PixCell: Unilocular adipocyte and lipid tracer for immunofluorescent images

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Installation Requirements

MATLAB_R2023 or newer: https://www.mathworks.com/products/matlab.html

Follow Installation Prompts

Required Toolboxes:

Imaging toolbox

Image processing toolbox

Computer vision toolbox

Mapping toolbox

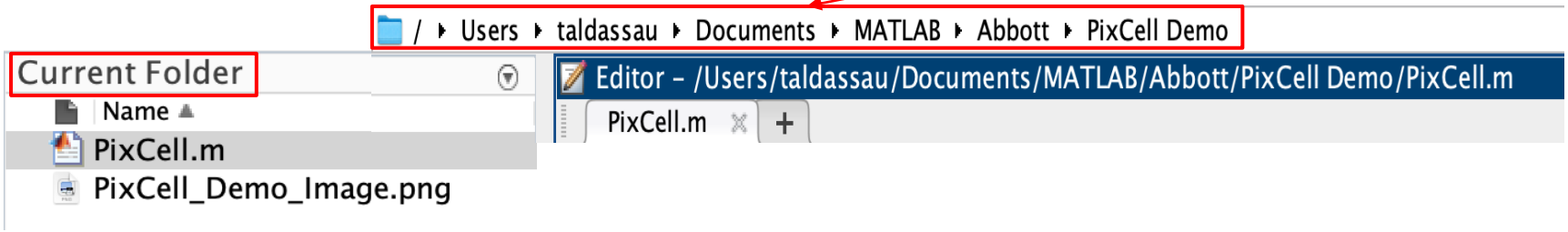
If you need to install these toolboxes you can either:

Rerun the **MathWorks Installer** to add any additional products not currently included in your installation. You do not need to reinstall MATLAB if you select the same installation folder.

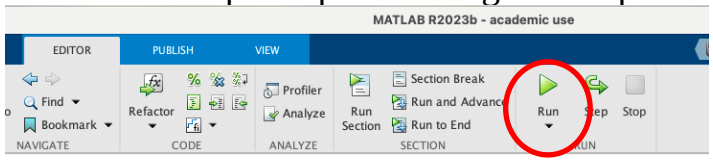
Use the **Add-On Explorer** in MATLAB, if your license permits usage of it. MATLAB has an online tutorial for this: https://www.mathworks.com/videos/add-on-explorer-106745.html

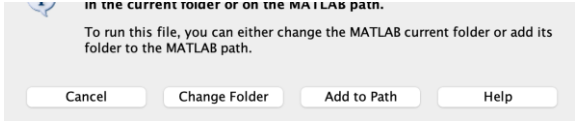
PixCell Installation and Running the Script

1. Download PixCell m-file from GitHub repository: https://github.com/rabbottlab/pixcell/
2. Drag m-file into your MATLAB folder on your computer
3. Launch MATLAB and make sure that the m-file is in your current folder/file-path



1. Make sure that the images you are analyzing are in the current folder
    a. If you have a folder of images make sure that the PixCell m-file is in that folder
        a. If it is not, you will be prompted to either change the MATLAB current folder or add its folder to the MATLAB path upon running the script
2. Press RUN

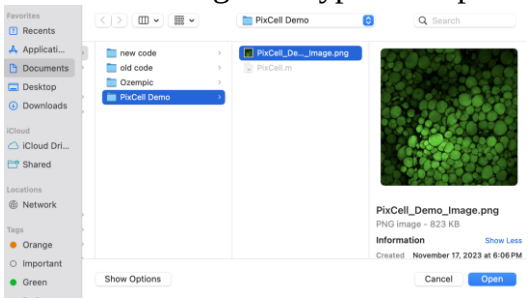




PixCell Workflow Continued

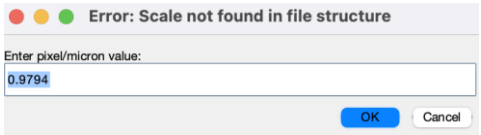
Select your image in the pop-up and click Open

a. PixCell works with almost all image file types but specifically searches for *.jpg, *.tif, .*png, and *.gif



If your image metadata does NOT contain scale you will be asked to enter a pixel/micron value. Enter the pixel/micron value and click OK.

1. Please enter a numeric value or the program will keep asking you to re-enter until it receives a valid entry
2. Note that this scale will be used for all the calculations on the selected image (different images may have different scales)

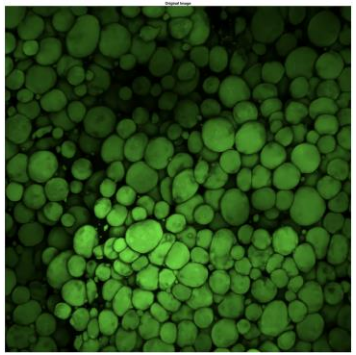


PixCell Workflow Continued

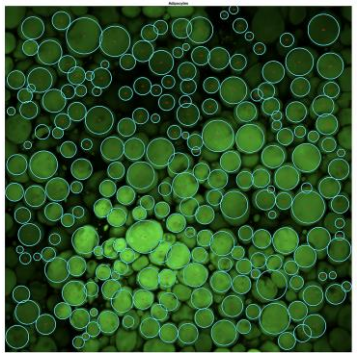
1. At this point, within seconds, a new folder (same name as the selected image) will be created in your Current Folder
    a. Contains:
        i. 4 images
            1. Original_image.png
                a. The image you selected
            2. Adipocytes.png
                a. Circled adipocytes (blue):
                    i. Circle size calculated from adipocyte size
                b. Numbered adipocytes (red text) : this number correlates to the number in the Adipocyte_data excel sheet
            3. ExtracellularLipids.png
                a. Extracellular lipids marked (magenta)
            4. AllLipids.png
                a. Adipocytes and extracellular lipids marked
        ii. 3 Excel sheets with raw pixel measurements and converted (µm) measurements
            1. Adipocyte_data.xlsx
                a. Corresponds to Adipocytes.png
            2. ExtracellularLipid_data.xlsx
                a. Corresponds to ExtracellularLipids.png
            3. AllLipid_data.xlsx
                a. All data combined

2. By cross-comparing the circled adipocyte image and the Excel sheet
    a. Eliminate any inconsistencies like two cells circled as one
    b. Remeasure specific cells in ImageJ

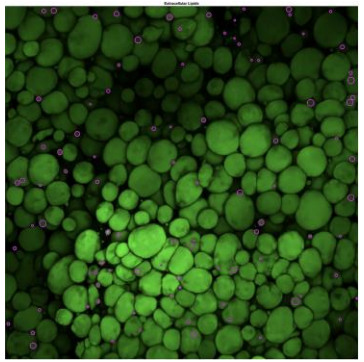
1



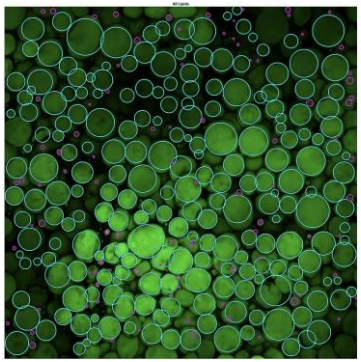
2



3



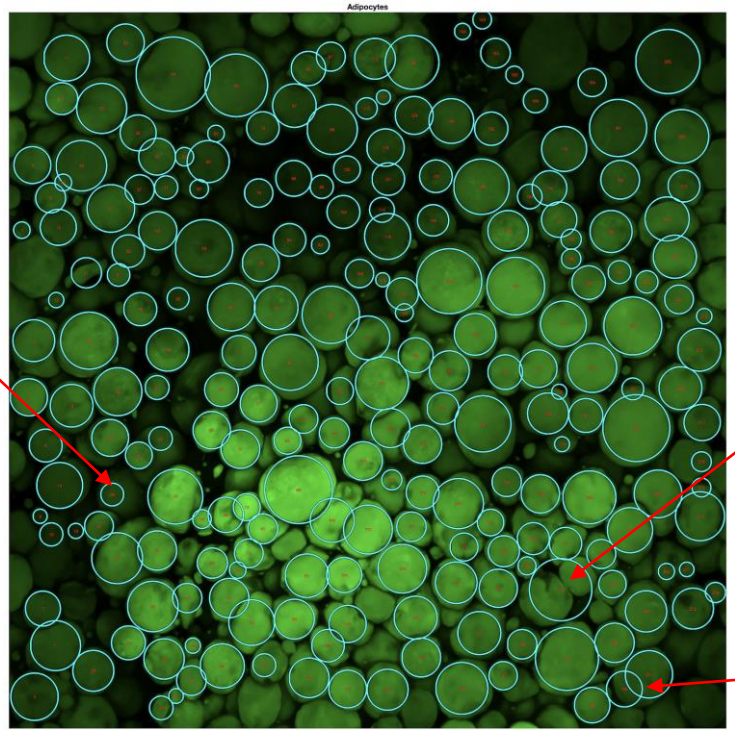
4



Examine Results:

Example of Things to Look out For:

Too small



2 cells counted as 1

1 cell split into 2

Areas for Potential Optimization

Stats

Convert to microns

```
% Find and store lipids in new table, artifacts are also removed
lipid_size = 20;
minimum = 5;
idx = cells.converted_diameters <= lipid_size;
lipids = cells(idx, :);
lipidx = lipids.converted_diameters >= minimum;
lipids = lipids(lipidx, :);

% Remove lipids from cell table
cells = cells(cells.converted_diameters >= lipid_size, :);

% Check to see if anything is too big to be an adipocyte
max_size = 300;
idx2 = cells.converted_diameters <= max_size;
cells = cells(idx2, :);

% Update Centers and radii variables
uniqueCenters = cells.uniqueCenters;
radii = cells.diameters / 2;
```

Users can alter the maximum and minimum diameter of objects detected as well as define what size should be the cut-off for an adipocyte

Masking

```
% Mask high filter cells (fix scalar adjustment)
filtered_bw = insertShape(img, 'FilledCircle', [uniqueCenters(:, 1), uniqueCenters(:, 2), radii * 1.18], 'Color', 'black', 'Opacity', 1);
```

Users can alter the scale of the masked lipids based on their needs

High-pass

```
i = im2gray(img); % grayscale

bw = imbinarize(i,"adaptive"); % Thresholding

bwborder = imclearborder(bw); % suppresses lighter structures connected to the border
```

Users can apply a sensitivity metric when thresholding if they need to adjust what is foreground versus background