SCHERING
making medicine work

Live cell imaging in Target Validation and Lead Optimization

S. Prechtl

AD-HTS / High-Content Analysis

Assays and Cellular Targets (ACT) 2006
October 30 - November 1, 2006 - Green Valley Ranch Resorts - Las Vegas, NV

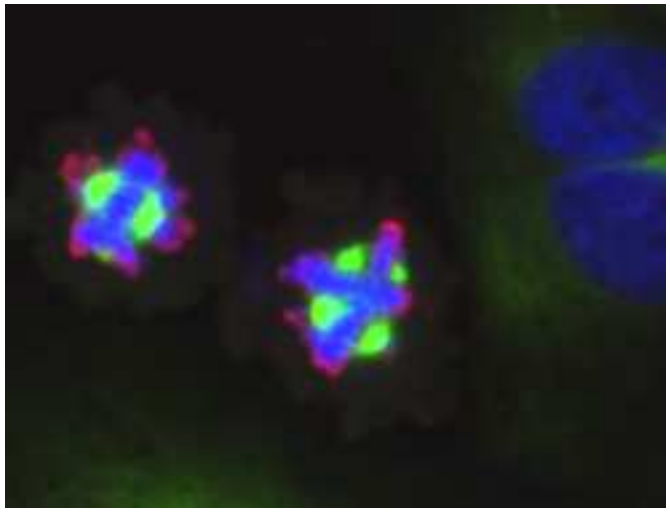
cell systems in modern drug research

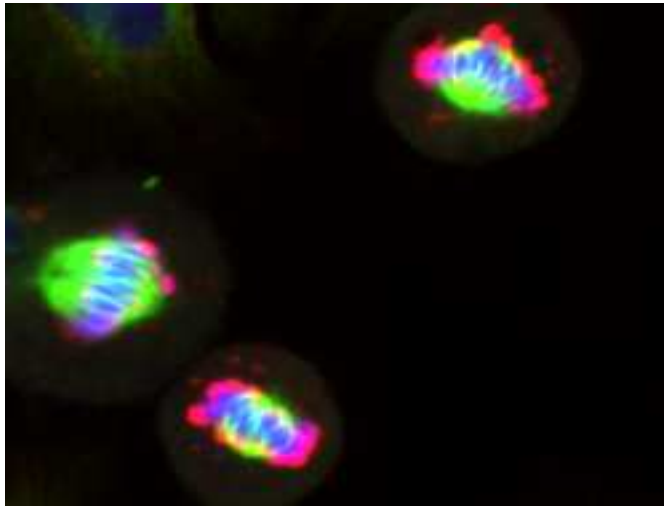
- highly recommended scientific tools
- examination of patho-physiological mechanism
- investigation of compound interactions

⇨ within the cellular context

High-Content Analysis

- automated high-resolution imaging & image analysis
  - ⇨ exploits the detailed insight into cell system
- analysis of structural and metabolic changes
  - ⇨ functional high–quality information about targets and potential drug candidates
- quantification of complex drug actions at the level of the individual cell
  - ⇨ providing statistically significant results of greater reliability





HCA assays are applicable throughout nearly all stages of the drug discovery process

- target validation, lead discovery, lead optimization, secondary screening, *in vitro* toxicology studies, preclinical studies

HCA assays have the potential to accelerate drug discovery process by opening current bottlenecks

- performing higher-throughput cell-based assays providing high-quality data
- time-saving multiplexed / multilinear designed assays
- identifying genes or proteins involved in diseases
- qualifying drugability and therapeutic benefits of selected compounds
- identifying possible side-effects within cellular context

equipment consists of

fully automated confocal HT imaging system, MT epi-fluorescence imager, motorized fluorescence microscope

OPERA
Evotec Technologies

Discovery 1
Molecular Devices

Axiovert 200
ZEISS

application panel

multiplex network

cell viability	proliferation apoptosis
cell cycle	DNA content mitotic index classification (G1 / S / G2 / M)
cell differentiation	monopole spindle formation tube formation cell migration micro nuclei (in progress)
enzyme activity	protein-protein interaction protein translocation
GPCR activation	β-arrestin, ET_A-R (evaluated)
time lapse studies	phenotype characterisation dynamic analysis (project started)

cell systems

HeLa
CHO
DU145
PC3
LNCap
MCF7
T47D
BT474
SK-OV-3
U-2 OS
HUVEC
MVEC
PAEC
S49
Jurkat
prim. MΦ

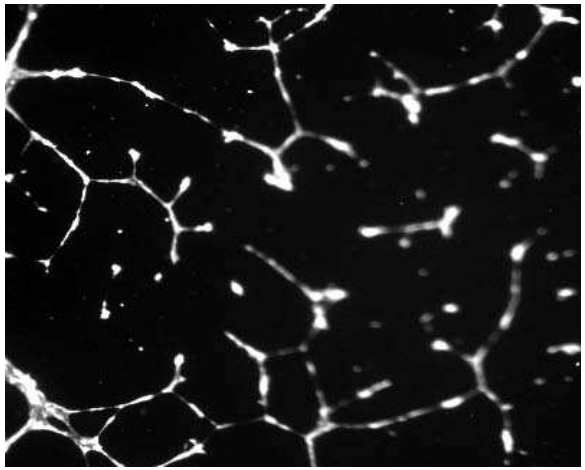
tube formation

– cellular model to quantify the differentiation of endothelial cells into capillary-like structures -> compounds modulating angiogenesis

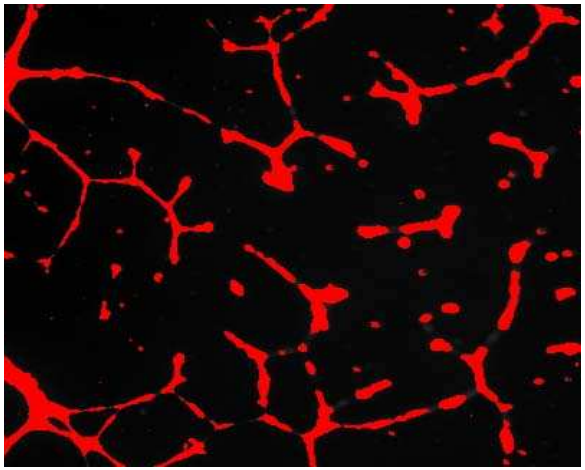
=> automated *in vitro* tube formation assay enabling the screening of potential angiogenesis stimulators and inhibitors

– quantification of the extent of tube formation by measuring the total tube length

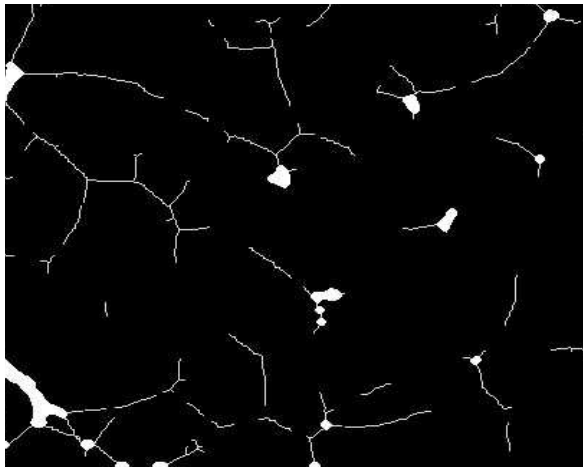
tube image



thresholding

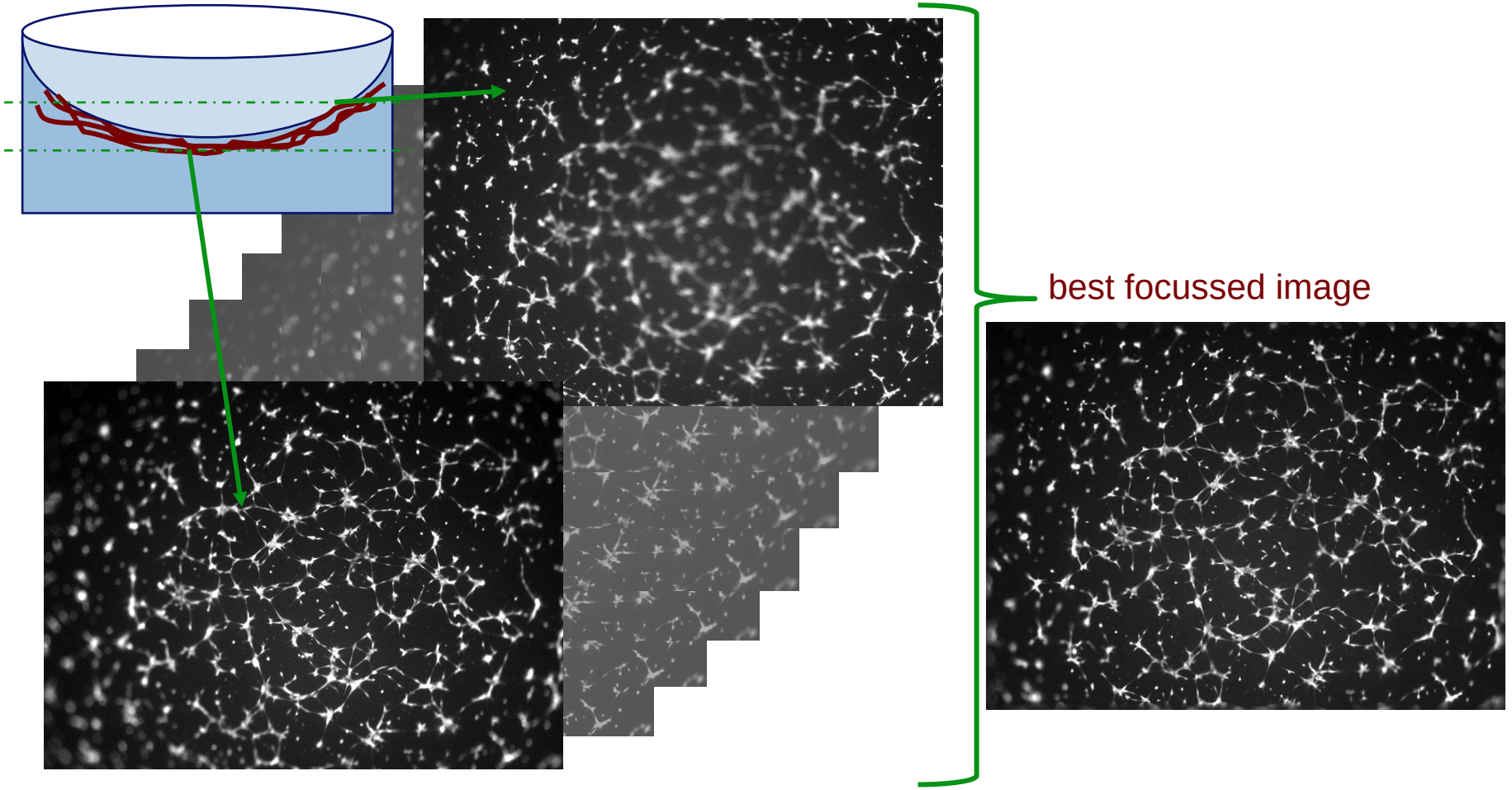


analysis image



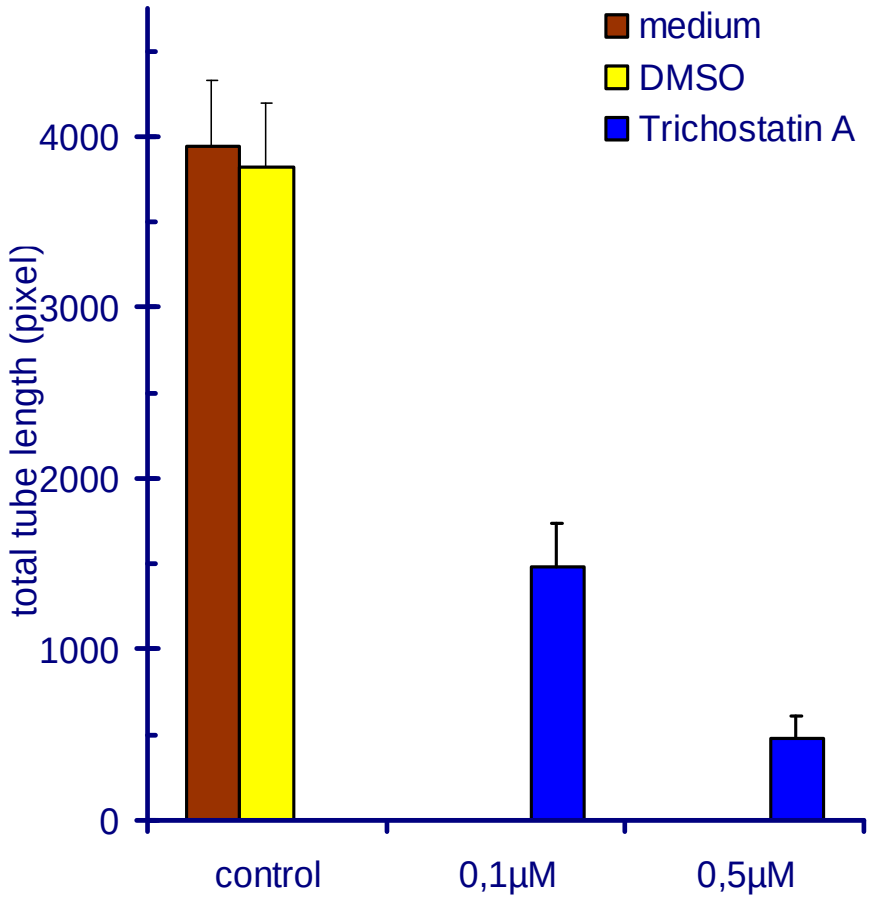
“best focus” algorithm

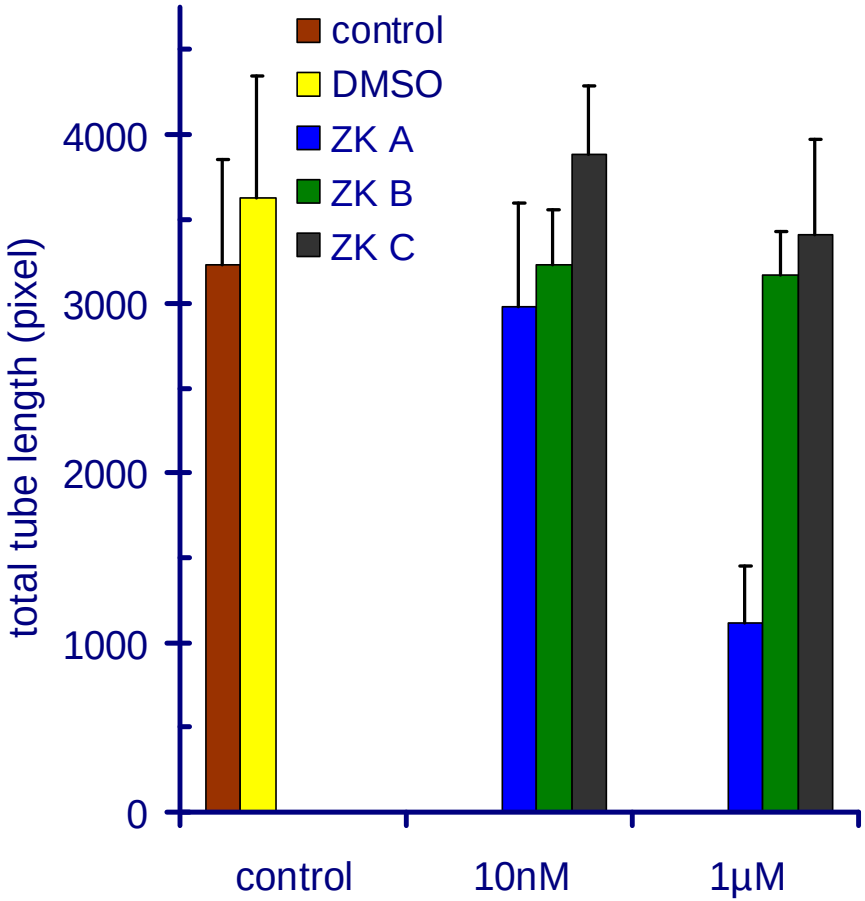
tube formation



MVEC

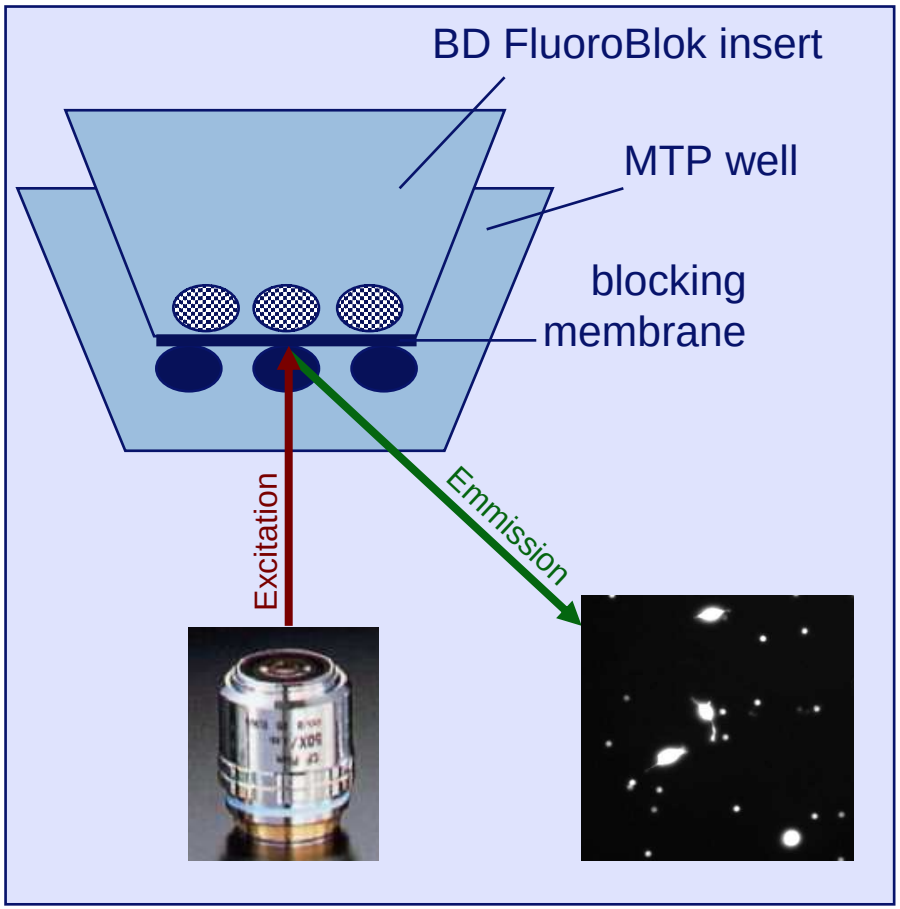
tube formation

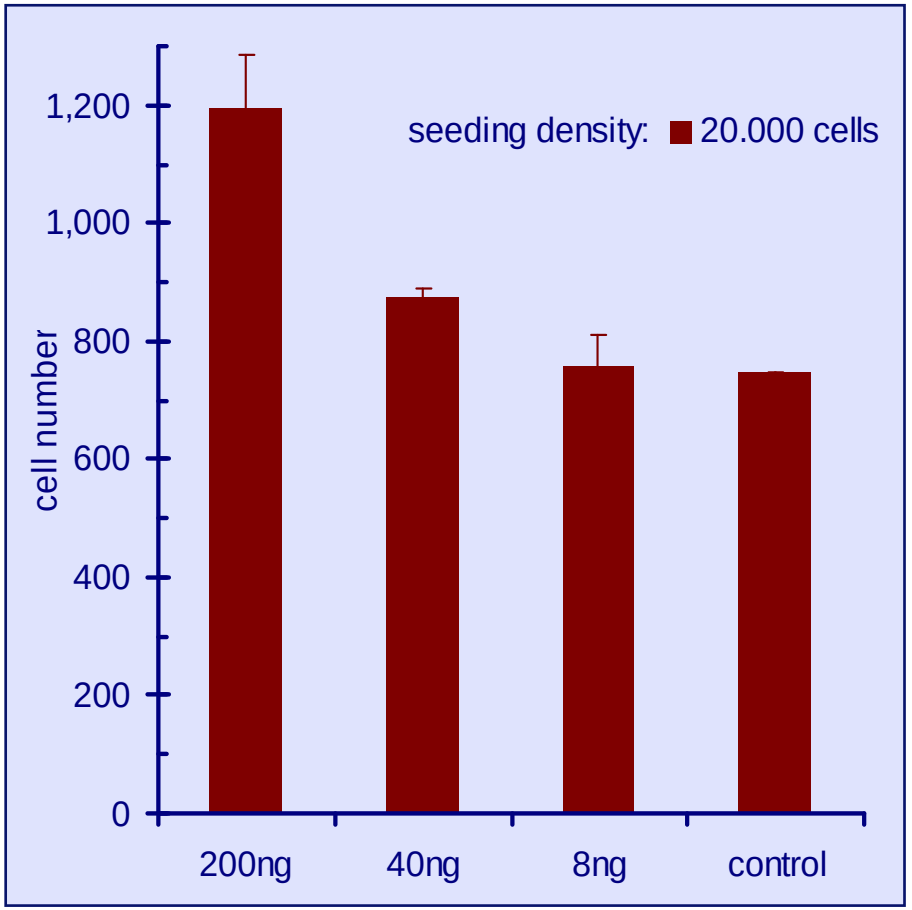




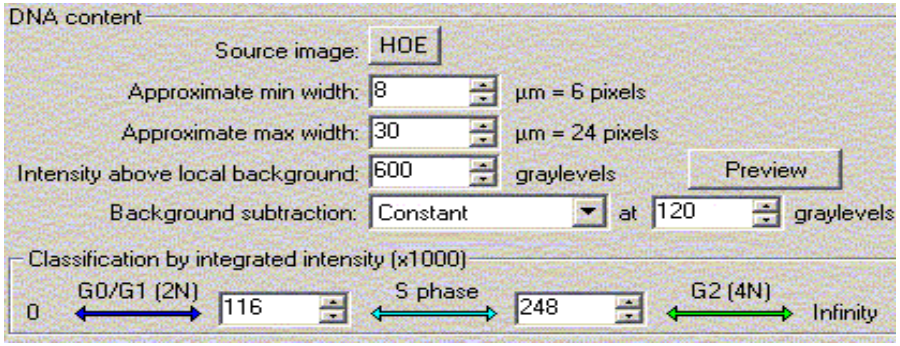
migration

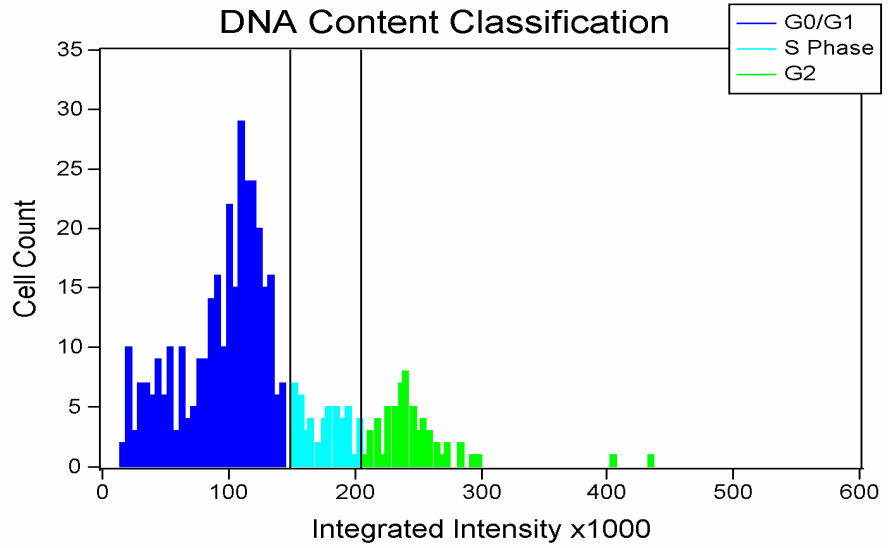
MVEC





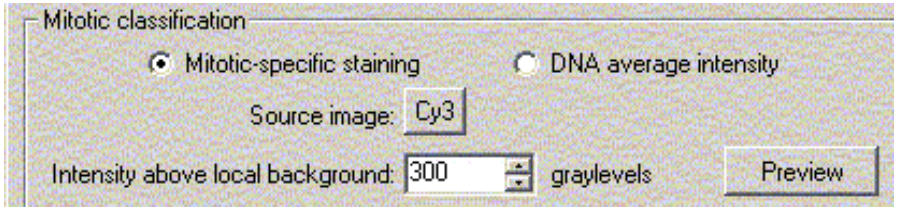
DNA content

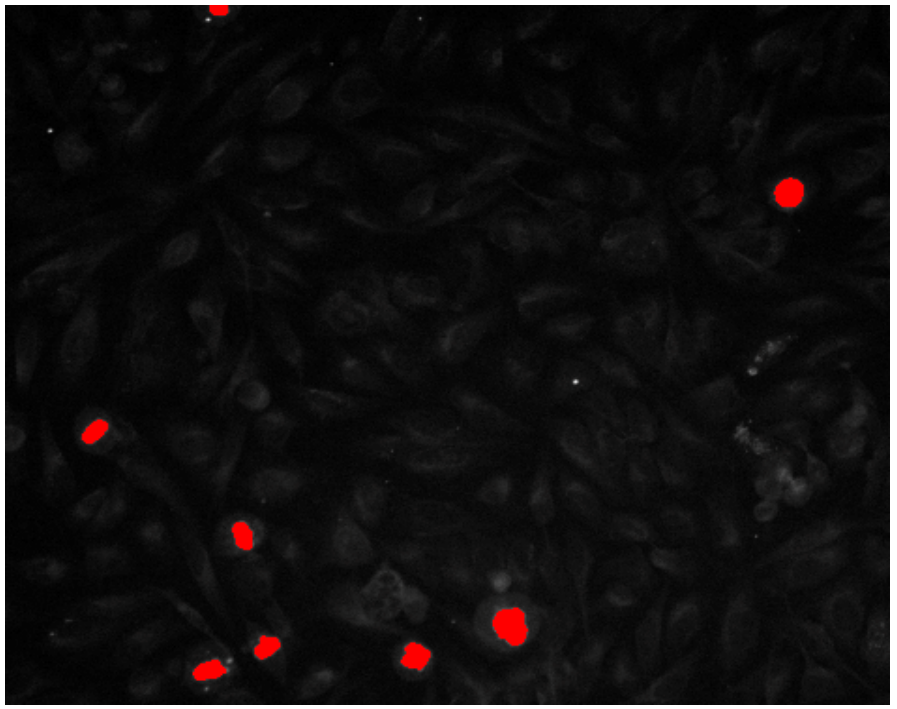




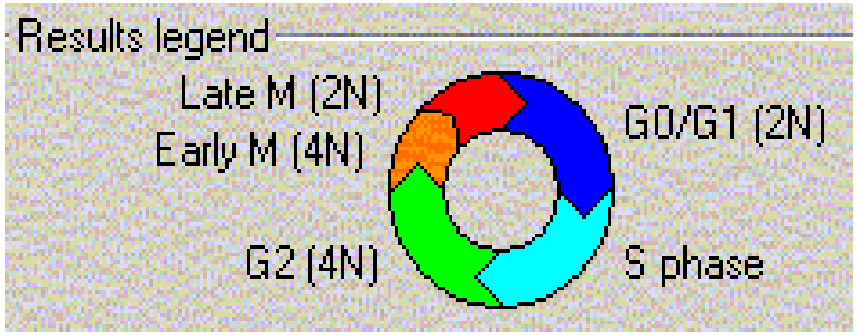
= summed intensity values of all pixels in a object

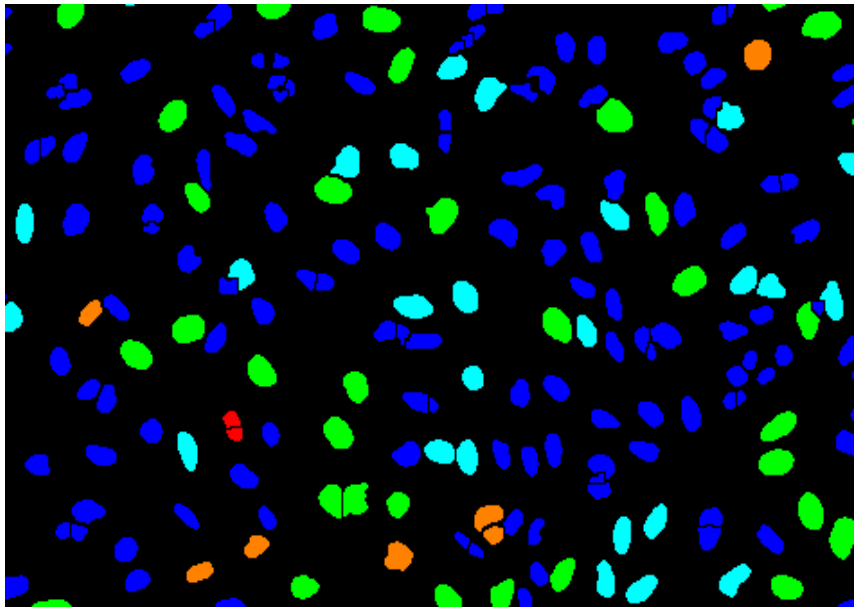
mitotic index

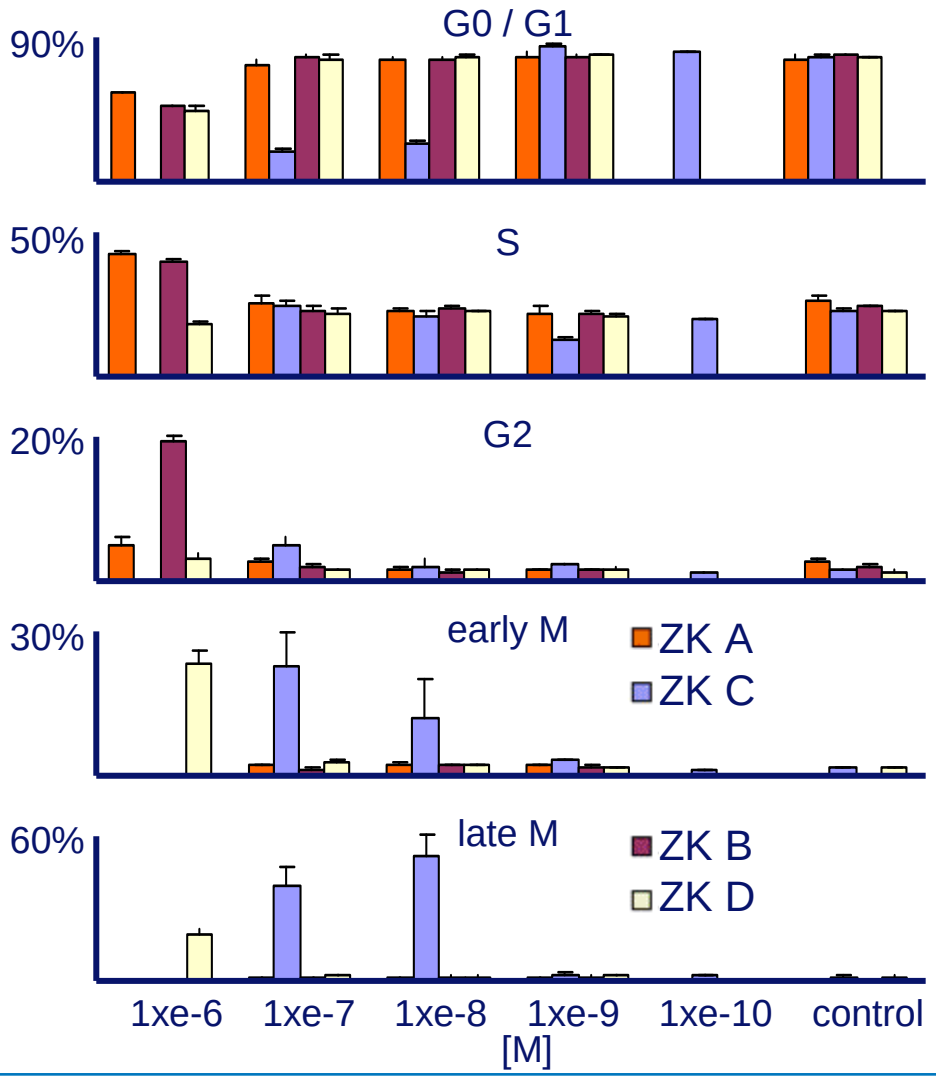




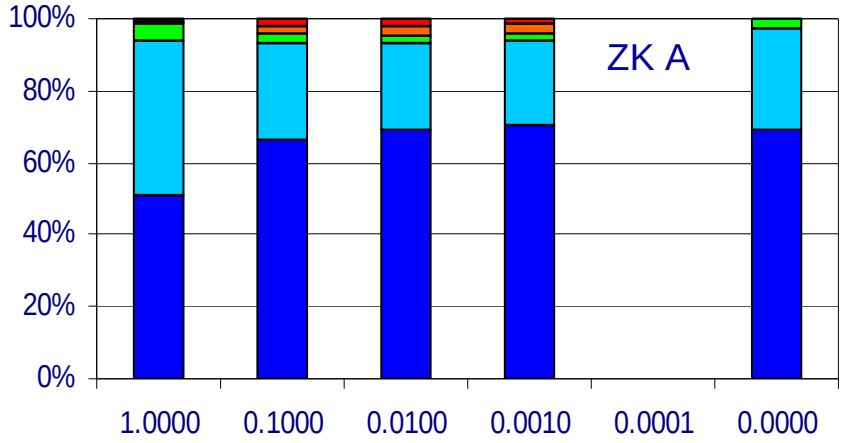
cell cycle classification

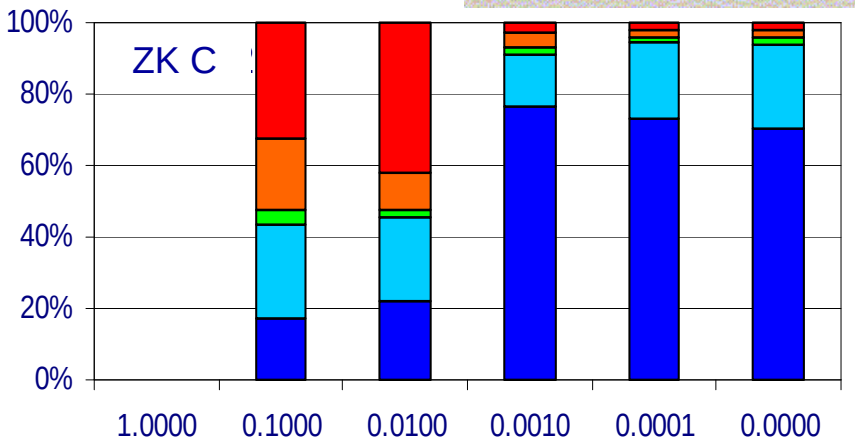


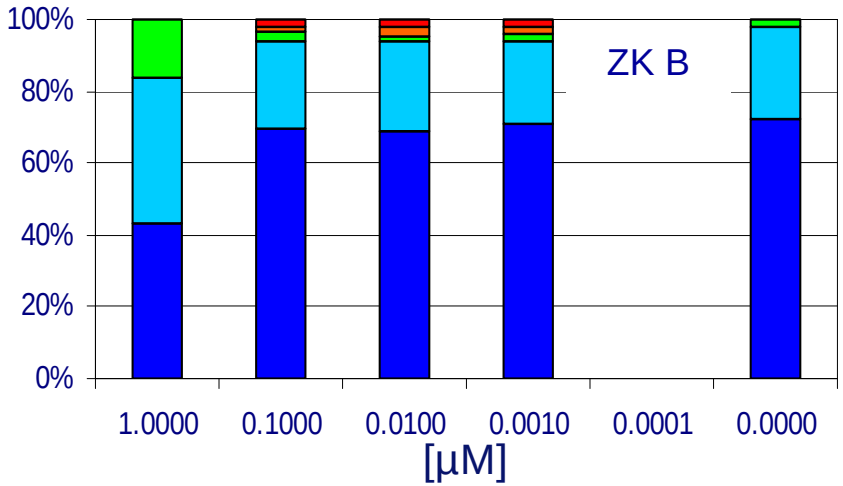


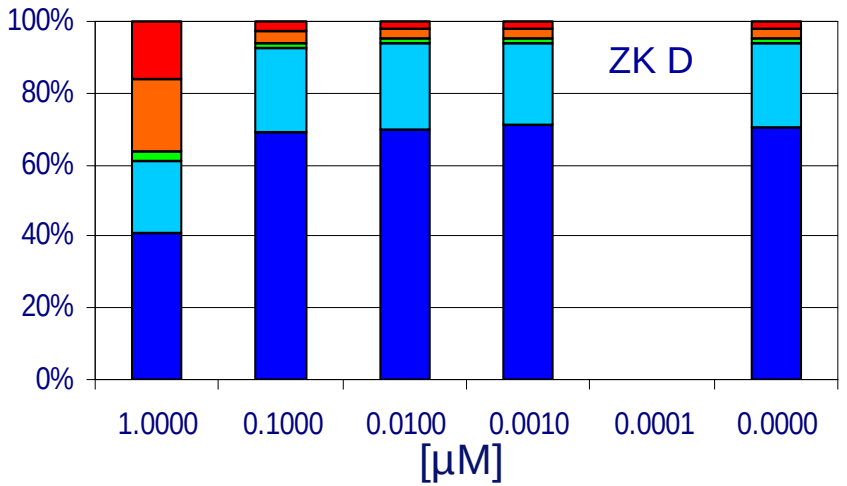


cell cycle classification

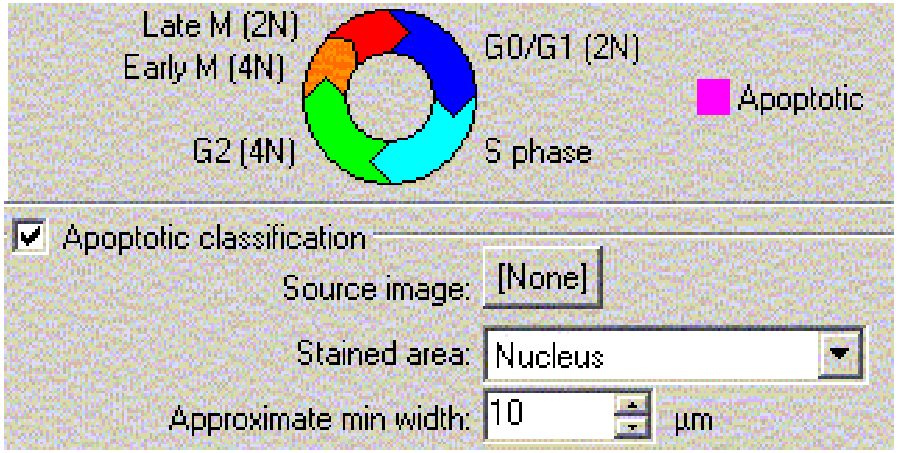


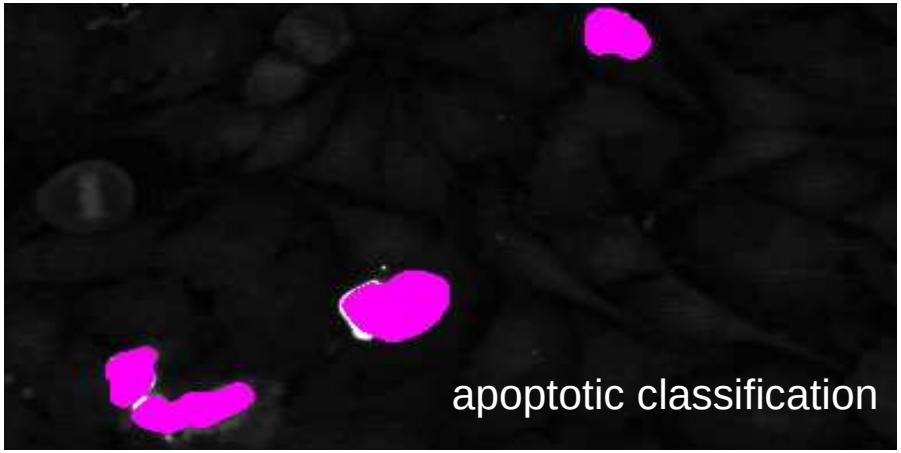


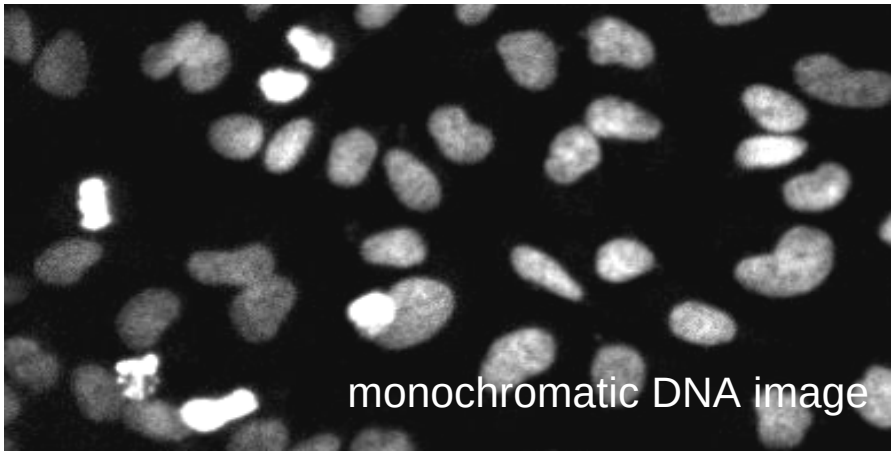


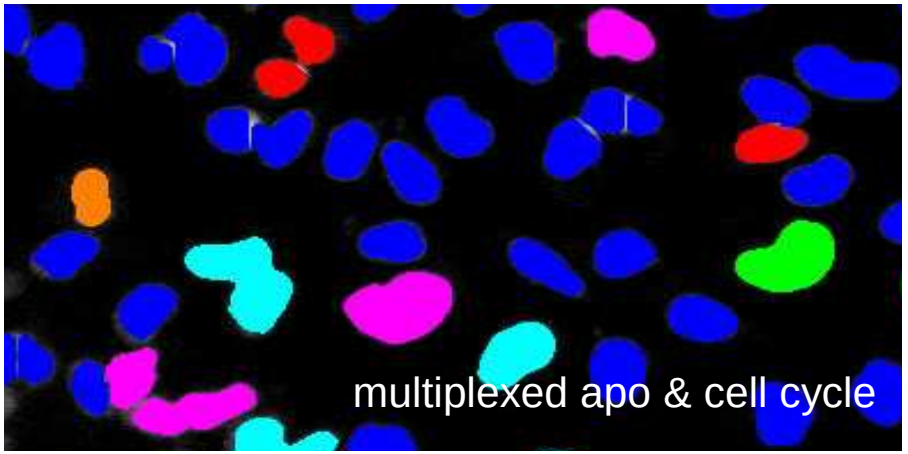


apoptotic classification



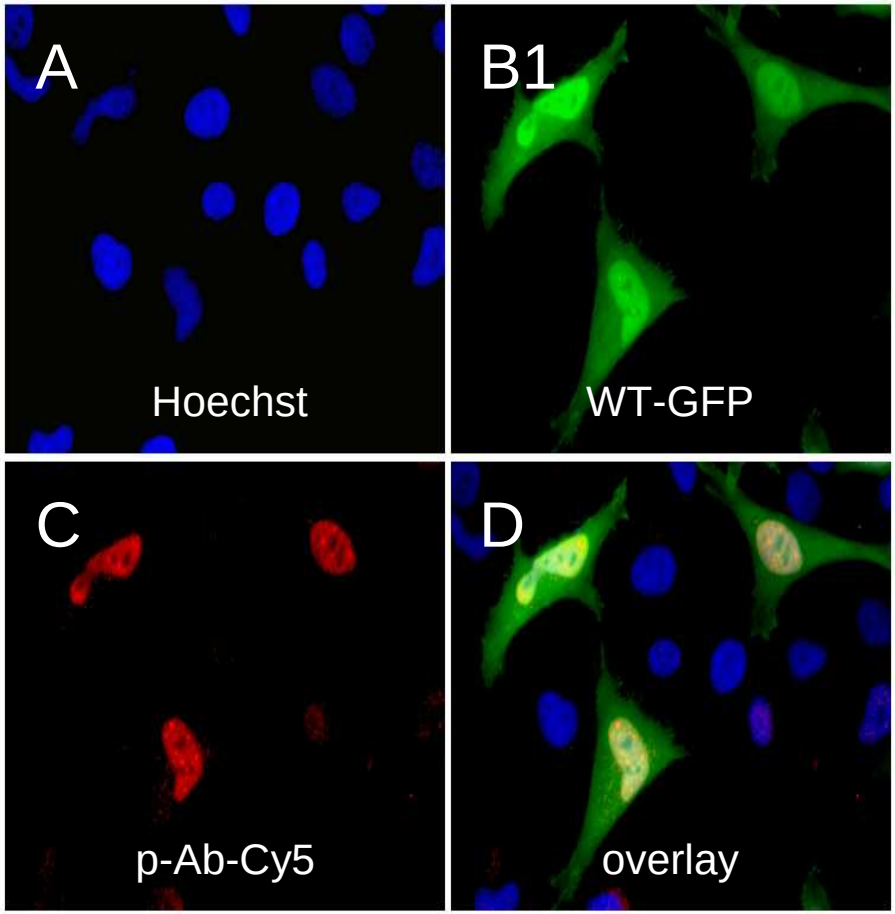


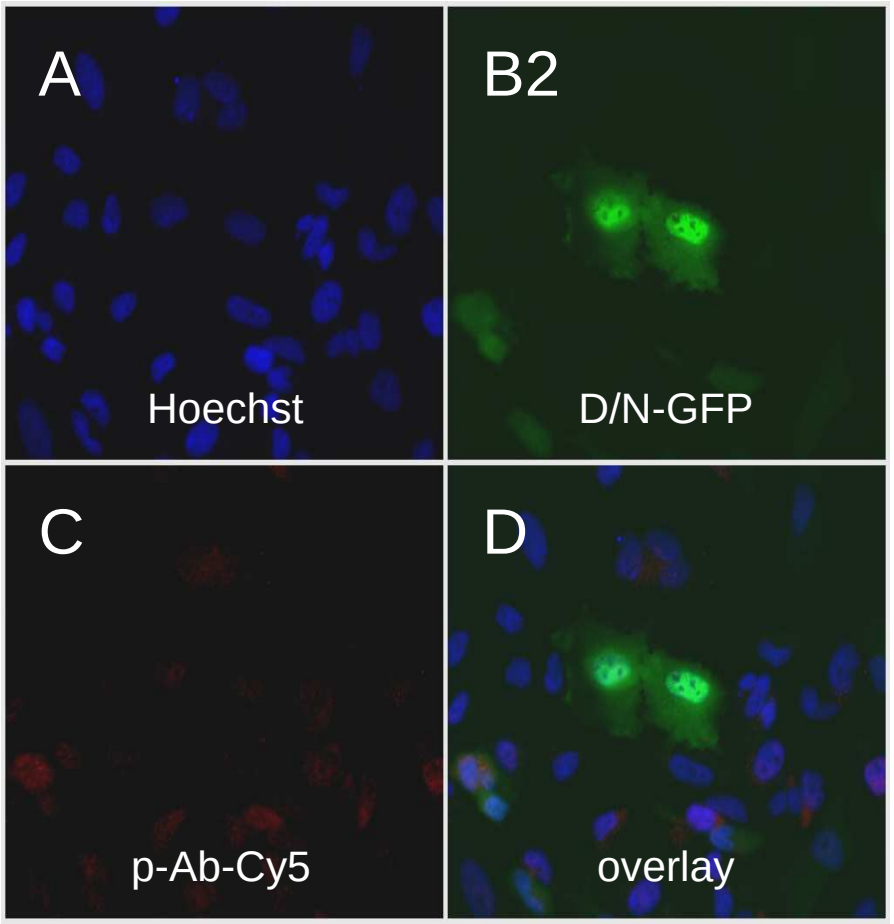




protein-protein interaction

kinase vs. substrate



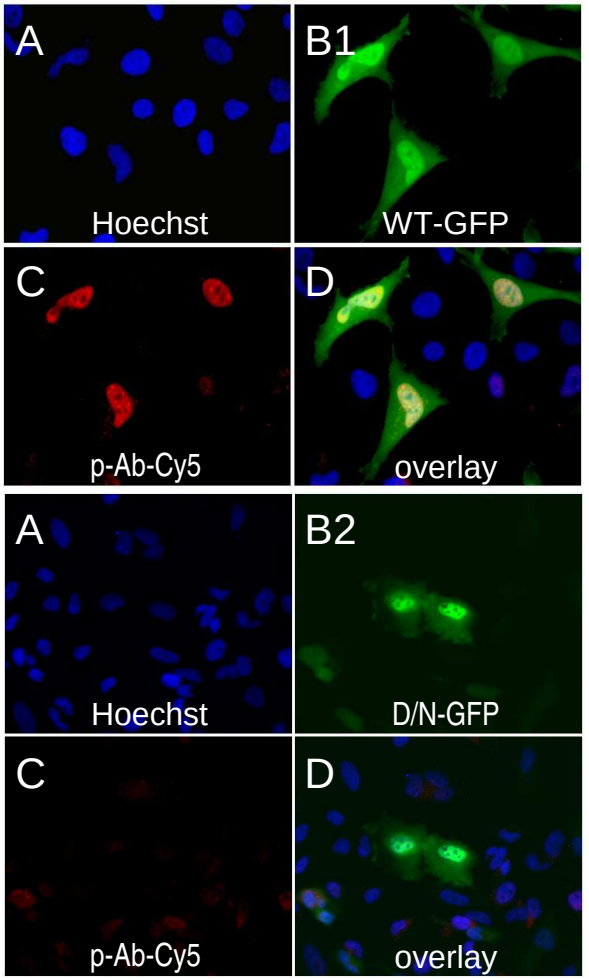


protein-protein interaction

kinase vs. substrate

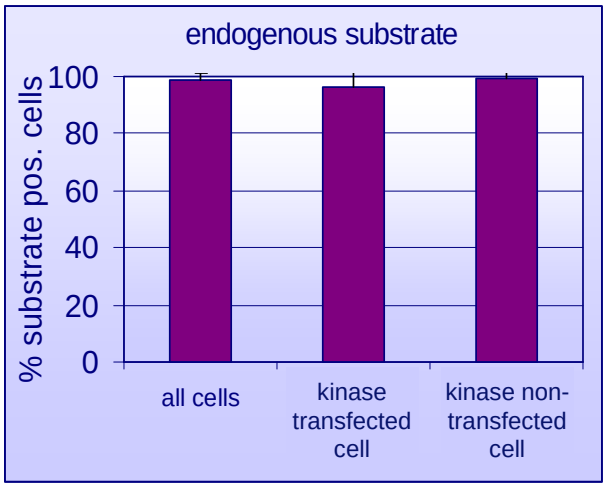
transient transfection of GFP-tagged kinase (WT or D/N) and co-staining of phosphorylated known nuclear substrate

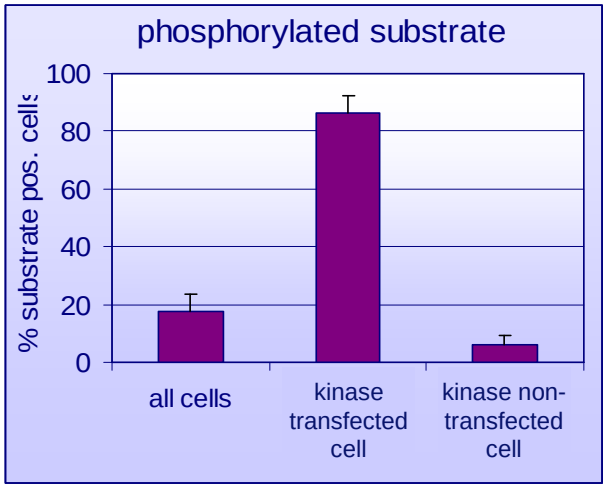
- ►HOECHST dye (A)
  - visualisation of total number of nuclei
- ►GFP expression (B)
  - identification of transfected cells
  - distribution pattern of GFP-kinase
  - B1: WT // B2: D/N
- ►phosphorylated kinase substrate (C)
  - identification of substrate that has been phosphorylated at specific phosphorylation site
- ►superimposition (D)
  - documentation of kinase dependent phosphorylation of substrate at specific site: only in WT-GFP positive nuclei

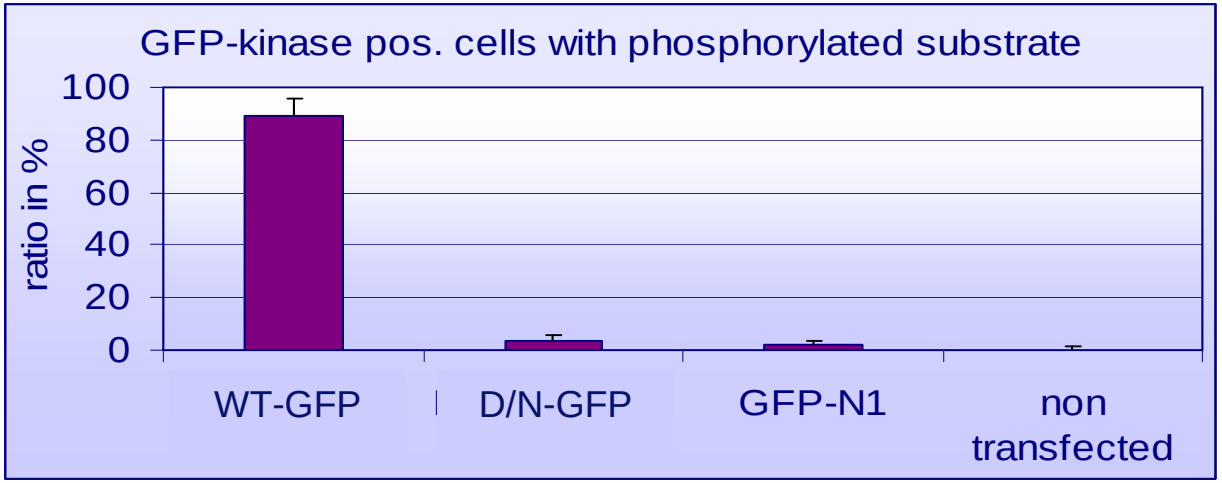


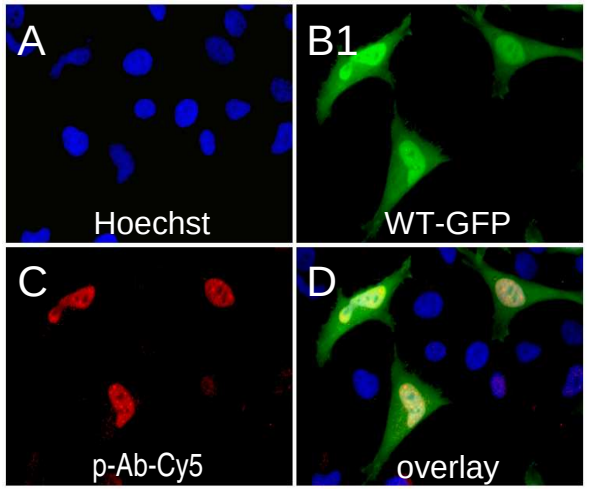
protein-protein interaction

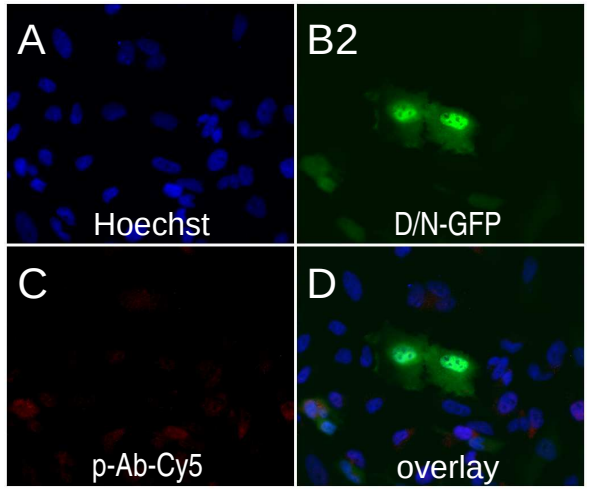
kinase vs. substrate







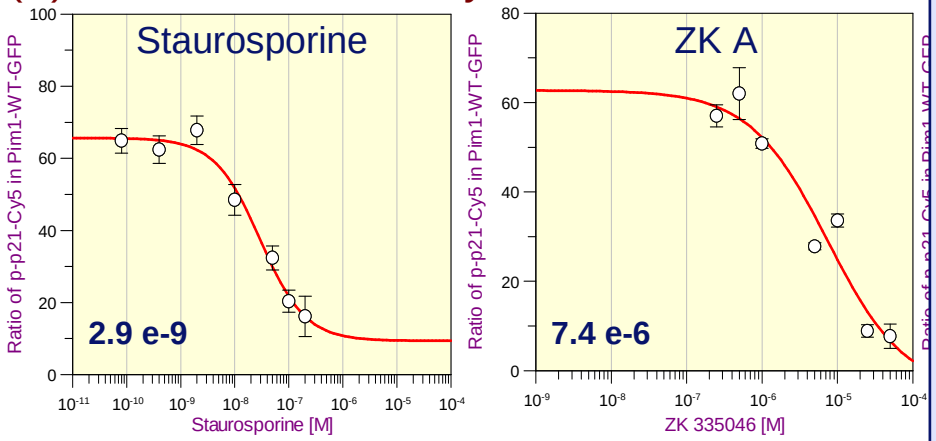




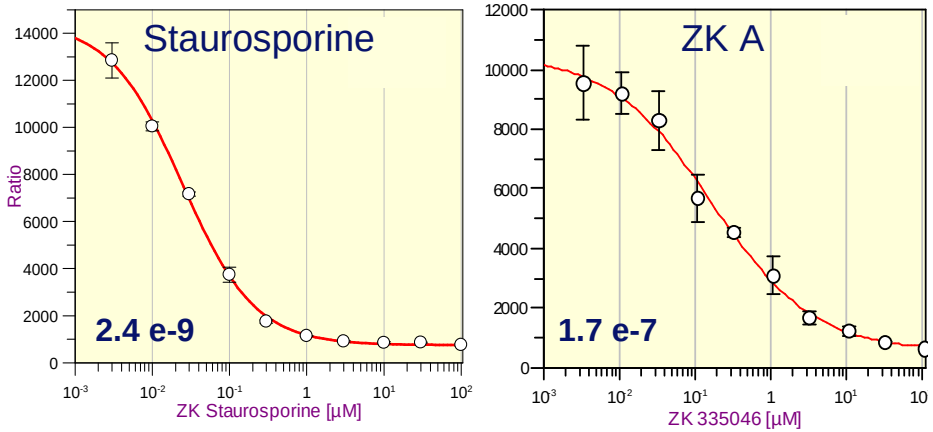
protein-protein interaction

kinase vs. substrate

(A) functional HCA assay



(B) HTRF assay



- functional HCA assay & biochemical HTRF assay revealed the same rank order for IC_{50} values of selected compounds
- IC_{50} values determined by HCA assay differ in 1 order of magnitude
- results from HCA assay contain
  - compound specificity
  - tox & site effects
  - cell permeability
  - MDR effects

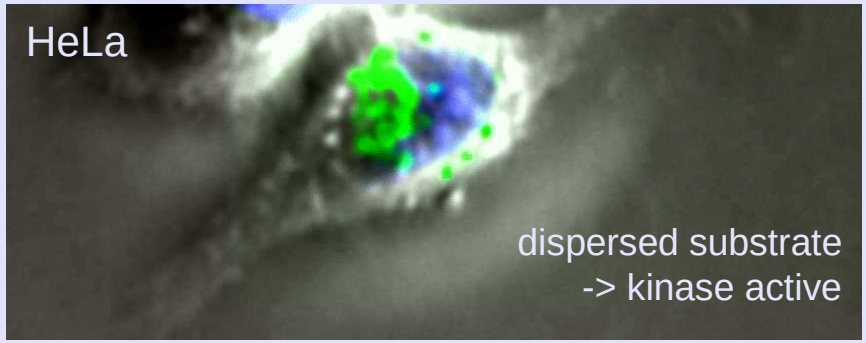
⇨ results from HCA assay are of greater value

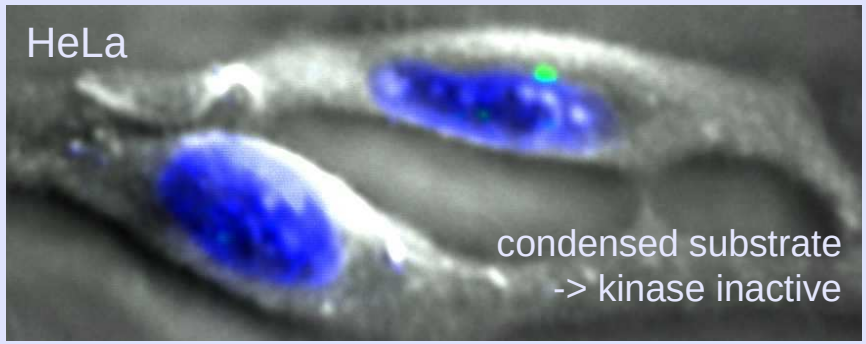
protein-protein interaction

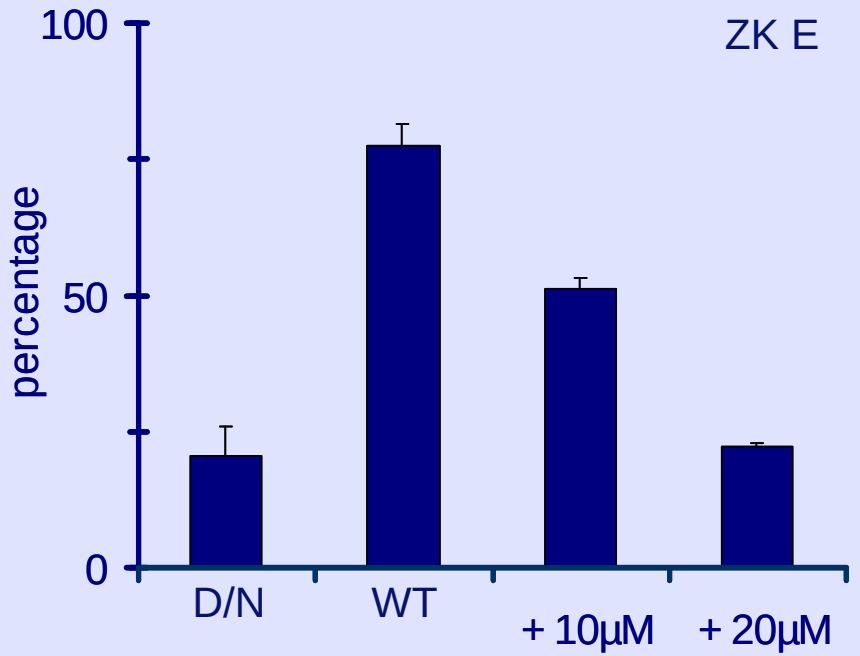
kinase vs. substrate

phosphorylation of substrate by kinase leads to a specific morphological alteration

- identification of kinase inhibitors
- quantification of potency



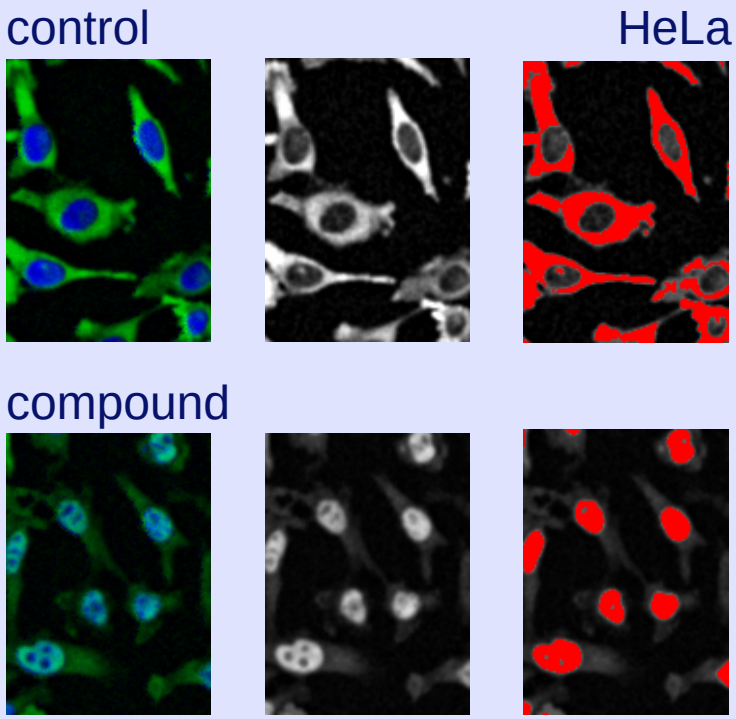


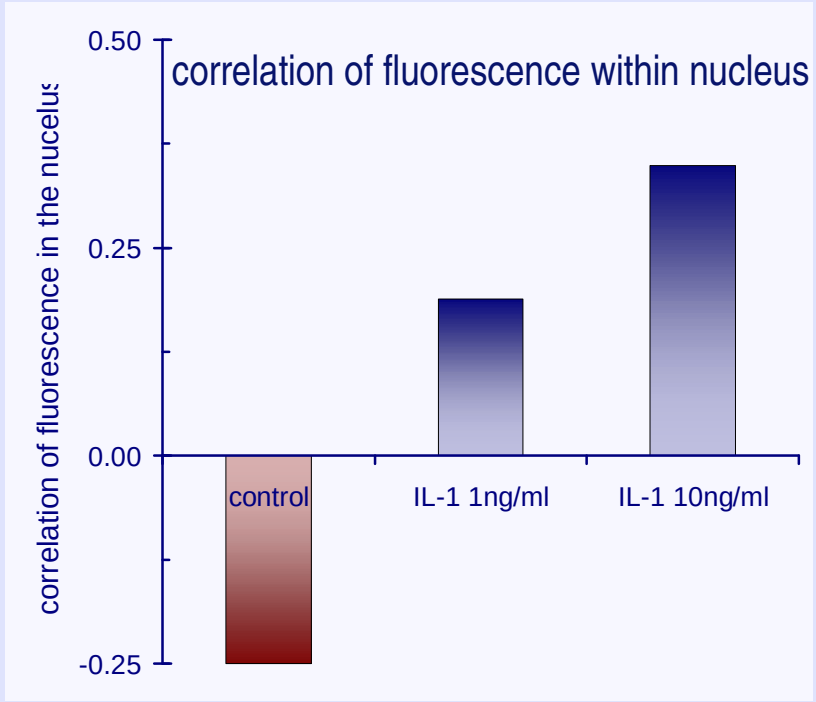


protein translocation

protein activation often correlates with dislocation from origin to point of interaction

- phenotypical parameter which can be utilized by HCA
- identification of inhibitors / inductors





receptor internalization

GPCR activation

Transfluor

- technology which can be used to screen for ligands or drugs regulating GPCR activity
- GPCR desensitization occurs very early in a common pathway and is coupled with binding to β-arrestin
- can be monitored by cycling from membrane to cytoplasm and spot formation

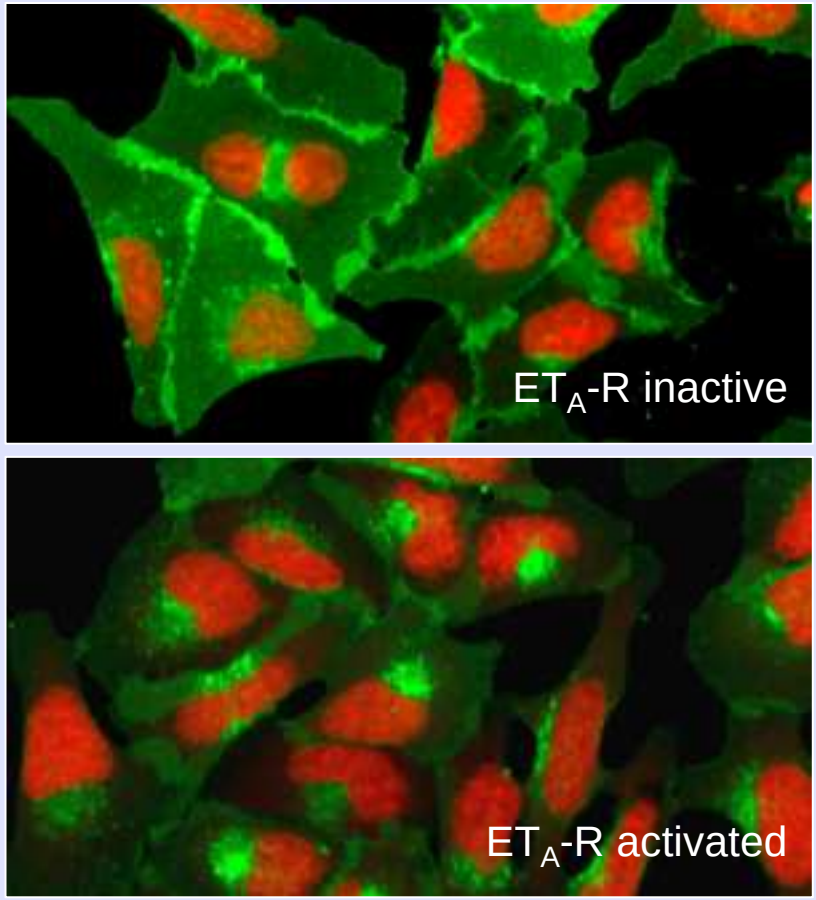
Endothelin A receptor internalization (ET_A-R EVOTEC Technologies)

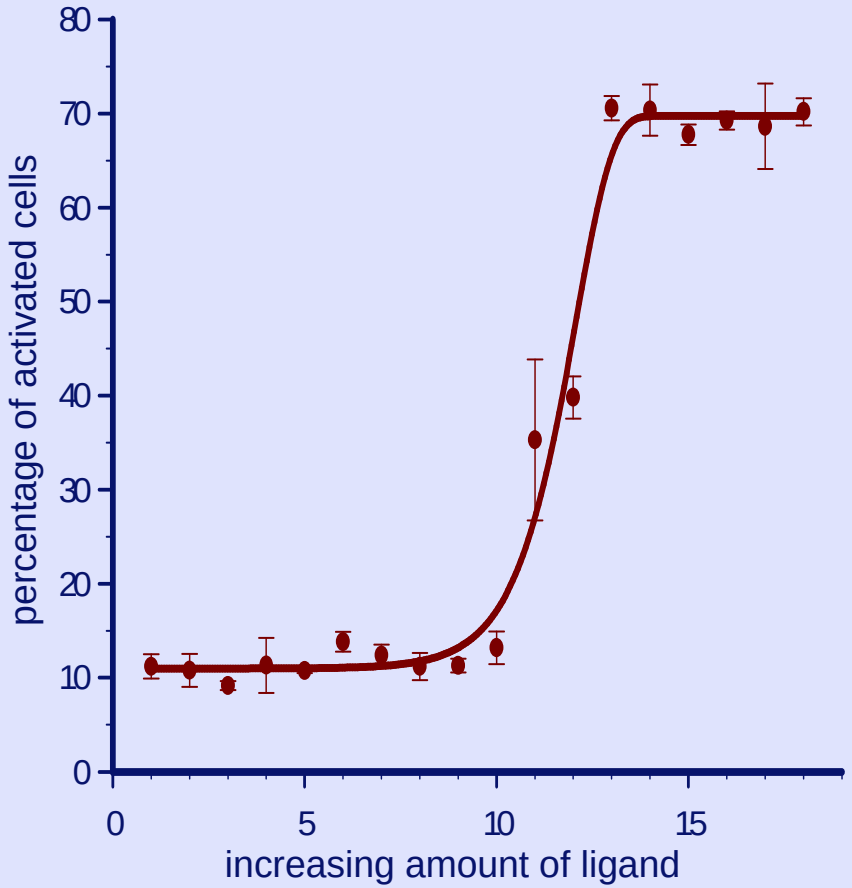
- after agonistic stimulation, ET_A-R-GFP translocates from membrane to cytoplasm
- accumulation of ET_A-R-GFP in endosomes can be quantified by HCA

⇨ functional receptor internalization assays can be utilized for primary HTS

receptor internalization

OPERA





EVOTEC Technologies

time lapse studies

utilization of dynamic HCA approaches during preclinical drug research

- enlighten complex subcellular processes and pathways
- establish a profound knowledge data base
- improve specificity of compounds, efficiency of drugs and compliance

live-cell assays

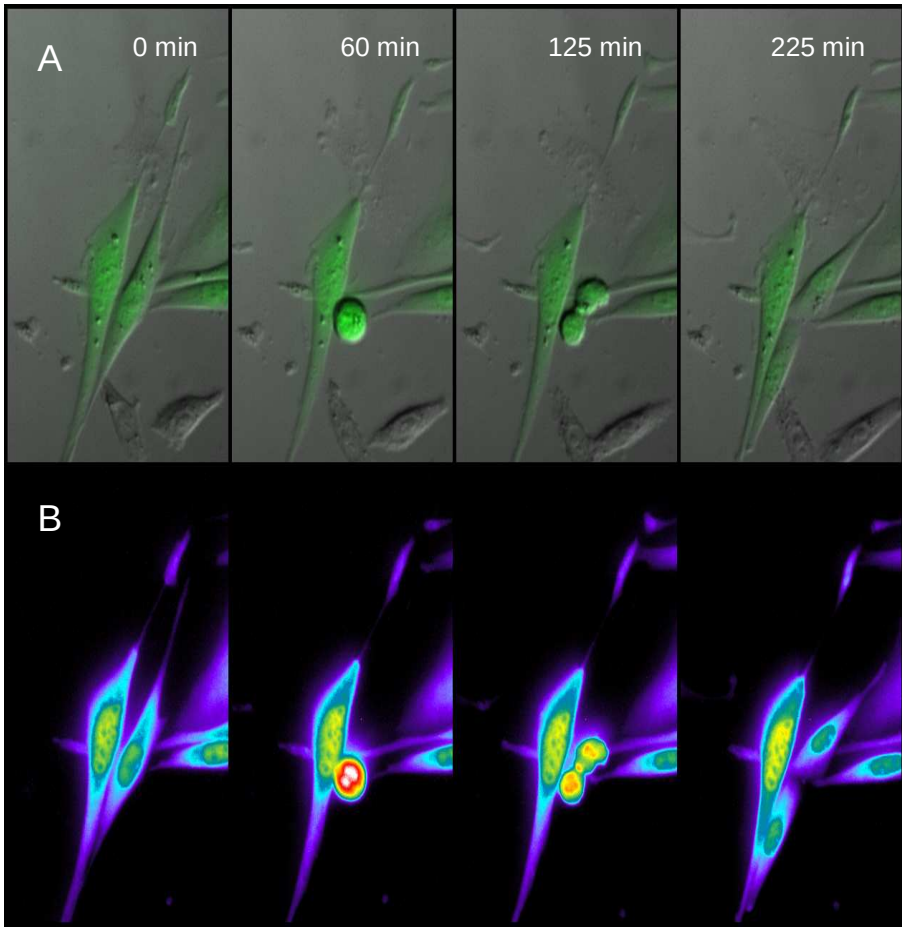
- increasingly requested during target validation & lead optimization
- identification of important check points e.g. during cell cycle progression
- improve the understanding of cellular mechanisms
- qualify compound specificity in selected cell systems
- document the uniqueness of drug candidates

⇨ require improved data management, data storage, data handling & automated data analysis solutions

cell cycle kinase

GFP-labeled kinase

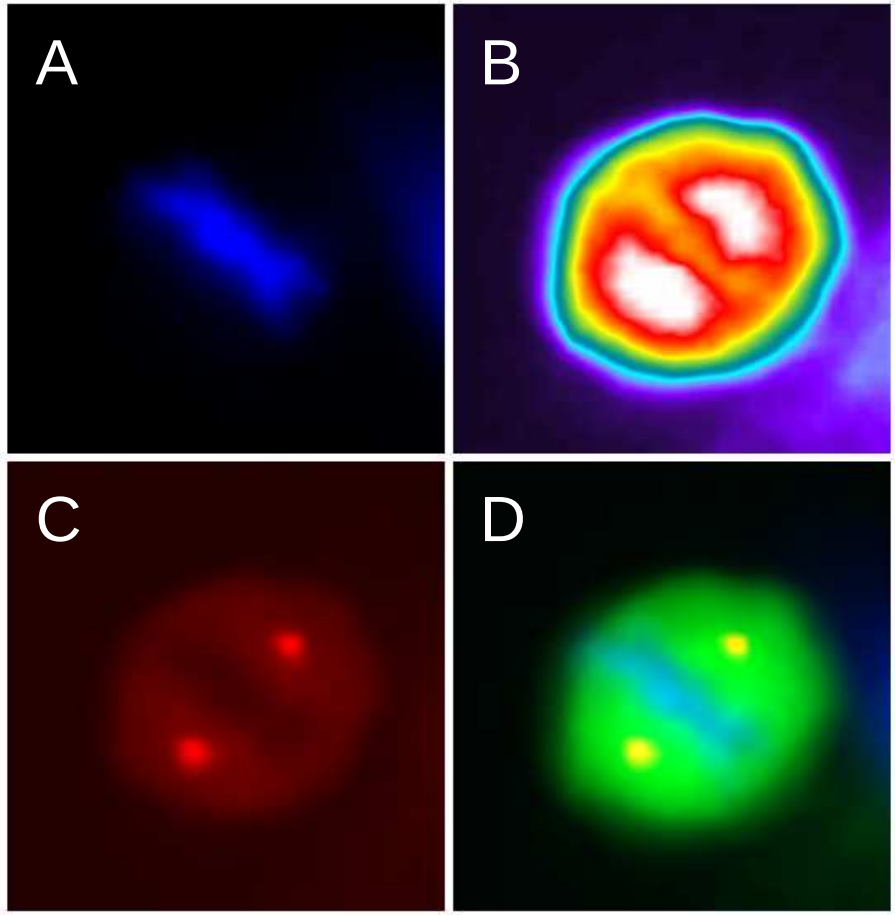
- intracellular localization during cell cycle progression
  - superimposition (A) of DIC and GFP channel
  - intensity profile of GFP fluorescence (B)
- interphase (0 min)
  - predominant nuclear localization
- mitosis
  - localisation in specific subcellular regions
- metaphase (60 min)
  - bipolar localisation
- cytokinesis (125 min)
  - area of the reassembling nuclei and region of the contractile ring



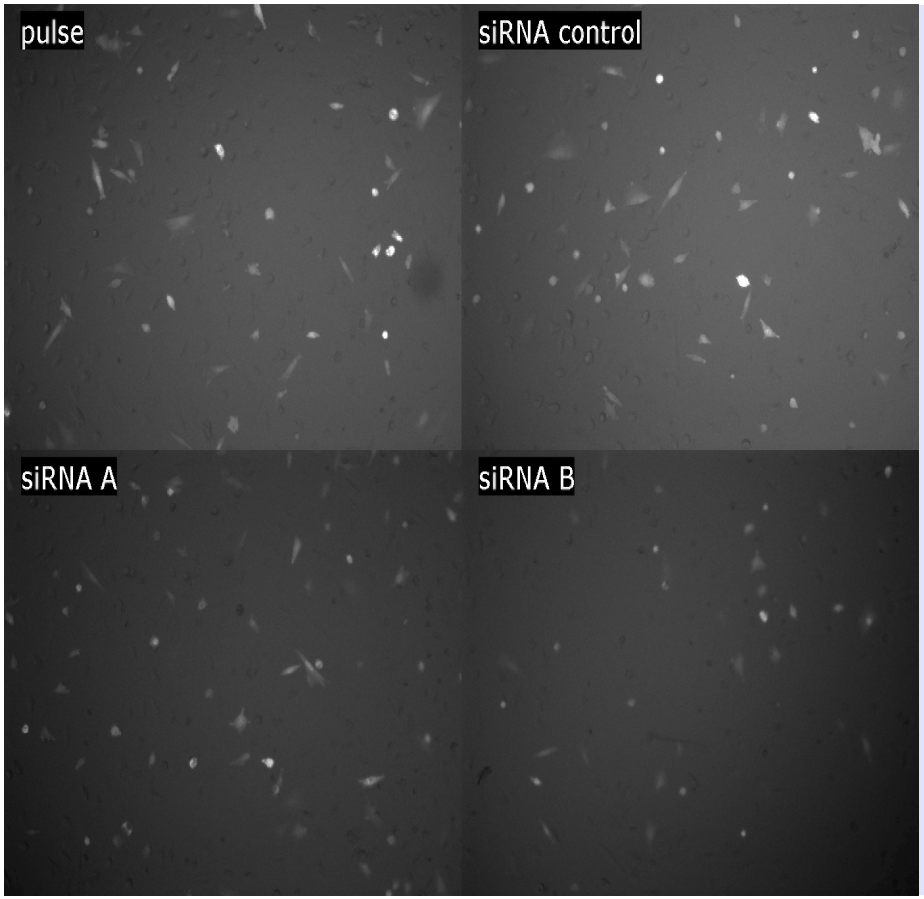
cell cycle kinase

GFP-labeled kinase

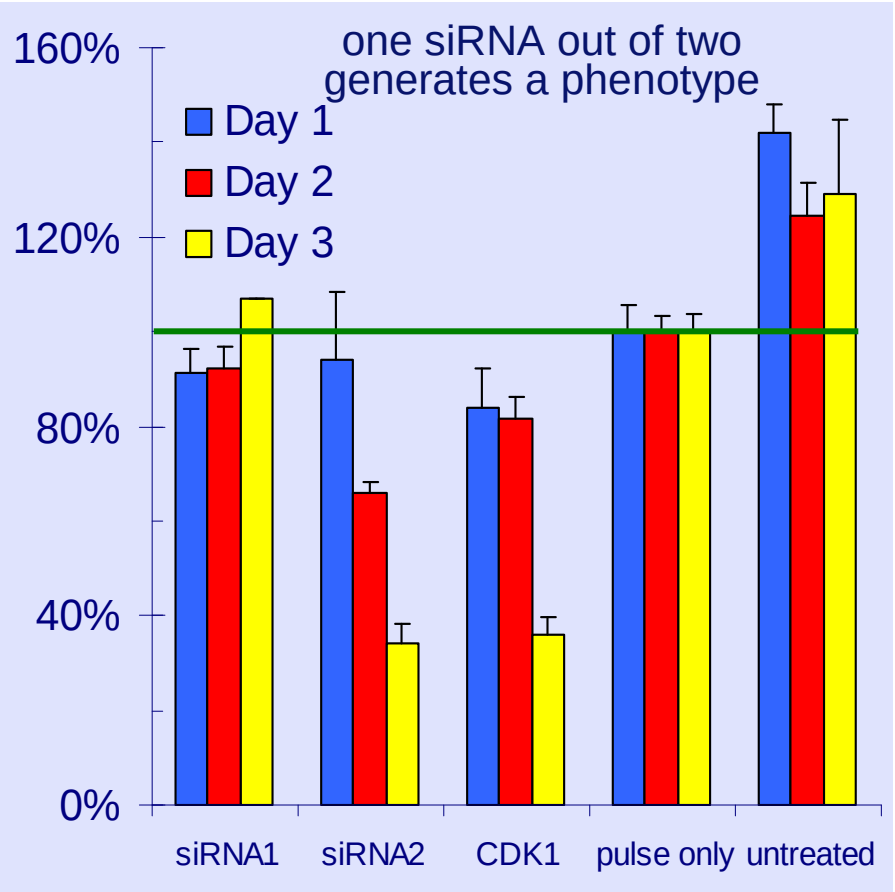
- intracellular localization of GFP-tagged kinase during metaphase
- chromosomes (A)
  - condensed localisation in equatorial plate
- GFP-tagged kinase (B)
  - intensitiy profile of GFP expression
- γ-tubulin (C)
  - bipolar localisation of spindle poles
- superimposition (D)
  - indication of a predominant localisation of GFP-kinase fusion protein around spindle poles during metaphase



siRNA knockdown of a GFP-kinase in CHO cells



siRNAi k.d. of endogenous kinase in MCF7 cells



in collaboration with Claudia Merz

time lapse studies

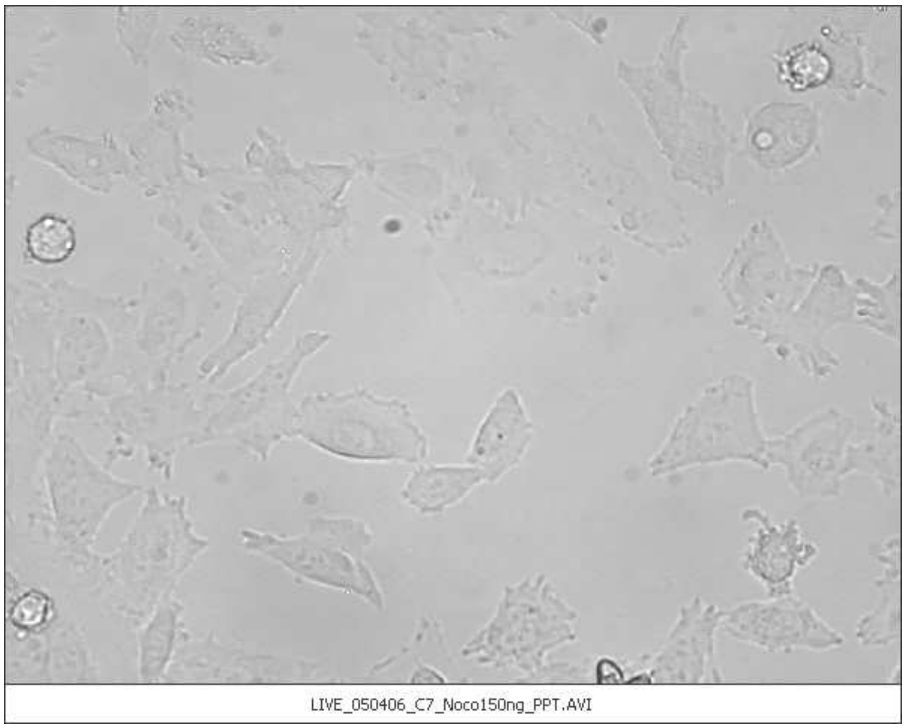
cell cycle kinase

inhibition of mitotic spindle check point protein leads to G2/M arrest

- documented by Nomarski contrast method, 24h monitoring, images taken every 5min

HeLa - ZK D

HeLa - Nocodazole

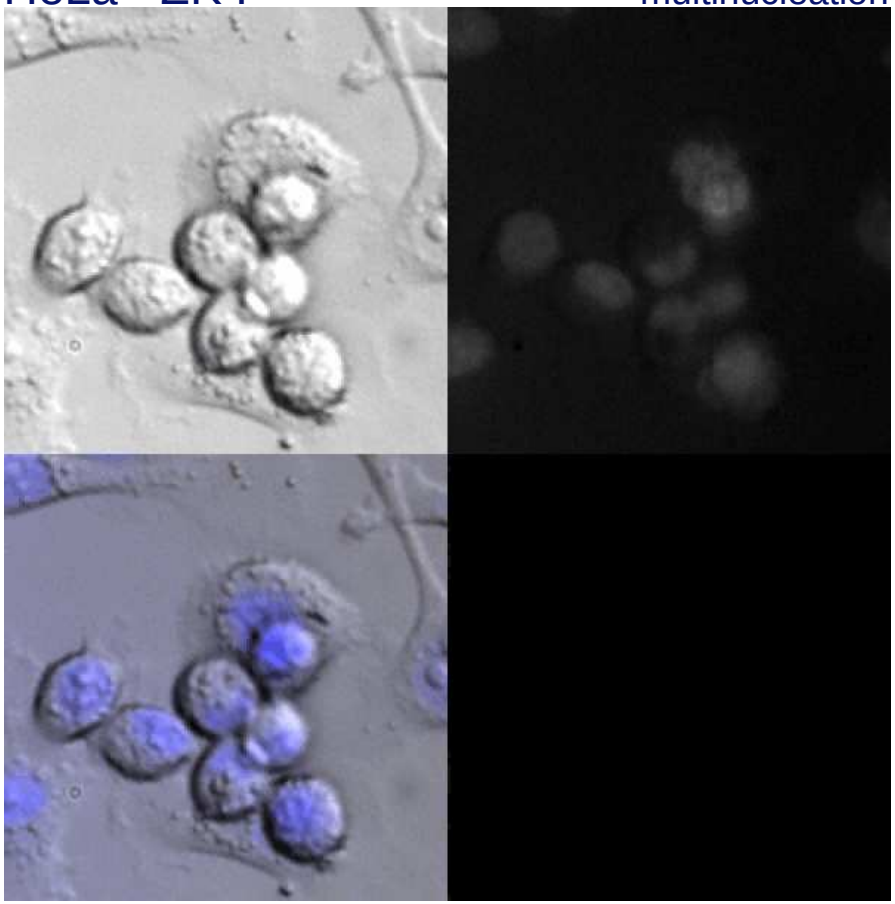


time lapse studies

cell cycle kinase

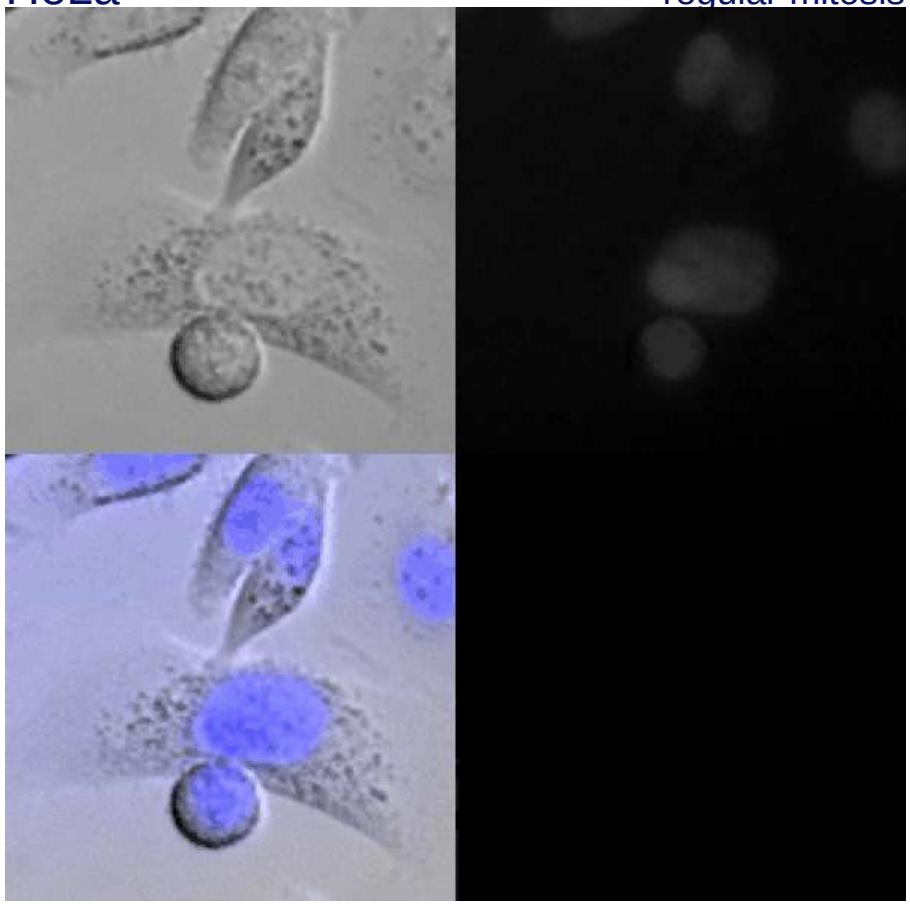
HeLa - ZK F

multinucleation



HeLa

regular mitosis



time lapse studies

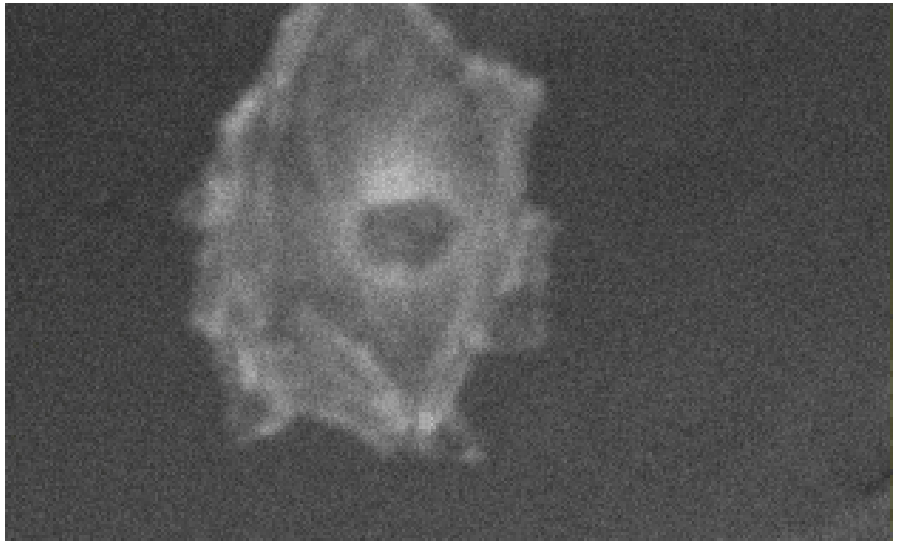
Discovery 1

stably transfected cells expressing AFP tagged cell compartment or target protein

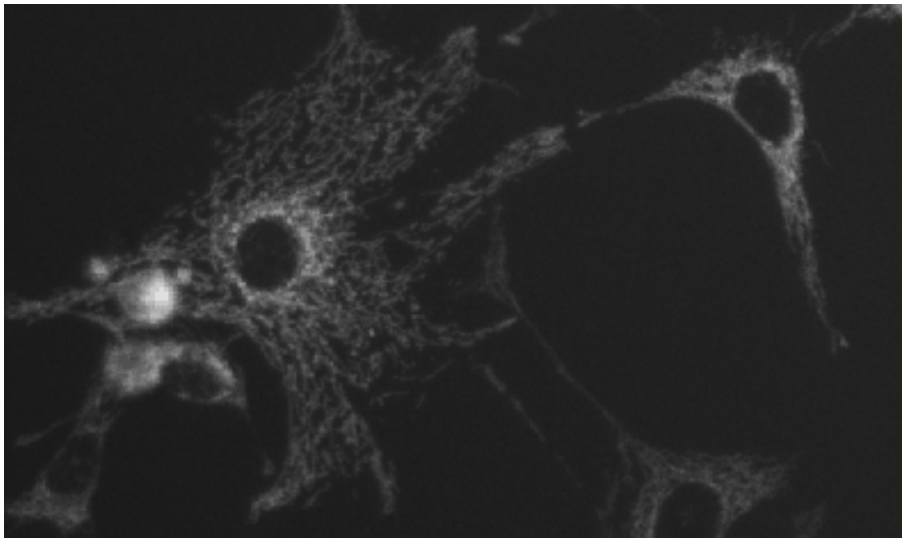
- ➢ phenotypical characterisation ⇨ improved black box screening
- ➢ functional pathway analysis ⇨ quantification of compound specificity

⇨ accounting the dynamic and spatial context

PtK2-PhiYellow-Actin



HeLa-TurboGreen-Mitochondria



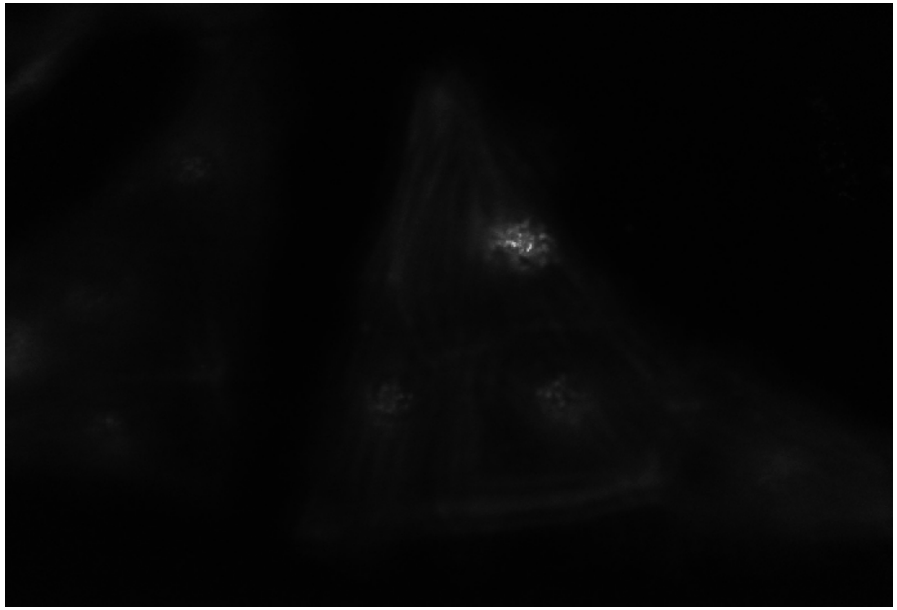
3D sectioning

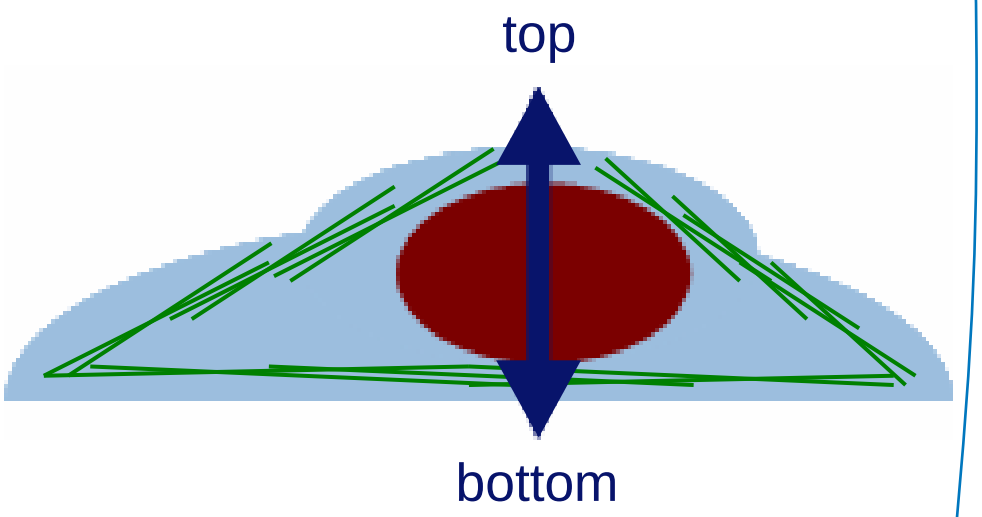
OPERA

confocal imaging enables the dissection of a cell into a stack of discrete planes

- cells can be analysed in a three-dimensional context
- 3D sectioning combined with time-lapse generates a powerful novel HCA tool

PtK2-PhiYellow-Actin 40x





supported projects

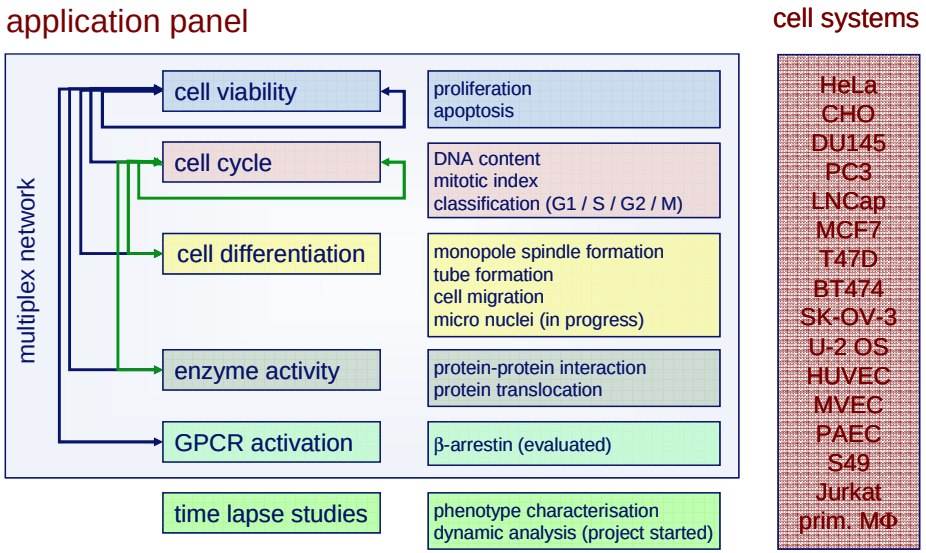
HT-RNAi project

– phenotypical screening: proliferation, apoptosis, DNA content, mitotic index, cell cycle classification, monopole spindle formation, etc.

Oncology

– siRNA / cDNA transfection: proliferation, apoptosis, cell cycle classification, protein-protein interaction, protein translocation, time lapse studies

applications



supported projects

applications

Gynecology & Andrology

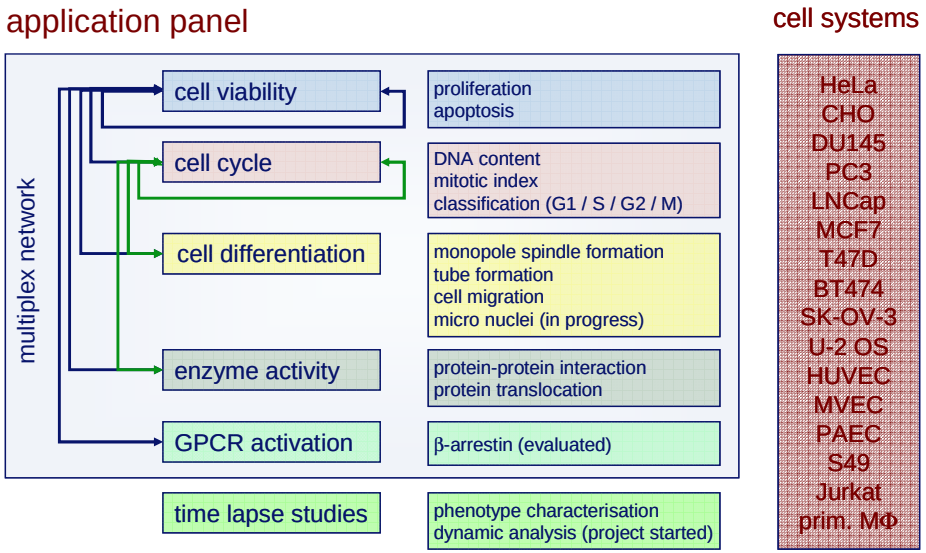
- proliferation, migration, primary macrophage classification

Inflammation

- proliferation, apoptosis

Oncology

- proliferation, apoptosis, tube formation, mitotic index, DNA content, cell cycle classification, protein-protein interaction, time lapse studies



supported projects

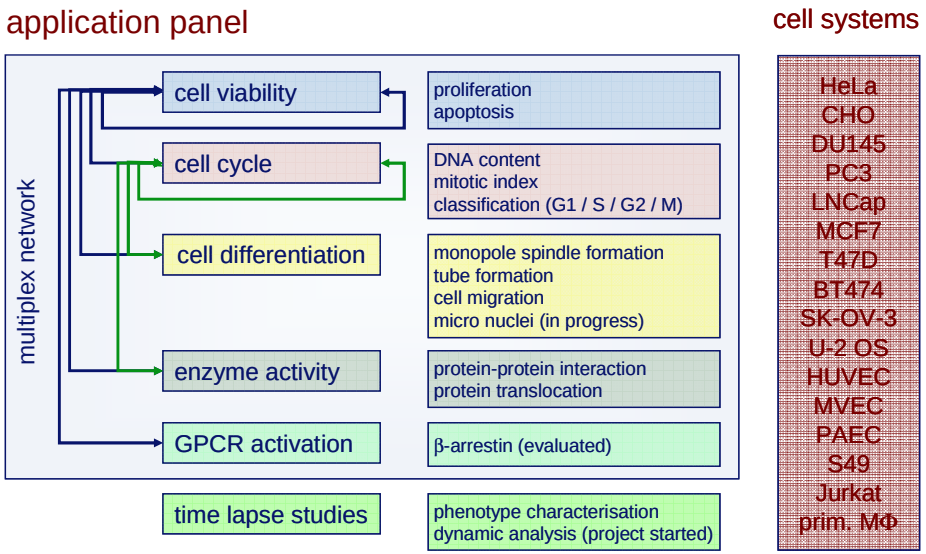
Inflammation

– proliferation, apoptosis

Oncology

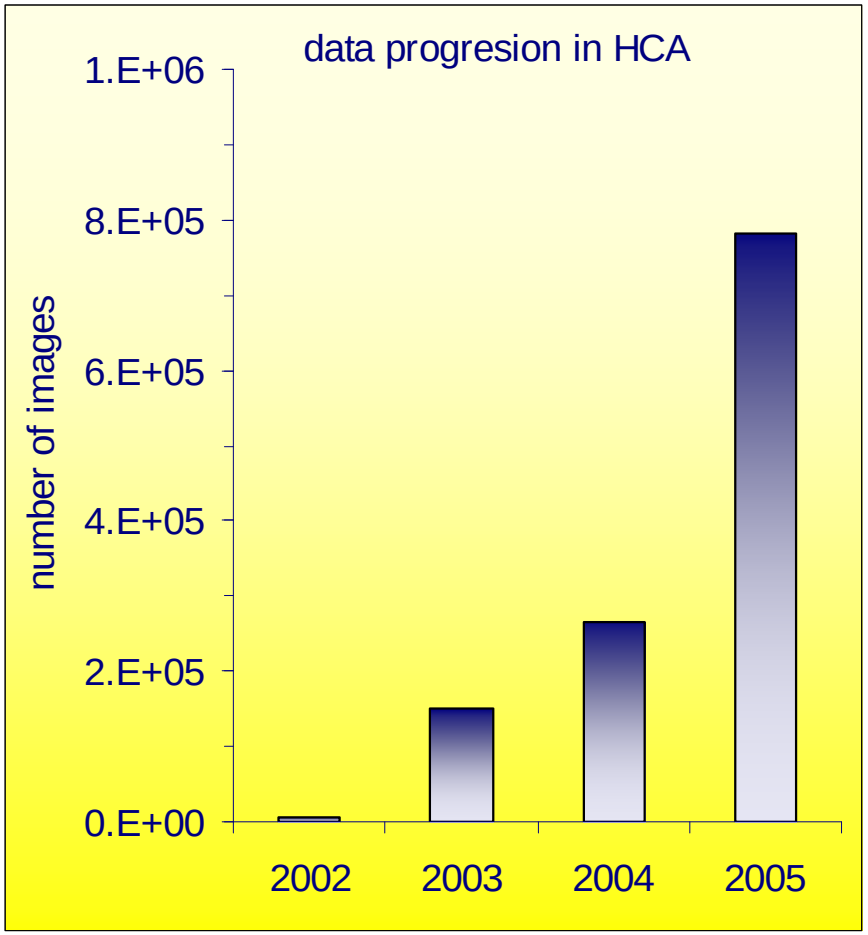
– proliferation, apoptosis, mitotic index, DNA content, cell cycle classification, protein-protein interaction, time lapse studies

applications



HCA at Schering AG in 2006

- broad panel of HCA applications established that meet CRBA needs
  - statistically secured and relevant data confirmed HCA as a reliable technology
- highly requested technology
- Target Validation
  - RNAi k.d. & transient transfection studies for all major target families
- LD / LO support
  - multiplexed & functional assays established -> target specific mechanistic profiling
- contributions to primary HTS possible
  - target oriented assays, improved black box



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