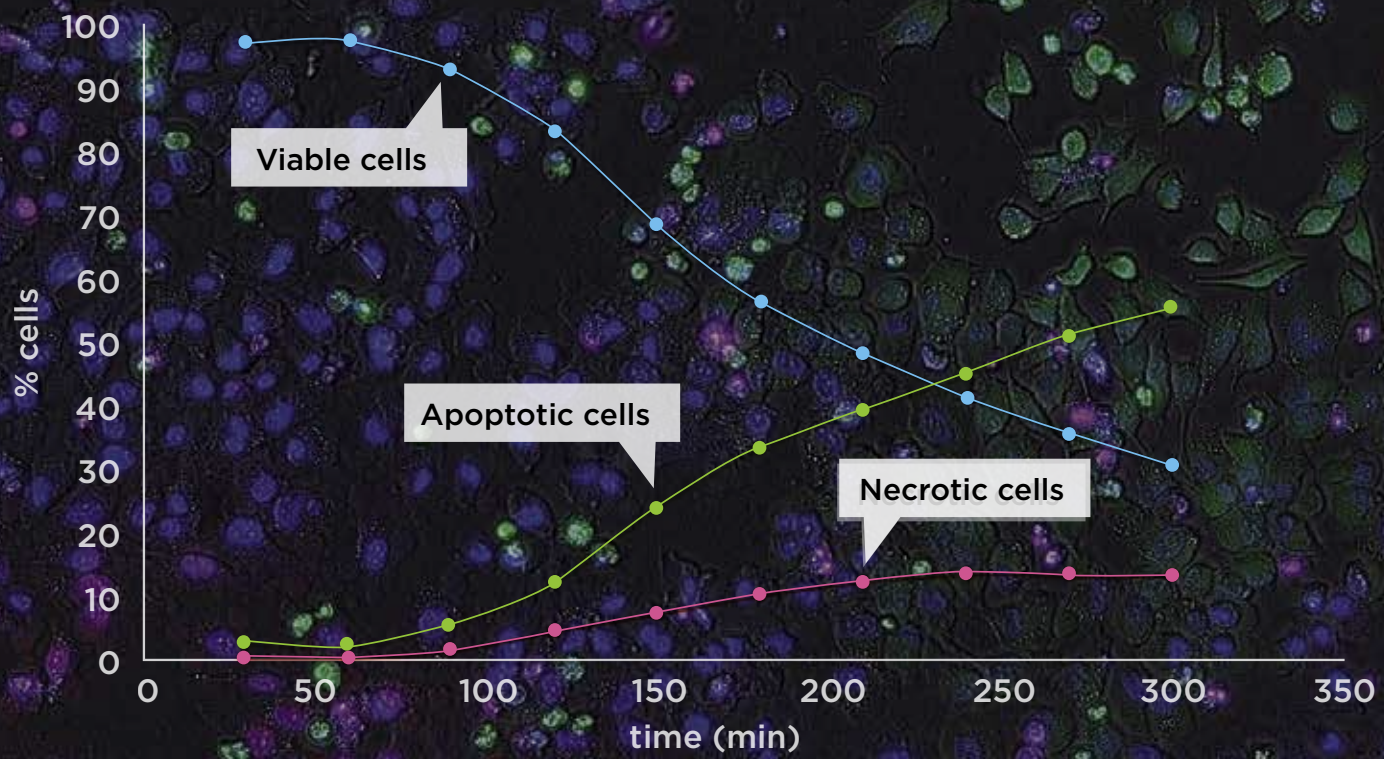
Spark® Cyto.

LIVE CELL PLATE READER WITH REAL-TIME IMAGE CYTOMETRY



Spark Cyto is a multimode plate reader combining bright field and fluorescence imaging with industry-leading detection technologies to enable real-time image cytometry, unlocking new possibilities for your cell-based research.

Your cells don't stay static when you leave the lab, so your research requires a dynamic instrument that ensures you never miss a critical biological event. Spark Cyto works in real-time with integrated cell incubation capabilities, and uses parallel data acquisition and analysis to deliver meaningful insights for cell-based assays.

With Spark Cyto, you now have the ability to unite qualitative and quantitative information into unique multiparameter data sets faster than before.

More insights delivered in real time, and more cells analyzed

Spark Cyto brings together a unique combination of patent-pending technologies to ensure you can truly investigate your entire cell population. It gives you the ability to record the whole well area of a 96- or 384-well microplate with just one image – no tiling or distortion – meaning you never miss a cell.

Magnification	Numeric aperture
2x	0.08
4x	0.13
10x	0.30

A dedicated optical set-up for live-cell cytometry in microplates, from 6- to 384-well formats

Using three objectives, five LEDs (bright field and fluorescence excitation), a multiband filter set and a CMOS camera, Spark Cyto eliminates pixel shifts and delivers high quality images in a flash.

Spark Cyto combines three magnification levels with four channels for fluorescence and bright field imaging, enabling high quality cell analysis for a wide range of applications.

LED colors	Spectral range
Blue	381–450 nm
Green	461–530 nm
Red	543–611 nm
Far red	626–800 nm
Bright field and digital phase contrast	

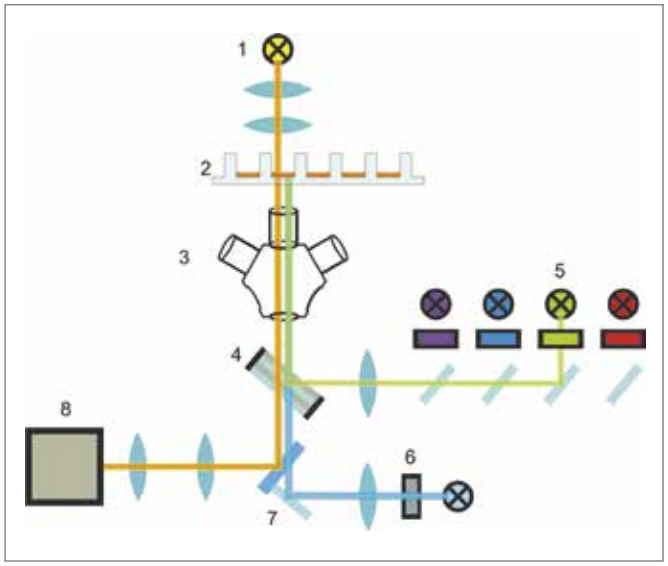


Figure 1: Schematic diagram of imager module. (1) LED for bright field; (2) microplate with sample; (3) objective; (4) multiband filter set; (5) LEDs and excitation filters for fluorescence; (6) Autofocus unit; (7) reflection mirror; (8) CMOS camera.

Autofocus enabled – stay focused on your research

Spark Cyto uses a patent-pending LED-based autofocus system to deliver high quality images while offering uncompromised speed for scanning. The autofocus system projects an extended grid pattern onto the sample surface, which minimizes the impact of potential distortions from isolated impurities. This fast, simple and effective autofocus comes as standard on every instrument, so you'll never miss an image.

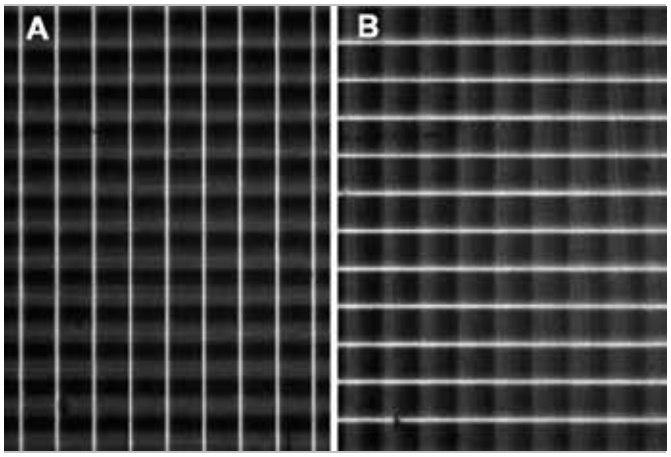


Figure 2: Grid patterns for fast autofocusing. A) vertical grid; B) horizontal grid.

One single image can tell the whole story

Spark Cyto captures the whole well (96- and 384-well plates) with a single image, giving you a real picture of your research.

It is based on a proprietary patent-pending approach where image acquisition with the 2x (96-well plates) and 4x (384-well plates) objective is combined with a large camera chip and advanced imaging algorithms to give you accurate results.

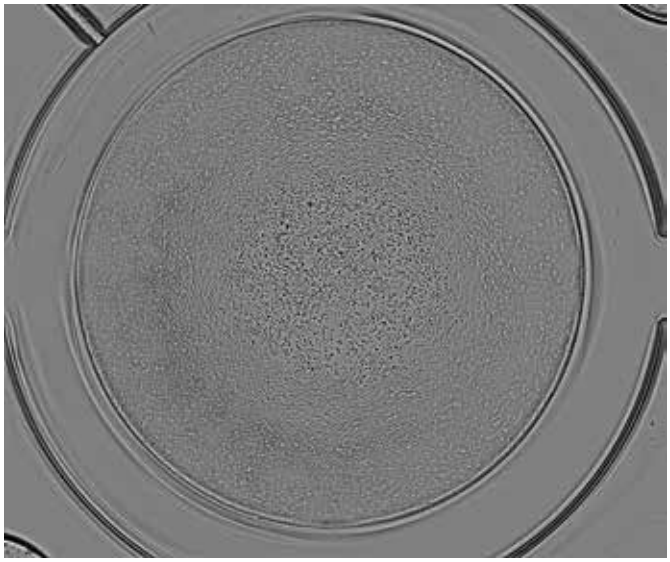


Figure 3: Single image of an entire well from a 96-well plate. No tiling or edge-to-edge optical distortion leads to superior results when analyzing cell populations.

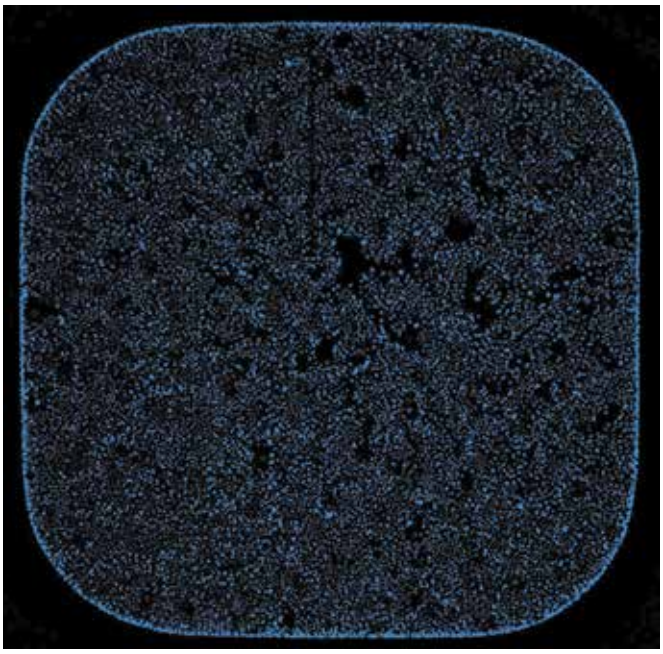


Figure 4: Whole well imaging in 384-well plates enables fast and accurate nuclei counting of cells stained with Hoechst 33342.

Applications.

Predefined applications for the most common cytometric assays:

- confluence
- nuclei counting
- transfection efficiency
- cell viability
- cell death

Confluence

Use the bright field imaging channel to provide a quick overview of a well's cell density. Cell confluence is calculated automatically by the software, and displayed as a yellow overlay for easy visual confirmation. In addition, you can use the roughness factor as a simple indicator of cell death.

Nuclei counting

Optimized for Hoechst 33342, this function provides an easy method for cell counting using any blue fluorescent dye with nuclear DNA binding capabilities.

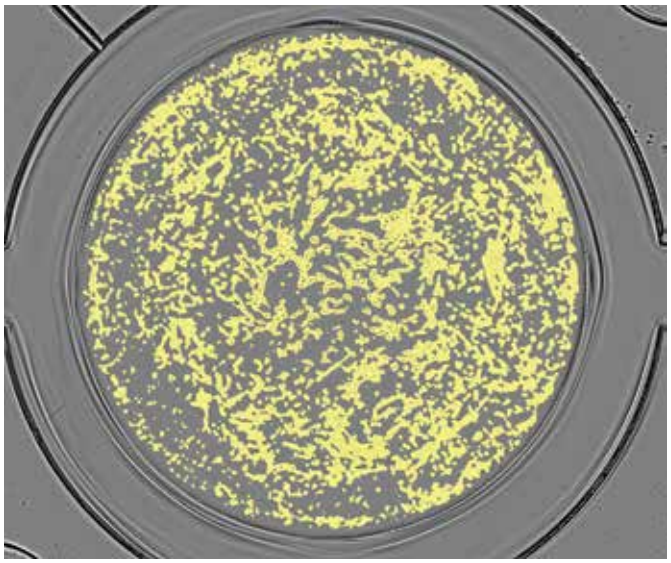


Figure 5: Whole well image from a 96-well plate, acquired with the 2x objective; NHDF cells with confluence evaluation mask.

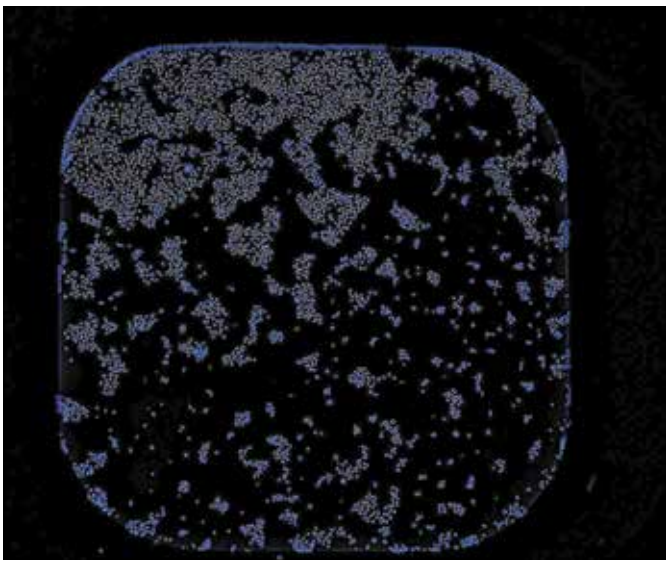


Figure 6: Whole well image from a 384-well plate, acquired with the 4x objective; CHO cells with nuclei counting mask.

Transfection efficiency

This feature can automatically determine transfection rates for cells containing green fluorescent protein (GFP) – a widely used reporter for gene expression – and counter-stained with Hoechst 33342 (blue). The green and blue images are overlaid and analyzed to determine the transfection efficiency in the cell population.

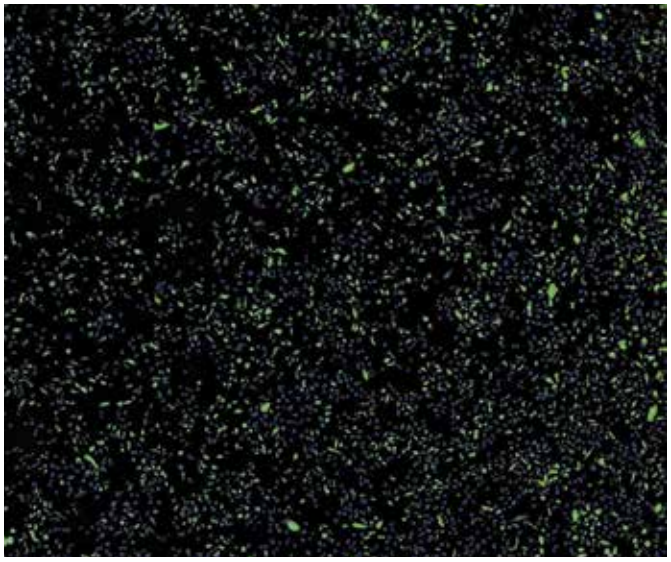


Figure 7: Centered image of CHO cells cultured in a 96-well plate, acquired with the 4x objective, showing an overlay of the blue and green channels.

Cell viability

Spark Cyto's preset cell viability application relies on a common double staining approach to discriminate between live (green) and dead (red) cells in a population. Using two fluorescent dyes, such as calcein AM (live cells) and propidium iodide (dead cells), you can image and analyze your population in minutes.

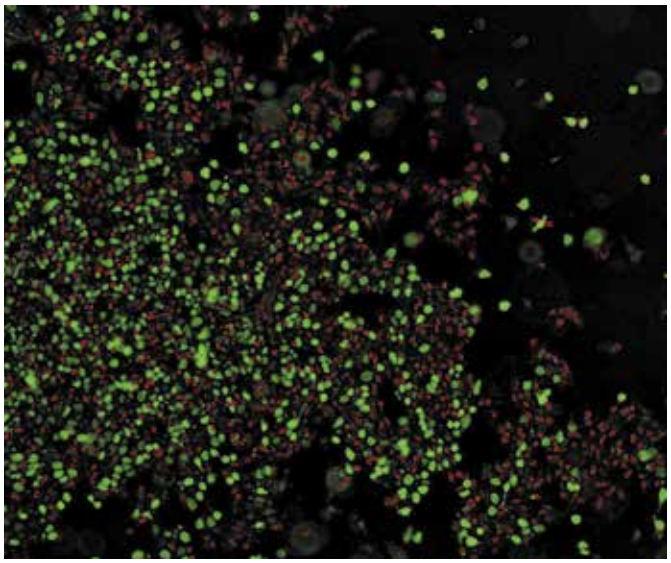


Figure 8: Centered image of HeLa cells cultured in a 24-well plate, acquired with the 10x objective, showing an overlay of the bright field, green and red channels.

Cell death

Spark Cyto can detect cell death, and discriminate between apoptosis and necrosis, using differential staining:

Hoechst 33342 (blue) – nuclear stain

Propidium iodide (red) – necrotic cell stain

Annexin V-FITC / Alexa Fluor® 488 (green) – binds to the early apoptosis marker phosphatidylserine

Using a proprietary algorithm, the software can uniquely identify three object classes:

- Blue objects – cell nuclei
- Blue/red objects – necrotic cells (for live:dead cell ratio)
- Blue/red/green objects – apoptotic cells (for apoptotic:necrotic cell ratio)

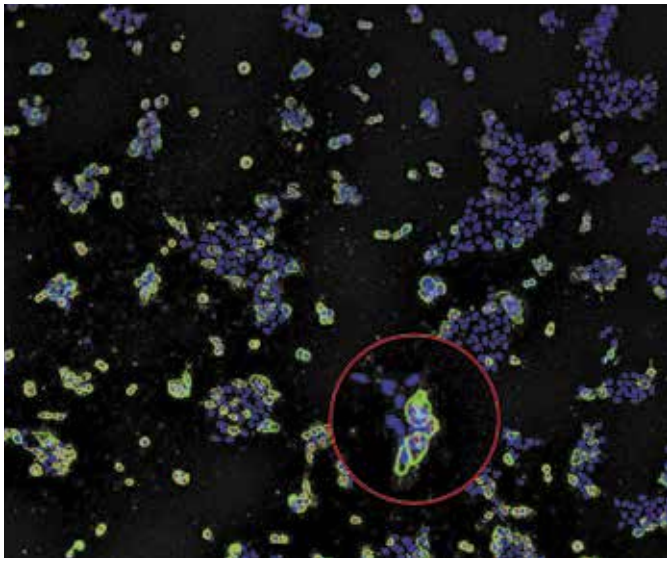
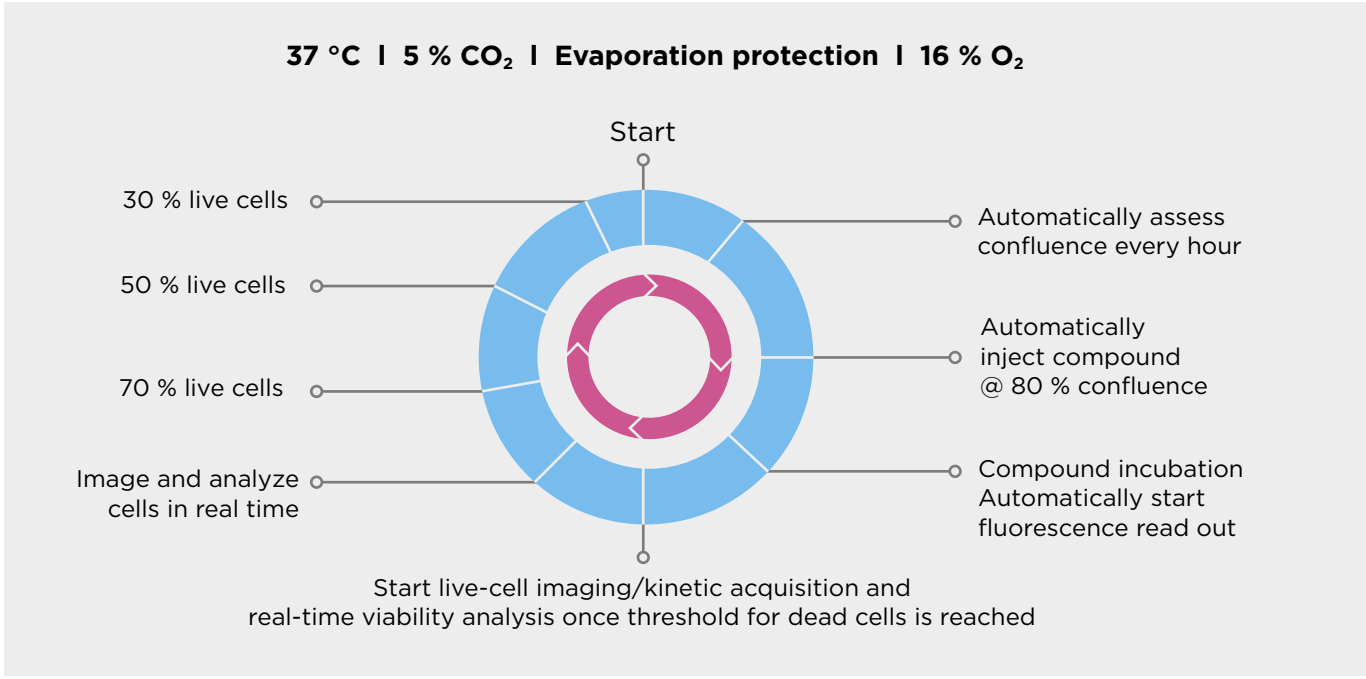


Figure 9: Image of A431 cells cultured in a 96-well plate, acquired with the 10x objective, showing an overlay of the blue, green and red channels.

Automation of live-cell experiments with Real-Time Experimental Control (REC™)

REC grants you the ability to create novel experimental workflows and unlock new research possibilities for multiplexed data. The system combines standard detection technologies and imaging capabilities with proprietary software to enable kinetic experiments to be performed automatically. For example, the system can inject a reagent or start a fluorescent measurement once a user-defined population status or signal threshold is reached, such as a confluence of 80 percent.



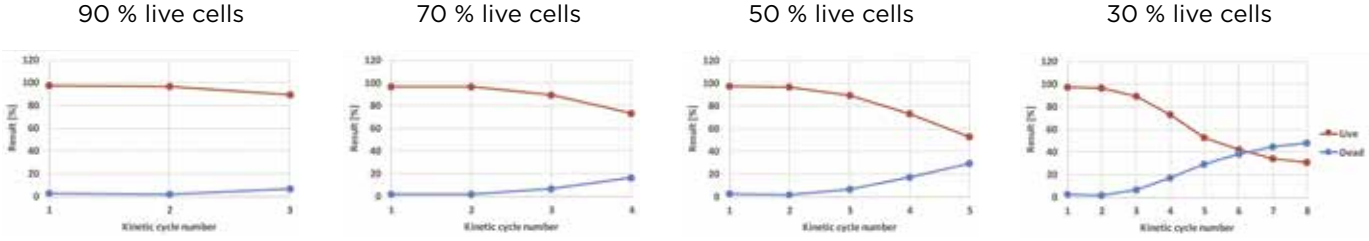
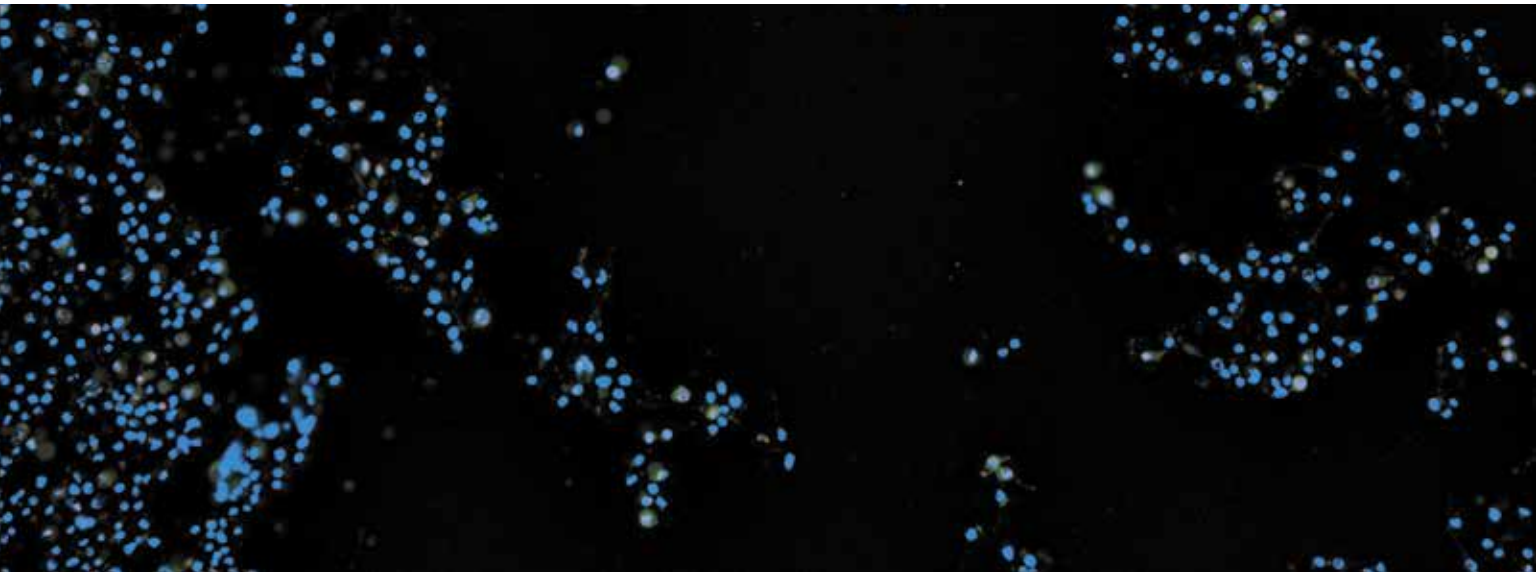


Figure 10: Automatic kinetic measurements for live/dead cells with an interval of 2 h over a time period of ca. 20 h.



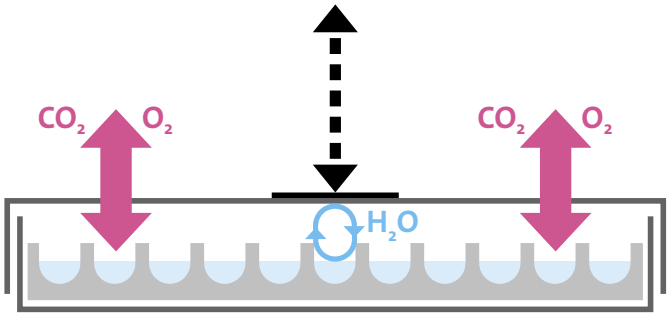
Complete environmental control comes as standard

Spark Cyto is equipped with a unique environmental control system that allows you to maintain a stable environment for your assays, effectively eliminating the risk temperature fluctuations or evaporation could pose to your results. Spark Cyto is the only instrument to put these features right at your fingertips:

- Uniform temperature control (up to 42 °C)
- Dynamic gas control (CO_2 and O_2)
- Humidity control via Lid Lifter™ and Humidity Cassette

Humidity control for optimal evaporation protection

Maintaining humidity levels of 95 percent or higher is essential for unimpaired cell viability and growth, and miminizing evaporation is essential for maintaining consistent concentrations during long-term assays. Spark's Humidity Cassette is a cost-effective solution to minimize evaporation.



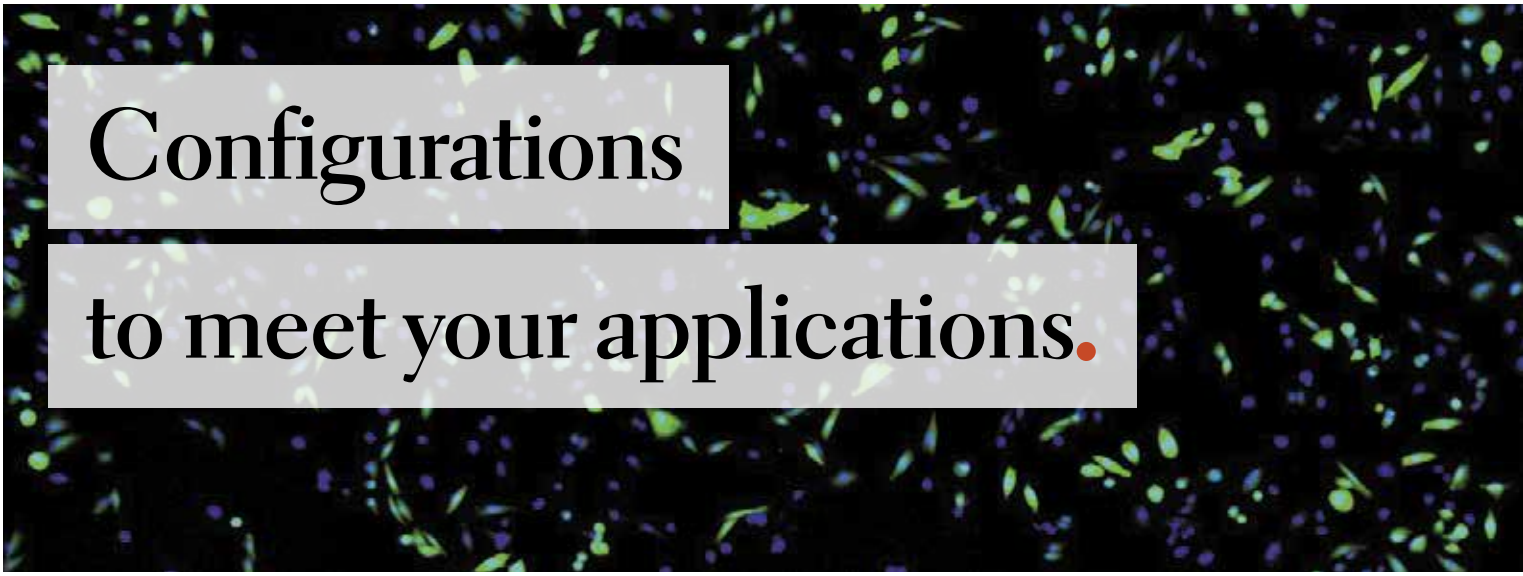
Lid Lifter

Spark's integrated and patented lid lifting function establishes an ideal environment for long-term kinetic assays and reduces the risk of sample contamination. Whether you want to dispense reagents without the need for manual intervention or maintain optimal environmental conditions without compromising evaporation protection, Spark Cyto is the only reader to offer this benefit.

More parameters measured

The system's Method Editor offers unique options for researchers looking to customize their assays:

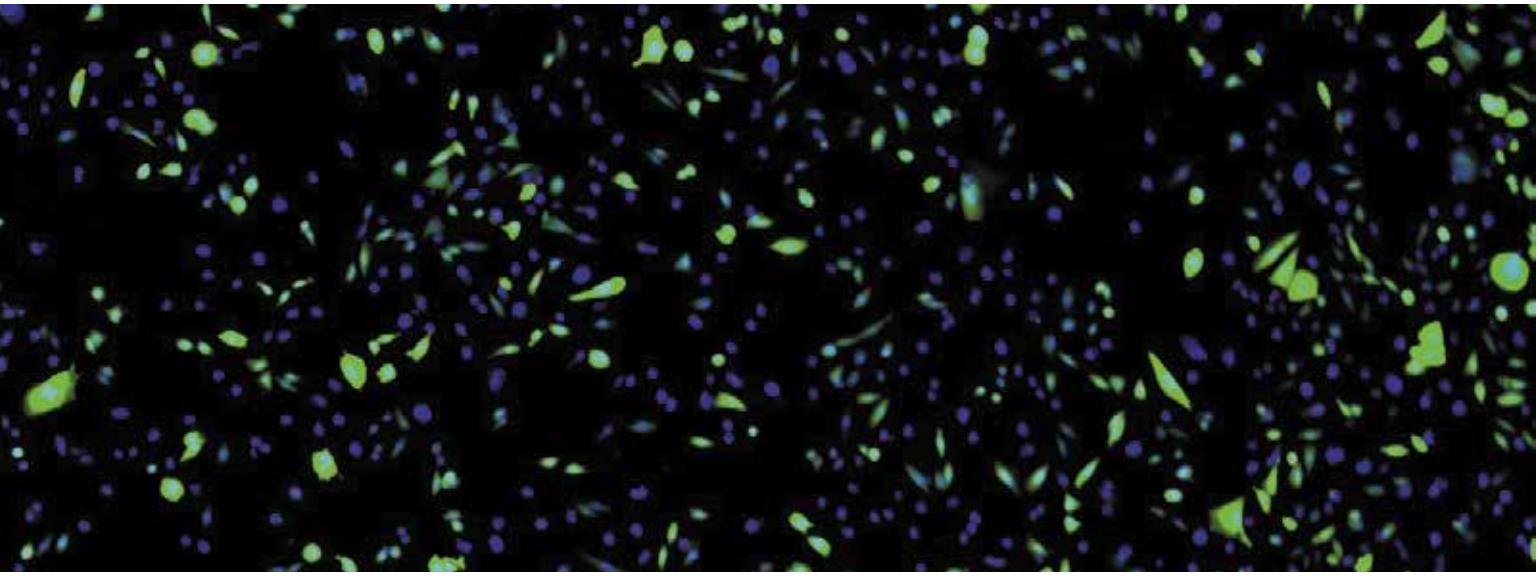
- **User-defined protocols** – for automated image acquisition and analysis
- **Imaging only** – allowing acquisition and export of files to any third-party image analysis software, such as ImageJ or CellProfiler



Configurations to meet your applications.

No matter the configuration of your Spark Cyto, you have a fully equipped system ready for live-cell imaging cytometry.

Capabilities	SPARK CYTO 300	SPARK CYTO 400	SPARK CYTO 500	SPARK CYTO 600
Fluorescence imaging	●	●	●	●
Bright field imaging	●	●	●	●
Digital phase contrast imaging	●	●	●	●
Absorbance UV/vis monochromator	●	●	●	●
Fluorescence – standard	●			
Fluorescence – enhanced		●	●	●
Fluorescence filter top/bottom	●		●	●
Fluorescence monochromator top/bottom		●		●
Fluorescence variable bandwidth		●		●
Fluorescence polarization			●	●
Fluorescence dichroic mirrors		●	●	●
Luminescence	●	●	●	●
Luminescence multicolor and scanning			●	●
Alpha technology				●
Lid Lifter	●	●	●	●
Heating	●	●	●	●
CO_2 and O_2 control	●	●	●	●



Spark Cyto sets a new standard for fluorescent imaging microplate readers by offering the following features with every configuration:

- Lid Lifter
- Integrated gas control (CO_2/O_2)
- Heating
- LED-based autofocus
- Objectives (2x, 4x, 10x)
- 5-LED excitation, 4 color channels
- Digital phase contrast
- SparkControl™ software
- Image Analyzer™ software
- Instrument control unit

All four configurations can be equipped with additional options:

- Reagent dispensers with heating and stirring
- Humidity Cassette
- NanoQuant Plate™
- QC tools for IQ/OQ services
- Spark-Stack™ microplate stacker

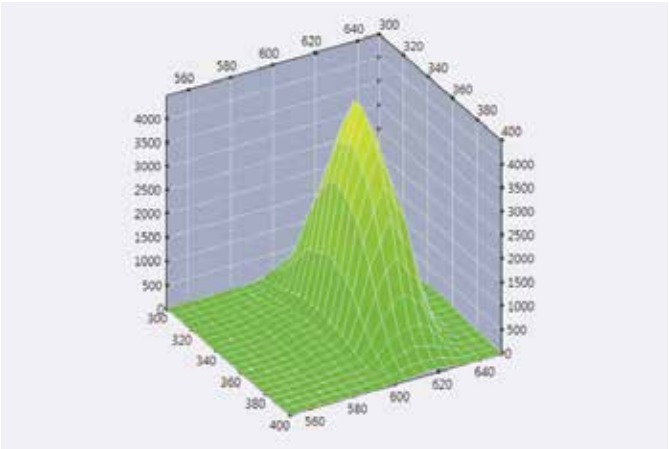
Figure 11: The NanoQuant Plate allows parallel quantification and analysis of up to 16 nucleic acid or protein samples, in volumes as little as 2 µl.

Figure 12: You have full control of the environmental conditions during a run, including the temperature and the CO_2 and O_2 levels inside the reader.

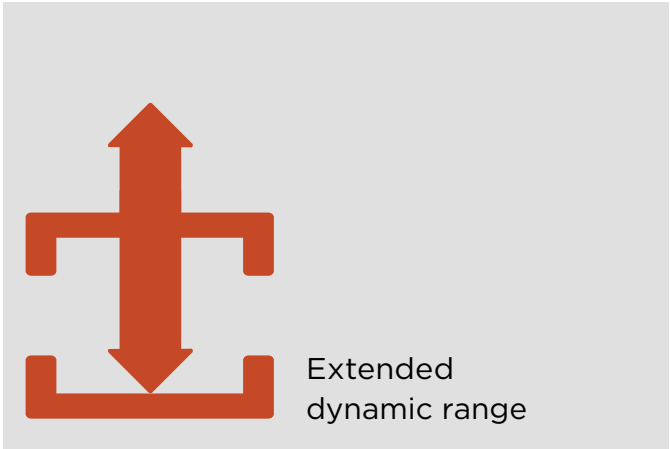
Figure 13: Reagent dispenser with heating and stirring enhances application flexibility: Spark injectors offer a heating and stirring option for the reagent storage. This is especially beneficial for cell based applications, minimizing cold shock caused by reagent addition and enabling automated dispensing of viable cells within the reader.

SPARKCONTROL

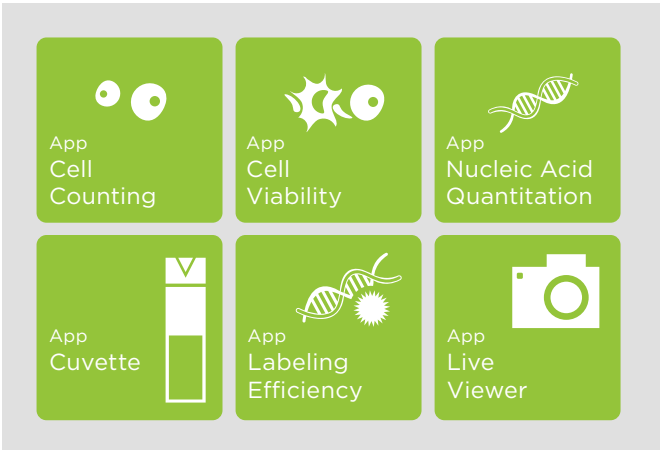
Software designed for long-term studies.



3D scanning accelerates assay development by providing simultaneous excitation and emission scans. This can help to identify changes in the spectral properties of fluorescent probes or characterize unknown fluorescent samples more quickly and easily.



Detect even very low signals with the Spark **extended dynamic range**. This function automatically adjusts the gain settings during a measurement run, allowing the detection of very low signals without compromising on sensitivity. All results are automatically correlated and displayed within one single data set.



One-click applications streamline your workflows, getting you from sample to results faster than ever before.

Safeguard your kinetic assays using automated gain regulation to avoid fluorescence measurements running into saturation. Measurements with different gain settings are then automatically correlated, allowing evaluation of the entire dataset.

SparkControl enables automation of long-term kinetic assays, providing a hands-off solution for complex experimental set-ups. The imaging stripe can be combined with any other programming stripe, making it effortless and straightforward to create multiplex assays. The software uses an icon-driven, 'drag and drop' approach, making it suitable for users at any skill level.

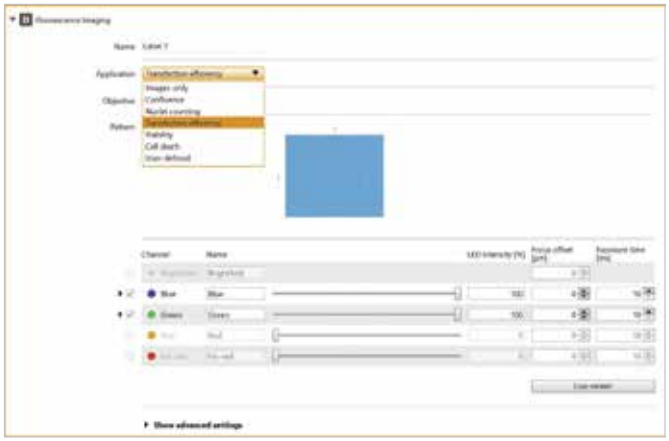


Figure 14: The fluorescence imaging stripe in SparkControl's Method Editor.

Image Analyzer

Images acquired with the Spark Cyto can be automatically processed with Image Analyzer, Tecan's proprietary imaging software package. Image Analyzer offers you an array of customization options, making it easy to adjust and optimize imaging parameters such as cell size, segmentation and cell gating. Predefined analysis reports provide comprehensive and effortless documentation of your experiments.

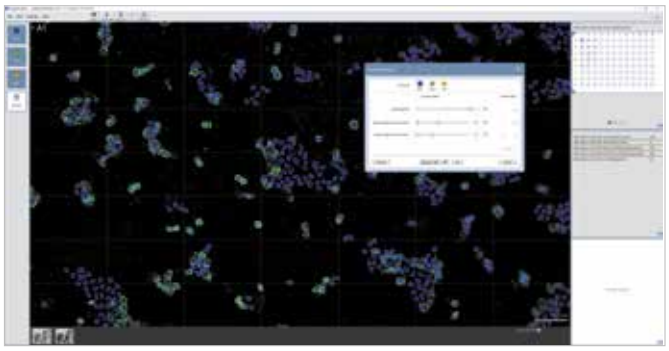


Figure 15: Spark Cyto's Image Analyzer offers easy data analysis for object segmentation, gating and object counting.

Optimized plates for optimal results

Tecan offers proprietary transparent tissue culture plates in 24-, 48-, 96- and 384-well formats. The Spark Cyto's capabilities are specifically optimized for use with Tecan's cell culture plates, reducing optical artifacts and ensuring optimal results.

Figure 16: Tecan's 96-well transparent cell culture plate. The optics of the Spark Cyto are optimized for these plates, helping to guarantee the best possible image quality.

Consumables.

At Tecan, we work continually to ensure that our instruments meet your application requirements. We offer a broad range of consumables tailored to your application and laboratory needs.

Figure 17: Tecan microplates come in transparent, white, and black. Available in 24-, 48-, 96- and 384-well formats.

Tecan microplates
Performance assured with Tecan microplates, for absorbance, fluorescence and luminescence measurements, as well as cell imaging. We offer a selection of polystyrene, medium-binding microplates in ANSI/SLAS-formats.

- Optimal plate height and height tolerance limits allow the Spark reader's optics to be moved as close as possible to the plate, avoiding well-to-well signal crosstalk
- The imaging algorithm of the Spark reader is developed and tested in combination with Tecan microplates, assuring good performance
- Microplate well diameter is optimal for the Spark reader – critical in confluence assessments

Lid Lifter discs
The Lid Lifter is a convenient solution that helps researchers to increase workflow automation to decrease hands-on time for long-term incubation and in-between measurements, and further reduce sample evaporation. Simply add the sample to a Tecan microplate, cover with a lid with a Lid Lifter disc attached, place in the Spark reader and incubate for as long as required. The Spark Lid Lifter will remove the lid from the plate for readings at specified time intervals.

Figure 18: Lid Lifter discs come in 50 pcs/box.

Capabilities*.

Applications

- Nuclei counting
- Transfection efficiency
- Cell viability
- Apoptosis
- Confluence assessment
- Cell migration and wound healing
- ELISAs
- Low-volume DNA/RNA quantification
- Nucleic acid labeling efficiency
- Protein quantification
- Reporter gene assays
- HTRF®, DELFIA® and LanthaScreen®
- Transcreener®
- DLR®
- BRET – including NanoBRET®

Detection modes

- Fluorescence imaging (blue, green, red, far red)
- Bright field imaging
- Digital phase contrast imaging
- Absorbance – incl. UV/vis
- Fluorescence top and bottom
- Time-resolved fluorescence (TRF)
- Full spectral scanning capability for all measurement modes
- FRET
- TR-FRET
- Fluorescence polarization (FP)
- Luminescence – glow, flash, multicolor, scanning
- AlphaScreen®, AlphaLISA® and AlphaPlex®

Additional options

- Reagent dispensers with heating and stirring
- Humidity Cassette
- NanoQuant Plate
- QC tools for IQ/OQ services
- Spark-Stack microplate stacker

*Capabilities depend on the Spark Cyto configuration

Typical performance values+

Fluorescence imaging and cytometry

Imaging technologies	Fluorescence, bright field, digital phase contrast			
Imaging methods	Single color, multicolor, end-point, kinetics, whole-well			
Sample formats	6- to 384-well ANSI/SLAS-format microplates			
Camera sensor	Grayscale, 5 Mpixel, CMOS Sony			
Objectives	2x (NA 0.08), 4x (NA 0.13), 10x (NA 0.30)			
Optical properties	Objective	Pixel resolution	Optical resolution	Field of view
	2x	3.45 µm	4.50 µm	8.47 x 7.09 mm
	4x	1.72 µm	2.77 µm	4.24 x 3.54 mm
	10x	0.69 µm	1.20 µm	1.69 x 1.42 mm
Channels	Bright field, four fluorescence channels (blue, green, red, far-red)			
Autofocus	Proprietary astigmatism-based technology			
Field of view	Whole-well, 96- and 384-well imaging with a single image (2x and 4x objectives)			
Applications	4 pre-defined applications: Confluence, transfection efficiency, cell viability and cell death (apoptosis via annexin V-FITC), plus user-defined applications			
Image collection rate	≤12 min for 96-well plate, whole-well image with 2x, bright field and digital phase contrast			
	≤15 min for 96-well plate, center image with 10x, bright field, digital phase contrast + 1 fluorescence channel			
Analysis speed	≤20 min for 96-well plate, whole-well image with 2x, bright field and digital phase contrast including real time confluence assessment			

Fluorescence – enhanced

Light source	High energy xenon flash lamp
Spectral range	Ex: 230–900 nm Em: 280–900 nm
Wavelength accuracy	Ex: <0.5 nm; Em: <0.5 nm
Wavelength reproducibility	<0.5 nm
Bandwidth	Adjustable from 5–50 nm
Optical mirrors	50 %, 510, 560, 625 nm built-in; 410, 430, 458, 593, 660 nm user-selectable dichroics
Well scanning	Up to 100 x 100 data points

FI (fluorescence intensity)	Limit of detection[1]
Filter - top	≤8 amol/well (10 µl; 1,536-well)
Fusion* - top	≤15 amol/well (10 µl; 1,536-well)
Mono - top	≤20 amol/well (10 µl; 1,536-well)
Filter - bottom	≤180 amol/well (10 µl; 1,536-well)
Fusion - bottom	≤200 amol/well (10 µl; 1,536-well)
Mono - bottom	≤220 amol/well (10 µl; 1,536-well)

FP (fluorescence polarization)[2]

Spectral range	300–850 nm
Precision - Filter	≤1.25 mP
Precision - Fusion	≤2.0 mP
Precision - Mono	≤2.5 mP

TRF (time-resolved fluorescence)[3]

Limit of detection - Filter	≤0.5 amol/well (20 µl; 384-well SV)
Limit of detection - Fusion	≤0.6 amol/well (20 µl; 384-well SV)
Limit of detection - Mono	≤0.7 amol/well (20 µl; 384-well SV)

Fastest read time

384-well plate (FI)	≤22 sec
1,536-well plate (FI)	≤34 sec

Fluorescence – standard

Light source	Dedicated xenon flash lamp
Spectral range	Ex: 230–900 nm Em: 280–900 nm
Wavelength accuracy	Ex: <1 nm; Em: <2 nm
Wavelength reproducibility	<1 nm
Bandwidth	Fixed @ 20 nm
Optical mirrors	50 %; 510 nm dichroic
Well scanning	Up to 100 x 100 data points

FI (fluorescence intensity)	Limit of detection[1]
Filter - top	≤25 amol/well (100 µl; 384 well)
Fusion - top	≤35 amol/well (100 µl; 384 well)
Mono - top	≤50 amol/well (100 µl; 384 well)
Filter - bottom	≤500 amol/well (200 µl; 96 well)
Fusion - bottom	≤700 amol/well (200 µl; 96 well)
Mono - bottom	≤800 amol/well (200 µl; 96 well)

FP (fluorescence polarization)[2]

Spectral range	300–850 nm
Precision - Filter	≤1.5 mP
Precision - Fusion	≤2.5 mP
Precision - Mono	≤3.0 mP

TRF (time-resolved fluorescence)[3]

Limit of detection - Filter	≤4.0 amol/well (100 µl; 384-well)
Limit of detection - Fusion	≤6.5 amol/well (100 µl; 384-well)
Limit of detection - Mono	≤10 amol/well (100 µl; 384-well)

Fastest read time

96-well plate (FI)	≤13 sec
384-well plate (FI)	≤30 sec

Absorbance (enhanced or standard)

Light source	Dedicated xenon flash lamp
Spectral range	200–1,000 nm
	OD range 0–4 OD
Scan speed (200–1,000 nm)	≤5 sec
Wavelength accuracy	<0.3 nm
Wavelength reproducibility	≤0.3 nm
Wavelength ratio accuracy (260/230)	<0.08
Wavelength ratio accuracy (260/280)	<0.07
Precision @ 260 nm	<0.2 %
Accuracy @ 260 nm	<0.5 %
Limit of detection (nucleic acids)	<1 ng/µl

Plate formats for all read modes – enhanced

1-1,536 wells; NanoQuant Plate; Cuvettes; Roboflask®

Plate formats for all read modes – standard

1-384 wells; NanoQuant Plate; Cuvettes; Roboflask

Luminescence (enhanced or standard)

Spectral range	370–700 nm
Limit of detection – Glow[4]	≤225 amol/well (25 µl; 384-well SV)
Limit of detection – Flash[5]	≤12 amol/well (55 µl; 384-well)
Dynamic range	>9 orders of magnitude
Multi-color luminescence	38 spectral filters;
	OD1, OD2, OD3 attenuation filters

AlphaScreen (enhanced or standard)

Limit of detection	<100 amol/well bio-LCK-P[6]; 20 µl
	<2.5 ng/ml Omnibeads[7]; 20 µl
Uniformity	≤3.0 %
Z´value	>0.9
Fastest read times[8]	≤2 min (384-well plate)
	≤1 min (96-well plate)

Gas Control Module (GCM™)

Adjustable concentration range – CO_2	0.04–10 % (vol.)
Adjustable concentration range – O_2	0.1–21 % (vol.)
Concentration accuracy – CO_2	<1 % (vol.)
Concentration accuracy – O_2	<0.5 % (vol.)

Reagent injectors

Syringe sizes	0.5 ml; 1 ml
Pump speed	100–300 µl/sec
Injection volume	5–2,500 µl; step size: 1 µl
Dead volume	≤100 µl
Injection accuracy and precision	≤0.5 % at 450 µl

Temperature control	Ambient +3 °C up to 42 °C
Uniformity	<0.5 °C

Shaking

Linear, orbital, double-orbital;
variable amplitudes and frequencies

+Specifications are subject to change. Performance values represent the average observed factory tested values.

*Fusion Optics: a combination of filter and monochromator on the excitation and emission sides

1) Detection limit for fluorescein

2) FP detection limit @ 1 nM fluorescein

3) Detection limit for europium

4) Detection limit for ATP (144-041 ATP detection kit SL, BioThema)

5) Detection limit for ATP (ENLITEN® Kit)

6) (PE# 6760620; P-Tyr-100 assay kit)

7) (PE# 6760626D; Omnibeads)

8) Including temp. correction

Spark Cyto multimode reader is For Research Use Only.

For product specifications refer to operators manual.

Live-cell imaging in real time: **www.tecan.com/SparkCyto**

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401217 V1.0, 2019-06, 30164988

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