Institut Pasteur

Imagopole

EuCAI founding meeting

Trinity College, Dublin, Feb. 18th 2013

Organized by Peter Hovarth & Anthony Davies

IBiSA INFRASTRUCTURES BIOLOGIE SANTÉ ET AGRONOMIE

IMAGOPOLE
Pôle de Dynamique Moléculaire et Fonctionnelle

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

Functional analyses of molecular & cellular dynamics

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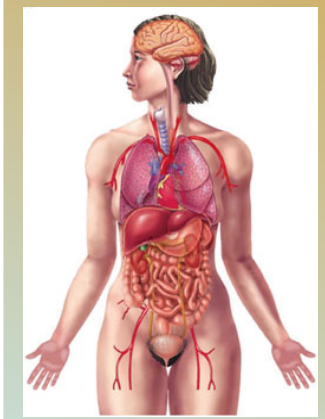
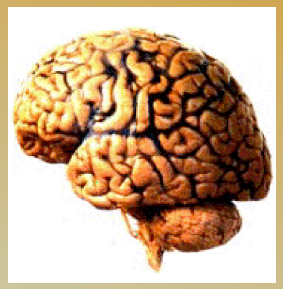
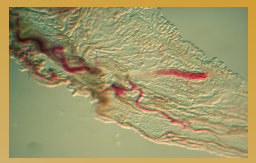
Tissue/organism | **Cellular** | **Molecular**

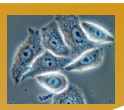
Bedside → **Bench**

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...

Imaging describes an emergent set of tools for gathering data to leverage knowledge on the mechanism and charateristics of biological functions across manifold dimensions in space and time...

Biology is a scale problem...

Integrative Experimental Biology
Imaging *functional & molecular dynamics*

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Tissue/organism	Cellular	Molecular
Intravital imaging-2Pi/SHG	Multi-D fluorescence	Cryo-Electron-Microscopy
In situ cell imaging/tracking	Bioluminescence	CLEM- Correlatoive EM
Ex-vivo tissue imaging	Imaging Flow Cytometry	FRET/FLIM/FCS/FRAP/FLIP
Histopathology/QWBA/MALDI	Tomo-Electron microscopy	*STORM/PALM/STED/+???...*
In Vivo BL/FL/FMT/Spectral imaging	Hyper-Spectral	AFM/FL

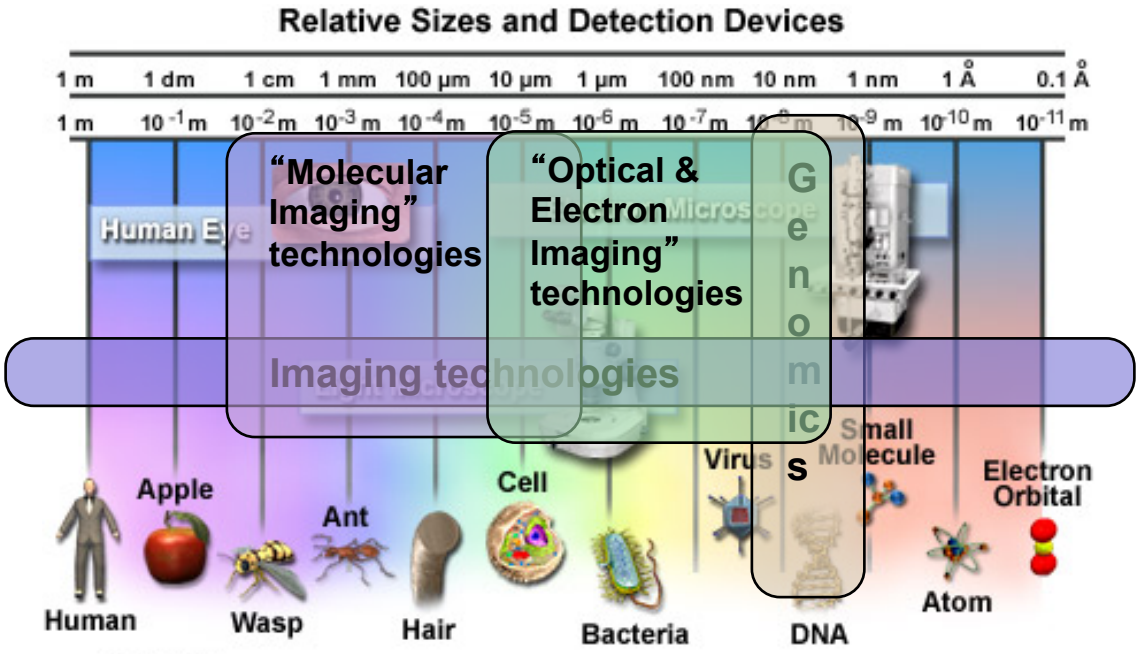

Figure adapted from Florida State University "Expressions" web-site

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IBiSA INFRASTRUTURES BIOLOGIE SANTÉ ET AGRONOMIE

Integrative Experimental Biology

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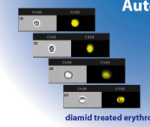
High Content Screening (HCS)

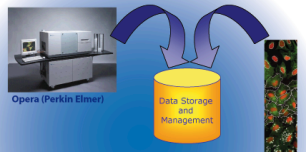
Imaging Cytometry

Cells in suspension

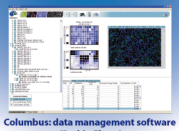
Automated Image Analysis on a Per-Cell basis



Multi-well Plates



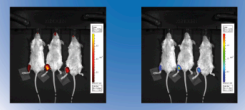
High Content Analysis (HCA)



In Vivo Optical Analysis

Whole Animal

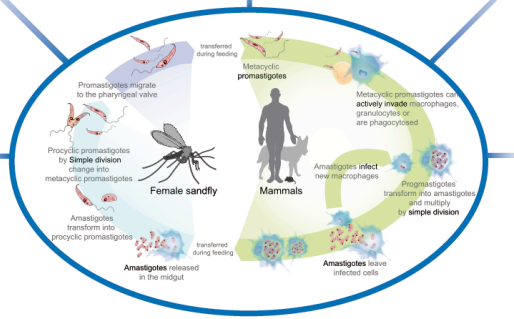
Correlative Analysis



Cytometry

Cell Phenotype

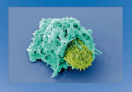
Cell population Analysis by Scatter Plots



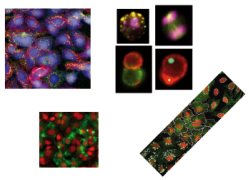
Electronic microscopy

Ultra Structural Level

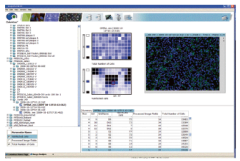
High Resolution Imaging



Quantification

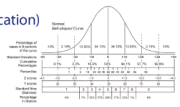


Analysis by readout model

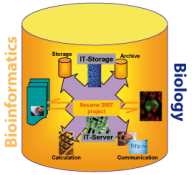


Validation & Statistics

b Correlation analysis (Realtionship between HCA readouts)
b Normalisation
b Multivariate Analysis
b Cluster analysis of readouts (Classification)
b Hit/Event selection Procedure



Data Storage & Management



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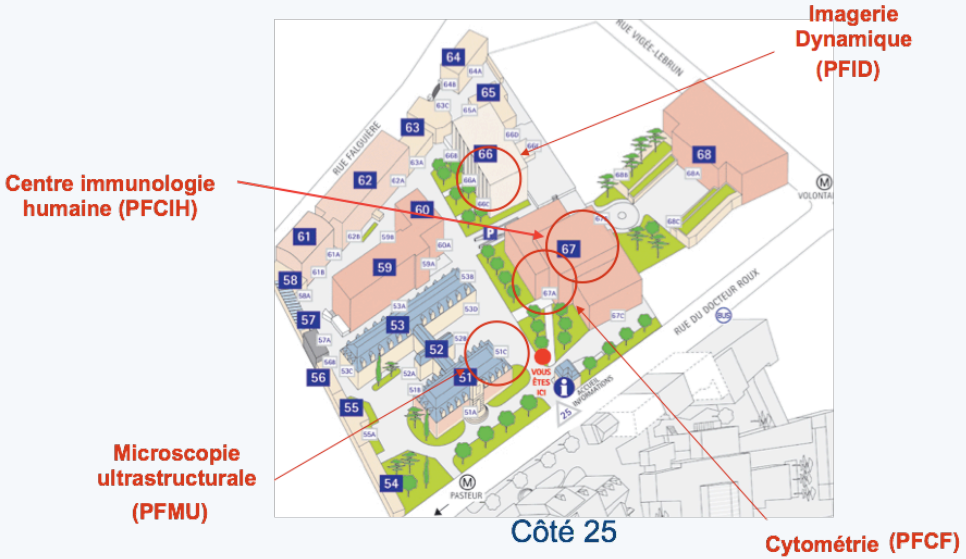
www.imagopole.org

Imagopole

Imaging functional & molecular dynamics

Institut Pasteur



Imagopole mission: *Develop and apply scientific imaging technologies, in the context of experimental frameworks aimed at understanding of biological processes, and their usurpation by infectious disease*

www.imagopole.org

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Defining a technical platform group

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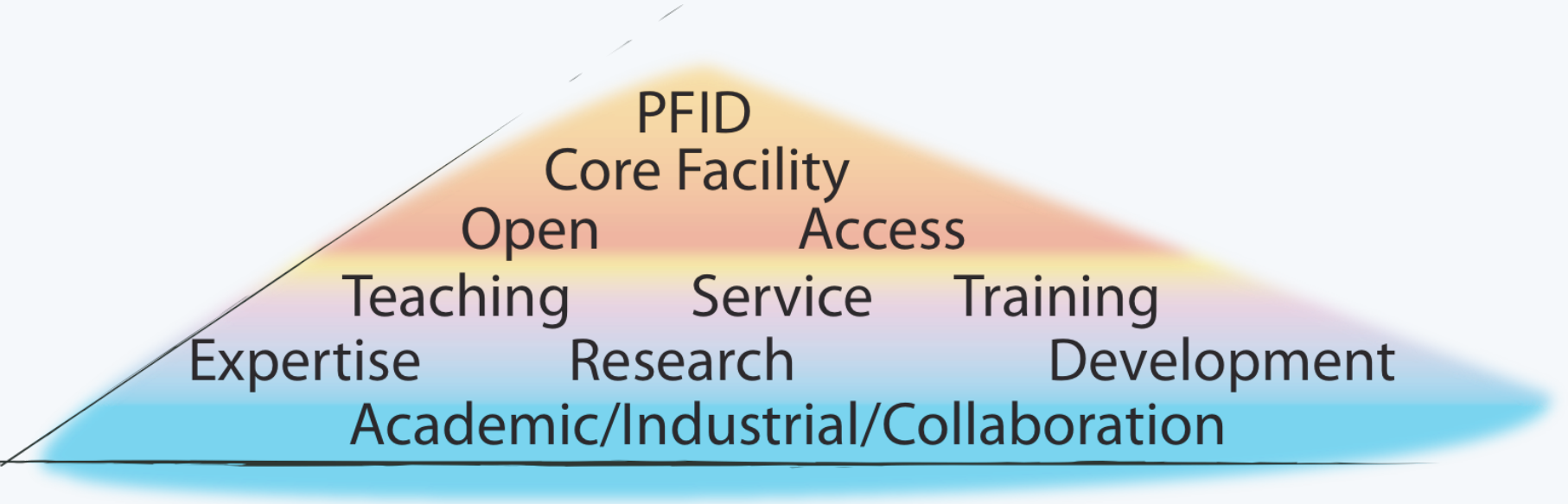
Technology Platform philosophy:

platforms have a different focus to research units

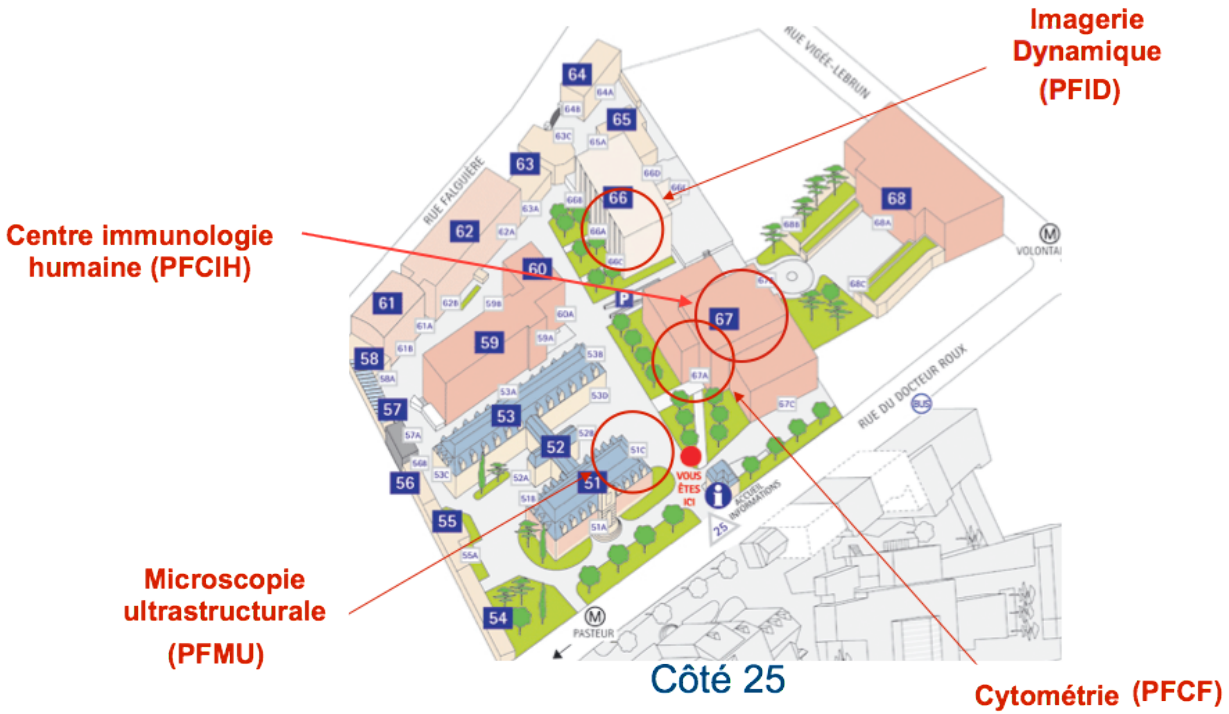


Imagopole

Imaging functional & molecular dynamics

Institut Pasteur

- ***Ultrastructural Microscopies***
- ***Imaging & Flow Cytometry***
- ***Dynamic optical imaging***
- ***Intravital & in vivo imaging***
- ***Image processing & analysis***
- ***Bio-informatics, & statistics***
- ***Molecular imaging***
- ***Translational research***
- ***Reagents, Contrast Agents***



35 Permanent scientific, engineering, technical staff including 7 temporary staff (post-docs, students etc); 40 major equipment installations (imaging microscopes, scanning & flow cytometry, cryoEM etc); Informatics comprising 100 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

Imagopole

Key facts & figures

- ISO 9001 Certification for quality in service standards (Dec 2007)
- IBiSA national scientific platform label (Nov 2009)
- >700 registered users, >45,000 hours/year "burn-time"
- 10% External users
- 4 Patents, 1 Software copyright (1 spin-out Stratocore.com)
- >25 peer reviewed high-impact scientific articles a year
- Global annual budget millions euros (salaries, consumables, equipment)

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Upon request…

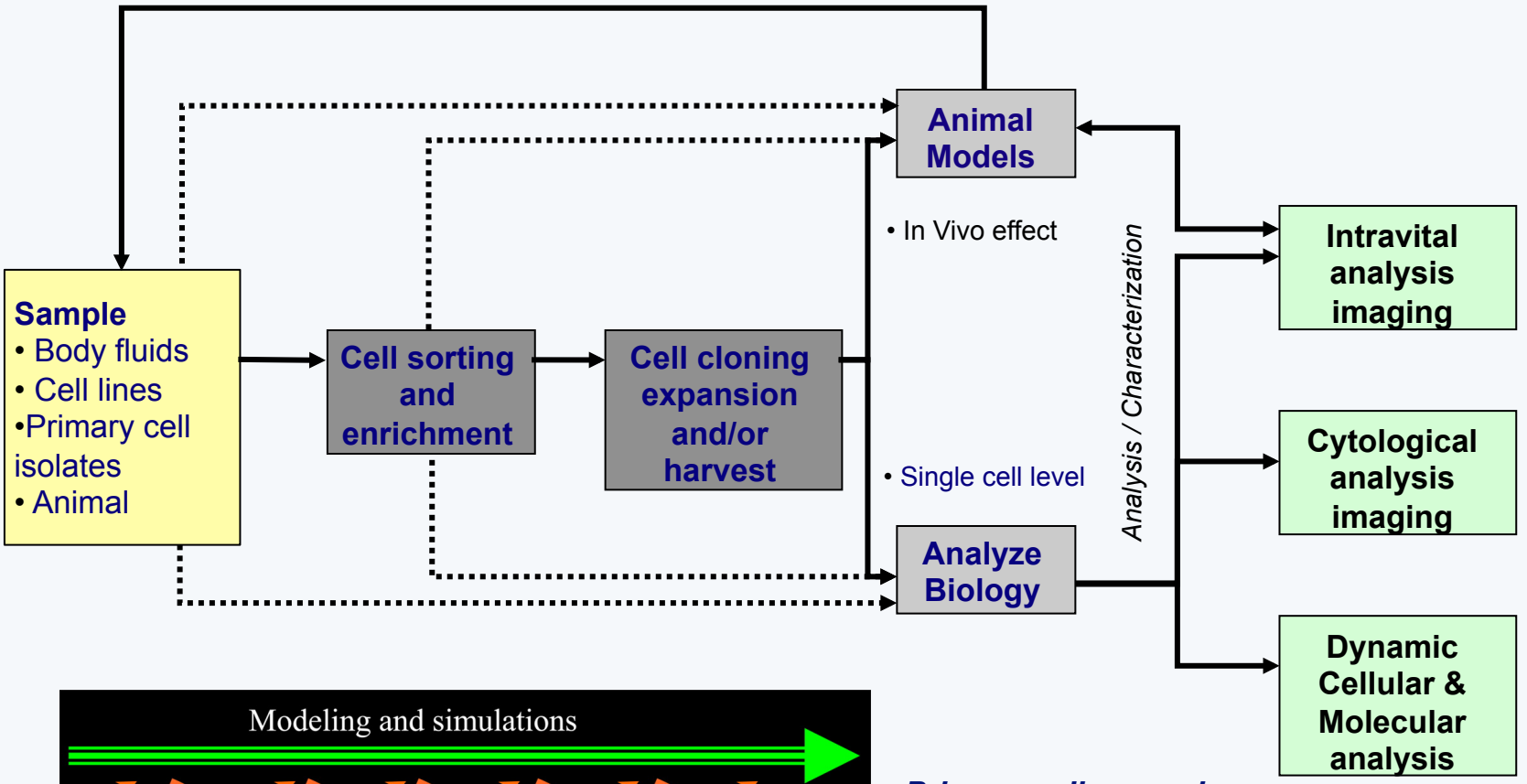
Stratocore

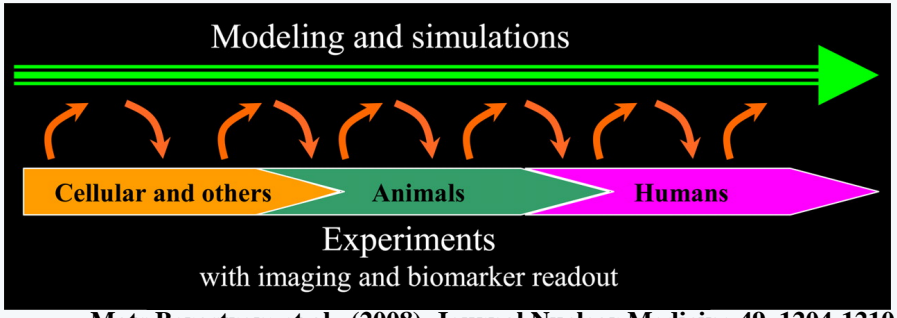
Core facility resource management

http://stratocore.com

Integrative Experimental Biology

Institut Pasteur



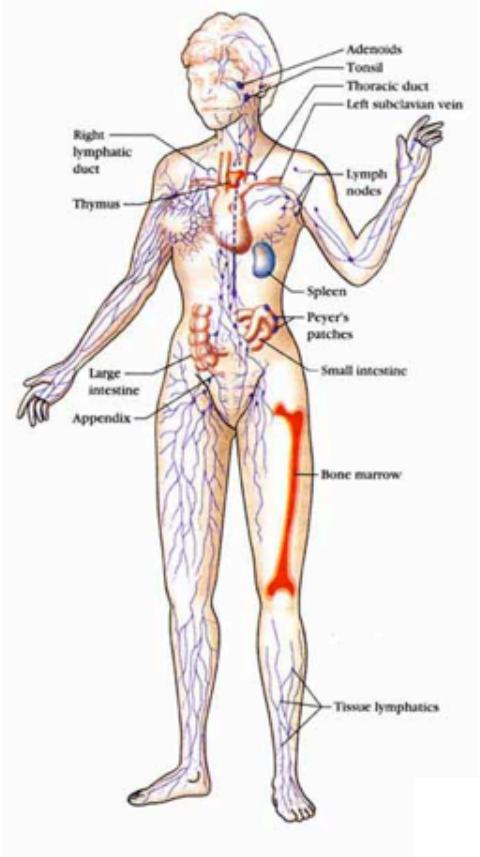


Mats Bergstrom et al., (2008), Journal Nuclear Medicine 49, 1204-1210.

- *Primary cell screening*
- *Sub-clone isolation for screening*
- *Quality control*
- *Isolation of transgenically modified pathogens*
- *Animal models for in vivo & intravital imaging*

A systems approach to understanding the Immune System

Genetic & Environmental Determinants of Immune Phenotype Variance



- How variable is the « immune response » across a population of healthy individuals?
- How can we account for this variation – genetic, epigenetic *versus* environmental control?
- How have genetically-controlled immune mechanisms contributed to our survival?
- How do perturbation of these mechanisms contribute TODAY to immune-related pathologic conditions?

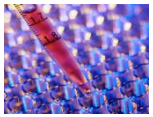
A path towards personalized medicine

- Host response to infectious agents
- Response to adjuvants and vaccines
- Relationship between commensals and immunity

1000 healthy donors included: 3 visits (V0, V1, V2)

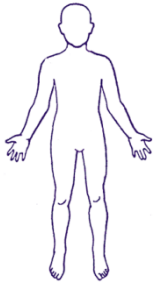
Clinical data

Serology



Whole blood 20ml

Daily Transport 18C-25C

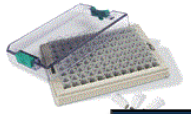


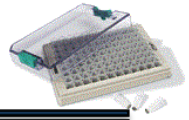
Whole blood 50ml

x40 stimulations

plasma

stabilized RNA in TruC





Nasal/Faeces swabs

Skin biopsy 1st visit

Daily Transport 4C

BioTrial

weekly transport -80C

CIH Pasteur

Flow cytometry

DNA

magPix

RNA profile

Epidemiology | Cellular phenotyping | Genome Sequencing | Proteomics | Transcriptome Profiling | Microbiota Profiling | Pluripotent stem cells

Imagopole: *Pole for functional molecular dynamics*

Institut Pasteur

Imagopole mission:

Apply cutting edge "imaging" science & technologies to studies on the dynamics of biological processes and their usurpation by infectious disease

"Imaging" describes an emergent set of experimental tools...

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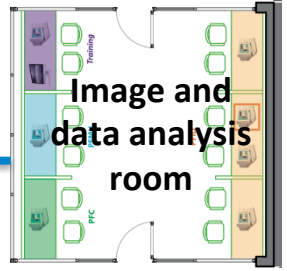
Plateforme d'imagerie dynamique



OFFICES

LSM700 inv

LSM700 Upright

APOTOMES

inv.

Upright

04c

PFID Develop.

Open P1 space

SP5 CONFOCAL

BIOSTATION

12b

SPINNING DISK VOX

P2+

12a

Sas P2+

High Content Analysis

OPERA

Liquid Handling Robot JANUS mini

13b

Super Resolution

ELYRA

13d

PFID Development

FAST ACQUISITION

Wide Field

13e

DEFINITE FOCUS

FLIM

08c

08e

08d

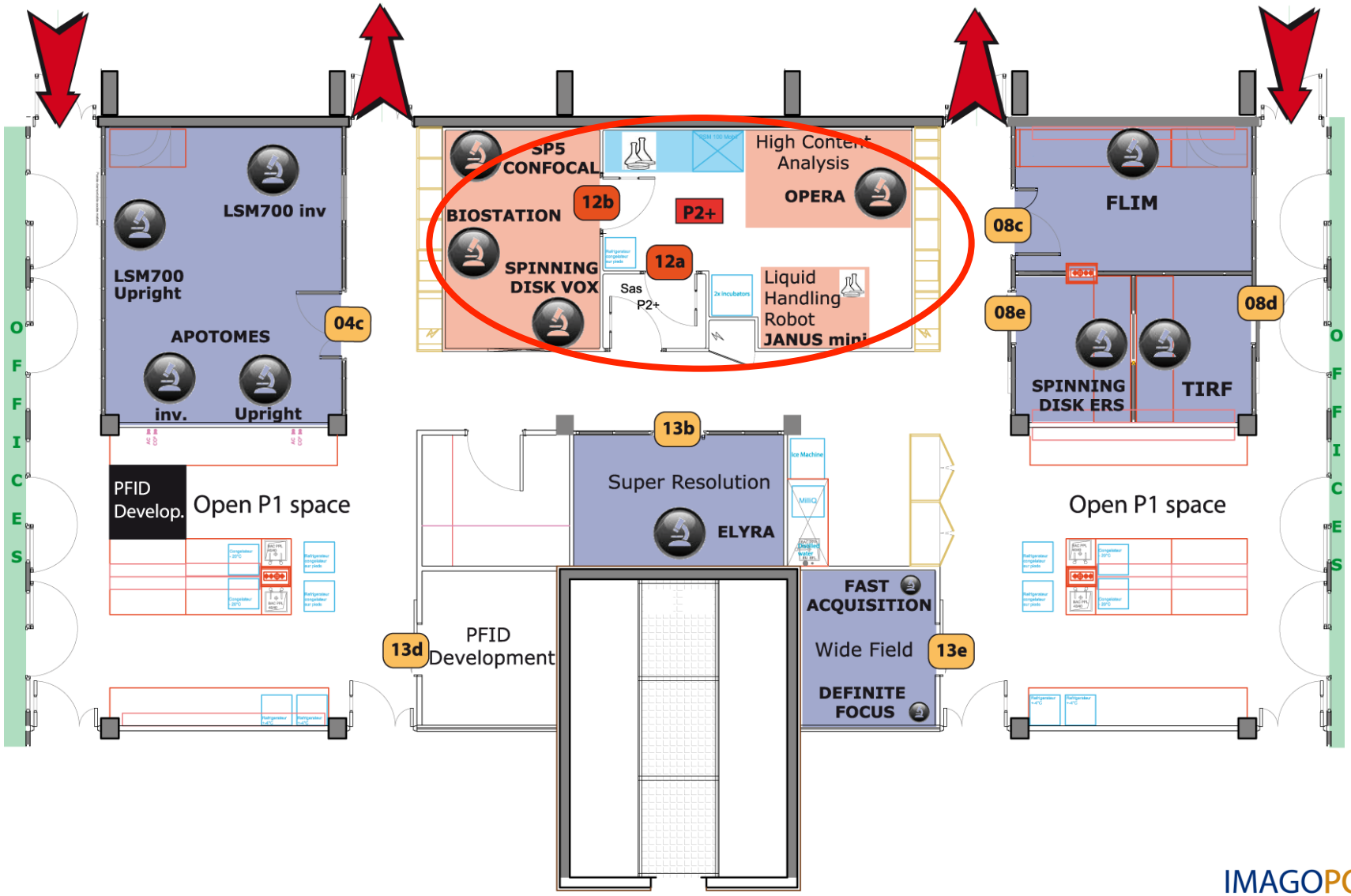
SPINNING DISK ERS

TIRF

Open P1 space

OFFICES

BSL2+ Equipment for live cell imaging in relevant models



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Small Animal Imaging → Towards relevant biology

Institut Pasteur

New animal facility: BIME level -2 (BSL3)

2x IVIS Spectrum

- Filters 20nm band pass from 400nm to 800 nm for fluorescence and bioluminescence
- 5 mice at a time
- second system equipped with a CT scanner

1 FMT (PerkinElmer)

- 3D NIR fluorescence
- 1 animal at a time

Possible under development system (longer term)

- Combined Fluorescence and bioluminescence

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HCS of *Klebsiella pneumoniae* mutants library

Joelle Mounier & Régis Tournebize (P. Sansonnetti, Molecular microbial pathogenecity)

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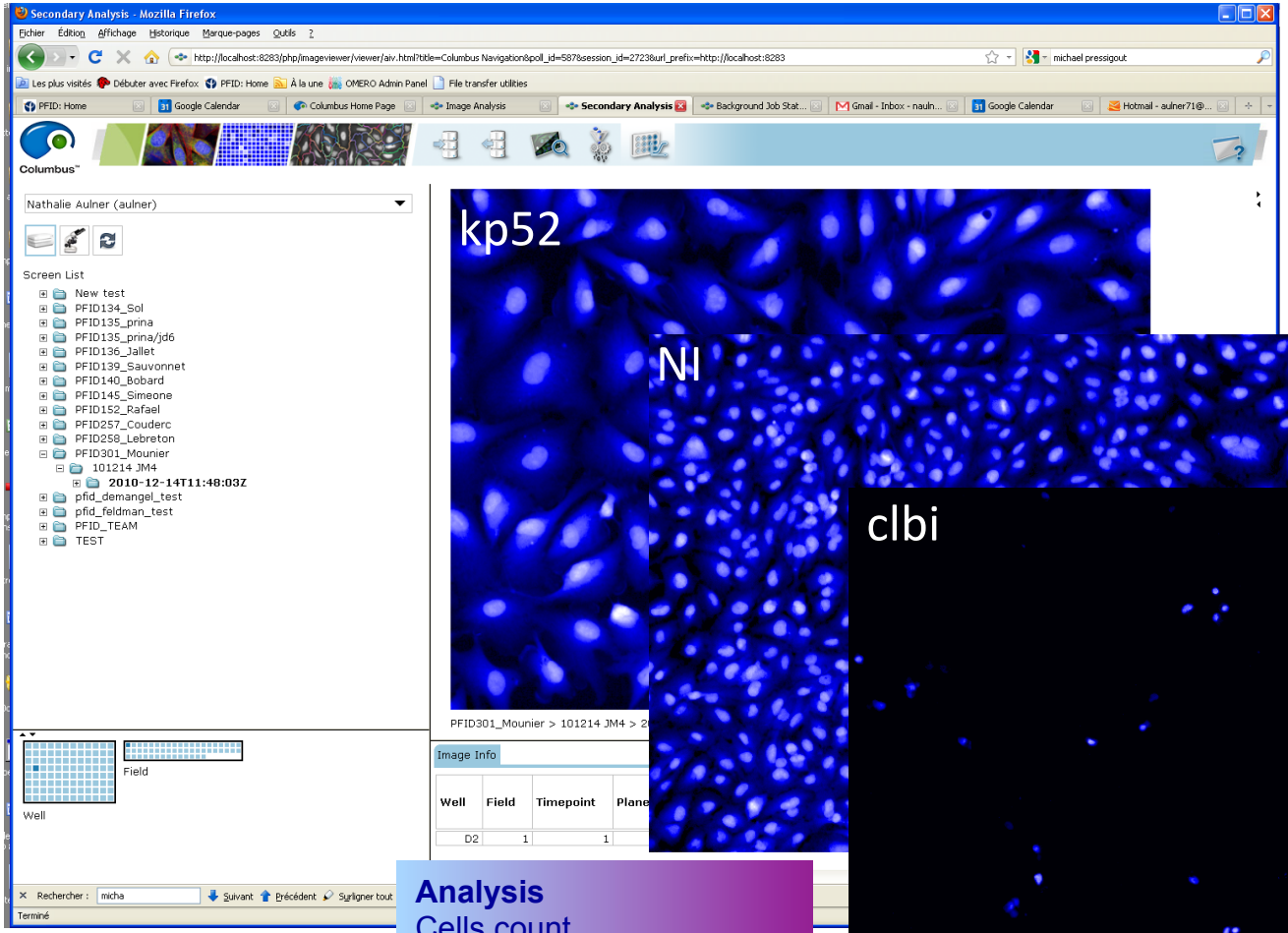
Acquisition 10x bin2
Hoechst
HC Cell Mask Blue



Analysis
Cells count
Fitness of cell population
Cells Size
Subpopulation analysis
…

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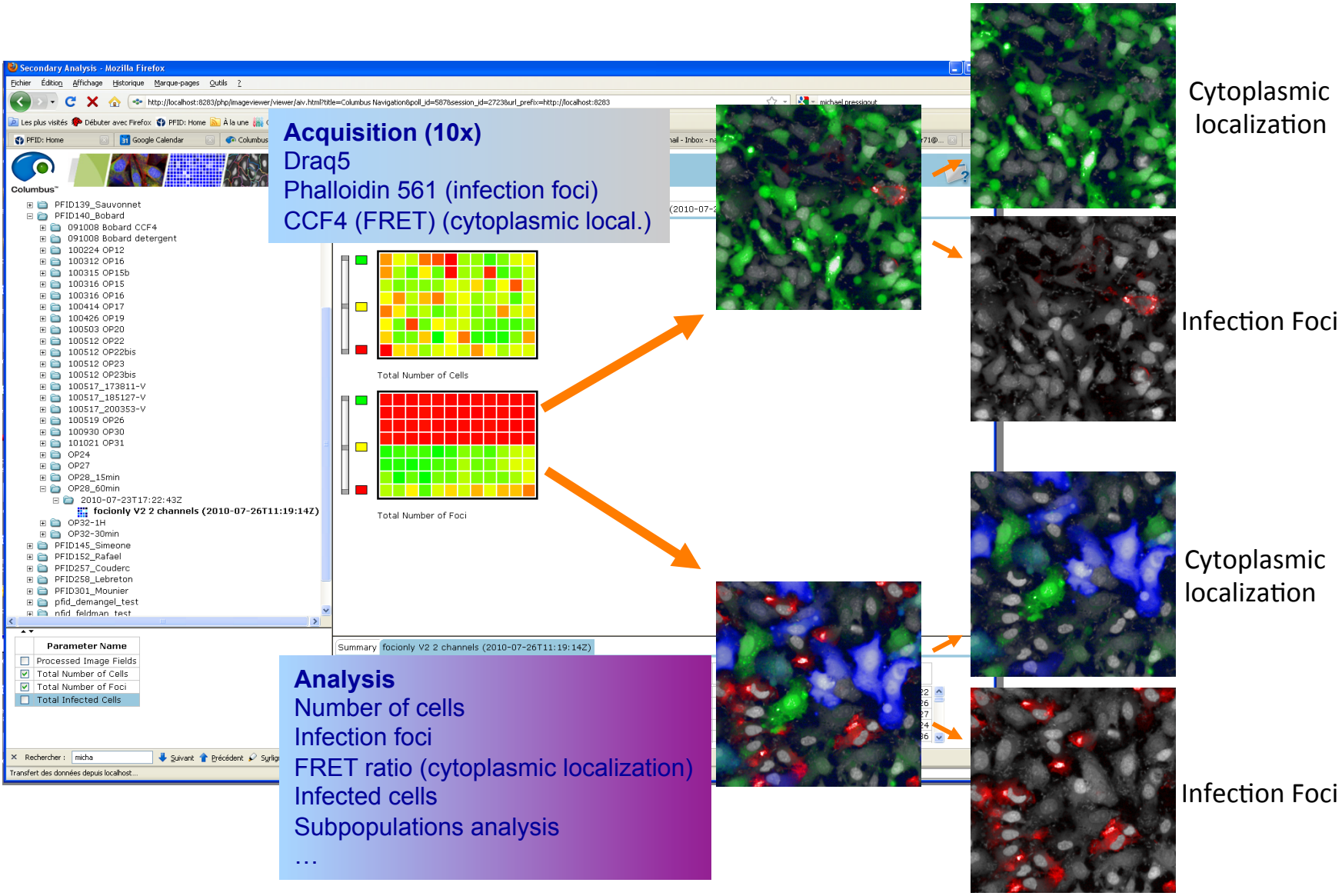
Development of a high throughput siRNA screening for deciphering shigella's infection behaviour

Nora Mellouk (J. Enninga, Host-Pathogen Interactions Dynamism)



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Research & Development

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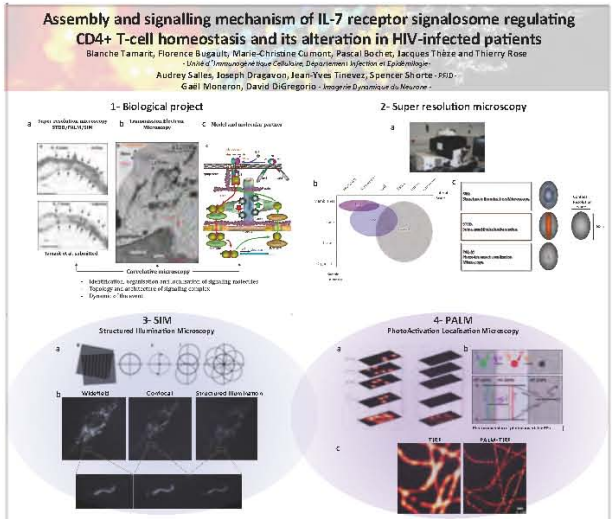
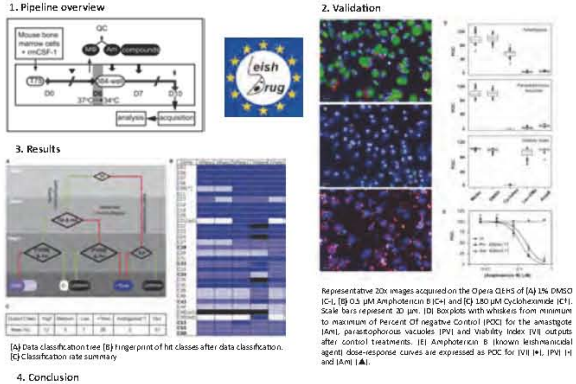
Assembly and signalling mechanism of IL-7 receptor signalosome regulating CD4+ T-cell homeostasis and its alteration in HIV-infected patients

Blanche Tamarit, Florence Bugault, Marie-Christine Cumont, Pascal Bochet, Jacques Thèze and Thierry Rose
- Unité d'Immunogénétique Cellulaire, Département Infection et Epidémiologie -
Audrey Salles, Joseph Dragavon, Jean-Yves Tinevez, Spencer Shorte - PFID -
Gaël Moneron, David DiGregorio - Imagerie Dynamique du Neurone -



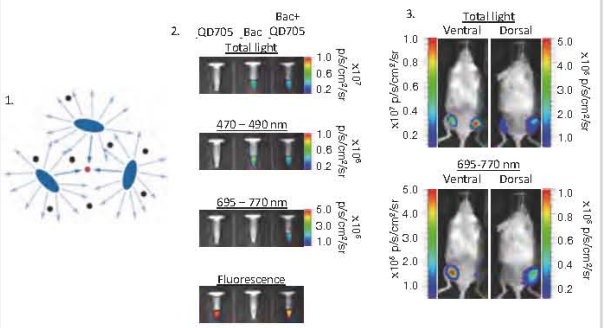
High content analysis of primary macrophages hosting *Leishmania* amastigotes: application to leishmanicidal agents identification

Nathalie Aulner, Anne Danckaert, Eline Rouault-Hardoin, Julie Desrivot, Olivier Helynck, Pierre-Henri Commere, Hélène Munier-Lehmann, Gerald F. Späth, Spencer L. Shorte, Geneviève Milon and Eric Prina
Institut Pasteur 25-28 Rue du Docteur Roux F-75015 Paris [1] Institut Pasteur, Imagopole[1*], [2] Institut Pasteur, Laboratoire Immunophysiologie et Parasitisme, Département de Parasitologie et Mycologie , [3] Institut Pasteur, Unité Chimie et Biocatalyse, Département de Biologie Structurale et Chimie, [4] CNRS UMR 3523, [5] Institut Pasteur, Unité Parasitologie moléculaire et Signalisation, Département de Parasitologie et Mycologie. * CNRS URA 2581, * Present address: Galápagos Sasu, Parc Biocitech - 102 Avenue Gaston Roussel 93230 Romainville, France



Fluorescence by Unbound Excitation from Luminescence

Joe Dragavon[1], Abdessalem Rekiki[1], Alexandra Holland[1], Ioanna Theodorou[1], Régis Tournebize[2,3], Spencer L. Shorte[1]
(1) Imagopole, PFID, Institut Pasteur, Paris
(2) Unité de Pathogénie Microbienne Moléculaire, Institut Pasteur, Paris
(3) Unite INSERM U786, Institut Pasteur, Paris



FUEL takes advantage of an epifluorescent interaction between a living luminescent light source (i.e. bioluminescent bacteria) and a fluorophore (**Figure 1**) as a means to significantly increase the production of red photons (**Figure 2**). When applied to *in vivo* conditions, an overall increase in both the total and red photons is observed (**Figure 3**), increasing the efficacy and the contrast of bioluminescence imaging.

Dragavon, J.; Blazquez, S.; Rekiki, A.; Samson, C.; Theodorou, O.; Rogers, K.; Tournebize, R.; Shorte, S. *In vivo* Excitation of Nanoparticles Using Luminescent Bacteria. *PNAS.* 2012, *109*, 8890-8895.

Dragavon, J.; Blazquez, S.; Samson, C.; Rogers, K.; Tournebize, R.; Shorte, S. Validation of a Method for Enhanced Production of Red-Shifted Bioluminescent Photons *in vivo*. *Proc. of SPIE.* 2011, *7902*, 790210-1-790210-9.

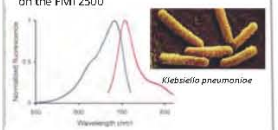
Special thanks to the Pasteur Foundation, the Institute Carnot-Pasteur MI Program 11, the Conny-Maeva Charitable Foundation, Ile de France

Fluorescence Tomography Imaging of bacterial infection

Abdessalem Rekiki[1], Samantha Blazquez[1], Marie-Anne Nicola[1], Joe Dragavon[1], Spencer L. Shorte[1], Philippe Sansonetti[2], Régis Tournebize[2,3]
(1) Imagopole, PFID, Institut Pasteur, Paris
(2) Unité de Pathogénie Microbienne Moléculaire, Institut Pasteur, Paris
(3) Unite INSERM U786, Institut Pasteur, Paris

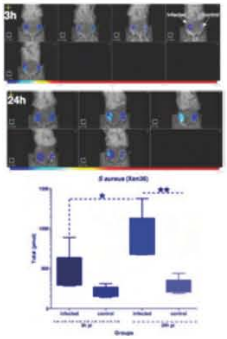
Lung infection : *Klebsiella pneumoniae*

- IFP1.4 is a near-infrared fluorescent protein
- IFP1.4 is expressed by K. pneumoniae under the control of a Lac promoter
- Balb/C mice were infected via the intranasal route with IFP-KP
- Mice are imaged 1 and 2 days post-infection on the FMT2500



Subcutaneus infection : *Staphylococcus aureus*

- Balb C female mice (7-8 weeks)
- Injected IM with $1*10^7$ CFU/mouse
- 3 and 24h post infection, mice were injected with 5 nmoles of Bacterisense
- 1h after agent injection, mice were imaged on the FMT2500
- BLI: mice were imaged at 0, 3 and 24h PI



Fluorescence Tomography Imaging quantitatively permits 3D imaging of bacteria infection *in vivo*

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Pilus structure in *Streptococcus agalactiae* : Immuno-labeling in SEM and TEM

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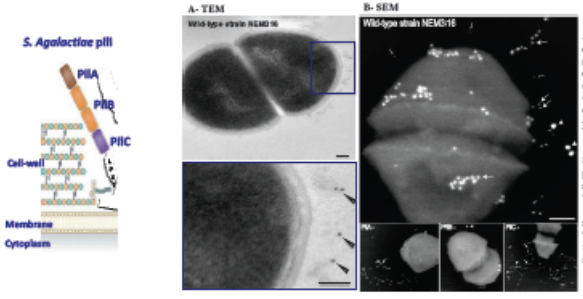
Adherence to host epithelial cells is the first critical step of the infectious process. Most bacterial pathogens have long filamentous structures know as pili which are often involved in the initial adhesion of bacteria to host tissues. The pilus of *S. agalactiae* is composed of three structural subunit proteins : Gbs 1478 (pilA), Gbs1077 (pilB) the major component and Gbs 1474 (pilC) analyses by immuno-electron-microscopy (IEM) using antibody conjugated with gold particles. We analysed a capsular type III polysaccharide (mAb 39) by TEM and we examinated the pilus arrangement by SEM .



A- The mAb 39 decorated the external layer of the capsule and its thickness in strain NEM316 was examinated to be 60nm on ultrathin sections in TEM. The type III capsular polysaccharide (mAb 39) was labeled by 5 nm gold particles. (scale bar = 50nm)

B- Triple-labeling experiment in SEM to detect simultaneously the pilB pilus, the pilA-associated adhesin, and the capsule labeled with antibodies conjugated to 20 nm, 10 nm, and 5 nm gold particles, respectively. (scale bar = 50nm) The PilB pilus backbone as a carrier for surface display of the pilus-associated adhesin PilA.

By using IEM we showed that the pilus is essential for optimal display of the pilus associated adhesin and overcomes the masking effect of the capsule. This method is a process currently used to localize proteins of interest and combine with the high resolution of SEM or TEM permitted to associated an immuno-localisation with a surface morphology or ultrastrutural details.

"Dual role for pilus in adherence to epithelial cells and biofilm formation in Streptococcus agalactiae." Mallet A, Konto-Ghiorghi Y, Mairey E, Duménil G, Caliot E, Trieu-Cuot P, Dramsi S. PLoS Pathog. 2009 May 8

Tokuyasu method applied to label an autophagy receptor and Chikungunya virus

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The Tokuyasu method uses a mild aldehyde fixation, which retains both morphology and antigenicity. The use of sucrose prevents ice crystal formation during the subsequent freezing procedure which hardens the sample and it can be cut into thin sections. The protein labelling at room temperature with antibodies allows the study of protein distribution at high resolution.

Essential steps of the Tokuyasu procedure for sectioning and immunolabeling.

1 Tissue processing
Aldehyde fixation is followed by sucrose infusion and freezing

2 Cryosectioning
Frozen sections are cut with dry knives (glass or diamond)

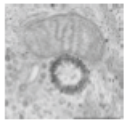
3 Section retrieval
Sections are picked up thawed on a droplet surface, and deposed onto grids

4 Immuno-incubation
Grids, section side down, are passed over successive drops of solutions to perform immunolabeling.

5 Final contrasting, embedding and drying
Grids floating on methylcellulose/uranyl acetate mixture are looped out, the excess fluid drained away, and the sections become embedded in the thin film after drying in the air

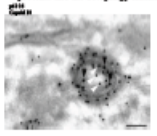
(Liou et al., 1996)

Morphology of the intracellular Chikungunya replication sites



Chikungunya virus (CHIKV) is a recently re-emerged arbovirus responsible in 2006 for a massive outbreak in India and islands of the indian ocean.
HeLa cells were infected with CHIKV for 20h and prepared for classical TEM. Small vesicles containing and surrounding by nucleocapsid were detected. Scale bar =100nm.

We used the Tokuyasu technique to study the spatial relationship of viral proteins and the autophagy marker p62.



HeLa cells were infected with CHIKV for 20h and fixed for immuno EM. Small vesicles containing, and surrounded by nucleocapsid were detected. P62 (black arrowheads) and capsid (white arrowheads) were labelled with appropriate primary antibody followed by proteinA-gold of different sizes respectively.
Scale bar =100nm.

By immuno EM we showed co-localisation of the autophagy receptor p62 and the capsid of Chikungunya virus.

Cryo-CLSEM: Cryo-correlative Light & Scanning Electron Microsopy: Application to Tunneling Nanotubes

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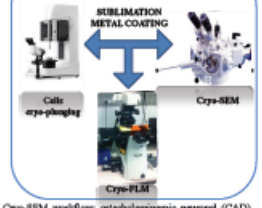
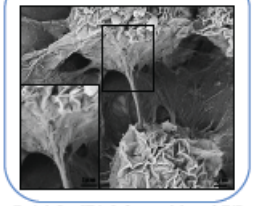
Recently Tunnelling Nanotubes (TNT) have emerged as a new way of inter-cellular communication for the transfer of various proteins, organelles & pathogens between cells. TNTs are transient, thin, very fragile membrane structures, detached from the substrate, that do not easily survive conventional electron microscopy preparation methods involving dehydration and resin embedding. Thus we used cryo-preparation and imaging methods to preserve their structural integrity and to observe them close to their native state. Specifically in this part of the project we use a Cryo-CLSEM approach, that combines fluorescent and surface ultrastructural information in cryo-conditions, to characterise the surface morphology of TNTs and their connection to the cell bodies.

A. Cryo-SEM Workflow



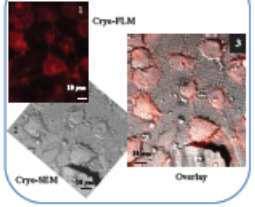
Cryo-SEM workflow: catecholaminergic neuronal (CAD) cells were grown on EM grids & then cryo-fixed in liquid ethane. For cryo-SEM observations, the grid was sublimated and coated with Au/Pd inside the cryo transfer module installed on a JEOL 6700F and then imaged at -150°C. For Cryo-CLSEM, cells were first imaged using a cryo-holder which permit to image cells with fluorescence in cryo conditions.

B. Classical Cryo-SEM



Classical Cryo-SEM. Surface morphology of TNTs between two CAD cells: a single TNT is composed of several small tubes. The insert shows the TNT's attachment to the cell plasma membrane at higher magnification.

C. Cryo-CLSEM



Cryo-CLSEM. 1. CAD cells were grown on EM marker grids, labeled with a fluorescent membrane dye (wheat germ agglutination) & plunge-frozen in liquid ethane. TNTs were identified & imaged by cryo-fluorescence. 2. The same cells are recovered in the SEM and imaged by cryo-SEM. 3. Overlay of cryo-fluorescence and cryo-SEM pictures.

Virus Associated Pyramids in *Sulfolobus islandicus*: A TEM study for their formation

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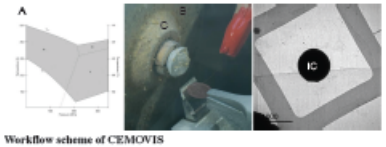
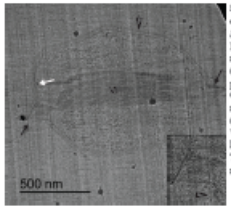
The Archeal virus *Sulfolobus islandicus* rod shaped virus 2 (SIRV2) induces lysis of the host cell to release their virions. In the case of SIRV2 the virions assemble in the cytoplasm, where the major capsid protein forms the outer structure of the linear virion with the double stranded viral DNA in the interior. Parallel to virion assembly the formation of Virus Associated Pyramids (VAPs) takes place at the membrane of the infected cells [1]. We used Cryo-Electron Microscopy Of Vitreous Sections (CEMOVIS) to visualise the morphological changes during this infection process. CEMOVIS consists of cryo-immobilisation, cryo-sectioning, and low dose cryo-electron microscopy and offers a higher resolution than chemical prepared samples.



Workflow scheme of CEMOVIS
(A) Detail of the phase diagram of water with the minimal melting temperature (Tm) and the minimal nucleation temperature (Tn). In the gray area supercooled water can exist. Around 200 MPa, the melting point of water has a minimum. This sets out the best situation for high pressure freezing to vitrify the sample. Adapted from [3]. (B) Sectioning of the frozen sample. With an eyelash the sections are pulled over the grid. (C) The sections are observed in the cryo-microscope close to -180°C under low dose conditions. The picture shows an overview of the boundary between two sections. The different gray level between the two sections reflects the thickness of them. IC = ice crystal.



SIRV2 clusters in the cytoplasma
S. islandicus 10 p.i. with SIRV. Before lysis of the cell and release of the viral particles (VP), the VPs are densly packed in the cytoplasma. Concurrently at the cellular membrane (white arrow) VAPs (black arrow) are induced. The VAPs protrude through the S-layer (arrowhead) of the cell. The insert shows a higher magnification of a VAP.

References:
[1] Bize A, Karlsson EA, Ekefjärd K, Quax TE, Pina M, Prevost MC, Forterre P, Tenaillon O, Bernander R, Prangishvili D. Proc. Natl. Acad. Sci. (2009) p.11306-11.
[2] This work was supported by the Programme Blanc of the Agence Nationale de la Recherche (Grant ANR-09-Blan-0288.01). T.Q. is supported by a fellowship from the Ministère de l'Enseignement supérieur et de la Recherche of France.
[3] Vinchurkar D, Gruber W, Studer D. Methods Cell Biol. (2008) p. 151-164

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