High-resolution X-ray phase-contrast tomography from single-distance radiographs applied to developmental stages of Xenopus laevis

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Abstract. Considering a pure and not necessarily weak phase object, we review a noniterative and nonlinear single-distance phase-retrieval algorithm. The latter exploits the fact that a well-known linear contrast-transfer function, which incorporates all orders in object-detector distance, can be modified to yield a quasiparticle dispersion. Accepting a small loss of information, this algorithm also retrieves the high-frequency parts of the phase in an artefact free way. We point out an extension of this highly resolving quasiparticle approach for mixed objects by assuming a global attenuation-phase duality. Tomographically reconstructing two developmental stages in Xenopus laevis, we compare our approach with a linear algorithm, based on the transport-of-intensity equation, which suppresses high-frequency information.

1. Introduction

Since its invocation [1–5] phase-contrast X-ray microtomography has evolved into a routine 3D imaging method which is used in particular at modern synchrotrons. Reasonable signal-to-noise ratios at low dose depositions in essentially pure-phase objects and quantitative imaging suggest a great potential for developmental biology. Here we are concerned in particular with single-distance propagation based phase-contrast tomography using a parallel, monochromatic beam. The experimental setup is undemanding and, within in the Fresnel regime, easily adaptable to geometric magnification [6]. Presently, we report on results obtained for fixed stages of early embryogenesis (pure-phase X-ray but optically opaque objects) in wild-type *Xenopus laevis*, but we also provide an outlook on *in vivo* time-lapse analysis [7].

While 3D *in vivo* tracking of fluorescently marked cell parts in translucent embryos is well developed in visible-light microscopy [8, 9], 3D microimaging of cells in opaque living samples is in its infancy. Here, imaging setups and phase retrieval algorithms should minimise residual radiation doses at acceptable signal-to-noise ratios and resolution levels, and for useful lengths of the time-lapse series. This excludes redundancies in the acquired data needed by certain *linear* models to determine the object transmission (multiple distances [10, 11], ptychography [12]). On the other hand, a large object-detector distance z is beneficial to generate high contrast

at a limited exposure time in single-distance, propagation-based phase contrast imaging. Also, strong phase variations take place in projections through entire embryos. As a consequence, the retrieval of phase maps encoding subcellular structure information poses a nonlinear and nonlocal problem. In [13, 14] this problem was addressed by a noniterative quasiparticle approach: Nonlinear corrections to the linear and local "dispersion" between Fourier transformed intensity and phase are shown to respect certain characteristics of the contrast-transfer function for a large range of propagation distances and upscalings of a weakly varying phase map. Here we present more experimental evidence for the validity of this quasiparticle approach and point out an extension to include absorptive effects.

2. Single-distance phase retrieval for strong phase objects and large propagation distances

Let us present a brief review of the quasiparticle approach [13, 14] for pure-phase objects before we apply it to experimental data. Also, we would like to point out an extension assuming global phase-attenuation duality [15].

Up to quadratic order in the exit phase map $\phi_{z=0}$ an important relation between intensity I_z and object transmission $I_o \exp(i\phi_{z=0})$ [16, 17], specialised to a pure-phase object, predicts the following representation of the intensity contrast $g_z \equiv \frac{I_z - I_{z=0}}{I_{z=0}}$ [14]:

$$
(\mathcal{F} g_z)(\vec{\xi}) = 2\sin(s) \, (\mathcal{F} \phi_{z=0})(\vec{\xi}) - \cos(s) \int d^2\xi' \, (\mathcal{F} \phi_{z=0})(\vec{\xi'})(\mathcal{F} \phi_{z=0})(\vec{\xi} - \vec{\xi'})
$$

$$
+ e^{is} \int d^2\xi' \, e^{-\frac{4\pi^2 iz \vec{\xi} \cdot \vec{\xi'}}{k}} (\mathcal{F} \phi_{z=0})(\vec{\xi'})(\mathcal{F} \phi_{z=0})(\vec{\xi} - \vec{\xi'}) + O((\mathcal{F} \phi_{z=0})^3), \quad (1)
$$

where $s \equiv \frac{2\pi^2 z \vec{\xi}^2}{k}$, $k = \frac{2\pi}{\lambda} = \frac{2\pi E}{hc}$, λ is the wave length of the monochromatic, parallel X-ray beam, E is the energy of its photons, h and c denote Planck's quantum of action and the speed of light in vacuum, respectively, and $\mathcal{F}$ denotes 2D (transverse) Fourier transformation, $\vec{\xi}$ being the 2D transverse wave vector. To linear order $(\mathcal{F} g_z)(\vec{\xi})$ exhibits zeros at $|\vec{\xi}|_n \equiv \sqrt{(kn)/(2\pi z)}$ ($n = 0, 1, 2, \cdots$). Upscaling $\phi_{z=0}$ as $\phi_{z=0} \to S\phi_{z=0}$ ($S > 1$) away from the regime, where the linear order in Eq. (1) represents a good approximation, it was shown in [13, 14] that the effects on $|\mathcal{F} g_z|_{S>1}$ are twofold: (i) the former zeros $|\vec{\xi}|_n$ of $|\mathcal{F} g_z|$ become minima of $|\mathcal{F} g_z|_{S>1}$, and (ii) these minima grow much slower than the maxima when increasing S. Starting at a critical value S_c of S, which typically corresponds to maximal phase variations of about 3.5, this behaviour no longer holds. Namely, the minima $|\vec{\xi}|_n$ rapidly start to move away from their formerly fixed positions, and a sinusoidal modulation of $|\mathcal{F} g_z|_{S>1}$ no longer persists. Thus, within the window $1 \leq S \leq S_c$ and in taking into account the linear order in $\phi_{z=0}$ only phase retrieval can be performed by replacing the left-hand side of Eq. (1) by:

$$
(\mathcal{F} g_z)(\vec{\xi})_{S>1} \to \Theta\left(\left|\sin\left(\frac{2\pi^2 z}{k}\vec{\xi}^2\right)\right| - \epsilon\right) \times (\mathcal{F} g_z)(\vec{\xi}). \quad (2)
$$

Here $\frac{2\pi^2 z}{k}\vec{\xi}^2 > \frac{\pi}{2}$, Θ denotes the Heaviside step function, and ϵ is a threshold ($0 < \epsilon < 1$) such that regions about the minima $|\vec{\xi}|_n$ of $|\mathcal{F} g_z|_{S>1}$ are centrally cut out from $(\mathcal{F} g_z)(\vec{\xi})_{S>1}$. Division by the zero of $\sin(s)$ at $s = 0$ still requires regularisation which is achieved by letting

$$
\sin(s) \to \sin(s) + \alpha, \quad (3)
$$

where $0 < \alpha \ll 1$. For a given value of α (which mimics the effects of nearly homogeneous absorption under a duality assumption, see below) the retrieval result was shown to be practically independent of ϵ within a broad range of values $\epsilon \ll 1$ [13] For $\epsilon \to 1$ the result is close to the

one obtained using the linearised transport-of-intensity equation (linearised TIE) [18,19]. Also, for $\epsilon \ll 1$ the obtained phase map conforms to the resolution of the linear-order result in the regime of the latter's applicability.

We expect that this quasiparticle approach can be extended to include attenuation $\exp(-B_{z=0})$ under a global phase-attenuation duality assumption [15] (homogeneous chemical composition): $\phi_{z=0} = -\frac{1}{\alpha} B_{z=0}$ where $\alpha \equiv \frac{\delta}{\beta}$ is a positive, real constant determined by the real increment δ and the imaginary part β of the refractive index n. Under this assumption the linear order on the right-hand side of Eq. (1) transmutes into: $2(\sin(s) + \alpha \cos(s)) (\mathcal{F} \phi_{z=0})(\vec{\xi}) = 2\sqrt{1+\alpha^2} \sin(s + a) (\mathcal{F} \phi_{z=0})(\vec{\xi})$ where $a \equiv \alpha \arcsin\left(\frac{1}{\sqrt{1+\alpha^{-2}}}\right)$. Thus, for $a \neq l\pi$ $(l = 0, 1, \dots)$ no regularisation at $s = 0$ is required in the phase retrieval. By the duality assumption, a simultaneous rescaling $\phi_{z=0} \to S \phi_{z=0}$ and $B_{z=0} \to S B_{z=0}$ does not change the values of α and a. As a consequence, the zeros of $\sin(s+a)$ are not affected, and we expect a quasiparticle approach to hold as in the pure-phase case. It shall be mentioned that a multi-distance phase-retrieval approach was proposed in [20] which relaxes the assumption of a global phase-attenuation duality for the prior to iterative phase retrieval.

3. Developmental stages of Xenopus laevis

Let us now present and compare 3D reconstruction results based on single-distance phase retrieval using the linearised TIE [19] and the quasiparticle approach. The embryos imaged are of the wild type, were stored in ethanol, and embedded in a 3% agarose solution for the experiment. The latter was contained within an polyethylene tube. A parallel-beam setup for propagation based phase retrieval was used, and 1600 tomographic projections were recorded in a stepwise fashion using a camera-synchronised fast-shutter system (beamline ID19@ESRF). The estimated transverse coherence length at the sample is $90\,\mu$m for $E = 20\,$keV which is about twice the radius of the largest (endodermal) cell type at developmental stage 10.5 in Xenopus laevis. A hot-pixel filter was applied to all intensity maps (including flat and dark fields), and object images were flat- and dark-field corrected. In both phase-retrieval algorithms the same value of the regularisation parameter α was employed: $\alpha = 10^{-2.5}$. A filtered-backprojection (FBP) algorithm [21] with a linear ramp filter was used to reconstruct phase tomograms for both approaches, linearised TIE and quasiparticle. For a pure-phase object and zero noise any phase-retrieval algorithm, which is identical to linearised TIE as $\xi \to 0$, produces zero mean in the retrieved phase at a finite and global value of α [22]. Obviously, the quasiparticle approach is in this category. Because line integration is a linear operation we conclude that 3D reconstruction only yields the variation $\Delta\delta$ of the real increment of the complex refractive index n about its mean value. Notice that in any case, due to the application of the ramp filter in FBP a potential, the mean value of ϕ is ignored in the tomographic reconstruction of δ. The reconstruction of $\Delta\delta$, however, depends on the prescribed value of α which influences low frequencies. To determine a physical value for α one could reconstruct $\Delta\delta$ across the boundary between a homogeneous medium 1 and a homogeneous medium 2 of known, nontrivial values δ_1 and δ_2, respectively. Matching $\Delta\delta \equiv \delta_1 - \delta_2$ with the reconstructed $\Delta\delta_{\text{rec}}(\alpha)$, yields the physical value α_{phys}.

In Fig. 1(a) a 3D rendering of and a slice through the reconstructed fixed Xenopus laevis embryo in its 4-cell developmental stage are shown. Figs. 1(b) and (c) depict a region of interest within the same slice based on phase retrieval using linearised TIE and the quasiparticle approach, respectively. For this particular sample and setup the improvement of resolution between the former and the latter roughly is a factor of four. This is explained by the fact that linearised TIE simply inverts a regularised form of the Laplacian thus invoking a contrast-transfer function $\propto \vec{\xi}^2$, or $(\mathcal{F} \phi_{z=0})(\vec{\xi}) \propto \vec{\xi}^{-2}(\mathcal{F} g_z)(\vec{\xi})$. Thus the information at high frequencies is suppressed. The quasiparticle approach, on the other hand, invokes at bounded contrast-transfer function treating high and low frequencies on equal footing. In the present work, we

refrain from performing the above-sketched determination of α_{phys}. However, $\Delta\delta$ are typically 10% of $\delta_{\text{H}_2\text{O}}$ which is a reasonable variation. In Fig. 2 we represent a Xenopus embryo at stage

(a)   (b)   (c)   (d)
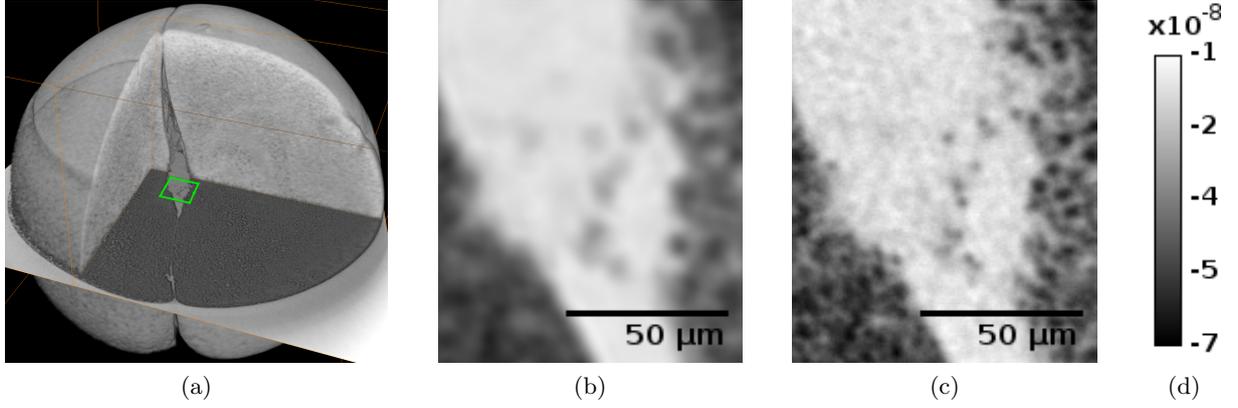
Figure 1. (a) 3D rendering of and slice through reconstructed Xenopus laevis embryo at 4-cell stage subject to quasiparticle phase retrieval. Region of interest within this slice subject to phase retrieval using (b) linearised transport-of-intensity equation and (c) quasiparticle phase retrieval. Experimental parameters are $E = 20\,\text{keV}$, $z = 0.945\,\text{m}$, exposure time $2\,\text{s}$, monochromaticity $\frac{\Delta E}{E} = 10^{-4}$, and effective detector pixel size of $\Delta x = 0.745\,\mu\text{m}$. The colorbar indicates the deviation $\Delta\delta$ from the mean real increment $\langle\delta\rangle$ of the real increment δ in the refractive index n. Notice that $\Delta\delta$ depends on the choice of α in Eq. (3), see discussion in text. The measurement was performed at beamline ID19@ESRF.

10.5 (start of gastrulation) in analogy to Fig. 1 but half the detector resolution. Also here an improved resolution could be achieved using the quasiparticle approach.

(a)   (b)   (c)   (d)
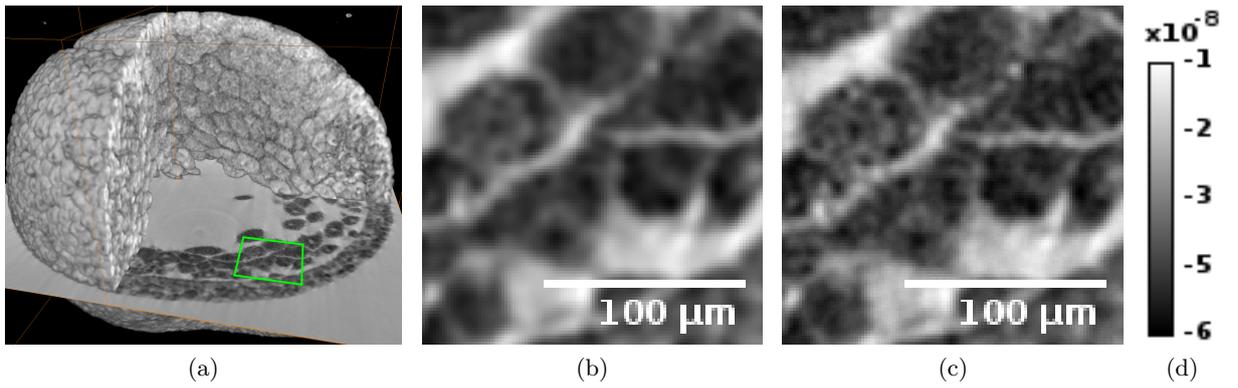
Figure 2. Image sequence in analogy to Fig. 1 but now for an embryo at developmental stage 10.5 (start of gastrulation), an effective detector pixel size of $\Delta x = 1.4\,\mu\text{m}$, $z = 0.949\,\text{m}$, and an exposure time of $2\,\text{s}$. The slice is a midhorizontal plane, perpendicularily intersecting the vegetal-pole animal-pole axis. The colorbar indicates $\Delta\delta$. The measurement was performed at beamline ID19@ESRF.

4. Summary

In this paper we have reviewed the quasiparticle approach of [13, 14] to the single-distance, propagation-based phase-retrieval problem in Fresnel theory for pure and strong phase objects

and large propagation distances. It is suggestive that this approach can be extended to include intensity modulations due to absorption by assuming a global phase-attenuation duality. We have, relying on X-ray phase-contrast data, presented 3D reconstructions of the electron density of early developmental stages in fixed *Xenopus laevis* embryos (pure-phase objects). Reconstructions are based on the conventional approach of a linearised transport-of-intensity equation as well as the quasiparticle approach to phase retrieval yielding improved spatial resolutions in the latter case.

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