**Reply to Reviewers' comments:**

**Reviewer #1:**

*Summary:*

The authors outline a technique that has been very influential in pushing forward our understanding of nervous system structure, organization and development, namely the manual labeling of neurons with DiI. They rightly assert the importance and continued usefulness of this method.

The execution is clearly presented in a way that should allow novices to get set up and started. The supporting figures are absolutely wonderful - very clear and visually extremely pleasing.

*Minor Concerns:*

I would like the authors to consider the following, which I think might improve this manuscript further.

Page 6: 3) Filling of injection micropipettes: it might be helpful to point out that 'dry' absolute EtOH is needed, prepared by using 'molecular sieve' or similar for absorption of any residual humidity. 99.6% EtOH stocks can be sufficiently humid to cause DiI precipitation, which should be avoided at all cost.

**Reply**: a note has been added accordingly.

Page 6: Equipment: the objectives required are 'dipping' objectives, i.e. do not require a cover glass between lens and specimen, as 'immersion' objectives would.

**Reply**: We point out in the text: 'This objective should be designed for use without a coverslip'

On filter sets, it might help to provide notes on the excitation and emission maxima of DiI and a range of filter sets that would do the trick: e.g. sets suitable for Cy3 imaging, but also for Texas Red, Rhodamine or Alexa 568 / CF568 fluorophores (i.e. DiI has a pretty broad spectrum).

**Reply**: We added the maxima of DiI and several filter sets that will work beside the Cy3 set.

On the foot switch: perhaps to state that this can be helpful, but I doubt it is necessary. In my view, to facilitate use of this method, everything should be done to simplify as much as possible, especially on the equipment side, which will be pretty specific and expensive for most labs. In other words, it would help to clearly differentiate between the essential items (and why these are essential), perhaps suggest cheaper alternatives, and the useful add-ons.

**Reply**: A sentence was added that makes clear that we performed the experiment also without electric shutter. Regarding the other items in this chapter we think they are all necessary.

Step 4.9: Add that after application of DiI specimens should be kept in a humidified chamber protected from light to avoid bleaching.

**Reply**: A corresponding statement was added.

On DAB conversion:

Here it would be good to point out possible pitfalls; e.g. is the quality of DAB important? Do specimens need pre-incubation with DAB prior to photoconversion? Will objectives be damaged by a DAB precipitate? What about toxicity of DAB and its disposal? How does fluorescence loss during the procedure relate to gain of DAB precipitate?

**Reply**: A new paragraph addressing these points has been added to the chapter on „Photoconversion“.

Table of reagents:

Most of the "labels" may need changing to "labeling" or similar - please check.

**Reply**: 'labels' were changed to 'labeling'

**Reviewer #2:**

*Summary:*

This is an important technique with excellent cellular resolution that has been used successfully over many years and continues to be important currently as shown by several excellent recent publications from the Technau lab.

*Minor Concerns:*

The authors might want to include a reference to the most recent paper from the Technau lab that uses this technique (i.e. Kunz et al., 2012).

**Reply**: We added the reference.

In part 6.1 a step 4.10 is mentioned, but this step does not exist.

**Reply**: 4.10 was corrected to be 4.9

Figures 5C and 5D are mixed up.

**Reply**: We uploaded the correct Figure.