Getting better diffraction

On-line crystal dehydration

MX-School Feb/09

Juan Sanchez-Weatherby

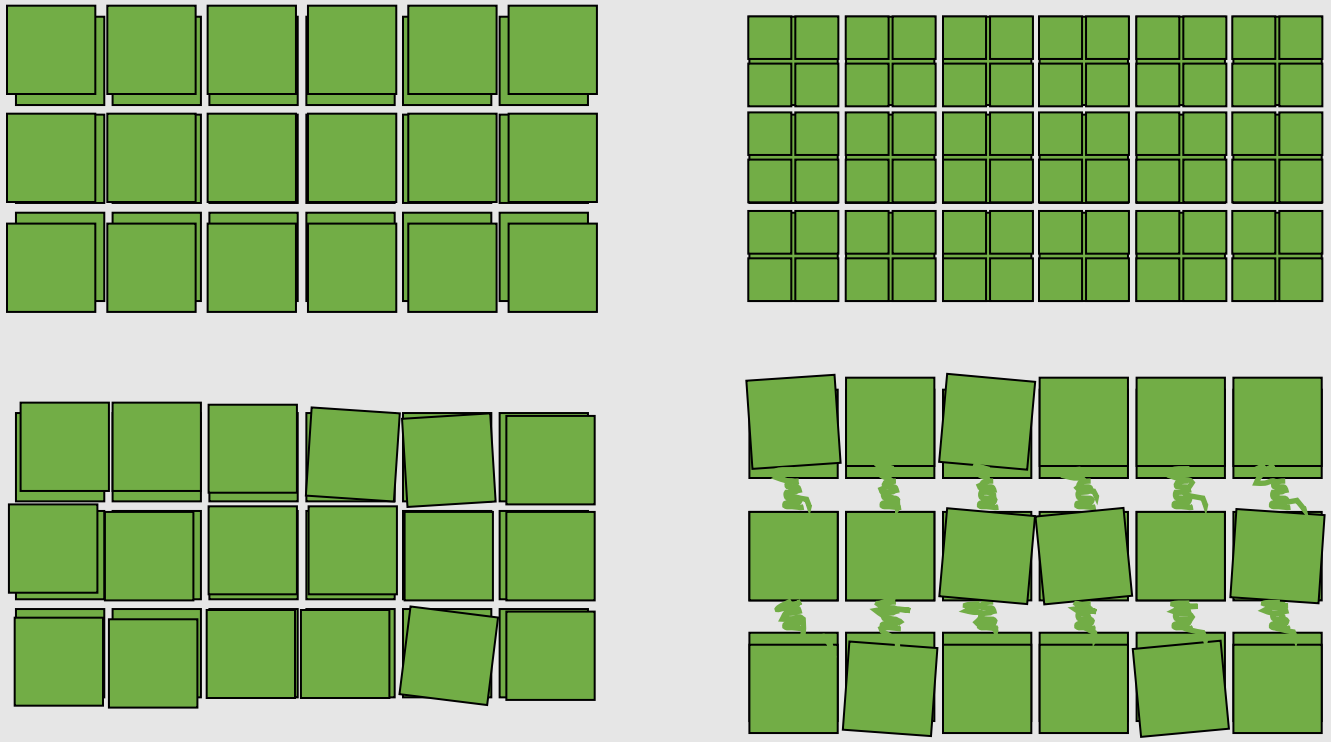
EMBL

SPINE 2 COMPLEXES

Introduction

1. Crystal dehydration is a possible method for improving crystal diffraction.

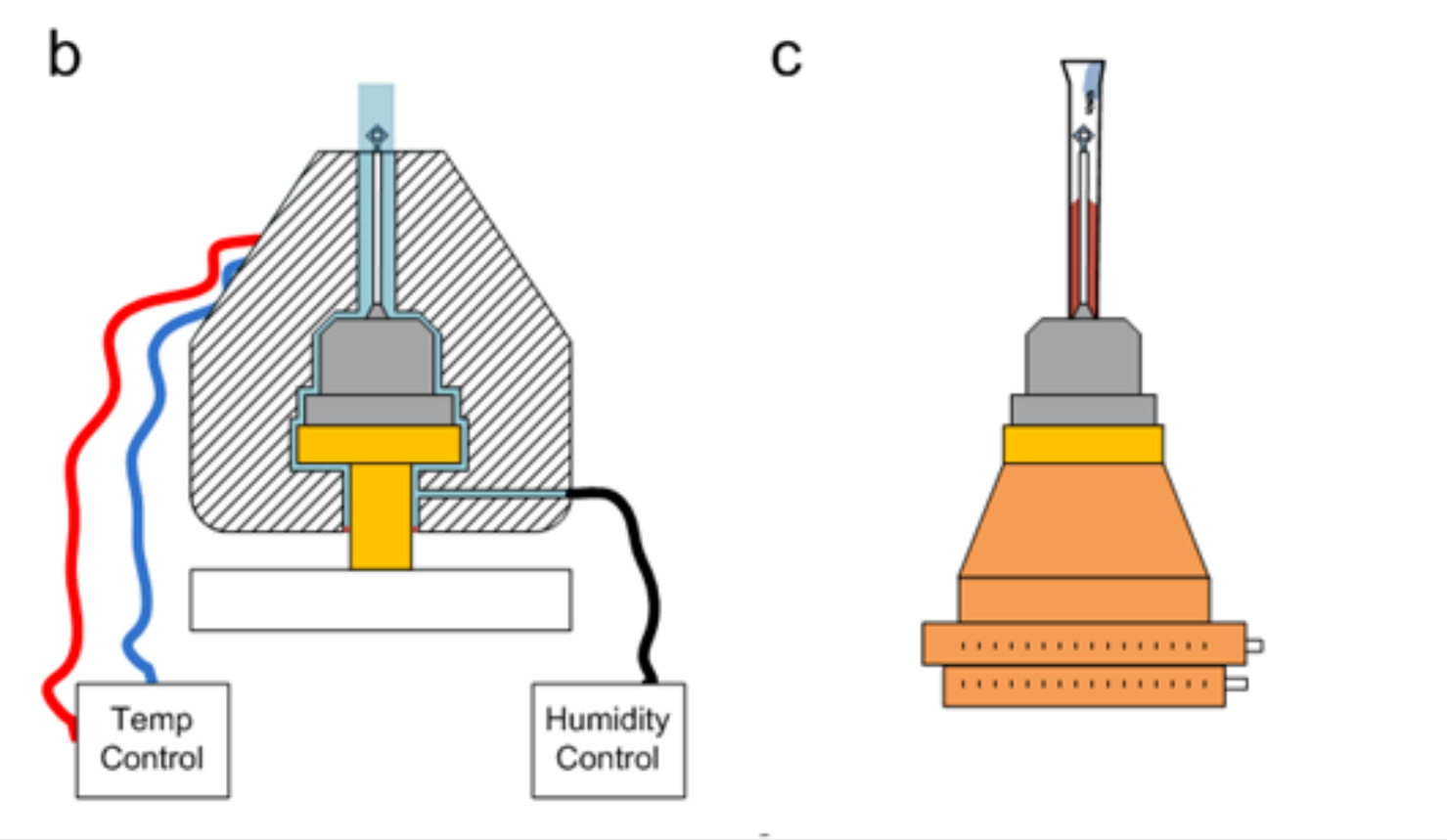
- Improving protein/solvent ratio
- Inducing different packing (SG, UC, …)
- Improving the internal order of the lattice (mosaicity, diff power)
- Changing the behavior towards cryo-solutions



Introduction

2. There are several methods for dehydration

- Crystal soak
- Vapor diffusion
- Annealing



Introduction

3. The MiTeGen MicroRT system
- Vapor diffusion
- Capillary
- Non standard mount

4. Proteros FMS system
- Controled airstream
- Free Mounting
- Bulky

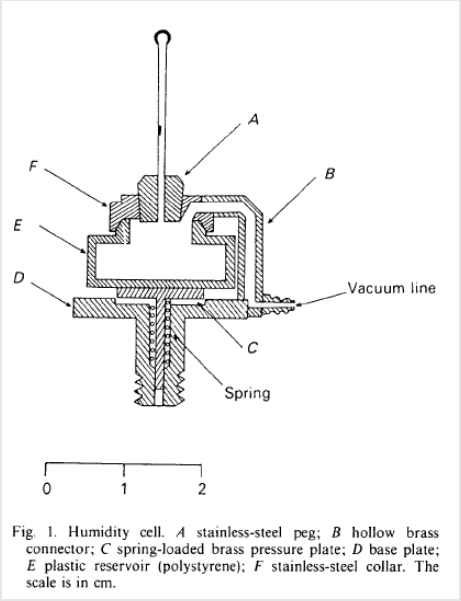
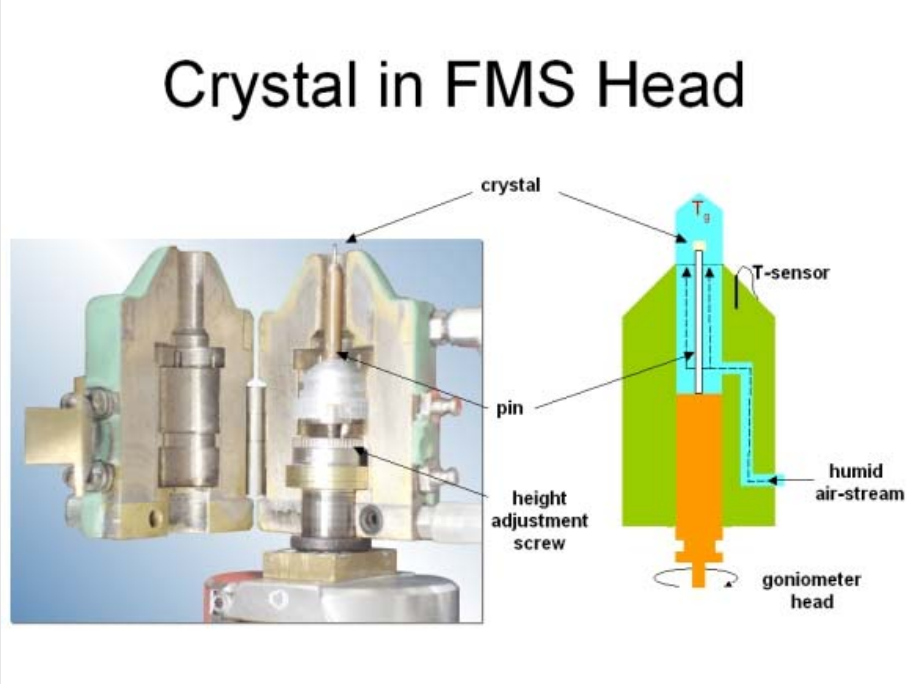
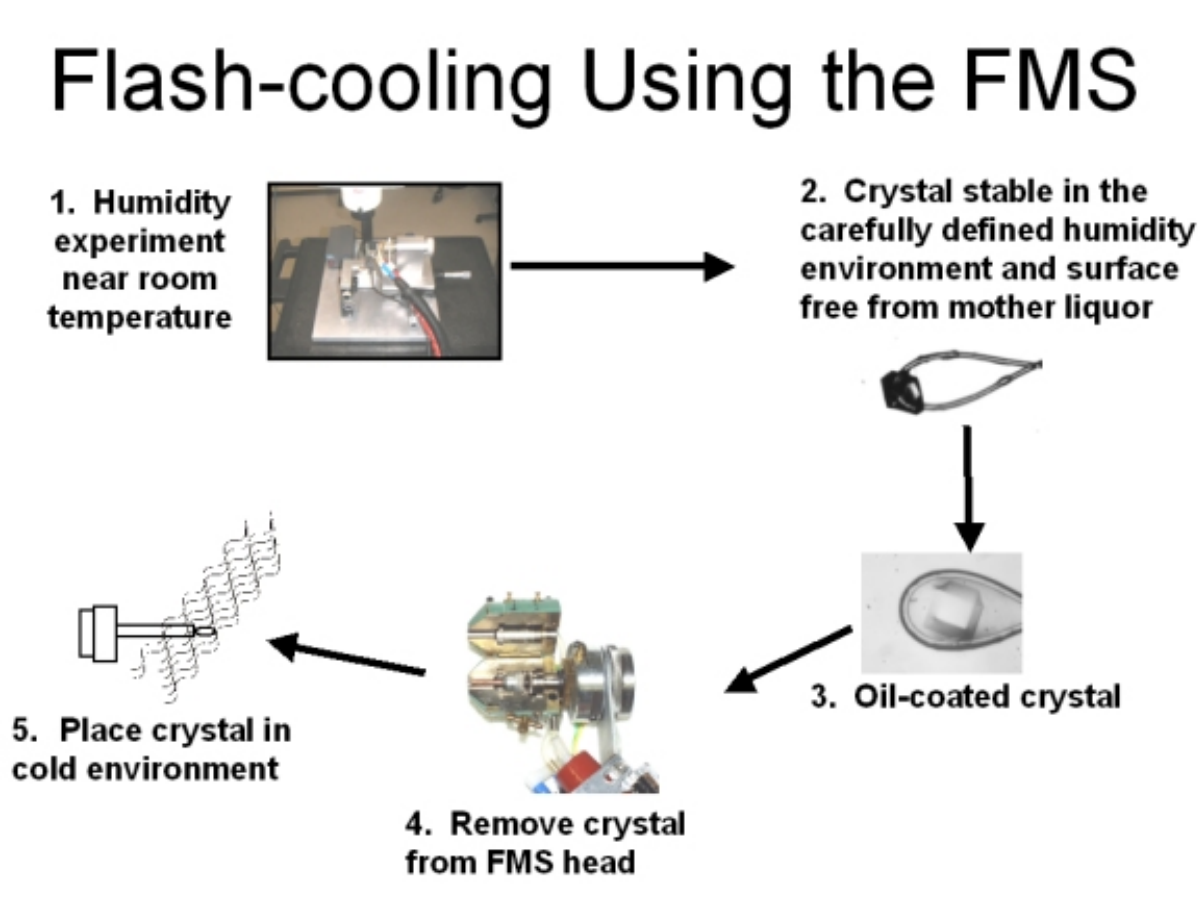


Fig. 1. Humidity cell. *A* stainless-steel peg; *B* hollow brass connector; *C* spring-loaded brass pressure plate; *D* base plate; *E* plastic reservoir (polystyrene); *F* stainless-steel collar. The scale is in cm.



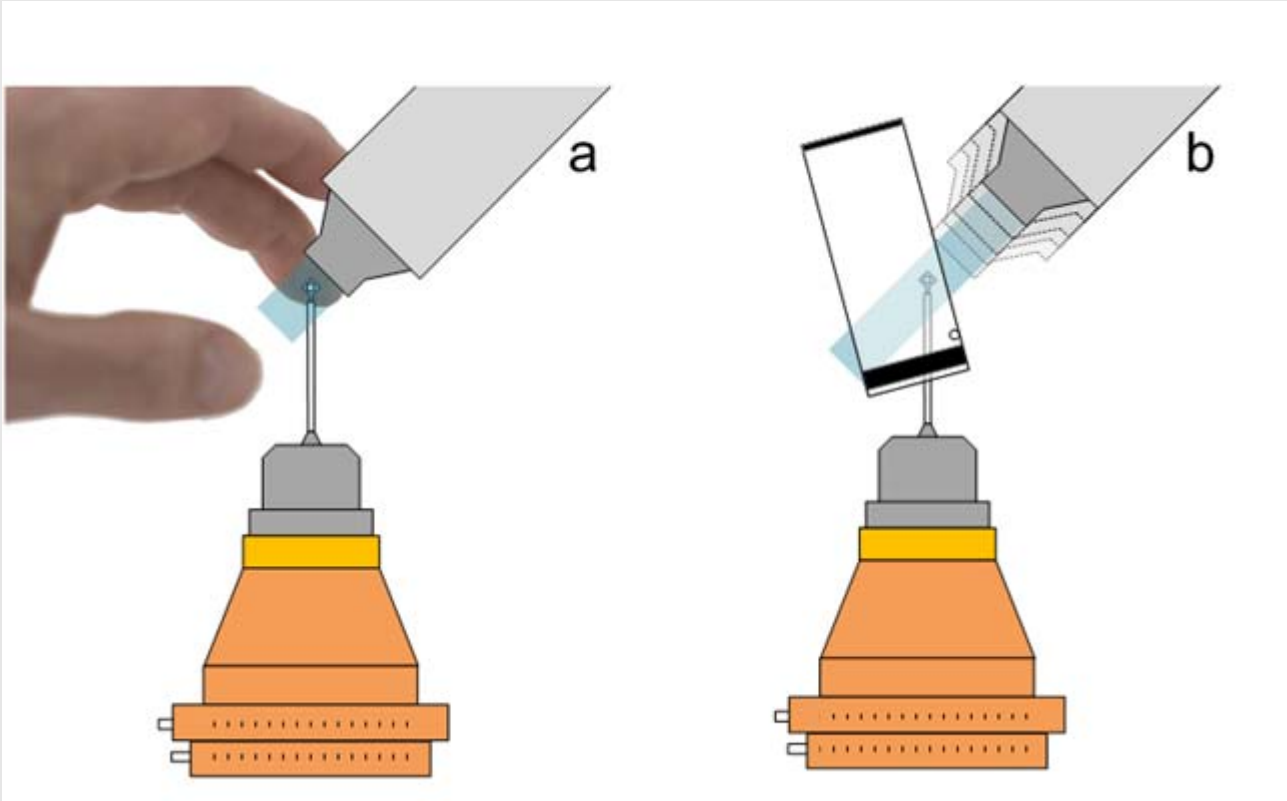
Introduction



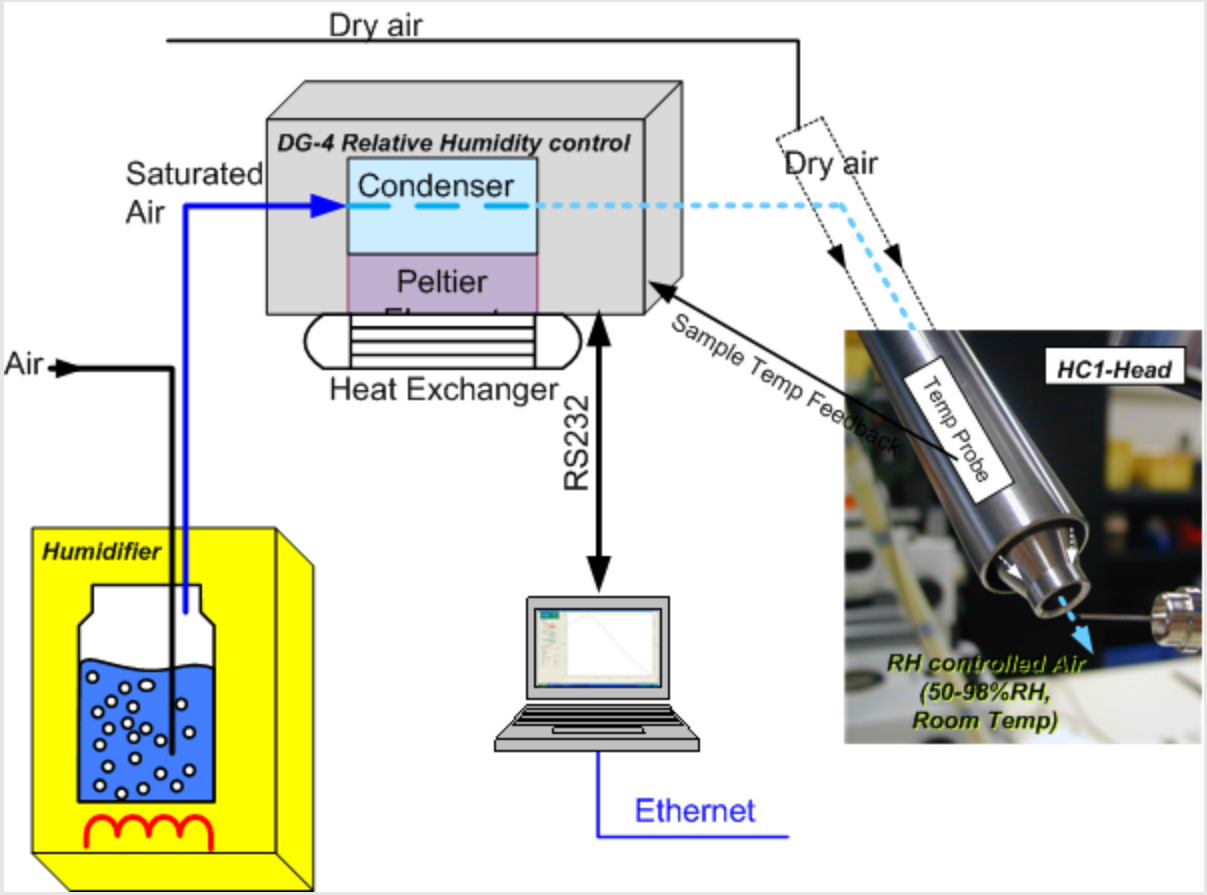
Aims and Objectives

1. Design a more user and beamline friendly machine
2. Based on simpler but robust technology
3. With similar, or better, performance to the FMS
4. Develop the methodology for its use
5. Make it available to users at the ESRF

The Concept



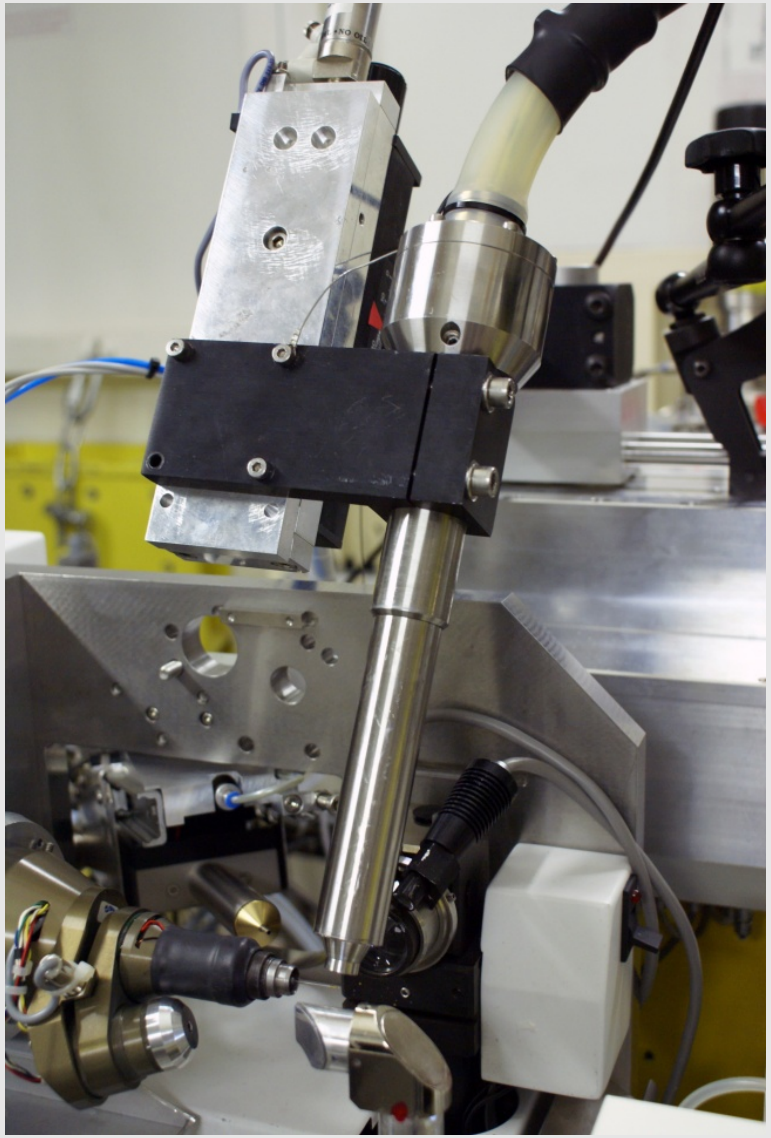
The HC1b

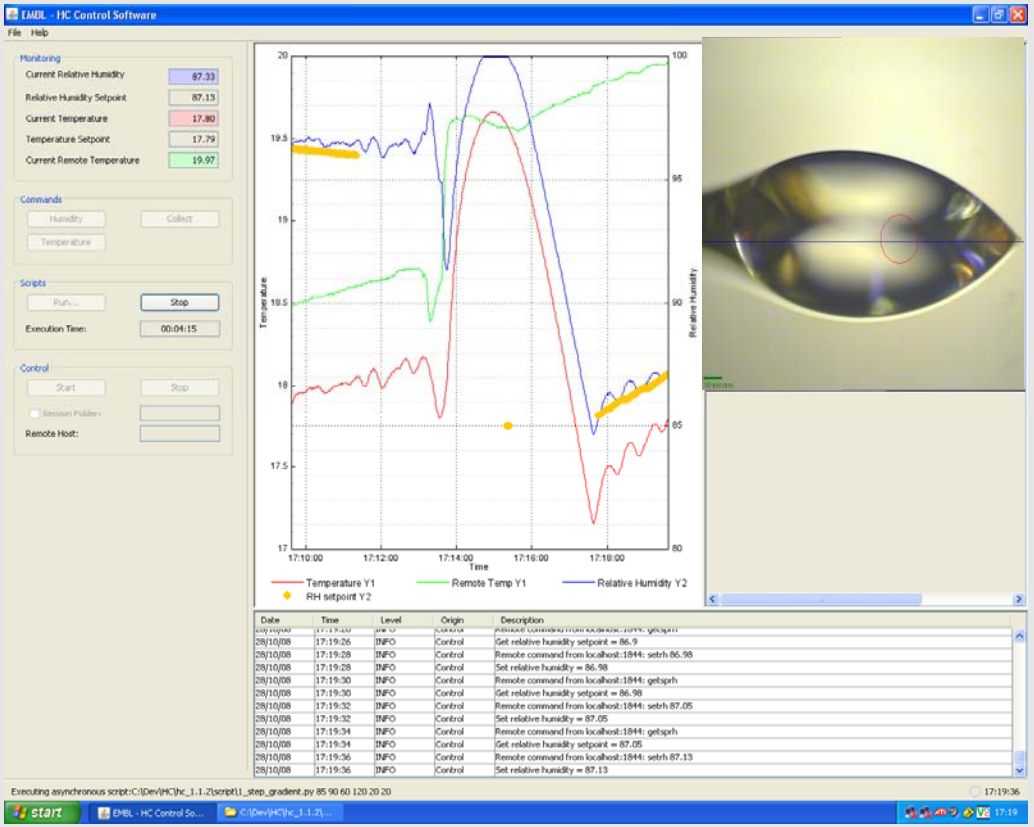

Dry air
DG-4 Relative Humidity control
Saturated
Air
Condenser
Peltier
Dry air
Air
Heat Exchanger
Sample Temp Feedback
HC1-Head
Temp Probe
RS232
Humidifier
RH controlled Air
(50-98%RH,
Room Temp)
Ethernet

The HC1b in place

- Mounted on BM-14, ID14-1 or ID14-2
- Ready for normal users at the ESRF (Rolling)

The HC1b in place





- Java/Python Software
- Total integration of Data/Picture/Humidity
- Automatic droplet area recognition for optimisation
- Modular and easily adaptable to any beamline

Samples

- The experiments are, for the moment, carried out at room temperature
- Crystals need to be brought in their plates to the synchrotron or crystallised onsite
- Crystals need to be stable between 25 and 28 °C
- Crystals that have been cryo-cooled already are not suitable for these experiments
- The crystallisation condition cannot include high amounts of volatile compounds
- Very delicate crystals (photosensitive, O2 sensitive, radiation sensitive ...) may have problems

Data collection

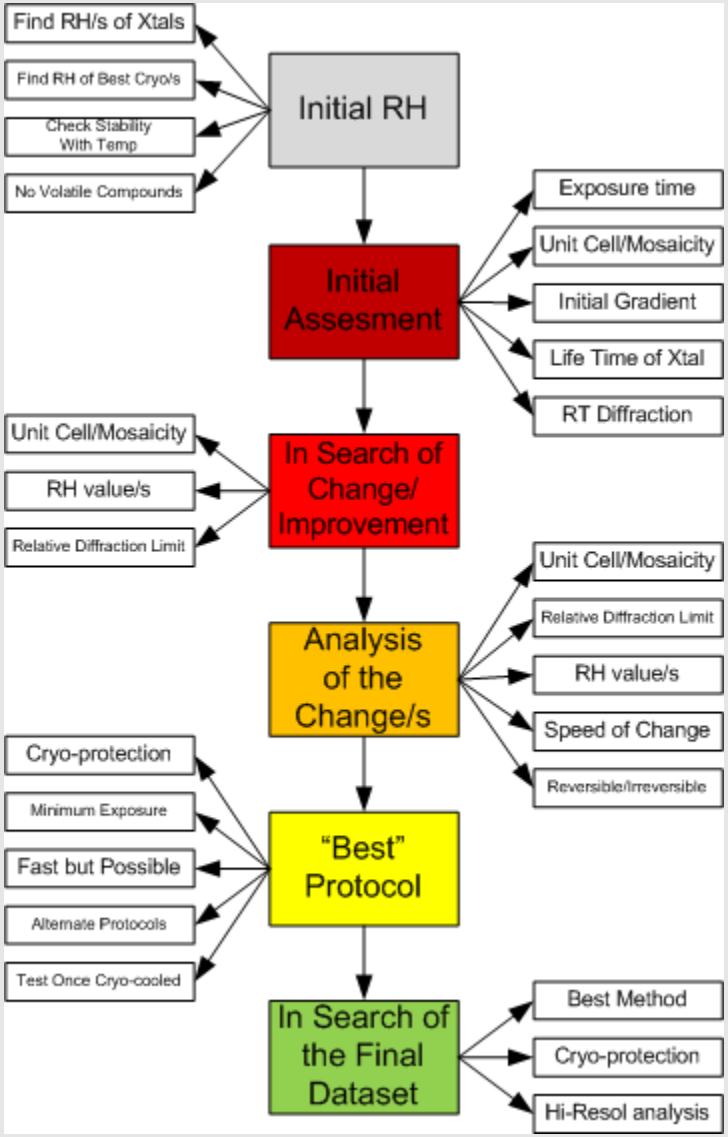
- Exposure time and flux need to be minimised to prevent radiation damage
- Crystals need to have enough scattering power to be able to index the image/s
- These experiments are time and crystal consuming so that a good stock of crystals is necessary

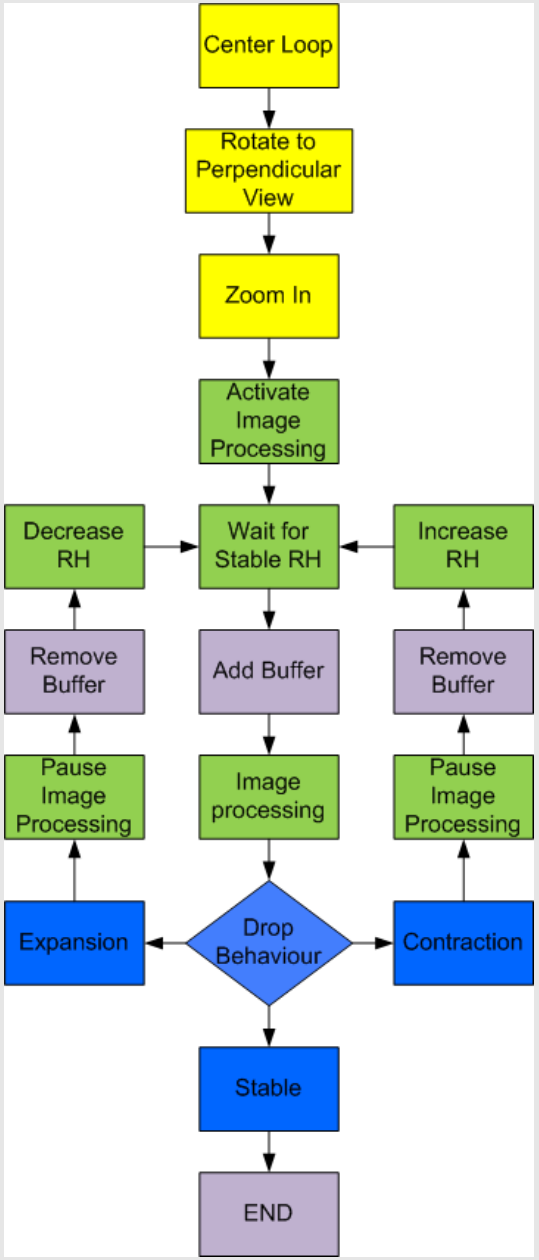
Better Crystals?

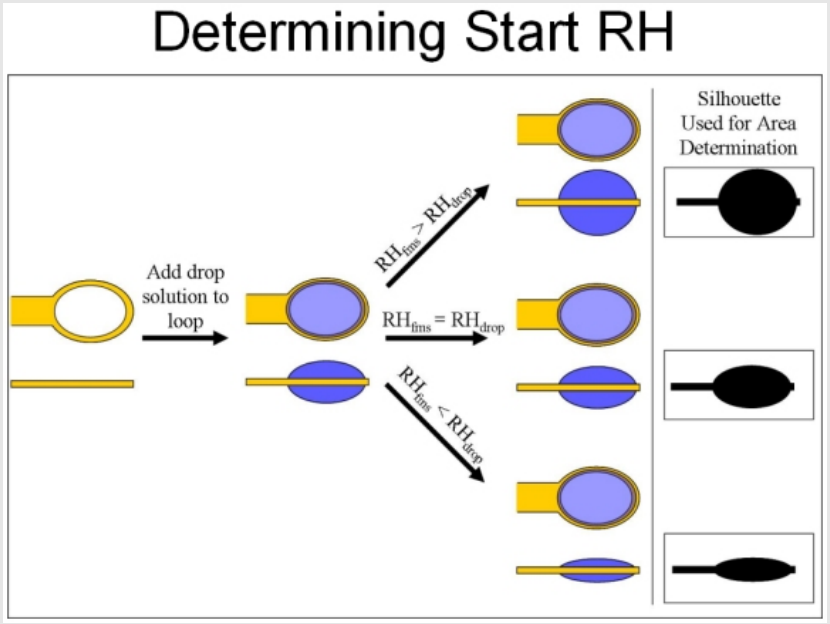
- Crystals that may have shown to be able to crystallise in alternative space groups
- Crystals that show very variable behaviour upon cryo treatment
- Low symmetry SG's
- High solvent content
- Highly mosaic crystals

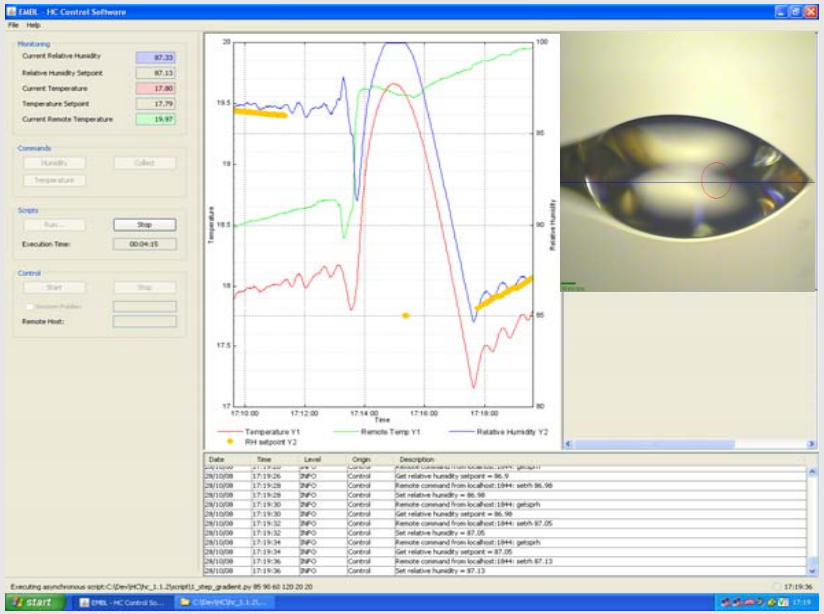
But…

- Even if a suitable diffraction is achieved cryo-cooling can be difficult and will require further testing
- Some of these experiments may be also performed off-line at a later date.
- This is an experiment not a routine synchrotron data collection


Find RH/s of Xtals
Find RH of Best Cryo/s
Check Stability With Temp
No Volatile Compounds
Initial RH
Exposure time
Unit Cell/Mosaicity
Initial Gradient
Life Time of Xtal
RT Diffraction
Initial Assesment
Unit Cell/Mosaicity
RH value/s
Relative Diffraction Limit
In Search of Change/ Improvement
Unit Cell/Mosaicity
Relative Diffraction Limit
RH value/s
Speed of Change
Reversible/Irreversible
Analysis of the Change/s
Cryo-protection
Minimum Exposure
Fast but Possible
Alternate Protocols
Test Once Cryo-cooled
"Best" Protocol
Best Method
Cryo-protection
Hi-Resol analysis
In Search of the Final Dataset

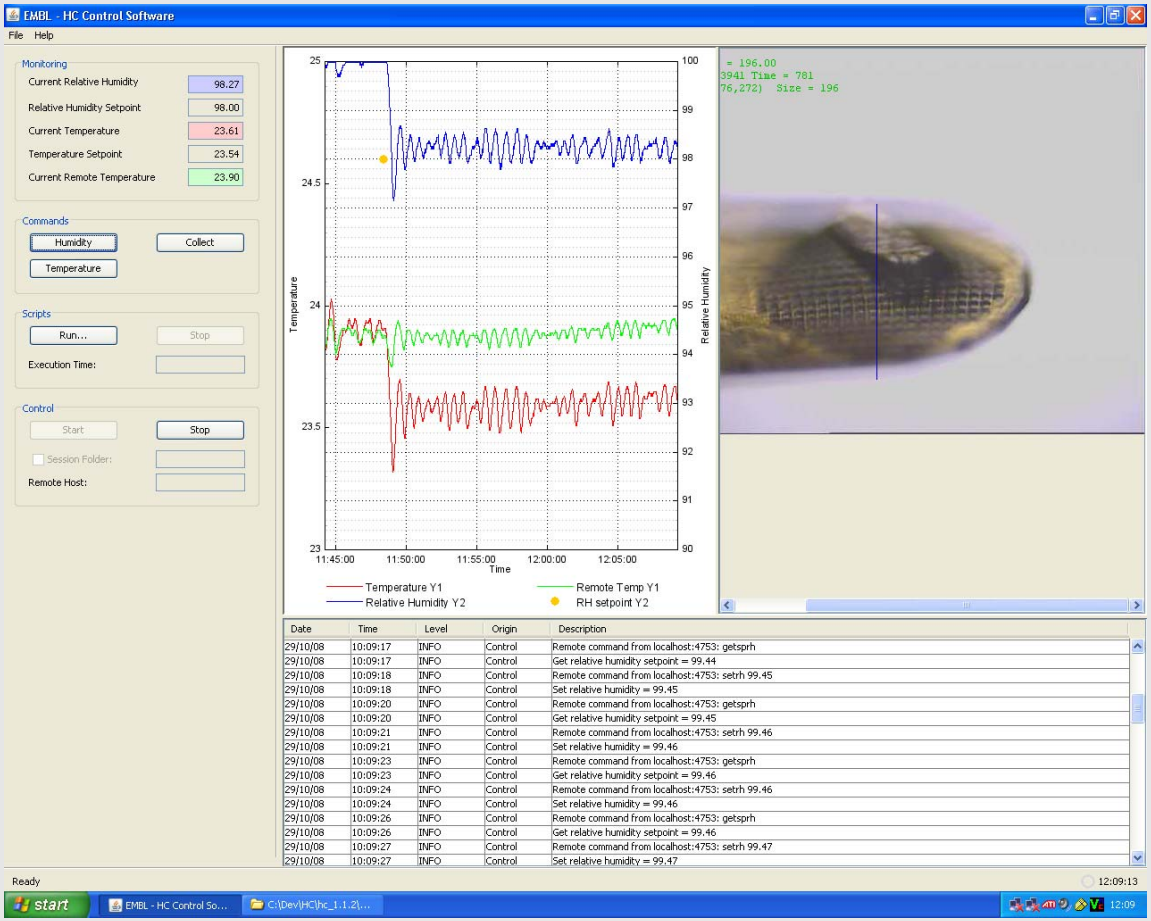


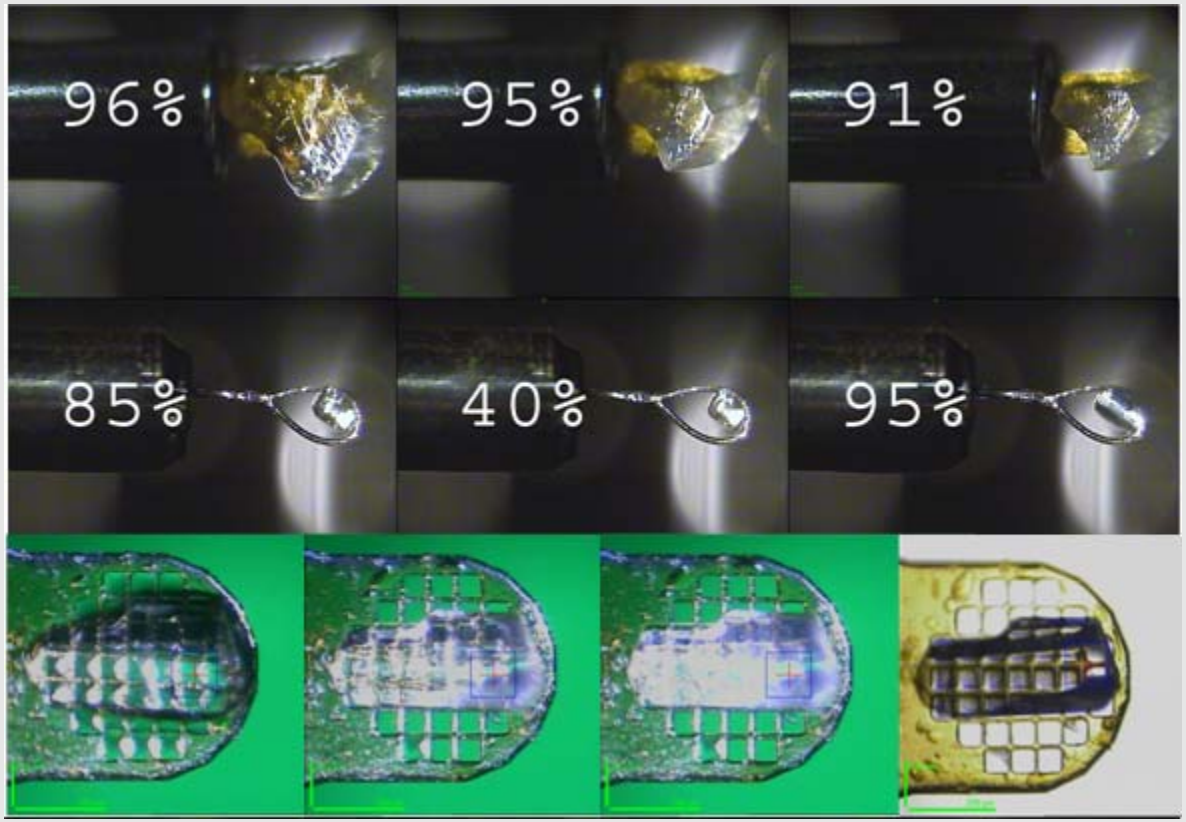


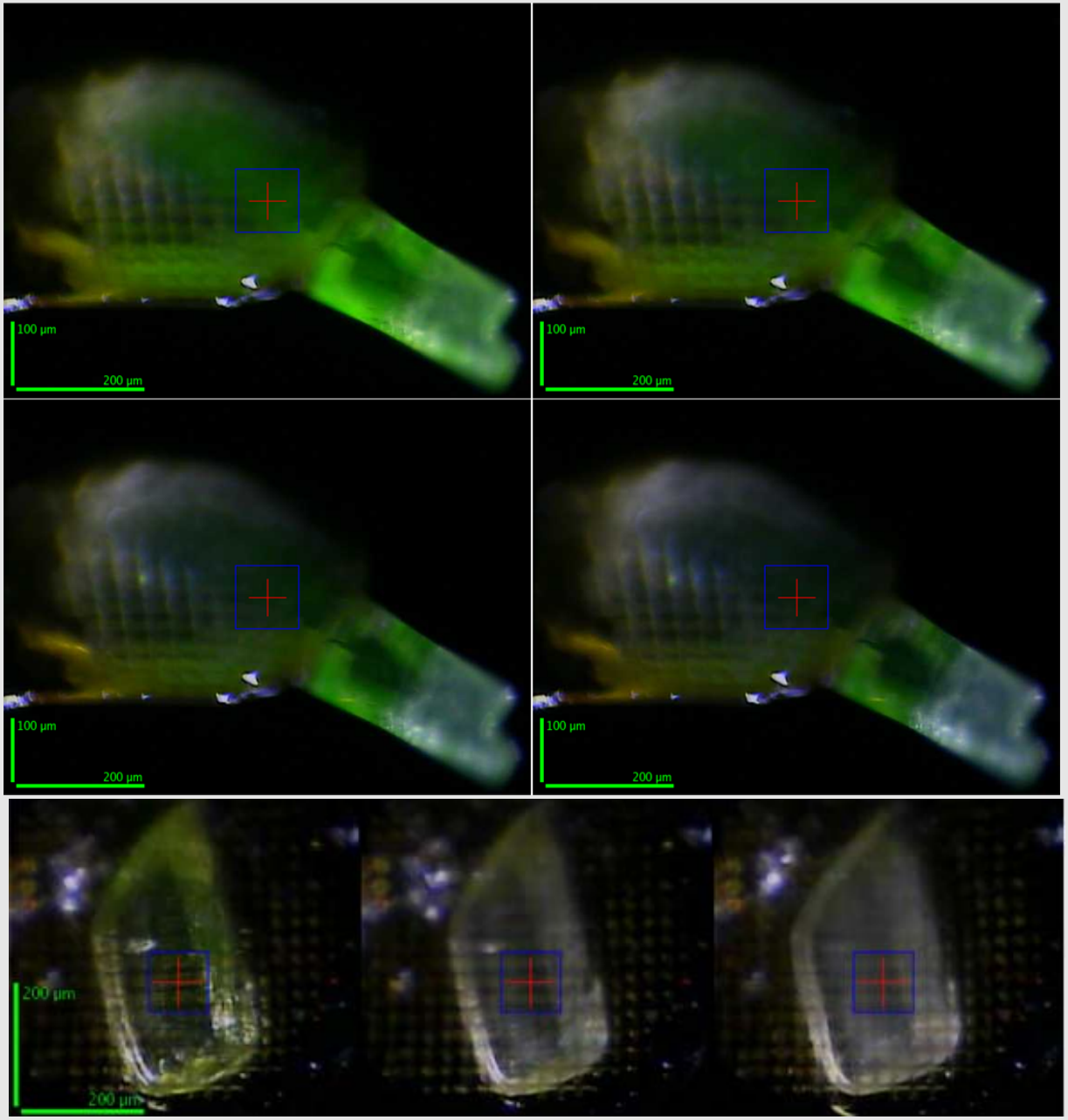


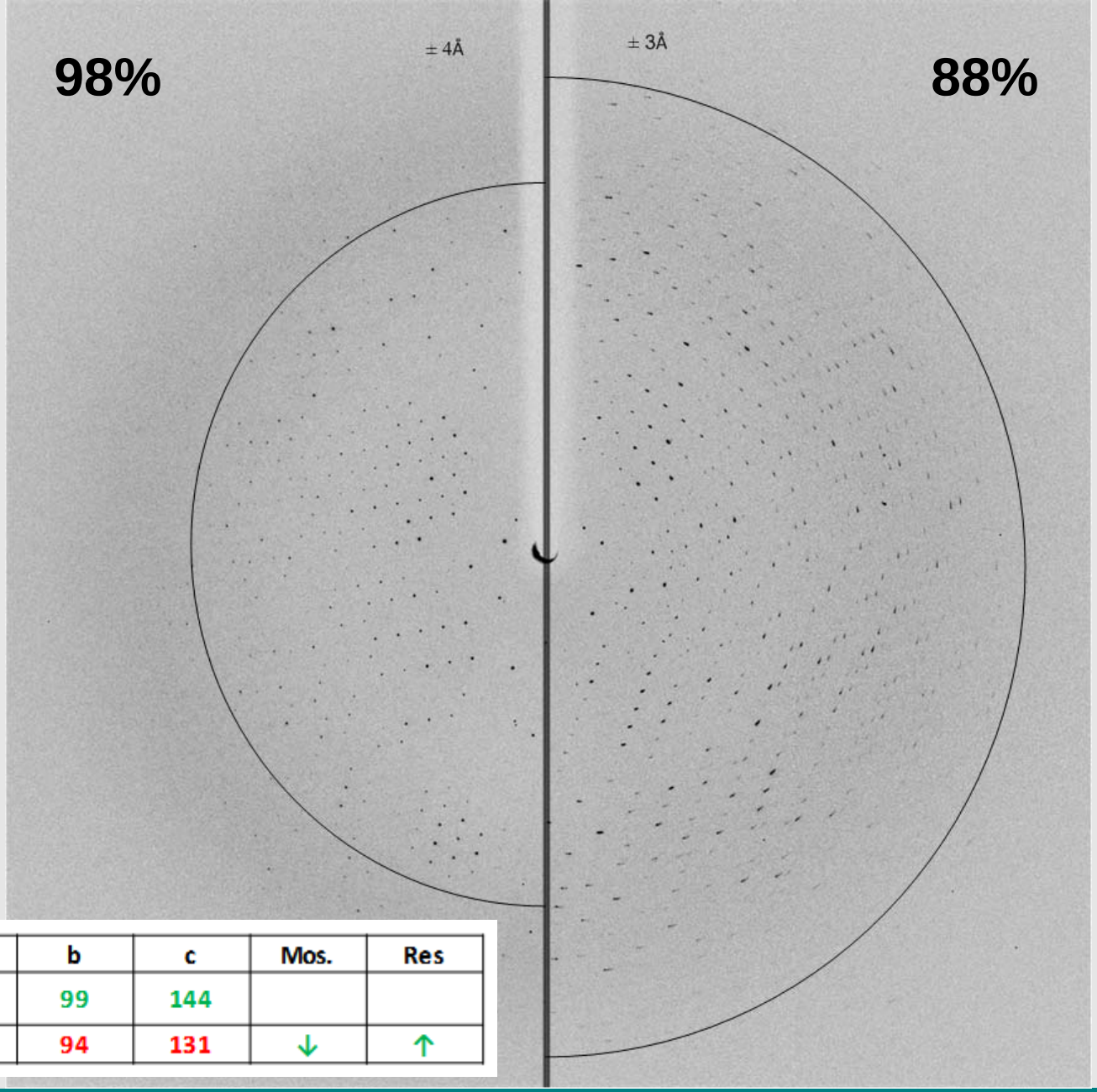
SPINE COMPLEXES

EMBL

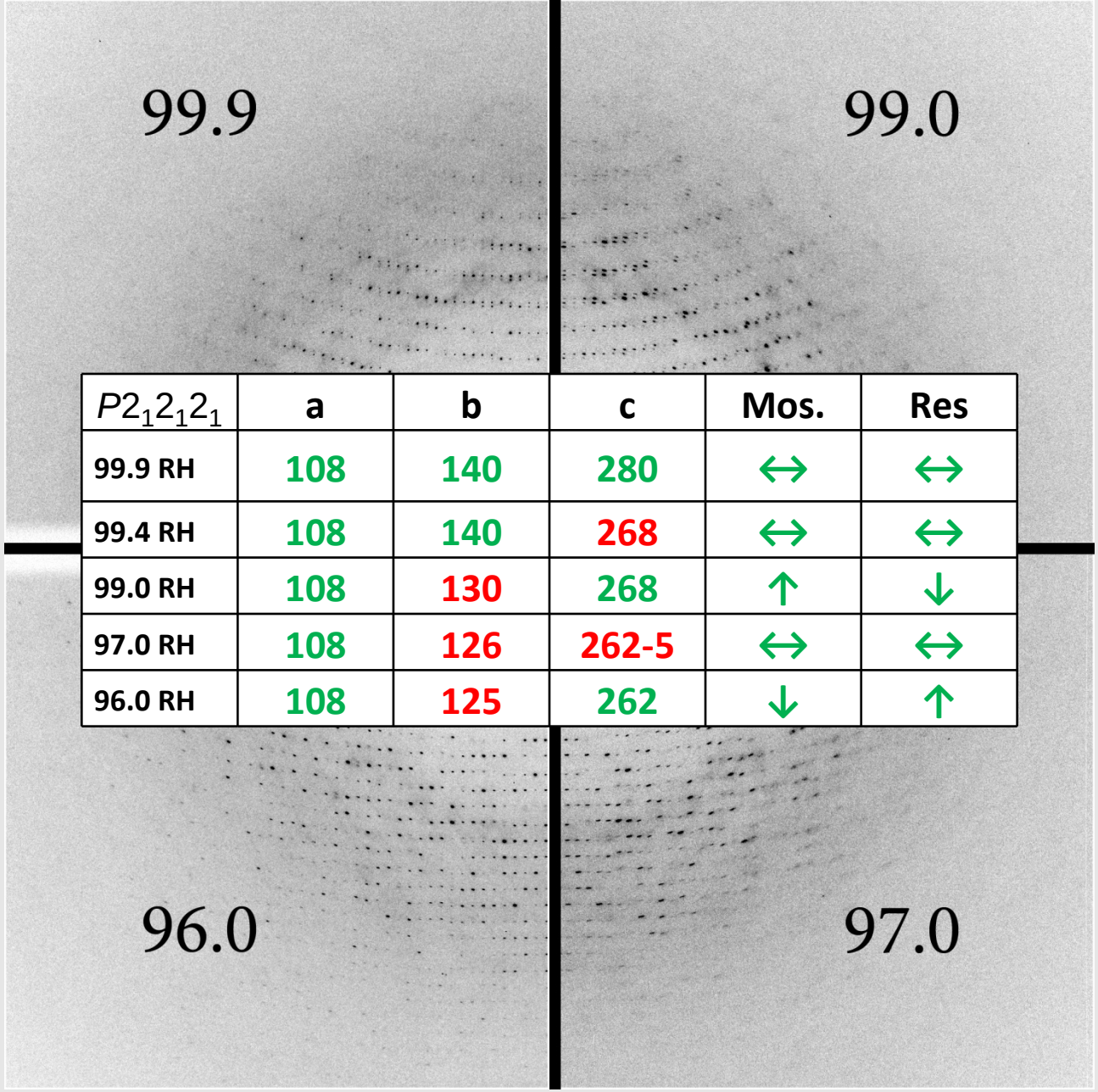

EMBL - HC Control Software
File Help
Monitoring
Current Relative Humidity 98.27
Relative Humidity Setpoint 98.00
Current Temperature 23.61
Temperature Setpoint 23.54
Current Remote Temperature 23.90
Commands
Humidity
Collect
Temperature
Scripts
Run...
Stop
Execution Time:
Control
Start
Stop
Session Folder:
Remote Host:
Temperature
Relative Humidity
Time
11:45:00
11:50:00
11:55:00
12:00:00
12:05:00
Temperature Y1
Relative Humidity Y2
Remote Temp Y1
RH setpoint Y2
Date Time Level Origin Description
29/10/08 10:09:17 INFO Control Remote command from localhost:4753: getsprh
29/10/08 10:09:17 INFO Control Get relative humidity setpoint = 99.44
29/10/08 10:09:18 INFO Control Remote command from localhost:4753: setrh 99.45
29/10/08 10:09:18 INFO Control Set relative humidity = 99.45
29/10/08 10:09:20 INFO Control Remote command from localhost:4753: getsprh
29/10/08 10:09:20 INFO Control Get relative humidity setpoint = 99.45
29/10/08 10:09:21 INFO Control Remote command from localhost:4753: setrh 99.46
29/10/08 10:09:21 INFO Control Set relative humidity = 99.46
29/10/08 10:09:23 INFO Control Remote command from localhost:4753: getsprh
29/10/08 10:09:23 INFO Control Get relative humidity setpoint = 99.46
29/10/08 10:09:24 INFO Control Remote command from localhost:4753: setrh 99.46
29/10/08 10:09:24 INFO Control Set relative humidity = 99.46
29/10/08 10:09:26 INFO Control Remote command from localhost:4753: getsprh
29/10/08 10:09:26 INFO Control Get relative humidity setpoint = 99.46
29/10/08 10:09:27 INFO Control Remote command from localhost:4753: setrh 99.47
29/10/08 10:09:27 INFO Control Set relative humidity = 99.47
Ready
12:09:13
start
EMBL - HC Control So...
12:09


96%
95%
91%
85%
40%
95%

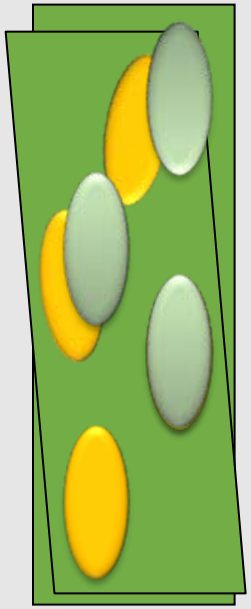

100 µm
200 µm
100 µm
200 µm
100 µm
200 µm
100 µm
200 µm
200 µm
200 µm



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



$P2_12_12_1$	a	b	c	Mos.	Res
99.9 RH	108	140	280	↔	↔
99.4 RH	108	140	268	↔	↔
99.0 RH	108	130	268	↑	↓
97.0 RH	108	126	262-5	↔	↔
96.0 RH	108	125	262	↓	↑



Old space group

P21

a	b	c	alpha	beta	gamma	volume
77.3400	100.6000	83.6400	90.000	106.500	90.000	623954.8

2 assym units

N(mol)	Prob(N) for resolution	Prob(N) overall	Vm A**3/Da	Vs % solvent	Mw Da
1	0.0013	0.0024	12.11	89.85	25757.00
2	0.0013	0.0030	6.06	79.69	51514.00
3	0.0032	0.0356	4.04	69.54	77271.00
4	0.0667	0.2343	3.03	59.38	103028.00
*5	*0.4363	0.5194	2.42	49.23	128785.00*
*6	0.4717	0.1980	2.02	39.07	154542.00*
7	0.0183	0.0049	1.73	28.92	180299.00
8	0.0013	0.0024	1.51	18.76	206056.00

12/24

5 to 6 heterodimers per assymmetric unit !

NEW Space group!

C222 or C2221

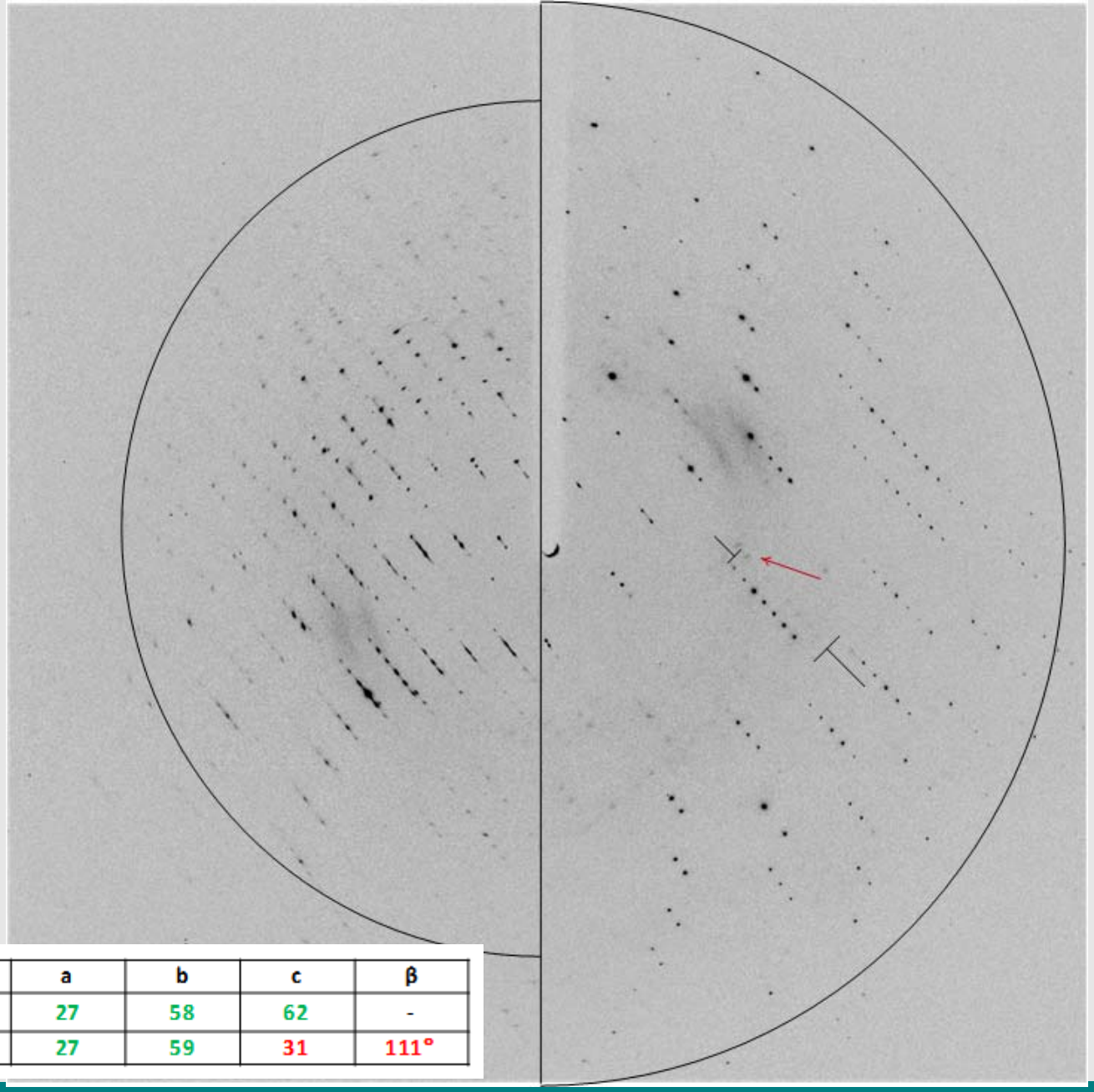
a	b	c	alpha	beta	gamma	volume
89.9200	123.7000	96.5200	90.000	90.000	90.000	1073601.9

8 assym units

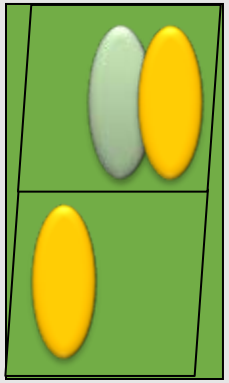
N(mol)	Prob(N) for resolution	Prob(N) overall	Vm A**3/Da	Vs % solvent	Mw Da	
1	0.0045	0.0123	5.21	76.39	25757.00	
*2	0.9223	0.9760	2.61	52.79	51514.00	*
3	0.0732	0.0117	1.74	29.18	77271.00	

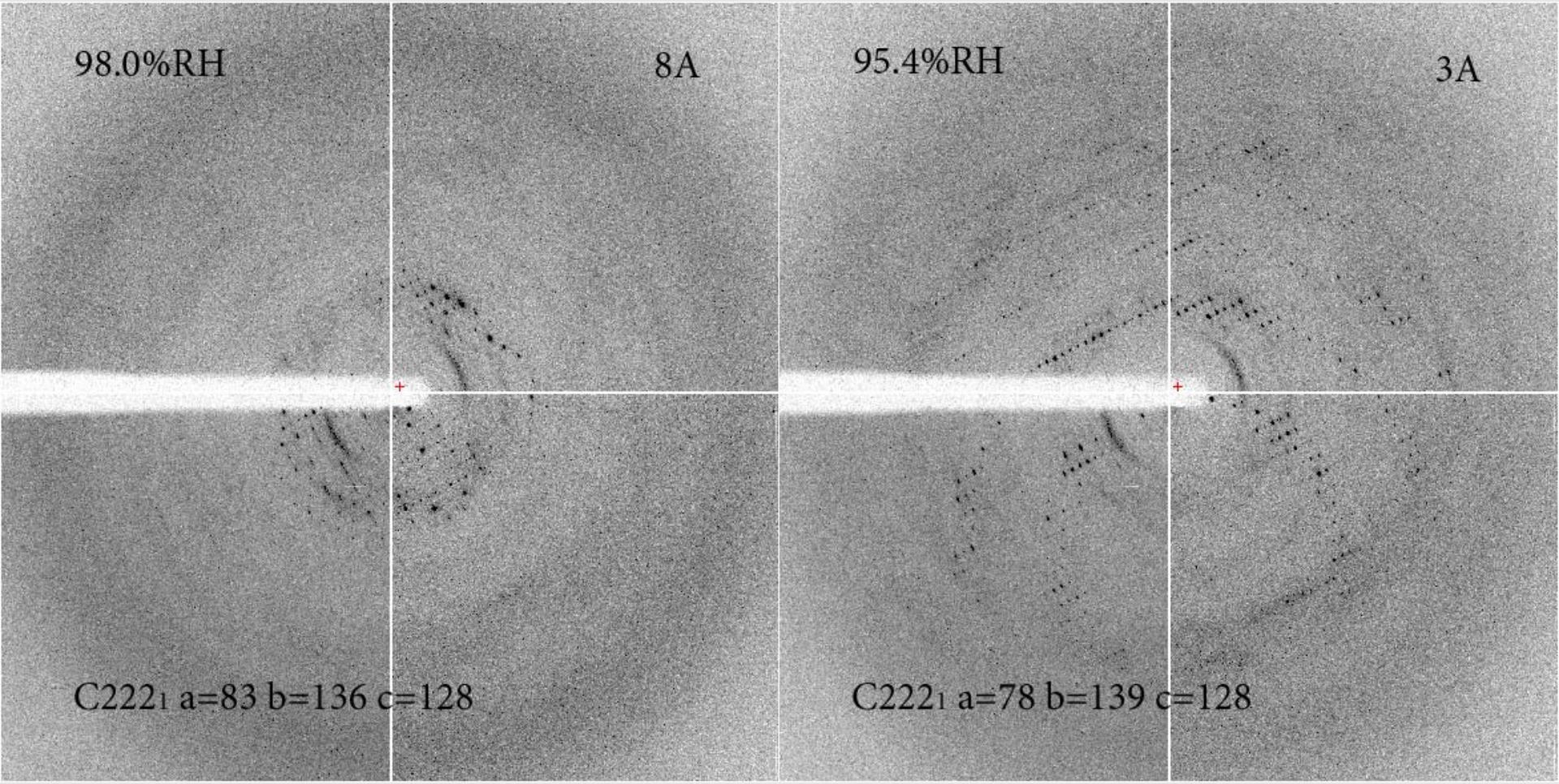
4/32

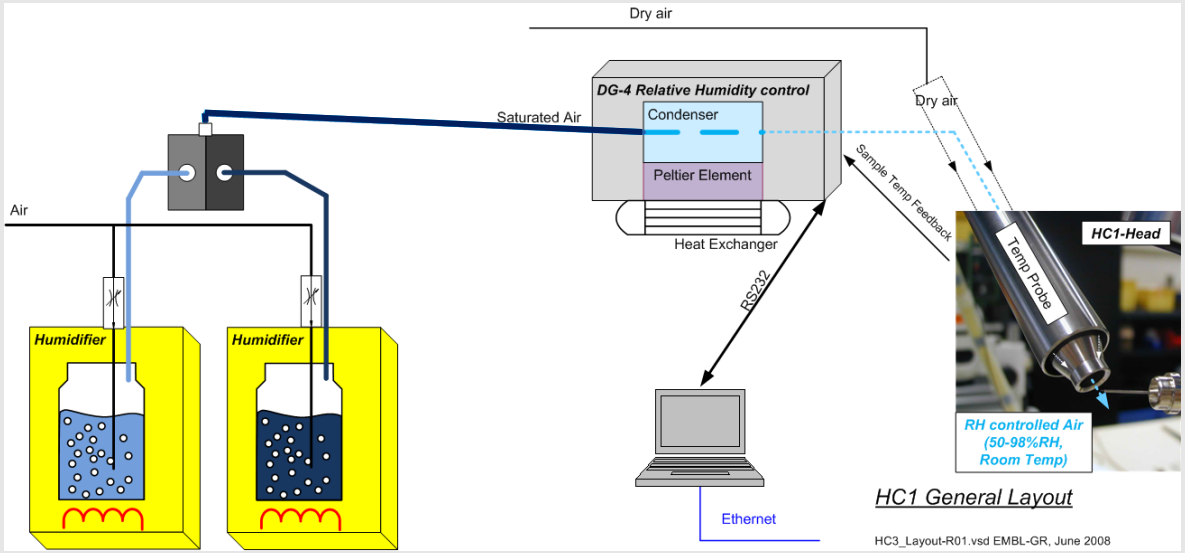
2 heterodimers (mass of one dimmer 25.75 kda)

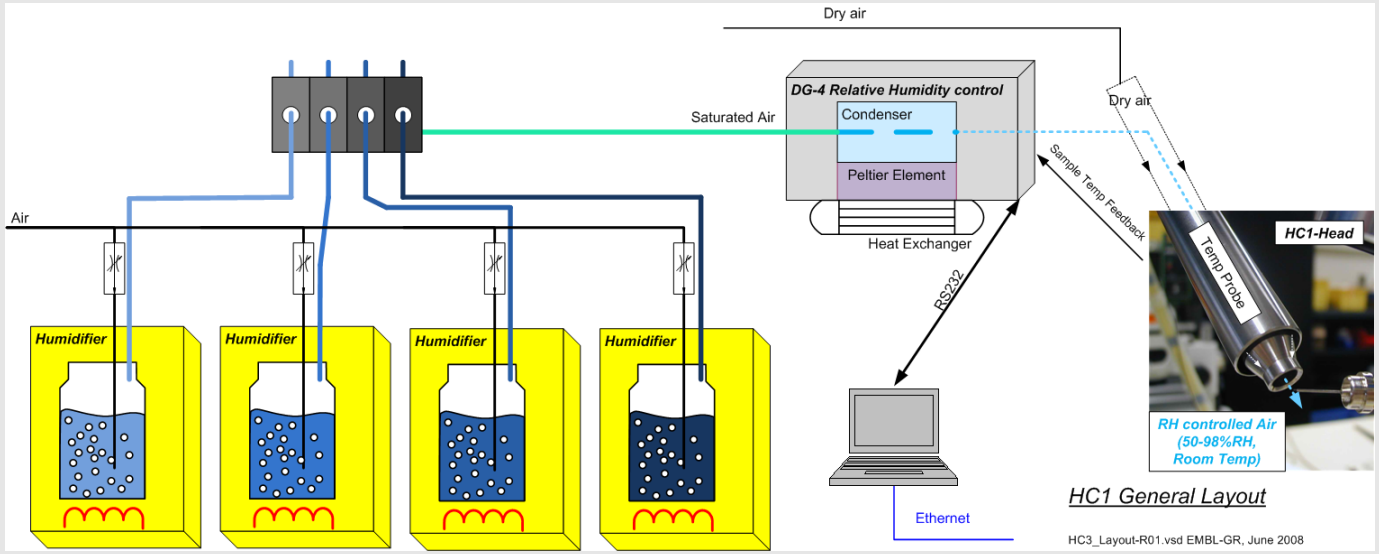


	SG	a	b	c	β
98-70 RH	$P2_12_12_1$	27	58	62	-
<65 RH	$P2_1$	27	59	31	111°

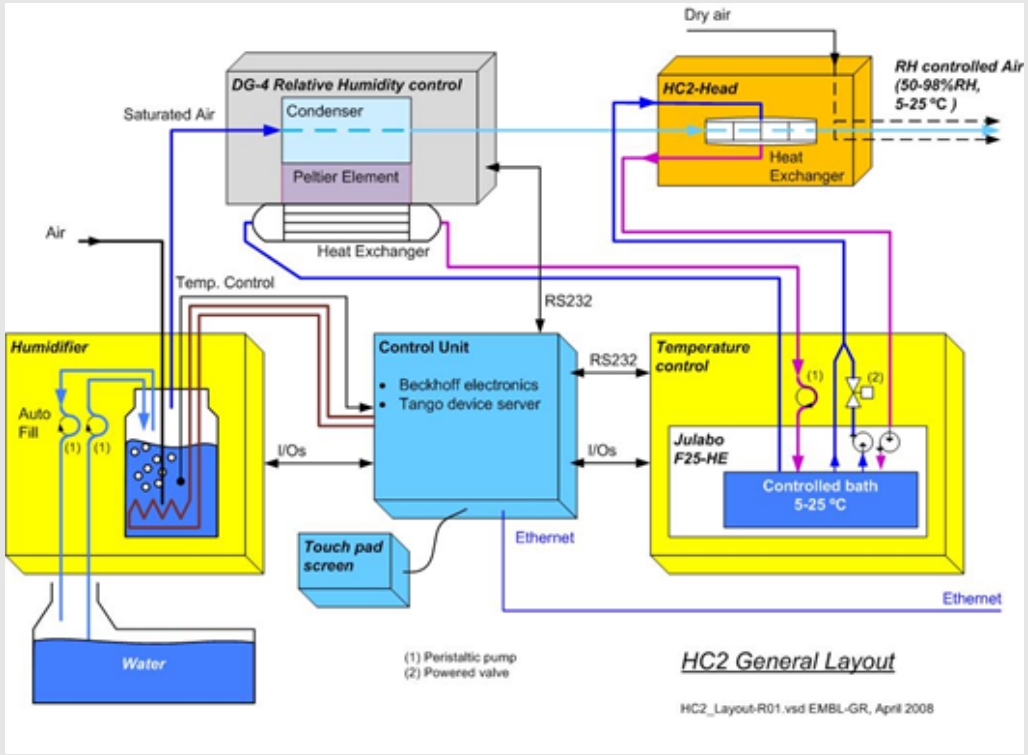



98.0%RH
8A
95.4%RH
3A
C222₁ a=83 b=136 c=128
C222₁ a=78 b=139 c=128


Dry air
DG-4 Relative Humidity control
Condenser
Peltier Element
Heat Exchanger
Saturated Air
Dry air
Sample Temp Feedback
Air
Humidifier
Humidifier
RS232
HC1-Head
Temp Probe
RH controlled Air
(50-98%RH,
Room Temp)
Ethernet
HC1 General Layout
HC3_Layout-R01.vsd EMBL-GR, June 2008

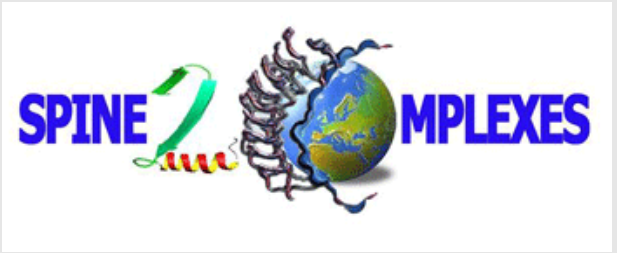

Dry air
DG-4 Relative Humidity control
Condenser
Peltier Element
Heat Exchanger
Saturated Air
Dry air
Sample Temp Feedback
Air
Humidifier
Humidifier
Humidifier
Humidifier
RS232
HC1-Head
Temp Probe
RH controlled Air
(50-98%RH,
Room Temp)
Ethernet
HC1 General Layout
HC3_Layout-R01.vsd EMBL-GR, June 2008

3nd Prototype (HC2)



- **5-7 °C**
- **Improved stability and performance**

Acknowledgements



Instrumentation Group
Florent Cipriani
Franck Felisaz (Design and build)
Alexandre Gobbo (HC1 Software)
Julien Huet (Switch, technical help)
Raphael Moya (Build)

Synchrotron Crystallography Group
Andrew Mc Carthy
Sandor Brockhauser (linux/windows)
Hassan Belrahali (“Genix” Setup, …)
Raimond Ravelli (FMS)

BM-14
Martin Walsh
Hassan Belrahali
Hugo Caserotto

ID14-1/14-2
Matthew Bowler
MX-Group

HT-X Lab
Josan Marquez (Data collection & thinking)
Florine Dupeux (Crystallography)
Martin Roewer (Crystallography)

EMBL-Labs
Annie Simon
Virginie Vaultier

Sample donation
Carlo Petosa (crystal search)
Meindert Lamers (DNA Pol III)
Ricardo Pires (Alix V)
Anna Yaremchuck (?)
Majida El Bakkouri (RavA & ad3)
Nicolas Tarbouriech (El9?)
Marchello Clerici
Esko Oksanen

External users
Jim Barber/ Karim Maghlaoui
Cataldo Tarricone
Lauris Kemp
David Marçal
Pavel Plevka
Bin Liu
John Walker/Matthew Bowler
Jean Baptiste Artero
D Bourgois/Virgile Adam …..