Spectroscopic analyses for the characterization of bioceramics (with a hint to cells and tissues…).

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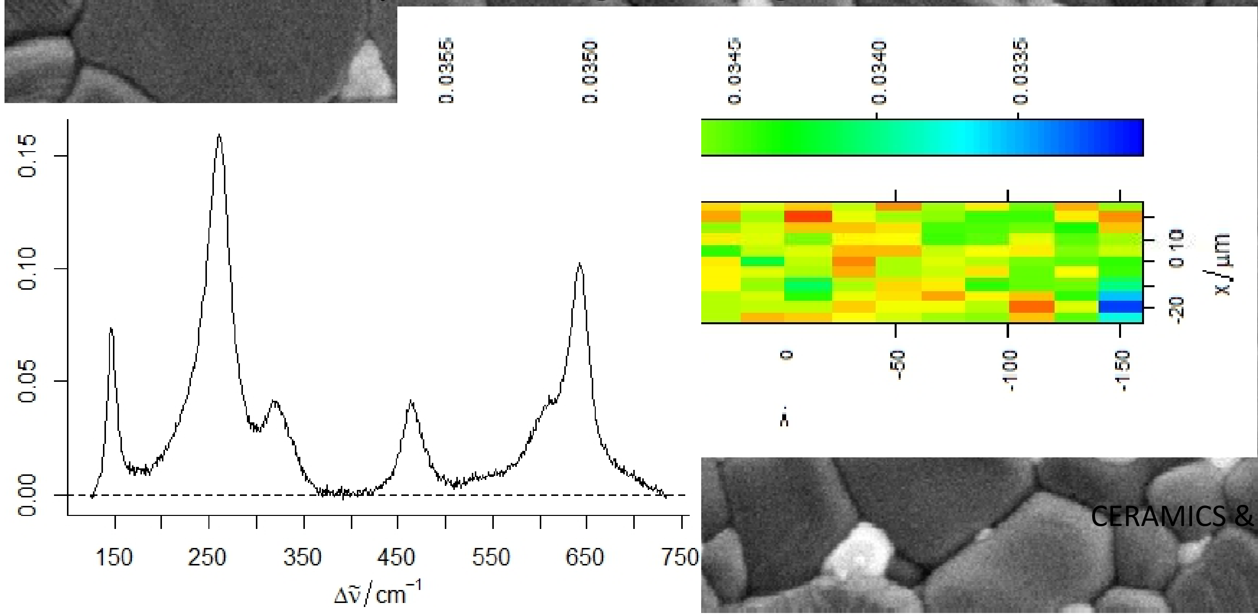
Dept. of Engineering and Architecture, University of Trieste, Italy



CERAMICS & GLASS SCIENCE & TECHNOLOGY Summer school
Application to Bioceramics and Bioglasses
Madrid, Spain, June 17-19° 2015

Outline

The principle of the Raman effect and the instrumentation

Applications: single spectra

Is the bone healing?
Hydroxyapatite: relax and don't get acid…
The eternal dilemma of Miss zirconia: tetragonal or monoclinic?
Gee! You are not aging well, are you, Miss?

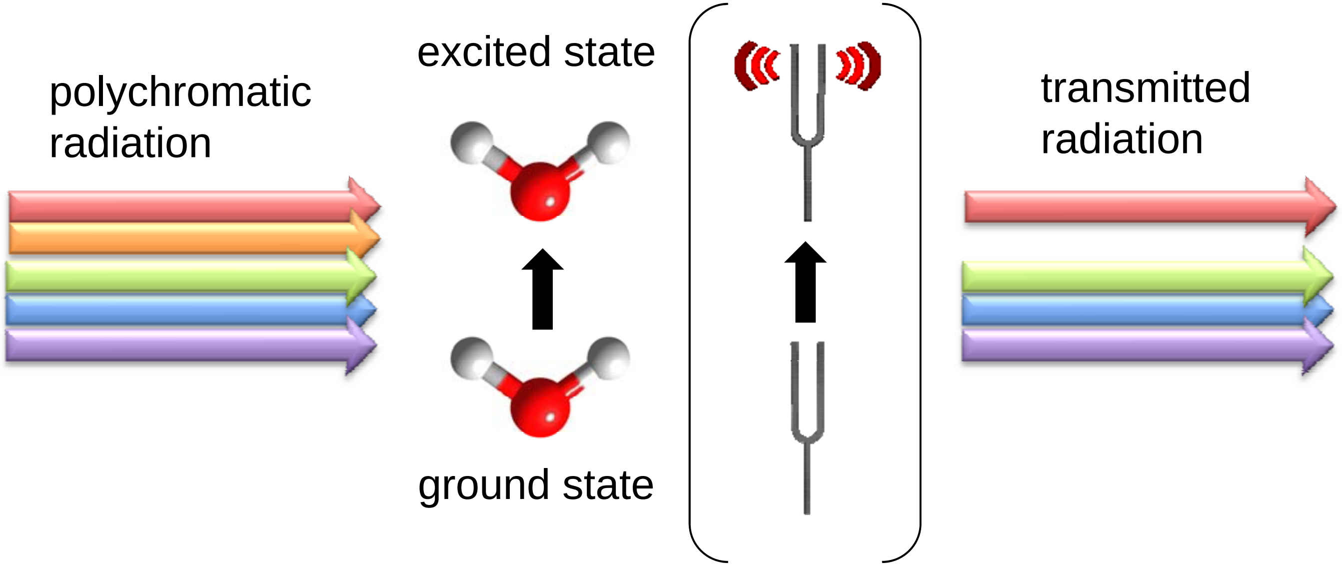
Applications: we like images…

… more zirconia, middle age!
Stress do change, even in rigid alumina!
The garbage of the malaria bacteria
Inside a living organism: ever living tardigrades
Cartilage

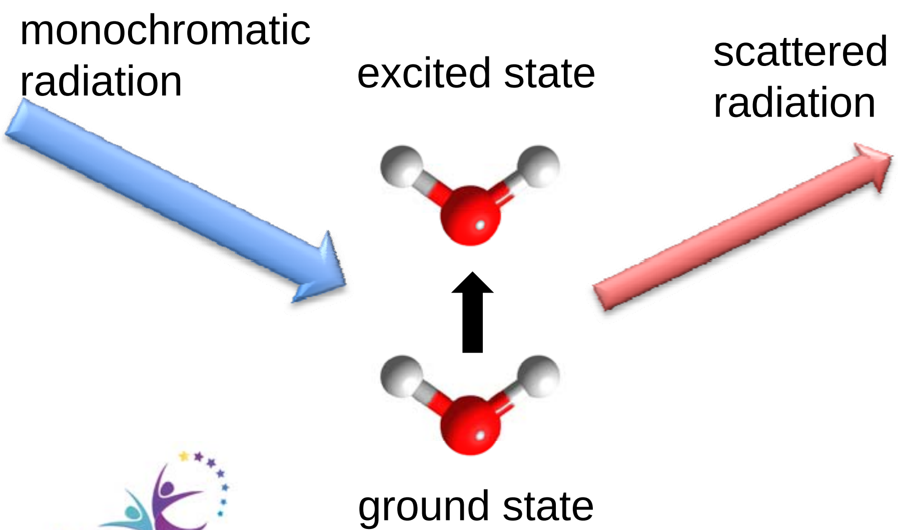
Conclusions

Origin of the Raman effect: molecular vibrations

absorption
(IR)



scattering
(Raman)

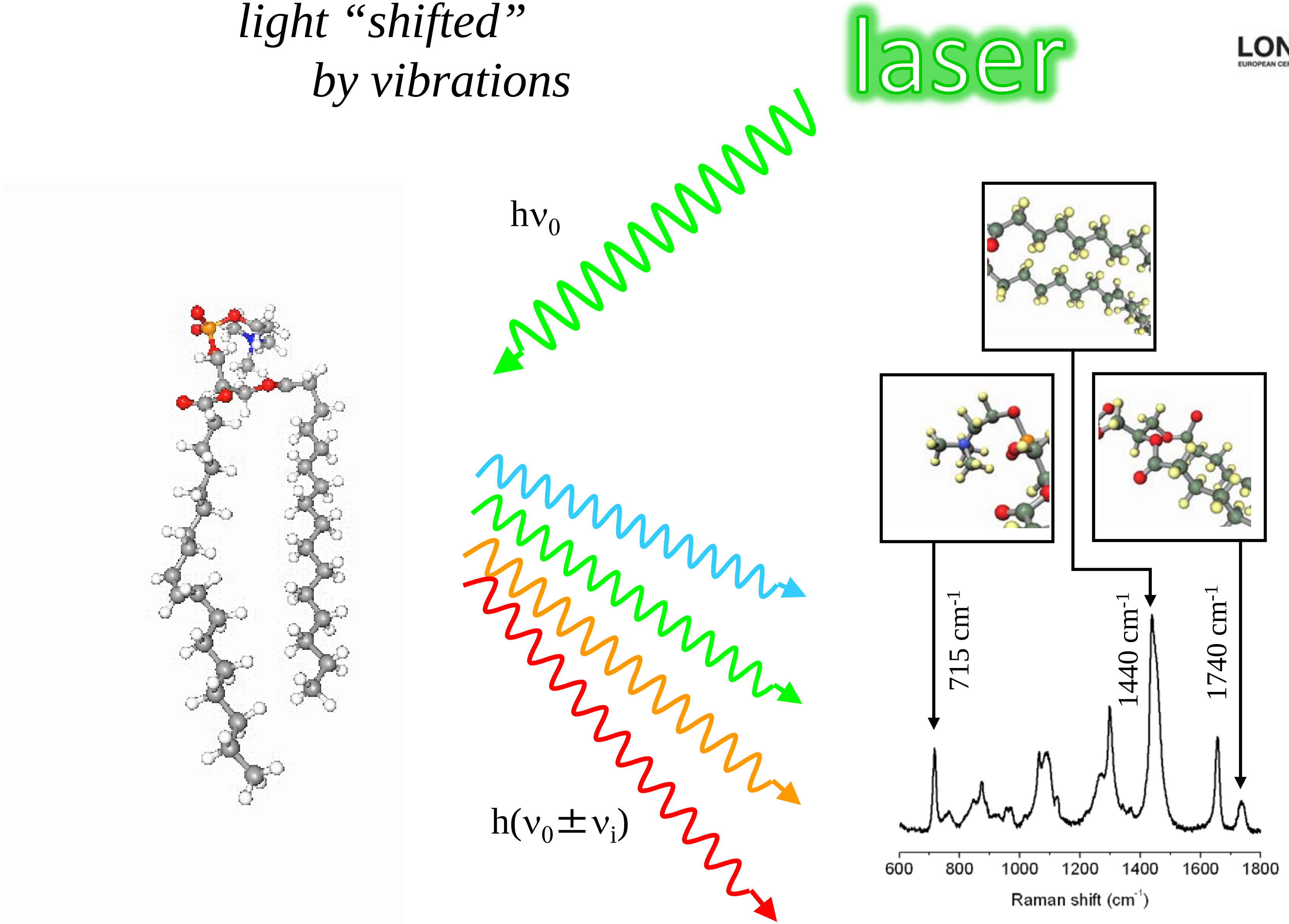


Chandrasekhar Venkata Raman
(1888-1970)

The ***Raman Effect****:*

light "shifted"

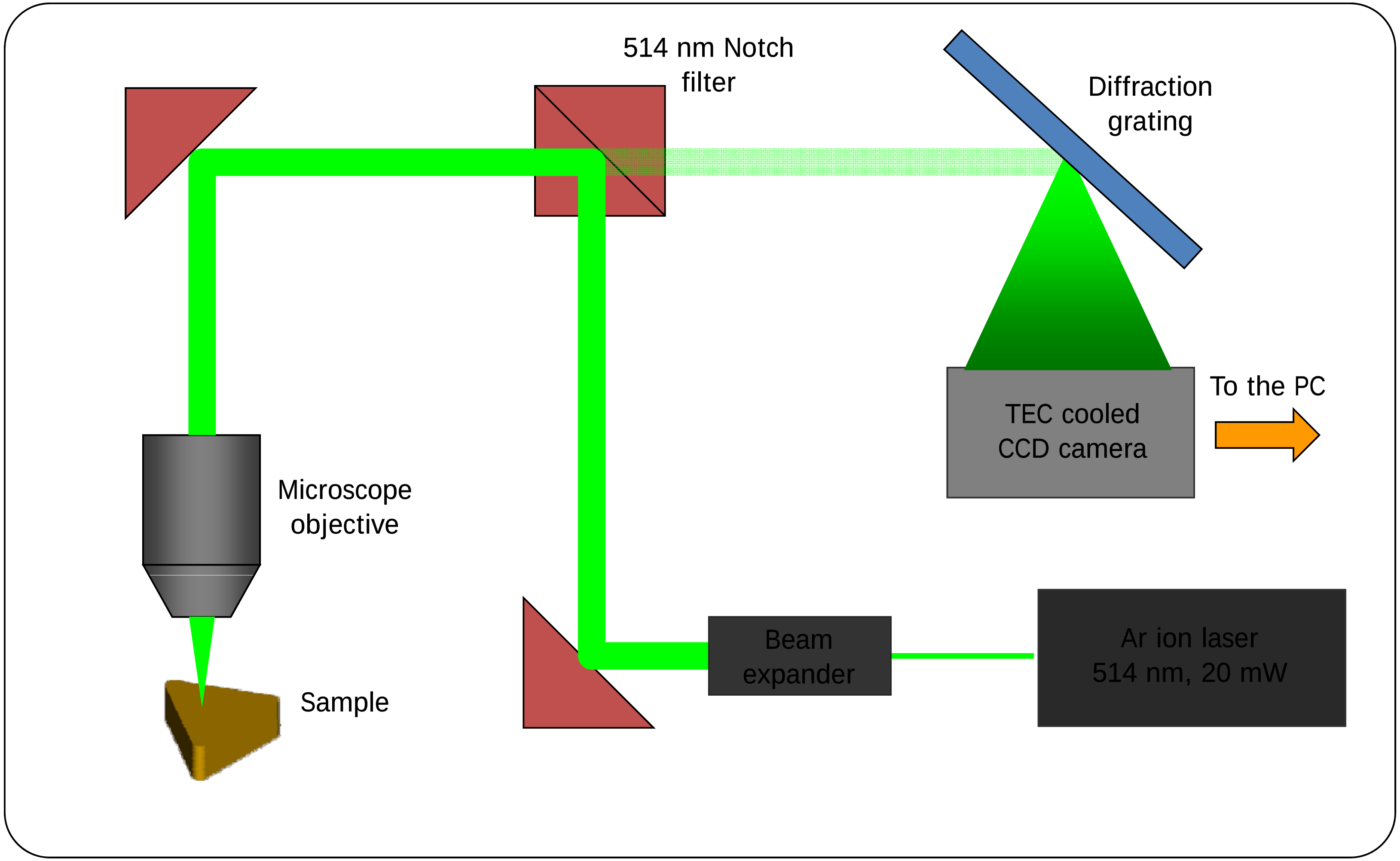
by vibrations



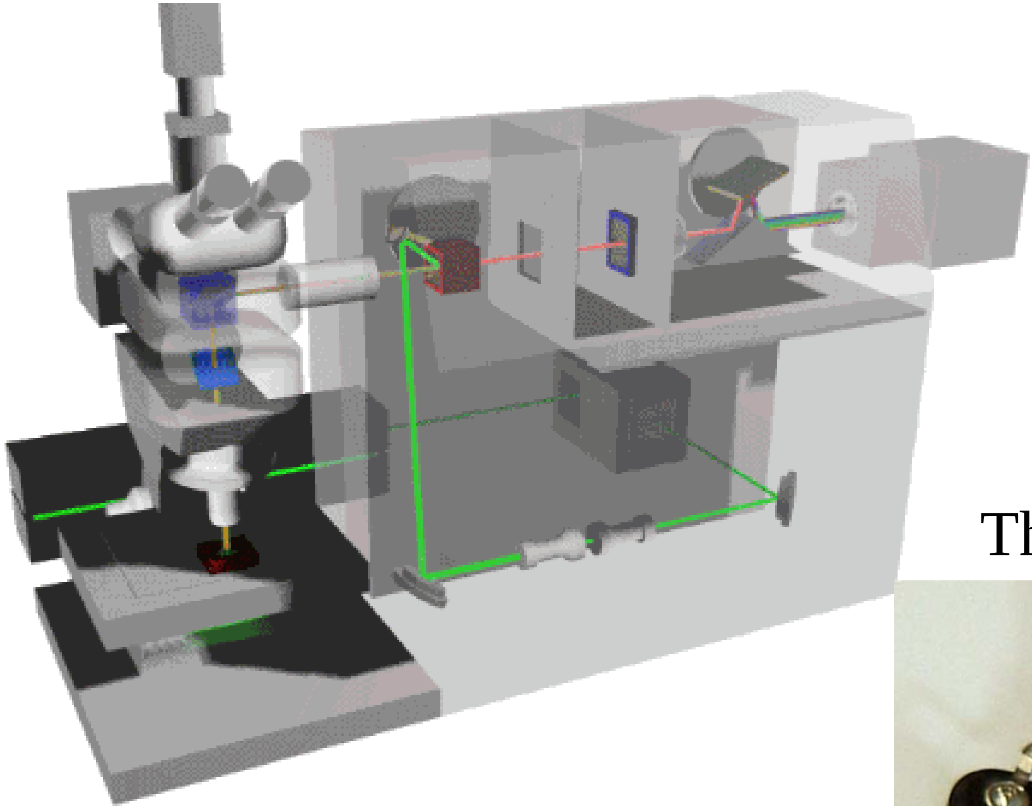
Raman spectrum

Typical set-up for Raman microscopy

(back-scattered configuration);
-lateral (spatial) resolution ≈1 μm
-spectral resolution ≈ 1cm^{-1}



Schematic



Now

The holy grail

LONG life
EUROPEAN CERAMIC IMPLANTS PROJECT

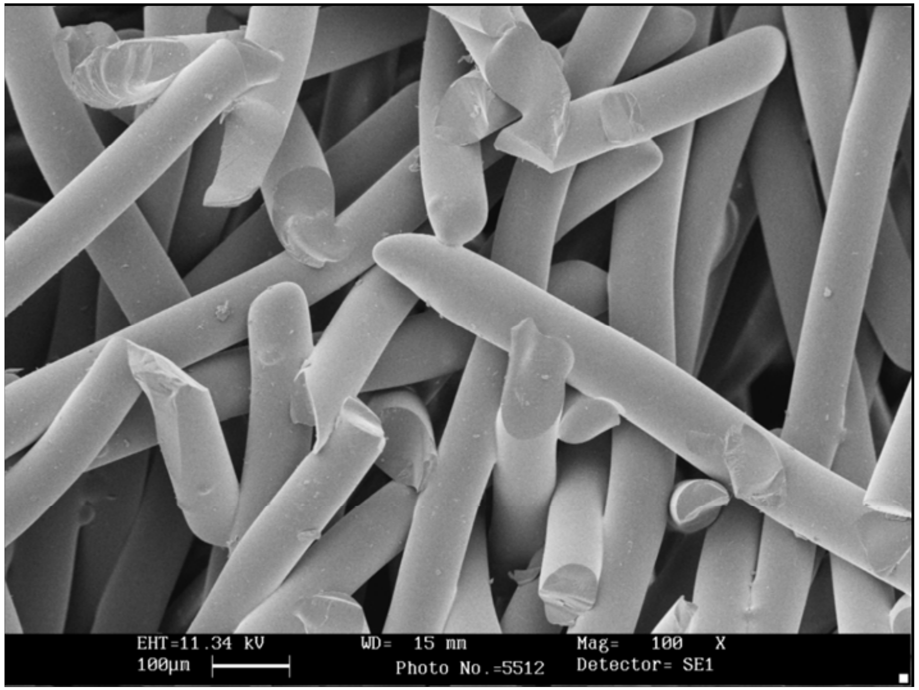
BIOACTIVE GLASS LWR (Large Working Range):

	Na_2O	K_2O	MgO	CaO	B_2O_3	P_2O_5	SiO_2
Mol %	12,1	10,1	5,0	14,8	0,9	0,9	56,2

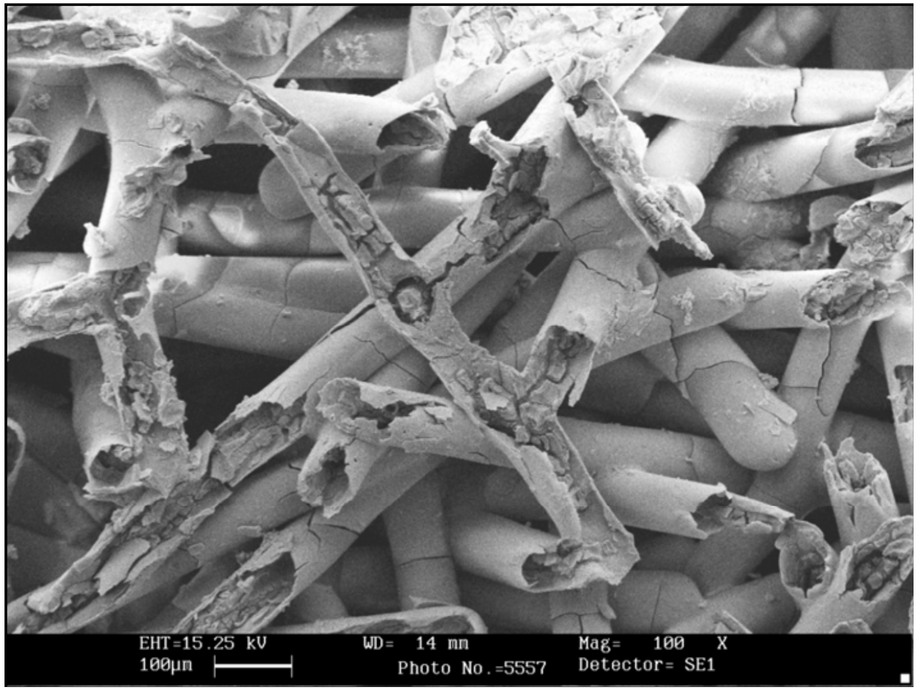
Starting from fibers (75 μm in diameter and 3 mm long), three-dimensional porous scaffolds are obtained by sintering.

Subsequently scaffolds are immersed in Simulated Body Fluid (SBF) to monitor possible formation of hydroxil-carbonate-apatite (HCA).

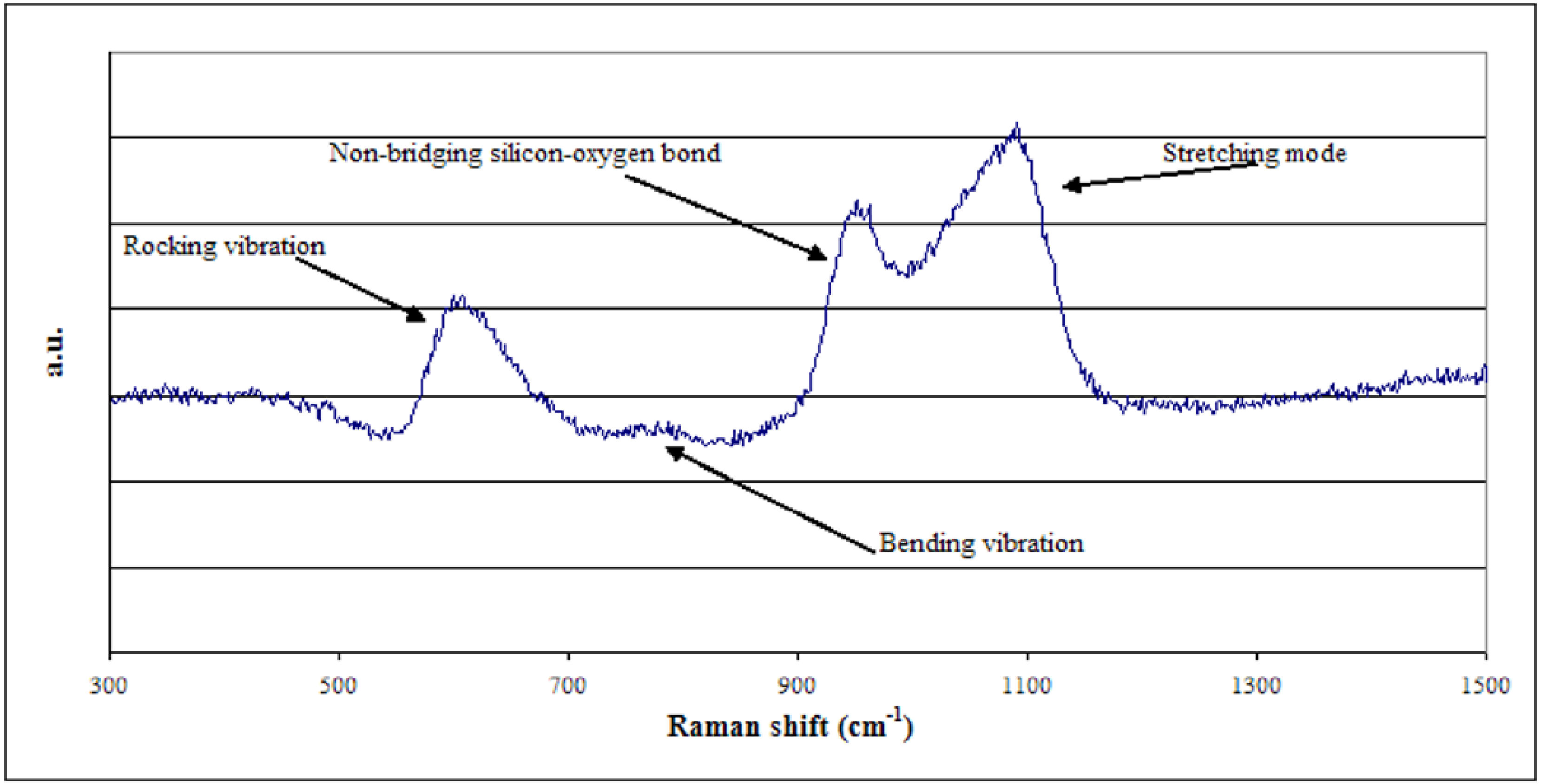
Pristine scaffold



Scaffold after 3 weeks of immersion in SBF at 37°C (no stirring))



RAMAN SPECTRUM OF PRISTINE SCAFFOLD (AMORPHOUS SILICA)



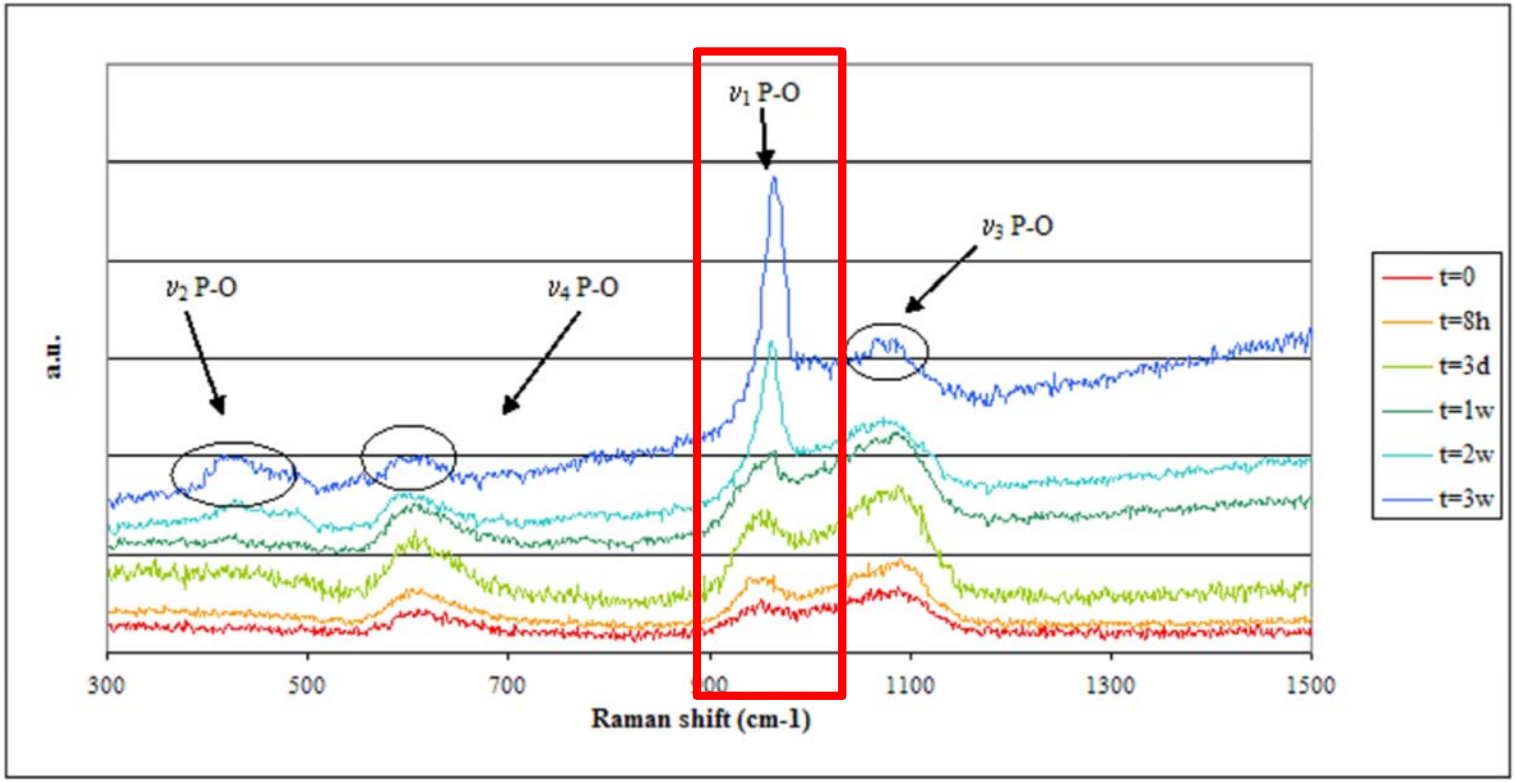
Raman bands: 1064-1183 cm^{-1} bond stretching vibration

783-837 cm^{-1} bond bending vibration

453-490 cm^{-1} bond rocking vibration

900-950 cm^{-1}, 1100 cm^{-1} O-Si-O , O-Si-NBO symmetric vibrations

Raman Spectral evolution of scaffolds treated in SBF at 37°C for different times



ν_1: 962 cm^{-1} symmetric stretching vibration of P-O: FORMATION OF HYDROXYAPATITE!

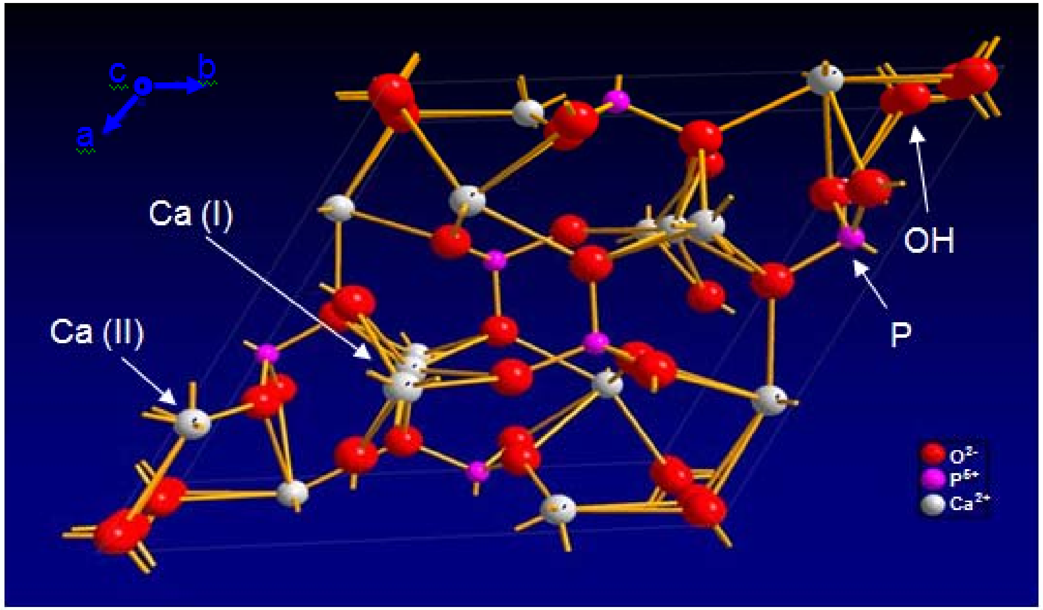
V. Sergo et al., Journal of Applied Biomaterials & Biomechanics 2006; Vol. 4 no. 2: 102-109
Best contribution award , International meeting of Biomaterials Societies, Ischia 2005:

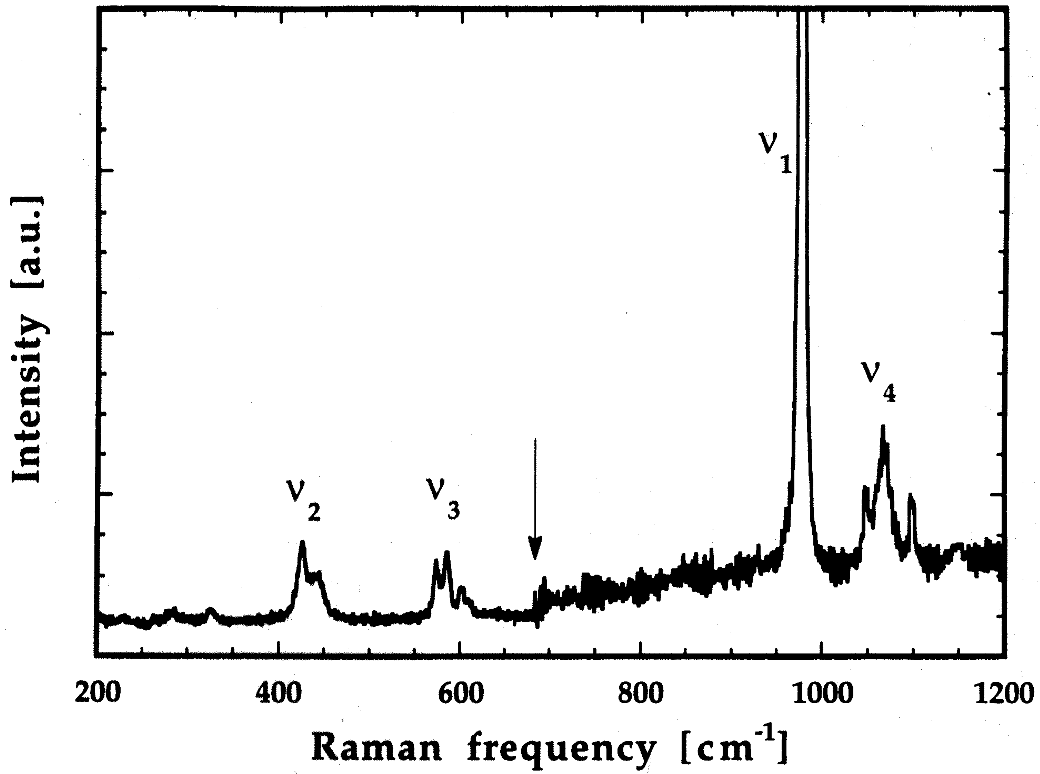
Hydroxiapatite contituents of bones and teeth, $Ca_5(PO_4)_3(OH)$

https://www.youtube.com/watch?v=yz7mihE1iM8

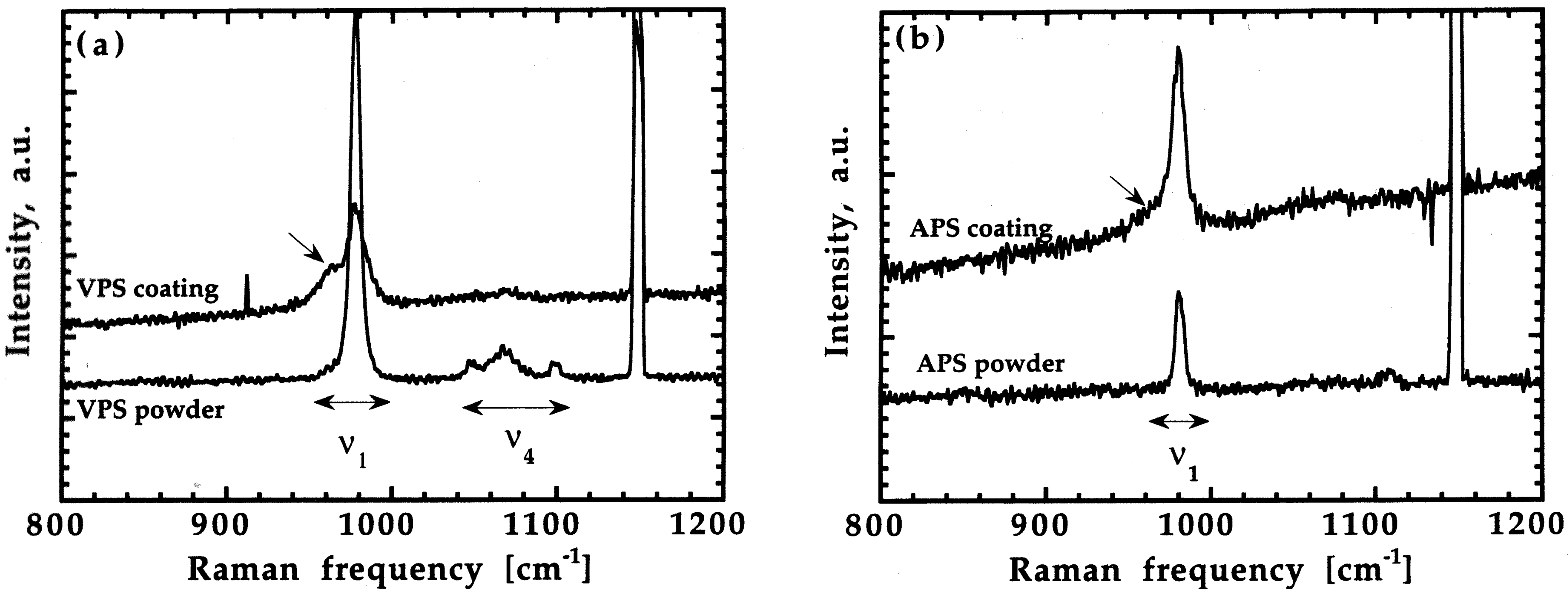
HydroxyApatite

Structure....





...and Raman spectrum



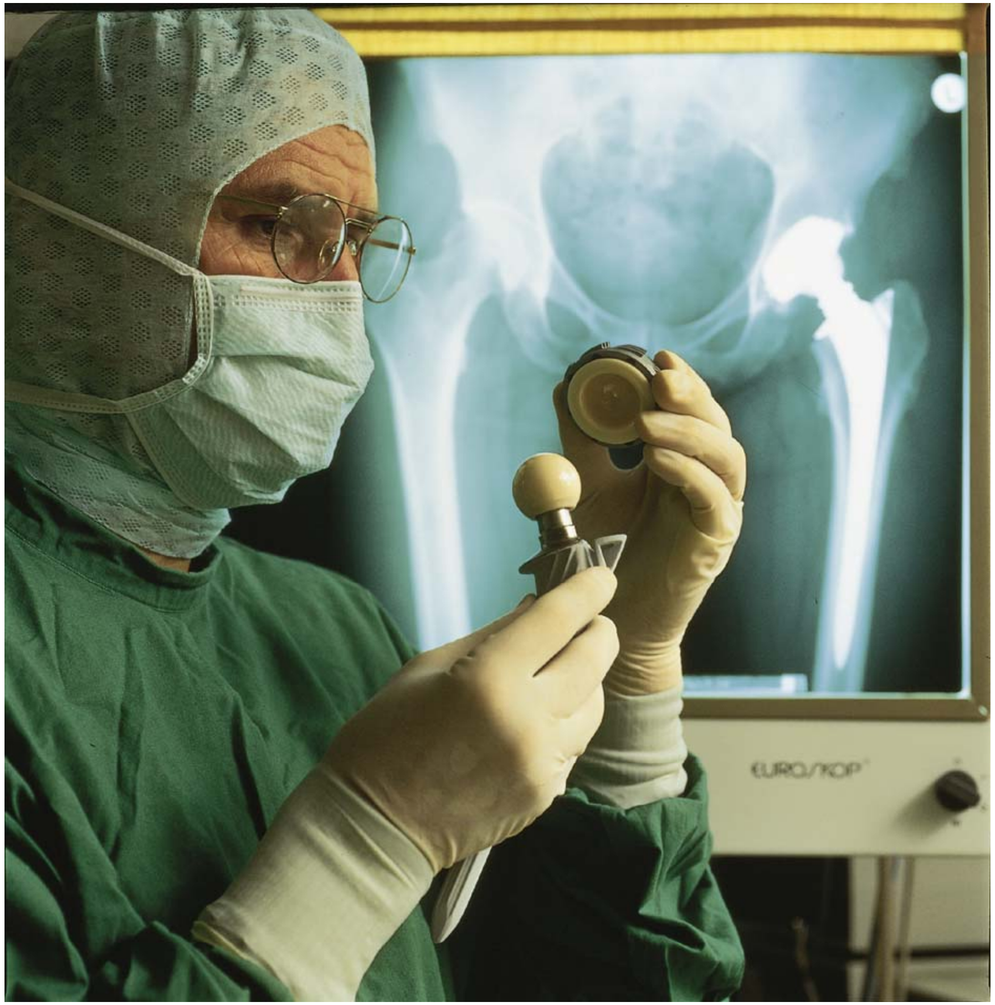
(a)
VPS coating
VPS powder
ν1
ν4
Intensity, a.u.
800
900
1000
1100
1200
Raman frequency [cm-1]
(b)
APS coating
APS powder
ν1
Intensity, a.u.
800
900
1000
1100
1200
Raman frequency [cm-1]

Dissolution thermodynamics of HydroxyApatite HA

Now, 160 Mpa of stress difference translates in a change in OH^- concentration of 5 times!

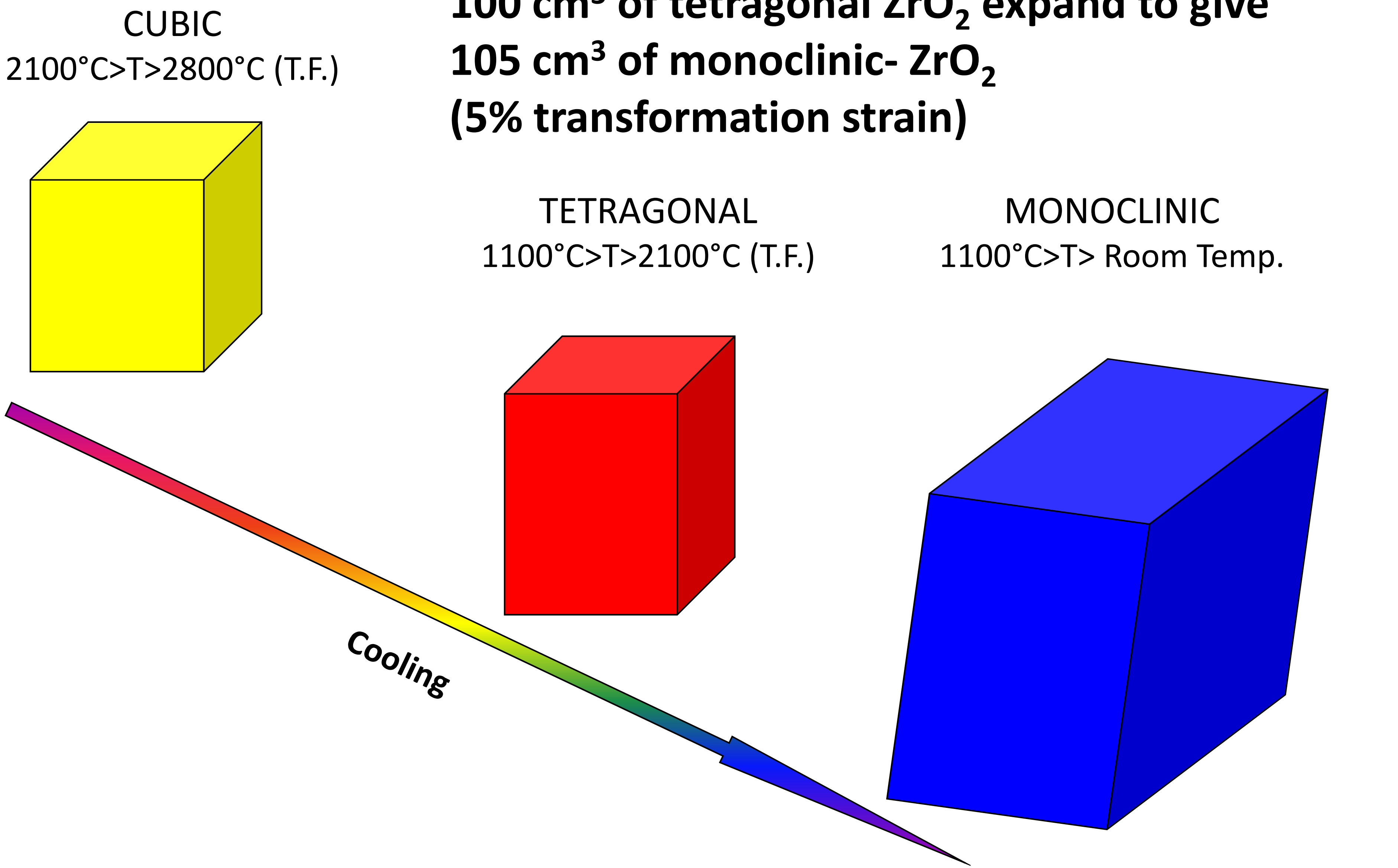
Conclusion: residual stresses can play an important role in the dissolution/reprecipitation behavior of ceramic biomaterials and on the local pH of biofluids!

Ceramic hip joint prostheses



NB: The material is ZIRCONIUM OXIDE, ZrO_2.

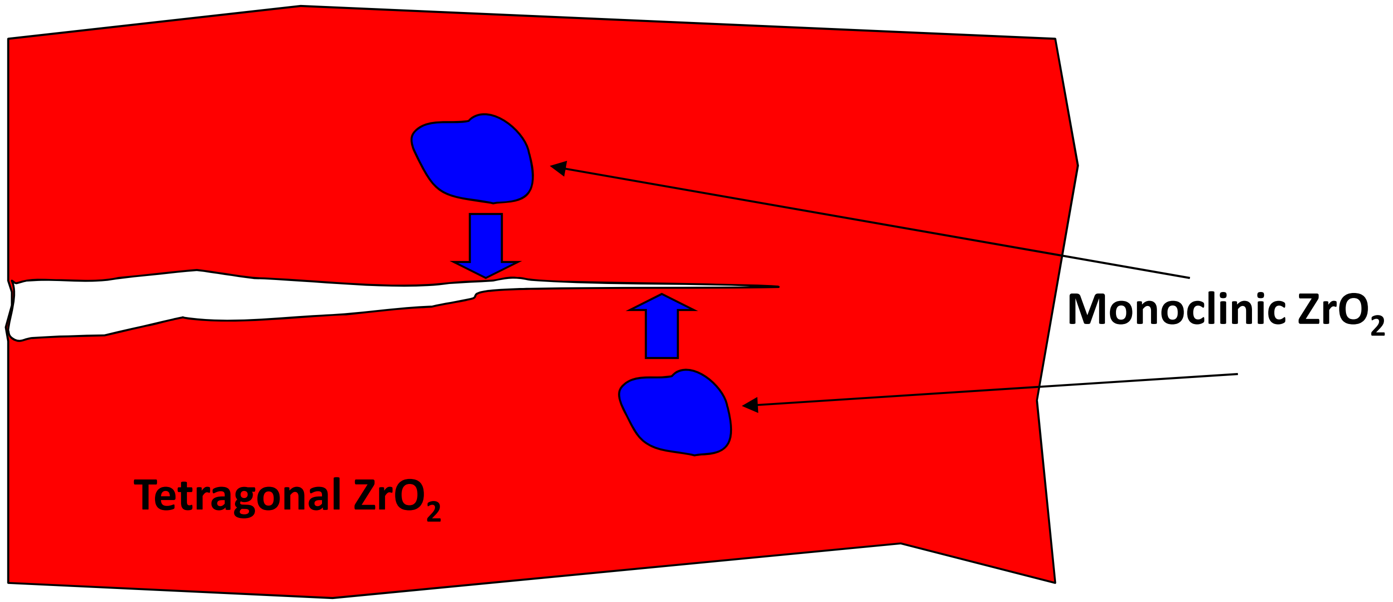
100 cm^3 of tetragonal ZrO_2 expand to give 105 cm^3 of monoclinic- ZrO_2 (5% transformation strain)



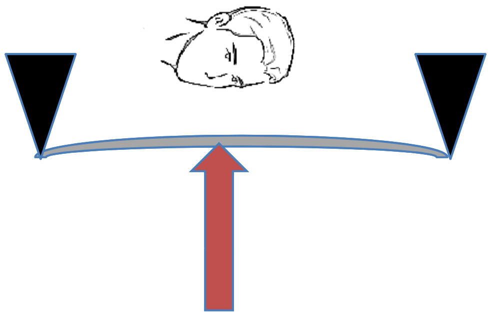
First consequence:The transformation toughening of ZrO_2

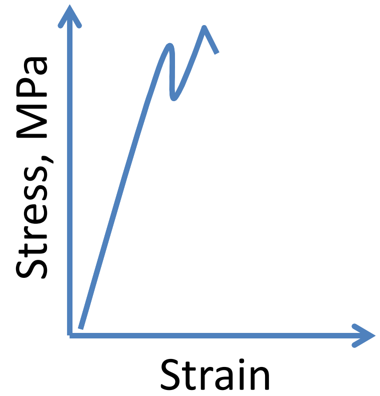
At the crack tip, tetragonal ZrO_2 particles transform to monoclinic; they try to expand and, consequently,

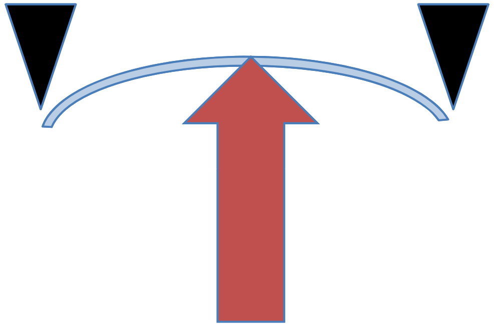
EXERT A CLOSING PRESSURE ON THE ADVANCING CRACK!!

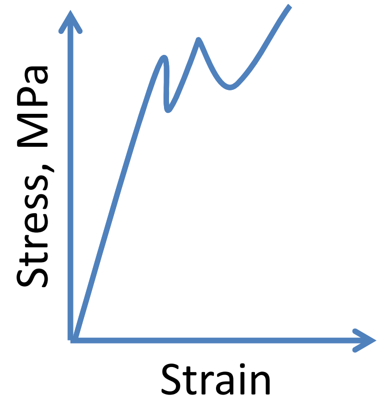


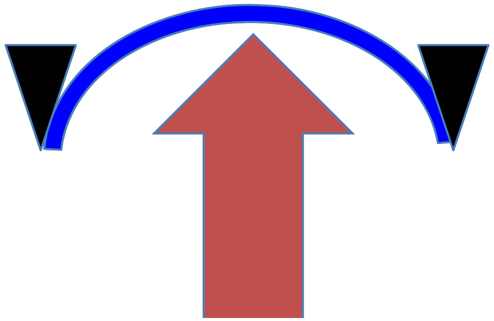
2nd consequence: pseudo-plasticity

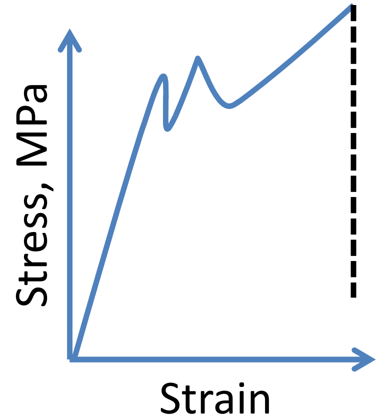




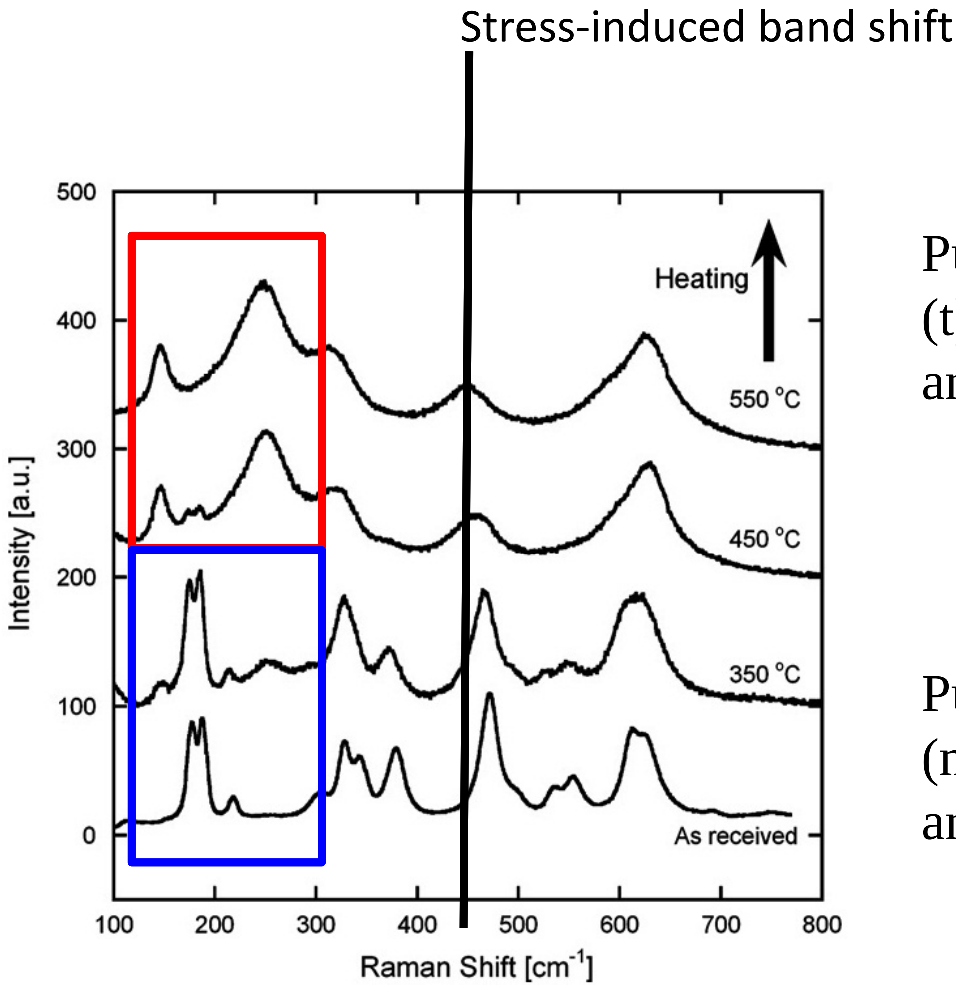




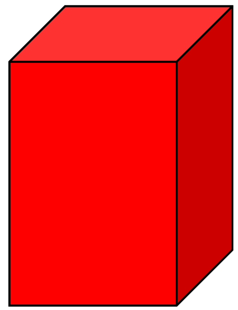




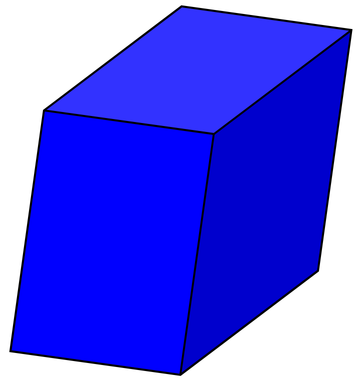
Raman spectroscopy of zirconia



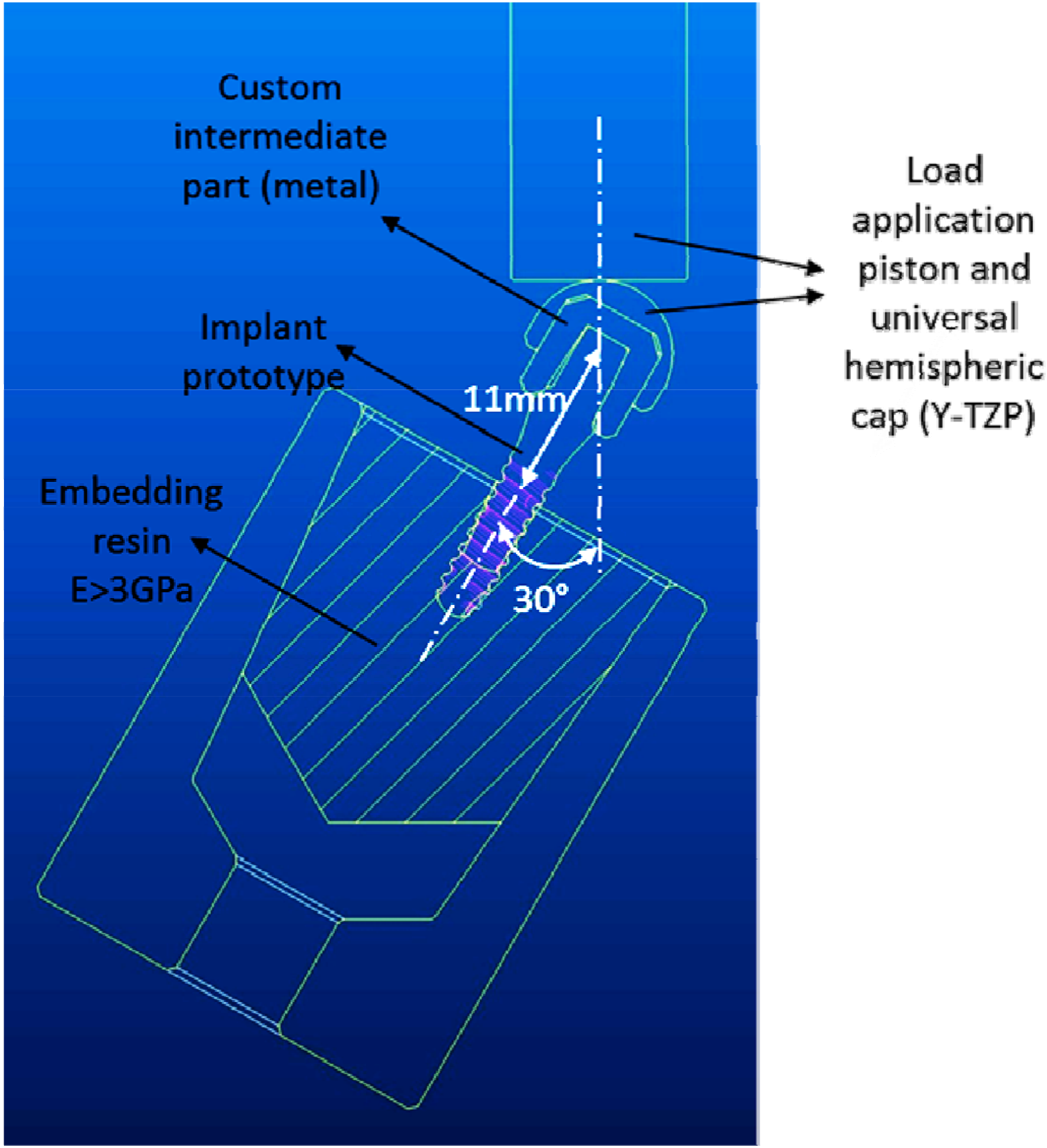
Purely tetragonal (t) (peaks at 147 and 265 cm^{-1})



Purely monoclinic (m) (peaks at 181 and 190 cm^{-1})

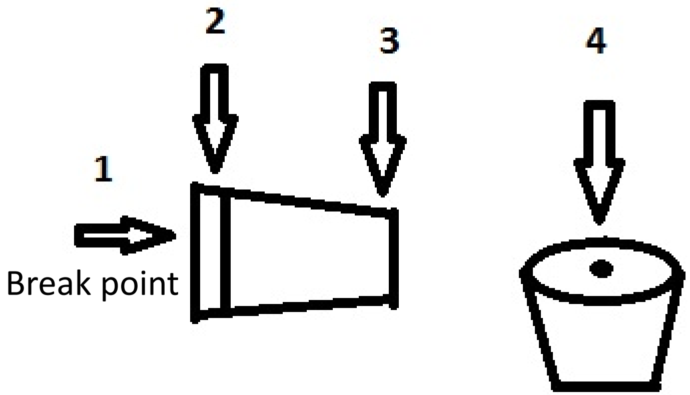


Lughi and Clarke
Acta Materialia 2007

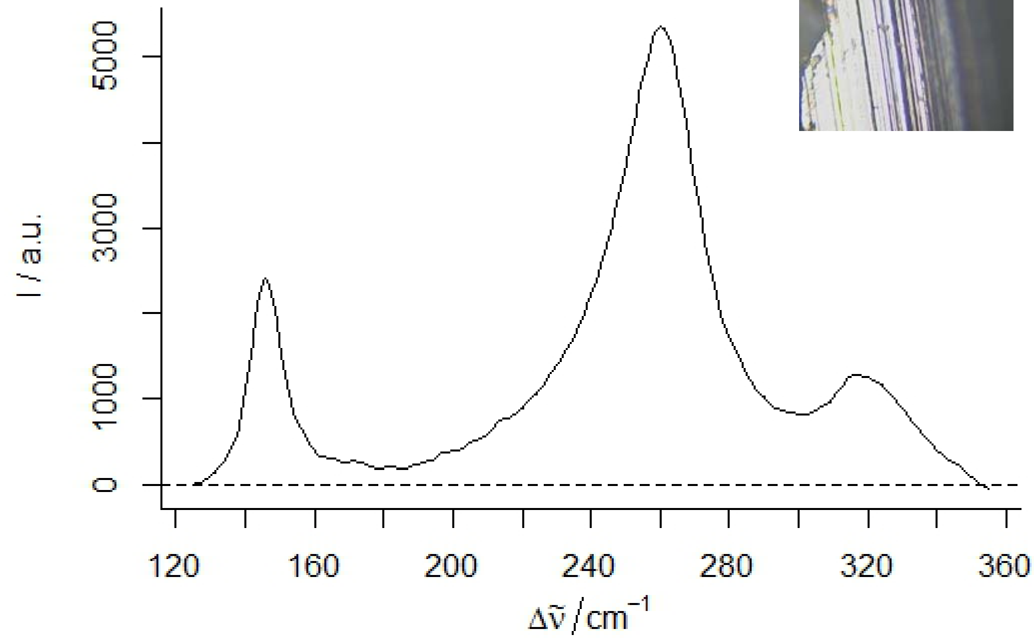


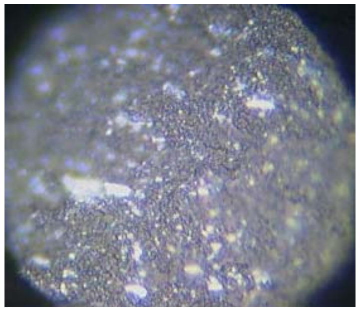
Custom intermediate part (metal)
Load application piston and universal hemispheric cap (Y-TZP)
Implant prototype
11mm
Embedding resin E>3GPa
30°

Mounted screw SAMPLE: T14033_S26
Laser used: 633 nm

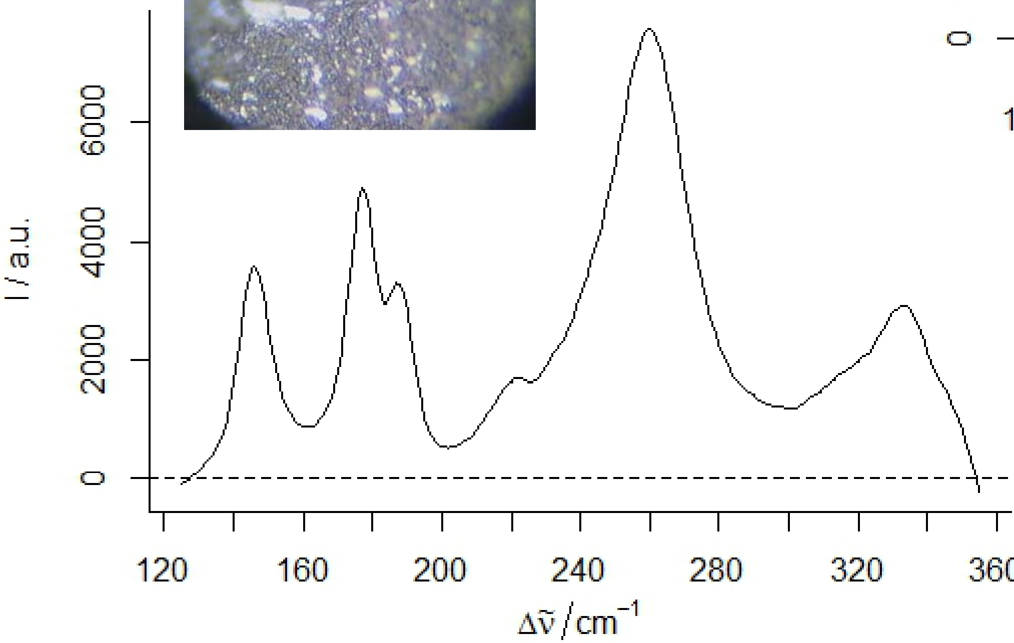


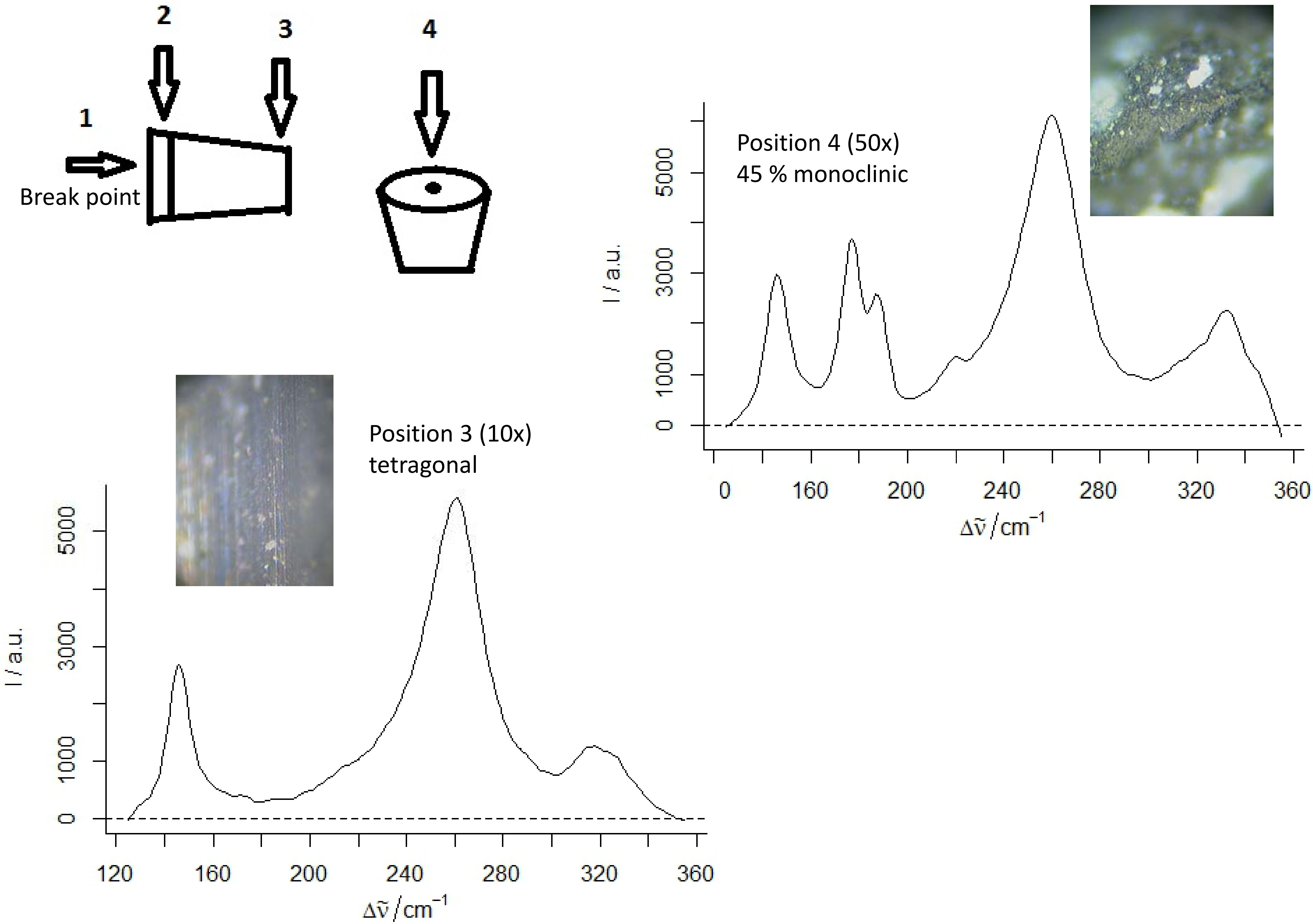
Position 2 (10x)
tetragonal





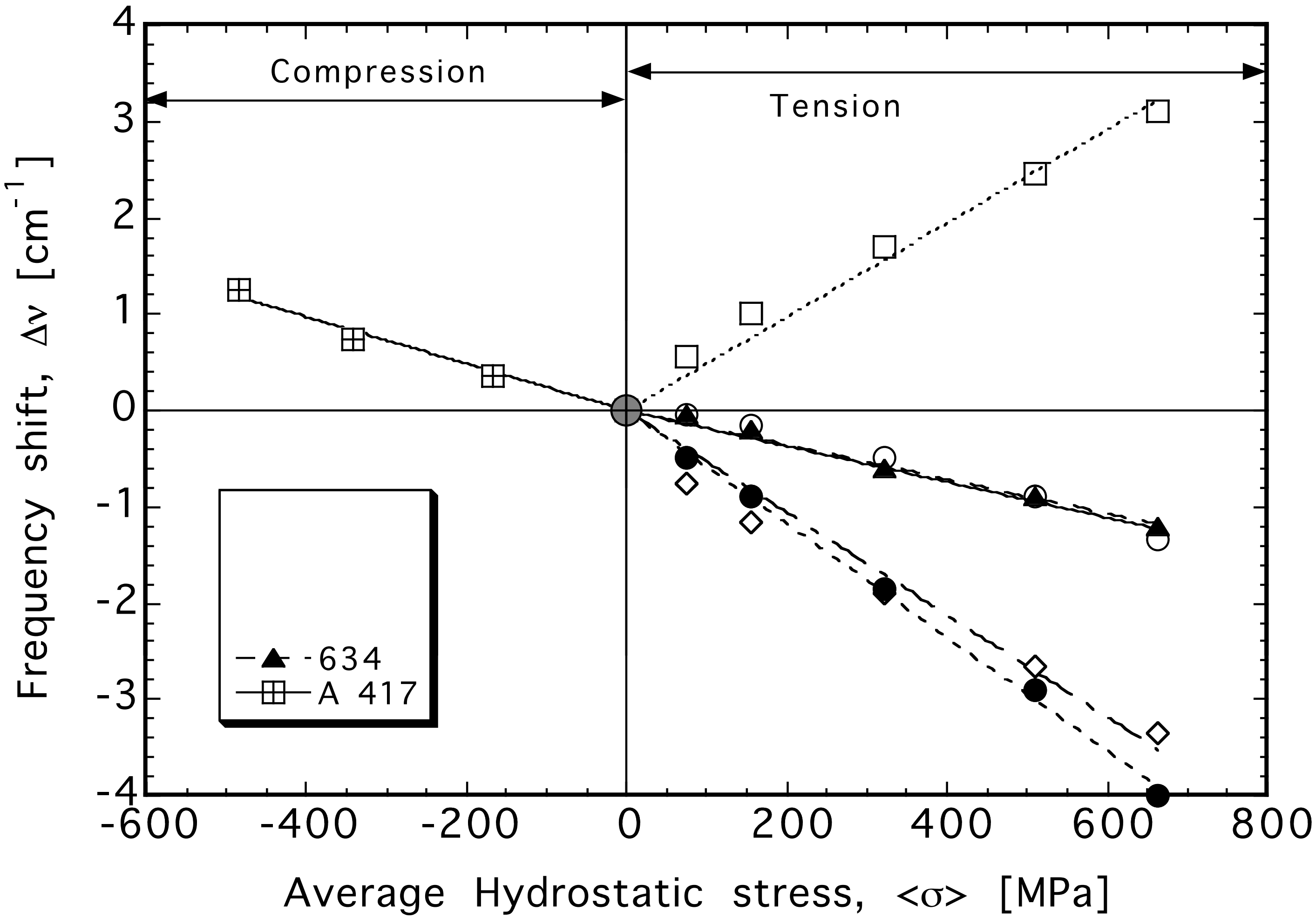
Position 1 (50x)
47 % monoclinic



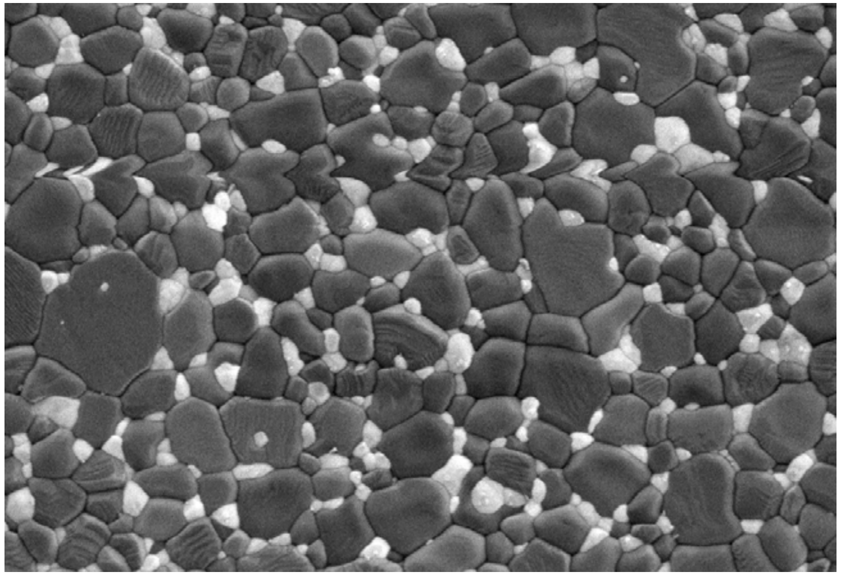


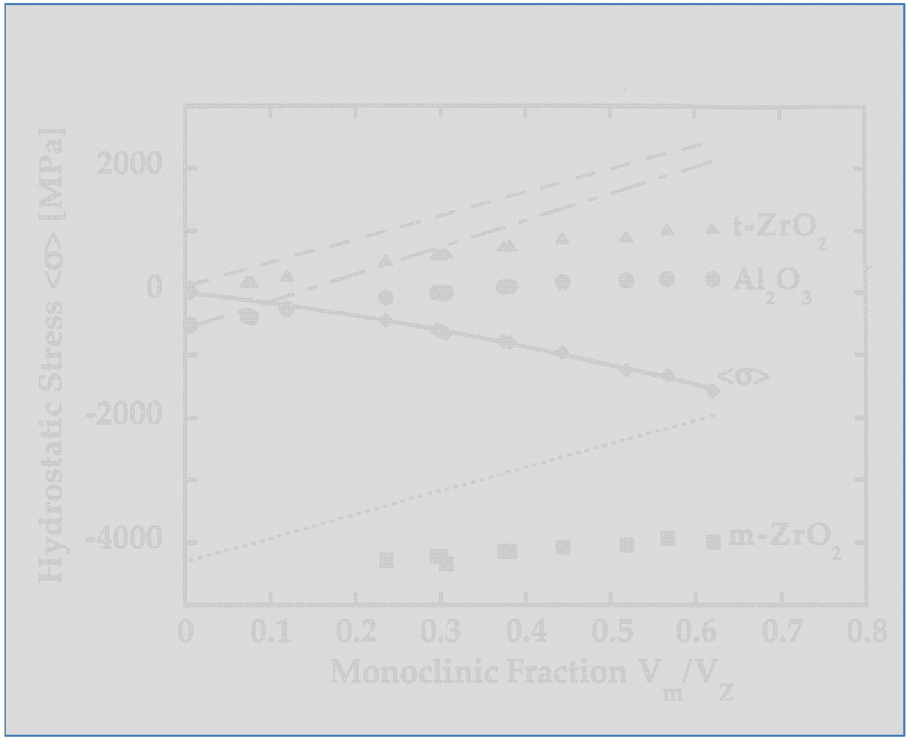
1
2
3
4
Break point
Position 4 (50x)
45 % monoclinic
I / a.u.
0
1000
3000
5000
0
160
200
240
280
320
360
$\Delta\tilde{\nu}$ / cm^{-1}
Position 3 (10x)
tetragonal
I / a.u.
0
1000
3000
5000
120
160
200
240
280
320
360
$\Delta\tilde{\nu}$ / cm^{-1}

Each band shifts differently under stress: i.e. it has its own Piezo-Spectroscopic coefficient (reported here is the case of *t*-zirconia (in tension).

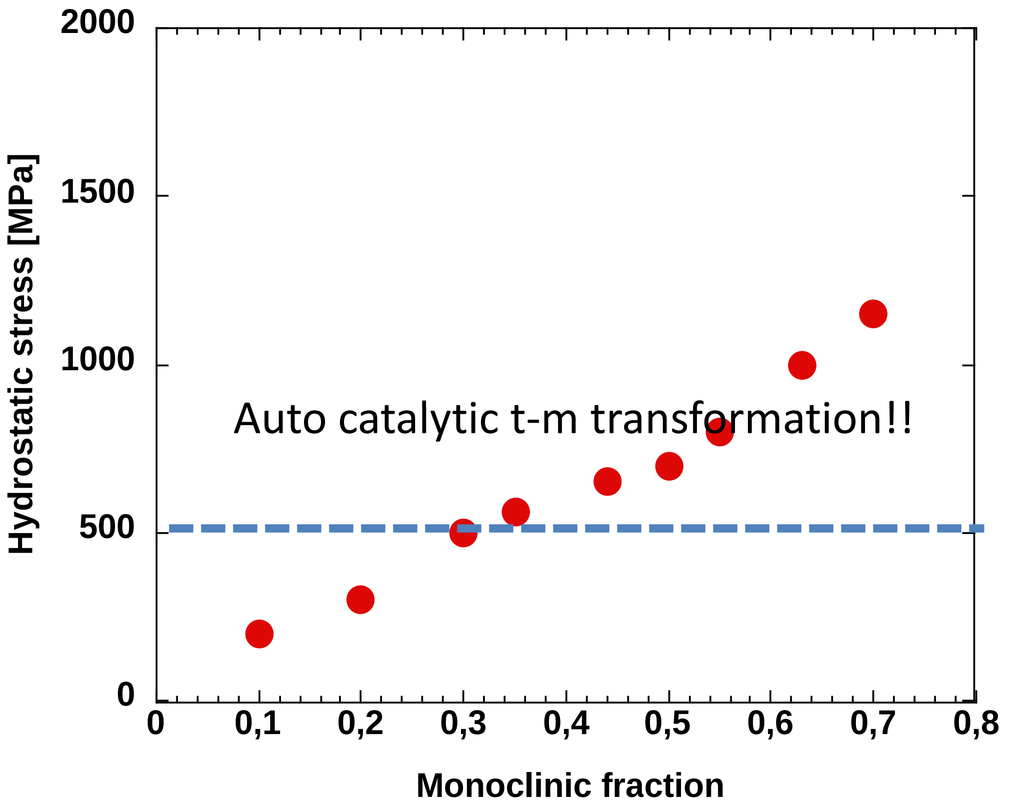


H. Tomaszewski, J. Strzeszewski L. Adamowicz and **V. Sergo**, J. Am. Ceram. Soc., 85 (11) 2855-57 (2002).



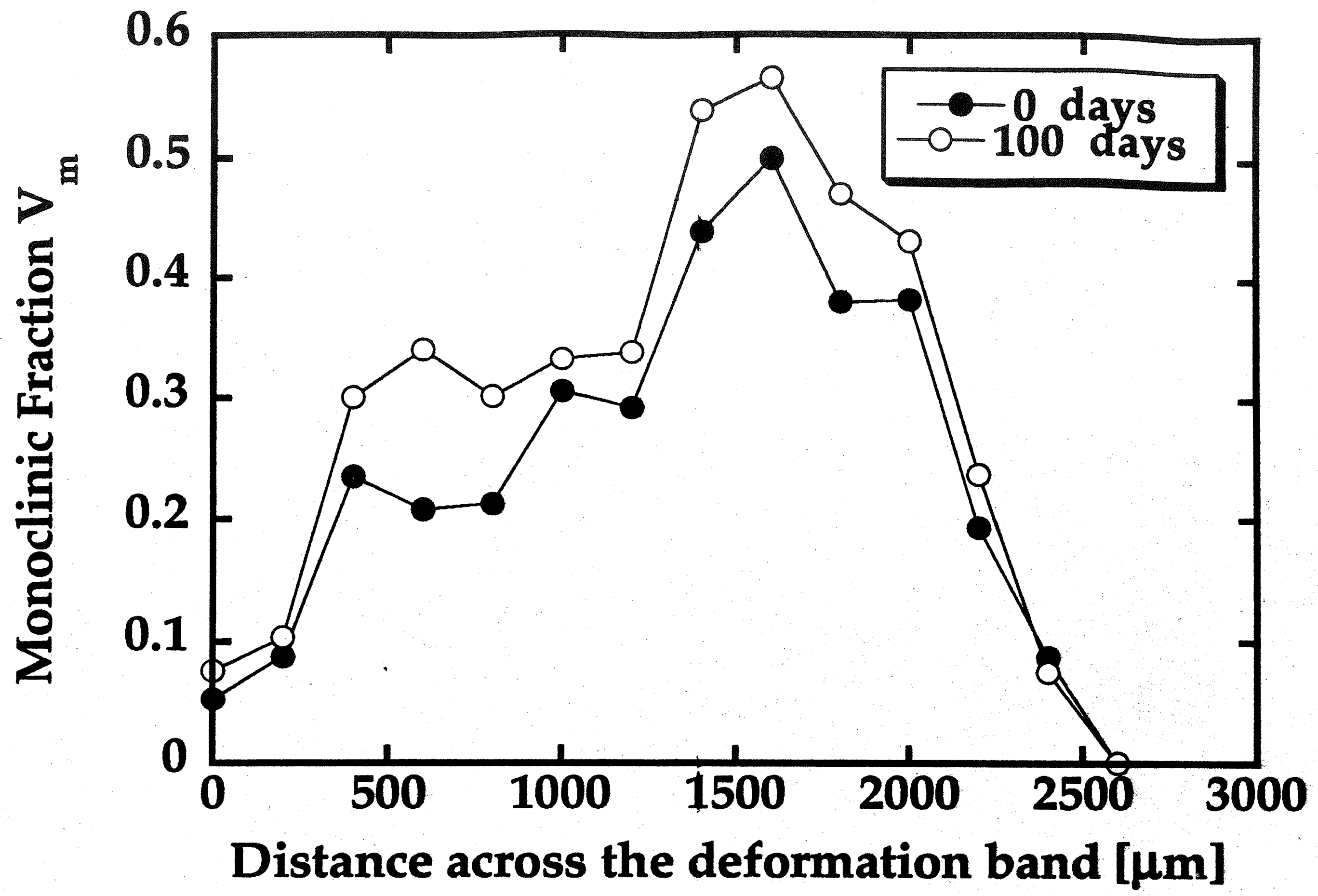


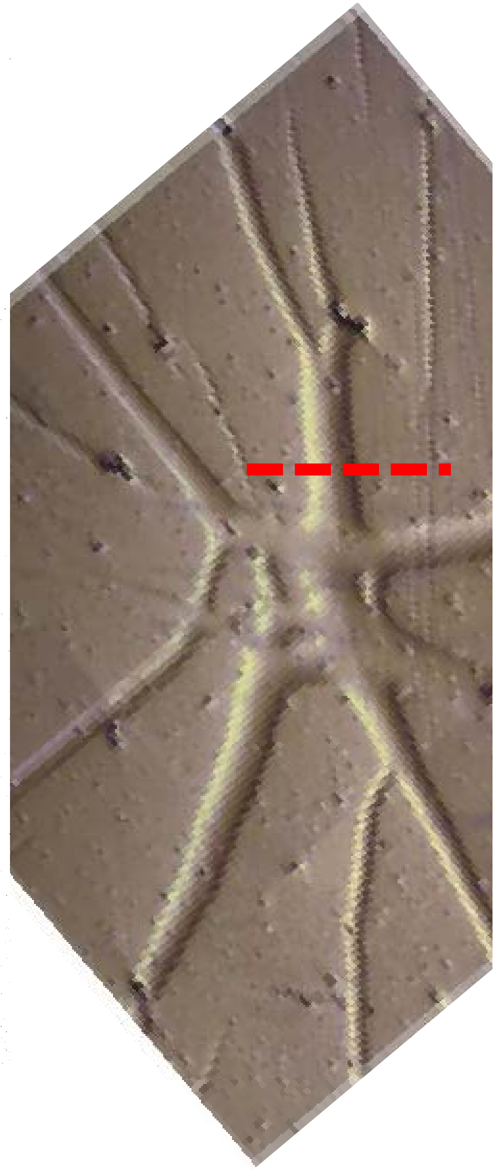
Residual stress in the remaining tetragonal phase of zirconia



Sergo et al, J Am Ceram Soc, 1995

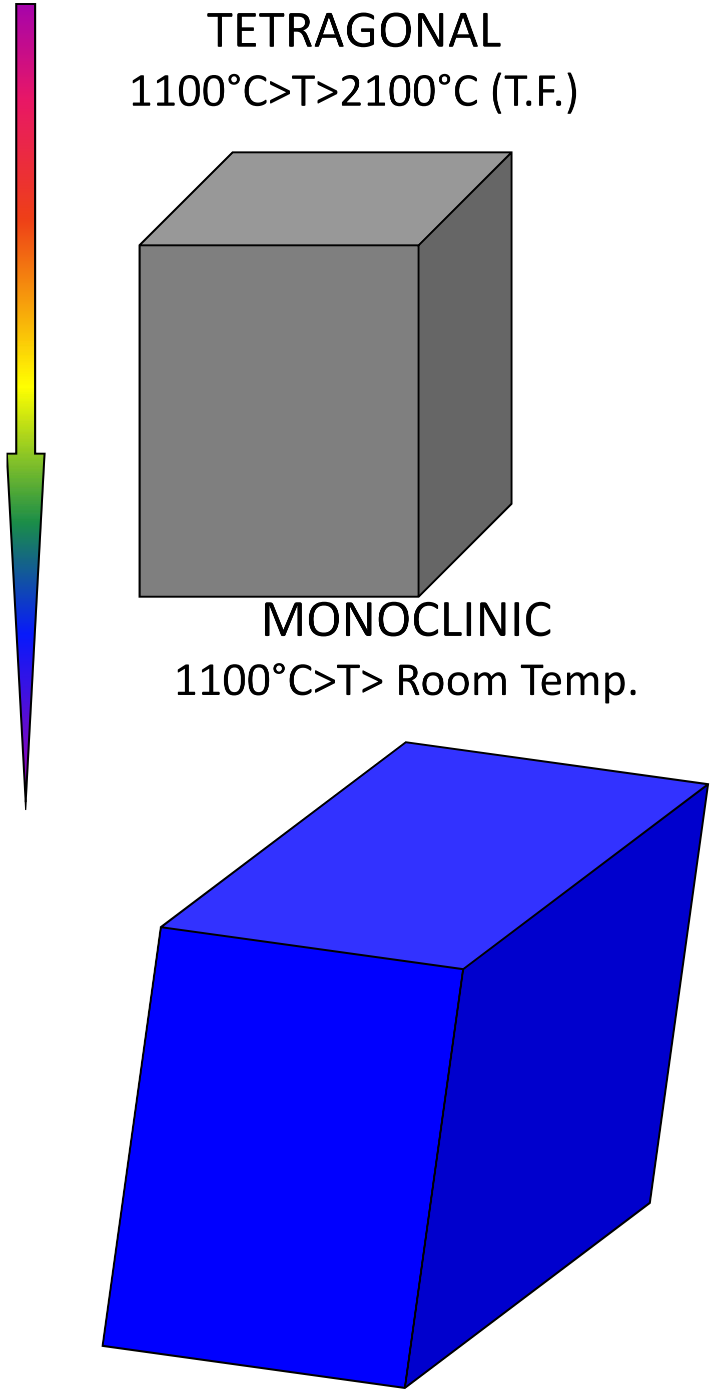
Monoclinic fraction increases more rapidly if there is already some monoclinic, even at room temperature!

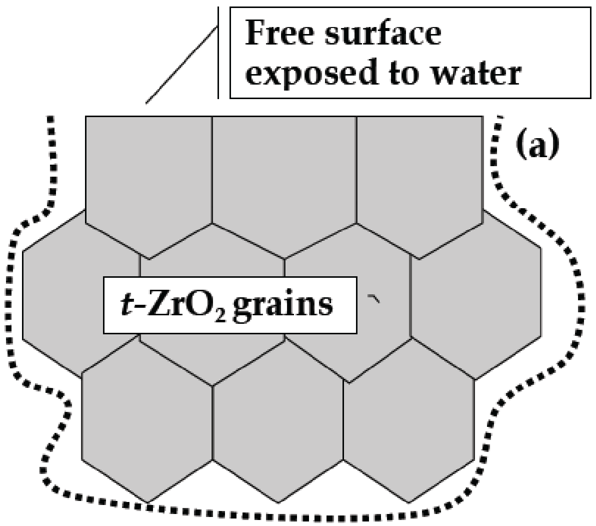


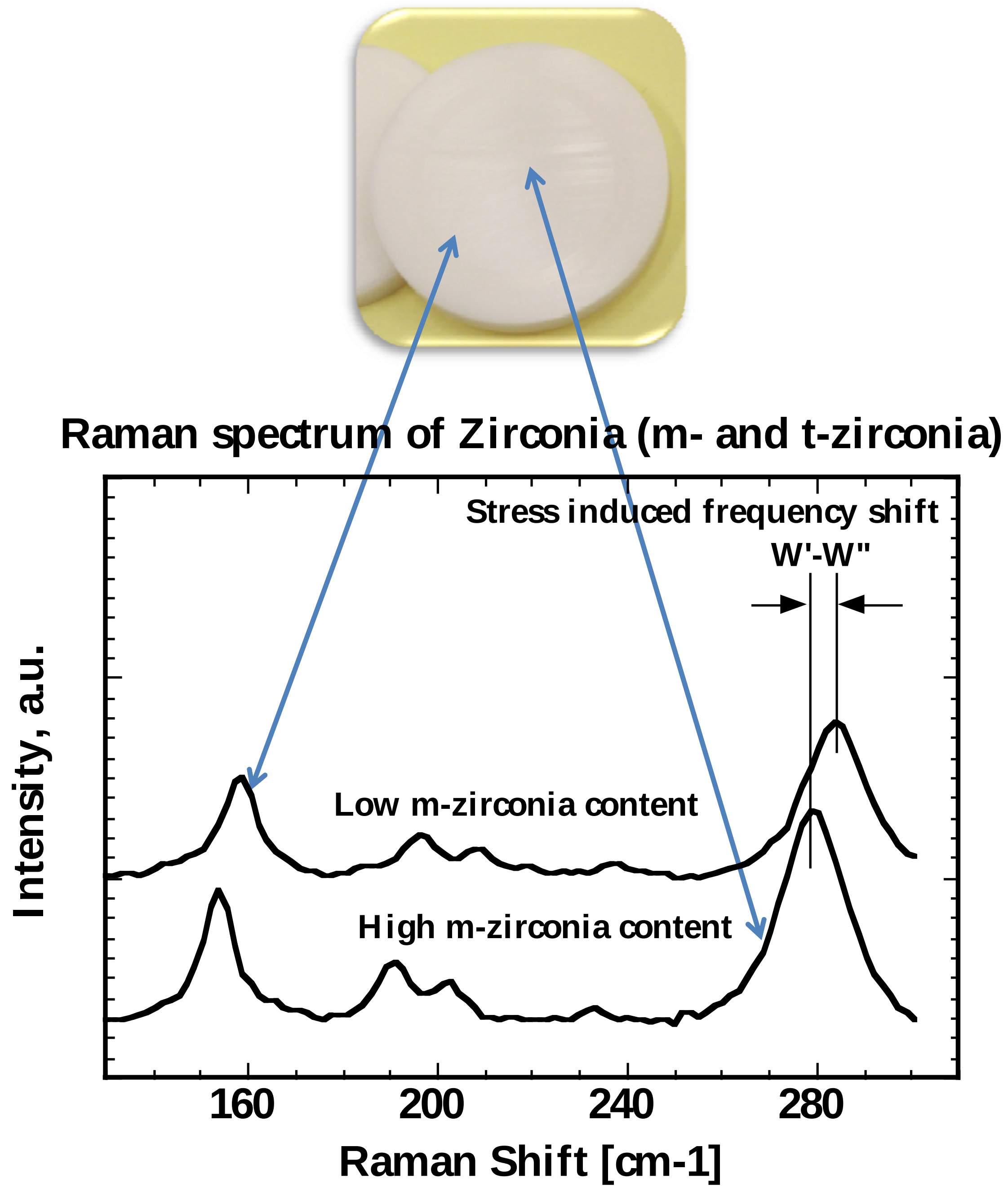


Sergo and Clarke, J Am Ceram Soc, 1995

Aging problem of zirconia

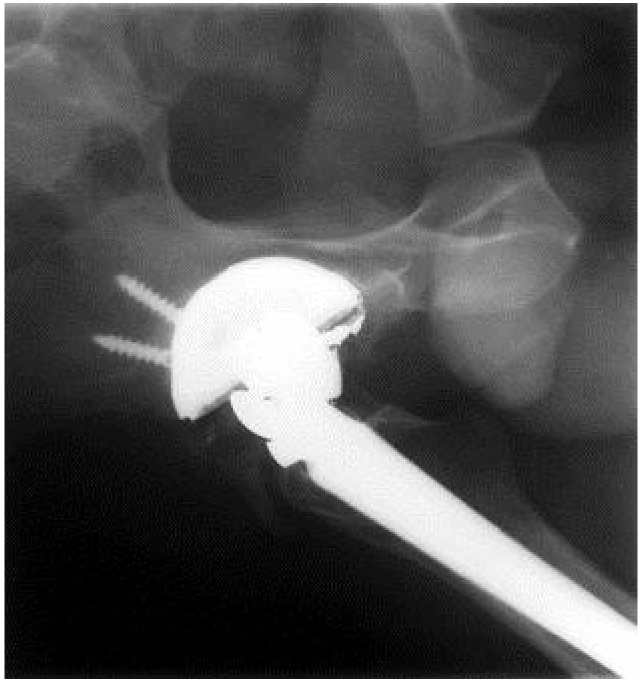


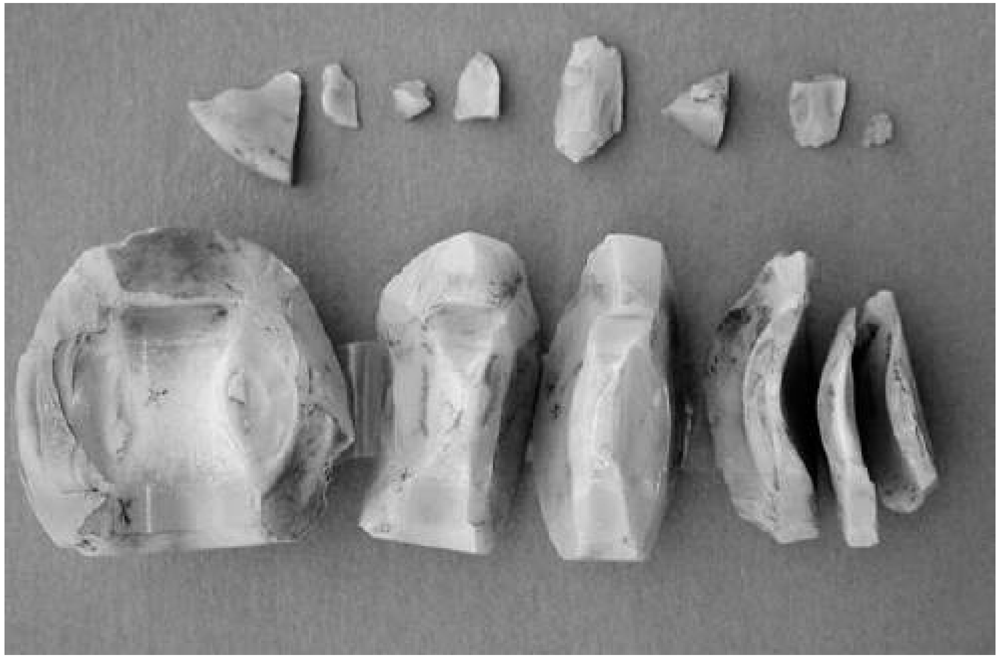




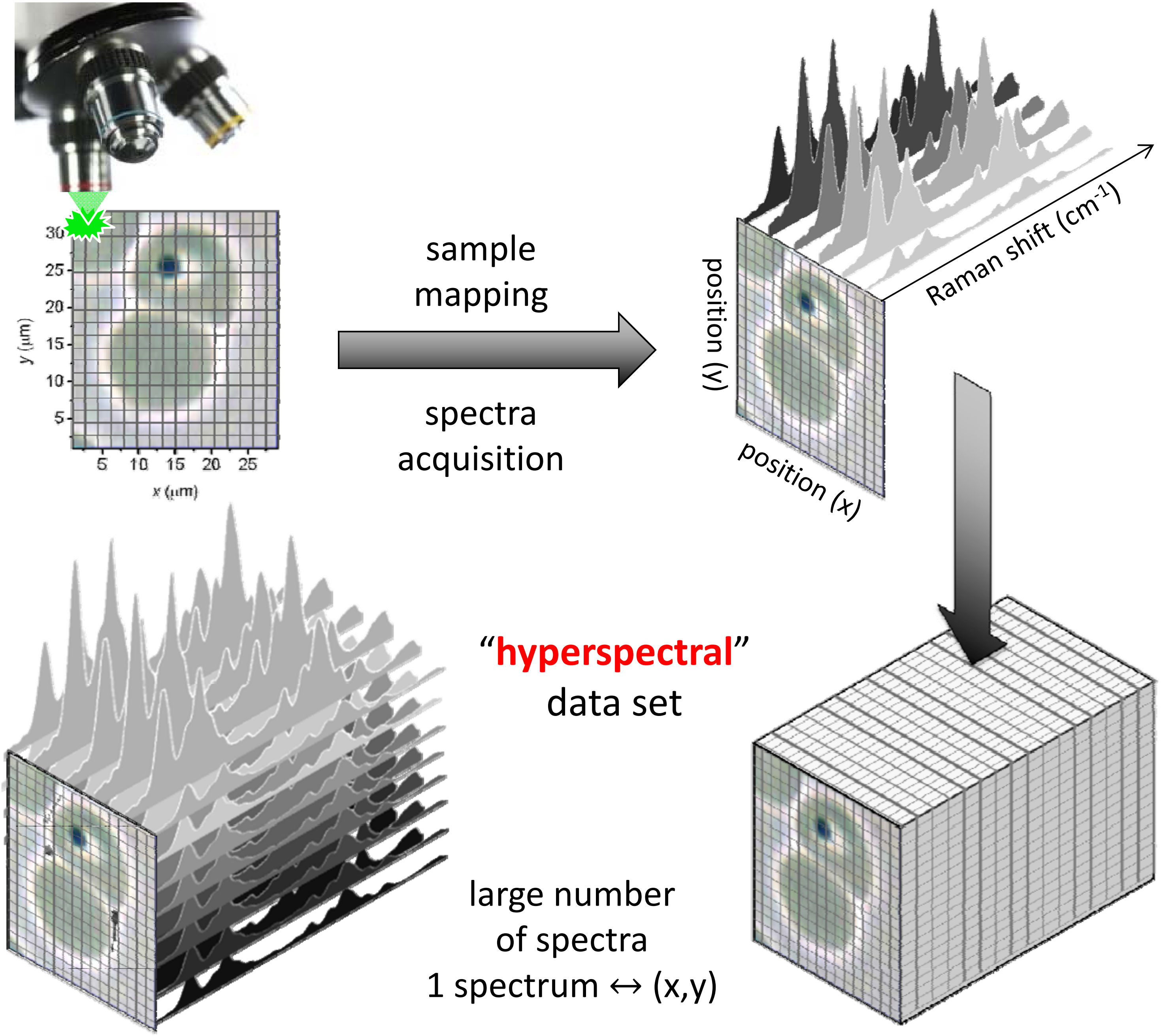
Raman spectrum of Zirconia (m- and t-zirconia)
Stress induced frequency shift
W'-W"
Low m-zirconia content
High m-zirconia content
Intensity, a.u.
160
200
240
280
Raman Shift [cm-1]

Fracture of zirconia ball heads for hip joint prostheses: the Prozyr® affaire

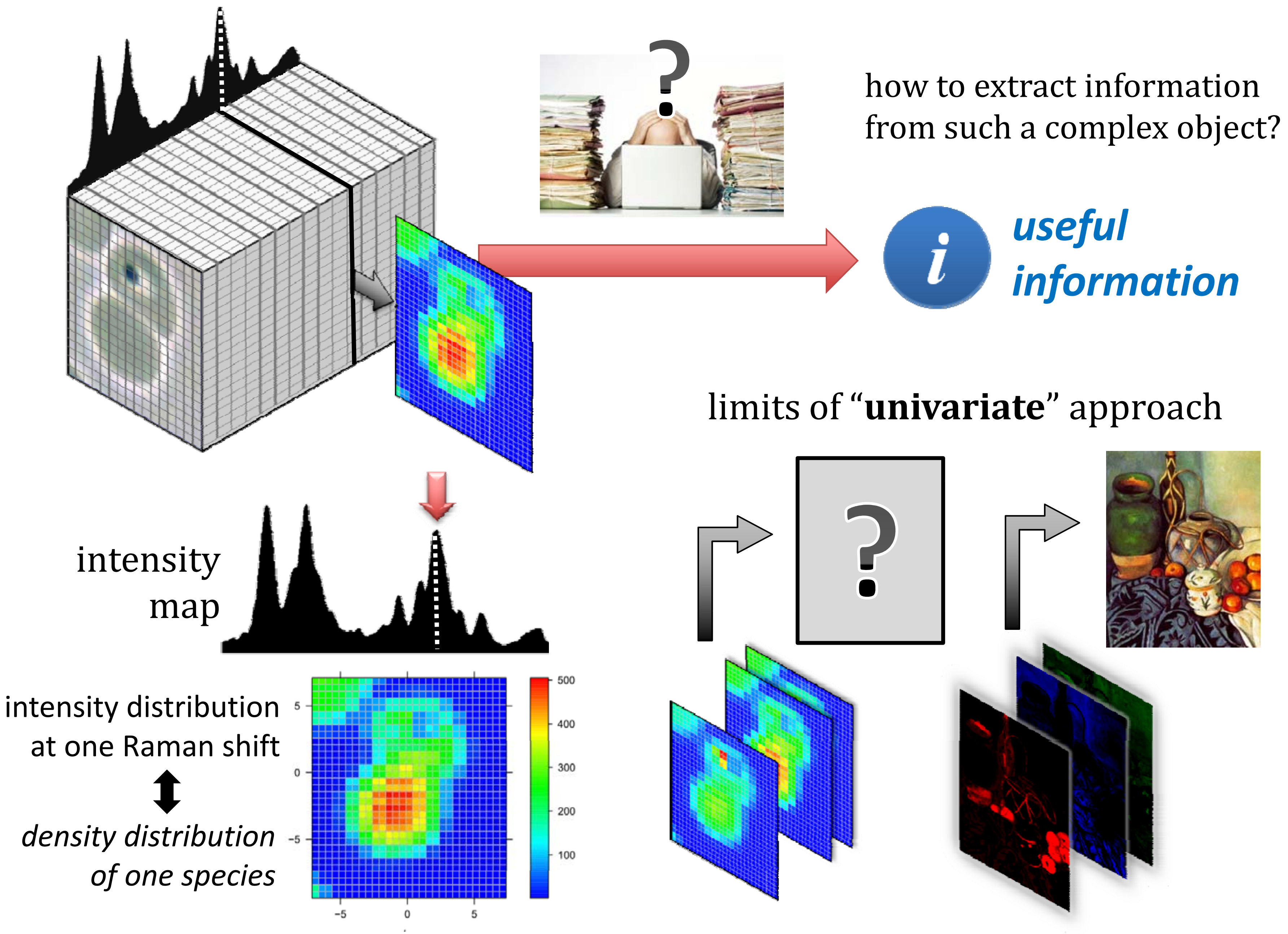


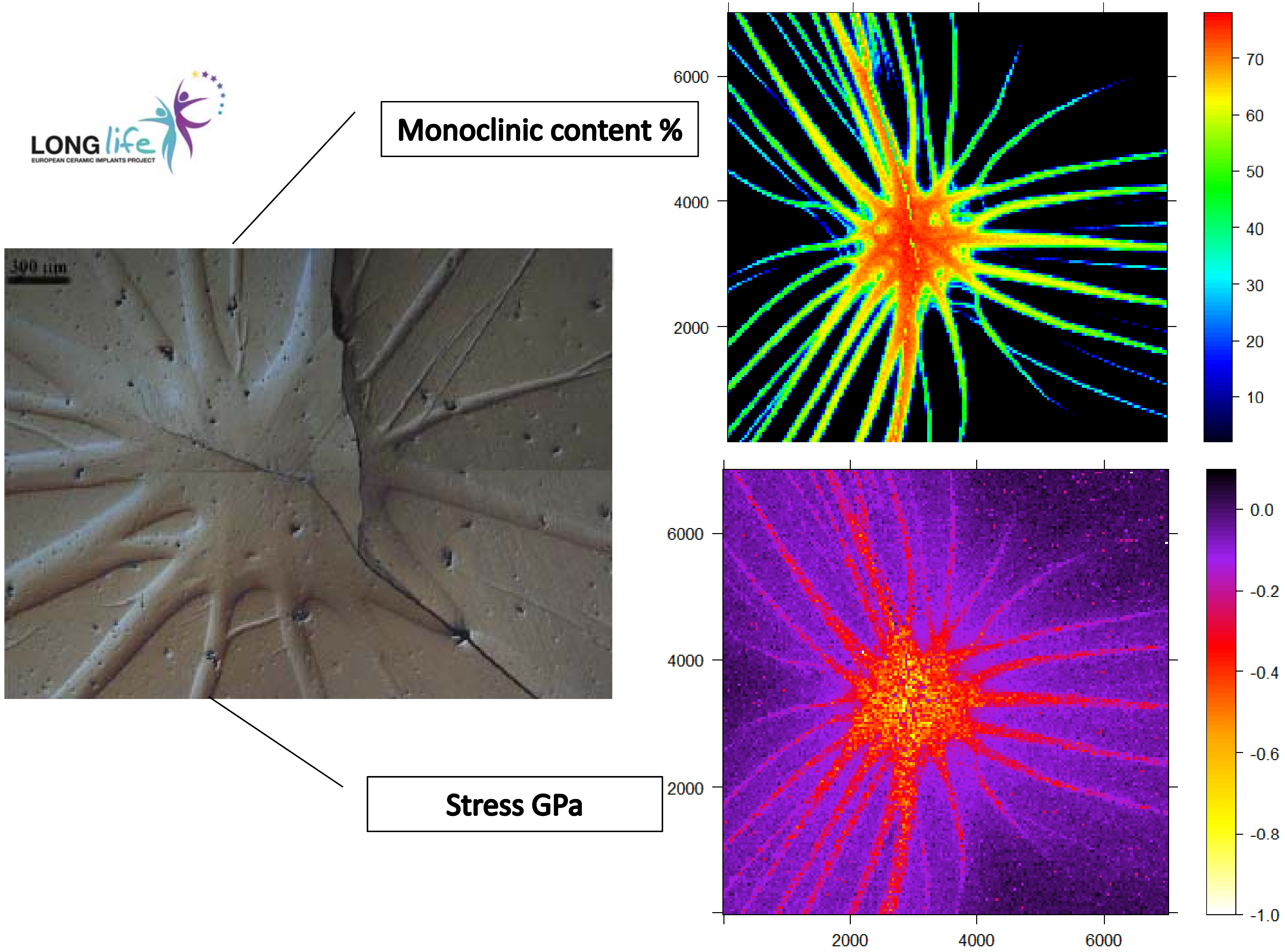


St. Gobain Desmarquest reported 162 known failures requiring revision, and that 9,051 heads were involved in the affected batches. Of those 9,051 heads involved, it is unknown how many have already been implanted. The company recalled several batches by production numbers and, due to a class action, filed for bankruptcy and disappeared.



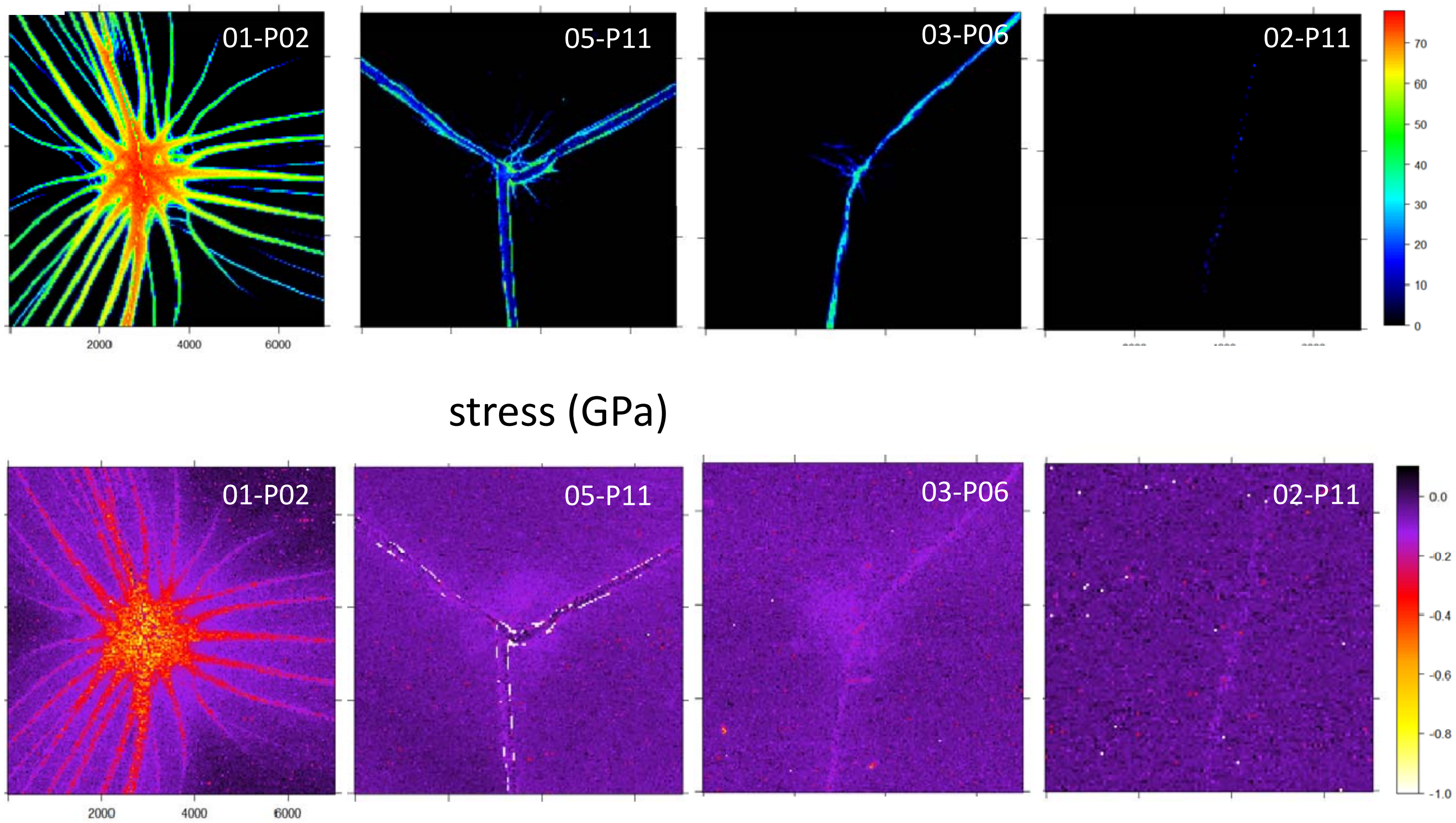
30
25
20
15
10
5
y (µm)
5 10 15 20 25
x (µm)
sample
mapping
spectra
acquisition
position (y)
position (x)
Raman shift (cm-1)
"hyperspectral"
data set
large number
of spectra
1 spectrum ↔ (x,y)


how to extract information
from such a complex object?
useful
information
limits of "univariate" approach
intensity
map
intensity distribution
at one Raman shift
density distribution
of one species
500
400
300
200
100
5
0
-5
-5
0
5


300 μm
Monoclinic content %
6000
4000
2000
70
60
50
40
30
20
10
Stress GPa
6000
4000
2000
2000
4000
6000
0.0
-0.2
-0.4
-0.6
-0.8
-1.0

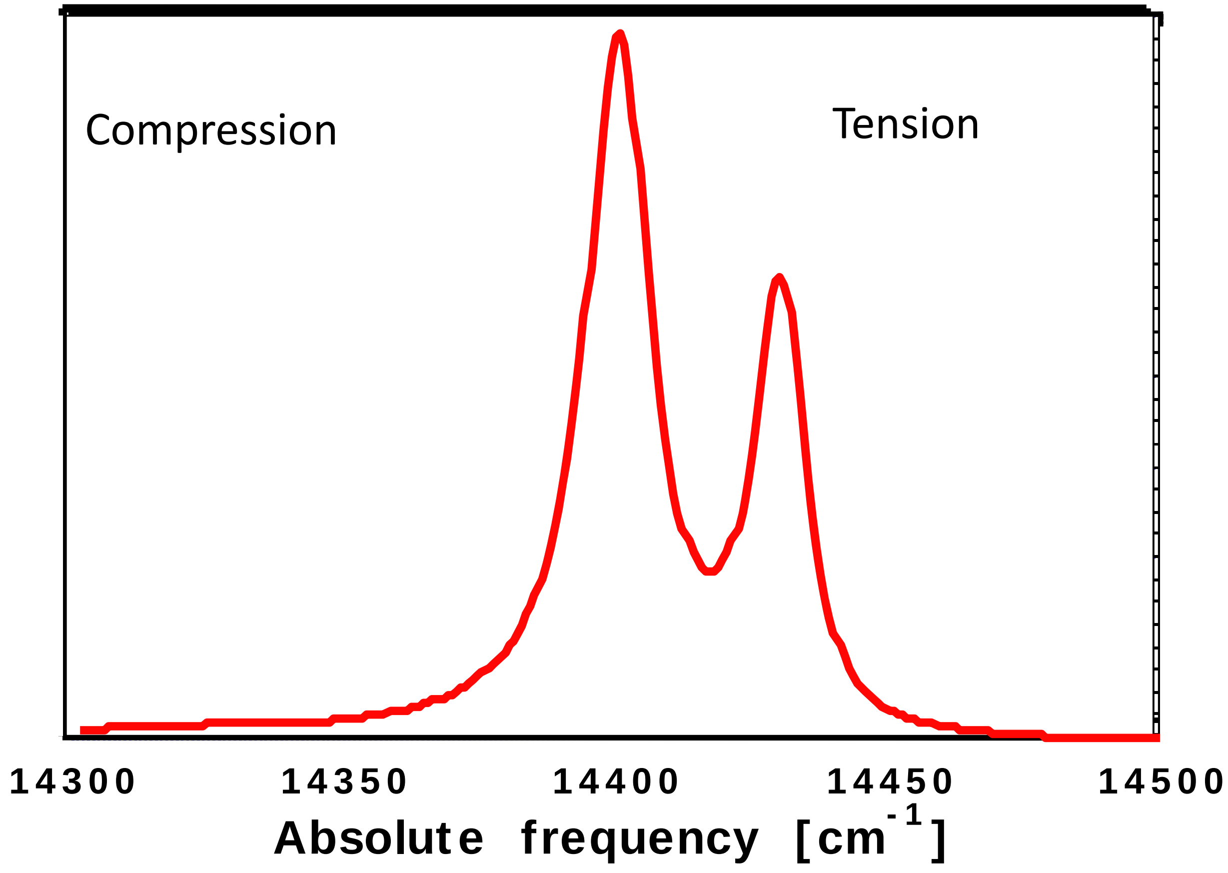
Behaviour of different ZrO_2 solid solution

monoclinic (% vol)



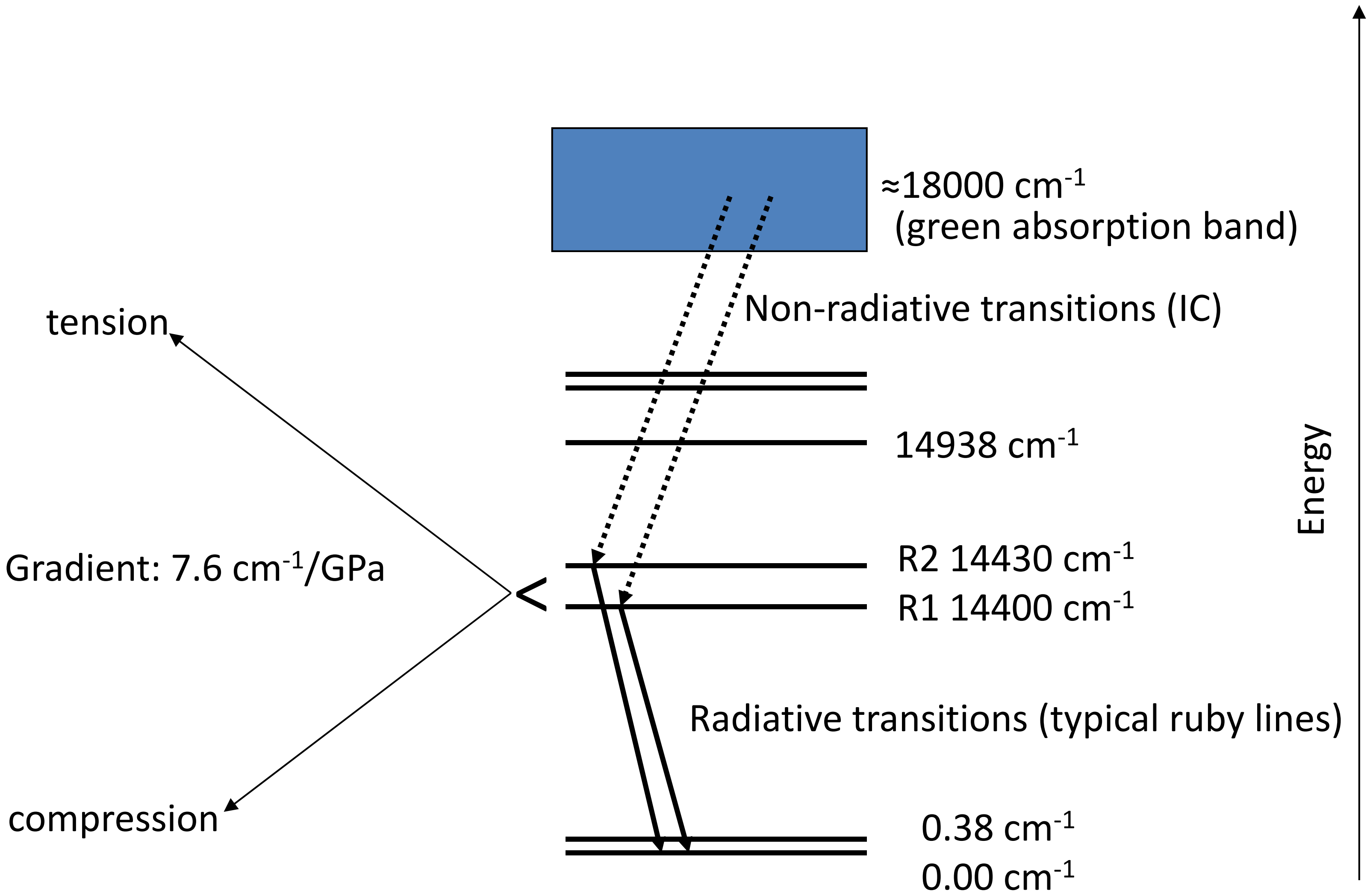
ZA_8Sr_8-Ce10.5	ZA_8Sr_8-Ce11	ZA_8Sr_8-Ce11.5	ZA_8Mg_8
(10.5Ce-TZP/ 8vol%Al_2O_3/ 8vol%$SrAl_{12}O_{19}$)	(11Ce-TZP/ 8vol%Al_2O_3/ 8vol%$SrAl_{12}O_{19}$)	(11.5Ce-TZP/ 8vol%Al_2O_3/ 8vol%$SrAl_{12}O_{19}$)	(10Ce-TZP/ 8vol% Al_2O_3/ 8vol%$CeMgAl_{11}O_{19}$)

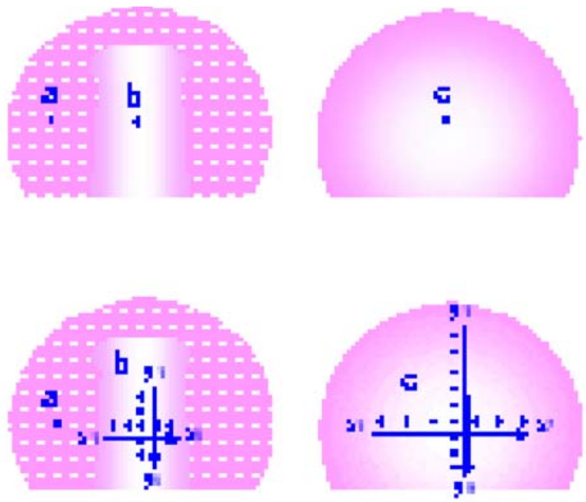
Typical fluorescence spectrum of Al_2O_3 (ruby red, due to substitutional Cr^{3+} ions)



Fluorescence Piezo-spectroscopy

Best technique for alumina-based materials: TGO under TBC's, ball heads for hip-joint prostheses, cutting tools, sapphire fibers-reinforced composites, etc.





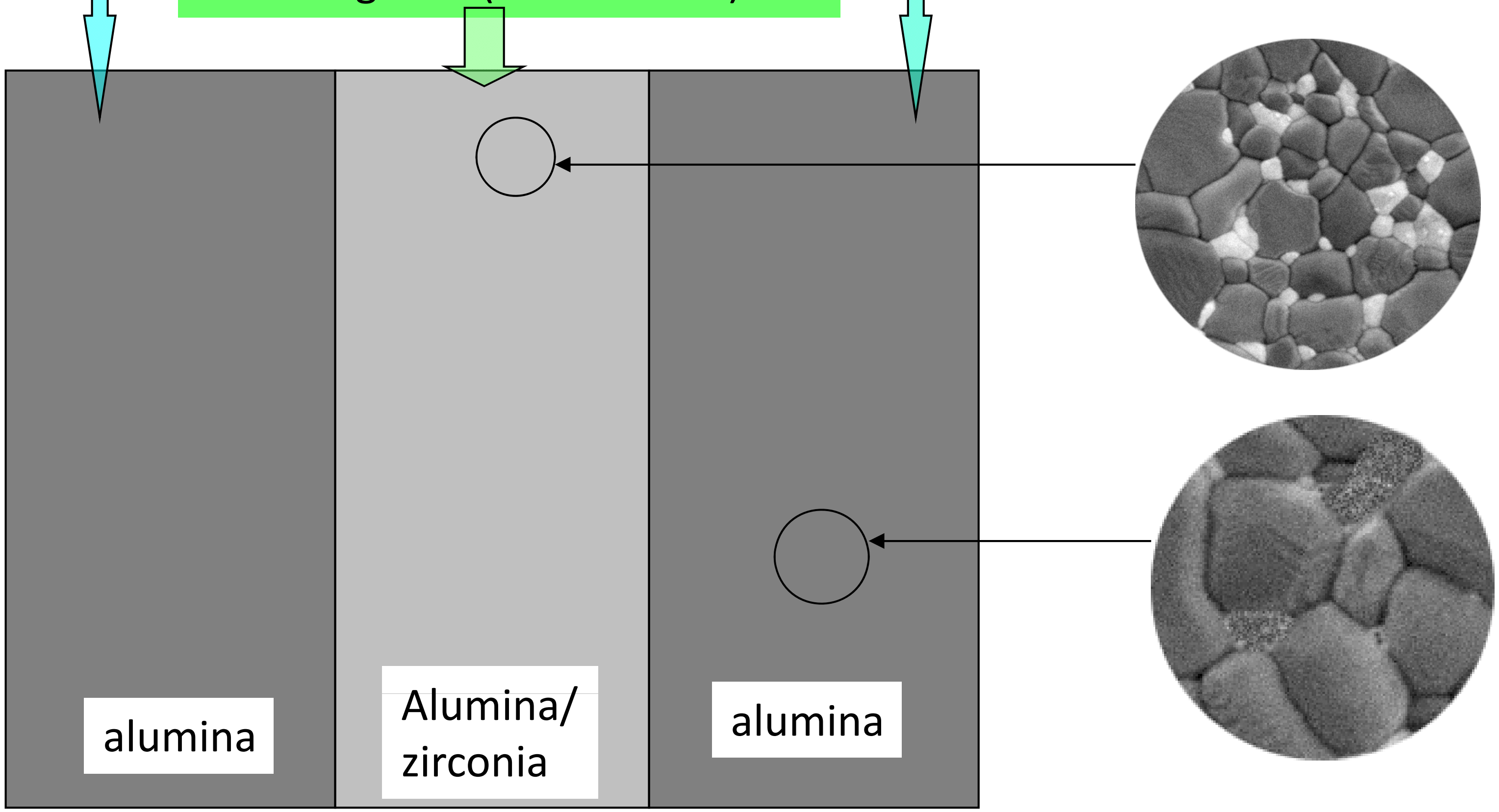
Spot analysis						
	7778		7759		7823	
	Stress (MPa)	Moniclinic Content (%)	Stress (MPa)	Moniclinic Content (%)	Stress (MPa)	Moniclinic Content (%)
a	-105	23	-118	24	-105	19
b	-39	14	-39	14	-39	11
c	-53	6	-53	11	-53	10

Evolution of stresses in laminate composites of alumina/*t*-zirconia

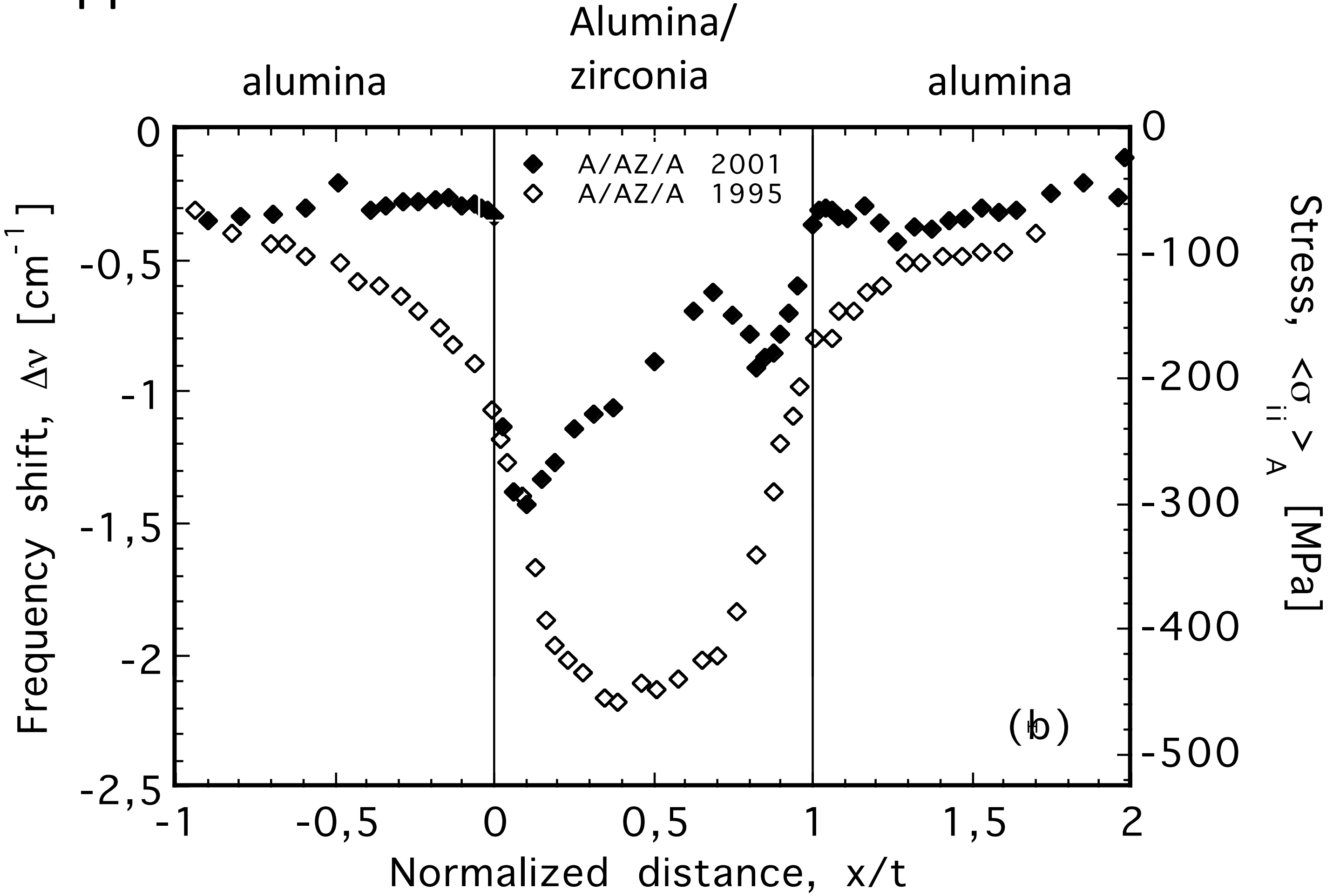
Stress due to lamination process (macro-stress only)

Superposition of stresses due to lamination (macro-stress) and intimate mixture of alumina and zirconia grains (micro-stress)

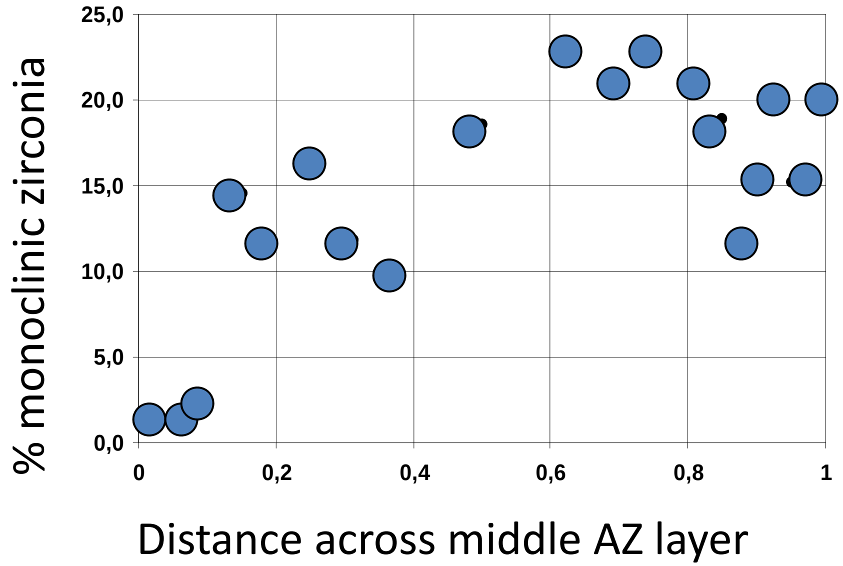
V. Sergo et al.,
J. Am. Ceram. Soc., 80 (7) 1633-1639 (1997).



The stress in alumina is compressive and symmetrical in 1995; after 6 years the stress is lower and not symmetrical any more.
What did happen?



Over 6 years t-zirconia (which is subject to residual tensile stress) has partly transformed to the monoclinic polymorph, and this has had the net effect of decreasing the residual compressive stress in alumina.



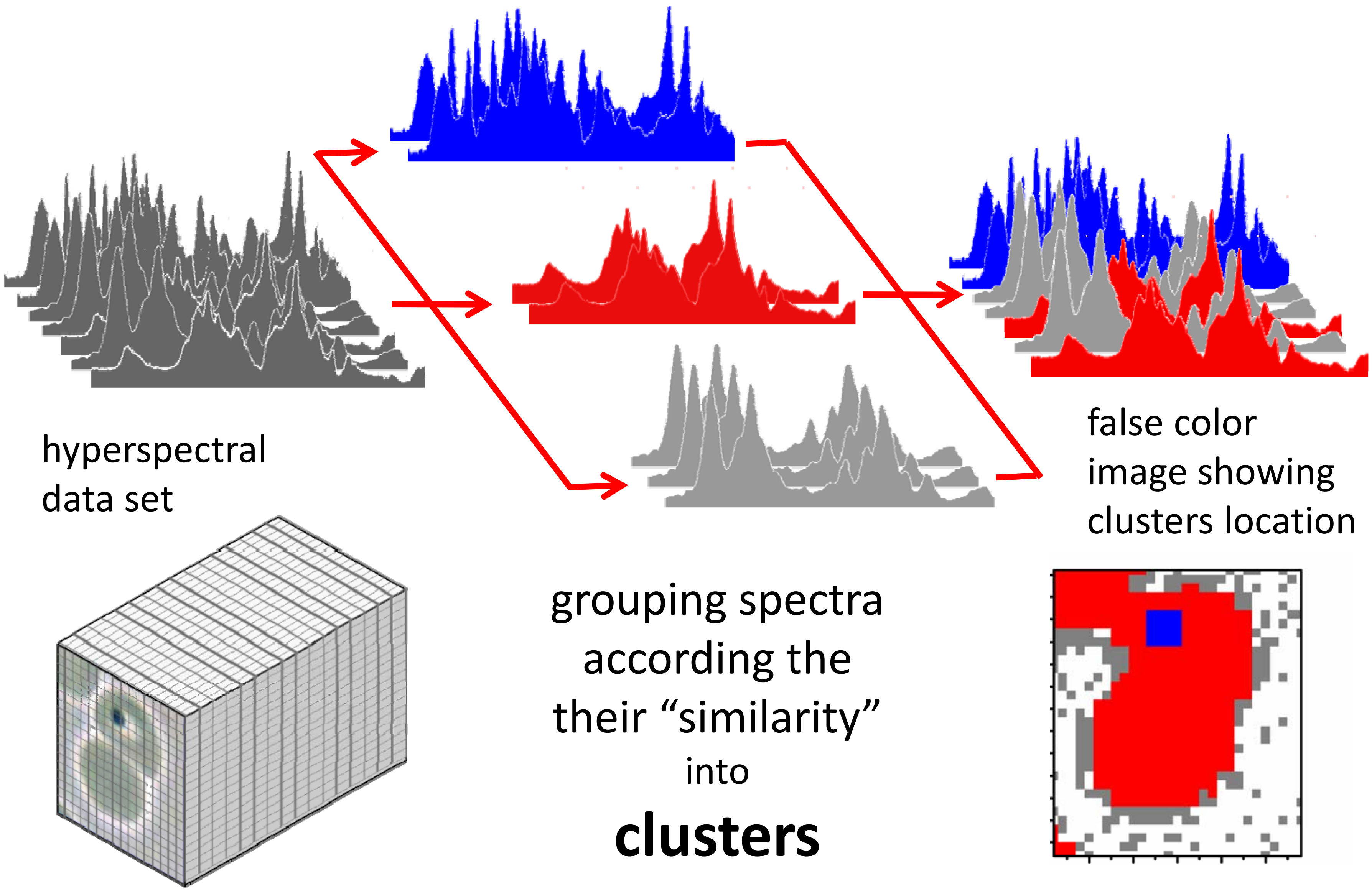
Note: The t-m transformation took place at Room temperature!

V. Sergo, J. Am. Ceram. Soc., 87 (2) 247-53 (2004)

Classification by similarity
(i.e. by distance)

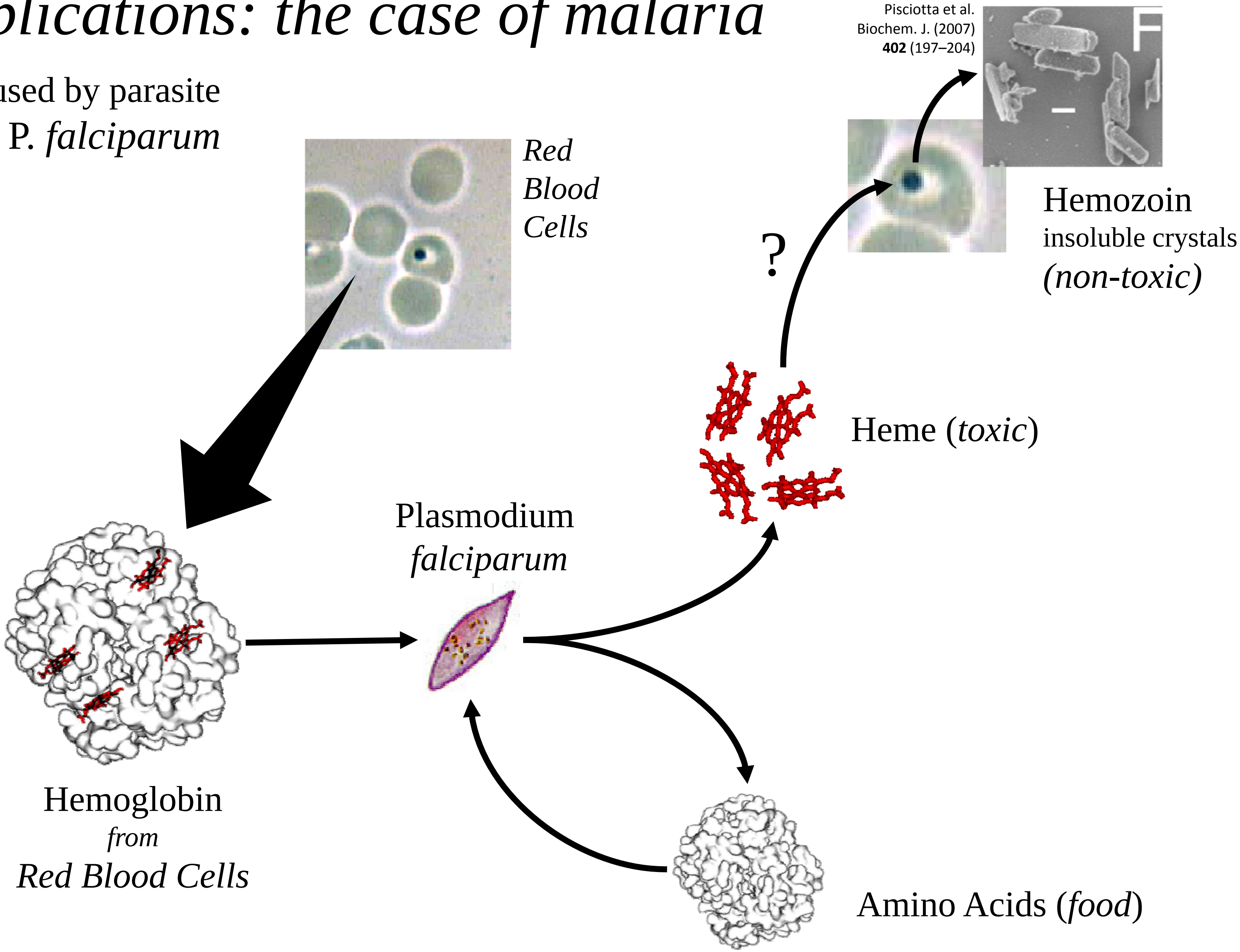
- Candies
  - Soluble in saliva
    - Sweets
      - Fruit sweets
        - Orange sweet
        - Strawberry sweet
        - Banana sweet
      - Gummy sweets
        - Gummy green little bear
        - Gummy orange dice
        - Gummy red dice
        - Gummy red sweet
      - Hard sweets
        - Red & white sweet
        - Blue sweet
    - Marzipan
      - Square marzipan
      - Round marzipan
    - Chocolate
      - Round chocolate candy
      - Square chocolate candy
      - Milk chocolate bar
      - Bitter chocolate bar
  - Insoluble in saliva
    - Chewing-gum
      - Flat Chewing-gum
      - Round Chewing-gum
      - Heart-shaped Chewing-gum

multivariate imaging with cluster analysis

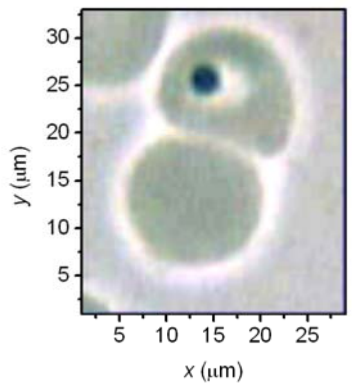


applications: the case of malaria

caused by parasite
P. *falciparum*

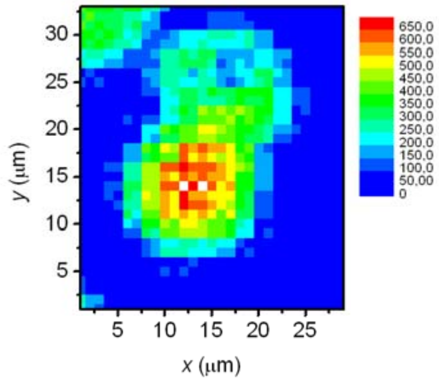


bright field image



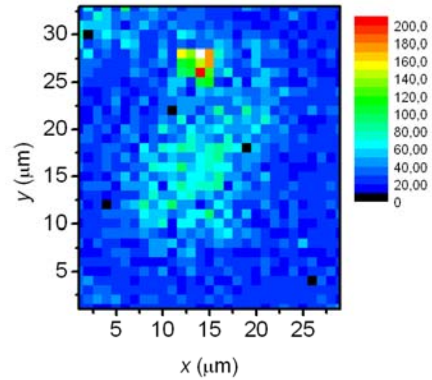
density distribution of Hemoglobin

Raman intensity @1640 cm^{-1}



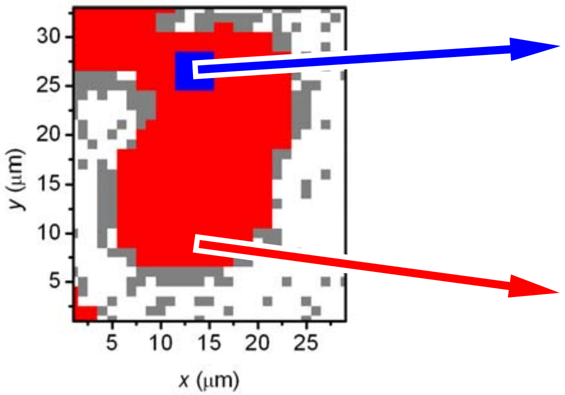
density distribution of Hemozoin

Raman intensity @1630 cm^{-1}

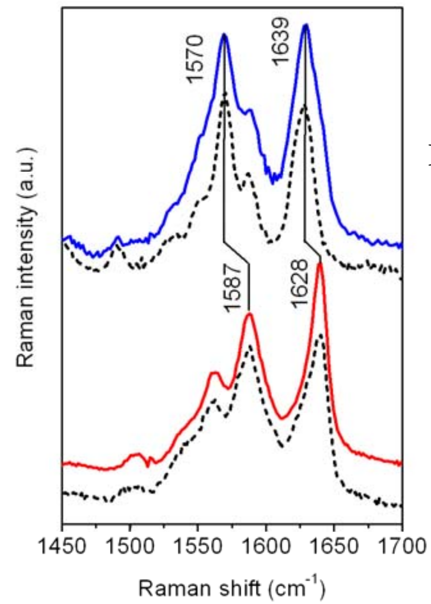


hierarchical cluster analysis

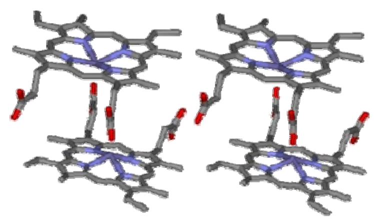
(blue:hemozoin, red:hemoglobin)



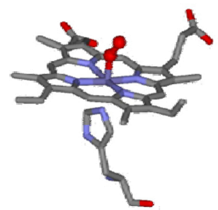
average spectra



Hemozoin



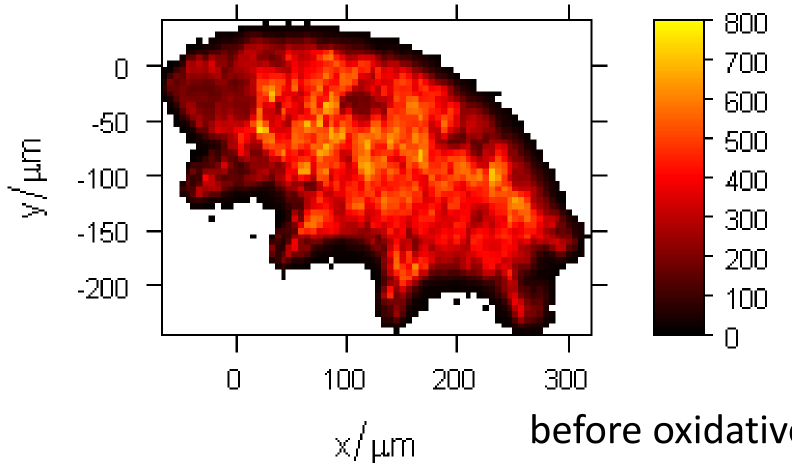
Hemoglobin



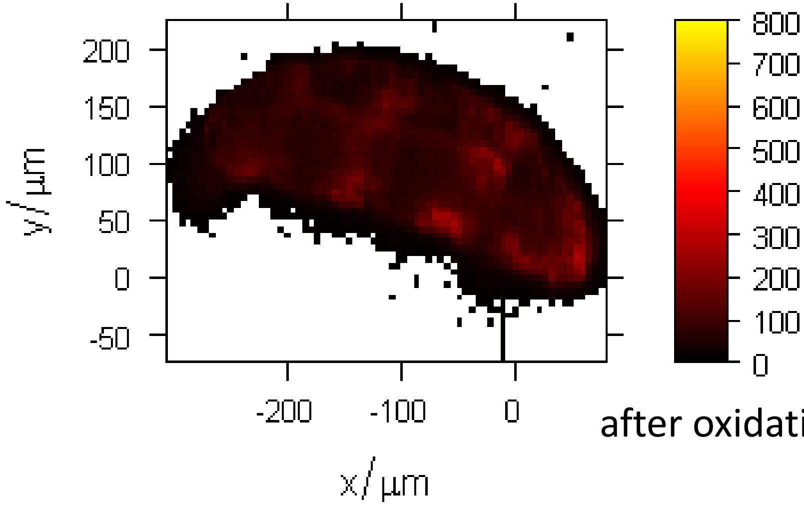
In collaboration with the University of Milan
Department of Publich Health-Microbiology-Virology
S.Finaurini, D.Taramelli

exc@514nm, 1mW, 10s/p, 60x N.A.1.00 W, step 0.5µm
Bonifacio et al. Anal.Bioanal.Chem. 2008, in press

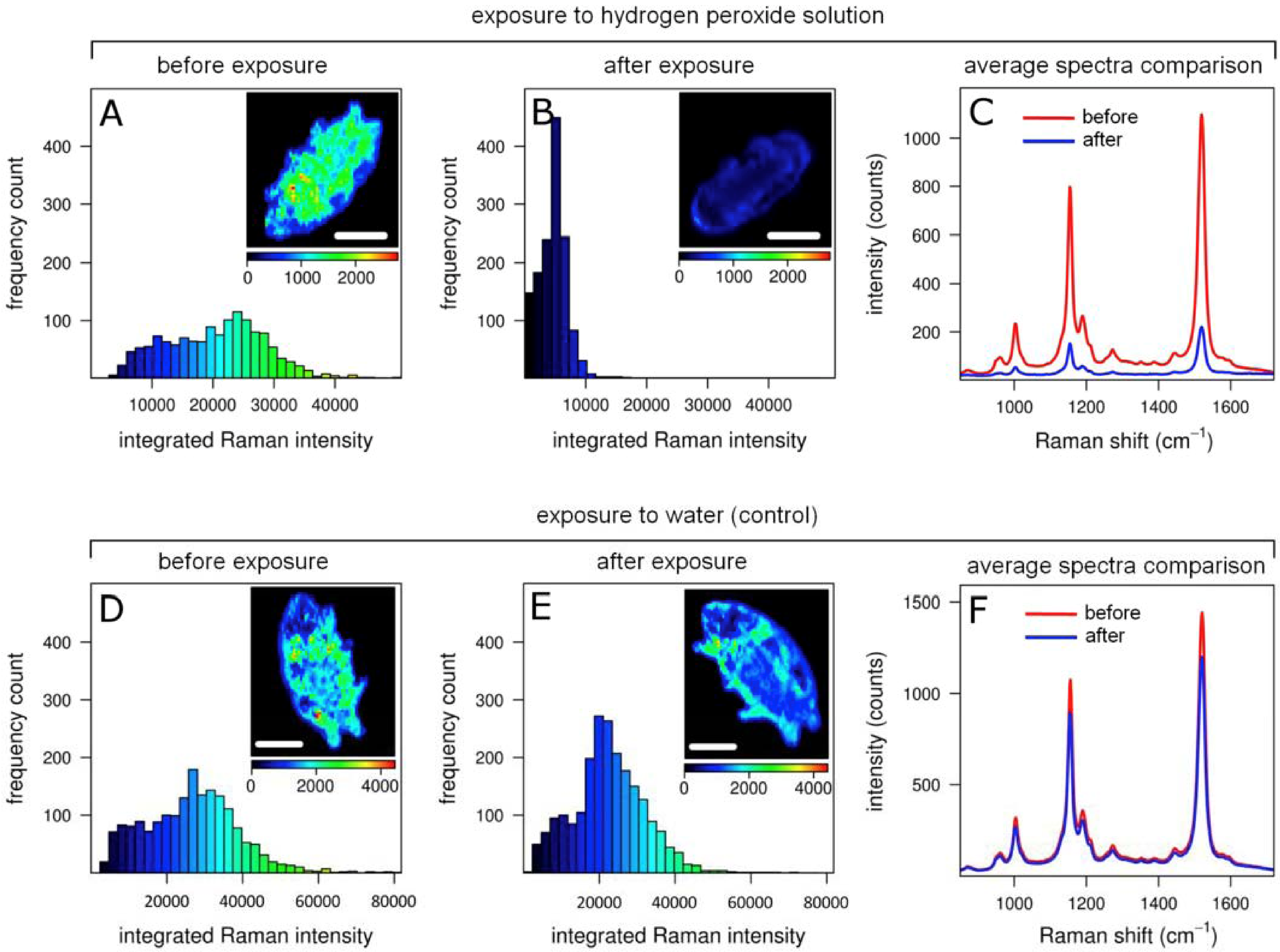
Imaging of small living organism:
carotenoids decrease in tardigrades upon oxidative stress



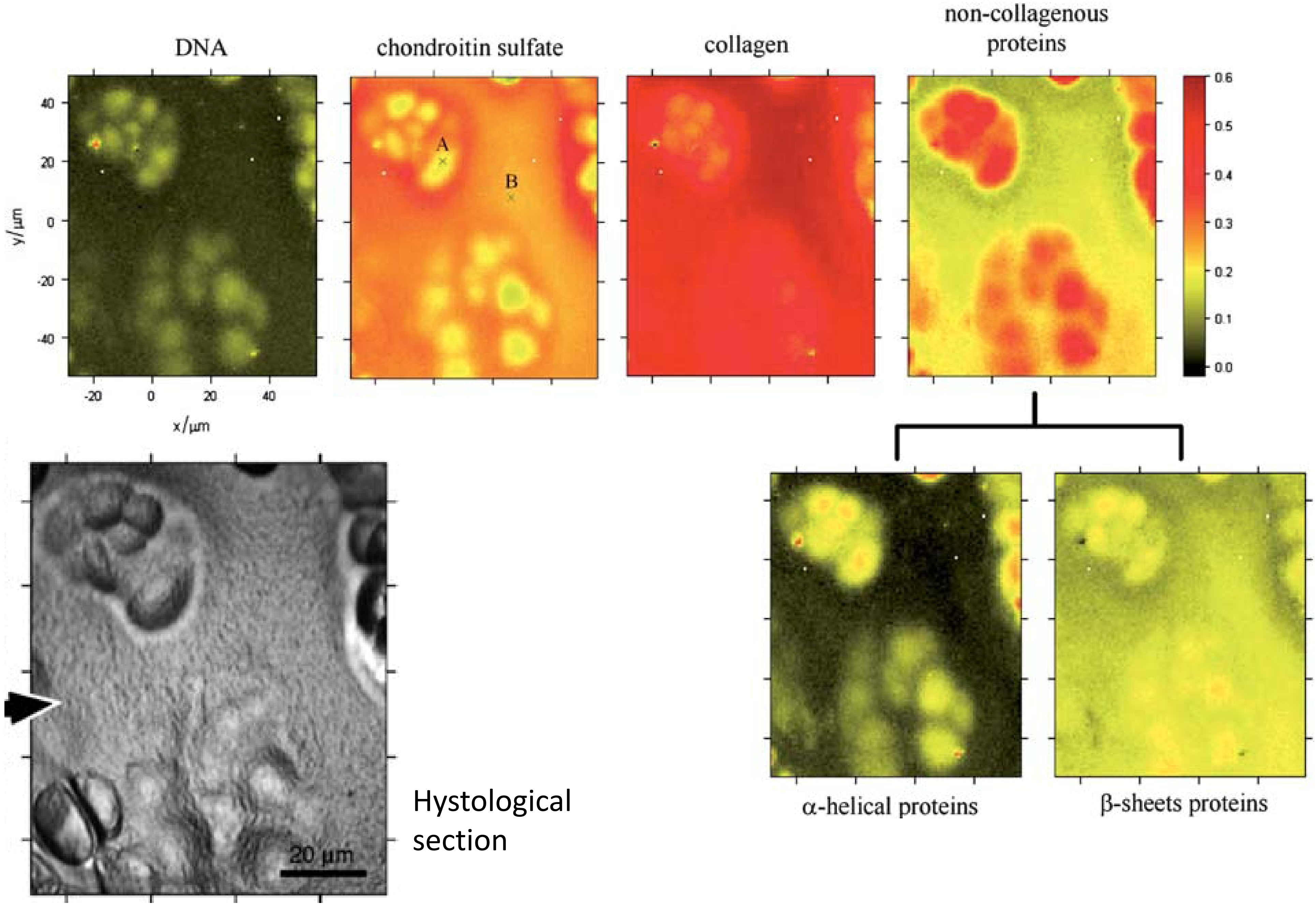
before oxidative stress



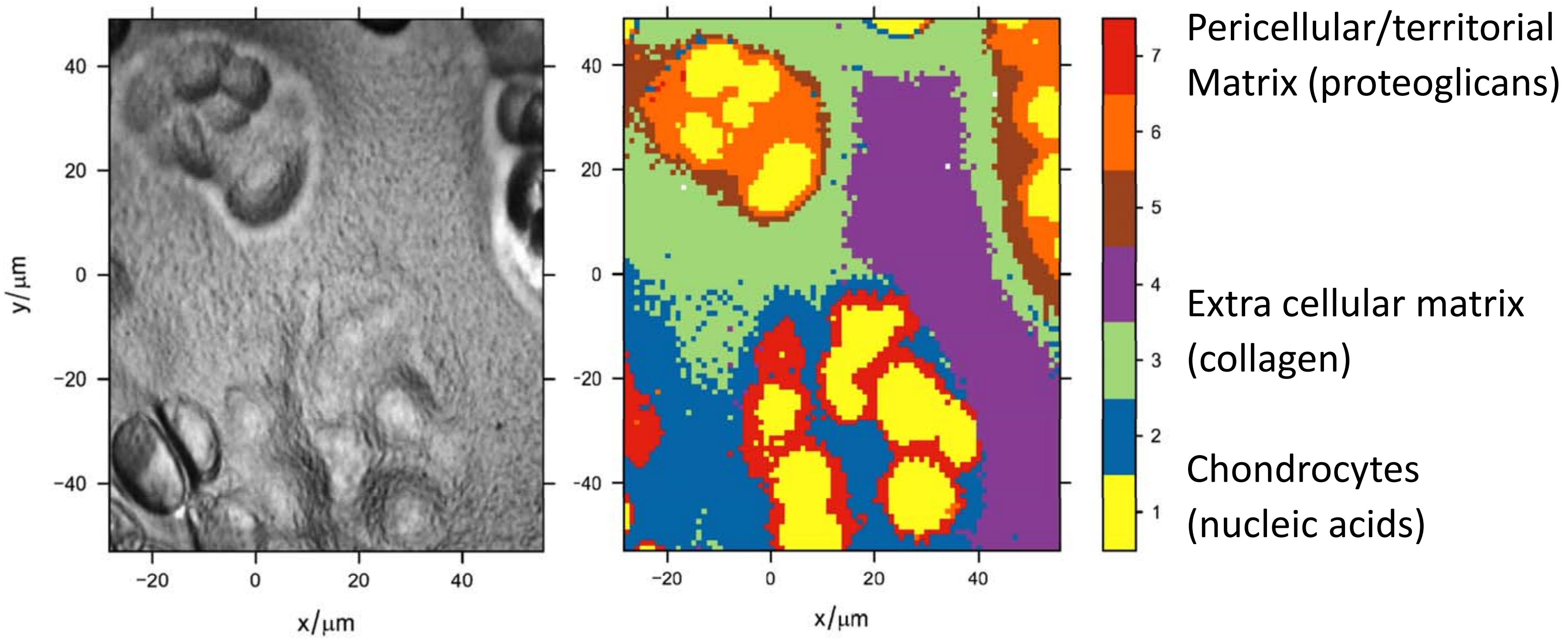
after oxidative stress


exposure to hydrogen peroxide solution
before exposure
after exposure
average spectra comparison
A
B
C
before
after
frequency count
integrated Raman intensity
intensity (counts)
Raman shift (cm^{-1})
exposure to water (control)
before exposure
after exposure
average spectra comparison
D
E
F
before
after
frequency count
integrated Raman intensity
intensity (counts)
Raman shift (cm^{-1})

Cartilage analysis



More sophisticated mapping applied to cartilage tissue



That's all volks, THANK YOU!