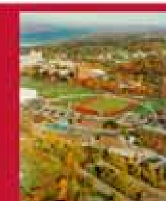
Cryopreservation of Structural Integrity under High Pressure

Chae Un Kim

Cornell High Energy Synchrotron Source

Science at the Hard X-ray Diffraction Limit
June 6 , 2011

Cornell University MacCHESS
Macromolecular Diffraction at Cornell High Energy Synchrotron Source



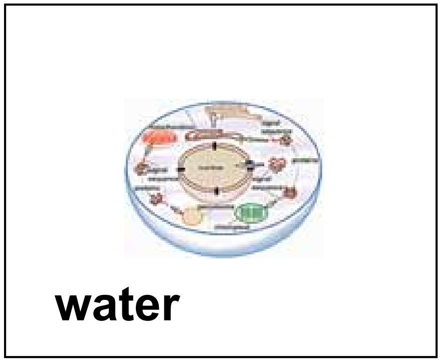
High Pressure Cryocooling

&

X-ray Diffraction Microscopy (XDM)

of

Biological Samples

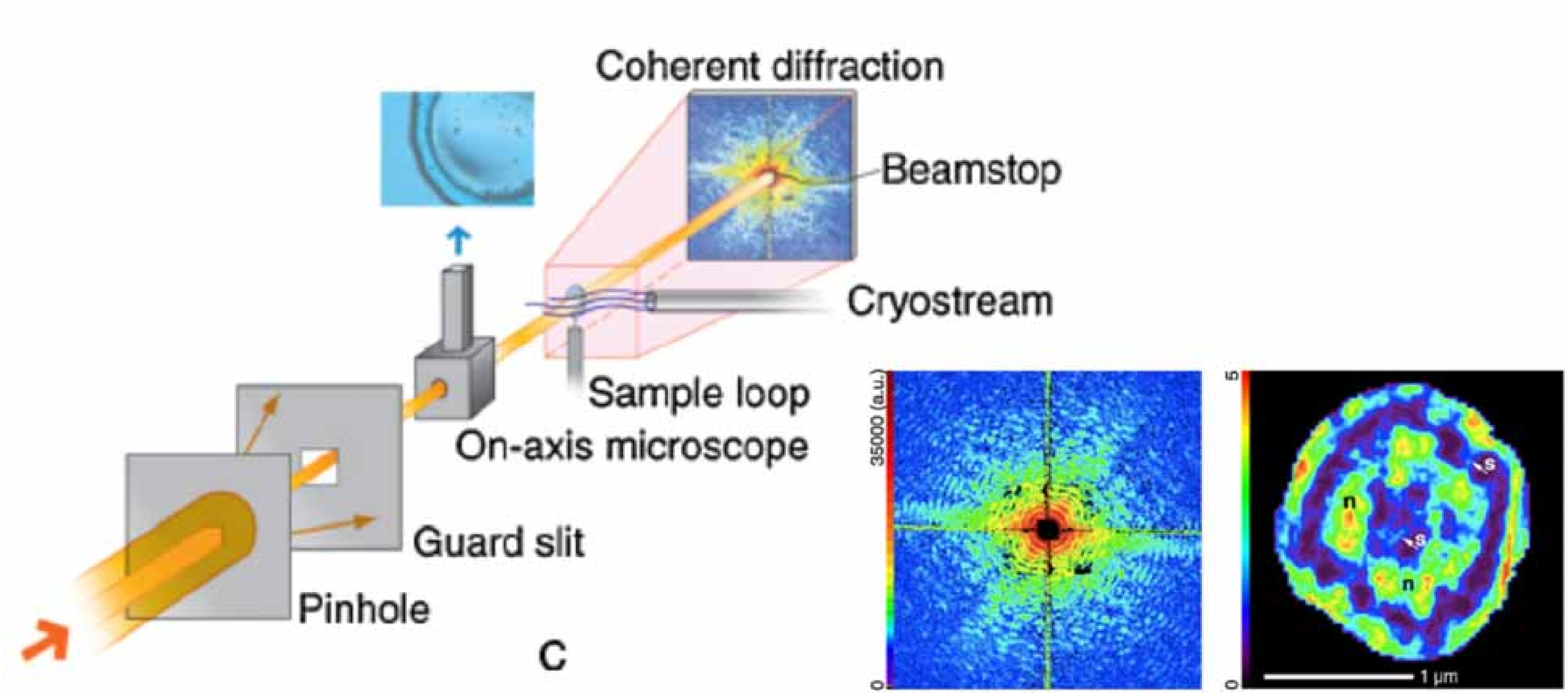


XDM of Biological Samples

X-ray Diffraction Microscopy (XDM)

- **High penetration power of X-rays**
- **Biological cells (a few μm) at 10 nm or higher resolution**
- **Fills the gap between light microscopy (low resolution, ~ 200 nm) and electron microscopy (only thin samples, ~ 0.5 μm)**
- **Lens-less method**
- **Image resolution is limited by X-ray diffraction**

Experimental Setup



Lima *et al*. (2009), PRL **103**, 198102

Oversampling phasing method

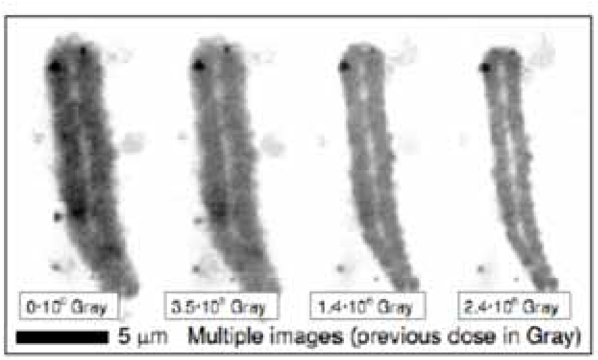
Sample Preparation

Dehydration

- Chemically fixed or freeze-dried
- Structure degradation/distortion during dehydration process
- Mass loss at 10^6 Gray (Gy)

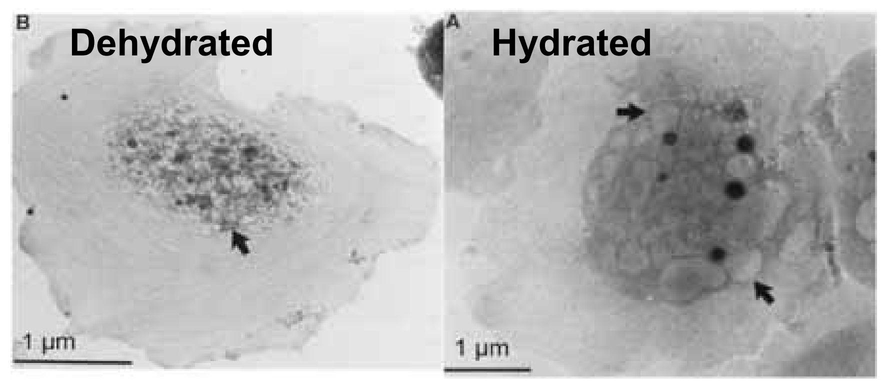
Freezing in hydrated state

- Plunge-freezing at 1 bar or high-pressure freezing
- Structure close to native state
- Less radiation damage



William et al, *Journal of Microscopy*, **170**, pp 155-165 (1993)

- Mass loss at 10^6 Gray (Gy).
- To reach 10 nm, radiation dose > 10^8 Gy



OToole *et al*. (1993), J. Stuct. Bio. **110**, 55

Recent Progress

1. Miao *et al*. (2003), PNAS 100, 110-112.
2. Shapiro *et al*. (2005), PNAS 102, 15343-15346.
3. Jiang *et al*. (2008), PRL 100, 038103.
4. Song *et al*. (2008), PRL 101, 158101.
5. Nishino *et al*. (2009), PRL 102, 018101.
6. Nelson *et al*. (2010), PNAS 107, 7235.
7. Jiang et al. (2010), PNAS 107, 11234.

Dehydrated

8. Huang *et al*. (2009), PRL 103, 198102.
9. Lima *et al*. (2009), PRL 103, 198103.

Frozen hydrated

What should be done during freezing ?

Cryopreservation Concerns

Crystallization must be suppressed during freezing !!

Otherwise,

1. Cell structure damage via

- Solution effect → osmotic shock

- Mechanical force due to expansion, etc.

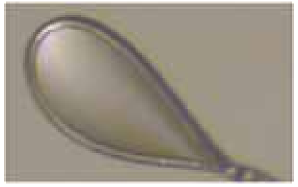
2. Parasitic scattering due to density fluctuation

→ Applying high pressure is helpful to suppress crystallization

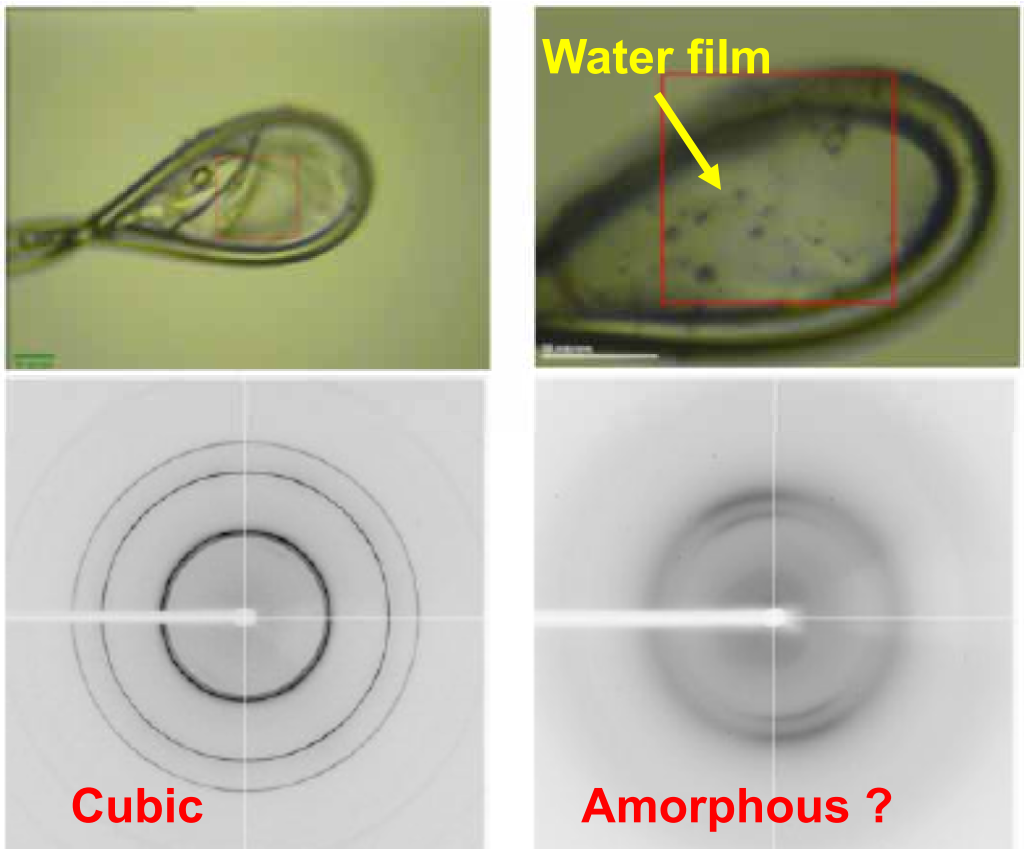
Pressure Effects on Freezing

	1 bar	2 kbar
Crystallization	Fast	Slow
Freezing rate	> 10,000 K/s	~ 100 K/s or slower
Cryo-protectants	High concentration	Low concentration
Ice phase	Amorphous (Low-density)	Amorphous (High-density)

Freezing target: 10 % glycerol solution film (~ 10 um thick)



Plunge-freezing at 1 bar



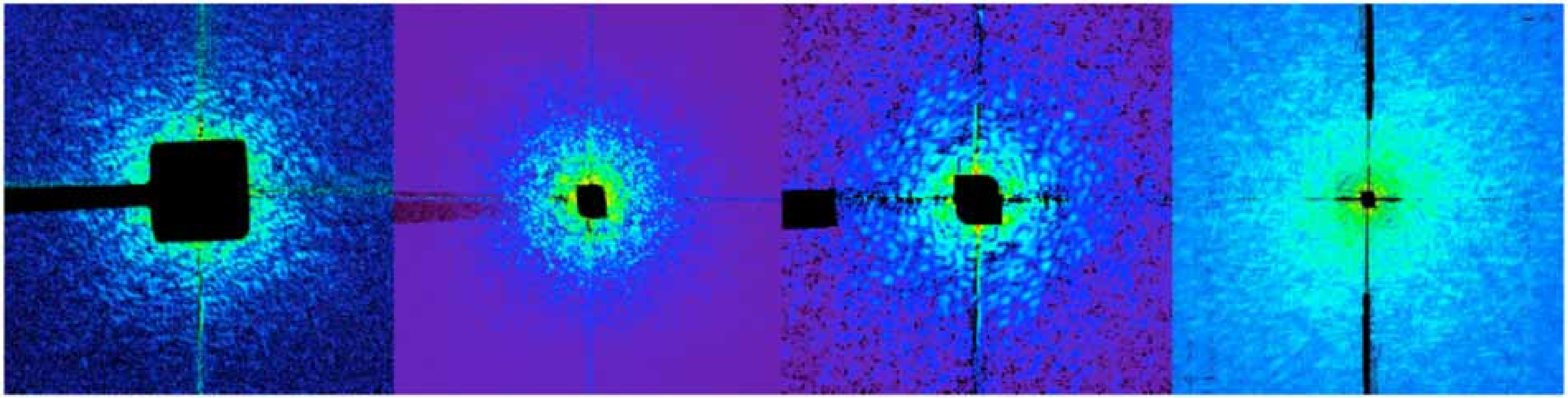
1. **10 % glycerol is added**
2. **Plunge-frozen in liquid ethane**
3. **Often cubic ice**
4. **Sometimes diffuse scattering**

→Amorphous or micro-cubic ice phase ?

Source: Enju Lima (BNL)

Low Success in Reconstruction

Nice speckles but no image reconstruction !!!



Source: Enju Lima (BNL)

May be due to the cubic ice producing parasitic scattering

→ High pressure cryocooling

High Pressure Cryocooling

1. Crystal cryoprotection & diffraction phasing

1) Kim CU, Kapfer R, Gruner SM (2005), *Acta Cryst.* D**61**, 881-890.
2) Kim CU, Hao Q, Gruner SM (2006), *Acta Cryst.* D**62**, 687-694.
3) Kim CU, Hao Q, Gruner SM (2007), *Acta Cryst.* D**63**, 653-659.
4) Kim CU, Chen Y-F, Tate MW, Gruner SM (2008), *J. Appl. Cryst.* **41**, 1-7.

2. Scientific studies

1) Albright RA, Ibar JL, Kim CU, Gruner SM, Morais-Cabral JH (2006), *Cell*. **126**, 1147-1159.
2) Barstow B, Ando N, Kim CU, Gruner SM (2008), *Proc. Natl. Acad. Sci.*, **105**, 13362-13363.
3) Barstow B, Ando N, Kim CU, Gruner SM (2009), *Biophys J*., **97**, 1719 -1727.
4) Domsic JF, Avvaru BS, Kim CU, Gruner SM, Agbandje-McKenna M, Silverman DN, McKenna R (2008), *J Biol Chem*, **283**, 30766-30771.
5) Kim, CU, Barstow, B, Tate, MW, Gruner, SM (2009), *Proc. Natl. Acad. Sci.,* **106**, 4596-4600.

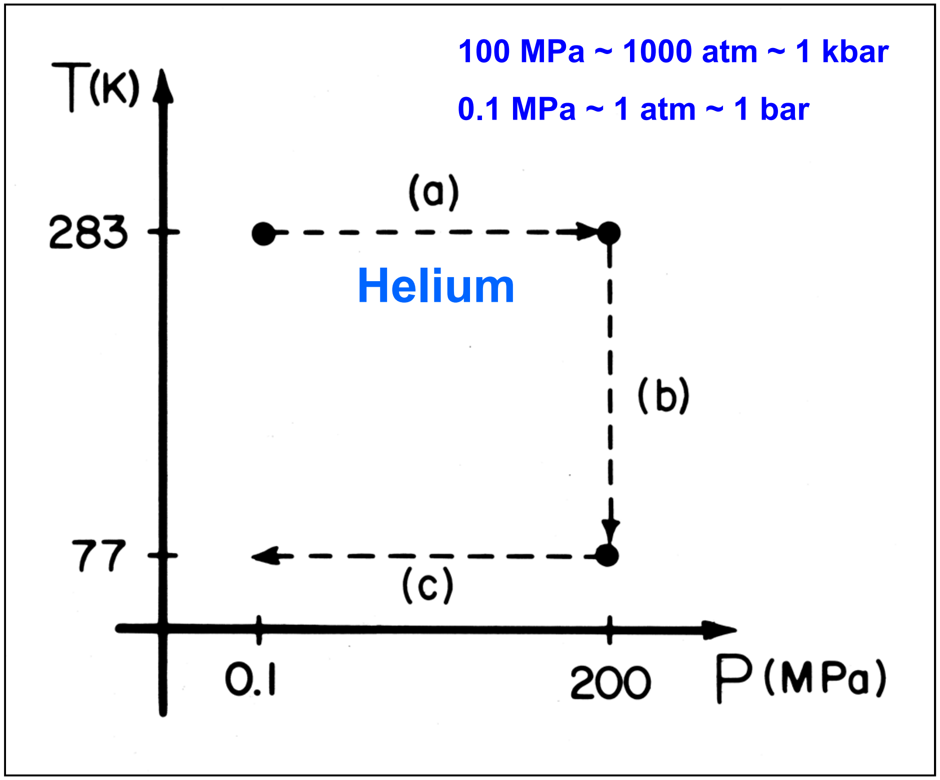
Apparatus at Gruner Lab

Apparatus at CHESS

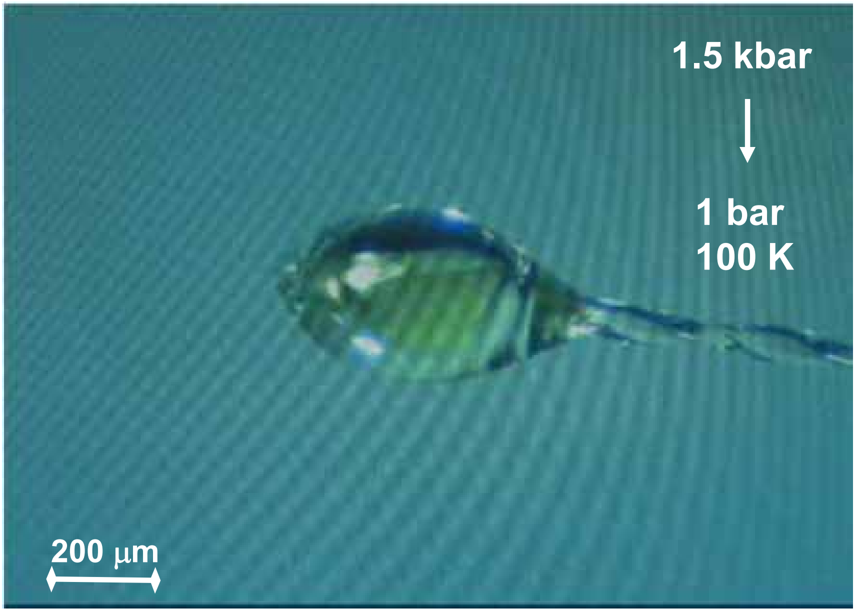
1. **Double protection with ½" steel plates.**
2. **All high pressure lines are enclosed.**
3. **Weight ~ 3000 lbs.**

High Pressure Cryocooling

Procedure



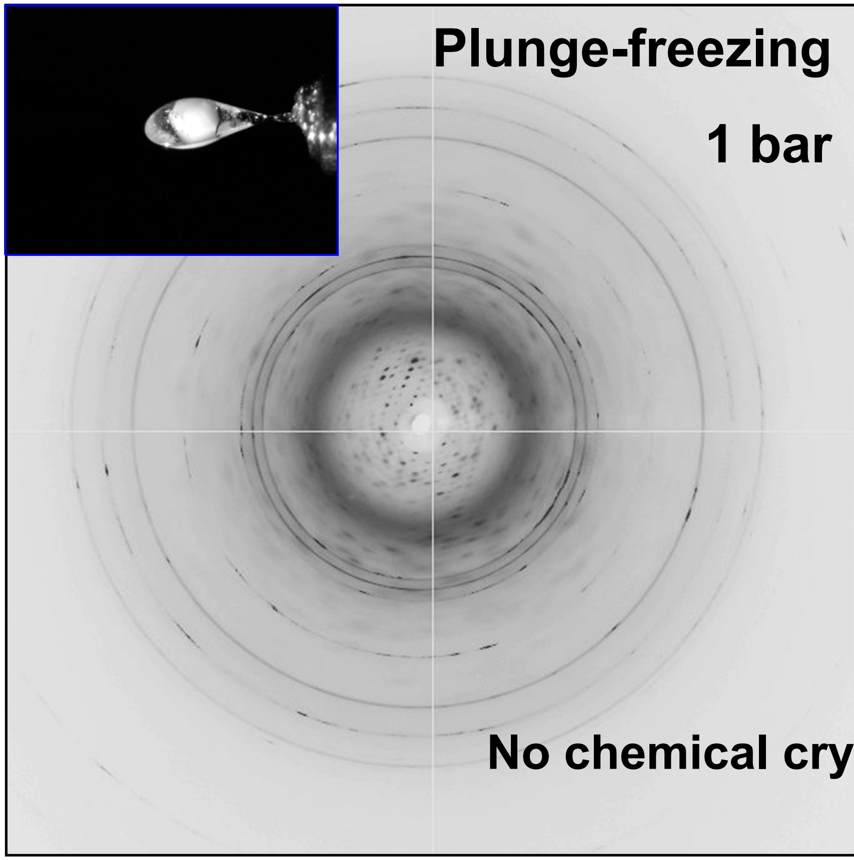
Example

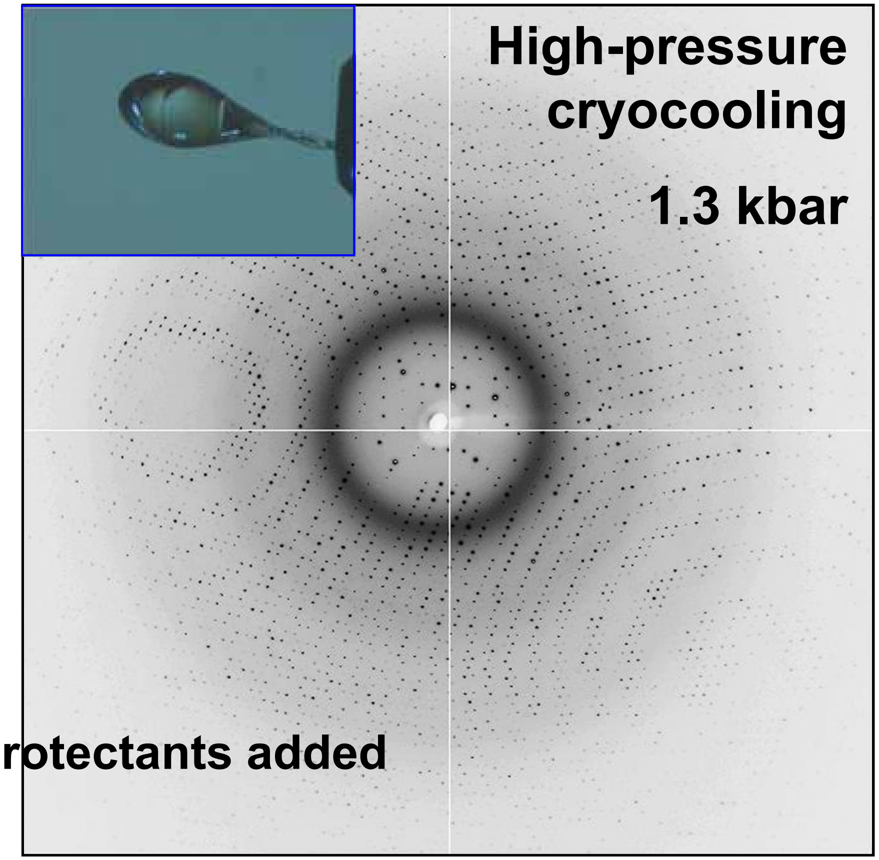


→ **Nice X-ray diffraction**

Chae Un Kim, Raphael Kapfer & Sol M. Gruner (2005). *Acta Cryst*. D**61**, 881-890.

Glucose Isomerase





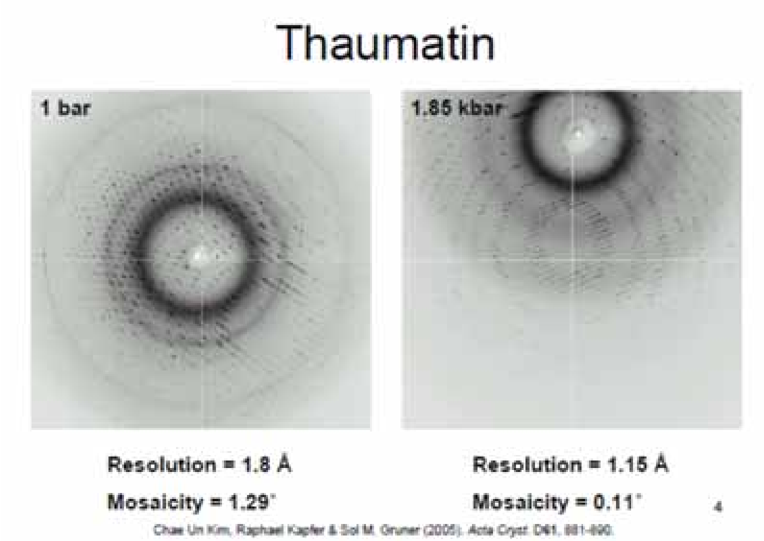
No chemical cryoprotectants added

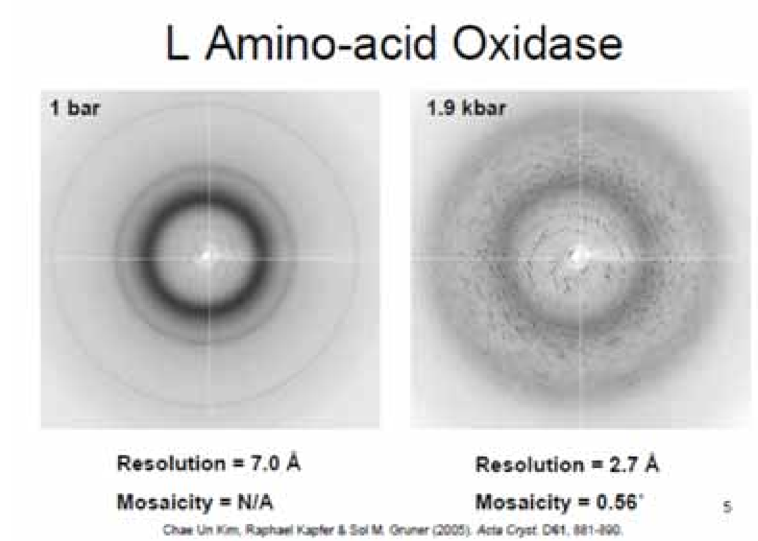
Resolution = 5.0 Å
Mosaicity = N/A

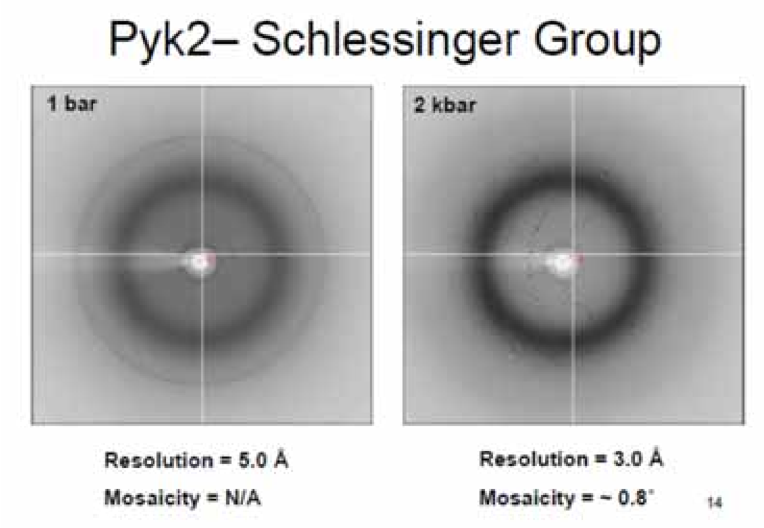
Resol. = 1.1 Å (1.3 Å for 3 crystals)
Mos. = 0.39˚ (0.48˚ for 3 crystals)

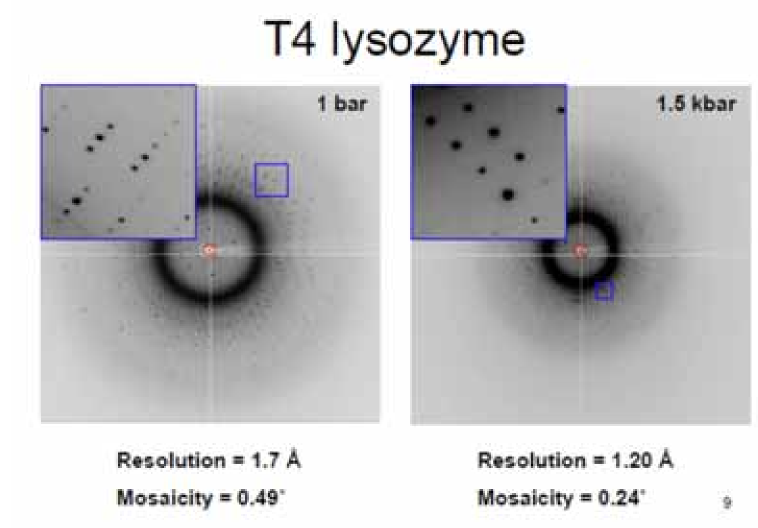
Chae Un Kim, Raphael Kapfer & Sol M. Gruner (2005). *Acta Cryst*. D**61**, 881-890.

Examples

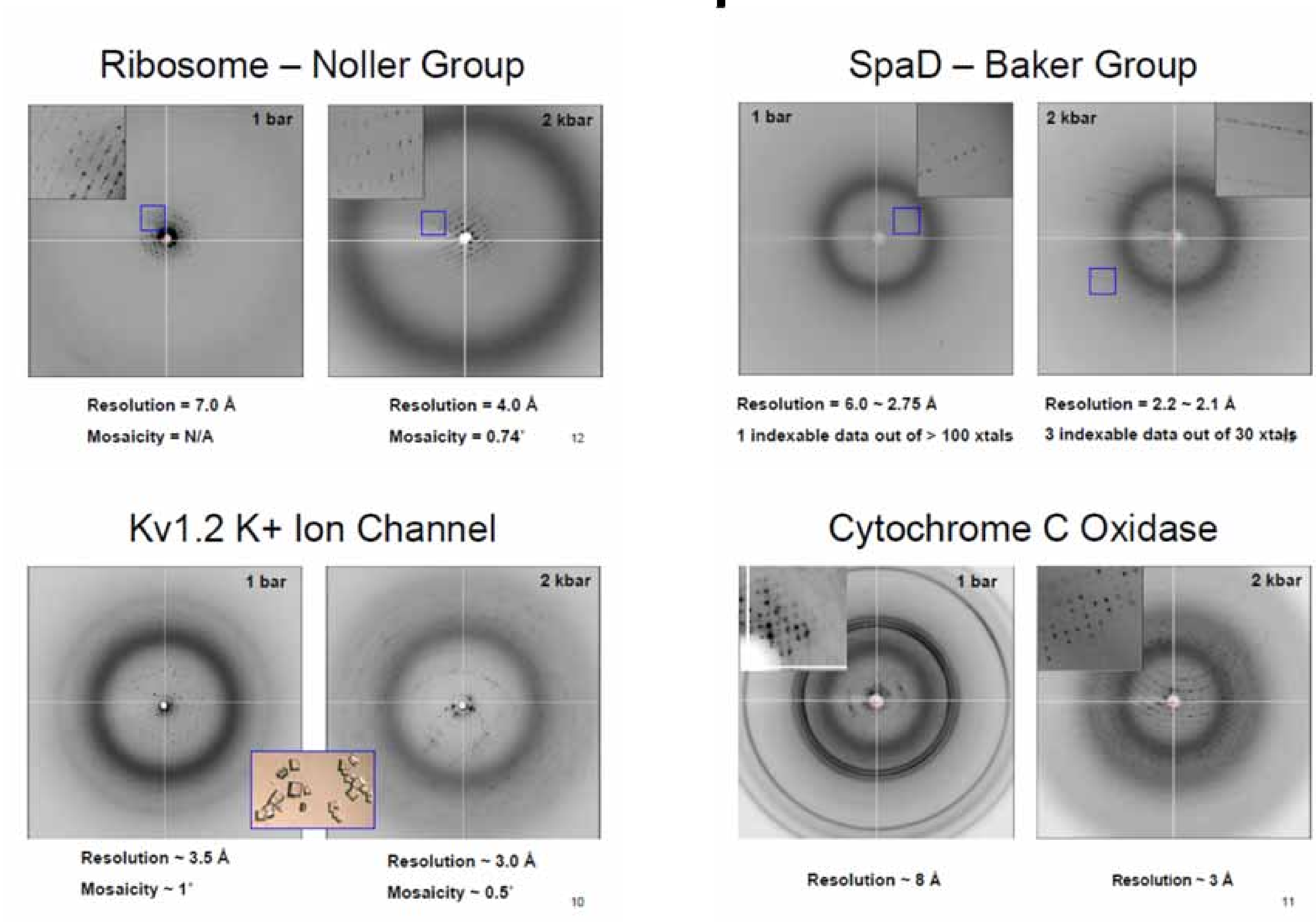

Thaumatin
1 bar
1.85 kbar
Resolution = 1.8 Å
Mosaicity = 1.29°
Resolution = 1.15 Å
Mosaicity = 0.11°
4
Chae Un Kim, Raphael Kapfer & Sol M. Gruner (2005). Acta Cryst. D61, 881-890.

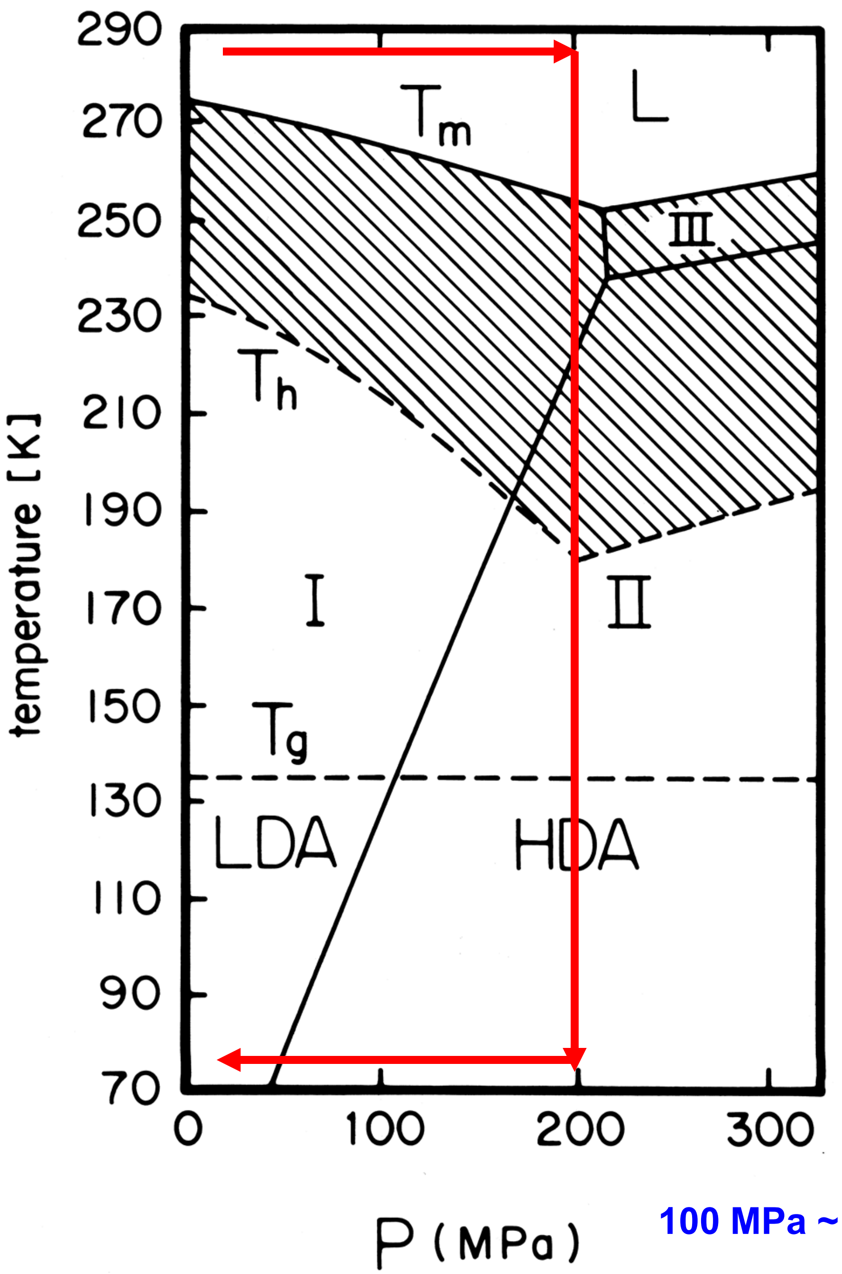

L Amino-acid Oxidase
1 bar
1.9 kbar
Resolution = 7.0 Å
Mosaicity = N/A
Resolution = 2.7 Å
Mosaicity = 0.56°
5
Chae Un Kim, Raphael Kapfer & Sol M. Gruner (2005). Acta Cryst. D61, 881-890.


Pyk2– Schlessinger Group
1 bar
2 kbar
Resolution = 5.0 Å
Mosaicity = N/A
Resolution = 3.0 Å
Mosaicity = ~ 0.8°
14


T4 lysozyme
1 bar
1.5 kbar
Resolution = 1.7 Å
Mosaicity = 0.49°
Resolution = 1.20 Å
Mosaicity = 0.24°
9

Examples


Ribosome – Noller Group
1 bar
2 kbar
Resolution = 7.0 Å
Mosaicity = N/A
Resolution = 4.0 Å
Mosaicity = 0.74˚
12
SpaD – Baker Group
1 bar
2 kbar
Resolution = 6.0 ~ 2.75 Å
1 indexable data out of > 100 xtals
Resolution = 2.2 ~ 2.1 Å
3 indexable data out of 30 xtals
Kv1.2 K+ Ion Channel
1 bar
2 kbar
Resolution ~ 3.5 Å
Mosaicity ~ 1˚
Resolution ~ 3.0 Å
Mosaicity ~ 0.5˚
10
Cytochrome C Oxidase
1 bar
2 kbar
Resolution ~ 8 Å
Resolution ~ 3 Å
11



Phase Diagram of H_2O

1. **Liquid water: L**
2. **Crystalline ice: I, II, III**
3. **Hatched region: supercooled liquid water**
4. **Amorphous ice:**

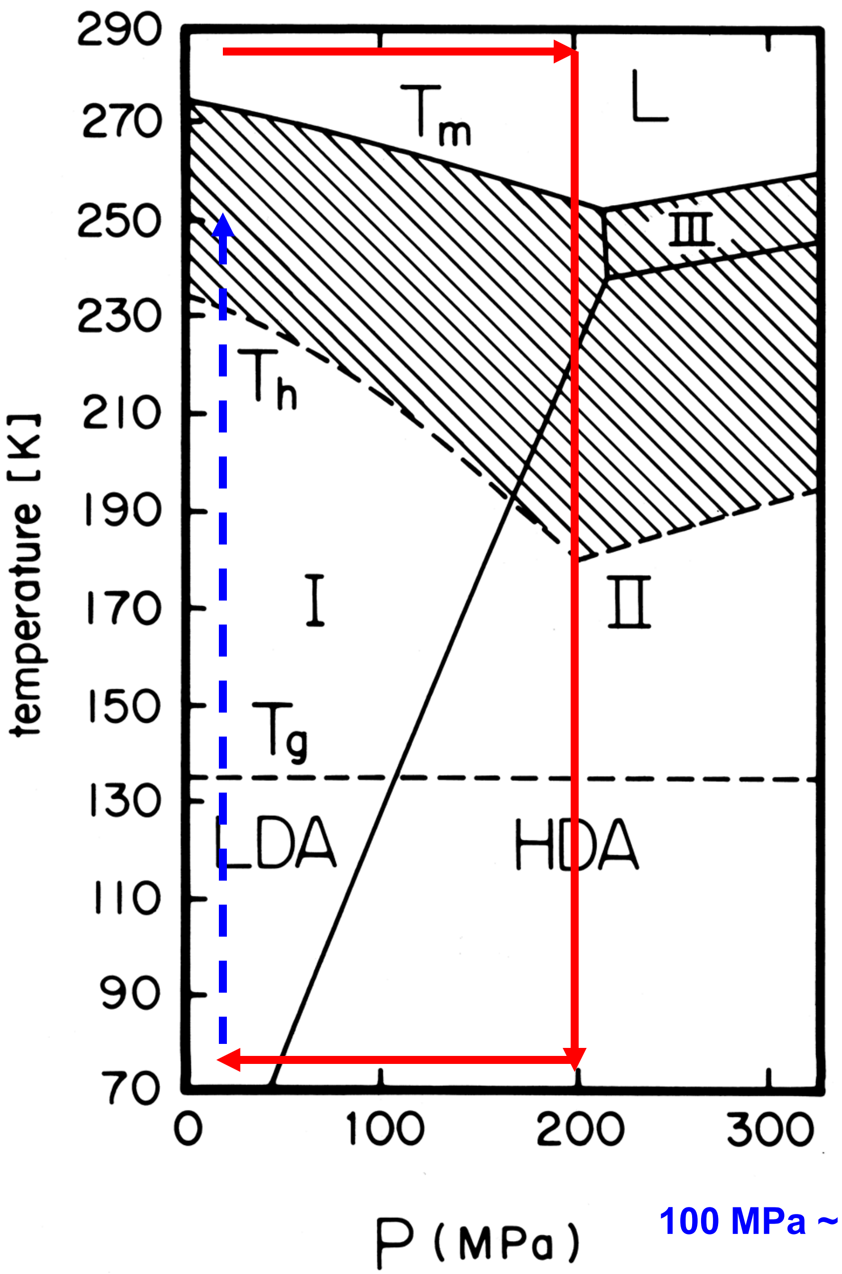
   Low density amorphous (LDA)

   High density amorphous (HDA)

Note: Phase boundary is an estimate

100 MPa ~ 1000 atm ~ 1 kbar

Chae Un Kim, Raphael Kapfer & Sol M. Gruner (2005). *Acta Cryst*. D**61**, 881-890.



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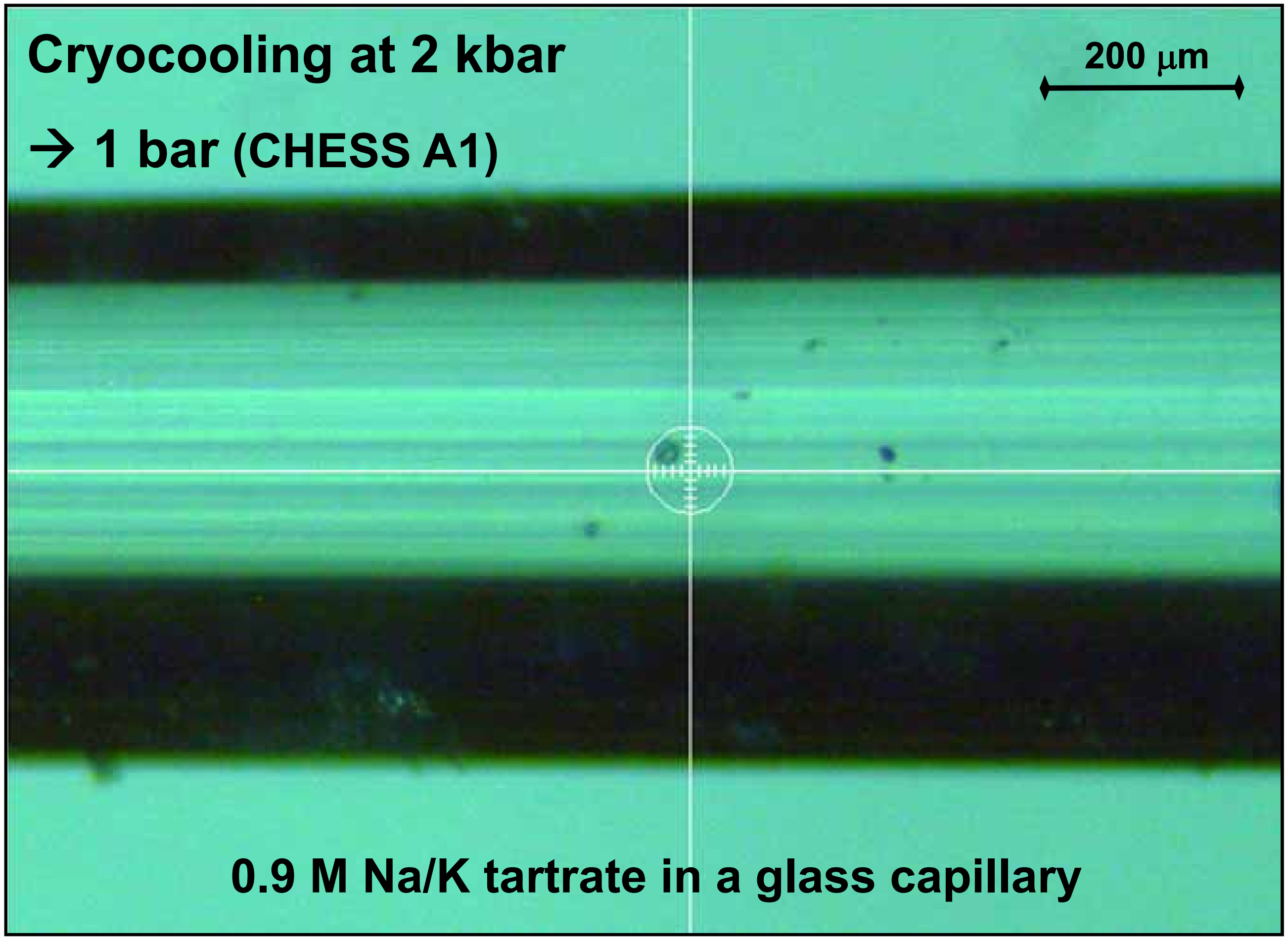
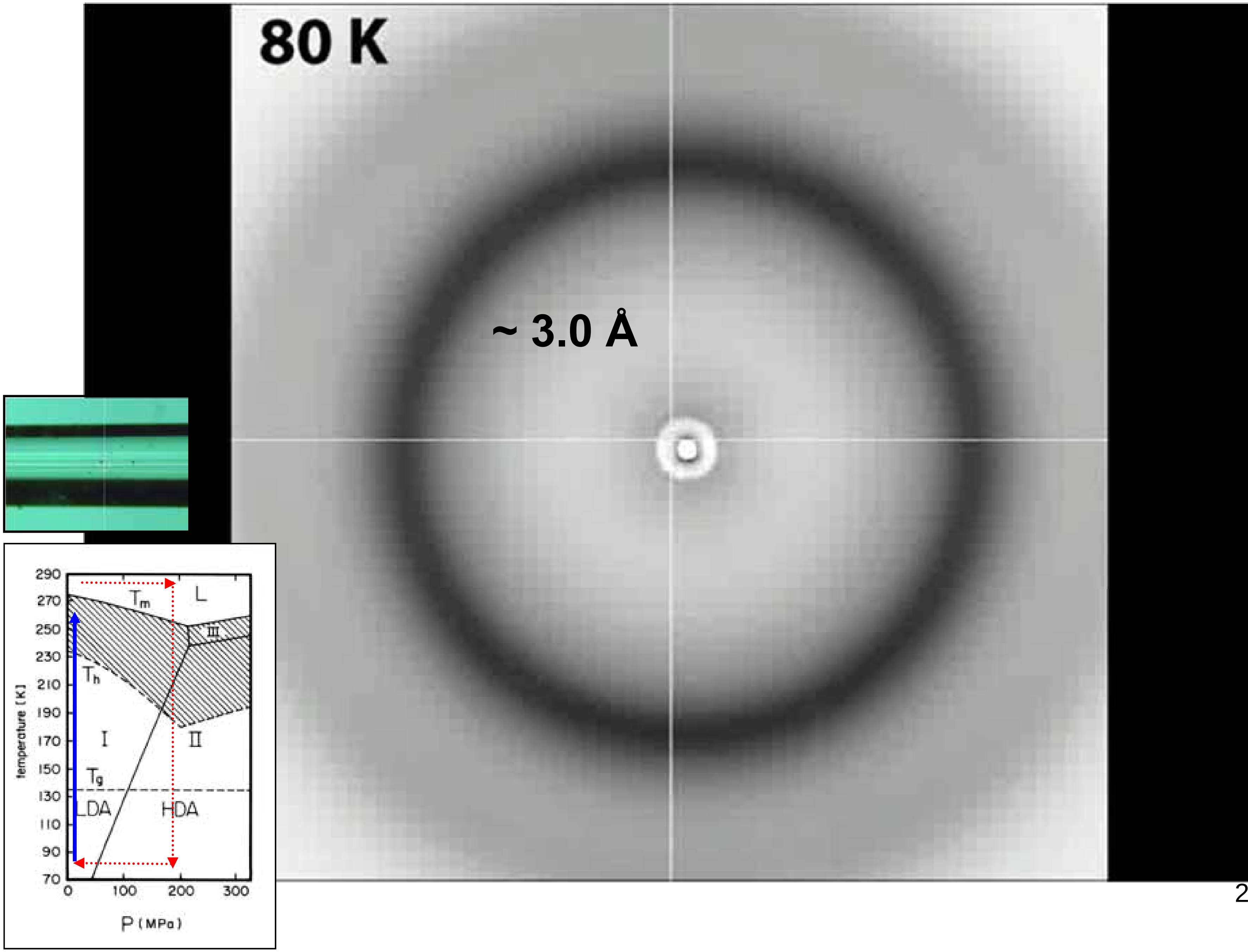
Bulk Solution



Glass capillary : Inner diameter of 500 μm, wall thickness of ~ 10 μm

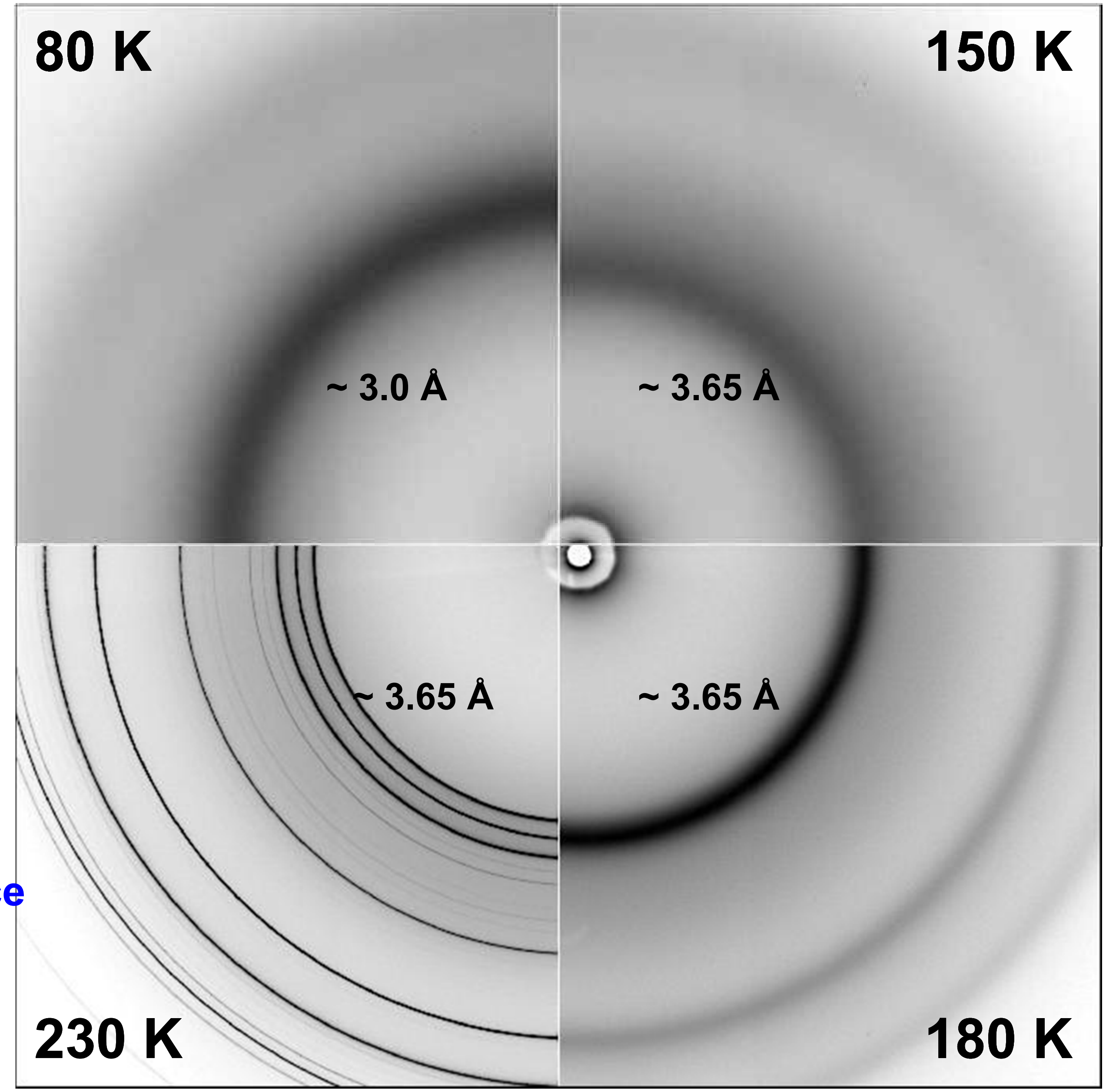

80 K
~ 3.0 Å
290
270
250
230
210
190
170
150
130
110
90
70
temperature [K]
0
100
200
300
P (MPa)
Tm
L
III
Th
I
II
Tg
LDA
HDA

HDA ice

1.17 g/cm^3

LDA ice

0.94 g/cm^3



Hexagonal ice

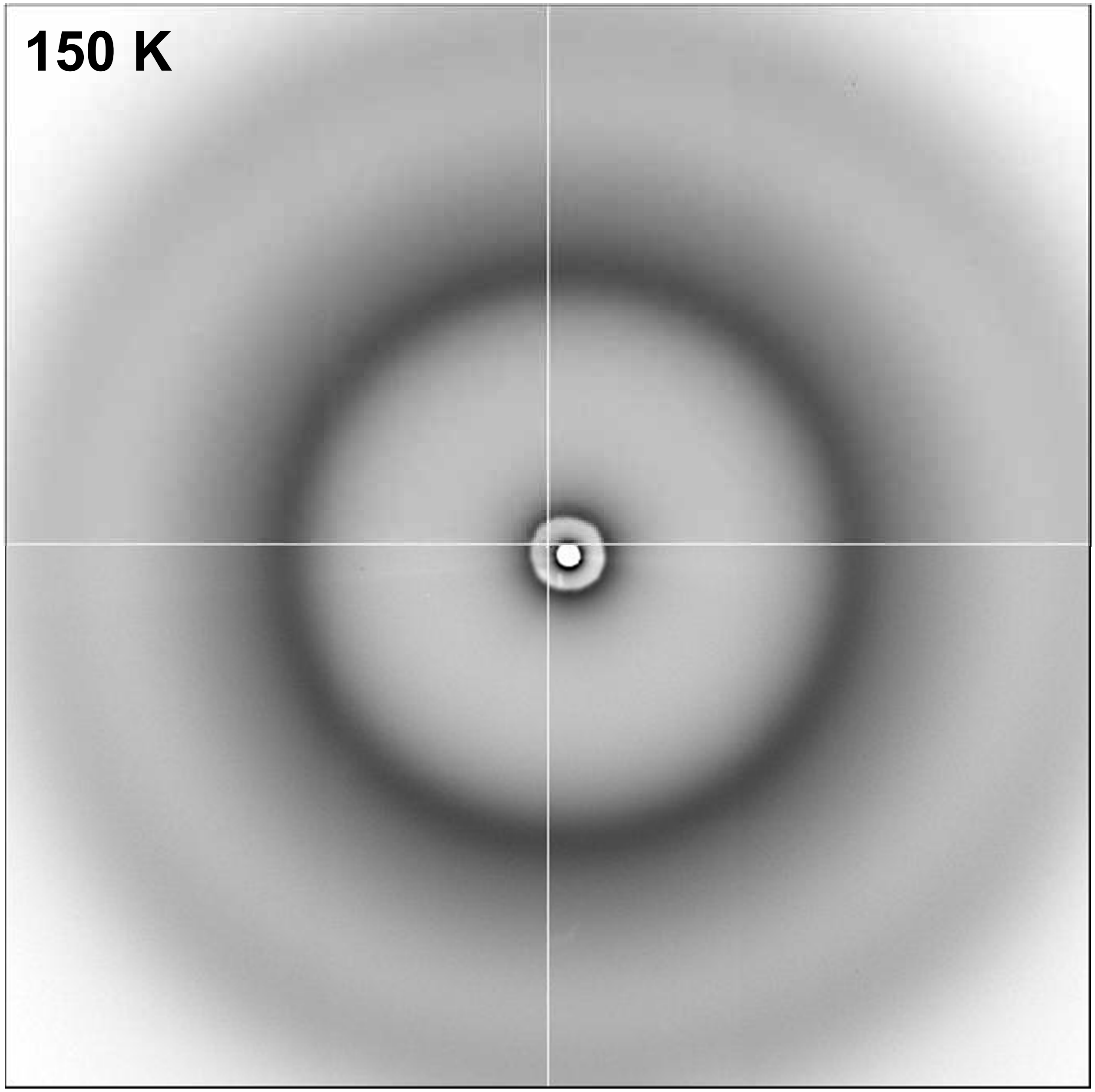
0.94 g/cm^3

Cubic ice

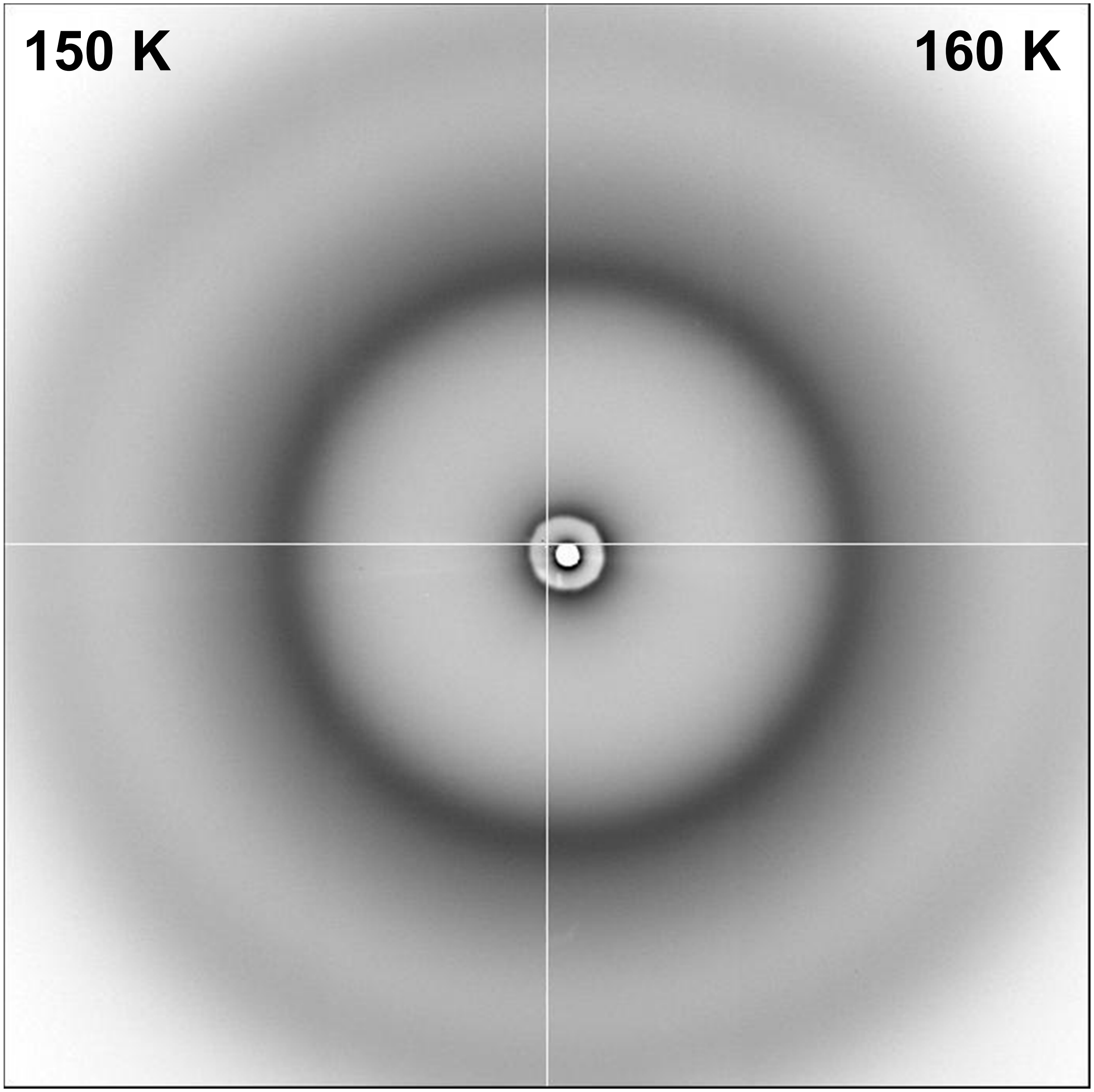
0.94 g/cm^3

Chae Un Kim, Yi-Fan Chen, Mark W. Tate & Sol M. Gruner (2008). *J. Appl.Cryst*. **41**, 1-7

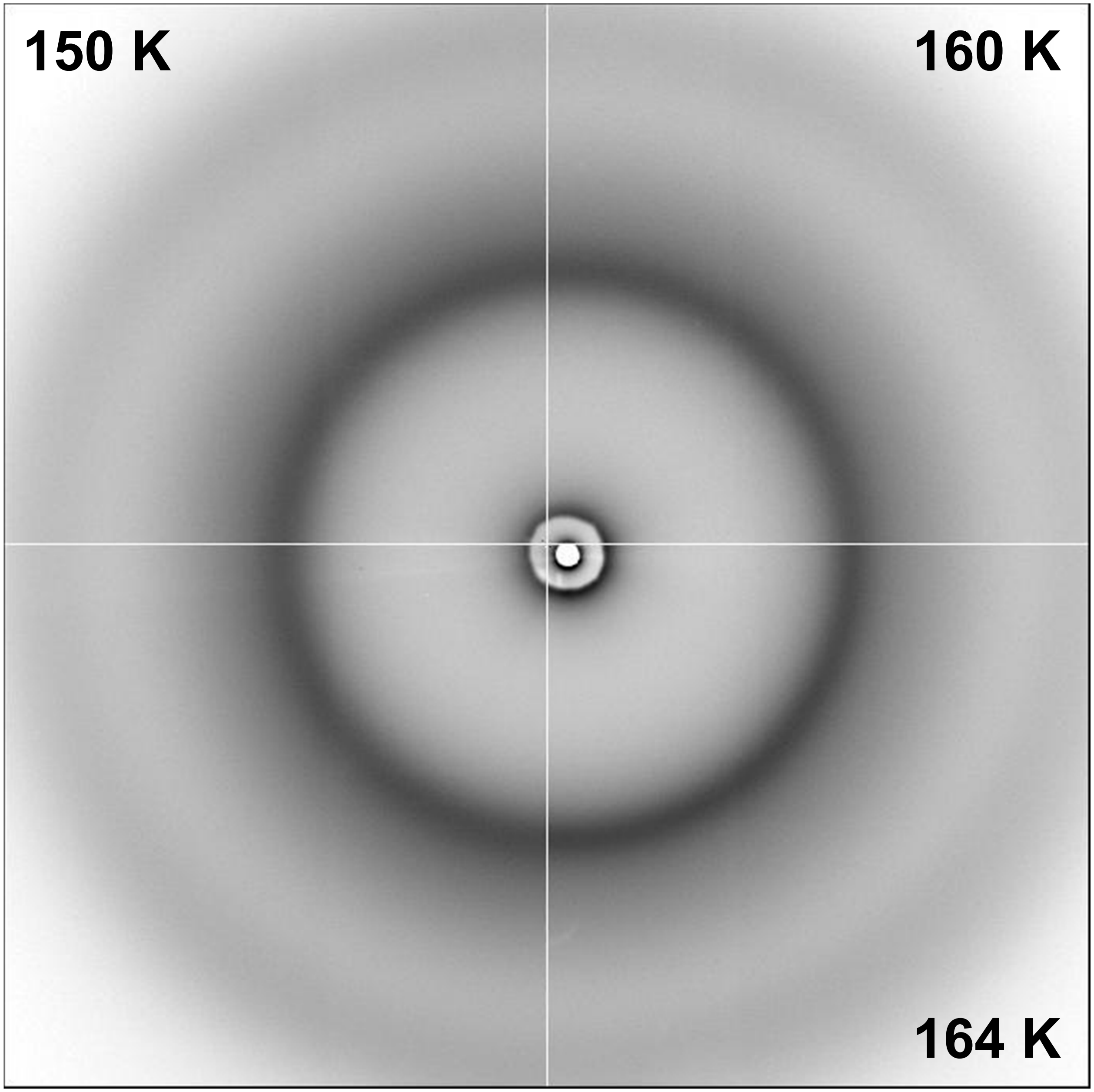
LDA ice

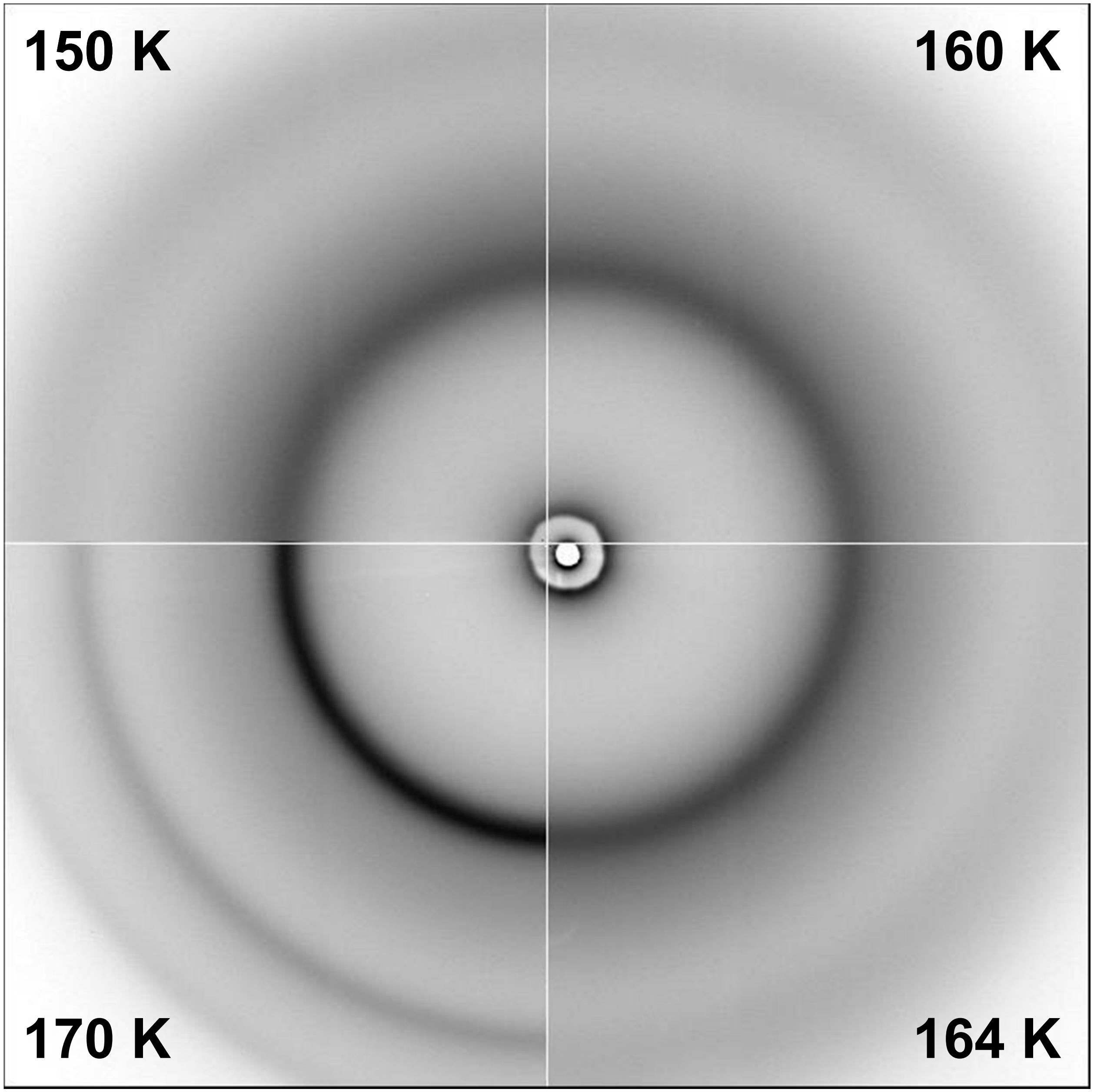


LDA ice



LDA ice





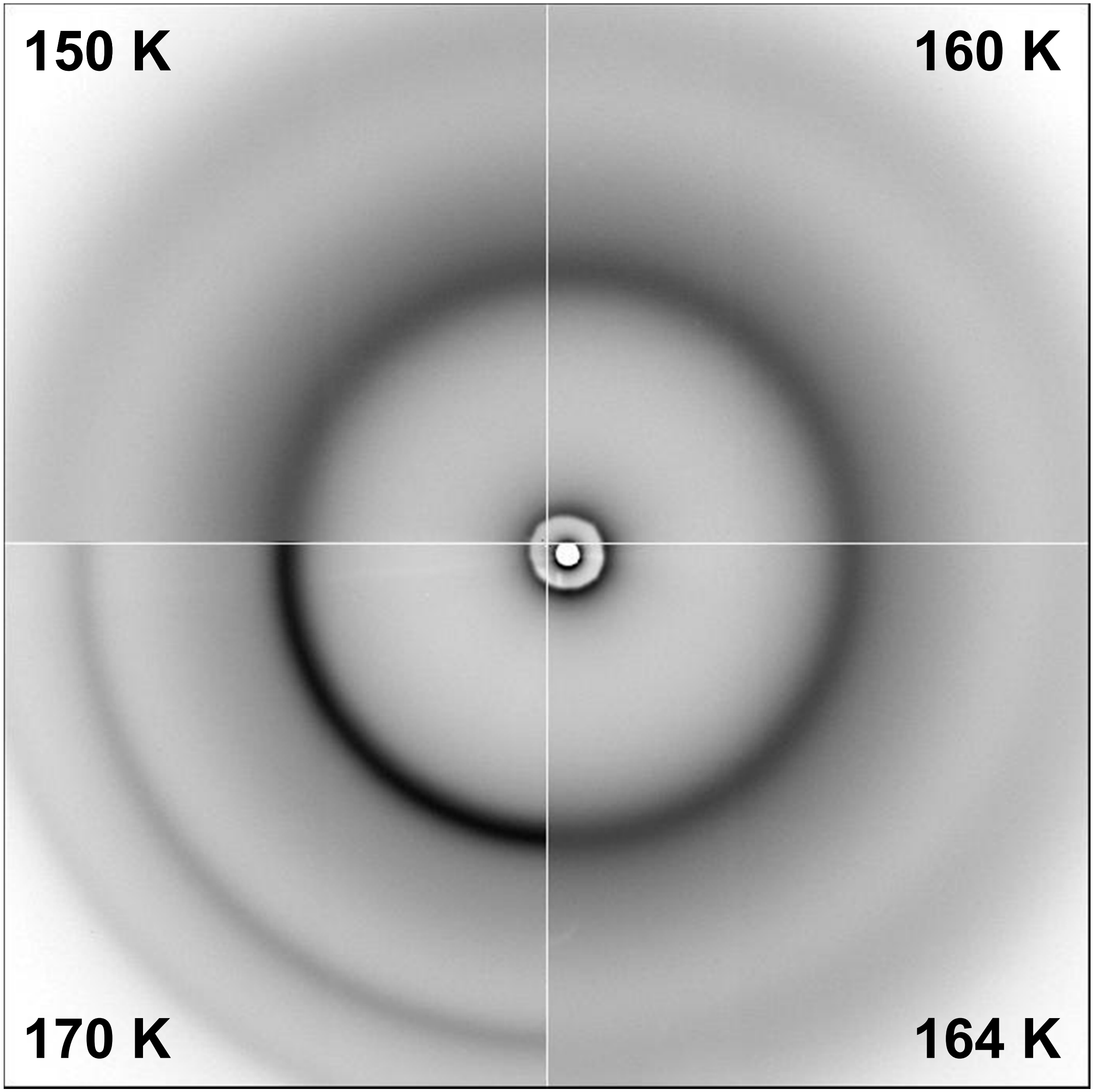
LDA ice

???

Cubic ice

???

LDA ice



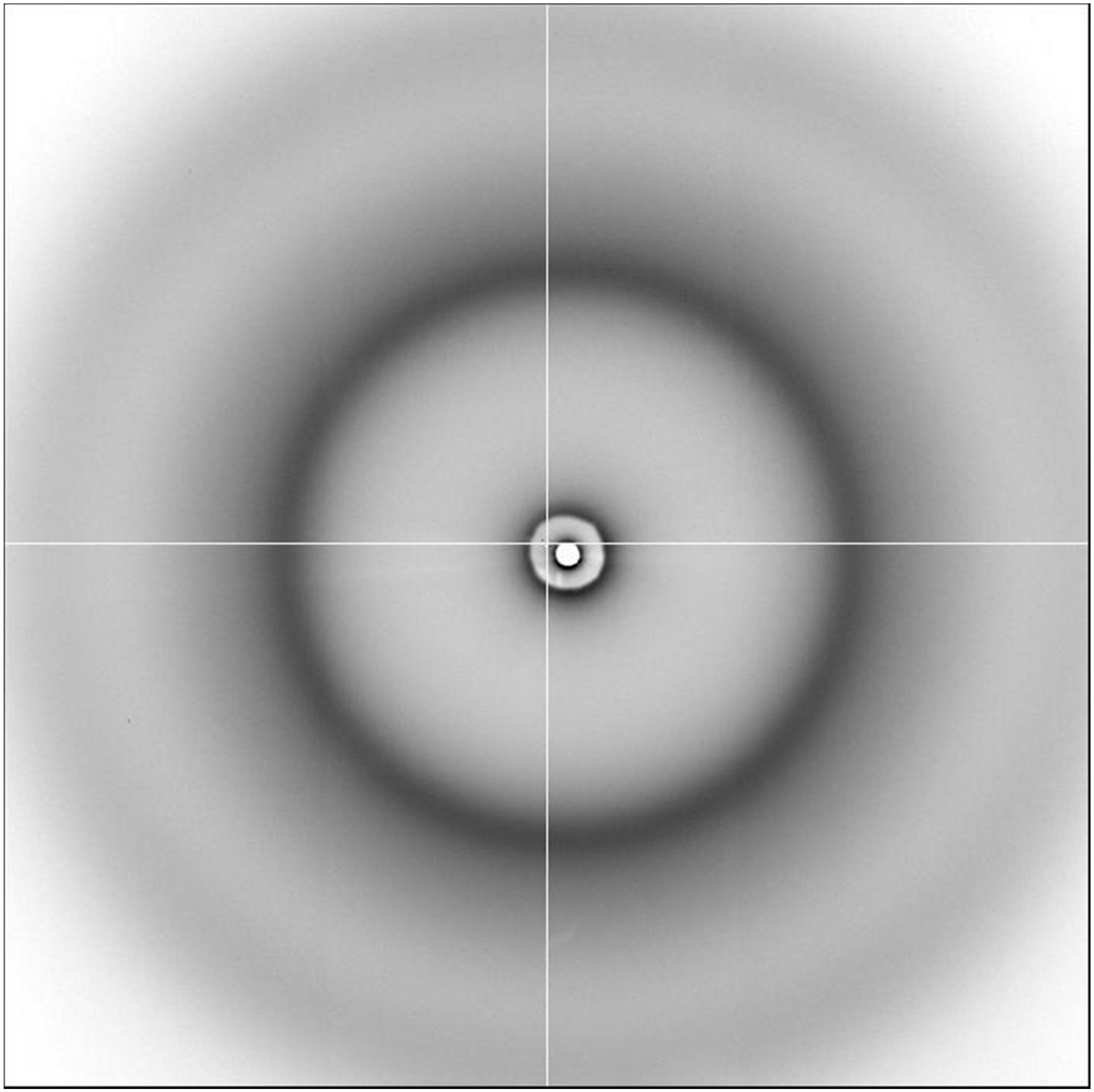
LDA +

Cubic ice

Cubic ice

LDA +

Cubic ice

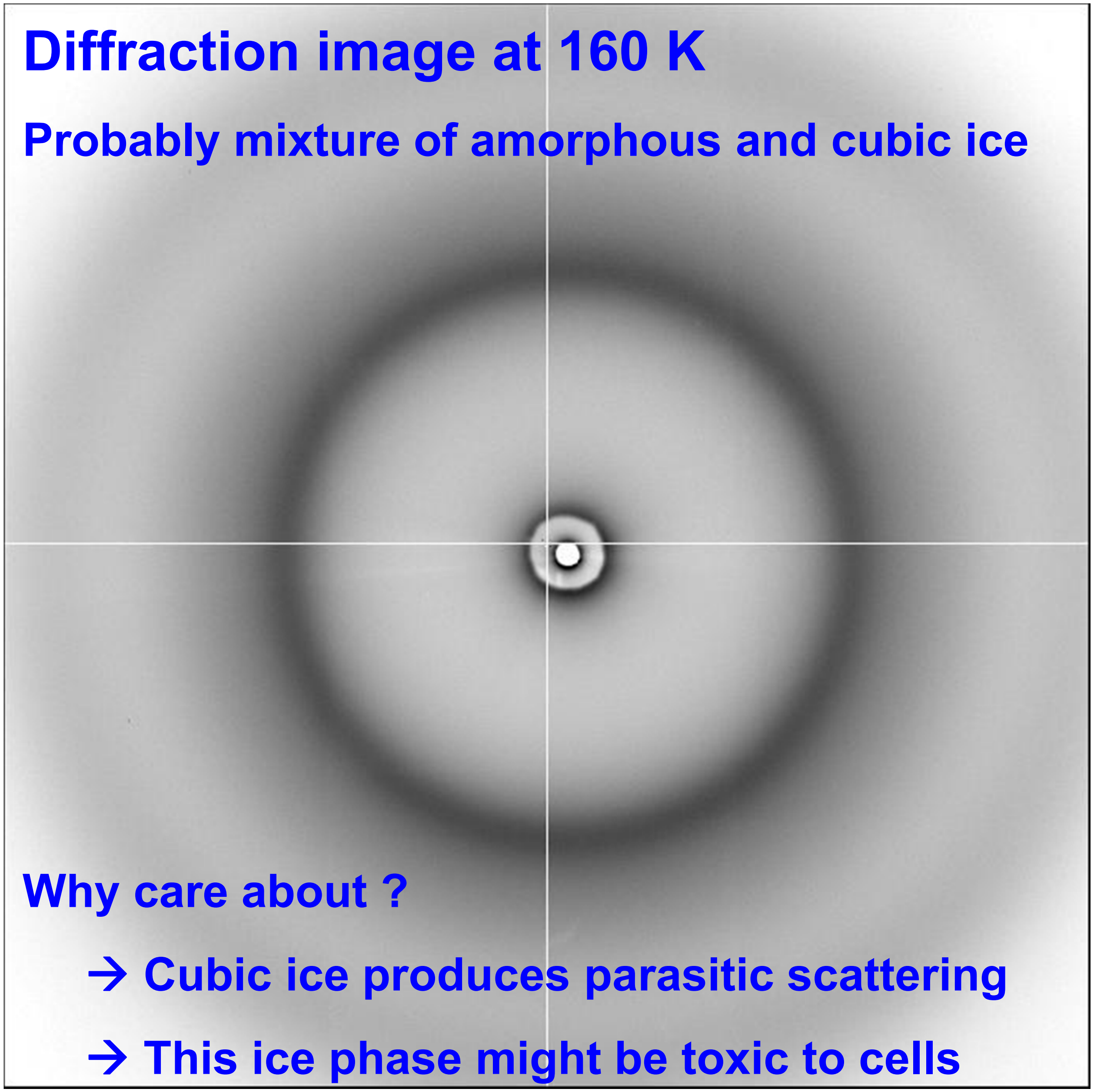


LDA

???

Diffraction image at 160 K

Probably mixture of amorphous and cubic ice

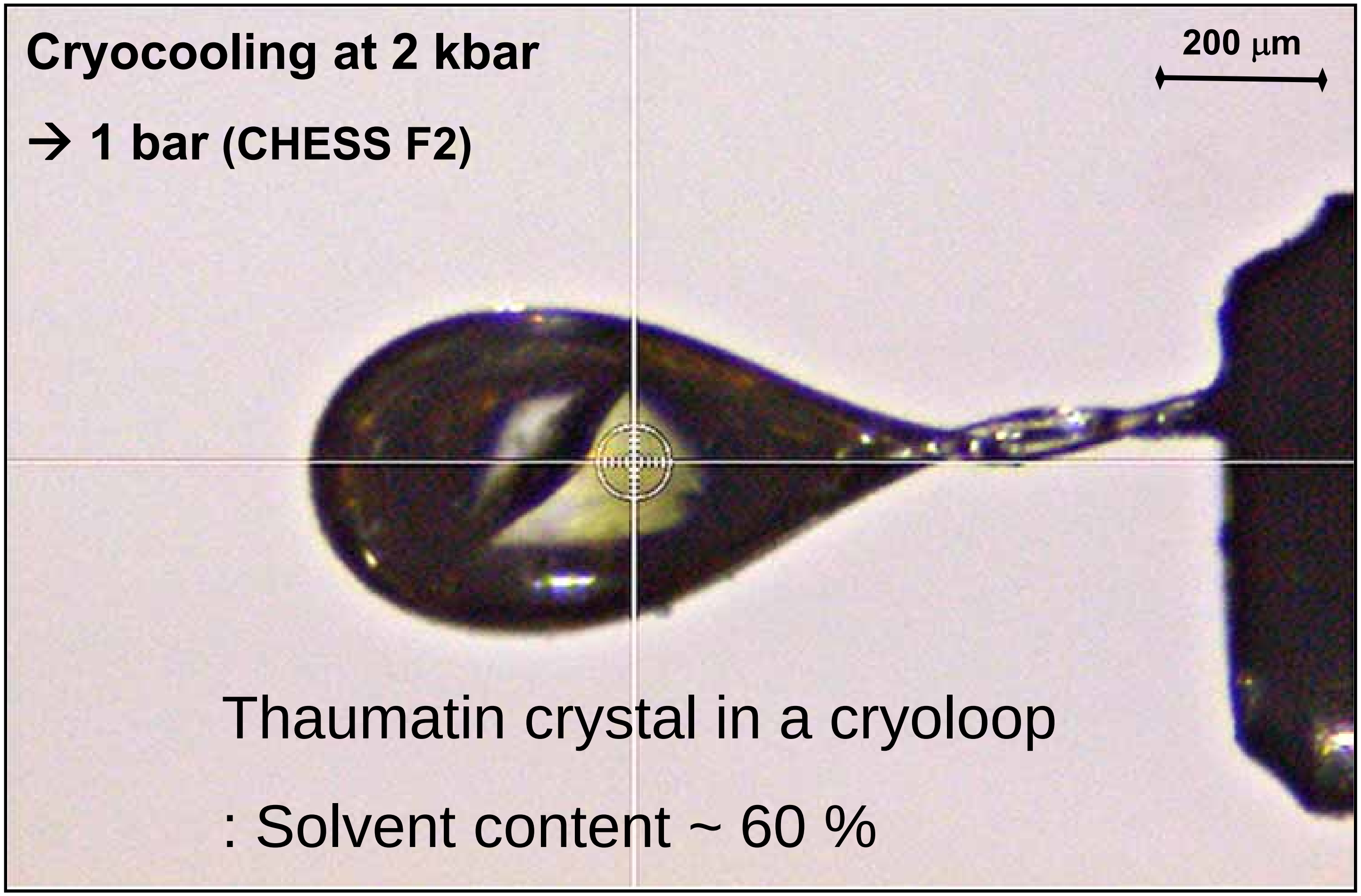


Why care about ?

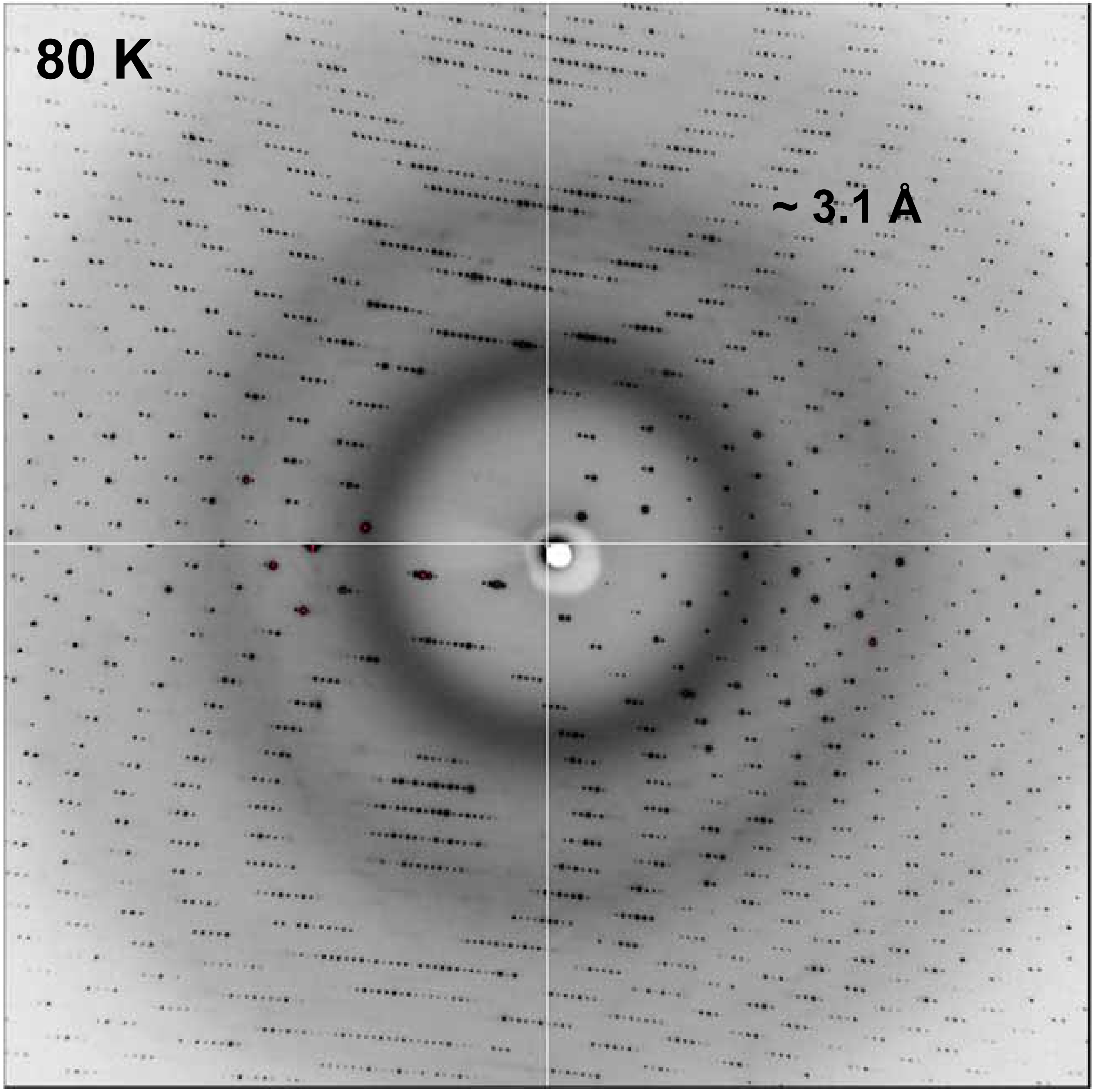
→ Cubic ice produces parasitic scattering

→ This ice phase might be toxic to cells

Protein Crystal

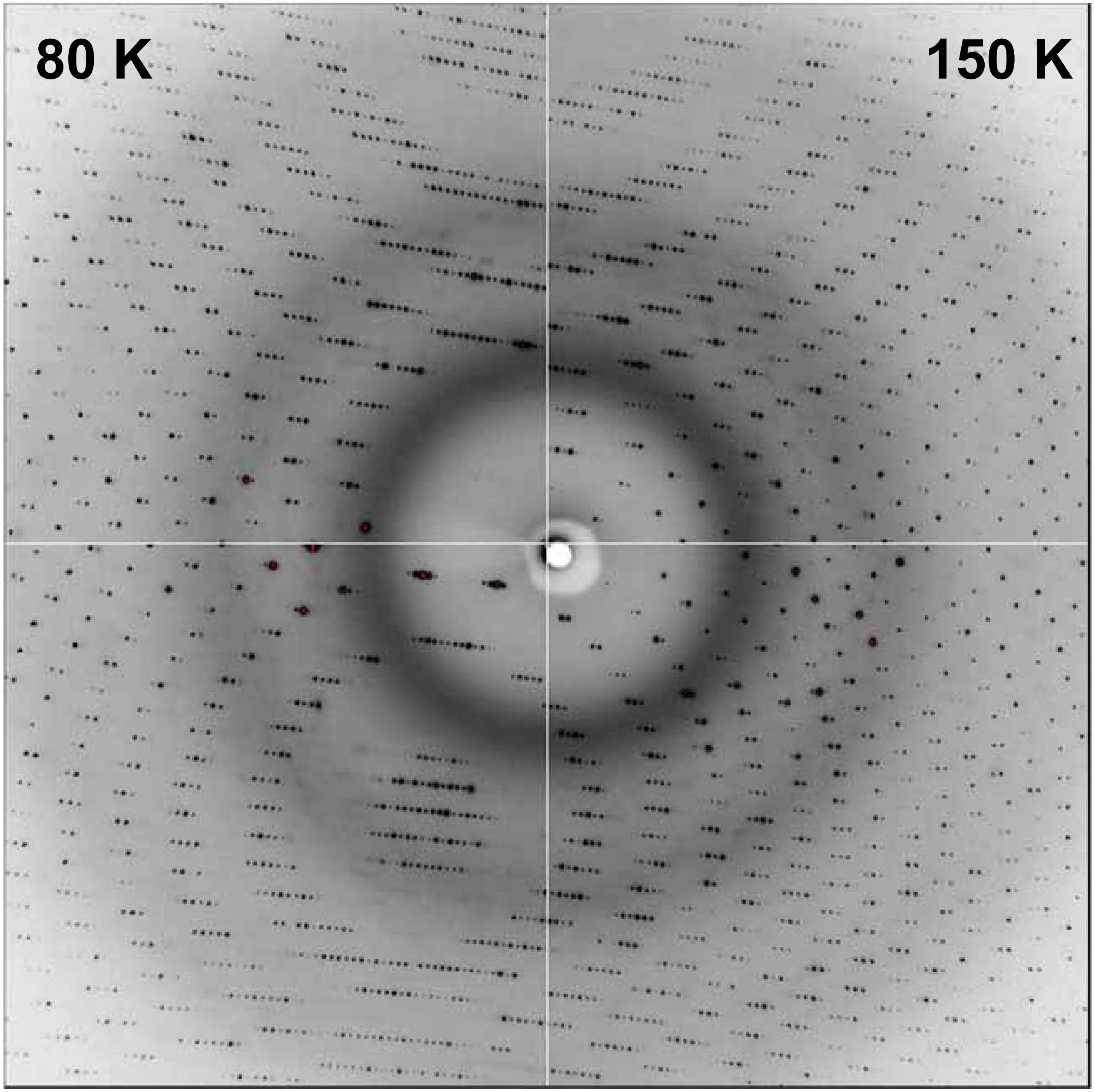


HDA ice



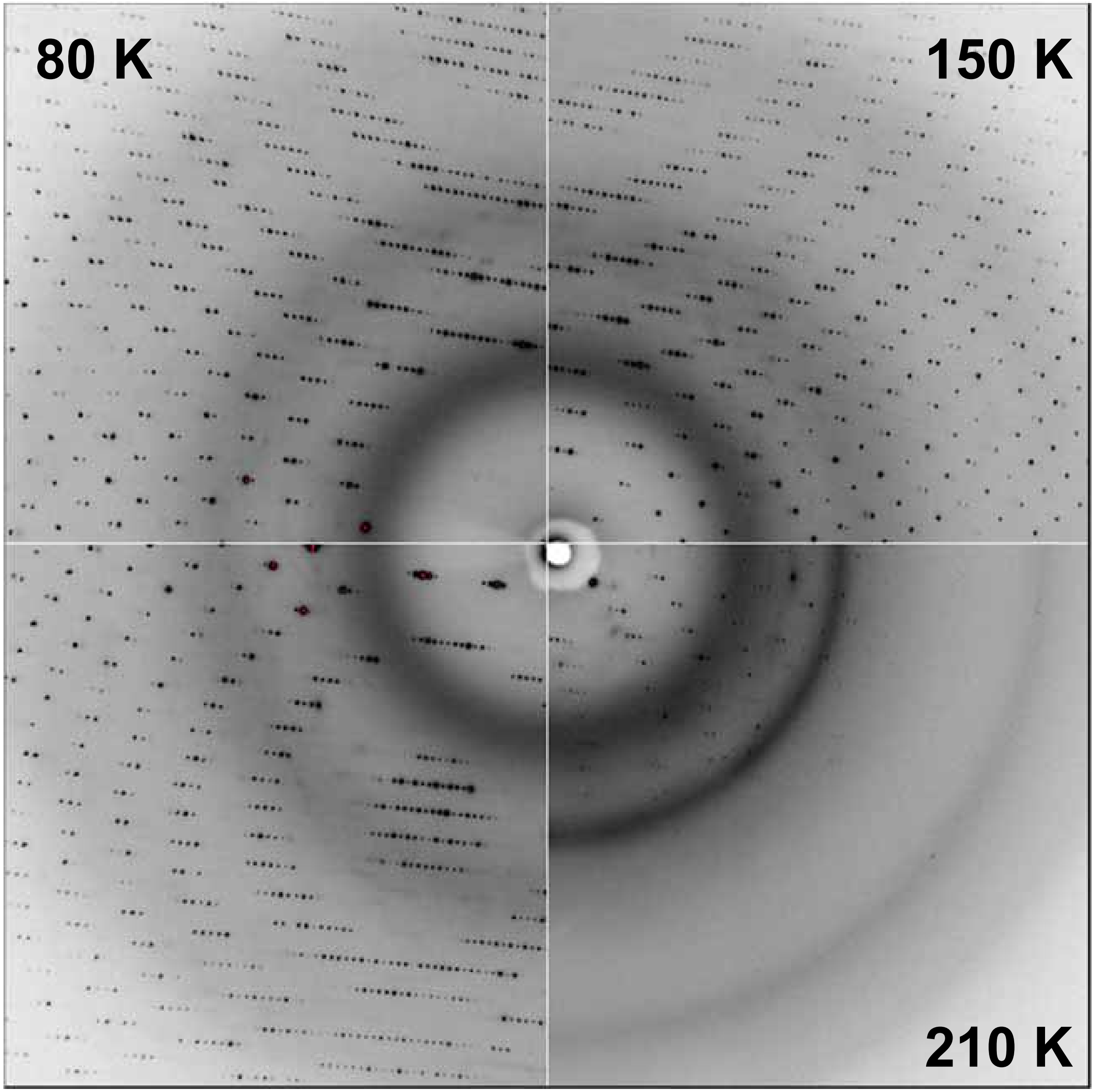
Chae Un Kim, Yi-Fan Chen, Mark W. Tate & Sol M. Gruner (2008). *J. Appl.Cryst*. **41**, 1-7

HDA ice



LDA ice

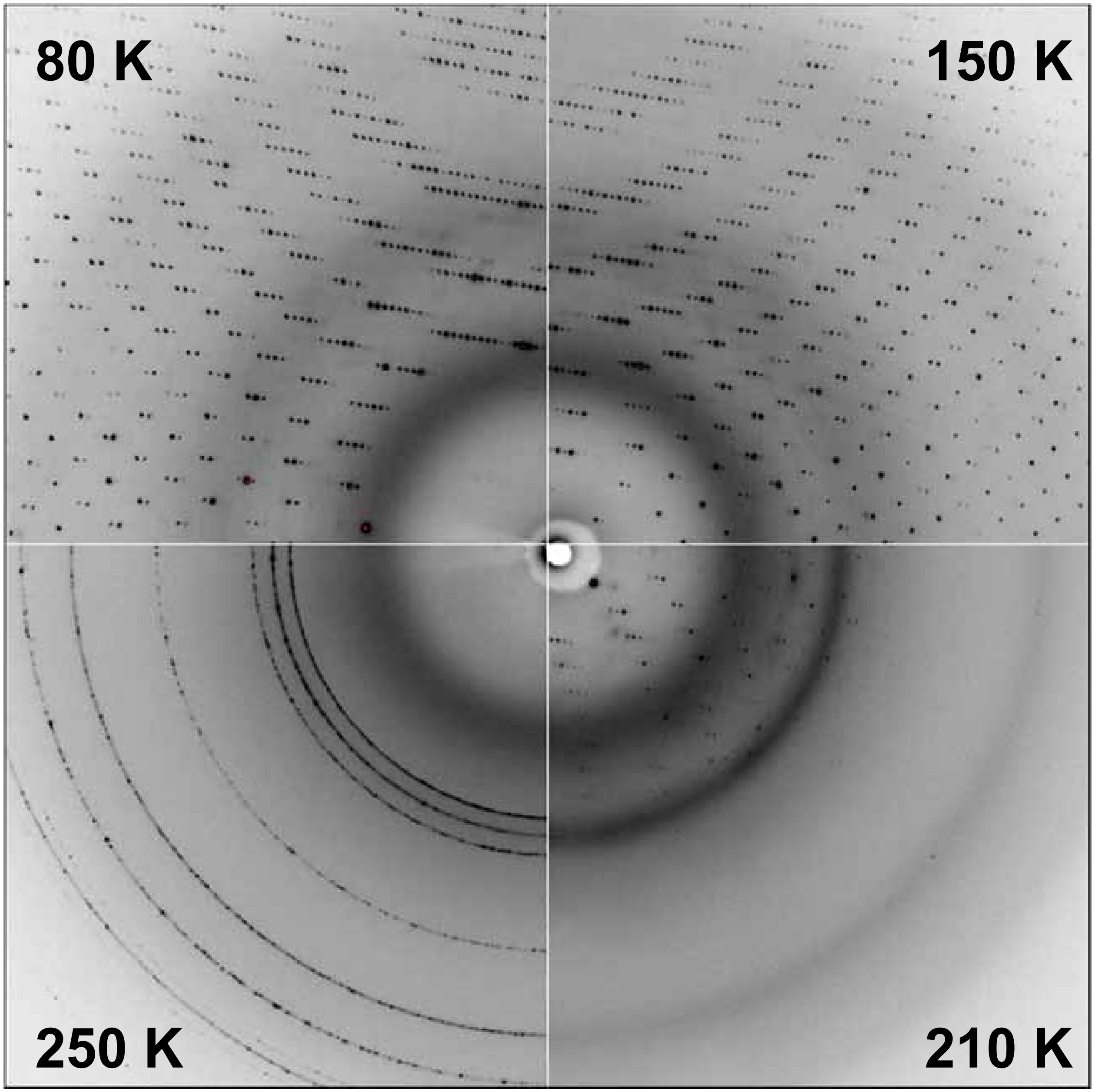
Chae Un Kim, Yi-Fan Chen, Mark W. Tate & Sol M. Gruner (2008). *J. Appl.Cryst*. **41**, 1-7



Chae Un Kim, Yi-Fan Chen, Mark W. Tate & Sol M. Gruner (2008). *J. Appl.Cryst*. **41**, 1-7

HDA ice

LDA ice

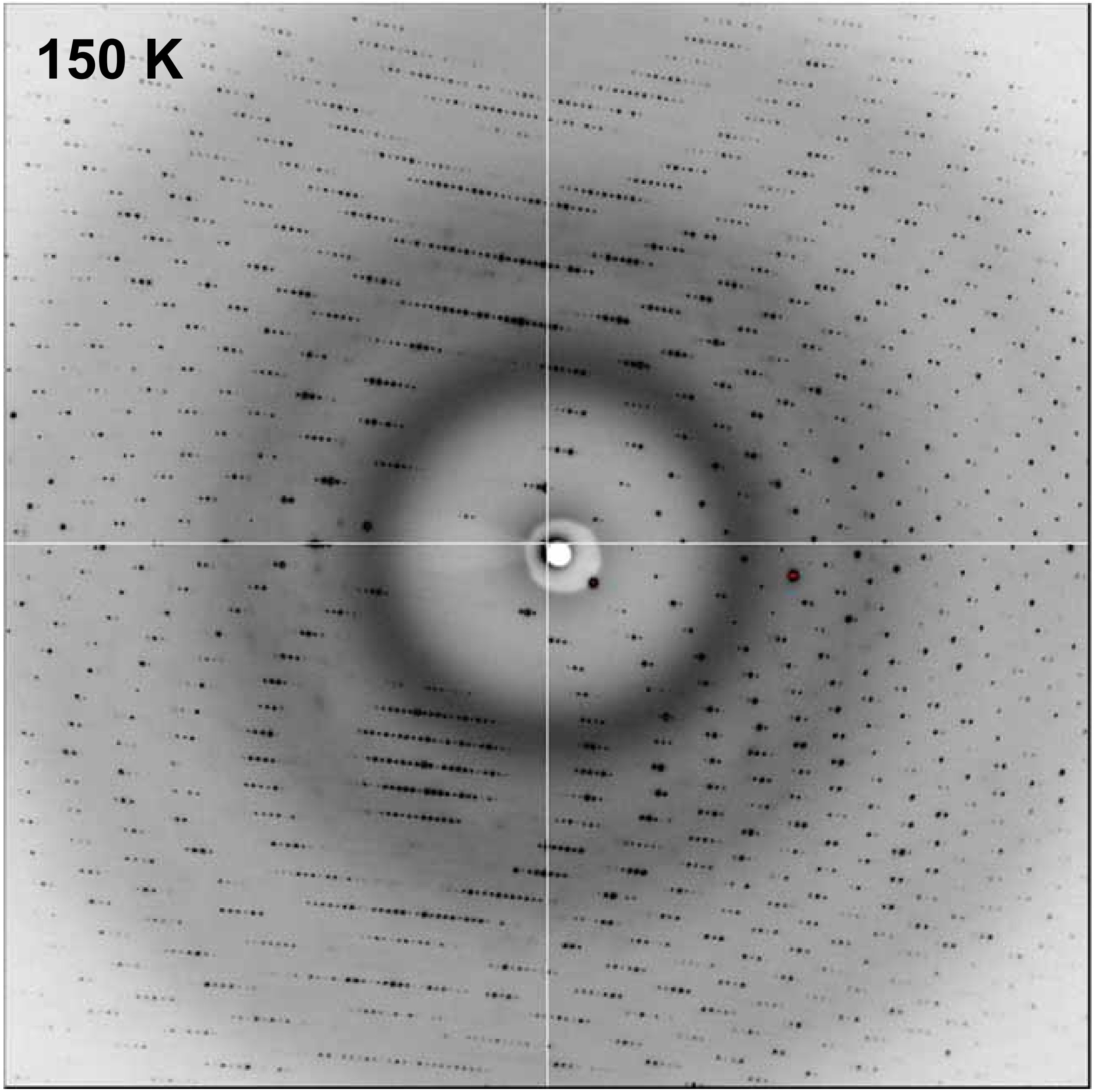


Hexagonal ice

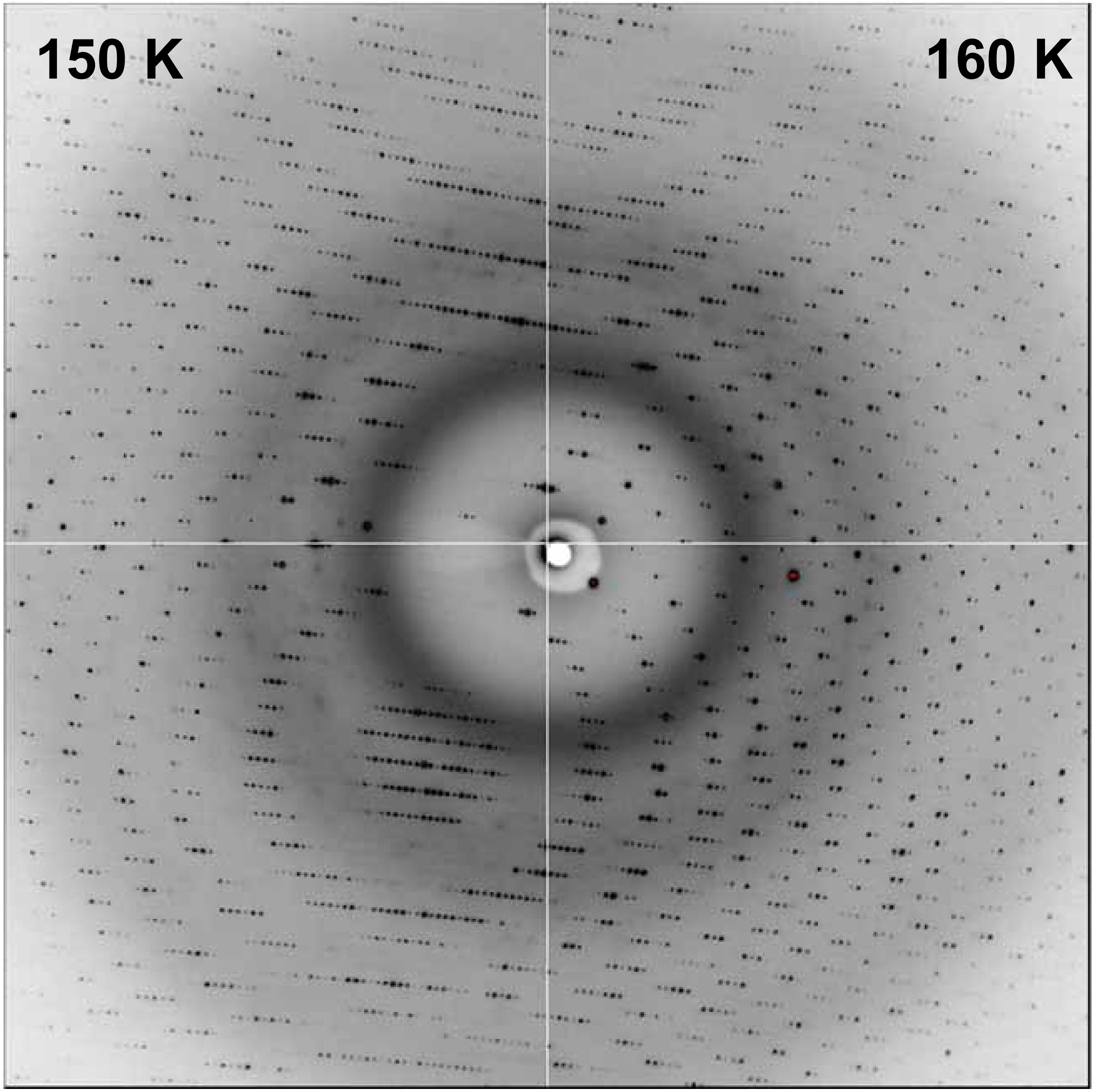
Cubic ice

Chae Un Kim, Yi-Fan Chen, Mark W. Tate & Sol M. Gruner (2008). *J. Appl.Cryst*. **41**, 1-7

LDA ice

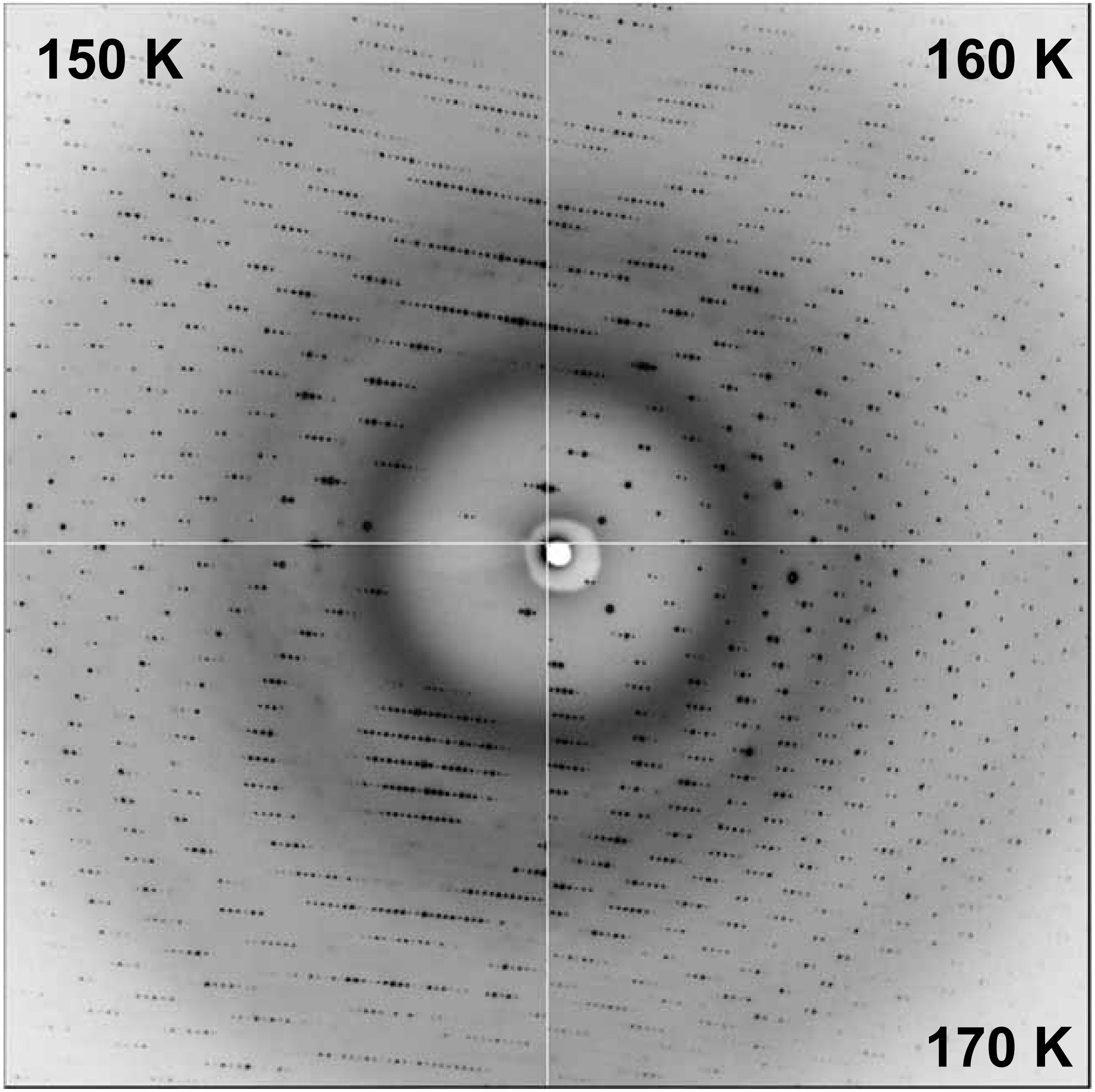


LDA ice



LDA +

Cubic ice



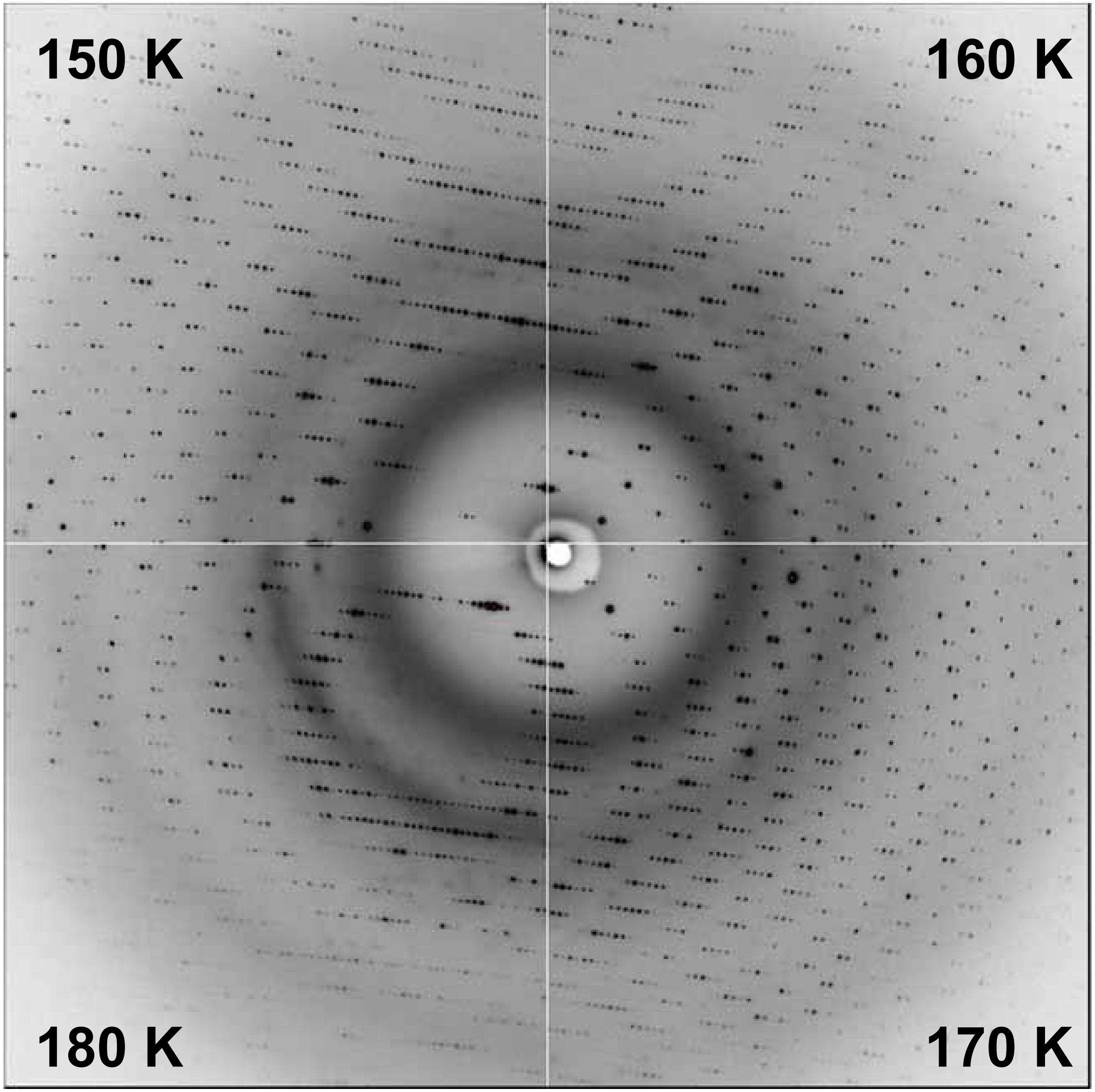
LDA ice

LDA +

Cubic ice

LDA +

Cubic ice



LDA ice

LDA +

Cubic ice

Cubic ice

LDA +

Cubic ice

Implications

1. **Wide angle X-ray diffraction does not recognize the cubic ice formation until it is quite much developing.**

2. **Plunge-freezing at ambient pressure may end up with the mixture of amorphous and cubic ice phases.**

   → Low convergence in image reconstruction

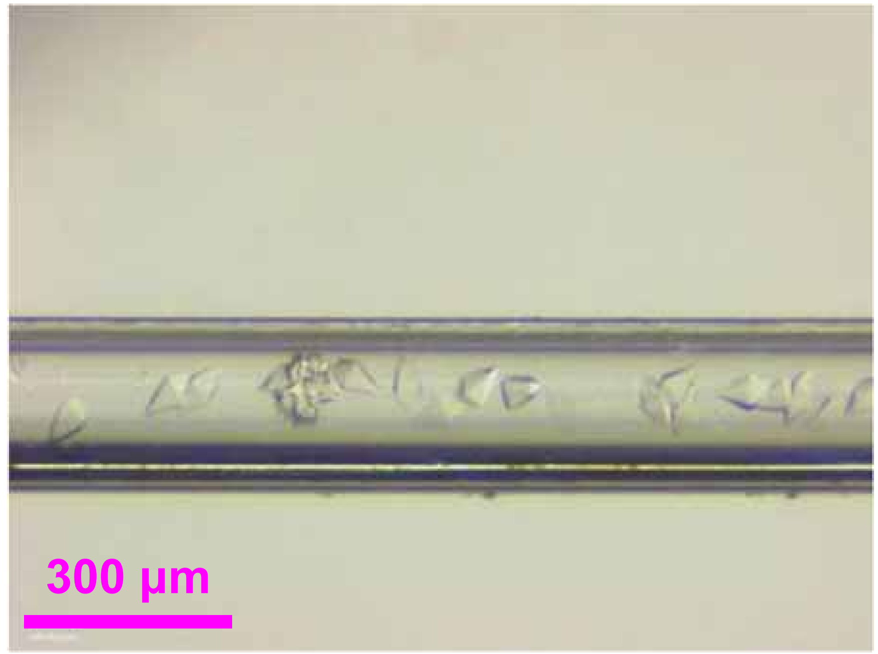
On the other hand,

High pressure cryocooling starts with high-density amorphous phase, ensuring amorphous phase.

Methods for Crystal Hydration

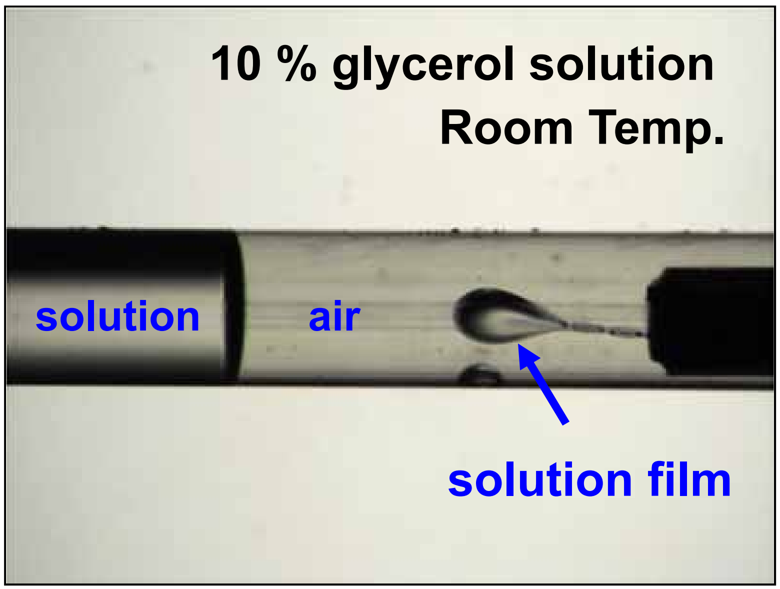
Oil-coating

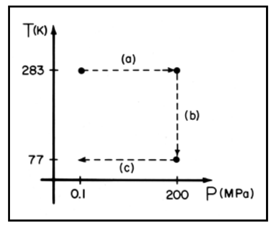
Capillary Hydration



Application for XDM

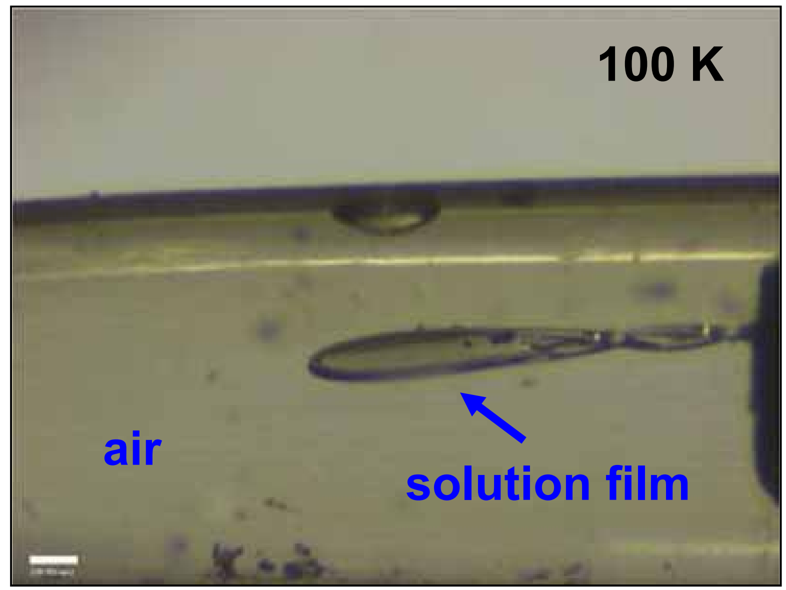
Before





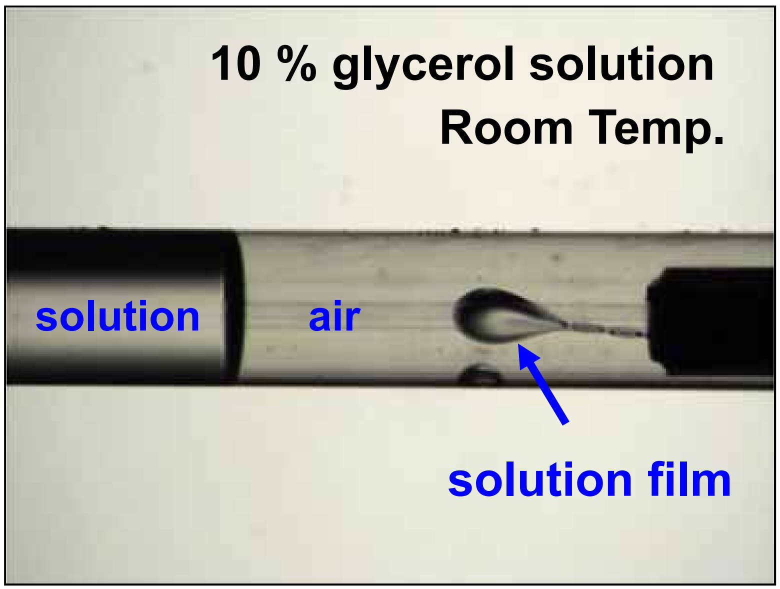
2 kbar

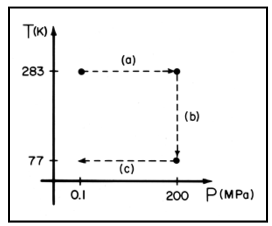
After



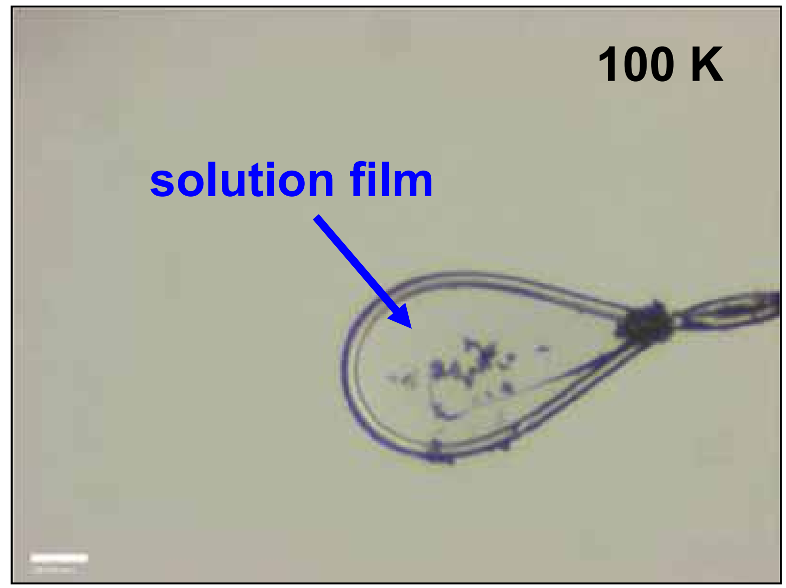
Application for XDM

Before | **After**



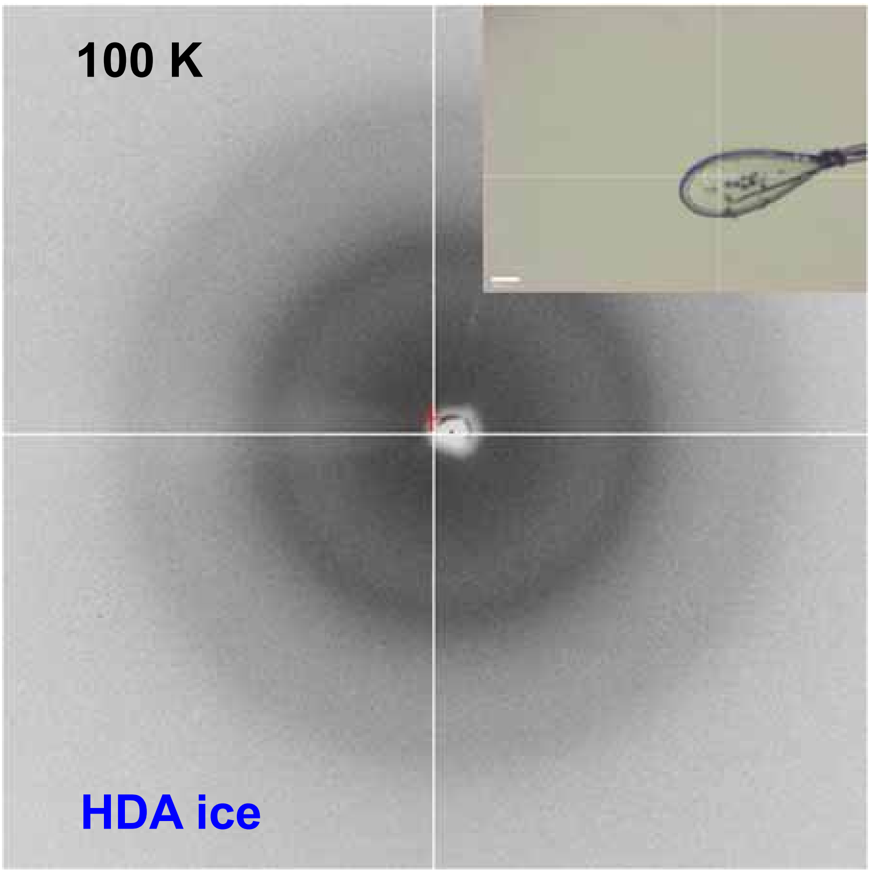


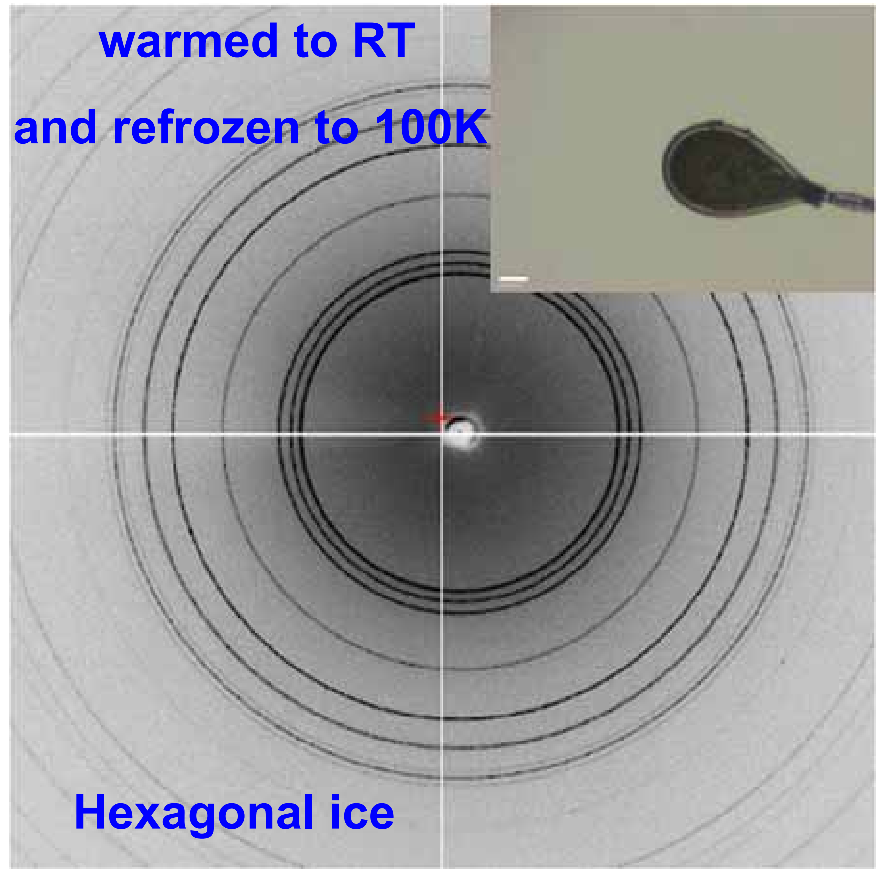
2 kbar



Background scattering from oil and capillary can be removed !!

Application for XDM

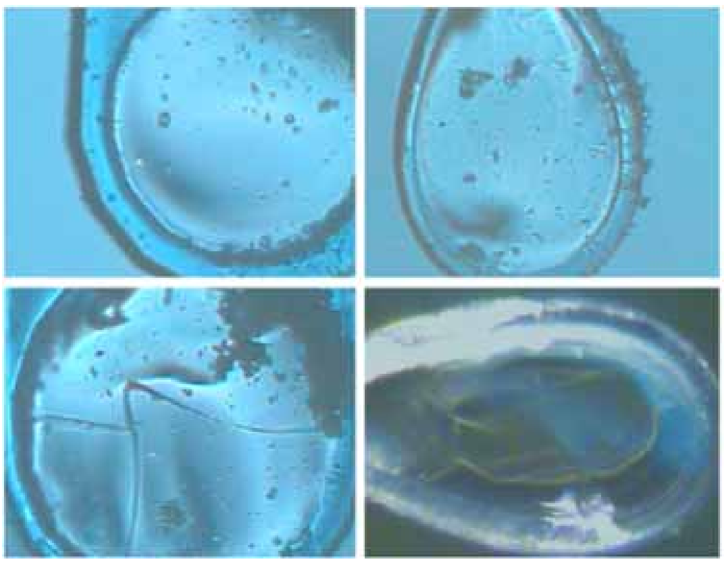




Collaboration w/ Enju Lima (BNL)

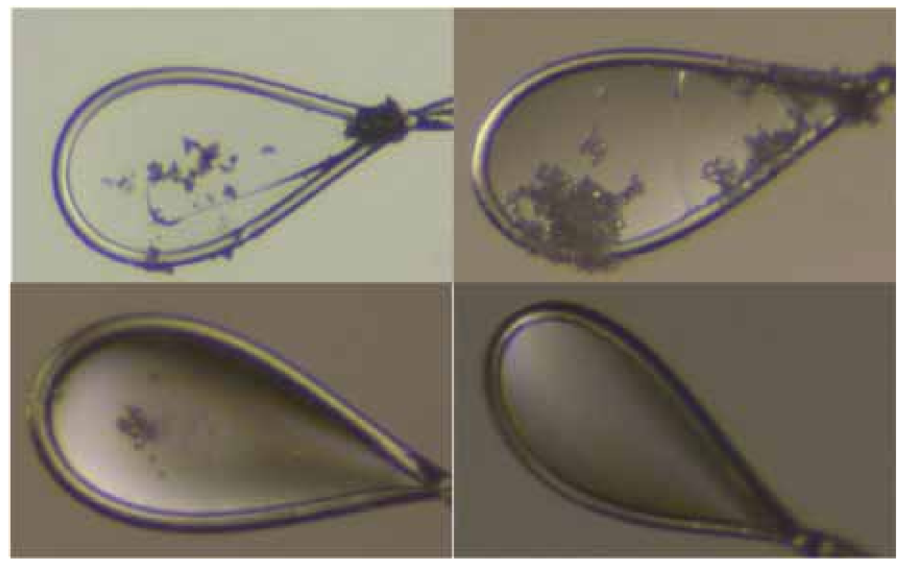
Samples under Optical Microscope

Plunge-freezing at 1bar



Source: Enju Lima (BNL)

High-pressure Cryocooling



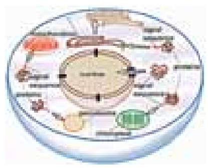
Source: Chae Un Kim (CHESS)

First Trial in ESRF

First Trial in ESRF

Collaboration w/ Enju Lima (BNL)

Data Collection

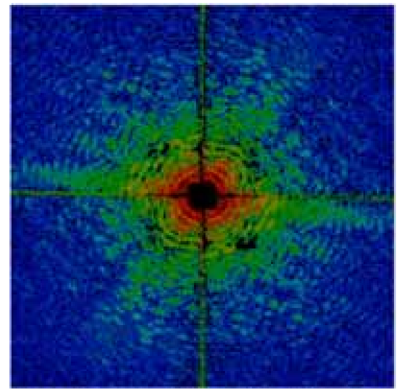


HDA

XDM

→

80 K



$(\rho_{cell} - \rho_{HDA})$

$= (1.35 - 1.17)$

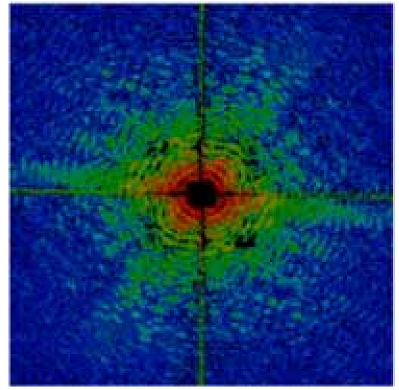
$= 0.18\ g/cm^3$

Data Collection

HDA

XDM

80 K

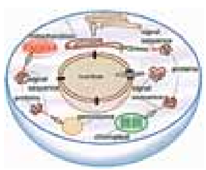


$(\rho_{cell} - \rho_{HDA})$

$= (1.35 - 1.17)$

$= 0.18\ g/cm^3$

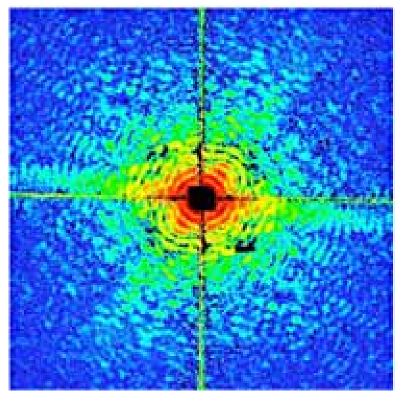
Warming



LDA

XDM

150 K



$(\rho_{cell} - \rho_{LDA})$

$= (1.35 - 0.97)$

$= 0.38\ g/cm^3$

$I_{gain} = (0.38/0.18)^2 = 4.46$!!

Remaining Questions

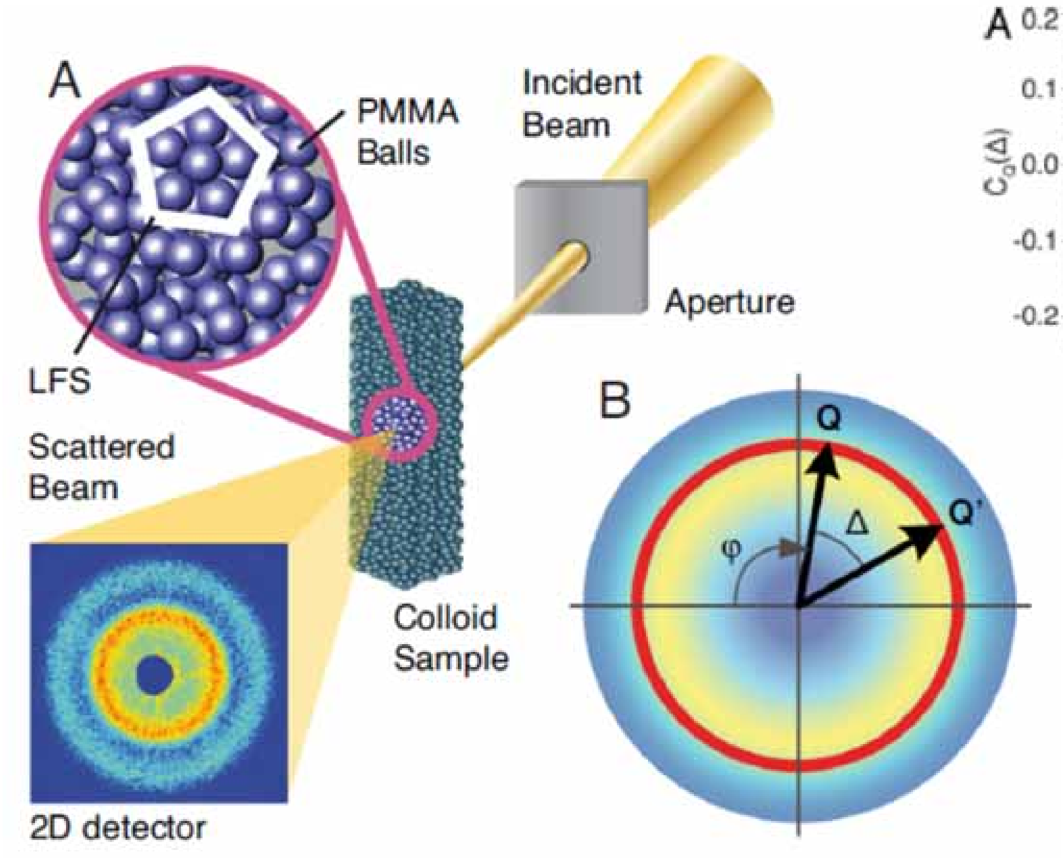
1. **Is high-density amorphous (HDA) ice homogeneous in nm scale ?**

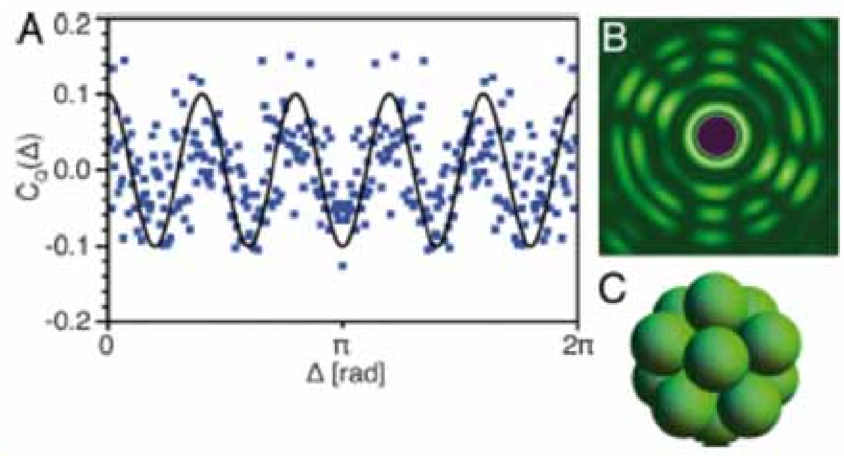
2. **Is low-density amorphous (LDA) ice homogeneous in nm scale ?**

3. **Is LDA ice purely amorphous, not micro-cubic phase ?**

→ Coherent nano X-ray beam would be helpful !!

X-ray Cross Correlation Analysis



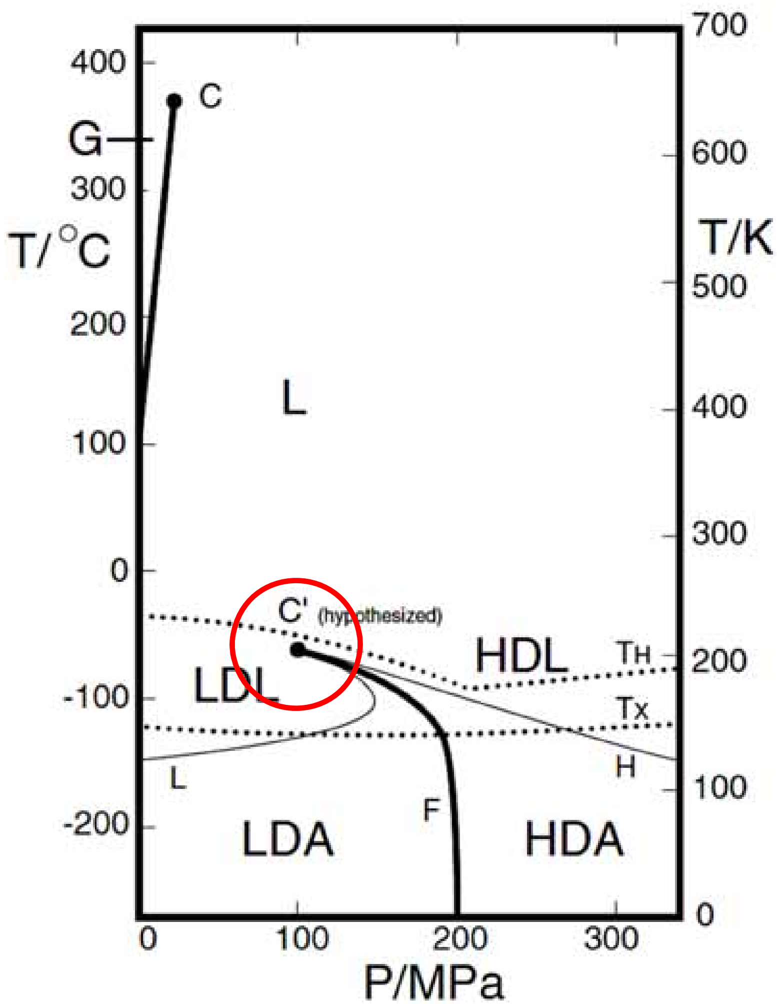


The analysis may reveal the local structure/symmetry of amorphous ice !!

ERL/USR sources would facilitate the study !!

Wochner *et al*. (2009), PNAS **106**, 11511

Water and 2nd critical point



1. **Water shows mysterious thermodynamic properties when supercooled**
2. **2nd critical point was proposed in low T & high P**
3. **2nd critical point involves HDA/LDA and their liquid counter-parts**
4. **No structures of HDA/HDL**

Mishima & Stanley (1998), *Nature* **396**, 329.

Summary

1. Amorphous ice is required for freezing biological samples in hydrated state.

2. High pressure cryocooling has potential for XDM of biological samples.

3. ERL/USR sources would be helpful to study water structures during phase transition.

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PSI

The End