Scaling ROIs

**Purpose:** For Iniya’s paper we want to subtract the density maps for one subject between two time points or all subjects between the two time points.

**Background:**

* 19 subjects each with visit 1 (300um ROI) and visit 2 (500um) foveal ROIs for a total of 38 foveal ROIs
* All of the ROIs were scaled based on their original AO montage
* However, in order to subtract density maps we need to get all of the ROIs to a common scale

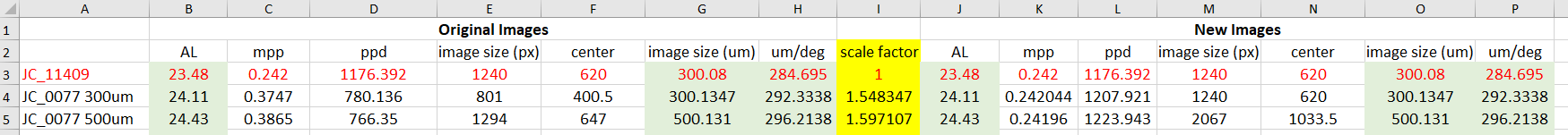
**Steps of what Emma did:**

1. Determine the smallest mpp scale of all 38 ROIs. We will call this the targeted ROI
   1. JC\_11409 300um – mpp is 0.2420
2. Make an excel doc to convert the images into a new scale
   1. Column A: subject ID and ROI size (found in ROI file name)
   2. Column B: Axial length (found in ROI file name)
   3. Column C: mpp scale (found in ROI file name)
   4. Column D: ppd scale (found in ROI file name)
   5. Column E: image size in pix (open TIF in imageJ to see this)
   6. Column F: center pixel (divide total pixels by 2)
   7. Column G: image size in um (mpp\*image size in pix; multiply columns C and E); should be close to 300 or 500um)
   8. Column H: um/deg (multiply AL by 291, divide product by 24) – this is the inherent scale based on a 24mm eye
   9. Column I: divide the mpp of the ROI by the mpp of the targeted ROI
      1. In the example below: divide 0.3747/0.2420

A screenshot of a computer

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* 1. Column J: axial length (same as column B)
  2. Column L: ppd of the new image (ppd of the original image \* scale factor; multiply columns D and I)
  3. Column M: image size in pix of the new image (image size of the original image \* scale factor; multiply columns E and I) – I also rounded this to the nearest whole number
  4. Column N: center pixel of the new image; divide the image size in pixels of the new image by 2
  5. Column O: Image size in um (same as Column G)
  6. Column K: divide the new image size in um (which is the same as the old) by the new image size in pixels (Column O/Column M)
  7. Column P: um/deg of the new image (axial length\*291/24) (Column J\*291/24); should be the same as column H
  8. Here is what the entire excel scaling sheet should look like (the first row in red is the target image):



* 1. And zoomed in for more detail:

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1. Copy the original TIFs, coords, and LUT file to a folder.
   1. Within that folder, make two folders:
      1. Calculations for Scaled Coords – when we do math on the coords files, we save the files with all the math here
      2. Scaled ROIs and Coords – here is where all the scaled ROIs and scaled coords will go
2. Scale the TIFs
   1. The only TIF/coords pair that you will not change is the targeted ROI (JC\_11409 300um in this case)
   2. In the folder, right click on a TIF, and click open with photoshop
   3. Go to Image > Image Size
   4. In the document size dimensions, change the drop down menu to percent. Make sure the height/width link is turned on
   5. For document size width and height, type in the scale factor for that TIF that you determined from the excel sheet. From what is listed on the excel sheet, the scale factor has to be multiplied by 100 when you type it into photoshop to change the size of the TIF
      1. For example, if the scale factor is 1.548347, the scale you type into photoshop would be 154.8347
      2. The image size of the target image is 1240. When you type in the scale factor into photoshop, make sure that all of the 300um images are also 1240 pixels. If it is not, force scale it to 1240 pixels to change it. **When you do this, record the percentage scale that photoshop used to achieve the target pixel size. You need to record that this is different than the theoretical scale factor (column I in excel doc) because the photoshop scale, not the theoretical scale, will have to be used to scale the coordinate csv.**
      3. For 500um images, use the same steps as above and change the image size by doing the percentage of the scale factor. To double check that the pixel size is correct, do 500/0.242 (mpp of the target image). The result is 2066 pixels. So, for all of the 500um images after you use the percentage to scale in photoshop, double check that they are all 2066 pix. If not, force scale to 2066. **Again, if you force scale, record that the photoshop scale is different than the theoretical scale.**
   6. Save As a TIF into the Scaled ROIs and Coords folder. Rename the TIF with the new mpp that you calculated on the excel doc. Add “\_scaled” to the end of the file name
      1. **The new mpp should be the same as the target image for all tifs**
         1. In this case 0.2420
      2. **The ppd scale will be different whether you force scaled in photoshop or not**
         1. **If you did NOT force scale, the ppd is column L in the excel doc (the original PPD\*theoretical scale factor column I)**
         2. **If you DID force scale, update the scale factor in column I in the excel doc with the scale from photoshop** 
            1. **If your percent scale was 159.66 in photoshop, the scale factor would be 1.5966 in the excel doc**
            2. **This should auto-update column L and slightly change your ppd. Use this ppd calculated from the photoshop scale factor in instances where you force scaled the tif.**

A screenshot of a computer code

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* 1. Repeat this for all TIFs that you need to scale.

1. Scale the coords
   1. Open up the original csv for the TIF
      1. There will be x,y coordinate pairs in columns A and B respectively
   2. In cell C1, type “=A1-the center pixel of the original image”
      1. In this screenshot, it would be the value in column F
         1. So for JC\_0077 300um, you type “=A1-400.5” into column C
      2. Double click the bottom right corner of the cell in column C so it copies for all coordinates

A screenshot of a computer

Description automatically generated

* 1. In cell D1, type “=B1-the center pixel of the original image”
     1. It is the same (column F) as the screenshot above
     2. Drag this function down column D so it copies for all coordinates
  2. Columns E and F (steps e and f below) will be multiplying the scale factor by the offset from the center coordinate. **Pay attention to these instructions because they differ for the ROIs that you had to force scale in step 4.**
  3. In cell E1, type “=C1\*scale factor”
     1. **If you did NOT have to force scale the image to 1240 or 2066 in photoshop, use the value in column I (screenshot below) as the scale factor.** 
        1. For JC\_0077 300um (second subject listed in screenshot below) you would type “=C1\*1.548347” into column E
     2. **If you DID force scale the image in photoshop, use the recorded photoshop scale as the multiplied scale factor.** 
        1. **For example, if your photoshop scale was 159.66 when you force scaled an image, you would type “=C1\*1.5966” in cell E1.**
     3. A screenshot of a calculator

        Description automatically generatedDrag this function down column E so it copies for all coordinates
  4. In cell F1, type “=D1\*scale factor”
     1. **Same rules apply here, if you did NOT force scale, use the scale factor in column I; if you DID force scale, use the photoshop scale**
     2. Drag this function down column F so it copies for all coordinates
  5. In cell G1, type “=E1+the center pixel of the new image”
     1. **This value should either be 620 for 300um images or 1033 for 500um images because all final images are either 1240 or 2066 pixels.**
        1. For JC\_0077 300um (second row for the screenshot below) you would type “=E1+620”
     2. A screenshot of a computer

        Description automatically generatedDrag this function down column G so it copies for all coordinates
  6. In cell H1, type “=F1+the center pixel of the new image”
     1. **This value should either be 620 for 300um images or 1033 for 500um images because all final images are either 1240 or 2066 pixels.**
     2. Drag this function down column H so it copies for all coordinates
  7. Save As with “\_scaled\_calcs” to the Calculations for Scaled Coords folder
     1. That way if there is a mistake when you are checking the new coords docs, you can go back to this one
     2. Make the file type an excel doc instead of a csv; when you save as a csv, you can’t see the function in the cells

1. Open a new excel doc
   1. Copy columns G and H from the scale calculations doc into columns A and B of this new doc
   2. A screenshot of a computer code

      Description automatically generatedSave As with the new scaled TIF name and add “\_scaled\_coords” to the end of the file name. Save into the Scaled ROIs and Coords folder
2. Verify that the scale calculations worked
   1. Open mosaic
   2. Drag in the new scale TIF with its coords
   3. Drag around the TIF and make sure that the coords are relatively centered on the cells
3. Make a new LUT file
   1. Open a new excel doc
      1. In column A copy/paste the scaled file names of the TIFs
      2. In column B copy/paste the axial length measures for each subject (found on the original LUT or on the first excel doc where you do the scale calculations for the new images)
      3. In column C copy/paste the scaled ppd of the new images (from the first excel doc where you did the calculations to determine the scale of the new images)
   2. Save As a csv into the Scaled ROIs and Coords folder