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Phase contrast X-ray microtomography of the *Rhodnius prolixus* head: Comparison of direct reconstruction and phase retrieval approach

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HIGHLIGHTS

- We used SR-PhC-μCT.
- We compared slices of *R. prolixus*, with and without phase retrieval.
- The comparison suggests the complementary nature of the two approaches.

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ABSTRACT

We have used phase-contrast X-ray microtomography (PPC-μCT) to study the head of the blood-feeding bug, *Rhodnius prolixus*, which is one of the most important insect vector of *Trypanosoma cruzi*, ethiologic agent of Chagas disease in Latin America. Images reconstructed from phase-retrieved projections processed by *ANKA phase* are compared to those obtained through direct tomographic reconstruction of the flat-field-corrected transmission radiographs. It should be noted that the relative locations of the important morphological internal structures are observable with a precision that is difficult to obtain without the phase retrieval approach.

1. Introduction

Application of X-ray microtomography (μCT) on insects is quite recent (Hörnschemeyer et al., 2002, 2006; Beutel et al., 2008; Eberhard et al., 2010; Zhang et al., 2010) and its transposition to use phase contrast synchrotron X-ray microtomography (SR-PPC-μCT) is even more recent (Betz et al., 2007; Heethoff and Norton, 2009; Hönnicke et al., 2010; Kim et al., 2011; Ameida et al., 2012). A review on real time phase contrast X-ray imaging was given by Socha et al. (2007) but even after, Westneat et al. (2008) has published an excellent review on synchrotron X-ray imaging and outlined the many uses for anatomical imaging of living organisms.

The various methods of phase-contrast X-ray microtomography (PPC-μCT) can be classified as either qualitative or quantitative. The former class, utilized by most practitioners of phase contrast imaging, is primarily interested in qualitative (e.g., morphological) characteristics of the phase-contrast image produced by a given sample in a given imaging system. The latter class of imaging, commonly termed phase retrieval or phase reconstruction, seeks to infer quantitative phase and amplitude information from a given set of phase contrast images. This information may subsequently be related to the quantitative structural properties of a sample with which the phase-retrieved wave field has interacted (Paganin et al., 2002).

In this work, the phase-amplitude retrieval approach from images obtained using PPC-μCT was considered. The 2D radiographic projection frames were processed with *ANKAphase* software (Weitkamp et al., 2011) based on the single-distance phase-retrieval method developed by Paganin et al., 2002. *ANKA phase*

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computer program is written in Java (Java Consortium, 2010) and can be run either as a stand-alone application or as a plugin to ImageJ (National Institutes of Health, 2010), a widely used scientific image-processing program. This is of particular interest for scientific fields where excellent volume renderings are required, for example in biology.

Our focus in this study is the hematophagous insect, *Rhodnius prolixus*, which can transmit Chagas disease after feeding on humans in Central and South America where the insects are endemic (Rabinovich and Himschoot, 1990). The parasite, *Trypanosoma cruzi*, the ethiologic agent of Chagas disease, multiplies and differentiates in the intestinal tract of *R. prolixus. T. cruzi* often infects humans when it is passed out of the insect in the primary urine after the blood meal. In hematophagous insects, feeding triggers a long-term neuroendocrine response.

It is generally accepted that larvae of *Rhodnius prolixus* pass through a Head Critical Period (HCP) for ecdysis related to their feeding time (Wigglesworth, 1934). This period depends on a factor originating from the head, named prothoracicotropic hormone (PTTH)-ecdysone pathway, which interferes with *Trypanosoma cruzi* survival and development in its vectors (Fig. 1). Therefore, the neuroendocrine system has an important role in the transmission of the disease (Paluzzi et al., 2008). As a means to further understand the neuroendocrine system, preserved muscles and neurohemal sites within the head of *R. prolixus* were assessed by PPC-μCT.

2. Materials and methods

2.1. Sample preparation

Rhodnius prolixus (Hemiptera: Reduviidae) were reared and maintained at 28 °C and between 60% and 70% relative humidity. Fifth-instar nymphs were fed on rabbit blood using a membrane apparatus as previously described by Azambuja and Garcia (1997). Three days after blood meal, insects were immobilized at 4 °C for 10 min and bounded on a polystyrene table with entomological pins and transversally cut at the junction between prothorax and mesothorax segments of body. The anterior fragments were fixed and maintained at room temperature in a solution containing 1% glutaraldehyde and 5% sucrose in 0.1 M cacodylate buffer, pH 7.2 until using (not more than one month).

2.2. The SYRMEP beamline

The experiments were carried out at the third generation synchrotron radiation source of the ELETTRA synchrotron radiation Facility at the SYRMEP (Synchrotron Radiation on Medical Physics) beamline. A complete description of the beamline can be found in Abrami et al. (2005). The detector system is comprised of a 12/16-bit CCD camera (KODAK KAI-11000 CCD sensor), with 4008×2672 pixels^2, 4.5 mm pixel size CCD camera with a field of view of 18×12 mm^2, coupled to an intensifier screen with no magnification (1:1). The detection system was positioned at 10 cm away from the sample so that phase contrast technique could be performed. The projections were acquired on a range from 0 to 180°, in steps of 0.2°, resulting in 900 projections, with average acquisition time of 4 s per projection. The energy of the monochromatic beam was set to 12 keV.

The 2D radiographies are normalized by using flat and dark images in order to take into account incident beam non-uniformities and different efficiency of the detector elements, as well as to correct for fixed pattern noise. Tomographic raw images were reconstructed using an imaging processing software (SYRMEP_Tomo_Project) developed in the SYRMEP laboratory (Montanari, 2003) which uses Interactive Data Language (IDL). The reconstruction was performed using filtered back projection with Shepp Logan filter.

A standard procedure for processing a set of projections with *ANKAphase* requires, as user input, only the image file paths, the parameters of the experimental set-up (energy, distance, pixel size) and the estimated X-ray optical properties of the sample, δ (decrement of X-ray refractive index) and β (the absorption coefficient). Once the parameters are set, the full set of projection images can be processed.

3. Results and discussion

Until now, the internal structure of the head of *Rhodnius prolixus* is well known from histological methods. Previously, we studied the microanatomy of *Rhodnius prolixus* using SR-PPC-μCT (Almeida et al., 2012). Here, reconstructed tomographic slices in the ventral (Fig. 2) and sagittal (Fig. 3) planes are shown using phase retrieval approach.

In Fig. 2b, not only is the *protocerebrum* clearly visible, but also important neurohemal sites: the *corpora cardiaca* and the *corpus allatum* (Wigglesworth, 1948). In both Fig. 3a and b, muscles of pharynx are also visible. Details of the striation in the muscle, which depend on their orientation relative to the virtual cutting plane, can be observed more clearly in Fig. 3a. It is expected because of the resulting slices with phase retrieval approach exhibit an “area contrast” rather than an “edge-enhancing contrast” than enables a better visualization of the different details and structures (Weitkamp et al., 2011).

In Fig. 3b it can be seen other structures such as the *deutocerebrum*, the *tritocerebrum* and the prothoracic ganglion, unattainable with previously used technique. Further, in Fig. 3b, it is possible to access information on organs syntopy, such as the *deutocerebrum* lies laterally and ventrally to the brain, bellow the *protocerebrum* and the *tritocerebrum* lies medial to the *deutocerebrum*.

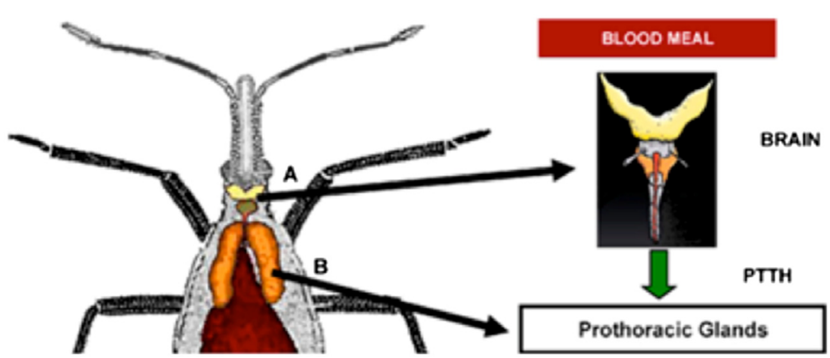

Fig. 1. A comprehensive scheme showing brain (protocerebrum) and the prothoracic glands of *Rhodnius prolixus*. Adapted from Garcia et al. (2007).