

Technology that counts



NucleoCounter® SP-100™

Fast and precise semen Analysis with
the NucleoCounter® SP-100™



Introducing the NucleoCounter® SP-100™

The NucleoCounter® is maintenance and calibration free, offering extreme ease of use and reliable operation for the preparation of insemination products for animal husbandry.

Introduction

ChemoMetec A/S has developed and patented a novel technology which addresses the problems of conventional routine semen analysis. The integrated fluorescence microscope in the NucleoCounter® SP-100™ is designed to detect signals from individual sperm cell nuclei. This result of the NucleoCounter® SP-100™ represents direct cell concentration of the sample which offer considerable accuracy compared to indirect methods.

NucleoCounter® SP-100™ is intended for use in semen collection centers and processing facilities offering accurate, simple and reliable semen analysis as means for product optimization and quality control.

Key benefits of the NucleoCounter® SP-100™ are 45 sec. analysis time, high accuracy and simple integration into any work environment.



Simple as 1-2-3

The system consists of the NucleoCounter® SP-100™ fluorescent microscope, the SP1-Cassette™ containing immobilized dye and Reagent S100 for dilution and sample preparation. The SemenView™ PC software application for optional documentation, image viewing and data processing is delivered with the instrument, however NucleoCounter® SP-100™ can also be

operated as a stand-alone instrument. Furthermore the NucleoCounter® SP-100™ is available in a configuration which allows direct connection to an optional printer for documentation.

NucleoCounter® SP-100™ is suited for the analysis of semen from several species such as boars, bulls and stallions.

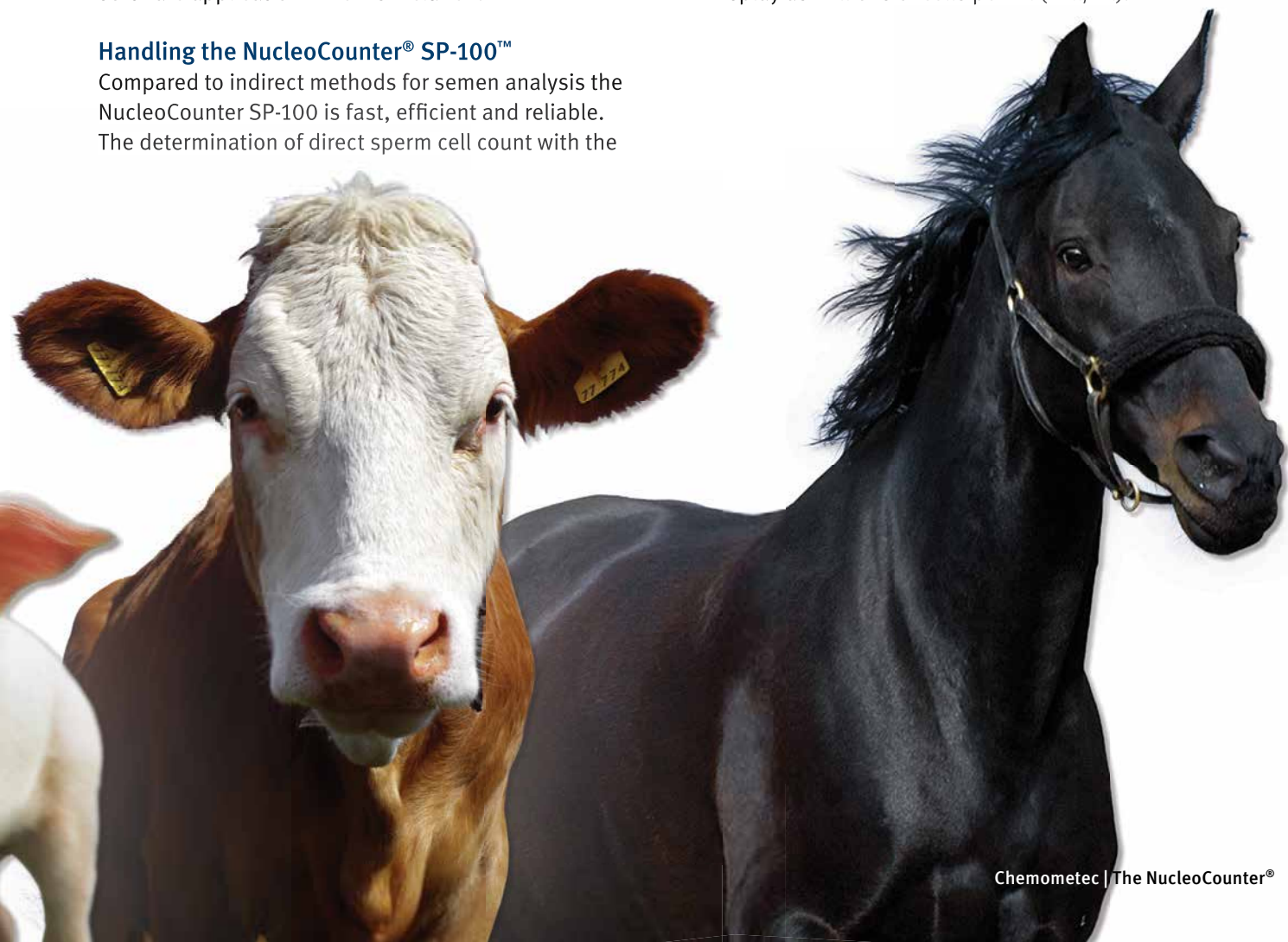
The NucleoCounter® SP-100™ – Cell Counting System

The core of the system is a novel integrated fluorescence microscope, comprising an excitation light source of light emitting diodes (LED's), optics (including lenses, excitation and emission filters), and a charged coupled device (CCD) camera. The fluorescent microscope is optimized to excite the nuclei staining dye, Propidium Iodide (PI), with intense green light and subsequently detect the red light emitted from DNA in the sperm cells with a CCD camera. Detected signals are correlated to direct sperm count, which is presented to the user in the built-in display. Optionally, the image and result can be transferred to a PC for viewing and documentation using the SemenView™ software application which is included.

Handling the NucleoCounter® SP-100™

Compared to indirect methods for semen analysis the NucleoCounter SP-100 is fast, efficient and reliable. The determination of direct sperm cell count with the

NucleoCounter SP-100 involves sample preparation and sample analysis. During sample preparation the sample is diluted and the plasma membrane of the sperm cells disrupted, thus exposing the DNA of the individual cells to the nuclei staining dye. The cell lyzate is subsequently loaded into an SP1-Cassette where the DNA of the nuclei is stained. The SP1-Cassette is then placed into the NucleoCounter SP-100 instrument for analysis. During analysis the fluorescent signal from the PI bound to DNA of the individual cell nuclei is detected and the cells counted in the built-in computer. The sperm cell count of the sample is presented in the NucleoCounter SP-100 display as millions of cells per ml (mill/ml).



The NucleoCounter® SP-100™

– Cell Counting System



- 1 Sample preparation - Time: 10 seconds**

Pipette a representative semen sample (50.0 μ l) into a sample vial. Add a volume of reagent (10.00 ml). This dilutes the semen sample and prepares the cells for staining (201 fold dilution). (Dilution factor depends on species).



- 2 Sampling - Time: 5 seconds**

Load the SP1-Cassette with the cell lysate by immersing the tip of the cassette into the cell lysate and aspirate the sample.



- 3 Sample analysis - Time: 30 seconds**

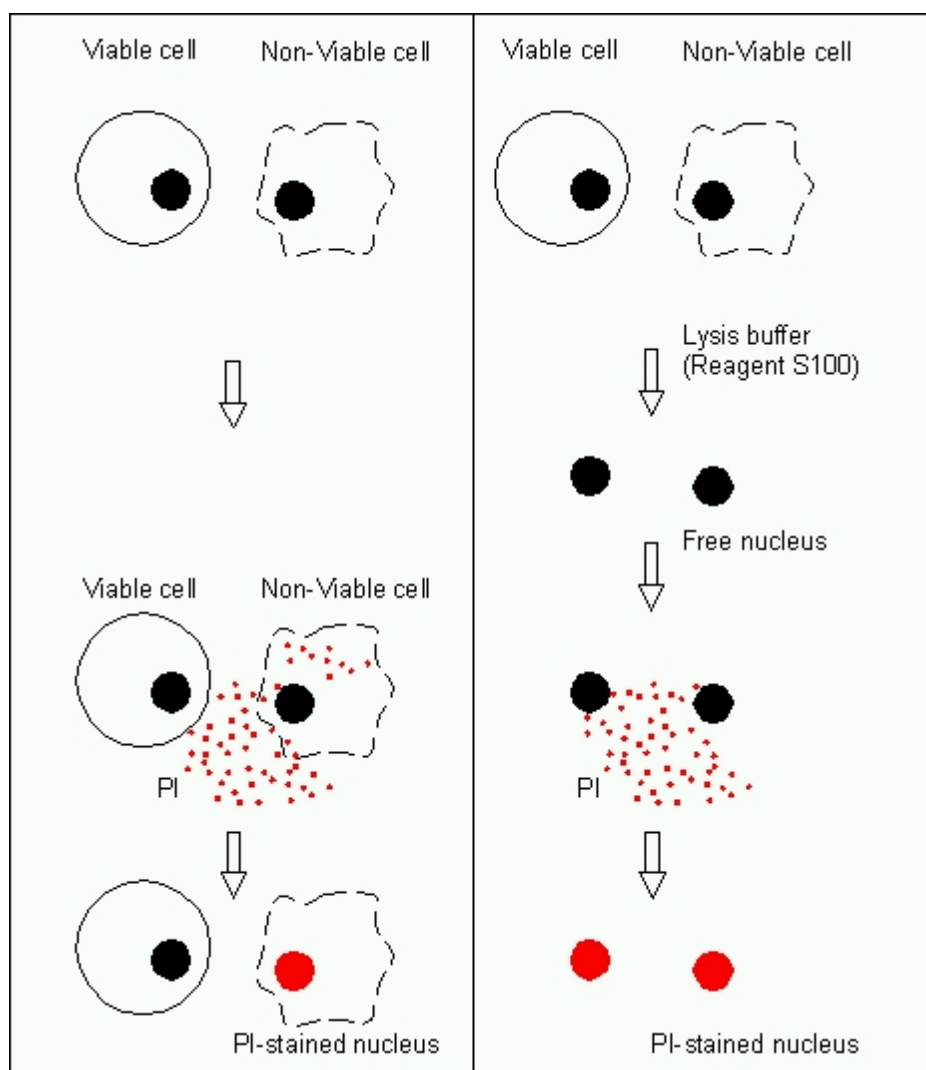
Place the SP1-Cassette in the NucleoCounter SP-100 instrument and press the "Run" button. After approximately 30 seconds the total cell concentration appears in the display. The data is optionally presented on a PC using the SemenView software application.

Cell viability

Cell Viability (the membrane integrity) can be measured with the NucleoCounter SP-100 in a two-step procedure by measuring the difference between the total cell count and the non-viable cell count (see the figure below).

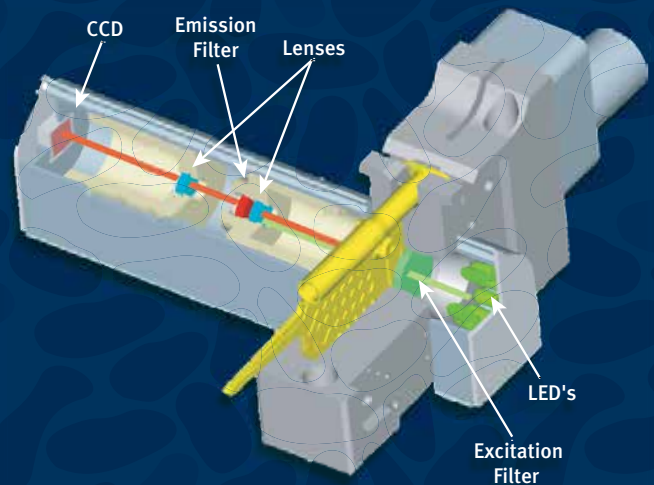
When a sample is diluted with Reagent S100 a lysis of the viable cell membrane takes place rendering all the cell nuclei susceptible to staining with propidium iodide (PI) resulting in a total cell count.

Non-viable cells diluted with PBS (Phosphate Buffer Saline) are however permeable without treatment and are therefore stained directly with PI, resulting in a non-viable cell count. The difference between the two measurements provides the viable cell count - or % Viability.

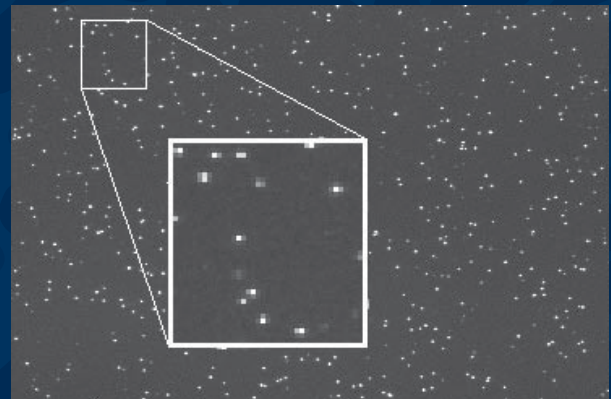


Integrated Fluorescence Microscope

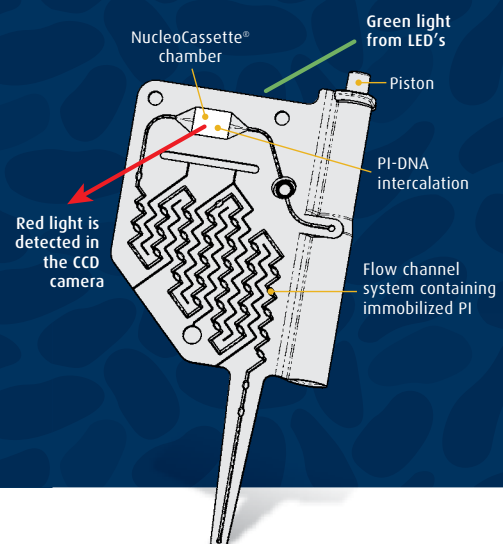
The interior of the NucleoCounter is a fluorescence microscope. The SP1-Cassette is placed into the fixture. LED's emit green light in order to excite the PI-DNA intercalation. The excitation filter sorts the green light and the emission filter sorts the PI-DNA emitted red light. The CCD camera placed to the left registers the red light and the signals are correlated to the cell count. The magnification is approximately x1.3.



The patented method of low magnification microscopy offer several advantages compared to other methods for particle counting. The large view area allows a large sample volume to be analyzed in a single image. The limited size of the imaged cells, relative to the size of the image elements (enlarged section in the illustration) demands only limited quality of the imaging system and allows cells to be imaged for a long time since slight movement of the cells during exposure is not reflected in the image.



The cell nuclei are stained within the SP1-Cassette. After placement in the fixture of the NucleoCounter SP-100 instrument, the stained mixture is transferred to the SP1-Cassette chamber. Green light excites the PI-DNA intercalation and the red light emitted is registered in the CCD camera for correlation into individual cell count.



The NucleoCounter® SP-100™

– Accurate and Precise Cell Counting

Accuracy

The counting of sperm cells in the SP-1 Cassette with the NucleoCounter SP-100 system is operator independent. The NucleoCounter technique assures that the volume of the sperm cell lyzate analyzed using the SP1-Cassette is accurately known for each measurement. Each NucleoCounter SP-100 instrument is carefully calibrated during production assuring optimal accuracy. The construction of the NucleoCounter SP-100 instrument offers great stability rendering NucleoCounter SP-100

maintenance and calibration free. Thus the NucleoCounter SP-100 offers unique long-term accuracy compared to other direct and indirect methods for semen analysis.

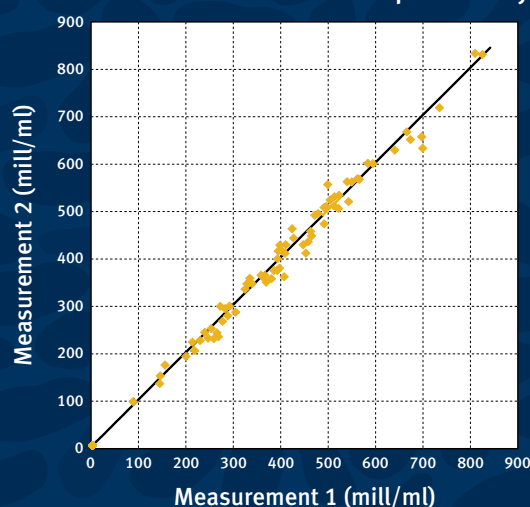
Precision

Approximately 1 µl of sperm lyzate is analyzed in one measurement. Thus a high number of sperm cells are counted in each analysis assuring high precision compared to many other methods.

Repeatability

An illustration of the superb repeatability of the NucleoCounter SP-100 is given in the repeatability graph. A total of 75 boar semen samples were measured in duplicate and the results are plotted against each other. The thin lines adjacent to the diagonal line represent the theoretical limits of 95% significance of the difference between duplicate measurements (Poisson distribution).

NucleoCounter® SP-100™ Repeatability



Reproducibility

For the investigation of reproducibility 15 standard NucleoCounter SP-100 instruments were compared to a master instrument. Variance analysis (ANOVA) of repeatability and differences between instruments showed no significant effect between instruments ($p=0.88$). This suggests that the reproducibility of the NucleoCounter SP-100 is solely determined by the repeatability error.

Precision of NucleoCounter® SP-100™ is equal to Reproducibility.

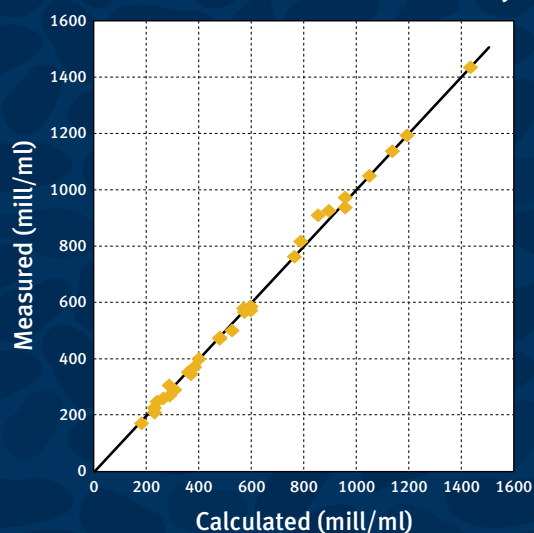
Typical values when measuring boar sperm count (200 fold dilution).

Cell Count (mill/ml)	Repeatability CV (%)	Reproducibility CV (%)
200	3.8	3.8
400	3.1	3.1
800	2.7	2.7

Linearity

The response of the NucleoCounter SP-100 is linear over an extensive range. This is illustrated in the linearity graph which shows results obtained from the measurement of accurately diluted boar semen samples. Each point on the graph is the average of 2 measurements.

NucleoCounter® SP-100™ Linearity



The NucleoCounter® SP-100™

– key benefits

Simple and fast

Analysis takes less than 30 seconds. The procedure is simple and easy to learn by all laboratory staff. The NucleoCounter SP-100 requires no daily cleaning or calibration and is maintenance free.

High Accuracy

The NucleoCounter SP-100 offers accurate count of individual sperm cells. Reproducibility is substantially equal to repeatability.

High Precision

The precision of NucleoCounter SP-100 is high. The large number of cells counted generally renders precision of about 3% (one standard deviation).

Integration

The small size and simple and fast operation makes the NucleoCounter SP-100 ideal for integration in any existing production environment.

Direct Cell Counting

The results of the NucleoCounter SP-100 are direct estimates of cell concentration as opposed to methods based on indirect determination.

Calibration free

The optical system of the NucleoCounter SP-100 is calibrated during production. This system is not altered during the life-time of the instrument. Each SP1-Cassette is calibrated individually during production

Maintenance free

The NucleoCounter SP-100 is constructed using durable components such as Light Emitting Diodes (LED's) as light source and Charge Coupled Device (CCD) as detector.

Safe sample disposal

The SP1-Cassette is disposable and can be disposed of safely as laboratory waste.

Great reliability

Great consideration has been given to reliability in the development of the NucleoCounter® SP-100™ instrument.

User-safety

The potentially hazardous propidium iodide is safely enclosed in the SP1-Cassette™ thereby providing optimal safety for the operator.

Specific and established method

MiBy using the DNA binding fluorescent propidium iodide the cell count is based on a very specific signal.

Small loading volume

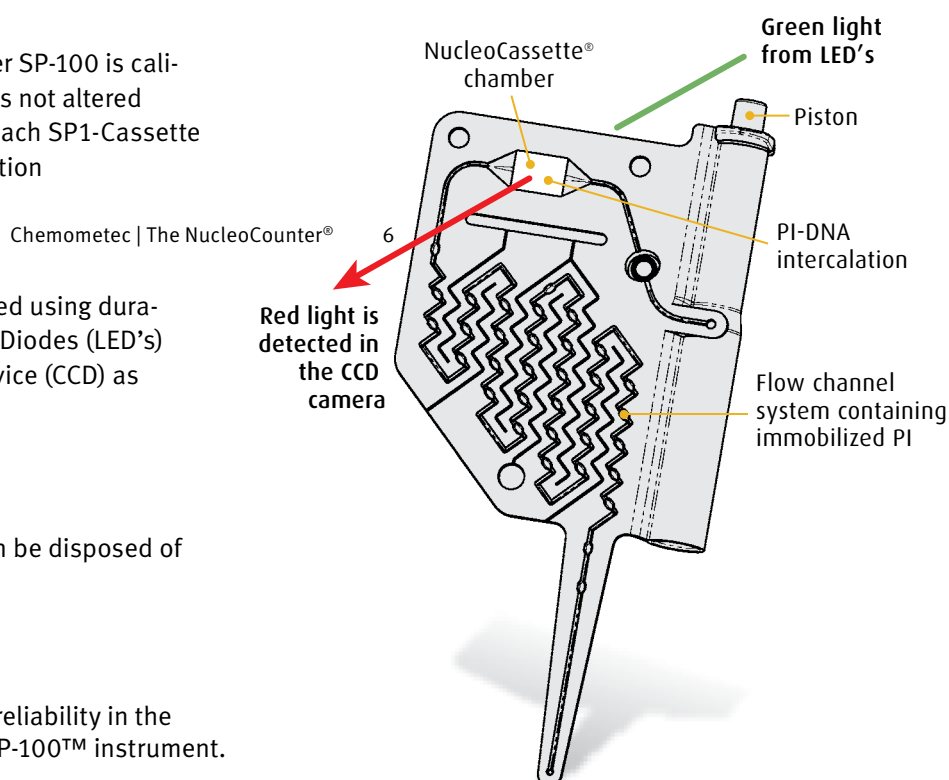
Minimum 10 µl but typically 50 µl semen sample is required to perform an analysis.

Documentation

The included SemenView software application is ideal for data storage and documentation purposes.

Compact instrument

The NucleoCounter® SP-100™ fits into any laboratory facility due to its small size (38x26x22) and low weight (3 kg). The low weight and small size makes it also ideal for use in the field.



Technical specifications

The NucleoCounter® SP-100™

Specificity	The NucleoCounter SP-100 counts sperm cell nuclei stained with the DNA specific fluorescent dye, propidium iodide.		
Analysis time	After pressing "Run" on the instrument the result will be displayed within 30 seconds.		
Measurement range (ejaculates)	5 mill./ml to 5 bill./ml - depending on dilution factor.		
Operation	Menu-controlled by means of keyboard and LCD display.		
Physical data	Weight	3 kg	
	Height	26 cm	
	Width	38 cm	
	Length	22 cm	
Power	External 11 – 13 VDC Power Supply		
Power consumption	Peak	25 W	
	Ready mode	2.5 W	
	Standby	2 mW	
Operation conditions	For indoor use. Maximum relative humidity (RH) 80% for temperatures up to 31°C decreasing linearly to 65% RH at maximum temperature of 35°C; minimum temperature 15°C.		
USB	USB, version 1.1. Note: Support of USB Hubs varies, dependent on model.		

The NucleoCassette™

Reagent	Each SP1-Cassette contains approximately 2.8 µg propidium iodide (PI).
Storage	Store the SP1-Cassettes in a sealed bag at max. 30°C.
Stability	Stable for minimum 12 months.

SemenView™ software

System requirements	Windows 2000 operating system or newer including Windows 7 and Windows XP 64-bit versions. Mac is not supported.
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The information contained herein is to the best of our knowledge accurate and complete. However cell species and cell environments may vary in property. Therefore systematic and/or random deviation between estimates obtained by the NucleoCounter® SP-100™ and other cell counting methods may occur. As such, nothing contained or stated herein, including results obtained from use of the NucleoCounter® SP-100™ or SP1-Cassette™, shall be construed to imply any warranty or guarantee. ChemoMetec A/S and affiliated companies shall not be held liable for damages, and customers shall indemnify ChemoMetec A/S and affiliated companies against liability flowing from use of potentially inaccurate data generated by the NucleoCounter® SP-100™. It is recommended that all results obtained with the NucleoCounter® SP-100™ be validated against appropriate reference methods and/or traditional laboratory methods at regular intervals. For installation and operation of NucleoCounter® SP-100™ refer to appropriate documentation.

