Nanoparticles Research Accelerated by Digital Solutions Platform

End-end Microscopy Workflow in One Single Platform

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Engineered nanoparticles have a wide range of industrial applications including catalyst research, electronics, inks and pigments, coatings, cosmetics, filtration, energy materials, pharmaceuticals and biomedical applications. With nanotechnology applications likely to develop rapidly in these and other industries, advancing nanoparticles research will play an important role in improving the quality of life [1].

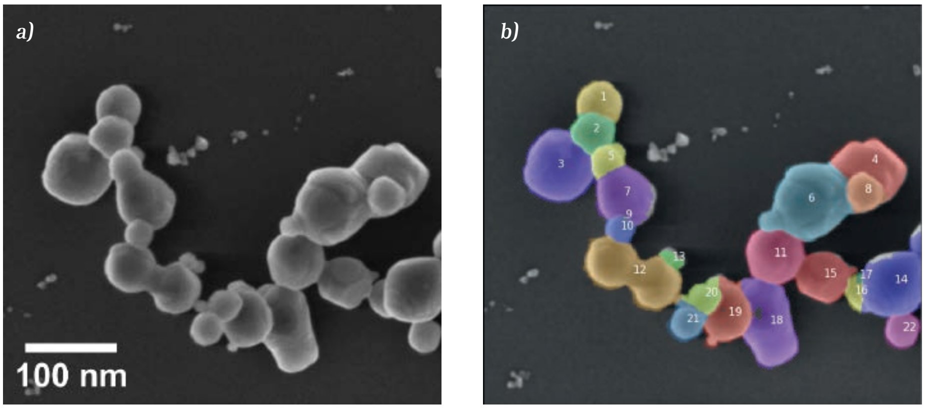

Fig. 1: (a) Original image of particles obtained at 2 kV beam energy, using the Inlens SE detector and (b) Image of separated particles using machine learning and subsequent processing.

Advances in processing routes allow researchers to design the structure of the materials to achieve unique properties. It has been concluded that bulk properties such as vibrational behavior, mechanical performance, magnetic response and catalytic activity are highly dependent on the size of nanoparticles. This applies in such diverse materials as silicon nanowires, silica nanoparticles in paper, magnetic materials and gold nanoparticles supported on glassy carbon [2]. To engineer materials at the nanoscale, optimize the synthesis process, and innovate new products and applications, industry requires accurate and detailed nanoparticles size characterization using both advanced microscopy and image analysis.

Limitations and Challenges in Nanoparticles Analysis

There are many traditional methods to determine particle size and particle size distribution (e.g. sieving or laser scattering) but there are still many challenges that need to be addressed to seamlessly analyze nanoparticles in a single workflow. Researchers [3] working on nanoparticle analysis using electron micrographs stated some of the most common challenges that still exist in this research area: 1) separating and classification of individual nanoparticles from an agglomeration of particles and 2) automating analysis of what can typically be hundreds of nanoparticles in a single micrograph.

Conventional image segmentation methods use the watershed approach after initial thresholding by greyscale or RGB values. However, in the case of separating agglomerated particles, the problem is more challenging and identifying the boundaries between individual particles still remains a complex problem. Therefore, measurement of nanoparticle size is still a highly manual process which is often not repeatable or reliable. To address this need, an end to end automated workflow is proposed that combines image acquisition, machine learning advanced image segmentation, particle analysis, and report generation - all on a digital solutions platform, Apeer. Apeer enables the combination of easy-to-use modules into workflows that can be personalized to address the most challenging research problems.

Bringing Fragmented Software Together

In the following example, Zeiss GeminiSEM 500 [4,5] was used to acquire a high-resolution micrograph of particles from the sparks of ferrocerium collected on a silicon substrate (fig. 1a). These were imaged using Secondary Electron (SE) imaging, a technique which is extremely sensitive to surface topography. It is this variation in surface topography which (during visual inspection) can be used to separate otherwise overlapping particles. The manifestation of such features in an image is complex and they can be challenging to extract using traditional computer vision techniques. In this study we use machine learning trainable image segmentation with Zeiss ZEN Intellesis. Instead of performing standard image processing operations on the 2D image, it classifies images on local and non-local greyscale, gradient and texture features, effectively creating a 33-dimensional hyper-image.

A custom image processing and analysis module was then created using the open-source python package Sci-Kit Image (https://scikit-image.org). This module first removes noise from the segmented phase by removing particle centers smaller than 9 pixels [2]. The (separated) particle centers were labelled and then grown using a watershed algorithm operating over a distance map radiating away from each particle center, bounded by the segments of the background phase (fig. 1 b).

Once the particles have been separated and indexed, measurements such as diameter, perimeter, area, length, etc. can be performed on individual nanoparticles. Graphs of interest are automatically generated in Apeer. Figure 2 illustrates an example - the particle area distribution of the nanoparticles shown in figure 1.

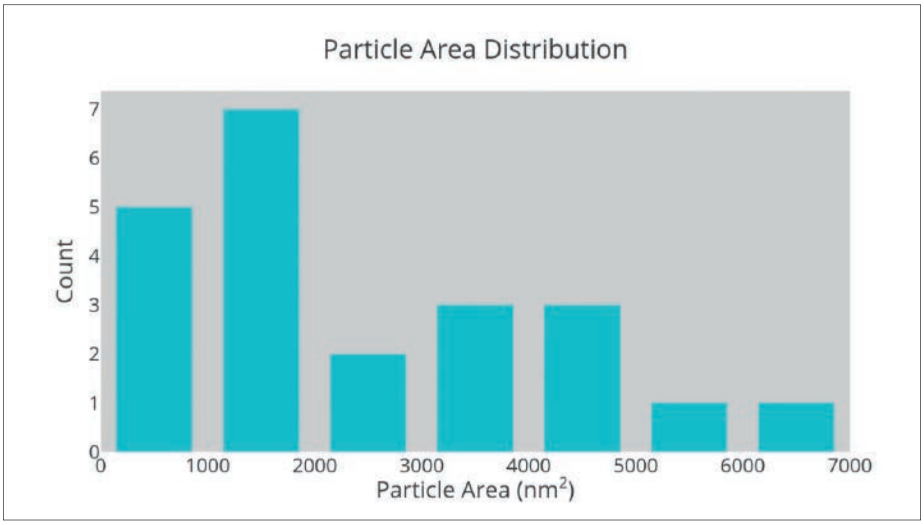

Fig. 2: Particle area distribution of separated particles.

One of the principal challenges associated with complex image processing and analysis workflows is modularity. Single software packages, while offering powerful analytical capabilities, do not provide an end-to-end complete solution for every application. As such, multiple disparate packages must be used (or even created from scratch) making workflow replication at an industrial scale extremely challenging. Apeer provides a platform where such custom or disconnected components can be defined, developed and connected to create complete workflows, ready to be shared for use. Detailed analytical workflows can be made without the requirement of a deep expertise in computer vision. Figure 3 shows the workflow used for the above nanoparticles segmentation and analysis in Apeer interface.

The modular architecture of Apeer allows for custom analysis components to continue being used without either requirements for ongoing maintenance or uncertainty about code stability or operability.

Conclusion

The size and size distribution of nanoparticles strongly affects the quality, properties and applications of these materials, but in the case of overlapping nanoparticles, characterization still lacks precision and automation, requiring many hours of manual particle counting.

Accurate and automated measurement of nanoparticles was made possible by a combination of both advanced microscopy and image analysis techniques –Zeiss GeminiSEM 500 was used to acquire high-resolution images and Zeiss ZEN Intellesis was used for advanced image segmentation of these FE-SEM images. Using the information achieved through image segmentation, a new platform, Apeer, is now available to allow the integration of customized modules in a full workflow, to gain flexibility and save research time.

In this application, area distribution of individual overlapped nanoparticles was automatically determined in a single end-to-end workflow, using Apeer. The described workflow can be used to optimize synthesis processes of nanoparticles and better understand the relationship between size of nanoparticles and material properties. This allows researchers to expand the use of these materials to novel industrial applications.

References

[1] Buzea, Cristina, Ivan I. Pacheco, and Kevin Robbie: Nanomaterials and nanoparticles: sources and toxicity, Biointerphases 2.4: MR17-MR71 (2007)
[2] Guisbiers, Grégory, Sergio Mejía-Rosales, and Francis Leonard Deepak: Nanomaterial properties: size and shape dependencies, Journal of Nanomaterials (2012)
[3] Park, Chiwoo, *et al.*: Segmentation, inference and classification of partially overlapping nanoparticles, IEEE transactions on pattern analysis and machine intelligence 35.3: 1-1 (2013)
[4] 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: 1586-1587 (2016)
[5] Alfonso, C., *et al.*: Low kV High Resolution Scanning Electron Microscopy Study of Si Nanowire Surfaces, Microscopy and Microanalysis 21.S3: 1261-1262 (2015)

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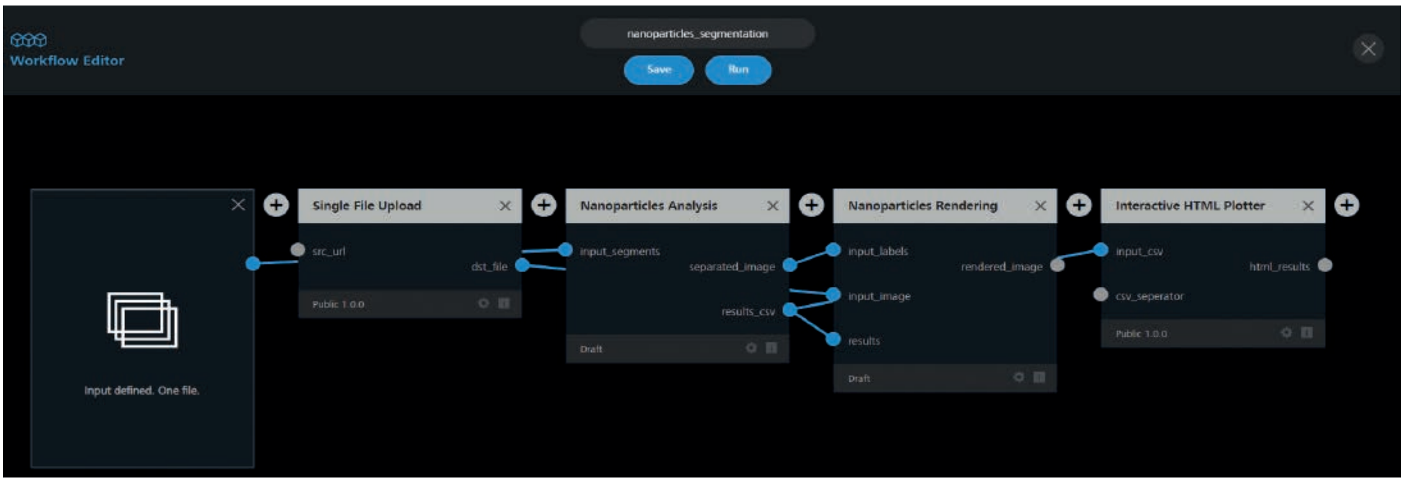

Fig. 3: Apeer interface for nanoparticle segmentation and analysis, showing how modules can be connected to create a full workflow.