—Photogravure—

Ultra-high-resolution Images of Nestin and Vimentin in Pancreatic Carcinoma Cells Using 2 Novel Microscopy Systems

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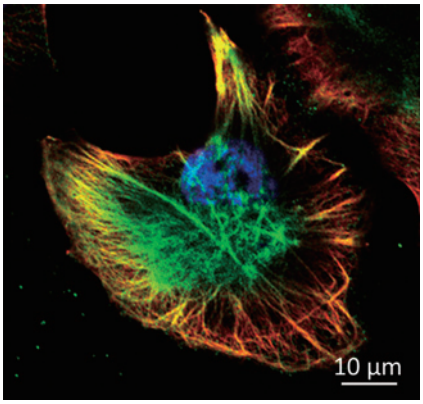

Fig. 1

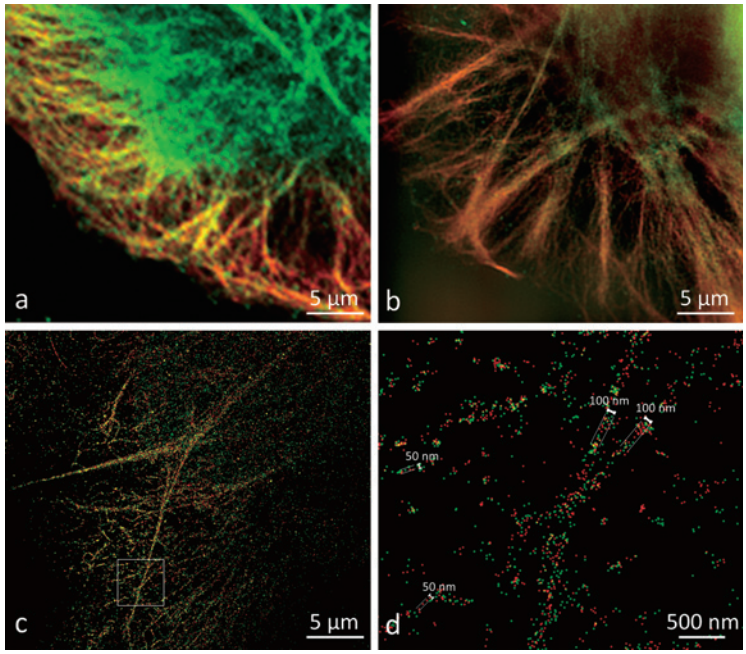

Fig. 2

Nestin, a type VI intermediate filament protein, heterodimerizes with vimentin, desmin, or internexin and forms intermediate filaments 10 nm in diameter[1]. Because the limit of resolution of conventional microscopes is approximately 250 nm, the ability to demonstrate the locations of these proteins has previously been restricted. In the present study, we determined the locations of nestin and vimentin in PANC-1 human pancreatic carcinoma cells by means of 2 novel ultra-high-resolution imaging systems: structured illumination microscopy (SIM, 100-nm resolution) and stochastic optical reconstruction microscopy (STORM, 20-nm resolution)[2,3]. The SIM system improves resolution by processing moiré images arising from samples excited by structured illumination. STORM makes high-resolution images with the center of mass of individual molecules determined with high precision. Although confocal laser scanning microscopy (CLSM) has demonstrated the filamentous expression of nestin and vimentin **(Fig. 1)**, it produces uninterpretable images at high magnification **(Fig. 2a)**. A corresponding-magnification image captured with SIM clearly resolved filamentous structures, and nestin and vimentin appeared to be merged **(Fig. 2b)**. STORM images revealed the filamentous network created by aggregates of small dots indicating nestin and vimentin molecules **(Fig. 2c, d)**. The nestin and vimentin dots

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were randomly distributed within filamentous lines tens of nanometers to one hundred nanometers thick (**Fig. 2d**), and some of them were merged (yellow signals), indicating that the 2 molecules were co-assembled and formed heterodimers and mixed polymers. In conclusion, SIM and STORM may help us to resolve the morphology of molecular-scale cellular structures.

Fig. 1 An image of nestin and vimentin in PANC-1 pancreatic carcinoma cells captured with a CLSM (Digital Eclipse C1, Nikon Corporation, Tokyo, Japan). After fixation with 4% paraformaldehyde and treatment with 0.1% Triton X-100 to improve antibody penetration, cells were incubated with a mixture of mouse anti-nestin antibody (dilution, 1 : 50, R&D Systems, Inc., Minneapolis, MN, USA) and rabbit anti-vimentin antibody (dilution, 1 : 20, Epitomics, Burlingame, CA, USA), followed by incubation with a mixture of Alexa Fluor 488 donkey anti-mouse IgG antibody, Alexa Fluor 568 goat anti-rabbit IgG antibody (dilution, 1 : 1,000, Molecular Probes, Eugene, OR, USA), and DAPI (dilution, 1 : 1,000, Dojindo, Kumamoto, Japan). Green, nestin; red, vimentin; blue, nucleus.

Fig. 2 A comparison of CLSM, SIM, and STORM images of nestin (*green*) and vimentin (*red*) in PANC-1 pancreatic carcinoma cells. **a**: CLMS image. **b**: SIM image (N-SIM, Nikon Corporation). Sample preparation for SIM was the same as that for CLMS. SIM illuminated the sample with a sinusoidal striped patterning of the excitation light and generated a series of images in the form of moiré fringes. These images were computationally reconstructed into a high-resolution image. **c**: STORM image (N-STORM, Nikon Corporation). **d**: This STORM image shows that nestin and vimentin molecules are within tens of nanometers of each other. White bars with numeric values indicate the thickness of a filament or a bundle of filaments. STORM images were prepared with the following procedure: After incubation with primary antibodies, samples were incubated with secondary antibodies conjugated with photo-switchable fluorophores that could be switched between a fluorescent and a dark state. All fluorophores were first put in the dark state, and then only a small set of the fluorophores were switched to the fluorescent state. The individual fluorescences were captured with a digital STORM camera; each dot indicates the center of a single fluorescence source. Finally, the super-resolution image was reconstructed from a number of dots obtained from repetition of the above process. The white box (**c**, *inset*) is expanded in **d**.

References

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