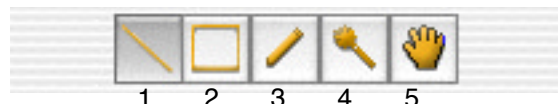# Carnoy

2.0

easy-to-use software specifically designed to carry out measurements on digital LM, SEM and TEM images.

Your workplace

Tool buttons

The orange buttons are the tool buttons. You can select a tool by clicking one of these five buttons. The selected button has a grey background.
1. Line tool: use this tool to drag lines to measure length
2. Rectangle tool: use this tool to drag rectangles to measure surface area of rectangular objects
3. Pen tool: the pen tool is a freehand selection tool: area and perimeter of selections can be calculated.
4. Wand tool: with this tool, you can measure the surface area and perimeter of irregular objects
5. Hand tool: use this tool to move the image when it is too large to fit in its window

Shortcut buttons

1. Open image: presents you the standard open dialog
2. Calibrate: click this button when using manual calibration or choosing a magnification (see the paragraphs on calibration)
3. One Click Calibration (OCC): click this button and then click somewhere on your scale bar for fast calibration
4. Threshold: this button opens a dialog to set the threshold (see the paragraph on thresholding)
5. Analyze particles: performs an automated particle analysis
6. Add scale bar: adds a scale bar to your image (see the paragraph on adding a scale bar)
7. Restore image: restores the image to its original state

Buttons for managing data
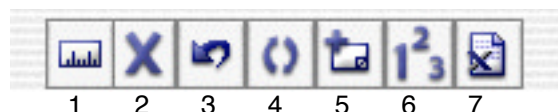
1. Measure: adds the current measurement to the data list
2. Clear all: deletes all measurements in the data list
3. Undo measurement: removes the last measurement
4. Redo measurement: select a measurement, redo it and then click this button to replace the previous value with the new one
5. Insert / edit comment: insert a comment or change it
6. Statistics: displays basic statistics of selected data
7. Export to file: exports your data list to a tab delimited text file

Working with images

Open a new image by clicking the **Open image** button, or by choosing Open from the File menu. You can zoom in / out on images by moving the zoom slider on the toolbar or by choosing Zoom in / out from the Window menu. When an image is larger than its window, you can move around using the scrollbars or the Hand tool. Click the **Hand tool** button on the toolbar to drag the image.

You can also open an image on the Internet from within Carnoy*. Choose Open URL from the File menu and type the image's URL (e.g. www.kuleuven.ac.be/bio/sys/carnoy/images/ss1.jpg). You can carry out measurements on this image as if it was on your local computer. This function might come in handy if you need to measure type specimens, available on a website. (* = not available for Windows).

Calibrating images

To carry out useful measurements, Carnoy needs to know the relation between pixels and a real world unit (e.g. cm, μm,...). There are three methods to calibrate images in Carnoy. If your image has a scale bar (SEM and TEM images), you can use the first two methods. If your image doesn't have a scale bar (LM images), you can use the third method.

One Click Calibration (OCC)

With OCC you can click a continuous scale bar. Carnoy will highlight the scale bar and a sheet will be presented prompting for the length and unit of the scale bar. OCC only works with a horizontal scale bar. If your image has a vertical scale bar, choose Rotate from the Edit menu.

1. Click the **OCC** button
2. Click somewhere on the scale bar
3. Enter the length and unit of the scale bar

You are now ready to carry out measurements.

Manual calibration

When for one or the other reason you can't use OCC, you can calibrate your image manually.

1. Click the **Calibrate** button
2. Drag a line over the scale bar (you can zoom in for maximal precision)
3. Enter the length and unit of the scale bar

You are now ready to carry out measurements.

Selecting a magnification

Use this method when using LM images or images that don't have a scale bar.

You will have to take a picture of a calibration slide or an object of known length at every magnification of your microscope. Repeat the following steps for every magnification:

1. Click the **Line tool** button
2. Drag a line over the object of known size (you can zoom in for maximal precision)
3. Note the pixel size (displayed in the status bar)
4. Go to the Preferences in the Application menu (Macintosh) or to Options in the Analyze menu (Windows)
5. Create a new magnification and enter its name (Click the **New Magnification** button)
6. Enter the number of pixels corresponding to one unit and enter the name of the unit
7. Click the **Save Magnification** button

For example, if your object measures 10 μm and the pixel size was 150 (step 3), enter 15 in the pixels field and enter μm in the unit field.

When done for every magnification, open a picture, and click the **Calibrate** button. Select the Magnifications tab and choose a magnification from the popup menu to calibrate the image.

Note: Fixed calibration only works if you don't resize images in a graphics program prior to measuring in Carnoy. Also, if you take pictures at different pixel sizes (e.g. 640 x 480, 800 x 600, ...), you must do a separate calibration, not only for every magnification you use, but also for every pixel size you use.

Measuring length

Once your image is calibrated, you can start length measurements.

1. Click the **Line tool** button
2. Drag a line over the object you want to measure (you can zoom in for maximal precision). The measurements (pixel and real world values) are permanently updated in the status bar
3. Click the **Add measurement** button to add the current value to the length/area column of the data list

If this is your first measurement on this image, Carnoy will add the name of the image file to the data list, so you can keep track of which measurements were made on which image. You can change the name of the image file by clicking on it.

If you want to redo a measurement, repeat steps 1 and 2, select the measurement you want to redo and click the **Redo measurement** button.

Measuring segments

Carnoy offers the possibility to measure curved objects.

1. Click the **Line tool** button
2. Click the points of the path along which you want to measure while pressing the alt-key. The measurements (pixel and real world values) are permanently updated in the status bar
3. Click the **Add measurement** button (Mac) or shift-click it (Windows) to add the current value to the length/area column of the data list

To create a new path, click the image once without pressing the alt-key and start again.

Measuring area and perimeter

Carnoy can measure rectangular and irregular areas.

To measure a rectangular area:

1. Click the **Rectangle tool** button
2. Drag a rectangle over the object you want to measure (you can zoom in for maximal precision). The measurement (pixel and real world values) are permanently updated in the status bar
3. Click the **Add measurement** button to add the current value to the length/area column of the data list

You can move a rectangle: click inside the rectangle and drag it.

To measure the area and perimeter of an irregular object, you can use either the Pen tool, or the Wand tool. When you have to measure a lot of objects it's more convenient to use the Wand method. If the colors of the objects you want to measure are very similar to the background colors of the image, you will get better results with the Pen method.

The Wand method

To measure the area and perimeter of an object with the Wand button, you first have to set the threshold (see the section on Thresholding).

1. Click the **Threshold** button and move the slider so that the object you want to measure is white and the background is black
2. Click the **Wand tool** button
3. Click somewhere on the object you want to measure
4. The measurement (pixel and real world values) are shown in the status bar
5. Click the **Add measurement** button to add the current values to the area and perimeter columns of the data list

If you want to add other areas to the current value, hold the shift key while clicking objects.

The Pen method

1. Click the **Pen tool** button
2. Draw a closed area (you can zoom in for maximum precision). If drawing a selection does not work well, increase the pen width in the Preferences.
3. Click the **Add measurement** button. The area and perimeter of the selection will appear in the area and perimeter columns of the data list. The selection itself (the colored pixels) are included in the area value.

If you want to clear the selection, click the **Restore image** button.

If this is your first measurement on this image, Carnoy will add the name of the image file to the data list, so you can keep track of which measurements were made on which image. You can change the name of the image file by clicking on it.

To restore the image to its original state, click the **Restore image** button.

Thresholding

Thresholding is used to segment an image into objects of interest and background on the basis of gray level. Objects you want to measure are displayed in white and background is black. The more you increase the threshold value, the more objects will be colored white and will be included in further analysis. As you vary the threshold, the effect is continuously displayed (after a few seconds). If the objects you want to measure are darker than the background, you can invert the image before thresholding (Edit - Invert Image).

To restore the image to its original state, click the **Restore image** button.

Analyzing particles

With the analyze particles function, Carnoy counts and measures the surface of particles on a calibrated, thresholded image.

1. Click the **Threshold** button and move the slider so that the object you want to measure is white and the background is black
2. Click the **Analyze particles** button
3. Enter the diameter (pixel size) of the smallest and largest particle Carnoy has to analyze
4. Mark the checkbox if you want to measure the perimeter for every particle (measuring the perimeter will increase analysis time)

Carnoy will measure the surface area, perimeter (if you have clicked the checkbox) and the longest horizontal axis of every particle that fits your criteria. Every particle will be numbered and this number corresponds to the number next to every value in the data list.

To restore the image to its original state, click the **Restore image** button.

Adding comments

Every measurement can be annotated. Select the measurement from the data list and click the **Insert / edit comment** button or just double click the measurement. Enter the comment in the text field and click the **Save** button. To remove the comment, click the **Clear** button and the **Save** button. An annotated measurement has an asterisk (*) next to it. You can add the same comment to several measurements: select them all before clicking the **Insert / edit comment** button.

Adding a scale bar

When your image is calibrated, but does not have a scale bar, you can add one.
1. Click the **Add scale bar** button
2. Enter the scale bar's length, pixel height and color
3. Mark the checkbox if you want to add a legend
4. Click the **Add** button
To restore the image to its original state, click the **Restore image** button.
You can set the default scale bar options by choosing Preferences from the Application menu (Macintosh) or Options from the Analyze menu (Windows). The scale bar options are on the General tab.

Analyzing data

Carnoy is able to export your data to a spreadsheet for further analysis. However, you can perform basic statistics from within Carnoy. Select the measurements you want to use (use Shift or Apple keys for multiple selections) and select Statistics from the Analyze menu. Carnoy gives you the minimum, mean, maximum, and standard deviation for the selected data. You can calculate statistics for length/area, perimeter and longest horizontal axis by choosing the right category from the popup list.

Exporting data

Carnoy exports data to a tab-delimited file, which can be read by every text editor, spreadsheet, or database. Simply click the **Export measurements** button to export the entire data list. The newly generated file will open in Excel by default, but you can open it with another application by dragging the file onto it. Before quitting Carnoy, the program checks for unsaved measurements and presents a dialog box if needed.
You can copy measurements to the clipboard by selecting them (use Shift or Apple keys for multiple selections) and choosing Copy from the Edit menu. Select Edit - Paste in a spreadsheet.

Exporting images

You can export images to a lot of file formats, such as BMP, PICT, Photoshop, JPEG, PNG, SGI, TGA, TIFF and Quick-Time image files. Select Save As from the File menu.

Contacting the author

We welcome your suggestions, comments and bug reports. Send feedback to: peter.schols@bio.kuleuven.ac.be

Citing Carnoy

You can refer to Carnoy in publications. Please use the following reference: Schols, P. & E. Smets. 2001. Carnoy: analysis software for LM, SEM and TEM images. Leuven: distributed by the authors. http://www.carnoy.org.

Registering Carnoy

Carnoy is shareware. If you like the program, please donate $15 to enable further development. You can register Carnoy by choosing Register Carnoy from the Help menu. You can pay with Visa or Mastercard: enter your information and click send. The information you send will be encrypted using a secure RC4 encryption algorithm. You will receive a serial number within 24 hours. Click Enter Serial Number from the Help menu and paste your serial number.

List of shortcut keys

Windows users press the Control Key in stead of the Apple key.

Apple + O	Open Image
Apple + S	Save As
Apple + W	Close Window
Apple + E	Export Results
Apple + P	Print Image
Apple + X	Cut
Apple + C	Copy
Apple + V	Paste
Apple + I	Invert Image
Apple + R	Restore Image
Apple + B	Add Scalebar
Apple + 1	Calibrate
Apple + 2	Measure
Apple + 3	Insert Comment
Apple + Z	Undo Measurement
Apple + Y	Redo Measurement
Apple + T	Threshold
Apple + A	Analyze Particles
Apple + J	Statistics
Apple + L	Clear Data
Apple + +	Zoom In
Apple + -	Zoom Out
Apple + 0	Actual Size

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