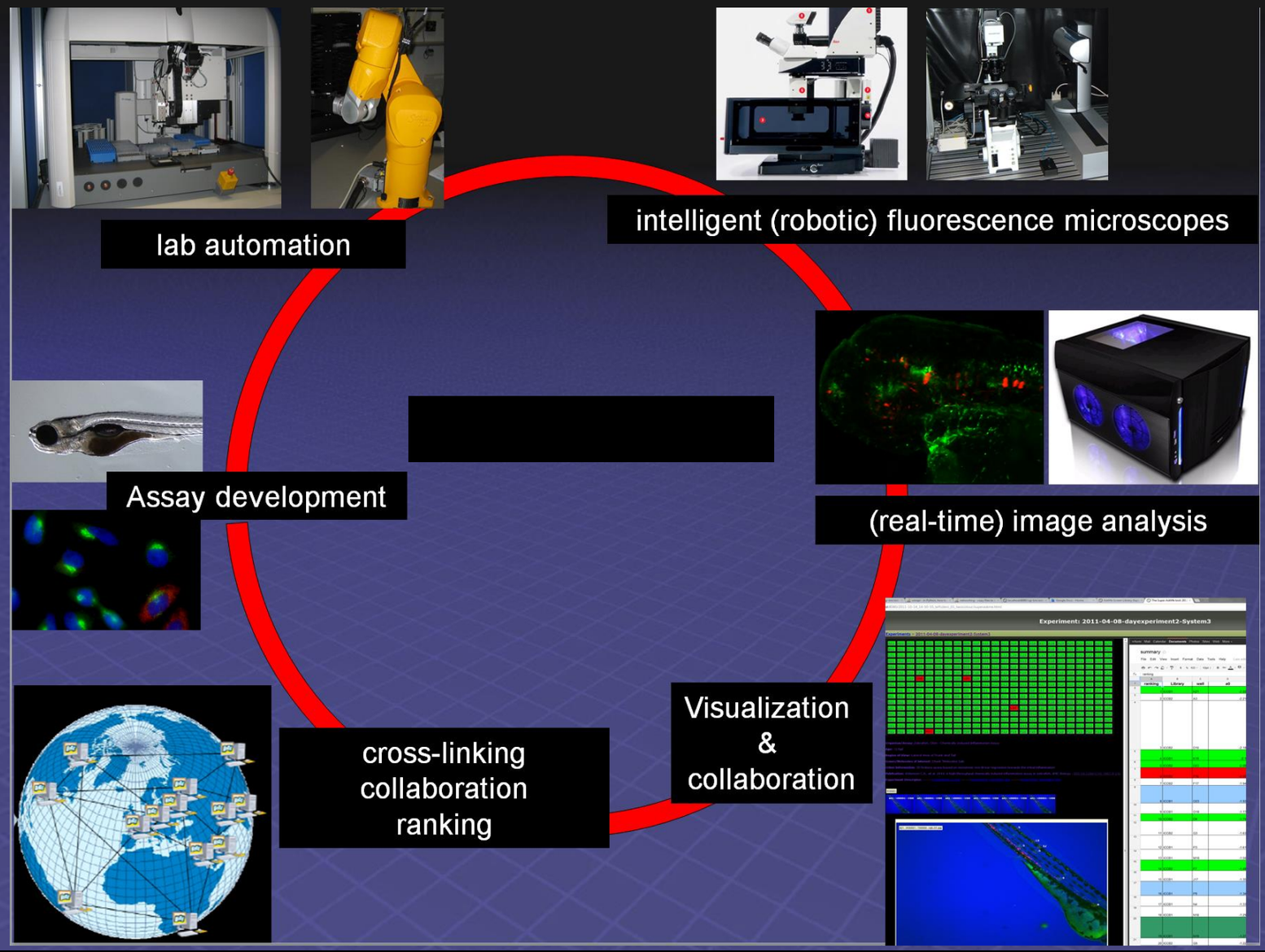
next generation high content screening platforms -
ideas → photons → bytes → ~~answers~~ → questions → ideas



Karlsruhe Institute of Technology

EMBL

ACQUIFER let's see

urban.liebel@kit.edu - u.liebel@acquifer.de

Step 0
Three messages...

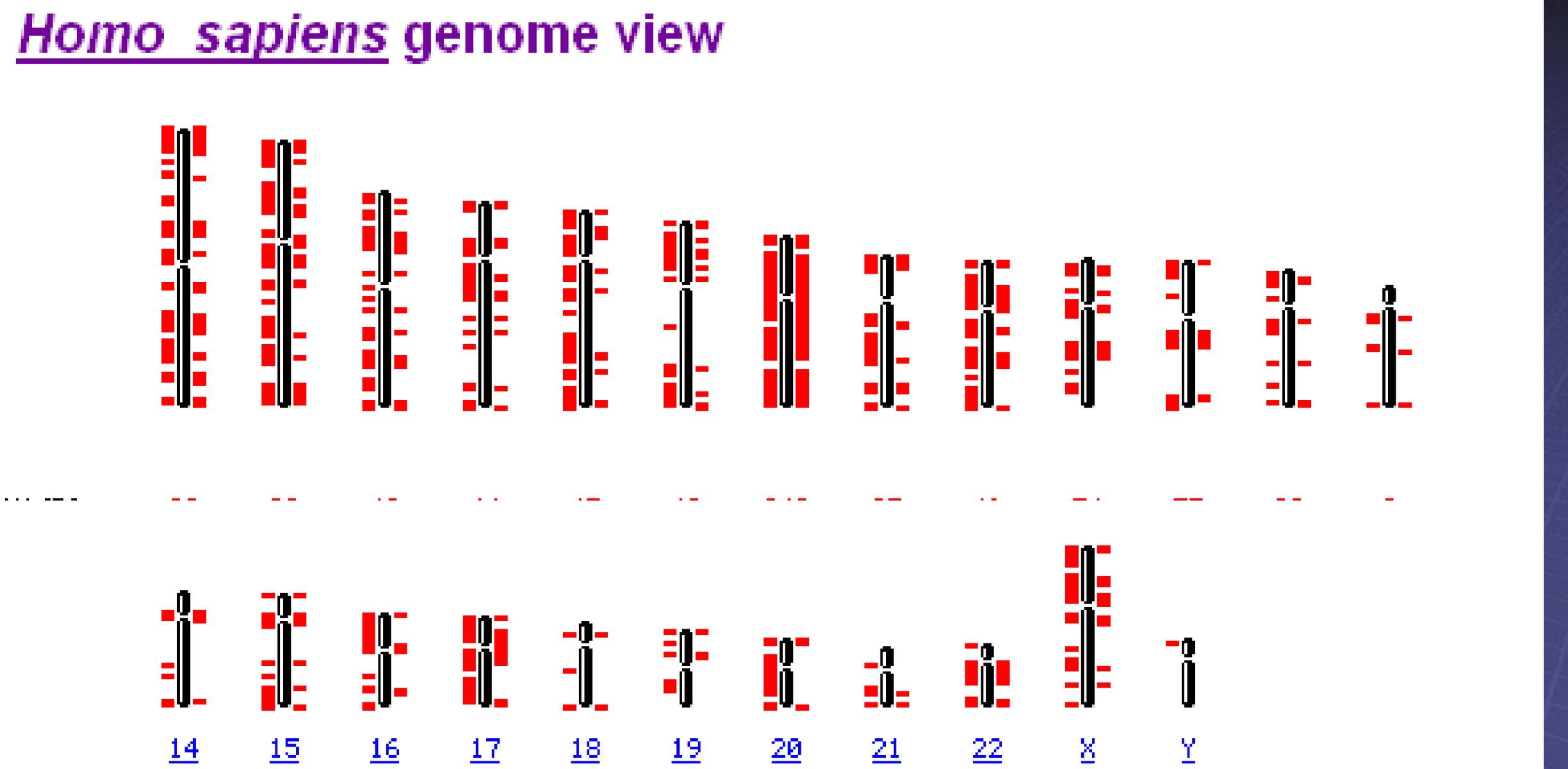
a) Life science BigData platforms are pure FUN ☺

b) Everything i am about to show is connected and influences BigData at each and every level...

c) Life science BigData platforms are a TEAM sport (which require the combination of several disciplines)

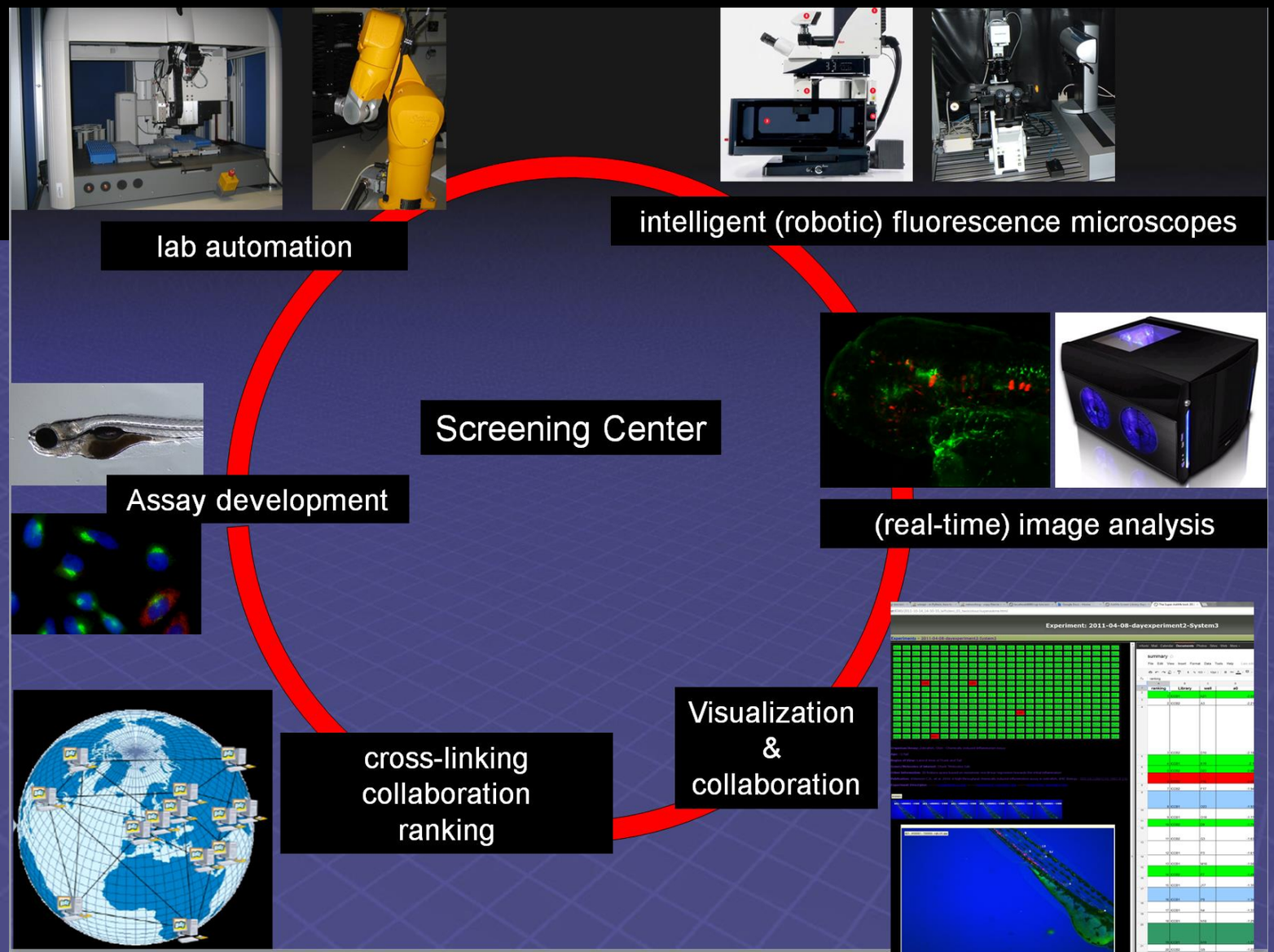
One of the motivations for image based screens..

~ 7900 unknown genes…from ~ 23.000



Step 1

Transforming Question into model organism



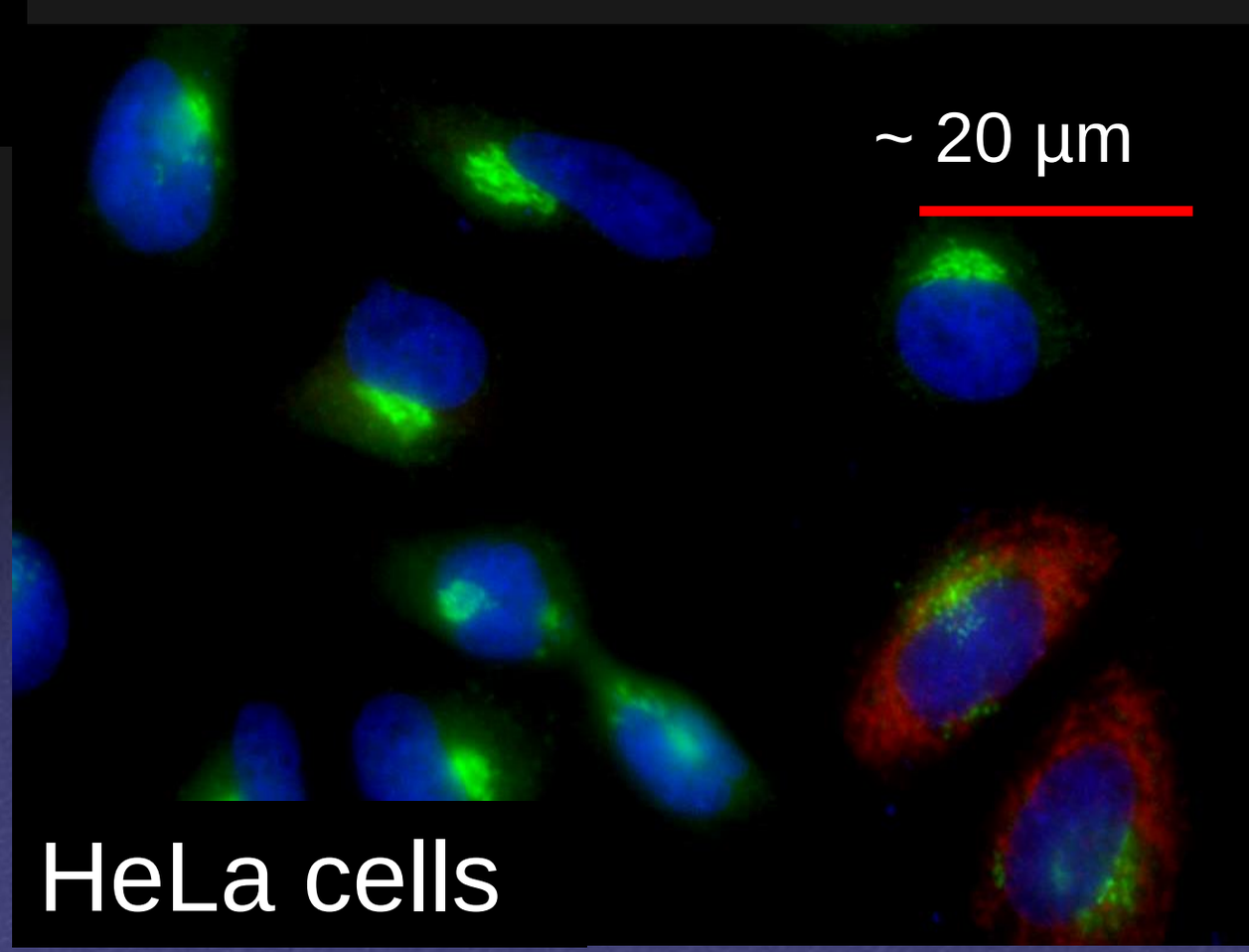
Microscope Sample “range”:

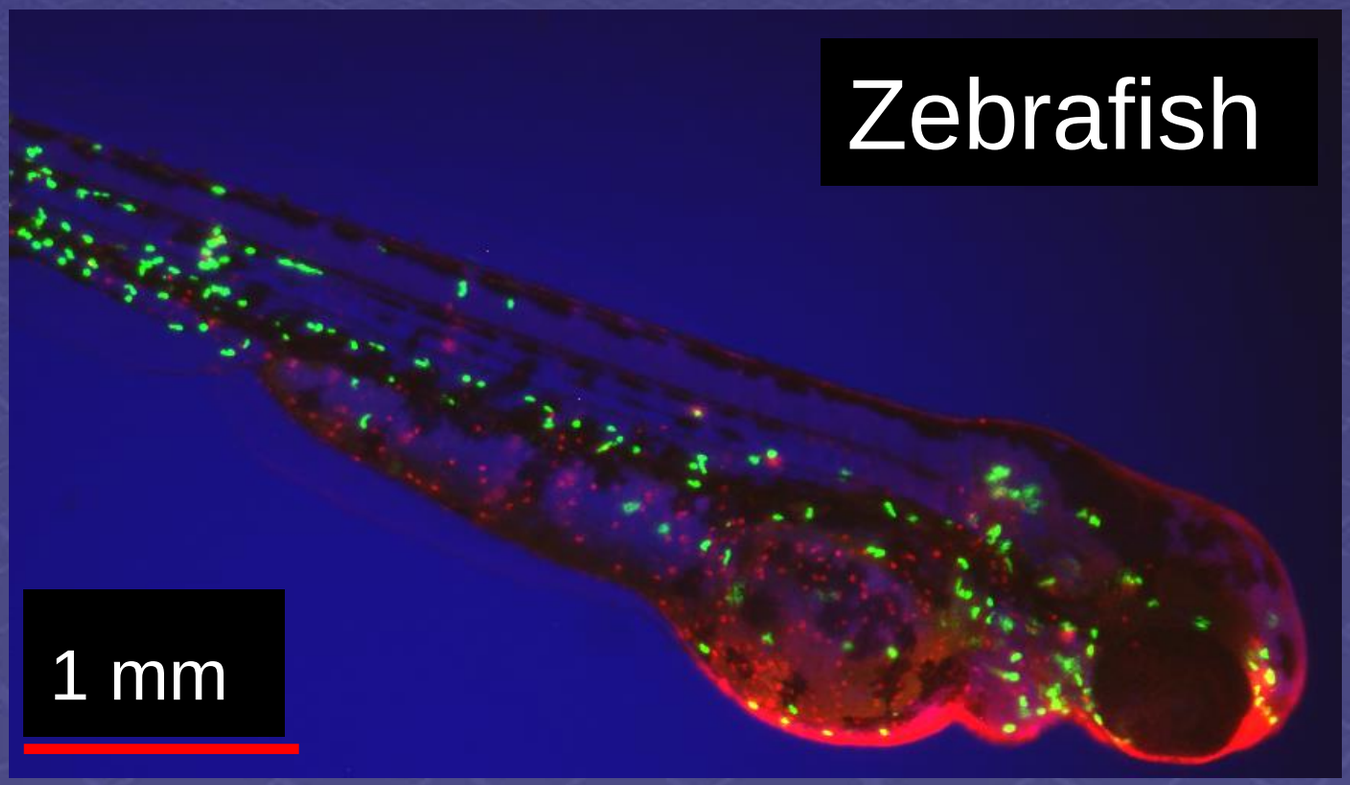
format: 2D – 3D – 4D - 5D

object size: 300nm – 5 mm

type: Fixed cell, live cell

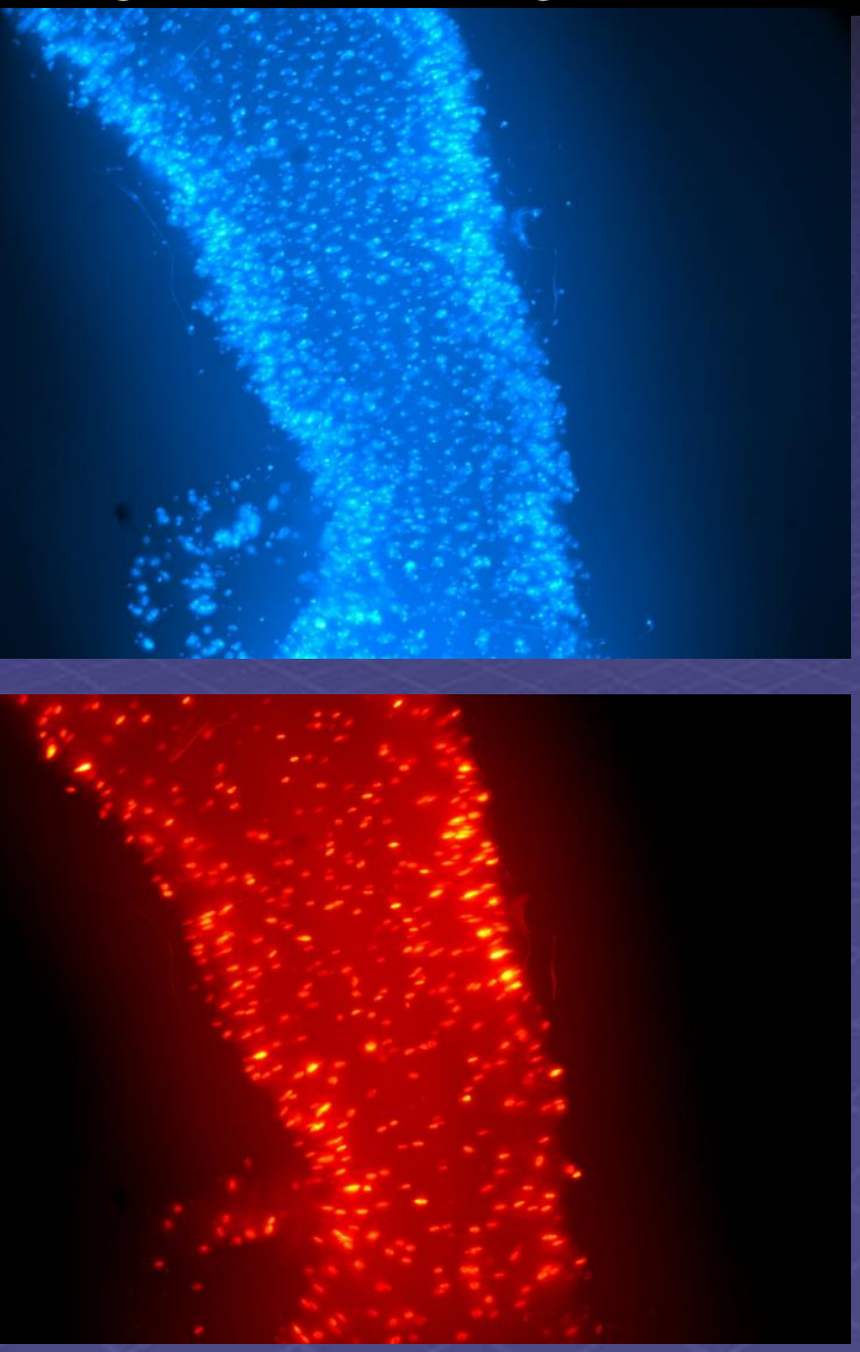
→ Convert question → photons



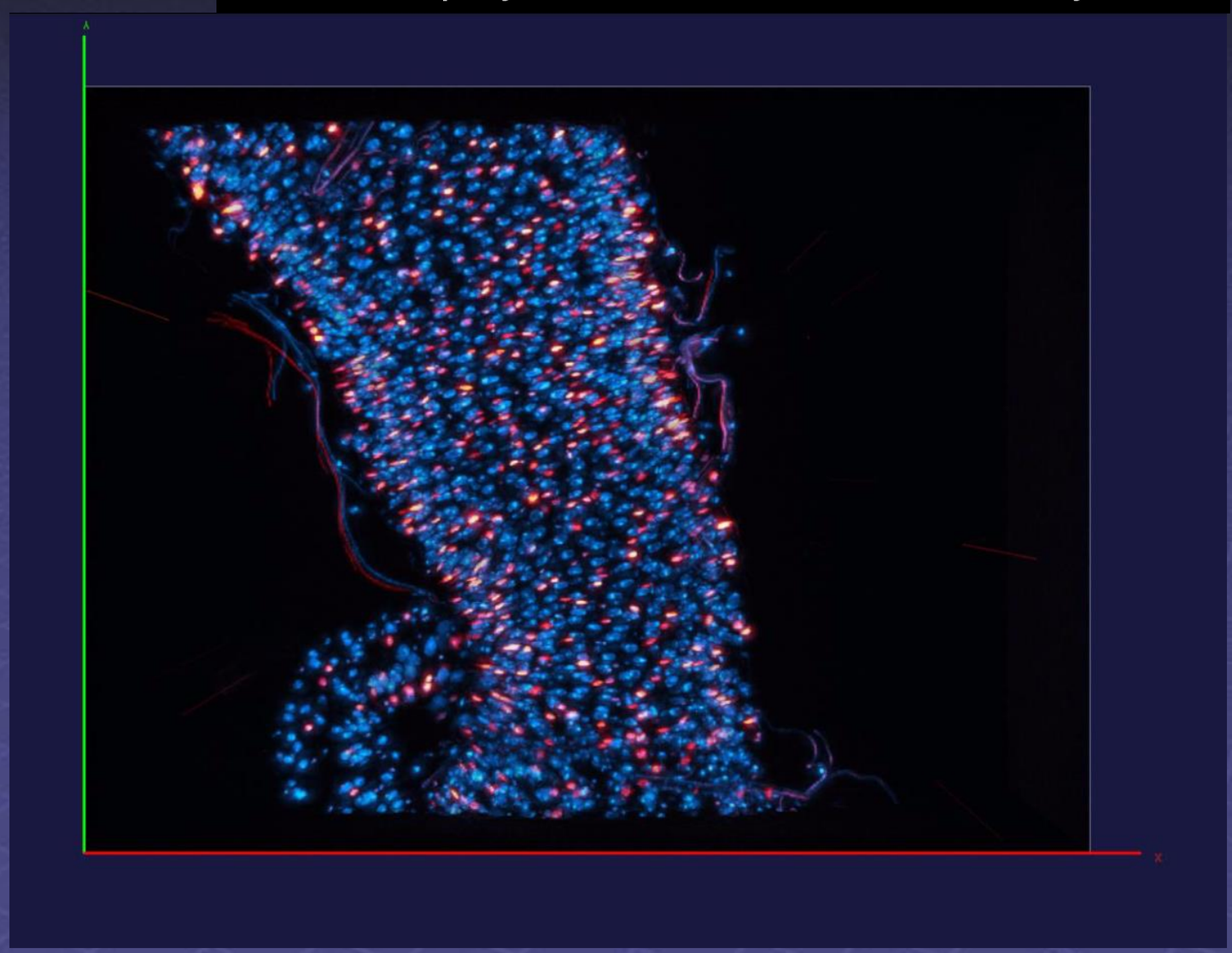


(Fruit fly) Drosophila tissue imaging
gut stem cell screen

Image restoration using batch deconvolution



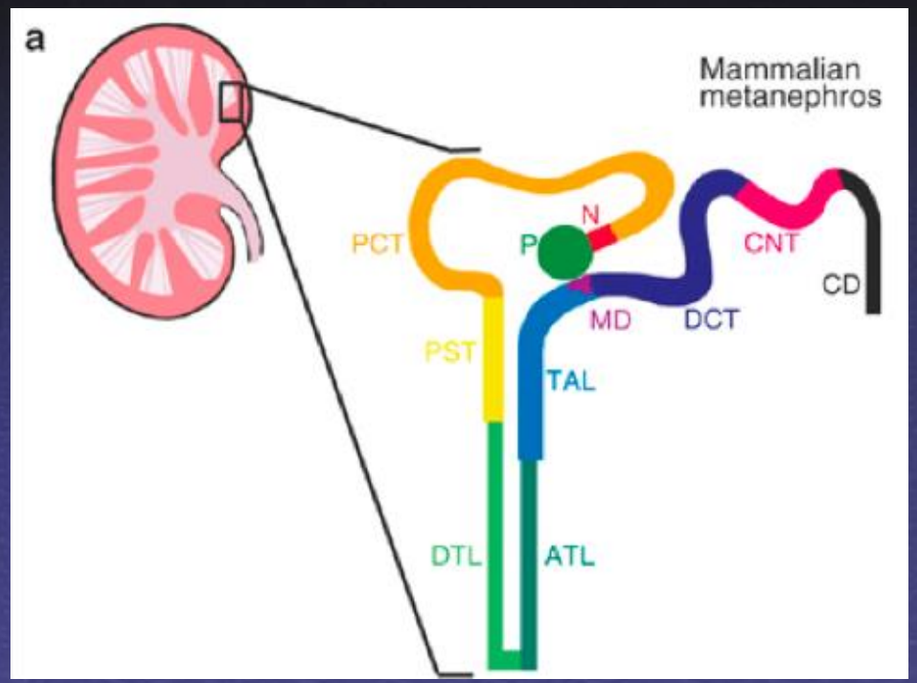
Maximum projection of 150 z-slices, 20x objective

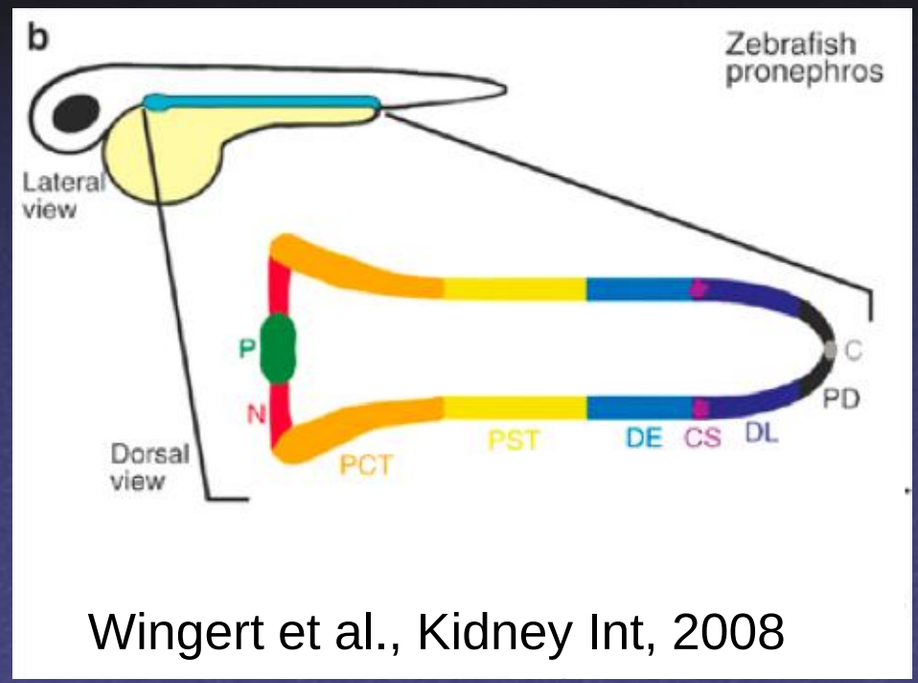


In collaboration with A. Böttcher, M. Boutros, DKFZ and S. Streichan, EMBL

The zebrafish pronephros as a model system

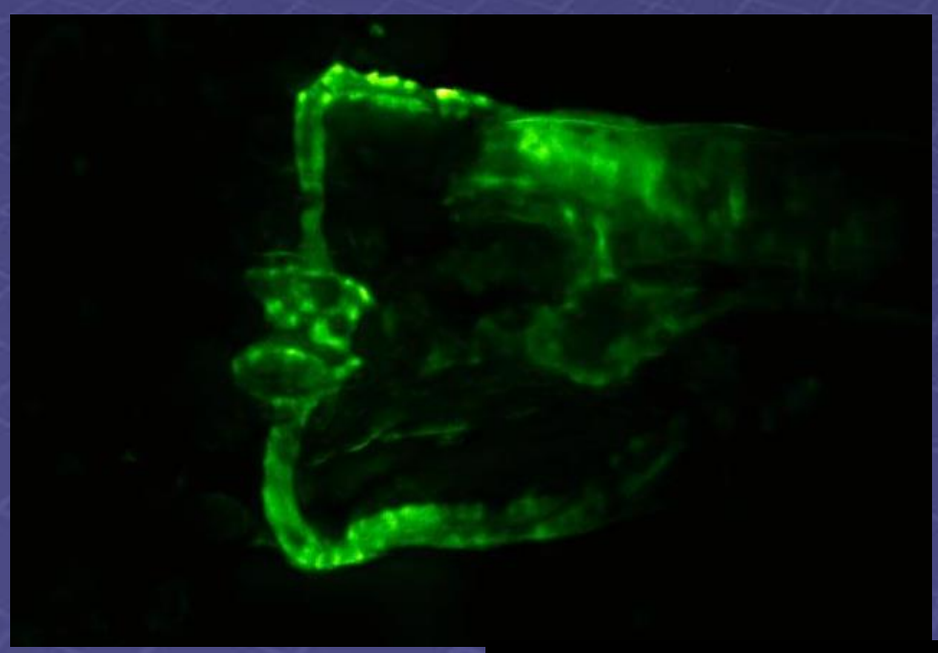
Nephron segmentation in mammals and zebrafish larvae





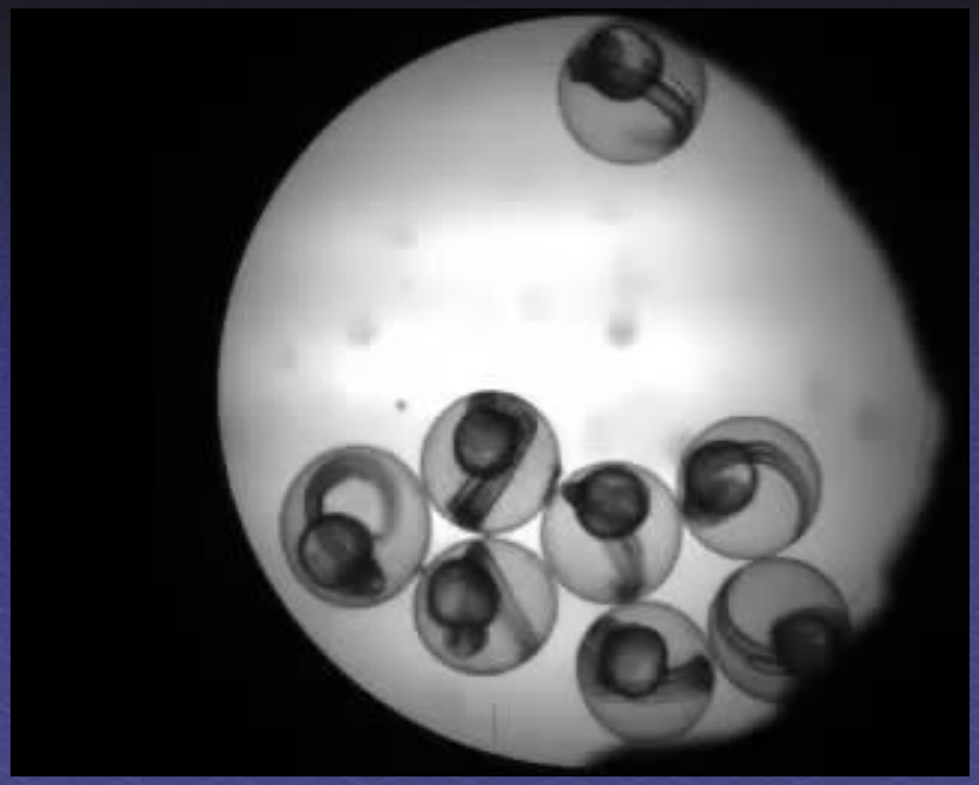
Wingert et al., Kidney Int, 2008

Pronephros specific GFP expression in wt1b:gfp transgenic line



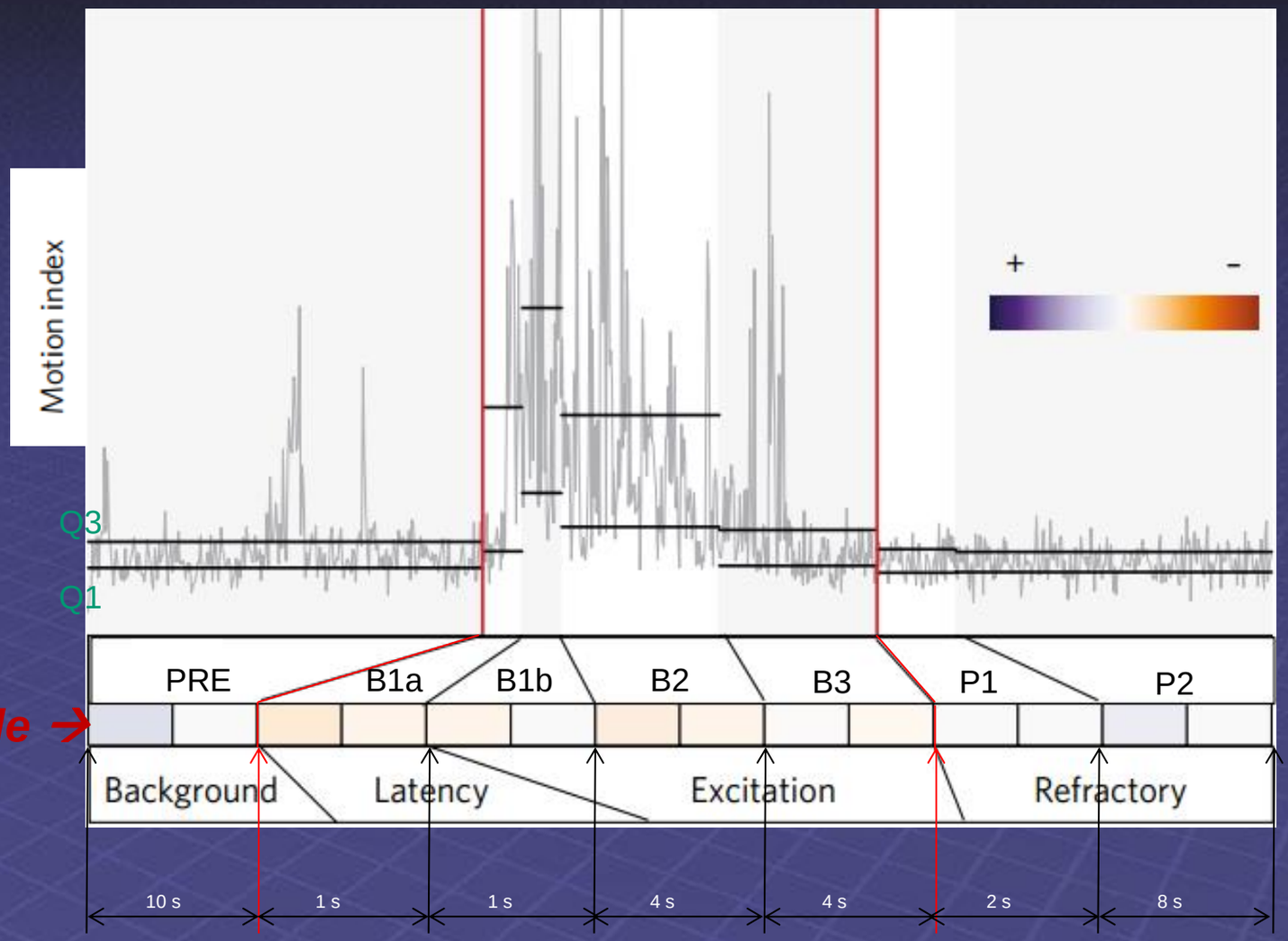
Jens Westhoff / Jochen Gehrig

Photo Motor Response (PMR) Assay:



Zebrafish embryos in a well, no drug used, lights flashed and motion tracked

Barcode →

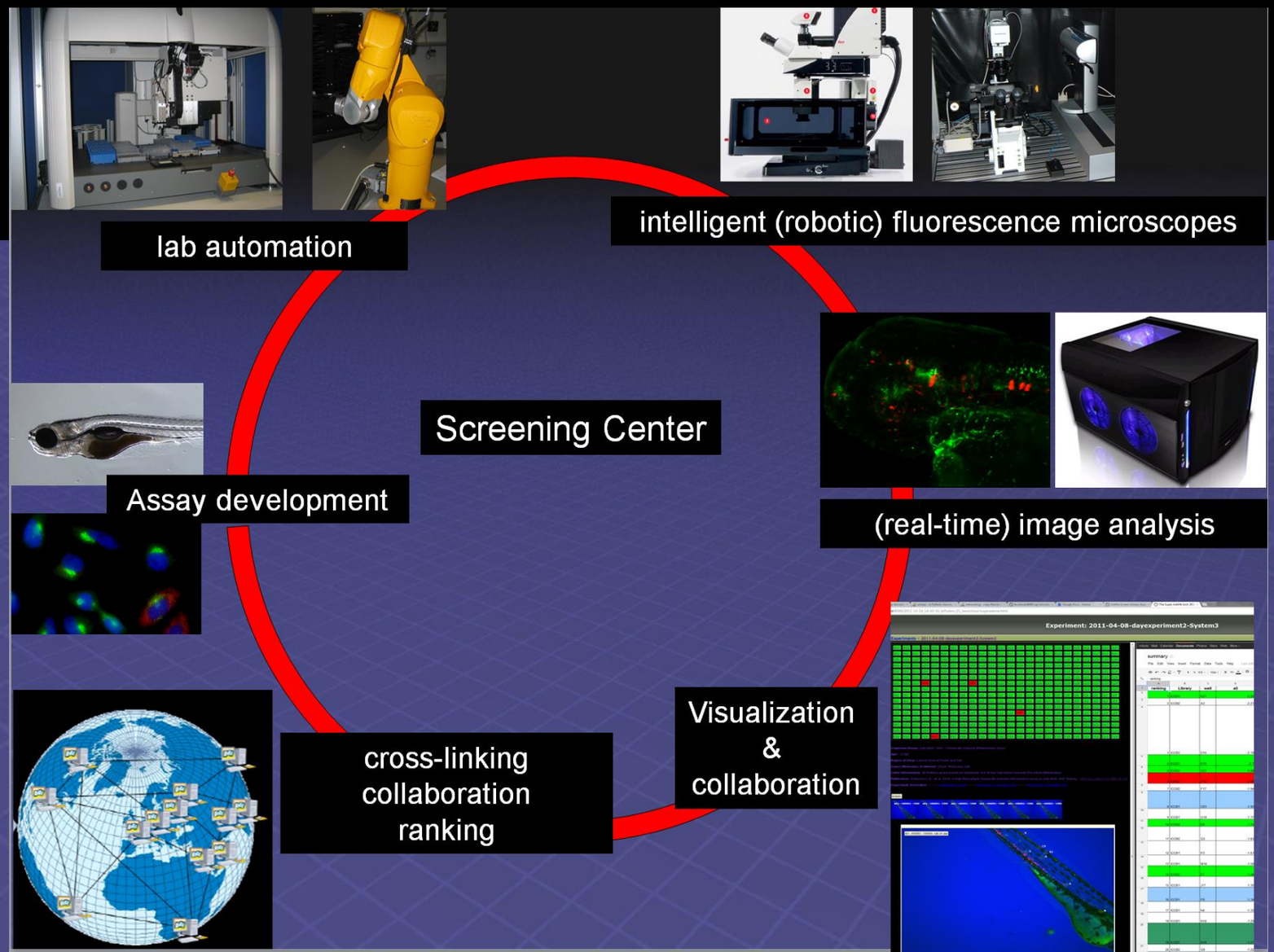


Complex behavior reduced to a string of numerical features

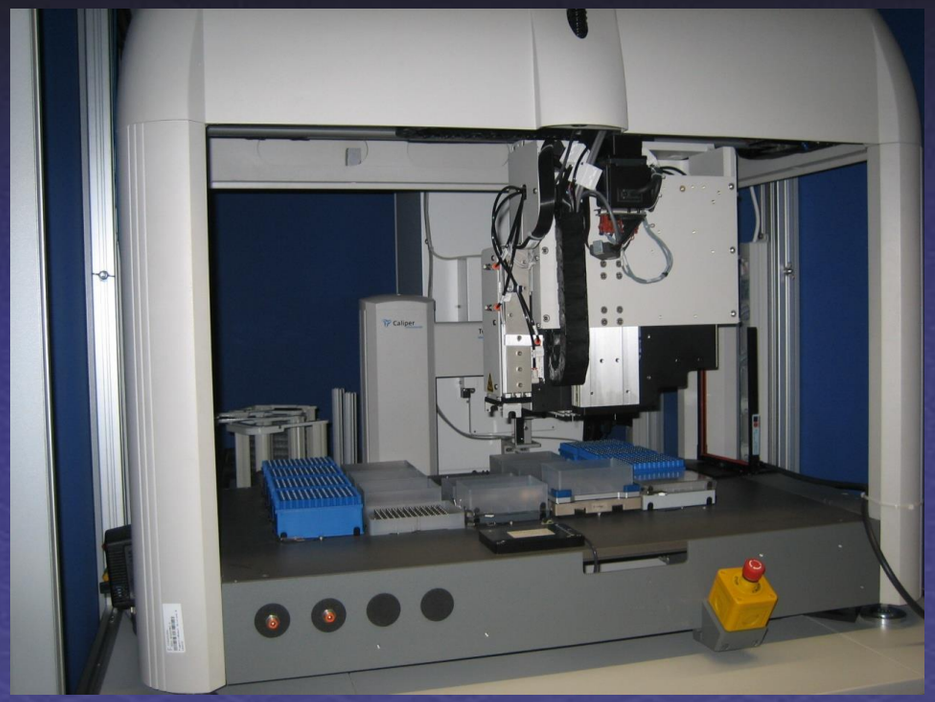
Kokel D., et al. (2010) Nature Chemical Biology

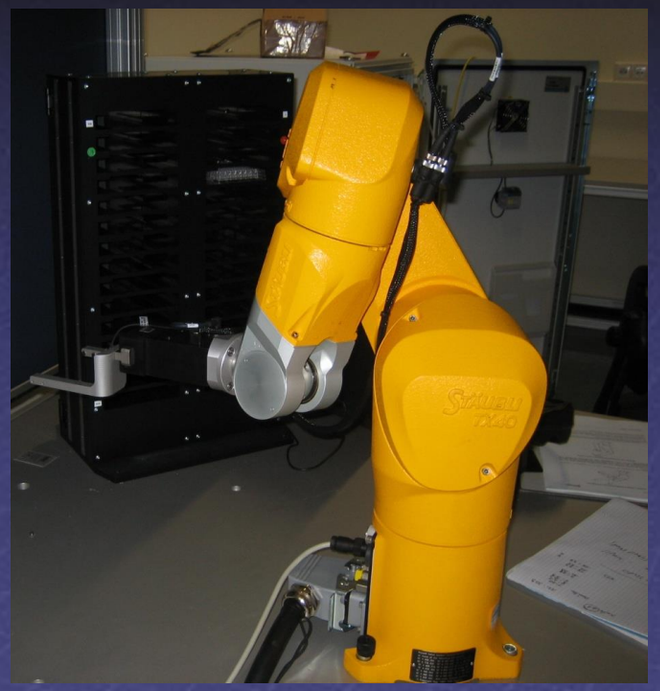
zebrafish “server racks” – require heating ;-)

Step 2
lab automation



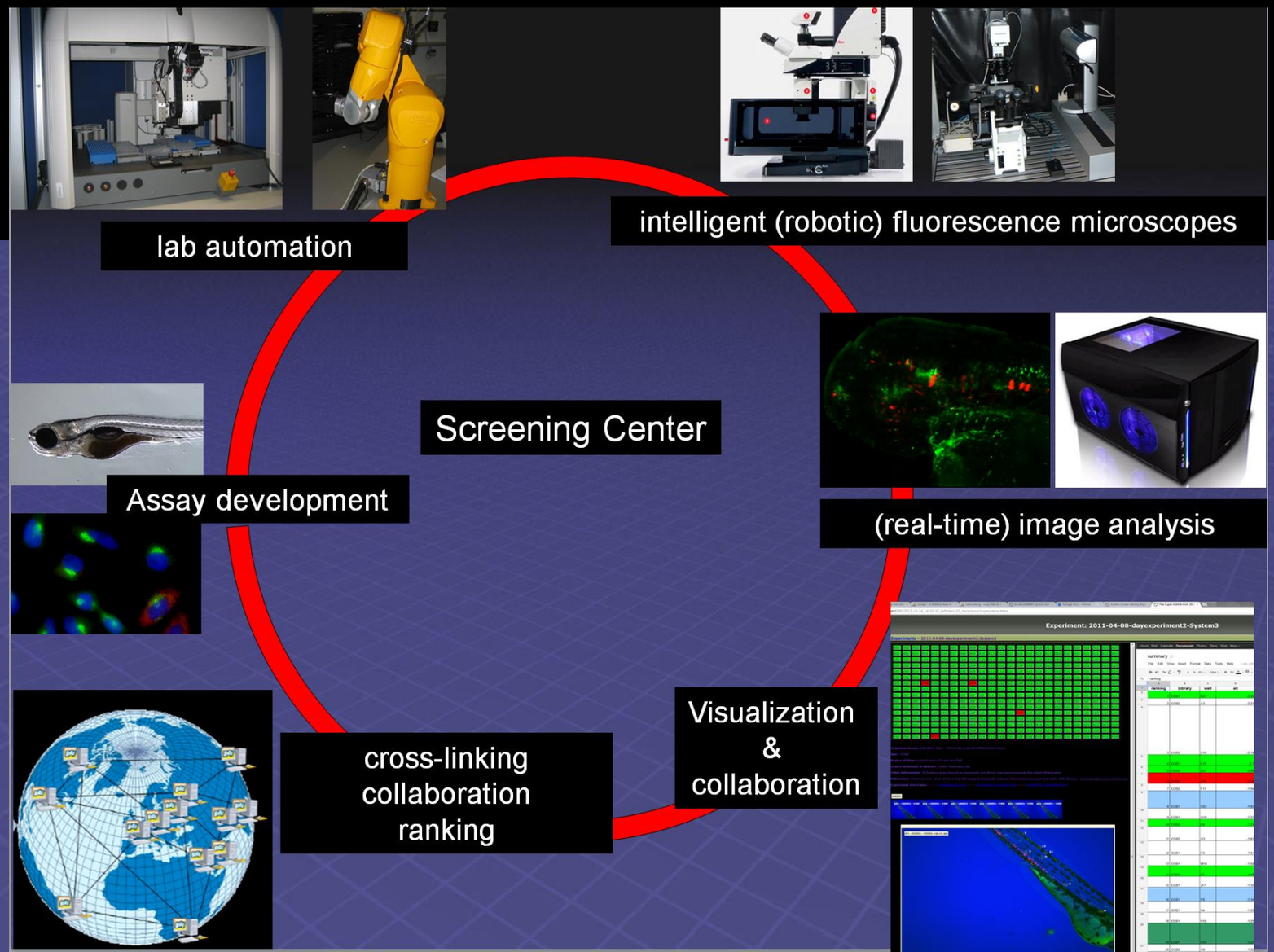
Sample preparation/robotics is not a bottleneck…
(still expensive though…)





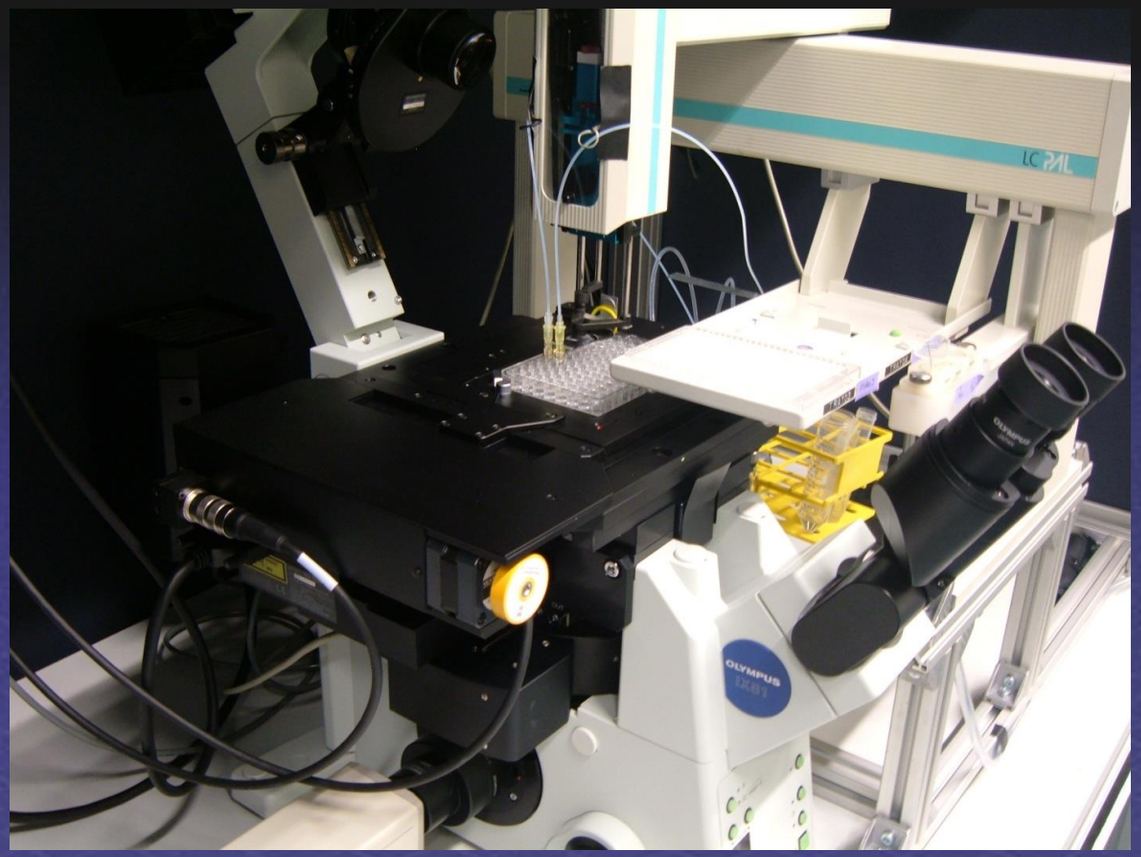
Step 3

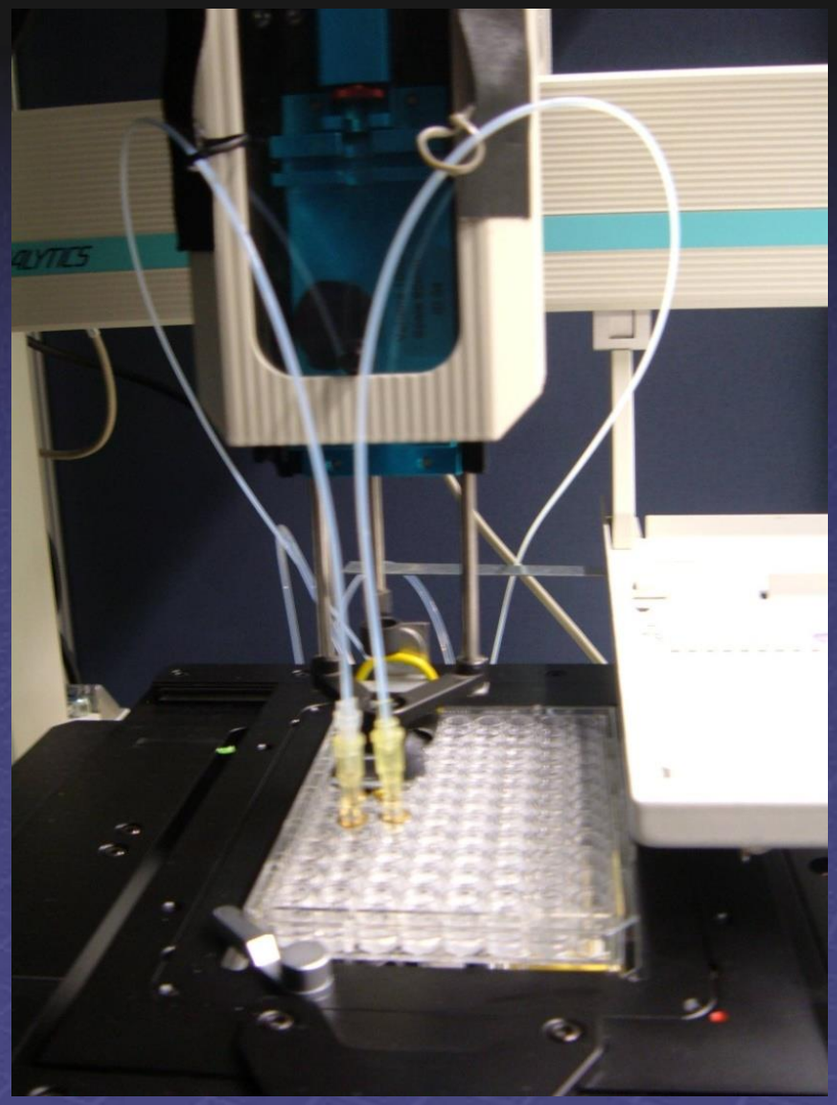
Converting Photons → Bytes/images

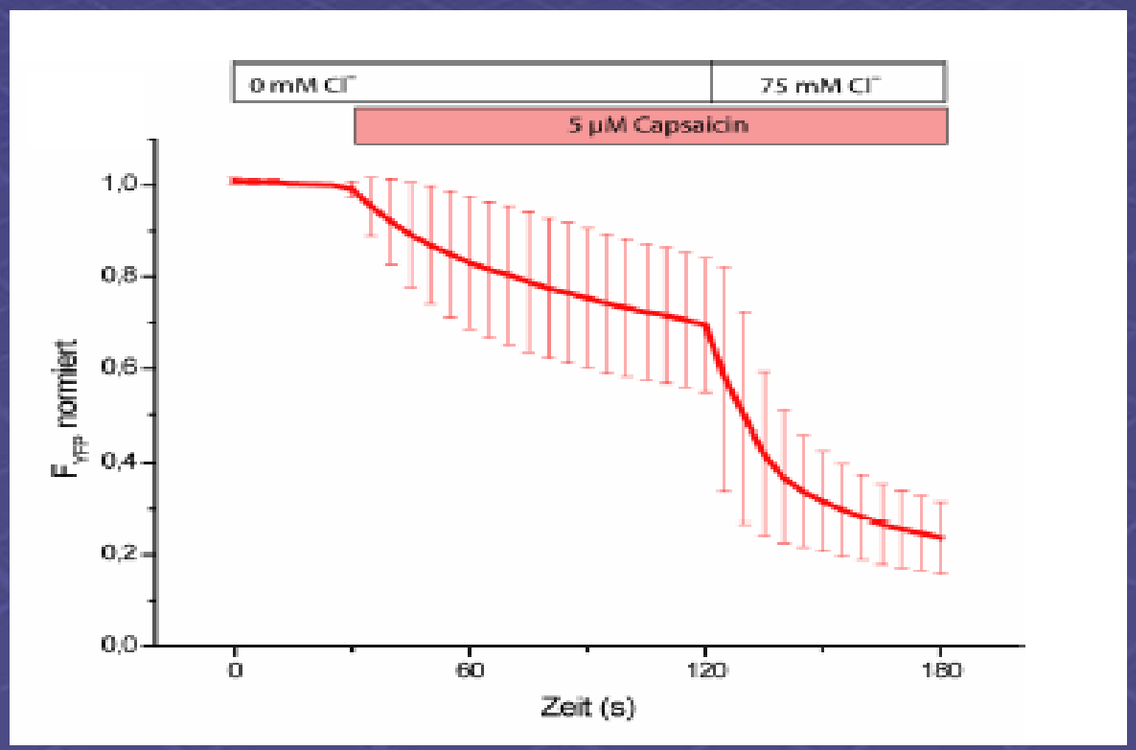


Automated microscopy…the beginning

Liquid handling robot





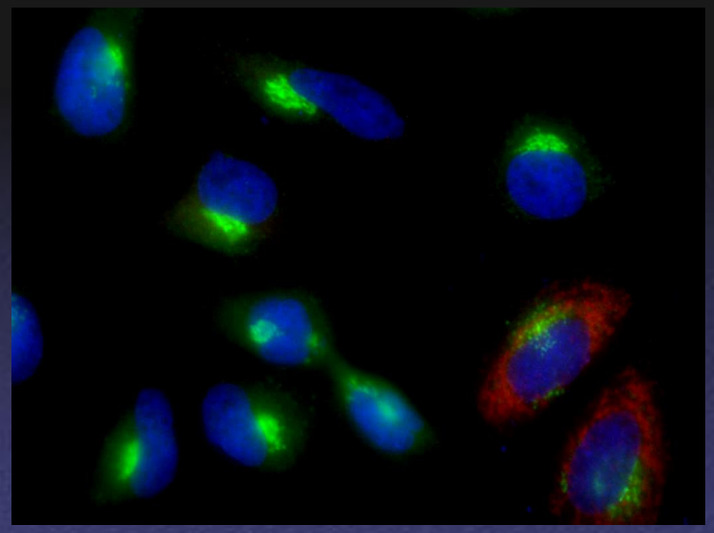


Semir Jeridi

(High Content Screening) microscopes

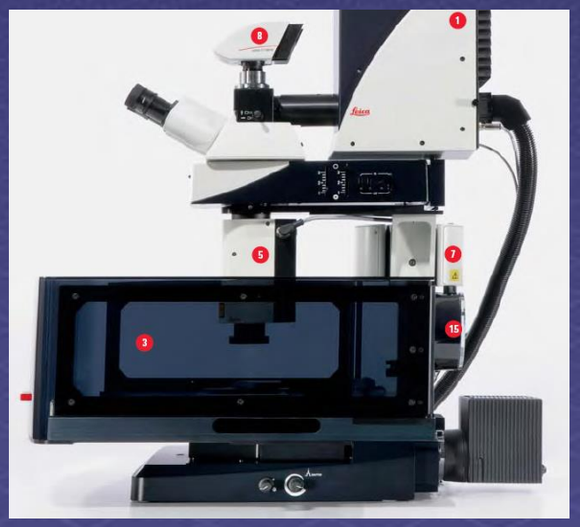
+++ throughput
- resolution
+ large specimen

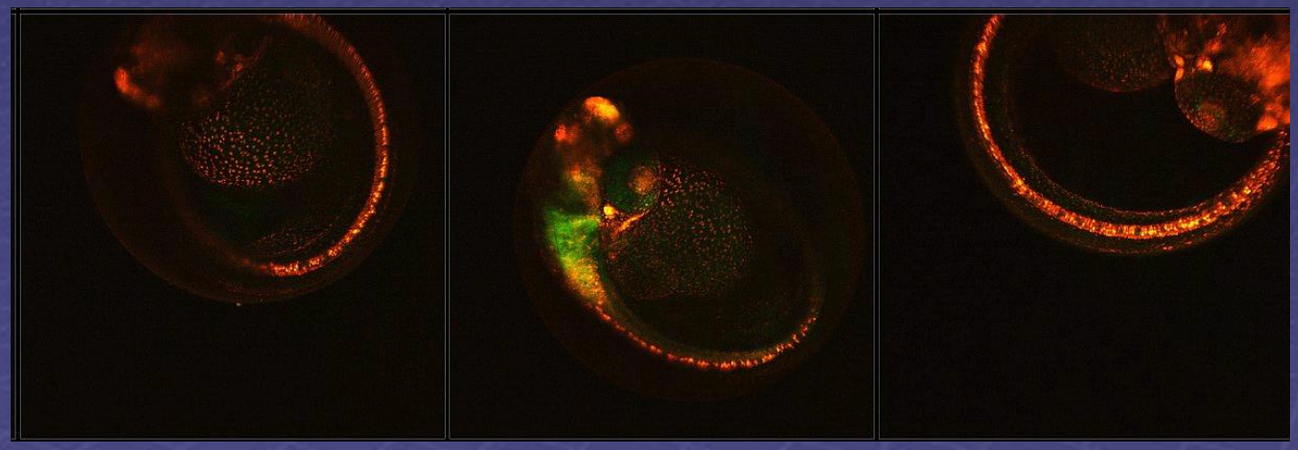
2004



+ throughput
+ - resolution
++ large specimen

2007

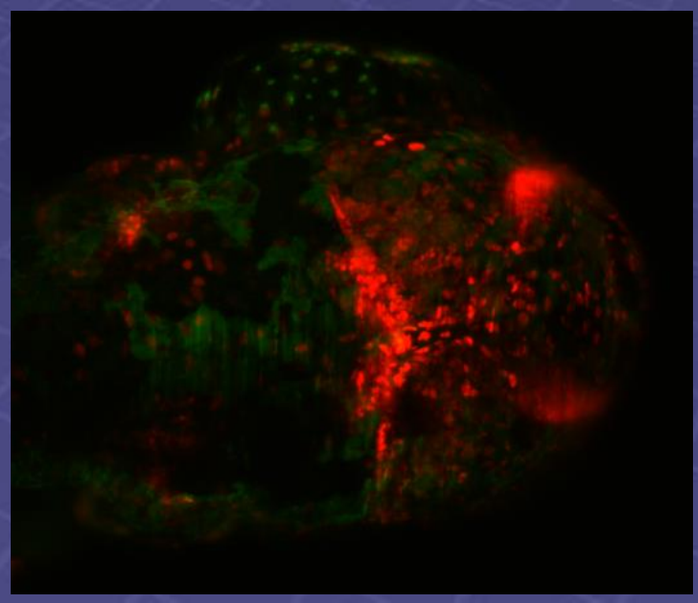




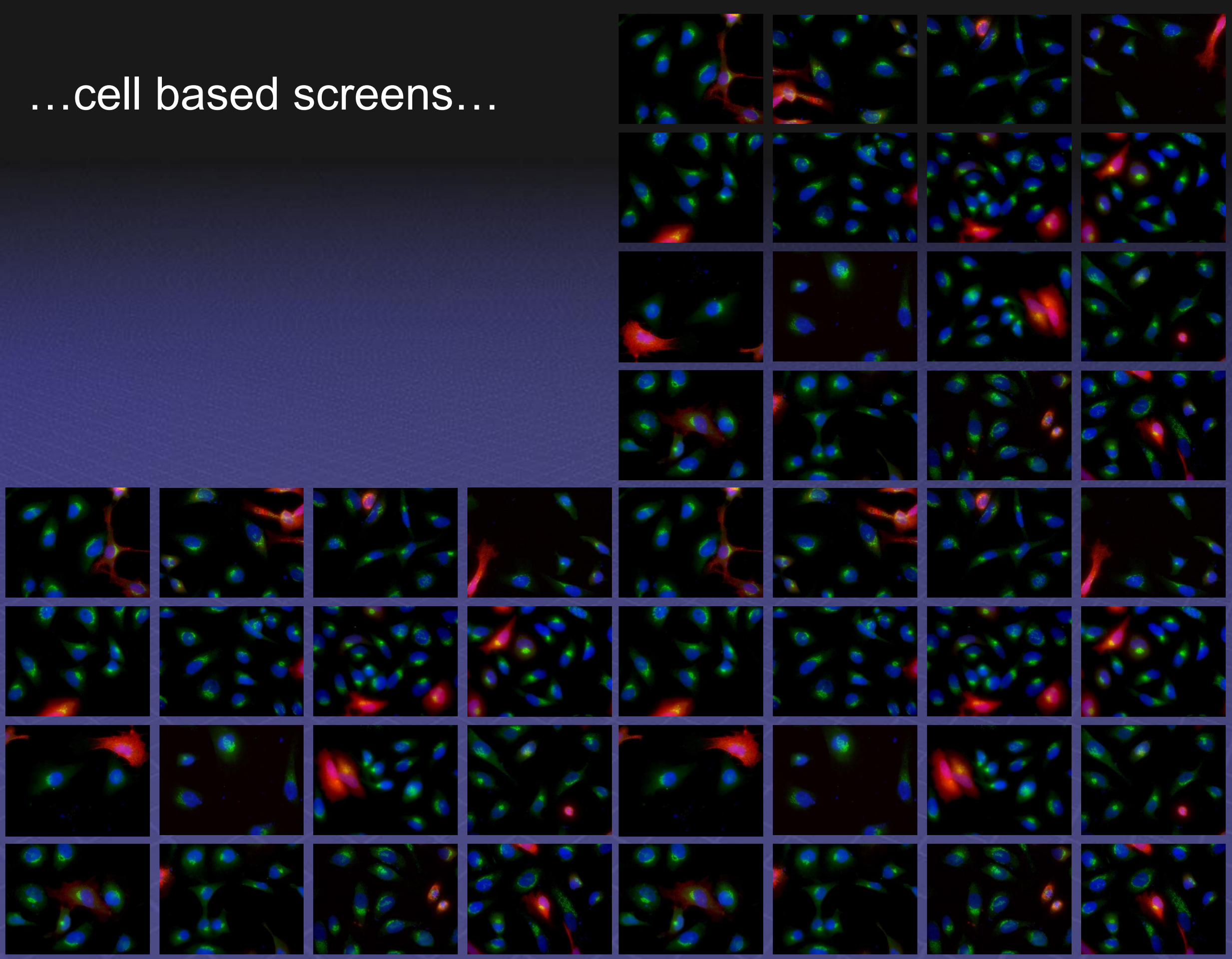
- - throughput
+ resolution
+ + large specimen

2009

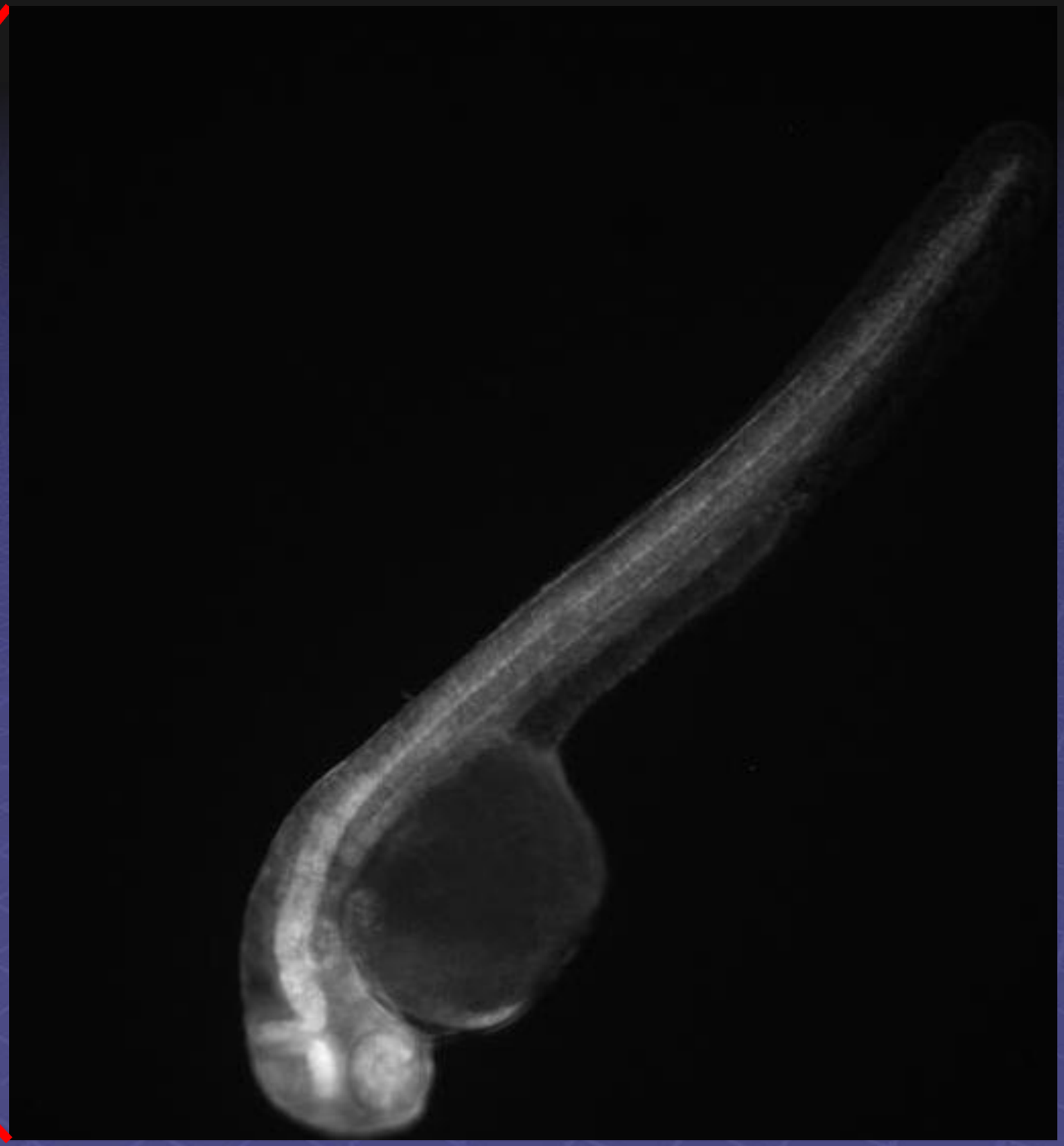
Light-sheet mic: Keller / Hufnagel



…cell based screens…

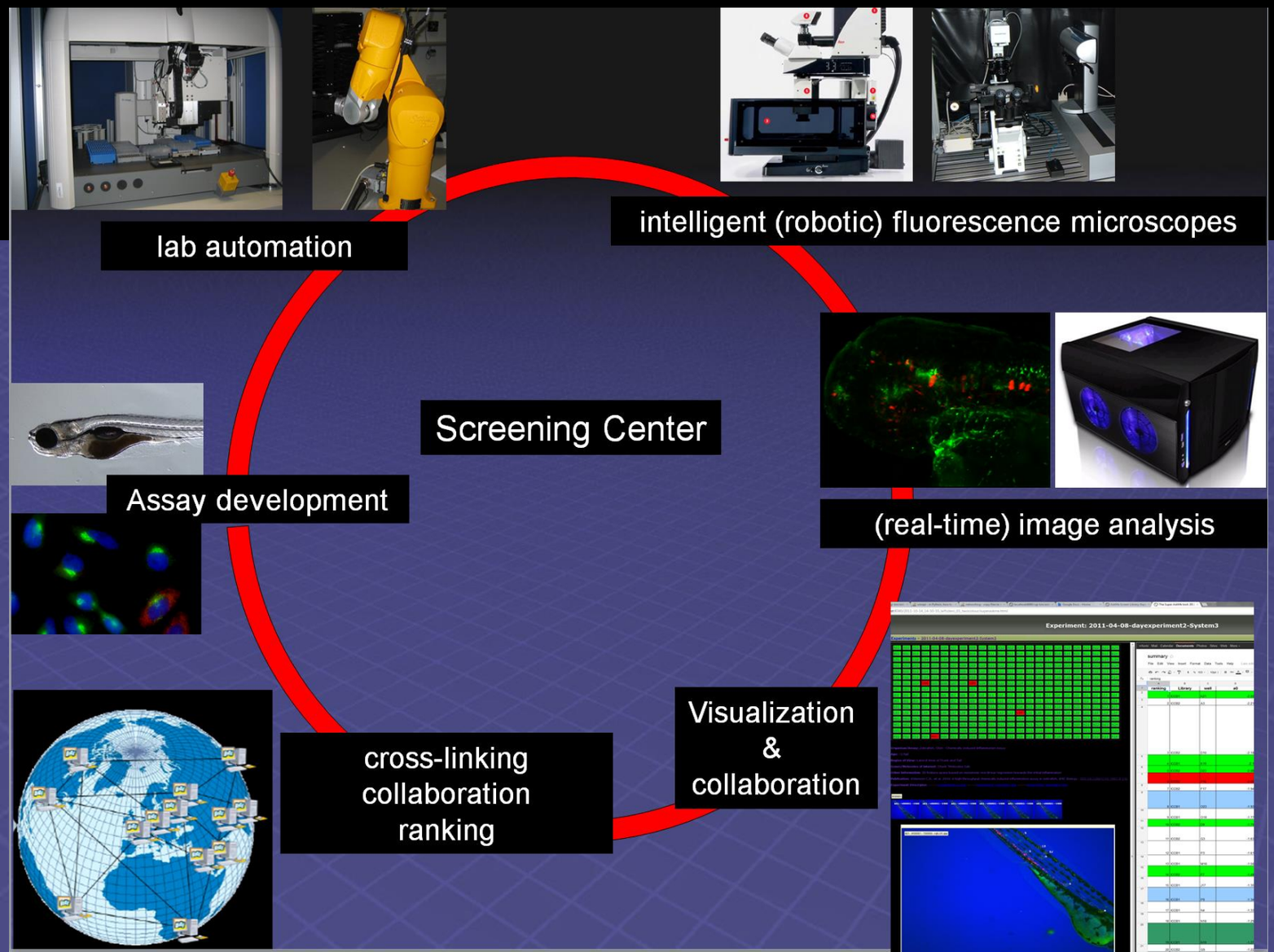


Zebrafish in 96 well plate …



Step 3.1

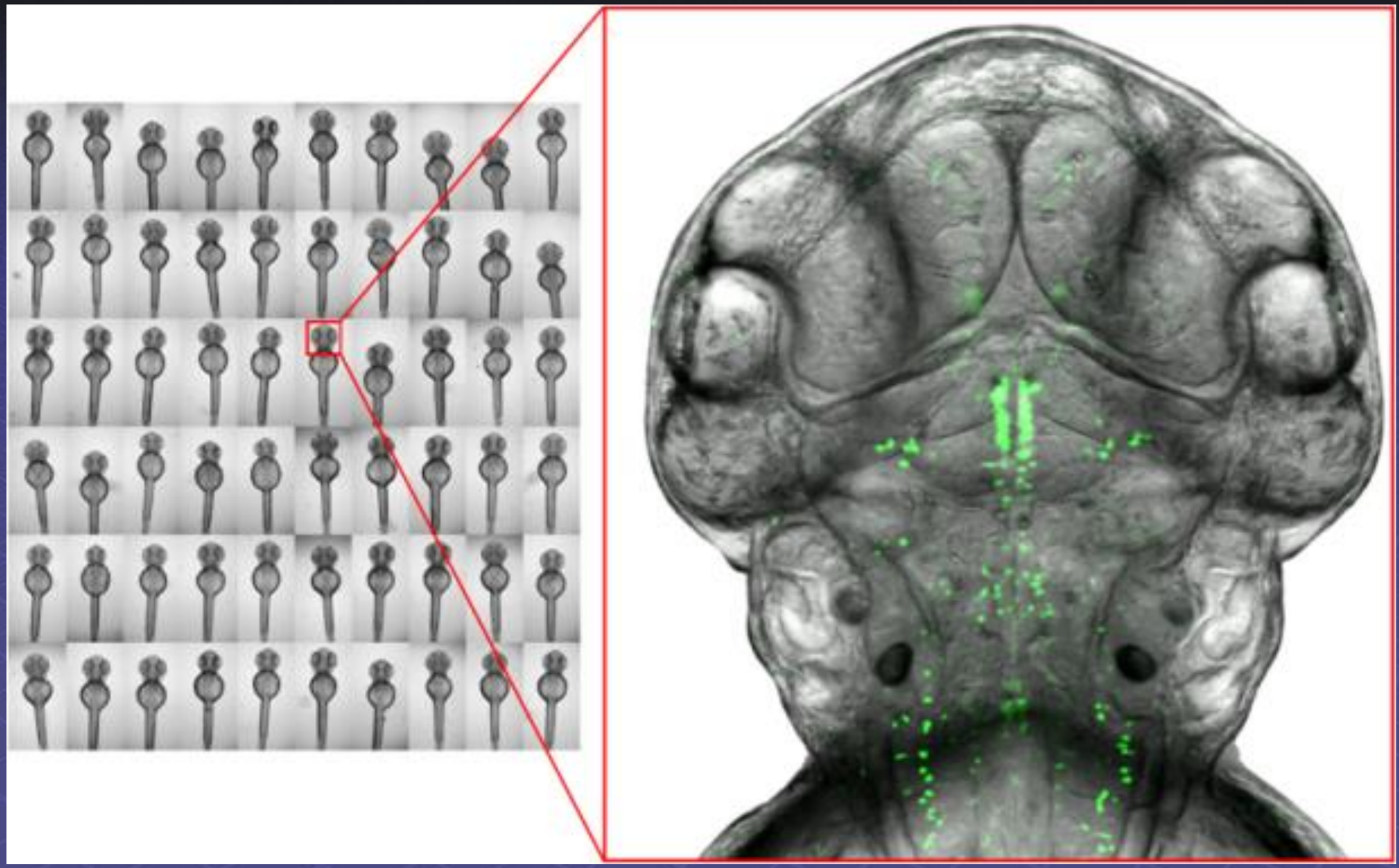
Smart imaging machines avoid bigData ...



Automated dorsal imaging of zebrafish embryonic brains

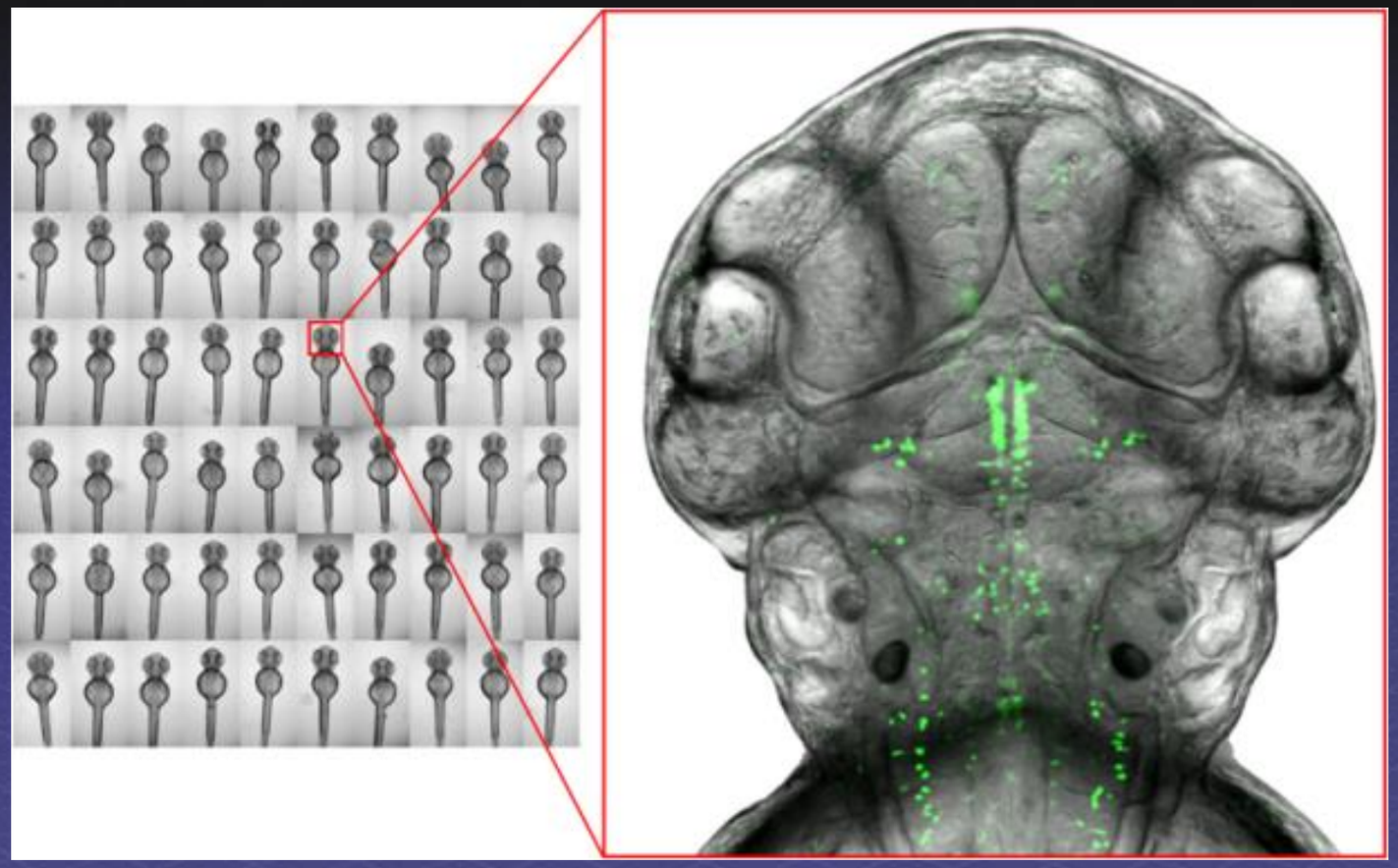
1. Pre-screen

2. High-res screen

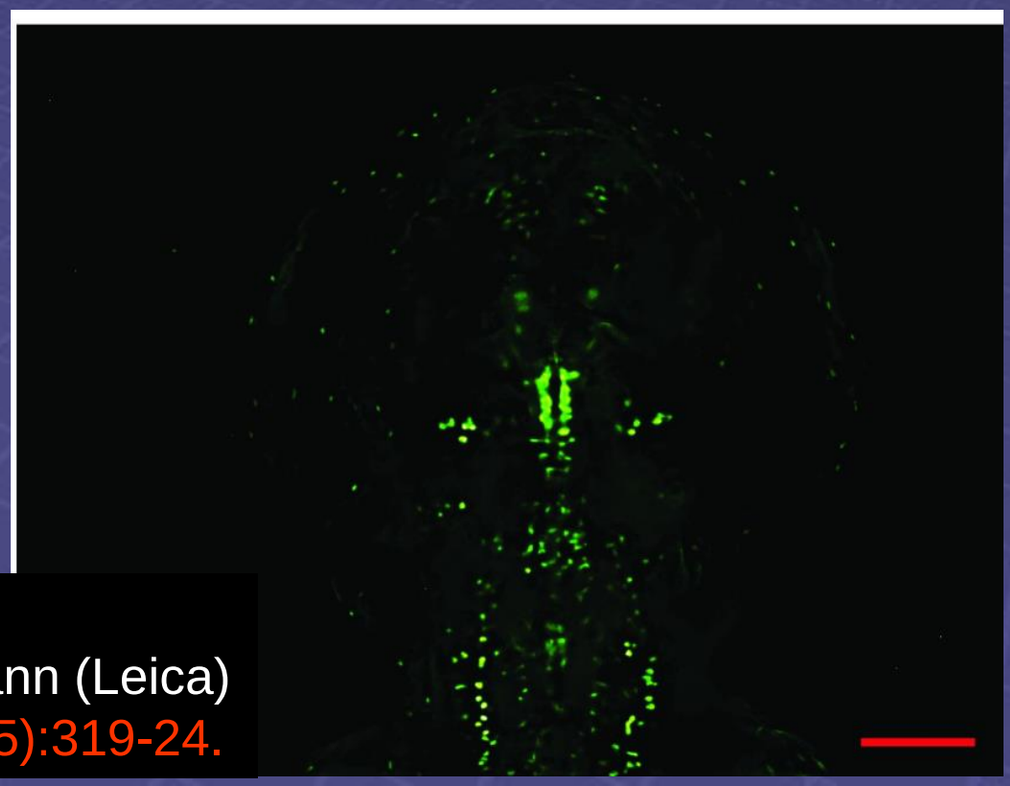


Smart dorsal imaging of zebrafish embryonic brains

1. Pre-screen



2. High-res screen

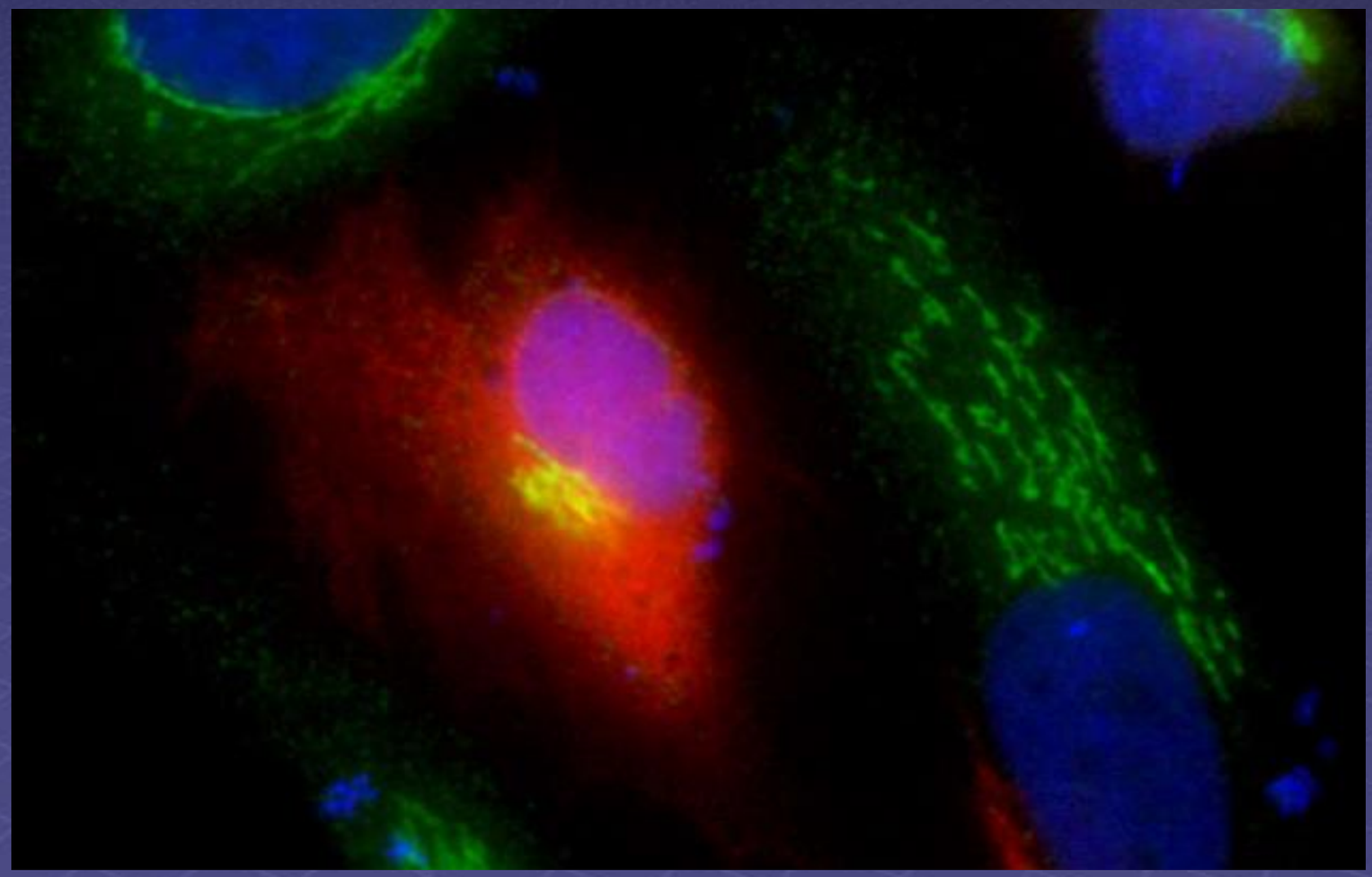


Widefield Scan^R microscope, *ETvmat2:gfp (Wolfgang Driever)*
Jochen Gehrig, Ravi Peravalli, Dominic Luetjohann, Frank Sieckmann (Leica)
Nat Methods. 2011 Mar;8(3):246-9 ; Biotechniques - 2011 May;50(5):319-24.

Smart imaging hardware tools

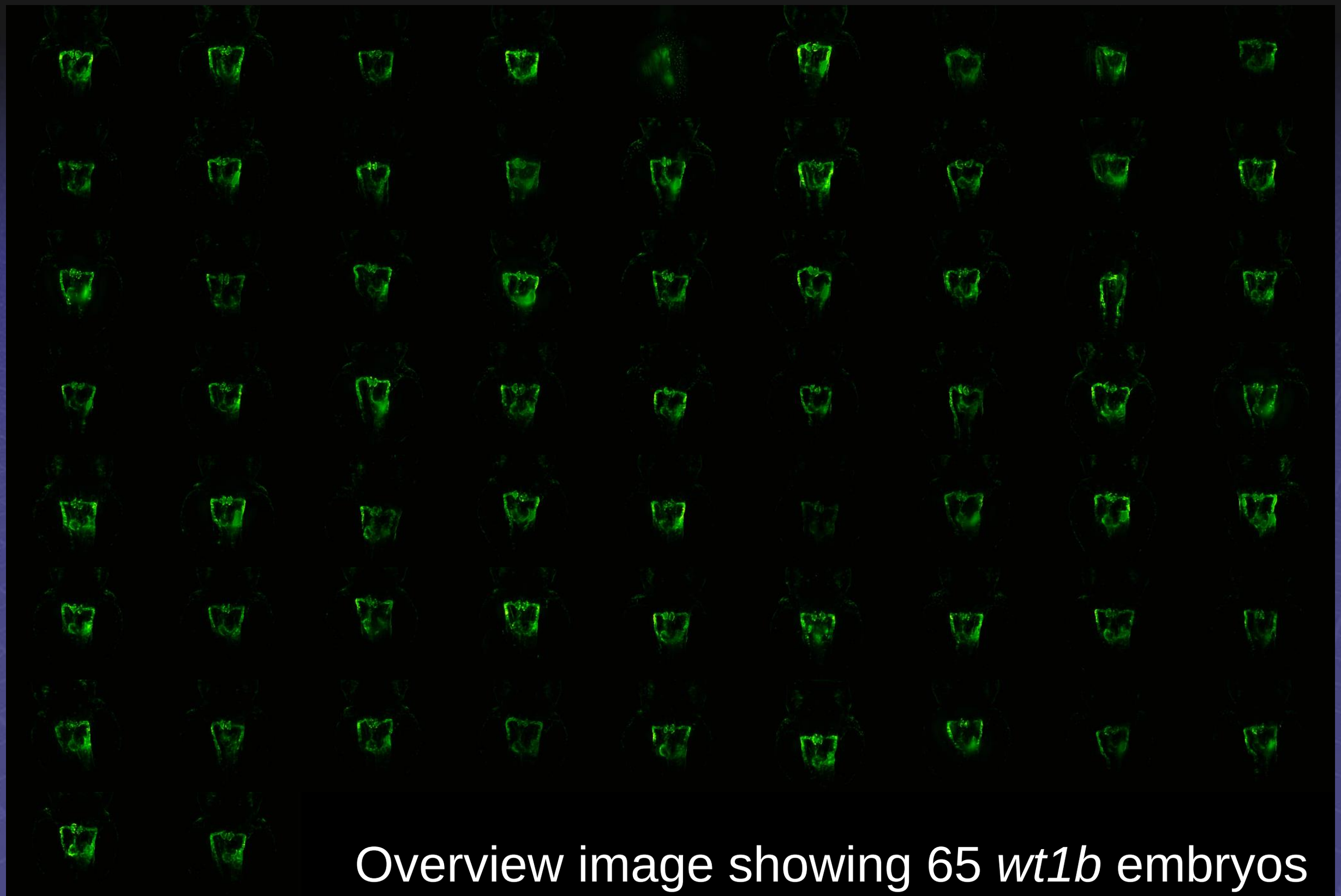
example: Automated immersion objectives

a) low resolution pre-scan
b) identify structures (via real-time image processing)
c) start high resolution experiment



2007 collaboration with Leica Microsystems Automated water objective

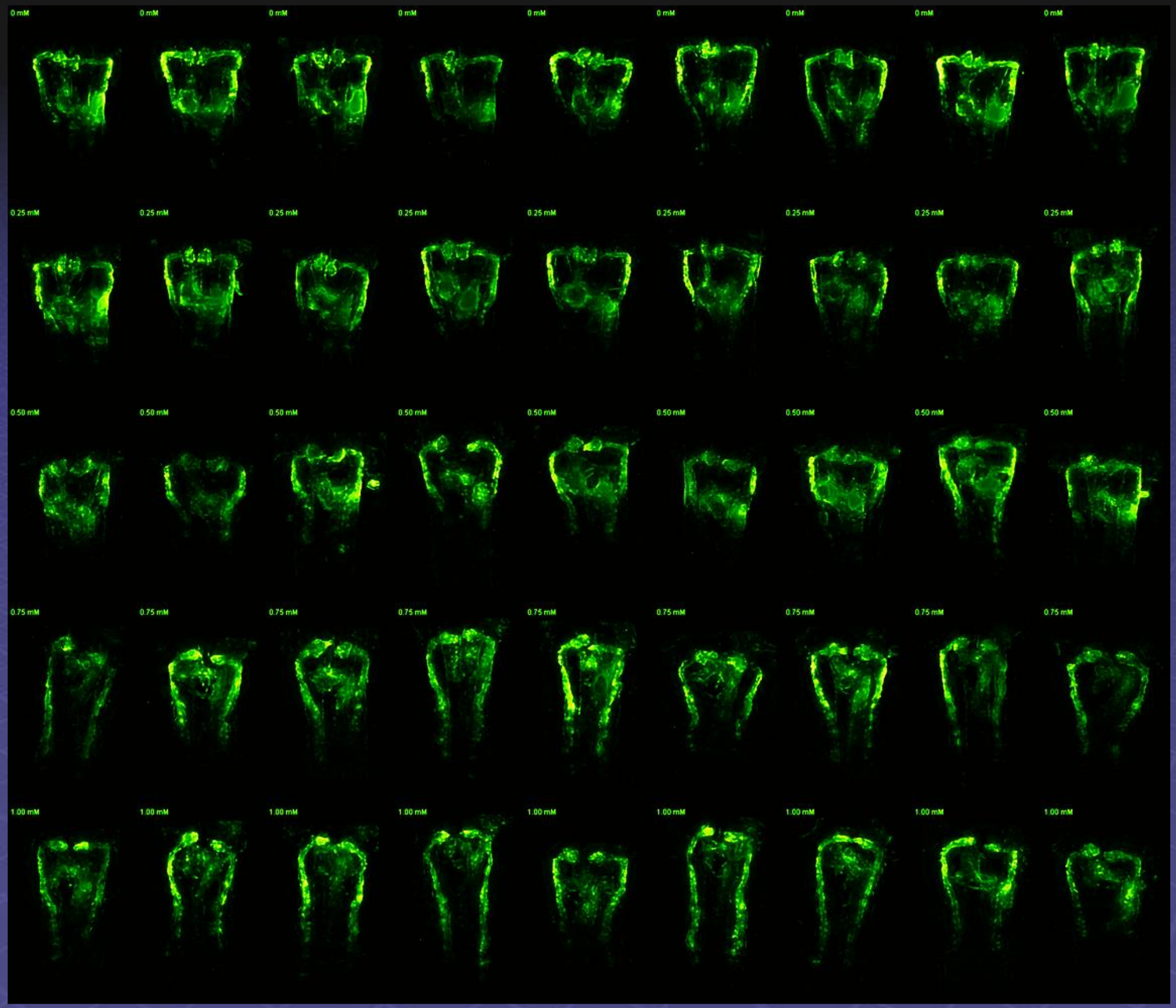
Automated imaging of kidneys of *wt1b* zebrafish embryos using intelligent automated microscopy



Overview image showing 65 *wt1b* embryos

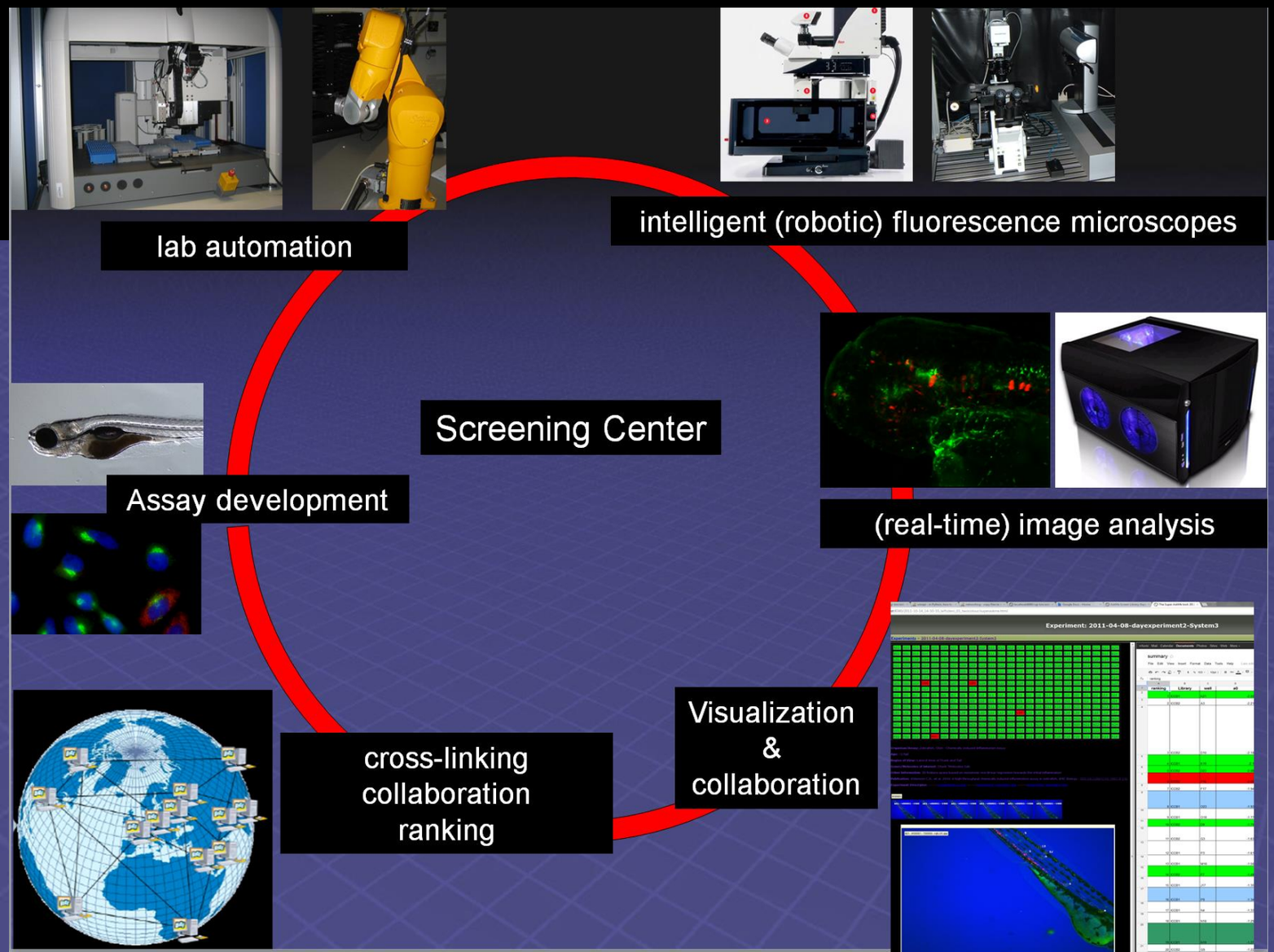
Evaluation of dose dependent toxicity of compounds

concentration



Step 3.2 ...next generation...

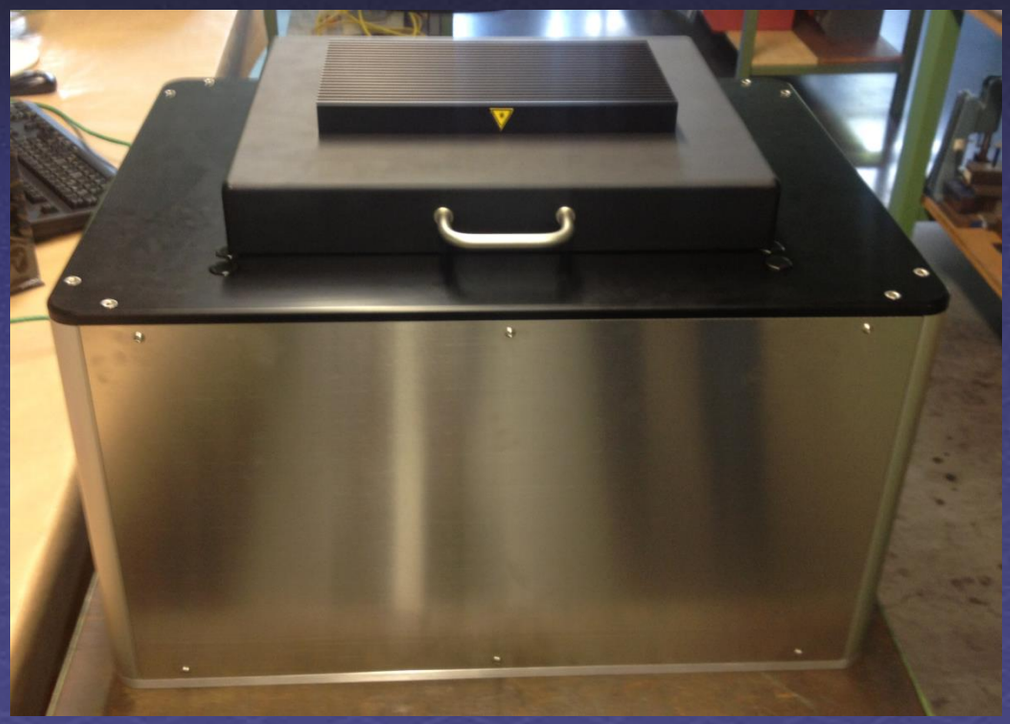
Machine grade ...microscopes



Modern (High Content Screening) machines / microscopes

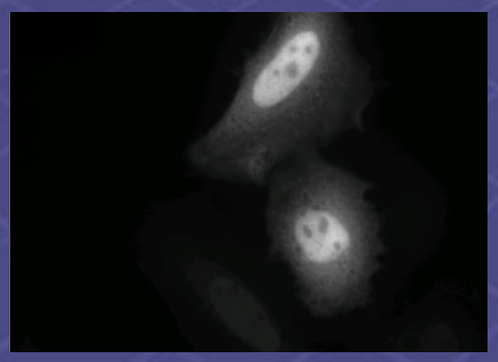
+++++ throughput
++ resolution
++ large specimen

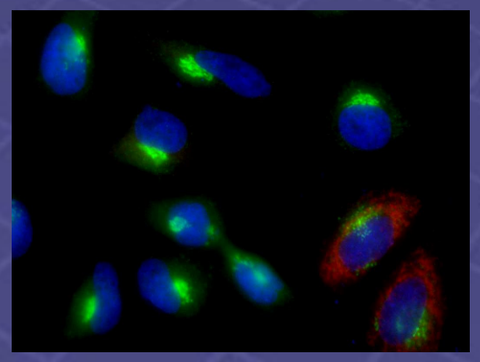
2012

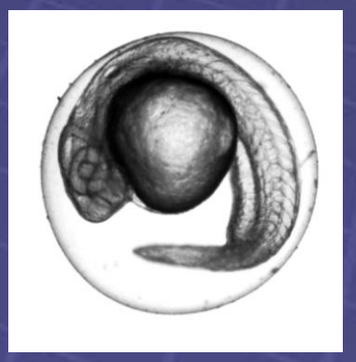


+

Imaging machine + Realtime data (pre-)processing







„machine grade imaging devices“

Examples:

→ sCMOS cameras: 1-100 frames/second → ~ 4-800 MByte/sec

→ Multi camera systems

→ stable light sources (HIGH power LEDs)
→ allow data integration from several machines

→ Industrial grade mechanics (linear motors, position encoders etc

→ Realtime controlled devices (µsec domain)
→ Speed (obviously)
→ Experiment reproducability (e.g. RT controlled camera trigger)

...more & more labs adapt to machine grade imaging devices
→ data rates increases dramatically

Step 4

Understand !!! photons & bytes

high end detector or (maybe) better image pre-processing?!

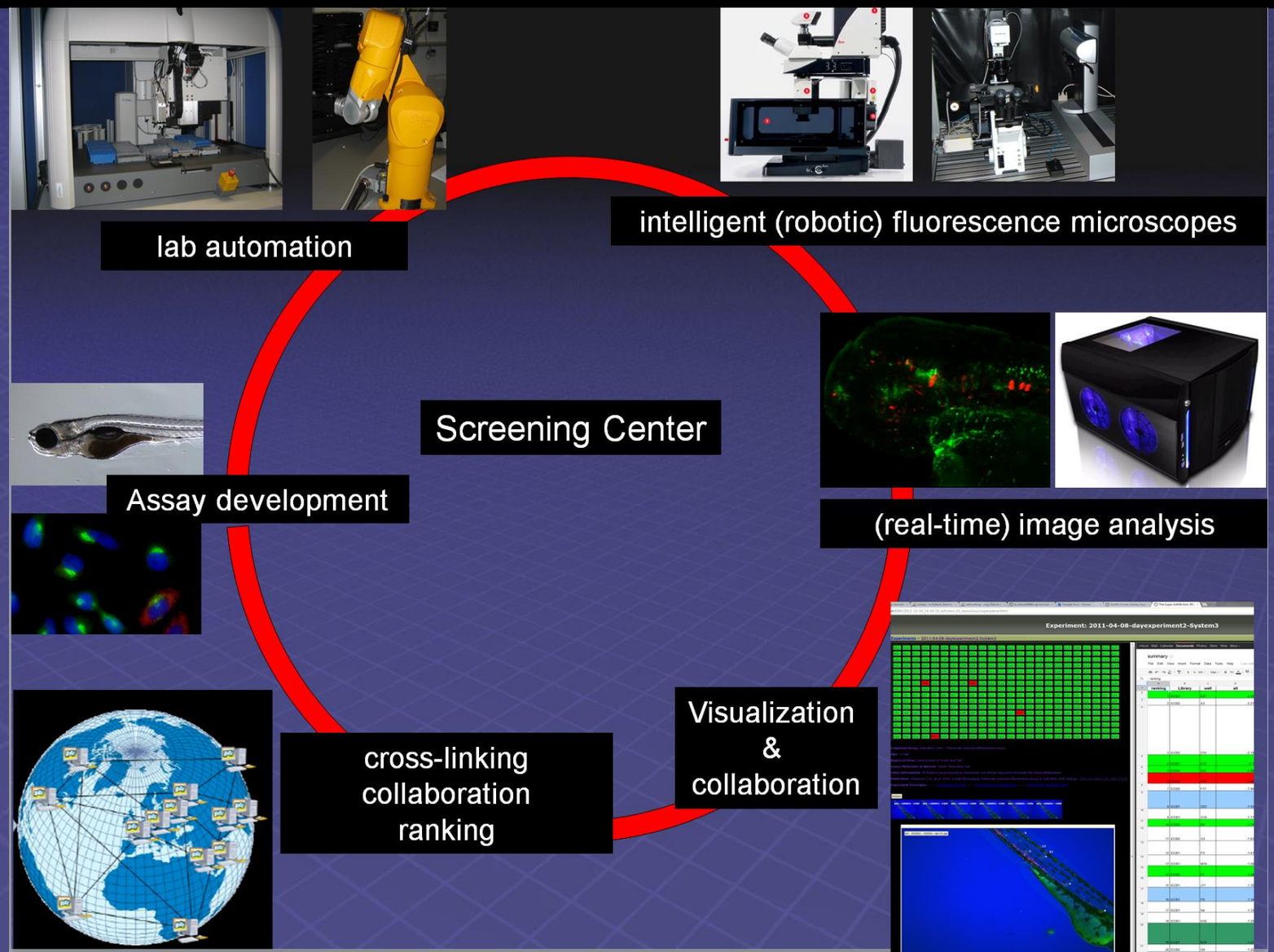
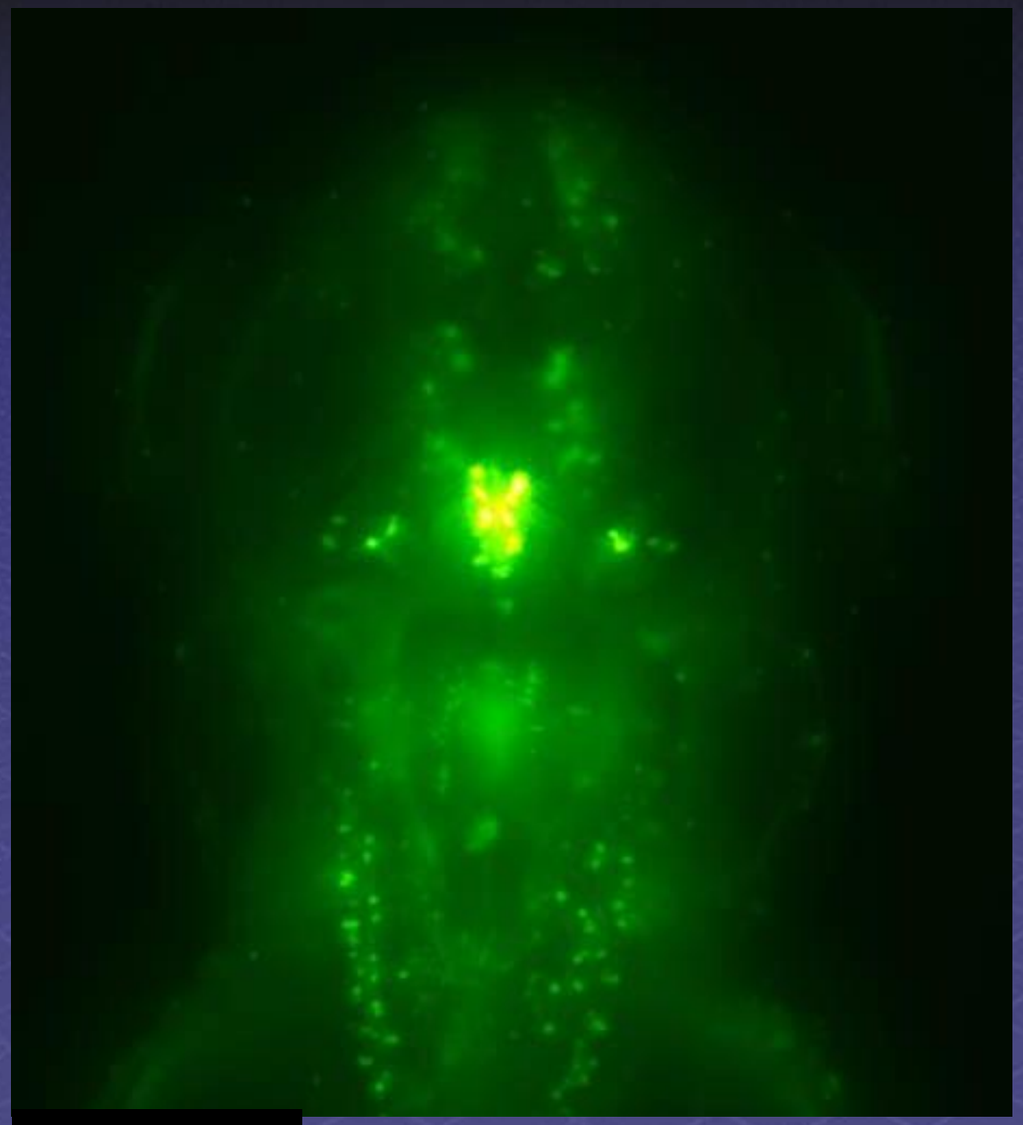
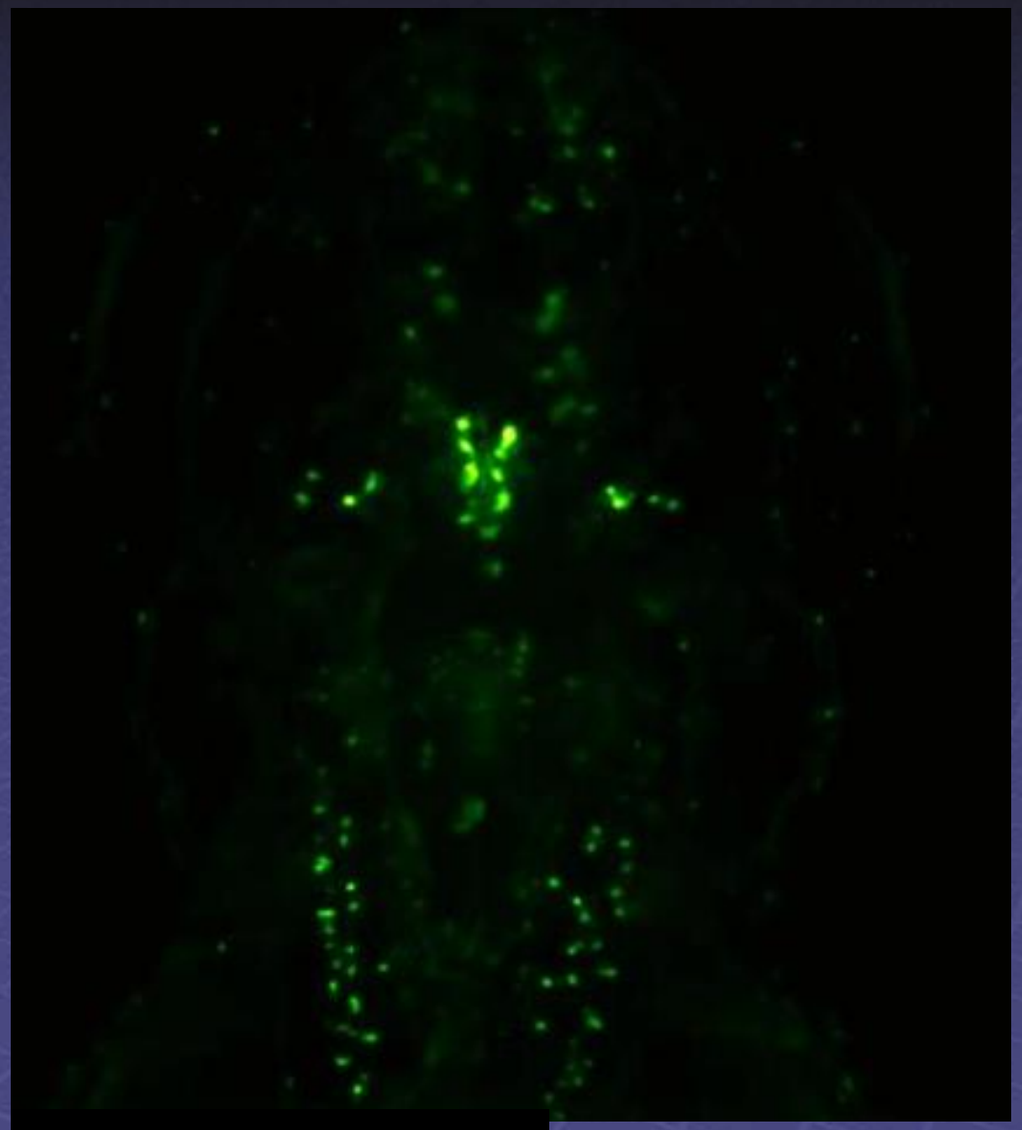


Image restoration of widefield z-stacks using deconvolution: Deblurring



raw data

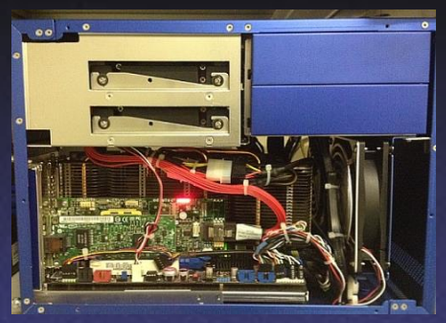


deconvolved data

ETvmat2:gfp, 48hpf, dorsal view, maximum projection - 60 z-slices - 5 µm distance
800x600 µm field of view, Jochen Gehrig

...(our) history of high speed image processing

+



2007 : pure SSD (raid)
+ fast ~1TByte buffer - storage

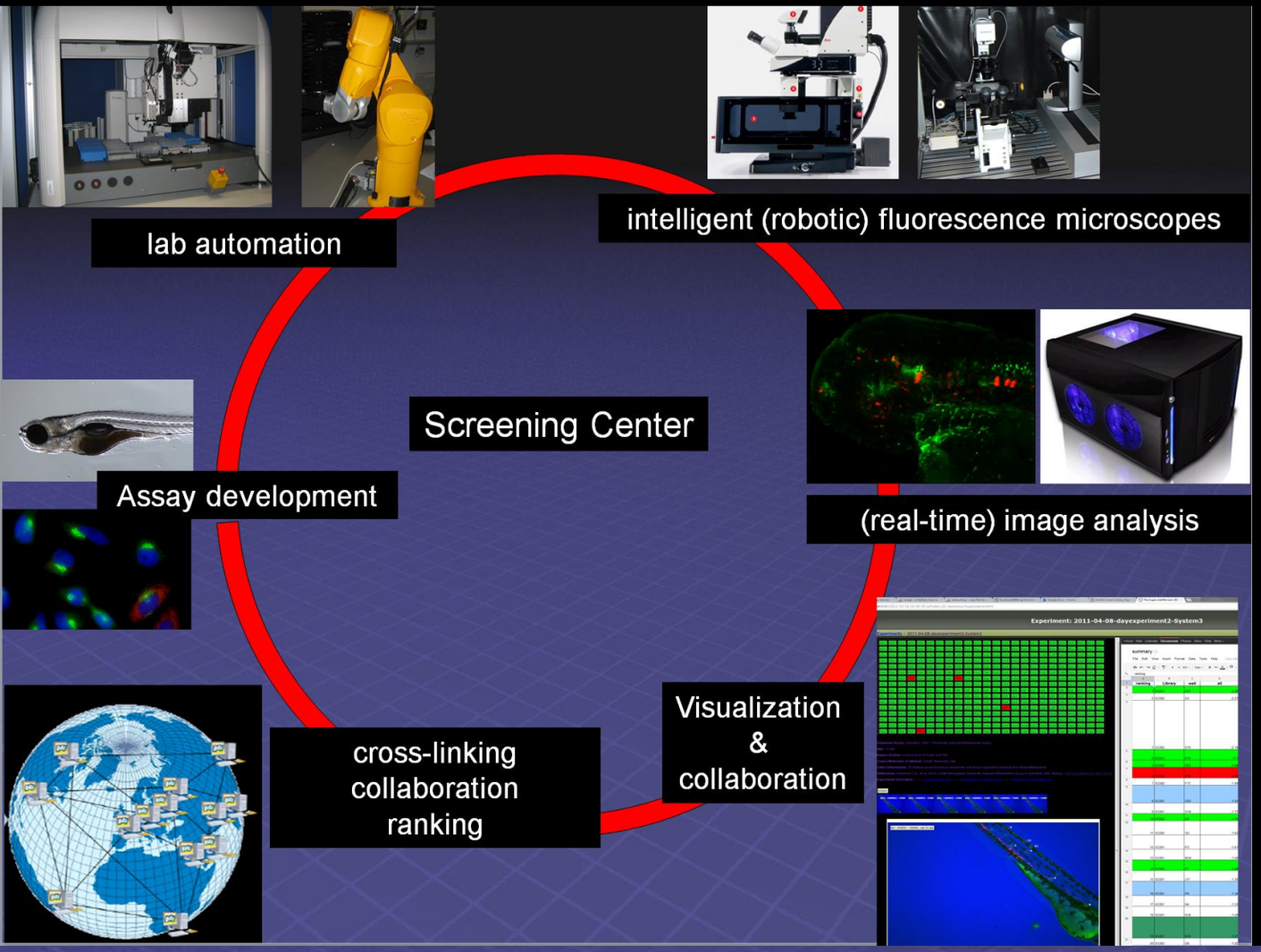
2009 : pure disk RAID: up to 13 disks / enclosure
~ 400-700 MByte/sec – data stream programming!!!

2011: mixed architecture:
SSD „cache“ (PCIe SSD card!) + disk RAID – storage

→ Balance image processing software / hardware

Step 3.3
..what we need is not so much...

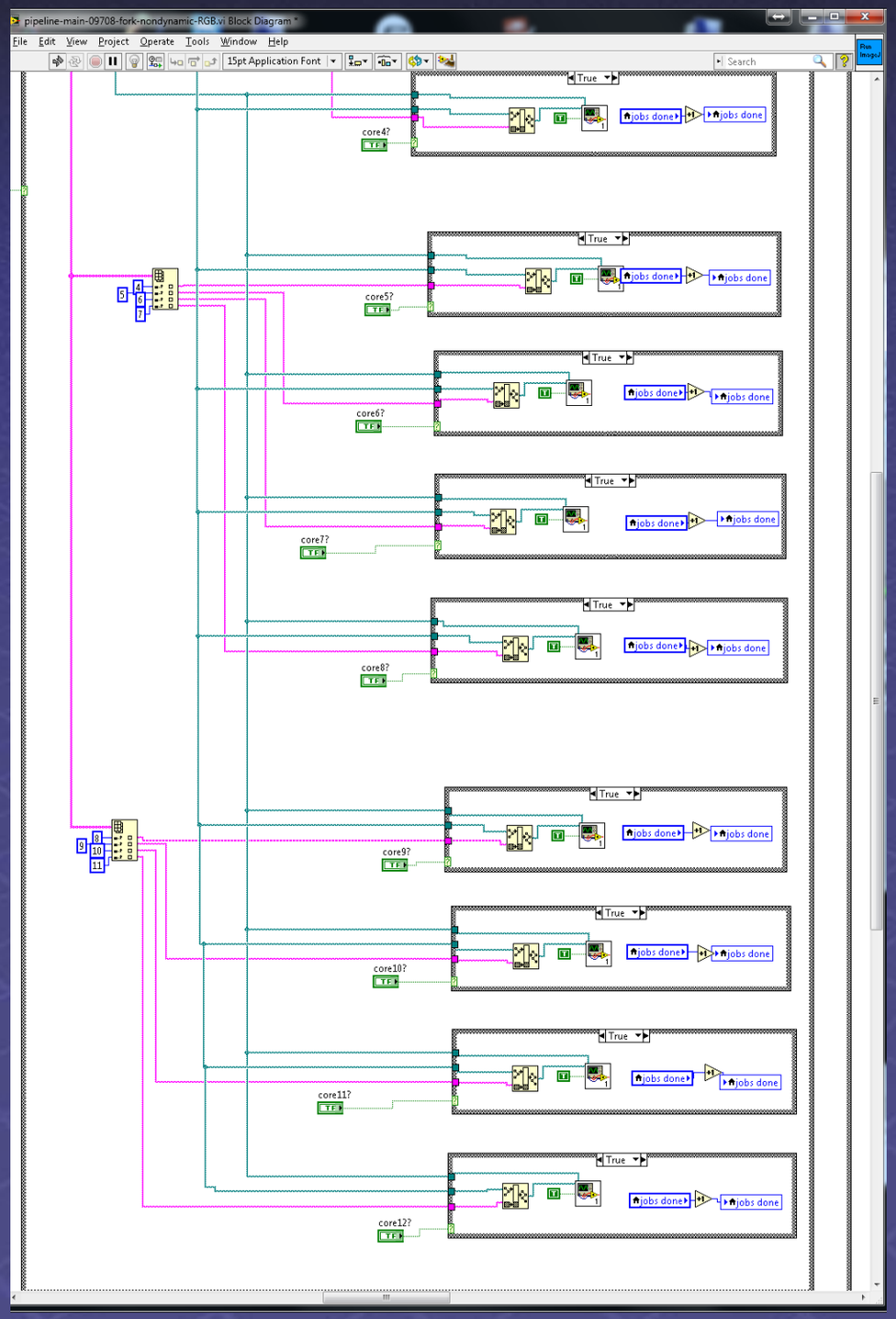
…faster CPUs...more cores...
faster RAIDs...
more bandwith...no latency...high gfx
...efficient cooling...less energy..
easy maintenance...apps...backup
(in a small form factor w/o noise ☺)



The „Future“ …

→Parallel detectors → > Bytes
→Higher resolution → > Bytes
→Faster Devices → > Bytes
→Multiple sites & Collaborations → > Bytes

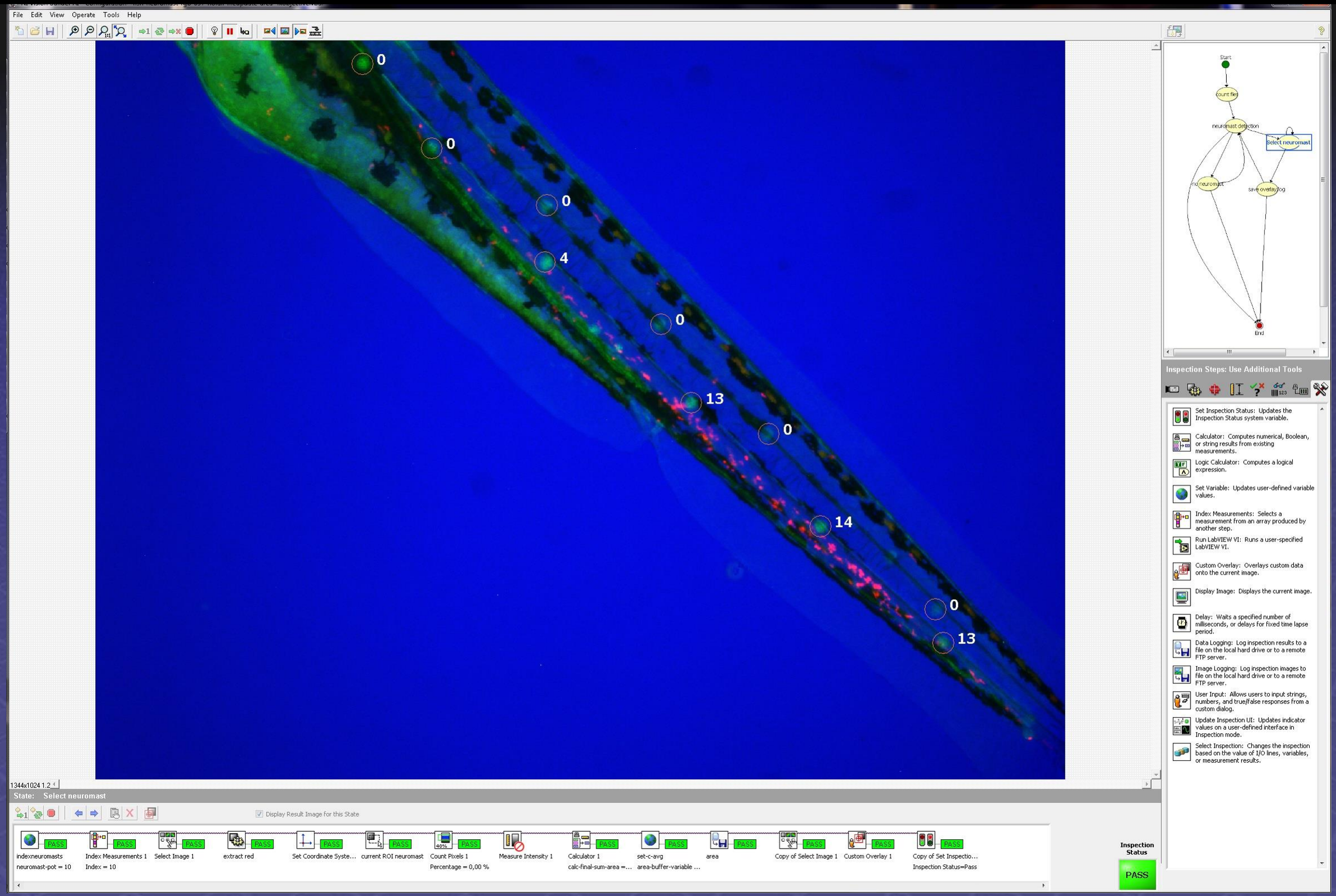
...rapid prototyping – example load balancing of open source image processing software



Snapshot of 12core load balancing – LabView / Fiji

…Rapid prototyping example : inflammation

Vision

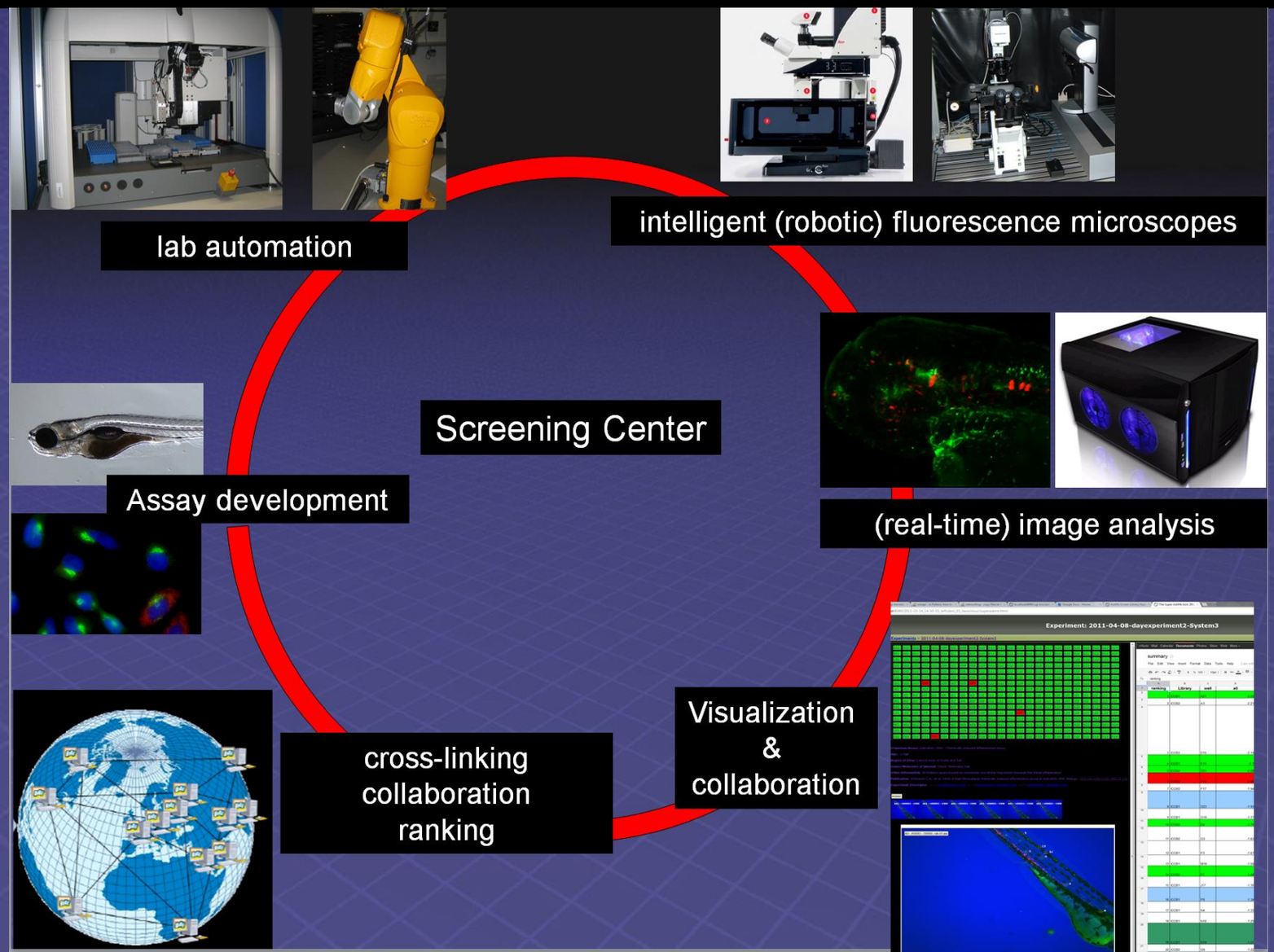


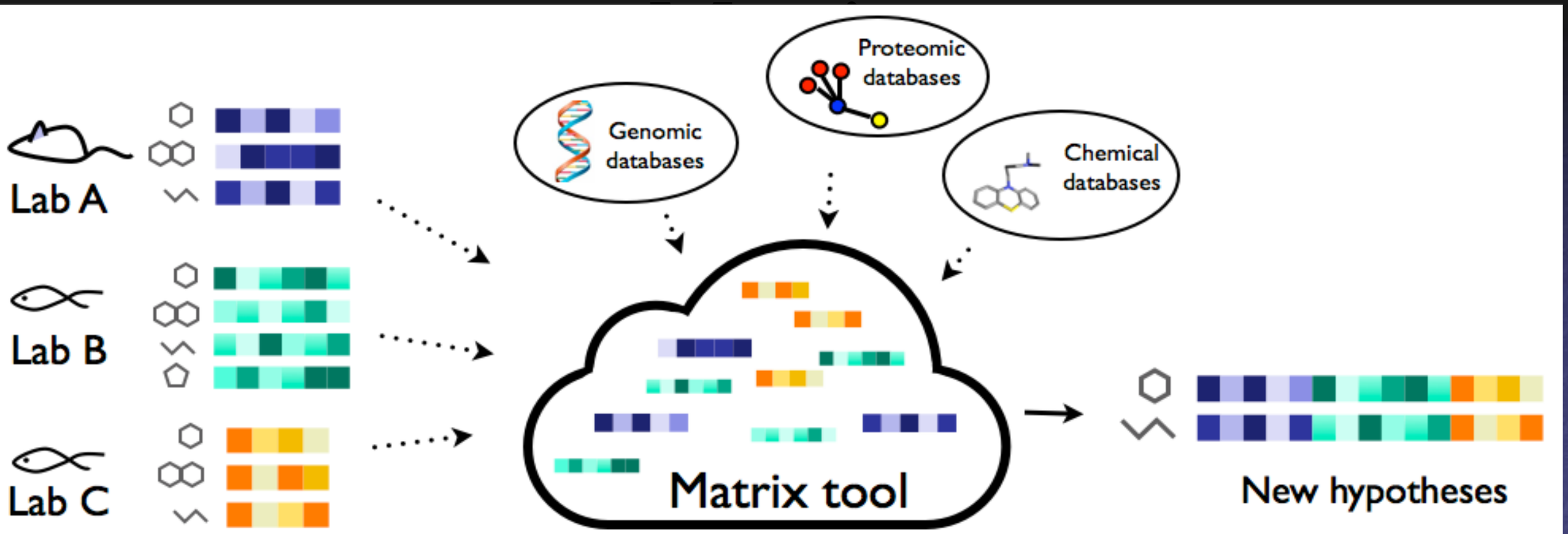
Clemens Grabher, Christine Wittmann - BMC Biol. 2010 Dec 22;8:151.

Step 5

Share & integrate

search & find...would we complex enough





- Integrate analyzed results from several assays/projects
- Interactive data filtering and faceted navigation
- Structured visualization and presentation of the data
- Serendipity exploration

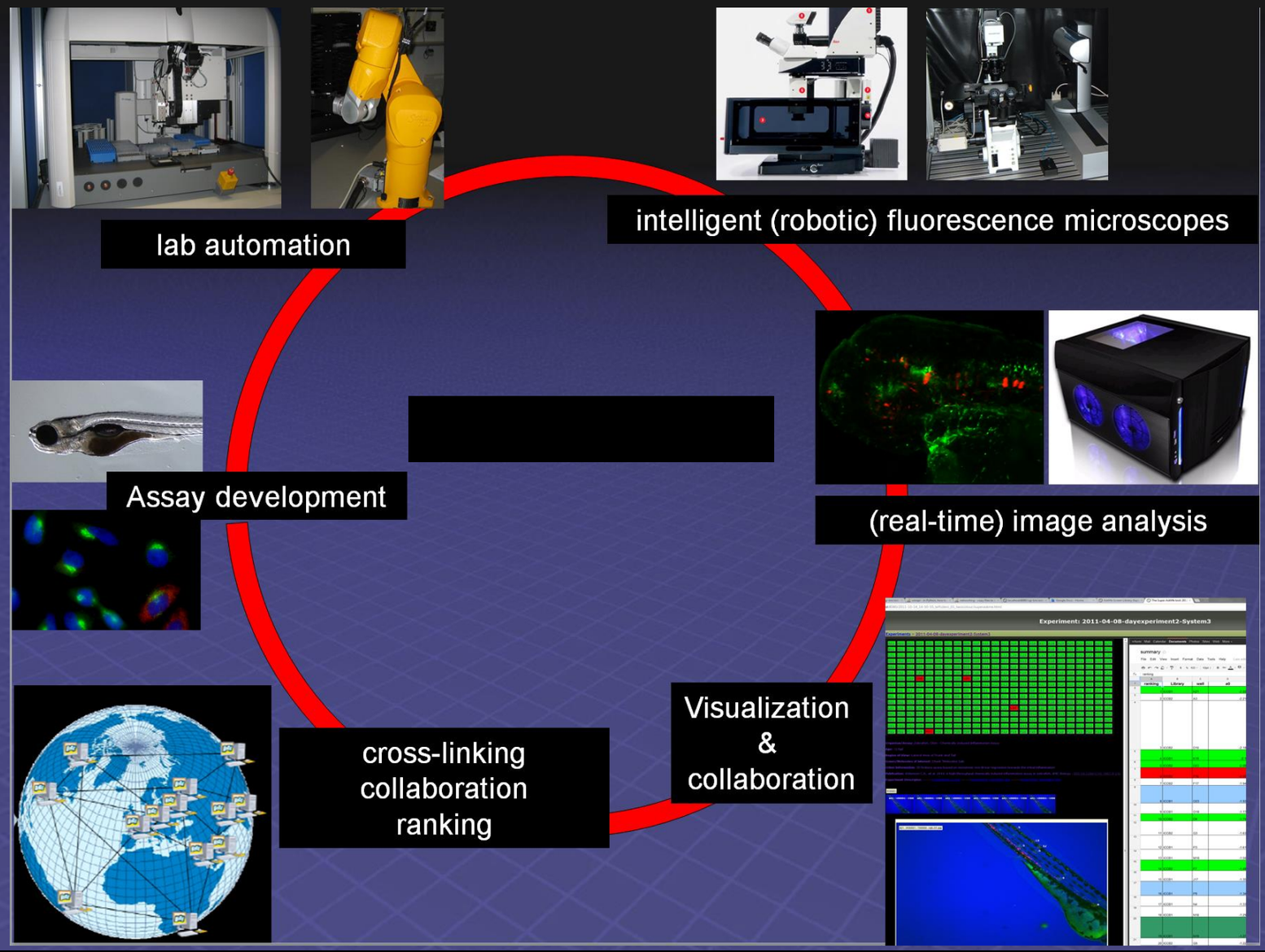
Kokel D., Rennekamp A., Shah A.H., Liebel U. & Peterson R.T. (2012) Trends in Biotechnology, in press.

~ 1230 (bioinformatic) databases

some DBs require IPI Ids,
some UniGene, Protein Ids,
Some sequence data,

some genes have 60 Synonyms,
Many of the existing Dbs have a unique viewing mode

next generation high content screening platforms -
ideas → photons → bytes → ~~answers~~ → questions → ideas



Karlsruhe Institute of Technology

EMBL

ACQUIFER let's see

urban.liebel@kit.edu - u.liebel@acquifer.de