Confocal Quantitative Image Cytometer CQ1

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Application Note

Quantitative characterization of metaphase cellular events using 3D imaging

Introduction

In conventional monolayer cell culture systems, cell nuclei show dynamic and cyclic changes in their shape and height. This phenomenon is pronounced during metaphase (M-phase).

Generally, confocal optical microscopes have superb capability for capturing images of the fine structures of cells by narrowing the depth-of-field. However, this confocal mechanism has difficulties acquiring quantitative information from the entire thickness of cells because they exclude light from out-of-focus areas (Fig 1A).

The CQ1's confocal image acquisition mechanism with the distinctive CSU® unit has a function to sequentially acquire fine cell images along the Z-axis and capture information from the entire thickness of cells which include heterogenic populations of various cell cycle stages (Fig 1). In addition, saved digital images can be useful for precise observation and analysis of spatial distribution of intracellular molecules.

The CQ1's capability to seamlessly analyze images and obtain data for things such as cell population statistics to individual cell morphology will provide benefits for both basic research and drug discovery targeting M-cell cycle phase.
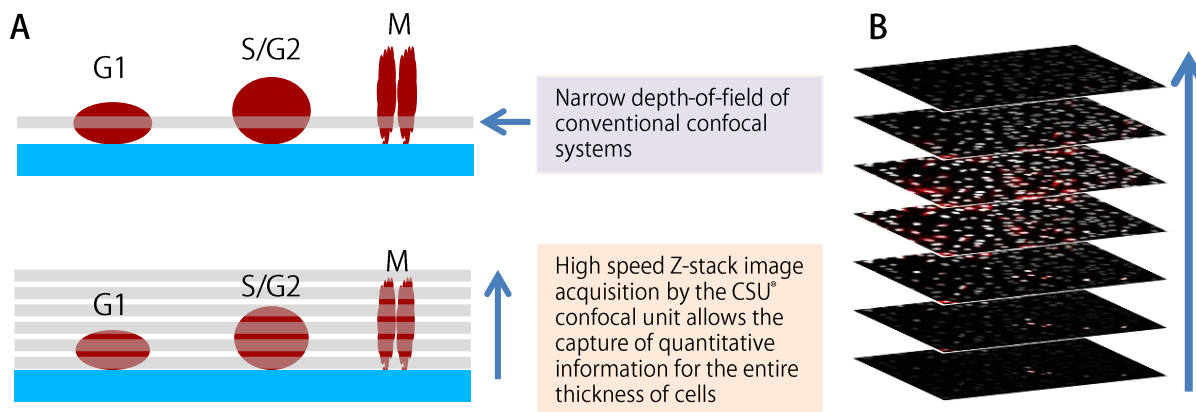

Figure 1. The advantage of CQ1's image acquisition feature.
A) Morphological changes of cell nuclei along the cell cycle progression and thin depth-of-field of confocal optical systems; **B)** Schematic illustration of Z-stack image acquisition by the CQ1.

M-phase analysis

Sequentially acquired cell images along Z-axis were created using the SUM (summation intensity projection) feature of the CQ1 software (Fig 2A). Cell cycle analysis was carried out by using SUM images (Fig 2B). Morphologies of selected M-phase cell nuclei on the scattergram can be confirmed visually (Figs 2C and 3).
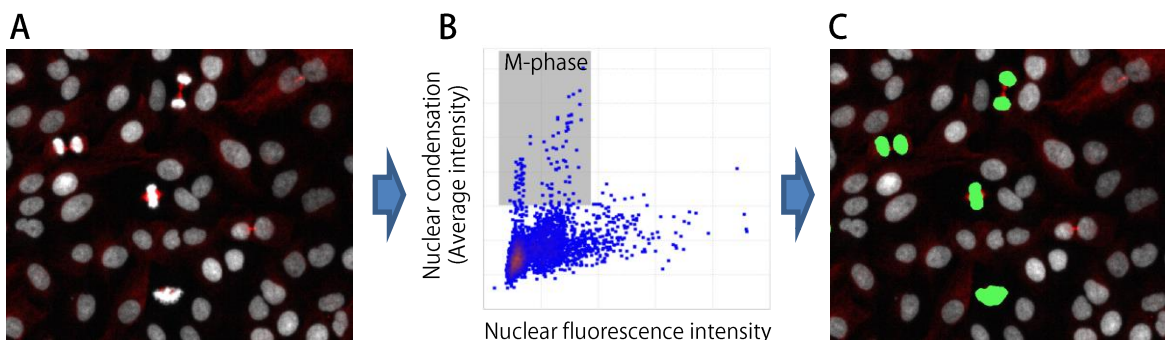

Figure 2. Cell cycle analysis by the CQ1 with Z-stack image acquisition.
Monolayer culture of A549 lung cancer cells in a 96-well glass bottom plate were fixed then their cell nuclei and alpha-tubulin molecules were fluorescently labeled with Draq7 (gray) and immunofluorescence (red), respectively.
A) SUM image composed from 11 layer Z-stack graphical files. Objective lens: 20X, number of image field: 6 fields, Number of cells analyzed: approximately 3×10^3
B) Scattergram of cell cycle analysis from SUM images and selection of M-phase cells (gray square).
C) Selected M-phase cell nuclei (highlighted by green).
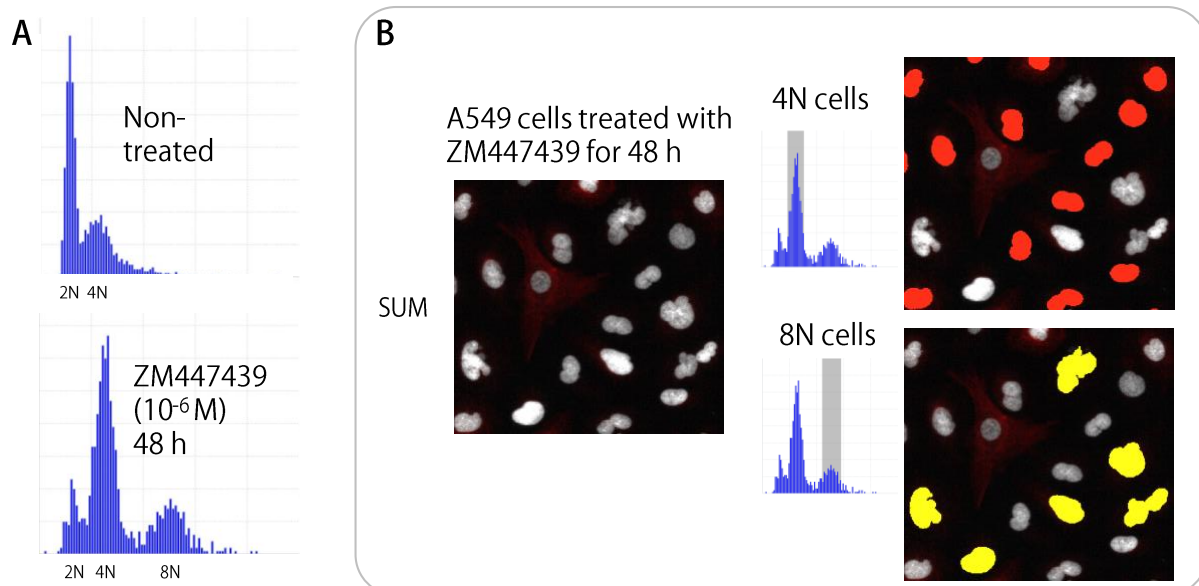
SUM

MIP

3D
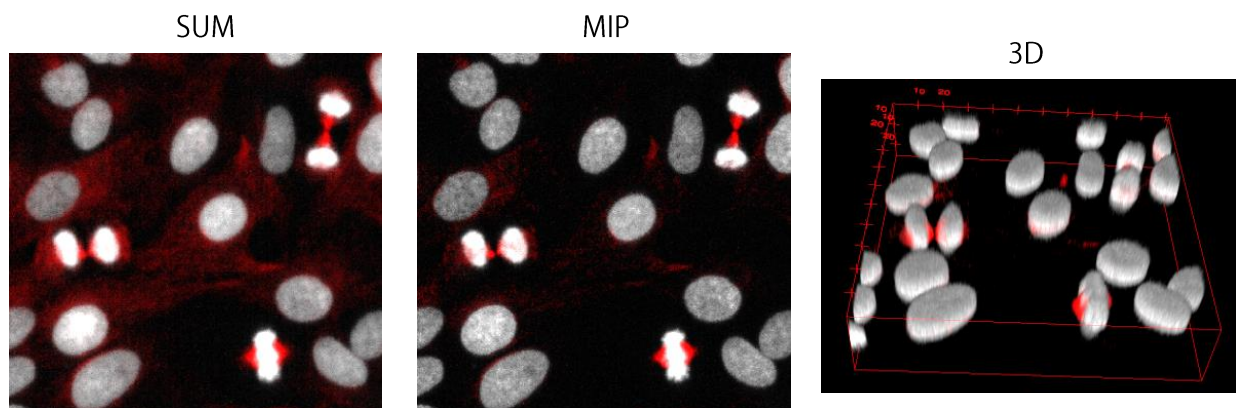
Figure 3. Three display styles of Z-stack images. Enlarged Z-stack images shown in Fig 2. SUM (summation intensity projection) is suitable for quantitative light intensity acquisition from the entire thickness of cells and that, MIP (maximum intensity projection) is superior for analysis of fine cellular structures and intracellular distribution of fluorescently labeled molecules. Typical M-phase characteristics (e.g. chromosomal condensation and spindle formation) were observed clearly.

Effect of M-phase inhibitor

Intracellular protein molecules governing M-phase progression, like Aurora kinases or TACC3, have been recognized as significant targets for cancer chemotherapeutic agents. A small molecule, ZM447439 is a specific Aurora kinase inhibitor and exerts suppressive activity against M-phase progression. In addition, affected cells with this chemical enter an additional genome replication but fail to divide resulting in the generation of polyploid nuclei.

By performing cell cycle analysis by SUM-synthesized images of ZM447439 treated cells, apparent accumulation of 4N or 8N were detected on a histogram (Fig 4A). In addition, these polyploid cells showed non-uniform chromosome condensation and lack of spindle formation implying inhibitory effects on Aurora kinase-related molecular events (Fig 4B).

A

Non-treated

2N 4N

ZM447439 (10^{-6} M) 48 h

2N 4N   8N

B

A549 cells treated with ZM447439 for 48 h

SUM

4N cells

8N cells

Figure 4. Polyploidy analysis by the CQ1.
A) Non-treated or ZM447439-treated A549 cells were stained and analyzed as described in Figure 2.
B) SUM image representation of drug-treated cells and selection of polyploid cell nuclei.

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