



Cell Counting in 30 Seconds



Fig. 1: The NucleoCounter

The NucleoCounter from ChemoMetec presents a novel use of the technique of fluorescence microscopy for counting cell nuclei stained with Propidium Iodide. The instrument is a result of an interdisciplinary collaboration between electronics, mechanical engineering, chemistry, and cell biology. Abandoning the safe waters of conventional thinking, our research team has built a small, powerful and highly advanced fluorescence Microscope based on a CCD camera and integrated image analysis.

Worldwide there are a few approved methods for counting cells. One traditional method of cell counting is to lyse all cells and make a nucleus stain with for example Crystal Violet or Trypan Blue. With fluorescence microscopy, it is possible to use a fluorescent dye for staining the nuclei. A traditional fluorescence microscope, however, is generally expensive.

The NucleoCounter makes use of the fluorescent DNA probe Propidium Iodide (PI). PI offers several advantages, such as enhanced signal intensity when bound to DNA compared to non-bound PI, a property, which improves signal to noise ratio, especially when the background (medium) contains cell debris.

The NucleoCounter Instrument

The NucleoCounter is a small and compact fluorescence microscope, which allows the implementation of fluorescent application, comparable to conventional systems. The NucleoCounter has now made it possible for even small cell laboratories to perform a fast, objective and reliable automatic cell count using a nuclei staining concept that worldwide has been accepted for decades. NucleoCounter therefore also supports the "Point Of Care"-concept that states that a result of an analysis should be conceived as close (and quickly) as possible to the analysed specimen.

What is Inside the NucleoCounter?

The NucleoCounter system comprises a LED-based excitation light source, excitation and emission filters, optics, a CCD camera, and advanced electronics and integrated image processing combined in a novel and revolutionary manner. All this is built into a single unit, which is simple in operation and allowing a

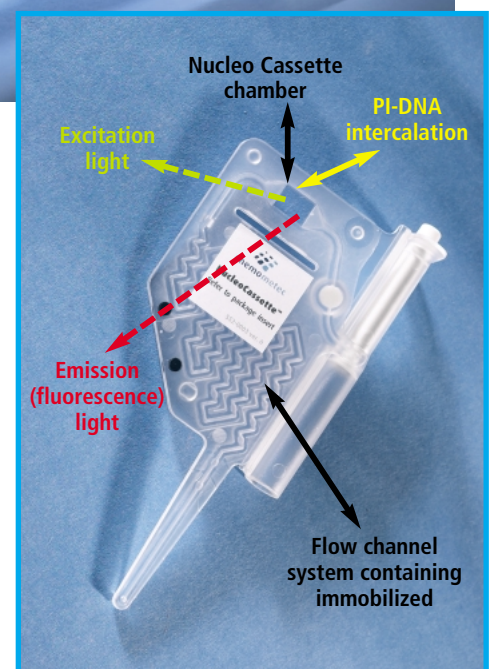


Fig. 2: NucleoCassette depicted indicating immobilization of Propidium Iodide and flow of sample to reading chamber. NucleoCassette depicted indicating immobilization of Propidium Iodide and flow of sample to reading chamber.

cell count to be carried out within 30 seconds! The images can be saved on a computer for documentation purposes through the built-in USB port.

Sample Pre-Treatment

Prior to analysis the cells are lysed using a simple lysing buffer. The addition of the lysing buffer and the mixing takes only few seconds and then the sample is ready for analysis. Cells often form aggregates or clusters. This phenomenon is a challenge to any cell counting method. The NucleoCounter offers an extremely



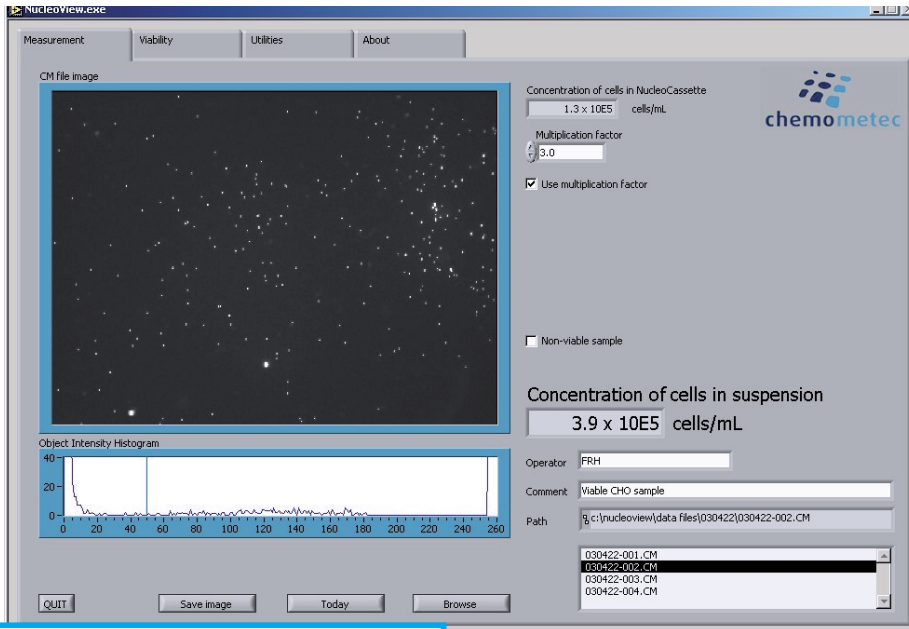


Fig. 3: The NucleoView software

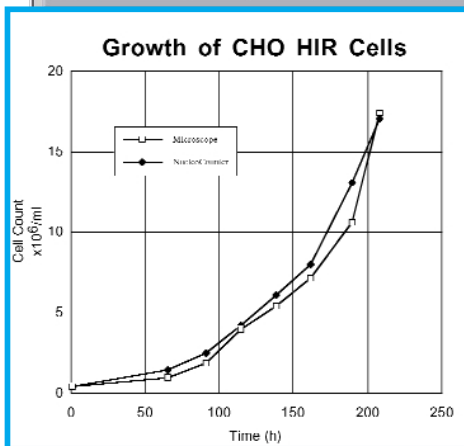


Fig. 4: Growth of CHO HIR cells

fast and reliable method for the disaggregation of the sample since only the nuclei of the cell are analysed. Therefore it is not necessary to preserve the cell membrane for the analysis. The disaggregation is achieved in only few seconds. A typical sample pre-treatment procedure is therefore completed within 30 seconds.

The NucleoCounter Cassette

The loading of a hemacytometer with a cell sample and the rinsing of the chamber after counting is no longer necessary. The NucleoCounter uses an ingenious disposable plastic device called the NucleoCounter Cassette. The cassette contains the nucleus dye PI on a form ready to be used. The cassette also contains means for the aspiration of the sample and mixing with PI, without any further handling of hazardous material. After mixing the cell suspension with PI, the sample mixture reaches a compartment that is similar to the chamber in a hemacytome-

ter. The main difference is that up to ten times volume is now available for analysis. This offers an inherent improvement of the statistical quality of the cell count since about ten times as many cells are counted. After a successful analysis performed by the NucleoCounter, the cassette can be disposed together with the used biological material.

NucleoCounter – Key Benefits

- Calibration free – the physical properties of the system are preserved.
- Fast Analysis – analysis is performed in about 1 minute, including pre-treatment.
- High Precision – CV is typically less than 5%.
- Objectivity – no more variation between technicians or laboratories.
- Simplicity – minimum training of the operator.
- Reliability – no internal flow system to be contaminated or blocked.
- High Specificity – only nuclei will be stained and counted.
- Not Cell Specific – the cell count is based on the number of nuclei, not morphology or cell size.
- Software – the NucleoView software facilitate easy documentation and transfer of data

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