LACTOSCAN COMBO
Operation Manual

Accurate Somatic cells counting & Ultrasonic milk analyzer in one device
Simple. Fast. Accurate. Reliable. Portable.

Available online at http://lactoscan.com/editor/ufo/manuals/COMBO/IM_COMBO_EN.pdf

Operation Manual of LACTOSCAN Combo 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 LACTOSCAN COMBO. However, it can be changed without any preliminary consultation or notification.

Last edited: 30.08.2018

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 11.
3. Always make sure the main switch is in position "OFF" before connecting it to the electrical network.
4. Never touch the power cable with wet hands.
5. 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.
6. In order to prevent a possible electrical shock, make sure, that the supply is well grounded.
7. 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.
8. Use only the authorized accessories (USB flash drive, LACTOCHIP, wireless keyboard and mouse).
9. 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.
10. 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

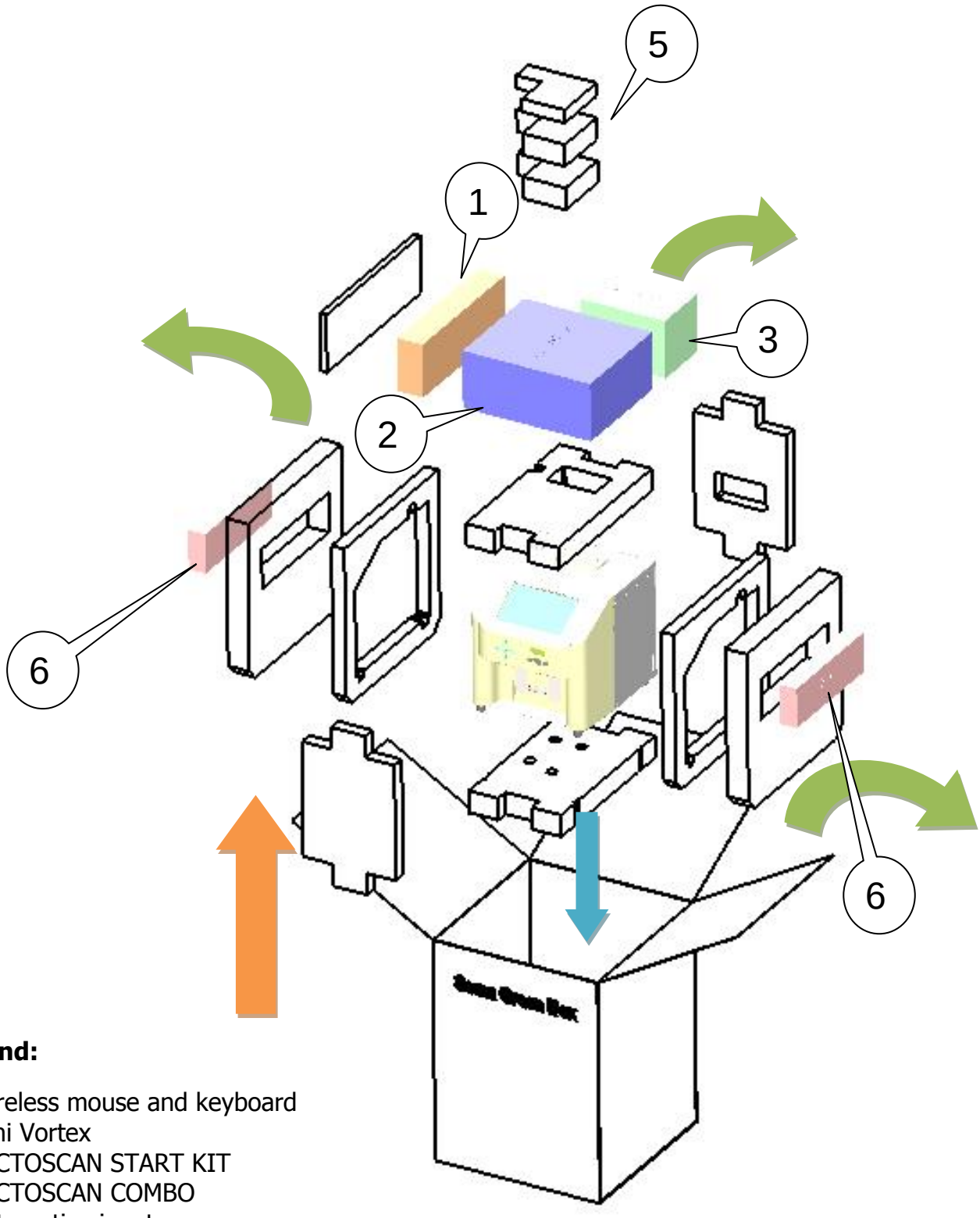
Object	Warning
Embedded tablet battery	There is a risk of explosion if the battery is being changed improperly. This battery not to be changed by the customer. Please contact the authorized service.
Box	Do not disassemble the box. There are not adjustable components inside the box. If there is an issue, 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 LACTOCHIP it is necessary to throw it in the appropriate bin.
Operator	Must have the basic knowledge on the procedure on cell counting.

Content:

Unpacking of LACTOSCAN COMBO

Unpack carefully the LACTOSCAN Combo following the sequence illustrated below:



Legend:

1. Wireless mouse and keyboard
2. Mini Vortex
3. LACTOSCAN START KIT
4. LACTOSCAN COMBO
5. Automatic pippets

Content of the kit

The Somatic Cell Counter LACTOSCAN Combo is sent with the following items:

Object	Amount
LACTOSCAN COMBO	1
LACTOSCAN COMBO STARTER KIT	1
LACTOSCAN COMBO 14V power cable	1
LACTOSCAN COMBO Operation Manual	1
Automatic pipette up to 100 µL – set at 8 µL	1
Automatic pipette up to 100 µL –set at 100 µL	1
USB wireless keyboard with batteries	1
USB wireless mouse with batteries	1
Mini Vortex	1
Plastic sample holder	2
Spare Pipes	1
Alkaline cleaning solution Lactodaily	1
Acidic cleaning solution Lactoweekly	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, 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 LACTOSCAN COMBO by visiting www.lactoscan.com/device_registration. 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 LACTOSCAN COMBO.

Purpose of the device:

LACTOSCAN COMBO is designed to be used only for analysis of milk in milk collecting centers, laboratories, etc. It is not manufactured for testing human milk or animal but with therapeutic or diagnostic usage.

Environment conditions

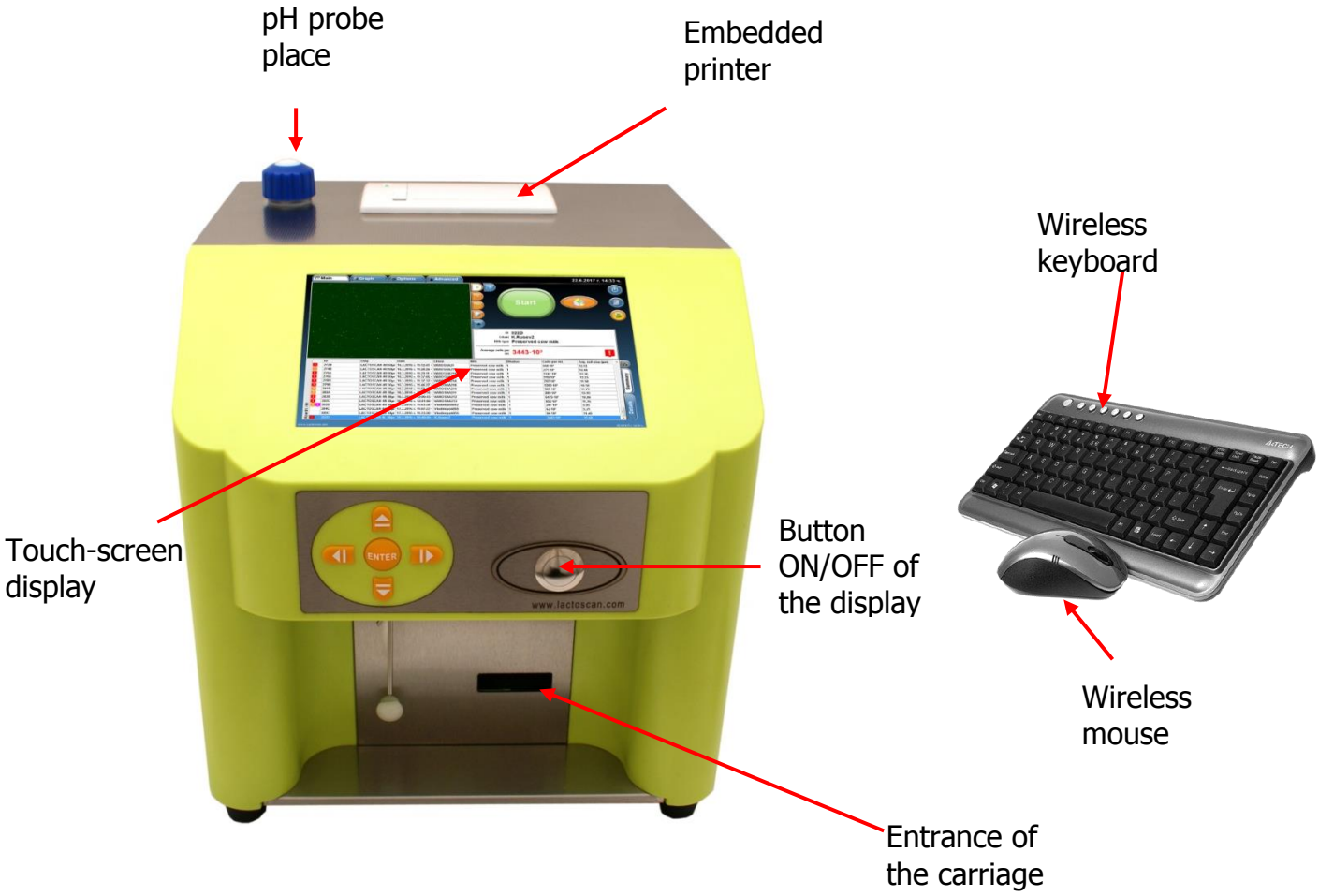
In order to work properly and stable for a long period, LACTOSCAN COMBO needs to be installed in a room meeting the following environmental conditions:

- Indoor temperature 10-35 ºC. It is not recommended to place it in a room where the temperature is below 4 ºC.
- Prevent exposing the device to direct sun light.
- Prevent exposing to a continuous vibration.
- Relative humidity 0-95%.
- In a place free of corrosive gazes 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.

LACTOSCAN COMBO

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 LACTOCHIP with the analyzed sample.

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

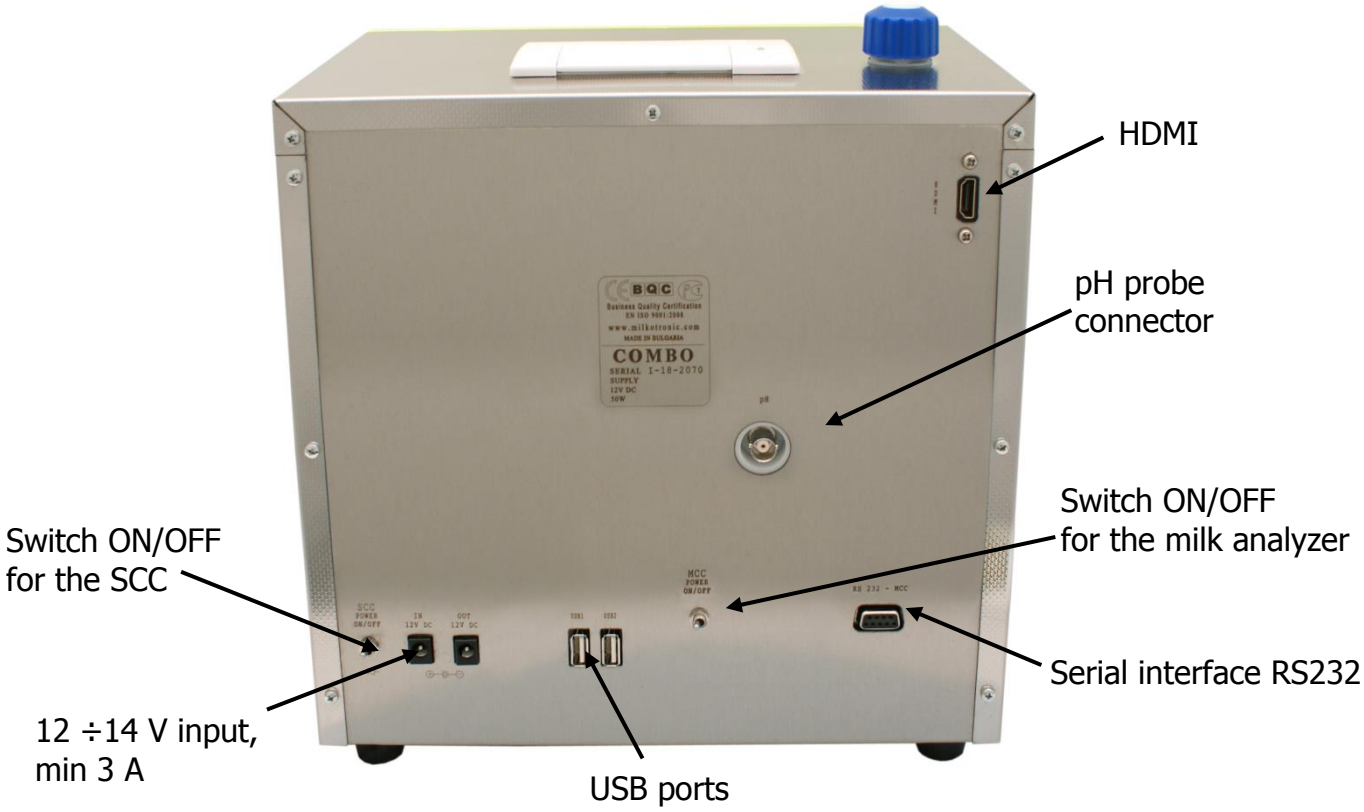
Embedded printer. It allows printing the results after analysis or the results of previous tests.

pH probe place. To place the pH probe inside.

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

Wireless keyboard and mouse. Make entering data easier.

Back panel:



Switch ON/OFF for the SCC. It is used to power on / off the SCC part of the device.

12 ÷14 V input, min 3 A. Power supply. Always connect only the powering adapter, supplied with the device.

Attention!
Do not connect the power supply of the device if the toggle switch ON/OFF is not on "Off" position.

USB ports. For connection of USB flash-drive and wireless keypad, mouse and external printer.

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

Serial interface RS232. Used only in service center. Please do not connect something to it.

Switch ON/OFF for the milk analyzer. It is used to power on / off the SCC part of the device.

pH probe connector. It is used to connect the pH probe cable towards the device.

HDMI. It is used to connect an external monitor towards the device.

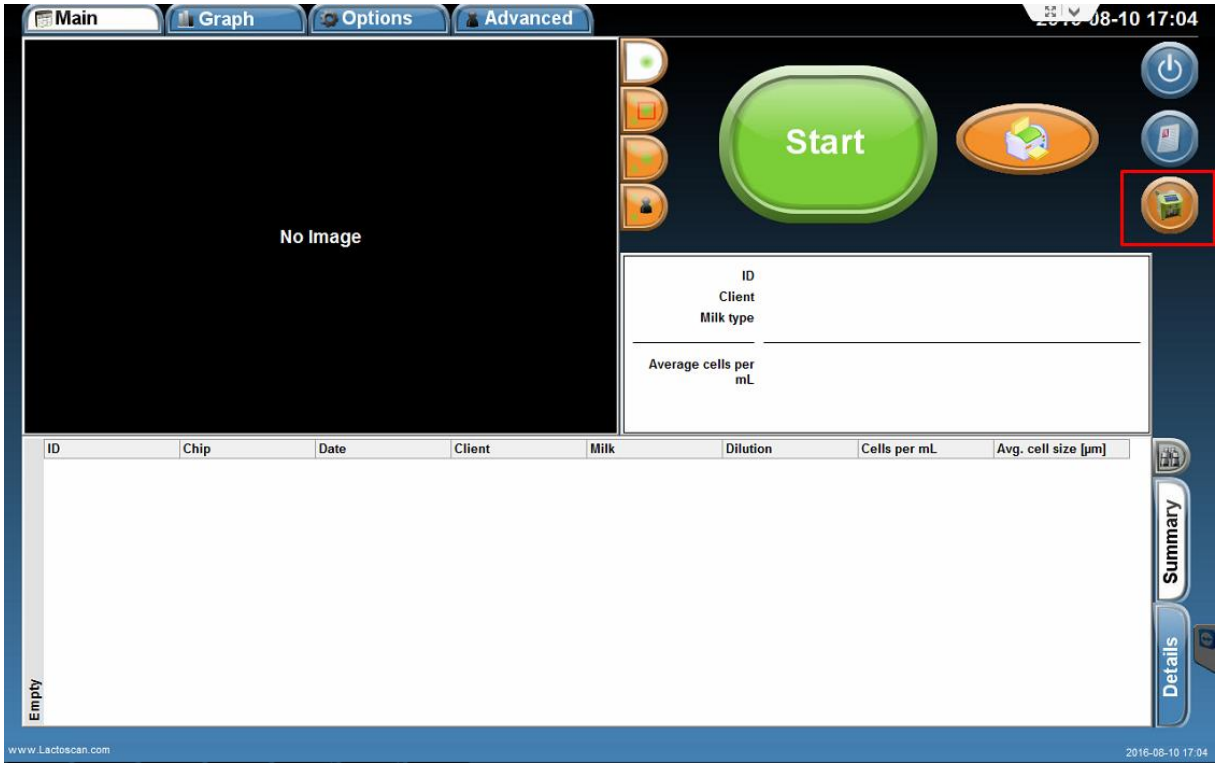
Specifications of LACTOSCAN COMBO:

LACTOSCAN COMBO		
CHARACTERISTICS	**Type:**	Somatic Cells Counter & Ultrasonic milk analyzer
	Dimensions (HxWxL):	39 cm x 30 cm x 26 cm
	Weight:	6.000 kg
	Working power:	100-230 VAC, 2.5A, 120V
	Frequency:	50/60 Hz
	Power supply:	12 VDC, 5A, 60W
	Working place:	Inside only!
	Working temperature:	15-35 °C
	Working humidity:	0-95%

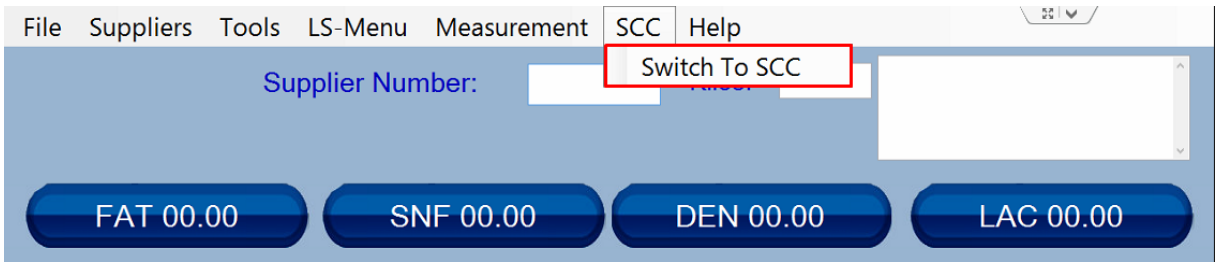
LACTOSCAN COMBO Somatic cell counter and ultrasonic milk analyzer in one device.

The Operation manual consists of 2 separate descriptions for the both devices. Here's how to switch between the Somatic cell counter and the Ultrasonic milkanalyser:

If you are in the SCC, by pressing the icon in the red rectangle with picture of the device will switch to the milk analyser:



If you are in milk analyzer's mode:

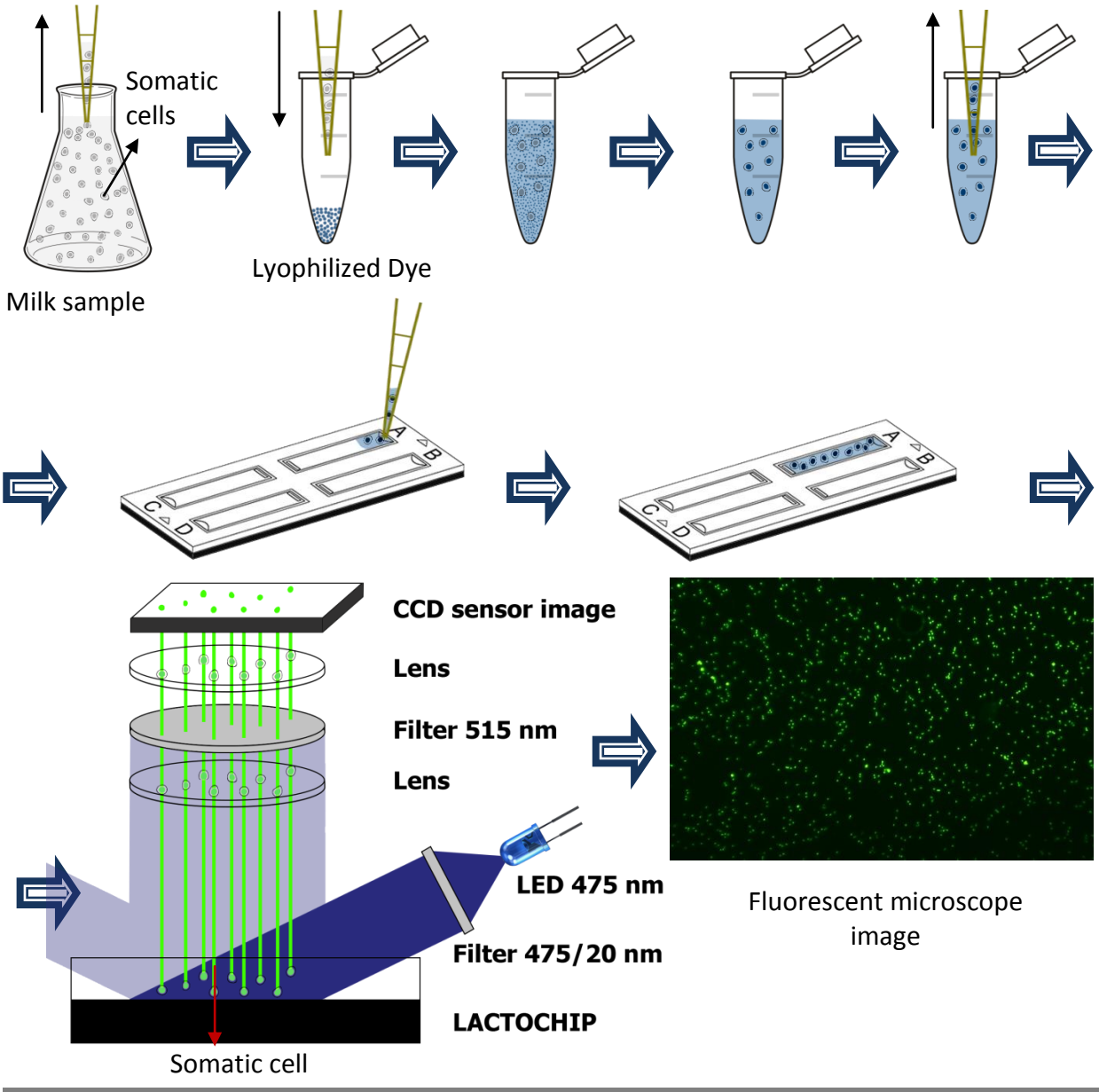


I. Somatic cell counter (SCC) of LACTOSCAN COMBO

Introduction of the Somatic cell counter

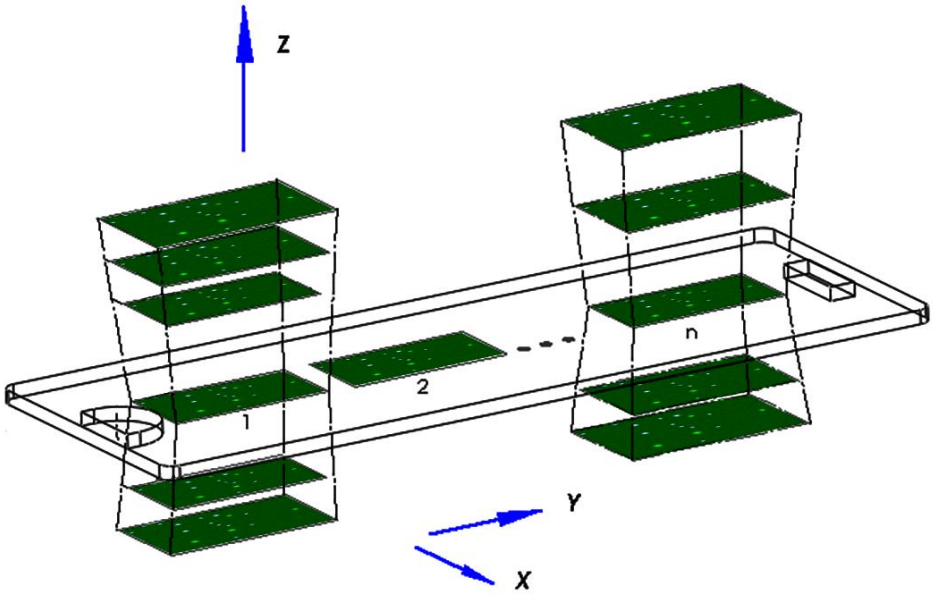
The LACTOSCAN COMBO's SCC is based on fluorescent microscope technique of counting cells. Thanks to the fluorescent dye, LED optics and CCD filming Technologies, the analysis of the milk is precise, reliable and fast.

In order the somatic cell to be counted by with SCC, the sample is mixed with SOFIA GREEN dye. It is only 8 µL that is needed to be pipette onto the single LACTOCHIP. After that, the CHIP is loaded in the device. The analysis is being conducted during a period between 10 seconds and 2 minutes and the duration depends on the number of filmed fields. The system of SCC is focusing automatically on the CHIP and the dyed cells are filmed by the sensitive CCD camera. This algorithm of analysis of digital images determines the number of the fluorescent cells and counts their concentration and size. The result is automatically displayed.



Working principle of SCC

Unique, 3D, multi-image, patent application protected, sequential scanning process, based on a precise fluorescent optics and low magnification, images analysis software, LACTOSCAN SCC is fast, precise and reliable counter of somatic 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 IDF/ISO 13366, of all the filmed images is displayed. The whole process, after placing the LACTOCHIP in the cartridge, is automatic.

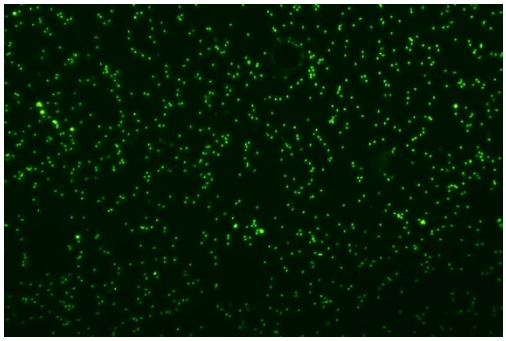


SCC compared with a standard methods for Somatic Cells Counting

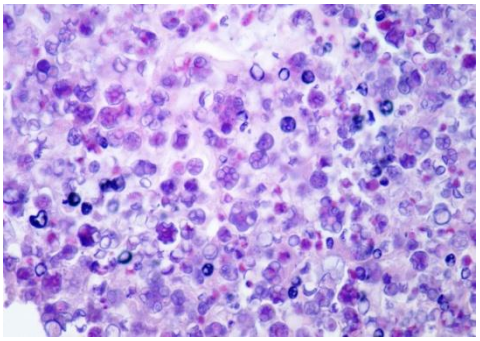
The below described data are based on validation and check of SCC done by the "Biotechnologies" body of University "Prof. AsenZlatarov", Bulgaria

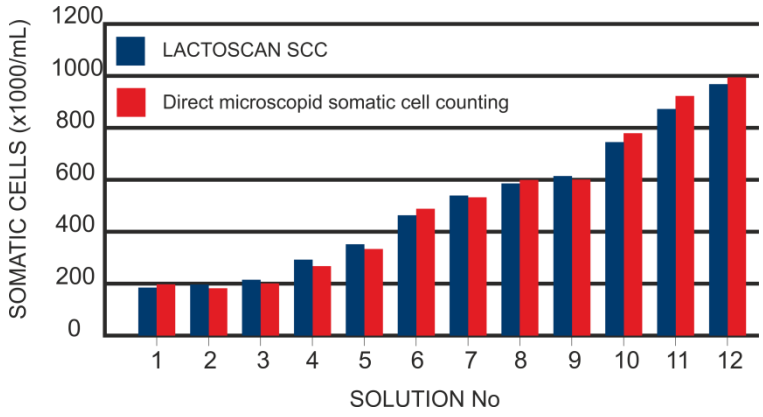
1. Accuracy of analysis – comparison between SCC and Direct microscopic counting of somatic cells (DMCSC)

Images of somatic cells (SCC)



Images of somatic cells (DMCSC)

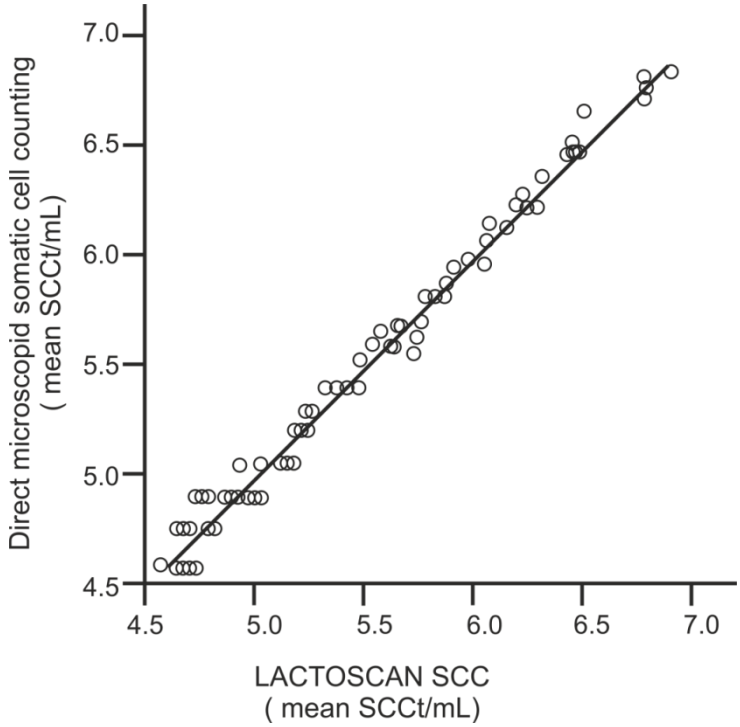




SCC		Standard method	
NSC (cells/ml)	***CV %**	**NSC (cells/ml)**	***CV %**
100 000	5%	100 000	7%
500 000	3%	400 000	5%
1 000 000	2%	600 000	4%

*Coefficient of variation

2. Compatibility – ratio between data from SCC and DMCSC



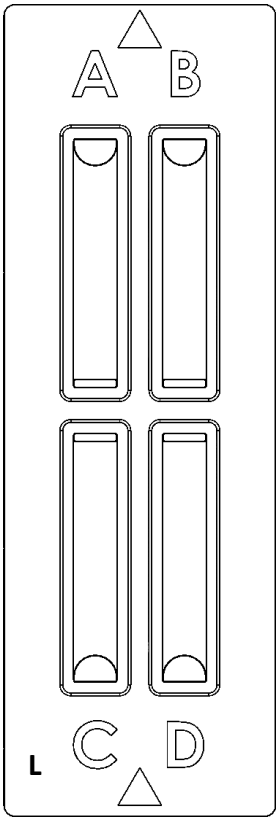
Description of LACTOSCAN SCC KIT

Types of LACTOSCAN SCC KIT:

- LACTOSCAN SCC STARTER KIT 100 tests – consists of 25 disposable LACTOCHIP x4, 100 micro test tubes with SOFIA GREEN lyophilized dye and 200 tips for automatic pipette. The expiration date of the set is one year after its manufacturing date.
- LACTOSCAN SCC KIT x4 400tests–consists of 100 disposable LACTOCHIP x4, 400 pcs micro test tubes with SOFIA GREEN lyophilized dye and 800 tips of automatic pipette. The expiration date of the set is one year after its manufacturing date.

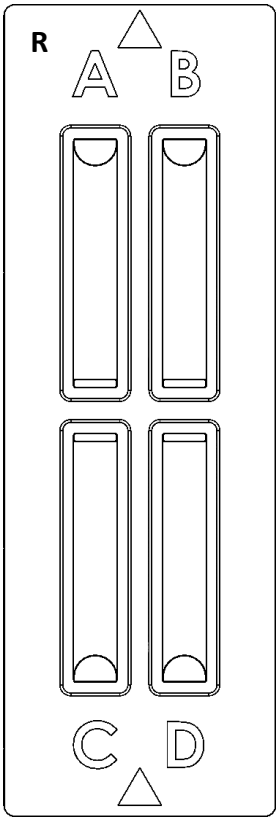
Description of LACTOCHIP

LACTOCHIP is a disposable chip, specially designed to be used with LACTOSCAN SCC. It is made of ABS and PMMA.



LACTOCHIP 4L

LACTOCHIP x4 has four separate closed chambers (A, B, C и D), that allow analysis of four different samples. Each chamber's capacity is 8 µL. LACTOSCAN SCC makes 16 pictures of the sample in each chamber and then it performs the analysis via a specific algorithm.



LACTOCHIP 4R

Attention!

Before starting work with LACTOSCAN SCC KIT always check LACTOCHIP letter. It can be L or R. Letter L indicated LACTOCHIP 4L and letter R indicated LACTOCHIP 4R. The LACTOCHIP letters match with the already put in the program LACTOCHIPs data. One LACTOSCAN SCC KIT included only LACTOCHIPs with letter L or only with letter R.

Specifications:

LACTOSCAN COMBO'S SCC		
CHARACTERISTICS	**Type:**	Somatic Cells Counter
	Dimensions (HxWxL):	25.5 cm x 38 cm x 30 cm
	Weight:	7.300 kg
	Working power:	100-230 VAC, 2.5A, 120V
	Frequency:	50/60 Hz
TECHNICAL SPECIFICATION	**Power supply:**	14 V, 5A, 60W
	Working place:	Inside only!
	Working temperature:	15-35 °C
	Working humidity:	0-95%
	Time of analysis:	From 10 sec to 2 min in dependence of the number of pictures taken

LACTOSCAN SCC STARTER KIT 100 tests		
PHYSICAL CHARACTERISTICS	**Dimensions (HxWxL):**	355 mm x 255 mm x 135 mm
	Weight:	1.500 kg
	Number of LACTOCHIP x4:	25 pcs
	Number of SOFIA GREEN lyophilized dye:	100 pcs

	Number of tips for the automatic pipette:	200 pcs

LACTOSCAN SCC KIT x4 400 tests

Physical characteristics	**Dimensions (HxWxL):**	355 mm x 255 mm x 135 mm
	Weight:	1.500 kg
	Number of LACTOCHIP x4:	100 pcs
	Number of SOFIA GREEN lyophilized dye:	400 pcs
	Number of tips for the automatic pipette:	800 pcs

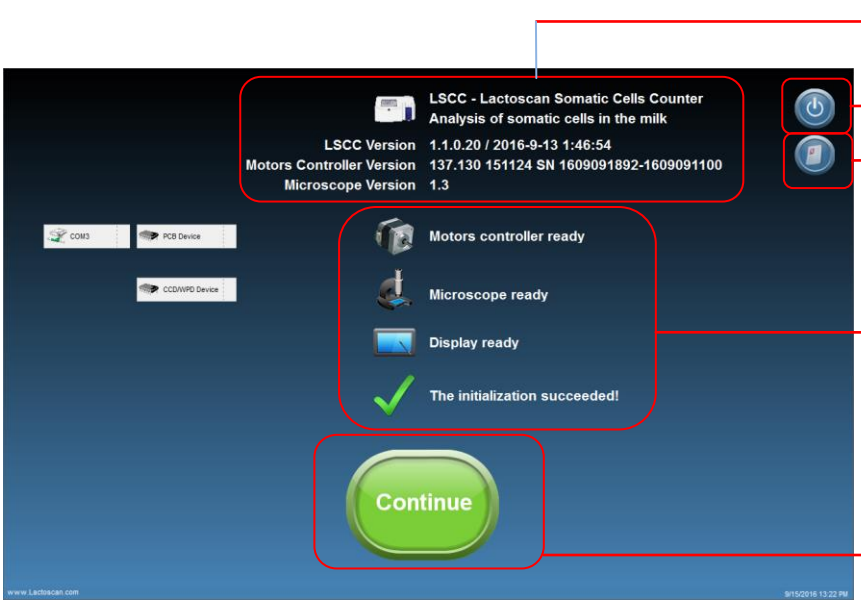
LACTOCHIP x4

Physical characteristics	**Material:**	ABS and PMMA
	Dimensions (HxWxL):	2 mm x 25 mm x 75 mm
	Weight:	0.005 kg
	Volume of the camera:	8µL
	Thickness of the microfluidic chip	50 µм

Description of the software displays

LACTOSCAN SCC software consists of the following displays:

- **Display "Initial"**



Device's ID

Exit button

User manual

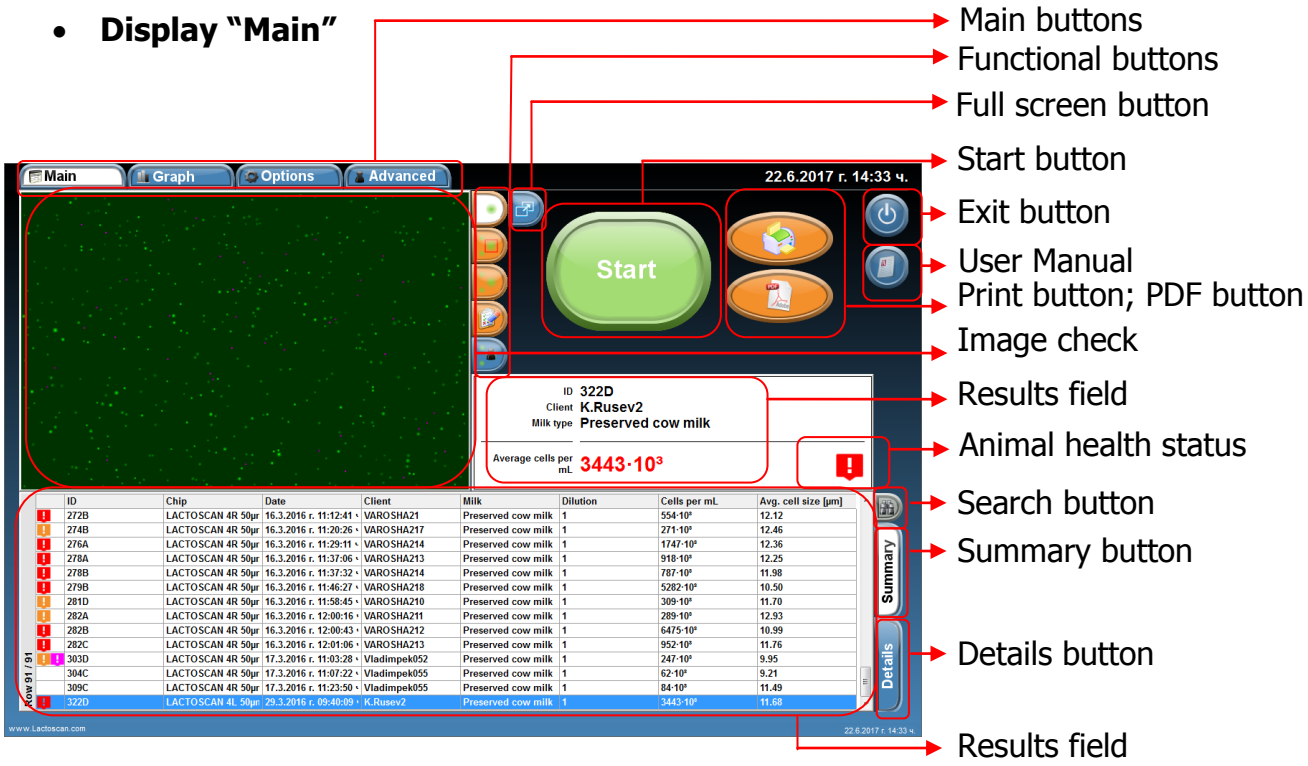
Summary

Continue button

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 LACTOSCAN SCC is opened in PDF file.
Summary	Information about the condition of the device after self-diagnosing 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.

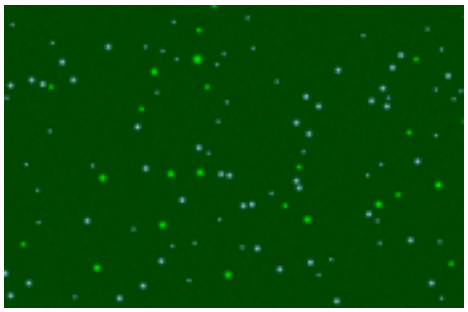
- **Display "Main"**



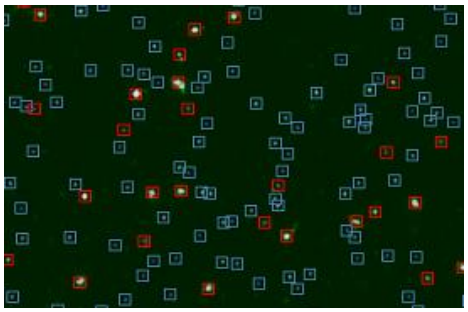
Main display

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.

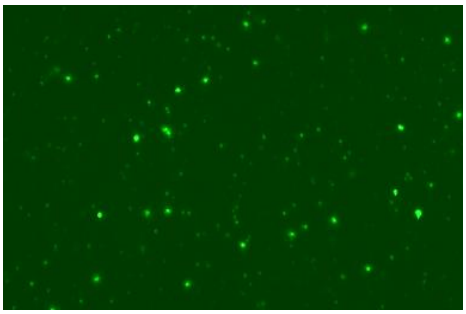
Shows software edited image on which mastitis cells are colored in green, yeast cells in blue and the background is presented completely smooth.

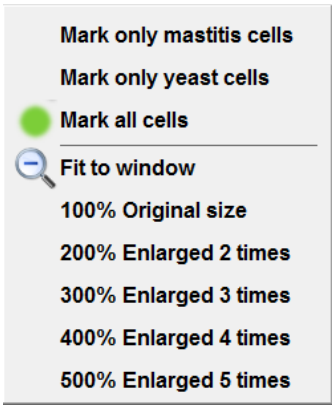


Software marks all the counted cells. Mastitis cells are marked with red square, yeast cells in blue square.



Shows the real image

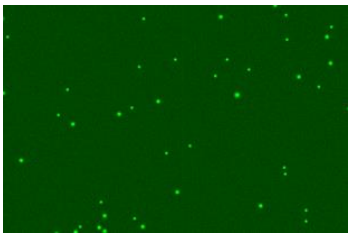




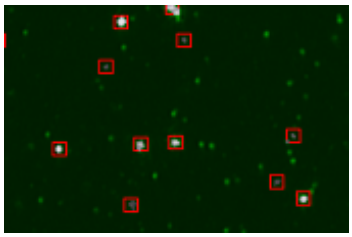
Allows user to select whether to see only mastitis cells, yeast cells or all cells and to zoom the image:

Marked only mastitis cells

Software edited image

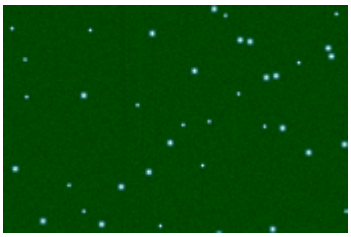


Real Image

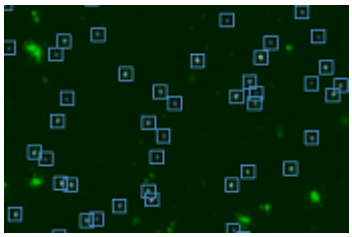


Marked only yeast cells

Software edited image

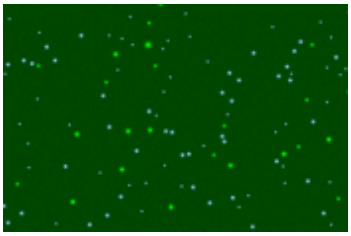


Real Image

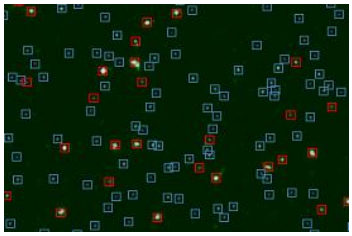


Marked all cells

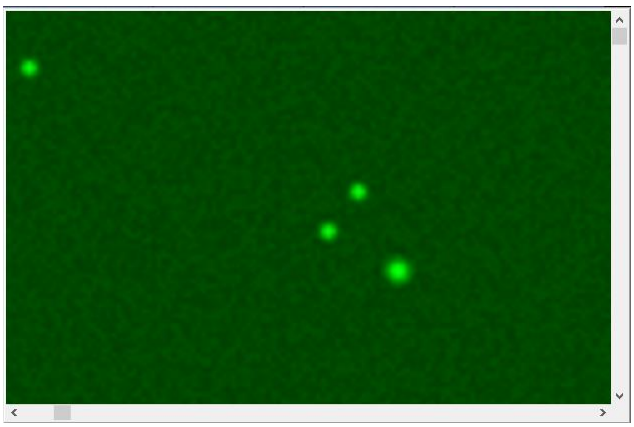
Software edited image



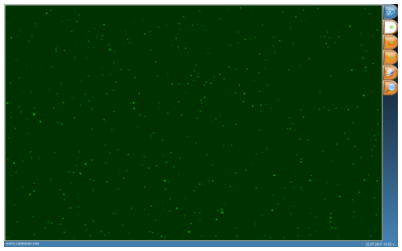
Real Image



Zoom



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



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

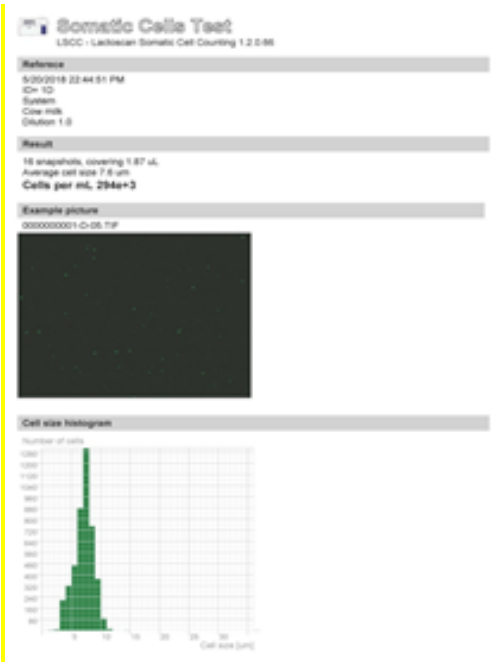
Starts the sample analysis

Turns off the device

By pressing this button the User manual for LACTOSCAN SCC is opened in PDF file.

Switches to print page

Shows a report from the made analysis in PDF format.

	
Image check	Shows the density of the somatic cells in the selected sample
Results field	Summary of the selected analysis
	The flags show the following animal health status: 1.  - Sub-clinical mastitis 2.  - Clinical mastitis 3.  - Presence of yeast cells in milk sample. Possible yeast mastitis. A test for yeast mastitis must be performed.
	Opens the search panel
	Switches to a table with summarized information about the performed analysis
	Switches to a table with detailed information about the already performed analysis

- **Display "Options"**

Allows the operator to set options of analysis according to the local or regional requirements for the different types of milk.

Options display

Main buttons	Switch main displays of the software
Parameters of measurement	Sets the min. and max. size of the counted cells for the different milk samples
Precautious levels	Sets a precautious level of sub-clinical and clinical mastitis according to the animal species
Laboratory offset	Coefficient for corrections
Apply and store	Applies the set options and their storage
Set default options	Sets default options of the measurement parameters and precautious levels
Advanced option	Opens menu with options needed for service purposes only
Focusing	Pressing the button switches to Setting focus menu

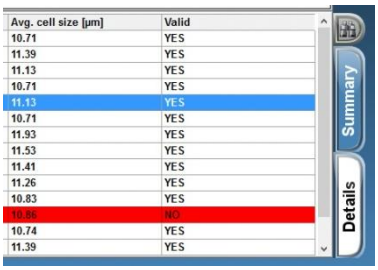
- **Display "Graphics"**

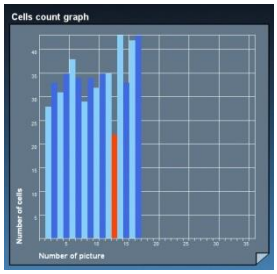
Graphics display

Main buttons	Switch main displays of the software
Summary	Summary of the data used to create the graphics
	Switches the source of information used to create the graphics
Min and Max size of the cell fields	Sets the border size of the cells included in the final analysis result by pressing the arrow buttons
Graphics	Graphical representation of the analysis data. "Size of the cell graphic" presents the data for the number of the cells according to their size. "Sample graphic" shows the size and number of cells that are taken into consideration during counting the analysis results.
Average size	Average size of the cells
Green line	Shows the average size of the cells

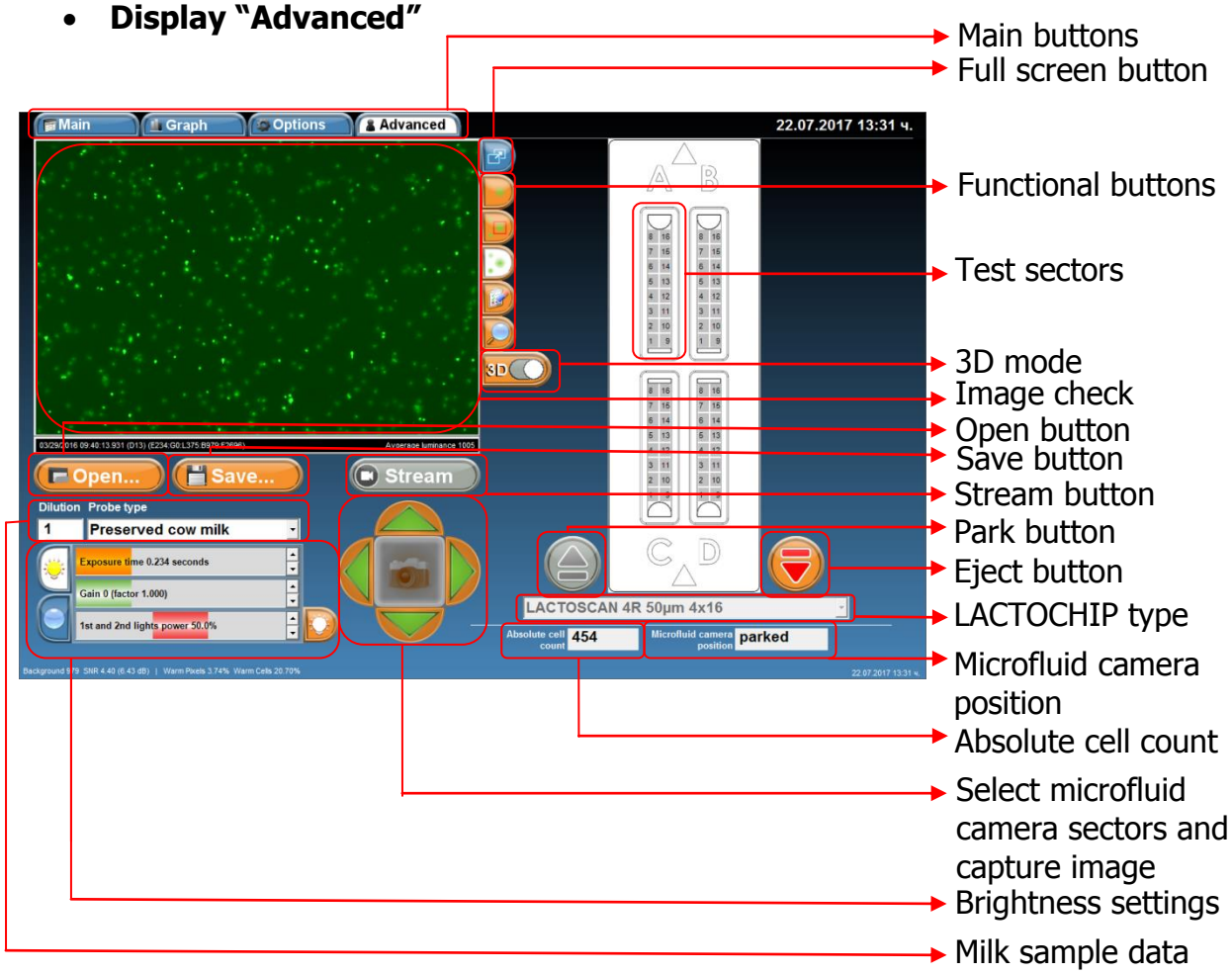
Shows an image which is not included when calculating the final result. In the table of details for the corresponding result, the image is marked with NO in column VALID, because of unacceptable deviation from the average of results derived from other images.

Red column





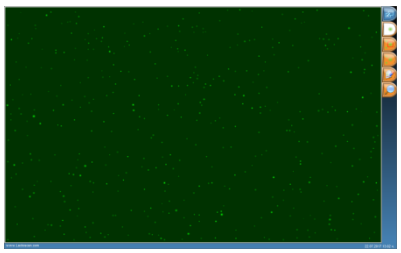
- **Display "Advanced"**



Advanced display

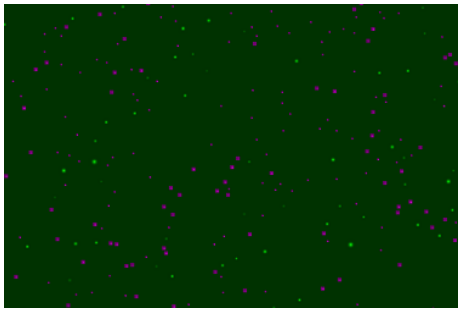
Main buttons Switch the main displays of the software

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

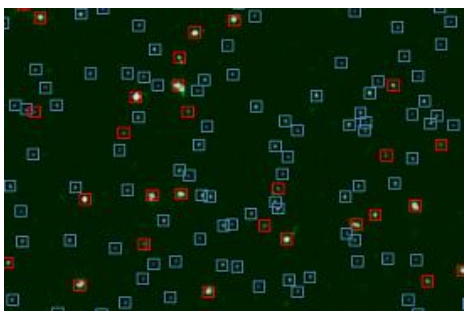


Functional buttons Allows the user to mark and unmark cells, to select cells, to zoom the image by pressing on the buttons.

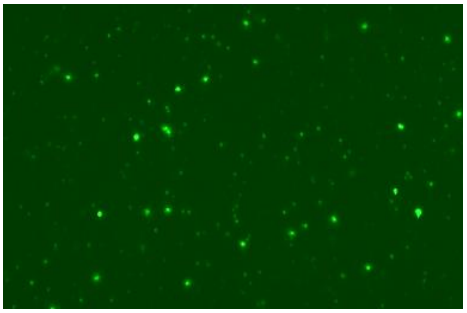
Shows software edited image on which mastitis cells are colored in green, yeast cells in blue and the background is presented completely smooth.



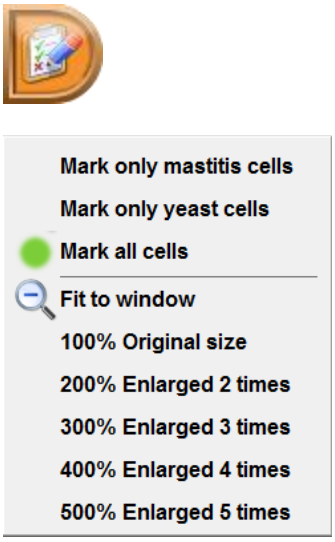
Software marks all the counted cells. Mastitis cells are marked with red square, yeast cells in blue square.



Shows the real image

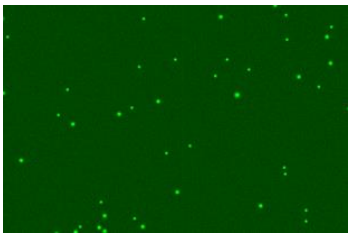


Allows user to select whether to see only mastitis cells, yeast cells or all cells and to zoom the image:

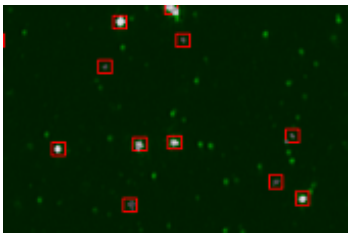


Marked only mastitis cells

Software edited image

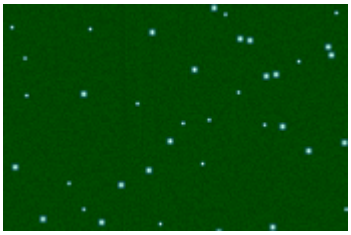


Real Image

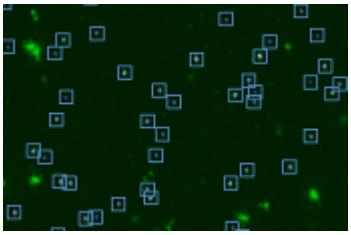


Marked only yeast cells

Software edited image

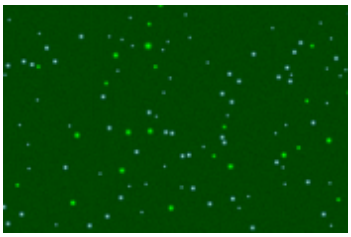


Real Image

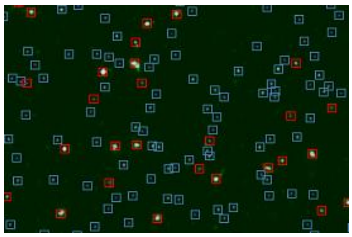


Marked all cells

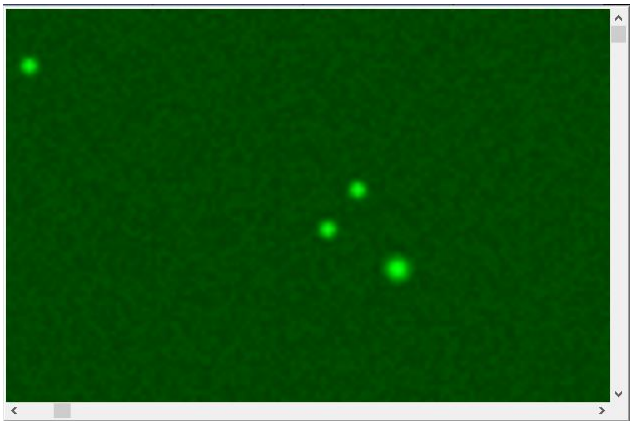
Software edited image



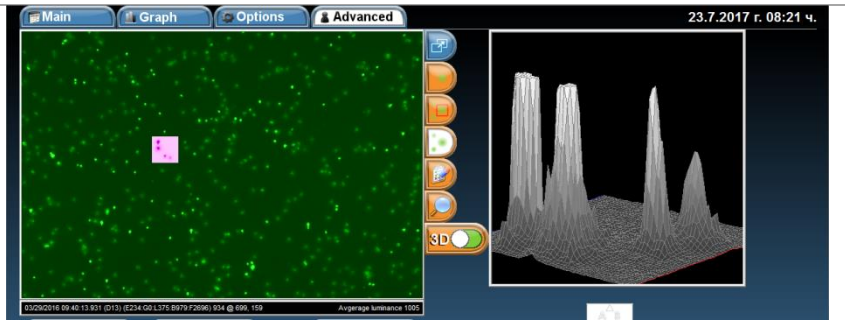
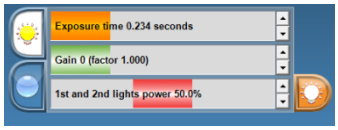
Real Image



Zoom



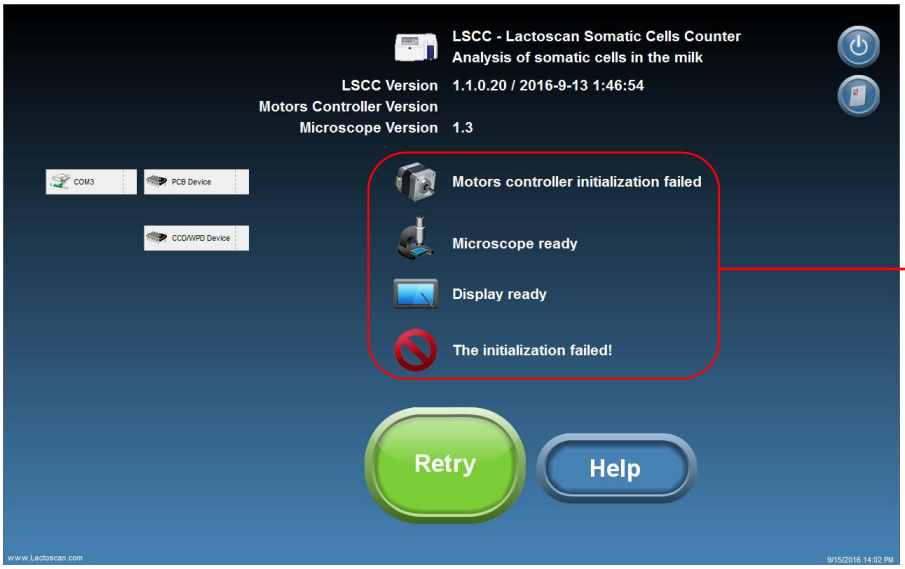
Test sectors	Allow user to select the exact sector from the microfluid camera which he wants to test and examine in advance mode.
	Examine the test image in 3D mode.

	
Image check	Shows the density of the somatic cells 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 LACTOCHIP.
	Ejects the cartridge with LACTOCHIP.
	Selects the LACTOCHIP type.
	Shows the position of the cartridge with the microfluid camera / LACTOCHIP.
	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 LACTOSCAN SCC

Working sequence when preparing the LACTOSCAN SCC 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 analyzer. 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 analyzer and wait a minute until it charges.
5. Turn on the display by using the ON/OFF key placed at its upper side.
6. LACTOSCAN SCC 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.

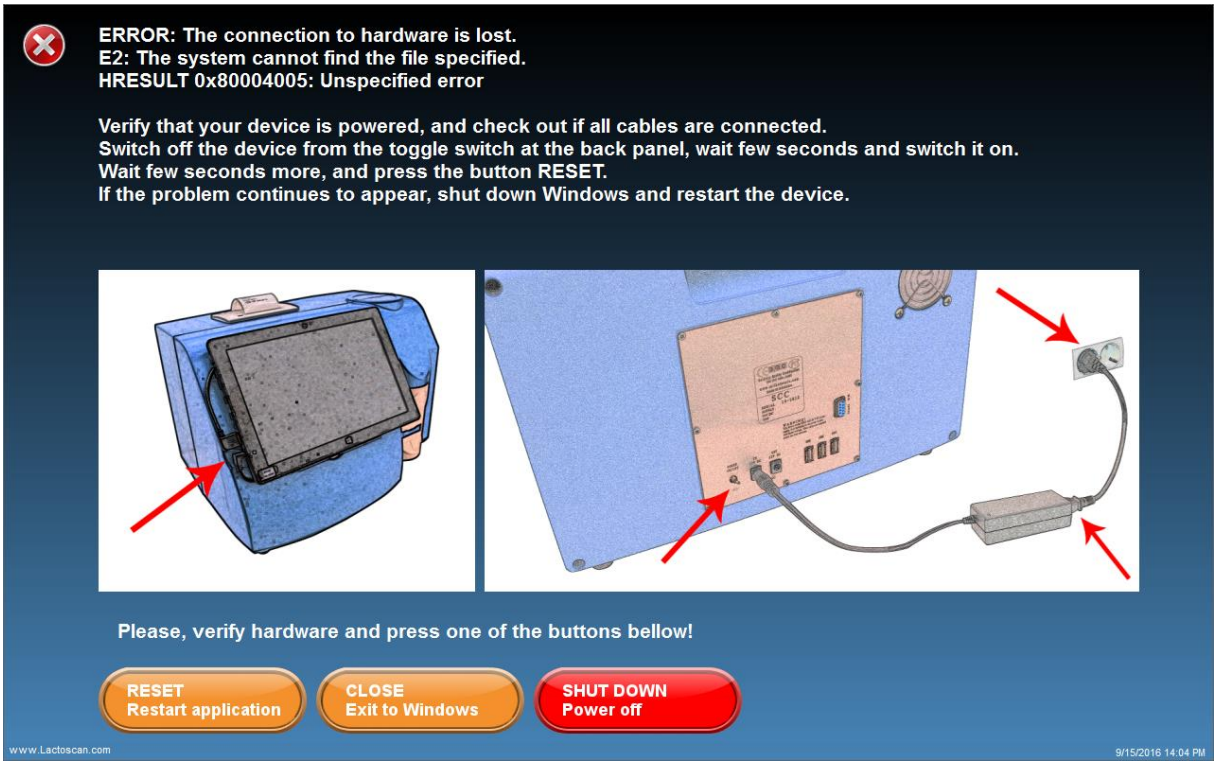


Issues related to the optics, mechanics and operation

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.



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

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

Preparation of sample for analysis

To prepare a sample for analysis are needed:

- Raw milk;
- LACTOSCAN SCC KIT;
- 2 pcs. automatic pipettes.

Attention!
To obtain results with reference accuracy, the requirements for proper sampling specified in ISO 707 | IDF 50 must be met.

Attention!
Measurement accuracy depends on the correct and consistent implementation of all stages of sample preparation and good mixing of the sample. To minimize the difference in reporting the results of several tests on the same sample of milk, always stir well before taking the sample. The sample is representative only when stirred well.

Stages:

1. Preparation of the raw milk:
It is mandatory a raw milk, just milked or preserved with room temperature 15-25^{0}C. The necessary min. volume is 30 mL.

Attention!
According to **INTERNATIONAL STANDARD ISO 13366-1 | IDF 148-1:2008**, to obtain the best results, you should observe the following principles:

If the samples are without preservative, they should be measured within 6 hours after milking.

If samples cannot be measured in the course of these 6 hours, they must be preserved with Bronopol ($C_3H_6BrNO_4$), Potassium Dichromate ($K_2Cr_2O_7$) or Formalin (CH_2O) in amounts specified in the standards for sampling for analysis. The final concentration of Bronopol shall not exceed 0,05 g per 100 ml of test sample. The final concentration of potassium dichromate shall not exceed 0,1 g per 100 ml of test sample. They may be stored in a refrigerator at 4 °C ± 2 °C for no longer than 6 days.

We recommend using Bronopol as a milk preservative!

Before measuring samples, they must be heated up to 40 °C and cooled down to 20 °C and then stirred thoroughly with Vortex mixer. When samples are kept in a refrigerator, the fat globules float to the top and majority of the leukocytes adhere to them, and therefore the somatic cells go up together with fat globules. Often if the sample is not heated up to 40 °C and cooled down to 20 degrees, it is not possible to be mixed thoroughly, leading to uneven

distribution of somatic cells in the sample volume. Then the measurements will vary.

Preserved and stored in a fridge sample is suitable for measuring no longer than 5-6 days.

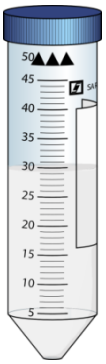
The sample must be no more than 50 ml, and must not fill the bottle with the sample to the cap in order to allow easier mixing with Vortex mixer or by hand.

Attention!
If the analysis is not conducted within 3-4 hours after milking, it is necessary to preserve the milk. When the raw milk is preserved is recommended to use preservatives **formalin, bronopol** or **potassium dichromate**.

Attention!
If the preserved milk is chilled below 10 ^{0}C, it must be tempered naturally to room temperature 15-25 ^{0}C. Freshly milked milk is not necessary to be chilled or heated.

Attention!
Do not use for analysis raw or preserved milk with acidity above:

- 18 °T (Therner)for cow milk
- 17 °T for buffalo milk
- 16 °T for goat milk
- 22 °T for sheep milk



Attention!
In case of measurement of milk with Fat more than 5 %, for example buffalo milk, it is necessary the milk sample to be diluted with water in ratio 1:1. Then 100 µL of it is taken and added to the lyophilised dye. Adding water prevents difficulties in milk samples entry into the microfluidic chamber.

Using the Mini Vortex mixer stir the raw milk sample. For stirring, place the tip of the container in the stirrer, press and keep it pressed for 1-2 seconds, then remove it (see 1, 2, 3.). Repeat it 3-4 times paying attention during the stirring process the sample not to reach the cap of the container.


1.1


1.2


1.3

2. Pipetting 100 µL raw milk in micro-tube with SOFIA GREEN lyophilized dye:

Take one micro tube containing SOFIA GREEN liquid dye, open it, and place it on the rack.

Attention!

Before start working with the automatic pipettes, read carefully their Instruction Manual. Make several trials sucking and pipetting water in order to understand when exactly the first and second stop of the working button is reached.

Take preliminary set to 100µL automatic pipette. Make sure that the front cone of the pipette is clean. Place it vertically over one of the tips on the working rack and place the cone of the pipette in the opening of the tip by slightly pressing it.

From initial position (see 2.1), press the working button of the pipette till the first stop is reached (see 2.2), keep it pressed and dip 2-3 mm of the tip in the milk (see 2.3). Smoothly release the working button and take out the tip from the liquid. Touch the walls of the bottle to remove the excessive milk (see 2.4, 2.5).


2.1


2.2


2.3


2.4


2.5

Pipette milk in the opened micro-tube on the rack by smoothly pressing the working button of the pipette from the initial position to the first stop (see 2.6, 2.7). After a short period press the button to the second stop (see 2.8). In this way you'll empty the tip and will guarantee precise pipetting. Always pipette the milk without dipping the tip in the lyophilized dye.

Release the button to its initial position (see 2.9).


2.6


2.7


2.8


2.9

3. Stirring the sample:

Close the micro-tube containing SOFIA GREEN dye and milk sample. Take it from the rack and place the tip of the micro-tube in the opening of the stirrer Mini Vortex. Press and hold it pressed for 1-2 seconds and remove. Repeat 8-9 times being careful while stirring the solution not to reach the cap of the micro-tube. (see 3.1, 3.2 and 3.3)

3.1

3.2

3.3

4. Interaction of milk with dye:

1 minute is needed for this interaction. If it is less 1 minute or more than 20, the analysis result may be with deviation 2-3%.

5. Repeated stirring the sample:

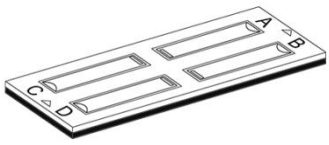
Take the micro-tube containing the sample from the rack and place its tip in the opening of the stirrer Mini Vortex. Press and keep it pressed for 1-2 seconds, remove. Repeat 2-3 times, paying attention place the tip of the container in the stirrer, press and keep it pressed for 1-2 seconds, then remove (see 3.1, 3.2, 3.3.). Repeat it 3-4 times paying attention during the stirring process the sample not to reach the cap of the container.

Attention!

If more than 5 minutes elapse after the milk was placed inside in micro-tube with SOFIA GREEN dye, stir with Mini Vortex mixer the sample once again, before filling in the LACTOCHIP.

6. Pipetting 8 µL sample in the micro-fluidic camera of the LACTOCHIP x4:

Open one LACTOCHIP x4.



Attention!

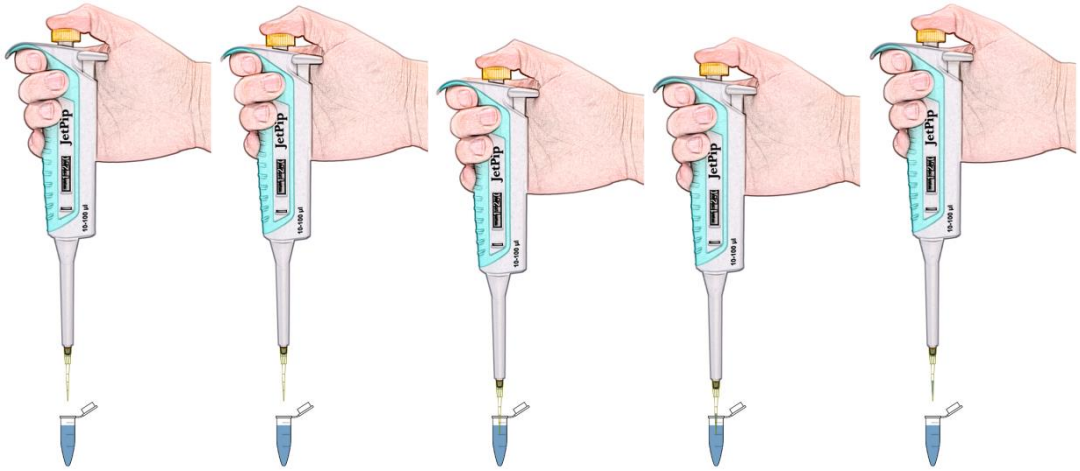
Do not touch the upper surface of the LACTOCHIP x4. Always hold its side edges.

To eject the solution into the micro-fluidic camera of LACTOCHIP x4, take preliminary set to 8 µL automatic pipette.

Make sure that the front cone of the pipette is clean. Place it over one of the tips on the rack and place the cone of the pipette in the opening of the tip by slightly pressing it.

Open the micro-tube containing the solution.

From initial position (see 6.1), press the working button of the pipette till the first stop is reached (see 6.2), keep it pressed and dip 2-3 mm of the tip in the solution (see 6.3). Smoothly release the working button to the initial position. Take out the tip from the liquid by touching the walls of the bottle to remove the excessive solution (see 6.4, 6.5).



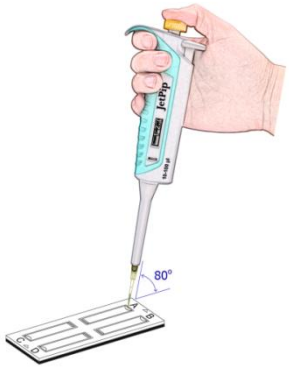
6.1 6.2 6.3 6.4 6.5

Now there's 8 µL solution in the tip.

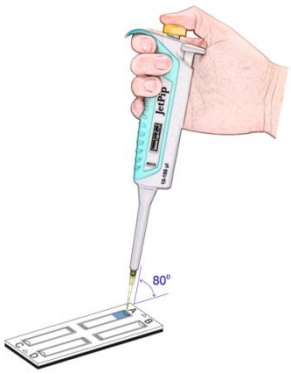
Take the LACTOCHIP x4 by holding its side edges.
Pipette the solution at an angle of approximately 80^0 to the filling opening in semicircular shape. Pipetting is done by smooth pressing the working button of the pipette from the initial position to the first stop (see 6.6, 6.7). Hold the button at the first stop, remove the pipette from the LACTOCHIP and smoothly release the button to the initial position (see 6.8).
In this way you'll empty the tip and precise pipetting will be assured. Release the button to its initial position.

Attention!
Do not use the second stop in order to avoid air entering into the micro-fluidic camera.

Attention!
Avoid forming bubbles in the micro-fluidic camera and splashes during pipetting the sample.



6.6



6.7


6.8

In order to load the rest of the micro-fluidic cameras of the LACTOCHIP x4 / LACTOCHIP x2, repeat the procedure described in points 1 to 6 by consecutively filling the micro-fluidic cameras from A-D.

Attention!

It is recommended to use the all microfluidic cameras at once. If you use only 1 or 2, store the LACTOCHIP x4, paying attention not to contaminate it with dust or other pollution as it will lead to false results of the analyses.

Attention!

It is recommended to place the loaded LACTOCHIP x4 in the device and to start analyses within 1 minute. Delay may lead to inaccurate results due to evaporation of the sample and air entering it.

7. Starting analysis:

Place the loaded with sample LACTOCHIP x4 in the cartridge of the LACTOSCAN SCC.
Using the software, start the analysis.

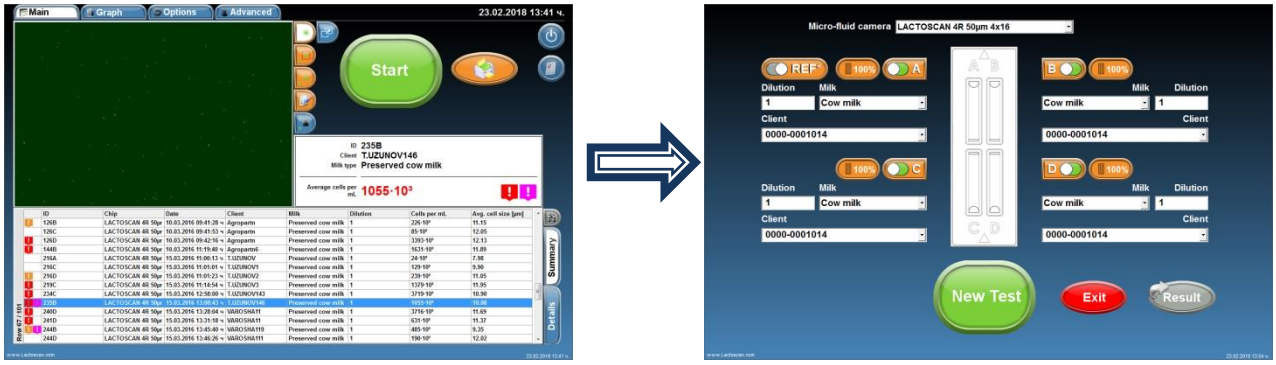
8. Disposal:

Using the button for removing the tip, leave the tip inside the micro-tube with the sample. Dispose the micro-tube with the sample residue, the tip and used for analysis LACTOCHIP x4 in suitable container.

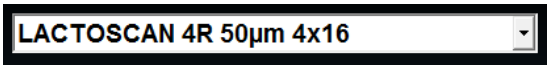
Sample analysis

1. Press 

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

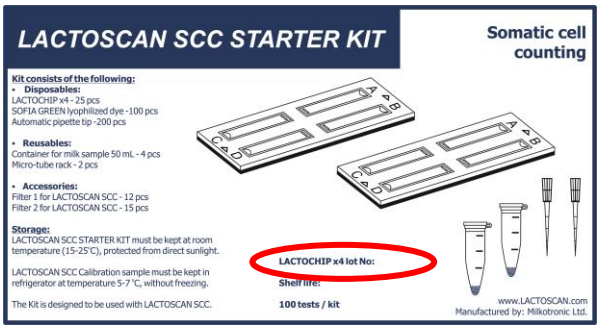


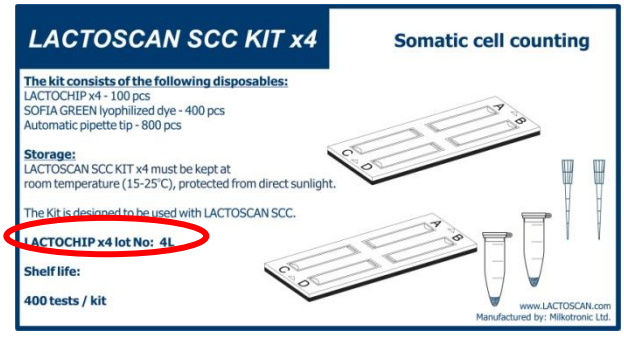
2. Choose the type of chip from the drop down menu

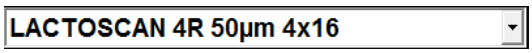


Attention!

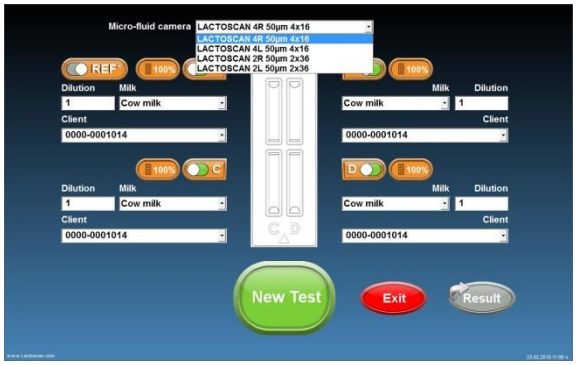
Before starting work with LACTOSCAN SCC KIT always check LACTOCHIP letter. It can be L or R. Letter L indicated LACTOCHIP 4L and letter R indicated LACTOCHIP 4R. The LACTOCHIP letters match with the already put in the program LACTOCHIPs data. One LACTOSCAN SCC KIT included only LACTOCHIPs with letter L or only with letter R.

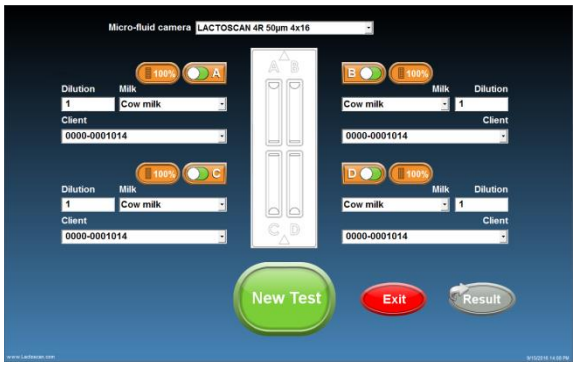




In our example we choose 

for LACTOCHIP 4X16 with thickness of the four micro fluidic chambers from 50µm.

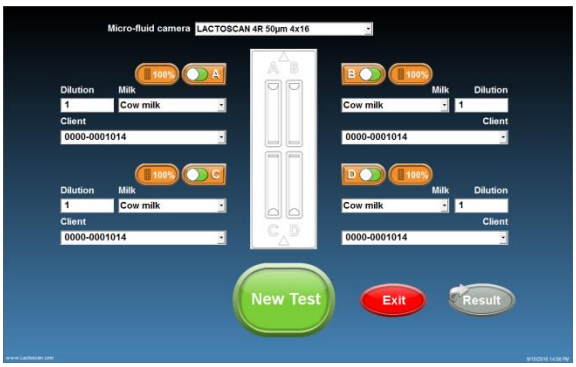


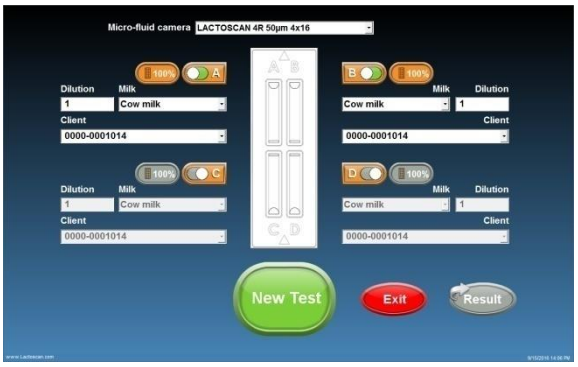


**3. All the micro fluidic chambers of the LACTOCHIP are selected. If you want to use only 1 or 2, using button 

deselect the chambers which you will not be using in this test.**

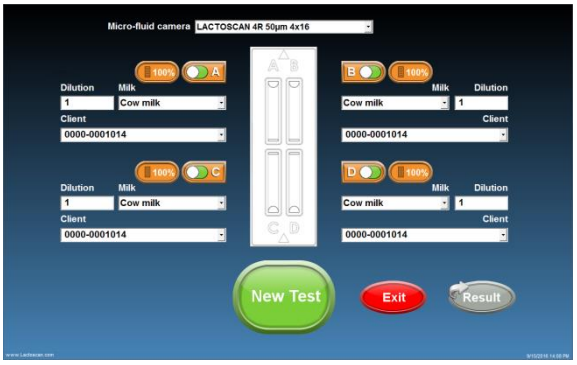
In our example we deselect Sample C and D.

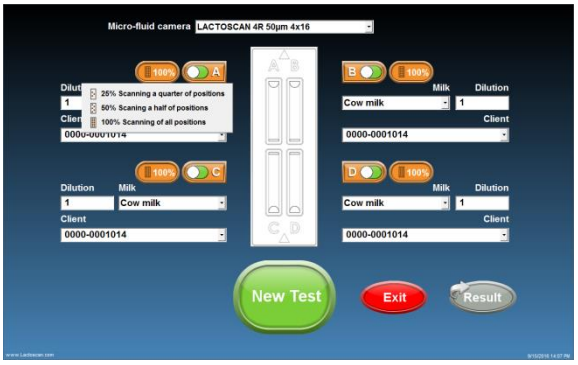




**4. For a quick analysis, press button 

and select a test from the drop-down menu. You can select from test "Scanning 25% of positions", test Scanning 50% of positions", test "Scanning 100% of positions".**





5. Complete the value of the dilution's multiplier in field 1 **under Dilution.**

Attention!
The value of the multiplier for dilution depends on the fat content of the milk.

For milk with fat:

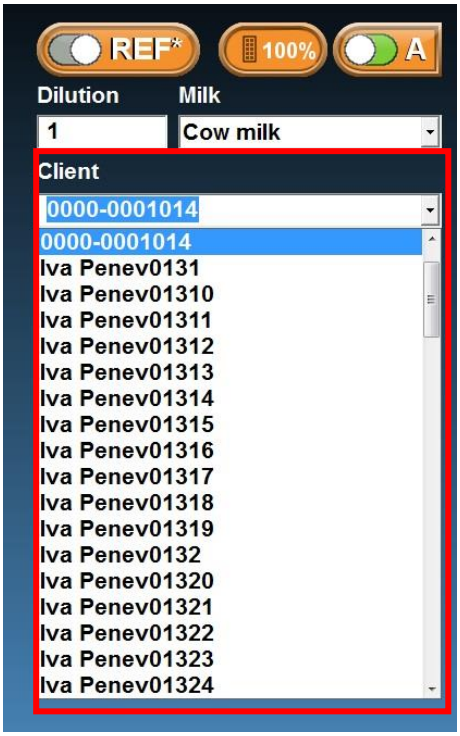
- Up to 5 %, the value of the multiplier for dilution is 1, as the milk is added directly to the lyophilized dye in the micro tube.

- Above 5 %, the value of the multiplier for dilution is 2, because, for example, in case of measurement of milk with Fat more than 5 %, for example buffalo milk, it is necessary the milk sample to be diluted with water in ratio 1:1. Then 100 µL of it is taken and added to the lyophilised dye. Adding water prevents difficulties in milk samples entry into the microfluidic chamber.

Attention!
Don't forget to change the multiplier for dilution in the field "Dilution" when working with milk, containing fat over **5%** and add distilled water to the lyophilized dye.

6. Choose the type of milk from the drop-down menu.

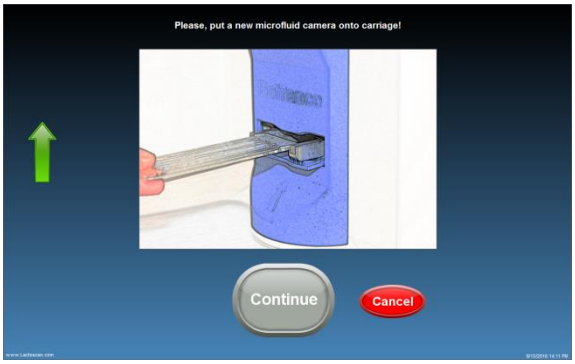
7. Enter the name of the supplier or choose from the drop-down menu.



8. After the information for all the samples is entered, press 

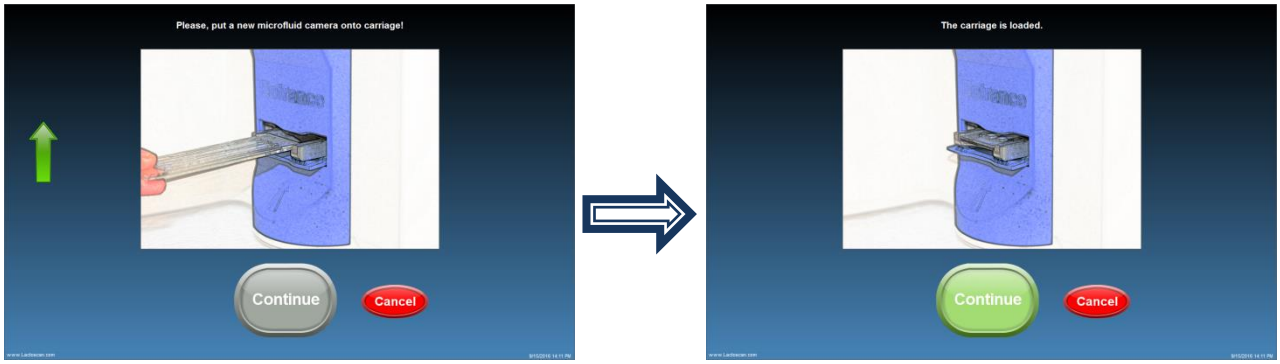
in order to proceed with the analysis. On the screen a message will be displayed, showing that the cartridge for placing the LACTOCHIP comes out of the door. Pressing 

will revert to the Main screen.

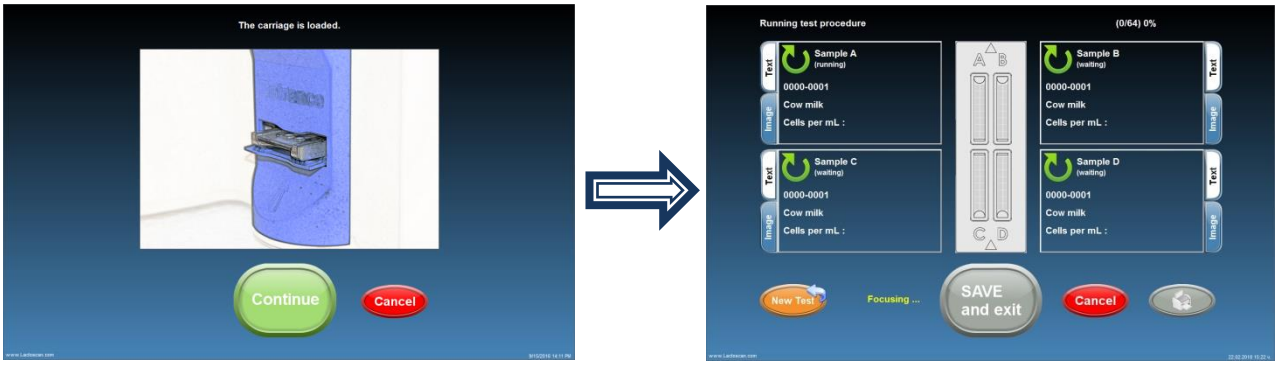


9. Place LACTOCHIP into the cartridge and press 

.

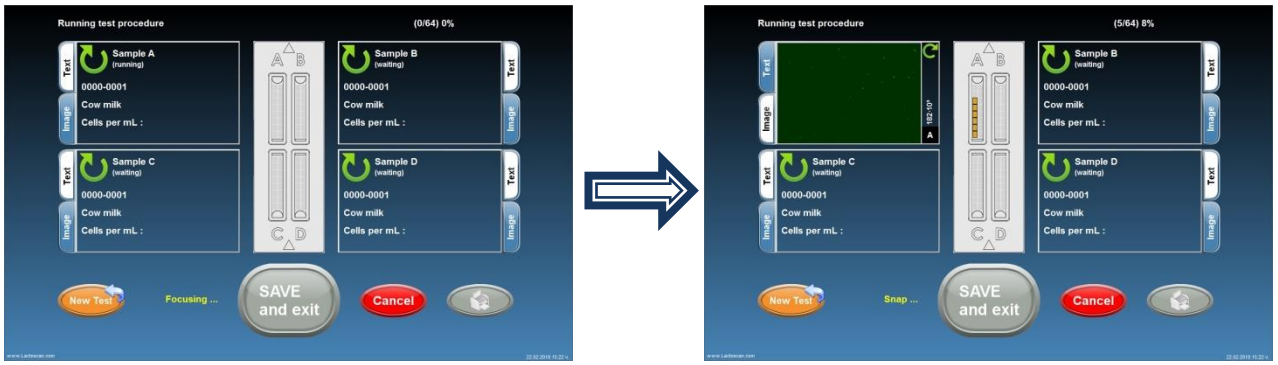


10. The device is making the analysis of the samples.



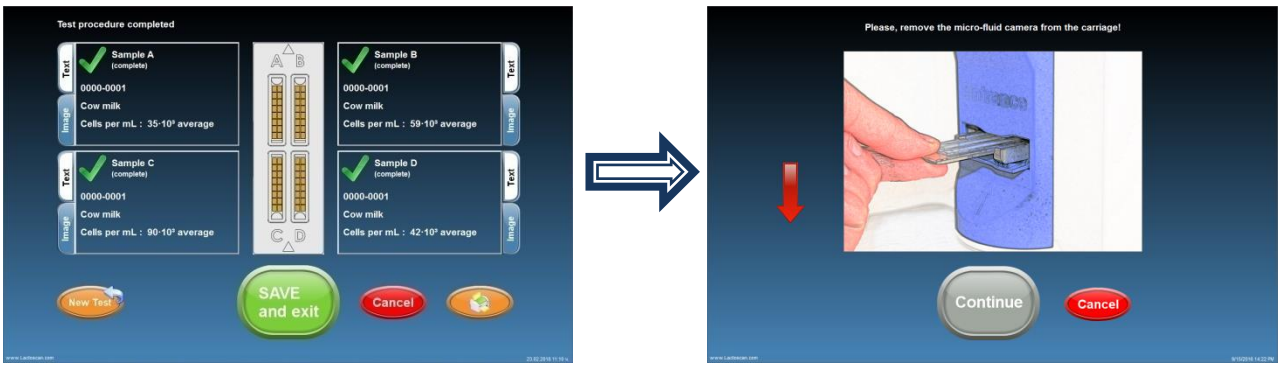
11. If you want to see test image in real time, press 

.

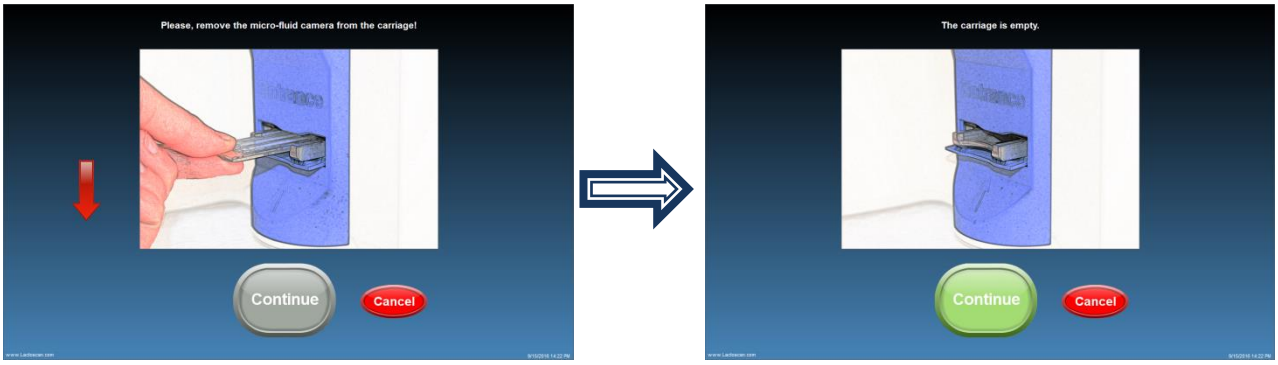


12. In order to print the results, press 
.

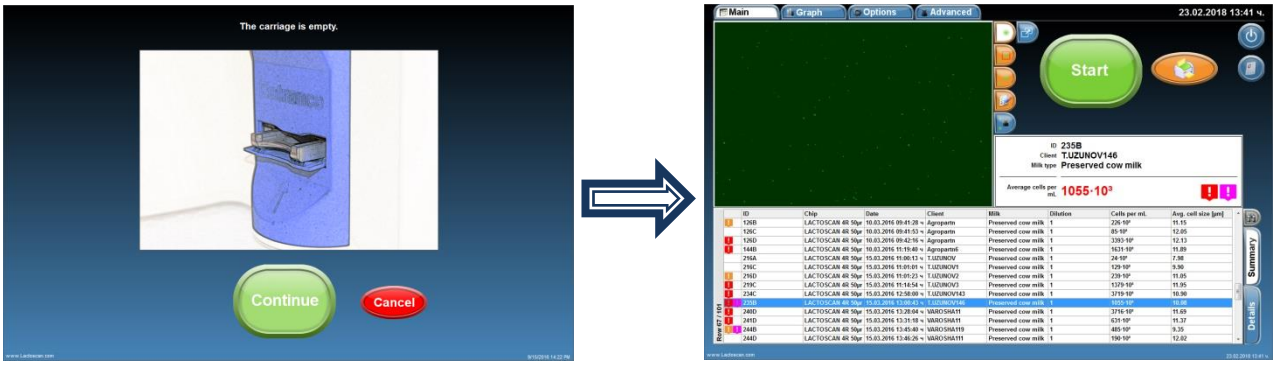
13. In order to save the results and continue the analysis of new samples, press SAVE and exit or press Cancel, to continue with the analysis of new samples without saving the results.



14. Take the LACTOCHIP out.



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



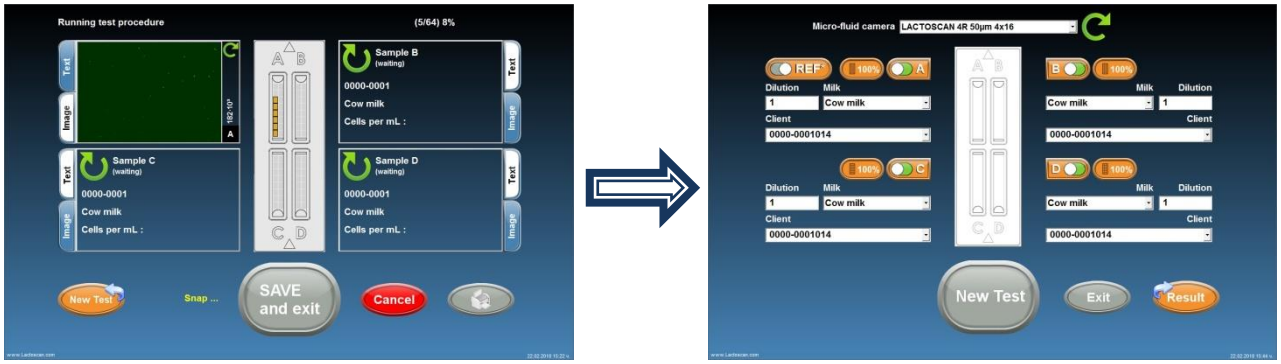
Data samples entry during the analysis

The software of LACTOSCAN SCC allows the entry of data for new samples during the process of analyzing the samples.

In order to do this:

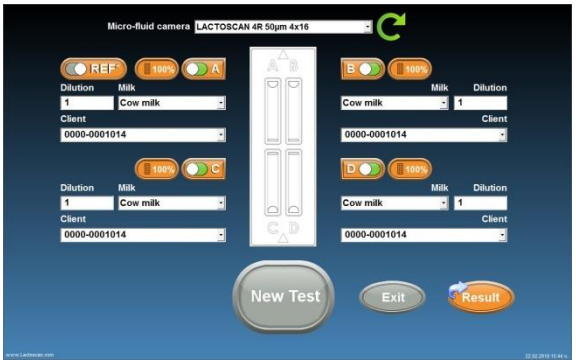
1. Press 

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



The data for the samples which are being analyzed 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.

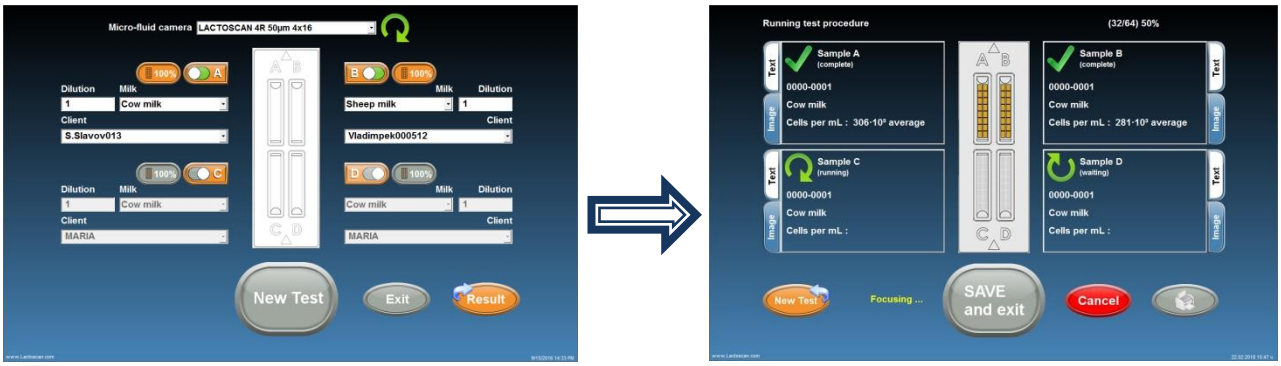


If 

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 

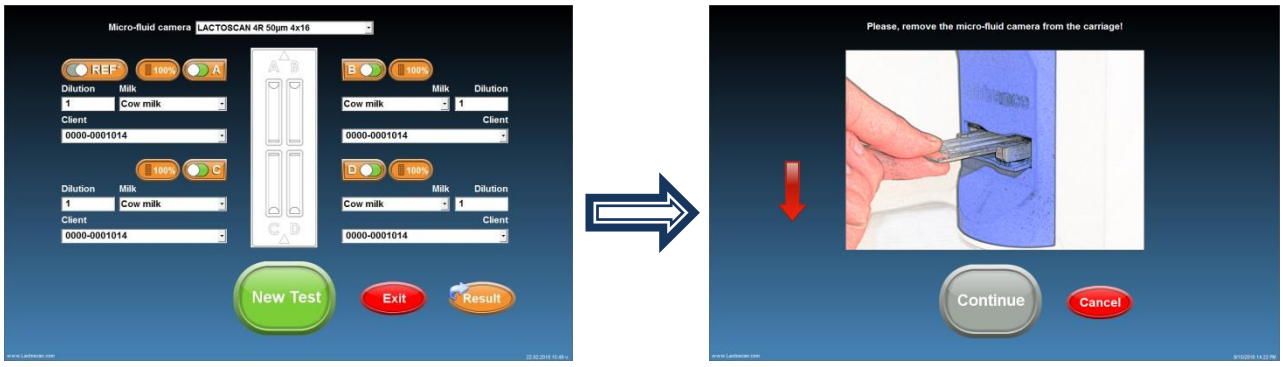
to become active, or go back to the screen with the current analysis.

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

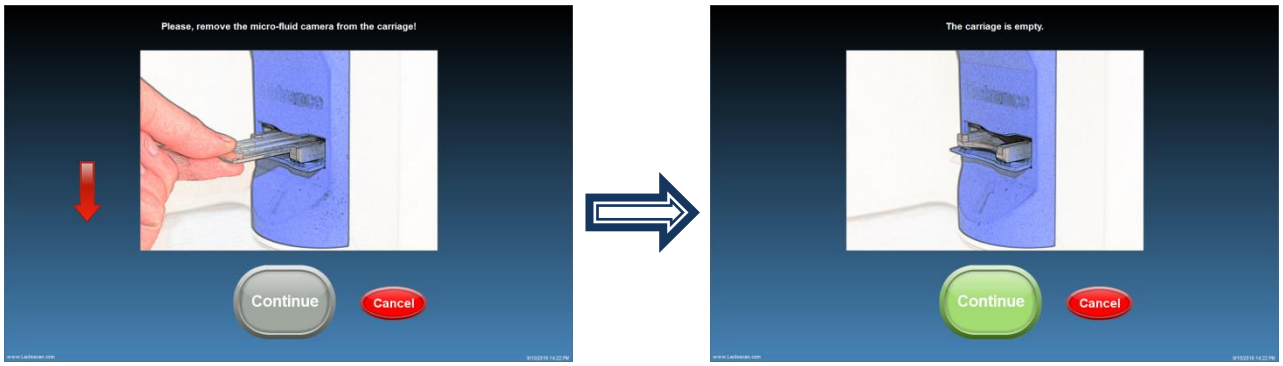


4. Press New Test, in order to proceed directly to the new analysis.

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

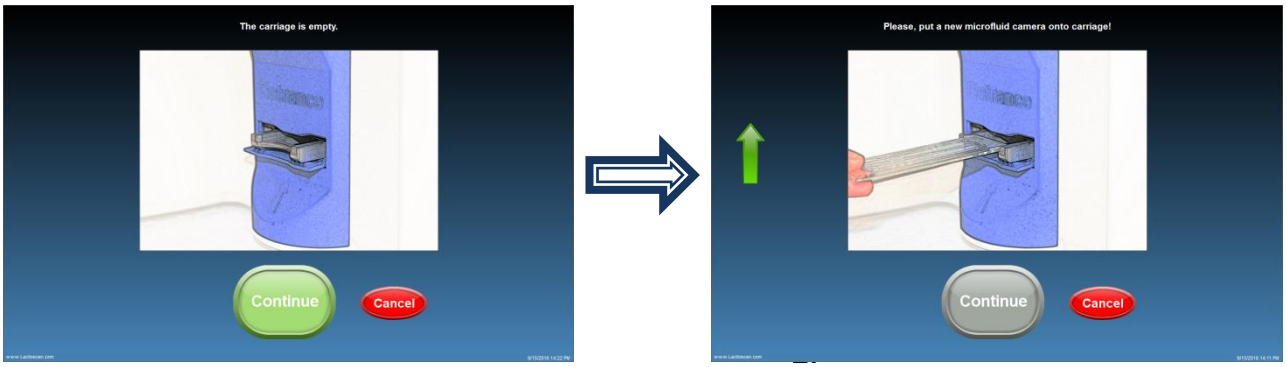


5. Pull out the LACTOCHIP with the samples from the previous analysis.

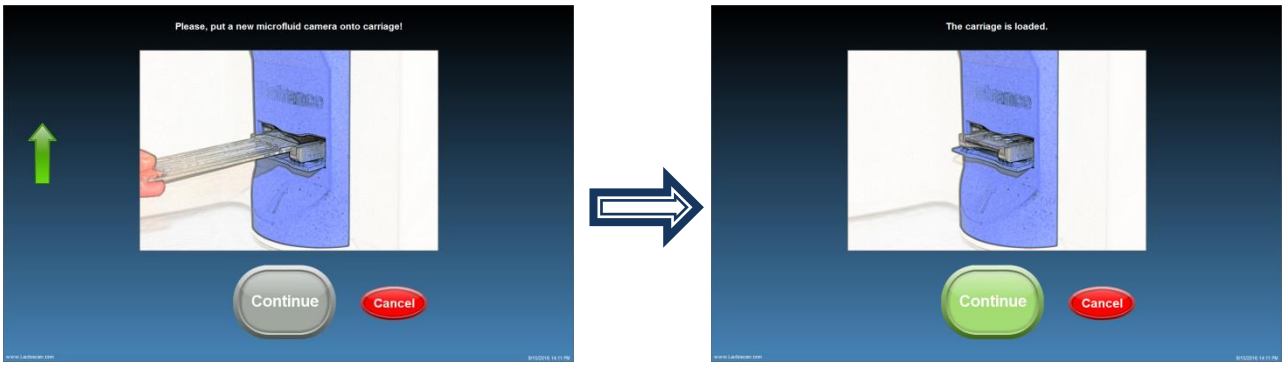


6. Press 

. The software prompts for placing the LACTOCHIP with the samples for which data are entered.

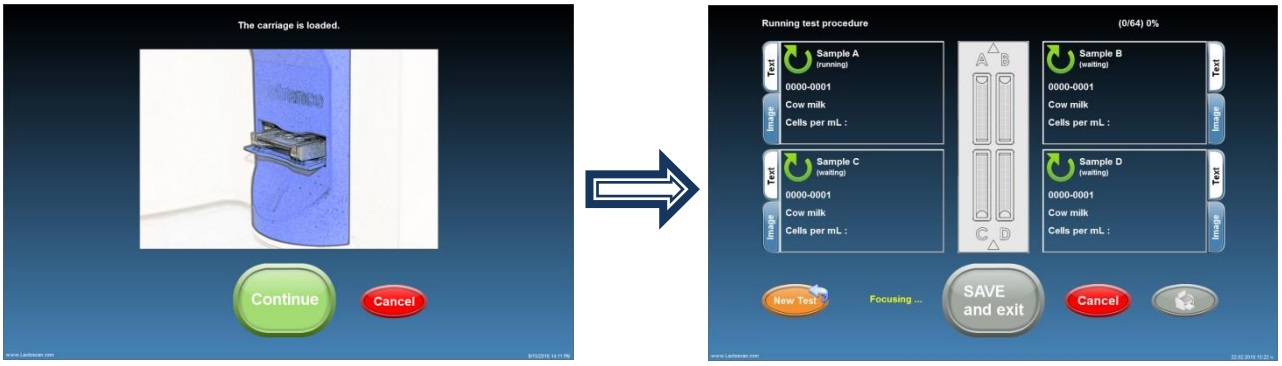


7. Place the LACTOCHIP with the samples with entered values.



8. Press 

in order to start the analysis.



Reference sample testing

To receive results with reference accuracy from somatic cell counting using LACTOSCAN SCC the following steps must be done:

1. Samples taking:
Samples must be taken with accordance to the recommended sampling method given in ISO 707|IDF 50.

2. Samples storage:
Samples must be stored with accordance to the recommendations in ISO 13366-1 | IDF 148-1:2008, page 4:

"7.1 Storage

Prior to testing or preservation, store the test samples at a temperature of 4 °C ± 2 °C.

Analyse the test samples within 6 h after sampling. In the case of longer storage, add chemical preservatives such as boric acid, bronopol or potassium dichromate. The final concentration of boric acid shall not exceed 0,6 g per 100 ml of test sample. The final concentration of bronopol shall not exceed 0,05 g per 100 ml of test sample. The final concentration of potassium dichromate shall not exceed 0,1 g per 100 ml of test sample. Store the thus preserved test samples at a temperature of 4 °C ± 2 °C for no longer than 6 days.

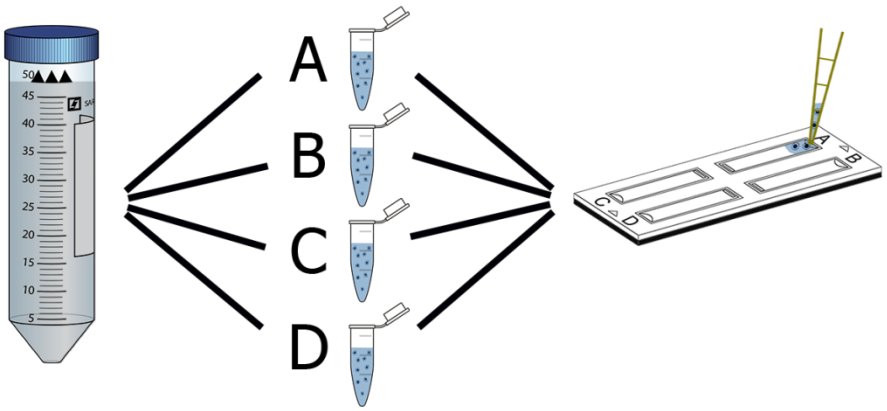
For environmental reasons, it is recommended to restrict the use of potassium dichromate to samples that require a long shelf life only."

Note! Bronopol is the recommended preservative for preparing samples for analysis with LACTOSCAN SCC.

3. Sample Preparation procedure recommended by IDF Reference laboratory:
Heat the sample in a water bath with the temperature between 38 and 40°C (100 – 104°F) for a period of 10 minutes. Shake vigorously for 2 minutes and then emerge the vial in the water bath for an additional 10 minutes. Cool the sample to the temperature of 20 °C.

4. Testing:

To do this the following consumables will be required: 1 x X4 cartridge, 4 x eppendorf tubes with Sofia Green dye and 8 x pipette tips.



- After sample preparation take 100µL of sample and put it into an eppendorf tube. Do this a total of 4 times for the 4 cameras of the chip.
- Incubate for 5 minutes while mixing frequently.
- After incubation do a final mixing and take 8 µl of the sample and put into the X4 cartridge – do this a total of 4 times, each time with sample from a new eppendorf tube.

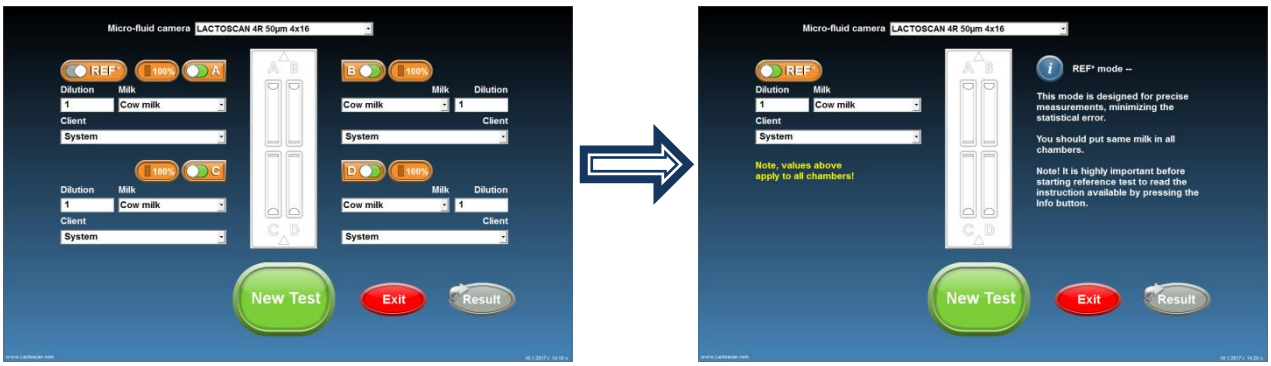
The procedure for preparing single sample for testing is described in section **Preparation of sample for analysis**.

Note! A new tip must be used for preparing each of the 4 samples and a new tip must be used for putting the sample in each chamber A, B, C, D.

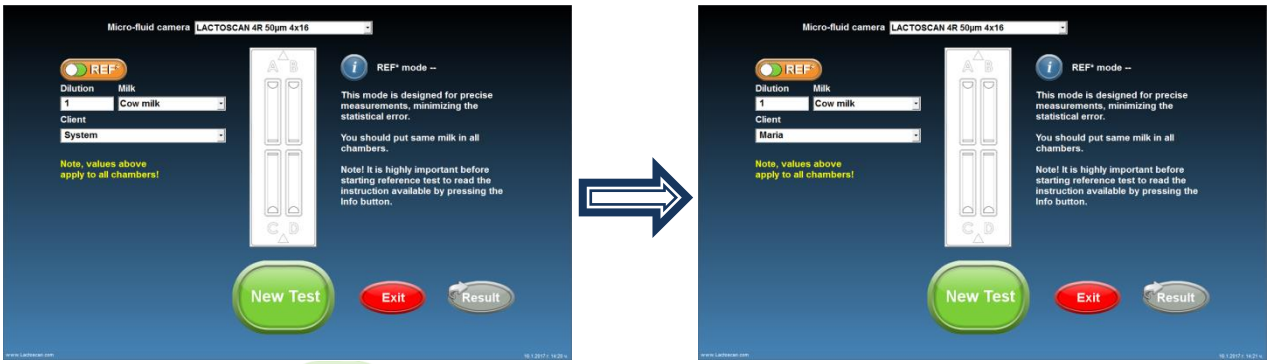
- Let the cartridge sit for 30 seconds before testing in order the cells to stop moving inside the chambers.

- In testing mode, press button 

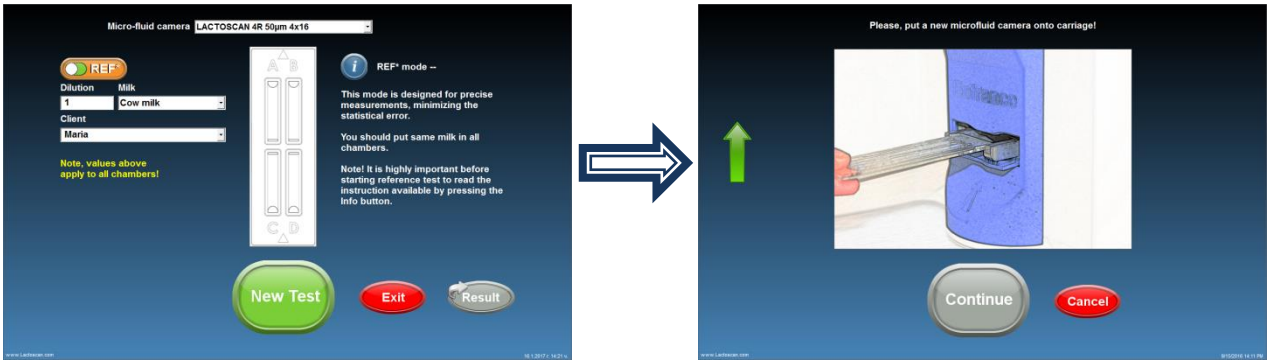
to select "Reference testing mode".



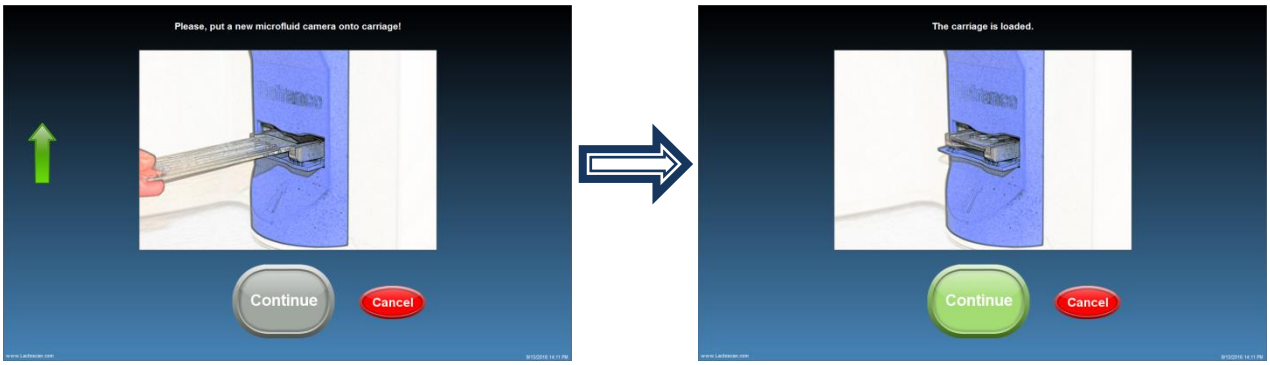
- Fill in the data for the tested milk sample.



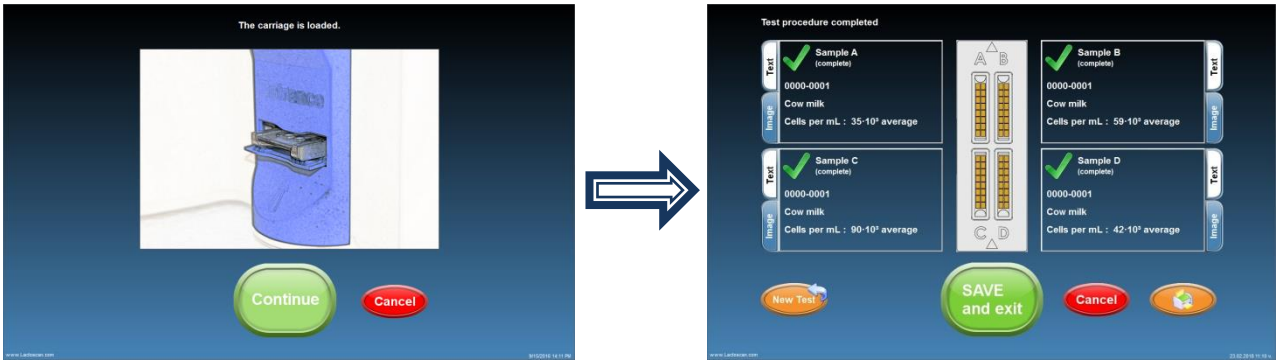
- Press button  to start the testing.



- Place LACTOCHIP into the cartridge and press  .



- The device is making the analysis of the sample.

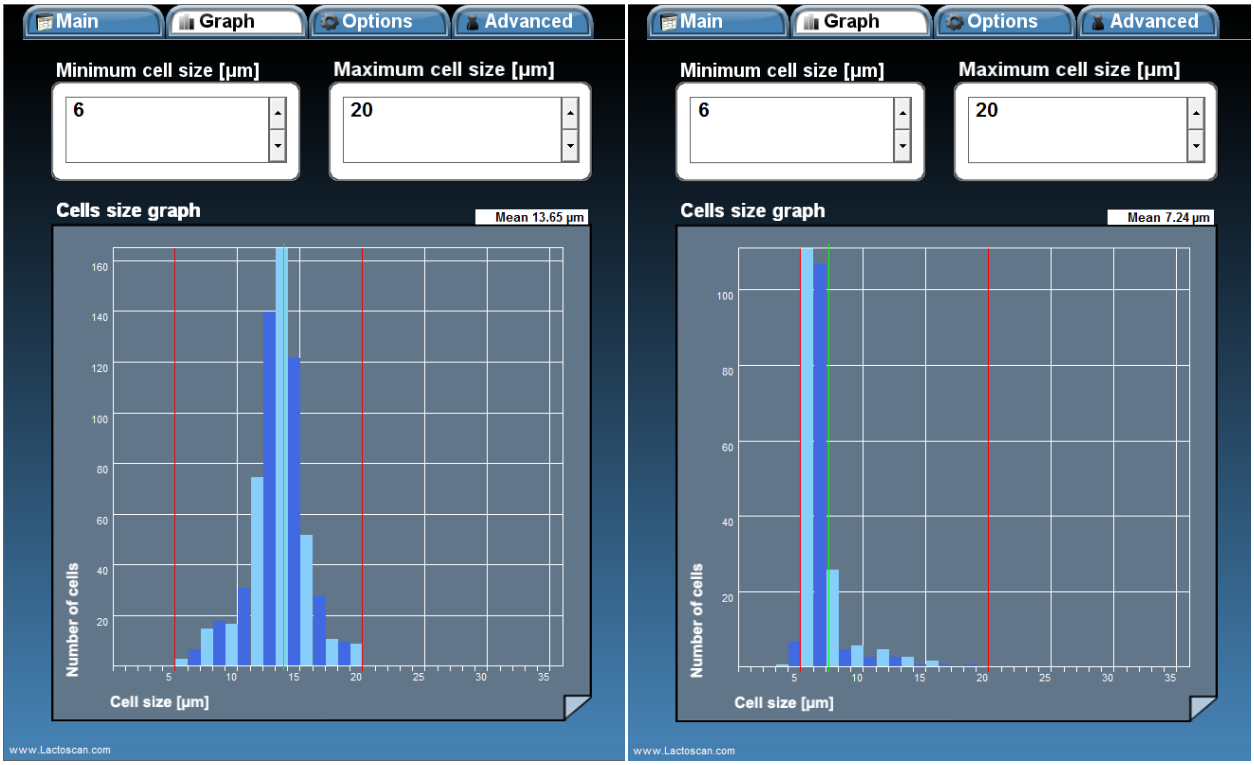


LACTOSCAN SCC performs the test and gives final result. The final result is an automatically calculated average result from the results of the tests of chambers A, B, C, D.

5. Coefficient of variation (CV) %:

Coefficient of variation (CV) % is measured in Reference mode following the instruction for Reference testing. It is determined by analysis of standard reference milk samples from **certified dairy laboratories**. To determine the reference coefficient of variation (CV) % only such standard reference milk sample must be used. If nonstandard milk samples are used, a deviation of the CV% may be received. The standard reference milk samples do not include a large amount of cell fragments and do not include yeast cells. The presence of yeast cells in milk can easily be determined by examining the graph of the analyzed milk sample. Below you will find examples for graph of normal milk without yeast cells and graph of milk with yeast cells:

Normal milk **Milk with yeast cells**



6. Calculating Coefficient of variation (CV) %:

To calculate the Coefficient of variation (CV) %, following the above procedure, you have to test 10 times milk sample with somatic cell concentration 50000-200000 cell/ mL, to test 10 times milk sample with somatic cell concentration 400000-600000 cell/mL and to test 10 times milk sample with somatic cell concentration 800000-1400000 cell/mL. Then to fill in the received results in the table below.

Sample tables:

Empty

Raw data	Sample 50000-200000 cell/mL 1	Sample 400000-600000 cell/mL 2	Sample 800000-1400000 cell/mL 3
Measurement 1			
Measurement 2			
Measurement 3			
Measurement 4			
Measurement 5			
Measurement 6			
Measurement 7			
Measurement 8			
Measurement 9			
Measurement 10			
Mean value			
Standard deviation			
Coefficient of variation %			

Filled in

Raw data	Sample 50000-200000 cell/mL 1	Sample 400000-600000 cell/mL 2	Sample 800000-1400000 cell/mL 3
Measurement 1	142 000	571 000	1 153 000
Measurement 2	161 000	563 000	1 164 000
Measurement 3	147 000	548 000	1 146 000
Measurement 4	157 000	530 000	1 142 000
Measurement 5	142 000	543 000	1 146 000
Measurement 6	145 000	584 000	1 122 000
Measurement 7	151 000	558 000	1 100 000
Measurement 8	150 000	584 000	1 154 000
Measurement 9	140 000	539 000	1 120 000
Measurement 10	145 000	570 000	1 155 000
Mean value	**148000**	**559000**	**1140200**
Standard deviation	6815	18708	19904
Coefficient of variation %	4,60	3,35	1,75

Formulas for calculating:

Mean value – The all 10 measurements were summed and then divided into 10 (number of samples).

Standard deviation in statistics, typically denoted by σ, is a measure of variation or dispersion (refers to a distribution's extent of stretching or squeezing) between values in a set of data. The lower the standard deviation, the closer the data points tend to be to the mean (or expected value), μ. Conversely, a higher standard deviation indicates a wider range of values.

$$s = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(x_i - \overline{x})^2},$$

Where:

xi is one sample value

$\bar{x}$ is the sample mean

N is the sample size

Coefficient of variation: CV, % = Standard deviation x 100/mean value.

The above table can be downloaded from: https://autocellcount.com/page/coefficient-of-variation

7. LACTOSCAN SCC setting:

For measuring standard reference milk samples from certified dairy laboratory and calculating the Coefficient of variation (CV) %, LACTOSCAN SCC must be set as follow:

- Minimum cell size in micrometer : 6
- Maximum cell size in micrometer : 20

The Menu Options allows the user to set the analysis parameters for different types of milk.

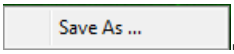
Saving image from the analysis as .BMP file

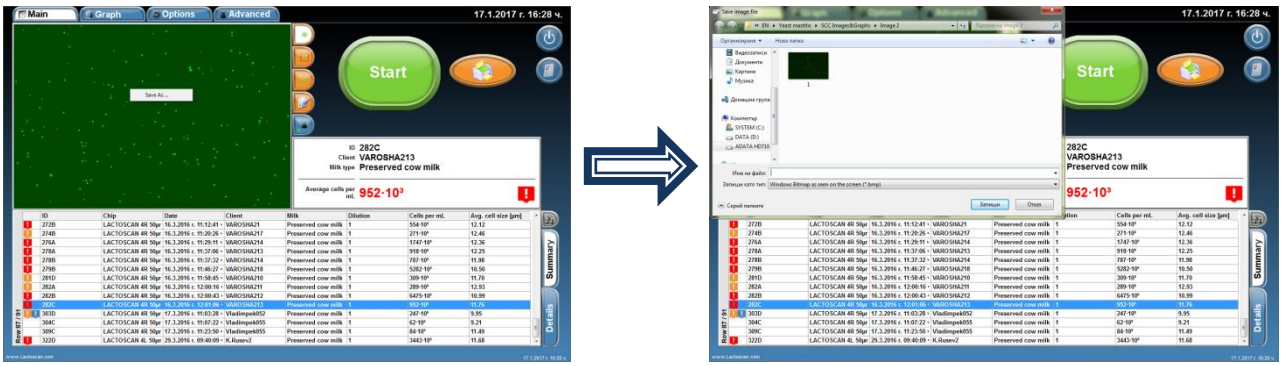
LACTOSCAN SCC allows Users to save an image from already done analysis as .BMP file. The .BMP file is easy for further process and can be opened by or imported in any Windows program.

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  .



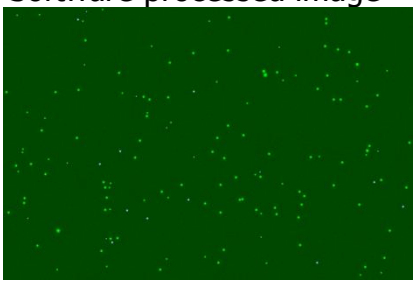
3. Select the folder where you want to save the image, write the name of the image, select the .BMP file format from the dropdown menu and press button SAVE to finally save the image as .BMP file.

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.

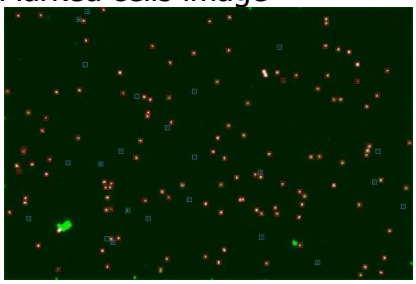
Before saving the image, the User can select a visualization mode such us Software processed image, Marked cells image, Raw image and to save in .BMP file the image as it is seen in the software.

Examples:

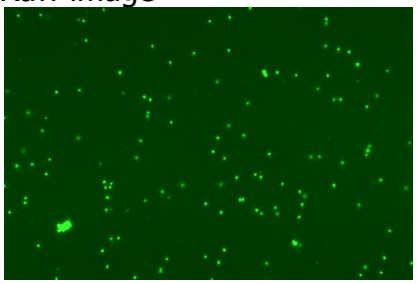
Software processed image



Marked cells image



Raw image



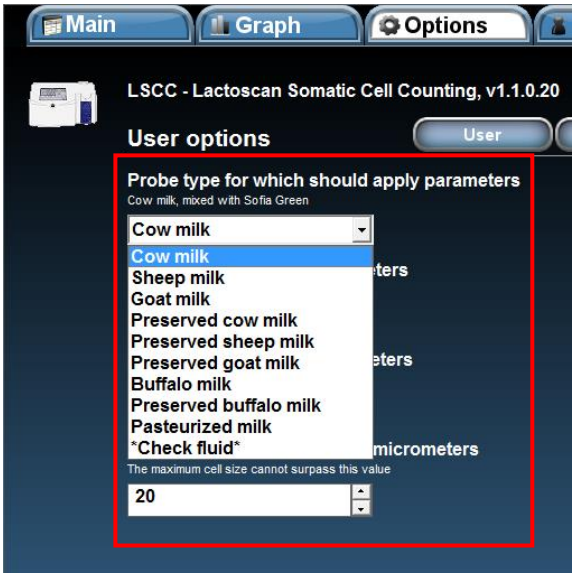
Setting the analysis' parameters

The Menu Options allows the user to set the analysis parameters for different types of milk.

1. Setting the minimum and maximum size of the cells:

1.1. Choosing the sample of milk, for which the set parameters have to be applied.

With the assistance of the drop-down menu, choose the sample of milk for which change of the pre-set parameters is needed.



1.2. Filling in the required parameters.

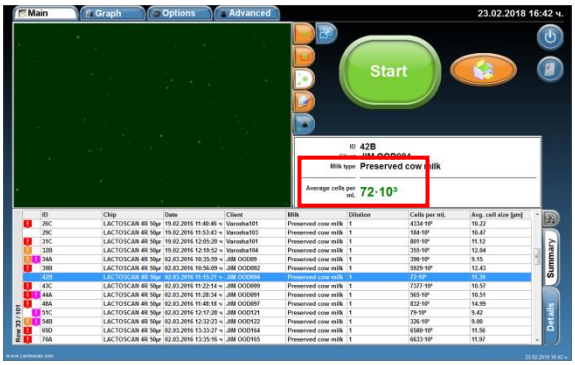
When the milk sample is chosen, pre-set values of the minimum and maximum size of the cells, which will be counted appear below it. With up and down arrows or by manual entry of value, the range of the counted cells may be changed according to the certain type of milk.

1.3. Setting the limits of the clinical and subclinical mastitis.

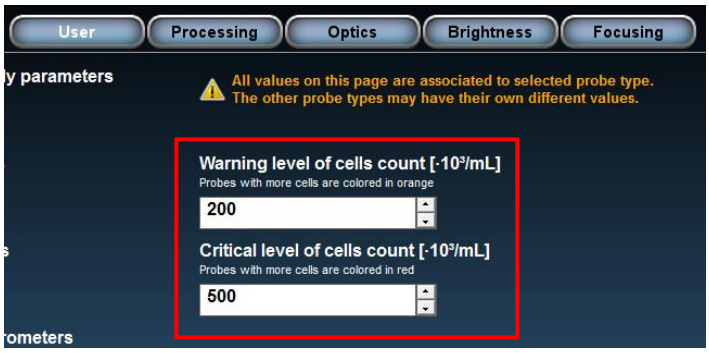
According to the breed of the animal, the local and state requirements in the different countries, the limits of the clinical and subclinical mastitis may differ.

For example in Germany it is accepted that the healthy animals are these, which milk contains somatic cells up to 100 000 pcs/ml. Over 100 000 pcs/ml up to 400 000 pcs/ml the animal has a subclinical mastitis. And over 400 000 pcs/ml the animals has a clinical mastitis.

By coloring the analysis result in green, orange and red, the device signalizes for the health condition of the animals.



Setting the limits of the subclinical mastitis is made by changing the value in the field "Warning level of cells count". Changing of the value is made with up and down arrows or by setting the value manually.
Setting the limits of the clinical mastitis is made by changing the value in the field "Critical level of cells count". Changing of the value is made with up and down arrows or by setting the value manually.



1.4. Saving the entered settings

By press the button 

, all entered settings are saved in the program. After being saved, the button changes its color from green to grey.

2. Setting the Laboratory offset in percentages:

It is accepted that there is a difference of the results from analysis of one and same milk sample between two laboratories. According to ISO 13366-1:2008 "Milk – Enumeration of somatic cells – Part 1: Microscopic method (Reference method) », Annex A Collaborative trail, Table A. 1 – Results from interlaboratory test, the deviation between two laboratories can be up to 20%. If LACTOSCAN SCC is checked by using reference samples from local laboratory, a difference in the results between the reference samples value and the results from LACTOSCAN SCC can be received. In this case, a correction using the option Laboratory offset can be done by filling in a number indicating the percentages of correction.

The Laboratory offset number can be:

0 (zero) – meaning that no correction is done.
+N (positive number) – meaning that positive correction in percentages % is done. The final result of the analysis is increased with the set percentages of correction.
– N (negative number) – meaning that negative correction in percentages % is done. The final result of the analysis is decreased with the set percentages of correction.

Attention!
To determine the need of correction and to do a correction, the instructions below must be strictly followed:

- For determining the deviation, to be used **only reference milk samples from IDF accredited laboratory**.

- To be used minimum 3 different reference samples with the following ranges of cells concentration:

1st Reference sample cell concentration range: **50 000 – 200 000 cell/mL**
2nd Reference sample cell concentration range: **400 000 – 600 000 cell/mL**
3rd Reference sample cell concentration range: **800 000 – 1 200 000 cell/mL**

- Reference samples must be measured in **Reference mode** of LACTOSCAN SCC following the procedure for Reference sample testing.

- The received results from the analyses to be filled in the Excel table for calculation of Laboratory offset available at:
http://lactoscan.com/editor/ufo/files/Laboratory_offset_table.xlsx

Or to be written in a table shown below:

Raw data	Sample 50000-200000 cell/mL 1	Sample 400000-600000 cell/mL 2	Sample 800000-1400000 cell/mL 3
Measurement 1			
Measurement 2			
Measurement 3			
Measurement 4			
Measurement 5			
Measurement 6			
Measurement 7			
Measurement 8			
Measurement 9			
Measurement 10			
Mean value Reference sample value Difference in percentages			
Average deviation			

- The Mean values (Average results) for each sample to be calculated using the formula below:

$$MV = \frac{M1+M2+M3+M4+M5+M6+M7+M8+M9+M10}{10}$$

Where:

MV – Mean value (Average result) from the results of the 10 measurements of one and same reference sample

M1,M2,M3,M4,M5,M6,M7,M8,M9,M10 – Results from the ten measurements of the one and same reference sample

- The received Mean values (Average results) from the calculation of all three samples to be compared with the reference sample values from the labels of the analyzed reference samples. The difference in percentages between each the Mean value and the Reference sample value to be calculated using the formula below:

$$D1\% = 100*(RSV1-MV1)/\ RSV1$$

Where:

D1% - the difference in percentages for 1st reference sample
RSV1 – 1st Reference sample value from the label
MV1 – Mean value for 1st sample

- **The difference in the received results for the three samples must be only positive (+) value or only negative (-) value for all the three of them**. **If you**

receive positive (+) and negative (-) values, this means that there is an inaccuracy in the measurement, according to the value of the reference sample, written on the label of the sample. In such cases no corrections should be done, until the correctness of the laboratory samples is proven.

- If the results from the three samples are all positive (+) or all negative (-), then the deviation in percentages has to be calculated. This is done using the formula below:

$$D\% = \frac{D1\%+D2\%+D3\%}{3}$$

Where:

D% - the average deviation of the three samples
D1% - the difference in the received results for the first sample in percentages
D2% - the difference in the received results for the second sample in percentages
D3% - the difference in the received results for the third sample in percentages

After calculating the average deviation for the three samples, the received number can be filled in the field for Laboratory offset.

Sample table with results from calculation of Laboratory offset:

Raw data	Sample 50000-200000 cell/mL 1	Sample 400000-600000 cell/mL 2	Sample 800000-1400000 cell/mL 3
Measurement 1	142 000	571 000	1 153 000
Measurement 2	161 000	563 000	1 164 000
Measurement 3	147 000	548 000	1 146 000
Measurement 4	157 000	530 000	1 142 000
Measurement 5	142 000	543 000	1 146 000
Measurement 6	145 000	584 000	1 122 000
Measurement 7	151 000	558 000	1 100 000
Measurement 8	150 000	584 000	1 154 000
Measurement 9	140 000	539 000	1 120 000
Measurement 10	145 000	570 000	1 155 000
Mean value	**148000**	**559000**	**1140200**
Reference sample value	156000	565000	1230000
Difference in percentages	5,13	1,06	7,30
Average deviation	4,50		

Excel formulas for calculating:

Mean Value: =AVERAGE(Measurement 1: Measurement 10)
Reference sample value: fill in the value from the label of the reference sample
Difference in percentage: =100*(Reference sample value-Mean value)/Reference sample value

Average deviation: =(Difference in percentages for sample 50000-200000 cell/mL + Difference in percentages for sample 400000-600000 cell/mL + Difference in percentages for sample 800000 -1400000 cell/mL)/3

To do this:

2.1. From Main menu go to Options menu:

2.2. Enter the calculated deviation number.

Note! If you have received 5% deviation, you should enter number 5. If you have received -5% deviation, you should enter number -5.

2.3 Press button 

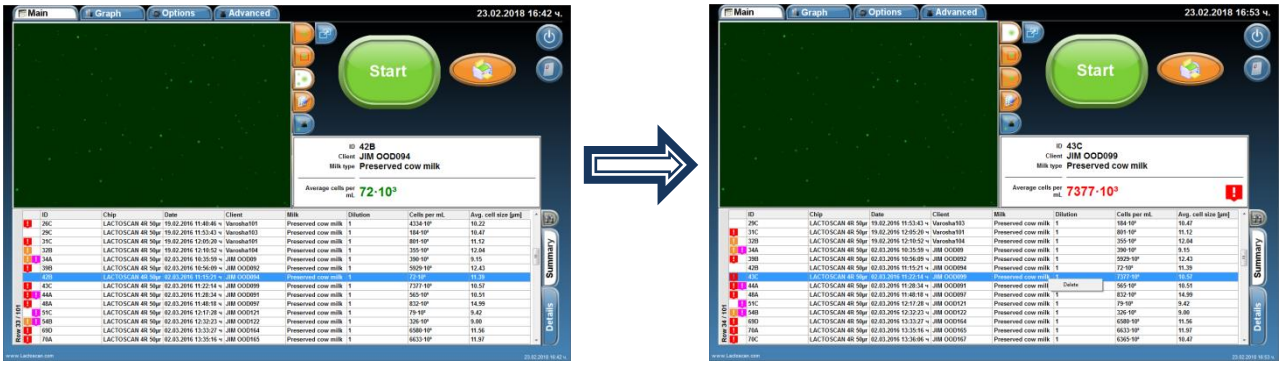
.

To restore the factory settings, number 0 must be filled in the Laboratory offset field.

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 analyzes, the entire database to be deleted to make space for storing the results of the upcoming analysis.

To clean the Database:

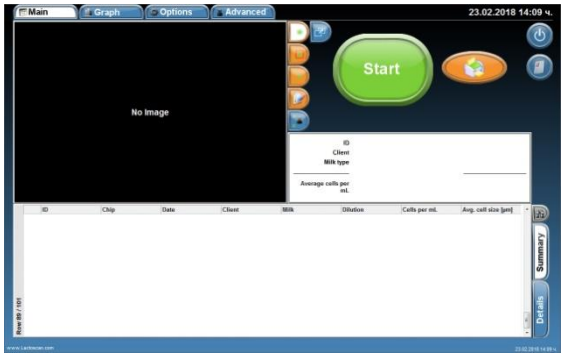
1. Go to menu Options

2. Press button 

3. From the drop-down menu select Clean Database

4. Press Clean Database

Attention!
Once deleted, the results cannot be restored!



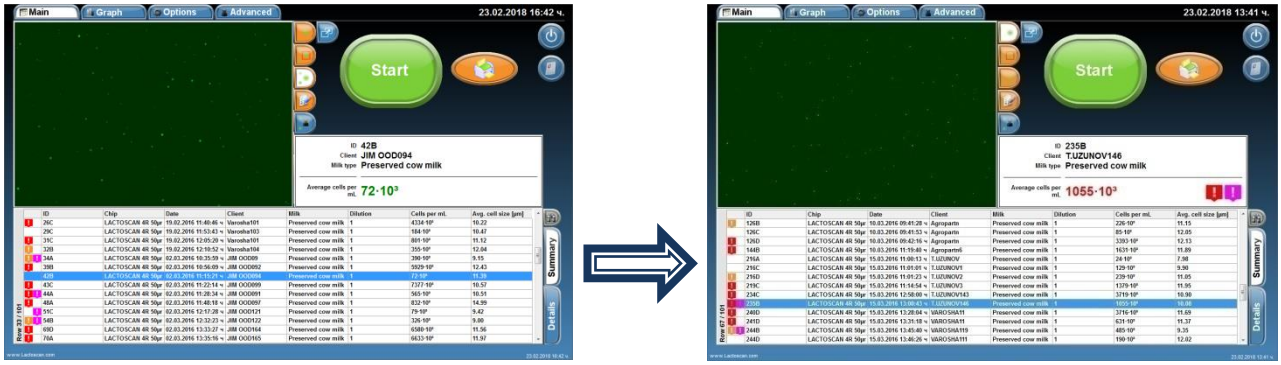
Printing the results from the data base

LACTOSCAN SCC 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.

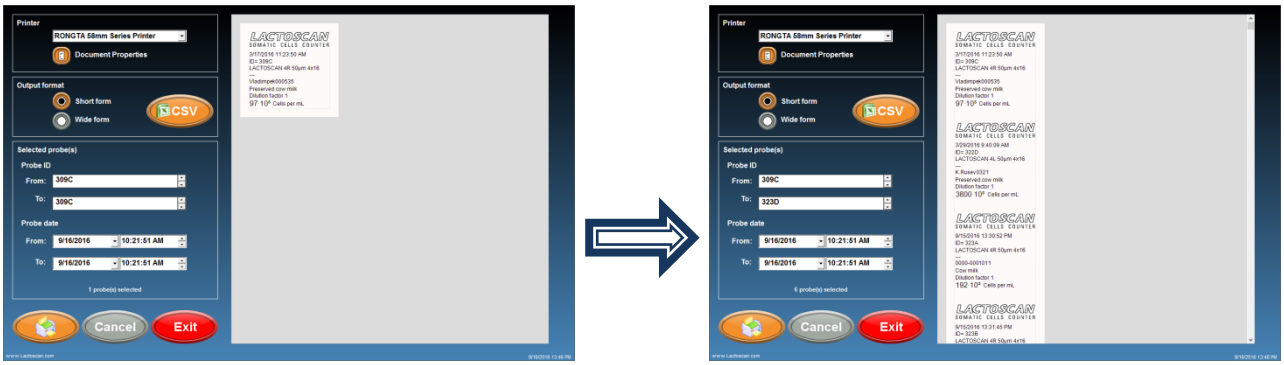
It is marked by pushing onto the information for the relevant analysis in the field "Summary".



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 LACTOSCAN SCC will print the results on the integrated printer in a short form.

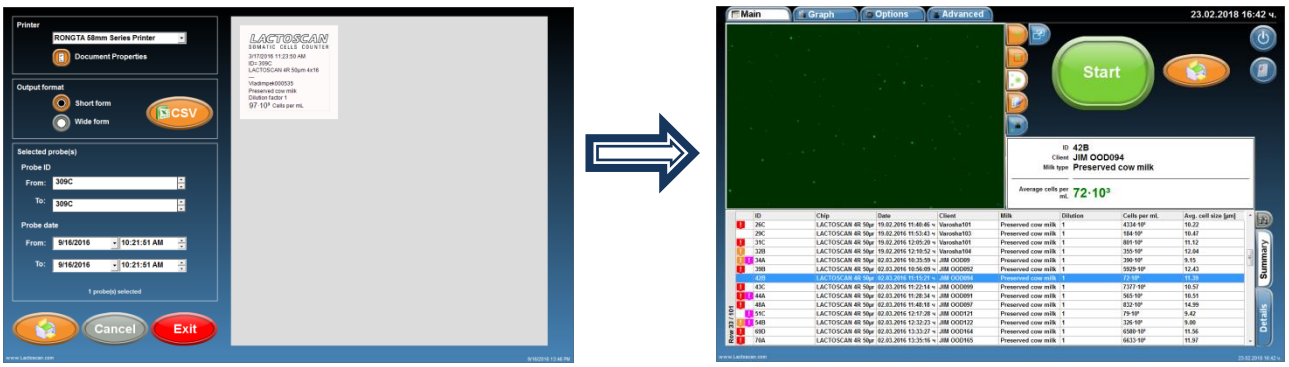
4. Push the arrows from the right side of the results ID if changing or adding new results for printing is needed.



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  **.**

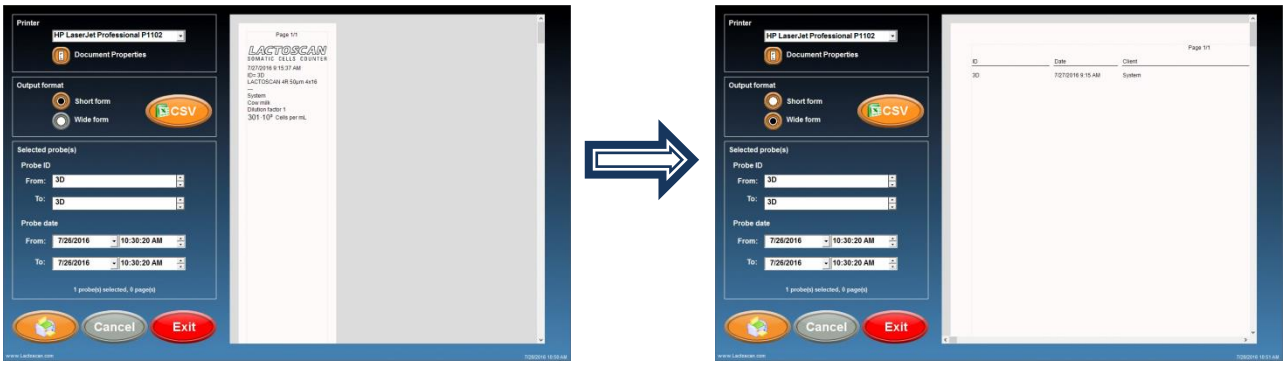


Printing the results on an external printer:

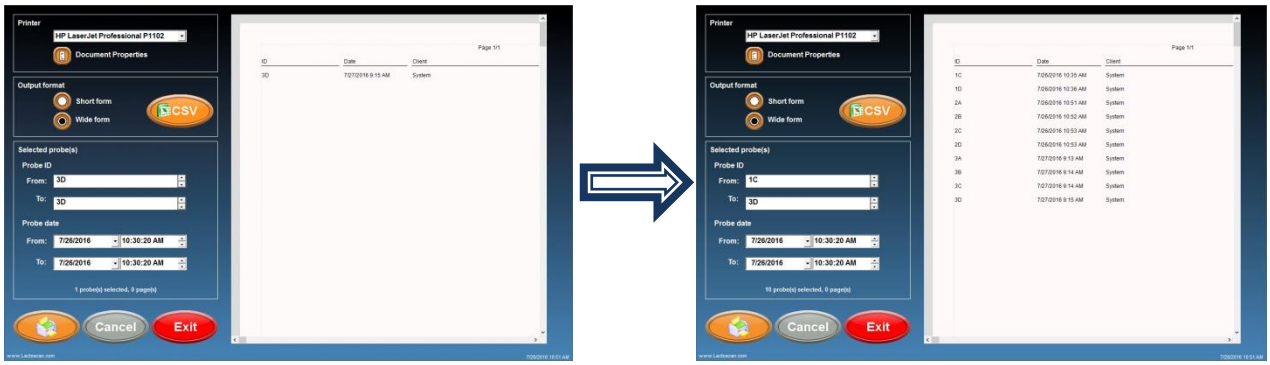
1. Before start printing, make sure that the external printer is connected and installed.

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.**

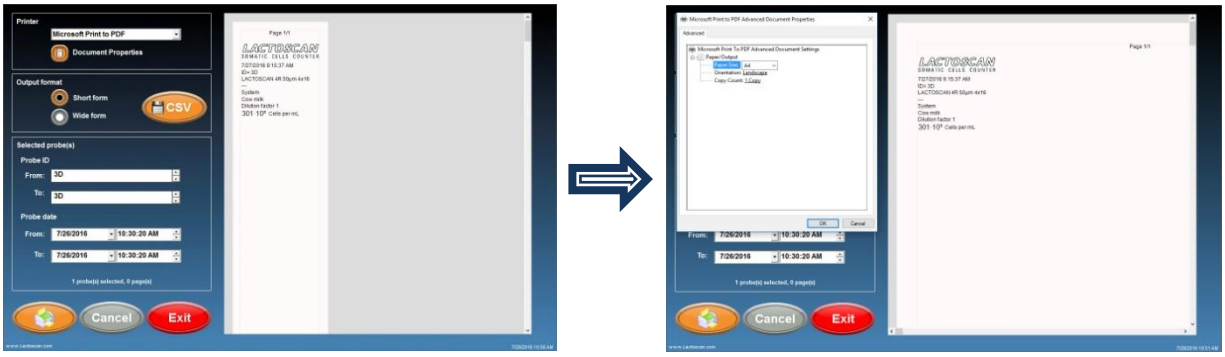
6. To go back to the Main menu, press  **.**

Saving the results in PDF file:

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

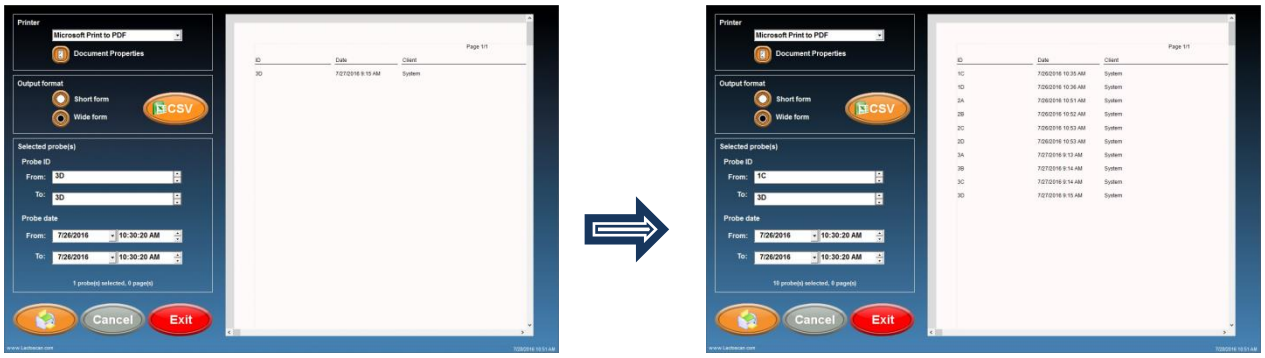
2. Before saving, check the orientation of the page.

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.

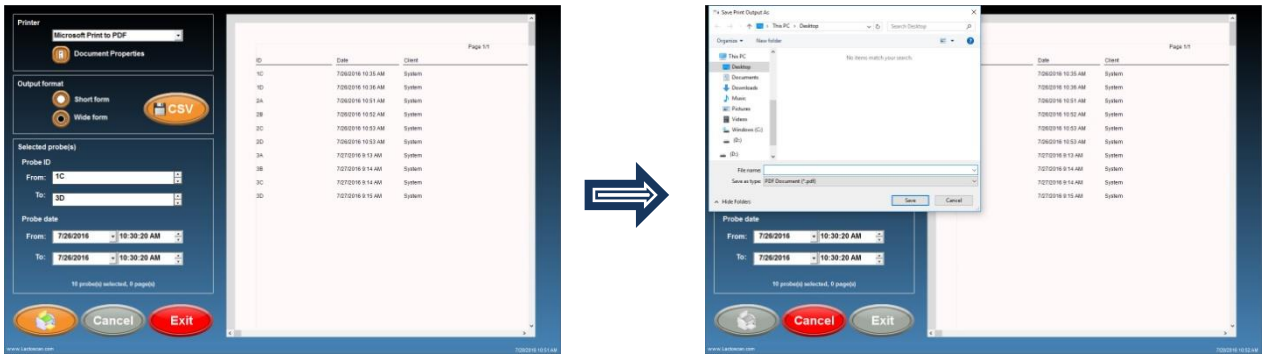


3. Choose Wide form for printing the results in a table.

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



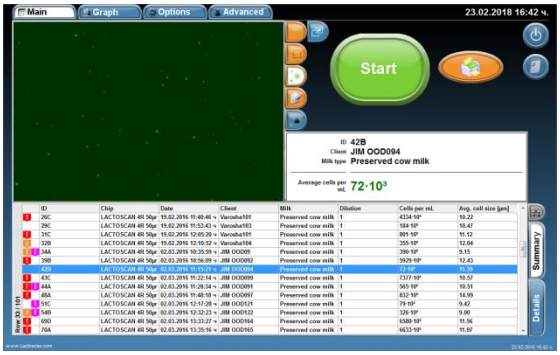
5. Press button  **to save the file.**

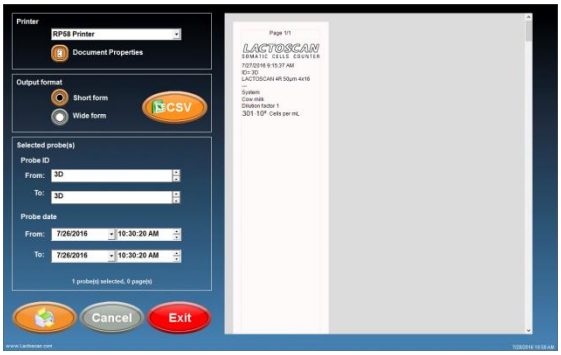


6. Select the folder where you want to save the file, write the name of the file and press button SAVE to finally save the results in PDF file.

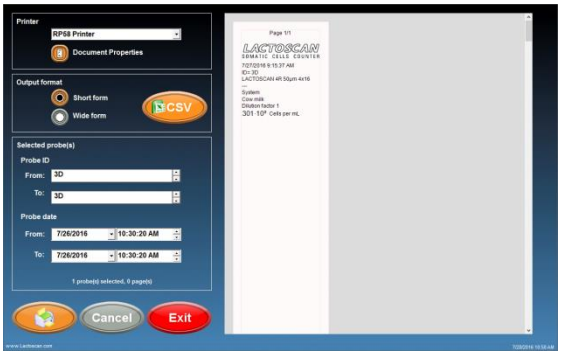
Saving the results in Excel file:

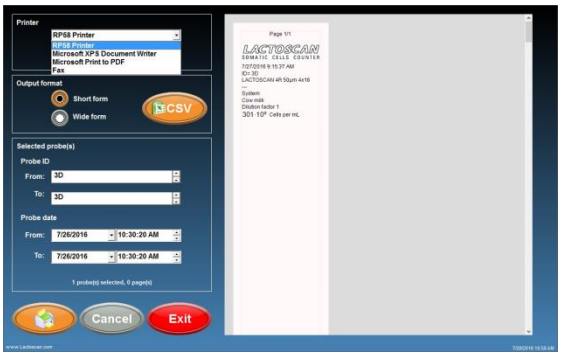
1. Go to printing mode by pressing the button  **.**



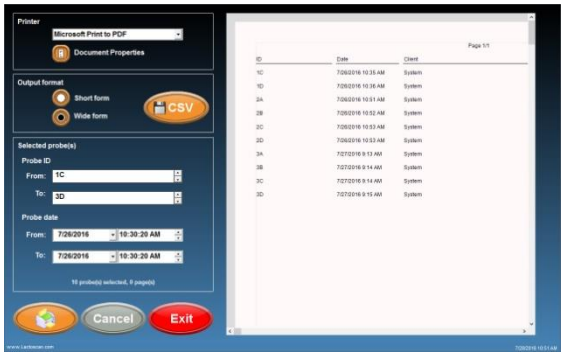


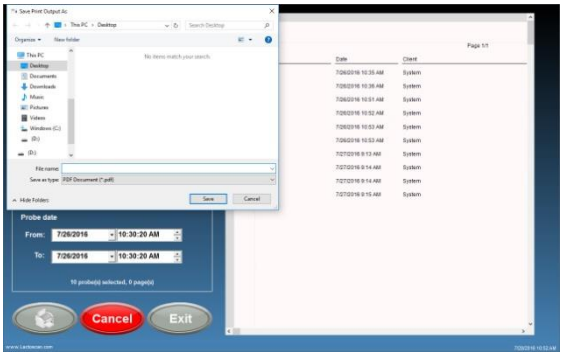
2. Add the results you want to be included in the Excel file.



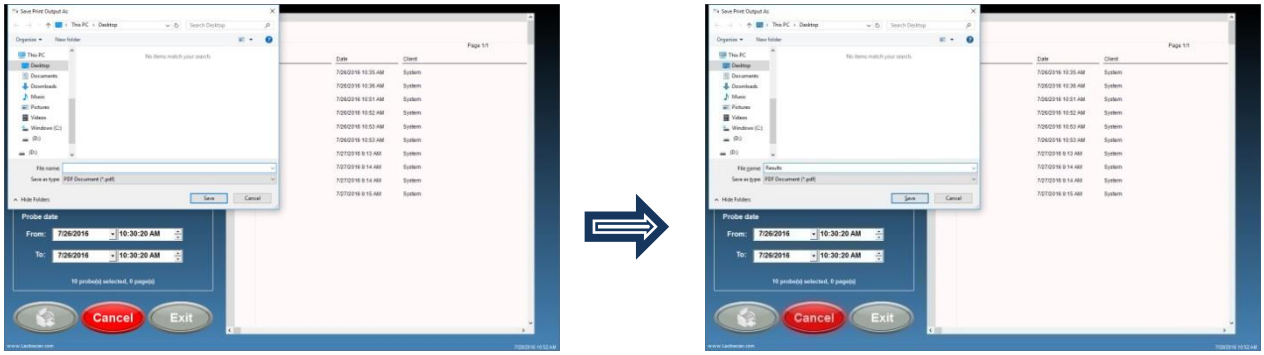


3. Press button  **to save the file.**





4. 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 LACTOSCAN SCC

Focusing of the device must be done every 3 months.

In order to focus the device:

1. Preparing cow milk sample with 300-500x10^{3}somatic cells per milliliter.

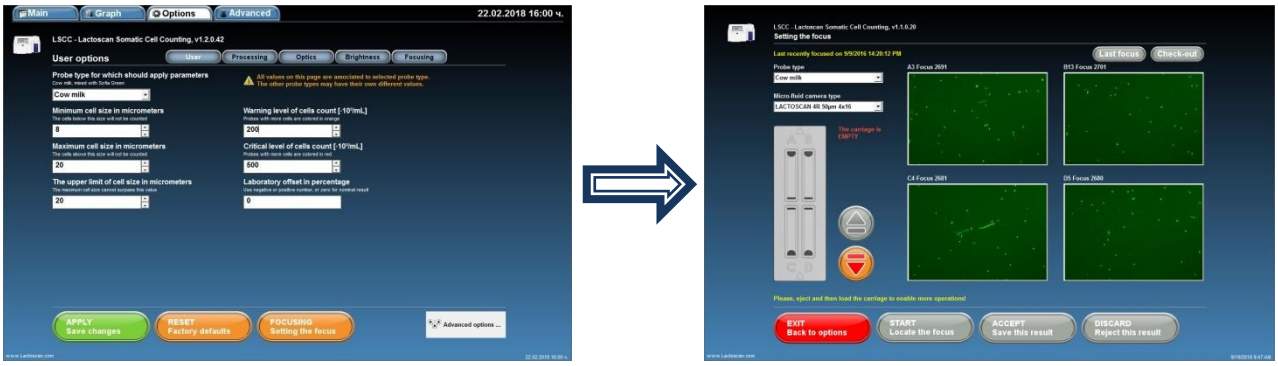
It may also be used a cow milk sample from a previous analysis with a result between 300x10^3 and 500x10^3 somatic cells per milliliter.

If such a cow milk sample is not available, it must be used such cow milk sample for which it is supposed that the presence of somatic cells is between 300x10^{3}and 500x10^3 somatic cells per milliliter. The cow milk sample must be prepared for analysis as per the instructions from section "Preparing of the sample for analysis"**.**

2. Focusing of LACTOSCAN SCC

2.1. From the screen "Options" press the button 

, in order to proceed to the screen "Focusing". In the four squares the last found focuses are seen.

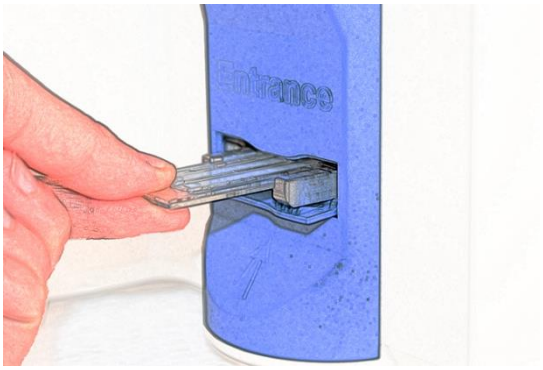


2.2. From the drop-down menu "Type of sample", choose cow milk.

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

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

2.5. Place the loaded with the milk sample LACTOCHIP in the cartridge.

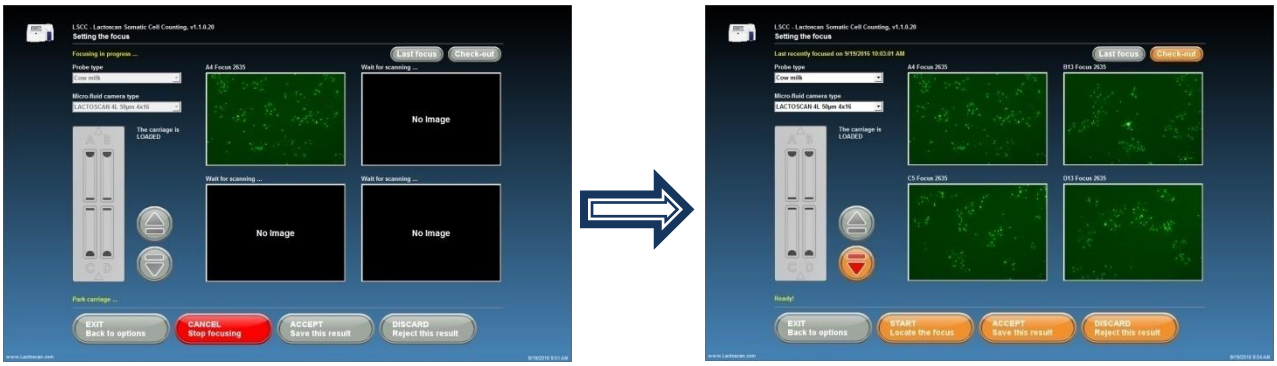


2.6. Press the button  .

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

2.7. Press the button  , to start focusing.

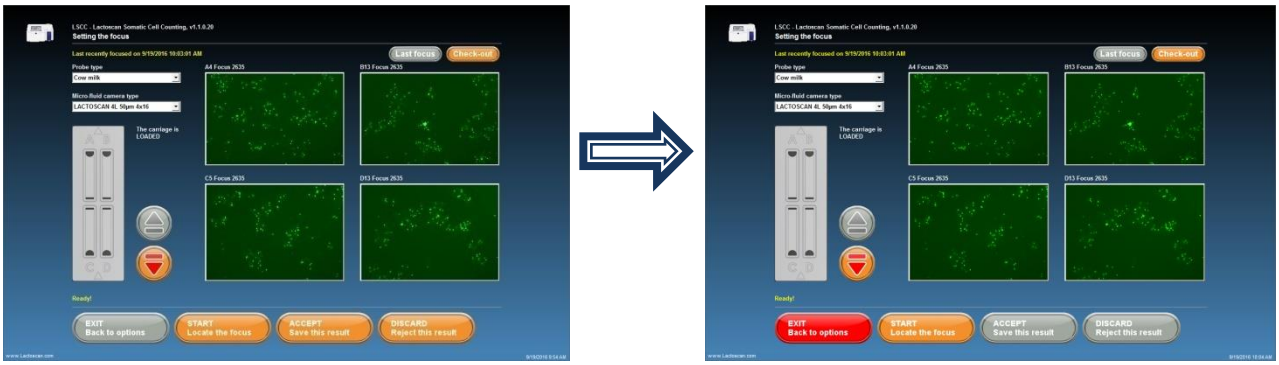
The device starts searching the focus of the four main points.



2.8. Press button 

, in order to save the new focuses or button 

, to reject them.



2.9. Press the button 

, in order to check the found focuses.

Work in Advanced mode:

1. Press , to eject the cartridge.

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

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

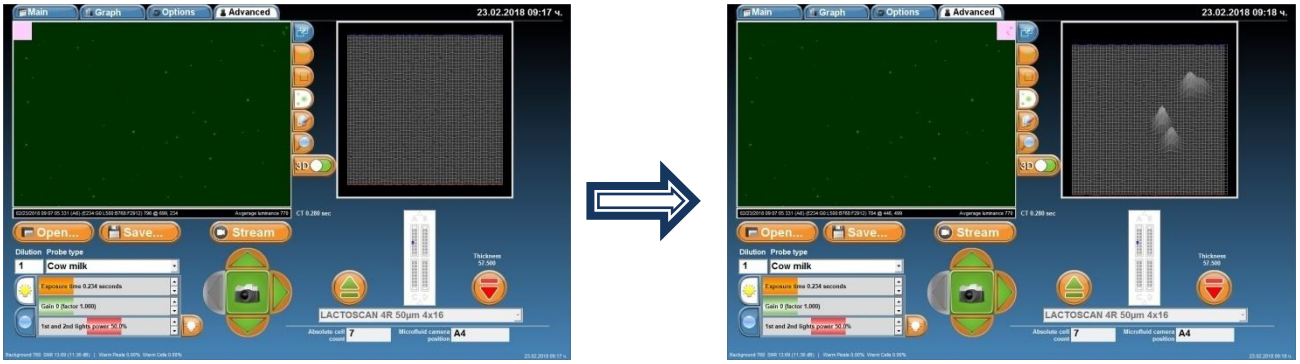
4. Choose a sector in the microfluidic camera, which is needed to be displayed.

In the sample it is microfluidic camera A, section 4

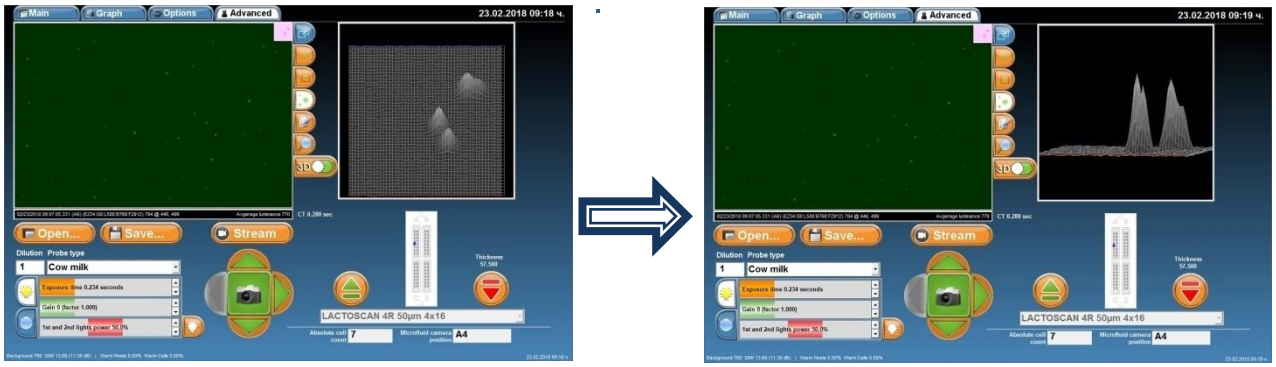
5. To make a picture and see the image of the chosen section, press  **.**

6. 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.**

7. 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.

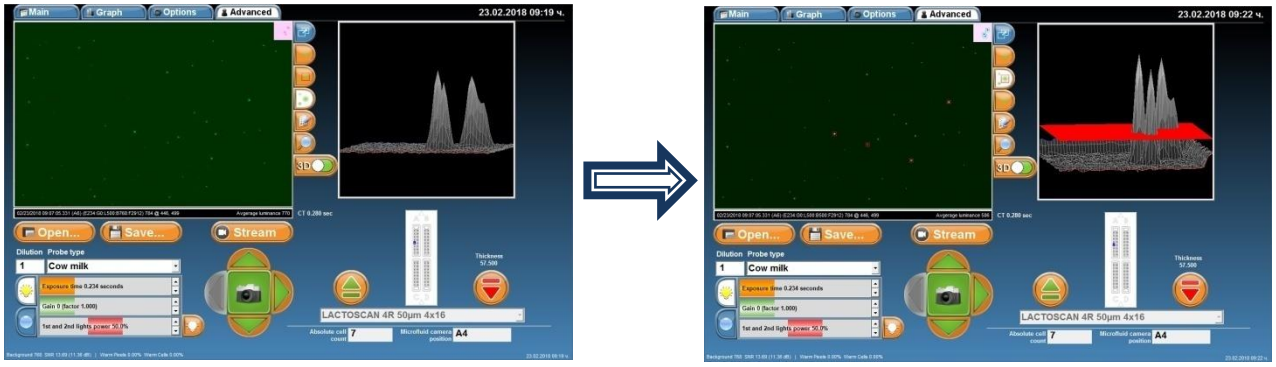


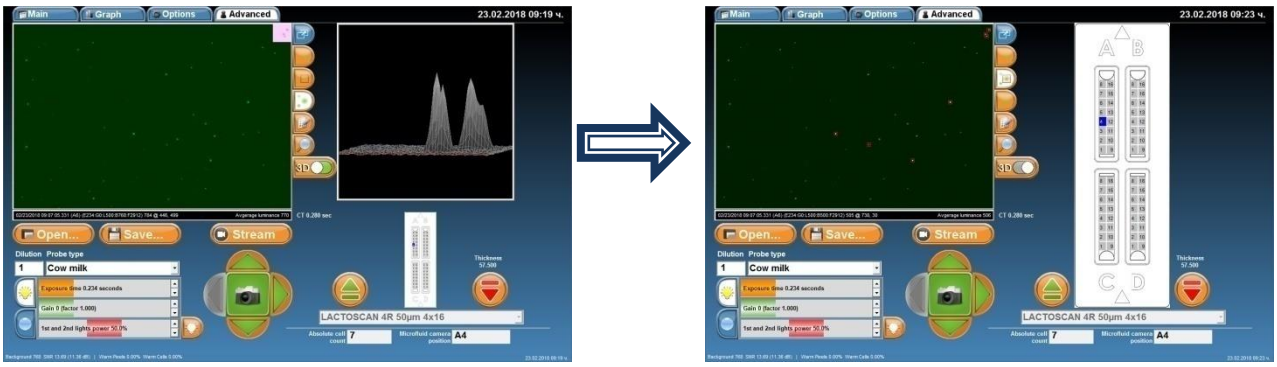
8. All the sides of the 3D graphics could be seen by pressing on it and moving up/down/left/right until the required field is seen. The mouse can be used by positioning on the 3D graphics; pressing and keeping the right button pressed and moving the mouse.



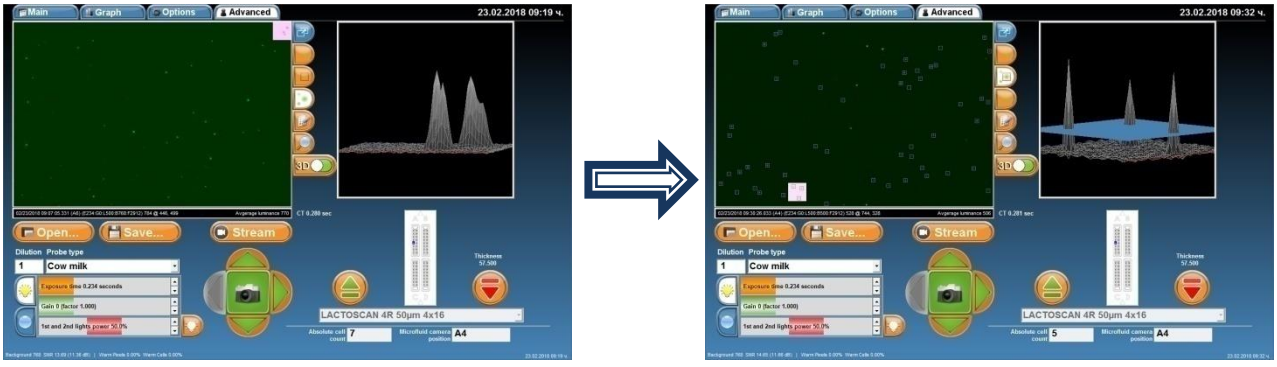
9. To see all cells of the image marked and to see the threshold level for mastitis cells, yeast cells or all cells, press button  **.**

Mastitis cells

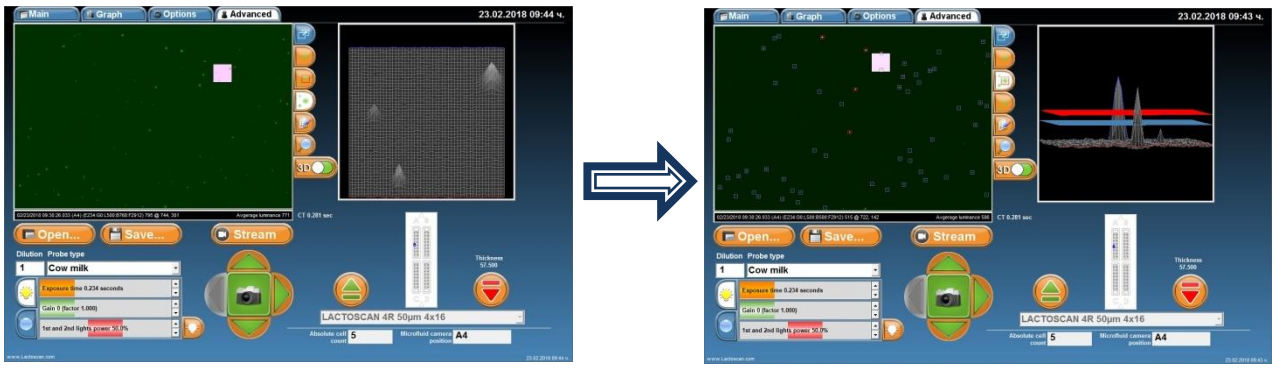


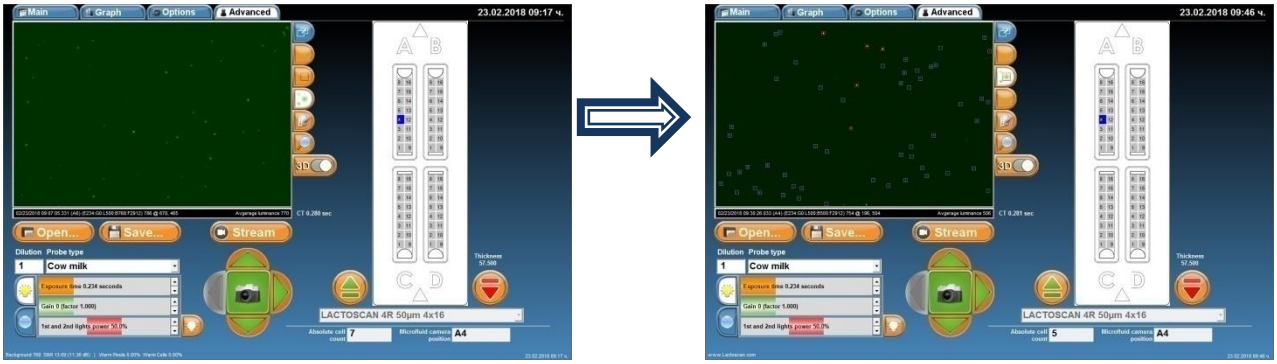


Yeast cells

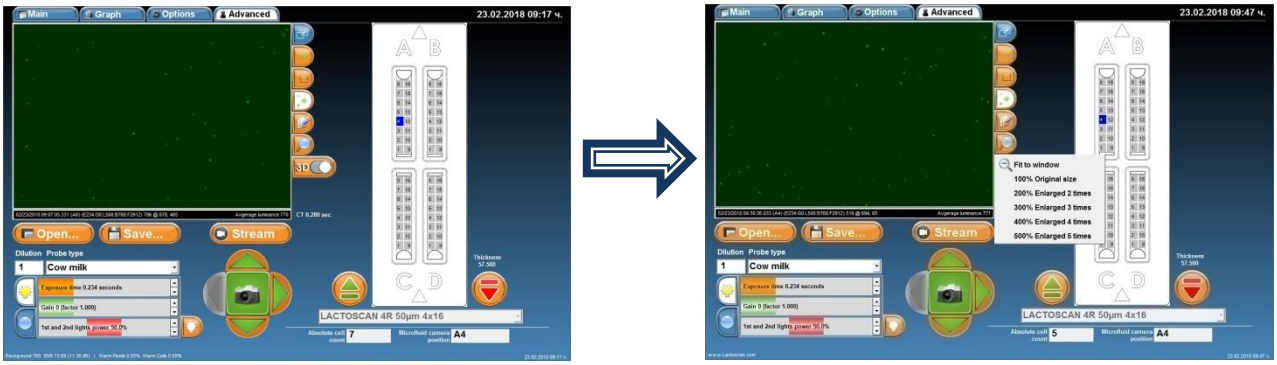


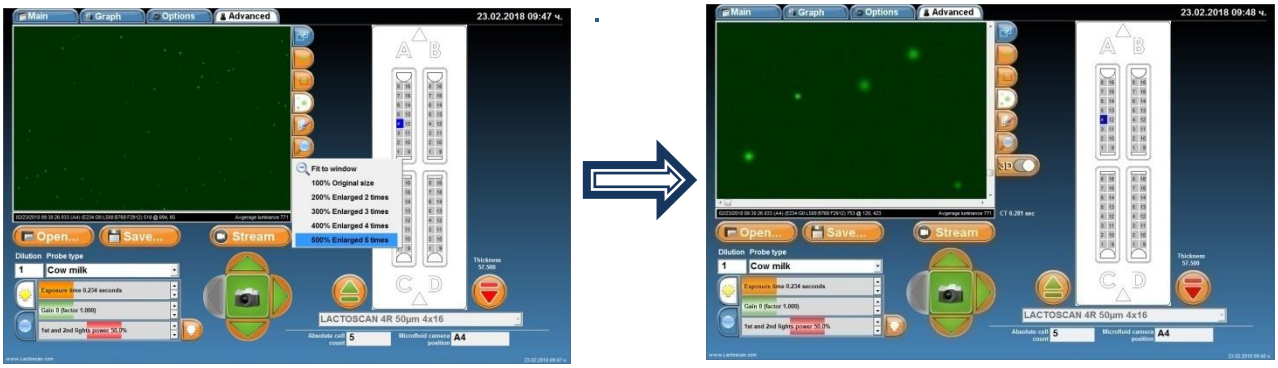
All cells





10. For magnification, press  **and choose % of magnification.**

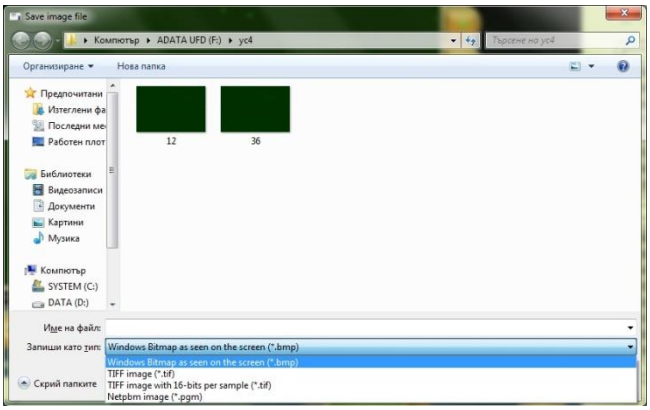




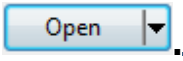
11. To save the image of the section, press 

. Write name of the file, choose where to save it and press 

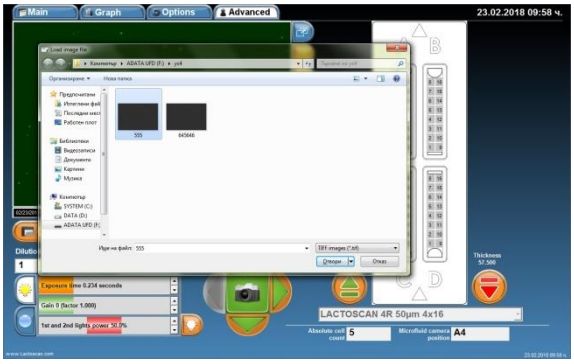
The User can select the format of the saved image. Possible formats are .TIFF and .BMP. The .BMP format is easy for further process and can be opened by or imported in any Windows program.



12. To open an image from previous analysis, press 

. Open the directory where the image was saved, click on it and press 

.



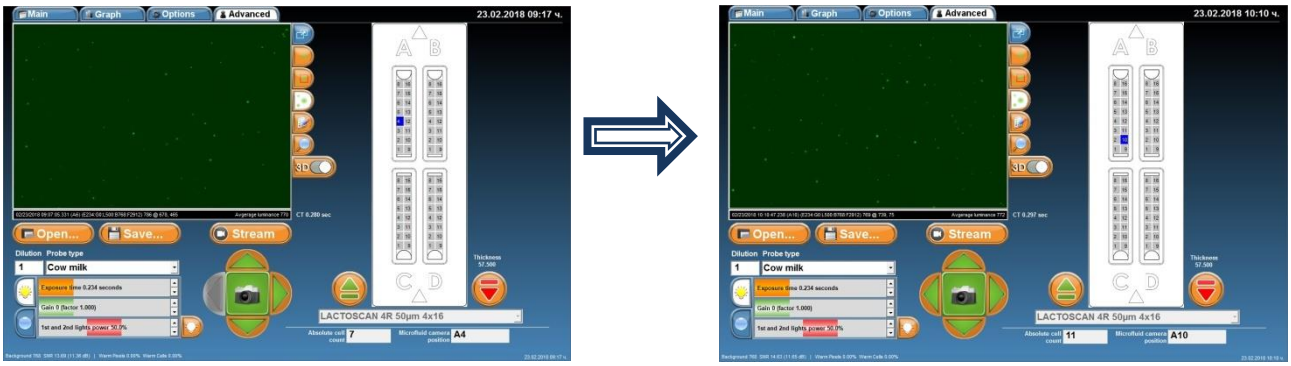
13. Button 

– after pressing the button 

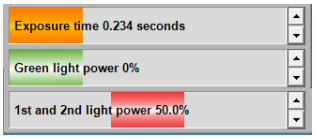
, mode Movie is activated and LACTOSCAN SCC starts making consecutive pictures as the time for changing the image depends on the set exposition time. For example, if it is set to 1.945 sec. exposition time, images on the display will be changed in approximately 1.945sec. Mode Movie is used when a number of sections of the microfluidic camera need to be seen. Instead of pressing the button  **after each new section chosen, LACTOSCAN SCC 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 (CCD GAIN).**

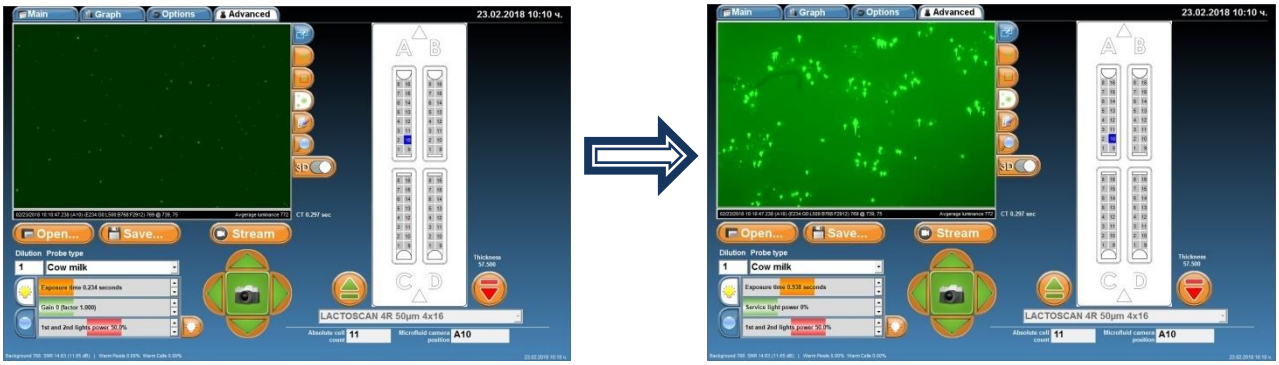
14. By pressing  **, different sections of the microfluidic camera can be chosen. Another section can be chosen by pressing its corresponding number. To make a picture and see the image of the newly chosen section, press**  **.**

In the example: section 10, microfluidic camera A.

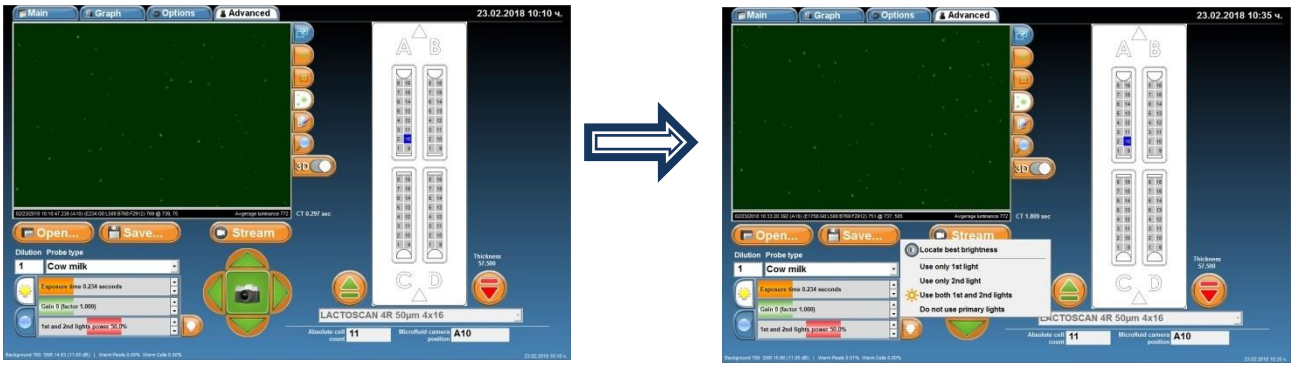


15. For setting the intensity of the LED 1 and 2 (if they are available) and exposition time, press  **.**

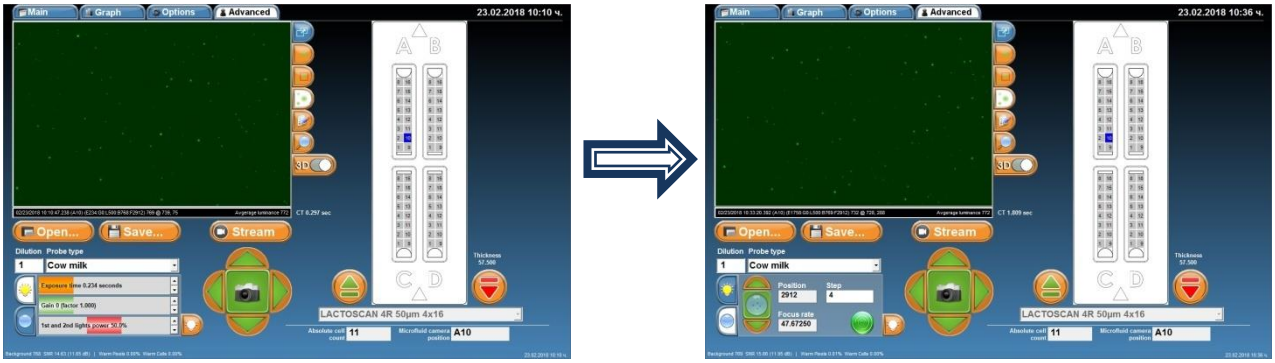
Using the buttons,  the needed values can be set.



16. To switch off/on one or both LEDs or to locate best brightness, press  **.**



17. To see the buttons used for setting the focus of the microscope, press  **.**



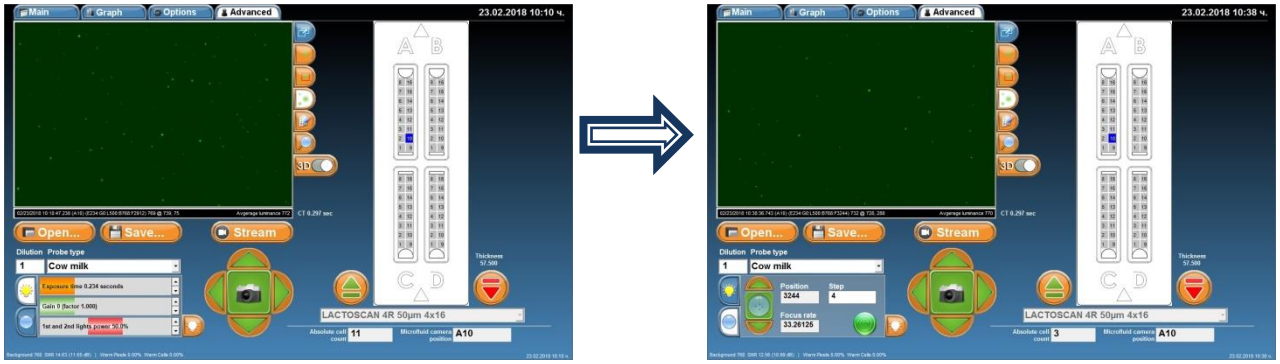
18. For autofocus, press  **.**

19. By pressing  **and**  **, manually set the autofocus 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.



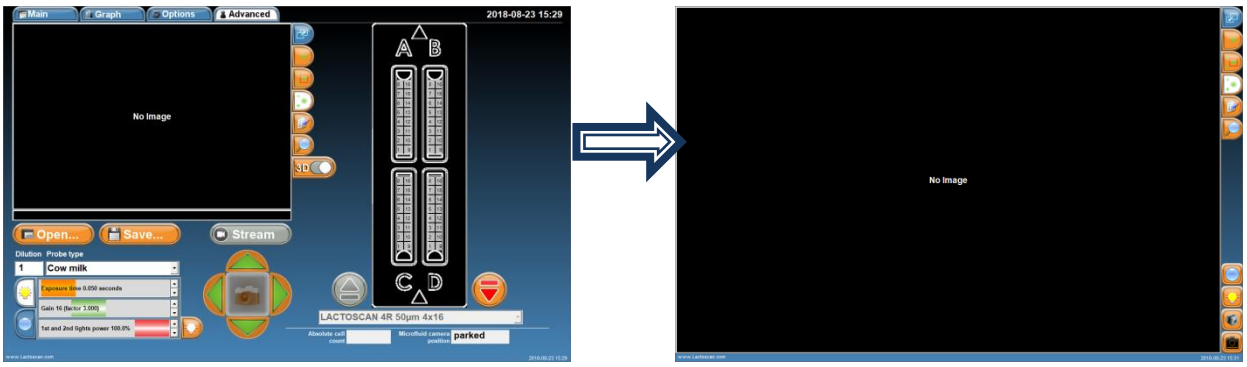
20. In order to see the graphics of the results of the current image, before passing to menu Graph, the dilution and type of the sample must be entered. Then press 

and  **.**

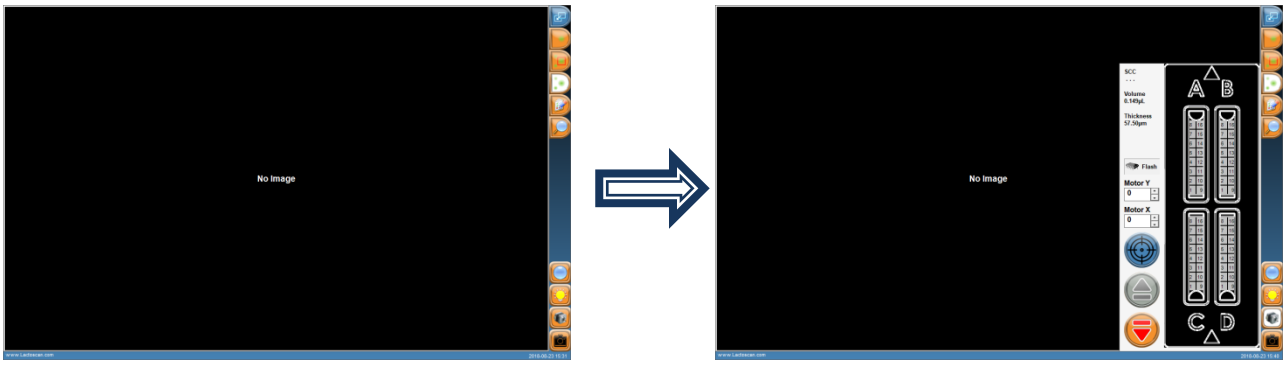
Check of LACTOSCAN SCC magnification using a graduated scale

The check of LACTOSCAN SCC magnification is done with the use of a special LACTOCHIP with a graduated scale.

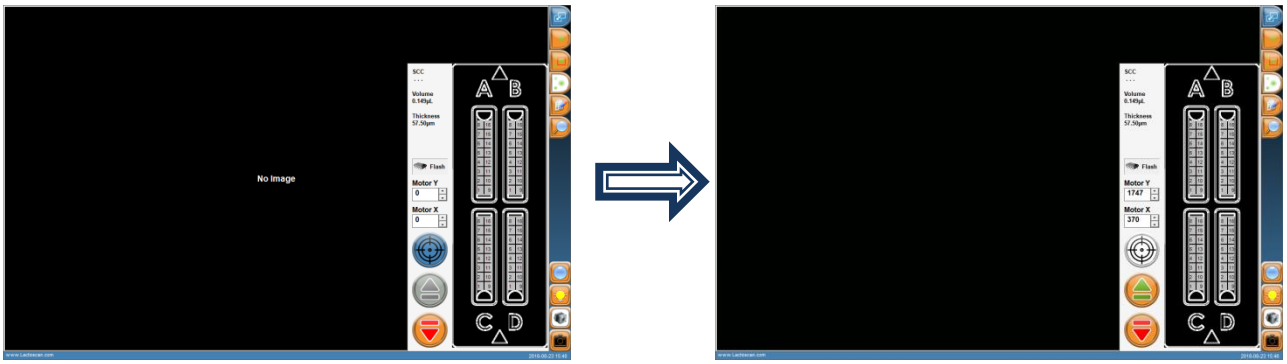
1. In order to perform the check, in menu"Advanced" press the button .

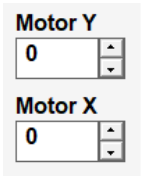


2. Press the button to open the menu for positioning of LACTOSCAN SCC microscope on the graduated scale.

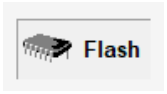


3. Press to position the microscope on the graduated scale.



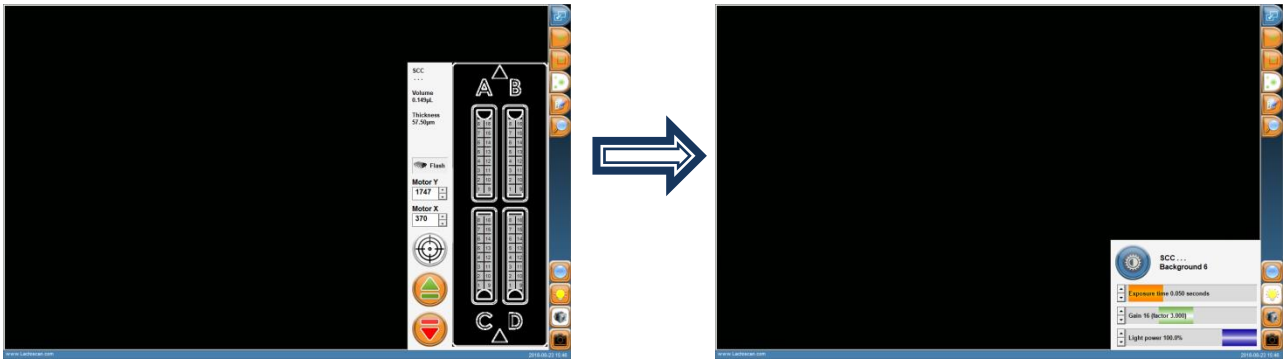
3.1. Use Motor Y and Motop X 

for a more precise positioning of the microscope on the graduated scale.

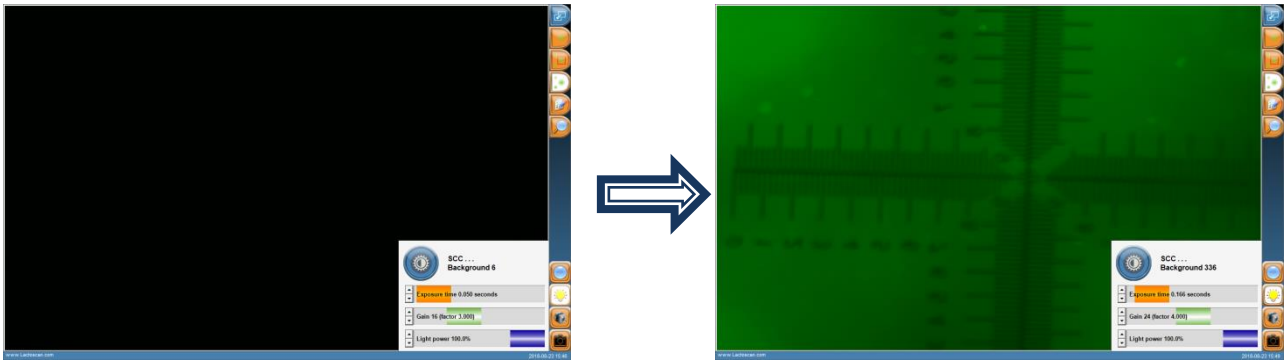
3.2. Button Flash 

opens a menu, which is used only by a trained technician. All changes, made in this menu, may lead to malfunctions of the device.

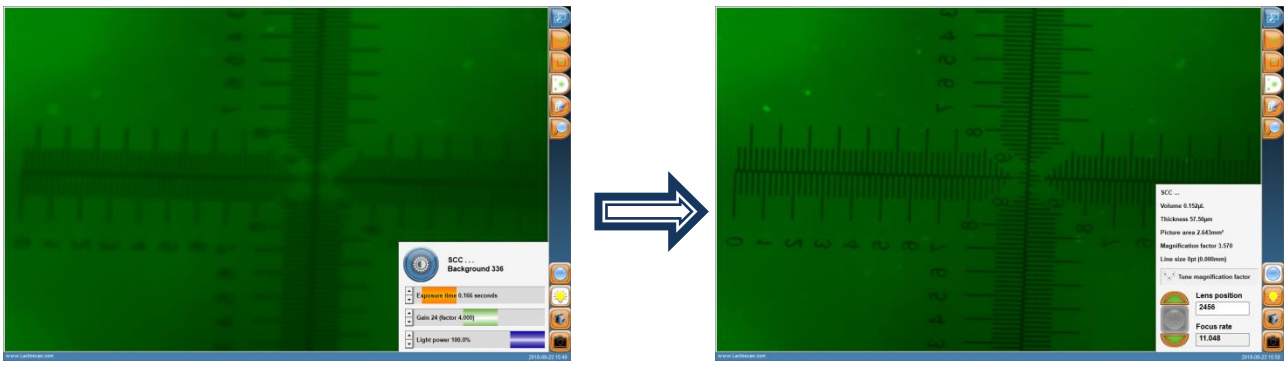
4. Press the button  to open the menu for setting of the intensity of LED.



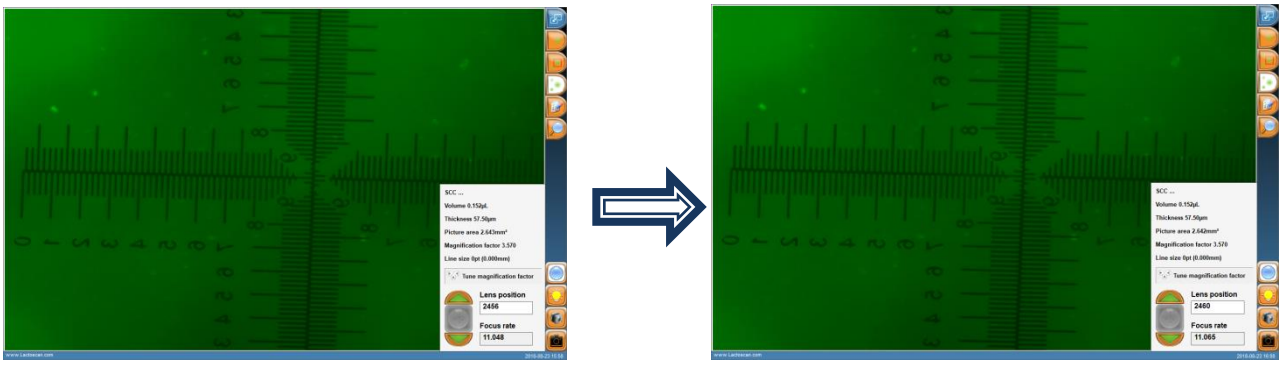
For an automatic finding of the best intensity for the LED, press the button  . If the image doesn't seem light enough or the graduated scale seems too dark, use the sliding button to set the exposition.



5. Press the button  in order to proceed to the menu for check.



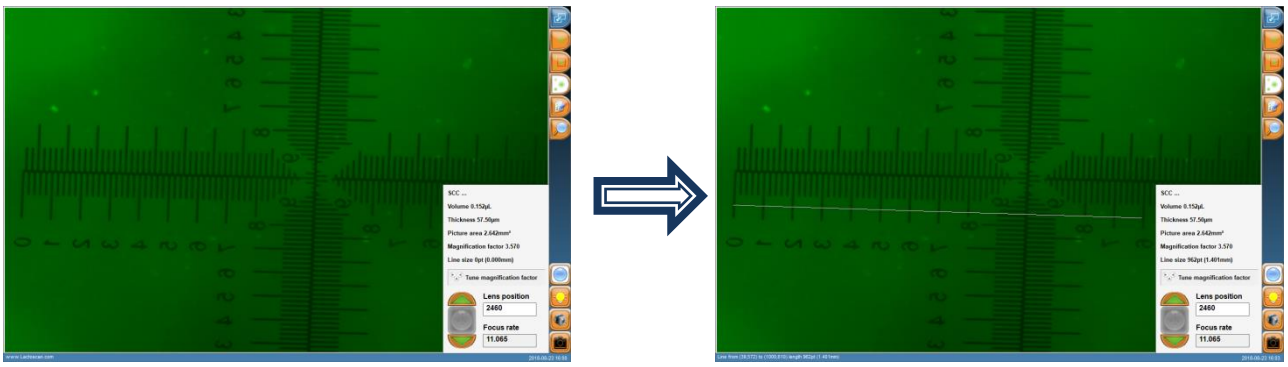
5.1. By using the buttons  and  , choose the best Lens position, at which the Focus rate is the highest.



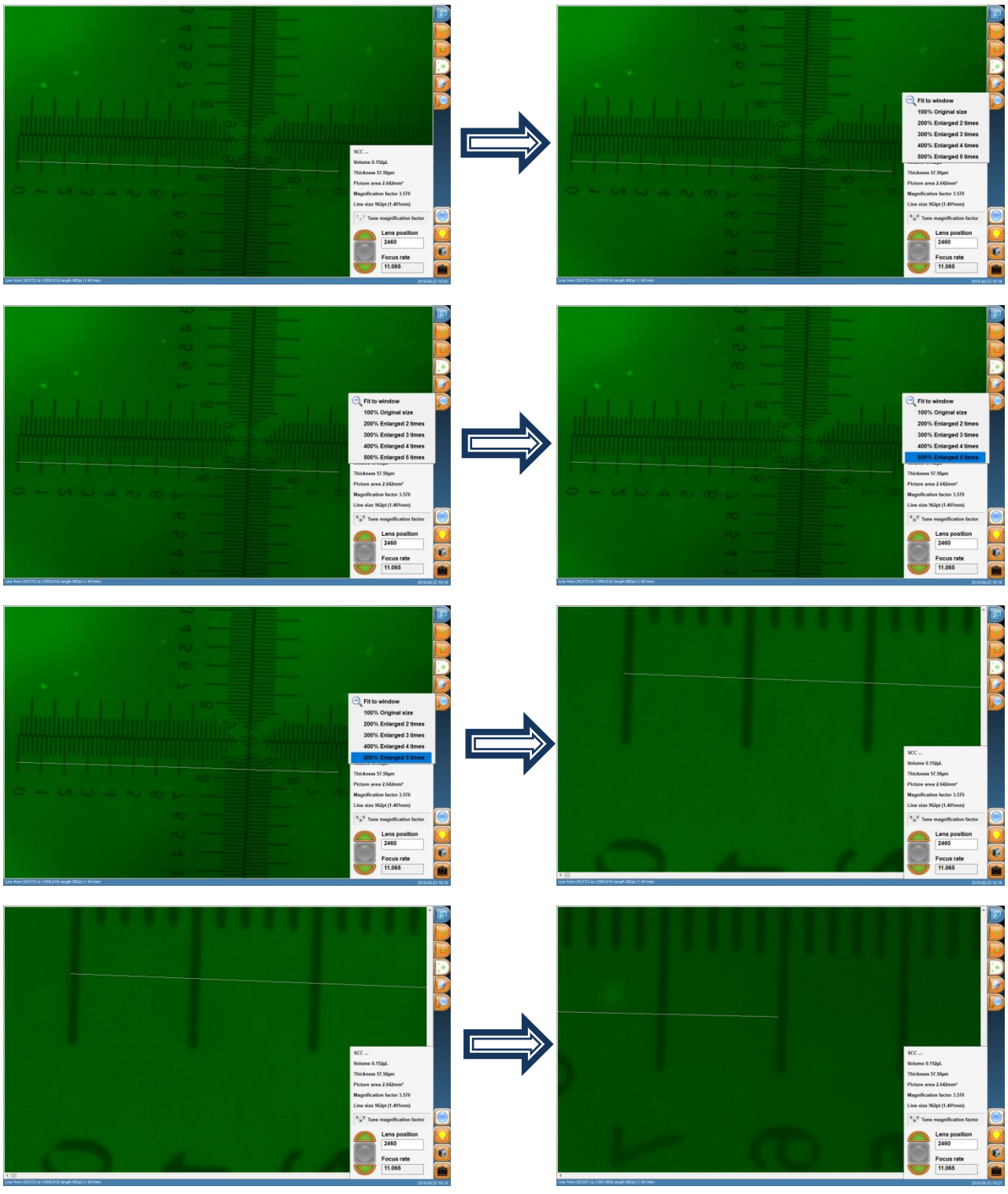
The values for Focus rate are different at a different intensity of the LED.

Position the mouse on the left horizontal scale, on position 0. Press the right button of the mouse and drag it to position 6 of the right horizontal scale. This way you will draw a measuring line.

For example: The Line size must be 1.400 mm ±0,01÷0,03 mm. When the Line size is different from 1.400 mm ±0,01÷0,03 mm, then the LACTOSCAN SCC magnification is not set. It is necessary to contact LACTOSCAN service center.



5.2. In order to be sure that the line is correctly positioned on position 0 and position 6 from the scale, press the button  to zoom.



5.3. The button  opens a menu, which is used only by a trained technician. All changes, made in this menu, may lead to malfunctions of the device.

Updating the information for the types of LACTOCHIP available in the software

Updating the types of LACTOCHIP 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 LACTOSCAN SCC

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 work with the SCC.

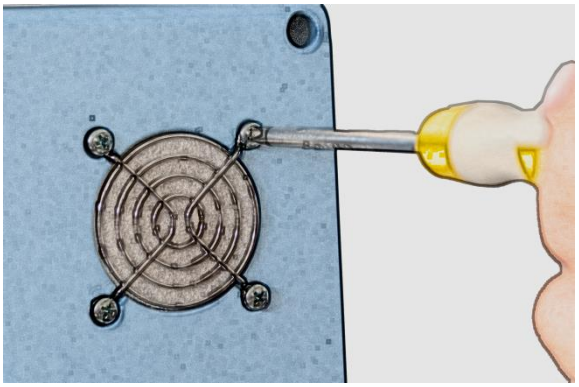
4. To go back to the program, press RETURN Back to application **.**

Replacing LACTOSCAN SCC filters

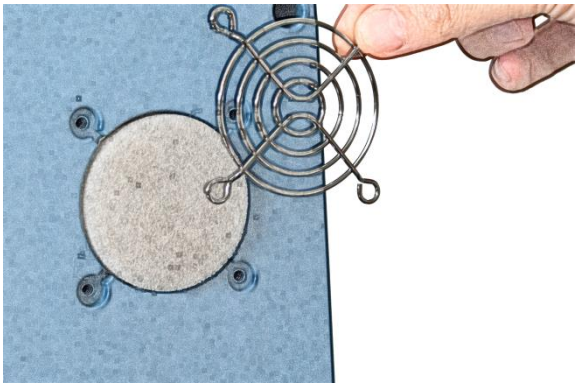
The filters, situated at the rear panel and at the bottom of the LACTOSCAN SCC need replacement each month on regular basis or in case it is noticed that they are obviously dirty.

Replacing the filters of the rear panel:

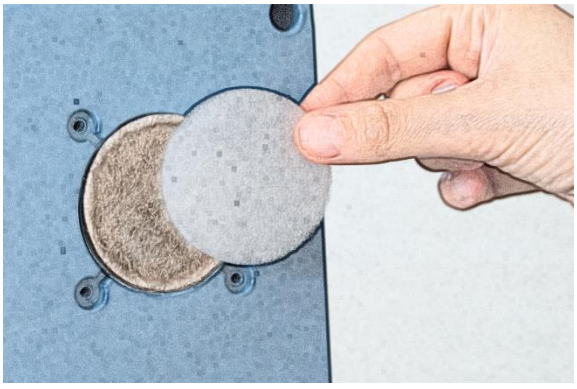
1. Unscrew the screws of the filter's grid.

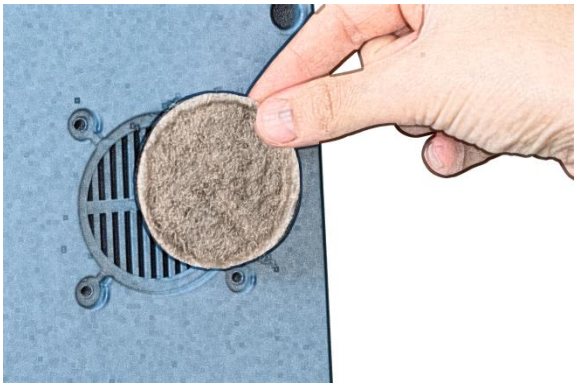


2. Remove it.



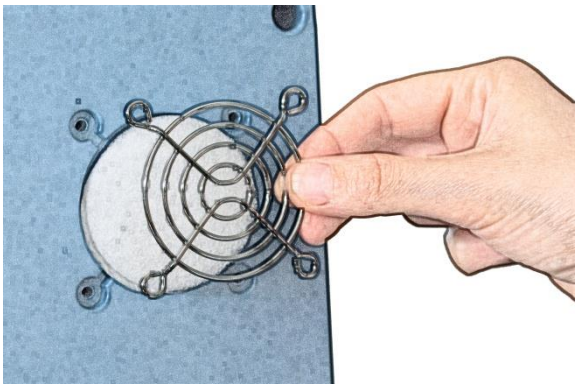
3. Remove the filters.



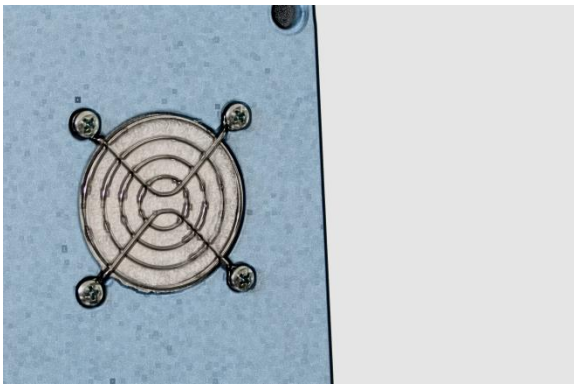


4. Place the new filters.


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 LACTOSCAN SCC filters.

Maintenance and cleaning

LACTOSCAN SCC needs a periodical auto focus.

Change the filters of LACTOSCAN SCC once a month.

Clean the box of LACTOSCAN SCC 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 LACTOSCAN SCC 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 LACTOSCAN SCC 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.

LACTOSCAN SCC - determination the number of the somatic cells in raw milk - method

The number of somatic cells (SCC) is one of the internationally recognized standards for milk quality control and is also a useful indicator for mastitis presence.

There are several different methods for determination the total number of somatic cells in milk (SCC). Each method is based on different feature and exploitation characteristics, but none of them is capable of determining the total number of somatic cells. In case of perfect conditions it is possible to determine the SCC of a milk sample, but there are several theoretical and practical problems, that make it impossible, especially when automation and speed of analysis are required. Because of the random positions of the cells in the sample, each result, unless total sample is tested, may only indicate a part of the SCC.

When determining the SCC it is necessary to maintain the requirement of ISO 13366-1:2008 "Milk – Enumeration of somatic cells – Part 1: Microscopic method (Reference method) » standard. Since the cells in milk are located based on the Poason law, there is a determined minimum quantity of cells, which needs to be counted in order to achieve the needed level of exactness (page 6 of the standard). For authentic determination of SCC it is necessary to be counted not less quantity of somatic cells than the mentioned in the following table:

Concentration of somatic cells thousands/ml	Coefficient of variation, CV%	Min. quantity of counted cells
< 150	10	100
150-250	7	200
250-400	6	300
≥ 400	5	400

The Poisson distribution is based on the formula: M = V = s2,

Where:

M – average value – (number of counted cells)

V – Dispersion

s – Standard deviation

The coefficient of variation (CV) would be equal to $CV = \frac{S}{M} * 100\%$

In order to achieve the needed precision, with the different methods of counting, some limitations appear, linked with the volume of the effectively measured milk. This way, if the measured sample contains 100 000 somatic cells in 1 ml, in order to achieve the needed precision (CV<10) there must be counted not less than 100 cells, in no less than 1 µL undiluted milk. In order to achieve higher precision of measurement – for example CV<5%, it is necessary the amount of measured milk to be substantially greater – to count at least 400 cells that may be contained in 4 µL milk.

An important moment is the correct identification of the cell, if it occurs, and rejection of each object or particle in the milk, which look like but is not a somatic cell. This is an important factor for determination the precision of each method for counting SCC. A key moment is the possibility of measuring the size of the mentioned cells. The question of selectiveness is further complicated by the fact that most milk samples are analyzed in a place, away from the place of milking, after a period of time. Since the milk is a biological medium with active enzymes and microorganisms, this may lead to a change in the number of cells and their morphology, even if they're preserved.

As a solution to the above, there is general agreement as the reference method for determining the "true" number of somatic cells in milk to accept direct microscopic analysis (DMSCC) using methylene-blue or fluorescent dyes. The method is protected in IDF / ISO standard 148. There are some aspects of this method, though, that limit its feasibility from practical point of view, like long procedure and intensive training of the operator, in order to provide objective selectiveness or precision.

Significant disadvantage of direct microscopic analysis (DMSCC) is the tendency to color artifacts and with the potential problem of cell aggregation and limited sample volume gives rise to uncertainty in the number of cells. Especially clearly this is expressed in the milk with a low content of the somatic cells (up to 300 000 / ml).The accuracy, precision and repeatability of the results of the DMSCC method depend a lot on the operator's training and abilities, notwithstanding the used equipment or protocol.

There are other methods for determining the SCC. Typical SCC methods are counting the SCC by

Similar Standard Method, based on Direct Fluorescent image low magnification microscopic recognition (Lactoscan SCC by Milkotronic Ltd., C-Reader "ADAM" by Digital Bio Technology; NucleoCounter SCC 100 by Chemometec; DCC by DeLaval);

Fluorescent flow cytometry ISO 13366-2:2006 (IDF 148-2: 2006) Milk -- Enumeration of somatic cells -- Part 2: Guidance on the operation of fluoro-opto-electronic counters (Somacount 150 by Bentley Instruments; SomascopeTM by Delta Instruments).

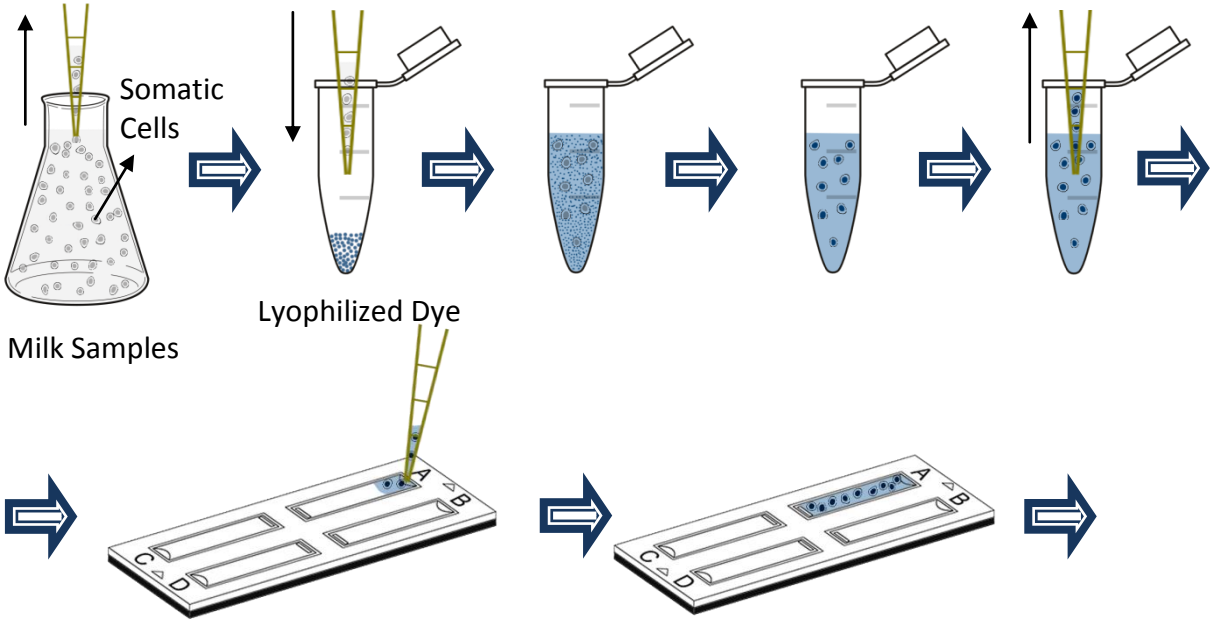
Based on disc cytometry – fluorine-optical counter with spinning disc (FossoMatic4000TM by Foss Electric). In milk testing laboratories the number of somatic cells is usually determined by automatic electronic machines, which may be exact and reliable instruments.

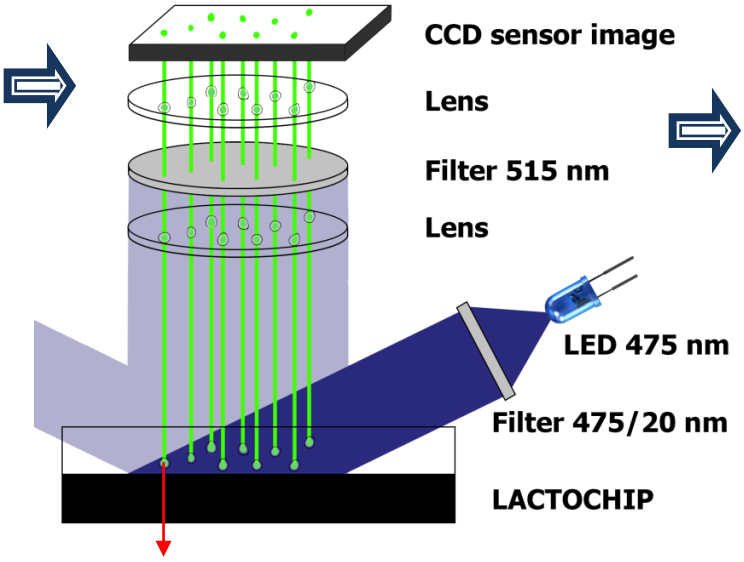
With them the SCC is quickly and cheaply determined.

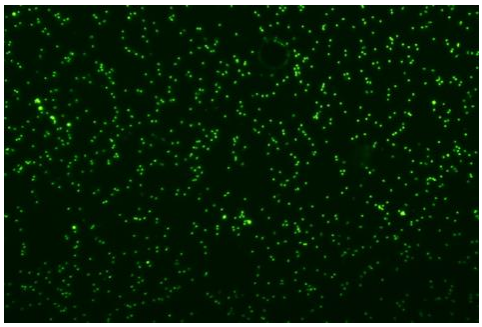
There are also indirect methods, where the SCC is determined after the interaction of the milk with certain reagents, for example the use of California mastitis tests. They're with lower accuracy and repeatability.

Milkotronic Ltd. has developed a portable Lactoscan SCC based on direct fluorescent, low magnification microscopic somatic cell counting. Lactoscan SCC uses a very sensitive fluorescent dye Sofia Green, LED optics и CMOS technologies for capturing in order to make the cells analysis more accurate, reliable and fast. Mostly, the objective selectiveness of Lactoscan SCC is combined with the permanent high stability of the different mechanical, electronic, optical and chemical components of the system, which provides almost identical results during the whole duration of machine's use. On top of that, the production of the machines provides high level of uniformity between the devices. This is a unique feature of Lactoscan SCC which actually offers identical results when we consider the measurement of one and the same sample, notwithstanding which machine is used, what is the location, who is using the machine, and in what moment the analysis is being made, in case that the characteristics of the samples have not changed.

In order to count the somatic cell with Lactoscan SCC, the milk sample is mixed with the dying reagent, containing fluorescent dye Sofia Green. Only 8 µL from the dyed sample is pipetted on the measuring chamber of disposable LACTOCHIP. The chip is loaded into the device and for a period between few seconds and 2 minutes, depending on the measuring mode, the analysis is done. Lactoscan SCC system focuses automatically on the chip and the dyed cells are captured by the sensitive CMOS camera. The analysis algorithm of digital images determines the number and dimension of the fluorescent cells and counts their concentration. The results are automatically shown on the display, also on printer, with possibility to save the results and generate reports from the results.







Fluorescent microscopic picture

Comparison between the direct microscope analysis (DMSCC) and analysis with LACTOSCAN SCC.

A comparison test with measuring of 14 reference samples of raw cow milk treated with glycerin, thimerosal and dimethyl sulfoxide was conducted in the laboratory "Buluritest", Scientific Research Sector, University "Prof. d-r AssenZlatarov"- Burgas, Bulgaria. The total amount of somatic cells in each reference sample of cow milk has been analyzed by the direct microscopic analysis (DMSCC) and by LACTOSCAN SCC.

Direct microscopic analysis, as a control method, was conducted according to ISO 13366-1:2008 «Milk –Enumeration of somatic cells – Part 1: Microscopic method (Reference method)».

10 microliters of the analyzed milk samples were applied on a marked area of 1 cm^2 from the surface of the glass slide obtaining smear, which was dried. After drying, the smears were colored with a solution of methylene blue and propidium iodide fluorescent dye (PI). After that, the colored cells were counted with microscope. The amount of the counted cells in a specific surface (1 cm^2) was multiplied with the working coefficient so the amount of somatic cells in 1 ml to be determined.

The counting was made by counting 50 fields in one smear, while moving the lens of the microscope consequently vertical and right. Therefore from each milk testing sample were analyzed 10 smears, 50 fields were counted in each of them.

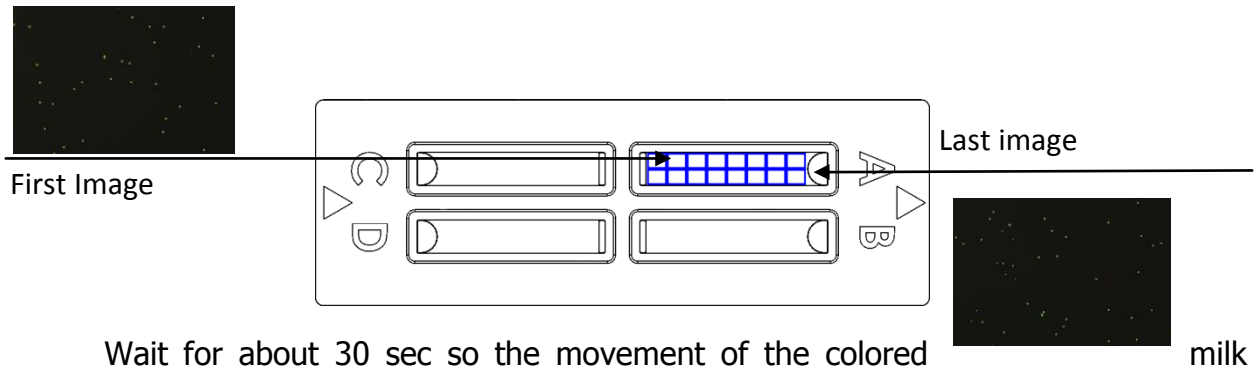
Determination of SCC by using **fluorescent-microscopic counter Lactoscan SCC** was conducted according to the company records.

The essence of the method is based on the fact that after demolition of the cytoplasmic membrane of the somatic cells in the milk, under the influence of the used by us dried lysogenic buffer reagent, the cores of the cells become accessible to the action of the used fluorescent dye SOFIA GREEN, which colors the nuclear DNA. The light signals

from the colored cells are detected by the detector of the so called CMOS-camera, i.e. on the elements of the CMOS-camera are formed images, where the emitted energy from every cell is displayed as an illuminated ball. By automatic moving of the mechanism on the axes X and Y, the device captures a maximum of 70 images. The images are analyzed with the help of the embedded software and in this way the amount of the somatic cells is determined. The whole process after placing a LACTOCHIP with a sample in the carriage is automatic.

Performing the analysis

Slowly stir the milk sample while avoiding foaming. The temperature of the sample may be in the range from 10 to 40 ºC. With the automatic micropipette put 100 µL of the stirred milk in an Eppendorf with dried lysis coloring buffer reactive. Slowly stir for a couple of seconds by a multiple pipetting with the automatic micropipette or by using Vortex stirrer. After a stay of about a minute, the content in the Eppendorf is stirred again and 12 µL of the colored milk are being pipetted in the microfluidic camera of Lactochip x2.



Wait for about 30 sec so the movement of the colored milk in the microfluidic camera can stop and after that put the loaded chip into the carriage of Lactoscan SCC. Now follows an automatic analysis of the sample up to visualizing of the results (expressed in thousands of cells in 1ml) on the display of the device. These results along with all captured images are automatically stored in the data base of the device.

Analysis of the results

The number of somatic cells in the analyzed milk sample is reported by the results, obtained on the display of the device and are expressed in thousands of cells/ml. Repeatability results of every milk testing sample are evaluated by the coefficient of variation (CV), calculated for each of the two methods SCC. The accuracy and repeatability of the system Lactoscan SCC and the average comparison and regression analysis of the SCC data between Lactoscan SCC and the direct microscope analysis (DMSCC) were also established on the base of the obtained results.

Results

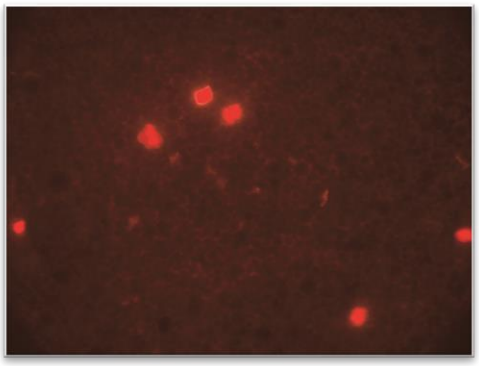
1. Accuracy of the system Lactoscan SCC

The accuracy of our method for defining SCC with the help of Lactoscan SCC, based on the technical abilities of the device with its 2 versions for 4 and 9 microliters of measuring is:

Number of cells 1 ml	Lactoscan SCC mode 4 microliters		Lactoscan SCC mode 9 microliters		Minimum requirement of the standard ISO13366-1	
	Counted cells	CV%	Counted cells	CV%	Counted cells	CV%
100000	400	5,0	900	3,3	100	10,0
500000	2000	2,2	4500	1,5	400	5,0
1000000	4000	1,6	9000	1,1	400	5,0
1500000	6000	1,3	13500	0,9	400	5,0

Image comparison of the direct microscopic somatic cell counts (DMSCC) and Lactoscan SCC

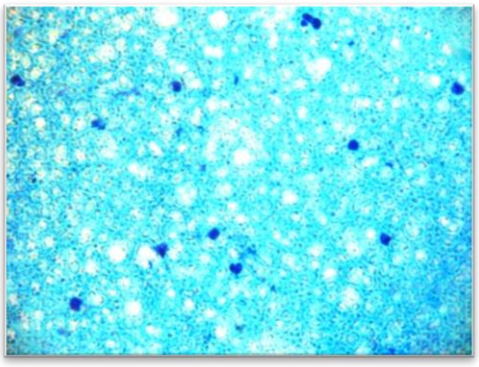
A) Stained somatic cell image with propidium iodide (DMSCC)



Somatic cell concentration: **700x10^3/ml**

Magnification: **1:1000**

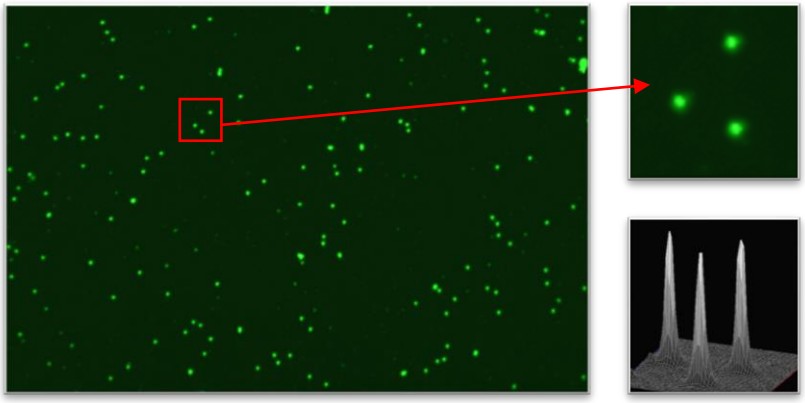
B) Stained somatic cell image with methylene-blue (DMSCC)



Somatic cell concentration: **1200x10^3/ml**

Magnification: **1:1000**

C) Stained somatic cells with Sofia Green dye captured by Lactoscan SCC.



Digital zoom e: 1:20

3D view

Somatic cell concentration: **1200x10^3/ml** Magnification: **1:4**

For an analysis with version 4 microliters, the implied accuracy is significantly better than the minimum requirements of the standard. For a chip with a version 9 microliter of the tested milk sample, the result is the maximum possible accuracy for electronic devices.

The measurement results for 14 milk samples are summed up in the following table.

	Lactoscan SCC				DMSCC				
Milk test samples	**Average cell/ml**	**Standard deviation**	**Coefficient of variation %**	**Number of counted cells in 50 fields**	**Average cell/ml**	**Standard deviation**	**Coefficient of variation %**	**Number of counted cells in 50 field**	**Necessary visual fields**
1	48 200	2974	6.17	190	50 315	5701	11.33	7.5	667
2	105 400	5015	4.76	411	108 009	10701	9.91	16.1	311
3	148 000	6815	4.60	569	165 033	12329	7.47	24.6	407
4	244 500	9180	3.75	952	258 954	12728	4.92	38.6	389
5	338 300	12472	3.69	1320	348 178	20846	5.99	51.9	289
6	453 400	15254	3.36	1768	461 554	30135	6.53	68.8	291
7	559 000	18708	3.35	2189	554 805	28646	5.16	82.7	242
8	677 100	20469	3.02	2604	621 891	24301	3.91	92.7	216
9	776 070	22944	2.96	3019	777 531	37612	4.84	115.9	173
10	978 900	26917	2.75	3871	989 524	47568	4.81	147.5	136
11	1 091 000	26541	2.43	4275	1 104 242	43037	3.90	164.6	122
12	1 140 200	19904	1.75	4502	1 179 379	40102	3.40	175.8	114
13	1 485 000	24855	1.67	5844	1 463 154	61846	4.23	218.1	92
14	1 668 900	30700	1.84	6598	1 661 730	53859	3.24	247.7	81

The linear correlation between the values of SCC in the 14 milk test samples with a progressive increase of SCC from 50 000/ml to 1 650 000/ml (R 2, 0,995; 95% CI, 0,990 ~ 0,999; p <0.01), measured by DMSCC and Lactoscan SSC is presented in Fig. 2

In comparison with the data from SCC for the 14 milk test samples, measured by DMSCC, the system Lactoscan SCC shows an acceptable and similar repeatability and accuracy of the conventional device for SCC in the correlation analysis of Pearson.

At low values of SCC in the milk, it is necessary in the method of DMSCC to be watched much more than 50 visual microscope fields in order to fulfill the requirement of the standard. Like this, for example, for the tested milk with SCC 50 000/ml with the method of DMSCC were counted from 6 to 9 cells in 50 microscope fields, and with the use of Lactoscan SSC - from 171 to 212 cells from a single measurement.

Comparison results between Lactoscan SCC and DMSCC are presented in the graphs below:

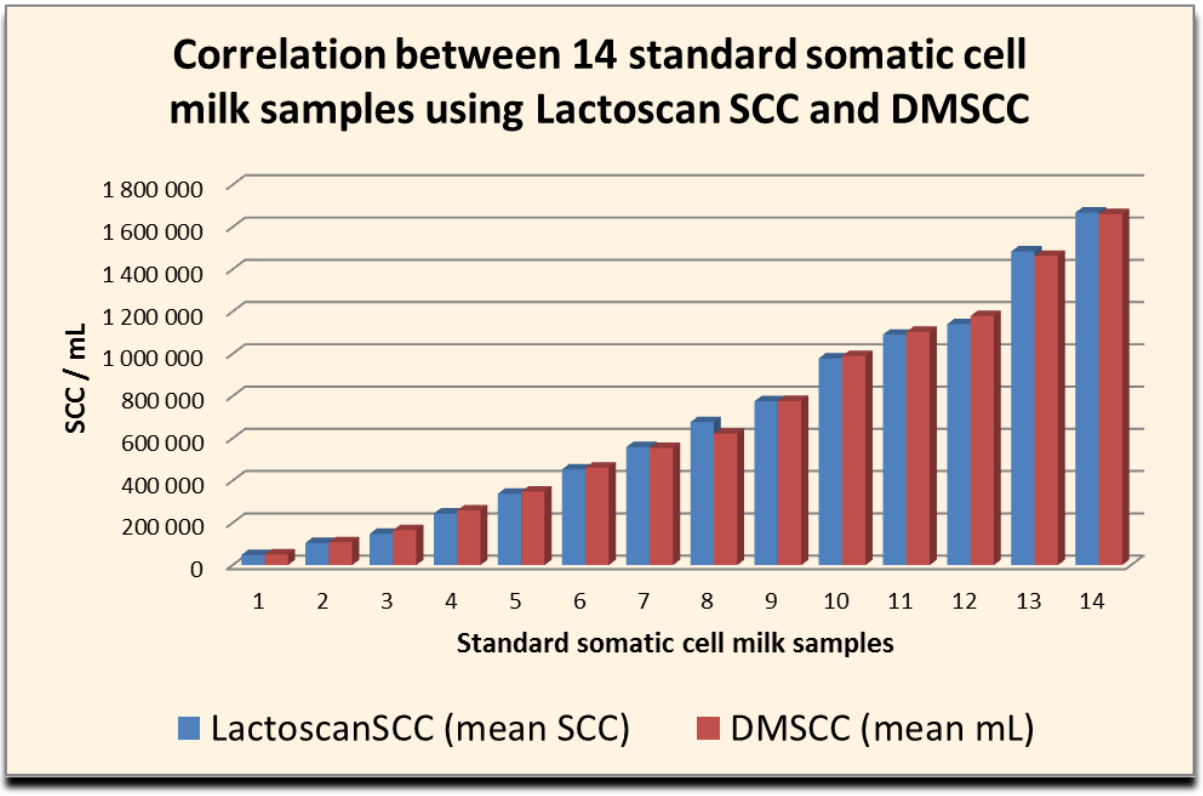


Figure1

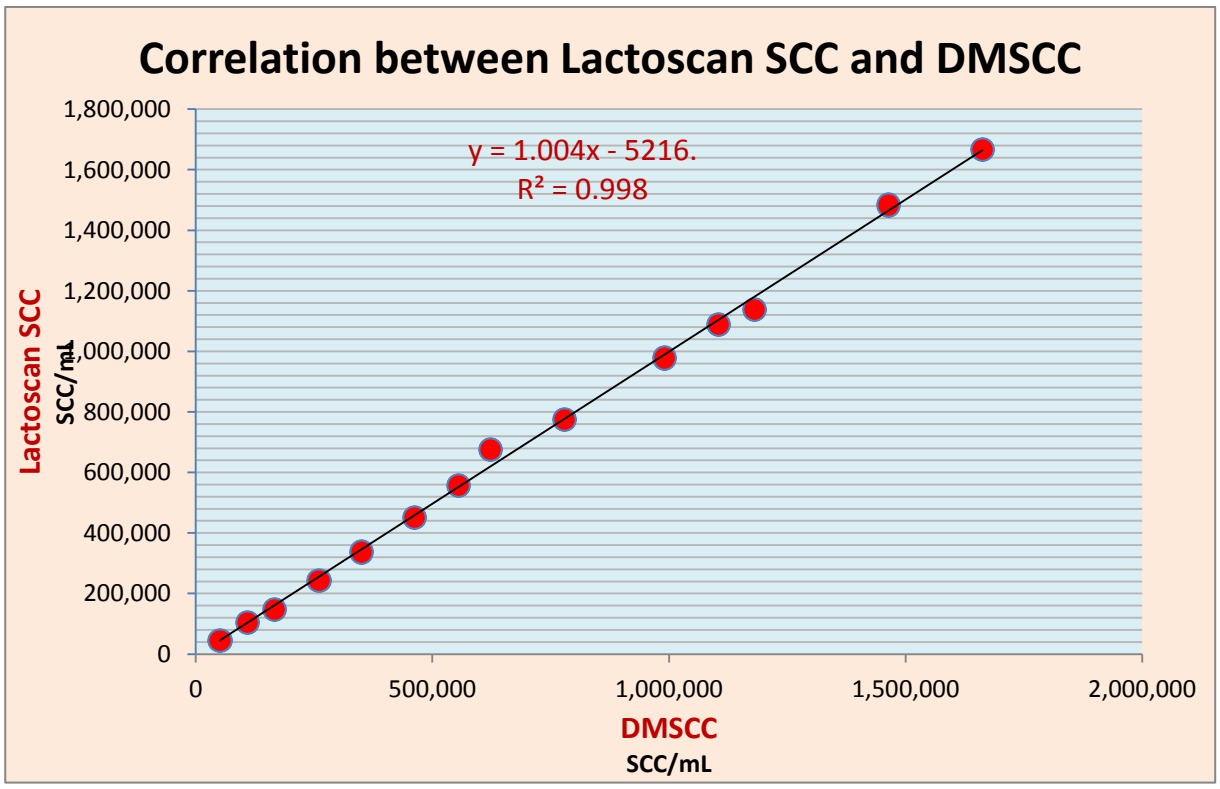


Figure2

The common data for the repeatability of SCC in each of the 14 milk test samples with the use of DMSCC are summed up in the following table:

Concentration	1	2	3	4	5	6	7	8	9	10	Arithmeticaverage	Standard deviation	Coefficient of variation %
50 000	53 669	53 669	46 960	46 960	60 378	46 960	40 252	46 960	53 669	53 669	50 315	5701	11,33
100 000	107 338	100 630	100 630	120 756	93 921	120 756	114 047	107 338	120 756	93 921	108 009	10701	9,90
150 000	154 299	181 133	181 133	161 007	161 007	167 716	140 881	161 007	167 716	174 425	165 033	12330	7,47
250 000	261 637	268 346	248 220	261 637	254 928	268 346	268 346	261 637	268 346	228 094	258 954	12729	4,92
350 000	348 849	362 267	368 975	301 889	322 015	362 267	348 849	348 849	355 558	362 267	348 178	20846	5,99
450 000	483 022	462 896	509 857	436 062	456 188	409 227	442 770	442 770	489 731	483 022	461 554	30135	6,53
550 000	529 983	570 234	516 565	523 274	576 943	583 652	536 691	583 652	536 691	590 360	554 805	28646	5,16
650 000	603 778	597 069	597 069	630 612	657 447	630 612	623 904	630 612	590 360	657 447	621 891	24302	3,91
750 000	838 580	724 533	711 116	805 037	764 785	791 620	778 202	791 620	771 494	798 328	777 531	37612	4,84
950 000	1 059 965	979 461	925 792	992 879	919 084	999 587	979 461	1 046 548	1 033 131	959 336	989 524	47569	4,81
1 100 000	1 153 886	1 113 634	1 046 548	1 113 634	1 086 800	1 113 634	1 046 548	1 160 595	1 059 965	1 147 177	1 104 242	43038	3,90
1 150 000	1 113 634	1 160 595	1 153 886	1 160 595	1 133 760	1 227 681	1 214 264	1 194 138	1 220 972	1 214 264	1 179 379	40102	3,40
1 450 000	1 549 696	1 442 358	1 408 814	1 435 649	1 395 397	1 589 948	1 428 940	1 469 192	1 475 901	1 435 649	1 463 154	61847	4,23
1 650 000	1 650 325	1 744 246	1 596 656	1 697 286	1 737 538	1 603 365	1 697 286	1 616 782	1 636 908	1 636 908	1 661 730	53860	3,24

The common data for the repeatability of SCC in each of the 14 milk test samples with the use of Lactoscan SCC in measuring of 4 µL samples are summed in the following table:

Milk samples	1	2	3	4	5	6	7	8	9	10	Arithmetic average	Standard deviation	Coefficient of variation %
1	43 000	46 000	48 000	46 000	50 000	54 000	50 000	47 000	49 000	49 000	48 100	2974	6,17
2	112 000	101 000	103 000	103 000	112 000	99 000	100 000	111 000	108 000	105 000	105 400	5015	4,75
3	142 000	161 000	147 000	157 000	142 000	145 000	151 000	150 000	140 000	145 000	148 000	6815	4,60
4	251 000	242 000	243 000	230 000	232 000	249 000	242 000	243 000	253 000	260 000	244 500	9180	3,75
5	343 000	335 000	330 000	366 000	322 000	339 000	335 000	328 000	350 000	335 000	338 300	12473	3,69
6	440 000	436 000	474 000	472 000	453 000	446 000	432 000	469 000	450 000	462 000	453 400	15255	3,36
7	571 000	563 000	548 000	530 000	543 000	584 000	558 000	584 000	539 000	570 000	559 000	18709	3,35
8	715 000	675 000	648 000	686 000	682 000	671 000	679 000	683 000	643 000	689 000	677 100	20469	3,02
9	800 700	800 000	802 000	774 000	773 000	763 000	733 000	750 000	789 000	776 000	776 070	22944	2,96
10	940 000	934 000	975 000	1 006 000	987 000	1 007 000	993 000	967 000	970 000	1 010 002	978 900	26918	2,75
11	1 102 000	1 071 000	1 064 000	1 077 000	1 083 000	1 080 000	1 081 000	1 083 000	1 115 000	1 154 000	1 091 000	26541	2,43
12	1 153 000	1 164 000	1 146 000	1 142 000	1 146 000	1 122 000	1 100 000	1 154 000	1 120 000	1 155 000	1 140 200	19904	1,75
13	1 517 000	1 494 000	1 466 000	1 505 000	1 497 000	1 501 000	1 466 000	1 484 000	1 489 000	1 431 000	1 485 000	24855	1,67
14	1 694 000	1 677 000	1 647 000	1 663 000	1 702 000	1 705 000	1 677 000	1 639 000	1 607 000	1 678 000	1 668 900	30701	1,84

As it is seen, the intervals are within those, recommended from the International milk association for somatic counters (to150 000 CV %< 10: from 150 000 to 250 000 CV %<7: from 250 000 to 400 000 CV%<6 and over 400 000 CV%<5). With the use of Lactoscan SCC, based on the microscope counting of somatic cells with a plastic chip for analyzing of the milk, the values of SCC are less variable than with DMSCC. Nevertheless, the obtained ratio of SCC measured by the system Lactoscan SCC, in the milk test samples to those, measured by DMSCC, vary from 0,897 ~ 1,089. Therefore, the values of SCC in the milk test samples, defined with Lactoscan SCC are acceptably repeatable.

Comparison of CV% for the 14 milk test samples for the results, obtained from the both methods of analysis – by the use of Lactoscan SCC and the direct microscope analysis (DMSCC) is presented in the graph below:

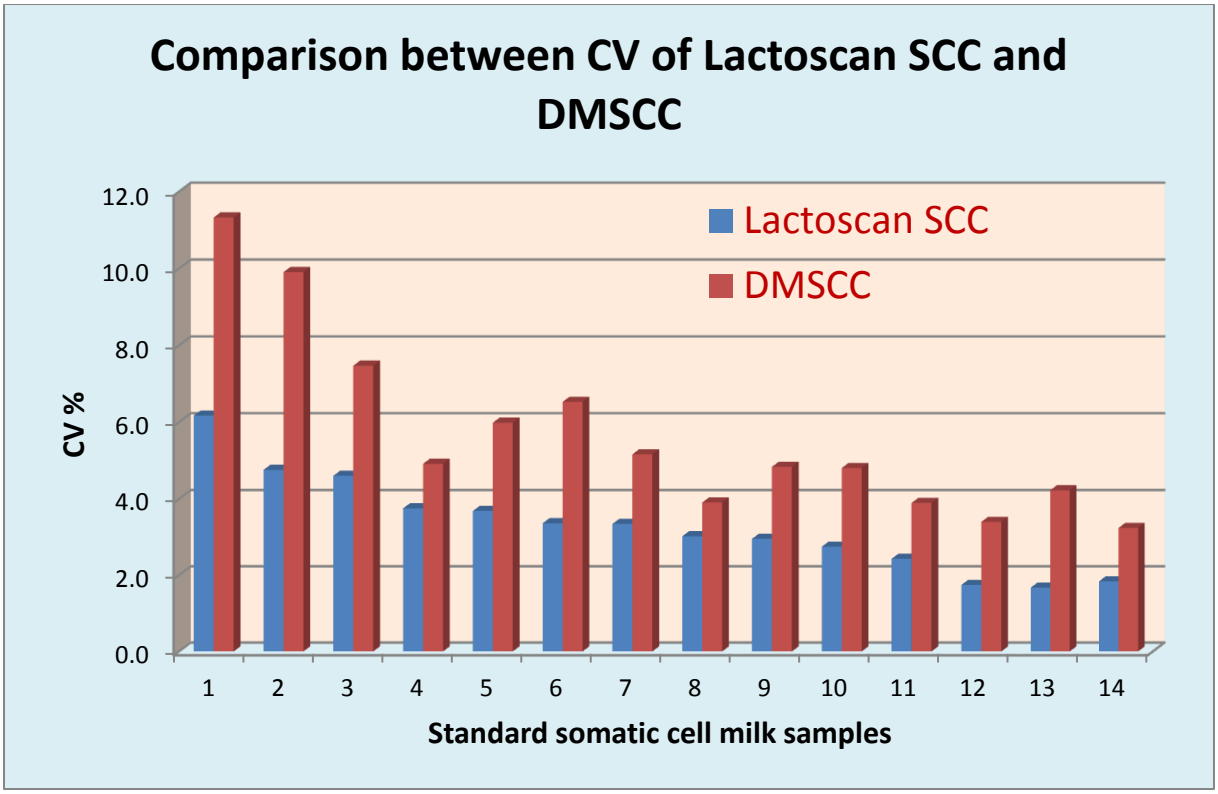


Figure 3

Comparison of the potential accuracy of the devices, based on Direct Fluorescent image low magnification microscopic recognition, starting from the declared data from the producers about the effective volume of the measured milk and the results from the Normal distribution of Poisson.

Number of cells 1 ml	Lactoscan SCC mode 4 microliters		Lactoscan SCC mode 9 microliters		C-Reader "ADAM" max 9 microliters		NucleoCounter SCC 100 1 microliter		DELAVAL DCC 0.1 microliters	
										
	Total cells	**CV%**	**Total cells**	**CV%**	**Total cells**	**CV%**	**Total cells**	**CV%**	**Total cells**	**CV%**
50 000	200	7,07	450	4,71	450	4,71	50	14,14	5	44,72
100 000	400	5,00	900	3,33	900	3,33	100	10,00	10	31,62
300 000	1200	2,89	2700	1,92	2700	1,92	300	5,77	30	18,26
600 000	2400	2,04	5400	1,36	5400	1,36	600	4,08	60	12,91
1 000 000	4000	1,58	9000	1,05	9000	1,05	1000	3,16	100	10,00
1 500 000	6000	1,29	13500	0,86	13500	0,86	1500	2,58	150	8,16

Comparison on the base of some of the main features of the devices, based on Direct Fluorescent image low magnification microscopic recognition, starting from the declared data from the producers.

	Lactoscan SCC	DMSCC	C-Reader "ADAM"	NucleoCounter SCC 100	DELAVAL DCC
					
Feature	Milkotronic Ltd.		DigitalBio Technology	Chemometec	DeLaval
Staining Method	Fluorescent dye	Fluorescent/Exclusion dye	Fluorescent dye	Fluorescent dye	Fluorescent dye
Reagent	Sofia Green	EtBr/MB	PI	PI	PI
Microscope	Fluorescent (4 x)	Light (1000 x)	Fluorescent (4 x)	Fluorescent (0,7x)	Fluorescent (4 x)
Sample Volume Needed	100uL	1 mL	100uL	100uL	60 uL
Dilution	no	no	1:2	1:2	no
Measured Volume	**9 µL**	**0,149 µL**	**9 µL**	**1 µL**	**0,1 µL**
Captured Images	max 70	~ 50	90	1	1
Machine Analysis Time	max 120 samples/hour	-	max 60 samples/hour	up 100 samples/hour	max 60 samples/hour
Cell Density Range	1 x 10E4 ~ **20 x 10E6**	1 x 10E4 ~ 10 x 10E6	0 ~ 10x10E6	1 x 10E4 ~ 2 x 10E6	1 x 10E4 ~ 2x 10E6
Image Processing	Fluorescent image recognition	Human	Fluorescent image recognition	Fluorescent image recognition	Fluorescent image recognition
Calibration	No	No	No	No	No
Applications	**Count, Cell size**	**Count, Cell size, Morphology**	**Count, Cell size**	**Only Count**	**Only Count**
Process Time	Fast	Very slow	Fast	Fast	Fast

Personal Error	N			Y	N	N	N
Method	Similar Standard Method			Standard Method	Similar Standard Method	Similar Standard Method	Similar Standard Method
Repeatability	100 000 cell/ml 400 000 cell/ml 1 000 000cell/ml	**4 µl** CV(%)- **5%** CV(%)- **3%** CV(%)- **2%**	**9 µl** CV(%)- **3%** CV(%)- **2%** CV(%)- **1%**	<150 000 cell/ml CV(%) - **10%** 150 000-250 000 cell/ml CV(%) - **7%** 250 000-400 000 cell/ml CV(%) - **6%** > 400 000 cell/ml CV(%) - **5%**	100 000 cell/ml CV(%) - **3%** 400 000 cell/ml CV(%) - **2%** 600 000 cell/ml CV(%) - **1%**	100 000 cell/ml CV(%) - **10%** 400 000 cell/ml CV(%) - **5%** 1 000 000cell/ml CV(%) - **3%**	100 000 cell/ml CV(%) - **12%** 400 000 cell/ml CV(%) - **8%** 1 000 000cell/ml CV(%) - **7%**
Sample Prep.	Simple			Complex	Simple	Simple	Simple
Calibration	N			-	N	N	N
Cell Types	Somatic Cell			Most Cells	Somatic Cell	Somatic Cell	Somatic Cell
Throughput	High			Low	High	High	High
Device Price	Low			-	Low	Low	Low
Peripheral Device	Chip, dry Stain/ Lysis reagent			Microscope, Reagent	Chip, Stain Solution	Cassette, Lysis Buffer	Cassette, dry Stain/ Lysis reagent
Device Size	Small			-	Small	Small	Small
Weight	5 kg			-	10 kg	3 kg	4 kg
Reagent				Manual prep	Stain Solution	Coated in Cassette, Lysis Buffer	Coated in Cassette, Lysis Buffer

It should be noticed that Lactoscan SCC increases the images 4 times, and such magnification is the most suitable and this provides an opportunity for measuring the size of the particles and respectively for more correct differentiation, which has a determining influence for the accuracy of the measurement. For comparison NucleoCounter SCC 100 has only 0.7 magnifications which don't allow a visual differentiation of the cells.

It is necessary to add as well the large volume of measurement = up to 9 µL, which provides an opportunity for measurements with values of CV, that are significantly better than the standard requirements. The guaranteed high accuracy of measurement with Lactoscan SCC in combination with the competitive price of the device and the lowest on the market price of consumables / the price of consumables was limiting till today the use of this kind of devices / makes it currently extremely attractive.

Comparison on some of the main features of Lactoscan SCC with the devices, based on fluorescent flow cytometry, starting from the declared data of the producers:

	Lactoscan SCC			**Somacount 150**	**SomascopeTM**
					
Feature	Milkotronic Ltd.			Bentley Instruments	Delta Instruments
Staining Method	Fluorescent dye			Fluorescent dye	Fluorescent dye
Reagent	Sofia Green			EtBr/0,083 mg	DAPI
Microscope	Fluorescent (4 x)				
Sample Volume Needed	100uL			3,5 ml	2 ml
	No			1:2	
Measure Volume	**max 9 µL**			max 10µL	max 10 µL
Captured Images	max 70			-	
Machine Analysis Time	max 120 samples/hour			max 150 samples/hour	max 400 samples/hour
Cell Density Range	1 x 10E4 ~ 20 x 10E6			0 ~ 10x10E6	0 ~ 10x10E6
Image Processing	Fluorescent image recognition			Fluorescent flow cytometry	Fluorescent flow cytometry
Calibration	No			Y	Y
Applications	**Count, Cell size**			Count	Count
Process Time	Fast			Fast	Fast
Personal Error	N			N	
Method	Similar Standard Method			-	-
Repeatability	100 000 cell/ml 400 000 cell/ml 1 000 000cell/ml	**4 µl** CV(%)-**5%** CV(%)-**3%** CV(%)-**2%**	**9 µl** CV(%)-**3%** CV(%)-**2%** CV(%)-**1%**	100 000 cell/ml CV(%) - **5%** 300 000 cell/ml CV(%) - **3%** 500 000 cell/ml CV(%) - **2%**	100 000 cell/ml 400 000 cell/ml 1 000 000cell/ml
Sample Prep.	Simple				
Calibration	N			Y	Y
Cell Types	Somatic Cell			Somatic Cell	Somatic Cell
Throughput	High			High	High
Device Price	Low				
Peripheral Device	Chip, dry Stain/ Lysis reagent				
Device Size	Small				
Weight	5 kg			33 kg	10 kg

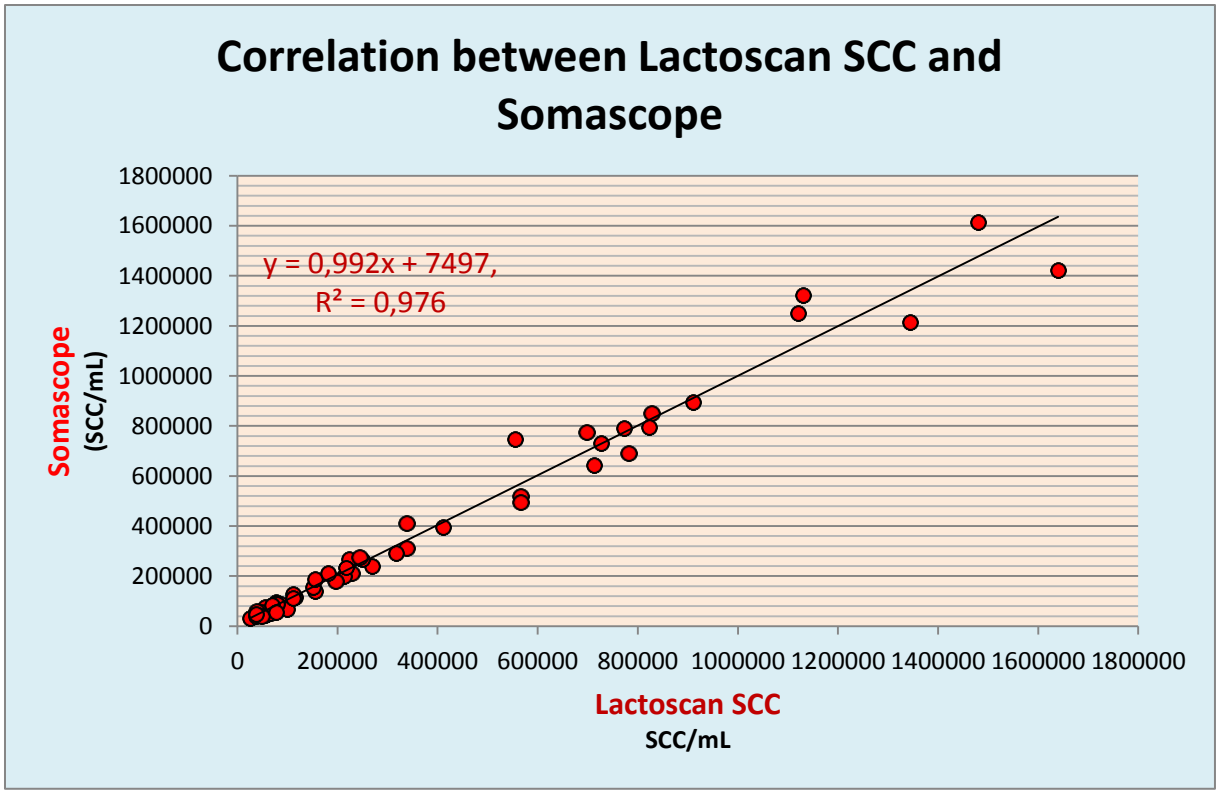


Figure 1

Comparison between Lactoscan SCC and FOSS device based on disc cytometry-fluoride-optoelectronic counter with rotating disc, based on the data declared by the producer.

Feature	**Lactoscan SCC**  Milkotronic			Fossomatic 5000TM  FOSS
Repeatability	100 000 cell/ml 400 000 cell/ml 1 000 000cell/ml	**4 µl** CV(%)-**5%** CV(%)-**3%** CV(%)-**2%**	**9 µl** CV(%)-**3%** CV(%)-**2%** CV(%)-**1%**	100 000 cell/ml CV(%) - **7%** 300 000 cell/ml CV(%) - **5%** 500 000 cell/ml CV(%) - **4%**

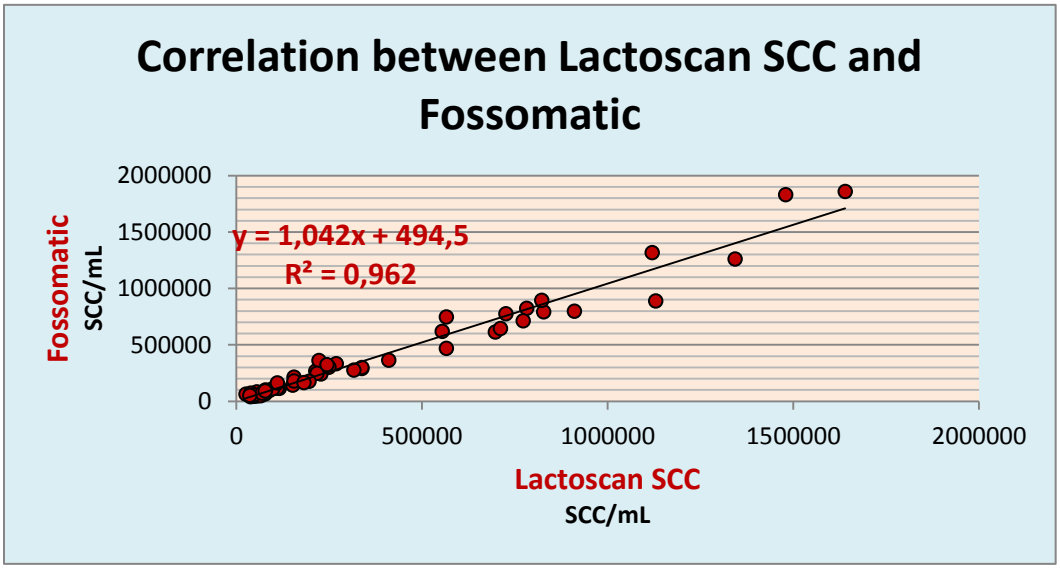


Figure2

Conclusion

A method for defining the number of somatic cells in samples of cow milk with the help of Lactoscan SCC has been developed.

With the help of LACTOSCAN SCC can be AUTHENTICALLY defined the SCC in the raw or preserved with bronopol, boric acid, sodium azide, potassium dichromate and other milk preservatives.

The device can REPRODUCIBLY be used for defining the number of somatic cells both in milk testing laboratories and in dairy farms and milk processors.

Work with Lactoscan SCC is with HIGH REPRESENTATIVENESS (by a single measurement can be defined the SCC in 4 or 9 microliters of milk, while for DMSCC this quantity is 0.149 microliters).

The repeatability indexes for Lactoscan SCC doesn't inferior to other devices with fluorescent microscopy of the other globally presented companies (Foss Electric, Bentley Instruments, and Delta Instruments etc.)

With the device Lactoscan SCC it can be worked easily, simple, fast, accurately and reliably and its portability makes it extremely comfortable to use not only in laboratories but also in the farms with aims to improve the quality of raw milk.

Detection of Yeast mastitis in raw milk samples using LACTOSCAN SCC

The topic of the Yeast mastitis and in general the presence of yeast cells in fresh raw milk is very important for all who work in the dairy sector: dairy farmers, veterinarians, analytical laboratories, scientific researchers, manufacturers of equipment for somatic cell counting and others, which is though barely illuminated in researches and practice.

Its importance is determined by a number of reasons like:

1. Increased and uncontrolled use of antibiotics which stimulates the growth of yeasts in the udder;

2. The presence of yeast mastitis has increased over the last decade;

3. In some countries, more than 10% of the animals suffer from such kind of mastitis.

The main cause of yeast mastitis are: Candida albicans, Candida catenulata , Candida glabrata. Usually these yeasts are also found in milk from animals that have not developed the symptoms of yeast mastitis and at least in the standard ISO 13366-1 IDF 148-1 Milk — Enumeration of somatic cells — Part 1: Microscopic method (Reference method) should be pointed what to be done if such cells come under the microscope. This is important because they are with the size of 5-6 micrometers and should be listed by the size criteria which says that all leukocytes, epithelial cells and cell fragments of a size greater than 4 microns are counted.

"INTERNATIONAL STANDARD ISO 13366-1 IDF 148-1

Milk — Enumeration of somatic cells — Part 1: Microscopic method (Reference method)

8.2 Determination

8.2.1 Reading optimization

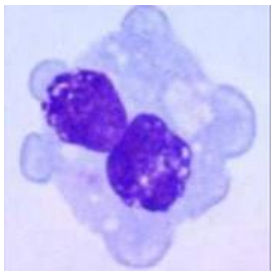
Using the microscope (5.3), count the cell nuclei in the obtained smear (8.1.1 or 8.1.2) of fields, entirely filled with milk smear only. Choose the best magnification (from 500x to 1 000x), in order to have an average maximum number of 20 cells in each field.

The cells possess a stained nucleus. The cells generally are 8 µm or larger. Do not count cells less than 4 µm (see Figure 1). Count fragments only if more than 50 % of

nuclear material is visible. Count cell clusters as one, unless the nuclear unit(s) is (are) clearly separated.

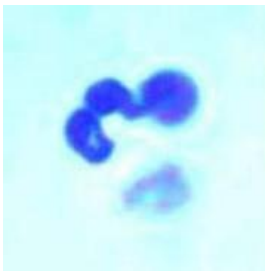
See also Figures 2 and 3

Figure 1 — Examples of cells



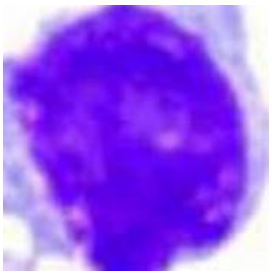
Macrophage
8-30 µm

The relation between cytophasma/nucleus is big. Phagocytosis, antigen presentation, secretion chemoattractants



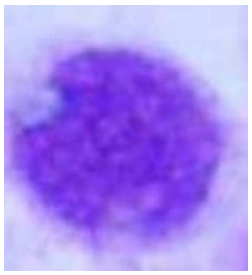
PMN
10-14 µm

90 % acute mastitis
60 % chronic. The relation between cytoplasma/nucleus is small. Phagocytosis. First line of defense against mastitis



Lymphocyte
5-10 µm

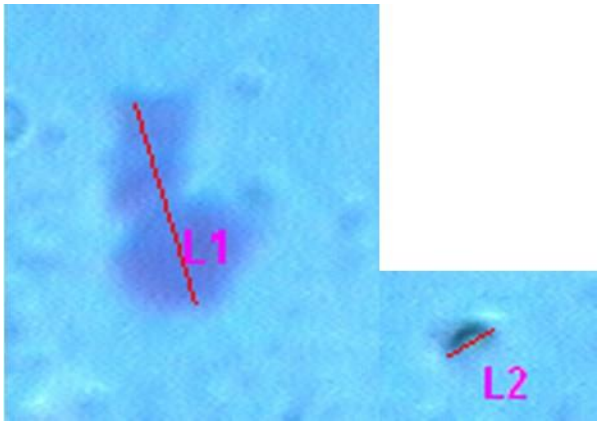
The relation between cytoplasma/nucleus is small. Nucleous intensively stained
T helper
T suppressor B cell



Epithelial cell
10-14 µm

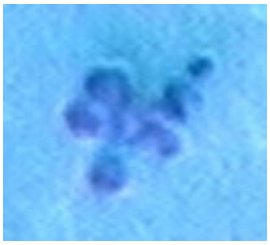
Nucleus round. Cytoplasma weakly stained

Figure 2 — Examples of cells from bulk cows' milk (1 000 x magnification)

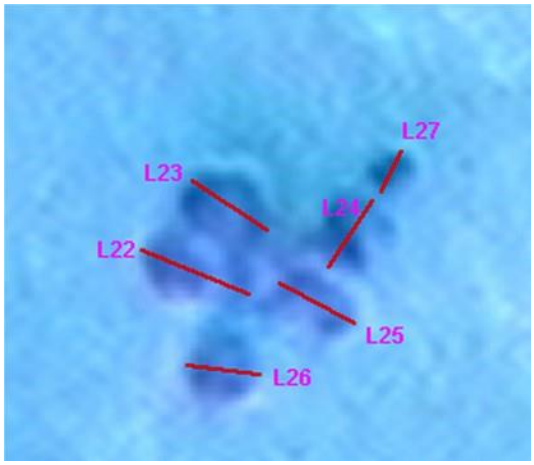


Cell lengths: L1 = 9,79 µm and L2 = 2,77 µm

Figure 3 — Examples from cells from bulk cows' milk



(500x magnification)



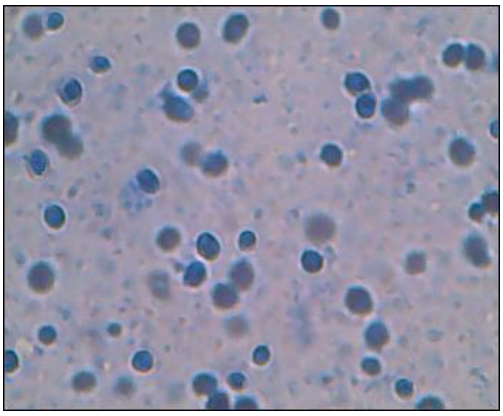
(1 000x magnification)

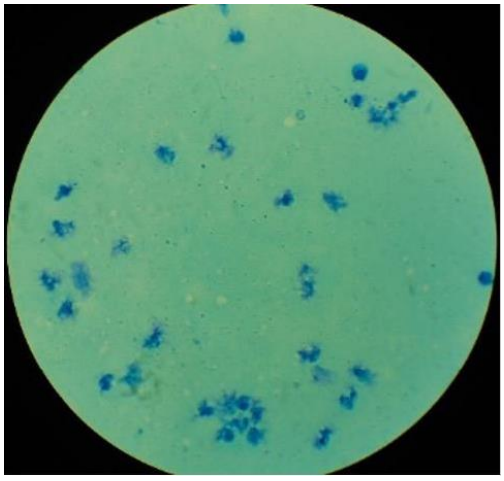
Cell length: L22 = 9,08 µm; L23 = 8,27 µm; L24 = 4,95 µm; L25 = 7,39 µm; L26 = 6,37 µm and L27 = 3,58 µm.

In the example of a cluster, as shown in Figure 3, five cells have to be counted. L27 is omitted because its diameter is less than 4 µm."

Nothing is said about the existence of yeast cells in milk, which looks similar to the leucocytes and attention has to be paid in order not to be added to the total number of somatic cells. And if in a referent laboratory the laboratory technician, who has never, been taught to recognize the yeast cells from leucocytes, can count and add their number to the total number of somatic cells in reference milk samples, which then to be used for calibration of electronic counters.

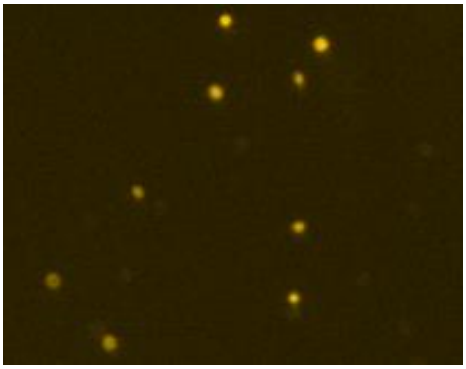
The images below show yeast cells dyed with the same reagents used in the ISO 13366-1 IDF 148-1:





Yeast cells dyed in methylene blue in a milk sample

and



Yeast cells dyed in propidium iodide in a milk sample

It is clear that they can be counted as somatic cells and can affect on accuracy in determining the number of somatic cells in milk from there and on determination of the mastitis' type: healthy animal, sub-clinical bacterial mastitis or clinical bacterial mastitis, from there to affect on the manner and the type of treatment /treatment with antibiotics assist the propagation of the yeast/, on milk price and others.

Until now no one indicated the number of yeast cells per ml milk that does not lead to yeast mastitis as until now IDF has not advised methods /reference DMSCC or devices/ for differential cell count in milk, by which you can determine which cells are with leukocyte origin, which are with epithelial, which with yeast origin and differential counting of leucocytes /determination of neutrophils and macrophages separately as a criterion for the development of mastitis/.

Only in 2016 talks started about such requirements for the reference method and the new reference methods, possessing such features.

In IDF documents:

1. SCSA S15 Improvement of the reference method for SCC:

"Thoughts on a new method

Properties

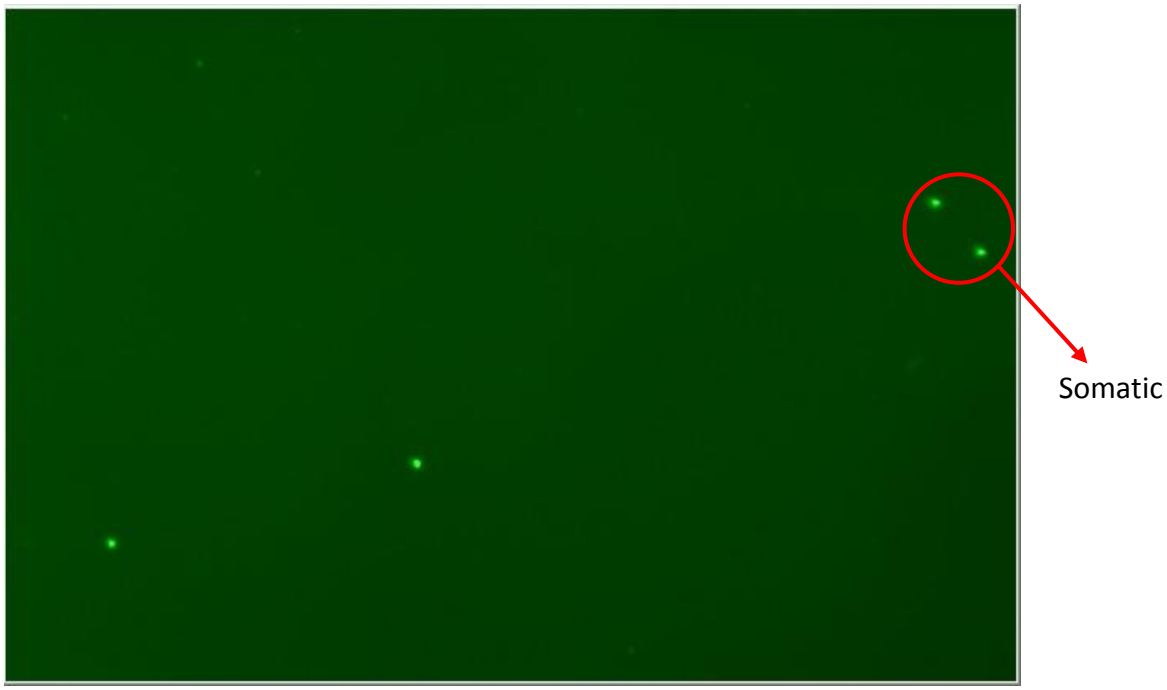
- **Clear identification of cells**
- Measurement of the same cells as today's reference method (no change in definition or measurement level)
- Fair repeatability
- Improved performance
- Easy to use for characterization of reference material
- **Ideally applicability for differential SCC**"

On the other hand, the electric methods for somatic cell counting are monopolized by 2-3 companies. Basically they are using flow-cytometry in its oldest version, compared with the medical flow-cytometry, without determination of cell size and their differentiation as it is in medical fluorescent flow-cytometers. In practice, they can measure the yeast cells, but manufacturers do not deepen in the problem. They set apparatus not "see" most of the yeast cells by setting threshold /level of the separation of the signals from the fluorescent cells is higher than the level which emit yeast cells/, as they are small than 4-6 μm, and possess less DNA. Because these devices are like big black boxes, the operators have no visual control as monitoring under a microscope and the presence of yeast goes unnoticed.

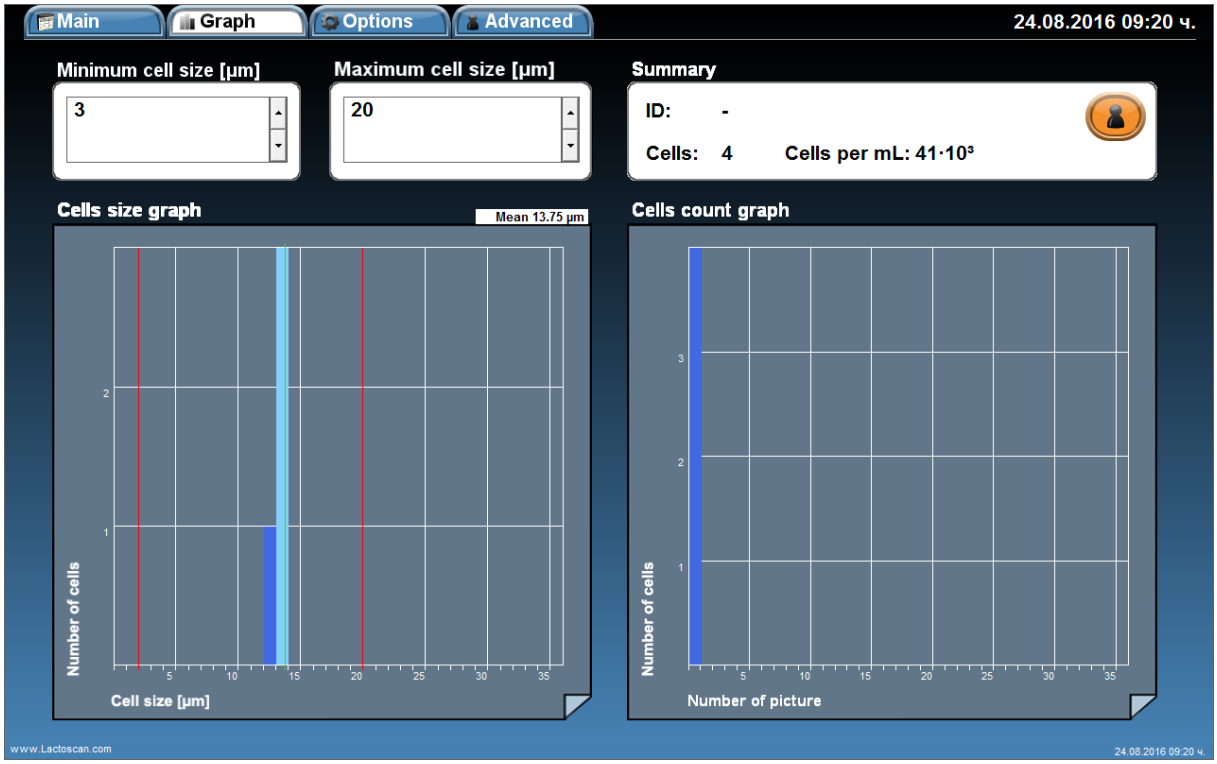
In LACTOSCAN SCC, for the first time there is a possibility to automatically count the number of yeast cells in milk samples when determining the number of total somatic cells with reference accuracy.

Below there are images, downloaded from LACTOSCAN SCC devices, located in different parts of the world. When in the milk there are only leukocytes, induced by bacterial mastitis or only a normal level of leukocytes / macrophages, neutrophils and lymphocytes / the average cell size is in the range 9-14 μm.

Image 1. Milk sample from a healthy animal

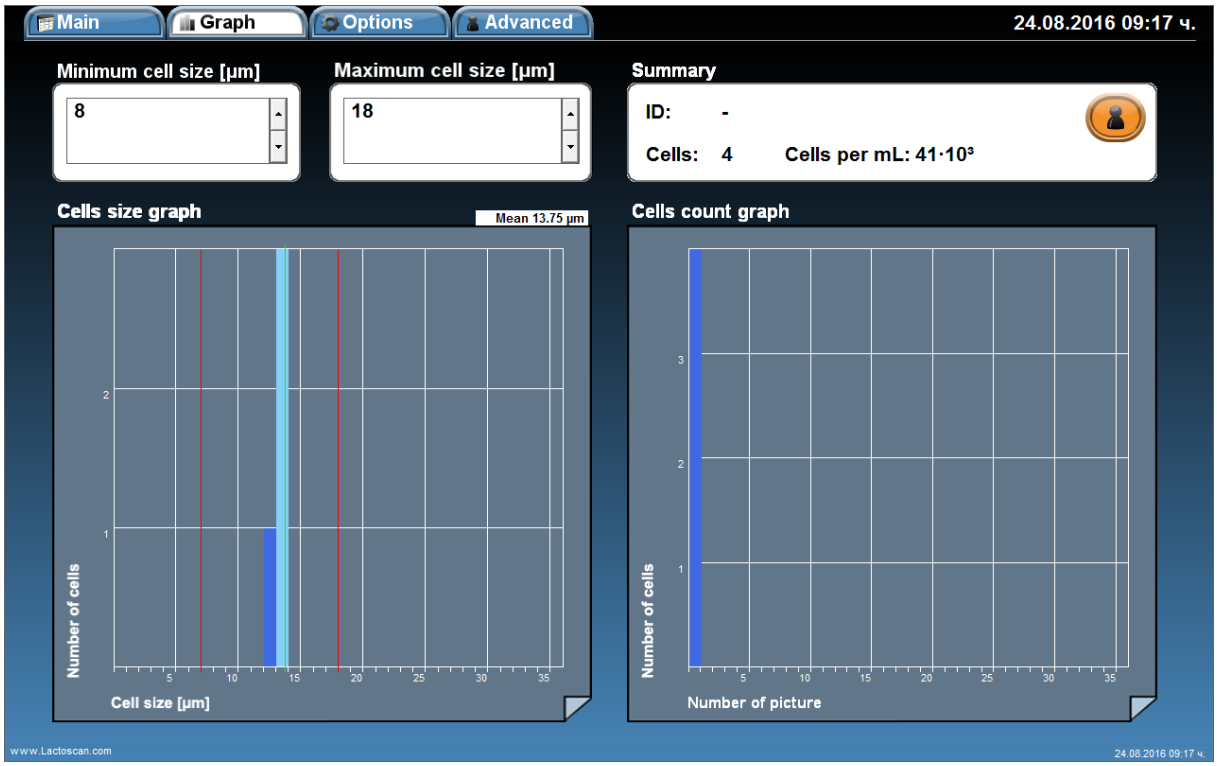


Graphic from Image 1. Minimum cell size set to 3 µm



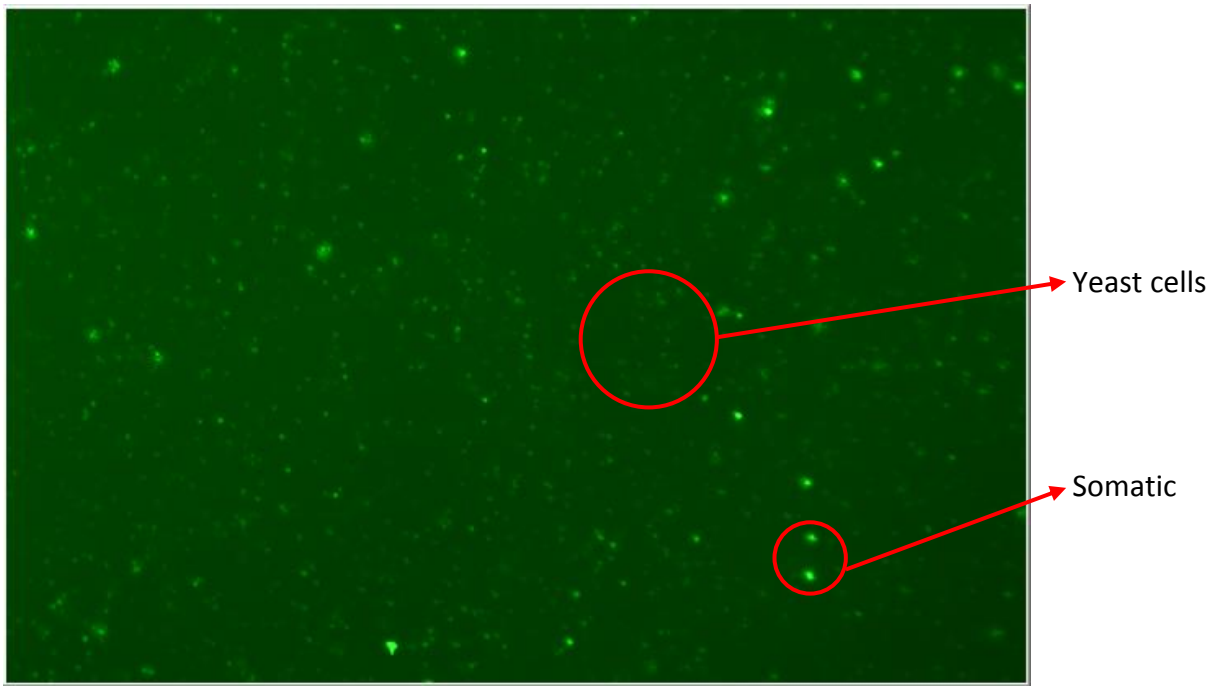
When the cell size range is set from 3 to 20 µm, the counted cells are 4, equivalent to 41x10^3. The cells size is between 13 and 14 µm. The average cell size is 13.75 µm and there are no small cells with size under 9 µm. The cell size peak is at 14 µm.

Graphic from Image 1. Minimum cell size set to 8 µm

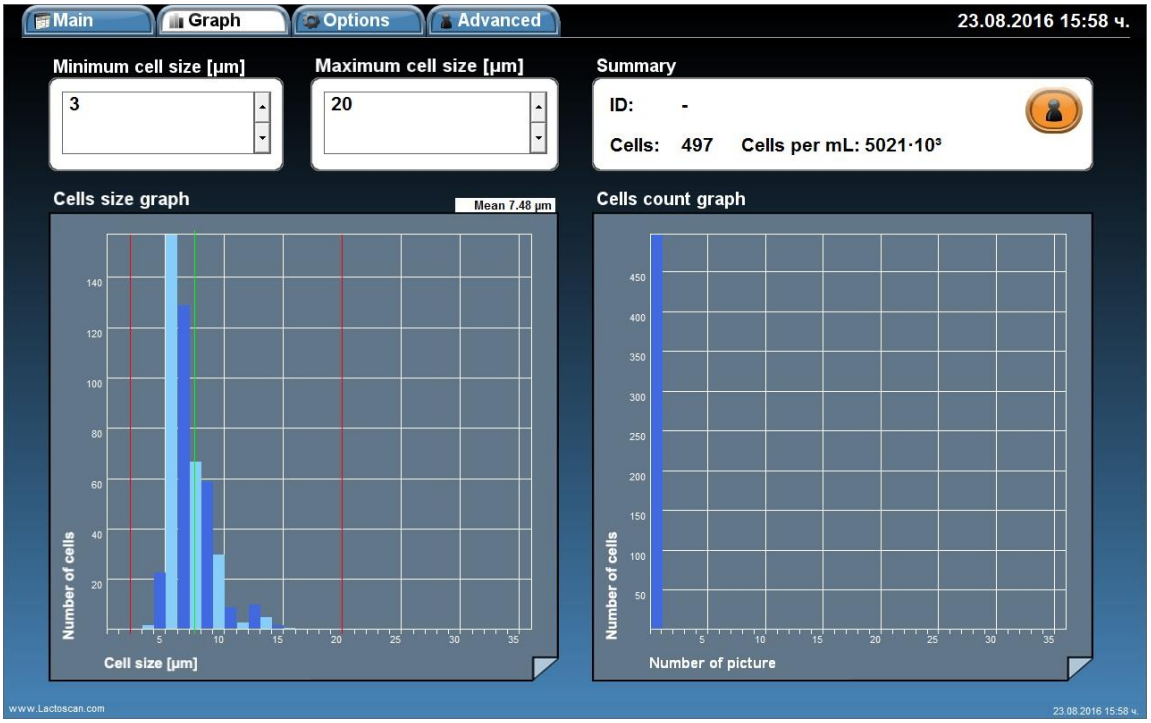


When the cell size range is set from 8 to 20 µm, the counted cells are 4, equivalent to $41x10^3$. The cells size is between 13 and 14 µm. The average cell size is 13.75 µm and there are no small cells with size under 9 µm. The cell size peak is at 14 µm. According to the graphics, it can be concluded that there are only somatic cells in the milk sample. There is no change in the final result when the cell size range of the counted cells is set to from 3 to 20 µm and when set from 8 to 20 µm.

Image 2. Milk sample from an animal with yeast mastitis

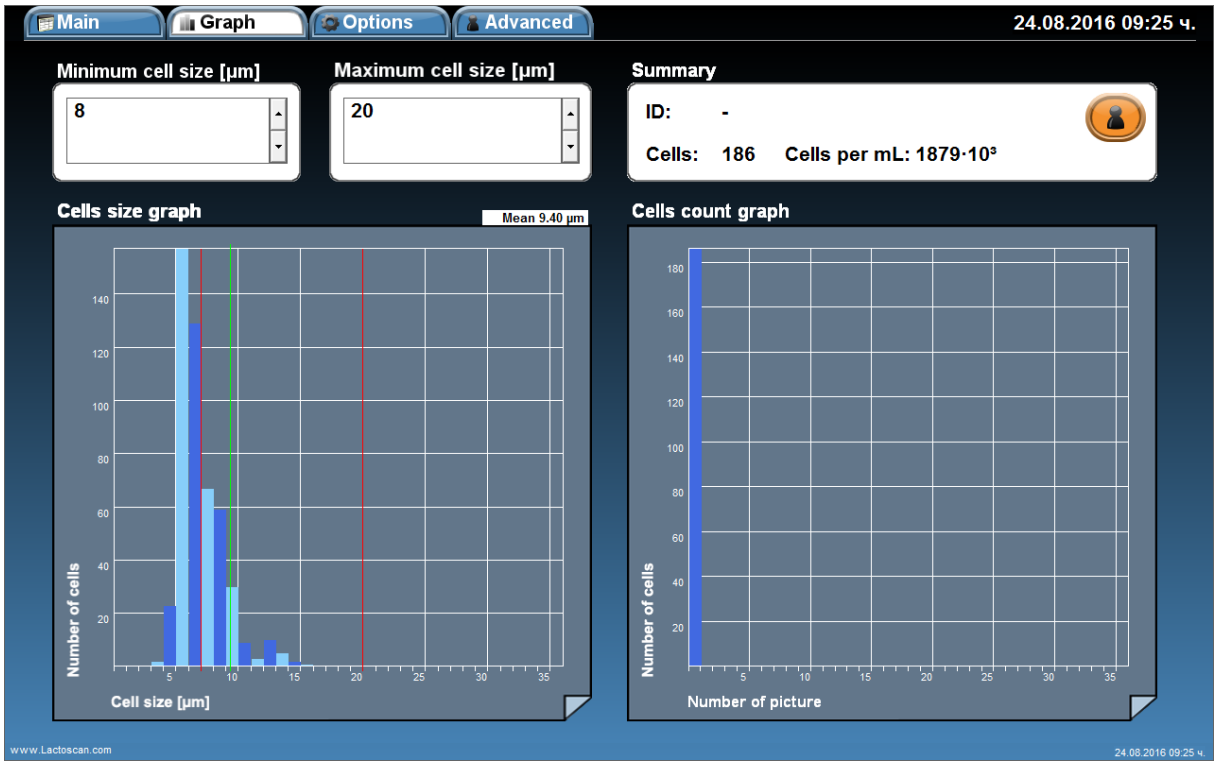


Graphic from Image 2. Minimum cell size set to 3 µm



When the cell size range is set from 3 to 20 µm, the counted cells are 497, equivalent to 5021x10^3. The cells size is between 4 and 15 µm. The average cell size is 7.48 µm. Most of the cells are with size under 9 µm. The cell size peak is at 7 µm.

Graphic from Image 2. Minimum cell size set to 8 µm



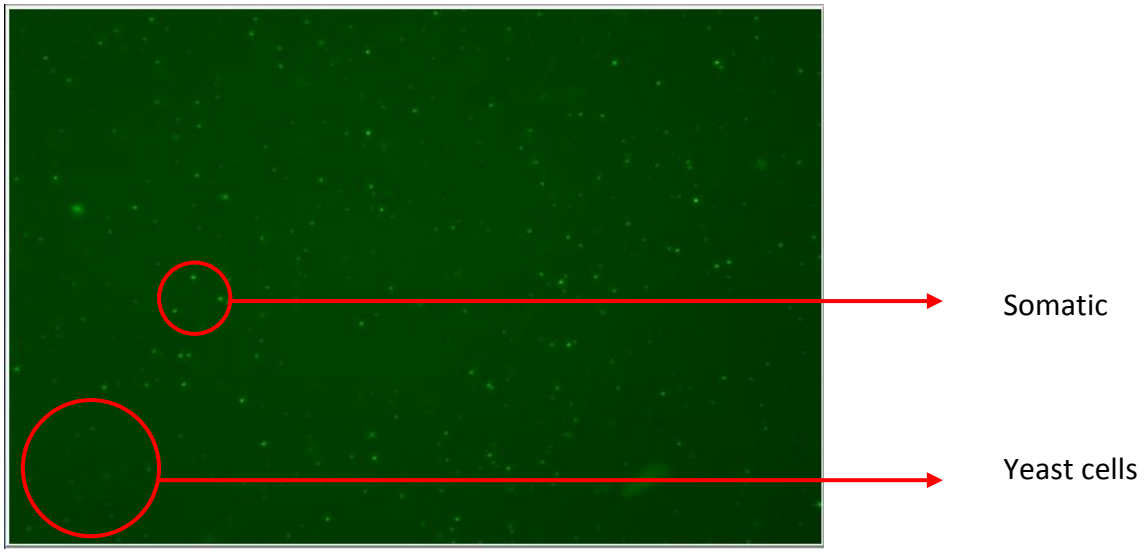
When the cell size range is set from 8 to 20 µm, the counted cells are 186, equivalent to 1879x10^3 cell/mL. The size of the cells which are included in the final result is between 8 and 15 µm. The average cell size is 9.40 µm. The cell size peak is at 8 µm.

If we compare the results from both graphics, we can conclude that on the image there are 67% yeast cells with size between 4 – 8 µm and 37% somatic cells. The higher number of yeast cells, 311 counted cells = 3142 x10^3 cell/mL, is a confirmation for presence of yeast mastitis.

The number of somatic cells, 186 counted cells = 1879x10^3 cell/mL, is a confirmation of presence of clinical mastitis.

According to the results, we can conclude that the analyzed milk sample is from an animal with yeast mastitis and clinical mastitis at the same time.

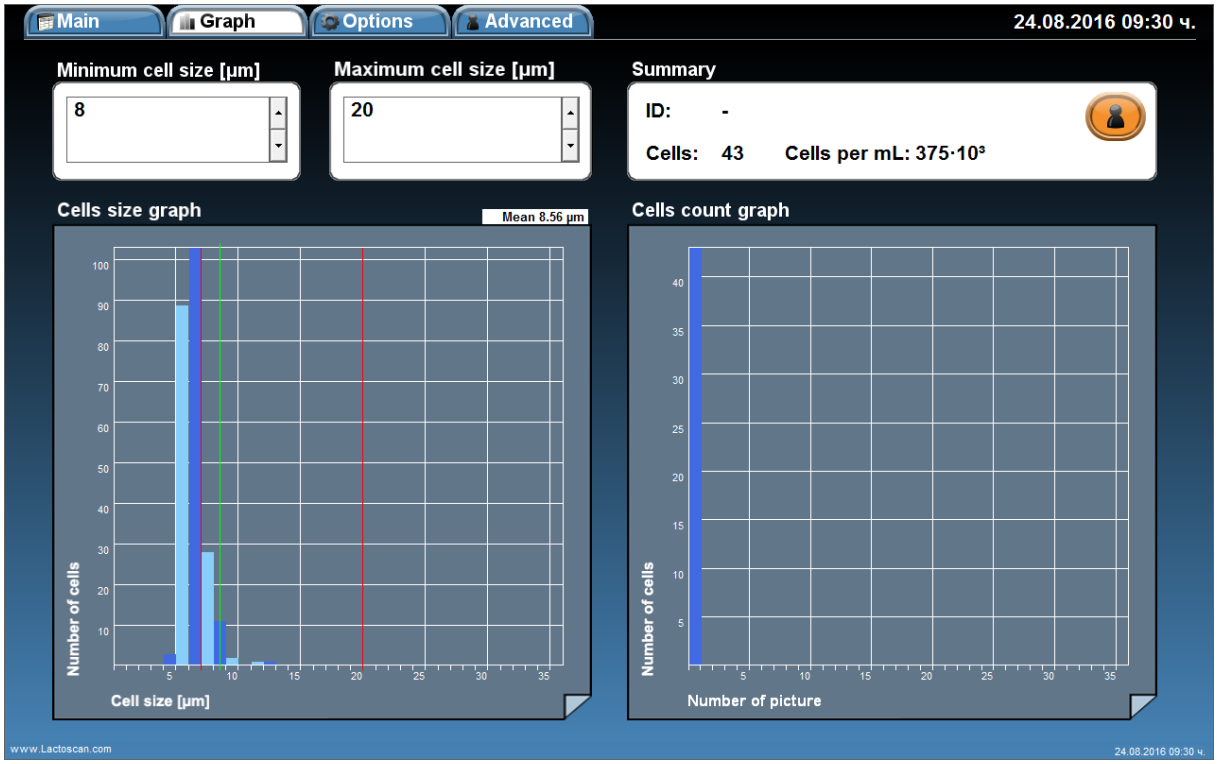
Image 3. Milk sample from an animal with yeast mastitis



Graphic from Image 3. Minimum cell size set to 3 µm

When the cell size range is set from 3 to 20 µm, the counted cells are 238, equivalent to 2074x10^3 cell/mL. The cells size is between 5 and 13 µm. The average cell size is 6.88 µm. Most of the cells are with size under 9 µm. The cell size peak is at 7 µm.

Graphic from Image 3. Minimum cell size set to 8 µm



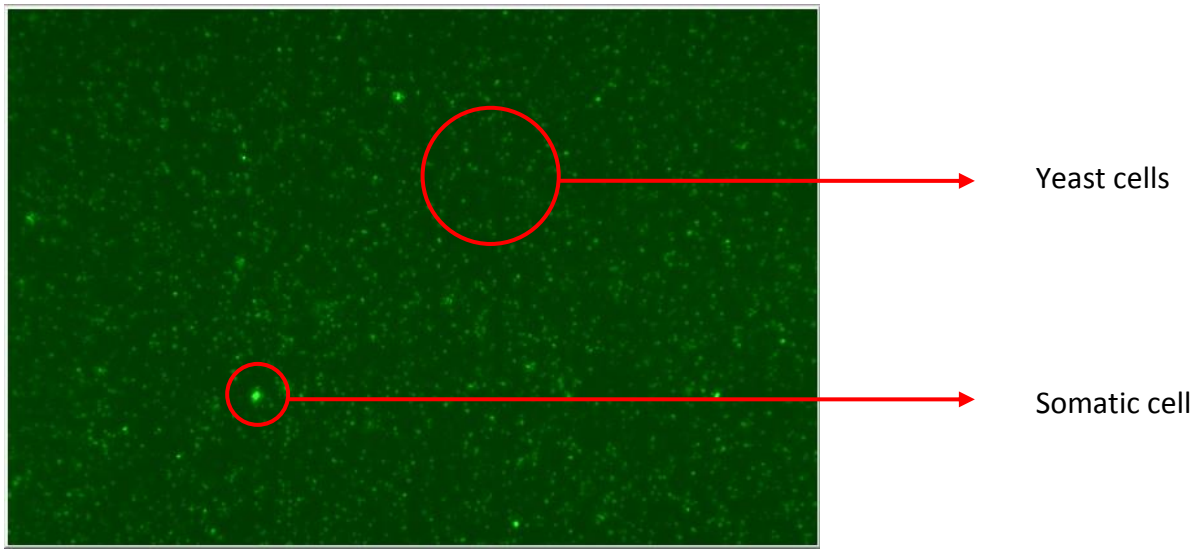
When the cell size range is set from 8 to 20 µm, the counted cells are 43, equivalent to 375x10^3 cell/mL. The size of cells which are included in the final result is between 8 and 15 µm. The average cell size is 8.56 µm. Most of the cells are with size 8-9 µm. The cell size peak is at 8 µm.

If we compare the results from both graphics, we can conclude that on the image there are 82% yeast cells with size between 5 – 8 µm and 18% somatic cells. The higher number of yeast cells, 195 counted cells = 1699x10^3 cell/mL , is a confirmation for presence of yeast mastitis.

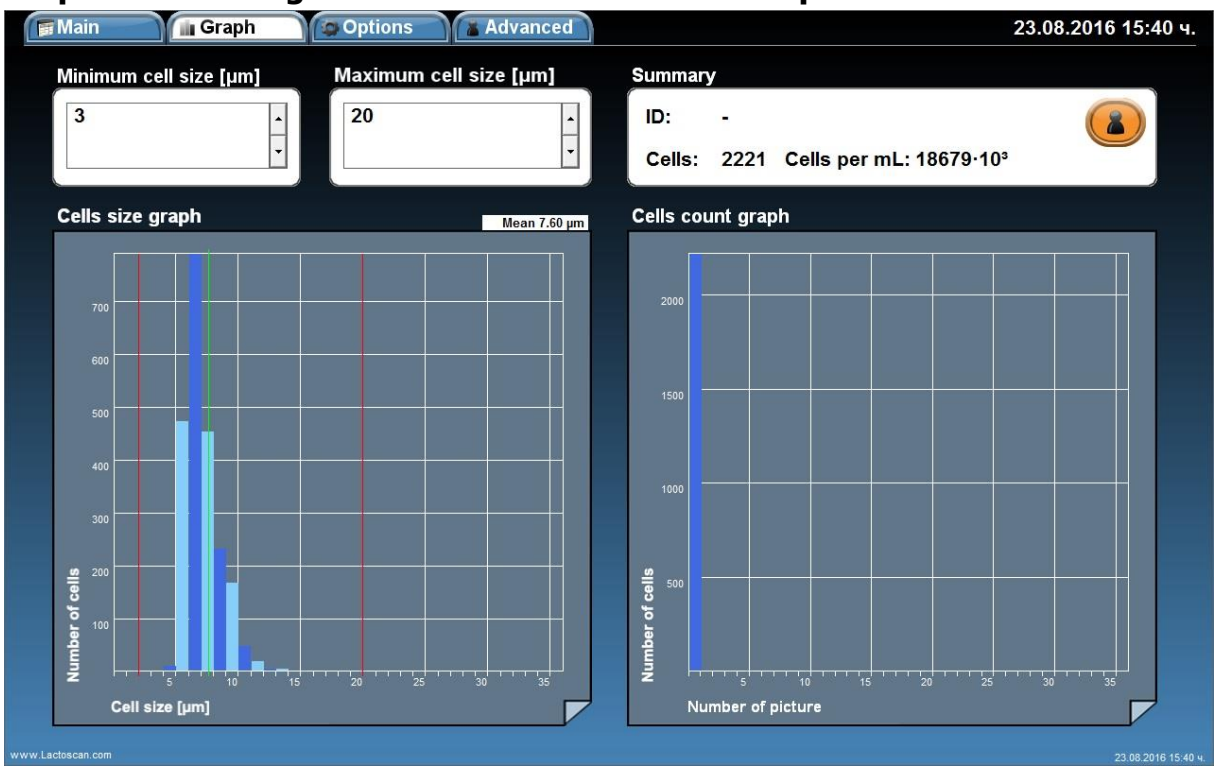
The number of somatic cells, 43 counted cells = 375x10^3 cell/mL, is a signal for possible development of sub-clinical mastitis.

According to the results, we can conclude that the analyzed milk sample is from an animal with yeast mastitis and possible development of sub-clinical mastitis at the same time.

Image 4. Milk sample from an animal with yeast mastitis

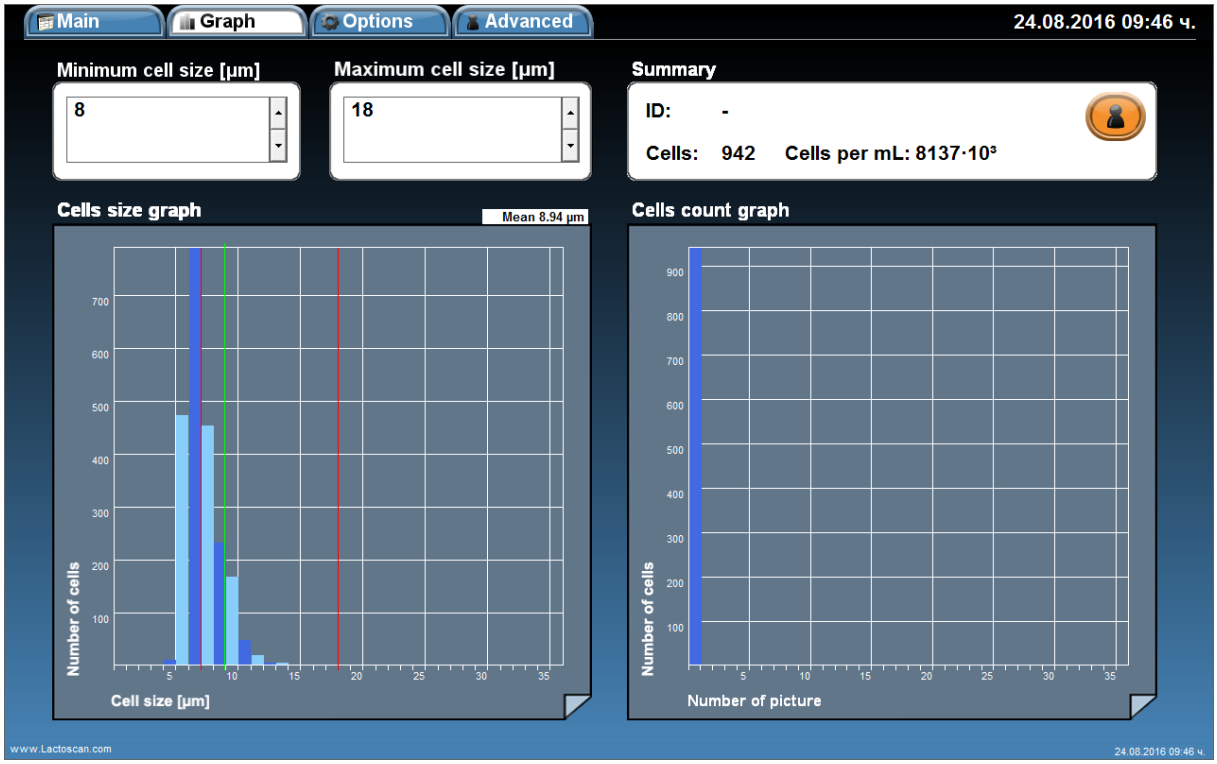


Graphic from Image 4. Minimum cell size set to 3 µm



When the cell size range is set from 3 to 20 µm, the counted cells are 2221, equivalent to 18679x10^3 cell/mL. The cells size is between 5 and 14 µm. The average cell size is 7.60 µm. Most of the cells are with size 6-8 µm. The cell size peak is at 7 µm.

Graphic from Image 4. Minimum cell size set to 8 µm



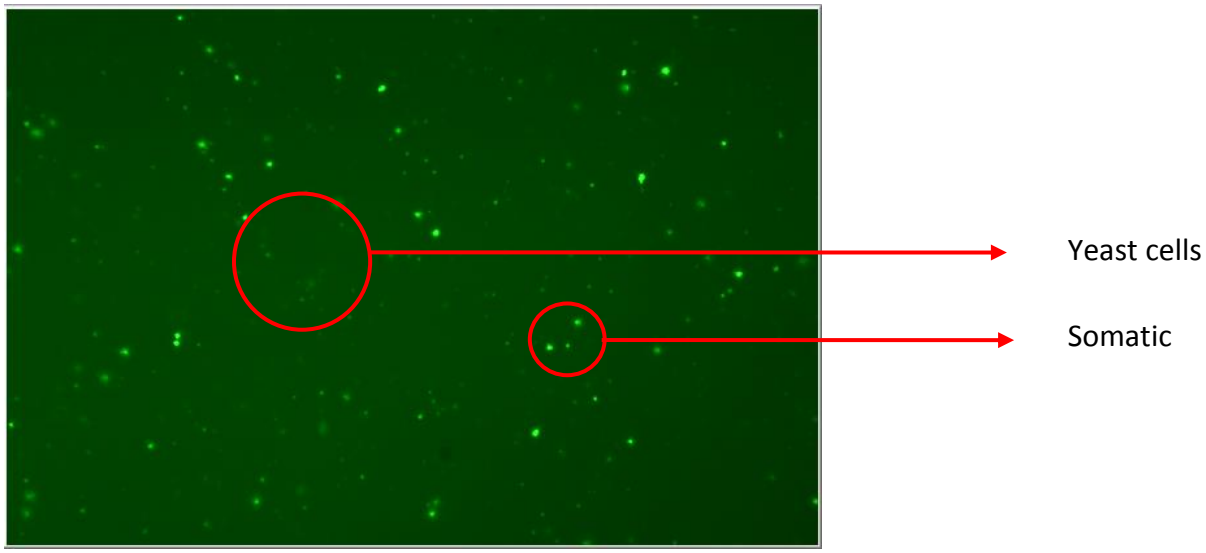
When the cell size range is set from 8 to 20 µm, the counted cells are 942, equivalent to 8137x10^3 cell/mL. The size of the cells which are included in the final result is between 8 and 14 µm. The average cell size is 8.94 µm. Most of the cells are with size 8-10 µm. The cell size peak is at 8 µm.

If we compare the results from both graphics, we can conclude that on the image there are 66% yeast cells with size between 5 – 8 µm and 44% somatic cells. The higher number of yeast cells, 1279 counted cells = 10542 x10^3 cell/mL, is a confirmation for presence of yeast mastitis.

The number of somatic cells, 942 counted cells = 8137x10^3 cell/mL, is a confirmation for presence of clinical mastitis.

According to the results, we can conclude that the analyzed milk sample is from an animal with yeast mastitis and clinical mastitis at the same time.

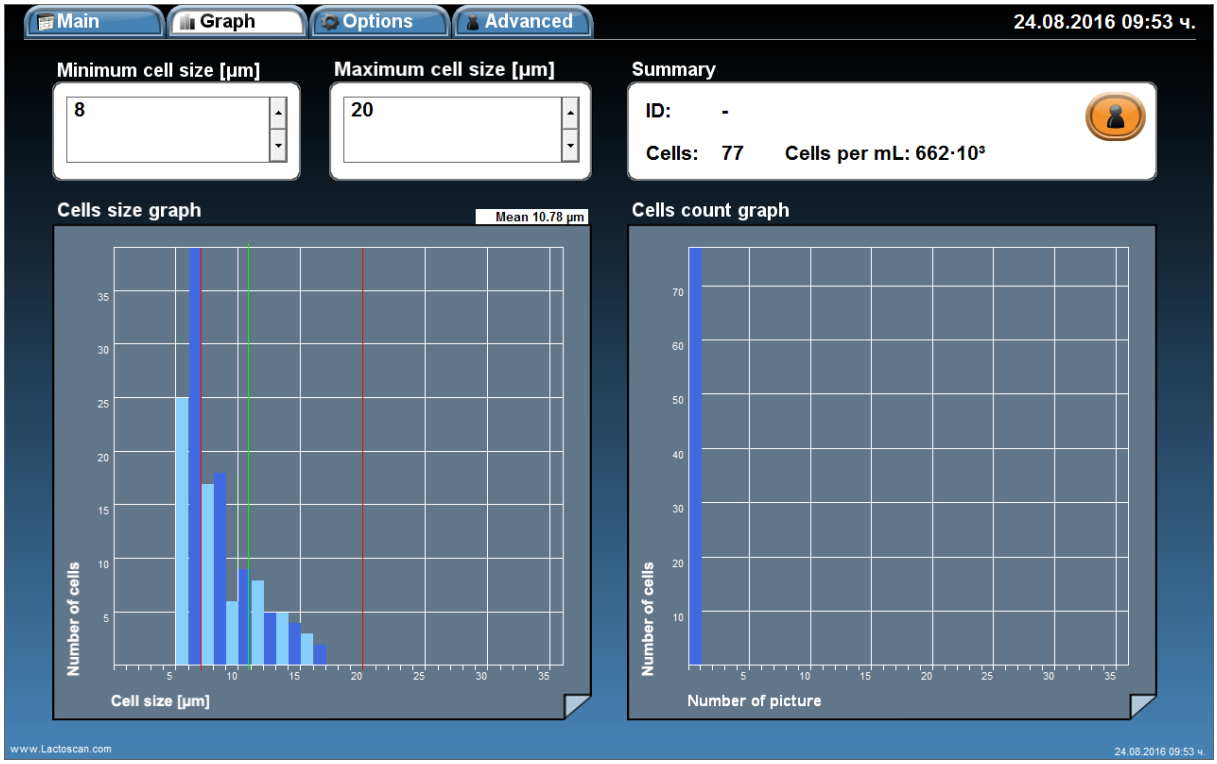
Image 5. Milk sample from an animal with yeast mastitis



Graphic from Image 5. Minimum cell size set to 3 µm

When the cell size range is set from 3 to 20 µm, the counted cells are 141, equivalent to 1213x10^3 cell/mL. The cells size is between 6 and 17 µm. The average cell size is 8.89 µm. Most of the cells are with size 6-11 µm. The cell size peak is at 7 µm.

Graphic from Image 5. Minimum cell size set to 8 µm



When the cell size range is set from 8 to 20 µm, the counted cells are 77, equivalent to 662x10^3 cell/mL. The size of cells which are included in the final result is between 8 and 17 µm. The average cell size is 10.78 µm. Most of the cells are with size 8-9 µm. The cell size peak is at 9 µm.

If we compare the results from both graphics, we can conclude that on the image there are 55% yeast cells with size between 6 – 8 µm and 45% somatic cells. The higher number of yeast cells, 64 counted cells = 551 x10^3 cell/mL, is a confirmation for presence of yeast mastitis.

The number of somatic cells, 77 counted cells = 662x10^3 cell/mL, is a confirmation for presence of clinical mastitis.

According to the results, we can conclude that the analyzed milk sample is from an animal with yeast mastitis and clinical mastitis at the same time.

In the results that we received from customers in Columbia, we found a lot of animals from one herd with average cell size 7-8 µm. We did researches of such animals and the researches emphatically have shown that the increased number of small cells /4-7 µm/ always is due to the existence of yeasts in milk. The yeasts have such cell size.

In researches for India and Brazil it is said that up to 12% of the animals have yeast mastitis. Probably the percentage for the other part of the world is close to 12% in some areas.

The big problem is that if these cells are counted as it is required in the standard for somatic cell counting /all cells with size above 4 μm are counted for somatic cells/, though the boarder of 500 000 cells indicating the existence of bacterial mastitis will surpass many times not due to somatic cells, but mainly due to the counted yeast cells, which proliferate very fat in milk and even the antibiotic treatment helps for this proliferation.

Very easily the vet can err for such milk samples and to accept that they have to treat mastitis for bacterial mastitis but it could have been yeast mastitis. For this reason, LACTOSCAN SCC is set the range for somatic cell counting from 8 to 20 μm for cow milk. This way cells with cell size 4-6-7 μm are divided from somatic cells. Of course, this way cells, mainly small lymphocytes with similar cell size, are not included in the final result but generally they are only 1% from the total number of lymphocytes and body cells entered in milk.

Below there are photos of Petri dishes with such milk sample (average cell size is 6-7-8 μm), cultured in a special culture medium for yeast, showing the proliferation of yeast in them. The microbiological cultures and the results are clearly visible from the photos:

Photo 1. Milk from a healthy animal

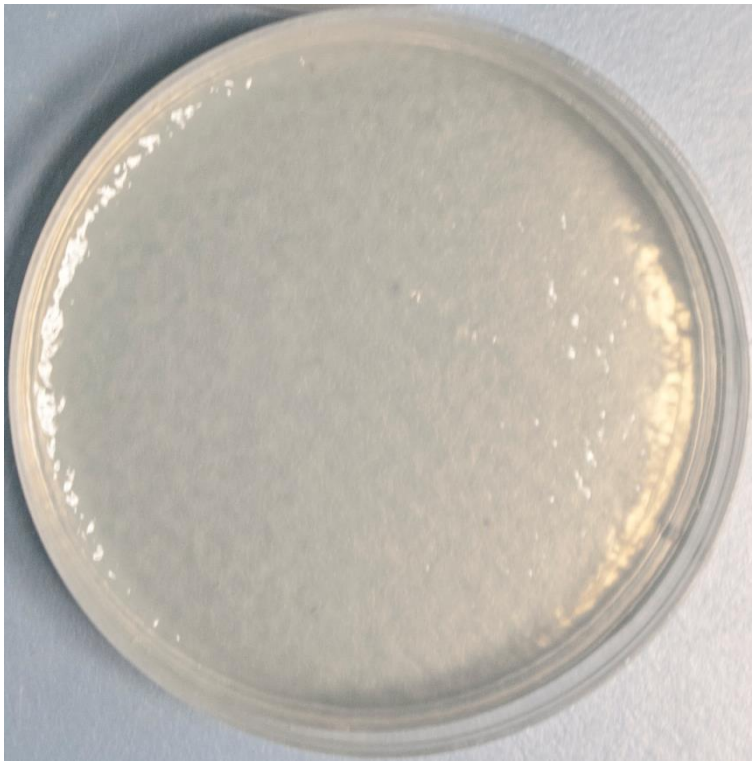


Photo 2. Milk from an animal with yeast infection

Photo 3. Milk from animal with yeast infection

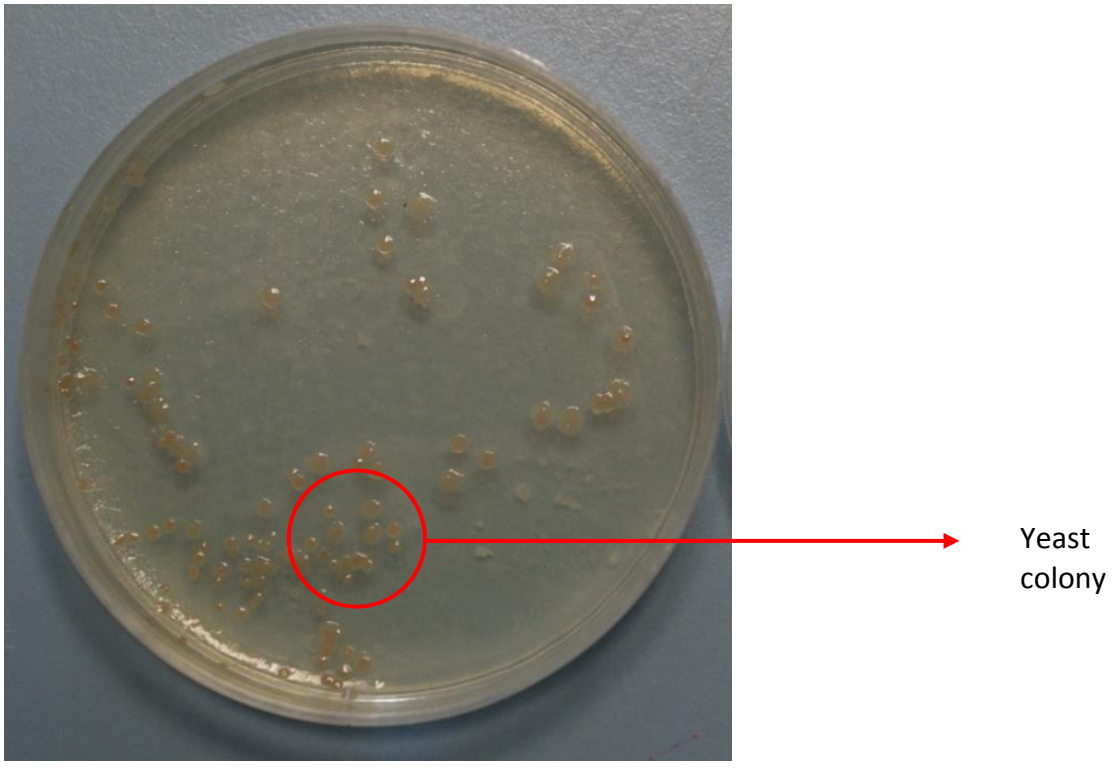
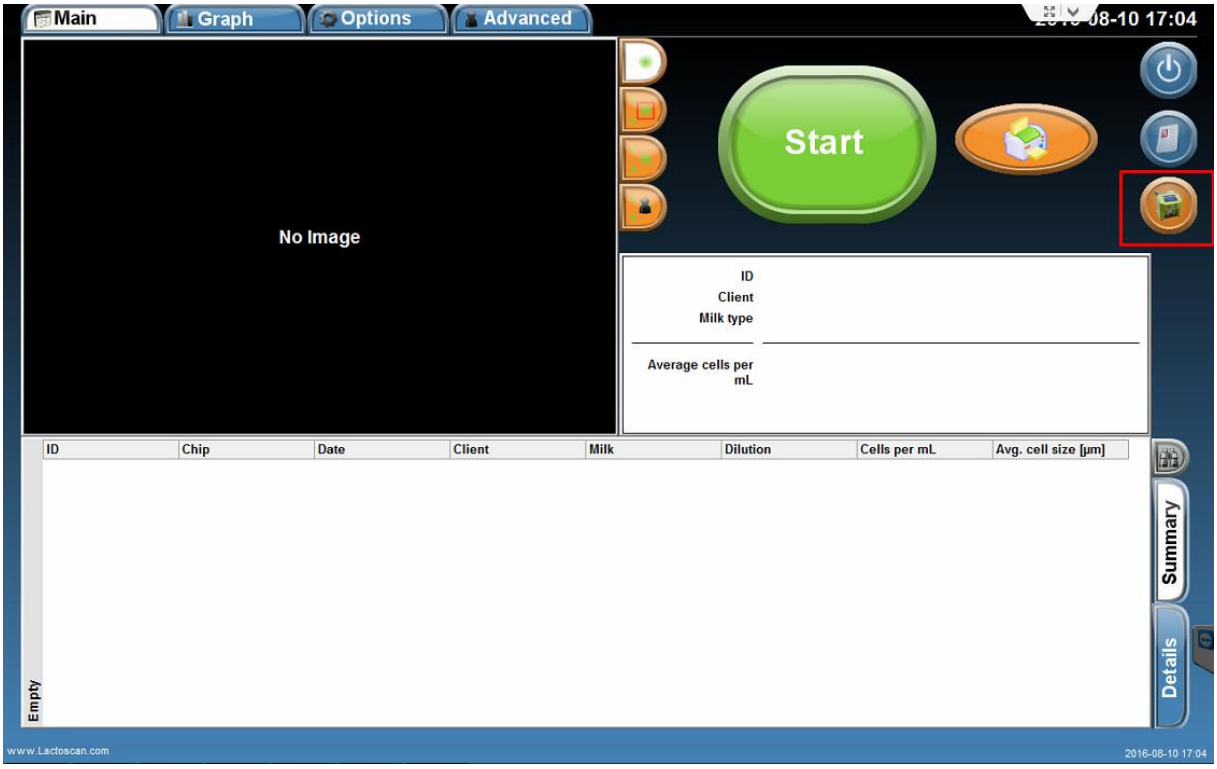


Photo 4. Milk from an animal with yeast infection

II. ULTRASONIC MILK ANALYZER

If you are in the SCC mode, by pressing the icon in the red rectangle with picture of the device will switch to the milk analyser mode:



Specification of LACTOSCAN COMBO's Ultrasonic milk analyzer

Switching Adapter

- **Input**: 100-240 V ~1.6 Amax.
  50-60 Hz
- **Output:** +12 V⎓3 A min.
- **Output power:** 36-42 W

Measurement modes

- cow milk ☒
- sheep milk ☐
- UHT milk ☐
- goat milk ☐
- buffalo milk ☒
- cream 25% ☐
- cream 45% ☒
- whey ☐
- recovered milk ☐
- other /pasteurized milk/ ☐

In the table below the configuration of the milk analyzer including spares and accessories, which are delivered on customers request are listed:

№	Description a) included in the set: ☒ b) not included in the set (may be additionally bought): ☐	Item №	pcs	☒ / ☐
1.	1 sample measurement time	60 sec		☐
		30 sec		☒
2.	RS232 Interface Cable - Analyser-IBM PC	LSS006		☒
3.	pH measuring system	LSS009	1	☐
4.	pH probe with cable and holder	LSS010	1	☐
5.	Buffer solution Ph 60 ml (pH7.00±0.01/20˚C)	LSS011	1	☐
6.	Buffer solution pH 60 ml (pH4.00±0.01/20˚C)	LSS012	1	☐
7.	Milk conductivity measuring system	LSS013	1	☒

8.	Buffer solution conductivity 50 ml (5.02 (±5%) mS/cm (18±0.1˚C)	LSS014	1	☐
9.	RS232 Interface Cable - Milk Analyser – Serial Printer/IBM PC	LSS018	1	☐
10.	High-fat measurement function	LSS020	1	☒
11.	Spare O-ring for the pH probe		1	☒

1. FUNCTION

The function of the milk analyzer is:

- **to make quick analysis of milk on fat (FAT), non-fat solids (SNF), proteins, lactose and water content percentages, temperature (^{o}C), freezing point, salts, total solids, as well as density of one and the same sample directly after milking, at collecting and during processing.**
- **Total integration of the milk sample's measured parameters with the processes for storing, processing and sending the results towards the corresponding receiver.**

2. TECHNICAL PARAMETERS

2.1. Working modes characteristics:

The program of the milk analyzer has four working modes.

2.1.1. Measurement mode milk / dairy product – first type

2.1.2. Measurement mode milk / dairy product – second type

2.1.3. Measurement mode milk / dairy product – third type

These modes have been calibrated on customers' request for 3 milk types from the following: cow, sheep, UHT, buffalo, goat, camel milk, cream, ice cream mixtures, whey, recovered milk, etc. before leaving the production facilities and the text on the display will be for the corresponding types.

2.1.4. Cleaning

2.2. Measuring range:

Fat	.....from 0.01% to 25%
SNF	.....from 3% to 15%
Density **	.....from 1000 to 1160 kg/m^3
Proteins	.....from 2% to 7%
Lactose	.....from 0.01 % to 6 %
Water content	.....from 0 % to 70 %
Temperature of milk	.....from 1^{o}C to 40^{o}C
Freezing point***.....	.....from – 0,4 to – 0,7^{o}C
Salts	.....from 0,4 to 1,5%
PH*.....	.....from 0 to 14
Conductivity *.....	.....from 3 to 14 [mS/cm]
Total Solids*	.....from 0 to 50 %

* Option, on customers' request

** Density data are shown in an abbreviated form. For example 27.3 have to be understood as 1027.3 kg/m^3. To determine the milk density, write down the result from the display and add 1000.

Example: result 21,20; density = 1000 + 21,20 = 1021,2 kg/m^3

The abbreviated form of the density is used also when entering data for samples in working mode **Recalibrate**, for example:

If the measured sample density is 1034.5 kg/m3, then in the menu for entering the samples parameters used for calibration, across the parameter Den = , you have to enter 34.5.

**** Please, carefully read Appendix Freezing Point.

2.3. Accuracy:

Fat	.....± 0.10%
SNF	.....± 0.15%
Density	.....± 0.3 kg/m^3
Proteins	.....± 0.15%
Lactose	.....± 0.20%
Water content	.....± 3.0%
Temperature of milk	.....± 1°C
Freezing point.....	.....± 0.001°C
Salts	.....± 0.05%
PH	.....±0.05%
Conductivity	.....±0.05
Total solids	.....± 0.17%

The difference between two consequent measurements of one and the same milk could not exceed the maximum permissible absolute error.

2.4 Correct ambient conditions:

Maximum permissible absolute error is guaranteed in case of normal ambient conditions:

Air temperature.....	.....from 10°C to 40°C
Relative humidity	.....from 30% to 80%
Power supply	.....220V (110V)

Extent of contamination at normal environmental conditions.....2

Maximum permissible absolute error values in point 2.3 are in dependence on the correctness of the corresponding chemical method, used for component content determination. In point 2.3. are used the following reference methods: Gerber – for fat, gravimetric – for SNF, Kjeldahl – for protein. The boundary for maximum variation of repeatability when the power supply voltage is from +10 to – 15% from the nominal voltage values (220 V) have to be no more than 0.8 accuracy according point 2.3. The analyzer is used in conditions free of outer electrical and magnetic fields (except the magnetic field of the Earth) and vibrations.

2.5. Continuous working time:

.....non-stop

2.7 Milk sample volume per one measurement:

.....15 cm^3 (= 25 ml)

3. Qualification of raw milk, thermally treated milk, other dairy products and derivatives

3.1. Taking samples and preparation for analysis

In order to receive reliable results in qualification of milk, dairy products and derivatives are needed: precise samples taking; correct samples storing (in need to be preserved); correct preparation before making measurement. The rules and requirements for this are described in details in *Appendix Preparing Samples.*

3.2. Making the measurement.

3.2.1. Preparing the analyzer for working mode

3.2.1.1. Put the analyzer on the working place, providing good ventilation and not in the vicinity of heat providing devices or sources. The temperature in the premises has to be in the boundaries 10-30^{o}C.

3.2.1.2. Switching on the analyser is done with a switch at the rear panel of the analyser. The tablet, controlling the analyser is powered by its power switch. Wait for Windows to be loaded. The operator has an access to all the resources of the system, where can be set, if necessary - printer (for example standard A4 printer), WiFi or connection to other peripheral devices (for example mouse and keypad, flash memory etc.). There's an USB Hub, which ports are mounted on the back panel of the analyser. These are standard USB ports, connected to the tablet (controlling computer).

There are 2 ways of control of the tablet/software:

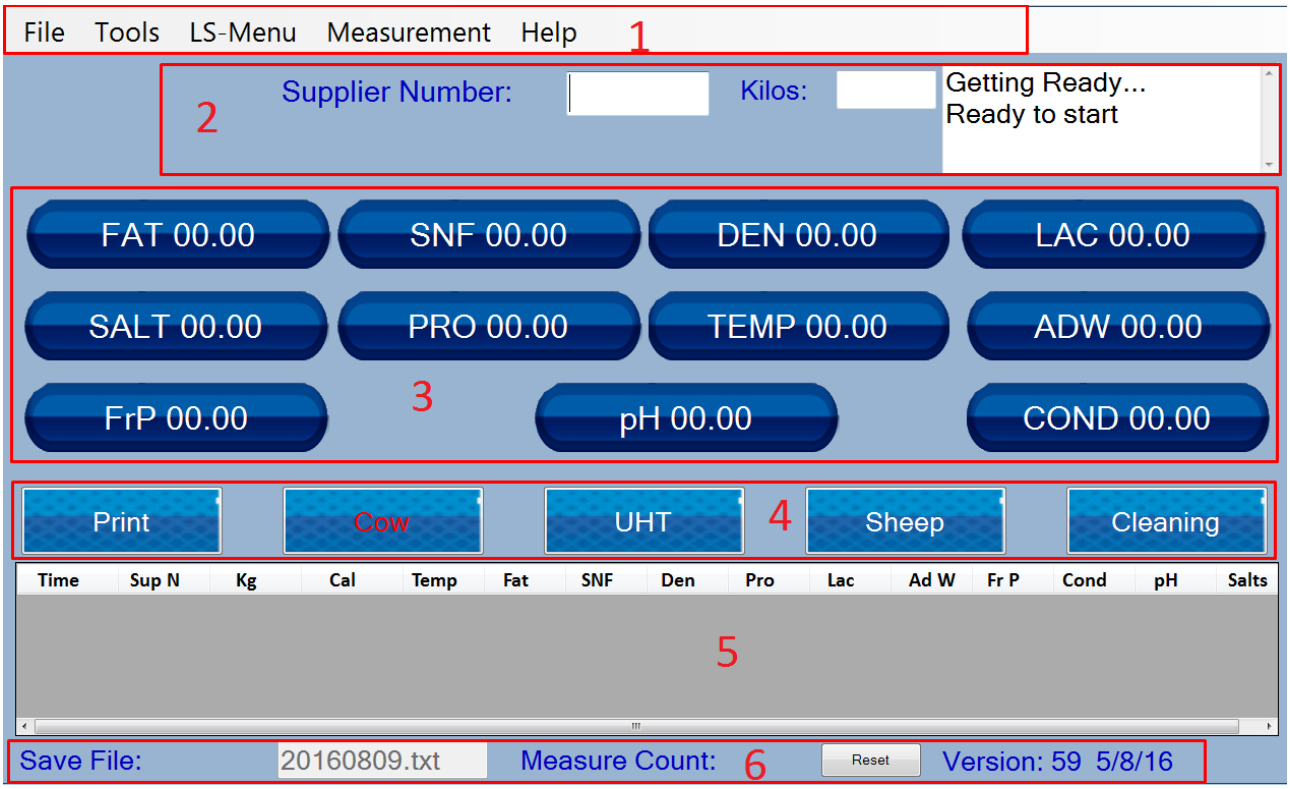
A. By direct touch on the buttons on the display (using the possibility of the touch screen of the tablet) and buttons on the front panel (if the version is with such buttons);

or

B. With the mouse and/or the keyboard, working like a standard computer.

Version A is suitable for daily work for measuring and cleaning the analyser, while version B is recommended for setting and servicing.

The software can be started directly after Windows has been loaded or by pressing an icon, in dependence of the custom's requirement. After the software is loaded, the main menu appears on the display:



Description of the main screen:

Position 1. System menu – the upper one on the display. It is described with the file Supplier Tools LS-Menu Help. Serves for direct control of the settings/test/service mode of the analyser.

Position 2. System Status – includes groups Supplier, Liters and information tab, imitating the display of the standard (analyser without a tablet) Lactoscan (upper right corner). Serves for entering the ID of the deliverer, liters/ kgs which were delivered and shows the current information, describing the exacution of the specific commands sent from the tablet towards the PCB of the analyser.

Position 3. Tab for measurement results – Fat=00.00, SNF=... Information tab and could not be changed by the operator. It serves for displaying the measurement results. The last measurement results stay active till the next measurement is started.

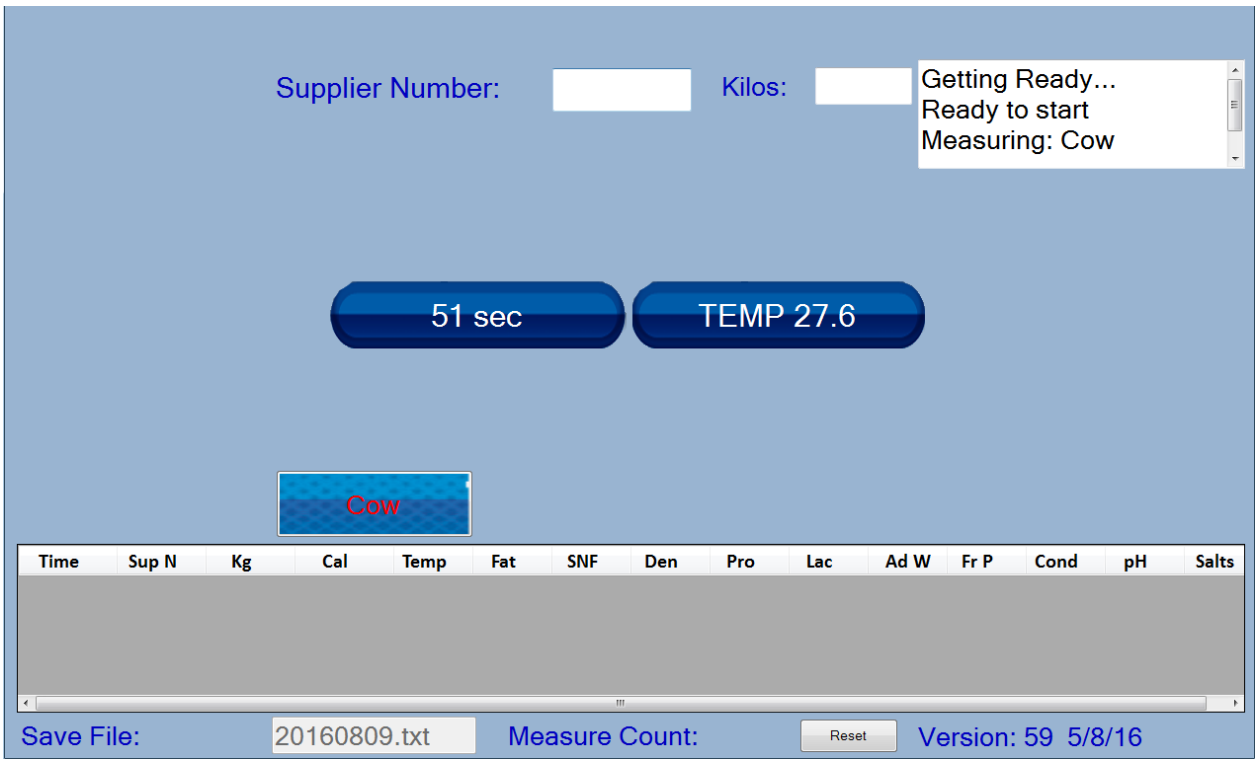
Position 4. Control buttons. The buttons at both ends are with fixed names – Print and Clean – for starting the corresponding commands. The rest 3 are with changeable names, because they show the name of the corresponding calibration (type of milk). By pressing them, a measurement on the chosen calibration is started. If the analyser is with outer keyboard, mounted in the box of the device (a number of buttons with the corresponding designations), by pressing the buttons left/right is moving the choice of the calibration. Using this keyboard, the chosen calibartion is started by pressing the button Enter.

Position 5. Archive tab, type List Box at the lower part of the display. It shows the last 10 measurement results. The meaning of the results is described in the list over them.

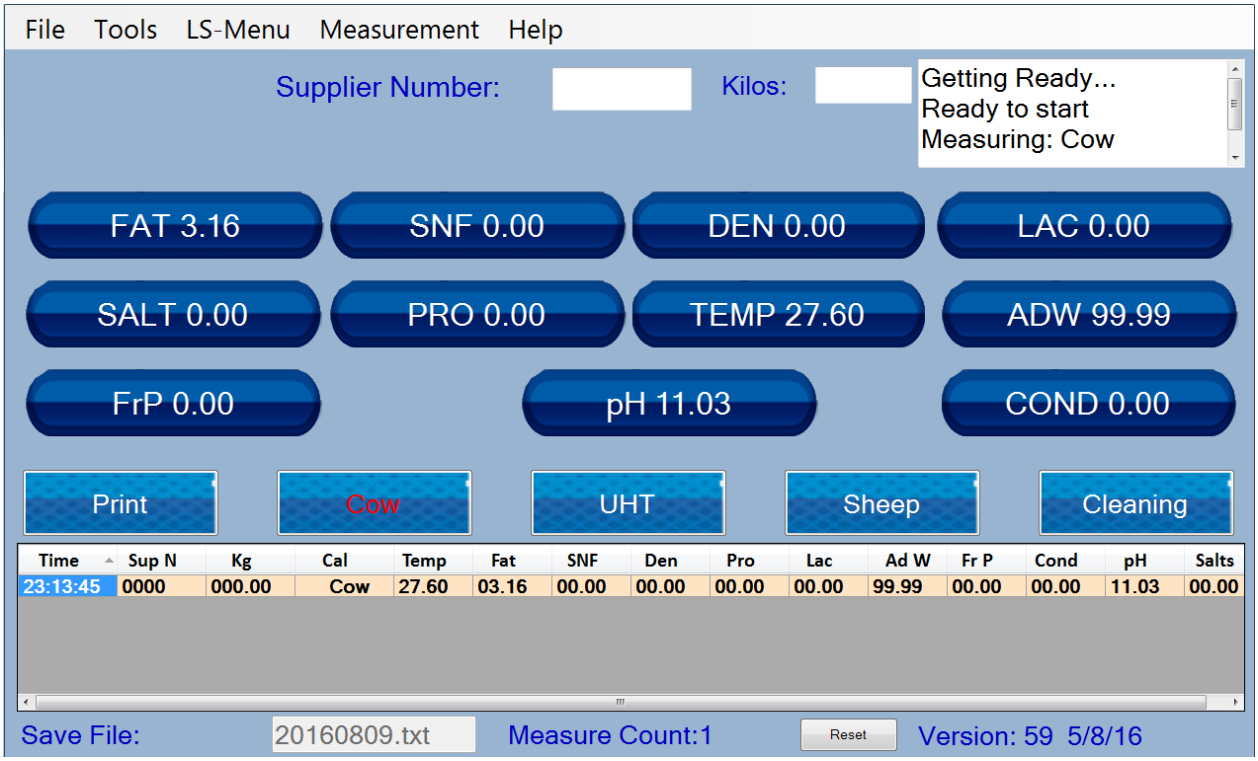
Position 6.Information line. It is at the bottom of the display. The group Save File shows the name of the file, where the results will be saved. It is generated automatically, based on the current date. If needed, it ca be changed. The group Meas Count – shows the number of the measurements already done. At the right of this line is displayed the version of the PCB (Main PCB – on which the measurement system is based) and software in it.

3.2.2. Working with the analyser in measurement mode.

This is the main working mode. It is needed a milk to be poured in the glass, to place it in the recess of the analyser, to choose the needed calibration and start it. The sample is sucked, display is changed as follows:



The calibration used at the moment remains on the display. The previous results are hidden. First appears the temperature of the sample. A clock/timer appears in the middle of the display, showing in seconds the remaining time till the appearance of the measurement results. After measurement completion, the results appear in the tab for the measurement results as well as in the archive tab i.e. the initial start display with valid results appears. The results are archived to a text file and can be used for later result analysis. If there is a internet connection(e.g. WiFi, 3G modem, etc.) the results can be send to a cloud, where they can be stored or further analysed



If there's serial printer connected (loaded with paper, with closed cover), the results are printed out. The analyser is ready for the next command (it is in Idle mode) i.e. ready for the next measurement. Button Print is active – by pressing it a copy of the results can be printed out (it is advisable one printout to remain in the milk collecting center, while the second one to be given to the deliverer).

4. CLEANING THE ANALYZER

This procedure prevents gathering milk fat residues and milk stone on the sensor. The milk stone consists of milk solids, calcium, iron, sulphates, magnesium, etc. All these substances form layer on the pipe and sensor's walls, which leads to deviations inthe measurement results and blocking up the piping.

The company-producer recommends usage of the chemicals, supplied with the analyser – alkaline and acidic (Lactodaily and Lactoweekly). You may order them separately or together with the analyser. Try to use only these chemicals for cleaning the analyser.

In case you missed to order these chemicals, the alternative is to use alkaline and acidic cleaning solutions for dairy equipment by one the companies, producing such chemicals, as for example:

http://www.diversey.com

http://www.ecolab.com

http://www.calvatis.com

Do not use chemicals not intended for usage in the milking systems or vessels in the dairy sector. Pay special attention to the concentration of the acidic chemical. **Increased concentration may damage the measuring sensor.**

4.1. Periodically cleaning (rinsing) the analyzer

It is done in the process of routine work of the analyzer. Its aim is to prevent drying up and adhesion of different milk components in the milk analyzer's measuring system.

4.1.1. Periodical cleaning frequency.

It is easy to understand what is the period on which the rinsing could be done as the analyzer reminds you when it is necessary. This is done by a sound signal in 1-second cycle after the set time intervals elapse:

- 55 min. after switching on the power supply of the analyser, but idle work;

- 15 min. after the last measurement of real milk sample.

*Idle Mode is that part of the standard working mode, when the analyser is not making measurements. There's embedded in the analyser system for measurement of the idle time. The idle time is measured starting from the last action of the operator. In dependence of it (what the operator last did), are taken decisions regarding the cleaning.

There are 2 options:

Option A: If the analyser:

1. Was only switched on but was not started in measurement mode,
2. Or the last action was cleaning,
3. Or the last action was measuring sample with very low Fat (similar to water)

Then the signal for cleaning is started after 55 min.

Option B: If the last thing done with the analyser was measurement of normal milk sample, the signal for cleaning is started after 15 min.

After cleaning completion, new measurement takes place in above described time intervals.

4.1.2. Making the rinsing

After above message is received put in the recess of the analyzer a sample holder with alkaline cleaning solution or water.

Press Clean to start the rinsing mode.

In this mode the analyzer makes 8 cycles and stops.

Already used solution is poured out of the analyser. Now the device is ready for the next measurement. In case of doubt that the analyzer is still not well cleaned, the procedure Cleaning may be executed repeatedly.

4.2. Complete cleaning

4.2.1. Complete cleaning frequency

This cleaning is done after finishing the work with the analyzer at the end of the working day or if it is obvious that the measuring system of the analyzer is contaminated in case of intensive work with it. It is done with alkaline cleaning solution.

Preparation of 3 % alkaline solution of Lactodaily for circulation cleaning in the milk analyzer:

1. Take the package 100 g concentrated chemical Lactodaily

2. In appropriate vessel (for example bucket) pour 1 l water.

3. Add the powder and then again water up to 3 l.

For a single cleaning cycle you need only 25 ml cleaning solution. We recommend you to prepare working solutions of cleaning chemicals, enough for normal work for 1 week, because, during their stay unused, the working solutions loose their strength and also is difficult to store them.

Then follow the instruction for milk analyzer cleaning.

4.2.2. Cleaning

4.2.2.1.Rinsing milk residues

Fill in the glass with water. Put it in the recess of the analyser and start command Cleaning from the main menu. After finishing it pour out the contaminated water.

4.2.2.2.Cleaning with alkaline cleaning solution

Fill in the glass with warm (50-60 C) alkaline cleaning solution. Put it in the recess of the analyser and start the command Clean. After finishing it, pour out the contaminated liquid.

4.2.2.3.Rinsing with water

Fill in the glass with water. Put it in the recess of the analyser and start command Clean. After finishing it pour out the contaminated water. Now the device is ready for work.

4.2.2.4. Cleaning with acidic solution

It is recommended to be done every day.

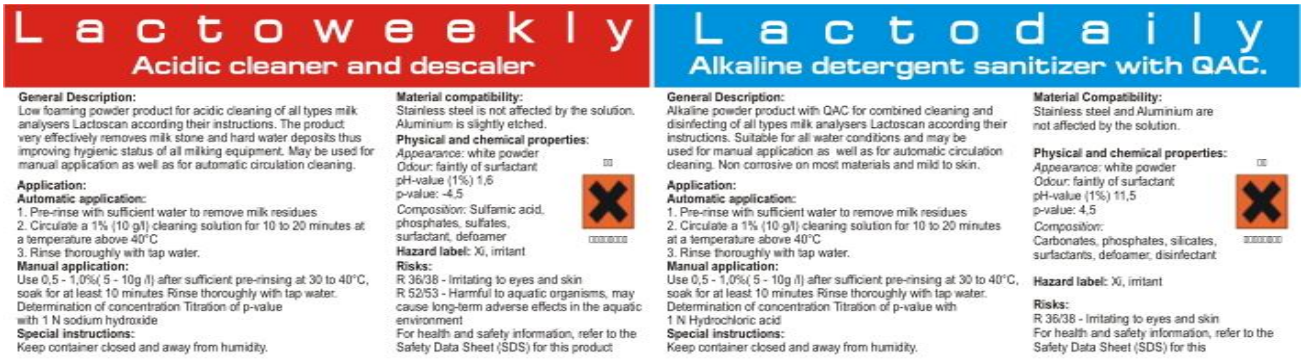
Preparation of 3 % acidic solution of Lactoweekly for circulation cleaning in the milk analyzer:

1. Take the package 100 g concentrated chemical Lactodaily

2. In appropriate vessel (for example bucket) pour 1 l water.

3. Add the chemical and then again water up to 3 l.

Labels for the cleaning chemicals



Lactoweekly
Acidic cleaner and descaler

General Description:
Low foaming powder product for acidic cleaning of all types milk analysers Lactoscan according their instructions. The product very effectively removes milk stone and hard water deposits thus improving hygienic status of all milking equipment. May be used for manual application as well as for automatic circulation cleaning.

Application:
Automatic application:
1. Pre-rinse with sufficient water to remove milk residues
2. Circulate a 1% (10 g/l) cleaning solution for 10 to 20 minutes at a temperature above 40°C
3. Rinse thoroughly with tap water.
Manual application:
Use 0,5 - 1,0%(5 - 10g /l) after sufficient pre-rinsing at 30 to 40°C, soak for at least 10 minutes Rinse thoroughly with tap water. Determination of concentration Titration of p-value with 1 N sodium hydroxide
Special instructions:
Keep container closed and away from humidity.

Material compatibility:
Stainless steel is not affected by the solution. Aluminium is slightly etched.
Physical and chemical properties:
Appearance: white powder
Odour: faintly of surfactant
pH-value (1%) 1,6
p-value: -4,5
Composition: Sulfamic acid, phosphates, sulfates, surfactant, defoamer
Hazard label: Xi, irritant
Risks:
R 36/38 - Irritating to eyes and skin
R 52/53 - Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment
For health and safety information, refer to the Safety Data Sheet (SDS) for this product

Lactodaily
Alkaline detergent sanitizer with QAC.

General Description:
Alkaline powder product with QAC for combined cleaning and disinfecting of all types milk analysers Lactoscan according their instructions. Suitable for all water conditions and may be used for manual application as well as for automatic circulation cleaning. Non corrosive on most materials and mild to skin.

Application:
Automatic application:
1. Pre-rinse with sufficient water to remove milk residues
2. Circulate a 1% (10 g/l) cleaning solution for 10 to 20 minutes at a temperature above 40°C
3. Rinse thoroughly with tap water.
Manual application:
Use 0,5 - 1,0%(5 - 10g /l) after sufficient pre-rinsing at 30 to 40°C, soak for at least 10 minutes Rinse thoroughly with tap water. Determination of concentration Titration of p-value with 1 N Hydrochloric acid
Special instructions:
Keep container closed and away from humidity.

Material Compatibility:
Stainless steel and Aluminium are not affected by the solution.
Physical and chemical properties:
Appearance: white powder
Odour: faintly of surfactant
pH-value (1%) 11,5
p-value: 4,5
Composition:
Carbonates, phosphates, silicates, surfactants, defoamer, disinfectant
Hazard label: Xi, irritant
Risks:
R 36/38 - Irritating to eyes and skin
For health and safety information, refer to the Safety Data Sheet (SDS) for this

The following procedure is executed:

1. Rinsing the milk residues:

Fill in the glass with water. Put it in the recess of the analyser and start command Clean. After finishing it pour out the contaminated water.

2. Cleaning with acidic solution

Fill in the glass with warm (50-60 C) acidic cleaning solution. Put it in the recess of the analyser and start the command Clean. After finishing it, pour out the contaminated liquid.

3. Rinsing with water

Fill in the glass with water. Put it in the recess of the analyser and start command Clean. After finishing it pour out the contaminated water. Now the device is ready for work.

Please, pay attention that, when the analysers gives a signal for need of cleaning 15 min after the last measurement of real milk samples or 55 min. after being powered and not used, cleaning is made ONLY with alkaline solution in concentration 1-3%.

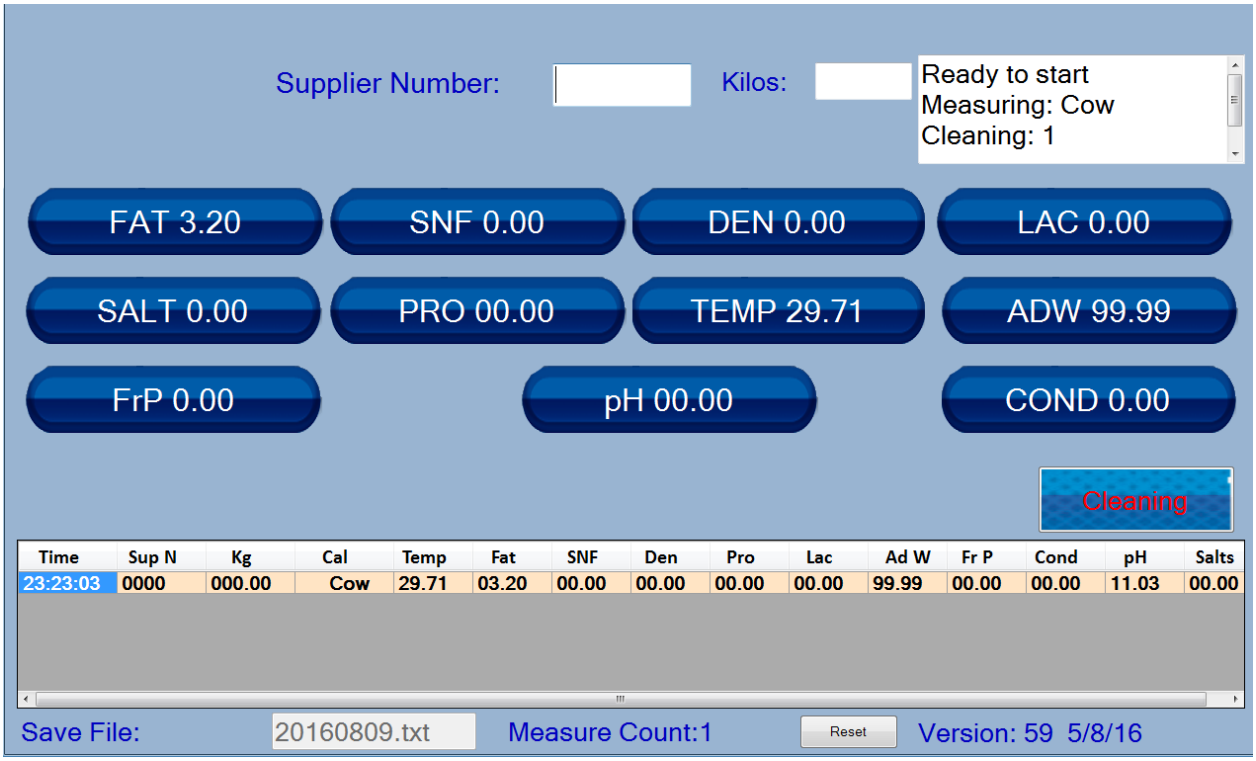
During the basic/final cleaning consequence is: alkaline solution – water – acidic solution - water

IMPORTANT

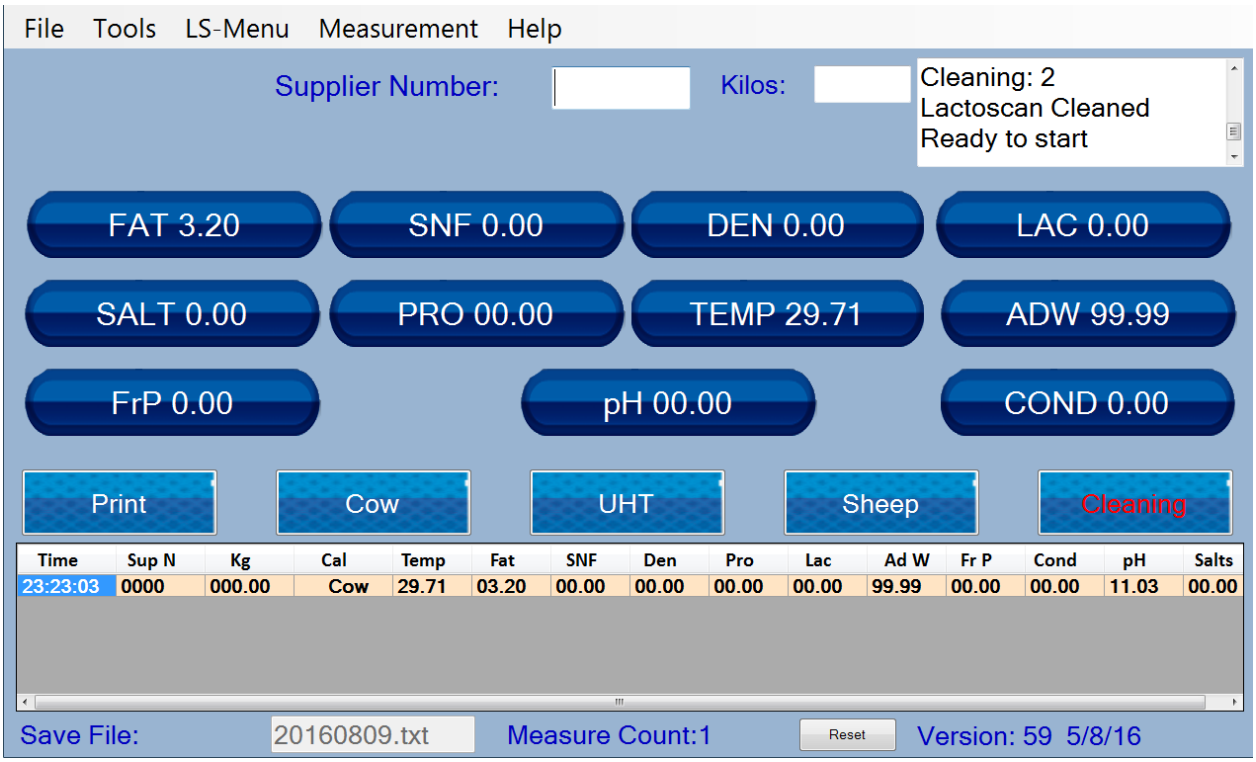
THE MAIN REASON FOR MALFUNCTIONING OF THE DEVICE IS THE BAD CLEANING OF THE SYSTEM AFTER MAKING ANALYSIS.

In case of malfunction due to the bad cleaning of the analyser your guarantee is not valid anymore and any repair has to be paid.

By pressing the button Clean, a cleaning procedure is started.



After the cleaning cycles are finished, the analyser returns "Lactoscan Cleaned" message and "Ready to start"



5. Menus' description.

These functions are NOT for everyday use. They are used in case some parameters of the analyser to be changed, after careful acquaintance with these commands and how to use them

Service mode menus are selected from the main menu system at the top of the screen. For easier work with the service menu we recommend using a mouse and a keyboard. Structure of the menu system:

5.1 Menu File

System Setup

Purpose: To select the communication ports. Used in production conditions, or if necessary in the service conditions

Reset System

Purpose: To reset the hardware of the device - the specialized board. It is equal to switching the power Off / On.

Exit

Purpose: Exit from the software, controlling the analyser and returning to windows desktop. It is used in cases when the tablet needs settings to be made- for example connecting to a WiFi network, connecting an external printer, etc.

ShutDown

Purpose: Exitfrom the software and shutting down the tablet. It is used to turn off the system. After pressing shutdown, turn off the pcb using the switch on the back panel.

5.2 Menu Supplier

Add Supplier

Purpose: To enter the details of a new supplier of milk. It is used in case of building or using a report system.

Edit Supplier

Purpose: To edit / change data for already introduced suppliers of milk.

5.3 Menu Tools

LSCal

Purpose: To start an external software tool, used for the calibration of the device. The way of work with the tool LSCal.exe is described separately in the service documentation. After completion of the calibration, to resume work with the program press the button "Finish".

Calibration

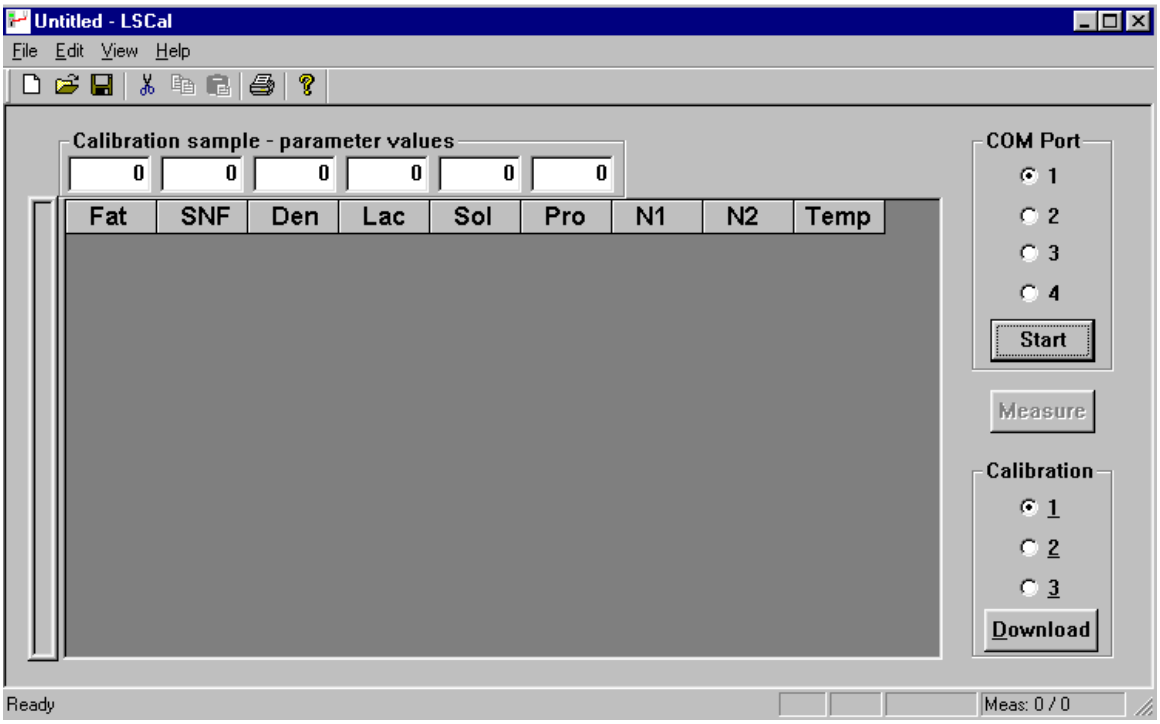
The program LSCal.exe is used to calibrate Lactoscan Milk Analyzers- WLS Version. Two samples are used – with high and low fat, the third measurement group is measurement with water. Measurements are taken in the following order: high fat sample, low fat sample, water.

Note:

Before starting the calibration procedure, the operator has to be acquainted with the analyzer's manual, mainly in its part preparation of samples, described in service manuals.

Program control:

After the program is started the display shows the following:



Radio button:

COM Port

1 – COM 1 connection

2 – COM 2 connection
3 – COM 3 connection
4 – COM 4 connection

Calibration

1 – Calibration cal 1
2 – Calibration cal 2
5 – Calibration cal 3

Control buttons:

Start - Starts the communication, this means the device is ready for work

Measure - starts a measurement in Calibration mode.

Download - saving the new calibration in the device

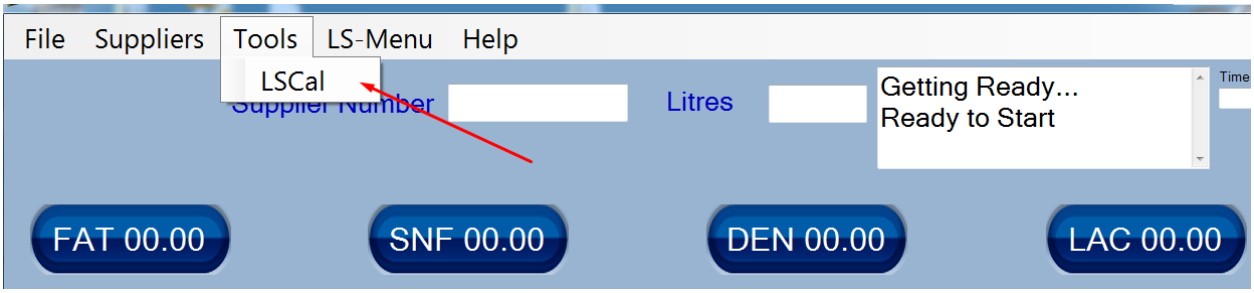
Edit boxes:

Group Calibration sample – parameter values - set the sample parameters values for each parameter at a time.

Colomns N1, N2, Temp - the results of the measurements in calibration mode, used for making the calculations of the new calibration.

Workflow

This program receives the control from the main program by selecting the Tools menu and choose LSCal.



It opens the software tool for calibration. The operator chooses the Com Port, and presses the button Start. After that inputs the parameter values in the fields. With the button Measure starts a new measurement of the sample in calibration mode.

Details of the calibration process are described in the service documentation.

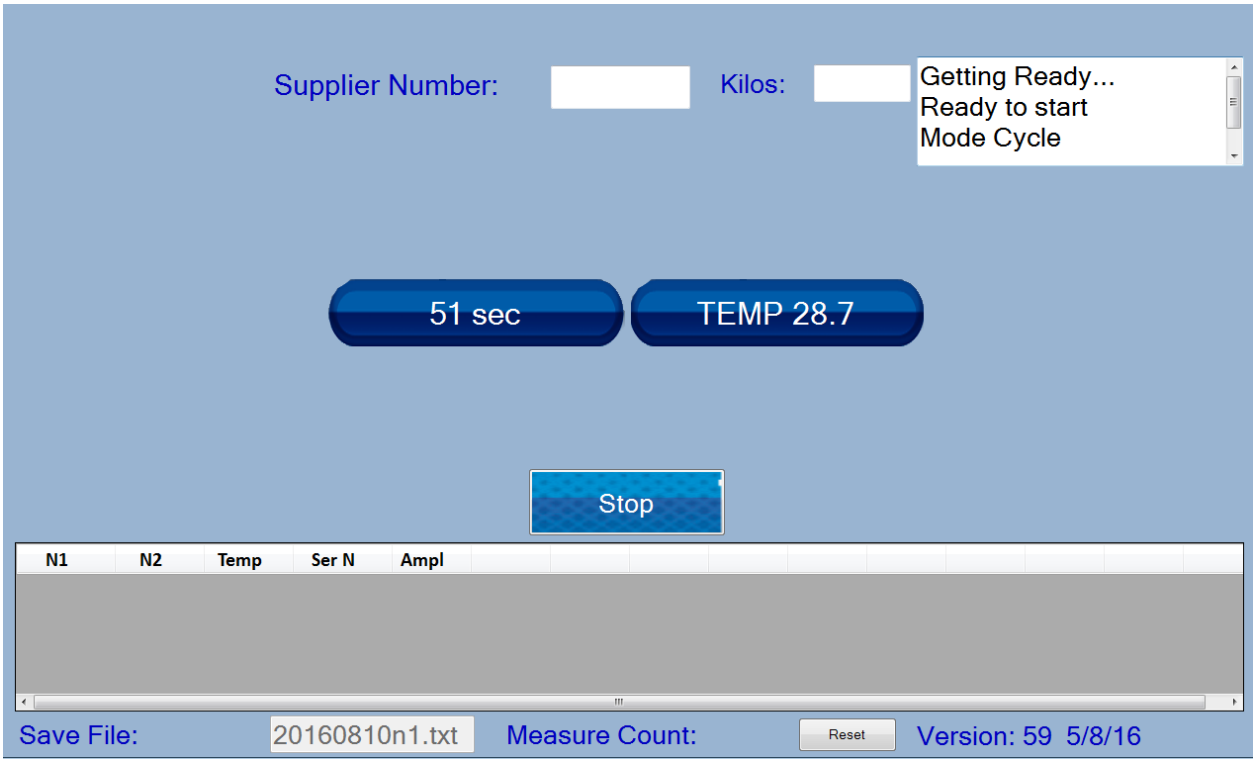
After finishing the calibration, close the LSCal program and press button Finish of the main program to return to normal use of the analyser.

5.4 Menu LS-MENU

Special Modes

Cycle

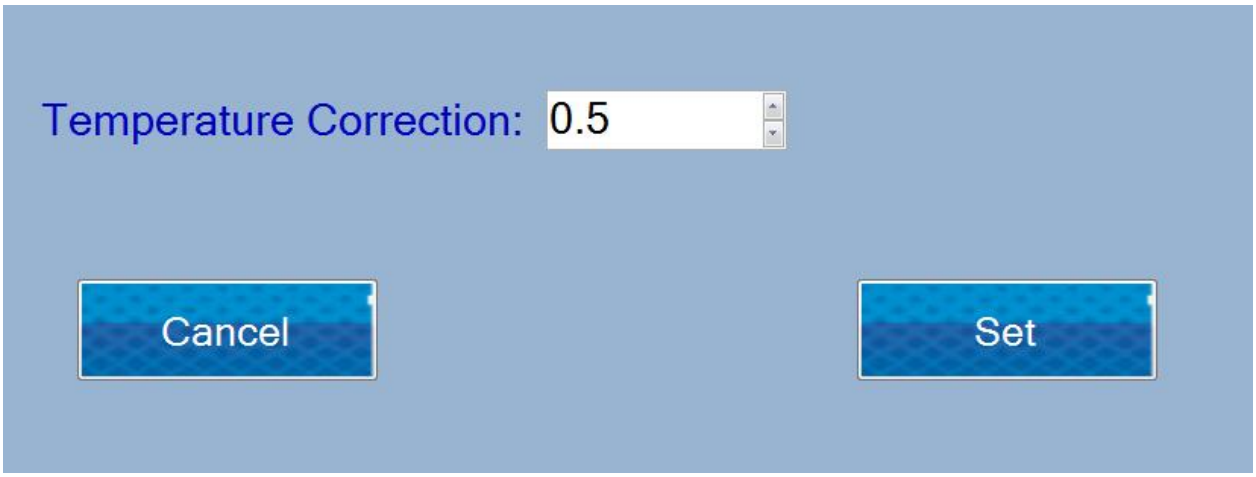
Purpose: Starts the unit in cyclic measurement of the ultrasound. The results appear in the results field, in place of Fat, SNF ... The results are remembered in a file. The mode is used for industrial purposes or for service work by qualified professionals. This type of measurement is not used in everyday laboratory work.



5.5 Menu Correction

Temperature Correction

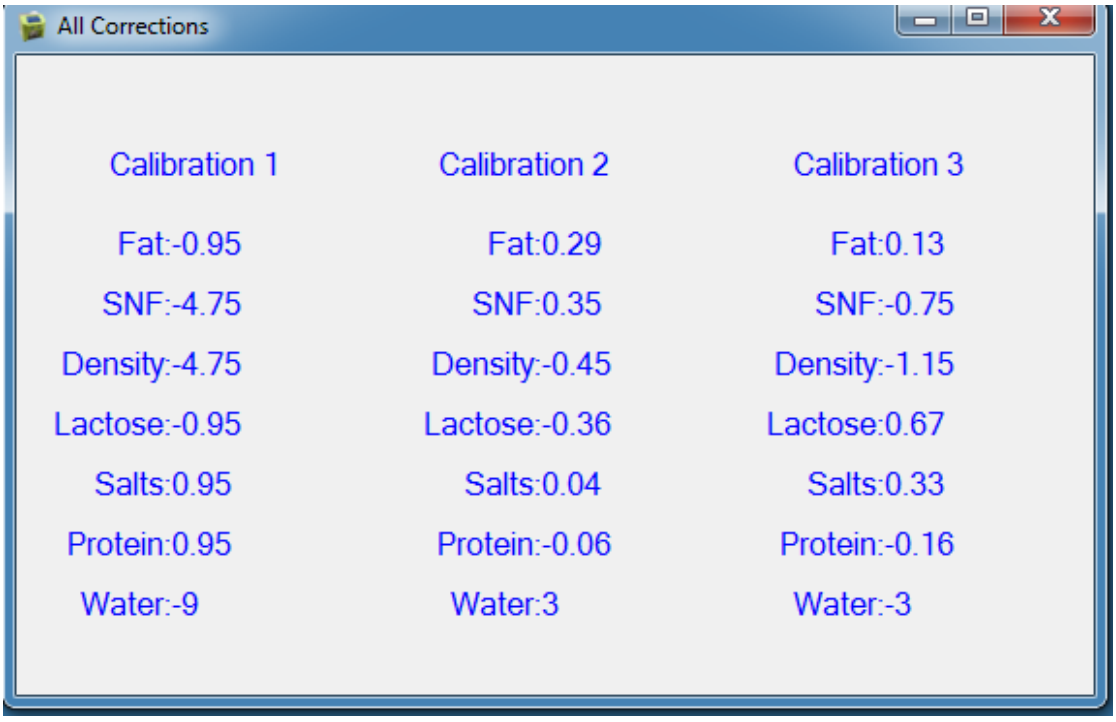
Purpose: To correct the measured sample temperature. Used by trained service specialists. The measured temperature can be varied within + - 9.9 degrees.



Correction

All Corrections

Purpose: Provides information about all existing corrections of the system parameters. The window is informative, for editing adjustments proceed to the selection of calibration and selection of parameters to be adjusted (see below)

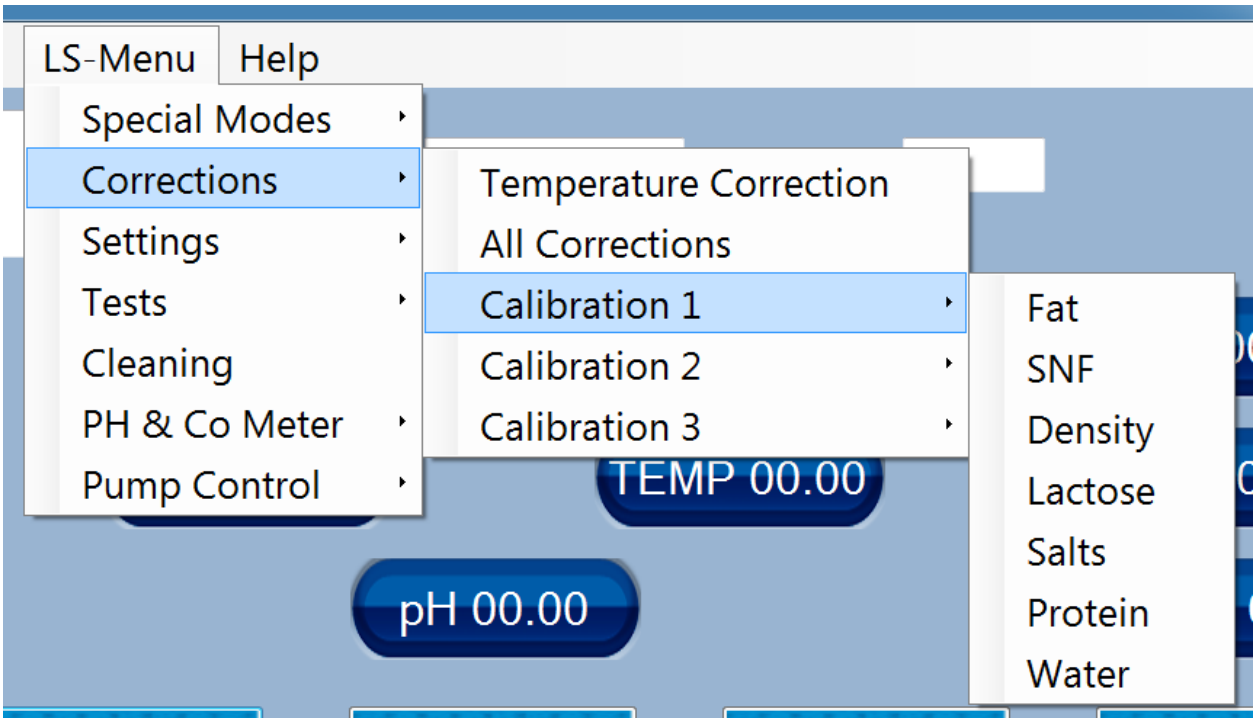


Corrections

Calibration

Parameter:

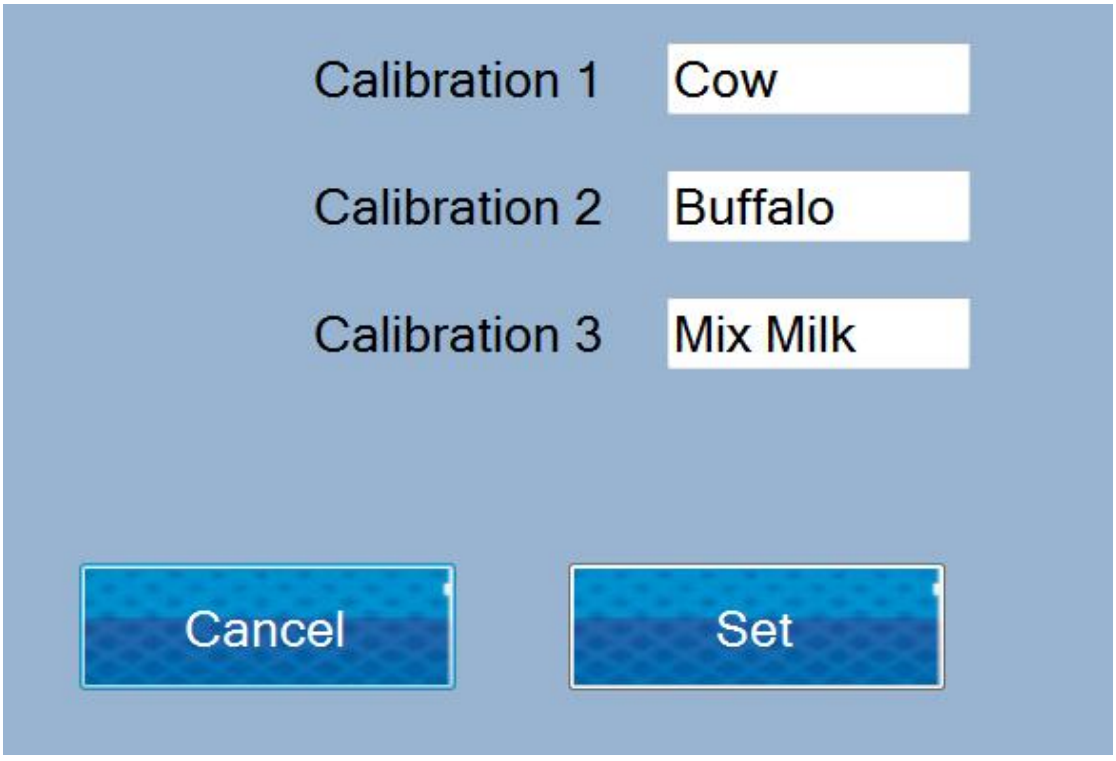
Purpose: The menus are used for selecting which parameter of which calibration will be corrected.



5.6 Menu Settings

Set Calibration Names

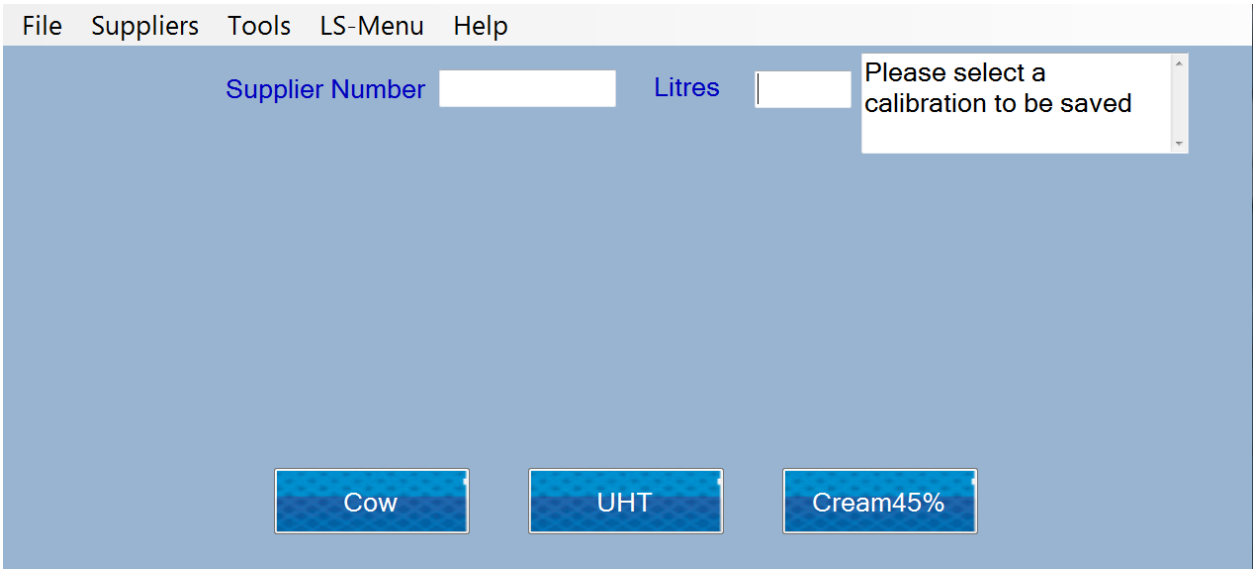
Purpose: To edit the names of the calibrations. The operator can introduce a free string of 8 alphanumeric characters. After starting, the screen displays the following, the operator must follow the instructions of the program:



Using the keyboard, the operator inputs the names of calibrations. Memorize them by pressing the Set button or refuse corrections by pressing button Cancel.

Save Calibration

Purpose: To save the calibration to a file in the computer. Select the calibration to be saved



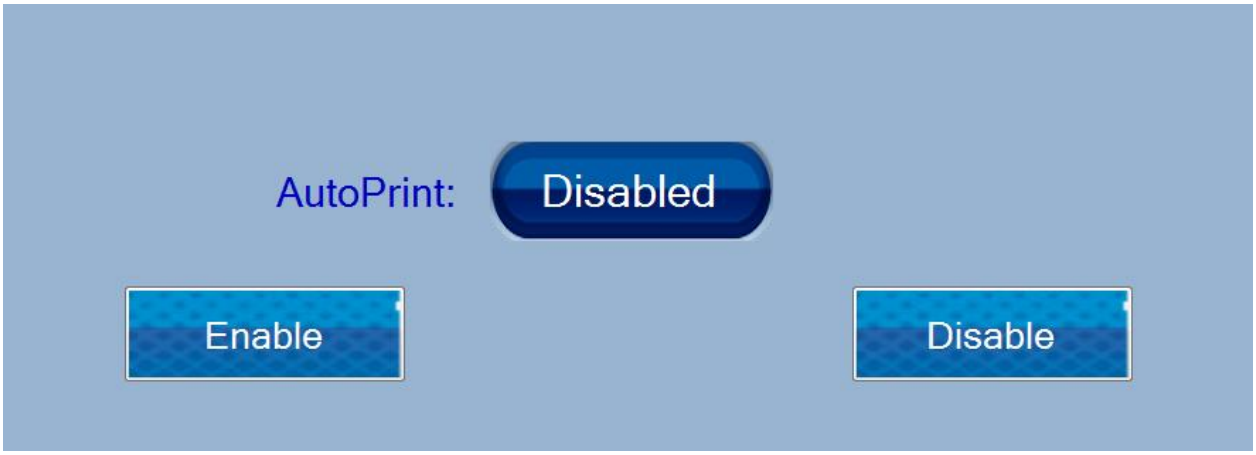
Restore Calibration

Purpose: Restore an already saved to the tablet calibration

*Note: Each calibration is specific to the analyser. You can save a calibration and restore it only to the same machine

Autoprint Enable/Disable

Purpose: To select whether the device will automatically print the results after finishing the measurement

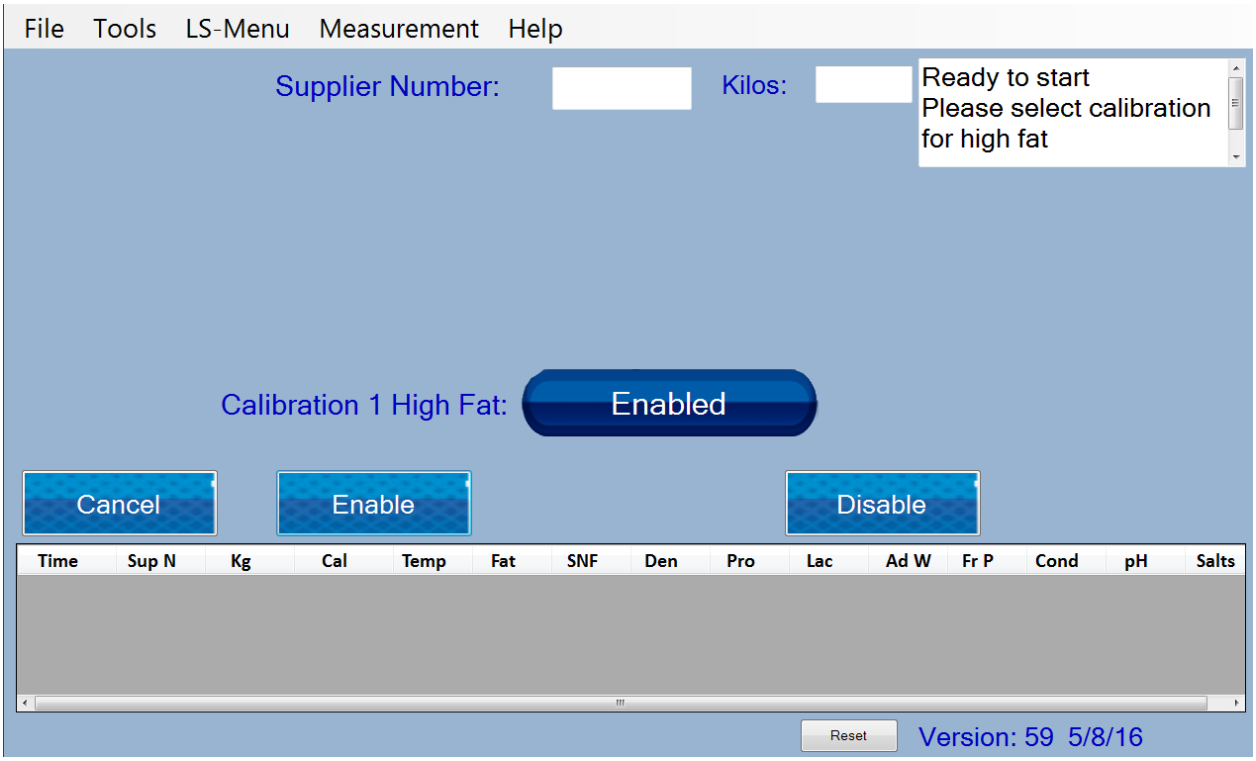


Format Results:

Purpose: To select the format of the results – main or large. The device comes with factory settings for Large results. The machine should be set for operating with Large Results

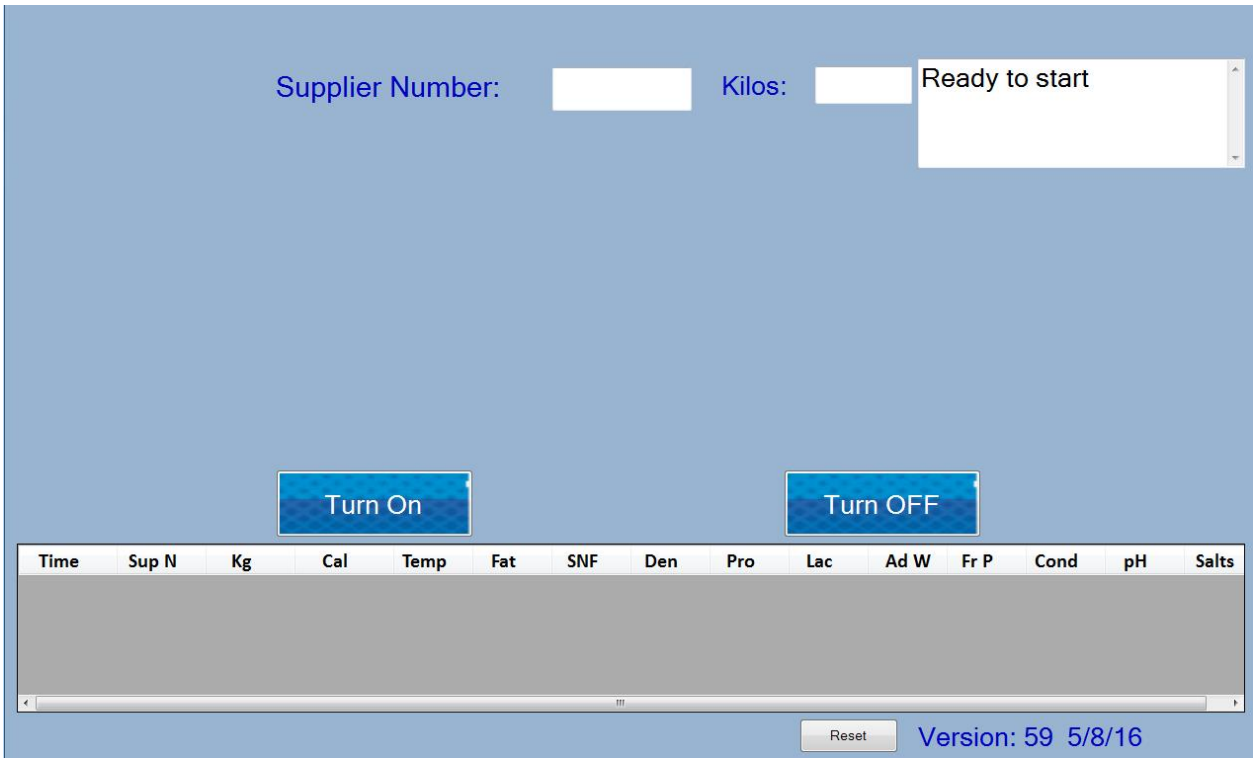
High Fat Control

Purpose: To enable/ disable high fat measurement for a specific calibration for machines with High Fat Option. When selected from the menu, choose a calibration:



High Fat Speed For Calibration

Purpose: For machines **with** High Fat Option. Turn on High Fat speed for calibration, before attempting a calibration with high fat samples



5.7 Menu Tests

Test Pump

Purpose: To test of the pump unit. Used in service conditions by qualified specialists. To end the test press the button "Finish".

Ultrasound

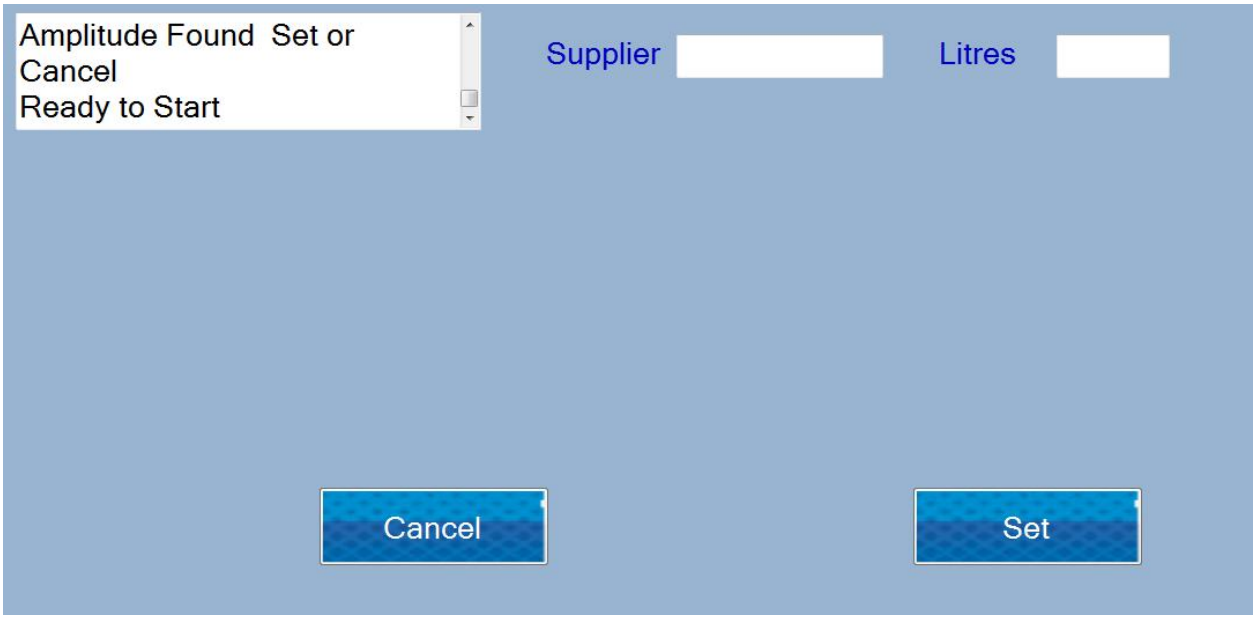
Purpose: To set the measuring system of the device. Used in service conditions by qualified specialists. The procedure is similar to that used in standard devices without integrated tablet. Current messages are displayed in the

field System Status (top left corner of the display)

To finish the procedure, press the button "Finish".

Set Amplitude

Purpose: To set the metering system of the device. Used in service conditions by qualified specialists. The procedure is similar to that used in standard devices without integrated tablet. Current messages are displayed in the field System Status (top left corner of the display). When the procedure is finished apparatus brings a message that the amplitude is found and asks the user to set this amplitude or cancel the procedure



5.8 Menu Cleaning

Starts cleaning of the analyser. It is equal to pressing the button Clean from the main menu.

5.9 Menu pH & Co Meter

pH Meter En/Dis

Purpose: To turn on / off pH measurement in the process of measuring other parameters. It is available for devices with manufactured hardware option pH.

pH Test

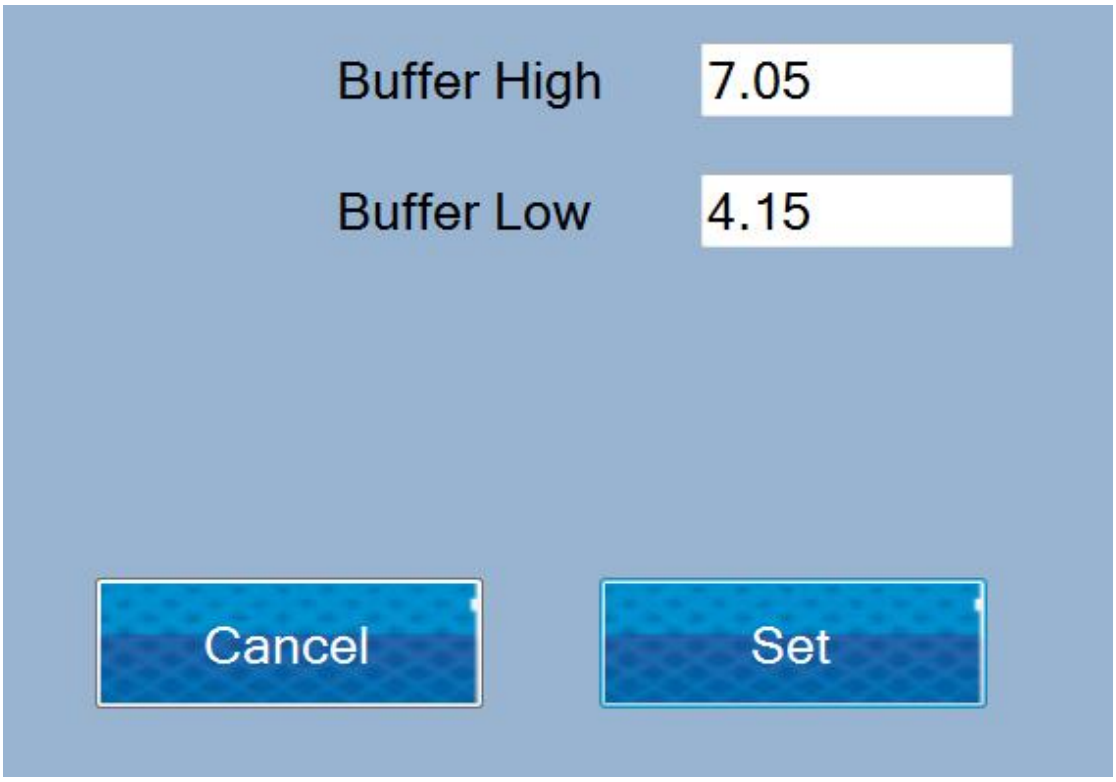
Purpose: To set a pH measurement system of the device (if the device is manufactured with an optional pH). Used in service conditions by qualified specialists.

pH Measure

Purpose: For off-line measurement of pH. In this case the analyser works only as pH meter, without measuring other parameters of the sample.

pH Meter Calibration

Purpose: To calibrate the system for measuring the pH. After starting, the screen displays the following, the operator must follow the instructions of the program:



The operator inputs the values of the buffers used for calibration. Selection of buffers and principles of work with the pH meter are described in the document WLS-TechManual. By pressing the Set button to go to the actual calibration. Reports of this process is displayed in the System Status, in the upper right corner of the screen. By pressing the Cancel button procedure is terminated and transferred to the main screen.

Co Meter En/Dis

Purpose: To turn on / off the conductivity measurement in the process of measuring other parameters. It is available for devices with manufactured hardware option conductivity.

Co Meter Test

Purpose: To set up the measuring system to the conductivity of the device (if the device is manufactured with an option conductivity). Used in service conditions by qualified specialists.

Co Meter Calibration

Purpose: To calibrate the system for measuring conductivity. After starting, the screen displays the following, the operator must follow the instructions of the program:

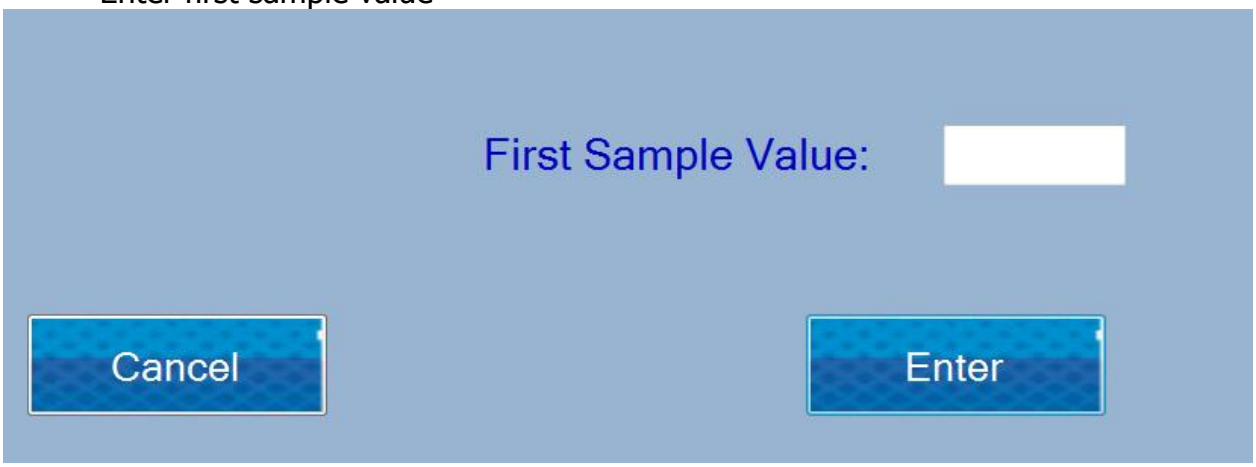
The operator enters the values of the buffer that will be used for calibration. Selection of buffer and principles of working with the system for measuring conductivity are described in the document WLS-TechManual. Pressing the Enter key passes to the actual calibration of the measurement system. Reports of this process is displayed in the System Status, in the upper right corner of the screen. By pressing the Cancel button procedure is terminated and transferred to the main screen.

Sample calibration

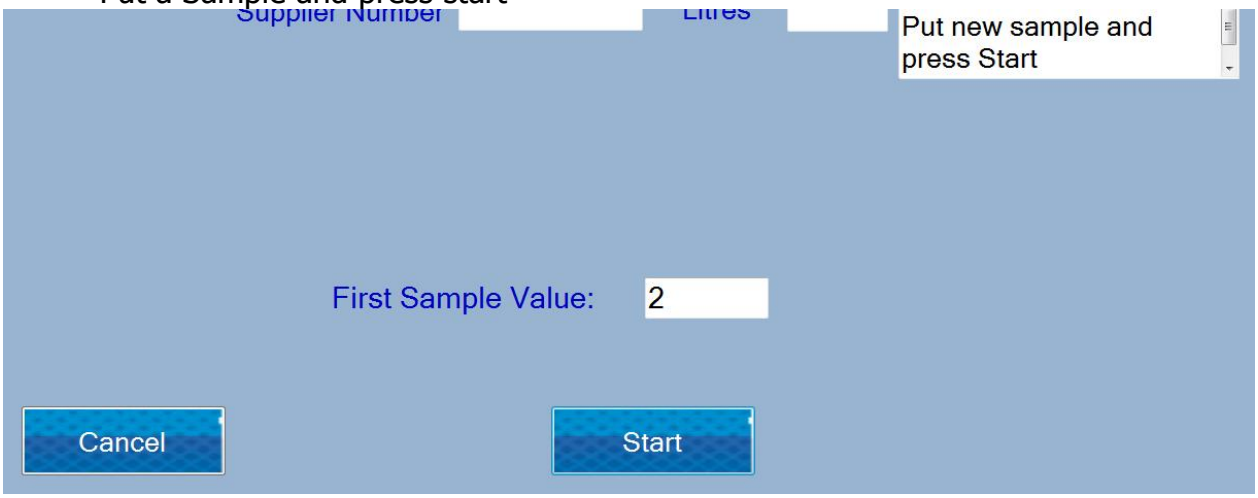
Purpose: To make a precise calibration of the Conductivity Meter. For the calibration are used 5 conductivity buffers

The procedure is as follows:

- Enter first sample value



- Put a Sample and press start



- Repeat the same steps for the rest of the buffers.

For each sample five measurements are made.

5.9 Menu Pump Control

Purpose: The following menus are used for test of the pump unit. Used in service conditions by qualified specialists.

Pump In

Pump Out

Stop

5.11 Menu Measurement

Discard Last measurement

Purpose: Discard the last measurement from the daily report

5.12 Menu Help

LS Identity

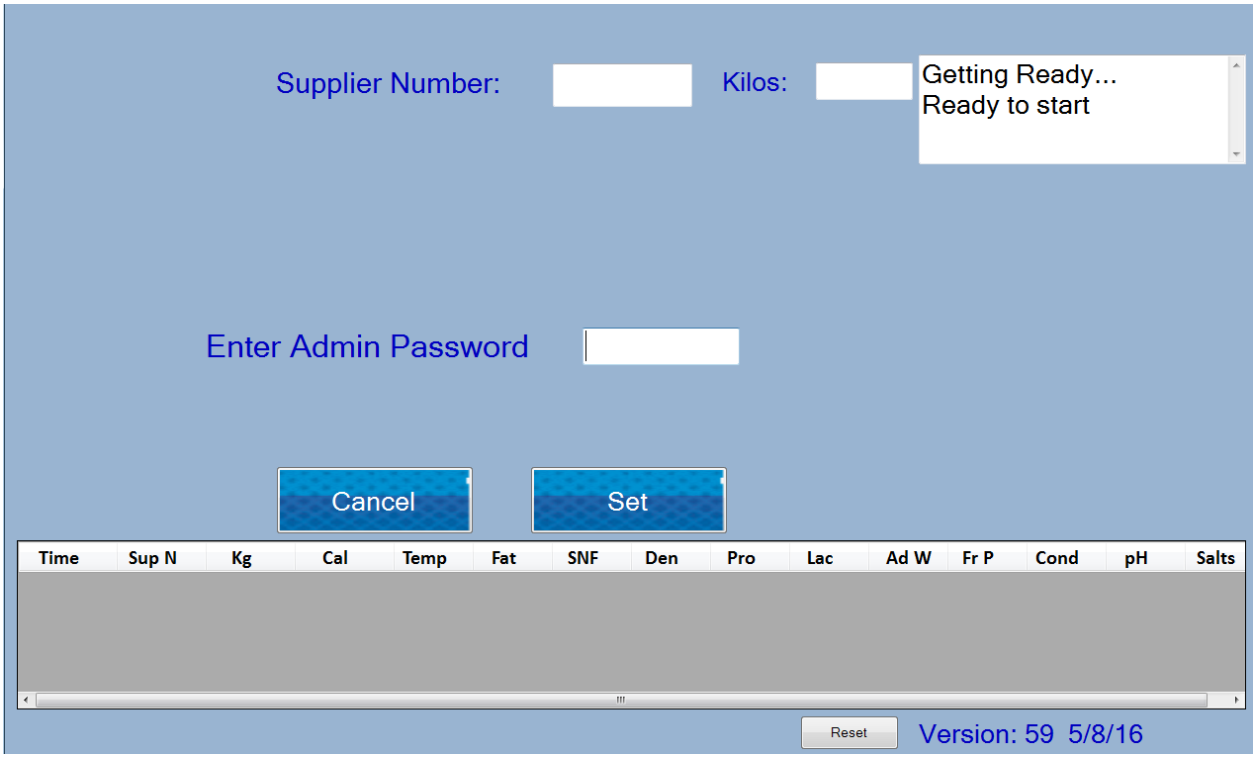
Purpose: Displays information about the hardware of the device - serial number version of the program board.

About

Purpose: Displays information about the program in the tablet control hardware appliance.

Admin Mode

Purpose: Enter admin password for enabling admin menu. For receiving your admin password please contact your Milkotronic representative



5.13 Menu Admin Mode

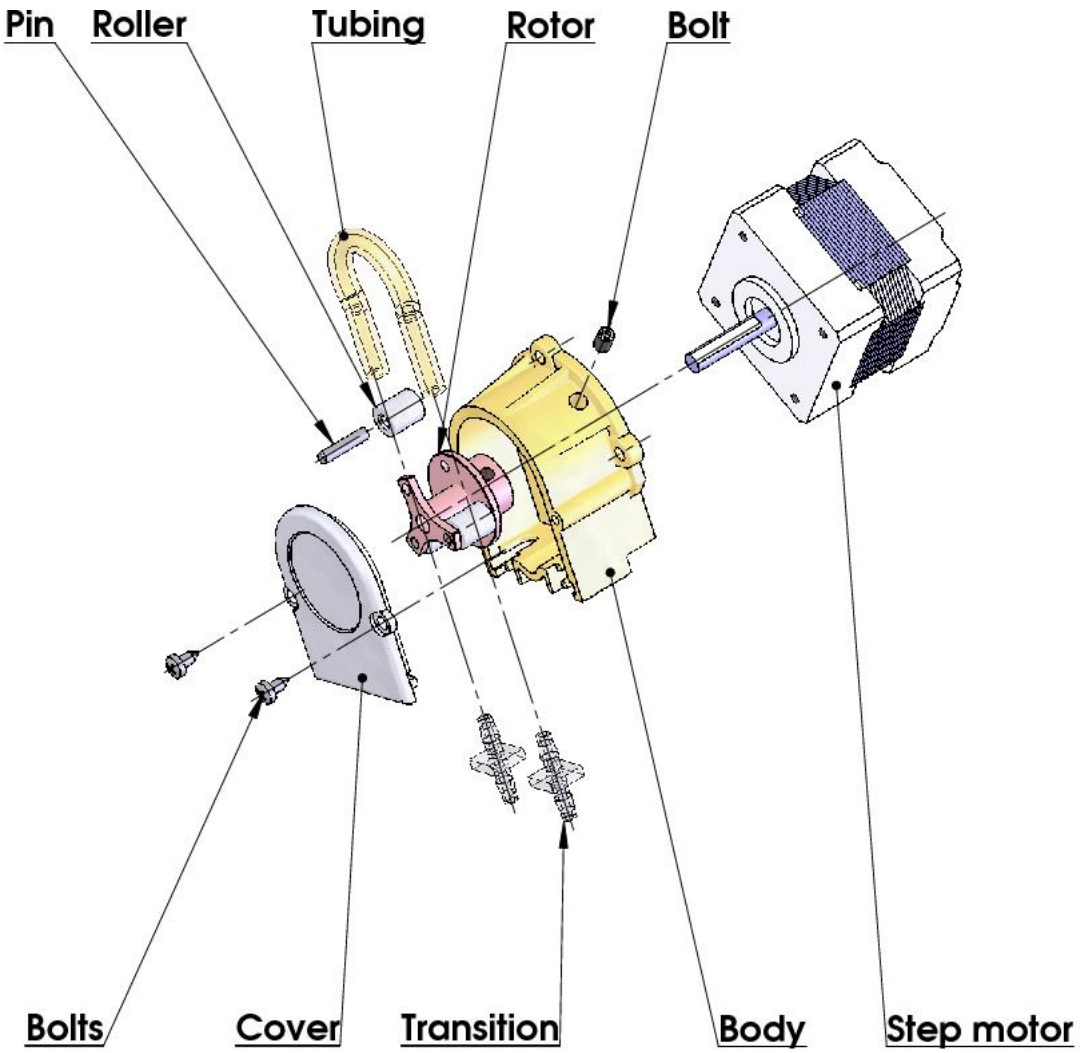
Restore Factory Settings

Purpose: Return the machine in the state of which it was produced.

NOTE! This procedure will erase all user data such as calibration corrections, new calibrations and will restore the machine to the state in which it was received from Milkotronic.

6. Peristaltic pump service

Peristaltic pump



7. Possible malfunctions and error messages, troubleshooting

In the table below are described the possible malfunctions during the milk analyzer's exploitation and ways for their repair/remedy. If the problem persists after all recommended measures are taken, please, connect the nearest service center for help. Do not forget to tell the analyser's identity.

To receive the analyzer's identity, refer to point 3.2.1.3.

Error message	Possible problem /cause	Repair/remedy
2 MA overheated Accompanied by a continuous sound signal	Overheated milk analyzer	Immediately switch off the analyzer. Pay attention the analyzer to be situated away from direct sunlight or heating devices. Wait 5-10 minutes the device to cool down or to be normalized the ambient temperature and switch it on again.
3 Empty Camera	Insufficient quantity of the milk sample sucked in the system or air in the sample	The analyzer is ready to measure the next sample. In order to avoid the future appearance of the same error message, please, check the following: - The sample is prepared according the instructions and there aren't air bubbles in it. - There is a real suction of the sample after starting measurement, i.e. it is obvious that the level of the milk sample in the sample holder decreases. In other case – there is damage in the suction system. - Avoid the end of the suction pipe to be above the surface of the liquid (not dipped enough). - Avoid curdling of the milk sample. Clean immediately if there is a sample curdled in the system. - In mode Measurement, after starting the measurement, remove the sample holder and see if there is no milk poured back in the sample holder.
4 Sample Overheat	Sucked overheated	The analyzer is ready to measure the next sample. In order to avoid the future appearance of the same

	sample	error message, please, check the following: -The sample is prepared according the instructions and its temperature does not exceed the maximum permissible sample's temperature. -Complete the procedure for checking the analyzer in case of error message **Empty Camera.**

8. Making corrections and recallibration of the device

In the process of work with the analyser there is a possibility the results to start differing between the data for some of the measuring parameters when measured with the milk analyzer and the corresponding reference method of analysis (Gerber for fat, Kjeldhal for proteins etc). In order to establish the possible discrepancy and to correct the readings of the milk analyser do the following:

8.1. Taking samples and preparation of samples for checking the accurracy of the milk analyser, making corrections and recalibration

This is a basic moment for the correct checking the accuracy of the analyser and for making correct and precise correction and calibration. It is accomplished according Appendix Sampling and preparation of samples for verification the accuracy of the milk analyzer, making corrections and recalibration.

8.2. Determination the type of the discrepancy:

8.2.1. Making measurements

Make measurements with different samples (not less than 3) with known values of a separate parameter (for example fat content), determined by the known reference methods of analysis (for example Gerber's method for determination of fat content). For more accuracy it is recommended among these samples to be also such with values, close to the lowest and highest bounds for the measured parameters.

Make 5-time measurement for each of the samples. Calculate the average value for each sample parameter, without taking into consideration the first measurement for each sample.

8.2.2. Analysing the measurement results

Make comparison between the values of the parameter from the reference sample and measured with the analyser. Make analysis of the difference received.

8.2.2.1. If the received differences are relatively constant value for samples with different content of the analysed parameter, it is necessary to make correction.

For example

M% of the reference samples:	2,20	3,00	3,80	4,60	5,20
M%average when measuring					
with the analyser:	2,38	3,17	4,01	4,79	5,42
Difference:	0,18	0,17	0,21	0,19	0,22

Conclusion: the difference is relatively constant value and correction is possible to be done with – 0,2 % (see Corrections, p6.3.3)

8.2.2.2. If the differences are not a constant value it is necessary recalibration to be done.

For example.

M% of the reference **Conclusion**: It is obvious that the difference is variable value and recalibration have to be done.

8.3. Making corrections

8.3.1. Possible corrections, limits and changing steps

Every parameter from each calibration may be separately corrected. Below is the table with possible corrections, limits and changing steps:

samples: 2,20 3,00 3,80 4,60 5,20

M% when measured with the

analyser: 2,022,933,764,755,44

Difference: -0,18 -0,07 -0,04 0,15 0,24

Parameter	Increasing	Decreasing	Step
FAT	0.95%	0.95%	0.01%
SNF	4.75%	4.75%	0.05%
Density	4.75%	4.75%	0.05%
Lactose	0.95%	0.95%	0.01%
Salts	0.95%	0.95%	0.01%
Proteins	0.95%	0.95%	0.01%
Added water	9.00%	9.00%	1.00%
Sample's temperature	9.90°C	9.90°C	0.1°C

Corrections are described in a dedicated software tool.

8.3.3.4. Making verification

After the corrections are made put the milk analyser in working mode and make several times measurement of reference samples with known values of the corrected parameter. If the difference between the values of the parameter from the reference

methods and milkanalyser are in the limits for the parameter it may be considered that the correction is successfully made.

If the discrepancy between the measurements from the milk analyser and classical methods is bigger than is necessary to make second correction according above described way.

If after the second correction the results are unsatisfactory we recommend making a calibration of the analyser.

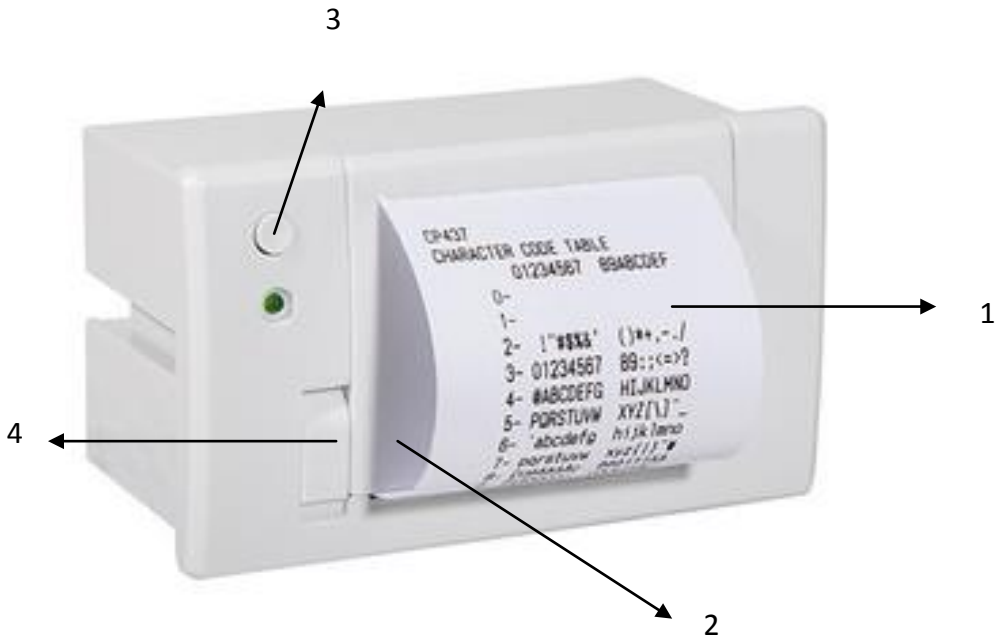
When making corrections or calibrations be 100% sure in the accuracy of the reference methods result.

9.Basic printer characteristics

Paper Working	Loading way	easy
	Cutting way	divulsion（dentiform）
Printer performance	Print method	Direct thermal print and auto paper feed
	Printing density	384 dots/line
		8 dots/mm
	Printing width	48 mm
	Max Printer Speed	85 mm/s（max.）
	dot distance	0.125 mm
Drawing/Bar code	word size（W x H mm）	0.75×1
	ASCⅡ Character（W x H dots）	16 lattice：8×16; 24 lattice：12×24
	ASCⅡ Size（W x H mm）	16 lattice：2×4； 24 lattice：1.5×3
	drawing/bar code	suitable
Testing Method	lacking paper testing	photoelectricity sensor
Control System	printer interface	TTL/RS232
	buffer	32k
	Command System	ESC printer command/WH printing command
Power	Woking Voltage	DC3.5V--9V (from7.2-5v,with highest speeed and excellent effect)
	Average electricity	less than 1.5A
	Max electricity	less than 3A
Printer Mechanism	printing head lifespan	50km
Paper	paper item	thermal paper

	paper width	58mm
	papaer thickness	65±5um
	diameter of paper roll	30mm
Physics Specification	Environment adaptability	0～55℃；-20～55℃
		10～90%RH
	Storage temperature	–25～70℃
		10～90%RH
	Dimension (W x H x D mm)	103mm×57mm×57mm

9.1 Printer control panel



1. *Printout*
2. *Paper roll compartment*
3. *Printout button*
4. *Cover opening button*

9.2 Changing the roll

To change the paper rolls proceed as follows:

1. Press the button as it is shown on the figure.

2. Position the paper roll making sure it unrolls in the proper direction.
3. Tear off the paper and close the cover

9.3 Consequence of actions when printing

The embedded printer is automatically switched on with switching on the device. After finishing the measuring procedure the results are automatically printed out. If there is a need of repeated printing of the same results the operator has to press the button Print on Main Display (each pressing the button Print - the results will be printed out). If you do not need to print out the results after the measurement is completed, just open the lid of the printer.

10. Additional possibilities of the analyser

10.1. Connecting to 12 V DC power supply.

If there is a need the analyser to work on place without electrical supply available, then it could be powered by car battery or other 12 V DC external power supply. Use the 12 V power supply cable (art. number 30030).

10.2. Measuring high fat samples (option).

The standard device measures samples up to 25% fat.

On customer's request, the device could be produced with possibility to measure samples up to 45% fat. The customer can choose which calibration to have this possibility and which not, as well as during the process of exploitation to change the measuring mode i.e. to pass from measuring normal fat percentage towards high and vice versa.

What the operator sees during these passes is the difference in the speed of sucking the sample. For that purpose, the high-fat sample has to be preliminary heated up to 30C +- 3C.

Appendices

Appendix 1: Preparation of samples for milkanalysers' calibration

For calibration are needed samples of cow milk with the following parameters:

		Low Fat	High Fat	Middle
1	Cow	2,2%	5,2%	3,6%

For the calibration are needed:

1. Distilled water

2. Min. 3 milk samples with known values for fat, SNF, protein, density, lactose, salts.

Calibration samples have to be with low, middle and high values of the analyzed components. Samples have to be representative for given milk type. Volume of the sample has to be enough for making min 5 measurements for each sample – not less than 1,00 l. Changes in the analyzed parameters in the samples have, if possible, to cover the whole measuring range – i.e. used samples to be with low, middle and high content of the analyzed components.

Methods of milk samples preparation for calibration.

For milk sample with middle value of the analysed components we recommend to use milk taken from not less than 10 animals from most common in the region breed.

Sample with low and high value are prepared on the following way:

1. Pour the fresh milk with FAT at about 3.7% in a separating funnel.
2. Leave the funnel with the milk in refrigerator for 12 hours at temperature +5-+8 ° C.
3. Draw the substratum of the separated milk in a vessel, mix it well, pour it and heat it in water-bath up to 20°C.
4. Pour the upper layer in another vessel.
5. Determine the concentration of the measured components (FAT, protein, SNF, density, lactose, solids) by using certified methods.

The analyser's accuracy depends only on the correctness of the chemical analysis of the components in the samples and the normal acidity during calibration!

It is recommended the first cow milk sample with low fat content to be with the following parameters:

2-2,3% FAT; 8.7-9% SNF; 3,3-3,5 % Protein; 4,8-4,9% Lactose; 0,75 Salts; 1030-1033 kg/m3 Density.

The second cow milk sample with high fat content to be with the following parameters:

5-5,3% FAT; 8.4-8,79% SNF; 3,1-3,2% Protein; 4,6-4,7% Lactose; 0,7 Salts; 1028-1029 kg/m3 Density.

If, after milk's separation you do not obtain samples in the requested range, then, by adding milk with high fat value into the low fat milk sample you can obtain necessary value-2,3%

Analogous to this, by adding low fat milk sample into a milk sample with high fat value you may receive 5,3%

Samples with medium values are received by mixing low fat and high fat samples in necessary proportion.

If there is a need of longer sample storing they have to be preserved; the most commonly used preservative is potassium dichromate (K2Cr2O7) - 1 g for 1 000 ml.

When using samples, stored shortly, preliminary pour the sample from one vessel to another in order to distribute the milk components evenly paying attention not to form foam in the sample.

When the samples are stored for a longer period it is recommended to warm it up to 35-45 °C, and the vessel to be shaken carefully. In case that there is a cream stuck on the vessel's surfaces – remove it. The sample is poured from vessel to vessel several times and is cooled down (advisable to 20 °C /.

If there is separated liquefied fat or white particles with irregular form on the vessel's walls reliable results could not be received.

Because it is very difficult both lactose and salts to be measured but they are substantial and influence in great extend when determine added water. That's why it is better both lactose and salts to be calculated by using SNF results.

The milk must be for sure without added water.

If you are unable to make the analysis of milk in certified methods in a pinch you can use the following formulas:

DETERMINATION OF THE BASIC PARAMETERS IN THE MILK SAMPLE BY USING FORMULAS IS NOT AS PRECISE AS USING THE ARBITRARY METHODS, BUT IS SUITABLE FOR USAGE IN FIELD WORK.

1. Determination some of the parameters by formulas

There is dependence between the different parameters in milk and its density, which may be expressed with mathematical equation. On this base different formula, tested and confirmed by the classical laboratory methods for analysis, are developed. We recommend the following:

2. SNF determination.

For determination of SNF the correlation dependence exists between the milk's density, fat and SNF in the milk. When the density and the fat are known, the SNF can be calculated.

There are several formulas with different applicability.

A/ When the salts and fat are known

SNF is calculated by subtracting the fat percentage from the salts.

SNF = Salts – F (%)

Where

Salts – salts in (%),

F – fat content in (%),

This formula is used for determination of SNF in whey, buttermilk, and cream.

B/ Known quantity of fat and density (most commonly used method when maximum accuracy is needed).

We recommend the following formula:

$$SNF = \frac{0{,}075 * F\% + 100 - 100 / density}{0{,}378}$$

This is a universal formula and actual for milk of almost all kind of cows and sheep all over the world.

3. Determination of lactose content

We recommend the following formulas:

A/ for cow milk

Lact. = SNF * 0,55 (%)

Where

SNF – content of SNF in percentages (%),

0,55 – constant coefficient.

B/ for sheep milk

Lact. = SNF* 0,45 (%)

Where

SNF –solids-non-fat content in percentages (%),

0,45 – constant coefficient.

This is an actual coefficient for sheep breeds on the territory of the Balkan Peninsula.

4. Determination of salts content

We recommend using the following formulas:

A/ for cow milk

Salts = SNF* 0,083 (%)

Where

SNF – solids-non-fat content in percentages (%),

0,083 – constant coefficient.

B/ for sheep milk

Salts = SNF * 0,075 (%)

Where

SNF – solids-non-fat content n percentages (%),

0,075 – constant coefficient.

This is an actual coefficient for sheep breeds on the territory of the Balkan Peninsula.

5. Determination of total proteins content

We recommend using the following formulas:

A/ for cow milk

Protein = SNF * 0,367 (%)

Where

SNF - solids-non-fat content in percentages (%),

0,367 – constant coefficient.

B/ for sheep milk

Protein = SNF * 0,475 (%)

Where

SNF – solids-non-fat content in percentages (%),

0,475 - constant coefficient.

This is an actual coefficient for sheep breeds on the territory of the Balkan Peninsula.

Appendix 2. Freezing point determination

1. Methods for determination.

The milk analyzer determines the freezing point of each sample and the quantity of added water. The milk analyser does not measure the freezing point, but calculates it from the components it depends on. The basic components in the milk are water, solids, lactose, FAT, proteins, minerals (salts) and acids. The freezing point depends only on the diluted in the milk components and quantity of the solvent (in the milk it is water). The ultrasonic technology allows direct measurement of FAT, proteins, lactose + salts (the soluble components, only influencing the freezing point), and the quantity of the solvent in % is determined by 100 % – total solids %, total solids = lactose % + FAT % + proteins % + salts % + acids %.

Without understanding the meaning of the freezing point – determined or shown from the milk analyzer added water result easily may lead to a mistake for the value of this parameter.

2. The basic freezing point.

Milk freezes at lower temperature than water. The average freezing point of the raw milk in the most regions is at about -0,540°C. The average reading for your region is called "basic" freezing point.

The freezing point of milk is a "physiological constant". This does not mean that it will not vary. In fact feed, breed, season, time of lactation, climate, whether the sample is taken at the beginning, middle or end of lactation – all these factors will have an effect on the freezing point of the individual sample. This means that there is an average value of all these numbers. The more samples used in obtaining this average, the more reliable it is as a base. Or the basic freezing point is an average of freezing points of milk, taken from many cows. When a laboratory checks a producer, it is only comparing the average of the producer's cows against a larger area average.

The Health authorities establish the basic freezing point or agriculture departments in some regions, sometimes by universities, separate dairy producers, or their associations. Frequently, tolerances have been established on top of a basic freezing point to allow some variations in the milk as well as device or operator variations.

Withoutmentioningthebasicfreezingpoint, the Association of Official Analytical Chemists now recommends an upper limit freezing point at -0,525°C (2,326 standard deviations above the most recently determined North American average of –0,5404°C), below which there will be at 95%confidence that will show 99% of all freezing point determinations on unwatered milk:

"if the freezing point is –0,525°C or below, milk may be presumed to be free of water or may be confirmed as water free by tests, specified below. If the freezing point is above –0,525°C, milk will be designated as "presumptive added water" and will be confirmed as added water or added water free by tests specified below. Evaluate

extreme daily fluctuations in the freezing point of herd, pooled herd, or processed milk for presence of added water".

"Presumed added water", as described above, must be "confirmed" by means of tests on authentic milk samples obtained as specified in the AOAC METHODS.

After determination the freezing point of your sample via the milk analyzer, the added water is calculated using the following formula:

$$AddedWater = \frac{FrPo\,\mathrm{int}_{Base} - FrPo\,\mathrm{int}_{Calc}}{FrPo\,\mathrm{int}_{Base}} * 100[\%]$$

Where:

FrPointBase is the basic freezing point

FrPointCalc is measured freezing point

Note:

If the freezing point is not correctly determined, the result for the added water is not valid. In this case results for FrPoint and AddWater are not shown on the display and on the printout from the printer. If the density of the measured sample is 0, the result for AddWater is not valid and is also not shown on the display and the printouts.

Sample:

First variant

If you've entered for milk analyzer basic freezing point -0.520°C (according article 5.9 of the EU Milk Hygiene Directive 92/46/EEC), measured freezing point –0.540°C, using the above pointed formula you'll receive –3,8%. Because it is not possible the added water to be negative value, the milk analyzer indicates 0% added water. The reason for this is the tolerance in the basic freezing point, reasons for which are described below.

If in the same milk we add 3,8% water, and the basic freezing point is the same, the milk analyzer will measure freezing point –0.520°C, and will indicate again 0% added water.

Second variant

If you've entered for the device basic freezing point –0.540°C, measured freezing point –0.540°C, the milk analyzer will indicate 0%. When you add 3,8% water, the device will indicate 3,8%-added water.

Fromtheabovementioned follows that it is very important to enter correct basic freezing point in the device.

The device's results for added water may give information about doubt of added water in the milk and the exact value of this added water may be determined after a

"cowshed sample" is taken and the result for the freezing point, measured by the milk analyzer of the "cowshed sample" is entered as basic freezing point in the formula for calculation of added water.

Then the result from this formula will give us the absolute value of the added water for the corresponding milk supplier.

Appendix 3. pH measuring

1. General information

PH probe is a unit, measuring the solution acidity or alkalinity degree. It is measured on scale of 0 to 14. The term pH is derived from "p", the mathematical symbol for the negative logarithm, and "H", the chemical symbol of Hydrogen. The formal definition of pH is the negative logarithm of the Hydrogen ion activity.

2. pH Electrode

For pH measurement the milk analyzer needs a combination electrode, compatible with most pH electrodes that have BNC connectors and zero potential (the pH where the mill volt output of the electrode equals 0) near 7 pH.

2.1. Electrode part

The electrode is the most important part of the pH measurement. The electrode glass membrane is fragile and must be handled with care. To protect the glass membrane and to maintain activation, a protective rubber cap containing a suitable storage solution covers the glass membrane.

2.2. Electrode care & Electrode maintenance

pH Electrodes are susceptible to dirt and contamination and need to be clean regularly depending on the extent and condition of use. At no time should one touch or rub the glass bulb as this causes the build-up of electrostatic charge.

2.3. Storage

For best results, always keep the pH bulb wet. An optimal storage solution for combination electrode is pH 4 buffer with 225 grams of KCl per liter. Table salt, NaCl, can be used if KCl is not really available. Other pH buffers or tap water are also acceptable storage media, but avoid storage in de-ionized water. The protective rubber cap filled with the buffer solution provides ideal storage for long periods.

2.4. After Use

After measurement is completed, follow the sequence below for storage.

- Wash the electrode and reference junction in de-ionized water.

- Close the refilling hole by returning its rubber sleeve or stopper cap. (Necessary for only refillable electrode).

- Store the electrode as mentioned above (see section Storage).

2.5. Electrolyte Replacement (for refillable electrode only).

The reference electrolyte needs to be refilled when the electrode has been used for a long period, or when the internal electrolyte has dried up. To accomplish this, follow the procedure described below.

- Remove the protective rubber cap or sleeve;
- Remove the protective rubber sleeve to expose the filling port of the electrode;
- Remove the old reference electrolyte with a syringe;
- Fill the new reference electrolyte.

2.6. New electrolyte preparation:

- Open the KCl container;
- Add in de-ionized water until it reaches the level of 20 ml;
- Close the container and shake it to dissolve the KCl;
- Add in fresh electrolyte until it reaches the level of the refilling port. The reference electrolyte used should be 3M(Mol) KCl;
- Replace the rubber sleeve.

2.7. Re-use the electrode.

- Rinse the liquid junction with de-ionized water.

If these steps fail to restore normal electrode response, you may attempt to rejuvenate it (See: Electrode Rejuvenation).

2.8. Electrode cleaning

Electrodes which are mechanically intact can often be restored to normal performance by one or combination of the following procedures.

- Salt deposits:

Dissolve the deposit by immersing the electrode in tap water for ten to fifteen minutes. Then thoroughly rinse with de-ionized water. Wash the electrode pH bulb in a little detergent and water. Rinse electrode tip in with de-ionized water.

- Oil/Grease films:

Wash electrode pH bulb in a little detergent and water. Rinse electrode tip with de-ionized water.

- Clogged Reference Junction:

pH electrodes have junction, which allows the internal fill solution of the measuring electrode to leak out into the solution being measured. The junction can become clogged by contamination in the solution. If a clogged junction is suspected it is best to clear the junction.

Heat up the diluted KCl solution to 60-80°C. Place the sensing part of the pH electrode into the heated KCl solution for approximately 10 minutes. Allow the electrode to cool while immersed in some unheated KCl solution.

- Protein Deposits

Prepare 1% pepsin solution in 0.1 M HCI. Allow the electrode to stand in this solution for five to ten minutes. Rinse the electrode with de-ionized water.

2.9. Electrode activation

Generally, if the procedure of storage and maintenance had been closely followed, the electrode can

be used immediately. However, should the electrode response become sluggish, it may be possible that the bulb has dehydrated.

The bulb can be dehydrated by immersing the electrode in an ideal storage solution (e.g. buffer pH 4 solution) for 1-2 hours. If this fails, the electrode may require re-activation. If the above procedure does not reactivate the electrode to acceptable status, try rejuvenation the electrode by following the procedure outlined below.

2.10. Rejuvenation Procedure

Dip and stir the electrode in freon or alcohol for 5 minutes.

Leave the electrode in tap water for 15 minutes.

Dip and stir the electrode in concentrated acid (HCI, H_2S_4) for 5 minutes.

Leave the electrode in tap water for 15 minutes.

Dip and stir in strong base (NaOH) for 5 minutes.

Leave the electrode in tap water for 15 minutes.

Test with standard calibration solution.

Finally, test with standard calibration buffer solution to see if the electrode yields acceptable results. You may repeat again for better response (maximum 3 times). If the response does not improve, then the electrode has completed its useful life. Replace with a new electrode.

2.11. Electrode Lifespan

pH electrodes have a finite lifespan due to their inherent properties. How long a pH electrode will last will depend on how it is cared and the solution it is used to

measure. Even if an electrode is not used it still ages. Electrode demise can usually be characterized by a sluggish response, erratic readings or a reading, which will not change. When this occurs an electrode can no longer be calibrated. pH electrodes are fragile and have a limited lifespan. How long an electrode will last is determined by how well is maintained and the pH application. The harsher the system, the shorter the lifespan. For this reason it is always a good idea to have a back-up electrode on hand to avoid any system down time.

3. Buffer Solutions

Buffers are solutions that have constant pH values and the ability to resist changes in that pH level. They are used to calibrate pH measurement system.

PH buffer solution description (Pharmacopoeia standard)

Use only this types standard buffers for calibration!

Description	pH 7.00±0,01/20ºC	pH 4.00±0,01/20ºC
Composition	Potassium dihydrogen phosphate, Di-sodium hydrogen phosphate	Borax, Sodium hydroxide solution
Temperature parameters	10ºC - 7.06 25ºC - 6.99 20ºC - 7.00 30ºC - 6.98 40ºC - 6.95 50ºC - 6.91	10ºC - 4.00 25ºC - 4.00 20ºC - 4.00 30ºC - 4.00 40ºC - 4.00 50ºC - 4.05

4. pH Electrode Calibration

pH Electrodes are like batteries; they run down with time and use. As an electrode ages, its glass changes resistance. For this reason, electrodes need to be calibrated on a regular basis. Calibration in pH buffer solution corrects for this change.

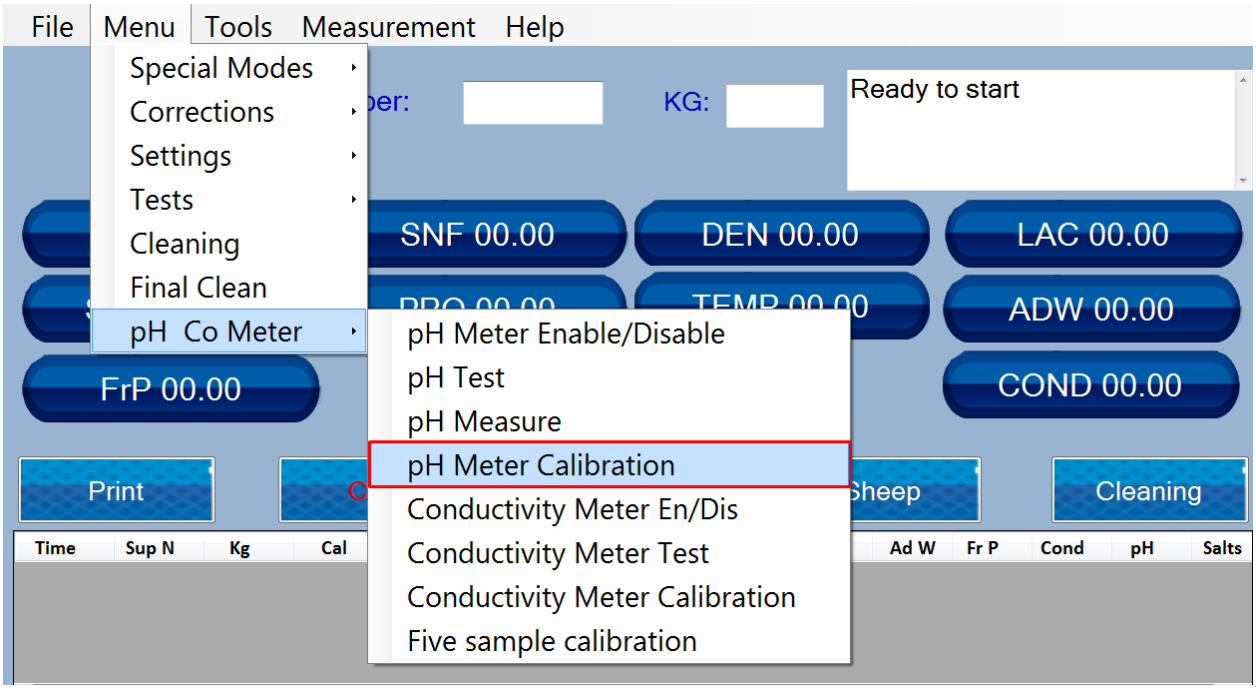
Calibration is an important part of electrode maintenance. This assures not only that the electrode is behaving properly but that the system is operating correctly.

Usually pH meters require calibration at 3 specific pH values. One calibration is usually performed at pH 7, second and third are typically performed at pH 4 and pH 10.

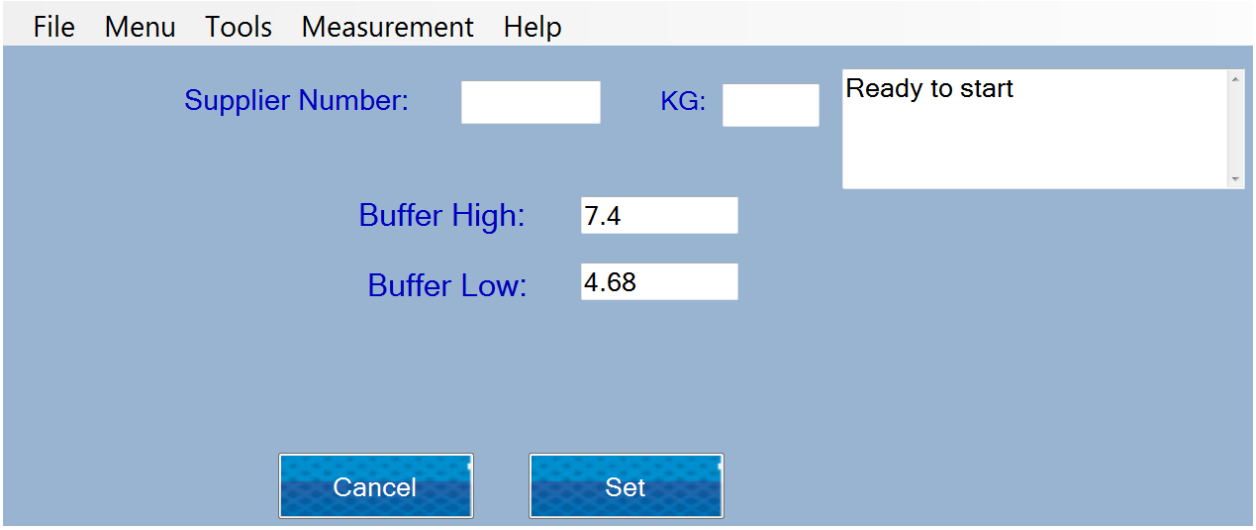
It is best to select a buffer as close as possible to the actual pH value of the sample to be measured. Use standard calibration buffers that the temperature and the sample solution are the same.

Use the operation manual for the corresponding pH meter.

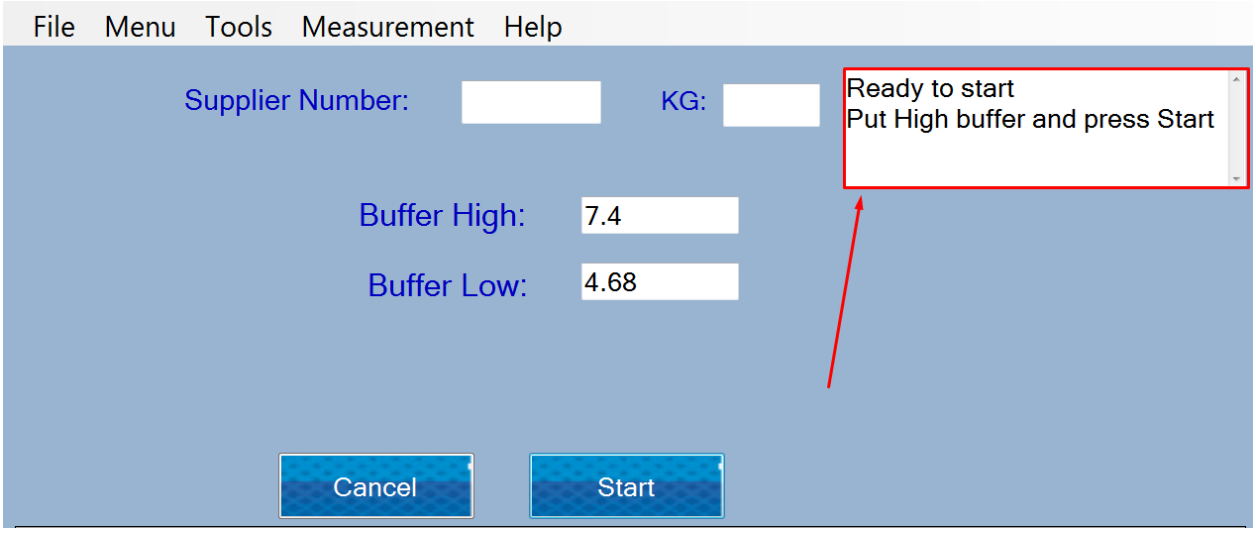
To start the calibration procedure select Menu -> pH & Co Meter ->pH Meter Calibration



Input the values of the buffers, that are going to be used and press “Set”

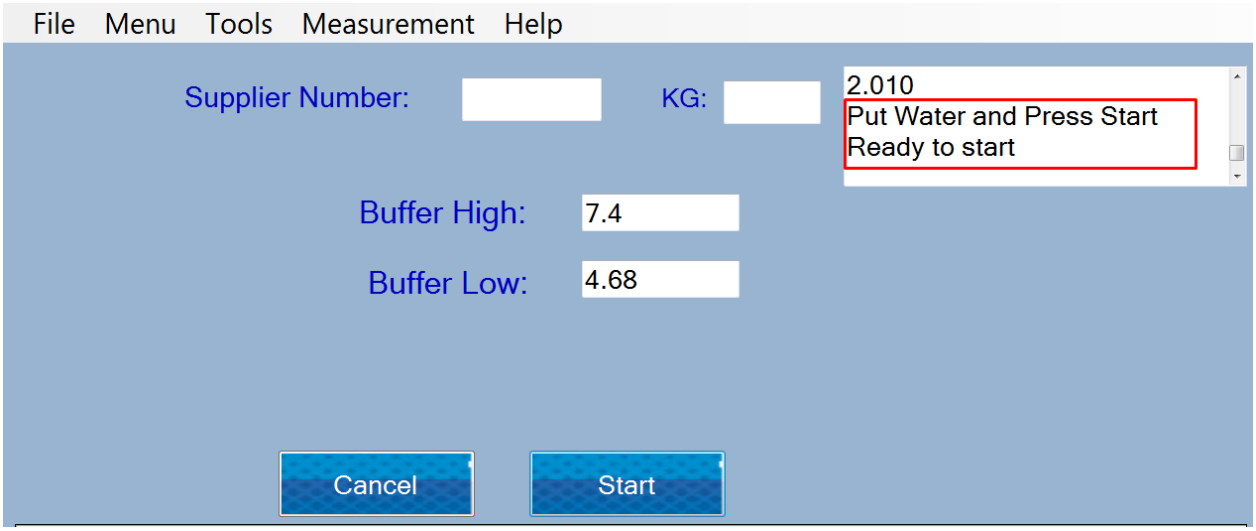


Follow the instructions, received in the field

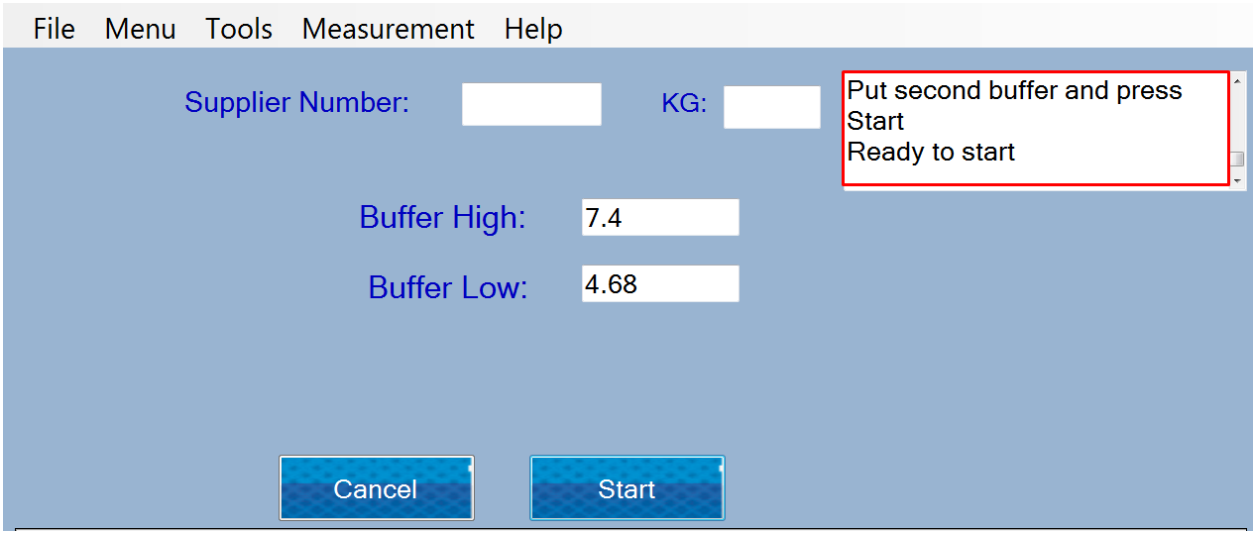


Wait for couple of measurements and press “Stop”. The buffer should be returned in the glass.

After that water should be placed in the machine



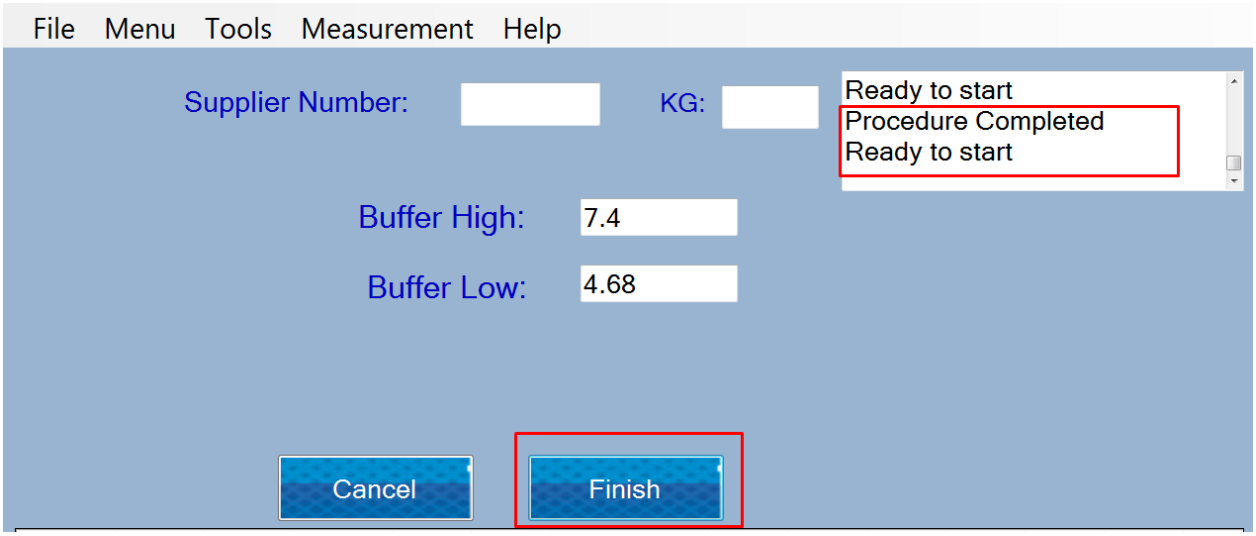
Next step is the second buffer:



Wait for couple of measurements and press “Stop”. The buffer should be returned in the glass.

Again place water in the machine

After the rinsing the machine returns “Procedure completed message” and the operator should press “Finish” button to complete the procedure



For Sensorex pH electrodes, originally supplied with the milk analyser read the following information:

Temperature compensations

The output of pH electrodes varies with temperature in manner, predicted by theory. When needed, Sensorex can supply electrode holders with build-in automatic temperature compensators. The need of automatic compensation depends on the temperature variation, the pH value being measured. At pH of about 7 there is no error due to temperature and, of course, at a constant temperature there is no error. As shown in the following table, the pH error due to temperature is a function of both the temperature and the pH value being measured. At a pH of about 7 there is no error due to temperature and, of course, at a constant temperature there is no error. The more the temperature changes from the ambient calibration temperature and the more the pH departs from 7 the greater is the pH error.

pH temperature error table

°C	pH										
	2	3	4	5	6	7	8	9	10	11	12
5	.30	.24	.18	.12	.06	0	.06	.12	.18	.24	.30
15	.15	.12	.09	.06	.03	0	.03	.06	.09	.12	.15
25	0	0	0	0	0	0	0	0	0		0
35	.15	.12	.09	.06	.03	0	.03	.06	.09	.12	.15
45	.30	.24	.18	.12	.06	0	.06	.12	.18	.24	.30
55	.45	.36	.27	.18	.09	0	.09	.18	.27	.36	.45
65	.60	.48	.36	.24	.12	0	.12	.24	.36	.48	.60
75	.75	.60	.45	.30	.15	0	.15	.30	.45	.60	.75
85	.90	.72	.54	.36	.18	0	.18	.36	.54	.72	.90

0 pH Error Range Less than .1 pH Error Range

5. PH helpful hints

For greatest accuracy in pH measurement, follow these guidelines:

Use the same technique to measure samples, which was used for calibration.

Be consistent with stirring rates, times and conditions.

Calibrate with buffers, which are close in temperature to that of the sample.

Calibrate the pH electrode regularly, e.g. once an hour for accuracy to within 0.01 pH, or once a day for accuracy to within 0.1 pH.

Use fresh buffers for calibrations. Avoid contamination of the stock buffer solution and do not use it beyond the expiry date.

Keep all connections dry.

Immerse the electrode far enough into the solution to insure the reference junction is below the surface.

Allow adequate time for the electrode to stabilize in standards and samples before taking a reading.

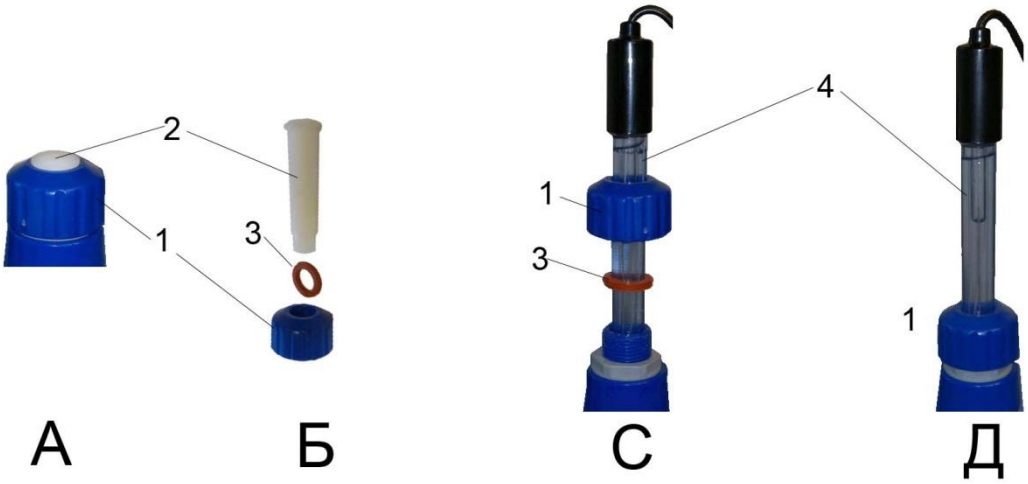
Clean the electrode periodically. Allow more time for aged electrodes.

Do not use the pH electrode in solutions of fluoride ion at low pH. This will etch the glass membrane.

Sulphide vapors can permeate the electrode wick and contaminate the reference element. Minimize contact in such environments and change the reference electrolyte frequently.

6. Preparation for pH measurement

When the analyzer is with pH measuring option, it is received from the customer with pH probe packed separately and there's a stopper on its place. If you need to measure pH follow the procedure below:



1. Loosen the nut anti-clockwise (1).

2. Pull up the stopper (2)

3. Carefully place the pH probe paying attention not to remove the sealing O-ring (3)

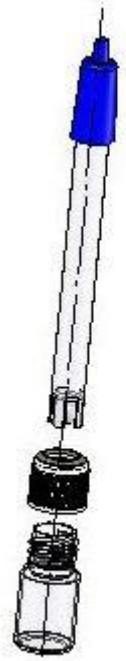
4. Place the probe with the nut in the hole (C & D) and tighten it.

It is very important to close the nut tightly, paying attention not to allow air to enter the system.

If you are working with the analyser regularly (each day) do not remove the probe after work.

If you 'll not use the analyser more than 2 days, you must take out the probe and to place the stopper back.

The pH probe must be stored separately as per the instructions of point 2.3 Appendix 3.



7. PH measuring.

Measuring pH is an additional feature of the analyser and is optional.

Remove the protective rubber cap of the pH electrode. Take care to handle it appropriate in order not to be damaged. Use de-ionized or distilled water to rinse the electrode before usage. Fill in the sample holder with milk, put it in the recess of the analyser and dip the pH electrode into the milk sample, ensuring complete dip of the electrode in the sample. Stir gently for homogenization of the sample.

Measuring can be done in two modes:

Off line by starting the menu **pH & Co Meter | Measuring**, when the analyser works only as a pH meter.

On line automatic pH measuring, when measuring the rest of the sample's parameters.

When starting work with pH meter first connect the probe/sensor, and then the power supply of the device.

Having in mind the characteristics of the process of pH measuring it is necessary to dip the pH probe in the sample and then start measurement.

Appendix 4. Conductivity measuring

1. Method of determination.

Conductivity (or Electrolytic Conductivity) is defined as the ability of a substance to conduct electrical current. It is the reciprocal of the resistance.

In a healthy animal*, the mean value of electric conductivity is:

Milk type	Conductivity values
Cow milk	between 4 to 6 mS/cm (18°C);
Sheep milk	between 3 to 5 mS/cm (18°C);
Buffalo	between 2,5 to 5 mS/cm (18°C);

*These values depend on the geographical region, the breed and on other factors.

Milk conductivity changes on the concentration of ions in the milk:

Added water, sugar, proteins, insoluble solids	Decrease the ion's concentration. Milk conductivity decreases.
Added salts	Increase the ion's concentration. Milk conductivity increases. Increase the ion's concentration. Milk conductivity increases. Often the milk is falsified by adding salt: towards milk with good characteristics: fat 4%, SNF 8,8, conductivity 4,5 are added salt and water. Then the results are changed to 3,2 and 8,8, conductivity 10. In other words adding water regulates the increased value of SNF and density till normal (within the boundaries/parameters) and even the fat is normal. By the values of these parameters may be determined if the sample is falsified, but the only characteristic, proving this is conductivity, which is out of boundaries nevertheless added water. But be careful, as the falsification is not the only possible reason for conductivity increasing. The other possibility is mastitis that's why we recommend using another (chemical) method for checking it.
Significantly extreme value (6,5 - 13,00 mS/cm (18°C)	Should indicate the development of mastitis. Infections damage the tissue of the udder. This allows sodium and chlorine ions from the blood to be released into the milk. The concentration of ions in the milk is thereby raised, and it can more easily conduct an electrical current - the conductivity of the milk increases.

Milk conductivity can be used as tests for degree of water evaporation in condense milk production.

Milk conductivity change notifies of powder (dry) milk solution rate.

2. Conductivity measurement

Conductivity measurement is additional possibility of the analyser and is delivered on customers request/

3. Corrections in conductivity measurement.

Please, follow the instructions on the display of the tablet.

4. Conductivity calibration buffer preparation

In order a standard buffer for conductivity measuring to be prepared follow the instruction below:

- Take the packet with the powder buffer.
- Carefully shake the packet in order to gather the powder at the bottom.
- Cut one end of the packet.
- Empty its content in a measuring mug with 1 l volume, paying attention all its content to be emptied.

  For standard buffer: 5,02 ms – 3,056 г

- Add 600-700 ml distilled water, which was preliminarily deaerated in vacuum dryer or boiled and then cooled down to 20 °C.
- Shake the mug till the powder is fully dissolved.
- Add distilled water to the mark.

Warranty card

LACTOSCAN COMBO

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:

„Milkotronic" Ltdpreservestherighttochoosethemethodsfor checking the deviceinordertoestablishthevalidityofthewarranty. Devices with expired warranty are not subject to a free warranty service.

Service card

Customer:

Service report:

Service entry date	Damage	Date of receipt	Signature

Contacts

For more information or technical support, visit our website www.lactoscan.comor contact us:

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Last edited: 18.01.2018