Computer image-based automatic cell counting system: High-throughput onboard device for the future scientific drilling

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Direct microscopic count of acridne orange-stained cells (AODC) showed that marine subsurface sediments harbor remarkable populations of microorganisms. However, the discrimination of life forms with AODC method required professionally trained shipboard scientists and was difficult to perform high-depth resolution onboard counting with statistically mean values. In addition, high backgrounds of mineral auto-fluorescence and/or non-specific binding of fluorescent dye cause serious problem in eye-dependent cell discrimination. We have constructed a newly developed computer-based automatic cell count system. Using SYBR Green I fluorescent dye, the image calculation of fluorescent spectra enable us to objectively discriminate cell-derived signals from the backgrounds (Morono *et al.*, ISME J, 2009). Furthermore, the integration of an automatic slide-loader and infrared LED light-camera monitoring system made it possible to perform stable, eye-independent, high-throughput and high-resolution counting operation. If the cell counting system and device is deployed on the drilling platforms, it will be very useful to onboard microbiological study of cores in the future scientific drilling.

Reference

Morono Y, Terada T, Masui N, Inagaki F, (2009) Discriminative detection and enumeration of microbial life in marine subsurface sediments. *ISME J.* 3: 503-511

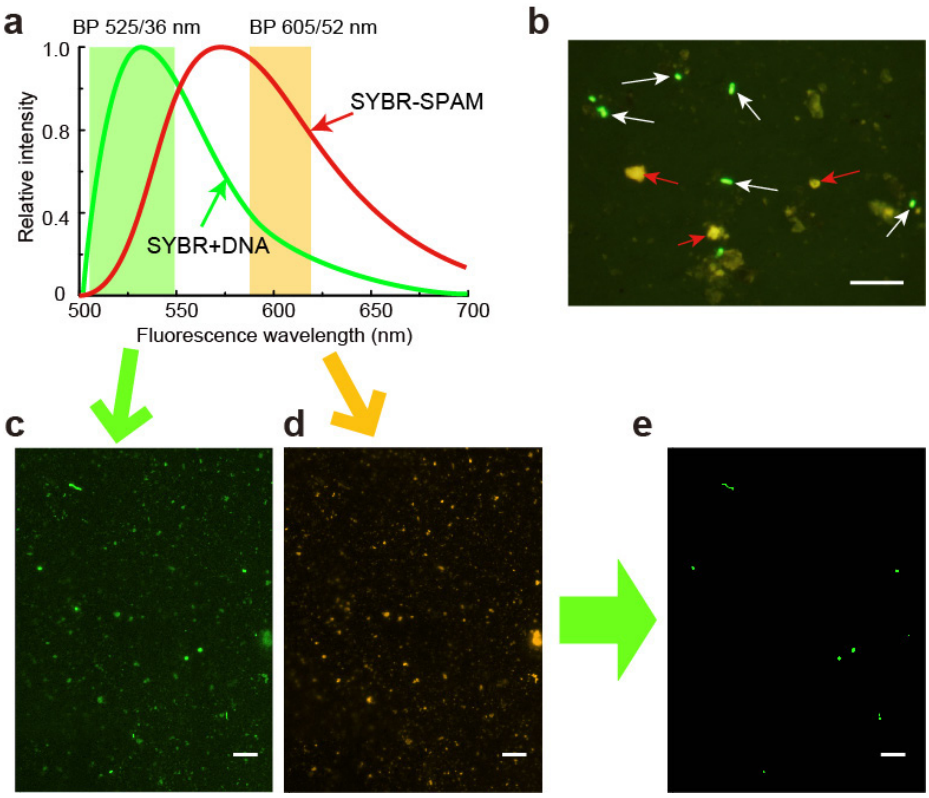

Figure 1. Difference of SYBR-I fluorescence spectrum with intracellular DNA or SYBR-SPAM, and discrimination of cell-derived SYBR Green I fluorescence from background signals using image analysis. (a) Spectrum patterns show "red shift" of SYBR-I fluorescence. When SYBR-I binds to SYBR-SPAM (red line), fluorescent spectra shift to longer wavelengths than SYBR-DNA complex (green line). Green and orange shading areas show the wavelength range of 525/36, 605/52 (nm of center wavelength/bandwidth) band-pass filters, respectively. (b) Examples of fluorescence-producing cellular and non-cellular objects stained with SYBR-I. Red arrows, yellowish SYBR-SPAM. White arrows, green *E. coli* cells. The image was obtained using a long-pass filter of cut-off wavelength 510 nm. (c to e) Image analysis to distinguish cell-derived SYBR-I signals from SYBR-SPAM in natural marine sediments (core 1H-1 of Site C0006 Hole E in the Integrated Ocean Drilling Program Exp. 316). Fluorescent microscopic images taken using band-pass filters of 525/36 (c) and 605/52 (d). Relative intensity profiles of green/red fluorescence (e) show only cell-derived fluorescent signals without background fluorescence. Bars: 10 µm.

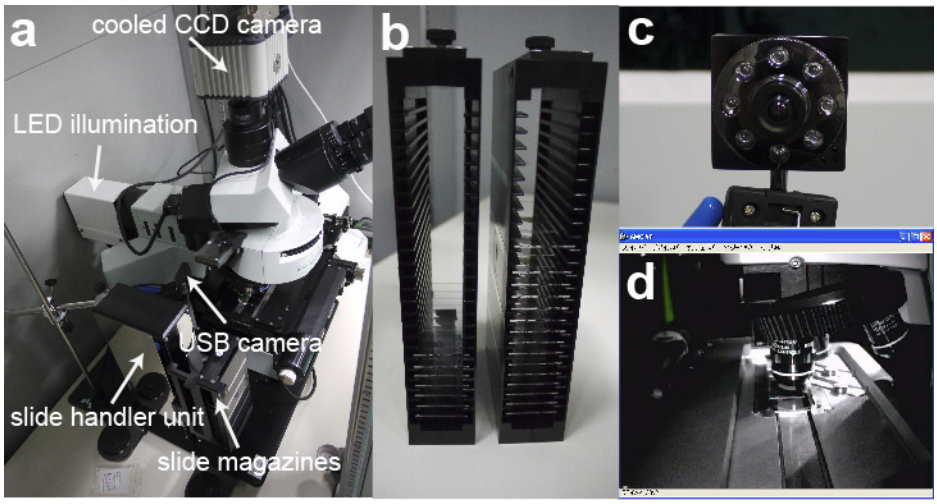

Figure 2. Newly constructed automatic microscopic system with slide handler. (a) Overview of the system. All the units are operated in a dark and cool (15℃) chamber. (b) Slide magazines capable of carrying 25 slides each. (c) USB-connected monitoring camera with infrared LED. The system is monitored without opening the door of the chamber (d).

Table 1. Correlation of the lower detection limit and the corresponding time required for obtaining images, amount of sediment, number of images, and number of filters. For future exploration to limits of life, further development on obtaining higher sensitivity is needed.

Lower limit of detection (cells/cm^3)	1.3 x 10^4	1.3 x 10^3	1.3 x 10^2	13	1.3
Required time (hour)	1	10	100	1000	10000
Required sediment volume (cm^3)	0.000228	0.00228	0.0228	0.228	2.28
Image fields (fields)	450	4500	45000	450000	4500000
Area of analysis (cm^2)	0.163	1.63	16.3	163	1630
Number of requred filters (filters)	1	1	7	66	652