Quantitative Imaging in Biology

Many hats for the computational biologist: (Nature Biotechnology, Vol. 31, Number 11, Nov. 2013)

« - data analyst
- data curator
- database developer
- statistician
- mathematical modeler
- bioinformatician
- software developer
- ontologist
- and **many more** »

A trend :

- "preuve par l'image" is a powerful tool nowadays provided that it is statistically valid

A picture is worth a thousand words?

A question :

- articulating genotype – phenotype analyses and at multiscale level: from the cell to the whole organism

A need :

- « High-Throughput Screening », « High-Content Analysis » « Open&Big Data »

PLOS Computational Biology
Translational Bioinformatics: PLOS Computational Biology presents an educational resource for an emerging field
A Peer-Reviewed, Open Access Journal, PLoS Comput Biol. 2013 Apr;9(4):e1003043. doi: 10.1371/journal.pcbi.1003043. Epub 2013 Apr 25.

Chapter 17: bioimage informatics for systems pharmacology.

Automated High Resolution Fluorescence + robotics

- coupled hardware/software for systematic and cheap study of morphological changes observed over a large population of cells under various constraints like drugs

→ *statistically valid :-)*

« Bioimage informatics » = Image Analysis + Informatics + Biology + etc.

- automatize the extraction if interest organelles in digital images (segmentation)
- automatize this interest organelles and their representation (+ diffusion Open Data)
- for a rapid analysis ? objective ?

Many more challenges to overcome : scientific and technologic

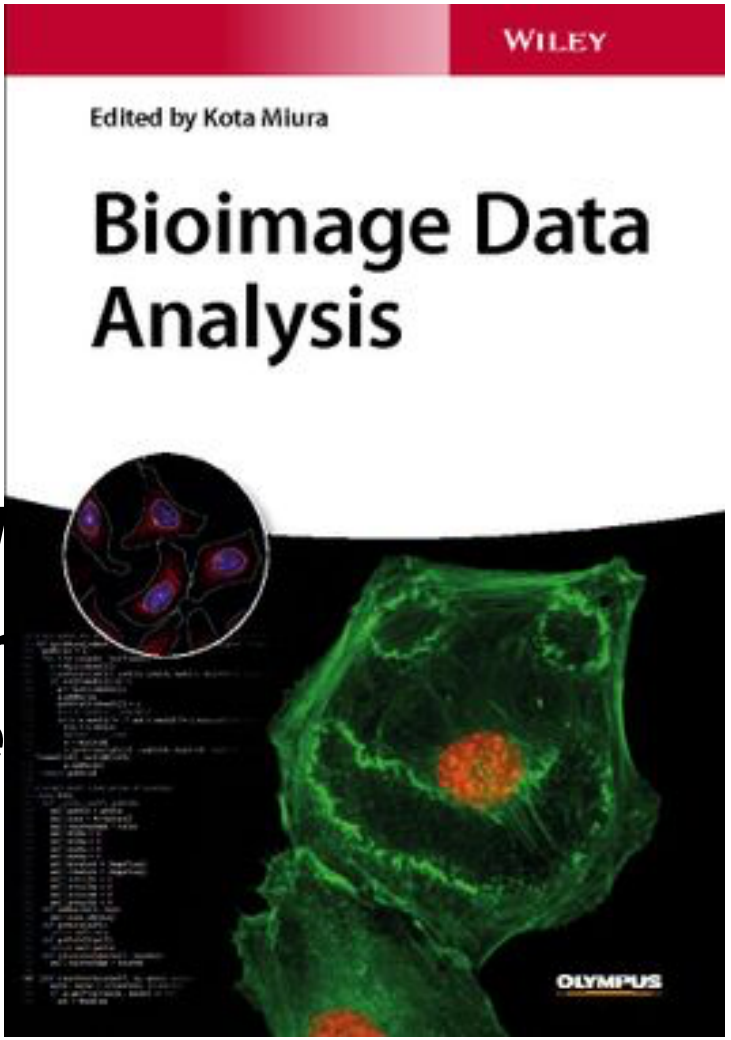
- «High-Throughput Screening » and « High-Content Screening » : identifying components and genes leading to targeted phenotypic changes

Extracting biological bio-markers is also related to quantitative image segmentation, major challenge in image processing and analysis

- **NeuronStudio etc.**
- **CellProfiler**

Related to pattern recognition and machine learning and computer vision and to "big data" : 1 image = millions/billions pixels (multispectral, 3D + time, 5D etc.)

- **Digital pathology challenge**



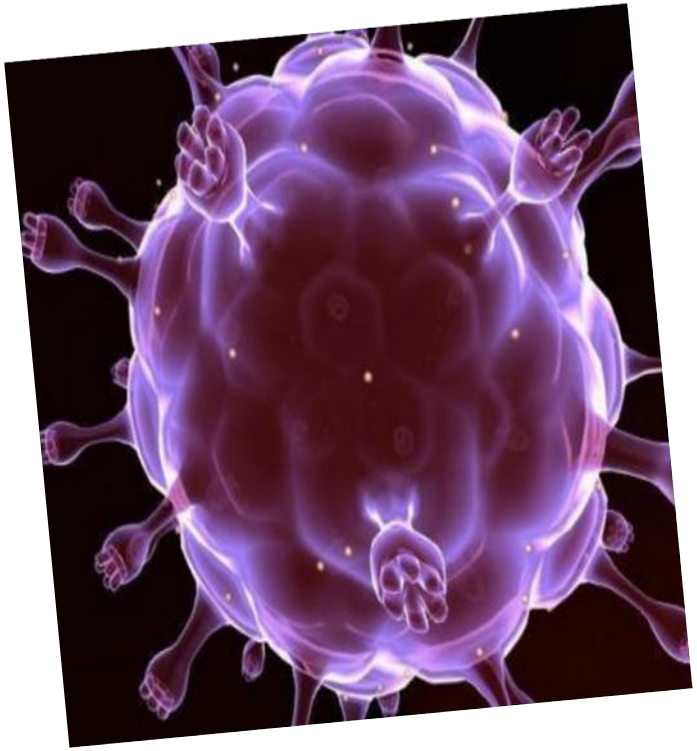
And “big data” : 1 image = millions/billions pixels

Digital pathology : a new challenge challenge

"C'est ça, le principal critère. On avait quelque chose que l'on a pu transmettre en série et maintenir. Et nous **avons vu** qu'il s'agissait d'un rétrovirus non seulement **à partir de son aspect visue**l mais aussi par voie biochimique, par l'activité de transcriptase inverse qui est vraiment spécifique des rétrovirus.[...] Nous avons un atlas. Quand on est un peu habitué, on sait distinguer ce qui est un rétrovirus de ce qui n'en est pas un. **On peut faire la distinction en se fondant sur la morphologie mais il faut une certaine habitude."**
Interview du Prof. Luc Montagnier dans Luc Montagnier a-t-il découvert le VIH ? par Djamel Tahi, Continuum, hiver 1997.
http://www.sidasante.com/journal/dtintlm.htm

« Il est vrai que l'habitude peut parfois permettre de faire la distinction entre des particules ressemblant à des rétrovirus et des particules ressemblant à des virus en s'appuyant sur les traits morphologiques. Cependant, il existe des particules qui ne sont PAS des virus ni des rétrovirus et qui ont néanmoins des traits morphologiques identiques à ceux des rétrovirus. Par conséquent, on ne peut pas conclure à partir de simples considérations morphologiques que les bourgeonnements ou les particules détachées de la cellule sont des rétrovirus. Les cultures de tissus provenant de patients souffrant du SIDA contiennent une pléthore de particules ressemblant à des virus (particles virus-like) **dont le diamètre va de 65 à 250 nm, qui ont des formes coniques ou allongées, avec des noyaux centrosymétriques ou tubulaires, ou même avec des noyaux doubles ou un mélange de noyaux.** »
Commentaire par Eleni Papadopulos-Eleopulos et al.

A long time ago...



Microscopy and biology today

- classical microscopy quantifying the number, size, shape and motion of cells

- going further : super resolution + fluorescence = 2 recent Nobel Prizes : chemistry to Osamu Shimomura, Martin Chalfie and Roger Tsien 8 october 2008 for GFP discovering and in 2014 to Eric Betzig, Stefan W. Hell and William E. Moerner

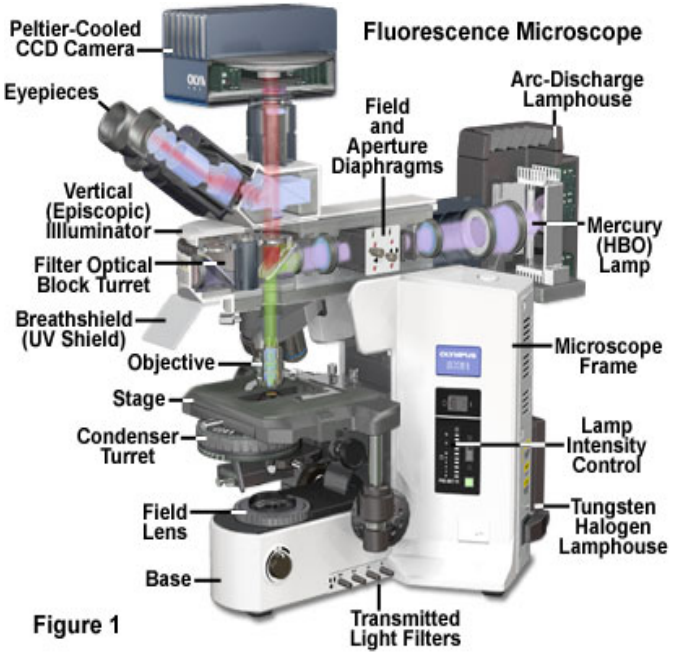
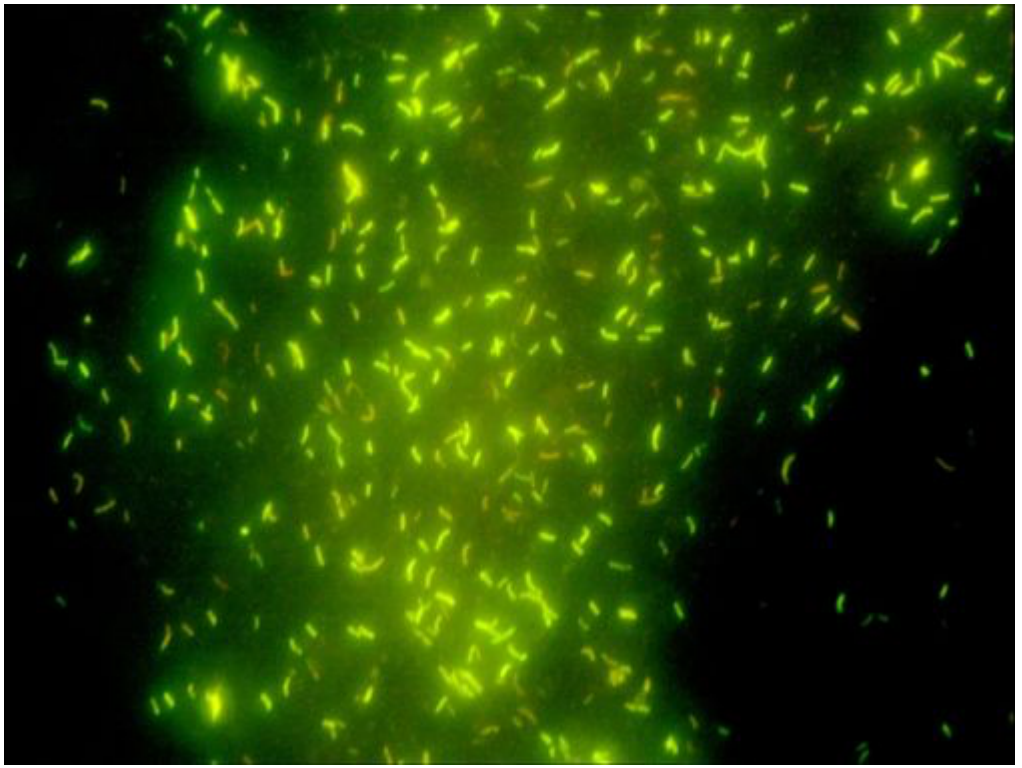
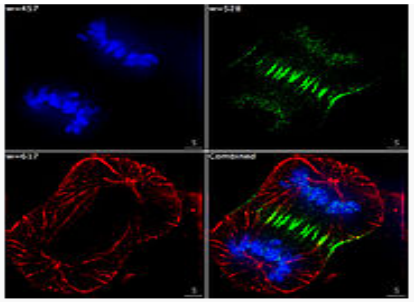


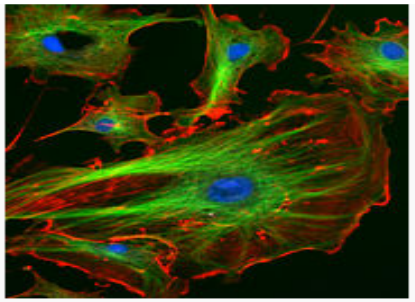
Figure 1



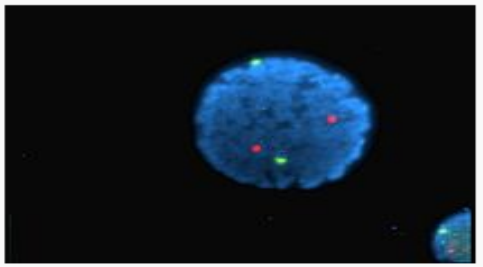
https://fr.wikipedia.org/wiki/Microscopie_%C3%A0_fluorescence



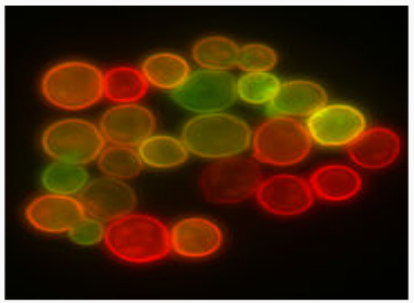
Imagerie en épifluorescence de trois composantes d'un cellule cancéreuse humaine en cours de division. L'ADN apparaît bleu, une protéine dite INCENP apparaît en vert, et les microtubules en rouge. Chaque fluorophore a été imagé séparément, avec une longueur d'onde d'excitation spécifique et des filtres, puis une image a été recomposée, à partir des photos prises par la caméra CCD.



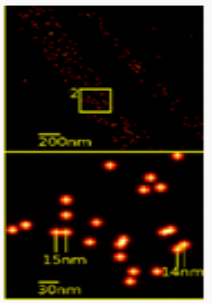
Cellules endothéliales d'une artère pulmonaire bovine (noyaux colorés en bleu avec du DAPI, microtubules marqués en vert par un anticorps lié au FITC et filaments d'actine marqués en rouge par de la phalloïdine liée à la protéine TRITC).



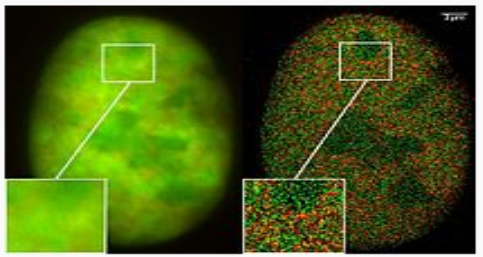
Noyau de lymphocyte humain coloré au DAPI avec les chromosome 13 (vert) et 21 (rouge) par des sondes hybridées aux centromères (hybridation in situ en fluorescence).



Membrane cellulaire de levure, mise en évidence de structure (en jaune) obtenue par fusion de protéines de la membrane avec deux marqueurs fluorescents (RFP et GFP).

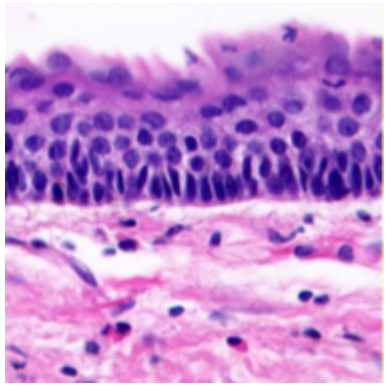


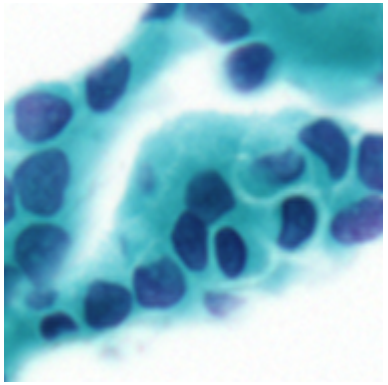
Détection de la molécule YFP dans une cellule cancéreuse humaine, à une échelle nanométrique.

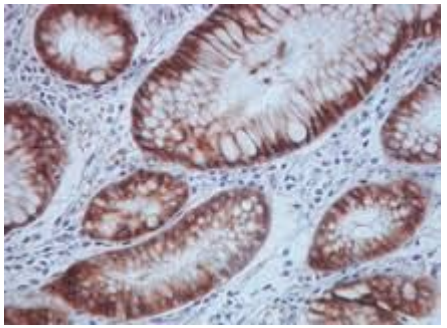


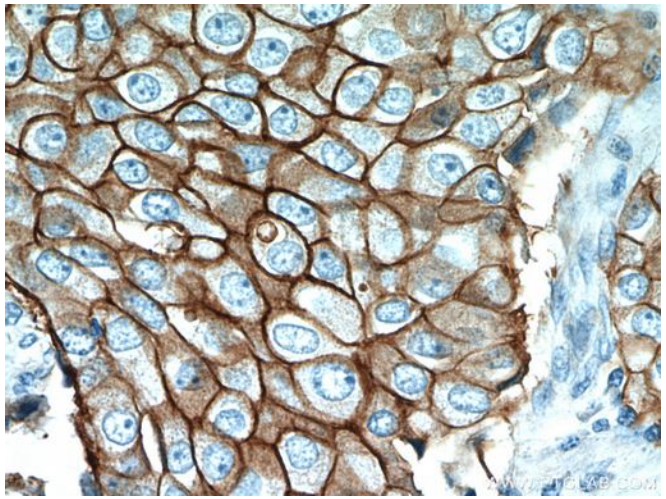
Noyau d'une cellule de cancer des os (technique dite *Co-localzation microscopie ou 2CLM* pour les anglophones). Image obtenue par fluorescence de marqueurs fusionnés avec les protéines GFP et RFP, pour 120 000 molécules

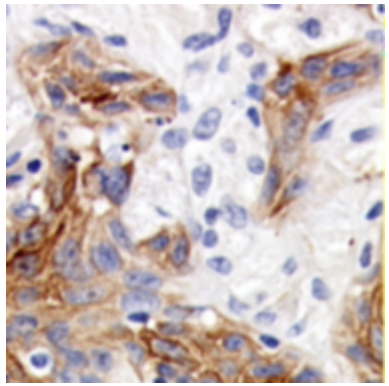
***But still challenges also in brigth field microscopy with* immunohistochemical markers**



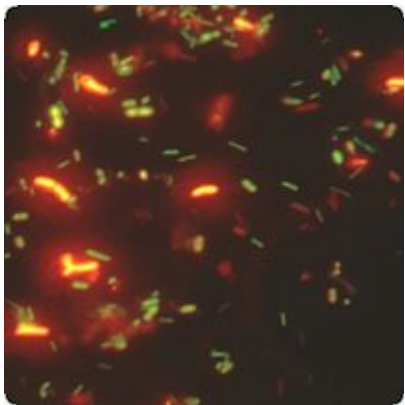


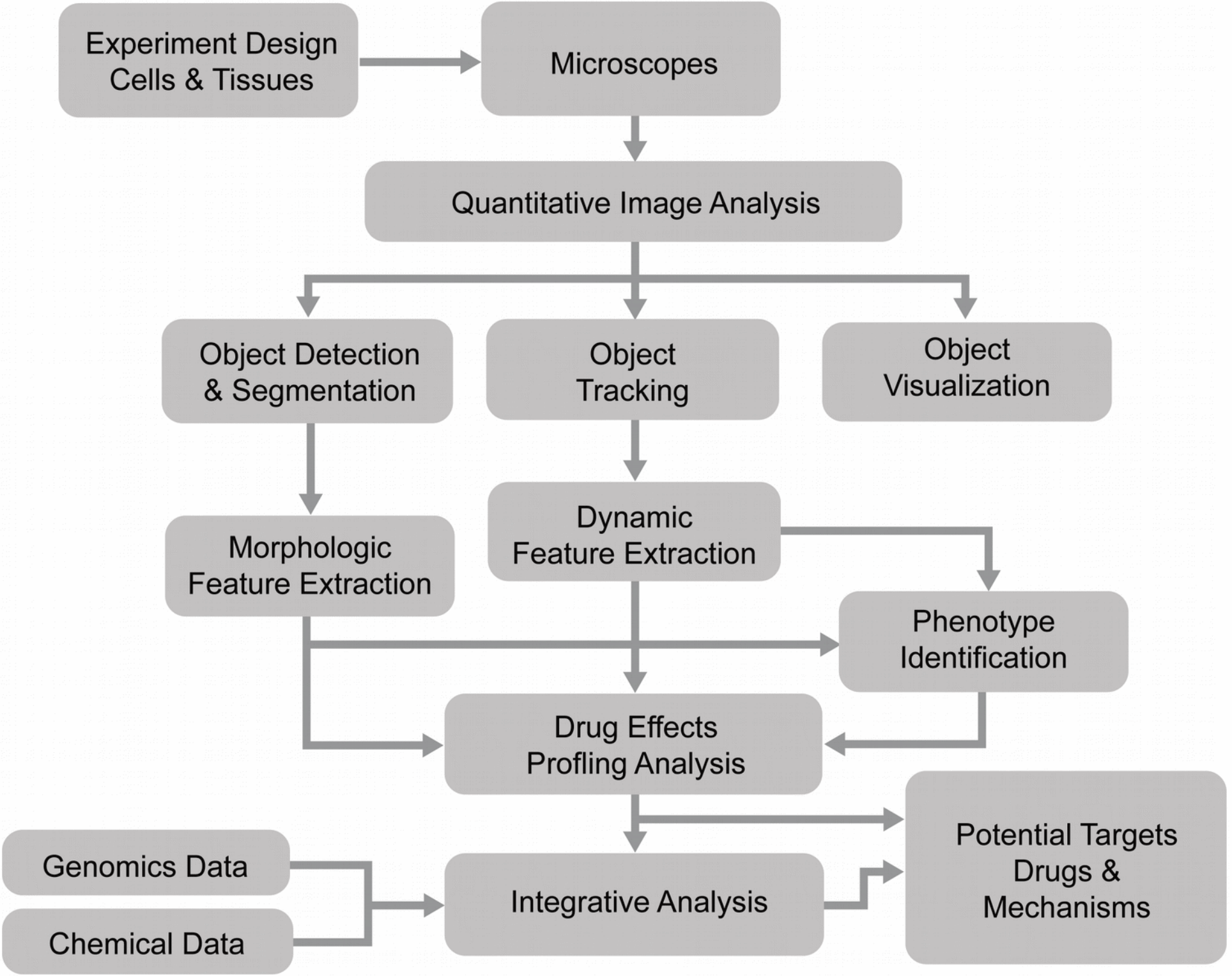






Images 5D of thousands of cells, 3D acquisition, *time-lapse sequence*, « multiple fluorescent probes » etc.




Experiment Design
Cells & Tissues
Microscopes
Quantitative Image Analysis
Object Detection
& Segmentation
Object
Tracking
Object
Visualization
Morphologic
Feature Extraction
Dynamic
Feature Extraction
Phenotype
Identification
Drug Effects
Profling Analysis
Genomics Data
Chemical Data
Integrative Analysis
Potential Targets
Drugs &
Mechanisms

Name	Link	Basic Functions
ImageJ	http://rsb.info.nih.gov/ij/	General image analysis with rich plugins
Fiji (A distribution of ImageJ)	http://fiji.sc/	Bioimage analysis with rich plugins
CellProfiler	http://www.cellprofiler.org/	Bioimage analysis with rich analysis pipelines
CellProfiler Analyst	http://www.cellprofiler.org/	Screening data analysis with machine learning approaches
Icy	http://icy.bioimageanalysis.org/index.php	Bioimage analysis
BioimageXD	http://www.bioimagexd.net/	3D Bioimage analysis and Visualization
PhenoRipper	http://www.phenoripper.org	Bioimage analysis for rapid exploration and interpretation of bioimage data in drug screening
FarSight	http://www.farsight-toolkit.org/wiki/Main_Page	Dynamic Biological Microenvironments from 4D/5D Microscopy Data
Vaa3D	http://penglab.janelia.org/proj/v3d/V3D/About_V3D.html	Bioimage visualization and analysis
Cell Analyzer	http://penglab.janelia.org/proj/cellexplorer/cellexplorer/What_is_Cell_Explorer.html	*C. elegans* image analysis
AceTree and StarryNite	http://starrynite.sourceforge.net/	*C. elegans*' embryo cell tracking and lineage reconstruction
Ilastik	http://www.ilastik.org/	Image classification and segmentation
Image Quantitators (ZFIQ, DCellIQ, GCellIQ, NeuriteIQ, NeuronIQ)	http://www.methodisthealth.com/bbpsoftware	A set of image analysis software packages for cell tracking in time-lapse images, and RNAi cell, neuron, neurite and Zebrafish image analysis
CellCognition	http://cellcognition.org/software/cecoganalyzer	Cell tracking in time-lapse image analysis
TLMTracker	http://www.tlmtracker.tu-bs.de/index.php/Main_Page	Cell tracking in time-lapse image analysis
NeuronJ	http://www.imagescience.org/meijering/software/neuronj/	Neurite Tracing and Quantification
NeurphologyJ	http://life.nctu.edu.tw/~microtubule/neurphologyJ.html	Neuron image analysis
NeuronStudio	http://research.mssm.edu/cnic/tools-ns.html	Neuron image analysis
CellOrganizer	http://cellorganizer.org/	Synthetically model and simulate fluorescent microscopic cell images
SimuCell	http://www.simucell.org	Synthetically model and simulate fluorescent microscopic cell images
PatternUnmixer	http://murphylab.web.cmu.edu/software/PatternUnmixer2.0/	Model fundamental sub-cellular patterns
μManager	http://valelab.ucsf.edu/~MM/MMwiki/	Control of automated microscopes
ScanImage	http://openwiki.janelia.org/wiki/display/ephus/ScanImage%2C+Ephus%2C+and+other+DAQ+software	Control of automated microscopes
OME	http://www.openmicroscopy.org/site	Image Database Software
Bisque	http://www.bioimage.ucsb.edu/bisque	Image Database Software
OMERO.searcher	http://murphylab.web.cmu.edu/software/searcher/	Content-based bioimage search
KNIME	http://www.knime.org/example-workflows	Workflow system for data analytics, reporting and integration

doi:10.1371/journal.pcbi.1003043.t001

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CellProfiler	http://www.cellprofiler.org/	Bioimage analysis with rich analysis pipelines
CellProfiler Analyst	http://www.cellprofiler.org/	Screening data analysis with machine learning approaches
Icy	http://icy.bioimageanalysis.org/index.php	Bioimage analysis
BioimageXD	http://www.bioimagexd.net/	3D Bioimage analysis and Visualization
PhenoRipper	http://www.phenoripper.org	Bioimage analysis for rapid exploration and interpretation of bioimage data in drug screening
FarSight	http://www.farsight-toolkit.org/wiki/Main_Page	Dynamic Biological Microenvironments from 4D/5D Microscopy Data
Vaa3D	http://penglab.janelia.org/proj/v3d/V3D/About_V3D.html	Bioimage visualization and analysis
Cell Analyzer	http://penglab.janelia.org/proj/cellexplorer/cellexplorer/What_is_Cell_Explorer.html	*C. elegans* image analysis
AceTree and StarryNite	http://starrynite.sourceforge.net/	*C. elegans*' embryo cell tracking and lineage reconstruction
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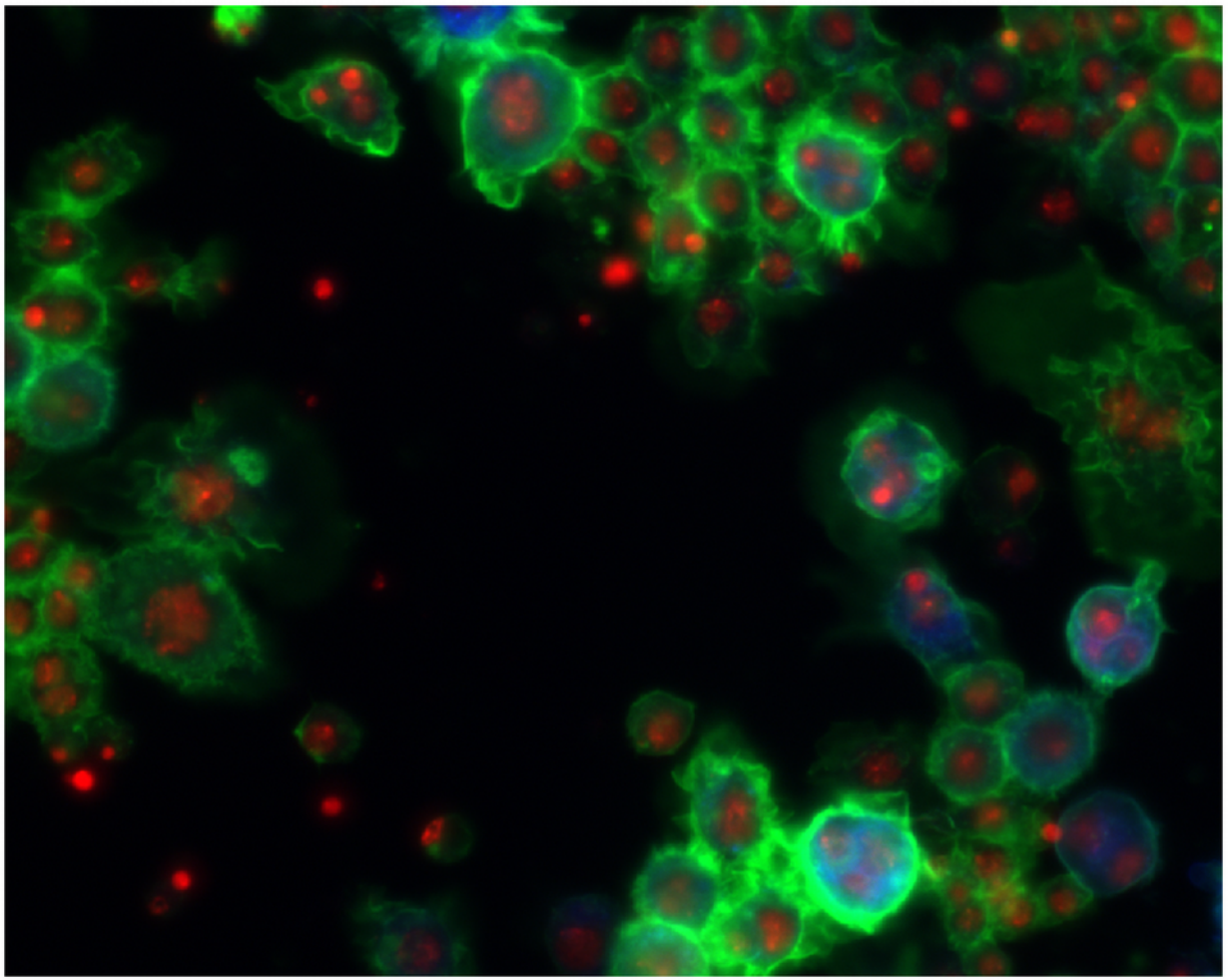
NeuronJ	http://www.imagescience.org/meijering/software/neuronj/	Neurite Tracing and Quantification
NeurphologyJ	http://life.nctu.edu.tw/~microtubule/neurphologyJ.html	Neuron image analysis
NeuronStudio	http://research.mssm.edu/cnic/tools-ns.html	Neuron image analysis
CellOrganizer	http://cellorganizer.org/	Synthetically model and simulate fluorescent microscopic cell images
SimuCell	http://www.simucell.org	Synthetically model and simulate fluorescent microscopic cell images
PatternUnmixer	http://murphylab.web.cmu.edu/software/PatternUnmixer2.0/	Model fundamental sub-cellular patterns
μManager	http://valelab.ucsf.edu/~MM/MMwiki/	Control of automated microscopes
ScanImage	http://openwiki.janelia.org/wiki/display/ephus/ScanImage%2C+Ephus%2C+and+other+DAQ+software	Control of automated microscopes
OME	http://www.openmicroscopy.org/site	Image Database Software
Bisque	http://www.bioimage.ucsb.edu/bisque	Image Database Software
OMERO.searcher	http://murphylab.web.cmu.edu/software/searcher/	Content-based bioimage search
KNIME	http://www.knime.org/example-workflows	Workflow system for data analytics, reporting and integration

doi:10.1371/journal.pcbi.1003043.t001

Case studies : Molecule discovery (drugs) and therapeutic targets

1. Multicolor cell imaging-based studies

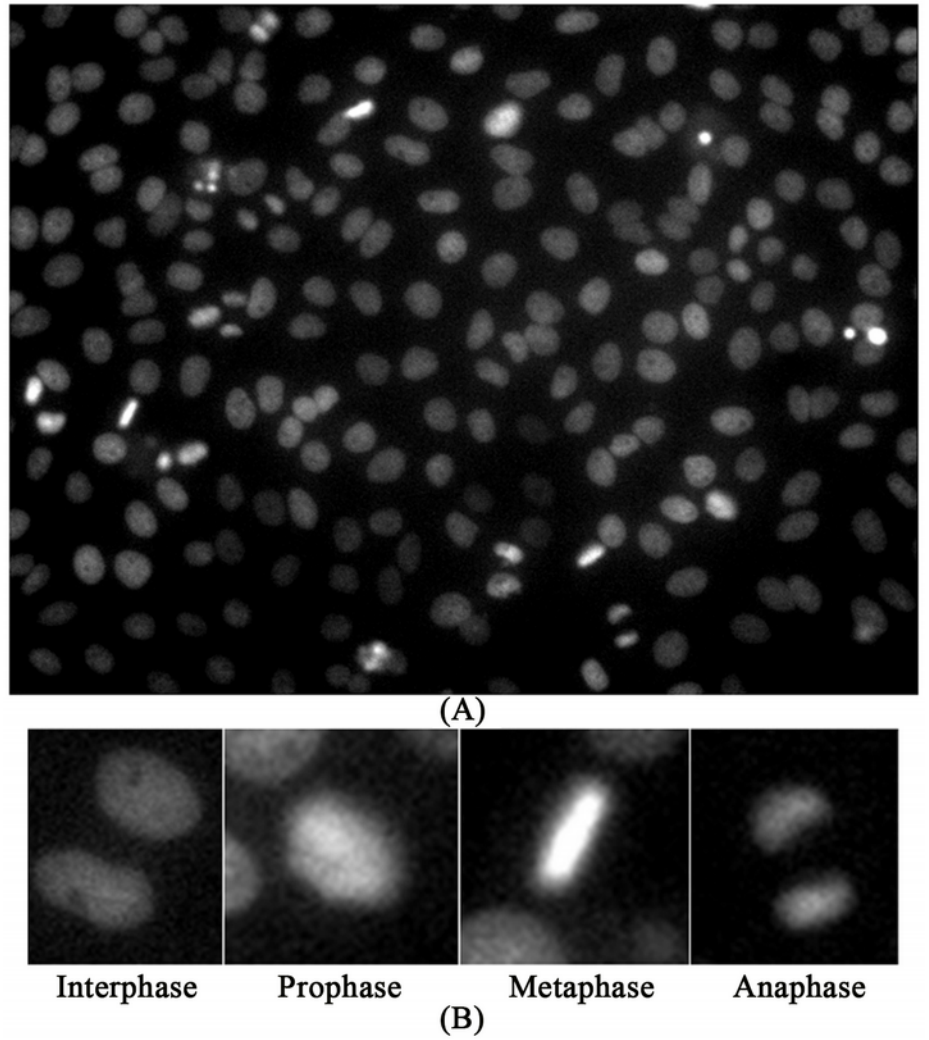
ex. Phenotypic changes of proteins inside individual Drosophila Kc167 cells treated with RNAi libraries



A representative image of Drosophila Kc167 cells treated with RNAi. The red, green, and blue colors are the DNA, F-actin, and α-tubulin channels.
doi:10.1371/journal.pcbi.1003043.g002

Case studies : Molecule discovery (drugs) and therapeutic targets

2. Live-cell imaging-based studies for cell cycle and migration regulatory discovery



Examples of HeLa cell nuclei and cell cycle phase images.
(A) A frame of HeLa cell nuclei time-lapse image sequence;
(B) Example images of four cell cycle phases.
doi:10.1371/journal.pcbi.1003043.g003

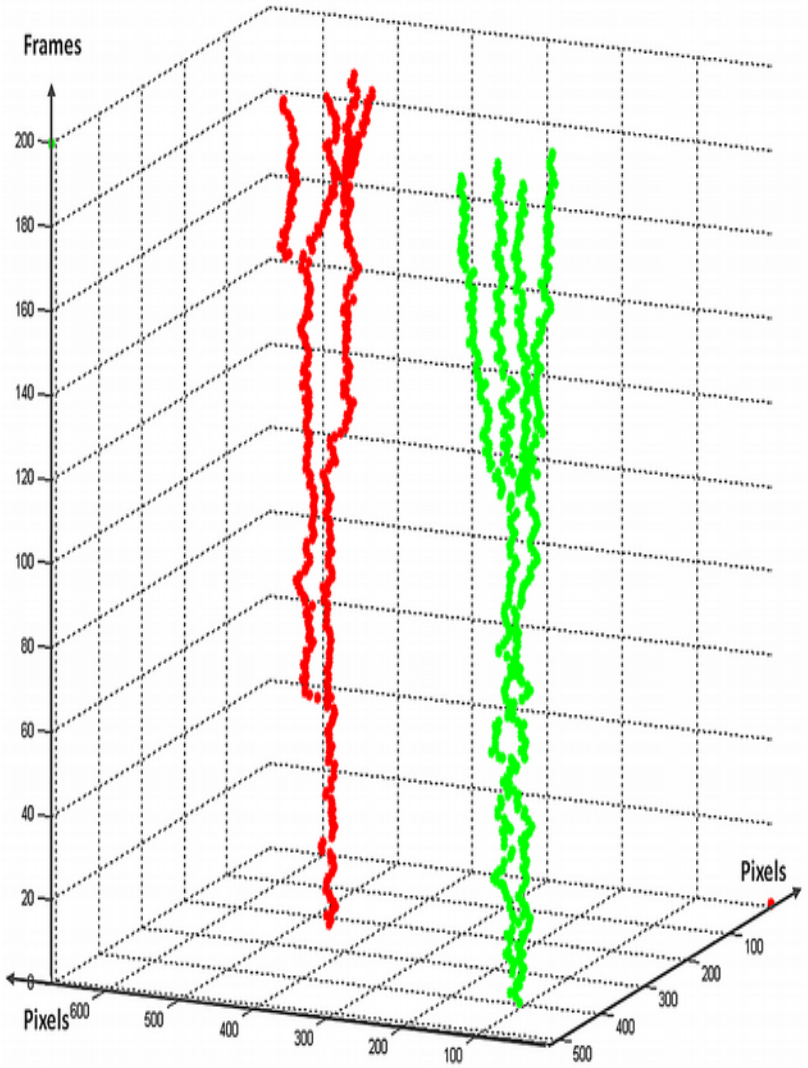
- 600 genes among 22 000 human genes regulating mitosis : identified by using image of live cells and RNAi processing (project www.mitocheck.org extended with the project www.mitosys.org)

- Imagery *in vivo of* HeLa cells used for new elements regulating mitoses

-*time-lapse imaging* to study the stem cells dynamics a,d predict the cell fate of neuronal progenitor cells

Case studies : Molecule discovery (drugs) and therapeutic targets

4. *Caenorhabditis elegans (C. elegans)* Imaging-based studies and embryogenesis



- Excellent biological model biologique: a few hundreds of cells ony to study cellular development and organisation.

- Embryogenesis study with *time-lapse imaging* and tracking of cells evolving to study genes involved in the development process.
- Atlas of *C. elegans* quantifying the nuclear localization and satistics over spatial pattern of development built upon a stack of confocal images with the software CellExplorer. *CellProfiler* = « *image analysis pipeline » to segment cells and quantify protein expression profile of specific proteins like clec-60* for various individuals *C. elegans* submitted to various treatments.