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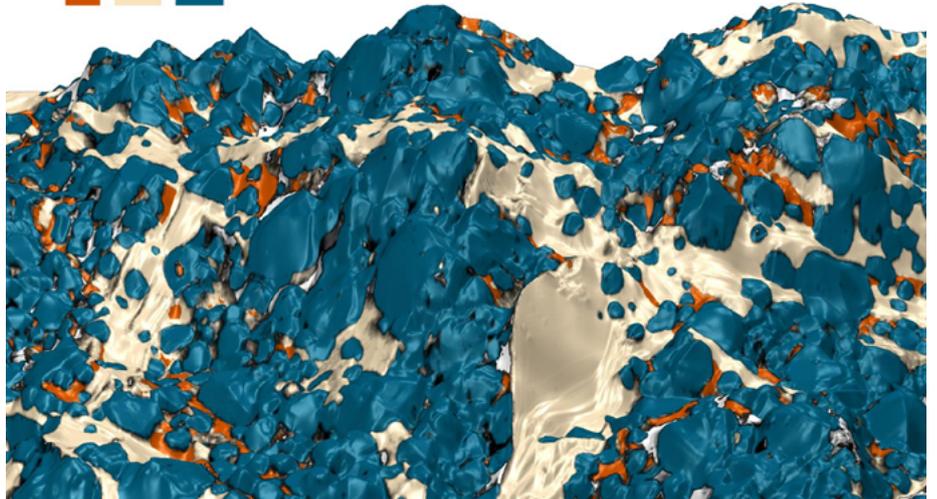
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## REVEALING CORRELATIONS BETWEEN TOPOGRAPHY & CHEMICAL COMPOSITION

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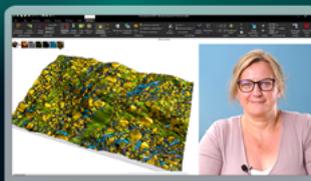
The study of the functional capabilities resulting from the combination of surface shape and texture with chemical composition is key to manufacturing the materials of tomorrow.

A new workflow is emerging for bringing to light correlations between topography from SEM images and EDS/EDX maps, all within a single software package.

[... Turn to page 2 ...](#)

Register for our  
**WEBINAR**

Spectroscopy data preparation, analysis and correlation with MountainsSpectral®



MountainsSpectral® is a dedicated solution for processing & combining images and other data from spectroscopic techniques.

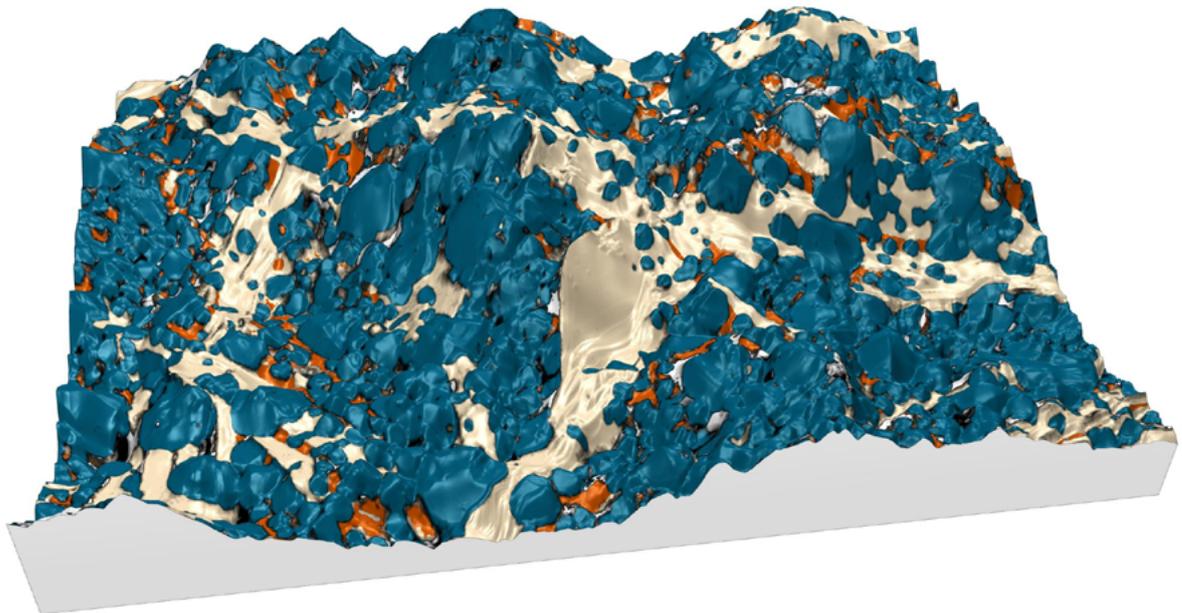
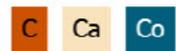
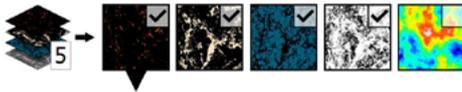
Join us for a look at some of the key features including data preparation and correlation : [bit.ly/MountainsSpectral-webinar](http://bit.ly/MountainsSpectral-webinar)

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## CORRELATIVE ANALYSIS: HOW TO COMPARE 3D TOPOGRAPHY & CHEMICAL COMPOSITION



The vast majority of SEMs (Scanning Electron Microscopes) come equipped (or may be equipped) with EDS (Energy Dispersive x-ray Spectroscopy, also known as EDX) which allows quantitative analysis of chemical elements present in the sample. Exciting recent developments in MountainsSpectral® software now open up possibilities for combining and comparing these characteristics with surface topography in order to better understand material behavior. **Renata Lewandowska**, product manager for spectral applications at Digital Surf, explains the new tools and their applications.



*Above. 3D model of surface topography with overlay of chemical composition on a Cobaltite sample. Courtesy of Emmanuel Guilmeau, CRISMAT (Caen, France), Jean-Claude Ménard, JEOL France.*

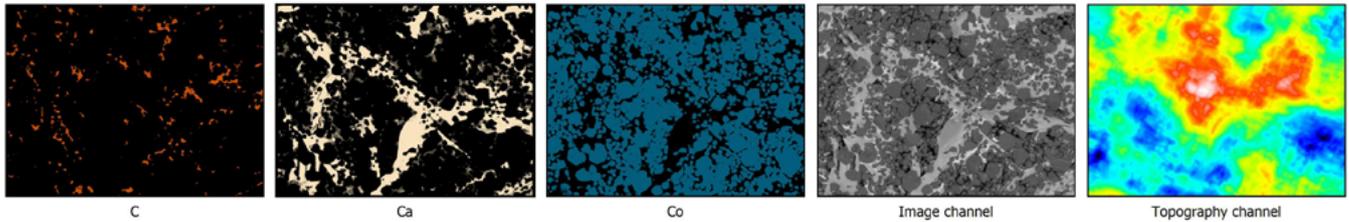
### 3D RECONSTRUCTION

Several years ago, the Digital Surf team unveiled a new, revolutionary method for quickly building a 3D model from 2 successive scans or four 4-quadrant images from an SEM.

**SEE THE FULL METHOD IN OUR WEBINAR**  
[bit.ly/3D-reconstruction](https://bit.ly/3D-reconstruction)

### COLOCALIZATION TOOLS FOR OVERLAYING SPECTRAL DATA

Very often, users of SEM can also generate EDS maps of the sample they are studying. However, bringing together these datasets of different nature and at different scales to study them collectively can be particularly tricky.



Above. Grid view of all data (background and overlays) used to perform colocalization.

MountainsSpectral® now offers a streamlined method for performing this task, all within a single software package.

## CONNECTING BACKGROUND DATA WITH OVERLAYS

The first step in the process is to select the desired background and create a **Colocalization study**. This could simply be a 2D image but in the case presented here (a Cobaltite sample imaged with SEM and EDS), a 3D topography was used.

In the next step, users can choose to add overlay images. In the present case, three EDS chemical maps were chosen as overlay images.

Transparency settings can be manipulated to check positioning of the overlays on the background (particularly useful when the two datasets are not at the same scale).

Several methods are available to position overlays more accurately. In this example, we used the **Use points** method to identify similar structures on the two datasets and create pairs of points to be colocalized with each other.

Finally, we used the **Composite rendering** tool in MountainsSpectral® to choose which EDS maps to work with and mix colors from these.

## SHOWCASING DATA IN 3D

Visualizing and manipulating data in 3D can really help us better interpret data analyzed. Luckily, a variety of methods are available for generating a 3D view in MountainsSpectral®.

The **Generate 3D view** tool in the Colocalization study is one way to display your data in 3D with close to zero effort. This technique uses the

topographic values from the reconstructed SEM image and pastes the color image from the colocalization on top.

As an alternative, using the **Generate whole content** feature, it is possible to obtain all colocalization datasets (background and overlay images) as a **Multi-channel image**. This has the extra advantage of opening up other possibilities for processing this data (access to Operators and Studies on Multi-channel images).

This method also gives more flexibility for adjusting composition once the colocalization is performed.

Whichever method you end up using, once the 3D view has been generated, you benefit from a multitude of enhancement options: amplify height representations, modify rendering and gloss and define the ideal lighting conditions for your sample.

Finally, the **Animated view** allows you to simulate flight over the data, following one of several predefined paths. All these are excellent tools for creating an outstanding presentation.

## CORRELATION MADE EASY

Beyond creating an esthetically pleasing image, the workflow described in this article allows us to make correlations between different kinds of instrument data (SEM & EDS images).

The ability to easily hide and unhide the various chemical maps (calcium, carbon etc.) on 3D data allows us to show and understand relationships between the different components.



### SEE CORRELATION IN ACTION

There's nothing like a demonstration to better get your head round new tools and how they work!

Join us on September 8, 2022, when Renata will be showing correlation step-by-step in our webinar on "Spectroscopy data preparation, analysis and correlation with MountainsSpectral®" [bit.ly/MountainsSpectral-webinar](https://bit.ly/MountainsSpectral-webinar)

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## PERFORMING THICKNESS ANALYSIS WITH MEASUREMENTS FROM ANY PROFILOMETER



Thickness is an important parameter for many applications as it affects the functionality, quality and cost of manufactured products. New tools released recently in Mountains® software give users access to a wealth of options for calculating, quantifying and analyzing thickness. **Christophe Mignot**, Digital Surf CEO, explains more to *Surface Newsletter*.

### DATA REQUIREMENTS FOR ANALYZING THICKNESS

Typically, a profilometer produces a single surface measurement. In order to analyze thickness, it is necessary to dispose of at least two surfaces, for example the upper face and the lower face of a membrane.

Several methods allow users to obtain these two measurements:

- ▶ **measuring two surfaces successively** using a profilometer, SEM or AFM (applications in nanotechnology, including MEMS)
- ▶ **measuring two different layers of the same sample simultaneously** using optical techniques, in particular confocal microscopy (applications in transparent films, coatings etc.)
- ▶ **measuring a flexible surface using a profilometer equipped with a dual sensor** (eg. banknote, plastic packaging).

### INDUSTRIAL APPLICATIONS OF THICKNESS ANALYSIS

Controlling thickness is essential in many domains of industry.

- ▶ **Checking thickness homogeneity of sealing membranes** (eg. radial shaft seals) helps ensure efficiency during operation and longer life.
- ▶ **Measuring varnish thickness** is useful in fine art conservation for assessing previous treatments applied to paintings.
- ▶ **Thickness analysis of transparent or semi-transparent layers** is widely used in quality control of composite plastic materials.
- ▶ **Controlling thickness of MEMS** accelerometers in airbag systems is used to check performance.

- ▶ **Devices where resonance is important** at any scale, from musical instruments to quartz crystals: here again, thickness is an essential functional parameter as it is directly linked to resonant frequency.

### TOOLS FOR STUDYING THICKNESS IN MOUNTAINS® 9.2

Mountains® 9.2 released in May 2022 provides a comprehensive set of tools for performing thickness visualization and analysis.

#### Align thickness pairs

As previously mentioned, in most cases, a prerequisite to thickness analysis is the alignment of two measured datasets.

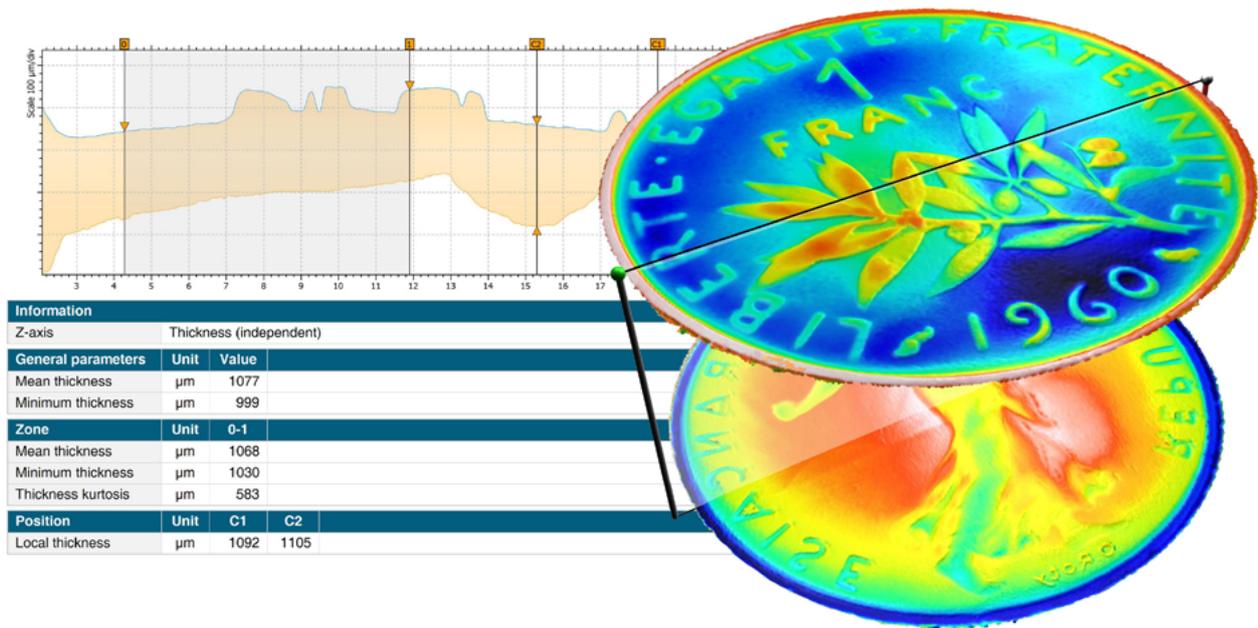
Thanks to Mountains® capacity for managing a large number of data types, these datasets can be simply two separate surfaces or they can originate from surface-image studiabiles, series of surfaces, multi-channel images etc.

A new operator allows users to align and adjust these surfaces and generate a series of two surfaces corresponding to the two sides of the membrane (or layer) on which thickness mapping is required. A wide variety of settings are available to cover the needs of the various types of source measurements mentioned previously.

#### Examine thickness in 3D or in cross-section

Once aligned, the membrane, composed of an upper and lower face, can be visualized in stunning Mountains® 3D.

Cross-sectioning can be performed directly on the 3D view, in order to obtain the thickness parameters on a particular zone.



**Above.** 3D thickness visualization and two-profile 2D analysis of upper and lower membranes of a one franc coin (the "Align for thickness analysis" operator was applied on these two surfaces measured in succession).

### Carry out 2D analysis on a double profile

In Mountains®, thickness membranes are considered as series of two surfaces. This means a vertical cross-section can easily be obtained using the existing **Extract Profile** operator.

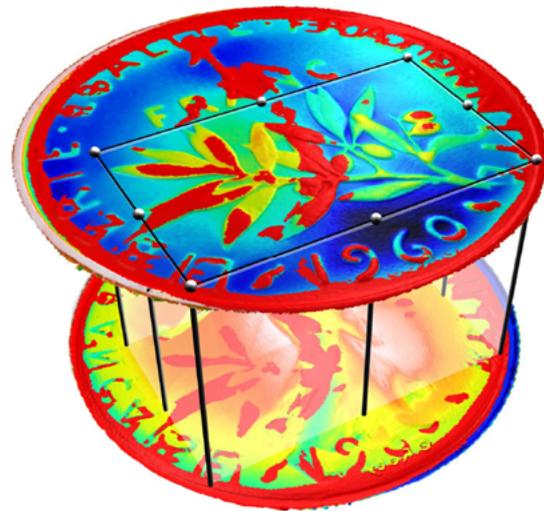
By moving the cursors (orange in the image above), interactive thickness analysis can be performed (values in the table below the profile are updated instantaneously).

### Generate thickness parameters

As usual in Mountains®, users can access a wide range of parameters for their analysis. For quantifying thickness, these include:

- ▶ mean thickness
- ▶ minimum/maximum thickness
- ▶ standard deviation
- ▶ skewness, kurtosis etc.

Using thresholds, it is also possible to color-highlight areas outside tolerances (see right).



**Above.** Areas outside thickness tolerances are highlighted (here in red) on the 3D view of data.



## HOW CAN I ACCESS NEW TOOLS FOR THICKNESS?

The **Thickness Analysis** module is now available as an option on any Mountains®-based product allowing topographic analysis. If your Mountains® product is supplied by an instrument manufacturer, please contact them to check if the module is listed in their catalogue.

The Thickness Analysis module is only available with version 9.2 onwards. This version is freely available to users with an active Software Maintenance Plan (SMP). If you don't have an active SMP, please get in touch to arrange an update by emailing [sales@digitalsurf.com](mailto:sales@digitalsurf.com).

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## USING 3D RECONSTRUCTION IN SEM TO REVERSE-ENGINEER THE HUMAN OVARY



During cancer treatment, chemo- and radiotherapy can potentially be toxic for the ovaries, ultimately leading to sterility. In this context, the demand for a transplantable artificial ovary (TAO) emerges as a procedure for fertility restoration in cancer survivors unable to benefit from other available solutions. **The gynecology research team based at the Catholic University of Louvain (Belgium)** recently conducted the first attempt to “reverse engineer” the human ovary. Emna Ouni, one of the researchers involved in the project, shares their findings with *Surface Newsletter*.

In biology, reverse engineering refers to the concept of taking apart a process or mechanism in order to understand it, re-engineer it (perhaps in a new way) and apply it to the biological world.

### DECONSTRUCTING TO RECONSTRUCT

In this project, the micro-environment of the human ovarian cell (extracellular matrix or ECM) was deconstructed to better understand its proteomic, architectural and mechanical cues and its role in ovarian activity from pre-puberty until the menopause. The data uncovered will enable the TAO to be a future life-changer for many female patients, making motherhood a possibility after cancer survival.

“If the cell is the basis of life, the extracellular matrix is the basis of multicellular life”. Based on this statement, I have been an advocate of the role ECM plays in fertility for the last 5 years.

In this study, my colleagues and I shed light on one of the ECM’s overlooked features: topography.

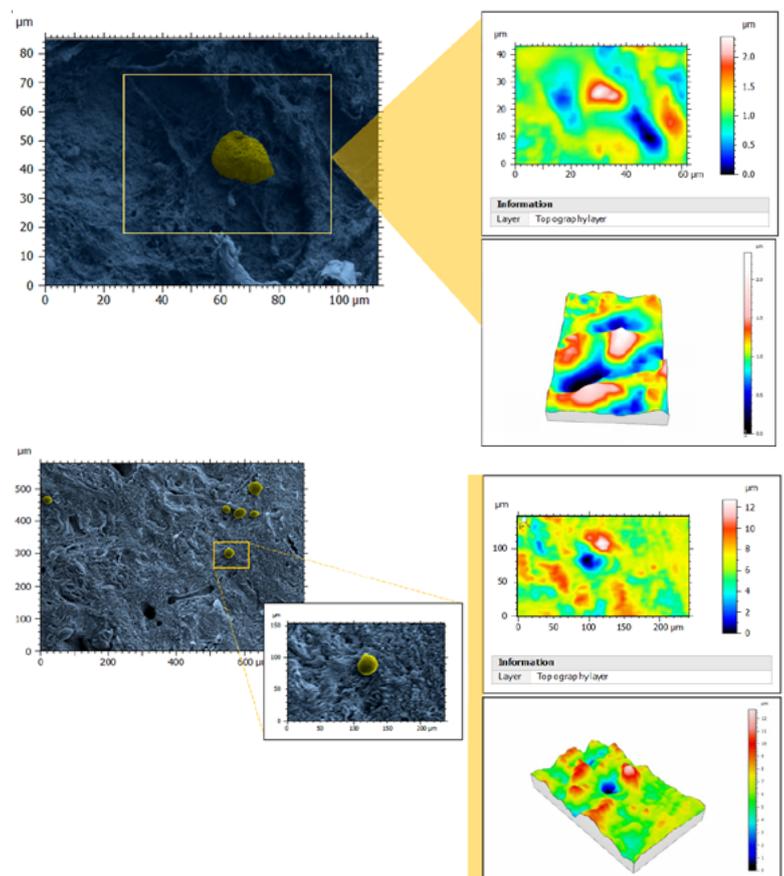
### CREATING A TOPOGRAPHICAL BLUEPRINT

The rationale for exploring the link between topography and fertility is that native ECM displays physical topography at the nanoscale and thus features of similar size on engineered substrates may be a better mimic.

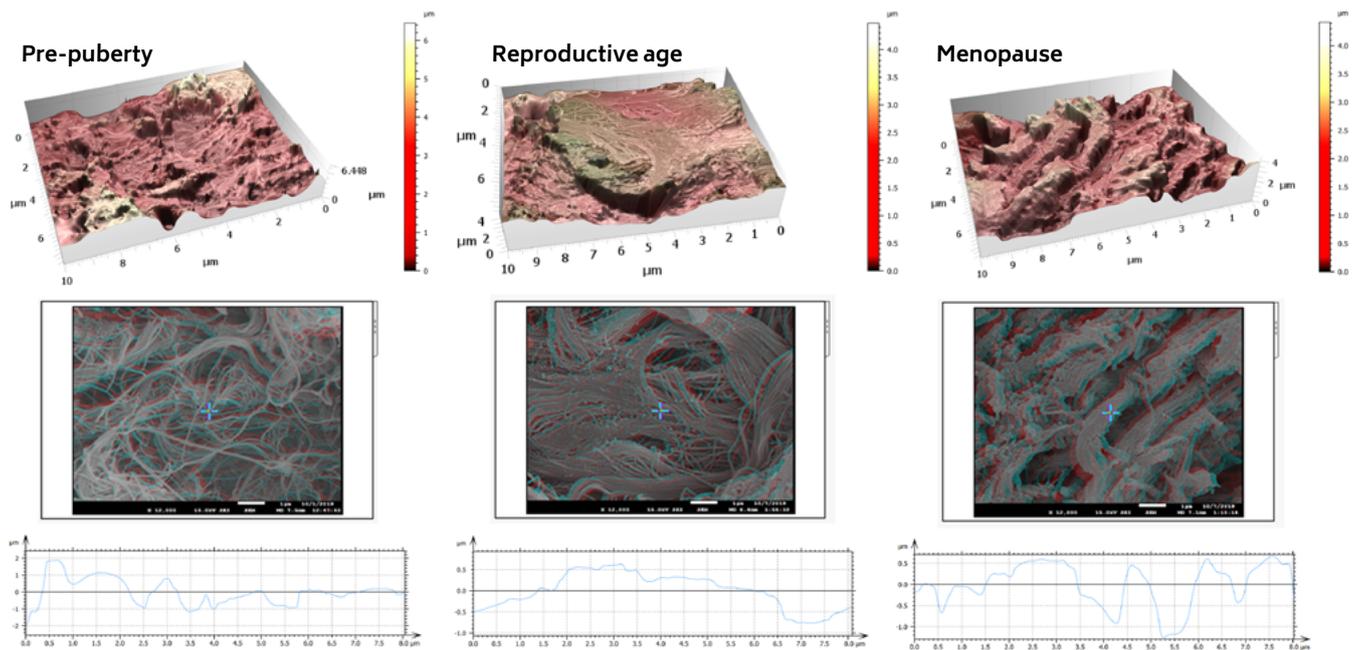
Furthermore, emerging *in vitro* studies demonstrate that ECM surface features play a role in a plethora of biological processes: cell differentiation, cell shape and morphology,

cell adhesion, mechanotransduction, tumor propagation, dormancy and even ECM composition.

To provide the first description of these structural features, ovarian tissues from prepubertal, reproductive-age and menopausal patients were analyzed by scanning electron microscopy (SEM) at different magnifications to capture: fibril (nanoscale), fiber (microscale) and fiber-bundle



**Above. Topography of perifollicular ECM before and after menopause.** SEM images were colorized with SMILE VIEW™ Map software, based on MountainsSEM®, to highlight the presence of follicles (yellow) and distinguish them from the ECM (blue) and 3D renderings were obtained.



**Above. Architecture, topography and biomechanics synergy.** 3D models of surface topography of ovaries obtained from stereoscopic reconstruction of two SEM images. Color scales indicate the height range on each surface.

(mesoscale) organization and diameters, as well as pore number and area, as a reflection of ECM network tightness.

Based on a stereoscopic reconstruction of SEM captures created by tilting the sample +5° along the X-axis, 3D renderings were generated using SMILE VIEW™ Map software based on Mountains®. The team was able to extract surface topography and measure micro-roughness on the same tissue regions used to characterize ECM architecture.

## RESULTS

In the course of these analyses, the mechanical features of ovarian tissue recorded by atomic force microscopy (AFM) was linked to its nanotopography, ultrastructure and organization. The team discovered that soft ovarian tissue from reproductive-age patients was characterized by a significantly smoother tissue surface ( $99 \text{ nm} \pm 33$ ) compared to the rougher prepubertal ( $228 \text{ nm} \pm 87$ ) and menopausal ( $238 \text{ nm} \pm 28$ ) tissues, which are more rigid. This points to a possible synergy

between tissue rigidity and surface roughness.

## CONCLUSION

Overall, in the human ovary, the team found that topography, in completion to biomolecular cues, synergizes with the ECM elasticity to influence ovarian tissue remodeling from prepuberty until menopause. Considering age as the most prominent fertility biomarker to date, the unique topological features that we succeeded in recording and reporting in our blueprint of the human ovarian ECM can be linked to female fertility, namely follicle activation and dormancy.

Moreover, the analyses demonstrated that ovarian ECM at reproductive age represents an ideal micro-environment for ovarian cell and follicle function and development due to its unique topological and mechanical features. Thus, the study provided the ECM cues necessary to replicate ovarian tissue properties in a functional engineered ovary and represents a reliable reference for comparing physiological and pathological ovarian conditions based on ECM status.



### ABOUT THE AUTHOR

Emna Ouni is currently a PhD bioengineer at the Gustave Roussy Institute (Paris, France).

### INSTRUMENTS AND SOFTWARE USED

JEOL JSM-7500F field emission scanning electron microscope + SMILE VIEW™ Map software, based on Mountains®.

### FURTHER READING

**A blueprint of the topology and mechanics of the human ovary for next-generation bioengineering and diagnosis.** E. Ouni, A. Peaucelle, K. T. Haas, O. Van Kerk, M.M. Dolmans, T. Tuuri, M. Ojala, C. A. Amorim. Nature Communications: [doi.org/10.1038/s41467-021-25934-4](https://doi.org/10.1038/s41467-021-25934-4)

## 5 COMMON MISTAKES TO AVOID IN PRESENTATIONS & PAPERS

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With many conferences beginning to restart in person following the pandemic, we've noticed the return of a few classic mistakes in conference presentations and papers. Digital Surf's senior surface metrology expert **François Blateyron** gives a short review of the main errors that authors should avoid in order to provide sound and replicable results.

### 1. NEVER REPORT PARAMETER RESULTS WITHOUT FILTERING CONDITIONS

All too often, scientific papers report surface texture parameters (usually profile parameters such as Ra or Rq) without any filtering conditions.

Although ISO 4288 or ISO 21920-3 admit default conditions that can therefore be omitted, they are not always applicable, due to the small size of the measured parts.

For clarity, parameters should always be provided with the cut-off value and type of filter used. Otherwise, one can obtain any required parameter value simply by tuning the cut-off value, which makes results totally meaningless!

Moreover, with the new ISO 21920 standard, parameters are no longer averaged, so the reference standard used should always be stated in the paper.



**Takeaway #1:** Always provide filtering conditions alongside parameter values.

#### Read more:

- ▶ [guide.digitalsurf.com/en/guide-default-specifications.html](https://guide.digitalsurf.com/en/guide-default-specifications.html)
- ▶ [guide.digitalsurf.com/en/guide-iso-21920-parameters.html](https://guide.digitalsurf.com/en/guide-iso-21920-parameters.html)

### 2. DON'T LIMIT ANALYSES TO Ra OR Sa. EXPLORE OTHER PARAMETERS.

Surface texture is too often expressed simply as Ra or Sa parameters.

Before jumping on the Ra bandwagon, ask yourself: what am I trying to achieve? What kind of function do I want to characterize? If you're looking to analyze average roughness height, use Rq

#### ISO 25178 - Roughness (S-L)

F: None		
S-filter ( $\lambda_s$ ): Gaussian, 2.5 $\mu\text{m}$		
L-filter ( $\lambda_c$ ): Gaussian, 0.25 mm		
Height parameters		
Sq	1.806 $\mu\text{m}$	Root-mean-square height
Ssk	-0.436	Skewness
Sku	3.197	Kurtosis

**Above.** Mountains® parameter tables automatically display filtering conditions.

instead, which is more closely related to physical phenomena. If you want to characterize a particular function or performance on a surface, try exploring other parameters that will likely be more "pertinent" and better correlated to the process.

In particular, parameters related to height distribution (Rsk and Rku) or to material ratio (Rmr, Rdc) are usually more significant. Many other parameters offer useful insight, for example Rk, Rdq, Sdr, etc.



**Takeaway #2:** Take the time to think about which parameter is most appropriate for your application.

#### Read more:

- ▶ [guide.digitalsurf.com/en/guide-surface-function.html](https://guide.digitalsurf.com/en/guide-surface-function.html)
- ▶ [guide.digitalsurf.com/en/guide-iso-13565-parameters.html](https://guide.digitalsurf.com/en/guide-iso-13565-parameters.html)

### 3. AVOID USING SPACING PARAMETERS ON STOCHASTIC PROFILES

The most common spacing parameter, Rsm, is sometimes used in contexts where it cannot provide sound results. Spacing parameters are



Feature parameters that should be used on periodic, pseudo-periodic or structured profiles and not on stochastic profiles.

Here also, filtering conditions are critical to avoid removing wavelengths of interest.



**Takeaway #3:** Only use spacing parameters on (pseudo-)periodic or structured surfaces

#### Read more:

- ▶ [guide.digitalsurf.com/en/guide-lateral-features.html](https://guide.digitalsurf.com/en/guide-lateral-features.html)
- ▶ [guide.digitalsurf.com/en/guide-iso-12085-parameters.html](https://guide.digitalsurf.com/en/guide-iso-12085-parameters.html)
- ▶ [guide.digitalsurf.com/en/guide-stochastic-vs-deterministic.html](https://guide.digitalsurf.com/en/guide-stochastic-vs-deterministic.html)

## 4. AVOID USING DEFAULT CUT-OFFS

Users are sometimes lost when they have to select cut-offs, and this is why default values are used so frequently. But it is important to remember that filtration is an operation that affects results and even changes the conclusions of a study.

The cut-off value depends on the material or process signature on the surface. It cannot be chosen only with respect to instrument capabilities or measurement length.

Users should carry out explorations to determine at which scales or which wavelengths their material or process will exhibit characteristics of interest. The chosen cut-off value may not necessarily be the default one, nor the same as that used by

another user working on another application.



**Takeaway #4:** Investigate at which bandwidths your study is relevant.

#### Read more:

- ▶ [guide.digitalsurf.com/en/guide-filtration-techniques.html](https://guide.digitalsurf.com/en/guide-filtration-techniques.html)
- ▶ [guide.digitalsurf.com/en/guide-bandpass-filters.html](https://guide.digitalsurf.com/en/guide-bandpass-filters.html)

## 5. DO NOT REPORT Sdr OR Sdq WITHOUT STATING SURFACE RESOLUTION

Some parameters are calculated with respect to neighboring points, which means that depending on the spacing (lateral resolution), they provide different values.

This is indeed the basis of scale-sensitive fractal analysis (SSFA) where Sdr varies with scale. This applies, in particular, to Rdq/Sdq (mean slope) and Rdr/Sdr (developed area), and even on Spc/Svc (curvature). These parameters are very sensitive to resolution and to noise.



**Takeaway #5:** Sdr and Sdq parameters should always be accompanied with lateral resolution indications.

#### Read more:

- ▶ [guide.digitalsurf.com/en/guide-fractal-analysis.html](https://guide.digitalsurf.com/en/guide-fractal-analysis.html)
- ▶ [guide.digitalsurf.com/en/guide-areal-field-parameters.html](https://guide.digitalsurf.com/en/guide-areal-field-parameters.html)



### ADDITIONAL RESOURCES

- ▶ **Digital Surf Surface Metrology Guide:** [guide.digitalsurf.com](https://guide.digitalsurf.com)

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## A BUSY SPRING SCHEDULE FOR THE DIGITAL SURF TEAM

### Control 2022, Stuttgart

The return of the Control international trade fair for quality assurance in Stuttgart May 3-6 kicked off the spring show season and definitively marked the restoration of some kind of post-Covid "normality".

With over 550 exhibitors spread out over the six halls of the Messe, there were many opportunities to finally catch up with old acquaintances and meet new ones.

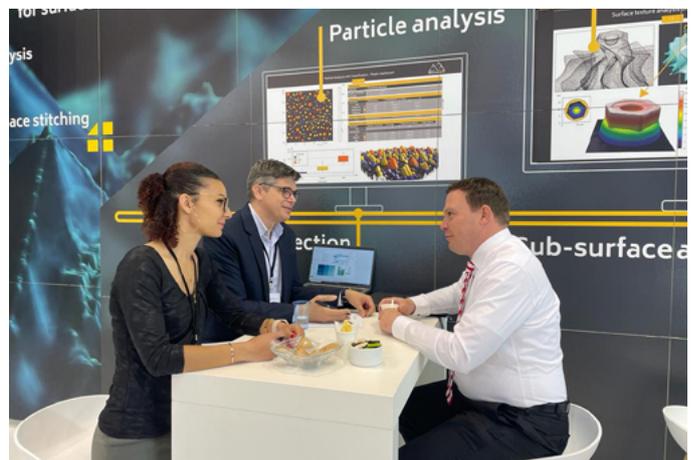
The Digital Surf team was present *en force* with Arnaud, Nicolas, Anne, Cyrille, François, Bertrand and Sophie on stand #3418 in hall 3.

Mountains® software version 9.2 including an extensive new toolbox for thickness analysis was demonstrated to the many visitors we were thrilled to welcome to our stand.

As usual, it was great to see our partners' software solutions, based on the Mountains® platform in action alongside their surface measuring instruments throughout the fair.

Although visitor numbers were somewhat down on previous years, Control 2022 was an excellent vintage with many fruitful meet-ups with clients and partners after two years of home office.

See you in 2023!



### CIRP Conference on Surface Integrity, Lyon

Anne and François were on hand in the beautiful city of Lyon, France June 8-10 last for the 6th CIRP Conference on Surface Integrity (CIRP CSI 2022).



The latest version of MountainsMap® software was showcased for applications involving the analysis of surface integrity and surface performance of parts including advanced materials (metal, composite etc.) and coatings.

A very engaging few days!

### Met & Props, Glasgow

Anne, François and Clare traveled to Glasgow June 27-30 for the 23rd International Conference on the Metrology and Properties of Surfaces.

As main sponsor, the Digital Surf team was thrilled to be able to meet up with the global surface metrology community at this event, organized by our friends at the EPSRC Future Metrology Hub at the University of Huddersfield.

Thanks to all speakers and delegates!



## WHAT'S HOT ONLINE



### POPULAR ON LINKEDIN

We were thrilled to receive a visit from our valued partner Louise Hughes of Oxford Instruments at our headquarters recently. It was great to finally be able to discuss in person the Relate software package for correlative analysis based on Mountains®: [bit.ly/3PaDBgC](https://bit.ly/3PaDBgC)



Have you been to our YouTube channel lately?



Check out our channel for tutorials on Mountains® software basic and advanced features for profilometry, electron microscopy, scanning probe microscopy and spectroscopy!

[bit.ly/2U2I2za](https://bit.ly/2U2I2za)



### SEEN ON FACEBOOK

Our team was at the Met & Props conference in Glasgow recently where they got to meet up with the surface metrology community, attend amazing presentations and give one lucky winner this delicious Scottish hamper. Did you get the answer to our question? [bit.ly/3OPjx3l](https://bit.ly/3OPjx3l)



### Surface Newsletter

Know a friend or colleague who would be interested in receiving the *Surface Newsletter*?

Let us know:

[contact@digitalsurf.com](mailto:contact@digitalsurf.com)

The newsletter is available for download on our website

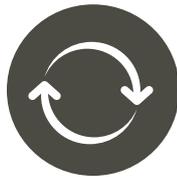
[www.digitalsurf.com](http://www.digitalsurf.com)

# Useful LINKS



## TRY MOUNTAINS® 9

Take Mountains® for a test drive  
Visit [www.digitalsurf.com/free-trial](http://www.digitalsurf.com/free-trial)



## CONTACT US FOR AN UPDATE

Contact [sales@digitalsurf.com](mailto:sales@digitalsurf.com) for information about updating Mountains® 8 or earlier versions to the latest Mountains® version



## WATCH A MOUNTAINS® TUTORIAL

Get the most out of Mountains® software by watching one of our video tutorials [www.digitalsurf.com/tutorials](http://www.digitalsurf.com/tutorials)



## LEARN SURFACE METROLOGY

Dive into our free online surface metrology guide and learn about characterizing surface texture in 2D and 3D  
[www.digitalsurf.com/guide](http://www.digitalsurf.com/guide)



## CATCH UP WITH US

### Microscopy & Microanalysis (M&M) meeting

August 1-4, 2022 | Portland, Oregon, USA

### DPG-Tagung

September 6-8, 2022 | Regensburg, Germany

JASIS | September 7-9, 2022 | Tokyo, Japan

SciX | October 4-5, 2022 | Cincinnati, Kentucky, USA



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### Surface Newsletter, July 2022

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