Localized Biosensing with Topas Microstructured Polymer Optical Fiber

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Microstructured optical fibers (MOFs) have become a driving force in the development of new devices in many areas, including the sensing of gaseous, chemical and biological samples. Such fibers offer an efficient interaction though evanescent waves between the guided light and a sample positioned into the holes.

Of particular interest are microstructured polymer optical fibers (mPOFs),[1] which have a lower melting temperature, more flexible hole patterns, easier manipulation and improved biocompatibility compared to their silica-based counterparts. Biocompatibility is the key issue for developing mPOF biosensors, because it allows for the use of simple immobilization procedures.[2]

We fabricated mPOFs from Topas cyclic olefin copolymers (Topas COCs).[3] In contrast to the most commonly used polymethyl methacrylate (PMMA), Topas has no monomers, and its moisture absorption is 100 times lower than PMMA, allowing better conditions for the drawing of optical fibers.

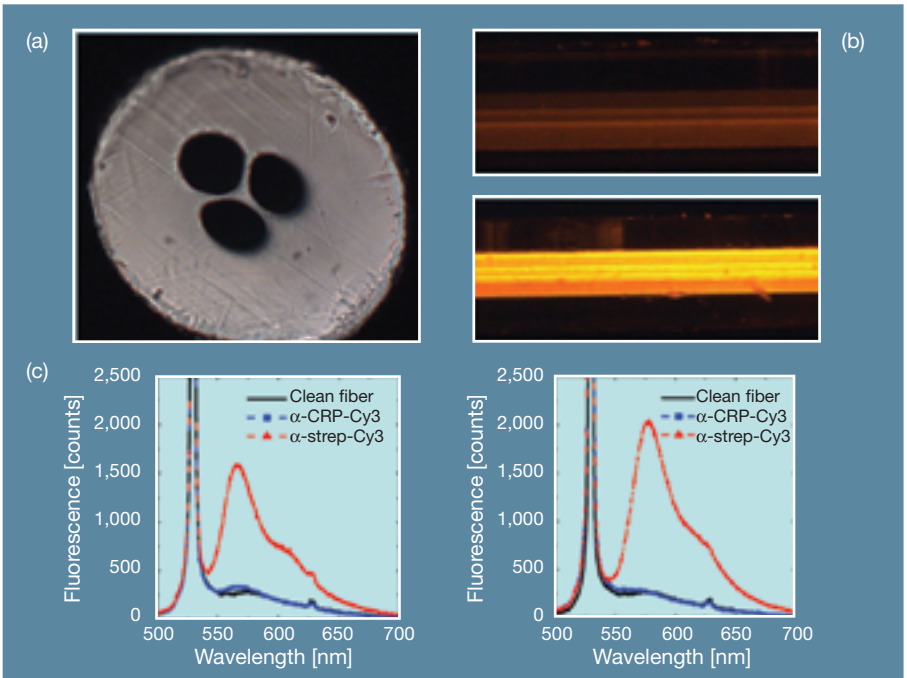
In addition, we developed a novel concept for fiber optical biosensing based on the biochemical properties of Topas.[4] Our concept allows for one or more localized sensor layers to be defined in predetermined sections of the fiber.

Topas is a chemically inert material; direct binding of biomolecules on its surface is difficult. However, commercially available antraquinon (AQ) linker molecules can attach to the Topas surface when activated by UV light[5] and subsequently accept sensor layers. In contrast, PMMA will always accept sensor layers.

A 30-cm-long piece of a three-hole mPOF was used to demonstrate the concept. The fiber was filled with AQ linker solution. After an incubation period of one hour, half of it was illuminated with a UV lamp.

To confirm the localized binding of the AQ molecules to the surface and hence the localized definition of a sensing layer in only one end of the fiber, we used a well-known antigen-antibody binding detection procedure,[2] where the sensing layer is built from streptavidin molecules. The biosensor was then exposed to an aqueous solution of fluorophore-labeled α-streptavidin. The results of the capture process were investigated under an epifluorescence microscope.

A UV mask can be used to define localized sensor layers inside a Topas mPOF. We believe this technique can contribute to the development of multi-antibody mPOF-based biosensors by definition of different sensor layers in the same fiber.



(a) Microscope picture of the end facet of a three-hole of a Topas mPOF. The outer diameter is 220 µm; hole diameter is 50 µm; and core diameter is 12 µm. (b) Epifluorescence microscope pictures of 2-mm sections of the end of a Topas mPOF biosensor without (top) and with (bottom) a UV-activated sensor layer. Fluorescence is only observed from the activated fiber end (bottom). An excellent differentiation between the two parts was observed, proving that the sensor layer was only present where the AQ molecules have been activated by UV light. (c) Fluorescence measurements from the 3-hole Topas mPOF biosensor from (a) showing selective detection of (left) α-streptavidin and (right) α-CRP. This shows that selective detection of antibodies can be achieved with such a biosensor.

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Tracking a Single Organelle with Two-Photon Protein Conversion

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Two-photon excitation by femtosecond (fs) laser pulses in the near-infrared region has potential applications in the imaging and manipulation of organelles within cells. Femtosecond lasers in the near-infrared region offer attractive advantages, including high spatial resolution, deep penetration into thick samples and reduced photon-induced damage.

Tracking organelles within a living cell provides spatial and temporal information about them. Fluorescent proteins allow visualization of organelles; however, whole organelles in cells are visualized by fluorescence labeling. A site-specific organelle of interest can be discriminated by photo-activated fluorescent proteins that exhibit pronounced light-induced spectral changes.[1,2,3,4] Most such proteins require light in the UV or visible region for activation via one-photon excitation. However, UV light can be phototoxic to some biological cells. To address this, we have reported on selective labeling of a single organelle using two-photon conversion of a photoconvertible fluorescent protein with near-infrared fs laser pulses.[5]

We used tobacco BY-2 cells whose mitochondria were labeled with Kaede, a cloned fluorescent protein extracted from the open brain coral *Trachyphyllia geoffroyi*. Kaede has an emission color that can be irreversibly changed from green to red by exposure to UV light.[3,4] We used a confocal microscope to simultaneously image unphotoconverted (green) and photoconverted (red) fluorescence from Kaede. Femtosecond laser pulses of 750 nm and a repetition rate of 76 MHz were focused at the mitochondrion.

Two-photon photoconversion by the fs laser irradiation showed that the fluorescence from a single mitochondrion was converted from green to red. A volume of the mitochondrion of approximately one femtoliter was photoconverted around the focal volume. The results demonstrate that the site-specific labeling of a single organelle can be performed in three-dimensional space.

We tracked the movement of one mitochondrion among several hundreds of mitochondria in a living BY-2 cell by observing time-lapse photoconverted red fluorescence of Kaede. Investigation of the interaction between mitochondria and cytoskeletons and mitochondrial transport by tracking a mitochondrion in plant cells suggested that the directional movement of mitochondria strongly depends upon actin filaments, whereas microtubules are not required for mitochondria movement, indicating that the movement is regulated by myosin motors.

This site-specific organelle labeling technique will open the door to biological and medical applications such as vesicular transport, axonal transport, chromatin dynamics, signal transduction and signal pathway. ◮

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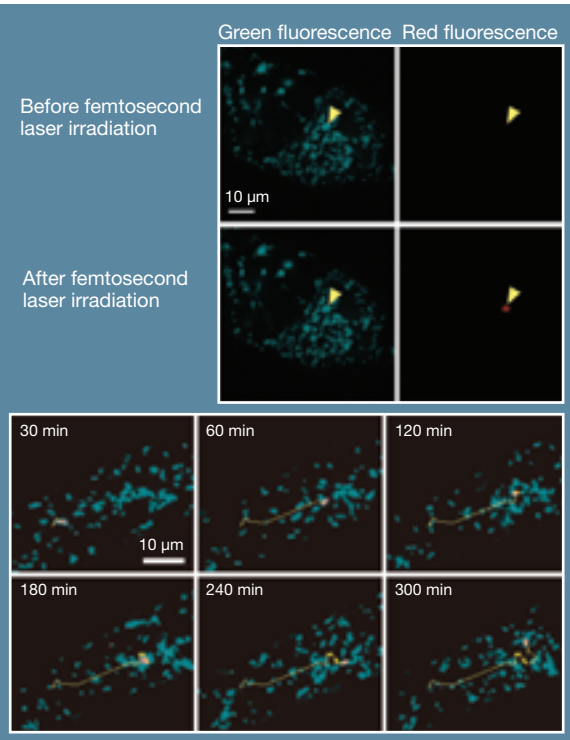

(Top) Selective photoconversion of mitochondria with Kaede in a fixed BY-2 cell. Left column, stacked 3D confocal images of green fluorescence obtained through bandpass filter (510 to 540 nm); right column, stacked 3D confocal images of red fluorescence obtained through bandpass filter (560 to 600 nm) before and after fs laser pulse irradiation. A single mitochondrion was photoconverted from green to red by 750-nm pulses with an energy of 0.053 nJ/pulse (exposure time: 1 s). A target mitochondrion is indicated by the yellow arrow. (Bottom) Tracking a mitochondrion in a living BY-2 cell. Time-lapse stacked confocal images along *z* axis (0.5-µm steps, total 13 slices) after marking a mitochondrion by two-photon excitation. The movement of the mitochondrion labeled by two-photon conversion could be tracked for 5 hours. The trajectory of the labeled mitochondrion is shown by the yellow line.