GETTING BETTER DATA DIFFRACTION

On line crystal dehydration

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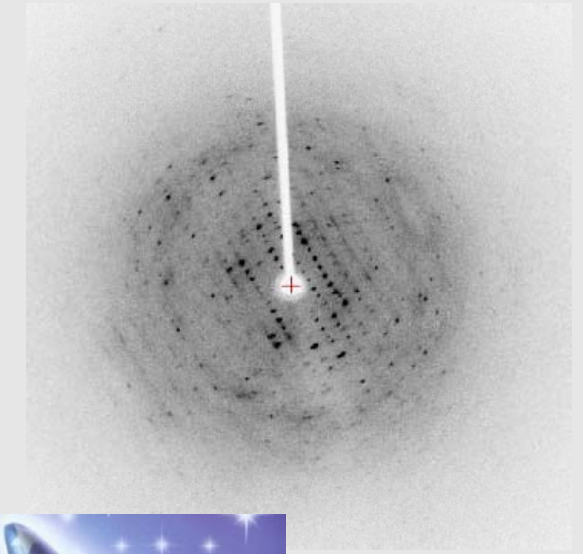
MX School “Getting the most from the ESRF MX Beamlines”
February 2010

EMBL

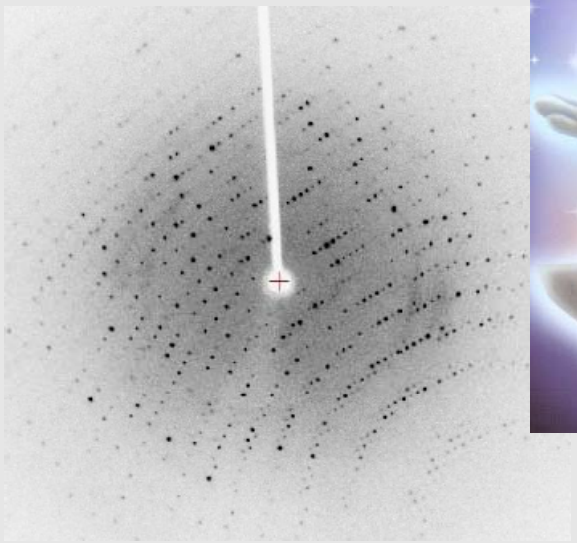
SPINE 2 COMPLEXES

Introduction

How to improve crystal diffraction ?



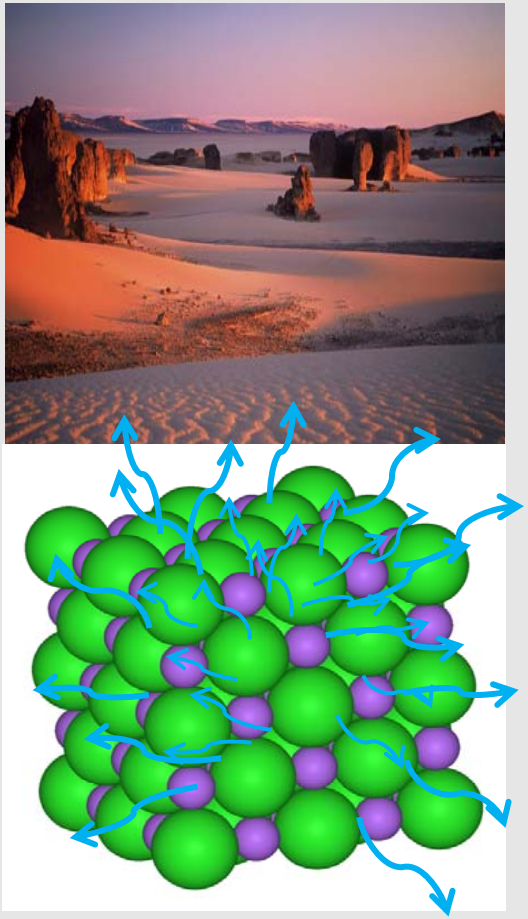
- Soaking with different compounds
- Cross-linking
- Crystal annealing
- Dehydration



Introduction

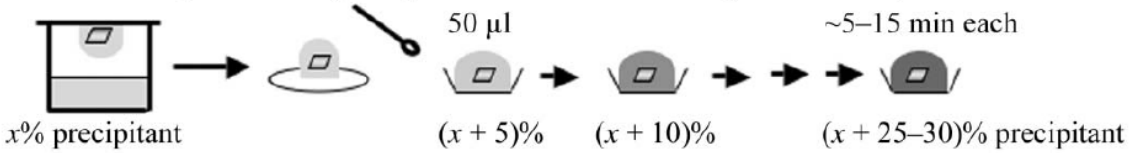
Crystal dehydration

- Improves protein/solvent ratio
- Induces different packing
  (unit cell, space group)
- Improves the internal order of the lattice
  (mosaicity, diffraction power)
- Changes the behavior towards cryo-solutions

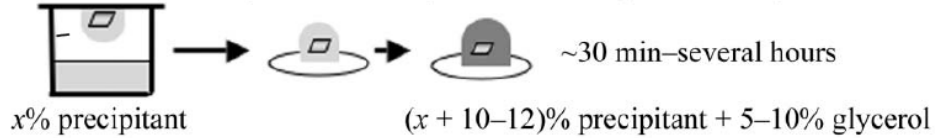


How to dehydrate protein crystals ?

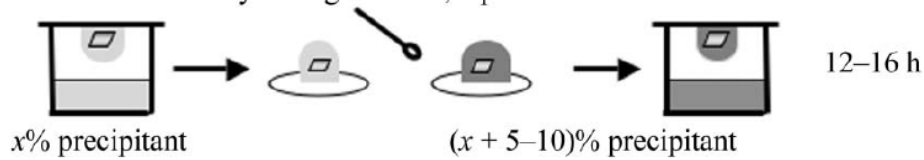
Method 1: Serial transfer to increasing concentration of precipitant; dehydrate either over reservoir or exposed to air (example increments and soaking times shown)



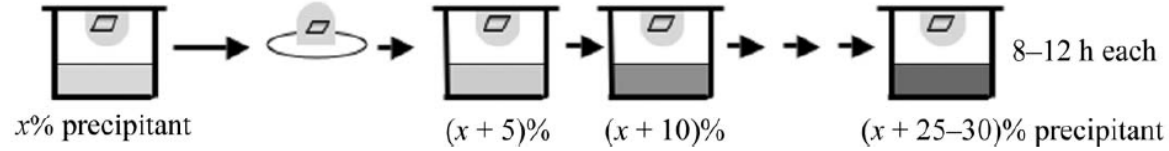
Method 2: Add dehydrating solution to crystallization drop and air dehydration



Method 3: Transfer to dehydrating solution, equilibrate over reservoir



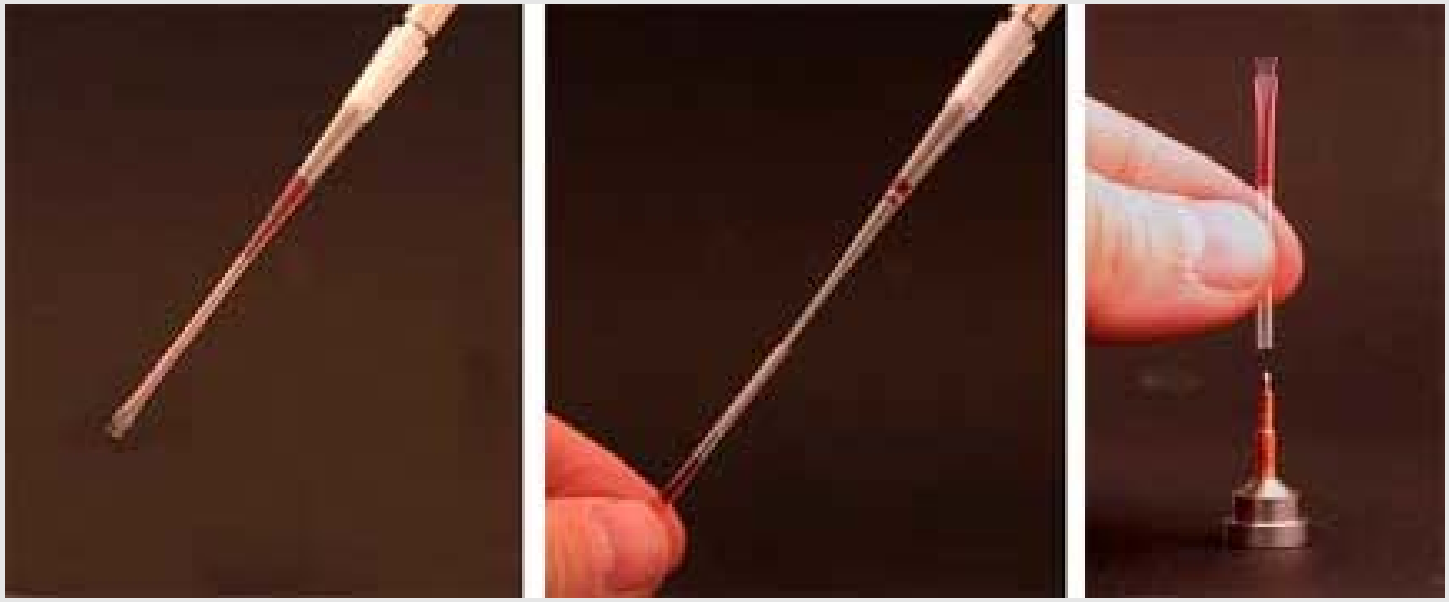
Method 4: Transfer cover slip to reservoirs containing serial increase of dehydrating solution



Heras B., Acta Cryst. D61 (2005) 1173-1180

How to dehydrate protein crystals ?

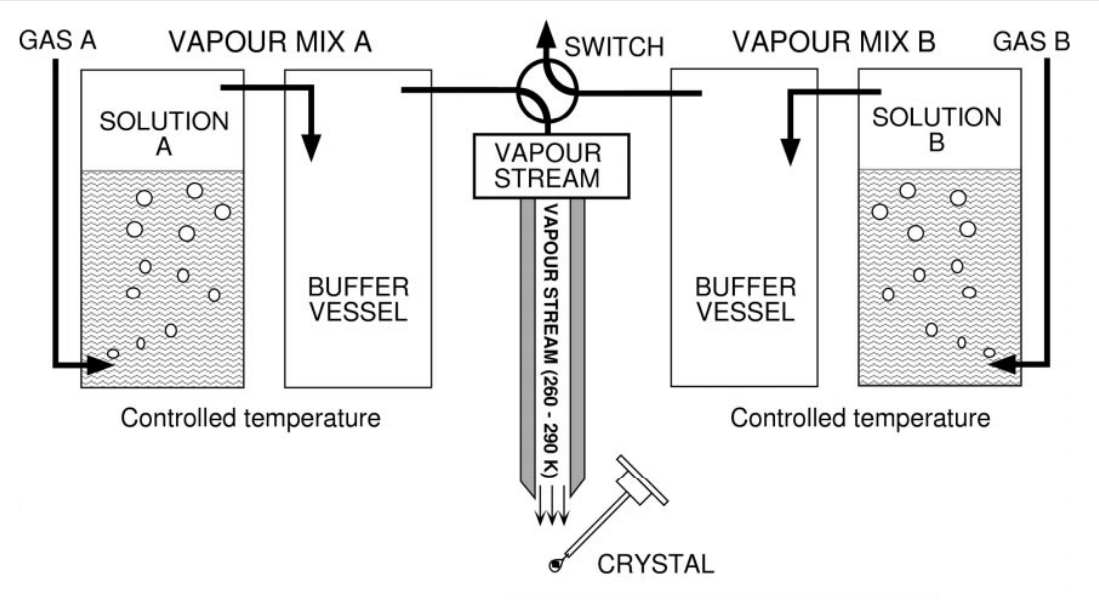
The MiTeGen MicroRT system

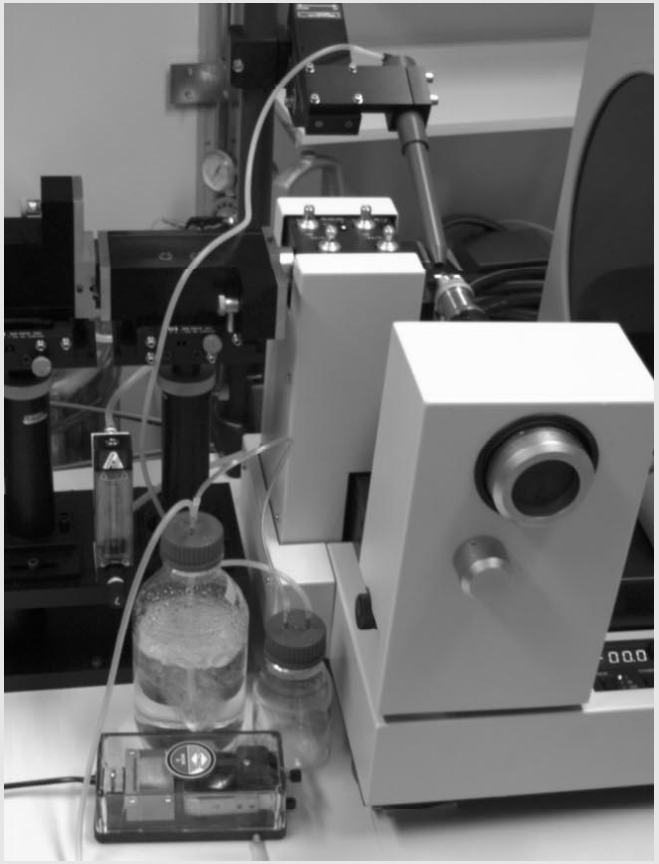


The crystal is equilibrated against a saturated salt solution of known RH during 1-2 hours.

How to dehydrate protein crystals ?

Dehydration using a vapor stream

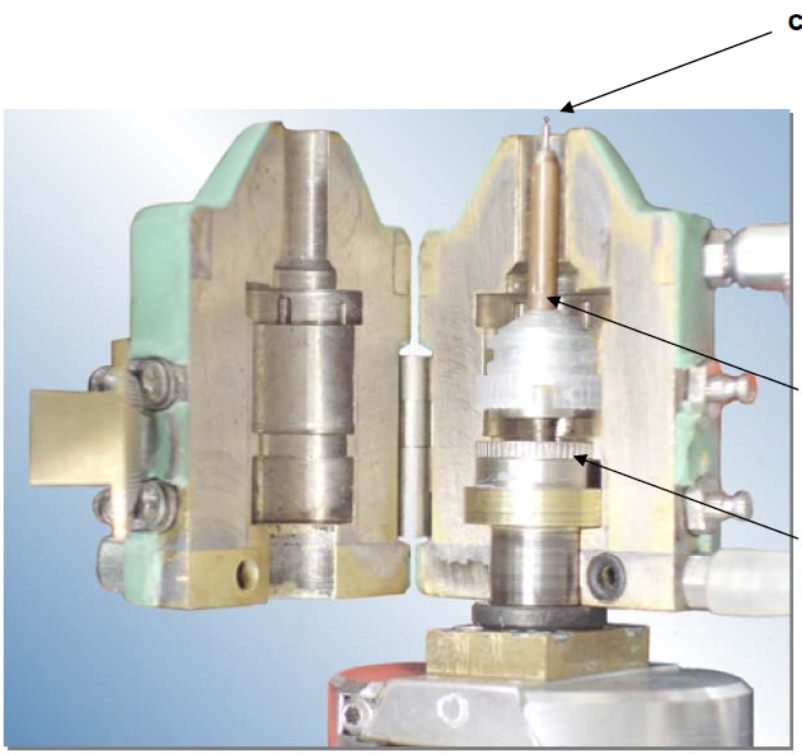


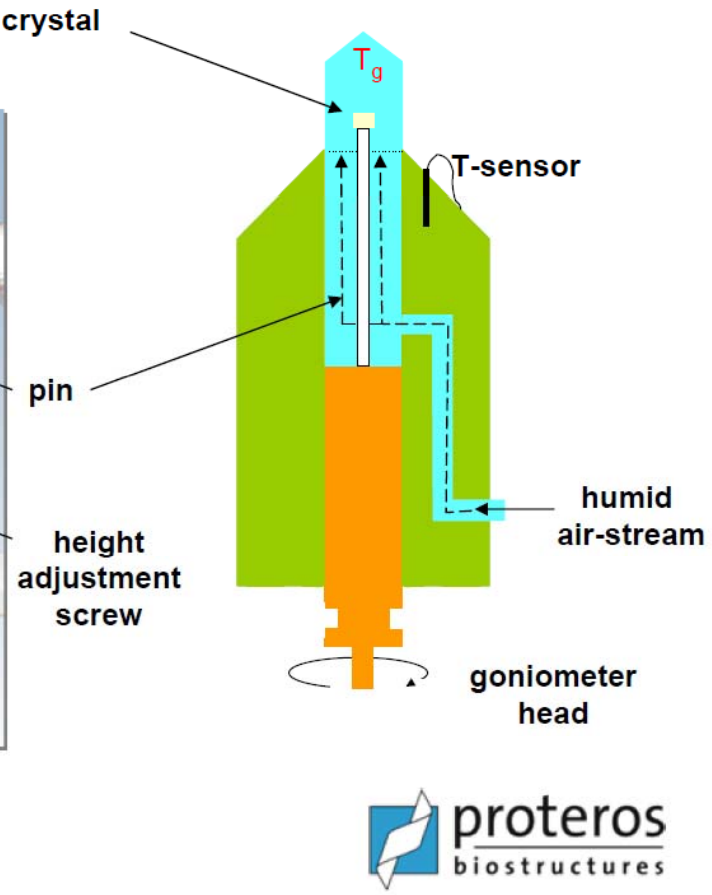


Sjögren T., Appl. Cryst. 35 (2002), 113-116

How to dehydrate protein crystals ?

Free Mounting System





proteros
biostructures

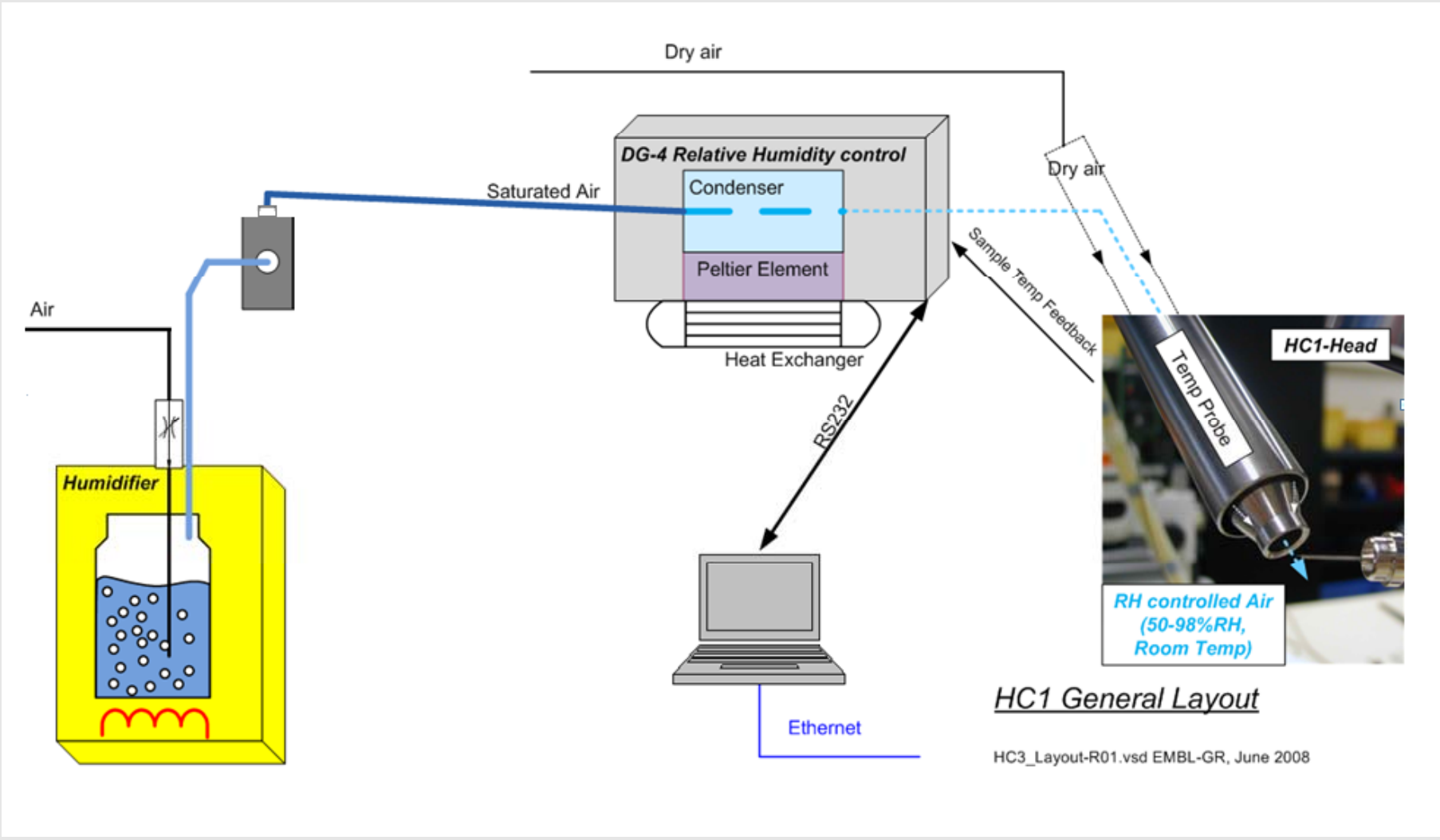
How to dehydrate protein crystals ?

The humidity control device (HC1b)

by the EMBL Diffraction Instrumentation Team

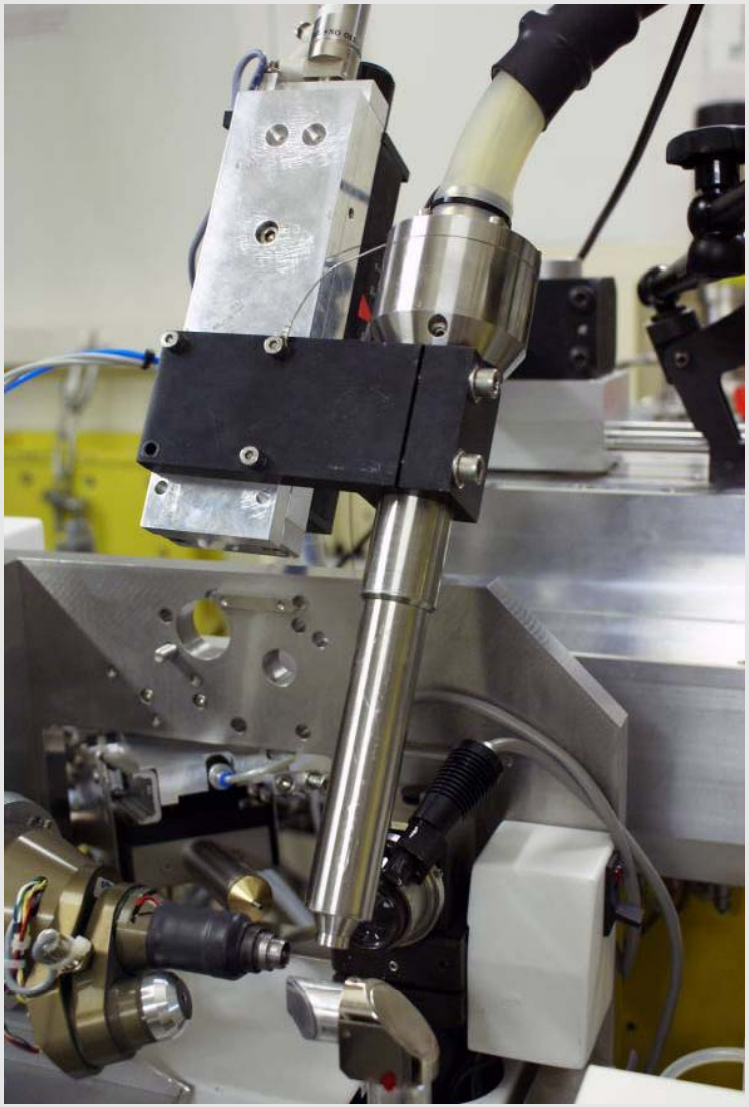
- Designed to be user friendly and completely adapted to MX beamlines
- Based on simpler but robust technology
- With similar, or better, performance to the FMS
- Available to all users of the ESRF

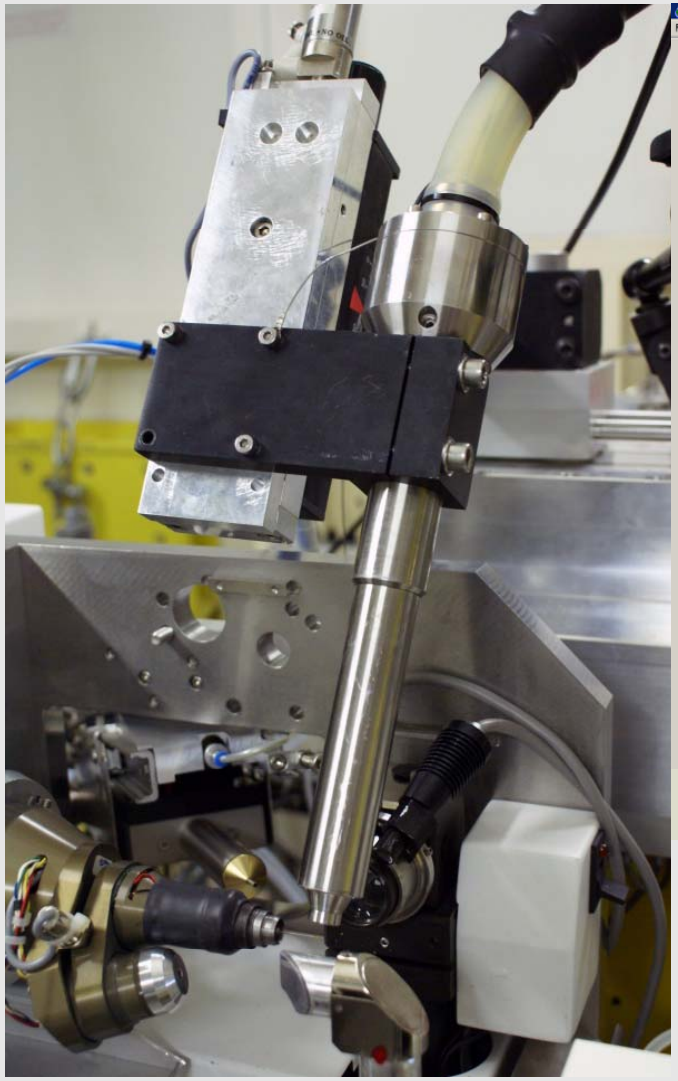
The humidity control device (HC1b)

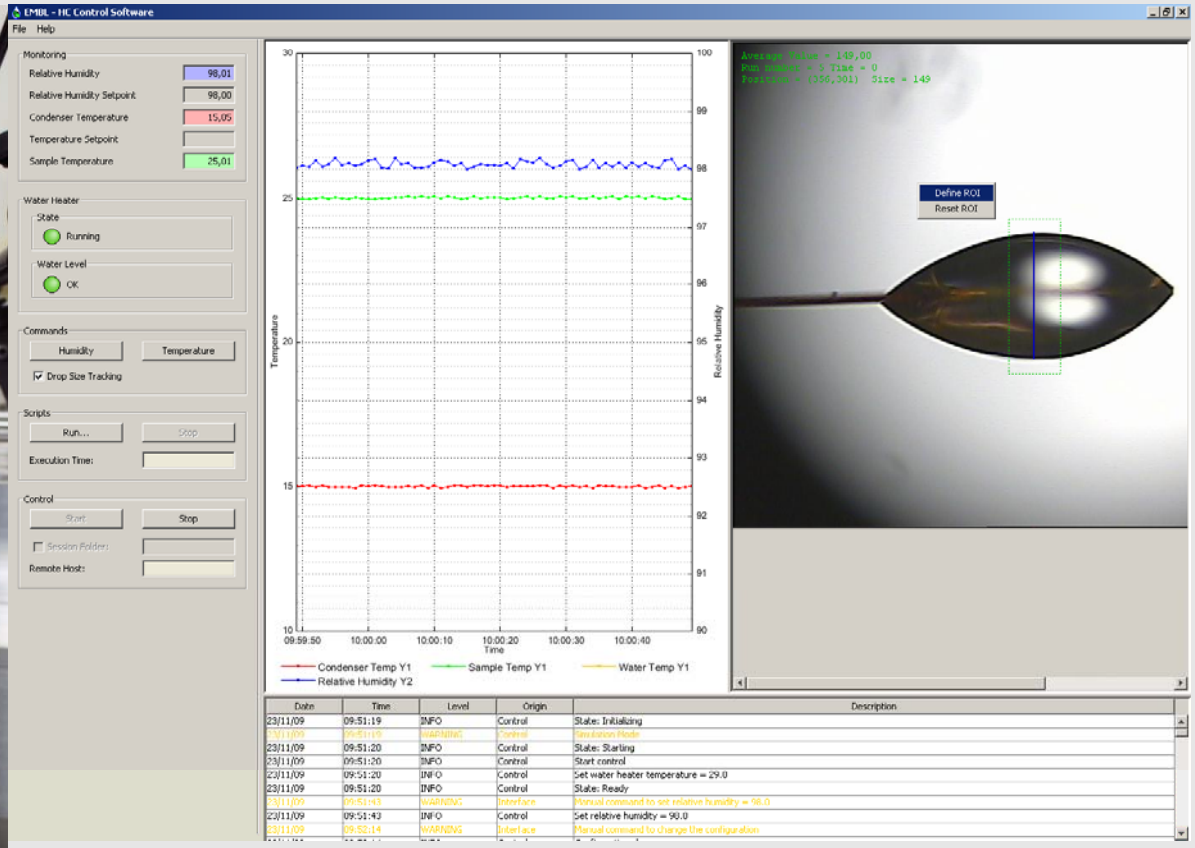


The device on site (BM14, ID14-1 or ID14-2)


NC
AIR PRESSURE
NC
DG4 RS232
POWER SUPPLY







Java/Python Software

Automatic droplet area recognition for optimization

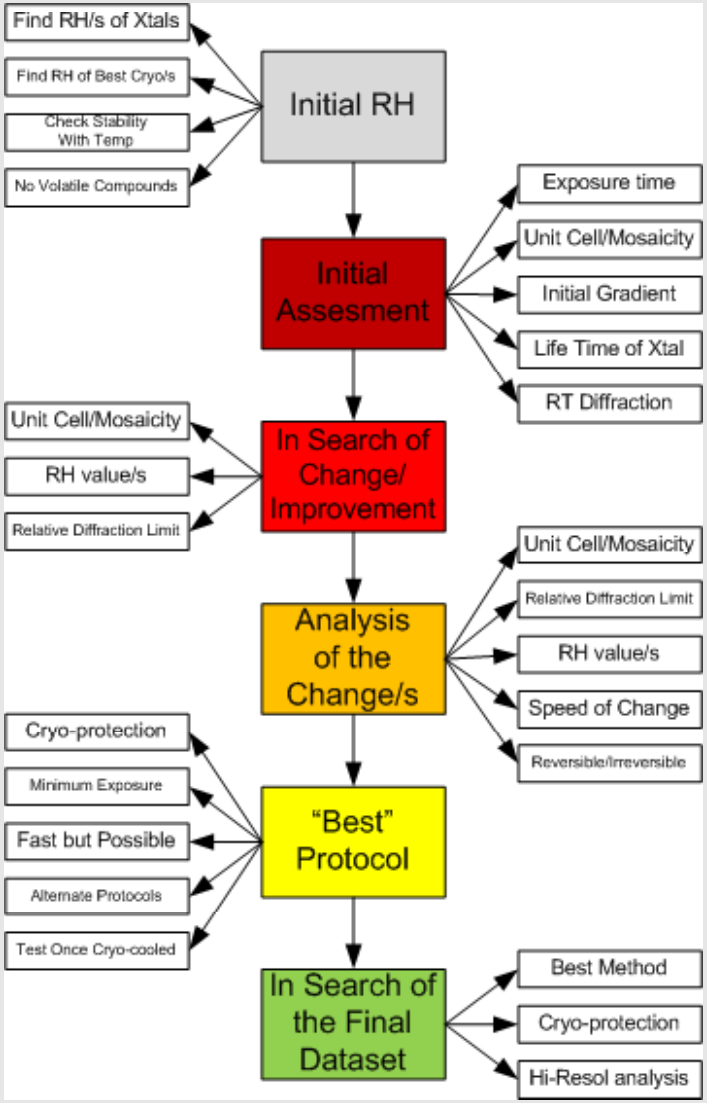
Samples

- The experiments are, for the moment, carried out at RT
- Crystals need to be stable between 20 and 25 °C
- Crystals need to be brought in their plates to the synchrotron or crystallized on site
- The crystallization condition can’t include high amounts of volatile compounds

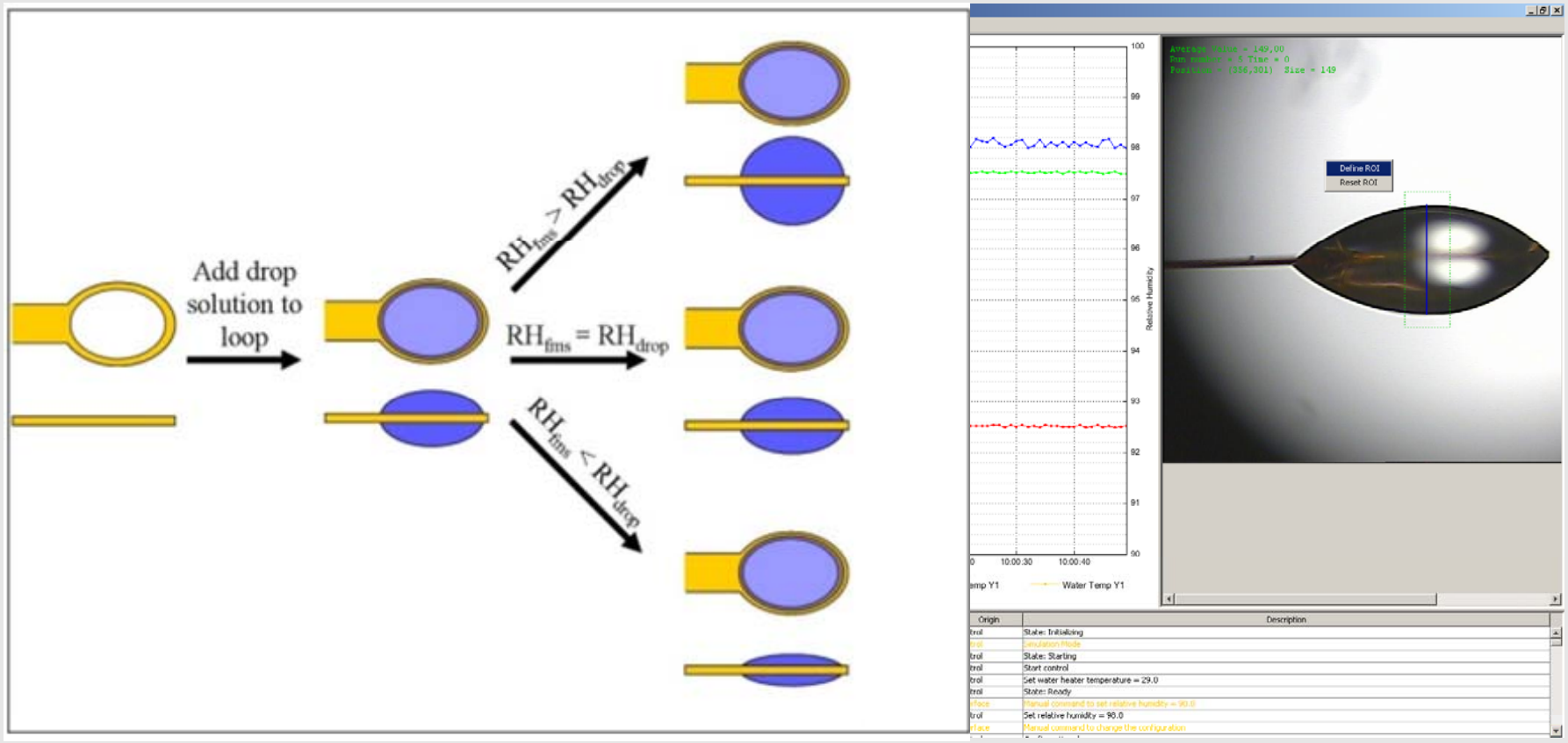
Samples

- Crystals need to have enough scattering power to be able to index the image/s
- Exposure time and flux need to be minimized to prevent radiation damage
- These experiments are time and crystal consuming so that a good stock of crystals is necessary

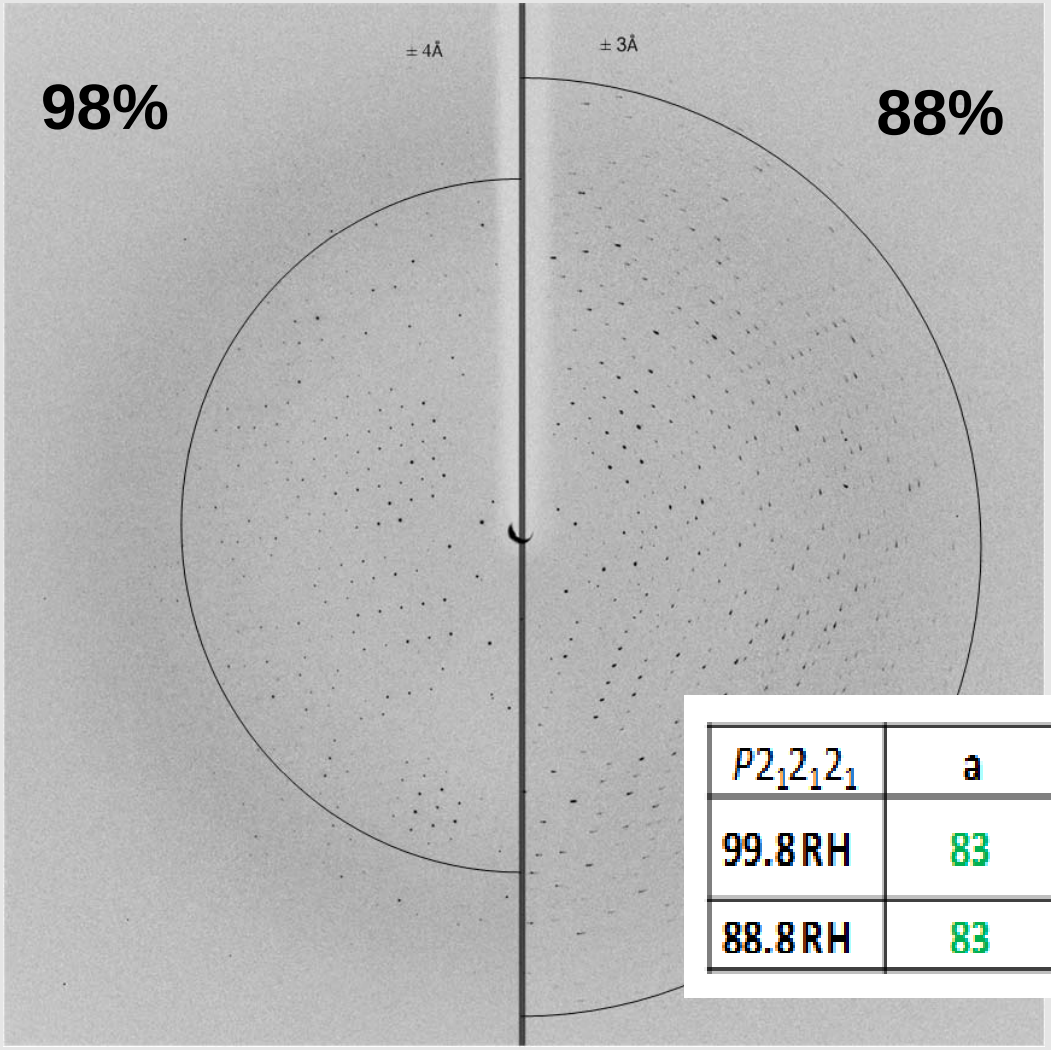
The dehydration experiment workflow



Determining the initial RH value

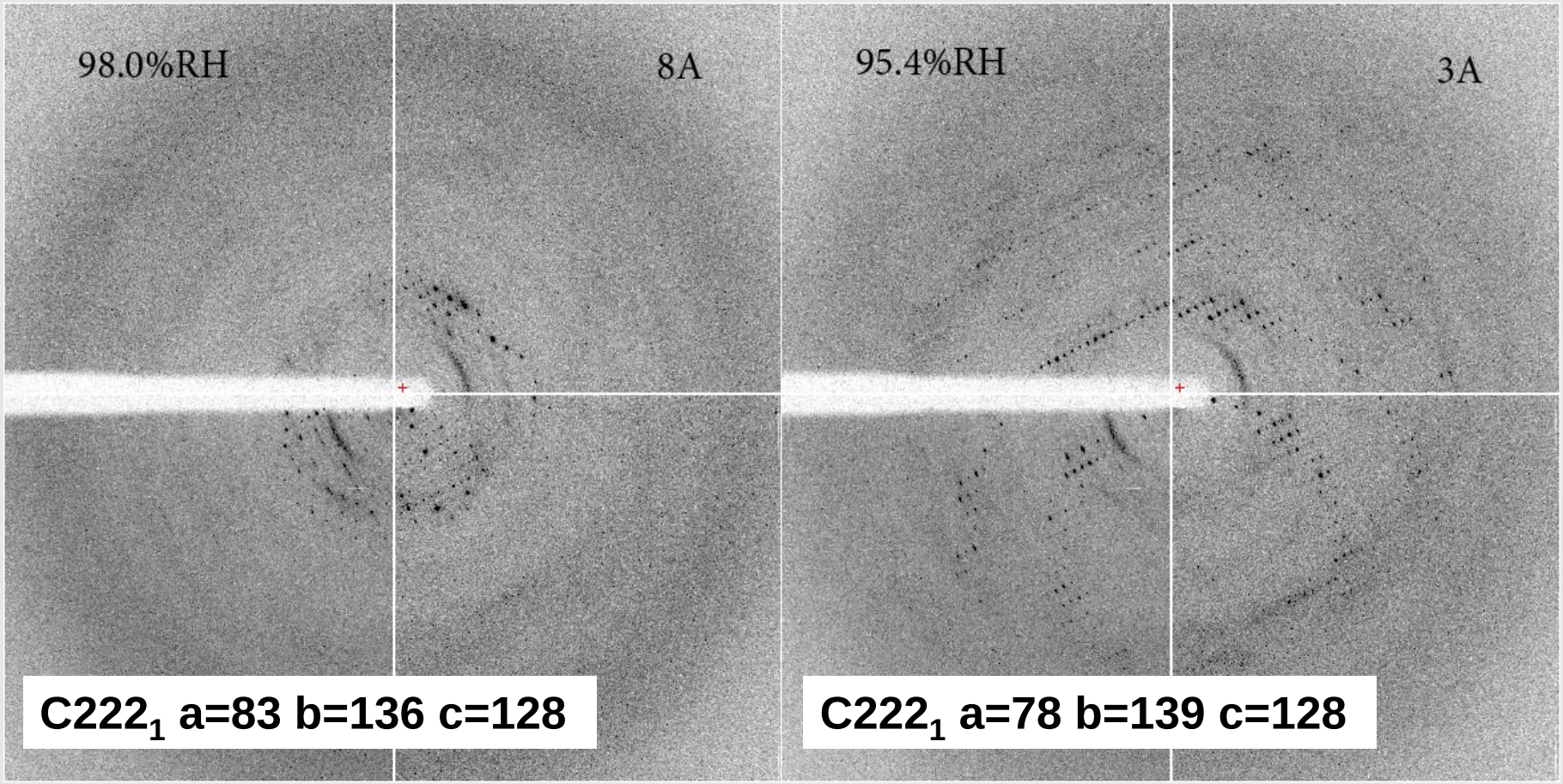


“Real life” cases ....

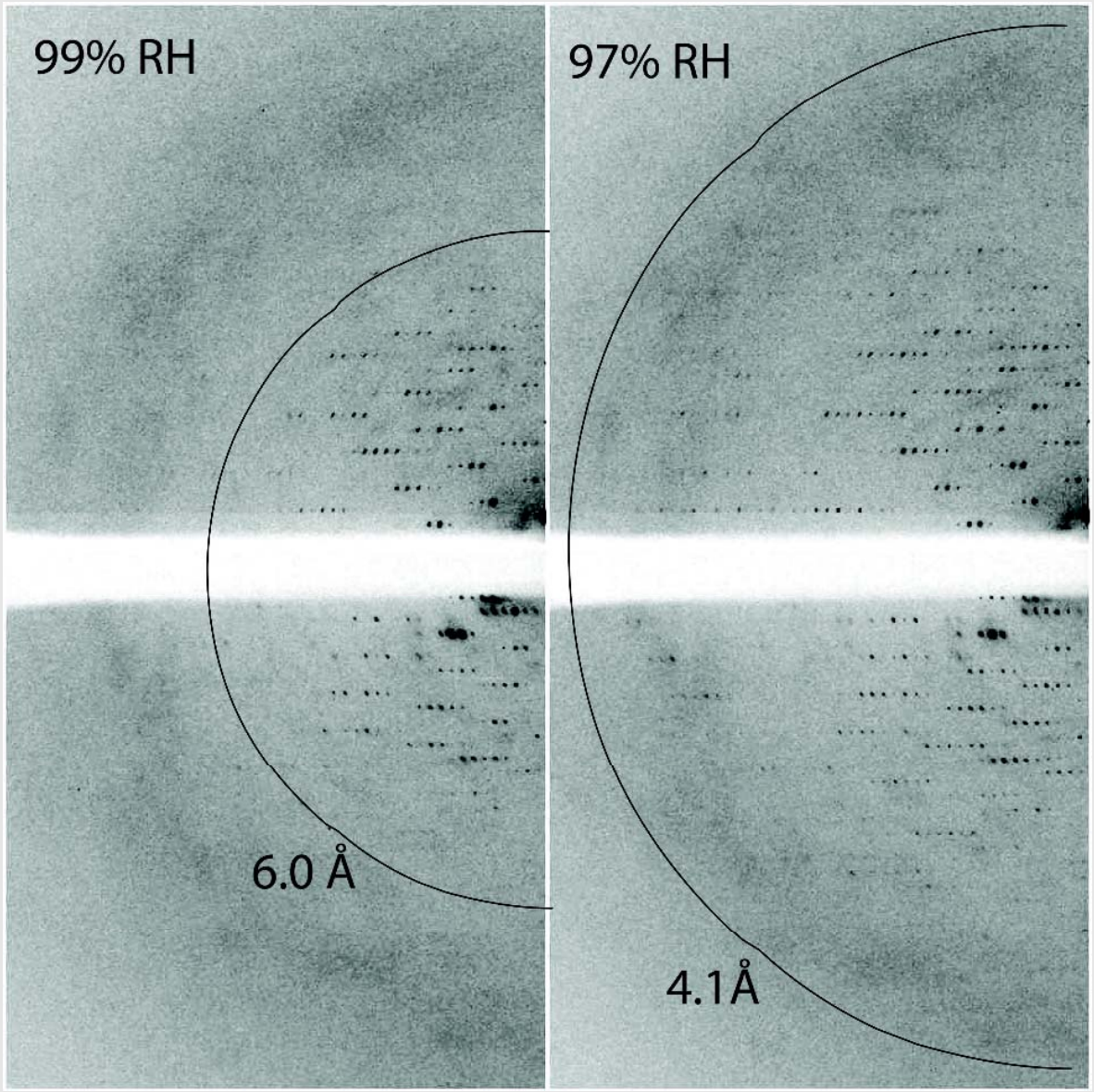


DNA polymerase III

$P2_12_12_1$	a	b	c	Mos.	Res
99.8 RH	83	99	144		
88.8 RH	83	94	131	↓	↑



Chromatin-modification complex

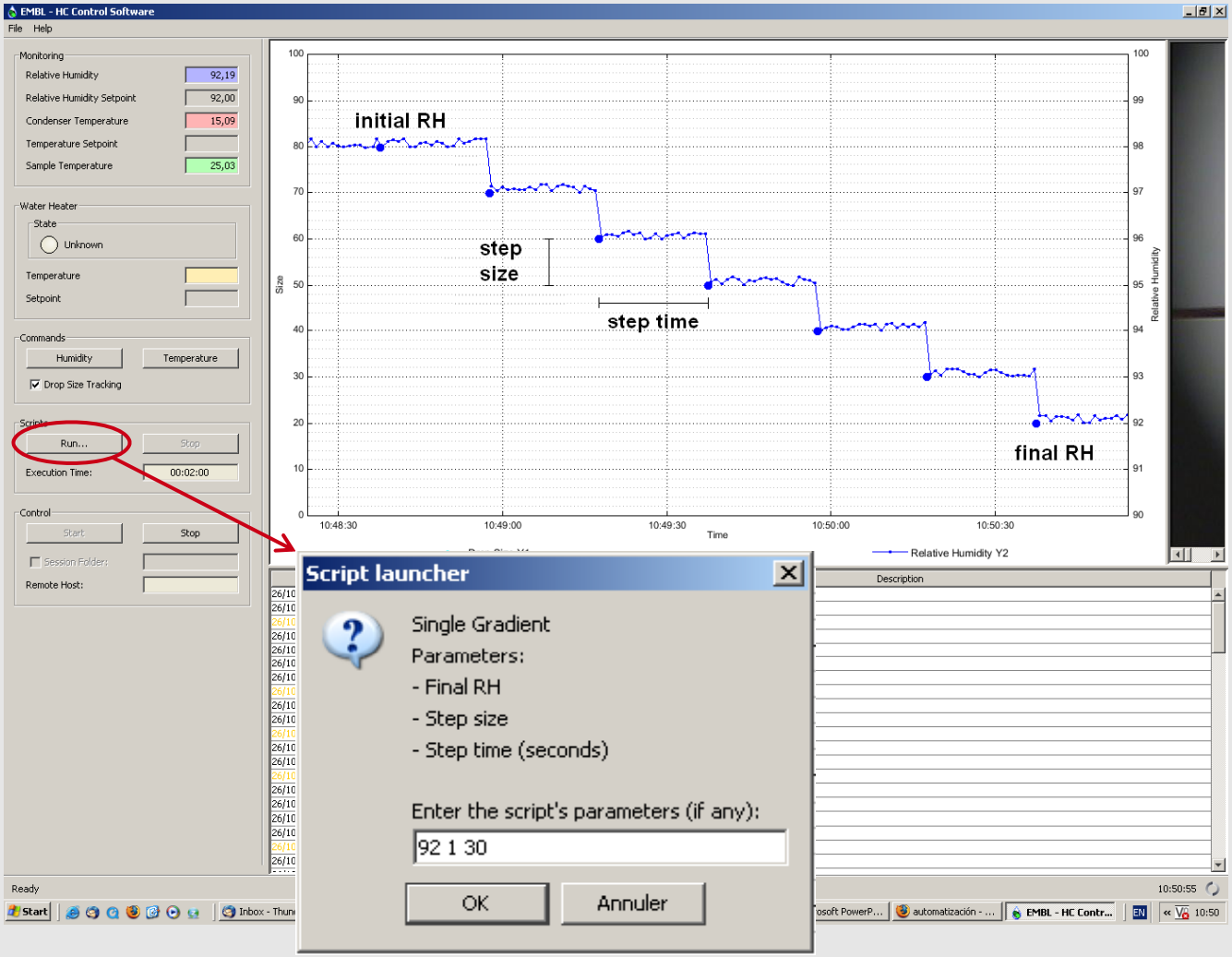


Photosystem I

Multiprotein complex
~ 500 kDa


What's next ?

HC1b Automation



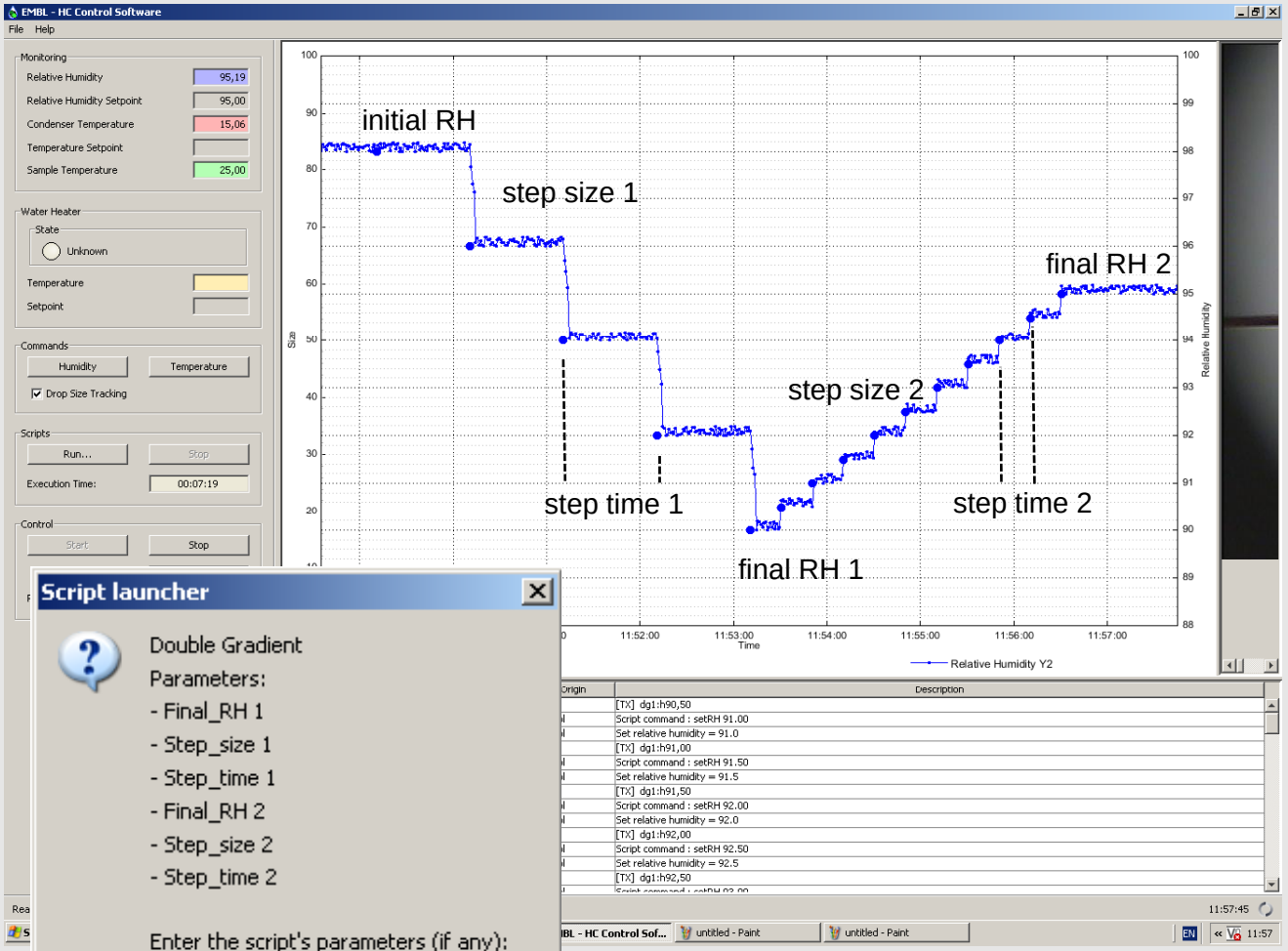
Prebuilt scripts

Flexible single gradient

Variables:

- final RH value
- step size
- step time

HC1b Automation



Prebuilt scripts

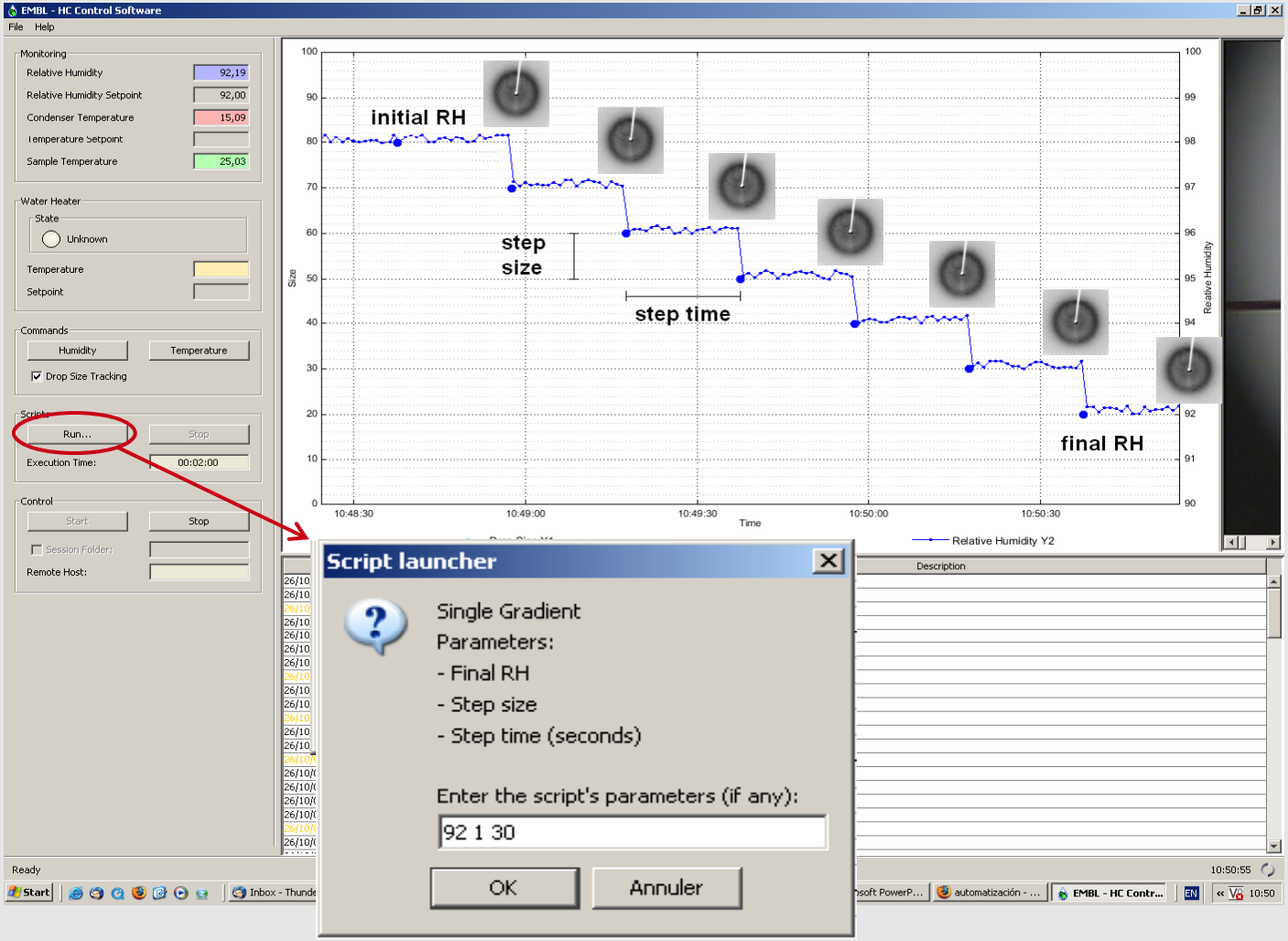
Flexible double gradient

Variables:

- final RH 1
- step size 1
- step time 1

- final RH 2
- step size 2
- step time 2

HC1b Automation



HC1b Survey

HC1b HUMIDITY CONTROL DEVICE SURVEY

BEAMLINE: ☐ USER ID: ☐ DATE : ☐

E-MAIL ADDRESS: ☐ INSTITUTE: ☐

USUAL CRYSTAL PARAMETERS AT CRYO TEMPERATURE

SAMPLE NAME/ACRONYM		MOLECULAR WEIGHT (kDa)		
PROTEIN TYPE	☐ SOLUBLE	☐ MEMBRANE-BOUND		
SAMPLE TYPE:	☐ DNA/RNA	☐ PROTEIN	☐ PROTEIN-DNA/RNA	☐ PROTEIN-PROTEIN
CRYSTALLISATION CONDITIONS				
USUAL CRYO PROTECTANTS				

The HC1b Partners

SPINE2COMPLEXES

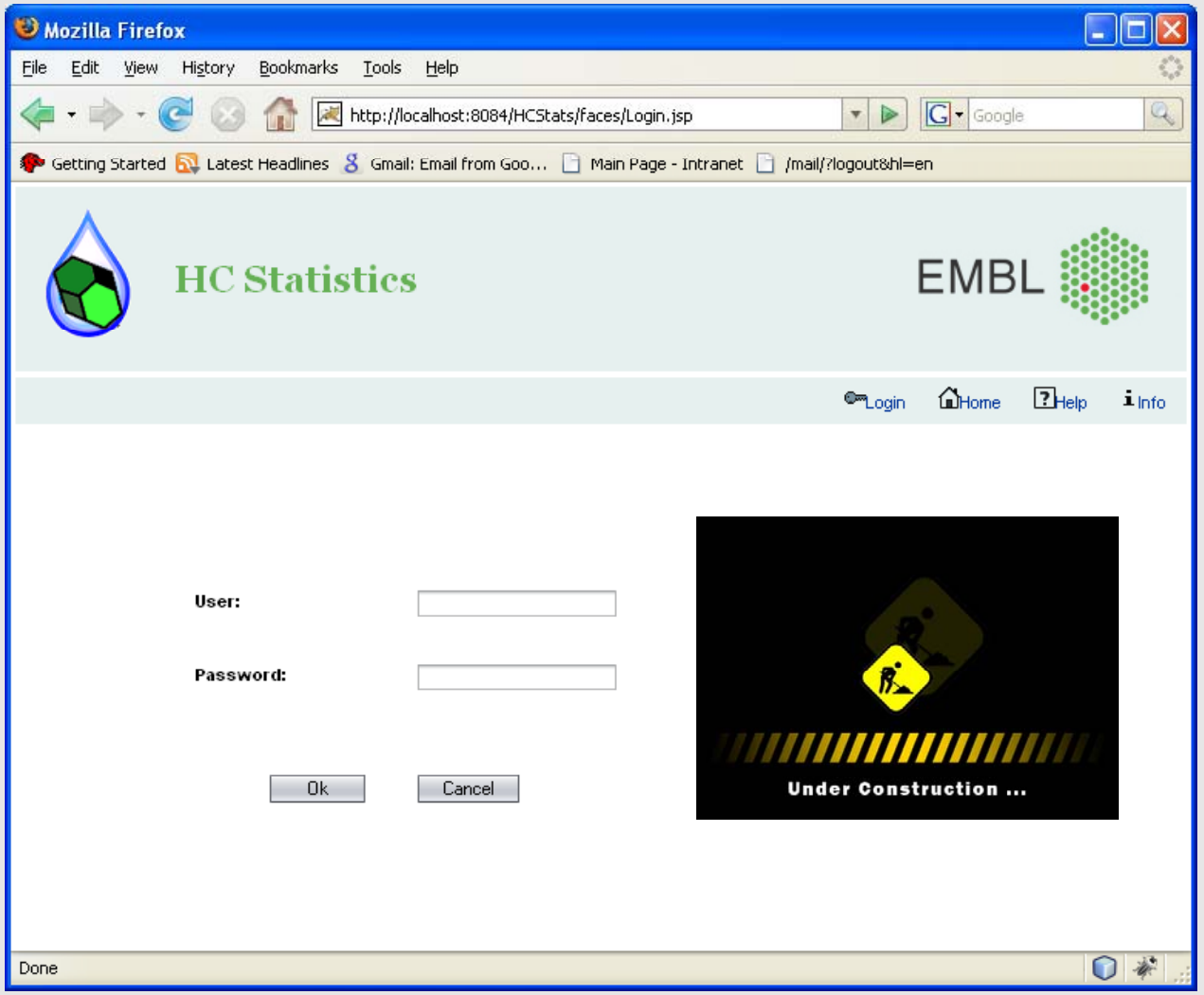
diamond

ESRF

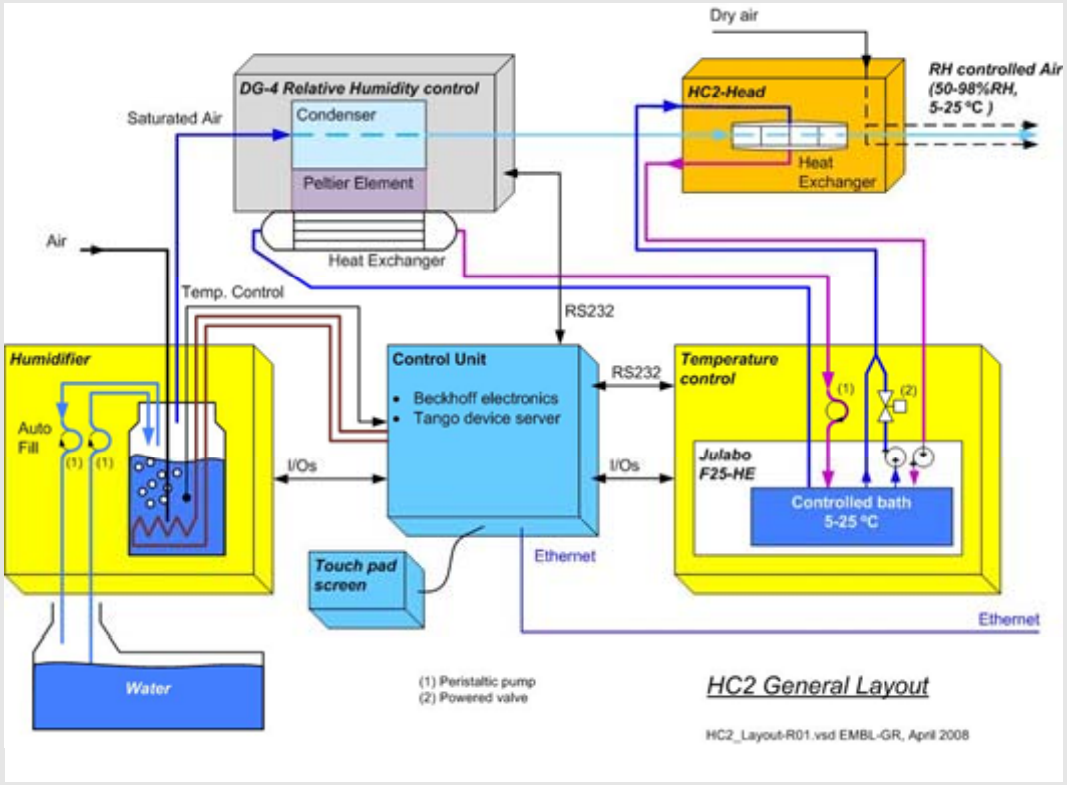
EMBL Grenoble

MAX-lab

HC1b web server



Next generation HC



Sample T control between 4°C and room temperature

Aknowledgements

EMBL Grenoble

Diffraction Instrumentation Team

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