



Create in-focus images of uneven samples using optical Z-stacks

It is a challenge to obtain high-quality, in-focus optical microscope images when studying uneven and rough samples. Often the height variation of the sample is greater than the depth-of-field of the objective lens. This leads to out-of-focus regions in optical images and multi-image montages. This can limit your understanding of the sample, or how the image can be used for future work. For example, you may need sharp in-focus images of particles on a surface for automated particle analysis.

The Z-stack optical imaging feature within Renishaw's WiRE™ software generates high depth-of-field optical images of the sample. It keeps all points of the image or montage perfectly in-focus, regardless of how uneven the surface is. It complements Renishaw's LiveTrack™ technology, which keeps the sample in focus during Raman measurements and when you are viewing the sample. Your Raman samples no longer have to be flat, extending the variety of samples that you can measure.

The benefits of Renishaw's Z-stack software feature

- This easy-to-use feature captures images in user-defined steps in the Z plane.
- The software determines the optimal focus for each point on the surface and uses the individual captured images to generate a high-quality in-focus image of the surface.
- It can be used to capture images in one field-of-view or generate a montage to image a larger area.



Example of a polymer image generated using Renishaw's Z-stack software feature

Figure 1 (a-d) shows four images of a polymer captured using the Z-stack software feature. The sample's surface height varies over a range of 30 μ m and different sections of the sample are in-focus in each image. (e) The final image produced by the Z-stack software feature combines the images so that all points of the sample are in-focus.

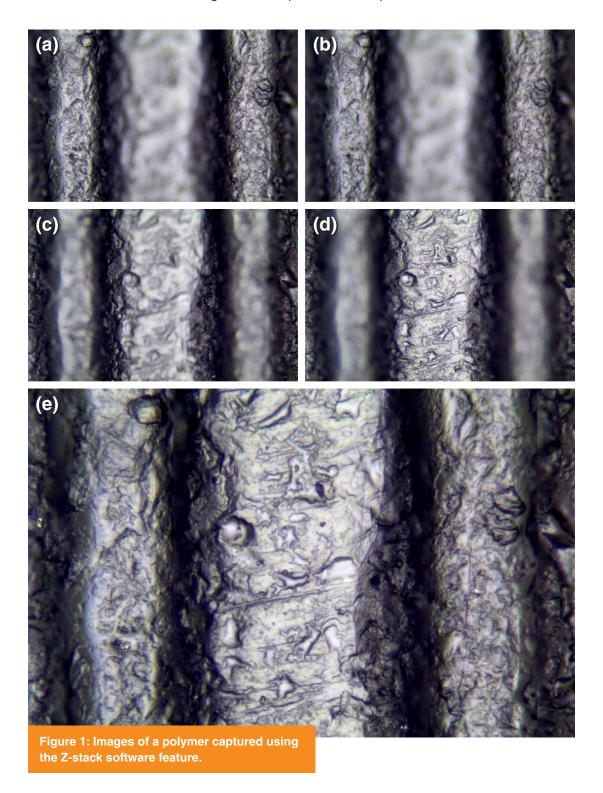






Figure 2: In-focus 2D image of a lactose particle generated using the Z-stack software. The height variation from the surface to the top of the particle is approximately 50 μ m. Imaged using a 20 \times objective (depth of field \sim 3 μ m).

Suitable for a very wide range of samples

- Analyse large and small samples the image size is not limited to the field of view of the microscope.
 Montaged images are only limited by the total travel of the microscope stage.
- Fully user controllable you can define both the number of images captured in the z plane and the step size.
- Use any illumination generate high resolution images using bright field, dark field or other commonly used illumination options.
- Edit with ease built in editing options to control pixel size and sensitivity ensure you are able to generate the best images.
- Maintain perfect focus you can select a step size in z much smaller than the objective depth of field (as small a 0.01 μm). This ensures your sample is perfectly in-focus.

Combine Z-stack software with Renishaw's LiveTrack focus-tracking technology to create in-focus topography images

You can use the Z-stack software in conjunction with Renishaw's LiveTrack focus-tracking technology. LiveTrack keeps the laser in-focus at the sample surface. It therefore enables a detailed 3D surface to be generated. Optical images, including the Z-stack in-focus image, can then be overlaid onto the 3D surface. Together, this enables accurate optical and morphology viewing of the sample surface in 2D and 3D.

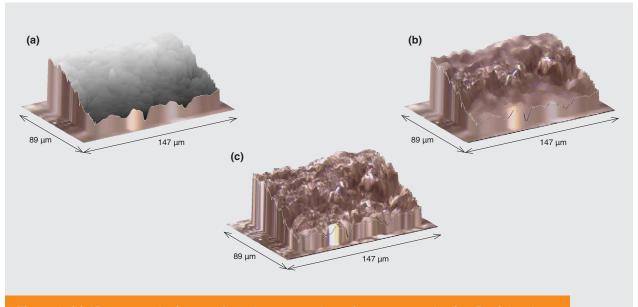


Figure 3: (a) 3D topography image of a polymer sample surface generated using Renishaw's LiveTrack software (b) topography image with the normal white light image superimposed (c) topography image with the in-focus image obtained using the Z-stack software superimposed.





Want to learn more? Contact our spectroscopy team to discuss your specific requirements.

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