

CASY[®]

Cell Counter + Analyser System

Model TT

Operator Manual

innovatis AG CASY[®]-Technology

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2. Introduction

CASYS[®]-Technology is a German development based on extensive experience gained in analytical working methods in the fields of cytobiology, microbiology, biochemistry and physics. Its ergonomic and easy operation were developed specifically for day-to-day, routine use. Standardized cell counting methods, viability checks, aggregation correction, volumetric measurement with high measuring range dynamics all make the CASYS[®] an indispensable tool for the qualitative and quantitative assessment of cell cultures.

2.1. The CASYS[®] measuring principle

CASYS[®]-Technology of Schärfe System combines a proven particle measurement technique, referred to as the *Resistance Measurement Principle*, with a modern method of signal evaluation, *Pulse area analysis*.

Pulse area analysis was developed in 1987 by Schärfe System on the basis of Digital Pulse Processing (DPP), a process employed for the digital recording of electrical signals for more than 40 years. For cell analysis, DPP is combined with a high scanning rate and a highly-standardized method of analysing cell signals. This combination facilitates unprecedented measuring range dynamics and guarantees maximum resolution and excellent reproducibility for all measuring parameters.

Measurement is performed by suspending the cells in CASYS[®]ton, an electrolyte developed specifically for cell counting and aspirating them through a precision measuring pore of defined geometry at a constant flow speed. The precision measuring pore is designed as a hole within an artificial precious stone, which is cast inside the capillary body. During the measurement process, a pulsed low voltage field with 1 MHz is applied to the measuring pore via two platinum electrodes. The electrolyte-filled measuring pore represents a defined electrical resistance. During their passage through the measuring pore, the cells displace a quantity of electrolyte corresponding to their volume. Since intact cells can generally be considered isolators, an increased level of resistance is achieved over the measuring pore. This resistance is a dimension for the volume of the cells. By contrast, dead cells whose membrane no longer acts as an electrical barrier, are recorded by the size of their cell nucleus. It is important that the cells are passing through the measuring pore individually.

The measuring signal is scanned by CASYS[®] at high frequency. As well as the amplitude of the measuring signal, CASYS[®] also records the shape of the signal as a whole. CASYS[®] calculates the integral of the measuring signal from the individual measurements. The calculated signal areas are evaluated by a standardized method and accumulated in a multi-channel analyser (pulse area analysis). A diameter-linear size distribution with a resolution of 400 display channels is calculated from a linear-volume, original distribution with 524,288 measuring channels. This distribution forms the basis for the calculation of all other measuring parameters.

The signal amplifier technology developed specifically for CASY® guarantees excellent measuring range dynamics (> 1 : 70000 in terms of volume and > 1 : 40 in terms of diameter respectively). Measuring range dynamics is described as the ratio of the smallest to the largest particles that can be analysed simultaneously. This guarantees that the entire physical measuring range of the used measuring capillaries from approx. 2% to approx. 80% of the measuring pore diameter is always in view, from very small debris particles to very large cell aggregates.

2.2. CASY® Model TT

CASY® Model TT combines a high degree of ergonomics with functionality. At the push of a button, the electronics control the measuring stand, record the measuring data, evaluate the measuring data via a multi-channel analyser, calculate and display the measuring results on the control panel. All measuring parameters can be set through a minimum of steps. For each measuring capillary, up to 20 settings can be stored for various cell types or various applications and remain available the next time the device is switched on. The user can focus his concentration on his scientific work.

To output the data, a HP-compatible printer (PCL) can be connected directly to the device. The data for display, recording and printout purposes can also be exported to MS-Excel™ (PC) through a fully automated process. This requires CASY®excell, which is available from *innovatis AG*.

3. Device configuration

CASY® Model TT comprises the following functional units:

- Measuring stand consisting of measuring unit, pressure systems and mains power supply (see Figure 1).
- Measuring capillaries (see Figure 1).
- Control panel with graphic display and 13 control keys (see Figure 2).

Measuring unit, pressure system and mains power supply are accommodated in three separate metal cylinders. This guarantees maximum protection for the highly-sensitive measuring electronics against interference and enables the measuring capillaries to be arranged in one large, open sampling chamber.

3.1. Measuring unit

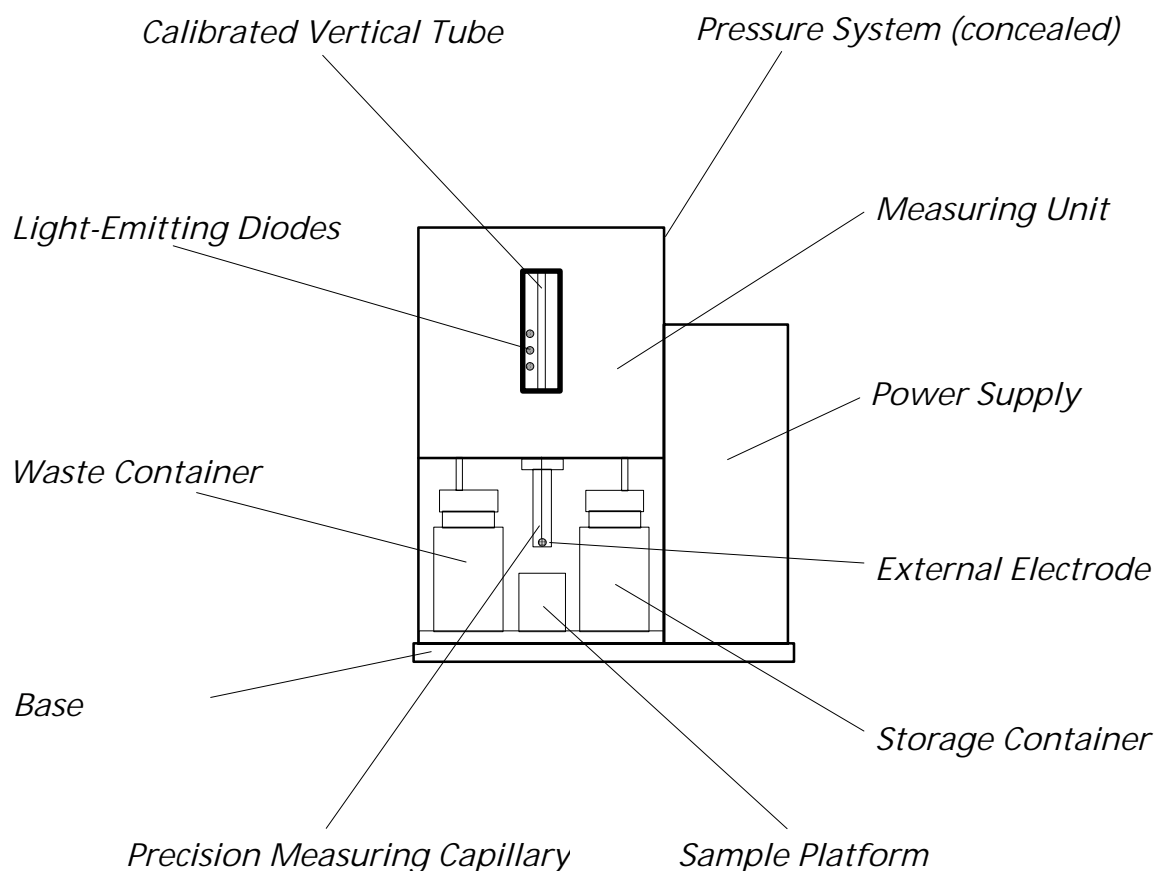
The measuring unit (see Figure 1) contains the measuring electronics, the fluid system and the valves required to control the measuring process. The close monitoring of the measuring volume (200 µl or 400 µl) required to determine the concentration is carried out by a light-barrier monitored, calibrated precision vertical tube. CASY® handles larger measuring volumes (maximum 4 ml) by employing a multiple measurement system. The results of

the individual measurements are accumulated. CASY® operates almost dead-volume free, i.e. the required sample quantity is only slightly larger than the measuring volume. The control and monitoring of the measuring process are designed to exhibit maximum protection against interference. Any faults that do occur are automatically detected by CASY® and notified to the user.

The sampling chamber accommodates the following elements:

- Measuring capillary
- External electrode
- Sample platform
- Waste container (left)
- Storage container (right)

Figure 1: Schematic of CASY® Model TT, measuring stand, frontal view



The measuring unit is controlled by the control panel (see 3.4 and Figure 2). An inspection window, which also enables the vertical tube to be monitored visually, is situated above the sampling chamber. The inspection window accommodates three light-emitting diodes, which indicate the filling level of the vertical tube. The green light-emitting diode indicates the start of a measurement, the lower red light-emitting diode indicates when the 200 µl level is reached and the upper red light-emitting diode indicates when the 400 µl level has been reached.

Before the start of each measurement, the contents of the measuring capillary are emptied into the waste container. The sample volume is then aspirated through the measuring capillary under constant low pressure. The progress of the meniscus can be tracked in the inspection window. The time required to reach the light barriers depends on the diameter of the used measuring capillary and is precisely monitored by CASY®. This process enables potential faults resulting from measuring capillary blockages to be automatically detected. During the measurement process, the vertical tube is also monitored for disturbances caused by air bubbles.

Blockages can be removed by running a clean cycle, which, in addition to refilling the internal fluid system, also actively counter-flushes the measuring pore (see 4.4). The required CASY®-ton is sucked in from the storage container and then emptied into the waste container. The entire volume of the internal fluid system is approx. 2 ml.

3.2. Measuring capillary and external electrode

The *external electrode* (see Figure 1) is from platinum, arranged before the measuring capillary, can rotate freely and be pulled downwards if necessary.

The *measuring capillary* (see Figure 1) is from highly resistance plastic. It is secured to the sampling chamber cover by an cap nut. At the lower end of the capillary body, an artificial precious stone is cast with the precision measuring pore. The measuring pore has a cylindrical geometry with a diameter to length ratio of 1:1. The axial arrangement of the hole minimises the risk of blockage and prevents impurities from accumulating inside the capillary body.

To change the measuring capillary, first carefully rotate the external electrode forwards. Then release the cap nut and remove it with the measuring capillary by pulling downwards. In order to prevent the measuring capillary becoming contaminated with grease and dust, try to avoid touching its lower end, which is immersed in the sample.

To re-assemble, screw in the cap nut manually (do not use a tool)! Ensure that the tube extending inside the measuring capillary is not buckled or pinched during assembly. Then, carefully rotate the external electrode, without bending, and return to its original position. Ensure that the background is monitored before commencing the measurement (see 4.4).

If you have to change the measuring capillaries several times in one day, store the unused ones in particle-free CASY®ton.

Note:

Unused measuring capillaries must always be cleaned thoroughly before being stored. Dried residues in the measuring pore can result in subsequent blockages. This can be prevented by placing the measuring capillaries in CASY®clean and leaving overnight. The following day, flush the measuring capillaries carefully using CASY®clean and then flush repeatedly with distilled water. Blow the fluid from the measuring capillaries and store in a clean place.

Caution:

The measuring capillaries should be cleaned using only the *CASY®clean* agent specified by *innovatis AG*. Other agents may destroy the measuring capillaries. *Innovatis AG* is not liable for any damage caused by using alternative cleaning agents.

3.3. Pressure system and power supply

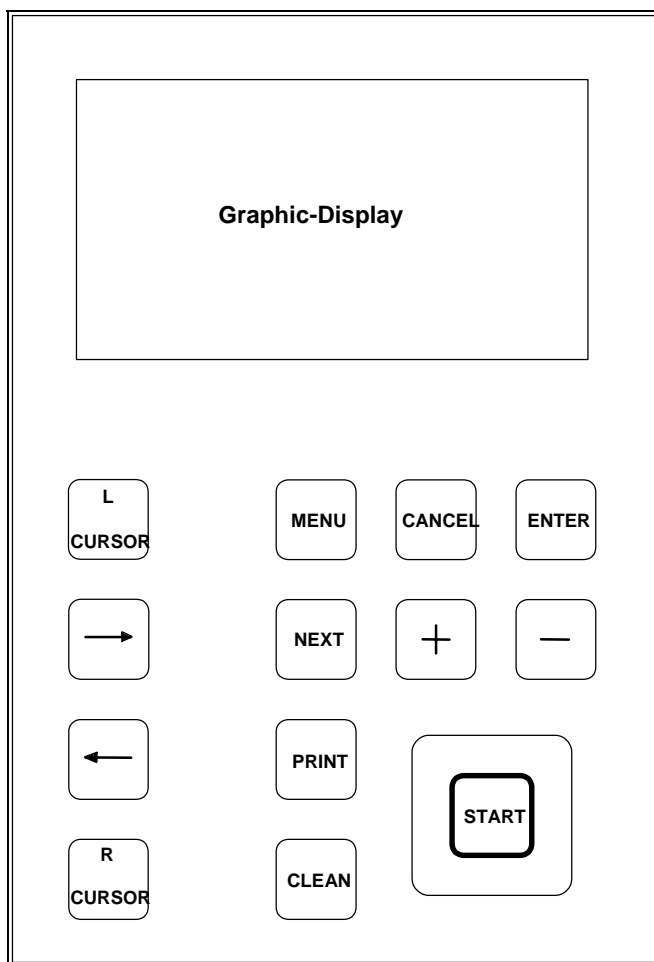
The *pressure system* is accommodated in its own cylinder situated behind the measuring unit (concealed in Figure 1). A no-maintenance vacuum pump, combined with the latest pressure sensors, guarantee that the vacuum required to aspirate the sample remains constant, irrespective of any external pressure. The pressure system also interacts with the valves on the measuring unit to control all fluid movements required to empty the vertical tube, replenish the fluid system and counter-flush the measuring capillary.

The *power supply* for the CASY® measuring electronics is housed in the cylinder arranged outside to the right (see Figure 1). Special filters ensure that the mains power supply is afforded maximum interference protection.

3.4. Control panel

The control panel (see Figure 2) consists of a graphic display and a membrane keyboard exhibiting 13 keys. In measuring mode, the graphic display shows the size distribution and indicates the measuring results. All measuring and output parameters can be displayed and adjusted through the menu control.

The keyboard is divided into three functional areas. The top right-hand area accommodates the keys used to enter the measuring and output parameters. The bottom right-hand area accommodates the keys used to control the measuring unit and output the results to the printer. On the left are the keys used to position the evaluation and the normalization cursors. All keys have a clearly defined pressure point.



Definition of key functions:

MENU:	Starts the menu control.
CANCEL:	Cancels a measurement or entry.
ENTER:	Confirms an entry.
NEXT:	Switches from graphic to numerical display. Continue to next number. Switches to set the second cursor pair.
+ :	Increases number / change selection.
- :	Decreases number / change selection.
PRINT:	Output to printer and/or serial interface (CASY®excell).
CLEAN:	Starts one or several cleaning cycles.
START:	Starts a measurement.
L-CURSOR:	Positions left cursor
→ :	Shifts cursor to the right.
← :	Shifts cursor to the left.
R-CURSOR:	Positions right cursor

Figure 2: CASY® Model TT, Control Panel

4. Start-up

4.1. Choosing the location

CASY® Model TT is a precision measuring instrument. To ensure fault-free measuring operation, you must choose the location according to the following criteria:

- Place the instrument on a solid, dry work table that is exposed to neither strong mechanical vibrations nor high-volume noises (e.g. ultrasonic baths).
- Choose the installation site so that CASY® is protected from mechanical damage and water and solvent spillage.
- Choose a room that is as smoke and dust-free as possible and that has a constant room temperature of between 15°C and 32°C
- Do not place CASY® in the vicinity of devices that generate strong electrostatic or electromagnetic fields. This includes specifically computer monitors that work with high-voltage picture tubes, brush motors, flickering fluorescent lamps, arcing contacts, water baths, gas chromatographs etc. In the event of any alternative site being unavailable, maintain the greatest possible distance from such devices.

4.2. Cabling

Connect your CASY® to an interference-free measuring instrument socket. Under no circumstances should you operate CASY® from the same socket as laboratory equipment that works with high switching loads (autoclaves, centrifuges, agitators etc.). You must also ensure that all laboratory equipment situated in the same vicinity is interference-free/fault-free. The following connection cables and/or control elements must be connected.

- Data line for control panel (9-pin sub-D plug).
- Mains power cable.
- Printer, if available (25-pin sub-D plug).
- Serial transfer cable for PC connection (CASY®excell), if available.

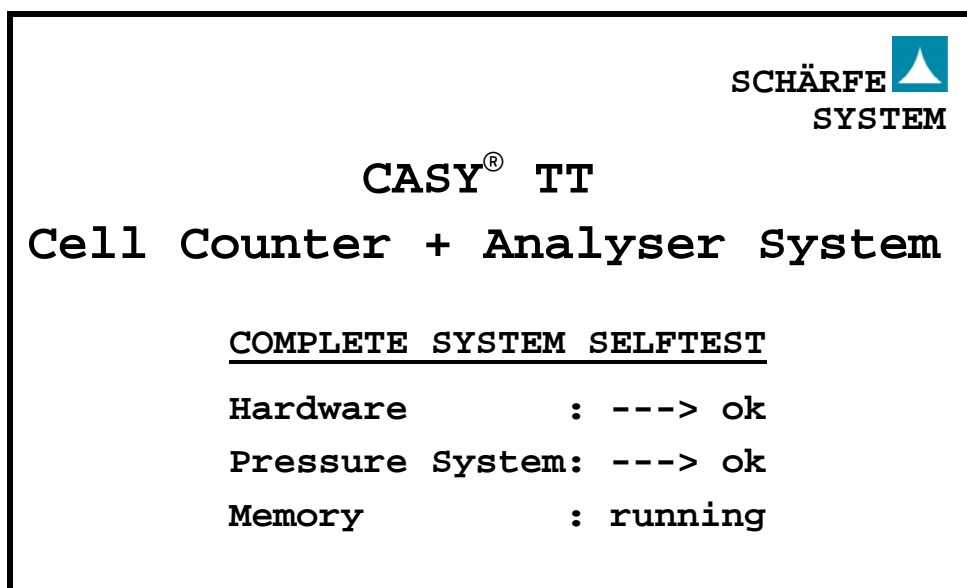
All connections are clearly labeled to prevent confusion.

Caution:

Before switching on for the first time, ensure that the mains voltage set on the *voltage selector* on the rear of the device is consistent with your mains power supply. If it is necessary to convert the mains power supply, please observe the information on the primary fuses (see 8.3). *Innovatis AG* accepts no liability for damage caused by operating the device from an inappropriate power supply.

4.3. Switch-on

Fitted to the rear panel of CASY® is a green power switch, which when turned to ON, illuminates and indicates that the device is live. After being switched on, CASY® automatically performs all the required initialisation procedures. Specifically, the system is subjected to a complete self-test during which the hardware, pressure system and all memory modules (program, calibration, setups) are checked. The following screen appears when the system is powered up:



When the initialisation procedure is complete, your CASY® Model TT will display an empty size distribution screen. The previously selected measuring capillary and previously used setup is loaded automatically.

Caution:

Before switching on your CASY®, ensure that the control panel is connected and properly bolted to the measuring stand. Removing the plug from the control panel when the measuring unit is switched to ON may result in damage to the graphic display electronics. *Innovatis AG* accepts no liability for damage caused by severing a live connection between the measuring unit and the control panel.

4.4. Preparing for measurement

CASY® is factory-calibrated for common cell culture media. Preparing for measurement is therefore limited to the following steps:

- Replenishing the Storage container with clean CASY®ton.
- Emptying the waste container.
- Fitting (see 3.2) and selecting (see 6.5) the required measuring capillary where necessary.
- Selecting an appropriate setup (see 6.3) or manually inputting the parameters (see 6.4)
- Monitoring the background.

You must monitor the background of your CASY® at regular intervals. Problems resulting from choosing an unsuitable location and also those relating to impurities in the system manifest themselves in too high a background.

Background monitoring

1. Place a CASY®cup containing CASY®ton below the measuring capillary.
2. Press and hold the *CLEAN* key down until the clean counter indicates 3. Releasing the key triggers three clean cycles.
3. Place a new CASY®cup containing CASY®ton below the measuring capillary.
4. Press the *START* key to initiate a measurement.

Note:

To guarantee that the CASY®cup is fixed, you can rotate the sample platform one turn so that the recess is no longer pointing forwards. This secures the cup.

Depending on the measuring capillary used, the background should not exceed the following values:

150 µm measuring capillary: < 100 Counts/ml (typically 10 - 50)

60 µm measuring capillary: < 200 Counts/ml (typically 20 -100)

45 µm measuring capillary: See instructions on how to use the 45 µm measuring capillary.

Too high a background may result from impurities in your CASY®ton or not having used a new CASY®cup. Filter the CASY®ton through a sterile filter with a pore size of $\leq 0,8 \mu\text{m}$ and use a new CASY®cup for every measurement. Repeat steps 1 to 4 with freshly filtered CASY®ton. If the system fails to become clean even after several attempts, you should carry out the *Weekly Cleaning* routine (see 5.1). If this exercise fails to produce the desired result, please check that the location of your CASY® satisfies the criteria indicated in 4.1.

Caution:

You must ensure that all the vessels used to prepare the samples, the CASY®ton used to flush the system and dilute the samples and also any CASY®cups used are *free of grease and particle impurities*. For routine preparation of the measuring samples, use a dispenser (10 ml) with integral filter ($\leq 0,8 \mu\text{m}$) wherever possible.

To avoid incorrect measurements, CASY®cups should be used once only.

Instructions on how to use the 45 μm measuring capillary:

The 45 μm measuring capillary has been optimised for microbiological applications. A special process for signal detection is used to ensure that very small cells near the system's physically determined lower measuring limit are reliably recorded. This process works reliably from a *minimum concentration of 20,000 cells/ml* upwards. The background displayed when running empty with filtered, de-gassed CASY®ton (up to 3000 counts/ml) is of no consequence for measurements above the minimum concentration.

Your CASY®ton *must be de-gassed on a daily basis* for measurements using the 45 μm measuring capillary. Owing to the higher capillary resistance and the higher measuring vacuum, failure to de-gas may lead to false measuring results due to gas bubbles being present in the capillary. To de-gas the CASY®ton, you will need an ultrasonic bath and a vacuum pump (suction cylinder with water jet pump). De-gas for at least 10 minutes to guarantee fault-free measuring operation.

During the measurement process, you must use the protection shield provided. The protection shield guarantees that the very small measuring signals recorded by the 45 μm capillary cannot be changed by external interference. The protection shield is connected to the right or the left ground socket in the roof of the sampling chamber by the corresponding cable. Before a measurement is started, insert the protection shield into the sampling chamber up to the limit stop, so that the sample is shielded on all sides.

5. Caring for your CASY®

Your CASY® is designed to be low-maintenance; the fluid system however requires regular attention to guarantee fault-free work over several years. Remember that you are working with biological material. Residues in the fluid system or growth of bacteria, algae or yeast cells may lead to blockages in the measuring pore, too high a background or other faults during operation. To avoid such errors, you must follow the cleaning procedure described below at least once per week. The recommended agent, *CASY®clean*, is available from *innovatis AG*.

Caution:

The system should be cleaned using only the *CASY® clean* agent specified by *innovatis AG*. Never use aggressive cleaning fluids on *CASY®*. *Innovatis AG* accepts no liability for damage caused by using other cleaning agents!

5.1. Weekly cleaning routine

1. Fill two *CASY®* cups with *CASY® clean*.
2. Place one *CASY®* cup under the measuring capillary.
3. Replace the storage container holding *CASY® ton* (right-hand cylinder) with the second *CASY®* cup.
4. Run three cleaning cycles (hold the *CLEAN* key down and select 3 cycles).
5. Perform a triple measurement using a measuring volume of 400 µl (*START*).
6. Leave your *CASY®* for at least 3 hours in *CASY® clean*.
7. Replace the cleaning solution with two *CASY®* cups containing *distilled water*.
8. Run three cleaning cycles (hold the *CLEAN* key down and select 3 cycles).
9. Perform a triple measurement using a measuring volume of 400 µl (*START*).
10. Repeat steps 7 to 9 to remove the cleaning solution completely.
11. Repeat steps 7 to 9 using *CASY® ton*.
12. Your *CASY®* is now operational again.

Duty of care:

Never leave samples under the measuring capillary! On completion of a series of measurements, place a *CASY®* cup containing fresh *CASY® ton* under the measuring capillary and run at least 3 clean cycles (hold the *CLEAN* key down and select 3 cycles).

Before switching off, always monitor the device's background (see 4.4). Run a measurement using fresh *CASY® ton*.

5.2. Cleaning the cylinders and the sampling chamber

Clean the surface of your *CASY®* regularly using a damp micro-fibre cloth. It is important to remove salt solution spillages immediately. To do this, raise the sample platform and remove by pulling forwards. Methylated spirit can be used to remove organic impurities. Under no circumstances, however, may other organic solvents be used.

5.3. How to set CASY® out of operation

If your CASY® has not to be operated for several weeks, the fluid system should be drained. Before shutting down, the fluid system must be cleaned (see 5.1, Weekly Cleaning Routine). Follow this procedure to shut-down the system:

1. Carry out steps 1 to 10 of the *Weekly cleaning routine*.
2. Replace the two CASY® cups containing distilled water with two empty CASY® cups.
3. Select the *Service Menu* option from the main menu.
4. Execute the *Dry Liquid System* command from the *Service Menu*.
5. Empty the waste container and the two CASY® cups.
6. You can now switch off your system.
7. Ensure that the system is adequately protected from dust whilst it is shut down.
8. You should begin the *Start-up* procedure with the *Weekly cleaning routine*.

Caution:

You must ensure that when the CASY® fluid system contains a saline solution, it is not allowed to dry out. This will prevent malfunction or damage to the fluid system and valves caused by salt crystals.

6. Operation of CASY® Model TT

6.1. General

CASY® is operated using 13 control keys. Operation is divided into three distinct areas, each of which reflects the spatial arrangement of the keys (see Figure 2.).

- Entering parameters
Upper right-hand keys: *MENU, CANCEL, ENTER, NEXT, +, -*
- Measurement operation and output of results
Lower right-hand keys: *START, PRINT, CLEAN.*
- Evaluation of the size distribution with various cursor positions
Left-hand keys: *L-CURSOR, →, ←, R-CURSOR.*

Because up to 20 device settings can be permanently stored for each measuring capillary, parameter setting in most cases can be done by selecting an appropriate setup. Each time CASY® is switched on, the previously used setup and the previously used measuring capillary are loaded. All other setups are readily available through the

menu function. In addition, the measuring and output parameters can be modified to match the actual measurement and do not have to be stored in one of the setups.

6.2. Menu Control - MAIN MENU

All CASY® settings are selected with the *MENU* key. Pressing *MENU* displays the *MAIN MENU*.

```
MAIN MENU                TT-2AB-9999    15.04.2002  10:15
-----
*   Select Setup
    Edit Setup
    Select Capillary
    Set Date and Time
    System Settings
    Service Menu
-----
USE MENU TO SELECT AND PRESS ENTER TO OPEN.
USE CANCEL TO LEAVE SCREEN.
```

All menu control screens have the same structure. The name of the screen in upper case letters is contained in the left part of the header, which is separated by a horizontal line: *MAIN MENU*. Displayed to its right are the serial number of your CASY®, the date and time. The lower border, also separated by a horizontal line, contains instructions on how the screen and individual keys operate.

Use the *MENU* key to select individual menu items. Pressing *ENTER* opens a menu item. Pressing *CANCEL* closes the *MAIN MENU*. If you overshoot the item you wish to select, you can move backwards by pressing and holding down the *MENU* key.

Irrespective of which menu control screen you are in, pressing *CANCEL* repeatedly returns you to the *MAIN MENU* and then to the size distribution display.

6.3. MAIN MENU - Select Setup

The *Select Setup* option opens the *SETUP SELECTION* screen. This screen enables you to select one of 20 permanently stored setups for measuring capillary currently in use.

```

SETUP SELECTION      TT-2AB-9999   15.04.2002  10:15
-----
                Capillary: 150 µm

Setup No:   00 01 02 03 04 05 06 07 08 09
                *
                10 11 12 13 14 15 16 17 18 19
-----
USE NEXT TO SELECT SETUP AND PRESS ENTER TO LOAD.
USE CANCEL TO LEAVE SCREEN.

```

The setups are numbered from 00 to 19. Each setup stores a complete set of parameters for the selected measuring capillary. The setups already in use to store parameters are tagged with ★. If no setup is logged, the system works with the factory-default set of parameters.

Pressing the *NEXT* key selects a setup. You may select only those setups that have been logged and tagged with ★. Pressing and holding down the *NEXT* key enables you to move the selector mark backwards. Pressing *ENTER* displays the selected setup.

Further details on the setup screen can be found under *Edit Setup* (see 6.4). Check whether the selected setup is matching your needs. Beside the measuring and evaluation parameters, each setup has a distinct name which you can define and which is displayed in the top right-hand corner of the setup screen.

Press *ENTER* again to load the setup displayed. If you do not wish to load the setup displayed, you can return to Setup Selection by pressing *CANCEL*.

Note:

The setup parameters cannot be changed at this point. To edit a loaded setup, use the *Edit Setup* menu item described below (see 6.4).

6.4. MAIN MENU - Edit Setup

Edit Setup opens the setup screen. All the parameters provided by CASY® to define the measuring conditions, display the size distribution, calculate and output the measuring results, can be edited in this screen. The active parameters are always displayed. Any setup you wish to edit must first be loaded through *Select Setup* (see 6.3).

```

SETUP NUMBER: 01      NAME: Chondrozyt
-----
Capillary   : 150 µm      X-Axis : 50 µm
Sample Vol  : 400 µl      Cycles : 2
-----
Dilution   : 1.000e+02
Y-Axis      : Auto
Eval.Cursor : 11.20 - 50.00 µm
Norm.Cursor : 6.80 - 50.00 µm
%Calculation: %Via      Debris : On
Aggr.Correct: Auto      P.Vol: 0.000e+00 fl
Interface   : Par       P.Feed : On
Print-Mode  : Manual    Graphic: On
-----
USE MENU AND NEXT TO STEP.  USE +/- TO EDIT.
USE CANCEL TO LEAVE SCREEN OR ENTER TO CONFIRM CHANGES.

```

The setup screen is divided into three areas. The header, separated by a horizontal line, contains the *SETUP NUMBER* (00-19) and the *NAME* (setup name) you have defined. If the active parameters do not correspond to any of the stored setups, two forward slashes // are shown in place of the setup name and *NOT SAVED* in place of the setup name.

The block below, separated by another horizontal line, contains all the *measuring parameters*. These parameters must be defined before a measurement takes place. They may not be retrospectively changed.

The main block below contains all *evaluation parameters* and also the *output parameters*, which define the settings for outputting via a directly connected printer or via the serial interface. The parameters can be modified following a measurement.

When a setup screen is opened, the input marker is located initially on the *Dilution* input field. The current input position is indicated by a flashing, horizontal line below the characters which can currently be changed. From this point, you can use the *MENU* key to move forwards or backwards between the input fields. To move forwards, press the *MENU* key briefly. The input marker skips forward to the next available input position. To move

backwards, keep the *MENU* key pressed until the input marker reaches the position you require. Both methods move you to the start or the end of the screen when the last or the first parameter respectively is reached.

When inputting numbers, you can move forwards from number to number using the *NEXT* key. To increase or decrease a number selected using *NEXT*, use the *+* and *-* keys.

For all other parameters, the *+* and *-* keys can be used to select various pre-defined values. This also applies for the individual characters of the setup name.

NAME (Setup name)

Each setup can be assigned a specified name. Each name can be up to 10 characters long (letters and numbers). The setup name is output with the numerical measuring results both on the display and on the printout.

Measuring parameters

The measuring parameters must be set before the measurement takes place. They cannot be modified once the measurement has been carried out. To change the measuring parameters, the sample must always be subjected to a second measurement!

Capillary: The capillary belonging to the processed setup is displayed here for information only. You cannot change its value. To select a different capillary, you must use the *Select Capillary* command in the *Main Menu*.

X-axis: The range of the size distribution to be shown in the graphic display can be set in increments of 5 μm or 10 μm . An additional range of 0 - 3 μm is available for the 45 μm measuring capillary. All scales start at 0 μm . This guarantees optimum comparability of the measuring results.

Sample Vol: Enter here the measuring volume (200 μl or 400 μl). The set measuring volume is automatically considered as the cell concentration is calculated. For all measuring capillaries with a pore size \geq 100, you should in principle use the 400 μl setting.

Cycles: Enter here the number of repeat measurements (1-10). The individual measurements are automatically accumulated. The maximum measuring volume is 4.0 ml (10 x 400 μl). The set measuring cycles are automatically considered as the cell concentration is calculated.

Evaluation parameters

Dilution: The dilution factor is used to calculate the cell concentration in the original sample. It is entered in exponential format (e.g. for dilution 200, enter: 2.000e+02). Pressing *NEXT* enables the input marker to move from number to number. Holding this key down causes the input marker to move backwards.

Y-axis: The *Auto* setting automatically modifies the Y-axis scale according to the distribution maximum. The Y-axis scale can also be set in predefined steps between 10 and 7500. This enables the scale to be manually modified to the level of the distribution maximum. It is advisable to modify manually, if the peak of interest within the size distribution is not the maximum.

Eval.Cursor: The right and left-hand evaluation cursors (shown in the size distribution as a continuous line) determine the size range to be used to calculate the measuring result. For all percentage calculations (% viability, % counts or % volume), the evaluation cursors define the size range for which the percentage of the standard range (see *Norm.Cursor*) is calculated. The **+** and **-** keys can be used to position the evaluation cursor. Pressing *NEXT* enables you to shift the input marker between the numbers before and after the decimal points. If the marker is positioned before the decimal point, **+** and **-** causes a change by 10 measuring channels, if it is positioned after the decimal point, the keys cause a change by one measuring channel. One measuring channel corresponds to 1/400 of the scale range selected under the X-axis (see measuring parameter). In order to modify the evaluation cursor whilst visually monitoring the size distribution, you may also use the control panel arrow keys (see 7.6).

Norm.Cursor: The right and left-hand normalization cursors (shown in the size distribution as a dotted line) determine the size range for which all percentage calculations indicate 100% (% viability, % counts or % volume). Parameters *Total/ml*, *TotCnt/ml* and *TotVol/ml* are also calculated for this range. In the % viability setting, the left-hand normalization cursor also defines the size range for determining *Debris/ml*. The procedure for setting the normalization cursor is the same as that described for the evaluation cursor. The normalization cursor can also be modified using the control panel arrow keys whilst visually monitoring the size distribution.

%Calculation: In addition to the *Off* setting (percentage calculation disabled), three other percentage calculation settings (*%Via*, *%Cnt* and *%Vol*) are available.

%Via: This setting calculates the viability of a cell sample in percentage terms. It is assumed that the evaluation cursor is set to the viable cell size range and the normalization cursor covers the size range of all cells (dead + viable).

%Cnt: This setting calculates the percentage counts in the size range of the evaluation cursor in relation to the size range of the normalization cursor, which is set to 100%.

%Vol: This setting calculates the percentage bio volumes in the size range of the evaluation cursor in relation to the size range of the normalization cursor, which is set to 100%.

Debris: This parameter can be either *On* or *Off*. It can be set to *On* only if parameter *%Via* is selected from *%Calculation*. When set to *On*, all counts left of the normalization cursor are summed up and output as debris/ml taking into account measuring volume and dilution factor.

Aggr.Correct: This parameter defines whether and how the *aggregation correction* of your count results should be performed. *Off*, *Manual* and *Auto* are the available settings. In the *Auto* setting, the aggregation factor of a sample is automatically calculated as the quotient of mean volume and peak volume. The calculation is based on the following assumptions:

- a) The evaluation cursors are set to the viable cell size range, including all aggregates.
- b) The size distribution of the viable cells exhibits a clear maximum.
- c) The measurement is supported by adequate statistical reliability (> 1000 count results in the viable cell range).

If your samples do not match Item b) and/or c), you can specify the volume of the individual cells manually. To do this, you must select the manual setting and enter the mean individual volume of your sample using parameter *P.Vol* (see below). This setting is always recommended if the volume of your cells does not change significantly during the course of an experiment. The aggregate correction facility is normally used in conjunction with the *%Via* percentage calculation.

P.Vol: This parameter defines the mean volume of individual cells in your samples for the manual aggregation correction (see *Aggr.Correct*) in terms of femtolitres. The value is entered in exponential format.

Output parameters

Interface: You can specify through the interface, whether after pressing *PRINT*, the data should be output through the parallel output – *Par*, through the serial output – *Ser* or through both simultaneously – *P + S*. The *P + S* setting enables you print the data, whilst simultaneously transferring it to a connected computer. The connected printer must be PCL capable. Hewlett-Packard printers and all compatible products satisfy this requirement. To export data to a PC, *innovatis AG* offers *CASY® excell*, a program which exports and displays your measuring results directly to MS-Excel™. More precise details on the serial transfer of data can be found in the Appendix (see 8.2).

P.Feed: This parameter can be either *On* and *Off*. When set to *On*, a page is output to the printer after each print command. When set to *Off*, the first page is sent to print only when full. For outputting numerical results (*Graphic* to *Off*) and printing setups, this setting allows up to three printouts to fit onto one page. You can print the page at any time in advance with the *Page Feed* command on your printer. When the size distribution is printed (*Graphic* to *On*), *P.Feed* is ignored.

Print Mode: The print mode can be set to either *Manual* or *Auto*. When set to *Auto*, the data is automatically output after every measurement. When set to *Manual*, you must press *PRINT* after a measurement to output the data.

Graphic: This setting determines whether the size distribution is also to be printed as data is output to the printer. The only possible settings are *On* and *Off*.

You can abort parameter input at any time by pressing either *ENTER* or *CANCEL*. If you press *CANCEL*, your entries are discarded and the previous settings are restored. Pressing *PRINT* will printout all setup parameters for reference purposes. Pressing *ENTER* gives you the option of saving your new settings in one of the 20 setups.

Note:

A warning message will appear if a measurement is shown in the display and you have changed one of the measuring parameters *X-axis*, *Sample Vol* or *Cycles*. You will be reminded that the current measuring result may be lost if you confirm the message with *ENTER*. At this point, you can press *CANCEL* to return to the setup screen. The changes you have made to the measuring parameters will then be reset.

```
SAVING SETUP          TT-2AB-9999   15.04.2002  10:15
-----
                Capillary: 150 µm
Setup No:   00 01 02 03 04 05 06 07 08 09
            *
            10 11 12 13 14 15 16 17 18 19
-----
USE NEXT TO SELECT SETUP AND PRESS ENTER TO SAVE.
USE CANCEL TO WORK WITH NEW PARAMETERS WITHOUT SAVING.
```

You must save your entries if you want the settings to be available next time you switch on CASY®. To do this, press *NEXT* to select one of the 20 setups and then press *ENTER*. The setup currently loaded will be offered as a default setting. You may select both setups that have already been used and tagged with ★ and also unused setups. If you select a setup tagged with ★, its stored settings will be overwritten with your new settings. If you select an unused setup, it will appear tagged with ★ and will be available for selection next time *Select Setup* is opened.

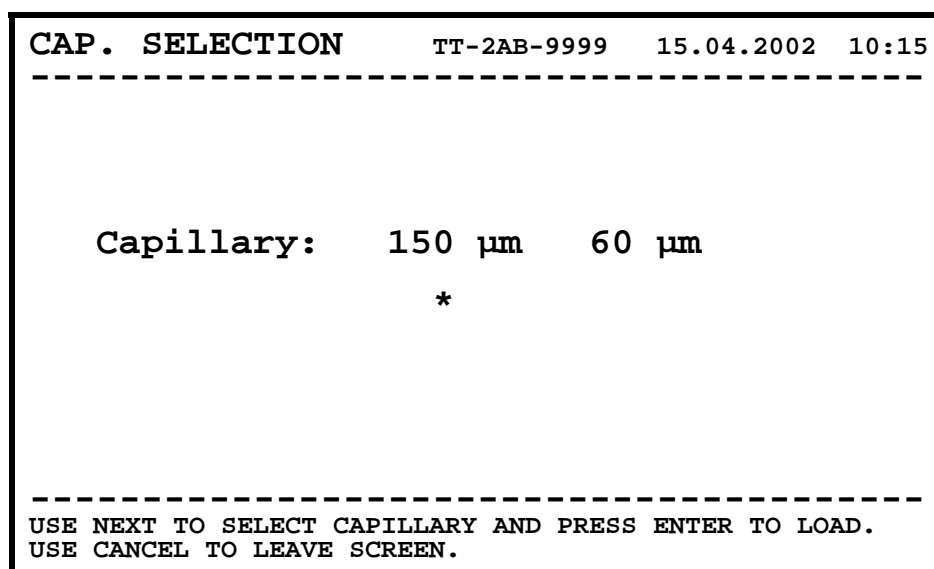
If you do not wish to save your settings, press *CANCEL*. The new settings are used until you select an alternative setup or switch off your CASY®. They will then no longer be available. As a setup name for unsaved settings, *NOT SAVED* appears and the setup number is replaced by two forward slashes //.

Note:

If you try to overwrite a logged setup, a warning message will appear. You will be reminded that pressing *ENTER* will permanently delete this setup's existing settings. Pressing *CANCEL* enables you to return to the *Saving Setup* screen in order to select a free setup for saving.

6.5. MAIN MENU - Select Capillary

The *Select Capillary* option opens the *CAP SELECTION* screen that enables you to switch the system to an alternative capillary size. Depending on how your system is configured, between one and three entries are available for different measuring capillaries.



Pressing *NEXT* enables you to switch between the measuring capillary sizes available on your system. The selected measuring capillaries are tagged with ★. Press *ENTER* to load the calibration for the selected measuring capillaries. The setup previously used with this measuring capillary is activated. Please note that previously displayed measuring data are lost when a capillary setting is changed. Pressing *CANCEL* enables you to exit the screen without changing the settings.

6.6. MAIN MENU - Set Date and Time

Your CASY® has an integral Real-Time-Clock. The *Set Date and Time* function opens the *DATE AND TIME* screen, in which you can set date and time. You have the option of changing the date display from EU format "DD.MM.YYYY" to US format "MM/DD/YYYY".

Use the *MENU* key to move between settings and press *NEXT* to skip to the individual figures used in the date and time. Use the **+** and **-** keys to change the settings. Press *ENTER* to confirm your settings or *CANCEL* to exit the screen without accepting any changes.

```

DATE AND TIME      TT-2AB-9999  15.04.2002  10:15
-----
Date Format: dd.mm.yyyy

Date:              15.04.2002

Time:              10:15:00

-----
USE MENU AND NEXT TO STEP.  USE +/- TO EDIT.
USE CANCEL TO LEAVE SCREEN OR ENTER TO CONFIRM CHANGES.

```

6.7. MAIN MENU - System Settings

System Settings contains settings relevant to the entire system. The *Graph Parameter* function allows you to specify up to four measuring parameters that you wish to be displayed in the upper section of the size distribution screen. The *Used Setups for Capillary* function allows you to delete setups not needed anymore.

```

SYSTEM SETTINGS    TT-2AB-9999  15.04.2002  10:15
-----
1. Graph Parameter: Conc. Range      CON
2. Graph Parameter: Aggregation      AGG
3. Graph Parameter: Viab.Cells/ml    LML
4. Graph Parameter: %Viability       VIA

Used Setups for Capillary 150 µm:
Setup No:  00 01 02 03 04 05 06 07 08 09
           *  *  *           *
           10 11 12 13 14 15 16 17 18 19
                   *           *
-----
USE MENU AND NEXT TO STEP.  USE +/- TO EDIT.
USE CANCEL TO LEAVE SCREEN OR ENTER TO CONFIRM CHANGES.

```

All measuring results, date and time and the used setup can be selected as *Graph Parameters*. Each parameter has its own abbreviation consisting of up to three characters, which is used to display the relevant value in the size distribution screen. The following values are available:

<i>Bezeichner</i>	<i>Abkürzung</i>	<i>Bezeichner</i>	<i>Abkürzung</i>	<i>Bezeichner</i>	<i>Abkürzung</i>
not used	---	Viab.Cells/ml	LML	Peak Diameter	PDI
Conc. Range	CON	%Viability	VIA	Mean Volume	MVL
Counts	CNT	Debris/ml	DML	Peak Volume	PVL
Counts >	C>	Volume/ml	VML	Date	DAT
Aggregation	AGG	%Volume	%V	Time	TIM
Counts/ml	CML	Total/ml	TML	Setup	SET
%Counts	%C	Mean Diameter	MDI		

You can step to the settings of the 4 parameters by pressing *MENU*. The required parameters are selected from the predefined values as indicated in the table using the + and – keys.

If *not used* is selected, the corresponding position in the upper section of the size distribution screen remains empty.

To delete redundant setups, use the *MENU* key to set the input marker in the area called *Used Setups for Capillary....* You can only delete setups for the measuring capillary currently selected using *Select Capillary*. The diameter of the active measuring capillary is displayed behind *Capillary*. Pressing *NEXT* enables you to shift the input marker between all setups tagged with ★. Hold the *NEXT* key down to move the selection marker backwards. If you wish to delete a setup, press the – key. The setup to be deleted is now tagged with *D* instead of ★. You can cancel your selection at any time by pressing the + key at the relevant position. The *D* is replaced by ★. Follow this procedure to select all the setups you wish to delete.

When you have made all the changes you require, press *ENTER* to accept the new settings. Pressing *CANCEL* enables you to leave the screen and return to the old settings.

Note:

If you have selected certain setups for deletion, a warning message will appear. You will be reminded that pressing *ENTER* will permanently delete these setups tagged for deletion. Pressing *CANCEL* enables you to return to the *SYSTEM SETTINGS* screen to revise your selection. Deleting the currently active setup will also delete the current measuring data.

6.8. MAIN MENU - Service Menu

The *Service Menu* contains the *Dry Liquid System* command you require to shut-down your CASY® properly (see 5.3). It also contains two menu items that enable you to check that the system electronics are functioning correctly.

```
SERVICE MENU          TT-2AB-9999   15.04.2002  10:15
-----
*  Dry Liquid System
   Start Selftest
   Generate Testpattern

-----
USE MENU TO SELECT AND PRESS ENTER TO START.
USE CANCEL TO LEAVE SCREEN.
```

Start Selftest performs the self-test known from System Start (see 4.3). This serves to monitor the hardware, the pressure system and all memory functions in the system (program, calibration, setups). The individual checks are confirmed by displaying *OK*. If an error is detected, the corresponding error message will appear (see 8.1).

The *Generate Testpattern* feature creates a test size distribution and performs a standardized evaluation using specified parameters. You will find a test printout, generated during the final factory check, in your system documentation. This command verifies that the evaluation electronics function properly and all calculations produced by the evaluation software are correct.

Use the *MENU* and *ENTER* keys to select and start the individual commands. Pressing *CANCEL* enables you to exit the Service Menu.

7. Performing and evaluating measurements

Working with your CASY® is very simple. The system is ready to start a measurement as soon as it is switched on. However, please observe the following before you start to measure your samples:

- On initial start-up or after moving the system, check the points cited in Section 4.1 (Choosing the location). And Section 4.2 (Cabling).
- Follow the steps described in Section 4.4 (Preparing for measurement). Specifically:
 - Fitting (see 3.2) and selecting (see 6.5) the required measuring capillary where necessary.
 - Selecting an appropriate setup (see 6.3) or manually inputting the parameters (see 6.4).
 - Background monitoring (see 4.4).

Please note that after power-up, the system will automatically load the previously used setup for the previously used measuring capillary. Further information on how to manually specify parameters or create a new setup can be found in Section 7.5.

7.1. Preparing samples

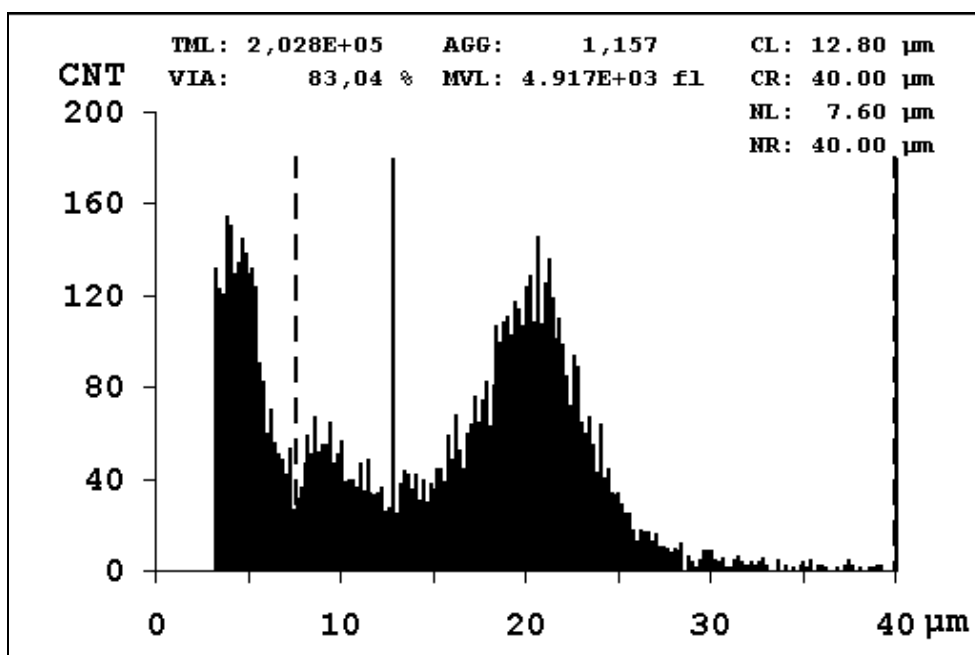
- Fill a CASY®cup with 10 ml CASY®ton.
- Add an aliquot of the cell suspension to be examined (normally between 10 and 200 µl).
- Secure the lid and mix the sample by tilting the CASY®cup three times. Carefully avoid the formation of bubbles or foam!

Notes:

- a) Your CASY® can measure only the samples you provide. The quality of the measuring result depends on how well you prepare your samples. Standardize the sample preparation procedure as far as possible (used cell culture media, timing of sample preparation, duration and intensity of cell re-suspension, carrying out of the required dilution steps etc.)
- b) You should always freshly prepare your samples just before the measurement. Depending on the sensitivity of the cell type, there is no guarantee that your cells in CASY®ton will remain unchanged in terms of cell count, cell volume, aggregation and viability over long periods. If a sample remains in CASY®ton for longer than two minutes, you must remix it before the measurement.
- c) Always use new CASY®cups. This ensures that your measuring results cannot be impaired by high background caused by contaminated measuring cups.

7.2. Measuring samples and displaying size distributions

- Rotate the recess in the sample platform until it points forwards and remove the CASY® cup from below the measuring capillary.
- Place the sample to be measured below the measuring capillary and ensure that the external platinum electrode is immersed in the sample.
- Rotate the sample platform somewhat to the side again.
- Press *START* to perform a measurement using the current parameters. CASY® automatically works through all the necessary steps. After a short term, the size distribution for your sample appears in the display.



This example shows the size distribution *for a eucaryotic cell line*.

- To the left of the left-hand normalization cursor appearing as a dotted line (< 7.6 µm), you will see the *Debris Peak*.
- Between the left-hand normalization cursor and the left-hand evaluation cursor shown by a solid line (7.6 µm to 12.8 µm), you will see the peak for the *dead cells*. In the case of dead cells, the cell membrane no longer acts as an electrical barrier. Therefore dead cells are shown by the size of their nucleus.
- To the right of the left-hand evaluation cursor, the peak for the *viable cells* begins followed by *cell aggregates*.

Note:

The size distribution for your samples may appear differently, depending on your applications, your cells and the used measuring capillaries.

7.3. Numerical measuring results

Depending on the *Graph Parameters* set on your system (see 6.7), you will find the most important measuring results at the top of the size distribution. You will also see, in the top right-hand corner, the current setting of the evaluation cursors *CL* and *CR* and the normalization cursors *NL* and *NR*, which are used as a basis to calculate the measuring results.

Pressing *NEXT* enables you to display the entire numerical evaluation of the measurement data. Pressing *NEXT* again will return you to the size distribution.

```

SET :      TUMOR-03 |DIL : 5.000E+01 |CAP :      150 µm
DATE:      15.04.2002 |SVOL: 3 x 400 µl |SCAL:      40 µm
TIME:      10:15 |AGG :  MANUAL |NORM: 7.60- 40.00 µm
SN :      TT-2-XX-9999 |PVOL: 4.249E+03 fl |EVAL: 12.80- 40.00 µm
-----
Counts      :      2911 |ConcRng:      OK
>###µm/ml: 1.500e+02 |Aggreg : 1.157
Viable/ml: 1.684e+05 |%Viable: 83.04 %
Total/ml : 2.028e+05
Debris/ml: 7.483e+04
Volume/ml: 1.431e+07 fl
MeanVol   : 4.917e+03 fl |MeanDia: 21.10
µm
PeakVol   : 4.250e+03 fl |PeakDia: 20.10
µm

```

The numerical results display is divided into two distinct areas. The header, which is separated by a horizontal line, contains a complete documentation on all *measuring parameters* used for the measurement. The main section contains all the *measuring results* for the set cursor ranges.

Note:

This example shows the format of the numerical display for settings *%Calculation = %Via* and *Aggr.Correct = Manual*. Details on possible settings can be found in Section 6.4. Depending on your settings, some of the parameters visible in this example may not appear and/or alternative parameters are shown!

Measuring parameters in the header of the numerical display

<i>SET:</i>	The setup used for the measurement in <i>NAME-##</i> format. If the current setup is not stored, <i>NOT SAVED</i> will appear here.
<i>TIME:</i>	The time at which the measurement was performed.
<i>DATE:</i>	The date on which the measurement was performed.
<i>SN:</i>	The serial number of your CASY®.
<i>DIL:</i>	The dilution factor used to calculate the sample concentration.
<i>SVOL:</i>	The sample volume used for the measurement, indicated as cycles x measuring volume.
<i>AGG:</i>	The type of aggregation correction used (<i>Off</i> , <i>Manual</i> or <i>Auto</i>).
<i>PVOL:</i>	The peak volume entered for the manual aggregation correction. This value is not shown if the aggregation correction is set to <i>Off</i> or <i>Auto</i> .
<i>CAP:</i>	The diameter of the used measuring capillary.
<i>SCAL:</i>	The scale of the X-axis (size axis) in μm .
<i>NCUR:</i>	The position of the two normalization cursors used for data evaluation.
<i>ECUR:</i>	The position of the two evaluation cursors used for data evaluation.

Measuring results for the active cursor settings

The calculation of the aggregation factor, the viable cell concentration, the percentage viability, the total cell concentration and the debris concentration assumes that the evaluation cursor is set to the size range of the viable cells, including all aggregates and that the normalization cursor is set to the size range of all cells (dead + viable) (see 6.4 and 7.5). The actual measured sample volume and the entered dilution factor are taken into account in the calculation of all values indicated in ml.

<i>Counts:</i>	Number of particles/cells counted in the size range of the evaluation cursors.
<i>ConcRng:</i>	Indicates whether the measured concentration lies within the permissible range. Possible indications are <i>Ok</i> or <i>Too High</i> . The permissible maximum concentration depends on the used measuring capillaries (see below).
<i>> ###$\mu\text{m/ml}$:</i>	<i>###</i> indicates the current scale of the X-axis (size axis). The parameter indicates the particle concentration in the size range above the size distribution shown. It can be used to check that the correct size scale has been selected.
<i>Aggreg:</i>	The aggregation factor calculated for the sample from the mean volume and peak volume. Depending on the <i>Aggr.Correct</i> parameter setting, the aggregation correction is calculated from the peak volume currently determined or the manually entered peak volume.
<i>Viable/ml:</i>	Concentration of viable cells in the sample in the evaluation cursor size range.
<i>%Viable:</i>	Percentage of viable cells in the sample.

<i>Total/ml:</i>	Total cell count of the sample (dead + viable) in the normalization cursor size range.
<i>Debris/ml:</i>	Concentration of particles in the size range to the left of the left-hand normalization cursor.
<i>Volume/ml:</i>	Concentration of particle/bio-volume in the evaluation cursor size range.
<i>MeanVol:</i>	Mean volume of all particles/cells in the evaluation cursor size range.
<i>MeanDia:</i>	Mean diameter of all particles/cells in the evaluation cursor size range.
<i>PeakVol:</i>	Volume at the maximum of the size distribution expressed in fl in the evaluation cursor size range.
<i>PeakDia:</i>	Maximum of the size distribution expressed in µm in the evaluation cursor size range.

Depending on the selected setting (see 6.4), the following parameters may also be displayed:

<i>Counts/ml:</i>	Particle concentration in the evaluation cursor size range.
<i>%Counts:</i>	Percentage of particles in the evaluation cursor size range relative to the normalization cursor size range (100%).
<i>TotCnt/ml:</i>	Particle concentration in the normalization cursor size range.
<i>%Volume:</i>	Percentage of volume in the evaluation cursor size range relative to the normalization cursor size range (100%).
<i>TotVol/ml:</i>	Volume in the normalization cursor size range.

Note:

The calculation of the peak diameter *PeakDia* and the peak volume *PeakVol* is based on a sliding weighted mean value. The algorithm will perfectly match each peak width through a five-stage iteration and guarantees an accurate calculation of the maximum, even for very flat peaks and for measurements with low cell concentrations. The peak volume determined in this way is also used to automatically calculate the aggregation factor.

7.4. Points to be observed when performing measurements

For the measuring operation, use only *CASY® ton*, *CASY® cups* and *CASY® clean* specified for the *CASY®* by *innovatis AG*. Using alternative measuring solutions, cups and cleaning agents may lead to incorrect measuring results and cause damage to the *CASY®* fluid system. *Innovatis AG* accepts no liability for damage caused by using alternative consumables.

A smooth measuring operation depends on the system undergoing the regular *Weekly Cleaning Routine* (see 5.1.) using *CASY® clean* (see Section 5.1.)!

You must ensure that your *CASY®* is also kept clean between cleaning cycles. Specifically that cells never remain in the fluid system for long periods! The daily background monitoring procedure (see 4.4) and the visual check of the vertical tube will indicate when the system is becoming contaminated.

The vertical tube is a calibrated glass pipette through which CASY®, controlled by a light barrier, aspirates the set measuring volume. The vertical tube is visible in the inspection window at the front of the measuring unit. During the measurement, you can visually track the rising level of the fluid meniscus. Depending on the level in the vertical tube, one or several light diodes (*Green* = start of measurement, *Red 1* = 200 µl measuring volume, *Red 2* = 400 µl measuring volume) illuminates. Judge the status of the vertical tube as you would a glass pipette. If you see that the fluid is no longer running cleanly and drops of fluid remain suspended on the edge, the calculation of your sample concentration may be adversely affected. In such cases, run a cleaning cycle using CASY®clean (see 5.1.).

Changing the samples

Provided you measure samples of the same cell type in a similar concentration range, you do not have to flush the system when the samples are changed. If you expect larger concentration differences or wish to perform measurements with a different sample type, before a new measurement, place a CASY®cup containing freshly filtered CASY®ton below the measuring capillary and flush the system using the *CLEAN* function .

Sample concentration

For correct measurement results the concentration of the diluted cell suspension in the CASY®cup should not exceed certain maximum values. The concentration limit depends on the size of the measuring capillary:

150 µm measuring capillary:	<	20 000 counts/ml
60 µm measuring capillary:	<	100,000 counts/ml
45 µm measuring capillary:	<	250,000 counts/ml

Higher concentrations than specified result in *coincidence errors*, i.e. an increased probability that two particles will be in the measuring pore at the same time. CASY® will evaluate two particles being in the measuring pore at the same time as if they were one particle with a correspondingly larger volume. The calculated particle count will therefore be too low and the size distribution will shift towards the larger volumes.

Note:

The coincidence limits do not depend on to the positions of your evaluation cursor settings. They are always determined for the entire physical measuring range of the used measuring capillary (approx. 2% to 80% of the measuring pore diameter). In the case of cell samples, this includes cell debris as well as cell aggregate with a diameter above the scaling range selected for the size axis (X-axis). This guarantees that the cell count and cell volume are always measured correctly.

You are not required to check yourself whether the concentration of your samples is too high. CASY® displays the following warning message if the coincidence limit values indicated above are exceeded.

```
ERROR SCREEN          TT-2AB-9999   15.04.2002  10:15
-----
          Concentration too high !

          Please dilute your sample
          Measured Counts/ml      :    34345
          Max Counts/ml allowed  :    20000

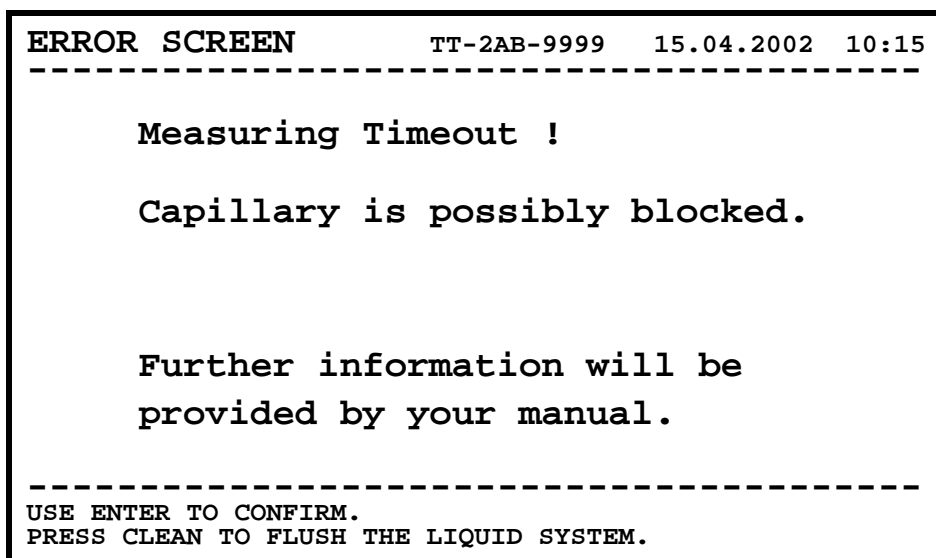
          Further information will be
          Provided by your manual!

-----
          USE ENTER TO CONFIRM.
          PRESS CLEAN TO FLUSH THE LIQUID SYSTEM.
```

You must confirm this message by pressing *ENTER*. The measuring result is displayed, but the *ConcRng* parameter is shown as *Too high*. To obtain valid measuring results (cell count, cell volume), you must adequately dilute your sample and repeat the measurement.

Measuring pore blockages

During the measurement, CASY® exactly monitors the rise times of the fluid column. This procedure ensures the reliable detection of even film-like deposits (protein, lipids etc) in the measuring pore, which shrink rather than actually block the pore, resulting in incorrect volume calculation. If the rise time exceeds a critical limit, the following error message appears:



Most blockages occurring during the measuring operation result from the temporary presence of very large particles (e.g. aggregates, tissue remnants) in front of the measuring pore, which act like a “valve flap”. You can repeat the measurement immediately after confirming the error message by pressing *ENTER*. Shaking the CASY®cup slightly often helps to remove contamination from the outside of the measuring pore. If the blockage remains, you can usually remove the contamination easily using the *CLEAN* function.

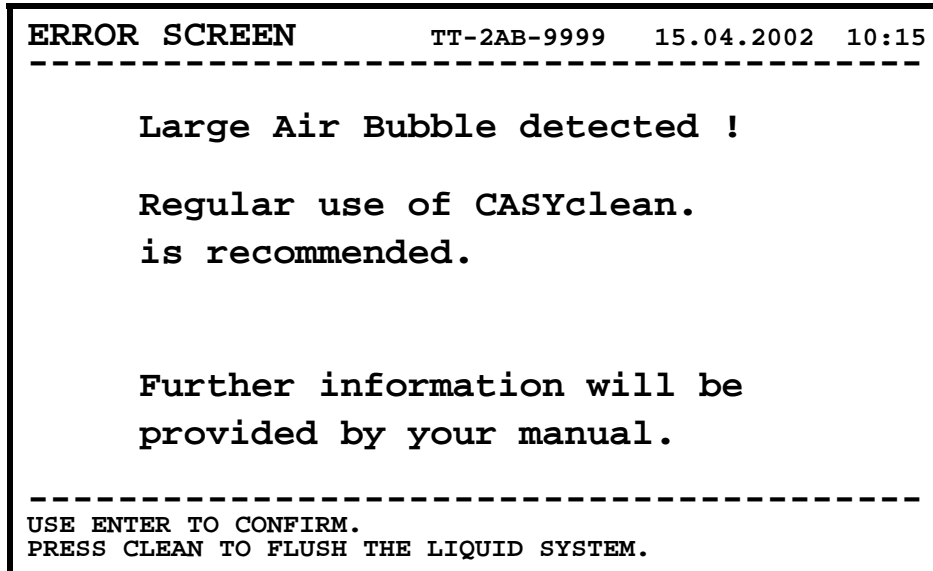
Caution:

During the *CLEAN* cycle, fluid is flushed under pressure from inside the measuring capillary through the measuring pore into the sample cup. To prevent your sample becoming diluted or contaminated, replace it with a CASY®cup containing freshly filtered CASY®ton before you run a *CLEAN* cycle.

If even repeated *CLEAN* cycles fail to remove a measuring pore blockage, you can unscrew the measuring capillary (see 3.2) and try to flush the measuring pore through from the top using a syringe. If necessary, place the measuring capillaries in CASY®clean and leave overnight.

Air bubbles

CASY® precisely monitors the incidence of “critical” air bubbles in the rising fluid column within the vertical tube. Large air bubbles affect the accuracy of the sample volume measurement and may therefore result in the cell concentration being measured incorrectly. If an air bubble exceeds a critical size during a measurement, the following error message appears:



If you observe the vertical tube in the inspection window at the front of the measuring unit, you will be able to see the air bubble. If the air bubble rises to the fluid meniscus and disappears, it will not affect subsequent measurements. You can repeat the measurement immediately after confirming the error message by pressing *ENTER*. If, however, the air bubble remains suspended in the vertical tube, you must remove it by replenishing the fluid system using the *CLEAN* flushing function.

Deposits within the fluid system sometimes become noticeable through frequent air bubble error messages. If necessary, run a CASY®clean cycle (see 5.1) to remedy the problem. Ensure that the system is flushed thoroughly with distilled water to remove any remnants of CASY®clean!

Note:

If, during the measurement, only small air bubbles are evident within the vertical tube, no action is necessary. Small air bubbles – which are higher in number with small measuring capillaries – are caused by the process of electrolysis at the inner electrode. These are quite normal, no error message is triggered and the measurement is not affected.

Empty Storage container

Should CASY® fail to completely replenish the fluid system during a *CLEAN* cycle, the following error message appears:

```
ERROR SCREEN          TT-2AB-9999  15.04.2002  10:15
-----
Error-Code:  PR-8

Liquid System could not be filled
during Clean!

Please check, that there is enough
CASYton in the storage container.
Further information will be
provided by your manual.

-----
PRESS ENTER TO CONFIRM OR PRINT TO GET A HARDCOPY
PRESS CLEAN TO FLUSH THE LIQUID SYSTEM.
```

Ensure that there is sufficient CASY®ton in the storage container (right hand container). Replenish the CASY®ton if necessary and run another *CLEAN* cycle. If the error message re-appears even when the storage container is sufficiently full, the pressure system may be mal-functioning or the internal tube system is blocked. In this case, contact innovatis AG or your nearest dealer.

7.5. Setting the measuring range and the cursor positions

Accurate measuring results rely on the measuring range and cursor positions being correctly adjusted. It is particularly important for evaluation of the data, that all samples to be compared are measured with identical settings. It is generally advisable to specify the measuring range and the cursor positions for a particular cell type or a certain application after the appropriate preliminary tests have been performed in a setup. For the required preliminary tests, the cursors can be controlled manually using the control panel keys.

Depending on your application, completely different settings may be required. It is usually simple to derive the correct setting logically from the displayed size distribution. If you have any questions regarding the settings for your application, please contact the *Application Support* at *innovatis AG*. We will be pleased to help.

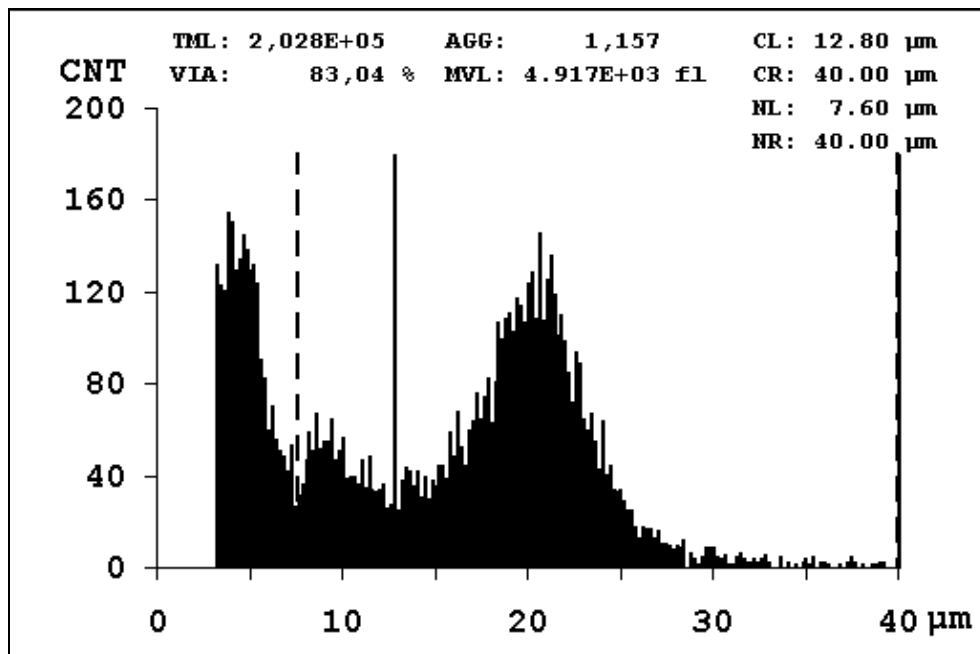
The settings for the measuring range and the cursors for monitoring the status of eucaryotic cells is described below.

Measuring range

Select the measuring range so that even the largest anticipated cell aggregates can be recorded within the displayed size distribution. For most eucaryotic cells, a measuring range of between 0-40 µm and 0-60 µm has proven optimum. If you are not sure whether your cells will aggregate more intensely under different experimental conditions, you should select a larger rather than a smaller range.

Evaluation and normalization cursors

To monitor the status of cell cultures, the right-hand cursors (evaluation and normalization cursors) must always be positioned at the right-hand edge of the size distribution. This guarantees that all cell aggregates are included in the calculations. To specify the position of the left-hand evaluation cursor and the left-hand normalization cursor, you must first perform a measurement on a culture with high viability. Then kill an aliquot of your cells and measure the size distribution of these dead cells. To finally set your cursors, it is recommended that you perform a measurement on a mixture consisting of 70% viable cells and 30% dead cells. You should see a size distribution similar to the one in the measuring example below.



- Set the left-hand normalization cursor at the lowest point on the size distribution between cell debris and dead cells.
- Set the left-hand evaluation cursor at the lowest point on the size distribution between dead and viable cells.

This setting should ensure that all measuring results and calculations for this cell type are correct. When you have created the correct settings for your various cell types, you should save them as setup and assign them a typical



name. This guarantees that the same cell type will always be measured with the same settings, i.e. that the measurements are and remain comparable.

7.6. Manual cursor control using control panel keys

You can use the manual cursor control keys located on the left-hand side of the control panel to change the position of the cursor whilst visually monitoring the size distribution. If you are in the numerical results display or menu control, you must first move to the display.



Using the *L-CURSOR* and *R-CURSOR* keys activates the mode to manually set the cursor. This mode is identified by the presence of a ★ in the top right-hand corner of the screen, just before the cursor position to be adjusted using the arrow keys. The evaluation cursor setting is always active after starting manual cursor control.

Alternate between *L-Cursor* and *R-Cursor* to activate the setting for the left and/or right-hand evaluation cursor. You can switch to the normalization cursor setting by pressing *NEXT*. Now you can use the *L-Cursor* and *R-Cursor* keys to activate the adjustment for the left and right-hand normalization cursor respectively. Pressing *NEXT* again returns you to the evaluation cursor setting.

The  and  keys move the active cursor right and left respectively. The selected cursor moves across the size distribution accordingly. At the same time, the numerical display of the cursor position is constantly updated in the top right-hand corner of the size distribution. Pressing *L-Cursor*, *R-Cursor* and *NEXT* enables you to select a different cursor to move at any time.

The manual cursor setting mode can be terminated by pressing either *ENTER* or *CANCEL*. If you press *ENTER*, the new cursor positions are accepted as active settings and the calculation of the displayed measured values is updated. If you press *CANCEL*, the previous cursor position settings are restored. All new changes are rejected.

Note:

While you remain in the manual cursor control mode, only the *L-Cursor*, *R-Cursor*, , , *CANCEL* and *ENTER* keys are active. You must terminate manual cursor control before you can use other control panel functions.

7.7. Outputting the results

The data can be output via either a parallel or a serial interface. The *parallel interface* is provided to connect a *PCL-capable printer* (PCL = Printer Control Language). All Hewlett-Packard printers and compatible products satisfy this requirement (see Appendix 8.3., Technical Data). The data can be transferred to a computer for further editing via the *serial interface* (see Appendix 8.2, Data output via the serial interface).

To export size distributions and measuring results into MS-Excel™ (PC) through a fully automated process, you will require *CASY®excell*, a program available from *innovatis AG*.

If you have set the *Print Mode* parameter to *Auto*, the data are automatically output after each measurement via interfaces specified in the current parameters (see 6.4). If *Print Mode* is set to *Manual*, you must start to output the measuring results by pressing *PRINT*.

The *Graphic* parameter applies only to the printed output. Depending on whether the *Graphic* parameter is set to *On* or *Off*, only the numerical results or both the numerical results and the size distribution are output. The numerical output has the following format:

CASY Model TT	Date	: 15.04.02	Serial Number	: TT-2-AX-9999	
	Time	: 10:15	Setup	: Tumor-03	
Comment	:	_____			
User/Siganture	:	_____			
Capillary	: 150 µm	Conc. Range	: OK	Volume/ml	: 1.431e+07 fl
X-Axis Scale	: 40 µm	Counts	: 2911		
Eval Cur. left	: 12.80 µm	Counts> 50µm/ml	: 1.500e+02		
Eval Cur. right	: 40.00 µm	Aggr. Correct	: 4.249e+03 fl	Mean Diameter	: 21.10 µm
Norm Cur. left	: 7.60 µm	Aggr. Fact.	: 1.157	Mean Volume	: 4.917e+03 fl
Norm Cur. right	: 40.00 µm	Viable Cells/ml	: 1.684e+05	Peak Diameter	: 20.10 µm
Sample Volume	: 3 x 400 µl	Total Cells/ml	: 2.028e+05	Peak Volume	: 4.250e+03 fl
Dilution	: 5.000e+01	% Viability	: 83.04 %	Interface	: Parallel
%Calculation	: Viability	Cell Debris/ml	: 7.843e+04	Print Mode	: Manual

The *Comment* and *User/Signature* rows are intended for hand-written comments on the measuring results and a signature where appropriate.

The size distribution is inserted between the *User/Signature* and the *Measuring Results* rows if the *Graphic* parameter is set to *On*.

Note:

This example shows the format of the numerical printout for settings *%Calculation = %Via* and *Aggr.Correct = Manual*. Details on possible settings can be found in Section 6.4. Depending on your settings, some of the parameters visible in this example may not appear and/or alternative parameters are shown!

If you have selected *Par* or *P + S* as an interface, a printer must be connected to the parallel interface. If you have omitted to switch on your printer or if it is not correctly connected, the following error message appears when you try to output data.

```
ERROR SCREEN          TT-2AB-9999   15.04.2002  10:15
-----
Error-Code:  CM-1

Printer does not respond!
Be sure that your Printer is
switched on and connected.

-----
PRESS ENTER TO CONFIRM OR PRINT TO GET A HARDCOPY
```

Press *ENTER* to confirm the error message, switch the printer on and check that it is correctly connected. Having eliminated the cause of the error, you can resume outputting the data by pressing *PRINT*.

8. Appendix

8.1. Error messages

CASY® permanently monitors all its system functions and reports error messages as soon as a fault is detected:

```
ERROR SCREEN          TT-2AB-9999   15.04.2002  10:15
-----
Error-Code:  XX-##

Please shut off your CASY
For at least 5 seconds.
If error persists note the code
and contact Schärfe System
or your local distributor.

-----
PRESS ENTER TO CONFIRM OR PRINT TO GET A HARDCOPY
```

Generally, an *error code* consisting of two letters followed by a hyphen and a one or two figure number is output (e.g.: *PR-7*).

In special cases, a combination of up to 12 hexadecimal characters may be output, which are arranged in 3 blocks separated by colons.

If you receive an error message, first try to switch off and then restart your CASY®. Errors can sometimes be triggered by *power supply faults*. If no error message appears when you restart the system, no further problems should occur. If the same error message appears during the self-test or measuring operation, you should *note the error code*. You can also output the error message on the connected printer by pressing *PRINT*.

The following errors may be caused by *false handling* and can usually be easily cleared.

- CM-1:* Check that the printer is correctly connected and switched on.
- PR-7* Check that the selected capillary setting corresponds to the measuring capillary in use.
- PR-8* Check that the storage container is full of CASY® ton.

All other error messages that have an error code are caused by faults within the system and cannot be cleared. The same applies to the errors listed above, if they cannot be cleared by the actions described. Please contact the Service Department at *innovatis AG* or your nearest dealer to clarify further procedure.

8.2. Data output via the serial interface

You can transfer all the data relevant to a measurement via the serial interface to a connected computer. This data includes all the measuring parameters specified in the active settings, the content of the 400 size channels and all the values calculated from the current cursor settings.

To enable CASY® to output the measured data via the serial interface, the *Interface* parameter must be set to *Ser* or *P + S* in the current setup. If the *Print Mode* is set to *Auto*, the data is automatically exported after every measurement. If *Print* is set to *Manual*, the data is output by pressing *PRINT* on the control panel. The data is exported in text format (ASCII) and the individual values are separated by CR/LF.

To be able to accept the data, your computer requires a free serial interface. The connection is established by a straight serial transfer cable with a minimum of three signal lines (TxD, RxD and SignalGround).

CASY® uses the following *Transfer parameters*:

Baudrate:	19200
Parity:	None
Databits:	8
Stopbits:	1

To export size distributions and measuring results into MS-Excel™ (PC) through a fully automated process, you can obtain the *CASY®excell* program from *innovatis AG*. The package also includes the serial connection cable required for data transfer.

Format of serial data transfer

Row	Name of Parameter	Format	Comments
1	Serial Number	@@@@@@@@@@@@	
2	Setup Name	@@@@@@@@@@@@	
3	Setup Number	##	00 - 19
4	Time	##:##	24 h format
5	Date	dd.mm.yyyy oder mm/dd/yyyy	depending on the selected format
6	Dilution Factor	#####e+##	
7	Measuring Cycles	##	1 - 10
8	Sample Volume (µl)	###.##	corrected based on volume calibration
9	Mode of Aggregation Correction	#	0=Off, 1=Manual, 2=Auto
10	Manual Aggregation Correction (fl)	#####e+##	single cell volume
11	Percent Mode	#	0=Off, 1=Viab, 2=%Cnt, 3= %Vol
12	Debris Mode	#	0=Off, 1=On
13	Capillary Diameter (µm)	###	
14	X-Axis Scale (µm)	###	
15	Left Normalization Cursor (µm)	###.##	
16	Right Normalization Cursor (µm)	###.##	
17	Left Evaluation Cursor (µm)	###.##	
18	Right Evaluation Cursor (µm)	###.##	
19	Y-Axis	####	Auto or 10 - 7500
20 - 419	Counts in the 400 Size Channels	#####	
420	Counts	#####	within evaluation cursor range
421	Concentration Range	#	0=Ok, 1=Too High
422	Counts > Upper Scaling Limit/ml	#####e+##	
423	Aggregation Factor	###.###	
424	Counts/ml	#####e+##	within evaluation cursor range*
425	Total Counts/ml	#####e+##	within normalization cursor range*
426	Viable Cells/ml	#####e+##	within evaluation cursor range*
427	Total Cells/ml	#####e+##	within normalization cursor range*
428	Cell Debris/ml	#####e+##	on left side of left normalization cursor*
429	Volume/ml in fl	#####e+##	within evaluation cursor range
430	Total Volume/ml in fl	#####e+##	within normalization cursor range *
431	%Counts	###.##	based on normalization range=100%*
432	%Viability	###.##	based on normalization range=100%*
433	%Volume	###.##	based on normalization range=100%*
434	Mean Volumen in fl	#####e+##	within evaluation cursor range
435	Peak Volumen in fl	#####e+##	within evaluation cursor range
436	Mean Diameter in µm	###.##	within evaluation cursor range
437	Peak Diameter in µm	###.##	within evaluation cursor range
438	Check Sum	XXXXXXXX	4 bytes hexadecimal

Depending on your setup settings, some data tagged with ★ is output as *not calculated*.

8.3. Technical data on CASY® Model TT.

<i>Function:</i>	Electronic Cell Counter + Analyser System for determining cell count and cell size distributions, aggregation and viability.
<i>Dimensions:</i>	Height: 39 cm Width: 31 cm Depth: 39 cm
<i>Weight:</i>	14.5 kg (including control panel)
<i>Ambient conditions:</i>	maximum operating temperature: 10°C to 40°C recommended operating temperature: 15°C to 32°C
<i>Power supply:</i>	230 V~ / 50 Hz 115V~ / 60 Hz
<i>Power consumption:</i>	max. 112 VA
<i>Fuses (primary):</i>	230V: 2 x 800 mA 115V: 2 x 1.6 A
<i>Fuses (secondary):</i>	SI1: T 160mA SI2: T 1.6A SI3: T 400mA SI4: T 2.5 A SI5: T 400mA
<i>Volume measuring tube and pressure system:</i>	Calibrated, opto-electronically controlled volume measuring tuben with no-maintenance, sensor controlled pressure system. Mercury-free.
<i>Measuring principle:</i>	Electrical resistance measurement in 1 MHz, low voltage field with pulse area analysis by Schärfe System. Distinctive measuring channels: > 512.000.
<i>Volume resolution:</i>	1 in 512,000
<i>Displayed size channels:</i>	400
<i>Measuring range:</i>	0.7 µm to 120 µm
<i>Measurement dynamics:</i>	Ratio of smallest to largest particles that can be analysed simultaneously: > 1 : 70,000 in terms of volume, . > 1 : 40 in terms of diameter
<i>Typical analysis time:</i>	10 sec
<i>Typical sample volume:</i>	5 µl – 100 µl
<i>Measuring volume:</i>	200 µl – 4 ml

<i>Analysis/results:</i>	Analysis of the size distribution using two cell-specific, adjustable cursor pairs: Cell count: absolute, relative -%-, Cell viability: absolute, relative -%-, Cell aggregate: Aggregation factor, aggregation correction, Cell volume: Volume, mean volume, total volume (bio-mass)
<i>Interfaces:</i>	Serial, RS232 (DB9) Parallel (DB25) Only EIC or UL-tested devices can be connected to the RS232 and printer interfaces.
<i>Printer output:</i>	PCL (Hewlett- Packard compatible printer) Printout of: Size distribution Numerical result Setups Error messages
<i>Data export:</i>	ASCII, fully-automatic export into MS-Excel™ using CASY®excell
<i>Technical features:</i>	Permanent, highly stable calibration for biological media – certified by innovatis AG, identical for all CASY® systems. Recording of measuring results, including date, time, setup name, device serial number and all device settings. System self-test checking all measuring-specific parameters before and after each measurement. Electronic monitoring of the measuring pore. Automatic flushing of the measuring pore. Automatic detection and display of operating and system errors. Integrated service functions for fast error diagnosis. Fan-less system suitable for use in clean rooms.
<i>Operation:</i>	Menu-driven operation and control of all functions through a movable control panel with membrane keyboard and graphics display. All cell-specific device settings can be saved as setup. 20 setups per measuring capillary – each assigned a different name. Output of results and size distribution to display and printer. Export function for computers via the serial interface.
<i>Maintenance:</i>	If cared for on a regular basis, CASY® is virtually maintenance-free. If a service becomes necessary or requested by the user, contact innovatis AG or your nearest dealer. Servicing may only be carried out by engineers who have been trained and approved by innovatis AG.

CASY® is a registered trademark of innovatis AG.

Subject to technical alteration, Reutlingen, April 2002.