SUMMER 2018

Newsletter

SURFACE

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TAKE SEM IMAGE ANALYSIS
TO NEW HEIGHTS

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Mountains® 8 sneak preview

Mountains® 8 is on the horizon with features for scanning electron microscopy to be revealed during the Microscopy & Microanalysis Exhibit in Baltimore, MD, USA on August 6-9.

But what exactly is the added value of using specialized software for your scanning electron microscopy image analysis ?

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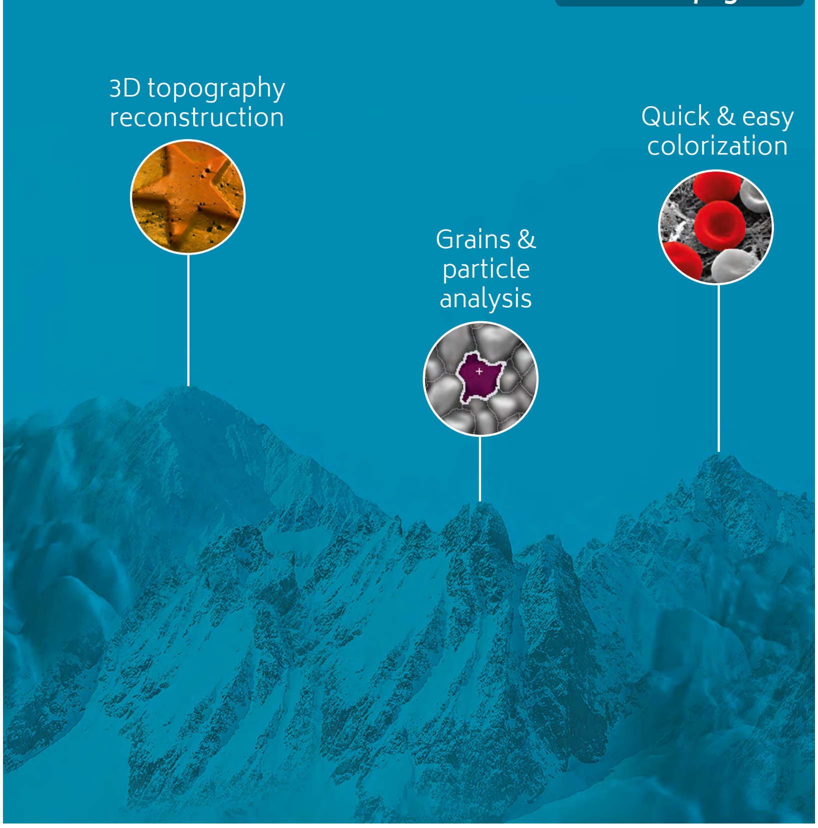


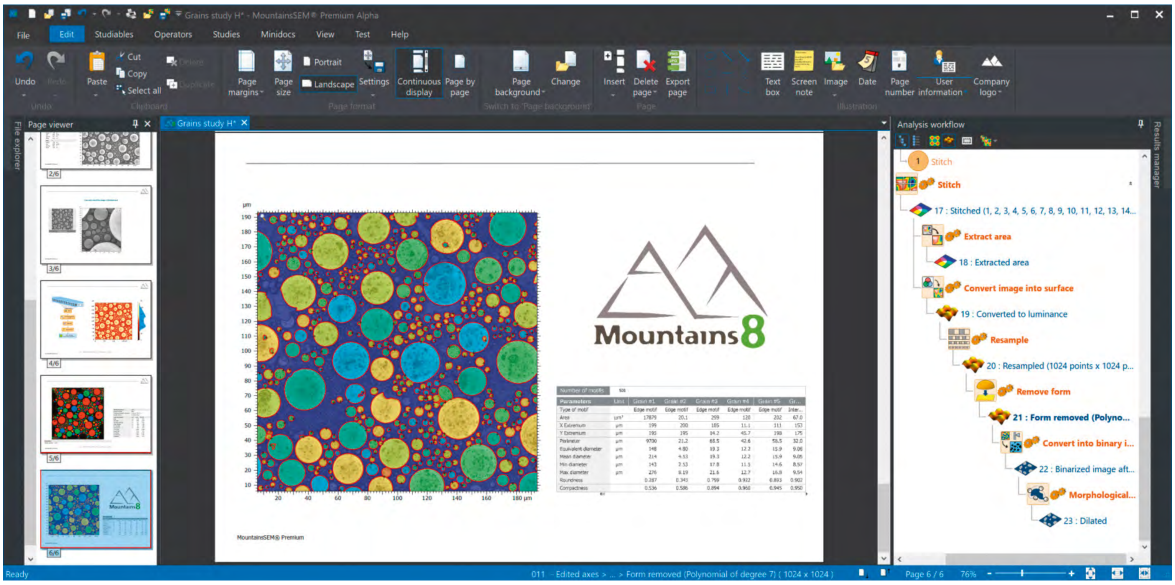
Meet us
EVENTS

We look forward to seeing you at:

- M & M - Booth #425 - Aug. 6-9, 2018 - Baltimore, MD, USA
- JASIS - Booth #6A-609 - Sept. 5-7, 2018 - Tokyo, Japan
- IMC19 - Booth #4 - Sept. 9-14, 2018 - Sydney, Australia

TAKE YOUR SEM IMAGE ANALYSIS TO NEW HEIGHTS

Mountains® 8 is on the horizon with features for scanning electron microscopy to be revealed during the **Microscopy & Microanalysis Exhibit** in Baltimore, MD on August 6-9. But what exactly is the added value of using specialized software for your SEM image analysis?



1 - SCIENTIFIC SOFTWARE WITH A DOCUMENT LAYOUT

Imagine being able to organize the different steps of your SEM image processing (original images, distance measurements, particle statistics etc.) on one or several pages and being able to publish these directly in different formats?

This is exactly what Mountains® software for SEM allows you to do, enabling you to put your data to use right away.

(This also avoids "window deluge", a common disadvantage of many scientific software programs).

2 - TOTAL TRACEABILITY

Thanks to Mountains® unique analysis workflow, you can see all the analysis steps already applied to your data and instantly revert back to any step in the process.

Edit any step and all dependent steps will automatically be updated.

3 - AUTOMATION: LET THE SOFTWARE DO THE WORK

Many users working with scanning electron microscopes find themselves performing repetitive analysis routines, following the same steps again and again.

Why not automate your repetitive SEM work and speed up your analysis process with Mountains® powerful tools? These include: templates, Minidocs (macros) and the statistics feature.

4 - TRUST THE EXPERTS

Digital Surf has almost 30 years experience developing surface imaging & metrology software for the global industrial and scientific community.

We invest heavily in research and development. Many of the algorithms used in our products are unpublished and result from our own research.

Our team of experts constantly test and improve the quality of Mountains® and ensure the software is compliant with current scientific norms and methods.

5 - COMPATIBLE WITH ALL SEMS

We have partnerships with leading SEM manufacturers (such as JEOL, Hitachi, Zeiss, Thermo Fisher Scientific including FEI). This means Mountains® software is available as standard or as an option with most new SEMs purchased.

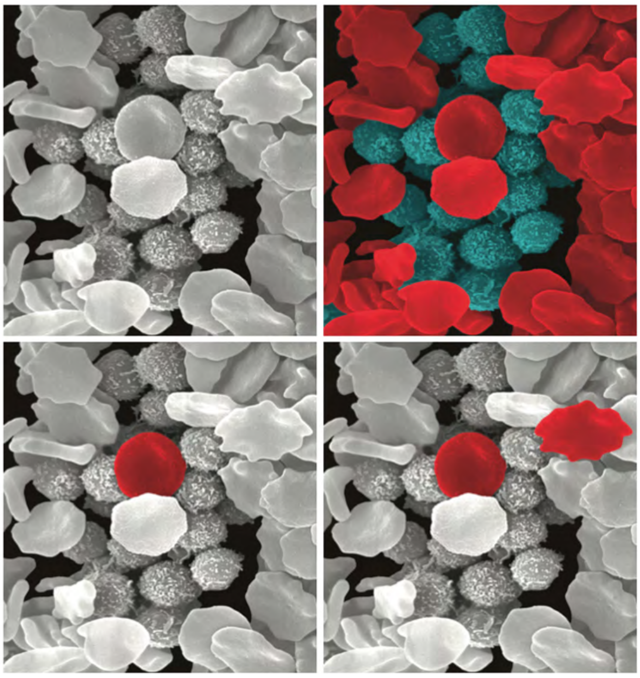
In some cases, Mountains® can be seamlessly integrated with image acquisition software, speeding up the process flow.

To add to this, Mountains® is capable of processing data from any brand of electron microscope.

6 - CLICK AND COLORIZE

Colorizing SEM images is a technique that has been around for a long time so what's new here?

Well, the sheer speed with which you can take your image from black and white to color. In literally just a few clicks, objects in the image are automatically detected and colorized by the software.



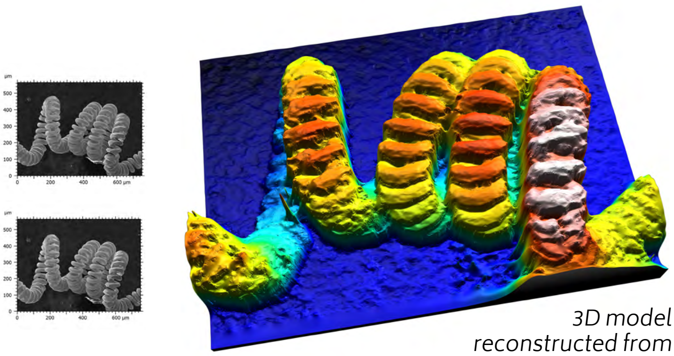
7 - MEASURE ANYTHING

Sometimes obtaining measurements from your SEM data can be complicated. Mountains® makes calculating distances, angles, areas and volumes quick and accurate. You can also analyze dimensions of extracted profile contours and cross-sections.

8 - ENHANCED 3D RECONSTRUCTION OF SEM DATA

Ever wondered what your scanning electron microscopy images would look like in 3D?

Mountains® offers you several techniques for switching from standard 2D images to "topographic" images. Version 8 algorithms have again been improved to make this easier and quicker than ever before.



3D model reconstructed from 2 SEM images

9 - PORE & PARTICLE ANALYSIS

This improved Mountains® feature allows you to quickly identify and quantify features in virtually any SEM image.

Methods based on thresholding, watershed and circle detection make it possible to detect objects of almost any type (particles, pores, grains, surface defects, cells, contamination, pits, pillars etc.)

10 - SEE YOUR SEM DATA FROM EVERY ANGLE

Mountains® 8 will bring a new dimension to 3D visualization of images by enabling you to see them from any angle.

Using the multiple-image reconstruction tool, you can build a model from series of SEM images in stunning high definition 3D. A wide range of customizable rendering types, materials and lighting options are available. You can zoom in/out, rotate, make a movie and export the reconstructed model directly for 3D printing.

UNDERSTANDING STEM CELL STRUCTURE: A NEW METHOD

Using scanning electron microscopy and Hitachi map 3D software based on Mountains® technology, cell biology scientists at the University of Miyazaki (Japan) defined a new method for examining stem cell architecture. Their findings were recently published in the academic journal *Nature* .

A NEW METHOD FOR INVESTIGATING STEM CELLS

Recent advances in biomedical research, such as the production of regenerative organs from stem cells, require three-dimensional analysis of cell and tissue architectures.

High-resolution imaging by electron microscopy is understood to be the best way to elucidate complex cell/tissue architectures, but this method requires very laborious and time-consuming sample preparation.

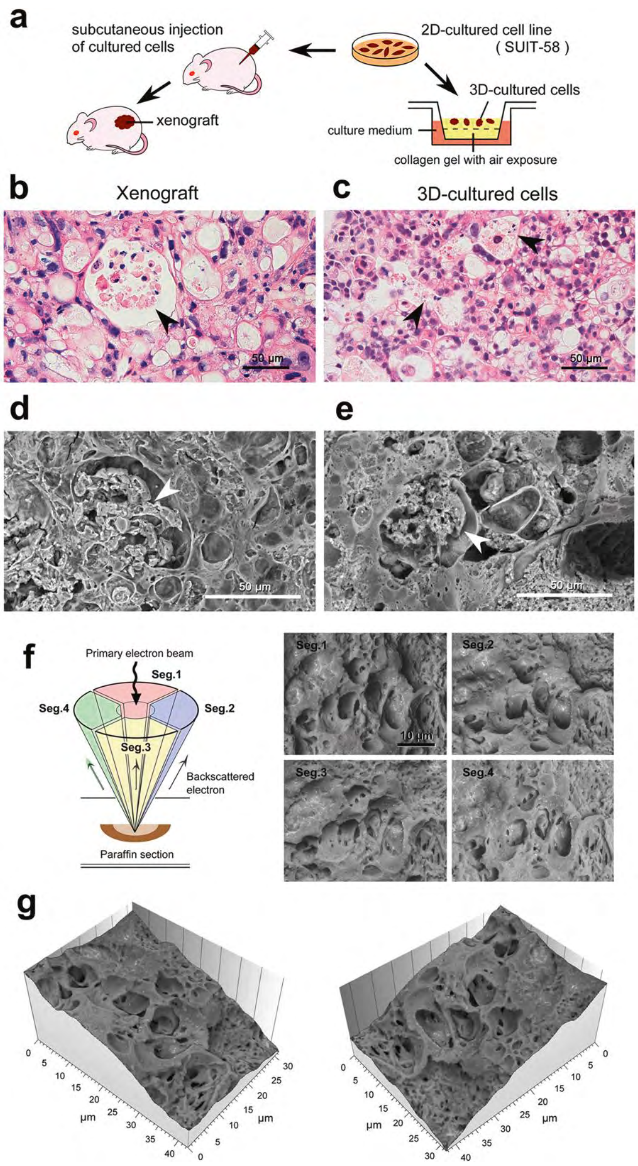
A team of cell biologists at the University of Miyazaki in Japan, led by Akira Sawaguchi recently presented a new three-dimensional method for assessing cell/tissue structures. This involves embedding cells in paraffin and visualizing 30-μm-thick paraffin sections using backscattered electron imaging in a low-vacuum scanning electron microscope.

APPLICATIONS IN ONCOLOGICAL EXPERIMENTS

This new method offered a new wealth of insight in an experiment on pancreatic tumor cells. The so-called "three-dimensional culture system" helped scientists confirm the similarity between xenografts (cells transplanted into mice) and three-dimensionally cultured cells (figures a-e).

Cells were imaged using a scanning electron microscope, equipped with a four-quadrant detector (figure f). These images were processed using Hitachi map 3D software which allowed the construction of a 3D topographic model (figure g). The microstructure, composed of vacuoles (enclosed compartments) containing necrotic cell debris, could be clearly visualized.

It is thought that this technique is applicable in many other domains of medical research and could even enable scientists to re-examine valuable samples prepared decades ago.



Above: *application of the new method for oncological experiments using SUIT-58 cell line. (a) Illustration of the comparative experiments between xenograft and three-dimensionally cultured cells. (b,c) Light micrographs of H&E stained 5 μm sections and (d–g) Thick PS-LvSEM micrographs of 30 μm sections. Note the similar profiles of vacuole formation enclosing necrotic cell debris (arrowheads). (f) Illustration of the four-segment BSE detectors (left) and collected micrographs by each detector (right). (g) Topographic images reconstructed from the raw micrographs of the four-segment BSE images.*

Read full article in Nature: doi.org/10.1038/s41598-018-25840-8

MOUNTAINSMAP® IN THE CLASSROOM

Our readers may recall the winners of our 3D printing contest last fall who were none other than the fourth grade class at the Victory World Christian School. *Surface Newsletter* was curious to see how the class was using MountainsMap®, more commonplace in research labs and industry than in the school classroom.

STEM (Science, Technology, Engineering, and Mathematics) is a growing movement in education, in particular in the United States but also around the world. STEM-based learning programs are designed to encourage students' interest in one day pursuing a career in these fields.

Victory World Christian School (Georgia, USA) is one of those schools providing an advanced STEM educational program to students from a young age.

In the STEM lab, where students are encouraged to participate in a very "hands-on" way, one can find a scanning electron microscope equipped with MountainsMap® software.

Sophia Chin, the school's STEM coordinator explained to *Surface Newsletter* that the presence of a SEM in the classroom helps "further develop practical applications with more advanced science equipment by exploring theoretical examinations. These examinations frame a working knowledge, which empowers deeper learning."

The winning samples in last year's contest were firstly of an aloe plant sample imaged in a study on the importance of growing healthy plants for people in the community. The second, hot chocolate powder, saw the students investigating characteristics of various different powders.

Above: *Students show off MountainsMap® colorization capabilities.* ***Below:*** *The VCWS STEM class of future scientists. Acknowledgments: Jeff Le May and Sophia Chin.*

In another recent experiment, students investigated how bees pollinate plants. After imaging a bumble bee with the scanning electron microscope, MountainsMap® was used to convert the gray-scale image into a color image in order to make visualization of different features easier. Students were able to see spiral shapes on the bee's legs which helps the pollen adhere to the insect's body. The final step was to print out a 3D model of the imaged sample.

Well done kids - you are the scientists of tomorrow and almost certainly among the youngest MountainsMap® users!

FROM THE IMAGE FOLDER OF AN AFM PRACTITIONER

Dr. Sergei Magonov is an experienced and respected figure in the world of atomic force microscopy (AFM). With over three decades of practice working with instruments from several different manufacturers, Sergei tells Surface Newsletter why highly-specialized tools for preparation, visualization and quantification are the key to understanding AFM data.

Commercial AFM instruments mostly come equipped with software focused on the collection of data. Off-line data treatment capabilities and quantitative examination are typically limited.

This is why it is, in my opinion, essential that AFM users work with specialized software packages such as those based on Digital Surf's Mountains® platform.

I myself have enjoyed working with Mountains® for a number of years. To demonstrate its value, the following article presents a few examples of AFM image processing and analysis taken from studies of single macromolecules and heterogeneous materials.

VISUALIZATION AND QUANTIFICATION OF SINGLE MACROMOLECULES

High-resolution profiling of nanoscale objects with an AFM probe has fascinated researchers since images of single DNA strands and their double helical structure were first observed in the early 90s. Since 1996, in addition to natural macromolecules, synthetic polymer chains are also regularly studied using AFM.

The chemists who create these macromolecules greatly appreciate being able to directly visualize the architecture of synthesized polymer chains deposited on an atomically-flat substrate from their dilute solutions.

However, a little data preparation is necessary to make this possible. And that's where software like Mountains® comes in.

PREPARING AFM DATA FOR ANALYSIS

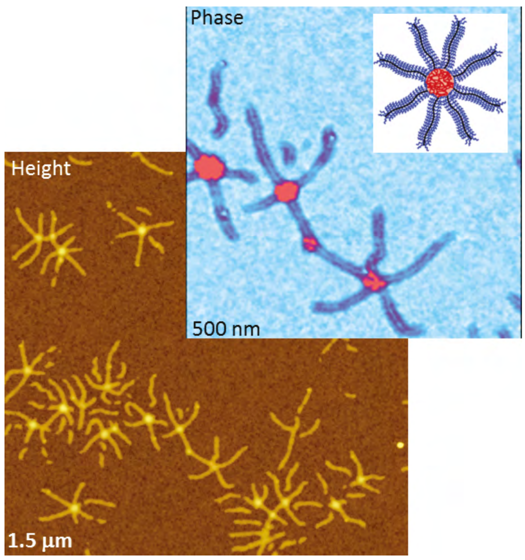
It is often necessary to subject the raw height images of single macromolecules to processing such as leveling with the exclusion of raised structures and form removal which eliminates occasional sample tilt and tube-scanner bulging.

In Mountains®, these common procedures are very time-efficient and user-friendly. The software offers a broad choice of user-definable color palettes.

The resulting processed images are well suited to quantitative analysis and provide, for example, statistics on macromolecule length (an important characteristic of polymers, related to molecular weight distribution).

Also vital is the examination of chain conformation and changes caused by different factors: temperature, environment etc.

The height image below illustrates polymer macro-molecules absorbed on a mica surface. Further analysis of these images can be performed for example using the Motifs analysis tool.



AFM height & phase images of brush macro-molecule.
Top right: *sketch of the brush macromolecule in "spoke-wheel" configuration.*

1 - CHARACTERIZING MEMBRANE MORPHOLOGY

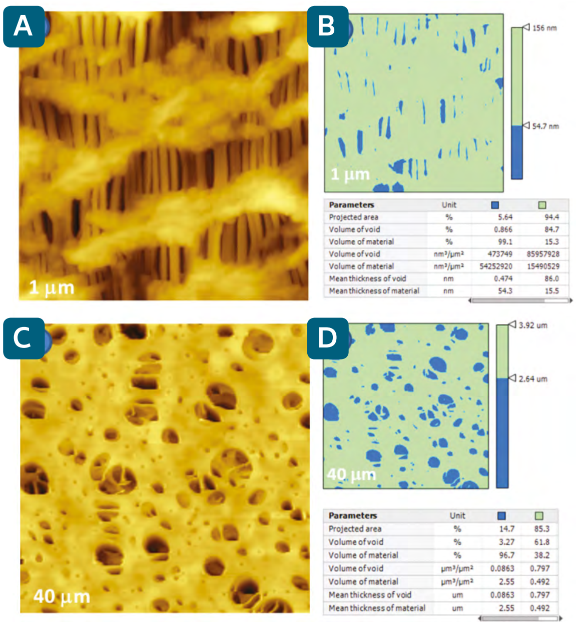
Membranes are important functional components for a variety of applications from batteries to biochemistry.

Size of pores, pore distribution and morphology are valuable characteristics for defining a membrane's overall performance.

Figure A below shows the surface morphology of a Celgard microporous polymer film containing numerous nano-size voids originated in fibrillar regions separated by densely packed lamellar regions.

To quantify morphology, one can apply the Mountains® Slices tool (figure B), which provides projected areas, volumes and mean thickness of the voids and surrounding material. The procedure is user-controlled with a choice of one or two color-coded threshold levels separating features of interest.

Figures C & D show a similar analysis routine applied to an industrial nitrocellulose membrane with features much larger than those of the Celgard film. The size of the pores varies from tens of nanometers to several microns.



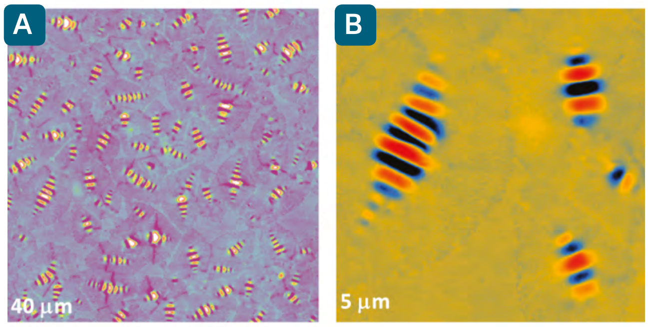
Parameters	Unit		
Projected area	%	5.64	94.4
Volume of void	%	0.866	84.7
Volume of material	%	99.1	15.3
Volume of void	nm³/µm²	473749	85957928
Volume of material	nm³/µm²	54252920	15490529
Mean thickness of void	nm	0.474	86.0
Mean thickness of material	nm	54.3	15.5

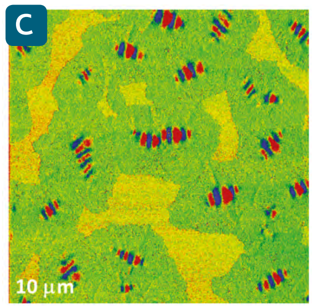
Parameters	Unit		
Projected area	%	14.7	85.3
Volume of void	%	3.27	61.8
Volume of material	%	96.7	38.2
Volume of void	µm³/µm²	0.0863	0.797
Volume of material	µm³/µm²	2.55	0.492
Mean thickness of void	um	0.0863	0.797
Mean thickness of material	um	2.55	0.492

2 - EXAMINATION OF BITUMEN COMPOSITION

Bitumen is broadly used for road pavements and as roofing material. Technological properties of this material depend on its composition and morphology, which can be examined with AFM phase imaging and mapping of local dielectric response.

Typically, bee-like structures resulting from surface stress during cooling from high temperature can be observed in height images of bitumen surface regions. As their profiles are corrugated, the leveling of such images is facilitated by automatically excluding features below and above the average level (figures A & B below).





Their heterogeneous morphology and different domains can only be faintly seen here. However phase images are more sensitive not only to topographical features but also to differences in mechanical and adhesive local properties (figure C). Here, the color-coded contrast differentiates bee-like structures and two kinds of surrounding surface domains.

Composition was then quantified using Slices analysis applied to both height and phase images. Comparison of these allowed us to identify the bee-like features with surrounding domains as wax and the other regions as polar asphaltene material, these being two of the multiple chemical constituents of bitumen.

RESOURCES

- *S. S. Sheiko, F. Sun, A. Randal, D. Shirvanyants, K. Matyjaszewski and M. Rubinstein, Nature 2006, 440, 191.*
- *S. Magonov, J. Alexander, M. Surtchev, A. M. Hung and E. H. Fini, Journal of Microscopy 2016, 265, 196.*

ABOUT THE AUTHOR

Dr. Sergei Magonov was attracted to field of scanning probe microscopy while working at the University of Freiburg (Germany) in the late 80s. In 1995, he joined Digital Instruments (Santa Barbara CA, USA). His expertise in STM and AFM further developed and he later spearheaded applications at Veeco Instruments, Agilent Technologies and NT-MDT. His contributions to research on the subject include a book, 16 chapters/reviews, over 200 peer-reviewed papers, 6 US patents and more than 40 application notes. As of December 2017, Sergei is a member of SPM Labs LLC where he is involved in the development of AFM instrumentation and novel applications.

HOW CAN I CHARACTERIZE LATERAL FEATURES?

Most surface texture parameters are related to heights. But in some applications lateral features and their spacing are as (if not more) important.
Which parameters should be used to characterize these properties and how is this done?
François Blateyron, Digital Surf's ISO expert has the answer.

Common roughness parameters such as Ra, Rq, Rsk (and their areal equivalents Sa, Sq, Ssk) integrate heights calculated from the reference surface. Even material ratio parameters such as Rmr, Rdc, Rk (or Smr, Sdc, Sk) are based on the Abbott-Firestone curve, built from the height or depth distribution. What is more, these parameters take into account all the points of the profile (or surface): they are "field parameters". They do not tell us anything about the lateral position of the points nor about features of interest on the surface.

The only lateral information that is used in roughness analysis is the filter cut-off:

- either the small-scale cut-off λs or the main cut-off λc used to separate roughness from waviness on a profile
- or the areal equivalent with S-Filter and L-Filter applied to produce scale-limited surfaces.

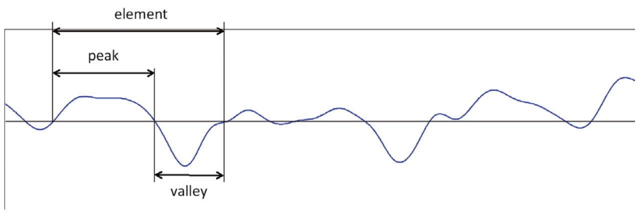
However, some workpiece functions rely on the lateral size of structures or features on the surface or on the lack of periodicity on the surface. For example, the grained surface of a laptop case is important for its feeling and aspect. The perceived quality depends on the average height of grains but also, and probably more importantly, on the lateral distribution of the grains on the surface (their relative distance) or their lateral size (diameter or dimension).

On the other hand, a shaft rotating at high speed should not exhibit periodical variations on its surface otherwise it creates noise and generates heat, likely resulting in failure after a certain time. If the amplitude of the noise depends on heights, its frequency depends on the wavelength of the period.

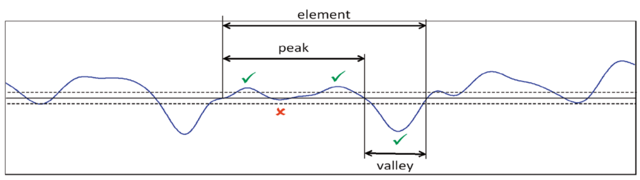
WHICH PARAMETERS CAN BE USED TO CONTROL LATERAL SIZES ON A PROFILE?

Spacing of elements

As far as profiles are concerned, very few parameters are designed for characterizing lateral sizes. One well-known example is RSm, mean spacing of elements, defined in ISO 4287. An element is defined on a profile as a peak followed by a valley or a valley followed by a peak. Both peaks and valleys are defined between two zero-crossings, on the mean line.



To avoid taking into account small peaks or valleys resulting from noise or roughness, vertical and lateral discrimination is introduced. For example, the second element on the example profile will count only as one peak by joining the two humps together because the valley in between is too small (see below).



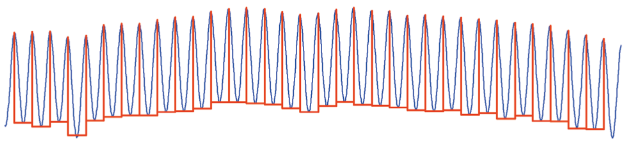
By calculating the mean of all element lengths, we can estimate the mean spacing of a periodical structure. This is also useful on semi-periodical profiles, where the dispersion of element lengths is reduced by the averaging process. This parameter is therefore useful for specifying a dimensioning tolerance along the surface (lateral characteristics) instead of in heights.

However, the initial definition of RSm is somewhat ambiguous, because it is specified on a sampling length, and because the use of discriminations is not clearly explained. Each manufacturer has implemented their own understanding of this parameter so it is frequent to have discrepancies in RSm values on semi-periodical profiles or more frequently on non-periodical profiles.

RSm is given in length units on the X axis, usually in millimeters. RSm can be used to assess the length of a periodic structure. In particular, it is often used to calibrate the lateral amplification coefficient of scanning profilers with the help of a grating or a sinusoidal or triangular calibration artefact (e.g. Rubert & Co models 521 or 525).

Spacing of motifs

R&W motifs are defined in ISO 12085. They are usually referred to as "French motifs". The beauty of this method is that it decomposes the profile into motifs that are a peak-valley-peak triplet. Two adjacent motifs share one peak.



After a pass to reduce non-significant features, motifs are characterized with parameters such as R, mean height of motifs, or AR, mean length of motifs. The latter is similar to RSm and should give the same value on periodical profiles. An interesting complement is the parameter SAR which is the standard deviation of all motif lengths. It provides an indication on whether the profile is periodical, semi-periodical or non-periodical.

Motifs can also be calculated on the waviness profile (envelope profile) in which case the corresponding parameters are W, AW and SAW.

Spectral analysis

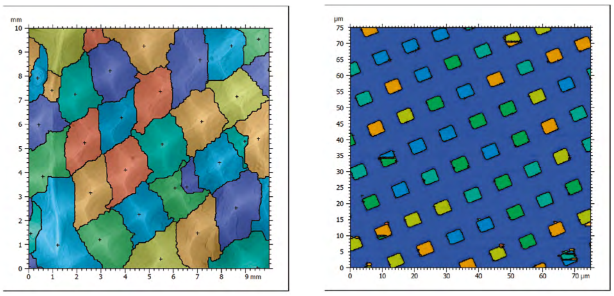
Periodical structures can also be analyzed with Fourier analysis. A periodic structure will generate a high spectral peak which can be characterized, for example, by the Averaged Power Spectral Density study in MountainsMap®.

WHAT ABOUT LATERAL CHARACTERISTICS ON A SURFACE?

Most textured and structured surfaces cannot be accurately studied with profile measurements and require areal measurements parameters. Surprisingly, the only official areal parameters that are purely lateral in ISO 25178-2 are Sal and Str. The isotropy parameter Str indicates whether the surface is homogeneous in all directions or has dominant directions. A structured surface will tend to have high Str values. Sal is called "autocorrelation length" and is supposed to characterize the size of the texture cell that best represents the lateral surface characteristics. However, this parameter is not understood very well and not really supported by proven studies.

Feature parameters

Feature parameters are calculated from specific features (points, lines, areas) extracted from the surface by a segmentation algorithm defined in ISO 25178-2 and ISO 16610-85. In particular, hills or dales are the areal equivalent of motifs, i.e. an area enclosed by a contour that represents an identified feature of the surface. This can also be a structure when using a gradient prefilter.



The two examples above show texture cells (left) or structures (right) detected by the watershed algorithm. Motif characteristics can be evaluated individually or as a whole with mean values. Several parameters are of interest here: area and diameters. The ISO standard only mentions Sha (area of hills) and Sda (area of dales) but other parameters can be calculated such as the equivalent diameter or the min/max/mean diameter. They are all provided in MountainsMap® as part of the Grains & Particles module.

CONCLUSIONS

ISO standards offer plenty of parameters based on heights, material ratio or slopes, but very few for characterizing lateral features such as size or diameter. There is probably room for improvements in order to offer metrologists and designers the tools for a better specification of manufactured parts. Segmentation allows the detection of individual features that can be analyzed in the x,y plane from their contour. Many lateral quantities can be derived from the contour and inter-feature characteristics can also be calculated, such as pitch.

RESOURCES

- *ISO 4287:1996 GPS – Surface texture: Profile method - Terms, definitions and surface texture parameters*
- *ISO/CD 21920-2:2017 GPS – Surface texture: Profile method – Definitions and parameters*
- *ISO 12179:2000 GPS – Surface texture: Profile method - Calibration of contact (stylus) instruments*
- *ISO 25178-701:2010 GPS – Surface texture: Areal - Calibration and measurement standards for contact (stylus) instruments et ISO 25178-2:2012 GPS – Surface texture: Areal - Terms, definitions and surface texture parameters*
- *ISO 16610-85:2013 GPS – Filtration - Morphological areal filters: Segmentation filters*
- *F Blateyron, Feature parameters, chap. 3 in Characterization of Areal Surface Texture, Springer*
- *N Senin, L Blunt, Characterisation of individual areal features, chap. 8 in Characterization of Areal Surface Texture, Springer*
- *J Blanc et al, Surface characterization based upon significant topographic features, J of Physics, conf series*
- *Rubert & Co material measures: www.rubert.co.uk*
- *Surface Metrology Guide: www.digitalsurf.com/guide*

EVENTS & PRODUCT HIGHLIGHTS

CONTROL 2018

Great to see so many of our customers and partners at the Control Trade Show for Quality Assurance in Stuttgart, Germany on April 24-27.

With over 800 exhibitors from 31 countries and in excess of 28,000 visitors, Control is more than ever the place to be to see and hear the hottest trends in the world of metrology.

The Digital Surf stand saw a strong stream of visitors over the four days and elsewhere on the exhibit floor Mountains® software was on show on many of our partners' stands demonstrating its high versatility in different applications.

Thanks to all for making it another great year!

NEW LOGO & NEW WEBSITE

Visitors to www.digitalsurf.com will have noticed some sizable changes recently.

Firstly our logo has been redesigned to reflect more accurately who we are and what we stand for today and in particular our expertise in surface metrology and microscopic topography analysis.

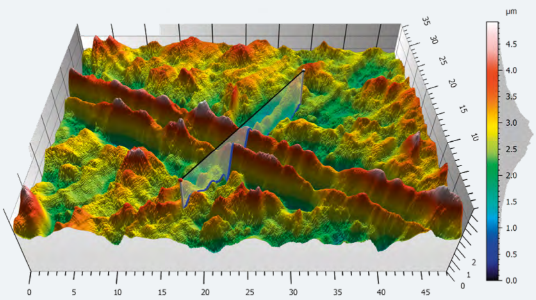
Furthermore, the look and structure of our website has been updated and is now fully responsive across all devices including mobile, making information and resources easier to access.

Digital Surf

PRODUCT NEWS

JEOL and Digital Surf partner launch SMILE VIEW™ Map software

Tokyo, Japan & Besançon, France, May 18, 2018: leading high-tech equipment company JEOL and Digital Surf, creator of Mountains® surface and image analysis technology, announced the release of SMILE VIEW™ Map software for users of JEOL's cutting-edge scanning electron microscope (SEM) systems.



This new release will be of great benefit to researchers and engineers working in a wide range of application areas including nanotechnology, metals, semiconductors, ceramics, medicine and biology.

Read the press release: goo.gl/agYuAT

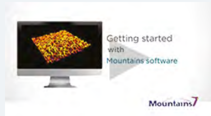
WHAT'S HOT ONLINE

Seen on Facebook

5/9/18: Great to see the May issue of Microscopy Today (published by the Microscopy Society of America) in print.

An article on ground-breaking techniques for adding color and 3D to scanning electron microscopy images by our CEO Christophe Mignot featured on the cover and inside on pages 12-17.

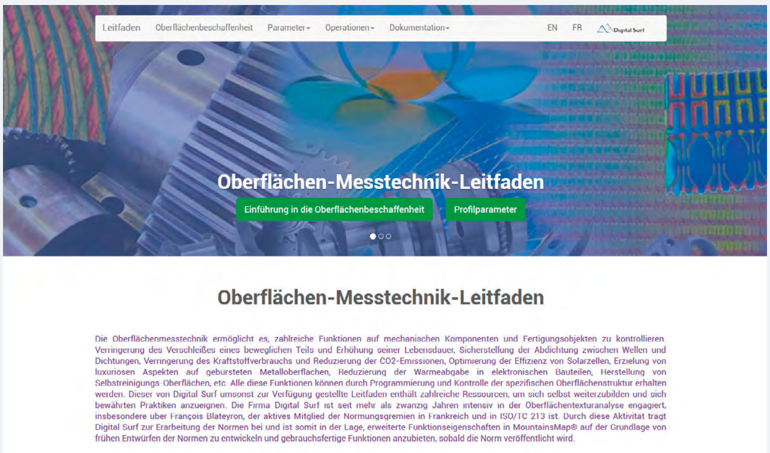
goo.gl/sZT1Lz



Have you visited our YouTube channel recently?

Discover tips for getting started in Mountains®, tutorials for SEM image reconstruction & colorization and much more!

www.youtube.com/channel/UC5cyEQHs-9IWZdn0p-cJcJA



Popular on the Digital Surf website

Ever had a question on Surface Metrology and were too scared to ask? Never fear – help is at hand. Our free online metrology guide covers topics such as areal surface texture, profile parameters and filtration techniques. And the good news is: as well as the English and French versions, it is now available in German too!

See more: www.digitalsurf.com/guide

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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MountainsMap® Premium Software with all the options, free for 30 days!

CONTACT US FOR AN UPDATE
Contact us for information about updating MountainsMap® 6 or earlier software to the latest version of MountainsMap® 7

WATCH A MOUNTAINS® TUTORIAL
Get the most out of Mountains® software by watching one of our tutorial videos

LEARN SURFACE METROLOGY
Dive into our free online surface metrology guide and learn how to characterize surface texture in 2D and 3D

MEET DIGITAL SURF
Microscopy & Microanalysis - Booth #425
Aug. 6-9, 2018 - Baltimore Convention Center, Baltimore, MD, USA

Japan Analytical & Scientific Instruments Show - Booth #6A-609
Sept. 5-7, 2018 - Makuhari Messe, Tokyo, Japan

19th International Microscopy Congress (IMC19) - Booth #4
9-14 Sept. 2018 - Int. Convention Centre, Sydney, Australia

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