# **CFP Manual Annotation Instructions**

## Equipment check

1. You will need a mouse and mouse pad for selecting/editing the pixels for segmentation.
2. You will also need a large monitor, and preferable be able to perform manual segmentation **not** on a laptop screen but a typical desktop monitor.

## ITK-SNAP setup

1. Go to [ITK-SNAP Version 4.x Downloads](http://www.itksnap.org/pmwiki/pmwiki.php?n=Downloads.SNAP4) and click the link for ITK-SNAP 4.2.0 (stable version) windows Self-Install Package (64-bit).
2. Fill in the form and select next. On the next page and select the download. It will take you to another page and the download should start.
3. Navigate to the downloaded .exe file and double click to begin install process.
4. Select next, agree to the terms and conditions and select next. Tick the box next to ‘Do not add SNAP to the system PATH’, keep selecting next until the setup is complete, press finish.
5. Navigate to the SNAP program and open, it will prompt you if you would like to enable automatic updates, select yes.
6. **(OPTIONAL)** Go to [RSNA 2016 ITK-SNAP Training - Intro & Manual Segmentation - YouTube](https://www.youtube.com/watch?v=-tjVN5GwjKg&feature=youtu.be) and watch the ~15min video. This is a training video to learn how to use SNAP, they give an example in 3D but we will be annotating in 2D. The slides for the training video can be found here: [RSNA-manual-Guido-2.key (itksnap.org)](http://www.itksnap.org/pmwiki/uploads/Train/RSNA2016-Manual-Guido-Final.pdf).

## Load in image, label properties, main tools

1. Go to File > Open Main Image. Use the browse button to navigate to the image. Under File Format select ‘Generic ITK image’. Select Next and Finish.
2. Click the ‘A’ in the top right corner of the top left window in it to expand the view to full size and set the window to full screen (*below*).
   1. If the image is not already shown in RGB mode (it may appear by default as grayscale and low contrast), go to Tools > Layer Inspector and under the General tab select RGB Display in the Display Mode drop-down list.
3. Go to Segmentation > Open Segmentation and select browse to navigate to the corresponding binary vessel mask. Under File Format select ‘Generic ITK image’. Select Next and Finish.
4. Next, we need to set up some labels. Click Segmentation > Import Label Descriptions and select browse to navigate to the AutoMorphalyzer/manual\_annotations folder where you will find three text files whose names begin with *LabelProperties*:
   1. If correcting the artery-vein segmentation map, upload *LabelProperties\_AV.txt.*
   2. If correcting the binary vessel segmentation map, upload *LabelProperties\_Binary.txt.*
   3. If correcting the optic disc/cup segmentation map, upload *LabelProperties\_OD.txt*

Once selected for upload, the file should be automatically detected as a text file (if not, select Text File in the File Format drop-down list). Click Ok.

1. Using *LabelProperties\_AV.txt,* your Segmentation Labels toolbar you will now see different colour labels for:
   1. Clear Label (Black), which is your “rubber”
   2. Artery (White)
   3. Vein (Dark Blue)
   4. Overlap (Gray)

For *LabelProperties\_Binary.txt* there is just the Clear Label (Black) and Vessel (Blue).

For *LabelProperties\_OD.txt* there is just the Clear Label (Black) and Optic disc (Blue) and Optic cup (White).

1. Your window should look something like below (if correcting the AVOD segmentation map).A screenshot of a computer

   AI-generated content may be incorrect.

Magnifying glass

A screenshot of a computer screen

AI-generated content may be incorrect.

Paint brush

Label editor

Paintbrush size/shape options

Label selector

## Main tools

1. Note, on this toolbar there is also an opacity option to change the transparency of the labelling which can be helpful for manual segmentation. Use your mouse scroll to toggle quickly between opacity levels.
2. On the Main Toolbar, the magnifying glass can be used to zoom into the portion of the image you would like to annotate. If you hover your mouse over this icon it provides instructions on how to do that.
3. We recommend all annotating is done using the Paintbrush tool, seen on the Main Toolbar

## Segmentation

1. Optic disc-cup:
   1. Select the Paintbrush tool. This is a paint brush that allows you to ‘colour in’ pixels. You can change the size and shape of the brush.
   2. Select the “Optic disc” (or “Optic cup”) segmentation label and make sure you are painting over All Labels.
   3. You may use the magnifying glass freely. Holding the right mouse-click will zoom in and out for you.
   4. You may alter the opacity to aid segmentation.
   5. You can select a Paintbrush Brush size freely, and you may want to start with a large size to cover the core region of the optic disc.
   6. I would recommend selecting the circular Brush Style, which is helpful for annotating edges.
   7. Annotate the optic disc including the optic disc cup and rim.
   8. If you over-annotate, you can select “Clear Label” as the active label and “Optic Disc” as the label to Paint Over. This prevents you from rubbing out any vessels and only rubbing out optic disc labels.
2. Artery-vein / Binary vessel segmentation:
   1. Select the Paintbrush tool. This is a paint brush that allows you to ‘colour in’ pixels. You can change the size and shape of the brush.
   2. Select one of “Artery” or “Vein” (in the artery-vein setting) or “Vessel” (in the binary-vessel setting) as the Active Label and you can select “All Labels” as the Paint Over label or select the other vessel type to paint over for more controlled corrections.
      1. In the former, this lets you colour new pixels as artery/vein/vessel which is helpful for fixing disconnected vessels/false negatives.
      2. The latter is helpful to speed up the annotation process – you can select a large Brush Size for large vessels and paint over them freely without worrying about annotating any of the fundus tissue adjacent to them.
   3. You can use the magnifying glass freely. Holding the right mouse-click will zoom in and out for you.
   4. Use the “Clear Label” to correct false positives, i.e. rub these pixels out.
   5. You can select a Paintbrush Brush size freely, and you may want to start with a large size to cover the larger vessels more quickly.
   6. I would recommend selecting the circular Brush Style, which is helpful for annotating edges.
   7. You can alter the opacity to aid segmentation.
3. Some tips:
   1. You can select the “Clear label” as a “rubber”, in case you label parts of the image mistakenly. This can also be done with the right mouse-click.
   2. Ctrl+Z will undo the most recent Paintbrush annotation.
   3. Press 2 or 4 to toggle between pan/zoom (2) and brush tool (4)
   4. Regularly reduce the “Overall label opacity” on the Segmentation Labels” toolbar to sanity check any selected regions to make sure you are happy with your selections.
4. Some rules we follow for artery-vein classification
   1. Alternating rule: It is common for the distribution between arterial and venule retinal vasculature to appear on the fundus in an alternating like fashion (see image above, particularly around the macula).
   2. Thickness rule: It is generally known that veins are thicker than arteries (see image above, blue vs. white).
   3. Brightness rule: It is also generally known that veins appear darker than arteries.
   4. Intersection/junction rule: If it appears that retinal vessels are intersecting (and forming at least a 4-ended junction), it’s very likely that an artery and vein are crossing (see image above).
   5. Note that for any AV-crossings, the label “Overlap” (light blue) should be used to represent the fact that for those pixels both an artery and vein are present. This is important for feature measurement to ensure veins and arteries are complete.

## Saving segmentations

1. Once you’re happy with your annotation, save the segmentation into an “annotations” folder within the overall output folder (i.e. whatever “output\_directory” is in the “config.txt” file) by going to **Segmentation > Save Segmentation Image** and browsing to “{output\_directory}/annotations”. You may need to create this folder manually. See below for file naming conventions:
   1. **Add the postfix “\_binary\_vessel” if editing the binary vessel segmentation mask**
   2. **Add the postfix “\_artery\_vein” if editing the artery-vein segmentation mask**
   3. **Add the postfix “\_optic\_disc” if editing the optic disc/cup segmentation mask.**
   4. **Change “.png” to “.nii.gz**”, (saving the file format as NiFTI)

For example, if you edited the artery-vein segmentation mask for the file “example.png”, you would save this out as “example\_artery\_vein.nii.gz” in the “{output\_directory}/annotations” folder. **This is crucial for AutoMorphalyzer to identify an artery-vein manual annotation for this “example.png”**. Select Finish.

1. You can do step 2 & 3 as you go along. If you need to finish an annotation later follow step 2 and 3 and close SNAP. When you want to come back to it, load the image as in (1) described earlier in this document and load the segmentation by going to Segmentation > Open Segmentation and select the file to open and go to Segmentation > Import Label Descriptions and select the .txt file to open and continue annotating where you left off.
2. Close the ITK-Snap window with the CFP and newly generated segmentation map **before re-running AutoMorphalyzer.**

**Note:** When you run AutoMorphalyzer again, you don’t need to change the directory paths in config.txt.