

A whole new world of colour (and 3D) for electron microscopy

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The Scanning Electron Microscope (SEM) is widely used in various fields of industry and science because it is one of the most versatile imaging and measurement instruments. SEMs allow users to see details 1,000 times smaller than those obtained using conventional microscopy.

On the downside, images provided by SEMs are two-dimensional and black-and-white. This is of course normal, as SEM technology uses electrons and not photons - or light rays - for visualization. And, where there is no light, there is no colour.

Yet, colour is in itself something that is very important to us humans. And not just from an aesthetic point of view. Even a long time ago, human perception of colour helped our ancestors live and survive, by allowing them to distinguish between different objects with potentially different properties. It was – and continues to be - very important to be able to identify a ripe fruit hidden in amongst the green leaves of a tree!

And so it follows that, when it comes to researching the nanoscopic world using SEMs, in order to speed-up object recognition and understanding of a specimen, researchers can spend hours of their time manually "colourising" the images they obtain.

But all that could soon change thanks to the increasing power of computer software and graphics, the technology surrounding electron microscopes is gradually moving towards both colour and 3D. In short, electron microscopy is becoming more "human".

From black-and-white to colour

In a SEM image, the value of each pixel corresponds to a single number, representing the number of electrons re-emitted by the surface at that particular location. This number is usually represented as a

greyscale value, and the overall result is a black-and-white image, in keeping with those produced using historical photographic methods.

So the question is, how does one go about colourising such an image?

Of course, each number representing a pixel can be arbitrarily matched to a colour via a lookup table. This is known as "false colour". Using this approach obviously doesn't add any additional information to the image, but it can allow better visualisation of different regions (or phases) of a sample. In SEM, this is the case when it is applied to images obtained from a BSE (back-scattered electrons) signal. (See Figure 1)

A colourised BSE image can also be superimposed onto the image obtained from the SE (secondary electron) signal. The result is an image in which the texture and composition of a sample are both highlighted thanks to variations in colour. This is what is called a "DDC-SEM" (density-dependent colour scanning electron micrograph). (See Figure 2)

Another approach, for SE images, is to manually colourise objects. Once a SEM image is obtained, researchers can spend many hours identifying, isolating and colourising each individual object, so that readers of their future publications are able to instinctively comprehend and interpret them.

The problem being, of course, that this is usually a very long and tedious operation. Each object has to be manually separated from the others, for example

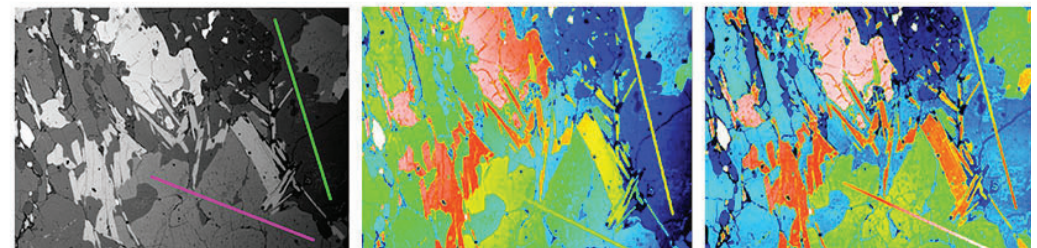


Figure 1. BSE images of a mineral specimen. From left to right: BSE image from a SEM displayed in greyscale - False colour image obtained by arbitrarily matching a colour to each grey level - After mathematical correction of grey level drift, each colour represents a different material that can then be quantified.

Image courtesy of the School of Geosciences, University of Edinburgh.

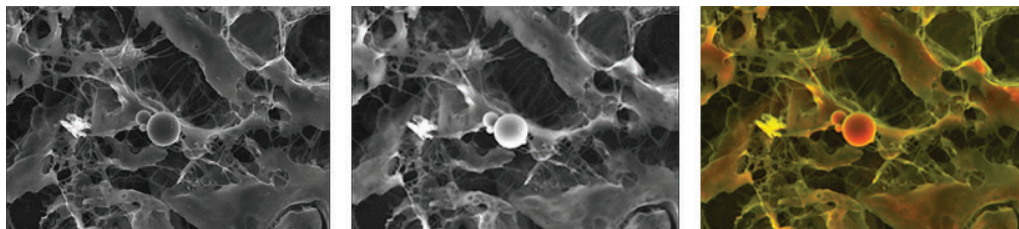


Figure 2. Image of calcified particles in cardiac tissue. From left to right: SE image – BSE image – DDC-SEM image obtained by superimposing the two previous images. This colourisation technique helps reveal the composition and texture of the sample.
Image author: Sergio Bertazzo / CC BY-SA 4.0.

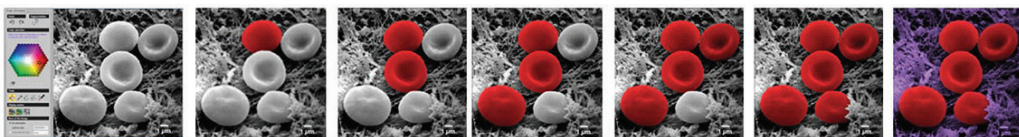


Figure 3. Image of erythrocytes (red blood cells): each stage of the colourisation process using MountainsMap® software is shown above. Only one mouse click is necessary to pass from one step to another, whereas the same operation could potentially take hours using photo-editing software.

Despite appearances, this method involves over 30 successive mathematical operations performed by the software in order to distinguish different objects in the image.

Of course, whether or not two adjacent objects should be separated can remain open to interpretation. For this reason, the software also allows users to define and modify boundaries around objects as they please.

Image courtesy of Thierry Thomasset – Université technologique de Compiègne

using image-editing software.

One new technique however, allows users to colourise images very quickly and simply. (Figure 3)

From 2D to 3D

There are several possibilities for switching from standard two-dimensional (SE) images to “topographic” images (i.e. those representing X, Y and Z, or height, coordinates). And, as we will see, colour is very closely linked to this process.

The most metrologically-accurate approach to 3D reconstruction of surface features is stereophotogrammetry. This technique consists of taking two SEM images of the same object from two different angles. Height information can then be calculated trigonometrically. The only drawback of this method is that it requires having a SEM which

allows specimen tilt and calls for two successive images of the sample to be taken.

In the case of Figure 4, the reconstructed 3D model is colourised by applying a colour palette and playing with transparency levels.

Another simpler method for performing reconstruction involves stereo-photometry and requires the use of a four-quadrant detector. Heights on a surface are no longer calculated trigonometrically, but by interpreting the differences between the 4 signals. The advantage of this method is that it only requires taking one “shot” of the sample, since all four images are created simultaneously by the four detectors. Unlike the previous example, it is not necessary to take two successive scans.

Of course, in this type of reconstruction, it is only

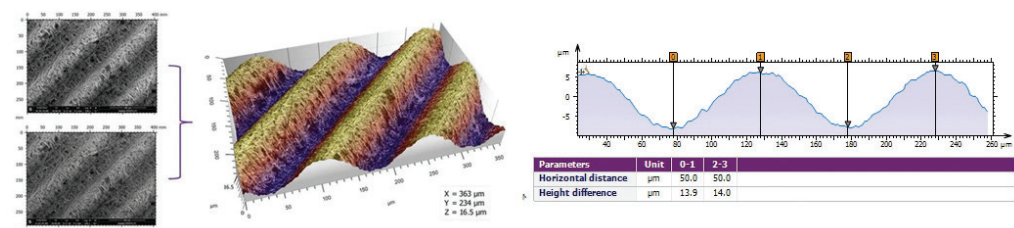


Figure 4. Images of a physical reference specimen. Metrologically-accurate topographic reconstruction obtained from two SEM images using stereophotogrammetry. The topography obtained allows the quantification of surface features (step height, volume, angles, flatness ...).

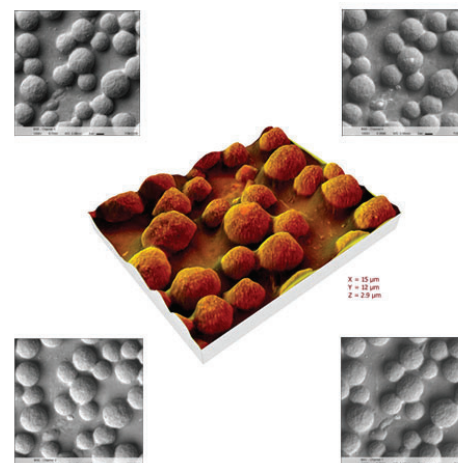


Figure 5. Topographic image of ink toner particles obtained from a four-quadrant SEM.

possible to reconstruct the visible part of the sample (i.e. that facing the electron gun). In other words, “cliffs” and overhangs are not taken into account. This method can only be metrologically-accurate if all the slopes of the object are visible.

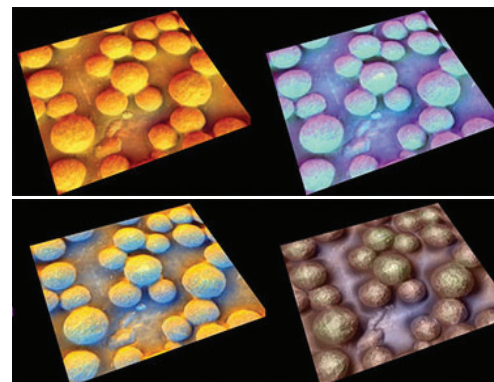


Figure 6. Reconstructed 3D models of the same image. Surface details and texture are highlighted by associating a colour with each image pair.

The presence of several signals does, however, allow users to simulate surface lighting. A different colour can be associated with each image pair, resulting in a much clearer perception of surface features.

Last but not least, another method is pushing the boundaries even further, making it possible to produce a colour 3D model from a single SEM image.

This 3D reconstruction from a single SEM image can only be obtained under certain conditions.

The surface of the specimen must be of smooth composition and not overly porous. Also, contrary to the 3D reconstruction techniques using two or four SEM images, the height values obtained using this technique have no metrological value. However, it is a major advantage and convenience to be able to work with a single image, even in low resolution, as is the case here with an image downloaded from the internet.

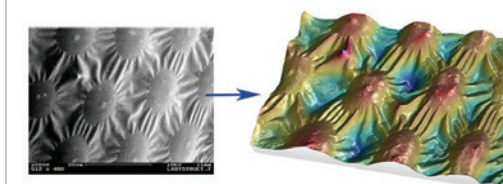
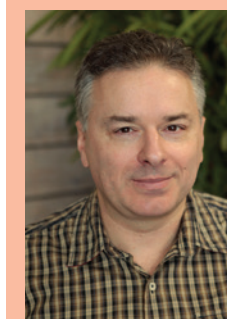


Figure 7. Image of spots on a ladybird. Left: original SEM image featuring image noise and a strong shadow effect on the left. Right: the same image rendered in 3D, using only the information contained in the first image. Note that the colours applied here are those traditionally used to express height on a physical map: the lowest points are blue (like the sea), the planes are green, the mountains red and the highest points are white (snow-capped peaks).
Image courtesy of Chris Supranowitz, University of Rochester

With all these new tools now available, the days of flat, greyscale SEM images may soon be a thing of the past. In the same way our ancestors sought out ripe fruit in the forest, researchers from all fields of science are beginning to use these techniques to find and highlight the smallest details contained in their data. Bacteria, cells, nanowires and all sorts of tiny surface features are brought to life in 3D and in colour.

All images processed using MountainsMap software. See: www.digitalsurf.com

About the author



Christophe Mignot is a software engineer and CEO of the surface analysis and metrology company Digital Surf which he founded in Besançon (France) in 1989.