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

High Content Analysis for the study of infectious diseases at the Institut Pasteur

N. Aulner, A. Danckaert, P-H. Commere, B. David-Watine, J. Dragavon, A. Holland, M-A. Nicola, E. Perret, A. Rekiki, F. Rueckel, P. Roux, A. Salles, I. Theodorou, J-Y.Tinevez and S.L. Shorte
Imagopole, Institut Pasteur, 25 Rue du Dr Roux, 75724 Paris Cedex 15, France
Nathalie.aulner@pasteur.fr

High Content Analysis (HCA) is an emerging state of the art that has its roots in over one hundred years of biological imaging. It is only recently that its power as a scientific experimental tool has begun to be harnessed, facilitated mainly by a new generation of post genomic imaging, signals processing and computer science technologies. Arguably, among the most important of these systems tools is the engineering of image acquisition and processing into an unbroken, and fully-automated sample handling workflow, making possible the acquisition and analysis of literally hundreds of thousands of images per day. Whereas a typical High Content Screening (HCS) application would tend to find the best compromise between throughput, speed and resolution, our approach focuses on more subtle features such as high resolution analysis of details cell features and live cell imaging to measure dynamics of cell functions.

Here we present the strategy we have implemented at the **Institut Pasteur** in Paris for **HCA of infectious diseases** and its niche application where it concerns the needs of academic researchers wanting to use such techniques for basic sciences, and especially cellular microbiology and infection. Indeed, among academic life sciences researchers somewhat habituated to routine live cell microscopy since twenty-five years, HCA/HCS while potentially very powerful remains poorly adapted, as yet, mostly due to reasons that can be grouped under the adage "**quality versus quantity**", or more specifically "**content versus throughput**". Basic scientists tend to want both content and throughput without compromise because fundamental studies on biology require readouts to approximate the **verity of biological processes**, and "quality" in this sense means **relevant biology**. Studies on infection are especially demanding because infectious processes are complex and exigent, requiring very precise molecular, physiological and biochemical metabolic processes to successfully engage before a pathogen may realize its infectious destiny in vivo. Accordingly, it is a major challenge to establish **cell-based paradigms for studying infection**, and a constraint that today has brought the community of scientists working in the various areas of microbiology and infection to help lead the way in defining the emerging paradigm of HCA for life sciences research. In our capacity as a large integrated imaging facility (www.imagopole.org) specializing in studies on cellular microbiology and infection, we have recently come to examine the practical articulation between routine application of advanced microscopic imaging, and the cutting-edge technologies for cell-based high-content analysis and high-throughput imaging.

Bio-Imaging Technologies in infectious diseases investigations

High Content Screening (HCS)

Multi-well Plates

Opera (Perkin Elmer)

Data Storage and Management

High Content Analysis (HCA)

Columbus: data management software (Perkin Elmer)

Imaging Cytometry

Cells in suspension

ImageStream (Amnis)

Ideas analysis software (Amnis)

Automated Image Analysis on a Per-Cell basis

diamid treated erythrocytes

In Vivo Optical Analysis

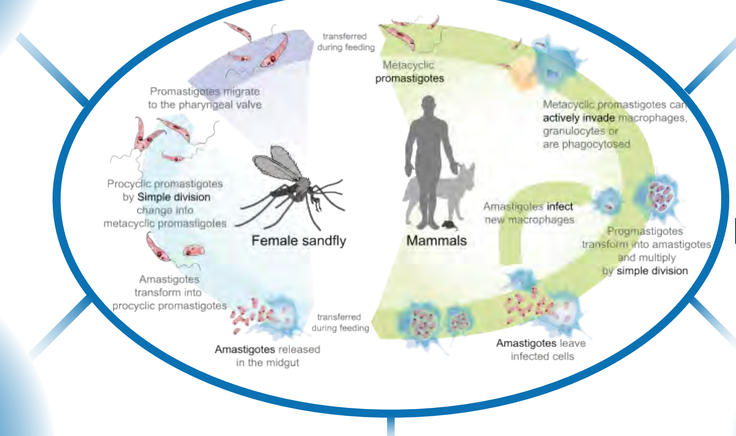
Whole Animal

IVIS100 (Caliper)

Correlative Analysis

Fluorescence

Bioluminescence



Cytometry

Cell Phenotype

Gallios (Beckman Coulter)

Kaluza analysis software (Beckman Coulter)

Cell population Analysis by Scatter Plots

Electronic microscopy

Ultra Structural Level

MEB JSM 6700F (Jeol)

High Resolution Imaging

Macrophage and Lymphocytes infected by HIV

Super resolution Imaging

Super resolution Level

Super Resolution Imaging

Widefield Confocal SIM

Mitochondria in Muntjac cells

Quantification

Analysis by readout model

Validation & Statistics

Correlation analysis
Normalisation
Multivariate Analysis
Cluster analysis (Classification)
Hit/Event selection Procedure
....

Data Storage & Management

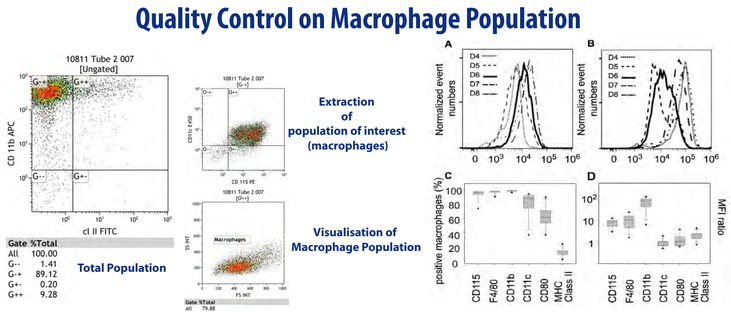
Bioinformatics — Biology

High content analysis of intramacrophagic Leishmania amastigotes propagation studies

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

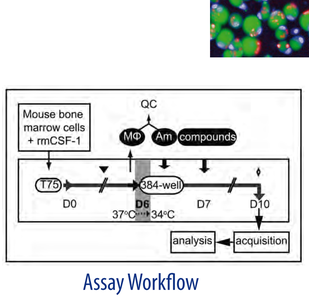
In mammals- wild rodents and humans -, the rapid and long term establishment of parasites such as Leishmania/L. amazonensis strictly relies on the subversion of dendritic leucocyte and macrophage lineages, the latter being the dominant population where the Leishmania amastigote, the only developmental stage found in mammalian hosts, develops. Using living highly virulent amastigotes directly extracted from mouse cutaneous sites and primary macrophages derived from mouse bone marrow cells, we established a high content image analysis assay with the objective to screen compounds that selectively target intra-macrophagic amastigotes without displaying toxicity for the macrophages. The validation of a miniaturized assay, relying on automated biological material and imaging probe handling, image acquisition, data storage and analysis is described/presented. Based on robust statistical methods and quality control metrics, the data analysis pipeline allowed the classification of compounds based on the visual phenotype of macrophage cultures observed after a few days of treatment with compounds.

Quality Control on Macrophage Population



HCS/HCA Pipeline

Sample Preparation & Plate Design → Acquisition → Data Analysis → Output Choice → Quality Control → Hit Validation

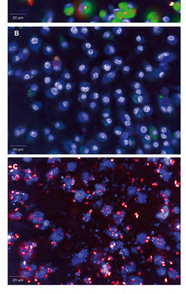


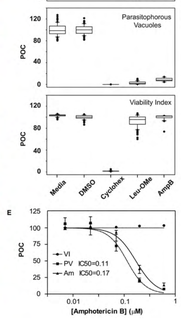
Assay Workflow

Acquisition

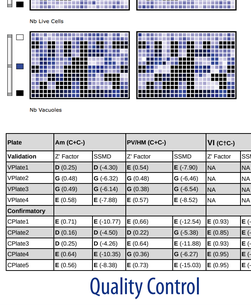
Nuclei
Vacuoles(PVs)
Parasites

Image Analysis





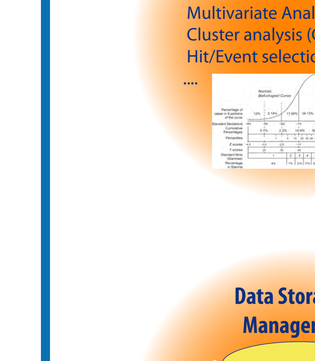
Data Analysis



Quality Control

Plate	Am (C+/C-)		PV/HM (C+/C-)		VI (C1/C-)	
Validation	Z' Factor	SSMD	Z' Factor	SSMD	Z' Factor	SSMD
VPlate1	D (0.25)	D (-4.30)	E (0.54)	E (-7.90)	NA	NA
VPlate2	G (0.48)	G (-6.32)	G (0.48)	G (-6.46)	NA	NA
VPlate3	G (0.49)	G (-6.14)	G (0.38)	G (-6.54)	NA	NA
VPlate4	E (0.58)	E (-7.88)	E (0.57)	E (-8.52)	NA	NA
Confirmatory						
CPlate1	E (0.71)	E (-10.77)	E (0.66)	E (-12.54)	E (0.93)	E (-50.11)
CPlate2	D (0.16)	D (-4.50)	D (0.22)	G (-5.38)	E (0.85)	E (-27.23)
CPlate3	D (0.25)	D (-4.26)	E (0.64)	E (-11.88)	E (0.93)	E (-51.79)
CPlate4	E (0.64)	E (-10.35)	G (0.36)	E (-6.27)	E (0.95)	E (-81.29)
CPlate5	E (0.56)	E (-8.38)	E (0.73)	E (-15.03)	E (0.95)	E (-69.57)

Filtering



C

Output Class	High	Medium	Low	~Toxic	Ambiguous(*)	Out
Rate (%)	12	3	7	25	2	51

Results table

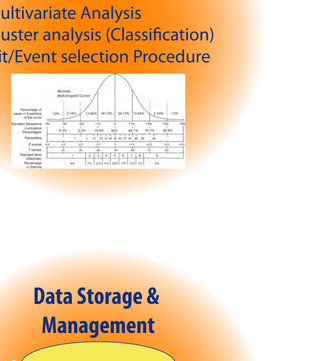


Plate pairwise comparison

	VPlate1	VPlate2	VPlate3	VPlate4	CPlate
VPlate1		100 (97)	91 (87)	75 (62.5)	87.5 (78)
VPlate2			91 (84.5)	75 (59.5)	87.5 (87)
VPlate3				78 (69)	84.5 (78)
VPlate4					72 (56)
CPlate					

Images contribution: J. Dragavon and S. Blazquez (Bioluminescence - PFID), A. Salles (superresolution - PFID) (Electronic Microscopy, PFMU), I. Safeukui Noubissi (Imaging Cytometry, Unité d'Immunologie Molécula ire des Parasites)