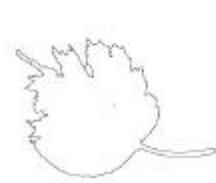
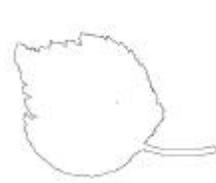


Examples of Image Analysis Using ImageJ

Area Measurements of a Complex Object

Problem: Determine the photosynthetic (i.e., green) portion of a variegated leaf.

	<ul style="list-style-type: none"> ◆ Convert scanned color image of leaf to grayscale: Image → Type → 8-bit ◆ Set measurement scale: Draw a line over a 50 mm section of the ruler then Analyze → Set Scale In <i>Set Scale</i> window enter 50 into the 'Known Distance' box and change the 'Unit of Measurement' box to mm , check 'Global' ◆ Draw a new line and confirm that the measurement scale is correct.
	<ul style="list-style-type: none"> ◆ Threshold the leaf image using the automated routine: Process → Binary → Threshold <p>The automated threshold includes only the dark green areas.</p>
	<ul style="list-style-type: none"> ◆ Calculate area of green portion: Enclose the leaf with the rectangular selection tool Analyze → Analyze Particles Enter 500 as the minimum particle size, toggle 'Show Outlines' and click 'OK' Outline of analyzed area is automatically drawn. Data window gives an area of 2001.3 mm².
<p><i>(See bottom of page for an alternative method for measuring areas.)</i></p>	
	<ul style="list-style-type: none"> ◆ Threshold new image of leaf using manual settings: Image → Adjust → Threshold and play with sliders to include all of leaf <i>or</i> Image → Adjust → Brightness/Contrast, click 'Auto', click 'Threshold', play with the slider until all of leaf is include, click 'Apply' <p>The manual threshold setting includes all of the leaf area.</p>
	<ul style="list-style-type: none"> ◆ Calculate area of entire leaf: Enclose the leaf with the rectangular selection tool Analyze → Analyze Particles Enter 500 as the minimum particle size and click 'OK' Outline of entire leaf is automatically drawn. Data window gives an area of 2447.8 mm².

This analysis suggests that about 82% of leaf surface is dark green. These values should be manually confirmed before beginning a 'production run' of measurements.

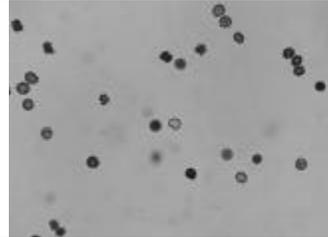
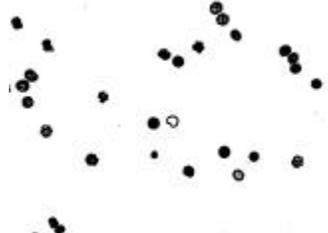
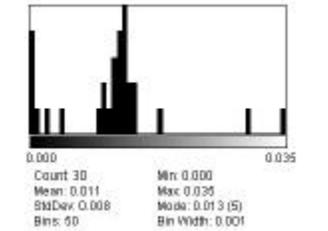
An alternative procedure for measuring areas:
Analyze → Set Measurements, check 'Limit to Threshold'
Analyze → Measure
 This procedure is simpler but does not draw an outline of the measured area.

(more on reverse side)

Examples of Image Analysis Using ImageJ (continued)

Particle Counting and Analysis.

Problem: Count and determine the size distribution of a collection of echinoderm embryos.

	<ul style="list-style-type: none"> ◆ Separately photograph a stage micrometer and the embryos at the same magnification. Set the measurement scale: Draw line over a know distance on the micrometer then Analyze → Set Scale In <i>Set Scale</i> window enter the distance value into the 'Known Distance' box and Change the 'Unit of Measurement' box to mm , check 'Global' ◆ Confirm that the measurement scale is correct. <p>This image was improved by simplifying a cluttered background: (Process → Subtract Background)</p>
	<ul style="list-style-type: none"> ◆ Open the color image of the embryos. ◆ Convert the image to grayscale: Image → Type → 8-bit
	<ul style="list-style-type: none"> ◆ Threshold the image using the automated routine: Process → Binary → Threshold
	<ul style="list-style-type: none"> ◆ Analyze Particles: Analyze → Analyze Particles Enter 20 as the minimum particle size, toggle 'Show Outlines', check 'Size Distribution' and click 'OK' <p>Thirty embryos are counted, numbered and outlined. The data window lists the area (in mm²) for each embryo. These data could be copied to a spreadsheet.</p>
	<p>An analysis of particle size distribution is also shown in a new window.</p>

As mentioned in the previous example, this technique should be manually validated before collecting experimental data.