Topic Introduction

Imaging Methods in *Xenopus* Cells, Embryos, and Tadpoles

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Xenopus is an excellent vertebrate model system ideally suited for a wide range of imaging methods designed to investigate cell and developmental biology processes. The individual cells of *Xenopus* are much larger than those in many other vertebrate model systems, such that both cell behavior and subcellular processes can more easily be observed and resolved. Gene function in *Xenopus* can be manipulated and visualized using a variety of approaches, and the embryonic fate map is stereotypical, such that microinjections can target specific tissues and cell types during development. Tissues, organotypic explants, and individual cells can also be mounted in stable chambers and cultured easily in simple salt solutions without cumbersome environmental controls. Furthermore, *Xenopus* embryonic tissues can be microsurgically isolated and shaped to expose cell behaviors and protein dynamics in any regions of the embryo to high-resolution live-cell imaging. The combination of these attributes makes *Xenopus* a powerful system for understanding cell and developmental processes as well as disease mechanisms, through quantitative analysis of protein dynamics, cell movements, tissue morphogenesis, and regeneration. Here, we introduce various methods, of both fixed and living tissues, for visualizing *Xenopus* cells, embryos, and tadpoles. Specifically, we highlight protocol updates for whole-mount in situ hybridization and immunofluorescence, as well as powerful live imaging approaches including methods for optimizing the time-lapse imaging of whole embryos and explants.

BACKGROUND

For the last century, advances in cell and developmental biology have been propelled by leaps in imaging and microscopy technologies. Our knowledge of vertebrate development, generally in amphibians, and more specifically in *Xenopus*, has benefited greatly from these leaps. For instance, methods for lineage tracking, unique to amphibians (Vogt 1929; Keller 1975, 1976; Lane and Sheets 2002), have been central to our foundational understanding of fate, commitment, and specification. The emergence of electron microscopy provides unparalleled descriptions of cytoarchitecture that form the basis of our understanding of gastrulation (Keller 1980; Winklbauer et al. 1992) and neurulation (Schroeder 1970). Key arrangements of germ layers, organs, and cells in the embryo and larva were exposed in an atlas laid out after use of plastic embedding and sectioning (Hausen and Riebesell 1991). Fluorescence imaging of fluorophore-conjugated dextrans (e.g., Keller and Tibbetts 1989) followed by the introduction of confocal microscopy and fluorescent proteins (e.g., Wallingford et al. 2000) afforded advances in live-cell imaging in a range of preparations from single cells to organotypic explants to whole embryos. New imaging modalities, from magnetic resonance imaging (MRI; Jacobs and Fraser 1994) to X-ray phase contrast microscopy (Moosmann et al. 2013) to optical coherence tomography (OCT; Boppart et al. 1996) are routinely showed in

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developing *Xenopus*. Advanced imaging tools leveraged with fate maps and stage descriptions of Nieuwkoop and Faber (1967) have provided a rich framework for posing and testing mechanistic hypotheses on the genetic regulation and molecular pathways that were subsequently uncovered and that continue to be the subject of lively investigation.

Efforts to decrypt the cell and molecular mechanisms responsible for patterning, morphogenesis, and organogenesis have been made possible by advances in sample preparation. Whole-mount RNA in situ hybridization has exposed gene expression patterns, whereas the creation of synthetic fluorophores and discovery and refinement of fluorescent proteins has made possible localization and protein dynamics in vivo. Whole-mount RNA in situ hybridization methods (Harland 1991) were rigorously adopted by the field and continue to advance, revealing patterns of gene expression, making possible the construction of gene regulatory networks. Fluorophores combined with antibody technology and fluorescent proteins have revealed the effector networks that integrate these gene regulatory networks with cell–cell communication and microenvironmental cues to guide cells to their targets and effect the construction of physiologically functional organs.

The protocols in this collection reflect newer techniques and detailed methods of sample preparation that have not been well-covered in past collections. In each of the sections below, we will also recommend previously published methods for imaging and sample preparation as these remain excellent resources for improving technique and troubleshooting methods.

IMAGING OF FIXED EMBRYOS AND TADPOLES

Whole-mount, half-mount, and sectioned preparations of fixed *Xenopus* embryos provide access for high-resolution studies of gene expression patterns, tissue as well as subcellular protein localization, cell shape, and tissue microanatomy. Whole-mount RNA in situ hybridization adapted northern blot techniques for use in a permeabilized *Xenopus* embryo (Harland 1991) and was further adapted to deposit tyramide fluorescent substrates, allowing single-cell gene expression studies (Davidson and Keller 1999; Vize et al. 2009). In the first protocol, new techniques are introduced to improve methods to visualize gene expression patterns simultaneously with protein localization. Recent years have seen development of novel technologies to enhance fluorescent RNA in situ methods using RNAscope (ACD RNAscope) and Hybridization Chain Reaction (HCR3.0, Molecular Instruments). In this protocol, Protocol: **Whole-Mount RNA In Situ Hybridization and Immunofluorescence of *Xenopus* Embryos and Tadpoles** (Willsey 2021), Willsey et al. (2020) includes an update on the Harland protocol as well as a protocol for HCR and whole-mount immunofluorescence. The adaptations of these protocols to late stages of *Xenopus* are particularly helpful for characterizing the molecular anatomy of the larval brain and shows great potential for other organ systems where efforts to dissect tissue–tissue interactions are aided by preserving 3D context.

High-resolution imaging of the cell and subcellular structures are enhanced when the tissues are fixed and sectioned to preserve protein–protein interactions and protein localization. Such preparations can eliminate the need for antibodies to penetrate deep tissues and reduce background fluorescence that accompanies thick-tissue imaging. In Protocol: **Cryosectioning and Immunostaining of *Xenopus* Embryonic Tissues** (Ossipova and Sokol 2021), Ossipova and Sokol provide an update on the methods of fixation, embedding, sectioning, and immunostaining that best preserve the delicate epitopes of *Xenopus* early embryos. Immunofluorescence staining of thin frozen sections remains a central tool in visualizing the location and status of endogenous proteins in *Xenopus* embryos.

IMAGING OF LIVING TISSUES

One of the greatest benefits of using *Xenopus* is that it is particularly ideal for live imaging of dynamic processes at multiple scales, ranging from whole embryos to individual cells to even subcellular

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processes (Joshi et al. 2012). Embryonic development is external, the embryos and tissues can be cultured at room temperature without incubation nor CO_2, and the cells are quite large compared to other vertebrates. Thus, *Xenopus* is a powerful model system for in vivo time-lapse studies of cell and developmental biology. Here, we summarize the various types of protocols that have been used for imaging living tissues in *Xenopus*.

Previously published protocols have described the strengths of using *Xenopus* for low-magnification imaging of whole embryos, which, in combination with manipulation of gene functions, have provided numerous insights into the control of morphogenetic processes such as gastrulation and neural tube closure (Wallingford 2010; Danilchik 2011). Moreover, previous protocols have detailed methods for high-magnification time-lapse imaging using confocal microscopy (Kieserman et al. 2010). Protocol: **Chambers for Culturing and Immobilizing *Xenopus* Embryos and Organotypic Explants for Live Imaging** (Chu and Davidson 2021) describes the preparation of special chambers for immobilizing *Xenopus* embryos and embryonic explants for live-cell and tissue imaging.

There are many different cell and tissue types that can be imaged in living intact *Xenopus* embryos and tadpoles. For example, Protocol: **Imaging the Dynamic Branching and Synaptic Differentiation of *Xenopus* Optic Axons In Vivo** (Santos et al. 2020) demonstrates how individual fluorophore-labeled neurons can be imaged in living tadpoles to visualize optic axonal arbors and formation of neuronal circuits. Not only can confocal microscopy be used to image individual cells during development, but fluorescent fusion proteins and molecules can also be imaged to follow their dynamics. For example, fluorescent probes for Rho GTPase activity have been used while imaging large blastomeres of the early *Xenopus* embryo to uncover new insights into epithelial cell biology (Stephenson and Miller 2017). Furthermore, Protocol: **Whole-Brain Calcium Imaging in Larval *Xenopus*** (Offner et al. 2020) describes how calcium imaging can be performed in larvae to provide information regarding the spatiotemporal activity of the entire brain.

Although live imaging of *Xenopus* embryos can be illuminating, one caveat is that the yolk opacity of *Xenopus* cells can sometimes interfere with traditional types of deep imaging in intact embryos. However, almost any type of *Xenopus* cell and/or tissue can also easily be cultured ex vivo. For example, high-resolution live imaging of mucociliary organoids from *Xenopus* have been used to investigate mechanisms of mucociliary signaling and morphogenesis (Walentek 2018; Kang and Kim 2020). Protocol: **Live Imaging of Cytoskeletal Dynamics in Embryonic *Xenopus laevis* Growth Cones and Neural Crest Cells** (Erdogan et al. 2020) describes how live imaging of fluorophore-tagged cytoskeletal proteins allows for monitoring the dynamic nature of cytoskeleton components in cultured embryonic neuronal growth cones and neural crest cells.

Finally, although the majority of live imaging studies of *Xenopus* have used confocal microscopy, OCT is another powerful method for live imaging tissue structures in vivo. OCT is a rapid, label-free imaging modality (Boppart et al. 1996) that can acquire 2D and 3D data in real time to assess cardiac and facial structures, as described in Protocol: ***Xenopus* Tadpole Craniocardiac Imaging Using Optical Coherence Tomography** (Deniz et al. 2021).

Over the past several decades, *Xenopus* cells, embryos, and tadpoles have been used for powerful imaging studies to dissect key processes in cell and developmental biology. As new imaging techniques become available, the many benefits of the *Xenopus* model system (external and rapid development, fate mapping and gene manipulation, easy culture conditions) will ensure that *Xenopus* will remain an ideal system for continued investigation of the molecular and cell biological mechanisms governing cell and developmental processes.

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REFERENCES

Boppart SA, Bouma BE, Brezinski ME, Tearney GJ, Fujimoto JG. 1996. Imaging developing neural morphology using optical coherence tomography. *J Neurosci Methods* **70:** 65–72. doi:10.1016/S0165-0270(96)00104-5

Chu C-W, Davidson LA. 2021. Chambers for culturing and immobilizing *Xenopus* embryos and organotypic explants for live imaging. *Cold Spring Harb Protoc* doi:10.1101/pdb.prot107649

Danilchik MV. 2011. Manipulating and imaging the early *Xenopus laevis* embryo. *Methods Mol Biol* **770:** 21–54. doi:10.1007/978-1-61779-210-6_2

Davidson LA, Keller RE. 1999. Neural tube closure in *Xenopus laevis* involves medial migration, directed protrusive activity, cell intercalation and convergent extension. *Development* **126:** 4547–4556. doi:10.1242/dev.126.20.4547

Deniz E, Mis EK, Lane M, Khokha MK. 2021. *Xenopus* tadpole craniocardiac imaging using optical coherence tomography. *Cold Spring Harb Protoc* doi:10.1101/pdb.prot105676

Erdogan B, Bearce EA, Lowery LA. 2020. Live imaging of cytoskeletal dynamics in embryonic *Xenopus laevis* growth cones and neural crest cells. *Cold Spring Harb Protoc* doi:10.1101/pdb.prot104463

Harland RM. 1991. In situ hybridization: an improved whole-mount method for *Xenopus* embryos. *Methods Cell Biol* **36:** 685–695. doi:10.1016/S0091-679X(08)60307-6

Hausen P, Riebesell M. 1991. *The early development of Xenopus laevis: an atlas of the histology*. Springer Verlag, New York.

Jacobs RE, Fraser SE. 1994. Magnetic resonance microscopy of embryonic cell lineages and movements. *Science* **263:** 681–684. doi:10.1126/science.7508143

Joshi SD, Kim HY, Davidson LA. 2012. Microscopy tools for quantifying developmental dynamics in *Xenopus* embryos. *Methods Mol Biol* **917:** 477–493. doi:10.1007/978-1-61779-992-1_27

Kang HJ, Kim HY. 2020. Mucociliary epithelial organoids from *Xenopus* embryonic cells: generation, culture and high-resolution live imaging. *J Vis Exp* **161:** e61604. doi:10.3791/61604

Keller RE. 1975. Vital dye mapping of the gastrula and neurula of *Xenopus laevis*. I. Prospective areas and morphogenetic movements of the superficial layer. *Dev Biol* **42:** 222–241. doi:10.1016/0012-1606(75)90331-0

Keller RE. 1976. Vital dye mapping of the gastrula and neurula of *Xenopus laevis*. II. Prospective areas and morphogenetic movements of the deep layer. *Dev Biol* **51:** 118–137. doi:10.1016/0012-1606(76)90127-5

Keller R. 1980. The cellular basis of epiboly: an SEM study of deep-cell rearrangement during gastrulation in *Xenopus laevis*. *Development* **60:** 201–234. doi:10.1242/dev.60.1.201

Keller R, Tibbetts P. 1989. Mediolateral cell intercalation in the dorsal, axial mesoderm of *Xenopus laevis*. *Dev Biol* **131:** 539–549. doi:10.1016/S0012-1606(89)80024-7

Kieserman EK, Lee C, Gray RS, Park TJ, Wallingford JB. 2010. High-magnification in vivo imaging of *Xenopus* embryos for cell and developmental biology. *Cold Spring Harb Protoc* doi:10.1101/pdb.prot5427

Lane MC, Sheets MD. 2002. Rethinking axial patterning in amphibians. *Dev Dyn* **225:** 434–447. doi:10.1002/dvdy.10182

Moosmann J, Ershov A, Altapova V, Baumbach T, Prasad MS, LaBonne C, Xiao X, Kashef J, Hofmann R. 2013. X-ray phase-contrast in vivo microtomography probes new aspects of *Xenopus* gastrulation. *Nature* **497:** 374–377. doi:10.1038/nature12116

Nieuwkoop PD, Faber J. 1967. *Normal tables of Xenopus laevis (Daudin): a systematic and chronological survey of the development from the fertilized egg till the end of morphogenesis*. North-Holland Biomedical Press, Elsevier, Amsterdam.

Offner T, Daume D, Weiss L, Hassenklover T, Manzini I. 2020. Whole-brain calcium imaging in larval *Xenopus*. *Cold Spring Harb Protoc* doi:10.1101/pdb.prot106815

Ossipova O, Sokol SY. 2021. Cryosectioning and immunostaining of *Xenopus* embryonic tissues. *Cold Spring Harb Protoc* doi:10.1101/pdb.prot107151

Santos RA, Jr RR, Cohen-Cory S. 2020. Imaging the dynamic branching and synaptic differentiation of *Xenopus* optic axons in vivo. *Cold Spring Harb Protoc* doi:10.1101/pdb.prot106823

Schroeder TE. 1970. Neurulation in *Xenopus laevis*. An analysis and model based upon light and electron microscopy. *J Embryol Exp Morphol* **23:** 427–462.

Stephenson RE, Miller AL. 2017. Tools for live imaging of active Rho GTPases in *Xenopus*. *Genesis* **55:** 10.1002/dvg.22998. doi:10.1002/dvg.22998

Vize PD, McCoy KE, Zhou X. 2009. Multichannel wholemount fluorescent and fluorescent/chromogenic in situ hybridization in Xenopus embryos. *Nat Protoc* **4:** 975–983. doi:10.1038/nprot.2009.69

Vogt W. 1929. Gestaltungsanalyse am Amphibienkeim mit ortlicher Vitalfarbung II. Teil: Gastrulation und Mesodermbildung bei Urodelen und Anuren. *Wilhelm Roux'Arch Entwicklungsmech Org* **120:** 384–706. doi:10.1007/BF02109667

Walentek P. 2018. Manipulating and analyzing cell type composition of the *Xenopus* mucociliary epidermis. *Methods Mol Biol* **1865:** 251–263. doi:10.1007/978-1-4939-8784-9_18

Wallingford JB. 2010. Low-magnification live imaging of *Xenopus* embryos for cell and developmental biology. *Cold Spring Harb Protoc* doi:10.1101/pdb.prot5425

Wallingford JB, Rowning BA, Vogeli KM, Rothbacher U, Fraser SE, Harland RM. 2000. Dishevelled controls cell polarity during *Xenopus* gastrulation. *Nature* **405:** 81–85. doi:10.1038/35011077

Willsey HR. 2021. Whole-mount RNA in situ hybridization and immunofluorescence of *Xenopus* embryos and tadpoles. *Cold Spring Harb Protoc* doi:10.1101/pdb.prot105635

Willsey HR, Xu Y, Everitt A, Dea J, Exner CR, Willsey AJ, Harland RM. 2020. The neurodevelopmental disorder risk gene *DYRK1A* is required for ciliogenesis and control of brain size in *Xenopus* embryos. *Development* **147:** dev189290. doi:10.1242/dev.189290

Winklbauer R, Selchow A, Nagel M, Angres B. 1992. Cell interaction and its role in mesoderm cell migration during *Xenopus* gastrulation. *Dev Dyn* **195:** 290–302. doi:10.1002/aja.1001950407

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