Time-Resolved Lanthanide Imaging and Microarray: Towards Reliability and Quantification

Kazuko Matsumoto[1], Hiroko Kimura[2], Masahiro Mukaida[3], Ken-ichi Yoshida[4]

[1] *Department of AppliedChemistry, Tokyo University of Technology, Tokyo, Japan*
[2] *Juntendo University School of Medicine, Tokyo, Japan*
[3] *Department of Forensic Medicine, National Defense Medical College, Saitama, Japan*
[4] *The University of Tokyo School of Mdecine, Tokyo, Japan*

E-mail: kmatsu@yf6.so-net.ne.jp (KM)

Although recently a number of new imaging technologies have been developed for life science, diagnostics, and medicine, several improvements are still desired in conventional fluorescence imaging; (i) removal of autofluorescence and false fluorescence signal, (ii) more environment-independent quantitative signal intensity, and (iii) photo-stability of label materials for better signal quantification. Long luminescence life times of Eu^{3+} and Tb^{3+} complexes (more than 100 micro seconds) are suitable as labeling reagents for time-resolved luminescence imaging and other variety of bio detections such as immunoassay and bio-chips. We have developed several Eu^{3+} and Tb^{3+} complexes, and proved that time-resolved detection using the lanthanide complex labels gives 1 to2 orders better detection limit in many applications.[1] In the present study, a time-resolved microscopy for cell and tissue imaging has been developed, and the characteristics of the system was evaluated by using Eu^{3+} complex specifically designed for stable luminescence even in phosphate-buffer and for use of DNA labeling.[2] Quantitative luminescence intensity analysis of tissue images was conducted for comparison of a time-resolved image using the Eu^{3+} complex and a conventional non-time-resolved image using commercial dye Alexa Fluor 488.

The Eu^{3+} labelling complex DTBTA-Eu^{3+} has a very high stability constant (log K) of 25.0 and can be used without luminescence intensity loss even in phosphate buffer and for DNA labelling. Such high stability of the metal complex is beneficial for less-environment-dependent luminescence in tissues and cells. The time-resolved microscopy in the present study consists of an Olympus microscope, a CCD camera, a flash Xe lamp, and a laboratory-made high-speed shutter placed in front of the CCD. Fig. 1 shows comparison of rat kidney specimens. Image A and B show location of a stress-marker nitrotyrosine (NT) in kidney. Fig. 1A is non-time-resolved image using Alexa Fluor 488 (green), whereas Fig. 1B is a time-resolved image using DTBTA-Eu^{3+} (red). Fig. 1C and 1D are the results of image analysis. The C1 and C2 rectangular areas correspond to areas of tissue's tubular structure (C1), and no tubular structure (C2). In both areas, luminescence shoing NT is not observed. Rectangular areas P correspond to location of NT. In Fig. 1C and 1D, the gradation intensity (horizontal axis) and the pixel number (vertical axis) in each areas are plotted to show the intensity distribution within each area. As show in Fig. 1C and 1D, signal peak intensity distribution in P is overlapped on non-specific and autofluorescence signals (C1 and C2) in Fig. 1C, whereas in Fig. 1D, the signals of C1 and C2 are separated from signal P. This analysis quantitatively proves that in time-resolved image, autofluorescence and non-specific signals can be removed from the measured signal intensity

and true luminescence intensity due to label can be estimated.

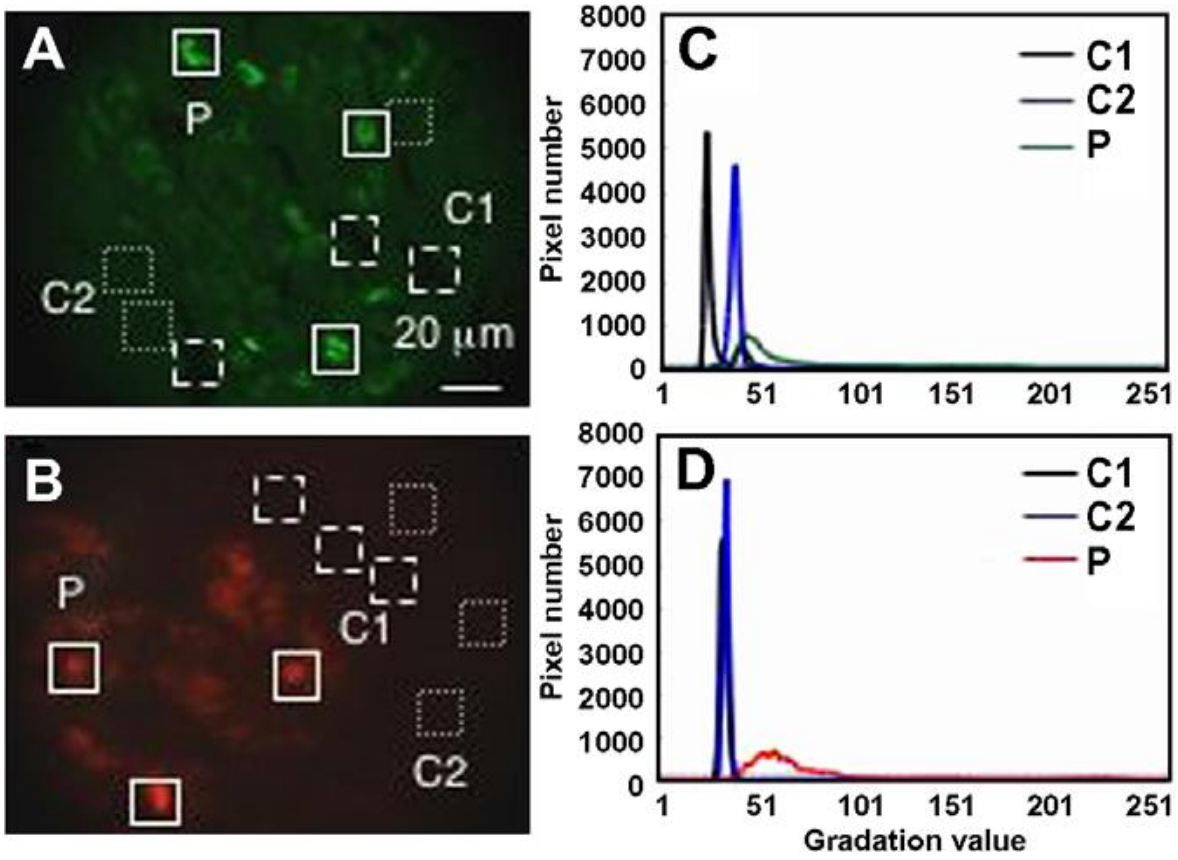

Figure 1. Non-time-resolved image using Alexa Fluor 488 (A), time-resolved image using DTBTA-Eu^{3+} (B), intensity-pixel number distribution in each rectangular area for non-time-resolved (C) and time-resolved (D) mode.

For DNA microarray, similar microscope was constructed as above microscopy, but image intensifier CCD was used. Comparison of non-time-resolved mode using Cy5 dye and time-resolved mode using DTBTA-Eu^{3+} showed that cynamic range of the concentration calibration line is 2.5 orders of magnitude in non-time-resolved mode, whereas the range is 6 orders of magnitude in time-resolved mode.

References:

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