



Quantum dot (Qdot) labeling of gene expression in fresh frozen brain tissue using high-throughput *in situ* 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 multiprobe labeling. The intrinsic photostability and tunability of the Qdot nanocrystal 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 streptavidin conjugated Qdot to the biotinylated tyramide (see figure 1 below).
- For sequential labeling, this last step is repeated using an alternative TSA conjugation; prior to replication of the previous steps, a series of blocking steps, which include: avidin/biotin and a peroxidase inhibitor are added to ensure no cross-talk between the two targeted genes (example shown in Fig. 4A).
- Finally, the tissue is counterstained with either DAPI (a blue nuclear counterstain) or Qnuclear™ Deep Red stain (a near infrared nuclear counterstain) and coverslipped prior to scanning.

Qdot Labeling Mechanism - Digoxigenin (DIG) Hapten Example

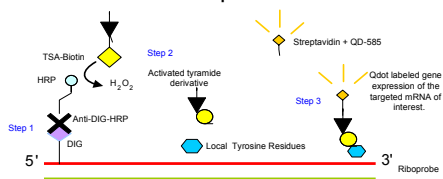


Figure 1. Labeling mechanism for gene expression *in situ*.

Results:

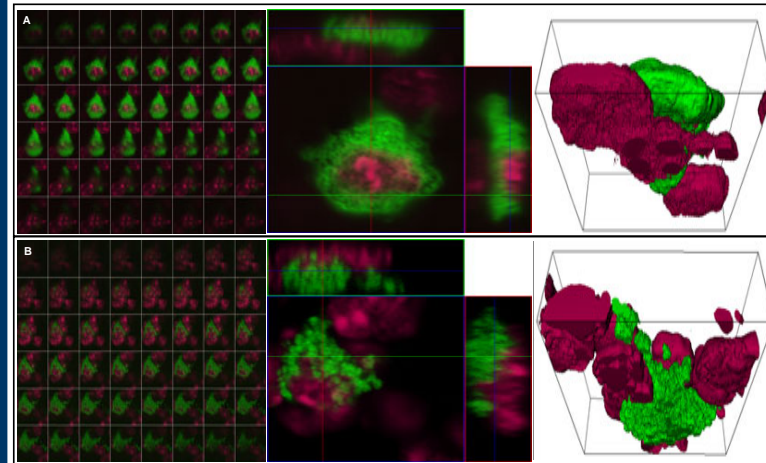


Figure 2: Adjacent brain sections from C57BL6/J adult mice expressing Neuropeptide Y (NPY) were labeled (green) with either A. Alexa Fluor 488 streptavidin conjugate or B. Qdot 525 streptavidin conjugate for the purpose of comparing depth of penetration (see methods). The nuclear dye, Qnuclear™ Deep Red stain (magenta), was used for spatial reference. Laser-scanning confocal microscopy was used to generate 3-D image reconstructions showing expression of NPY throughout the brain sections. Comparative analysis demonstrated that although the Qdot conjugate is 4 – 5x greater in diameter than the Alexa Fluor conjugate, the same effective depth of penetration was achieved. Additionally, brightfield and epifluorescence images were acquired to confirm the total thickness of the section and relative depth of penetration observed in confocal images. The tissue thickness and the label penetration was measured to be approximately 18µm for both Alexa Fluor and Qdot conjugates (data not shown).

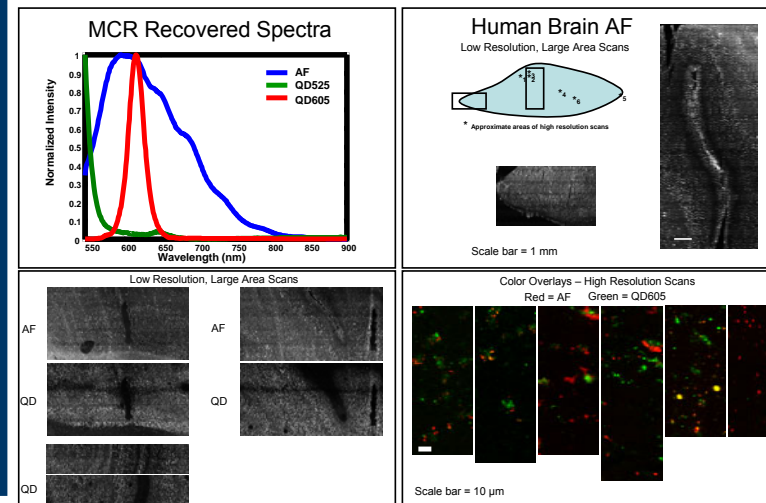


Figure 3: Shown are both high and low resolution 2-D hyperspectral images of Growth Associated Protein 43 (GAP-43) in the primary visual cortex of the human brain. Hyperspectral analysis provides a mechanism for spectrally separating endogenous autofluorescence (lipofuscin) routinely observed in postmortem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

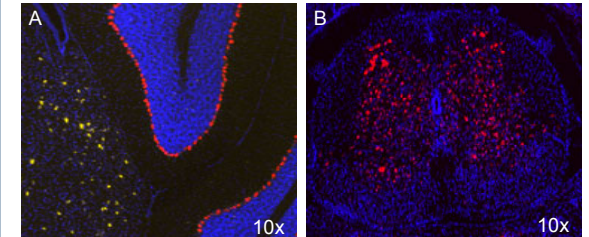


Figure 4: A. Shown is a multi-labeled Qdot image of Neuropeptide Y (NPY) and Calbindin 1 (Calb1) in a C57BL6/J mouse brain. Calb1 expression can be seen labeled in red (streptavidin-QD655) within the Purkinje layer of the cerebellum. NPY cortical expression is shown in Gold, (streptavidin-QD585). B. Mesoderm Specific Transcript Homolog (mouse) (Mest) expression in the spinal cord of a P4 C57BL6/J mouse strain, labeled with streptavidin conjugated QD 655. DAPI (blue) was used as the counter stain for anatomical reference.

Discussion

The photostability and narrow emission spectra 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 nanocrystal and subsequent multiplexing 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 an unprecedented signal-to-noise ratio. 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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