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Image Cytometry: A New Approach to Advance Cell Analytics without FLOW

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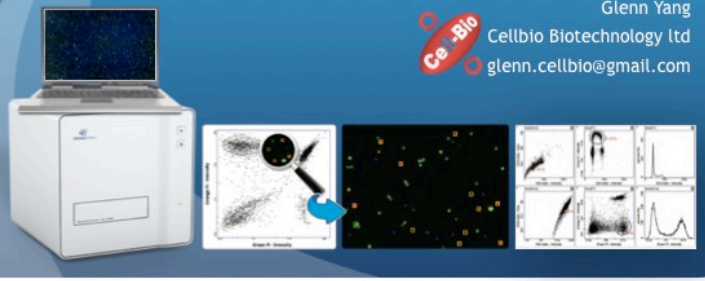

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Cytometry ? A Definition

- Cytometry is a general name for a group of biological methods used to ***measure various parameters of cells***.Parameters which can be measured by cytometric methods are :
  - Cell number
  - Cell size
  - The stage of the cell cycle
  - The DNA content of the cell
  - The existence or absence of specific proteins on the cell surface or in the cytoplasm
  - More

Cyto-Meter

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2 Major Platform

	FLOW Cytometry	IMAGE Cytometry
State	FACS ,a well known method exist >30 years	A new approach raised last 15 years
Target	Liquid base	Slide base
	Floating	Fix static
	Suspension cell(e.g. blood)	Adherent cell
Measure	Scatter information Fluorescents intensity	Morphological information Fluorescents intensity
Throughput	High 10^4~10^6 cells are analyzed	Low~Mid 10^2~10^4 cells are analyzed

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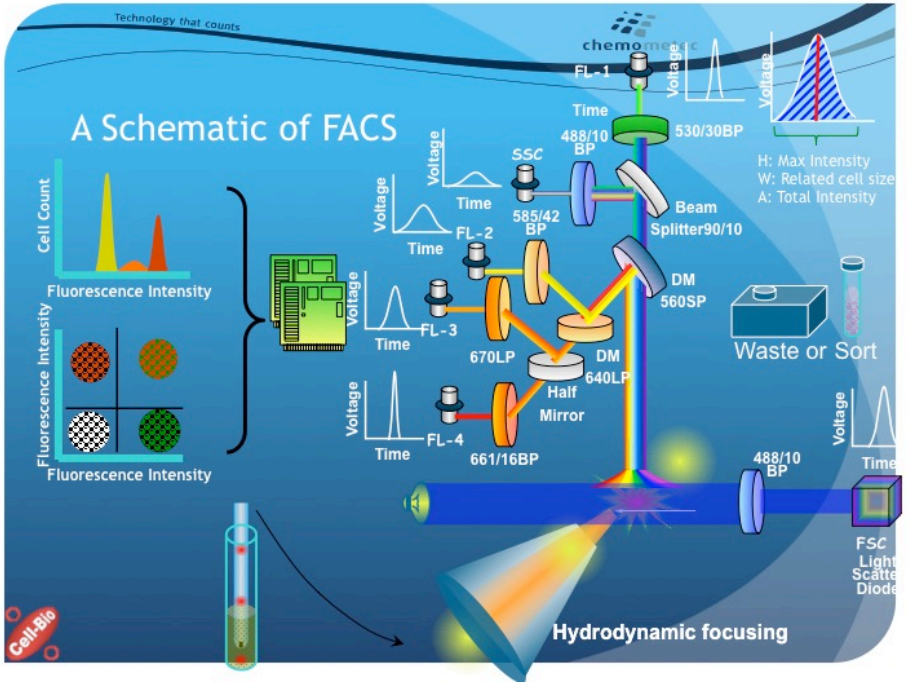
A Schematic of FACS



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Benefit and limitation of FACS

- Well known technology
- High Throughput
- High sensitivity
- Multichannel
- Statistical data
- Plenty Application
- Sorting target cell

- FLOW PROBLEM:
  - Single suspension cell only
  - Flow speed v.s C.V
  - Clogging
  - Washing & rinsing
  - Maintenance & calibration
- High sample volume and number
- No morphological information
- No distribution information
- Expensive
- Experience-steep learning curve

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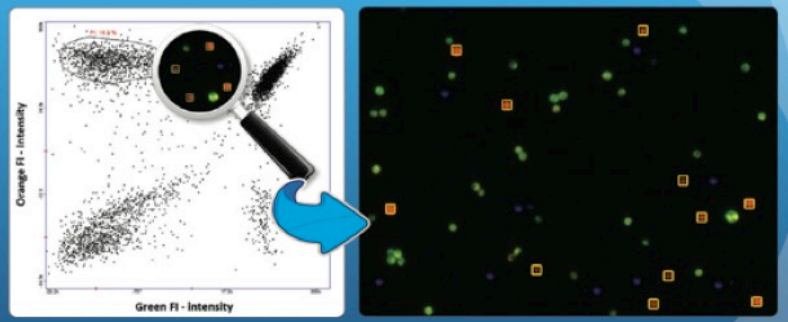
Cytometer without FLOW



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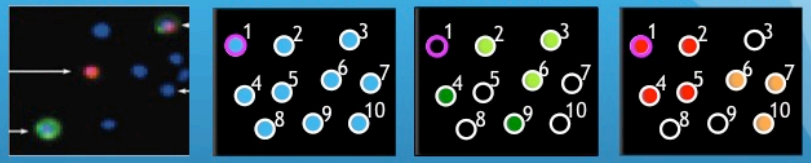
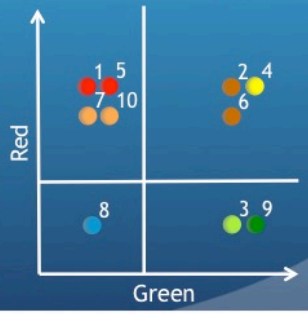
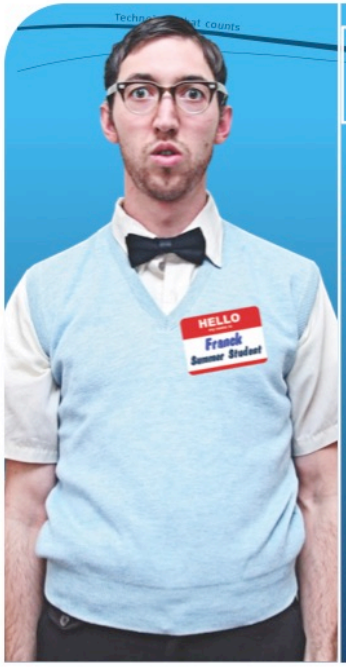
Principle of Image cytometry equivalent to FACS

Merge color | Counterstain | Green Channel | Red Channel



	Blue	Green	Red
Cell#1	+	-	+
Cell#2	+	+	+
Cell#3	+	+	-
Cell#4	+	+	+
Cell#5	+	-	+
Cell#6	+	+	+
Cell#7	+	-	+
Cell#8	+	-	-
Cell#9	+	+	-
Cell#10	+	-	+





Dear Scientists!

Now you can trust your advanced cell analysis to your new summer student with *Chemometec NC3000*!!!

- Easy to use
- Validation protocol
- Consistence
- Gentle learning curve
- No FLOW problem
- No maintenance
- No Calibration

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Why can I trust NC-3000?

Reliable hardware	Validated sampling media	Validated reagents	Validated assay protocols

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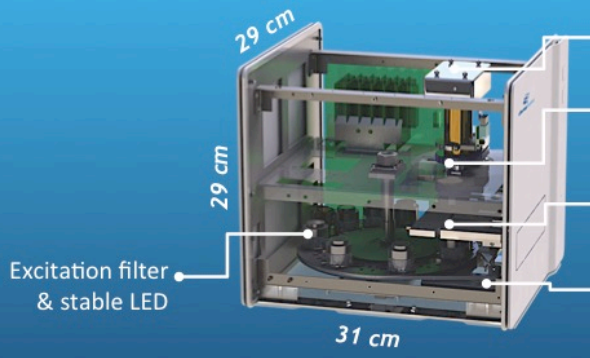
Reliable hardware



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Validated sampling media

- Pre-validate volume
- Capillary
- High quality glass
- Auto florescence-free glu

	A8 Slide	A2 Slide	Cassette
Chambers per slide	8	2	1
Loading volume	9 µl/well	30 µl/well	60 ul
Field of view	1-6	1-20	1-4
Analyzed volume	0.8-4.8µl	0.8-16µl	1-4µl
Counting event per well	320-32000	800-80000	200-20000
Application	High throughput Image cytometry	High precision Image cytometry	User independent Cell counting and Viability check

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Chemometec Pre-Validated Solution

Solution 2 - Propidium Iodide Staining Sol. - 25 mL
Solution 3 - DAPI Staining Solution - 25 mL
Solution 5 - VB-48•PI•AO - 1 mL
Solution 7 - JC-1 - 1mL
Solution 8 - DAPI•PBS Staining Solution - 25 mL
Solution 10 - Lysis Buffer, 100 mL
Solution 11 - Stabilization Buffer , 100 mL
Solution 12 - DAPI, 1mL
Solution 13 - AO•DAPI - 1 mL
Solution 15 - Hoechst - 1 mL
Solution 16 - Propidium - 1 mL
Solution 17 - Blood Lysis Buffer 25 mL

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Chemometec Pre-Validated Protocol

- Viability and Cell Count
- Two-step Cell Cycle
- Cell Cycle of Fixed Cells
- Mitochondrial Potential-JC-1
- DNA Fragmentation
- GFP Transfection Efficiency
- Annexin V & PI
- Caspase 3/7, 8 & 9

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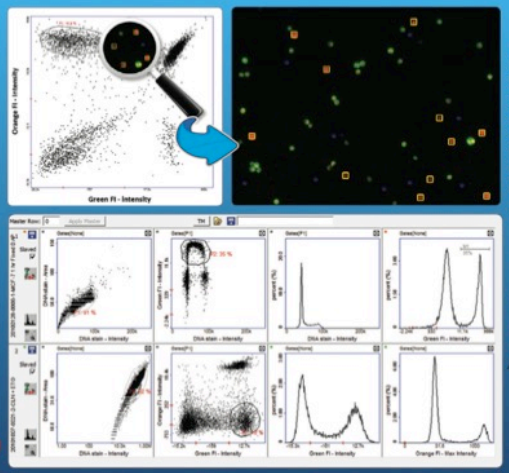
Data Display
Image analysis equivalent to FACS



After image acquisition cell data are presented in the PlotManager as either scatter plots or histograms or both. In the plot manager data can be further processed and analyzed, and facilitates e.g. counting, gating and spectral compensation. Cells in selected gates can be visually inspected in the image.

All data can be exported as FCS3.0 file format allow user use external programs such as FCS Express 4 Image Cytometry etc.

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Application

- Cell absolute count & viability
- Cell cycle
- Apoptosis
- Transfection efficiency
- FlexiCyte User Adaptable protocol

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User Work Flow

Prepare cell sample

Turn on NC-3000™ and PC

Place cassette/NC-Slide in sample tray

Select type of application and press run

Computed data are displayed on PC

What happens inside the NC-3000™

NC-3000™ selects appropiate exposure time, LEDs and filters

NC-3000™ captures image(s)

NC-3000™ calculates data

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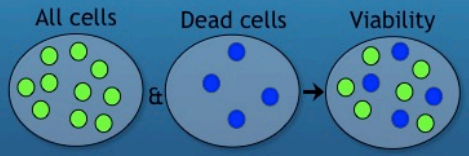
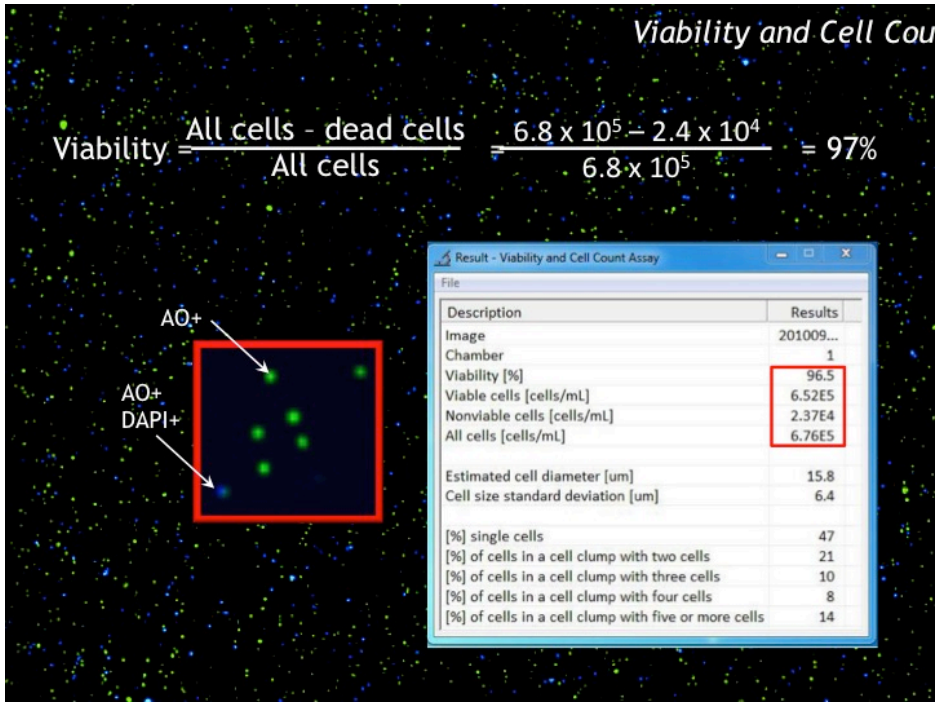
Viability and cell count assay

- Harvest cells
- Load cells into Via1-Cassette
- Image cytometry

- ✓ Analysis < 1 min!
- ✓ No pretreatment, no calibration
- ✓ High accuracy, high reproducibility
- ✓ Automatic data collection
- ✓ Objective analysis





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2-step cell cycle assay

- Lysis buffer + DAPI
- Stabilization buffer
- Image cytometry

- 5-minute analysis
- Acquisition, analysis and data presentation in one step
- No cell detachment, no RNase treatment, no calibration
- Standardized results

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Fixed cell cycle & DNA fragmentation assay

- Ethanol fixation
- Wash with PBS
- Stain cells with DAPI
- Image cytometry

- Robust and simple protocol with predefined settings
- Acquisition, analysis and data presentation in one step
- No RNase treatment, no calibration
- Standardized results

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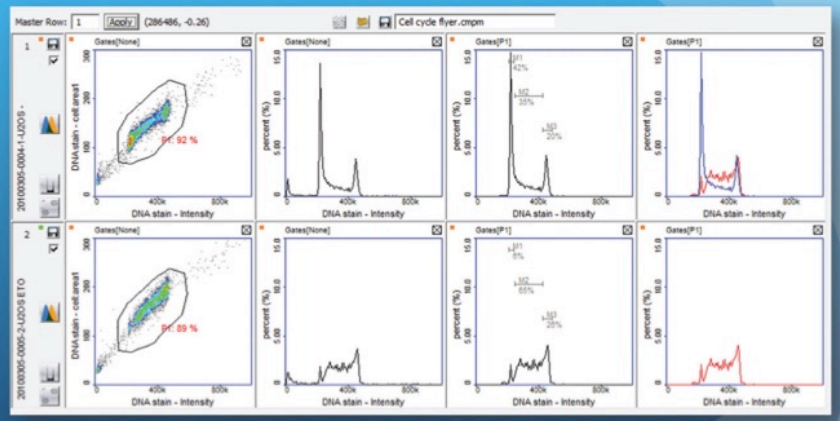
Cell cycle & DNA fragmentation assay



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Apoptosis - time line

Time	Cell function changes		Corresponding assay
Start ↓ End	Mitochondrial membrane potential	↓	Mitochondrial potential Assay
	Mitochondrial transition pore	↑	
	Phosphatidyl translocation		Annexin V
	Caspase activity	↑	Mulitiple FLICA assay
	Metabolic activity	↓	Vitality assay
	DNA condensation	↑	Combined Hoechst /PI stain
	Plasma membrane integrity	↓	Viability and cell count, DAPI, PI
	DNA fragmentation	↑	DNA Fragmen-taion assay

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Add JC-1 staining solution (10 min.)

↓

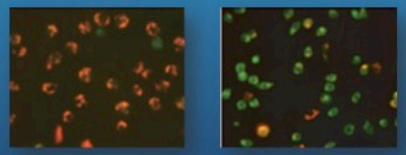
Wash and resuspend cells in DAPI staining solution

↓

Image cytometry

Mitochondria Potential

- Fast, automated, pre-defined and robust
- Acquisition and analysis in one step
- No calibration
- Easy discrimination between polarized (healthy) cells, depolarized (apoptotic) cells and necrotic/late apoptotic cells



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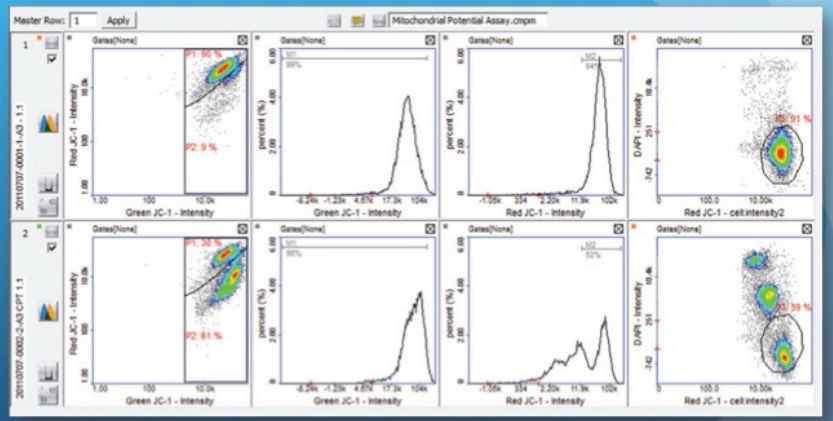
Mitochondrial Potential



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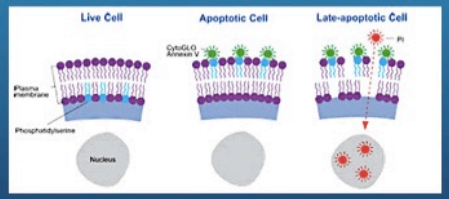
Add Annexin V-FITC conjugate and Hoechst (15 min.)

Wash and resuspend cells in PI-containing buffer

Image cytometry

Annexin V assay

- Fast automated single cell analysis
- Acquisition, analysis and data presentation in one step
- Well-recognized assay
- User friendly protocol with predefined settings
- Standardized results
- No calibration



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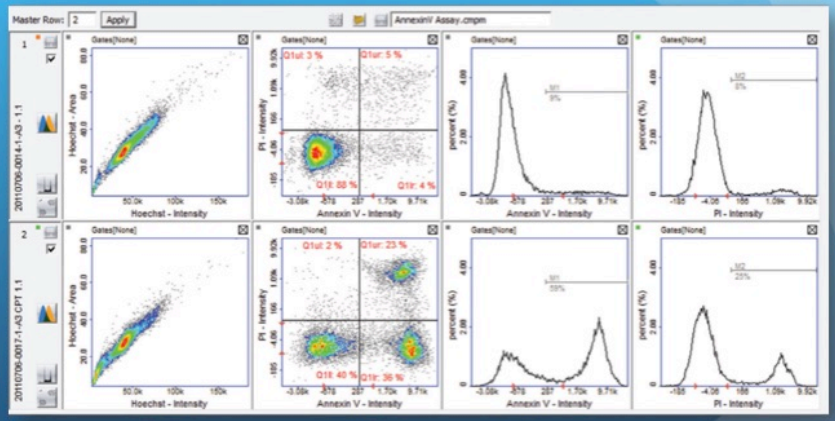
Annexin V assay



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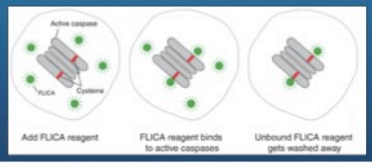
Add FLICA Reagent & Hoechst 33342 (1 hr.)

Wash and resuspend cells, add PI

Image cytometry

Caspase 3/7, 8 & 9

- Fast, automated, pre-defined and robust
- Well-recognized assay
- No calibration
- Easy measurement of Caspase 3/7, Caspase 8 or 9 information on single cell level
- Provide information about early, late apoptotic and necrotic cells
- Standardized results



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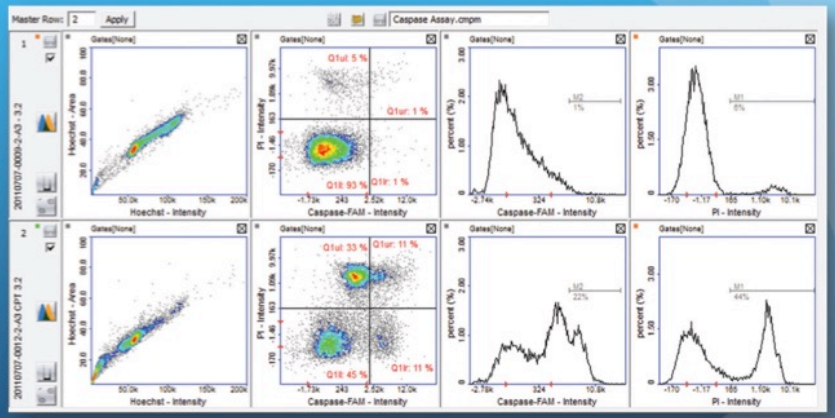
Caspase 3/7, 8 & 9



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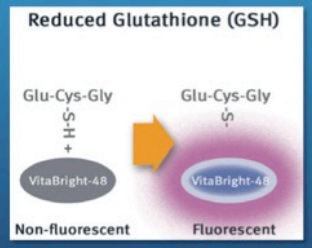
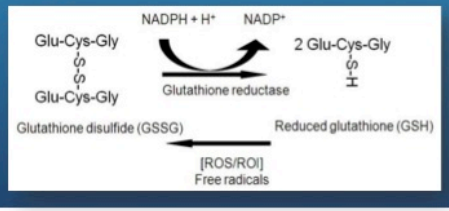
Add VitaBright-48

Image cytometry



Cell vitality assay

- Evaluation of cellular health, oxidative stress and indirect detection of apoptosis
- Fast, automated, pre-defined and robust
- Automated detection of changes in the level of reduced thiol
- Assay < 1 minute!



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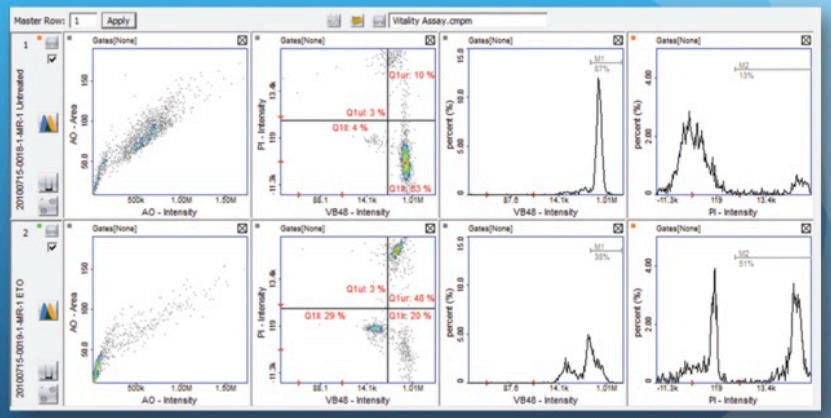
Cell vitality assay



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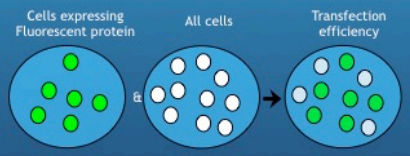
Harvest transfected cells

Add viable stain (VB-48)

Image cytometry

GFP Transfection Efficiency

- User friendly protocol with predefined settings
- Fully automated analysis procedure
- GFP intensity histogram displayed
- Objective analysis - standardized results
- Assay < 1 min!



$$\% \text{ transfection efficiency} = \frac{6}{10} \cdot 100\%$$

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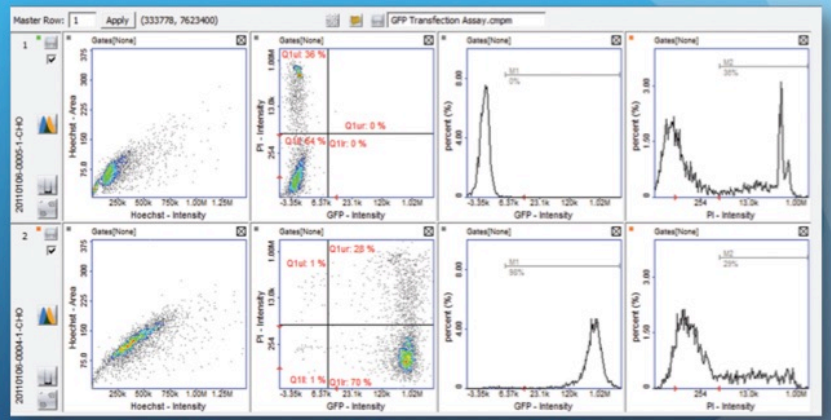
GFP Transfection Efficiency



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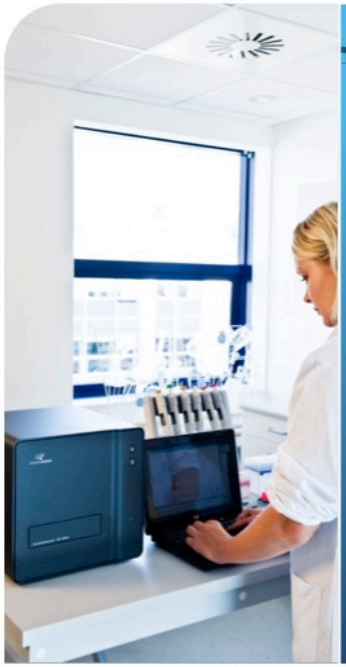

FlexiCyte™

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User Adaptable Protocol

1~3 marker analysis just so easy!

	Blue Channel	Green Channel	Orange Channel	Red Channel
Light source Emission filter	Ex365 Em470/55	Ex475 Em560/35	Ex530 Em675/75	Ex630 Em740/60
Non-fixed cells	Hoechst-33342	Biomarker 1	Biomarker 2	Biomarker 3
Fixed cells	DAPI	Biomarker 1	Biomarker 2	Biomarker 3
Recommended fluorophores	NA	e.g. AlexaFluor488, Atto488, DyLight488, FluoProbes488,CF488a	e.g. AlexaFluor568, Cy3, atto565, DyLight549, FluoProbes547H, CF568	e.g. AlexaFluor647, Cy5, atto647N, DyLight649, FluoProbes647H, CF647
Classical fluorophores	NA	e.g. FITC, PE	e.g. PE, TRITC	e.g. APC
Nucleic acid stains	NA	e.g. AO	e.g. PI, 7-AAD, EtBr	e.g. Draq5, Draq7, Nuclear-ID Red
Fluorescent proteins	NA	GFP and green and yellow variants	DsRed, RFP and orange/red variants	FlexiCyte

Ex: 365nm ; Em: 430/20nm Em: 470/55nm Em: 475/15nm Em: 560/35nm Em: 675/75nm Em: 630nm LP Em: 740/60nm	Ex: 405nm ; Em: 475/15nm Em: 530/15nm Em: 560/35nm Ex: 530nm ; Em: 630nm LP Em: 740/60nm Em: 675/75nm Ex: 630nm : Em: 740/60nm
Ex: 475nm ; Em: 560/35nm Em: 580/25nm Em: 675/75nm	

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Summing Up...

The NucleoCounter® NC-3000™

- 7 colors, 9 filters from UV to far red
- Create user adaptable protocols with FlexiCyte™
- Easy to operate - no training, calibration or service
- No flow, no rinsing, no clogging
- Superior data visualization with the PlotManager
- Export your data to FCS Express 4 Image Cytometry
- Get Visual inspection of your cells
- Predefined assays, including high-speed cell count
- Low costs per sample

AUTOMATED IMAGE CYTOMETRY

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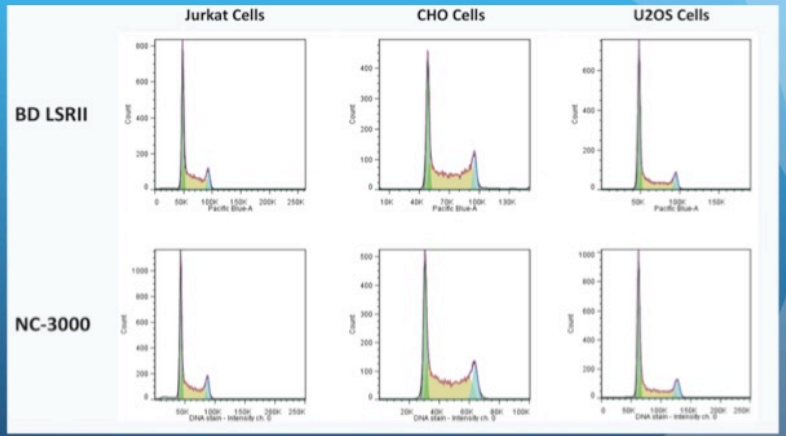
FAQ 2: Can we trust the data ?

Comparison Study of Chemometec NC3000 v.s BD LSRII



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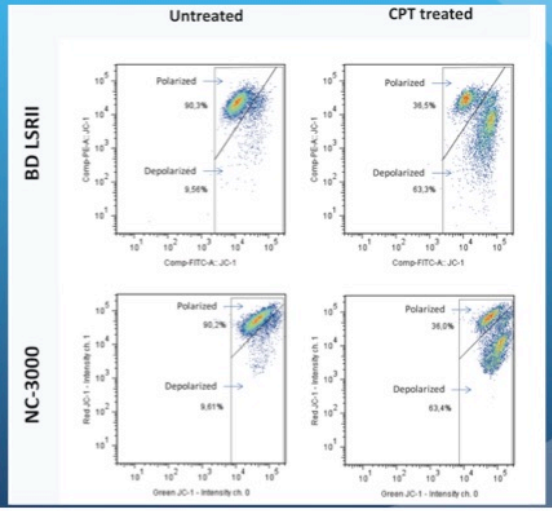

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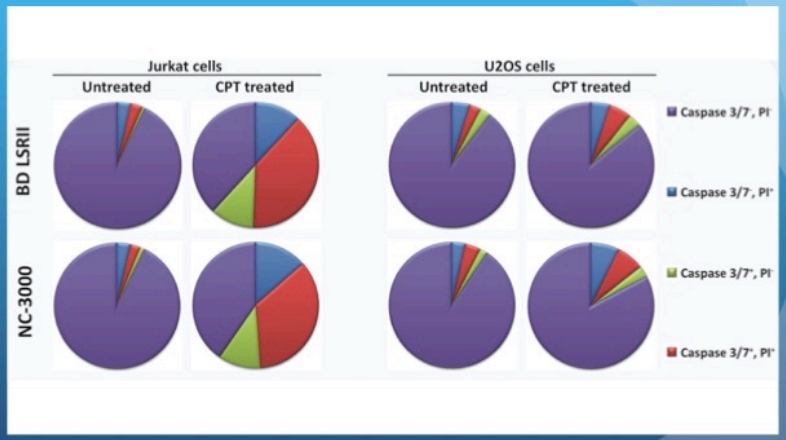

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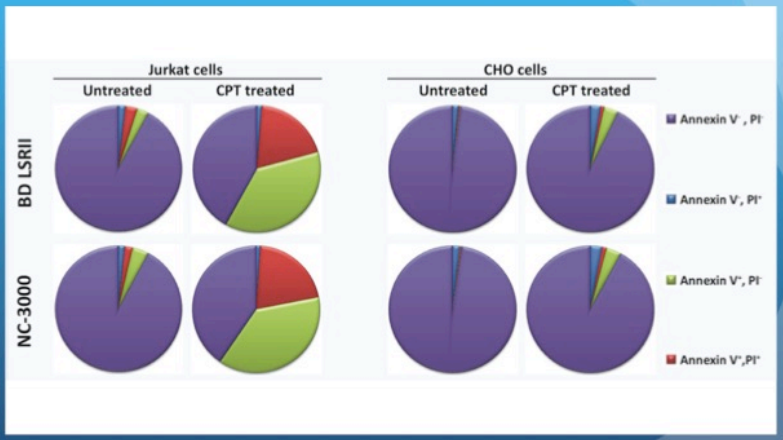

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