Università degli Studi di Napoli "Federico II"

PhD Thesis
in
Novel Technologies for Materials, Sensors and Imaging

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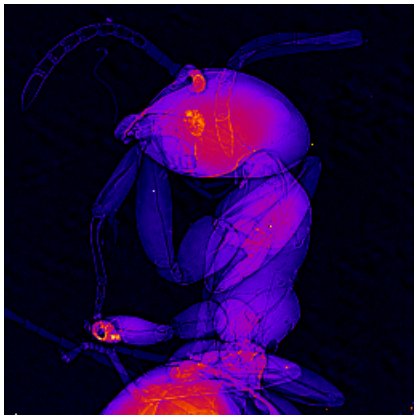
Single Photon Counting X-Ray Micro-Imaging of Biological Samples

Paola Maria Frallicciardi



Supervisor:

Prof. Paolo Russo

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Abstract

In this thesis we compared the experimental technique of Single Photon Counting (SPC) imaging to the charge integrating Flat Panel (FP) detector imaging, for X-ray biomedical imaging applications. In particular, we investigated the application of SPC detector for the X-ray micro-imaging and X-ray volumetric Computed Tomography (CT) technique.

The motivation for such a research arises from the potential advantages of the single photon counting technology. In fact, this detection modality allows to have an efficient suppression of the electronic noise, scatter radiation rejection and immunity for afterglow effect of scintillator-based detectors, thanks to a read-out scheme able to discriminate photons with energy above a chosen threshold. This means that, during the exposure, the signal increases but not the noise, leading to excellent values of the image quality parameters such as the Signal-to-Noise Ratio (SNR) and the Contrast-to-Noise Ratio (CNR). In SPC imaging, each interacting photon is counted as one single event, independently of its energy, so that soft X-rays are equally weighted compared to the harder ones. This results into a high Contrast (C) also for low attenuating objects, such as soft tissues in an organism or small biological samples. On the contrary, charge integrating detectors (and FP detector among this class of devices) integrate both signal and noise, and high energy photons bring a larger weight than low energy ones. These high energy photons, however, contribute less to the detectability (SNR) and to the visibility (C) of low contrast samples, since material attenuation generally decreases with increasing energy.

The aim of this thesis is to experimentally demonstrate the feasibility of planar, real-time and tomographic X-ray imaging, with special interest for small biological samples, both *in vivo* and *post-mortem*, utilizing an SPC detector. Since the use of this technology is regarded as an alternative to the more commonly employed charge integrating systems, a comparison with an FP detector, in terms of image quality parameters (SNR, C, CNR) evaluation has also been done.

In this thesis we have used the SPC silicon (300 μm thick) pixel detector Medipix2 (*www.cern.ch/medipix)*.

Planar images of insects, plants, seeds have been acquired at a resolution level of about 4 μm with high image quality. Small anatomical and organic structures as insect respiratory system, insects' feelers details, leaf stoma and webbed veining features have been visualized in great detail, also taking advantage of phase contrast enhancement effects. In fact, thanks to the favourable experimental conditions - micrometric focal spot size (5 μm), suitable source-to-sample and sample-to-detector distances and SPC detector pixel size (55 μm) - the X-ray phase shift has been exploited jointly with the X-ray absorption, allowing the visualization of low-attenuating samples.

The Medipix2 detector read-out time, the high acquisition rate (100 kHz count-rate per pixel) and the possibility of arbitrarily setting the exposure time, make Medipix2 suitable for real-time imaging of moving objects at a reasonable frame-rate of few frames per second. This detector represents a non-invasive tool for *in vivo* investigations of small insects' life and allows entomologists to follow a biological sample through all its evolutional processes for longitudinal studies. As an example of live X-ray imaging, X-ray planar images of a living parasite, while moving in its natural environment (its host's body), have been acquired with a frame rate of 2 frames/s, with high contrast resolution. A series of planar images of the same sample, acquired in different periods of time, show the living object in several stages of its natural biological evolution, making clearly visible the morphological changes in the animal's body anatomy. 3D X-ray micro-imaging of the living parasite inside its host has been done with a voxel resolution of 17 μm x 17 μm x 21 μm. After the study the sample was still alive for further investigations.

The achieved spatial and contrast resolutions, both in 2D and in 3D images, can be regarded as adequate to detect the main morphogenetic changes in outer anatomy, as well as for observation of inner anatomy features of small insects and organic samples. The dynamics of biological processes, as well as of biological growth and changes can also been satisfactorily followed. The possibility of tomographic imaging enables either to virtually cut the specimen into 2D slices or to have a comprehensive visualization of its 3D model for a non-invasive investigation.

The results obtained are comparable to those achieved with modern integrating systems that use large facilities as a synchrotron light source but, in addition, allow for routine and highly sensitive investigations in laboratories.

A comparison study has also been carried on between the experimental SPC detector and a commercially available Flat Panel integrating detector (CsI:Tl scintillator coupled to a CMOS flat panel), in terms of image quality for planar and tomographic imaging. The two detection technologies have been compared on 2D and 3D imaging of both phantoms and biological samples. Image quality parameters have been evaluated on images acquired with the two detectors in the same experimental conditions (geometry, X-ray tube energy, exposure, etc.), showing in all the investigated cases a higher performance of the SPC technology. We observed that the SPC technique decreases significantly the fluctuations in the signal noise, permitting a higher image quality in a large attenuation range and for low X-ray energies (40 kVp tube voltage), as used for micro-imaging on biological samples.

To sum up our results, we believe that an SPC detector permits for high quality X-ray imaging. Moreover, its image quality performance is higher when compared to the charge integrating FP detector one, as far as both planar and tomographic images are concerned.