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, CO₂ concentration) control error occurs, BioStation CT can notify the users of the error by e-mails.

**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.
**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.

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**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.

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**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.

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**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.

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**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

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**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.
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.

Smooth vessel transportation
The wave 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.)

Environment data recording
The culture environment is constantly monitored and recorded. The environment data can be accessed at any time.

Easy operations

Vessel installation
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.

Imaging parameter setting
Easy touchscreen operation
Time-lapse imaging configurations such as magnification, imaging point, fluorescence channel and stage motion speed can be set.

Scheduling
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

- **24-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

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

- **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

- **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

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

- **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

- **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

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

- **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

- **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

Captured image view

- **Imaging date and time**
- **Sample name**
- **Micro image thumbnail**
- **Imaging point within the vessel**

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-iPS Auto Identification

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: 2x)

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 correlation coefficient of 0.904 is high.

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

Alkaline Phosphatase (AP)-positive Colony Counting

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

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

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
Differentiation research

**Direct Induction (Chondrocytes)**

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).

**Differentiation Induction (Skeletal muscle)**

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

**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

**Differentiation Induction (Skeletal muscle)**

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Courtesy of Professor Noriyuki Tsumaki, Department of Cell Growth and Differentiation, Center for iPS Cell Research and Application (CiRA), Kyoto University


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

(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.

Mobility Analysis

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 PFA.


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

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)

Scratch Assay

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

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
Controlled via a network-linked PC (with Internet Explorer®
web browser)

**Incubator volume**
460 L

**Temperature control**
Direct control via heater panels
37 ºC, controlled directly via heater panels

**Humidity control**
Via aerosol spray humidifier
Range: 70% to 95%, 1% increments

**CO₂ concentration control**
CO₂ supply: by external CO₂ gas cylinder connection
Range: 0% to 20%, 0.1% increments

**O₂ 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 cm², 75 cm²

**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

**Power source**
Voltage: 115, 230 VAC ± 10%
Power consumption: 1300 VA (max.)

**Weight**
Approx. 470 kg

**Operating environment**
Temperature: 15 ºC to 28 ºC
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.

### Dimensions

**Nitrogen generator**

**Oxygen regulator**

**Dimensions diagram**

**Hypoxic culture units dimensional diagrams**

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

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Cover image: courtesy of Dr. Ronald McKay, NIH

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