IMAGOPOLE
Pôle de Dynamique Moléculaire et Fonctionnelle

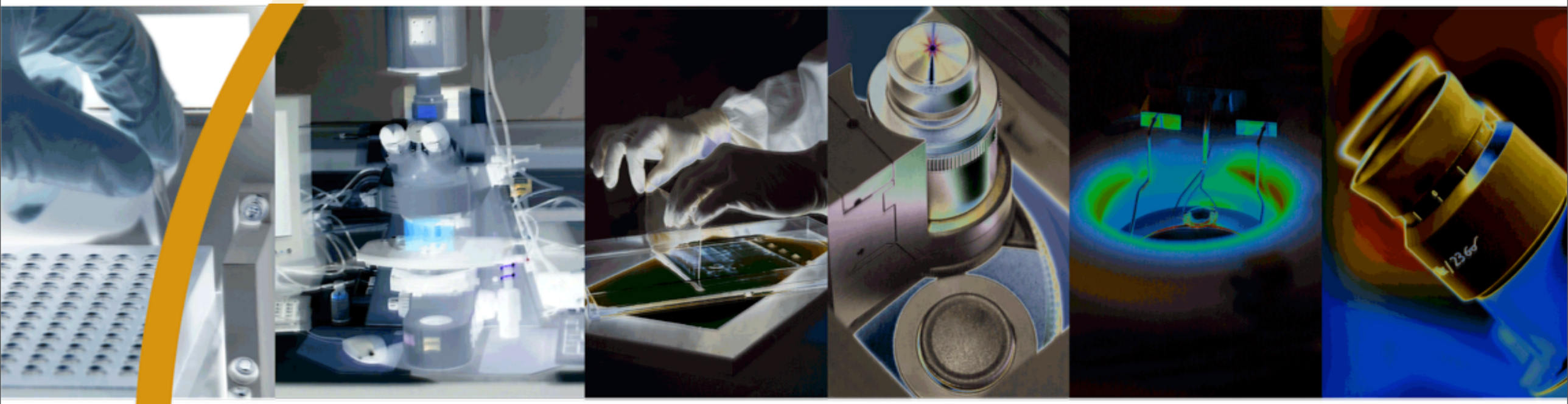
Plate-forme de Cytométrie en Flux
Plate-forme d'Imagerie Dynamique
Plate-forme de Microscopie Ultrastructurale
Centre d'Immunologie Humaine

120ème anniversaire
Institut Pasteur

Open Microscopy Environment European users meeting 2009

Jason Swedlow (Organiser)
& Spencer Shorte (Host)

Institut Pasteur, Paris, 19-20 May 2009



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Biology is an ill-posed ontology problem...

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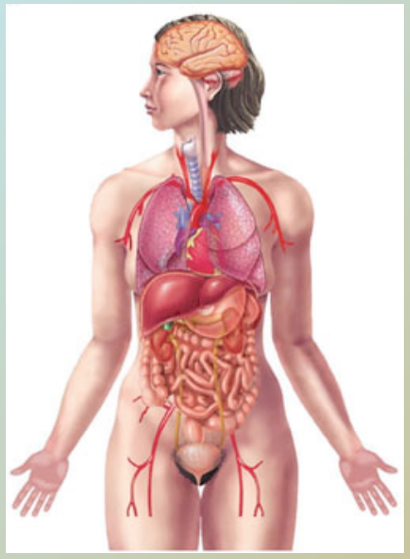
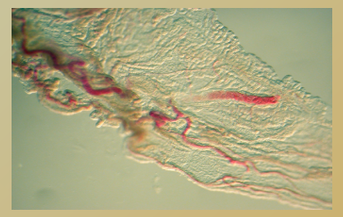
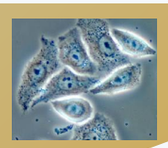
Tissue/organism — **Cellular** — **Molecular**

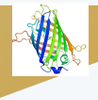
Bedside ⟷ **Bench**

Host — **Organ** — **Tissue** — **Cell** — **Molecule**









Molecular technologies (-omics) allow to quantify genomic, proteomic, transcriptome information that requires functional annotation...

Imaging technologies allow to qualify (describe) complex functional phenotypes *in situ*, inside intact cells and tissues...

...the link between imaging & -omics is emergent:
Functional Molecular Dynamics

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The world's first ever imaging platform was founded at the Institut Pasteur !

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Le service du microcinematography 1929

Hemogregarine Hepatozoon

Chef du Service: Jean Comandon (1877-1970)
Pioneer of microcinematography

Defining: "Functional Molecular Dynamics"
The relationship to imaging

Dynamic imaging was first used in early 1900s to teach doctors how to distinguish syphilis causing spirochetes…

- **living spirochetes incubated in chicken blood and imaged using "microcinematography"; identification of dynamic motility phenotype unique to syphilis causing strains: "frequent direction change…"**

PFID: diverse optical imaging modalities:
A technology tool-box...

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www.pfid.org

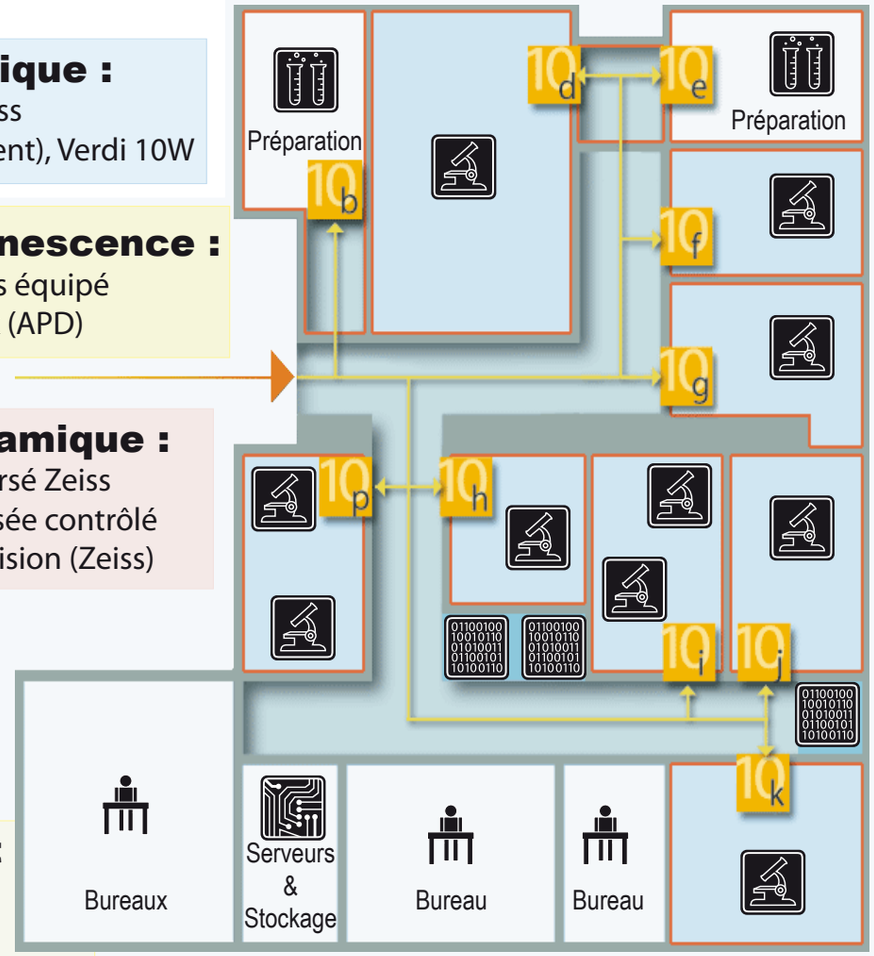
Imagerie Multi-Photonique :
Confocal LSM510 META Zeiss
avec laser multiphoton Mira 900 (Coherent), Verdi 10W

Imagerie par Bioluminescence :
Microscope inversé Zeiss équipé
d'un détecteur Photek (APD)

Imagerie Dynamique :
Microscope inversé Zeiss
avec platine motorisée contrôlé
par le logiciel AxioVision (Zeiss)

Imagerie en Lumière Structurée :
Microscope inversé équipé
d'un module Apotome (Zeiss)

Postes de Traitement et d'Analyse :
Déconvolution (Huygens2)
Reconstruction 3D (Imaris, Volocity, OsiriX)
Traitements (ImageJ, AxioVision, LSM510)
Analyse de formes (Cellenger)



Imagerie Confocale/FCS :
Microscope Confocal inversé
LSM510 Confocor2 Zeiss
avec module FCS
(Fluorescence Correlation Spectroscopy)

Imagerie Dynamique Confocale :
Confocal Nipkow Disk
Perkin Elmer UltraView RS sur
microscope inversé (Zeiss)

Imagerie Dynamique Rapide :
Microscope inversé Zeiss équipé d'un
monochromateur Poly IV (TILL Vision)

Imagerie en Lumière Structurée :
Microscope droit équipé
d'un module Apotome (Zeiss)

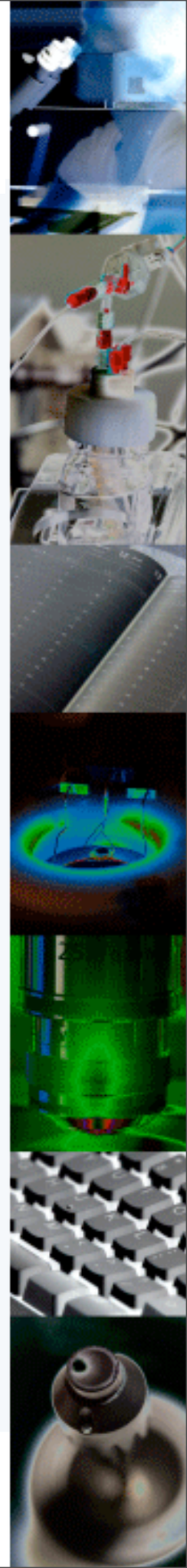
Recherche & Développement :
¶ Imagerie par micro-rotation de cellules
uniques vivantes non-adhérentes
Projet Automation
(SVI, Andor Tech., Evotec Tech., ENS Cachan, Helsinki University et Institut Pasteur)
www.pfid.org/automation
¶ FRET en temps réel (Cairn Res., Optical Insights):
FRET polarisé et FRET stochiométrique

Serveur de Calcul et Stockage :
Serveur AMD64 Opteron
Capacité stockage de 10To

Imagerie Confocale Spectrale :
Microscope Confocal inversé
LSM 510 META Zeiss

Systèmes localisés en dehors de la plate-forme

Imagerie Dynamique Rapide: Microscope inversé équipé d'un poly IV (TILL Vision). Laboratoire P3
Imagerie Dynamique Confocale: Microscope Confocal droit Révolution (Andor Technology)
Imagerie Confocale: Microscope Confocal Leica TCS 4D
Imagerie par Bioluminescence du petit animal: Système Xenogen IVIS 100



Imagopole: Diverse imaging modalities

Expert resources for imaging in close proximity

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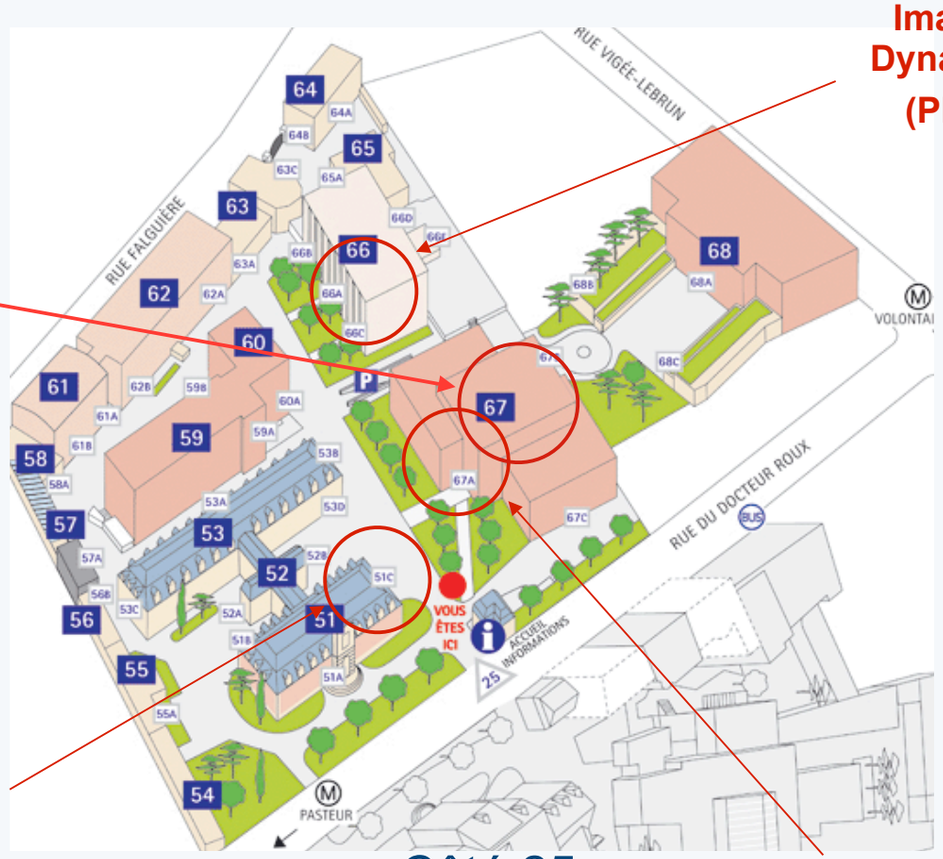
- ***Ultrastructural Microscopies***
- ***Flow Cytometry***
- ***Dynamic Imaging***
- ***Image analysis***
- ***Bio-informatics, computer sciences & IT***
- ***Translational studies***

Centre immunologie humaine (PFCIH)

Imagerie Dynamique (PFID)

Microscopie ultrastructurale (PFMU)



Côté 25

Cytométrie (PFCF)

25 Permanent scientific, engineering, technical staff + 7 temporary staff (post-docs, students etc); 30 heavy equipment installations (imaging microscopes, scanning & flow cytometry, cryoEM etc); Informatics comprising 35-50 active clients, numerous file-, web-, and calculation servers, 10TB local RAID storage; 120TB FAS 6070 NetApp SAN storage a variety of "imaging" softwares: Huygens-SVI, Metamorph, Imaris, Osirix, imageJ, Definiens, 3i etc

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Imaging versus Genomics:

"Walking the walk & talking the talk..."

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Imaging is a fundamental tool for gathering data to leverage knowledge on the mechanism and characteristics of biological function

Tissue/organism	Cellular	Molecular
Intravital imaging	Multi-D fluorescence	Cryo-Electron-Microscopy
In situ cell imaging/tracking	Bioluminescence	CLEM
Ex-vivo tissue imaging	Flow cytometry	FRET/FLIM/FCS/FRAP
Histopathology	Electron microscopy	*STORM/PALM (???)*

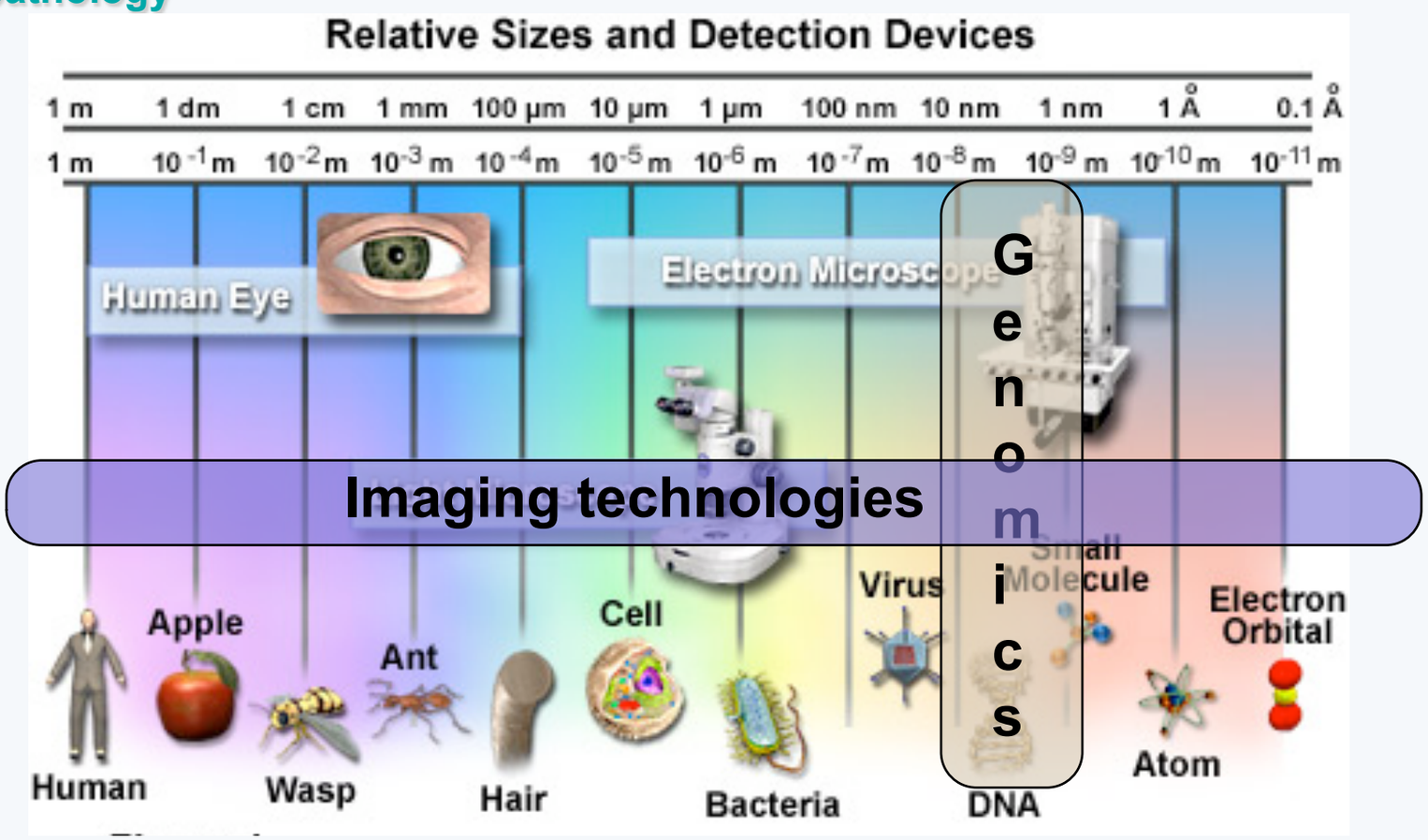

Figure adapted from Florida State University "Expressions" web-site

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Imaging infection:
Relating diverse imaging readouts…

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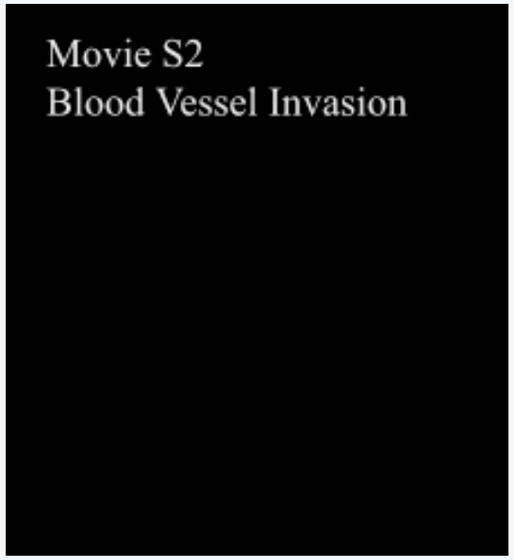
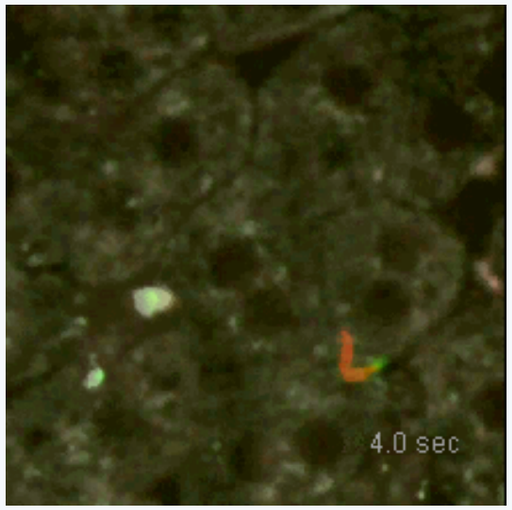
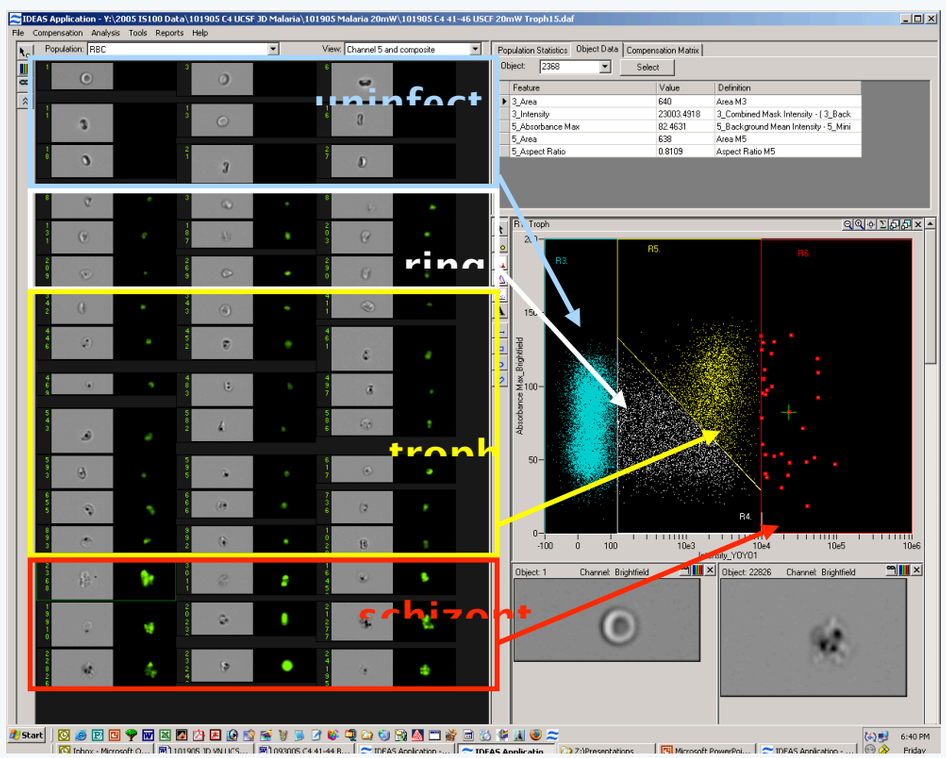
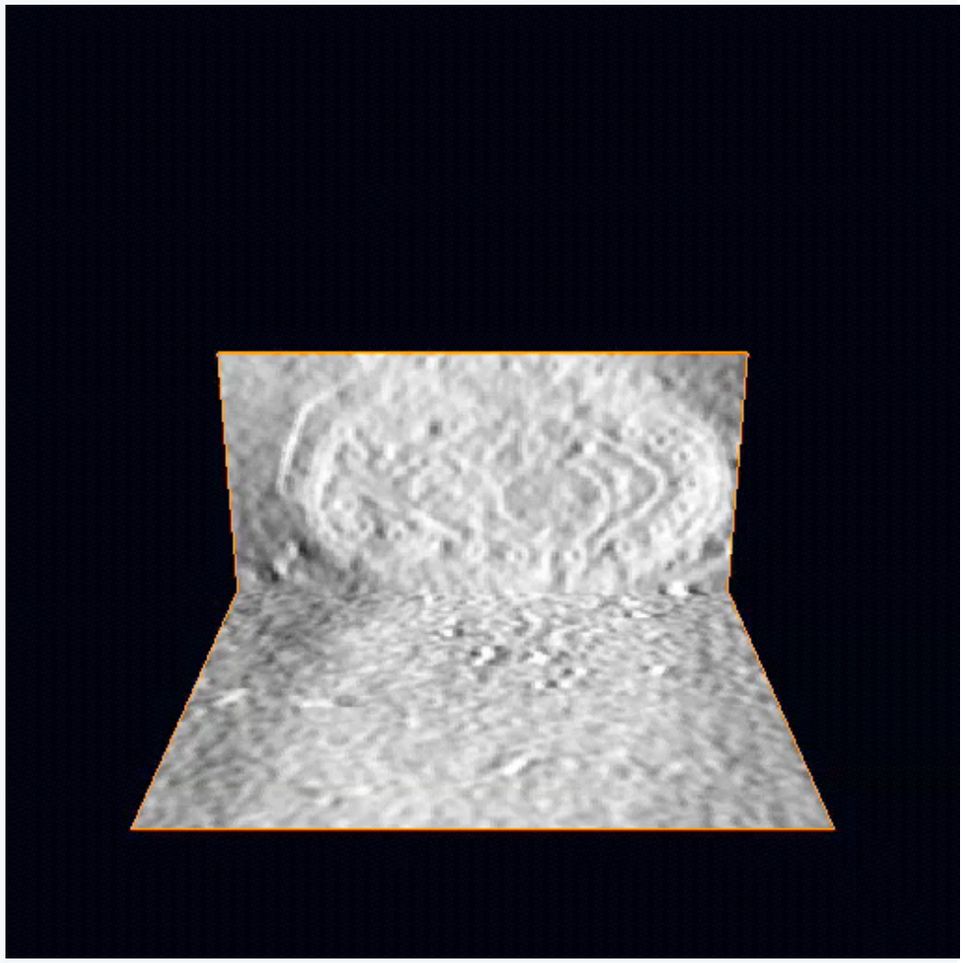
Functional Molecular Dynamics allows to qualify complex functions & phenotypes according to quantifiable criteria, usually by dynamical time-lapse or snap-shot "image" data acquisition (ideally in situ):





- **Information/data independent from mechanistic knowledge of the observed phenomena**
- **Uses multi-parameter descriptors, often partial and/or incomplete… semantic, statistical or population based relevance**





M. Cyrklaff , F. Frischknecht

Imagopole

Functional molecular dynamics

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Centre d'Immunologie Humaine

- **ISO 9001 Certification for quality in service standards (Dec 2007)**
- **>650 registered users, >35,000 hours/year "burn-time"**
- **3 Patents, 1 Software copyright**
- **>30 peer reviewed high-impact scientific articles a year**
- **Global annual budget 2,5 million euros (1,5 million euros salaries)**

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Imagopole - Dynamic imaging

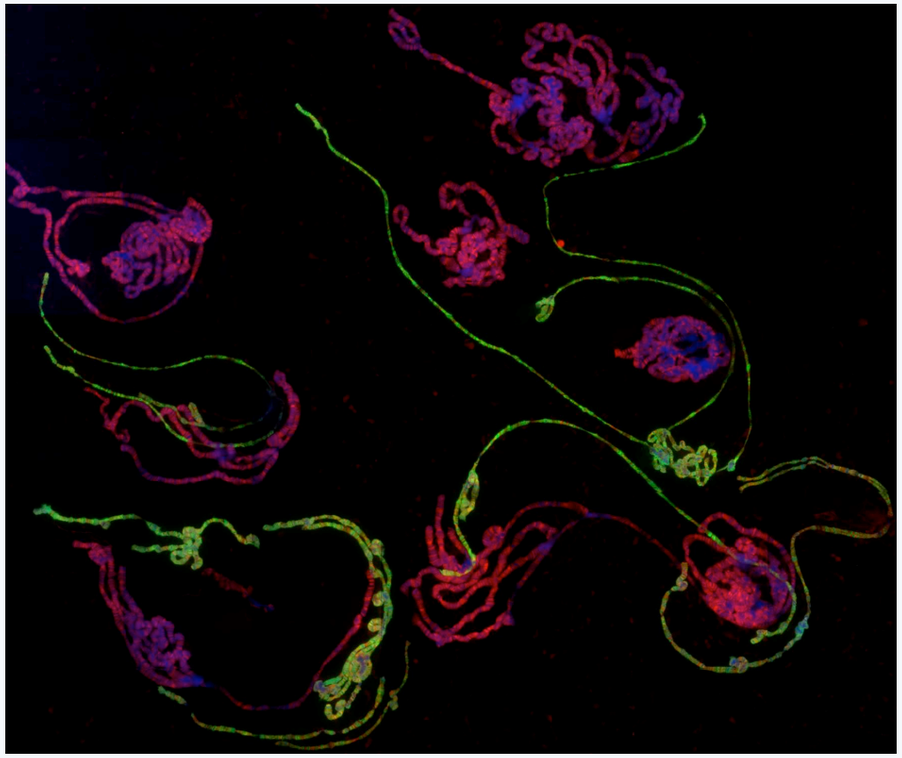
Plateforme d'imagerie dynamique (PFID)

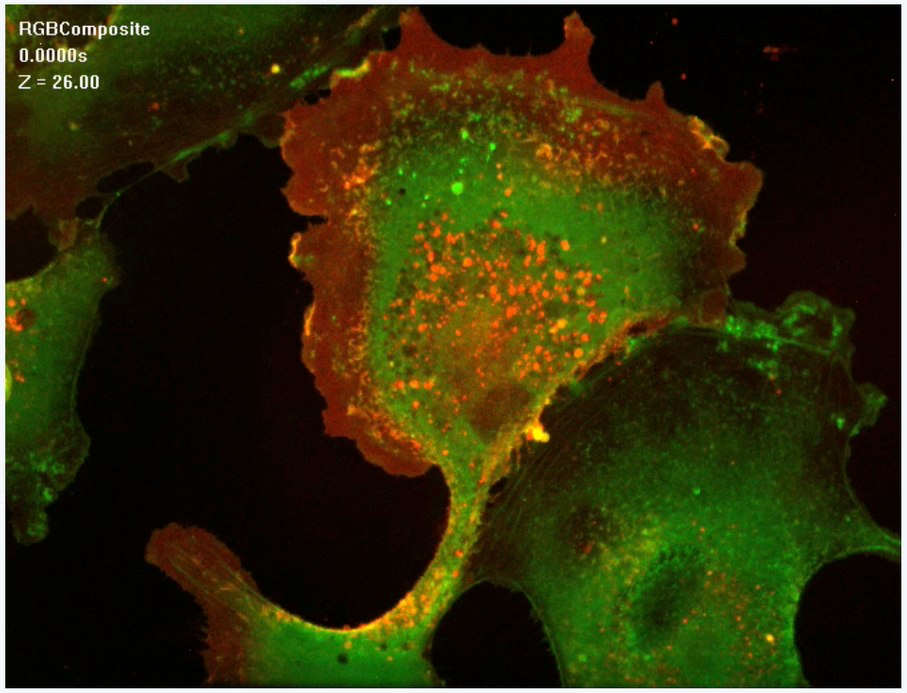
120ème anniversaire

Institut Pasteur

Overview and key facts

- **Dedicated resources for image acquisition, analysis, and data management.**
- **PFID Team: 8-9 engineers/researchers**
- **>20 imaging systems**
- **Multi-D & dynamic imaging; 3D/4D visualization; bio-informatics (statistics & image analysis); intravital imaging; two-photon**
- **Level for biologic security: P2/P2+/P3; A1/A2**





Annual:

- **>15 000h acquisitions**
- **350 users (autonomous and assisted)**
- **>150 specific trainings to give autonomic access to systems**
- **Approximately 10 % of acquisitions assisted by PFID Team**
- **6-10 co-authored research articles**

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Imagopole - Ultrastructural imaging

Plateforme microscopie ultrastructurale (PFMU)

120ème anniversaire

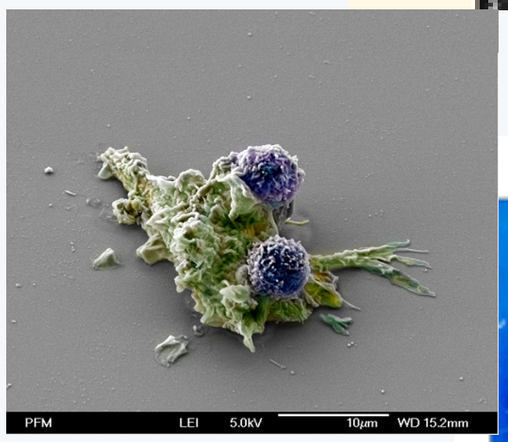
Institut Pasteur

Overview and key facts

➡ **10 peoples (5 engineers and 5 technicians)**

- **Morphological analysis on biological samples in transmission electron microscopy (TEM) and scanning electron microscopy (SEM)**
- **Implementation and development of new technology in cryomethods , cryofixation by immersion and by high pressure freezing , cryosubstitution, cryosectioning, and cryomicroscopy**

- **5 transmission electron microscopes (TEM) and 1 scanning electron microscope (SEM)**
- **9 systems for biological samples preparation**



Annual (based upon 2008):

- **10500 hours per year for using systems**
- **28 autonomous users**
- **10 specific training per year to give autonomous access to systems**
- **46 publications during the last 4 years**

Correlative light electron microscopy (CLEM), and super-resolution optical methods e.g. PALM/STORM & STED

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Imagopole - Cytometry

Plateforme cytometrie en flux (PFCF)

Overview and key facts

➡ 5 permanent staff, 2 temporary

➡ Flow cytometry analysis, fluorescence activated cell sorting; scanning cytometry, imaging cytometry and high-content analysis

➡ 4 flow analyzers, 2 flow sorters; 2 magnetic bead sorters; 1 image-based flow analyzer; 1 high-content visual screening system (Perkin Elmer, *Opera*)

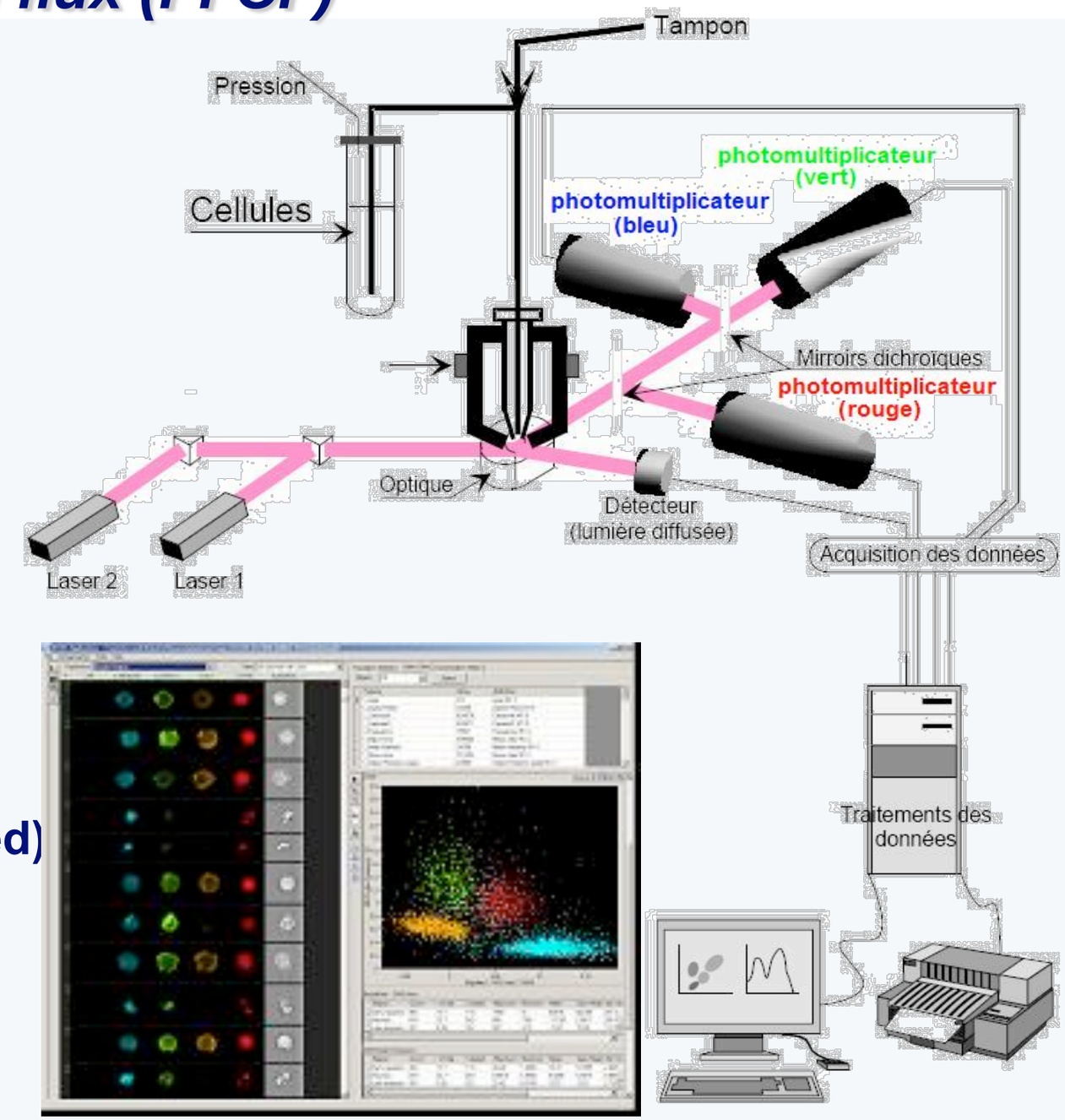
➡Over 2500 hours sorters (1500 hours assisted)

➡ Over 1000 hours autoMACS (autonomes)

➡Over 5000 hours flow analysis:

- ➡120 trainings (1100 heures environ);
- ➡250 hours acquisition / analyses assisted

➡ number of users 250



Imaging cytometry in flow & suspension, and scanning image cytometry

Imagopole – Translational research and human immunology
Center for Human Immunology (PFCIH)

120ème anniversaire
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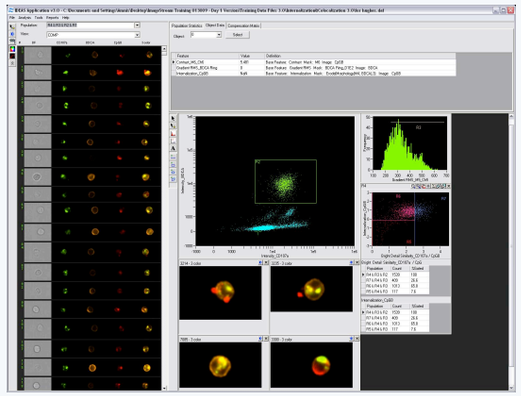
Overview and key facts

- A translational research center dedicated to investigators engaged in human subject studies

- Aim: facilitate the development of collaborative projects between basic scientists and clinicians (bedside-to-bench & back)
- Center of Excellence of the Federation of Clinical Immunology Societies (FOCiS)



CIH staff: 7 team members
(including 2 research engineers, 1 clinical project manager and 1 ARC)

- Advices and help with application of existing technologies

- Trainings for autonomous users on technologies available in the laboratory

- Help scientists with each step of setting up a clinical project

Space and equipment
- Office space and BSL P2+ laboratory = infrastructure developed to host initiation and conduction of translational research projects;
- 4-laser cell sorter, imaging cytometer (ImageStream, Amnis), magnetic cell sorter, Luminex, 4-laser cytometer, fully equipped tissue culture laboratory

10 project-members; 30 users

Access to interesting cohorts, primary tissue samples, cell selection & clonal propagation/amplification

www.pasteur.fr/CIH
cih@pasteur.fr

Outlook and perspectives:
High-content imaging, for chemical & genomic research applications, in studies on infectious disease

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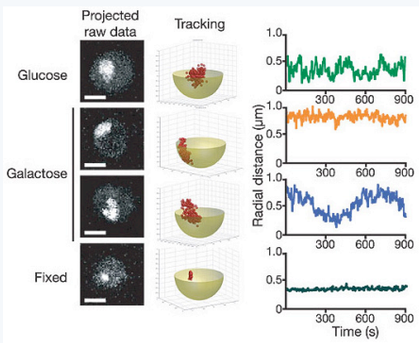
Towards objective approaches in Imaging: Automation

Sample Preparation

Image Acquisition



Data Analysis

Automation

- **Reproducibility**
- **Statistical significance & robust quantification (biological heterogeneity)**
- **Feature rich High-content Information**
  Measuring a maximum of parameters

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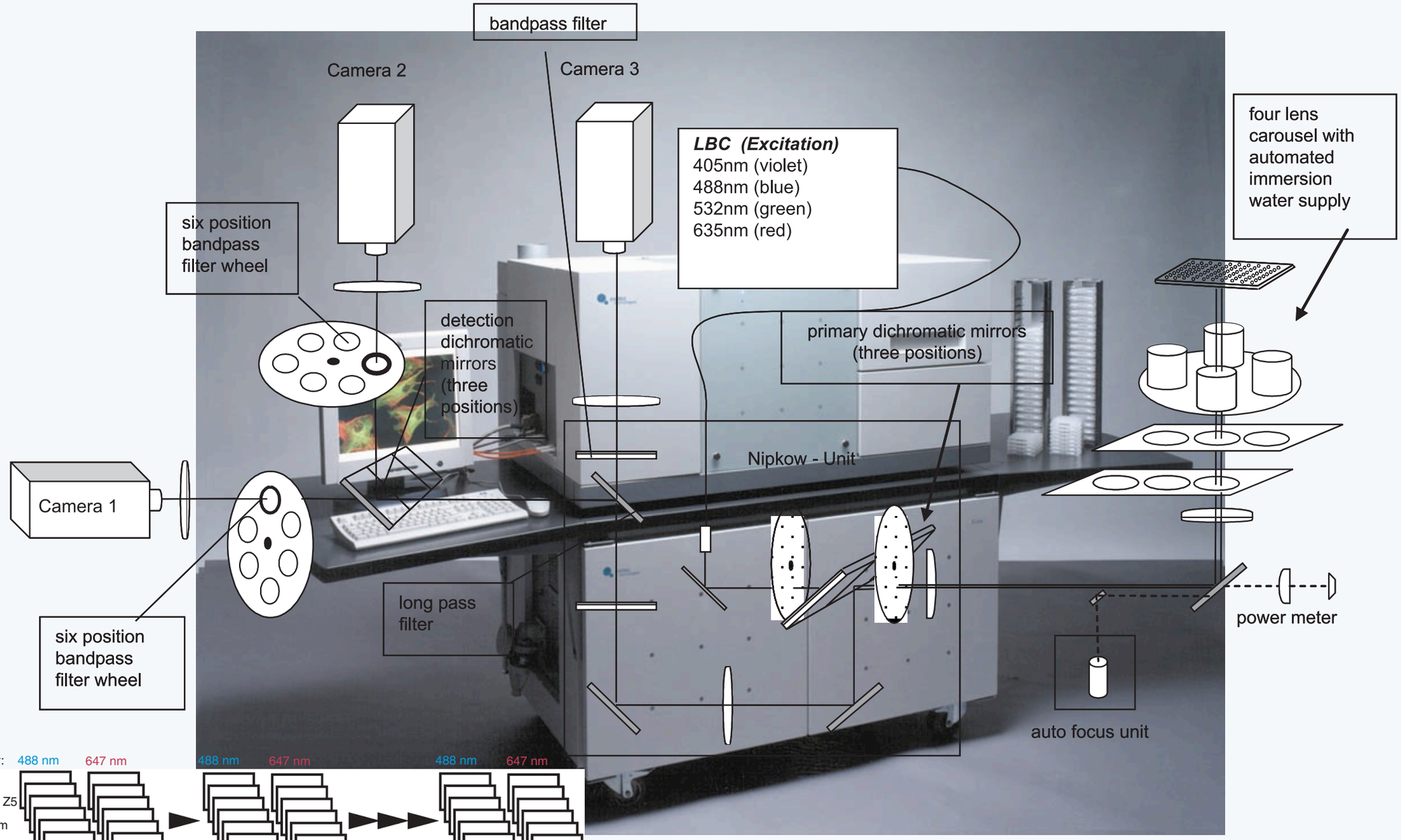
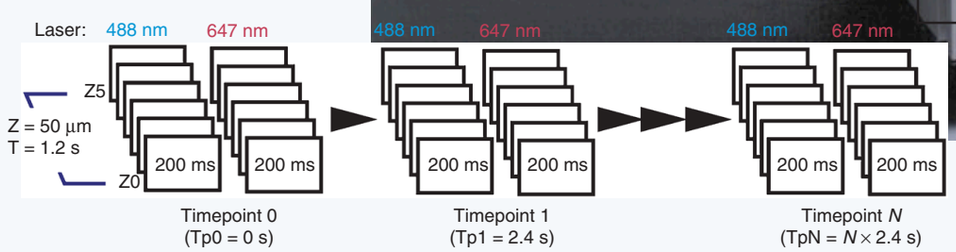
Outlook and perspectives:

High-content imaging, for chemical & genomic research applications, in studies on infectious disease





Automated multi-dimensional fluorescence imaging

G. Gradl et al.

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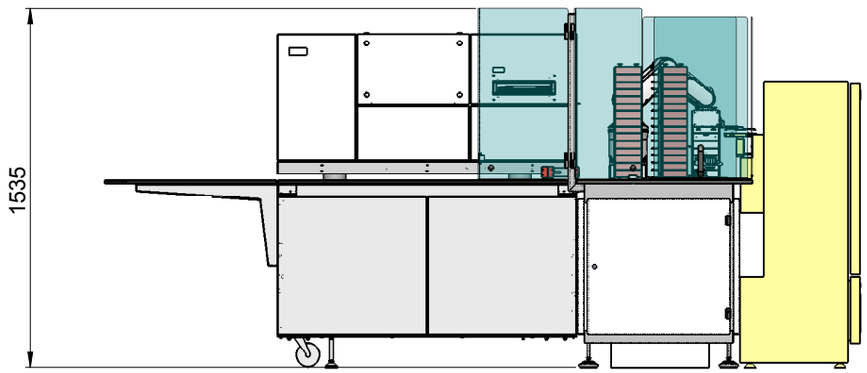
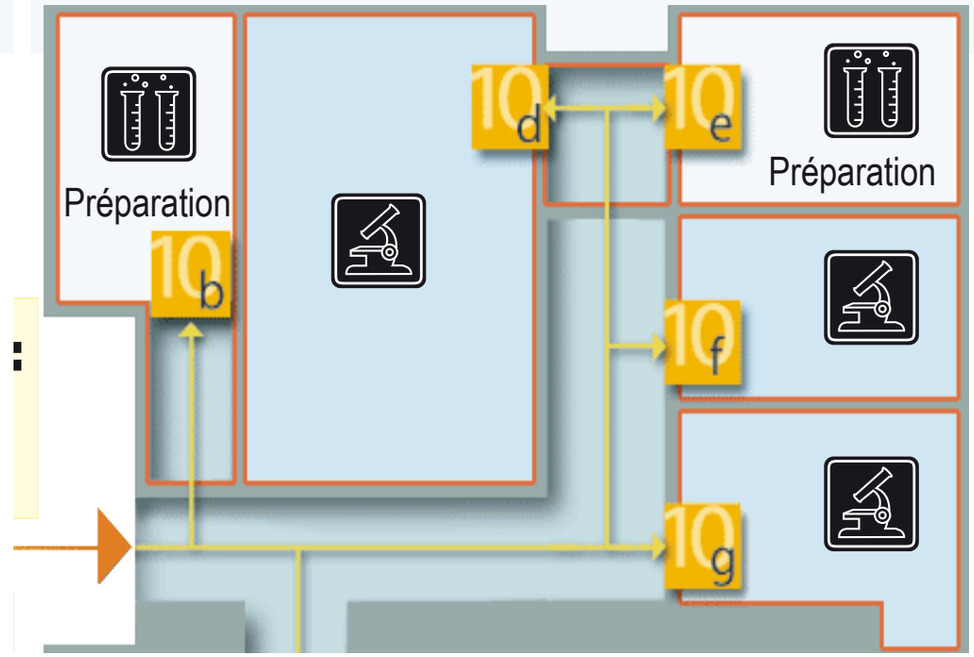
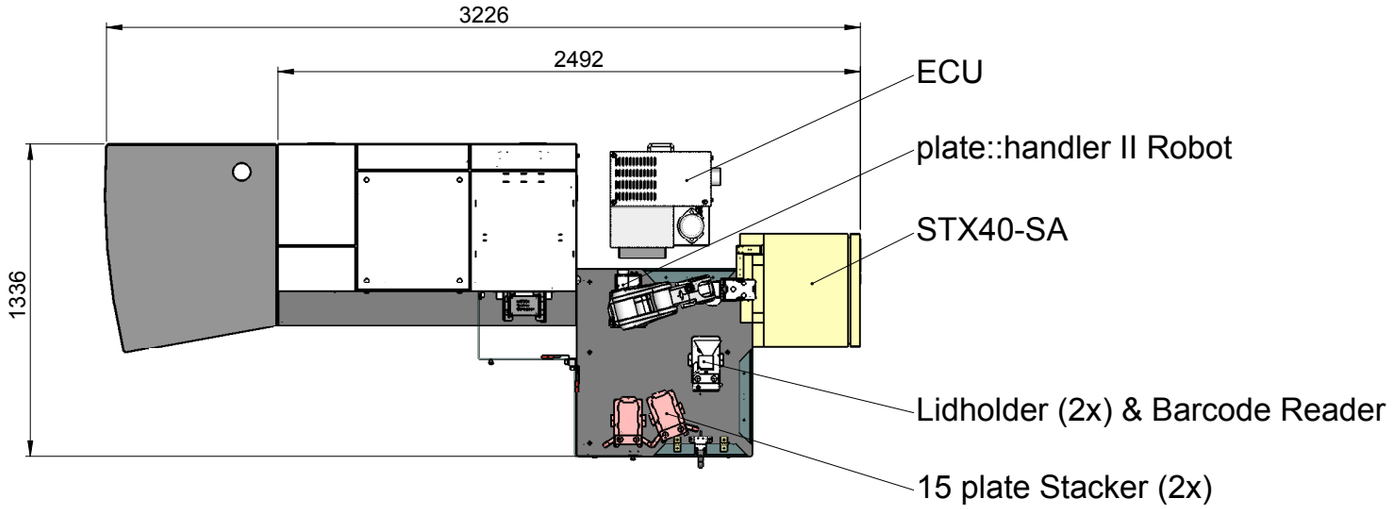
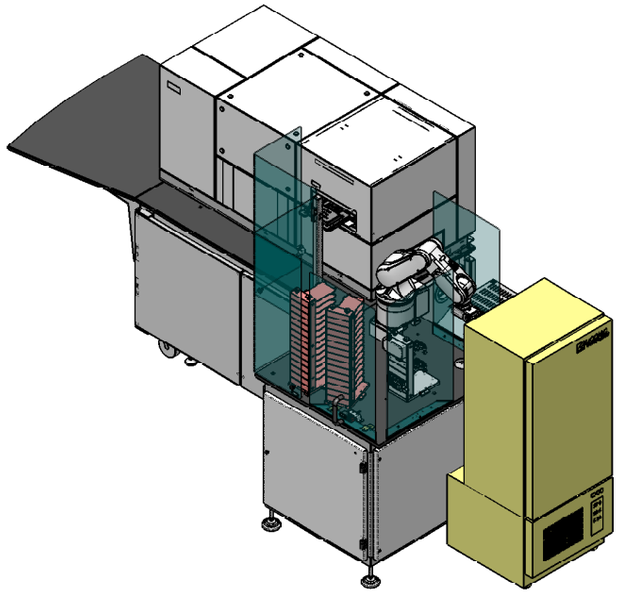
Outlook and perspectives:

High-content imaging, for fundamental research & chemical/genomic screening applications









Program SESAME2007- "Imagopole"
Installation 16 March 2009, into PFID P2+ laboratory environment
Chef du projet Dr. Nathalie Aulner

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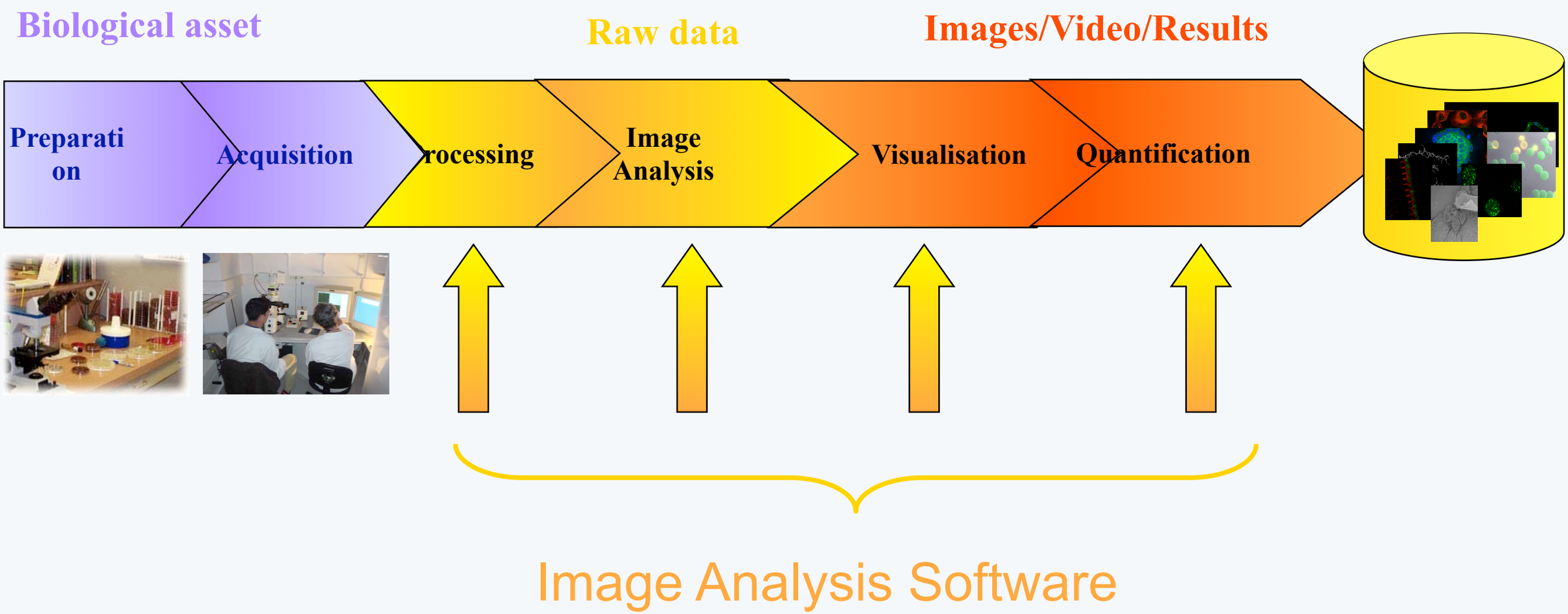
Perspective: Informatic workflow Imagopole



Outlook and perspectives:
Pipelining high-content analysis

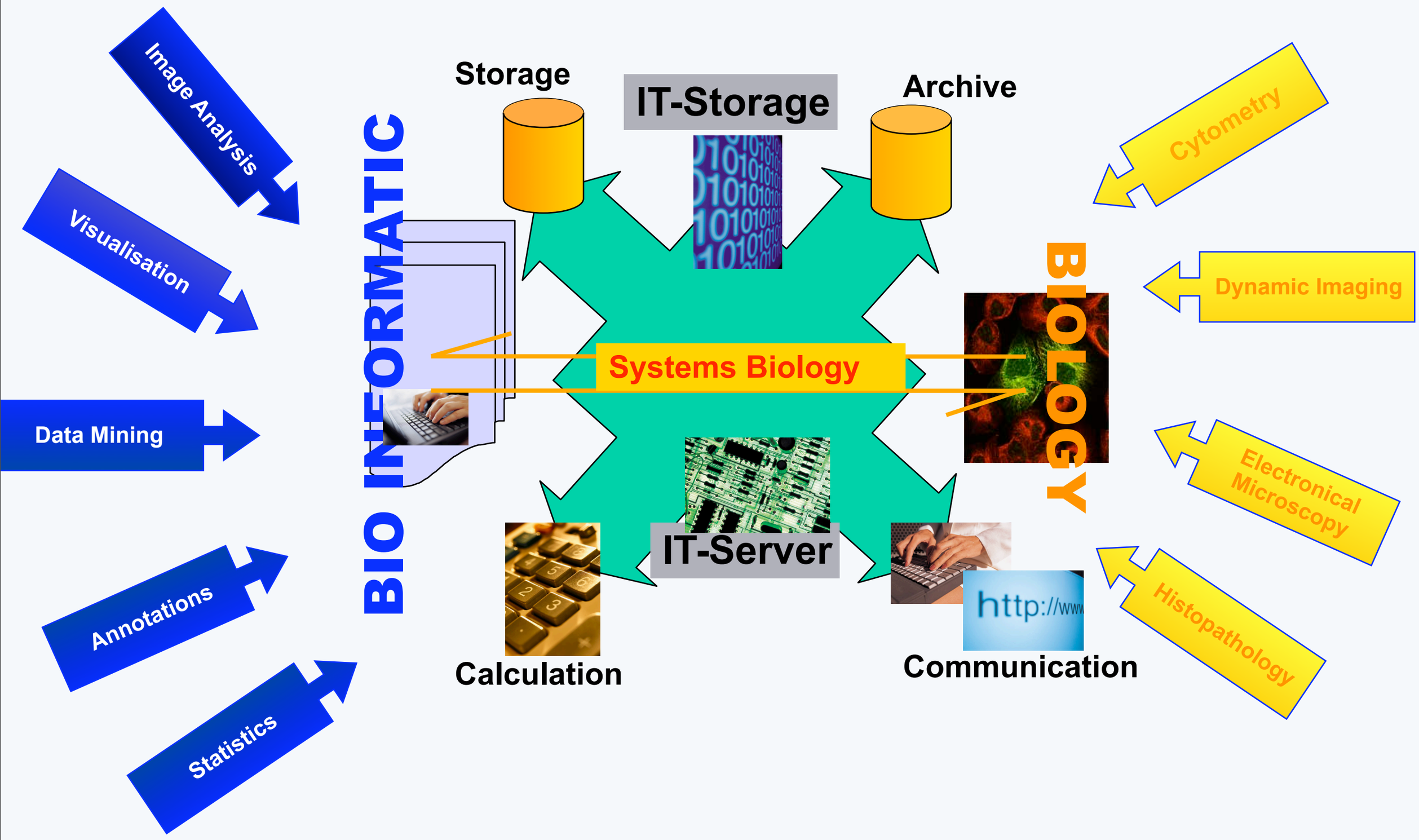


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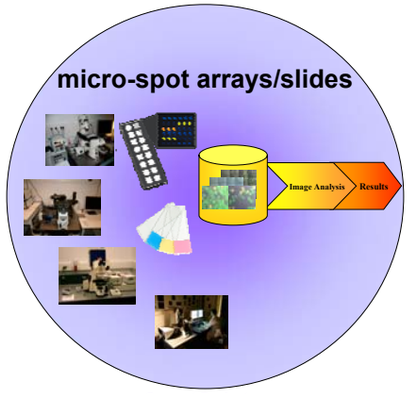
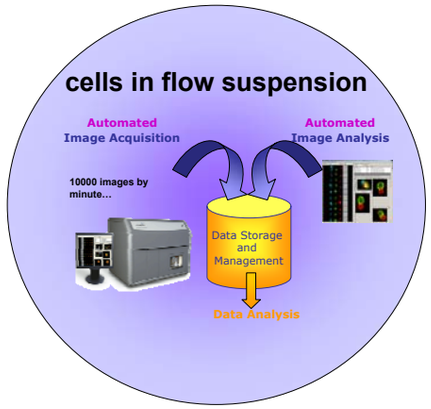
Image database & knowledge mining

Imaging Cytometry

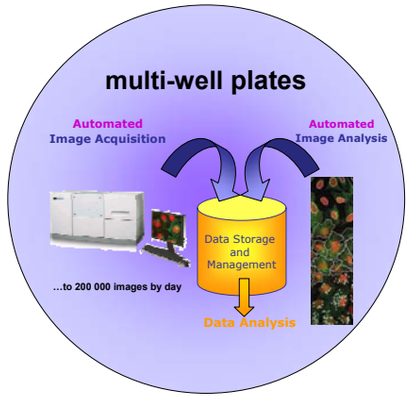
High Content Screening (HCS)



Current Projects
- Claire Forestier (G. Spaeth)
- Christelle Langevin (C. Zurzolo)
- Alexandre Bobard (J. Enninga)
- David Del Alamo (F. Schweisguth)
- Pauline Menager (M. Lafon)
- Olivier Juffroy (J. Theze)
- Nathalie Duval (B. Robert)
- Mounia Lagha (M. Buckingham)
- Corinne Jallet (N. Tordo)



High Throughput Screening (HTS)



Future Projects
- Alcover Andres (A. Alcover)
- Ali Amara (F. Arenzana)
- Clerc Philippe (P. Avner)
- Delmas Olivier (H. Bourhy)
- Duval Nathalie (B. Robert)
- Fabre Emmanuelle (B. Dujon)
- Feldman (O. Schwartz)
- Colucci Emma (P. Herbomel)
- Jory Aurélie (S. Tajbakhsh)
- Marin Karima (P. Herbomel)
- Menard Robert (R. Menard)
- Levraud Jean-Pierre (P. Herbomel)
- Lucas (P. Forterre)
- Michel (S. Pellegrini)
- Mostowy Serge (P. Cossart)
- Prina Eric (G. Milon)
- Sauvonnet Nathalie (A. Dautry)

Data Storage & Management

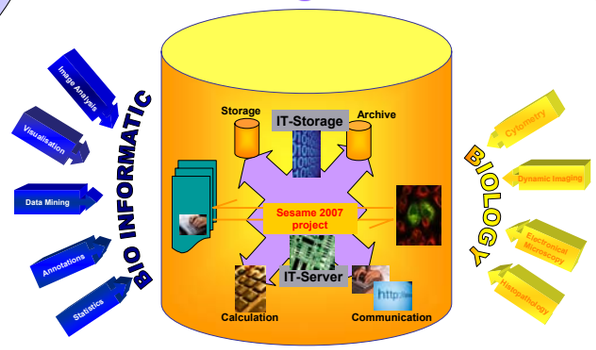


Image Database

OMERO implementation

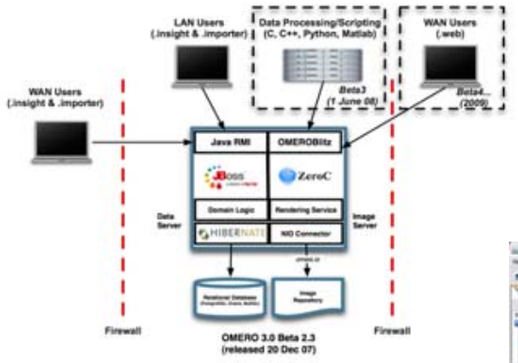
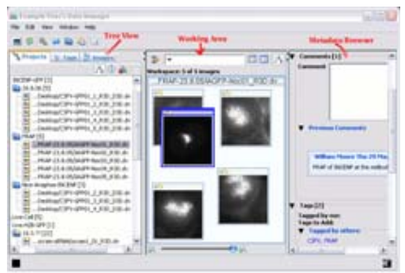
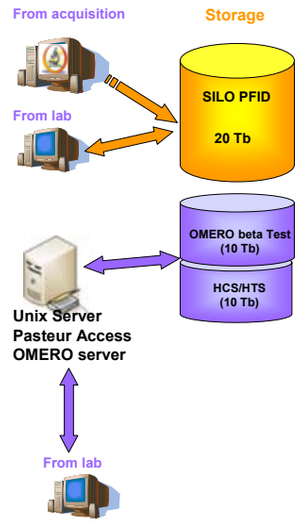


Image Viewer



Sesame 2007 Project



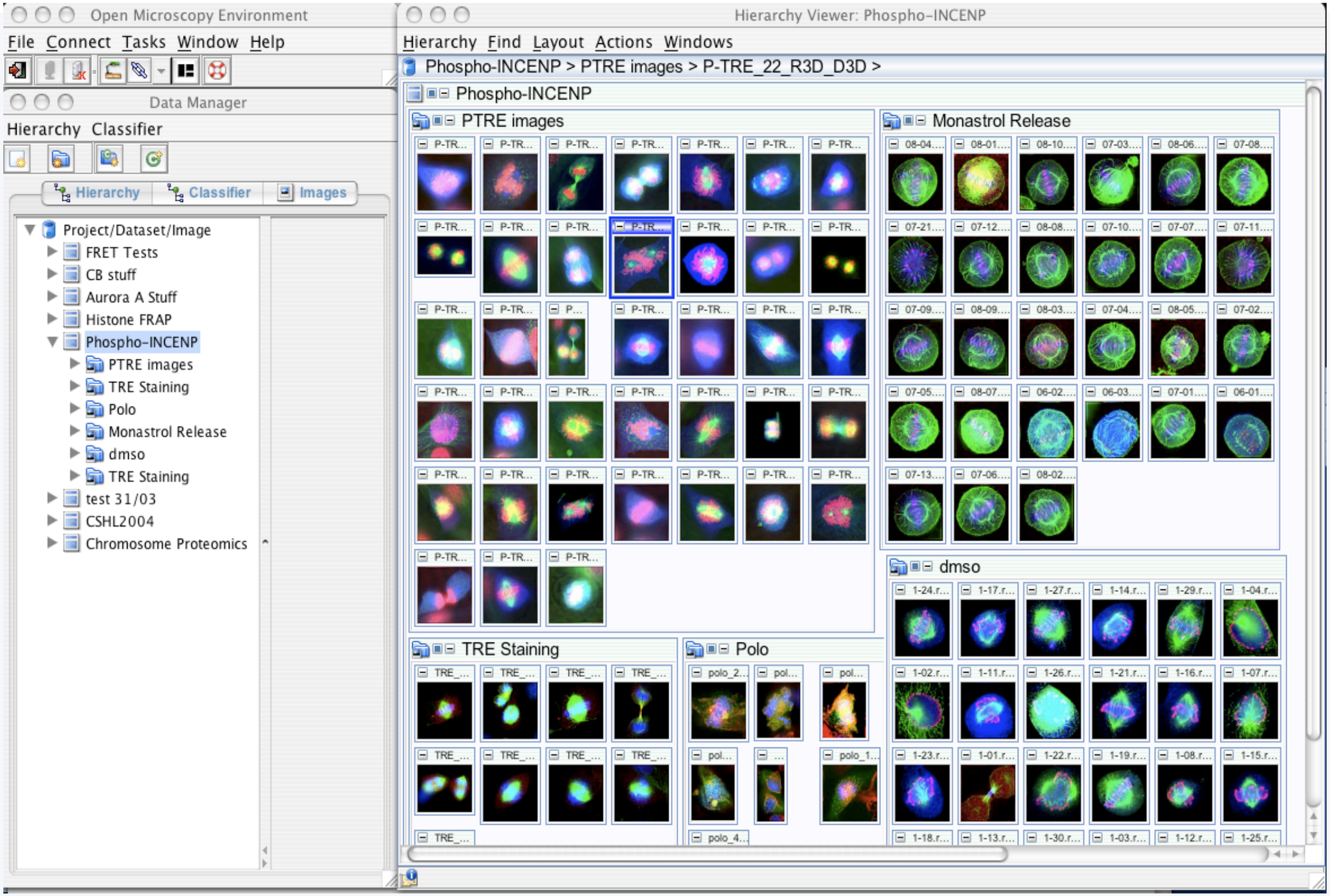
1) Microscopie électronique ultrastructurale par tomographie & "Correlative Light Electron Microscopy" (CLEM) (Dr. Anna Sartori)
2) Systèmes automatisés de criblage par l'imagerie à haut débit et haute résolution pour des cellules uniques adhérentes ou non-adhérentes (« Automated high-throughout/high-content single cell analysis in adherent and non-adherent cells ») (Dr Olivier Renaud).
3) Création d'un système centralisé et sécurisé de stockage et de partage d'information pour l'imagerie dans le domaine de la recherche en biologie (Dr Anne Danckaert).

OMERO Beta testers

Unit Name	Chef	Size (Tb)
Biologie Cellulaire des Lymphocites	Alcover	1
défense innée et inflammation	Chignard	1
interactions bactéries-cellules	Cossart	1
Biologie des Interactions Cellulaires	Dautry	1
Dynamique Interactions Hôte-Pathogène	Enninga	1
Macrophages et Développement de l'Immunité	Herbomel	1
Biologie et Génétique du Paludisme	Menard	1
Génétique des déficits sensoriels	Petit	0.5
Virus et immunité	Schwarz	0.5
PF-Imagerie-Dynamique	Shorte	1
Trafic membranaire et pathogénèse	Zurzolo	1
Total (Tb)		10

IMAGOPOLE
Pôle de Dynamique Moléculaire et Fonctionnelle
Plate-forme de Cytométrie en Flux
Plate-forme d'Imagerie Dynamique
Plate-forme de Microscopie Ultrastructurale

OME/OMERO…



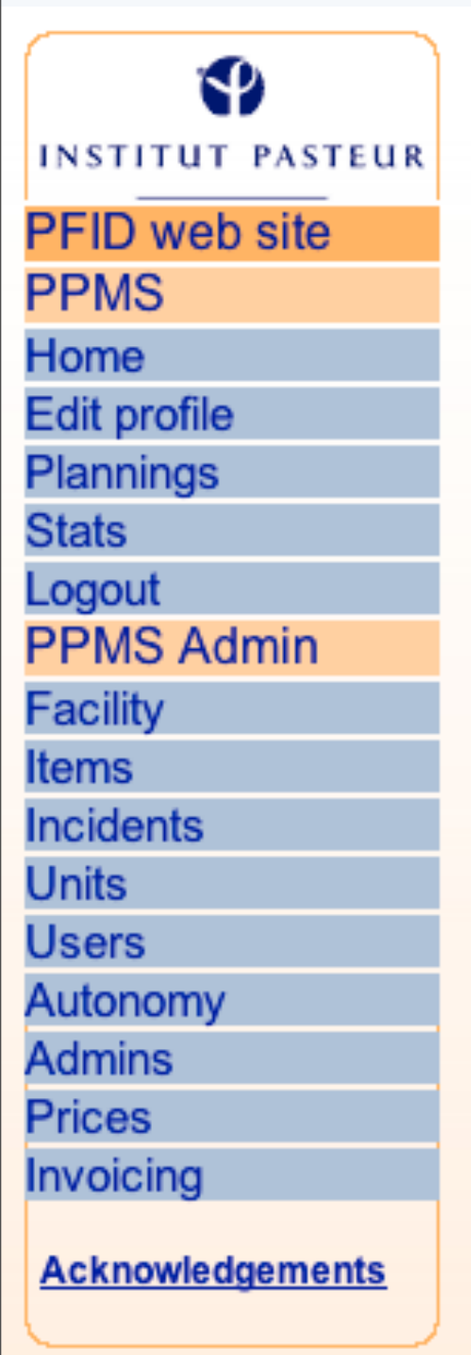
- **Screenshot from OME/OMERO** www.openmicroscopy.org

Imagopole - Resources management

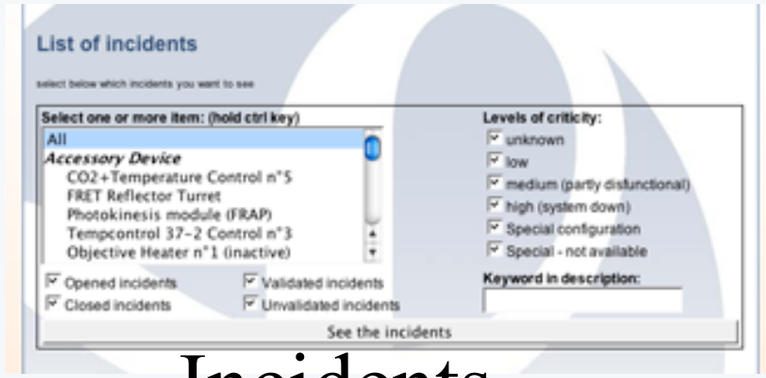
Pasteur platform management system (PPMS)

120ème anniversaire

Institut Pasteur



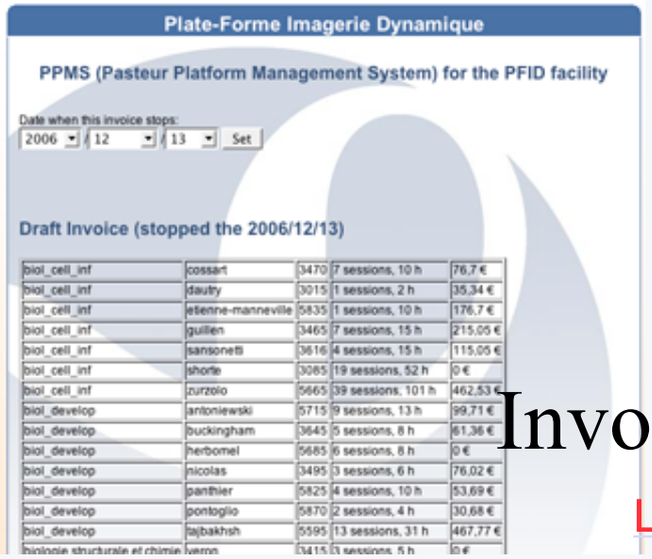
Booking



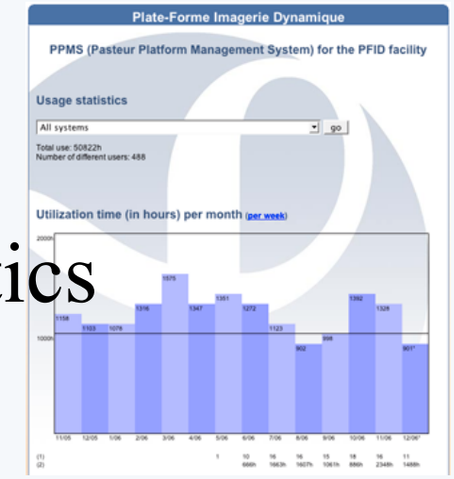
Incidents

User profile

PPMS



Invoicing



Statistics

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Acknowledgements

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