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IN THIS ISSUE

NEW SEM APPLICATION
3D RECONSTRUCTION OF EAR BONE EROSION
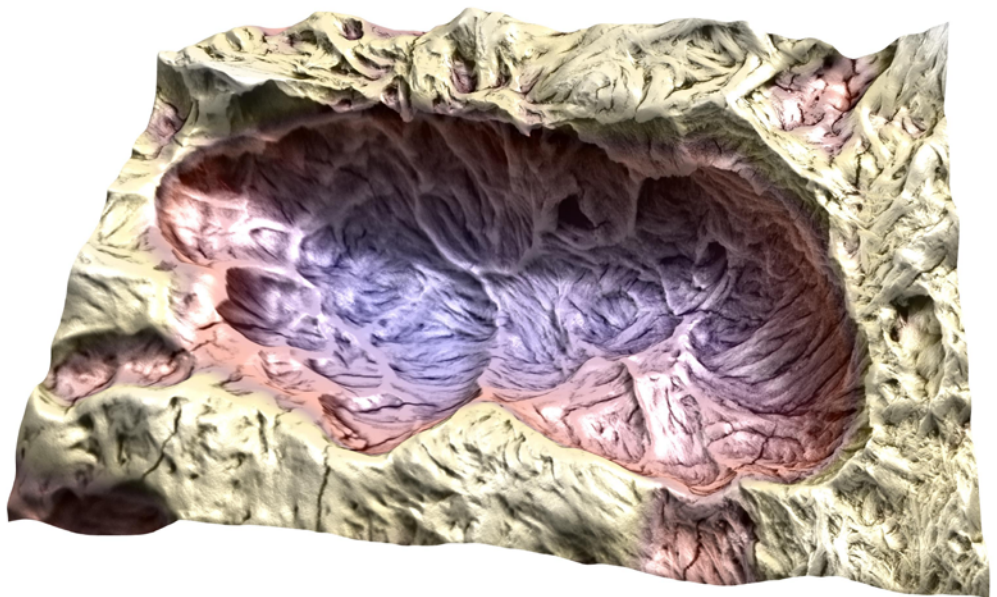
RECONSTRUCTION FROM 4-QUADRANT SCAN ALLOWED QUANTITATIVE ANALYSIS

Morphological data including volume calculations generated from 3D reconstruction allowed Italian researchers to identify cells responsible for bone erosion in a pathology of the middle ear, opening up new horizons for understanding and treatment of the disease.

Watch our WEBINARS

Digital learning resources

With many still working from home, digital resources are more useful than ever.

Our Mountains® webinars are online, available for you to watch, as many times as you need and from wherever your workspace is at the moment. bit.ly/2Xqqro3

SEM IMAGE 3D RECONSTRUCTION
OF EAR BONE EROSION

In police investigations and crime novels, in order to discover the culprit, it is often necessary to find and analyze fingerprints first.

A team of researchers at the Sapienza University of Rome, Italy used this same approach to solve an enigma relative to the ear pathology cholesteatomas (CHO), a condition in which an epithelial mass forms in the middle ear, causing inflammation, pain and hearing loss. The mystery of this pathology: which was the cell responsible for eroding the middle ear incus bone?

Incus bone erosion is considered a typical characteristic of advanced CHO. However, it is still a matter of debate whether resorption lacunae[1] on the surface of incus bones are caused by osteoclast[2] action.

To address this issue, the research group at the Sapienza observed resorption bays in the neck of an osteoporotic human femur (indubitably of osteoclastic origin) and those present in incus bone affected by CHO.

4-QUADRANT RECONSTRUCTION FOR QUANTITATIVE ANALYSIS

"We used BSE images from our scanning electron microscope Hitachi VP-SEM SU 3500, equipped with a four-quadrant BSE detector that allows us to obtain four images simultaneously in only one scan" said **Prof. Michela Relucenti**, who led the study. "The four pictures obtained were reconstructed into a 3D image using Hitachi map 3D software based on Mountains® and processed to extract quantitative information."

"Obtaining this type of data was extremely useful in implementing the morphological classification parameters generally used to characterize the formation and resorption of bone surfaces.

In fact, acquiring quantitative data of resorption lacunae, such as area, mean depth and volume allowed us to compare lacunae derived from different sources (femur and incus) and finally, assess if they had the same origin.

Resorption lacunae area, mean depth and volume were calculated with Hitachi map 3D.

For further detail, we selected a single lacuna on the 3D image reconstruction and performed automatic measurements of the area, the mean depth and the volume."
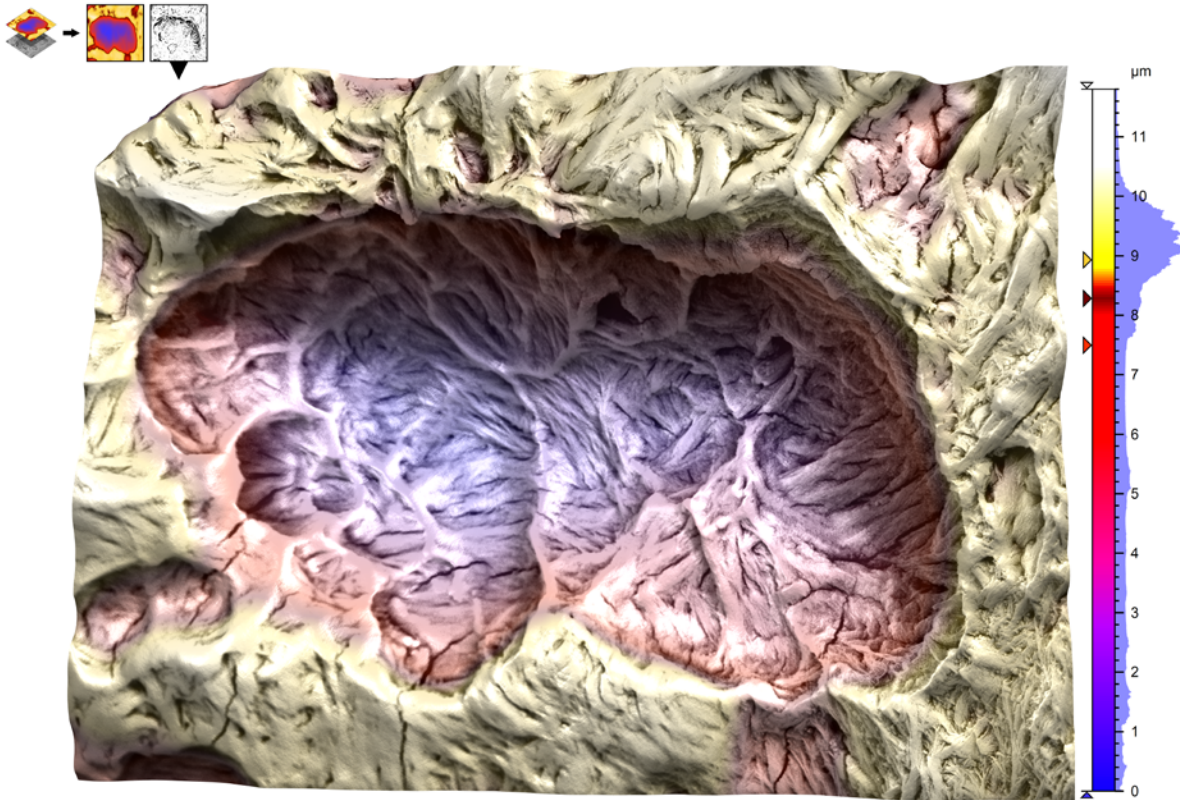
ANALYZING RESULTS & IDENTIFYING THE CULPRIT

"The data, collected and statistically analyzed, showed that no significant differences exist between the two groups.
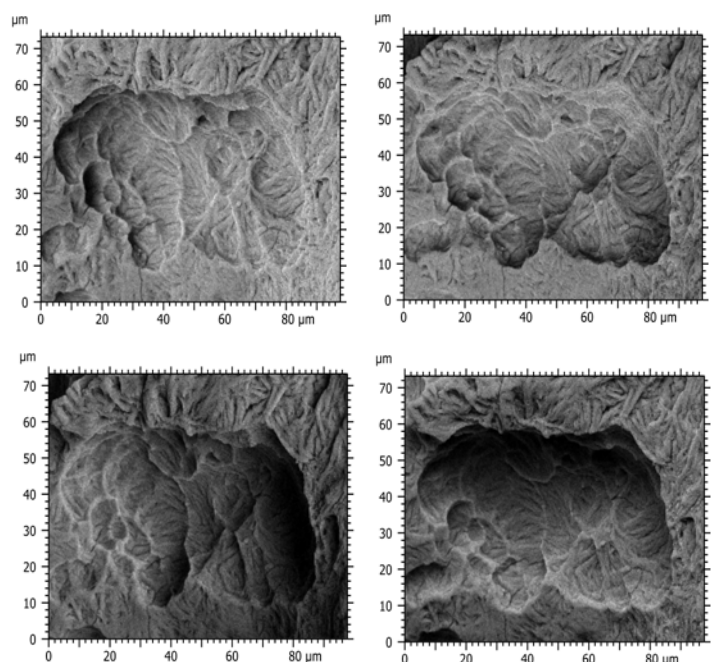
Above. Four SEM images of a CHO incus bone resorption bay formed by several lacunae.

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[1] A concavity found in bones undergoing resorption.
[2] A type of bone cell that breaks down bone tissue.

Above. Color-coded 3D reconstructed model generated using Hitachi map 3D software from four SEM images of an incus bone eroded surface. The resorption bay contains several lacunae.

Therefore, if the fingerprints are the same, then the culprit is the same: in this case the osteoclast!

This quantitative approach implementing morphological characterization allowed us to state that surface erosion of the incus was due to osteoclast action."

NEW UNDERSTANDING & TREATMENT OF MAXILLO-FACIAL PATHOLOGIES

The novel scanning characterization approaches used in this study allowed for the first time the 3D imaging of incus bone erosion and its quantitative measurement, opening up a new era of quantitative SEM morphology on biological samples.

New therapeutic options for inhibiting bone erosion development in CHO were also brought to light.
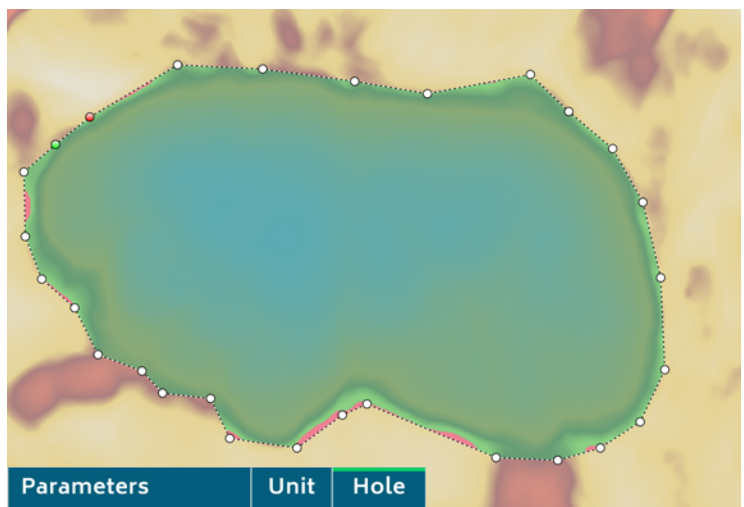
Parameters	Unit	Hole
Projected area	µm²	3407
Volume	µm³	15641
Max. depth/height	µm	9.099
Mean depth/height	µm	4.591

Above. A delimited single lacuna from which Hitachi map 3D software calculated morphological parameter values.

READ MORE

SEM BSE 3D Image Analysis of Human Incus Bone Affected by Cholesteatoma Ascribes to Osteoclasts the Bone Erosion and VpSEM dEDX Analysis Reveals New Bone Formation. Michela Relucenti, Selenia Miglietta, Gabriele Bove, Orlando Donfrancesco, Ezio Battaglione, Pietro Familiari, Claudio Barbaranelli, Edoardo Covelli, Maurizio Barbara and Giuseppe Familiari In: Scanning, Volume 2020.

INSTRUMENTS AND SOFTWARE USED

*Hitachi VP-SEM SU 3500 Scanning Electron Microscope + **Hitachi map 3D software** based on **MountainsSEM®**.*

5 THINGS TO KNOW ABOUT CONTOUR PROFILE ANALYSIS

The **Contour Analysis study in Mountains®** allows users to calculate and show distances, angles, radii etc. on a measured profile and check for deviations. This tool is particularly useful in quality control, for studying the form of an object or for comparing shapes to CAD drawings and is essentially applied to profiles measured on mechanical parts. Following the recent broadcast of a webinar on this topic, here's a quick introduction in the form of 5 useful tips.

1. TWO DEDICATED TOOLBARS FOR EASY ACCESS

When conducting a Contour Analysis study with Mountains®, two **dedicated toolbars** will appear on each side of your study. These panels provide easy access to the different tools available.

On the left-hand side, you will find tools for creating and manipulating different elements. On the right hand-side one you will find all of the calculation tools available for this type of analysis.

2. CREATING POINTS, SEGMENTS, ARCS AND MORE

Creating matching elements, such as points, segments, arcs and circles on measured contours is pretty simple. Just head over to the left-hand toolbar to find the multitude of options available.

Numerous automatic detection features are implemented in Mountains® in order to make analysis more efficient and easier to conduct. For instance, with the **Create Lowest Point** tool, you can simply click on your profile and Mountains® will find the lowest point for you and create it.

With the **Auto-Connect tool** you can join multiple segments of your profile in just one click.

3. CALCULATING DISTANCES ANGLES AND VALUES

Calculating horizontal, vertical or oblique distances on different elements is also possible in Mountains®, this can be done with the measuring tools located in the toolbar on the right.

This toolbar is also home to tools for measuring angles, diameters and radii.
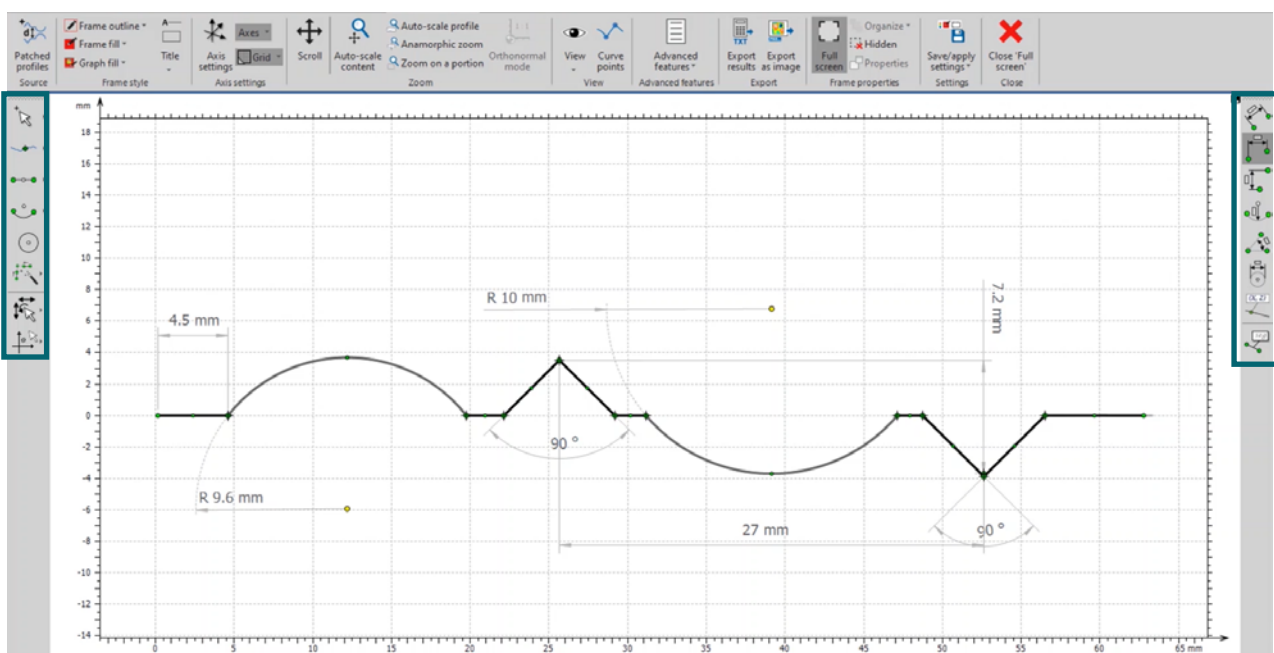
Above. Contour Analysis of a horizontal profile studiable in Mountains®. Tools for creating and manipulating elements are to be found on the toolbar to the left, while tools for calculation are on the right.
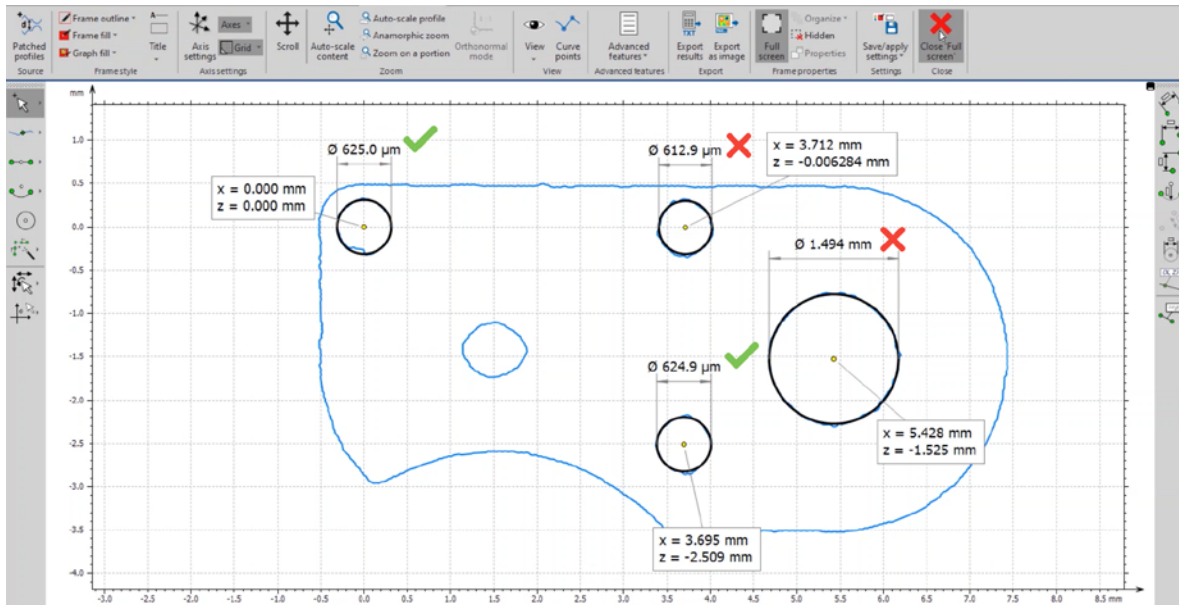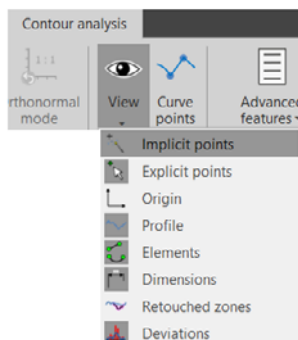
Above. A planar contour extracted from a surface studiable of a mechanical piece with pass/fail tests.

4. GENERATING PLANAR CONTOURS FROM SURFACES

With the **Extract Planar Contour Operator**, it is possible to apply Contour Analysis to the shape of a surface. Mountains® uses Watershed segmentation to identify and extract this shape. Local height and area thresholds are adjustable, which gives users full control over how contours are detected. Once you are satisfied with your settings, the software will generate a contour profile ready to be analyzed.

Did you know implicit points are created automatically by Mountains®?

To display them go to the View icon, located in the ribbon, then click on **Implicit Points**. The coordinates of your elements will appear when you hover over them with the cursor. You can also display coordinates by using the **Add Point Coordinates tool**, located on the right-hand toolbar.

5. DISPLAYING PASS/FAIL TESTS

In Mountains® it is possible to display pass/fail tests directly in the Contour Analysis study. You can do so by clicking on your reference value, then on **Define Tolerance Limits** situated in the ribbon. A Tolerance limits management window will open up with the values available in your study. Select those you wish to track, then click on Track Limits of Result and set a limit for each.
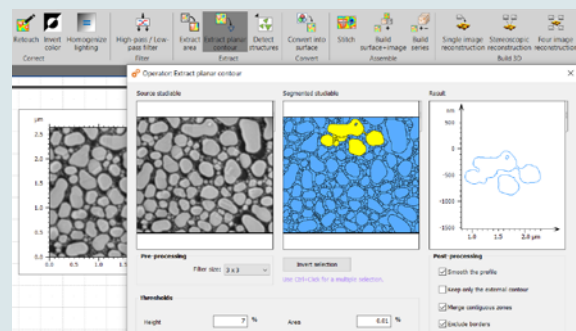
CONTOUR ANALYSIS FOR SEM IMAGES

The Contour Analysis study can also be applied to SEM images. To do so, simply load your image into Mountains®, select the Extract Planar Contour option and click on the segments you wish to study.

LEARN MORE

▶ *All tools shown are available in the Mountains® Contour Analysis module: www.digitalsurf.com/optional-modules/contour*
▶ *Watch our webinar on Contour Analysis with Mountains®: www.gotostage.com/channel/digital-surf*

PERFORM A PARTICLE ANALYSIS
ON MICROSCOPY IMAGES

Particle analysis is used by microscopists across many fields of industry and research ranging from quality control of structured materials (metal, alloys, etc.) to characterize nano-structure assemblies.

Mountains® provides several feature detection methods, a range of graphical representations and classification tools. Mathieu Cognard, recently appointed product manager for SPM applications at Digital Surf, gives *Surface Newsletter* a guided tour.
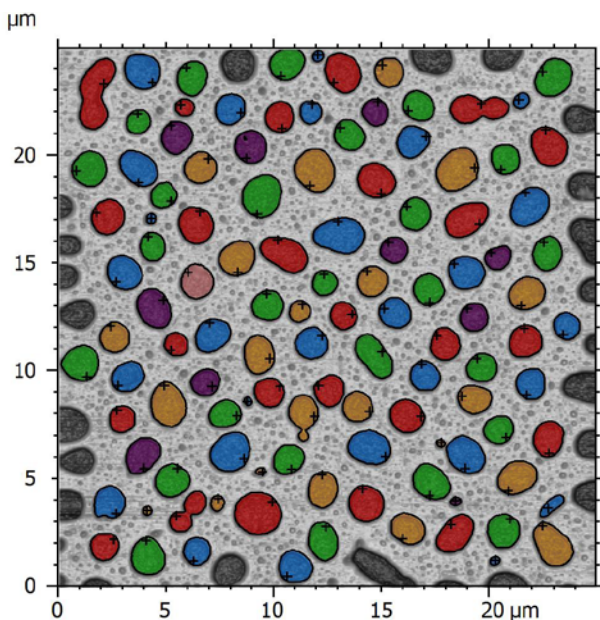
Information		
Method	Threshold detection	
Layer	Height_Trace (1 / 6)	
Threshold 1	37.1	nm
Number of particles	113	
Coverage	29.7	%
Density	180800	Particles/mm²

Parameters △	Projected area	Maximum depth
Unit	µm²	nm
Particle #4 🟩	1.85	29.0
Particle #5 🟦	0.193	26.6
Particle #6 🟥	2.32	30.7
Particle #7 🟦	2.12	30.5
Mean	1.64	29.5

***Above.** View of the Particle analysis study in Mountains®. On the left, detected particles can be visualized in color-coded classes. On the right, parameters for the whole surface and for individual particles are displayed.*

The Particle analysis study is used to detect, quantify and classify particles, pores, grains, and other image features (of any shape and size) that have boundaries.

PREPARING THE SURFACE BEFORE DETECTION

First of all, it's important to know a little bit about your surface and decide which features you want to detect (particles, pores etc.) This will help you choose the right detection method.

Leveling the surface is also necessary in order to obtain a flat and horizontal background, especially if you are going to use the Threshold detection method. Remove noise and filter the surface if necessary.

ACCESS TO THE TOOLS AND DISPLAY OPTIONS

The Particle analysis tools can be found in the Studies tab (see below). Clicking on the icon will produce a new Study in the Document.
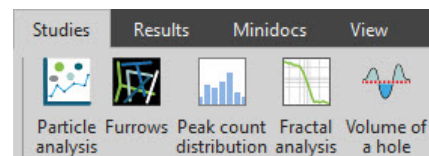
By default, the Table of Results is shown below the image of the detected layer. However, it can also be displayed to the right of the panel to improve visualization (see image above). Just right-click on the table and select Show results on the

right side. To save this setting as default, you can press Alt+S. Press F11 for full screen-mode.

PARTICLE DETECTION

Several different methods for detecting particles are available in Mountains®. Each method will detect different types of features automatically and generate specific parameters.

Threshold detection

Detect particles (or pores) that stand out clearly on a flat background, using one or two thresholds. Surface roughness must be smaller than the height of the particles (or smaller than the depth of the pores).
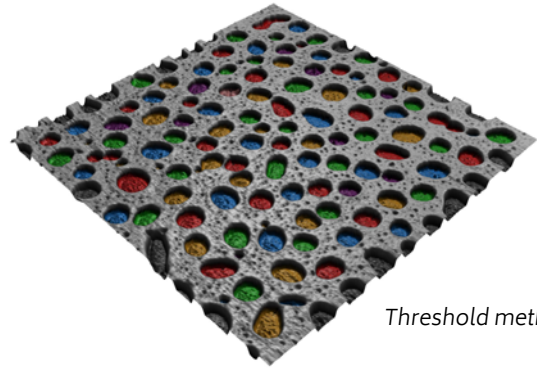
Threshold method

Watershed detection

Detect contiguous dale or hill motifs using watershed segmentation as defined in ISO25178-2 and fully segment the surface. The algorithm calculates watersheds that divide or partition hill or dale motifs and locates their peaks and pits.
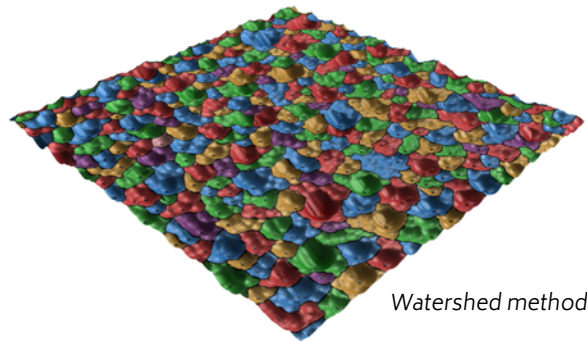
*Watershed method*

Edge detection

Detect features on an uneven background, using watershed segmentation after the application of an edge detection filter. This method can also be used if the features are connected or not clearly separated. It also works for detecting grain boundaries of polished metal or ceramic surfaces.
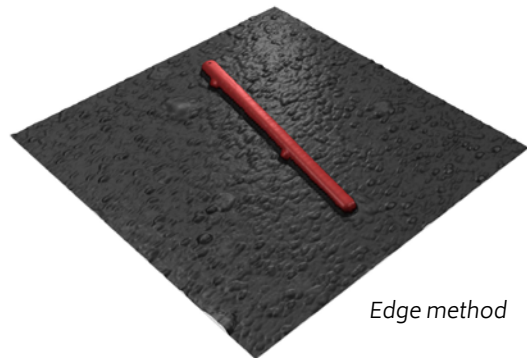
PARTICLE CLASSIFICATION

Mountains® also offers a powerful tool for particle classification. This tool can create several classes defined by one or several criteria. Particles can be classified according to their specific characteristics. To learn more about Particle analysis and classification, visit our website or watch the dedicated webinar (see below right).

Edge method
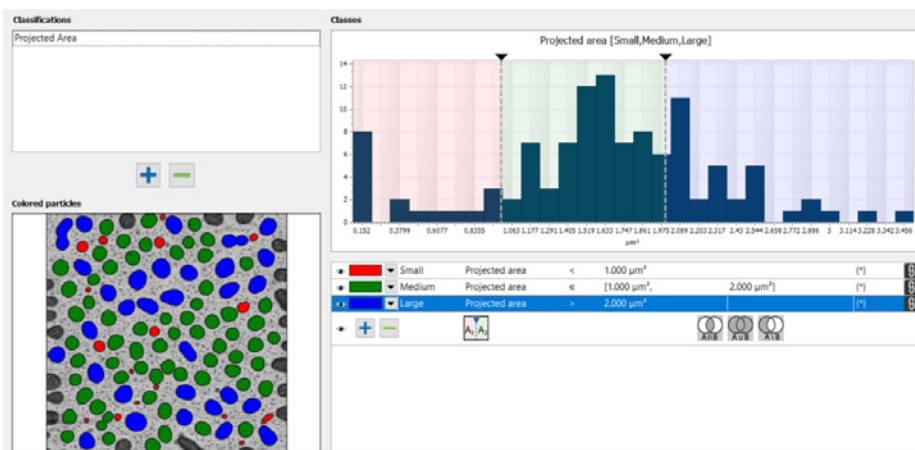
Above. *Particles can be classified based on multiple criteria.*

WATCH THE WEBINAR
Want to know more? Our in-depth webinar on particle analysis is available on demand: www.gotostage.com/channel/digital-surf

Mountains® webinar
Contour Analysis

HOW CAN I BE SURE MY RESULTS CAN BE TRUSTED?

"

Users and instrument makers worldwide rely on metrology software to help them improve their products or understand material properties. At Digital Surf, we are responsible for providing them with **accurate and compliant results**. However, proving that an algorithm is correct is not so straightforward. François Blateyron, senior expert on surface metrology, discusses this complex issue.

Users tend to compare the results they get with other sources, other commercial software packages or so-called "reference software" results provided by National Metrology Institutes (NMI). **But when results differ, which ones can be trusted?** A recent study shows that NMI software packages[1] do not provide comparable results for common parameters and do not cover all parameters and filters. Therefore, they cannot be used as a reference by everyday users.

TOWARDS AN INDEPENDENT REFERENCE

There is a strong need for an independent reference that would not put too many constraints on the production of algorithms but would allow developers to measure the accuracy of their implementations. Developers should remain free to make their own implementation, their own optimizations for speed (or their own approximations when the program runs on less powerful hardware.)

Such independent references can only be provided by Nature and in particular, Maths. Most parameters are now defined for the continuous case by an equation. By defining a surface or a profile (a studiable) also as an equation and combining with the parameter equation, a resulting formal equation for the reference parameter value can be calculated that is highly accurate. For example, a simple sine wave profile can be defined as:

$$z(x)=A \sin(x)$$

with A the half amplitude and $x \in [0; 2n\pi]$. We can set the length L to be a multiple of the chosen period, all in the same unit (for example, μm). The parameter can then be calculated, for example by substituting the surface equation into the parameter equation:

$$Pq = \sqrt{\frac{1}{L} \int_0^L z^2(x)dx}$$

$$Pq = \sqrt{\frac{1}{L} \int_0^L A^2 sin^2(x)dx} = A\sqrt{\frac{1}{2n\pi}\left[\frac{x - \cos(2x)}{2}\right]_0^{2n\pi}}$$

$$= A\sqrt{\frac{1}{2n\pi}\left[\frac{(2n\pi - 1) - (0 - 1)}{2}\right]} = A/\sqrt{2}$$

In order to test the parameter algorithm alone, it is tested without filter, without leveling or any other operation. Filters and form removal are tested separately, and the full process is taken as a series of black boxes, each tested independently with a mathematical reference.

This means that $Pq=Pt/(2\sqrt{2})$ for a sine wave ($A=Pt/2$) and that it is independent from the period. For example, for an amplitude $Pt=1\mu m$, we should obtain $Pq \approx 0.353553\mu m$ and the result can be calculated with as many decimal places as needed.

The accuracy of the parameter calculation in a software package depends, of course, not only on the algorithm but also on the number of points in X and digitization in Z of the discrete profile. So, discrete studiables generated for the purpose of validation should introduce the least amount of uncertainty and allow the evaluation of the sole contributions of the algorithm to the final uncertainty (or deviation).

GENERALIZING MATHEMATICAL REFERENCE VALUES

For several years, Digital Surf has invested in research on new methods to validate parameters and filters. In collaboration with the University of Nottingham and the National Physical Laboratory (UK), we work to develop reference pairs for each parameter from common standards. A reference pair is 1) a definition of a mathematical studiable paired with 2) a formal expression of the parameter when calculated on the given studiable.

This mathematically intensive work allowed Digital Surf to validate field parameters from ISO 25178[2] and more recently profile parameters from ISO 21920 (and by extension from ISO 4287 and ISO 13565) and the Gaussian filter[3]. This work allowed Digital Surf to validate and improve Mountains® algorithms and identify which parameters are sensitive to which characteristics.

AREAL PARAMETERS

Several synthetic surface equations were defined, from simple sine waves in X and Y to Bernouilli or Chebychev polynomials with various orders, and their corresponding equations were passed through each parameter definition, to obtain the formal resulting equation. Then a numeric approximation was calculated, by assigning a value to the various equation terms (amplitude, polynomial coefficients etc.) to obtain a value to which the value calculated in Mountains® could be compared. Then, a discrete version of the surface was generated at various resolutions and loaded into Mountains® to calculate the parameters and obtain deviations. To the right is an example of results for the "4-term cosine" surface. The accuracy is usually better than ±0,001% on large surfaces, which is more than necessary for most metrological results.

	Expected Value	Mountains 4000 x 4000 High V res	Δ	Mountains 8000 x 8000 High V res.	Δ
Sq	0,84391002989	0,8439100693	0,00000%	0,8439100366	0,00000%
Ssk	0,12138411815	0,1213844145	0,00024%	0,1213841942	0,00006%
Sku	2,101085801812	2,1010854425	0,00002%	2,1010857188	0,00000%
Sp	1,913568060702	1,9135682800	0,00001%	1,9135681100	0,00000%
Sv	1,658843053989	1,6588371200	0,00036%	1,6588404800	0,00016%
Sz	3,572411114691	3,5724054000	0,00016%	3,5724085900	0,00007%
Sa	0,715320000000	0,7153227485	0,00038%	0,7153226801	0,00037%
Sdq	0,0025907034111	0,0025902278	0,01836%	0,0025904705	0,00899%

It was not always possible to obtain the formal expression of all parameters with all surface equations, as some calculations were too complex. But in the end, it was possible to validate all field parameters, including slope (Sdq), developed area (Sdr), material ratio (Smc, Smr) with derived families, Sk, Spk, Svk etc. and volume parameters Vmp, Vmc, Vvc, Vvv.

A test on various Abbott curve shapes, suitable for the calculation of functional parameters was carried out. A dozen shapes were generated from the same polynomial equation and material ratio parameters were tested for different points on the curve. Functional parameters, were also validated. In most cases, deviation was always below 0.1% and usually much less. See bit.ly/3ef7Akx.

The trickiest parameters were those based on autocorrelation (Sal, Str) where synthetic equations had to be generated in the autocorrelation domain instead of the space domain. This required a special test procedure in the source code of Mountains® to validate the thresholding at s=0.2 and the accurate

calculation of the smallest and largest radius on the autocorrelation lobe. By varying the equation, it was possible to generate lobes that were oblong or round, thin or large and test algorithm robustness.

So far, the missing references are for Feature parameters which are based on a topological algorithm instead of a linear equation. The only solution here is to carry out a code review to ensure that the algorithm is correctly implemented. Reference values will be necessary in the future for these parameters as well.

PROFILE PARAMETERS

Following the first 4-year research work focusing on areal parameters, an extension was agreed during Luke Todhunter's post-doc year to apply the same method to profile parameters (those defined in ISO 21920) and also to the Gaussian filter[3] (profile and areal) and form fitting, including leveling.

CONCLUSION

Users have legit questions and expectations on the compliance and accuracy of parameter results provided by Mountains®. Thanks to the method developed over the last four years, we now have the tools to validate most areal and profile parameters. Ideally, an official certification for our software, similar to a UKAS or DKD certificate, would be necessary but this is not yet possible (only instruments and material measures can be certified). However, we can provide the necessary mathematical background for our algorithms.

Mountains® is not only the most complete surface metrology software package, it is also the most accurate and compliant.

[1]Todhunter L, Leach R K, Lawes S D A, Blateyron F, 2017, *An analysis of Type F2 software measurement standards for profile surface texture parameters, Measurement Science and Technology*, 28(6).

[2]Todhunter L, Leach R K, Lawes S D A, Blateyron F, Harris P, 2018, *Development of mathematical reference standards for the validation of surface texture parameter calculation software*, Journal of Physics, conference series, 1065(8).

[3]Todhunter L, Leach R K, Blateyron F, 2020, *Mathematical approach to the validation of surface texture filtration software*, Euspen 20th international conference, Geneva.

DIGITAL RESOURCES & PRODUCT HIGHLIGHTS

NEW WEB CONTENT FOR GERMAN, FRENCH & CHINESE AUDIENCES

Digital Surf is thrilled to announce that its website is now available in French and German!

We invite you to have a look at our "Software & Solutions" pages and check out all the Mountains® features dedicated to profilometry, electron microscopy and scanning probe microscopy in your own language.

Descriptions of Mountains® Optional modules pages are also available in French and German. Here, customers can have a look at the add-on tools available to suit the needs of various research and industrial projects.

Our Surface Metrology Guide is available in three languages as well. Get a free, detailed introduction to surface texture analysis & parameters, and stay informed about recent work in standardization committees.

Be sure to visit our website to discover a lot more!

In French: www.digitalsurf.com/fr

In German: www.digitalsurf.com/de

We are also pleased to announce that a dedicated presentation page in Chinese is now available on our website. Chinese users can now read more about Digital Surf, Mountains® software applications and visit our Tencent video channel.

In Chinese: www.digitalsurf.com/cn
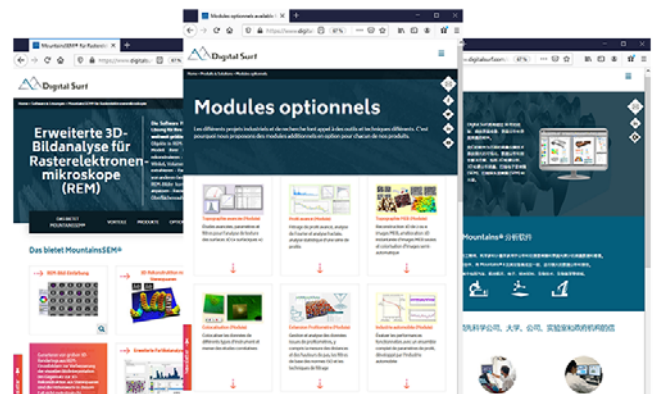
LAUNCH OF RELATE SOFTWARE

### OXFORD INSTRUMENTS AND DIGITAL SURF JOINT ANNOUNCEMENT

Jun 4, 2020: leading provider of high technology products Oxford Instruments and Digital Surf, creator of the industry-standard Mountains® surface and image analysis software platform, announced the release of Relate software for users of Oxford Instruments' leading-edge tools for materials characterization.

This software will bring great value to Oxford Instruments' users working in R&D across a wide range of academic and industrial applications including semiconductors, renewable energy, mining, metallurgy, and forensics.

Relate software lets users get more out of their data by supplying accurate, innovative tools for visualizing, analyzing and reporting, in particular:

▶ Correlation of spectrometry (EDS and EBSD) data with AFM data and microscopy images with easy-to-use tools for rapid manual and semi-automated correlation of multiple images.

▶ 3D and 2D visualization of composite data sets showing EDS, EBSD and EM image layers combined with topography and material properties as measured by AFM, helping to reveal the micro- and nano-characteristics of a sample.

Full press release: bit.ly/31WjUUk
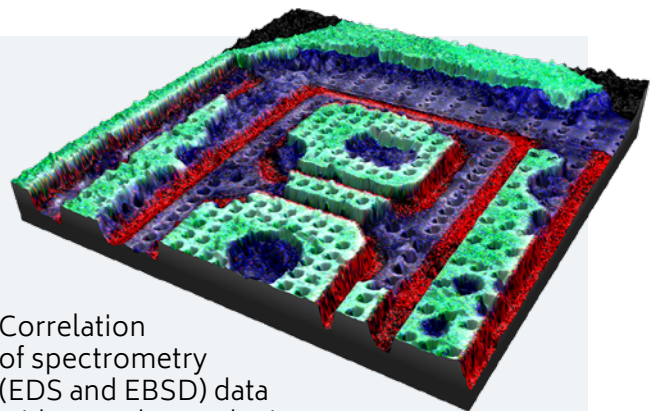
WHAT'S HOT ONLINE
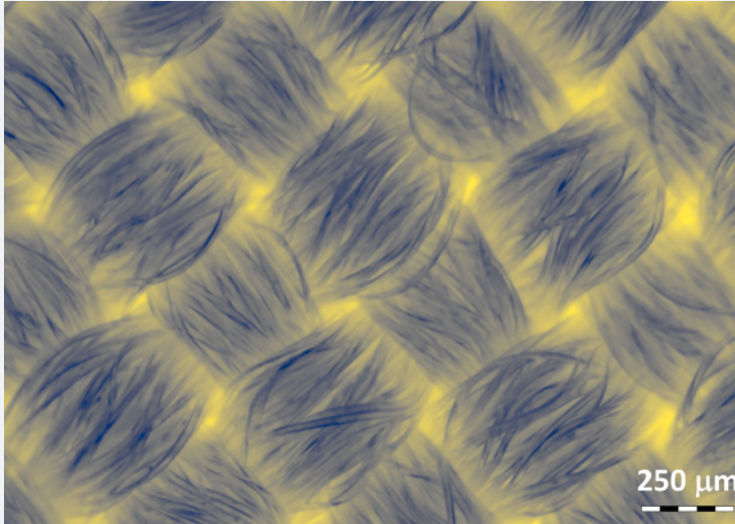
POPULAR ON FACEBOOK

July 1, 2020: This image shows the polyester fibers of a face mask. It was published in a recent study by the NIST (US National Institute of Standards and Technology) and the Smithsonian Institute on the filtration efficiency of face coverings against COVID-19.

To see more detail, the image was converted to a two-toned image using Mountains® software. The yellow areas indicate thinner regions where virus-size particles are more likely to pass through.
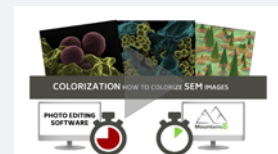
bit.ly/396AsKY

SEEN ON LINKEDIN

May 5, 2020: All 50 members of the Digital Surf team got together 'virtually' for a general meeting during lockdown. Since then, on-site operations have gradually resumed to normal.

bit.ly/3iFwrBn

Have you visited our YouTube channel recently?

Watch our new Mountains® video tutorials to help you use version 8 features.

Check them out:

www.youtube.com/
playlist?list=PLK5Jlf_
LO8rjhB8ILDbKNm
bSAA6xfMYcm

Surface Newsletter

Know a friend or colleague who would be interested in receiving the *Surface Newsletter*?
Let us know:
contact@digitalsurf.com

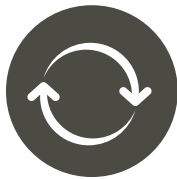The newsletter is available for download on our website
www.digitalsurf.com

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MEET DIGITAL SURF

► **Microscopy & Microanalysis (M&M) 2020 Virtual Meeting**
   Exhibit dates: Aug 4-6, 2020
   www.microscopy.org/mandm/2020/

► **JASIS - Japan Analytical & Scientific Instruments Show**
   Nov 11-13, 2020
   Makuhari Messe, Tokyo, Japan
   www.jasis.jp/en/

Digital Surf

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IMPRIM'VERT