command line. Otherwise, you can open the ContactAnalysis.m file and hit the "run" button.
3. When you do this, the main GUI window will open:

4. Use the "browse" button to open your file(s). Allowed formats are multi-channel .oib or single channel .tiff or .tif images.

5. Once you have selected your image, click the "upload" button to begin the image upload process.
6. Once the upload process is finished, you will see that the name box has been populated with generic names for each channel. You may either use these names or change them by typing new ones in the boxes.

Channels for analysis (numbers, sep

Enter names, one per line:

NewName1
ch
chan3
chan4

X p

Y p

Display   Pick Point

7. The x and y pixel scale boxes will also be populated if this information is available in your image's metadata. If it is not available, or if the information automatically populated in these fields is incorrect, you will have to type in the correct pixel size in nm.

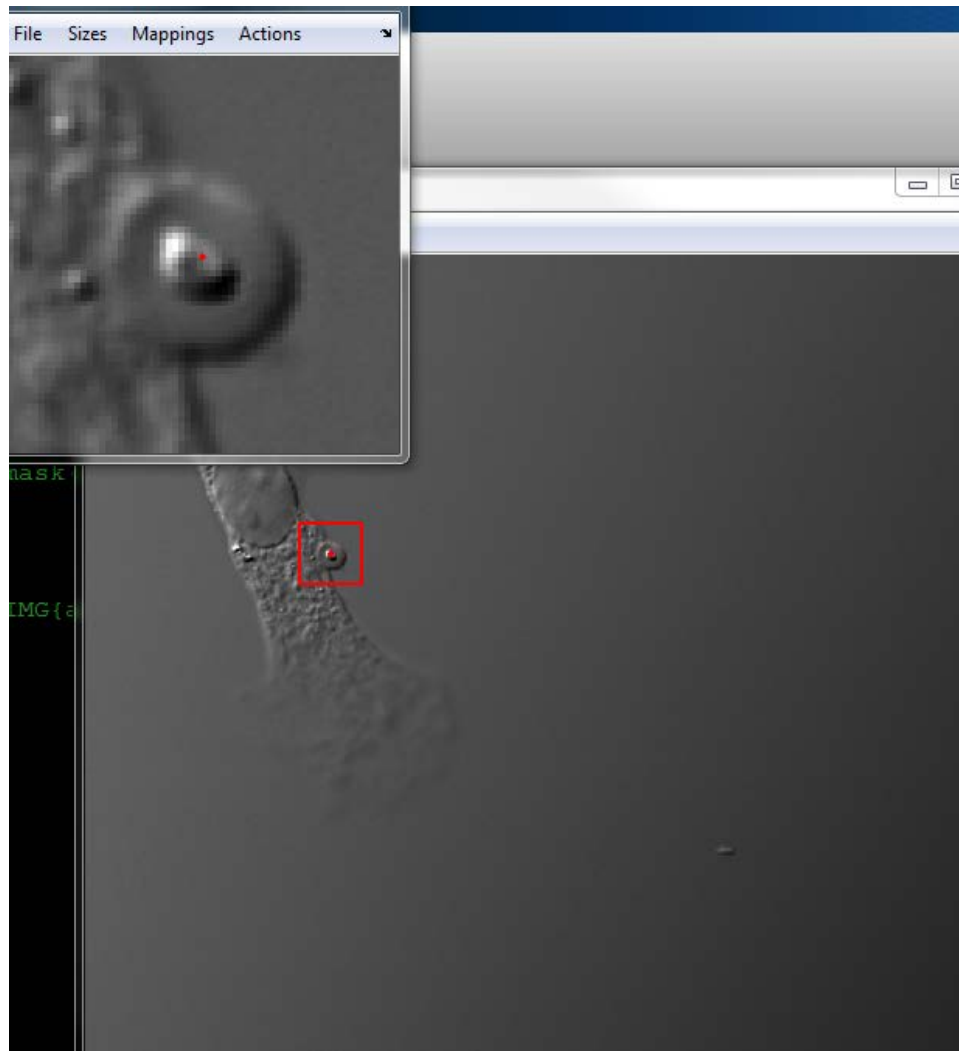| Z-Step (nm): | 250 | Box Size (px): | 150 |
| X pixel size (nm): | 103 | Voxel Scale: | 3 |
| Y pixel size (nm): | 103 | Radius of Sphere (um): | |

8. Fill in the correct z step for your data (this is not automatically populated) as well as the voxel scale box. The voxel scale refers to how fine the sub-voxels for the spherical conversion are. Making this number larger means finer division of voxels and a more accurate spherical surface, but it may significantly increase processing time.
9. Next, indicate which channels will be used for ROI selection, creating the contact region mask, and for quantitative analysis.

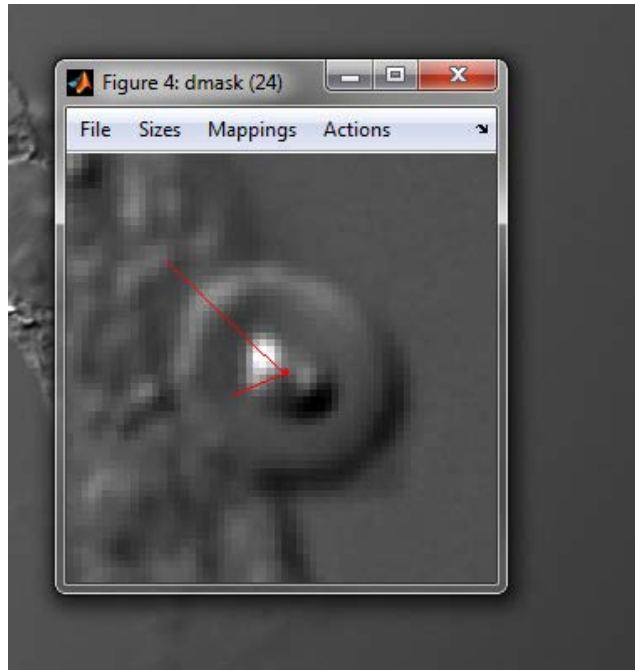| Channel for ROI selection (number): | 4 |
| Channel to use for mask (number): | 2 |
| Channels for analysis (numbers, separated by commas): | 1,3 |

10. When you have filled in all of the information, click the "display" button to display the selected image channel. It will open in a new window.
11. Use the 'n' and 'p' keys to scroll through the image stack until you find the midplane of the yeast (or other spherical object) you wish to analyze.
12. Then set the box size so that it is large enough to completely enclose the yeast and click the "pick point" button.
13. The mouse cursor will turn into a crosshairs. Use this to select the center of the yeast.
14. A new window with the newly cropped region will open with a red marker to indicate the point that you clicked.

15. Now you must draw the inner and outer radii for the analysis. NOTE: The intersection point of these two lines will become the new center point of the yeast region. THEY MUST INTERSECT.

Draw the outer radius first, double click to end the line, then click again and draw the inner radius.

16. At this point you can either click the "save variables" button to save the ROI and the radii for batch processing, or you can hit the "run" button to run analysis on this image. Click the "show figures" checkbox to display figures generated during analysis.
17. When you click "run" or "save variables" you will be prompted to select a directory in which to save the program output or variables.