

## Development of a Robust, Quantitative Fluorescence *In Situ* Measurement System for Cellular Biomolecules

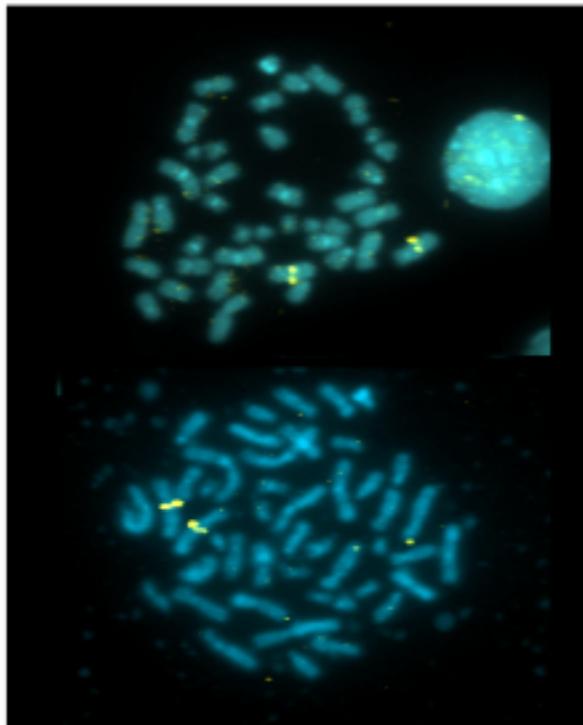
*Better measurement tools are needed for quantitating specific proteins and DNA sequences in single cells. NIST is developing new applications of quantum dot (qdot) fluorescence technology for the detection, measurement and localization of medically-important protein and DNA biomarkers in cancer. Highly-specific affinity reagents are coupled to fluorescent qdots, and used to probe single cancer cells. Qdot probes have certain key advantages over chemical fluorophores. The new methods are expected to enable innovation in several business sectors including health care and homeland security.*

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The most critical analytes in biomedicine can be simplistically conceptualized to nucleic acids and proteins. In most cases, protein and nucleic acid detection systems use ensemble methods which, in effect average the values of various nucleic acid and protein biomarkers. While this appeals to chemists, the fact of biological heterogeneity among cells demands cell-by-cell quantitation. This means not only single molecule detection (SMD), but SMD and quantitation superimposed on cell type.

NIST scientists first demonstrated FISH analysis using quantum dots, a technique expected to greatly improve cancer biomarker detection.

A significant measurement problem with SMD in cells has been the nonquantitative nature of classically used organic fluorophores. Most fluorophores photobleach, rendering replication difficult, if not impossible. Whereas qualitative imaging has met needs in the clinical community in the past, the increasingly sophisticated interplay among genes and proteins means that, to extract optimal value from cell measurements for medical diagnostics or evaluation of drug effects on cells, quantitation is key for the future. In addition, quantitation on single analytes (proteins or genes or RNA species) will prove to be insufficient for clinical decision making of the future. Thus, in addition to quantitation, analyte multiplexing at the cell level will assume greater importance as the discoveries of the biotechnology revolution of the past 25 years undergo translation in service of medicine and the private sector in, for example, pharmaceutical drug development and evaluation.



With this future measurement need in mind, we have focused on a novel non-photobleaching detection tool that shows great promise for SMD and multiplexing at the level of the cell: quantum dots or semiconductor nanocrystals. This technology development and physical standards work is based on development of new nucleic acid probes labeled directly or via haptens such as biotin and digoxigenin, and novel IgY antibodies. Because generic applications of quantum dots would be too broad for a small research lab, we focus on two model systems: HER2, an FDA approved analyte of significant medical and financial proportions in breast cancer detection and management; and TMPRSS2, a promising prostate cancer biomarker early in its translational development stage. These antibody probes are detected with semiconductor nanocrystals and 3D imaging in a high throughput mode to generate data with associated performance metrics. Work has focused on HER2 gene (breast cancer), TMPRSS2-ETV1 fusion genes (prostate cancer) as needful metrology improvement programs for clinical cancer and quantitative histochemistry QA/QC. These studies led to a long term evaluation of cellular uptake of functionalized qdots as part of the nanotech and cellular biometrology competencies at NIST.

Our major accomplishments include: the first demonstration of fluorescence in situ hybridization (FISH) with qdots; development of a novel IgY chick antibody for human telomerase detection (Xiao patent submission); demonstration of high resolution imaging of cellular responses to WMD protectant roxithromycin.

A number of press interviews for breast cancer nanotech imaging work; qdot work has expanded to a number of different gene and protein analysis systems and has been the reason for several invited presentations (Barker, Xiao). Roxithromycin qdot work was selected as best paper in the November 2006 U. S. Army Science Conference. The applications work on qdots has positioned the lab, group and division among leading labs working on these novel fluorophores, and has established a track record in successful qdot metrologies in the bioscience community.

**Future Plans:** Qdot imaging of HER2 gene and protein in high throughput mode; imaging on tissue microarrays; completion of HER2 SRM.

**Patents:**

Xiao, Y. IgY antibody for human telomerase (patent submitted September 2006)

**Publications:**

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Xiao, Y., Telford W. G., Ball, J. C., Locascio L. E., Barker, P. E. (2005). pH, FISH and semiconductor nanocrystals. Nature Methods 2(10): 723, 2005. (IF=6.741, C=2)

Barker, P. E. (2006). Preface to standards for health-care: needs assessment and development. Cancer Biomarkers 1(2005) 207-208. (IF=not available; C=0)

Mueller, F., Houben, A., Barker, P. E., Xiao, Y., Kas, J., Melzer, M. (2006). Quantum dots: a versatile tool in plant science. J. Nanobiotechnology 5:4.  
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