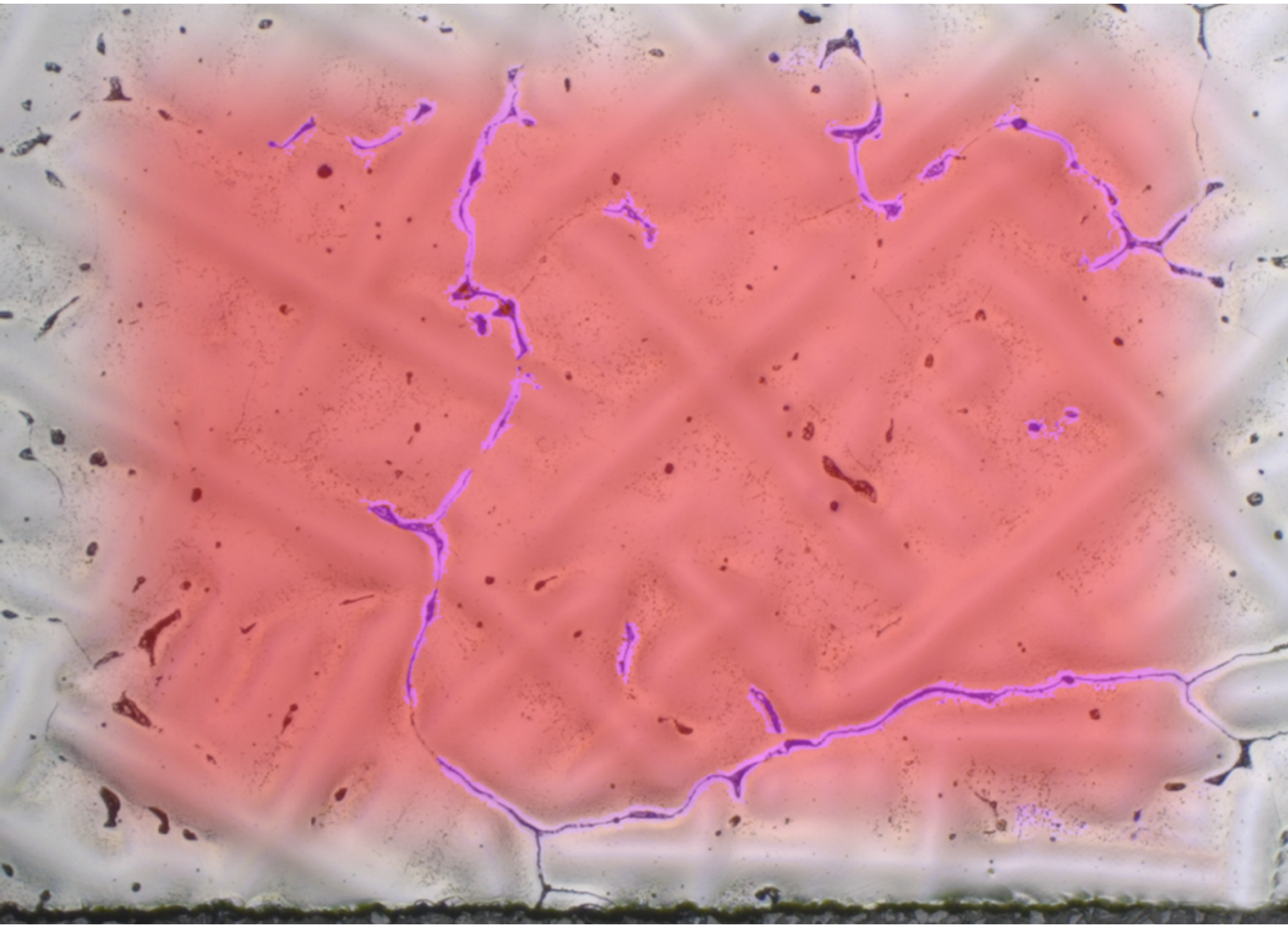
Application Note



# Advanced Segmentation for Industrial Materials

using Machine Learning

# Advanced Segmentation for Industrial Materials
## using Machine Learning

Authors: Dr. Roger Barnett and Dr. Alisa Stratulat
*Carl Zeiss Microscopy, Cambridge UK*
Dr. Matthew Andrew
*Carl Zeiss X-Ray Microscopy, Pleasanton USA*

Date: November 2018

**Introduction**

Since the development of the first microscope cameras, researchers and operators have sought to extract quantitative, actionable information from micrographs to further advance their research and improve their processes.

Every quantitative analysis of a set of micrographs involves some form of segmentation. Segmentation is the division of images into defined regions for subsequent categorization and analysis. A region could be a mineral fragment, a grain in a metal, a pore in a composite, an oil contamination on the surface, a blood cell – any area differentiable from a neighboring area. By analysing these regions or the borders between regions, we get useful information. There are several standards for determining useful microstructural properties by image analysis of segmented images – e.g. for grain size (ASTM E112),[1] graphite in cast iron (EN ISO 945-1),[2] inclusion content (ASTM E45)[3] and porosity in ceramic coatings (ASTM E2109).[4] These analyses, however, depend on the accuracy and reliability of the segmentation results.

**The Challenge**

Despite segmentation being the core of quantitative image analysis, segmentation is often difficult. Standard segmentation techniques involve defining regions based on thresholding their greyscale value or their color. This is frequently challenging as regions may have similar color and brightness and only be differentiable based on their texture, shape or their appearance under a particular contrast mode.

Another challenge is that, despite being obvious to a human operator, artefacts in the image such as scratches or tiny speckles may be mistaken for true features or relevant regions by a basic thresholding analysis. Working with multi-channel data (e.g. color data with red/green/blue channels or combined images with multiple channels each generated from a different polarization angle, different fluorescence channels, or spatially correlated datasets acquired from different instruments) often becomes more difficult as the complexity of region identification increases. Finally, time-consuming sample preparation is needed to generate high quality images, and for 3D data sets, a long data collection time is needed to reduce noise and create an output that can be easily characterised.

All of these difficulties mean that unless the system is simple, with clear image-wide contrast between regions, a skilled image processing specialist is required to create a usable workflow that combines appropriate digital filters and tools. Even then, this may not be possible and the operator must resort to manual analysis, which can result in biased analyses and much longer processing times.

***On the cover*** *Segmentation of cracking in Alloy 825 casting using machine learning with ZEISS ZEN Intellesis, shown in overlay. Sample provided by TWI Ltd.*

## Machine Learning – A solution to the segmentation problem

ZEISS ZEN Intellesis is a plugin for the ZEISS ZEN microscope control software. It is a data-agnostic guided machine learning system, which can be used alone or in conjunction with other software platforms for initial data generation or analysis/processing of a segmented image. The user defines a model, and then 'trains' the model by labelling regions on an image, set of images or part of a larger data set (2D or 3D). This is done by 'painting' the different classes or features of interest onto the image, as shown in Figure 1.

In this study we use ZEISS ZEN Intellesis, an interactive tool for machine learning based segmentation, which operates using the following principles. For every pixel in the image, a feature vector is created, a profile for each pixel that has a certain number of properties, generated from several intensity, texture and edge filters. A "forest of random trees" approach is then used to create a classifier, using these feature vectors, which best recovers the provided training labels. This classifier can then be applied across the extended image.

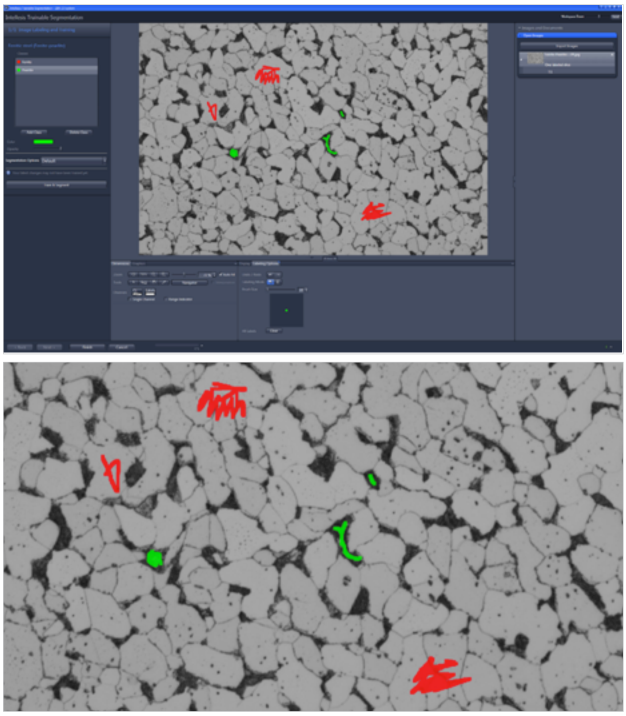


***Figure 1*** *ZEISS ZEN Intellesis training interface – identification of typical regions of interest in a ferritic steel: Ferrite in red and Pearlite in green.*

An iterative interactive interface is used to generate the training regions. This allows the user to paint on initial training regions on a portion of the image data. The interface then generates a segmentation algorithm for this region of the data set, and overlays the results onto the data. The user updates the labelling, improving the segmentation results that the machine learning has produced, and giving it additional labelled regions to generate an updated segmentation algorithm in an iterative manner. The user repeats the process as many times as needed to generate satisfactory results.

Once the algorithm has been trained on a small data set (e.g. two or three micrographs of a specific area of the sample) it can then be applied repeatedly to a much larger data set taken under the same imaging conditions, to automatically segment all micrographs and facilitate easy quantitative analysis, as well as repeatability of the results. ZEISS ZEN Intellesis will accept all standard 2D and 3D image formats as an input. It can accept single channel data (e.g. black and white images) or images with several channels (e.g. a composite image containing color light micrographs and a scanning electron micrograph of the same region).

ZEISS ZEN Intellesis supports even 6D datasets, like tile images with multi-channels and z-stacks over time.
Several practical applications of ZEISS ZEN Intellesis to industrial materials issues are shown below, from routine analysis to cutting-edge research.

## Phase Fraction Analysis in duplex stainless steels

Austenitic stainless steels are tough, relatively easy to weld and generally resistant to corrosion (hence their use in many domestic applications) but can be susceptible to stress-corrosion cracking in certain environments. Ferritic stainless steels are more resistant to stress-corrosion cracking but are comparatively brittle versus austenitic stainless steels and are harder to weld. Duplex stainless steels have carefully selected compositions which have high levels of chromium and other alloying elements that lead to a microstructure containing approximately equal amounts of ferrite and austenite. The synergy of the two disparate phases allows the structure to overcome several issues of the individual phases – the steel is relatively weldable but also resistant to stress-corrosion cracking. Duplex stainless steels are generally used for specific service environments, where corrosion resistance, mechanical strength and weldability are all needed.

The ratio of ferrite to austenite is affected by the composition but also by the thermal history. Welded regions and heat-affected zones may have different ratios of austenite to ferrite and thus different local properties. To understand/predict the steel behavior a metallurgist must determine the austenite/ferrite ratio in these regions. Metallographic preparation of duplex stainless steels is relatively straight-forward, to allow the austenite and ferrite to be visualized. However, it is difficult to etch the duplex stainless steel in a way that lends itself to automated analysis by thresholding, particularly where the austenite grain size varies dramatically.

Figure 2 shows an example micrograph of unwelded duplex stainless steel after color etching. Automated segregation of white austenite from brightly colored ferrite by thresholding based on RGB values is successful for the larger grains of austenite, but struggles on the smaller grains of austenite due to etching effects and color bleed from surrounding ferrite. Using machine learning, a user can successfully segregate the austenite from ferrite, even the smaller grains. There is always a degree of ambiguity determining the exact position of borders between one region and another in any image analysis operation, but this ensures that smaller regions are not missed. When measured using traditional thresholding, this field of view has 46.9% austenite, but when measured using segmentation by machine learning, this increases to 51.2% austenite.

**Determination of size distribution of nanoparticles**

Nanoparticles research plays a very important role in numerous industrial applications such as pharmaceuticals, biomedical applications, coatings, inks and pigments, energy materials and filtration. To engineer nanoparticles with unique properties, improve synthesis methods and innovate new products, the chemistry, size and shape of individual nanoparticles must be characterized. Even though there are bulk analytical techniques (such as sieving or laser scattering) to determine particle size distribution (though these methods may be limited by particle size and/or composition), auto-mated analysis of individual nano-particles in agglomerates still remains a challenge.

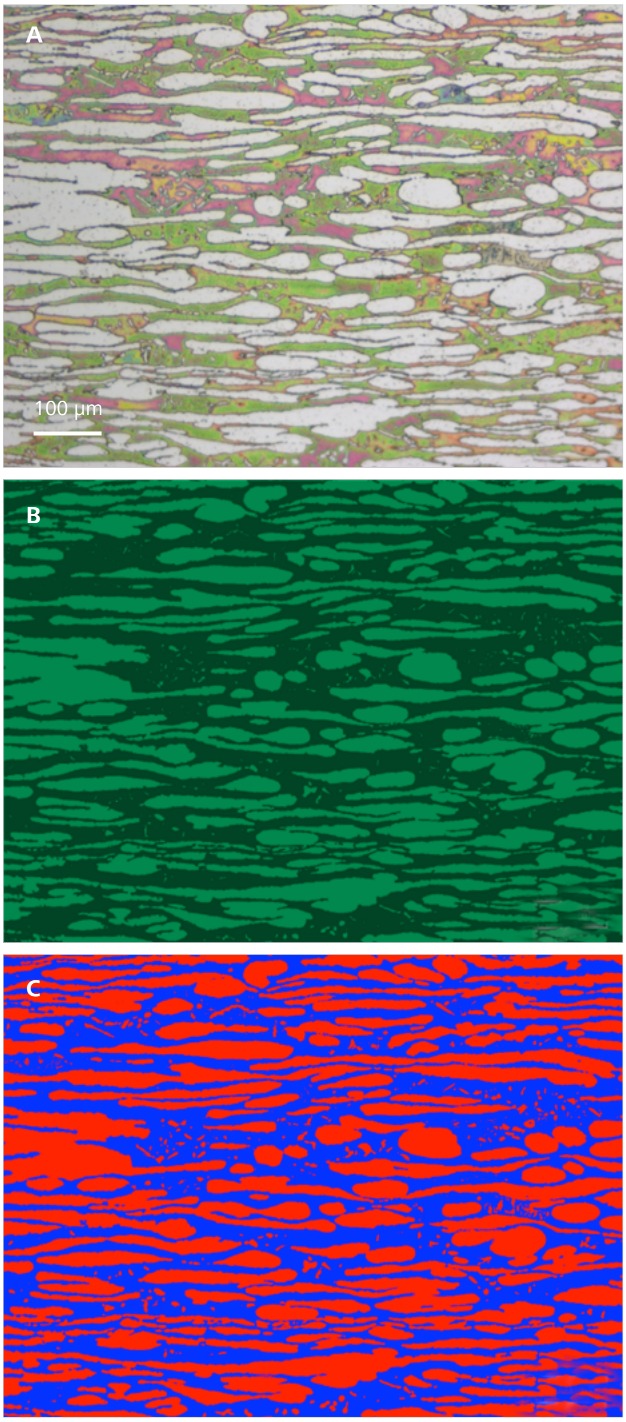



***Figure 2*** *(A) Cross-section of duplex stainless steel*
*(B) The same cross-section after segmented by thresholding on RGB values*
*(C) After segmentation using machine learning in ZEISS ZEN Intellesis (right).*
*Sample courtesy of TWI Ltd*

As traditional image segmentation algorithms fail to identify the boundaries between individual nanoparticles, this type of investigation is still performed by a human operator and often leads to inaccurate and inconsistent results.

Figure 3 shows an example of an end-to-end automated workflow used to separate individual nanoparticles in agglomerates and to determine their particle area distribution. Nanoparticles from the sparks of ferrocerium collected on a silicon substrate were acquired using Secondary Electron detector, on a ZEISS GeminiSEM 500.[5,6] Machine learning segmentation in ZEISS ZEN Intellesis was successfully used to identify three different classes: nanoparticles, boundary between nanoparticles and background. To further separate individual nanoparticles and determine the size distribution, an open-source python package *(https://scikit-image.org)* was created in APEER, the cloud-based digital microscopy platform for ZEISS (see below), for a seamless and personalized analysis. Using an automated workflow that combines advanced microscopy, machine learning image segmentation and analysis, characterization of individual nanoparticles in agglomerates was performed. This type of investigation helps researchers to better understand the relationship between material properties to further advance industrial research.

**APEER – Microscopy workflows simplified through easy to use modules**

Often, in industrial materials research, integrating complex image processing and analysis workflows is required. As shown in the nanoparticles example above, a series of tools and microscopy solutions were used in order to extract meaningful data from micrographs. While there are individual software packages that offer analytical capabilities, there is still the need for modularity and end-to-end personalized solutions that can be reused easily, in an automated manner.

APEER provides a digital common platform to overcome this challenge, such that microscopy users can build and combine pre-defined packages into unique workflows that can be shared with peers, to accelerate research and innovation. Individually created modules and workflows can be easily accessed and no expertise in computer vision is needed.

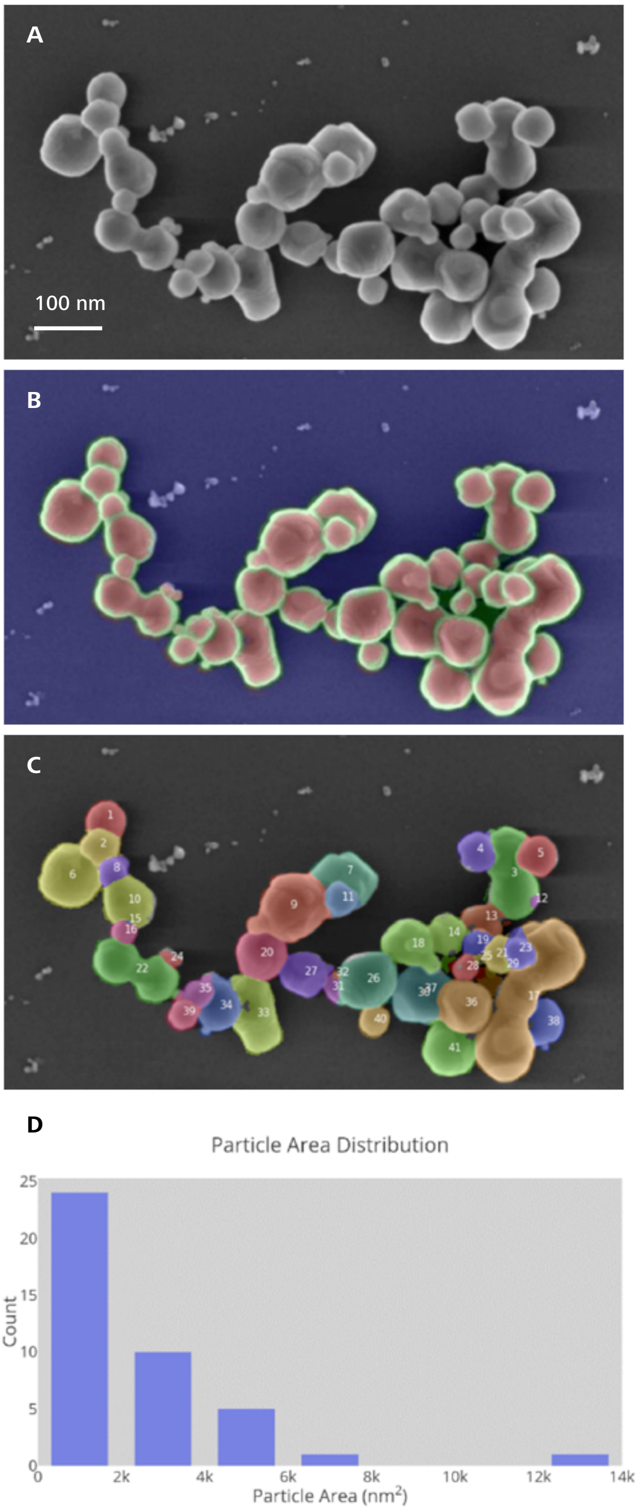



***Figure 3*** *Workflow of nanoparticles size distribution analysis.*
*(A) Original Scanning Electron Microscope (SEM) image of nanoparticles acquired at 2 kV using the Inlens detector.*
*(B) Image of segmented image using Intellesis showing background (blue), boundaries between particles (green) and nanoparticles (red).*
*(C) Image of separated individual nanoparticles using machine learning and further analysis.*
*(D) Particle area distribution of individually segmented nanoparticles.*

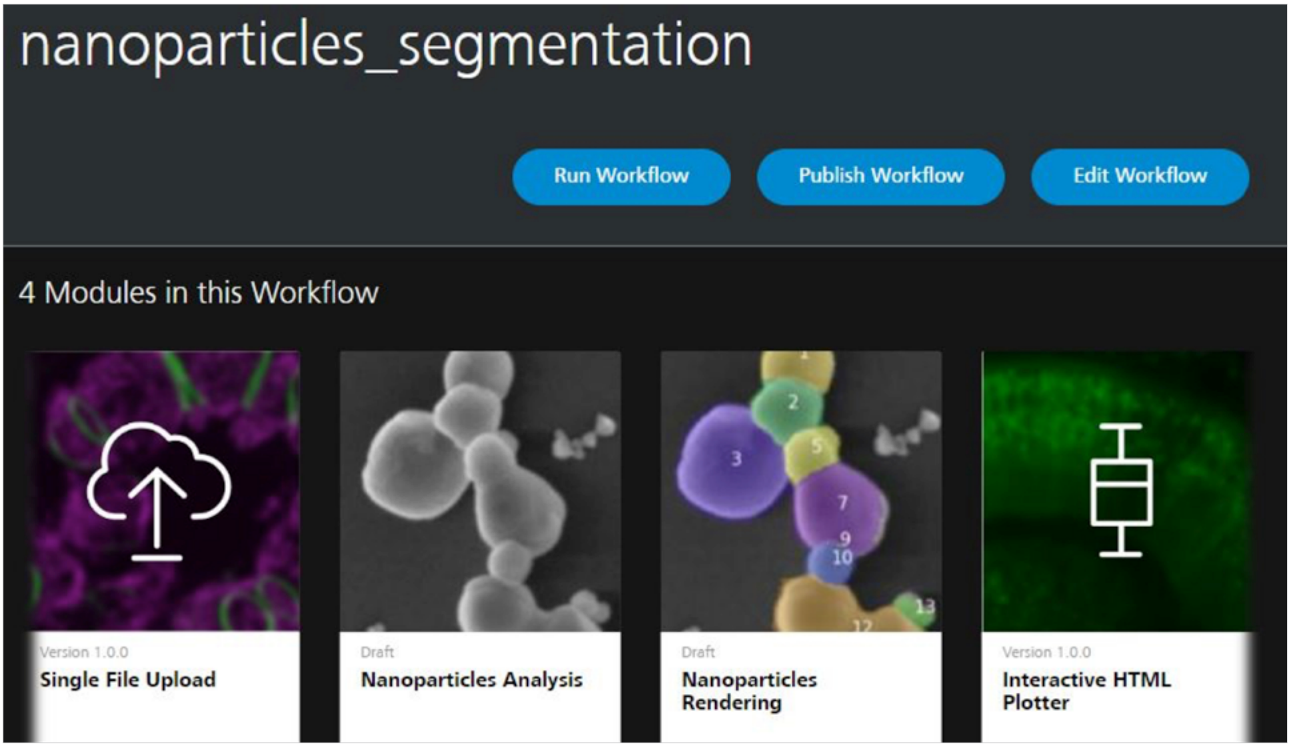



***Figure 4*** *APEER interface showing how modules can be connected to create a full workflow for nanoparticle analysis and segmentation*

An example workflow is illustrated in Figure 4, which was used for nanoparticles segmentation above. In this particular example, four different independent modules were connected to perform the analysis in simple steps, from uploading the image of interest to producing the personalized visualization of the results.

In industrial materials environment, researchers could benefit from APEER automatically performing routine tasks for improved efficiency, gaining flexibility in building pre-defined modules and saving time by using workflows targeted for specific jobs. In addition, APEER can also help researchers manage and keep tracks of their results by benefiting from a common integrated digital platform.

**Assessment of layer thickness**

Coatings and surface layers are used in a variety of industries – cosmetic, functional, protective or even just formed as a by-product. Examples include paint, galvanization, thermal spray coatings, corrosion scale, epoxy resins, physical vapor deposition, chemical vapor deposition and catalytic layers.

The thickness of the coating(s) and individual coating layers will affect the lifetime and performance. As such, measurement of the thickness of the coating is a key parameter – not just average thickness but minimum, maximum and thickness distribution over a known length. This can be done manually or automatically, and there are a number of Standards for coating measurement (e.g. ASTM B487-85[7]) depending on the specific application.

In some micrographs, coatings are clearly differentiable from their surroundings, with a color or greyscale value range that does not overlap with that of the substrate or mounting resin. Standard thresholding and image processing applications are sufficient to segregate the coating in these cases.

More often, the differences between layer(s) and substrate may be clear to the human eye but hard to automatically analyse. Figure 5 shows three examples. For the galvanized steel, the greyscale values of the coating match those of the resin in several locations. This makes subsequent image processing difficult.

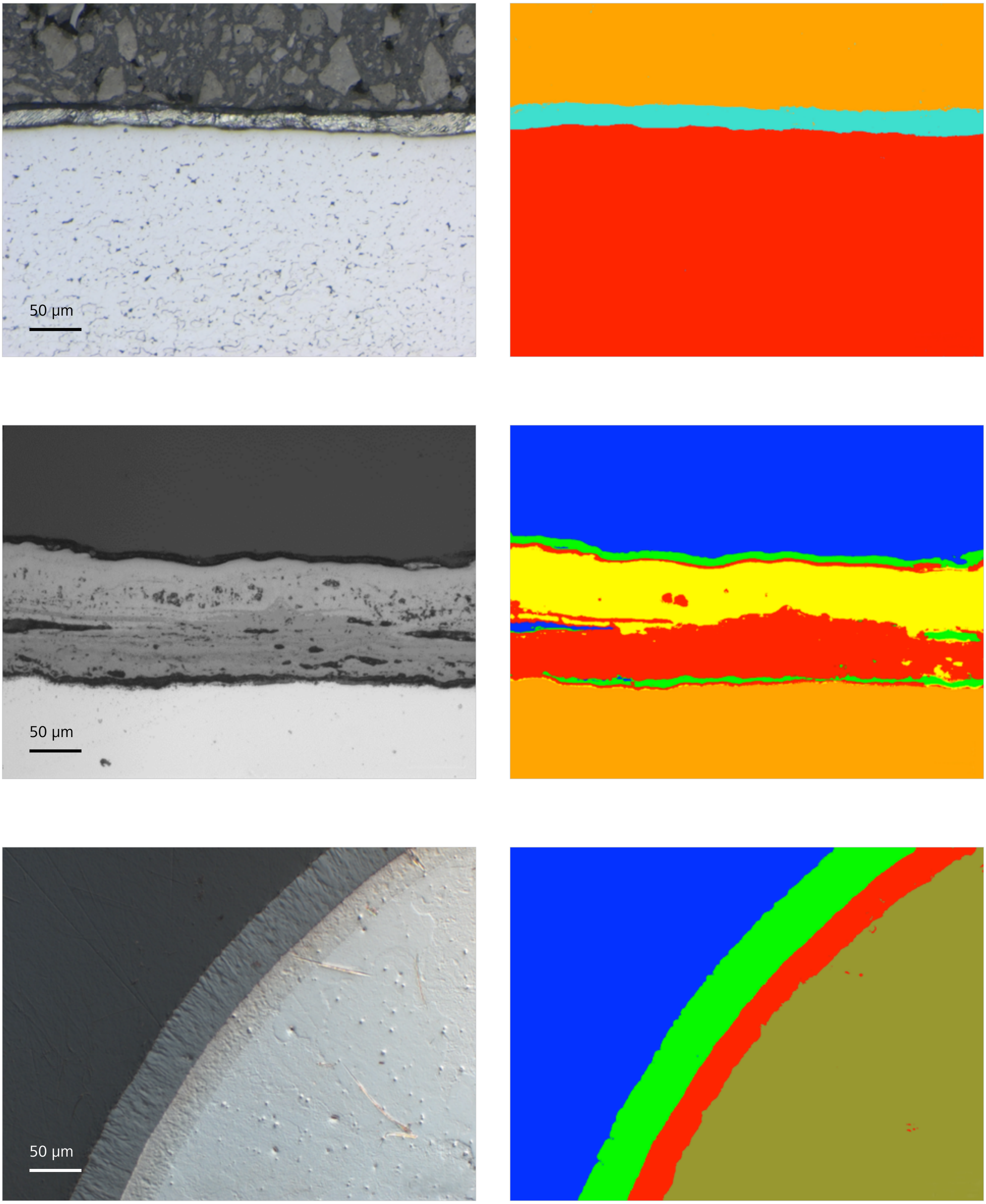
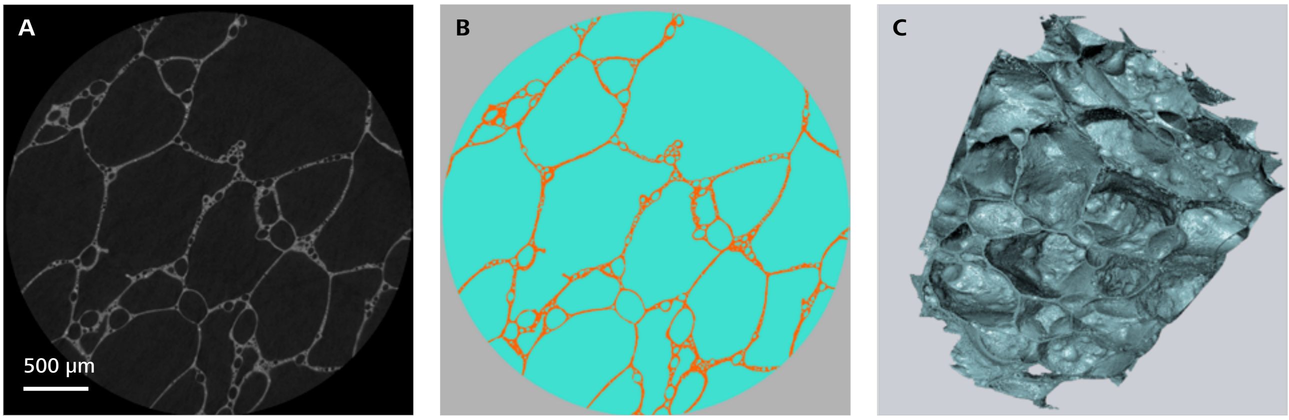



*__Figure 5__ ZEISS ZEN Intellesis segmentation of coating cross-sections. Each color on the segmented image represents a different coating layer.*
*(Top) Galvanised steel – bright field.*
*(Middle) High temperature corrosion scale on 9% chromium steel – bright field. Sample courtesy of TWI Ltd*
*(Bottom) Thermal spray coating, taken using C-DIC contrast.*




The high temperature corrosion scale can be segregated from the substrate, but individual layers within the scale are more difficult, particularly as the pores in one phase can match the greyscale value of the other.

For the ceramic sample, two separate challenges were present – the layer closest to the substrate differs from it primarily in terms of texture rather than greyscale values, and there are several scratches on the sample crossing boundaries between layers.

In all cases, after sufficient training ZEISS ZEN Intellesis was able to segregate the layers for subsequent quantitative measurement. This was true even where the sample was scratched or had other metallographic preparation artifacts.

**Segmentation of 3D data sets – Foam glass**

In order to better understand failure and fracture of materials, observe microstructural evolution in real time and perform physics simulations on real structures, 3D imaging techniques must be used. ZEISS ZEN Intellesis is also capable of segmenting 3D data sets (in a compatible format, e.g. .txm, .tiff or .czi), such as those generated by X-ray microscopy.

Labelling can be applied to any or all slices through the 3D dataset. The model trains on all labelled slices and thus tine inputs can come from any labels on any slices. It should be noted that there are no feature vectors operating within 3D – the feature vectors are created on a slice-by-slice basis. However, this effect may be mitigated by analysing the data with different slice angles and taking averages.


***Figure 6*** *ZEISS ZEN Intellesis segmentation of a foam glass.*
*(A) Virtual 2D slide of X-ray micrograph data set.*
*(B) Segmented microstructure showing pores in blue, glass walls in red.*
*(C) 3D model of the foam glass, using segmentation results.*
*(D) Image of a typical foam glass structure.*
*Sample courtesy of Martin Bonderup Østergaard, Dr. Rasmus R. Petersen and Prof. Yuanzheng Yue from Aalborg University, and Dr. Jakob König from Jozef Stefan Institute.*

An example of 3D data set segmentation is shown in Figure 6. The specimen is a foam glass insulator used in the construction industry. Researchers are interested in determining the porosity and internal structure of these materials, improving the synthesis process of mixing glass powder plus foaming agents and simulating its thermal properties using physics simulations on real 3D structures.
In order to extract this information, obtain homogeneous pore size distribution, minimize defects and increase insulation capability, image segmentation in 3D of raw data is needed. Then, ZEISS ZEN Intellesis was able to create a 3D representation of the sample. To do this, a model was trained to segment the internal structure such that both large pores and smaller pores present in the glass walls are identified to produce accurate results.

Comparison of classical image segmentation algorithms (global multi-Otsu thresholding or seeded watershed growing) with machine learning multivariant classification in ZEISS ZEN Intellesis has also been carried out on synthetic images produced from actual 3D data sets. All algorithms performed well under low noise levels but machine learning classification was much more noise tolerant than the other algorithms. Machine learning was also able to segment based on textural contrast, which the traditional algorithms were unable to do. The two traditional techniques achieved misclassification rates of above 50% in the textural contrast regions (at zero noise levels), whereas for machine learning this dropped to below 5% misclassification.[8]

**Grain size determination of metals and ceramics**

The properties of most engineering alloys and ceramics are strongly affected by the grain size and morphology. Various standards exist for measurement of grain size by light microscopy e.g. ASTM E112 [1] or by other methods including electron back-scatter diffraction such as ASTM E2627.[9] There is a fundamental factor common to all methods – differentiation of one grain from its neighbors. For light micrographs, this is facilitated by appropriate etching – either to highlight the grain boundary (typical in steels and nickel alloys) or by coloring each grain differently from its neighbor (e.g. some aluminium alloys under polarised light). Once individual grains have been identified, measuring their size/shape distribution is trivial. Figures 7 and 8 show examples of grain boundary detection in metals and ceramics using light and field emission scanning electron microscopy. The metal (Alloy 600) was polished to a 0.25µm finish and then electro-etched in a dilute sulfuric acid.

The grain boundaries are clearly visible, as are the twinning lines within the grains. However, the twinning lines are lighter than the grain boundaries, and grain boundary detection was straightforward using machine learning. The zirconia sample was more challenging – it was examined in the as-received (un-polished and un-coated) condition in a ZEISS Sigma 300 FE-SEM using secondary electron imaging at 1kV.

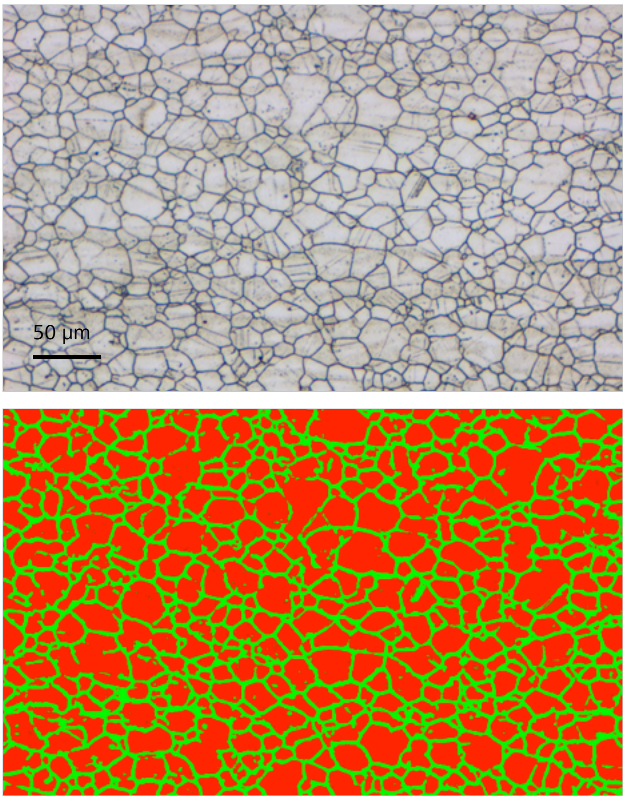



***Figure 7*** *(Top) Nickel Alloy 600 after metallographic preparation and electro etching. Brightfield imaging on a ZEISS Axio Imager Z2.m. (Bottom) ZEISS ZEN Intellesis segmentation of this image, showing grains in red and grain boundaries in green.*

Grain boundaries are visible, but there are significant variations in contrast across the sample, as well as several pores. Using machine learning in ZEISS ZEN Intellesis, it was possible to directly segment grain boundaries to permit determination of grain size/shape, while simultaneously detecting and measuring pore size/shape/distribution.

**Conclusions**

Image segmentation is an important step for industrial researchers, materials scientists and technicians who want to extract meaningful information from their 2D or 3D micrographs. They can enhance their research, improve productivity of routine tasks and increase their accuracy. Even though classical threshold-based methods and machine learning algorithms exist, using them effectively and accurately often requires image segmentation expertise.

The lack of automated image segmentation can result in operator-biased analysis as well as many hours of manual investigation. In fast moving industrial environments, a robust image segmentation platform that provides repeatability and accuracy of results while saving time, is essential.

ZEISS ZEN Intellesis brings all these advantages to organizations and individuals working on industrial materials and it can be fully integrated into the ZEISS ZEN software platform. From performing grain size analysis on metals or ceramics, size distribution of nanoparticles in agglomerates, layer and phase analysis of materials, to porosity and exporting 3D real structures for physics simulations, ZEISS ZEN Intellesis works efficiently on all standard image formats (both colour and greyscale) and provides a seamless image segmentation.

A free trial licence of ZEISS ZEN Intellesis is available from the ZEISS website *(https://www.zeiss.com/intellesis)* and it can be downloaded for use on the most challenging micrographs. Benefit from an easy to use and integrated ZEISS ZEN ecosystem, to get more information faster from industrial materials micrographs. Combine ZEISS microscopy solutions with machine learning image segmentation and modular workflows to bring innovation in your industry.

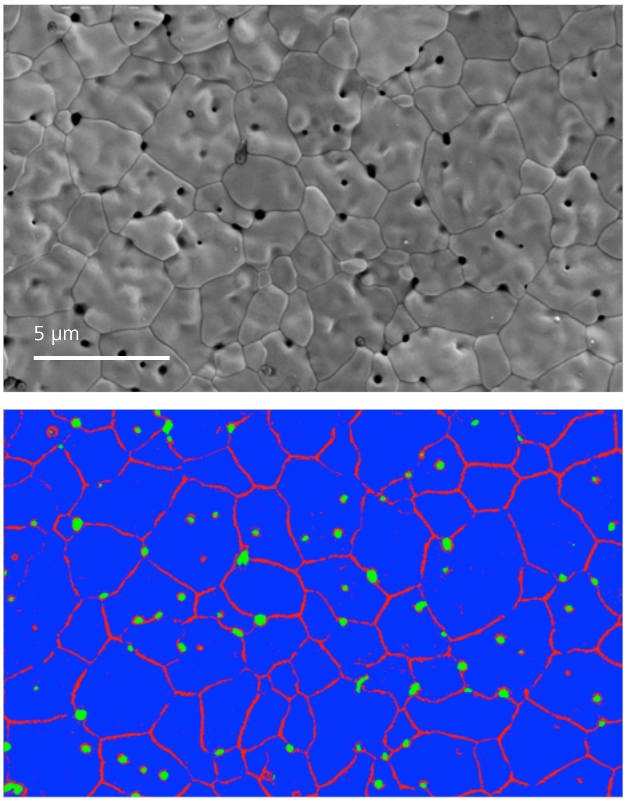



***Figure 8*** *(Top) Zirconia in the as-received condition, secondary electron imaging at 1kV at 30Pa in a ZEISS Sigma 300 VP.*
*(Bottom) ZEISS ZEN Intellesis segmentation of this image, showing grains in blue, grain boundaries in red, pores in green.*

**References**


[1] ASTM E112-13 "Standard Test Methods for Determining Average Grain Size"

[2] ISO 945-1:2017 "Microstructure of cast irons -- Part 1: Graphite classification by visual analysis"

[3] ASTM E45-18 "Standard Test Methods for Determining the Inclusion Content of Steel"

[4] ASTM E2109-01(2014) "Standard Test Methods for Determining Area Percentage Porosity in Thermal Sprayed Coatings"

[5] Zhou, Fang, et al. "Characterizing InGaAs/GaAs quantum dots using low-kV FESEM imaging and EDS analysis at the nanometer scale." *Microscopy and Microanalysis* 22.S3 (2016): 1586-1587.

[6] Alfonso, C., et al. "Low kV High Resolution Scanning Electron Microscopy Study of Si Nanowire Surfaces." *Microscopy and Microanalysis* 21.S3 (2015): 1261-1262.

[7] ASTM B487-85(2013) "Standard Test Method for Measurement of Metal and Oxide Coating Thickness by Microscopical Examination of Cross Section"

[8] Andrew M. "A quantified study of segmentation techniques on synthetic geological XRM and FIB-SEM images" *Computational Geosciences,* August 2018. https://doi.org/10.1007/s10596-018-9768-y

[9] ASTM E2627-13 "Standard Practice for Determining Average Grain Size Using Electron Backscatter Diffraction (EBSD) in Fully Recrystallized Polycrystalline Materials"

**Carl Zeiss Microscopy GmbH**
07745 Jena, Germany
microscopy@zeiss.com
www.zeiss.com/intellisis