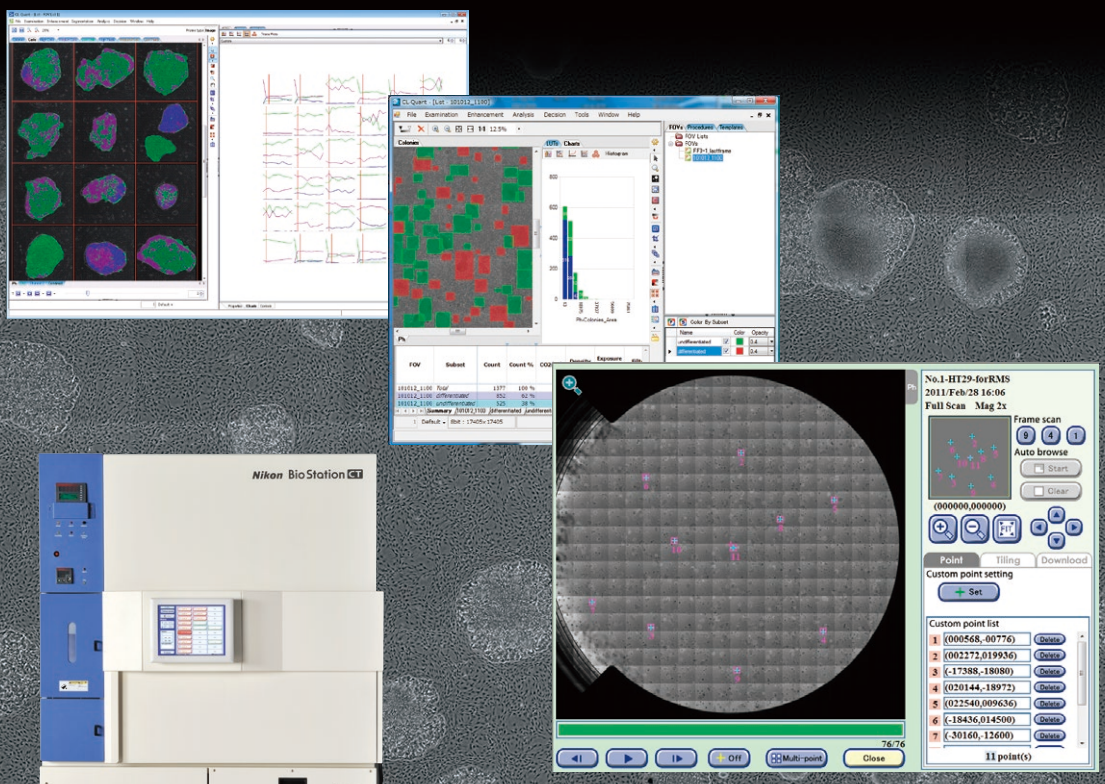
Nikon

Imaging Solutions for Stem Cell Research

Cell Culture Observation System

BioStation CT

Cell Tracker



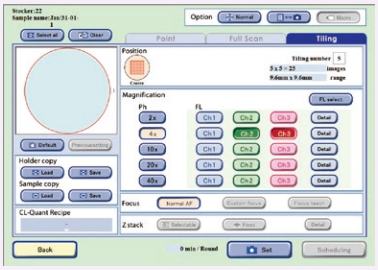
Stem cell screening inside the incubator

With conventional cell monitoring procedures, a culture vessel has to be taken out of the incubator for microscope observation, where cells are subjected to stressful environmental changes and vibration. Researchers then have to spend additional time repositioning the vessel to find the same observation points. Nikon's BioStation CT eliminates these problems by providing a stable environment so that the cultures don't suffer while they are being imaged and allowing for a complete trace of the same live cells, including stem cells.

Advanced basic functions

Automatic image capture

The autofocus mechanism allows the capture of in-focus images. Z-stack imaging in phase contrast observation, multi-sample imaging and multi-point imaging are possible with multiple magnifications. User-configured imaging conditions that can be saved in BioStation CT support the repeatability of observations.



Remote access

Configuring the imaging settings, scheduling a time-lapse experiment, and viewing the cell images are possible via a network. The captured data can be automatically downloaded to the user's local computer. This enables users to monitor the cell status away from the laboratory. When a culture environment (temperature, humidity, CO_2 concentration) control error occurs, BioStation CT can notify the users of the error by e-mails.

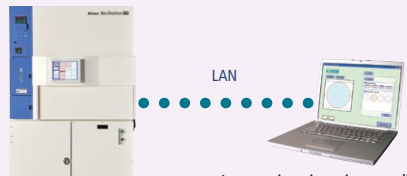
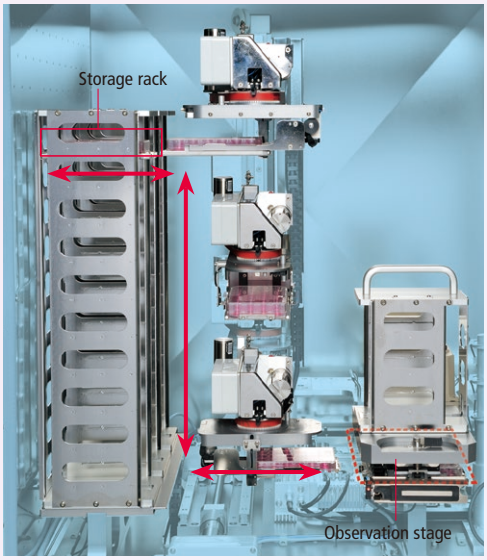


Image view, imaging condition change

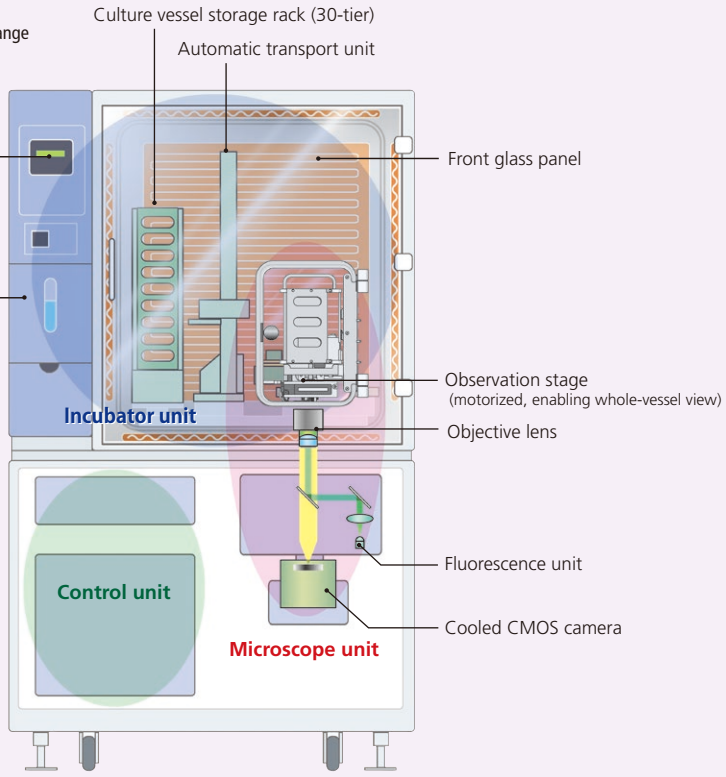
Error notification received

Automatic vessel transportation

BioStation CT incorporates a transport unit that provides stable vessel transportation within the heated and humidified incubation area. The high-precision motorized stage in the observation unit allows for automated imaging of the entire area of a well in all culturing formats.



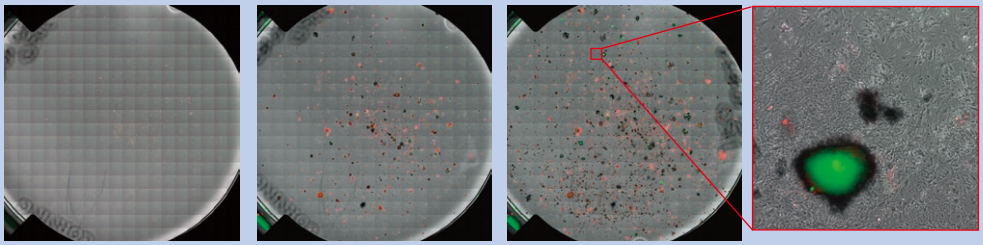
The transport unit carefully conveys the vessels from the storage rack to the observation stage in accordance with configured schedules.



Various functions

Full-well scan imaging and highly magnified image stitching

High-resolution full-well scans are reconstructed by stitching captured adjacent images. This enables clear detection of an iPS colony, which is difficult to detect because of its low induction efficiency, no matter where it forms in the vessel. The specified position of the vessel can be highly magnified with high resolution. BioStation CT also offers cell registration to allow for repeated visits to the same location. These time-lapse sequences can be created even when a vessel is removed from the BioStation CT for medium exchange.



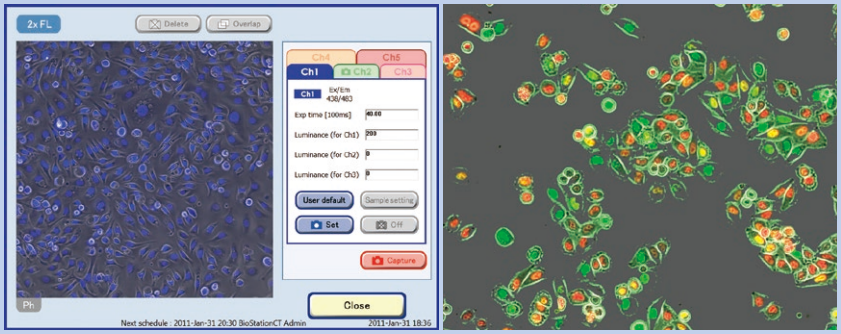
Day 5 of culture | Day 13 of culture | Day 18 of culture

Mouse iPS cells reprogramming

GFP: Nanog-GFP
DsRed: retrovirally transduced
Vessel: 100 mm culture dish
Magnification: 2x
Culture period: 3 weeks
Imaging interval: 4 hours
Courtesy of Dr. Hidemasa Kato, Saitama Medical University

Fluorescence observation

Long-life and low-cost LED illuminator is employed as a light source. Up to five fluorescence filter cubes can be mounted. Up to three channels can be used with simultaneous multi-channel acquisition. The expression of fluorescence proteins such as CFP, YFP, Kusabira Orange, DsRed, Texas Red and Cy5 can be observed effectively in fluorescence observation.



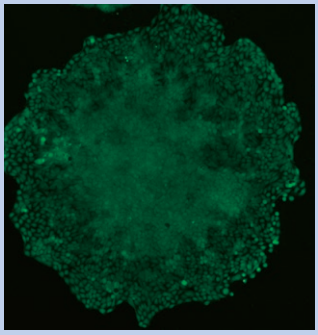
Reduced phototoxicity

The excitation period is shortened by synchronizing the camera exposure with the excitation illuminator. This prevents photobleaching of the specimen and minimizes the phototoxic damage on the cells.

High S/N ratio image acquisition

Thanks to the built-in cooled CMOS camera, low-noise images with an S/N ratio two times higher than conventional cameras can be acquired.

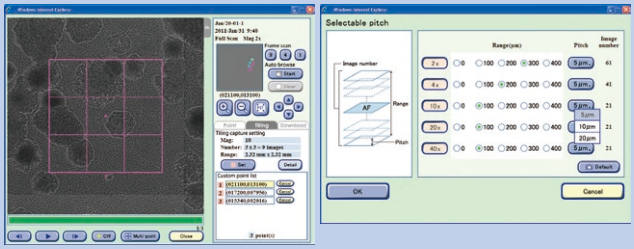
NEW



iPS colony acquired with a built-in camera

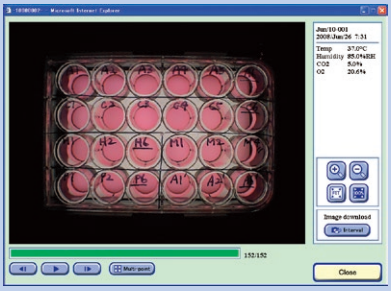
Micro observation

Phase contrast and fluorescence images can be captured with the high-sensitivity cooled CMOS camera. These images can be magnified in 2x, 4x, 10x, 20x and 40x. Up to 40 phase contrast images can be captured along the Z axis with the Z-stack function.

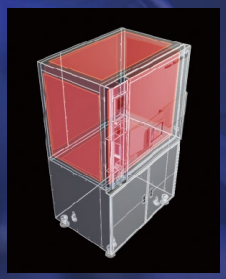


Macro observation

Brightfield image of the whole vessel provides users outside the BioStation CT with information such as handwritten information on the vessel, medium color and whether mold is growing or not. In addition, alkaline phosphatase stained cell counting is available as an option.



Stable culture environment maintenance

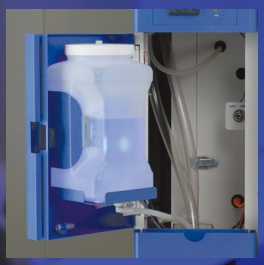


Precise temperature control

The inside temperature is directly controlled by panel heaters embedded in the incubator's six sides. This allows highly precise temperature maintenance.

Humidity control with air-flow type active aerosol spray humidifier

Distilled water is automatically sprayed inside the incubator to keep the optimum humidity. Water can be supplied to the tank without opening the incubator door. This air-flow type humidifier reduces contamination risks compared to the water bath type.

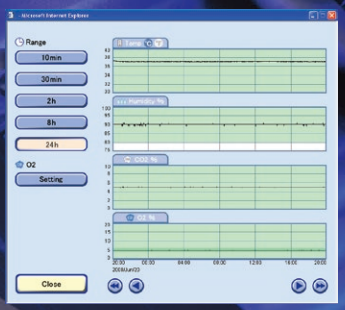


Hypoxic culture capability

Hypoxic culture observation is possible with the optional oxygen regulator and nitrogen generator.

Environment data recording

The culture environment is constantly monitored and recorded. The environment data can be accessed at anytime.



CO_2 incubator environmental graph screen

Smooth vessel transportation

The waver of liquid surface during the transportation is less than 2 mm. The drift and stress of cells are reduced.

Reduced contamination risk

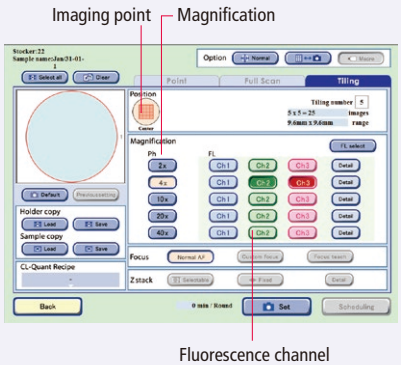
The incubator interior can be sterilized using hydrogen peroxide gas. (This is optional, and a 200 V power source is necessary.)

Easy operations

Vessel installation | **Imaging parameter setting** | **Scheduling**

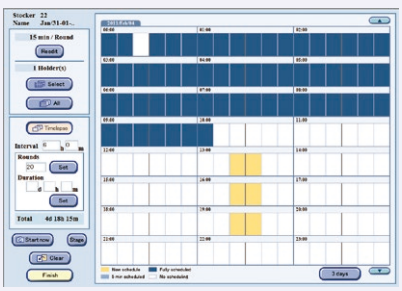
Culture vessel installation into the incubator

Vessels are placed in the incubator through a small door in the front glass panel, minimizing negative effects on the environment within the incubator.



Easy touchscreen operation

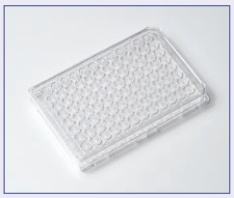
Time-lapse imaging configurations such as magnification, imaging point, fluorescence channel and stage motion speed can be set.



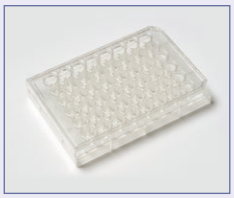
Time-lapse imaging schedule

The imaging interval and total period can be set. The shortest time-lapse imaging interval is one minute.

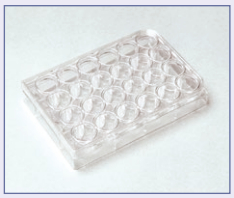
Compatible with various culture vessels



96-well plate
Up to 30 plates stored in a storage rack
Up to 25 observation points in a well



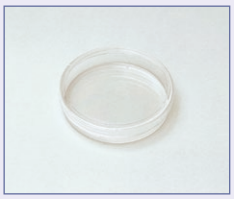
48-well plate
Up to 30 plates stored in a storage rack
Up to 25 observation points in a well



24-well plate
Up to 30 plates stored in a storage rack
Up to 25 observation points in a well

12-well plate
Up to 30 plates stored in a storage rack
Up to 25 observation points in a well

6-well plate
Up to 30 plates stored in a storage rack
Up to 25 observation points in a well



100 mm culture dish
Up to 30 dishes stored in a storage rack
Up to 25 observation points in a dish

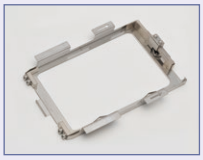
60 mm culture dish
Up to 60 dishes stored in a storage rack
Up to 25 observation points in a dish

35 mm culture dish
Up to 150 dishes stored in a storage rack
Up to 25 observation points in a dish

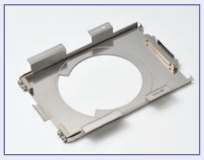
75 cm² culture flask
Up to 30 flasks stored in a storage rack
Up to 25 observation points in a flask

25 cm² culture flask
Up to 30 flasks stored in a storage rack
Up to 25 observation points in a flask

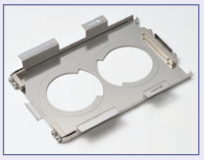
Tray holders for various culture vessel types



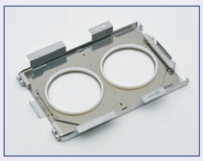
For well plate



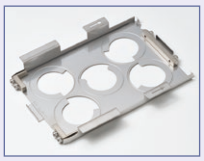
For 100 mm culture dish



For 60 mm culture dishes

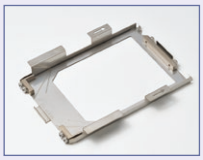


For nunc 60 mm culture dishes

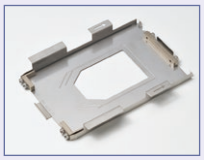


For 35 mm culture dishes

For Falcon 35 mm culture dishes

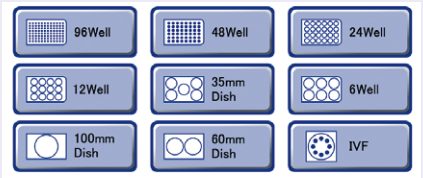


For 75 cm² culture flask

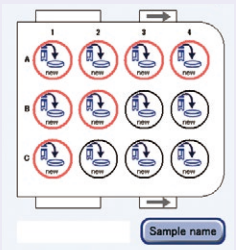


For 25 cm² culture flask

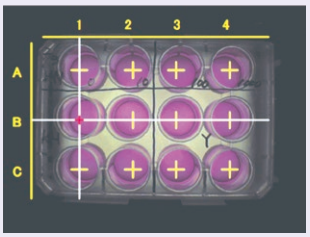
Different GUI for each vessel type



Vessel type icons



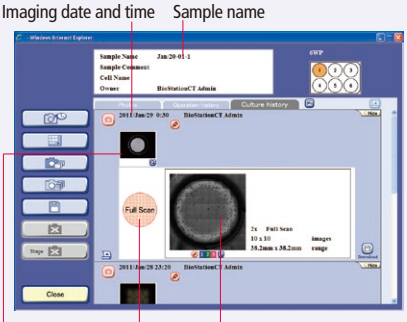
GUI for 12-well plate



Wells to be observed can be chosen on the touchscreen.

Captured image view

Imaging date and time Sample name

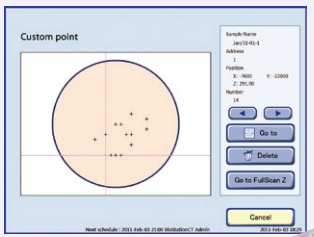


Micro image thumbnail
Imaging point within the vessel
Macro image thumbnail

Culture history data management

The time-course change of a specimen can be observed easily in sequentially displayed captured images.

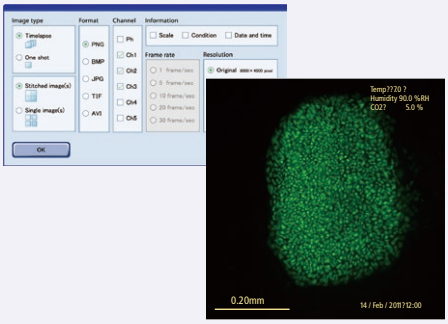
Medium exchange



High-precision repeatability

Accurate tracing of same cells, even after medium exchange, is possible using a dedicated tray holder, as BioStation CT records culture history, such as medium exchange and subculture, as well as X-Y positions for each vessel.

Data report



Reliable data management and documentation support

Obtained data is duplicated and protected using uninterruptible power supply. Observation information such as temperature, humidity and imaging date can be written and displayed on the captured image to simplify presentation document preparation.

iPSC/non-iPSC Auto Identification

CL Quant

Nikon co-developed an optional program for the BioStation CT with Kyoto University that automatically identifies colonies of iPS cells and counts them based on the structure of each colony. This method acquires data faster and increases its reliability. The iPS/non-iPS cell colony auto identification program saves times when evaluating large quantities of samples.

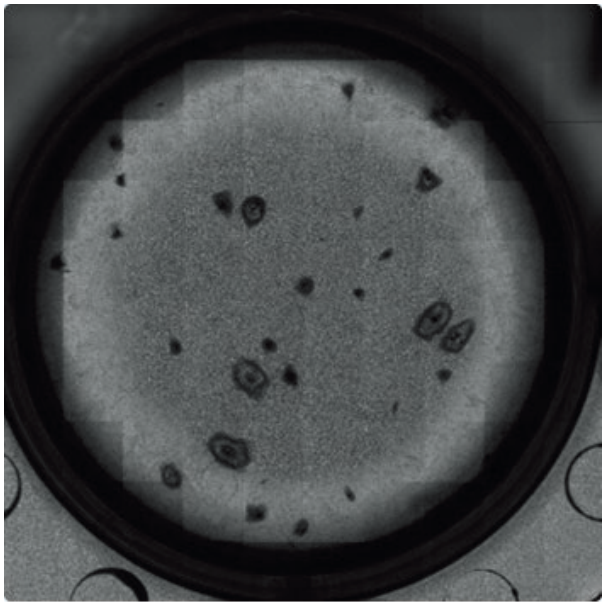


Image captured by the BioStation CT (magnification: 2×)

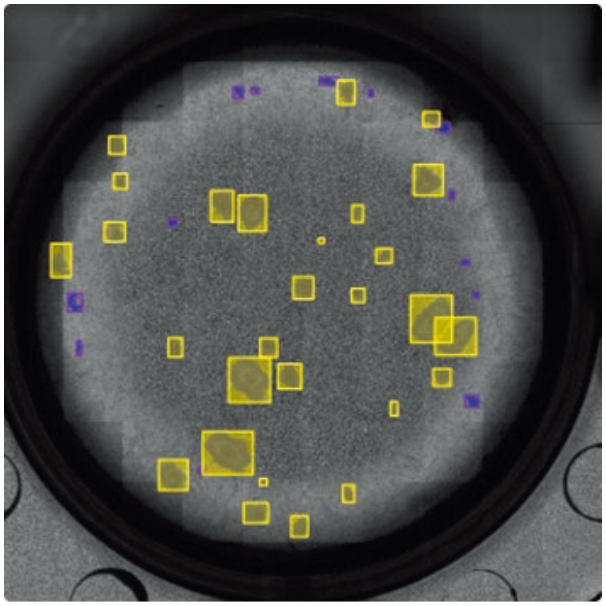
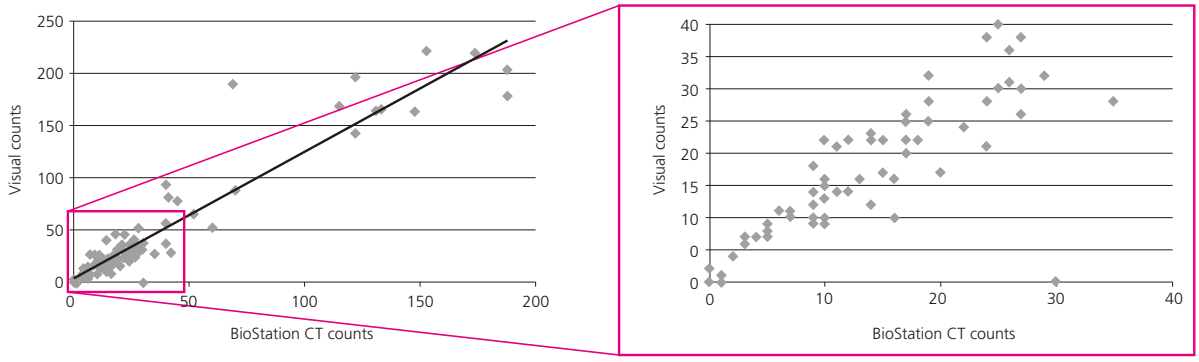


Image of iPS cells automatically distinguished from other cells using the iPS/non-iPS cell colony auto identification program

Showing the correlation between visual counts (vertical axis) and BioStation CT count (horizontal axis).

The graph below is an enlargement of the area in red in the graph on the left.



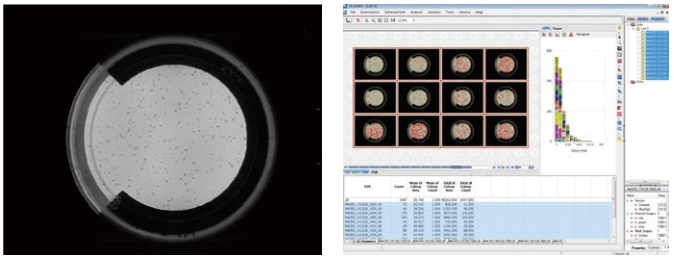
Counting colonies using the BioStation CT
Source: Tatsuya Yamakawa, CiRA, Kyoto University

$R^2 = 0.904$ The correlation coefficient of 0.904 is high.

Alkaline Phosphatase (AP)-positive Colony Counting

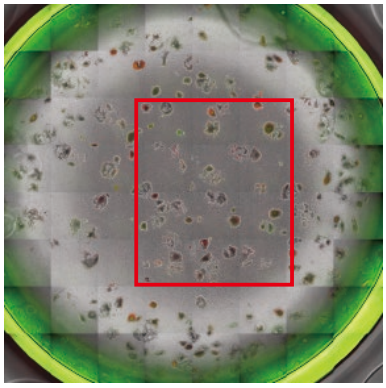
CL Quant

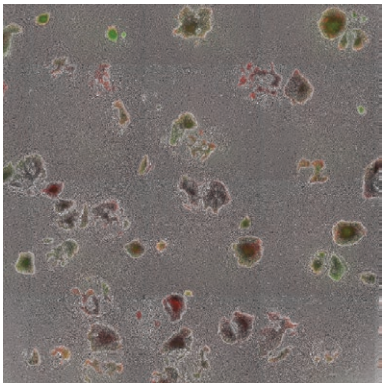
BioStation CT offers alkaline phosphatase-positive colony counting in macro images captured after AP staining, which enables valuation of the undifferentiated stem cell state.



AP-positive colony area comparison in 12 100 mm culture dishes
Courtesy of Dr. Kazutoshi Takahashi and Mr. Koji Tanabe, Department of Reprogramming Science, Center for iPS Cell Research and Application (CiRA), Kyoto University

Reprogramming

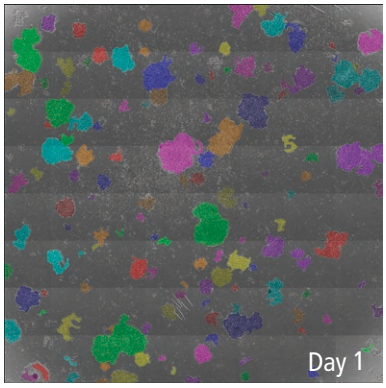


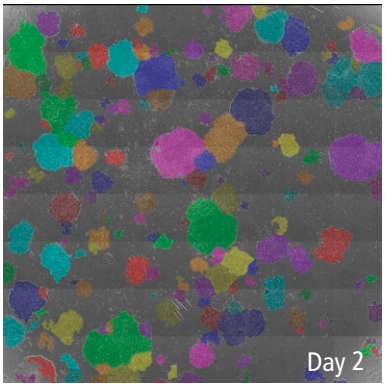


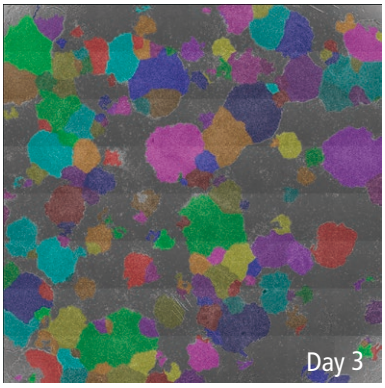
Murine embryonic fibroblasts expressing transgenic oct4-sox2-klf4-iresCherry and carrying an oct4-egfp reporter
Full well scan at 2X and magnified view of reprogrammed colonies in phase, GFP, and DsRed
Courtesy of Dr. Konrad Hochedlinger, Professor of Medicine, Harvard Medical School

iPS Colony Tracking Analysis

CL Quant

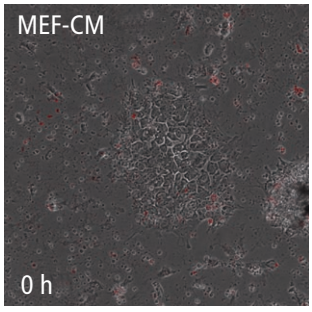


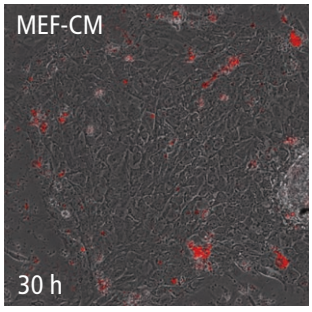


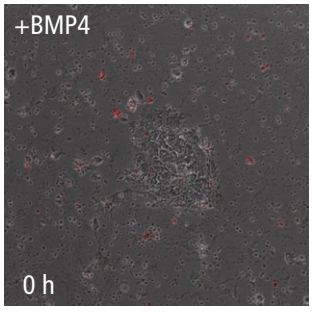


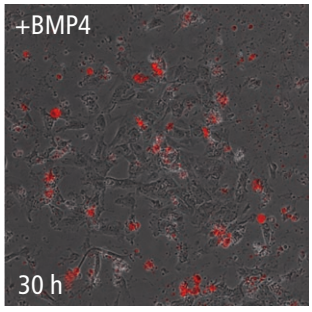
These whole images of 201B7 cell colonies grown in a 6-well-plate coated with fibronectin in the presence of drugs in hESF9 medium were measured by analysis software CLQuant.
This assay can detect each iPS colony by recognizing the boundary even when confluent.
Magnification: 4x
Culture period: four days
Imaging interval: 12 hours
Courtesy of Dr. Miho K Furue (Project Leader) and Mr. Masaki Kinehara (2010-2013), National Institute of Biomedical Innovation (Japan)

Apoptosis





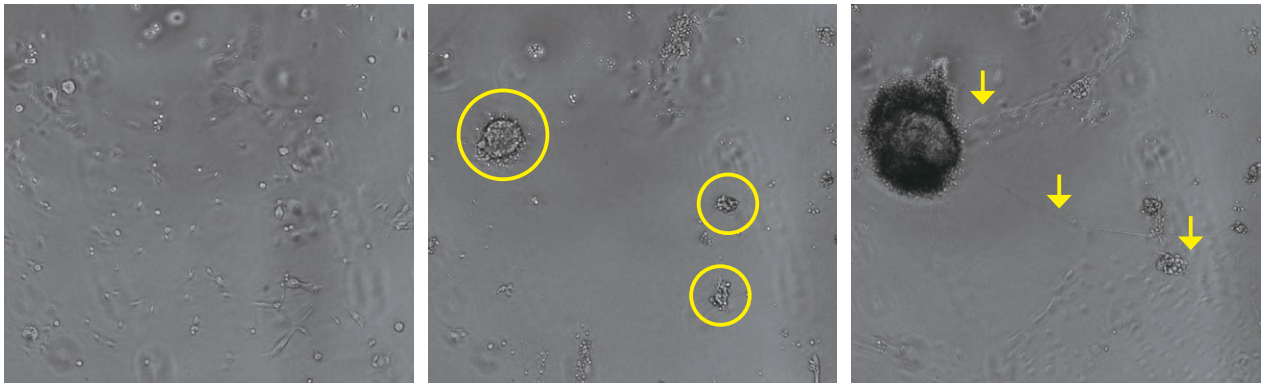




The apoptosis process of human ES cell line H9 cultured in the presence of MEF-CM on Matrigel® was observed. Annexin V (red fluorescence) was used as a detection probe for the cell membrane change that was caused by added BMP4.
Courtesy of Mr. Jamie McNicol, McMaster University

Neural Stem Cells Direct Differentiation

Fibroblast | **Neurosphere formation (neural stem cells)** | **Neurite elongation**



Day 0 | Day 9 | Day 18

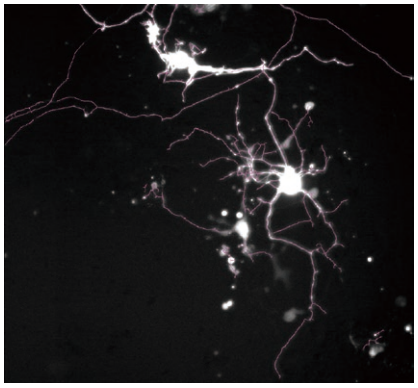
Imaging of the direct induction from mouse fibroblasts to neural stem cells and neurons

Magnification: 4x
Culture period: 18 days
Imaging interval: 4 hours

Stem Cells. 2012 Jun;30(6):1109-19
Courtesy of Prof. Hideyuki Okano and Dr. Takeshi Matsui Department of Physiology, Keio University School of Medicine

Dendrite Detection

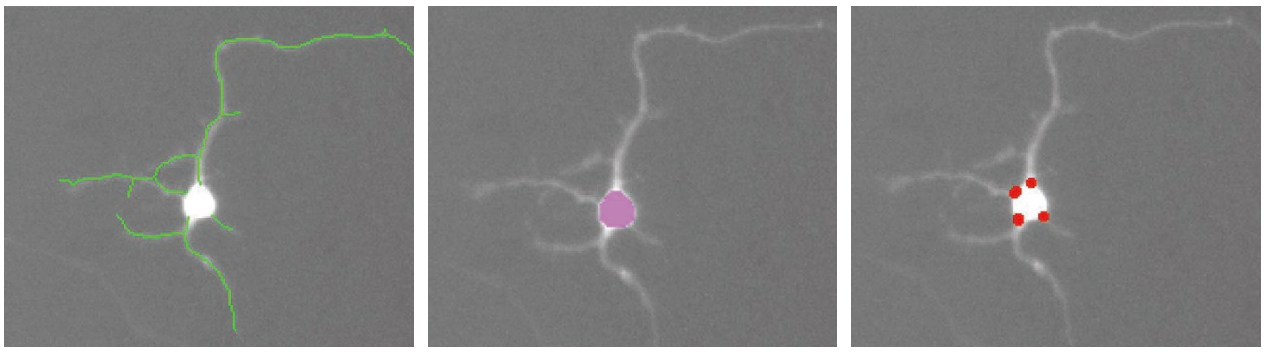
CL Quant



The neurons are generated by directed differentiation of human iPS cells to neurons. A plasmid containing GFP (under EF1 promoter) was transfected. The dendrite length was measured with the image analysis software CL-Quant. The software can detect the dendrite (green), cell body (purple) and branch points (red).

Magnification: 10x (fluorescence)
Culture period: 19 hours
Imaging interval: 10 min

Courtesy of Prof. James Ellis (Hospital for Sick Children-Toronto) and CCRM

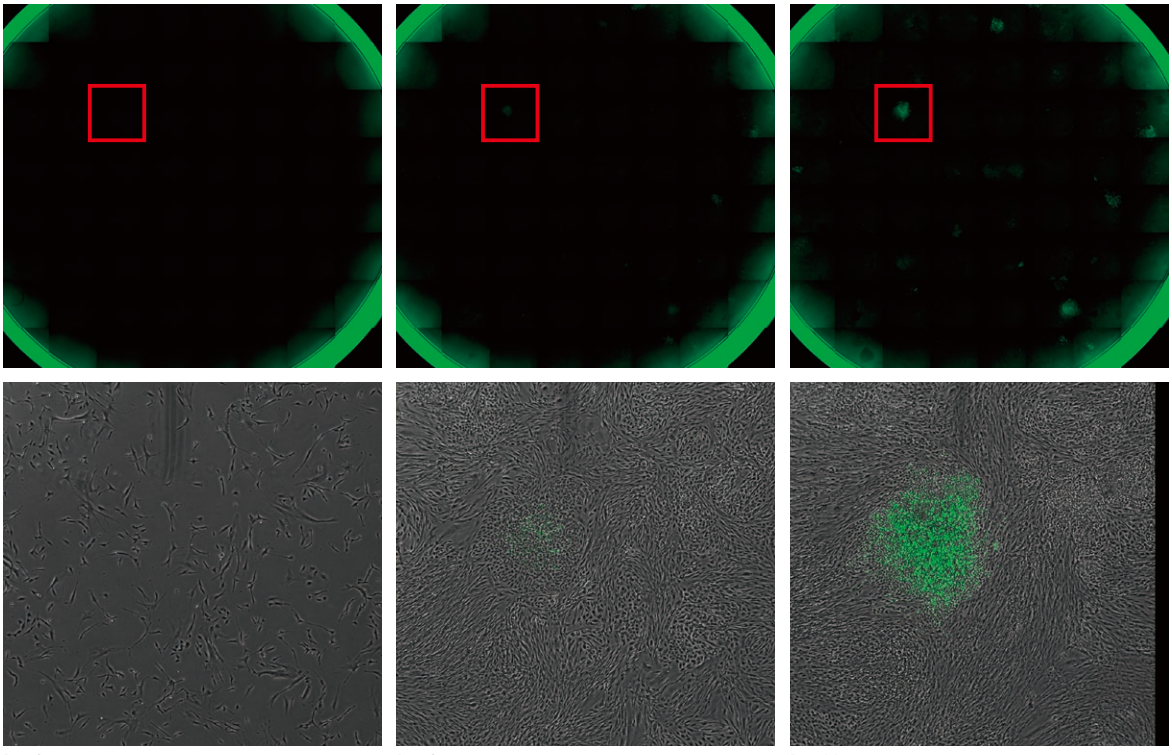


Dendrite | Cell body | Projection points

Direct Induction (Chondrocytes)

Whole-well fluorescence images of the 6-well plate

Merged images of phase-contrast and GFP images (2x magnification)



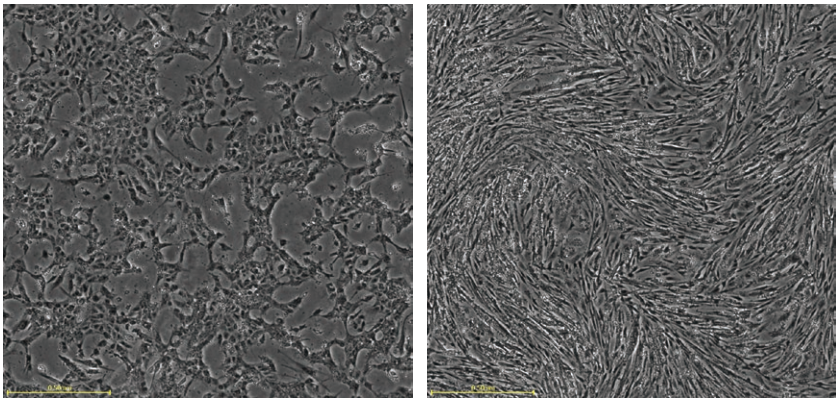
Day 3 | Day 8 | Day 14

The time-lapse imaging of direct induction of chondrogenic cells from Human Dermal Fibroblast (HDF) cultured by defined factors. The forced expression of two reprogramming factors (c-Myc and Klf4) and one chondrogenic factor (SOX9) can induce chondrogenic (iChon) cells from HDF culture without going through a pluripotent state. The human iChon cells expressed marker genes for chondrocytes (COL11A2-GFP).

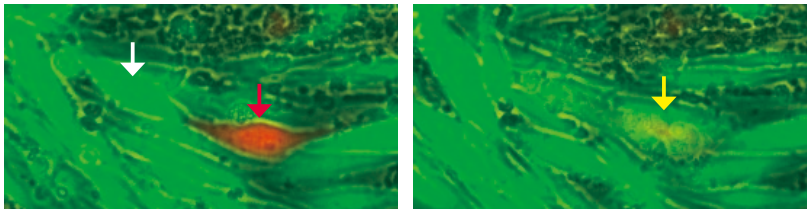
Courtesy of Professor Noriyuki Tsumaki, Department of Cell Growth and Differentiation, Center for iPS Cell Research and Application (CiRA), Kyoto University
PLoS ONE 8(10): e77365.

Differentiation Induction (Skeletal muscle)

Human iPSCs (MyoD-hiPSs) changed their shape uniformly to spindle-like during differentiation from Day 1 to Day 7.



Day 1 | Day 7



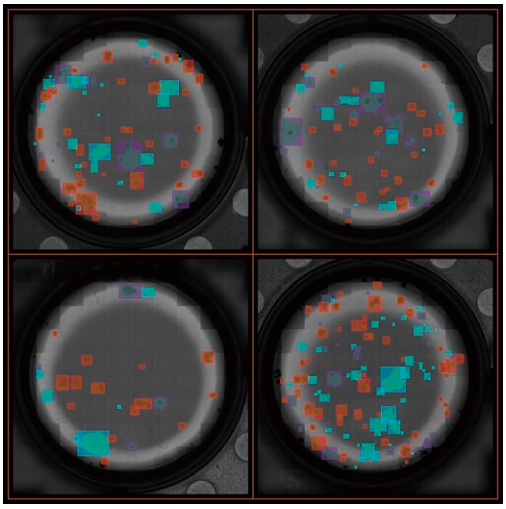
Functional assay for differentiated MyoD-hiPSCs. Serial photographs of differentiated MyoD-hiPSCs co-cultured with C2C12 cells (mouse myoblast cell line). A hiPSC-derived mCherry+ cell (red arrow) fused with a mouse-derived GFP+ cell (white arrow), resulting in a yellow cell (yellow arrow). This phenomenon is a characteristic of skeletal myocytes.

Courtesy of Dr. Hidetoshi Sakurai, Department of Clinical Application, Center for iPS Cell Research and Application (CiRA), Kyoto University
PLoS ONE 8(4): e61540

Hematopoietic Colony Forming Cell Assay

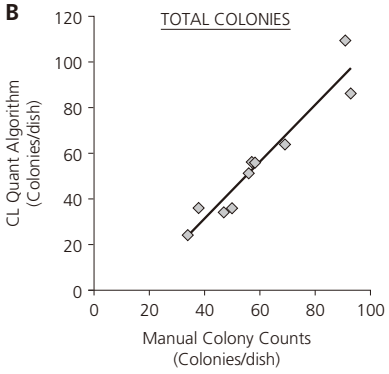
CL Quant

A

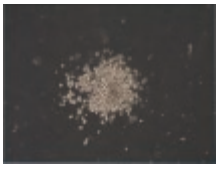


(A) End-point colony identification and enumeration using the CL-Quant algorithm was compared to manual colony scoring (n=10). (B) The CL Quant algorithm produced a strong correlation to the total colony numbers quantified by manual counts (R2 = 0.917). (C, D, E) Correlations are shown between the algorithm-generated counts and the manual counts for each of the three major colony types: (C) CFU-E/BFU-E; (D) CFU-G/CFU-M; (E) CFU-GEMM.

B



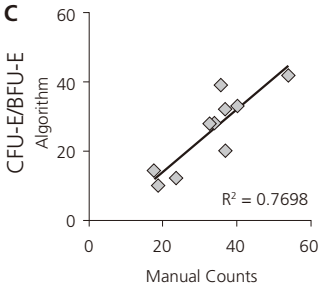
Red/dark colonies (CFU-E/BFU-E)
Colony forming unit-erythroid (CFU-E)
Burst forming unit-erythroid (BFU-E)



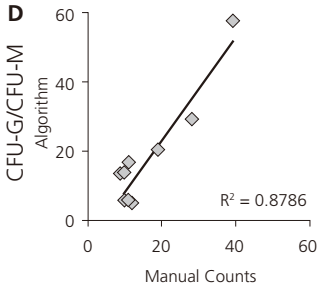
White colonies (CFU-M/CFU-G)
Colony forming unit-macrophage (CFU-M)
Colony forming unit-granulocyte (CFU-G)

Mixed colonies containing both types on left (CFU-GEMM)
Colony forming unit-granulocyte/erythroid/macrophage/megakaryocyte (CFU-GEMM)

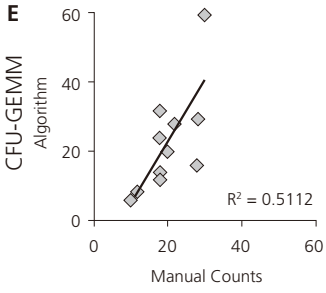
C



D



E

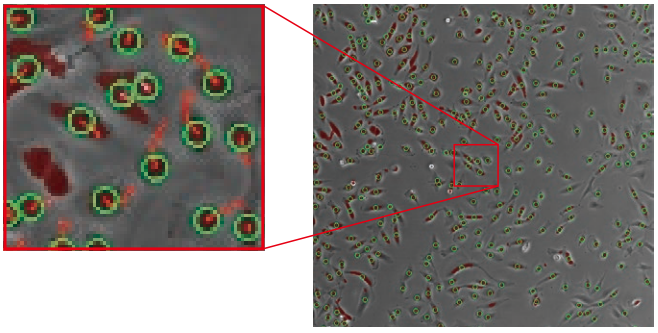


Mobility Analysis

CL Quant

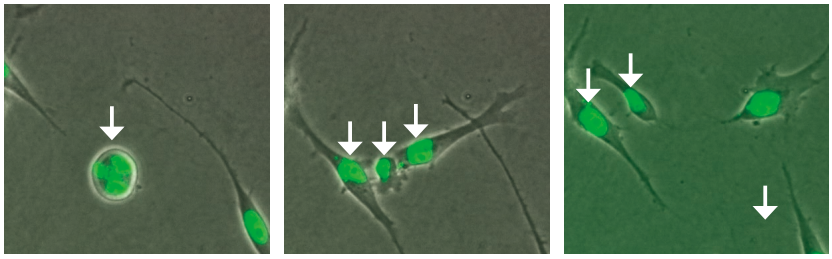
The distance of RCC4 cells (human renal cell carcinoma) was quantified by tracking (red line) the positions of cell centroids (green circle) using CL-Quant software. This assay could quantify the effect of adding Rapamycin or PP24.
J Urol. 2013 May;189(5):1921-9.

Courtesy of Dr. Shintaro Maru, Department of Renal and Genitourinary surgery, Hokkaido University



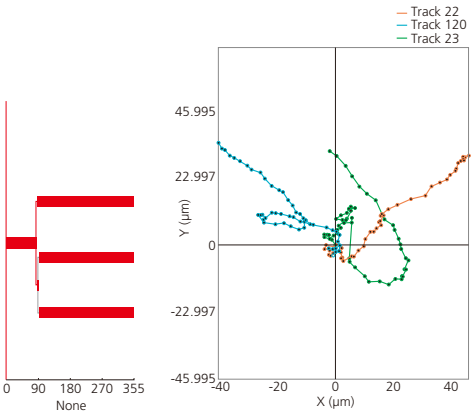
Lineage Analysis

CL Quant



Breast cancer cells (MDA-MB-231) migrating in a 3D matrigel. The cells stably express H2B-GFP which nicely shows the chromatin structures in the nuclei. Some cells divide into three daughter cells (white arrowhead) instead of two. Cell tracking, lineage analysis and directional analysis are possible when using image analysis software CL-Quant.

Courtesy of Ivar Noordstra, Department of Cell Biology, Utrecht University (Netherlands)

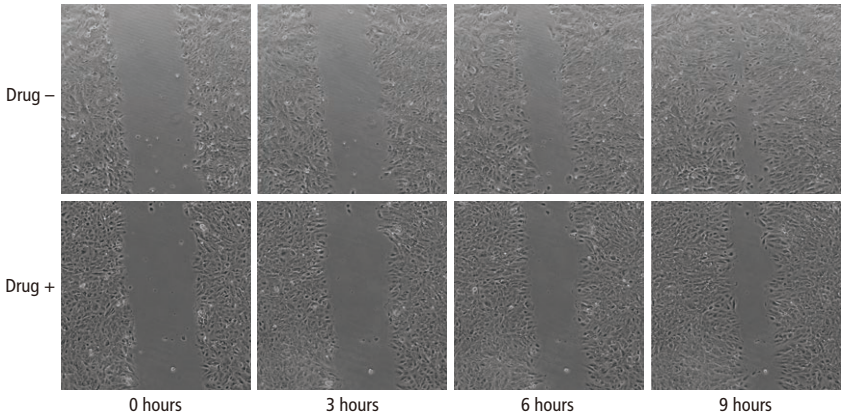


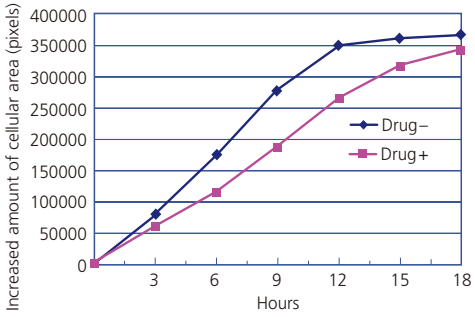
Lineage Analysis | Directional Analysis

Scratch Assay

CL Quant

The acellular areas are extracted from captured images, and the time course is quantified. This enables comparative analysis of cells' metastatic ability.





Inhibition of cell migration by the anti-cancer drug sunitinib (Sutent®) added to clear cell renal carcinoma cell line (KMRC-1) was quantified by scratch assay. Cellular areas in the images captured in three-hour-interval time-lapse observation by BioStation CT were quantified by image analysis software CL-Quant.
Courtesy of Dr. Naohisa Tomosugi and Dr. Shintaro Maru, Division of Nephrology, Kanazawa Medical University

NIS-Elements and the BioStation CT

NIS-Elements
High Content Analysis
Microscope Imaging Software

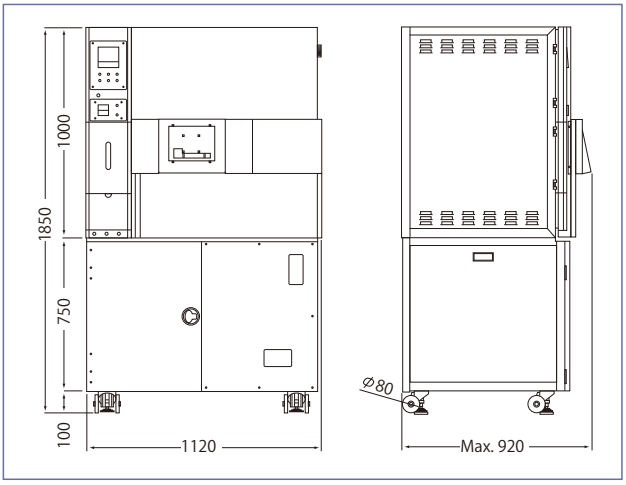
In addition to CL-Quant, all images acquired with BioStation CT can be analyzed using the Nikon software NIS-Elements in conjunction with the module HC/JOBS, giving high flexibility in analysis.

Specifications

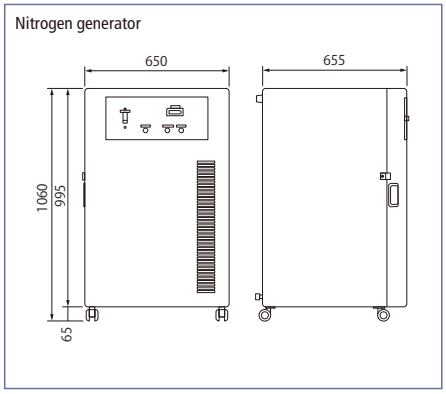
Operation	With touchscreen LCD Controllable via a network-linked PC (with Internet Explorer® web browser)
Incubator volume	460 L
Temperature control	Direct control via heater panels 37 °C, controlled directly via heater panels
Humidity controll	Via aerosol spray humidifier Range: 70% to 95%, 1% increments
CO_2 concentration control	CO_2 supply: by external CO_2 gas cylinder connection Range: 0% to 20%, 0.1% increments
O_2 concentration control (optional)	Via optional nitrogen gas generator Range: 0% to 20%, 1% increments
Compatible culture vessels	Culture dish: ø35 mm, ø60 mm, ø100 mm Well plate: 6-well, 12-well, 24-well, 48-well, 96-well Culture flask: 25 cm^2, 75 cm^2
Specimen storage rack	3 rows x 10 tiers (autoclave sterilizable)
Macro observation	Image capture of whole vessel with dedicated camera (bird's-eye view) Camera head: color CCD camera (1280 x 960 pixels) Brightfield: backlight illumination
Micro observation	Magnification: 2x, 4x, 10x, 20x, 40x Intermediate magnification: 0.5x, 1x, 2x, 4x Objective: 4x (Plan Apo DLL), 10x (Plan Fluor ADL) Camera head: cooled CMOS camera (1M pixels) Phase contrast: high-intensity red LED illumination, automatic phase ring changeover Epi-fluorescence: LED 438 nm, 472 nm, white light illumination (up to 5 fluorescence filter cubes mountable)
Observation range	X-Y: 120 x 90 mm Z: 4 mm
Z-axis focusing	Z-focus point is automatically detected by image contrast detection through Z-axis scanning
Observation	With touchscreen LCD or via network-linked PC
Power source	Voltage: 115, 230 VAC ± 10% Power consumption: 1300 VA (max.)
Weight	Approx. 470 kg
Operating environment	Temperature: 15 °C to 28 °C Humidity: max. 60% relative humidity (noncondensing)

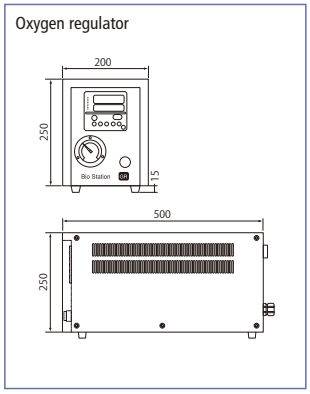
- BioStation CT does not have special components to protect the operator from infection.
- To decontaminate inside of incubator, use dry type hydrogen peroxide gas decontaminator.

Dimensional diagram



Hypoxic culture units dimensional diagrams





(Unit: mm)

Cover image: courtesy of Dr. Ronald McKay, NIH

WARNING TO ENSURE CORRECT USAGE, READ THE CORRESPONDING MANUALS CAREFULLY BEFORE USING YOUR EQUIPMENT.

Depending on the vessel used, the BioStation CT may not be able to focus on some areas.
External PC for data download is not included.
Repair of the machine under definite conditions is guaranteed by Nikon, but Nikon is not responsible for specimen and reagents.
Monitor images are simulated.
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NIKON CORPORATION
Shinagawa Intercity Tower C, 2-15-3, Konan, Minato-ku, Tokyo 108-6290, Japan
phone: +81-3-6433-3705 fax: +81-3-6433-3785
https://www.healthcare.nikon.com/en/

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Nikon Instruments Inc.
1300 Walt Whitman Road, Melville, N.Y. 11747-3064, U.S.A.
phone: +1-631-547-8500; +1-800-52-NIKON (within the U.S.A. only)
fax: +1-631-547-0299
https://www.microscope.healthcare.nikon.com/

Nikon Europe B.V. (headoffice)
Tripolis 100, Burgerweeshuispad 101, 1076 ER Amsterdam, Netherlands
phone: +31-20-7099-000 fax: +31-20-7099-298
https://www.microscope.healthcare.nikon.com/en_EU/

Nikon Instruments (Shanghai) Co., Ltd.
CHINA phone: +86-21-6841-2050 fax: +86-21-6841-2060
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Nikon Canada Inc.
CANADA phone: +1-905-625-9910 fax: +1-905-602-9953

Nikon France, Succursale de Nikon Europe B.V.
FRANCE phone: +33-1-4516-4516 fax: +33-1-4516-4505

Nikon Deutschland, Zweigniederlassung der Nikon Europe B.V.
GERMANY phone: +49-211-9414-888 fax: +49-211-9414-322

Nikon Italy, Branch of Nikon Europe B.V.
ITALY phone: +39-055-300-9601 fax: +39-055-300-993

Nikon Europe B.V., Amsterdam, Zweigniederlassung Schweiz (Egg/ZH)
SWITZERLAND phone: +41-43-277-2867 fax: +41-43-277-2861

Nikon UK, Branch of Nikon Europe B.V.
UNITED KINGDOM phone: +44-208-247-1717 fax: +44-208-541-4584

Nikon Österreich, Zweigniederlassung der Nikon Europe B.V.
AUSTRIA phone: +43-1-972-6111 fax: +43-1-972-6111-40

Nikon Singapore Pte Ltd
SINGAPORE phone: +65-6559-3651 fax: +65-6559-3668

Nikon Instruments Korea Co., Ltd.
KOREA phone: +82-2-6288-1900 fax: +82-2-555-4415

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