



GROUPEMENT NATIONAL DE
MICROSCOPIE ELECTRONIQUE A BALAYAGE
ET DE MICROANALYSES



En convention de coopération avec la Société Française de Physique

Le couplage MEB-FIB- μ Raman en conditions cryogéniques : nouvelle voie de caractérisations structurale et chimique des échantillons biologiques

Cryo SEM-FIB- μ Raman for the investigation of biological samples



Mouad Essani

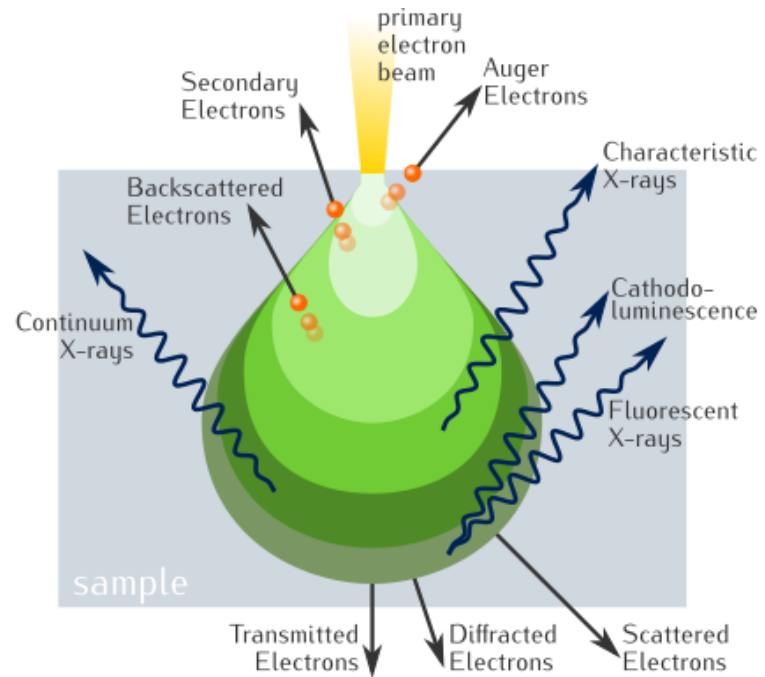


mouad.essani@lisa.ipsl.fr

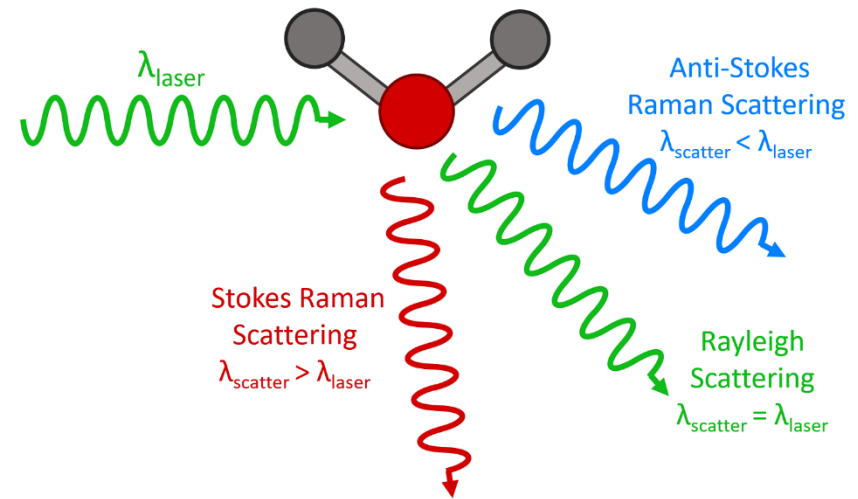
06/07/2023

Correlative scanning electron microscopy (SEM)-Raman

Some basic notions...



Electron microscopy



Raman spectroscopy

Some studies on correlative SEM- μ Raman



Micron

Volume 67, December 2014, Pages 50-64



Raman-in-SEM, a multimodal and multiscale analytical tool: Performance for materials and expertise

[Guillaume Wille](#), [Xavier Bourrat](#), [Nicolas Maubec](#), [Abdelatif Lahfid](#)



Microchemical Journal

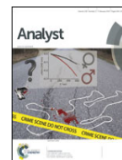
Volume 94, Issue 1, January 2010, Pages 65-72



Interfaced SEM/EDX and micro-Raman Spectrometry for characterisation of heterogeneous environmental particles – Fundamental and practical challenges

[Anna Worobiec](#), [Sanja Potgieter-Vermaak](#), [Alan Brooker](#), [Larysa Darchuk](#), [Elzbieta Stefaniak](#), [Rene Van Grieken](#)

Issue 3, 2014



From the journal:
Analyst

In-SEM Raman microspectroscopy coupled with EDX – a case study of uranium reference particles

[Elzbieta A. Stefaniak](#), [Fabien Pointurier](#), [Olivier Marie](#), [Jan Truyens](#) and [Yetunde Aregbe](#)



Preliminary Evaluation of Micro-Raman Spectrometry for the Characterization of Individual Aerosol Particles

[S. S. Potgieter-Vermaak](#) and [R. Van Grieken](#) [View all authors and affiliations](#)

Volume 60, Issue 1 | <https://doi.org/10.1366/000370206775382848>



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analytical
chemistry

Characterization of the Chemical Composition of Uranium Microparticles with Irregular Shapes Using Standardless Electron Probe Microanalysis and Micro-Raman Spectrometry

[Mouad Essani](#), [Emmanuelle Brackx](#), [Fabien Pointurier](#), [Faustine Berthy](#), [Emmanuel Excoffier](#), and [Philippe Jonnard](#)

Cite this: *Anal. Chem.* 2020, 92, 12, 8435–8443

Publication Date: May 29, 2020

<https://doi.org/10.1021/acs.analchem.0c01124>

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Some studies on correlative SEM- μ Raman



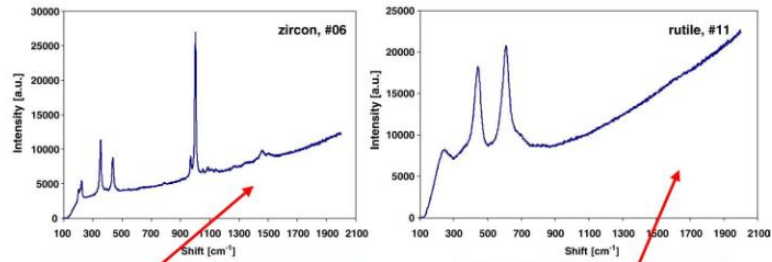
Microchemical Journal
Volume 94, Issue 1, January 2010, Pages 65-72



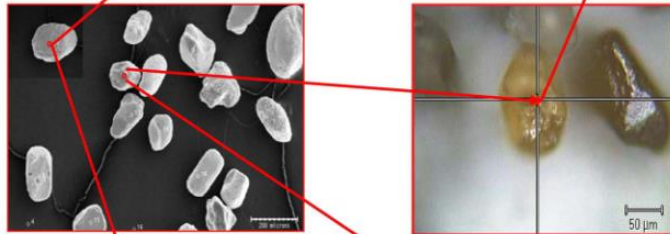
Interfaced SEM/EDX and micro-Raman Spectrometry for characterisation of heterogeneous environmental particles — Fundamental and practical challenges

Anna Worobiec^a, Sanja Polgieter-Vermaak^a, Alan Brooker^{a,b}, Larysa Darchuk^a, Elzbieta Stefaniak^a, Rene Van Grieken^a

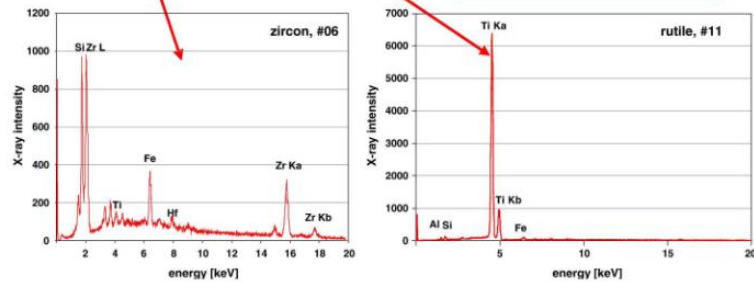
Raman



SEM



EDX



- Poor electrostatic separation of Zircon and TiO₂ attributed to enhanced conductivity of zircon by the presence TiO₂ phases

Some studies on correlative SEM- μ Raman



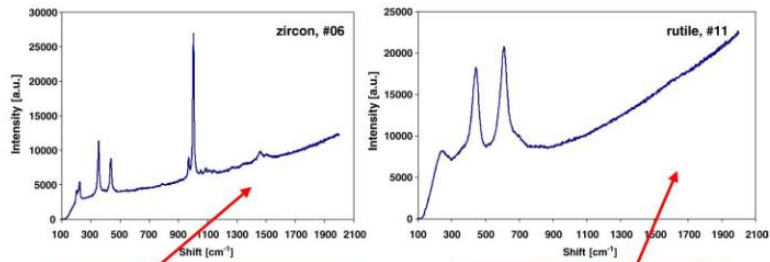
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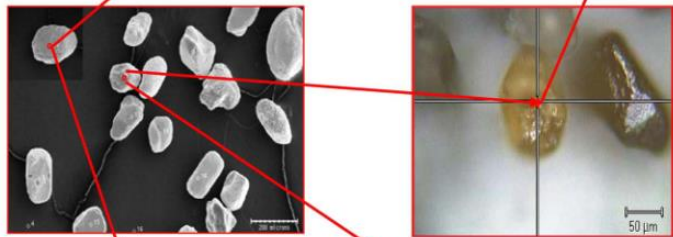
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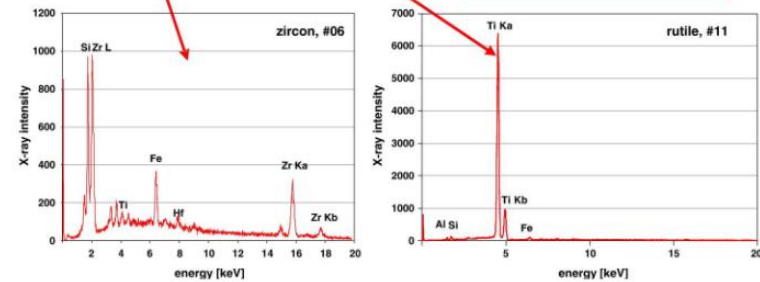
Raman



SEM



EDX



➤ Poor electrostatic separation of Zircon and TiO₂ attributed to enhanced conductivity of zircon by the presence TiO₂ phases



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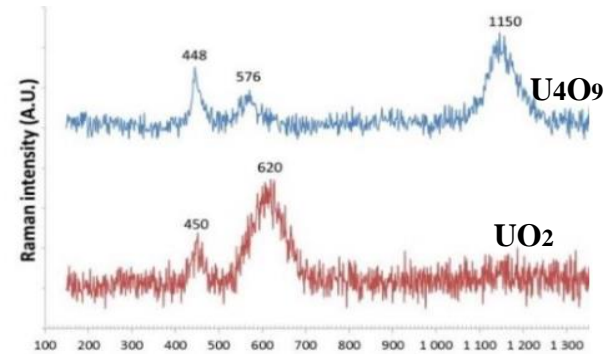
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analytical chemistry

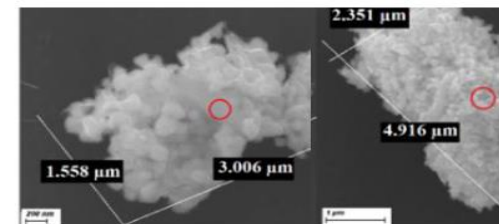
Mouad Essani^a, Emmanuelle Brackx, Fabien Pointurier, Faustine Berthy, Emmanuel Excoffier, and Philippe Jonnard

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Publication Date: May 29, 2020
<https://doi.org/10.1021/acs.analchem.0c01124>
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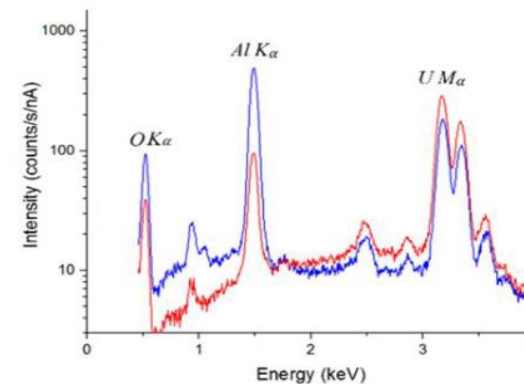
Raman



SEM



EDX



Average quantitative concentrations

	Cc %(U)	Cc %(O)
UO ₂	88.1	11.9
	85.6±1.1	14.4±1.1

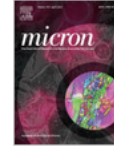
➤ MRS reveals U₄O₉ caused by oxidation of UO₂ -> in agreement with EDS results showing higher amount of O

Is it always beneficial to work with a SEM- μ Raman instrument ?









Micron

Volume 143, April 2021, 103029



The combination of Raman microscopy and electron microscopy – Practical considerations of the influence of vacuum on Raman microscopy

Harald Fitzek^a  , Karin Wewerka^b , Ute Schmidt^c , Hartmuth Schröttner^{a b} ,
Armin Zankel^{a b} 

Raman microscope has to operate in vacuum in a correlative Raman-SEM, which is a type of microscope combination that has recently increased in popularity. This work considers the implications of conducting Raman microscopy under vacuum, as opposed to operating in ambient air, the standard working regime of this technique. We show that the performance of the optics of the Raman microscope are identical in both conditions, but laser beam-sample interactions, such as fluorescent bleaching and beam damage, might be different due to the lack of oxygen in vacuum. The bleaching of the fluorescent background appears to be mostly unaffected by the lack of oxygen, except when very low laser powers are used. Regarding laser-beam damage, organic samples are more sensitive in vacuum than in air, whereas no definite verdict is possible for inorganic samples. These findings have practical implications for the application of correlative Raman-SEM, as low laser powers, or in extreme cases cryo-methods, need to be used for organic samples that appear only moderately beam sensitive under usual ambient air.

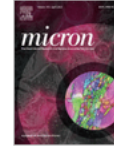
- **Laser beam-sample interactions (e.g. fluorescent bleaching and beam damage) might be different due to the lack of oxygen in vacuum.**
- **Laser-beam damage affects organic samples in vacuum more than in air.**

Is it always beneficial to work with a SEM- μ Raman instrument ?







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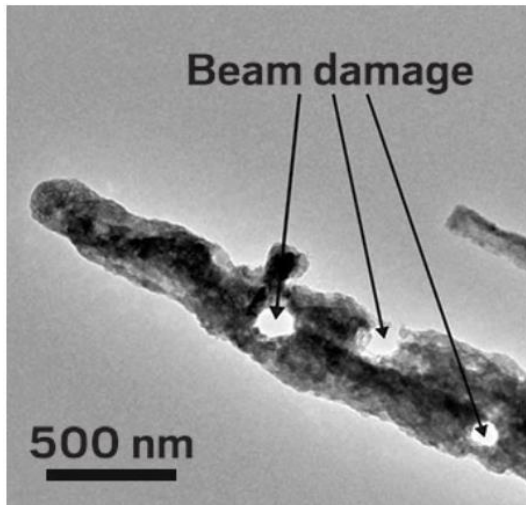
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Why cryo SEM ?

1st issue



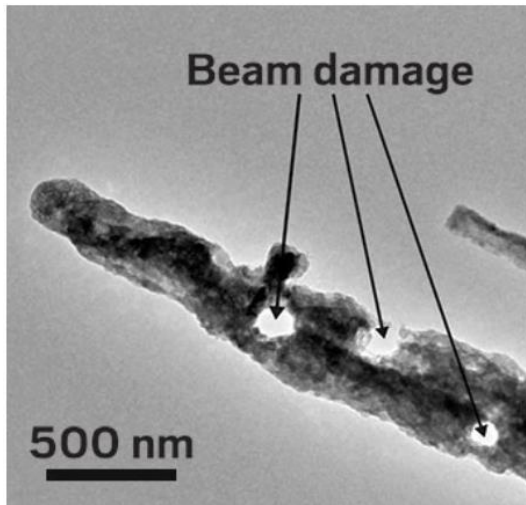
Beam damage on a sensitive lithium dendrite
([Yuzhang et al., 2017](#)).

- **Electron beam damage of sensitive materials**

Yuzhang Li et al. Atomic structure of sensitive battery materials and interfaces revealed by cryo-electron microscopy. *Science* 358, 506-510 (2017).

Why cryo SEM ?

1st issue

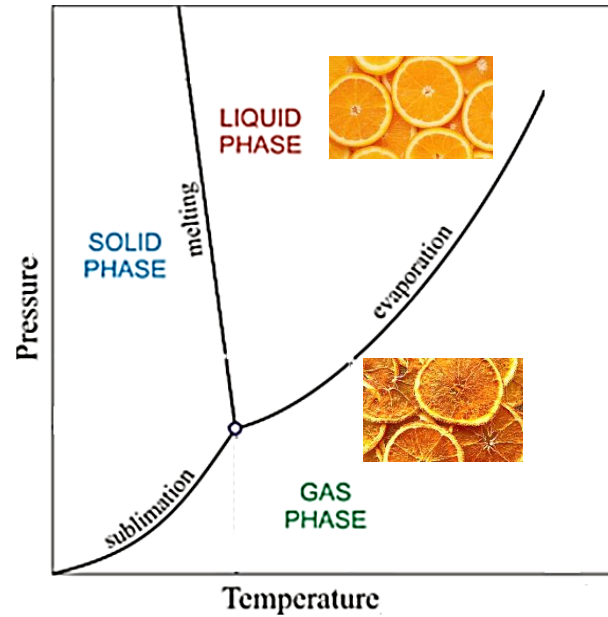


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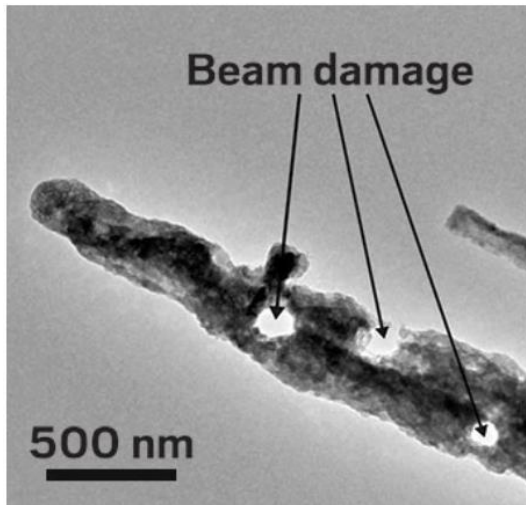
2nd issue



- **Dehydration caused by low pressure**

Why cryo SEM ?

1st issue

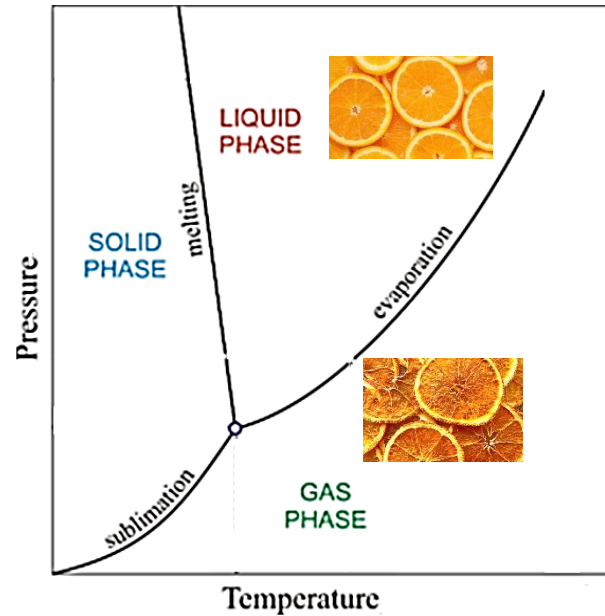


Beam damage on a sensitive lithium dendrite (Yuzhang et al., 2017).

- **Electron beam damage of sensitive materials**

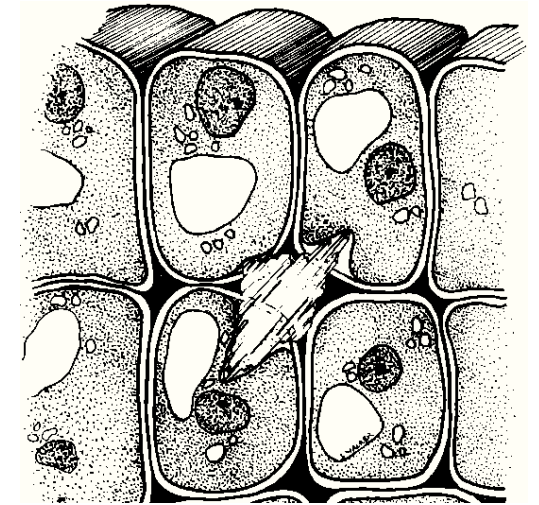
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2nd issue



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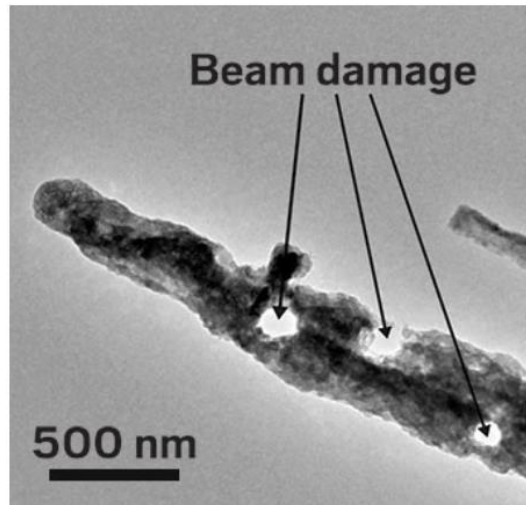
3rd issue



- **Ice crystals are destructive to soft materials**

Why cryo methods ?

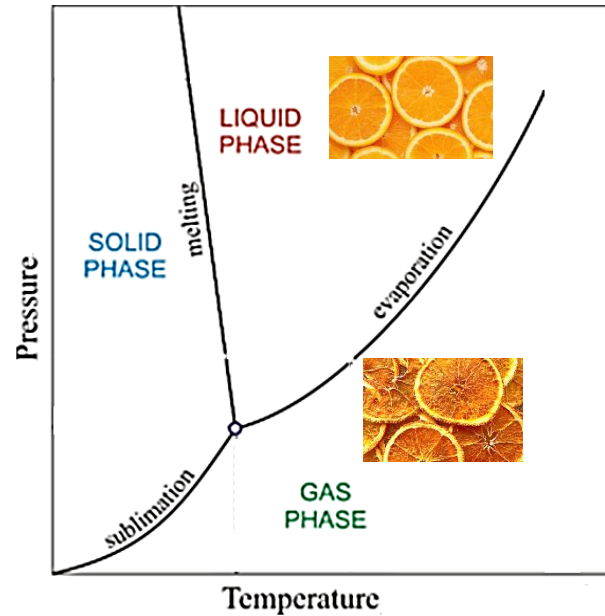
1st issue



Beam damage on a sensitive lithium dendrite (Yuzhang et al., 2017).

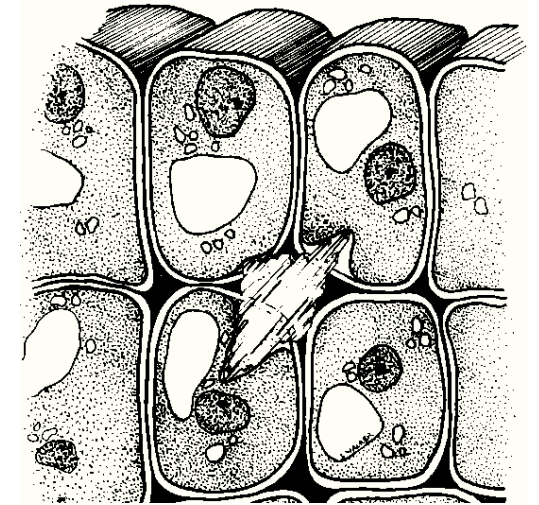
- **Electron Beam damage of sensitive materials**

2nd issue



- **Dehydration caused by low pressure**

3rd issue

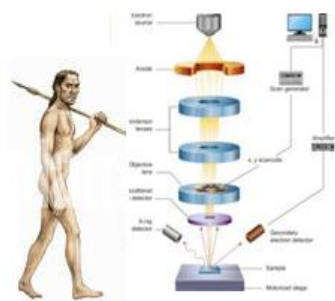


- **Ice crystals are destructive to soft materials**

- ✓ **Analysis under cryogenic conditions by preserving water in an amorphous state**

Correlative SEM-Raman is evolving quickly...

High Resolution SEM (HR-SEM)



~ 1973

HR-SEM with Raman



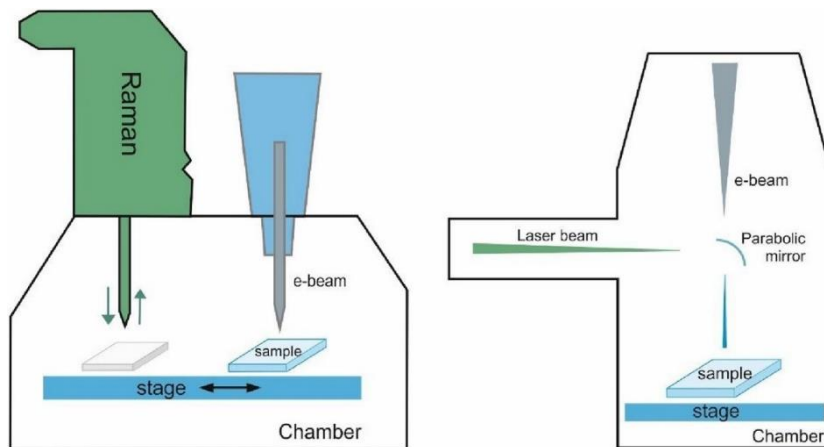
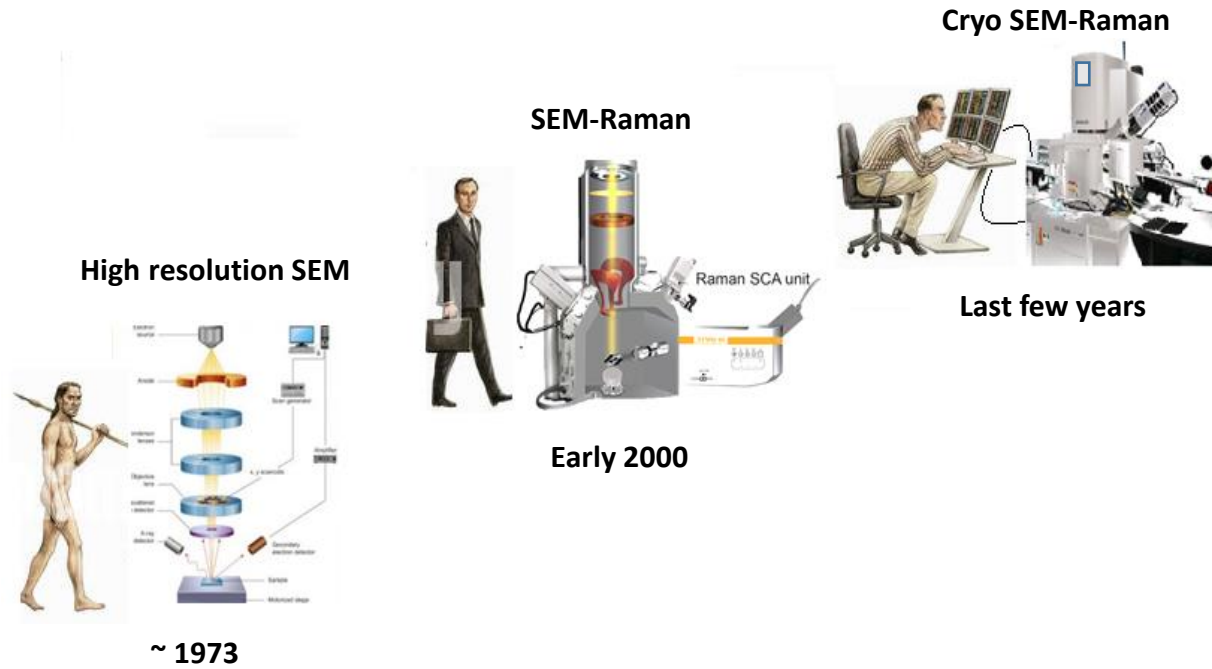
Early 2000

Cryo HR-SEM with Raman

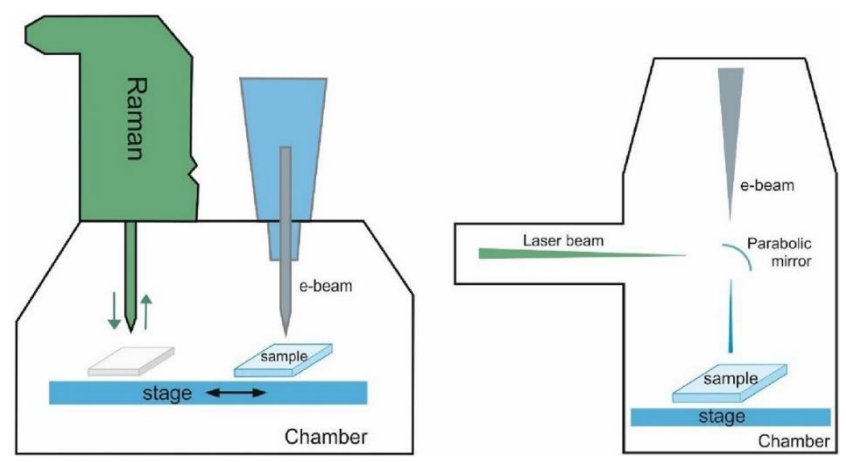
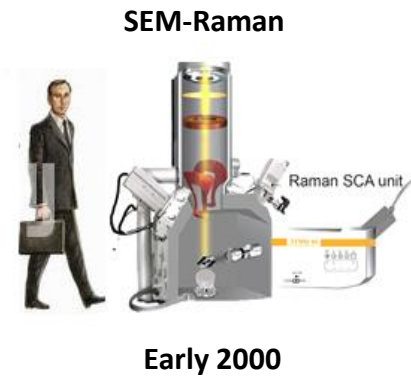
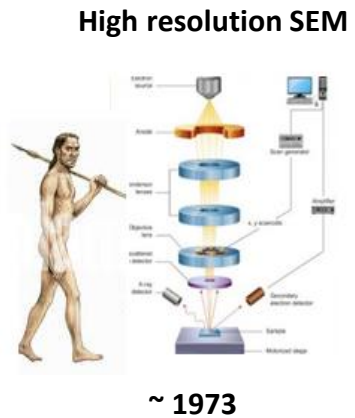


Last few years

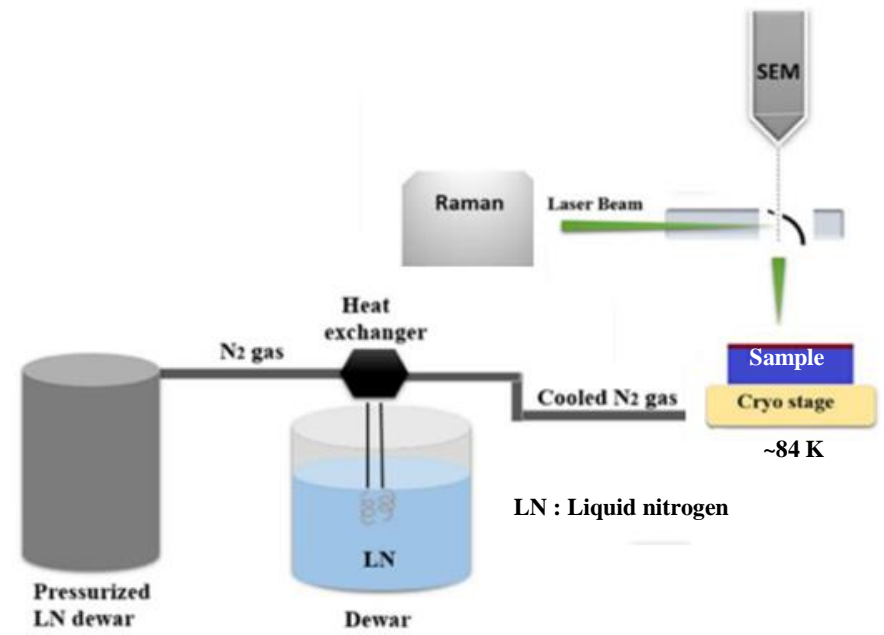
Correlative SEM-Raman is evolving quickly...



Correlative SEM-Raman is evolving quickly...



Under cryogenic conditions



On axis cryo Raman-SEM.

Cryo SEM- μ Raman on biological samples ! Some studies...



Volume 244, Issue 2
November 2011
Pages 122-128

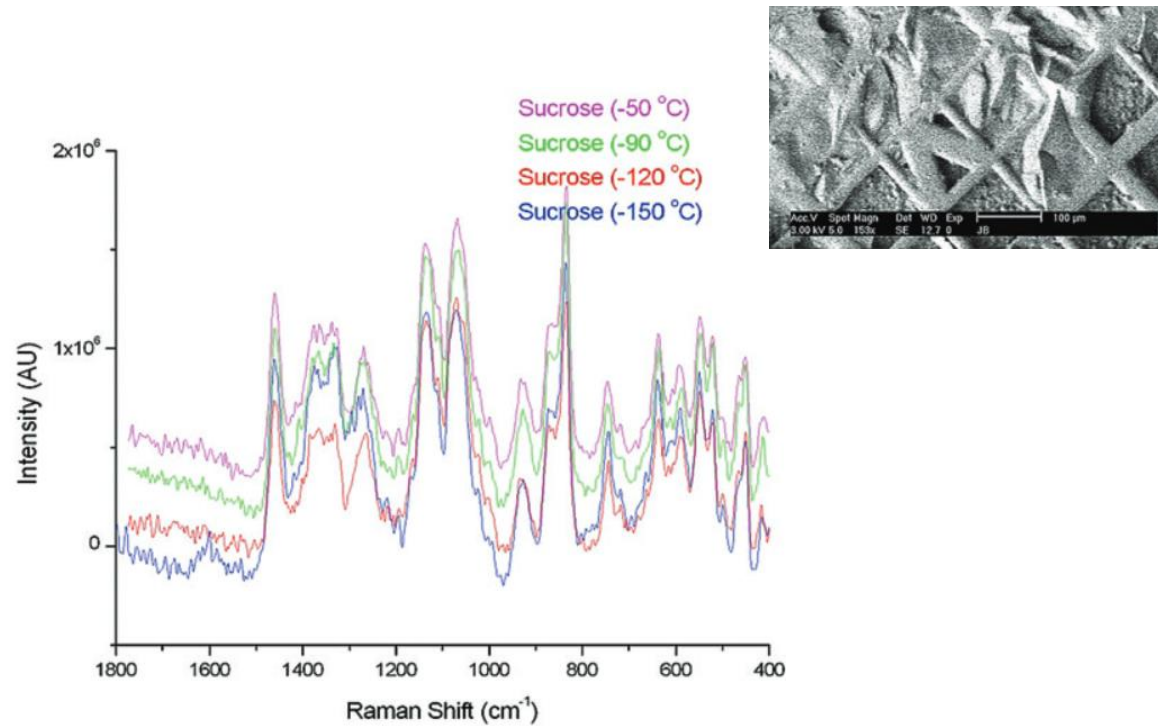
Journal of
Microscopy



The feasibility of Cryo In-SEM Raman microspectroscopy

J. HAZEKAMP, M. G. REED, C.V. HOWARD, A. A. VAN APELDOORN, C. OTTO

First published: 23 June 2011 | <https://doi.org/10.1111/j.1365-2818.2011.03515.x> |



- Successful characterization of sucrose in a cryo SEM-Raman instrument

From the journal

Analyst

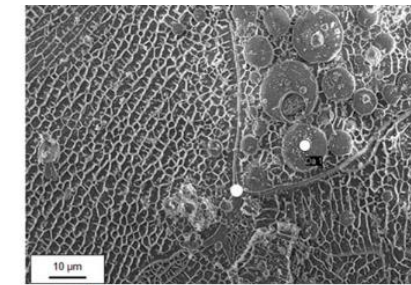
Issue 18, 2011

Analyst

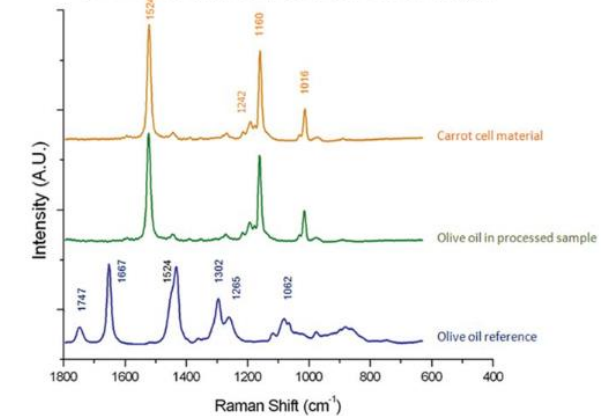


Carotene location in processed food samples measured by Cryo In-SEM Raman

Patricia Lopez-Sanchez,^a Stephan Schumm,^a Paul D. A. Pudney^b and Johan Hazekamp^a

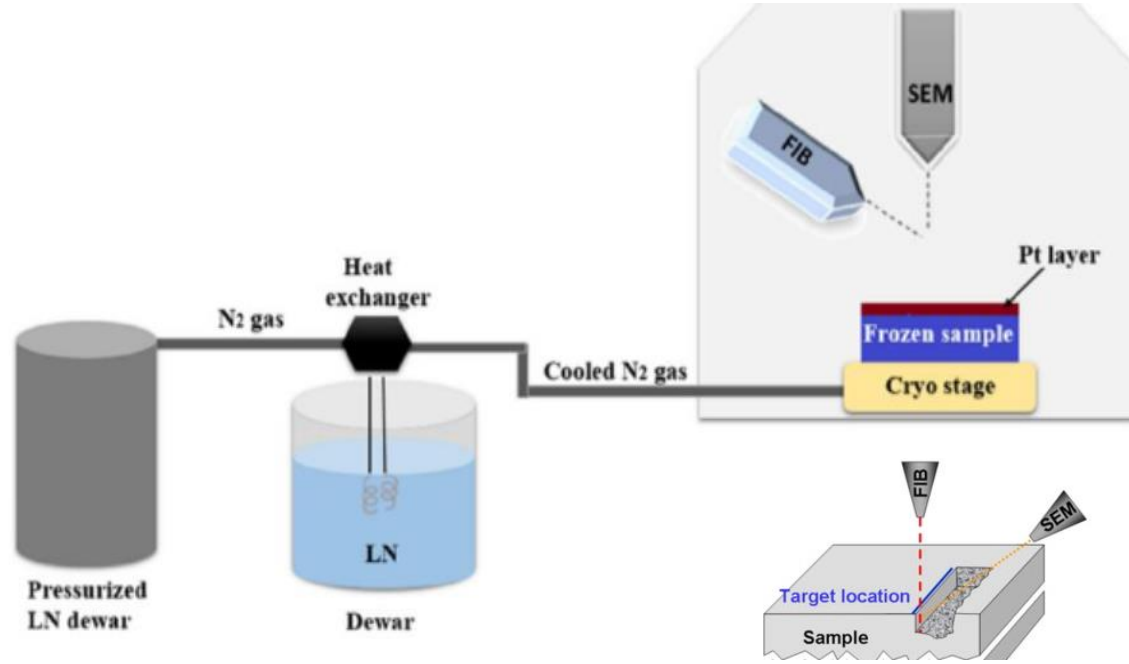


Carrot skin
+
Sorbic acid
+
Olive oil

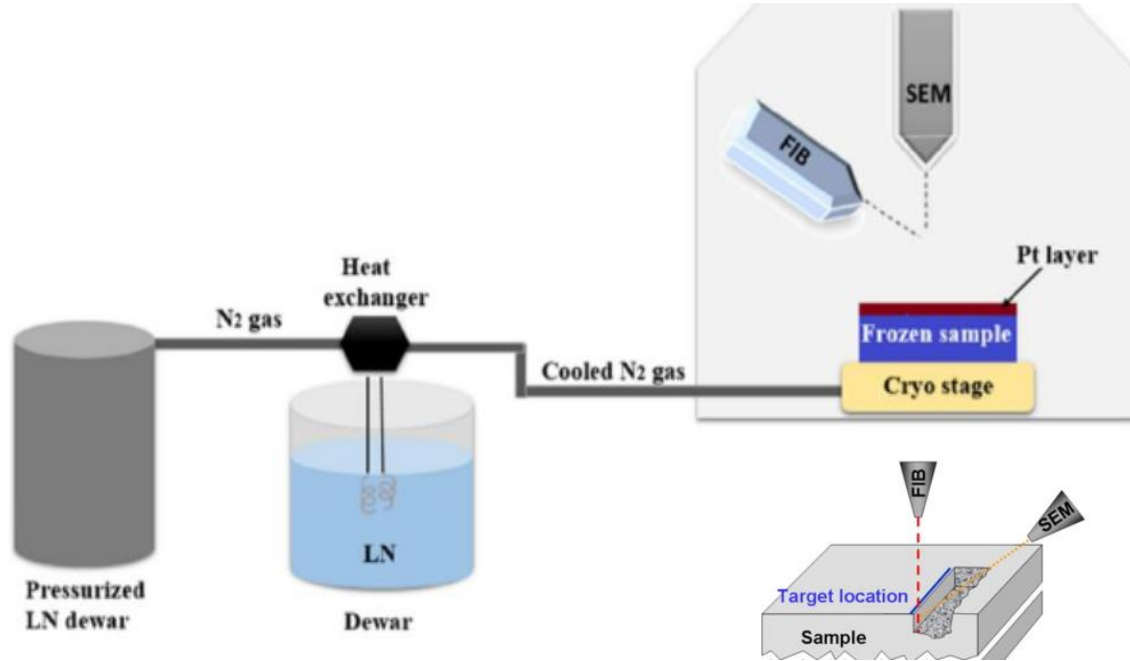


- Cryo SEM-Raman used to identify the distribution of lipophilic micronutrients in plant based food systems

With focused ion beam (FIB) the internal structure can be revealed...



With focused ion beam (FIB) the internal structure can be revealed...



The cryo SEM-FIB- μ Raman of the IMN 

With focused ion beam (FIB) the internal structure can be revealed...



Cryo preparation chamber

Cryo stage

Liquid nitrogen dewar



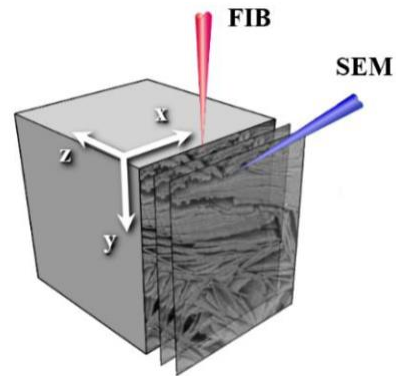
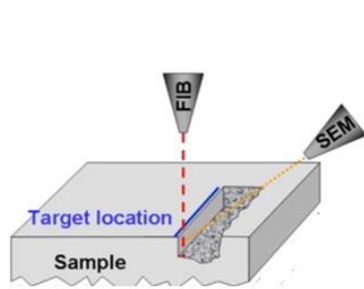
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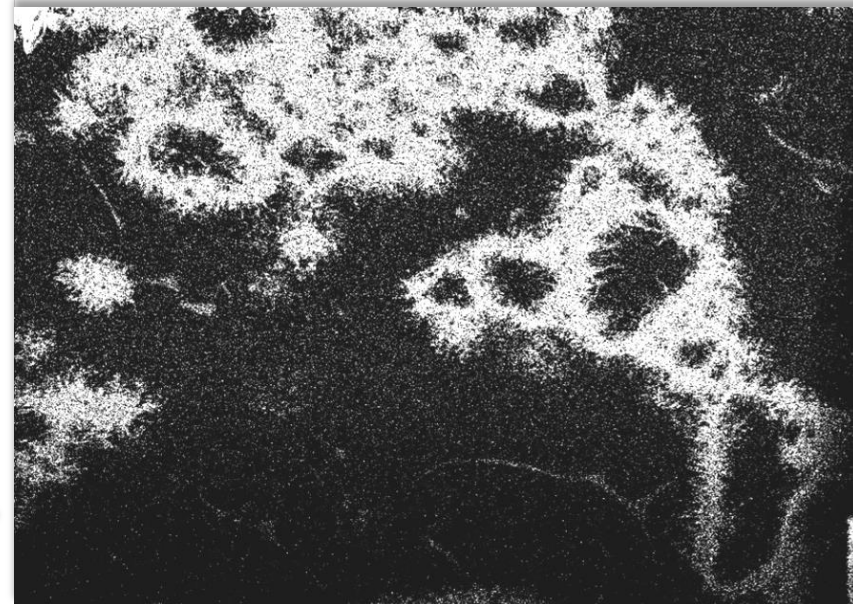
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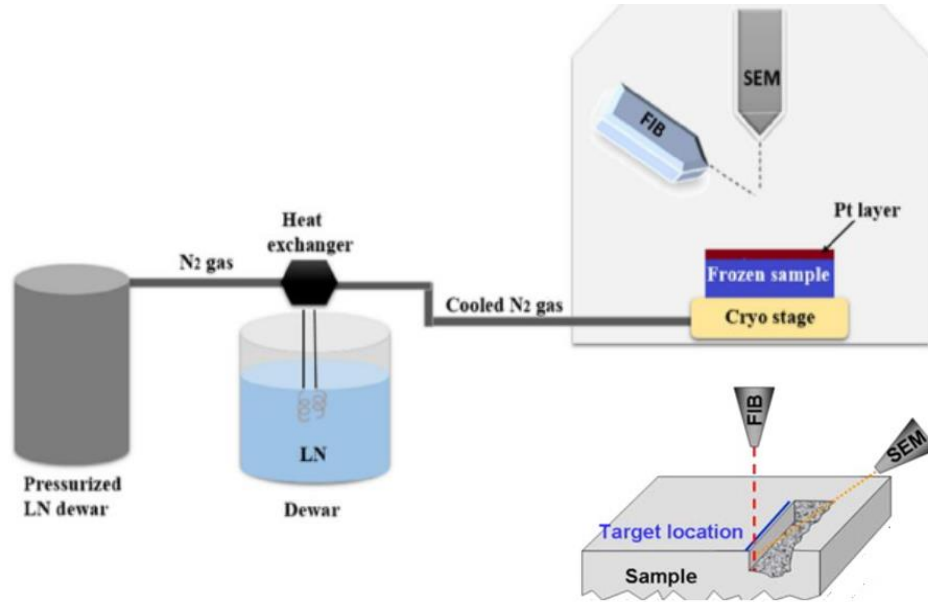
Liquid nitrogen dewar



Internal structure of calcium phosphate/gelatin mixture along the electron beam axis



With focused ion beam (FIB) the internal structure can be revealed...

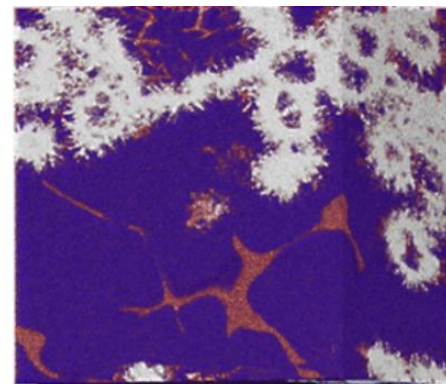


Probing the microporosity and 3D spatial distribution of calcium phosphate cement/hydrogel biomaterials using FIB/SEM at cryogenic temperatures

Mater. Adv., 2023
4, 2474-2486

[Mouad Essani](#), ^{*a} [Baptiste Charbonnier](#), ^b [Nicolas Stephant](#), ^a [Hilel Moussi](#), ^{ab} [Pierre Weiss](#), ^c [Jean Le Bideau](#) ^a and [Patricia Abellan](#) ^{*a}

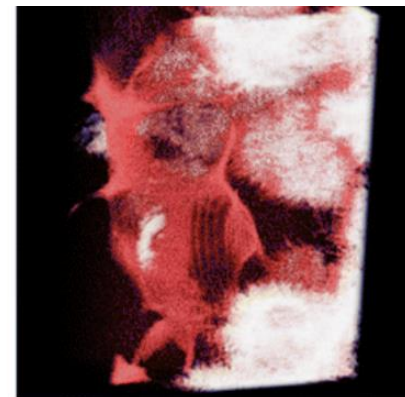
3D constructed volume of CDHA/gelatin mixture



1 μm



3D distribution of gelatin

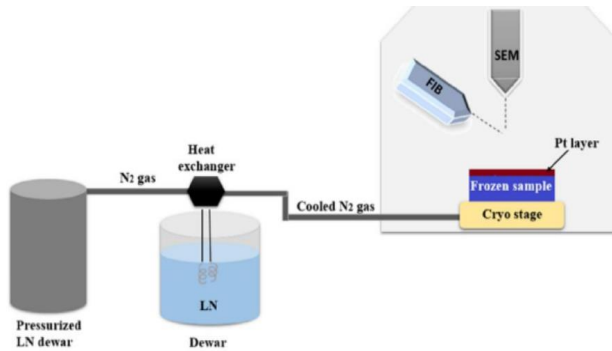


1 μm



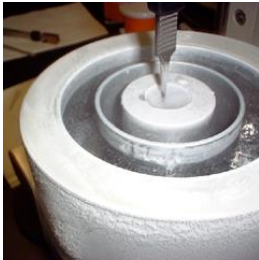
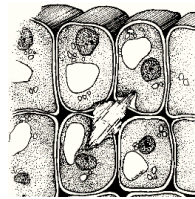
What about problems associated with cryo SEM ?

Plunge freezing in particular !!



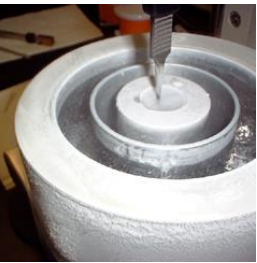
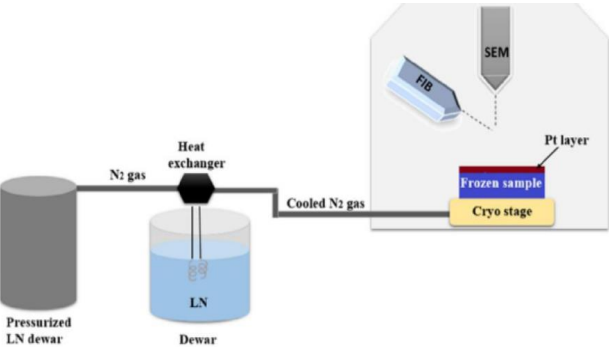
- Plunge freezing can lead to the formation of ice crystals

Cooling rate $\sim K/b^2$
K: thermal diffusion coeff
b: thickness depth



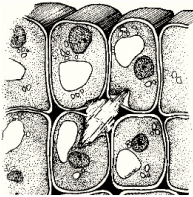
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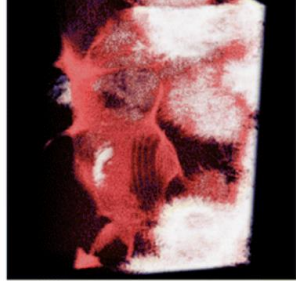
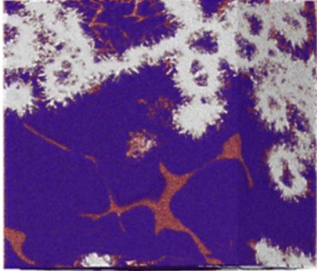


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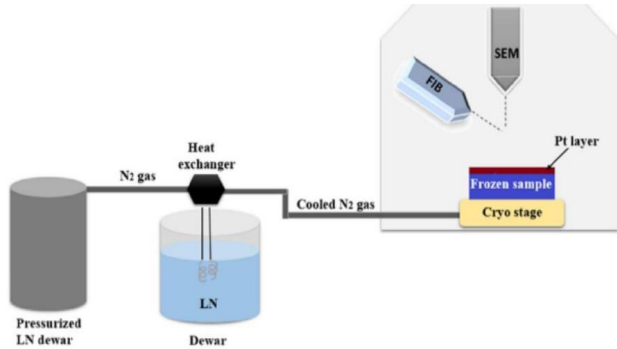
Is this the real internal structure ?



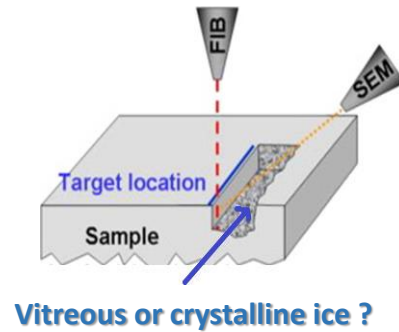
Gelatin
 Aq phase
 CDHA

What about problems associated with cryo SEM ?

Plunge freezing in particular !!

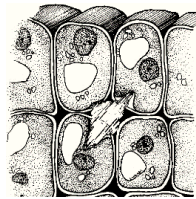


What is the depth of vitreous ice ?

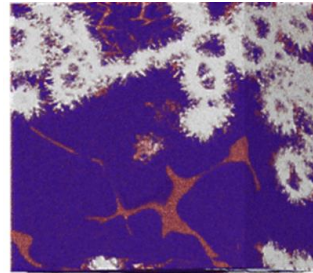


- Plunge freezing can lead to the formation of ice crystals

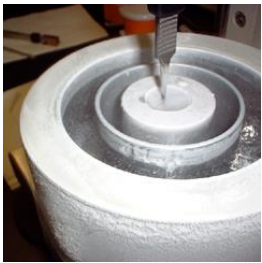
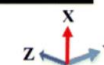
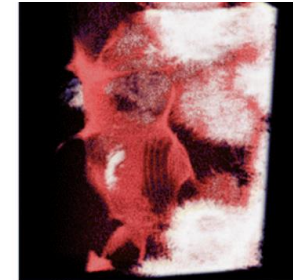
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 K: thermal diffusion coeff
 b: thickness depth



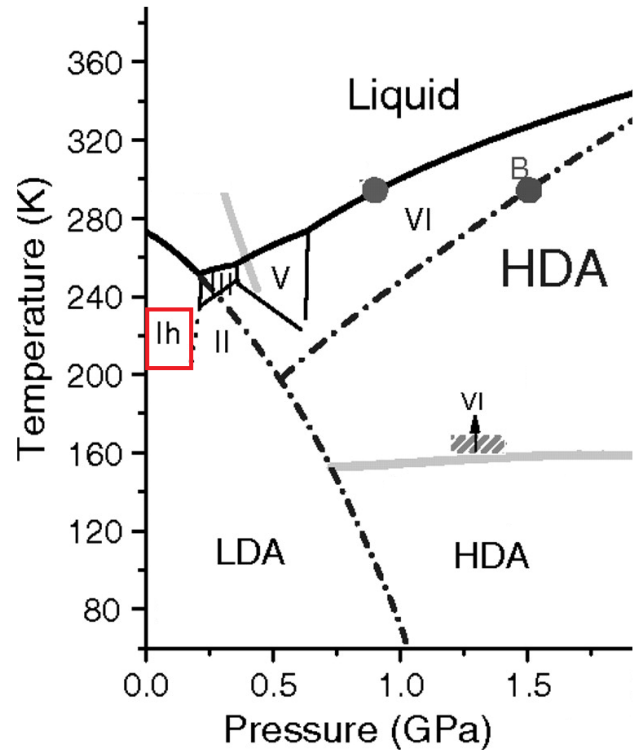
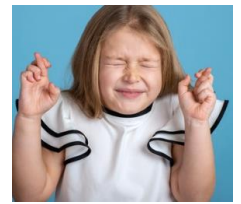
Is this the real internal structure ?



Gelatin
 Aq phase
 CDHA



If only there is a way to know the crystalline structure of ice !



Phase diagram of water

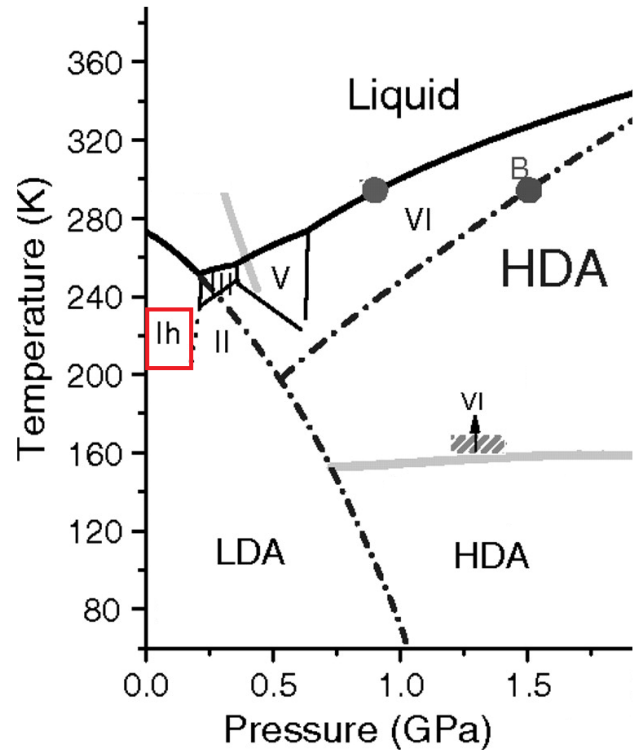
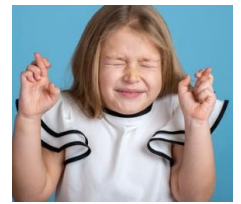
Ih : hexagonal ice crystal

II, V, VI, VI : Other forms of crystalline ice

LDA : low amorphous density ice

HDA : High density amorphous ice

If only there is a way to know the crystalline structure of ice !



Phase diagram of water

Ih : hexagonal ice crystal

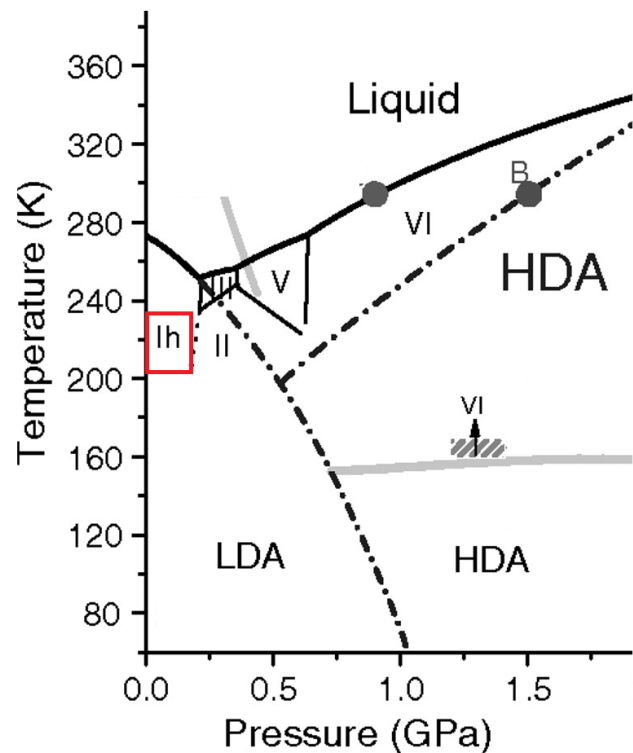
II, V, VI, VI : Other forms of crystalline ice

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Let's give Raman spectrometry a chance

If only there is a way to know the crystalline structure of ice !



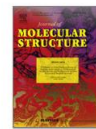
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Let's give Raman spectrometry a chance

J. Phys. C: Solid State Phys., 17 (1984) 5833-5850. Printed in Great Britain

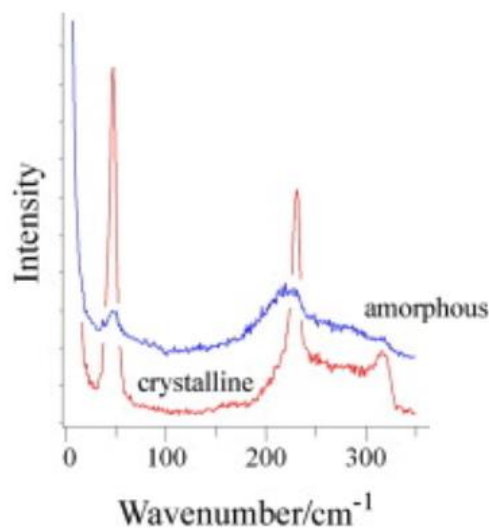


Journal of Molecular Structure
 Volume 976, Issues 1-3, 15 July 2010, Pages 174-180



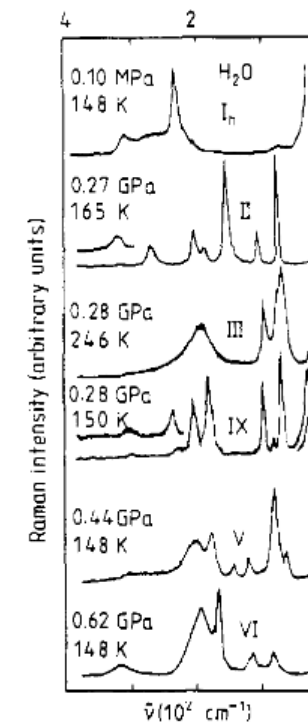
Water ice at low temperatures and pressures: New Raman results

Krešimir Furić^a, Vesna Volovšek^b

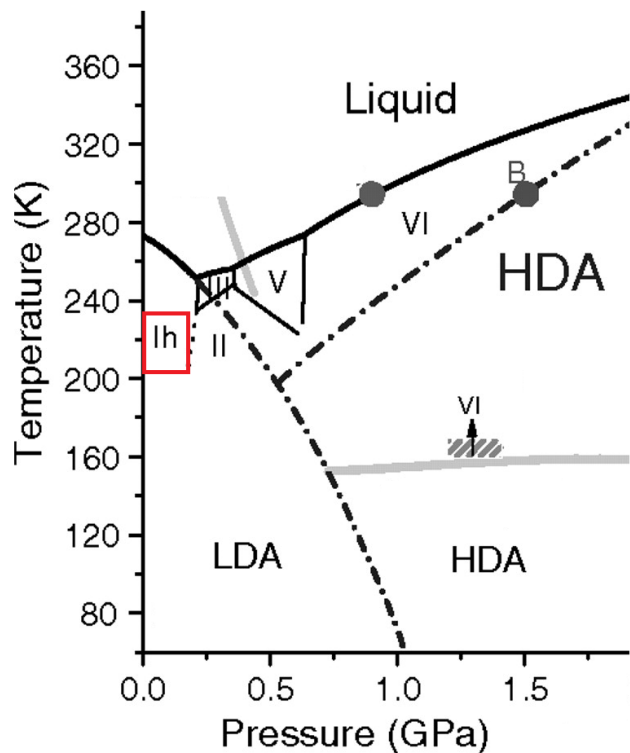


The Raman spectra of ice (I_h, II, III, V, VI and IX) as functions of pressure and temperature

B Minceva-Sukarova[†], W F Sherman and G R Wilkinson
 Department of Physics, King's College London, Strand, London WC2R 2LS, UK



If only there is a way to know the crystalline structure of ice !



Phase diagram of water

Ih : hexagonal ice crystal

II, V, VI, VII : Other forms of crystalline ice

LDA : low amorphous density ice

HDA : High density amorphous ice

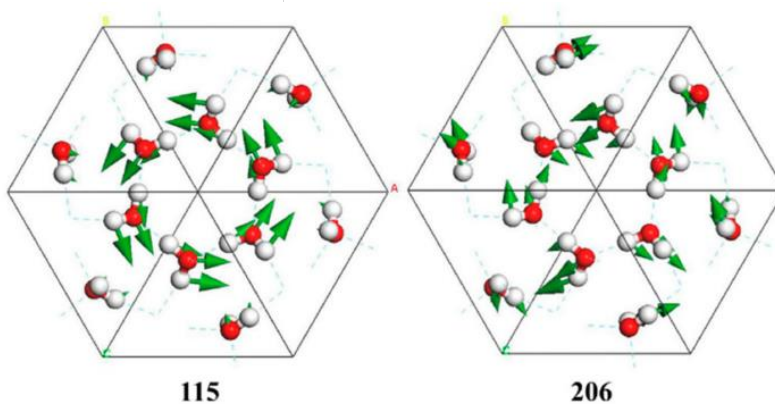
Translational modes at low frequency



Article

DFT Investigations of the Vibrational Spectra and Translational Modes of Ice II

Jing-Wen Cao, Jia-Yi Chen, Xiao-Ling Qin, Xu-Liang Zhu, Lu Jiang, Yue Gu, Xu-Hao Yu and Peng Zhang*

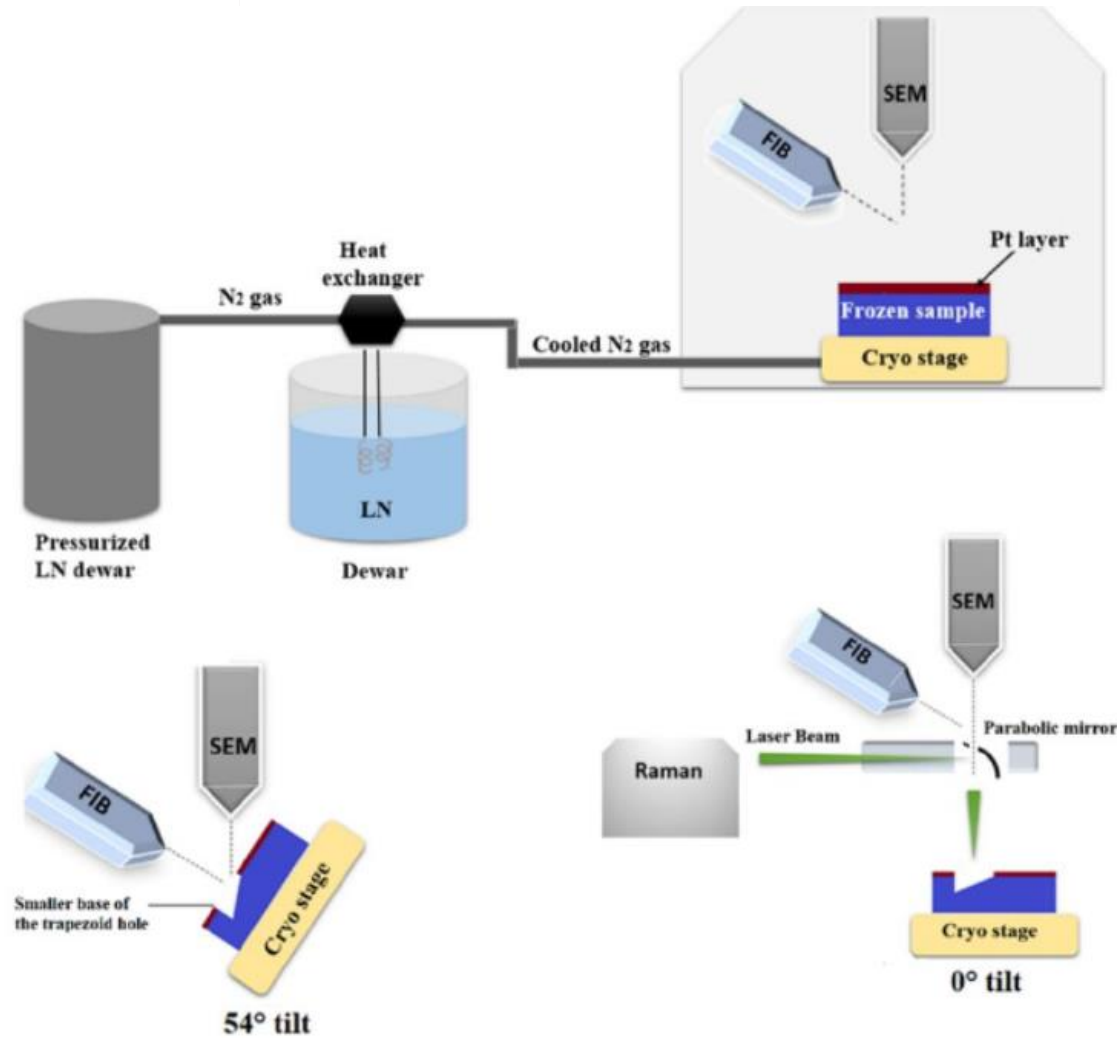


Low frequency Raman spectrometry can reveal the crystalline structure of ice

If only there is a way to know the crystalline structure of ice !



The SEM-FIB- μ Raman can be used to assess the crystalline structure in depth

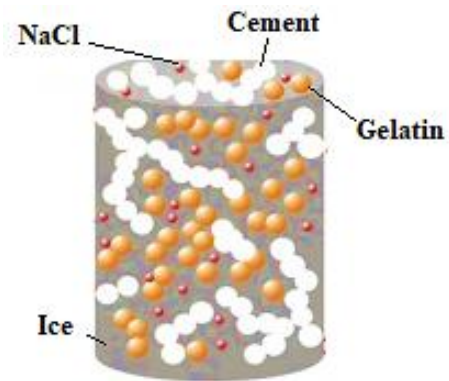


Let's test cryo SEM-FIB- μ Raman on a hydrated biomaterial...

Preparation of a CPC/gelatin sample used as injectable bone substitute

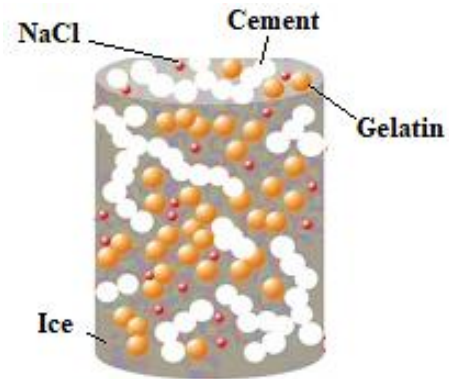


Cryo fixation of the hydrated sample
By plunge freezing



Let's test cryo SEM-FIB- μ Raman on a hydrated biomaterial...

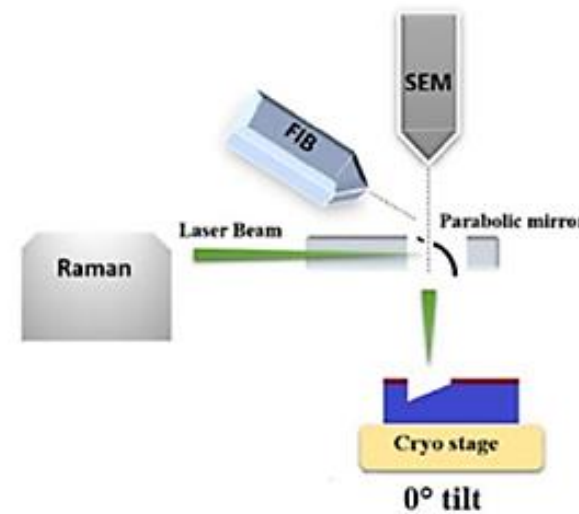
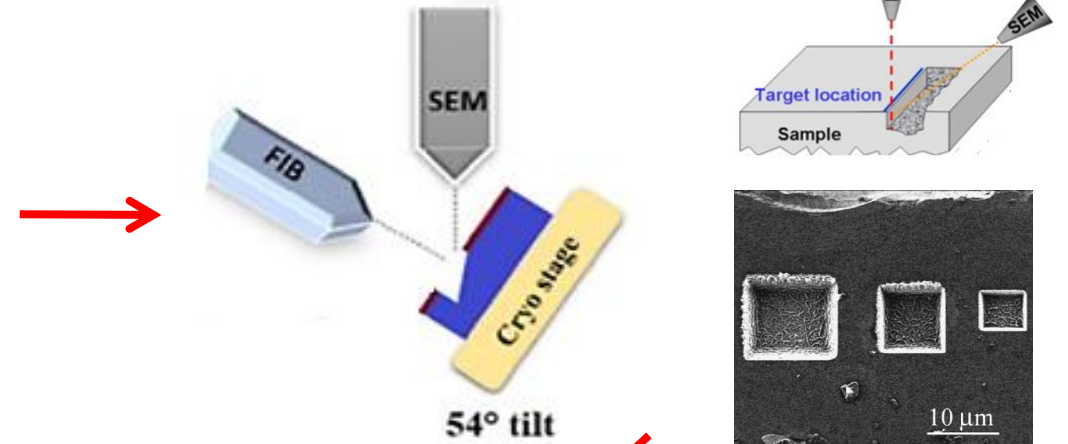
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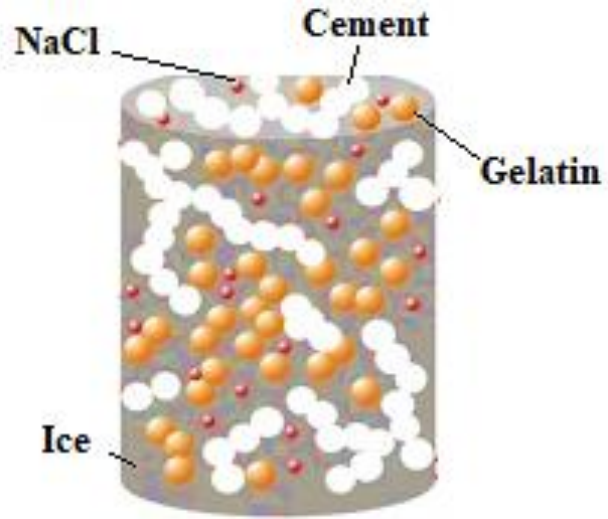
FIB milling of a trench of tens of μm



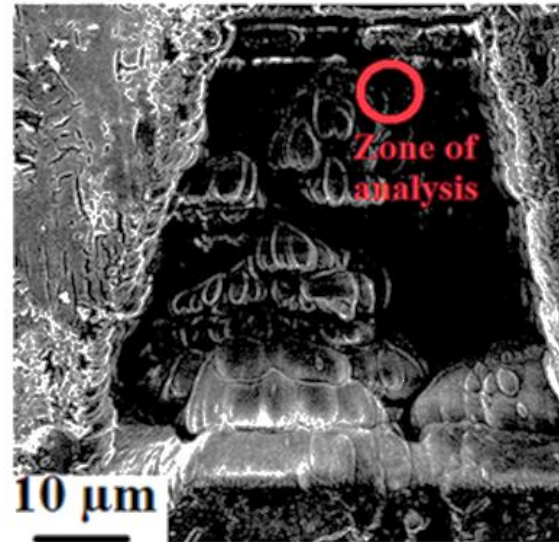
Investigation of the crystalline structure at a defined depth

Let's test cryo SEM-FIB- μ Raman on a hydrated biomaterial...

Measurements performed at 123 K



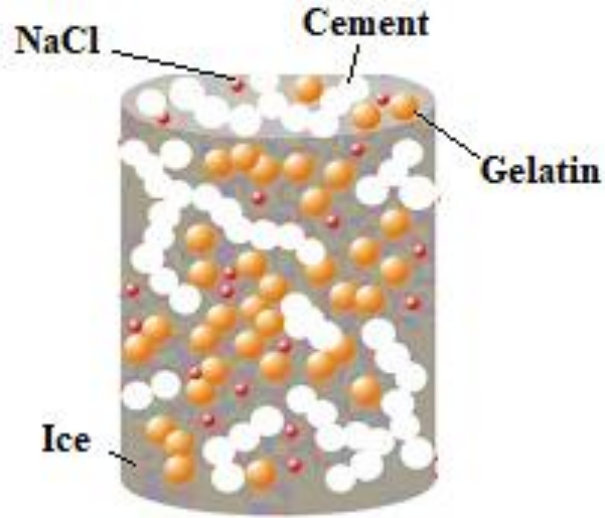
Forzen CPC/gelatine mixture



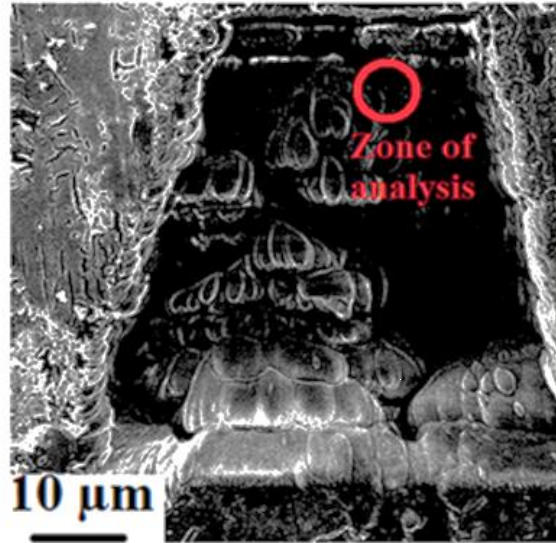
Trench with a depth of 20 μm and zone of MRS analysis.

Let's test cryo SEM-FIB- μ Raman on a hydrated biomaterial...

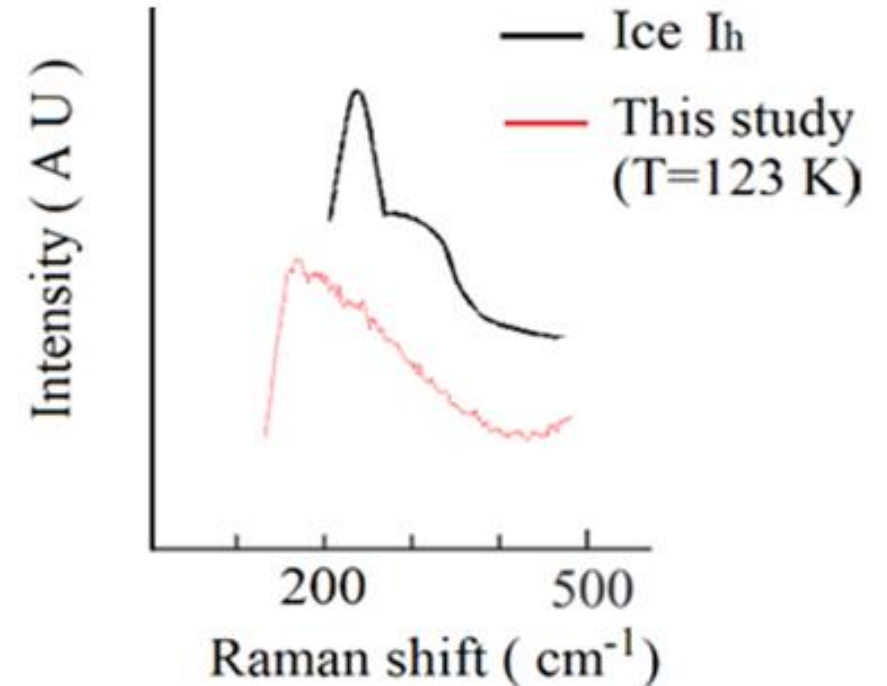
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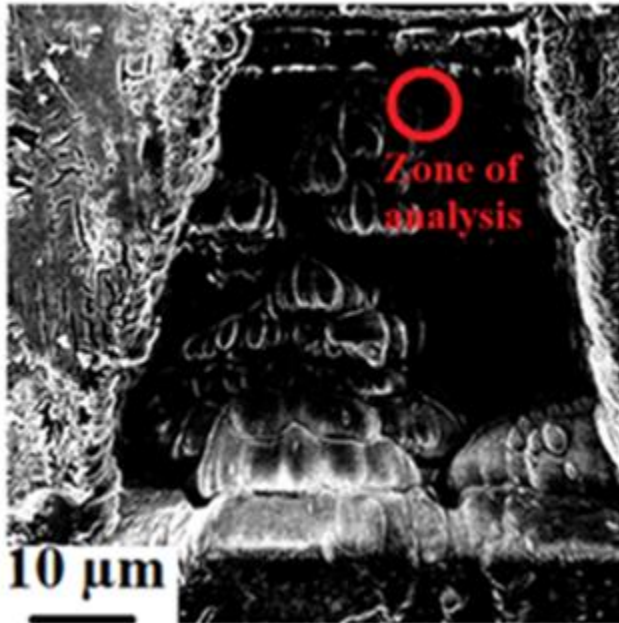
Trench with a depth of 20 μm and zone of MRS analysis.



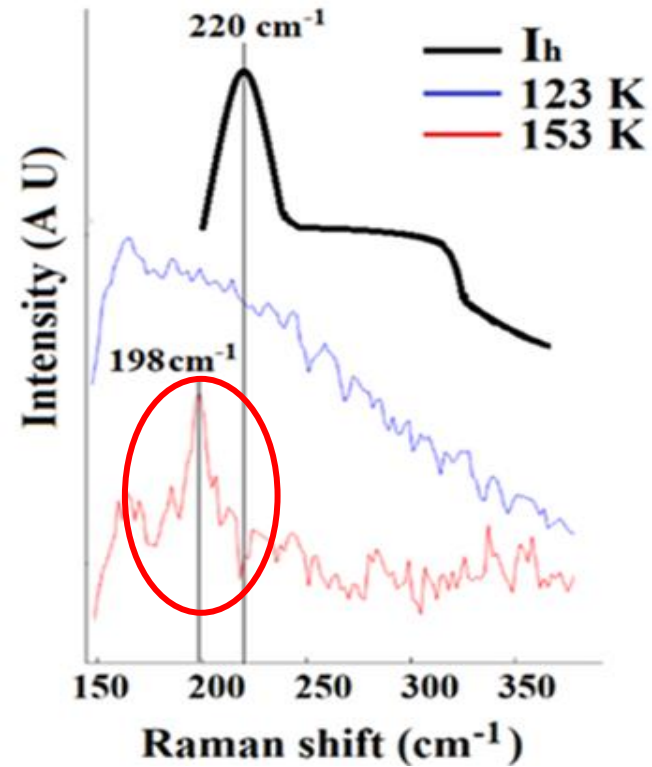
Raman spectrum (red) in comparison to that of crystalline hexagonal ice I_h (black)

Absence of sharp peaks suggesting a disordered liquid-like structure of ice

What if we heat the sample above the water T_g (i.e. T > 136 K) ?!



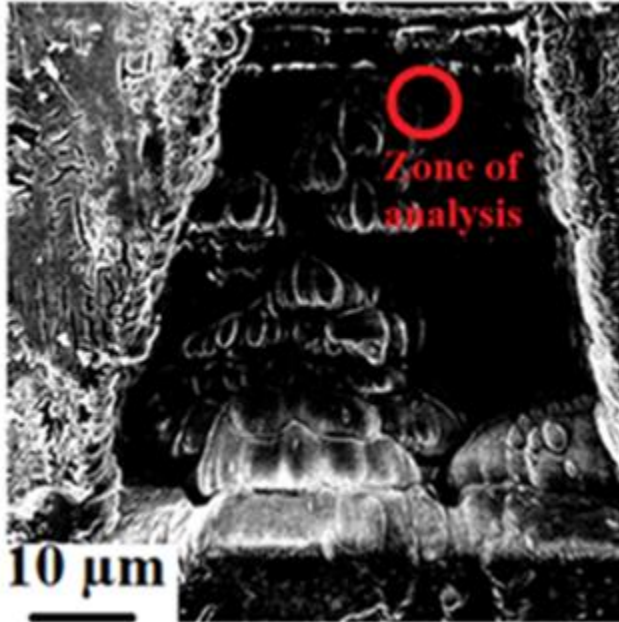
Trench with a depth of 20 μm and zone of MRS analysis.



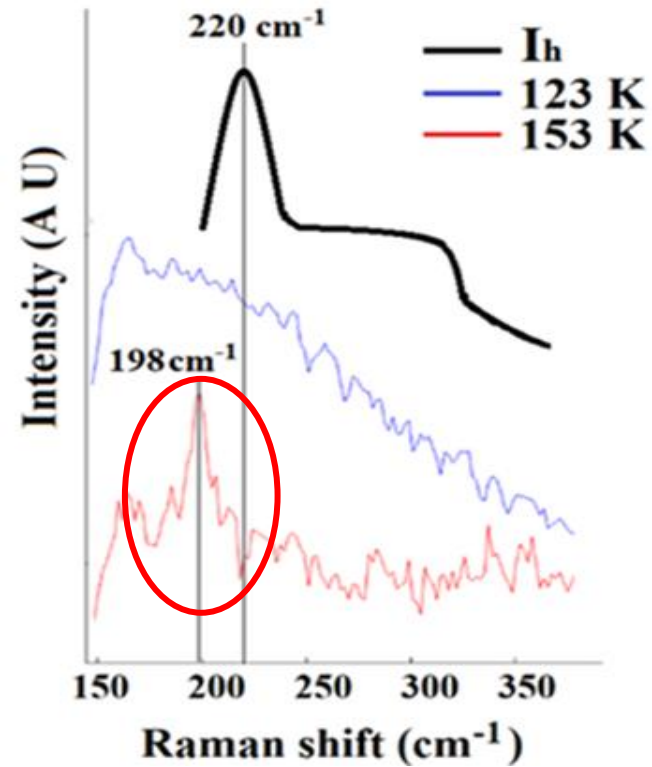
Raman spectra at 123 K and 153 K in comparison to that of hexagonal ice I_h

Formation of a crystalline structure after heating

What if we heat the sample above the water T_g (i.e. $T > 136$ K) ?!



Trench with a depth of 20 μm and zone of MRS analysis.



Raman spectra at 123 K and 153 K in comparison to that of hexagonal ice I_h

Is the downshift a temperature effect ?

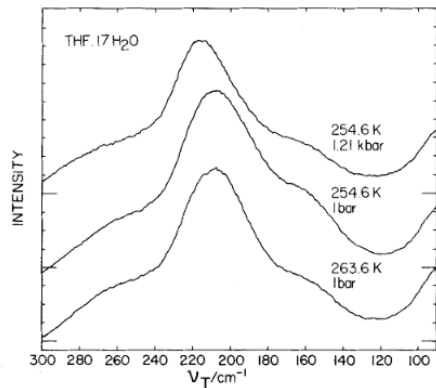
Formation of a crystalline structure after heating

Effects of temperature on the Raman signal of ice



Effect of pressure and temperature on the O—H and O—D stretching, and translational vibrations in the Raman spectrum of ice clathrate

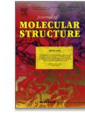
G. P. Johari & H. A. M. Chew



Raman spectrum of tetrahydrofuran (H₂O) ice clathrate in the translational vibration regions showing the effect of temperature and pressure on the frequency of the main peak.



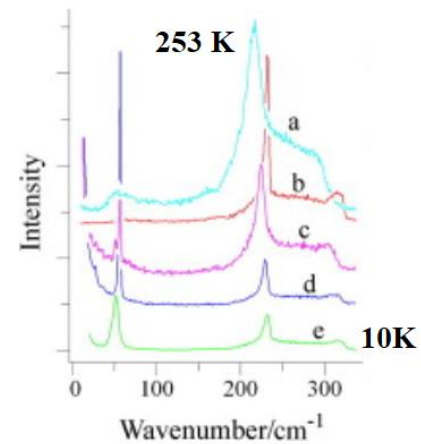
Journal of Molecular Structure
Volume 976, Issues 1–3, 15 July 2010, Pages 174–180



Water ice at low temperatures and pressures: New Raman results

Krešimir Furić^a, Vesna Volovšek^b

Hexagonal Ice



Translational modes in aqueous solutions shift to higher frequencies when the temperature decreases

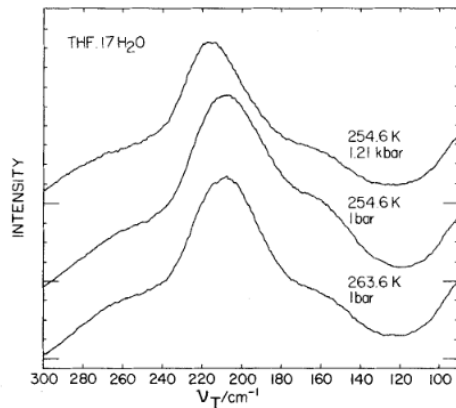
↓
Not Hexagonal Ice

Effects of temperature on the Raman signal of ice



Effect of pressure and temperature on the O—H and O—D stretching, and translational vibrations in the Raman spectrum of ice clathrate

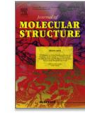
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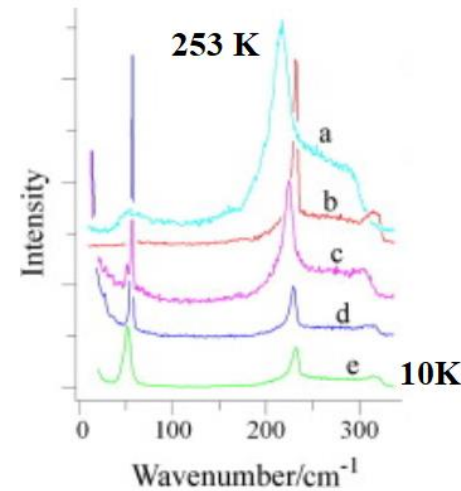
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Krešimir Furić^a, Vesna Volovšek^b

Hexagonal Ice



If not hexagonal ice then what is it ??

Translational modes in aqueous solutions shift to higher frequencies when the temperature decreases



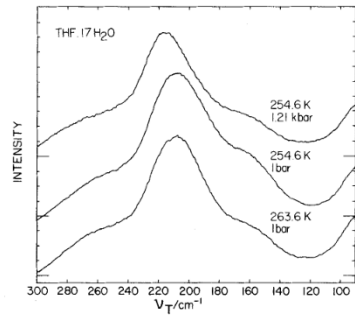
Not Hexagonal Ice

Effects of temperature on the Raman signal of ice

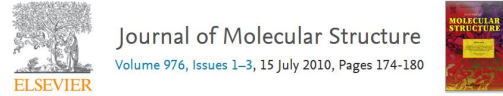


Effect of pressure and temperature on the O—H and O—D stretching, and translational vibrations in the Raman spectrum of ice clathrate

G. P. Johari & H. A. M. Chew



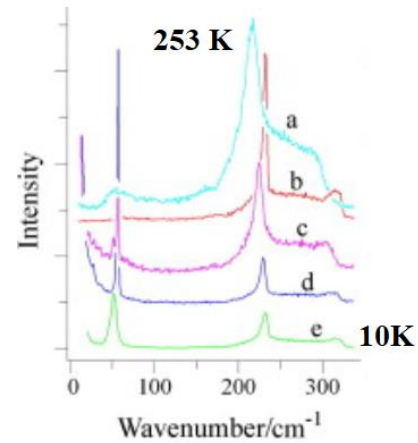
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Water ice at low temperatures and pressures: New Raman results

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Hexagonal Ice

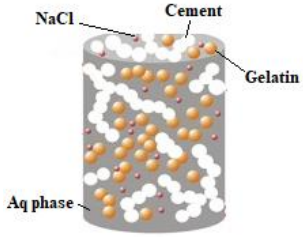


Translational modes in aqueous solutions shift to higher frequencies when the temperature decreases



Not Hexagonal Ice

What about the presence of NaCl in the aqueous phase?

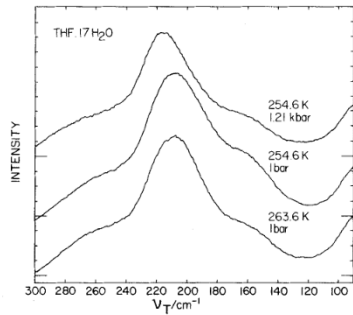


Effects of temperature on the Raman signal of ice

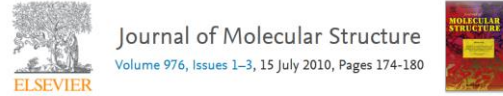


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G. P. Johari & H. A. M. Chew



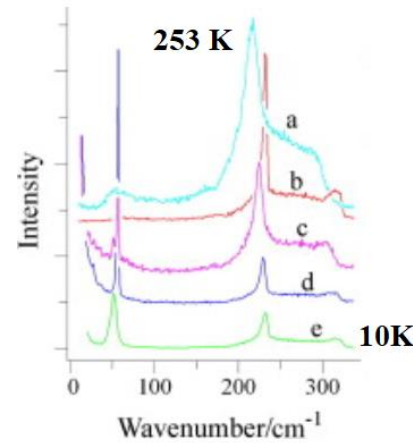
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Water ice at low temperatures and pressures: New Raman results

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Hexagonal Ice

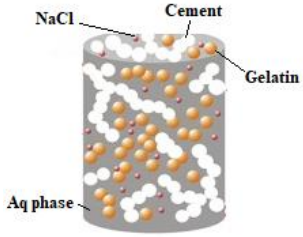


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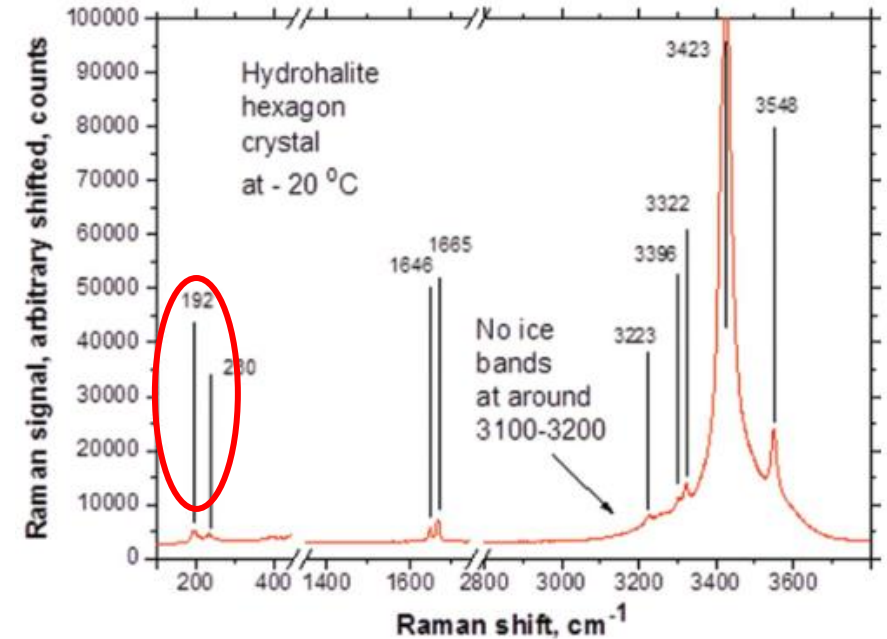
What about the presence of NaCl in the aqueous phase?



Applied Spectroscopy Reviews

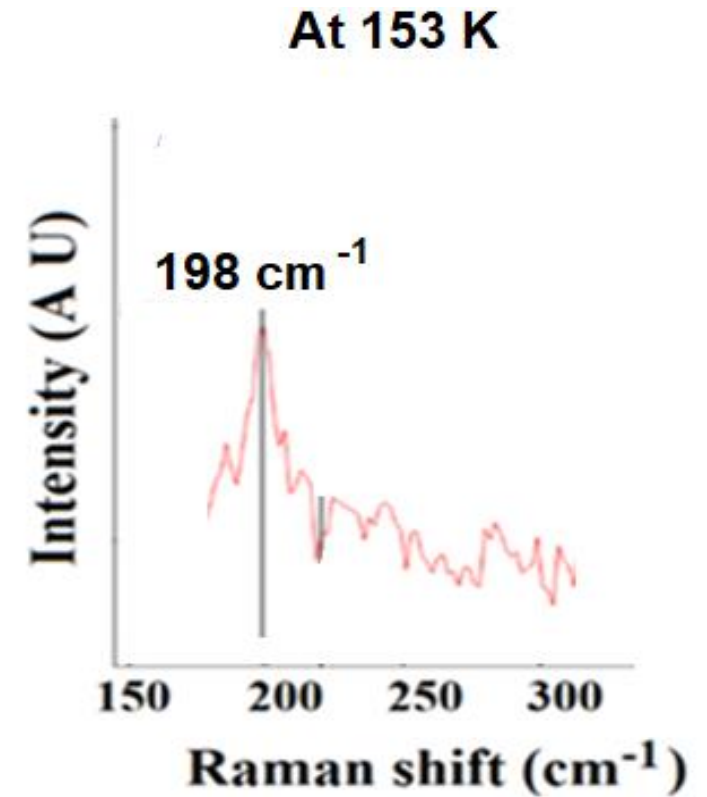
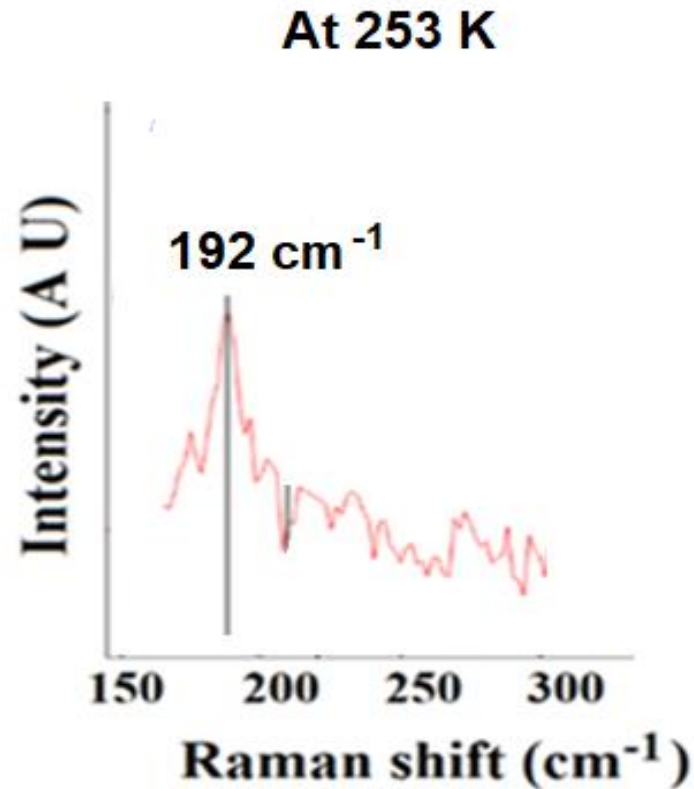
Raman detection of hydrohalite formation: Avoiding accidents on icy roads by deicing where salt will not work

Rolf W. Berg



Effects of temperature on the Raman signal of ice

Possible scenario...



Translational modes in hydrohalite crystals shifting to higher frequencies at lower temperature

Can we validate the temperature dependence of the Raman band ?!

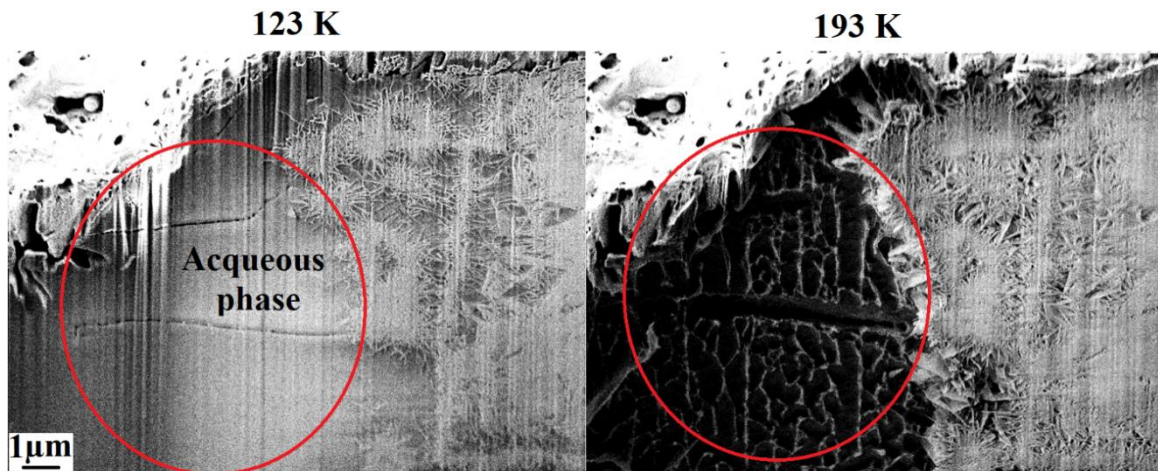
Not possible to warm the sample up to 253 K and verify the shift of the translational mode shift



Ice sublimation of the sample above 180 K

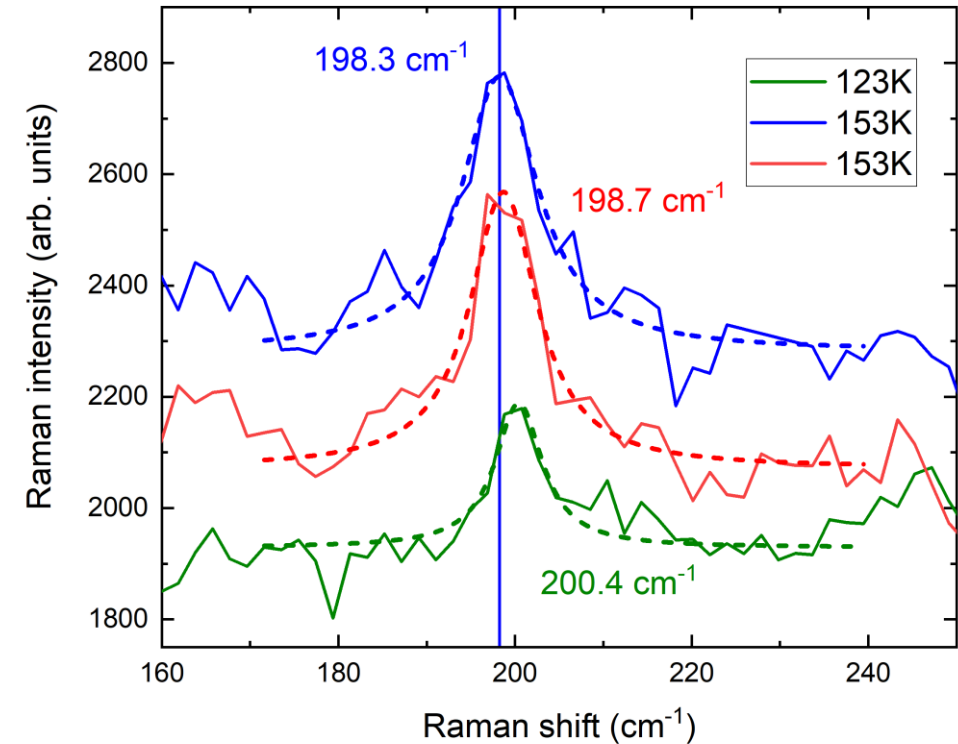
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Ice sublimation of the sample above 180 K

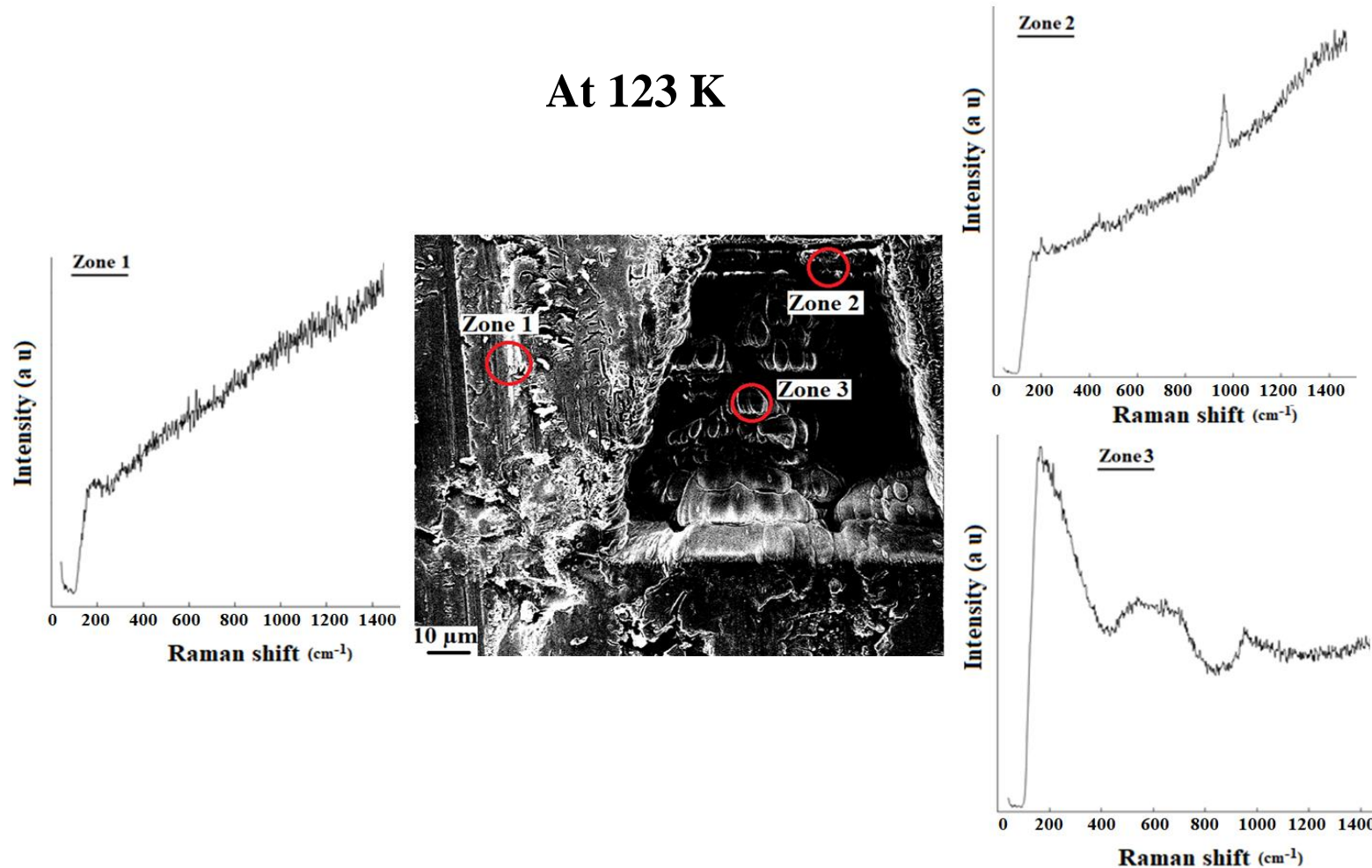
What about cooling the sample ?



A shift to higher frequencies of $\sim 2 \text{ cm}^{-1}$ is observed when cooling back the sample to 123 K

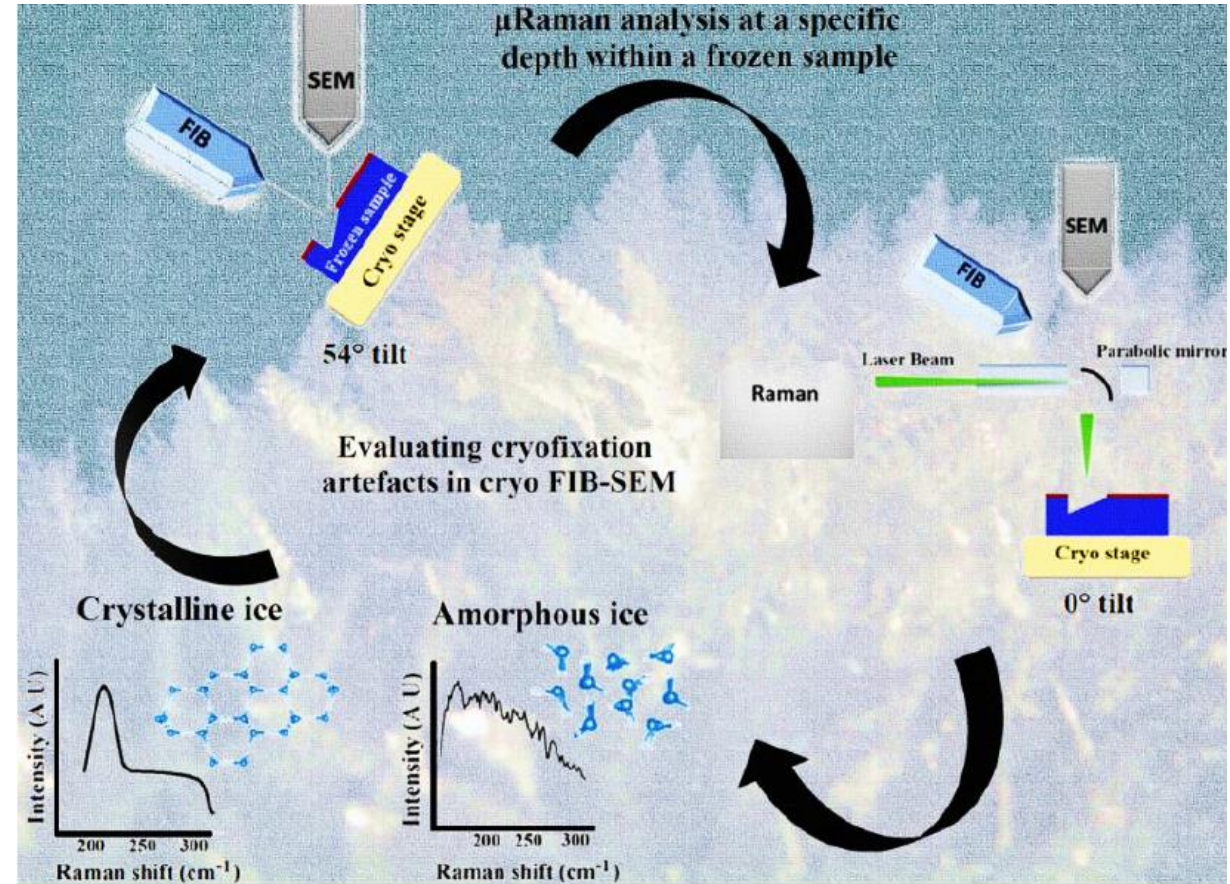
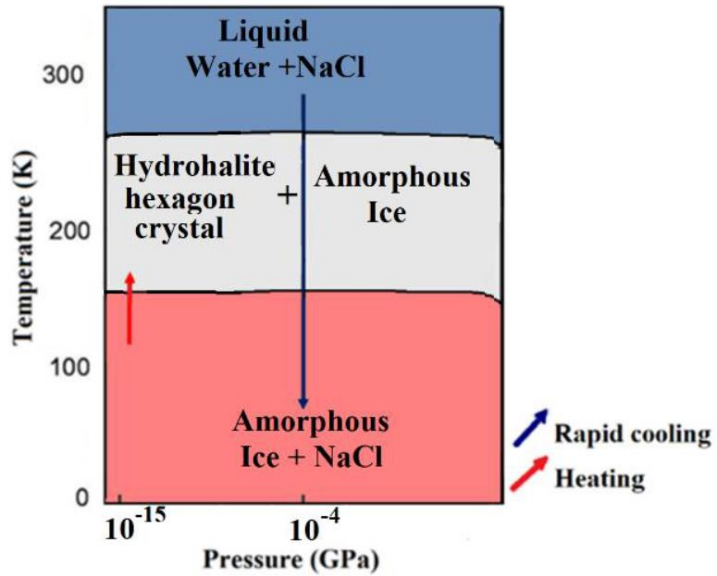
A bonus : FIB can be used to reduce fluorescence emission

At 123 K



- Local removing of metal coating while maintaining conductivity in the rest of the material
- Decrease of the fluorescence background for Raman analyses inside the FIB trench

Conclusion



ACS Publications
Most Trusted. Most Cited. Most Read.



Application of a Cryo-FIB-SEM- μ Raman Instrument to Probe the Depth of Vitreous Ice in a Frozen Sample

Mouad Essani*, Jean-Yves Mevellec, Baptiste Charbonnier, Philippe Moreau, Hilel Moussi, Pierre Weiss, Jean Le Bideau, Maxime Bayle, Bernard Humbert, and Patricia Abellan*

Cite this: *Anal. Chem.* 2022, 94, 23, 8120–8125

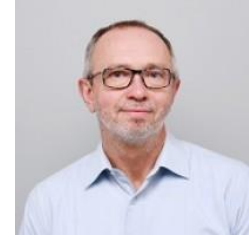
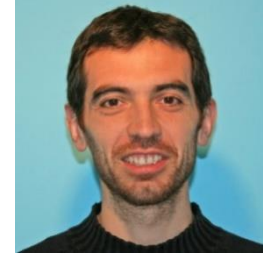
Publication Date: June 1, 2022

<https://doi.org/10.1021/acs.analchem.2c00245>

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Aknowledgment



P. Abellan, P. Morreau, E. Gautron, J. Le bideau, N. Stephant, N. Gautier, M. Dolatabadi, H. Roberge and all the CPC and PMN team...



The Raman team : M. Bayle, J-Y Mevellec, B. Humbert



The RMeS team : B. Charbonnier, H. Moussi, P. Weiss