Measuring a Ringed Region of Interest Using Fiji or ImageJ

Download the open source software Fiji from http://fiji.sc/wiki/index.php/Fiji It's essentially the same as ImageJ, but comes with many pre-installed plugins and can update itself automatically.
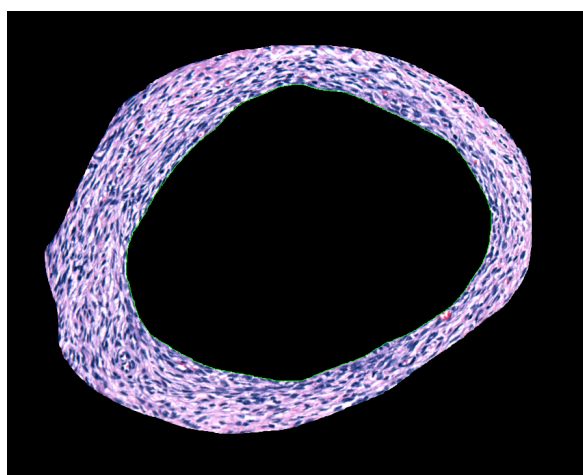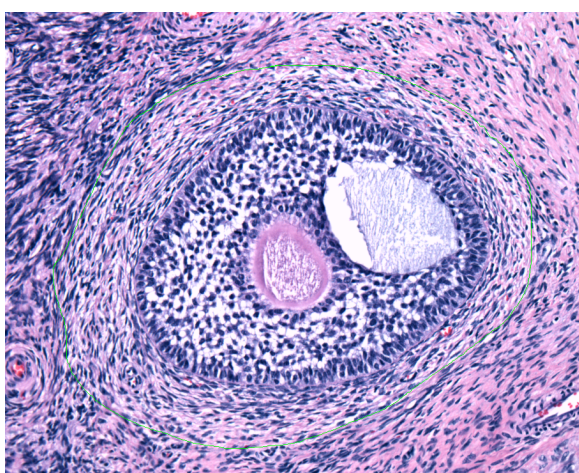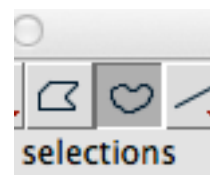
If you prefer, ImageJ can be downloaded at http://rsbweb.nih.gov/ij/.

Spatial Calibration

If you want 'real' dimensions in mm or µm for example, the image must be calibrated, otherwise dimensions are only in pixels. The calibration procedure is described in the pdf file at:
http://occm.otago.ac.nz/resources/Spatial-Calibration-of-an-Image.pdf

Selecting a Region of Interest (ROI)

Using the free hand selection tool from the main menu, draw around the outermost region of interest. Press 'Command t' to add this region to the ROI manager for later use.

Then, select Edit / Clear Outside to remove everything outside the selection area.

Next, use the selection tool again to trace the inner boundary of the centre region to remove. Press 'Command t' to add this region to the ROI manager for later use.
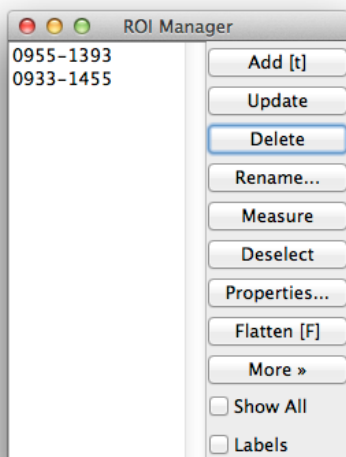
Press 'Delete' and the result will be similar to the example here.

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Measuring Area of Remaining ROI

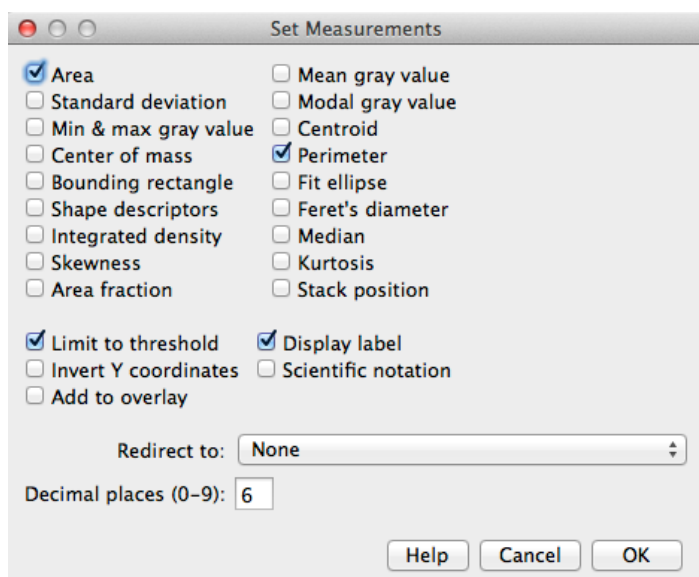If the ROI Manager window is not visible, go to Analysis / Tools / ROI Manager to find it.

The two ROIs saved earlier (the inner and outer traces) should be visible in the window. Highlight both ROI references in the list and click Measure.

The results table will appear as shown below. With this the active window, click File / Save and its contents will be save in an Excel file format. Further analysis, such as subtracting the inner region's area from the outer region's to give the area of the region of interest, needs to be done in a program such as Excel. Fiji just produces the raw data, its level of analysis varies between menus and plugins.

	Label	Area	Perim.
1	1506 slide 220 20x small healthy 5.tiff:0955-1393	39382.158249	730.863252
2	1506 slide 220 20x small healthy 5.tiff:0933-1455	21256.512268	536.126799

The variables which are measured (in this case area and perimeter) can be chosen by going to the Analyse / Set Measurements menu.

Always have Display Label ticked, so result tables show what the columns of data refer to.

It's also useful to have Limit to Threshold ticked if you want to count only features highlighted in the thresholding range.

Other parameters can be chosen as relevant to the sample and requirements.