Micron resolved *in vivo* tomographic time-lapse series of early neurulation in *Xenopus laevis* acquired by X-ray propagation based phase-contrast imaging

Alexander Rack,[1] Lukas Helfen,[1] Steffen Hahn,[2] Madeleine Hertel,[2] Xianghui Xiao,[3] Julian Moosmann,[4] Alexander Schober,[5] Jubin Kashef,[6] Marie-Claire Kratzer,[7] Angelica Cecilia,[6] Thomas van de Kamp,[2] Tomas Farago,[6] Alexey Ershov,[2] Maneeshi S. Prasad,[8] Tilo Baumbach,[2,6] Carole LaBonne[9], Iván Alexis Sánchez Salazar Chavarría,[6] Venera Weinhardt,[6,10] Kamel Fezzaa,[3] Ralf Hofmann[6]

1.European Synchrotron Radiation Facility, 6 rue Jules Horowitz, 38000 Grenoble, France
2. Laboratory for Applications of Synchrotron Radiation, Karlsruhe Institute of Technology, Postfach 6980, D-76128 Karlsruhe, Germany
3. Advanced Photon Source, Argonne National Laboratory, 9700 South Cass Avenue, Argonne, IL 60439, USA
4. Division of mathematics, KTH Royal Institute of Technology, SE-100 44 Stockholm, Sweden
5. Luxembourg Institute of Science and Technology (LIST), 5 Avenue des Hauts-Fourneaux, L-4362 Esch/Alzette, Luxembourg
6. Institute for Photon Science and Synchrotron Radiation, Karlsruher Institut für Technologie, Hermann-von-Helmholtz-Platz 1, D-76344 Eggenstein-Leopoldshafen, Germany
7. Fachbereich 17, Biologie, Karl-von-Frisch-Straße 8, D-35043 Marburg, Germany
8. School of Medicine, University of California, Riverside 900 University Ave. Riverside, CA 92521
9. Department of Molecular Biosciences, Northwestern University
Evanston, IL 60208, USA
10. Centre for Organismal Studies (COS),Universität Heidelberg, Im Neuenheimer Feld 230, 69120 Heidelberg, Germany

email: Steffen Hahn (uldjt@student.kit.edu), Ralf Hofmann (ralf.hofmann2@kit.edu)

Abstract

Induction, maturization, delamination, collective directional and individual migration as well as the invasion of target tissue by pluripotent Cranial Neural Crest Cells (CNCC) are essential processes anticipating differentiation subsequent to neurulation. They possibly represent useful models for cancer progression. During the collective migration stage local cell-cell interactions seem to be key to directionality. A quantitative *in vivo* assessment of temporal characteristics for the average rate of cell-cell contact formation within a given CNCC population via transient protrusions, associated changes in cell polarization, and of the chemical and mechanical interrelation between CNCC and adjacent tissue should provide some basic information to understand collective migration. Here we present micron spatially and ten-minute temporally resolved *in vivo* time-lapse series of 3D quantitatively reconstructed electron density, exhibiting the development of entire *Xenopus laevis* embryos throughout initial stages 17 to 23 (neurulation). Lengths of time-lapse series range from 20 to 70 min. Because the underlying tomographic stacks were acquired using X-ray propagation based phase contrast subject to quasi-monochromatic, hard synchrotron radiation, single and large propagation distances could be employed. Because of the increased signal-to-noise ratio at all frequencies contained within the intensity contrast and a good recovery of higher-frequency information for retrieved phase maps this allowed for the application of a comparably low dose at prescribed contrast and resolution goal. The here presented reconstructions rely on raw data collected at beamlines 32-ID of Advanced Photon Source and ID-19 of European Sychrotron Radiation Facility throughout the years 2012-2015.

Background & Summary

In *Xenopus laevis* the Cranial Neural Crest forms populations of highly motile and pluripotent cells – Cranial Neural Crest Cells (CNCC) – , which possibly are induced at early gastrulation along the border between neural plate and epidermis [1,2], see however [3], emigrate shortly before neural tube closure from the anterior neural plate, and migrate ventrally into the

pharyngeal pouches. From there, they start to move first as a cohesive sheet and later disseminate into single cells to eventually invade their target tissues. To understand migratory and invasive behavior of CNCC in *Xenopus laevis* to the point of influencability is important because of the conservancy of many underlying molecular mechanisms between this frog model system and humans. Regarding a successful molecular modelling of cancer progression [4,5], this is particularily pressing.

We have previously analysed [6,7] 3D time-lapse series, imaging gastrulation in *Xenopus laevis* in genetically unmodified embryos at subcellular resolution ($2\Delta x = (2-4)\mu\mathrm{m}$, Δx denoting the linear, effective size of a square pixel) by means of propagation based X-ray phase-contrast micro-tomography (XPCμT) [8-13]. To analyse developmental dynamics and structure evolution *in vivo* within optically opaque, early stage *Xenopus* embryos XPCμT is the 3D imaging modality of choice which, however, depends on the availability of hard ($E \geq 25\,\mathrm{keV}$), monochromatic, and spatially coherent X-rays of high photon flux densities ($\Phi \geq 10^{12}\,\mathrm{phs/mm^2/s}$). At present, there are about ten 3rd generation synchrotron radiation facilities worldwide whose X-ray sources and X-ray optics meet these requirements.

The here-provided data on time-lapse series of development throughout neurulation, although light on X-ray dose compared to conventional absorption tomography, still require several hundreds of grays per volume. This immediately raises the question of how severely developmental dynamics is impaired by such relatively high X-ray doses. There is not yet a conclusive answer. However, in [6] a comparison of blastupore radius closure rates in X-rayed and in control populations of *Xenopus* gastrulae indicates wildtype-like development up to ~ 2 hours of imaging where a sudden disintegration of the embryo occurs. We take this as supporting evidence for our hypothesis that the present time-lapse series represent wildtype-like development also throughout neurulation.

Methods

All methods concerning experimental, data processing, and image analysis procedures such as fertilization, embryo culture and scheduling, sample preparation and suspension, tomographic imaging (including determination of number of projections, X-ray energy, propagation distance, pixel size, monochromaticity, spatial coherence, photon statistics), intricacies of image pre-processing, phase retrieval, and tomographic reconstruction are described in [7] for *in vivo* XPCμT applied to *Xenopus* gastrulation. These procedures are, without any restrictions except for the development up to the more advanced stages of neurulation, adopted in creating the present data sets. Image analysis such as segmentation and motion study using optical flow algorithms, which future practitioners may want to apply, are also discussed in [7].

Large-distance phase retrieval algorithms [14], which were not yet used in [6], are applied to intensity data acquired at $z \geq 1\mathrm{m}$. Also, a potential source for degradation of spatial resolution – shake of monochromators through pump frequencies invoked by water cooling – which could not fully be eliminated in the data of [6], was absent in acquiring data at ID19 of ESRF. There, the use of a single-line undulator harmonic (low-K

operation plus absorption filters) at $E \sim 26$keV of band width $\Delta E/E \sim 0.03$ made further monochromatization unnecessary. Note that such a band width implies $z \geq 21.4$cm to guarantee full spatial resolution ($\Delta x = 1.6$ µm) in image formation by forward propagation [15]. This condition is always met with the propagation distances used at ID19.

Tab. 1: Overview of experimental parameters employed in acquiring raw data. Columns refer to: folder name for each time-lapse series, central X-ray energy E in keV, relative band width $\Delta E/E$, effective pixel sizes Δx in µm, propagation distances z in mm, exposure time per projection τ in ms, scintillator material (number denoting thickness in µm), CMOS camera type, number or projections N in each volume within a given time-lapse series, waiting time T inbetween tomographic exposures within a given time-lapse series, type of phase retrieval employed within all volumes of a given time-lapse series (TIE: transport-of-intensity based, QP x pi: quasi-particle based with ultraviolet cutoff at x pi). For all tomographic reconstruction, based on N phase maps per volume, Filtered Backprojection (FBP) was used.

name	E in keV	Delta E/E	Delta x(eff) in µm	z in mm	tau in ms	scintillator	camera	N	T wait in s	phase retrieval
stage17/2014_11_28_09h49_ESRF_ID19	26	$3*10^{-2}$	1.61	900	15	LuAg 100	Pco EDGE CAMERALINK (SN 429)	600	660	QP 2 pi
stage18/2012_10_12_09h00_APS_32ID	34.5	10^{-4}	1.1	700	30	LuAg 100	Pco DIMAX	834	600	TIE
stage18/2012_10_13_16h17_APS_32ID	34.5	10^{-4}	1.1	700	30	LuAg 100	Pco DIMAX	834	600	TIE
stage18/2013_07_30_02h25_APS_32ID	30	10^{-4}	1.1	700	15	LuAg 100	Pco DIMAX	500	480	TIE
stage18/2013_07_30_23h46_APS_32ID	30	10^{-4}	1.1	700	15	LuAg 100	Pco DIMAX	500	480	TIE
stage18/2014_11_28_11h49_ESRF_ID19	26	$3*10^{-2}$	1.61	900	15	LuAg 100	Pco EDGE CAMERALINK (SN 429)	600	660	QP 2 pi
stage19/2013_07_28_16h21_APS_32ID	30	10^{-4}	1.1	400	12	LuAg 100	Pco DIMAX	500	600	TIE
stage19/2014_07_18_07h48_APS_32ID	30	10^{-4}	1.3	715	60	LuAg 100	Pco EDGE 5.5	500	840	QP 1 pi
stage19/2014_11_27_20h19_ESRF_ID19	26	$3*10^{-2}$	1.61	900	15	LuAg 100	Pco EDGE CAMERALINK (SN 429)	600	660	QP 2 pi
stage19/2014_11_28_06h33_ESRF_ID19	26	$3*10^{-2}$	1.61	900	15	LuAg 100	Pco EDGE CAMERALINK (SN 429)	600	660	QP 2 pi
stage19/2015_06_17_23h40_ESRF_ID19	26.3	$3*10^{-2}$	1.59	3500	40	LuAg 100	Pco EDGE CAMERALINK (SN 429)	600	660	QP 2 pi
stage19/2015_06_18_01h45_ESRF_ID19	26.3	$3*10^{-2}$	1.59	3500	40	LuAg 100	Pco EDGE CAMERALINK (SN 429)	600	660	QP 2 pi
stage19/2015_06_19_08h39_ESRF_ID19	26.3	$3*10^{-2}$	1.59	5000	40	LuAg 100	Pco EDGE CAMERALINK (SN 429)	600	570	QP 2 pi
stage20/2014_11_28_18h02_ESRF_ID19	26	$3*10^{-2}$	1.61	900	15	LuAg 100	Pco EDGE CAMERALINK (SN 429)	600	660	QP 2 pi
stage20/2014_11_30_07h30_ESRF_ID19	26	$3*10^{-2}$	1.61	900	20	GGG 50	Pco EDGE CAMERALINK (SN 429)	600	660	QP 2 pi
stage20/2015_06_18_04h13_ESRF_ID19	26.3	$3*10^{-2}$	1.59	3500	40	LuAg 100	Pco EDGE CAMERALINK (SN 429)	600	660	QP 2 pi
stage20/2015_06_18_06h15_ESRF_ID19	26.3	$3*10^{-2}$	1.59	3500	40	LuAg 100	Pco EDGE CAMERALINK (SN 429)	600	660	QP 2 pi
stage21/2012_10_11_19h10_APS_32ID	34.5	10^{-4}	1.1	700	30	LuAg 100	Pco DIMAX	834	600	TIE
stage21/2012_10_12_11h45_APS_32ID	34.5	10^{-4}	1.1	700	30	LuAg 100	Pco DIMAX	834	600	TIE
stage21/2014_11_28_14h43_ESRF_ID19	26	$3*10^{-2}$	1.61	900	15	LuAg 100	Pco EDGE CAMERALINK (SN 429)	600	660	QP 2 pi
stage21/2014_11_29_09h37_ESRF_ID19	26	$3*10^{-2}$	1.61	900	20	LuAg 100	Pco EDGE CAMERALINK (SN 429)	600	660	QP 2 pi
stage21/2015_06_18_08h53_ESRF_ID19	26.3	$3*10^{-2}$	1.59	3500	40	LuAg 100	Pco EDGE CAMERALINK (SN 429)	600	660	QP 2 pi
stage23/2013_07_29_11h18_APS_32ID	30	10^{-4}	1.1	400	10	LuAg 100	Pco DIMAX	500	480	TIE
stage23/2014_07_18_23h30_APS_32ID	30	10^{-4}	1.3	715	60	LuAg 100	Pco EDGE 5.5	500	840	QP 1 pi
stage23/2014_11_28_04h12_ESRF_ID19	26	$3*10^{-2}$	1.61	900	15	LuAg 100	Pco EDGE CAMERALINK (SN 429)	600	660	QP 2 pi
stage23/2014_11_29_06h23_ESRF_ID19	26	$3*10^{-2}$	1.61	900	20	LuAg 100	Pco EDGE CAMERALINK (SN 429)	600	660	QP 2 pi
stage23/2015_06_18_10h52_ESRF_ID19	26.3	$3*10^{-2}$	1.59	3500	40	LuAg 100	Pco EDGE CAMERALINK (SN 429)	600	660	QP 2 pi
stage23/2015_06_18_12h39_ESRF_ID19	26.3	$3*10^{-2}$	1.59	3500	40	LuAg 100	Pco EDGE CAMERALINK (SN 429)	600	660	QP 2 pi
stage23/2015_06_19_02h58_ESRF_ID19	26.3	$3*10^{-2}$	1.59	5000	40	LuAg 100	Pco EDGE CAMERALINK (SN 429)	600	570	QP 2 pi

Tab. 1 gives an overview of experimental parameters employed to acquire and process (phase retrieval and tomographic reconstruction) all XPCµT *in vivo* data on *Xenopus* neurulation that is provided here in terms of time-lapse series of reconstructed electron density.

Code availability

We refrain from directly providing our MATLAB scripts for phase retrieval. However, should the need arise to perform phase retrieval from once own raw intensity data (not provided here) the authors are happy to work out and share adapted versions of their phase retrieval software upon request.

For tomographic-reconstruction software concerning Filtered Backprojection (FBP) a convenient and reliable package is the ASTRA Tomography Toolbox [16]. Commercial software for further image processing (segmentation, flow-field analysis) is quoted in [7].

Tab. 2: Overview of data structure. Columns refer to: folder name for each time-lapse series, size of time-lapse series, file type, dimensions of reconstructed slice (z-stack), number of reconstructed volumes per time-lapse series.

stage18/2013_07_30_23h46_APS_32ID	55	32-bit, tif (deflate)	1544	1480	1401	5
stage18/2014_11_28_11h49_ESRF_ID19	15	32-bit, tif (deflate)	960	1128	1009	4
stage19/2013_07_28_16h21_APS_32ID	94	32-bit, tif (deflate)	1476	1576	1451	8
stage19/2014_07_18_07h48_APS_32ID	9,9	32-bit, tif (deflate)	1176	1324	902	2
stage19/2014_11_27_20h19_ESRF_ID19	15	32-bit, tif (deflate)	906	1020	945	5
stage19/2014_11_28_06h33_ESRF_ID19	11	32-bit, tif (deflate)	825	870	1088	4
stage19/2015_06_17_23h40_ESRF_ID19	30	32-bit, tif (deflate)	1052	1064	971	8
stage19/2015_06_18_01h45_ESRF_ID19	23	32-bit, tif (deflate)	1084	1012	996	6
stage19/2015_06_19_08h39_ESRF_ID19	20	32-bit, tif (deflate)	1044	908	1011	6
stage20/2014_11_28_18h02_ESRF_ID19	13	32-bit, tif (deflate)	1059	927	961	4
stage20/2014_11_30_07h30_ESRF_ID19	17	32-bit, tif (deflate)	954	951	1021	5
stage20/2015_06_18_04h13_ESRF_ID19	25	32-bit, tif (deflate)	1000	1124	936	7
stage20/2015_06_18_06h15_ESRF_ID19	20	32-bit, tif (deflate)	1128	1128	771	6
stage21/2012_10_11_19h10_APS_32ID	55	32-bit, tif (deflate)	1488	1488	1201	6
stage21/2012_10_12_11h45_APS_32ID	111	32-bit, tif (deflate)	1824	1824	1401	7
stage21/2014_11_28_14h43_ESRF_ID19	16	32-bit, tif (deflate)	1161	879	876	5
stage21/2014_11_29_09h37_ESRF_ID19	10	32-bit, tif (deflate)	732	771	1025	5
stage21/2015_06_18_08h53_ESRF_ID19	29	32-bit, tif (deflate)	1120	1132	850	8
stage23/2013_07_29_11h18_APS_32ID	83	32-bit, tif (deflate)	1276	1219	1917	8
stage23/2014_07_18_23h30_APS_32ID	4,9	32-bit, tif (deflate)	940	848	862	2
stage23/2014_11_28_04h12_ESRF_ID19	9,8	32-bit, tif (deflate)	963	690	1057	4
stage23/2014_11_29_06h23_ESRF_ID19	7	32-bit, tif (deflate)	759	966	931	3
stage23/2015_06_18_10h52_ESRF_ID19	32	32-bit, tif (deflate)	1024	736	1551	8
stage23/2015_06_18_12h39_ESRF_ID19	16	32-bit, tif (deflate)	1068	700	1501	4
stage23/2015_06_19_02h58_ESRF_ID19	44	32-bit, tif (deflate)	1320	1024	1201	8
	936,6				32693	158

Data Records

All data on time-lapse series on reconstructed electron density in 3D are stored at the Xenbase Turbogfrog ftp server [17] under

KIT_neurulation_xenopus_laevis

according to the folder label structure used in the first columns (name) of Tab. 1 and Tab. 2. Here the first level of directories associates with the initial stage followed by a sublevel indicating date and initial time of acquisition as well as synchrotron facility and beamline.
We have deliberately refrained from providing raw projection data since their sheer amount would have implied a storage and maintainance effort too expensive on the scale of a public-access server. Only an apparently useful subset (~ 20TB) of our entire raw data was selected for reconstruction and is provided (~ 1TB due to trimming and lossless compression) by the Turbogfrog ftp server. For example, there are raw

data on Cadherin11mo one-sidedly manipulated neurulae which were not included into this subset. If future users express their interest in this data then it can be delivered.

Usage Notes

Once downloaded it is advantageous to convert the tiff files from *Deflate Official version ('Adobe-style')* compression format to *Uncompressed* format because of shorter access times in viewing volume stacks. *ImageJ or FiJi* [18] are particularily user friendly in scrolling through and manipulating entire volumes.

Ethics

All experiments at APS and ESRF were performed in accordance with a protocol approved by North-Western University's Committee on Animal Care and Use (Animal assurance number A328301) and by the Ministère de l'Enseignement Supérieur et de la Recherche of France, respectively.

Acknowledgements

We acknowledge the European Synchrotron Radiation Facility (ESRF) and the Advanced Photon Source (APS) for provision of synchrotron radiation facilities. Moreover, we would like to thank Francesco de Carlo and his X-ray imaging group at APS for his continuing support of our projects. This research was partially funded by COST action MP-1207 of the European Commission and the Swedish Foundation for Strategic Research unde rgrant number AM13-0049.

Competing interests

There is no conflict of interest.

References

[1] Sauka-Spengler, T. & Bronner-Fraser, M. A gene regulatory network orchestrates neural crest formation. Nat. Rev. Mol. Cell Biol. **9**, 557-568 (2008).

[2] Steventon, B. & Mayor, R. Early neural crest induction requires an initial inhibition of Wnt signals. *Dev. Biol.* **365,** 196-207(2012).

[3] Buitrago-Delgado, E., Nordin, K., Rao, A., Geary, L. & LaBonne, C. Shared regulatory programs suggest retention of blastula-stage potential in neural crest cells. *Science* **348**, 1332-1335 (2015).

[4] Sadaghiani, B. & Thiébaud, C.H. Neural crest development in the *Xenopus laevis* embryo, studied by interspecific transplantation and scanning electron microscopy. *Dev. Biol.* **124**, 91-110 (1987).

[5] Alfandari, D., Cousin, H., & Marsden, M. Mechanism of Xenopus cranial neural crest cell migration. *Cell. Adh. Migr.* **4**, 553-560 (2010).

[6] Moosmann, J, Ershov, A., Altapova, V., Baumbach, T., Prasad, M. S., LaBonne, C., Xiao, X., Kashef, J., & Hofmann, R. X-ray phase-contrast in vivo microtomography probes novel aspects of *Xenopus* gastrulation. *Nature* **497**, 374–377 (2013).

[7] Moosmann, J, Ershov, A., Altapova, V., Baumbach, T., Prasad, M. S., LaBonne, C., Xiao, X., Kashef, J., & Hofmann, R. Time-lapse X-ray phase-contrast microtomography for *in vivo* imaging and analysis of morphogenesis, *Nat. Protoc.* **9**, 294-304 (2014).

[8] Snigirev, A., Snigireva, I., Kohn, V., Kuznetsov, S., & Schelokov,I . On the possibilities of X-ray phase contrast microimaging by coherent high-energy synchrotron radiation, *Rev. Sci. Instrum.* **66**, 5486--5493 (1995).
[9] Wilkins, S. W., Gureyev, T. E., Gao, D., Pogany, A., & Stevenson, A. W. Phase-contrast imaging using polychromatic hard X-rays, *Nature* **384**, 335--338 (1996).
[10] Nugent, K. A., Gureyev, T. E., Cookson, D. F., Paganin, D. M, & Barnea, Z. Quantitative phase imaging using hard X rays, *Phys. Rev. Lett.* **77**, 2961--2964 (1996).
[11] Cloetens, P. Contribution to Phase Contrast Imaging, Reconstruction and Tomography with Hard Synchrotron Radiation: Principles, Implementation and Applications. PhD thesis Vrije Universiteit Brussel, (1999).
[12] Waller, L., Tian, L., & Barbastathis, G. Transport of intensity phase-amplitude imaging with higher order intensity derivatives. *Opt. Express* **18**, 12552--12561 (2010).
[13] Guigay, J.-P. Fourier transform analysis of Fresnel diffraction patterns and in-line holograms. *Optik* **49**, 121--125 (1977).
[14] Hofmann, R., Schober, A., Hahn, S., Moosmann, J., Kashef, J., Hertel, M., Weinhardt, V., Hänschke, D., Helfen, L., Sanchez Salazar, I. A., Guigay, J.-P., Xiao, X., & Baumbach, T. Gauging low-dose X-ray phase-contrast imaging at a single and large propagation distance. *Opt. Express* **24**, 4331--4348 (2016).
[15] Hahn, S.; Müller, Y., Hofmann, R., Moosmann, J., Öktem, O., Helfen, L., Guigay, J.-P., van de Kamp, T., & Baumbach, T. Spectral trasnfer from phase to intensity in Fresnel diffraction. To app. in *Phys. Rev. A* (2016).
[16] see *https://sourceforge.net/p/astra-toolbox/wiki/Home/*.
[17] see *ftp://xenbaseturbofrog.org/*.
[18] see *https://imagej.nih.gov/ij/* and *http://fiji.sc/*.