Critical insights for product development through 3D X-ray imaging

X-ray computed tomography (XCT) can non-destructively provide valuable microstructure information

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Importance of microstructure

Understanding microstructure is a significant part of the project development pipeline, from early manufacture of active pharmaceutical ingredients (APIs), formulation development, quality control and bioequivalence testing. Microstructure has strong links to processing and performance. For example, different API manufacturing processes may produce differing amounts of internal porosity [1] and the final porosity of tablets can affect their dissolution behavior [2]. For inhaled products, the microstructure of an emitted aerosol can affect product dissolution and therefore, performance of an inhaled product [3-5]. The aerosol microstructure for dry powder inhalers (DPIs) is intimately related to the interaction and structuring between the API and excipient lactose, which is created during blending. Therefore, along with the microstructure of the emitted aerosol, the microstructure of the pre-actuation formulation is also important [6].

Need for a complementary characterization method

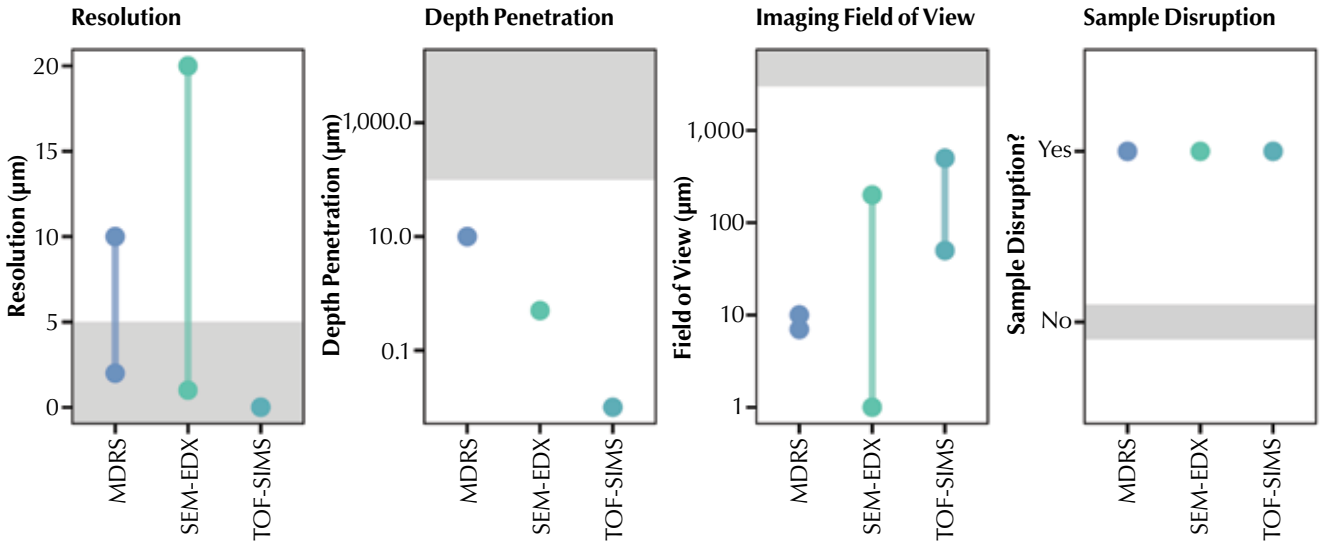
A number of microstructural characterization methods exist that can be used on pre-actuated formulations, including morphologically directed Raman spectroscopy (MDRS) [7], scanning electron microscopy with energy dispersive X-rays (SEM-EDX) [8] and time-of-flight secondary ion mass spectrometry (ToF-SIMS) [9]. Figure 1 summarizes the attributes of these methods. All of the methods can achieve a resolution below 5 µm needed to resolve the micronized API, although MDRS can often struggle with smaller-sized particles due to the 3 µm spot size limitation of the incident laser [10]. Notably, all of the methods are surface-level methods [11], which have a depth penetration well below 1 mm, and all of the methods require destructive preparation of the sample and therefore disturb the microstructure [12]. Each of these techniques are valuable in their own right, and each serves a slightly different purpose. For example, Raman spectroscopy is a speciation method, whereas ToF-SIMS provides chemical information at particle surfaces. Nevertheless, a complementary and orthogonal technique is also needed, which can provide information in a non-destructive manner, especially deeper than the surface.

X-ray computed tomography: A true 3D technique

One technique that has the potential to provide fully 3D, non-destructive details on pre-actuated formulations is X-ray computed tomography (XCT), which is also known as X-ray microscopy. It is the sister technique of medical CT and has grown in popularity across a diverse array of fields in materials science. Traditionally considered a density and morphology measurement technique, XCT techniques can also utilize diffraction, phase contrast and fluorescence. A previous article in *Inhalation* by this author [13] gave a detailed explanation of the XCT technique. Briefly, illuminating a sample with X-rays allows a radiographic projection similar to clinical X-ray radiographs, and by rotating the sample, the radiographs are taken from multiple angles. The illuminating X-rays beam may be produced either from an electron tube, as with laboratory XCT machines, or from synchrotron light, which is a much brighter

Figure 1

Summary of attributes of selected existing characterization techniques. The gray bars highlight desired attributes for characterization of pre-actuated formulations.



and more coherent X-ray source compared to electron tubes, thereby enabling much faster imaging. The radiographic projections are then mathematically reconstructed to produce a virtual volume representation of the sample. This virtual volume can be interrogated to provide valuable insight into the sample. For example, early work by Fu and colleagues [14] showed how the virtual volume could reveal the packing of powder beds, which could then be input into discrete element methods (DEM models).

The development of X-ray optics [15] has enabled higher resolutions more suitable for inhalation materials. For example, at sub-micron pixel sizes, X-ray computed tomography can reveal subtle differences in the structuring and positions of fine particles between milled and sieved lactose grades (Figures 2A and 2B) [12]. At nanoscale, the virtual volume can give unique insight into single particle structures, such as interior cracks within individual lactose crystals (Figure 2C) or spatial particle size distributions within a micronized lactose agglomerate (Figure 2D) [16]. These are truly 3D insights, which are not obtainable through the other aforementioned characterization techniques, and also not achievable through other utilization of X-rays, for example, using radiographic projections to measure fill-weights [17].

Revealing formulated structures

X-ray computed tomography can uniquely show the formulated structure between an API and excipient lactose [18, 19], as illustrated in Figure 3, which depicts lactose monohydrate (Lactohale® 100, LH100, DFE Pharma, Goch, Germany) blended with each of the following: micronized lactose (Lactohale 300, LH300, DFE Pharma), micronized terbutaline sulphate (micTBS, AstraZeneca, Cambridge, United Kingdom) and micronized fluticasone propionate (micFP, Coral Pharma, Mumbai, India). By interpreting both the absorption contrast and phase contrast present in the virtual volume, the excipient lactose has a different grayscale value compared to the micronized material, enabling segmentation into the two phases. However, the various micronized materials behave differently, which can be seen in the virtual volumes: LH300 is sparsely distributed throughout the blend, micTBS is shown to collect in aggregates, while micFP adheres to lactose carrier particles, forming layers around the excipient. The micFP blend contains particularly detailed particle structures due to the drug binding tightly with the excipient, and two different structures revealed by XCT are shown in Figures 4A and 4B. For comparison, SEM imaging of the same blend is shown in Figure 4C. The 3D structures can be probed through XCT, whereas SEM imaging can produce very detailed images but cannot see beyond the surface. This is particularly evident for the agglomerate in the center of Figure 4C, when compared to the agglomerate shown by XCT in Figure 4B.

A further advantage to XCT as a technique is that it allows quantitative analysis. For example, the structures shown in Figure 4 can be quantified using different metrics, with the drug-phase thickness for all drug-carrier units (or "clusters") in the micFP blends shown in Figure 5A, highlighting intra-sample variation in the cluster structures. Similarly, metrics can be used for comparisons between samples or between blends, with Figure 5B showing how the three blends highlighted in Figure 3 have different structures. In line with the qualitative observations, micFP forms thicker regions, whereas LH300 is very sparsely distributed.

Despite these promising results shown by XCT for non-destructive analysis of inhalation formulations, all of the results shown above required sample preparation by filling into Kapton-tube sample holders [12]. This filling process would certainly disturb the powder structure to some degree, and therefore, like all of the previously mentioned characterization techniques, XCT utilized in this manner would be considered destructive.

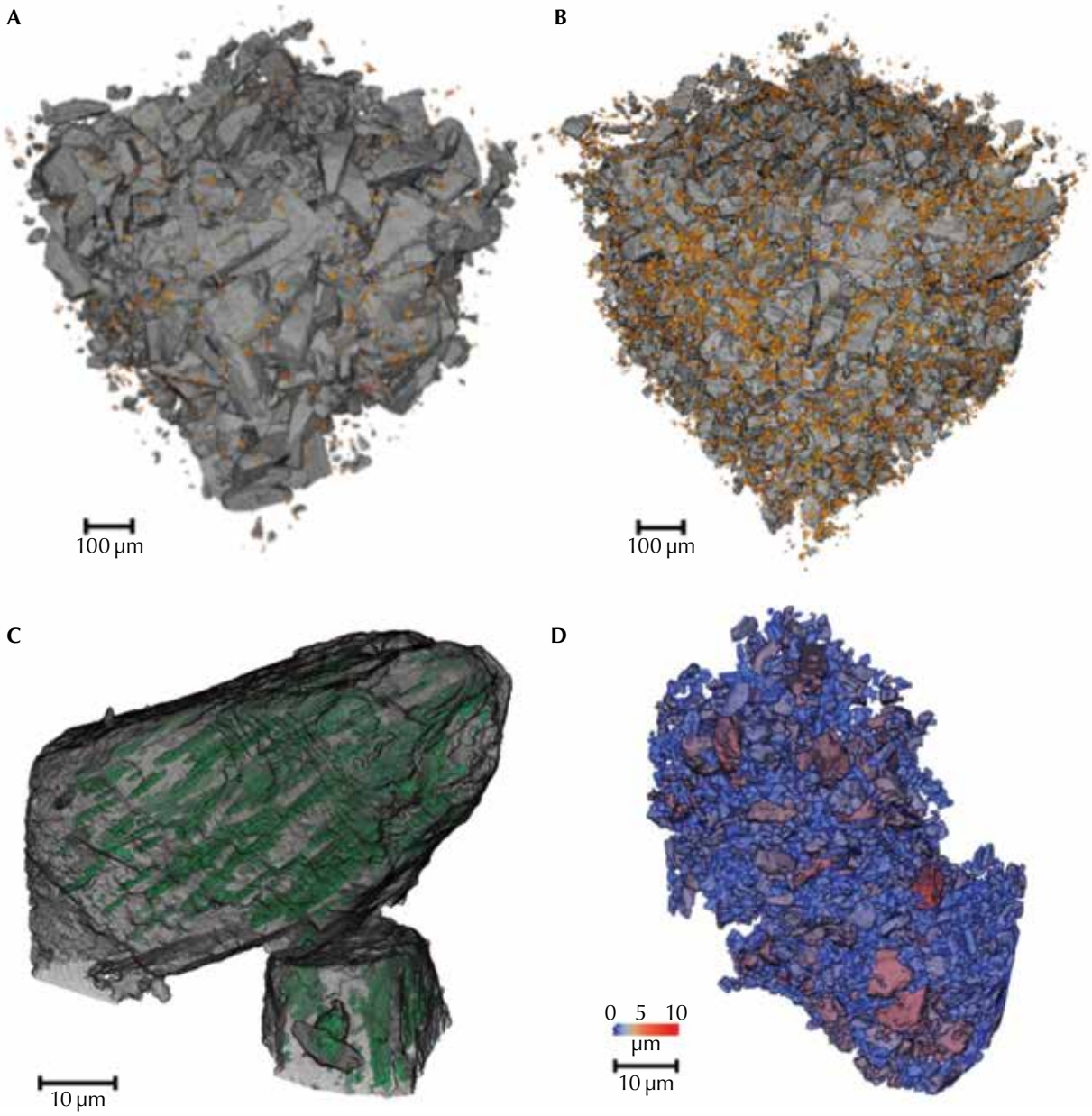
True non-destructive analysis?

Recent work has enabled viewing inside DPI blisters [20], notably without any need to open the blisters. Therefore, in the true sense, it allows non-destructive measurement of the powder structuring within the blister. Figure 6 shows visualizations of a Trelegy® Ellipta® placebo blister (GlaxoSmithKline, London, United Kingdom), revealing the powder bed structure inside. This enables calculation of powder bed statistics such as particle size distributions, powder packing densities and powder bed height.

Furthermore, the technique can reveal fine microstructural detail of real formulations. Figure 7 shows the powder bed within a Seretide® 250 blister (GlaxoSmithKline, London, United Kingdom) [6], with the excipient lactose colored grey and the drug-phase rendered in color. This measurement was taken *in situ*, i.e., inside of the blister, and therefore did not require any sample preparation; moreover, the blister could be re-used for performance testing to link the pre-actuated and post-aerosolized microstructure.

Figure 2

Valuable insight into lactose monohydrate, a common excipient for DPI products, and model inhalation blends: (A, B) Difference in fine particle (orange) density and spatial location between sieved (A) and milled (B) grades; (C) Cracks within single lactose crystals; (D) Spatial distribution of particle sizes within a micronized lactose agglomerate.



Navigating a complex landscape

Despite the considerable potential for X-ray computed tomography to complement other techniques with its non-destructive and 3D attributes, the XCT landscape is very complex, with a large technical barrier. Pharmaceutical materials provide very different challenges than other fields such as material science or geology where XCT is prevalent. Firstly, there is the challenge of sample preparation. In many cases, especially for nanoscale XCT, focused-ion-beam (FIB) milling is used to prepare material science samples, however, such preparation is too aggressive for pharmaceutical samples such as tablets and is completely unsuitable for powder samples or situations where non-destructive analysis is desired. Even direct use of glue to secure pharmaceutical compounds may react with the organic compounds present. Secondly, there is the challenge of instrument operation. Obtaining good data is paramount to useful results, but instrument operation is often different than in other fields. For example, pharmaceutical materials are often low Z materials and require low X-ray energies to maximize contrast, whereas many material science or geological samples require higher voltages for sufficient transmission.

Finally, there is the challenge of data interpretation. Often XCT of pharmaceutical materials contain a combination of absorption contrast and phase contrast. Naive interpretation may assign the bright peak in a phase fringe to be a highly dense material, whereas it simply marks the boundary of one particular material. Therefore, along with technical barriers, there can be limited results showing utility. Often when exploring XCT techniques, pharmaceutical scientists question firstly whether it will be effective with their samples, and secondly, whether it can add value to their project. Consequently, XCT has not penetrated deep into the pharmaceutical industry,

Figure 3

Visualization of different formulated blends, comprised for Lactohale 100 (LH100) blended with (A) Lactohale 300 (LH300); (B) Micronized terbutaline sulphate (micTBS); (C) Micronized fluticasone propionate (micFP). Figure adapted with permission from [6].

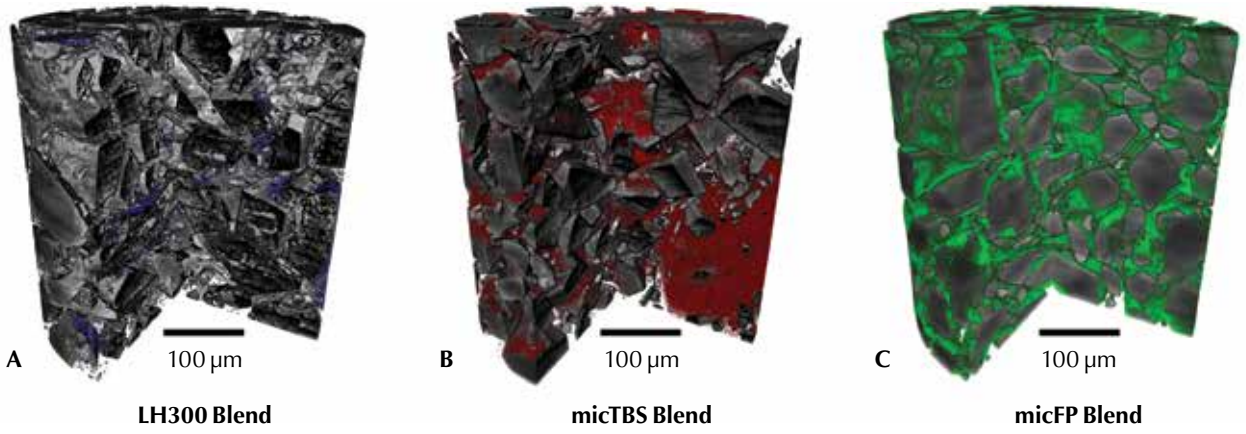
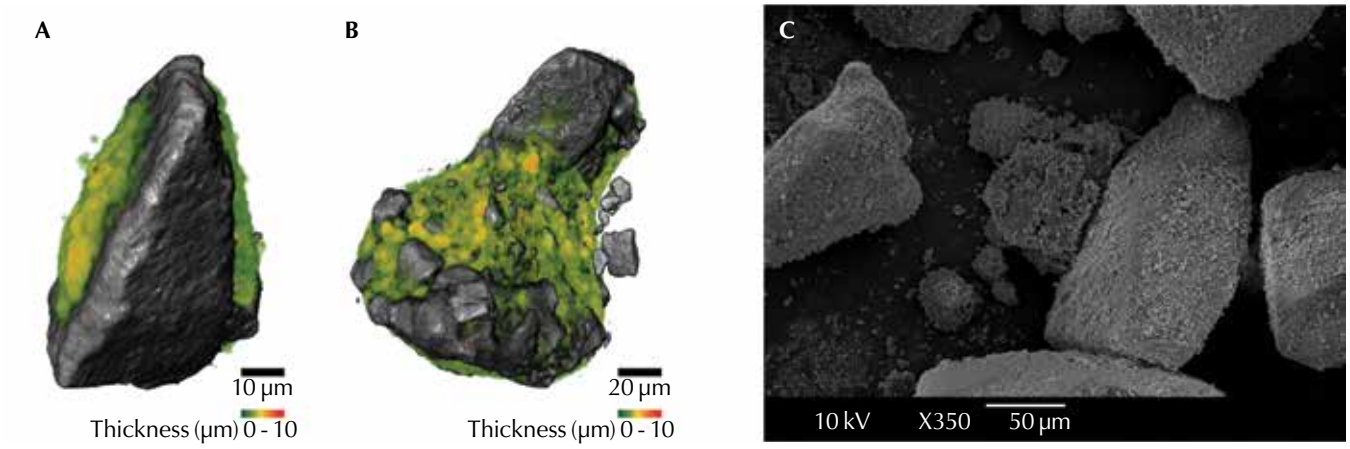


Figure 4

(A, B) 3D visualization from XCT of two particle structures formed in the blend of lactose monohydrate with micronized fluticasone propionate; (C) 2D-visualization of the same blend using SEM imaging. Adapted with permission from [18].



and understanding of the technique is limited, with resulting underutilization in comparison to other materials science disciplines.

Accessing XCT

With this in mind, investing in an instrument can be a very difficult business case, as it can be tough to justify the large capital outlay without firm scientific data demonstrating the utility. Even if a scientific case for XCT becomes demonstrable, there are important technical performance considerations that can be challenging to address without prior experience using XCT. For example, size resolution specifications are of importance in pharmaceutical science, but require a clear understanding of the differences between spatial, nominal and voxel size resolutions, as well as the ability to detect details for the samples of interest. Comparing resolution between different instruments can be challenging because there is no standardized approach [21], and comparisons require an understanding of the X-ray physics, X-ray

Figure 5

Quantitative analysis of the blends through XCT: (A) Thickness of the drug-rich phase across all clusters in the micFP blend. Adapted with permission from [18]; (B) Comparison of the drug-rich phase thickness across the entire sample for three different blends. Adapted with permission from [6].

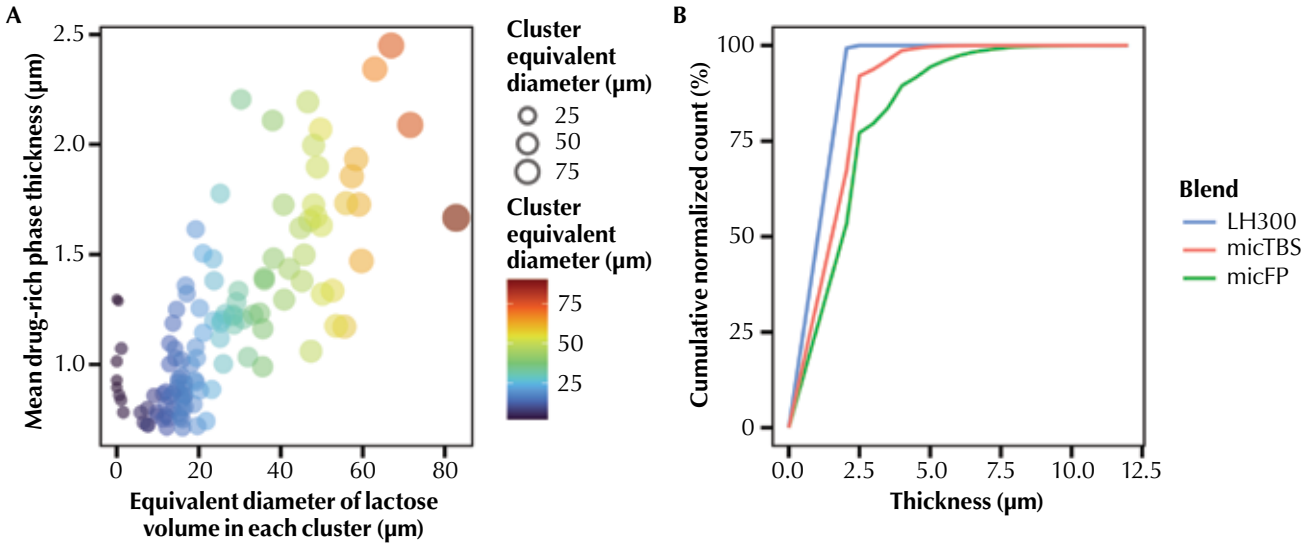


Figure 6

True non-destructive measurements inside an Ellipta placebo blister: (A) 3D view of entire blister; (B) View of blister with a cut away of the internal powder bed; (C) Single cross-sectional slice through the internal powder bed.

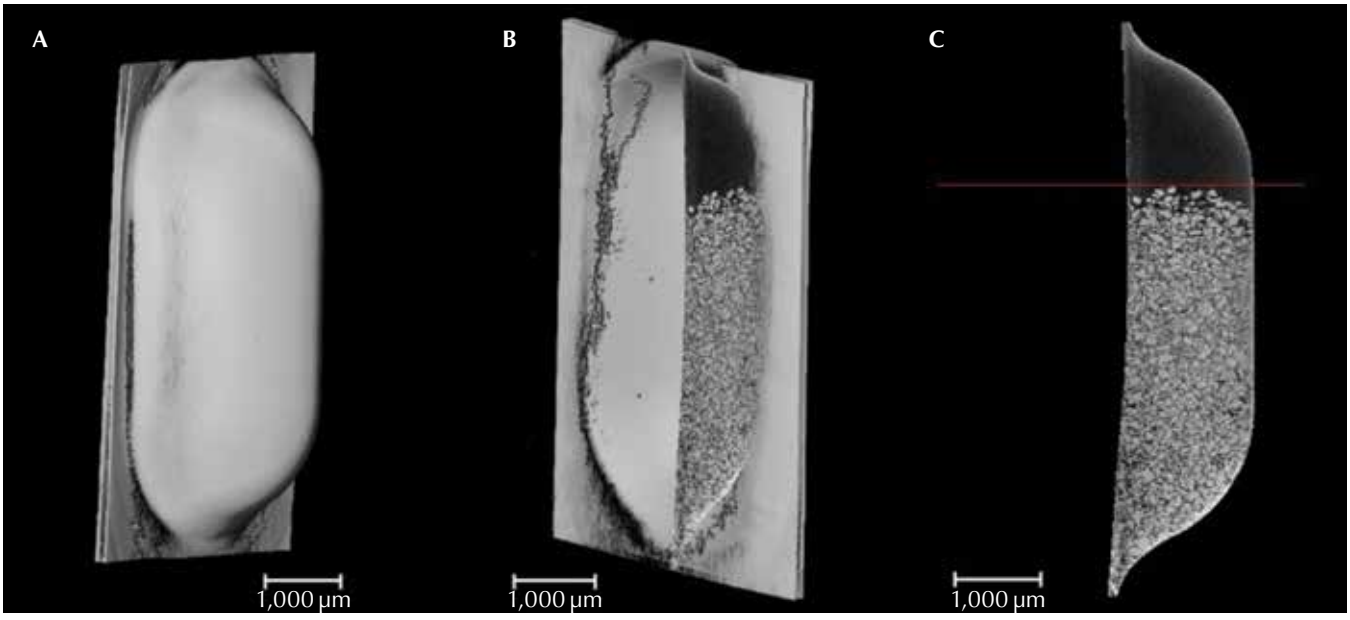
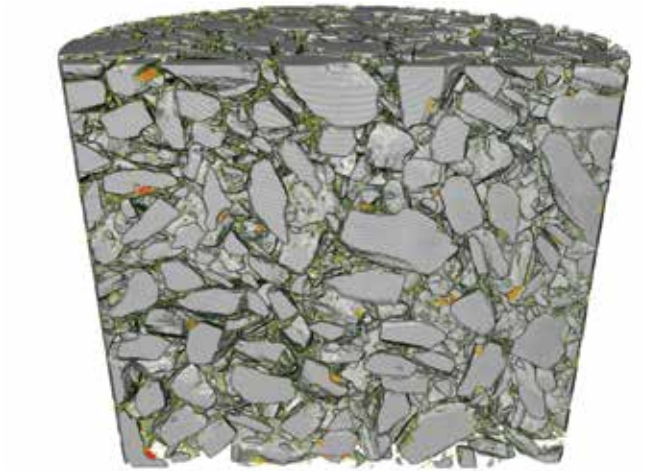


Figure 7

Microstructural view of a Seretide 250 blister, with lactose colored gray and the drug rendered in color.



optics and detector instrumentation of relevance to pharmaceutical materials across multiple instrument types. Assessing and validating the utility of different instruments can bring its own challenges, since the quality of data generated is affected by the sample preparation methodology. This can require a demo lab that has an ability to incorporate into the data generation process an understanding of the impact of handling and storage (e.g., humidity-mediated changes) on the measured pharmaceutical material properties. In most instances, the clarity needed to support an instrument purchase is difficult to obtain without engaging a XCT service provider since the low number of samples that can be run in demo labs is normally insufficient to meet QA-QC testing requirements of the pharmaceutical industry.

Accessing an XCT service provider can help bridge the gap in matching the technical capabilities of XCT technique with the application needs of the pharmaceutical industry. Services may include sample preparation, instrument selection, image analysis and data interpretation methods, chosen with specific understanding of the scientific problem that the formulator or pharmaceutical materials scientist needs to address.

When XCT expertise is coupled with pharmaceutical science to design the analytical program, it is possible to translate X-ray tomographic images and raw data into valuable microstructural insights with quantitative metrics that support formulation and manufacturing decisions.

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