



LabCellCount

Image analysis environment for automatic counting of cells, organisms or particles



Users manual



LABCELLCOUNT PROGRAM FOR AUTOMATIC COUNTING OF CELLS, ORGANISMS OR PARTICLES USERS MANUAL

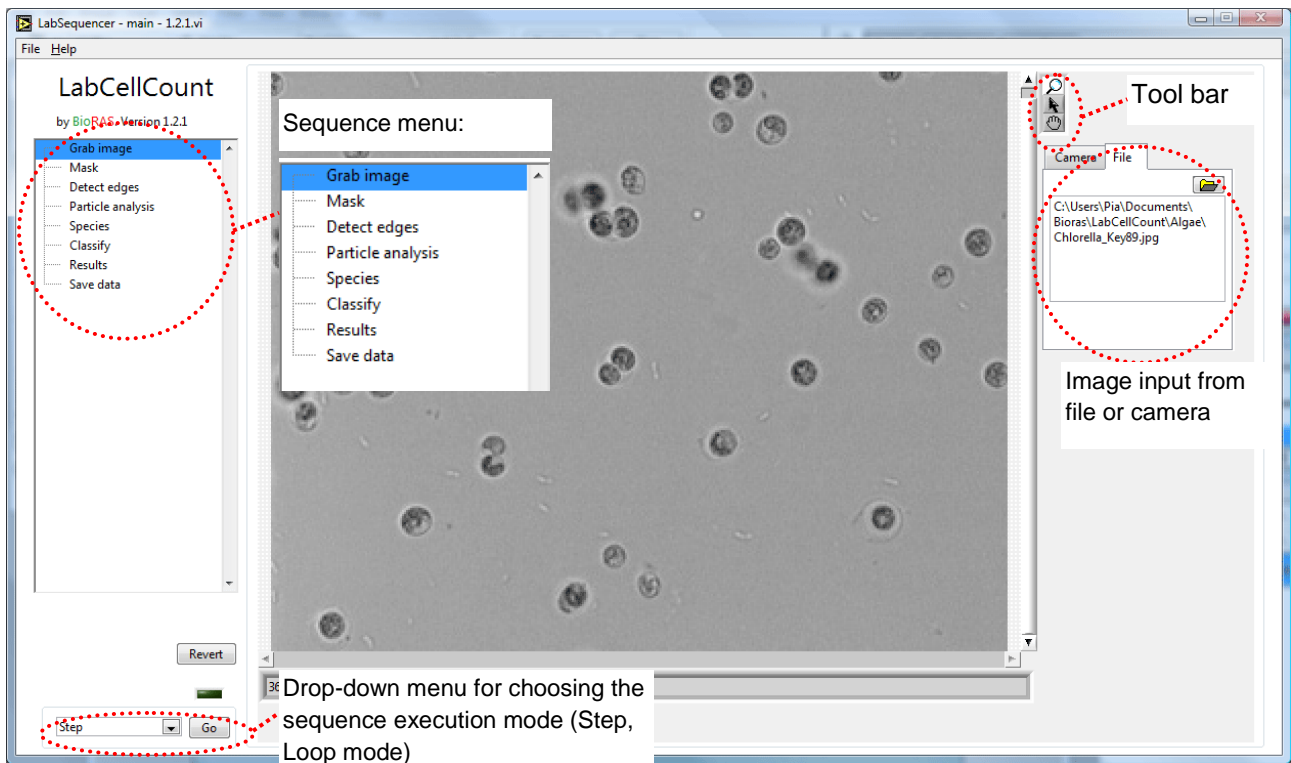
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OVERVIEW

- LabCellCount is an image analysis system for counting of particles, cells, organisms using image analysis.
- Analysis of digital images with good contrast and resolution, or images directly from a connected camera.
- Simple to operate, and easily be adaptable to different types of samples
- Built-in neural network, which can be trained to recognize various shapes.





START OF THE LABCELLCOUNT PROGRAMME

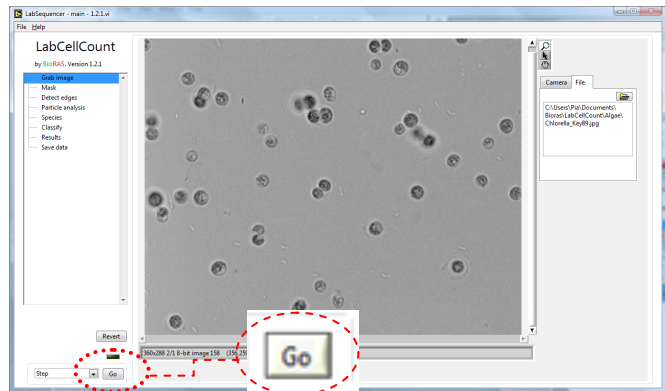
START LABCELLCOUNT

Open LabCellCount application from the application menu.

The application opens in the step where the picture is imported (Grab image).

Start-up of the program:

Start the program by clicking the "Go" button. The programme will start, and "Continue" and "Stop" button will be visible, if "Run sequence" or "Loop" is selected (see below).



CHOOSE BETWEEN RUN SEQUENCE, STEP OR LOOP

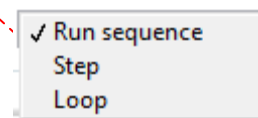
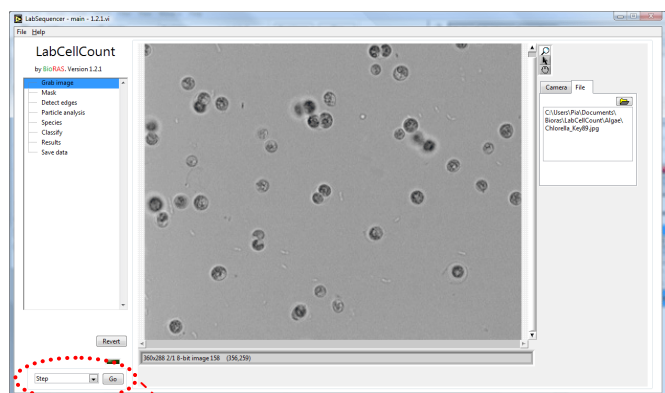
The menu at the bottom left lets users choose between three ways of running the program:

Run sequence: The entire program sequence will run through once. Start analysis with a click on the "Go" button. The sequence will stop at "Grab image" to display the image. Check that the image is OK, and click "Continue". The program will run through the menu, and stop at the "Results" step. Click "Continue" again to complete the job, the program is ready for the next sequence.

Step: Step through the program step by step by clicking "go" for each step. Step is used to customize and control the application settings.

Loop: This mode is similar to the "Run sequence" mode. In Loop mode the program will not stop at results a new sequence will start automatically, but will restart a new run automatically.

Tip: Instead of clicking the "Continue" button in the program use the keyboard "Enter".





CONTINUE, STOP, REVERT BUTTONS

In "Loop" or "Run sequence", the "Continue" and "Stop" buttons will appear. When the "Continue" button is activated, the entire program sequence is executed. Use the "Stop" button to abort the analysis.

In Step mode "Continue" will not appear. Each step of the sequence is executed individually, by clicking on "Go". The "Stop" button will appear during executing of each step.

Activate "Continue (Loop or Run mode):

- Mouse click on the button
- Use the "Enter" button on the keyboard

Stop-button:

- Stops the sequence
- When Stop is activated, Continue and Revert will disappear
- Continue by clicking "Go"

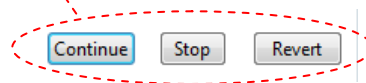
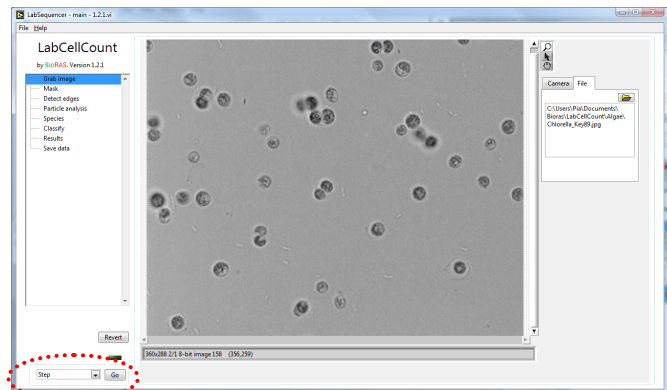
Revert:

- Go back to the last saved settings in the current step

LabCellCount always remembers the last used settings when closing the program and LabCellCount will start with the last used settings.

If settings are changed, it is possible to return to the last used settings by clicking the "Revert" button.

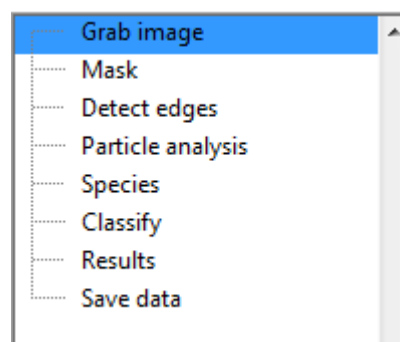
This only applies to the settings in the current step. The most secure way to return to a known protocol, in case of incorrect settings, is to store and retrieve settings via the "File" menu.



PROCESS

THE SEQUENCE MENU

The Sequence menu to the left in the program window is visible anytime during execution of the LabCellCount program. The sequence shows all steps in the image analysis process, and after running the program, it is possible to view all steps at any time, by clicking on one of the sequence steps.





GRAB IMAGE

In this step images are imported into the program.

The camera is connected: Images will be retrieved from the camera, as shown on the right, and the "Camera" tab will be open.

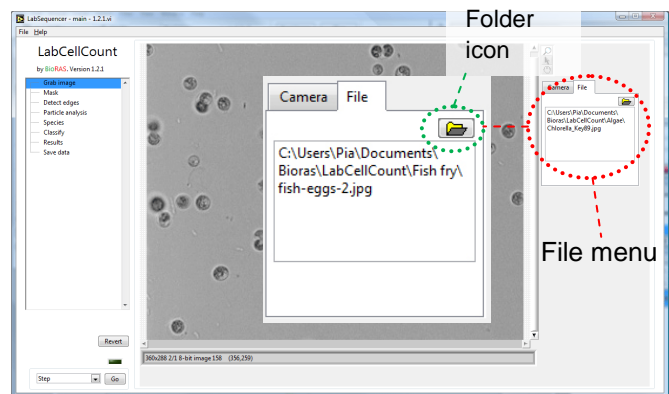
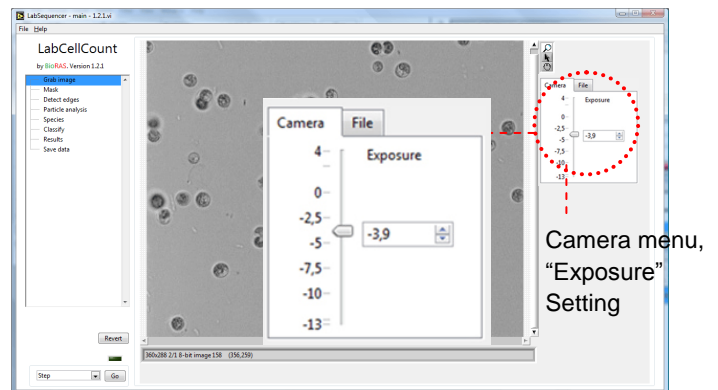
Please note that LabCellCount currently supports a limited number of cameras, please contact info@bioras.com for more information.

The Exposure on the camera should be set to fully open. After that adjust Exposure in the program, to obtain an image with suitable lighting. Set the exposure, by sliding the "exposure" slider until the bright areas of the image is light gray, without completely white parties, which may result in overexposure.

The value for Exposure can also be written directly into the "exposure" field.

The camera is not connected: The File tab will be opened instead. Stored images can be opened and analyzed. Choose pictures from image archives by clicking the folder icon, and specify the path on your computer to an image that you want to analyze. If the image is in an archive with multiple images, will LabCellCount analyze the images one after the other in alphabetical order.

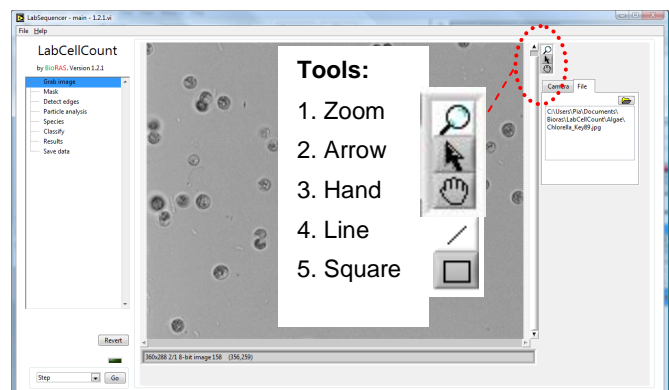
Important: LabCellCount makes a copy of the image with the location and file name specified in the "Save data" step (see the "Save data").



TOOLS (ZOOM, ARROW, OTHERS)

Tools vary slightly between sequences.

1. **Zoom in** (*enlarge image*): click in the image with the mouse; **Zoom out**: Hold down the shift key and click at the same time in the image window
2. **Arrow** for pointing in the image window
3. **Hand** for moving the image view
- 4,5. **Line and Square** are used in the "Mask" step to draw the mesh ring of land outside of the Petri dish (see below). The line tool can also be found in the Particle analysis step, where it can be used for calibration of pixel size.

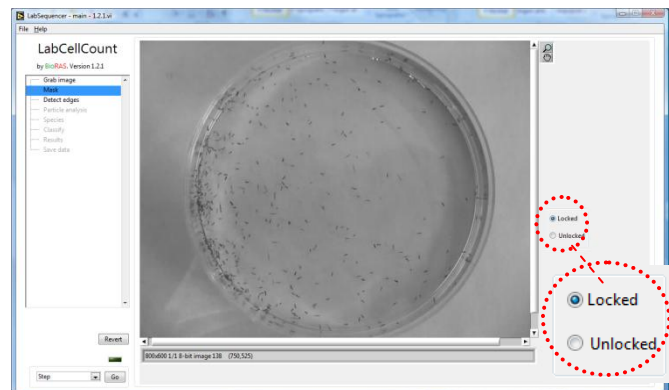




MASK

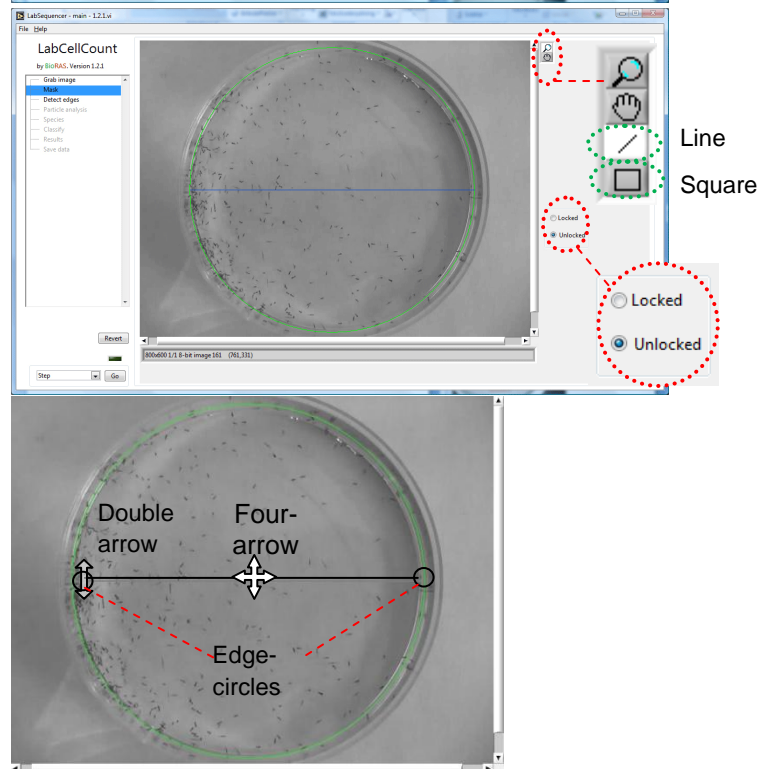
In the "Mask" a round or square shape can be drawn, in order to mask the area around that shape.

- The Mask is locked when "Locked" is enabled. By default, is Mask is "Locked"
- In order to change the mask, enable "Unlocked", whereby the Line and Square tools appear.



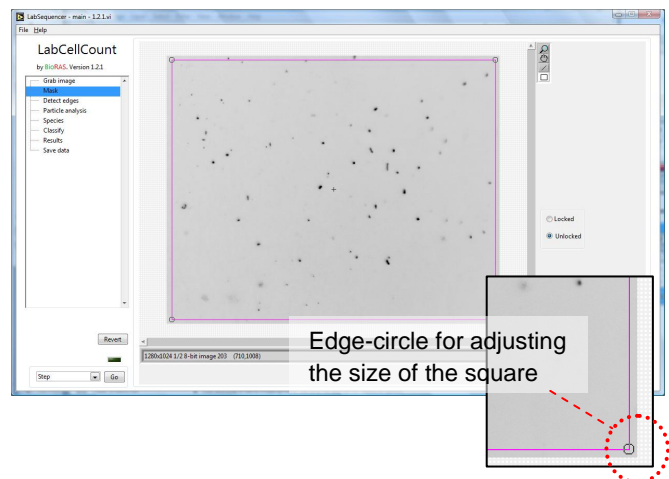
LINE TOOL FOR DRAWING OF CIRCLES

- The line is the diameter of a circle. Draw a line, and a circle will appear around it. The line tool can also be used to adjust an existing circular mask.
- In order to draw a circle, place the cursor at the edge i.e. a Petri-dish, as shown in the example.
- Draw a line across the dish, click the left mouse button at the beginning of the line, and release it at the end. To get a horizontal line, press the Shift button down at the same time.
- A circle will appear.
- Adjusting the circle:
 - Move the entire circle by moving the mouse over the line. A four-pointed arrow will appear. Click on the line, and drag the mouse to move the whole circle.
 - Change the radius of the circle by moving the mouse over one of the ends of the line. A double arrow and small circles at each end of the line will appear. Click on one of the ends; drag the mouse to change the circle's radius, as well as the line's direction.



SQUARE TOOL

- The square tool is used in a similar way as the circle tool.
- Draw a square by placing the mouse at the edge of the intended circle, left click, and draw a square. The square tool can also be used to adjust an existing circular mask, like described above.

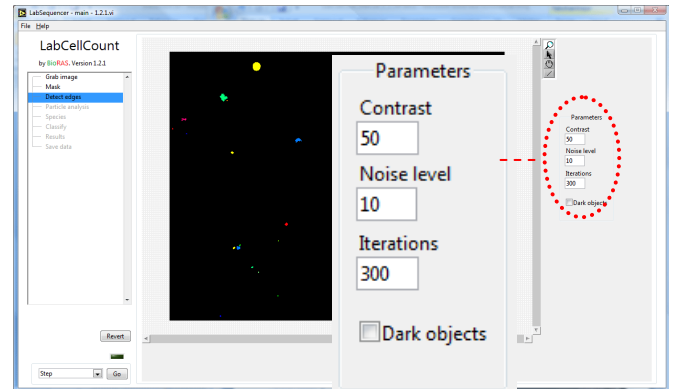




DETECT EDGES

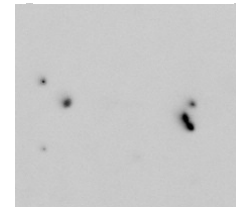
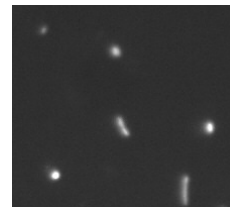
In this step, the program finds the particles to be counted. The following settings must be adjusted prior to analysis:

- Contrast, see below.
- Noise level, see below.
- "Dark objects" should not be marked, if particles are light against a dark background (default setting).
- "Dark objects": Check, if particles are dark against a light background.
- Number of iterations (repetitions) is set to 300 by default and should not be lower. Number of iterations should preferably correspond to half the distance in pixels between the particles in your image.



Light particles against dark background (default)

Dark particles against light background:



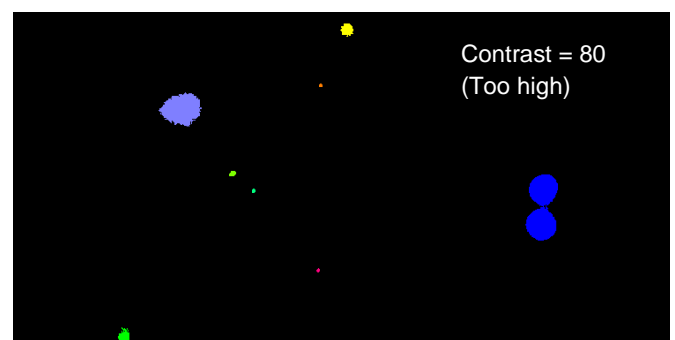
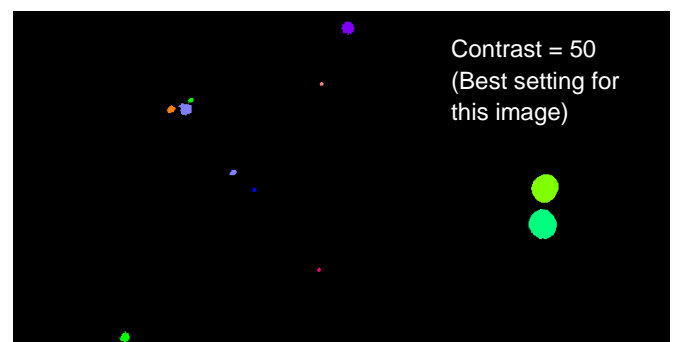
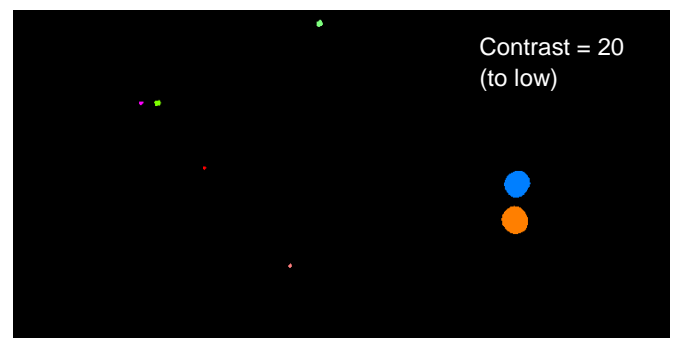
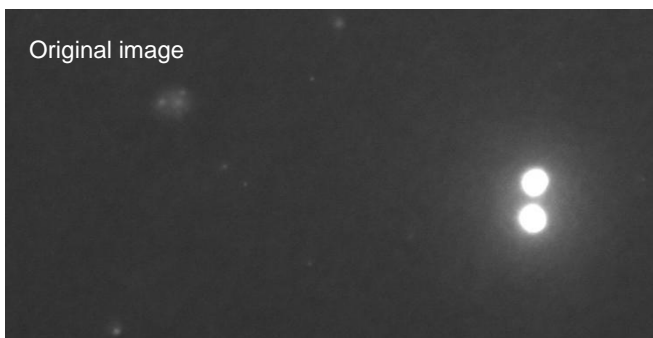
CONTRAST

Contrast will affect the program's ability to detect particles or outlines in the picture. Increasing "Contrast" increases the size of the particles, and more particles will be found.

The images to the right show the result of analysis of the image below with increasing Contrast.

If the Contrast is too low, the small particles will not be detected. If Contrast is too high, particles close to each other may be melting together. Therefore, it is important to find the best value for the Contrast for a given type of samples.

The best way to adjust Contrast is to compare with the original image. If particles are small, use the Zoom tool (Choose the magnification glass, and click in the image window. To zoom out, hold down Shift, and click the image window).





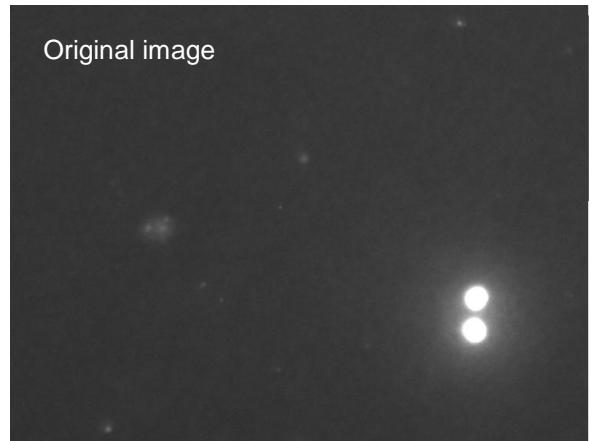
NOISE

The Noise parameter removes background noise and the weak particles.

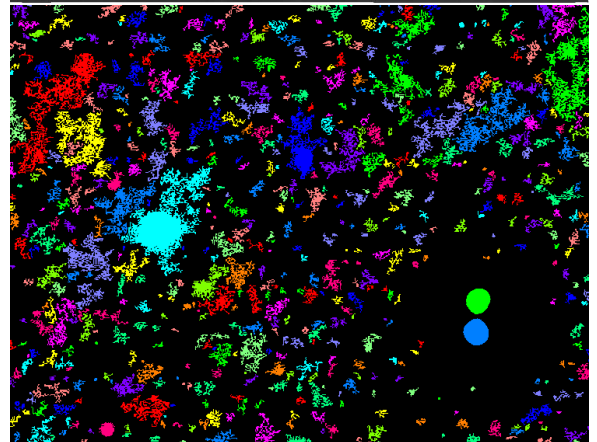
If background noise in the image is low or absent, should Noise should be set to a low value. If noise reduction is not required, Noise should be set to 1.

If the "Noise" is set too high, the weak particles will disappear along with the noise. The default value is 7.

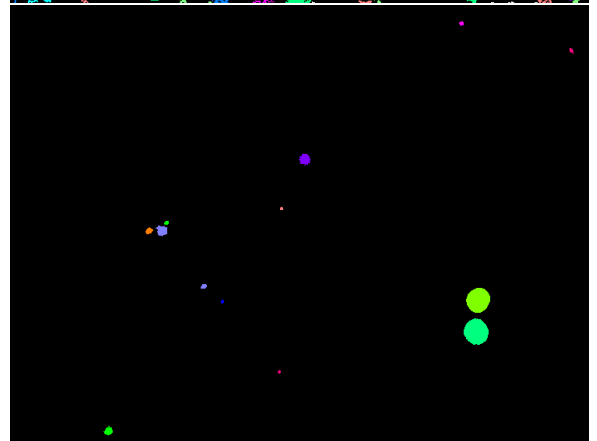
Original image



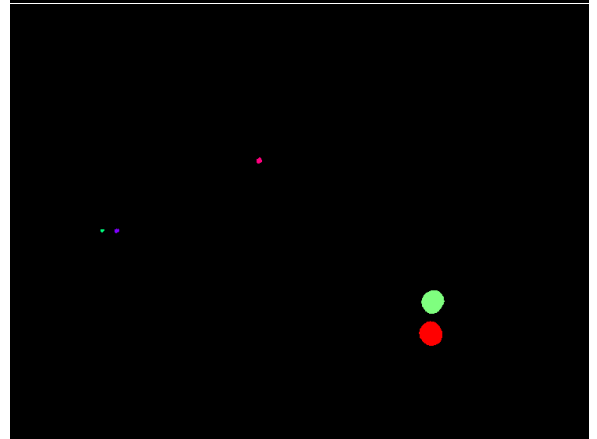
Noise = 1
Noise level too low



Noise = 10
Suitable noise
setting for this
image.



Noise = 20
Noise setting to
high for this
image. Some
particles were
removed by the
noise filter.





PARTICLE ANALYSIS

In this step, each particle is analysed, given an individual colour and measured. Measurements are by default in pixels. Measurements can be calibrated by changing the pixel size as needed.

Calibration of particle analysis can be done by loading an image of a ruler taken with the same camera and magnification as the images for analysis.

Run LabCellCount to the Particle analysis step. Measure the image of a ruler using the line tool, which gives the length of the line in pixels. Measure the pixel size by drawing a line between two bars. Read the length of the line by clicking on it (126 pixels in this example). Calculate the pixel size: Pixel size = length between bars/number of pixels.

For example, measuring a ruler showing 50 mm and the number of pixels is 126, the pixel size is 50 mm/126 = 0.396 mm/pixel.

This number should be written into the pixel size field. All results will be given in these units.

In the Measurements select which measurements are displayed as numbers in the picture:

- Colony number: Particle #
- Colony area: Area
- Colony edge: Perimeter
- Colony diameter: Length
- Colony length/width: Elongation

Sizes will be given in the units specified under Particle analysis.

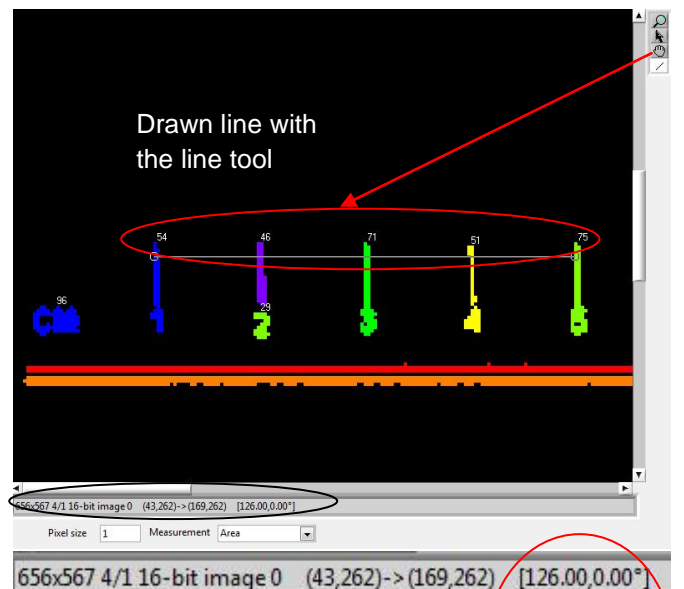
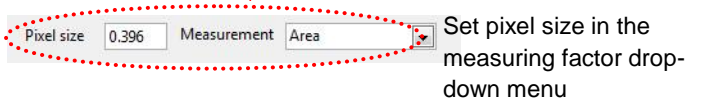
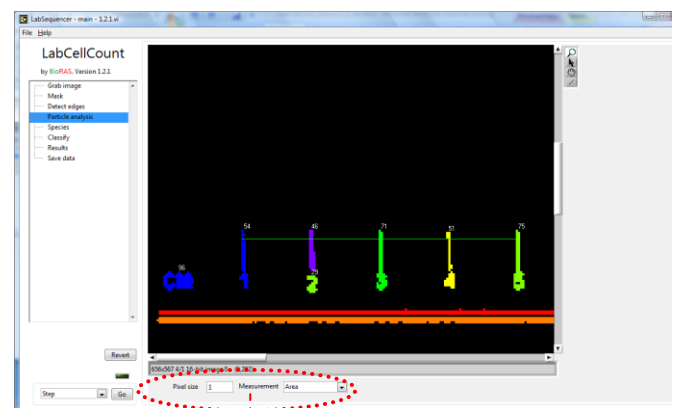
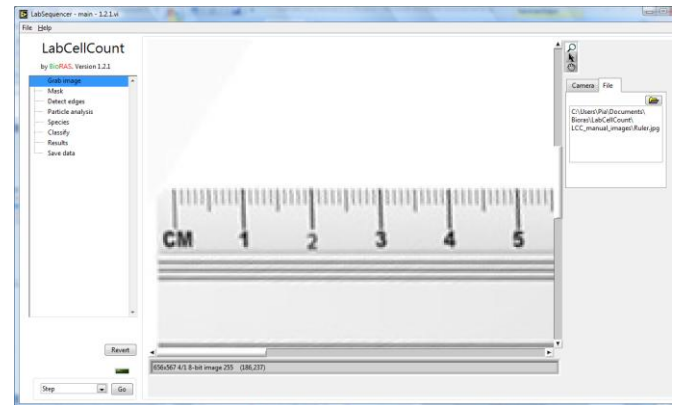


Image size in pixels;	Image format;	Line start position;	Line end position;	Line length in pixels and line angle
656x567	4/1 16-bit image 0	(43,262)	->(169,262)	[126.00,0.00°]



SPECIES






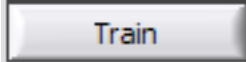
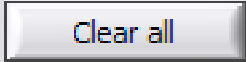
LabCellCount includes a neural network distinguishes countable particles from unwanted particles according to shape.

The neural networks should be trained to recognize particles and other structures. The system is set up to recognize round particles, and distinguish them from unknown particles.



TRAINING OF THE NEURAL NETWORK

The Neural network recognizes shapes. It is trained by naming a type of particles in the name field, and select particles with the given shape in the image with the Selection tool.

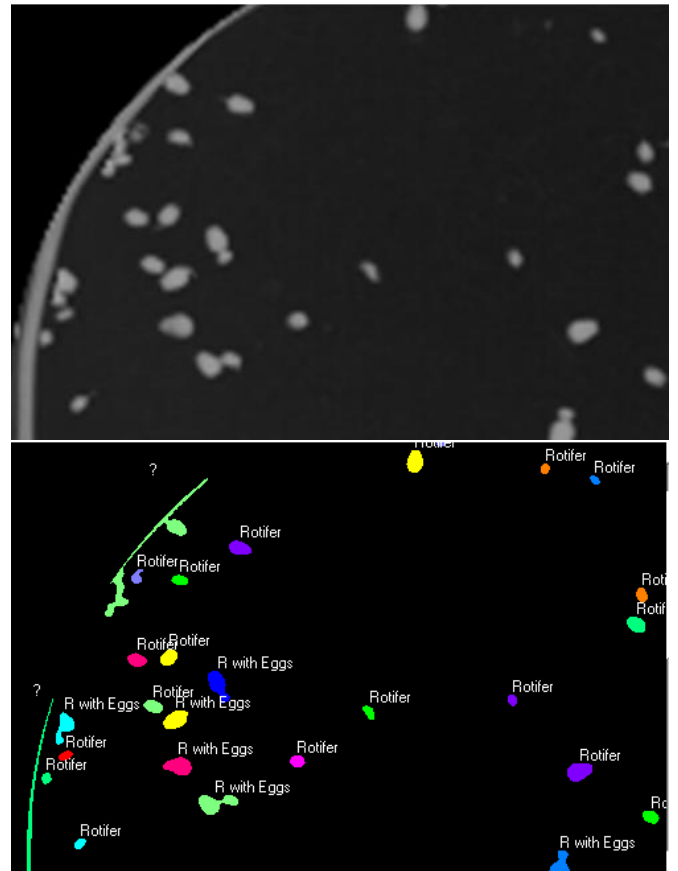
1. Fill in the name field with the name for the class or species of particles that will be chosen first (in the example the name was Rotifers).
2. Click on the selection tool
3. Drag a square around a particle 
4. When moving the cursor over the square, the centre will be marked by a cross . The cross marks the particle, make sure that it crosses it. The square can be resized by dragging the corners.
5. Select multiple particles by holding down the control button.
6. During particle selection zoom in () and out (shift+) can be used.
7. Click on the  button. The selected particles will be added to the Example count. After adding one selection, more particles of the same type can be selected and added to the example count.
8. Repeat the procedure for the next class of particles/organisms. This will enable LabCellCount to recognize more than one type of particles.
9. If the sample contains unwanted particles, that should not be counted, remember to train LabCellCount with these by creating an "Unknown" or "?" type of particles.
10. When a suitable number of particles have been chosen for each particle type (around 10 – 20 of each type), click the  button. Higher number of descriptors renders a better training result.
11. A training overview will be shown for a few seconds, showing targets and outputs. Ideally the output should be aligned with the target.
12. One or several Training sets can be saved (see below).
13. Start a new training set by clicking , to remove old training sets.



CLASSIFY

In the Classify step the particles are classified according to user defined categories. In this example the particles are divided into three categories:

- Rotifer
- R with Eggs
- ? – unwanted particles



RESULTS OF THE ANALYSIS

RESULTS, FILE NAME, FILE PLACEMENT

In the Results step the following is shown:

File information

- Placement of the file
- File name
- Other information about the file

Results of the analysis is shown as:

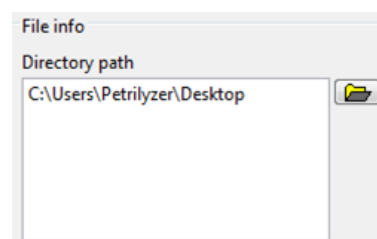
- Table
- Histogram



Results step. The numbers above refer to corresponding sections in the text.

1. DIRECTORY PATH

Select the desired location of pictures and result files from the analysis. Click the folder icon and select the location on your computer.



1.

2. BASE NAME OG INDEX

Base name field. Base name is the name of the file with Index number. Base name should be a name describing the test series (here called "Test").

Data for each Base name is written in the one data file, which can be any number of samples. When a new

2.



Base name is created, a new data file will also be created.

Index is the starting number for the sample counter. For each analysis increment the index number increases (this applies for the Run sequence and Loop mode). In Step mode the index counter is not counting up). The starting number is defined by the user. The index number increases automatically at the beginning of the analysis. Therefore, enter 0 to start a test run, with 1.

Base name	Index
Test1	1

3. FIELDS AND VALUES BOXES

In the Fields and Value boxes additional information about the samples can be added. Fields is the heading; Value is the value, which can be numbers or letters. The information will be part of the data set (see "Save data"). In order to include the headings in a data set, the Fields should be defined before a new data file is created (after defining a new Base name, and during the first analysis with the new Base name).

Fields are a part of the Settings, and the information will remain until new information for written into the boxes.

Fields and Values can be letters, numbers, or characters. Number formats should be compatible with spreadsheets or database formats, which the results will be imported into: numbers with optional number of decimal places. Scientific format will, for example show 0,00001 as 1E-5 (1 x 10 to the minus fifth power).

Fields	Values
Fortynding	1e-5
Volumer (ml)	1
Batch	5

3.

4. ACTION – ADD OR REMOVE DATA

Add to file: Data will be pasted into the data file (the default setting).

Omit from file: Data will not be added to the data file. This option can be selected if the analysis is not to be stored in the data file i.e. if there is something wrong with the analysis).

If "Omit from file" is chosen, LabCellCount will automatically go back to the "Add to file" setting in the next round of analysis.

Action
<input checked="" type="radio"/> Add to file
<input type="radio"/> Omit from file

4.

5. HISTOGRAM AND COUNT

The results are displayed as a histogram. The histogram shows the size distribution of the counted particles.

Select which category of particles that appears in the "Plot species" drop down menu of the histogram.

The total number of particles or particles of the selected category appears in the "Count" pane.



6. RESULTS TABLE

The results from each analysis are also shown in a table.

SAVE DATA

The save data step contains the data that is saved in the data file and export.

Data will be saved and exported **after** the "Results" step, only if the results are been accepted, and "Add two data" is activated.

The Save data step is not displayed when a sequence is run through. The Save data step is displayed only if it is chosen from the menu list to the left.

Data is exported in txt format, which can be read by Excel or databases. The following data will be saved::

- Image file: File name, as defined in the "File info" step
- Species: Designation of categories of particles
- Count: Number of particles
- < value: The number of particles that are smaller than the minimum size
- >value: The number of particles that are larger than the maximum size
- Mean Area: Average size (area)
- Stdev Area: Standard deviation of the average size
- Time: Date and time for the analysis
- Three columns, with extra information which is defined by the user (e.g. Sample volume, dilution, etc.)

FIL MENUE

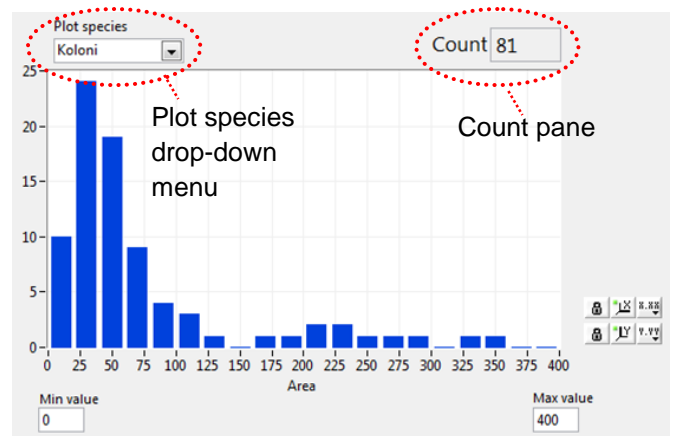
SAVE SETTINGS, REVERT AND OTHERS

Settings should typically only be adjusted and saved once for each type of samples. Various settings can be saved for different sample types.

File menu

In the File menu, LabCellCount settings can be saved and retrieved. Different settings for different types of samples can be saved.

Save settings: Go to the File menu and click "Save settings". Enter a filename and file location in the file system. All settings will be saved in the file. It is a good idea to choose a file name that describes the settings.



5.

Results

Species	#	<-#	#->	Mean A	Stdev A	Time
Koloni	89	0	1	57,1	78,5	02-10-2011 11:50
?	8	0	2	424	398	02-10-2011 11:50

6.

Species	#	<-#	#->	Mean A	Stdev A	Time
Koloni	89	0	1	57,1	78,5	02-10-2011 11:50
?	8	0	2	424	398	02-10-2011 11:50

File	Help
Save settings ...	Ctrl+S
Open saved settings	
Load settings ...	Ctrl+O
Factory default settings	

Page Setup...	
Print Window...	Ctrl+P

Exit	Ctrl+Q

- Save settings
- Open saved settings
- Factory default settings
- Setting for Page setup
- Print the current window
- End LabCellCount



Retrieve saved settings: Previously saved settings can be retrieved from a settings file by clicking "Load settings" and navigate to the file that contains the desired settings.

Return to default settings: Select "Default settings" from the file menu. LabCellCount is delivered with standard settings, which should be adjusted.

SHORTCUTS AND TIPS

CONTINUE BUTTON

Instead of clicking the "Continue" button for starting an analysis, the keyboard "Enter" can be used.

ZOOM IN AND OUT

Zoom in (*enlarge image*): click in the image with the mouse

Zoom out: Hold down the shift key and click at the same time in the image window
