Countstar
Smart Cell Analysis

Countstar® Rigel Series

The Fluorescence Cell Analyzer

Introduction

Introducing the Countstar Rigel Series, a line of instruments with an innovative combination of advanced technologies. The Countstar Rigel brings together the functionalities of digital microscopes, flow cytometers and automated cell counters into this intuitively designed systems. By combining bright-field and fluorescent imaging with classical dye-exclusion technologies, Rigel systems generate essential data about cell morphology, diameters, and aggregation in real time.

Our Countstar Systems acquire high-resolution images, the essential basis for sophisticated data analysis. With more than 2,000 systems installed worldwide, Countstar products have proven themselves as valuable tools in research, process development, and validated production environments.

The Countstar brand was inspired by the endless possibilities a person experiences when counting stars in the night sky. Following this approach, Countstar explores the limits of technology. Countstar was founded by ALIT Life Science, an emerging manufacturer of innovative equipment and consumables for the biological research community. Headquartered in the high-tech district of Shanghai, ALIT Life Science develops and produces the analytical equipment of the future.

CONTENT

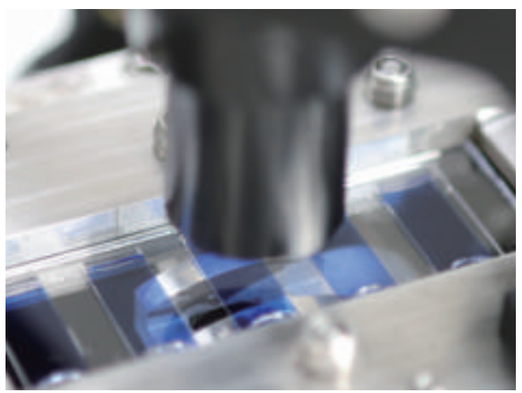
Countstar® Rigel Series Fluorescence Cell Analyzer

The Countstar Rigel system combines up to four separate fluorescence channels with a bright-field digital microscope. The Countstar Rigel analysers are modularly designed digital microscopes, image cytometers, and automated cell counters in one integrated bench-top instrument platform. This application-driven, compact, and automated imaging system provides an all-in-one solution for cell counting, cell viability, cell apoptosis, cell cycle and T/NK cell mediated cytotoxicity using our unique Countstar BioApps. The Countstar Rigel system provides standardized, GMP – compliant solution for cell quality control.

Features

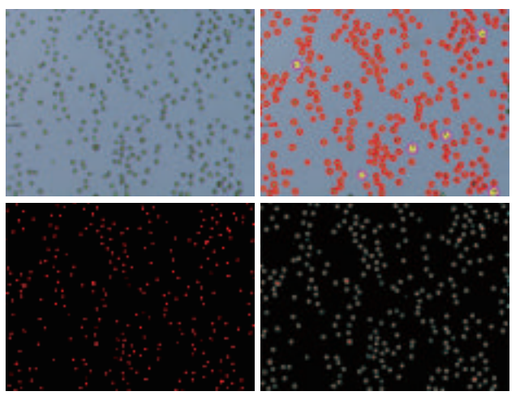
- Extremely compact, all-in-one design with a sensitive touchscreen
- Automated, consecutive analysis of five samples in a single sequence
- Verify results with the acquired images
- Minimal sample volumes (20μL)
- Compliant with cGMP and FDA 21 CFR Part 11
- Customizable BioApps allow multi-channel analyses
- Provided with a variety of validated BioApps for different assays such as AO/PI, Apoptosis, Cell Cycle, GFP Transfection, CD Marker, etc...

Our Patented Fixed Focus Technology



The Countstar Rigel contains an optical bench based on our "fixed focus" patent. There is no need at any time for the operator to focus when analyzing.

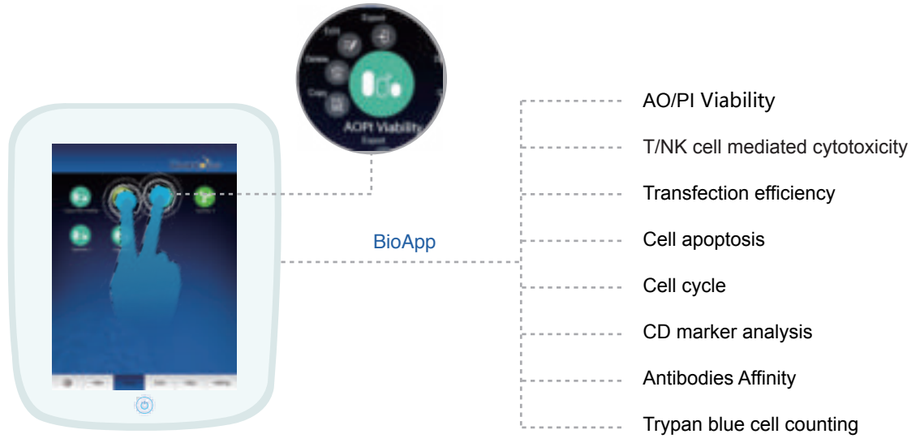
Our Innovative Image Recognition Algorithms



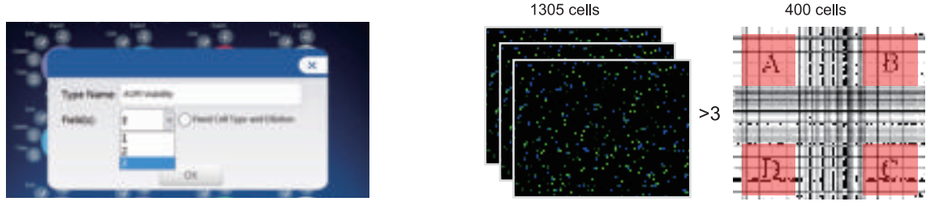
Our protected image recognition algorithms analyze more than 20 single parameters of each classified object.

All-in-one,compact design

User friendly and flexible BioApp-based cell analysis Protocols



Capable of analyzing up to 3 fields of view per sample with high repeatability from sample to



Up to 4 LED Wavelengths for up to 13 fluorescence channel combinations

- Available with up to 4 LED excitatiion wavelengths and 5 detection filters, allowing for 13 different combinations of fluorescent analysis.

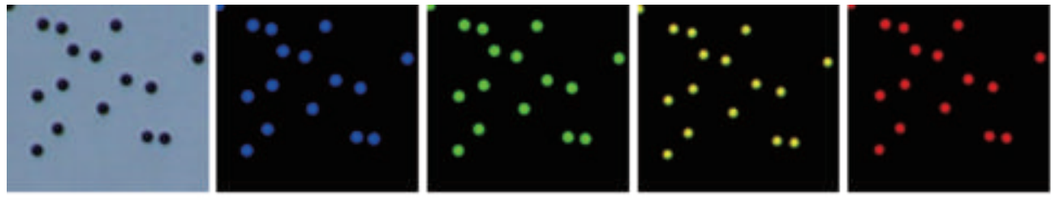
Specification	Excitation LED	375	480	525	620
Detectors	460/50	●			
	535/40	●	●		
	580/25	●	●	●	
	600LP	●	●	●	
	665LP	●	●	●	●

- Filter combinations of the Countstar Rigel series for popular fluorophores

Excitation Light(nm)	Emission Filter(nm)	Fluorescence Dye/Protein
480nm	535nm	AO, FITC, GFP, CFSE, Alex Fluor 488
525nm	600LP	PI, RFP, Alexa Fluor® 546, Fluor® 555, Cy®3, DsRed, Rhodamine Red, dTomato
480nm	600LP	ECD, PE-Texas Red, PE-CF594
375nm	460nm	DAPI, Hoechst, BFP
375nm	535nm	Amcyan, Brilliant Violet™ 510
375nm	600LP	Qdot® 605
375nm	580nm	Pacific Orange™, Brilliant Violet™ 570
480nm	580nm	PE
525nm	580nm	PI
375nm	665LP	Brilliant Violet™ 650
480nm	665LP	PC5, PC5.5, PerCP,PerCP-Cy5.5, PI, 7-AAD
525nm	665LP	7-AAD, Nile-Red, Alex Fluor 647-PE
625nm	665LP	APC, Alex Fluor 647, Alex Fluor 660

Countstar® Rigel S2
Countstar® Rigel S3
Countstar® Rigel S5
Countstar® Rigel S6

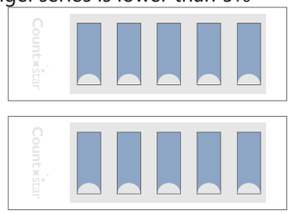
- Acquisition of bright-field and up to 4 fluorescent images automatically in a single test sequence



Bright Field | Ex:378nm Em:445nm | Ex:480nm Em:535nm | Ex:480nm Em:580nm | Ex:535nm Em:615nm

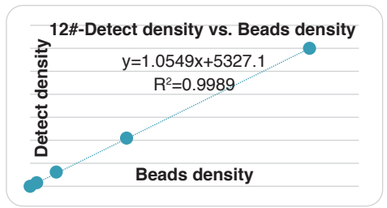
Stable Cell Counts/High Repeatability

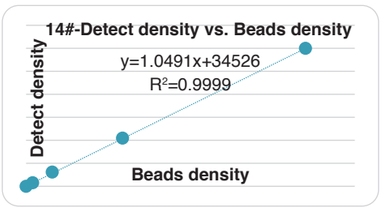
At the cell concentration range of 2.0×10^5/mL to 1.0×10^7/mL, the CV of cell concentration and viability analyzed by the Countstar Rigel series is lower than 5%

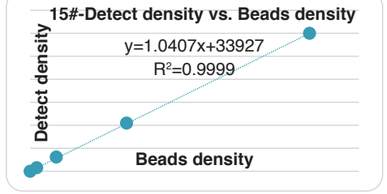


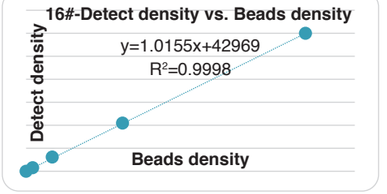
Stability test between chambers CV<5%
Stability test between slides CV<5%

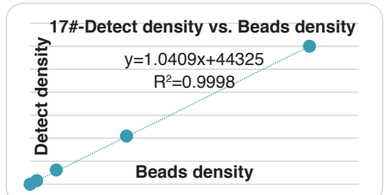
Stability test between Countstar® Rigel analyzers CV<5%

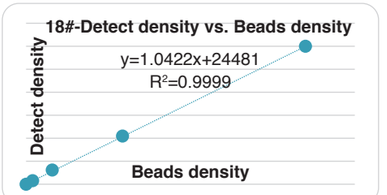












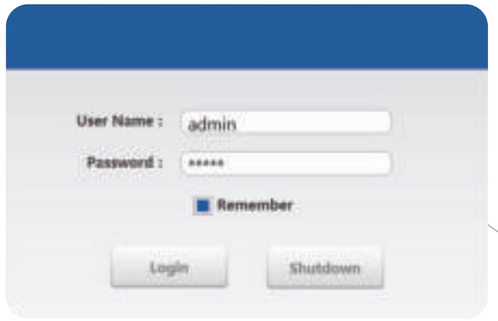
Accuracy and Reproducibility Test between 6 Countstar® Rigel analysers

Flow Cytometry Software (FCS. optional)

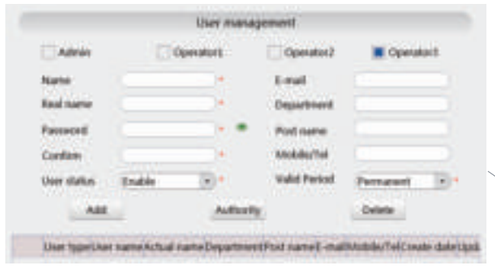
The FCS Express Image 6 was developed by De Novo Software to analyse and quantify fluorescence image data.
The optional De Novo FCS Express software transforms your images into highly dynamic data. The FCS software allows for in-depth analysis of populations using the Countstar BioApps and customizable fluorescence channels boost your experimental reach. Countstar Rigel together with FCS Express allows the user to efficiently analyze data for for cell apoptosis, cell cycle, transfection, antibodies affinity, CD marker, etc…

FDA 21 CFR Part 11

To meet the requirements of cGxP regulated environments, the Countstar Rigel systems can be operated in full compliance with the regulations of the FDA's 21 CFR Part11：



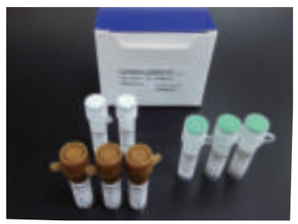
User Login



Four-level user access management

E-Signatures and Log Files

IQ/OQ/PQ Validation and Standard Particles



Standard Beads

IQ/OQ/PQ Document

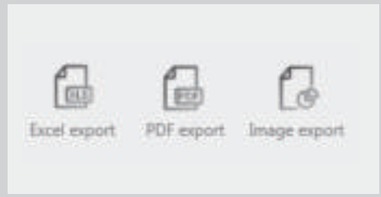
Data Management

The Countstar Rigel system uses a built-in database with a sophisticated and ergonomic design. It gives the operators maxiumum flexibility in regards to the data storage, while ensuring safe and traceable handling of results and images

Data Storage

With 500GB of hard disc drives, the Countstar® Rigel stores up to 160,000 complete sets of experimental data including images

Data Export

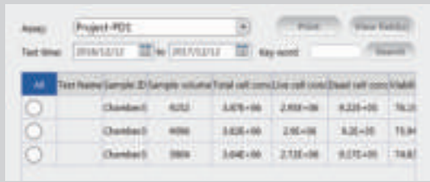


Choices for data output include PDF, MS-Excel, and JPEG files. All of which are easily exported using the included USB2.0 & 3.0 external ports

BioApp/Project Based Data Management

New experiment data are sorted in the database by their BioApp Project name. Consecutive experiments of a project will be linked to their folders automatically, allowing a fast and secure retrieval.

Easy Retrieval



Data can be selected by experiment or protocol name, analysis date, or keywords. All acquired data can be reviewed, re-analyzed, printed and exported in various formats.

The Countstar® Rigel Models

Countstar Rigel S2

The Countstar Rigel S2 System combines two fluorescence plus and a bright-field channel in its digital microscope . It provides an all-in-one solution for cell counting, cell viability, and transfection efficiency. The pre-configured assay protocols, named BioApps, guarantee for an easy execution of the tests. The BioApps have been optimized for the analysis of primary cell material, peripheral blood samples , CAR-T, NK, and MSC cells, monitored and tests frequently in cell based therapy.

Excitatiion light: 480nm, 525nm
Emission filter: 535nm, 600LP

Countstar Rigel S3

The Countstar Rigel S3 System combines three fluorescence and a bright-field channel. It combines a digital microscope, an image cytometer, and a cell analyzer in a single bench-top instrument. This application-driven, compact, automated cell imaging system provides an all-in-one solution for cell counting, cell viability and T/NK cell mediated cytotoxicity through the use of preconfigured biological applications (BioApps). They are designed to simplify routine cell lab tasks, which leaves you time to focus on your research!

Excitatiion light: 375nm, 480nm, 525nm
Emission filter: 460nm, 535nm, 600LP

Countstar Rigel S5

The Countstar Rigel S5 system increases the PE channel on the basis of Rigel S3, and can carry out a wide span of multi-color fluorescence experiments including rapid cell density and viability measurements, cell apoptosis courses (Annexin V / PI / DAPI), cell transfection efficiency (GFP / RFP / YFP) , CD marker phenotyping (DAPI / FITC / PE) , cell killing (Hoechst T33342/Calcein-AM/PI) and more.

Excitatiion light: 375nm, 480nm, 525nm
Emission filter: 460nm, 535nm, 580nm, 600LP

Countstar Rigel S6

The Countstar Rigel S6 is an automated image-based cell analysis platform, combing the characetristics of a digital fluorescence microscope, with a four-channel cytometer. In combination with DeNovo FCS Image 6 software,the Rigel S6 offers an in depth analysis of data. The Countstar Rigel S6 equipped with 4 fluorescence channels, allowing for 13 different fluorescent analysis combinations, supporting virtually any dye at different wavelengths.

Excitatiion light: 480nm, 525nm, 375nm, 620nm
Emission filter: 460nm, 535nm, 580nm, 600LP, 665LP

Experimental Assay	Countstar Rigel S2	Countstar Rigel S3	Countstar Rigel S5	Countstar Rigel S6
Trypan Blue Cell Count	√	√	√	√
Dual-fluorescence AO/PI method	√	√	√	√
Cell cycle(PI)	√*	√*	√	√
Cell Apoptosis(Annexin V-FITC/PI/Hoechst)	√*	√*	√	√
Cell Apoptosis(Annexin V-FITC/PI/Hoechst)		√*	√	√
GFP Transfection	√	√	√	√
YFP Transfection			√	√
RFP Transfection	√	√	√	√
Cell Killing(CFSE/PI/Hoechst)		√	√	√
Antibodies Affinity (FITC)	√	√	√	√
CD Marker Analysis(three channel)			√	√
CD Marker Analysis(four channel)				√
FCS Express Software	optional	optional	√	√

√*. This mark indicates that the instrument can be used for this experiment with the optional FCS software

Technical Specification

Diameter Range	3μm ~ 180μm
Concentration Range	1×10^4 ~ 3×10^7/mL
Objective magnification	5x
Imaging technology	1.4-megapixel CCD color camera
USB	1×USB 3.0 1×USB 2.0
Storage	500GB
Power supply	110–230 V/AC, 50/60Hz
Screen	10.4 inch ultrasensitive touchscreen
Weight	13kg (28lb)
Size(W×D×H)	Analyser: 254×303×453mm Package: 430×370×610mm
Operating temperature	+10 ℃ ~ +40 ℃
Working humidity	20% ~ 80%

Applications

Dual-fluorescence Viability(AO/PI)

Acridine orange (AO) and propidium iodide (PI) are nuclear staining and nucleic acid binding dyes. AO can penetrate the membrane of both dead and living cells and stains the nucleus, generating a green fluorescence. In contrast, PI can only permeate the disintegrating membranes of dead nucleated cells, generating a red fluorescence. The image based technology of the Countstar Rigel excludes cell fragments, debris and artifact particles as well as undersized events such as platelets, giving a highly accurate result. In conclusion, the Countstar Rigel system can be used for every step of the cell manufacturing process

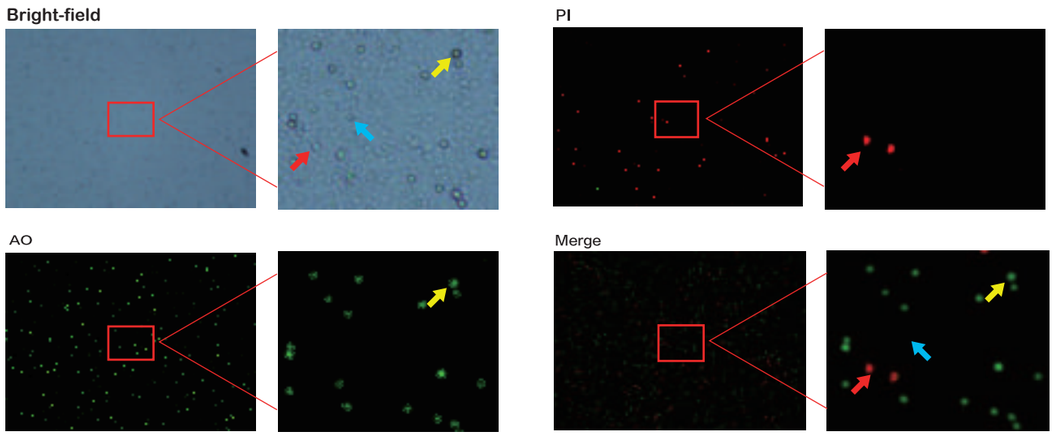
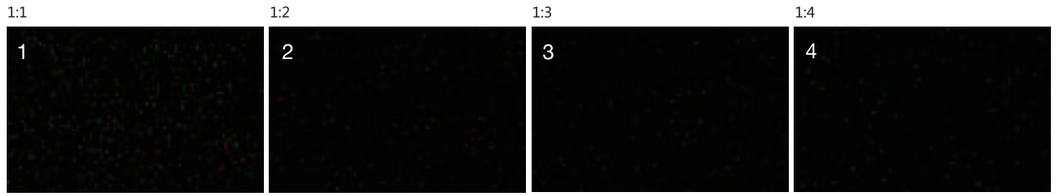
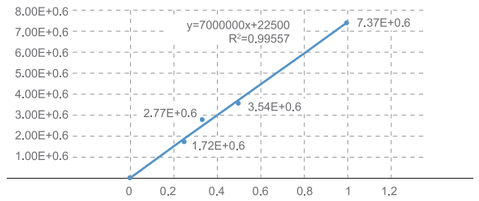


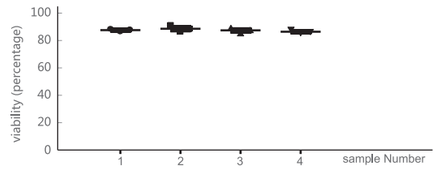
Figure 1 Images of PBMC stained by AO/PI dye, acquired by a Countstar Rigel S2



Images series of a dilution gradient of PBMC



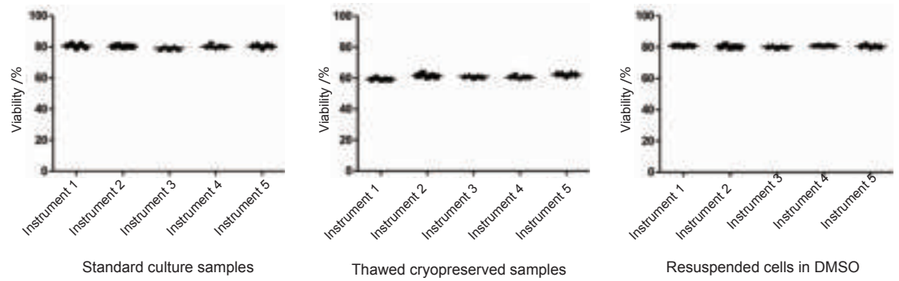
Results of a gradient dilution series of labeled PBMCs, analyzed by a Countstar Rigel S2. Data show an excellent linear correlation



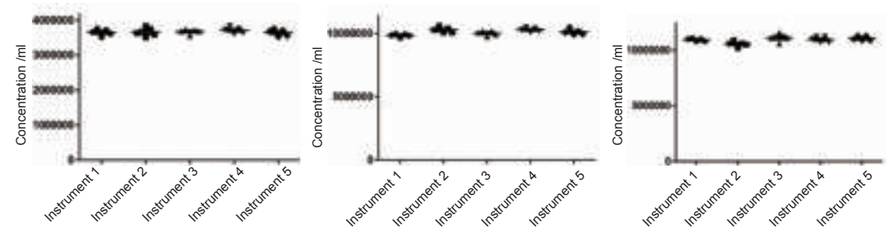
Check viability of aliquots in the gradient dilution series of PBMCs. Data show a very low CV between the different measurements

Figure 2 Determination of cell viability and concentration after a gradient dilution of PBMCs

T Cells at different stages(normal culture,thawed after cryopreservation,and resuspended in DMSO).Each sample was tested 5 times in five different Countstar Rigel S2 analysers



The stability of different stages of samples between 5 different instruments, CV of concentration result <5%



T/NK Cell Mediated Cytotoxicity

In the recently FDA-approved CAR-T cell therapy, genetically engineered T-lymphocytes bind specifically to the targeted cancer cells (T) and kill them. The Countstar Rigel analyzers are able to analyzed this complete process of T/NK Cell Mediated Cytotoxicity

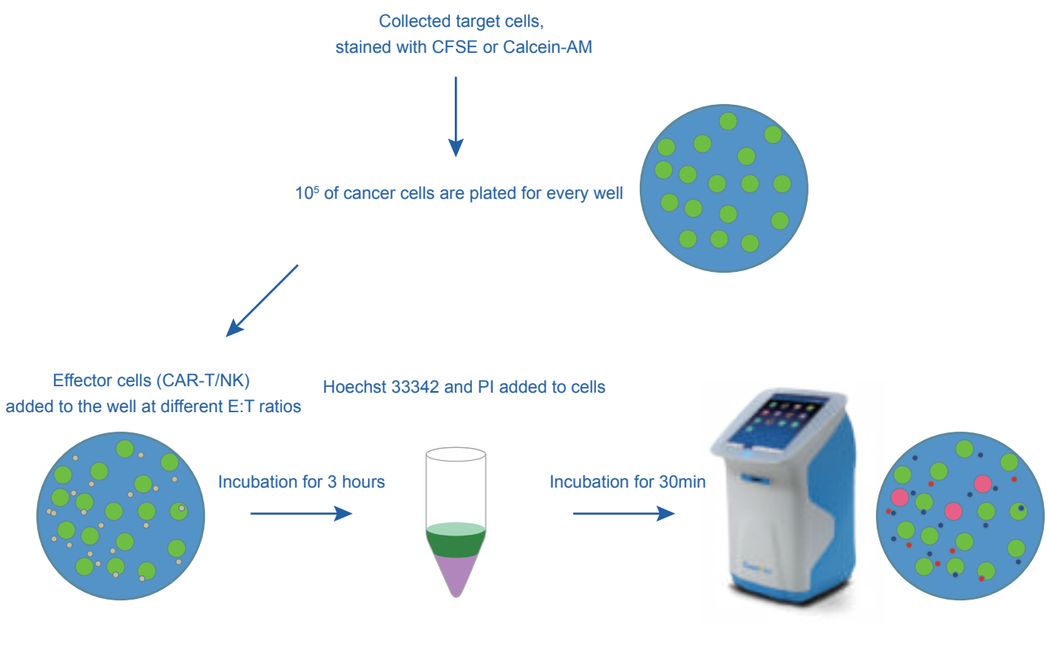
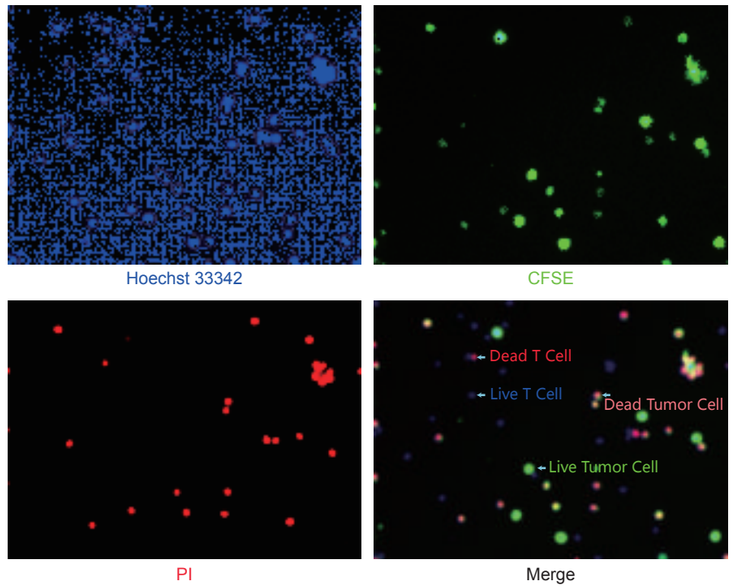


Figure 1. Experimental Procedure

（Alternative setup: if eGFP transfected target cells are used, an upstream staining by CFSE or Calcein-AM is not mandatory ）

Cytotoxicity studies are performed by labeling the target cancer cells with CFSE or transfecting them with GFP. Hoechst 33342 may be used to stain all cells (both T cells and tumor cells). Alternatively, target tumor cells can be stained with CFSE. Propidium iodide (PI) is used to stain dead cells (both T cells and tumor cells). Discrimination between different cells can be obtained using this staining strategy.



Live tumor cell: Hoechst 33342+CFSE+PI-

Live T cell: Hoechst 33342+CFSE-PI-

Dead tumor cell: Hoechst 33342+CFSE+PI+

Dead T cell: Hoechst 33342+CFSE-PI+

Figure 2 Images and merged overview of a cell killing experiment, acquired and analysed by the Countstar Rigel S3

Cytotoxicity% =（Number of live cells in the control group– Number of living cells in the target experiment）
/Number of live cells in the control group x 100%

E: T Ratio dependent Cytotoxicity of K562. As the E:T ratios increasing the dead tumor cell increases.

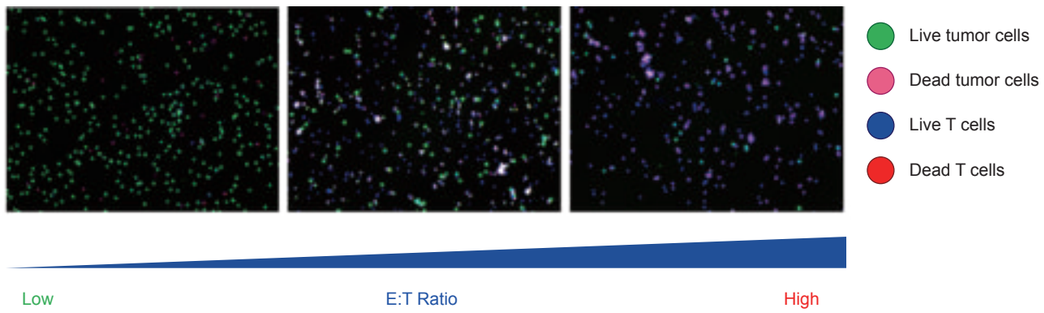
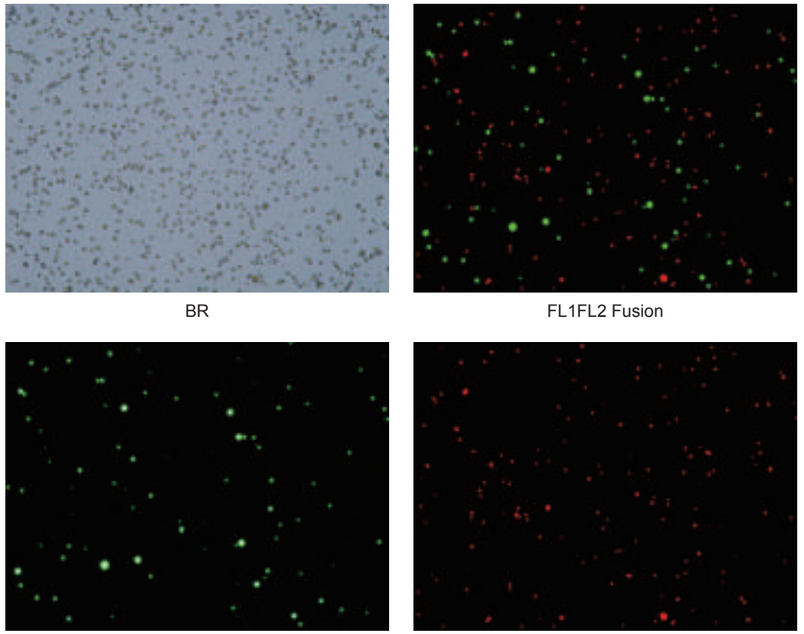


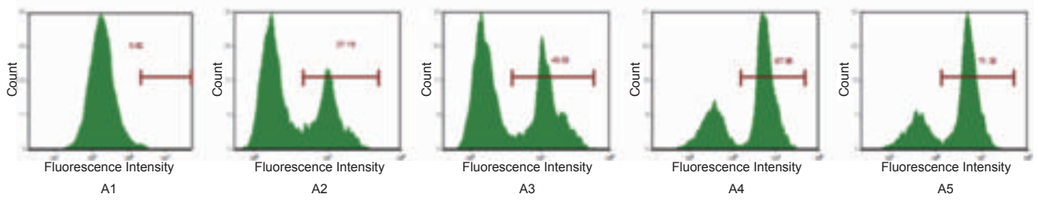
Figure 3 Fluorescent images of K562 elimination experiments with increasing effector to target ratios

GFP Transfection Efficiency

In molecular genetics, various model organisms, and cell biology, the GFP gene is frequently used as a reporter for expression studies. Currently, scientists are commonly using fluorescent microscopes or flow cytometers to analyze the transfection efficiency of mammalian cells. But handling the complex technology of an advanced flow cytometer demands for an experienced and highly qualified operator. Countstar Rigel enables users to easily and accurately perform a transfection efficiency assay without the operation and maintenance costs associated with traditional flow cytometry.



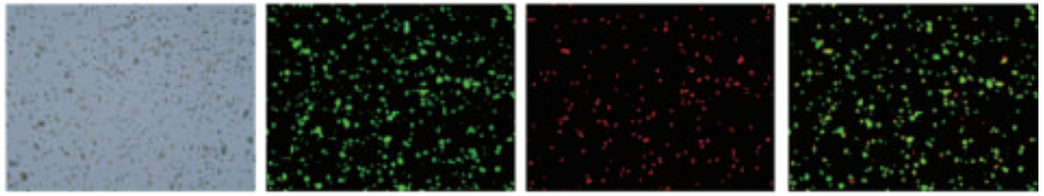
FCS Express Software used by the Countstar Rigel has enabled users to conduct image-based flow result



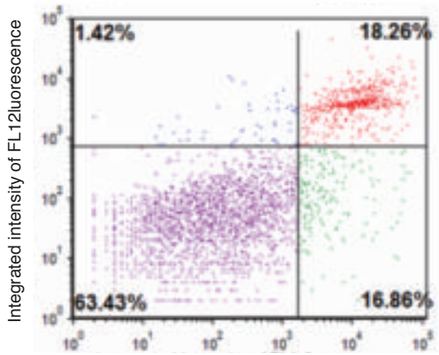
Cell Apoptosis

The progress of cell apoptosis can be monitored using FITC conjugated Annexin-V in combination with 7-ADD. Phosphatidylserine (PS) residues are normally located at the inner side of the plasma membrane of healthy cells. During early apoptosis, the membrane integrity gets lost and PS will be translocated to the outside of the cell membrane. Annexin V has strong affinity to PS and is therefore the ideal marker for early apoptotic cells.

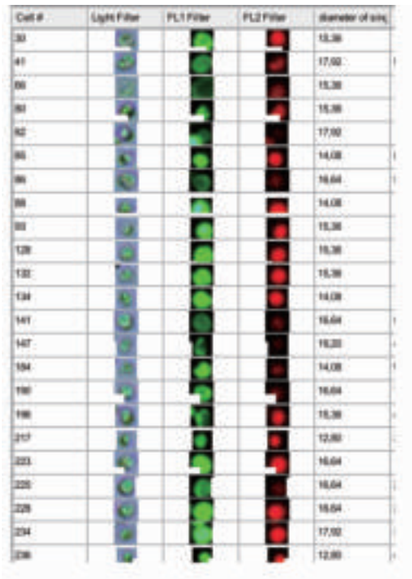
A



B



D



C

Cell types	% of gated Objects
live cells	63.43
apoptosis	16.86
necrosis	18.26
unclassified	1.42

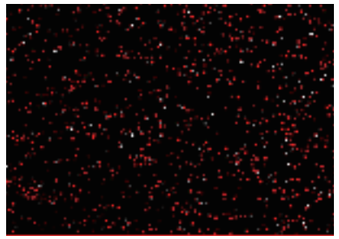
The Countstar Rigel was used to detect apoptotic processes in mesenchymal stem cells (MSCs). Figure A shows the bright field and fluorescent images of apoptosis induced MSCs. Figure B shows the gated scatterplot of Countstar Rigel data, analyzed by the DeNovo FCS Express Image software . Figure C shows the ratios of the different cell types detected. Figure D. Image of each single cell under bright field and fluorescent channels.

Cell Cycle

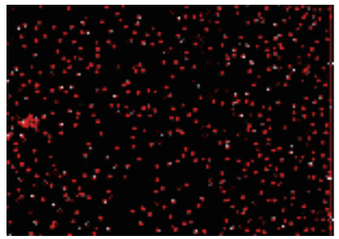
During cell division, cells contain increased amounts of DNA. Labeled by PI, an increase in fluorescence intensity is directly proportional to an accumulation of DNA. The differences of the fluorescence intensities of the single cells are the indicators of the actual status of the cell cycle.

MCF 7 cells were treated with 4μM of Nocodazole to arrest these cells at different stages of their cell cycle. The bright field images acquired during this test scenario allow to identify each single cell. The PI fluorescence channel of the Countstar Rigel identifies the DNA signals of single cells even in aggregates. The detailed analysis of the fluorescence intensities can be carried out using the FCS

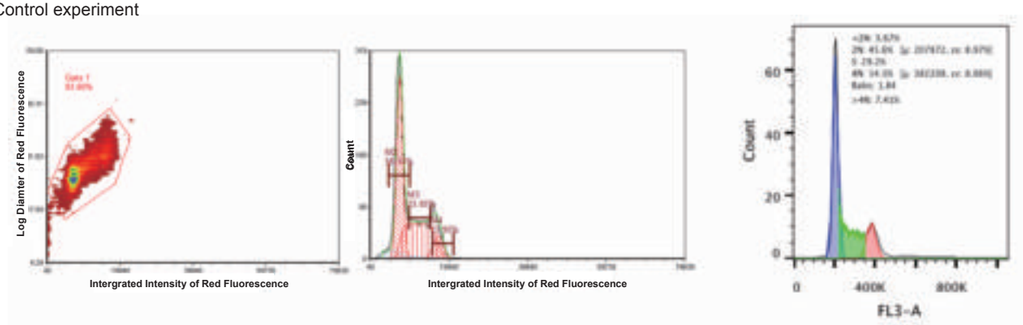
Control Group



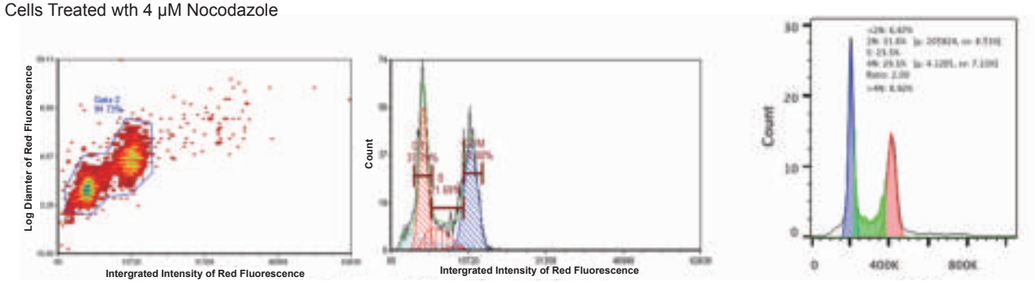
Cells treated with nocodazole , arrested in G2/M



Control experiment



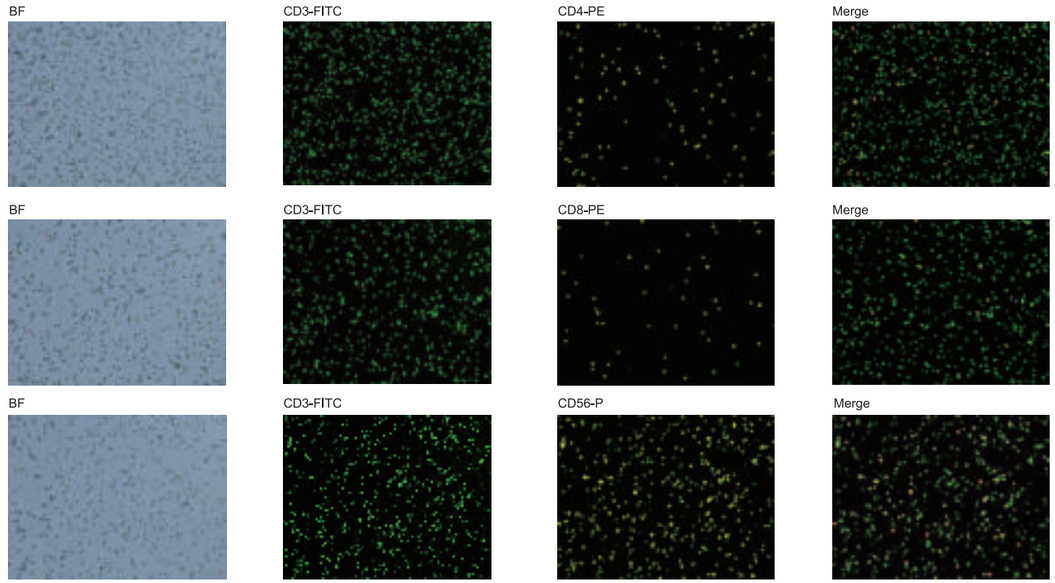
Cells Treated wth 4 μM Nocodazole



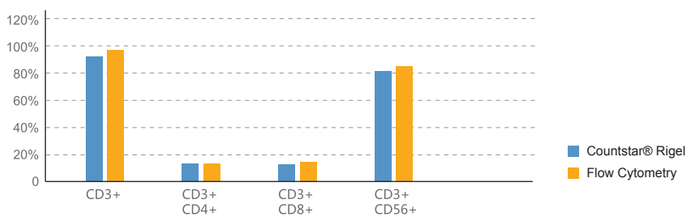
CD Marker Phenotyping

The Countstar Rigel models offer a faster, simpler and more sensitive approach to,immuno-based phenotyping of cells more efficient. With high resolution images and powerful integrated data analysis capabilities, the Countstar Rigel allows users to achieve consistently reliable results without the need for extensive complex control settings and fluorescence compensation adjustments

The Cytokine Induced Killer (CIK) cell differentiation demonstrates the outstanding performance quality of the Countstar Rigel analyzer in direct comparison to high class flow cytometers. PBMCs of mouse in culture were stained with CD3-FITC, CD4-PE, CD8-PE, and CD56-PE, and induced by Interleukin (IL) 6.Then analyzed simultaneously with Countstar® Rigel and Flow Cytometry. In this test, the CD3-CD4 , the CD3-CD8, and the CD3-CD56 were divided into three groups, to determine the proportion of different cell subpopulations



The proportion of different cell subpopulations of PBMCs, induced by Interleukin (IL)-6

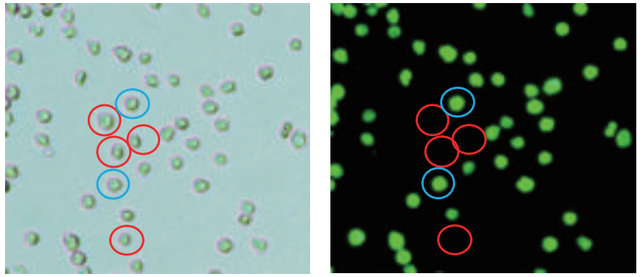


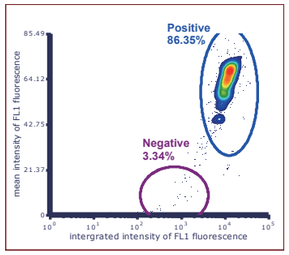
Data acquired by the Countstar Rigel (blue) show comparable results to the flow cytometry

Detection of Degenerated Cells by Immunofluorescence

Monoclonal antibodies producing cell lines will lose some positive clones during cell proliferation and passaging due to degradation or genetic mutations. A higher loss will significantly effect the productivity of the manufacturing process. The monitoring of the degradation plays an important role in the process control to shift the yield of antibodies to the optimum.

Most of the antibodies manufactured in the BioPharma industry can be detected by immuno-fluorescence labeling and analyzed quantitatively by the Countstar Rigel series. The bright-field and fluorescence channel images below clearly show those clones that lost their attribute to produce the desired antibodies. The more detailed analysis with the DeNovo FCS Express Image software confirms, that 86.35 % of all cells are expressing the immuno globulins, only 3.34 % are clearly negative.





Result analyzed by FCS Express

Antibodies Affinity

The antibodies affinity is usually measured by ELISA or Biacore, but these methods only detect the antibody with the purified protein, not for the in situ protein conformation. The antibody affinity of natively folded proteins can be detected by immunofluorescence method. Countstar Rigel can automatically capture the image and quantitate the fluorescence intensity which correlates with antibody affinity

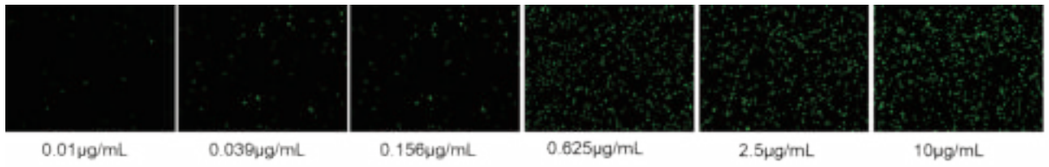


Figure 1 Fluorecent images of antigens , incubated with increasing concentrations of AB 4

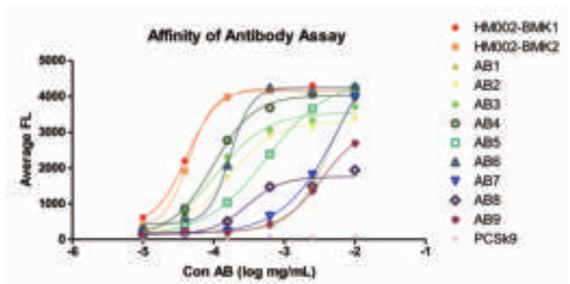
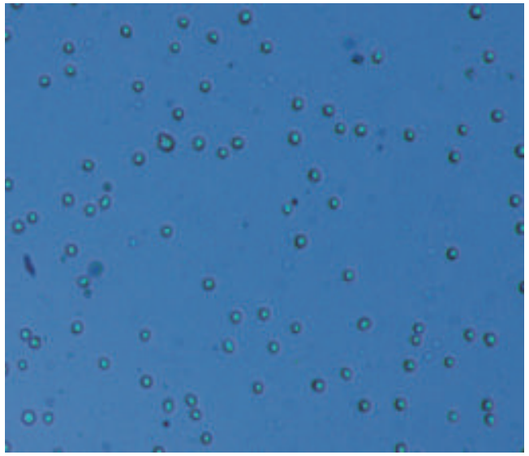


Figure 2 Fluorescence intensity in different antigen-antibody reactions under a gradient of various antibody concentrations

Trypan Blue Cell Counting

Trypan blue staining is still used in the majority of cell culture labs.

The Trypan Blue Viability and Cell Density BioApp can be installed on all Countstar Rigel models. Our protected image recognition algorithms analyze more than 20 parameters to classify each single object detected.



Cell image

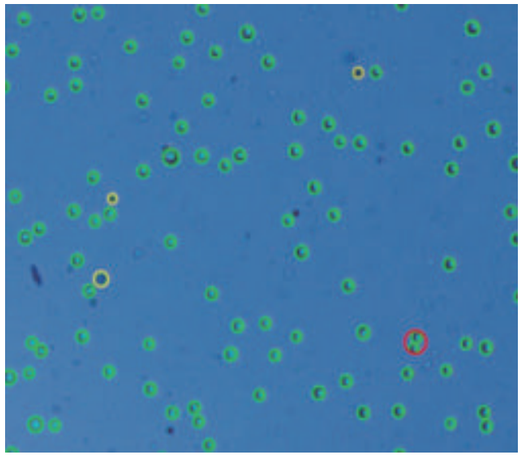


Image with detected and labeled cells

Comparison of concentration and viability of CHO by Countstar System and hemocytometer

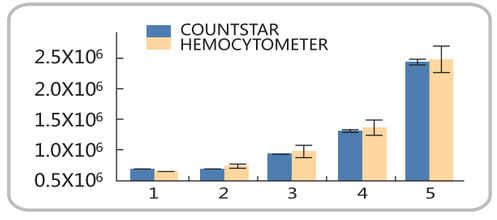


Figure 1 Countstar® Rigel and the hemocytometer concentration measurements show a similar result, but the result from Countstar® Rigel are more stable

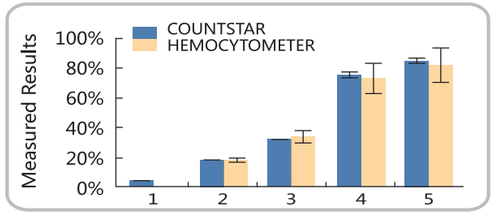
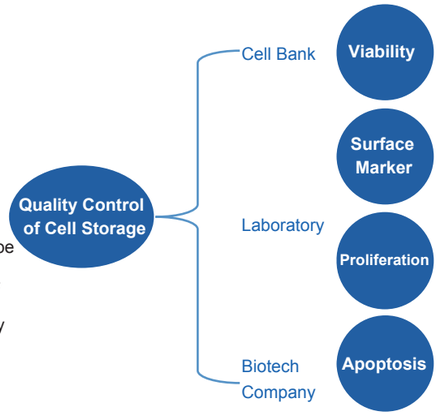


Figure 2 Countstar® Rigel and the hemocytometer vability measurements show a similar result, but the result from Countstar® Rigel are more stable

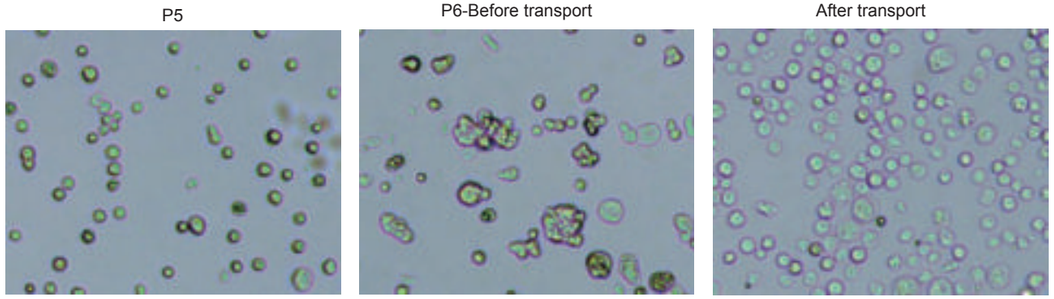
Cell Line Storage QC

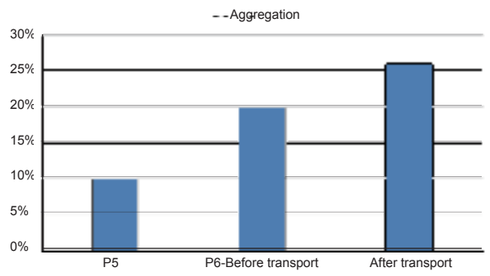
In cell storage, a sophisticated quality management concept ensures safe, efficient monitoring of all cellular products. This guarantees for a stable quality of cell cryopreserved for experiments, process development, and production.

The Countstar Rigel acquires high resolution images, anylzing various morphological characteristics of the cellular objects such as diameter, shape and aggregation tendency. Images of different process steps can be easily compared to each other. So variations in shape and aggregation can easily be detected , by avoiding subjective human measurements. And the Countstar Rigel database has a sophisticated mamagement system for storage and retrieval of images and data.



Monitoring morphological changes of MSCs during passaging and transporation





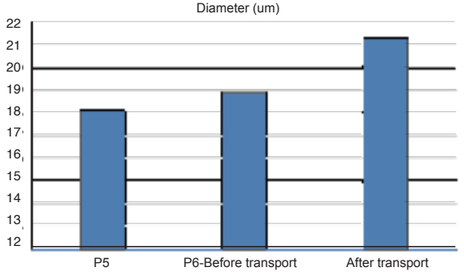
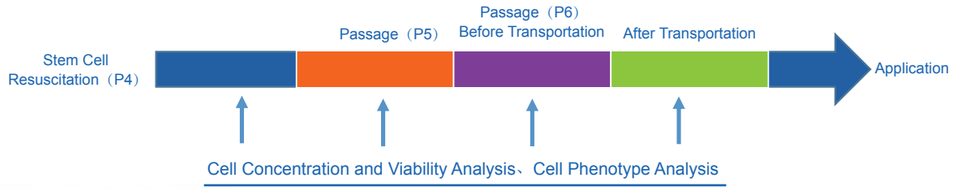


Figure 1 MSC morphological changes after the transportation

Stem Cell Research

In the field of stem cell research, from stem cell isolation to the actual application, the Countstar Rigel can be used at different process steps to check the quantity and quality of monitored cells.The Countstar Rigel with its highly sensitive optical system provides a fast and accurate tool for phenotyping the objects of interest. Measuring the cell concentration and viability of stem cells during their isolation, preservation and transport, is essential to guarantee a flawless final product.

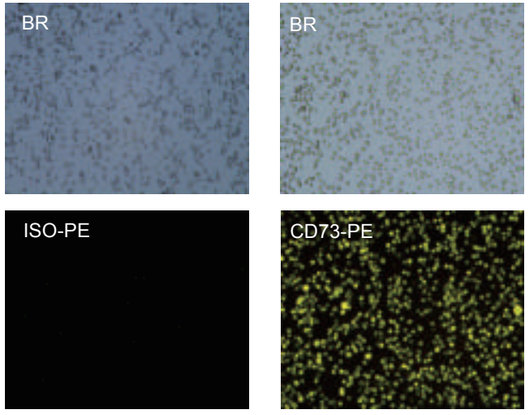


Countstar® Rigel complies with all the requirements of cGMP-regulated environments and FDA 21 CFR Part 11, providing you with a convenient, solution to help you build up a stable, standardized cell quality control system

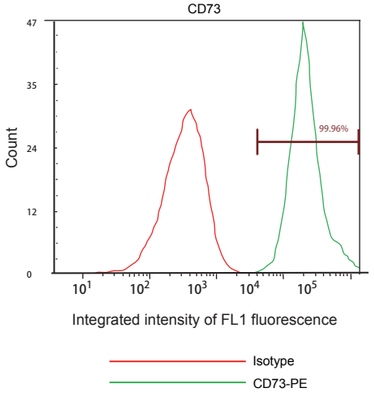
Build a Standardized Cell Quality Control System

- Easy customization of BioApps to individual stem cell lines
- Cell analysis result are comparable with FACS
- Flexible platform with pre-installed BioApps
- cGxP and FDA's 21 CFR Part 11 compliance
- Images provide additional proof of recorded data
- Easy harmonization of different Countstar Rigel analyzers

Identification of AdMSCs in Cell Phenotype



Analyzed Cell Phenotype of ADMSCs by Countstar Rigel



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