

# DULIA-bio 2019

2<sup>nd</sup> International workshop on  
Deep Underground Laboratory Integrating Activity in BIOlogy

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Laboratori Nazionali del Gran Sasso - Assergi (L'Aquila), Italy

## Exploring microbial biosignature in Mn-patinas of deep biosphere: a cross-disciplinary approach to investigate geomicrobiological interactions in Grotta Grande del Cervo (Abruzzo, Italy)

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# Deep underground

Subterranean environments are populated by a high diversity of microbes that use **unconventional energy sources** to perform their metabolic reactions.

**Microbial activity** reflects in alteration of rocks, formation of new structures, surface deterioration and cave expansion.

Subsurface fabrics, fossil bacteria preserved in minerals, mineralized nano-sized structures and biologically textures have been proposed as models for extraterrestrial **biosignatures**.



# Microbe-mineral interactions

Subsurface environments contain many **redox interfaces** and stable physical-chemical conditions, which enhance secondary mineral precipitation and microbial growth near starvation conditions.

Enigmatic microorganisms and unusual mineral features have been found associated with **secondary mineral deposits** or speleothems.

**Geomicrobiology** consists in studying past and present interactions between microorganisms and minerals and looking for traces of past microbe-mineral interactions recorded in ancient rocks.



# Biosignatures

One unique aspect of studying microbial processes in caves is the remarkable state of preservation of their biosignatures, traces that life leaves behind. These environments also delay the changes imposed by burial diagenesis.

In our geomicrobiological study we have discovered different structures and mineral types that appear to be biological in origin or the indirect result of biological activity.

A great research effort is needed in order to ascertain either the affiliation of these sheath-forming microorganisms and their metabolic capabilities, most likely related to the oxidation of mineral compounds, for tracing biological processes on early Earth.



# Study approach



Geo-chemical

Morfological

Molecular

Our primary goal was a detailed geo-chemical, mineralogical, geomicrobiological, morphological and microbiological characterization of secondary mineral deposits to understand their nature and origin. This involved an integrative, multidisciplinary and multi-scaled approach.

Aim of this work is to contribute to discover detectable macroscopic, microscopic, and chemical characteristics that are unequivocally biogenic traces.

Another target is to investigate which parameters control the interactions with minerals and their intensity so as to include those data in predictive models.

# Mn-patinas

Manganese is the **second-most abundant transition metal** in the Earth. Direct and indirect evidence for the biological formation of Mn coatings, crusts, and nodules can be found in diverse terrestrial and marine environments on the Earth's surface and in the subsurface, such as deserts, rivers and streams, springs, soils, ore deposits and **caves**.

Manganese oxides found in caves are **morphologically different** from the oxides in rock varnish. The manganese oxides from rock varnish are undulating and sometimes discontinuous and laminated with clay and quartz, most likely dust particles. The cave coatings, on the other hand, are thick and fluffy. Are cave oxides **protected** from the elements and have probably been growing undisturbed for perhaps hundreds of thousands—or millions?—of years.

Caves with active biological Mn oxidation show poorly crystalline formed from sheets of Mn oxide octahedra layers (**birnessite**) or 3 × 3 tunnels of octahedra (**todorokite**).



# Cave sampling

We examined the **geomicrobiology** of ferromanganese deposits in an epigenetic cave system, named **Grotta Grande del Cervo** and located in the cave-rich but poorly studied Central Apennine karst region.

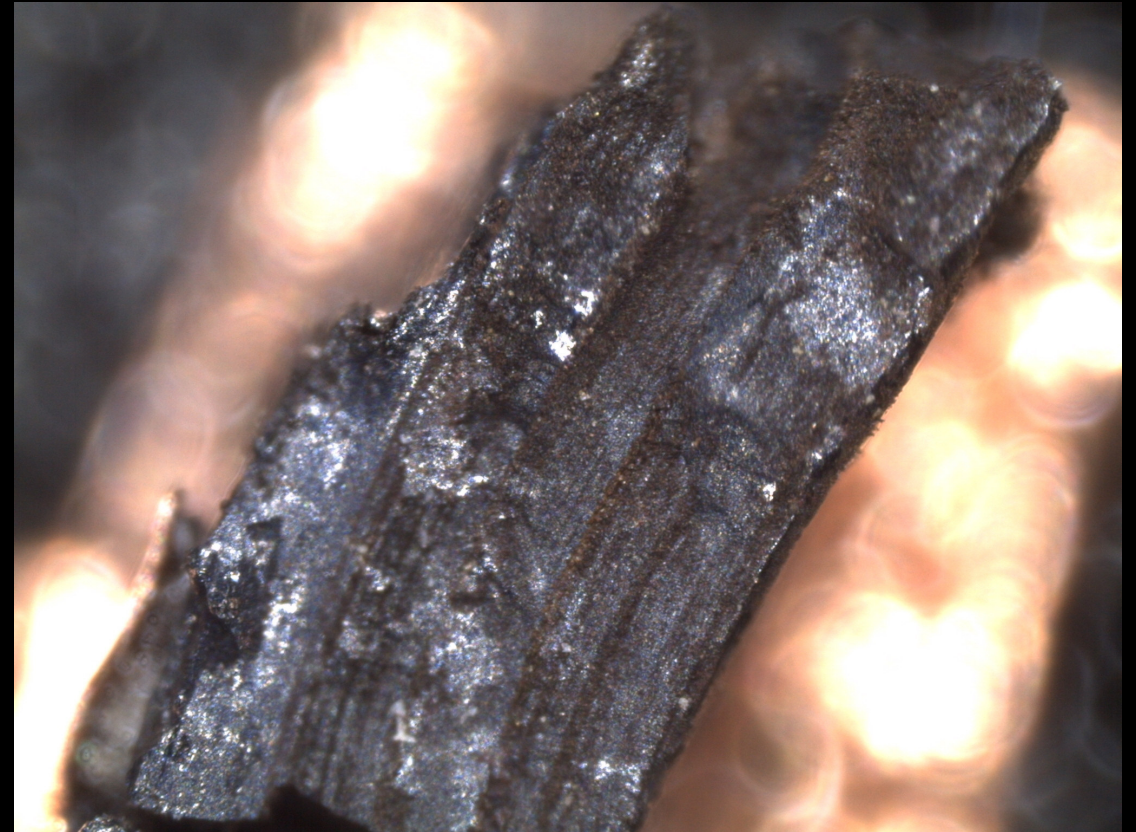
Black deposits are located in the currently unsaturated zone of the meander. These secondary deposits are uniformly red-brown and cover extensive areas of the **walls** and **floor**, overlying a zone of friable, altered bedrock, termed “**punk rock**”.



# Geo-chemical study

Crossing data through  
different techniques:

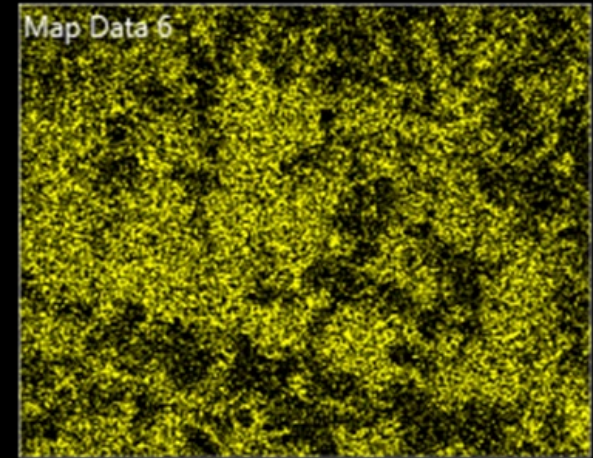
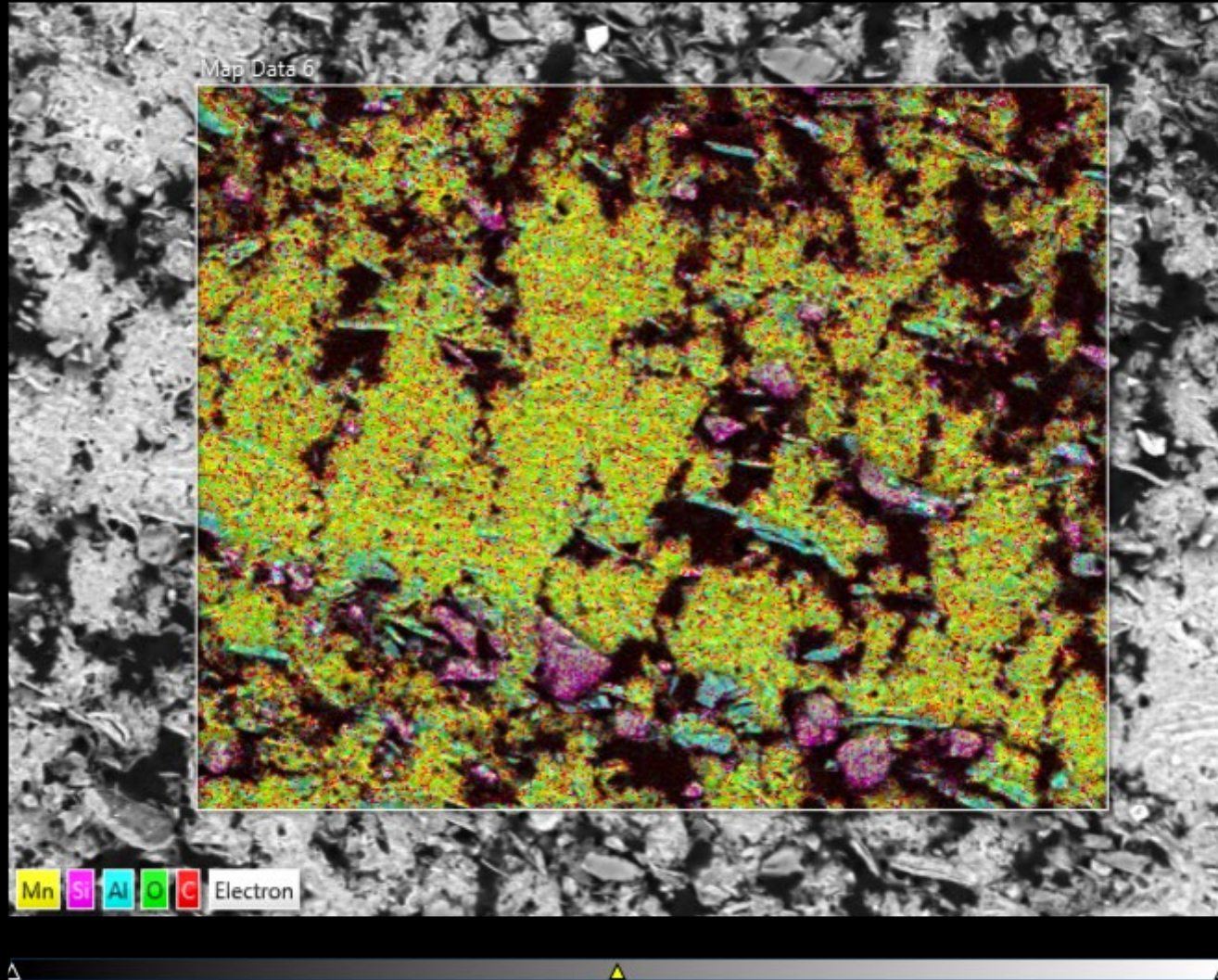
- SEM-EDS,
- XRPD,
- FT-IR
- Raman spectroscopy
- $\mu$ -XRF



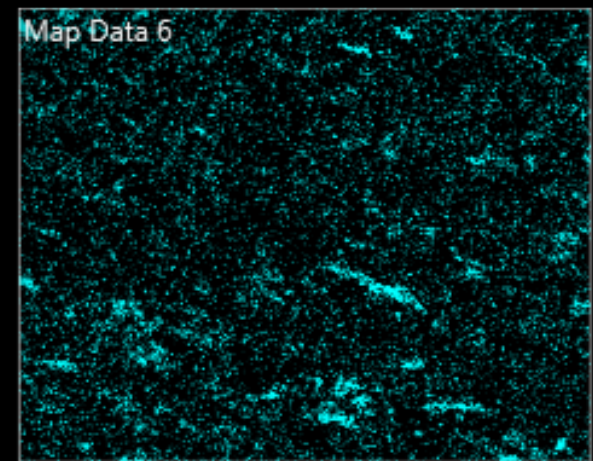
A proper mineralogical characterization of the samples was achieved. They consist of a very fine mixtures of **todorokite**  $[(\text{Ca}, \text{Na}, \text{K})(\text{Mn}^{4+}, \text{Mn}^{3+})_6\text{O}_{12} \cdot n\text{H}_2\text{O}]$ , a Mn oxide that can be related to **biological processes**, quartz, phyllosilicate-like minerals and calcite.



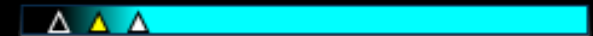
# Micro-analyses



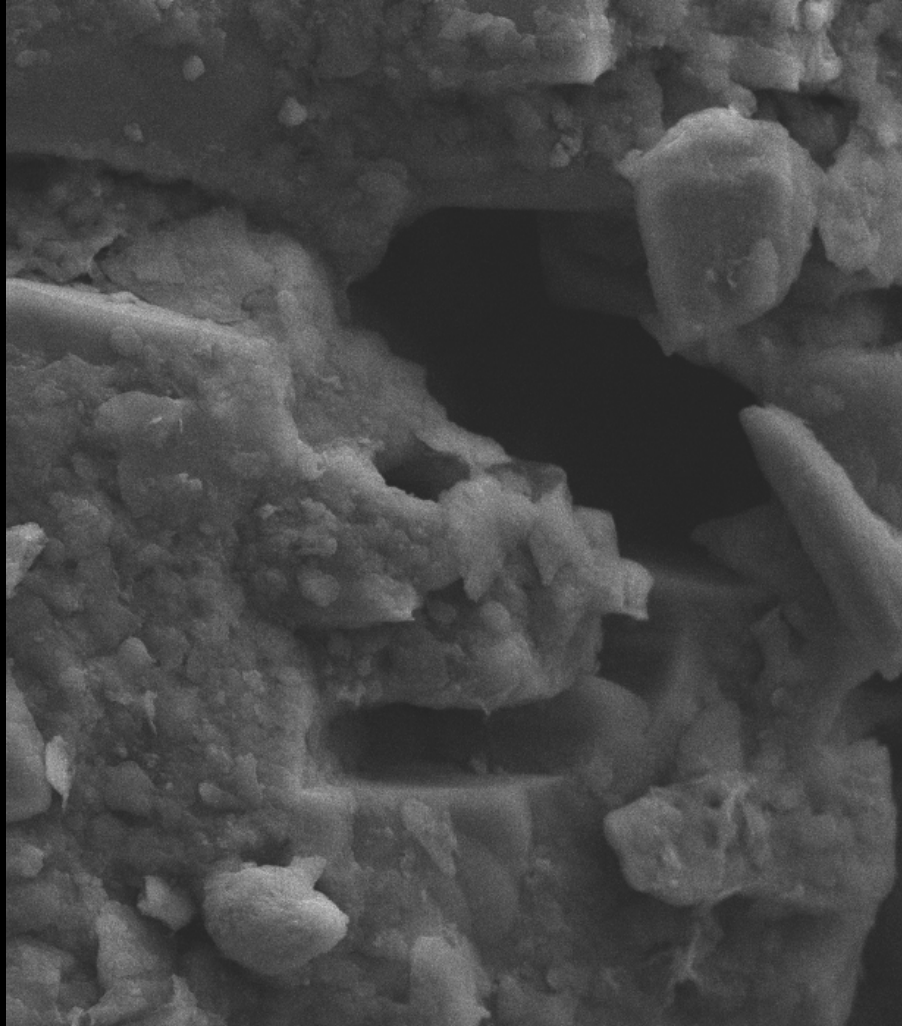
Mn



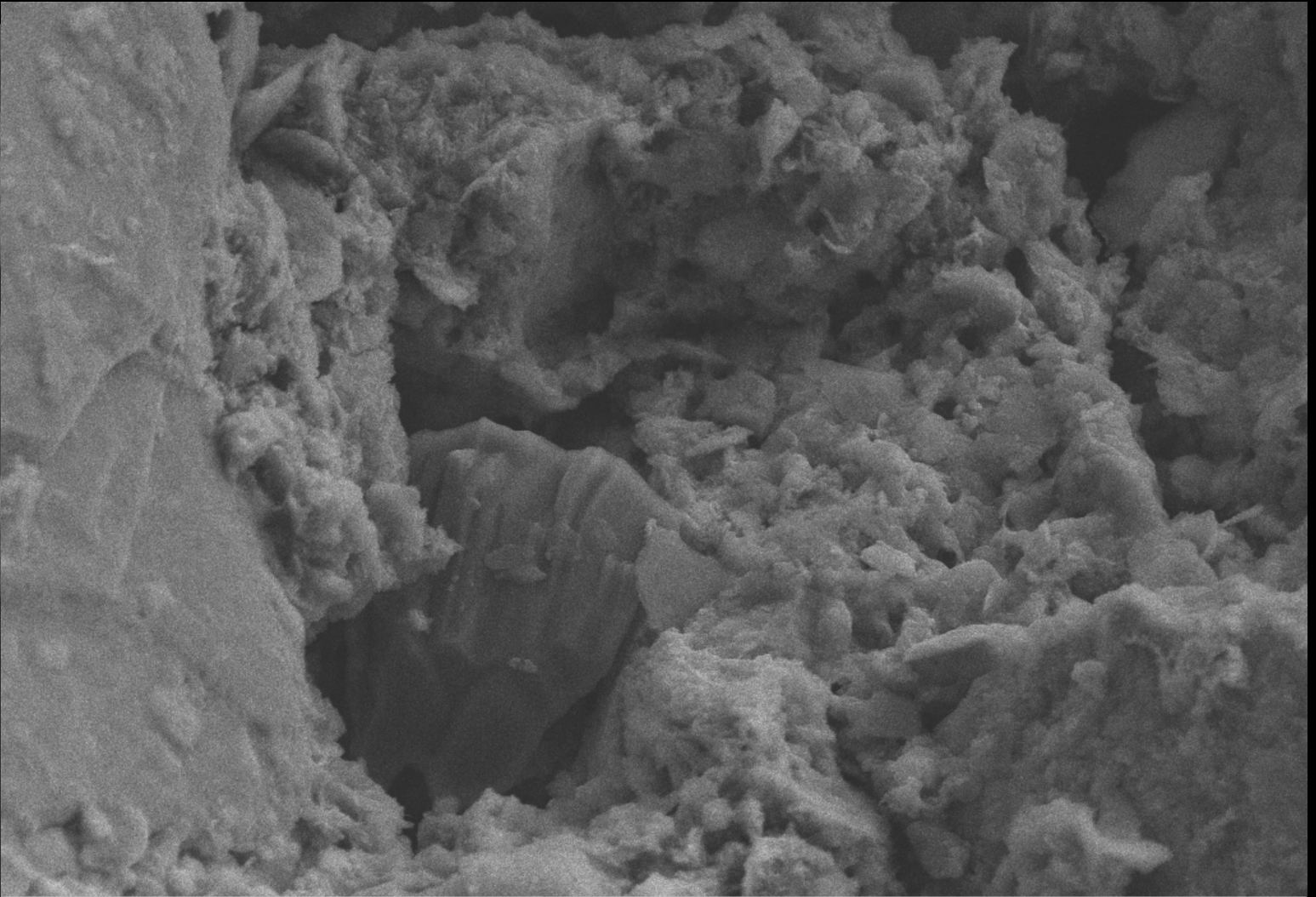
Al



# Scanning Electron Microscopy(SEM)

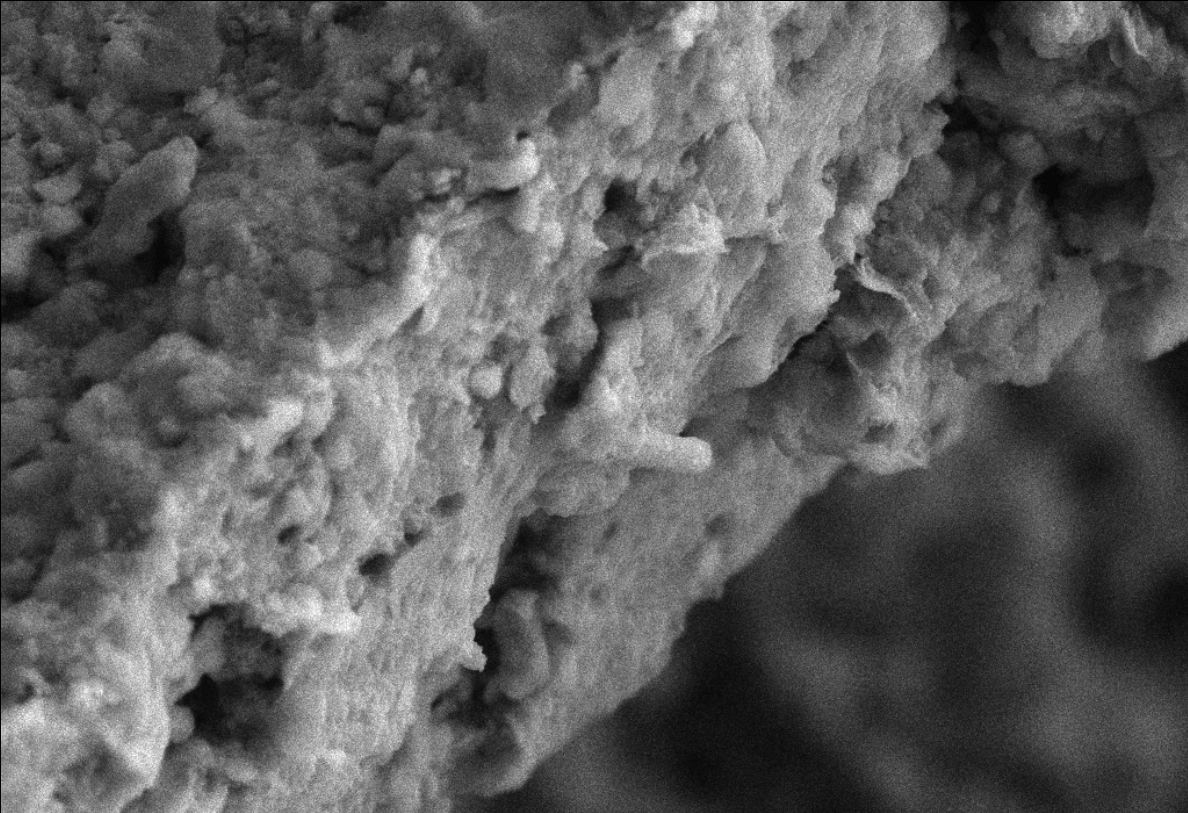


**ZEISS**  $1\ \mu\text{m}$  WD = 6.7 mm EHT = 7.00 kV  
Mag = 15.00 K X Noise Reduction = Frame Avg ESB Grid is = 600 V

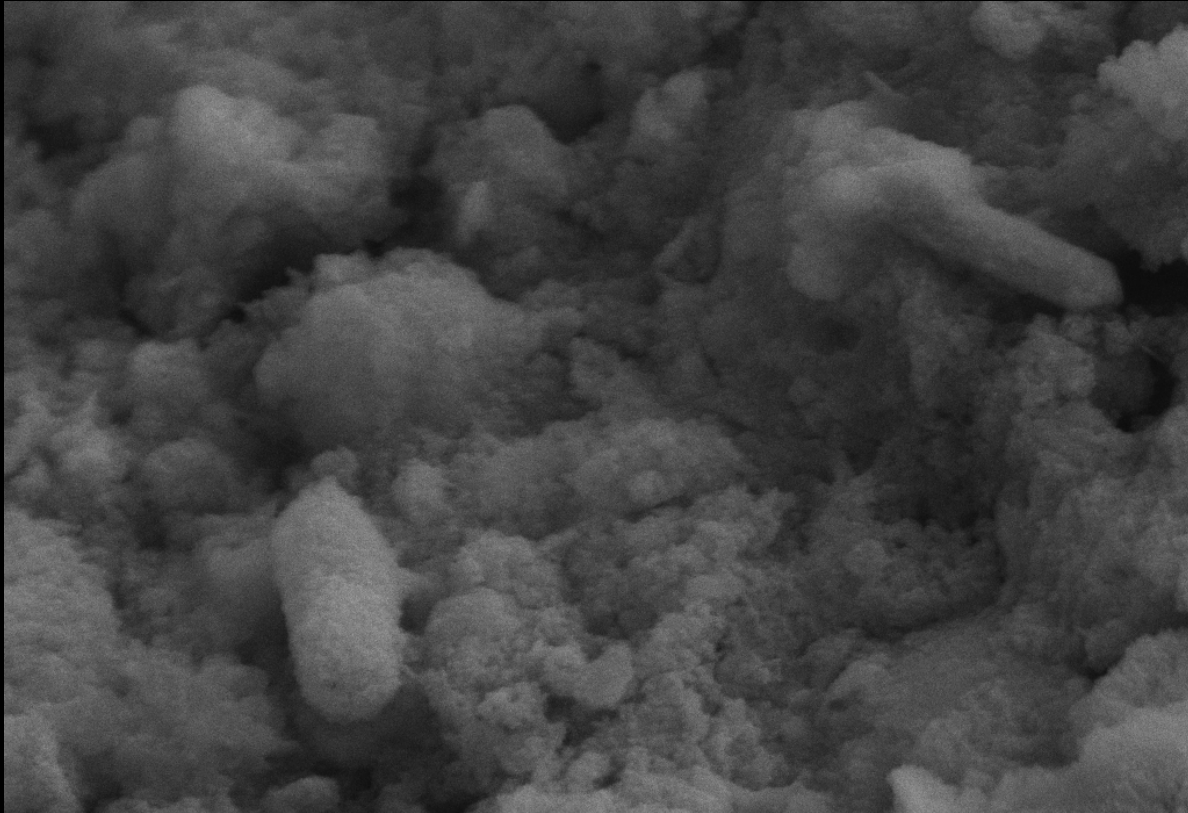


**ZEISS**  $1\ \mu\text{m}$  WD = 7.1 mm EHT = 7.00 kV Signal A = SE2  
Mag = 10.00 K X Noise Reduction = Frame Avg ESB Grid is = 600 V Date :17 Oct 2019 Time :15:19:18  
System Vacuum = 1.94e-06 mbar

# Scanning Electron Microscopy (SEM)

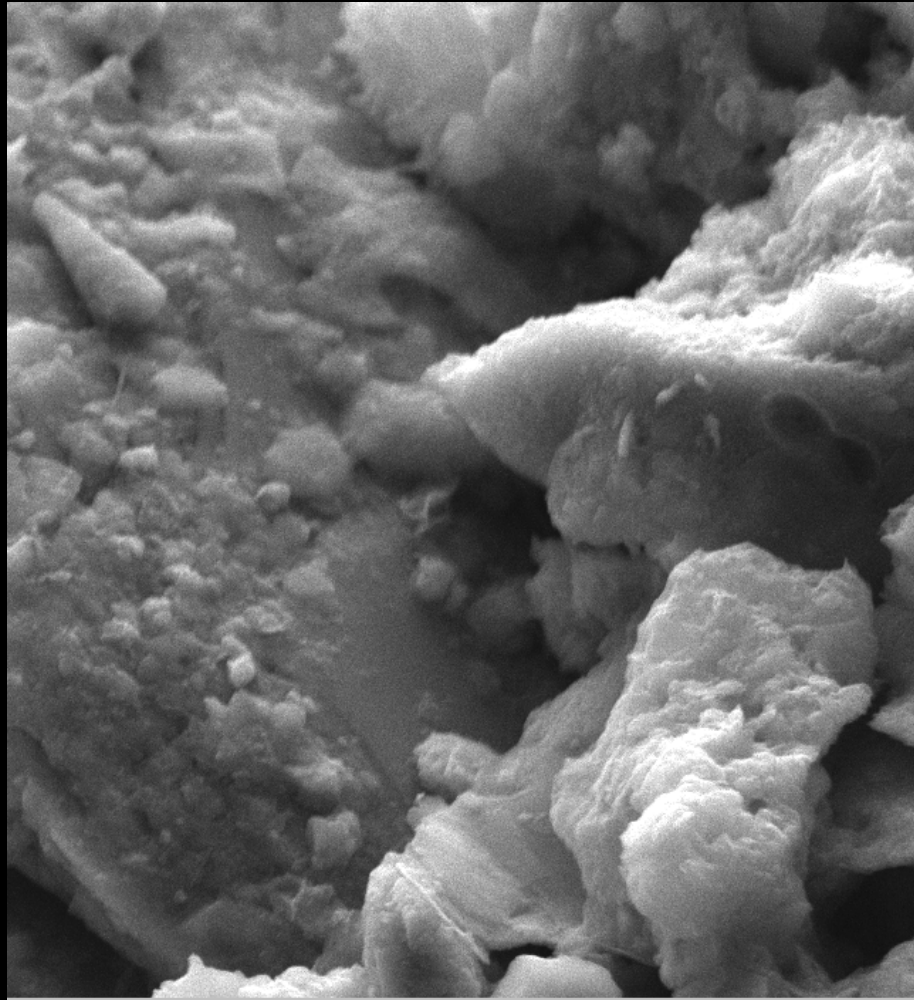


**ZEISS** *Mag = 15.00 K X* *1  $\mu$ m* *WD = 6.7 mm* *EHT = 7.00 kV* *Signal A = SE2* *Date :17 Oct 2019 Time :12:19:21*  
*Noise Reduction = Frame Avg* *ESB Grid is = 600 V* *System Vacuum = 2.34e-06 mbar*

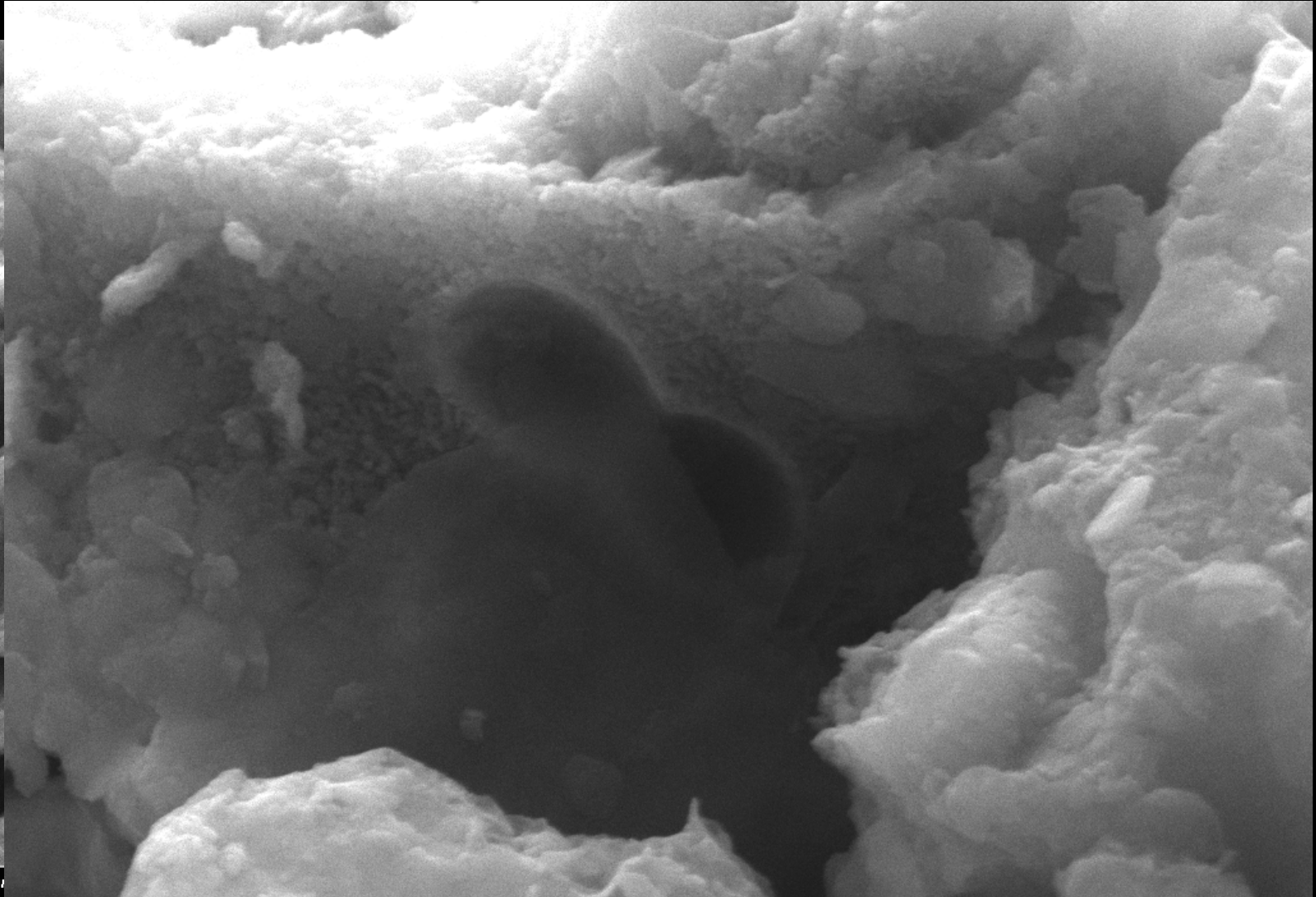


**ZEISS** *Mag = 45.00 K X* *200 nm* *WD = 6.7 mm* *EHT = 7.00 kV* *Signal A = SE2* *Date :17 Oct 2019 Time :12:09:41*  
*Noise Reduction = Frame Avg* *ESB Grid is = 600 V* *System Vacuum = 2.48e-06 mbar*

# Scanning Electron Microscopy(SEM)

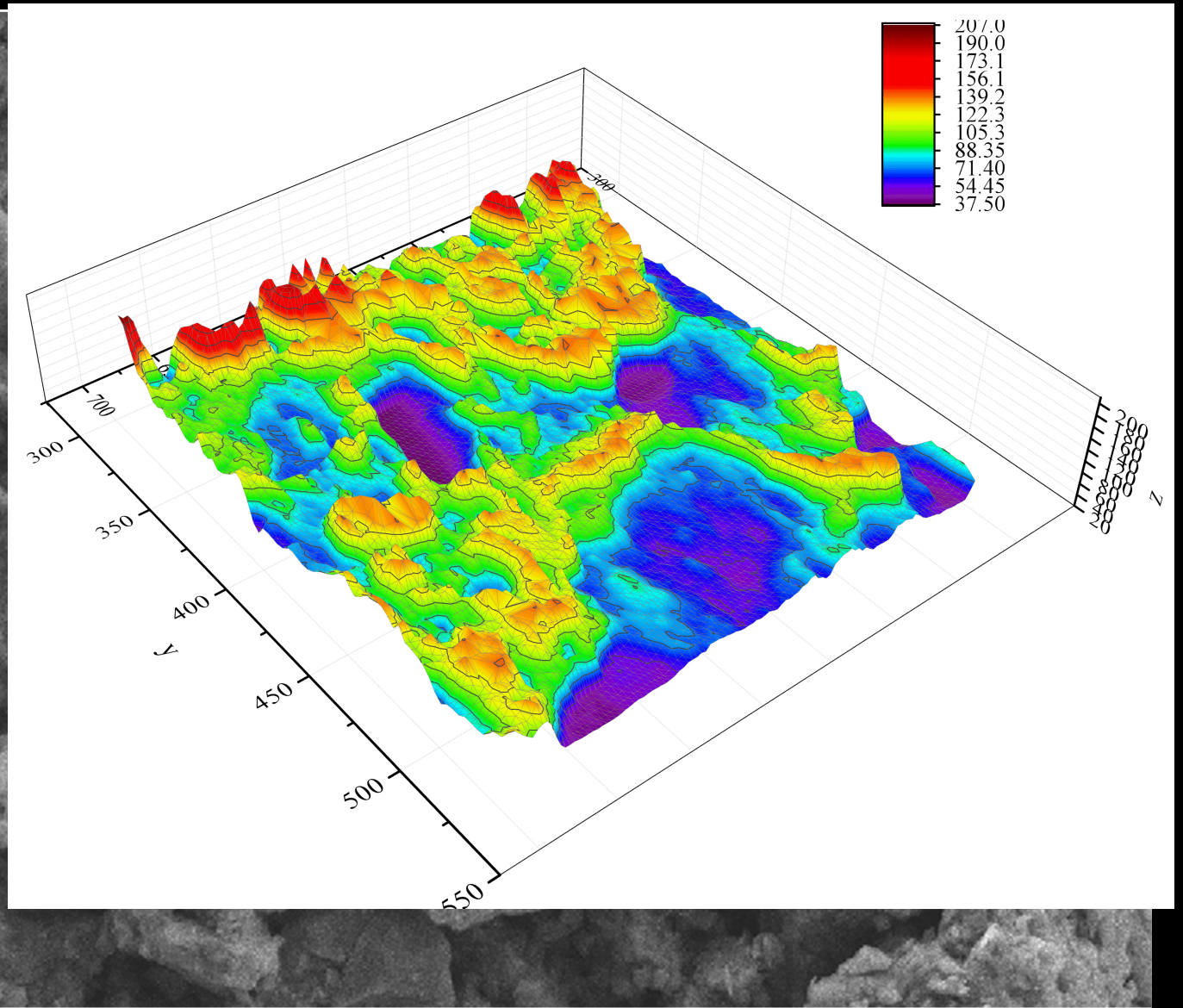
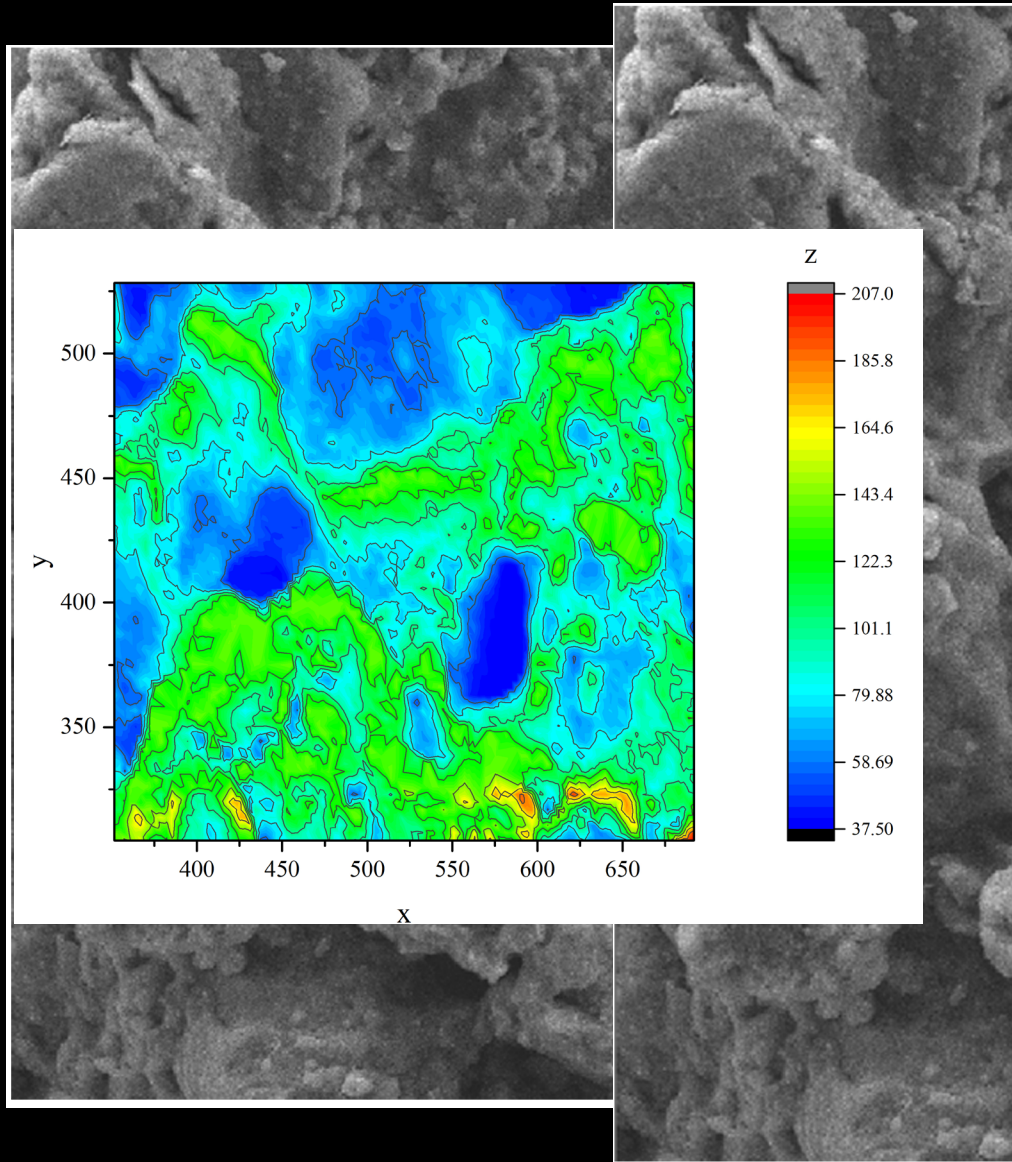


**ZEISS** Mag = 20.00 K X 1 μm WD = 7.5 mm EHT = 7.00 kV Signal A = SE2  
Noise Reduction = Frame Avg ESB Grid is = 600 V



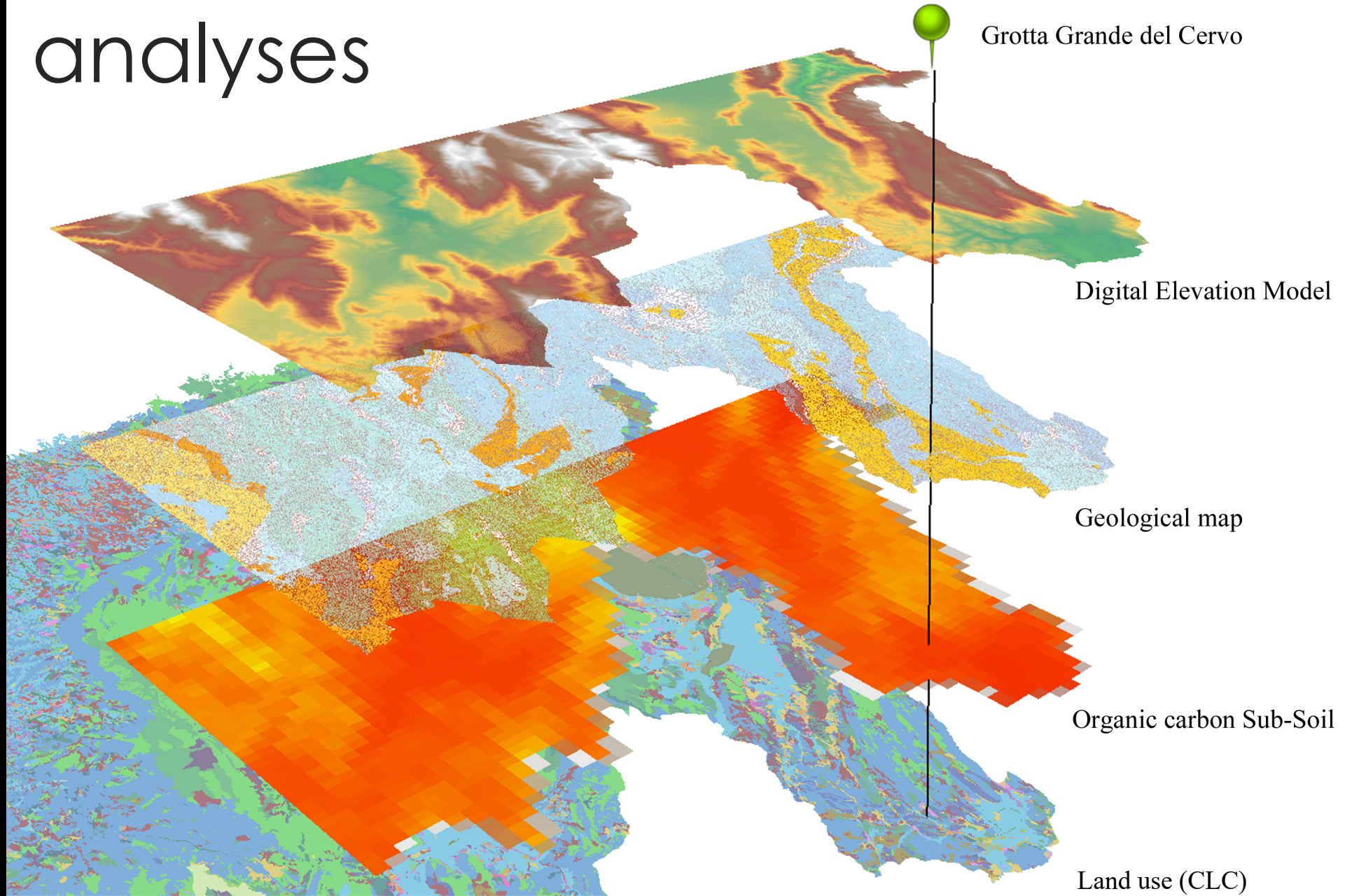
**ZEISS** Mag = 70.00 K X 200 nm WD = 7.5 mm EHT = 7.00 kV Signal A = SE2  
Noise Reduction = Frame Avg ESB Grid is = 600 V Date :17 Oct 2019 Time :11:24:01  
System Vacuum = 4.01e-06 mbar

# 3D SEM images



# GIS

# analyses



# Future perspectives

Sequencing analyses of specimens are undergoing.

Our data support the hypothesis that microorganisms may contribute to the formation of manganese mineralizations in cave environment, providing new encouraging insights in the understanding of the Mn cycle and over the processes of energy acquisition in unfavourable conditions.



# Future perspectives

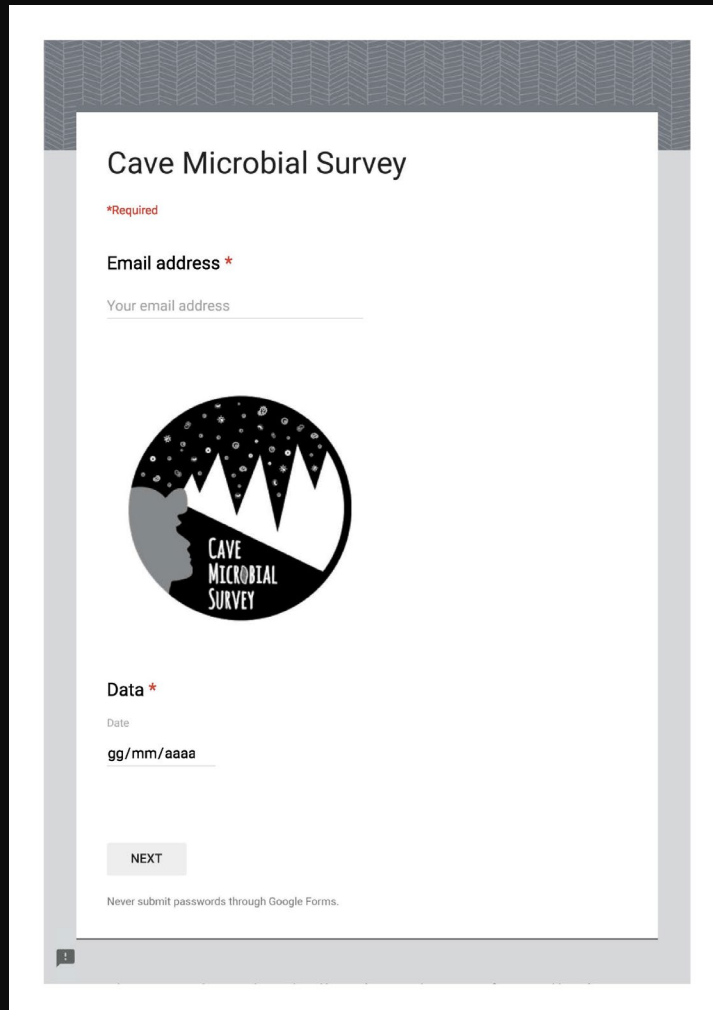
The key to establish the microbial origin for rock black deposits is to obtain more data from the ferromanganese laminae. Within Italy, as well as worldwide, new caves continue to be discovered, which may provide potentially unique geochemical environments to be examined.

Literature on biogenically mediated mineral structures from caves is limited. In this regard, a better understanding of the mechanisms involved may provide bases for defining a new type of fossil structure of biogenic origin that can be recognized in geologic records.





# Cave Microbial Survey




Cave Microbial Survey

\*Required

Email address \*

Your email address



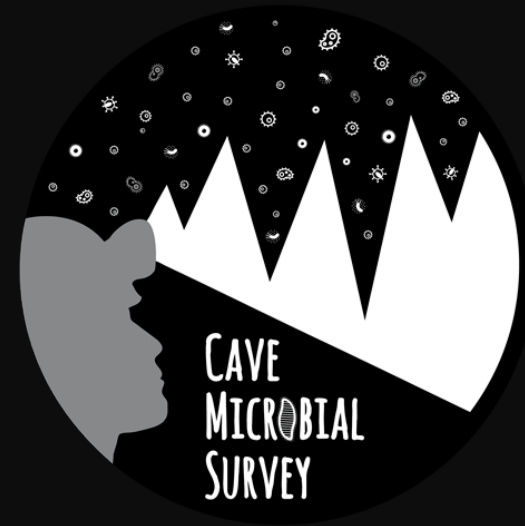
Data \*

Date

gg/mm/aaaa

Never submit passwords through Google Forms.

By establishing **national and international collaborations with different world-leading research groups**, it could be possible to perform an inter- and cross-disciplinary approach, essential to obtain significant results.

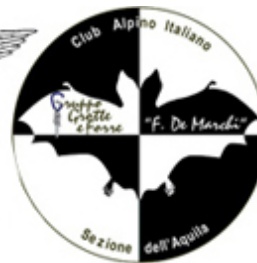


<https://vaccarelliilaria.wixsite.com/cavemicrobialsurvey/donate>

# Acknowledgments



PERSEPHONE  
esplorazioni



**GRUPPO GROTT E FORRE FRANCESCO DE MARCHI**  
CLUB ALPINO ITALIANO - SEZIONE DELL'AQUILA