Available online at www.sciencedirect.com
ScienceDirect
Nuclear Instruments and Methods in Physics Research A 582 (2007) 208–211
NUCLEAR INSTRUMENTS & METHODS IN PHYSICS RESEARCH Section A
www.elsevier.com/locate/nima

Characterization of diffraction enhanced imaging contrast in plants

T. Kao[a,*], C.-J. Liu[b], X.-H. Yu[b], L. Young[c], D. Connor[a], A. Dilmanian[d], C. Parham[a,e], M. Reaney[f], Z. Zhong[a]

[a] National Synchrotron Light Source, Brookhaven National Laboratory, Upton, NY 11973, USA
[b] Biology Department, Brookhaven National Laboratory, Upton, NY 11973, USA
[c] Department of Plant Science, University of Saskatchewan, Saskatoon, Canada S7N 5A8
[d] Medical Department, Brookhaven National Laboratory, Upton, NY 11973, USA
[e] Biomedical Research Imaging Center, University of North Carolina, Chapel Hill, NC 27599, USA
[f] Department of Applied Microbiology and Food Science, University of Saskatchewan, Saskatoon, Canada S7N 5A8

Available online 17 August 2007

Abstract

Diffraction Enhanced Imaging (DEI) was used to characterize the extinction contrast coefficient of plant tissues. Single-cells were imaged with planar DEI. The organized structures of the plant cell walls were found to exhibit significant extinction contrast and were readily visualized using DEI. Extinction properties of different tissue types in plant roots, stems and seeds were characterized by DEI in Computed Tomography (DEI–CT) mode at the peak of the analyzer-rocking curve. DEI–CT yields different extinction coefficients for different cell types, resulting in high-contrast, high-resolution images of internal anatomical structures of plants.

PACS: 87.59.−e; 87.62.+n; 07.85.Qe

Keywords: Diffraction enhanced imaging; Computed tomography; Rocking curve; Extinction contrast; Plant; Cell wall structure

1. Introduction

Diffraction Enhanced Imaging (DEI) is a new X-ray imaging modality described in detail by Chapman et al. [1], which utilizes a perfect silicon analyzer crystal to provide contrast in addition to X-ray attenuation. The sharp rocking curve of the analyzer crystal converts X-ray angular change to intensity change. The analyzer crystal can be tuned to the peak of its rocking curve such that it creates contrast due to the rejection of small angle scattering from the sample (extinction contrast) [2]. In addition to extinction contrast and attenuation, DEI imaging on the shoulder of the rocking curve uses the X-ray refraction of the sample as an additional contrast mechanism [3,4]. Previous studies using DEI have demonstrated enhanced contrast as compared to conventional X-ray radiography, especially for low density, poorly absorbing materials such as soft tissues in animals [5–7]. The non-destructive imaging of seeds using DEI at sub-lethal absorbed radiation dosages has also been demonstrated [8,9]. These studies suggest the potential for using DEI to complement biochemical analysis when screening collections of plants with gene knockouts or mutations and for the study of dynamic developmental events. The understanding of the link between DEI contrast mechanisms and the cellular structure of the plant is necessary for evaluating the potential for DEI in plant systems. In this study, we characterized the extinction contrast of typical plant structures by DEI in Computed Tomography (CT) mode [10].

2. Experimental method

Experiments were carried out at beamline X-15A at the National Synchrotron Light Source (NSLS) at Brookhaven National Laboratory (BNL) [2]. A double crystal silicon monochromator, 111 or 333, was used to select 20 or 40 keV X-rays from the white X-ray beam supplied by the synchrotron to form the beam striking the sample.

*Corresponding author.
E-mail address: kao@bnl.gov (T. Kao).

0168-9002/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.nima.2007.08.137

An additional analyzer crystal, similar to the type used in the monochromator, was placed between the sample and the detector. The analyzer has a triangular shaped reflectivity profile or rocking curve. A Photonic-Science (Photonic Science Limited, UK) X-ray camera, with a 9-μm pixel size and a 36 mm × 24 mm imaging area, was used to detect the X-rays.

For DEI–CT at 40 keV, the 111 analyzer was tuned to the peak of the rocking curve in order to reject X-rays that are scattered by more than a few micro-radians, thus maximizing extinction contrast [10]. For each CT image, 800 (seed, stem, and silique) or 2000 (carrot, celery, and onion) projections were acquired with a sample rotation step size of 0.45° and 0.18°, respectively. The integration time for each projection was 0.5 s, resulting in a total imaging time of 9 or 17 min per image, for small and large subjects, respectively. The X-ray flux incident on the sample was 3×10^9 ph/s/cm^2, corresponding to a dose rate of 1 mGy/s, delivering a dose of 0.4–1 Gy to the small and large subjects depending on the number of projections used. This is 10–25 times less than typical tolerable dose, about 10 Gy, by plants. For planar DEI, a 1 min imaging time was used to collect data at the peak of the rocking curve from a Si(3 3 3) analyzer crystal.

3. Plant material and sample mounting

Carrot (*Daucus carota*) root, onion (*Alium cepa*) bulb and celery (*Apium graveolens*) petiole were purchased from a local grocery store. Three-centimeter sections of these samples were held in a cylindrical Perspex sample chamber filled with distilled water to minimize surface refraction. The sample chamber itself was mounted on a Huber (Blake Industries) rotational stage. *Arabidopsis thaliana* plants were grown in the greenhouse. A single inflorescence stem still attached to the plant was mounted onto a vertical Perspex sheet using adhesive tape and imaged in the air in CT-mode. A still-green silique was excised from the plant and mounted and imaged in a similar manner.

4. Results and discussion

4.1. Planar DEI

An onion peel was imaged using planar DEI at 20 keV with a silicon [3 3 3] analyzer crystal on the peak of the rocking curve. The scan time was nearly 1 min to cover an area of 36 mm × 24 mm. The peak image of an onion bulb epidermal peel was recorded using planar DEI (Fig. 1). Single cells are defined by the darker pixels, which correspond to cell walls. This correspondence was confirmed with optical microscopy of the same sample. Darker pixels correspond to greater extinction contrast by the cell walls as the attenuation caused by a single layer of cells was not measurable at 20 keV. Similar contrast is seen in DEI planar images of leaves (not shown). In each of these cases, cell wall structure appears to be responsible for enhanced extinction contrast.

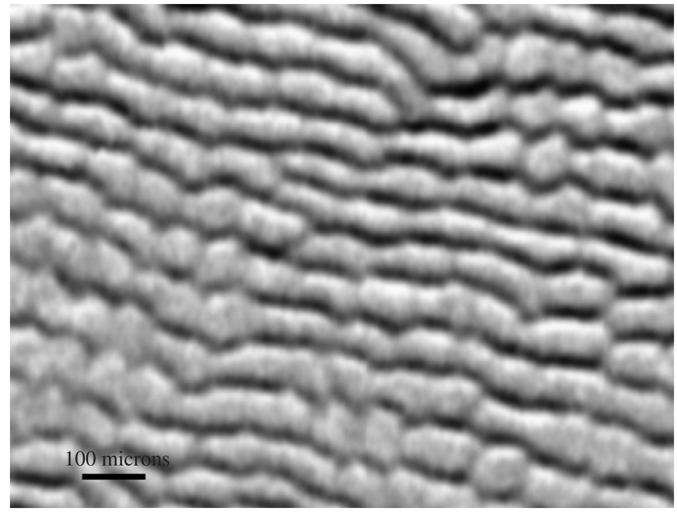

Fig. 1. DEI planar radiograph at 20 keV, 333 analyzer. Darker grayscale corresponds to less X-ray intensity, or more apparent absorption.

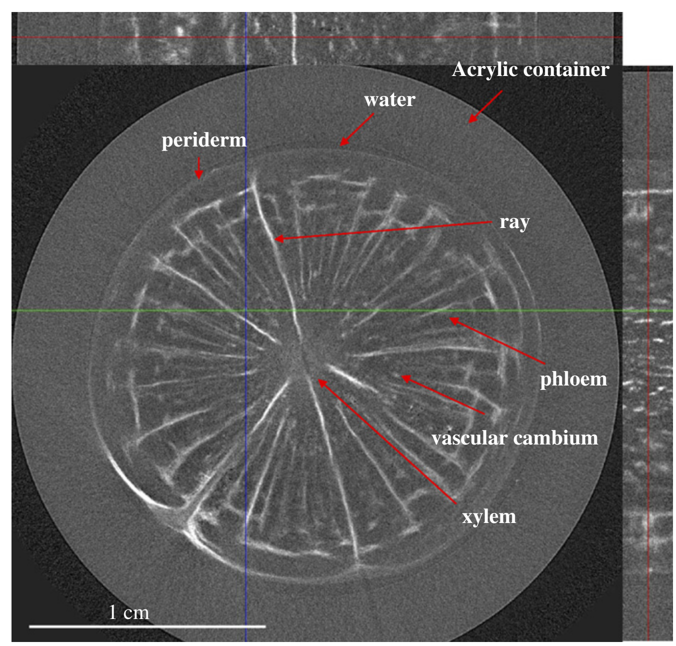

Fig. 2. DEI–CT of a carrot. The top and right images show tangential cross-sections. The center image shows the axial view. Brighter color corresponds to larger apparent absorption.

4.2. DEI–CT

DEI–CT of the carrot was obtained at 40 keV using silicon 111 reflection with the analyzer on peak of the rocking curve. Fig. 2 shows typical axial and tangential cross-sections of the reconstructed 3-D apparent absorption map. Anatomical features, such as rays, cambium, phloem and xylem are clearly visible and identified.