NEW

# CYTOQUBE™

Light-Sheet Microplate Cytometer

C15200-01RGBU

## Next Generation 3D Screening

HAMAMATSU

PHOTON IS OUR BUSINESS

C Y T O Q U B E™

# Advancing cell-based assays and drug screening with CYTOQUBE®

CYTOQUBE® is a Light-Sheet Microplate Cytometer that performs high-speed fluorescence imaging and analysis for both 2D and 3D cell-cultured samples. It is capable of scanning 1536, 384 and 96 well microplates, acquiring whole-well 3D fluorescence images.

During the image acquisition process, fluorescence intensity and 3D morphological information of the objects (cells, spheroids, or organoids) are analyzed, and the results are displayed upon completion of the scan.

Our unique patented technology, Zyncscan®, enables high-throughput cell assays with ease of use and higher reliability.

It contributes to the efficiency of drug screening, toxicity/safety evaluation, and translational research using cells, spheroids, organoids, and bioprinting samples.

## Fast 3D fluorescence imaging

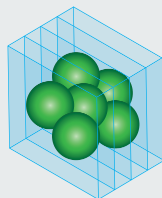


Image acquisition and analysis for 2D and 3D samples
(Observable height range: 400 µm or less)

## High-throughput analysis

Get 3 color results in
**21 min/plate**

- The same time applies for all plate formats: 1536, 384, 96.
- Analysis based on 3D imaging is performed in parallel
- Whole-well imaging
  (up to 400 µm in height from the bottom of the well)

## Key challenges addressed with CYTOQUBE®

### Focus adjustment

- Need to reduce focus adjustment time
- Need to ensure all samples are in focus
- Need to reduce photobleaching effect

### 2D / 3D Imaging

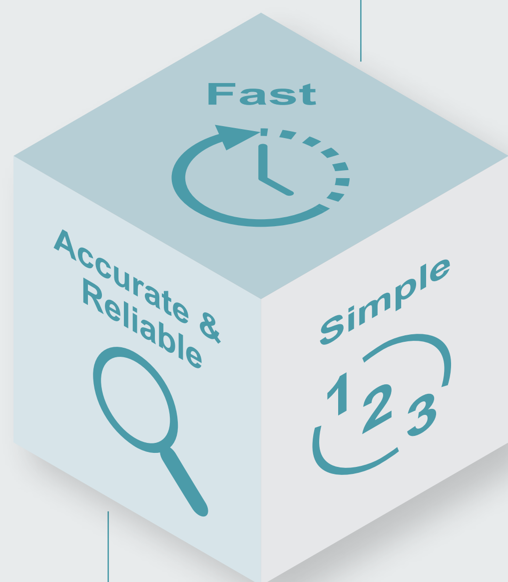
- Need to perform 2D/3D imaging and analysis in high throughput
- Need to image cells in U-bottom plates
- Need to reduce photobleaching effect in 3D imaging

### Analysis

- Need analyzed results faster and easier
- Need to automate scanning and analysis
- Need more quantitative 3D information

CYTOQUBE™

# Three key features




## High-throughput

P4

- Rapid scanning speed
- Parallel processing for scanning and analysis

## Simple scanning and analysis settings

P5

- Easy setting
- Intuitive user interface
- Complete scanning & analysis software in one package

## Enhanced realism and accuracy in 3D fluorescence imaging

P6

- Real-time background separation
- Seamless 3D fluorescence images

## New technology using light-sheet optics

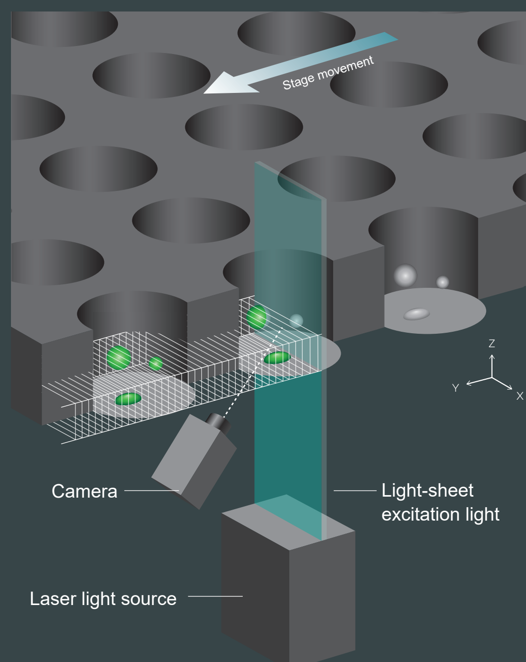
The patented Zyncscan® technology developed by Hamamatsu Photonics is a new technology that combines optical and image processing technologies based on light-sheet optics.

The light-sheet optics of Zyncscan® technology emit a sheet of excitation light from directly below the sample and capture XZ tomographic fluorescence images from an oblique downward direction. By continuously acquiring XZ tomographic fluorescence images while moving the microplate in a direction orthogonal to the light-sheet, 3D fluorescence images are constructed within a few minutes per color.

In parallel with the acquisition of XZ tomography fluorescence images, background fluorescence is removed in real time by three-dimensionally separating background fluorescence and sample fluorescence from the actual XZ tomographic fluorescence image acquired.

### Advantages of Zyncscan®

- High-speed scanning of whole wells in a wide field of view
- Z-axis optical performance equivalent to 10× confocal
- Focus free
- Real-time background separation
- Seamless 3D fluorescence images



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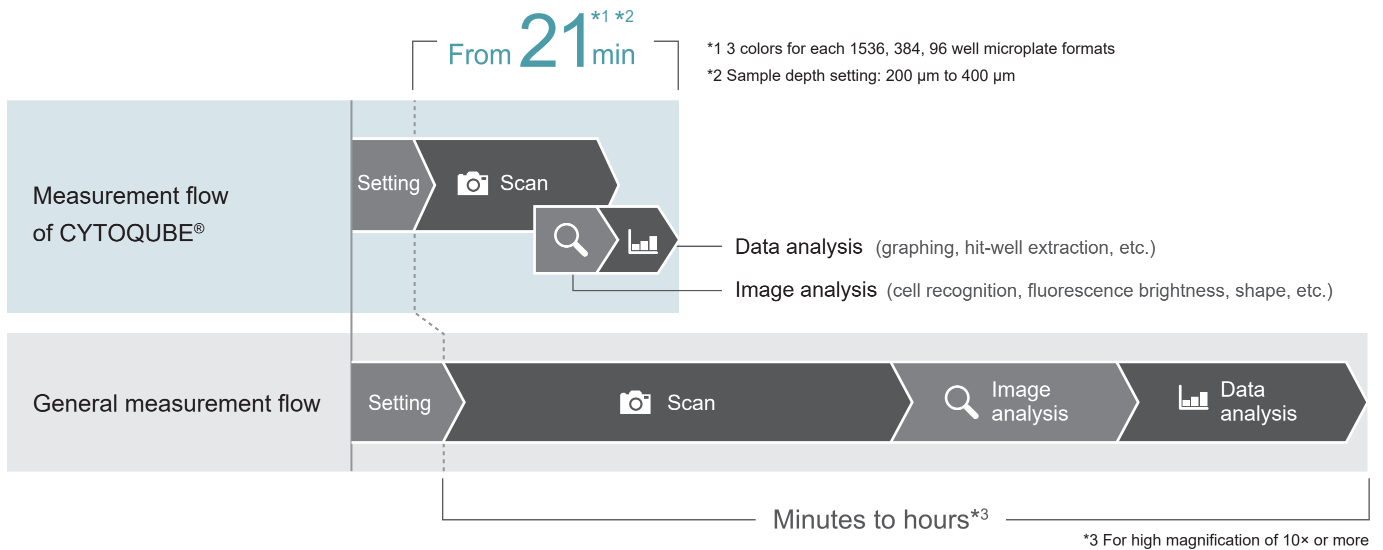
Fast

# High-throughput

## Measurement flow




## Rapid scanning speed

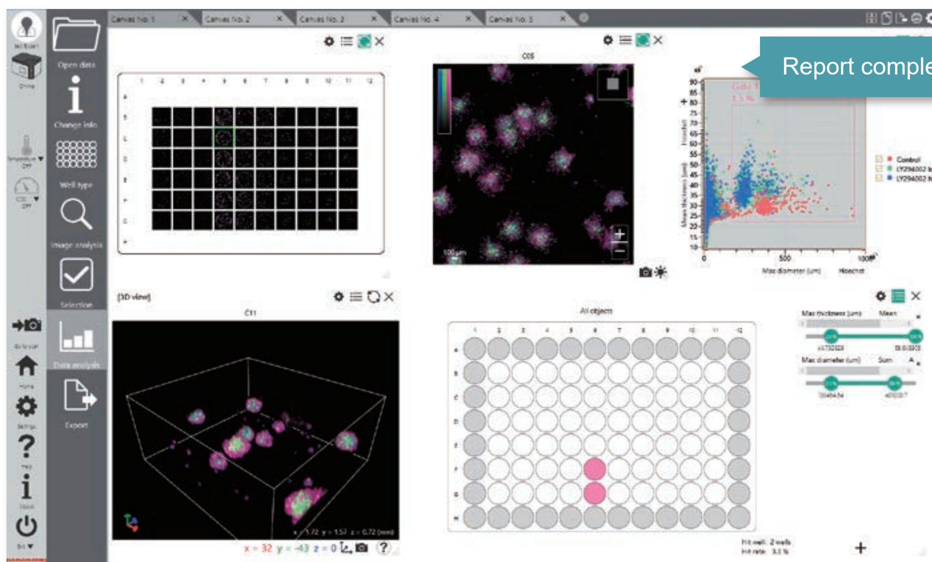
Acquire whole-well and whole-plate fluorescence images within a few minutes per color, regardless of plate format (1536, 384, 96 wells) or sample depth.

| | Scanning to analysis time*1 | |
|---|---|---|
| | 2D sample*2 | 3D sample*3 |
| 1 color | 4 min to 11 min | 7 min to 11 min |
| 2 colors | 8 min to 22 min | 14 min to 22 min |
| 3 colors | 12 min to 34 min | 21 min to 34 min |
| 4 colors | 16 min to 45 min | 28 min to 45 min |

*1 Whole plate measurement
*2 Sample depth setting: 0 μm
*3 Sample depth setting: 400 μm

## Parallel processing for scanning and analysis

The parallel processing of fluorescence image construction and image analysis has significantly shortened the time from scan start to data analysis result output.



Report completed as soon as scanning is finished

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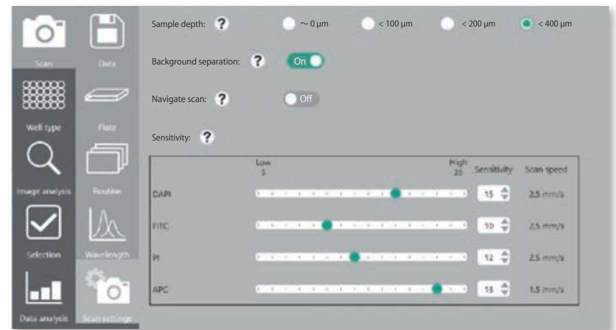
Simple 1 2 3

# Simple scanning and analysis settings

## Easy setting

The settings have been simplified to make it easy to use. Unnecessary settings such as 'focus adjustment' or 'exposure time adjustment' are not included. The optimal sensitivity can be set automatically with the Navigation Scan function. Additionally, you can save the entire process from scanning to result display as a protocol file, making it easy to reproduce scanning and analysis conditions.



**Only three settings are required for scanning:**

1. Sample depth (4 choices)
2. Background separation (ON or OFF)
3. Sensitivity (can be set automatically)

## Intuitive user interface

Settings required for image analysis can be set intuitively, as necessary settings are summarized for each application.

**Example image analysis settings:**

### Find objects/Spheroid

Fluorescence intensity and 3D morphological information (height, thickness, volume) measurements of individual cells and individual spheroids/organoids are analyzed and results are displayed.

### Cell invasion

The well is divided into multiple regions, and measurements are performed in each region.

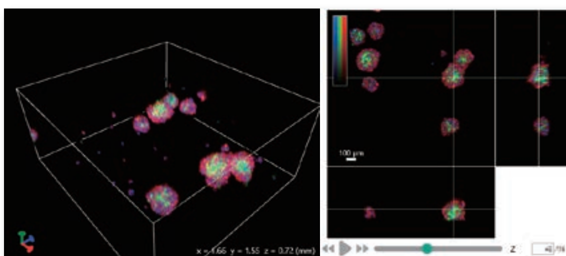
### Neighboring cells

Find the number of adjacent cells and spheroids/ organoids.

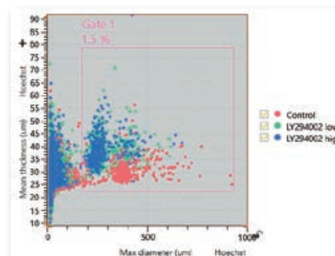
## Complete scanning & analysis software in one package

A single software can be used to check 2D/3D fluorescent images of each well, calculate EC50/IC50, create various graphs, identify hit wells, and generate simple reports.

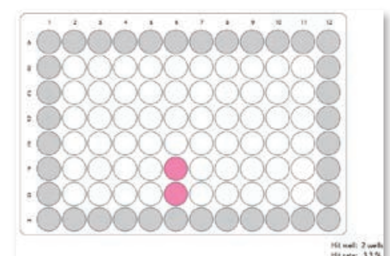
3D fluorescent image display



Scatter plots & gate settings



Hit-well identification



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Accurate & Reliable

# Enhanced realism and accuracy in 3D fluorescent imaging

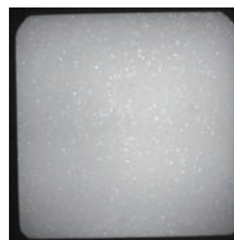
## Real-time background separation

Background fluorescence, derived from autofluorescence of the culture medium or fluorescent dyes in the solution, and fluorescence from the sample are separated from the acquired XZ tomographic fluorescence images, in real-time, effectively removing only the background fluorescence in three dimensions.

**No medium wash-out needed**

A549 cells cultured with DMEM medium, containing serum and fluorescent dye, were measured both in a fluorescence microscope and with CYTOQUBE® to compare the images.
Fluorescent dyes: Annexin V-Alexa Fluor 488

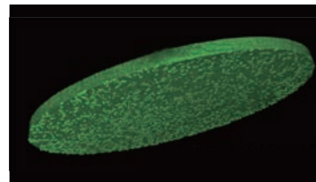
Fluorescence microscope



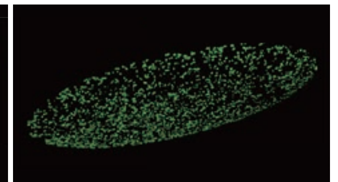
CYTOQUBE® (background separation ON)

**Separation of background signal at ultra-high level**

Real-time background separation OFF



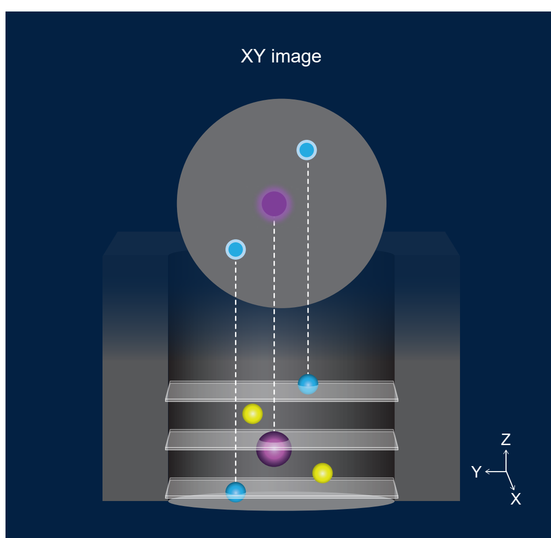
Real-time background separation ON



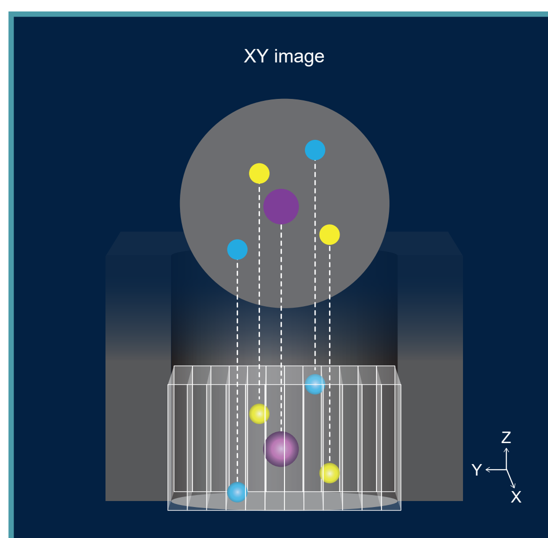
## Seamless 3D fluorescent images

In a typical confocal microscope, multiple 2D XY images at different depths are acquired to construct a 3D fluorescence image.
To obtain more accurate 3D information, a larger number of image acquisitions is required.
CYTOQUBE® consistently provides seamless 3D fluorescence images using Zyncscan® technology, ensuring precise 3D information without the need for extensive image acquisition.

Typical confocal microscope




CYTOQUBE®



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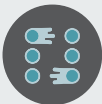
# Applications

## 3D samples

Spheroids, organoids,
patient-derived tissue samples,
bioprinting samples, etc.

## 2D samples

Established cells,
primary cultured cells,
differentiated cells derived from iPS cells,
etc.

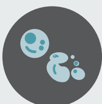

Migration/Invasion assay

Morphological analysis

Analysis of the number of adjacent cells

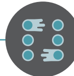
Live/dead assay

Reactive Oxygen Species (ROS) measurement


Apoptosis assay

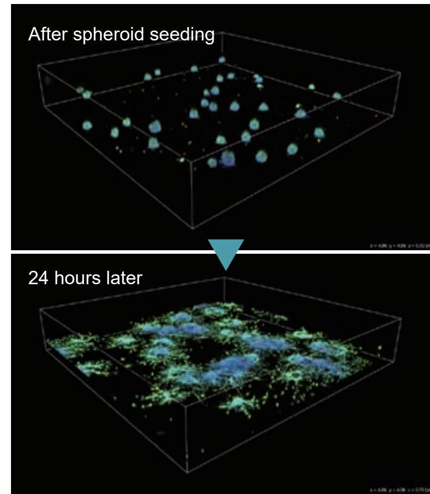
Angiogenesis assay

Fluorescence measurement of cell surface markers

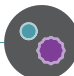


### Migration/Invasion assay

Cancer cell (U-87 MG) spheroids of approximately 200 µm diameter were seeded into a 96 well microplate and cultured in a medium containing Matrigel. After 24 hours, we observed the migration/infiltration of cells from the spheroids.

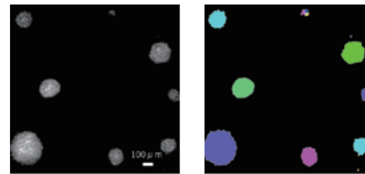



Hoechst 33342 (blue), Calcein (green), Propidium Iodide (red)



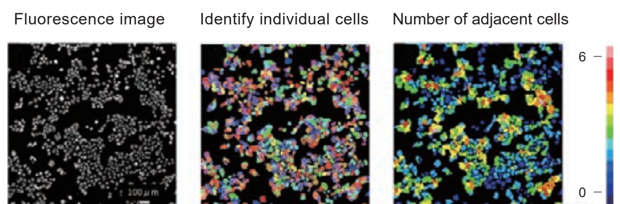
### Morphological analysis

The brightness, diameter, volume etc. of individual cells, spheroids and organoids can be quantified.



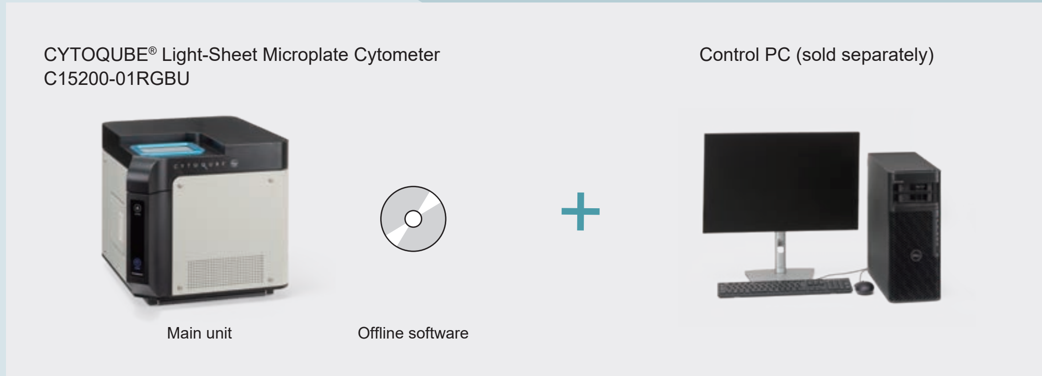
### Analysis of the number of adjacent cells

The analysis assesses the quantity of neighboring cells, including their fluorescence intensity, brightness, area, etc. These measured values enable the evaluation of cell-to-cell contact and aggregation.




Scan here for application notes and other information.

## System configuration




## Specifications

### CYTOQUBE® Light-Sheet Microplate Cytometer

| Type number | | C15200-01RGBU |
|---|---|---|
| Scanning method | | High-speed 3D fluorescence measurement method using light-sheet illumination (Zyncscan®) |
| Excitation laser | | Select from 405 nm, 488 nm, 561 nm and 637 nm (up to 4 types can be selected) |
| Fluorescence filter | | Select from 458 nm, 531 nm, 600 nm and 680 nm (up to 6 sets can be installed) |
| Imaging device | | Scientific CMOS image sensor |
| Image resolution | | 2.75 μm (X) × 2.75 μm (Y) × 6.215 μm (Z) |
| Maximum observable height*1 | | 400 μm |
| Focus | | Focus free (Zyncscan®) |
| Microplate | | 1536, 384, 96 well microplates (SBS standard format) |
| Setting environment | Temperature setting range | +30 °C to +45 °C (ambient temperature: +25 °C) |
| | $CO_2$ concentration monitoring range | 0 % to 10 % (with $CO_2$ inlet) |
| Barcode reader | | 1D barcode (reading position: both sides of the short side of the microplate)*2 |
| Power supply | | AC 100 V to AC 240 V |
| Power consumption | | Approx. 700 VA |
| Operating environment | Ambient operating temperature | +20 °C to +30 °C |
| | Ambient operating humidity | 30 % to 80 % (with no condensation) |

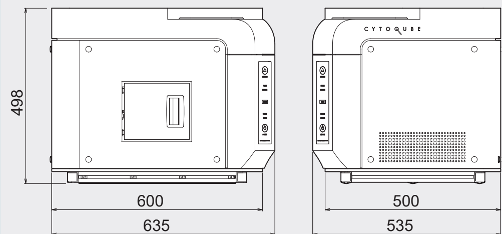
*1 Height from the bottom of the well when the flatness of the bottom of the microplate is ± 50 μm in the short side direction
*2 1536, 384 and 96 plates less than 9.5 mm thick may not read properly.
* For automation function, please contact our sales representatives.

### CYTOQUBE® software

| Type number | | U15134-01 |
|---|---|---|
| Analysis item (at each fluorescence wavelength of individual cells / spheroids) | | Fluorescence intensity per unit volume<br>Morphology of cellular objects (diameter, thickness / height, volume) |
| Display item | Data | Table, heat well map, hit well map, 2D dot plot, histogram, dose response curve |
| | Image | 2D image (at well unit or whole plate), 3D image (at well unit) |
| Data output format | | Measurement data (.csv), snapshot image (.tiff / .jpeg / .bmp / .png), 3D movie (.avi / .wmv), original image (.tiff) |

## Dimensional outline (Unit: mm)

Weight: Approx. 70 kg
(Main unit when 4 types of excitation lasers are installed)




### Laser safety

CYTOQUBE® is classified as a Class 1 laser product, conforming to international laser safety standards (USA: 21 CFR 1040.10 CDRH, other areas: IEC 60825-1). These standards mandate manufacturers to provide preventive safety measures. Hamamatsu lasers are appropriately classified, and they come with necessary safety measures and labeling. During operation, users must also adhere to preventive safety measures as per laser-related regulations.

Description Label (Sample)

Caution Label


- CYTOQUBE and Zyncscan are registered trademarks of Hamamatsu Photonics K.K. (China, EU, Japan, Switzerland, UK, USA).
- The product and software package names noted in this brochure are trademarks or registered trademarks of their respective manufacturers.
- Subject to local technical requirements and regulations, availability of products included in this brochure may vary. Please consult your local sales representative.
- The product described in this brochure is designed to meet the written specifications, when used strictly in accordance with all instructions.
- Specifications and external appearance are subject to change without notice.

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