DIFFUSION PROPERTIES OF LIPOPHILIC DYES WITHIN AND BETWEEN ADJACENT CELL MEMBRANES

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INTRODUCTION

- Loss-of-function (knockout) or other mouse mutants exist or are being generated for all 27,000 + genes of the mouse genome (NIH initiative).
- Studying brain development in those mutants will provide unsurpassed insights into how the mammalian brain develops. We are particularly interested in the development of hearing.
- Studies will require effective use of multicolor labeling systems to minimize the costly breeding of double (1:16) and triple (1:64) null mutants.
- DiI (Godement et al., 1987; www.probes.com) is the most commonly used lipophilic neurotracing dye (over 1600 citations in PubMed as of 5/06), but options for 2 and 3 color labeling in fixed tissue have until recently been very limited (Maklad & Fritzsch, 2003).
- In this Poster we present a series of new lipophilic neurotracers available in coated filter format (www.ptiresearch.com) that readily allow up to 5 color analyses of neuronal development.

Double Labeling Helps Establish Relative Topology

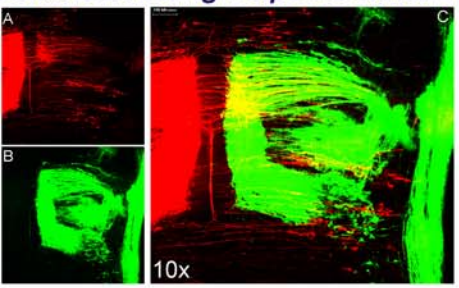

Figure1: Double labeling can be used to study overlapping cell and fiber distribution in a embryonic mouse hindbrain. Using the red-emitting DiI and the near infrared NeuroVue Maroon (green) allows imaging to the single cell level and fully segregated labeling of two channels (A,B). Combining the single channels shows that only the DiI labeled inner ear efferent cells are in the contralateral brain and that only their fibers cross the floor plate(C). NeuroVue Maroon labeled whole facial motoneurons by injection in the facial nerve are only visible ipsilateral to the labeled side.

Fluorescence Spectra of eGFP and NeuroVue (NV)Dyes

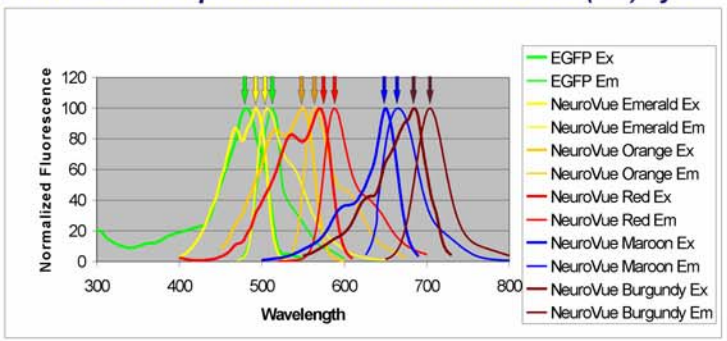

Figure2: Fluoresence excitation and emission profiles of five dyes and EGFP. These 6 different fluorescence signals allow spectral detection with color unmixing at laser lines 488, 543, 633nm in single photon mode. Peak excitation and emission for each dye and eGFP is indicated by arrows. Note that overlapping spectra require linear unmixing for complete segregation.

Triple Color Labeling of Cortical Afferents

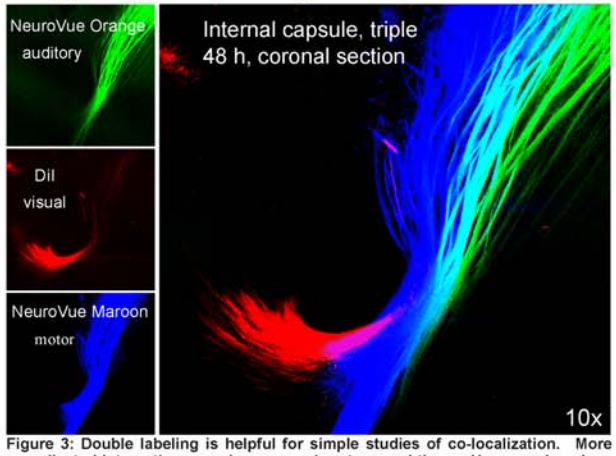

Figure 3: Double labeling is helpful for simple studies of co-localization. More complicated interactions require more colors to reveal them. Here we show how three different colored lipophilic tracers can be used to simultaneously reveal the distribution of corticofugal fibers in the internal capsule. Lipophilic tracers were applied to points 72hours prior to sectioning.

Combined neurotracing and eGFP expression

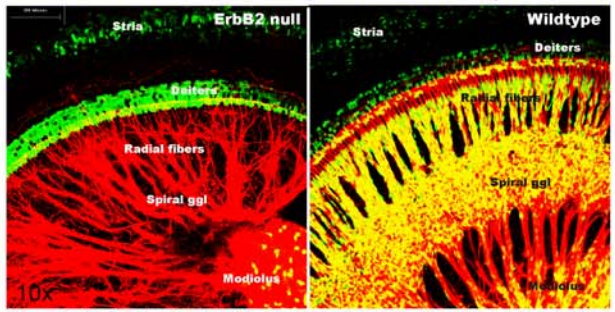

Figure 4: NV Red tracing after injection into the cochlear nuclei can be combined with gene expression using eGFP. Here we show the effect of a null mutation of ErbB2 on the presence of Schwann cells (green labeled cells near spiral ganglion) that carry the PLP-eGFP transgene. Note that in ErbB2 null mice all Schwann cells have disappeared and no eGFP positive cells are near the radial fibers. PLP-eGFP expression in the supporting cells of the organ of Corti (Deiters) is independent of ErbB2. Note that tracer labeled fibers are not targeted properly. Morris et al., 2006 in press.

Linear Color Unmixing Studies

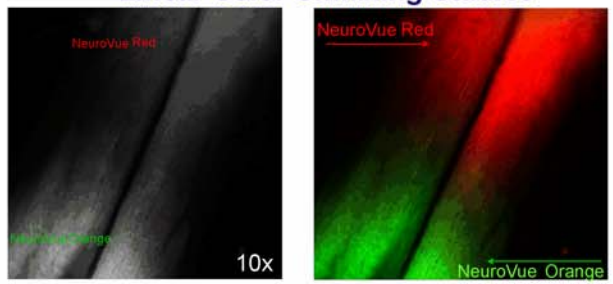

Figure 5: Filters alone cannot fully segregate emission of NV Orange from NV Red or of NV Maroon from NV Burgundy after injection into intercostal nerves (left panel). Using linear color unmixing on the Zeiss LSM 510 META confocal system allows complete segregation of NV Orange from NV Red (right panel) and NV Maroon from NV Burgundy (Fig. 6). The unmixed colors can be combined to show the relative distribution of dyes (right panel).

6 Dye Tracer Studies with Color Unmixing

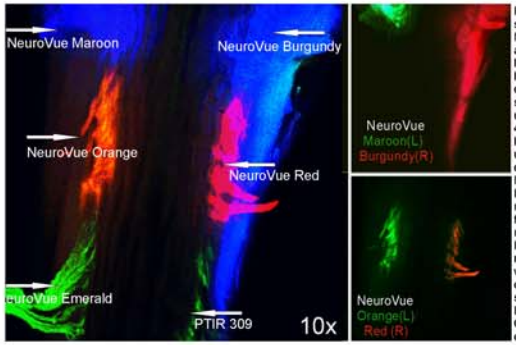

Figure 6: Good segregation of all 5 NV dyes can be achieved after parallel labeling with dye labeled filters at different intercostal sites (left panel), using excitation with 488/543/633nm laser lines and color unmixing to distinguish NV Maroon from NV Burgundy (upper right) and NV Orange from NV Red (lower right). A sixth dye, PTIR309 (ex. max. 513 nm, em. max. 533 nm) was also tested but could not be fully segregated from NV Emerald or NV Orange even using color unmixing.

Take Home Message: *Novel fluorescent lipophilic tracers allowing simultaneous labeling with up to 5 different neuronal tract tracers thus enhancing co-localization analysis.*

Lipophilic Tracers May Detect Contact Formation

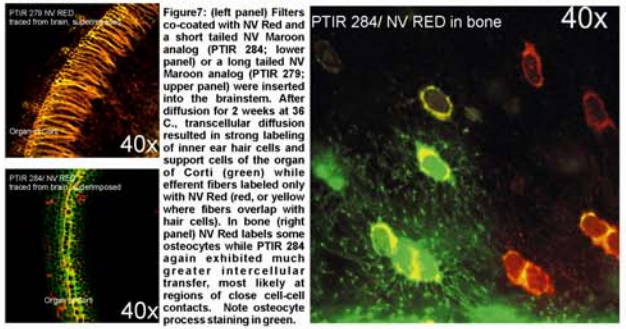

Figure7: (left panel) Filters co-coated with NV Red and a short tailed NV Maroon analog (PTIR 284; lower panel) or a long tailed NV Maroon analog (PTIR 279; upper panel) were inserted into the brainstem. After diffusion for 2 weeks at 36 C., transcellular diffusion resulted in strong labeling of inner ear hair cells and support cells of the organ of Corti (green) while efferent fibers labeled only with NV Red (red, or yellow where fibers overlap with hair cells). In bone (right panel) NV Red labels some osteocytes while PTIR 284 again exhibited much greater intercellular transfer, most likely at regions of close cell-cell contacts. Note osteocyte process staining in green.

Take Home Message: *Transcellular diffusion may be helpful in studying onset of connection formation or existing contacts in difficult to access tissue (ear and bone).*

SUMMARY AND CONCLUSIONS

- Multicolor neurotracing studies can detail developing connections in null mutants that are difficult to address using other methods.
- Up to 5 dyes of different spectral properties can be used simultaneously with each other and/or EFGP for multicolor neurotracing studies.
- Transcellular diffusion, long regarded as an undesirable artifact, may be useful for the study of connection formation or existing contacts in hard-to-access tissue such as bone or heart.

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For details of staining and imaging methods, see Fritzsch et al Brain Res Bull 66 (2005) 249-258.