Raman Spectroscopy in Biogeology

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UNIVERSITÉ DE LYON

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Outline

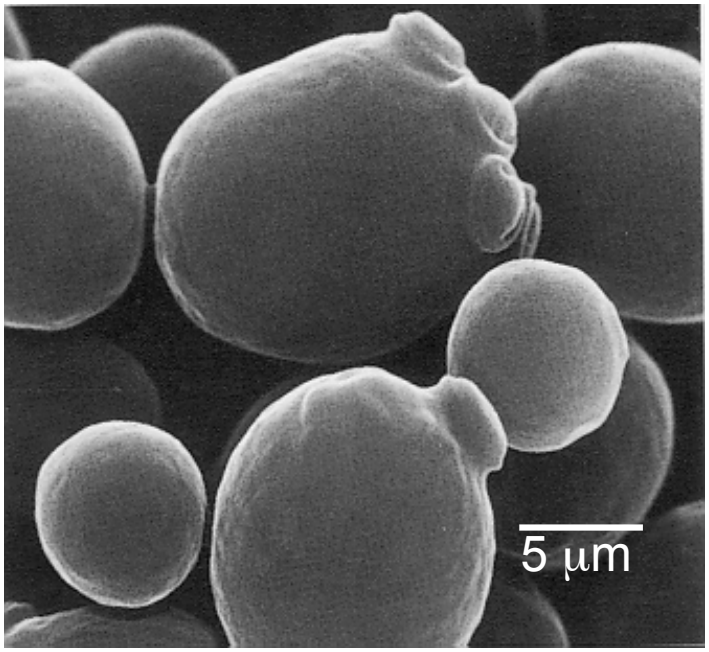
- Quantitative Raman spectroscopic analysis of microbial metabolic activity
- Raman spectroscopy for probing live cells
- Measurements of minute amount of biological and biotic products

Quantitative Raman spectroscopic analysis of microbial metabolic activity

The fermentation

by the baker yeast *Saccharomyces cerevisiae*

as a function of pressure



The yeast *S. cerevisiae* at high hydrostatic pressure

0.1 MPa
Optimal growth pressure

20-50 MPa
Cell cycle arrest

40-60 MPa
Internal acidification
Induction of stress transcriptional factors

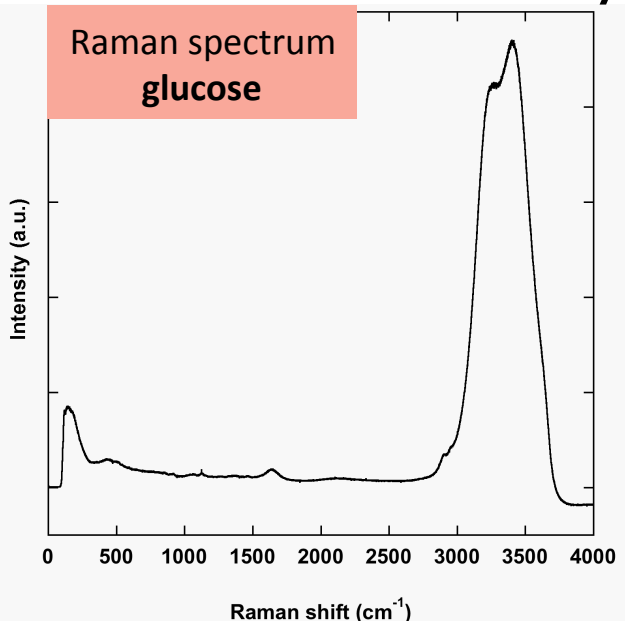
70-200 MPa
Induction of stress transcriptional profile

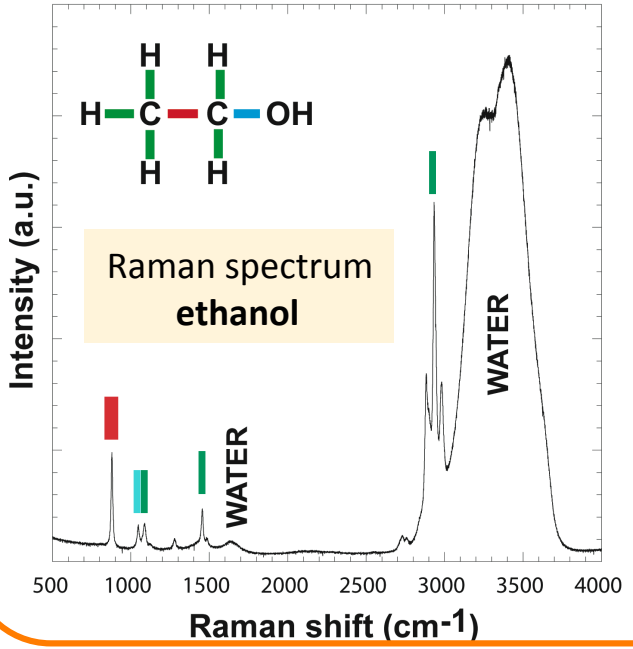
≥220 MPa
Death

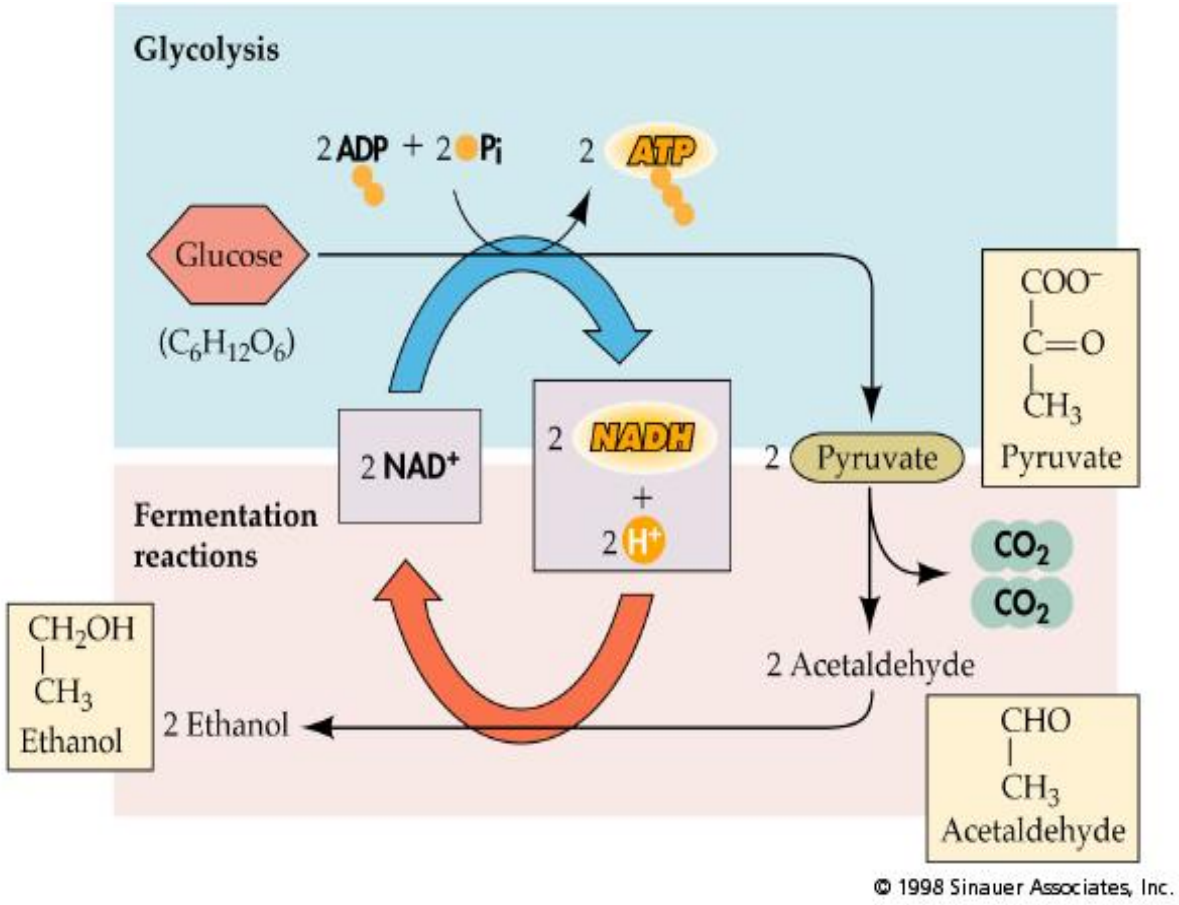
- The **eukaryotic model** for high-pressure studies
- No metabolic data for *S. cerevisiae* under high pressure
- Alcoholic fermentation well constrained at ambient pressure
- Ethanol easily detectable by Raman spectroscopy
- Arrest of alcoholic fermentation predicted at ca. 50 MPa (Abe *et al.* 2004)

Fernandes, et al., 2007

Characterization of alcoholic fermentation
by Raman spectroscopy



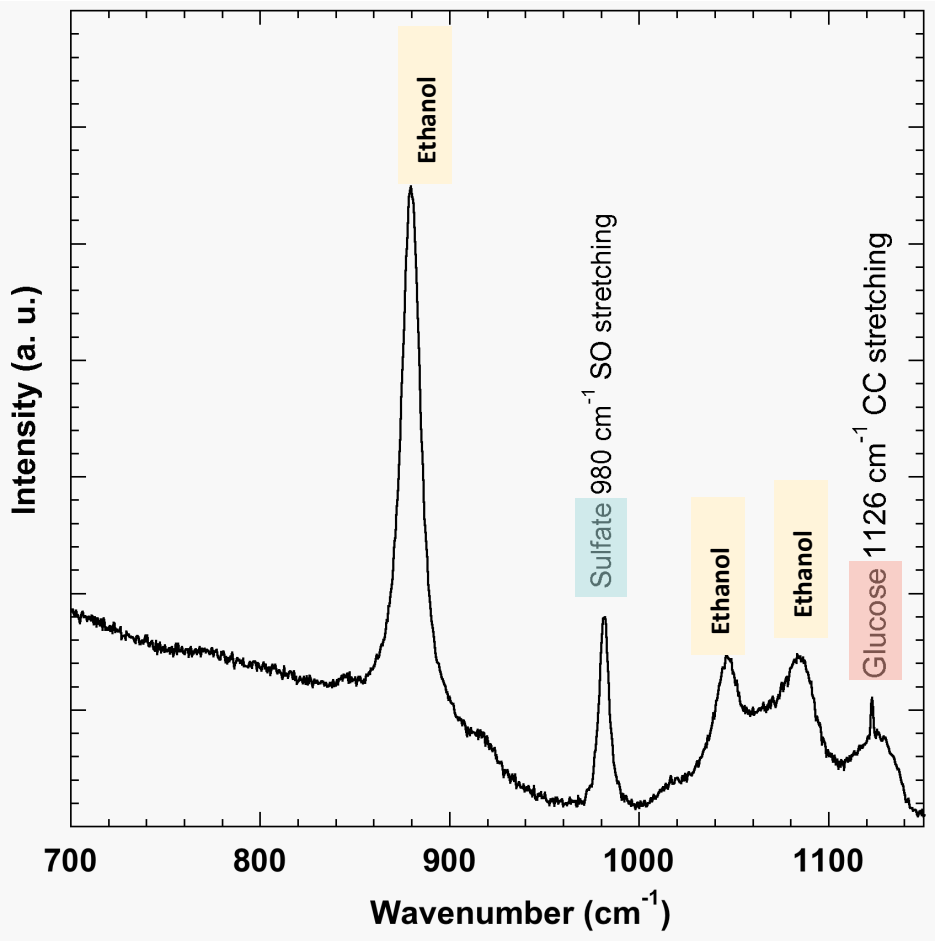




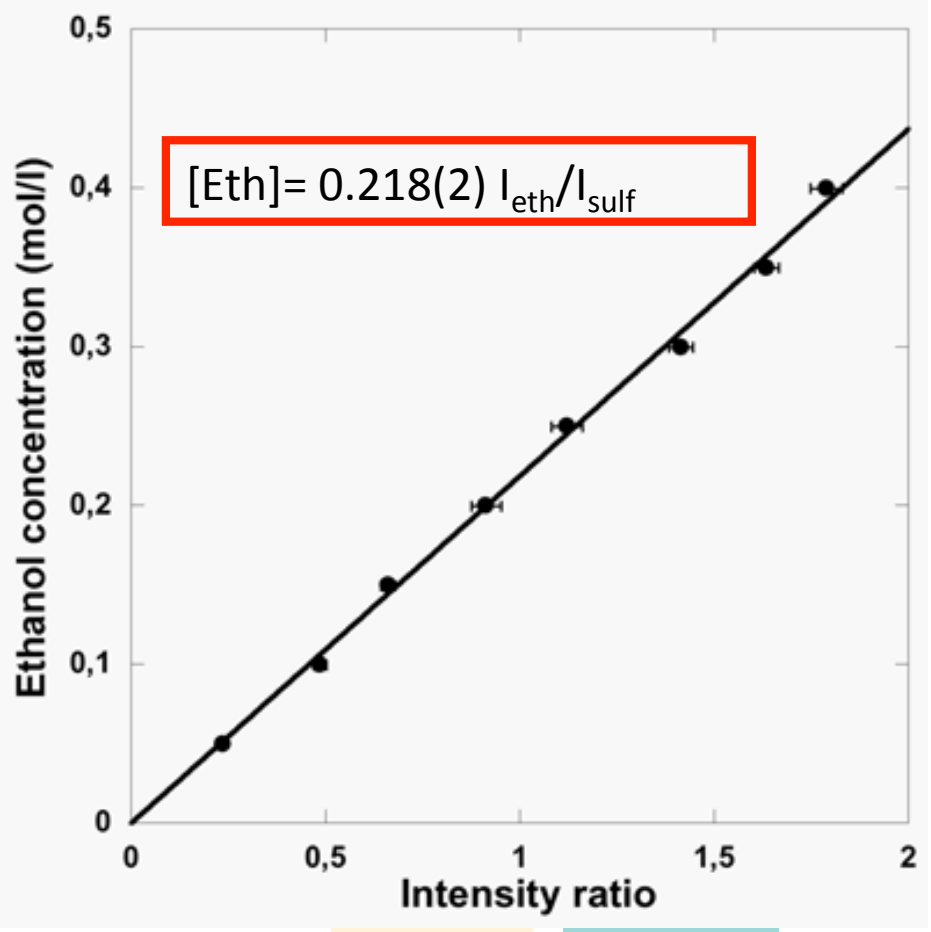
Symmetric C---C stretching mode of ethanol @ 883 cm^{-1}

Quantification of ethanol by Raman spectroscopy

… in the low-fluorescence culture medium



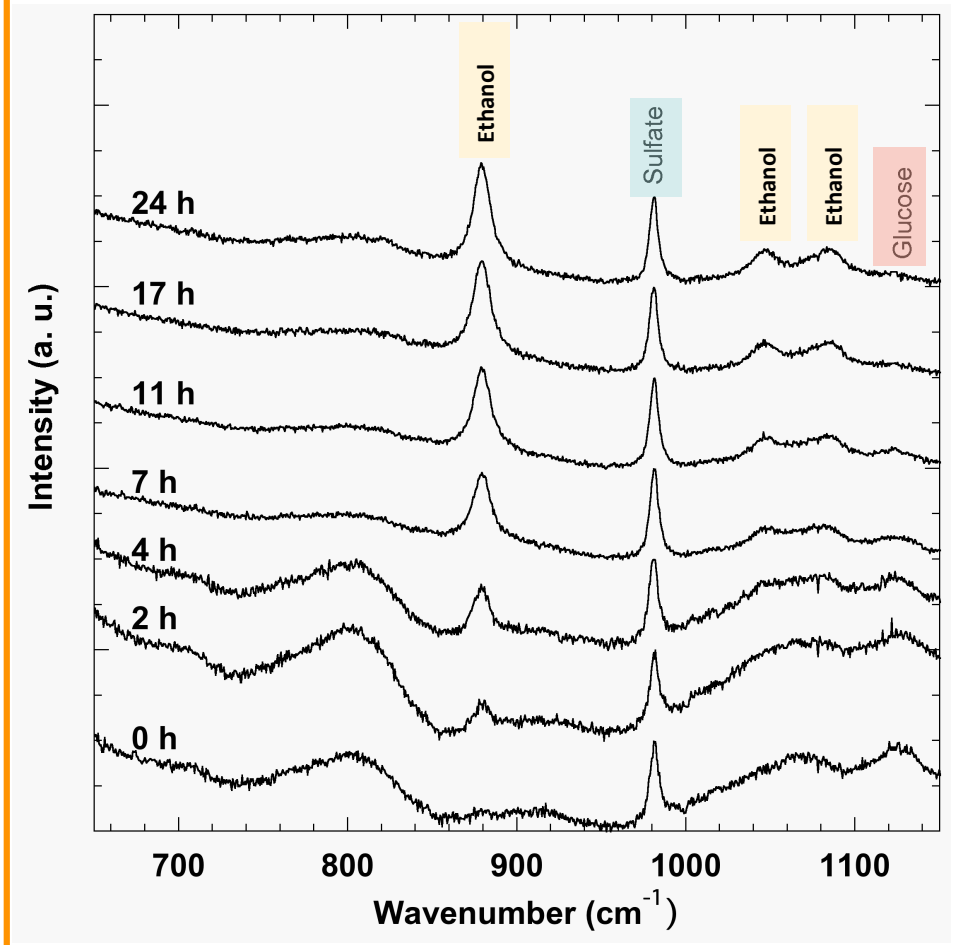
Calibration of
the normalised intensity of ν_s ethanol
as a function of ethanol titration



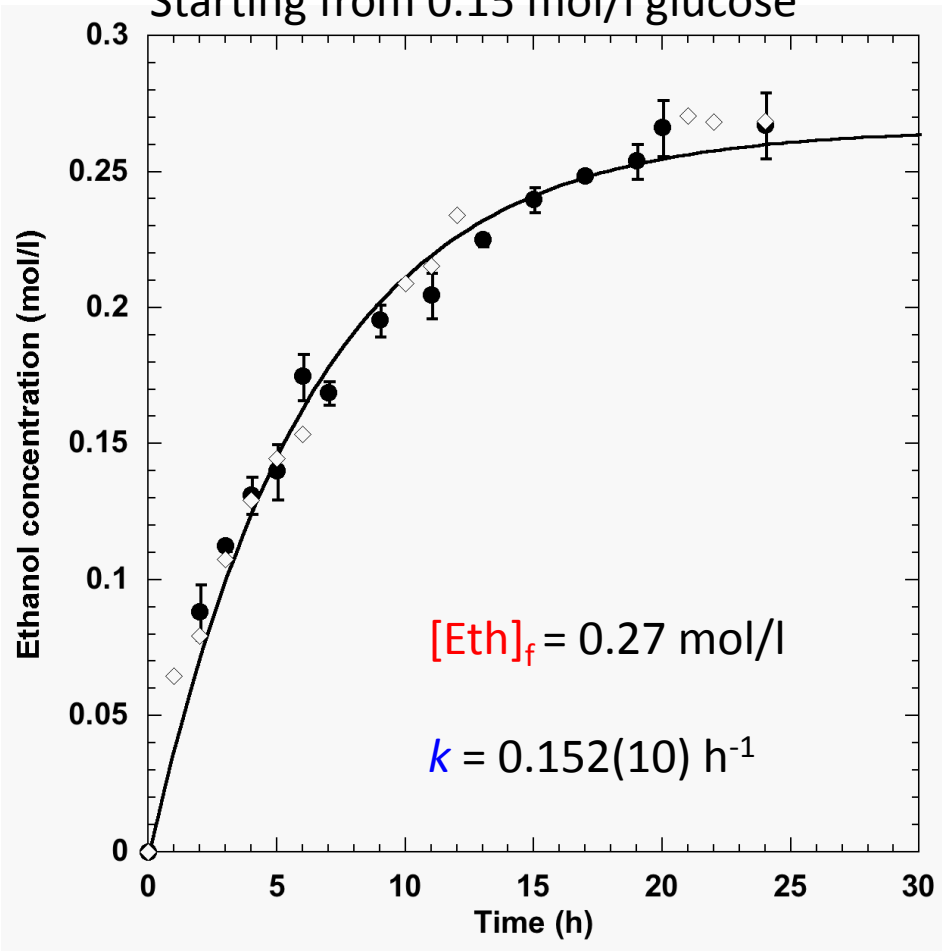
$I_{eth,\ 883}$ / $I_{sulf,\ 980}$

Experimental details:
- Jobin Yvon® HR800 spectrometer
- Ar laser, 514.53 nm, 40-50 mW
- 10x20 s acquisitions

Fermentation of *S. cerevisiae*, at ambient pressure



Starting from 0.15 mol/l glucose

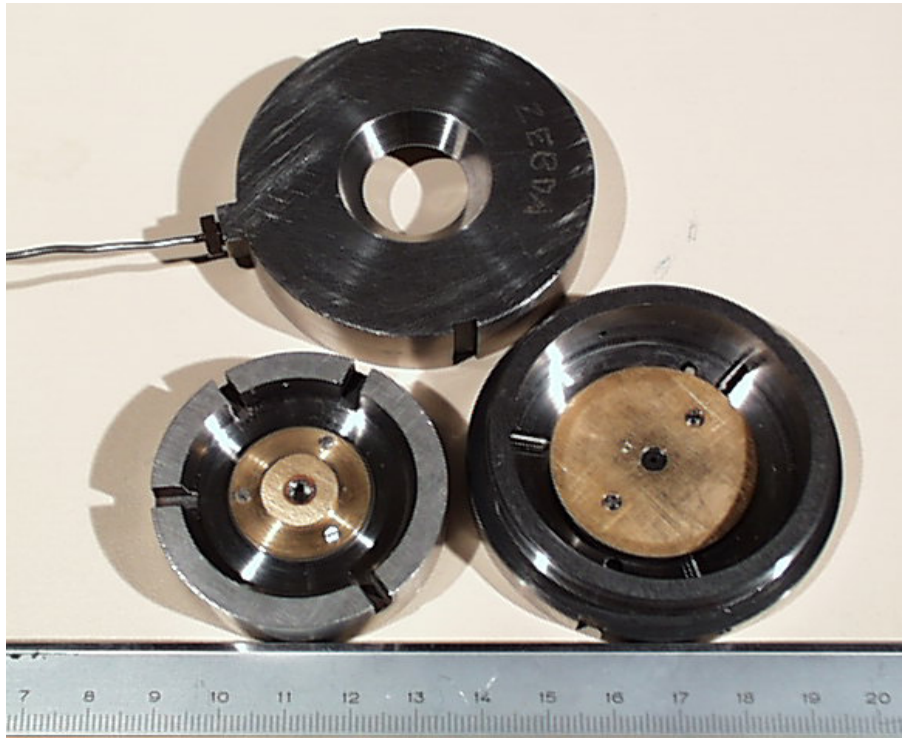


First order kinetic reaction

$[Eth] = 2[Glc](1-e^{-kt})$

Picard, A. et al., 2007. Extremophiles

Raman spectroscopy in a 'low'-pressure DAC



Coll. with J.C. Chervin, at IMPMC, Paris

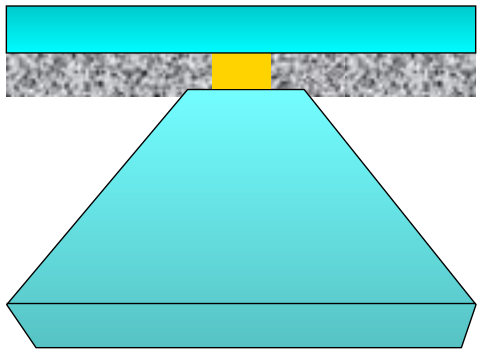
Pressure and temperature range

1.5 GPa

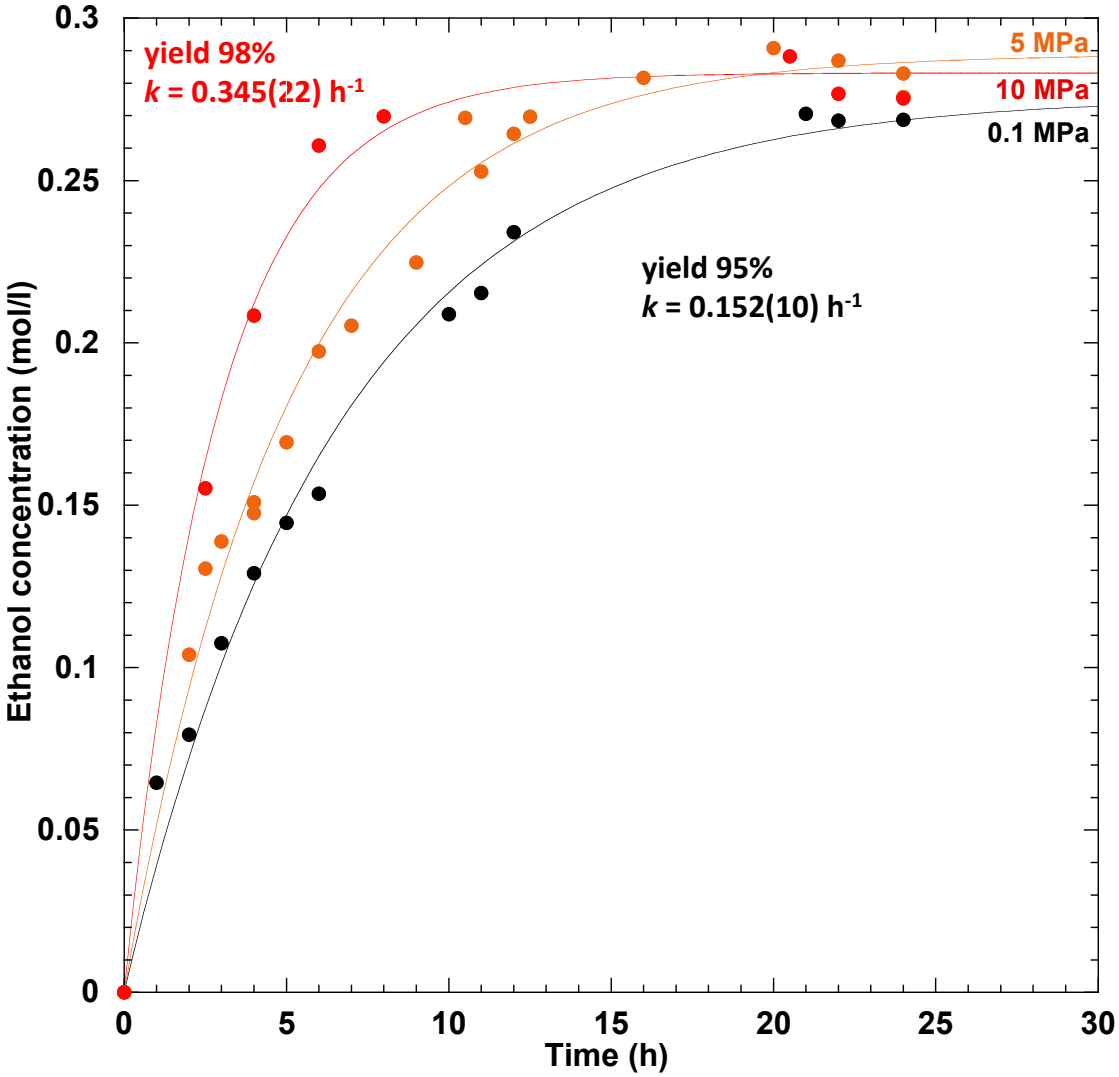
300 °C

sample 60 nl

- Diamond window 0.4 - 0.6 mm thick
- Ni gasket, 0.3 mm thick, 0.5 mm across
- Diamond anvil 2.2 mm thick, 1.4 mm culet



Fermentation of *S. cerevisiae* in the DAC, as a function of pressure



Observations : ambient to 10 MPa

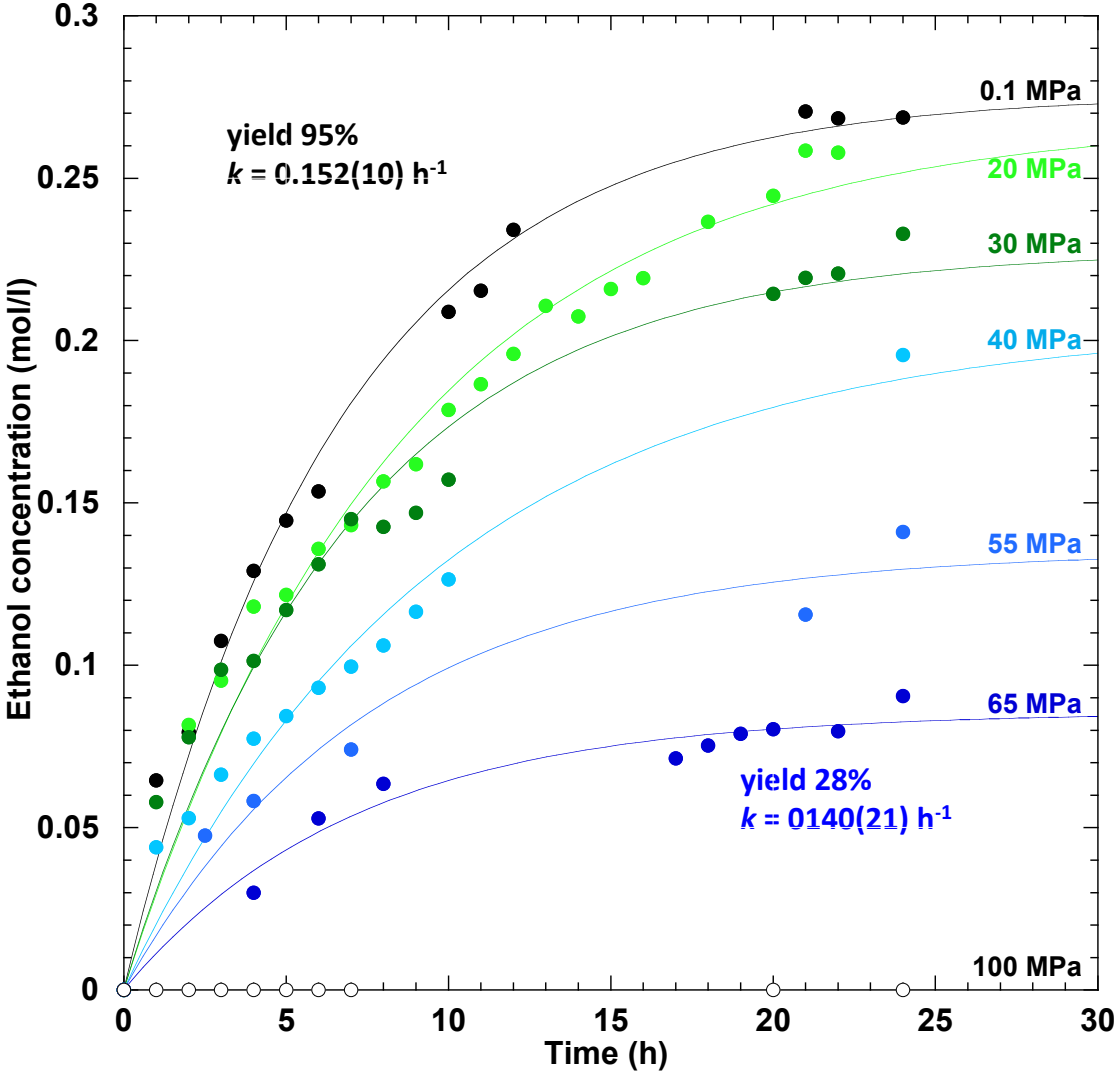
- reaction twice-thrice faster
- yield almost at the theoretical limit

Interpretation

- enhanced uptake of glucose
- enhanced activity of one/several enzymes of the glycolysis and/or fermentation pathways.
- no measurable lag phase tends to exclude pressure-induced increase in protein synthesis.
- more efficient expellation ethanol from the cell under pressure, due to an increase of passive diffusion.

Picard, A. et al., 2007. Extremophiles

Fermentation of *S. cerevisiae* in the DAC, as a function of pressure



Observations : above 10 MPa,

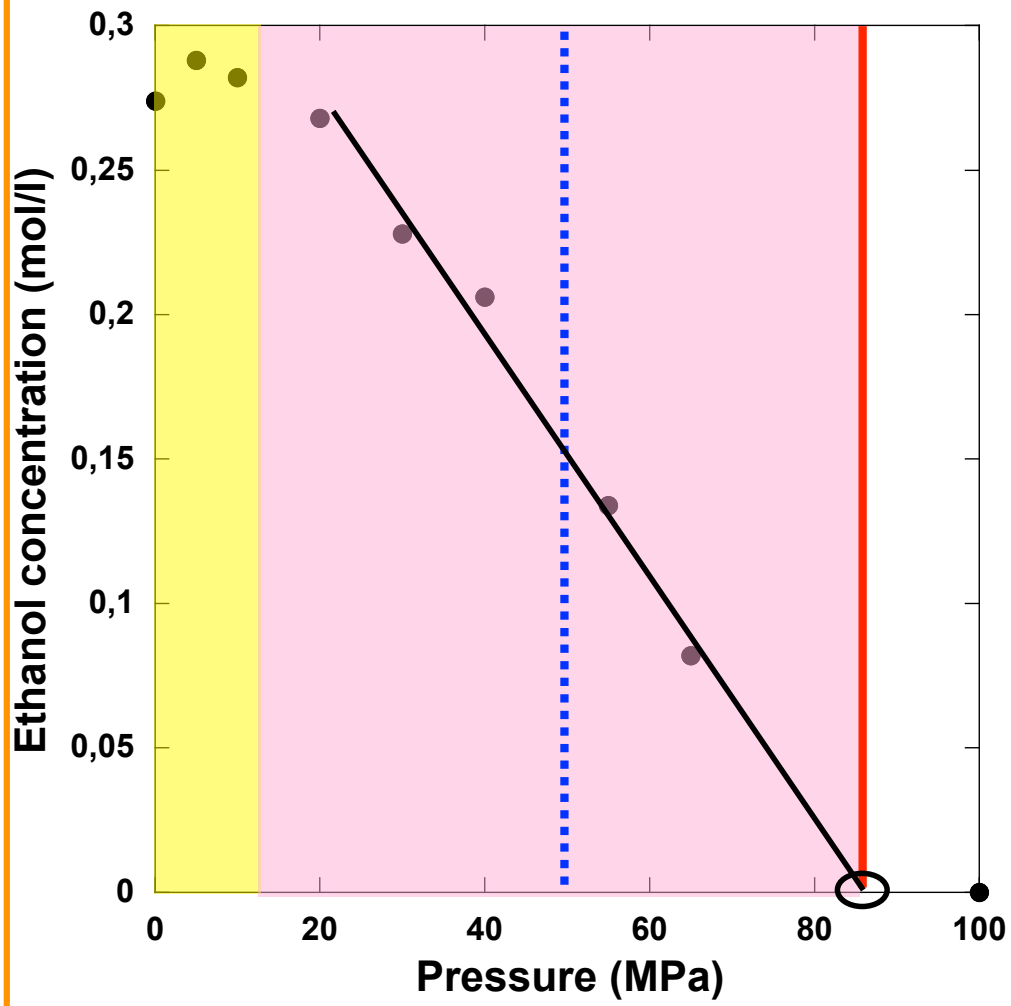
- yield decreases
- reaction rate almost constant

At 40 MPa, yield of 68%
similar to Abe & Horikoshi (1997)

Alcoholic fermentation stops between 65 and 100 MPa

Picard, A. et al., 2007. Extremophiles

Fermentation of *S. cerevisiae* in the DAC, as a function of pressure



Maximal pressure for ethanol fermentation calculated at **87±7 MPa**

37 MPa higher than the predicted value by Abe *et al.* (2004) and than pressure limit for growth

0-10 MPa

Activated steps of ethanol fermentation:

- Increased glucose import?
- Activation of glycolysis or fermentation pathway enzymes?
- Facilitated excretion of ethanol?

20-87 MPa

Decrease of final ethanol production:

- Loss of only 1 log after 24 hours at 70 MPa
- Progressive inhibition of enzymes?

Uncoupling of growth and metabolism

Raman spectroscopy for probing live cells

- CARS

Coherent anti-Stokes Raman Scattering

- Resonant Raman Scattering

- SERS

Surface Enhanced Raman Scattering

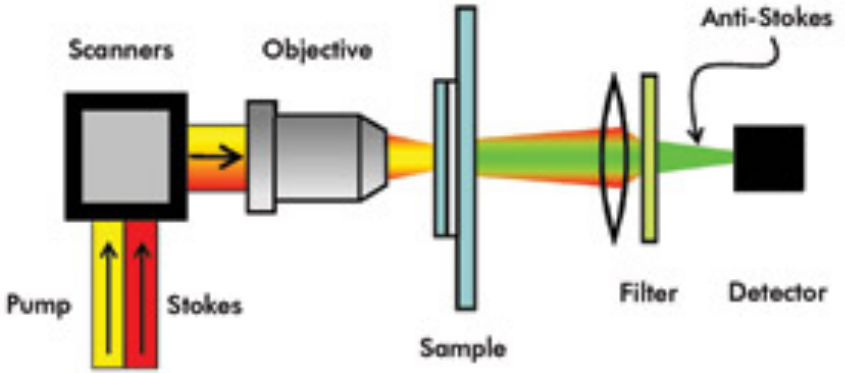
For 2D mapping

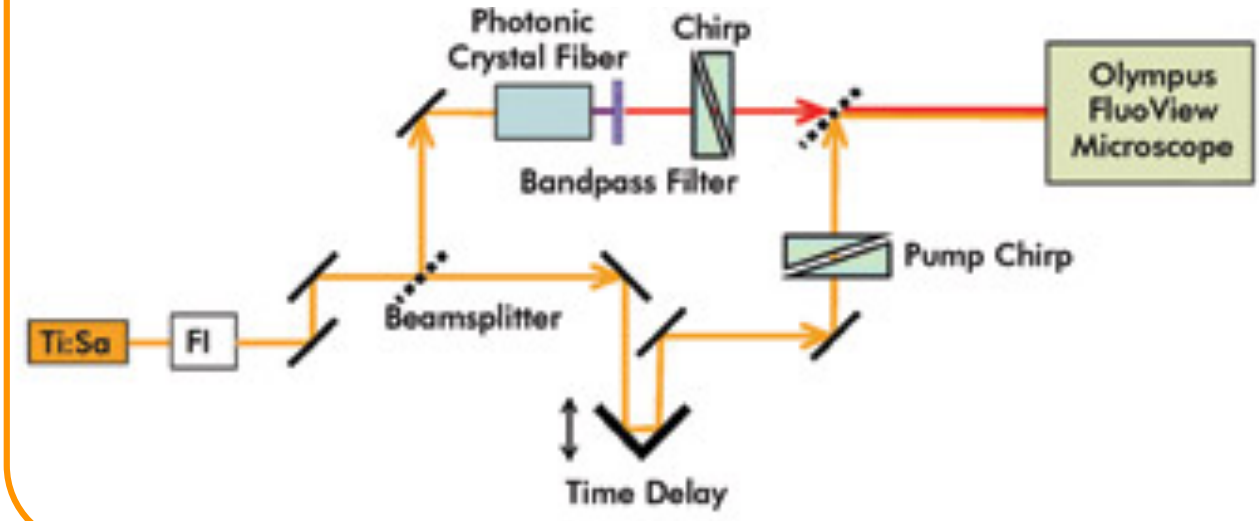
Coherent anti-Stokes Raman Scattering

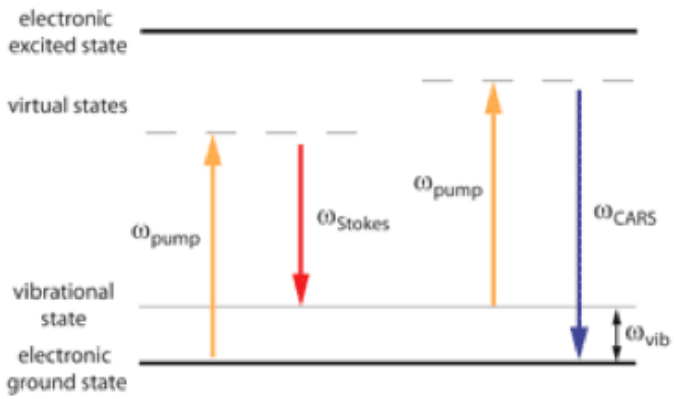
CARS microscopy provides label-free imaging

Non linear spectroscopy
A simple CARS microscope.
A beamsplitter splits pulses from an isolated femto-second Ti:sapphire or Nd:vandate laser.

Half goes to a photonic crystal fiber to generate the Stokes pulses, followed by a bandpass filter, before being recombined on a dichroic mirror.



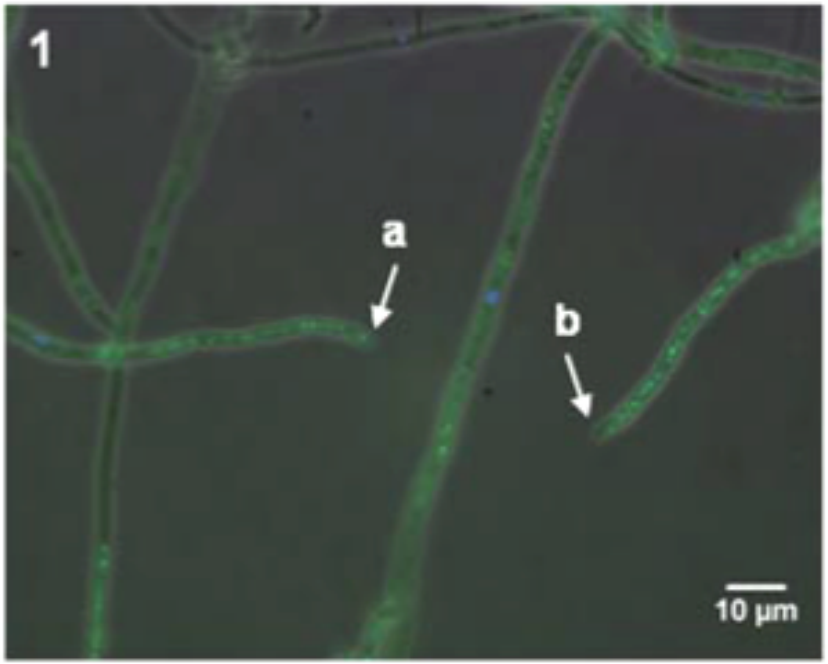


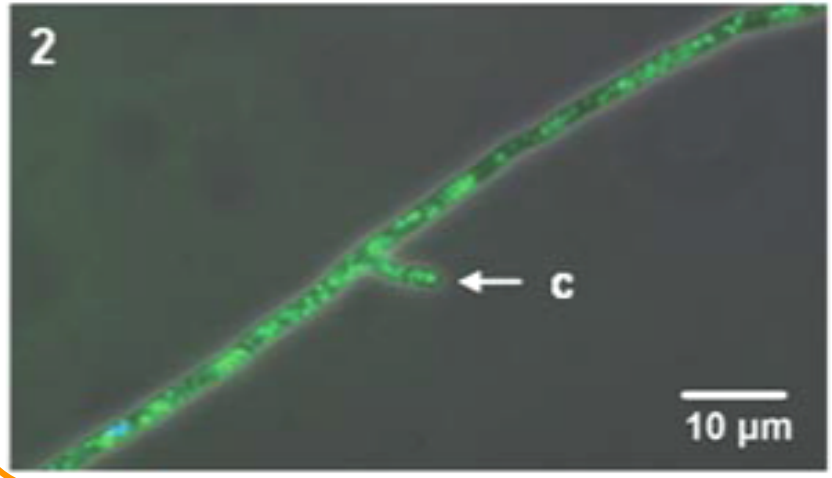


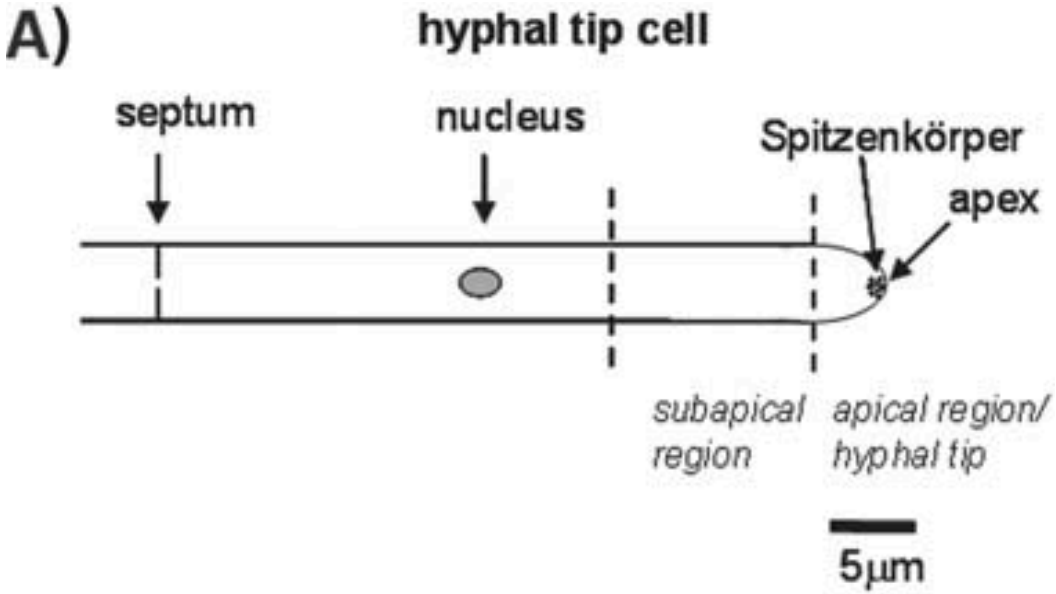
$$\omega_{CARS} = 2\omega_{pump} - \omega_{Stokes}$$

p://www.photonics.com/Article.aspx?AID=40014

Analysis of cytochrome distribution







The cytochrome distribution in hyphal tip cells of the fungi *Schizophyllum commune*

Walter et al. 2010, Analyst

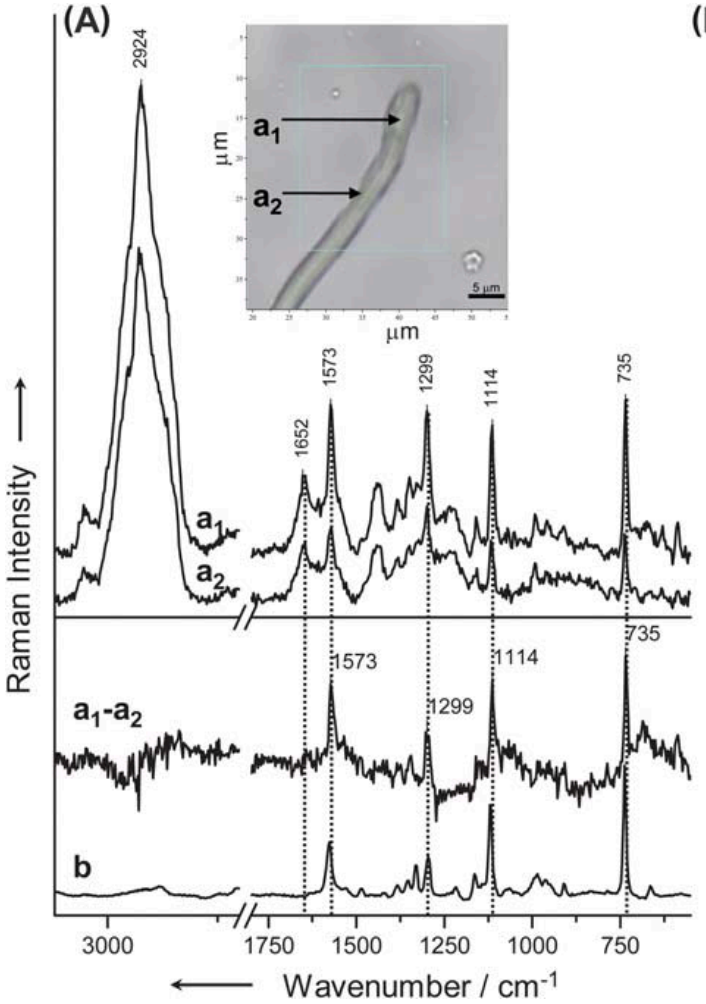
Resonant Raman spectra of a hyphal tip cell of *S. commune*

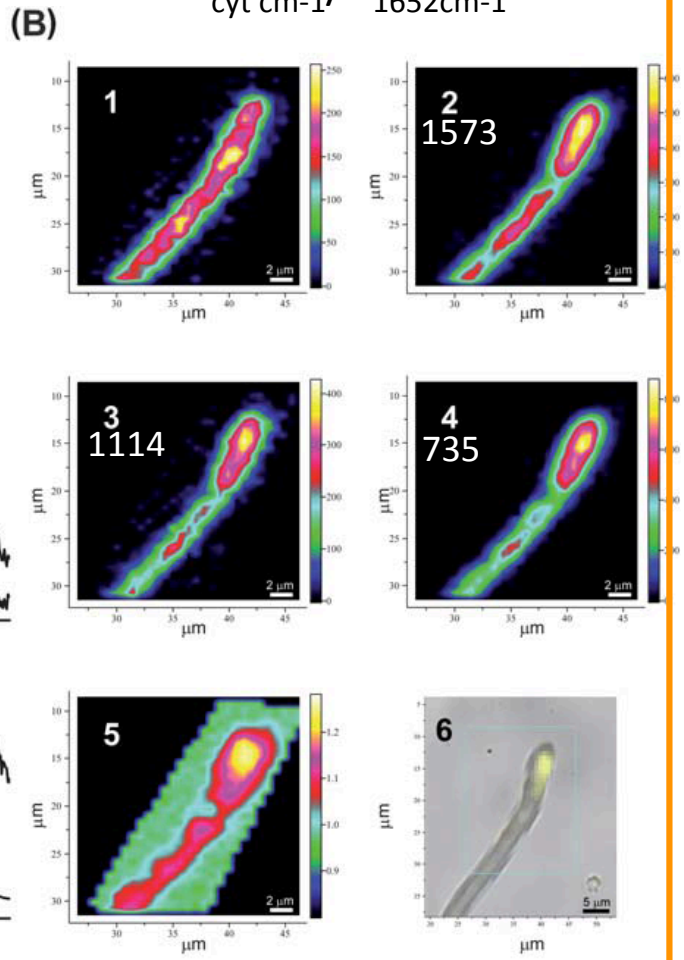
- 2924 cm^{-1}
asymmetric CH-stretching vibration of methylene groups from all cell constituents

- 1652 cm^{-1}
protein, lipid and polysaccharide vibrations

- 1573, 1299, 1114, 735 cm^{-1}
cytochrome vibrations

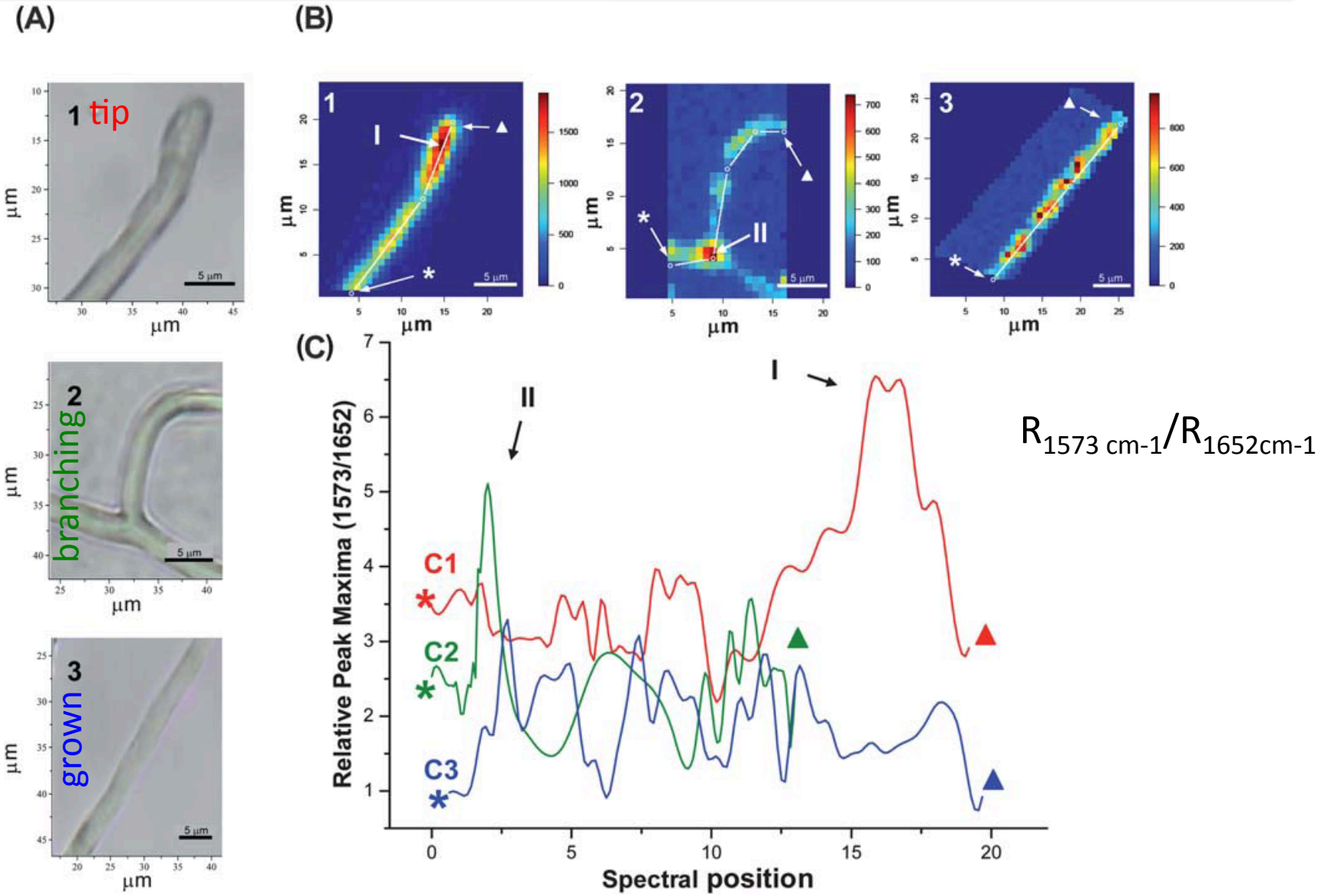
- Spatial resolution 0.7 μm
λ_0 = 532 nm **resonant** with the electronic absorption of cytochrome





Walter et al. 2010, Analyst

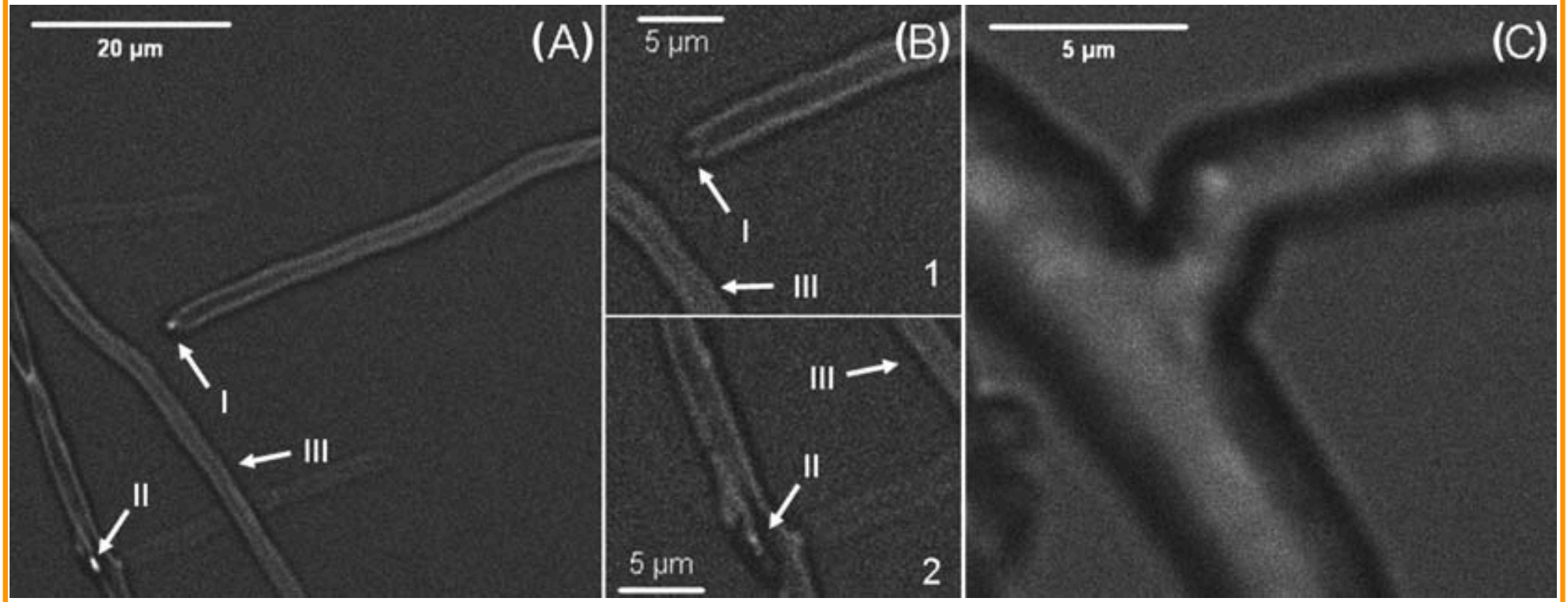
Resonant Raman spectra of a hypha of *S. commune*



$R_{1573\ cm\text{-}1}/R_{1652cm\text{-}1}$

Walter et al. 2010, Analyst

CARS images of the fungal hyphae



@1572 cm^{-1}
cytochrome marker band
spec. resolution 20 cm^{-1}

@1552 cm^{-1} under non resonant conditions

@2990 cm^{-1}, CH vibration
spatial resolution 30x30 nm^2
spec. resolution 110 cm^{-1}

In less than 25 s

Walter et al. 2010, Analyst

Measurements of minute amount of biological and biotic products

- by SERS

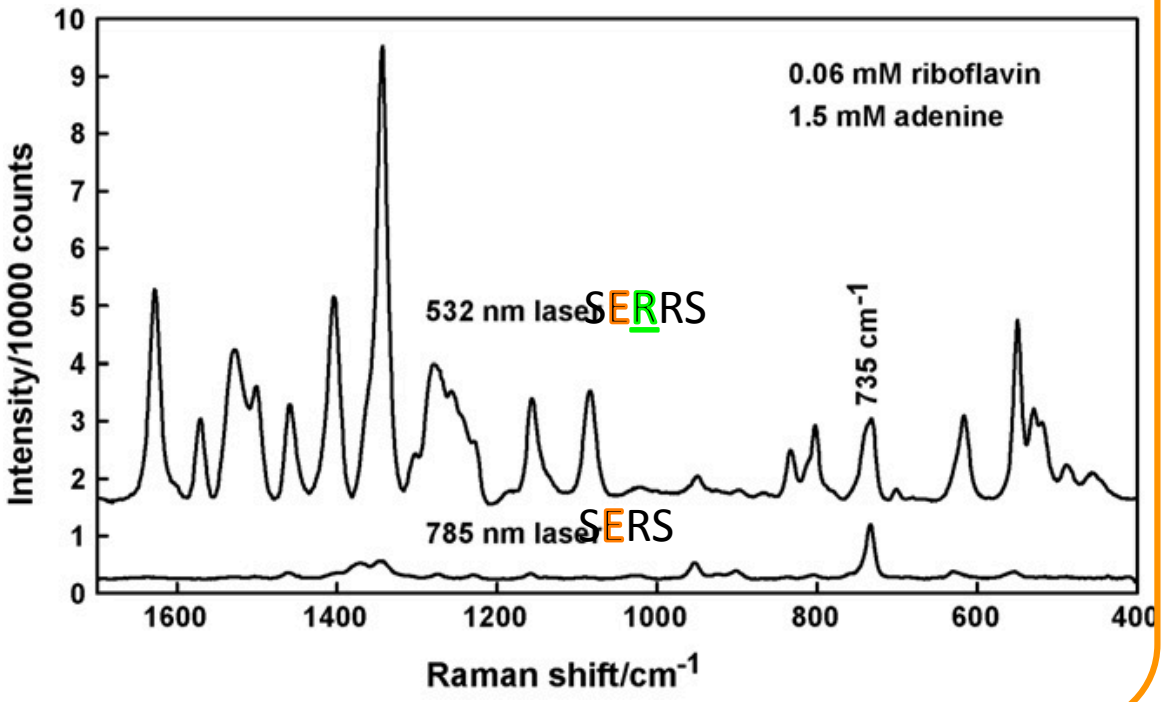
Surface Enhanced Raman Spectroscopy

- by SERRS

Surface Enhanced Resonant Raman Spectroscopy

relies on the enhancement
of the EM field
by a substrate

… requires contact between
the analyte
and metal nanoparticles



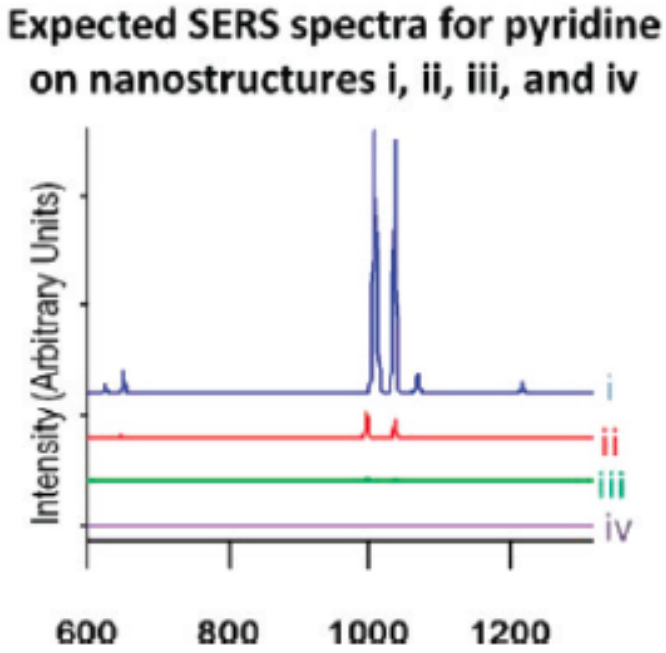
Halvorson et al. 2010, Environ. Sci. Technol.
Smith-Palmer et al. 2010, Vibrational Spectroscopy

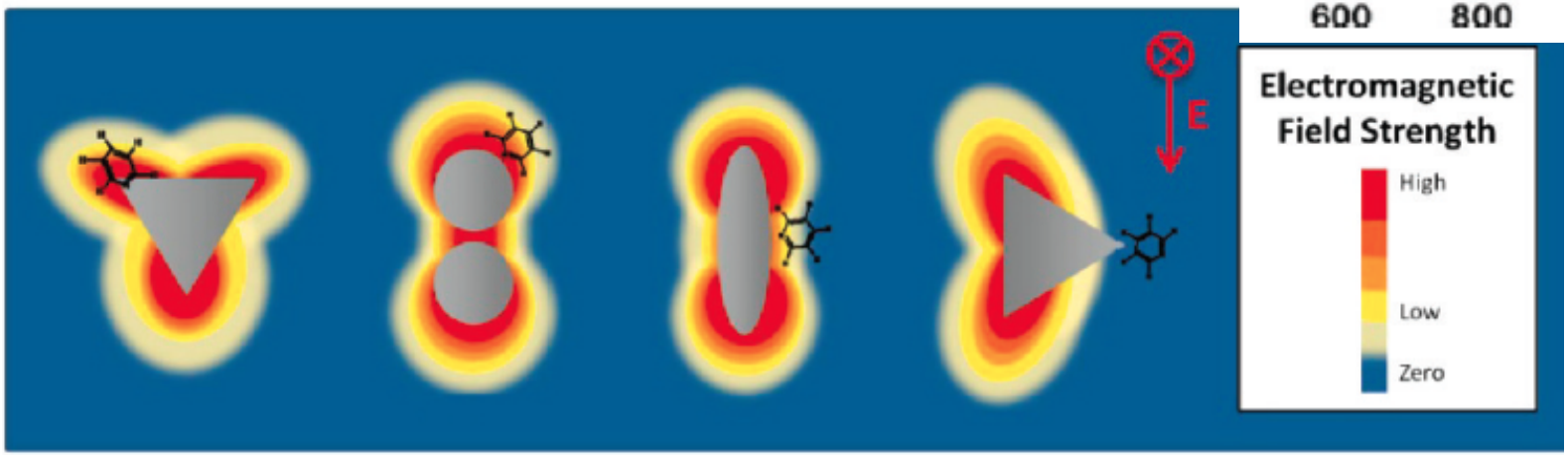
SERS & SERRS

✓ EM enhancement occurs when
the incident laser excites surface plasmons
(electrons at the metal surface that collectively oscillate upon excitation)
thereby creating an electromagnetic field
extending up to 20 nm from the metal
enhancement x10^4 up to 10^{11}

✓ CT enhancement when transfer of electrons between the analyte and metal, x10-100

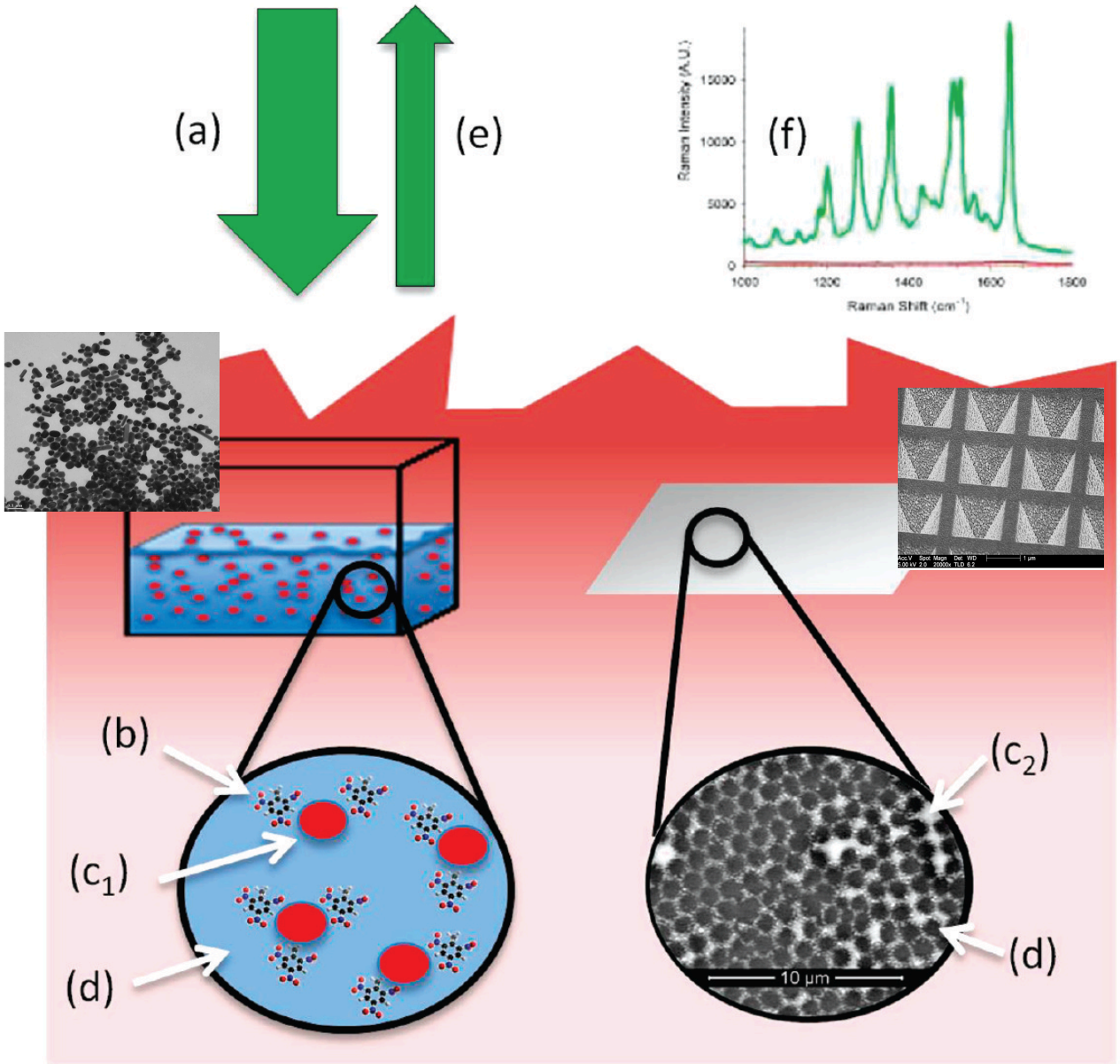
✓resonance enhancement if the laser wavelength falls near an absorption wavelength of the sample

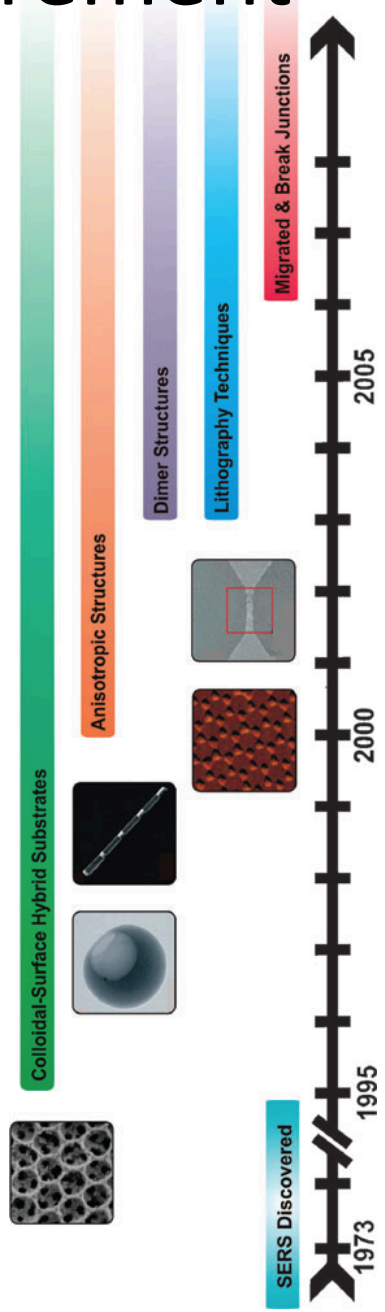




Halvorson et al. 2010, Environ. Sci. Technol.
Smith-Palmer et al. 2010, Vibrational Spectroscopy

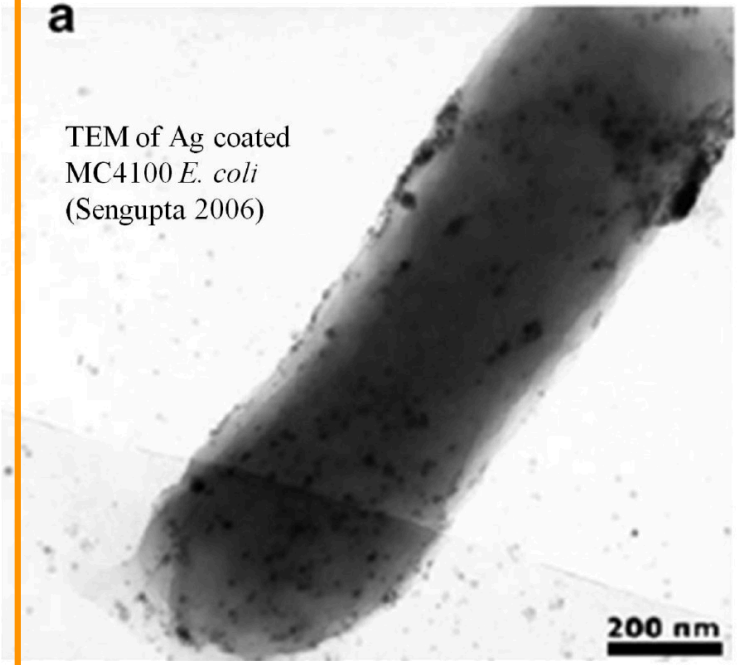
Components of a SERS measurement

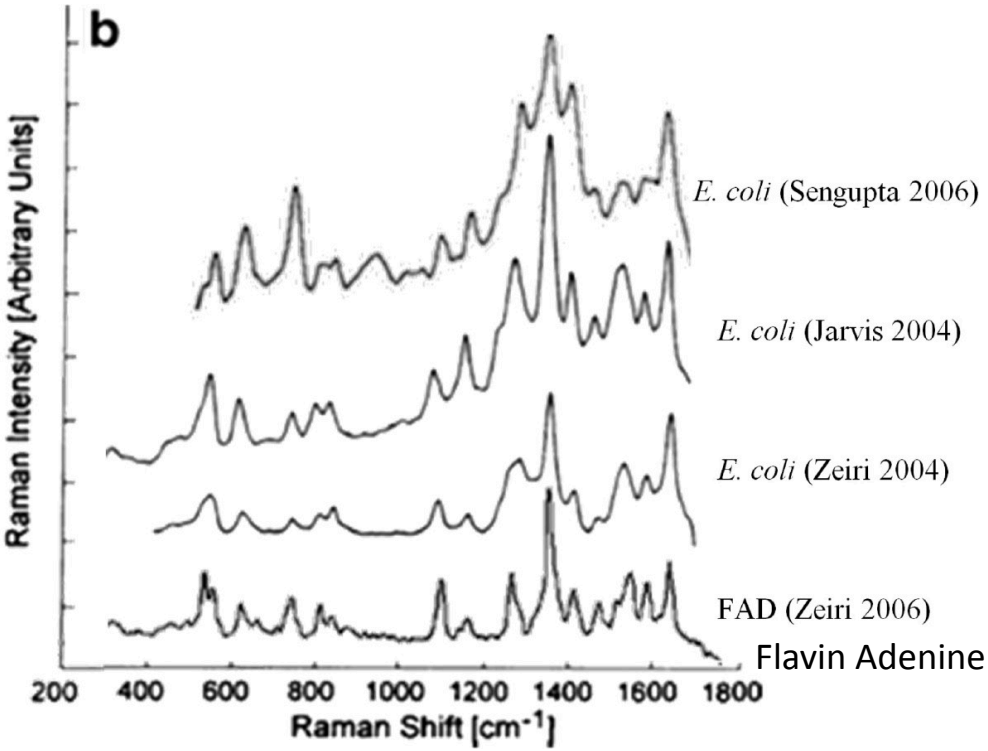




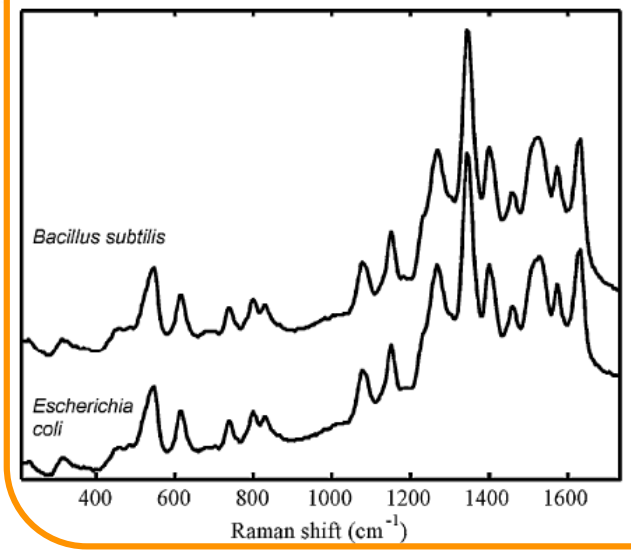
Halvorson et al. 2010, Environ. Sci. Technol. ; Banholzer et al. 2008 Chem. Soc. Rev.

SER spectra of bacteria @332 cm^{-1}



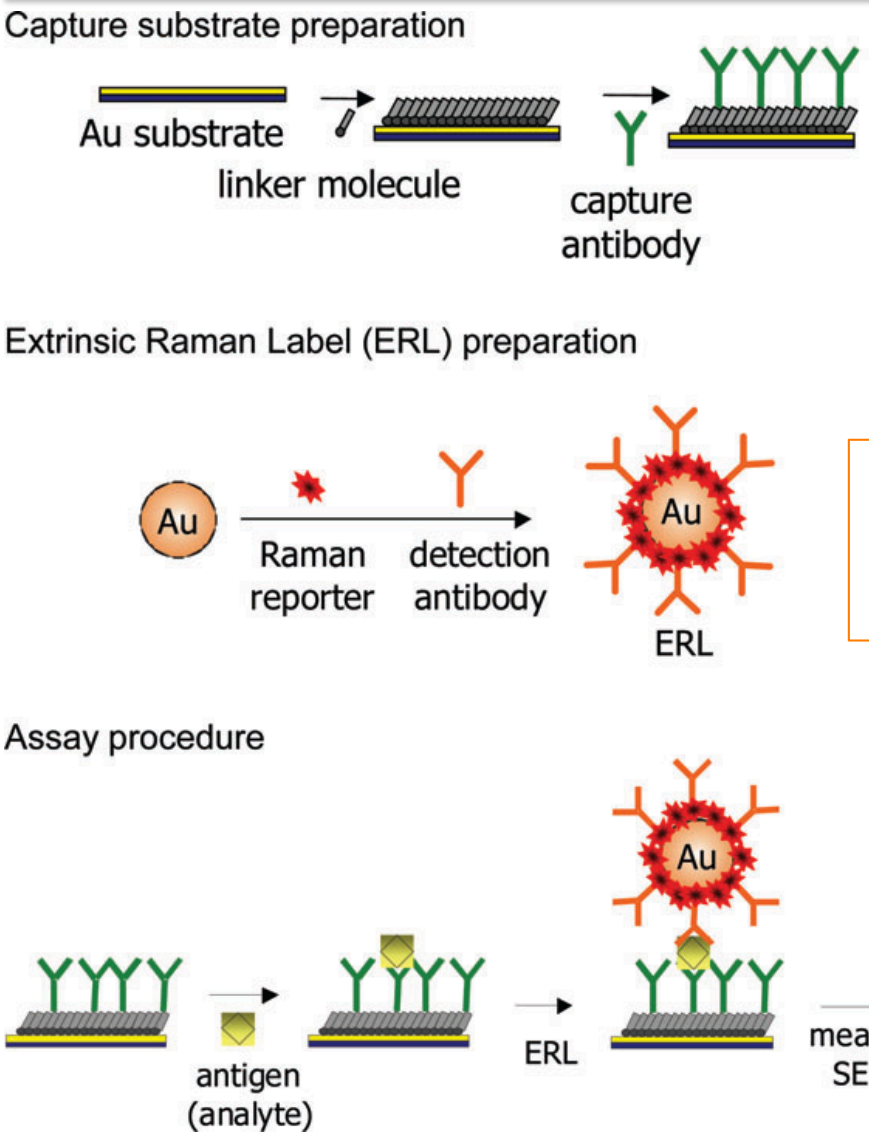


Flavin Adenine Dinucleotide



Halvorson et al. 2010, Environ. Sci. Technol.
Jarvis & Goodacre, 2008, Chem. Soc. Rev.

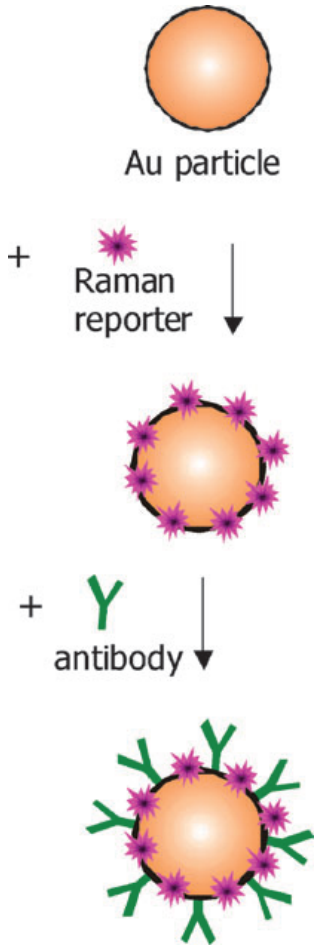
SERS-based immunoassays



Immunoassay platform

With ***femtomolar*** detection of the analyte

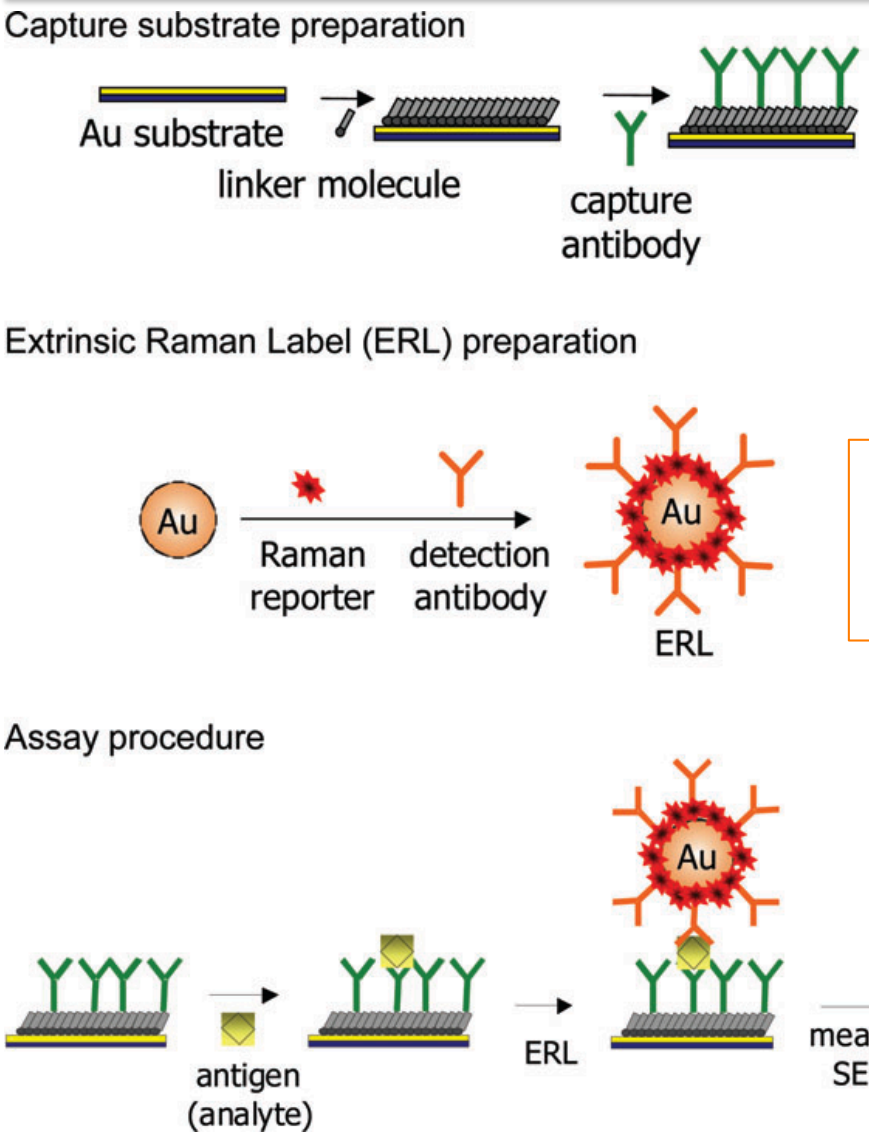
With 10^3-10^5 reporters on each particle



SER(R)S of the extrinsic Raman labels (ERLs)

Tutorial review by Porter et al. 2008, Chem. Soc. Rev.

SERS-based immunoassays



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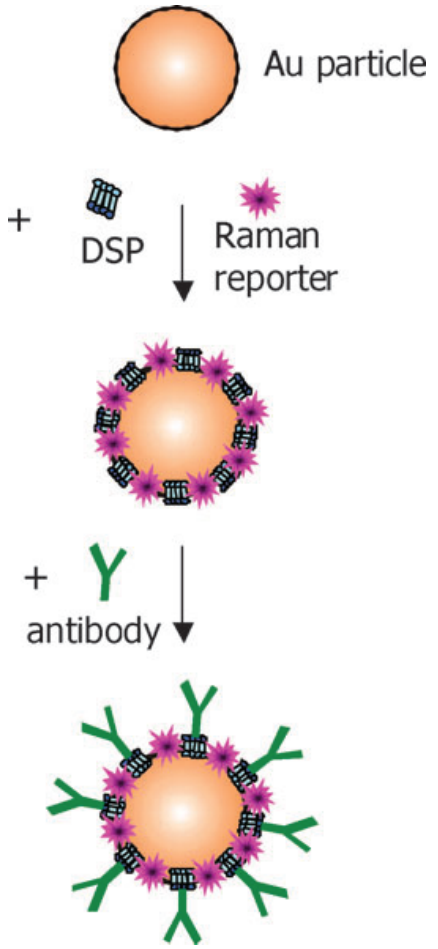


With
10^3-10^5 reporters
on each particle

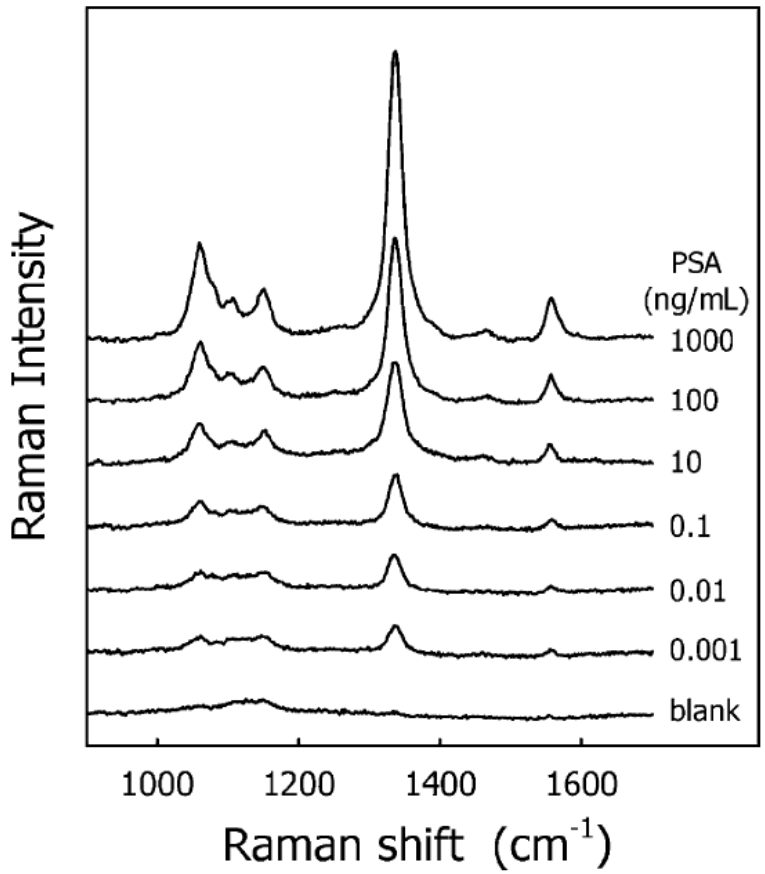
SER(R)S of the extrinsic Raman labels (ERLs)

Immunoassay platform

Tutorial review by Porter et al. 2008, Chem. Soc. Rev.

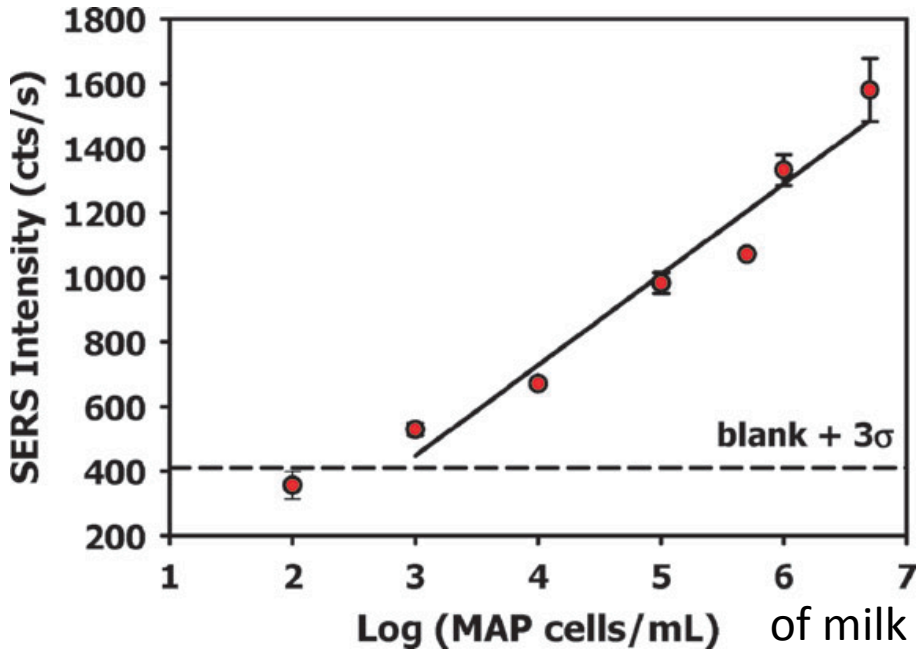
SERS-based immunoassays, examples

early disease diagnosis
detection of prostate specific antigen (PSA)
a 33 kDa glycoprotein



ERL: 30 nm gold part. with a monolayer of DSNB
30s acq; LOD= 1 pg.mL^{-1} or 30 fg

Pathogen detection
Mycobacterium avium subsp. paratuberculosis
(MAP)

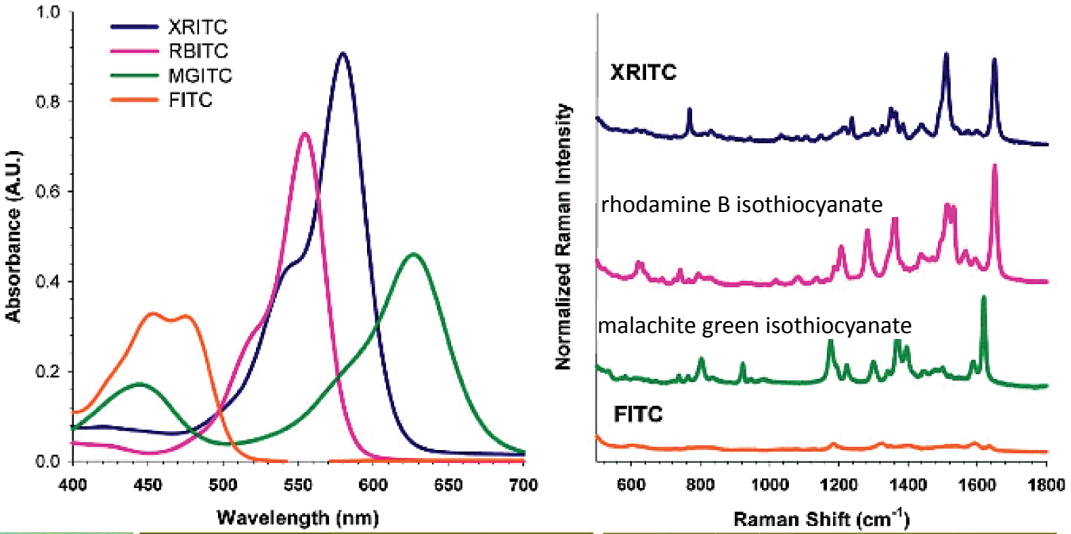


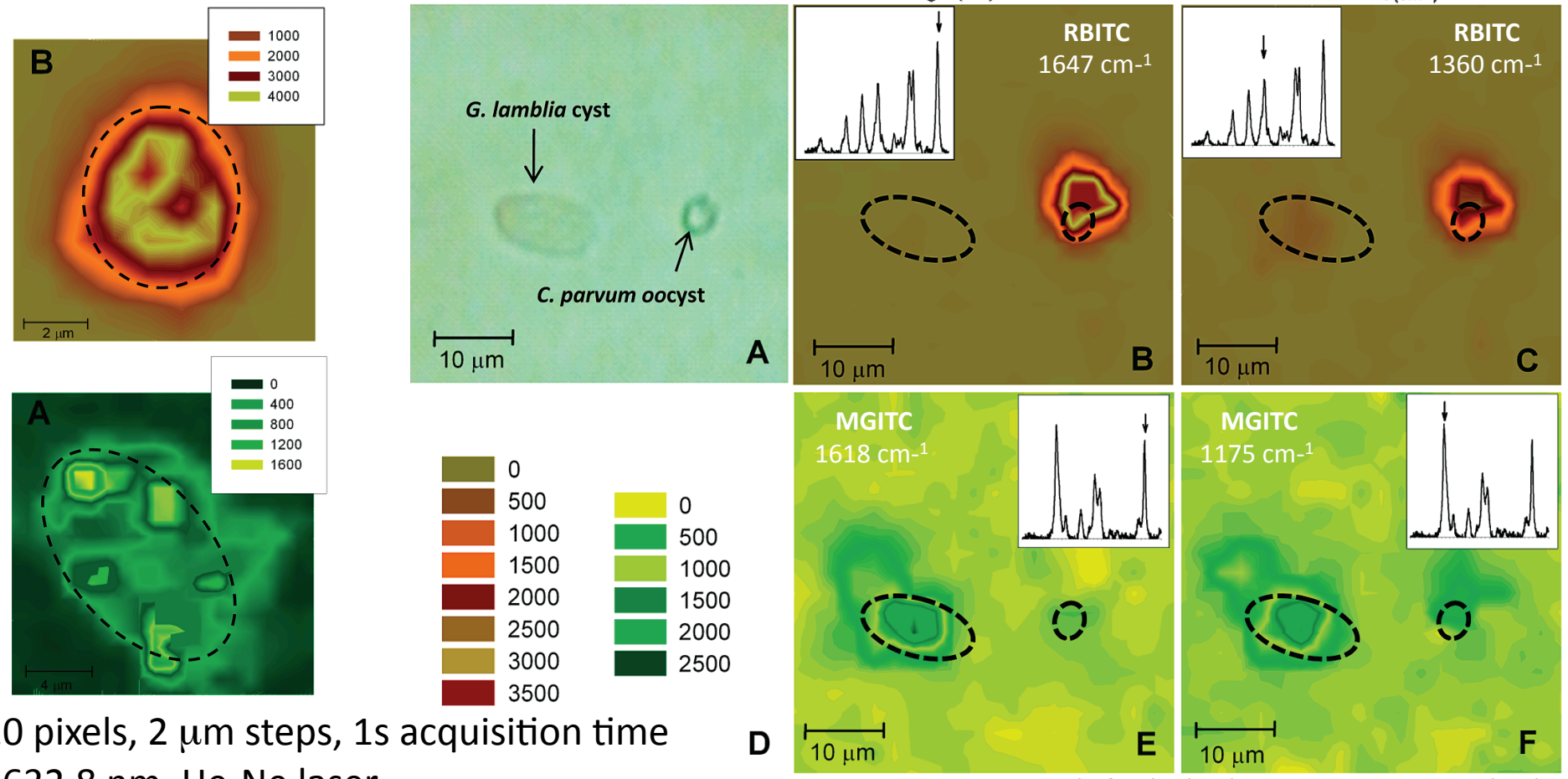
of milk

Classical culture method: 12-14 weeks
SERS (sample preparation, antigen extraction, ERL incubation, and readout): 24hrs
LOD 1000 MAP mL^{-1}

Tutorial review by Porter et al. 2008, Chem. Soc. Rev.

An exemple of SERRS immunogold labeling for aquatic pathogens



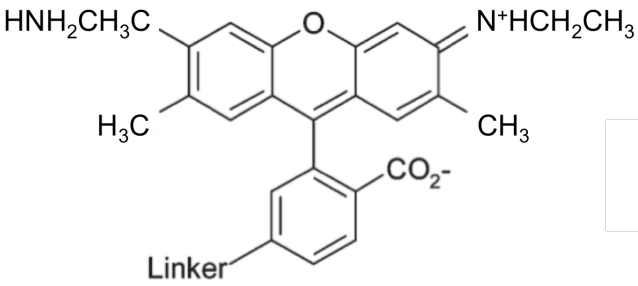


20x20 pixels, 2 µm steps, 1s acquisition time

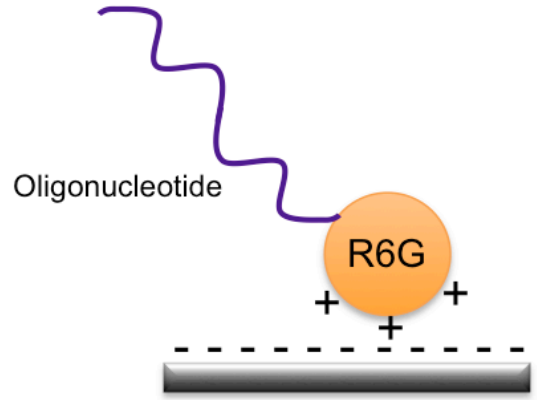
λe = 632.8 nm, He-Ne laser

Rule & Vikesland 2009, Environ. Sci. Technol.

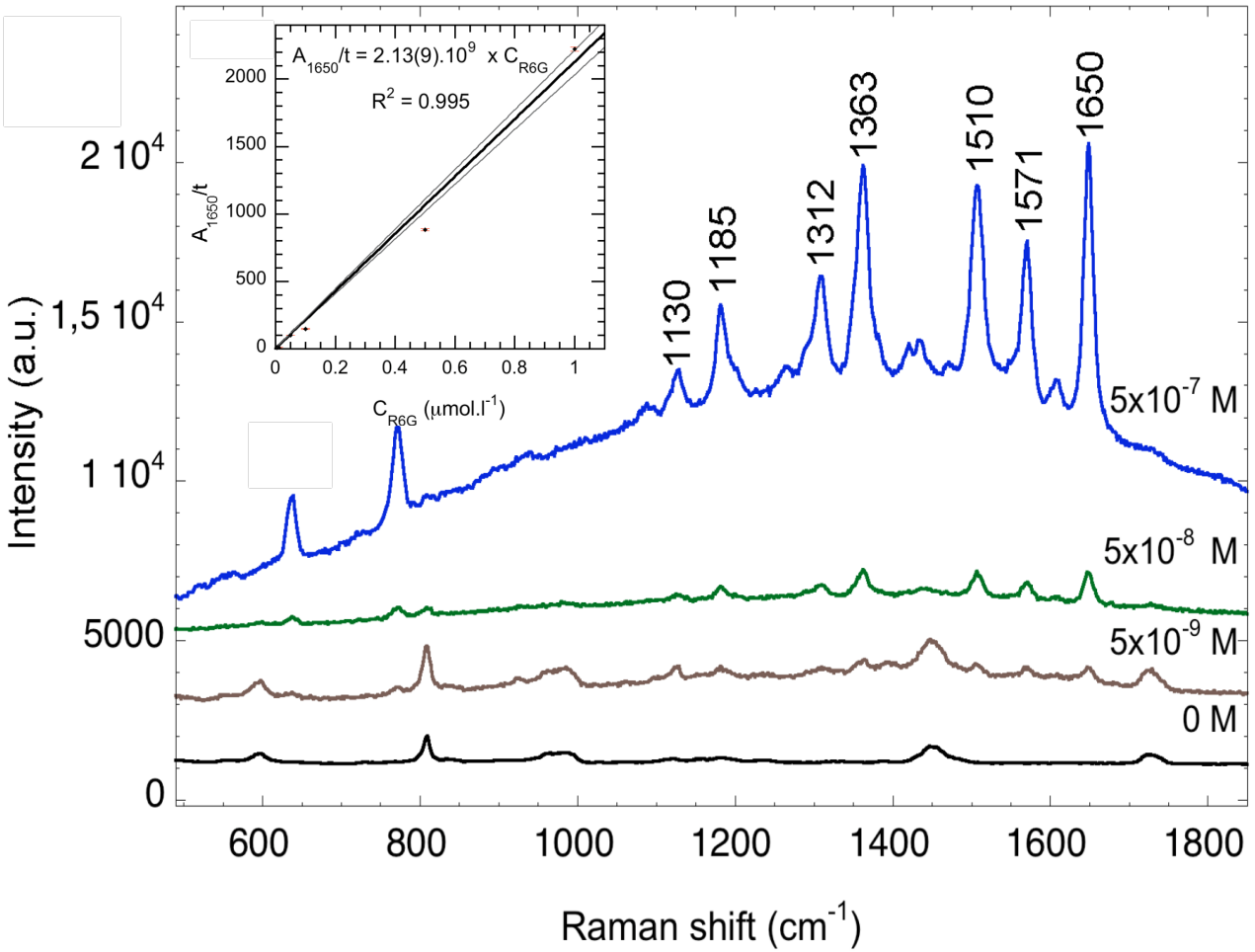
SERRS detection of minute amount of DNA



Rhodamine 6G (R6G)



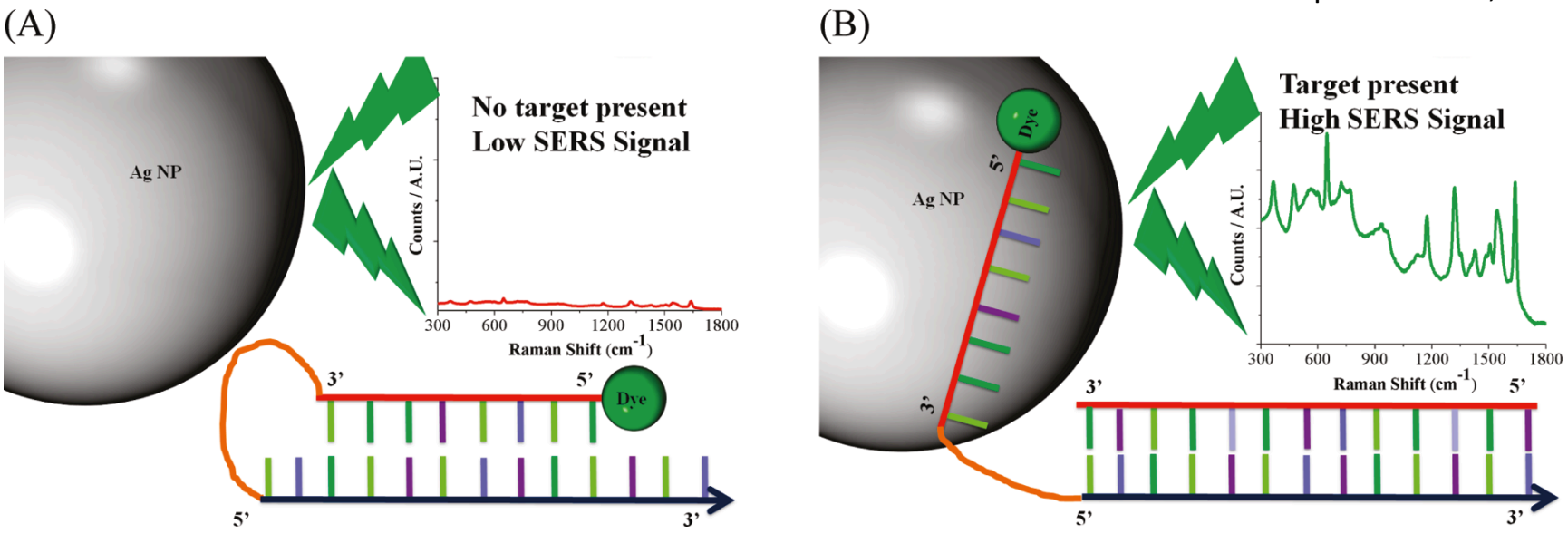
R6G-labeled oligonucleotide



Feuillie et al, 2011, PlOs One

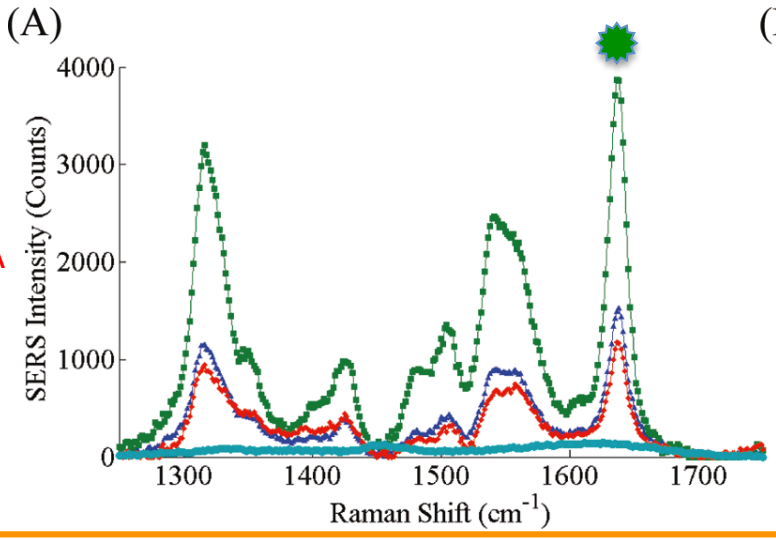
SERRS on single-stranded DNA

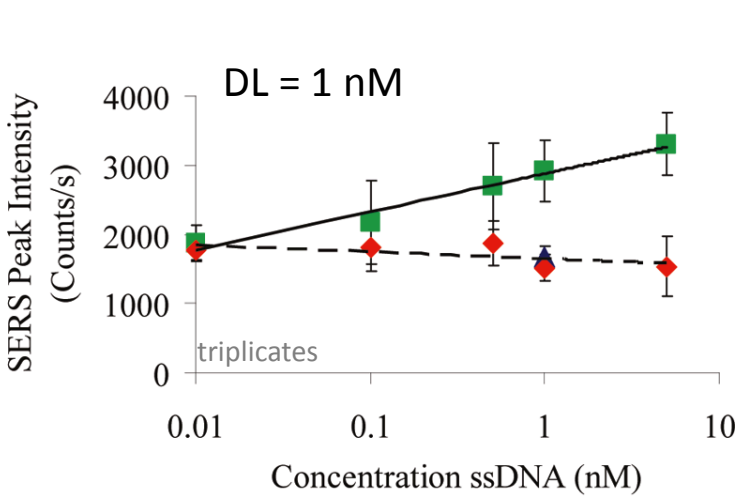
Van Lierop et al. 2011, Anal. Chem.



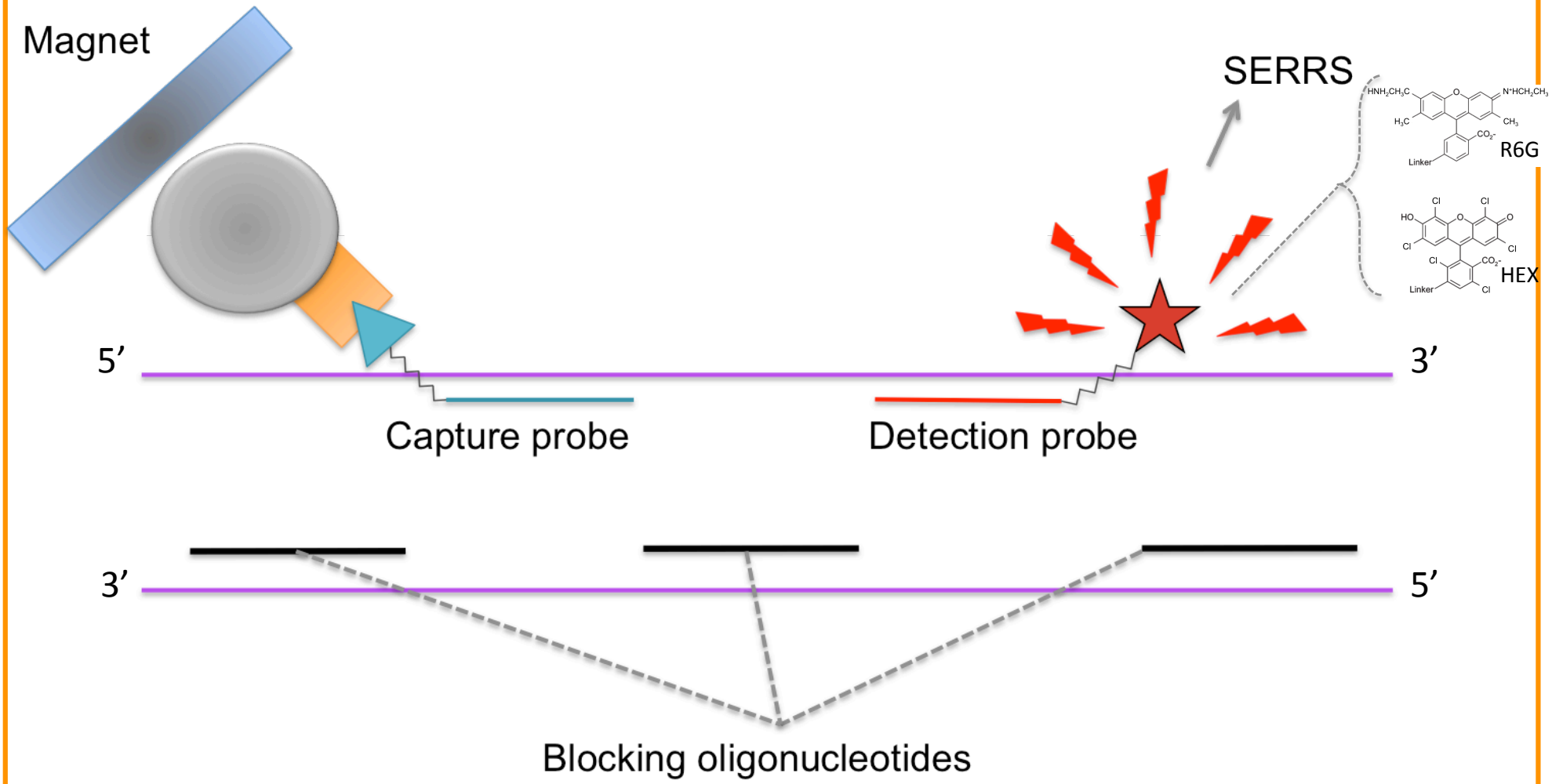
Blank
SERS primer
+ 1nm target DNA
+1 nM non-target DNA

3x 1s





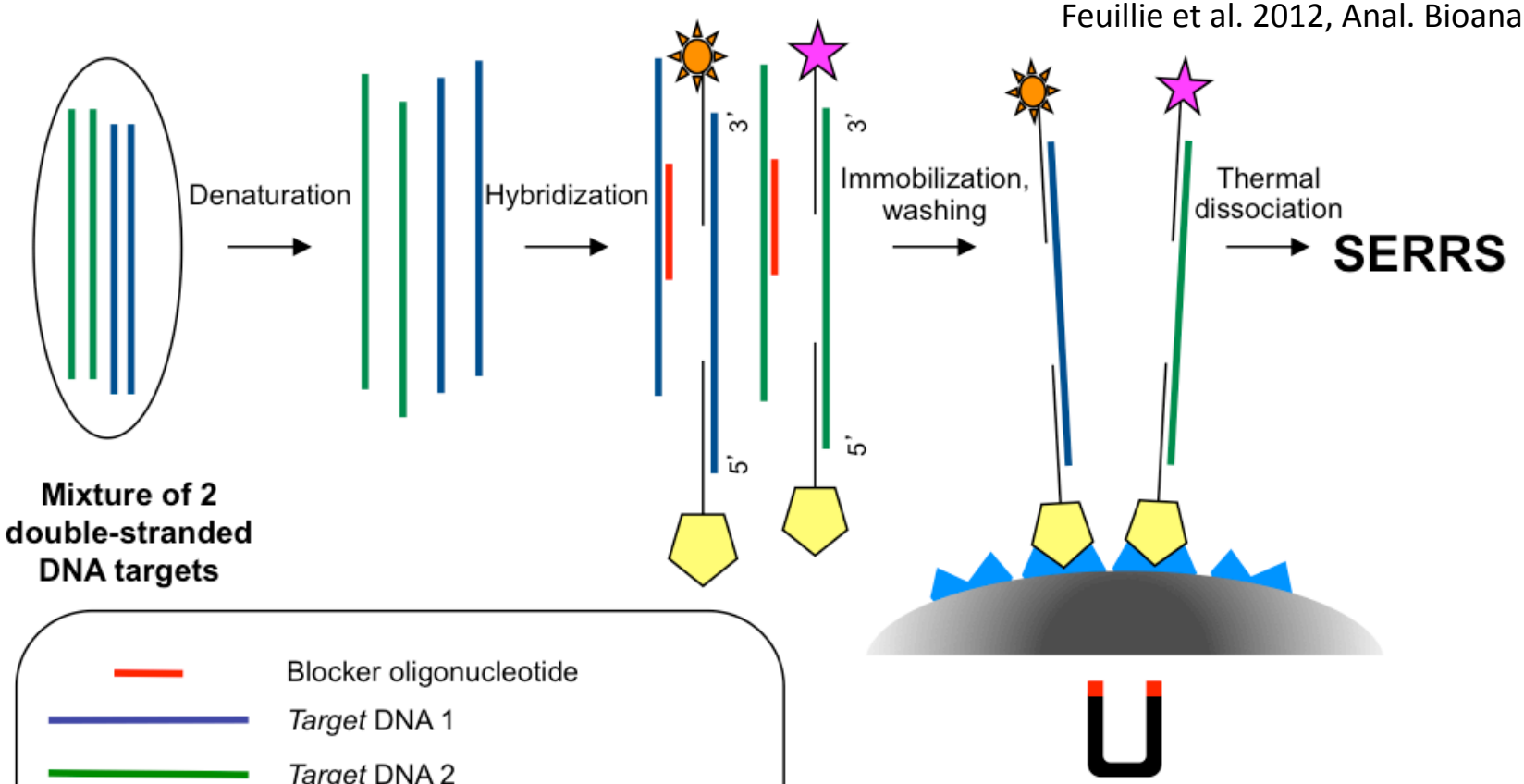
SERRS on double-stranded DNA



Feuillie et al, 2012, Anal. Bioanal. Chem.

A SERRS hybridization assay for ds DNA

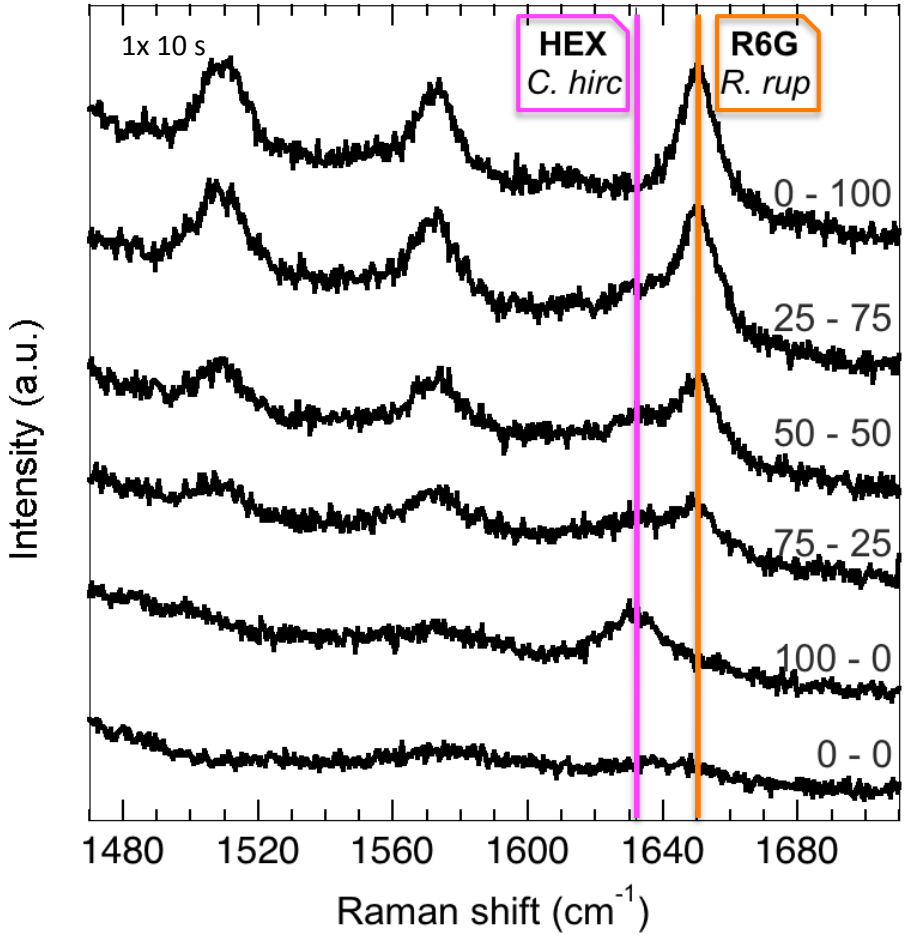
Feuillie et al. 2012, Anal. Bioanal. Chem.



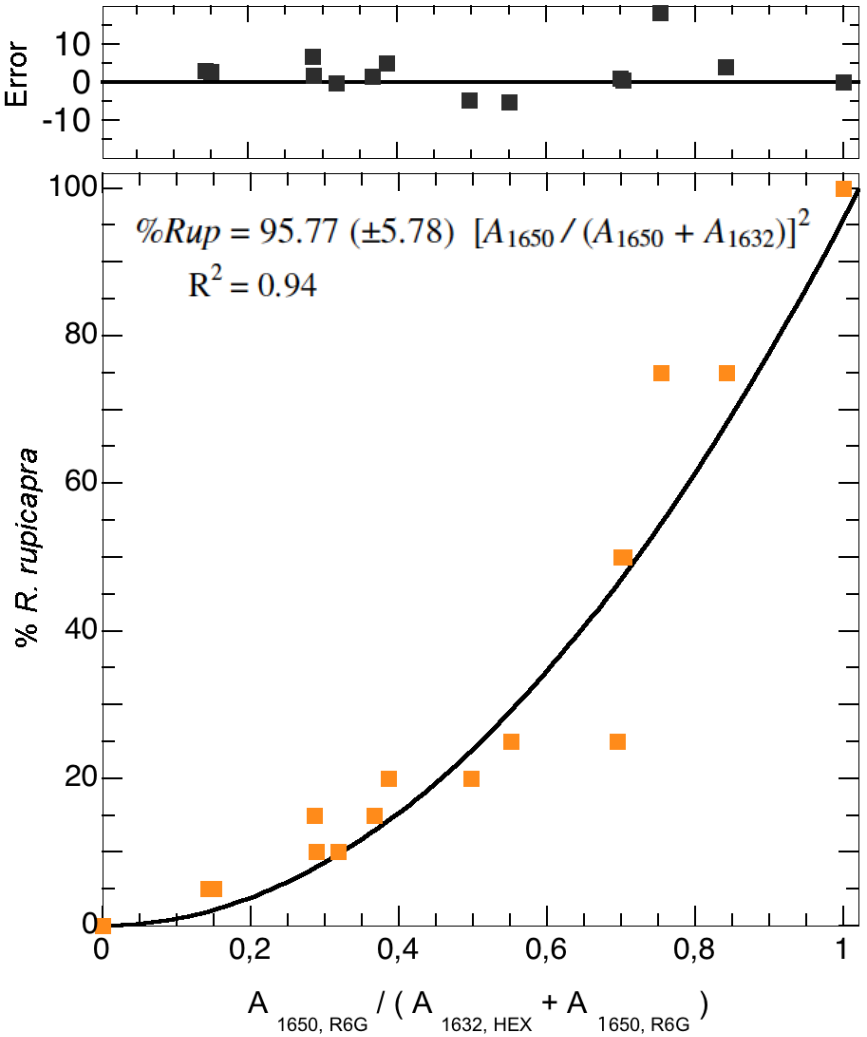
Rupicapra rupicapra

Capra hircus

Simultaneous detection of 2 species



Identification of both sequences
Total DNA amount = 5 10^{-8} M
DOL = 4 10^{-10} M



Quantification of their relative amount

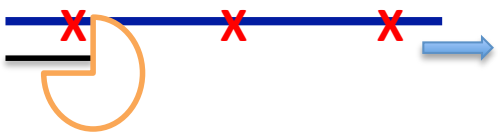
Detection of degraded DNA

Nomenclature
N5’ / N3’
N5’ / I3’
II5’ / N3’
N5’ / IV3’
V5’ / N3’
I5’ / I3’
II5’ / I3’
I5’ / IV3’
V5’ / I3’
II5’ / IV3’
V5’ / IV3’

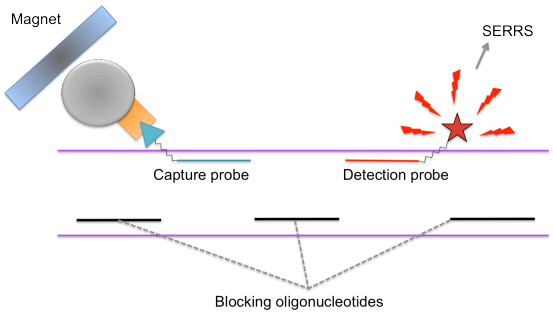
- Double-stranded DNA molecules of 139 bp
- Model blocking lesion : abasic sites
- Lesions distributed on both strands

N = no abasic site
I = 1 abasic site
II = 2 abasic sites …

Example : N5’ / IV3’

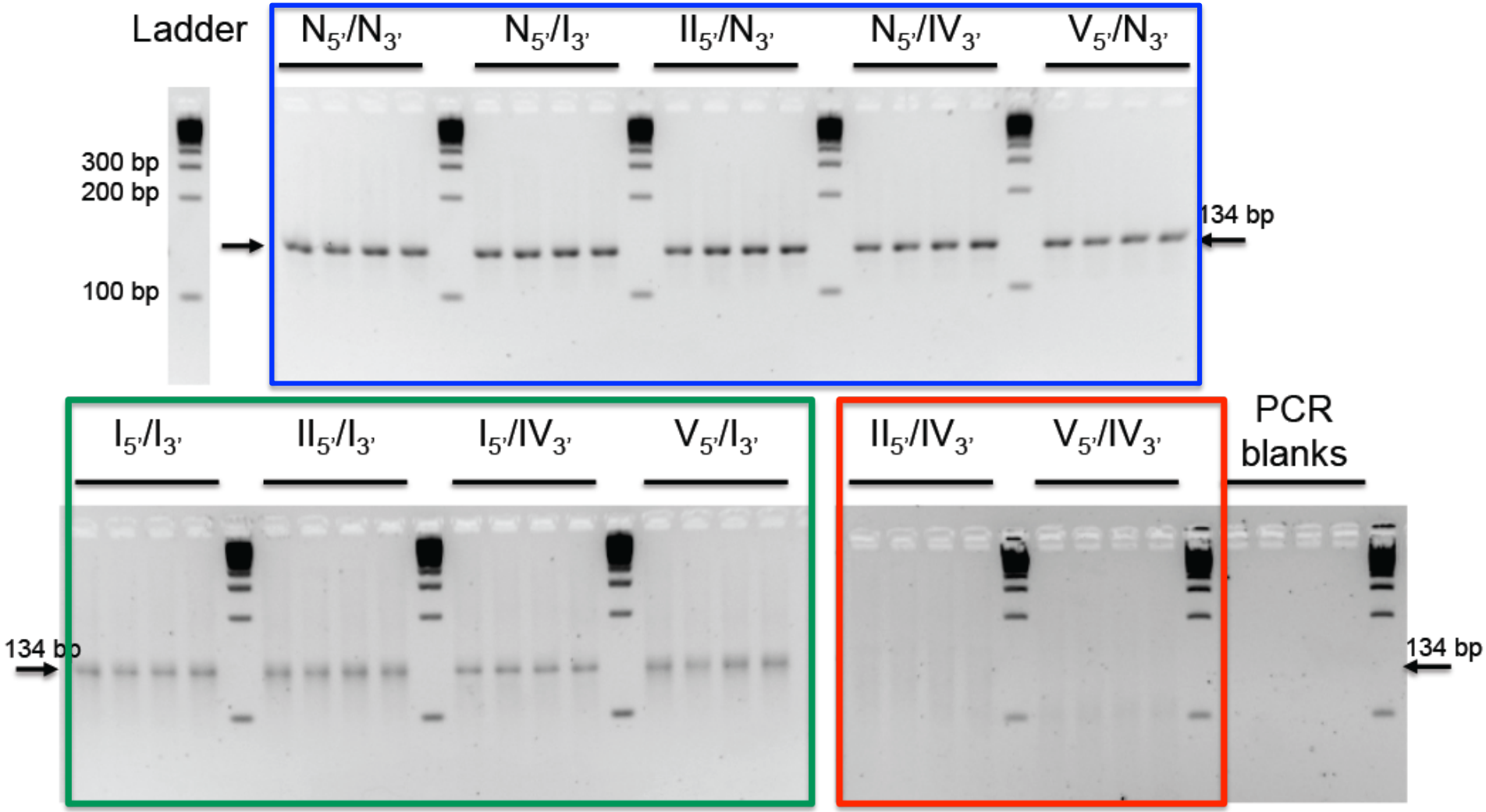


PCR vs SERRS-hybridization assay

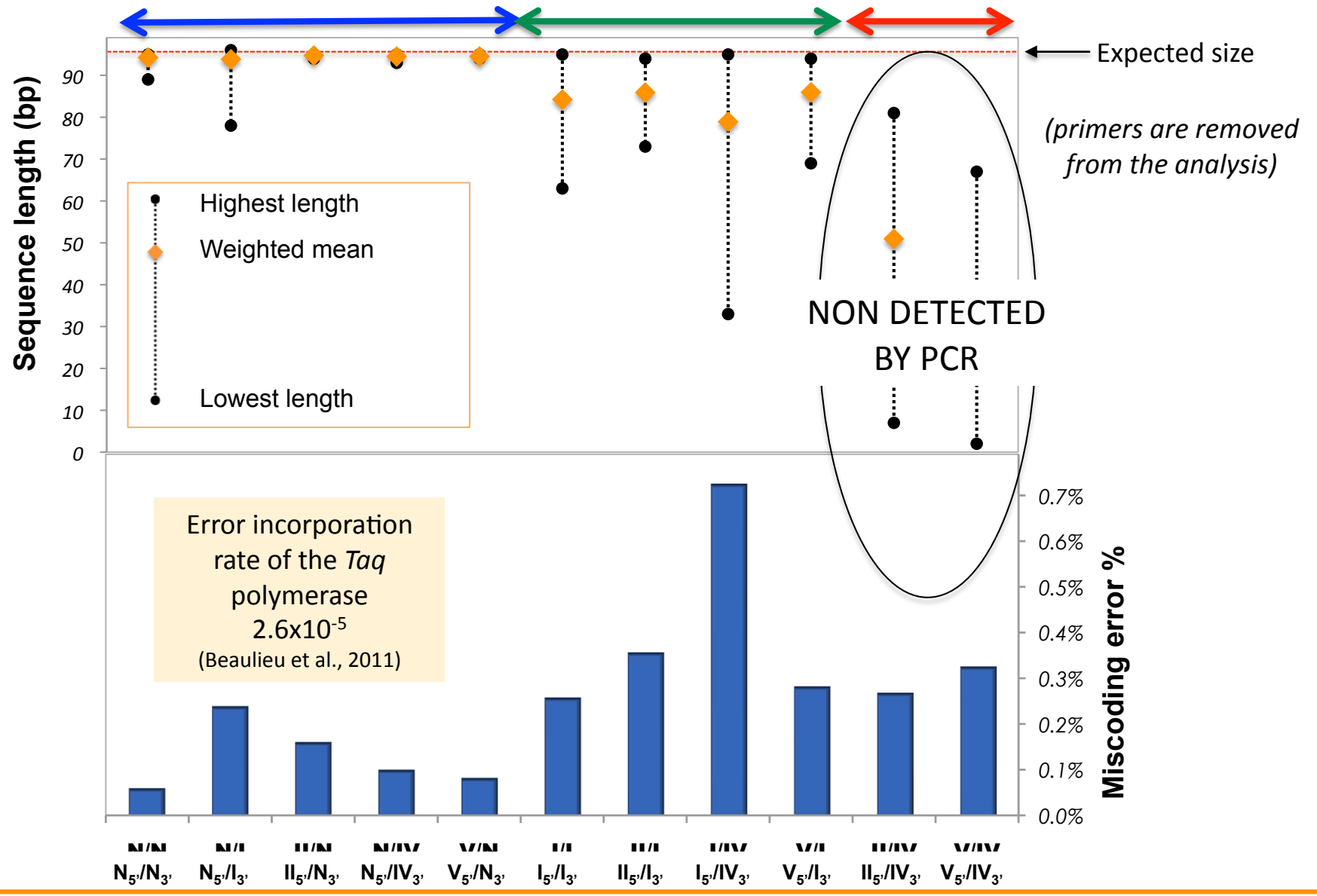


Feuillie et al., in press, PloS ONE

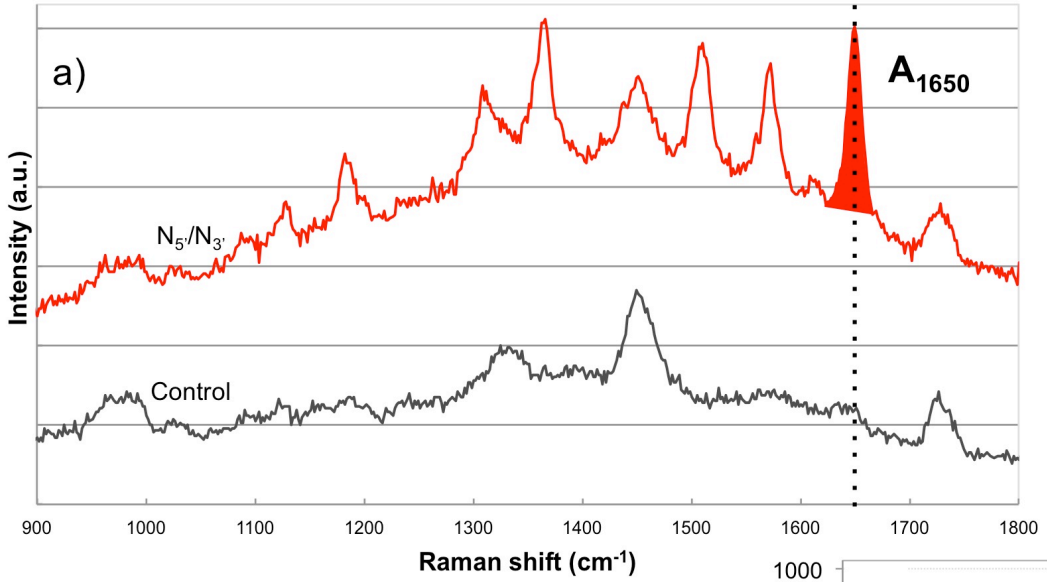
Detection of damaged DNA by PCR



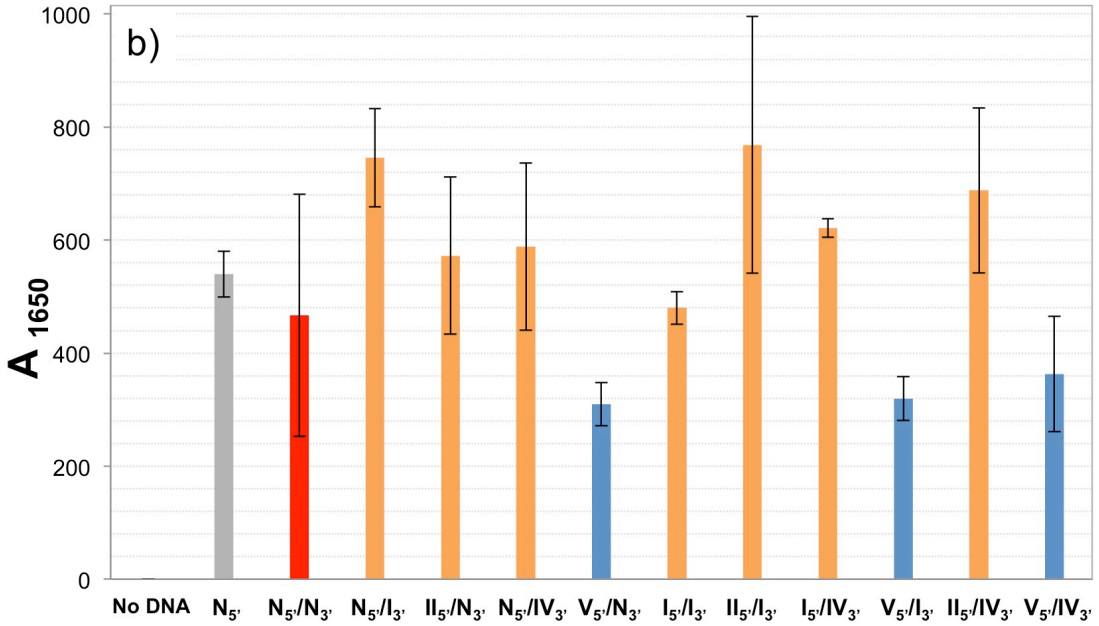
Detection of damaged DNA by PCR



Detection of damaged DNA by SERRS



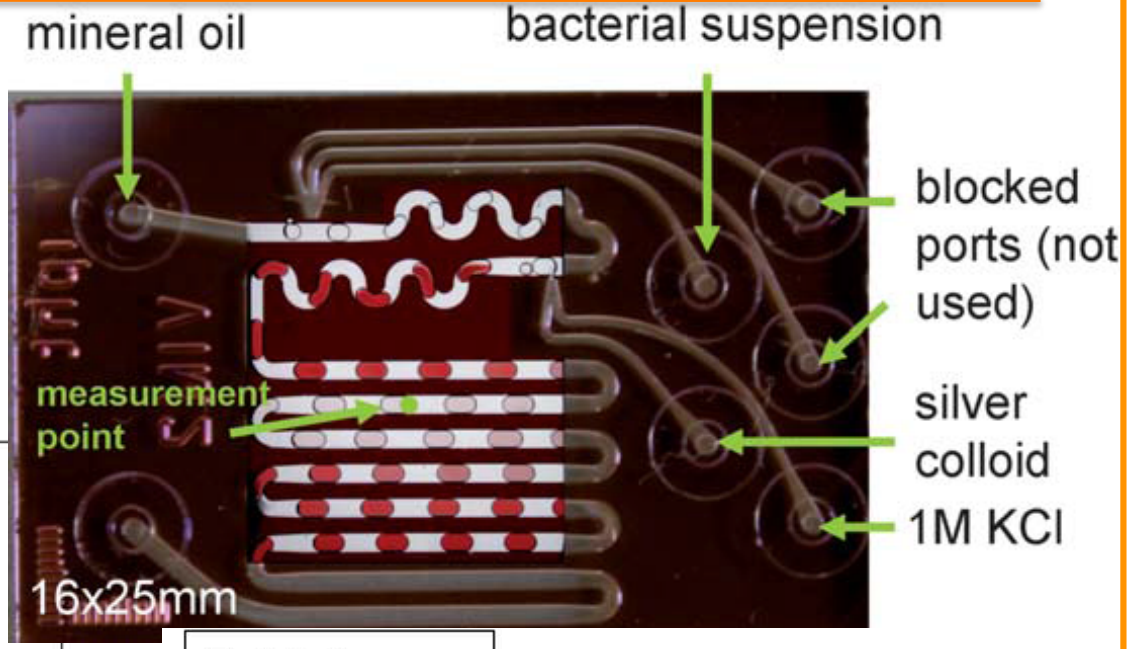
All molecules are detected by the SERRS-hybridization assay



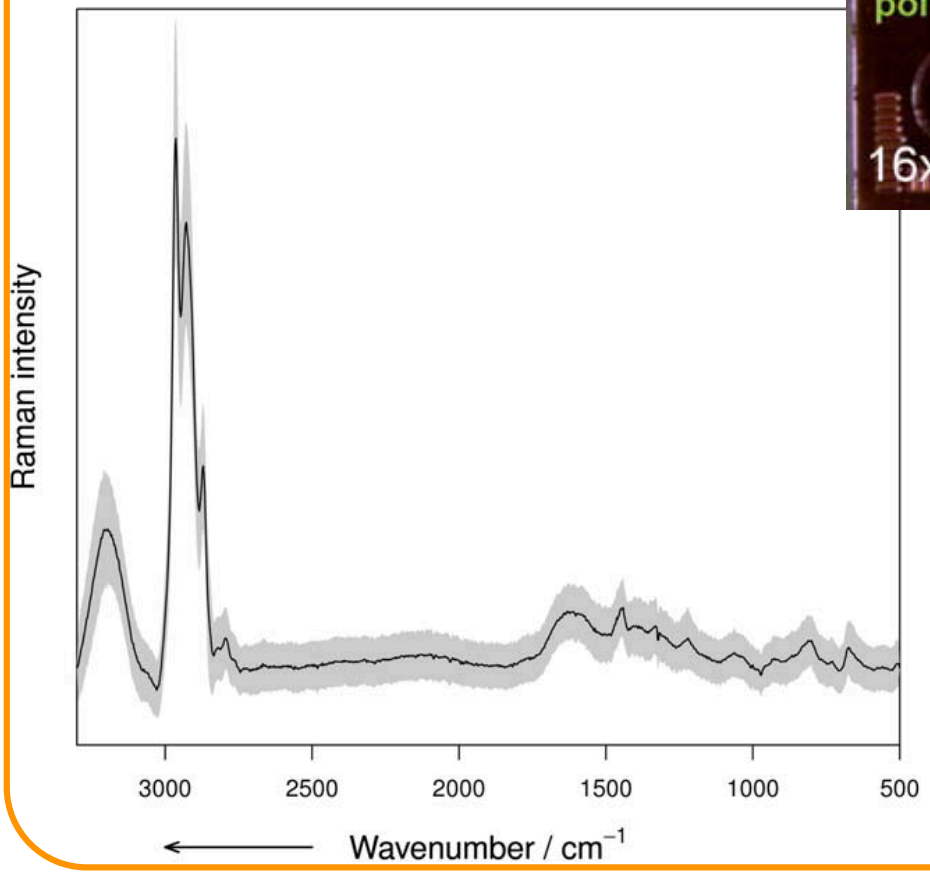
Feuillie et al. in press, PLoS ONE

On a Lab-on-a-chip device…

Walter et al. 2011, Lab Chip



Highly reproducible SER spectra of a strain of *E. coli*



Time table for data of one strain for database (1200 spectra for SERS and Raman)