3rd LEIBNIZ CONFERENCE OF ADVANCED SCIENCE

- SENSORSYSTEME 2006 -

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Miniaturisierte Raman-Sensoren zur Überwachung chemischer und biologischer Vorgänge

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Content

- Motivation
- What is Raman spectroscopy?
- Miniaturized Raman apparatus
- Special properties of Raman spectroscopy
- Raman spectroscopy in life sciences
- Conclusion

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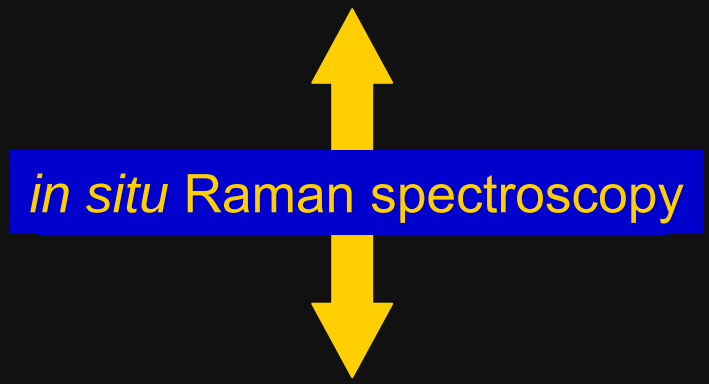
Motivation

State of a chemical process,
evolution of a biological systems,
the metabolism of molecules, etc.



detailed information on the molecular composition of the system
(if possible as a function of space and time)

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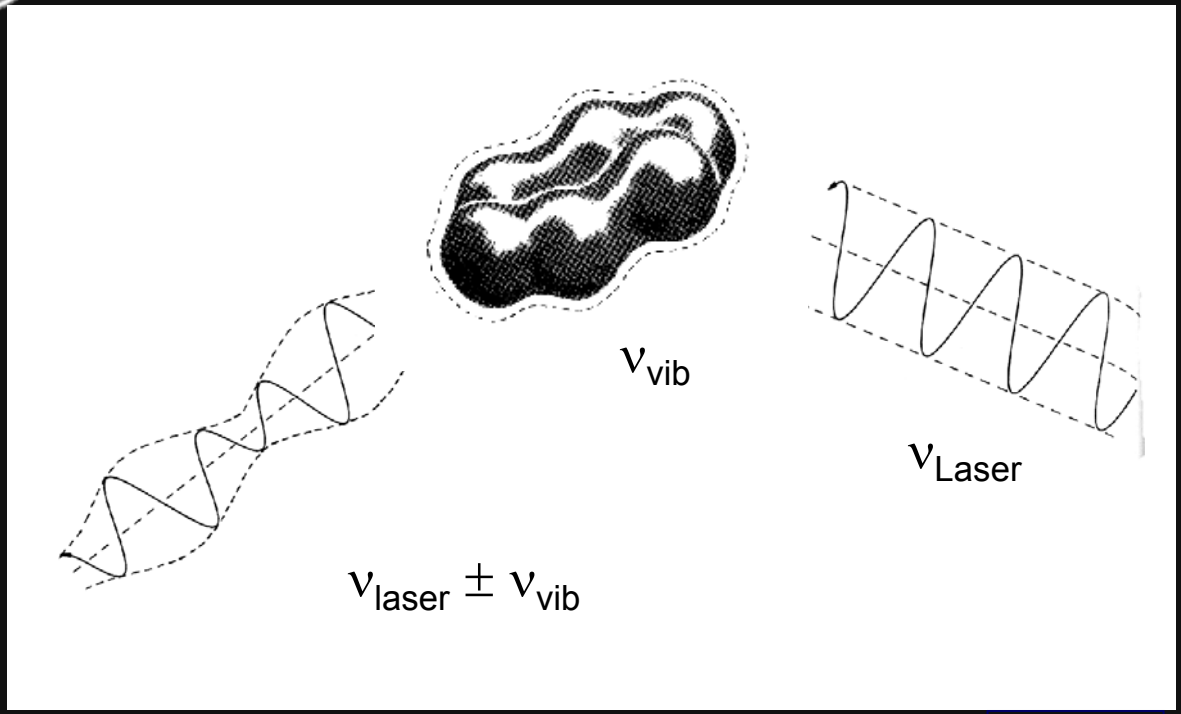
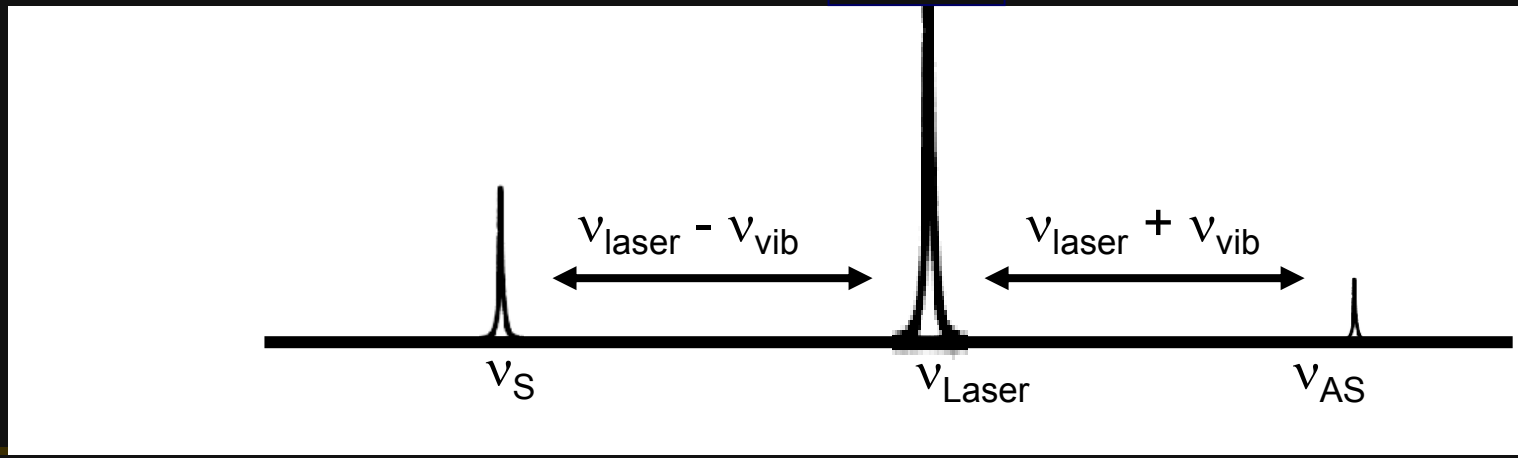
The Raman effect





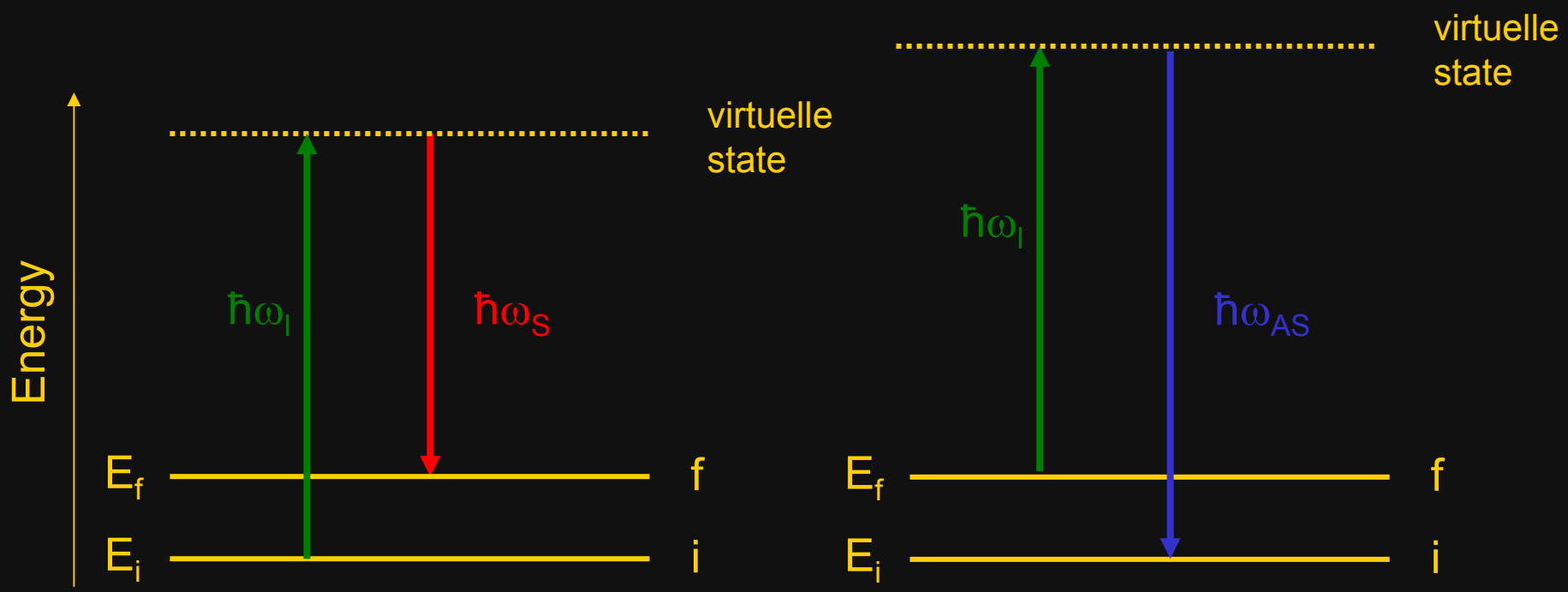
The Raman effect

scattering system with quantanized eigenstates E_f-E_i

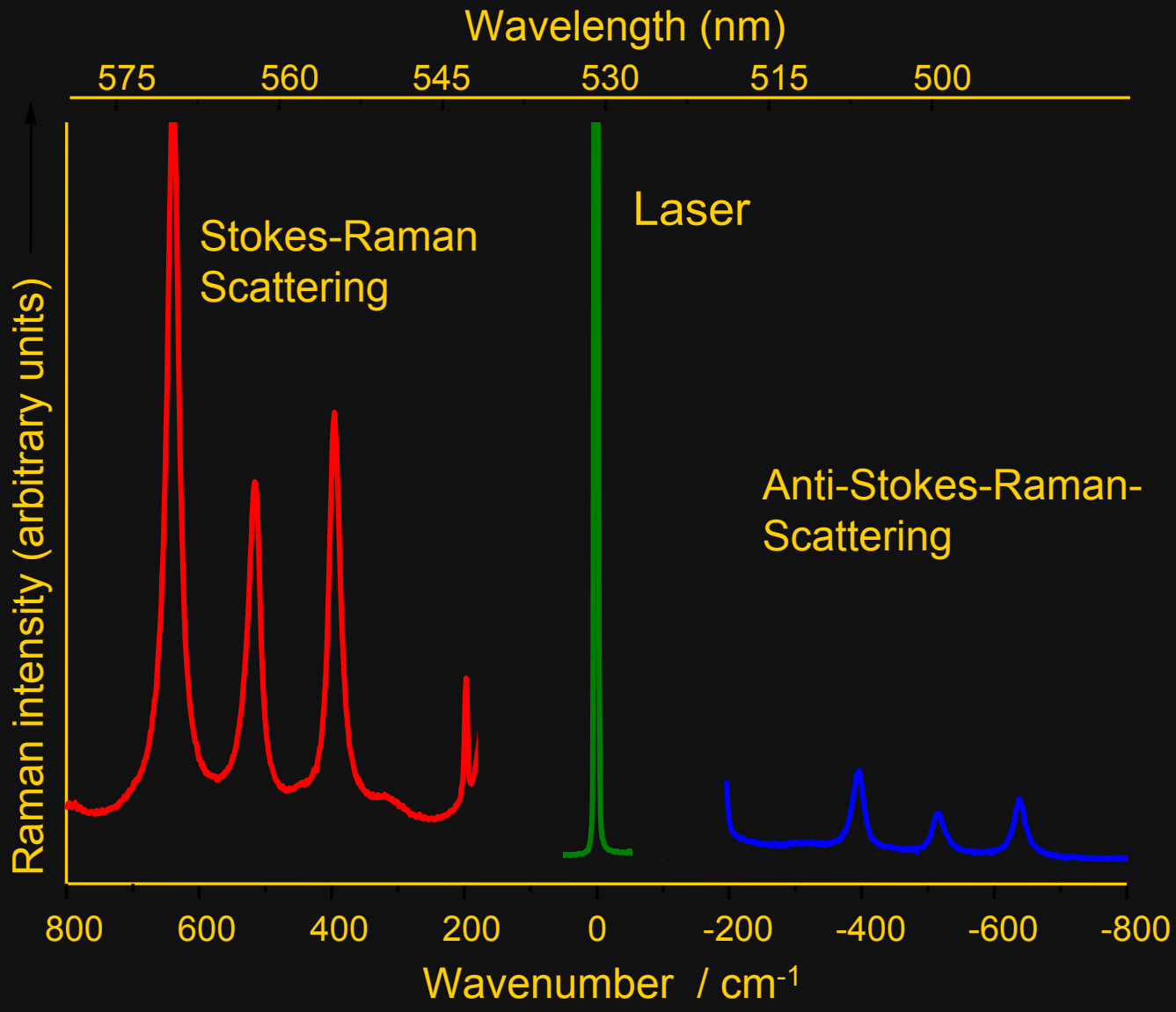
$\hbar\omega_R = E_f - E_i$

Stokes: $\hbar\omega_S = \hbar\omega_l - \hbar\omega_R$

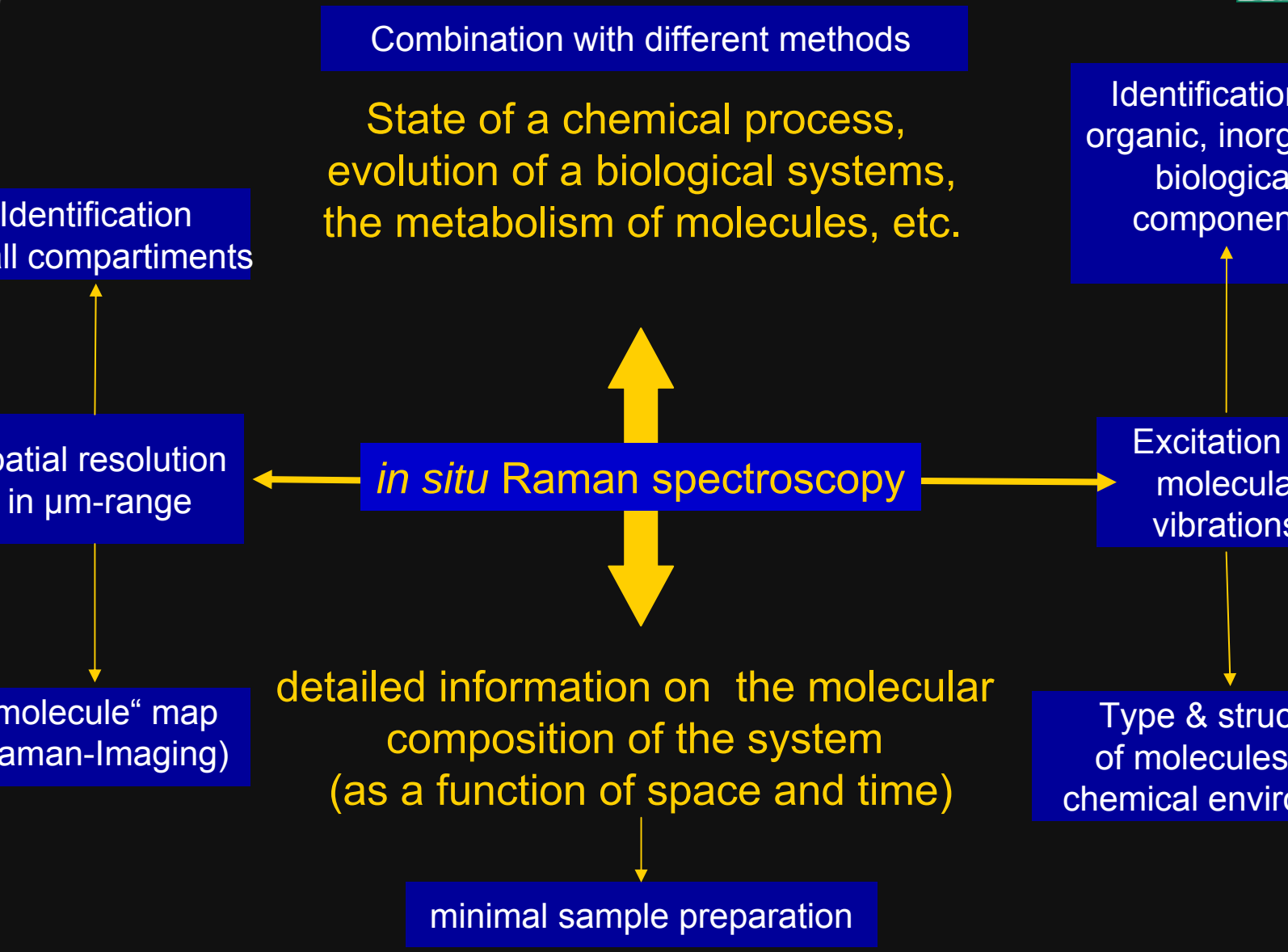
Anti-Stokes: $\hbar\omega_{AS} = \hbar\omega_l + \hbar\omega_R$



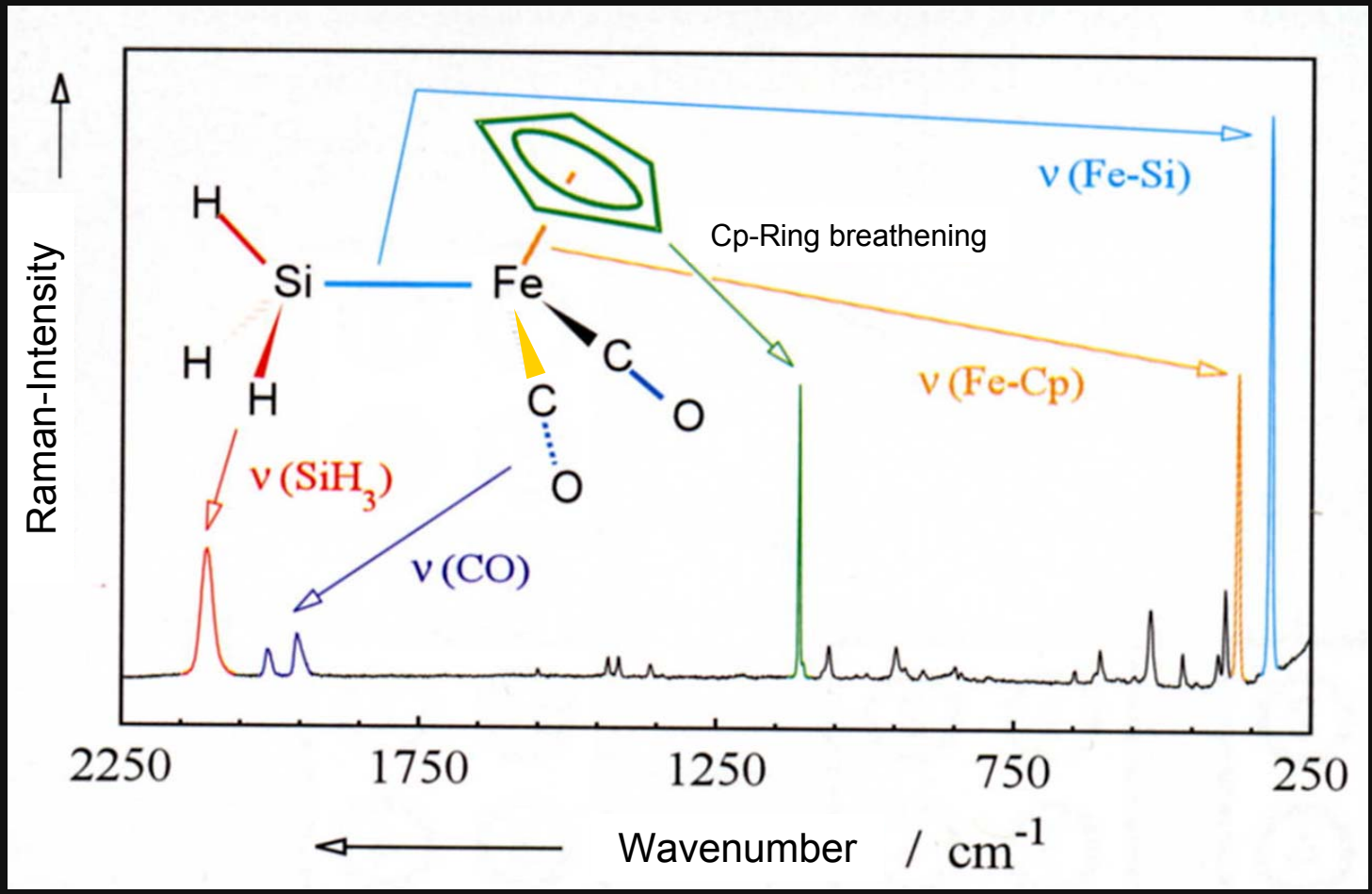
The Raman effect



Motivation

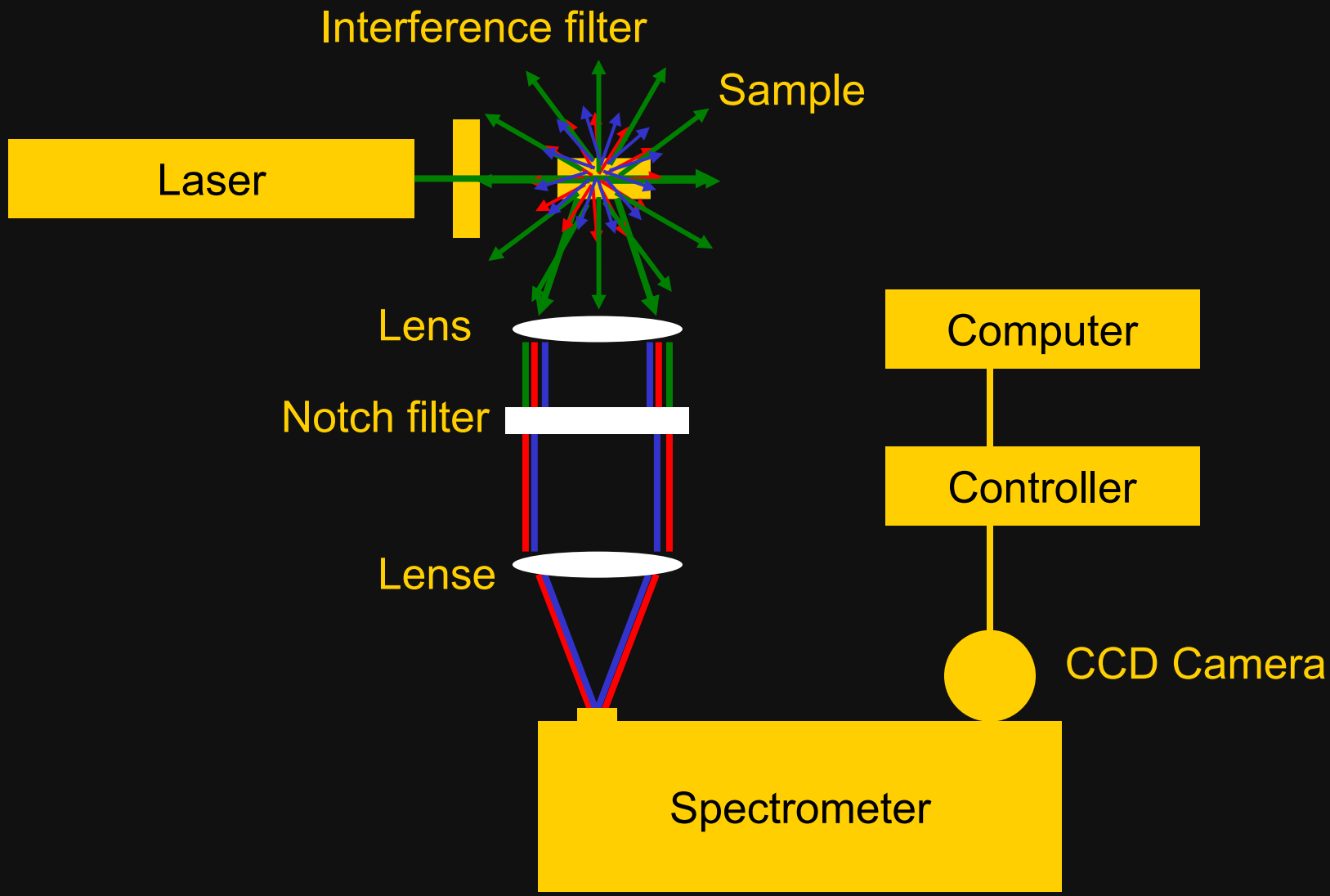

Combination with different methods
State of a chemical process, evolution of a biological systems, the metabolism of molecules, etc.
Identification small compartiments
Identification of organic, inorganic biological components
Spatial resolution in µm-range
in situ Raman spectroscopy
Excitation of molecular vibrations
"molecule" map (Raman-Imaging)
detailed information on the molecular composition of the system (as a function of space and time)
Type & structure of molecules and chemical environment
minimal sample preparation

Raman spectrum of a metal organic complex



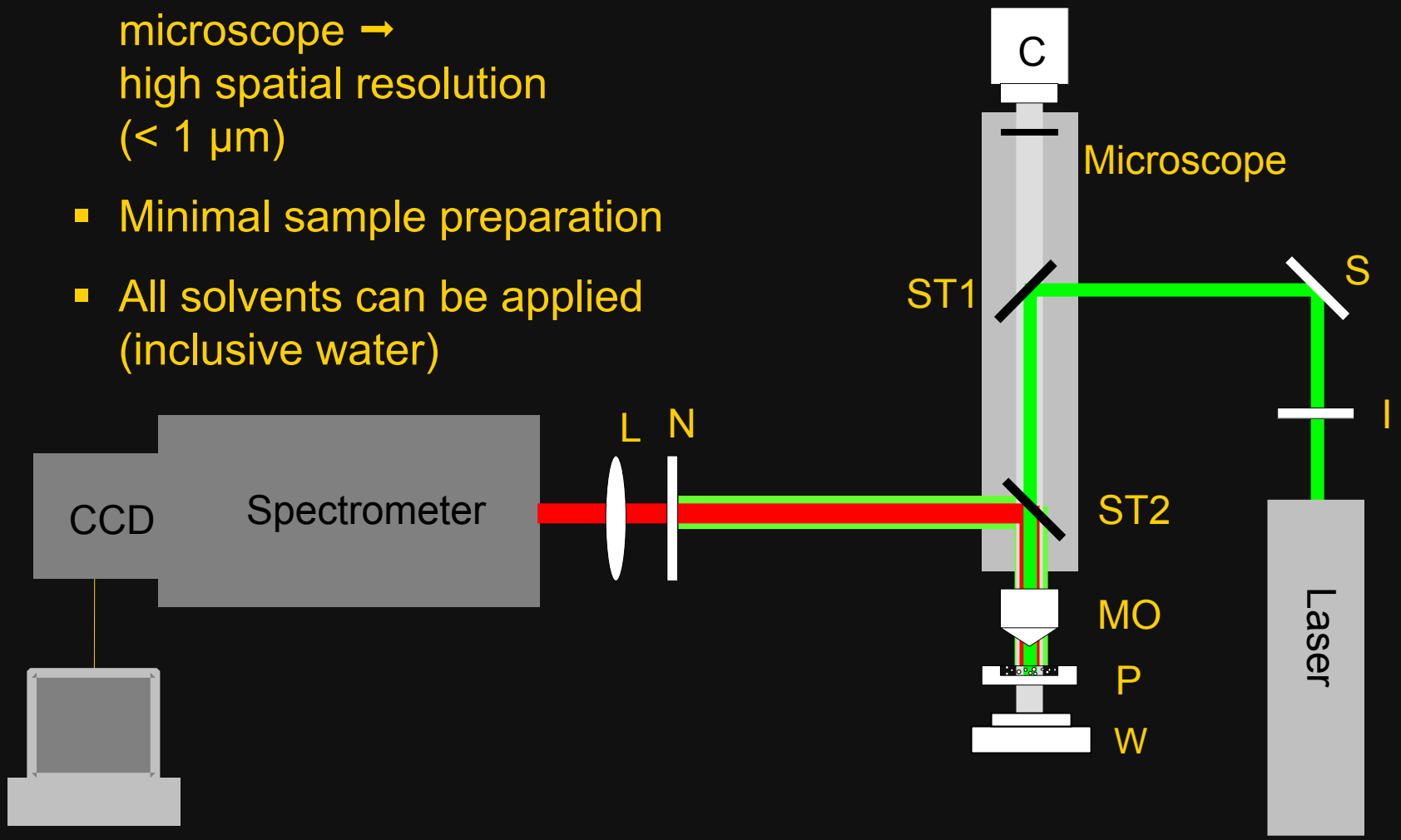
➞ Raman yields molecular fingerprint information

Conventional Raman set-up

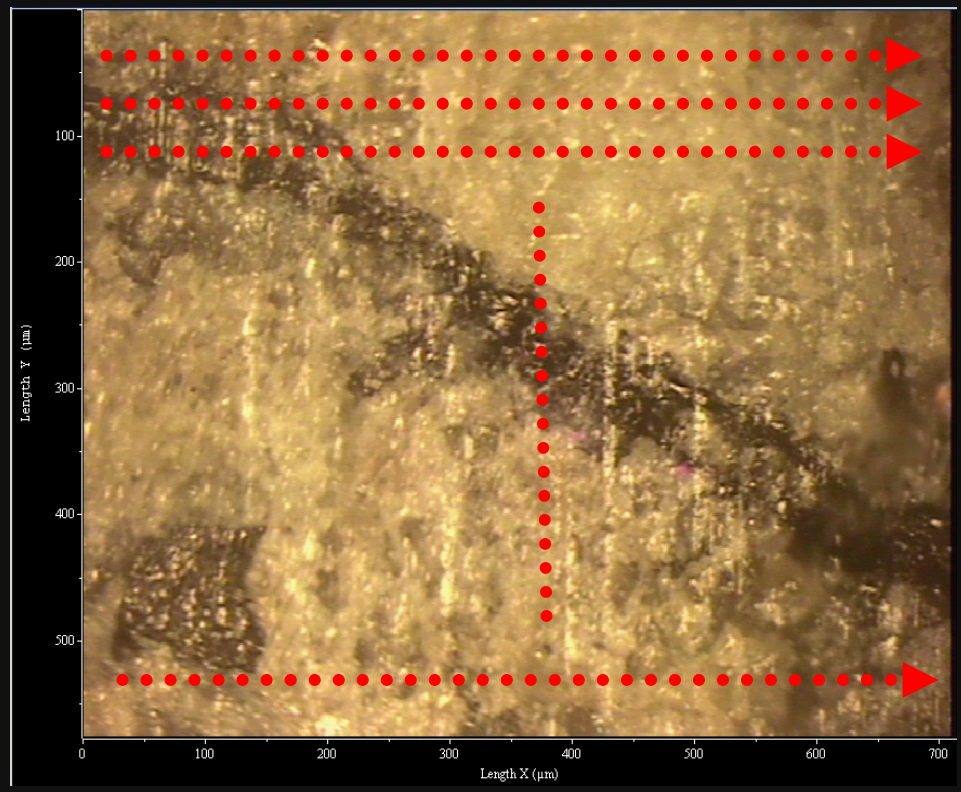


Raman spectroscopy

- High specificity
- Combination with a microscope ➞ high spatial resolution (< 1 µm)
- Minimal sample preparation
- All solvents can be applied (inclusive water)



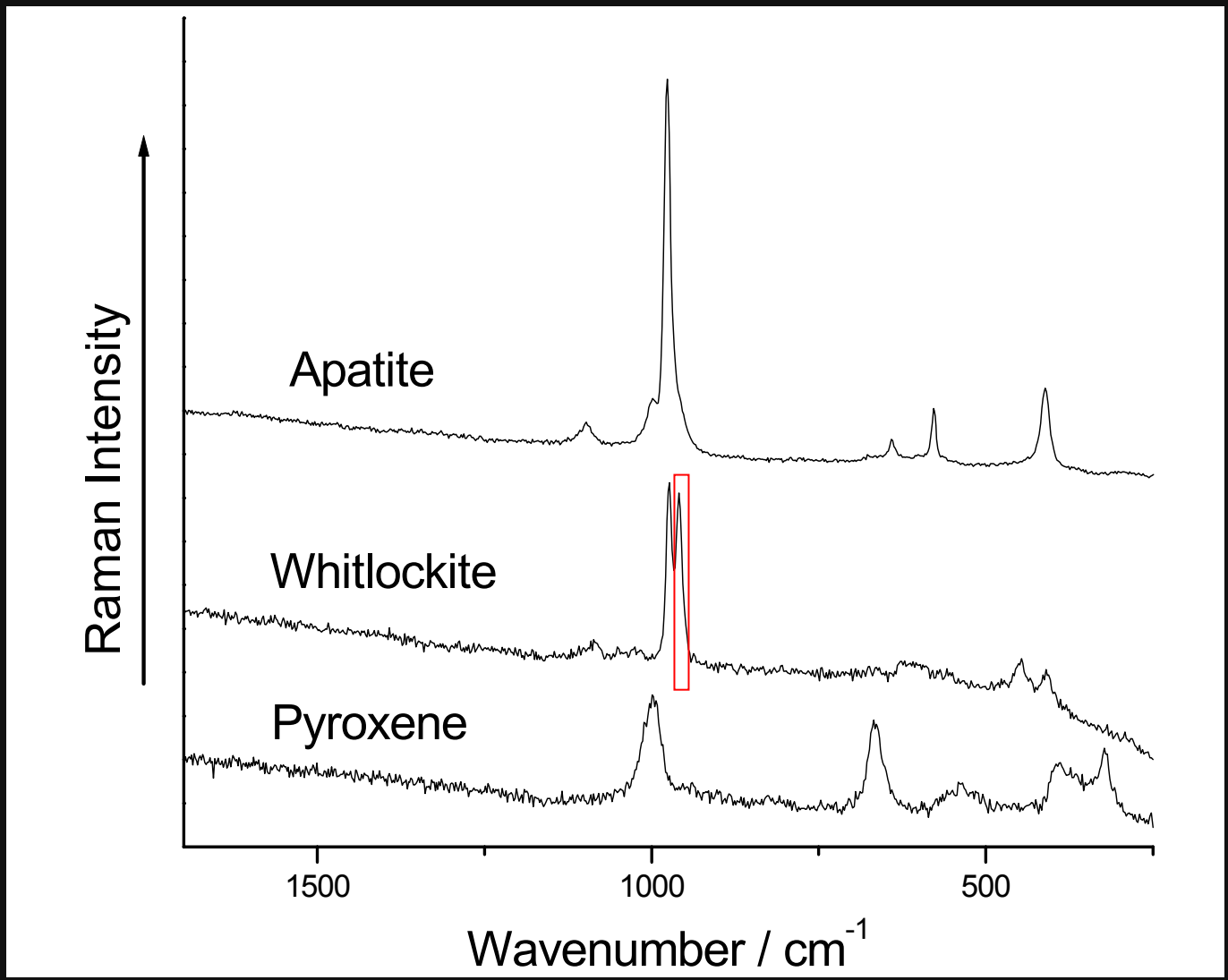
Raman Spectroscopy on Meteorites: Zagami



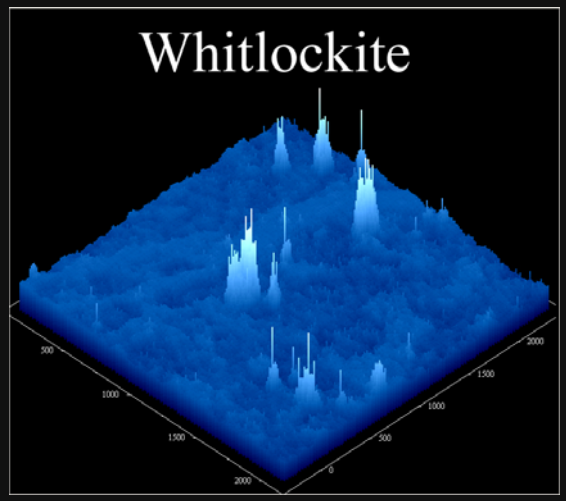
Surface scans:

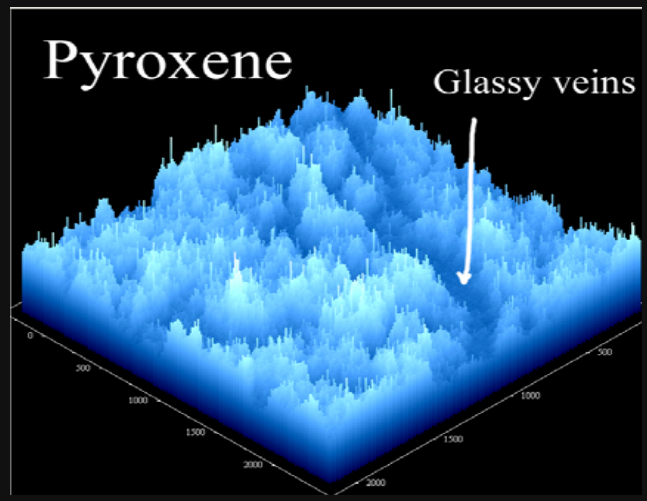
- 2,2 x 2,8 mm consisting of 90 x 110 measuring points (saptial resolution 25µm)
- 90 x 90 µm , 35 x 35 points grid, approx 3 µm spatial resolution

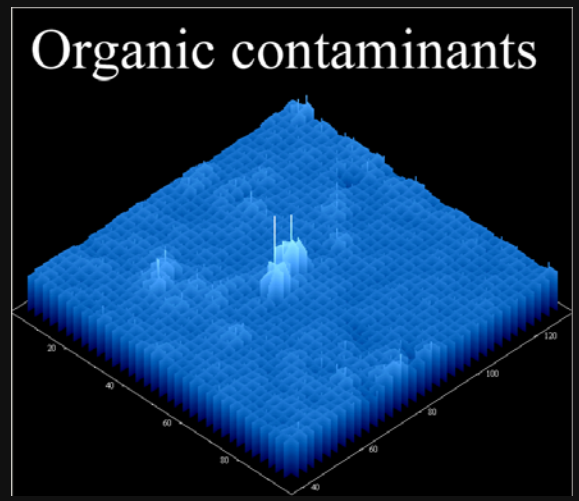
Raman Spectroscopy on Meteorites

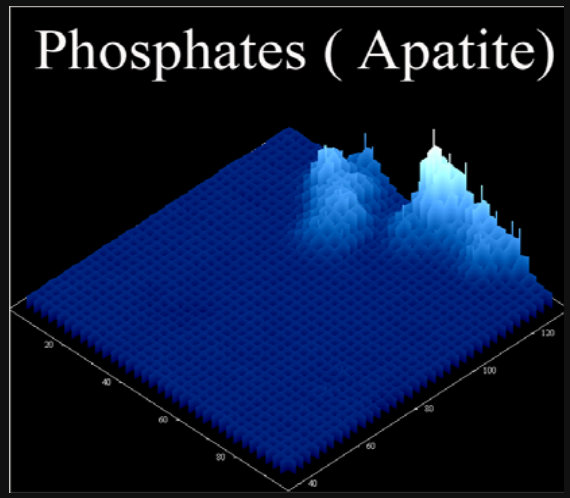


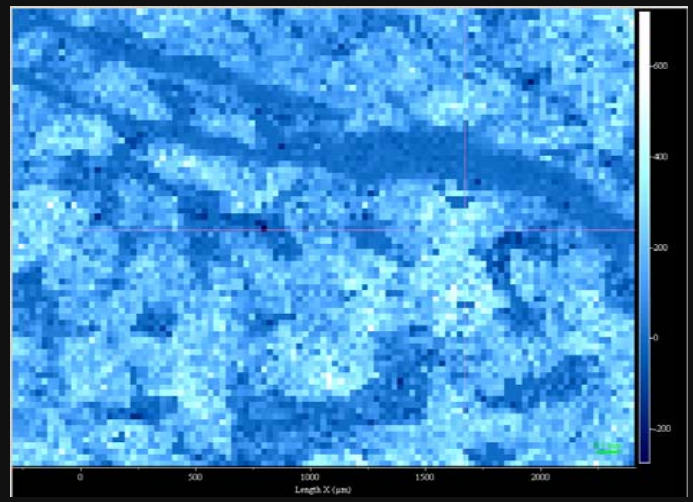
Surface Raman plots from Zagami

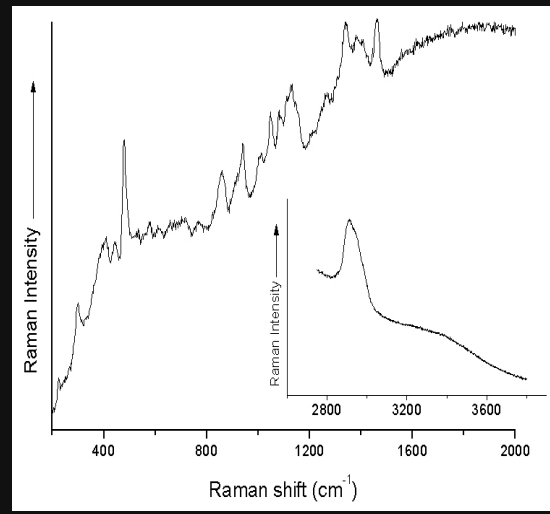












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What are now the requirement for Raman spectroscopy being used for mobile sensor (*in situ* planetary science)?

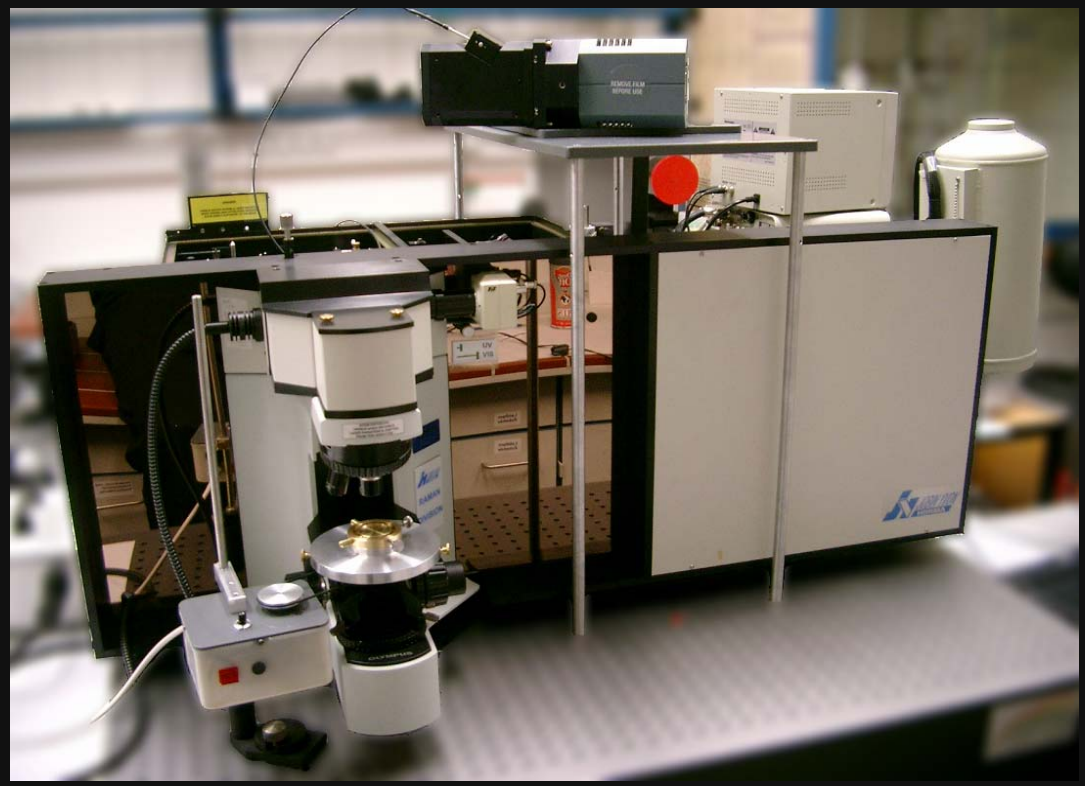
- a small, compact and robust Raman apparatus with an extreme low power consumption but with the performance of a large commercial device
- Spectral resolution between 5 to 8 cm^{-1}, spectral range from 150 up to 4000 cm^{-1}
- minimal sample preparation ➞ roughness of the sample surface should not effect the quality of the Raman spectra to much ➞ autofocus

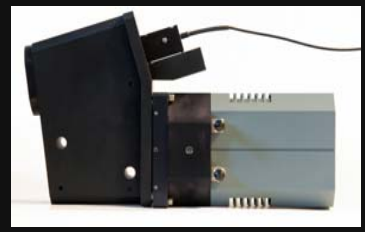
Miniaturization of Raman spectrometer

partner KAYSER-THREDE

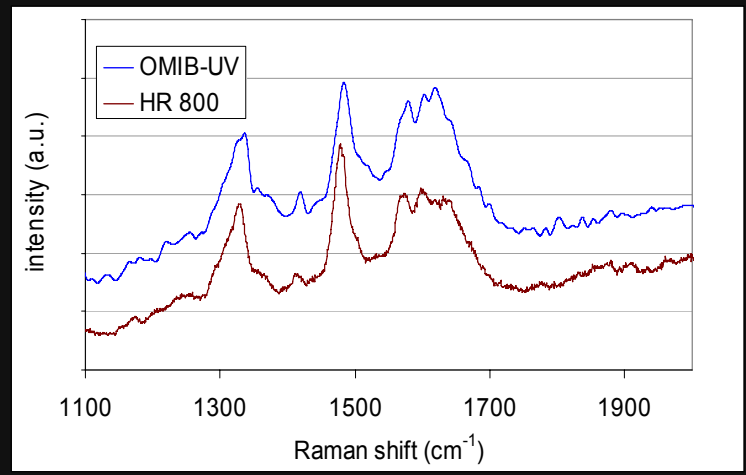
Funded by Bundesministerium für Bildung und Forschung

DLR





OMIB NIR spectral sensor

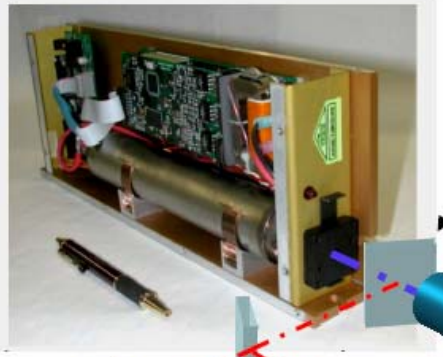


Raman spectra of bacteria: HR 800 von JY und OMIB-UV-Spektralsensor

Excitation:	from 245 nm to 325 nm
spectrale resolution	5 cm^{-1} @ 360 nm (0,065 nm)
spectral region:	up to 12000 cm^{-1}, 245 nm ... 360 nm
Throughput:	max. 50 %

Miniaturized UV-resonance Raman spectroscopy

Raman Laser:
commercially available hollow-cathode laser Laser @ 248 nm



dichroic beam splitter
UV laser 248 nm transmission
monitor 470 nm reflective

Laser-fiber coupling optics
(UV collimator)

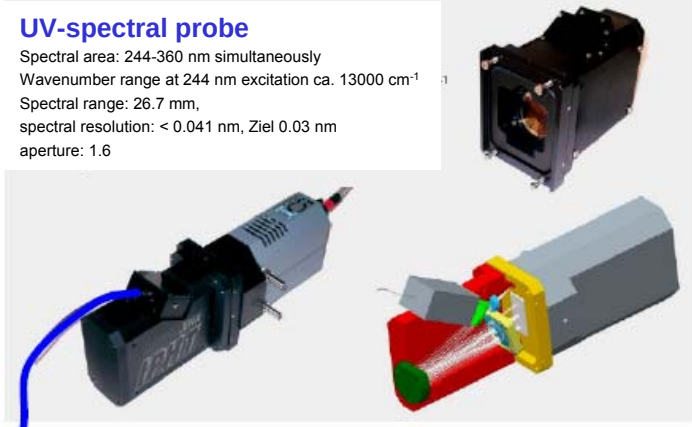
SCANNER
Laser 470 nm

SCANNER
beam splitter

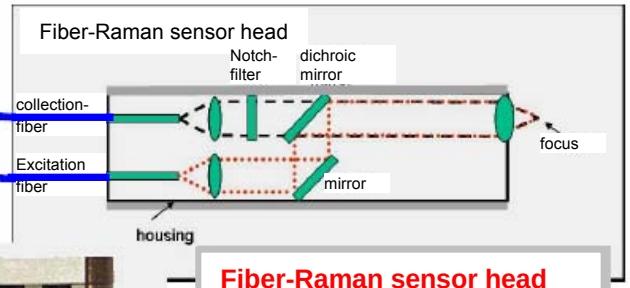
SCANNER
detector
Pin-Diode

Single mode
fiber ∅ 4µm (tbc.)

UV-spectral probe
Spectral area: 244-360 nm simultaneously
Wavenumber range at 244 nm excitation ca. 13000 cm^{-1}
Spectral range: 26.7 mm,
spectral resolution: < 0.041 nm, Ziel 0.03 nm
aperture: 1.6



multimode
Fiber ∅ 50 µm



Fiber-Raman sensor head for 248 nm excitation

X/Y/Z Piezo actuator / scanner (carries Raman sensor head)

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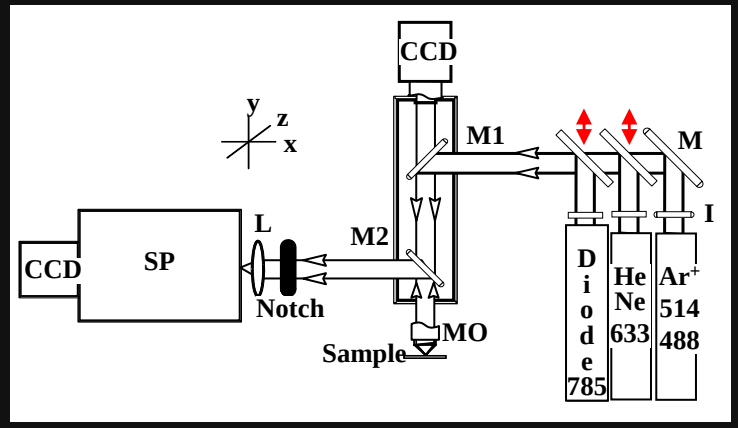
Special properties of Raman spectroscopy

- high specifity
- good spatial resolution (micro Raman)
- minimal sample preparation
- all solvents can be used



but:

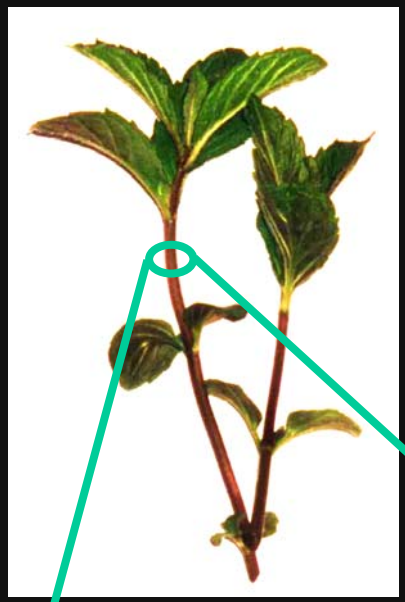
- biological but also other samples often show high fluorescence
- biological molecules appear often at low concentration level

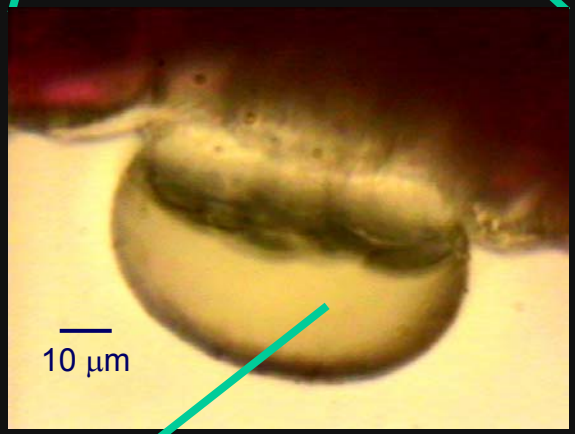
One Solution is for example:

→ **SERS** (***s***urface ***e***nhanced ***R***aman ***s***pectroscopy)

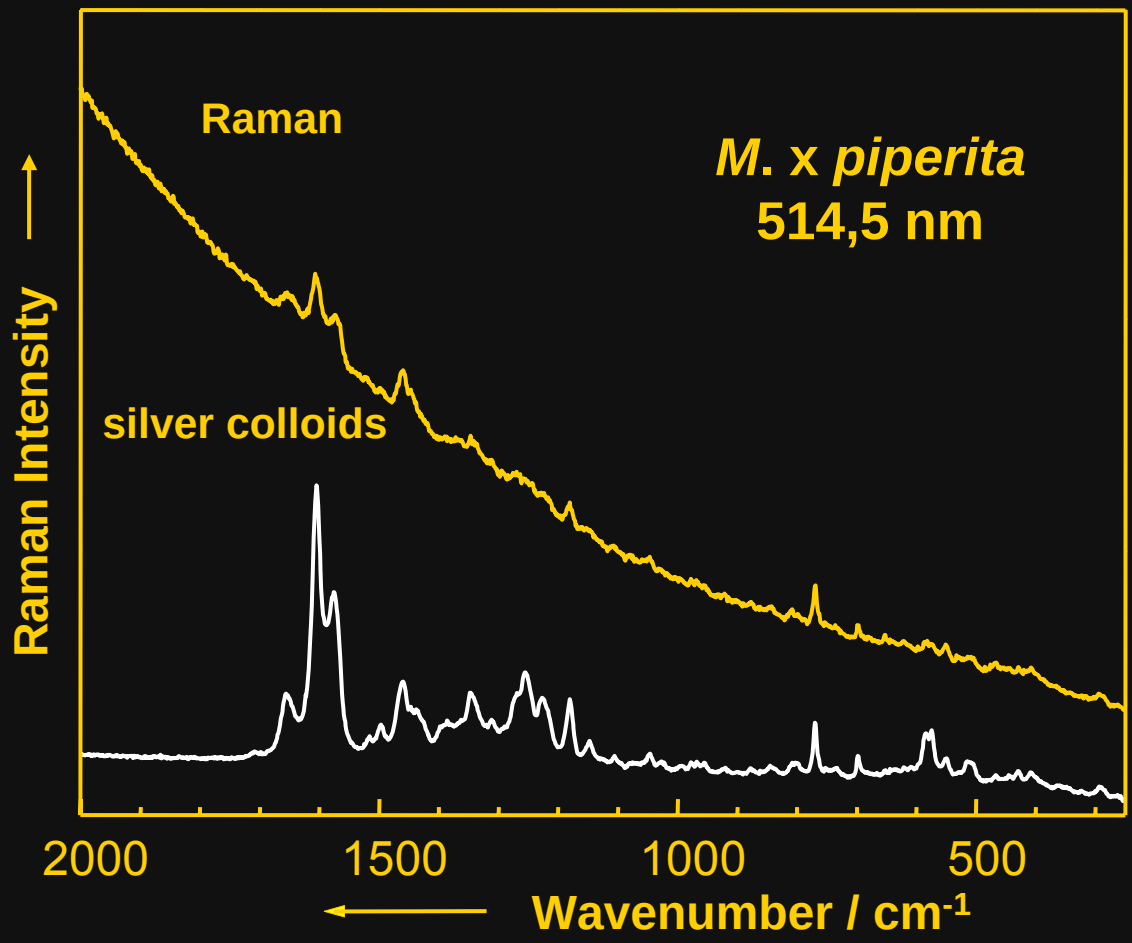
→ Resonance Raman spectroscopy

SERS quenches fluorescence

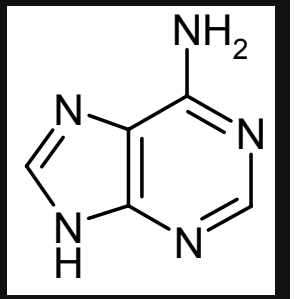


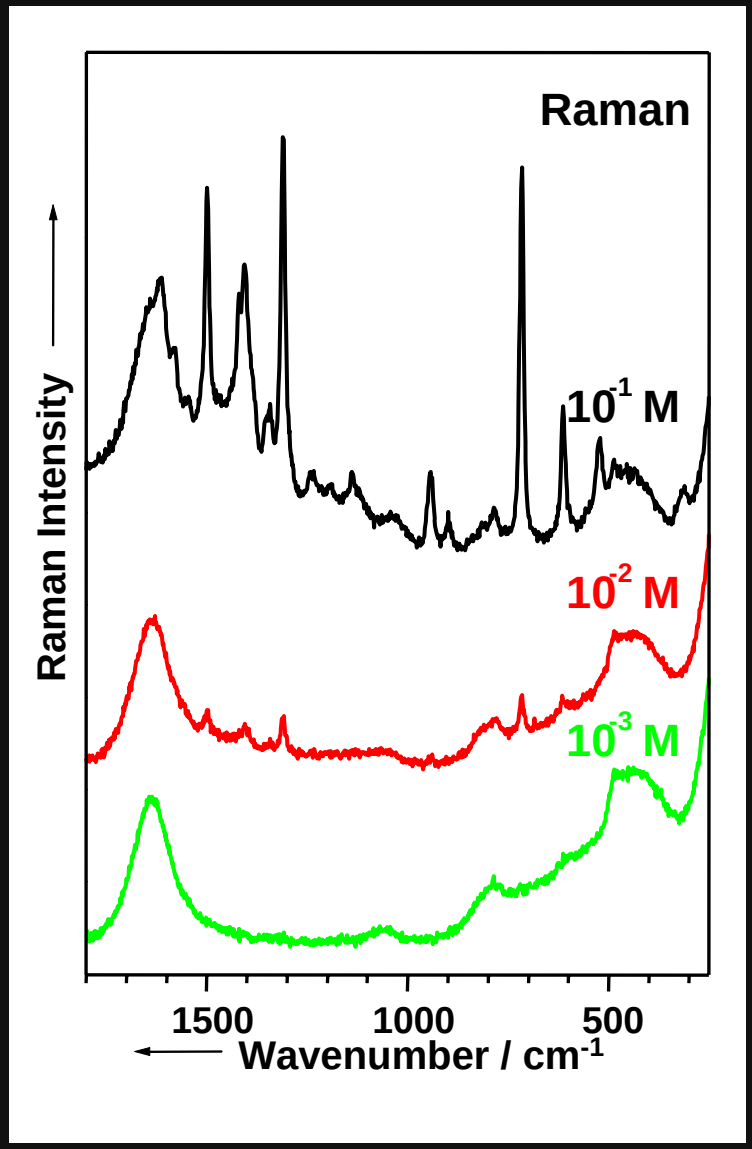


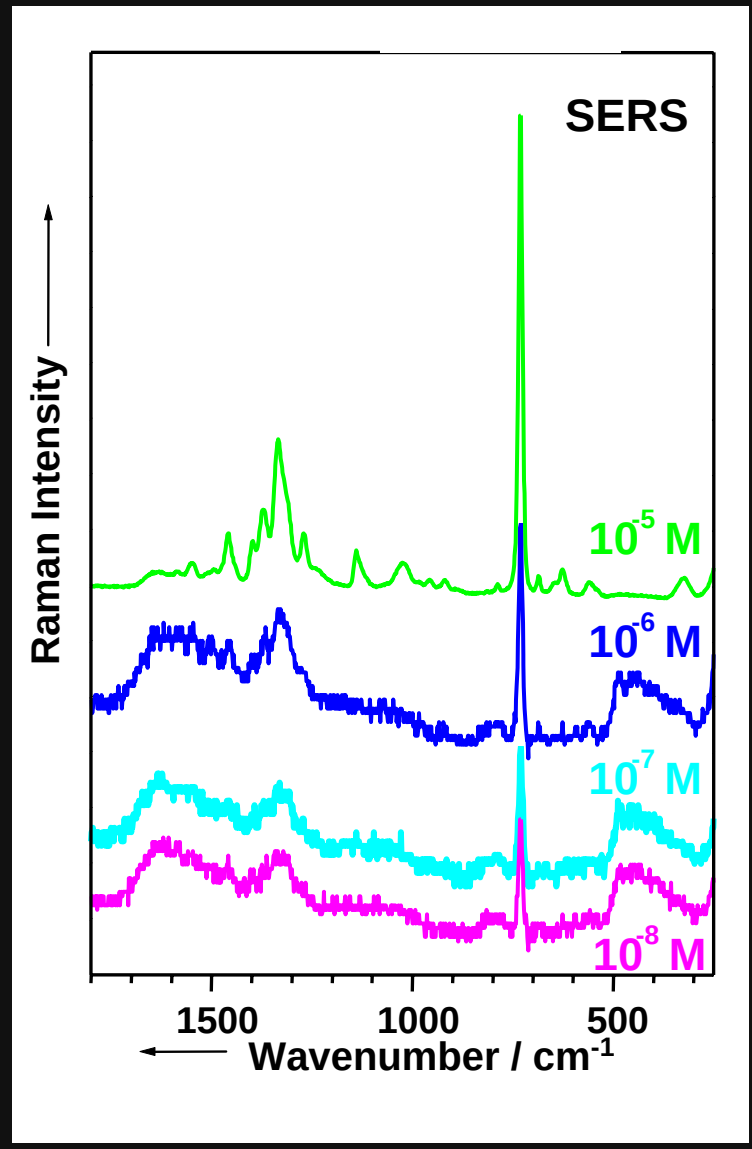
essential oil



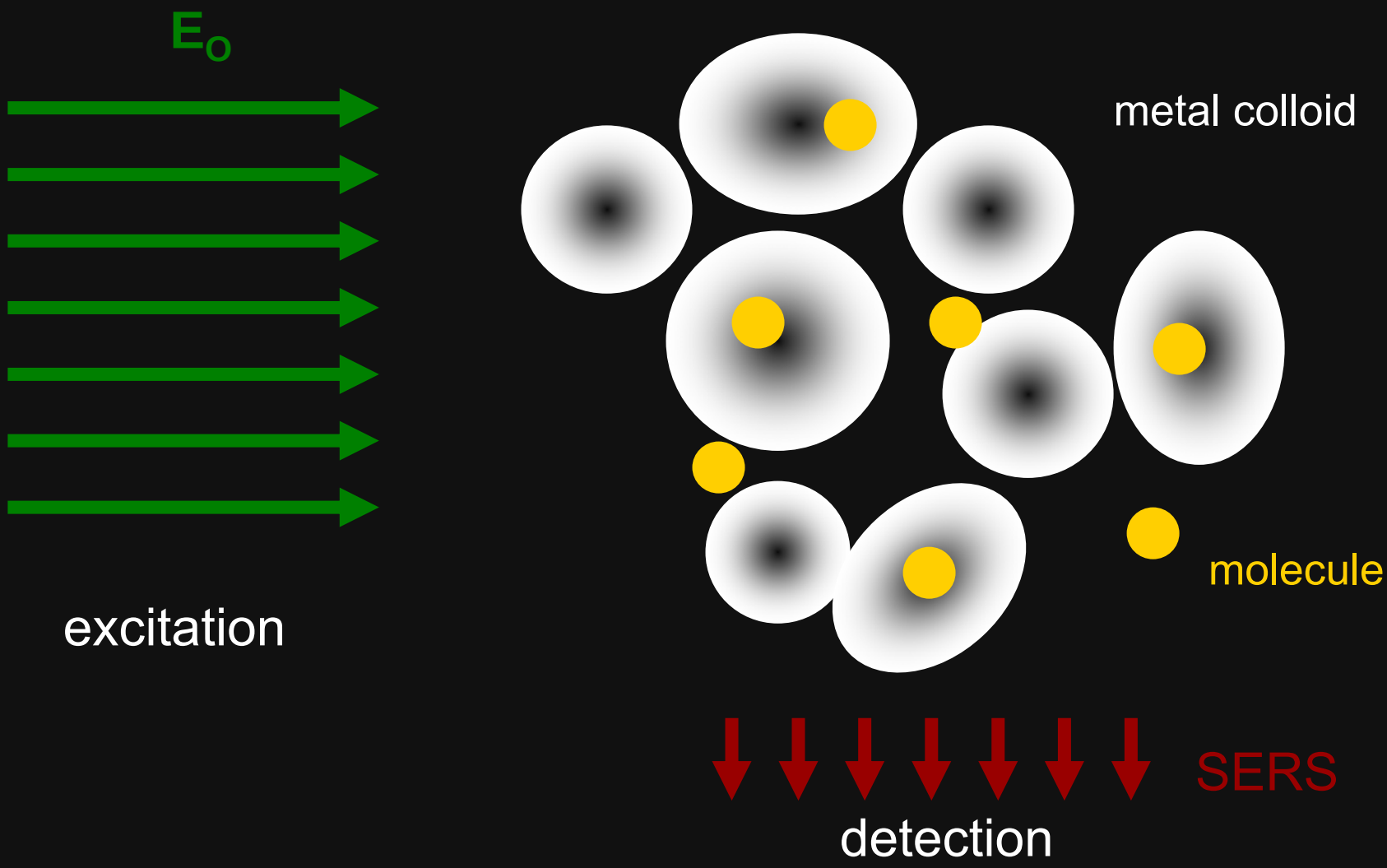
SERS improves the detection limit: Adenine



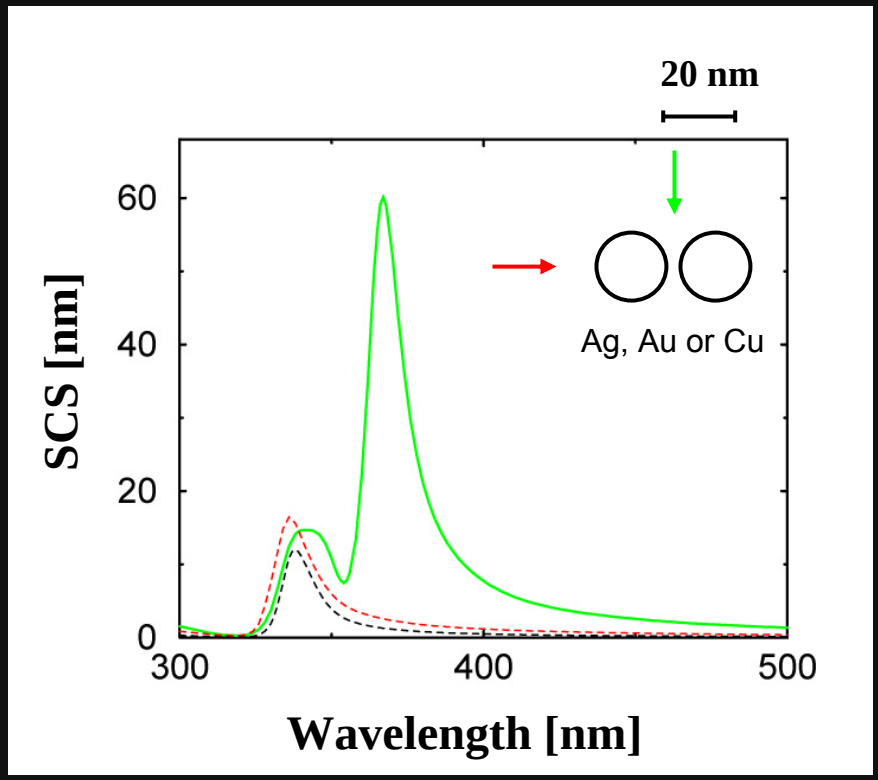


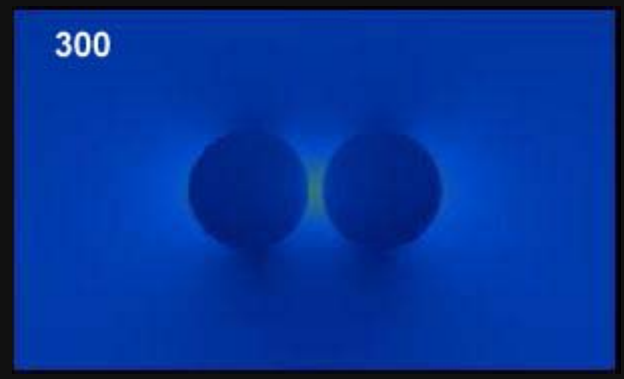


How does SERS work?
The SERS EM enhancement



SERS EM enhancement – coupling

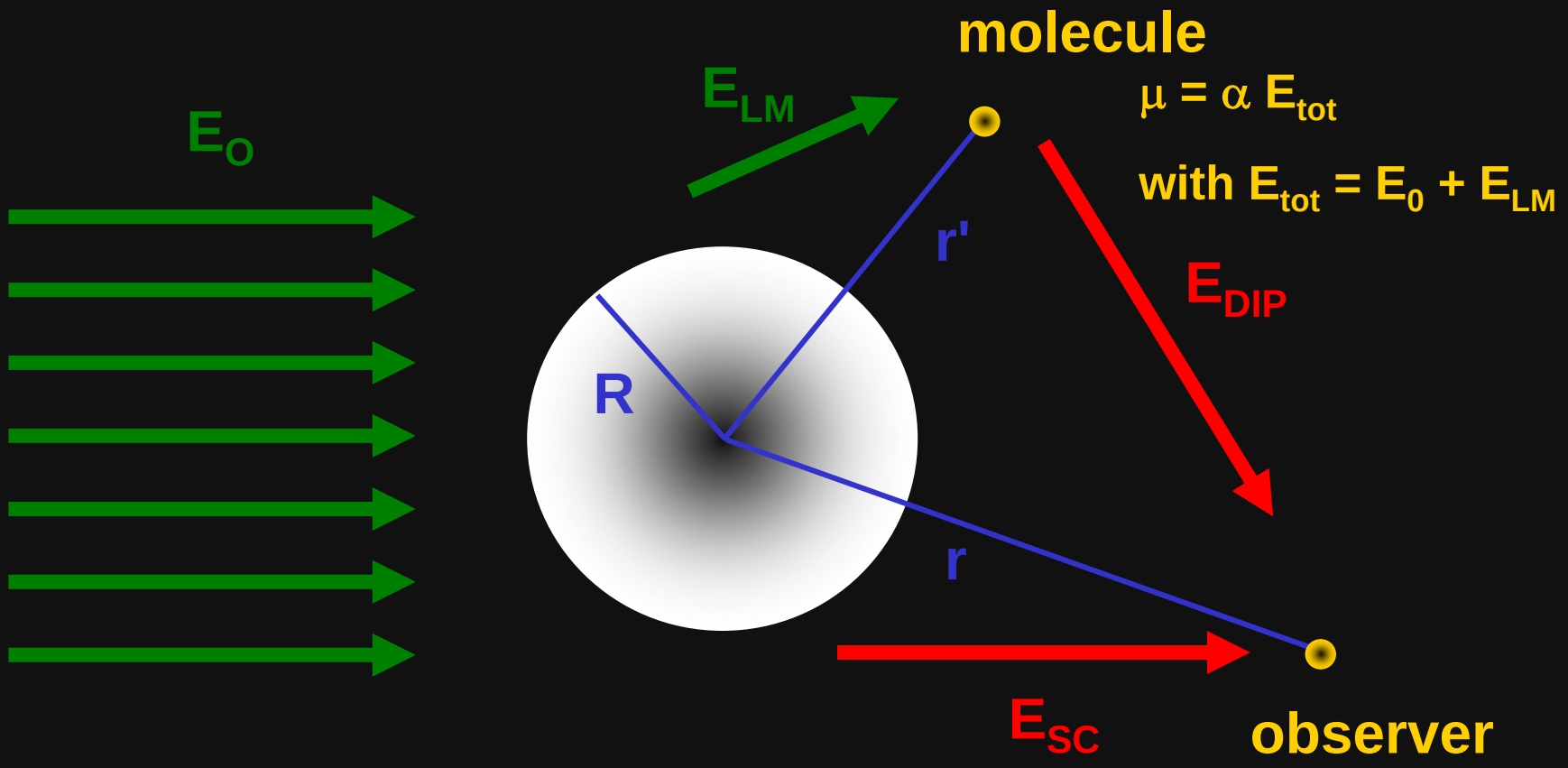




J. Kottmann *et al.*, IFH Field Theory Group, ETH Zürich

SERS: Raman spectroscopy utilizes optical properties of nanostructures

SERS EM enhancement

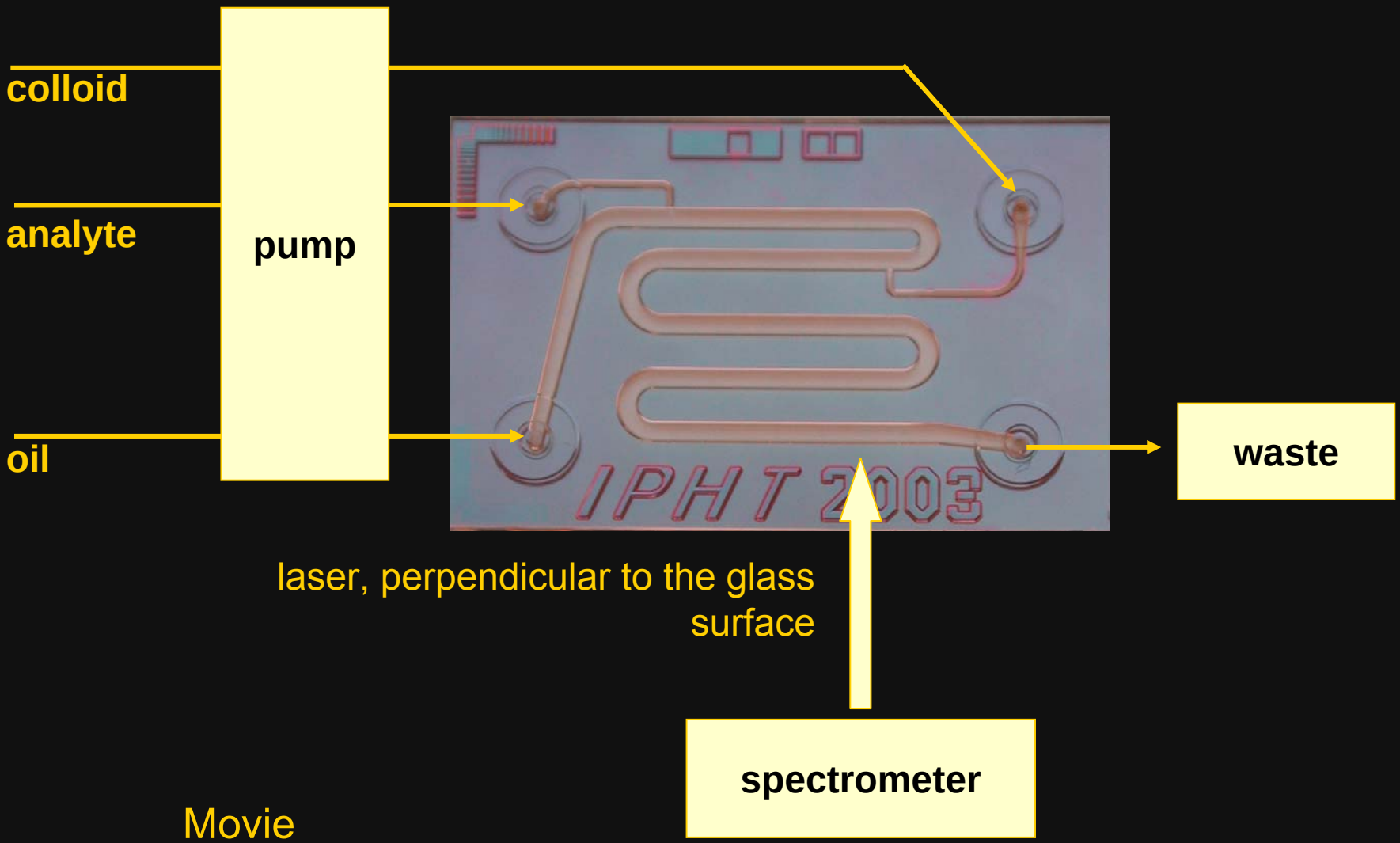


Raman intensity I_R

$$I_R \propto |E_R|^2$$

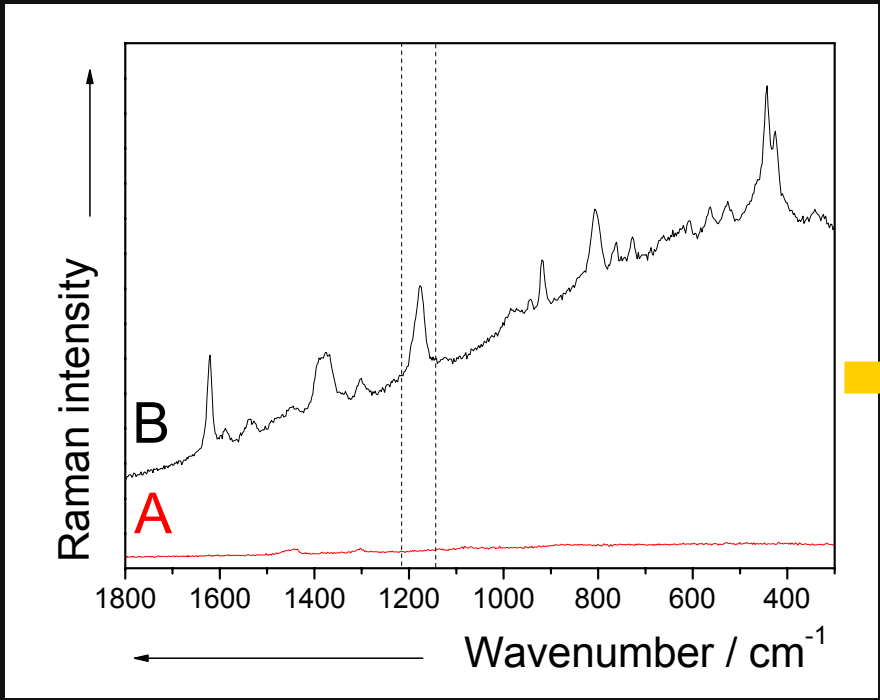
$$E_R = E_{DIP} + E_{SC}$$

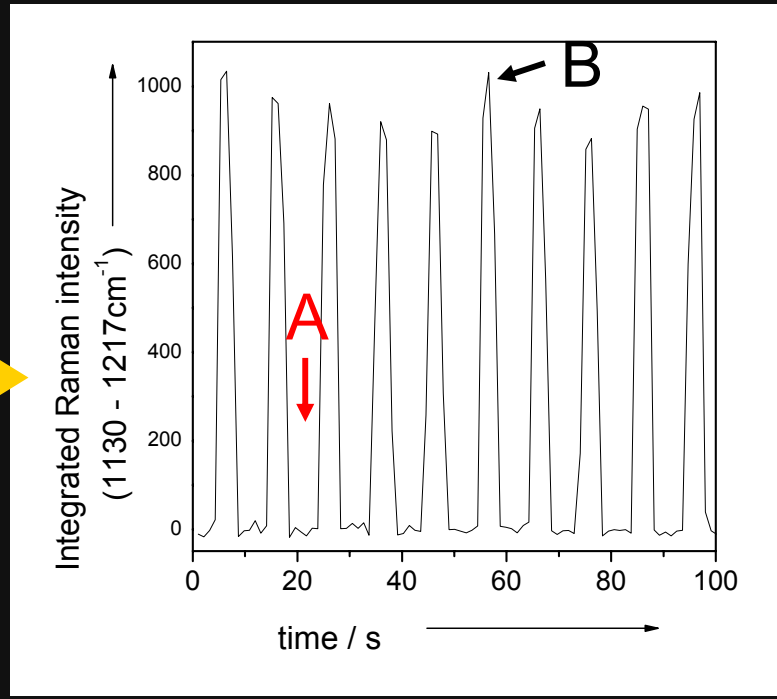
Setup and Cell design for reproducible SERS



Movie

Measuring principle



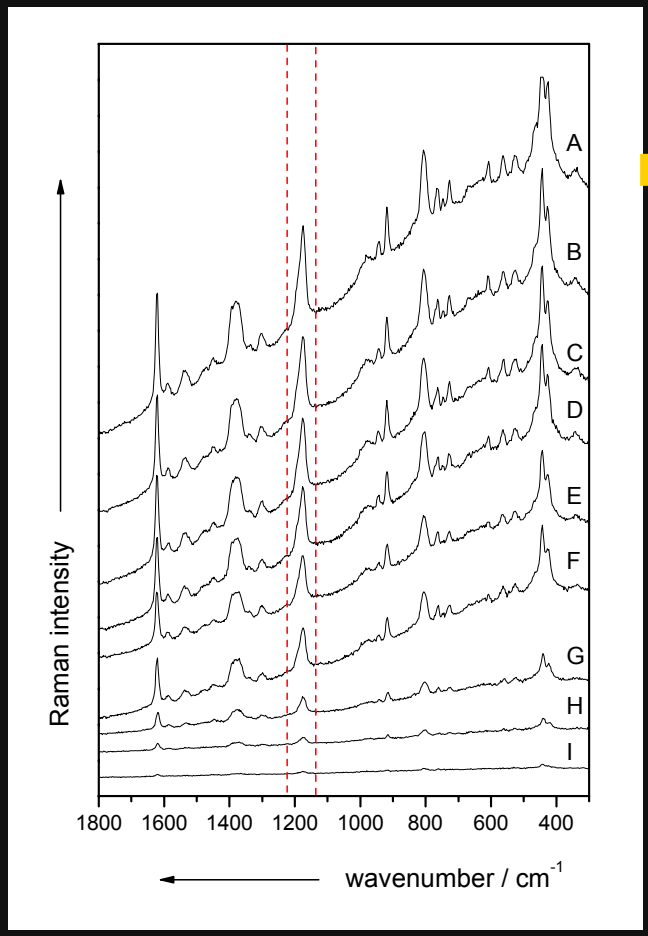


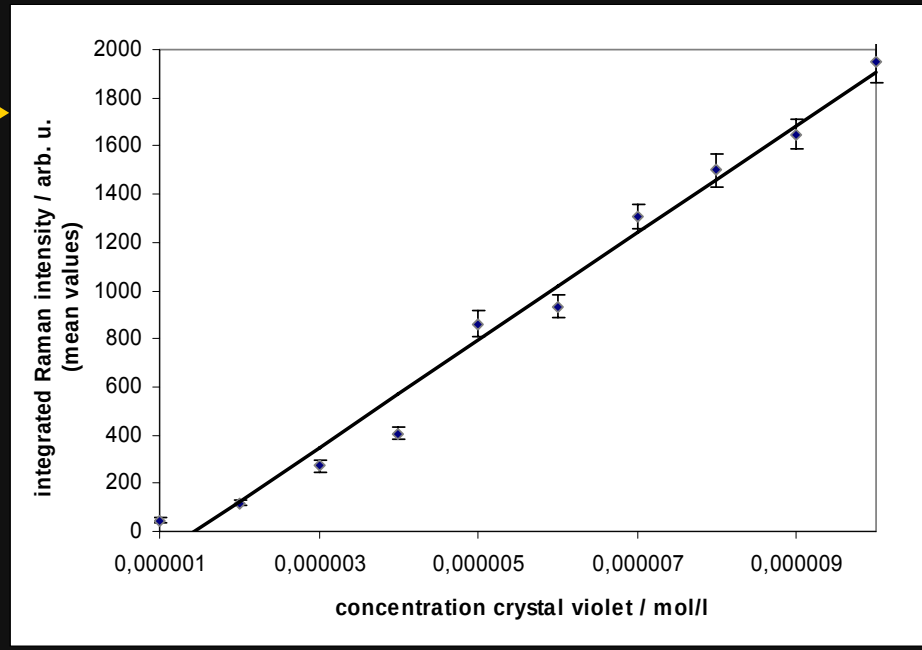
Spectrum A is measured in the separation medium tetradecane and Spectrum B in the aqueous droplet

Alternating peaks, showing the integrated Raman intensity in the wavenumber region between 1130 and 1217 cm^{-1}

Concentration dependence

Spectra of different crystal violet concentrations ranging from $1*10^{-5}$ M (A) to $1*10^{-6}$ M (I) are measured





linear dependence of the integrated Raman intensity against the concentration

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VDI

Bundesministerium für Bildung und Forschung

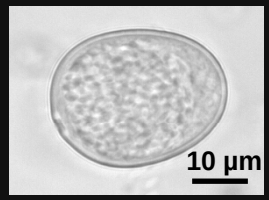
Identification of single microorganisms by micro-Raman spectroscopy

within the research project

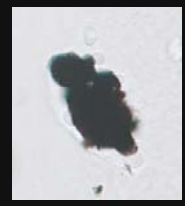
OMIB – Online Monitoring und Identification of Bioaerosols

Motivation - Bioaerosol

Pollen

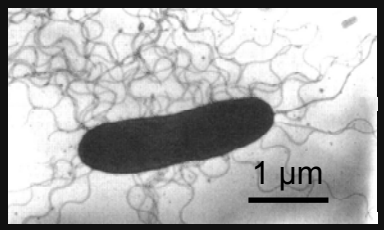


Dandruff,
Grit,
etc.

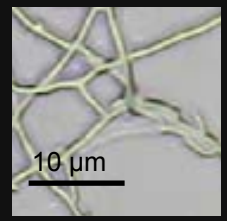


Allergies,
Diseases,
Food Spoilage,
etc.

Bacteria



Fungi, Yeast



Motivation – Bacteria investigations

- Hospitals:
  - ⇨ Formation of anti-biotic resistant bacteria “super bacteria”
- Pharmaceutical production:
  - ⇨ High cost if contaminations are found in products or the assembly line
- Food industry:
  - ⇨ Limited life time of products because of accelerate food spoilage

Goal / Vision:
Implementation of a real time method for the identification of bacteria (biotic contaminations) without a cultivation step on a single cell level.

OMIB in the field of clean room productions

Why clean room?

Bacteria

Bacillus pumilus (2)
Bacillus sphaericus (2)
Bacillus subtilis (2)
Micrococcus luteus (2)
Micrococcus lylae (2)
Staphylococcus aureus (3)
Staphylococcus cohnii (4)
Staphylococcus epidermidis (3)
Staphylococcus hominis (2)
Staphylococcus warneri (2)
Escherichia coli (4)
Pseudomonas aeruginosa (2)

Yeast

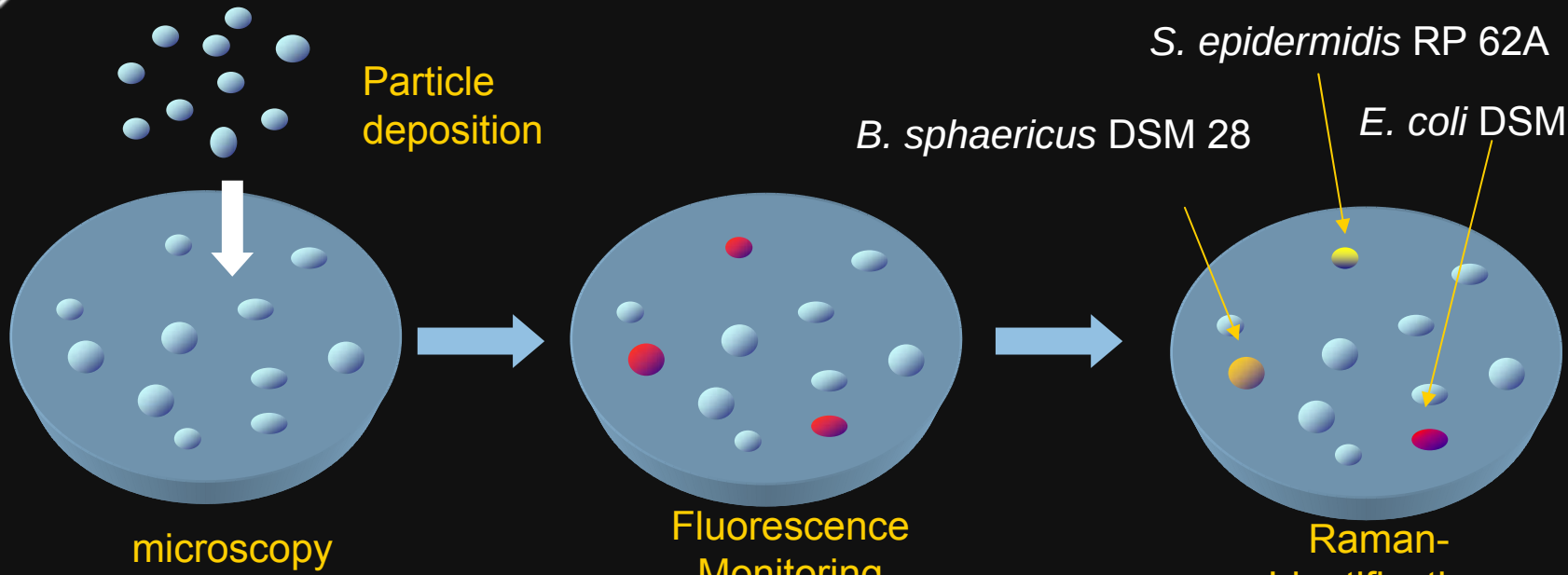
Candida albicans (2)
Rhodotorula mucilaginosa (1)
Saccharomyces cerevisiae (4)

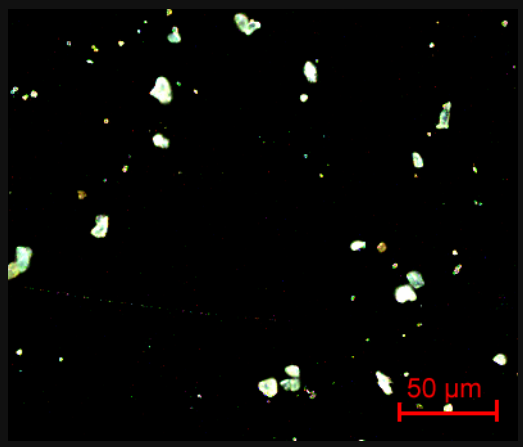
Fungi

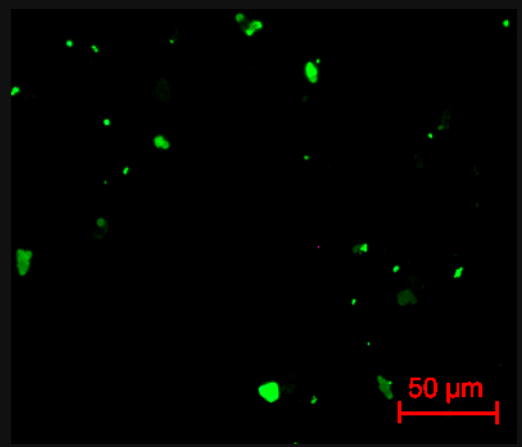
Aspergillus niger (2)

→ Limited amount of species needed to be identified!!!!

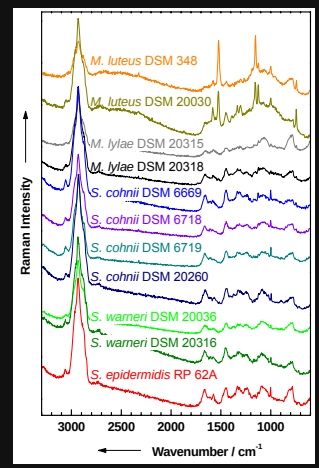
OMIB





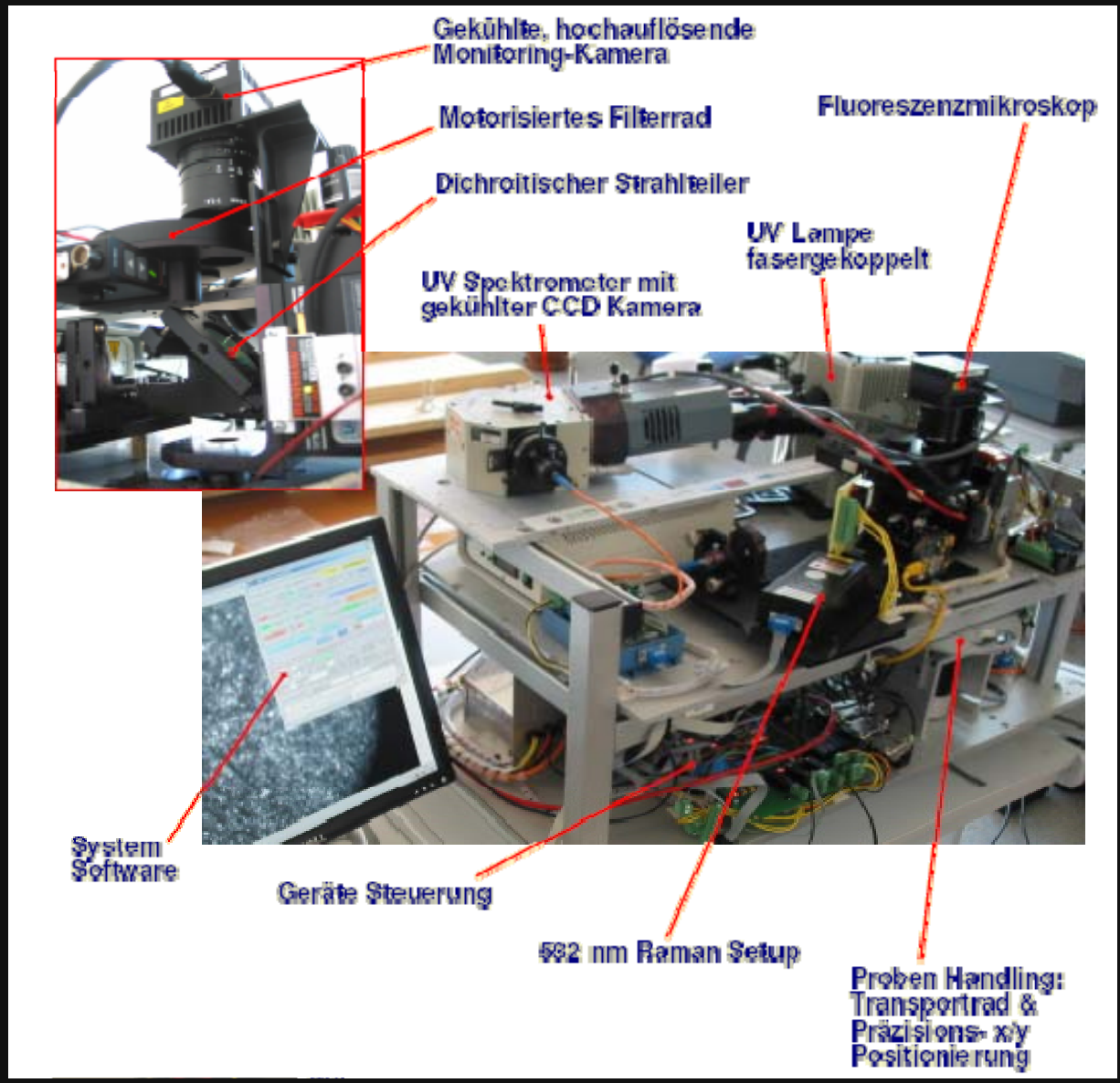


Identfication rate: ~ 90%



P. Rösch, M. Harz, M. Krause, R. Petry, K.-D. Peschke, O. Ronneberger, H. Burkhardt, A. Schüle, G. Schmautz, R. Riesenberg, A. Wuttig, M. Lankers, S. Hofer, H. Thiele, H-W. Motzkus, J. Popp, "Online-Monitoring and Investigation of Bioaerosols" in "*Biophotonics: Vision for a better Health care*", J. Popp, M. Strehle, eds., Wiley, **2006**.

System integration



Identification of a single microorganism (532 nm)

→ Data analysis by means of support vector machine

	Number of strains	*Number of spectra*	*Number of wrong classified strain spectra*	*Recognition rate for strains (%)*	*Number of wrong classified species spectra*	*Recognition rate for species (%)*
B. pumilus	2	100	19	80.5	7	92.7
B. sphaericus	2	95	17	81.8	11	88.2
B. subtilis	2	348	12	94.0	8	96.6
E. coli	7	666	178	73.1	8	99.1
M. luteus	2	667	10	93.5	7	96.6
M. lylae	2	40	1	97.5	1	97.5
S. cohnii	4	260	20	92.2	11	95.8
S. epidermidis	2	879	9	97.6	9	97.6
S. warneri	2	138	11	92.1	4	97.2
S. cerevisiae	3	42	7	80.7	5	86.9
Average recognition rate		**3235**		**85.6**		**95.4**

P. Rösch, M. Harz, K.-D. Peschke, O. Ronneberger, H. Burkhardt, A. Schüle, G. Schmautz, M. Lankers, S. Hofer, H. Thiele, H-W. Motzkus, and J. Popp, *Anal. Chem*. **2006,** *78*, 2163-2170.

Identification of a single microorganism (532 nm)

Identification of an independent dataset

Strain	Strain Number	Spectra Correctly	Identified as
Bacillus subtilis DSM 347	8	8	
Bacillus sphaericus DSM 28	8	8	
Bacillus sphaericus DSM 396	7	7	
Escherichia coli DSM 423	7	7	
Escherichia coli DSM 498	7	7	
Escherichia coli DSM 1058	20	17	*E. coli* DSM 499, *E. coli* DSM 423, *E. coli* DSM 2769
Micrococcus luteus DSM 20030	6	6	
Micrococcus lylae DSM 20315	5	5	
Micrococcus lylae DSM 20318	5	5	
Staphylococcus cohnii DSM 6669	8	8	
Staphylococcus cohnii DSM 6718	5	5	
Staphylococcus cohnii DSM 6719	5	5	
Staphylococcus cohnii DSM 20260	7	7	
Staphylococcus epidermidis RP 62A	7	7	
Staphylococcus epidermidis 195	20	18	*S. warner*, *E. coli*
Staphylococcus warneri DSM 20036	5	5	
Identification	**130**	**125**	

Content

- Motivation
- What is Raman spectroscopy?
- Miniaturized Raman apparatus
- Special properties of Raman spectroscopy
- Raman spectroscopy in life sciences
- Conclusion

Conclusion / Outlook

***In-situ*-Raman-Spectroscopy:**

- Identification of the chemical composition of
  - inorganic / mineralogical
  - organic, biological materials
- spatially resolved investigations in the area of ~1 µm
  - identification of inclusions & single microparticles
  - Raman-mapping → mineralogical map
- minimal sample preparation
- no limitation for a state of aggregation

UV excitation

→ minimization of fluorescence
→ significant increase of quantum yield (ω^4)
→ increased spatial resolution for Raman mapping experiments

SERS technique

→ avoid fluorescence
→ increased sensitivity

Raman spectroscopy
Powerful technique, which can easily be miniaturized!

Thanks!

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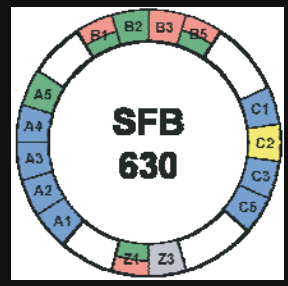
- Industrie



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