Visualization and analysis of pharmaceutical solids by X-ray microscopy

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1. Introduction

A current trend in drug delivery systems is the use of multicoated or orodispersible tablets. These new systems increase bioavailability and can improve patient compliance by removing the need to swallow. The functionality of these structured tablets is sensitive to fluctuations in the manufacturing process. The chemical formulation is important, but now the physical properties of the active ingredients and other compounds in the tablet have become important for the development, design, process optimization and quality control.

A prospective technique for the visualization and analysis of the 3-dimensional structure of pharmaceutical solids is sub-micron X-ray microscopy.

Pharmaceutical applications of this technique have been limited for two primary reasons. The first reason is that the penetrating power of the X-rays used by the conventional systems is too high for small organic samples composed of the light elements such as carbon, hydrogen, oxygen and nitrogen. The small size and elemental composition of these samples make them too "transparent" to the X-rays of conventional systems, and thus limits the application areas of the existing technology to heavier samples such as electronics devices (silicon, gallium arsenide) or bones (calcium and phosphorus). The second reason is that pharmaceutical applications require both high resolution and a wide field of view, concurrently. Specifically, a resolution of a few microns is needed over the whole tablet, several millimeters, to evaluate granule deformation or micro-cracks caused by compaction during tableting. Until now, it has been difficult to satisfy these two conflicting requirements by X-ray analysis.

Fig. 1. The nano3DX.

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In order to make high resolution, large field of view measurements possible, Rigaku has developed the nano3DX (Fig. 1), a novel X-ray microscope capable of analyzing the sub-micron structures of granules and tablets. This article reports the result of the measurements on several over-the-counter (OTC) tablets to evaluate the effectiveness of the system for pharmaceutical applications.

2. The nano3DX

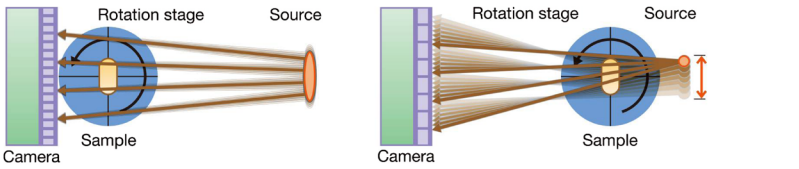
The nano3DX, Rigaku's new X-ray microscope, consists of a high-intensity X-ray beam module with the ability to easily change target materials, a new high-resolution X-ray detector (effective pixel size of 270 nm to 4.32 μm), and a high-precision automatic sample stage (spatial resolution of 50 nm or better). The optics of the system are schematically shown in Fig. 2(a). In an actual measurement, the sample is fixed to the automatic sample stage, located just front of the detector window, and aligned via the automated XYZ stage. An X-ray transmission image is taken, and the sample stage is rotated by a small angular increment. The process is repeated until the sample is rotated by 180 degrees. The multiple images are then processed using a sophisticated algorithm to produce a three-dimensional tomogram.

The major advantages of the nano3DX include high contrast, high 3D resolution, and a wide field of view, all of which are important in evaluating pharmaceutical solids. In the following sections, we describe how the nano3DX achieves all goals.

2.1. X-ray energy and image contrast: High sensitivity to density differences

A laboratory source generates X-rays by bombarding a metal target with a stream of high velocity (energy) electrons. The first type of X-ray radiation is continuous, or bremsstrahlung, radiation with energy spread over a wide range. The other type of radiation is characteristic X-ray radiation. The important feature of characteristic radiation is the production of discrete spectral lines specific to the target material. The bremsstrahlung has higher energy components than the characteristic X-rays from a same target, and thus they generally have a higher penetrating power than the latter.

Conventional X-ray microscopy systems utilize continuous radiation because it provides a large total radiant flux. These conventional systems use a tungsten (W) target, which radiates relatively intense continuous X-rays. These systems also use a high acceleration

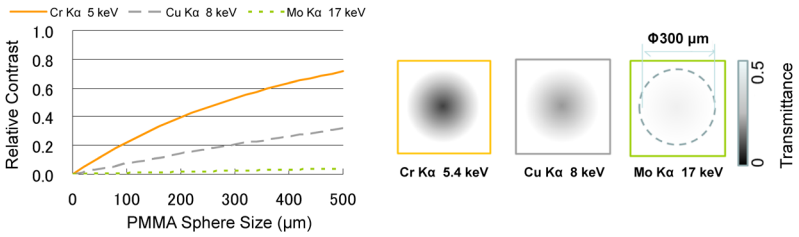


Source size: several tens of microns
X-ray Power: 1 kW or higher
Peak Energy: selectable among 5.4 keV, 8.0 keV, 17.5 keV, etc.
Pixel Size: 0.27 µm or larger
Field of View: 0.9 mm x 0.7 mm or wider

Source size: less than 1 microns to several microns
X-ray Power: several watts
Peak Energy: several tens of kilovolts
Pixel Size: several tens of microns
Field of View: 0.3mm x 0.3mm (with an effective pixel size of 0.3 µm)

(a) nano3DX (b) Conventional X-ray micro-CT

Fig. 2. Geometry Comparison between the nano3DX and a conventional X-ray microscope.



(a) PMMA sphere size vs relative image contrast. (b) Calculated transmittance through a ∅300 μm PMMA sphere.

Fig. 3. Calculated transmission of X-rays of different energies through poly (methyl methacrylate) (PMMA).

Table 1. Radiation types and appropriate applications.

Radiation / Purpose	Sample size	Sample type	Objects to be observed
Cu / high sensitivity, high resolution observations	Several tens of microns–several millimeters	Granules	Coatings, excipients, micron-size voids
		Tablet fragment	
Mo / thick sample observation	10 mm or smaller	Film-Coated tablet	Coating thickness
		Tablet	Internal cracks
		Orodispersible tablet	Granule shape and its variation
		Capsule	

voltage, typically several tens of kilovolts, to increase the continuous X-ray flux, which is proportional to the square of the acceleration voltage. A drawback to the use of continuous radiation is that it is more difficult to observe microstructures made of light elements because of the excess penetrating power.

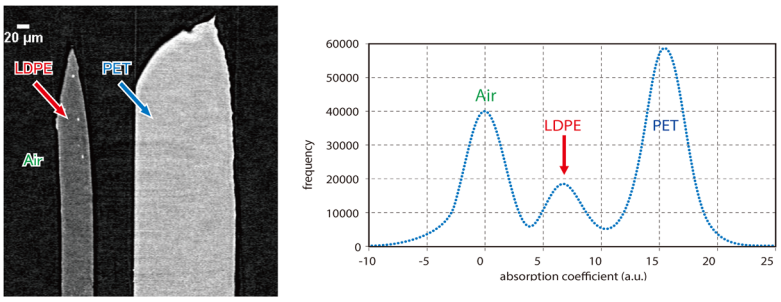
The nano3DX, unlike the conventional systems, utilizes characteristic X-rays. It is equipped with a Rigaku high-intensity X-ray source, which allows users to choose either Cr, Cu, Mo, or W target, in order to generate X-rays best suited for the sample. With Cu radiation for example, the nano3DX can provide images of granules from several tens of microns to several millimeters in diameter with a density resolution of 0.13 g/cm^3. Mo radiation, which has a higher penetrating power than Cu radiation, is suitable for non-destructive observation of tablets of about 10 mm in diameter. Suitable applications of the two radiations are summarized in Table 1. For lighter and thinner samples, the Cr radiation may give better contrast as shown in Fig. 3.

As a result of the ability of the nano3DX to change wavelengths as appropriate for a given sample, it can quantitatively distinguish light materials (those materials consisting of carbon, hydrogen, oxygen and nitrogen) if their difference in density is 0.13 g/cm^3 or greater as shown in Fig. 4. The flexibility of the nano3DX enables high quality measurements in the different stages in the drug manufacturing process including formulation, granulation, and final tablet product inspection.

2.2. High 3D-resolution CT measurement

An important performance indicator of the X-ray microscopy is the spatial resolution of the reconstructed 3D image. It is through the visualization and analysis of the 3D computer tomogram that full power of X-ray microscopy is realized.

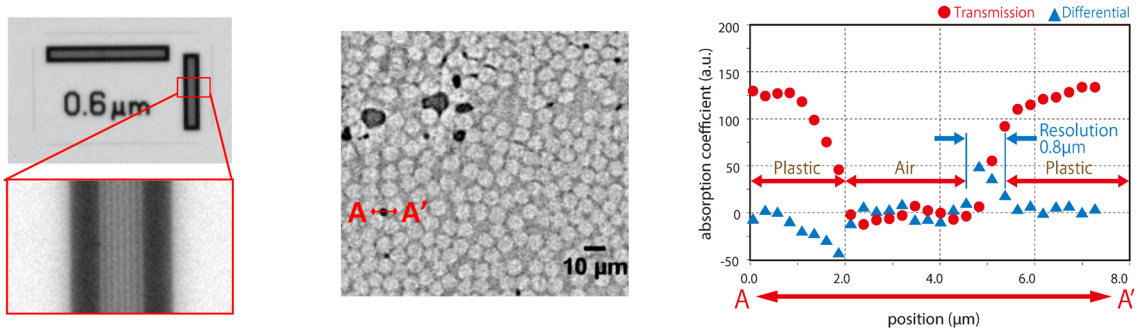
The 3D CT image quality, however, can be degraded by thermal drift of the focal position of the X-ray source. Obtaining a 3D CT image usually requires measuring



(a) A slice of a reconstructed CT image
Resolution: 1.08 μm/voxel.
Measurement time: 2 hrs.

(b) Histogram of absorption coefficient calculated from image density.

Fig. 4. CT image showing the absorption difference between air, LDPE, and PET. The density-difference sensitivity is estimated to be 0.13 g/cm^3 from the standard deviation of the peaks in the histogram.



(a) 2D resolution-chart image: Seven bright lines separated with six dark lines, all 0.6 μm wide, are resolved.

(b) A slice of a 3D image of carbon fiber reinforced plastic (CFRP) with voids.
Voxel size: 0.27 μm.

(c) Image-density profile along the Line A-A′.
Estimated 3D resolution: 0.8 μm or better.

Fig. 5. Experimentally observed resolution of a 2D projection and 3D CT images from the nano3DX operated at a 0.27 μm/voxel configuration.

hundreds or thousands of 2D transmission images. Thermal drift of the focal position during the long acquisition time can negatively impact the consistency in exposure conditions between those multiple images, resulting in poor 3D resolution of the reconstructed 3D CT image.

This problem can be especially serious with conventional X-ray microscopy systems because their geometry magnifies the effect of thermal drift. In these systems, the sample is placed close to the X-ray source to magnify the transmission image as shown in Fig. 2(b) in order to obtain high-resolution 2D images with a conventional detector with a typical resolution of about 10 μm/pixel. With this geometry, the effect of the thermal drift is also magnified.

The nano3DX, on the other hand, has a newly developed detector with very high resolution, down to 0.270 μm/pixel. Thus, the nano3DX does not need to utilize the principle of magnifying projection. As shown in Fig. 2(a), the sample is placed just before the X-ray detector to minimize the effect of thermal drift. With this new detector, the 2D and 3D resolutions of the nano3DX reach 0.6 μm and 0.8 μm, respectively, as demonstrated in Fig. 5.

2.3. Wide-field high-resolution projection image measurement

Certain X-ray microscopy applications require a large field of view and high resolution at a same time. A typical example is the investigation of the effect of compaction on tablet structure. In this application, the field of view must be large enough to cover the whole tablet and the resolution must be high enough to observe individual granules or micron sized cracks in the tablet.

With conventional X-ray microscopy systems, however, it has been necessary to sacrifice resolution in order to realize the needed large field of view. This constraint has made it difficult for the technique to provide the answers desired.

The nano3DX, on the other hand, is capable of observing a whole tablet non-destructively at a sub-micron pixel size (Patent Pending). Figure 6 shows an example of a nano3DX tablet image. The system is also capable of performing a two stage measurement where one obtains a high resolution, large field of view 2D image of a whole tablet and then performs a CT measurement over a defect area found in the 2D image.

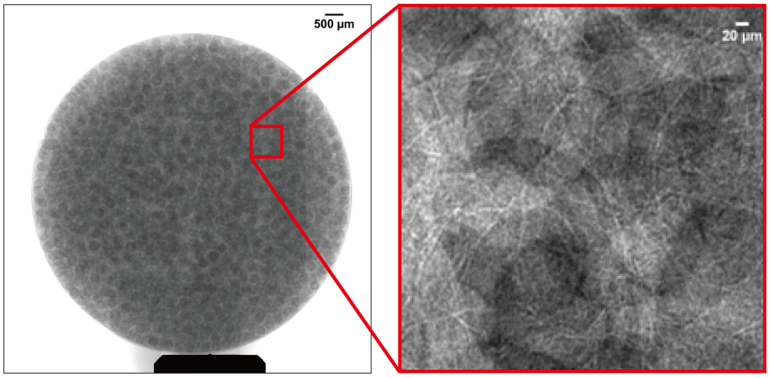
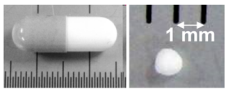
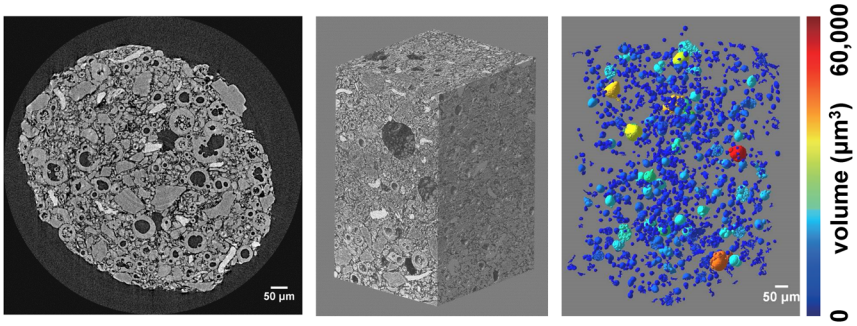


Fig. 6. Large field of view, high resolution projection image of an orodispersible tablet.
Field of view: 10 mm × 10 mm.
Image size: 18500 × 18500 pixels.
Pixel size: 0.54 μm.

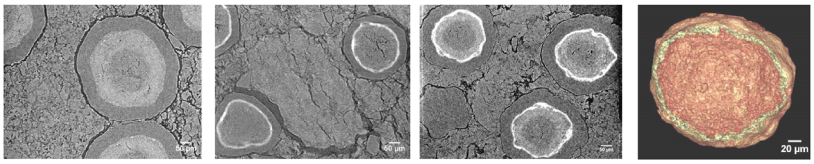


(a) Visible light images of the sample.



(b) Reconstructed CT image. (c) 3D rendered image. (d) Void-volume choropleth map.

Fig. 7. CT images and measured void-volume map of a single granule in a capsule.



(a) Brand A (reconstructed CT image).

(b) Brand B (reconstructed CT image).

(c) Brand C (reconstructed CT image).

(d) Coating of Brand-C tablet (3D rendered image).

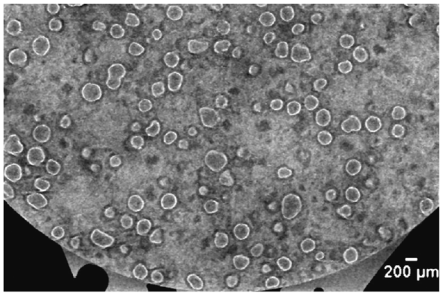
Fig. 8. High contrast CT images of different brands of orodispersible tablets. Structural differences are observed although the samples are claimed to have an identical drug action.

3. Test measurements and results

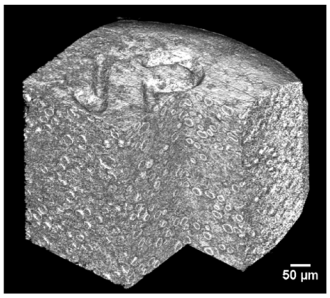
The authors performed test measurements on some OTC pharmaceutical materials using the nano3DX in order to evaluate the system's capability in this field. The images obtained are shown in Figs. 7–12, and the measurement conditions are summarized in Table 2.

The images in Figs. 7 and 8 show that the nano3DX succeeded in visualizing the 3D structure of the granules. This is difficult by conventional X-ray microscopy methods. It should be noted that the volume of the voids in the granules were measured and that cracks and other defects inside the tablet were observed. These results indicate that this technology can be useful as a tool in R&D for studying the granulation process in the pharmaceutical industry.

The images in Figs. 9–11 clearly show the irregularities in coating thickness, and the shape and the variation in the granules. It is expected that, by investigating the relation between the non-destructive CT images and the results of other physical measurements such as solubility experiments, the physical properties of the final products may be

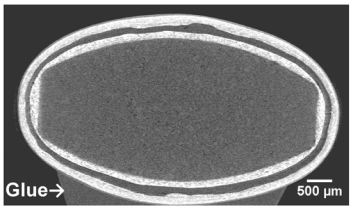


(a) Reconstructed CT image.

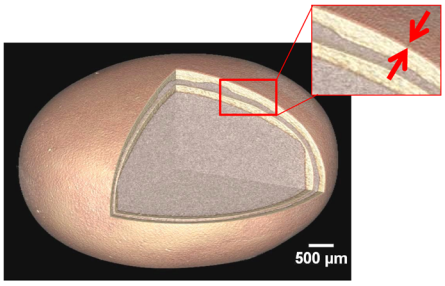


(b) 3D rendered image.

Fig. 9. Large FOV CT image of an orodispersible tablet. Variation between the granules in the tablet is observed. (greater transmission is shown as darker pixels.)



(a) Reconstructed CT image.



(b) 3D rendered image. A 15 μm-thick layer is visualized.

Fig. 10. Film-coated tablet images.

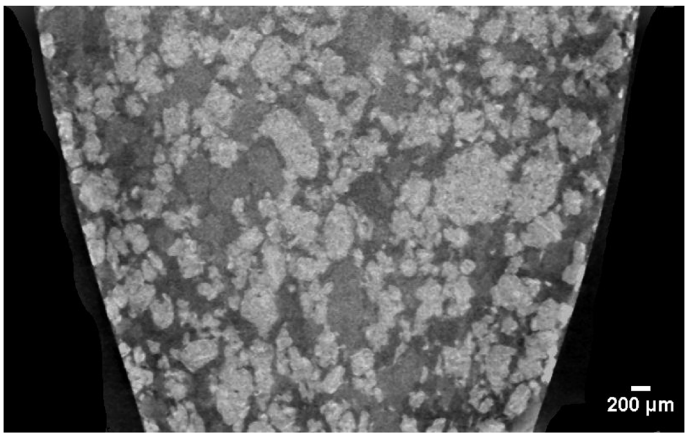


Fig. 11. Reconstructed CT image of a dietary supplement tablet (includes Mg, Ca and Zn).

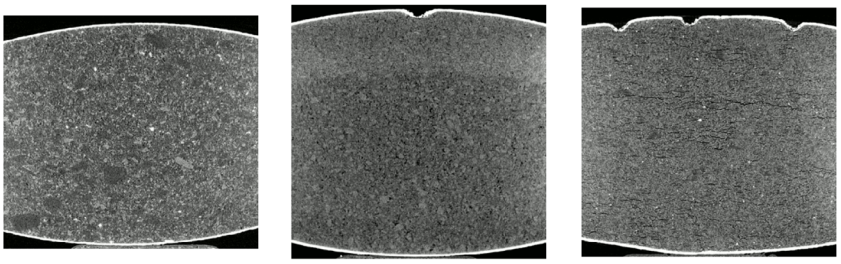
predicted from the parameters used in the production process.

The authors performed X-ray microscopy measurements on three different types of antihypertensive compound tablets, and compared the results. The A, B, and C specimens are film-coated antihypertensive tablets with sizes of 8.0×3.7 mm, 8.0×3.7 mm, and 8.5×3.9 mm, respectively. The formulation consists of amlodipine besylate and an angiotensin II receptor antagonist.

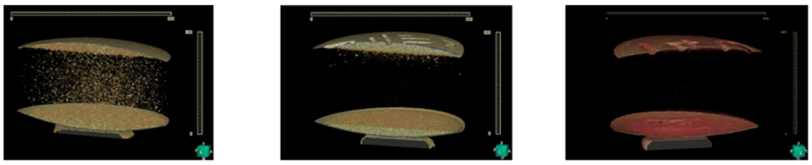
Figures 12 (a)–(c) show the reconstructed slice images of the tablets. The film-coat part of the tablets are extracted, and their rendered 3D images are shown in Figs. 12 (d)–(f).

Anan *et al.*[1] performed solubility tests, HPLC analyses, hardness tests and solubility tests on various brands of antihypertensive compound tablets including the three that have been measured in this work, and discussed the relationship between the conventional test data and the information obtained from nano3DX CT measurements. They concluded that the coat thickness and the grain size had a considerable influence on the disintegration rate of a tablet. A brief summary is shown in Table 3.

Although more investigation is still needed to clarify the correlation and determine causation, the information made available by the X-ray microscopy measurements could play an important role in the design and process control of pharmaceutical tablets.



(a) Tablet A. (b) Tablet B. (c) Tablet C.



(d) Tablet A. (e) Tablet B. (f) Tablet C.

Fig. 12. CT images of different brands of antihypertensive tablets with similar formulation (amlodipine besylate and angiotensin II receptor antagonist). (a), (b) and (c) are reconstructed slice images while (d), (e) and (f) are density-selective 3D rendered images visualizing the film coat materials.

Table 2. Observation conditions summary.

Fig. #	Sample / Measurement type	Radiation	Pixel size	Field of view	Obtained information
6	Orodispersible tablet / wide FOV projection	Cu	0.54 μm	$10\times10\,mm^2$	Deformation of granules by compaction
7	Granules in a capsule / high contrast CT	Cu	0.54 μm	$0.9\times0.9\times0.7\,mm^3$	Volume, surface area, and orientation of voids
8	Orodispersible tablet / high contrast CT	Cu	0.54 μm	$0.9\times0.9\times0.7\,mm^3$	Granule shape
9	Orodispersible tablet / wide FOV CT	Mo	8.64 μm	$7.1\times7.1\times5.4\,mm^3$	Size variation of granules; cracks
10	Film-coated tablet / wide FOV CT	Mo	8.64 μm	$7.1\times7.1\times5.4\,mm^3$	Coat thickness; inner structures; cracks
11	Dietary supplement tablet / wide FOV CT	Mo	8.64 μm	$7.1\times7.1\times5.4\,mm^3$	Mixing rate; volume and surface area of granules

Table 3. Summary of CT Observation, disintegration and hardness tests for the antihypertensive tablets A–C.

Tablet type	Coat thickness/μm	Grain size	Disintegration speed[1]
Tablet A	38	Coarse	Medium
Tablet B	50	Coarse	Slow
Tablet C	30	Fine	Fast

4. Summary

It has been demonstrated that the nano3DX, a new 3D X-ray microscope with high contrast and high spatial resolution with a large field of view, is capable of observing and quantitatively measuring the internal structures of pharmaceutical granules and tablets in a non-destructive manner. It is expected this technology will become a new tool for evaluating drug production processes by investigating the relationship between the 3D physical-shape information and other results of other evaluation including solubility tests.

Acknowledgement

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Reference

(1) Y. Anan, Y. Ito and H. Kanazawa: 57th Pharmaceutical Society of Japan Kanto Branch Meeting (Tokyo Oct. 26, 2013).