

EASYCOUNTER YC

Operation Manual

Accurate yeast cells counting in three simple steps
Simple. Fast. Accurate. Reliable. Portable.



Available online at: www.autocellcount.com

Operation Manual of EASYCOUNTER YC is made to help better understanding operation of the device. The Manual consists of step-by-step instructions for setting, use and maintenance of the device, preparation of samples, collection and processing of the received data.

The information in this manual is described as precise as possible and is applicable for the latest hardware and software versions of the EASYCOUNTER YC. However, it can be changed without any preliminary consultation or notification.

Safety measures

1. Always make sure that the power supply of the incoming current matches the supply in your region.
2. For working environment conditions see page 19.
3. This device works with aerial cooling, which means that its surface is heated up. When you install it, make sure there is nothing around it at 10 cm (4 inches) radius.
4. Never put any metal objects in the aerial fan, because this may cause electrical shock, physical or device damage.
5. Always make sure the main switch is in position OFF before connecting it to the electrical network.
6. Never touch the power cable with wet hands.
7. Always make sure that the grounding clamp on both the device and the wall outlet are well connected. The power cable must be connected to the grounding.
8. In order to prevent a possible electrical shock, make sure, that the supply is well grounded.
9. In case of damaged device, turn it off and contact the authorized service. Do not disassemble the device. If you do so, the warranty will not be considered valid.
10. Use only the authorized accessories (USB flash drive, CELLCHIP, wireless keyboard and mouse).
11. Use this device only the way that is indicated in this Operation Manual and in the documents related to its components. Each inappropriate use of the device is greatly concerning and may cause damages as the mentioned in the warning notes.
12. In case of smoke coming out of the device, turn in off and contact the authorized service

Safety symbols

The following symbols can be found on the device and in this document. Please, give them special attention and always use the device in the safest possible way.

Symbol	Meaning
	Attention and Warning
	Device is covering all the provisions of the EU
	Protection from change of the pole of the supply
	Plus and minus of the power receptacle

Warnings

Object	Warning
Embedded tablet battery	There is a risk of explosion if the battery is being changed improperly. This battery not to be changed by the customer. Please contact the authorized service.
Box	Do not disassemble the box. There are not adjustable components inside the box. If there is an issue, please contact the service.
Guidance	Do not try to service the device, unless this instruction is perfectly understood and discussed. If you do not pay enough attention to this instruction, this may lead to harm to the service supplier, electric shock during operation, mechanical or other risks.

Waste	After using the CELLCHIP it is necessary to throw it in the appropriate bin.
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Operator	Must have the basic knowledge on the procedure on cell counting.
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Introduction

Cell viability determination:

EASYCOUNTER YC is based on a fluorescence microscopic cell counting technique. Due to the fluorescent dye, LED optics, and CMOS capture technologies, cell analysis is accurate, reliable, and fast.

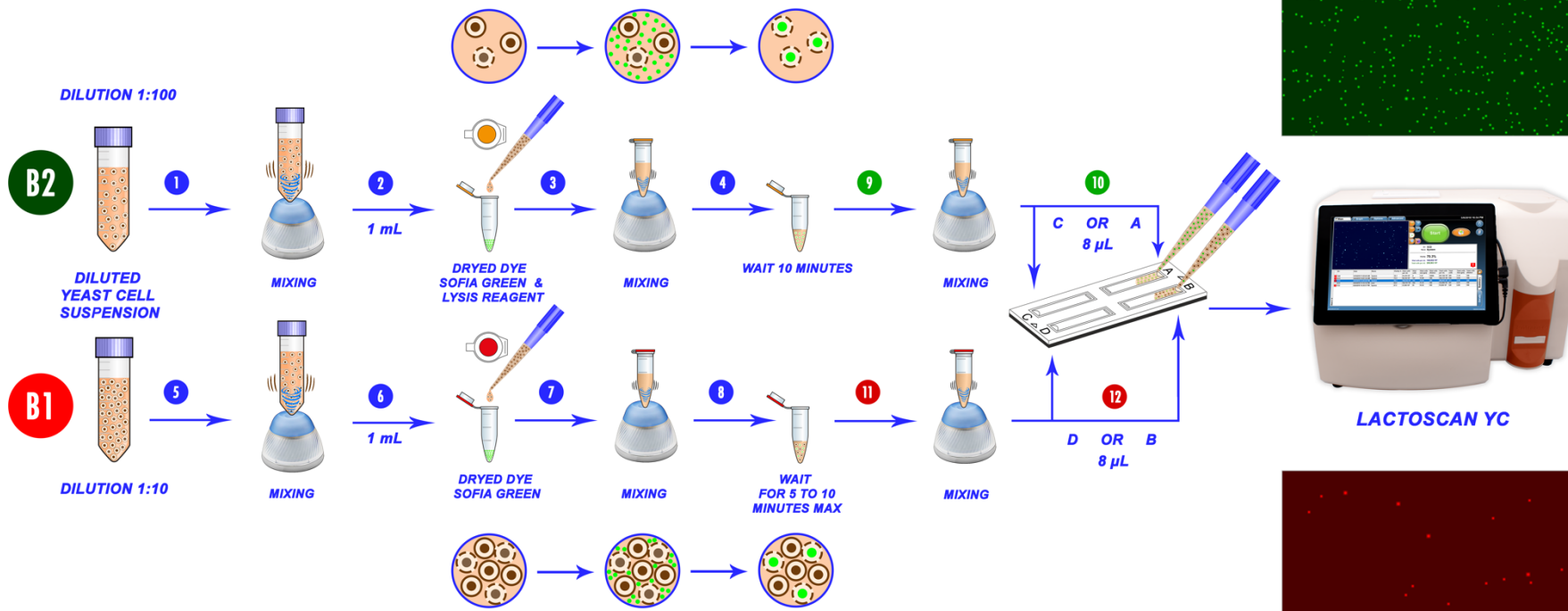
The system enables the user to obtain a total number of yeast cells and determine the viability of the yeast in the sample. The system considers individual cells in suspension using imaging technology.

EASYCOUNTER YC takes the individual cells into suspension by detecting the fluorescent signals of stained DNA in the cell nuclei. The fluorescent dye used is SOFIA GREEN. The cell membrane of viable cells is practically impermeable to SOFIA GREEN, whereas non-viable, dead and, to some extent, apoptotic cells are permeable to the dye. Thus staining with SOFIA GREEN on a sample of the cell suspension gives results on the number of non-viable cells in the sample. Determining the total number of cells requires membranes of viable cells to become permeable for SOFIA GREEN. This is achieved by mixing a cell suspension with a lysing reagent and SOFIA GREEN dye previously lyophilized into a microcentrifuge. The lysing reagent penetrates the cell walls, thus enabling all the cells in the suspension to stain. 8 µL of the ready sample with all staining cells is required to be pipetted directly in a camera A of single CELLCHIP. The other 8 µL from the sample with only dead stained cells is required to be pipetted in camera B of the same CELLCHIP. Then the chip is loaded into the appliance. The analysis takes place over a period of 10 seconds to 2 minutes, the length of which depends on the number of fields captured. The EASYCOUNTER YC system automatically focuses on the chip and the colored cells are captured by the sensitive CMOS camera. The algorithm for digital imaging analysis determines the number of fluorescent cells and calculates their concentration, size and viability. The result is automatically displayed on the screen.

B

VIABILITY OF YEAST CELLS

TOTAL CELL COUNT



- LIVE CELL
- DEAD CELL
- DEAD FLUORESCENT CELL

DEAD CELL COUNT

LIVE CELLS = (TOTAL CELLS – DEAD CELLS), CELLS/mL

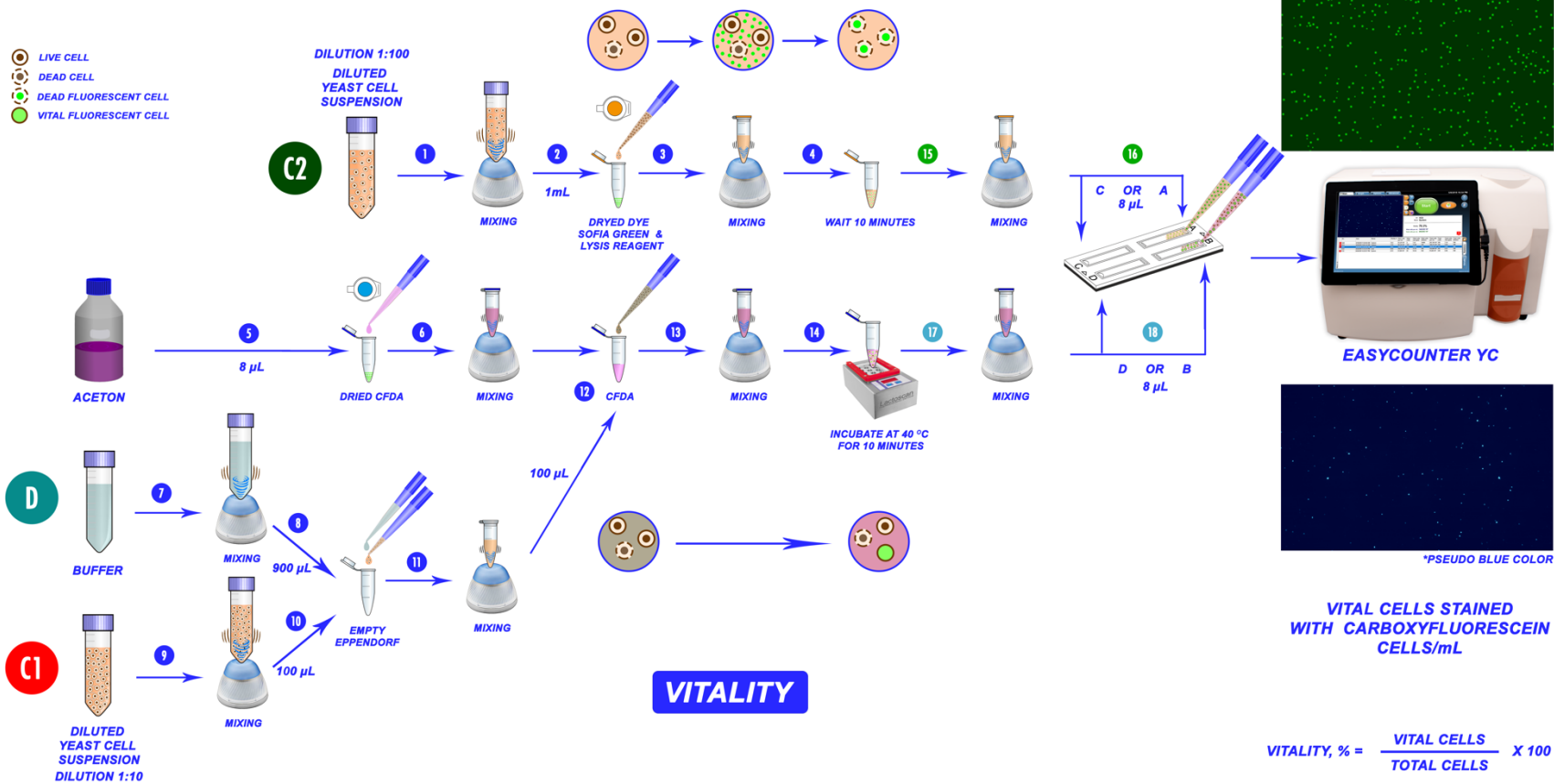
$$\text{VIABILITY, \%} = \frac{(\text{TOTAL CELLS} - \text{DEAD CELLS})}{(\text{TOTAL CELLS})} \times 100$$

Cell vitality determination:

In many cases, toxic effects of chemical or physical factors do not lead directly to cell death. Such factors may cause a number of morphological, intracellular, or metabolic alterations that will result in the inability of a cell to divide, yet the cell itself may still be alive. The method for assessing cell viability provide information only on alive and dead cells in the whole population, but do not provide information about the number of vitality cells. In this aspect, cell vitality defining the physiological capabilities of the cell is a very important parameter. Measurement of cell vitality is advisable for conducting effective control of the fermentation process at a significant concentration of inhibitory substances that are produced during the process. It is very important for controlling the metabolic activity of yeast cells in fermentation in the brewing industry, in the production of bioethanol, in the bakery industry, etc. The Yeast Vitality Method is based on the determination of the enzyme esterase activity by means of the esterase substrate 5-carboxyfluorescein diacetate (CFDA). In parallel with this analysis, it is important to determine the total number of cells in the yeast suspension. This can be achieved by using the Sofia-Green fluorescent dye, which only stains the dead cells. To determine the total number of cells, it is necessary to treat the cells so that the membrane of the living cells becomes permeable to Sofia-Green. This is achieved by mixing the cell suspension with a lysis reagent for 10 minutes. The combination of these two analyses results in an effective fermentation control and an increase in the yield and quality of the bio product obtained

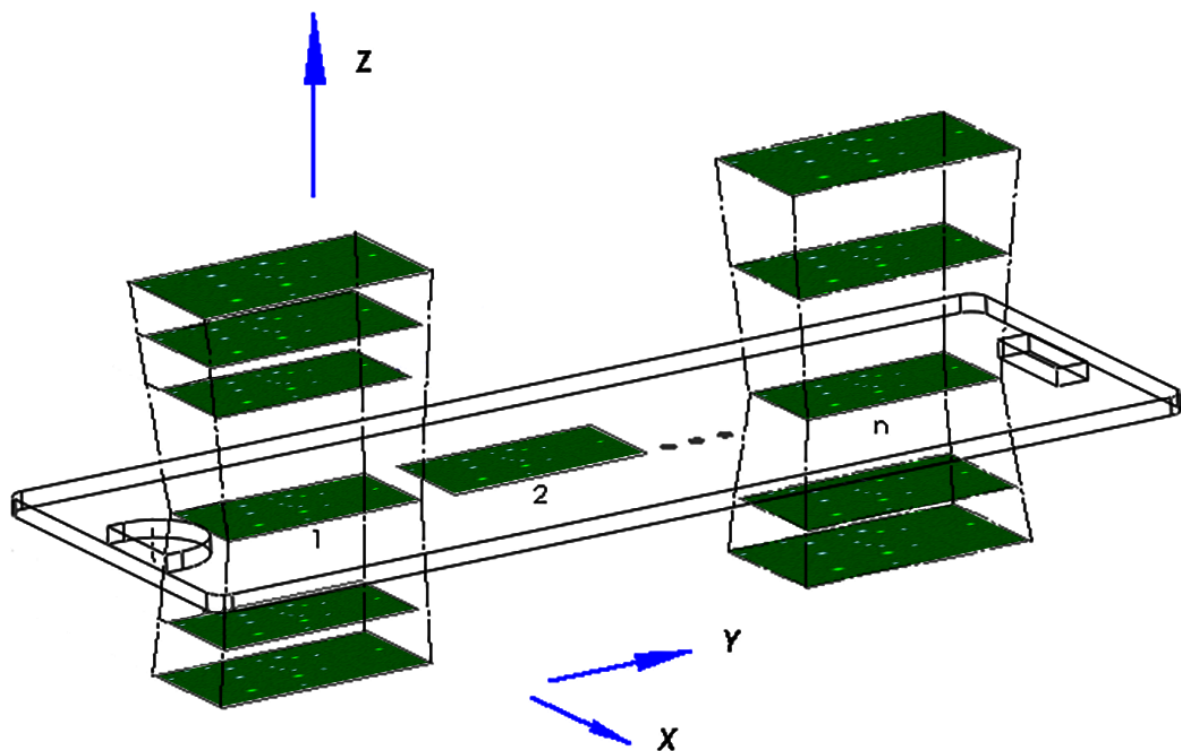
The 5-carboxyfluorescein diacetate (CFDA) esterase substrate is used to determine the vitality of yeast. CFDA penetrates the cell membranes of living cells, the diacetate groups are cleaved from non-specific esterases and a fluorescent, charged substrate form (carboxyfluorescein) is obtained that stains the cells in green and flows out of the cells very slowly. In parallel, in a separate sample with yeast cell suspension, the total number of yeast cells was determined using the fluorescent dye SOFIA GREEN. To determine the total number of cells, it is necessary for the membrane of living cells to become permeable to SOFIA GREEN. This is achieved by mixing the cell suspension with a lysis reagent for 10 minutes. SOFIA GREEN penetrates through damaged cell membranes, binds to core nucleus, and cells with damaged membranes turn green.

VITALITY OF YEAST CELLS

TOTAL CELL COUNT

Working principle of EASYCOUNTER YC

Unique, 3D, multi-image, patent application protected, sequential scanning process, based on a precise fluorescent optics and low magnification, images analysis software, Easycounter YC is fast, precise and reliable counter of yeast cells. Via automatic displacement of the mechanism on axles X – Y and liquid lens Z, the device is capturing maximum 60 images. After capturing, the images are being processed by the embedded software and the average result, calculated by using the formula from ISO 4833, of all the captured images is displayed. The whole process, after placing the CELLCHIP in the cartridge, is automatic.

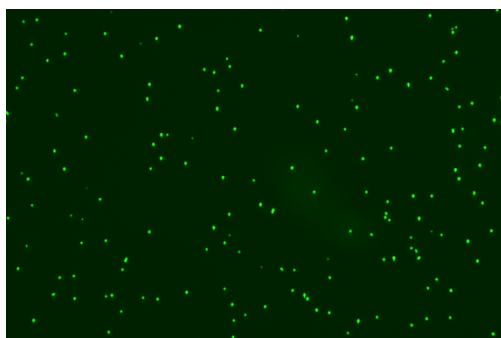


Comparison between EASYCOUNTER YC and standard method for yeast cells counting and determination of cell viability

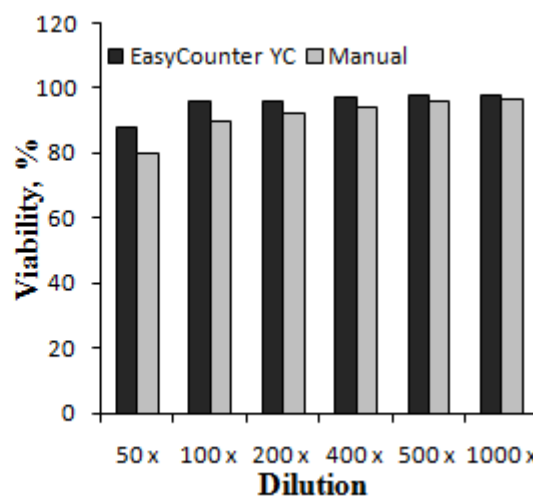
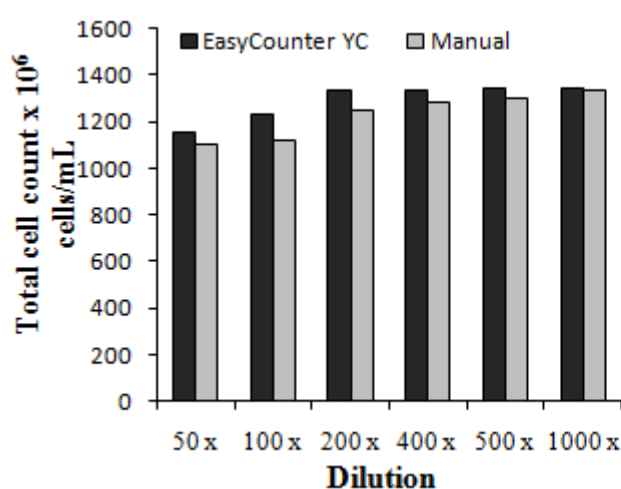
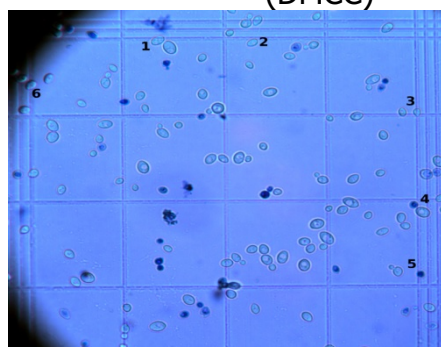
The below described data is based on validation and check of EASYCOUNTER YC done by —Biotechnology department, University —Prof. Asen Zlatarov, Bulgaria

1. Comparison between EASYCOUNTER YC and Direct microscopic counting of yeast cells.

Images of yeast cells
(EASYCOUNTER YC)



Images of yeast cells
(DMCC)



Determination of total cell count and cell viability using the fluorescent image cytometer EASYCOUNTER YC and direct observation under microscope at different dilutions of cell suspension with initial concentration 1.3×10^9 cells/ml.

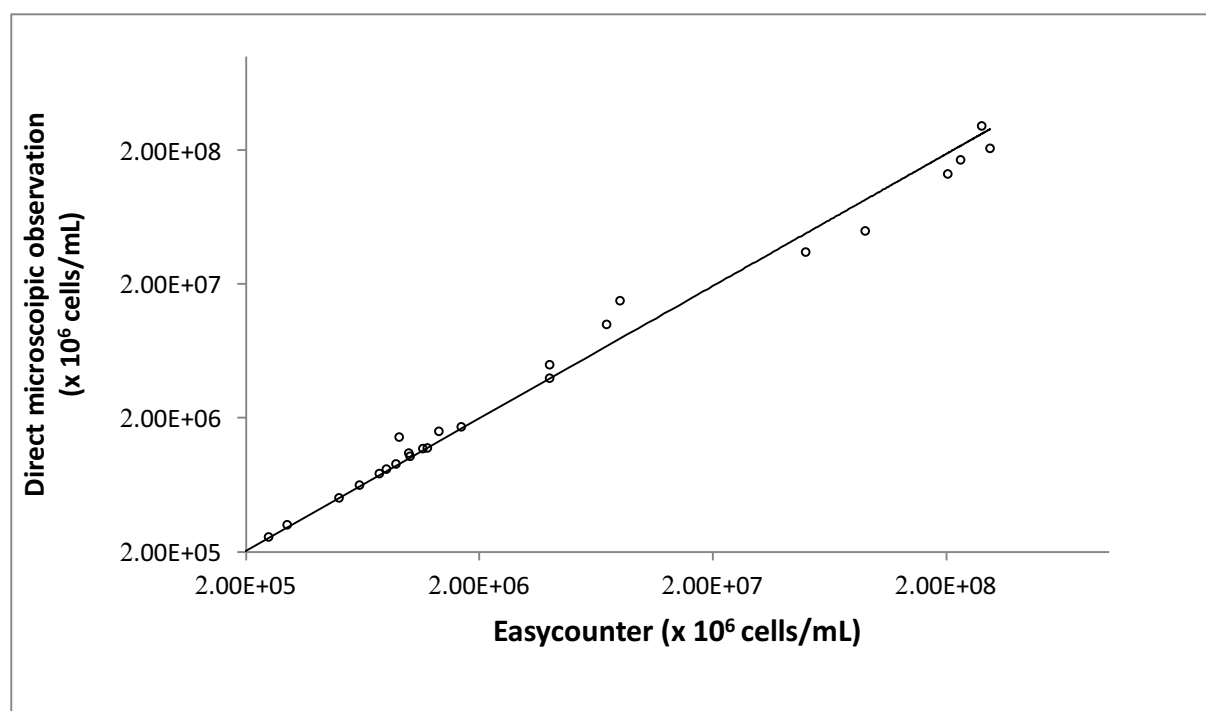
2. Correlation of data between EASYCOUNTER YC and Direct Microscopic Cell Counting (DMCC)

Coefficient of variation (CV, %) of the cell count determined using the fluorescent image cytometer EASYCOUNTER YC and direct observation under microscope at different dilutions of cell suspension with initial concentration 1.3×10^9 cells/ml.

The table shows that the coefficient of variation using EASYCOUNTER YC is 4-5 times lower than the classic microscopic counting, which convincingly shows the greater accuracy of the EASYCOUNTER YC instrument.

Dilution	Coefficient of variation (CV, %)			
	Total cell count		Viability	
	EASYCOUNTER YC	Direct Microscopic Cell Counting (DMBC)	EASYCOUNTER YC	Direct Microscopic Cell Counting (DMBC)
50 x	7.8	40	6.7	35
100 x	5.6	38	4.9	29
200 x	3.8	15	3.6	13
400 x	2.9	11	2.8	9.2
500 x	2.5	8	2.2	7.5
1000 x	3.8	7.3	4.8	6

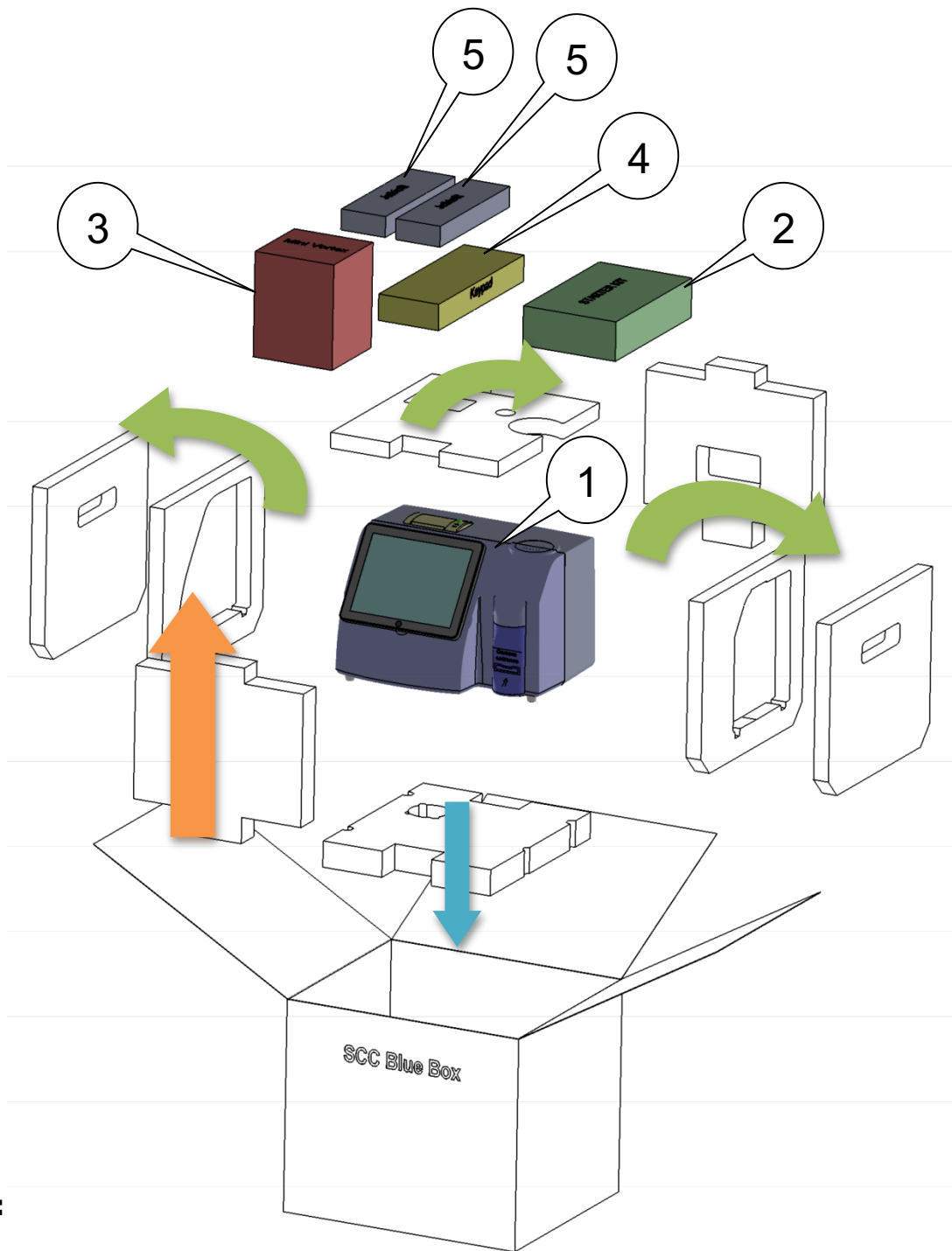
3. Correlation of data between EASYCOUNTER YC and Direct Microscopic Cell Counting (DMCC). Correlation of the results for cell count obtained using the fluorescent image cytometer EASYCOUNTER YC and direct observation under microscope. Correlation coefficient $R^2 = 0.94$.



Correlation of the results for cell count obtained using the fluorescent image cytometer EASYCOUNTER YC and direct observation under microscope. Correlation coefficient $R^2 = 0.94$.

Unpacking of EASYCOUNTER YC

Unpack carefully the EASYCOUNTER YC following the sequence illustrated below:



Legend:

1. EASYCOUNTER YC
2. EASYCOUNT YC STARTER KIT
3. Mini Vortex
4. Wireless mouse and keyboard
5. Automatic pippets

Product content

The EASYCOUNTER YC is sent with the following items:

Object	Amount
EASYCOUNTER YC - Yeast VIABILITY Counter	1
EASYCOUNT YC STARTER KIT	1
EASYCOUNTER YC 12V power adapter	1
EASYCOUNTER YC Operation Manual	1
Automatic pipette up to 100 µL – set at 8 µL	1
Automatic pipette up to 1000 µL	1
USB wireless keyboard with batteries	1
USB wireless mouse with batteries	1
Mini Vortex	1

When receiving the device, please make sure it is not damaged during transportation. Also make sure that all the parts, including the above mentioned, are in the box. In case of damage/missing parts, contact the carrier company. The warranty does not cover damages caused during transportation.



Attention:

Disregarding and not taking off all the transport brackets and Styrofoam may lead to a damage of the device. The transport brackets, Styrofoam, must be placed before transporting, in order to prevent a damage.

Device registration

Each customer can register the EASYCOUNTER YC by visiting www.autocellcount.com. The serial number is needed to be filled in, name of the owner and contacts data. After registration, notifications will be received about software updates and information about new analysis, which may be done with the EASYCOUNTER YC.

Purpose of the device

EASYCOUNTER YC is designed to be used for yeast analysis in the production of beer, bioethanol, bakery, research laboratories. It is not intended for human or animal therapeutic or diagnostic use.

Working environment conditions

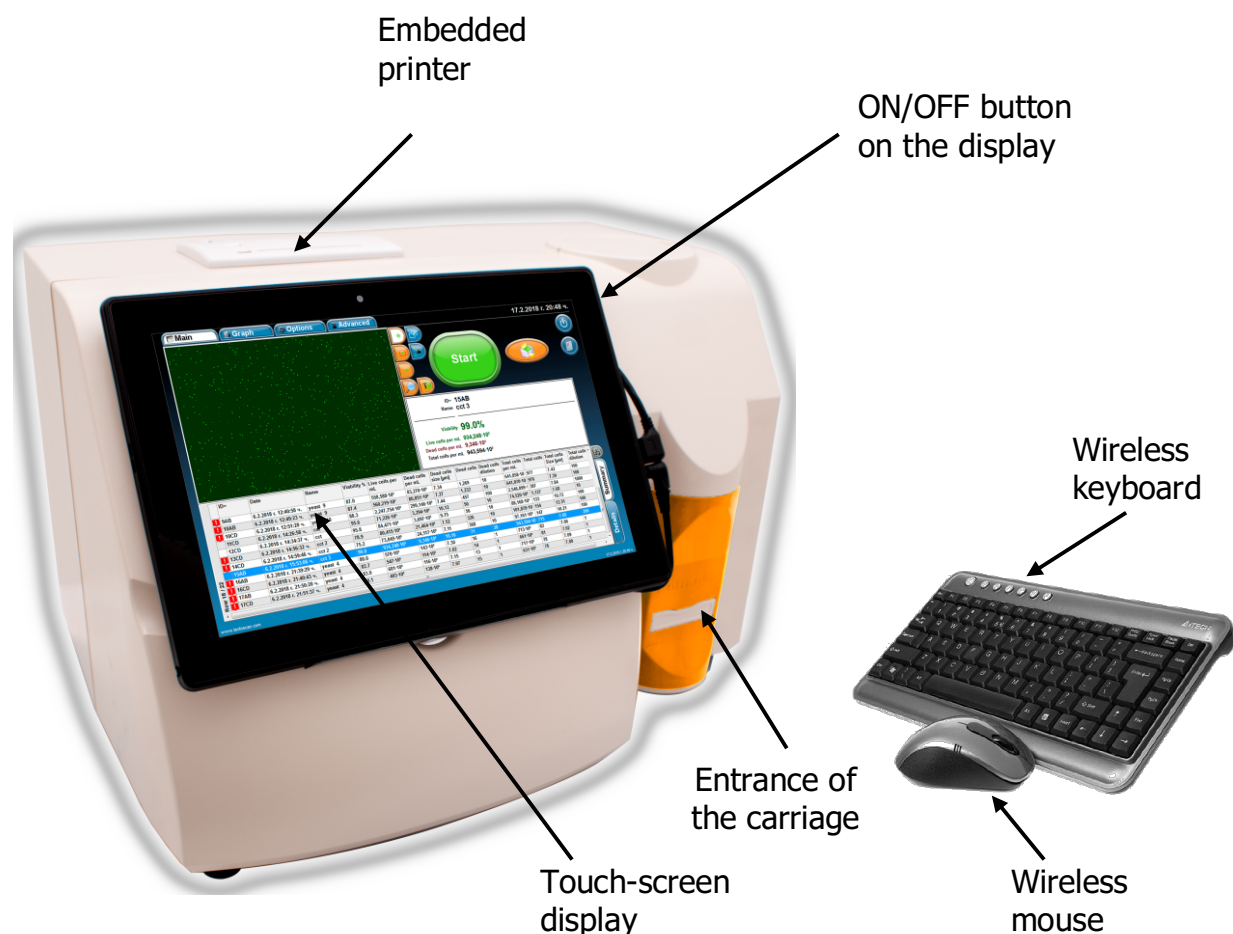
In order to work properly and stable for a long period, EASYCOUNTER YC needs to be installed in a room meeting the following environmental conditions:

- Indoor temperature 15-35°C. It is not recommended to place it in a room where the temperature is below 4°C.
- Prevent exposing the device to direct sun light.
- Prevent exposing to a continuous vibration.
- Relative humidity 0-95%.
- In a place free of corrosive gases or other corrosive substances.
- In a place free of dust.
- 10 cm (4 inches) is the minimum required distance for normal air flow.
- Do not place any heavy object on the device.

THE EASYCOUNTER YC

The front and upper panel, as well as the external accessories, are shown below.

Front panel



Entrance of the carriage. It opens automatically and ejects a stand for the CELLCHIP with the analysed sample.

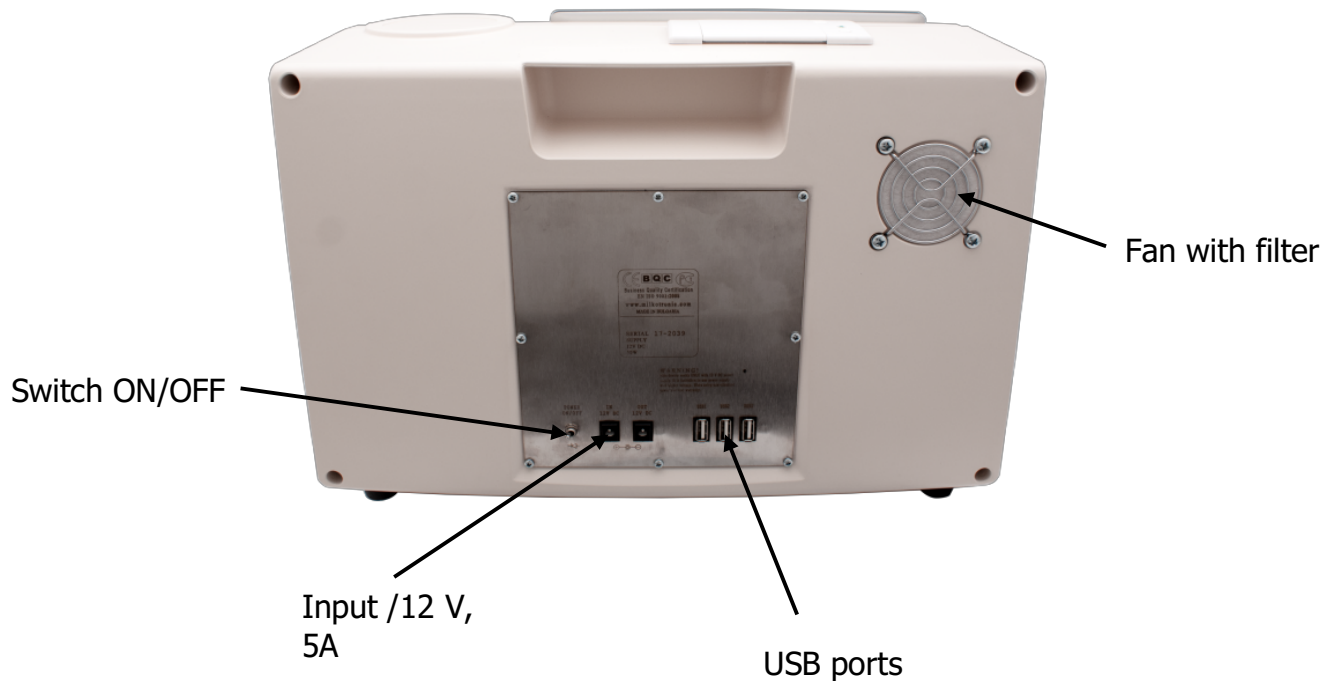
Touch-screen display. It operates the device. The necessary functions and results from the analysis are displayed there.

Embedded printer. It allows printing the results after analysis or the results of previous tests.

Wireless keyboard and mouse. Make entering data easier.

ON/OFF Button. It is used to turn on and off the display.

Back panel:



ON / OFF switch. This is the main switch of the device. It switches the device on and off.

12V input, min5 A. Power supply. Always connect only the original power adapter, that came with the device.



Attention!

Do not connect the power supply of the device if the toggle switch ON/OFF is ON! —Always make shure it is in OFF position.

USB ports. For plugging in a USB memory and wireless keyboard and mouse.



Attention!

Do not disconnect USB flash drive while the device is working.

Fan with filter. Cooling the inner volume of the device.

Description of EASYCOUNT YC KITS

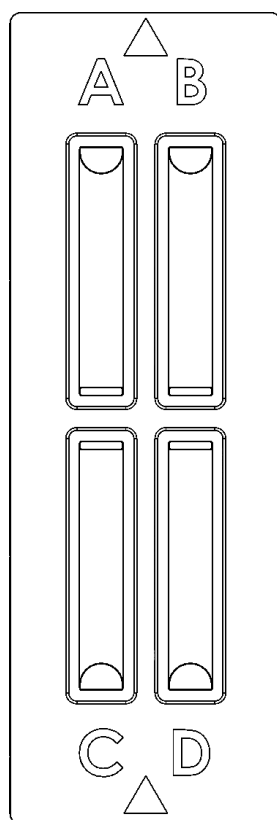
1. EASYCOUNT YC STARTER KIT Viability, 100 tests (starter kit) - contains 50 disposable CELLCHIP-s, 100 micrcentrifuge tubes with SOFIA GREEN lyophilized dye, 100 micrcentrifuge tubes with SOFIA GREEN and lyophilized lysis buffer, 200 pcs pipette tips with a volume of 100 μ l and 200 pipette tips with a volume of 1000 μ L. The set has a shelf life of 1 year from the date of manufacture.

2. EASYCOUNT YC KIT Viability, 200 tests - contains 100 disposable CELLCHIP-s, 200 micrcentrifuge tubes with SOFIA GREEN lyophilized dye, 200 micrcentrifuge tubes with SOFIA GREEN and lyophilized lyophilized buffer, 400 pipette tips with a volume of 100 μ l and 400 pipette tips with a volume of 1000 μ L. The set has a shelf life of 1 year from the date of manufacture.

3. EASYCOUNT YC KIT Vitality, 25 tests - contains 25 disposable CELLCHIP-s, 50 micrcentrifuge tubes with SOFIA GREEN and lyophilized lysis buffer, 50 pcs. brown-colored micrcentrifuge tubes with dried 5-carboxy-fluorescein diacetate (CFDA), 50 pcs. empty colored micrcentrifuge tubes for mixing the sample with the citrate buffer, 250 pcs. pipette tip with a volume of 100 μ L, 100 pcs. pipette tip - with a volume of 1000 μ L, 5 micrcentrifuge tubes containing salt in dry form to prepare the citrate-phosphate buffer pH 4 (Macilvaine buffer), 1 empty 15 mL volume tube required to dilute the citrate buffer. The kit has a shelf life of 6 months from the date of manufacture.

Description of CELLCHIP

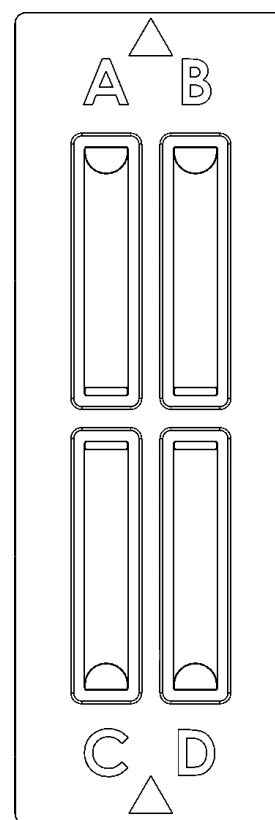
CELLCHIP is a disposable chip, specially designed to be used with EASYCOUNTER YC. It is made of ABS and PMMA.



CELLCHIP L

R

CELLCHIP has four separate closed chambers (A, B, C и D), that allow analysis of two different samples. Each chamber's capacity is 8 μ L. EASYCOUNTER YC YC makes 16 pictures of the sample in each chamber and then it performs the analysis via a specific algorithm.



CELLCHIP R



Attention!

Before starting work with EASYCOUNT YC KIT always check CELLCHIP letter. It can be L or R. Letter L indicated CELLCHIP 4L and letter R indicated CELLCHIP 4R. The CELLCHIP letters match with the already put in the program CELLCHIPS data. One Easycounter KIT includes only CELLCHIPS with letter L or only with letter R.

Specifications:

EASYCOUNTER YC

Type:	Yeast counter
Dimensions (HxWxL):	25.5 cm x 38 cm x 30 cm
Weight:	5.300 kg
Working power:	100-230 VAC, 2.5A, 120V

CHARACTERISTICS

Frequency:	50/60 Hz
Power supply:	12V DC, 5A, 60W
Working place:	Inside only!
Working temperature:	15-35 °C
Working humidity:	0-95%

TECHNICAL SPECIFICATION

Time of analysis:	Up to 2 min in dependence of the number of pictures taken
Concentrations of cells in the sample:	$1 \times 10^5 \div 1 \times 10^7$ cells/mL
Sample volume:	8 μ L
Firmware:	EASYCOUNTER YC Firmware (for Updates visit www.lactoscan.com/updates)
USB:	4 Gigabyte

OPTICS

Lens:	4 x
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LED:	5 W blue LED, 465 nm
Filter:	Excitation and emission filters
Camera:	CMOS camera
IEC 60825-1:	1993+A1; 1997+A2; 2001

EASYCOUNT YC STARTER KIT Viability 100 tests

<i>Physical characteristics</i>	Dimensions (HxWxL):	355 mm x 255 mm x 135 mm
	Weight:	1.500 kg
	Number of CELLCHIP x2:	50 pcs
	Number of SOFIA GREEN lyophilized dye:	100 pcs
	Number of SOFIA GREEN lyophilized dye and lyophilized lysis reagent:	100 pcs
	Number of tips for the automatic pipette with volume 100 µL:	200 pcs
	Number of tips for the automatic pipette with volume 100 µL:	200 pcs

EASYCOUNT YC KIT Viability 200 tests

<i>Physical characteristics</i>	Dimensions (HxWxL):	355 mm x 255 mm x 135 mm
	Weight:	1.500 kg
	Number of CELLCHIP:	100 pcs
	Number of SOFIA GREEN lyophilized dye	200 pcs
	Number of SOFIA GREEN lyophilized dye and lyophilized lysis reagent	200 pcs
	Number of tips for the automatic pipette with volume 100 µL:	400 pcs
	Number of tips for the automatic pipette with volume 1000 µL:	400 pcs

EASYCOUNT YC KIT Vitality 25 tests

<i>Physical characteristics</i>	Dimensions (HxWxL):	355 mm x 255 mm x 135 mm
	Weight:	1.500 kg
	Number of CELLCHIP:	25 pcs
	Number of SOFIA GREEN lyophilized dye and lyophilized lysis reagent	50 pcs
	Number of dried CFDA reagent	50 pcs
	Number of empty microcentrifuge tubes	50 pcs
	Number of tips for the automatic pipette with volume 100 µL:	250 pcs
	Number of tips for the automatic pipette with volume 1000µL:	100 pcs

CELLCHIP x4

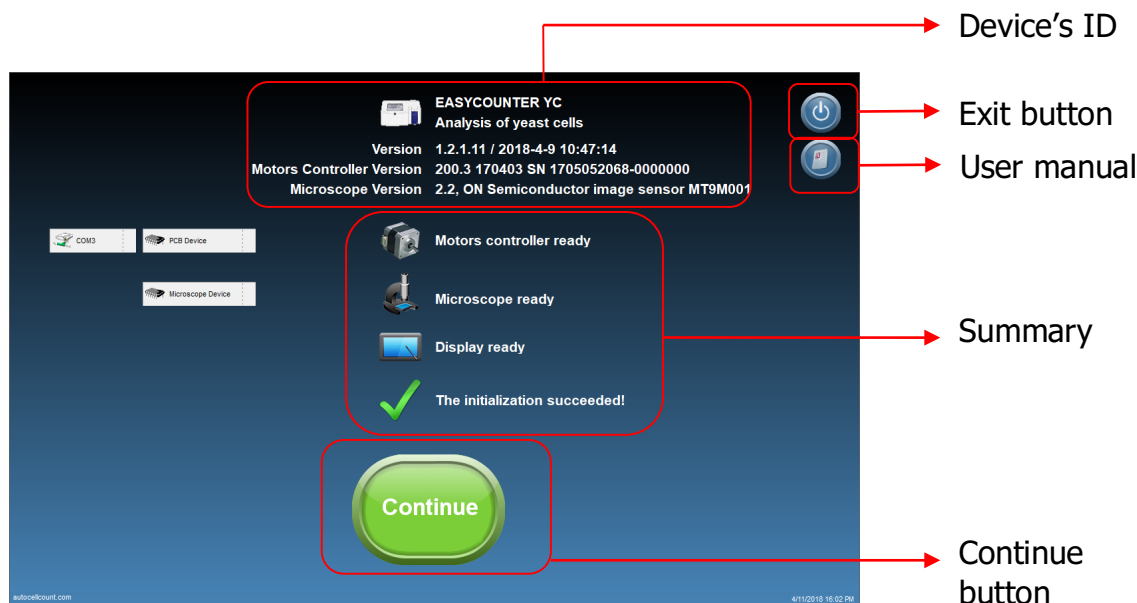
***Physical
characteristics***

Material:	ABS и PMMA
Dimensions (HxWxL):	2 mm x 25 mm x 75 mm
Weight:	0.005kg
Volume of the camera:	8 μ L
Thickness of the microfluidic chip	50 μ m

Description of the main software displays

EASYCOUNTER YC software consists of the following displays:

1. Display —'Initial'



Initial display

Device's ID

Information about the software versions of the device

Exit button

By pressing this button the operator switches to menu Switch off/Operational system, from where whether to turn off the device or the program can be chosen.



By pressing this button the User manual for EASYCOUNTER YC is opened in PDF file.

Summary

Information about the condition of the device after selfdiagnosing test.



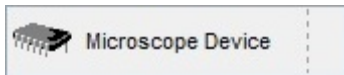
By pressing this button the operator continues to the main display of the software.



COM 3 must be selected, in order the device to work properly.

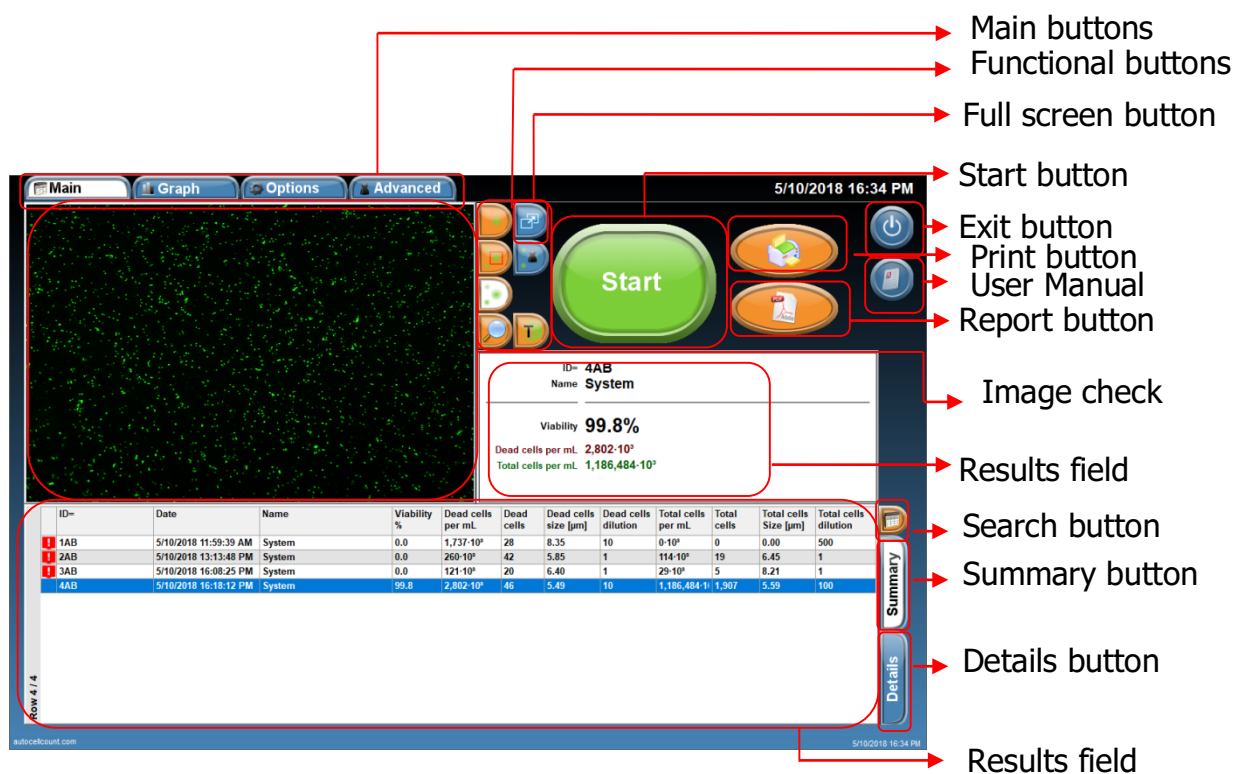


PCB Device must be selected, in order the device to work properly. For demonstrations choose «Simulator», this way only the software will be working, without the device itself.



Microscope Device must be selected, in order the device to work properly. For demonstrations choose «Simulator», this way only the software will be working, without the device itself.

2. Display —'Main'



Display —'Main'

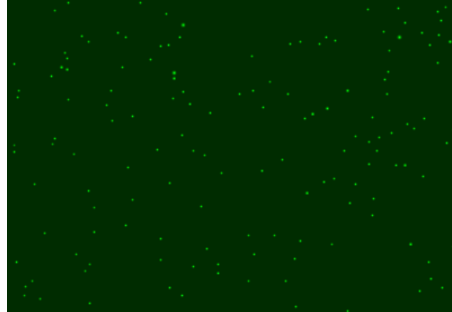
Main buttons

Switch the main displays of the software

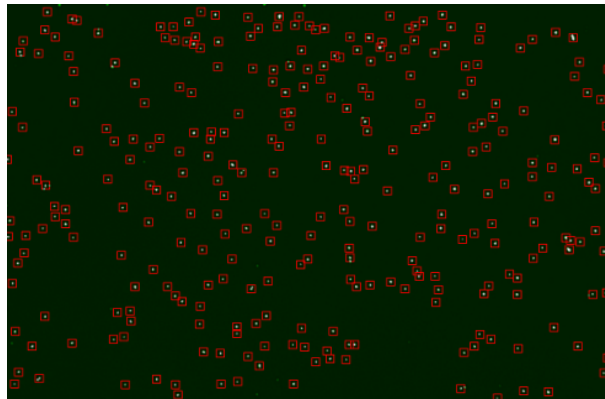
Functional buttons

Allows the user to mark and unmark cells, to select cells, to zoom the image by pressing on the buttons.

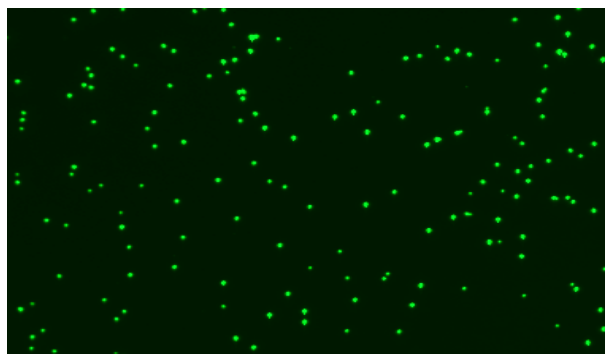
Shows software edited image on which cells are colored in green and the background is presented completely smooth.



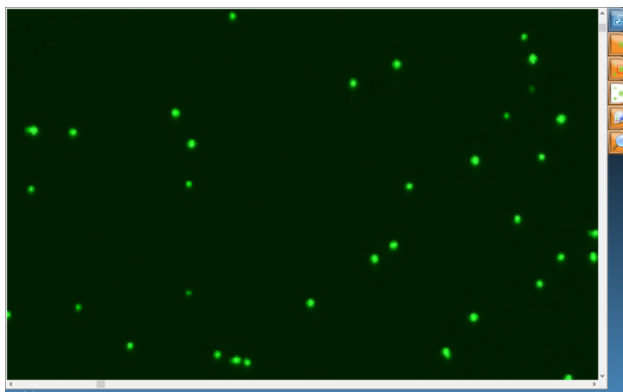
Software marks all the counted cells.



Shows the real unedited image



Zoom

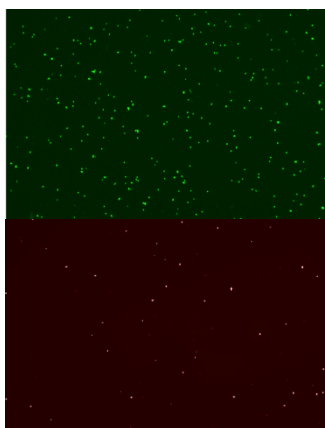


The button changes the image of total cells with image of dead cells or vital cells.

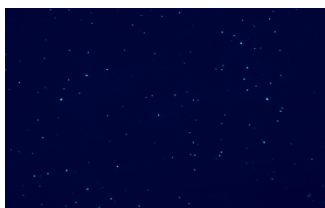


Total cells

Dead cells

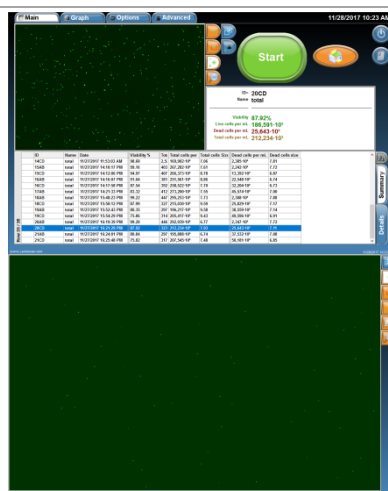


Vital cells



Opens the image in full screen and returns the image in Main mode





Transfers the image in Advanced mode where the user can examine it in details.



Starts the sample analysis



Turns off the device



Switches to print page



Shows report from the assay in PDF format.

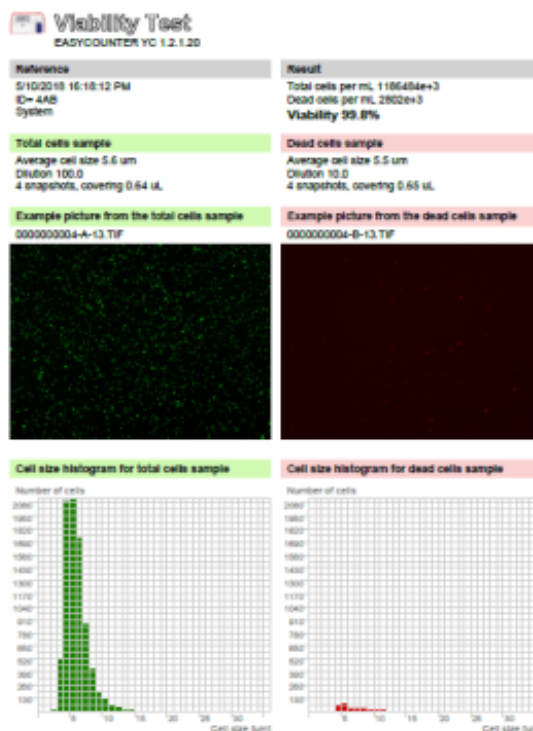


Image check

Shows the density of yeast cells in the selected sample

Result field

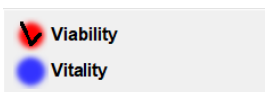
Shows the results from the assay and viability in percents



The flag shows warning for low viability – under 90 %.



Changes the type of analyses - Viability or Vitality.



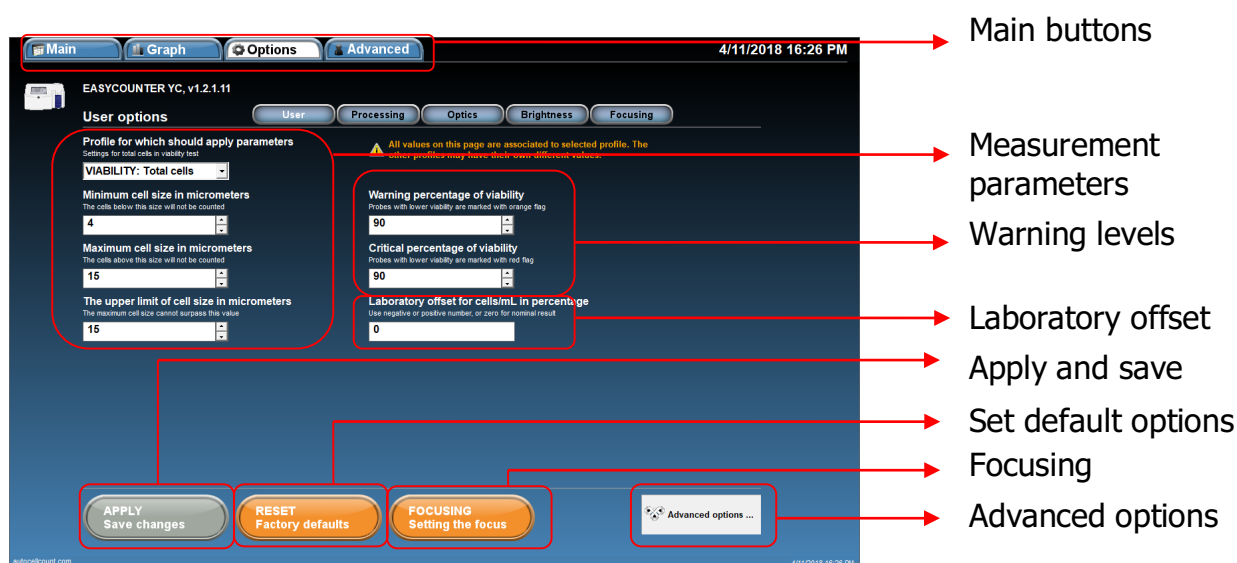
Switches to a table with summarized information about the performed analysis



Switches to a table with detailed information about the already performed analysis

3. Display "Options"

Allows the operator to set options of analysis .



Экран Опции

Main buttons

Switch main displays of the software

Measurement parameters

Sets the min. and max. size of the counted cells for the different samples

Warning levels

Sets the warning level for low cell viability in percents

Apply and save

Applies and saves the changes on the options

Default options

Sets default options of the measurement parameters and precautions levels

Advanced options

Opens menu with options needed for service purposes only

Focusing

Pressing the button switches to Setting focus menu

4. Display "Graph"



Экран Графики

Main buttons

Switch main displays of the software

Summary

Summary of the data used for graphics creation.



Changes the data source used for graphics creation.



Changes the data source used for graphics creation. Respectively for total cell count, dead cell count or vital cell count.

Minimal and maximal size of the cells

Sets size limit of the cells included in the final analysis result by pressing the arrow buttons.

Graphics

Graphical representation of the analysis data. —" Cell size" graphic presents the data for the number of the cells according to their size. —"Cell count" graphic shows number of cells on each image that are taken into consideration during counting the analysis results.

Average size

Average size of the cells

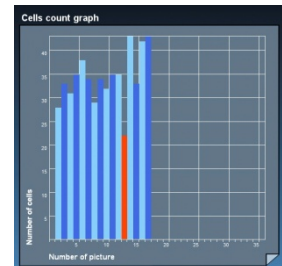
Green line

Shows the average cell size

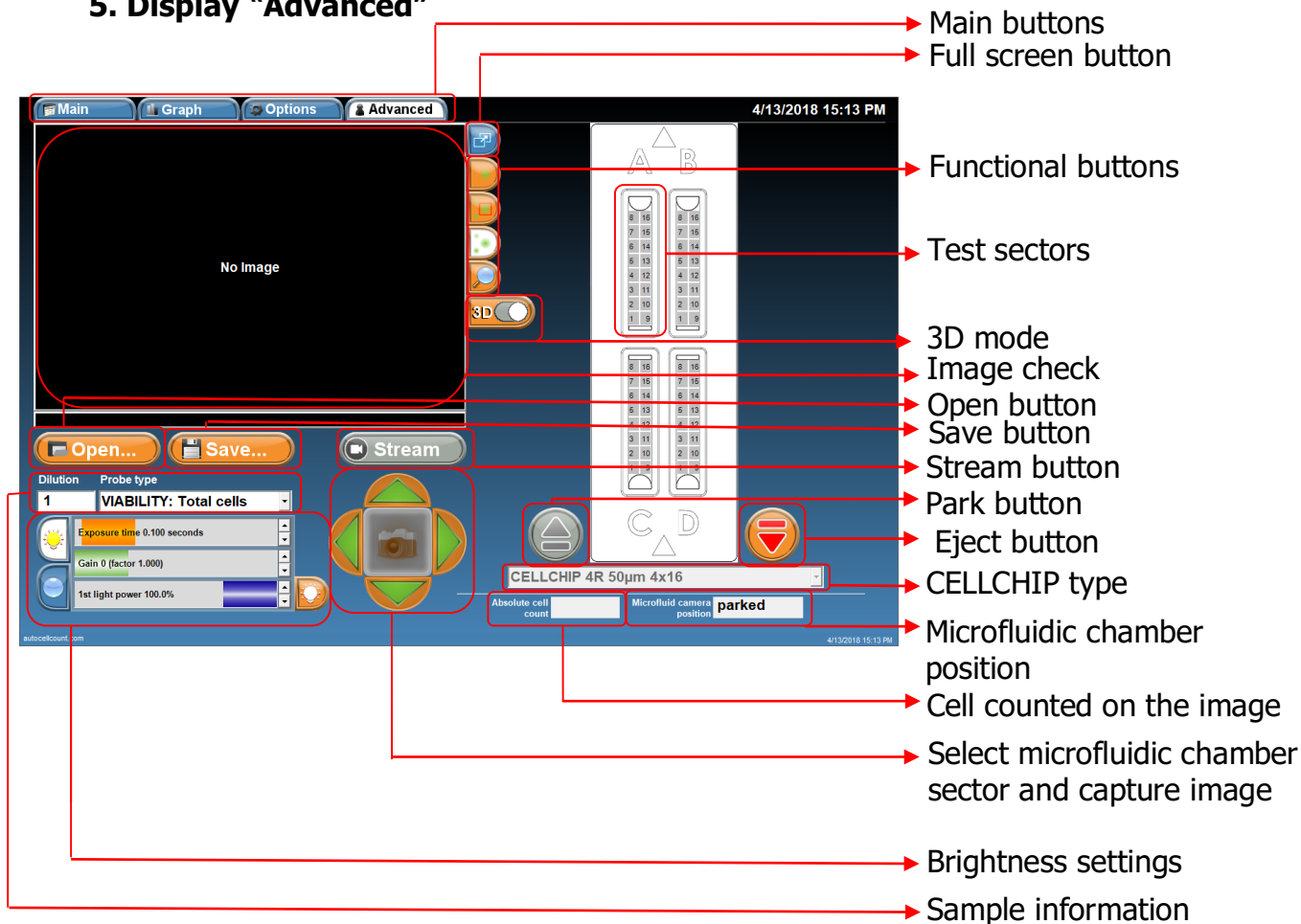
Image that is excluded from the calculations of the results. In the table of details for the corresponding result, the image is marked with "NO" in the column "VALID" because of the unacceptable deviation from the mean value of the result, obtained from the rest of the images.

Red column

Avg. cell size [µm]	Valid
10.71	YES
11.39	YES
11.13	YES
10.71	YES
11.13	YES
10.71	YES
11.93	YES
11.53	YES
11.41	YES
11.26	YES
10.83	YES
10.86	NO
10.74	YES
11.39	YES



5. Display "Advanced"



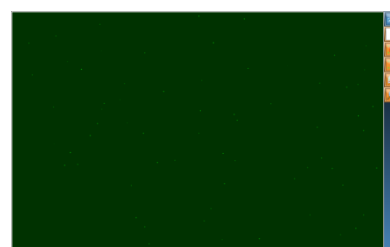
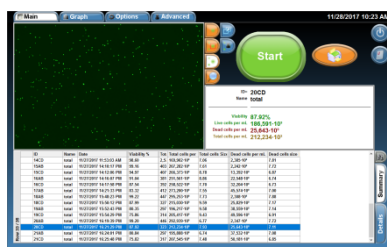
Display Advanced

Main buttons

Turns the main displays of the software



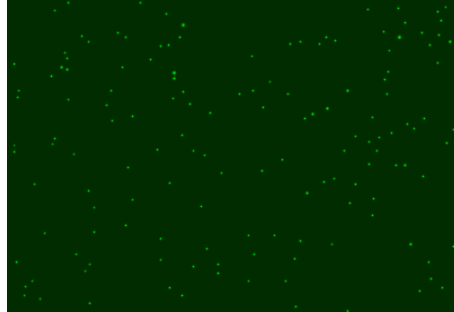
Opens the image in full screen and returns the image in Main mode



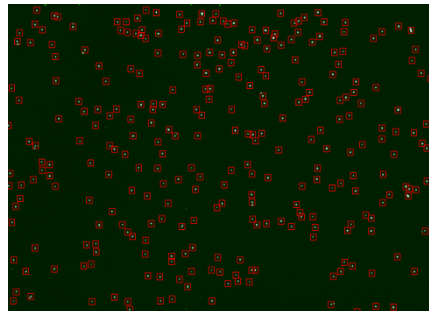
Functional buttons

Allows the user to mark/unmark cells, to select cells to zoom the image by pressing the buttons

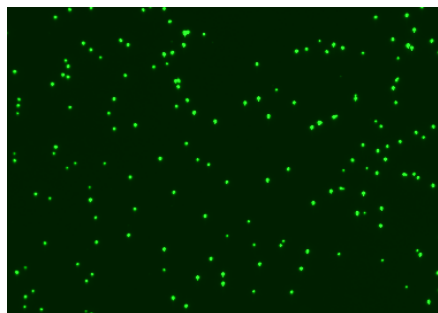
Shows software edited image on which cells are colored in green, and the background is presented completely smooth.



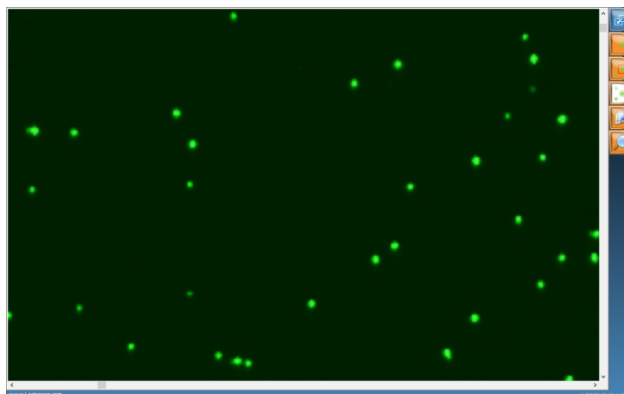
Software marks all the counted cells



Shows real unedited image



Zoom



Test sectors

Allows the user to select the exact sector from the microfluidic chamber that is wanted to be tested and explored in Advanced mode.

Examine the test image in 3D mode.

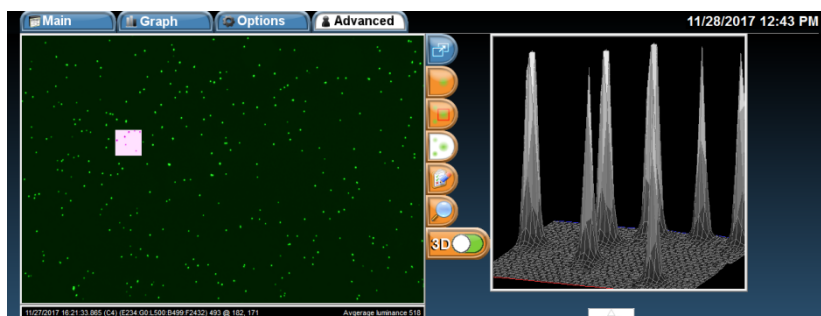


Image check

Shows cell density in the selected sector



Opens an image from previous testing for further examination in Advance mode.



Saves the shown image in the Image check field.



Parks the cartridge with the loaded CELLCHIP.



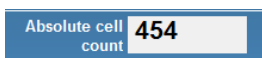
Ejects the cartridge with CELLCHIP.



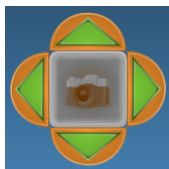
Selects the CELLCHIP type.



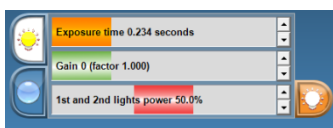
Shows the position of the cartridge with the microfluid camera / CELLCHIP.



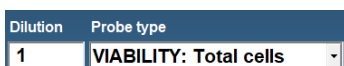
Shows the counted cell on the image from the selected test sector.



By using the arrows, the user can select the test sector of the microfluid camera and then by pressing capture button to make a photo of the sector.



Allows making manual or automatic changes of brightness, light power and focus.



Entering information about the dilution and probe type of the examined sample.

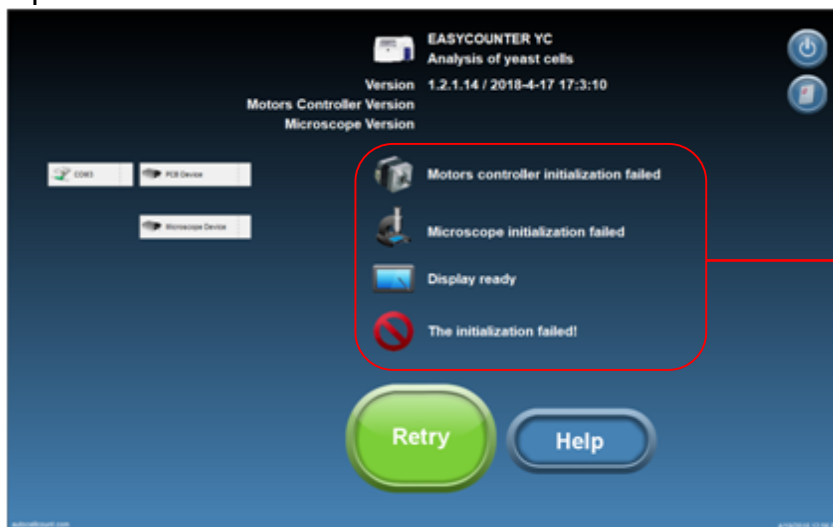
Preparation for work with EASYCOUNTER YC

Working sequence when preparing the EASYCOUNTER YC for work is described below:

1. Place the device on a flat, dry surface with nothing around it (4 inches radius)

2. Make sure the power supply is equal to the necessary for the equipment and connect the cable to the inlet on the back panel of the analyser. Using improper power supply may lead to serious damage of the device.
3. Place the plug into the electricity network.
4. Switched the ON/OFF key, placed at the back panel of the analyser and wait a minute until it charges.
5. Turn on the display by using the ON/OFF key placed at its upper side.
6. EASYCOUNTER YC launches the software and carries out an independent diagnostic test, including all the optic components.

If an issue is found during the check with any of the device's components, the program would not allow continuing to the Main screen menus and indicates which component is problematic.



Issues related to the optics, mechanics and operation

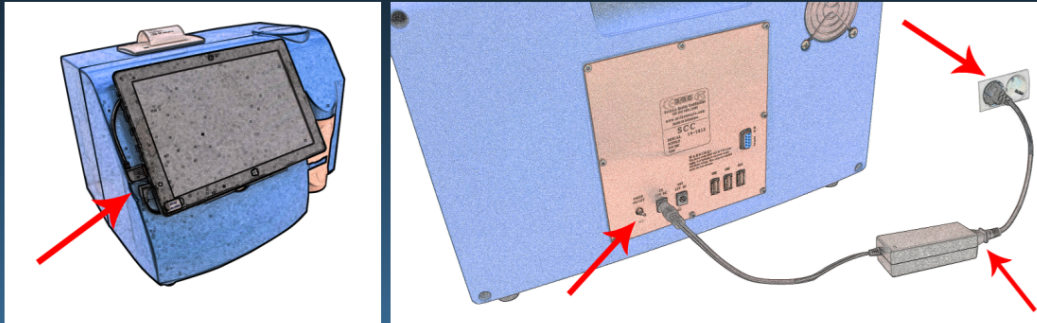
The issues may be caused by disconnection of cables, lack of power supply or software failure during the booting process. To eliminate them it is necessary to update the program by pressing the Retry button (try again).

7. Press button  , to open an instruction describing how to check the connections.



ERROR: The connection to hardware is lost.
E2: The system cannot find the file specified.
HRESULT 0x80004005: Unspecified error

Verify that your device is powered, and check out if all cables are connected.
 Switch off the device from the toggle switch at the back panel, wait few seconds and switch it on.
 Wait few seconds more, and press the button **RESET**.
 If the problem continues to appear, shut down Windows and restart the device.



Please, verify hardware and press one of the buttons bellow!

RESET

Restart application

CLOSE

Exit to Windows

SHUT DOWN

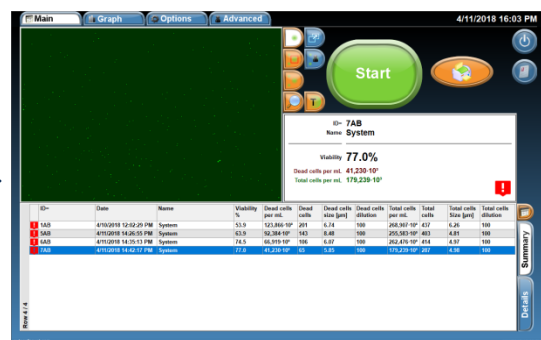
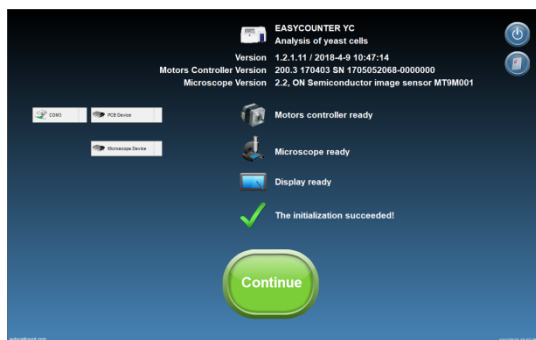
Power off

www.Lactoscan.com

9/15/2016 14:04 PM

If the issue is not resolved, please contact your local distributor or
www.autocellcount.com

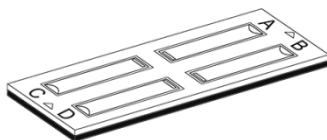
8. Switching to Main display menus, by pressing the button —Continue.



Preparing the sample for determination of Viability

I. For preparing one sample for analysis you will need:

- 1 pcs CELLCHIP



- 1 piece of transparent Eppendorfs with orange cap  with dried SOFIA GREEN fluorescent dye and lysis reagent



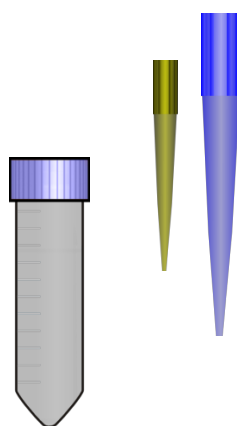
- 1 piece of transparent Eppendorfs with red cap  with dried SOFIA GREEN fluorescent



- 2 pcs of pipette tips - up to 100 μ L

- 2 pcs of pipette tips - up to 1000 μ L

- 2-4 pcs 15 ml tubes
- 1 piece of 100 μ L automatic pipette
- 1 piece of 1000 μ L automatic pipette
- 1 piece Stirrer Mini vortex



II. Principle of analysis

The EASYCOUNTER YC counts individual cells in suspension by detecting fluorescence signals of stained DNA in the cell nuclei. The staining dye used is SOFIA GREEN. The cell membrane of viable cells is virtually impermeable with regard to SOFIA GREEN, while membrane of non-viable, dead cells is permeable to SOFIA GREEN. The determination of the total concentration of cells requires that the membrane of viable cells is made permeable to SOFIA GREEN. This is achieved by mixing cell suspension with Lysis reagent. After Lysis reagent treatment (10 min), approximately 8 μ l of the all cell stained with YO-DEDM 1 and 8 μ l of the only dead cells stained with YO-DEDM 1 were loaded into the CELLCHIP. The CELLCHIP is placed in the EASYCOUNTER YC instrument where the cells are automatically enumerated.



ATTENTION!

The accuracy of the measurement depends on the proper and consistent execution of all the stages of the sample preparation and the good stirring of the sample. To minimize difference in counting the results for multiple testing of the same sample, always stir the sample thoroughly before taking part of its content. The sample is representative only when it is well mixed.

One very important factor is the rate of sample dilution. The concentration of the cell suspension to be transferred to the measuring chip and assayed must be within the optimum measuring range of the EASYCOUNTER YC apparatus. In order to provide a

statistically reliable result, the total cell count in the diluted cell suspension should be in the range of 1×10^5 to 1×10^7 cells/ml.

optimal range – from 1×10^5 to 1×10^7 cells/ml

measuring range - from 1×10^3 to 5×10^7 cells/ml

If cells concentration is in the range from 1×10^5 to 1×10^7 cells / ml, the initial sample is not diluted.

If cell concentration is higher than 1×10^7 cells/ml the initial sample is diluted with distilled water.

The rate of sample dilution for determination of dead cell count is 10 times lower than the rate of dilution for determination of total cell count; this is due to the fact that dead cells concentration in the sample is usually much lower than total cell concentration.

III. Determination of dilution rate

A. In case of unknown sample cell concentration

1. Take a 15 ml centrifuge tube containing 10 ml homogenized sample.
2. Place the tube in a centrifuge and balance the rotor with an opposing tube as necessary.
3. Centrifuge for 6 minutes at 4000 rpm.
4. Remove the test tube being careful not to disturb the sediment.
5. Measure the total volume in the tube, the volume of supernatant and from the difference - volume of sediment.
6. Calculate the percentage of sedimentation by the following formula:

$$\text{\% sediment} = \text{sediment volume} / \text{total volume} \times 100$$

7. . Determine the rate of sample dilution according to Table 1, depending on the determined % sediment.

B. With known indicative sample cell concentration

Usually, in beer, wine and bioethanol production it is known in what range cell concentration varies at different stages of production. In this case, it is not necessary to determine % spun solids, but the sample is directly diluted with distilled water. Table 1 shows how many times the initial sample is diluted according to cell concentration in the sample. The rate of sample dilution for determination of dead cell count is 10 times lower than the rate of dilution for determination of total cell count, this is due to the fact that dead cells concentration in the sample is usually much lower than total cell concentration.

Table 1. Dilution of the cell suspension depending on the initial cell concentration in order to reach the optimal measuring interval for total and dead cell count determination.

Spun solids	Total number of cells in the initial sample, cells/ml	Dilution of the initial sample, how many times	
		for a total number of cells (transparent Eppendorf with orange cap)	for dead cells (transparent Eppendorf with red cap)
≥ 80%	1500 - 2500 x 10 ⁶	1000	100
50-80%	500 - 1500 x 10 ⁶	500	50
5-50%	100 – 500 x 10 ⁶	100	10
≤ 5%	< 100 x 10 ⁶	20	2

If the concentration of the cells in the undiluted sample is below 1x10⁵ cells / ml, the concentration should be increased by centrifugation (> 10000xg, 15min) with subsequent resuspension of the pellets with PBS

IV. Measurement Procedure

1. Take two tubes (15 ml volume) and place in each of them 1 ml of the cell suspension using an automatic pipette. Add distilled water in order to achieve optimal dilution (Table 1), according to the indicative initial cell concentration. The sample in the first tube is diluted and used for determination of dead cell count. The sample in **the second tube is diluted 10-times more than first tube** and it is used for determination of total cell count.



ATTENTION!

When suctioning the cell suspension, hold the tip over the bottom of the tubes and observe particles from the medium not to impede the suction of the liquid sample. Even a partial occlusion of the tip entry may result in a reduction in the number of cells in the analysed sample.

If the cell suspension is with high density (sample from the propagator in brewing production), weigh 1 gram of suspension in each of the two 15 ml tubes at analytical balance using a special polystyrene foam stand and dilute with distilled water, **1000**

times to determine the total cell count and 100 times to determine the dead cell count.



ATTENTION!

Always indicate the rate of dilution in the tubes to know which tube to take when determining the dead cells and which ones to determine the total number of cells!

2. Take the two test tubes from the stand and homogenise them by placing the tip of each of them in the Mini Vortex stirrer opening. Press and hold the tip for 5 seconds and pull back. Repeat 8-9 times. (see 1.1, 1.2 and 1.3)



1.1



1.2



1.3

3. Take 1 ml of the diluted and homogenized sample in the 15 ml tube intended for determination of total cell count and put it **in the** transparent eppendorfs with orange cap  that contains lyophilized lysis reagent and fluorescent dye SOFIA GREEN.

4. Take 1 ml of the diluted and homogenized sample in the 15 ml tube intended for determination of dead cell count and put it **in the** transparent Eppendorfs with red cap  that contains lyophilized fluorescent dye SOFIA GREEN. .

Close both the tubes.

5. Take the transparent microcentrifuge tube from the stand and place the tip in the Mini Vortex stirrer opening. Press and hold the tip for 5 seconds and then pull it back. Repeat 8-9 times. Repeat this procedure for the other microtube (see 2.1, 2.2, 2.3). The transparent tube contains a lot of analytes and it takes more time to dissolve. Stir until complete dissolution of the dry analyte.



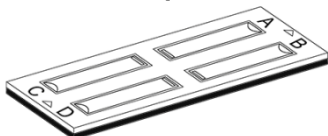
ATTENTION!

The transparent tube contains a lot of analytes and it takes more time to dissolve. Stir until complete dissolution of the dry analyte



6. Incubate both the samples for 10 min to let the sample interact with the lysis reagent and the fluorescent dye SOFIA GREEN.

7. Mix both the samples again using Mini Vortex. Press and hold the tip for 5 seconds and then pull it back. Repeat 2-3 times (see 2.1, 2.2 and 2.3).

8. Unpack one  CELLCHIP.



ATTENTION!

Do not touch the upper surface of the CELLCHIP. Always hold it on the side edges.

9. Load the two samples in the CELLCHIP microfluidic chambers by the 8 μ L pre-set automatic pipette. In the microfluidic **chamber A**, place 8 μ L of a sample **from the** transparent eppendorfs with orange cap  (**for total cell count**) and 8 μ L sample **from** transparent Eppendorfs with red cap  (**for dead cells**) in microfluidic **chamber B**.

Pipette the solution at an angle of approximately 80 ° to the semi-circle loop hole. Pipetting is done by gently pushing the pipette button from start position to first stop (see 3.1, 3.2). Hold the first stop button, withdraw the pipette from CELLCHIP and then slowly release the button to the initial position (see 3.3). **Do not use the second stop** to prevent air from entering the microfluidic chamber. In this way, you will completely empty the tip and ensure precise pipetting.



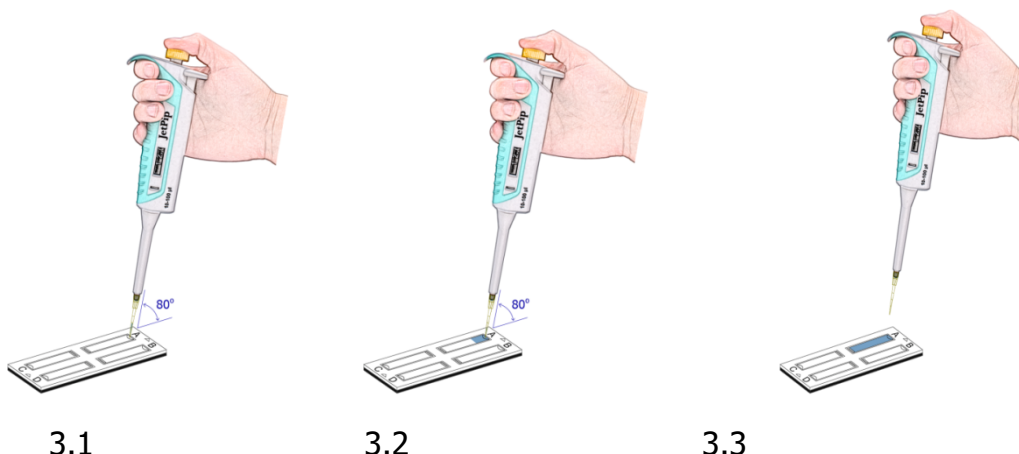
ATTENTION!

Always dispose of used tip and use a new tip for the next sample



ATTENTION!

Avoid the formation of air bubbles in the microfluidic chamber and reverse splashes during



To load the rest two chambers C and D, for measuring another yeast sample repeat the procedure described in point 1 to 9. Follow the sequence: the sample **from the** transparent eppendorfs with orange cap  should be loaded in **chamber C** and the sample **from** transparent Eppendorfs with red cap  in **chamber D**.

10. Place the loaded CELLCHIP in EASTCOUNTER YC cartridge.

11. Using the software proceed to sample analysis.



ATTENTION!

It is recommended to place the loaded CELLCHIP in the unit and start the analysis within 30-60 seconds. If the sample remains in the CELLCHIP in the apparatus longer, when you start the analysis it may result in inaccurate results due to evaporation of the sample and getting air into it.

V. Disposal of waste:

Using the automatic pipette second button, remove the tip into a suitable container. Discard the microfuge tube with the remaining sample, the tips, and CELLCHIP in a suitable container.

VI. Storage of EASTCOUNT YC KIT:

EASTCOUNT YC KIT should be stored at -10°C to +40°C and short-term to -20°C, protected from direct sunlight.

VII. Application: Working with an automatic pipette

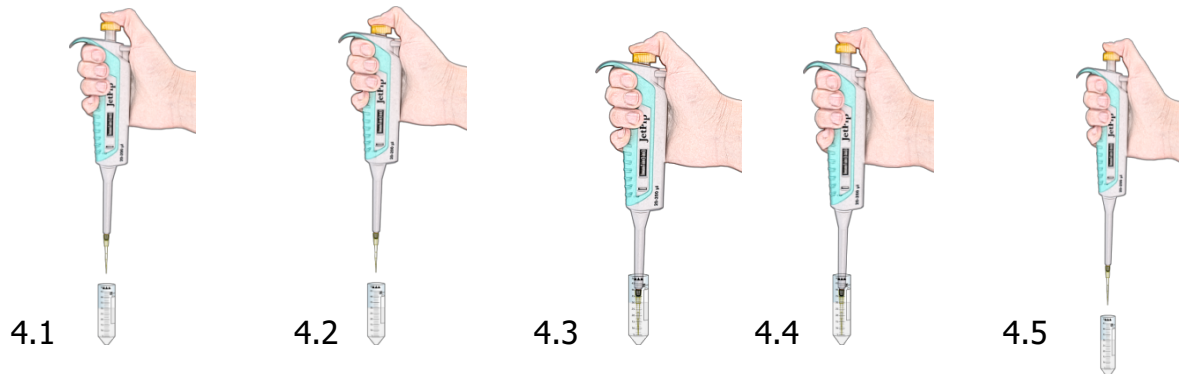


ATTENTION!

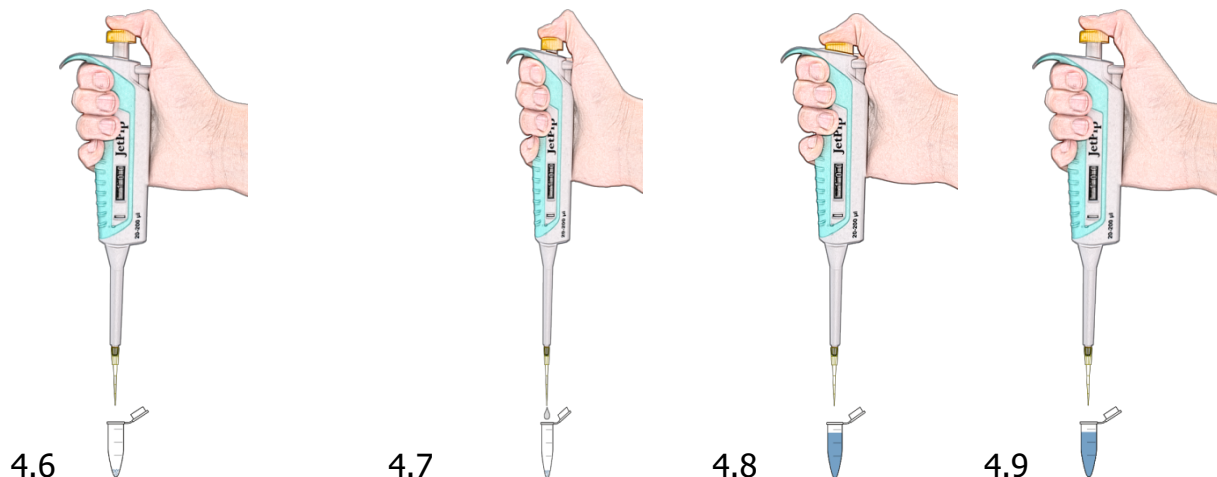
Before start operating the automatic pipettes, read the Instructions for Use of Automatic Pipette carefully. Make few attempts to suck in and suck out water to sense when you reach the first and second stops of the working button. Review the pipette manual video in the YOUTUBE at address: www.youtube.com/autocellcount.

Take the automatic pipette pre-set to a specific volume. Make sure the front pipette cone is clean. Place it vertically over a tip from the tips arranged on the work stand and put in the pipette tip by gently pressing.

From the initial position (see 4.1), press the pipette button until the first stop (see 4.2), hold it down and immerse the 2-3 mm tip of the pipette into the solution (see 4.3). Gently push out the operational button and remove the pipette from the liquid by touching the walls of the tube to remove excess yeast suspension (see 4.4, 4.5).



Pipette the yeast suspension from the tip into the open micro tube of the tripod by gently tapping the pipette button from the initial position to the first stop (see 4.6, 4.7). After a short pause, press the button next to the second stop (see 4.8). In this way, you will completely empty the tip and ensure precise pipetting.

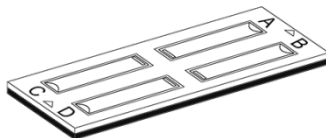


For more information and video instructions for working with EASTCOUNT YC KIT, visit www.autocellcount.com or www.youtube.com/autocellcount

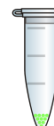
Preparing the sample for determination of Vitality

To prepare a sample for analysis are needed:

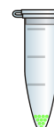
- 25 pcs CELLCHIP



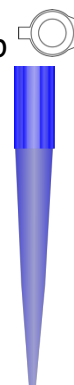
- 50 pcs transparent eppendorfs with orange cap  with lysis solution – lyophilized dye SOFIA GREEN



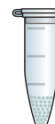
- 50 pcs transparent eppendorfs with blue cap  containing lyophilized 5-carboxyfluoresceindiacetate (CFDA)



- 50 pcs empty transparent eppendorfs with transparent cap  necessary for mixing the sample and the citrate buffer.
- 250 pcs automatic pipette tip – up to 100 µl
- 100 pcs automatic pipette tip – up to 1000 µl



- 5 pcs eppendorfs, containing dry components for buffer preparation – citrate-phosphate buffer pH 4 (Macilvaine buffer)

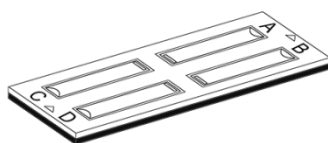


- 1 tube - volume 15 mL, for citrate-phosphate buffer preparation



Sample preparation for analysis

- 1 pc CELLCHIP



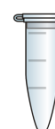
- 1 pc transparent eppendorfs with orange cap  with dry lysis solution and lyophilized dye SOFIA GREEN



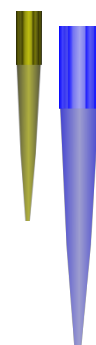
- 1 pc transparent eppendorfs with blue cap  containing dry 5-carboxyfluoresceindiacetate (CFDA)



- 1pc empty transparent eppendorfs with transparent cap  necessary for mixing the sample and the citrate buffer.

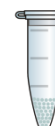


- 5 pcs automatic pipette tip – up to 100 μ l
- 2 pcs automatic pipette tip – up to 1000 μ l



- 1 pc automatic pipette up to 100 μ l
- 1 pc automatic pipette – volume 1000 μ l
- 1 pc Mini Vortex
- 1 pc Homogenized yeast suspension

- 1 eppendorf, containing dry components for buffer preparation – citrate-phosphate buffer pH 4 (Macilvaine buffer)



- 8 μ l acetone
- 1 empty tube - volume 15 mL, for citrate-phosphate buffer preparation



- 1 pc thermostatic device



Principle of analysis

The 5-carboxyfluorescein diacetate (CFDA) esterase substrate is used to determine the vitality of yeast. CFDA penetrates the cell membranes of living cells, the diacetate groups are cleaved from non-specific esterases and a fluorescent, charged substrate form (carboxyfluorescein) is obtained that stains the cells in green and flows out of the cells very slowly. In parallel, in a separate sample with yeast cell suspension, the total number of yeast cells was determined using the fluorescent dye SOFIA GREEN. To determine the total number of cells, it is necessary for the membrane of living cells to become permeable to SOFIA GREEN. This is achieved by mixing the cell suspension with a lysis reagent for 10 minutes. SOFIA GREEN penetrates through damaged cell membranes, binds to core nucleus, and cells with damaged membranes turn green.

Preparation of the reagent solutions for analysis

1. Preparation of the CFDA solution

Add 8 µL acetone to the transparent eppendorf containing the dried CFDA. Stir the mixture on Vortex.



Caution!

Do not add more than 8 µL of acetone as the test sensitivity is reduced.



Caution!

After receiving the kit, the 50 transparent eppendorfs with blue cap  containing dried 5-carboxyfluorescein diacetate (CFDA) should be stored frozen at $\leq -20^{\circ}\text{C}$, protected from light. If stored properly, this reagent should remain stable for six months. Before opening the eppendorfs, they should be warmed to room temperature.

2. Preparation of Buffer Solution

Add 1mL of distilled water to the eppendorf containing dry citrate buffer (Macilvaine buffer). Stir the mixture on Vortex. Pipette the entire obtained solution (1 mL) and transfer it to a 15 mL empty tube. Add 9 mL of distilled water to the tube. Store the prepared buffer in a refrigerator at 4°C .



Caution!

The shelf life of the prepared buffer solution is 10 days. To avoid clogging buffer salts are in dry form and are divided into four separate microcentrifuge tubes.

Dilution of the yeast sample for analysis

Diluted yeast sample 1. Yeast sample for determination of total cell count:

Dilute the initial yeast sample with distilled water in a 15 mL volume tube as described in the yeast viability assay, total cell dilution (see Table 1, EASYCOUNTER YC KIT VIABILITY).

Diluted yeast sample 2. Dilution of yeast vitality test:

Diluted yeast sample 2.1. Dilute the original yeast sample with distilled water in a 15 mL test tube with 10 times less dilution than the diluted yeast sample **1**.

Diluted yeast sample 2.2. To determine the vitality of the yeast, a 10-fold dilution of the diluted yeast sample 2.1 is performed. Take 100 µL of diluted yeast sample 2.1, place in a clear transparent eppendorf and add 900 µL citrate-phosphate buffer pH 4 (Macilvaine buffer).



Caution!

To provide a statistically reliable result, the total cell count in the diluted cell suspension should be in the range of 1×10^5 to 1×10^7 cells / mL:

- optimum interval of 1×10^5 to 1×10^7 cells / mL

Performing the analysis

Total Cell Count Assay

1. Take 1 mL of the diluted and well-homogenized yeast sample **1** for determination the total cell number and place it in transparent eppendorfs with orange cap  containing dried SOFIA GREEN dye and lysis reagent.
2. Close the transparent eppendorfs with orange cap .
3. Take the transparent eppendorfs with orange cap  from the tripod and place the tip in the Mini Vortex stirrer hole. Press and hold the tip for 1-2 seconds and then pull it off. Repeat 8-9 times.



Caution!

The transparent tube contains a larger amount of analytes and more time is necessary for dissolving. Stir until the dry analyte is completely dissolved.

4. Incubate the microtube for 10 minutes necessary for lysing and staining the cells with the SOFIA GREEN dye.
5. Mix the sample on the Mini Vortex mixer again. Press and hold the tip for 1 to 2 seconds and pull back. Repeat 2-3 times
6. Pipette 8 µL of the sample into CELLCHIP chamber A.

Assay for determination of vital cell count (Vitality)

1. Add 8 μ L of acetone to the dried CFDA in the transparent eppendorfs with blue cap .

2. Mix the sample on the vortex.

3. Pipette 100 μ L of the diluted yeast sample **2** for vitality determination and place it in the transparent eppendorfs with blue cap  containing the dissolved CFDA.

4. Mix the sample on the vortex.

5. Incubate the sample for 10 minutes at 40°C.



Caution!

The sample should not be incubated for more than 20 minutes as the staining of the cells decreases due to leakage of the dye from the cells.

6. Mix the sample on the vortex again.

7. Pipette 8 μ L of sample into CELLCHIP **chamber B**.

8. Place the CELLCHIP in the EASYCOUNTER YC and proceed to the assay.

9. Using the software, start the analysis.



Caution!

It is recommended to place the loaded CELLCHIP in the device and start the analysis within 20 - 60 seconds. The delayed placement of CELLCHIP in the apparatus and start of the analysis will result in inaccurate results due to evaporation of the sample.

Waste disposal:

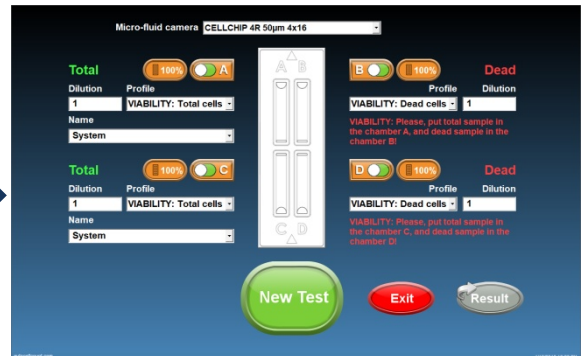
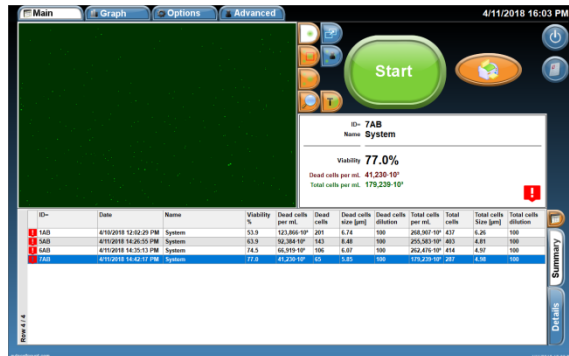
Using the automatic pipette button, remove the tip into a suitable container. Discard the microtubule with the rest of the sample and the used CELLCHIP in a suitable container.

Storage of EASYCOUNTER YC KIT VITALITY:

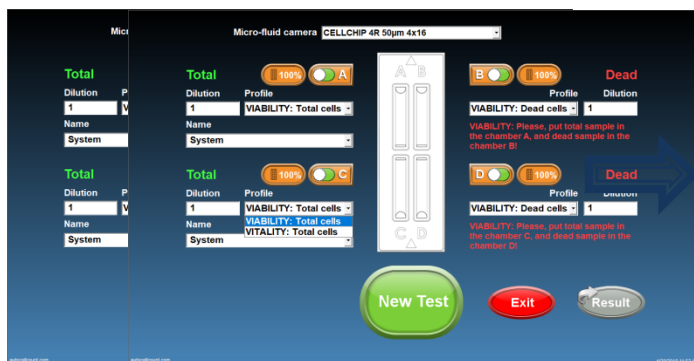
The 50 transparent eppendorfs with blue cap  with dried 5-carboxyfluorescein diacetate (CFDA) should be stored frozen at $\leq -20^{\circ}\text{C}$, protected from light. The remaining components of EASYCOUNTER YC KIT must be stored at -10°C to $+40^{\circ}\text{C}$, protected from direct sunlight.

Sample analysis

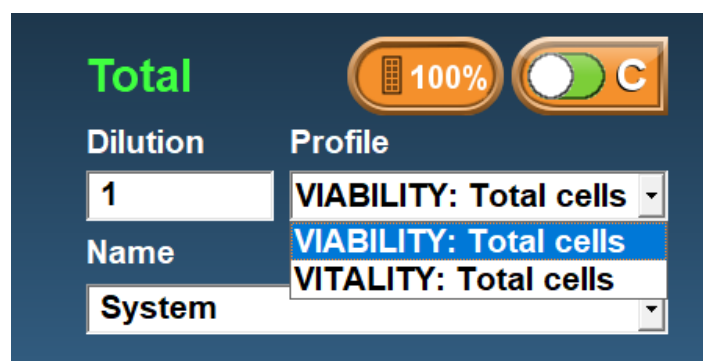
1. Press  on the Main screen in order to proceed with entering data of the sample.



2. Choose which mode you want to work in (Viability or Vitality)



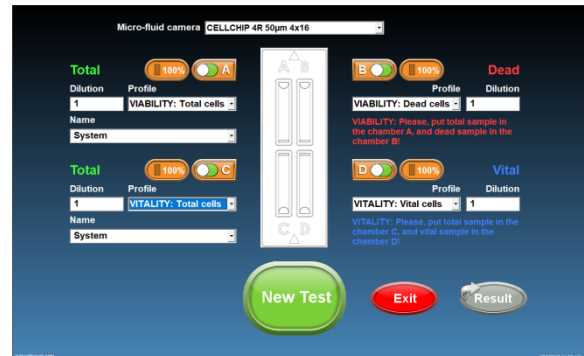
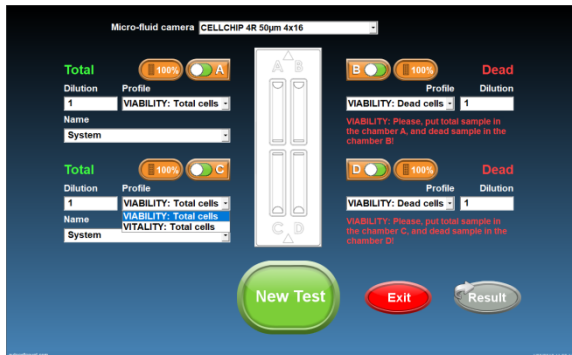
Select Viability or Vitality from the drop-down menu





Attention!

The software enables you to perform two Viability analyses, two Vitality analyses, or a Viability and Vitality analyse at the same time.



3. Choose the type of chip from the drop down menu

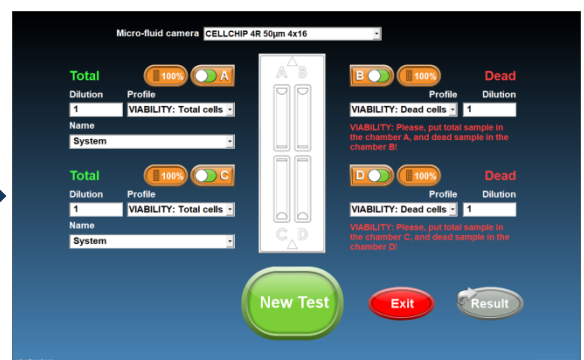
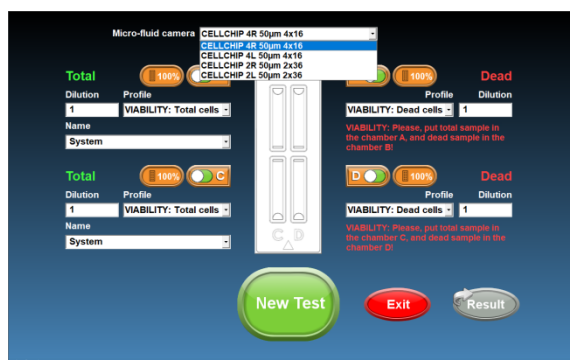
CELLCHIP 4R 50µm 4x16



Attention!

Before starting work with EASYCOUNT YC KIT always check CELLCHIP letter. It can be L or R. Letter L indicated CELLCHIP 4L and letter R indicated CELLCHIP 4R. The CELLCHIP letters match with the already put in the program CELLCHIPS data. One EASYCOUNT YC KIT included only CELLCHIPS with letter L or only with letter R

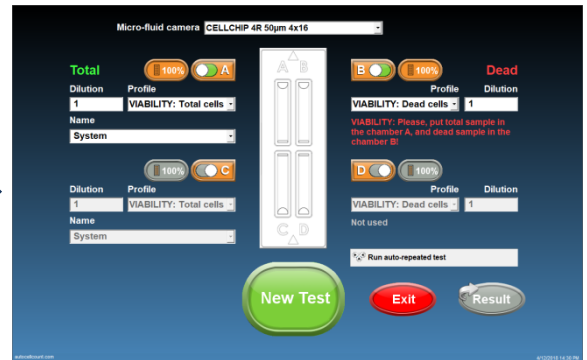
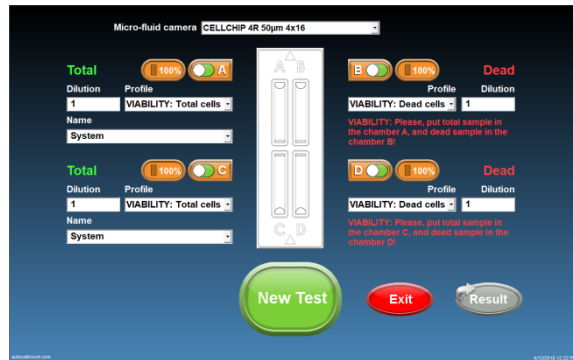
In our example we choose **CELLCHIP 4R 50µm 4x16** CHIP 4X16 with thickness of the four micro fluidic chambers 50µm.



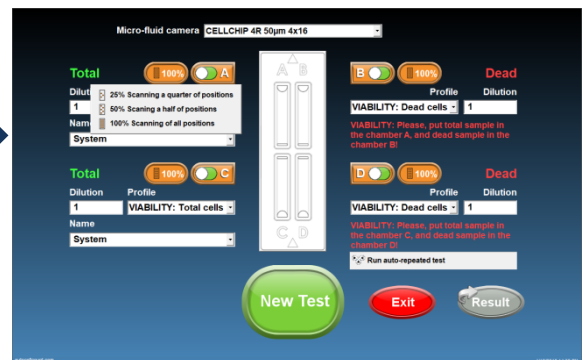
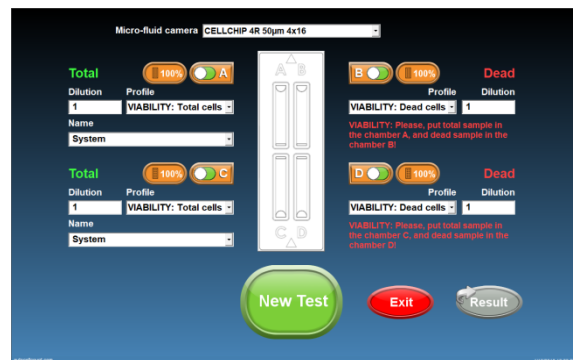
4. All the micro fluidic chambers of the CELLCHIP are selected. If you want

to use only 1 or 2, using button  deselect the chambers which you will not be using in this test.

In our example we deselect Sample C and D.



5. For a quick analysis, press button  and select a test from the dropdown menu. You can select from test "Scanning 25% of positions", test "Scanning 50% of positions", test "Scanning 100% of positions".



6. Complete the value of the dilution's multiplier in field  under Dilution.



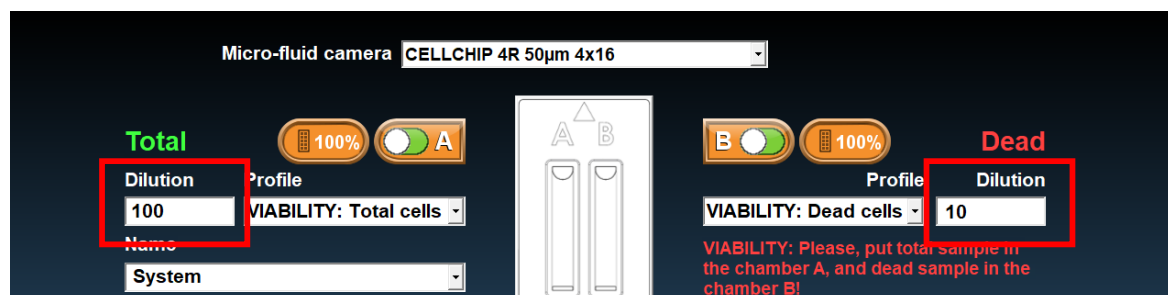
Attention!

The value of the multiplier for dilution depends on the density of the initial cell suspension

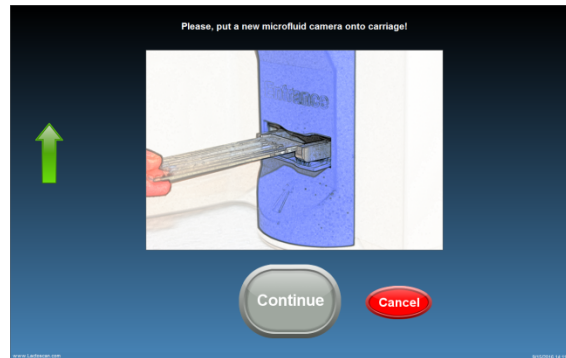


Attention!

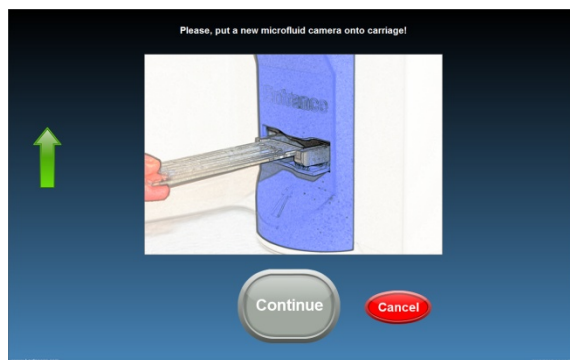
Do not forget to change the dilution multiplier in the field Dilution. This factor is different for Total cells, Dead cells and Vital cells.



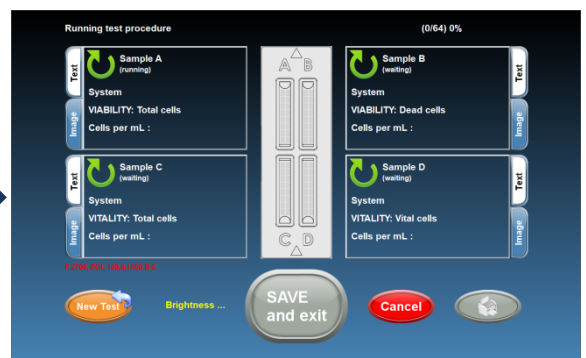
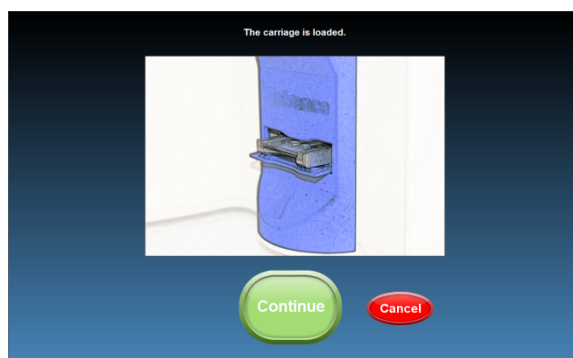
7. After the information for all the samples is entered, press  in order to proceed with the analysis. On the screen a message will be displayed, showing that the cartridge for placing the CELLCHIP comes out of the door. Pressing , will revert to the Main screen.



8. Place CELLCHIP into the cartridge and press .

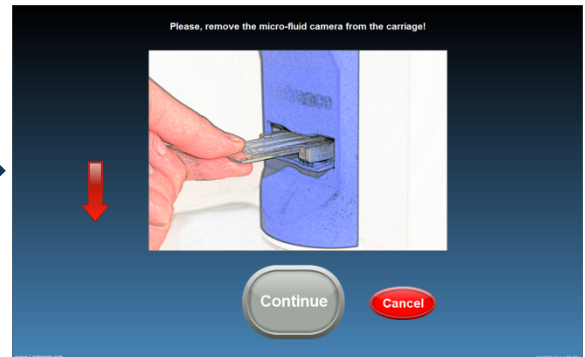
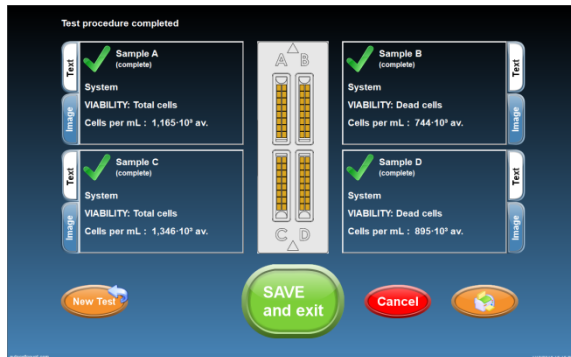


9. The device is making the analysis of the samples.

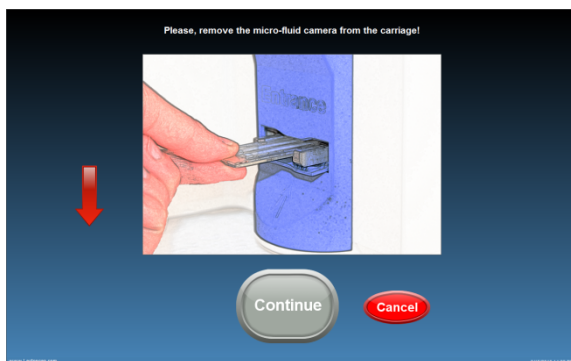


10. In order to print the results, press .

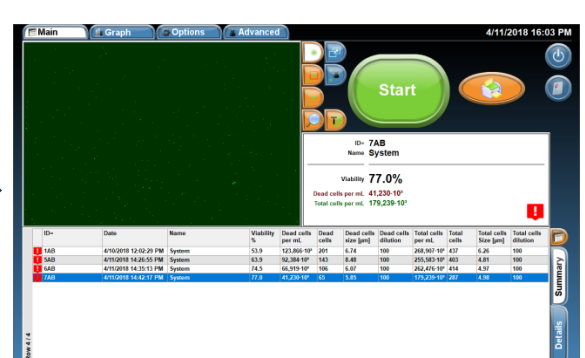
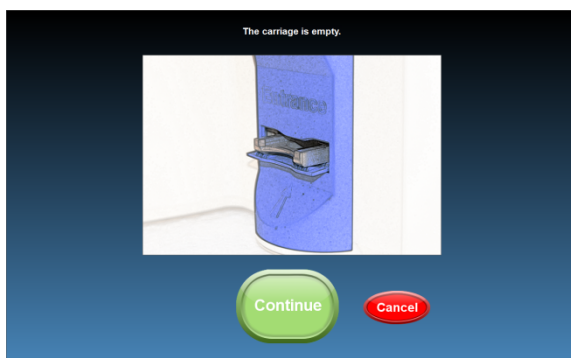
11. In order to save the results and continue the analysis of new samples, press **SAVE and exit** or press **Cancel**, to continue with the analysis of new samples without saving the results



12. Take the CELLCHIP out.



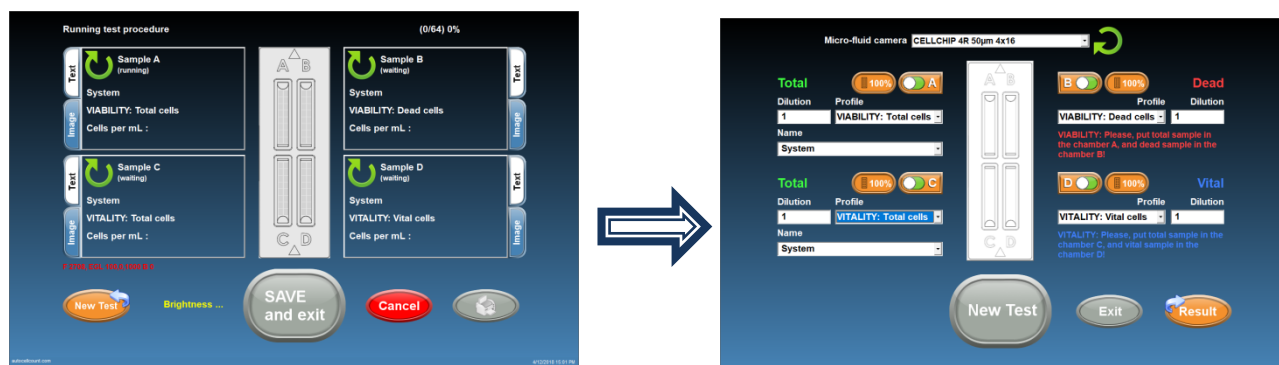
13. In order to go back to the Manual screen, press **Continue**.



Data samples entry during the analysis

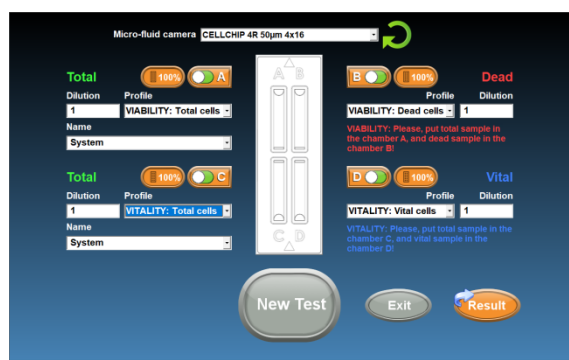
The software of EASYCOUNTER YC allows the entry of data for new samples during the process of analysing the samples. In order to do this:

1. Press  and will proceed to the form for data entry of the samples.



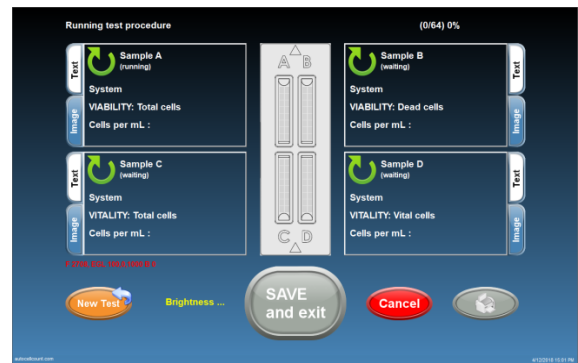
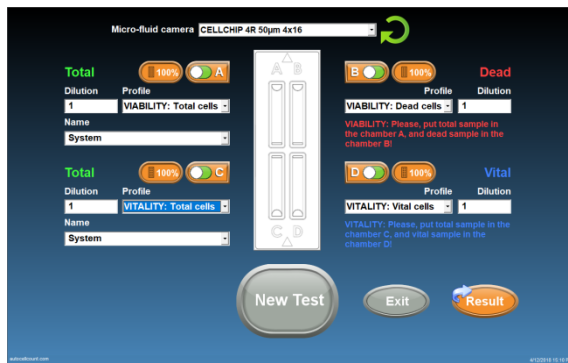
The data for the samples which are being analysed at the moment are saved in the form for facilitating the user's work, in case analysis of identical samples for different clients or identical species of animals have to be made.

2. Enter the data for the new samples.



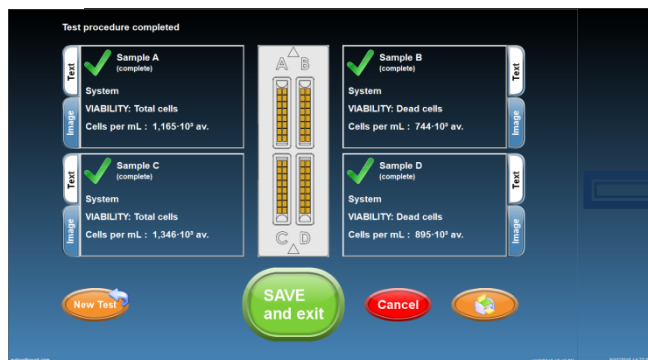
If  is grey, this means that the previous analysis has not been finished yet. In this case wait for the analysis to be finished and the  to become active, or go back to the screen with the current analysis.

3. Press , in order to go back to the screen with the current analysis.

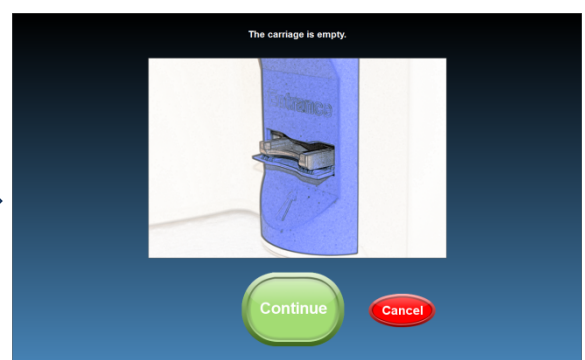
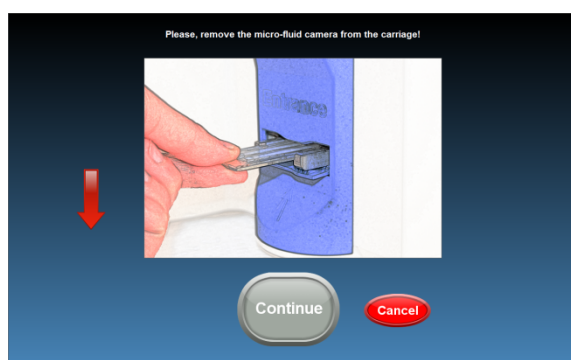


4. Press , in order to proceed directly to the new analysis.

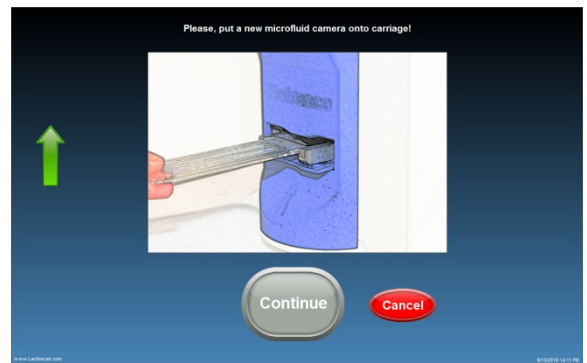
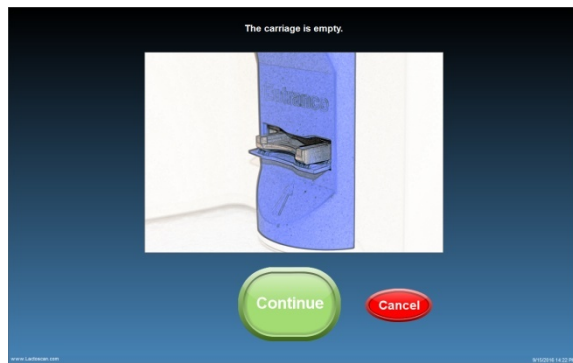
By pressing  the results are automatically saved in the data base of the device. And the software prompts to pull out the CELLCHIP with the samples from the previous analysis.



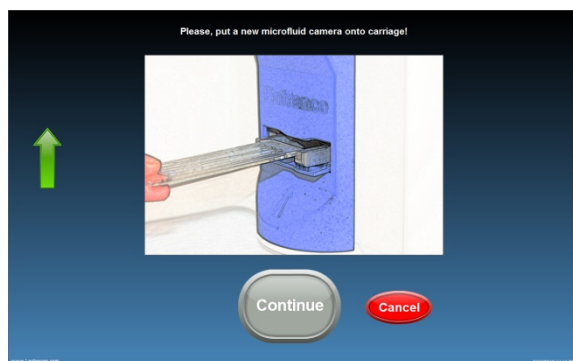
5. Pull out the CELLCHIP with the samples from the previous analysis.



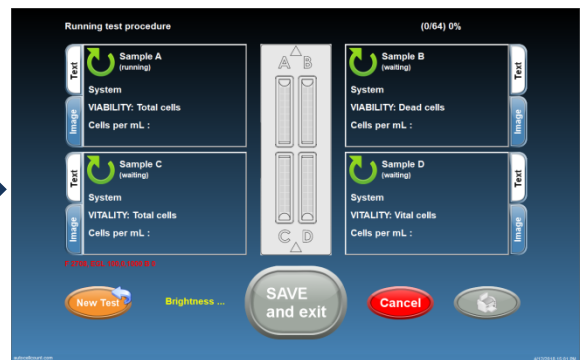
6. Press . The software prompts for placing the CELLCHIP with the samples for which data are entered.



7. Put the CELLCHIP with the samples with entered values.



8. Press  , to start the assay.



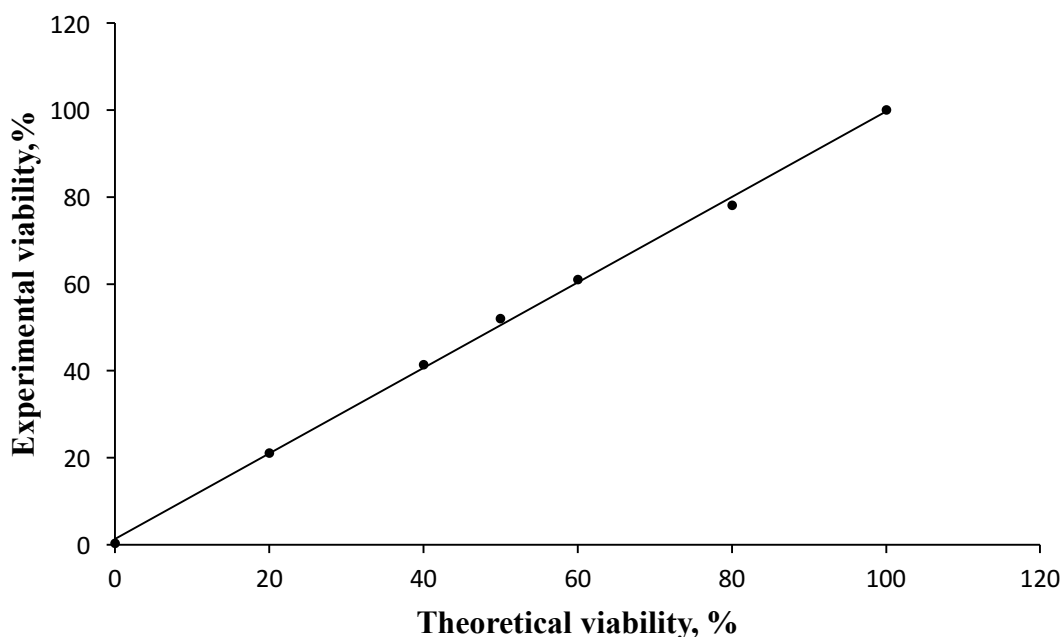
Reference sample testing

To obtain results with referent accuracy for yeast cell count using EASYCOUNTER YC, the following steps should be performed:

1. A representative yeast sample with concentration 5×10^6 cells/mL is used.
2. 2 mL of the sample are pipette in two separate tubes.
3. The yeast suspension in the first tube is heat-killed at 100 °C for 10 min.
The untreated suspension in the second tube is used for live cells source.
4. After that serial mixing of live and dead cells is performed to obtain sample with different % of viability (0, 25, 50, 75, 100 % viability).
5. 1 mL of each mix is pipette in a microcentrifuge tube containing lyophilized dye, vortexed and incubated for 3 min.
6. 8 µl of each sample is then placed in the CELLCHIP and analysed for determination of dead cell count.
7. The sample wit 0 % viability is used for total cell count determination.
8. The viability in % is calculated according to the following equation:

$$\text{Viability, \%} = \frac{\text{total cell count} - \text{dead cell count}}{\text{total cell count}} \times 100$$

9. The theoretically predicted viability is placed against the experimentally measured viability in a graphical dependance.



Linear range obtained using samples with different viability.

Comparison of theoretical and experimental viability should show high correlation coefficient $R^2 = 0.95 - 0.99$.

Calculating the Coefficient of Variation (CV)%

To calculate the Coefficient of Variation (CV)% following the above procedure, you must test 10 times each cell mixture. Put the measure values in table. Then calculate the coefficient of variation (CV),%.The precision of the determination of cell

concentration is dependent on the number of cells counted. It is normally assumed that the counting of random events follows the Poisson distribution, according to which the expected standard deviation is equivalent to the square root of the number of counted cells. The relative precision, expressed as Coefficient of Variation (CV) is therefore:

$$CV = \frac{SD \times 100\%}{M}$$

Where:

M – average value – (number of counted cells)

s – Standard deviation

Yeast viability in 5 standard samples (0, 25, 50, 75 and 100% viability) and the calculated coefficient of variation (CV)%

Measureme nt №	Sample 0% viability	Sample 25% viability	Sample 50% viability	Sample 75% viability	Sample 100% viability
1	0.13	24	52	73	99
2	0	23	54	77	95
3	0.08	25	56	72	98
4	0.13	27	51	78	93
5	0.2	25	55	71	96
6	0.18	26	50	76	95
7	0.24	24	48	70	98
8	0.13	25	52	78	97
9	0	23	54	71	94
10	0	26	49	77	93
Mean	0.19	24.8	52.1	74.3	95.8
Coefficient of variation, %	5.6	5	4.7	2.95	2.08

Saving image from the analysis as .BMP file

EASYCOUNTER YC allows Users to save an image from already done analysis as .BMP file. The .BMP file is easy for further process and can be opened by or imported in any Windows program.

To do this:

1. Select the analysis from the data base:

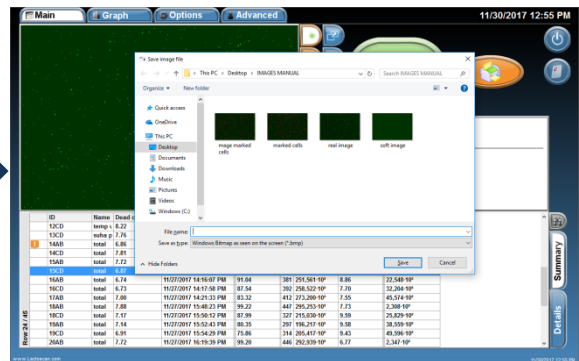
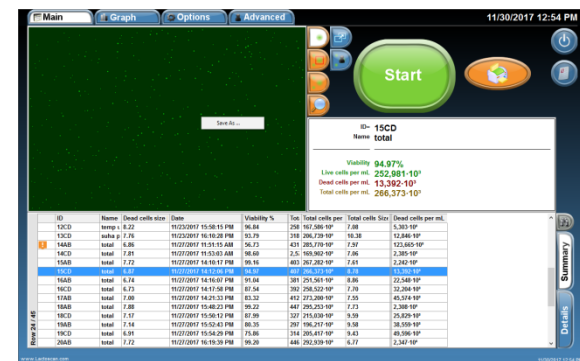


2. Go to the image and press the right button of the mouse on it.



3. Press the button

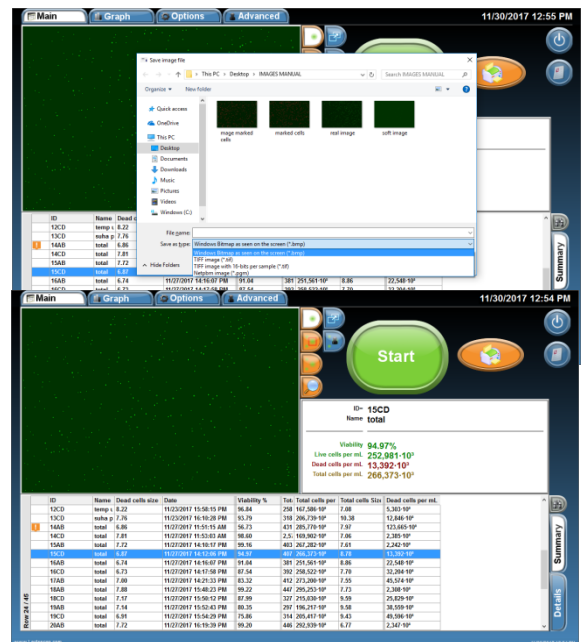
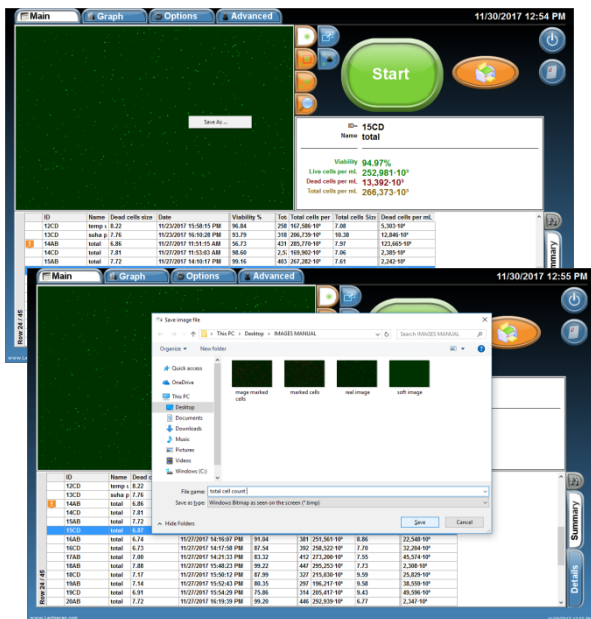
Save As ...



4. Select the folder where you want to save the image, write the name of the image, select the .BMP file format from the dropdown menu and press button SAVE to finally save the image as .BMP file.

EASYCOUNTER YC

OPERATION MANUAL

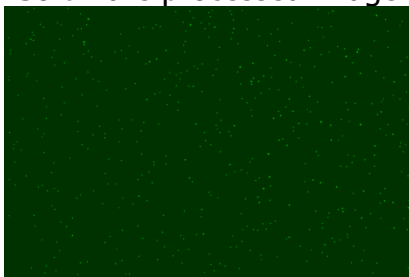


NOTE! The image can be saved in .TIFF file format as well. The .TIFF file is a RAW image format and the saved images can be processed in special software without a losing the quality of the initial taken image.

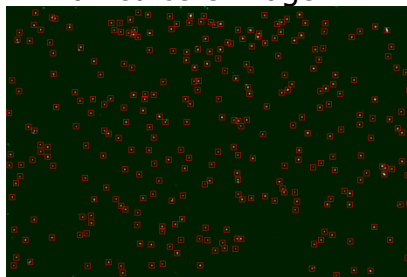
Before saving the image, the User can select a visualization mode such us Software processed image, Marked cells image, Raw image and to save in .BMP file the image as it is seen in the software.

Examples:

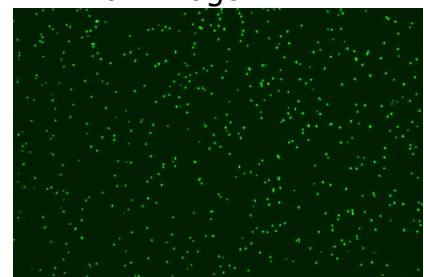
Soft ware processed image



Marked cells image



Raw image



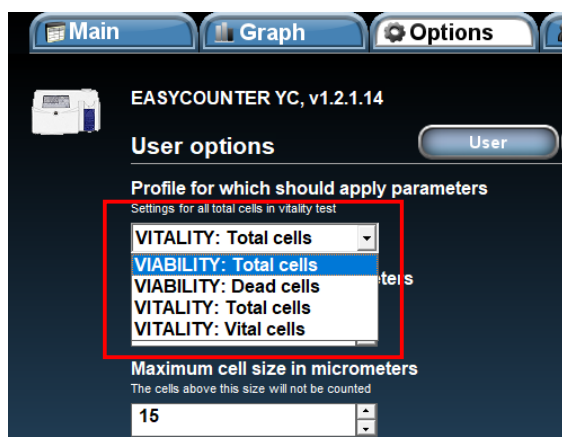
Setting the analysis' parameters

The Menu Options allows the user to set the analysis parameters



1. Setting the minimum and maximum size of the cells, which are going to be counted:

1.1. Choosing the sample for which the set parameters have to be applied.
With the assistance of the drop-down menu, choose the sample for which change of the pre-set parameters is needed.



1.2. Filling in the required parameters.

When the sample is chosen, pre-set values of the minimum and maximum size of the cells, which will be counted appear below it. With up and down arrows or by manual entry of value, the range of the counted cells may be changed according to the certain type of sample.

Deleting results from the data base

The User can delete results from the data base table. To do this:

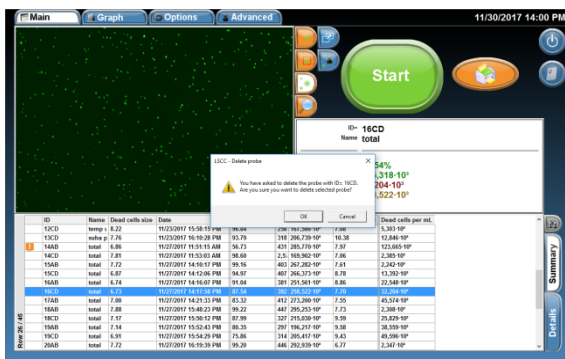
1. Select the result that you want to delete and press the right button of the mouse to appear button Delete.



2. Press the Delete button and confirm that you want to delete the selected result



3. The selected result is deleted.

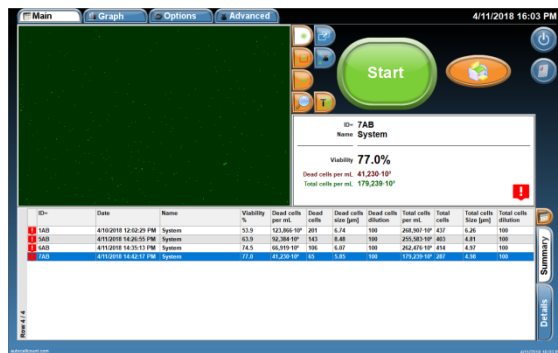


Cleaning of the Database

The software allows in accumulation of a large number of results of analyses, the entire database to be deleted to make space for storing the results of the upcoming analysis.

To clean the Database:

1. Go to menu Options



2. Press button

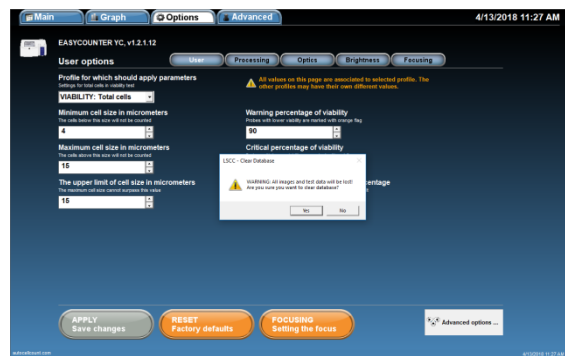


3. From the drop-down menu select Clean Database



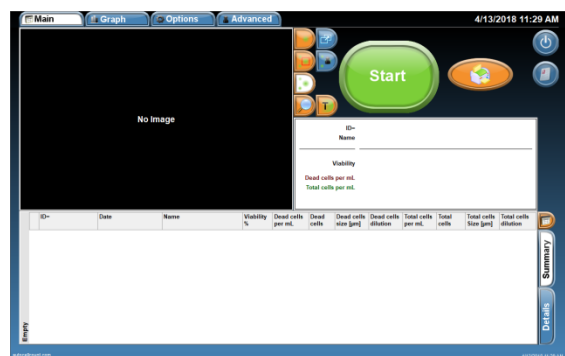
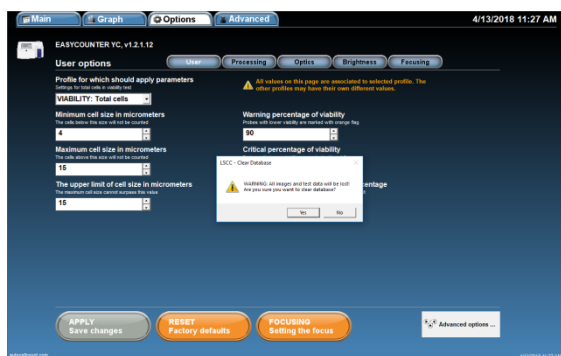
4. Press Clean Database





Attention!

Once deleted, the results cannot be restored!



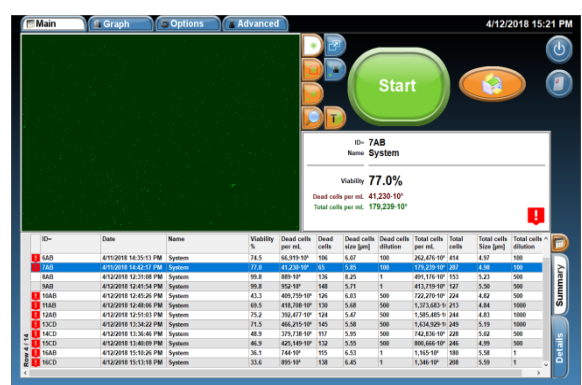
Printing the results from the data base

EASYCOUNTER YC gives the opportunity to print every single result in the data base of the device.

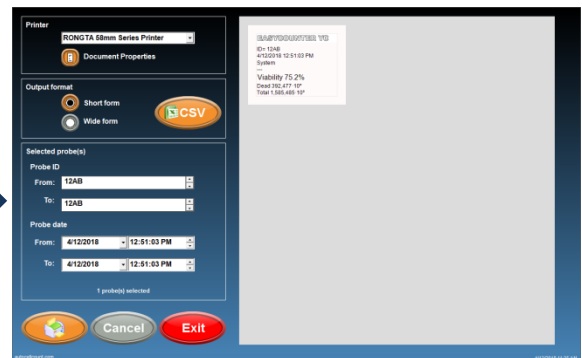
Printing the results from an integrated printer:

1. Choose the result which needs printing.

The user chooses a result as marking it by pushing onto the information for the relevant analysis in the field "Summary".

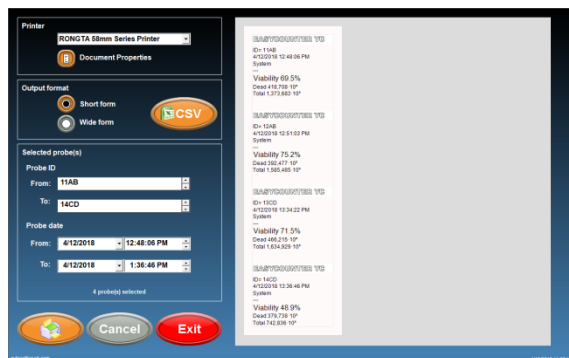


2. Press  , in order to proceed to the menu "Printing".

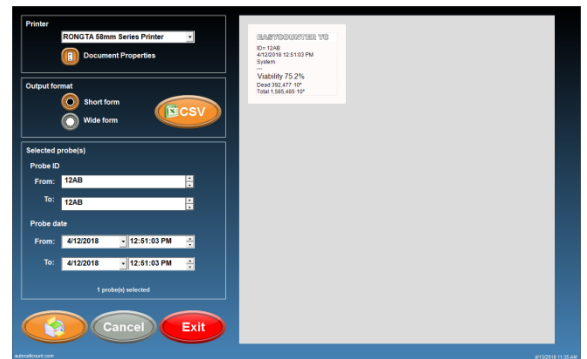
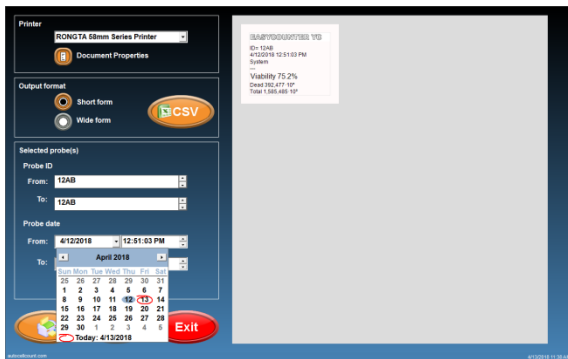


3. It is not necessary to choose a printer or form of printing. By default it is accepted that EASYCOUNTER YC will print the results on the integrated printer in a short form.

4. Push the arrows from the right side of the results ID if changing or adding new results for printing is needed.

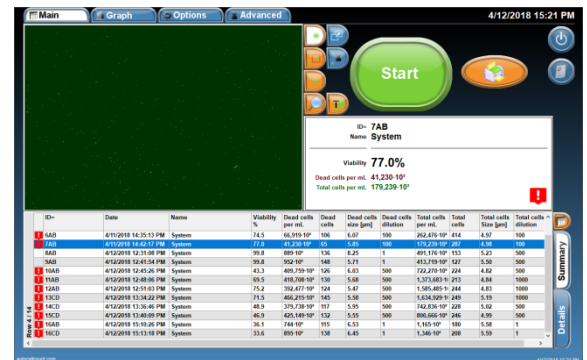
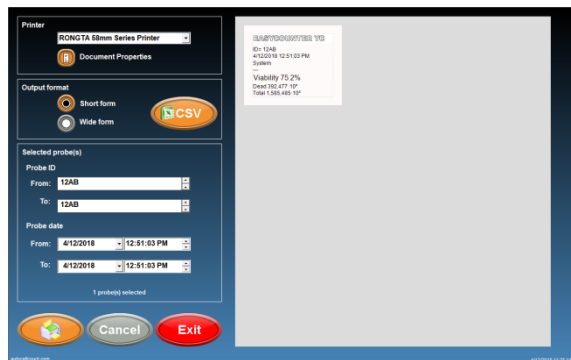


5. If printing the results from the analysis, made for a certain period of time is needed, then, with the assistance of a calendar the initial and the final date of the period can be set.



6. After the results for printing are chosen, press , in order to print them.

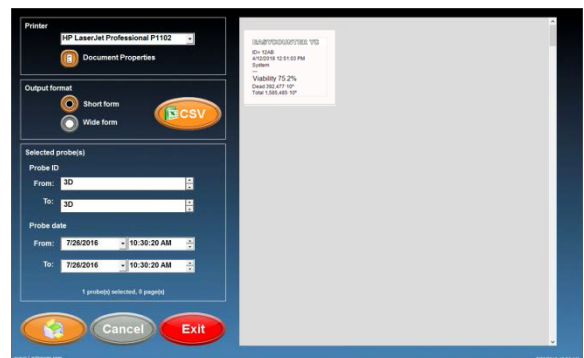
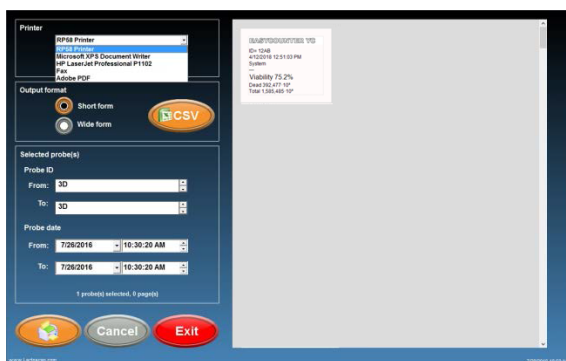
7. To go back to the Main menu, press .



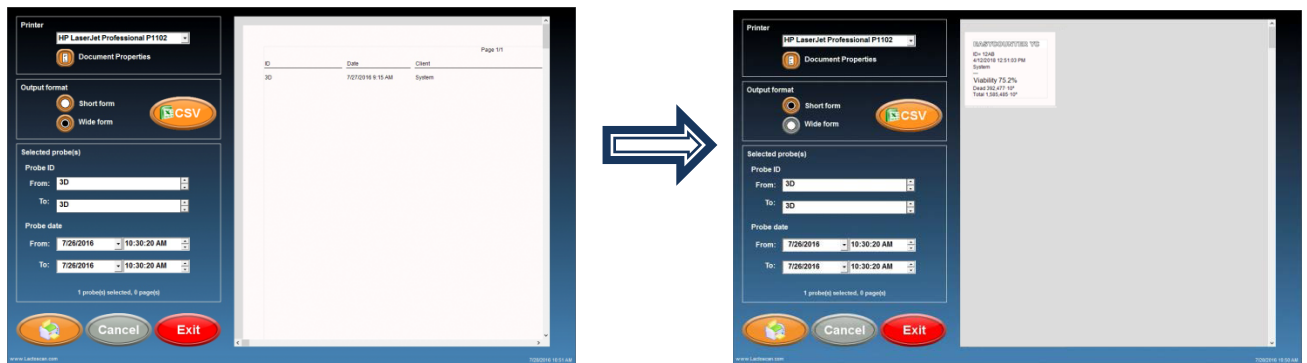
Printing the results on an external printer:

1. Before start printing, make sure that the external printer is connected and installed.

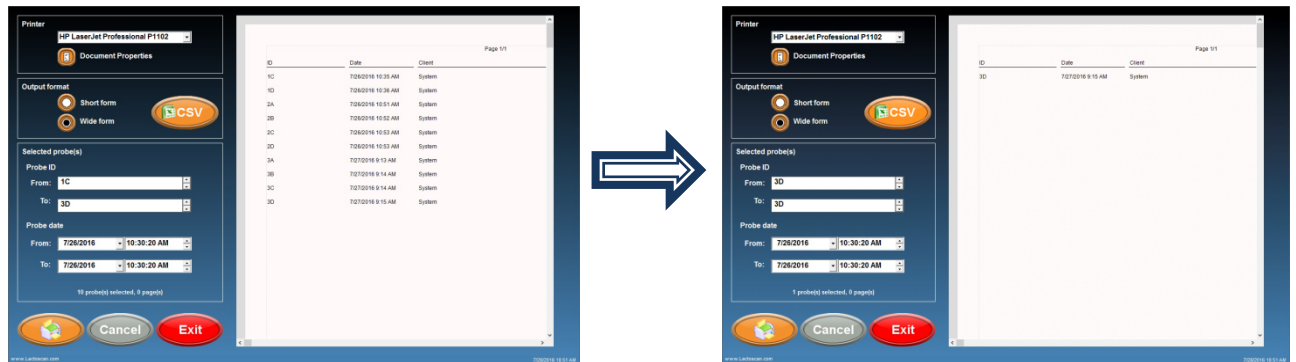
2. From the drop-down menu, choose the name of the external printer



3. Choose Wide form for printing the results in a table.



4. Press the arrows from the right side of the results ID, if changing or adding new results for printing is needed.

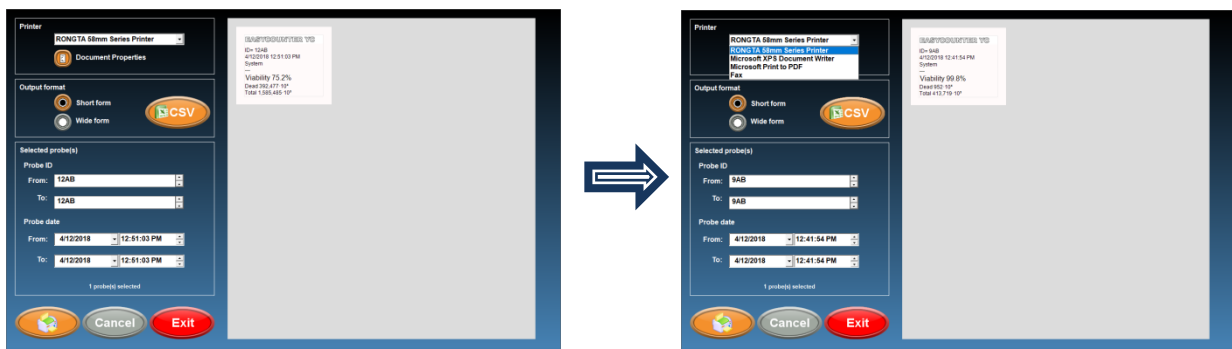


5. After the results for printing are chosen, press  , in order to print them.

6. To go back to the Main menu, press

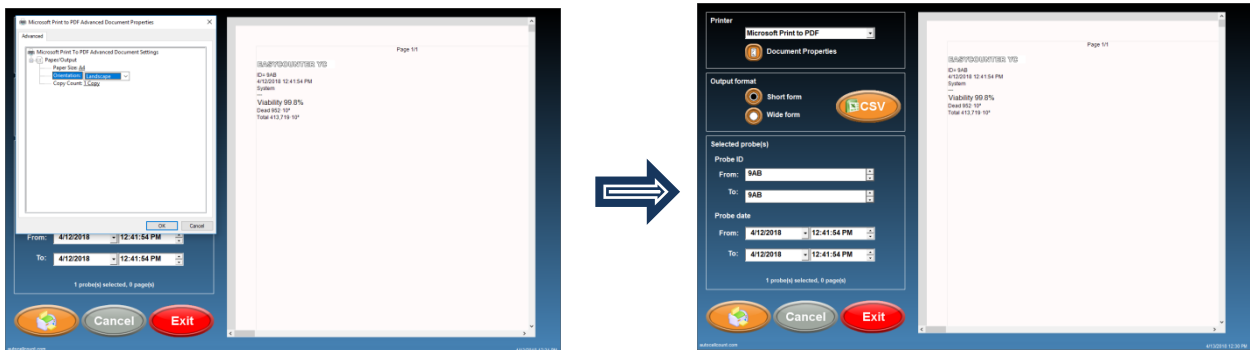
Saving the results in PDF file:

1. From the drop down menu, choose MICROSOFT PRINT TO PDF.

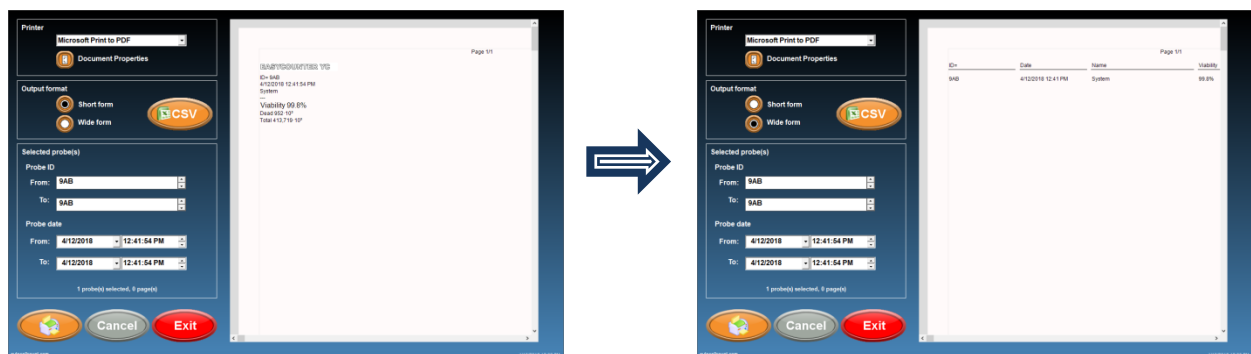


2. Before saving, check the orientation of the page.

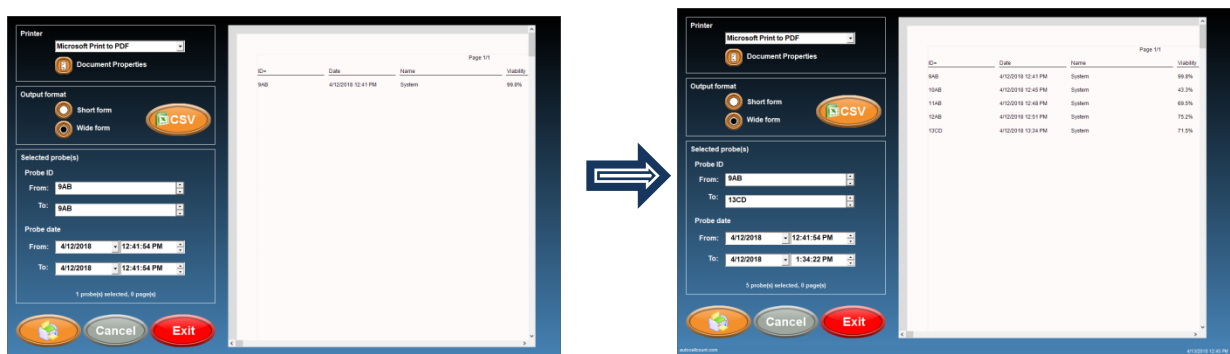
If you want to save the results in WIDE form, the orientation must be landscape and the paper size A4. To check the orientation, press Document properties.



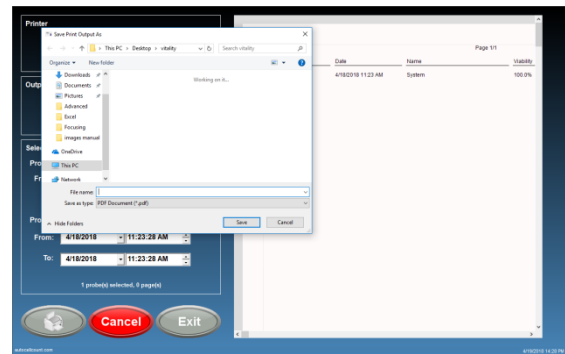
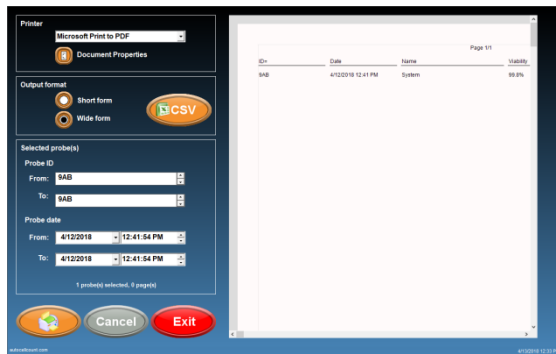
3. Choose Wide form for printing the results in a table.



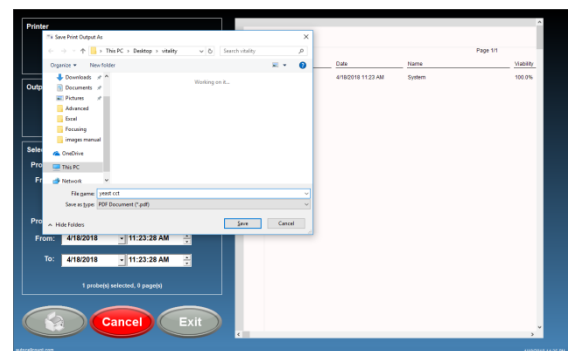
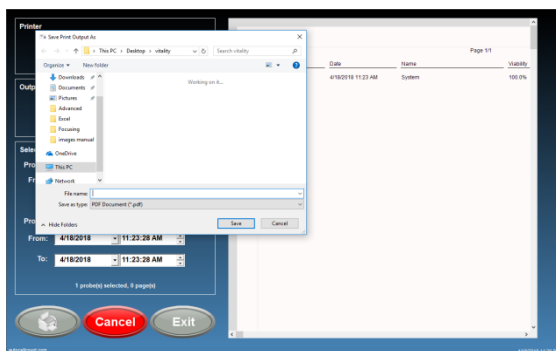
4. Add the results that you want to include in the PDF file.



5. Press button to save the file.



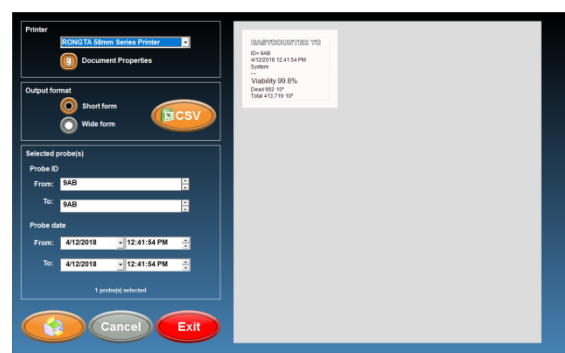
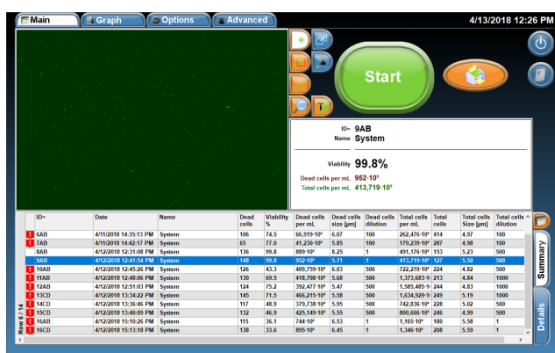
6. Select the folder where you want to save the file, write the name of the file and press button SAVE to finally save the results in PDF file.



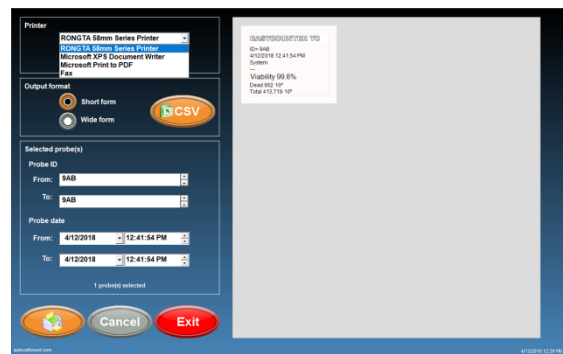
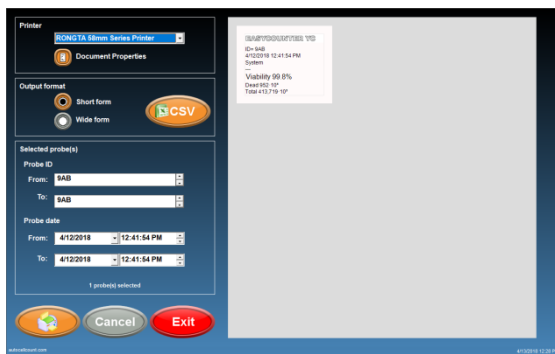
Saving the results in Excel file:



1. Go to printing mode by pressing the button

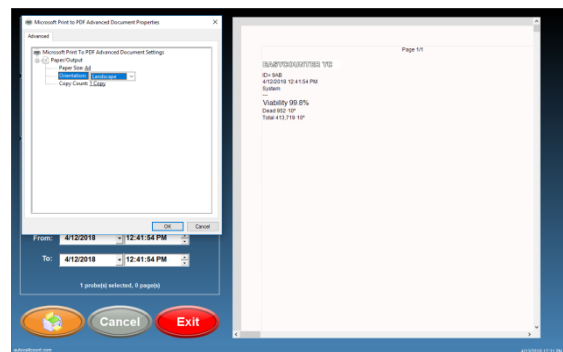
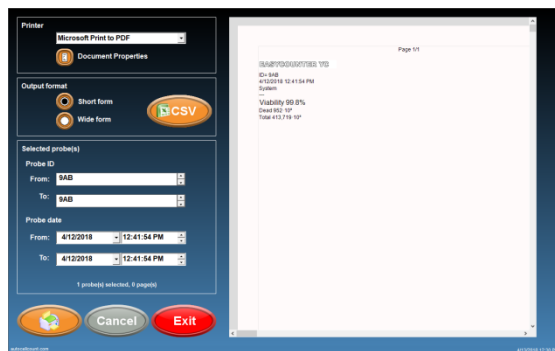


2. From the drop-down menu, choose MICROSOFT PRINT TO PDF.

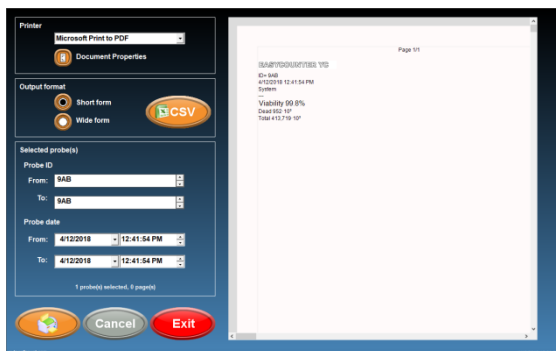


3. Before saving, check the orientation of the page.

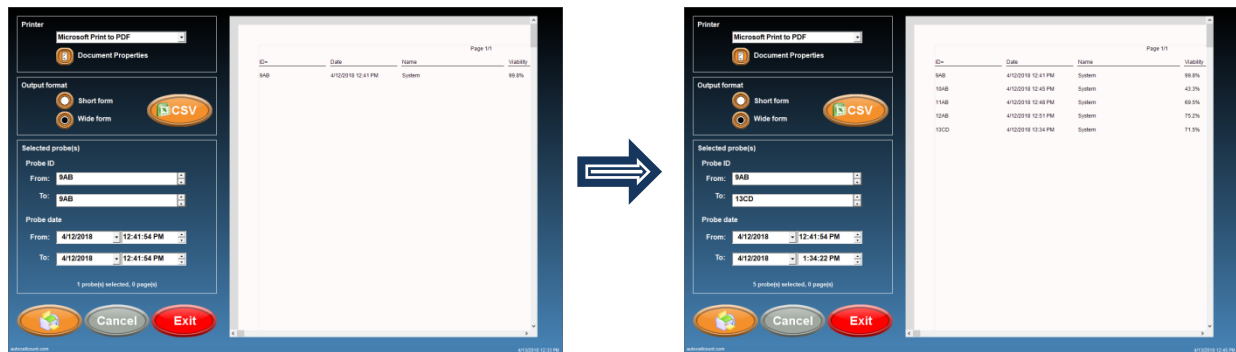
If you want to save the results in WIDE form, the orientation must be landscape and the paper size A4. To check the orientation, press Document properties.



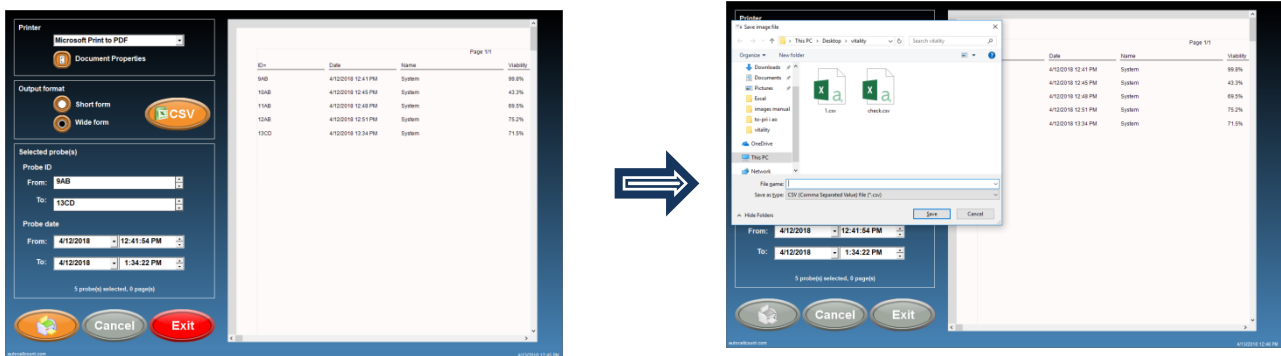
4. Choose Wide form to review the results in the Excel file.



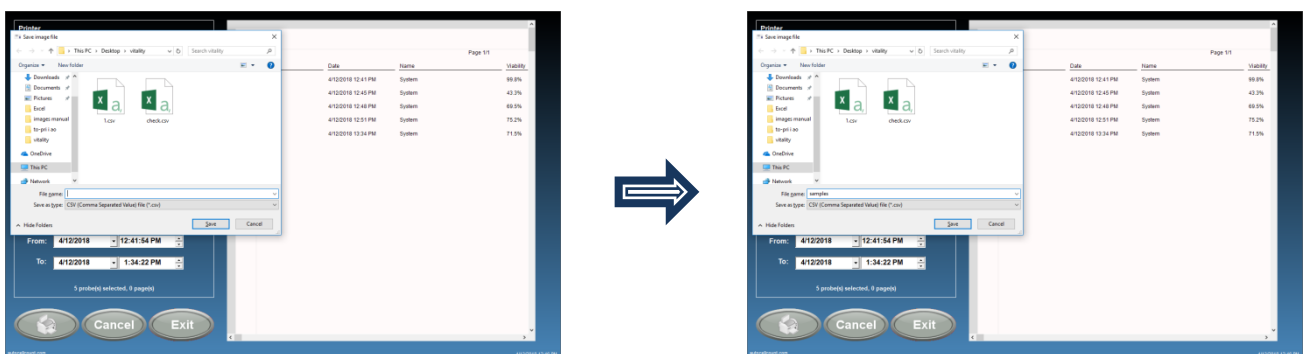
5. Add the results that you want to include in the Excel file.



6. Press button to save the file.



7. Select the folder where you want to save the file, write the name of the file and press button SAVE to finally save the results in Excel file.



Focusing of EASYCOUNTER YC

Focusing of the device must be done every 3 months.

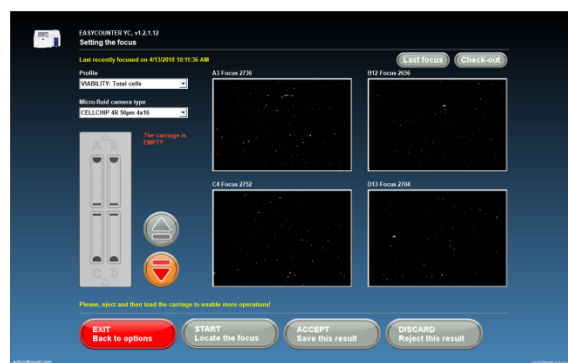
In order to focus the device:

1. Prepare a sample with 3×10^6 - 5×10^6 cells per milliliter (total number)

It may also be used a sample from a previous analysis with a result between 3×10^6 and 5×10^6 somatic cells per milliliter.

2. Focusing of EASYCOUNTER YC

2.1. From the screen "Options" press the button **FOCUSING Setting the focus, in order to proceed to the screen "Focusing". In the four squares the last found focuses are seen.**



2.2. Choose the type of the microfluidic camera which will be loaded with the sample.



2.3. Press the button , so that the cartridge can be shown.



2.4. Place the loaded with the sample CELLCHIP in the cartridge.

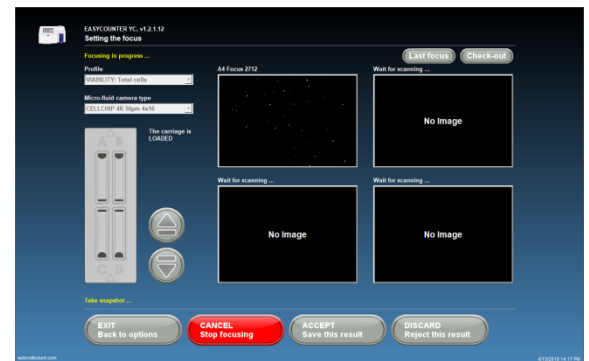


2.6. Press the button .

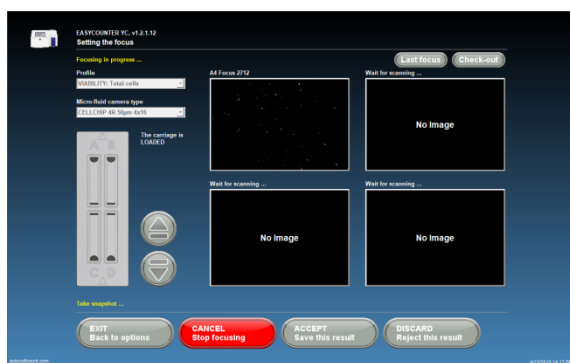


The device takes the cartridge in and focusing can be started.

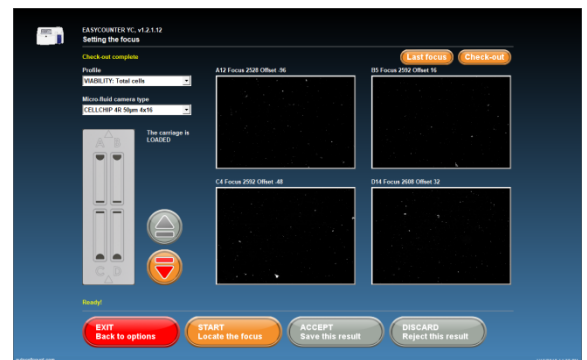
2.7. Press the button , to start focusing.



The device starts searching the focus of the four main points.



2.8. Press button , in order to save the new focuses or button , to reject them.

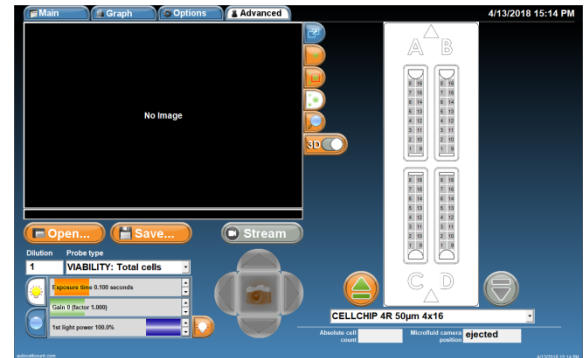
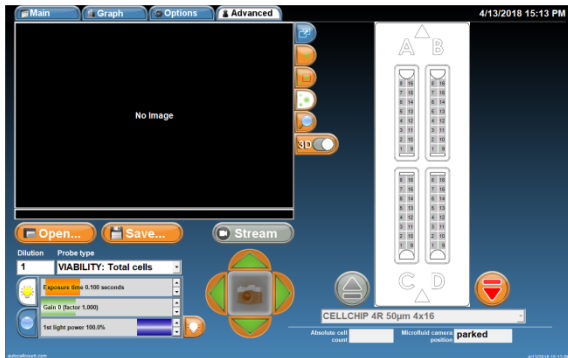


2.9. Press the button , to perform quick focusing procedure

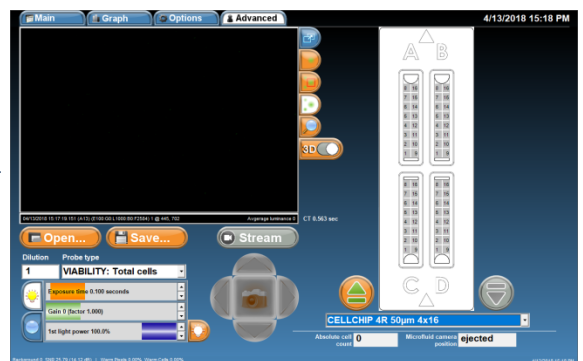
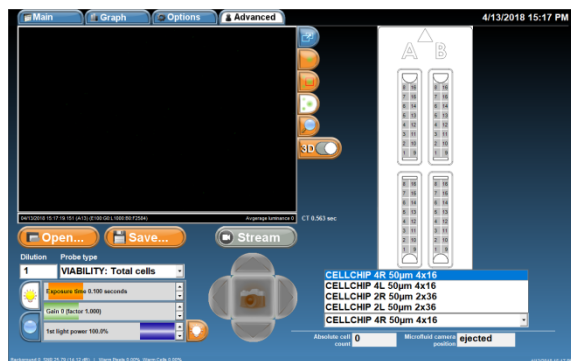


Work in Advanced mode:

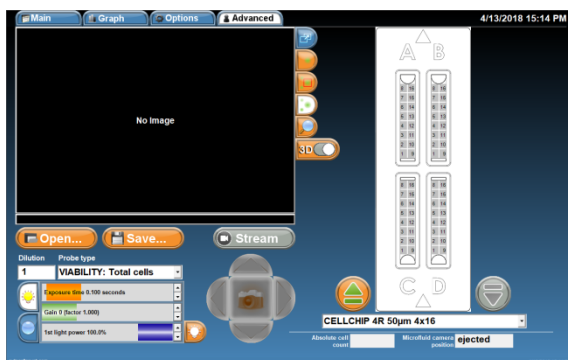
1. Press , to eject the cartridge.



2. Place the CELLCHIP with the sample in the cartridge and choose its type.

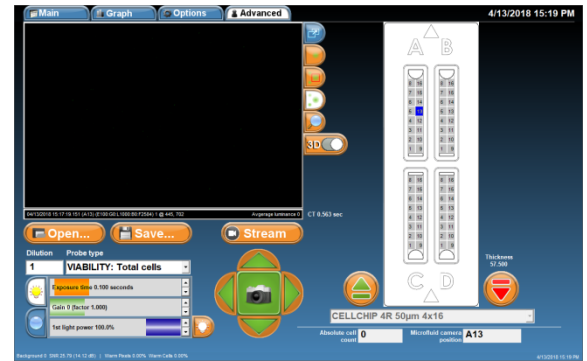
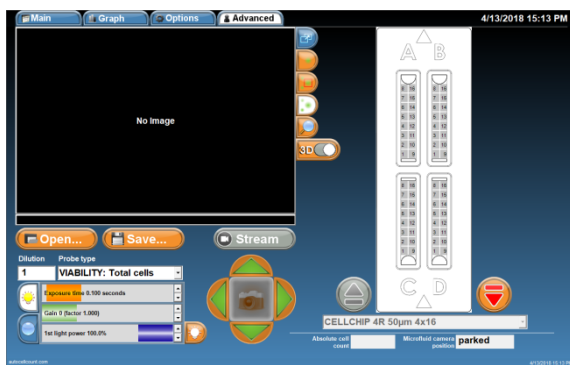


3. Press , to close the cartridge with the CELLCHIP in it.

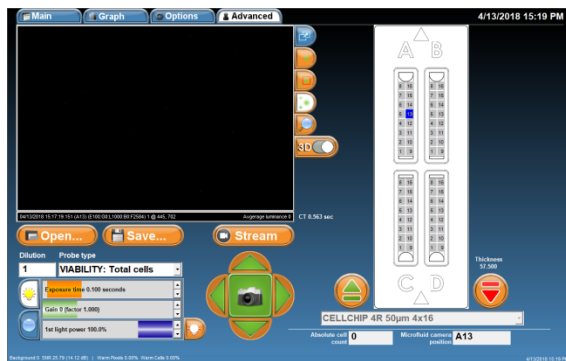


4. Choose a sector in the microfluidic camera, which is needed to be displayed.

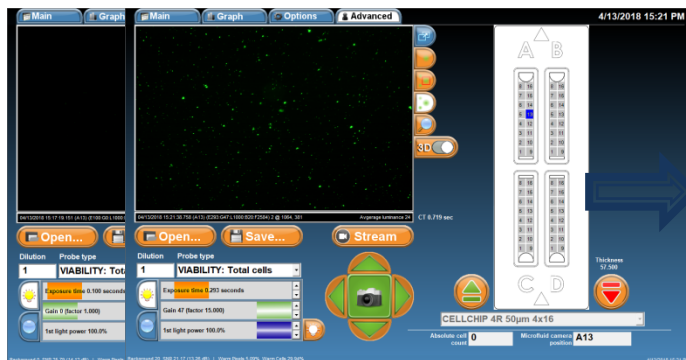
In the sample it is microfluidic camera A, section 13



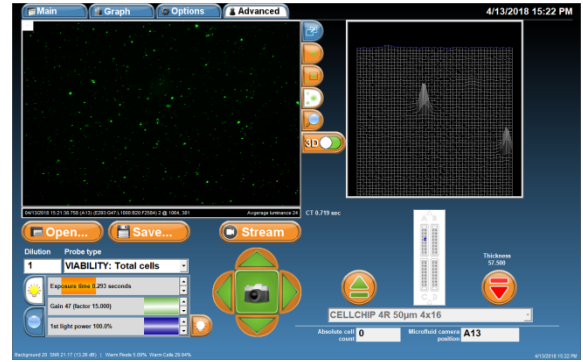
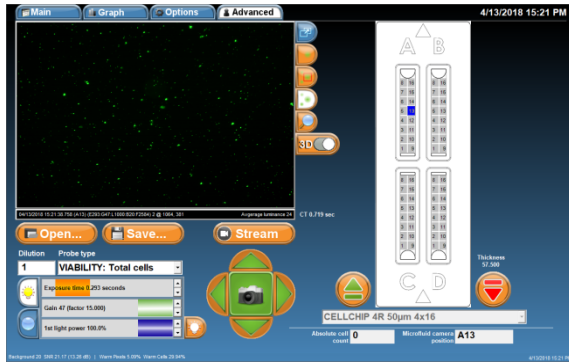
5. Select the type of the measured parameter:



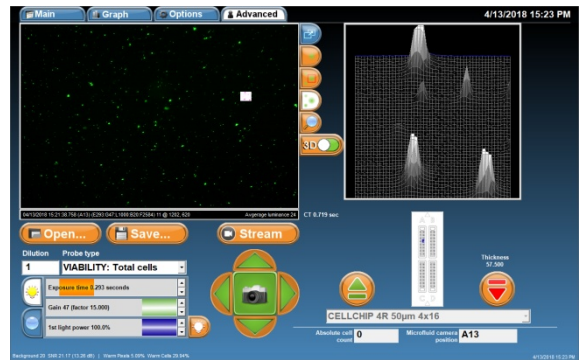
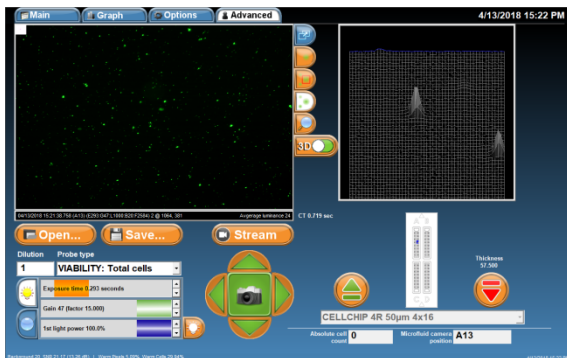
6. To make a picture and see the image of the chosen section, press



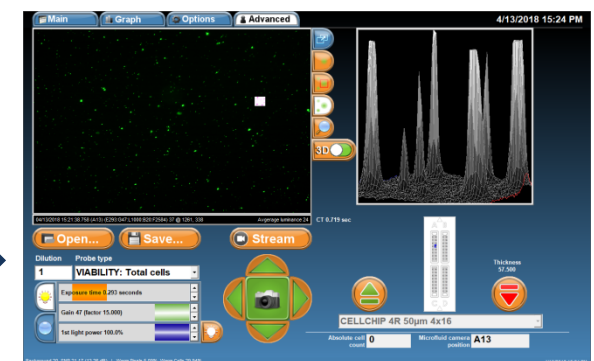
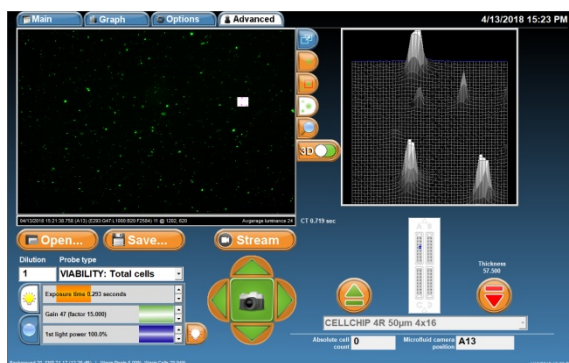
7. To see 3D graphics of the image, press . The 3D graphics shows the cells in form of columns, as the height of the column depends on its brightness.



8. In order to see a 3D graphics of group of cells from the image, move the grey square from the upper left corner on the needed group of cells. The mouse could also be used, position it on the grey square, press and keep pressed the right button and move the mouse over the image.

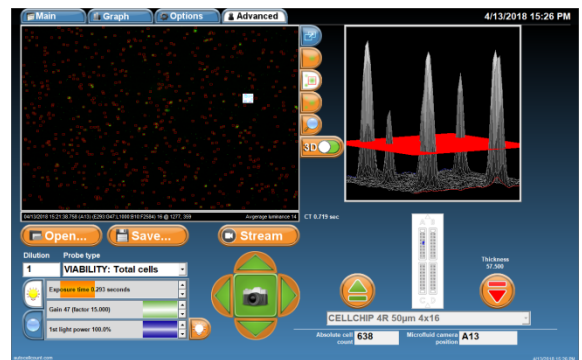
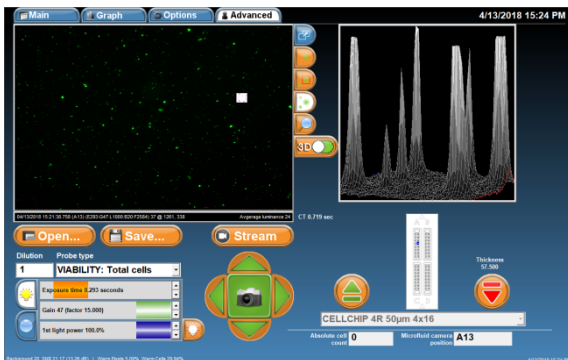


9. All the sides of the 3D graphics could be seen by pressing on it and moving up/down/left/right until the required field is seen. The mouse can be used by positioning on the 3D graphics; pressing and keeping the right button pressed and moving the mouse.

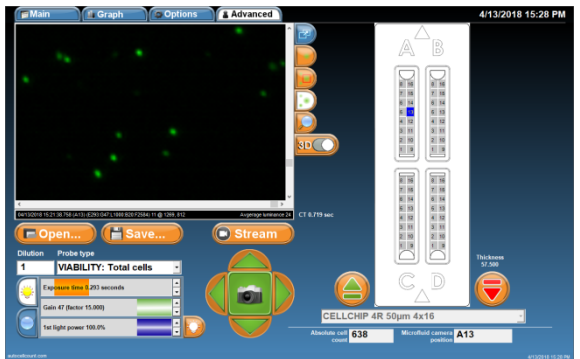
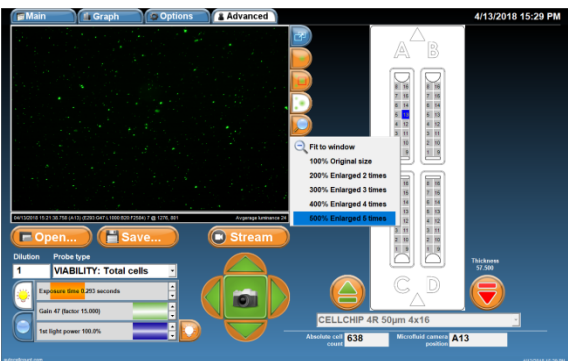
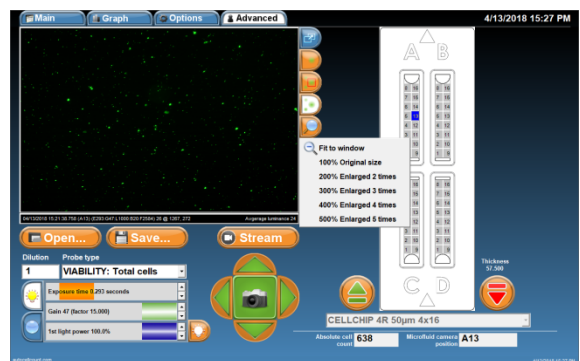
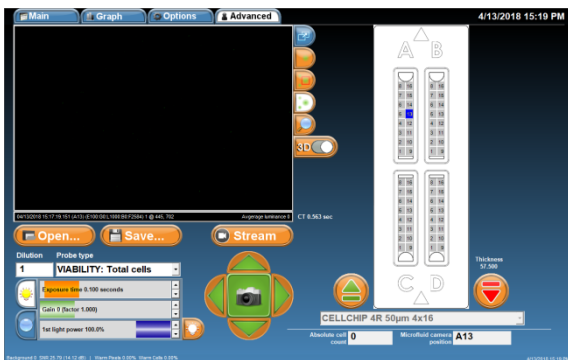


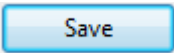
10. To see all marked cells of the image, press the button 

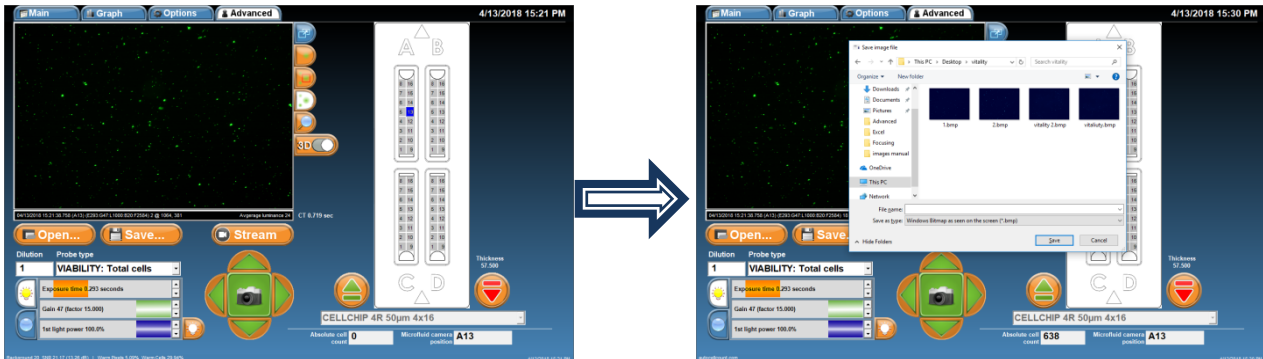
All counted cells



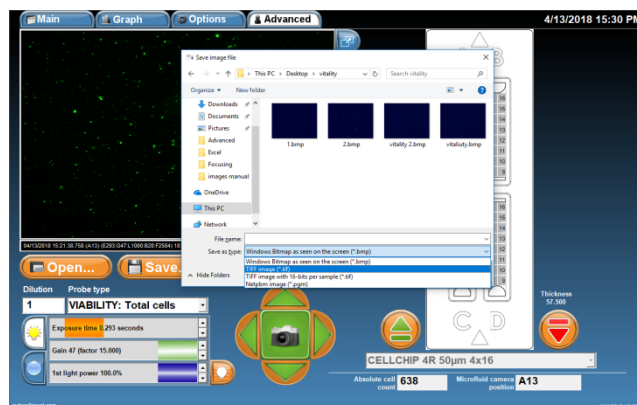
11. For magnification, press  and choose % of magnification.

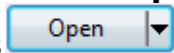


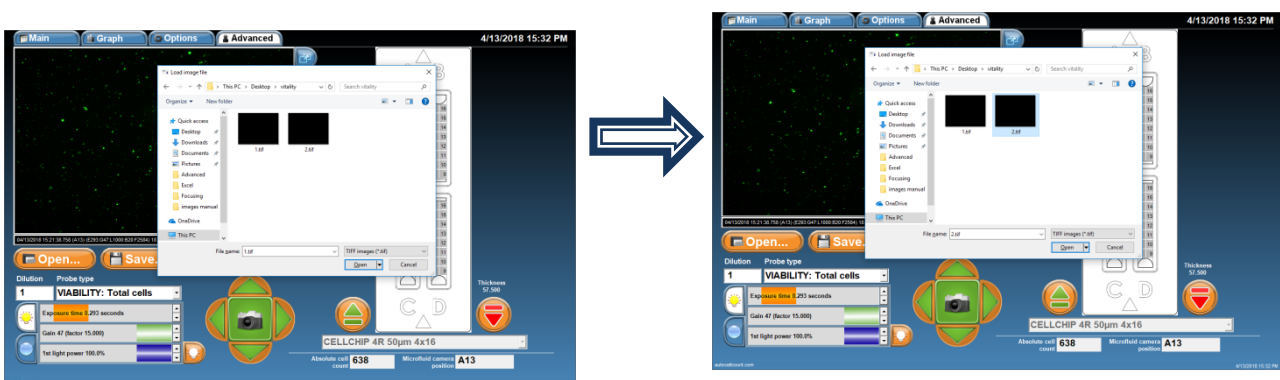
12. To save the image of the section, press  . Write name of the file, choose where to save it and press .



The User can select the format of the saved image. Possible formats are .TIFF and .BMP. The .BMP format is easy for further process and can be opened by or imported in any Windows program.

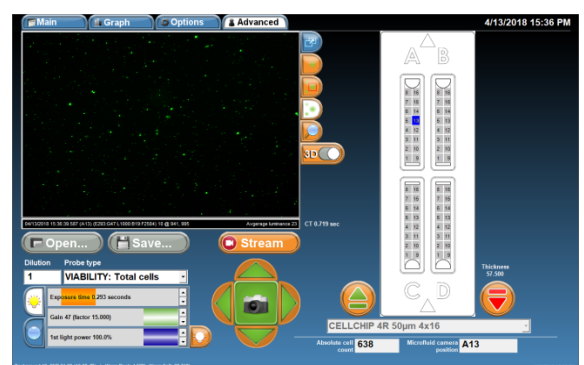


13. To open an image from previous analysis, press  . Open the directory where the image was saved, click on it and press .



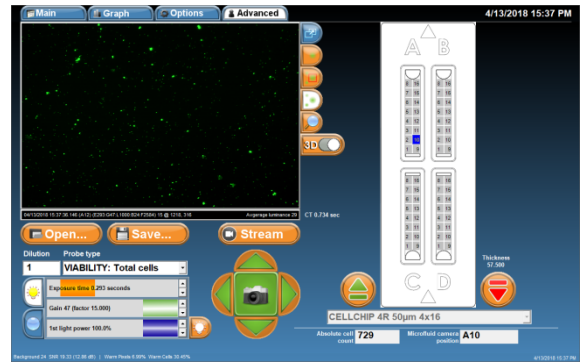
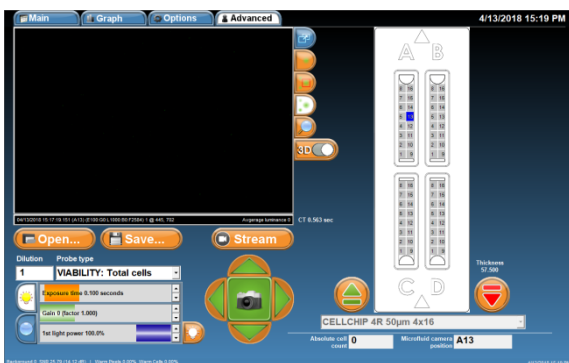
14. Button  **Stream** – after pressing the button , mode Movie is activated and EASYCOUNTER YC starts making consecutive pictures as the time for changing the image depends on the set exposition time. For example, if it is set to 1.945 sec. exposition time, images on the display will be changed in approximately 1.945sec. Mode Movie is used when a number of sections of the microfluidic camera need to be seen. Instead of pressing

the button  after each new section chosen, EASYCOUNTER YC is automatically filming the corresponding section. While the mode Movie is active, the user can not only choose a section of the microfluidic camera, but also change the intensity of the LED, exposition time, change the focus and gain sensitivity of the optical sensor (GAIN).



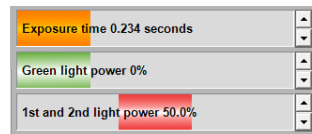
15. By pressing   , different sections of the microfluidic camera can be chosen. Another section can be chosen by pressing its corresponding number. To make a picture and see the image of the newly chosen section, press .

In the example: section 10, microfluidic camera A.

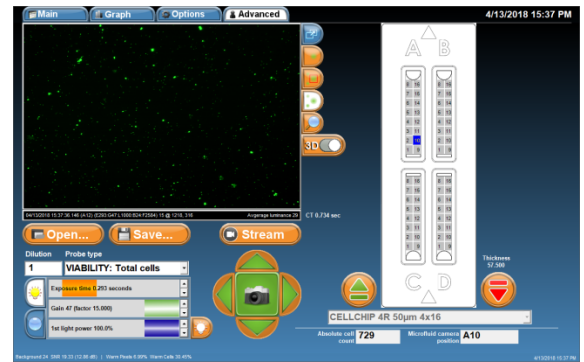
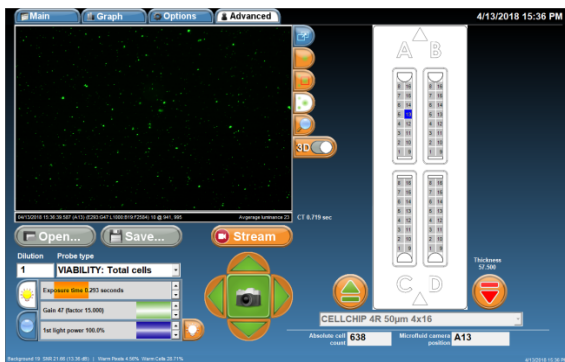


16. For setting the intensity of the LED 1 and 2 (if they are available) and exposition time, press .

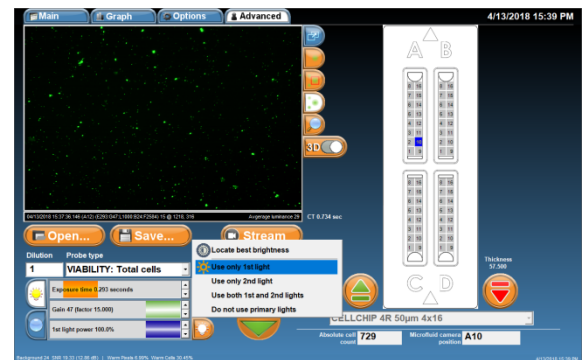
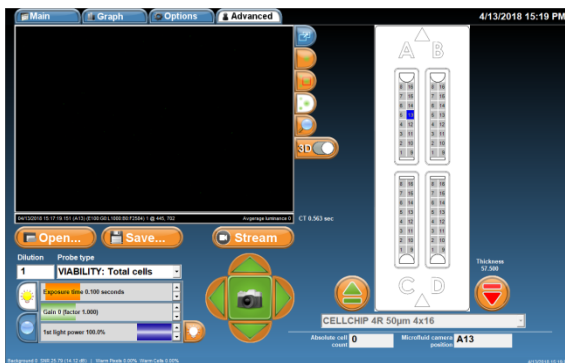
Using the buttons,



the needed values can be set

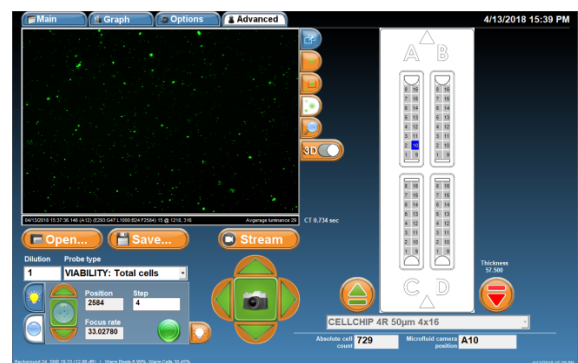
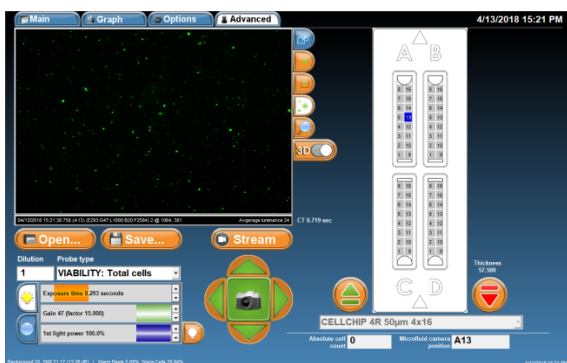


17. To switch off/on one or both LEDs, press .

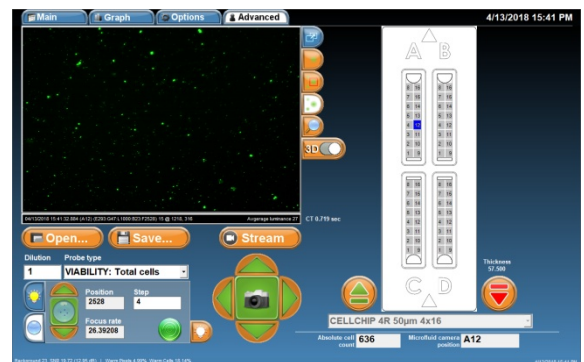
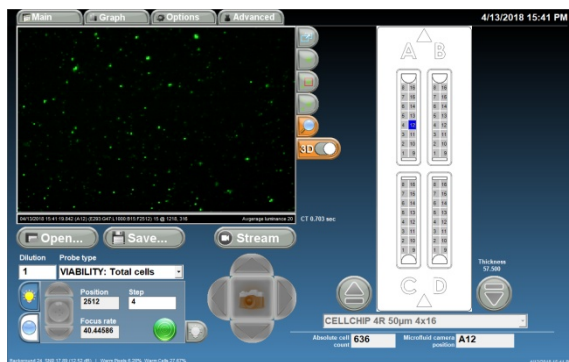


18. To see the buttons used for setting the focus of the microscope,

press .



19. For autofocus, press .

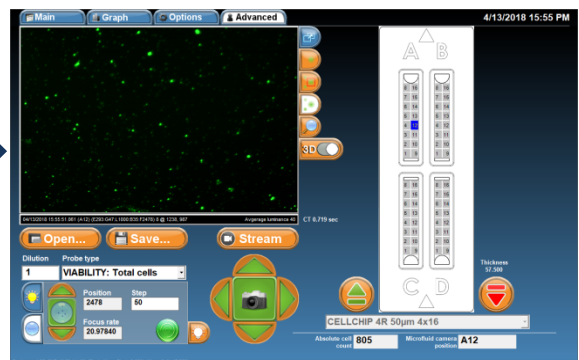
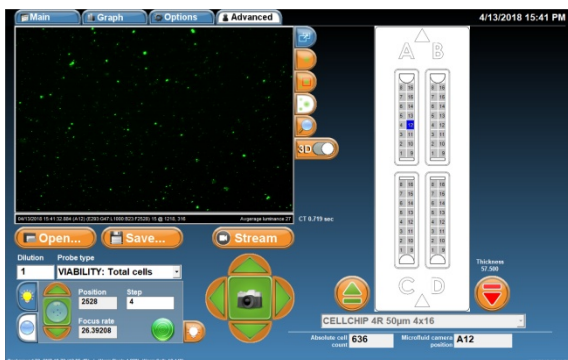


20. By pressing  and , manually set the focus position.



Attention!

In the tab "Step"  is shown the step with which the position of the focus will be changed. The value of the step can be changed by writing the corresponding number in the tab.

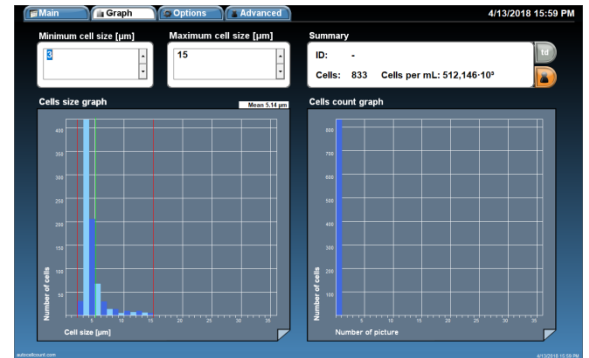
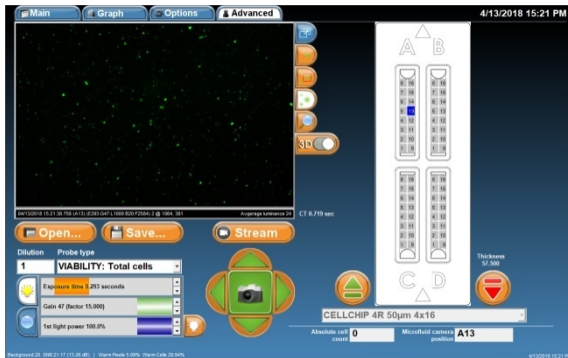


21. In order to see the graphics of the results of the current image, before passing to menu Graph, the dilution and type of the sample (Probe type)

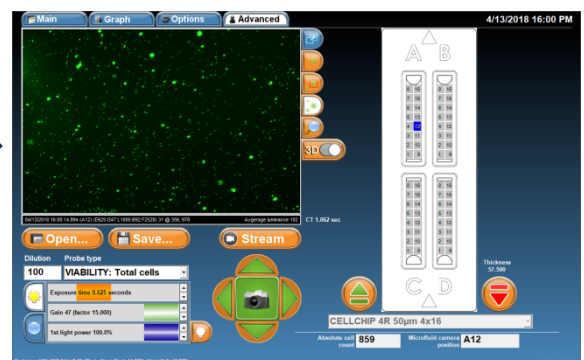
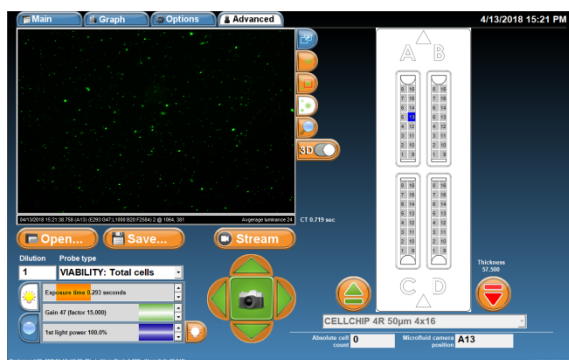
must be entered. After entering dilution and probe type, press



and after that press



22. The brightness of the image can be changed by changing the value of the Gain. The value can be in the range of 0 to 20 dB as it is recommended values bigger that 5 dB to be used. As the magnifications add noise to the image, too big magnification may lead to not clear image and inaccurate results.

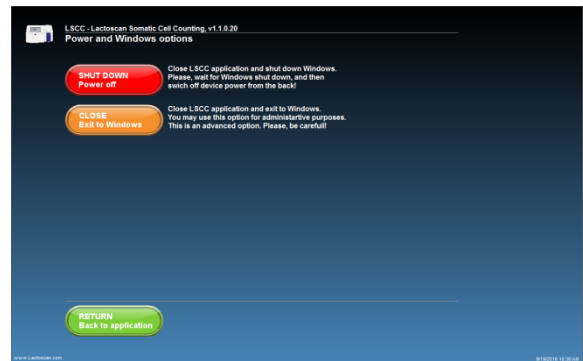
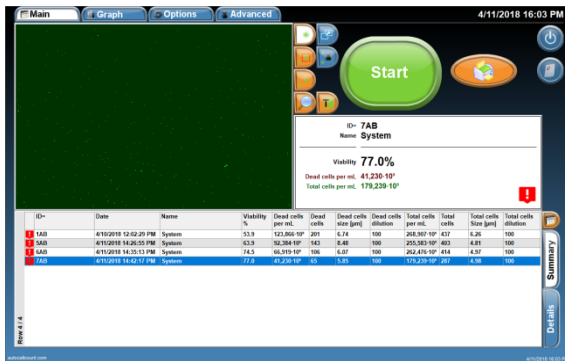


Updating the information for the CELLCHIP available in the software

Updating the types of CELLCHIP available in the software is made with the update file. If updating the information is needed, please contact our service.

Exit and switch off the EASYCOUNTER YC

1. In order to proceed to the screen Switch off, press the button



2. In order only to exit the device's program, proceed to the operational system Windows, and press **CLOSE Exit to Windows**.

3. In order to turn off the program and the operational system of the device, press **SHUT DOWN Power off**.



Attention!

Always turn off the device at the end of the working day or after finishing the measurements for the day.

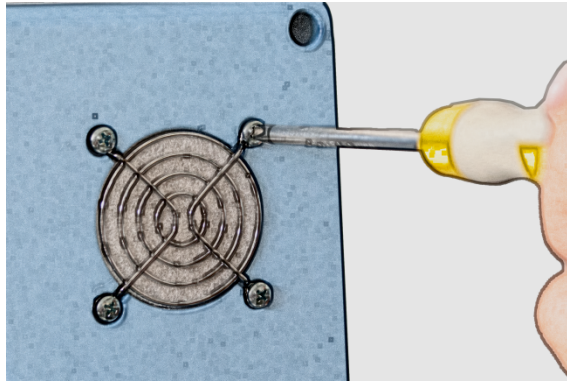
4. To go back to the program, press **RETURN Back to application**.

Replacing EASYCOUNTER YC filters

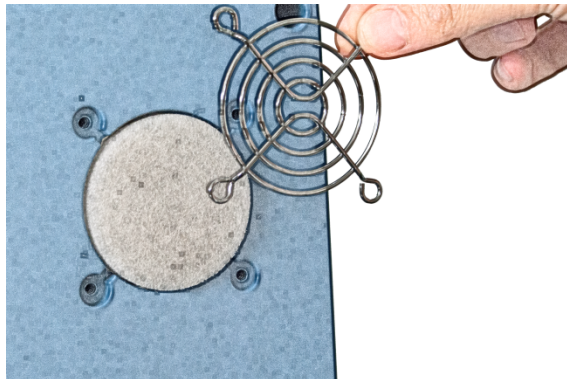
The filters, situated at the rear panel and at the bottom of the EASYCOUNTER YC , need replacement each month on regular basis or in case it is noticed that they are obviously dirty.

Replacing the filters of the rear panel:

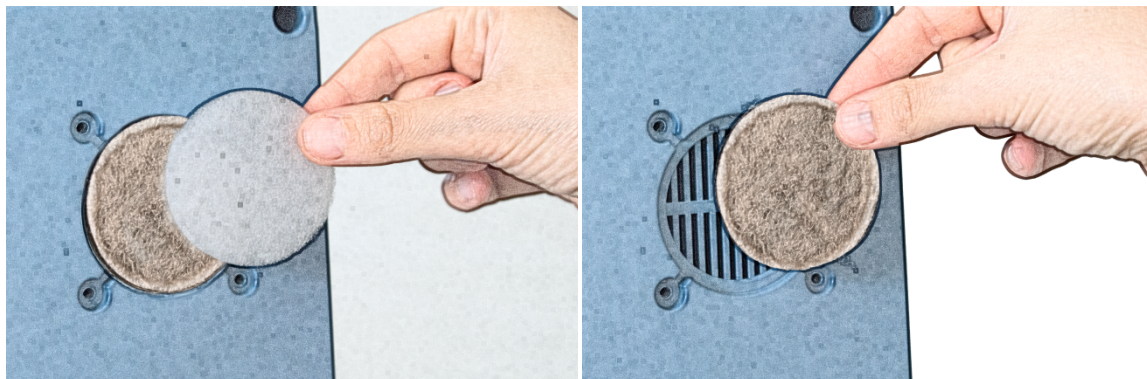
1. Unscrew the screws of the filter's grid.



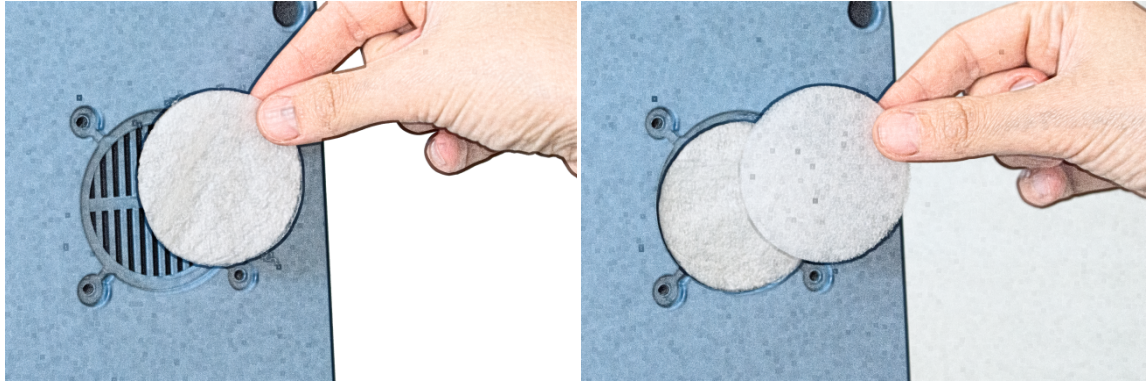
2. Remove it.



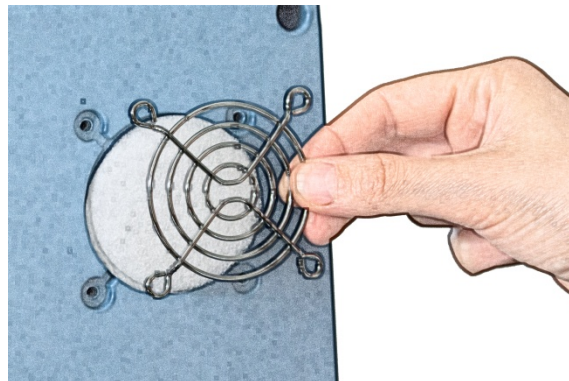
3. Remove the filters.



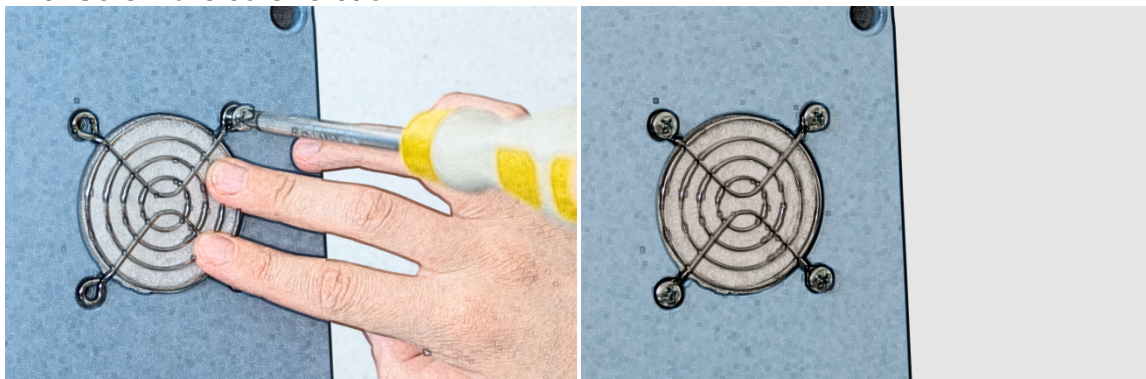
4. Place the new filters.



5. Place the grid back.



6. Screw the screws back.



In order to replace the filter at the bottom of the device, follow the instructions about replacement of filters at the rear panel, described in part "Replacement of EASYCOUNTER YC filters".

Maintenance and cleaning

EASYCOUNTER YC needs a periodical auto focus.

Change the filters of EASYCOUNTER YC once a month.

Clean the box of EASYCOUNTER YC with a soft cloth and alcohol or distilled water paying attention the cleaning liquid not to get into the power port, USB ports or through the door of the slide. Do not spill over or splash any kind of liquid directly on the device in order to avoid a power failure, when the device is on. Wipe the screen with a dry cloth right after cleaning.

Clean the sensor display of EASYCOUNTER YC with a soft lint-free cloth, moistened with a cleaning liquid for LCD displays without applying any force and being careful the liquid not to get into the buttons placed on the sides of the screen. Do not spill over a cleaning detergent directly on the screen and do not use any abrasive products, which may scratch the screen. Wipe the screen with a dry cloth right after cleaning.

Disinfect EASYCOUNTER YC by using a soft cloth and 70% alcohol paying attention cleaning liquid not to get into the power port, USB ports or through the door of the slider. Avoid using bleach because it may leave white leftovers on the device. Do not pour or splash any kind of liquid directly on the device in order to avoid a power failure when the device is turned on. Wipe the screen with a dry cloth right after the cleaning.



Attention!

Throw out the cloths in an appropriate waste container.

EASYCOUNTER YC – method for determination of total cell count and viability of yeast cells



Fermentation is the heart of beer production for all breweries, big or small. In essence, this process converts the fermentable carbohydrates in the wort into ethanol, CO₂, and various flavors and aromas. But fermentation is a complex interaction between environmental and microbiological components. Environmental components are related to the raw materials and processing conditions used during wort production, whereas microbiological components are related to the characteristics of the yeast used during fermentation and maturation. Starvation, the presence of ethanol, temperature or aging cause damage to the cell membrane, which, in turn may result in reduced yeast growth, loss of viability and a decrease in metabolic activity.

Assessment of the yeast cell state is very important in many areas of industrial microbiology.

Therefore, viability is most frequently determined by direct observation using microscopy and methylene blue.

Methylene blue staining is a bright-field staining procedure in which dead or nonviable cells are stained blue, whereas viable cells are able to exclude (or reduce) the dye and remain colorless. The sample is then analysed by light microscopy.

Although relatively inexpensive and easy to perform, the assay produces inconsistent results, with many observations of both overestimation and underestimation of viability. Moreover, the assay is subjective and the relatively small amount of the counted cells is not representative for the whole yeast population. Currently, the arising of automated cell counting instruments has provided the possibility of analysing a huge number of samples in a shorter time in addition to the benefits of reducing variability associated to human-error.

Total Cell Concentration Linearity and Reliability

Samples with different cell concentration were prepared by serial dilution and observed to determine EASYCOUNTER YC Image Cytometry reliability.

The serial dilutions of yeast suspension (*Saccharomyces carlsbergensis*) were stained with SOFIA GREEN following the protocol described previously and counted on the EASYCOUNTER YC to determine the linear range for reliable yeast concentration measurements. Plotting the measured concentration versus the theoretical concentration resulted in a linear relationship with an R^2 of 0.9968 (Figure 3). It was found that the optimal linear interval is from 1×10^5 to 1×10^7 cells / mL and the measurement range is from 3×10^3 to 5×10^7 cells / mL.

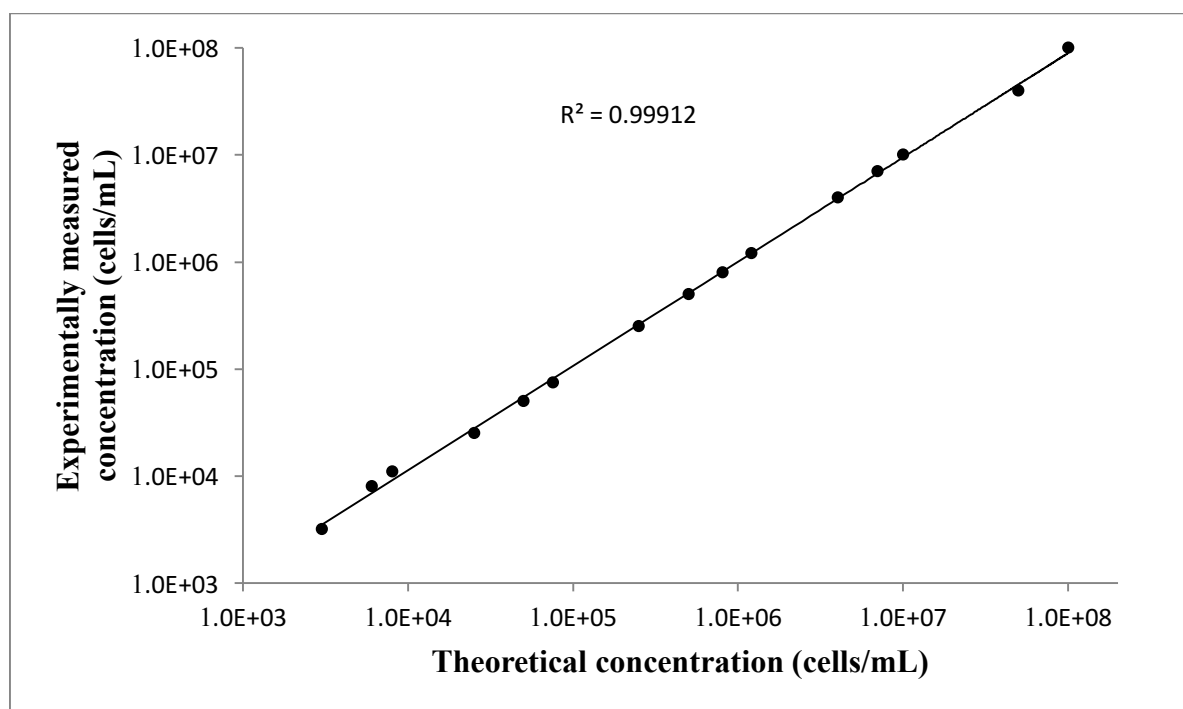


Figure 1. Cell concentration linearity measured by automated EASYCOUNTER YC image cytometry

In order to provide a statistically reliable result, it is recommended that the total number of cells in the diluted cell suspension is in the range of 1×10^5 to 1×10^7 . On Figure 4 are presented images of samples with increasing cell concentration from the optimal linear range (1×10^5 to 1×10^7 cells / mL).

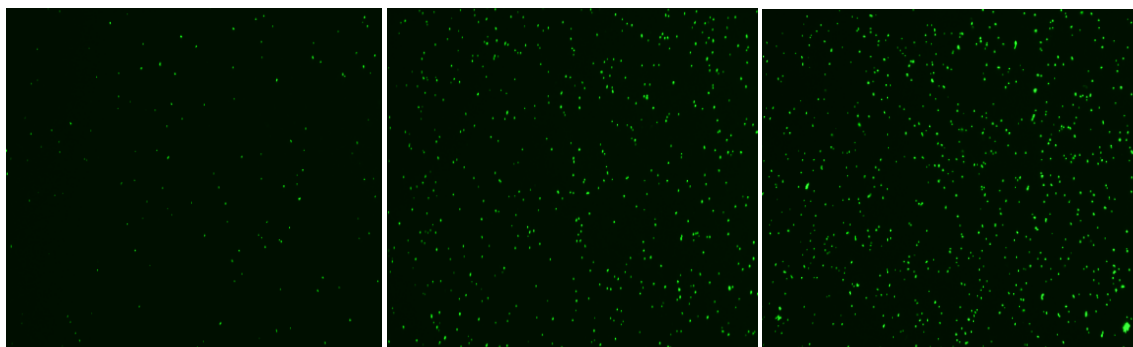


Figure 2. Images of samples with increasing cell concentration obtained with EASYCOUNTER YC

The coefficient of variation (CV,%) was calculated. Within the linear counting range of EASYCOUNTER YC is between 2.5% and 13% (Table 1).

Table 1. Coefficient of variation (CV, %) of measurements of different cells concentrations in the range from 3×10^3 to 1×10^8 by using automated EASYCOUNTER YC Image Cytometry and manual hemocytometric method

Cell suspension , cells/mL	Coefficient of variation, %		Cell suspension , cells/mL	Coefficient of variation, %	
	EASYCOUNTER YC	Manual microscopic counting		EASYCOUNTER YC	Manual microscopic counting
3×10^3	15	20	8×10^5	4	12
6×10^3	11	20	1×10^6	2.8	12
8×10^3	8	18	4×10^6	2.5	16
2×10^4	9	18	7×10^6	2.8	20
5×10^4	8	17	1×10^7	3.2	23
8×10^4	8	15	5×10^7	7	-
2×10^5	6	12	1×10^8	13	-
5×10^5	5	10			

The precision of the determination of cell concentration is dependent on the number of cells counted. It is normally assumed that the counting of random events follows the Poisson distribution, according to which the expected standard deviation is equivalent to the square root of the number of counted cells. The relative precision, expressed as Coefficient of Variation (CV) is therefore:

$$CV\% = \frac{SD * 100\%}{M}$$

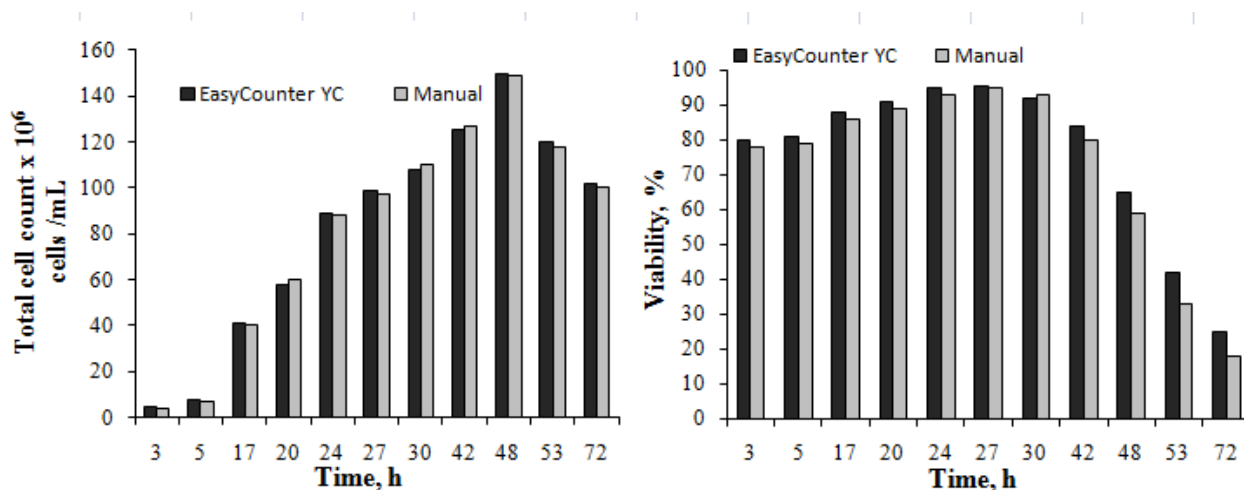
Where,

M – average count – (counted cells)

SD – standart deviation

Determination of total cell number and viability of yeast cells during cultivation

It was interesting to control the number of yeast cells and viability during cultivation process. It is very important to detect the time when reduction of cell viability begin. Beside that it is interesting if the morphology of cells play an important role on the cell images. For this reason the cultivation of IBI. Strain 53. (*Sacch. uvarum carlsbergensis*) in brewing wort was carried. The total number of yeast cells and viability results as a function of cultivation process time is shown measured by the cytometry and the manual microscopic method.



Total cell number and viability results as a function of cultivation process time. Results were obtained by both EASYCOUNTER YC image cytometry and manual hemocytometer.

Twelve samples were taken for 72 h. The highest total cell concentration was reached at the 48th h. The total concentration of yeast cells appeared to decrease

after the 48th h of the cultivation time course. In other side the viability increases to the 24th h, at 27th h is the same, and after the 27th h decreases, which may indicate cell death due to unfavorable growth conditions. It was observed that both methods showed a dramatic decrease in viability after 30 h of fermentation, which marked the end of the normal cultivation cycle in the process. The obtained results are important, because their measurement can ensure an effective control of cultivation process. From the images taken was made a conclusion that the cell morphology did not effect on the quality of images. The advantage of the novel cytometry method is the reduction of time spent on manual cell counting and that the method counts thousands of cells, which can improve the accuracy of the results.

Table 2. Coefficients of variation (CV, %) of cell measurement by EASYCOUNTER YC image cytometry and manual method during cultivation process

Time, h	CV, %			
	Total cell count		Cell viability	
	EASYCOUNTER YC	Manual microscopic counting	EASYCOUNTER YC	Manual microscopic counting
3	4.7	11.3	4.93	10.1
5	4.8	11.2	4.91	10.7
17	3.77	10.5	3.97	9.4
20	3.8	9.7	3.05	9
24	2.93	7.5	2.15	8
27	2.97	6.3	2.10	7.8
30	2.32	8.3	2.15	7.2
42	2.18	8.1	2.25	7

48	2.25	8.9	2.98	8.6
53	2.4	9.9	2.94	12.4
72	2.5	11.1	2.91	12

Table 2 summarizes the coefficient of variation (CV, %). In the microscopic method it ranged from 7-11%, as this percentage is higher for the small and large number of cells – 9-11%. In our case CV% ranged from 1.7 to 4.9, such as for 50 000 to 150000 cells the values were higher and the lowest was when the cells were more than 1×10^6 . The consistency between the two methods confirmed that the imaging cytometry method could generate accurate results.

Conclusion:

It was demonstrated a total cell count and viability detection method using the new EASYCOUNTER YC image cytometer and new DNA binding fluorescence dye SOFIA GREEN. The method was validated against the manual method using methylene blue. The proposed method can be used for assays involving simple cell counting and quality assurance for bioprocessing samples. The system presented here is an important tool for yeast concentration and viability measurements due to its greater throughput and robustness compared to manual counting with the potential to improve fermentation and yeast production performance in industrial setting.

Comparison of the potential accuracy of devices based on direct fluorescence image recognition with a low magnification microscope based on the manufacturer's reported data on the effective volume of measured sample and the results of the Poisson Normal distribution.

To ensure a more precise measurement, the counting area for counting cells is essential. The EASYCOUNTER YC camera in Camera A only has a recording area of 43 mm^2 , and this area is 43 times larger than the counting area of the standard haemocytometer (1 mm^2) and about 12 times larger than an area of other analog devices such as Countess, LUNA II -YF, NucleoCounter YC 100, Cellometer Auto T4, as they have only one counting area about 3.5 mm^2 , and EASYCOUNTER YC has 16 visual fields. In this way, the EASYCOUNTER YC provides five times more accurate, reliable results, and about 5 times lower the coefficient of variation compared to other devices.

Figure 1. Counting area. The counting area of the EASYCOUNTER YC instruments compared to a standard hemocytometer (43 times larger area) and Countess count area (12 times larger area)

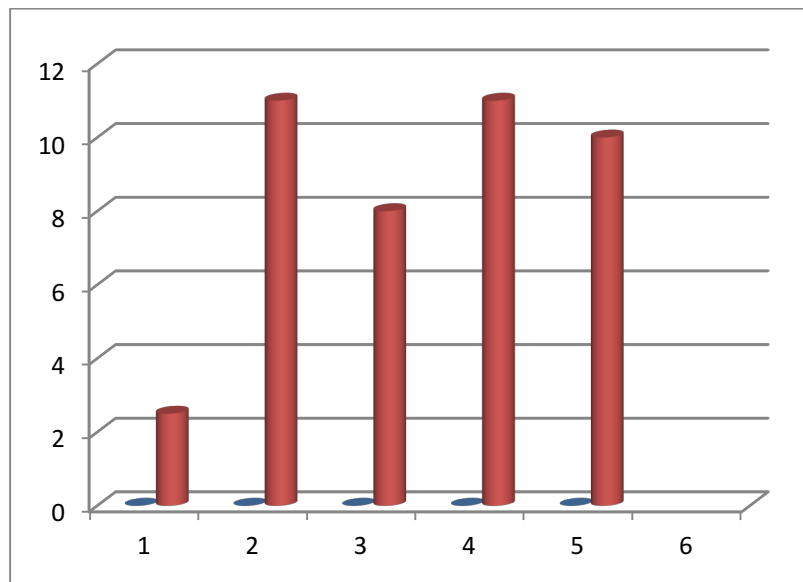
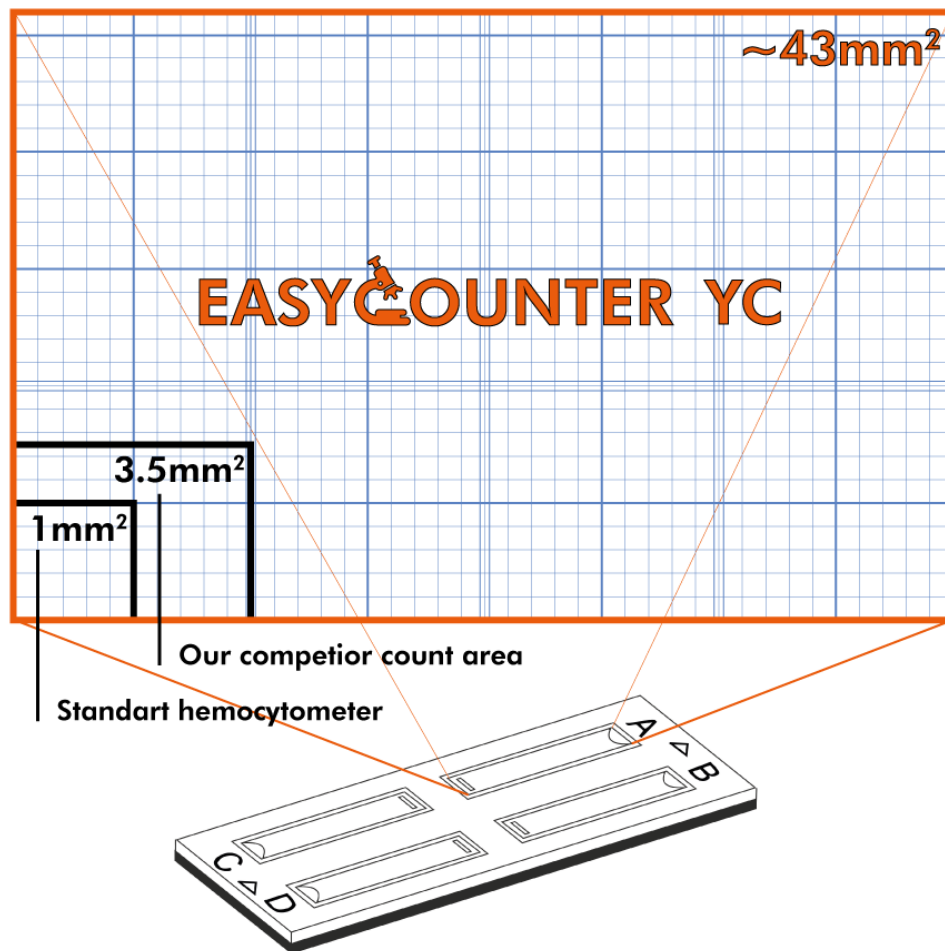


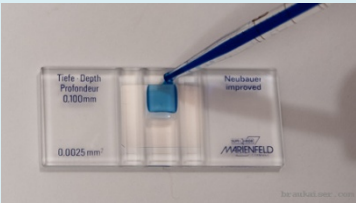
Figure 2. Comparison of coefficient variations of different cell counters: 1. EasyCounter - CV=2,5 %; 2. Countess – CV= 11 %; 3. LUNA II-YF –CV= 8 %; 4. NucleoCounter YC 100 – CV=11 %; 5. Cellometer Auto T4 10 – CV=10 %

We would like to note that EASYCOUNTER YC magnifies the images 4 times, such magnification is the most appropriate and this allows for particle size measurement and, therefore, for more accurate differentiation, which has a decisive influence on the accuracy of the measurement. For comparison, the NucleoCounter YC 100 has only 0.7 microscope magnification, which prevents visual differentiation of the cells.

It is also necessary to add the large volume of measurement - 8 μ L, which allows for measurements with CV values significantly better than the standard requirements. The guaranteed high measurement accuracy of EASYCOUNTER YC in combination with the competitor's price of the machine and the lowest purchase price of the consumable (the cost of consumables to date has limited the use of such devices) makes it extremely attractive to the present.

A comparison of some basic features of EASYCOUNTER YC with fluorescence flow cytometry based on the manufacturer's reported data is given in the following table:

A comparison of some of the key features of the devices based on direct fluorescence image recognition with a low magnification microscope based on the manufacturer's declared data is given in the following table.

					
	EASYCOUNTER YC	DMYC	LUNA II-YF	NucleoCounter YC 100	Cellometer Auto T4
Manufacturer	Milkotronic		Logosbiosystems	Chemometec	Nexcelom
Staining Method	Fluorescent dye	Methylene blue/non stained	Fluorescent dye	Fluorescent dye	Fluorescent dye
Reagent	SOFIA GREEN	MB	AO/PI	PI	AO/PI
Microscope	Fluorescent (4 x)	Light (1000 x)	Fluorescent (4 x)	Fluorescent (0,7x)	Fluorescent (4 x)
Sample volume	1000 μ l	1 mL	10 μ l	60 μ l	20 μ l
Count Area	43 mm ²	1mm ²	3,5 mm ²	3,5 mm ²	3,5 mm ²
Measuring volume	8 uL	0,149 μl	10 μl	1 μl	20 μl
Captured image	max 64	~ 50	90	1	1
Machine analysis time	max 90 samples/hour	-	Max 60 samples/hour	Max 120 samples/hour	Max 60 samples/hour
Cell density range	$1 \times 10^5 \sim 1 \times 10^7$	-	$5 \times 10^4 - 1 \times 10^7$ cells/ml	$5 \times 10^3 - 6 \times 10^6$ cells/ml	$2.5 \times 10^5 - 5 \times 10^7$ cells/ml
Image processing	Fluorescence image recognition	human	Fluorescence image recognition	Fluorescence image recognition	Fluorescence image recognition
Calibration	no	no	yes	no	yes
Application	Count, cell size, vitality	Count, morphology, vitality	Count, cell size, vitality	Count, cell size, vitality	Count, cell size, vitality
Process time	fast	Time consuming	fast	fast	fast

Conclusion

A method for determining the number of yeast cells using EASYCOUNTER YC has been developed.

With the EASYCOUNTER YC, it is possible to determine the total number of cells and their viability.

The apparatus may be REPRODUCTIVE to be used to determine the number of yeast cells as in

The EASYCOUNTER YC apparatus is operated with very high representativity, with a total cell count and viability determined in a 16 µL sample.

The repeatability indices of EASYCOUNTER YC are more reproducible than other fluorescence microscopes of other globally represented companies (Foss Electric, Bentley Instruments, Delta Instruments, etc.)

The EASYCOUNTER YC is easy, simple, fast, accurate and reliable.

Warranty card **EASYCOUNTER YC**

The warranty is valid for a period of 1 (one) year. The incorrect working, transporting and storage make the warranty invalid.

Serial №

Date of purchase:

Password:

Distributor:

Signature:

Stamp:

Milkotroni Ltd preserves the right to choose the methods for checking the device in order to establish the validity of the warranty. Devices with expired warranty are not subject to a free warranty service.

Service card

Customer:

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Service report:

Service entry date	Damage	Date of receipt	Signature

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