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High contrast radiography of normal and cataractous canine lenses

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Abstract
Cataract is a disease that degrades the transparency of crystalline lenses. The crystalline lens is a cellular structure that has a unique shape and protein composition. Cataract is associated with changes in the structure and composition of the lenses. Analyser-based x-ray phase contrast imaging (PCI) is a non-destructive technique that presents images with more contrast and details than the images acquired with conventional synchrotron radiography. Here, an analyser-based x-ray PCI set-up was optimized in the XRD2 beamline at Brazilian Synchrotron Light Laboratory for comparative studies on PCI and conventional synchrotron radiography, for non-cataractous (healthy tissue) and cataractous crystalline (diseased tissue) lenses. Refraction angle and apparent absorption contrast images (diffraction enhanced imaging—DEI) were also obtained. The present PCI and DEI images indicate that the healthy tissue shows enhanced shell structures, while in the diseased tissue these are almost absent. This is associated with the clinical case of total opacity of the cataractous crystalline lenses when it is exposed to visible light.

1. Introduction

Ultrasound imaging is a well-established technique for ophthalmological diagnosis [1, 2]. The crystalline lens is an individual layered tissue. Diseases related to the crystalline lenses are associated with changes in their structure and composition [3, 4]. Much work has been done to understand the structure of this tissue [5].

Conventional radiography is based on the x-ray attenuation contrast of different parts of a sample. Low contrast images are found when different details of an object have almost the same attenuation coefficient for the used x-ray energy. Enhanced contrast radiographic images can be obtained by using contrast agents. Alternatively, several techniques exploiting the real part of the refractive index, which is responsible for the phase shifts, in addition to the imaginary part that is responsible for the absorption, have been proposed to enhance the contrast. Such techniques are known as x-ray phase contrast imaging (PCI). Analyser-based x-ray PCI has been applied for *in vitro* studies with soft tissues, such as for breast cancer and articular cartilage [6–9]. Furthermore, such a technique may allow

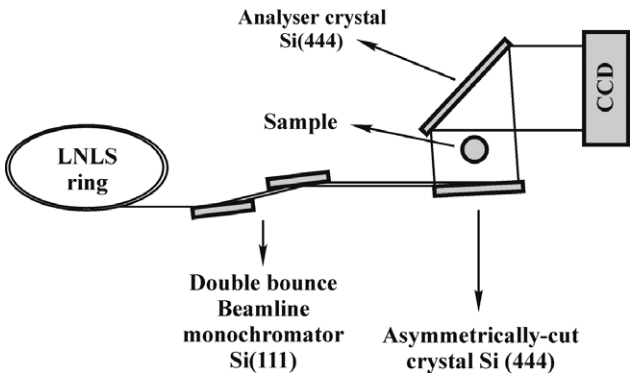

Figure 1. Schematic diagram of the DEI set-up in the XRD2 beamline LNLS.

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a low radiation dose for the analysed samples, when compared with conventional radiography. Three superposed types of contrasts are enhanced in PCI: refraction contrast, ultra-small-angle x-ray scattering (extinction contrast) and pure absorption contrast. Mathematical processing images, like diffraction enhanced imaging (DEI) [10, 11] can be used to obtain a refraction angle image, representing the spatial gradient of the refractive index [12], and also to obtain an apparent absorption contrast image that shows contrast arising only from pure absorption contrast and extinction contrast.

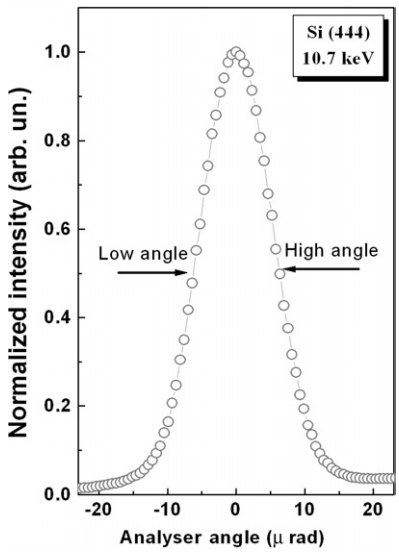

Figure 2. Rocking curve at 10.7 keV.

In this work a PCI set-up was used for comparative studies on PCI and conventional synchrotron radiography with non-cataractous (healthy tissue) and cataractous crystalline (diseased tissue) lenses. Images obtained with the DEI mathematical processing were also exploited. A description of the experiment followed by the results and conclusions will be presented.

2. Experiment

The experiment was carried out in the XRD2 beamline at the Brazilian Synchrotron Light Laboratory (LNLS). A double bounce Si(111) premonochromator upstream of the experimental hutch was used to select the energy at 10.7 keV. A non-dispersive set-up (figure 1) with an asymmetric Si(444) monochromator, as a first crystal, to expand the beam 25 times in the vertical scattering plane and a symmetric Si(444) as a Bragg analyser were mounted on a double axis diffractometer,

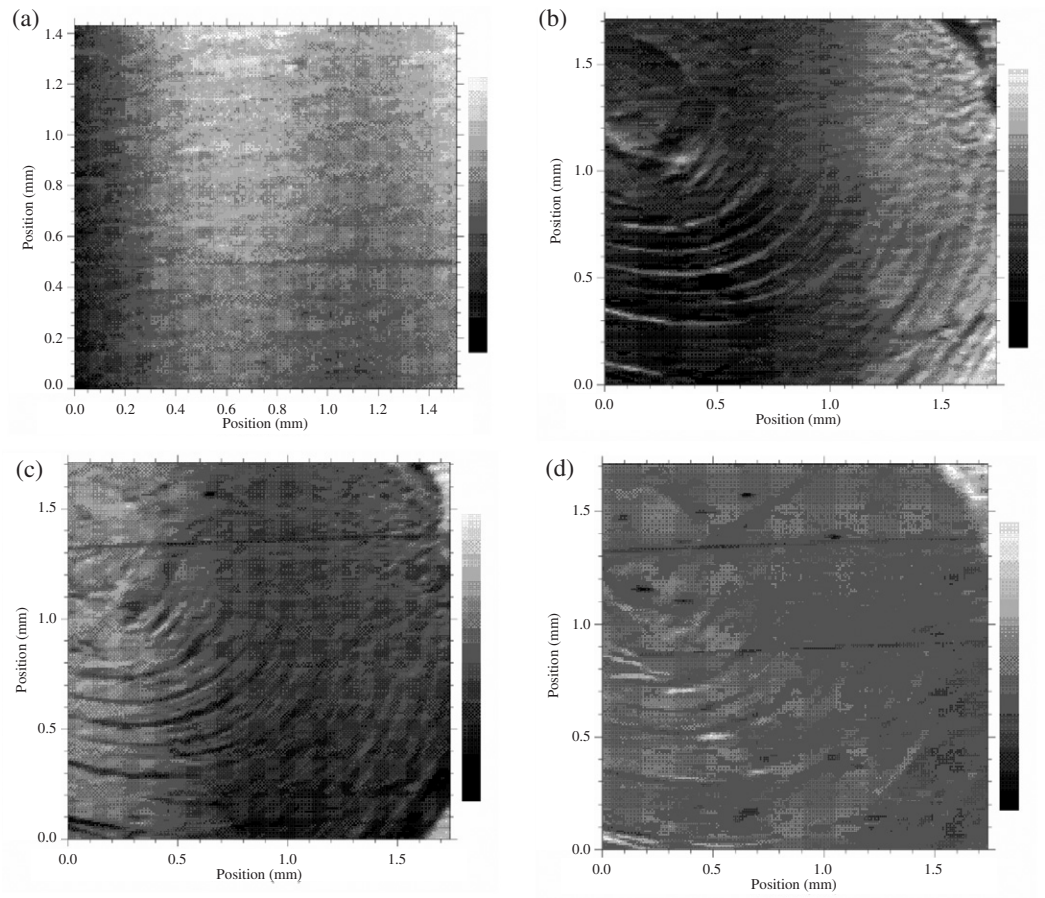

Figure 3. Images of healthy canine crystalline lens: (*a*) conventional synchrotron radiography, (*b*) details of structure at maximum slope angular position (low angle), (*c*) at maximum slope angular position (high angle) on the rocking curve of the analyser crystal and (*d*) refraction angle image obtained through DEI.

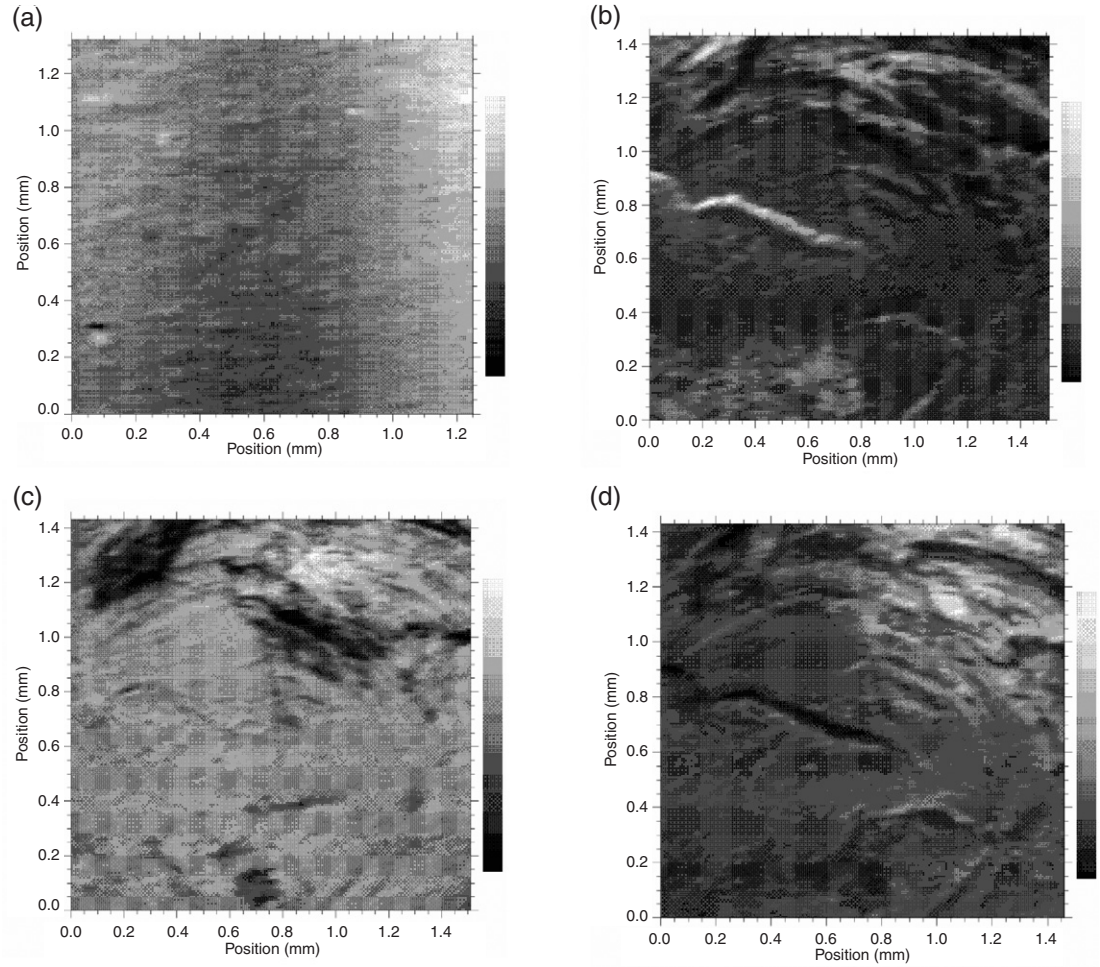

Figure 4. Images of the cataractous crystalline lens: (*a*) conventional synchrotron radiography, (*b*)–(*d*) PCIs at different angular positions on the rocking curve (on the top and on the slopes). The degradation of the structural integrity of the tissue (shell shaped) can be seen.

independently of the beamline monochromator, to ensure thermo-mechanical stability. The samples (crystalline lenses) were set between the two crystals. The images were acquired with a direct conversion 1242 × 1152 pixel (22.5 × 22.5 μm^2, each) CCD detector. PCIs were acquired at different angular positions on the rocking curve of the Si(444) analyser crystal. In its final form, all the PCIs were corrected pixel to pixel with background (darkfield image—shutter closed) subtraction of the flatfield correction (image of the beam without the sample). Refraction angle and apparent absorption contrast images through the pixel to pixel DEI algorithm were obtained. The procedure to obtain DEI images was described by Chapman *et al* [10]. Basically, two images (PCIs) were obtained on the maximum slope angular positions (high and low angle side) on the rocking curve (figure 2). For PCIs obtained at exactly 50% reflectivity points, the apparent absorption contrast image is simply the sum of the high and low angle PCIs, while the refraction angle image is the difference [12].

Conventional synchrotron radiographs were also acquired by just setting the sample after the crystals in contact with the CCD detector. Background subtraction and flatfield correction were also applied for these images.

3. Results and discussion

Comparative studies on PCI, DEI and conventional radiography with healthy and diseased canine crystalline lenses were done. Figure 3 shows a conventional synchrotron radiograph, PCIs at different angular positions of the analyser crystal and a refraction angle image (obtained with the help of the DEI algorithm) of a healthy canine crystalline lens. Details of the structure (shell shaped) of the lenses are not seen in the conventional synchrotron radiograph and are clearly seen in the PCI and refraction angle image. In figure 4 the images of a cataractous canine lens are shown. It was characterized by a total opacity of the lens when observed with visible light. Figure 3(*a*) shows the conventional synchrotron radiograph with an absence of detail. This is very similar to figure 4(*a*). PCIs acquired at different angular positions on the rocking curve and a refraction angle image are shown in figures 4(*b*)–(*d*). A drastic difference is noted when comparing these last images with those presented in figures 4(*b*)–(*d*). The structure (shell shaped) previously noted in figures 3(*b*)–(*d*) are not well defined in figures 4(*b*)–(*d*). These results show that the cataractous canine lens shows a complete degradation of structural integrity formed by concentric elliptical layers. The spatial resolution was limited by the pixel size of the CCD

detector ($22.5 \times 22.5\,\mu m^2$) because it is a direct conversion CCD and the optical elements between the sample and the CCD are only the highly perfect Si analyser crystal and the beryllium window in front of the chip detector.

4. Conclusions

Comparative studies on analyser-based x-ray PCI, DEI and conventional radiography with healthy and diseased canine crystalline lenses were done. Differences between the structure of healthy (non-cataractous) and diseased crystalline tissues (cataractous) were observed in PCI and DEI, but were not detected with conventional synchrotron radiography. Distinct changes in the structural integrity were observed in accordance with medical diagnostics.

Acknowledgments

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