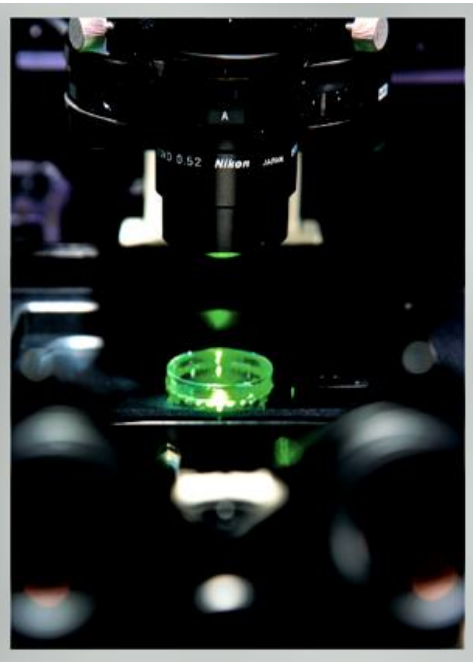
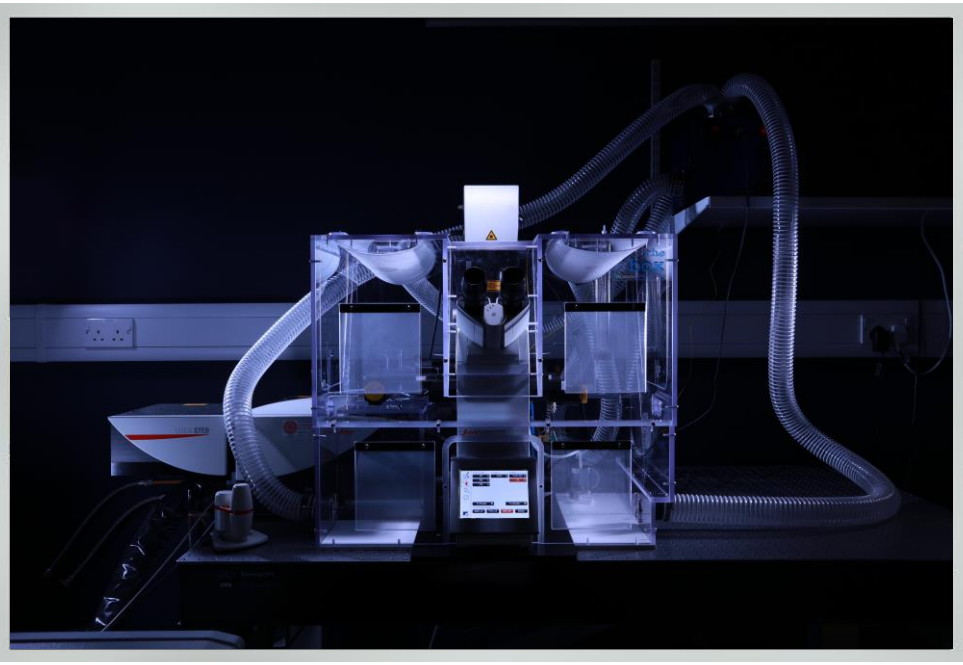
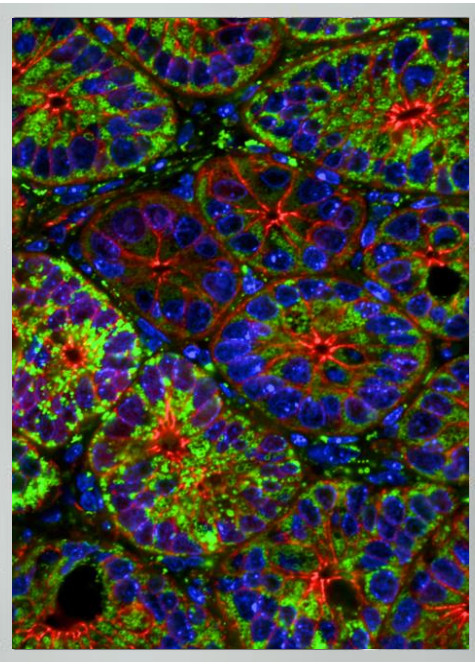
BioImaging facility update: *from multi-photon in vivo imaging to high-content high-throughput image-based screening*

Alex Laude
The BioImaging Unit

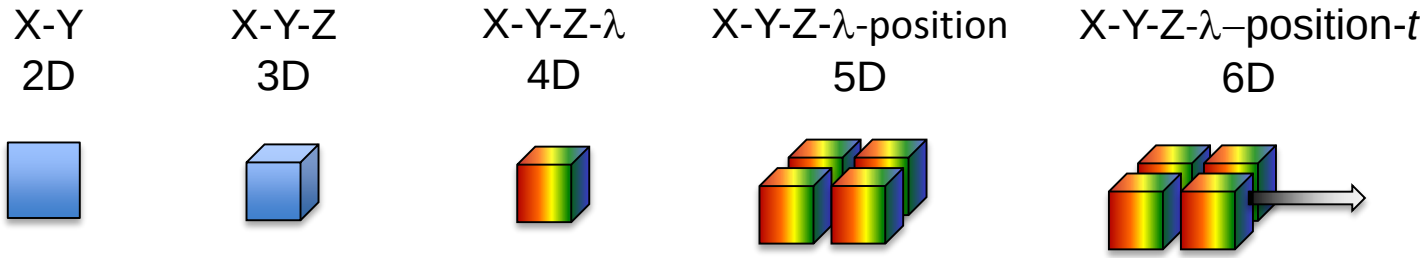






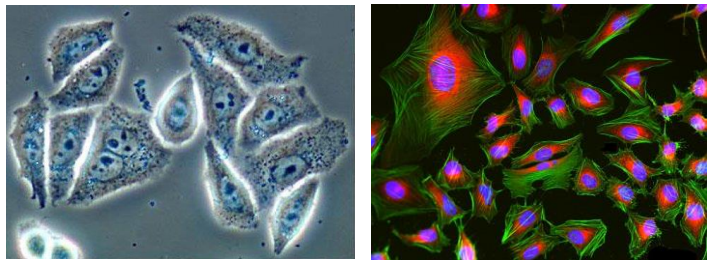
The BioImaging Unit

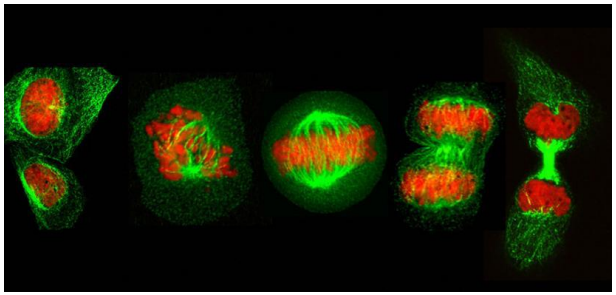
Multi-dimensional, multi-modal imaging *at the sub-cellular level*

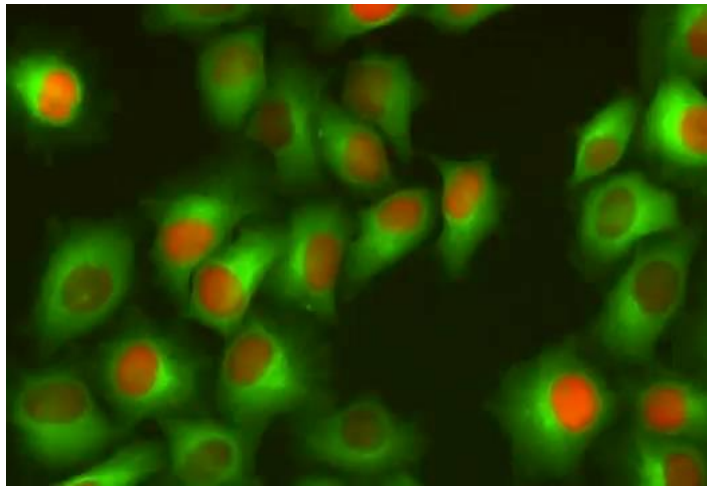


- **Brightfield, phase and DIC microscopy**
- **Wide field fluorescence microscopy**
  - ***multi-parametric (x5) analysis***
- **Confocal & Multi-photon microscopy**
- **Live-Cell imaging**

  Fast dynamic processes (>10fps)
  Longer lasting (days / weeks)





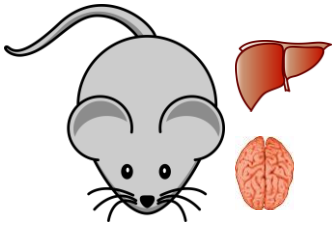


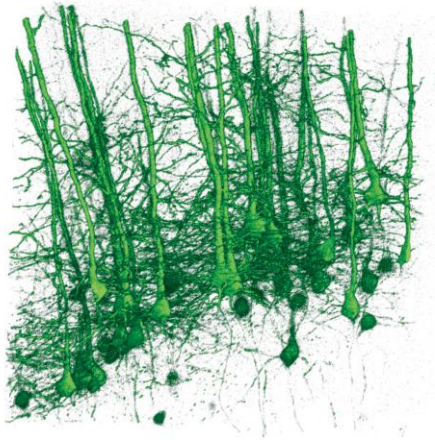
THE BioImaging Unit

New additions: Multi-photon & Super resolution imaging

- **Live-Cell multi-photon imaging inverted and upright configurations**

  Fast dynamic processes (>10fps)
  in vivo / ex vivo, tissue slice mm depth
  AiryScan technology

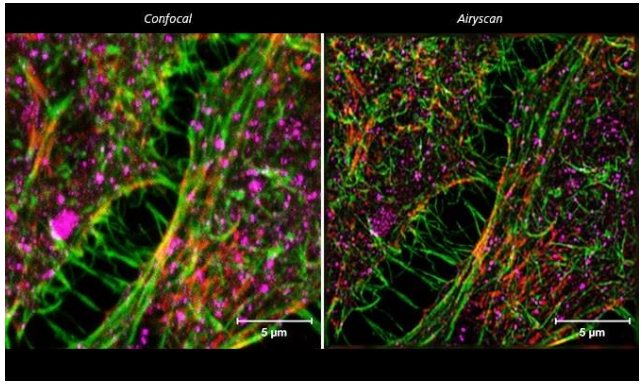


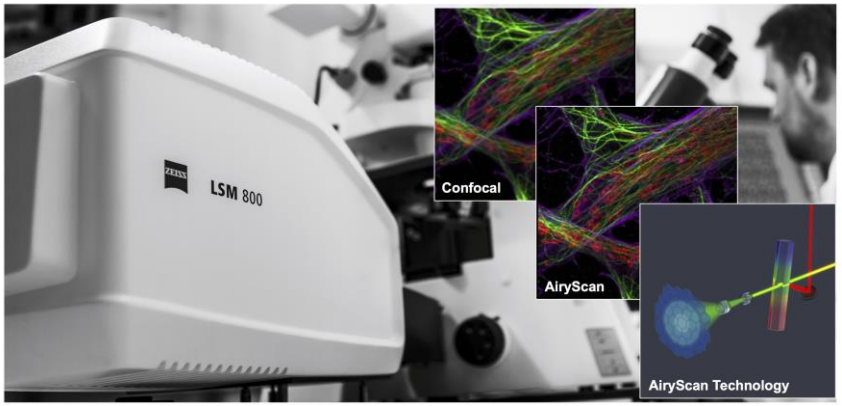


80% University (RIF) / 20% Wellcome funded

- **Live-Cell super resolution imaging**

  Fast dynamic processes (>10fps)
  AiryScan ~2x lateral resolution improvement





Wellcome funded

THE BioImaging Unit

Multi-dimensional, multi-modal imaging *at the sub-micron level*

X-Y
2D

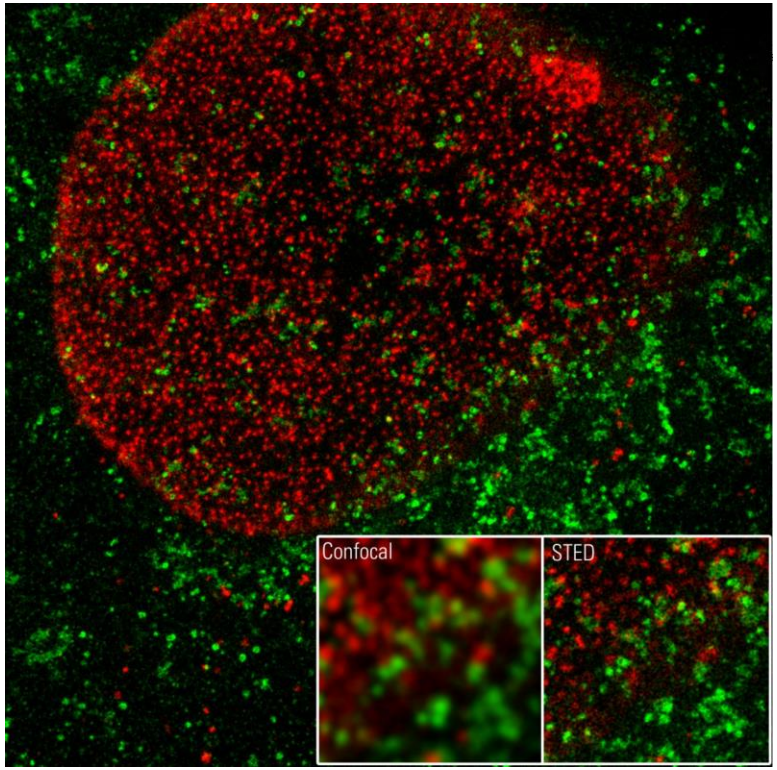
X-Y-Z
3D

X-Y-Z-λ
4D

X-Y-Z-λ-position
5D

X-Y-Z-λ–position-*t*
6D

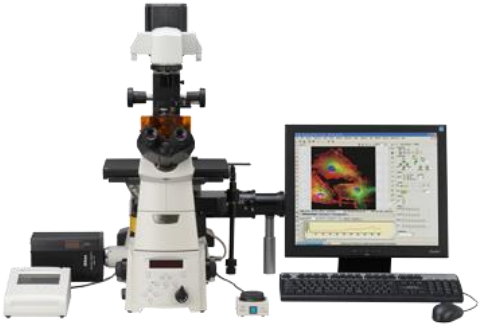
- **Super resolution microscopy (live & fixed)**
  - **STED ~ 50 nm**
  - **iSIM ~ 140 nm**
  - **AiryScan ~ 140 nm**



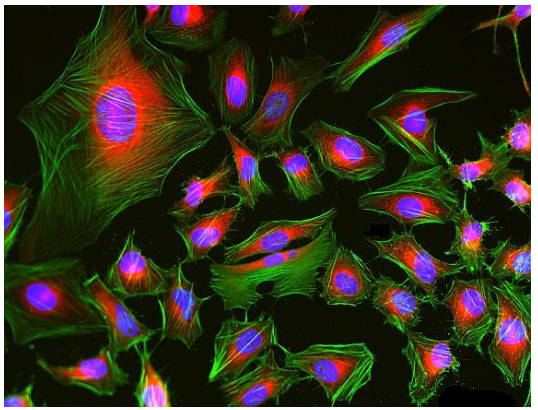
- **Commercial and open-source image analysis softwares.**
  Dedicated image analysis machines

THE BioImaging Unit

Choosing the right imaging modality: *the 'Triangle of Compromise'*



- Semi automated
- Low-throughput
- High cost per image

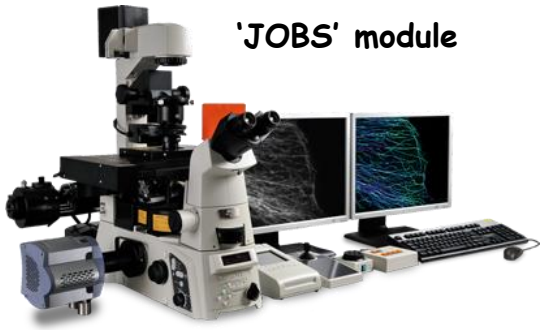


Experimenter bias

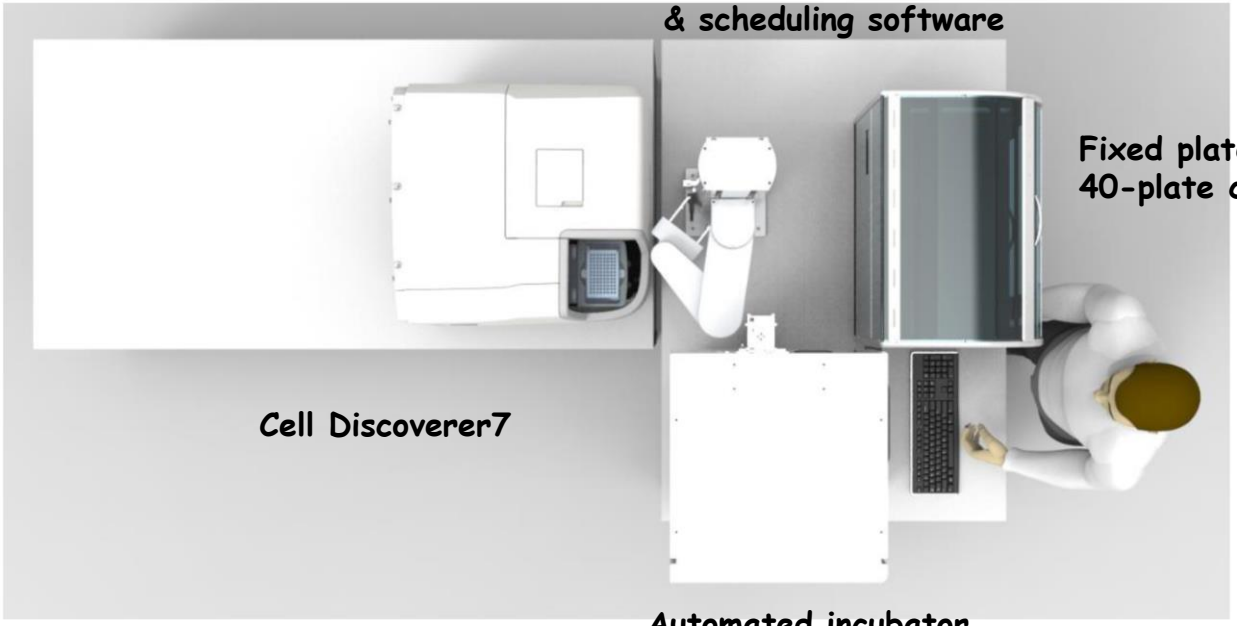
- Automate – save time & reduce bias

New additions: Automated, live-cell microscope – Zeiss Cell Discoverer7

Zeiss Cell Discoverer7



'JOBS' module



Robot loader
& scheduling software

Fixed plate storage
40-plate capacity

Cell Discoverer7

Automated incubator
20-plate capacity

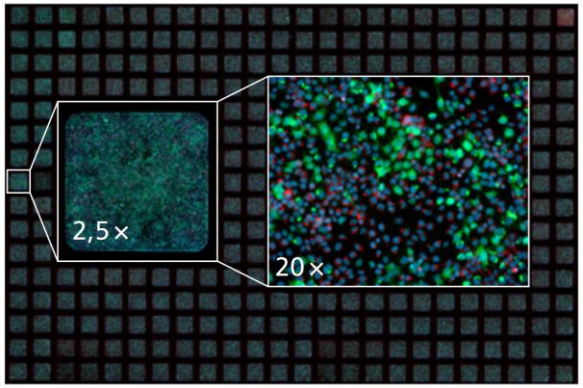
Wellcome funded

THE BioImaging Unit

New additions: Automated, live-cell microscope – Zeiss Cell Discoverer7

Acquiring an image

- Automate the process of image acquisition
  - Create acquisition workflow – building blocks
    - Automated plate recognition & alignment
    - Focus methods
    - How many images per well?
    - Selected at random, near the edge, in the middle?
    - Full well tile
    - Capture images based on cell number
  - Conditional acquisition -on the fly analysis
    - Image number of areas or only image areas that have an 'interesting' morphology or above threshold intensity. Cuts down on unwanted data.
    - Volume imaging? – deconvolution
    - Live or end point observation?
    - Add compounds, view a response?
  - Fluorescence slide scanner



THE BioImaging Unit

High-content imaging / screening / analysis

- Automated approaches yield rapid, unbiased acquisition of images containing many hundreds of cells
  - Gives us a view of the heterogeneity of a population in response to a given perturbagen.

- Screen a large number of genes or compounds for a phenotypic change
  - SiRNA & drug libraries already available in the Faculty
    - Dharmacon siGENOME smart pool library against 7500 human genes
    - MRCT drug library including FDA and natural product collection
    - A kinase targeting collection and an index collection – 20,000 compounds in total

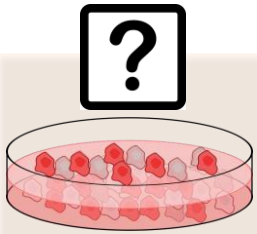
High-content imaging / screening / analysis - assay development

Setting up an assay…

- Understand what you want to measure?
  - Do you want to quantify a morphological change, viability or proliferation?
  - How are you going to do this?
    - Correct use of fluorescent labels tools / markers.
  - Is this possible within the context of the assay or the limits of the microscope?
- What resolution do you need?
  - lower magnification means more cells but less detail but do you need detail?
- What will the readout be?
  - And how robust is this? -experimental noise
- Develop and validate new or adopt and test an existing assay
  - Small-scale testing and validation of the assay
  - Scale up - plates multiple replicates
- How are you going to measure and quantify represent any changes?
  - 'Imaging informatics'
- DATA??
  - How are you going to store and organise your images?

THE BioImaging Unit

High-content imaging / screening / analysis - typical workflow



Cultured cells (live & fixed)
3D organotypic cultures

Drug
RNAi
Automated TC

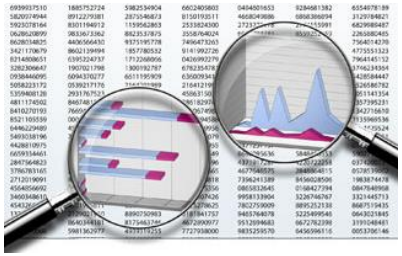
HTS facility

Fix
Stain

Assay

Sample prep

Image acquisition



Data mining

Data analysis

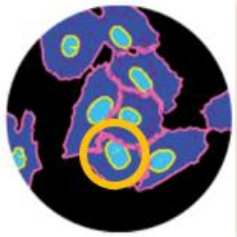


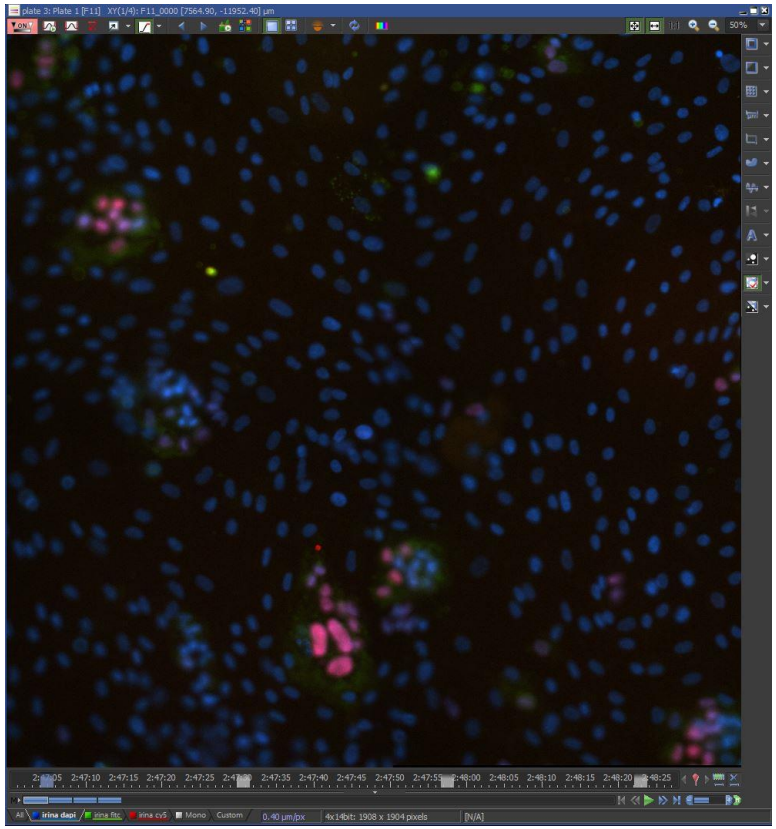
Image segmentation
Feature extraction

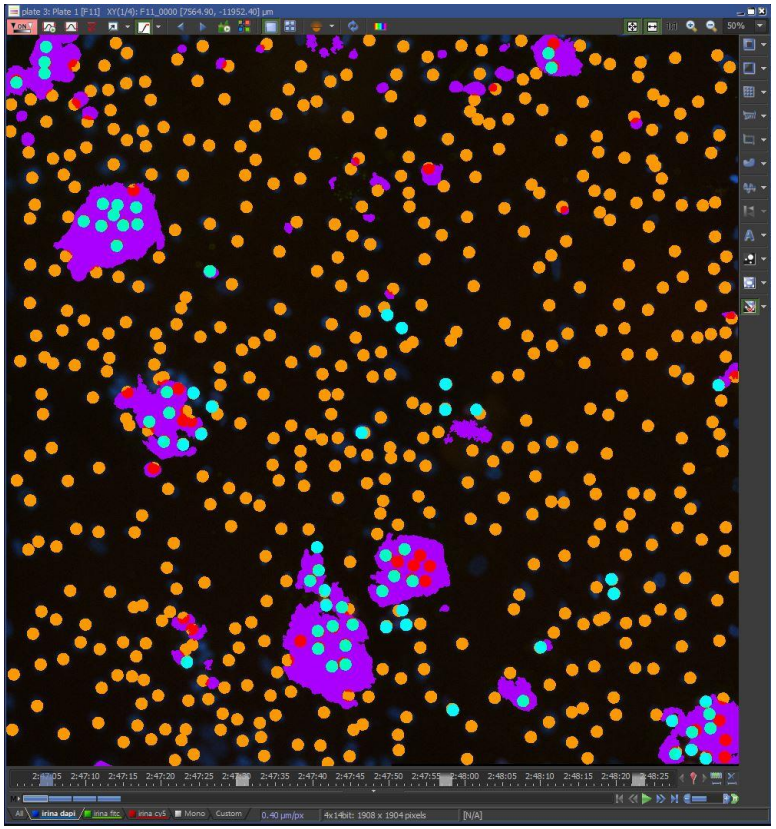
Image Analysis

THE BioImaging Unit

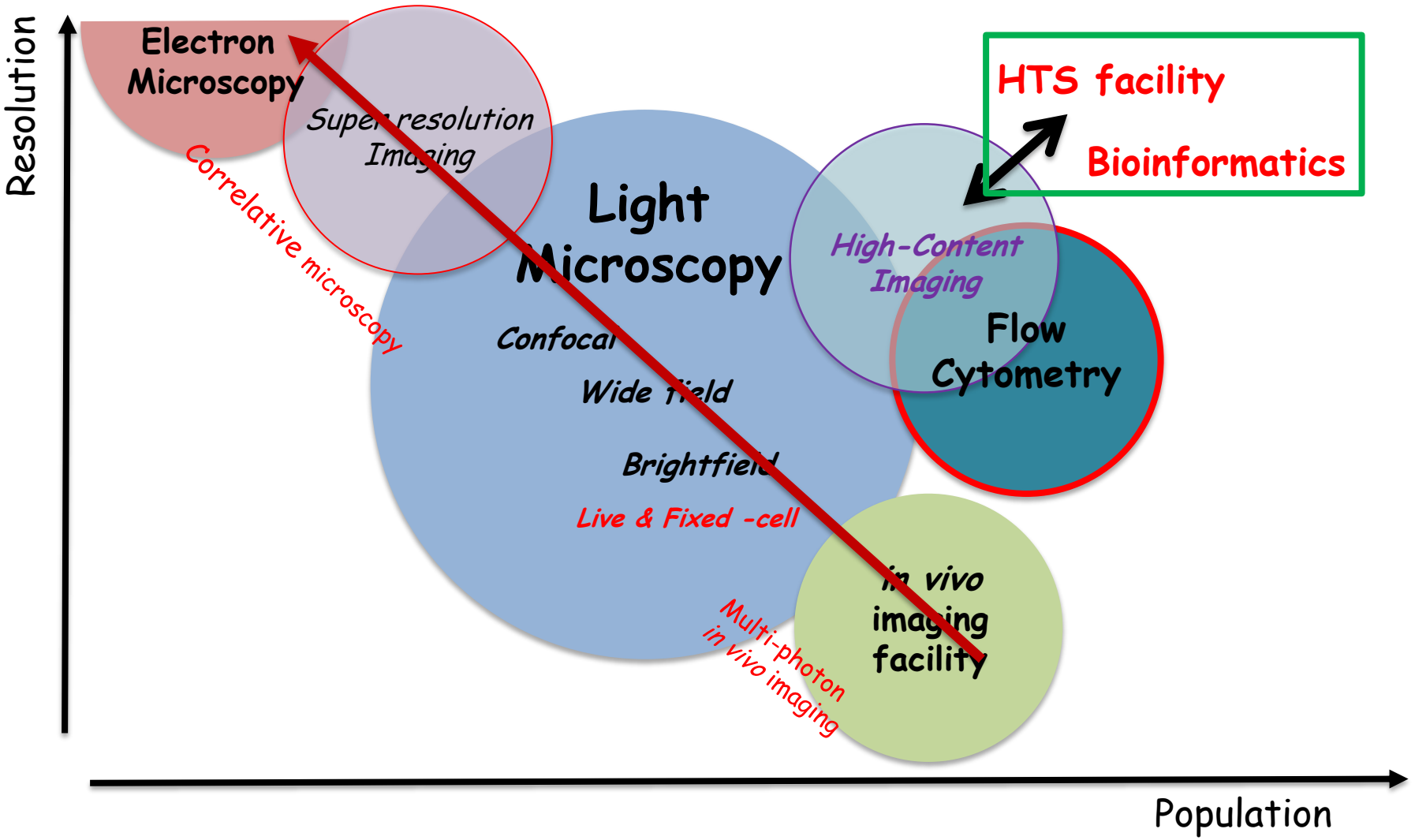
High-Content / Throughput Imaging - analysis

- Analysis softwares:
  - Commercial (Zeiss Zen, NIS Elements, PE Columbus) / open source software (Cell Profiler).
- Analysis methods:
  - Simple: cell count (nuclei), intensity-based segmentation
  - Complex: shape and texture analysis, spatial distribution (inter and intra-cellular).
  - Represent and display the data?





The BioImaging network



THE BioImaging Unit