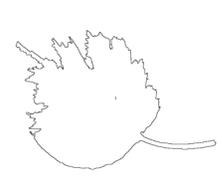
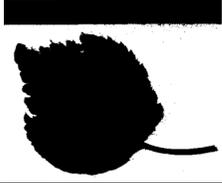
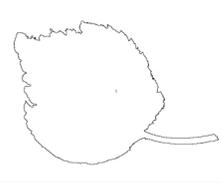


## Examples of Image Analysis Using ImageJ

### Area Measurements of a Complex Object

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

(Open leaf image via Select **File** → **Open Samples** → **Leaf**)

	<ul style="list-style-type: none"> <li>◆ Convert scanned color image of leaf to grayscale: <i>Image</i> → <i>Type</i> → <i>8-bit</i></li> <li>◆ Set measurement scale: Draw a line over a 50 mm section of the ruler then <i>Analyze</i> → <i>Set Scale</i> In <i>Set Scale</i> window enter 50 into the 'Known Distance' box and change the 'Unit of Measurement' box to mm, check 'Global'</li> <li>◆ Draw a new line and confirm that the measurement scale is correct.</li> </ul>
	<ul style="list-style-type: none"> <li>◆ Threshold the leaf image using the automated routine: <i>Process</i> → <i>Binary</i> → <i>Make Binary</i></li> </ul> <p>The automated threshold includes only the dark green areas.</p>
	<ul style="list-style-type: none"> <li>◆ Calculate area of green portion: Surround the leaf with the rectangular selection tool <i>Analyze</i> → <i>Analyze Particles</i> Enter 50 as the minimum particle size, toggle 'Show Outlines', check "Display Results" and click 'OK'</li> </ul> <p>Outline of analyzed area will be drawn. Data window gives an area of about 2000 mm<sup>2</sup> depending on the calibration setting.</p>
<p><i>(See bottom of page for an alternative method for measuring areas.)</i></p>	
	<ul style="list-style-type: none"> <li>◆ Threshold new image of leaf using manual settings: <i>Image</i> → <i>Adjust</i> → <i>Threshold</i> and play with sliders to include all of leaf in red and click 'Apply'</li> </ul> <p>The manual threshold setting includes all of the leaf area.</p>
	<ul style="list-style-type: none"> <li>◆ Calculate area of entire leaf: Enclose the leaf with the rectangular selection tool <i>Analyze</i> → <i>Analyze Particles</i> Use previous window settings and click 'OK'</li> </ul> <p>Outline of entire leaf is automatically drawn. Data window gives an area of about 2450 mm<sup>2</sup>.</p>

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'. After converting to a binary image, select *Analyze* → *Measure*

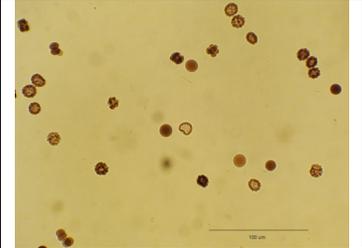
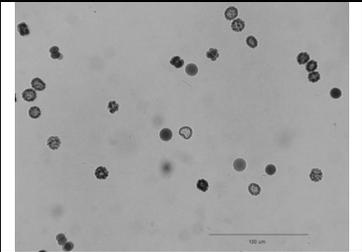
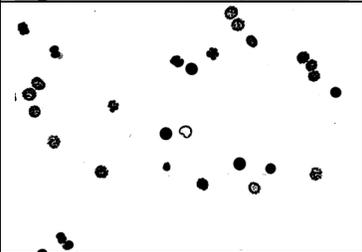
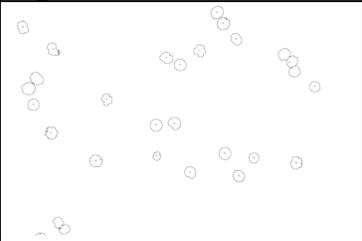
This procedure is simpler but does not draw an outline of the measured area.

**(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.  
(Open embryos image via Select **File** → **Open Samples** → **Embryos**)

	<ul style="list-style-type: none"> <li>◆ Draw line over the scale bar and select <b>Analyze</b> → <b>Set Scale</b> In <i>Set Scale</i> window enter 100 into the 'Known Distance' box and Change the 'Unit of Measurement' box to um , check 'Global'</li> <li>◆ Confirm that the measurement scale is correct.</li> </ul>
	<ul style="list-style-type: none"> <li>◆ Convert the image to grayscale: <b>Image</b> → <b>Type</b> → <b>8-bit</b></li> </ul>
	<ul style="list-style-type: none"> <li>◆ Threshold the image using the automated routine: <b>Process</b> → <b>Binary</b> → <b>Make Binary</b></li> <li>◆ Surround the scale bar with the rectangular selection tool and clear the contents (<b>Edit</b> → <b>Clear</b>)</li> </ul>
	<ul style="list-style-type: none"> <li>◆ Analyze Particles: <b>Analyze</b> → <b>Analyze Particles</b> Enter 20 as the minimum particle size, toggle 'Show Outlines', check 'Display Results', 'Summarize' and 'Record Stats' and click 'OK' Twenty five embryos are counted, numbered and outlined. The data window lists the area (in <math>\mu\text{m}^2</math>) for each embryo. These data could be copied to a spreadsheet.</li> </ul>
<p><b>Threshold: 0-0</b> <b>Count: 25</b> <b>Total Area: 3177.6 <math>\mu\text{m}^2</math></b> <b>Average Size: 127.1 <math>\mu\text{m}^2</math></b> <b>Area Fraction: 3.72%</b></p>	<p>A summary of the particle count is also shown in another data window.</p>

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