FIP -BM30A:
an automated beamline
for protein crystallography

Automated data collection (Xnemo)
Cryogenic Automated Transfer System (CATS)
Automated Data Processing (ADP)

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CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE

The FIP beamline

- installed on a Bending Magnet section (BM30A) at ESRF

- devoted only for protein crystallography

- tunable energy for anomalous scattering use (MAD/SAD)

- financed by:

Beam time
- 33% ESRF users
- 7 % Belgium users
- 60 % French users

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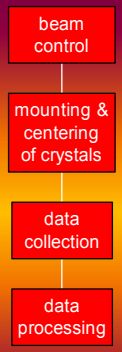
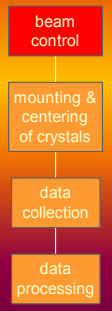
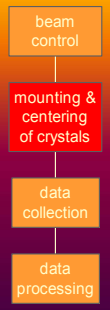
Automation of the crystallography experiment

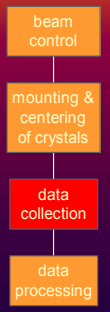




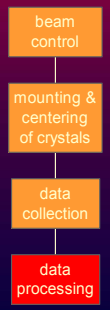
Automation of the beam control (1998)



Automation of mounting and centering (2003)

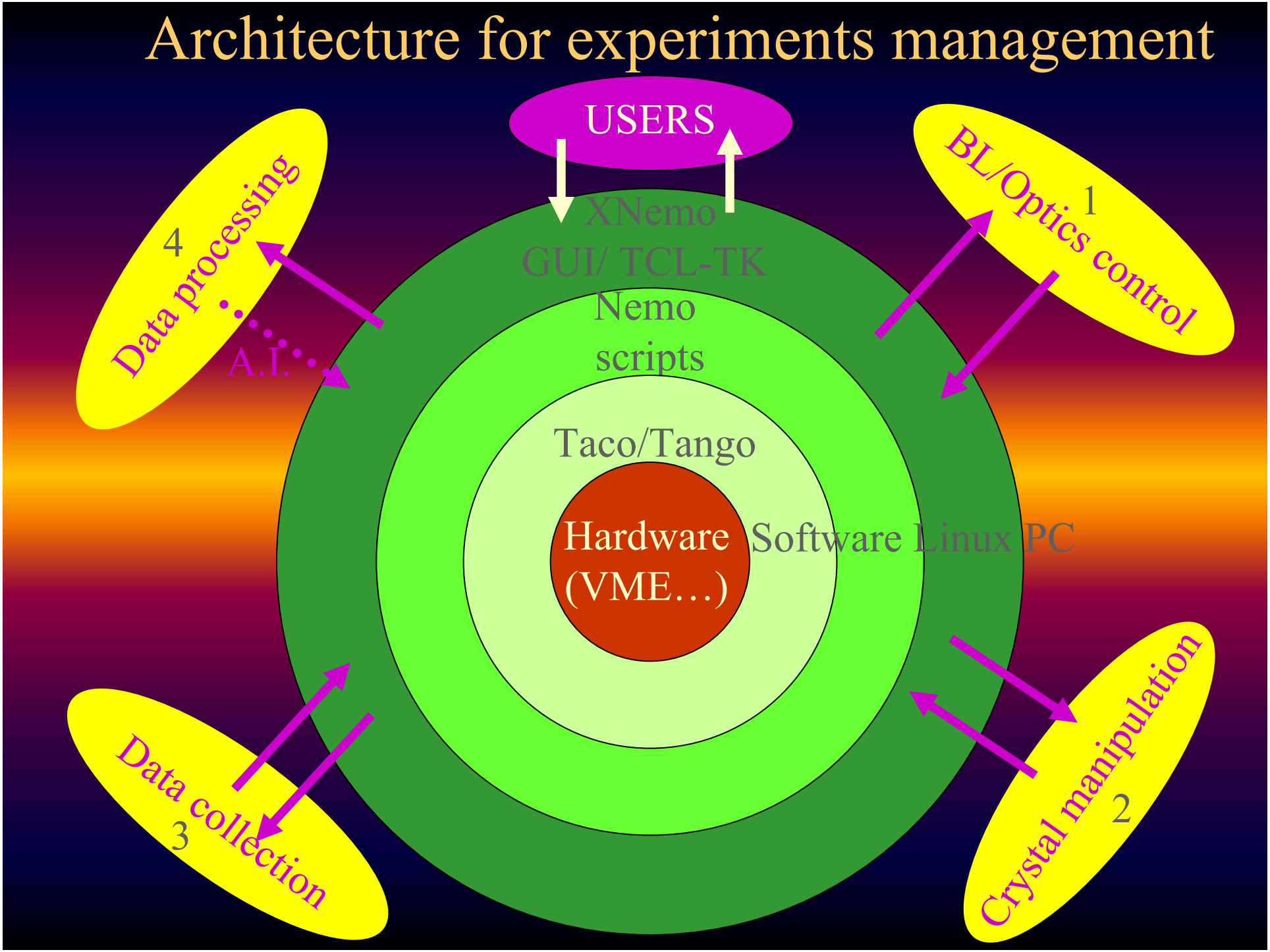


Automation of data collection (1999)

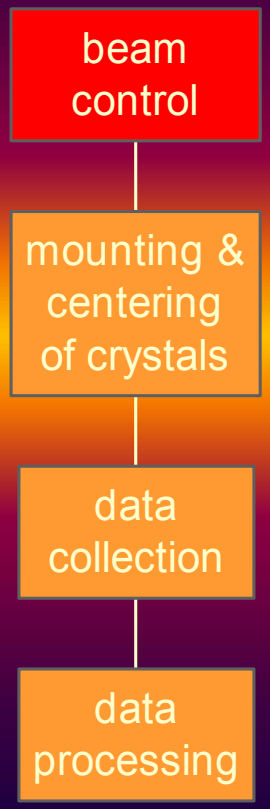


Automation of data processing (2001)

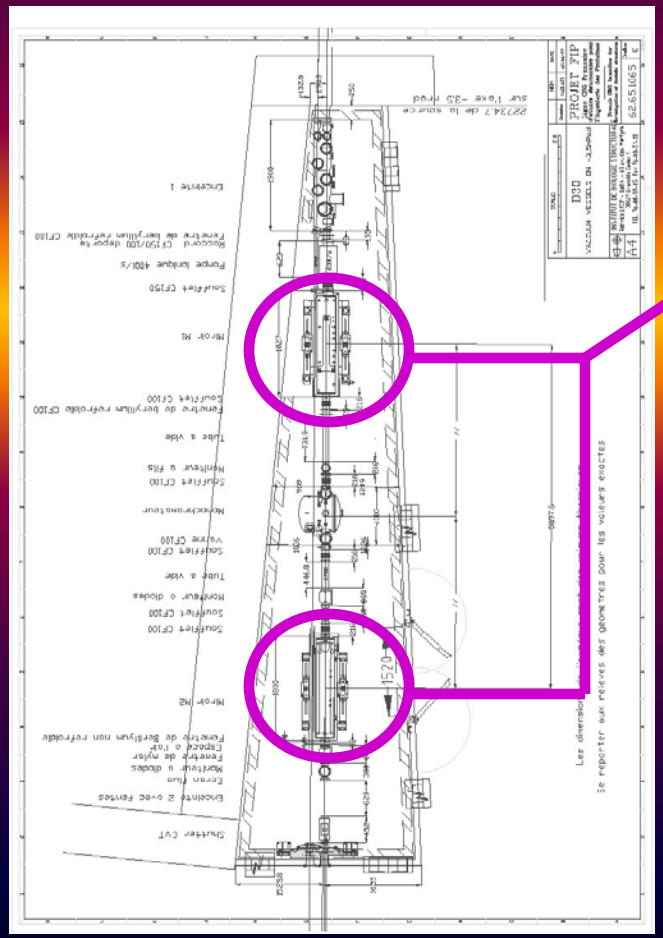
Architecture for experiments management

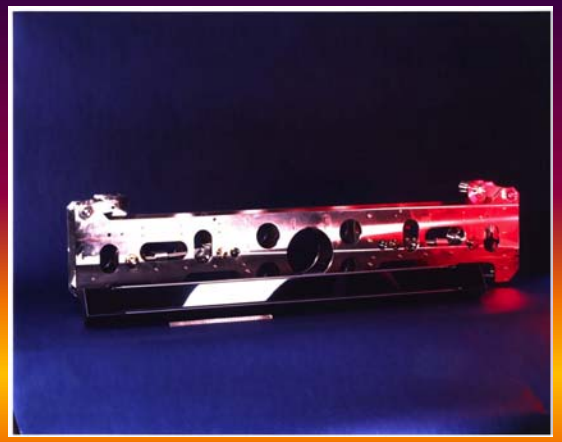


1- Automated experiment: beam control



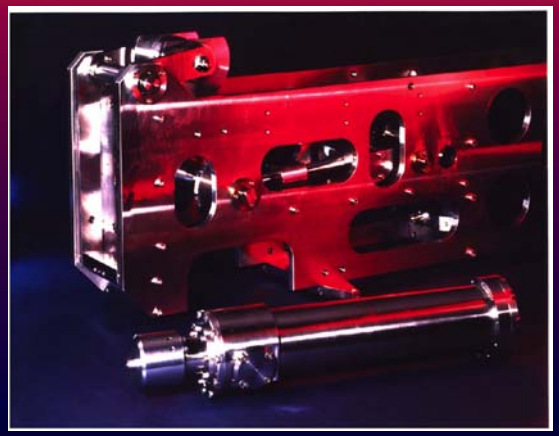
Two grazing angle mirrors





1.3 m long, 6 cm large
Si / Zerodur ™
water cooling (Ga bath)

vertical focusing
harmonics rejection
heat load reduction



beam control

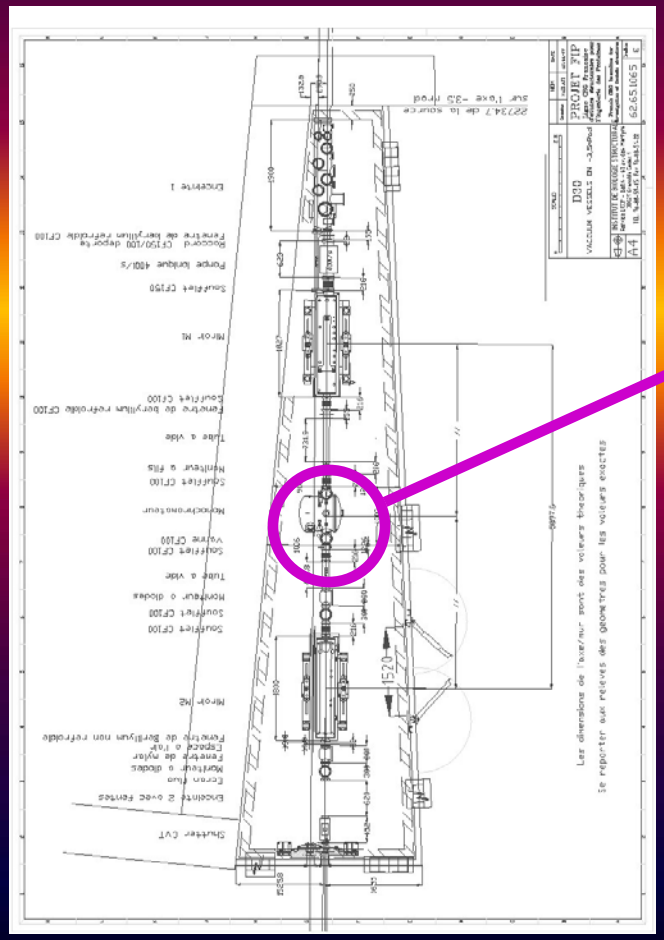
mounting & centering of crystals

data collection

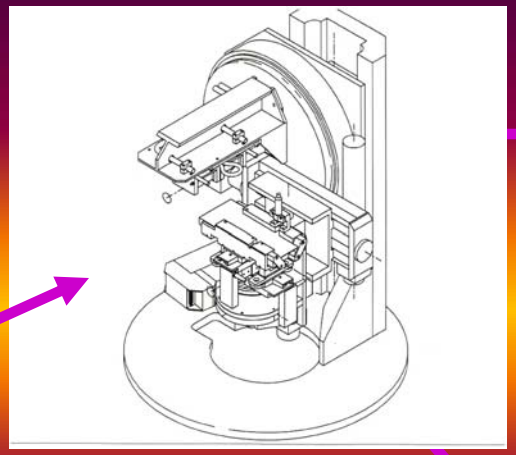
data processing

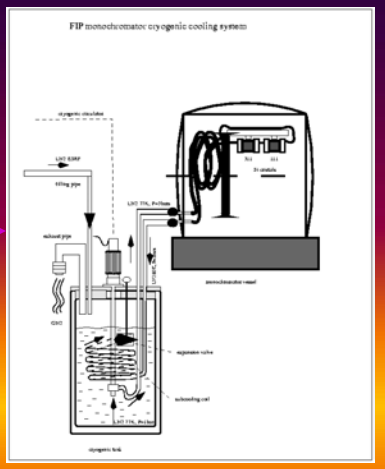
Two crystals monochromator

(13 motors!)



2Si(111)

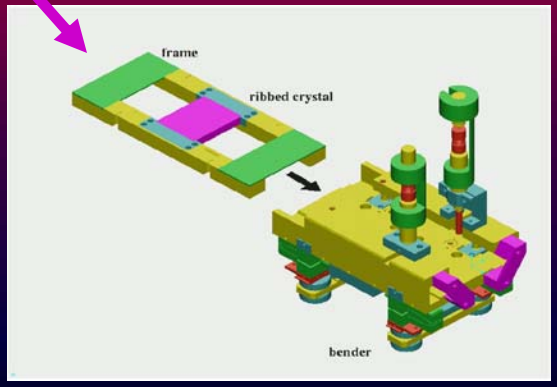




heat load reduction

Monochromatic:
7 keV < E < 17 keV
$\Delta E/E \sim 10^{-4}$

Focal spot:
300*300 μm^2



horizontal focusing

beam control

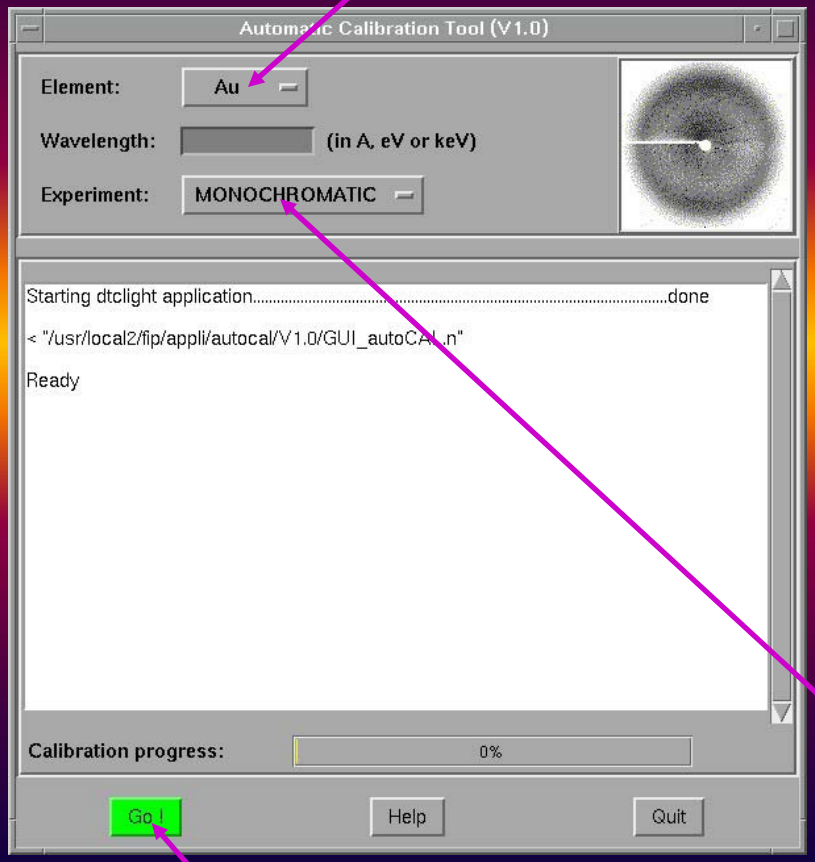
mounting & centering of crystals

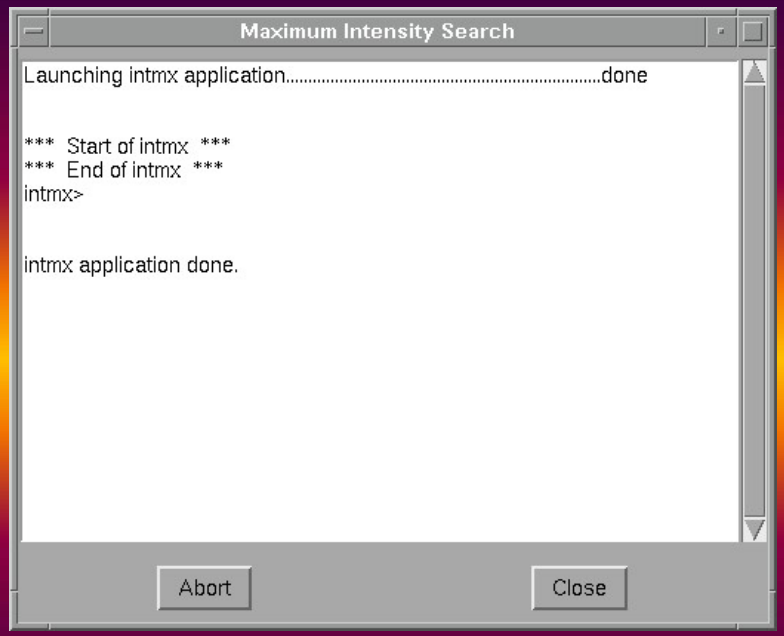
data collection

data processing

Setting up the X-ray beam

1. select edge or wavelength





2. theoretical position, or automated calibration with metal foil

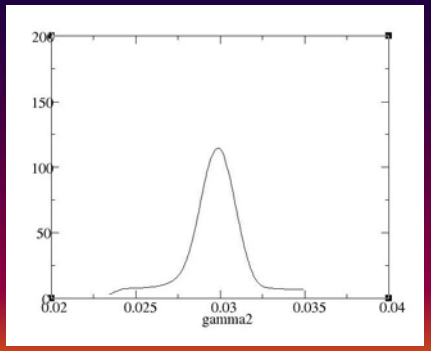
3. and then: Go...

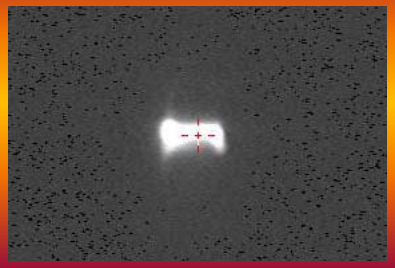
Setting up the X-ray beam

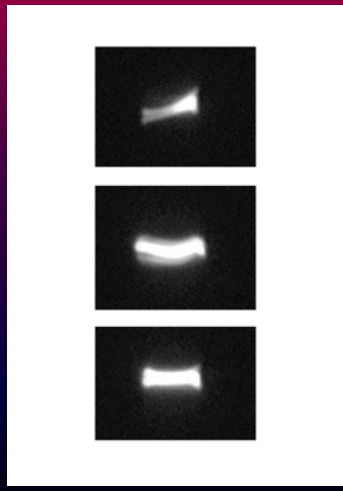
- optics geometry (theory)
  - * based on absolute position of origin
  - * tabulated corrections

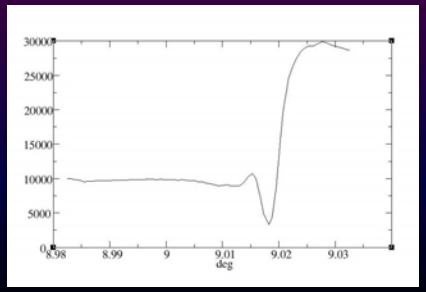
- beam optimization
  - * scans (before collimator)
  - * beam position on fluo.
  - * scans (after collimator)
  - * beam shape on fluo.

- energy calibration (optional)

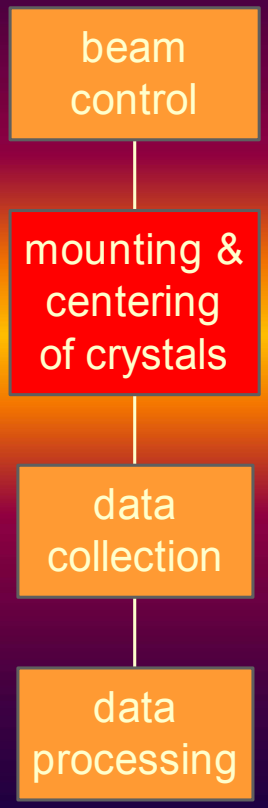








2- Automated experiment: sample mounting and centering



beam control

mounting & centering of crystals

data collection

data processing

The experiment

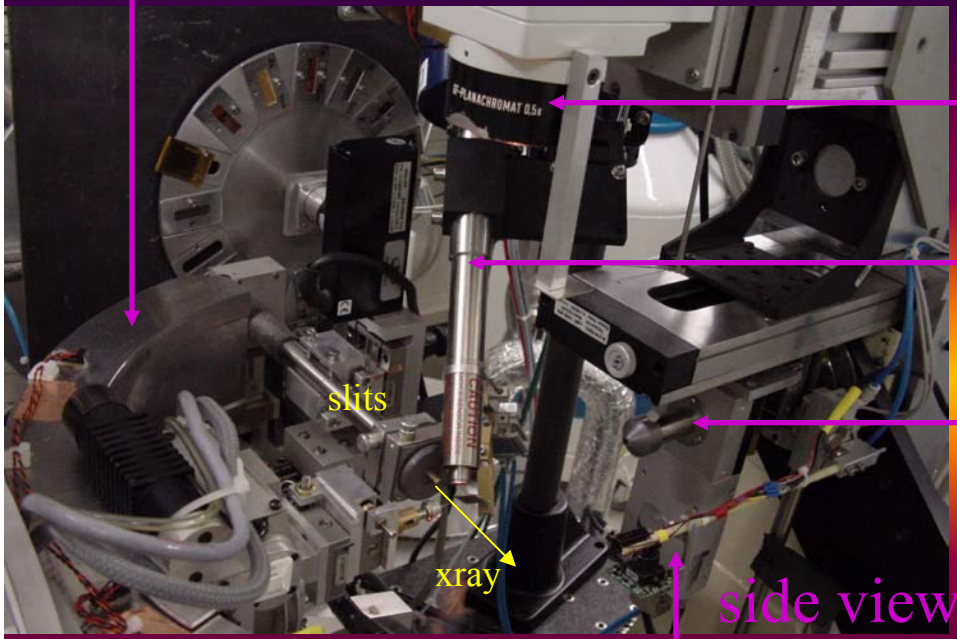
A 5-circle diffractometer including:

- sample orientation (MAD)

- microscope + video

- Oxford cryo. cooling

- fluorescence detector

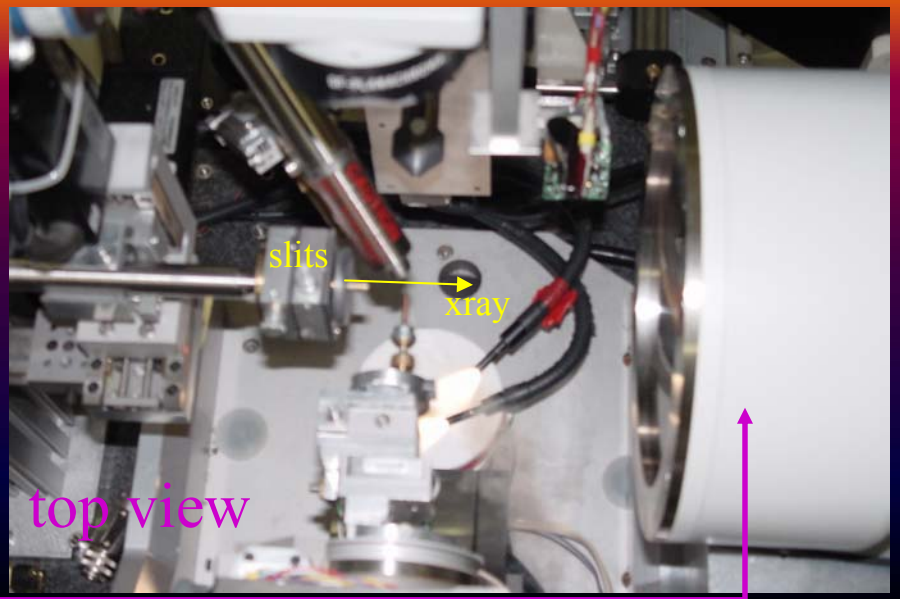


side view

- beam monitors

- 2-theta for high resolution

- Mar CCD detector



top view

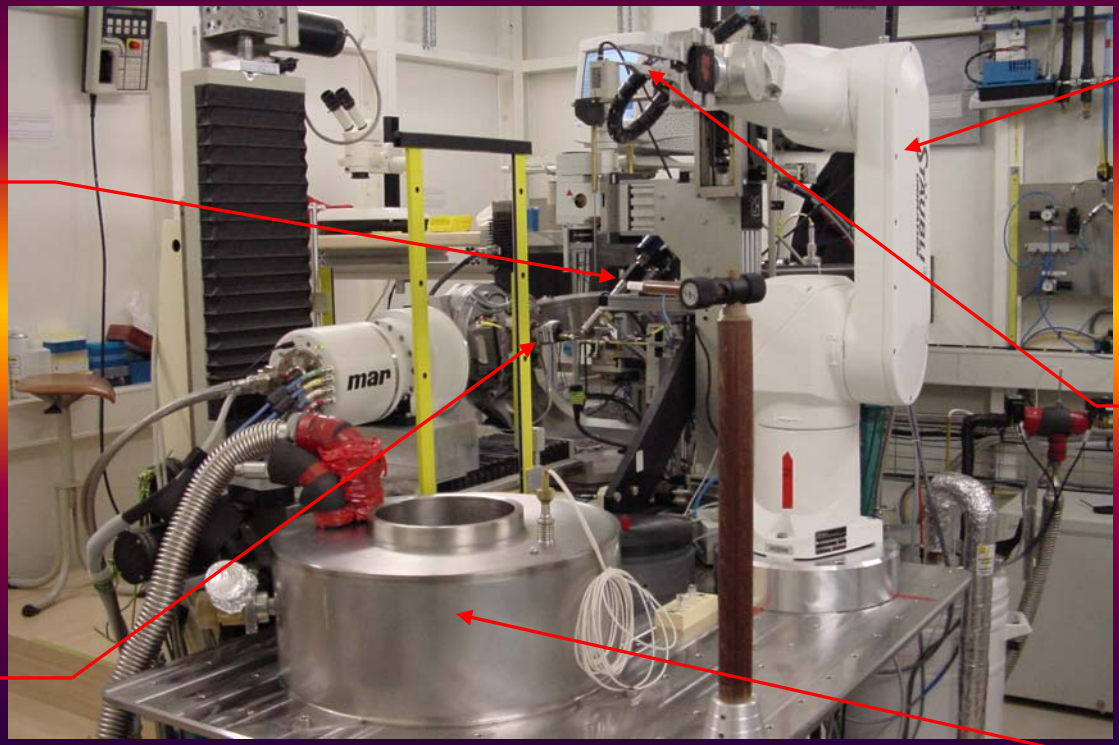
beam control

mounting & centering of crystals

data collection

data processing

Integrated system



Robot

Flipping tong

Actuator

Goniometer head

Storage Dewar

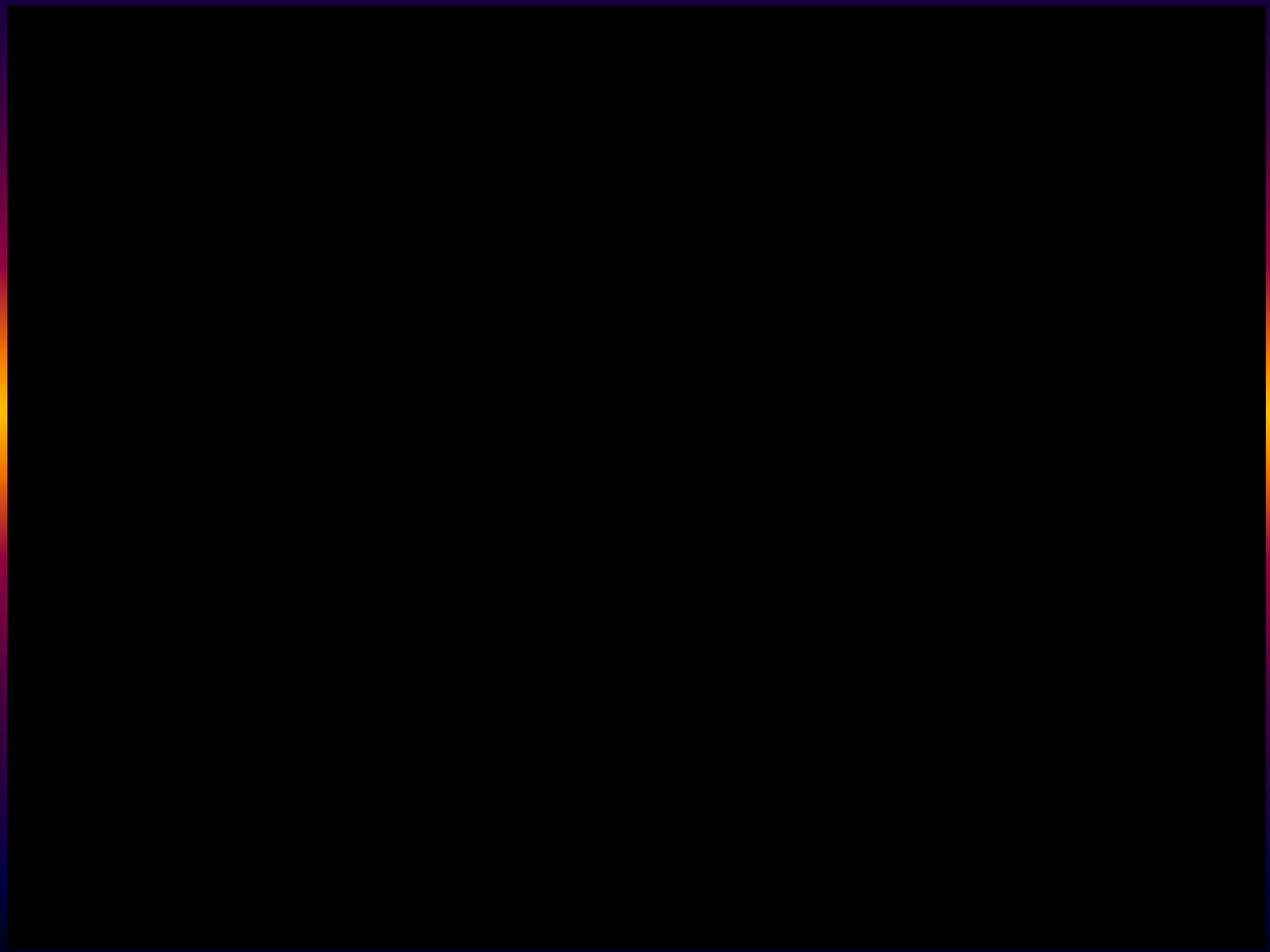
beam control

mounting & centering of crystals

data collection

data processing

Sample unmounting (magnetic cap)



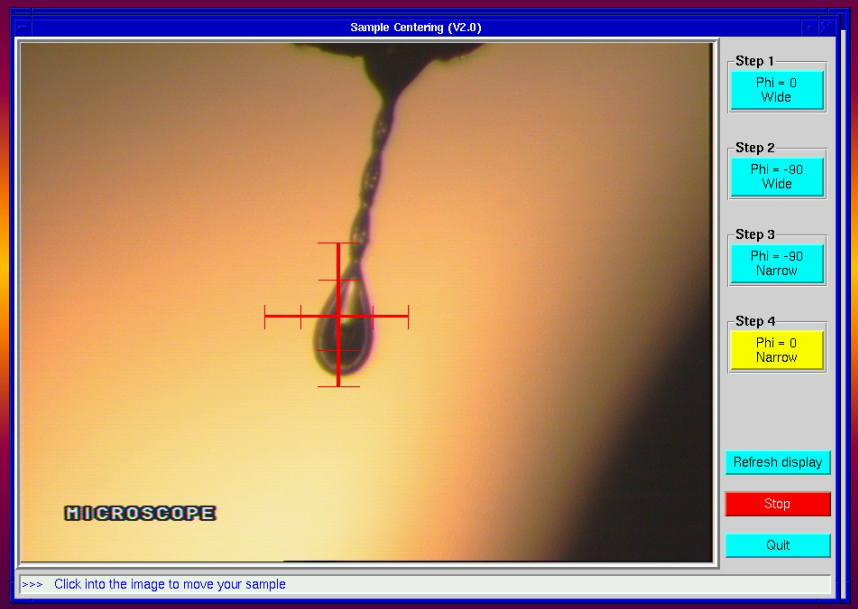
beam control

mounting & centering of crystals

data collection

data processing

Semi-automated crystal centering



… steps 1, 2, 3 and 4 in chronological order

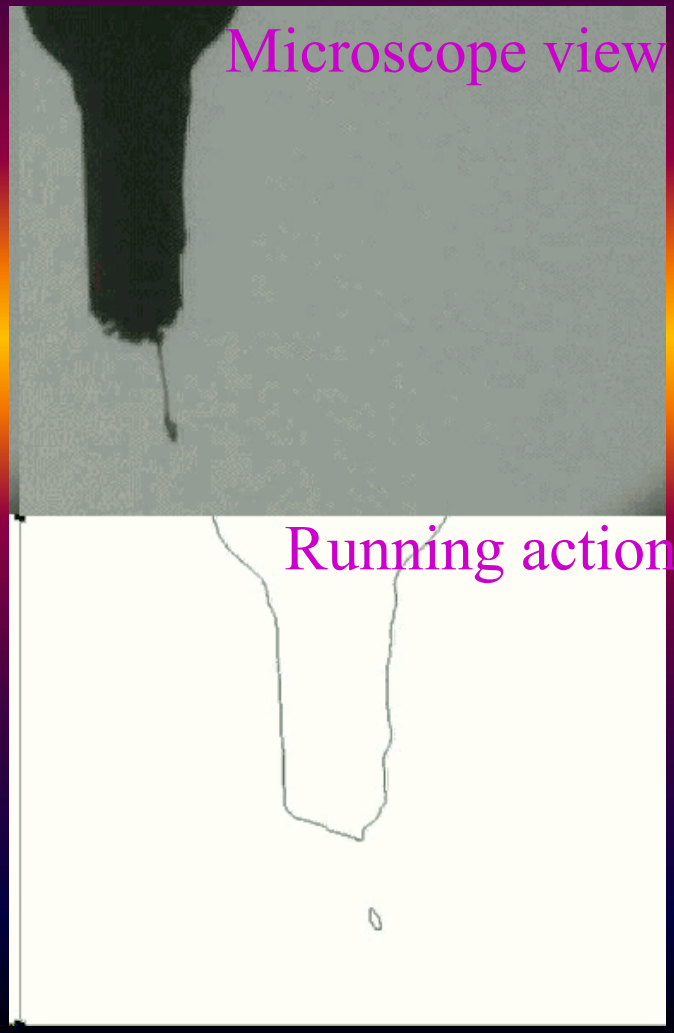
beam control

mounting & centering of crystals

data collection

data processing

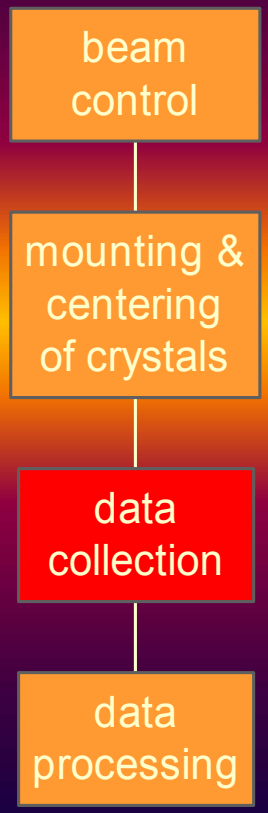
Automated crystal centering



Crystal recognition

standard **UV**

3-Automated experiment: data collection



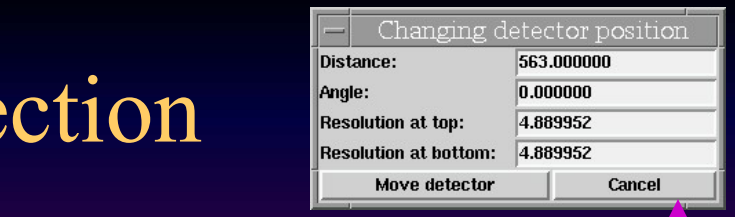
beam control

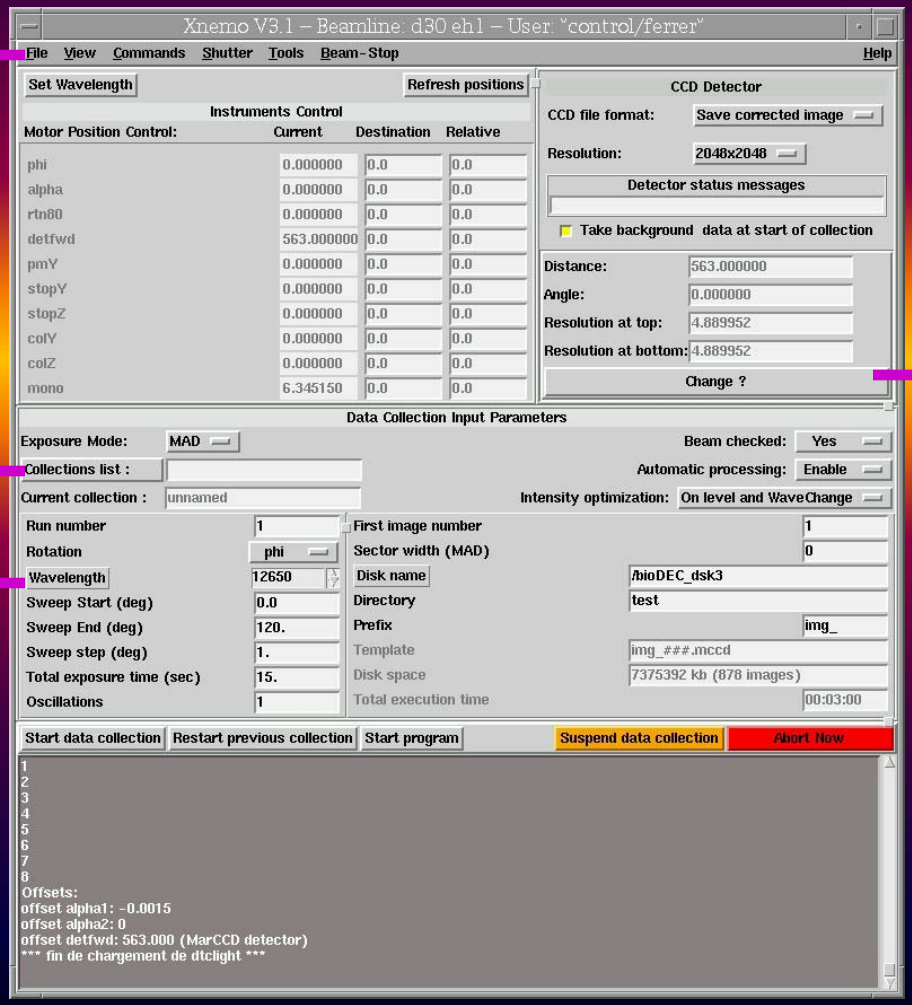
mounting & centering of crystals

data collection

data processing

Preparing data collection





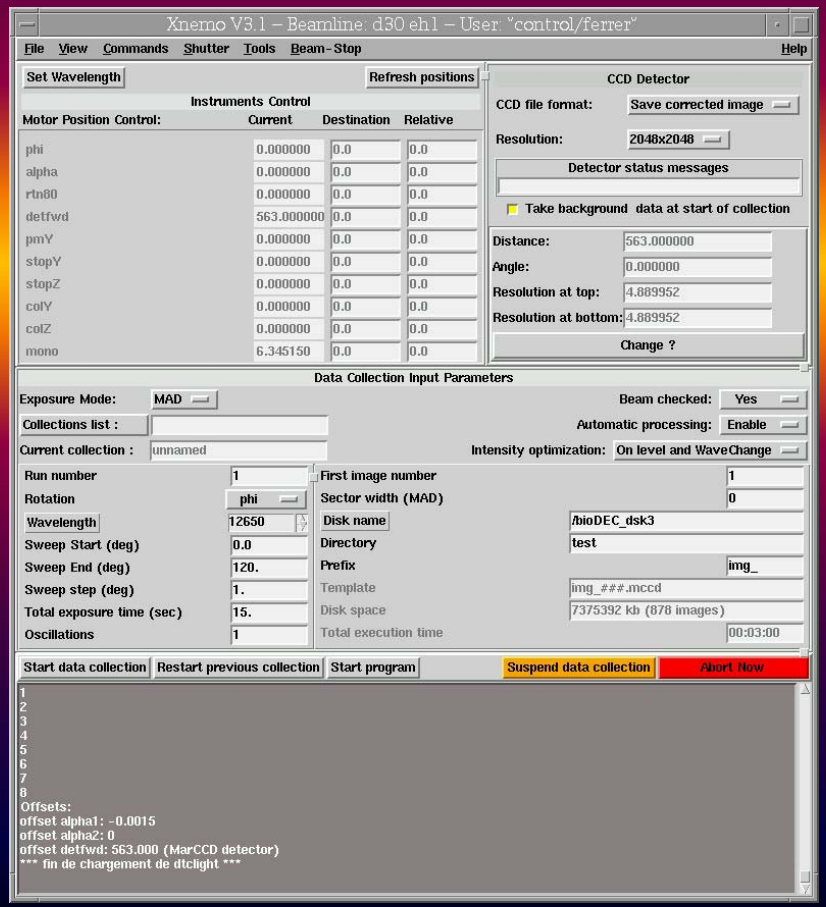
beam control

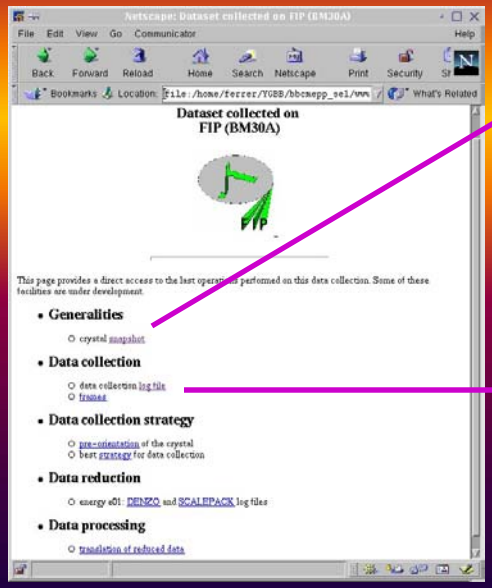
mounting & centering of crystals

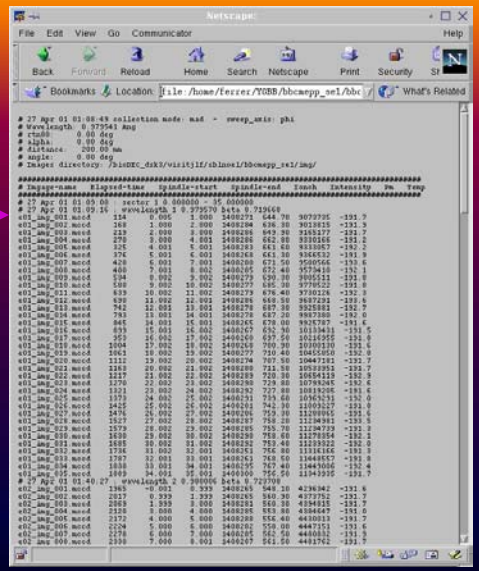
data collection

data processing

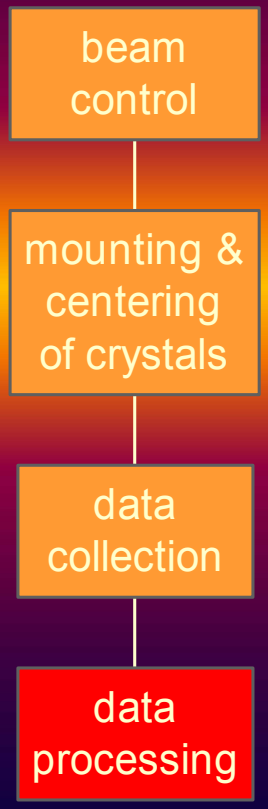
Xnemo

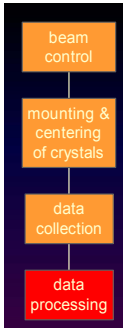






4- Automated experiment: data processing





adp: steps

peak search (MarSearch) ⇒ peak size ⇒ box size
indexation (denzo) ⇒ laue group, nbr. residues
mosaicity ⇐ *denzo log file* (*iterations*)
crystal reorientation (oXo)
strategy (strategy/best) ⇒ expected completness
integration (denzo)
partials ⇐ *interruptions*
scaling (scalepack) ⇒ anomalous signal
extinctions ⇒ final space group
Patterson map, … (CCP4) ⇒ max resol. for anomalous

site search (solve) molecular replacement (molrep)
heavy atoms
SAD phasing (solve)
solvent fl. (resolve)
model building (resolve)

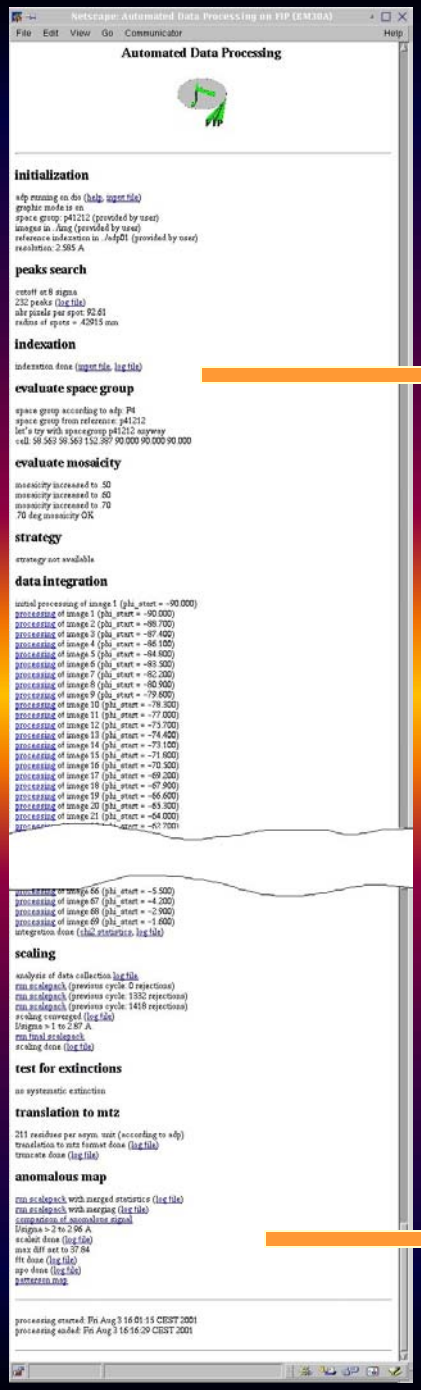
beam control

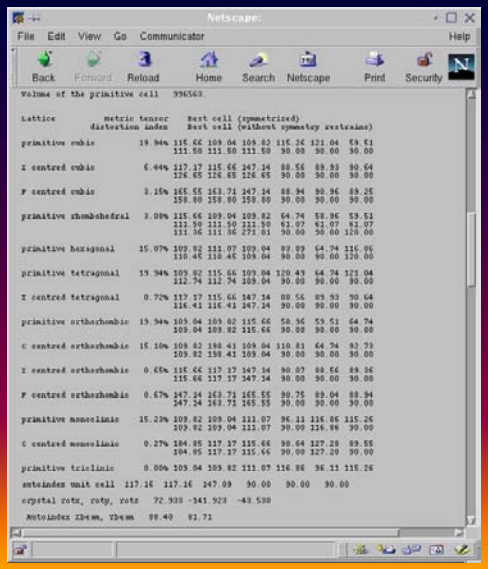
mounting & centering of crystals

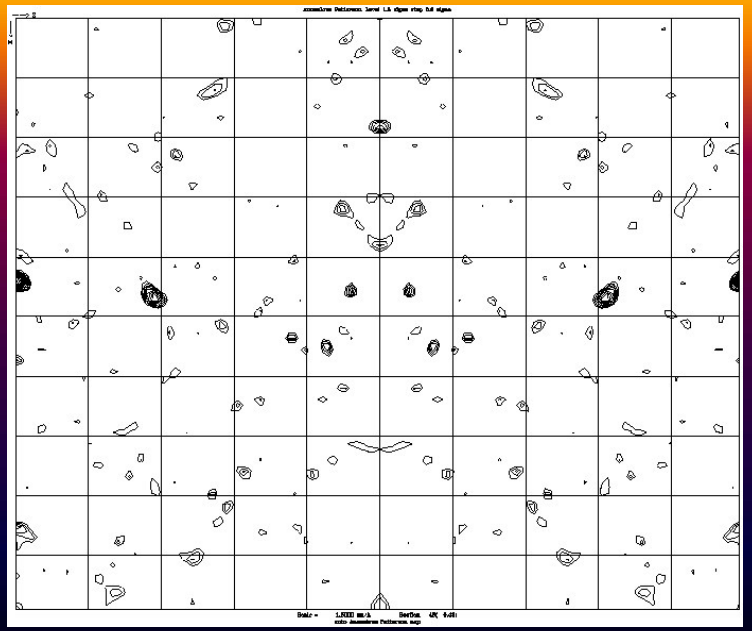
data collection

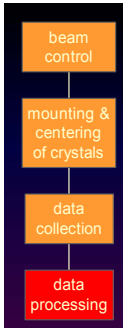
data processing

adp: log file









3.8 Å dataset processed by user *vs* adp

I	sigI	Average stat.	Norm. Chi**2	Linear R-fac	Square R-fac	
3356.8	285.7	267.0	0.451	0.125	0.083	⇐ user
3281.8	259.9	238.4	0.552	0.128	0.090	⇐ adp

% of reflections with I / Sigma less than

0	1	2	3	5	10	20	>20	total	
6.9	23.1	34.4	42.5	53.6	69.3	84.1	15.4	99.5	⇐ user
6.1	21.4	32.6	40.5	51.5	68.0	83.4	16.2	99.6	⇐ adp

Comparison of a typical dataset, processed by a crystallographer using *HKL* and with *adp*. Each data processing is illustrated with statistics calculated by *scalepack* (upper part: I, K^2 and R-factors; lower part: I/σ(I) and completeness).

beam control
mounting & centering of crystals
data collection
data processing

First structure solved in automated mode

Structure solved in
3h data collection
2h phasing/building

Data collection statistics:

p212121, 1 mol./asym. unit

completeness at 2.1 Å : 98.7%

<Rsym> : 4.2%

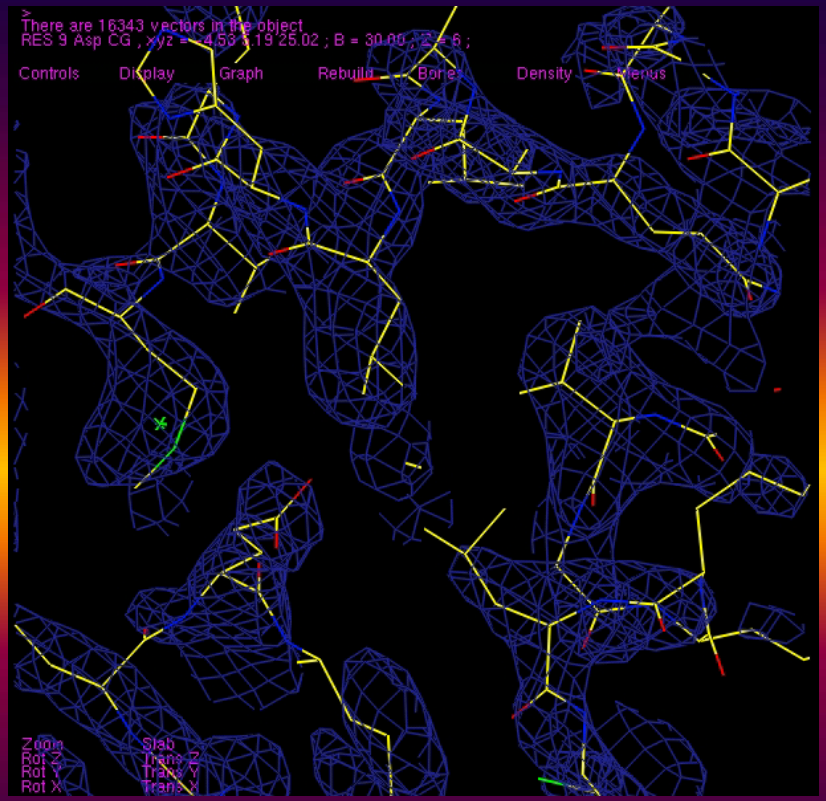
Rsym (last shell) : 12.5%

Phasing statistics (solve/resolve):

fig. of merit: 0.25

180/233 residues built autom.

80% of the model in 5 hours



Farnesoid X Receptor (FXR),
M. Downes et al.,
Molecular Cell 11 (2003), 1079

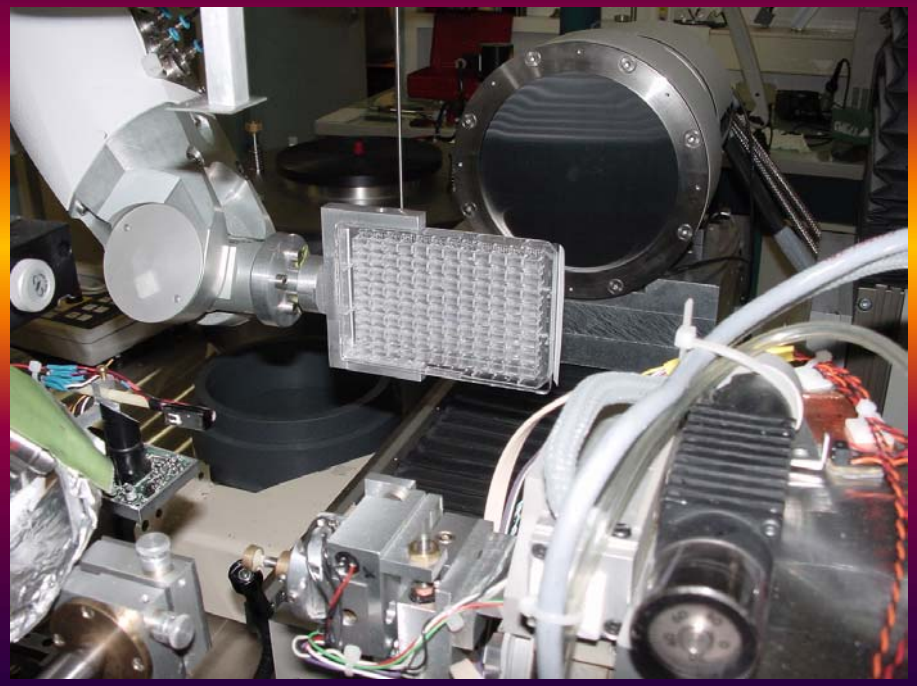
crystallization tray

crystallization analysis

Analysis of crystallization drops

Aim:

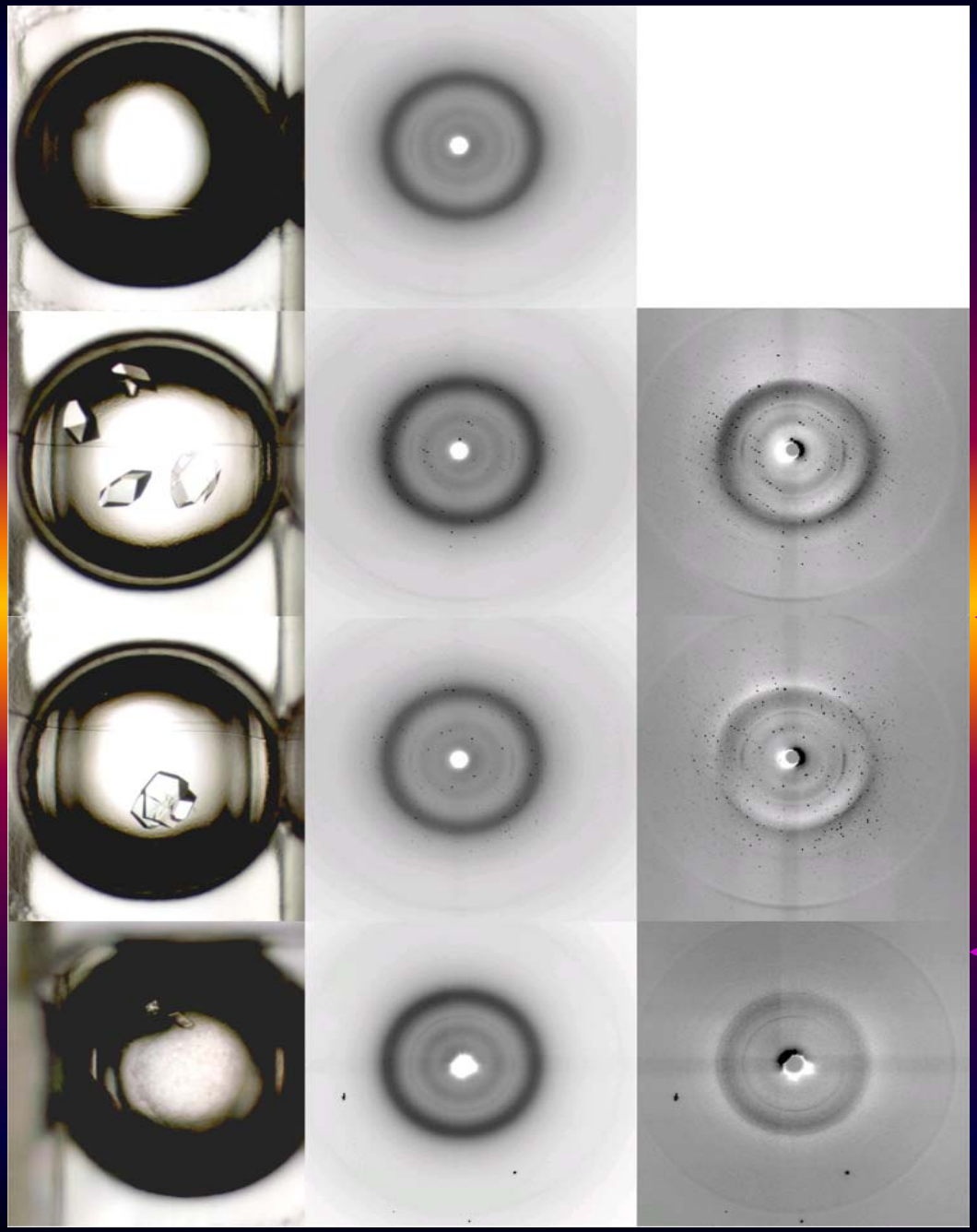
automated analysis of Greiner™ box
discrimination salt/protein
precipitate analysis ?



Means:

beam: 2x2 mm, 0.8 Å
oscillations: 1 deg
10 to 30 sec / drop (1 to 2 h per box)
image processing

Protein *vs.* Salt



Protein crystal

Salt crystal

Precipitate analysis !

Data collection *in situ*

Plate-drop	L-B3b	T-C12a	T-B4b	C-B1c	Y-A11a
sample	lysozyme	lysozyme	thaumatin	chalcone s.	kinase
Space group	p43212	p43212	p41212	p3121	c2
Resolution (Å)	1,8	1,8	2,2	3,0	2,2
Completeness (%)	89,8	85,1	95,1	70,0	48,2
Redundancy	2,6	2,8	2,4	2,6	1,3
I/σ	11,0	9,1	6,1	4,2	6,1
Rsym (%)	6,2	8,4	11,5	23,9	7,1
Rfree (%)	26,9	26,7	27,2	24,2	28,9

L. Jacquamet et al., *accepted in* Structure

J.-L. Ferrer	(beamline responsible)
L. Jacquamet	(crystallographer)
J. Joly	(software/hardware)
P. Charrault	(electronic)
M. Pirocchi	(vacuum)
J. Ohana	(robotics)
R. Kahn	(adviser)
F. Borel	(local contact)
S. Fieulaine	(local contact)
L. Serre	(local contact)
J. Dupuis	(local contact)
P. Israel-Gouy	(administration)
A. Bertoni	(machining)
M. Roth (retired)	(adviser)
P. Carpentier (IBS/BM16)	(instrumentation)
E. Fanchon (IBS/LCM)	(software)