FALL 2024

Newsletter

SURFACE

Surface imaging, analysis & metrology news from Digital Surf

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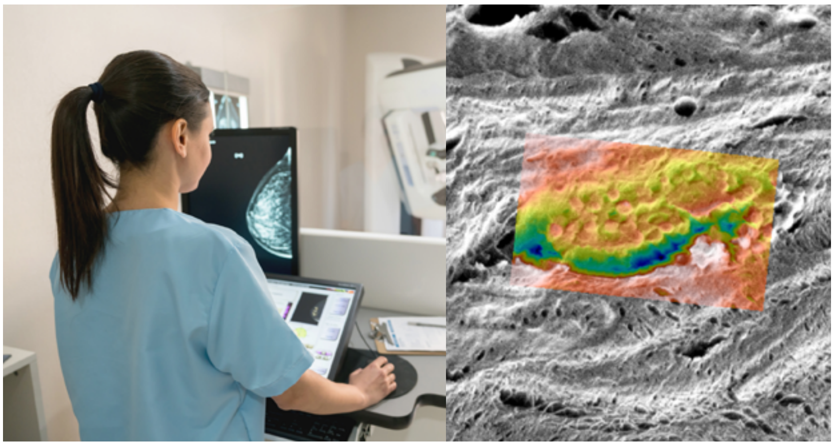
IN THIS ISSUE

NEW INSIGHTS INTO BREAST MICROCALCIFICATIONS USING COLOCALIZATION OF AFM-IR SPECTROSCOPY DATA



A recent study utilized advanced AFM-IR spectroscopy to explore the chemical complexity of breast microcalcifications (MCs) in unprecedented detail.

By combining SEM, µFTIR and AFM-IR techniques, researchers mapped out chemical variations within MCs at the nanoscale. This approach reveals insights that could reshape understanding of biomineralization in breast tissue, offering new perspectives in breast cancer research.

... Turn to page 4 ...

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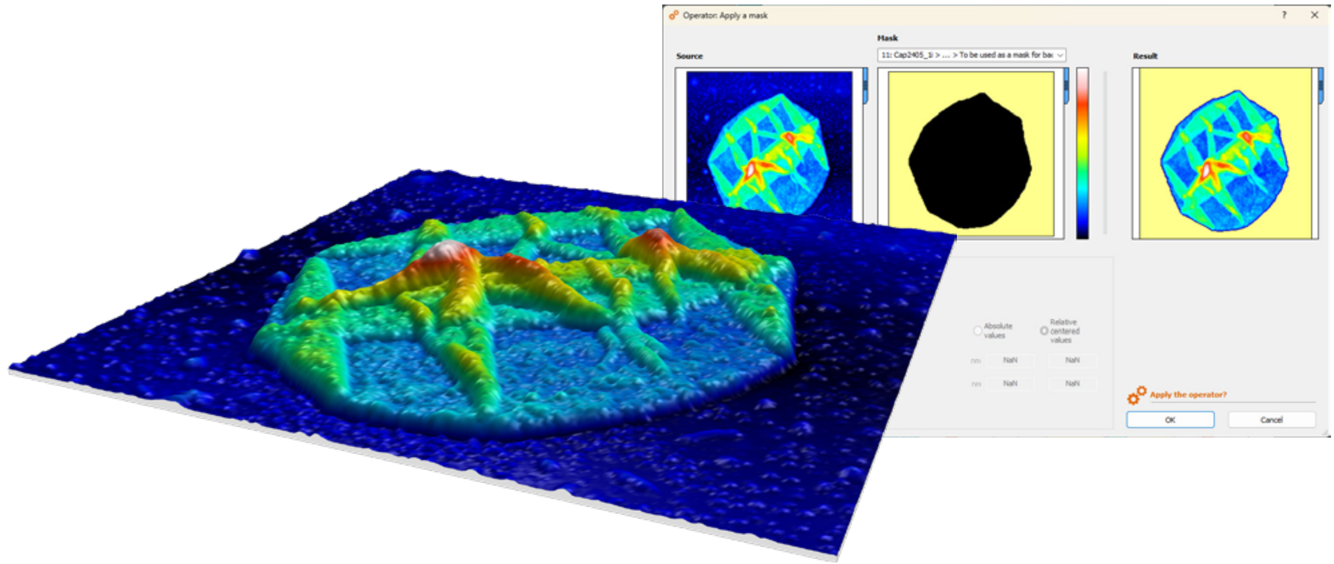
MOUNTAINS® 10.3 UPDATE: ANALYSIS, ENHANCED

In an exciting development for users of microscopy, profilometry and spectroscopy techniques, Digital Surf is set to launch **version 10.3 of its Mountains® analysis software** on November 19, 2024. This update introduces a plethora of new features designed to enhance the platform's analysis capabilities across various fields, from surface topography to spectral analysis.



Above. *New "Apply a mask" operator allows partitioning of surfaces, even if regions of interest have similar height values.*

CROSS-TECHNOLOGY FEATURES

One highlight of version 10.3 is the expansion of cross-technology features.

Users can now rename numerical results using customizable **aliases**, simplifying the organization of data and allowing for easier retrieval and export.

The update also introduces a powerful **Particle Analysis classification manager**, designed to ensure reliability by minimizing human error and enabling seamless collaboration. Users can save and share classifications, facilitating reproducibility and data-sharing across teams or even between laboratories.

Another useful addition is the ability to **set graduation scales** to conform to publication standards, facilitating the preparation of scientific papers.

Surface data can now be partitioned into several regions of interest by **applying masks**.

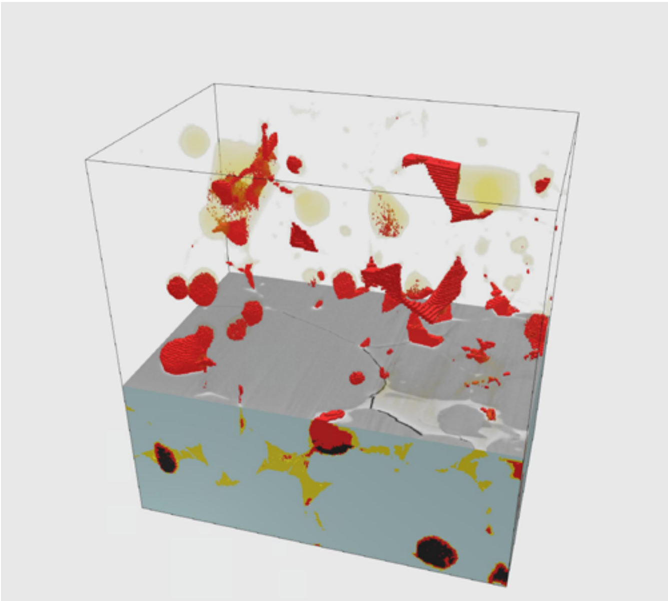
FEATURES FOR PROFILOMETRY

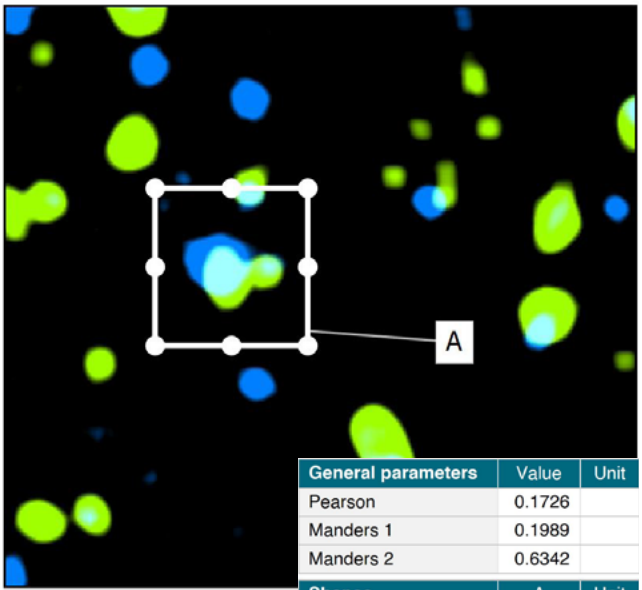
2D and 3D profilometry data analysis offers several important enhancements. Surface analysis now benefits from **improvements to the Map local properties operator** including new parameters such as surface variance and mean curvature.

Version 10.3 also sees **improved tools for defect removal**. Users can now correct outliers or asperities in series of profiles or surfaces more efficiently and access tools optimized for sloping or arc-shaped profiles.

Additionally, the new **ball screw analysis** feature allows detailed exploration of contour profiles measured on this type of mechanical component.

Improvements in **power spectral density (PSD)** analysis tools, which were initially launched in version 10.2, make multi-scale surface analysis even more intuitive. These tools now support ISO 10110-8 specification values and provide advanced visualization options.





General parameters	Value	Unit
Pearson	0.1726	
Manders 1	0.1989	
Manders 2	0.6342	
Shapes	**A**	**Unit**
Pearson	0.3900	
Manders 1	0.4083	
Manders 2	0.6593	

Above left. *Enhanced display and animation of materials in multi-channel cube data including volume electron microscopy data.* ***Above right.*** *Colocalization coefficient calculations.*

FEATURES FOR SEM

Version 10.3 debuts new functionalities for **volume electron microscopy, particularly FIB-SEM and EDX cubes**. These include **3D cutting planes** for more dynamic visualizations. Users can notably select crop options, adjust plane positions, and set transparency levels.

Advanced correction tools for handling grayscale drift in data slices are also now available, enhancing precision in multi-channel cube analyses.

FEATURES FOR SPECTROSCOPY

Spectral analysis workflows also get an upgrade in version 10.3. The release provides **new pre-processing techniques tailored for photoluminescence and cathodoluminescence data.** The Convert W-axis operator, new baseline correction methods and enhanced band analysis summaries make spectral data handling even more comprehensive.

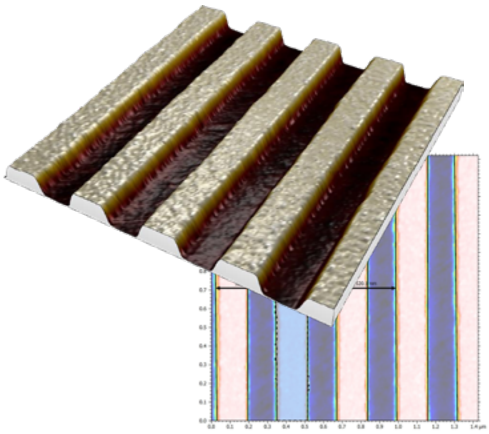
Another noteworthy feature is the ability to **convert images into spectral maps**, which paves the way to more robust data pre-processing options.

Mountains® now also supports **colocalization coefficient calculations**, useful in fluorescence microscopy for quantifying overlap between cellular components.

FEATURES FOR SPM

Scanning Probe Microscopy (SPM) specialists will appreciate the introduction of **line edge roughness (LER) analysis**, a useful feature for those working in semiconductor fabrication, on topographical data.

This feature allows quantification of the roughness of flanks on grooves, essential for process control.



Edges	LER		
Unit	nm		
Edge #1	10.27		
Edge #2	11.23		
Edge #3	9.147		
Edge #4	9.179		
Edge #5	9.996		
Edge #6	2.996		
Edge #7	10.29		
Edge #8	5.804		
Edge #9	8.245		
Peaks	**LWR**	**CD**	**Pitch**
Unit	nm	nm	nm
Band #1	18.63	172.1	321.7
Band #2	16.75	170.8	322.7
Band #3	12.20	168.2	320.1
Band #4	12.84	169.7	******
Trenches	**LWR**	**CD**	**Pitch**
Unit	nm	nm	nm
Band #1	16.60	149.6	320.4
Band #2	16.26	152.0	320.1
Band #3	13.11	151.9	321.6
Band #4	15.19	151.5	******

Above. *Calculate line edge roughness (LER) from topography.*

LEARN MORE & UPDATE

Check www.digitalsurf.com for full details of the v10.3 release (available Nov 19, 2024). Access to the new version is included for users with an active **Mountains® Software Maintenance Plan**. To find out more about your Maintenance options, please contact sales@digitalsurf.com

INFRARED NANOSPECTROSCOPY (AFM-IR) UNRAVELS CHEMISTRY OF BREAST MICROCALCIFICATIONS

The **AFM-IR lab at the Institute of Physical Chemistry, Paris-Saclay University (Orsay, France)** is the pioneering research group in InfraRed-Atomic Force Microscopy (AFM-IR). Founded by Alexandre Dazzi, the inventor of the AFM-IR technique, and now led by Ariane Deniset-Besseau, the group specializes in AFM-IR instrumental and methodological developments while continuously broadening the technique's field of applications. In particular, the team specializes in the **characterization of complex materials, ranging from astro to biosciences**, including studies on pathological calcifications such as breast microcalcifications (MCs), described here for *Surface Newsletter* by Margaux Petay, former AFM-IR lab PhD student.

A MULTIMODAL STRATEGY TO EXPLORE BREAST MICROCALCIFICATIONS

Breast microcalcifications (MCs) are calcium-based deposits found in breast tissue, ranging in size from hundreds of nanometers to millimeters. These inorganic deposits can be present in both cancerous and benign breast lesions, yet their formation mechanisms remain poorly understood.

Over the past decade, Raman, IR and O-PTIR spectroscopy have been increasingly used to study the chemical composition of MCs in breast biopsies. However, these techniques are limited to a spatial resolution of about a micron at best (Raman and O-PTIR), which restricts the chemical imaging of individual MCs - analyses that could give new insights into their formation processes.

To address these limitations, we developed a multimodal and multiscale strategy that integrates SEM, µFTIR and AFM-IR, enabling a comprehensive morphological and chemical characterization of MCs from the micro to the nanoscale level directly within breast biopsy samples (Fig.1). This approach involves the following sequential steps:

1. **SEM**: acquisition of micrographs at low, intermediate and high magnification. Localization of MCs within the tissue and morphological characterization at the micro and nanoscale
2. **µFTIR**: acquisition of hyperspectral images. Analysis of the chemical diversity among MCs across the tissue
3. **AFM-IR**: acquisition of topographical images, chemical maps at one wavenumber and hyperspectral images. High-resolution chemical imaging of individual MCs.

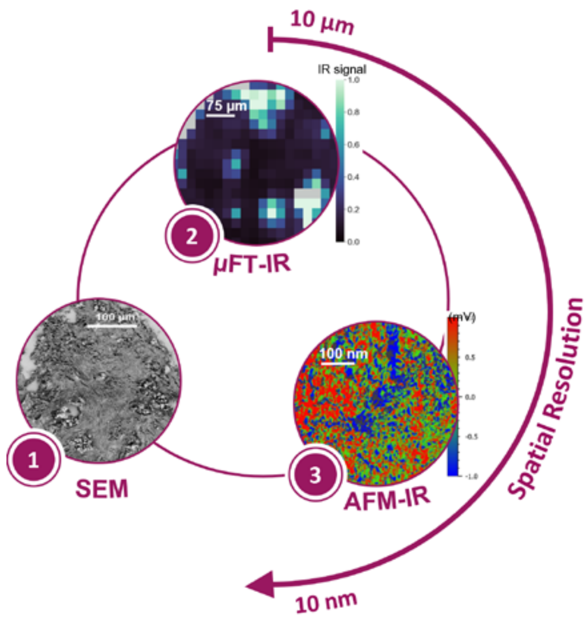


Fig. 1 *Summarized workflow of the multimodal and multiscale approach.*

COLOCALIZING MULTISCALE CHEMICAL AND STRUCTURAL INFORMATION

To accurately compare MC physicochemical properties across different scales - from the microscale to the nanoscale - a registration step is essential to align SEM, µFT-IR and AFM-IR

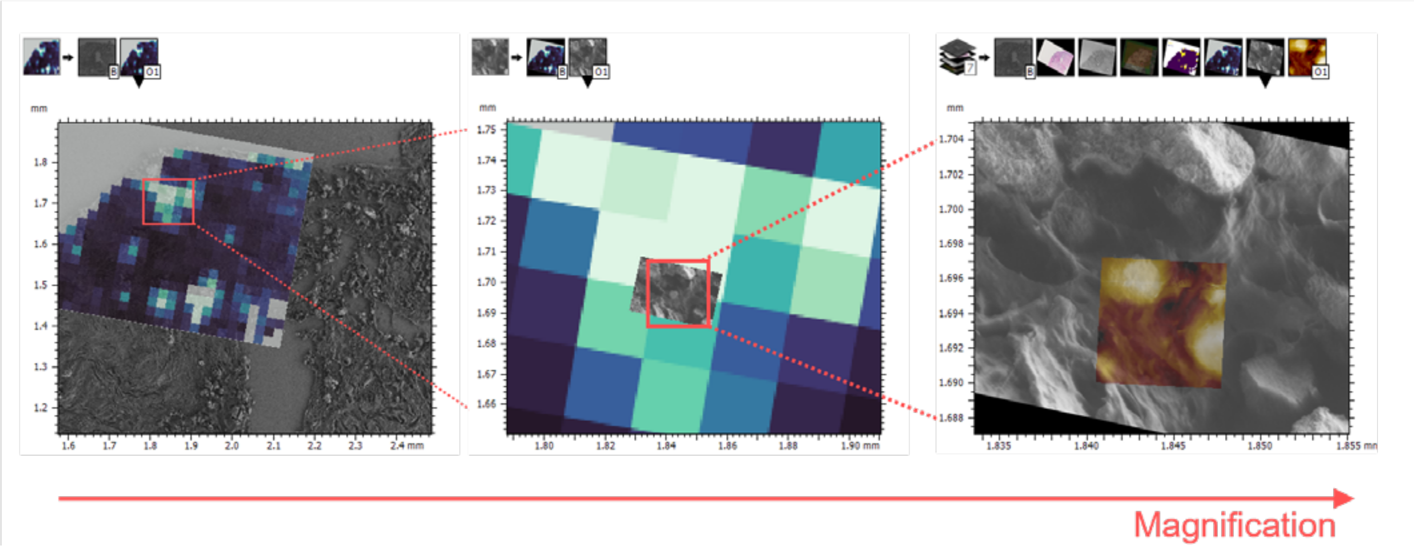


Fig. 2 µFT-IR and AFM-IR colocalization using SEM micrographs as reference images. From left to right: SEM cliché & µFT-IR 1655 cm^{-1} chemical map; µFT-IR & zoomed SEM micrograph and SEM micrograph overlay with AFM-IR topography.

measurements within the same spatial coordinate system. This process can be challenging due to significant differences in the spatial resolution of these techniques.

To achieve this, we used SEM micrographs at low, intermediate and high magnifications as reference images. These images were then employed to register the µFT-IR and AFM-IR datasets using the Mountains® software colocalization tool with manual point positioning. This approach ultimately enabled the creation of a multichannel study with colocalized SEM micrographs, µFT-IR chemical map and AFM-IR measurements (topography and chemical maps) (Fig. 3).

UNVEILING MICROCALCIFICATION HETEROGENEITIES AT THE NANOSCALE

This multiscale approach offers the advantage of capturing the chemical diversity of MCs across the biopsy thanks to FTIR microscopy, while AFM-IR investigates individual MCs, particularly those too small to be accurately identified and characterized using standard IR microscopy, as shown in Figure 3, with a 4-micron diameter MC.

This approach provides a global and detailed chemical description of MCs within breast biopsies, delivering a holistic view of calcifications that was not previously achieved. If applied to a broader cohort of samples, this strategy could enhance our understanding of the mechanisms driving biomineralization in breast tissue.

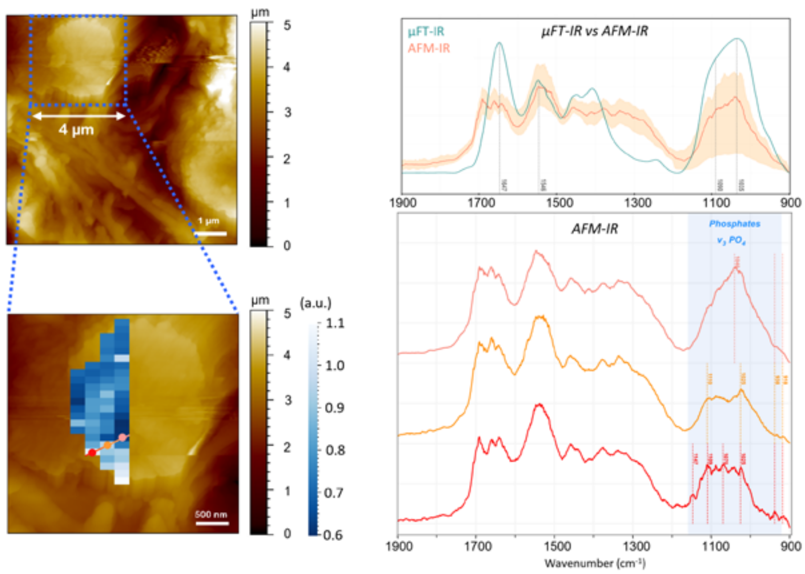


Fig. 3 AFM-IR analysis of a 4µm diameter MC. The chemical map overlaid with the topography corresponds to hyperspectral AFM-IR imaging showing 1109 cm^{-1} / 1035 cm^{-1} ratio, highlighting chemical heterogeneities within the MC's calcium phosphate lattice. Red, orange and pink dots locate positions where local AFM-IR spectra (right panel) were acquired.

INSTRUMENTS & SOFTWARE USED

Scanning electron microscope, µFT-IR, AFM-IR & Mountains® software.

READ MORE

- Multimodal and multiscale analysis of complex biomaterials: optimization and constraints of infrared nano-spectroscopy measurements, Petay M., Université Paris-Saclay, 2023, theses.hal.science/tel-04416918.
- Multiscale approach to provide a better physicochemical description of women breast microcalcifications, Petay et al., C. R. Chim, 2022, doi.org/10.5802/crchim.210.

BUILD A 3D MODEL FROM STANDARD SEM IMAGES

In this article, **Benoit Zupancic, product manager for SEM applications at Digital Surf**, delves into the world of 3D reconstruction from standard "2D" scanning electron microscopy (SEM) images. This technique, powered by advanced analysis software solutions such as MountainsSEM®, transforms SEM images into detailed 3D models, offering new insights on data in applications across a wide range of fields.

WHAT IS 3D RECONSTRUCTION FROM SEM IMAGES?

3D reconstruction from SEM images involves obtaining volume models of objects in the sample from standard two-dimensional images. When images are of good quality and reconstruction is done correctly, these methods can provide reliable height maps for precise analysis.

It's worth noting that this approach differs from volume techniques such as dual beam FIB-SEM, although MountainsSEM® software also offers a range of features for processing data from volume electron microscopy.

STEREOSCOPIC RECONSTRUCTION: AN OVERVIEW

One of the methods employed in obtaining 3D reconstruction is stereoscopic reconstruction.

Stereoscopic reconstruction creates height maps from two images taken at slightly different angles. This technique leverages computer vision to analyze proximity of objects to the detector using the disparity between images.

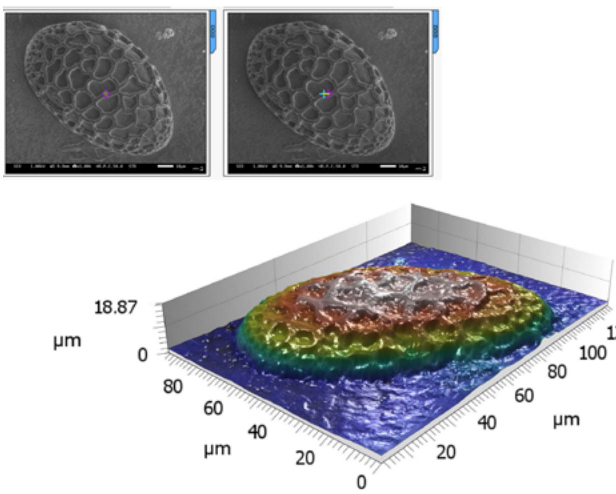
There are a few key points to remember when performing stereoscopic reconstruction:

- **Tilt stage use:** users should ensure the sample is tilted around one axis *only* for optimal results.
- **Eccentricity:** the sample's center of rotation must be aligned with the image center.
- **Image quality:** it is important to use samples with texture, avoid uniform or blurry areas, and maintain consistent brightness.

STEPS FOR STEREOSCOPIC RECONSTRUCTION

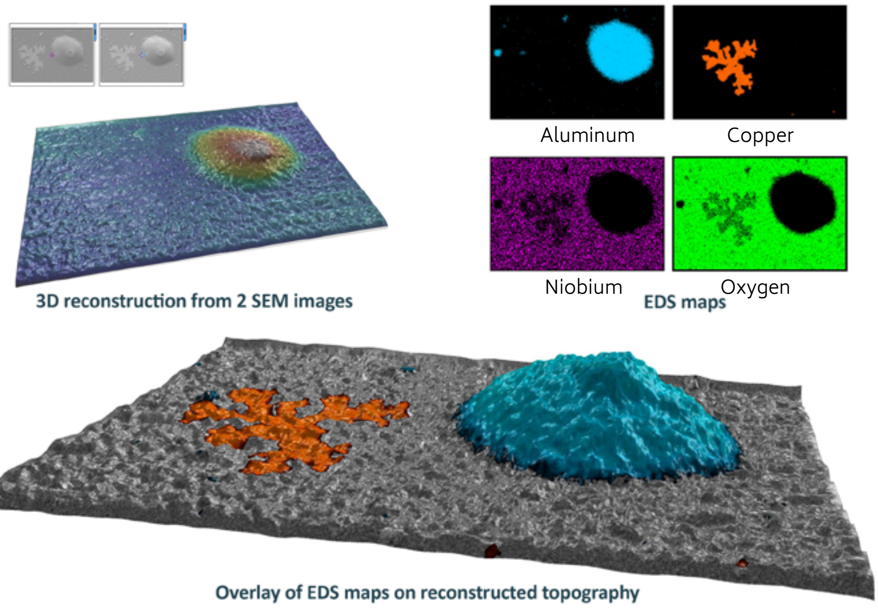
Once image capture is complete (ideally the two images should be acquired at tilt angles between 5 and 10 degrees), the workflow for obtaining a 3D reconstruction is surprisingly simple:

1. Load the images into MountainsSEM® software and select the "stereoscopic reconstruction" operator.
2. Set tilt direction and correct any image artifacts, for example using the "remove outliers" option.
3. Use the software to generate and customize the 3D model, adjusting settings like Z-scale amplification and transparency for better visualization of details.



***Above.** 3D reconstruction of a pollen sample obtained from two SEM images, in a matter of seconds using MountainsSEM®.*

Right. *Overlay of EDS compositional data on 3D reconstruction of two SEM images brings new insights in material science.*



ADVANCED TECHNIQUES AND APPLICATIONS

Beyond 3D reconstruction, MountainsSEM® software also supports correlative analysis and integration with other datasets, such as EDS mapping. This allows for detailed studies of phenomena in materials such as electromigration shown in the example on the right.

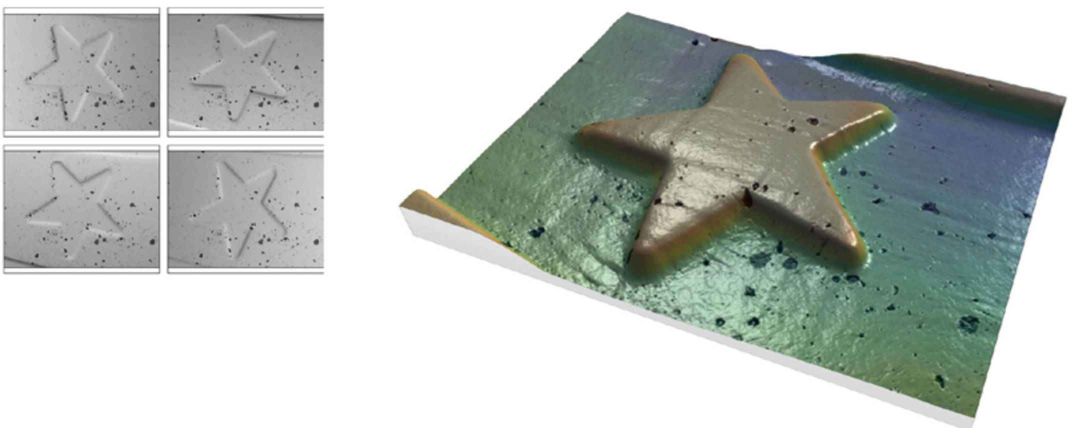
FOUR-IMAGE RECONSTRUCTION

Most SEMs come equipped with four-quadrant Backscatter Detectors (BSD). The good news is MountainsSEM® software also offers a 3D reconstruction technique based on the simultaneous acquisition of four images. This "shape from shading" method uses differences in illumination to construct height maps.

In the case of 3D reconstruction from four-quadrant images, it is important to regularly calibrate your instrument, using standards provided by SEM manufacturers, if you wish to obtain accurate height values.

The four-quadrant method is very useful in certain applications where tilting the object is not feasible, for example, when analyzing large wafers.

Whether you're an experienced SEM user or new to image reconstruction techniques, MountainsSEM® software is designed to make 3D reconstruction straightforward and accessible. Happy reconstructing!



Left. *3D reconstruction is also easily attainable using any SEM equipped with a four-quadrant detector.*

RECONSTRUCTION RESOURCES

- Watch the webinar www.digitalsurf.com/webinars/revolutionize-your-research-3d-topography-from-sem-images/
- Download a Free Trial of MountainsSEM® www.digitalsurf.com/free-trial
- 7 tips for producing SEM stereo pairs www.digitalsurf.com/blog/7-tips-for-producing-sem-stereo-pairs/

IS THE S-FILTER EQUIVALENT TO THE OLD λs FILTER?

Surface texture filtering has evolved with recent standards like ISO 25178 and ISO 21920, which introduced updated terminology. The traditional λs filter removed microroughness from profiles, but new concepts like the S-Filter and L-Filter now play distinct roles.

In this article, Digital Surf's senior surface metrology expert **François Blateyron** clarifies the actions of each filter and explains how users can apply them correctly in metrological analysis.

The λs filter, introduced in ISO 3274 and ISO 4288, was used on profiles to remove the microroughness that is composed of the shortest wavelengths, in order to obtain the primary profile which is the starting point of metrological analysis. Microroughness is mainly composed of instrumental and environmental noise and is of little interest.

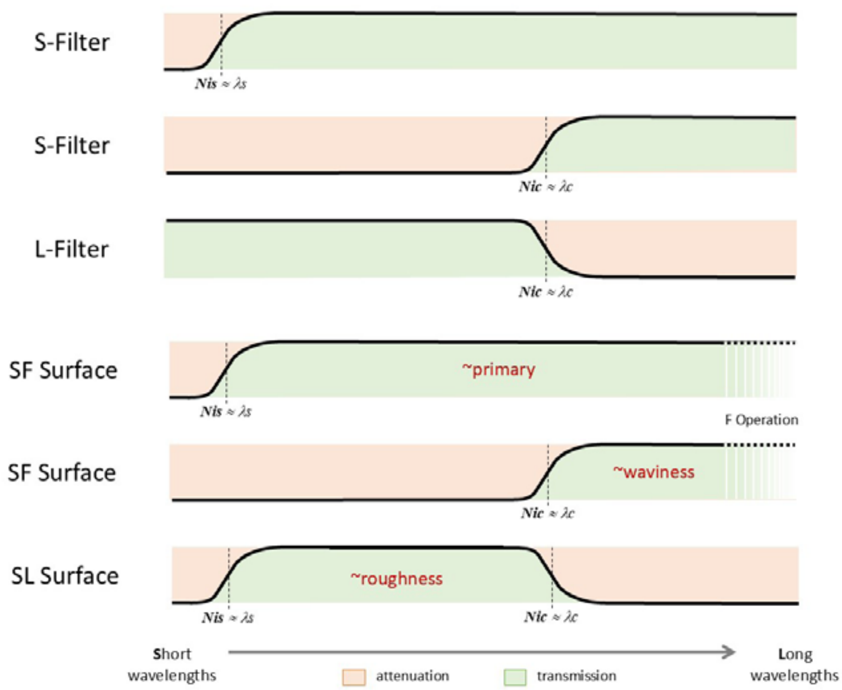
The development of the areal surface texture standard series, ISO 25178, provided the ideal opportunity to define new vocabulary, which was later transposed to the profile series ISO 21920.

This new vocabulary is sometimes a source of misinterpretation, in particular when users try to overlay the new concepts on the old terms too hastily. This article gives a detailed explanation of the new concepts with regards to filters and cut-offs, in order to help users to understand their meaning more precisely.

The new standards introduce two filter types, named with regards their action. As always, a filter is associated with a cut-off (also called nesting index) that defines at which wavelength the filter starts its action.

THE S-FILTER

The S-Filter attenuates **wavelengths shorter than the cut-off**. The letter S means "short" and underlines **its action of attenuation** of wavelengths that are shorter than the cut-off.



***Left.** The S-Filter attenuates wavelengths on the left side of the cut-off while the L-Filter attenuates wavelengths on the right side of the cut-off.*

***Left.** SF surfaces or SL surfaces are obtained after the respective filters/operations have been applied.*

But this "S" is not necessarily linked to the "s" of λs (which defines the cut-off value).

- The S-Filter uses a cut-off that can vary in value. **When the cut-off is small** and used to remove microroughness, it is called *Nis*, with the "s" matching the meaning of the "s" in λs.
- However, **when the cut-off is larger** (ie. used to create waviness by filtering out shorter wavelengths) it is called *Nic*, referencing λc, the cut-off wavelength separating roughness from waviness.

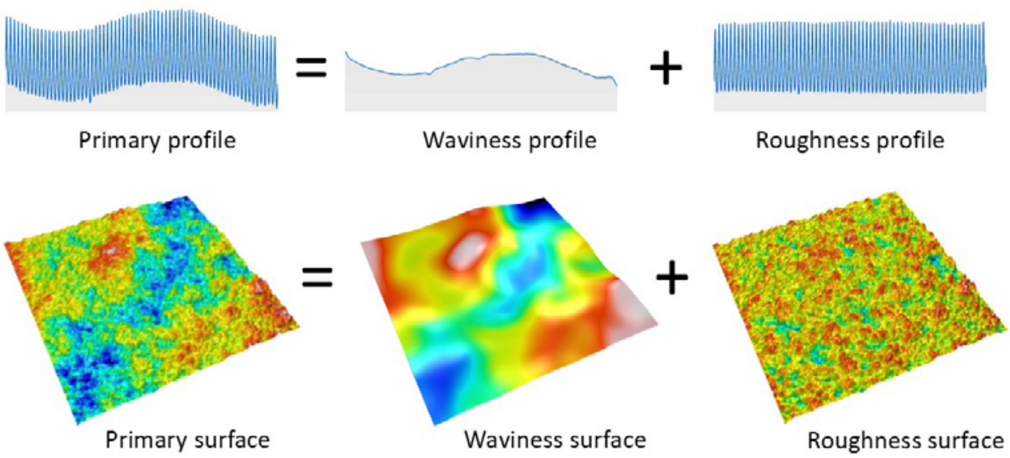
When we represent wavelengths on an axis, they go from the shortest wavelengths (on the left) to the longest wavelengths (on the right). However, the S-Filter always attenuates what is on the **left-hand side** of its cut-off, whatever its value.

In the case of an S-Filter associated with a *Nis* cut-off, it is a microroughness filter; in the case of an S-Filter associated with a *Nic* cut-off, it is a waviness filter.

THE L-FILTER

The second filter type, the L-Filter, **attenuates wavelengths that are longer than the cut-off** ("L" as in "long"). This filter attenuates what is on the **right-hand side** of its cut-off, again whatever its value. It is used to produce roughness, with a *Nic* cut-off.

When combining these filters with the form removal operation, or F-Operator, we can produce either an SF-Surface or an SL-Surface (**there is no LF-Surface**). However, the SF-Surface can be of two types, depending on the cut-off used for the S-Filter, λs or λc.



KEY TAKEAWAYS

- The concepts of S-Filter and L-Filter do not have the same meaning as λs and λc cut-offs.
- The *Nis* cut-off is similar to λs and can only be used with an S-Filter.
- The *Nic* cut-off is similar to λc and can be used either with an S-Filter (to produce waviness) or with a L-Filter (to produce roughness).
- The S-Filter attenuates wavelengths on the left side of the cut-off.
- The L-Filter attenuates wavelengths on the right side of the cut-off.

ASSOCIATED RESOURCES

- *Introduction to filtration* www.youtube.com/watch?v=2OMrfNUt0T4
- *Filtration techniques* guide.digitalsurf.com/en/guide-filtration-techniques.html
- *What cut-off should I use?* guide.digitalsurf.com/en/guide-what-cut-off-should-i-use.html

TRADE SHOW REVIEW

With a busy trade show calendar this year again, the Digital Surf team was thrilled to travel four continents to meet partners & customers. After a first stop at the **Forum des Microscopies à Sondes Locales** (Scanning Probe Microscopy meeting) in Lyon (FR) to meet the French speaking SPM community, we visited Stuttgart (DE) in May for the classic **Control international trade fair** dedicated to quality assurance. This exhibit was the opportunity to showcase the new features of Mountains® version 10.2.

We continued our journey in Morocco with the **Met & Props conference**. The International Conference on Metrology and Properties of Surfaces (taking place late May in Marrakech) was Digital Surf's first event on the African continent and was great experience for meeting our customers in the surface analysis & metrology field.

Above. *The Digital Surf booth at Control 2024.*

Above. *Anne & François at the Met & Props conference.*

The Digital Surf team was also very pleased to meet up with the Japanese analytical & scientific community at the **JASIS trade fair** (taking place from September 4-6 in Tokyo, JP). This huge event attracted over 20,000 visitors and 400 exhibitors and was a another big success.

Above. *Nicolas & Emmanuel at SciX 2024.*

Our team then headed to North America for the **SciX conference** which took place mid-October in Raleigh, North Carolina, USA. This conference brings together the spectral analysis community to discuss the latest developments in the chemical analysis & allied sciences fields. Our experts provided visitors with live demos of MountainsSpectral® software before coming back closer to home for a last stop in Munich, Germany for the **Nanoscientific Forum Europe** which took place from October 29-31. Attending as a sponsor, Digital Surf was excited to hold a scientific presentation during this European AFM user forum and discuss emerging analysis methods in nanotechnology.

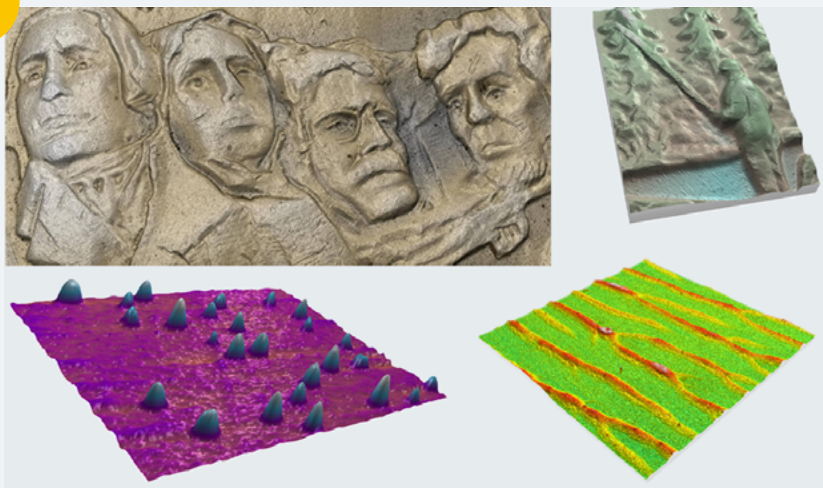
LATEST WEBINARS

Visit our website and check out our free webinars available to watch on-demand. Pick your preferred product technology and access all the webinars dedicated to this technology.

Among these webinars, you'll find the latest ones, dedicated to **3D topographic reconstruction from SEM images** and **compositional analysis in Raman spectroscopy**.

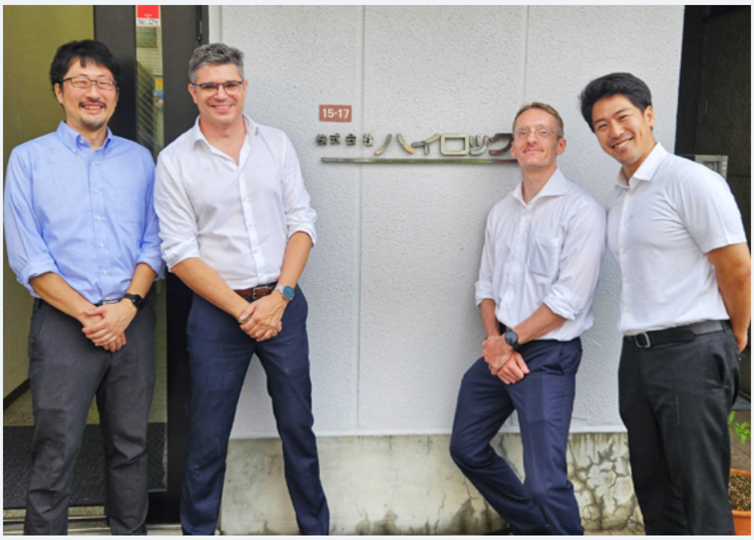
Don't miss the next one coming in December where you can learn more about **automation tools available in Mountains® software for particle analysis**. Register via the following link: bit.ly/4exVFxp

WHAT'S HOT ONLINE



LOVED ON LINKEDIN

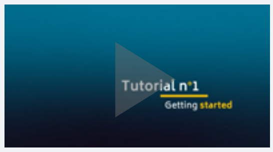
We recently hosted a contest where Mountains® users got to share their favorite 3D views and we're amazed by the spectacular images users sent in. We're featuring some of our faves above but you can see more on our LinkedIn page: bit.ly/4f1en1q



SEEN ON FACEBOOK

It has been quite a busy year for our team. Recently, Damien and Cyrille visited some of our incredible distributors in Japan. Rumor has it that Japanese delicacies were shared: bit.ly/4f23j4b

Have you been to our YouTube channel?



We have lots of quick, helpful videos, as well as tutorials on Mountains® software basic and advanced features, check them out: bit.ly/2U2I2za

Surface Newsletter

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and learn about characterizing surface texture in 2D and 3D
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2024 MRS Fall Meeting & Exhibit | Booth 716
Dec 1-6, 2024 | Boston, Massachusetts, USA

Pittcon conference and exposition
Mar 1-5, 2025 | Boston, Massachusetts, USA

Forum des microscopies à sondes locales
Mar 31 - Apr 4, 2025 | Spa, Belgium

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