**VEIN TRACING PROTOCOL**

|  |
| --- |
| We create a three-layer image, with the raw image as the base layer. On the second layer, make a yellow polygon that shows the silhouette of the leaf. On the third layer, make a red tracing of the leaf's veins. We can then take the yellow/red components of this image and analyze them automatically using a separate computer program. |
| 1.     Open one of the claheNAME.JPG images in GIMP. |
| 2.     Convert the image to RGB from grayscale in the Image/Mode menu. |
| 3.     Create two new blank layers, by shift-clicking on the new layer button (the sheet of paper icon) in the Layers palette. Make sure one of the new blank layers is selected. |
| 4.     Select the path tool from the tool palette, and make sure the 'polygonal' option is selected. |
| 5.     Set the foreground color to yellow by clicking on the top rectangle below the tools in the tool palette (html code ffff00). |
| 6.     Now use the path tool to make a polygon around the leaf. Leave out the petiole.  Select only the region you plan to trace (i.e. the whole leaf, or the section of the image that was chemically cleared without problems such as tears). |
| 7.     Click 'Selection from path'. |
| 8.     Fill the selection with the foreground color in the Edit-Fill menu. |
| 9.     Hide this layer and select the other empty layer by clicking on the eye icons in the Layers palette. |
| 10.   Change the foreground color to red (html code ff0000). |
| 11.   Select the pencil tool and make sure the brush size is set to 11 (Note: you may wish to change this to a smaller size). |
| 12.   Trace the leaf's veins using the red pencil tool, controlling the screen with the tracing tablet. You can zoom in and out with Control-minus or Control-shift-Equals. Control-z will undo, or you can use the eraser tool. |
| 13.   Use the layers palette to show all three layers, with the red tracing overlaid on the yellow silhouette  . |
| 14.   Go to the File/Save as menu, and save the image as a XCF file. |