Multiplexed and High-resolution Microscopy through the Transient Exchange of Fluorophores

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Statement of Purpose: Protein profiling techniques are often limited by the number of distinct protein biomarkers that can be simultaneously detected via immuno-fluorescence in cells or tissues, particularly when sample are precious and their sizes are limited. To surmount these problems, we have implemented a new technique called DNA-Catalyzed Molecular Biomarker Imaging and Amplification (DC-MBIA). Our method can substantially improve the sensitivity of fluorescence-based protein detection, while also facilitating exceptionally-high multiplexed detection capabilities. DC-MBIA combines two technologies: (1) engineered proteins and antibodies that are conjugated to DNA that enable oligonucleotide sequences to encode fluorophore labeling, (2) new classes of DNA-catalyzed biochemical circuitry that can react with these conjugates to create fluorescent reporting complexes that are trapped kinetically, but that can also be controllably exchanged between our conjugate probes. While these exchange reactions facilitate amplification of fluorescent signals and can increase the sensitivity of immunofluorescence methods, our 'transient' labeling scheme also bypasses issues associated with the spectral overlap of dye molecules. Furthermore, our methods enable fluorescent probes to be designed explicitly for super-resolution microscopy. Consequently, DC-MBIA enables many biomarkers to be detected and localized across a wide range of microscope resolutions all in the same biological sample.

Methods: A series of software-based tools were employed to design a library of DNA circuits that can function independently for our imaging experiments. Circuit components are prepared through separate annealing reactions and then isolated using native-PAGE gel techniques. Fluorescent dyes were incorporated into specific DNA strands allowing circuit complexes to carry fluorophores to their targeting site via a similar modality used by molecular beacons. However, additional circuit components were designed to deactivate reporting complexes of the system through sequence-specific strand-exchange reactions.

The function of the DNA circuits was assessed in three different experiments. First, custom designed DNA-microarrays were prepared to create spatially varying patterns of circuit targeting strands. Here, appropriately matched fluorescence signals report selective targeting in the arrays. Second, a custom-built fluorometer was designed and used to monitor catalytic reactions occurring on surfaces and at interfaces. Finally, a series of cellular imaging experiments were performed that harness DNA-conjugated antibodies and self-labeling peptides to confer circuit targeting to biomarkers. Antibodies are introduced to cultured cells using standard immunohistochemical techniques, while the self-labeling peptides were expressed after PEI-mediated transfections. In the later case, DNA targets were reacted with the expressed proteins after fixing and permeablizing cells. Afterwards, circuit reactions were introduced to create transient reporting probes.

Kinetic studies to evaluate the transient creation and removable of photo-switchable probes were also performed using a total-internal-reflection-fluorescence microscope (TIRFM). In these experiments, surface immobilized DNA-catalysts were reacted with DNA-circuits to create and remove optical probes for fluorescence intermittency localization microscopy (FILM). To evaluate the use of DC-MBIA for super-resolution microscopy, the number of reporting single-molecule point-spread functions (PSFs) was monitored in a sequence of images.

Results: Our microarray experiments verify the selective and independent function of all six circuits. Selective removal of dye molecules validates the multiplexed imaging capabilities of DC-MBIA. Furthermore, exchange reactions are found to be efficient, and facilitate the rapid removal of dyes using short incubation periods at ambient conditions.

Enhancements to super-resolution microscopy are also observed via increased PSF collection rates over existing methods. The exchange of targets again improves multicolor imaging capabilities of super-resolution microscopy methods.

Conclusions: Our ability to create transient reporting signals at the local targeting site of a biomarker can greatly increase the number of proteins that are analyzed in cells and tissues using a variety of fluorescent microscopy techniques. We have also demonstrated that our method can improve the fidelity of existing super-resolution microscopy methods.