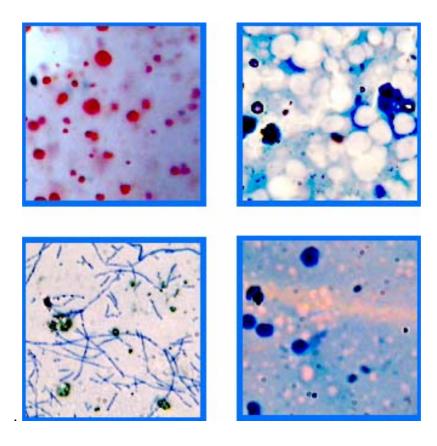


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# **EKOSCOPE**



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The system is designed to automate the Breed method of somatic cells counting in cow milk.

#### 1. General

## 1.1. Principle of the method

The direct microscopic method consists of examining, under a compound microscope, stained films of a measured volume of milk spread on glass slides over specified area. It enables the rapid estimation of the somatic cells counts of a sample of milk. The microscope is first calibrated so that the exact area of the microscopic field is known. Then a measured quantity of the milk (0.01 mL) spread over a measured area (one square centimeter) on a clean glass slide so that each microscopic field examined represents a quantitative aliquot of the sample. The milk is allowed to dry, and the film is fixed, defeated and stained with a suitable dye. The average number of somatic cells counts per microscopic field is determined after examining between 50 and 100 fields, depending on the microscopic factor and the number of somatic cells per field.

## 1.2. Advantages

- large number of samples to be prepared;
- high accuracy and repeatability in measuring of 0,01-mL quantities of sample;
- repeatability in staining of sample film;
- Accuracy: typically 10% (100 counted optical fields)
- Modular design allowing fast and easy maintenance.

## 1.3. Working stations

#### A. EKOSCOPE-FPS1 Films preparing station

EKOSCOPE-FPS1 makes all procedures, otherwise manual, to perform films preparation to the microscopic tests:

- Automatic milk transferring and spreading;
- Automatic films staining.

## B. EKOSCOPE-SCC1 - Films examining station

EKOSCOPE-SCC1 provides the enumeration somatic cells values, according to the official method with high accuracy and precision and performing all scientific examination directly on the display.

#### Specification:

- Biological microscope:
- Automatic system for moving the microscope's stage in transversal and longitudinal directions:
- Color CCD camera;
- PC;
- Software for managing the system:

# 1.4. Parts, accessories and spare parts

## 1.4.1. Parts

| Item | Description                           | Qty |
|------|---------------------------------------|-----|
| 1.   | EKOSCOPE-FPS1 Films preparing station | 1   |
| 2.   | Trinoculiar biological microscope     | 1   |
| 3.   | LCD P Computer - Flatop               | 1   |
| 4.   | CCD Camera                            | 1   |
| 5.   | CCD Camera - video cable              | 1   |
| 6.   | Bank 250 mL (empty)                   | 2   |
| 7.   | Milk mug                              | 4   |
| 8.   | Panel for slides                      | 10  |
| 9.   | Slides drying rack                    | 2   |
| 10.  | Storage Stand                         | 1   |
| 11.  | Dish                                  | 1   |
| 12.  | Cleaning pan                          | 2   |
| 13.  | RS232 Link cable                      | 1   |
| 14.  | User's guide                          | 1   |
| 15.  | CD - EKOSCOPE software tools          | 1   |
| 16.  | CD - Video Capture Drivers            | 1   |

## 1.4.2. Accessories

These accessories are provided only to start immediately work - there is all necessary for 5000 samples. They can be bought from any shop from lab. chemicals.

| Item | Description                                | Qty |
|------|--|-----|
| 1.   | Slides (set)                               | 5   |
| 2.   | Plastic gloves (set)                       | 1   |
| 3.   | Filter paper (set)                         | 1   |
| 4.   | Immersion oil                              | 1   |
| 5.   | Glass stirrer                              | 1   |
| 6.   | Work Solution (200 mL)                     | 2   |
| 7.   | Cleaning Solution – ethyl alcohol (200 mL) | 1   |
| 8.   | Microscope cleaner (50 mL)                 | 1   |
| 9.   | Pencil for slide marking                   | 1   |

# 1.4.3. Spare parts

| Item | Description  | Qty   |
|------|--------------|-------|
| 1.   | Fuse 1,6A T  | 1     |
| 2.   | Needle       | 1     |
| 3.   | Silicon tube | 0.5 m |

## 2. EKOSCOPE-FPS1 Films preparing station

## 2.1. Front view

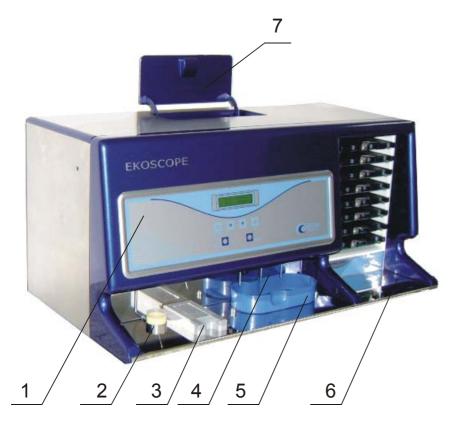


Fig. 1

- 1. Control panel
- 2. Milk mug position
- 3. Microscopic slide position
- 4. Film staining
- 5. Dish
- 6. Heating device
- **7.** Solution container position

# 2.1.1. Control panel

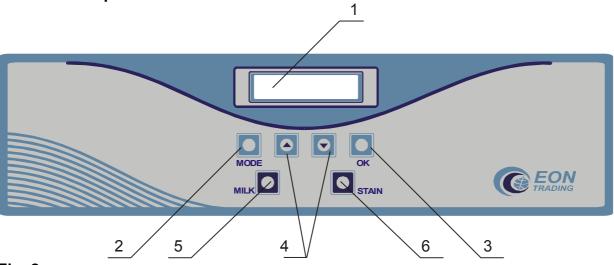


Fig. 2

- 1. LCD display
- 2. Select the work mode
- 3. Confirm the choice

- 4. Skip and search forward and backwards these buttons have one more function that is available only in work mode see section 2.3. Film preparation.
- 5. Short button available only in main mode see section 2.3. Film preparation
- 6. Short button available only in main mode see section 2.3. Film preparation

## 2.2. Rear panel

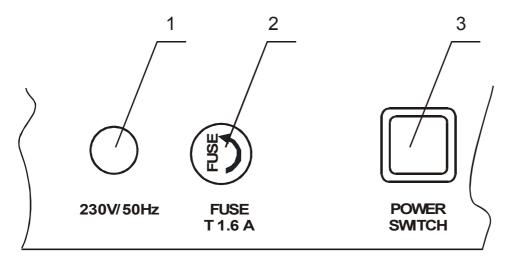


Fig. 3

- 1. 220 V AC power cable
- 2. Fuse
- 3. Power Switch

## 2.3. Film preparation

#### Switching on the system

Connect the AC power lead to the mains socket. Set the Power switch to "On".

Warning: The covers of the device should never be removed while the power leads are connected.

Warning: Under no circumstance should you try to repair the instrument's power lead yourself. In case of power lead damage, contact your dealer to make the repairs.

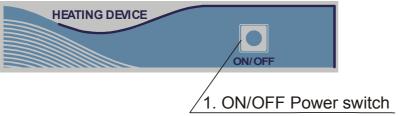
When the power is on, **PLEASE WAIT...** appears on the display. When the initialization is over in about 30 seconds, the system automatically enters into work mode and the display shows the following message:



## Switching on the heating device

Press ON/OFF button to switch the heater and a red light comes on. After 10 minutes the heating device will get to the working temperature – about 65 °C. The system controls automatically this temperature.

Fig. 4



## 2.3.1. Preparation of working solution

**Attention!** Working room must be under control in maintaining temperature stability, humidity and stable interference on the standard laboratory conditions. The solutions and slides can only be prepared and carried out by laboratory personnel.

## Working solution:

The producer provides prepared solution (for 5000 samples) to start immediately work. All needed chemicals can be bought from any shop for lab. materials.

**Attention:** This solution is a strong dye! Work in protective gloves.

| Methylene blue        | 1-2 g              |
|-----------------------|--------------------|
| Ethyl alcohol 99,99%* | 60 cm <sup>3</sup> |
| Xylene                | 40 cm <sup>3</sup> |
| Chilled acetic acid   | 6 cm <sup>3</sup>  |

**Attention!** Xylene is an organic solvent. Use only glass bottle to prepare this solution! The ethyl alcohol will be warmed up to 50-60 °C. Add the methylene blue and xylene. Cool the solution down to 4 °C, add the acetic acid and filter through a paper filter. Keep the solution in a well-closed bottle.

#### 2.3.2. Microscope Slides

## Preliminary treatment of slides

**Microscope Slides** – (size 2.6 x 7.6 cm) of clear glass - with etched margins to permit unmistakable identification of films. The slides should be clean and dry before use and should not contain any finger-prints or other residues on areas where milk films are to be placed.

**New slides** may be cleaned by soaking in strong cleaning solution (bichromate-sulphuric acid solution prepared by dissolving 50 g sodium bichromate in 200 ml of water in glass or earthen container and then adding cautiously 300 ml of concentrated sulphuric acid of commercial grade), rinsing in flowing tap water and then in distilled water.

**Used slides** should be soaked in hot or boiling alkaline detergent solution until all residues are removed, then rinsed in flowing tap water, dried and properly stored for reuse. Slides may be stored submerged in chloroform or alcohol and drained and dried at the time of use.

Flame slides just before use.

#### 2.3.3. Slide Identification

Legibly and indelibly identify each sample area on margin of slide.

#### 2.3.4. Milk Sample Agitation

Milk temperature must be 20-22°C.

- a. Mix samples by shaking 15 20 times in 10 sec. with 1 ft movement;
- b. Optional: Warm high fat samples to 40°C for no longer than 10 minutes prior to testing

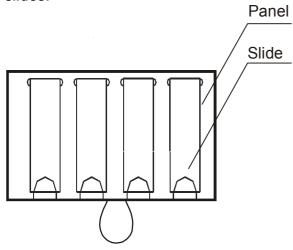
(discard after testing). Cool it to 20-22°C.

## 2.3.5. Film preparation

Pour the milk sample into the mug (approximately 2/3 from the volume of the mug) and place it on fig.1 – pos.2. (Optional: two smears may be prepared and examined for each milk sample).

Put a slide in fig.1 – pos.3. Mark the margin slide with a pencil. Press the short button **MILK** – fig. 2 – pos. 5. The needles will take a 0.01 mL milk portion and smear the milk at an area of size 10 mm x 10 mm.

Remove the mug and the slide. Place the slide on the Panel. The panel is designed for 4 slides.



## 2.3.6. Films drying

Fill up the panel with 4 slides and put it in the heating device. The correspondent timer is turned on and green light comes on. When the drying time finish the green light starts blinking. The initial timer time is 10 minutes. The films drying time is from 5 to 10 minutes. The drying time depends on the room temperature and humidity. If it is necessary to change the timer perform the following procedure:

- Switch on the heating device by pressing ON/OFF button;
- Press again the same button not releasing it for about 5 seconds;
- After button releasing all green lights start blinking. One blink corresponds to 1 minute.
- Press the ON/OFF to stop counting. For example, if you stop the counting after the sixth blink, the timer will be adjusted to 6 minutes.

#### 2.3.7. Staining of smears

**Attention!** Fill an empty bank (250 mL) with work solution and another with ethanol min. 90%. Open the cover on the top of the ECOSCOPE and put the 250 mL banks in the corresponding places. Tag the banks to the plastic tubes. Close the EKOSCOPE cover.

Put the Dish in Ekoscope film staining section - see fig. 1 pos. 4 and 5.

Perform the following procedure to fill the tubing with working solution:



for 3-4 seconds and then release it. The system starts pumping the solution.

Wait until a drop of solution falls from all of the nozzles and then press the same button to stop the procedure. Put a panel with already dried smears up in staining position and press button **STAIN** (fig. 2, pos.6)

This procedure doses an exact quantity of work solution.

**Attention!** Because the working solution is a strong dye, the **Ekoscope film staining section** is provided with a sensor that checks if there is a dish or panel in the section. In case of dish/panel missing a warning message will appear on the display.

# Push the di sh and try agai n!!!

## 2.3.8. Washing away the non-absorbed dye

Wait from 50 to 70 seconds and then take out the smears from the holding panel and dip them consecutively in 2 baths of potable water until full removal of the non-absorbed dye.

Put the smears in the slides drying rack until their dry up. Put filter paper in the rack grooves to blot the water.

**Attention!** Wash properly the smears! The non-absorbed dye will make the counting incorrect.

## 2.3.9. Cleaning out the EKOSCOPE

## 2.3.9.1. Cleaning out the milk needle

After the end of the working cycle the needle that spreads the milk portion should be cleaned with ethyl alcohol. Take care not to bend, damage and break the needle.

## 2.3.9.2. Cleaning out the work solution tubing

It is necessary to clean the system in the following cases:

- A. The interval between two consecutive stains is more than 1 hour:
- B. End of working day.

#### A. Cleaning out when the interval between two consecutive stains is more than 1 hour.

- Press the MODE button once. Press the search buttons ▲, ▼ to select CLEANING option. Confirm with OK.
- 2. Put the Dish in Ekoscope film staining section see fig. 1 pos. 4 and 5.
- 3. Press **OK** again to start cleaning. This procedure pumps out the dye and then washs the tubing with ethyl alcohol.
- 4. Done

Press the **MODE** button to resume work.

#### B. Ending the working day

- Press the MODE button once. Press the search buttons ▲, ▼ to select TURN OFF option. Confirm with OK.
- 2. Put the Dish in Ekoscope film staining section see fig. 1 pos. 4 and 5.
- 3. Press **OK**. This procedure checks the system and cleans it if it is necessary. Next all moving parts of system will be put in non-work positions.
- 4. When the message **SWITCH OFF...** is on the display press the Power switch Fig.3 –pos.3.
- 5. Done

#### 2.4. EKOSCOPE Menu

Press the **MODE** button once. Press the search buttons **△**, **▼** to select options:



| >Turn of f |
|------------|
| Settings   |
| Syst em    |
| Manual     |
| Cl eani ng |

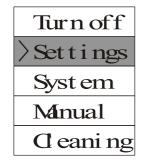
Press **OK** to confirm your selection.

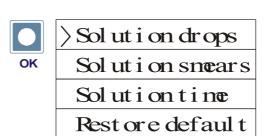
#### 2.4.1. Turn off

This option is described in section 2.3.9.2. Cleaning out the work solution tubing - Ending the working day.

## 2.4.2. Settings







#### 2.4.2.1. Solution drops

This option is for adjusting the quantity of the work solution.

**Important**: The number of the drops is set to 2 in the program and it is not advisable to change it!

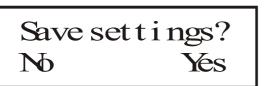
Press the **MODE** button once. Press the search buttons  $\blacktriangle$ ,  $\blacktriangledown$  to select **Settings** option. Press **OK** for confirmation. Press the search buttons  $\blacktriangle$ ,  $\blacktriangledown$  again to select this time the sub option **Solution drops** and press **OK**.

The display shows you the current setting of this parameter. You can change it from 0 to 24. By pressing ▲ the number of drops will be increased, ▼ will decrease them.

Button **OK** – confirm the change but without saving it in the system memory.

Press **MODE** to go back to Settings menu options. Now you can adjust another parameter from the **Settings** menu list.

Press **MODE** again to go back to the main menu. This time the system will ask you to save all settings:



Press **OK** to save settings or **MODE** to exit without saving.

Press the **MODE** button to resume work. The display will show:



The buttons MILK and STAIN will be active.

## 2.4.2.2. Solution smears

This option is for adjusting the needle movements for smearing the milk at an area of size 10 mm x 10 mm.

**Important**: The number of the smears is set in the program and it is not advisable to change it!

Press the MODE button once. Press the search buttons  $\blacktriangle$ ,  $\blacktriangledown$  to select **Settings** option. Press **OK** for confirmation. Press the search buttons  $\blacktriangle$ ,  $\blacktriangledown$  again to select this time the sub option **Solution smears** and press **OK**.

The display shows you the current setting of this parameter. You can change it from 0 to 24. By pressing ▲ the number of smears will be increased, ▼ will decrease them.

Button **OK** – confirm the change but without saving it in the system memory.

Press **MODE** to go back to Settings menu options. Now you can adjust another parameter from the **Settings** menu list.

Press **MODE** again to go back to the main menu. This time the system will ask you to save all settings:

Save settings?
No Yes

Press **OK** to save settings or **MODE** to exit without saving.

Press the **MODE** button to resume work. The display will show:



The buttons **MILK** and **STAIN** will be active.

#### 2.4.2.3. Solution time

This is an additional option that helps you to control the staining time. The timer is switched when you press the **STAIN** button. The symbol appears on the display for the time set in this option.

Press the MODE button once. Press the search buttons  $\blacktriangle$ ,  $\blacktriangledown$  to select **Settings** option. Press **OK** for confirmation. Press the search buttons  $\blacktriangle$ ,  $\blacktriangledown$  again to select this time the sub option **Solution time** and press **OK**.

The display shows you the current setting for this parameter. You can change it from 0 to 10 minutes. By pressing ▲ the time will be increased, ▼ will decrease it.

Button **OK** – confirm the change but without saving it in the system memory.

Press **MODE** to go back to **Settings** menu options. Now you can adjust another parameter from the Setting menu list.

Press **MODE** again to go back to the main menu. This time the system will ask you to save all settings:



Press **OK** to save settings or **MODE** to exit without saving.

Press the **MODE** button to resume work. The display will show:



The buttons MILK and STAIN will be active.

#### 2.4.2.4. Restore default

This option restores the producer's settings for all parameters.

Press the MODE button once. Press the search buttons  $\blacktriangle$ ,  $\blacktriangledown$  to select **Settings** option. Press **OK** for confirmation. Press the search buttons  $\blacktriangle$ ,  $\blacktriangledown$  again to select this time the sub option **Restore default** and press **OK**.

Press **OK** again to restore the default settings or **MODE** to go back to the **Settings** option.

Press the **MODE** button to resume work. The display will show:



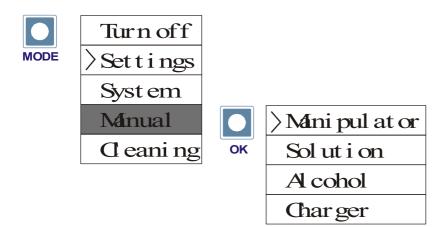
The buttons MILK and STAIN will be active.

#### 2.4.3. System

This is a manufacturer's mode only and it is protected by a password.

## 2.4.4. Manual

This option allows manual control of all systems gears.



## 2.4.4.1. Manipulator

This option moves needle gear up and down from one work position to another.

Press the **MODE** button once. Press the search buttons  $\blacktriangle$ ,  $\blacktriangledown$  to select **Manual** option. Press **OK** for confirmation. Press the search buttons  $\blacktriangle$ ,  $\blacktriangledown$  again to select this time the sub option **Manipulator** and press **OK**.

Press the arrow buttons ▲, ▼ to move the needles.

Press **MODE** to go back to **Manual** menu options. Press **MODE** again to go back to the main menu.

Press the **MODE** button to resume work. The display will show:



The buttons **MILK** and **STAIN** will be active.

#### 2.4.4.2. Solution

This option fills and empties the tubing with work solution.

Press the **MODE** button once. Press the search buttons  $\blacktriangle$ ,  $\blacktriangledown$  to select **Manual** option. Press **OK** for confirmation. Press the search buttons  $\blacktriangle$ ,  $\blacktriangledown$  again to select this time the sub option **Solution** and press **OK**.

Put the **Dish** in **Ekoscope film staining section – see fig. 1 pos. 4 and 5**.

**Attention!** Because the working solution is a strong dye, the **Ekoscope film staining section** is provided with a sensor that checks if there is a dish in the section. In case of dish missing a warning message will appear on the display.

Push the di sh and try agai n!!!

Press the arrow button ▲ for 3-4 seconds and next release it to fill the tubing. The system starts pumping the solution. Wait until a drop of solution falls from all nozzles and then press

the same button to stop the procedure. A short click on button ▲ doses an drop of the work solution.

Press the arrow button ▼ for 3-4 seconds to empty the tubing. The system starts pumping out the solution for 30 seconds. Press the same button to stop the procedure. A short click on button ▼ performs this procedure step by step.

Press **MODE** to go back to **Manual** menu options. Press **MODE** again to go back to the main menu.

Press the **MODE** button to resume work. The display will show:



The buttons **MILK** and **STAIN** will be active.

#### 2.4.4.3. Alcohol

This option fills and empties the tubing with ethyl alcohol (cleaning solution). Press the **MODE** button once. Press the search buttons  $\blacktriangle$ ,  $\blacktriangledown$  to select **Manual** option. Press **OK** for confirmation. Press the search buttons  $\blacktriangle$ ,  $\blacktriangledown$  again to select this time the sub option **Alcohol** and press **OK**.

Put the **Dish** in **Ekoscope film staining section – see fig. 1 pos. 4 and 5**.

**Attention!** The **Ekoscope film staining section** is provided with a sensor that checks if there is a dish in the section. In case of dish missing a warning message will appear on the display.

Push the di sh and try agai n!!!

Press the arrow button ▲ for 3-4 seconds and release it to fill the tubing. The system starts pumping the solution. Wait until a drop of Alcohol falls from all nozzles and then press the same button to stop the procedure. A short click on button ▲ doses an drop of the ethyl alcohol (cleaning solution).

Press the arrow button ▼ for 3-4 seconds to empty the tubing. The system starts pumping out the Alcohol. Press the same button to stop the procedure. A short click on button ▼ performs this procedure step by step.

Press **MODE** to go back to **Manual** menu options. Press **MODE** again to go back to the main menu.

Press the **MODE** button to resume work. The display will show:



The buttons MILK and STAIN will be active.

## 2.4.4.4. Charger

This option moves milk mug gear left and right from one work position to another.

Press the **MODE** button once. Press the search buttons  $\blacktriangle$ ,  $\blacktriangledown$  to select **Manual** option. Press **OK** for confirmation. Press the search buttons  $\blacktriangle$ ,  $\blacktriangledown$  again to select this time the sub option **Charger** and press **OK**.

Press the arrow buttons ▲,▼ to move the milk mug.

Press **MODE** to go back to **Manual** menu options. Press **MODE** again to go back to the main menu.

Press the **MODE** button to resume work. The display will show:



The buttons MILK and STAIN will be active.

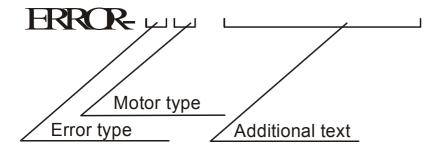
## 2.5. Errors Messages

**Attention:** In case of some problem, the system shows an error message on the display. The error can be caused by some mechanical or electrical problem. Often the power supply troubles provoke temporal errors.

The producer advises always first restart the system and if the problem remains, contact your dealer for repairs. Please give them the exact error message.

#### 2.5.1. Motors errors

In case of motors error the display will show the following message:



## Error type meaning:

1. Overload

2. Timeout

## Motor type meaning:

- 1. Milk manipulator motor (needle)
- 2. Charger motor (milk mug)
- 3. Pump motor (Solution)
- 4. Valve motor

## 2.5.2. EEPROM (IC Memory) error

In case of EEPROM reading/writing problem the display will show the following message:

Error - Manory

## 2.5.3. PCB damage

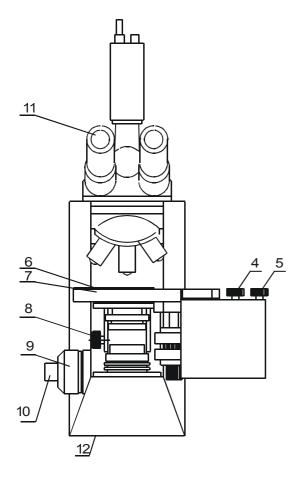
In case of PCB problem the display will show the following message:

Error communication Reset syst.

## 3. EKOSCOPE-SCC1 - Films examination

## 3.1. Microscope and camera assembling

## 3.1.1. Microscope installation



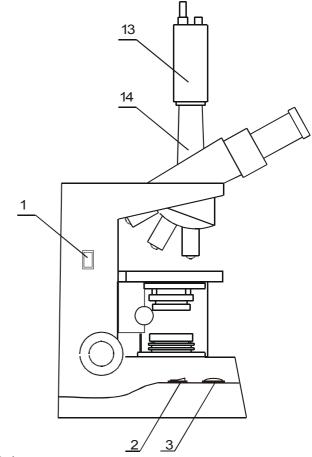
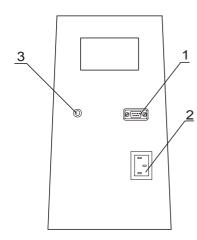


Fig. 4.1

- 1. Motors switch ON/OFF
- 2. Light switch ON/OFF
- 3. Brightness control
- 4. Stage adjustment X
- 5. Stage adjustment Y
- 6. Stage
- 7. Clips
- 8. Condenser/Condenser focus
- 9. Coarse focus control
- 10. Fine focus control
- 11. Eyepieces
- 12. Base/Light On the lower side of the microscope there is a lid and by opening it the lamp (6V/20W) can be changed when necessary
- 13. Camera
- 14. Camera tube

Assembly all microscope parts as it is shown on fig. 4.1.

## Microscope Back panel



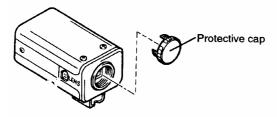
- 1. RS232 Connector
- 2. 220V Connector
- 3. 12V Connector
- Connect the AC power lead first to the 220V connector, next to the mains socket.

Connect the AC Adapter (Motors power supply) lead first to the 12V connector, next to the mains socket.

Connect the microscope to the computer RS port (COM1) by mean the RS cable.

#### 3.2. Video camera installation

The operation system works with CCD CAMERA, which transmits the image from the microscope to the computer. It is connected by a video cable to the computer and by means of power supply cable to 220 V AC.



- a. Remove the protective cap.
- b. Attach the camera to the microscope as it is shown on fig 1.
- c. Connect the video camera to the computer and to the power supply as it is shown on the fig.4.2

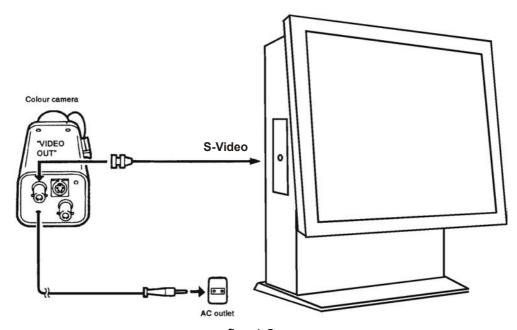


fig. 4.2

## 3.3. Adjustment of microscope for examination

From button pos. 2 (fig.4.1) switch on the light of the microscope. Switch on button pos.1 to turn on the stage motors. The potentiometer pos. 3 should be in max. lighted position in order to get a good image.

One or two drops of immersion oil (Refractive index 1.51-1.52 at 20°C) are dropped on the ready sample and the sample is put on the microscope table pos.7 (fig.4.1). The clamp pos.6 is placed. By means of the handle pos.4 and pos. 5 (fig. 4.1) it is oriented manually along the X and Y axes until the sample comes under the lens. The film is viewed by **1/40 lens**.

The condenser (pos. 8 - fig.4.1) regulates the intensity of the light by closing or opening the condenser diaphragm or by adjusting the height of the condenser. Closing the condenser diaphragm diminishes the resolution of the microscope, thus always open the diaphragm as wide as possible (or for two thirds of the maximum opening). Put the condenser (by means of the condenser handle) in higher position against the object glass. That is often almost the best situation. With handle pos.9 the film is oriented along for the lens to be roughly dipped in the oil and after that you start to turn back by pos.10-the fine setting carefully until the image comes out.

You can view it by the eyepiece pos.11 (fig.4.1) or on the computer screen (if the computer program is started), whatever is more convenient for the operator.

The sample is considered focused at the moment when somatic cells are clearly delineated. The sample is light blue or white in color and the somatic cells are dark blue.

**Attention!** After finishing the work with the microscope it is compulsory to clean the lens with filter paper, soaked in xylene. All parts greased with the immersion oil are also cleaned. Each sample you wish to preserve has to be cleaned with xylene and stored.

## 4. Computer Program

**SOMATIC CELLS COUNTER** is an electronic system for automatic, fast and efficient determining the number of somatic cells in the milk.

Its characteristic features are:

Capacity: 200..300 test/day

Accuracy of measurement: <u>+</u> 10 % (100 counted fields)

#### 4.1. Preparing the system for work

After loading the operation system, the program prepares the TV-capture to work with a CCD video camera. The preparation is automatic and lasts about 1 second.

Close the TV-capture program by pressing on the green round button on the screen and load the "Somatic cells counter" program by clicking on the icon. The computer is ready for work.

#### 4.2. Description of operation mode: Microscope

By starting the "Somatic cells counter" the program switches automatically into "microscope" mode.

That mode is used for:

- Pre-positioning and focusing the sample.
- Pre-view and evaluation of the sample.
- Save pictures, comments, date, time and name of sample.
- Create a set of sample pictures.
- Print pictures on a printer directly from the screen image as well as from earlier saved

sample sets and specimens.

- Export of pictures in various formats.
- Make references.

## 4.2.1. Pre-positioning and focusing the sample

The microscope focusing is done manually by the microscope mechanisms.

The microscope positioning can be done both manually and by the "Somatic cells counter" program.

In "Microscope" mode the positioning is done by means of a scrollbar for vertical (y) and horizontal (x) shifting located at the side of the displayed monitor.

Two ways for shifting electronically the optical fields are used.

The first one allows fine positioning of the desired sample part. Fine positioning can be achieved through the horizontal scrollbar buttons for shifting along (x) and the vertical scrollbar for shifting along (y).

The second method allows positioning on the next vision field of the sample in the desired direction.

Use the same scrollbars but click in the empty space between the buttons.

A red button in the displayed monitor does the shift from manual to electronic mode of microscope control.

**Attention!** When this button is red do not move manually the microscope stage. This will damage the motors. Press on with the mouse pointer the red button for releasing the motors and then move the microscope stage.

## 4.2.2. Preview and evaluation of the sample

Preview and evaluation of the sample are used mostly at the stage of preparing the sample for analysis and counting the somatic cells.

Quality of film, placing in initial counting position, set up of contrast and light of the sample are to be considered.

The microscope factor is set.

The microscope factor is selected from a falling window located on the display monitor.

## 4.2.3. Saving pictures, comments, date, hour and name of sample

The "Somatic cells counter" program makes it possible to take pictures of the current display of the microscope in "Microscope" mode; a lot of pictures to be collected in albums and catalogues; too be printed out both separately and by albums on a printer; to be exported to other programs in different formats; to put down comments to each selected album with the date and time of creating.

Select a sample and press the button <New Sample>.

The panels <Pictures> and <Comment> are empty and also for the Name of the sample (top center) there is "<unknown sample>".

To take a picture of the sample press <Add>.

To delete a picture of the sample press < Delete > from the picture panel.

In the adjacent box <Number 0> the number of added pictures is automatically displayed. Use the scrollbar to view all pictures.

For each sample you can enter a short note in the box <Comment>.

For a selected picture you can also enter a short note in the box <Comment>, if before that you save the picture as a separate sample.

In the box <Comment> data about the date and time of saving the sample are entered.

To save a sample first you have to create a folder in which to put that sample.

So, press the button <Folder> and enter a name of the folder.

Highlight the new folder and press the button <Save Sample>.

A box is opened in which you enter a name of the sample.

The name of the sample is loaded and displayed in the box <unknown sample>.

It is also displayed in the table under the panel <Samples> along with the number of pictures to the sample, the comment to the sample and the date and time of saving the sample.

## 4.2.4. Creating sets of samples with multiple pictures

It is necessary to classify all samples in folders created by your choice.

Press the button <Folder> and enter the name of the folder.

Create several folders according to your criterion – for example, for the various regions.

You can also create subfolders /for sub-regions/ by highlighting a selected folder and press <Folder>.

Highlight one folder and enter a sample in it by pressing the button <Save Sample>.

A box is opened in which you can enter the name of the sample.

The name of the sample is loaded and displayed in the box < unknown sample>.

It is also displayed in the table under the panel <Samples> along with the number of pictures to the sample, the comment to the sample and the date and time of saving the sample.

When you enter a specific period in the boxes <From> and <To>, in the table you will have all saved samples from a selected folder for the respective period of time.

## 4.2.5. Printing out pictures on a printer

You can print out pictures directly from the screen image or from earlier created sample sets or specimens.

## Directly from the screen image:

Select a specific image and press <Print>.

A list with sample name < unknown sample > is displayed and the selected image is displayed; you can view it and print it out by pressing the button with the printer.

#### Printing out pictures taken earlier is done in the following way:

Select a folder in which the sample with pictures for printing out is located.

Enter the period in which the sample has been taken /used when you have a larger data base/.

Highlight the sample.

Press <Load>.

All pictures saved in relation to the sample are loaded in the panel "Pictures". You can view them, delete a picture or change a comment.

When pictures are ready for printing, press <Print>.

A list with the sample name is displayed with all the pictures related to it and these can be viewed and printed out by pressing the button with the printer.

## Deleting a picture:

Highlight the pictures in the panel "Pictures" and press < Delete >.

#### Deleting a sample:

Highlight the sample from the table in the panel "Samples" and press <Delete>.

#### Deleting a folder:

Highlight the folder in the panel "Folder" and press < Delete >.

#### Renaming the folder:

Highlight the folder in the panel "Folder" and press <Edit>.

#### 4.2.6. Exporting pictures

Each image from the microscope screen can be exported in a bmp - format. To that end press <Export>.

## 4.2.7. Making references

When you enter a specific period in the boxes <From> and <To> all saved samples from a selected folder for the respective time period are displayed. The pictures to the respective can be viewed and printed out.

## **4.2.8. Summary**

- 1. Focus the microscope and select the microscope factor.
- 2. Create a few folders and subfolders according to your criteria.
- 3. Press <New Sample> for entering a new sample.
- 4. Select a picture and add it by <Add>.
- 5. Select several subsequent pictures and add them by <Add>.
- 6. Highlight the folder in which you will save that sample.
- 7. Save the name of the sample.

## 4.3. Describing an operation mode: SOMATIC CELLS COUNTER

## 4.3.1. Operation mode

Put a slide with a film on the microscope:

In "microscope" mode adjust the focus and the light for the sample.

Position the microscope (See Operation in "Microscope" mode) on the part of the sample you want to scan taking into consideration the scanning method you are going to use.

For example:

If you want to use method 1 for scanning , you will have to position the microscope in the right part of the sample.

Otherwise the microscope may appear out of the sample.

After you have focused the sample, positioned the microscope according to the method of scanning and assured yourself that the sample is well lighted with a good contrast – press the button "Somatic cells counter" from the main menu on the top center.

This is how you shift to the "Somatic cells counter" mode.

First select a folder in which to record the test results.

This is done in the bottom left panel.

The names of the folders are highlighted in yellow.

Select a folder by clicking on the desired one.

The selected folder is highlighted in blue.

Using the buttons "Folder", "Edit" and "Delete" on the same panel you can add new folders, change the names of existing ones and delete the unnecessary ones.

You can create both main Folders and Subfolders.

The content of the folders is in the table in the bottom right panel.

You can automatically use all samples for the day saved in a pre-selected folder.

To view recordings from a previous date you need to change the dates "From" and "To".

With the buttons "Load" and "Delete" you can go through saved samples and delete the unnecessary ones.

After selecting a folder in which to save the new test you have to determine how many optical fields will be scanned. For reliable results the number of optical fields has to be over 30.

There are about 90 optical fields along (y) and 110 fields along (x) on a 1 sq. cm sample.

In the box **Number of fields to count** enter the number of pre-selected optical fields.

Analyzed Optical Fields automatically displays the number of already analyzed fields.

**Somatic Cells Count /One Optical Field** automatically displays the average number of somatic cells counted in one optical field.

**Somatic Cells Count/ 1 cu. cm** – In this box you see the result of the count, i.e. the number of somatic cells per 1 cu. cm.

The selection of the number of optical fields to be tested is done by the center right panel "Number of fields to be analyzed".

After you have selected the number of analyzed fields you need to select a method of scanning.

This can be done by the top center panel.

Six buttons present the six adopted methods for counting somatic cells.

Press the button featuring the method preferred by you.

The scanning and counting process starts.

In the top right panel the direction and method of scanning are displayed and in the center right panel - the temporary and final results of the test. The result will be automatically saved. After some comment adding, press button "Save".

By the "Print' button on the same panel you can print out the test results.

With each scanning method the first 10 optical fields are automatically saved.

## 4.3.2. Demo operation mode

It is possible to illustrate the process of scanning and counting the somatic cells. By switching the DEMO button on the top center panel the Demonstration mode of operation of the somatic counter is activated.

In the same panel you can adjust the scanning speed.

This operation mode is for illustration of the process and it slows down the speed of somatic cells count up to 10 times.

#### 4.4. Settings

From the main menu select the button <Settings>.

It is used to set the company data, format of the video capture, to select a microscope step and set the microscope factor.

All settings are single and are made after installation of the system and starting to work with the program.

#### 4.4.1. Company information

Write the your company data.

#### 4.4.2. Setting the video capture

This option requires password. The password is 20040727.

Select the type of Capture sources Video – Aver Media.

Cancel the ticks of Want Audio and Want Audio Preview.

Select for BitmapPixel Format - pf24bit.

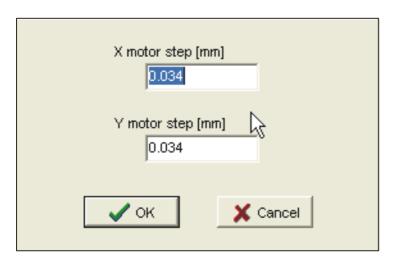
| <ul><li>✓ WantPreview</li><li>✓ WantCapture</li><li>✓ WantBitmaps</li></ul>  | Capture Sources  Video AVerMedia, AVerTV WDM ✓  Audio | OK<br>Cancel |  |  |  |
|--|---|--------------|--|--|--|
| <ul> <li>WantAudio</li> <li>WantDVAudio</li> <li>WantAudioPreview</li> <li>PreallocCaptureFile</li> <li>UseTempFile</li> </ul> | Compressors  Video  Audio                             |              |  |  |  |
| DV Resolution dont worry ▼ BitmapPixelFormat pf24bit ▼  Prealloc file size 1 → Mb  |   |              |  |  |  |
| CaptureFileName<br>TempCaptureFileName   | capture.avi 🚅   |              |  |  |  |

## 4.4.3. Setting the step of the microscope

This option requires password. The password is 20040727.

Enter the step along X and Y at which the microscope has to work.

The commonly used step along X= 0.034 mm and along Y=0.034 mm.



## 4.4.4. Setting the microscope factor

This option requires password. The password is 20040727.

The microscope factor is the number of optical fields in one film.

It is calculated on the basis of the size of the whole film and the size of one optical field. The calculation is automatic.

This is used to calculate the total number of somatic cells in 1 cu. cm.

That value is readily displayed in the box Somatic cells counter.

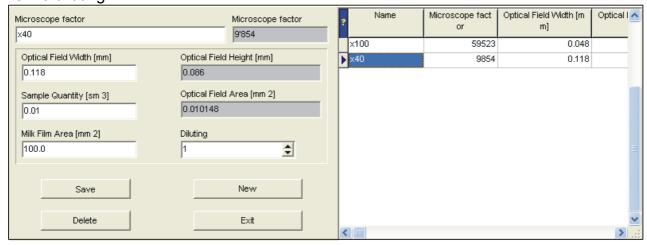
Enter the name of the microscope factor.

The given values have been measured by means of a object micrometer at 1/40 lens.

Optical Field Width – the width of one optical field, mm Sample Quantity – quantity of the sample, cu. cm Milk Film Area – area of sample film, sq. mm Diluting – rate of diluting

= 0.118 mm = 0.01 cu. cm =100 sq. mm =1

i.e. no diluting



These values must be changed only if the microscope lens is changed, i.e. lens with different magnification is used or the milk sample is diluted before making the film.

If you keep the same 1/40 lens but dilute the sample, you have to change the dilution rate. Dilution 1:1, Diluting = 2.

No dilution, Diluting = 1.

If you keep the sample dilution =1, but change the lens, it is necessary to change the size of the optical field by using the object micrometer.

Put the object micrometer on the slide place and with the immersion lens determine the size of one optical field having in mind that each mark on the object micrometer is equal to 10 microns.

The setting of the microscope factor is single only with the initial commencement of operation with the Somatic cells counter system.

## 5. Guarantee

Guarantee is one full year. Guarantee is void if warranty labels are removed. Under no circumstance you should try to repair Ekoscope yourself, as this will invalidate the guarantee. The guarantee conditions for device are as defined by our representative in the country of sale.

Serial N: E060222018

Date: 20.02.2006