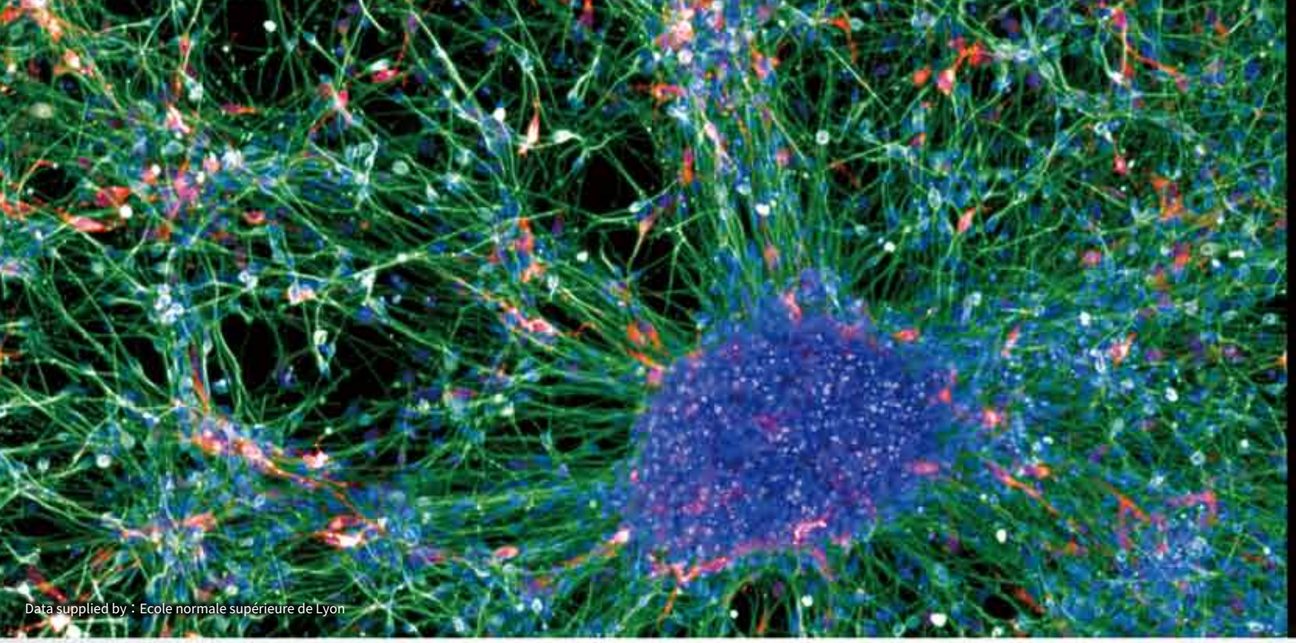
Co-innovating tomorrow™

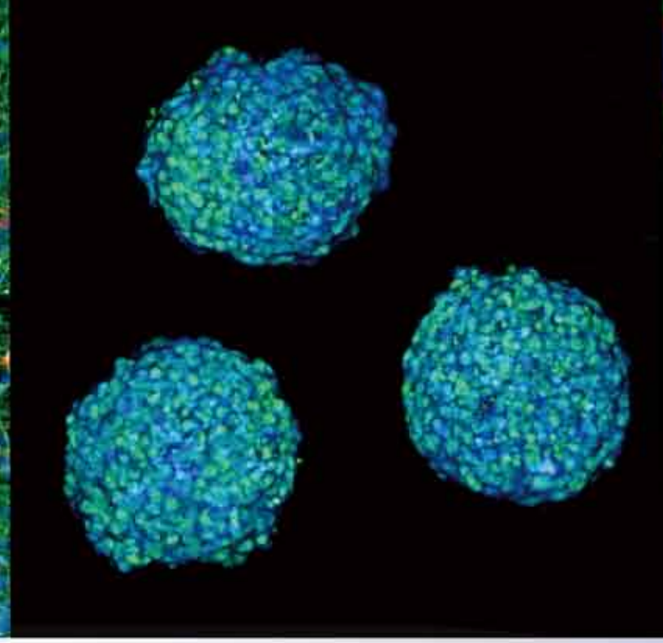
YOKOGAWA

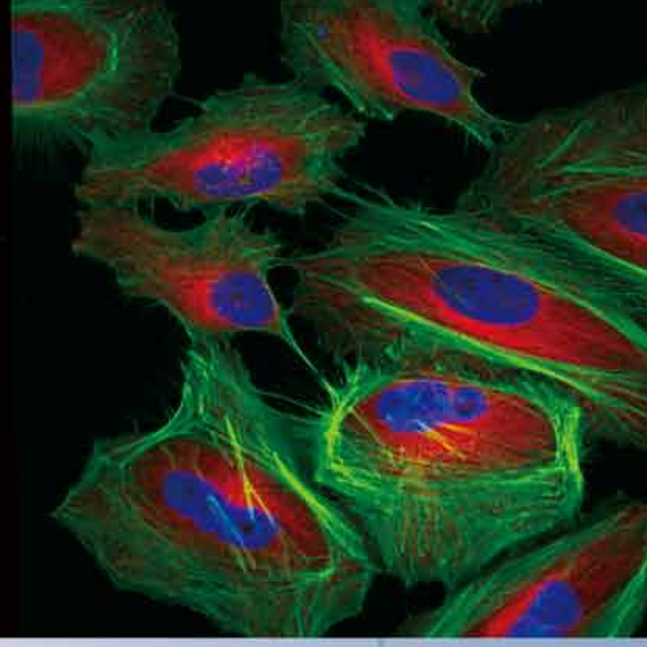
High content analysis software

CellPathfinder™

Bulletin 80K01B01-01E



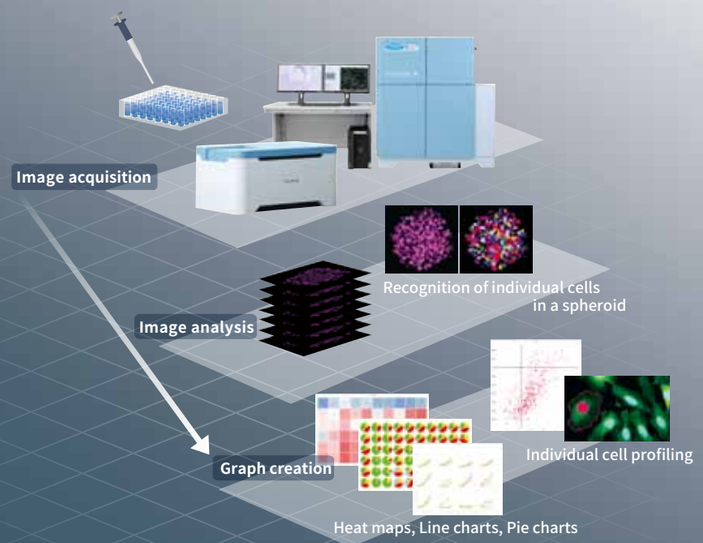




summary

Introduction

Compared with biochemical experiments, high content analysis (HCA), which analyze phenomena occurring in cells based on microscopic images, allows to acquire wide-ranging data in a single experiment, including not only the quantification of the target molecules but also the dynamics of them such as localization and the morphlogical information of cells as well as intracellular organelles.
In addition, the phenomena can be verified through actual images, and the non-specific phenomena that may result in false positives in traditional high throughput screening (HTS) can also be eliminated.
Moreover, after acquiring images once, HCA allows to analyze them any number of times from various angles, which provides an efficient and multidimensional understanding of cell's physiological phenomena. Owing to this, the necessity for HCA in basic research and drug discovery is increasing.
On the other hand, experimental systems which reproduce the environments similar to that in vivo such as live-cell systems using biomarkers and 3D culture, in order to capture phenomena occurring in the body, have become complicated.
Furthermore, label-free assays, which minimizes the effect on cells, have become popular in recent years especially in regenerative medicine.Thus the demand for the label-free analysis is increasing.



Solution

The CellPathfinder analysis software, with the intuitive and easy-to-use interface, guides the user from analyzing thousands of image data in various angles to visualize the result by creating graphs in the simple process.
In addition, the machine-learning function is provided as standard, increases the target recognition capability dramatically. It is also ideal for the analysis with the complex and high-degree-of-difficulty such as analyzing the data from 3D culture and live-cell imaging.
Furthermore, the machine-learning function is also highly usable for analyzing bright field images, it enables a wide range of the image analysis of not only fluorescence but also bright field.
The CellPathfinder is a powerful tool that broadens the capability of HCA.

CellPathfinder

CellPathfinder Resolves Difficulties

For high throughput screening Users

CellPathfinder resolves screening bottlenecks

- A specialized interface for inspecting multiple samples makes image comparison easy, improving efficiency.
- Advanced analysis using the machine-learning function is possible through simple operation, even for beginners.
- Various graph creation functions and simple image and video creation are available, reducing hassles at the time of reporting.

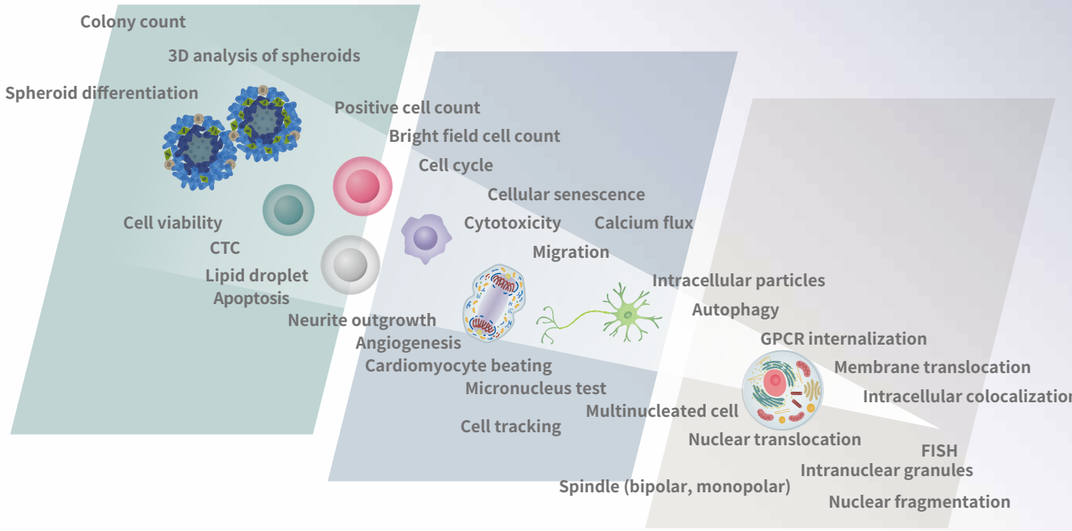
For cancer researchs and regenerative medicine researchers

CellPathfinder provides leading HCA through proprietary analysis technology

- Label-free analysis of sensitive samples is possible using Yokogawa's proprietary image generation technology "CE Bright Field"*1.
- Newly-developed easy-to-use machine-learning (standard function) makes previously difficult phenomena detection easy.
- Detection of rare events (CTC, etc.) with high speed and high accuracy.

*1 Refer to P. 7 for details on CE Bright Field.

Application example



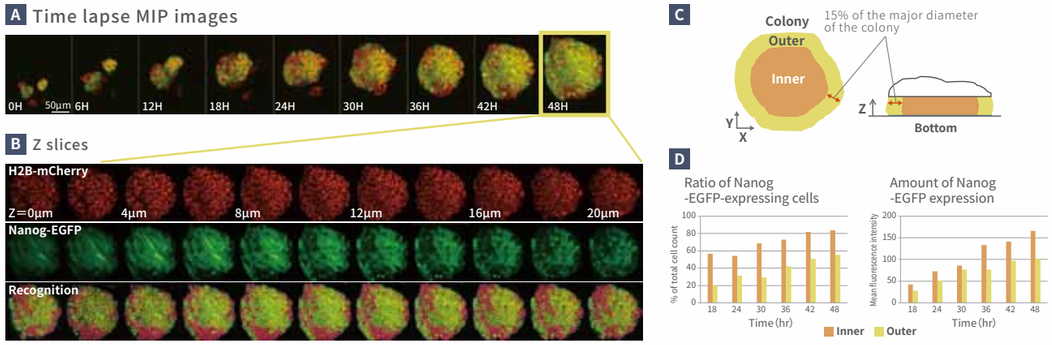
Application example

Regenerative Medicine Research

Differentiation of ES Cell Colonies

Time lapse | Quantitative | 3D | Multi-color

Analysis of not only colony size, but also the differentiation states of individual cells in the colony



3D time-lapse analysis of an ES cell colony. Images were acquired for 48hrs at 3D minutes intervals.
Cells expressing a differentiation marker Nanog-EGFP were analyzed.

A. Time lapse change of a colony B. Z slice images of a colony after 48 hrs
C. The colony was separated into two regions (outer and inner) based on the colony diameter, and 3D analysis was performed for Nanog-expressing cells in each region.
D. Nanog-EGFP expression levels for the inner and outer regions. Both regions show a continuous increase in expression level over time.

Objective lens：40x / Ex：488nm(Nanog-EGFP), 561nm(H2B-mCherry) / Time lapse: 48 hrs at 30 minute intervals

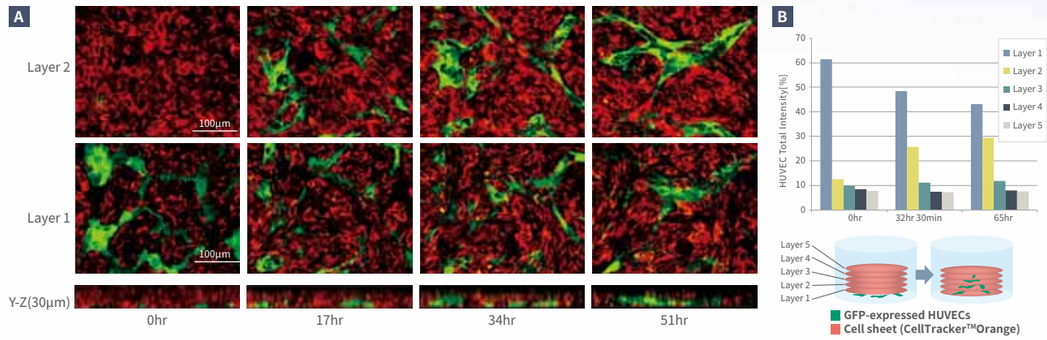
Data supplied by: Prof. Kyoji Horie, Department of Physiology II, Nara Medical University

Web site

3D Analysis of HUVEC Migration across Cell Sheets

Quantitative | 3D | Time lapse

3D analysis of HUVECs' upward migration through layered cell sheets



GFP-expressing HUVECs were seeded in the bottom layer of 5 layered cell sheets, and time lapse imaging was conducted over a duration of 67 hrs at 30 minute intervals. The total intensity of HUVECs in each layer was calculated.

A. HUVECs at representative time points for Layers 1 and 2, and YZ cross-sections.
B. Distribution of fluorescence representing HUVECs along different layers at 0 hr, 32 hr30 min and 65 hrs. HUVECs decreased over time in Layer 1; however they increased in Layer 2.

Objective lens：40x / Ex： 488 nm (HUVEC-GFP)， 561 nm (Cell sheet-CellTracker Orange) / Time lapse: 67 hrs at 30 minute intervals

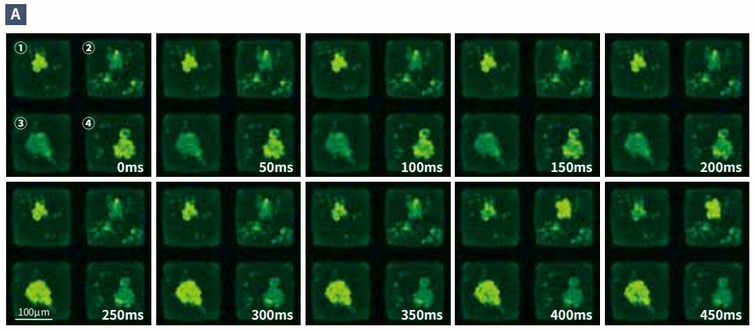
Data supplied by: Associate Professor Eiji Nagamori, Department of Biomedical Engineering, Graduate School, Osaka Institute of Engineering of Technology

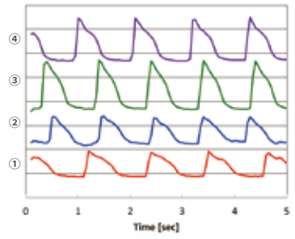
Web site

Imaging of Calcium Signals in iPSC-derived Cardiomyocytes

Quantitative | Time lapse | High speed

High speed time lapse (20fps) imaging of individual spheroids' calcium flux





iCell Cardiomyocytes (FUJIFILM Cellular Dynamics, Inc) were cultured on Elplasia (Kuraray Co., Ltd.).
After spheroids were formed, the culcium oscillation was visualized using a calcium indicator dye.
The mean fluorescence intensity for individual spheroid was quantified.

A. High speed time lapse of calcium signals in iPSC-derived Cardiomyocytes
B. Signal changes in individual spheroids 1 - 4

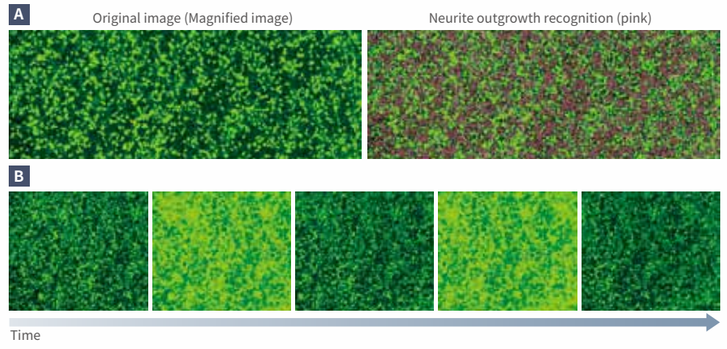
Objective lens：10x / Ex: 488 nm (Early Tox Cardiotoxicity Kit)/ Time lapse: imaged at 50 millisecond intervals

Web site

Neurite Outgrowth of iPSC-derived Neurons

Quantitative | Time lapse

Analysis of neurite outgrowth and spontaneous activity of iCells labeled with a calcium indicator



	Outgrowth Total Length (μm)	Outgrowth Total JunctionCount	Outgrowth Total BranchCount
Day20	166978.8	5384	18308

iCell DopaNeurons (FUJIFILM Cellular Dynamics, Inc) were cultured in a mixed culture medium of BrainPhys Neuronal Medium (STEMCELL) and Neuron Culture Medium (WAKO)

A. Analysis of neurite outgrowth after 20 days.
Total length of neurite outgrowth (Outgrowth Total Length), total junction count (Outgrowth Total Junction Count), and total branch count (Outgrowth Total Branch Count) were calculated.
B. Spontaneous activity after 20 days. C. The mean intensity at each time point was calculated.

Cell culturing conditions: cultured in Corning 96-well half-area plate (80,000/well)
Objective lens：10x / Ex:488（Cal-520AM）/ Time lapse: 299.5 seconds at 0.5 second intervals

Web site

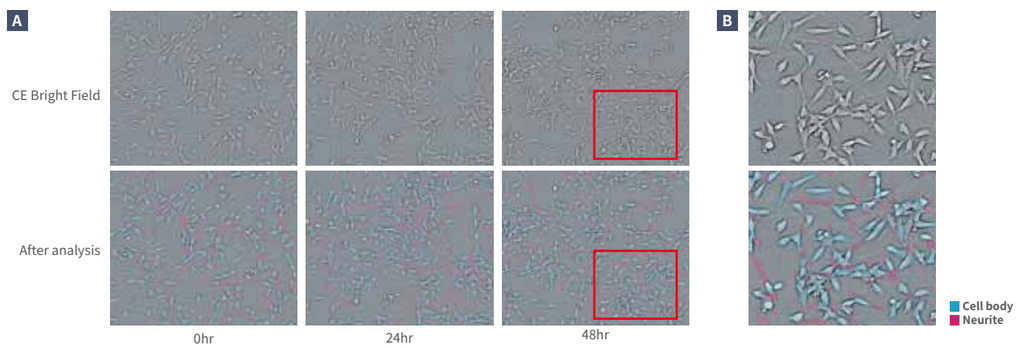
Application example

Cancer Research

SH-SY5Y Neurite Outgrowth

Quantitative | Time lapse | Label-free | Machine-learning

Analysis of cell proliferation and neurite outgrowth over time (without staining)



Time lapse imaging of SH-SY5Y cells was conducted over 67.5 hrs at 1.5 hr intervals, analyzed using bright field (CE Bright Field) image and the machine-learning function. Cell count, total length of outgrowth and junction count were calculated.

A. Top: Bright Field image (CE Bright Field) after 0, 24, 48 hrs.
Bottom: Image recognition.
B. Magnified image of the lower right part of A after 48 hrs. Neurites (pink) are accurately recognized.

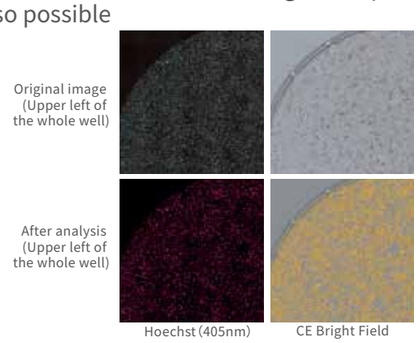
Objective lens: 10x / Bright field (CE Bright Field image created at the time of analysis) /
Time lapse: 67.5 hrs at 1.5 hr intervals

Web site

Monitoring of Cell Proliferation

Quantitative | Label-free | Machine-learning | Tiling

Counting of all cells in a whole well without staining
Creation of multi-field tiled images and precise recognition of cells near the edge of the 96-well is also possible



Cell Count

	1	2	3
Hoechst (405nm)	14351.6	10867.6	6944.3
CE Bright Field	14316.3	10847.6	6942.0
Ratio_CE Bright Field/405 (%)	**99.7**	**99.8**	**99.97**

n=3wells

Hela cells were cultured at three different densities. Nuclei were stained with Hoechst33342, and fluorescence images and bright field (CE Bright Field) images were acquired. Cells were counted using each image and the results were compared.
Very similar results were acquired for the CE Bright Field and nuclear stained images for all three densities.
Using CE Bright Field images cell number is properly counted without labeling even in a small wells of 96-well plates.

Objective lens: 10x / Ex: 405 nm, Bright field (CE Bright Field image created at the time of analysis) /Plate: Greiner 96-well plate

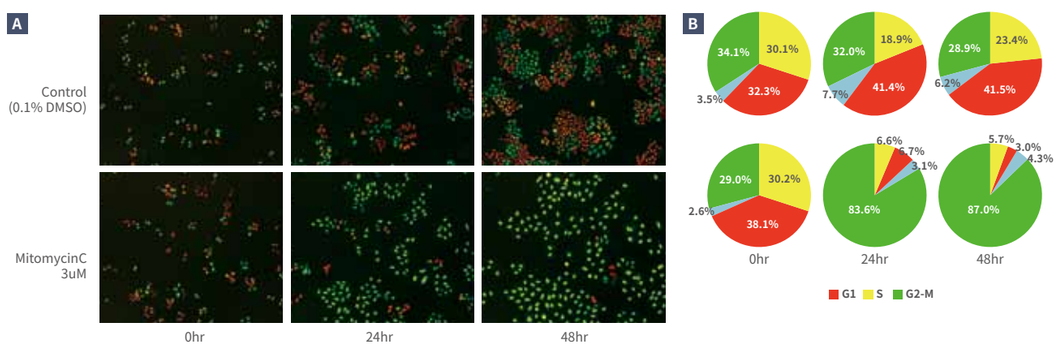
Web site

Web site

Cell Cycle

Quantitative | Time lapse | Multi-color

Cell Cycle phases categorized using Fucci



Time lapse imaging of Fucci-expressing HeLa cells was conducted over 48 hrs at 1 hr intervals. Gating was performed based on the mean fluorescence intensities of 488nm and 561nm channels for each cell. They were categorized into four phases of cellcycle and the cell count of each phase was calculated.

A. Marged images of 488nm and 561nm after 0, 24, 48 hrs. Cells in G1 phase decreased when Mitomycin C 3µM was added.
B. Ratio of cells in each cell cycle at each timepoint.

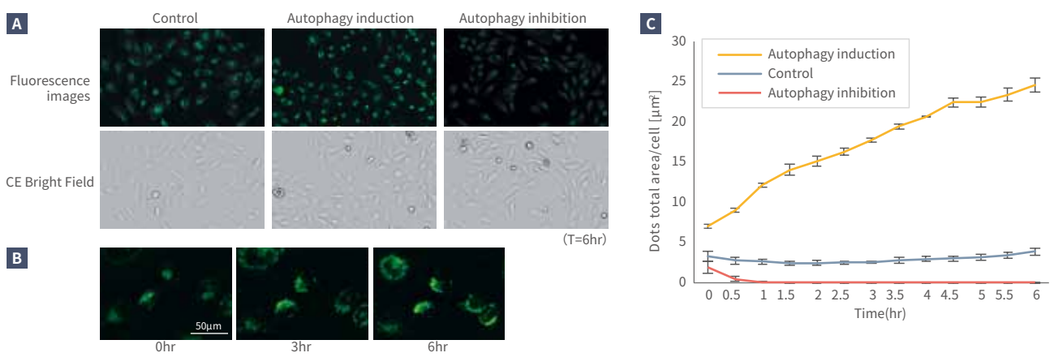
Objective lens: 10x / Ex: 488nm (Geminin-mAG1), 561 nm (Cdt1-mKO2) / Z range: 10µm, Z step: 5µm (Analyzed MaxIP images)/
Time lapse: 48 hrs at 1 hr intervals

Web site

Autophagy

Quantitative | Time lapse | Label-free | Machine-learning

Autolysosome formation analysis without nuclear staining



Hela cells cultured in a 96-well plate were treated with DAL Green (Dojindo Laboratories) and the media were replaced by three different culture media: a normal medium, autophagy inducer medium or an autophagy inhibition medium (Bafilomycin added to an inducer medium). Time lapse imaging was then conducted for 6 hrs and cell count, granule count, total area of granules are calculated.

A. Images of cells in each culture medium.
B. Formation of autolysosomes over time in the autophagy induction medium.
C. Change of autolysosome area per cell over time. Autophagy granules were detected using fluorescence images, and cells were counted using CE Bright Field images.

Objective lens: 20x / Ex: 405nm (DALGreen), Bright field (CE Bright Field image created at the time of analysis) / Time lapse: 6 hrs at 30 min intervals

Web site

Functionality Enabling Complex Analysis through Easy Operation

Machine-learning

Machine-learning functionality allows for unbiased digitization in experiments evaluated through appearance.
Automated shape recognition can be performed by simply clicking on the shape you wish the software to learn.

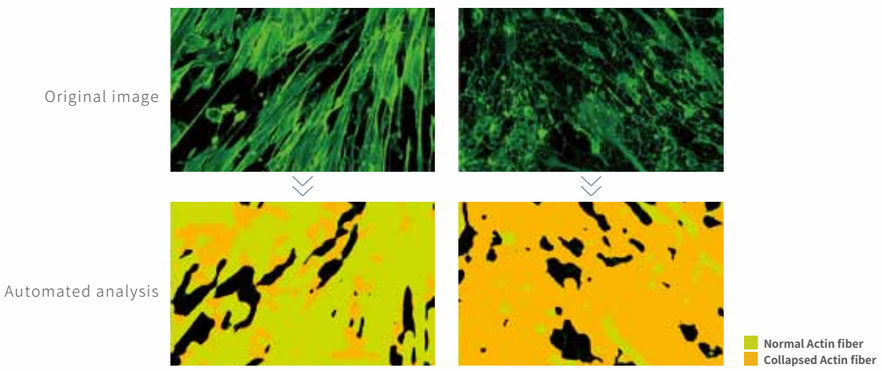
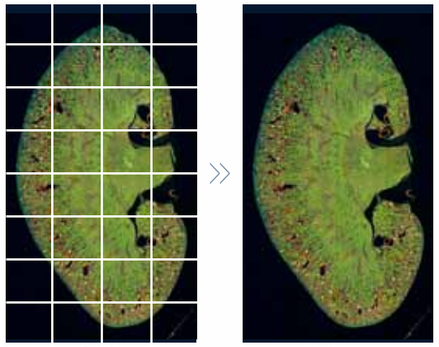


Image Stitching

Tiled images are generated through image stitching and analyzed, allowing for accurate quantification.
Ideal for analysis spanning across fields, such as of spheroids, tissue sections and neurites.



8x4 field image　　Tiled image

CE Bright Field（Contrast-enhanced Bright Field）

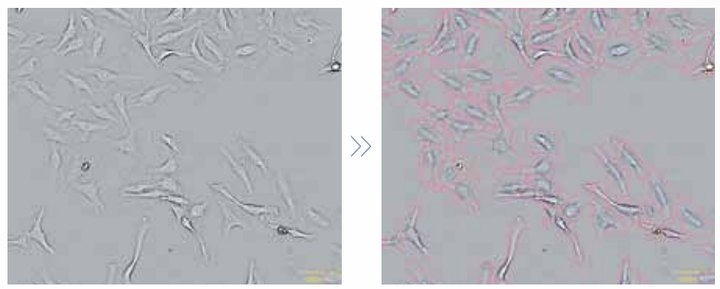
By using Yokogawa's "CE Bright Field" proprietary image creation technology, two types of images can be output from bright field images. The first is an image resembling a phase contrast image, created from a regular DPC (digital phase contrast), and is effective for cytoplasm contour recognition. The second is an image resembling a fluorescence image, effective for nuclear recognition.

A powerful tool for analysis without staining

Bright field imaging　　Phase-type　　Fluor-type

Label-free Analysis

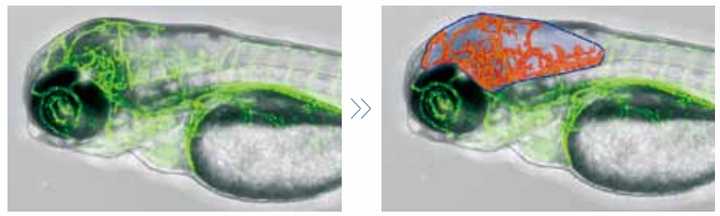
The recognition of samples without the use of labeling is possible using images created with CE Bright Field technology. Time, cost and effects on cells due to fluorescent labeling are eliminated from phenotype analysis.



CE Bright Field image　　Cell recognition image

Manual Region Specification

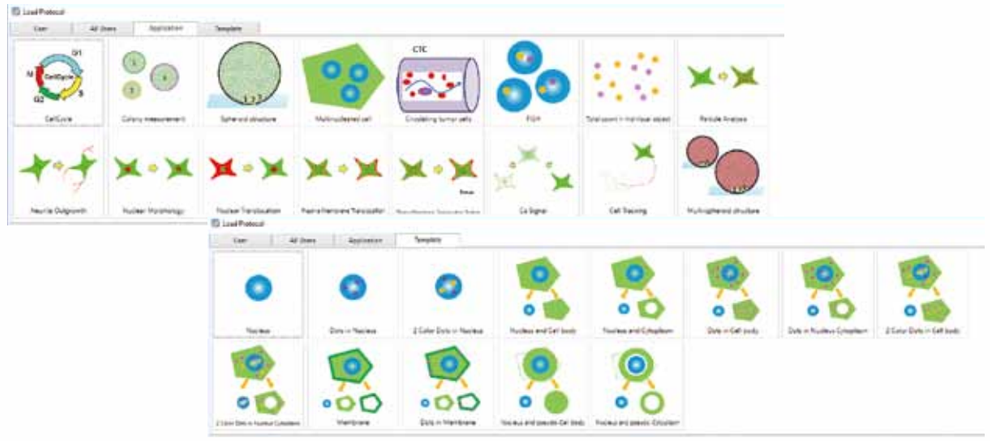
Manual specification of analysis regions is possible for complex samples that are difficult to identify through automated image processing. Facilitate the analysis of only the specified regions such as tissue section.



Data supplied by: Dr. Yasuhito Shimada, Department of Integrative Pharmacology, Graduate School of Medicine, Mie University

Abundant Pre-installed Analysis Menu

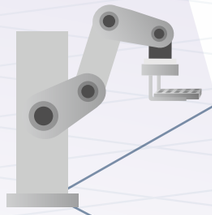
The analysis menu is displayed using easy-to-understand icons. Protocols can be loaded simply by clicking an icon. Beginners can start the image analysis easily.



Offering Total Solutions, from Acquisition to Analysis

Plate transport via robot, acquisition using CellVoyager or CQ1, data management using CellLibrarian, and image analysis using CellPathfinder.
We offer optimum combinations matched to users' needs and budgets.

Transfer



Realization of automated
plate transfer through cooperation
with a trusted robot manufacturer.

Acquisition

Confocal quantitative image cytometer CQ1

Space-saving benchtop design.
Easy-to-use confocal imaging system,
capable of automated imaging of multiple samples .
Compatible with live cell imaging.

High Throughput Cytological Discovery System CellVoyager

A high-end HCA system allowing high quality and high speed screening through the use of water immersion lenses and multiple cameras. Kinetic measurement is also possible, using a built-in pipetter.

Data Management

NAS

Large amounts of image data can
be saved and accessed via the network.
Data transfer is not required at the time
of analysis.

High content data management system CellLlibrarian

Situated at the heart of the CellVoyager high content analysis system, the data management system performs image storage and data management and analysis. The database is accessible via the Internet.

Analysis

High content analysis software CellPathfinder

Analysis image data of CQ1 and CellVoyager, graph creation, various types of data output.
Label-free analysis is also possible through CE Bright Field and machine-learning functionality.
The analysis menu features a wealth of preset functions, providing ease-of-use for beginners.

The Support System Underlying Our Total Solution

Prior to installation

- For trial imaging expreriments, our specialist staff can prepare samples
- Imaging and analysis of customer-created samples is also available
- Immediate support by our engineering team
- Using demo units, customers can test image acquisition and analysis
- Latest market trends are continuously monitored and reflected in our products

Delivery

- Delivery and setup performed by specialist staff
- Provide careful operation training to new users upon system installation

Customer Service

- Fault inspection and on-site repairs are performed immediately
- Periodic maintenance ensures equipment is always in ideal condition
- Analysis support
- Creation of complex analysis protocols
- Comprehensive phone and email support
- On-site support
- Provision of tutorials and basic manuals
- Provision of the latest information

Model	CVSW02
Standard applications	Cell count, Cell cycle, Colony measurement, Spheroid structure, Multinucleated cells, Nuclear fragmentation, CTC, FISH, Granule detection/localization, Neurite outgrowth, Membrane translocation, Nuclear translocation, Calcium flux, Cell tracking, etc.
Analysis functions	3D analysis, Tile analysis, Label-free analysis, CE Bright Field*1, Machine-learning, Texture analysis
Graphing functions	Line charts, Pie charts, Scatter plots, Heat maps, Histograms
Gating	Quadtree gating, Quadtree with hinge gating, Rectangle gating, Polygon gating, Linear gating, Machine-learning gating
Analysis modes	Single analysis, Batch analysis, Automated analysis
Output data formats	Numeric data: CSV Image data: PNG, JPEG, TIFF Video data: Windows Media Video (WMV), MPEG4
Compatible equipment	CellVoyager CV6000, CV7000, CV8000, CQ1
Accessories	Workstation Model: Dell Precision*2 CPU: Intel Xeon Memory: 128 GB HDD: system drive (C:) 4 TB, data drive (D:) 4 TB OS: Microsoft Windows 10 Pro 64 bit, Japanese/English Monitors: Two 24″ monitors with resolution of 1920x1200 *3

*1 Refer to P. 7 for details on CE Bright Field for.
*2 Dell Precision is certified in accordance with countries' laws and regulations by their manufacturer, Dell Corporation.
*3 Arranged by local distributors for overseas sales.

All functionality of CellPathfinder can be experienced free of charge

A time-limited trial version is available. There are no restrictions on the software functionality, so you are able to fully experience the functionality and operation of the commercial version.
If you have a CellVoyager*4 or CQ1, data acquired on these devices can be analyzed straight away using CellPathfinder.
There's also a number of basic manuals and tutorials, allowing you to begin analysis easily.

*4 Not compatible with CV1000

Please scan the QR code for more information.

YOKOGAWA

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