APPLICATION NOTE

Digital Sight 50M Monochrome Microscope Camera

High throughput cell proliferation assay with low magnification objective, utilizing high-resolution performance of Digital Sight 50M

Screening assays using multi-well plates such as 96 well-plates are commonly performed in drug discovery research and academia, and are used for acquisition of drug candidates and genetic analysis. In recent years, screening has become essential for searching for and optimizing candidate compounds, and quality control and increasing the speed of assays have become issues.

This application note introduces quantitative evaluation by image acquisition of the entire well and morphological analysis, as an application utilizing the high pixel count that is a feature of the Digital Sight 50M (DS50M) F-mount monochrome camera, equipped with a full-size image sensor (Fig.1a). Acquiring an image of the entire well in one shot provides the following advantages: (1) cell data for the entire well can be obtained in a single shot, and (2) uneven distribution of cells within the well can be understood. With these advantages, sufficient necessary quantitative data can be obtained and the status of the well can be verified, which is effective for stable and efficient acquisition of assay data. In addition, the high pixel count of the DS50M enables single shot imaging and analysis of the entire well, which greatly improves throughput.

In this case study, in order to evaluate the quantitativity of analysis and its effectiveness in terms of imaging throughput by means of the DS50M and a low magnification lens, a nuclear counting assay was performed using a high-sensitivity camera that is widely used for fluorescence observation, as a comparison target.

Keywords: DS50M, screening assay

Materials

- HeLa cells
- DMEM
- FBS
- Hoechst33482
- 96-well glass bottom multi-well plate (IWAKI)

Observation/analysis environment

- Microscope: Eclipse Ti2-E
- Objective: CFI Plan Apochromat Lambda D 2X, CFI Plan Apochromat Lambda D 20X, CFI Plan Fluor 4X
- CMOS camera: DS50M, competitor's camera A
- Software: NIS-Elements AR v5.42.01

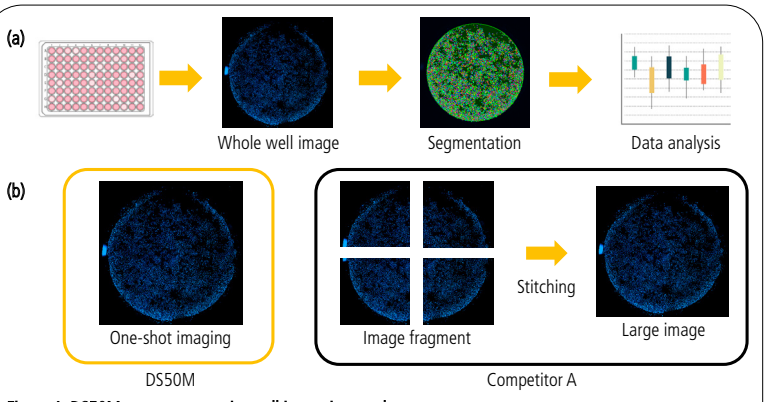


Figure 1: DS50M captures an entire well image in one shot
(a) Flow of quantitative analysis using entire well image
(b) The DS50M can image an entire well with a diameter of ø6.5 mm in one shot at a resolution that enables quantitative analysis (left). The Competitor A needs to obtain the image in fragments that are stitched together into a single image (right).

(a)

	DS50M	Competitor A
Full frame size [pixel]	9552 x 6360	2048 x 2048
Image sensor size [mm]	35.8 x 23.8	13.3 x 13.3
Pixel size [µm]	3.76	6.5

(b)

	DS50M 2x	Competitor A 2x
Objective	CFI Plan Apo Lambda D 2X	
Relay lens	1.8x	1x
Optical resolution / pixel size	2.63	0.85

(c)

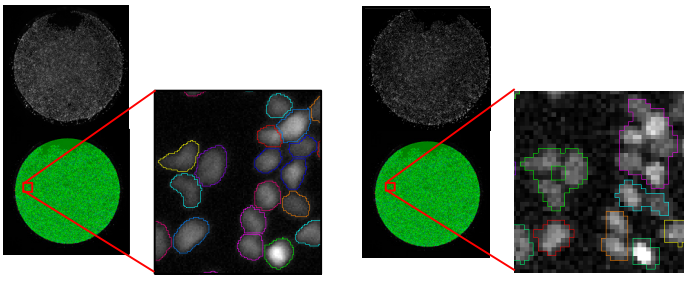
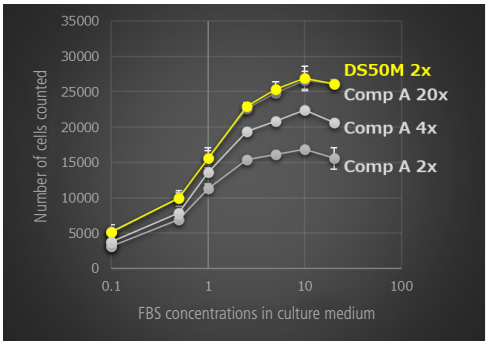


Figure 2: Nuclear segmentation from high-resolution whole-well images
(a) Specifications for image sensors of DS50M and Competitor A
(b) The objective used for imaging the entire well, relay lens, and ratios of the pixel pitch and spatial frequency are shown. DS50M satisfies the Nyquist criterion (>2).
(c) Comparison of nuclear segmentation from whole-well images captured by each camera, relay lens, and objective. NIS-Elements software was used for segmentation. For each condition, the upper row is a black and white image before analysis, the lower row is the image after analysis, and the green area indicates the range of the well. The images on the right are enlarged views, annotated with different colors for each recognized section.

(a)



(b)

	DS50M 2x	Competitor A 2x
Ratio to standard* [%]	101.1±8.6	63.5±8.9

*Based on images taken with Competitor A and CFI Plan Fluor 20X

(c)

	DS50M 2x	Competitor A 20x	Competitor A 2x
Z-factor	0.89	0.83	0.62

(d)

	DS50M 2x	Competitor A 4x
Imaging time of 96 wells	10 m 36 s	15 m 18 s

Figure 3: Quantification of number of nuclei

(a) Results of imaging and analysis using different cameras and objectives after 48 hours incubation with different FBS concentrations. The Y-axis indicates the number of nuclei counted and the X-axis indicates the FBS concentration of the culture medium on a logarithmic axis. DS50M 2x: DS50M and CFI Plan Apochromat Lambda D 2X, Competitor A 2x: Competitor A and CFI Plan Apochromat Lambda D 2X, Competitor A 4x: Competitor A and CFI Plan Fluor 4X, Competitor A 20x: Competitor A and CFI Plan Apochromat Lambda D 20X

(b) Quantification results of nuclei counted based on the analysis results of images taken with each camera. Ratios with respect to standard conditions are shown.

(c) Z factor under each imaging condition. The formula $Z\ \text{factor} = 1 - \frac{3(SD_{max} + SD_{min})}{|Ave_{max} - Ave_{min}|}$ was used for the calculation.

(d) Time required for imaging 96 wells under each imaging condition.

Methods

• Cell culture

HeLa cells were seeded in a 96-well glass-bottom multi-well plate (IWAKI) at a density of 1 x 10^4, and after 24 hours, the medium was replaced with DMEM medium adjusted to 20, 10, 5, 2.5, 1, 0.5, 0.1, 0% FBS and cultured for 48 hours.

• Image analysis

The cells were fixed with PBS containing 4% PFA and stained with PBS containing 1ug/ml Hoechst33482 for 30 minutes. Fluorescence signals were obtained using a Ti2-E inverted microscope (Nikon) with a fluorescence filter cube for DAPI, and CFI Plan Apochromat Lambda D 2X, CFI Plan Fluor 4X, CFI Plan Apochromat Lambda D 20X objectives, DS50M and Competitor A cameras. An entire well image was acquired in one shot when using the 2X objective, and was created by stitching together the acquired images when using the 4X and 20X objectives (Figure 1b). The analysis function of NIS-Elements was used to perform well region designation and segmentation, and to quantify the number of nuclei in the entire well.

Results

The DS50M has four times the number of pixels of Competitor A, and is equipped with a full-size image sensor with finer pixel sizes (Figure 2a). The DS50M used with the low-magnification CFI Plan Apochromat Lambda D 2X objective computationally satisfies the Nyquist criterion, indicating that high-resolution imaging is possible using a low-magnification lens (Figure 2b). Hoechst33482-stained images of HeLa cell nuclei in a well obtained with these cameras are shown in Figure 2c. Competitor A did not satisfy the Nyquist criterion with a low-magnification lens, and because of insufficient resolution, block noise made the shape of the nucleus unclear. On the other hand, the shape of the nucleus is smoothly represented by the DS50M. When segmentation is performed using data acquired by Competitor A, multiple nuclei are recognized as the same object, but with the DS50M, each nucleus is recognized individually. This suggests that the DS50M has sufficient resolution for morphological analysis and is effective for this assay system.

Next, to evaluate the quantitative performance of each camera in morphology analysis, the accuracy with which the number of nuclei in cells cultured with different FBS concentrations were quantified in each case was compared (Figure 3a). The number of nuclei obtained by analysis of the whole well image taken with Competitor A and a CFI Plan Fluor 20X served as the correct value, and was used to evaluate the analysis results of the whole well image taken with each camera and low-magnification objective. As a result, the quantification results of Competitor A showed a ratio of about 63.5±8.9% to the correct value, while those of the DS50M showed a value close to the correct value of 101.1±8.6% (Figure 3b). Calculating the Z-factor, a quality indicator of the assay system, the Z-factor of the DS50M with a low magnification lens was 0.89 and the Z-factor of Competitor A with a 2x lens was 0.62. The Z-factor of Competitor A with a high-magnification 20x lens was 0.88 (Figure 3c). These results demonstrate that the images obtained with the DS50M and a low magnification lens have comparable quality in terms of nuclear quantification to the images obtained with a high-magnification lens. In addition, the time required to acquire images of 96 wells was 10 minutes 36 seconds using the DS50M and a 2x objective, and 15 minutes 18 seconds using Competitor A and a 4x objective (Figure 3d). To obtain a whole well image with sufficient resolution using Competitor A, it is necessary to acquire multiple images with a 4x objective and stitch them together. On the other hand, the DS50M can acquire an image of the entire well in one shot, enabling high-speed acquisition.

Discussions / Summary

The DS50M camera equipped with a high pixel count full-size monochrome image sensor in combination with the CFI Plan Apochromat Lambda D 2X objective, which has excellent optical performance, enables high-resolution imaging of the entire well in a 96-well plate in one shot. Images obtained with the DS50M showed high nuclear quantification comparable to images obtained with a high-magnification objective. In addition, in order to obtain a quantifiable image of the entire well, it is usually necessary to stitch together images acquired with a high-magnification objective lens, such as a 20x lens, taking 2-3 hours to acquire images of all 96 wells; in contrast, the DS50M can acquire a high-resolution image of the entire well in one shot, making it possible to accelerate the assay.

Thus, the DS50M achieves assays with excellent quantitative performance and high throughput in cell screening using indexes such as proliferation ability and nuclear translocation, and is expected to be an effective tool for drug discovery assays that require efficient image acquisition.

Product Information

Digital Sight 50M monochrome microscope camera

An F-mount monochrome camera with a full-size sensor. The image sensor (9552 x 6360 pixels, pixel size 3.76 µm) enables a large FOV and high-resolution imaging. It delivers excellent results in assay systems that require quantitative performance and high throughput.