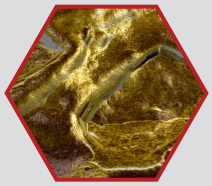
Combined SEM and AFM Particle Analysis of Vitamin C Using FusionScope®

Extract individual particle morphologies, surface roughness, and potential surface contaminations with correlative imaging and force-distance curve analysis.



Surface roughness of Vitamin C tablets, which can be observed by Scanning Electron Microscopy (SEM), plays a crucial role in defining its surface morphology and therefore its delivery rate and interaction time with external stimuli. Atomic Force Microscopy (AFM) then can be used to measure this roughness; however, since the size of these Vitamin C particles can range from several microns to less than hundreds of nm, the accurate positioning of an AFM probe onto specific individual particles becomes very challenging and time consuming. There are two reasons for this. First, traditional AFM geometry only allows a top-down view of the sample surface with optical microscopy (OM) and so the AFM tip and direct tip interaction with the sample surface cannot be observed easily. Second, OM in AFM cannot resolve structures below 200 nm very well (as compared to SEM). Using FusionScope's SEM-enabled Profile View along with its shared coordinate mapping, both challenges can be resolved simultaneously without having to change sample environment. This reduces transfer times and contamination exposure while minimizing possible time-based degradation of the particles.

Challenges to Solve

When creating tablets for health and pharmaceutical based ingestion, such as Vitamin C, information on several very important features is required. These features include Surface Morphology and Structure, Reaction to External Stimuli, Delivery Rate, and Contamination levels. To understand these features better, the particles/nanoparticles constructing the tablet are most often analyzed using a range of different techniques. All in all, this nanoparticle information must provide a clear understanding of the tablet to resolve technical challenges such as the ideal delivery rate to maximize the impact of the particle (in medicinal and supplement applications). However, this process must also be done in the most efficient manner possible, for example, by using minimum resources, maximizing throughput, and being very repeatable.

Current Solution

Surface roughness has become a more significant characteristic of nanoparticles, including Vitamin C, due to its impact on surface morphology. Responses and interactions to external stimuli can be understood with greater clarity, providing more precise information on exchange rate and delivery rate. The best way to measure this property, and one that has gained popularity most recently, is Atomic Force Microscopy (AFM). However, while positioning of the tip on Vitamin C particles on the scale of μm is straightforward, Vitamin C particles often can be as small as 60 nm. Therefore, positioning of an AFM using conventional optical microscopy (found on most AFMs) is very difficult to do. Additionally, since the surface of Vitamin C tablets on the nanometer scale as well as micrometer scale is very rough, positioning of the AFM using conventional approaches is very challenging resulting in contamination of the tip as well as being out of position.

Researchers in academia as well as in industry utilize a range of techniques to acquire this nanoparticle-based information including X-ray Diffraction (XRD), for crystallinity information, Energy Dispersive X-ray Spectroscopy (EDS) for direct chemical information, and Transmission Electron Microscopy (TEM) for porosity information.

At present, a very regularly used technique to acquire morphology and size-based information about the Vitamin C particles is Scanning Electron Microscopy (SEM). As well as direct morphology information about the particle itself, SEM can also be used to monitor morphology changes of the particle upon exposure to external stimuli – such as that of chitosan whereby Vitamin C is encapsulated by TPP-chitosan microspheres or hydroxiapetite based Vitamin C for treating bone infections. While surface structure information can be acquired through observation, size can be measured through extrapolating these images.

Our Approach

In this study, we present a comprehensive investigation of Vitamin C particles utilizing the FusionScope, a unique correlative analysis platform. FusionScope is a powerful tool that seamlessly combines SEM, AFM, and EDS in a single platform (and within a single user interface) offering high-resolution imaging and precise measurements at the nanoscale. By combining SEM imaging, AFM topography measurements, and force-distance curve analysis, we can extract individual particle morphologies, surface roughness, and potential contaminations on the particle surfaces. The ability to identify specific individual particles with the SEM, guide the cantilever tip to exactly the point of interest, and observe the AFM measurements using SEM-enabled Profile View, paves the way for enhanced analytical methodologies in particle research and analysis.

Workflow

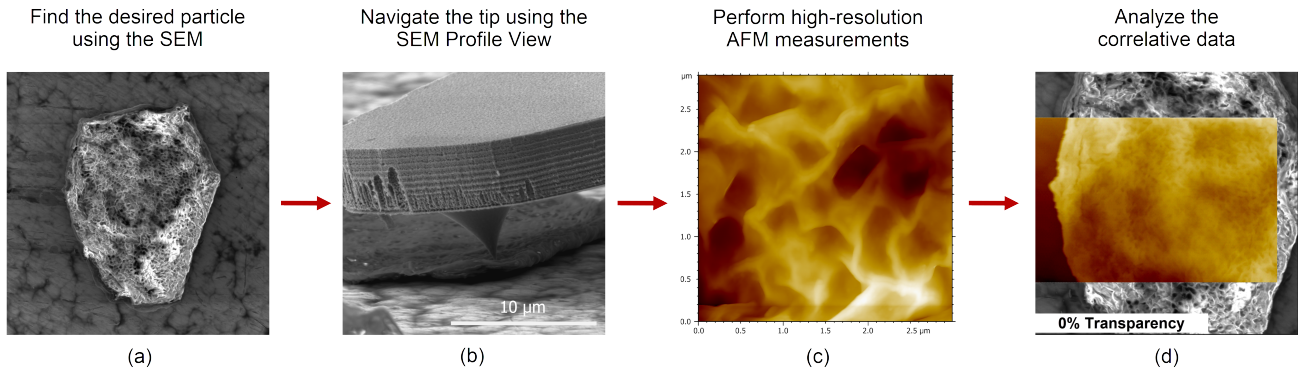


Figure 1: *Workflow to obtain correlative AFM-SEM data on Vitamin C particles using the FusionScope. (a) Identifying target Vitamin C particle. (b) Profile View of cantilever tip positioned on particle. (c) High-resolution AFM image of particle topography. (d) Correlated AFM/SEM image of particle.*

In the initial phase, the targeted Vitamin C particle can be easily identified and located with the SEM (Figure 1a). Charging often plays a major role in the SEM imaging of organic particles and can impede accurate measurements. To mitigate this challenge, a beam acceleration voltage of 3.5 kV was utilized alongside

working in Profile View (an 80° tilt of sample and AFM in respect to the SEM), effectively curbing the charging phenomenon experienced with these particles.

Users benefit from FusionScope's unified coordinate system of AFM and SEM, which allows for the automated and precise navigation of the AFM tip to the desired particle. Profile view gives direct line of sight to the cantilever tip region and ensures the positioning of the tip at the desired location on the particle (Figure 1b). High-resolution AFM measurements in amplitude modulation (AM) mode then can be performed (Figure 1c), with the SEM providing real-time visualization of the cantilever tip movement. Both SEM and AFM data can be directly correlated in the FusionScope software, making direct data analysis easy and straightforward (see Figure 1d).

Analyzing a Variety of Vitamin C Particles

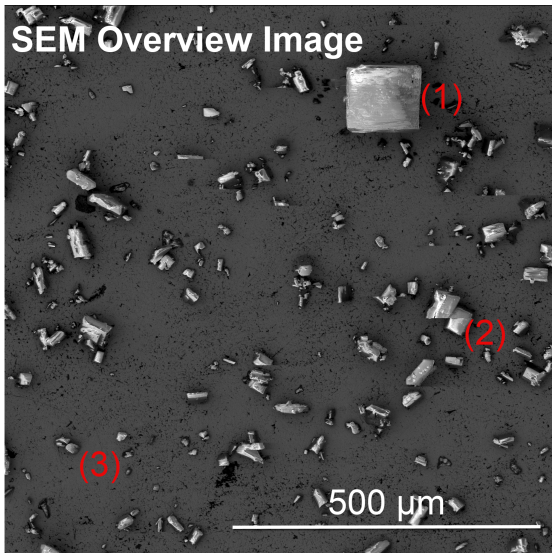


Figure 2: *SEM overview image of different Vitamin C particles. Large (1), medium (2), and small (3) particles can be identified.*

The SEM overview image (as depicted in Figure 2) reveals a variety of different particles exhibiting a diverse range of sizes. From notably sizable particles with diameters > 100 µm (1), to medium-sized particles with diameters > 10 µm (2), down to numerous diminutive particles (3), the spectrum of particle size is extensive.

To provide a comprehensive characterization of these distinct particles, this study focused on analyzing three particles showcasing different shapes and/or sizes to assess their surface roughness. Figure 3 displays the SEM top view of the three individual particles, whereby a different size and structure of the particles can easily be seen.

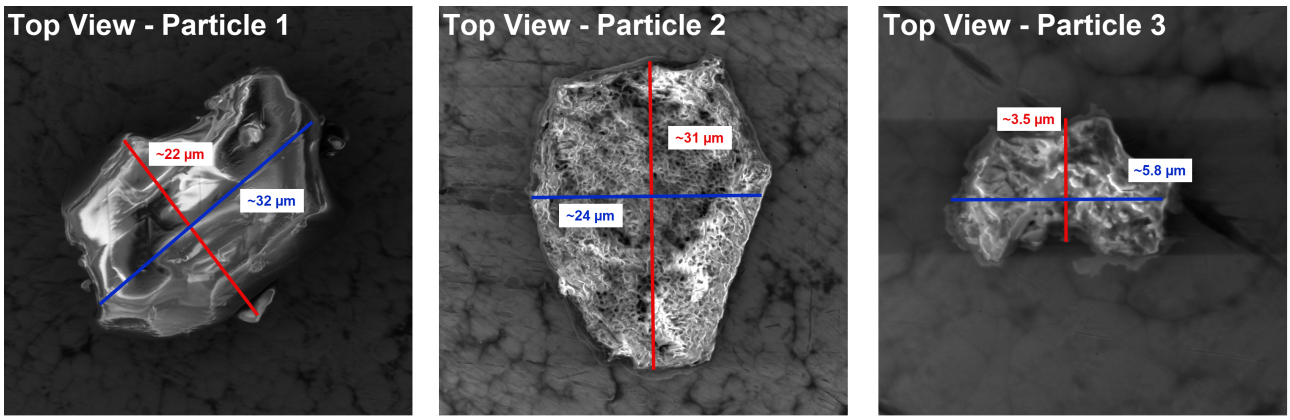


Figure 3: *SEM Top View of three different individual Vitamin C particles.*

Roughness Measurement Using the FusionScope

Utilizing FusionScope's unified coordinate system for both SEM and AFM, seamless navigation of the AFM tip toward each individual particle is facilitated (Figure 4). The near-orthogonal perspective of the sample and the tip further facilitates precise positioning, particularly advantageous when approaching rough or small structures, such as Particle 3.

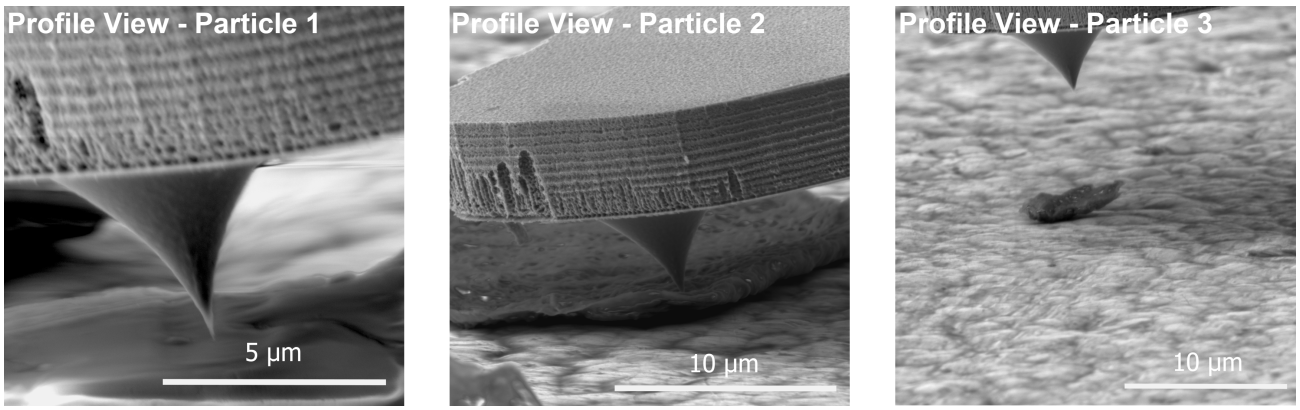


Figure 4: *SEM Profile View of the cantilever approaching three different particles.*

High-resolution AFM measurements in AM mode can then be performed on all three particles (see Figure 5). As could already be guessed from the SEM top view images Figure 3), Particle 2 has an internal structure that is not visible in Particles 1 and 3. The evolution of the AFM data reveals a sample roughness of 600 nm for Particle 1, 420 nm for Particle 2, and 150 nm for Particle 3.

This demonstrates the capability of the FusionScope to analyze the surface topography and roughness of individual Vitamin C particles easily and quickly, independently of their size or surface morphology.

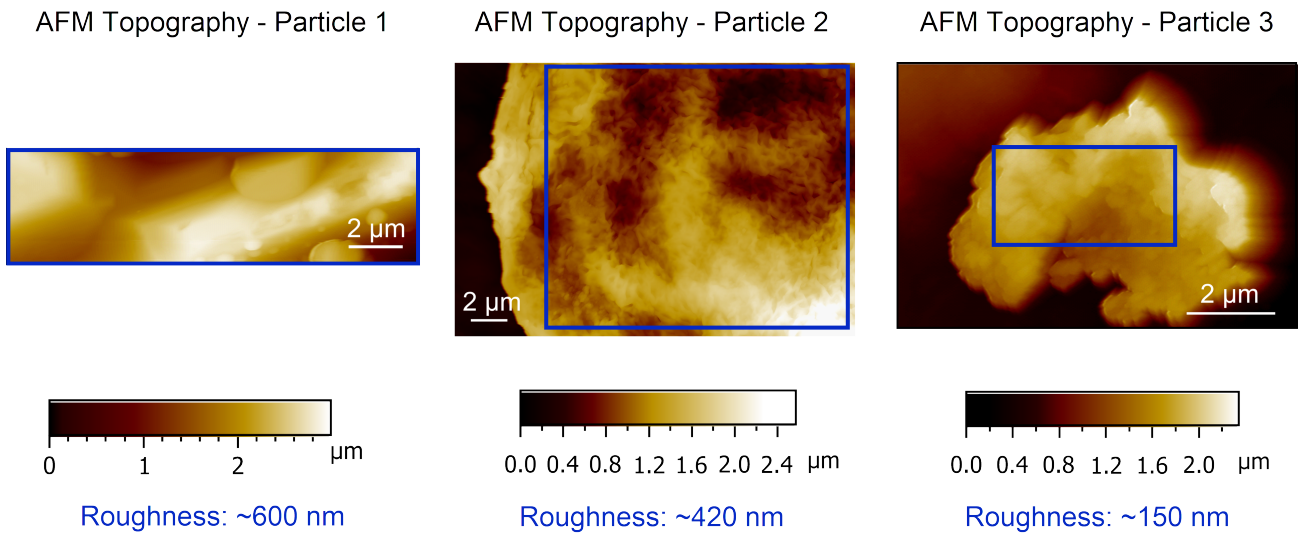


Figure 5: *AFM Topography data recorded in AM mode on each individual particle are shown. The blue rectangle indicates the area in which the roughness measurement was carried out. Particle roughness varies from ~600 nm (Particle 1), to ~420 nm (Particle 2), and ~150 nm (Particle 3).*

Using Phase Imaging and Force-Distance Curves to Investigate Possible Contamination

A common challenge encountered in particle preparation lies in the identification of specific contaminations. By combining phase imaging and force- distance curves on individual particles, FusionScope offers a solution to identify and analyze potential contaminations of the particle surface.

High- resolution AFM measurements of Particle 2 unveil a distinctive cave- like structure within its topography (see Figure 6 top left). However, it is noticeable that a phase contrast is present within the crater, indicating a different surface material of potential contamination (see Figure 6 top right). This becomes even more visible in the overlayed image of AFM topography and phase (see Figure 6 middle). Using Force-Distance Curves on the two different areas inside the crater structure reveals that the possible non- contaminated part (Figure 6 bottom left; Position 1) exhibits a greater hardness compared to the contaminated part (Figure 6 bottom right; Position 2). In addition, Position 2 also displays a stronger adhesion compared to Position 1. These two characteristics of the force- distance curves indicate that Particle 2 consists of different materials.

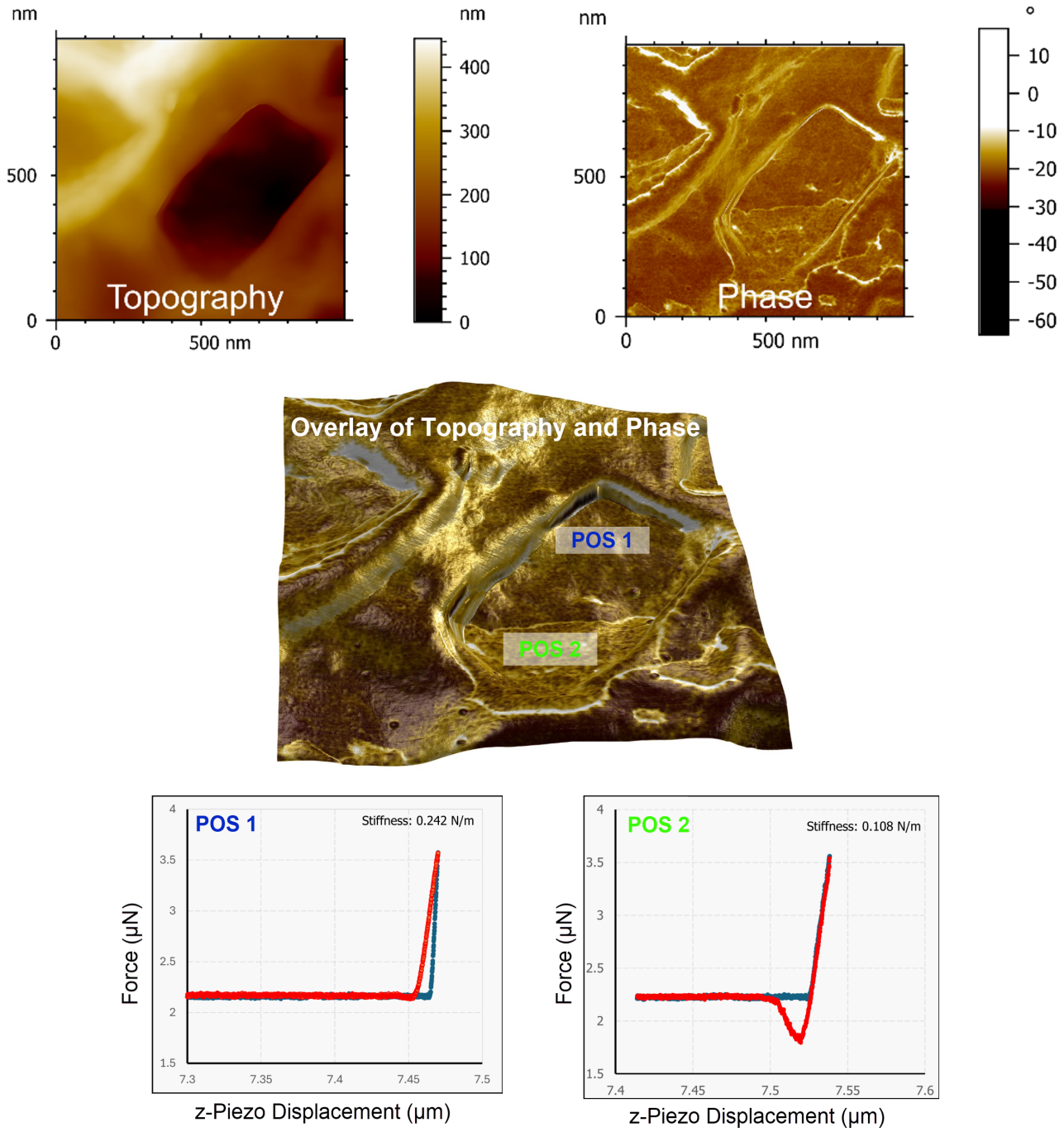


Figure 6: *(Top) High resolution AFM topography and phase image of Particle 2 indicates a possible contamination. (Middle) Overlay of AFM topography and phase image revealing the two different materials in the crater structure. (Bottom) Force-Distance Curves at Position 1 and Position 2 indicating different hardness and adhesion.*

Preventing Misinterpretation of Force-Distance Curves

The interpretation of force-distance curves to gain insights into the mechanical properties of a sample presents considerable challenges. Especially for small non-flat structures (e.g., Particles, Fibers,

Nanowires) the cantilever can move or damage the samples during the process of obtaining the force-distance data. Hence, the ability to observe the cantilever's movement during the force-distance curve becomes indispensable for identifying potential artifacts. Such an artifact is illustrated in Figure 7, where the whole force-distance curve is observed in Profile View with the SEM. As the cantilever approaches the sample surface (1) it contacts the sample (2). Then the cantilever first pushes the particle down onto the surface (3) before executing the real mechanical probing of the particle (4). This phenomenon culminates in the cantilever bending, as evidenced by the red curve (Figure 7b), a distortion that could inadvertently lead to misinterpretation of the slope as a real mechanical property of the sample.

By combining force-distance measurements with SEM Profile View, FusionScope can circumvent these misleading results and provide a comprehensive analysis of mechanical properties of small objects.

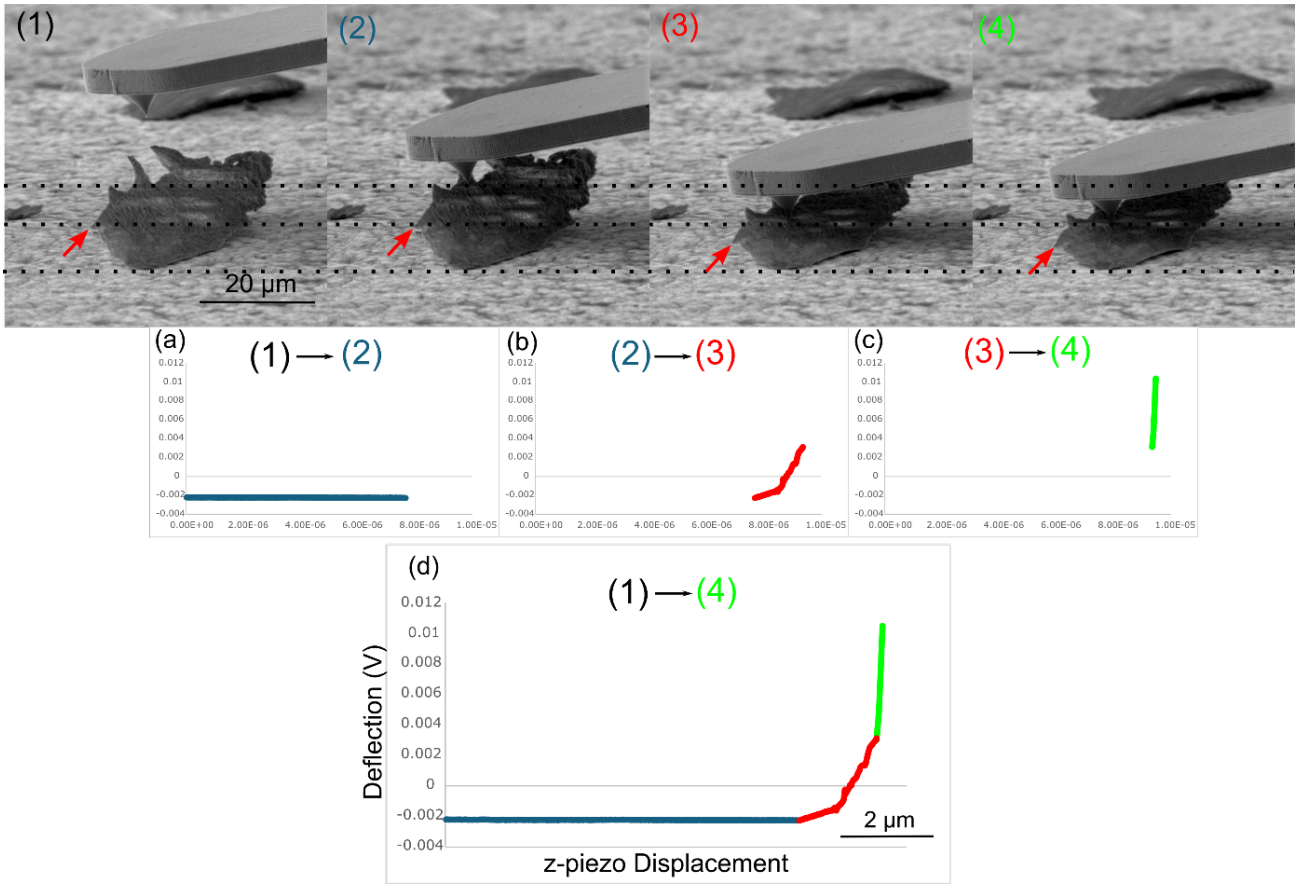


Figure 7: *SEM images 1 - 4 show the position of the cantilever during the acquisition of a force-distance curve. To illustrate the movement of the particle during the force-distance curve, horizontal dashed lines were added to the SEM images. The force-distance curve was divided into three sections (a-c). The force-distance curve (shown in (a)) in the range between images 1 and 2 shows constant deflection of the cantilever during the initial approach to the particle surface. The range between 2 and 3, on the other hand, shows an increase in the deflection (b). This deflection is because the cantilever pushes the particle downward onto the surface and does not exhibit any real mechanical data. The green curve in (c) shows the sequence of the force-distance curve between the SEM images 3 and 4. A very steep increase can be noticed since the particle is now completely pushed onto the sample surface. The complete force-distance curve is shown in figure (d).*

Outcomes

- We have reduced transfer time between different measurement systems by having all measurements conducted within one sample chamber and user interface.
- We have combined different measurement modes into a single system, making it simpler to use and learn, therefore you do not need an expert in each individual technique. A single user can learn fairly quickly how to use all the techniques on one platform.
- We have made the acquisition process of data, with AFM, very easy by being able to see the cantilever tip approaching the sample surface – minimizing measurement setup time as well as contamination or tip damage inspection time.
- We are able to perform measurements correlatively, using a joint coordinate system, thereby getting direct like-for-like data independent of time, at any angle of interest and very localized scales.
- We have created an easy to maintain system with strong service and support.