4 - 70 Efficiency of Dyes in STORM Influences the Result of Dual Color Experiment

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Stochastic Optical Reconstruction Microscopy (STORM) is one of the super-resolution microscopy, which provides resolutions down to a few tens of nanometers by exploiting the cycling of dyes between fluorescent and nonfluorescent states to obtain a sparse population of emitters and precisely localizing them individually[1]. The key material is the fluorescent dyes are photo-switchable thousands of times before they turn quenched completely. Fluorescent proteins and organic dyes are the two main kinds of fluorescent dyes in the experiment[2]. The former are widely used in the living cell, the latter are used in the fixed cells in the form of single dye or dye pair[3].

In our experiment, we choose Alexa 647 and Atto 550 for the dual color stain based on the paper published. HeLa cells cultured in glass bottom dishes were incubated for 30 min after irradiated by 1 Gy X-ray. Then they were fixed by 2% Paraformaldehyde (PFA) and cold 70% ethanol. Samples were immunostained with γH2AX and 53BP1 primary antibodies. Secondary antibody Atto 550 and Alexa 647 are divided into two group: one is γH2AX-Alexa 647 plus 53BP1- Atto 550, the other is γH2AX- Atto 550 plus 53BP1- Alexa 647. Then the sample were observed with a STORM microscope and analysed by the software provided by Nikon company. The results show that Alexa-647 has higher photons number emitted per cycle (about 1,000) than Atto-550 (about 800), and the durance is better. After exchange of the dyes, the results are completely opposite that Alexa 647 dye is spare and Atto 550 dye focuses at the center nomatter which kind protein was stained (Fig. 1). The preliminary results remind us that two equivalent fluorescent dyes are prerequisite in dual color experiment.

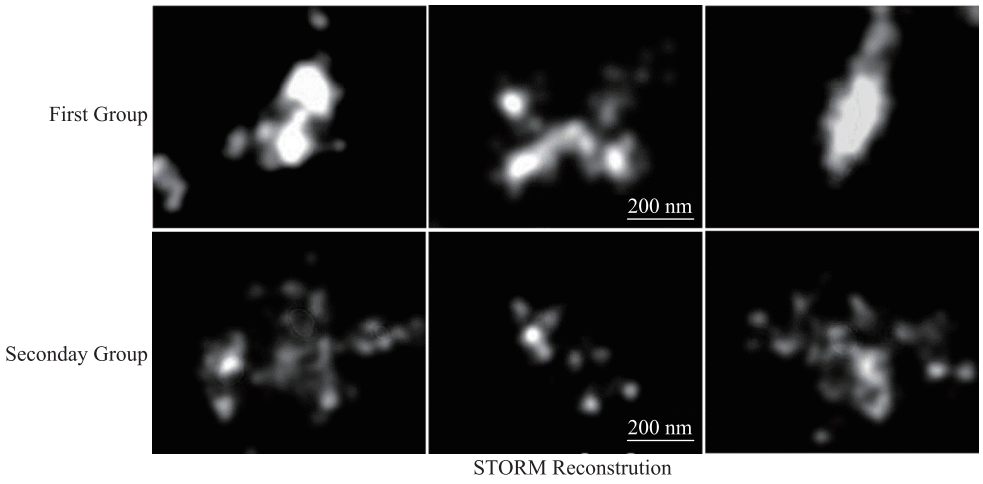

Fig. 1 The results of dual color stain of γH2AX and 53BP1 proteins. The upper and lower are results of exchange dyes. First group is γH2AX protein with Alexa 647 dye and 53BP1 protein with Atto 550 dye. Secondary group is γH2AX protein with Atto 550 and 53BP1with Alexa 647.

References

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