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Synchrotron Microtomography to Explore the Anatomy of Insects

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Abstract: Recent advancements in high intensity synchrotron sources have revolutionized the field of non-destructive imaging, particularly in biological sciences. Synchrotron radiation micro-computed tomography (SR-microCT) has emerged as a powerful tool for visualizing complex 3D structures, from dense materials to delicate biological specimens. This technique enables unprecedented spatial resolution, facilitating detailed analysis of sub-tissue structures within organisms without invasive procedures. Additionally, X-ray phase-contrast imaging (PCI) has enhanced the visibility of soft tissues by exploiting phase shifts, complementing traditional absorption-based methods. This paper highlights the capabilities of SR-PCI in biological research, demonstrating its application on millimeter-scale samples of *Aedes aegypti* mosquitos and *Drosophila melanogaster* fruit flies at prominent synchrotron facilities.

Keywords: Microtomography, Synchrotron radiation, *Aedes aegypti, Drosophila melanogaster*

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Microtomografia por luz síncrotron para explorar a anatomia de insetos

Resumo: Avanços recentes em fontes de luz síncrotron de alta intensidade revolucionaram a área de imagens não destrutivas, especialmente nas ciências biológicas. A microtomografia por luz síncrotron (SR-microCT) emergiu como uma ferramenta poderosa para visualizar estruturas 3D complexas, desde materiais densos até delicados espécimes biológicos. Esta técnica permite uma resolução espacial sem precedentes, facilitando a análise detalhada de estruturas em organismos sem procedimentos invasivos. Além disso, a imagem por contraste de fase de raios X (PCI) tem melhorado a visibilidade de tecidos moles ao explorar deslocamentos de fase, complementando métodos tradicionais baseados em absorção. Este trabalho destaca as capacidades do SR-PCI na pesquisa biológica, demonstrando sua aplicação em amostras de milímetros de mosquitos *Aedes aegypti* e moscas *Drosophila melanogaster* em instalações de síncrotron de destaque.

Palavras-chave: Microtomografia, Radiação síncrotron, *Aedes aegypti, Drosophila melanogaster*

1. INTRODUCTION

Mainly due to the complexity of the 3D structures of biological objects, developments of high intensity synchrotron sources fostered the use of the spatial resolution of Synchrotron radiation micro-computed tomography (SR-microCT) as a promising non-destructive tool for imaging a broad range of samples, from dense materials to soft biological specimens. New scientific problems can now be tackled with SR-microCT stressing the need for more multidisciplinary approaches that encompass X-ray optics,biology, and 3D structure analysis [1]. Characterisation and quantification of sub-tissue structures within a whole organism demand for non-destructive imaging methods, fast image acquisition and high-resolution 3D reconstruction. SR virtual histology has been demonstrated as a non-destructive alternative to conventional invasive approaches. Microtomographic visualization followed by 3D analysis at submicrometer resolution of soft tissues, which account for a major proportion of biological tissues, have shed light on the structural mechanism of biological function [2]. Traditional methods such as dissection and histological sectioning often require specimen destruction and may lead to structural deformation or loss of spatial context. In contrast, micro-CT imaging allows for non-destructive 3D visualization, preserving the anatomical integrity and enabling multiplanar analysis [3-4].

The ability of absorption-based X-ray imaging to provide important insight into the field of life sciences is limited by the difficulties of visualising weakly absorbing objects (e.g. soft tissues) without the reliance of exogenous contrast agents. The phase-contrast (PC) technique has been widely used in the scientific community, as it is a technique associated with radiography and microscopy and able to enhance contrast in soft tissues, specifically at the edges, showing details that could not be seen by the absorption technique [5-8].

X-ray PC imaging (PCI) methods might be more suited for structures with a low difference in absorption contrast due to their ability to involve a combination of phase and absorption contrast in terms of the complex X-ray refractive index in a single exposure [9, 10].

In this work we will describe the advantages provided by SR-PCI as a nondestructive 3D imaging technique in the study of biological samples in the absence of X-ray contrast agents. Millimetre-sized samples of *Aedes aegypti* mosquitos and *Drosophila melanogaster* fruit flies are presented to illustrate the technique for anatomical and structural investigations. These studies were carried out at the SYRMEP (SYnchrotron Radiation for MEdical Physics) beamline of ELETTRA, Trieste, Italy [11] and at MOGNO, the micro and nanotomography beamline, at Sirius, the 4th generation storage ring at the LNLS (Laboratório Nacional de Luz Síncrotron), Campinas, Brazil, [12].

2. MATERIALS AND METHODS

2.1 Fruit fly Drosophila melanogaster

Drosophila melanogaster is one of the most used model organisms for biomedical science since its low cost, rapid generation time, and excellent genetic tools have made the fly indispensable for basic research. From human disease modelling to the dissection of cellular morphogenesis, behavior and aging, this issue examines the current uses of flies and the influence of fly research on other models [13]. Previous studies in fixed samples of D. melanogaster, using a SkyScan 2211 (Multiscale X-ray NanoCT System, Bruker micro-CT, Belgium) at Oral Research Laboratory, University of Oslo, have found that that larval density leads to a tissue-specific effect on male reproductive organs [14].

Advances in SR-PCI technology, in terms of detectors, sub-micron spatial resolution, faster scan acquisition, computational analysis, and in the development of imaging protocols have been crucial for evaluation of non-destructively 3D anatomical and morphological data in life sciences. In order to improve quality for a better understanding of the *D. melanogaster,* we pursued new investigations involving the D. melanogaster head at SYRMEP beamline.

The specimens were mounted in Pluronic (F-127) hydrogel for stabilization, and were positioned at a sample-detector distance of 100 mm. Projections were recorded with a 16-bit, air-cooled sCMOS camera, average energy of 16 keV and the effective pixel size 1.5 µm pixel. Reconstruction was performed using the filtered back projection (FBP) algorithm, with careful parameter selection to guarantee the best contrast while minimizing information loss.

2.2 Aedes aegypti mosquito

The *Aedes aegypti* mosquitos are the main vectors of several arboviruses, responsible for the dengue, Zika, yellow fever and chikungunya. A recent study has shown that there are 215 countries potentially suitable for the survival and establishment of *Aedes aegypti* and globally, 146 countries reported at least one arboviral disease [15]. Millions of dollars are spent annually to eradicate these arboviral diseases. Until now, there is no effective remedy against these arboviruses, so disease prevention can be done in two ways. One is the reduction of mosquito infestation, a measure that has been promoted and carried out in recent years by research centers in some countries. And the other would be the use of an effective vaccine. However, the best way to eradicate it is still vector control [16].

In the preliminary experiments, *Aedes aegypti* of the Rockefeller strain was imaged at the SYRMEP beamline using a white (polychromatic) X-ray beam with the mean energy 20keV, in combination with a sample-detector distance of 15cm and exposure time per projection of 100ms. A 2.2µm effective pixel size was set. The eggs are provided by the Laboratory of Physiology and Control of Vector Arthropods - LAFICAVE of the Oswaldo Cruz Foundation - FIOCRUZ.

A second experiment was performed at MOGNO beamline, which was designed to obtain micro and nano-imaging using a quasi-monochromatic tender (22 and 39 keV) and hard (67.5 keV) X-rays with high photon flux. This beamline operates in a cone beam geometry, with variable field of view (150 µm - 85 mm), and spatial resolution (120 nm - 55 µm). MOGNO will be dedicated to operate in zoom tomography, where the sample can be investigated using low-and-high resolution, and 4D imaging through in-situ experiments [12]. In addition, a high-brightness synchrotron light source, such as Sirius, allows tomographic images to be obtained with better image contrast and greater spatial and temporal resolution. The images were obtained using an indirect detection system based on a sCMOS camera and a microscope, the energy was 22keV and the acquisition time was 1,5s per projection. By exploiting the variable field of view and continuous pixel demagnification, the samples were imaged in different resolutions, 1142 nm (in PC mode) and 2287nm (in zoom-tomography).

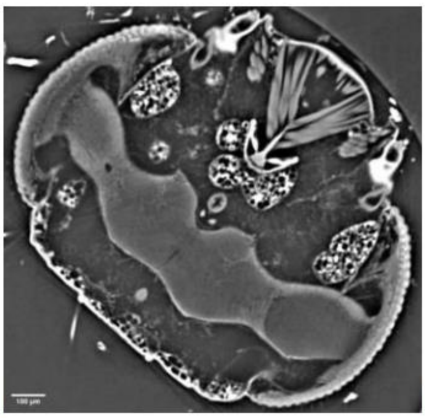
3. RESULTS AND DISCUSSIONS

3.1 Fruit fly Drosophila melanogaster

The microstructure of the specimens was observed and measured using the visualization and measuring features of the ImageJ software package. 3D reconstruction is shown in Figure 1a where the brain Veins can easily be identified (in orange). Figure 1b shows an example of the transverse cross section of D. melanogaster head.

Figure 1: (a) 3D rendering of the whole body of *D. melanogaster* (lateral view). (b) Virtual section through the head in the transverse sectional plane

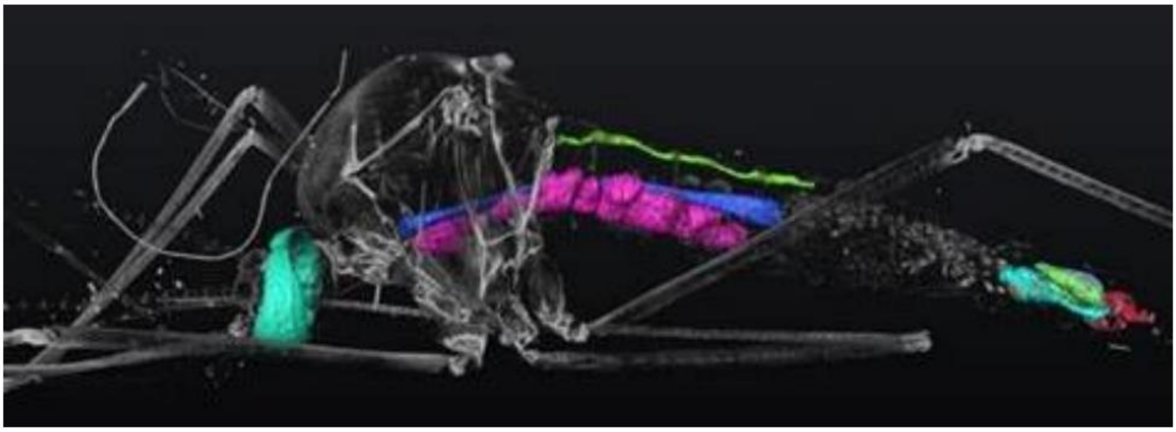

(a)


(b)

3.2 Aedes aegypti mosquito

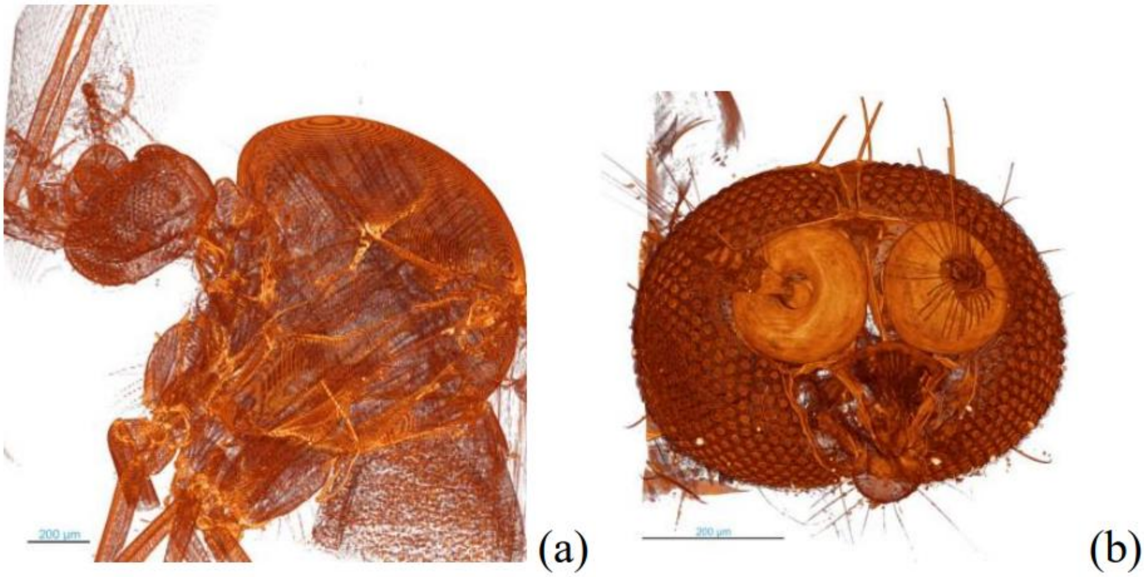
The images obtained at SYRMEP beamline allow us to visualize the entire body of this insect and to identify and segment different and important *Aedes aegypti* structures (Figure 2), which has never been done before with this technique. The identification and visualization of these specific organs provide valuable anatomical detail for studying the mosquito's physiology and vector competence. Segmenting structures involved in pathogen transmission and circulation enhances the utility of micro-CT data, allowing for targeted analyses relevant to disease control and functional morphology.

Figure 2: 3D rendering of the whole body of Aedes aegipti showing mainly the heart (green), the glands (pink) and the colon (red).



Regarding the available techniques, MOGNO works on absorption and propagation-based phase contrast imaging modes. In addition, due to the cone beam geometry, zoom in tomography will also be available, where the same specimen will be imaged in different resolution, without the need of resizing the sample. Figure 3a shows a 3D reconstruction of a *D. melanogaster* specimen (lateral view). In Figure 3b (zoom tomography) the compound eyes are visible on both sides of their head.

Figure 3: (a) Volume-rendered image (lateral view) of the whole body of *Aedes aegipti* specimen. (b) External section of *Aedes aegipti* head.



CONCLUSIONS

We showed the importance of SR-PCI in the study of biological samples, highlighting the ability to obtain detailed structural information, with high spatial resolution and depth, without the necessity of dissection processes. The potential of utilizing advanced synchrotron sources like the MOGNO beamline at Sirius (Brazil) presents exciting prospects.

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CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

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