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ACCOMPANYING MOVIES

Movies are freely available online at www.cshprotocols.org/imaging.

CHAPTER 15

Visualizing Cell Contacts and Cell Polarity in *Caenorhabditis elegans* Embryos

MOVIE 15.1. Contact between the C blastomere and the ABar blastomere directs the spindle orientation of ABar division in a wild-type *Caenorhabditis elegans* embryo. (Left) Contact between the C blastomere (membrane near contact outlined in blue) and the ABar blastomere (membrane near contact outlined in green). Focal depth (18 μm) of greatest contact is shown. (Right) Shortly after contact between the C and the ABar blastomeres, the mitotic spindle of the ABar blastomere (right line) is shifted toward the contact resulting in a cell division that is perpendicular to orientation of division of the neighboring ABpr blastomere (left line). Focal depth is 7.5 μm .

MOVIE 15.2. Disruption of the fate of the C blastomere using *pal-1(RNAi)* knockdown results in delayed and minimal contact between the C and the ABar blastomeres before division of ABar causing the ABar spindle to be misaligned. (Left) Contact is minimal and is delayed between the C blastomere (membrane near contact outlined in blue) and the ABar blastomere (membrane near contact outlined in green). Focal depth (14 μm) of greatest contact is shown. (Right) In lieu of a polarizing signal from the C blastomere, the ABar blastomere (right line) adopts the default spindle orientation and divides parallel with the ABpr blastomere (left line). Focal depth is 7 μm .

MOVIE 15.3. Laser killing of ABp creates a barrier between contact with the C and the ABar blastomeres in *Caenorhabditis elegans* embryos. The ABp blastomere (labeled with a red x) was laser killed at the four-cell stage of embryogenesis, just before the start of the movie. The corpse of ABp prevents contact between the C blastomere (membrane near closest contact outlined in blue) and the ABar blastomere (membrane near closest contact outlined in green). Focal depth (10.5 μm) of closest proximity between the two blastomeres is shown. As a result, the ABar blastomere aligns its spindle in the default orientation (line) and divides parallel with the other AB granddaughter cells (not shown).

CHAPTER 17

Imaging Retinal Progenitor Lineages in the Developing Zebrafish Retina

MOVIE 17.1. Time-lapse movie of *ath5:gapRFP* transgenic cells. The first cell to appear becomes brighter because of increased expression when it enters mitosis (red arrow). Both daughters (red and white arrows) can be followed until the end of the time lapse.

CHAPTER 18

Imaging the Development of the Fish Lateral-Line System

MOVIE 18.1. Time-lapse video of primordium migration in the blue-fin tuna, *Thunnus thynnus*. Nomarski optics, 1 frame per 30 sec. The embryos of this species are exceedingly fragile but can nevertheless be subjected to time-lapse analysis for short periods of time. In this case the trailing cells of a migrating primordium are slowing down and become organized in a "rosette," with the hair cells radially organized around the central pore through which the hairs will extend into the surrounding water.

MOVIE 18.2. Time-lapse video of primordium migration in zebrafish. The primordium and neuro-masts fluoresce in green owing to a *cldnb:gfp* insertion (Gilmour et al. 2002), and the sensory neurons fluoresce in red owing to a *nbt:dsred* insertion (Peri and Nüsslein-Volhard 2008). Afferent axons accompany the primordium all along its migration (Metcalf 1985; Gilmour et al. 2004). The upper red stripe is the spinal cord; the oblique fibers are motor axons.

CHAPTER 30

Live Imaging of Developing Hippocampal Neurons in Culture

MOVIE 30.1. Time-lapse sequence illustrating a hippocampal neuron undergoing axon specification in culture. Phase-contrast images were acquired every 10 min for a period of 13 h. Movie, 12.2 sec; total time, 13 h; play rate, x3750.

MOVIE 30.2. Time-lapse sequence illustrating dynamic changes in the accumulation of a constitutively active kinesin in stage 2. Phase-contrast (left) and fluorescence (right) images of a hippocampal neuron expressing KIF17¹⁻⁵¹¹-GFP were acquired every 15 min for a period of 6 h. Movie, 5 sec; total time, 6 h; play rate, x4320.

MOVIE 30.3. Visualizing the microtubule-based transport of Golgi-derived vesicles in the axon of a stage 4 hippocampal neuron. Cells were cotransfected with a GFP-tagged membrane protein (NgCAM) and an mCherry construct targeted to the lumen of vesicles in the default constitutive secretory pathway. Images were acquired continuously every 600 msec for 60 sec. Movie, 4.6 sec; total time, 60 sec; play rate, x13.

CHAPTER 41

Four-Dimensional Fluorescent Imaging of Embryonic Quail Development

MOVIE 41.1. Three-dimensional rotation of tiled, stitched z-stack. *Tg(Tie1:H2B::eYFP)* quail embryo somites, HH 12–13, near somite 10. Images were collected on a Zeiss META 510 two-photon microscope. Tiles were stitched using Zeiss LSM software with 10% overlap in x-y. The stitched stack was animated using Bitplane's Imaris 5.7.2 software. Objective, LD-Apochromat 40x/1.1 W Korr objec-

tive; stack size, 427.5 x 630.1 x 126 μm ; tiled stacks, 2 x 3 tiles; excitation, 860 nm (23%); emission, 505LP; initial frame, anterior on left, posterior on right.

CHAPTER 47

Quantitative Imaging of Gene Expression in *Drosophila* Embryos

MOVIE 47.1. The workflow developed to segment confocal images.

MOVIE 47.2. How to use the BREReA package to remove background, register the 1D expression patterns of segmentation genes, and construct reference data.

CHAPTER 49

From Confocal Imaging to 3D Model: A Protocol for Creating 3D Digital Replicas of Ascidian Embryos

MOVIE 49.1. Projection of a 3D time-lapse movie of *Phallusia mamillata* development from the 44-cell stage (stage 7 Hotta) to mid/late-gastrula stage (stage 12.5 Hotta) (Hotta et al. 2007). The movie starts in animal view, rotates along the anteroposterior axis from the 44-cell stage to the early-110-cell stage, and subsequently shows gastrulation and blastopore closure. The movie spans >2 h, for 10 successive stacks. For further reconstructions of time-lapse embryos and corresponding morphometric analysis, see Sherrard et al. (2010).

CHAPTER 52

X-Ray Microtomographic Imaging of Vertebrate Embryos

MOVIE 52.1. Volume rendering of a 9.5-d (Theiler stage-15) mouse embryo fixed in paraformaldehyde, stained with PTA, and embedded in 0.5% agarose before scanning. The window and transparency functions were adjusted to render the gel invisible.

MOVIE 52.2. Stage-11 chick embryo fixed in paraformaldehyde, stained with PTA, and embedded in 0.5% agarose. Note that the extra-embryonic membranes are intact.

MOVIE 52.3. Stage-14 chick embryo postfixed in osmium tetroxide, embedded for TEM sectioning, and scanned in the resin block.