Optical Sensors: Monitoring Cellular Chemistry

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Although a huge amount of information on cellular chemistry is already known, the ultimate dream for cell biologists and microbiologist for decades has been real time monitoring of chemical changes within a single living cell. Two of the biggest problems with real time monitoring of a cell are the small volume and the fragility of the system. A cell is an extremely complicated system whose survival depends upon all of the individual components functioning together. Any abrupt change in any one component can damage the entire system. Problems that exist with many of the probes used on cells are poisoning of the cell, disruption the cell's natural state and fouling of the probes by the cell's components. The optical sensors discussed here provide an opportunity for real time monitoring without many of these problems.

To date, there have basically been two methods used for monitoring intracellular chemistry, potentiometric sensors[1] and free molecular probes.[2] Optical sensors have not been used much because compared to potentiometric sensors (submicrometers and milliseconds), fiber optic sensors were large (100-1000 μm) and slow (seconds), making them unsuitable for the delicate and dynamic task of intracellular analysis. However, optical methods, primarily fiber optic sensors, have been used in many areas of analytical chemistry including environmental and industrial monitoring.[3,4]

Development of nanoscopic optical biochemical sensors (NOBS) has remedied most of the problems. NOBS are being fabricated in the micrometer to nanometer regime and at such small dimensions the response time is significantly reduced. Optical

fibers can easily be pulled to the micrometer size range[5,6] however incorporating a sensing element becomes problematic. There are several methods which have been tried including optical fibers dip coated with sensing matrix [7], optical fibers dip coated with sol-gels [8], and micropipettes filled with matrix.[9] Dip coating is a very popular method to fabricate fiber optic sensors. The problem with dip coating is that the resulting matrix in inhomogeous, both in thickness and composition. This can lead to difficulty in reproducibilty and calibration.

Two of the new NOBS methods will be discussed. Kopelman and coworkers developed a method using near field optics (NFO) and photopolymerization to fabricate submicrometer fiber optic sensors [10,11] which produces uniform and reproducible results. Kopelman and coworkers have also developed a new biochemical sensor called PEBBLE (probes encapsulated by biologically localized embedding).[12,13] These nano-optodes are designed to be delivered into an individual cell to monitor a given ion.

Fiber Optic Probes[10,11,14,15]

The fabrication of the NFO tips is fairly straightforward. The optical fiber is pulled to a suitable dimension and coated with aluminum. A laser is attached to the unpulled tip of the fiber and the other end is immersed into a solution of photopolymerizable polymer and any additional components. Finally the laser is turned on and the polymer is photopolymerized on the tip of the fiber. As a result of NFO theory, the solution only polymerizes in the near field region where the photon flux is the highest.[15] Therefore the growth of the polymer is well controlled and limited to only the tip of the fiber.

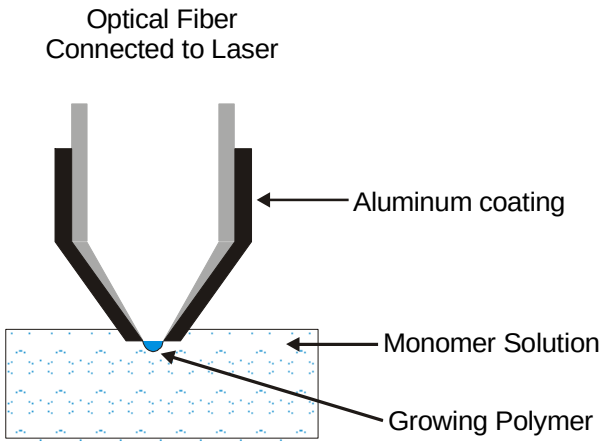


Figure 1: Photopolymerization process on a optical fiber tip

The fabrication process described above produces a well spatially resolved "super tip" as seen in figure 2. The "super tips" have excellent response times, reversibility and sensitivity. Response times depend on the size of the sensor; ranging

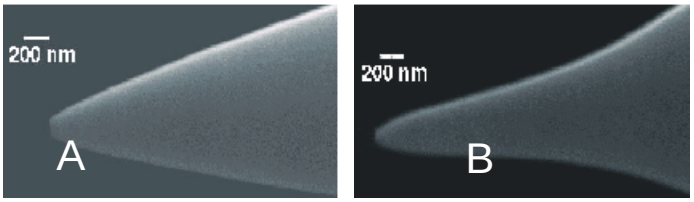


Figure 2: A) Pulled fiber optic tip B) Pulled fiber optic tip after photonanofabrication

from <20 ms for submicrometer sensors to 300-500ms for larger sensors. The response time is enhanced from other fiber optic sensors (40-120 s for 200μm) where mechanical confinement of the sensing matrix is used because the analytes have immediate access to the sensor tip. The reversibility is shown by the fact that the order of pH measurement does not affect individual intensities. For example when going from pH 6 to pH 7 and then from pH 8 to pH 7, the same intensities at pH 7 are observed. These sensors need only attoliters of sample and zeptomoles of analyte.

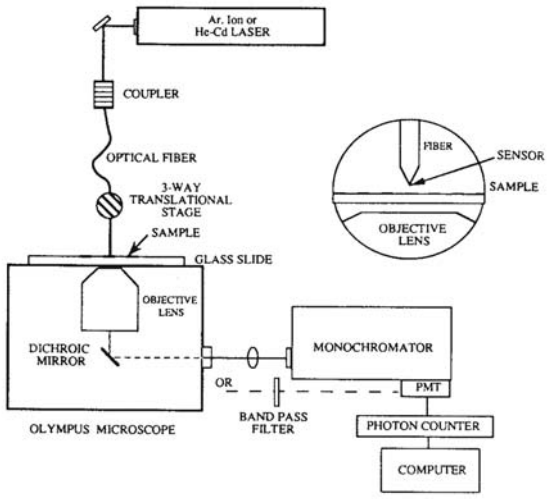
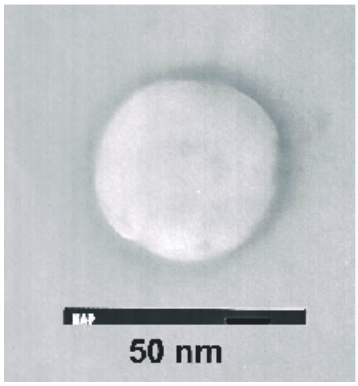


Figure 3: Schematic of instrumental set up

The technology has miniaturized the sensor however an inverted microscope is still used. The inverted microscope is used because signal intensity is increased an order of magnitude over back collection through the fiber. The set up, as shown in figure 4, consists of the laser, the fiber probe, the inverted microscope and a photomultiplier. The nanofabricated tips have been used in measuring intracellular chemistry for pH and Ca^{2+} in rat conceptuses[10] and smooth vascular muscle cells.[16]

PEBBLEs[12,13,17]

PEBBLEs are 20-200 nm spheres made from a polymeric matrix which encapsulates a fluorescent indicator and other components specific to a given ion. The matrices are similar to that of the NOBS but the polymer is not immobilized on the tip of an optical fiber it is a free sphere. The small size of the PEBBLEs is very advantageous since its volume is much less than



Figrue 4: STEM of a single PEBBLE

that of a cell; an 80 μm cell volume is 270,000 μm^3 whereas a 60 nm PEBBLE's volume is only 0.2 μm^3. [13] This is a significantly smaller volume than either fiber optic or potentiometric probes and the PEBBLEs are unencumbered by fibers or wire. Therefore the PEBBLEs are minimally invasive to the cell. The PEBBLEs have been characterized and they are spatially well defined making them easy to identify within a cell and easily distinguished from the autofluorescence of a cell.

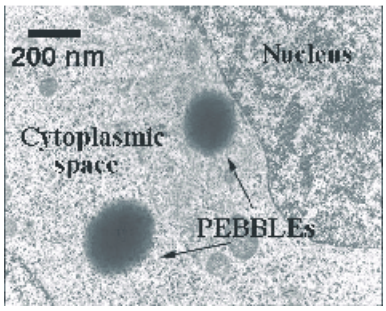


Figure 5: STEM of acrylamide PEBBLEs in neuroblastoma cell

The encapsulation of the fluorescent indicator into the PEBBLE polymer prevents several problems. First, there is very little leaching of the dye into the surroundings which can poison the cell or cause quenching of the fluorophore. Second, often a free fluorescent indicator dye will be affected by the cellular environment. Proteins are known to bind to the dyes [17,18] causing significant errors in fluorescence signals. This can lead to problems in calibration since in vitro measurements will not accurately reflect the cellular environment. However, PEBBLE calibration can be done in vitro and used in vivo.

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