Applied Radiation and Isotopes 70 (2012) 1340–1343

Contents lists available at SciVerse ScienceDirect

Applied Radiation and Isotopes

journal homepage: www.elsevier.com/locate/apradiso

# Phase contrast X-ray synchrotron imaging for assessing external and internal morphology of *Rhodnius prolixus*

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ARTICLE INFO

Available online 25 November 2011

*Keywords:*
Synchrotron X-ray imaging
Phase contrast
Microtomography
*Rhodnius prolixus*
Chagas' disease

ABSTRACT

PhC-SR-μCT is a nondestructive technique that allows the microanatomical investigations and 3D images reconstructions. This technique is performed in blood sucker, *Rhodnius prolixus*—one of the most important insect vectors of *Trypanosoma cruzi*, ethiologic agent of Chagas' disease in Latin America—was able to provide excellent information about the microanatomy of the thorax and head allowing a new tool for further studies of development and physiology of triatomine by a non-invasive method of observation.



## 1. Introduction

Conventional X-ray computed tomography (CT) is based on the difference in radiation absorption by different tissues. At the same time, a wide range of samples used in biology and medicine demonstrate very weak absorption contrast, nevertheless producing significant phase shifts in the X-ray beam. Enhanced contrast X-ray imaging can be achieved by exploiting the real part of the refraction index, which is responsible for the phase contrast. Imaging of soft tissue demands high sensitivity and contrast-enhancing techniques in order to facilitate reliably detection among the structures and patterns of small size and of similar element compositions (Jakubeka et al., 2007). The main requirement is a high quality source with high spatial coherence, which can be provided by microfocus X-ray tubes or by high brilliance and low emmitance third generation synchrotron radiation sources. High coherency of the beam provided by third generation of the synchrotron radiation allows phase contrast imaging in the very simple experimental setup (Snigirev et al., 1995; Wilkins et al., 1996).

The development of synchrotron X-ray sources and phase contrast imaging techniques have resulted in major advances in both the microscopic imaging of preserved specimens and the real time X-ray video of the internal processes of living organisms (Cloetens et al., 1996). Recently, a review on real time phase-contrast X-ray imaging was given by Socha et al. (2007) but even after Westneat et al. (2008) published an excellent review on synchrotron X-ray imaging and outlined the many uses for anatomical imaging of living organisms.

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-PhC-μCT) is even more recent (Betz et al., 2007; Jakubeka et al., 2007; Westneat et al., 2008; Heethoff and Norton, 2009; Weide and Betz, 2009; Hönnicke et al., 2010; Kim et al., 2011). A detailed knowledge of insect's anatomical structures is crucial for a better understanding of their function and evolution. Traditionally, in both morphology and anatomy, the internal structures of whole organisms or parts of them are accessed by dissecting or performing fixed tissues to histological serial sections. The main limitation of the two most frequently used techniques (stereomicroscope and SEM) is the fact that they allow only the acquisition of 2D images or of partial 3D data. Microtomographic scanning of specimens allows acquisition of real volumetric 3D data that can be then used for 3D rendering and virtual manipulation of the sample in dedicated software (Pouecha et al., 2010).

In this work we used SR-PhC-μCT to study the microanatomy of *Rodhinus prolixus* which is one of the most important insect

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doi:10.1016/j.apradiso.2011.11.028

vectors of *Trypanosoma cruzi*, ethiologic agent of Chagas' disease in Latin America (Garcia et al., 2007). Chagas' disease (Chagas, 1909, 1911), also called American trypanosomiasis, is a human tropical disease, which is endemic in large areas of South and Central America. Among the parasitic diseases, Chagas' disease is ranked as one of the most important in Latin America in terms of social and economic impact, affecting about 18 million people, with about 100 million people living in what are considered to be high risk zones, and approximately 300,000 new cases occurring every year with around 21,000 deaths annually (Schofield, 1994; Moncayo, 2003; WHO, 2002).

Although *R. prolixus* is one of the most well-know model in terms of both physiology and vector-parasite interactions studies, little is known about the structures of its internal organs and how some morphophysiological aspects of mainly the retrocerebral complex in different time of the life cycle are related to the development and reproduction of this insect vector. We investigate the potential and advantages of SR-PhC-μCT with emphasis given to the 3D digital reconstruction of head and thorax structures in order to get preliminary information about density and plasticity of the neuroendocrine tissues, which is responsible for the development and reproduction of the insect.

## 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 in a BOD incubator. Fifth-instar nymphs were fed on human blood using a membrane apparatus as previously described by Garcia et al. (1984). 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 0.1M cacodylate buffer, pH 7.2 until using (not more than one month).

### 2.2. 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. The beamline provides a monochromatic laminar-section X-ray with a maximum area of about $160 \times 5$ mm$^2$ at 20 keV, at a distance of about 23 m from the source. The system consists of a Si (111) crystal working at Bragg configuration. The useful energy range is 8–35 keV. The intrinsic energy resolution of the monochromator is about $10^{-3}$. Typical flux measured at the sample position at 17 keV is about $1.6 \times 108$ photons/mm$^2$ s with a stored electron beam of 300 mA as ELETTRA operates at 2 GeV (Abrami et al., 2005). A custom-built ionization chamber is placed upstream to the sample to determine the exposure on the sample. A micrometric vertical and horizontal translation stage allows the positioning and the scanning of the sample with respect to the stationary beam and a rotational stage allows CT acquisition with a resolution of 0.001°.

The detector system is comprised of a 12/16-bit CCD camera, with $4008 \times 2672$ pixels$^2$, 4.5 μm pixel size CCD camera with a field of view of $18 \times 12$ mm$^2$, coupled to an intensifier screen with no magnification (1:1). The CCD camera can move along the sample-detector axis, in order to set the desired sample-to-detector distance d. According to the choice of the sample-to-detector distance, one may distinguish between the absorption and phase sensitive regimes.

The detection system was positioned at 10 cm away from the sample position so that phase contrast technique could be performed. The tomographs were performed on a range from 0 to 180°, in steps of 0.2°, resulting in 900 projections. The 2D radiographies are normalized by using flat (images without the samples) and dark (background) images. This procedure allows one to take into account incident beam non-uniformities and to correct fixed noise due to the efficiency of the detector elements. 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.

## 3. Results and discussions

μCT can be used as a qualitative imaging technique which permits to section tissues in their natural state virtually, in any direction, with sub micrometer resolution and without time-consuming elaborate dissections (Betz et al., 2007). μCT approach allows observing and rotating the virtual reconstruction of the specimen It is then easy to orient it and to generate any interesting viewing angle in dedicated software (in the present case, ImageJ). The application of SR-PhC-μCT to insect anatomy demonstrated the ability to visualize fine details of the head and thorax of *R. prolixus* showing a 3D model external view of representative morphological characteristics of the genus, as both the clypeous (ante- and postclypeous) an ventral part of the cranium (i.e. gula) extremely elongated (Fig. 1a) as well as the labium (i.e. proboscis) curved backwards in the resting position with its labella (Fig. 1b and c). The labrum is short and has twice successive parts (Fig. 1a and b). The composed eyes are located

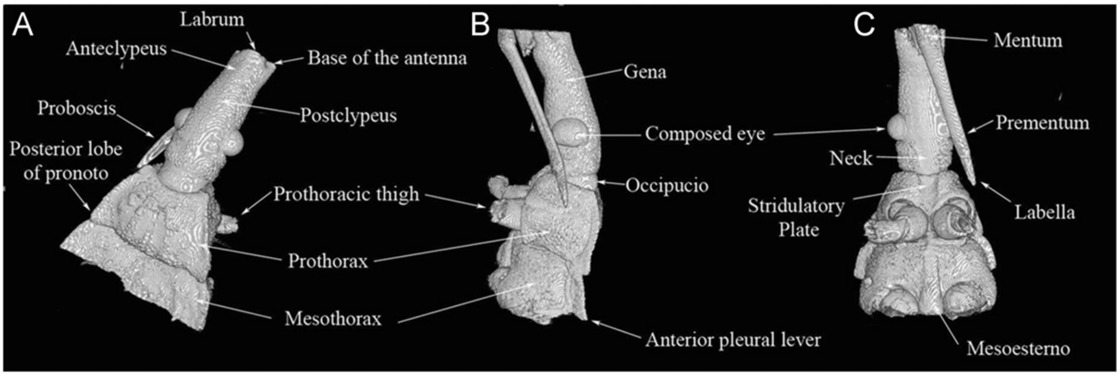


**Fig. 1.** Volume-rendered PhC-μC images showing external aspect of head and thorax of R. prolixus: dorsal (A), lateral (B) and ventral (C) views. Bar = 1000 μm.