Submission ID #: 61713
Scriptwriter Name: Bridget Colvin
Project Page Link: https://www.jove.com/account/file-uploader?src=18822513

Title: Simultaneous Live Imaging of Multiple Insect Embryos in Sample Chamber-Based Light Sheet Fluorescence Microscopes

Authors and Affiliations: Julia Ratke[1,*], Franziska Krämer[1,*], and Frederic Strobl[1]
*These authors contributed equally

[1]Physical Biology / Physikalische Biologie (IZN, FB15), Buchmann Institute for Molecular Life Sciences (BMLS), Cluster of Excellence Frankfurt, Macromolecular Complexes (CEF – MC)
Goethe-Universität, Frankfurt am Main (Campus Riedberg)

Corresponding Author:
Frederic Strobl
frederic.strobl@physikalischebiologie.de

Co-Authors:
julia.ratke@physikalischebiologie.de
franziska.kraemer@physikalischebiologie.de

© 2020, Journal of Visualized Experiments

Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or similar? **Y**

   From the authors: Our microscopy camera window on the right side is tilted by 90°. Either the right part can be rotated back (and shrunk in size to fit the other window) or it would maybe be a good idea to add floating text to the first time a screen capture is shown in the JoVE video that reads: "Note: camera window is tilted by 90° in the author's microscope user interface" or similar.

2. **Software:** Does the part of your protocol being filmed demonstrate software usage? **Y**

Authors recorded SCREEN capture videos with low frame rate and ask that all screen capture files be sped up x2-3

3. **Interview statements:** Considering the Covid-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interviewees wear masks until the videographer steps away (≥6 ft/2 m) and begins filming. The interviewee then removes the mask for line delivery only. When the shot is acquired, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. **Filming location:** Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Protocol Length
Number of Shots: **49**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Julia Ratke**: Sample chamber-based light sheet fluorescence microscopes are designed for high content rather than high throughput. Live imaging assays must therefore be performed sequentially and are thus affected by ambient variance **[1]**.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

REQUIRED:

1.2. **Franziska Krämer**: Our cobweb holder allows stacking and simultaneous imaging of multiple embryos, eliminating ambient variance and increasing throughput **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

OPTIONAL:

1.3. **Frederic Strobl**: Mounting embryos in the cobweb holder requires a certain finesse. An explanatory video is ideal for illustrating the sequence of the many short consecutive gestures **[1]**.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

© 2020, Journal of Visualized Experiments

Protocol

2. Light Sheet Fluorescence Microscope Calibration

2.1. For calibration of the sample chamber-based light sheet fluorescence microscope, first re-liquefy an agarose aliquot in a dry block heater-mixer at 80 degrees Celsius **[1]**.

2.1.1. WIDE: Talent placing aliquot into block heater-mixer

2.2. Allow the melted agarose to cool to 35 degrees Celsius **[1]** and transfer 50 microliters of the agarose to a 1.5-milliliter reaction tube **[2]**.

2.2.1. Talent placing agarose at 35 °C

2.2.2. Talent adding agarose to tube

2.3. Mix 0.5 microliters of fluorescent microsphere solution with the agarose at 1400 revolutions per minute for 1 minute **[1]** and fill the slotted hole of the cobweb holder with 10 microliters of the agarose-fluorescent microsphere solution mixture **[2][3]**.

2.3.1. Solution being mixed, with fluorescent microsphere solution container visible in frame

2.3.2. Hole being filled with solution, with solution container visible in frame

2.3.3. ADDED SHOT: LAB MEDIA: 2.3.2._2.4.1: 00:02-00:17 Authors recommended showing standard shot then showing SCOPE shot with voiceover. I would recommend showing SCOPE shot as inset if possible or just showing SCOPE shot (rather than one then the other) but whatever the video editor thinks looks best is fine

2.4. Aspirate as much agarose as possible until only a thin agarose film remains **[1]** and allow the agarose to solidify for 30-60 seconds **[2]**.

2.4.1. Agarose being aspirated

2.4.2. Shot of solidified agarose

© 2020, Journal of Visualized Experiments

2.5. Fill the sample chamber with autoclaved tap water **[1]** and slowly insert the cobweb holder into the sample chamber **[2]**.

2.5.1. Talent filling chamber with water

2.5.2. Talent inserting holder into chamber

2.6. Move the slotted hole in front of the detection lens and rotate the holder to a 45-degree position relative to the illumination and detection axes **[1]**. The cobweb holder should not be visible in the transmission light channel **[2]**.

2.6.1. Hole moved in front of lens and stage being rotated

2.6.2. SCREEN: 2.6.2.

2.7. Switch to the appropriate fluorescence channel **[1]** and adjust the laser power and exposure time so that the fluorescent microspheres provide a proper signal **[2]**.

2.7.1. Talent selecting channel, with monitor visible in frame

2.7.2. SCREEN: 2.7.2: 00:04-00:20

2.8. Specify a volume of view that covers the now transversely oriented agarose film completely and calculate the maximally possible axial resolution for the respective illumination and detection lens combination to define the z spacing **[1-TXT]**.

2.8.1. SCREEN: 2.8.1. **TEXT: Alternative: Use 4× lateral resolution as rough approximation** From authors: We try to stick to the multiplication symbol instead of the small „x“.

2.9. Then record a three-dimensional test z stack of the fluorescent microspheres **[1]** and compare the x, y and z maximum projections to the calibration chart **[2]**.

2.9.1. SCREEN: 2.9.1: 00:04-01:15

2.9.2. LAB MEDIA: Figure 2 From authors: It would be great if the A, B and C subfigures could fade in and out one after another. Else it might be difficult to see the rather tiny microspheres properly.

© 2020, Journal of Visualized Experiments

2.10. If the microspheres appear blurry, fuzzy, and/or distorted, adjust the positions of the illumination and/or detection lenses **[1]**.

2.10.1. LAB MEDIA: Figure 2 *Video Editor: please sequentially emphasize Figures 2A, 2B, and 2C*

3. Sodium Hypochlorite-Based Dechorionation

3.1. For embryo dechorionation, add 8 milliliters of autoclaved tap water into the A1, A2, A3, and B3 wells of one 6-well plate per line **[1]**. Then add 7 milliliters of autoclaved tap water and 1 milliliter of sodium hypochlorite solution into the B1 and B2 wells **[2]**.

3.1.1. WIDE: Talent adding water to well(s)

3.1.2. Talent adding NaOCl to well(s), with NaOCl container visible in frame

3.2. Position the first plate under a stereomicroscope **[1]** and insert the first embryo-containing cell strainer into the A1 well **[2-TXT]**.

3.2.1. Talent placing plate under microscope

3.2.2. Talent placing strainer into well **TEXT: See text for embryo collection details**

3.3. Wash the embryos under gentle agitation for 30-60 seconds **[1]** before moving the strainer to the B1 well **[2]**.

3.3.1. LAB MEDIA: 3.3.1. OR Videographer captured footage

3.3.2. Strainer being moved to well

3.4. Shake the plate vigorously for 30 seconds **[1]** before transferring the strainer to the A2 well **[2]**.

3.4.1. Talent shaking plate

3.4.2. Strainer being placed into A2 well

© 2020, Journal of Visualized Experiments

3.5. Wash the embryos for 1 minute under gentle agitation **[1]** and move the strainer into the B2 well for vigorous shaking until most of the embryos are completely dechorionated **[2]**.

3.5.1. LAB MEDIA: 3.5.1 OR Videographer captured footage

3.5.2. LAB MEDIA: 3.5.2: 00:00-00:12 From authors: Would it be possible to add a red circle or arrow to this SCOPE video pointing at detached chorion fragments as indicated in "61713-SCOPE-3.5.2_Suggestion.png"?

3.6. Then transfer the strainer to the A3 well for 1 minute of gentle washing **[1]** before storing the strainer of dechorionated embryos in the B3 well until the embryos of other lines have been treated as demonstrated **[2-TXT]**.

3.6.1. LAB MEDIA: 3.6.1.: 00:00-00:09

3.6.2. Talent placing strainer into A3 well **TEXT: Repeat dechorionation for each line of interest**

4. Embryo Mounting

4.1. To mount the embryos onto the cobweb holder, re-liquefy another aliquot of agarose as demonstrated **[1]**.

4.1.1. WIDE: Talent adding aliquot to 80 °C-block

4.2. When the agarose has cooled, place the cobweb holder under the stereo microscope **[1]** and add 10 microliters of the agarose onto the slotted hole **[2]**.

4.2.1. Talent placing holder onto bench

4.2.2. Talent adding agarose to holder

4.3. Use the pipette tip to spread the agarose over the slotted hole **[1]** before aspirating as much agarose as possible until only a thin agarose film remains **[2]**.

4.3.1. Agarose being spread

4.3.2. Agarose being aspirated

© 2020, Journal of Visualized Experiments

4.4. When the agarose has solidified, use a paintbrush to carefully pick **[1]** and transfer the embryos onto the agarose film **[2]**. Arrange them along the long axis of the slotted hole with their anterior-posterior axes aligned with the long axis of the slotted hole **[2]**.

4.4.1. LAB MEDIA: 4.4.1.

4.4.2. LAB MEDIA: 4.4.2.

4.4.3. LAB MEDIA: 4.4.3.

4.5. To stabilize the embryos, carefully pipet 1-2 microliters of agarose into the gap between the embryos and the agarose film **[1]**.

4.5.1. LAB MEDIA: 4.5.1.

4.6. When the agarose has solidified, slowly insert the cobweb holder with the mounted embryos into the image buffer-filled sample chamber **[1]**.

4.6.1. Holder being inserted into chamber

5. Comparative Live Imaging

5.1. To image the embryos, confirm that the cobweb holder is in a 45-degree position relative to the illumination and detection axes **[1]** and, in the transmission light channel, move the embryo into the center of the field of view. The cobweb holder should not be visible **[2]**.

5.1.1. WIDE: Talent checking holder position OR Additional shot showing holder being rotated 45°

5.1.2. SCREEN: 5.1.2.

5.2. Next, move the embryo with the microtranslation stage in z until the midplane of the embryo overlaps with the focal plane and the outline appears sharp. Without switching to the fluorescence channel, move the embryo 250 microns in both directions to specify the volume of view **[1]**.

5.2.1. SCREEN: 5.2.1: 00:06-01:20

5.3. If imaging along multiple directions is required, rotate the embryo appropriately **[1-TXT],** bring the embryo into focus and specify the volume of view as just demonstrated. ~~The cobweb holder supports up to four orientations in 90-degree steps~~ **[2]**.

5.3.1. SCREEN: 5.3.1_5.3.2: 00:06-00:49 **TEXT: Cobweb holder supports ≤4 orientations in 90° steps**

~~5.3.2. SCREEN: To be provided by Authors: Embryo overlapping with focal plane/coming into focus~~

5.4. Then repeat this procedure for all of the other mounted embryos **[1-TXT]**.

5.4.1. Talent adjusting stage **TEXT: Ensure topmost embryo remains in buffer when bottommost embryo in front of detection lens**

5.5. When the volume of view has been specified for all of the embryos, define the fluorescence channel and time lapse parameters and start the imaging process **[1-TXT]**.

5.5.1. SCREEN: 5.5.1. **TEXT: Consult supplementary metadata table for indicative values**

5.6. For assays that last several days, consider covering the sample chamber opening at least partially to reduce evaporation **[1]**.

5.6.1. Opening being covered

6. Imaged Embryo Retrieval and Cultivation

6.1. When the imaging assay has ended, carefully remove the cobweb holder from the sample chamber **[1]** and use a small paintbrush to detach the embryos from the agarose film and to place the embryos onto an appropriately labeled microscope slide **[2]**.

6.1.1. WIDE: Talent removing holder *Videographer: Important step*

6.1.2. LAB MEDIA: 6.1.2.

© 2020, Journal of Visualized Experiments

6.2. ~~Place the embryos onto an appropriately labeled microscope slide **[1]** and~~ Place the slide into a retrieval dish for incubation under the appropriate standard rearing conditions **[2]**.

~~6.2.1. SCOPE: To be provided by Authors: Embryo(s) being placed onto slide~~

6.2.2. Talent placing slide into dish in incubator

6.3. As the hatching time point approaches, check the retrieval dishes frequently **[1]** and transfer any hatched larvae to ~~individual rearing vials or~~ individual wells of a 24-well plate **[2]**. Fill the wells halfway with the appropriate growth medium **[3]**.

6.3.1. Talent checking dish From authors: This step is in, but with a *Tribolium* larva and should be placed before 6.4.1. From editor: I am not sure this note affects anything but am including it just in case.

~~6.3.2. Talent adding larvae to rearing vial~~

6.4. ~~Place *Tribolium* larvae in individual wells of a 24-well plate **[1]** and fill the wells halfway with the appropriate growth medium **[2]**.~~

6.4.1. Talent adding larvae to well OR LAB MEDIA: 6.4.1. Authors have suggested this shot for 6.4.1. (transferring larvae to individual wells) but I would consider using it for 6.3.1. (checking dishes)

6.4.2. Medium being added to well, with medium container visible in frame

6.5. Then incubate the larvae under the appropriate standard rearing conditions **[1]**.

6.5.1. Talent placing vial and/or plate into incubator or similar

6.6. When the observed individuals reach adulthood, provide suitable mating partners **[1]** and check for progeny after several days **[2]**.

6.6.1. Talent adding partner to vial or well

6.6.2. Shot of progeny From authors: There are two recordings made here. One where the progeny is “shaken out” of the flour and one close-up shot.

© 2020, Journal of Visualized Experiments

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see?
4.4., 6.1.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success?
4.4.2., 4.5.1.

Results

7. Results: Representative *Drosophila* and *Tribolium* Fluorescence Live Imaging

7.1. In this video, the fluorescence signal dynamics of three embryos derived from different transgenic *Drosophila* lines over a period of about 1 day can be observed **[1]**.

7.1.1. LAB MEDIA: Supplementary Video 1 *Video Editor: please emphasize three different embryos when mentioned or no animation*

7.2. A similar fluorescence pattern was expected, since all of the lines expressed nuclear-localized EGFP (E-G-F-P)-GFP under the control of presumably ubiquitous and constitutively active promoters **[1-TXT]**.

7.2.1. LAB MEDIA: Supplementary Video: 00:29 **TEXT: EGFP: enhanced fluorescent protein**

7.3. Comparative live imaging results, however **[1]**, revealed strong spatiotemporal differences in the expression patterns that were not secondary effects due to ambient variance **[2]**.

7.3.1. LAB MEDIA: Figure 4
7.3.2. LAB MEDIA: *Video Editor: please emphasize sequentially emphasize bright signal in top two rows of images, bright signal in middle two rows of images, and bright signal in bottom two rows of images*

7.4. In this analysis of three *Tribolium* embryos carrying the same piggyBac-based transgene **[1]**, comparative live imaging results of the serosa window closure process during gastrulation suggest that the second subline **[2]** provides a remarkably stronger overall signal than the other two sublines **[3]**.

7.4.1. LAB MEDIA: Supplementary Video 2
7.4.2. LAB MEDIA: Figure 5A *Video Editor: please emphasize signal in middle row of images*
7.4.3. LAB MEDIA: Figure 5A *Video Editor: please emphasize signal in top and bottom rows of images*

7.5. As these data indicate, the genomic context may also have a strong influence on the expression level of transgenes in *Tribolium* **[1]**.

7.5.1. LAB MEDIA: Figure 5A

Conclusion

8. Conclusion Interview Statements

8.1. **Julia Ratke**: Our approach is well suited to analyzing knockdown and knockout phenotypes, as it allows wild-type control embryos to be imaged simultaneously **[1]**.

8.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

8.2. **Frederic Strobl**: Our protocol can also be used to compare the morphogenesis of two or more insect species and to thus gain deeper insights into the evolution of development **[1]**.

8.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

© 2020, Journal of Visualized Experiments