Centre for Integrative Biology (CBI)

ICS

IGBMC

Image de synthèse réalisée par le Cabinet d'architecture CELNIKIER & GRABLI ARCHITECTES.

"Pipeline data collection at the IGBMC cryo-EM platform"

Ottilie von Loeffelholz (Klaholz Group)

Centre for Integrative Biology, IGBMC, Illkirch/Strasbourg

NeCEN, Netherlands

16.10.2018

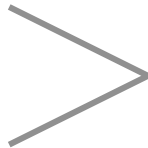Centre for Integrative Biology
(CBI)

ICS

IGBMC

Image de synthèse réalisée par le Cabinet d'architecture CELNIKIER & GRABLI ARCHITECTES.

<u>Driving scientific projects:</u> integrative structural biology of gene regulation:

- **transcription**
- **translation**   **nucleoprotein complexes, biomedical targets**
- **RNA**

Hosts the Strasbourg Instruct-ERIC / FRISBI infrastructures

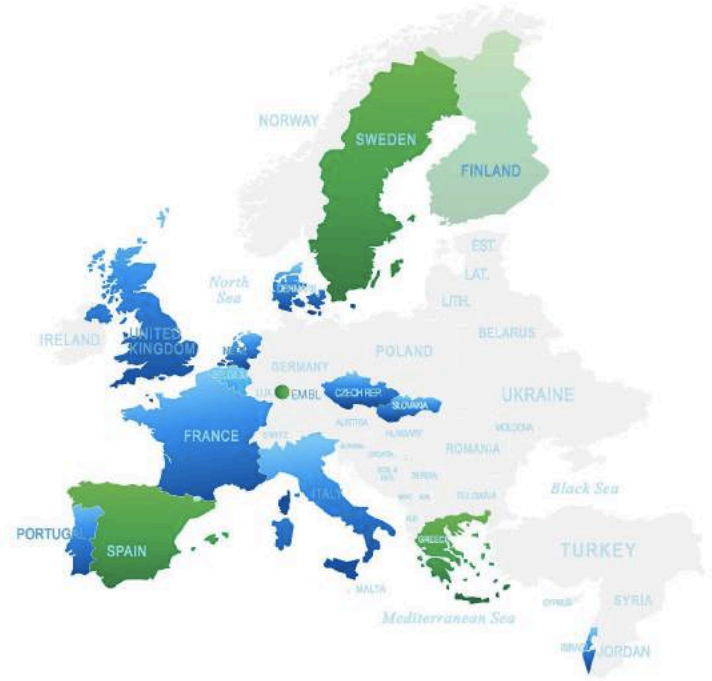**http://www.structuralbiology.eu/**

http://frisbi.eu

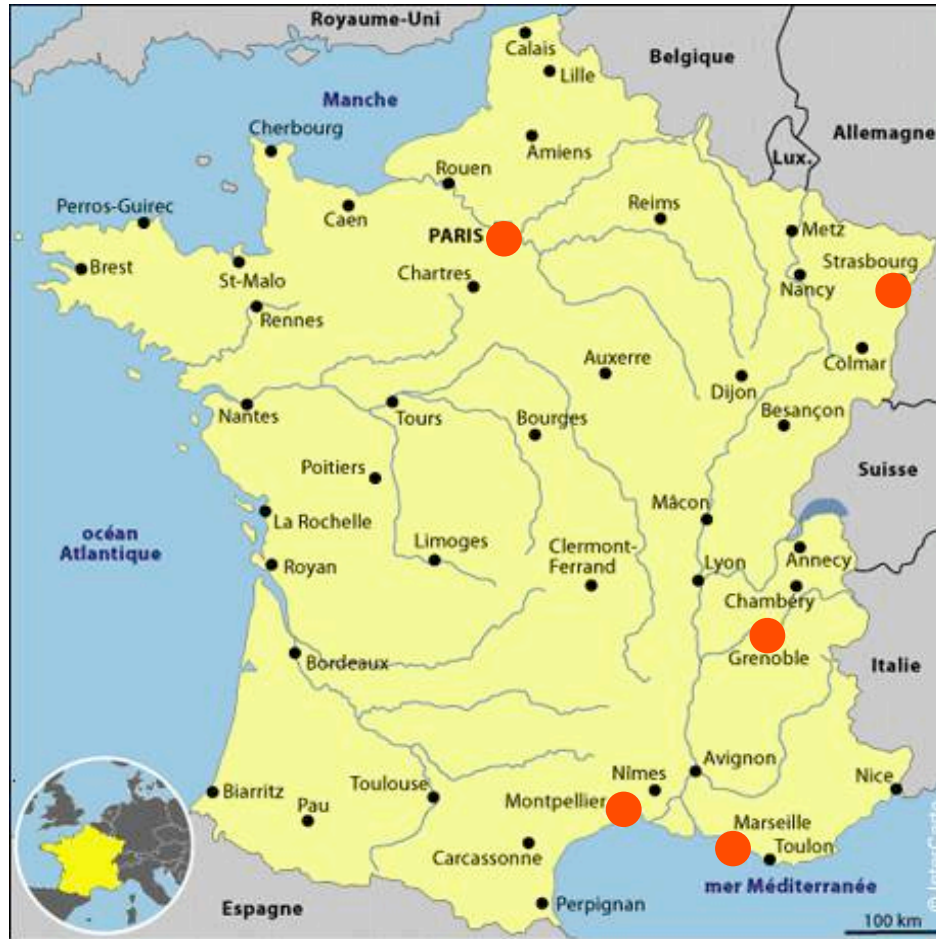# Instruct-ERIC: European infrastructure for Structural Biology

- integrated infrastructure with cutting-edge technologies

- scientific expertise

- training

- peer reviewed funded access for academia

- access for industry

http://www.structuralbiology.eu/

French Infrastructure for **I**ntegrated **S**tructural **BI**ology

5 FRISBI centers (Strasbourg, Grenoble, Montpellier, Marseille, South Paris)

Strasbourg and Grenoble are also Instruct centres

One Portal: http://www.frisbi.eu

Web site: accessible via the Instruct, CNRS, ReNaFoBis & ITMO websites

contact@frisbi.eu

72 access projects funded since 2015; specific calls for access
+ specific calls for transversal organization of training workshops

http://www.frisbi.eu
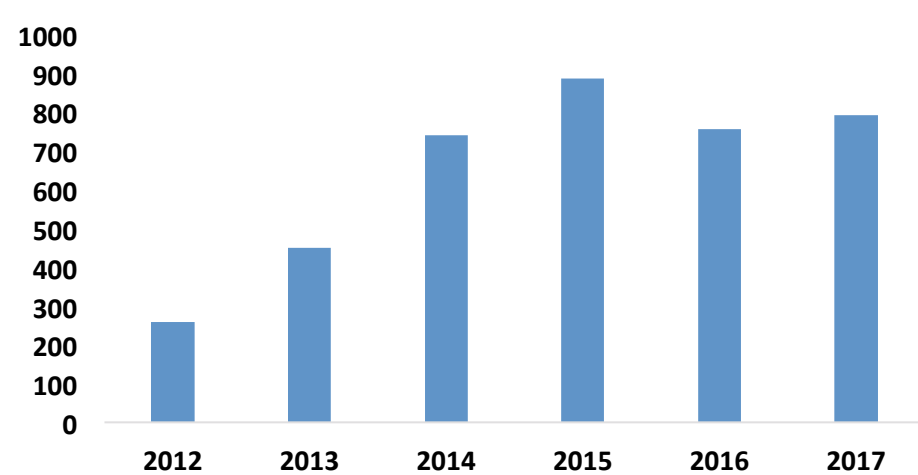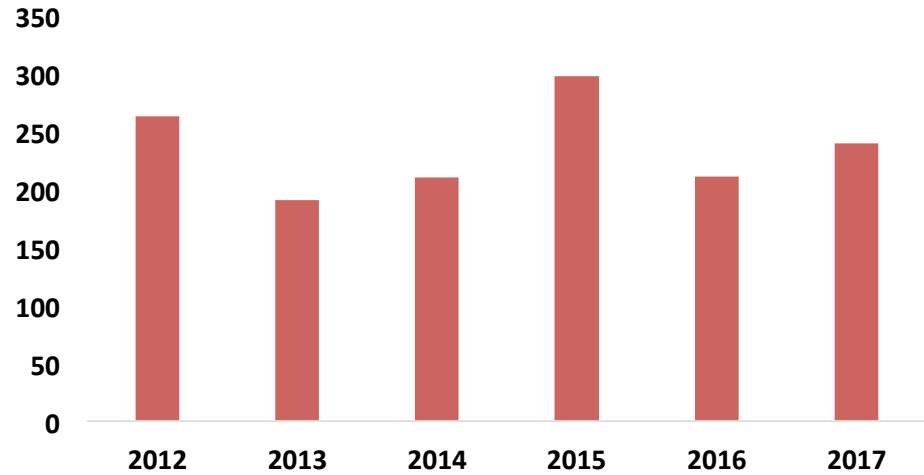
http://www.structuralbiology.eu/

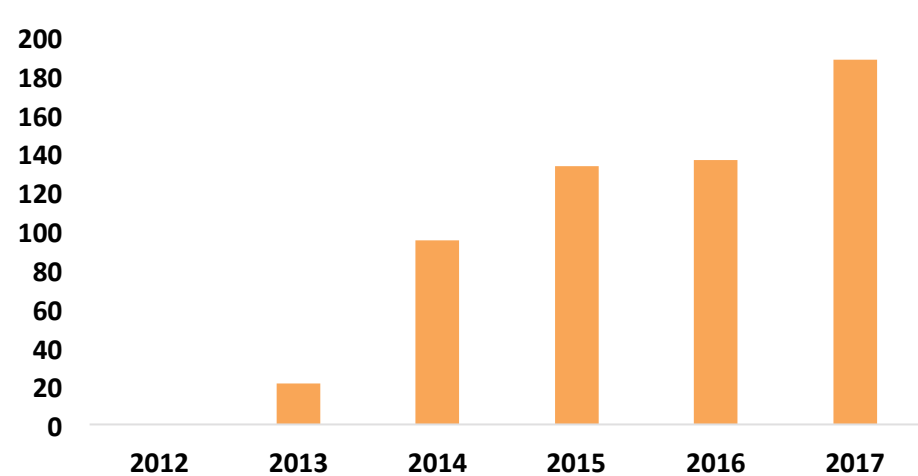# Increasing activity of the FRISBI infrastructure
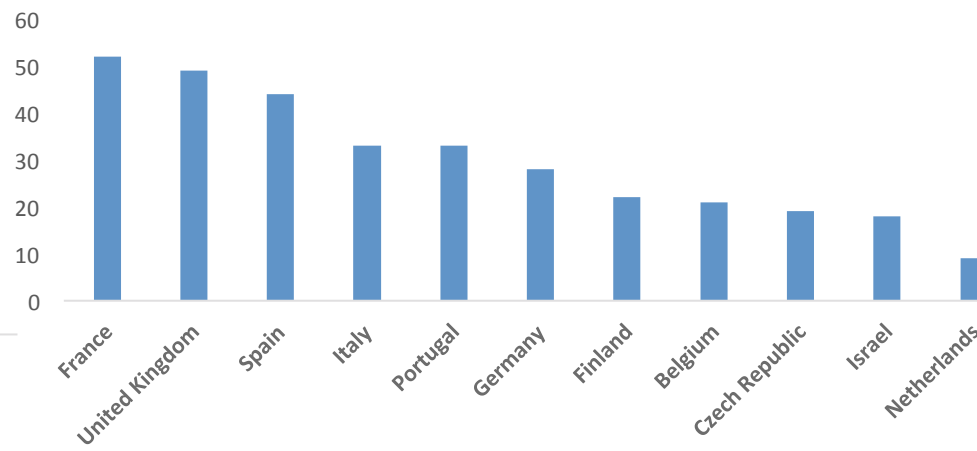
User projects (total 3877)

Users trained (total 1413)

Publications citing FRISBI (total 573)

France: first country in terms of access projects submitted

Training within the FRISBI infrastructure

National School in Integrated Structural Biology

Ile d'Oléron

Annual school since 2014

25 participants open to:
  - Master, PhD , Post-doc, CR, Engineer, Scientist from industry
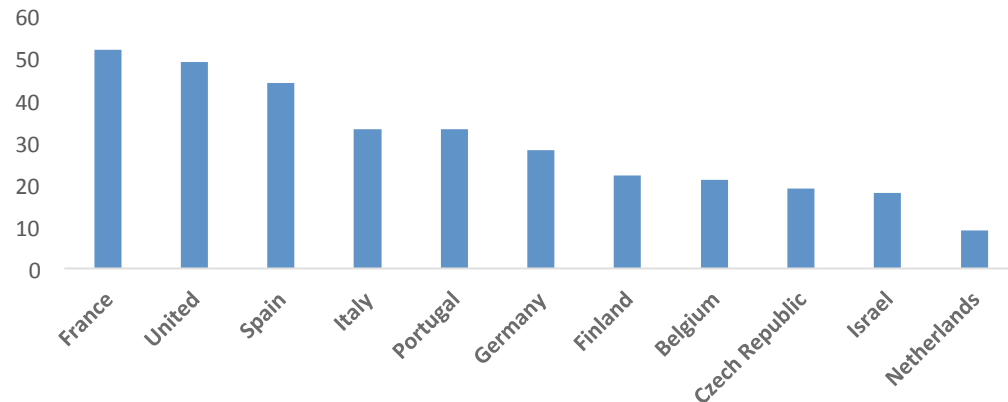 **- national and European scientist**

6ème Ecole RéNaFoBis - Oléron 2019
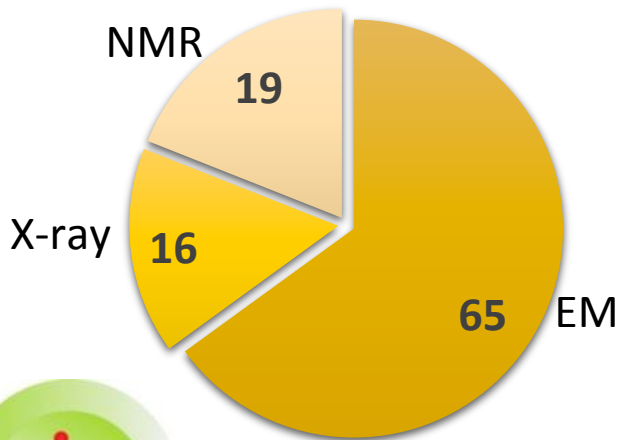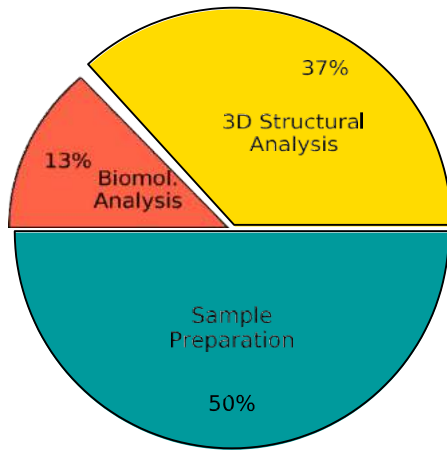
Importance of the FRISBI-Instruct link

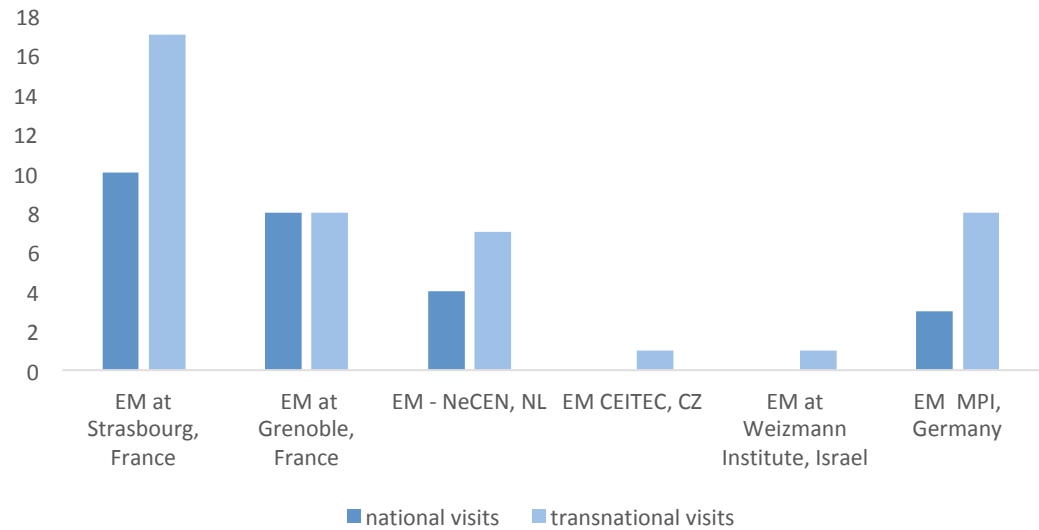**Number of French access project submitted to Instruct**
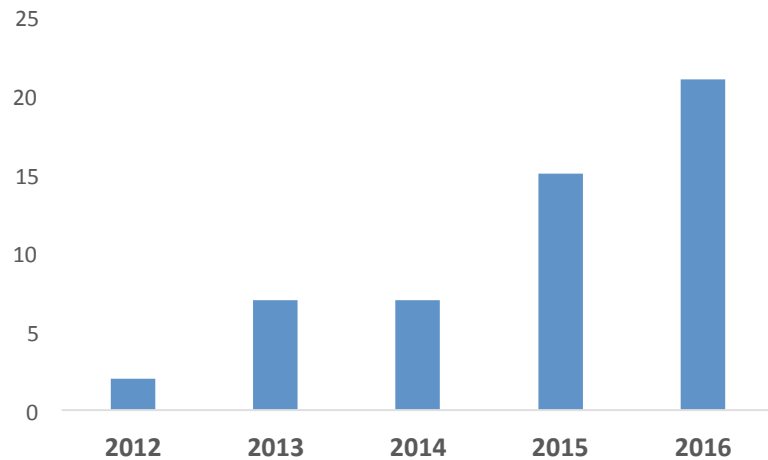
Countries in terms of access projects submitted

Instruct visits for different service technologies (2014-2017)

Distribution access to EM through Instruct 2014-2017

Strasbourg centre: platform for Integrated Structural Biology

6 modules:

- protein production facilities & biophysical characterisation

- crystallization facilities

- X-ray crystallography

- NMR

- cryo-EM

- super-resolution fluorescence microscope (GSDIM)

- dedicated computing resources + storage, on-the-fly processing:

  CBI/Mesocentre/DataCenter; co-financed FRISBI / CPER
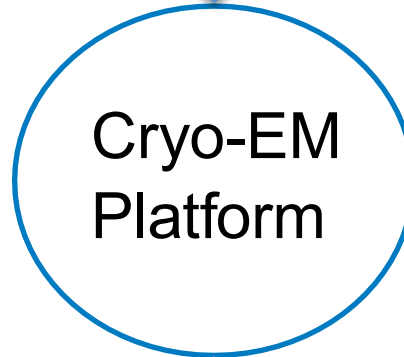

HP-computing

Per year:

~210 access projects

52 different research teams (internal + external)

60 scientists trained on the platform

Cryo-EM platform: Users

Internal users
73% of the access

Cryo-EM
Platform

**External users**
27% of the access

Cryo-EM platform: Services

Cryo electron microscopy

- Sample preparation
    - Pre-screening in negative stain
    - Vitrification using Vitrobot (TF)
    - Pre-screening in cryo conditions

- Data collection
    Single Particle Analysis (SPA)
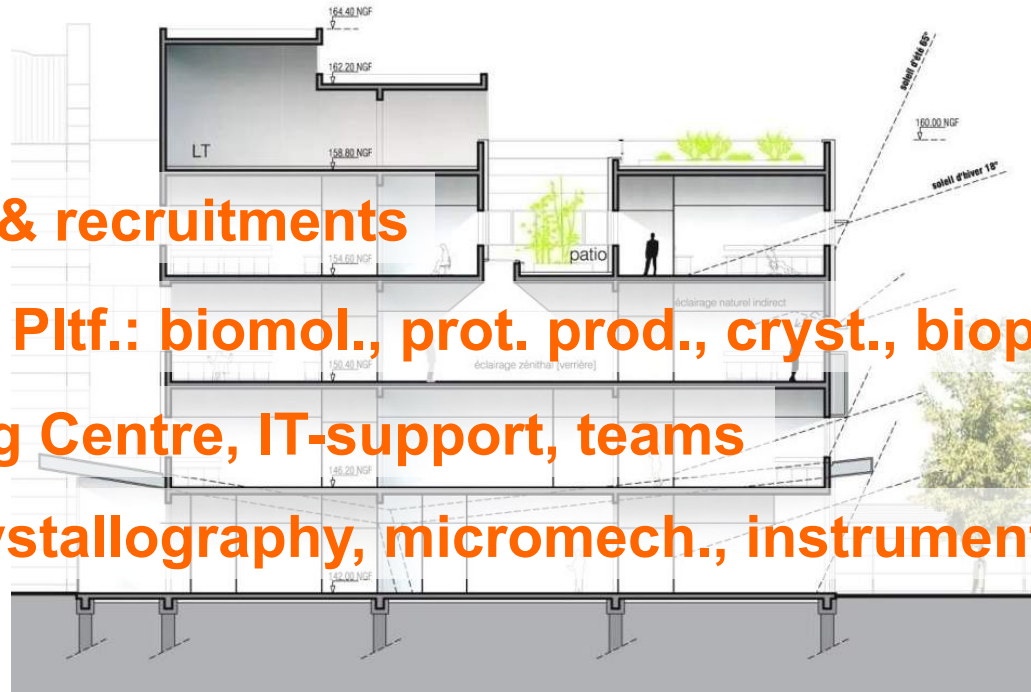   Cryo-electron Tomography (CET)

- Data pre-processing

Single Particle Analysis

Annu. Rev. Biophys. Biomol. Struct. 2002. 31:303–19
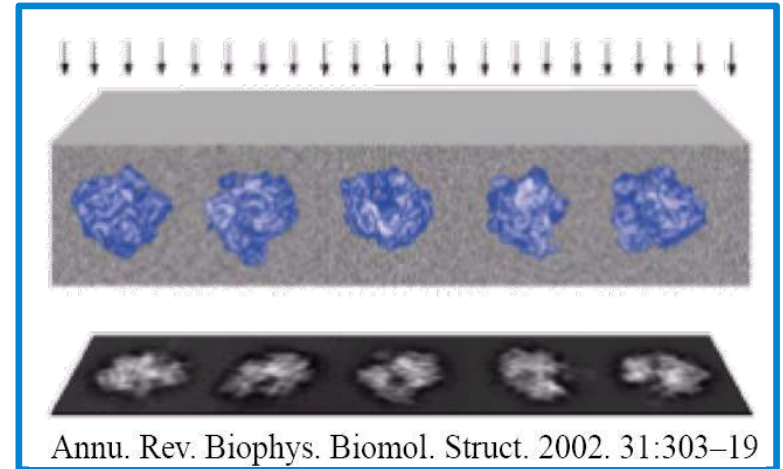
Cryo-electron Tomography
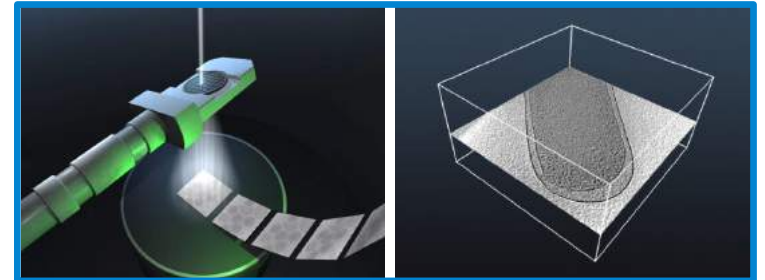
Centre for Integrative Biology (CBI), Strasbourg node

Teams & recruitments

Serv. & Pltf.: biomol., prot. prod., cryst., biophys., teams

Imaging Centre, IT-support, teams

EM, crystallography, micromech., instrumentation & teams

Integrative approach

National Centre for biomedical cryo-EM

Equipment, developments and know-how: high-resolution cryo electron microscopy facilities
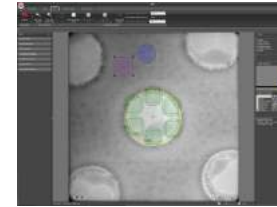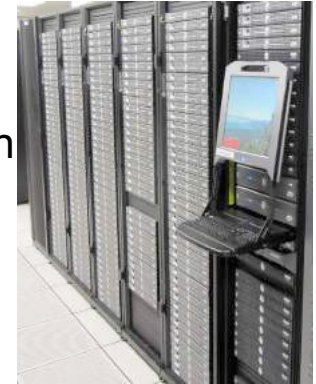
Sample freezing

Automated data acquisition

Sample transfer   Titan Krios   Polara

Direct electron detectors

HP-computing

24/7 access equivalent to beamline
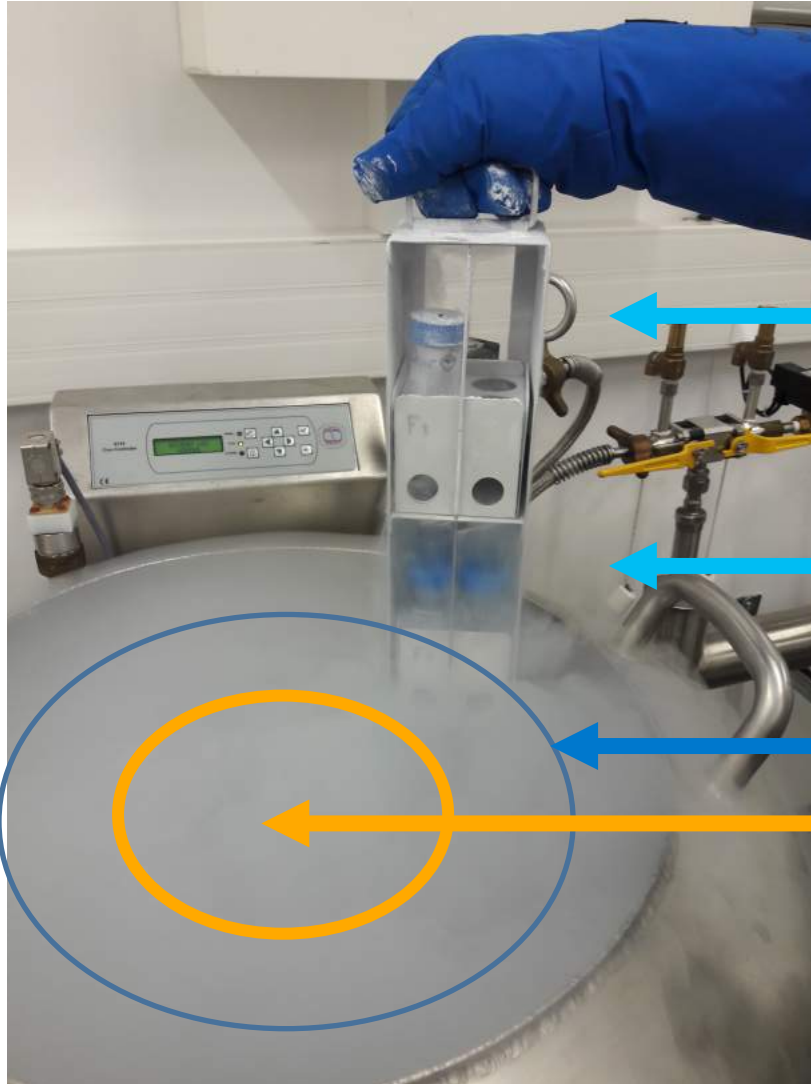
cryo-FIB/SEM

Fischione Plasma cleaner

National Centre for biomedical cryo-EM

Sample storage

daily access

long-term storage

cryo-EM

3D crystals

Instrumentation - Transmission Electron Microscopes

FEI Titan Krios

FEI™ Titan Krios.
- Electron Gun: 300 kV FEG
- GIF Energy Filter (Gatan)
- Cs corrector
- Volta Phase Plate
- Cameras: Falcon III (TM) and K2 summit (Gatan) (soon K3)
- Dual-axis tilt capabilities
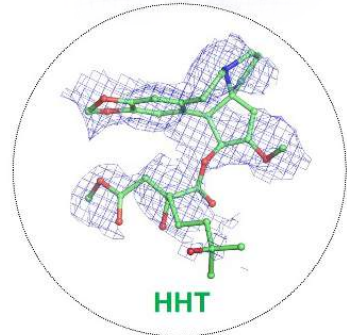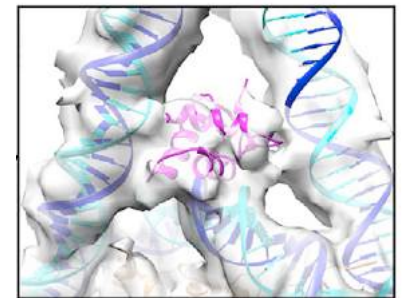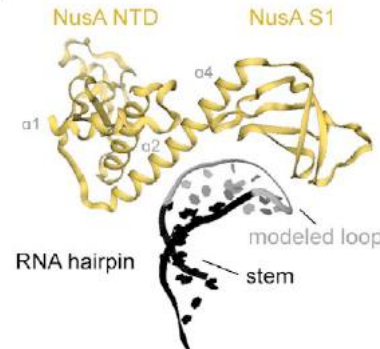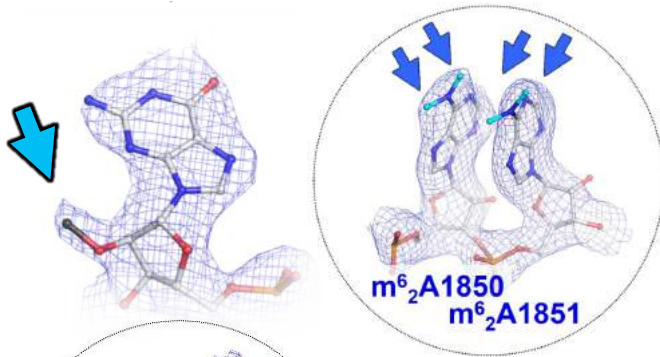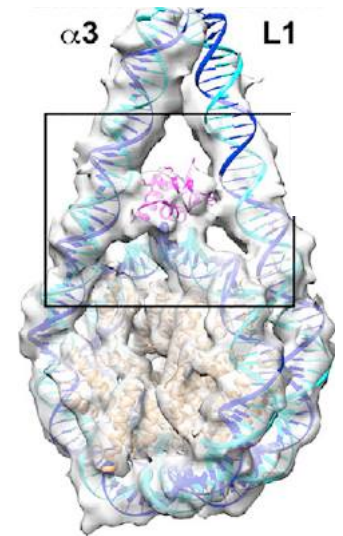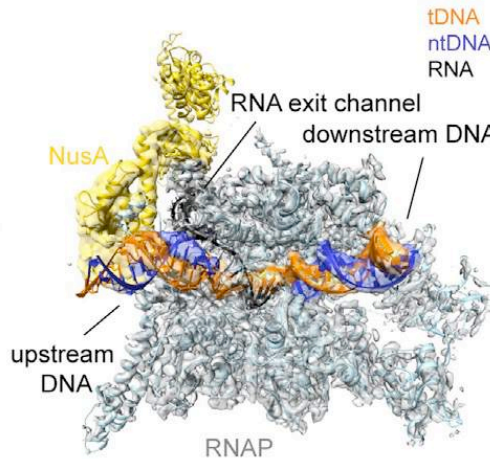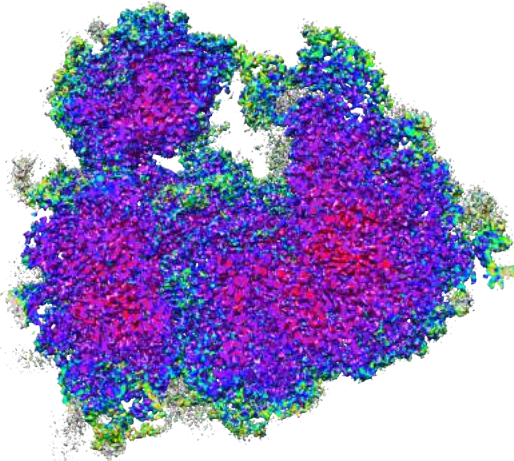
Tecnai F30 Polara
- Electron Gun: 300 kV FEG
- CMOS 4K*4K Falcon II (FEI)

Tecnai F20
- Electron Gun: 200 kV FEG.
- Gatan side-entry cryo-holder
- CCD 2K*2K (Gatan)

Cryo-EM Structures solved at the instruct ERIC IGBMC
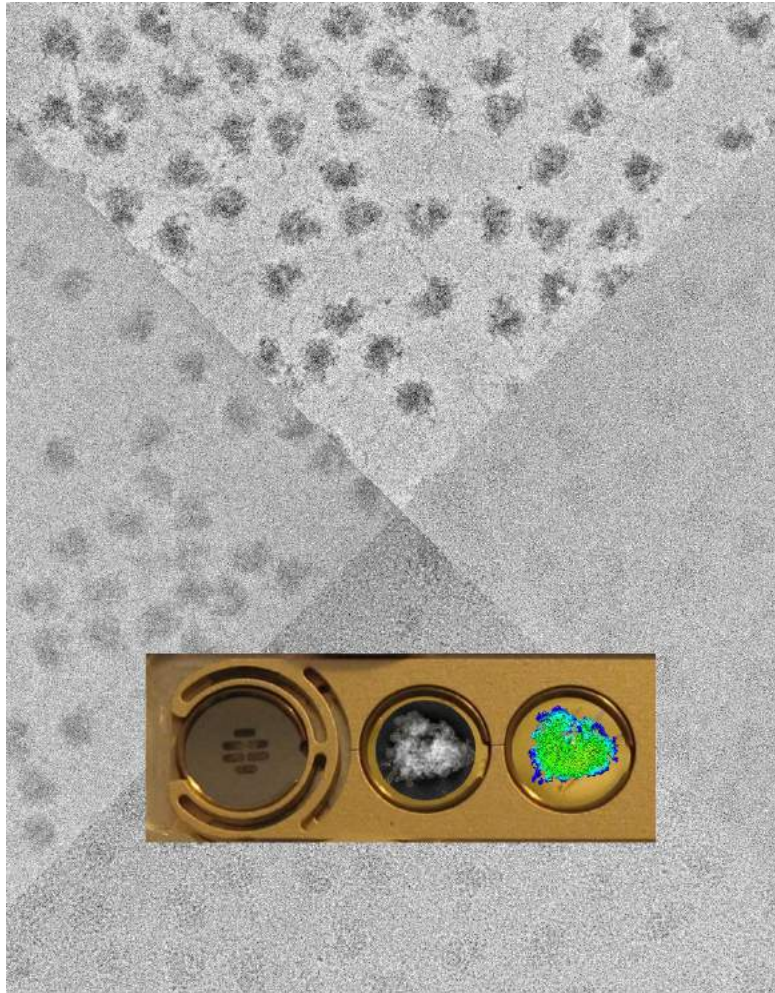
Recent highlights

Human ribosome:
Natchiar et al.,
Nature 2017.

RNA polymerase:
Guo et al.,
Mol Cell 2018.

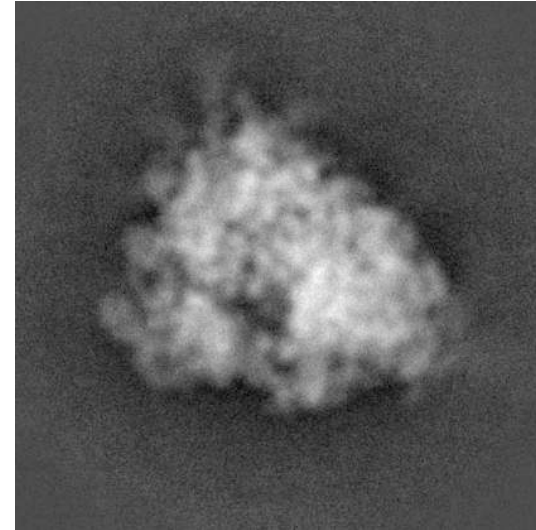Nucleosome complex:
Bednar et al.,
Mol Cell 2017.

Volta phase plate data collection facilitates image processing and cryo-EM structure determination

dfVPP data behave more robustly during image processing:

**particle selection, accuracy in alignments, 2D & 3D classifications, map interpretation**

MSA-based classification (3 particles, Imagic)

von Loeffelholz et al., JSB 2018.

SharpViSu, a pipeline for processing of super-resolution data

SharpViSu/ClusterViSu:

A pipeline for:

- Drift correction

- Chromatic aberration correction

- Voronoi-weighted image representation

- Resolution estimation (FRC)

latest dev.
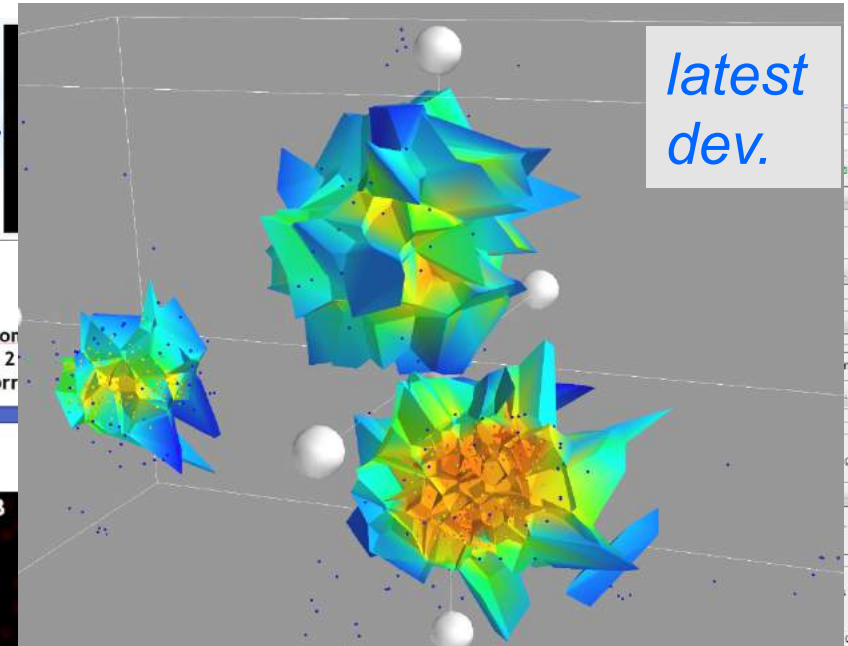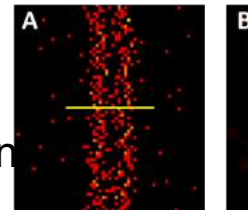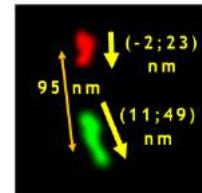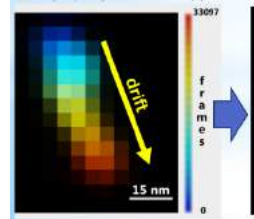
3D segmentation analysis
Andronov et al., Bioinformatics 2018.
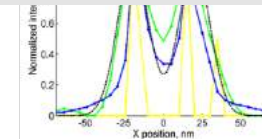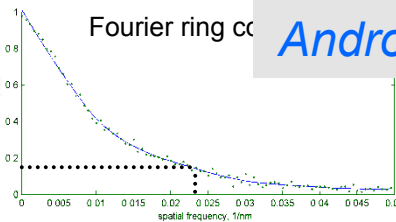
Andronov *et al.*, *Bioinformatics, 2016.*

Andronov *et al.*, *Sci. Rep., 2016.*

Andronov *et al.*, *Bioinformatics, 2018.*

Cryo-FIB: Lamella preparation of human cells

Zeiss

Researchers: Igor Orlov, Danièle Spehner and Andreas Schertel (Zeiss)

Software

EPU / Tomo4
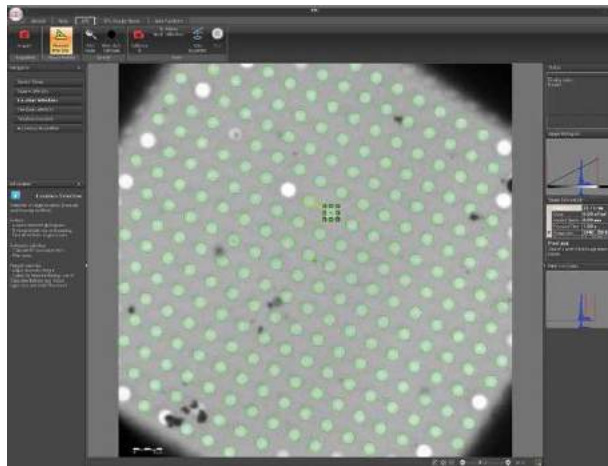
SerialEM
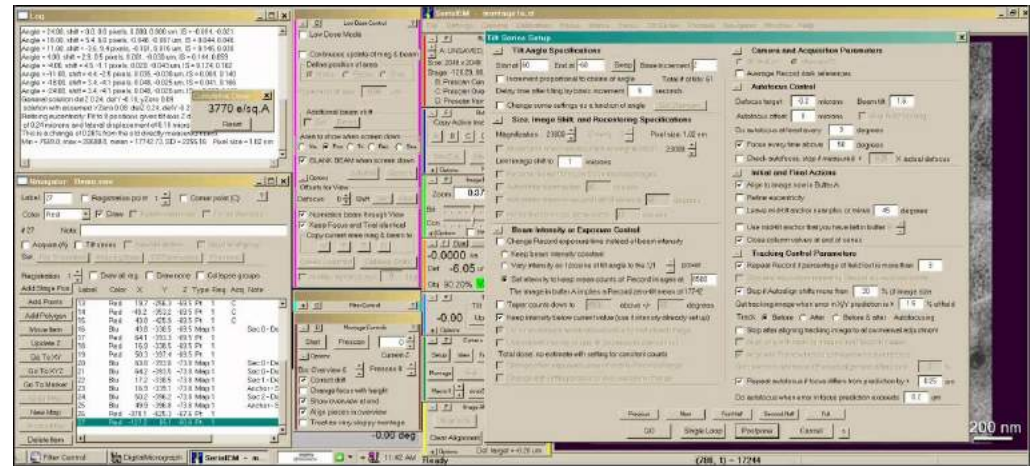
Mastronarde, D.N. 2003. SerialEM: A program for automated tilt series acquisition on Tecnai microscopes using prediction of specimen position. *Microscopy and Microanalysis* Vol. 9, Suppl. 2, 1182CD

Multi-shot functionality applying image shift
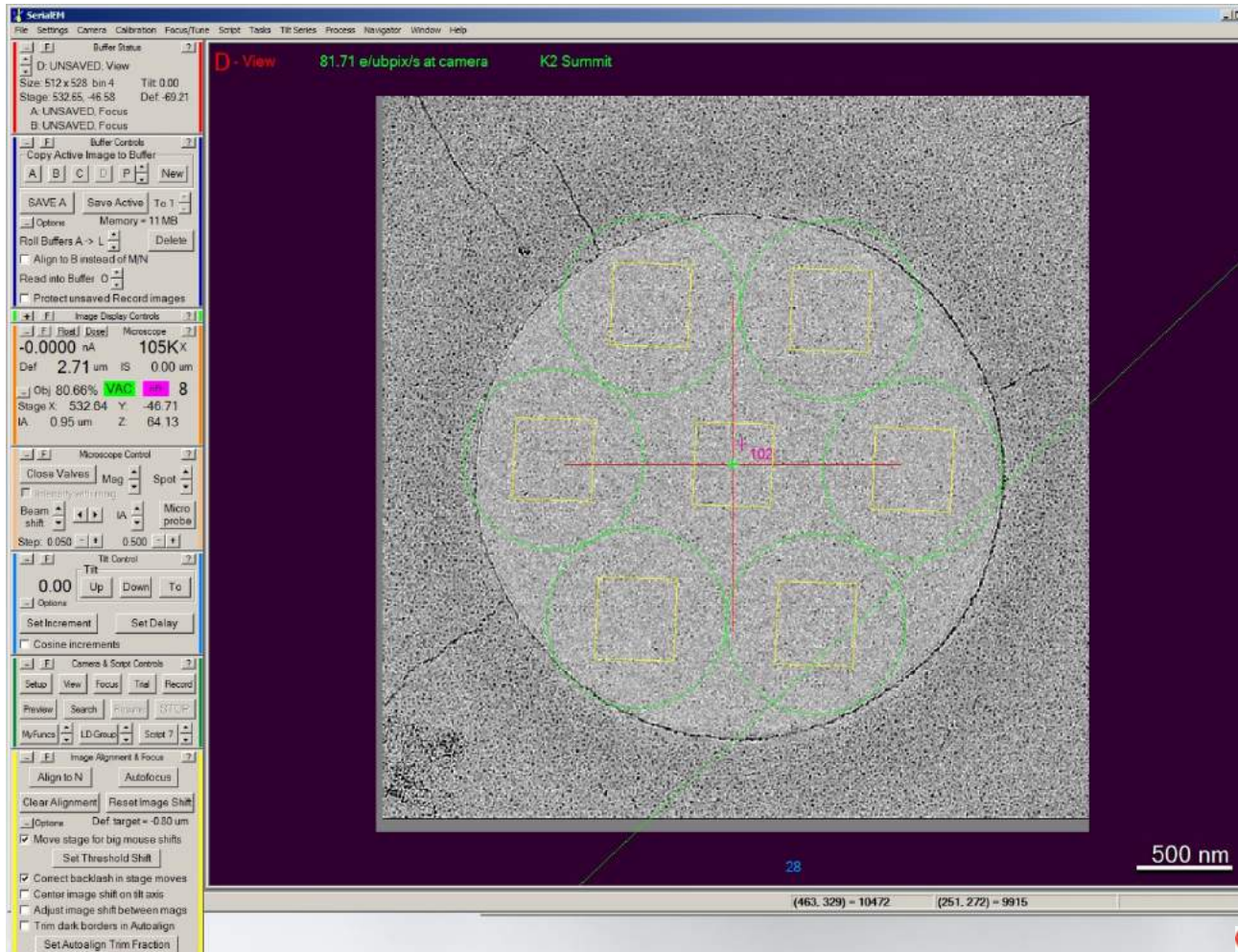
SerialEM v 1.3

Acquisition rate: ~95 images/h in super-resolution mode (tif compression).

Yield ~3300-3800 images in 40 h

On-the-fly processing for users

… particles picking and extraction, 2D classification, 3D classification and refinement

Pre-processing on-the-fly
Part of Instruct-ULTRA project

Gatan PC (10TB SSD Raid)

Acquisition: SerialEM (v. 3.7.0)

Stacks of 20-40 frames with K2 summit (Gatan) in counting super-resolution mode.

Images saved as TIF (1 stack of 32 frames ~ 250 MB)

Automatic synchronization

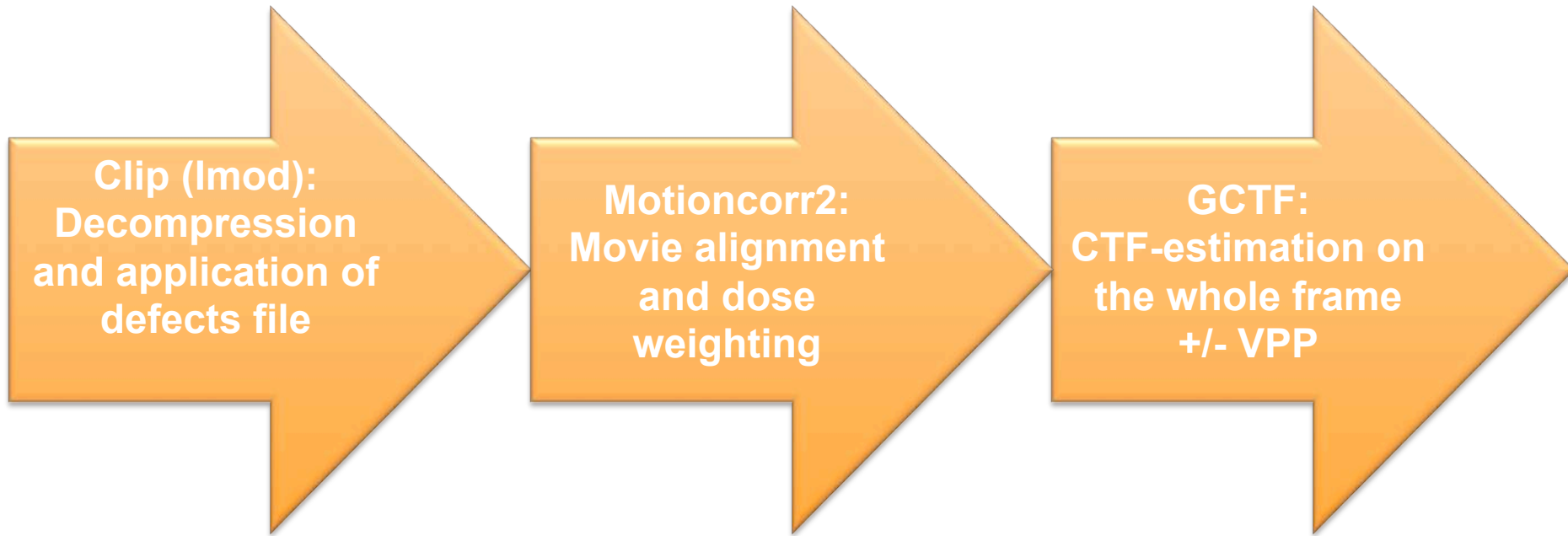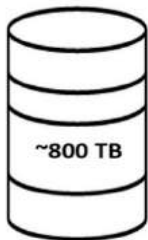**Gateway Titan PC (10TB)**

The user defines in a local web interface the parameters to be used with MotionCor2.

A script listens to kernel events from filesystem.
Each new TIF file is sent to the pre-processing machine

High-speed work storage

~800 TB

or

Pre-processing PC
(4 GPU Nvidia GTX 1080)

Start automatically Gctf

Results visualized with a local web interface to estimate image quality on-the-fly and change settings if necessary

One MotionCor2 job run per graphic card.

Acquisition rate at the Titan:
~1 micrograph per 50 seconds (Stage movements)
~1 micrograph per 37 seconds (Image Shifts)
The 4 Nvidia GTX 1080 can process in average 2 micrographs per minute

Conclusions

Improvements in data quality, analysis of smaller complexes, higher resolution and higher throughput are achieved by:

- upgrades in **hardware**, as direct electron detectors and the Volta Phase Plate.

- developments in data acquisition **software**, in VPP-data processing, and pipeline for on-the-fly processing as part of Instruct-ULTRA

Outlook

- Promote inter-platform networking
  Open/remote access through Instruct-ULTRA

- Creation of a French National Center for Biomedical Cryo-EM (2019-2024) in Strasbourg

- Participation to Instruct-ERIC, FRISBI and iNEXT2
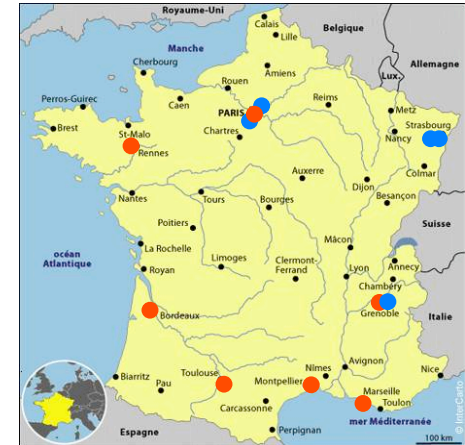
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Alberto Podjarny (Scientific coordinator Instruct)

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