Using ImageJ to Measure Leaf Area

There are two ways to measure leaf area in ImageJ, both require the leaf to be photographed with a rule in the image (or some other means of measuring the image). If the leaves are photographed on a white background and not touching anything else, then the "select" method can be used. This is the easier method and does not involve drawing round the leaf outline. If the leaf cannot be separated from other leaves by the programme, then you will need to draw around it using the "tracing" method. There are many other things that ImageJ can be used for, this is only a very simple introduction to the software.

1 The "select" method – note letters refer to the images in Figure 1.

     1. Open photograph in ImageJ by clicking OPEN and selecting the relevant photograph from the dialogue box (A).

     2. Select the straight line segment tool (B).

     3. Draw a 1cm line with the straight line selection tool using the ruler in the image for scale (C).

     4. Go to Analyse and select Set scale (D). In the dialogue box that opens fill in 1 cm (E). You must do this for each photograph as they were all taken at slightly different distances/angles.

     5. Go to Image and select Type. Select 32-bit (F). This will turn the colour image into a black and white one (G).

     6. Go to Image and select Adjust. Select Threshold (H). A new dialogue box will open showing the range of pixels in a histogram (I). The default setting is red and white, click on the drop-down box for colour and change this to black and white. Occasionally, the threshold bar may need to be adjusted to make sure that most of the leaf is black and that areas outside are white. This is achieved by moving the bar at the bottom of the threshold box and watching the black and white adjustment in the photograph until it is correct. This does not usually have to be done.

     7. Go to Analyse and select Set Measurement (J). Tick area and perimeter (K). There are many more things that can be measured, but you only need area for this practical. This only needs to be done once per session as the programme holds the information.

     8. Select the wand tool from the menu (L). Click on the edge of the leaf. The programme will draw a yellow line around the leaf image (M). It will use this as the limits of what will be measured.

     9. Once the leaf is enclosed by the yellow trace line, go to Analyse and click Measure Particles (N).

10. A dialogue box will open. Ensure that Display Results, Clear Results and Record Stats are ticked (O). This should only need to be done once in each session. Do not change any other settings.

11. A dialogue box will open with the requested measurements in the units defined by the initial scale measurement (P).

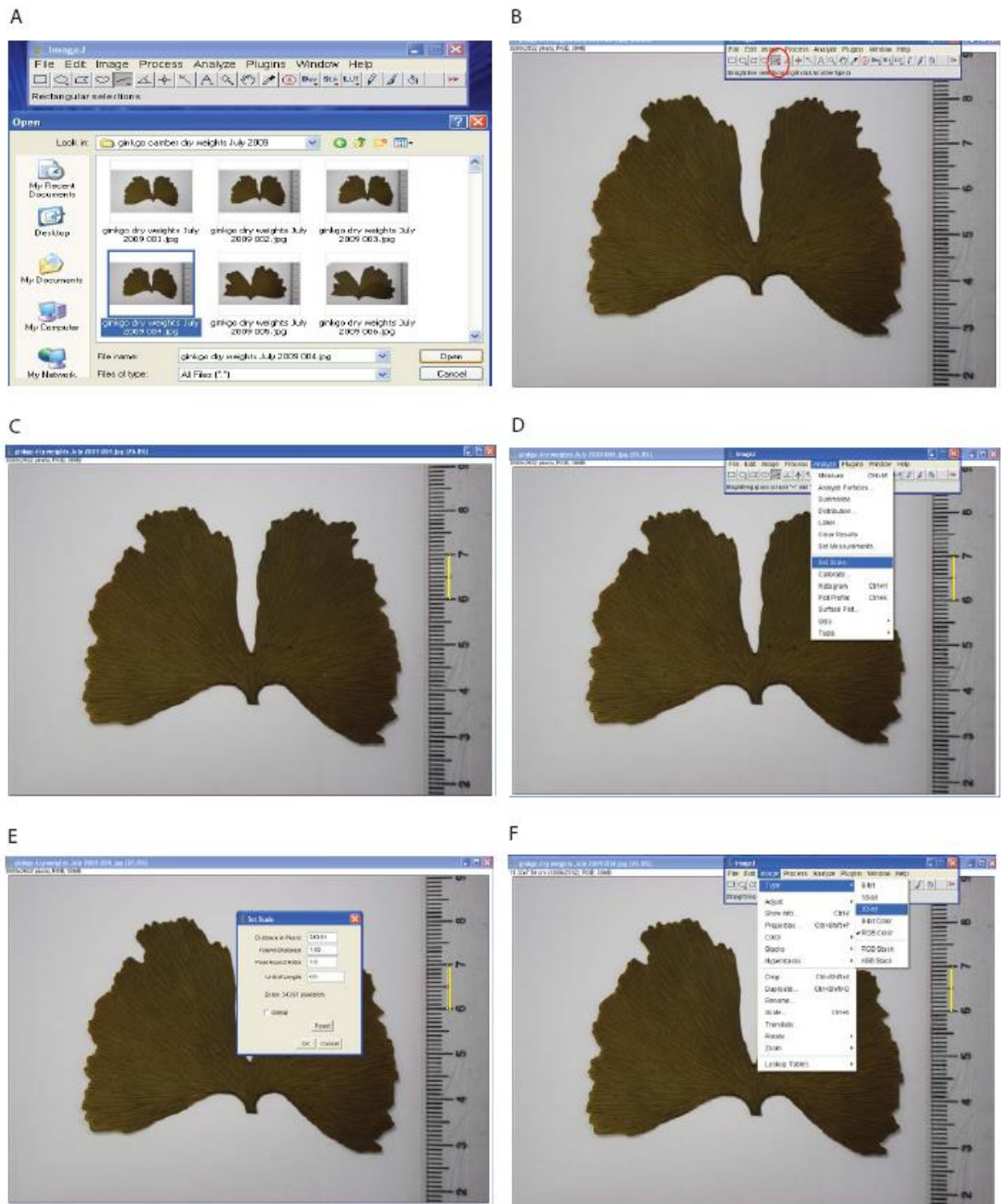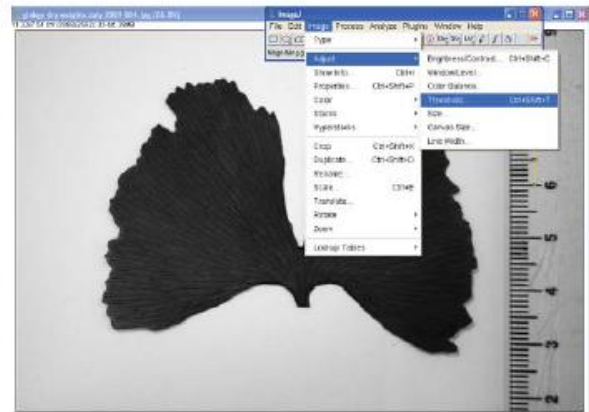12. All measurements in the dialogue box can be copied and pasted into Excel for analysis.

Figure 1: Step-by-step process of using ImageJ to measure a *Ginkgo biloba* leaf.
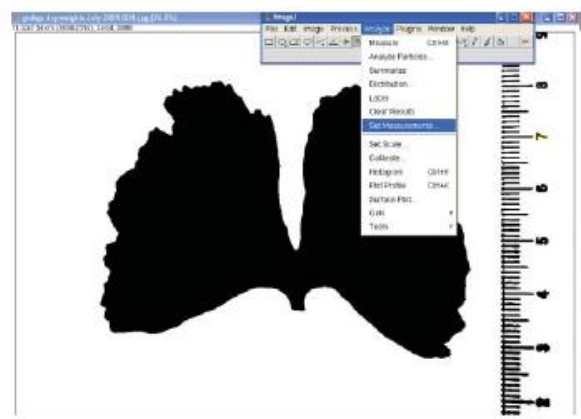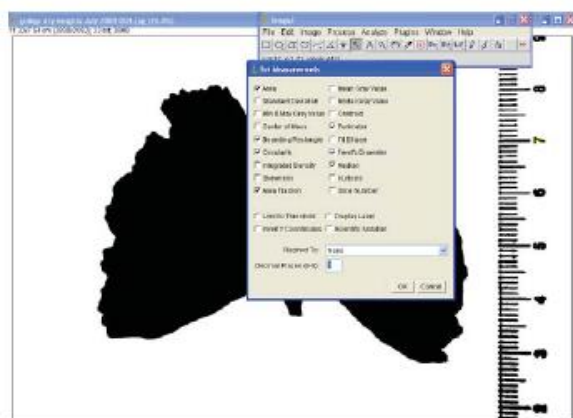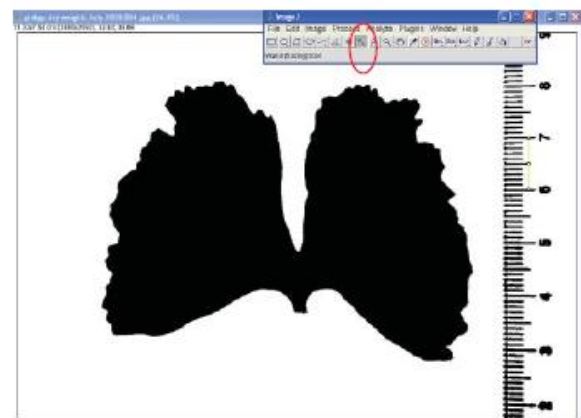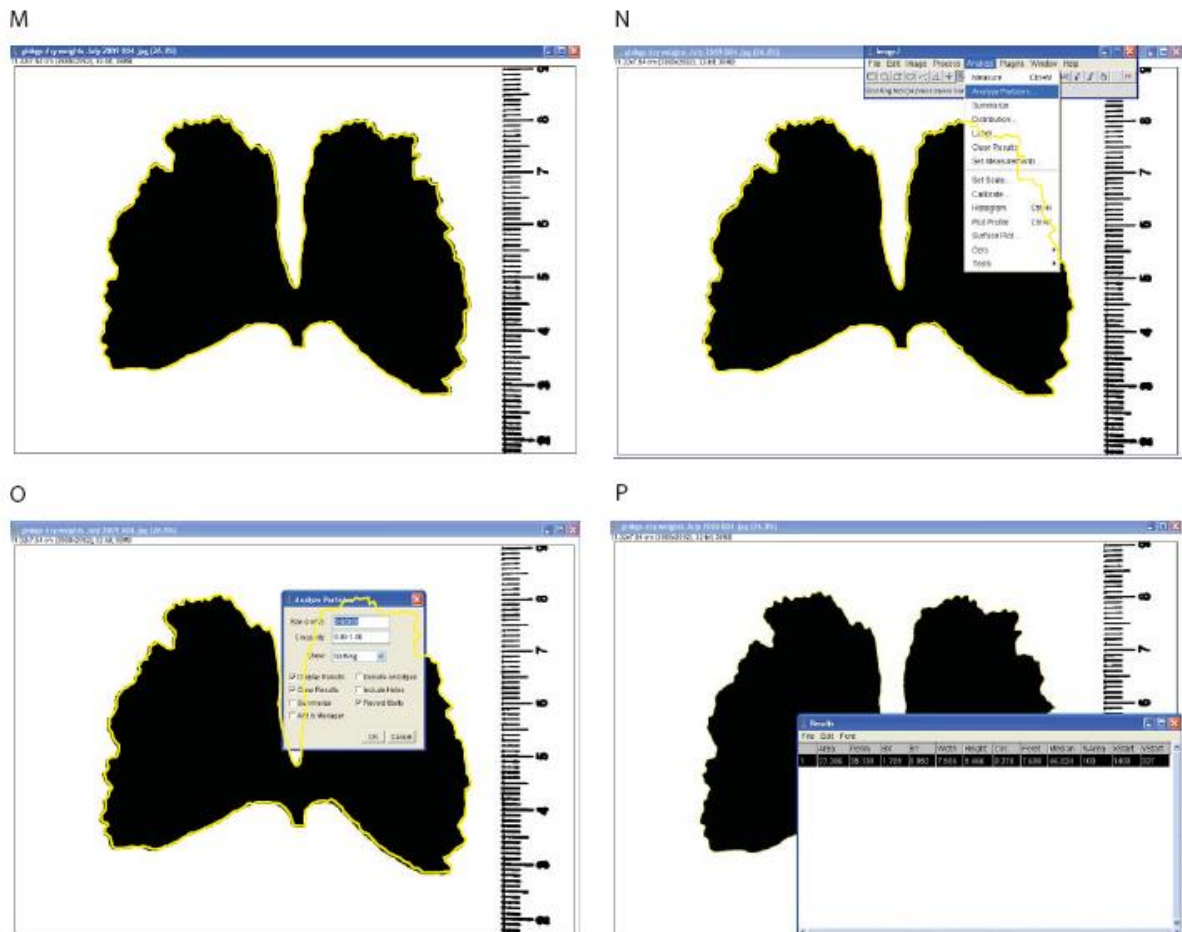
G

H

I

J

K

L

Figure 1 continued: Step-by-step process of using ImageJ to measure a *Ginkgo biloba* leaf.

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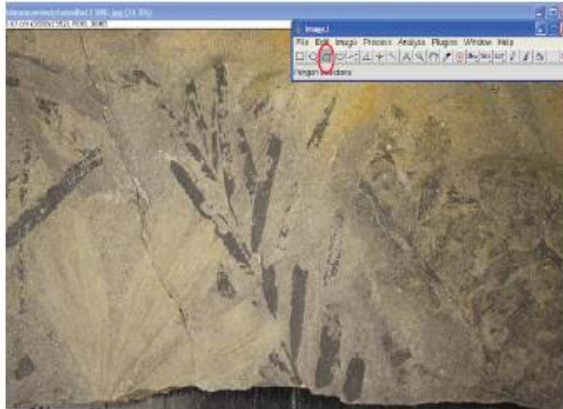
2 The "trace" method – note letters refer to the images in Figure 2. This method can be used when part of the leaf is slightly obscured or two leaves are in contact, meaning that the programme cannot distinguish between them. It is more time-consuming and requires you to be accurate in your tracing.

1. Open photograph in ImageJ by clicking OPEN and selecting the relevant photograph from the dialogue box (A).

2. Select the straight line segment tool (B).

3. Draw a 1cm line with the straight line selection tool using the ruler in the image for scale (B).

4. Go to Analyse and select Set scale (C). In the dialogue box that opens fill in 1 cm (D).

5. Go to Analyse, select Set Measurement (E). Tick area and perimeter(F).

6. Select the polygon selection tool (G) or the freehand selection tool. Either of these tools can be used to draw around an image. The freehand selection tool requires more precision on the part of the user and the polygon tool is therefore
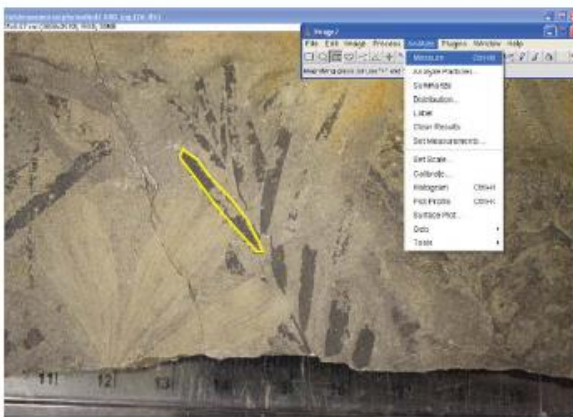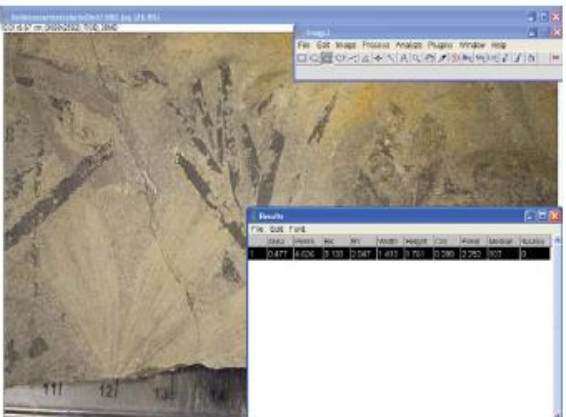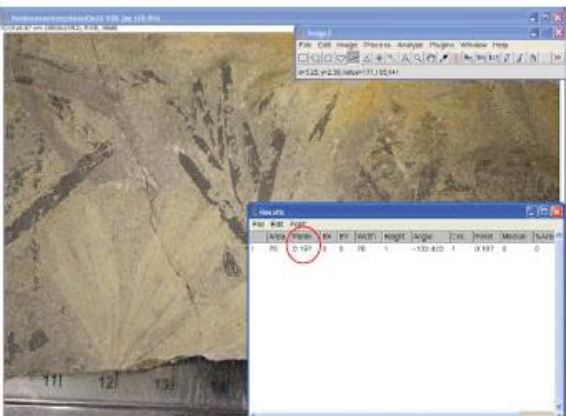
easier to use. Both tools generate the same results. Trace around the leaf (a fossil plant in Figure 2) with the tool (H).

7. Once the entire leaf is enclosed by the yellow trace line, go to Analyse and click Measure (I). A dialogue box will open with the requested measurements in the units defined by the initial scale measurement (J).

8. All measurements in the dialogue box can be copied and pasted into Excel for analysis.

Figure 2: Step-by-step process of using ImageJ to measure a fossil conifer leaf that could not be done using the "select" method.

Figure 2 continued: Step-by-step process of using ImageJ to measure a fossil conifer leaf that could not be done using the "select" method.