Quantum dot (Qdot) labeling of gene expression in fresh frozen brain tissue using high-throu[redacted] hybridization.

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Introduction

The Allen Brain Atlas 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 cISH platform, we have developed a high-throughput Qdot ISH platform. This new ISH tool provides a mechanism to systematically examine spatial gene expression patterns in mouse and human brain tissue 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 are cut fresh frozen at 25µm from C57BL6/J mice and post mortem human brain tissue.
- Once sectioned, they are then fixed, acetylated, and dehydrated prior to *ISH*.
- The sectioned tissue is placed in a micro-fluidic hybridization chamber and placed within a temperature controlled chamber on the deck of a Tecan EVO or RSP.
- The entire ISH process takes nearly 23 hours, all of which is accomplished in a scaled up high-throughput environment.
- Prior to hybridization, the tissue undergoes a gentle digestion with Proteinase K (PK), which allows larger molecules to penetrate through the cell membrane and bind with their target molecules.
- Digoxigenin (DIG), 2, 4-Dinitrophenol (DNP), or Fluorescein incorporated riboprobes are then hybridized to either mouse or human 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 binding of the Qdot to the targeted gene (see figure 1 below).

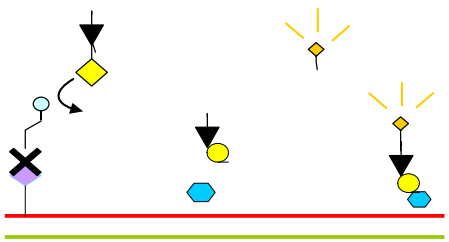

Figure 1. Labeling mechanism for gene expression *in situ*.

Results:

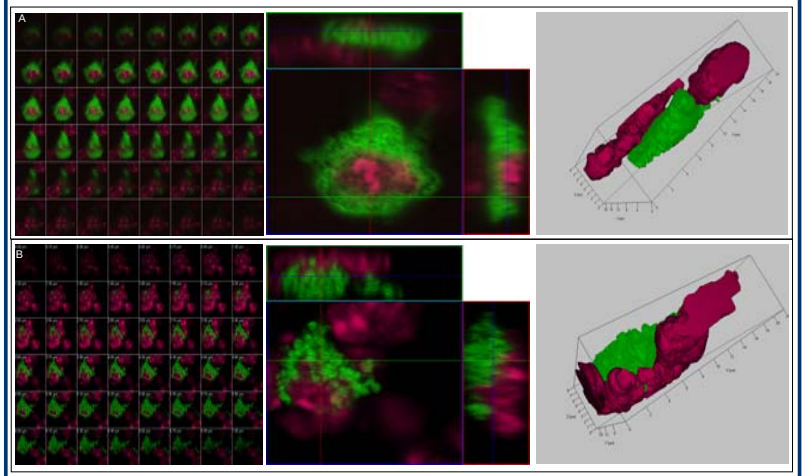

Figure 2: Here we show comparative cellular labeling of a single cell (green) expressing Neuropeptide Y (NPY) in a C57BL6/J adult mouse brain using confocal and 3-D analysis. Adjacent mouse brain sections were labeled with either A. Alexa Fluor 488 or B. Qdot 525 for the purpose of demonstrating depth of penetration. Analysis of these data demonstrates that although the Qdot is 4 – 5x greater in diameter to traditionally small Alexa Fluor dyes, we can still achieve the same effective depth of penetration in fresh frozen and subsequently fixed tissue.

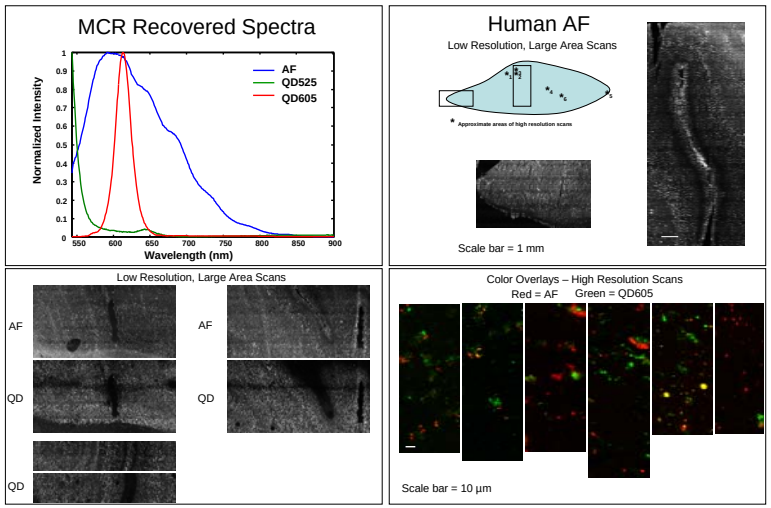

Figure 3: Shown are both high and low resolution 2-D hyperspectral images of Growth Associated Protein 43 (GAP-43) in the primary visual cortex of the human brain. Hyperspectral analysis provides a mechanism for spectrally separating endogenous autofluorescence (lipofuscin) routinely observed in postmortem human brain tissue.

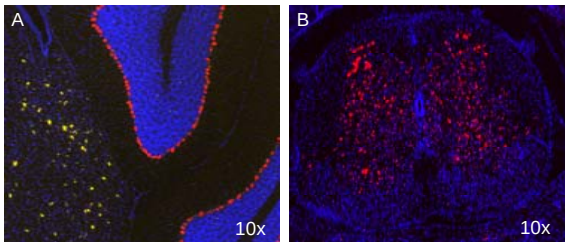

Figure 4: A. Shown is a multi-labeled Qdot image of Neuropeptide Y (NPY) and Calbindin 1 (Calb1) in a C57BL6/J mouse brain. Calb1 expression can be seen labeled in red (QD655) within the Purkinje layer of the cerebellum. NPY cortical expression is shown in Gold, (QD585). B. Mesoderm Specific Transcript Homolog (mouse) (Mest) expression in the spinal cord of a P4 C57BL6/J mouse strain, labeled with QD 655. 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 nanocrystal is an ideal technology for examining localized and sparse gene expression *in situ*. We were able to take advantage of the intrinsic properties of the Qdot to examine the spatial relationships between cells in both two and three dimensions within a single tissue section. Combining Qdot technology and high-throughput automation allows us to quickly and efficiently analyze the unique characteristics of gene families and cell types. This is a prerequisite to understand the molecular mechanisms of brain function and disease.

Summary

We have shown here that it is possible to detect gene expression not only in mouse and human brain tissue, but have also demonstrated that it can be adopted to other tissue types such as spinal cord. By adding a blocking reagent to the tissue between each detection step we are able to label and detect multiple genes on a single tissue section with unprecedented signal-to-noise. Here we have demonstrated a double label, but a triple and quadruple label are also achievable due to the spectral properties of the Qdot nanocrystals.

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