

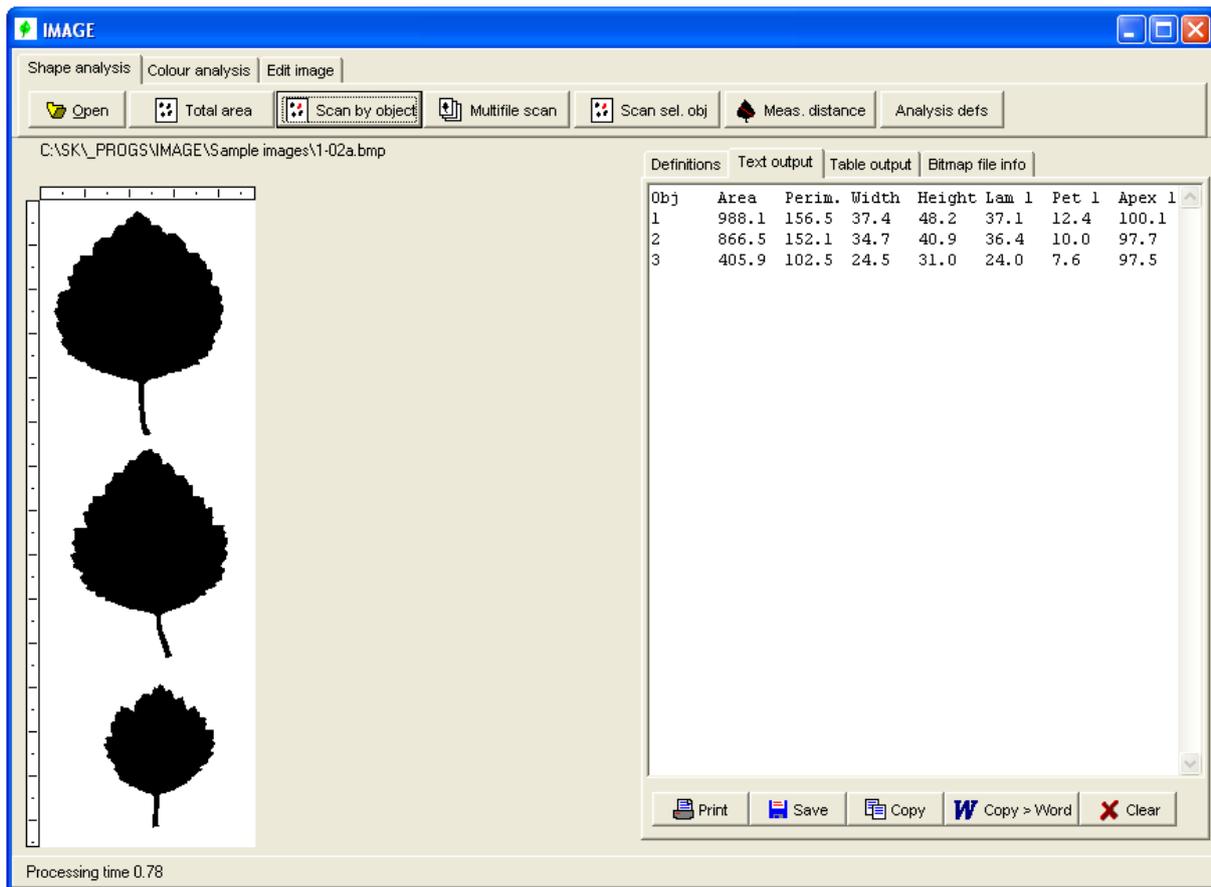
# IMAGE.EXE

## Image analysis program for measuring area, perimeter etc. on bitmap images

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The IMAGE program window after an image has been opened and analysed:

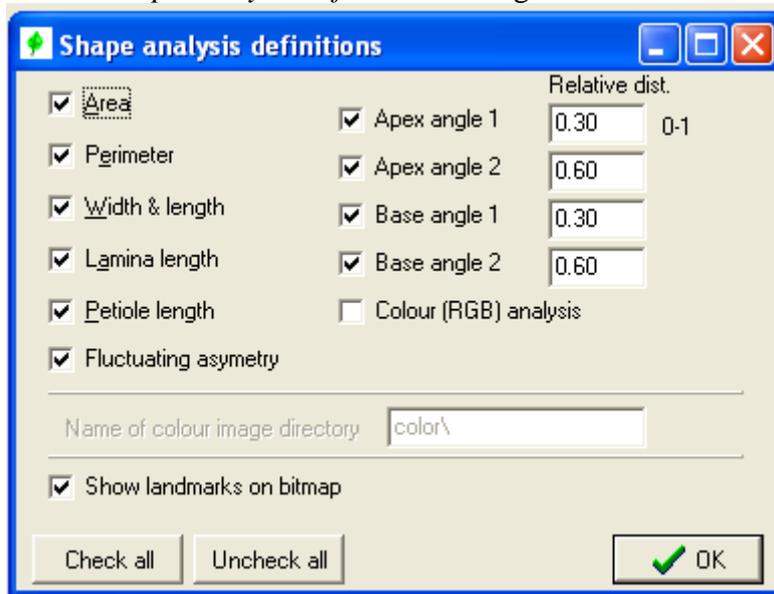


The scales above and to the left of the image indicates 1 cm and 0.5 cm distances from the upper left corner.

## The shape analysis tab

### General definitions

A number of shape and colour characteristics can be measured. Which characteristics to measure are defined in the *Shape analysis definitions* dialog:



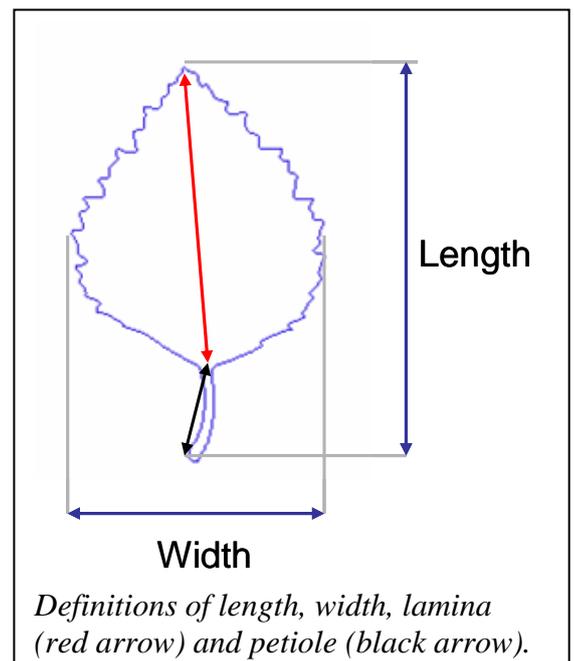
When checking the “Show landmarks on bitmap” the analysis is stopped twice for each object and the main five landmarks [(1) apex, (2) base, (3) left and (4) right most positions, and (5) petiole insertion on leaf disk) and if included in the analysis the basic measures of the asymmetry measures are shown on screen .

Note that the images to be analysed must be **black & white** (B&W)! Grey-scale or colour images can be converted to B&W under the *Edit image* tab (see below).

The scale can be defined under Definitions (see below) either by giving the resolution to which the bitmap was scanned with or by defining two points on the bitmap and the distance between them.

### Area and perimeter

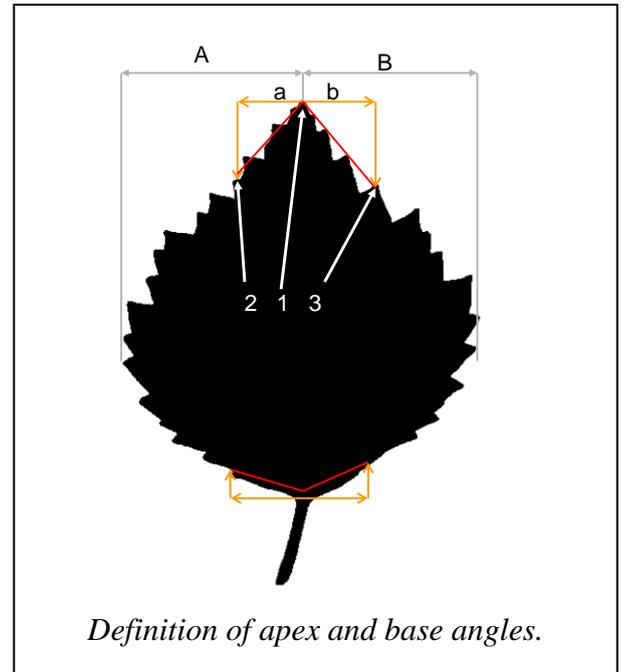
Area and perimeter are estimated from the number of pixels forming the area or the perimeter converted to mm or mm<sup>2</sup> using the scale definition. Length and width are defined as the horizontal and vertical distance between the extreme points of an object, respectively (c.f. figure). In contrast the estimate of lamina and petiole length equals the distance between the upper and lower extremes of these parts (c.f. the figure). Thus, lamina plus petiole lengths do not necessarily equal total length.



Definitions of length, width, lamina (red arrow) and petiole (black arrow).

### Apex and base angles

The apex angle is defined by the angle defined a line from point 2 to 1 and 1 to 3 in the figure to the right. Point 1 is the leaf apex (with the coordinates:  $x_1, y_1$ ). The other two points are computed from horizontal distance from leaf apex to the margins (marked A and B) and the relative distance ( $rd$ ) measures defined in the *Shape analysis definitions dialog* (c.f. above): The x coordinate of point 2,  $x_2 = x_1 - a$ , where  $a = rd * A$  and  $y_2$  is the y coordinate where a vertical line from  $x_2, y_1$  intercepts the leaf margin. Point 3 is analogously  $x_3 = x_1 + b$  [where  $b = rd * B$ ] and  $y_2$  is the y coordinate where a vertical line from  $x_2, y_1$  intercepts the leaf margin. The base angle is computed analogously using the point where the petiole is attached to leaf lamina.



### Fluctuating asymmetry FA

Is defined according to Kozlov et al (1996)<sup>1</sup> by the distance between the midvein (half-way between apex and base, i.e.  $2*a$  in the figure to the right = lamina height) and left and right leaf margin (L and R respectively):

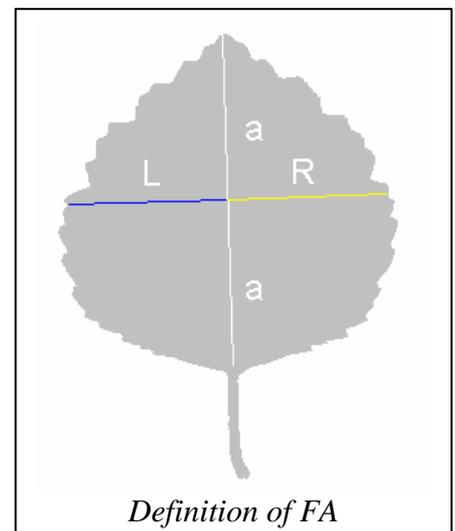
$$FA = (L - R)$$

and

$$\text{rel FA} = FA * 2 / (L+R)$$

where L and R are the maximum width of the lamina of the left and right side respectively (c.f. figure to the right). In the output FA is printed in millimetre units.

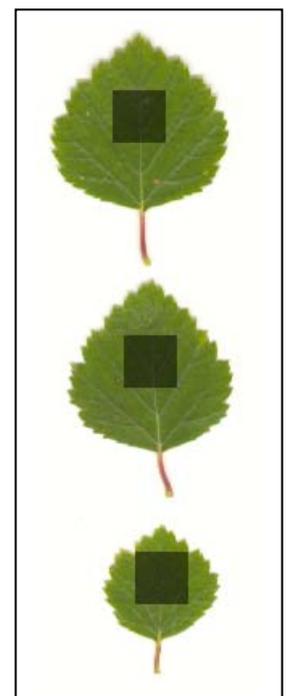
**Note:** this estimate of FA requires that Lamina length and/or Petiole length is checked in the shape analysis dialog above! If not FA, the position to measure widths is based on total length.



### Colour analysis

If a colour analysis is to be performed the program expects to find a colour image with the same name as the main (B&W) image but stored in a subfolder as defined in the definitions window (c.f. figure below). The colour images can be of any resolution: a fraction of the centre of each object is analysed using the relative position in the "main" (B&W) image. The colour analysis calculates the mean intensity (transformed to range from 0 to 1) of red, green, blue and "grey" for a square of  $33*33$  pixels in the centre of each object (c.f. the dark squares in the figure to the right). Any pixels within this area that are close to white (red+green+blue > 700, the intensity of each colour is coded in 256 steps from 0 to 255) are ignored. Grey is defined as (red+green+blue)/3. Thus a grey value of 1 equals white and 0 black.

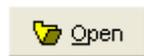
**Note:** these measures has been developed and tested only for some species of *Betula*. For leaf shapes a different leaf shape, some measures may malfunction. If checking the *Show landmarks* five landmark positions are



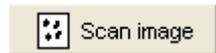
<sup>1</sup> Kozlov, M., Wilsey B.J., Koricheva J., and Haulioja E. 1996. Fluctuating asymmetry of birch leaves increases under pollution impact. - Journal of Applied Ecology 33: 1489-1495.

drawn on each object: (a) apex, (2) lowest point of petiole, (3) left-most leaf position, (4) right most position and (5) the position where the petiole connects to leaf lamina.

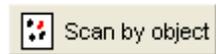
### *Shape analysis tab buttons*



Open: image file (BMP and JPG formats supported)



Scan image and estimate total area of all objects on the image



Scan image and estimate selected characteristics of each object on the image



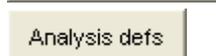
Perform "Scan image" on multiple files



Point-and-click on the object to analyse



Point-and-click to measure a distance on the image



Select the properties to be analysed (area, perimeter, length, width etc).

### *Sample images for shape analysis*

A sample B&W and corresponding colour images are found in the Sample image folders: *Sample images* and *Sample images\Colour* both found in the program folder.

## The colour analysis tab

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### General definitions

The colour analysis is performed using 24-bit colour resolution, i.e., 256 intensity steps each of red, green and blue (0-255). Bitmaps with higher colour resolution will be converted to 24-bit when loaded by the program.

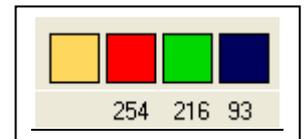
### Colour analysis tab buttons



Open: image file (BMP and JPG formats supported)



The intensities of the three colours are displayed on the screen (see picture on the right). The first box shows the composite colour while the three following boxes show the intensity of pure red, green and blue. Click to print the colour intensity values in the output.



Perform a pixel-by-pixel colour analysis of the entire image. The output consists of the number of pixels with a particular intensity of respective colour. See further details below.



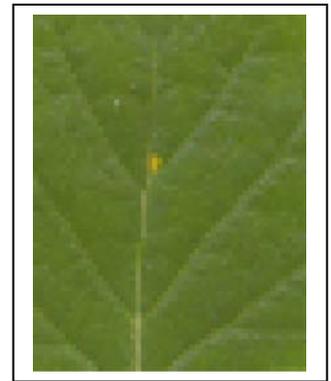
Perform the pixel-by-pixel colour analysis on multiple files.



Perform a colour analysis along a horizontal or vertical transect of the image printing the mean of a number of pixels defined by *Transect width*. See further details below.

*Sample output of colour analysis*

A colour analysis of the image to the right gives the following result:

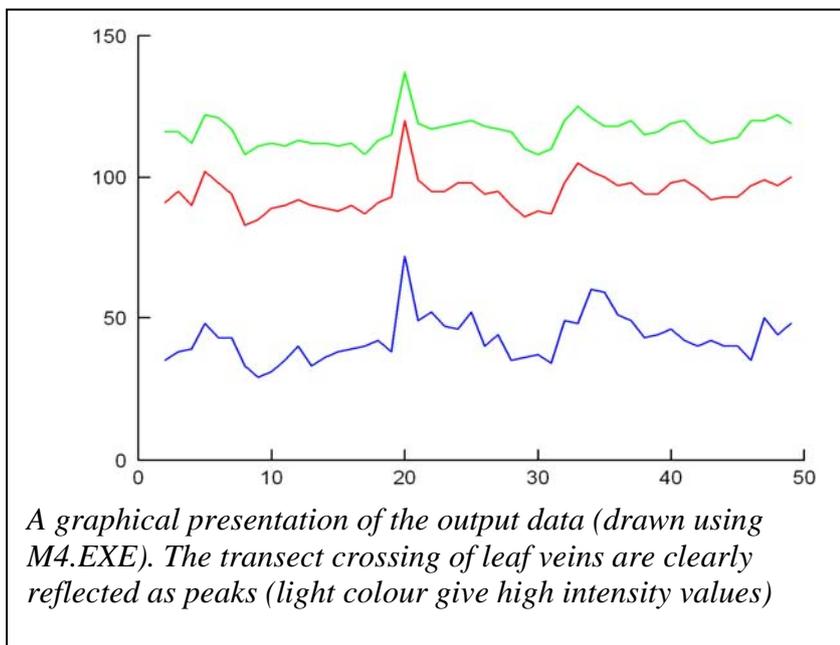
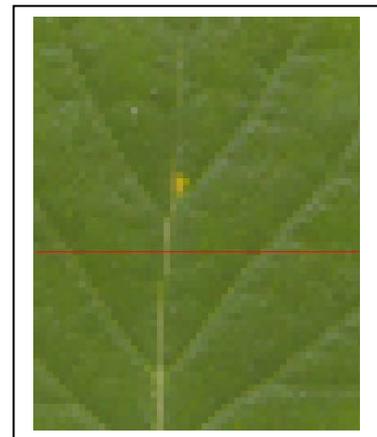


Col inten.	Red	Green	Blue
0	0	0	1
10	0	0	0
20	0	0	27
30	0	0	879
40	0	0	1694
50	0	0	522
60	0	0	182
70	0	0	92
80	400	0	15
90	1939	1	4
<b>100</b>	<b>894</b>	<b>212</b>	<b>1</b>
110	141	1906	0
120	18	1149	0
130	13	123	0
140	8	16	0
150	2	6	0
160	0	4	0
170	0	0	0
180	1	0	0
190	1	0	0
200	0	0	0
210	0	0	0
220	0	0	0
230	0	0	0
240	0	0	0
250	0	0	0
Mean	97.2	118.3	45.4

The colour intensity is summarised in 10-unit classes. For example, in the image above, there were 894 red, 212 green and 1 blue pixels having intensities between 100 and 109 (marked in **bold** in the table to the left). The mean pixel of this image have the colour signature red = 97.2, green = 118.3 and blue = 45.4.

A transect analysis of the image to the right (approximately) indicated by the red line give the following result (the first 8 lines only):

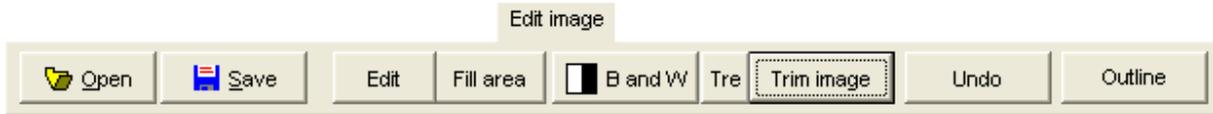
POS	Red	Green	Blue
2	91	116	35
3	95	116	38
4	90	112	39
5	102	122	48
6	98	121	43
7	94	117	43
8	83	108	33
.....			



## The edit image tab

Here simple editing of the image can be made. Note that the editor is simple and slow. For more extensive editing it is recommended to use other editors such as PAINT.EXE.

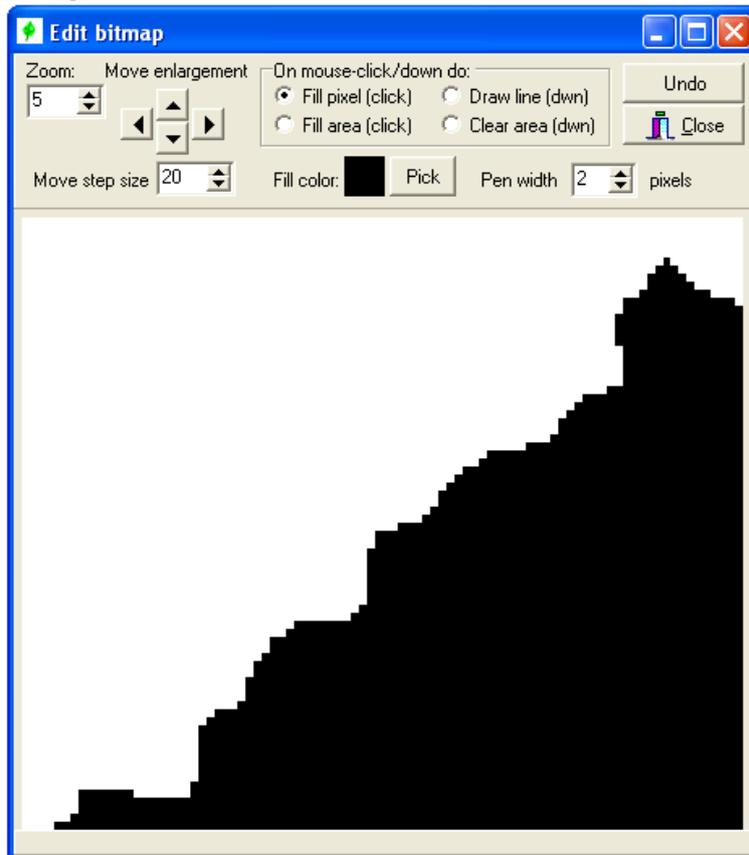
### The edit image tab buttons



Open and Save are used for loading or saving images as described above.



Opens a window where an enlargement of the image is shown, and simple editing can be made:



Click on a point on the image to fill the area with the area with the image colour (e.g. useful to fill in wholes created by herbivores in images of leaves)



Converts a colour or grey-scale image to black and white. The threshold between black and white can be adjusted by clicking button labelled *Tre*



Change the threshold definition used by *Convert to B and W* above.



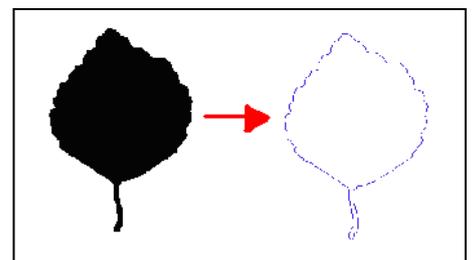
Allow the user to cut out a rectangle of the image for further analysis



Undo last change of the image.



Converts all objects on an image to outlines of the same objects.



## The definitions panel

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Here the colour of the objects to analyse can be defined and the colours indicating the progress of the analysis. Further the scale of the image (number of pixels per mm or inch), an optional correction term and the minimum object to analyse. All objects, such as dust marks, smaller than this size will be ignored.

Definitions

Object colour  Pick from bitm.

Object selected for analysis

Analysed area colour  Analysis completed

Resolution definitions

Use pixel units Estimate resolution

Resolution 1181 pix / 100 mm Pick resolution

Resolution 299 pix / inch

Correction term 0 o/oo

Minimum object size 20 pixels

Visual display of analysis (slow <---> fast)

Detailed  Found obj.  None

High bitmap resolutions result in small measuring errors but also long processing time.

## The text and table output panels

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All output is printed both as text and in a table. In the text output panel all numbers are separated by tab-characters and can thus easily be copied and pasted into a spreadsheet. The text editor has a button for copying the result to MS Word while the Table output can be copied directly to Excel (a new file is created each time the copy-to-Excel button is clicked). The table output can store a maximum of 100 000 lines of data.

## The bitmap file info panel

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Some basic info regarding the bitmap file is shown. The image can be exported to Word or be printed on the system default printer.