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X-ray coherent diffraction imaging of cellulose fibrils *in situ*

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***Abstract*— Cellulose is the most abundant renewable source of organic molecules on earth[1]. As fossil fuel reserves become depleted, the use of cellulose as a feed stock for fuels and chemicals is being aggressively explored. Cellulose is a linear polymer of glucose that packs tightly into crystalline fibrils that make up a substantial proportion of plant cell walls. Extraction of the cellulose chains from these fibrils in a chemically benign process has proven to be a substantial challenge [2]. Monitoring the deconstruction of the fibrils in response to physical and chemical treatments would expedite the development of efficient processing methods. As a step towards achieving that goal, we here describe Bragg-coherent diffraction imaging (CDI) as an approach to producing images of cellulose fibrils *in situ* within vascular bundles from maize.**

I. Introduction

BRAGG CDI [3] utilizes the intensity around a crystal Bragg reflection to reconstruct the shape of a crystal. The intensity of scatter around a Bragg reflection from a small crystal constitutes the square of the Fourier transform of the shape of the crystal [4]. The speckle about a single Bragg reflection is thereby adequate for the complete three-dimensional reconstruction of the crystal shape. Since this intensity is concentrated in a very small solid angle, the scattering from other constituents of the sample (such as lignin, membranes, cytoplasmic material) is effectively filtered from the acquired signal, making in situ imaging of cellulose within intact plant materials possible.

Collection of the three-dimensional distribution of intensity about the reflection requires an intense x-ray source with high coherent flux and coherence length large compared to the size of the crystal. We have used an x-ray beam ~2 microns in diameter produced at beam line 34-ID-C at the Advanced Photon Source. This beam line produces ~ 10^{10} photons per second. The position sensitive CCD detector used to collect the oversampled intensity is used at a specimen to detector distance of ~ 1 m at a scattering angle expected for the (0 1 2) reflection of cellulose. Once a Bragg reflection has been observed, the detector is translated along a long goniometer arm to a specimen to detector distance of ~ 3 m to collect the highly oversampled intensities about the Bragg reflection.

The resulting intensity distribution must be phased in order to produce an image of the crystal. It is now well established that the oversampling intrinsic to the data sets collected in this study provide sufficient information for reconstruction of a unique image (or, equivalently, a unique phase set) of the crystal giving rise to the scattering [5]. A number of algorithms have been developed to phase these oversampled intensities [6], [7]. They all utilize an iterative enforcement of the limited support of the diffracting object to refine an initial arbitrary phase set which, when combined with the measured intensities is used to compute an initial image. Convergence requires a phase solution consistent with both the finite support of the crystal (as can be determined by its autocorrelation function) and the observed intensities. Uniqueness in the presence of noise is routinely evaluated by comparing phase sets that are obtained using different starting phases.

Fig. 1. Vascular bundles in a fragment of corn stover. Cellulose fibrils are arranged approximately parallel to the long axis of vascular bundles. Centimeter-long segments of approximately two dozen vascular bundles were arranged along the surface of a copper cold finger through which liquid Nitrogen was circulated. A Beryllium dome was used to cover the sample which was evacuated in order to prevent the build-up of ice crystals on the sample.

II. RESULTS

Vascular bundles from maize (literally the stringy fibers

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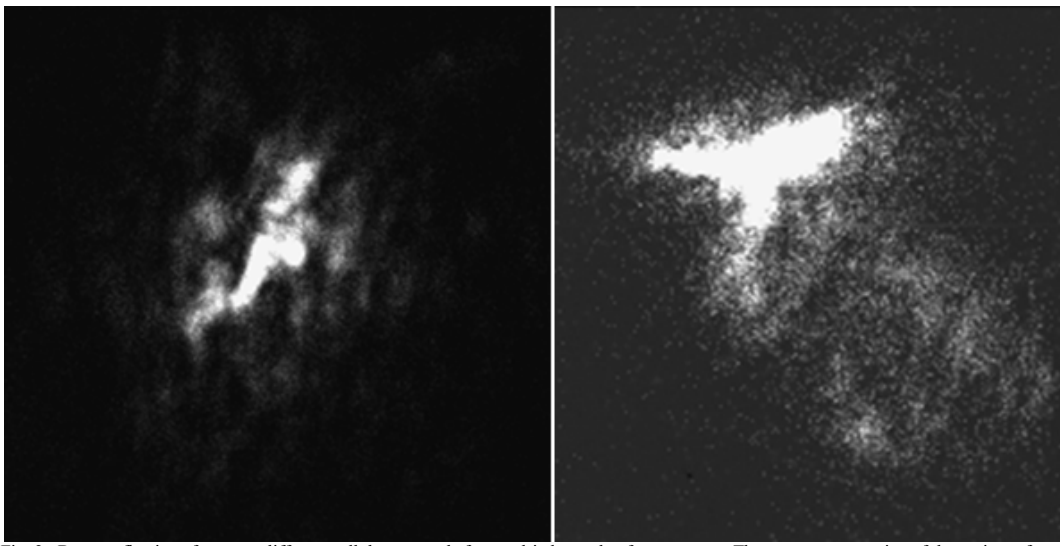

Fig. 2. Bragg reflections from two different cellulose crystals from a dried sample of corn stover. They are representative of the variety of different intensity distributions that were observed in individual samples, reflecting the diversity of crystal morphology present in the maize plant cell wall.

in corn stalks) were obtained from dried corn stover; from stover frozen immediately after harvesting; and from freshly frozen, immature maize. Figure 1 indicates the location of vascular bundles in a dried fragment of corn stover. Initial experiments on nanocrystalline cellulose (provided by Derek Gray (McGill) and John Simonsen (Oregon State)) demonstrated an unacceptable sensitivity to radiation when exposed at room temperature. Collection of a three-dimensional data set around a Bragg reflection can take 3-10 minutes. Comparison of multiple data sets collected from the same crystallite indicated a substantial reduction of intensity with exposure time. Consequently, subsequent data sets were collected with the sample maintained at liquid Nitrogen temperatures within an evacuated Beryllium dome (to prevent accumulation of ice crystals on the sample). Materials kept at cryogenic temperatures exhibited no detectable decrease in intensity after collection of multiple data sets, and successive data sets collected around the same Bragg peak were essentially identical.

Figure 2 includes representations of intensity speckle around the (0 1 2) reflection from cellulose crystals within two samples of corn stover. The intensity distributions are distinctly different from one another and representative of the variety of distributions observed. Cellulose crystals clearly exhibit a wide range of sizes and shapes as observed by coherent diffraction. Data derived from dried samples appeared rather different in form from analogous data from freshly frozen materials; the characteristics of the intensity distributions were clearly distinguishable. There were however, some clear similarities among the data sets. In particular, all reflections appeared to arise from crystallites ~ 300-600 nm across and from 500-2000 nm in length as judged from the distribution of speckles and the extent of the autocorrelation function calculated from the data.

Atomic force microscopy and electron microscopy of cellulose fibrils in maize [8], [9] have been used to identify 'macrofibrils', 50-250 nm in diameter and many microns in length. Coalescence of macrofibrils occasionally results in larger crystalline domains, and it is these larger domains that dominate the data sets we have collected to date. The data collection strategy, in which we scan the samples across the beam until we observe a Bragg reflection, biases our data to the larger crystallites. The length of crystallites as observed in Bragg CDI appears substantially less than that observed in scanning electron microscopy, AFM or TEM. That is due to the twisting of fibrils. CDI will only image the segment of the crystal that is 'spatially coherent', oriented in the same direction and diffracting towards the same Bragg reflection. Segments of the crystal twisted more than a very limited amount will scatter outside the sphere of reflection; will not contribute to the observed scatter; and will not be included in the reconstructed image.

Twisting of crystalline fibrils will give rise to a distribution of strain across and through the fibril. The imaginary part of the reconstructed image provides information on the distribution of strain in a crystal [10] and we anticipate deriving information about the magnitude of twist and strain from those data. In particular, the fibrils in the center of a twisted fibril should be under compression; those near the periphery should be extended [11], [12].

III. DISCUSSION

These results and analogous studies of collagen fibrils [13] demonstrate the feasibility of *in situ* Bragg CDI studies of crystalline fibrils of soft, biological materials. This is an under-characterized class of biomaterial that constitutes an important constituent of many connective tissues as well as pathological deposits such as those associated with neurological degeneration. Development of efficient protocols for data collection and algorithms for data analysis and phasing will help develop CDI into an important tool for expanding our understanding of a broad range of tissues.

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