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IT-3-P-2190 Super-resolution imaging of 3D-cultured cells by SAX microscopy

Yonemaru Y.[1], Yamanaka M.[1], Uegaki K.[1], Smith N. I.[1], Kawata S.[1], Fujita K.[1]

[1]Osaka University, Osaka, Japan

Email of the presenting author: fujita@ap.eng.osaka-u.ac.jp

We have proposed the use of saturated excitation (SAX) of fluorescence to improve the spatial resolution of confocal fluorescence microscopes [1]. SAX introduces the highly nonlinear relation between excitation and emission intensities. With using a focusing laser for fluorescence excitation, the nonlinear response is localized within the laser focus, therefore the extraction of fluorescence signal, which nonlinearly responds to the excitation intensity, can realize the spatial resolution beyond the diffraction limit [2]. Imaging of biological samples have been demonstrated firstly with a fixed sample [3] and recently applied to live cell observation with fluorescence proteins [4].

Since the spatial resolution in SAX microscopy is improved by the nonlinear relationship between the excitation and the emission, SAX microscopy has the imaging property similar to two-photon microscopy. The nonlinear relation between emission and excitation allows us to remove the background fluorescence signal generated at out-of-focus planes. With using this benefit, we have applied SAX microscopy to observed 3D-cultured HeLa cells [5, 6]. A cell cultured on a flat substrate expands its body and forms a thin and flat shape with approximately 10 μm thickness. On the other hand, a cell cultured in a 3D matrix (such as gel) has more freedom to expand in the 3D space and shape their bodies with a thickness of several tens of micrometers. Since many differences has already observed between cell functions in 2D and 3D cell culture and the 3D cell culture can provide a condition for cell growth closer to the nature, super-resolution imaging of 3D-cultured cell may become important in biological and medical researches in the near future.

Fig. 1a shows a SAX fluorescence image of actin in fixed HeLa cells cultured in 3D. The cells were cultured in gel and stained with ATTO Rho6G phalloidin. We scanned the entire cell cluster to obtain a 3D data set of fluorescence distribution in the sample to construct Fig. 1a as a projection of the data set. Fig. 1b shows the enlarged view of the dotted rectangle area in Fig. 1a. Fig. 1c shows the same area as Fig. 1b, but obtained by a typical confocal microscope without SAX. The comparison of Fig. 1b and 1c confirms the improvement of the spatial resolution and the image contrast by SAX.

Reference

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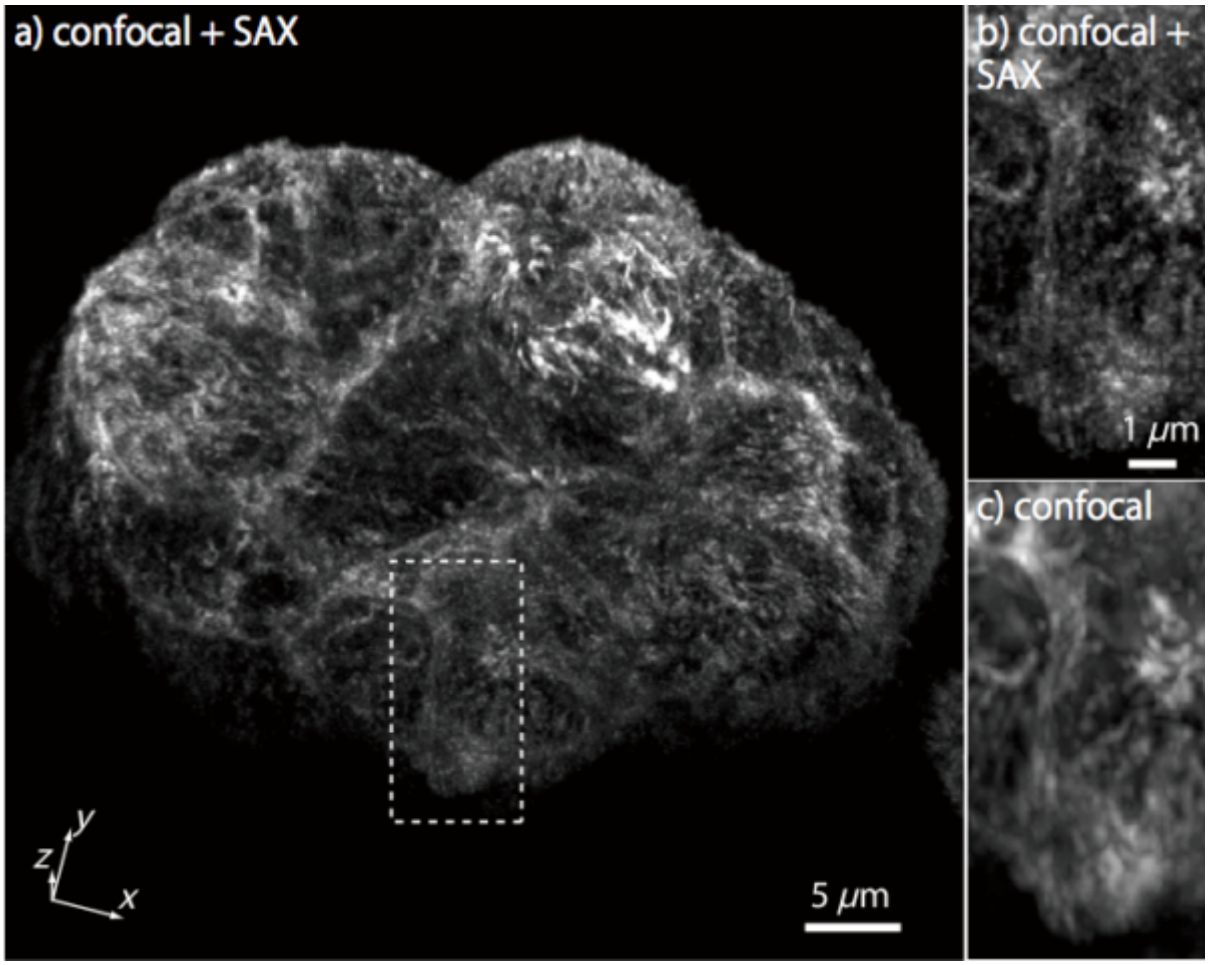

Fig. 1: a) SAX images of HeLa cells cultured in 3D matrix. Actin filaments were stained. b) the enlarged view of the area in the doted rectangle in a). c) the same area observed by a typical confocal microscope.