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***Film Date:***

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## Title: Cell Tracking Using Photoconvertible Proteins During Zebrafish Development

Authors, please fill out the brief questionnaire below.

A. Will you require assistance with video microscopy, such as filming a complex dissection or microinjection technique (Y/N, please specify steps by number. Also, please list make and model of your microscope)? Yes, 2.1, 2.2, 2.3, 2.4, 2.5, 4.1(now 3.6) (I perform these steps under stereomicroscope)

B. Does your protocol include detailed, step-by-step, descriptions of software usage (Y/N, please specify steps by number)? Yes, 3.2, 3.3, 3.4

C. Which steps of your protocol will viewers benefit most from having filmed? Please list 4-6 steps 2.5, 3.2, 3.3, 3.4, 4.1(3.6)

D. What is the single most difficult aspect of this procedure and what do you do to ensure its success?

The more difficult aspect of this procedure is to ensure that embryos survive. For this is important:

i) Use the minimal necessary amount of Diode (405 nm) laser light. We recommend to initiate tests using low laser power (e.g. 3 %, corresponding to 0.03 mW in the objective plan) combined with low numbers of iterations (e.g. 20), and to subsequently increase the laser power until complete photoconversion of the region of interest. (see Manuscript, protocol text, item 3.4, Note 2 for more details)

ii) In the bleaching procedure avoid the tissue near the yolk as it is sensitive to Diode laser (405 nm) pulses and can be lethal. (see Manuscript, protocol text, item 3.4, Note 2 for more details)

# 1. Introduction (Schematic Overview and Interview)

**A. Schematic Overview (read by voice talent at JoVE):**

Authors, please select from "Procedural Narrative" or "Conceptual Narrative" and complete the statements below. Please do not add additional steps. Then, attach your finished graphic overview. See accompanying instructions for details and examples.

***Procedural Narrative:***

The overall goal of this procedure is to track photo-switched cells in a living zebrafish embryo **(Intro).**

This is accomplished by first embedding an embryo expressing a tissue-specific photoconvertible fluorescent protein in low melting temperature agarose **(P1 Editor, place the embryo into the plate).**

Next, the protein within the region of interest is completely photoconverted **(P2 Editor, place the embryo in the box over the 20x lens then bring in the purple arrow and 405 nm).**

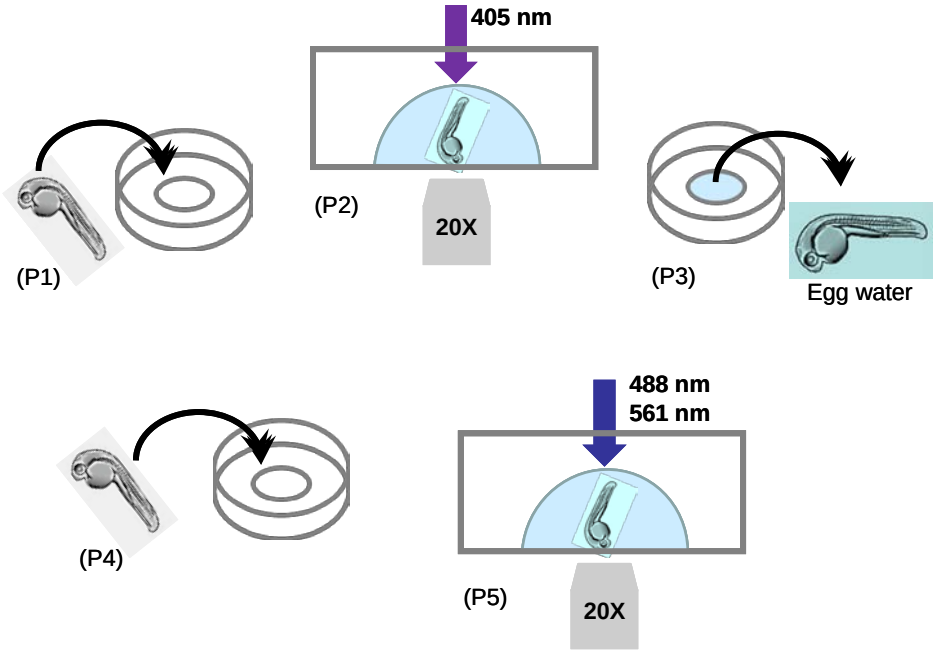
Then the embryo is carefully removed from agarose and maintained it in egg water at 28.5 °C until later stages **(P3 Editor, remove the embryo from the plate as in P3).**

Finally, the photoconverted embryo is again embedded in low melting temperature agarose **(P4 Repeat similar to P1 here).**

Ultimately results can be obtained that show that photoconverted cells can precisely be detected at later stages due to the stable presence of the photoconverted protein through confocal microscopy **(P5 Editor, repeat P2 with P5 and bring in Figure 1~~A and B~~ C here)). {Comment: only the figure 1C (24 hours post-photoconvertion) is ok here}**

*Paste a copy of your graphic overview here. The original file should be **adobe illustrator (preferred) or powerpoint** (see instructions) and should be uploaded through your online submission on the JoVE website.*

*Schematic overview (original file: 4350_Salim Seyfried_Schematic overview.ai):*



**B. Interview: (Said by you on camera. Don't forget to smile!)**

Authors: Below are statements we would like you to complete that are complementary to the information contained within the schematic overview. Only one statement should be chosen and completed per author who will be on camera demonstrating the protocol. In addition to choosing and filling out the appropriate statement, please enter the name of the individual who will say each line. **If individuals will be doing the demonstrations but not speaking in the introduction, please use statement 1.8 to introduce these demonstrators (ex PI introducing students).

1.1)

1.2) Author name ___Salim____ : This method can help answer key questions in the cell and developmental biology_____, such as elucidating tissue dynamics and cell behaviors as they occur during development .

1.3) **Author name __Salim_____: Demonstrating the procedure will be _Veronica Lombardo_____ a ___post doc _____(technician, post doc, grad student) from my laboratory (Add additional mention of demonstrators as necessary).

**Protocol (read by voice talent at JoVE):**

Authors: In order to ensure that your protocol can be filmed in a single day, the protocol text must be limited to 30 steps – each step being defined as 3 lines of 12 pt text in our formatting style below. This amounts to 3 pages of protocol text. The scope of the scripted protocol text should include only those aspects of the procedure that require visualization in order to be well understood.

***2.*** **Embedding Embryos for Photo Conversion Assay**

2.1. After in crossing transgenic zebrafish expressing a photoconvertible fluorescent protein, choose the brightest embryos which are homozygote and grow at 28.5°C.

2.1.1. WIDE Talent removes embryos from incubator; B need shot of talent placing embryo into incubator (after photoconversion) for 3.7.1 below

2.1.2. SCOPE Talent checks embryos for homozygotes

2.2. When they've reached the desired stage, use forceps to dechorionate them in a glass container with egg water.

2.2.1. SCOPE Talent removes chorion with forceps in egg water {Comment: 2 Takes with different size recorded}

2.3. To anesthetize the embryos, use a glass pipette or cut pipette tip to transfer them one at a time into Tricaine (TEXT: 0.16 mg/mL in E3).

2.3.1. SCOPE Talent picks up an embryo (SCOPE TAPE TC: 4:17)

2.3.2. MED/CU Talent transfers it into tricaine

2.4. Transfer an anesthetized embryo onto the cover of a culture dish and maintain the embryo within the smallest possible volume of medium.

2.4.1. ~~SCOPE Talent picks up embryo from tricaine~~ {Comment: This shot was not filmed}

2.4.2. CU Talent transfers it to the cover of a culture dish
2.4.3. SCOPE Talent removes excess medium (SCOPE TAPE TC: 5:14-5:55)

2.5. Transfer the embryo into agarose by pipetting it with 1% low melting temperature agarose with Tricaine at 30°C, and place it into a cover-glass-bottomed culture dish. Use a smooth plastic tip to orient the embryo.
2.5.1. SCOPE Talent pipettes embryo with 1% low melting temperature agarose with Tricaine (SCOPE TAPE TC: 5:55-6:40)
2.5.2. CU Talent places embryo into cover-glass bottomed culture dish
2.5.3. SCOPE Talent uses smooth plastic tip to orient embryos (SCOPE TAPE TC: 6:40-7:34)

2.6. When the agarose has polymerized, cover it with Tricaine solution.
2.6.1. CU Talent adds tricaine solution to polymerized agarose

## *3.* Photoconversion and Embryonic Development and Tracking Photoconverted Tissue

3.1. On an inverted confocal microscope equipped with 405, 488 and 561 nm laser sources and a 20x objective, use the 488 and 561 nm lasers (TEXT: see written protocol for more details) to generate a z stack of the entire structure of the specimen, which includes the region of interest.
3.1.1. Broll of microscope showing the lasers and objective
3.1.2. MED OVER SHOULDER Talent scans the region of interest to generate a z stack
3.1.3. SCREEN of z-stacks of the sample before photoconversion {Comment: this screen was added}

3.2. Select the *Time series* tool and set for 2 cycles with no interval, one for pre- and one for post photoconversion.
3.2.1. SCREEN Talent selects time series tool and sets for 2 cycles with no interval

3.3. Select the *Regions* tool and define one or more regions of interest for photoconversion.
3.3.1. SCREEN Talent selects the regions tool and defines regions of interest for photoconversion

3.4. Next, select the *Bleaching* tool and set for start bleaching after scan 1 of 2. Used here is a 30 mW (10% power) 405 nm diode laser carefully optimized for the minimal amount of laser light necessary for complete photoconversion which is achieved by varying the intensity of the laser light, the scan iterations of the region of interest, and the scan speed. ~~Take care to avoid the tissue surrounding the yolk as it is sensitive to laser pulses and can be lethal.~~ {Comment: This sentence is confusing here because in this case (photoconversion of head vessels) the yolk was not imaged. This is a general advice and it is described in the manuscript, protocol text, item 3.4, Note 2}
3.4.1. SCREEN Talent selects bleaching tool and sets for start bleaching after scan 1 of 2
3.4.2. SCREEN Bleaching is seen on the screen -
3.4.3. ~~SCREEN Brightfield scan showing where the yolk is - Videographer, have talent point out the yolk (Authors: perhaps this can be overlaid with the fluorescence?)~~ {Comment: This

screen was not necessary because in this case (photoconversion of head vessels) the yolk was not imaged}

3.5. Scan the sample with the 488 nm and 561 nm lasers to visualize any remaining green and the photoconverted red fluorescence of the photoconvertible fluorescent protein.

3.5.1. MED OVER SHOULDER Talent scans the sample with 488 and 561 lasers to see remaining green and photoconverted red

3.5.2. SCREEN of z-stacks of the sample after photoconversion {Comment: this screen was added}

3.6. After photoconversion, discard the solution and using a needle or smooth plastic tip, carefully remove the embryo from the agarose.

3.6.0. MED: Talent discards the fluid {Comment: this shot was added}

3.6.1. SCOPE Talent removes solution and removes embryo from agarose (SCOPE TAPE TC: 7:30)

3.7. Maintain the embryo in egg water in the dark at 28.5°C until the desired developmental stage.

3.7.1. WIDE Talent places embryo into incubator

3.8. Embed the embryo a second time then generate a z stack of the specimen, including the region of interest, again using the 488 and 561 nm lasers. Because this protocol does not affect normal development, it is possible to observe the photoconverted embryo at later stages.

3.8.1. SCREEN Talent scans the region of interest a second time after development

## *4.* Representative Photoconversion Results-(second to last section)

4.1. Here is an example of a photoconversion assay. Endothelial cells were tracked during zebrafish development, from 48 to 72 hours post fertilization, using a transgenic reporter line expressing KikGR (Pronounce "Kik G-R") within endothelial nuclei. As shown here, before photoconversion KikGR (Pronounce "Kik G-R") was expressed within endothelial tissue and was only detectable as green fluorescence using a 488 nm argon laser. Control scans with a 561 nm DPSS 561-10 laser for red fluorescence did not detect any photoconverted KikGR (Pronounce "Kik G-R") prior to the photoconversion.

4.1.1. LAB MEDIA Figure 1A

4.2. Subsequently, the region of interest was completely photoconverted using a 405 nm Diode laser, at 10% intensity, 20 iterations, and the head vessels were scanned again using the 488 nm and 561 nm lasers. In this panel, green KikGR (Pronounce "Kik G-R") had completely switched to red within the region of interest.

4.2.1. LAB MEDIA Figure 1B

4.3. To track the photoconverted endothelial cells at later stages, the embryo was observed at 24 hours post photoconversion, which is at ~72 hours post fertilization. Within endothelial tissue arising from the photoconverted region of interest, both non- and photoconverted KikGR (Pronounce "Kik G-R") proteins were observed due to the stability of the red fluorescent

photoconverted KikGR (Pronounce "Kik G-R") and synthesis of new green non-photoconverted KikGR (Pronounce "Kik G-R").

4.3.1. LAB MEDIA Figure 1C

**INSTRUCTIONS FOR AUTHORS:**
Please ensure that the representative results narration is appropriate and correctly describes your images, movies, or figures. Our editors have ensured that the results are written in our format.

We consider this section a critical aspect of the video, because here is where you provide validation for your experiments. For example, if this is a cell culture preparation, this section is where the video will show your cells at various time points following culturing. If this is an imaging prep, then this part is where you will show examples of your imaging experiments.

Please limit the extent of narration to no more than 2-3 lines of text per image or movie file being described. Figures with multiple panels submitted with the original protocol should be broken up so that each panel is a separate image. Like the schematic, each image or movie file supplied in the results should be referenced by annotation in parenthesis, however for the results, the specific filename should be given in parenthesis.

Below is an example of results text:

EXAMPLE REPRESENTATIVE RESULTS

5. Evaluation of Morpholino Injection and Knockdown

5.1 Representative results of both morpholino injection and mRNA injection are shown here. The uninjected control at 48 hours post fertilization looks normal, as expected
-LAB MEDIA: 0123_PIname_Figure1.tif (Replace 0123 with your jove video #)

5.2 However, embryos injected with the morpholino heg_e3i3_egfr1, which knocks down Heg isoforms containing the first of two EGF-like repeats, exhibit brain edema.
-LAB MEDIA: 0123_PIname_Figure2.tif

5.3 Injection of heart of glass mRNA also produced an obvious phenotype. At 24 hours post fertilization, the heads of the uninjected controls look normal
-LAB MEDIA: 0123_PIname_Figure3.tif

5.4 Conversely, some of the embryos injected with the mRNA exhibit cyclopia
-LAB MEDIA: 0123_PIname_Figure4.jpg

**Please visit the following URL to see an example of how the results will look when complete:**
http://www.jove.com/video/1597/results-example-mably?access=ksw0bprj

**5. Conclusion (said by authors on camera)**

Authors: Below are statements we would like you to complete that summarize and conclude the video. Only one statement should be chosen and completed per author who will be on camera demonstrating the protocol. In addition to choosing and filling out the appropriate statement, please enter the name of the individual who will say each line.

5.1 Author name _Veronica_: After its development, this technique paved the way for researchers in the field of _developmental biology_ to explore _cell biology_and tissue

remodelling (subdivision of field, disease, natural phenomenon) in zebrafish ( model organism, patient demographic, organ system).

***Provided Media***

*Authors, Please list all images, movie files, or 3-D rendered animations that can be included in the video per editor's request. The step in the script/video where these images will be inserted should be specified. For example:*

*6.2 – 0123_PIname_Figure1.tif - dual color imaging of tumor angiogenesis at 40X*
*6.2 – 0123_PIname_Figure2.tif - dual color imaging of tumor angiogenesis at 100X*

*Formats: For static images we prefer .tiff files at dimensions of at least 720X480 pixels and 300 dpi. The higher resolution, the better. Likewise any exported movie files should have at minimum these dimensions and be rendered to .mov, .mp4, or .avi files.*

*Insert your media filenames here.*

*1.A – 4350_Salim Seyfried_Schematic overview.ai*
*5.1 - 4350_Salim Seyfried_Figure1A.tif - images of green, red and merge channels of head vessels before photoconversion, scale bars: 50 μm*
*5.2 - 4350_Salim Seyfried_Figure~~2~~1B.tif - images of green, red and merge channels of head vessels after photoconversion, scale bars: 50 μm*
*5.3 - 4350_Salim Seyfried_Figure~~3~~1C.tif - images of green, red and merge channels of head vessels 24 hours post-photoconversion, scale bars: 50 μm*

***General Preparation***

*It's critical for a smooth and organized shoot that all reagents are accounted for, in advance.*

*Any overnight or long incubation steps should be recognized and specimens/samples be prepared in advance so that prior steps can be recorded and shooting can continue with pre-prepared specimens/samples.*

*All tubes/flasks should be pre-labeled neatly before we arrive.*

*Ex. Luciferase assay done in 96 well plates should be labeled with negative/positive control wells and experimental samples are labeled accordingly.*