RAMAN SPECTROSCOPY FOR DETECTION OF BIOLOGICAL MATTER IN MARS ANALOGUE MATERIAL

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ExoMars - Mission

ExoMars 2018 Scientific objectives:

“Searching for evidence of past and present life on Mars “

- Search for signs of former or recent life on Mars;
- Investigation of the planetary subsurface for a better understanding of the evolution and habitability of Mars
- Investigation of the surface environment and identification of risiks for future manned missions;

- RLS (Raman Laser Spectrometer – one of the Pasteur Payload Instuments onboard ExoMars 2018)

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Scientific Objectives of Raman Measurements on RLS:

- identify **organic compound and search for life**;
- identify the **mineral products and indicators of former biologic activities**;
- characterize **mineral phases** produced by **water related** processes;
- characterize **igneous minerals** and **their alteration products**;
- characterize **water/geochemical environment as a function of depth** in the shallow subsurface

Raman spectroscopy

Advantages:

- Detailled information about the structure of a system (molecule, crystal,..) complementary to LIBS and IR;
- Non-destructive method;
- Minerals as well as biological samples can be analysed;
- High spatial resolution -> very small samples are allowed;
- More or less no sample preparation;
- Samples can be a mixture of different kind of materials;
- With size of a few µm and less;

Problems:

- Fluorescence (-> other excitation wavelength);
- Interpretation of the measured spectra (for crystal structures);
- Lack of Raman databases

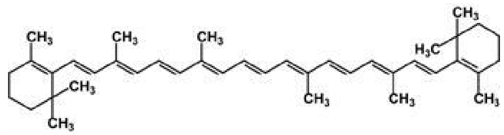
Objectives of the present investigation:

- To distinguish between the biological material and the mineralogical background;
- Cyanobacteria and methanogenes are chosen as candidates for potential life on Mars and Raman spectra of cyanobacteria and methanogenes are measured on Mars analogue material.
- The Mars simulant material can be assigned to phyllosilicatic and sulfatic Mars regolith.
- Appropriate measurement parameters for the determination of the mineral composition as well as the detection of biological material are derived.
- A measurement regime is proposed for mineral mixtures with cyanobacteria on the basis of the RLS instrument characteristics.

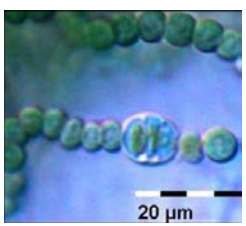
Sample Choice

Cyanobacteria

Carotene



- accessoric pigment in the photosynthesis apparatus;
- important in transfer of electrons to central chlorophyll molecules;
- has been developed very early in evolution of micro organisms probably also because of UV- shielding functions;



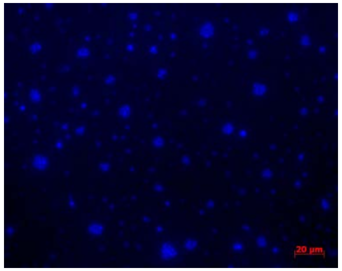
Light microscopic image of *Nostoc commune.*

Accummulation of cells of *Nostoc commune* in a biofilm.

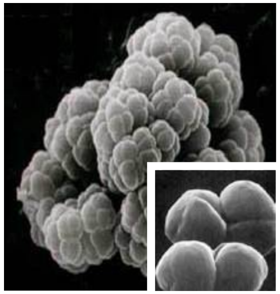
Methanosarcina, strain SMA 21

<<candidatus M. gelisolum>>

- **Methane producing archaea**
- **Strain from Siberian Permafrost (Island Samoylov)**
- **Single and multi cellular structures**



DAPI staining, fluorescence microscopy 100x



http://microbewiki.kenyon.edu/index.php/Methanosarcina

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Sample Choice

Martian Analogue Minerals

- terrestrial igneous rocks, phyllosilicates, carbonates, sulfates and iron oxides provided by the Museum für Naturkunde Berlin;
- structurally and chemically similar to those identified in martian meteorites and on Mars by recent orbiter and rover missions;
- two different mineral and rock mixtures:

1) **Phyllosilicatic** Mars Regolith Simulant (P-MRS)

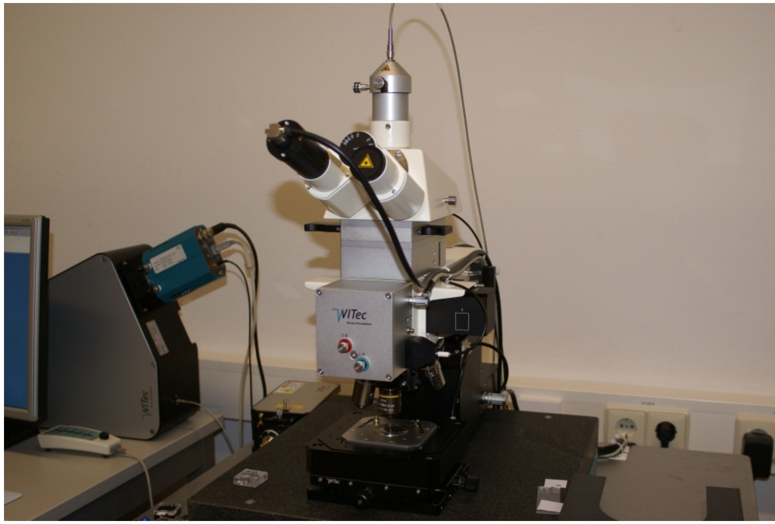
2) **Sulfatic** Mars Regolith Simulant (S-MRS)

Component	P-MRS [wt%]	S-MRS [wt%]
Pyroxene, Ilmenite, Plagioclase, Quartz (Gabbro)	3	32
Olivine	2	15
Quartz	10	3
Hematite	5	13
Montmorillonite	45	-
Chamosite	20	-
Kaolinite	5	-
Siderite	5	-
Hydromagnesite	5	-
Goethite	-	7
Gypsum	-	30

Böttger et al. Planetary and Space Science 60 (2012)

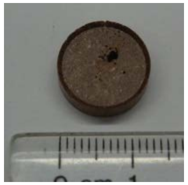
Experimental Setup and procedure:

- confocal Raman microscopeWitec alpha300 system;
- objective: 10xEPlan;
- excitation: 532 nm;
- power 1 mW on sample (expected for the RLS on ExoMars);
- spot size on the sample in focus <1.5 µm;
- Spectrometer UHTS 300:
    - 600 l/mm grating;
    - 4-5 cm-1 spectral resolution;
- room temperature under air at ambient pressure.



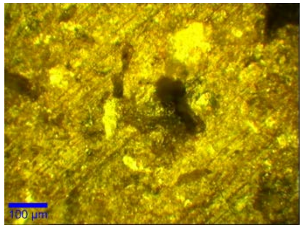
Sample Preparation: Cyanobacteria

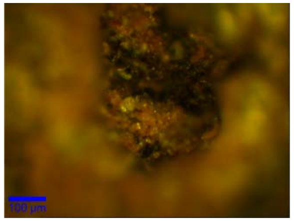
- Powders of grain size between 25 µm and 1000 µm;
- pressed with 4.5 MPa to pellets to get a smooth surface (less multiple scattering);
- colonies of a culture of *Nostoc commune* Strain 231-06 (Fraunhofer IBMT Potsdam) streaked on the Mars analogue minerals;
- single cell and cluster distribution close to natural biofilm conditions.



P-Mars

S-Mars





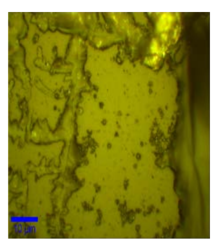
Images of samples of Mars analogue minerals with Cyanobacteria (upper row) and microscopic images (lower row)

For comparison – Raman measurements of **solid polished** samples of the mineral components of the Mars analogue mixtures.

Sample Preparation: Methanogenes

- **Spectra of Glass slide were checked by RAMAN**
- **Dry NaCl and $NaHCO_3$ (components of culture medium) on glass slide - Spectra of the salts were checked by RAMAN separately**
- **0.2 ml of liquid culture medium with methanogenes were dried out on glass slide and check by RAMAN**
- **Spectra of pure methanogene archaea cells has been extracted**
- **Pellet of S-MRS (Sulfatic Mars Regolith Substrate) and Raman spectra measurements**
- **0.2 ml of culture medium including methanogenes dropped and streaked on S-MRS and spectra check**

Salt/Methane producing archaea mixture on glass slide



S-MRS + methane producing archaea

Measurement procedure: Cyanobacteria

Raman spectra measurements of **cyanobacteria** on **mineral mixtures** assigned to **phyllosilicatic [P-MSR]** and **sulfatic [S-MSR] Mars** using excitation wavelength 532nm.

1. Measurements on solid, polished mineral samples
2. Measurements on mineral mixtures without and on pure cyanobacteria;
3. Biological materials were streaked over the surface of the pellets;
4. Measurements and scans on these pellets with varying integration time t and number of accumulations N.

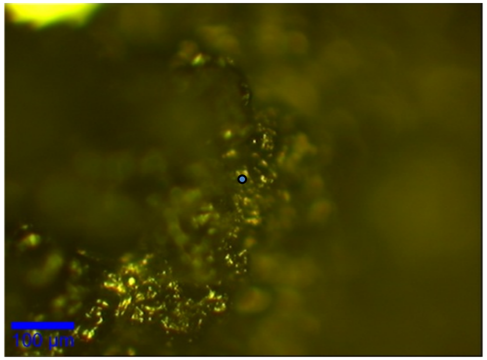
Böttger et al. Planetary and Space Science 60 (2012)

Pure Cyanobacteria – ß - carotene

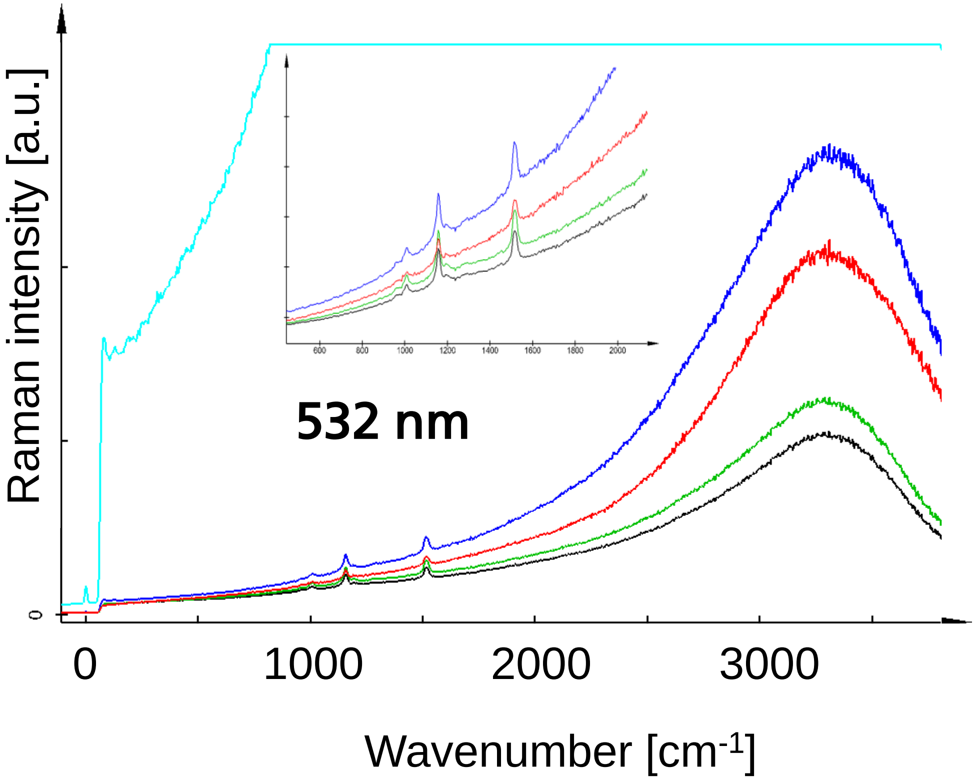
strong fluorescence of the cyanobacteria above 620 nm; resonance effect of β- carotene.

- Measurement time and iterations per spectrum:

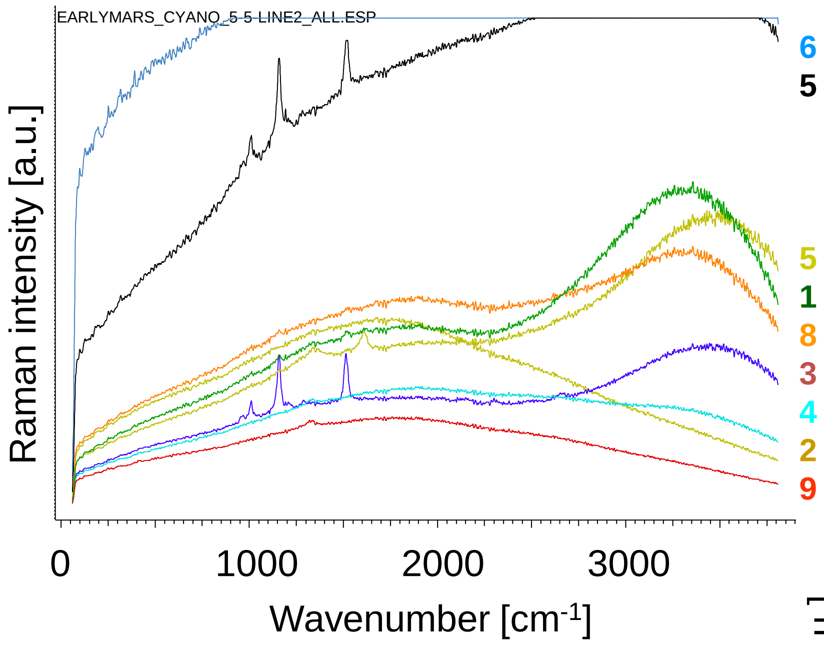
(1 s, 5 x), (1 s, 100 x), (1 s, 200 x), (5 s, 5 x), (100 s, 5 x)

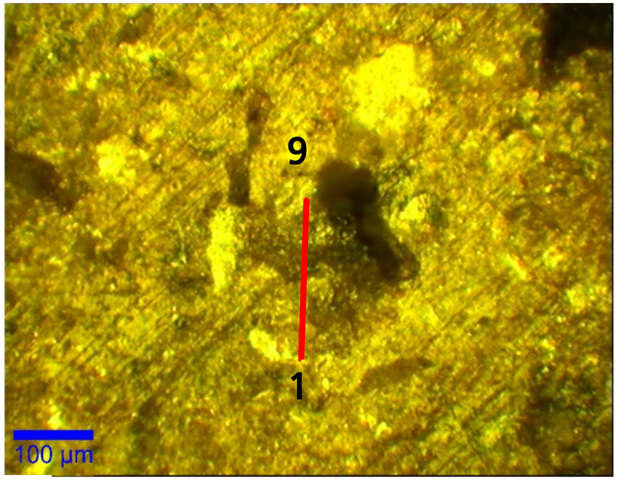


Microsopic picture (Witec)

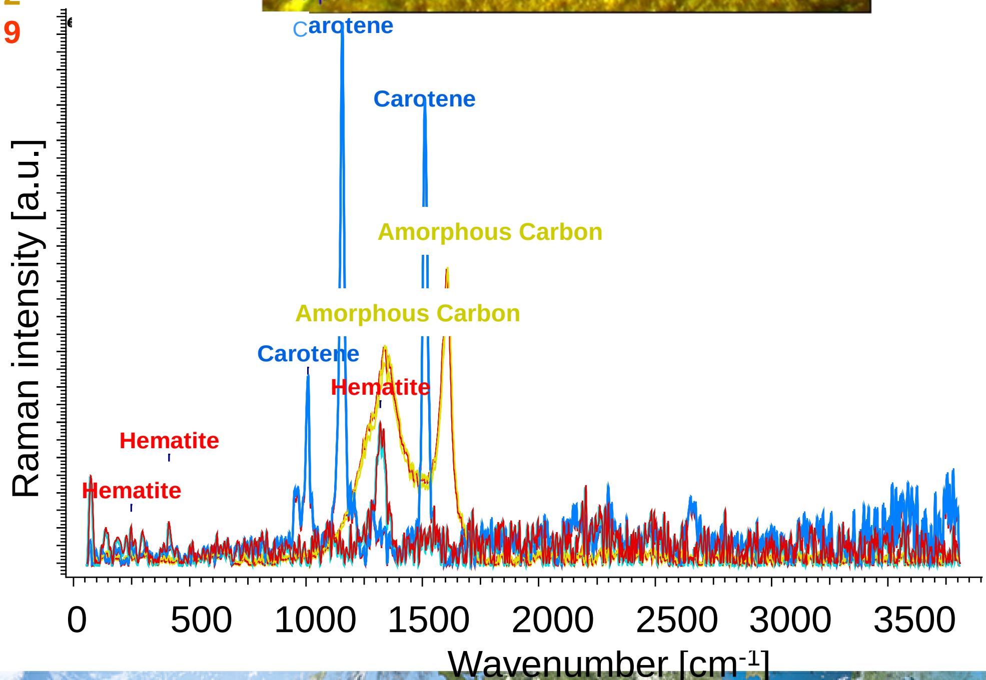


Raman spectra of P-MRS with cyanobacteria

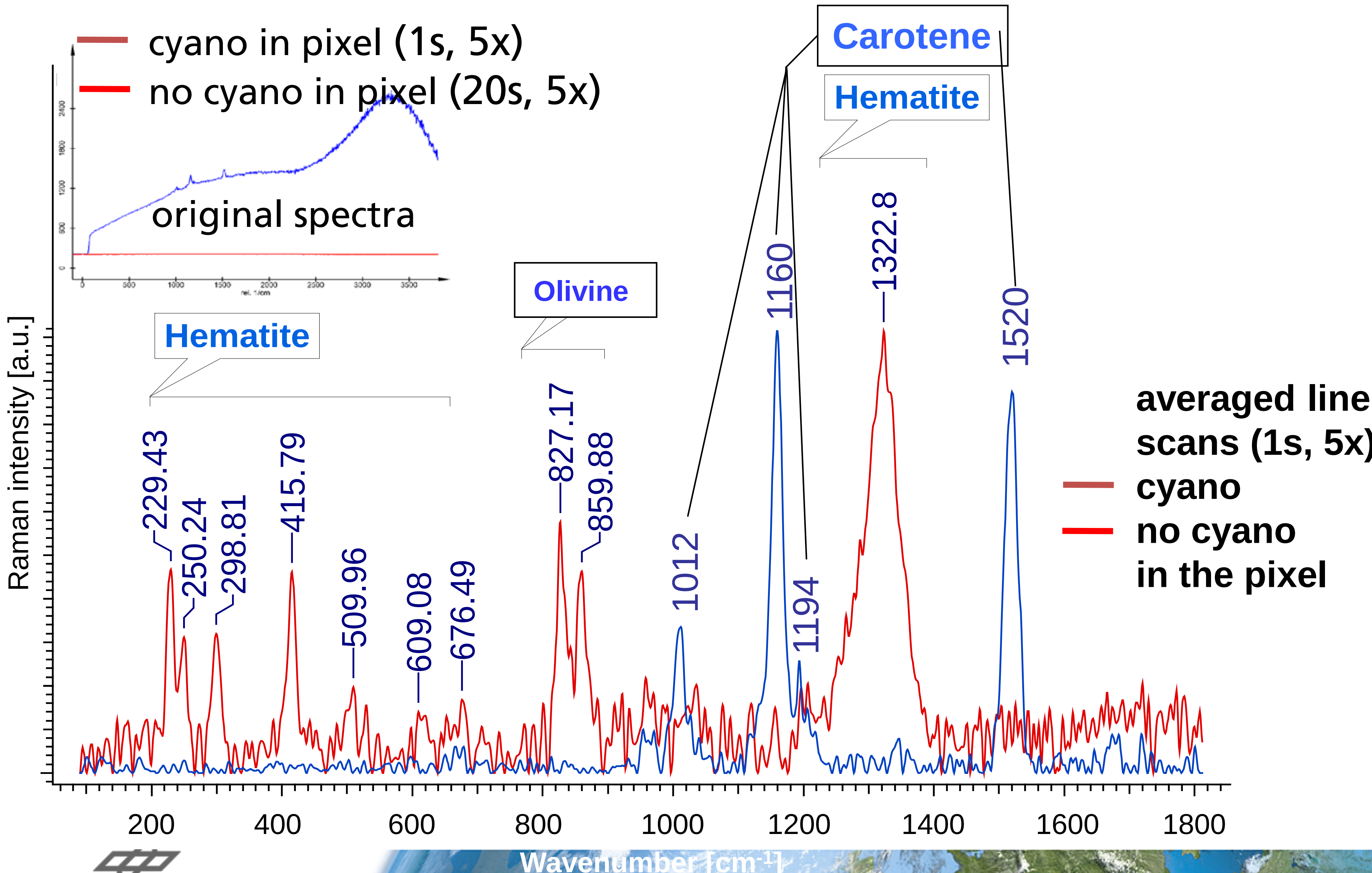




Typical Raman spectra of the line scan (20s, 5x).



Raman spectra of S-MRS with cyanobacteria



Measurement procedure: Methanogenes

Raman spectra measurements of **methanogenes** on **mineral mixtures** assigned to **sulfatic [S-MSR] Mars** using excitation wavelength 532nm.

1. Measurements on mineral mixtures as for cyanobacteria;
2. Measurements on culture medium and on glass slide;
3. Measurements of methanogenes on glass slide to get their pure spectrum;
4. Methanogenes were streaked over the surface of the pellet;
5. Measurements and scans on these pellets with varying integration time t and number of accumulations N.

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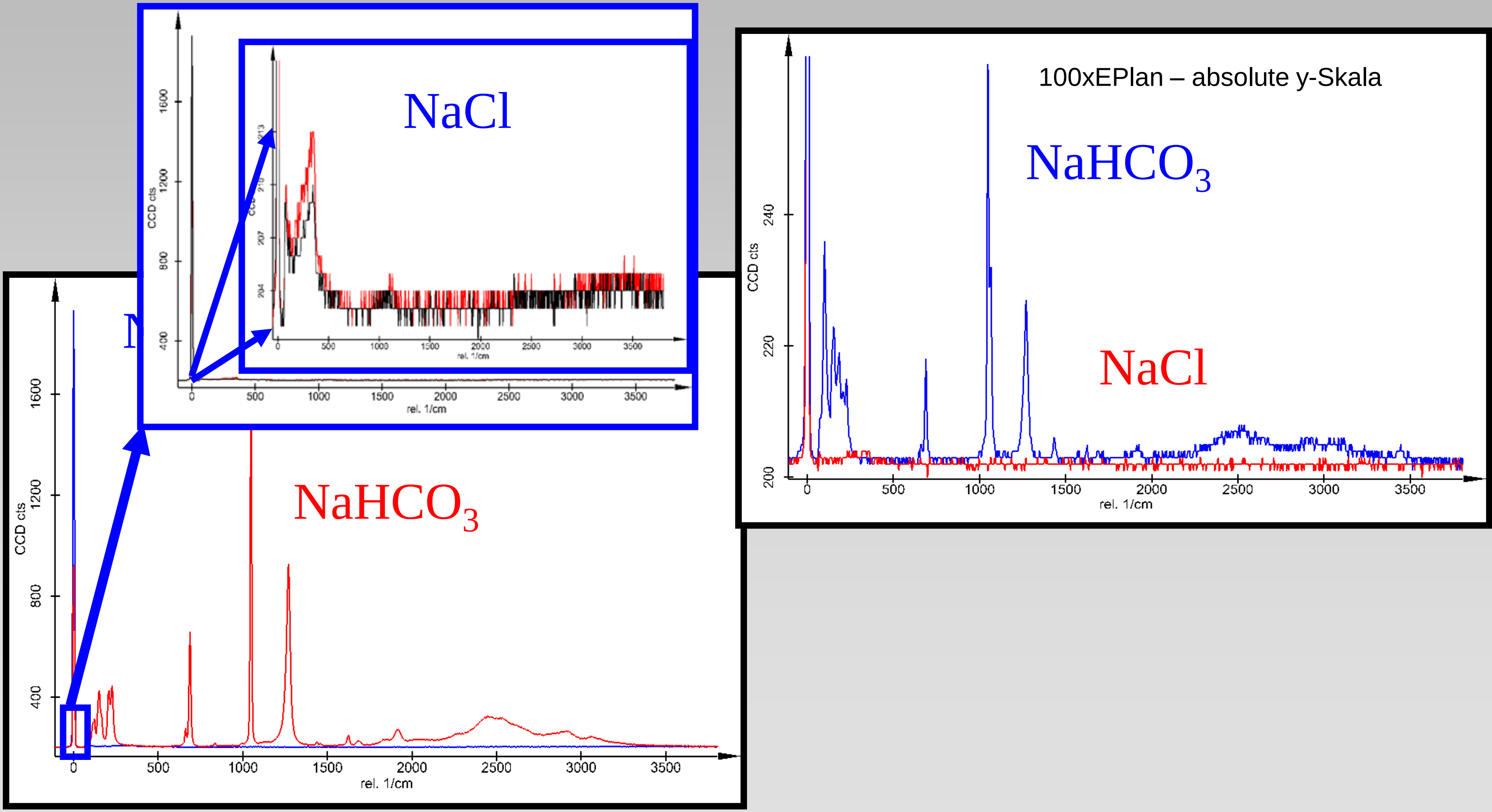
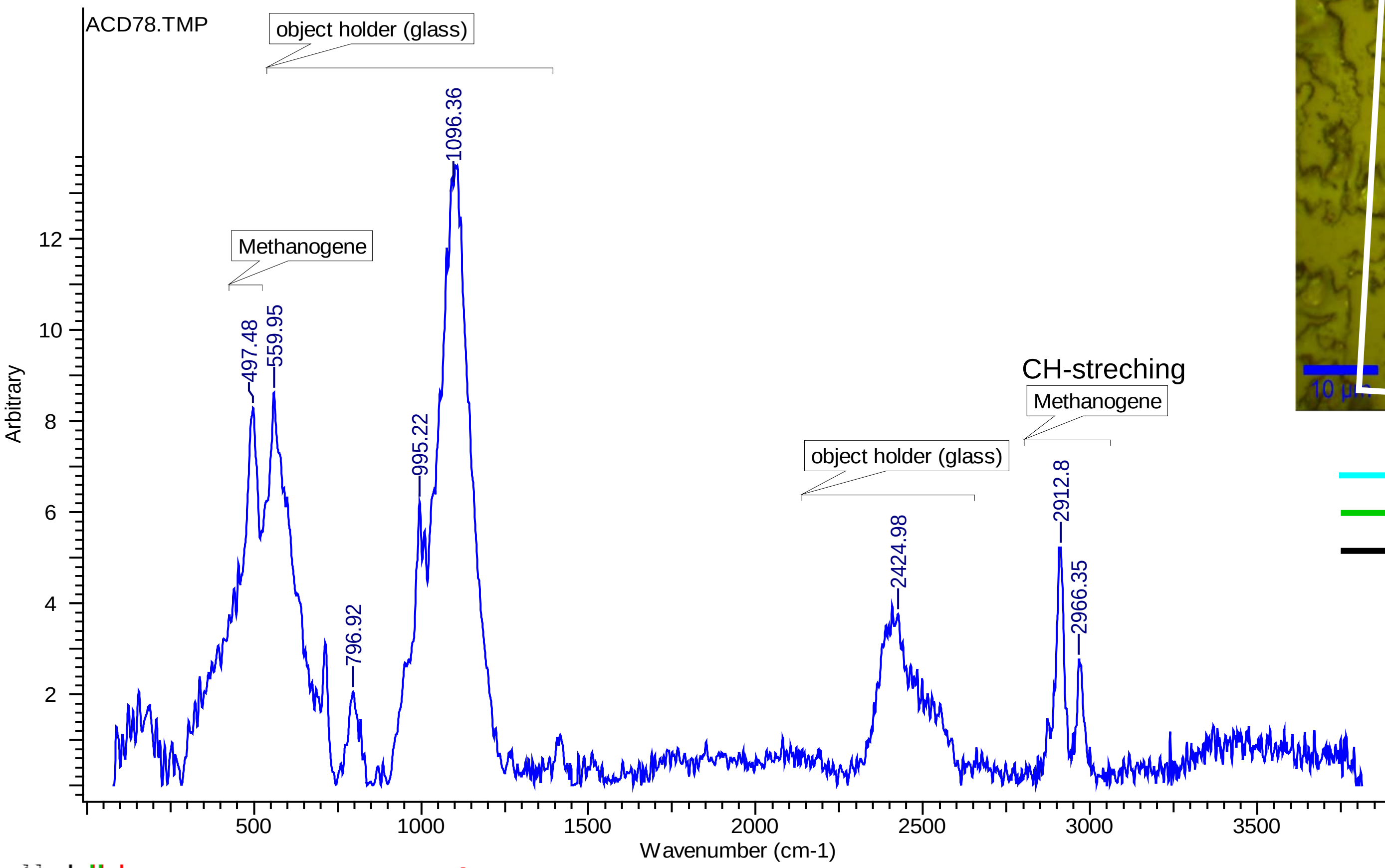
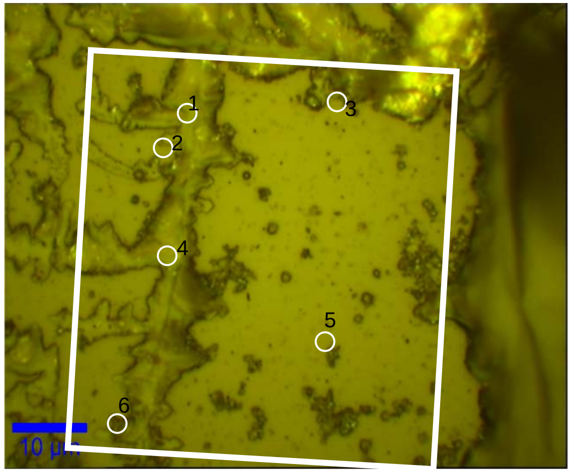
Comparison of spectra of NaCl and $NaHCO_3$



Raman spectra of methanogenes





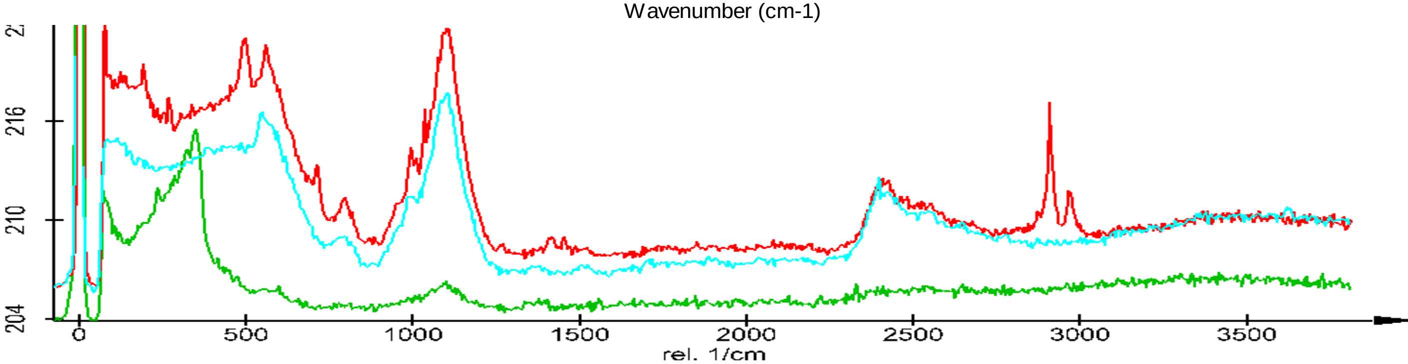
Glass slide (559, 1091, 2410 cm-1)

NaCl (341 cm-1)

$NaHCO_3$ (144, 210, 687, 1048, 1266 cm-1)

→Spectral lines specific for the methanogenic archaea:

→(478-494, 1637-1645, 2913-2917 cm-1).



Methanogene on S-Mars

including

NaCl	(341 cm-1)
$NaHCO_3$	(144, 210, 687, 1048, 1266 cm-1)
Methanogene	(254, 478-494, 1637-1645, 2913-2917 cm-1)

Methanogene SMA21 I

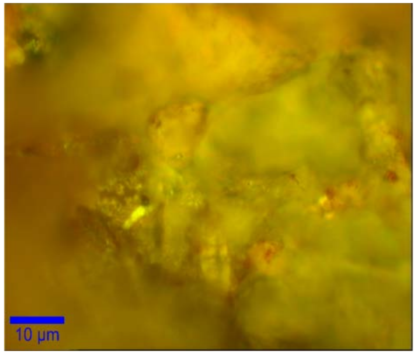
on S-Mars

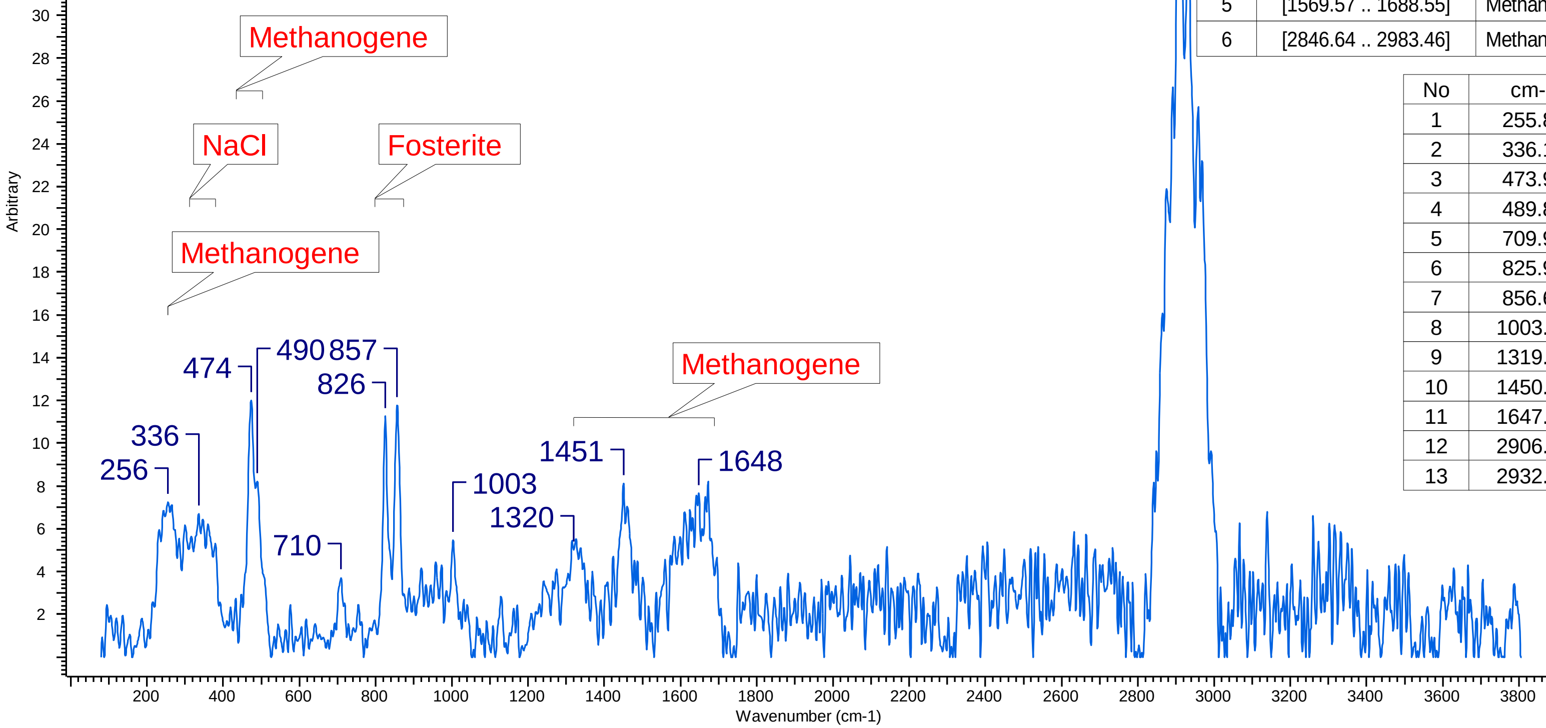
20101210

Time Series (CRR) (Average)

(10s, 50x)

1 mW Laserpower
100x EPlan





No	cm-1	Annotation
1	255.82	Methanogene
2	[312.33 .. 379.76]	NaCl
3	[435.28 .. 502.71]	Methanogene
4	[799.17 .. 874.52]	Fosterite
5	[1569.57 .. 1688.55]	Methanogene
6	[2846.64 .. 2983.46]	Methanogene

No	cm-1	Intensity
1	255.82	W
2	336.13	W
3	473.95	M
4	489.82	W
5	709.93	W
6	825.94	M
7	856.67	M
8	1003.42	W
9	1319.71	W
10	1450.59	W
11	1647.90	W
12	2906.13	VS
13	2932.90	VS

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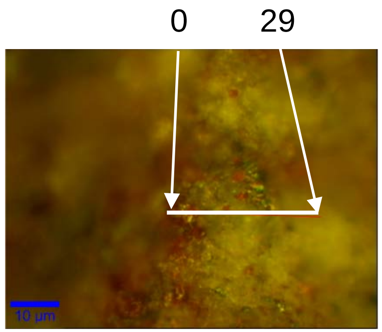
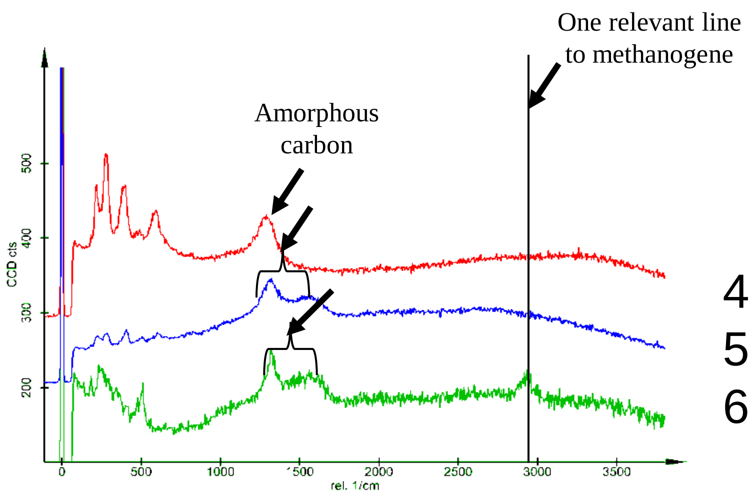
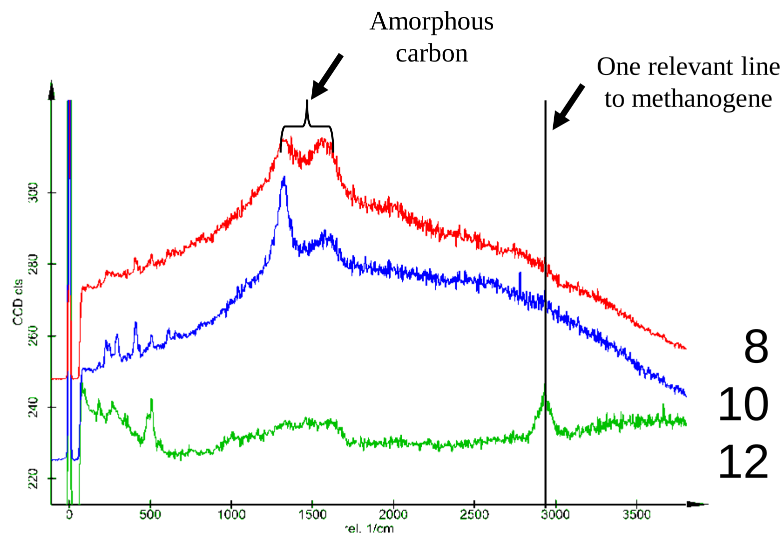
Methanogene on S-Mars: line scan



Methanogene SMA21 I
on S-Mars
20101210
LineScan (30Punkte)
(10s, 5x)
LWD 100x
1 mW Power of Laser

NaCl (341 cm^{-1})
$NaHCO_3$ (144, 210, 687, 1048, 1266 cm^{-1})
Methanogene (254, 478-494, 1637-1645, 2913-2917 cm^{-1})
Hematite + Goethite (228, 242, 297, 399, 541, 1318 cm^{-1})
amorphous carbon (1328, 1560 cm^{-1})





Summary

The Raman spectrum of the **cyanobacteria** is influenced by the Raman signal of the mineral background.

(1)β- carotene is the dominant feature in the spectrum - only short measurement time to avoid saturation of the spectrum of β- carotene;

(2)for P-MRS and S-MRS - longer integration time to identify the different mineral constituents of the sample;

Compaison of measurements on solid and crushed samples:

(1) Solid polished sample: 5 – 10 s

(2) Cyanobacteria: 1 s

(3) Pressed powder: 100 s good spectrum – 20 s interpretable spectrum

Methanogenes show a weaker but distinct spectrum, mainly characterized by the CH-streching region around 2900cm^{-1}

Summary and Outlook

- A possible measurement regime for mineral mixtures with **cyanobacteria**:
  (1) start with a measurement time of only a few seconds to find if cyanobacteria are present;
  (2) the time and the number of repetitions need to be increased until acceptable spectra of minerals are obtained;
  (3) if the laser power on the sample is 1 mW it was found that the measurement time should be selected between 1 s (for cyanobacteria) and 20 s (for minerals).

- Such regimes must be derived for the other biological samples.

- Further investigation of Raman measurement parameters will consider martian atmospheric pressure, composition and temperature.

Thank you for your attention!

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