Synchrotron Sources

Applications in Chemistry, Structural Biology, Medicine & Cultural Heritage

Firenze, October 2016

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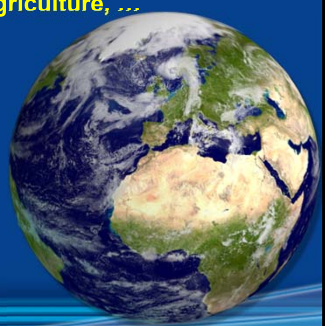
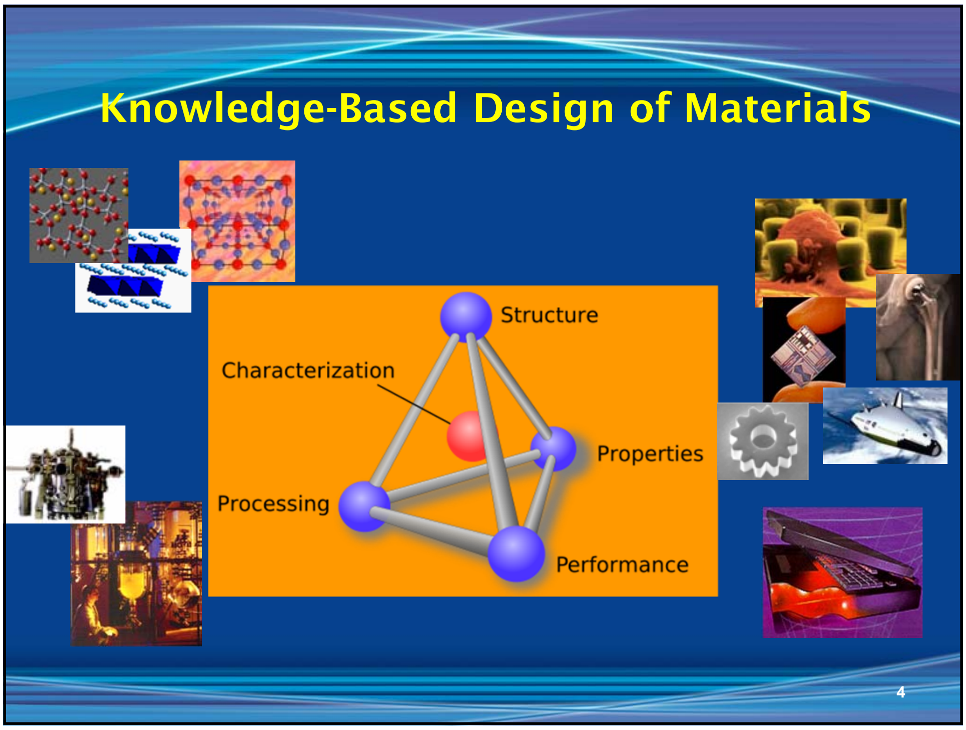
The Challenge for the Future of our Society

Identify models for sustainable development and Improve quality of life with a "durable" approach

- Environment and Climate Changes Mitigation and Understanding
    - global warming
    - pollution: atmosphere, water supplies, soil
    - food supplies and environmentally friendly agriculture, …
- Transport
    - energy sources alternative to oil: photovoltaic, wind, tides, ….
    - higher efficiency, limit waste, ….
- Health and Medicine
    - understand the mechanism of life from the molecular level
    - new drugs and treatments, …
    - ……



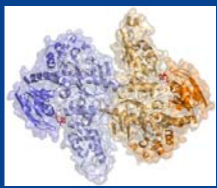

Investigating Materials
Technological progress
is often linked to progress
in understanding materials
Stone-age
Oil-age
Bronze-age
Silicon-age
Steel-age
3

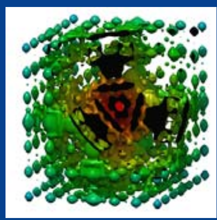

Knowledge-Based Design of Materials
Structure
Characterization
Properties
Processing
Performance
4

Investigating Matter and Materials

Matter is made of atoms – electrons and nuclei.

Neutron & Synchrotron Sources provide tools to understand materials on the atomic length- and time-scales, answering the fundamental and applied questions on the matter surrounding us.





Investigating Matter and Materials

Fields of application

Understanding matter links the many scientific disciplines :

Solid-state physics	Nuclear & particle Physics	Chemistry	Medicine	Life sciences
•Magnetic / electronic properties •Atomic structure	•Cosmology	•Structure / dynamics of new substances •Structure of interfaces	•Pharmaceutical molecules •New therapy protocols	•Protein cristallography •Protein dynamics

Neutrons

Particle beam (neutral subatomic particle)

Interactions with the nuclei and the magnetic moment of unpaired electrons (in the sample)

Scattered by all elements, also the light ones like the hydrogen isotopes

Deep penetration depth (bulk studies of samples)

Less intense beam measuring larger samples

Synchrotron Radiation

Light beam (electromagnetic wave)

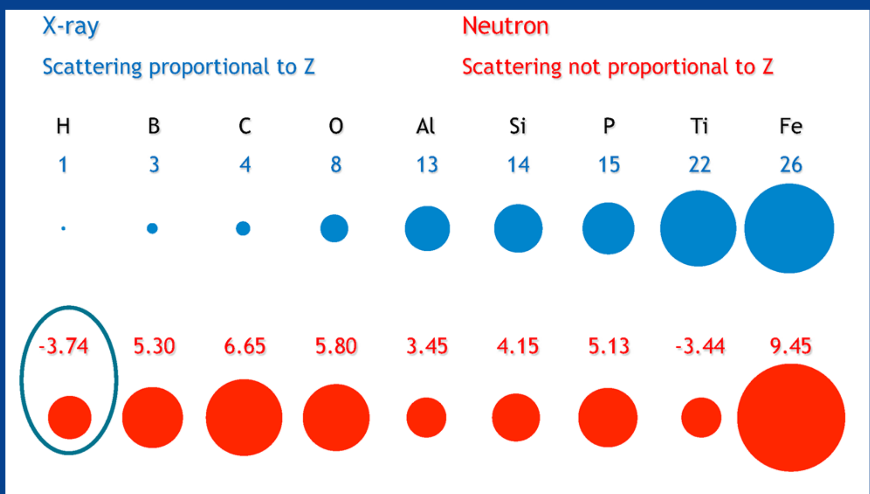
Interactions with the electrons surrounding the nuclei (in the sample)

Mainly scattered by heavy elements

Small penetration depth (surface studies of samples)

Very intense beam measuring small or ultra-dilute samples

Scattering: X-ray / Neutron



Neutrons

Applications:

- Magnetic structures & excitations
- Organic structures using the H-D isotope effect
- Bulk studies (strains, excitations)
- Low-energy spectroscopy e.g. molecular vibrations

Synchrotron Radiation

Applications:

- Protein-crystal structures
- Fast chemical reactions
- Surface studies (defects, corrosion)
- High-energy spectroscopy e.g. measurements of electron energy-levels

Major Neutron Sources in the World

Neutron sources:
12 in Europe, 6 in North America, 5 in Asia and Oceania

ILL: The most powerful neutron source in the world

ESS: European Spallation Source to be built in Lund (Sweden)

ILL Members and Their Financial Participation

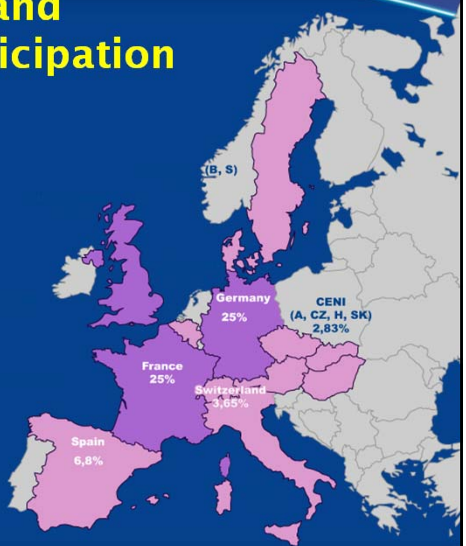
Associates: 75 %

France	25 %
Germany	25 %
United Kingdom	25 %

Scientific members: 21 %

- Spain: 6.80 %
- Switzerland: 3.65 %
- Italy: 4.46 %
- CENI, the Central European Neutron Initiative (Austria, the Czech Republic, Hungary, Slovakia) : 2.83 %
- BELSWENI, the Belgian-Swedish Neutron Initiative Consortium: 3.28 %
- Denmark: 0.24 %

Contracts : 4 %

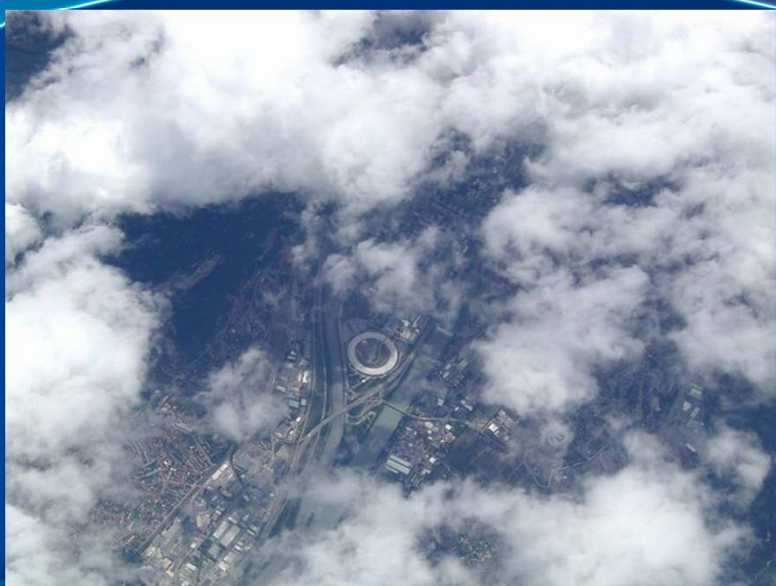


Major X-Ray Sources in the World

ESRF was the world's first 3rd generation hard X-ray source

Other hard X-ray sources: APS (USA) - SPring-8 (Japan) – Petra-III (D)

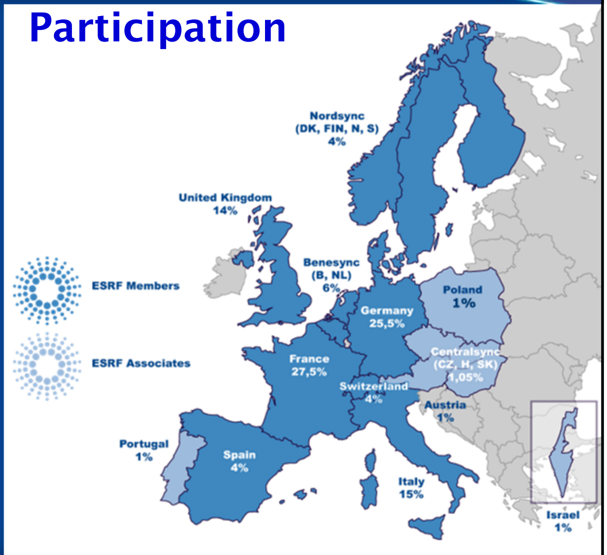
New national sources in Europe: Soleil (F) in 2006, Diamond (UK) in 2007, Petra-III (D), ALBA (E) in 2010, Lund ….



ESRF Members & Their Financial Participation

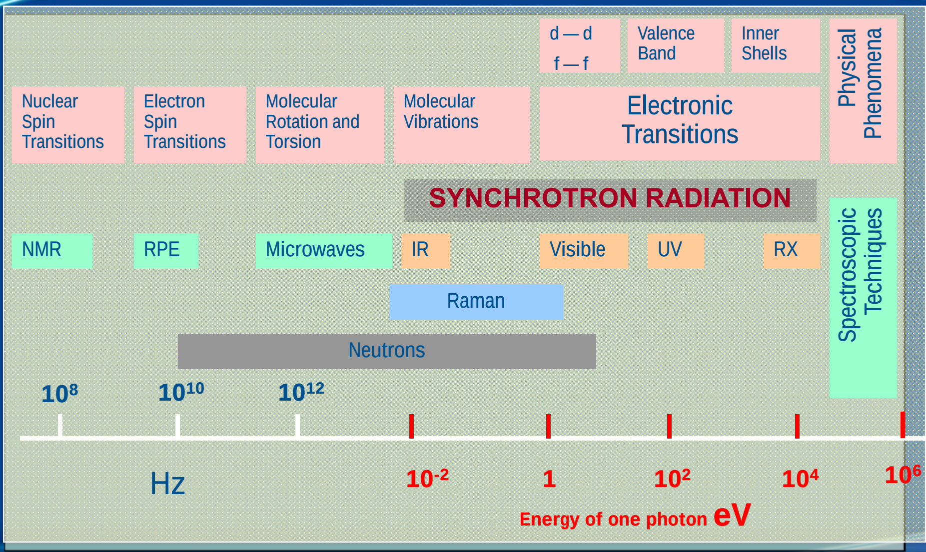
27.5% France
25.5% Germany
15% Italy
14% United Kingdom
4% Spain
4% Switzerland
6% Benesync
(Belgium, The Netherlands)
4% Nordsync
(Denmark, Finland, Norway, Sweden)

1% Portugal
1% Israel
1% Austria
1% Poland
1.05% Centralsync (Czech Republic, Hungary, Slovakia)



Synchrotron Radiation:

Intense (coherent) light from accelerated electrons



The Synchrotron Radiation

A synchrotron is an accelerator of electrons. The electrons are maintained in a circular ring by magnetic field and produce X-Rays tangentially to their trajectory.

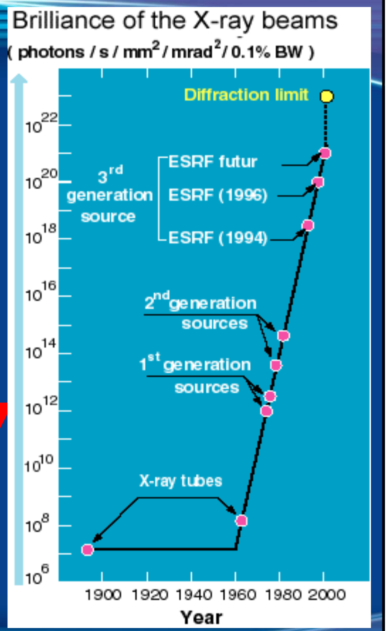
Electrons emitted by an electron gun are first accelerated in a ***linear accelerator*** (linac)** and then transmitted to a ****circular accelerator*** (booster synchrotron)** where they are accelerated to reach a high energy level.

These high-energy electrons are then injected into a circular ****storage ring*** ** where they circulate in a vacuum environment, at a constant energy, for many hours.

As they travel round the ring, the electron pass through different types of magnets, mainly bending magnets, undulators and focusing magnets.

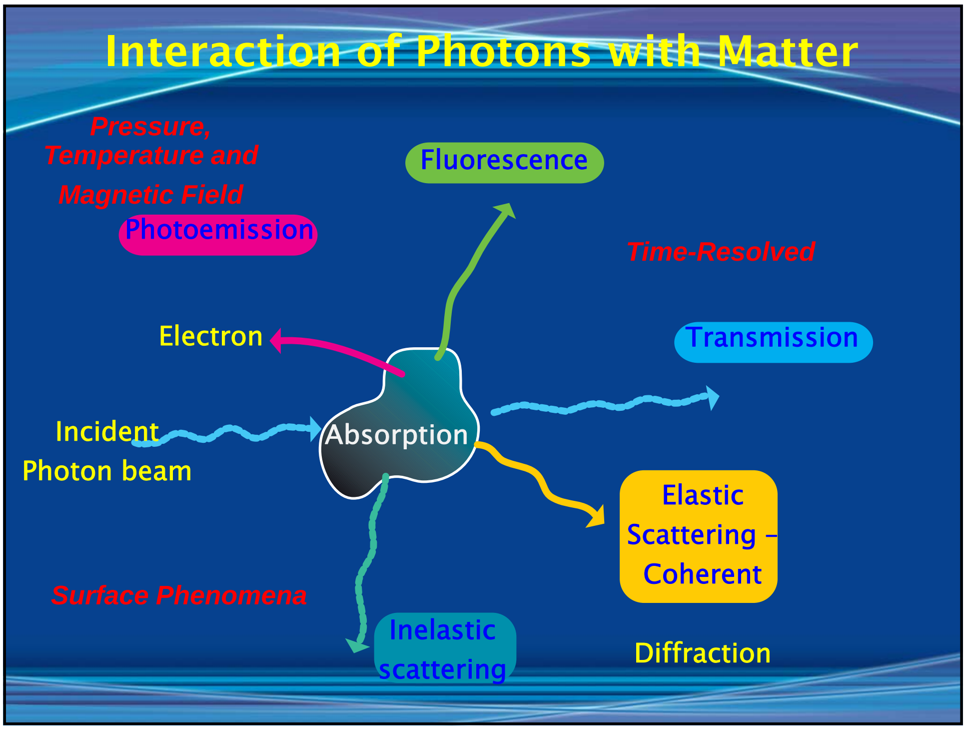
Synchrotron radiation is a universal tool, a swiss army knife for studying materials

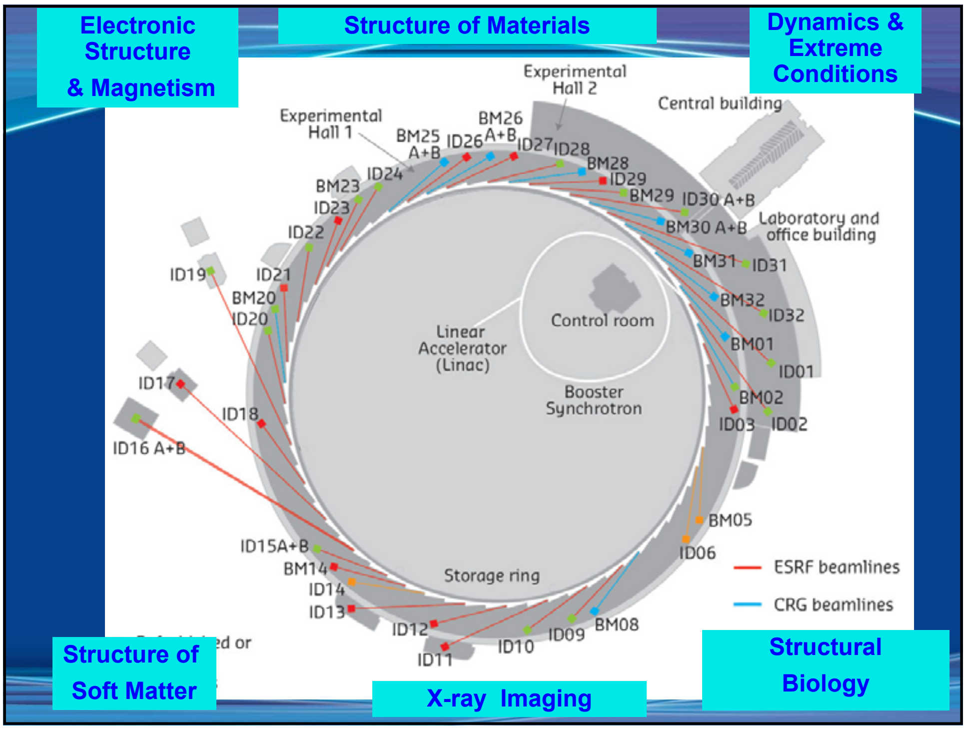
The tremendous success of the ESRF triggered the development of many 3rd generation synchrotron radiation sources in Europe

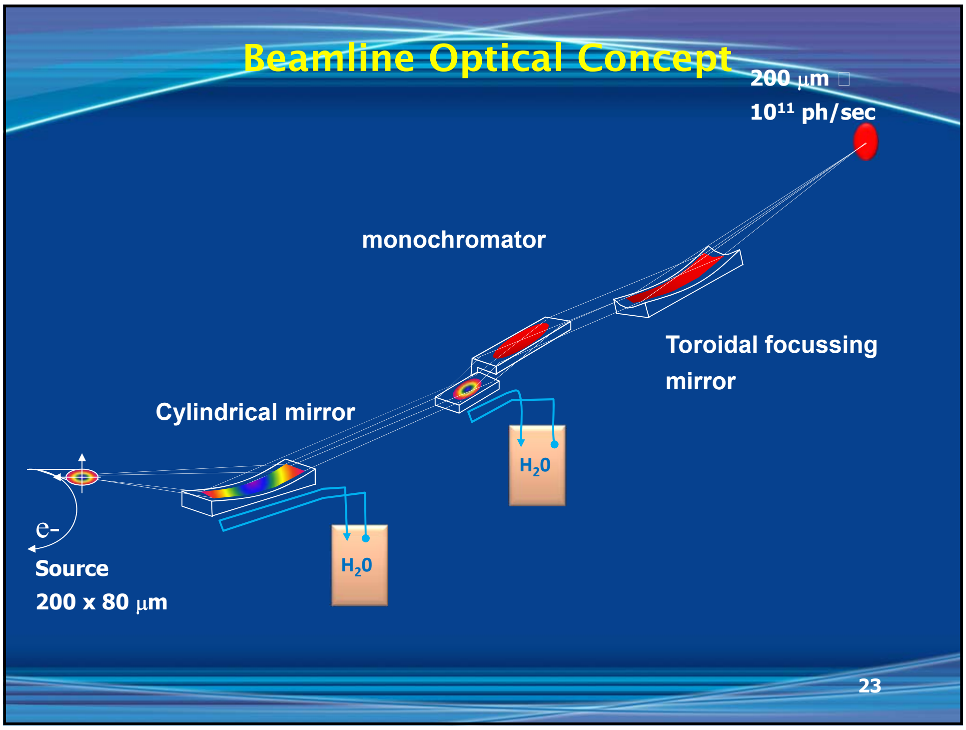


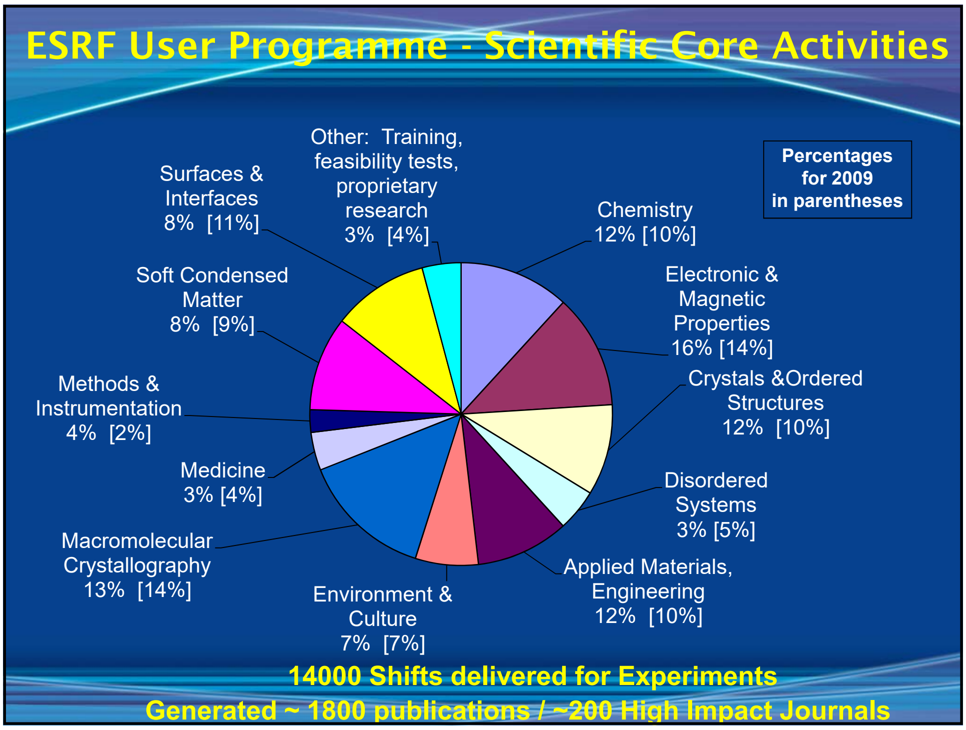
Vertical emittance : 3 pm
Horizonral emittance: 4 nm

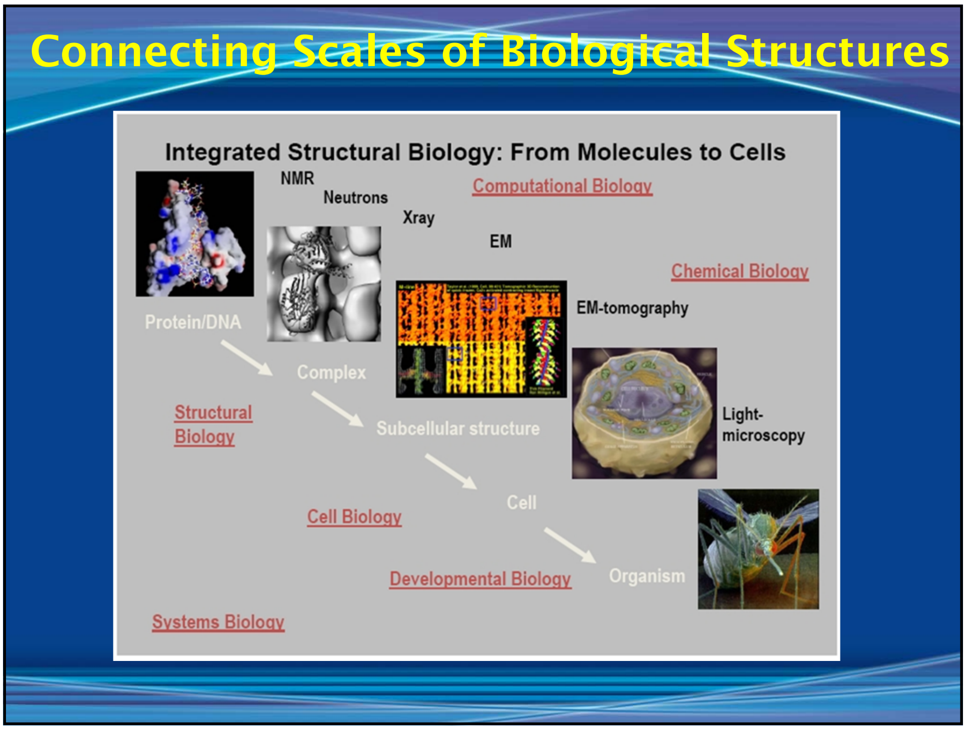
Availability (%)	**98.83**
Mean time between failures (hrs)	**178.7**
Mean duration of a failure (hrs)	**2.09**

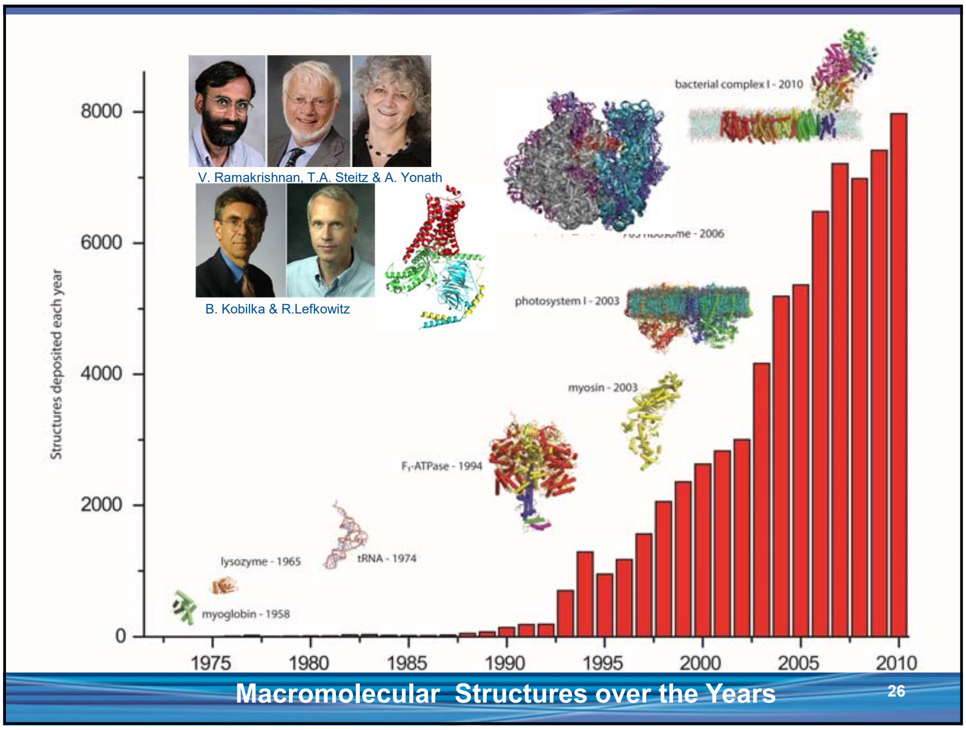

Interaction of Photons with Matter
Pressure, Temperature and Magnetic Field
Fluorescence
Photoemission
Time-Resolved
Electron
Transmission
Incident Photon beam
Absorption
Elastic Scattering – Coherent
Surface Phenomena
Inelastic scattering
Diffraction


Electronic Structure & Magnetism
Structure of Materials
Dynamics & Extreme Conditions
Experimental Hall 2
Central building
Experimental Hall 1
BM25 A+B
ID26
BM26 A+B
ID27
ID28
BM28
ID29
BM29
ID30 A+B
BM30 A+B
Laboratory and office building
ID24
BM23
ID23
ID22
BM31
ID31
ID19
ID21
BM20
ID20
BM32
ID32
BM01
ID01
Linear Accelerator (Linac)
Control room
ID17
ID18
Booster Synchrotron
BM02
ID03
ID02
ID16 A+B
BM05
ID06
ID15A+B
BM14
ID14
ID13
Storage ring
ESRF beamlines
CRG beamlines
ID12
ID11
ID10
ID09
BM08
Structure of Soft Matter
X-ray Imaging
Structural Biology


Beamline Optical Concept
200 µm
10^{11} ph/sec
monochromator
Toroidal focussing mirror
Cylindrical mirror
H_2O
H_2O
e-
Source
200 x 80 µm
23

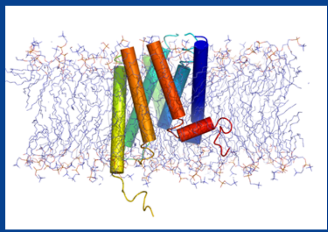

ESRF User Programme - Scientific Core Activities
Percentages for 2009 in parentheses
Other: Training, feasibility tests, proprietary research 3% [4%]
Surfaces & Interfaces 8% [11%]
Chemistry 12% [10%]
Electronic & Magnetic Properties 16% [14%]
Soft Condensed Matter 8% [9%]
Crystals &Ordered Structures 12% [10%]
Methods & Instrumentation 4% [2%]
Medicine 3% [4%]
Disordered Systems 3% [5%]
Macromolecular Crystallography 13% [14%]
Applied Materials, Engineering 12% [10%]
Environment & Culture 7% [7%]
14000 Shifts delivered for Experiments
Generated ~ 1800 publications / ~200 High Impact Journals

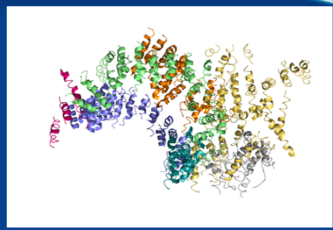

Connecting Scales of Biological Structures
Integrated Structural Biology: From Molecules to Cells
NMR
Neutrons
Xray
EM
Computational Biology
Chemical Biology
EM-tomography
Protein/DNA
Complex
Structural Biology
Subcellular structure
Light-microscopy
Cell
Cell Biology
Developmental Biology
Organism
Systems Biology


V. Ramakrishnan, T.A. Steitz & A. Yonath
B. Kobilka & R.Lefkowitz
bacterial complex I - 2010
photosystem I - 2003
myosin - 2003
F1-ATPase - 1994
tRNA - 1974
lysozyme - 1965
myoglobin - 1958
Structures deposited each year
8000
6000
4000
2000
0
1975
1980
1985
1990
1995
2000
2005
2010
Macromolecular Structures over the Years
26

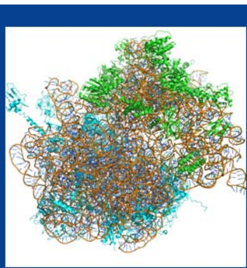
Crystals by Thousands…!

GPCR *1043 crystals*



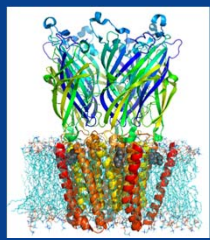


DNA depending Protein kinase > *2000*



Ribosome

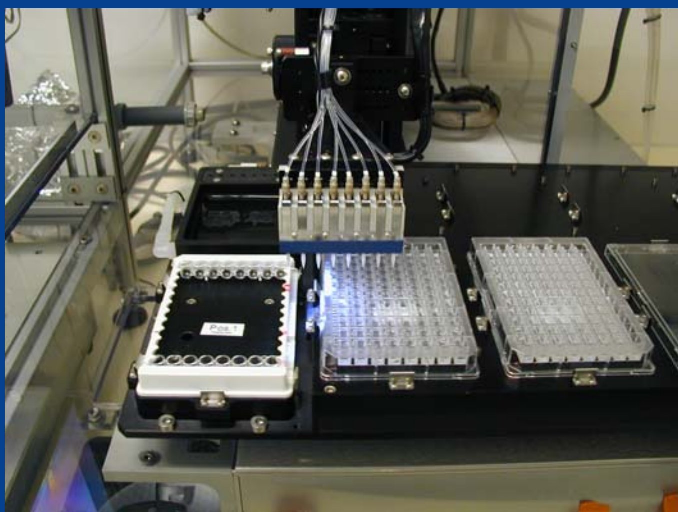
Thousands by thousands



> 1000

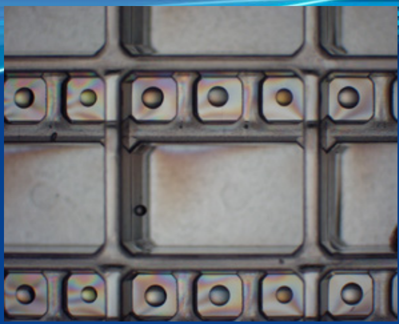
Anaesthetics bound to a pentameric ligand-gated ion channel

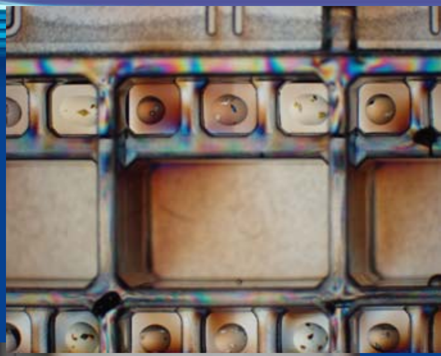
High Throughput Crystallisation

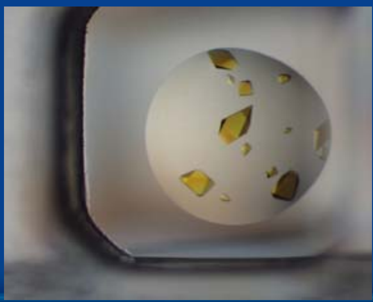


4°C

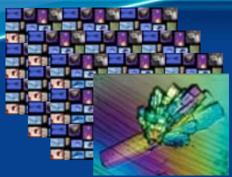
20°C







New Visions for Structural Biology

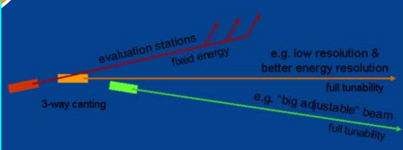


Building on unique ESRF know-how
- ♦ "second generation" beamline automation
- ♦ sample management
- ♦ robotics
- ♦ phasing

crystal evaluation & sorting
(including in-plate screening, dehydration experiments)

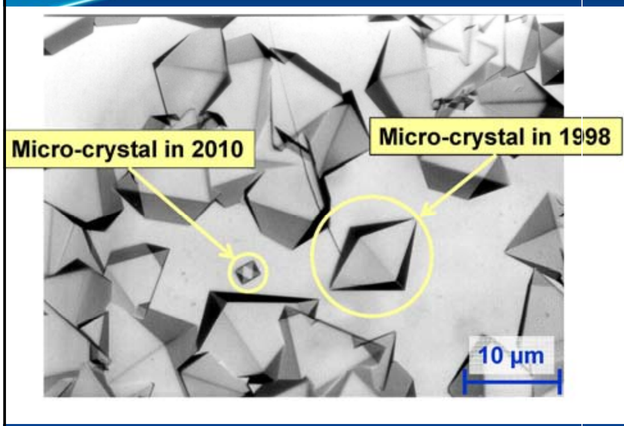
Specialised beamlines for
- ♦ low resolution (500 Å < d_{min} < 5 Å)
- ♦ flexible beam size
- ♦ long wavelength
- ♦ microbeam MAD/SAD
- ♦ small anomalous signals
- ♦ large unit cells (~1000 Å)

plus
- ♦ microspectrophotometry
- ♦ BioSAXS



- ♦ higher success rate for structure solution
- ♦ adaptability to specific data collection needs
- ♦ better beam time exploitation

Crystals Dimensions



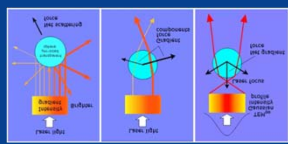
µ-focus beam lines:

ID23-2 (∅ ~ 5x7 µm)

ID 13 (70nm <∅< 1 µm)

Developping New Tools

Observation : AFM

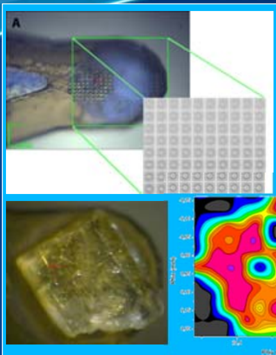


towards micro-fluidics

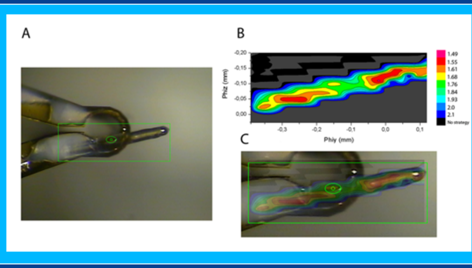
Optical tweezres

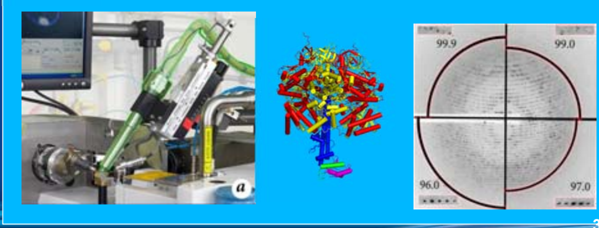
Dimensions of micro-crystals have changed!

New Approches….



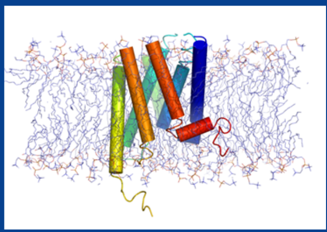
Cartography of the Diffracting Regions

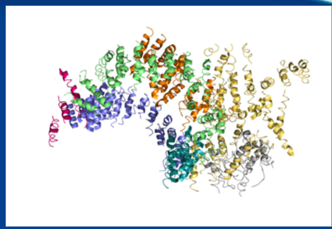




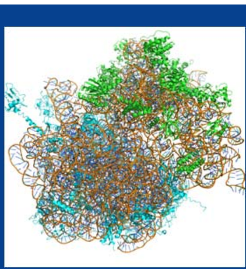
Crystals by Thousands…!

GPCR *1043 crystals*



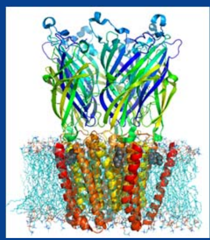


DNA depending Protein kinase *> 2000*



Ribosome

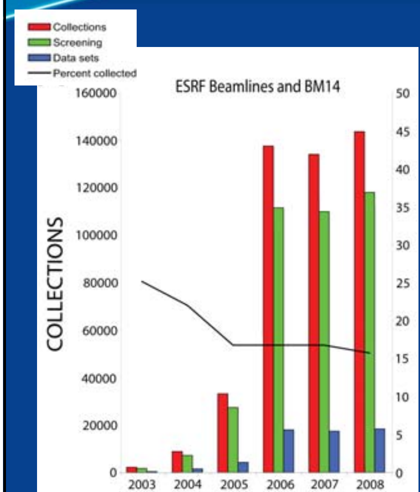
Thousands by thousands



> 1000

Anaesthetics bound to a pentameric ligand-gated ion channel

Evaluating prior Measuring…



A set of samples – eg several 1000 – Which one will answer the biological question being asked ?

Collect 2 diffraction images at (φ= 0 & 90°).

Characterise images – by eye or DNA – EDNA) for diffraction quality .

Rank samples against each other.

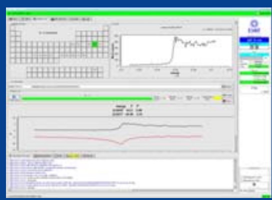
Collect data on the best samples

From Experiments to Measurements

Developping Tools for Automation

Sample Changer
(ESRF/EMBL)

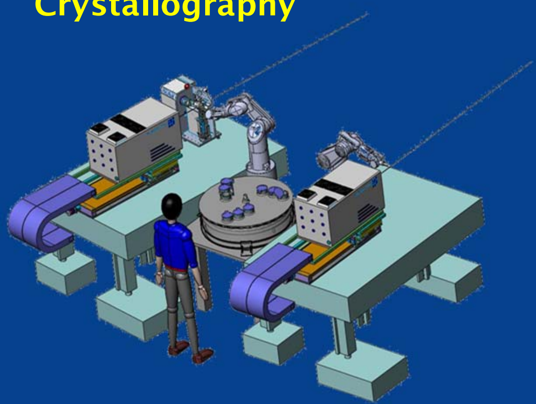
MXCuBE



DNA / EDNA

ISPyB

Massively Automated Sample Selection Integrated Facility for Macromolecular Crystallography



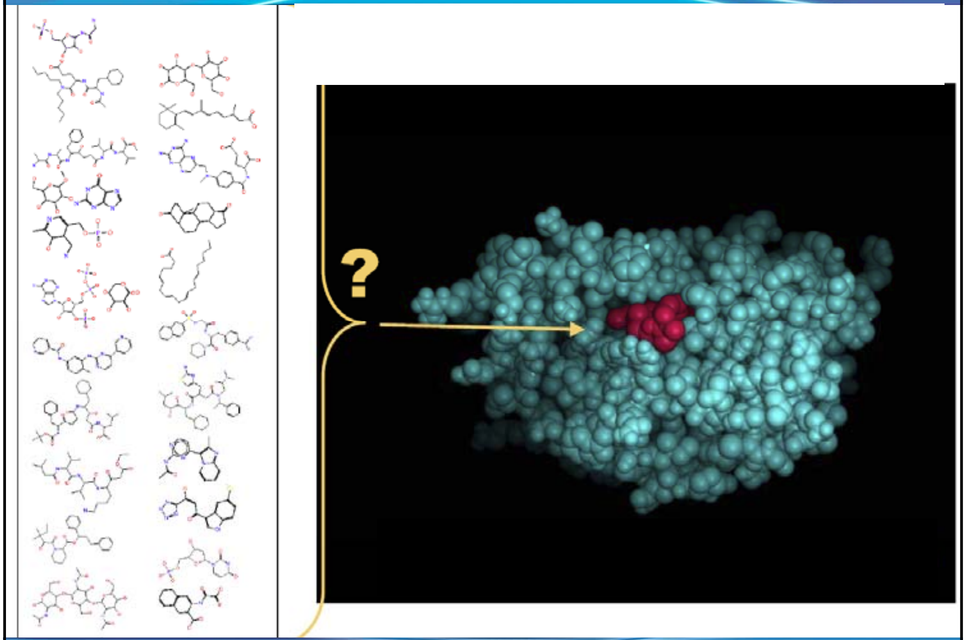
Capacity per Station: 1000 crystals per day

3 stations …. 600 000 crystals per year

Sphere of confusion < 20 μm

MASSIF (ESRF)

Screening Libraries......



Methodology
in silico-> in cellulo-> in cubo

Target 10^6

19824. 24951. 25457. 27592. 37553. 37627. 37641. 59620. 61610. 67436. 80313. 80997. 97920. 121861. 127133 . 156219 . 308835. 310325. 407628 . 601359. 5157. 54709 . 88916 .116702. 310326

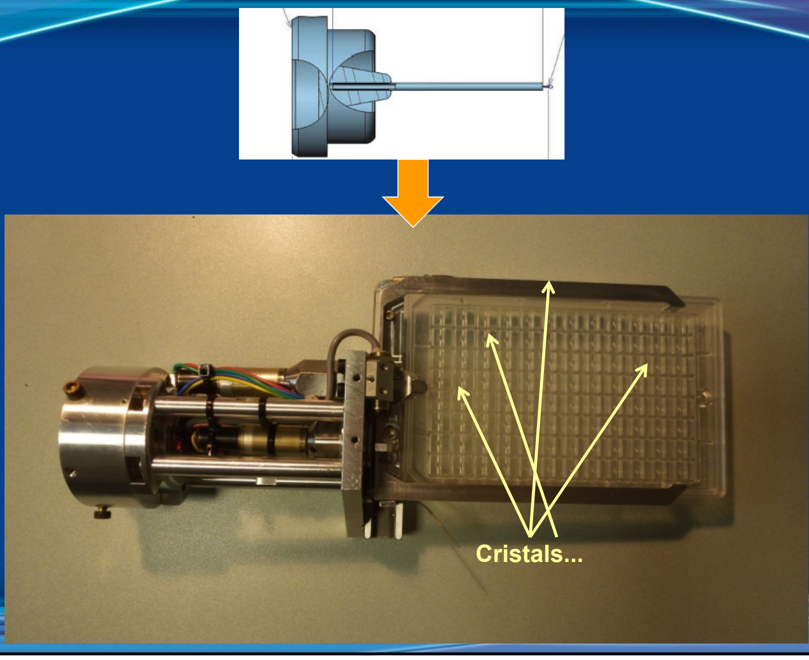
$x10^3$

In cellulo High Concentration Assay screening

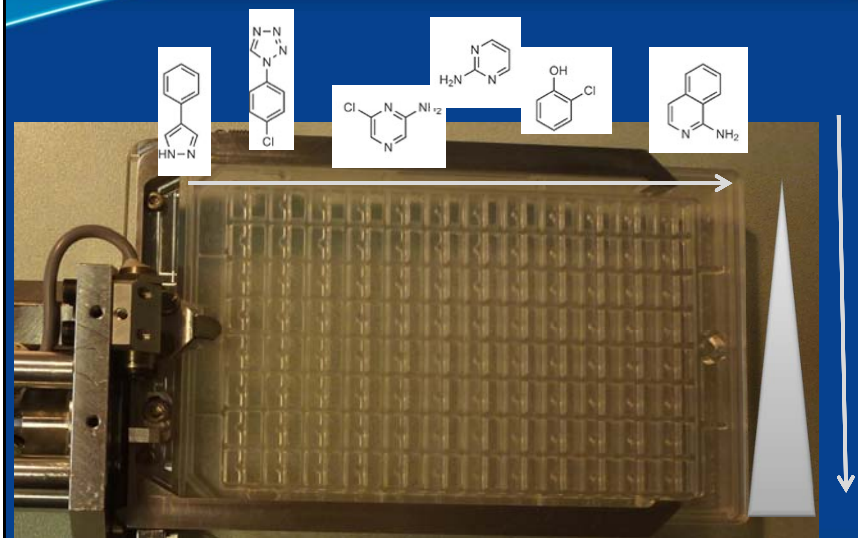
$x10^2$

In cubo screening !!!

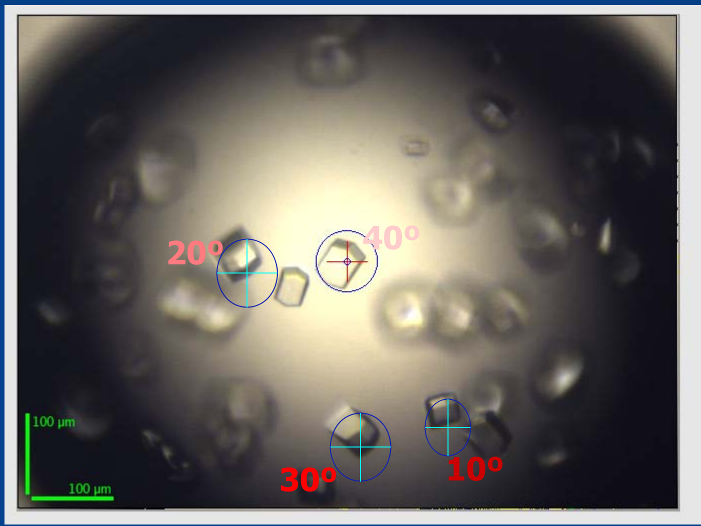
From Crystal to Crystals…



A new *in cubo* screening tool…

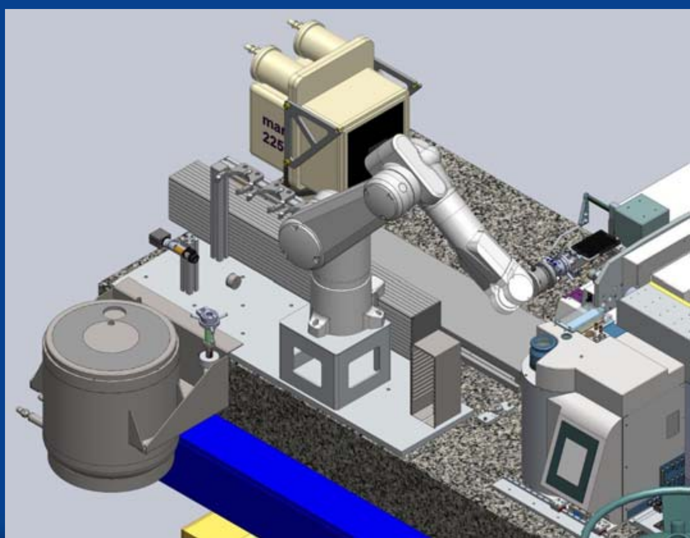


Finding the Crystals…..



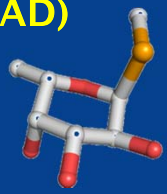
Excellent visualisation, 1 µm accuracy !

Employing Robotics for Drug Screenings



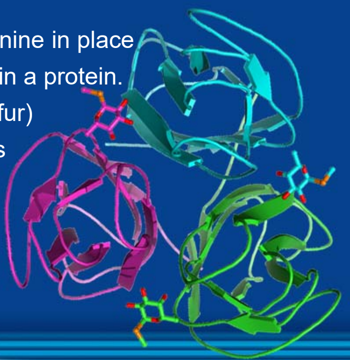
Seleno Methionine and Seleno Carbohydrate Multiple Wavelength Anomalous Dispersion (MAD)

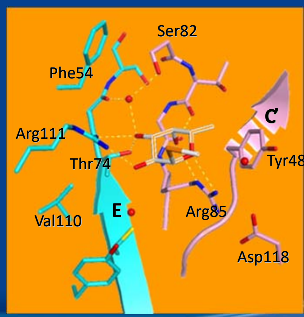
Anomalous diffraction is recorded at different wave lenghts of coherent X-ray light at a synchrotron facility near the absorption edge of an element in a crystal.



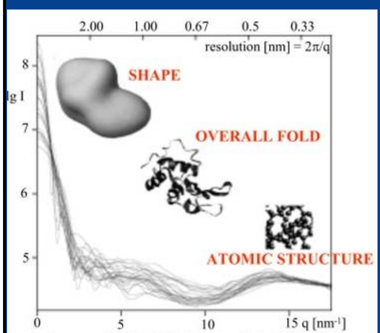
α-MeSe-L-Fuc

Introduce selenomethionine in place of methionine residues in a protein. Selenium (replaces Sulfur) has a strong anomalous signal at wavelengths obtained from synchrotron X-ray sources.

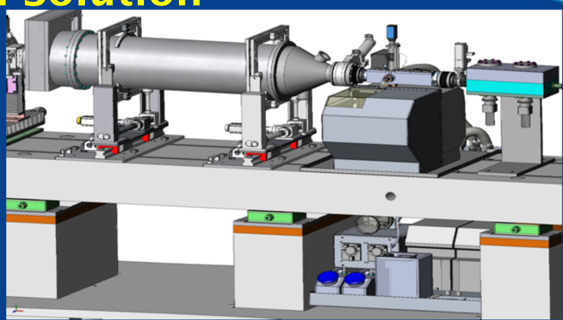




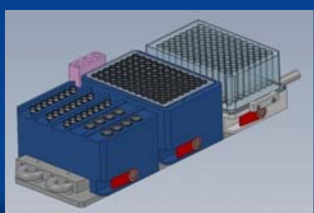
Conformation of Macromolecules In Solution



from D. Svergun and M. Koch, Biophysical methods (2002) 654-660



- Relative orientation of structural modules
- Comparaison 3D structures: Solution/Crystal
- *Ab initio* construction of macromolecular envelop



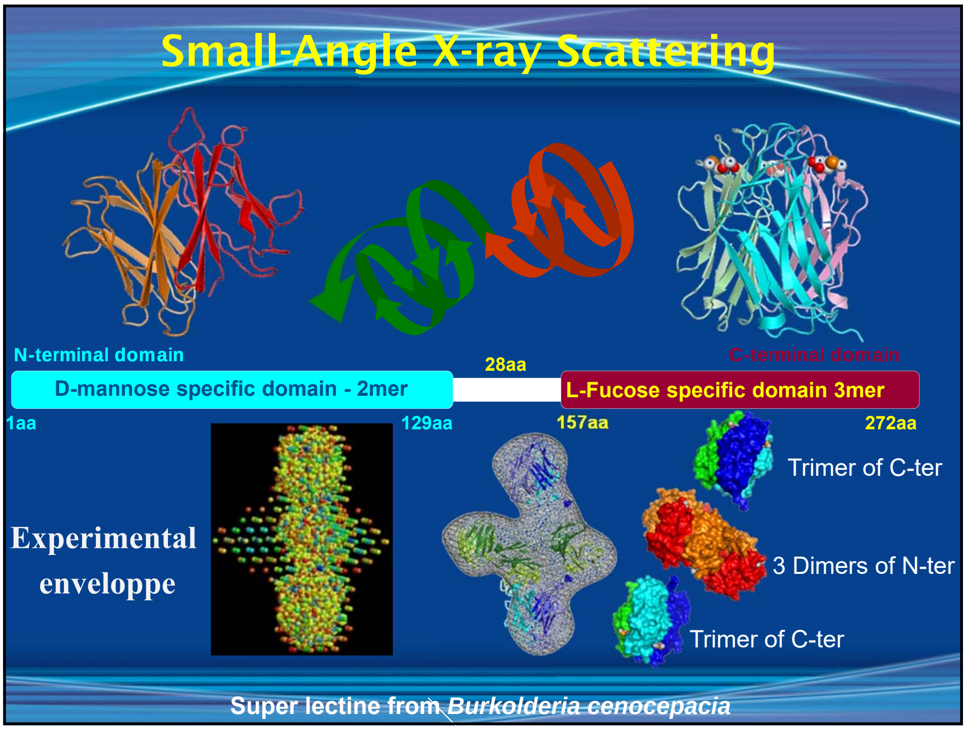
Concentration: 1-10mg/ml

Volume: 100μl

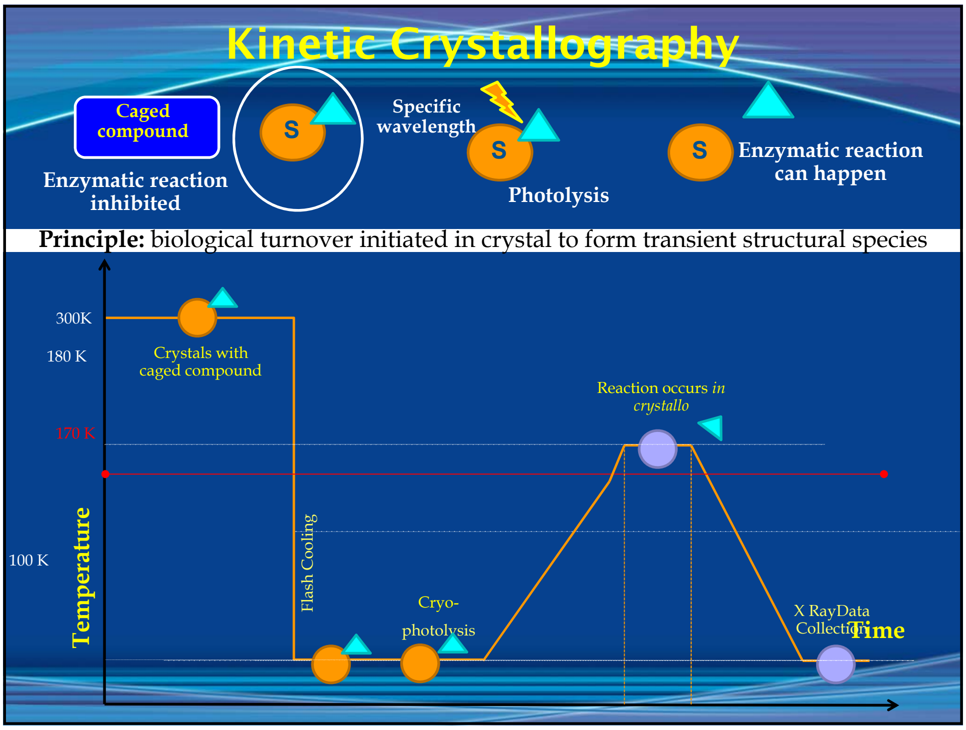
288 samples in the sample changer

Software for data Managemenyt ISPyB

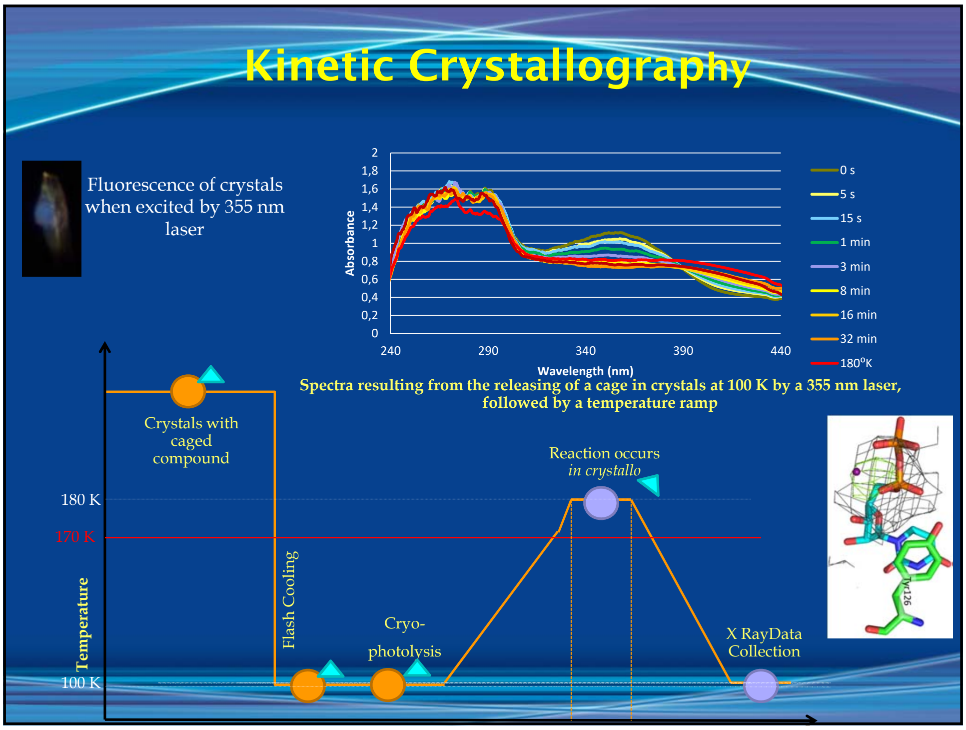
BioSASX (ESRF/EMBL)

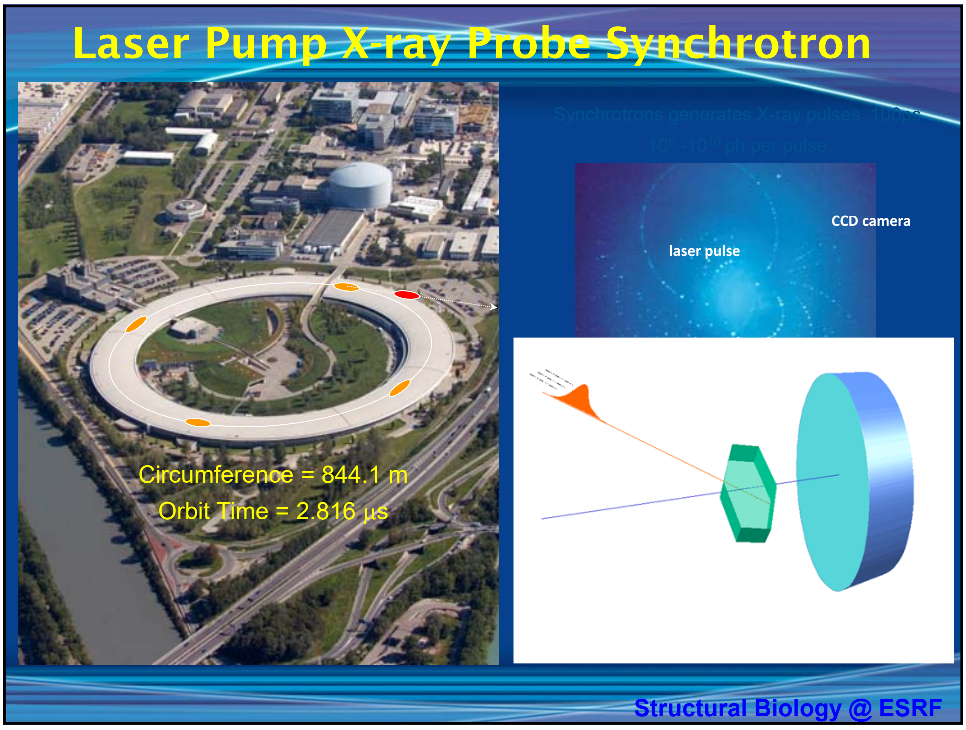


Small-Angle X-ray Scattering
N-terminal domain
C-terminal domain
28aa
D-mannose specific domain - 2mer
L-Fucose specific domain 3mer
1aa
129aa
157aa
272aa
Experimental enveloppe
Trimer of C-ter
3 Dimers of N-ter
Trimer of C-ter
Super lectine from *Burkolderia cenocepacia*

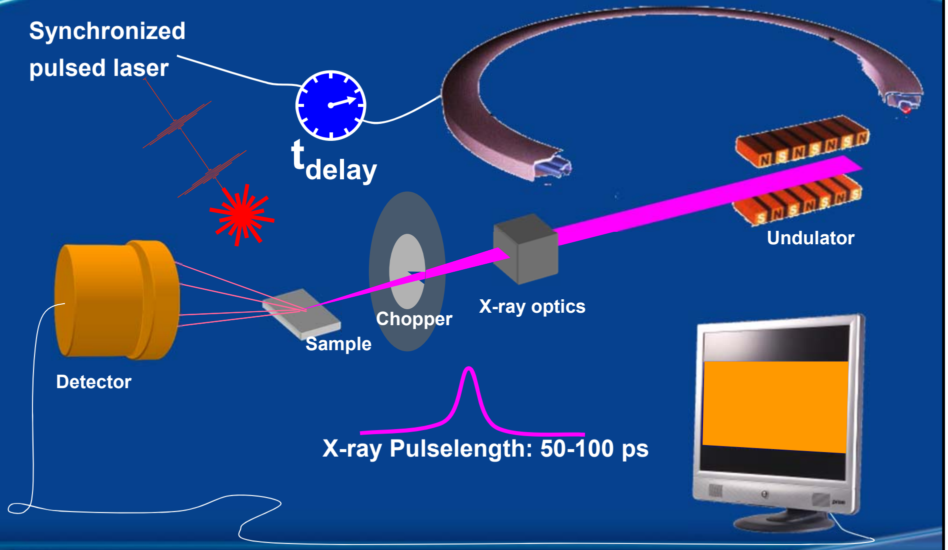


Kinetic Crystallography
Caged compound
S
Enzymatic reaction inhibited
Specific wavelength
S
Photolysis
S
Enzymatic reaction can happen
Principle: biological turnover initiated in crystal to form transient structural species
300K
180 K
170 K
100 K
Temperature
Crystals with caged compound
Flash Cooling
Cryo-photolysis
Reaction occurs *in crystallo*
X RayData Collection
Time


Kinetic Crystallography
Fluorescence of crystals when excited by 355 nm laser
Absorbance
Wavelength (nm)
0 s
5 s
15 s
1 min
3 min
8 min
16 min
32 min
180°K
Spectra resulting from the releasing of a cage in crystals at 100 K by a 355 nm laser, followed by a temperature ramp
Crystals with caged compound
Reaction occurs in crystallo
180 K
170 K
100 K
Temperature
Flash Cooling
Cryo-photolysis
X RayData Collection


Laser Pump X-ray Probe Synchrotron
Circumference = 844.1 m
Orbit Time = 2.816 μs
CCD camera
laser pulse
Structural Biology @ ESRF

Laser Pump X-ray Probe Synchrotron



BioMedical Research at the ESRF

Monochromatic and parallel X-rays for high resolution ($\geq$5 μm) medical imaging.

Monochromatic X-rays for brain tumor radiotherapy (SSRT)

Arrays of high energy microbeams for brain tumour treatment (MRT) and microsurgery.

Brain Tumours: Epidemiology

Malignant brain tumors: 100.000/year

~50 % are high grade tumors (in France: >3000 cases/year)

Morbidity: short life expectancy (2-36 months, median 11)

Children

Cancer of the Central nervous system is the second most common form of cancer, after leukemia

Sources: NIH 2004; J. Neuro-Onc., 2002 ; Childhood cancer information system

Radiotherapy, Neurosurgery, Chemotherapy are Only Palliative for High Grade Tumor*

ESRF:

Is *presently* the only SR source in the world for radiotherapy clinical trials:

- Dedicated biomedical BL
- Appropriated spectrum and fluency
- Strong connections with Hospital teams

Ultimate Goal of Radiotherapy

Cessation of tumor growth

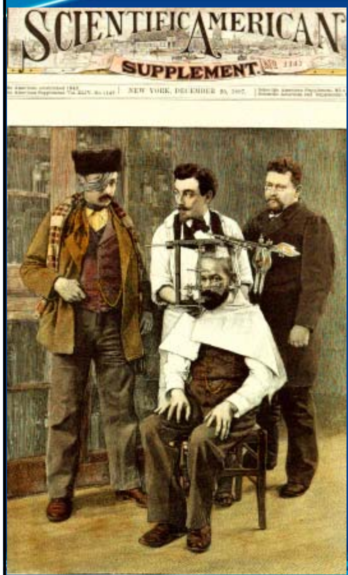
No radiotoxic side-effects

In practice: use of the highest doses tolerated by normal tissues in the vicinity of the tumor

Keywords:

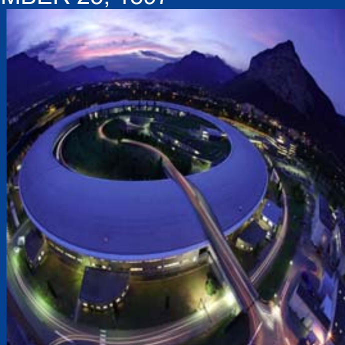
- Cure
- Tissue sparing

Stereotactic Synchrotron Radiation Therapy (SSRT)



NEW YORK, DECEMBER 25, 1897

100 YEARS



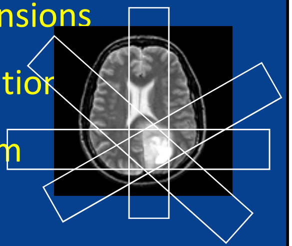
MEDICAL APPLICATIONS OF X-RAYS
FROM CROOKES TUBES TO SYNCHROTRONS

ESRF

APPARATUS FOR DETECTING WITH THE AID OF CROOKES TUBES,

Principles

– Tumor loaded with a high Z element
–(iodine, gadolinium) and/or chemotherapic drug
–(cis-platinum)--→ local dose enhancement.
– Beam size adjusted to the tumor dimensions
– Tumor positioned at the center of rotation
– Irradiation with kilo-Voltage X-ray beam



Preclinical studies

- Tumor model: F98 glioma
- Animal model: Fisher Rat

…..iodine…..

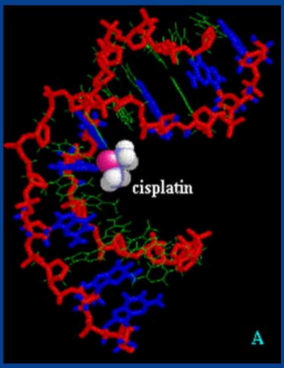
Tumor is irradiated (15 Gy) after infusion of iodine (2 ml, 350 mg/ml): bolus + continuous infusion

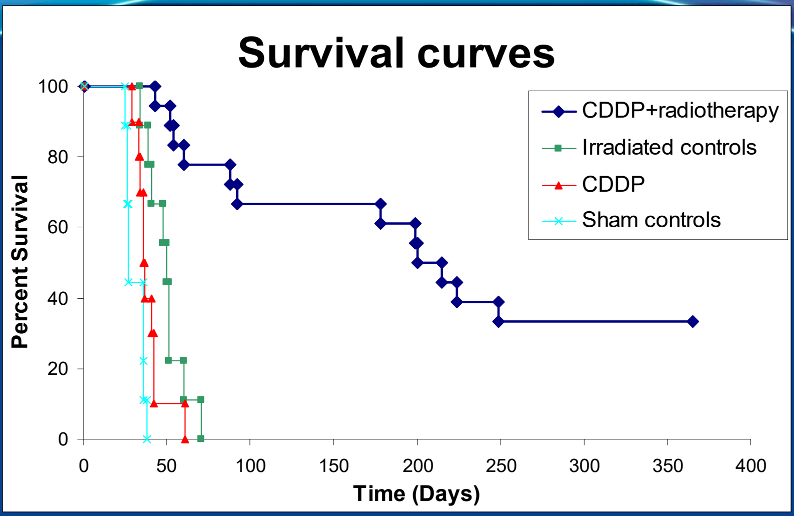
Increase of mean life span: +170 %

Chemotherapy + Radiation Therapy (bi-therapy)
Inoculation of a Platinum compound

- Cis-DiammineDichloroPlatinum (CDDP)
  - Chemotherapy agent
  - CDDP binds to DNA
  - Irradiation @78 keV

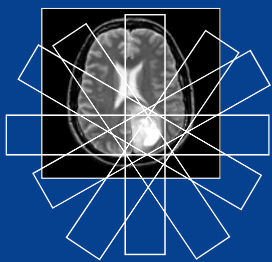
    15 Gy @ tumor

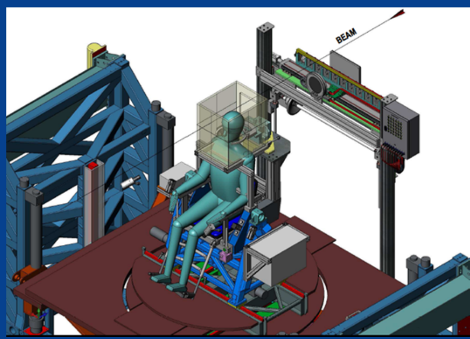




694 % Increase in life span relative to median survival time

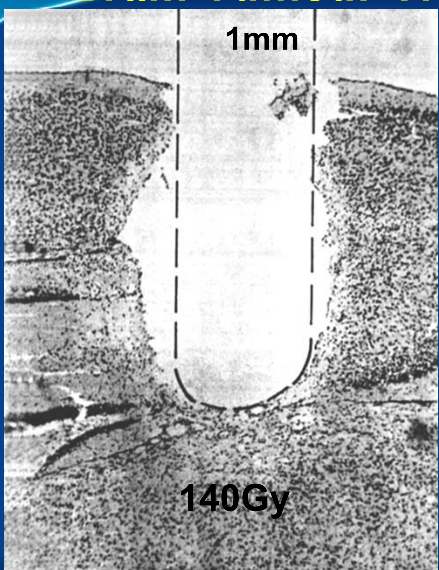
Monochromatic X-Rays for Brain Tumor Radiotherapy (SSRT)

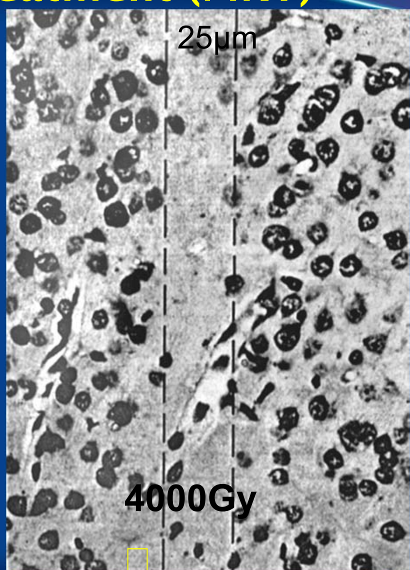




Phase I – Phase II Clinical Program

Arrays of High Energy Microbeams for Brain Tumour Treatment (MRT)





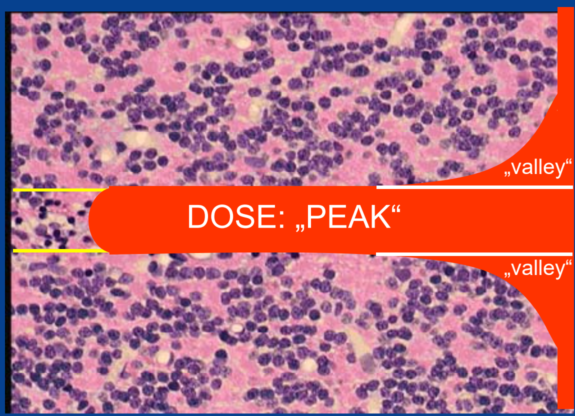
Mouse Brain, Visual Cortex

Rat Cerebellum after Micro-Radiation Therapy Irradiation

Entrance dose: 2000 Gy

25 µm-wide microplane

12 h after irradiation

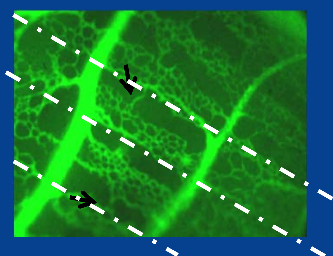


Microbeam Radiation Therapy

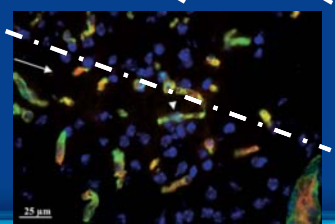
Tissue Sparing Effect of MBs

Piglet cerebellum (15 months later).The irradiation area was 15 x 15 mm^2 with 20–30 µm wide beams spaced of 210 µm intervals and entrance dose of **300 Gy**.

Radio-Resistance of Normal Vessels and Capillaries

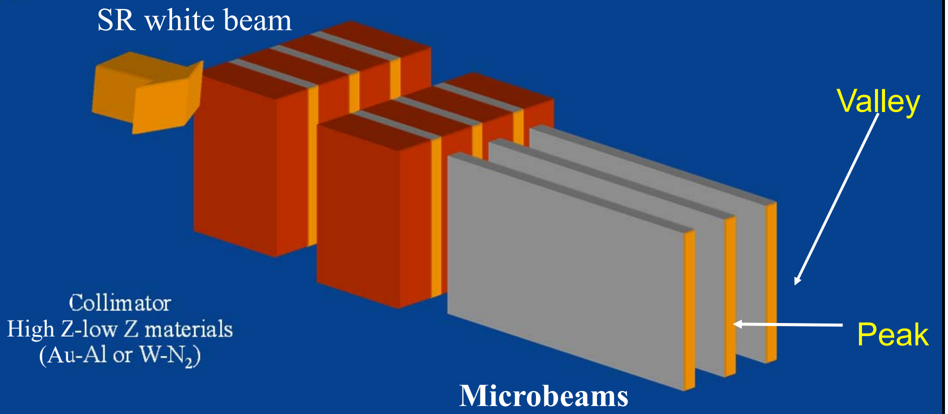


Chick chorioallantoic membrane (CAM). 24h after an irradiation exposure at 300 Gy. A model of an almost pure vascular system with immature vessels.



Parietal cortex mouse brain. Microbeams of 25 µm-wide, 211µm center-to-center spacing, delivered at 1000 Gy; 3 months later.

Arrays of High Energy Microbeams for Microsurgery



Microbeams: variable width (0-100 μm), 100-400 μm pitch

50-125 microbeam array to cover up to 5x5 cm^2

- **Spatial Fractionation of the Beam Delivered at High Doses**

Epilepsy: Epidemiology

- **It is a neurodegenerative disorder that affects people of all ages.**
- **Around 50 million people worldwide suffer from epilepsy.**
- **Nearly 80% of the people with epilepsy are found in developing regions.**
- **Symptoms of epilepsy are called seizures.**

Epilepsy: Treatment

Anti-epileptic drugs (AEDs)

Surgery

Resective:

Surgical removal of epileptogenic tissue

Non-resective (disconnective):

Multi subpial transections (MST)

Callostomy

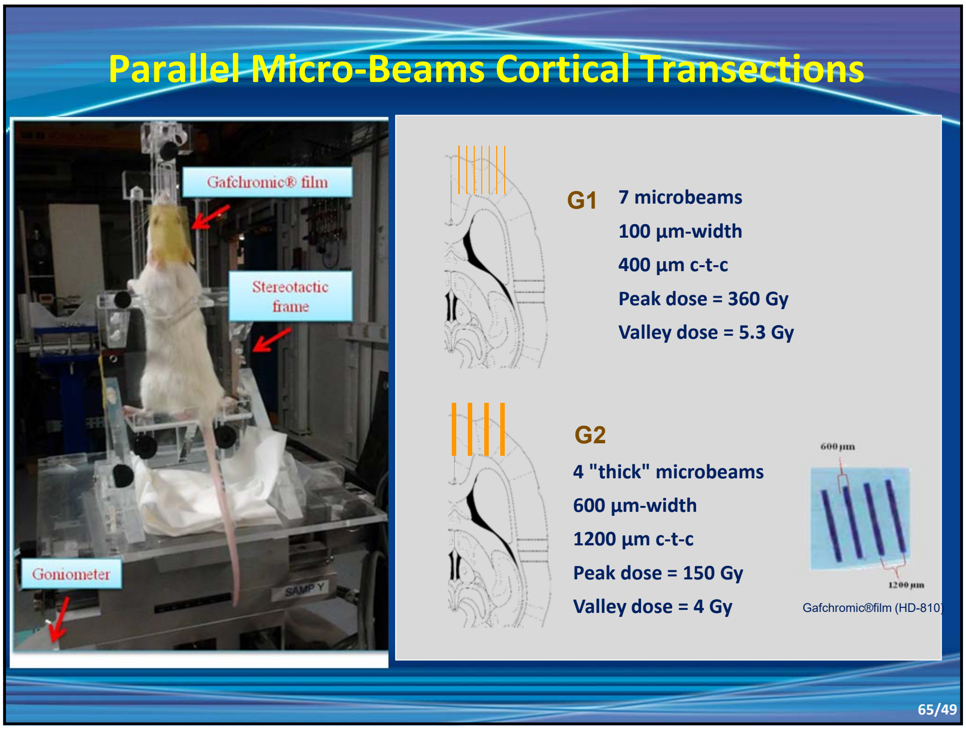
Implantation of neuromodulation devices

Vagus nerve stimulation (VNS)

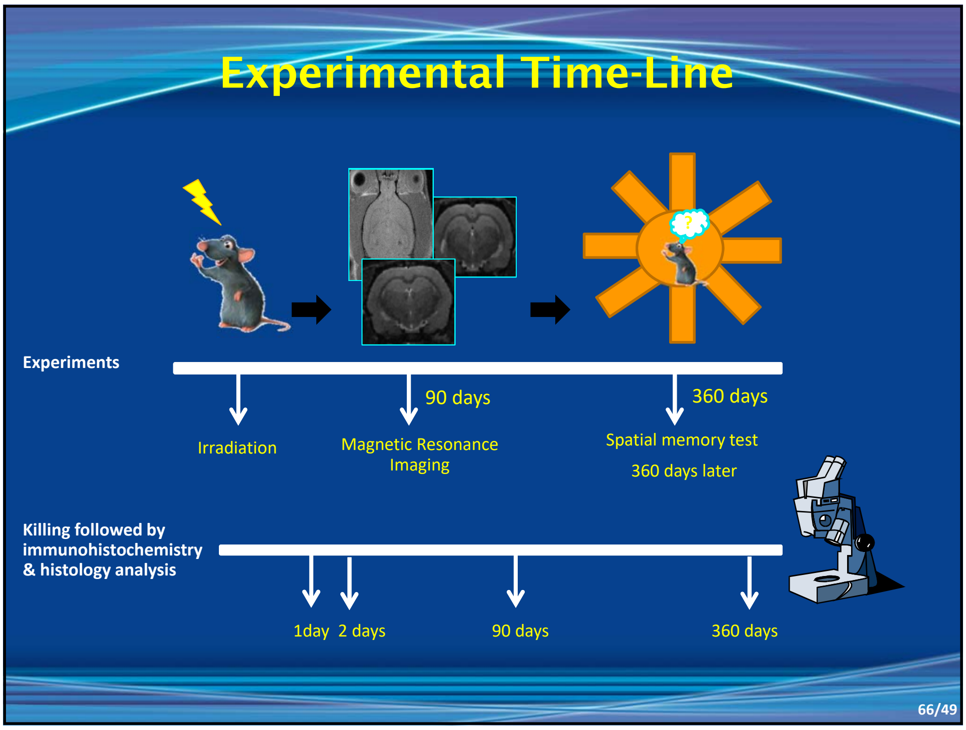
Deep brain stimulation (DBS)

Stereotaxic radiosurgery (SRS)

From: http://www.who.int/en/



Parallel Micro-Beams Cortical Transections
Gafchromic® film
Stereotactic frame
Goniometer
G1
7 microbeams
100 µm-width
400 µm c-t-c
Peak dose = 360 Gy
Valley dose = 5.3 Gy
G2
4 "thick" microbeams
600 µm-width
1200 µm c-t-c
Peak dose = 150 Gy
Valley dose = 4 Gy
Gafchromic®film (HD-810)
65/49



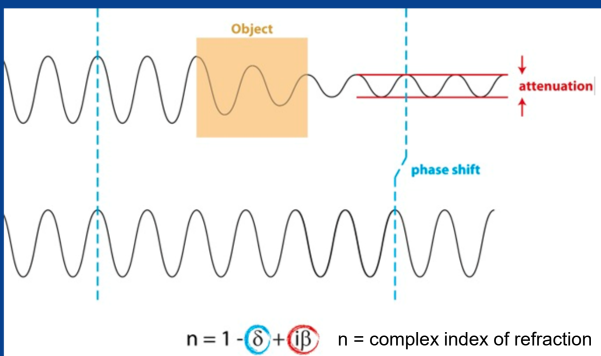
Experimental Time-Line
Experiments
Irradiation
90 days
Magnetic Resonance Imaging
360 days
Spatial memory test
360 days later
Killing followed by immunohistochemistry & histology analysis
1day
2 days
90 days
360 days
66/49

Preliminary Conclusions

- Micro-Beams delivered at different doses (from 150 to 600 Gy) are well tolerated by tissues and cell proliferation is partially affected.
- Micro-Beams towards the motor cortex do not cause paralysis in normal rat.
- Micro-Beams towards the dorsal hippocampus did not cause cognitive impairements after 1 year.

Dr. Erminia Fardone; Dr. Alberto Bravin, & Prof. Pantaleo Romanelli

X-Ray Phase Contrast Imaging

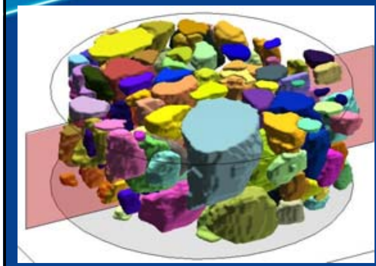


Monochromatic and parallel X-rays for high resolution (≥5 μm) medical imaging.

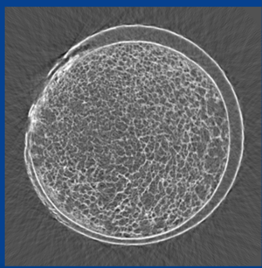
Brain vasculature
Holotomography

Technology provides enhanced soft tissue contrast and improved visualization of cancerous structures.

X-Ray Imaging at the ESRF



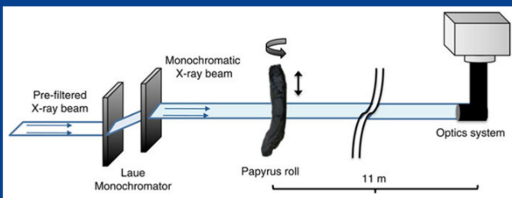
Diffraction contrast tomography:
grains in a polycrystal



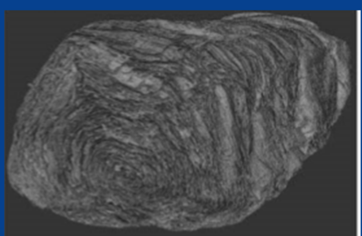
Bone, degraded cartilage

Phase contrast tomography of an insect in fossil amber

Revealing Letters in Rolled Herculaneum Papyri

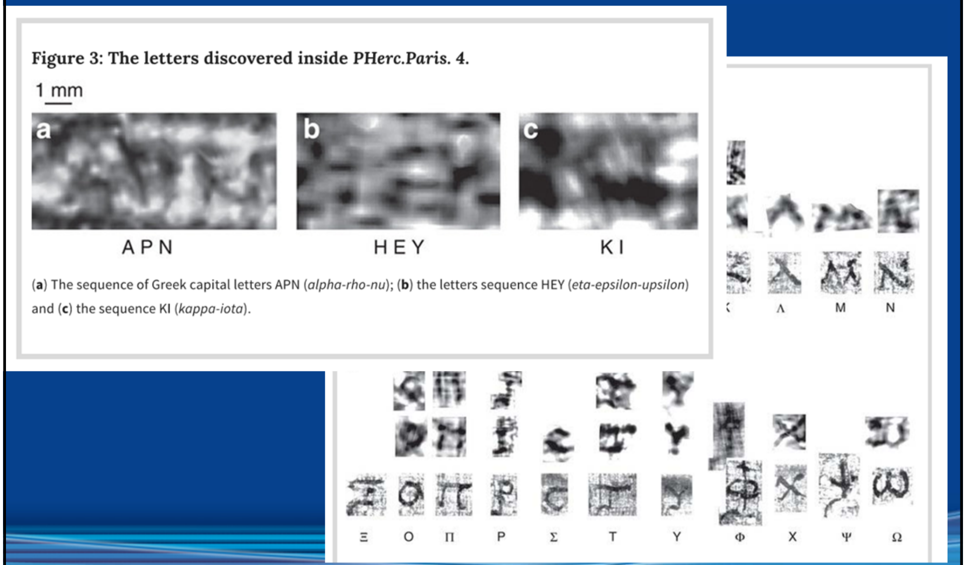


A pre-filtered synchrotron X-ray wavefront is monochromatized by a double Laue-Laue monochromator. The papyrus sample is fixed to a sample stage and rotates over 360 degrees. Due to the aspect ratio of the beam (5 mm high × 15 cm wide), the sample (16 cm in length) is moved after each scan by a distance equal to the height of the beam. After passing through the sample the X-ray beam is recorded by a FReLoN detector.



Volume rendition of the reconstructed papyrus showing the complexity inside the scroll where the papyrus convolutions were exposed to tremendous stress

Revealing Letters in Rolled Herculaneum Papyri



X-Ray Techniques

www.youtube.com/lightforscience

Infrared Microscopy
Extended X-ray Absorption Fine Structure (EXAFS)
X-ray Micro/Nano Diffraction
Nano Diffraction and Surface Diffraction
Hard X-ray Spectroscopy
X-ray Fluorescence Spectroscopy
Tomography
Powder Diffraction
X-ray Diffraction Imaging
Macro-Molecular Crystallography
SAXS & WAXS techniques
.....

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Thank you for your kind attention