APPLICATION NOTE

Volume Contrast, the analysis software of NIS-Elements

Cell cycle analysis of keratinocytes and label-free cell counting of mitotic cells using Volume Contrast

Keratinocytes are epidermal cells used in various studies such as cosmetics development, drug discovery, and regenerative medicine. In keratinocytes, autophagy induction is known to regulate melanosome degradation, which is being used to study whitening ingredients in cosmetics development. Psoriasis, an autoimmune disease, is known as a skin disease caused by abnormal cell proliferation of keratinocytes.

Analysis of the cell cycle and cell proliferation is being used to develop new drugs to suppress abnormal cell proliferation. The cell cycle can be estimated from the amount of DNA by measuring the fluorescence intensity of DNA in a nucleus stained with Hoechst 33342 or PI. In this application note, we introduce an example of analysis of the cell cycle from the sum intensity of the nucleus, using the binning function of the General Analysis 3 module in NIS-Elements imaging software. In addition, since cells have a different thickness during interphase and mitosis, we also introduce an example in which cells with different thicknesses were classified using the Volume Contrast module, and the numbers of cells during interphase and mitosis were measured without labeling.

Imaging flow

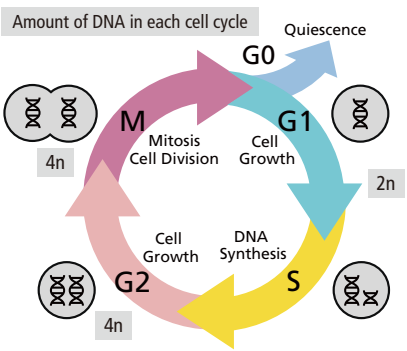
Sample preparation → Image acquisition → Image analysis → Data analysis

■ Observation device
- Microscope: Ti2-E
- Monochrome camera: DS-Qi2
- Fluorescent LED illumination system: D-LEDI
- Objective: CFI Plan Apochromat Lambda D 20X

■ Image analysis software and optional add-on module
- NIS-Elements AR
- General Analysis 3 (GA3)
- NIS-A EDF module
- Volume Contrast (VC)

Experiment overview

① Keratinocytes were fixed, nuclei were stained with Hoechst33342, and tubulin was immunostained.
② Images were acquired with a fluorescence microscope (Fig. 1, 2 and 4).
③ The fluorescence intensity of Hoechst 33342 was measured with GA3 to quantify the DNA content of the nucleus.
④ A histogram was created with Microsoft Excel®, and the cell cycle of each nucleus was classified into three thresholds: G0/G1 phase, S phase, and G2/M phase, from the sum intensity value of the nucleus (Fig. 3-A).
⑤ The classified "Hoechst Sum Intensity" thresholds were input to the Binning Function of GA3, and the ratio of the numbers of cells in the G0/G1 phase, S phase, and G2/M phase were analyzed (Fig. 3-B, Fig. 3-C).
⑥ The masks were color-coded for each cell cycle using GA3's "ColorByValue" Function (Fig. 4).



Since DNA synthesis starts in the S phase and the amount of DNA increases, the intensity of Hoechst 33342 increases.
In the G2 phase, the amount of DNA is about twice that in the G0/G1 phase (left figure). As the cells grow, the area of the nuclei gradually increases. (Fig. 2, left)
Due to this difference in the intensity (amount of DNA) and area of the nucleus, the cell cycle can be estimated from the sum intensity value of the nucleus.

Fig. 1: Stained image of nucleus (Hoechst 33342)
Since the amount of DNA in each nucleus is different, the fluorescence intensity is different.
Objective: 20X, Scale bar: 100 µm

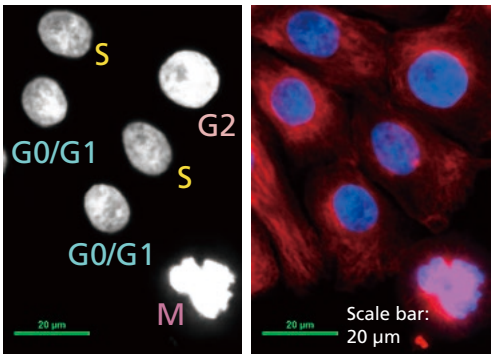


Fig. 2: Fluorescent images of nuclei and Tubulin
Left: Fluorescent image of Hoechst 33342 (nucleus)
Right: Merged fluorescent image of Hoechst 33342 and Tubulin

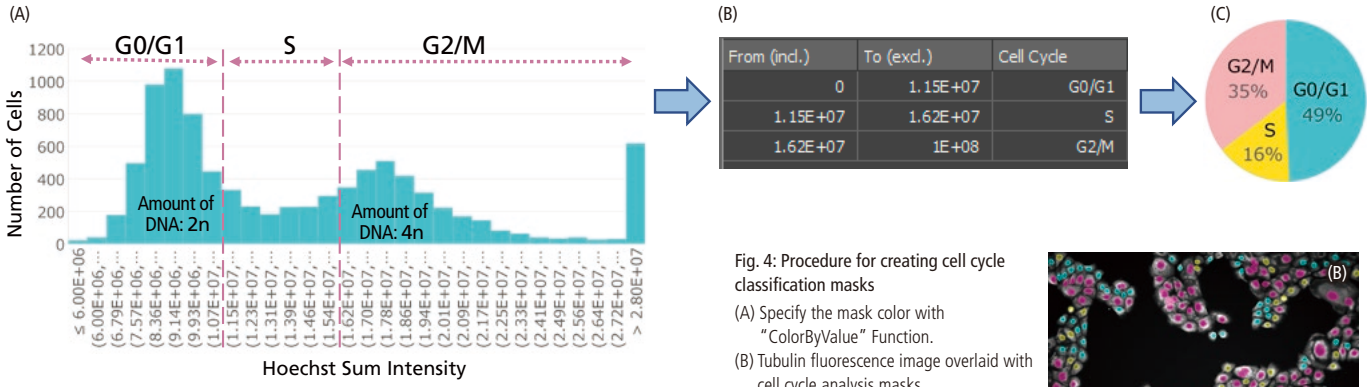


Fig. 3:Quantification of DNA content and cell cycle analysis by nuclear staining

(A) Histogram of sum intensity of nucleus (Hoechst 33342). The sum intensity value in the G0/G1 phase is about 9e6, while the sum intensity value in the G2/M phase is about 1.8e7, which is about twice that of the G0/G1 phase.

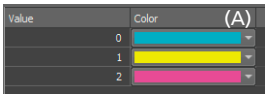
(B) The cell cycles were classified by inputting the sum intensity thresholds into the Binning Function.

(C) Percentage of cell numbers by cell cycle (a total of 6,157 cells were measured)

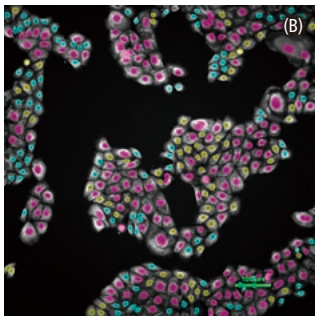
Fig. 4: Procedure for creating cell cycle classification masks

(A) Specify the mask color with "ColorByValue" Function.

(B) Tubulin fluorescence image overlaid with cell cycle analysis masks.



Light blue: G0/G1, yellow: S, Pink: G2/M



Objective : 20X, Scale bar: 100 µm

Label-free counting of interphase and mitotic cells based on cell thickness using Volume Contrast

Volume Contrast is an add-on module of NIS-Elements that constructs fluorescence-like phase distribution images from brightfield images captured at multiple z-depths.

Since interphase and mitotic cells have different thicknesses, cells with different thicknesses can be classified into interphase and mitotic cells using the difference in intensity value in the Volume Contrast image, enabling the cells in each phase to be counted.

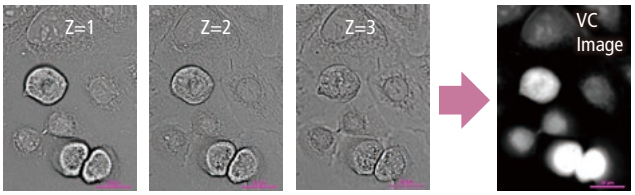
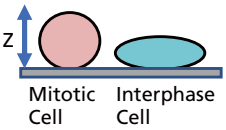
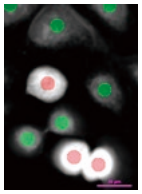


Fig. 5: Construction of a VC image from brightfield images

Left: Three Z-stack brightfield images (Z range : 3.25µm), right: VC image, lower right: VC image overlaid with cell count mask image



Red mask : Mitotic cells
Green mask : Interphase cells



Scale bar: 20 µm

Experiment overview

① Three brightfield images with different focal planes were acquired (Fig. 5, left).

② Fluorescence-like phase distribution images were constructed from brightfield images using the Volume Contrast function (Fig. 5, right).

③ Cells with different thicknesses were classified into interphase and mitotic cells based on the intensity value in the VC image, and cell count masks were created (Fig. 5, lower right).

④ The number of cells in interphase and mitosis was measured, and the proportion of mitotic cells was calculated (Fig. 6).

Summary

- The cell cycle can be analyzed from the sum intensity value of the nucleus (Hoechst33342).
- The Binning Function of General Analysis 3 can classify and aggregate the measured results by any numerical value.
- The ColorByValue Function can show the mask in different colors based on the specified measurement value.
- Cells with different thicknesses can be classified by the intensity value in the Volume Contrast image.
- Interphase and mitotic cells can be counted without labeling, using Volume Contrast images constructed from brightfield images.

Acknowledgments

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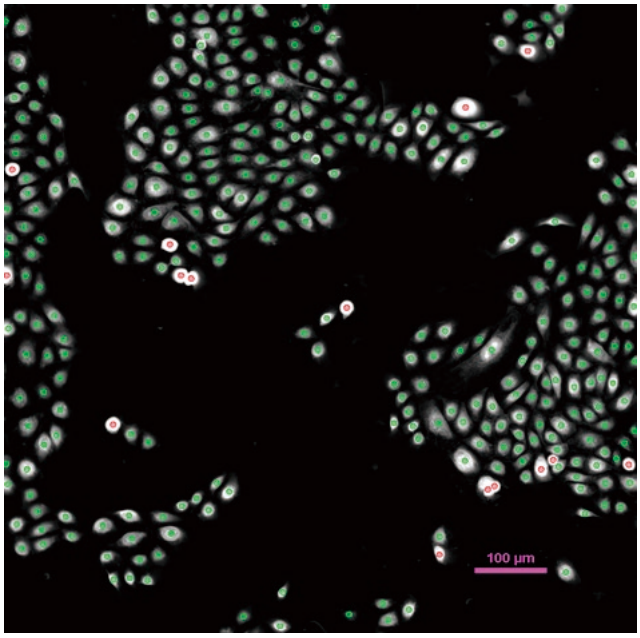

Fig. 6: VC image overlaid with cell count mask image

Red mask: Mitosis cells, Green mask: Interphase cells
Number of mitotic cells: 15 cells, Number of interphase cells: 321
Percentage of mitotic cells: 4.5%
Objective: 20X, Scale bar: 100 µm

Product Information

General Analysis 3 optional image analysis module of NIS-Elements imaging software

By combining analysis blocks, cell regions can be easily binarized and measured. This allows flexible image analysis according to your purpose.

Volume Contrast analysis module of NIS-Elements

Label-free quantitative phase analysis is possible without the need for special optical accessories.