

Area and frond number estimation in Image J

Principle

This protocol describes the method to estimate the frond surface area and frond number in a pot containing Lemna minor.

Taking pictures:

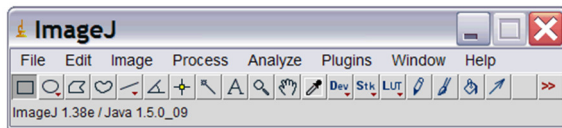
Each pot contains a small floating piece of plastic ruler of at least 10 mm

The pots are placed on a backlit surface (like a lightbox)

Pictures of the pots are taken with a conventional digital camera.



Software



Download ImageJ from website <http://rsbweb.nih.gov/ij/download.html>

<http://www.dentistry.bham.ac.uk/landinig/software/software.html>

save jar file includes source on your desk top, extract with winZIP and save tresh_colour.jar file into C:\Program Files\ImageJ\plugins\Analyze. Then reopen imageJ

Open picture by

File → open (or ctrl O)

Surface area estimation:

Set the picture scale:

Draw a line on scale of 10mm as correctly as possible

Then push Analyze → set scale

Fill in Known distance 10 and unit of length mm (press OK)

Amount of pixel is normally around 450 for 10 mm

Selecting the leafs

Image → adjust → color threshold select in hue so that green of plants is visible

Set Image → type → 8-bit (will change your color picture to greyscale)

Image → adjust → threshold: play with the setting until leaves are red press apply. Normally this will give you a nice selection of your leaves. If not for example when many white leaves (e.g. in very toxic conditions) are present you will probably will not get rid of the complete background. You can then either select part of your picture and press edit → cut to delete the selection or go to the next step with the selection (then only the area in the selection will be measured).

Analyse the leaf area

analyse → analyse particles. You will have a screen where in show you can change to outlines so you'll know what he measured. In size you can enhance to e.g 0.05mm-infinity: then you'll get rid of small impurities in the picture. Be sure to have include holes flagged OFF if not ImageJ will include holes in between plants that are in the same outline. Press OK and you'll have your area in mm². If you want you can save your results to excel.

Fronnd number counting:

Open your picture in ImageJ

Plugins → analyse → cell counter

At "Actions": click on keep original and initialize

Then click on type 1 (or 2, 3 ... if you prefer) and click on every frond: normally it will add a number to your picture and it will count in the cell counter screen.