Nanoscale arrays for direct RNA profiling in single cells and their compartments

Weihong Tan (PI), Steven Benner (co-PI), Leonid Moroz (co-PI), Steven Pearton (co-PI), and Harold Craighead (co-PI)

University of Florida and Cornell University

1. Real-time imaging of mRNA in single living cells using molecular beacons

Monitoring gene function and activity in living tissue and even in single living cells has long been a problem of great interest and difficulty in biomedical and basic life science research and development. We have used molecular beacons (MBs), coupled with our advanced imaging system, to monitor, in real time, genetic activity by targeting steady-state levels of specific mRNA molecules in the cytoplasm of single living cells. MBs serve as a highly sensitive, extremely selective, non-radioactive, easily detectable probe for monitoring real-time hybridization dynamics, making them optimal probes for intracellular studies. The techniques and methods developed in this study will be highly useful in detecting a variety of biomolecules inside living cells. We intend to use this mRNA monitoring technique to study gene expression and gene profiling studies which will complement with our efforts in nanoscale array research.

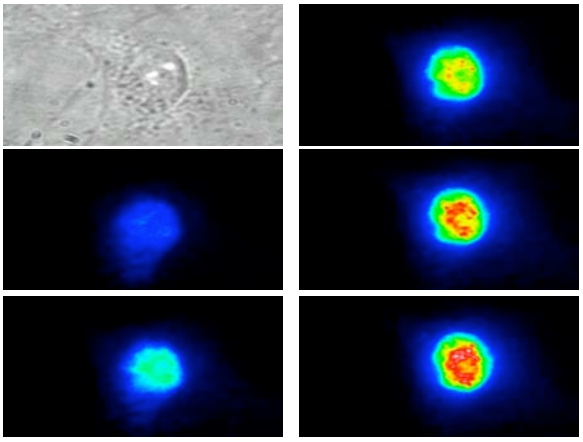

Figure 1. Hybridization of the β-actin MB2 to mRNA in living cells. Typical images of PtK2 cells after microinjection of MB specific for β-actin mRNA: optical image; fluorescence images at 3 minute intervals to 15 minutes (starting at 3 minutes) following injection of 10^{-6} M β-actin MB2. The fluorescence intensity was increased over time, which indicates the hybridization of MB2 by the mRNA inside the PTK2 cells. Cells were viable during the experiment.

We have used a conventional fluorescent imaging method to follow intracellular hybridization of β-actin mRNA. Kangaroo rat kidney cells (PtK2 cells) were microinjected with MB complementary to β-actin mRNA. Fluorescence images were taken at different time intervals depending on the hybridization rate inside the cell. We were able to take subframe images with a 30 ms dwell time. The fluorescence images

corresponding to the injected cells were used to determine the average fluorescence intensity that was recorded for following mRNA hybridization dynamics, shown in Figure 1. We performed extremely strict control experiments. We also observed that there was a large variation among the amounts of mRNA inside different cells of the same culture dish. More quantitative experiments are underway using a ratiometric imaging method based on one MB probe and another unrelated DNA probe labeled with a reference dye. Initial experiments showed that much better reproducibility and easier control for quantitative determination of mRNAs inside living cells can be obtained.

2. Nanofiber-based molecular filtration systems

We have developed a method for making nanofluidic filtration networks with controlled nanoscale dimensions. The method utilizes a new method for electrospinning polymeric nanofibers of thermally decomposable polymers. This deposition process creates random arrays of fibers of uniform dimension on a planar substrate. By coating the fibers with a sealing layer and then heating the system to remove the polymer a porous network is created. By using a transparent dielectric coating the filtration network may be integrated into a microfluidic chip system and utilized for optical detection of molecules. Figure 2 shows a fluorescence optical micrograph of dye solution filing an array of tubules encapsulated in a glass layer.

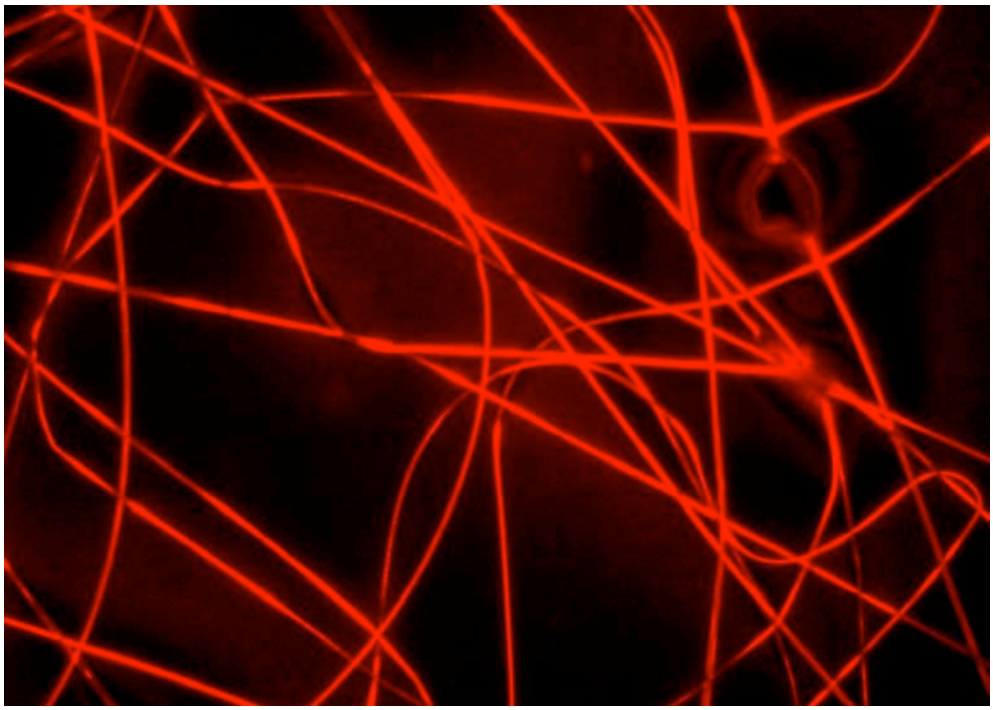

Figure 2. Fluorescence optical micrograph of dye solution filing an array of tubules encapsulated in a glass layer.

Our process integrates controlled nanofluidic structures with planar devices and couples well to microfluidic analytical systems. Unlike polymeric gels or packed beads this approach creates systems of uniform cross sectional dimensions, and the density and

thickness of the layer may be selected for a given application. The known and controlled dimension provides an opportunity for modeling the migration and separation of molecular species by differential motion through the network. The fiber deposition method utilized for this application was published as "A Scanning Tip Electrospinning Source for Deposition of Oriented Nanofibres", Jun Kameoka, Reid Orth, Yanou Yang, David Czaplewski, Robert Mathers, Geoffrey Coates and Harold Craighead, Nanotechnology, 14, 1124 (2003.

3. Outreach

We at University of Florida presented a general interest talk on "The Wonderful World of Nanoscience" and designed some simple hands-on experiments for participants in which they could measure resistance of nano-crystalline Si at different temperatures or in the presence of various types of light.

Figure 3. Participants in the 2003 Workshop "The Wonderful World of Nanoscience".

Corresponding PI:

Professor Weihong Tan
Department of Chemistry
University of Florida
Gainesville, FL 32611-7200
352-846-2410 (phone and fax)
e-mail: tan@chem.ufl.edu