Quantum dot (Qdot) labeling of gene expression in fresh frozen brain tissue using high-throughput *in situ* hybridization.

Z. Riley, E. Byrnes, S. Parry, S. Smith, N. Dee, J. Timlin*, A. Jones, A. Jeromin.
Allen Institute for Brain Science, Seattle WA 98103
*Sandia National Laboratories, Albuquerque NM 87185

Abstract:

The Allen Brain Atlas[1] is the first large-scale atlas of gene expression in the mouse brain, using chromogenic *in situ* hybridization (cISH) of over 20,000 genes. To overcome the limitations of a single label chromogenic cISH platform, we have developed a high-throughput multispectral Qdot ISH platform. This new ISH tool provides a mechanism to systematically examine spatial gene expression patterns in mouse and human brain aided by multispectral imaging. Here, we report the use of quantum dots for riboprobe labeling. The intrinsic photostability and tunability of Qdots is critical in providing superior signal-to-noise of the ISH signal and long-term stability, while minimizing photobleaching and allowing re-scanning of images.

Methods:

- Tissue sections were cut fresh frozen at 25µm from C57BL/6 mice and post mortem human brain tissue were fixed, acetylated, and dehydrated prior to *in situ* hybridization.
- Digoxigenin (DIG), 2, 4-Dinitrophenol (DNP), or Fluorescein incorporated riboprobes were then hybridized to either mouse or human brain tissue.
- The hybridized probe is detected *in situ* using a three phased detection approach, which includes an antibody (with conjugated peroxidase) to the hapten of choice, an amplification step (tyramide signal amplification), and finally the conjugation of the Qdot to the targeted gene see figure 1 below.
- All hybridization, detection and imaging steps are carried out automatically in a high-throughput environment using Tecan liquid handling robots and Leica DM 6000 B microscopes.

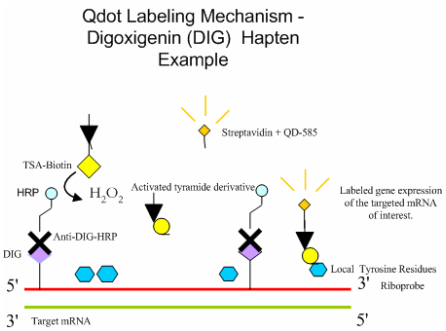

Figure 1. Mechanism for labeling gene expression.

Results:

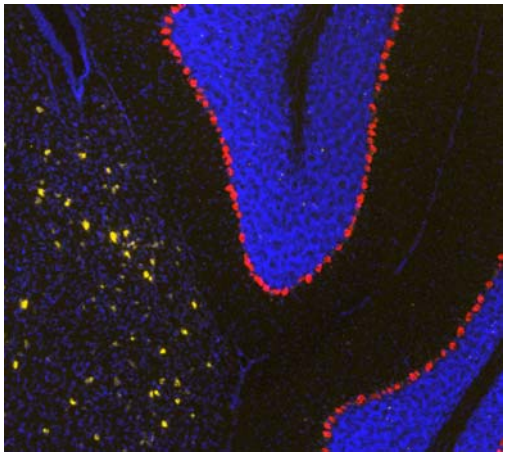

Figure 2: Shown is a 10x image of a C57BL/6 mouse brain. Calb1 expression can be seen labeled in red (QD655) within the Purkinje layer of the cerebellum. Neuropeptide Y (NPY) cortical expression is shown in Gold, (QD585). DAPI (blue) was used as the counter stain for anatomical reference. Each channel was captured using a Leica DM 6000 B in conjunction with a Q-Imaging Retiga 4000 R black and white camera. All images were captured with an exposure time of 100ms and then merged in Adobe Photoshop.

Discussion:

The photostability and tunability of the quantum dot nanocrystals is an ideal technology for examining localized and scattered gene expression *in situ.* We were able to take advantage of the intrinsic properties of the Qdot to examine the spatial relationships between multiple genes in both two and three dimensions[2] (not shown) within a single tissue section. Combining Qdot technology and high-throughput automation will allow us to quickly and efficiently analyze the unique characteristics of gene families and cell types. The data gathered from these studies should continue to help advance the field of neuroscience and disease research.

References:

[1] www.brainatlas.org
[2] Sinclair M.B., et. al., (August, 2006). *Hyperspectral confocal microscope.* Applied Optics; (45), 24, 6283 – 6291, 2006.