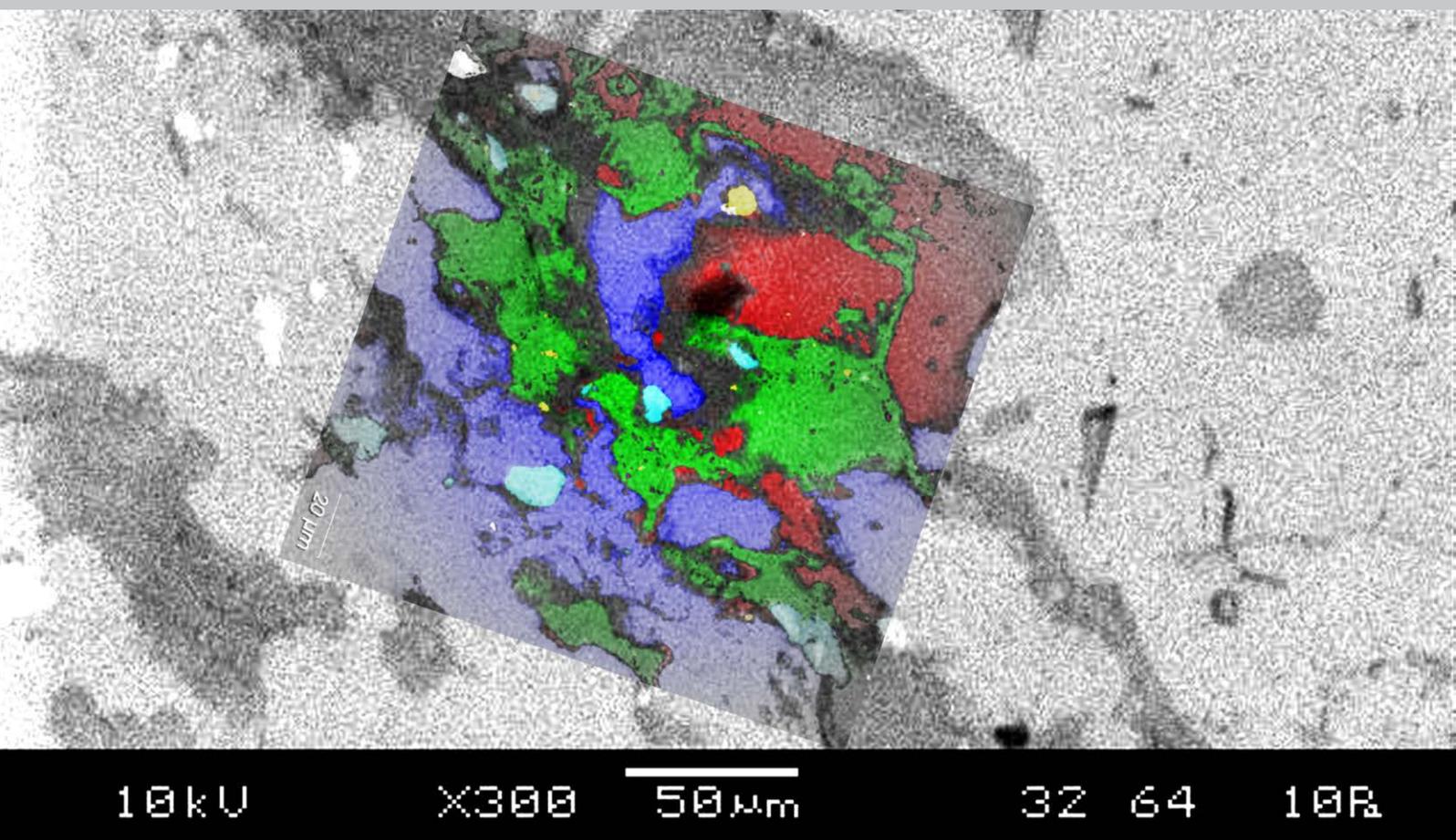


Correlate™ module in WiRE™ software



Overlay and register images and data from multiple microscopes within WiRE software

Get the most from your microscopes

Modern laboratories often have a range of different microscopes such as optical, fluorescence, confocal, scanning electron, infra-red and Raman. These—often complementary—imaging techniques are often used in isolation but, by combining the power of these techniques, more information, and a better interpretation of the sample, can be obtained. The Correlate module within Renishaw WiRE software enables you to easily transfer samples between microscope systems, and automatically visualise the combined results.

Raman microscopy has a spatial resolution of typically 1 μm or less. This is comparable to other optical microscopies, and intermediate between those of AFM/SEM and infrared microscopy. Raman images are therefore ideal for combining with images from other techniques.

Features of the Correlate module

The Correlate module has several powerful tools:

- Coordinate Manager to import and transform coordinates between different microscopy systems (e.g. scanning electron microscopes (SEM) and inVia™ Raman microscopes)
- Image Alignment Tool to align and overlay images from multiple microscopy systems
- Batch Measurements to automate the same Raman measurement at multiple positions
- Aspect Ratio Correction with image alignment and rotation for overlay

These tools can be used individually or in combination to support various workflows, as shown in the following examples.

Using the Correlate module

The Correlate module guides you to the regions of interest on your samples after you have transferred them between microscopes. You can then acquire data from the same locations and overlay the images for easier interpretation.

You simply record the XYZ coordinates of three or more reference points and any data-acquisition locations on your sample, using the first microscope, along with any images. Then transfer the sample to a second microscope and determine the new coordinates of the defined reference points and record them in the Correlate module's Coordinate Manager tool. This determines the coordinate transformation between the two microscopes and calculates any new analysis point coordinates for the second microscope. Data can then be obtained at these analysis locations or a transformed image can be used to navigate the sample under the second microscope to help define new analysis locations.

The Correlate module can also overlay images, with the ability to adjust opacity to best visualise the features of interest. The images are centred automatically for the different microscopes to ensure correct registration and accurate overlay. The magnification and rotation of the images is then adjusted to allow for the differences in microscopes. This powerful module enables you to accurately display and interpret complementary information, as shown in the following examples.

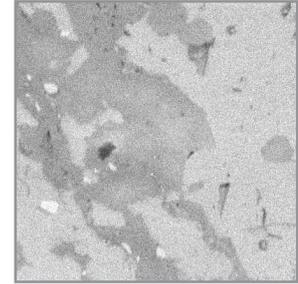


Figure 1. SEM image of a mineralogical section

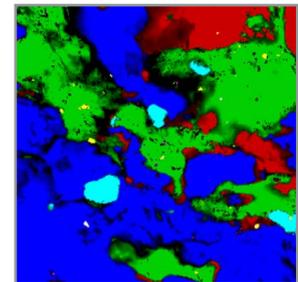


Figure 2. Raman image of a mineralogical section

Correlate microscope images with Raman images

The information in images from another microscopy system (e.g. atomic number in SEM images, topography in AFM images) can be correlated to the chemically specific information contained within Raman images.

Example workflow:

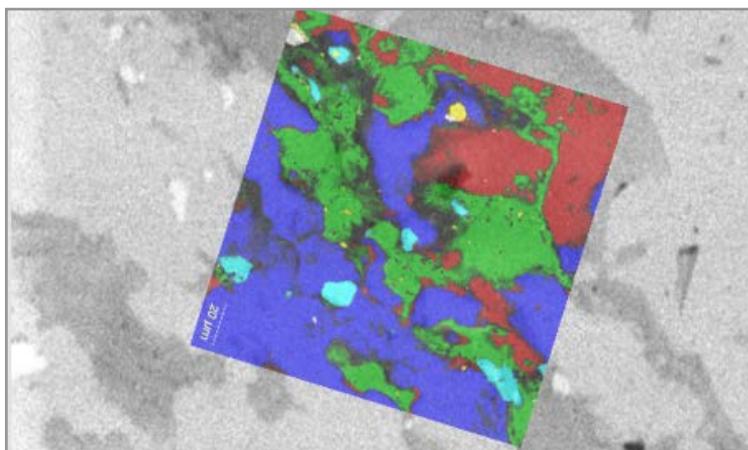
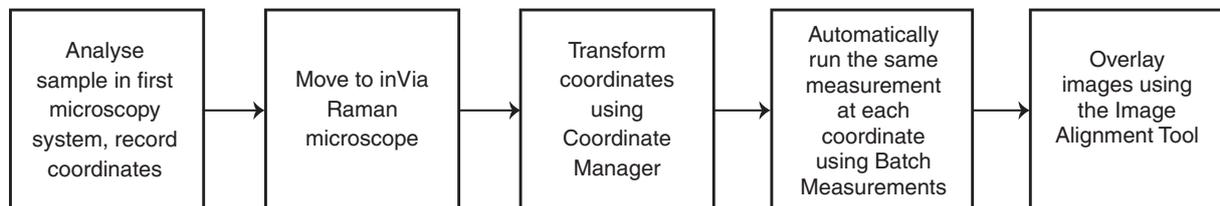


Figure 3. Example of correlation between SEM image (black and white) and Raman image (coloured) of a mineralogical section

The contrast of the high-resolution SEM backscattered electron image in Fig. 3 is based on atomic number. It reveals regions of differing composition.

The overlaid Raman image reveals the identity of these constituents.

Correlating multiple similar samples

In this case images of oesophageal tissue sections are correlated.

The samples are two adjacent, nominally identical, microtome slices. One was H&E stained for histopathology purposes, the other is unstained and has been analysed using Raman microscopy.

The combined image enables experts to compare and contrast the information from the two techniques.

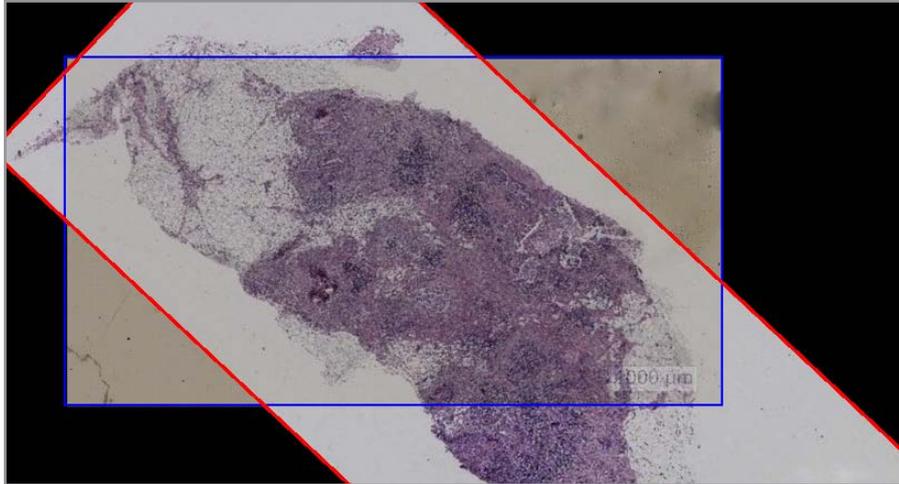
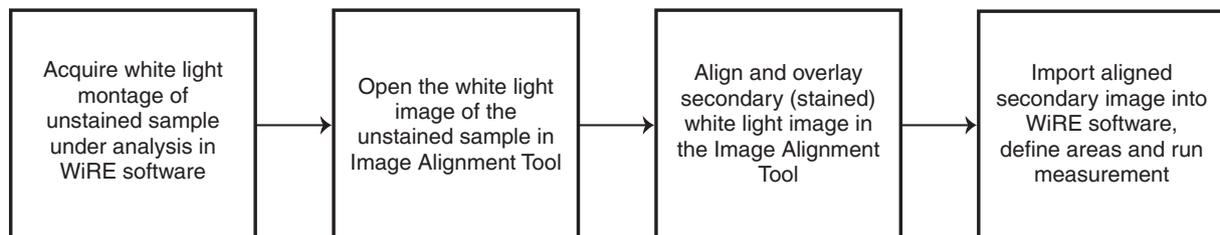


Figure 4. Example of correlation between stained and unstained serial tissue sections. The stained sample image (red border) is overlaid with the unstained sample (blue border)

Example workflow:

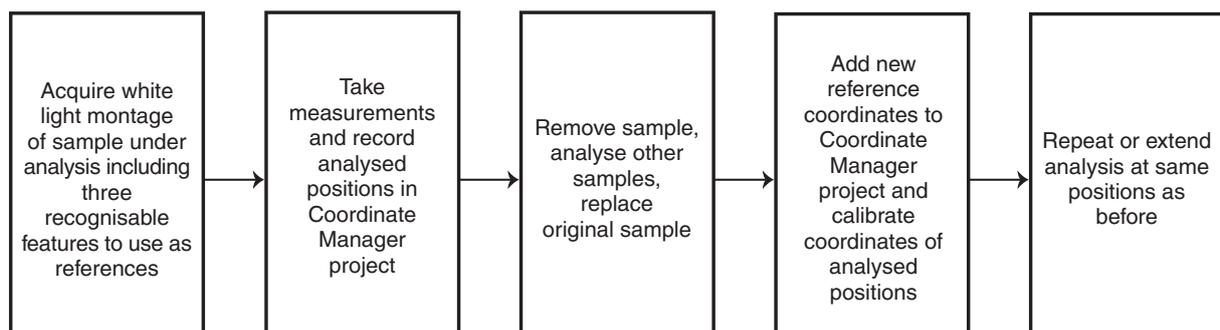


Example applications include pathological stained sections, any samples that utilise fluorescent tagging, and Raman data collection guided by SEM images.

Return to a sample

The features of the Correlate module can also be used solely with the inVia Raman microscope to allow easy removal and re-positioning of samples. For example, the sample can be moved between analysis sessions and the same positions located for further analysis. This makes the inVia Raman microscope available for analysis of other samples, and enables accurate re-positioning when returning to analyse the original sample.

Example workflow:



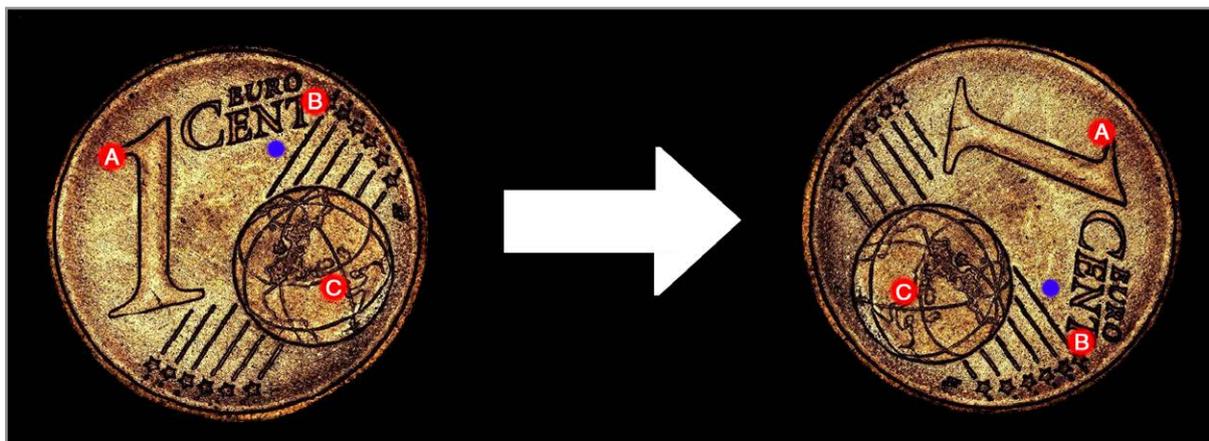


Figure 5. A coin that has been reanalysed using the Correlate module

The coin, shown in figure 5, was initially white light mounted and a particle analysed at the position shown in blue. Further analysis was required, so the coin was placed back in the inVia Raman microscope. Features in the white light montage of the coin were used as reference positions A, B and C. The same features were found in the moved sample and used to calibrate the coordinate of the analysed position, enabling it to be relocated for further analysis. This example shows the ability to find the same position on a sample after moving it, using the Correlate module and three recognisable features.

Renishaw. The Raman innovators

Renishaw manufactures a wide range of high performance optical spectroscopy products, including confocal Raman microscopes with high speed chemical imaging technology, dedicated Raman analysers, interfaces for scanning electron and atomic force microscopes, solid state lasers for spectroscopy and state-of-the-art cooled CCD detectors.

Offering the highest levels of performance, sensitivity and reliability across a diverse range of fields and applications, the instruments are designed to meet your needs, so you can tackle even the most challenging analytical problems with confidence.

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Please visit www.renishaw.com/raman for more information.

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