Label-free super-resolution imaging reveals the activities of cellular filopodia

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Super-resolution imaging based on nanometer topographical contrast is used to monitor the activities of cancer-cell filopodia in response to external electric stimuli. Filopodia are finger-like protrusions emanating from the outer edges of biological cells. Composed mainly of actin filaments, the responses of filopodia to external stimuli might have important roles in various cellular activities such as cell migration and neurite growth. Unfortunately, the diameters of filopodia are typically 100–300nm, which means they are only marginally observable by optical microscopy. Fluorescent labeling of the actin proteins is conventionally used to study these tiny structures. However, the labeling procedure and phototoxicity of the excitation light can lead to artificial results in living cells. Thus, a label-free method for visualizing cellular features smaller than the optical resolution limit is needed to study the behavior of these micro-structures.

In addition to fluorescence microscopy methods, topographic contrast provided by high-sensitivity measurement techniques offers an alternative by imaging features on a flat surface. This imaging modality, also known as surface profilometry, has been widely used in scanning probe microscopy. However, most high-sensitivity surface-profiling techniques require closed-loop control of the probe and therefore cannot be used for dynamic imaging in a field of view approximately $10 \times 10 \mu m^2$, the size of typical mammalian cells. In 2004, we proposed a super-resolution wide-field optical imaging technique based on nanometer topographic contrast and image restoration processing. The nanometer topographic contrast comes from structured-illumination sectioning microscopy and differential height measurement. On solid-state samples, we achieved lateral resolution nearly one-seventh of the illumination wavelength. Later, we demonstrated the observation on non-labeled filopodia of living lung cancer cells. We also identified a new protein that enhances filopodium growth and promotes cancer metastasis. Importantly, the signaling pathways of this protein might be a potential anticancer target.

On the basis of nanometer topographic contrast, as long as the signal-to-noise ratio is sufficiently high, other super-resolution approaches are feasible. In 2009, we demonstrated label-free structured-illumination microscopy using the contrast from surface height variations. This technique was named as structured-illumination nano-profilometry (SINAP). SINAP achieved...
lateral resolution \(\sim 0.3\) wavelengths with bright-field images. The imaging speed could be as high as 12 frames per second. Thus, SINAP is very suitable for analyzing the dynamics of filopodia.

To test the hypothesis that filopodia respond to variations in their microenvironment, we used microfluidic cell culture chips to culture lung cancer cells (see Figure 1).\(^7\) Two agar salt bridges were connected to the chip such that we could treat the cells with direct-current electric fields (dcEFs) and compare the growth of filopodia with and without the treatment. The other ends of the salt bridges were connected to a power supply through the silver/silver chloride electrodes in phosphate-buffered saline. The cells could be cultured in this chip for longer than four days. SINAP imaging revealed that the filopodia of lung cancer cells grow toward the cathode under the stimulation of a 180mV/mm dcEF (see Figure 2).\(^7\)

We immediately questioned which intracellular proteins were affected by the electrical stimulation. It is known that the epidermal growth factor receptors (EGFRs) have important roles in the electrotaxis of highly metastatic tumor cells,\(^8\) and that the signaling pathways of EGFRs are involved in filopodial formation.\(^9\) Thus, we compared the distributions of the intracellular EGFRs in fixed cells with and without the dcEF treatment. We found that, with dcEF treatment, intracellular EGFRs are biased toward the cathode,\(^7\) which is correlated with the cathodal-side growth of filopodia (see Figure 3). However, the detailed mechanisms of the electric-field-induced redistribution of EGFRs inside a cell require further investigation.

In summary, we used label-free super-resolution imaging to study the filopodial dynamics of cancer cells. Because the cells were not transfected by fluorescent proteins, and the illumination intensity was maintained at low levels, the filopodia demonstrated vivid responses to external stimuli. The label-free super-resolution imaging technique can also be applied to the studies of other cell behaviors in response to different environmental changes. For example, we are currently observing cell-to-cell coupling between neurons under electric field stimulation. We will also investigate the interactions between cellular filopodia and substrates of various degrees of stiffness.

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