Synchrotron Sources
Applications in Chemistry, Structural Biology, Medicine & Cultural Heritage

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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

Knowledge-Based Design of Materials

Structure
Characterization
Properties
Processing
Performance
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.

Fields of application
Understanding matter links the many scientific disciplines:

- **Solid-state physics**
  - Magnetic / electronic properties
  - Atomic structure

- **Nuclear & particle Physics**
  - Cosmology

- **Chemistry**
  - Structure / dynamics of new substances
  - Structure of interfaces

- **Medicine**
  - Pharmaceutical molecules
  - New therapy protocols

- **Life sciences**
  - Protein crystallography
  - 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

<table>
<thead>
<tr>
<th></th>
<th>X-ray</th>
<th>Neutron</th>
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<tbody>
<tr>
<td>Scattering proportional to Z</td>
<td>Scattering not proportional to Z</td>
<td></td>
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<tr>
<td>H</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>-3.74</td>
<td>5.30</td>
<td>6.65</td>
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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

Synchrotron X-rays:

• Allow observation of diffraction data very quickly. Good for systems that change rapidly
• Allow observation of diffraction data from small samples

Neutrons:

• Allow observation of hydrogen atoms and water – critical for biological systems
• Contrast variation
• Dynamics
• Allows exploitation of sample labelling (deuteration)
**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)

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**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 %
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)
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

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<th></th>
<th>98.83</th>
<th>178.7</th>
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<tr>
<td>Availability (%)</td>
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<td>Mean time between failures (hrs)</td>
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<tr>
<td>Mean duration of a failure (hrs)</td>
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Fluorescence

Transmission

Elastic Scattering – Coherent

Inelastic scattering

Absorption

Pressure, Temperature and Magnetic Field

Photoemission

Time-Resolved

Incident Photon beam

Surface Phenomena

Interactions of Photons with Matter

Electronic Structure & Magnetism

Structure of Materials

Dynamics & Extreme Conditions

Structure of Soft Matter

X-ray Imaging

Structural Biology
Beamline Optical Concept

- Source: 200 x 80 µm
- Cylindrical mirror
- Toroidal focusing mirror
- Monochromator
- H2O
- H2O

ESRF User Programme - Scientific Core Activities

14000 Shifts delivered for Experiments
Generated ~ 1800 publications / ~200 High Impact Journals

- Surfaces & Interfaces: 8% [11%]
- Soft Condensed Matter: 8% [9%]
- Methods & Instrumentation: 4% [2%]
- Macromolecular Crystallography: 13% [14%]
- Medicine: 3% [4%]
- Environment & Culture: 7% [7%]
- Chemistry: 12% [10%]
- Electronic & Magnetic Properties: 16% [14%]
- Crystals & Ordered Structures: 12% [10%]
- Disordered Systems: 3% [5%]
- Applied Materials, Engineering: 12% [10%]
- Other: Training, feasibility tests, proprietary research: 3% [4%]

Percentages for 2009 in parentheses
Connecting Scales of Biological Structures

Integrated Structural Biology: From Molecules to Cells

- NMR
- Neutrons
- X-ray
- EM
- Light-microscopy

Structural Biology

Complex
- Subcellular structure
- Cell
- Organism

Cell Biology

Developmental Biology

Systems Biology

Macromolecular Structures over the Years
Crystals by Thousands...!

GPCR 1043 crystals

DNA depending Protein kinase > 2000

Ribosome

Anaesthetics bound to a pentameric ligand-gated ion channel

Thousands by thousands

High Throughput Crystallisation

4°C
20°C

https://embl.fr/hxlab
10/18/2016

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

plus
♦ microspectrophotometry
♦ BioSAXS

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

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

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

New Visions for Structural Biology
Dimensions of micro-crystals have changed!

μ-focus beam lines:
- ID23-2 (Ø ~ 5x7 μm)
- ID 13 (70nm < Ø < 1 μm)

Developing New Tools
Observation: AFM
Towards micro-fluidics
Optical tweezers

New Approaches…

Cartography of the Diffracting Regions
GPCR 1043 crystals

DNA depending Protein kinase > 2000

Ribosome

Thousands by thousands

Anaesthetics bound to a pentameric ligand-gated ion channel

> 1000

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
Developing Tools for Automation

MXCuBE
Sample Changer (ESRF/EMBL)

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 \( \mu \text{m} \)

MASSIF (ESRF)
Screening Libraries......

Methodology

*in silico*> *in cellulo*> *in cubo*

Target

\[10^6\]

*In cellulo High Concentration Assay screening*

\[x10^3\]

*In cubo screening !!!*
From Crystal to Crystals...

A new *in cubo* screening tool...
Finding the Crystals.....

Excellent visualisation, 1 \(\mu\text{m}\) accuracy!

Employing Robotics for Drug Screenings
**Seleno Methionine and Seleno Carbohydrate**

**Multiple Wavelength Anomalous Dispersion (MAD)**

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

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.

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**Conformation of Macromolecules In Solution**

Concentration: 1-10mg/ml

Volume: 100μl

288 samples in the sample changer

Software for data management: ISPyB

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

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Small-Angle X-ray Scattering

N-terminal domain

D-mannose specific domain - 2mer

L-Fucose specific domain 3mer

C-terminal domain

Experimental enveloppe

Super lectin from *Burkholderia cenocepacia*

Kinetic Crystallography

Caged compound

Enzymatic reaction inhibited

Specific wavelength

Photolysis

Enzymatic reaction can happen

Principle: biological turnover initiated in crystal to form transient structural species
Fluorescence of crystals when excited by 355 nm laser

Spectra resulting from the releasing of a cage in crystals at 100 K by a 355 nm laser, followed by a temperature ramp

Crystallography

Kinetic Crystallography

Laser Pump X-ray Probe Synchrotron

Circumference = 544.1 m
Orbit Time = 2,816 us
Laser Pump X-ray Probe Synchrotron

Synchronized pulsed laser

Chopper

Sample

X-ray optics

X-ray Pulselength: 50-100 ps

Undulator

Detector

BioMedical Research at the ESRF

Monochromatic and parallel X-rays for high resolution (≥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
Principles

- Tumor loaded with a high Z element (iodine, gadolinium) and/or chemotherapeutic 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

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 mm² 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) 24 h 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, 211 µm center-to-center spacing, delivered at 1000 Gy; 3 months later.
Arrays of High Energy Microbeams for Microsurgery

SR white beam

Collimator
High Z-low Z materials
(Au-Al or W-N$_2$)

Microbeams
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)
Parallel Micro-Beams Cortical Transections

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)

Irradiation
Spatial memory test
360 days later

Magnetic Resonance Imaging
90 days
2 days
1 day

Experiments
Killing followed by immunohistochemistry & histology analysis

Experimental Time-Line

90 days
360 days
360 days
360 days
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 impairments 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

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

Figure 3: The letters discovered inside PHerc.Paris. 4.

(a) The sequence of Greek capital letters ΑΠΝ (alpha-phi-nu); (b) the letters sequence ΗΕΥ (eta-epsilon-upsilon) and (c) the sequence ΚΙ (kappa- iota).
Neutrons & Synchrotron Radiation for Science:

This school provides training for students, postdoctoral and senior scientists from European and non-European universities and laboratories, in the field of Neutron and Synchrotron Radiation for condensed matter studies (Biology, Chemistry, Physics, Materials Science, Geosciences, Industrial applications).

It includes lectures, practicals, tutorials, and visits of Large Facilities.

The ~4-week school is a yearly event, established in 1991.

www.hercules-school.eu

Thank you for your kind attention