Low Dose Scanning Transmission Electron Microscopy Techniques to Examine Small Molecular Crystals

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The solid-state structure of active pharmaceutical ingredients (APIs) is important to know due to the effects on the physiochemical, pharmacological and mechanical properties. Processing can affect these properties, for example, in amorphous solid dispersions (ASDs) residual crystallinity or recrystallisation from the polymer/API matrix can impact dissolution properties [1]. Transmission electron microscopy is a useful tool for nanoscale characterisation and has previously been used to analyse pharmaceuticals, however, organic crystals are extremely susceptible to electron beam damage [2, 3, 4]. Here we report on the use of low-dose scanning electron microscopy to image lattice defects through scanning moiré fringes (SMFs) and on preliminary work carried out using scanning electron diffraction (SED) to identify crystalline areas and the polymorphic form present.

Moiré fringes generated by the atomic lattice of a crystal interfering with a similarly sized scanning lattice produce crystal lattice fringe images at lower magnifications and electron fluence than would be required for direct imaging [5]. SMFs were acquired on the API furosemide, low fluence selected area electron diffraction was collected in bright field CTEM from areas containing strongly diffracting crystals (Figure 1a). The microscope was then operated in STEM and the scan direction rotated to align the scanning and crystal lattices. The magnification was changed so that the scan step size was similar in size to the aligned d-spacings. A bright field STEM image was then acquired (Figure 1b) using a probe current of 5 pA and pixel dwell time 10 µs resulting in an electron fluence of 1.8 $e^-/Å^2$, while still resolving high resolution details of the (001) lattice spacing. Minor variations in the size and angle of the SMFs were observed, suggesting strain due to defects within the crystal (Figure 1c and d).

In SED a nm-sized, non-convergent electron probe is scanned across a sample and an electron diffraction pattern collected at each probe position. This preliminary data was collected on a Medipix3 direct electron detector, at an emission current of 0.1 µA and spatial resolution of ~ 20 nm with a convergence semi-angle of ~0.6 mrad. Figure 2a shows a low fluence individual diffraction pattern collected from theophylline, while Figure 2b is a sum of all the diffraction patterns. This pattern can be indexed to the [011] zone axis of theophylline form VI. To identify which areas of the virtual bright field image (Figure 2c) are more crystalline, the Python library pyxem was used to identify peaks in each pattern, the number of peaks identified was then summed and plotted as a function of probe position. The resulting image is shown in Figure 2d and demonstrates a non-flat or defective crystal.

Further work could be done using SED by applying this technique to amorphous solid dispersions to identify and quantify any crystalline regions present within a sample using the same method shown in Figure 2d. In addition it may also provide further information on which polymorphs are present and on the underlying nanostructure.

References

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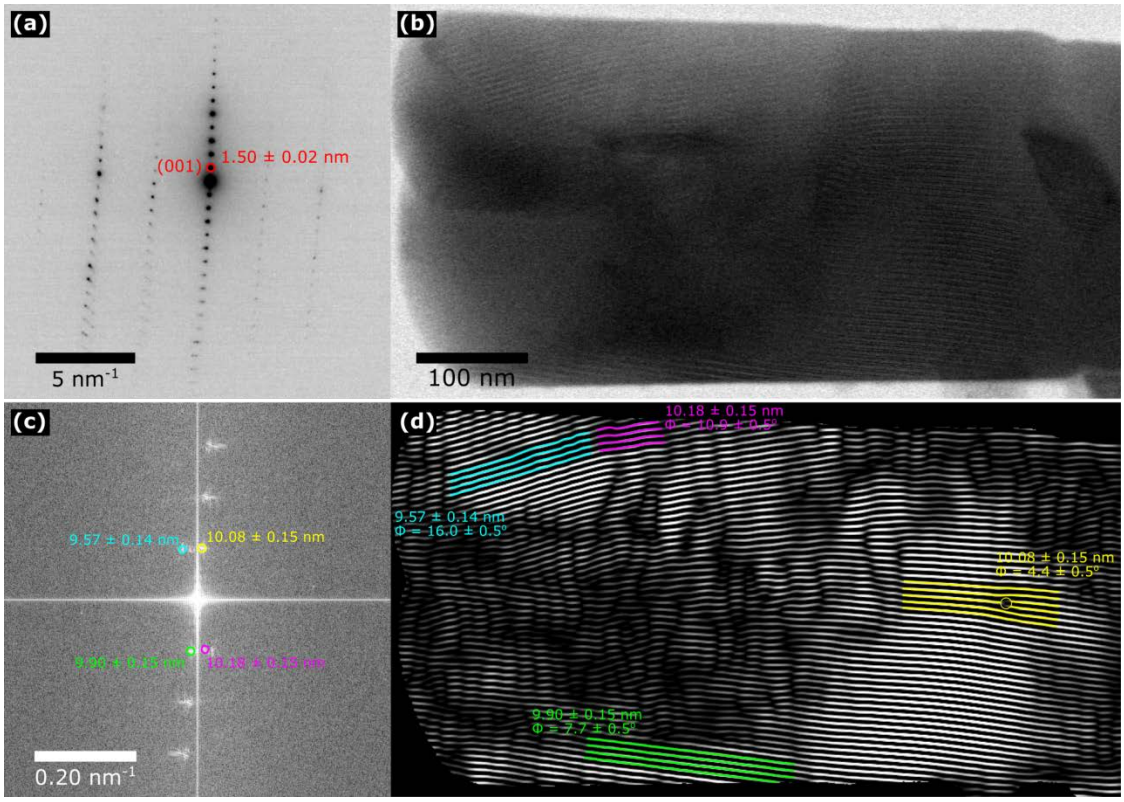

Figure 1: (a) SAED pattern of the furosemide (001) d-spacing equal to 1.49 nm. (b) BF-STEM image showing SMF from 1.49 nm d-spacing and the 1.32 nm scanning lattice, acquired at a magnification of 74 kX with a total electron fluence of 1.8 $e^-/Å^2$ (c) FFT of the image in (b); (d) Inverse FFT of (c) after applying Fourier filter around the first order spacings.

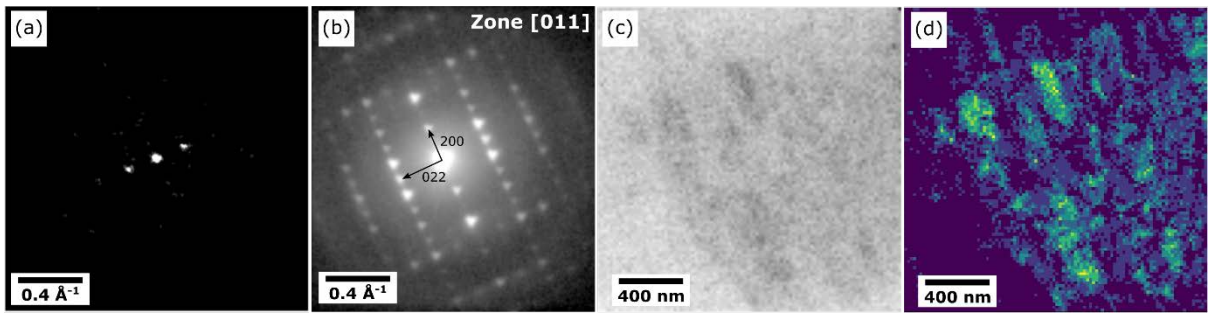

Figure 2: SED of Theophylline identified as form VI (a) Individual diffraction pattern collected near the bottom of the image in (c). (b) Log summed diffraction pattern. (c) Virtual bright field image. (d) Crystal map generated in pyxem by identifying peaks in each pattern then summing the number of peaks identified at each position to show the crystal distortion.