FLUORESCENT IMAGING: A NEUROTECHNIQUES JOURNEY

Erin L. Anderson,[1] Jeffery L. Twiss,[2] and Jill Turner[1]

[1]Department of Drug Discovery and Biomedical Sciences,
[2]Department of Biological Sciences,
College of Pharmacy at the University of South Carolina, Columbia, SC 29208, USA

UNIVERSITY OF SOUTH CAROLINA
College of Pharmacy

ABSTRACT

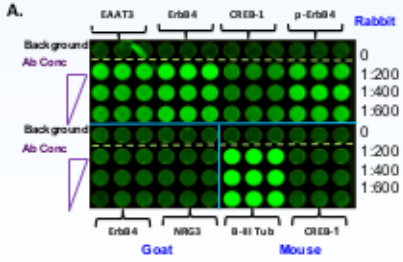
Many fluorescent techniques exist for the evaluation of protein and RNA levels. In neuroscience, analysis may need to occur on either a synapse or system level, making the nuances of detection especially critical. In this vein, three techniques were assessed for their application to the cellular mechanisms of nicotine dependence and withdrawal. First, primary neuronal culture was established to detect and quantify protein in a non-lytic cellular context via an In-Cell Western (in-plate) technique. Next, protein levels were analyzed in brain slices at a macromolecular level using far red (FR) and near IR (NIR) fluorescent probes, a technique that could speed assessment of tissue and save valuable microscope time. Finally, fluorescence in situ hybridization (FISH) to detect mRNA and/or lncRNA in brain slices was evaluated. We find that each approach possesses both weaknesses and merits, making informed choices regarding approach essential.

METHOD I.

Figure 1. In-Cell Western (ICW) with Primary Neuronal Culture

Rat cortex was isolated, dissociated and the cells plated (Fig. A) in NbActiv1 medium. The cortical neurons (Fig. B photo by BrainBits, LLC) were grown for 14 days in vitro (DIV) with AraC.

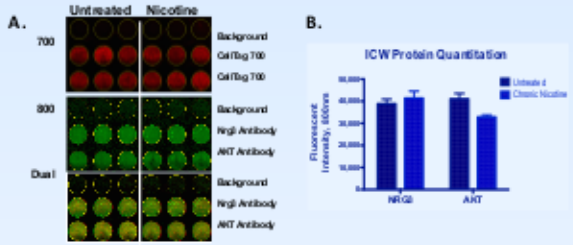
Figure 2. In-Cell Western (ICW) Primary Culture Antibody Validation



Antibodies validated for Western blot (WB) and IHC may not translate directly to ICW. For example, the ErbB4 antibodies were both valid for WB and IHC but only the rabbit ErbB4 antibody validated in ICW (Fig. A).

Figure 3. Chronic Nicotine Treatment Affects Protein Levels in the NRG3 Pathway

(Fig. A) An ICW assay on rat cortical neurons treated with chronic nicotine (1uM for 18 hours) probed with either NRG3 or AKT antibodies. The 700nm red channel contains CellTag 700, a non-specific cell stain. The 800nm green channel contains the antibodies to the proteins of interest. Protein quantitation of the ICW assay (Fig. B).

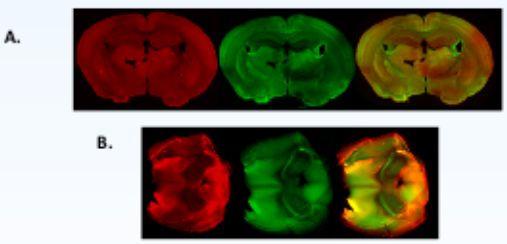


- **Advantage**: combined specificity and dual channel detectability
- **Advantage:** rapid data collection (multi-well format for multiple treatments read simultaneously)
- **Advantage:** high reproducibility
- **Disadvantage:** time and cost of primary culture generation

METHOD II.

Figure 4. In-Cell Western (ICW) for Macromolecular Screening

An ICW assay on coronal mouse brain slice (Fig. A slice 35um) or horizontal sections (Fig. B slice 40um) and treated with beta III tubulin (red 700nm channel) and ErbB4 (green 800 nm channel).

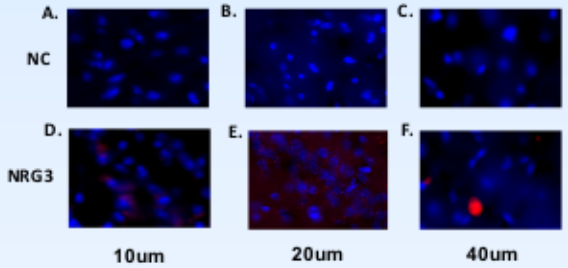


- **Advantage:** retention of native proteins with any post translational modifications in situ
- **Advantage:** rapid scan of multiple slides to identify regions of interest
- **Disadvantages:** lack of microscopic resolution and antibodies in FR/NIR that are non-standard for many wide-field microscopes

METHOD III.

Figure 5. Single Molecule Fluorescent In Situ Hybridization (smRNA FISH) Using Stellaris RNA Probes for mRNA Detection in Brain Slices

Mouse brain slices of varied thickness (10, 20 or 40um) were fixed, permeabilized, and hybridized with fluorescently tagged probes complimentary to 20bp regions along the length of NRG3 mRNA. The negative control (Figs. A, B, C non-specific probe) and DAPI stain (blue) shows very low background compared to the NRG3 Transcript 1 mRNA probe in red (Figs. D, E, F Quasar 670nm dye). The 10um slices showed the lowest non-specific binding and best resolution.



- **Advantage:** allows for identification and localization of single molecule mRNA in situ allowing for observations of cell-to-cell variability
- **Advantage:** allows for multiplexing
- **Advantage:** removes bias that could be introduced during isolation and amplification steps
- **Disadvantage:** requires costly probes and the method does not lend itself to the higher throughput available with RT-PCR

CONCLUSION

Overall, all of the methods possess both advantages and disadvantages and the choice of experimental approach will likely depend upon the particular question involved. For example, rapid screen of compound libraries would be best queried using the ICW with primary cells and a secondary screen in more complex systems. However, those questions requiring intact circuitry (ie, questions regarding plasticity), would better be utilized in Methods II and III.

ACKNOWLEDGEMENTS

This work was supported by grant funds from NIH/NIDA R00-DA-032681 and PhRMA Foundation.

I would also like to thank the Twiss lab, principally Ms. Liz Thames, Ms. Sharmini Miller-Randolph, Dr. Amar Kar, Dr. Pabitra K. Sahoo and Dr. Jeffery L. Twiss, for their time, technical expertise, ideas and support.