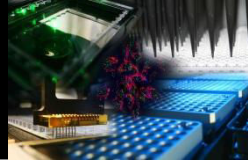
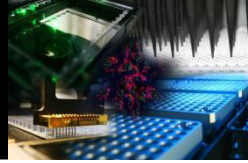


Galaxy Workshop Freiburg University

Automated Data Pipelines
&
Intelligent Microscopy

RNAi Screening Facility
BioQuant, Heidelberg University

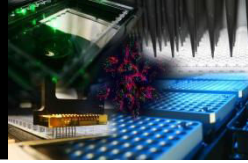
Jürgen Reymann

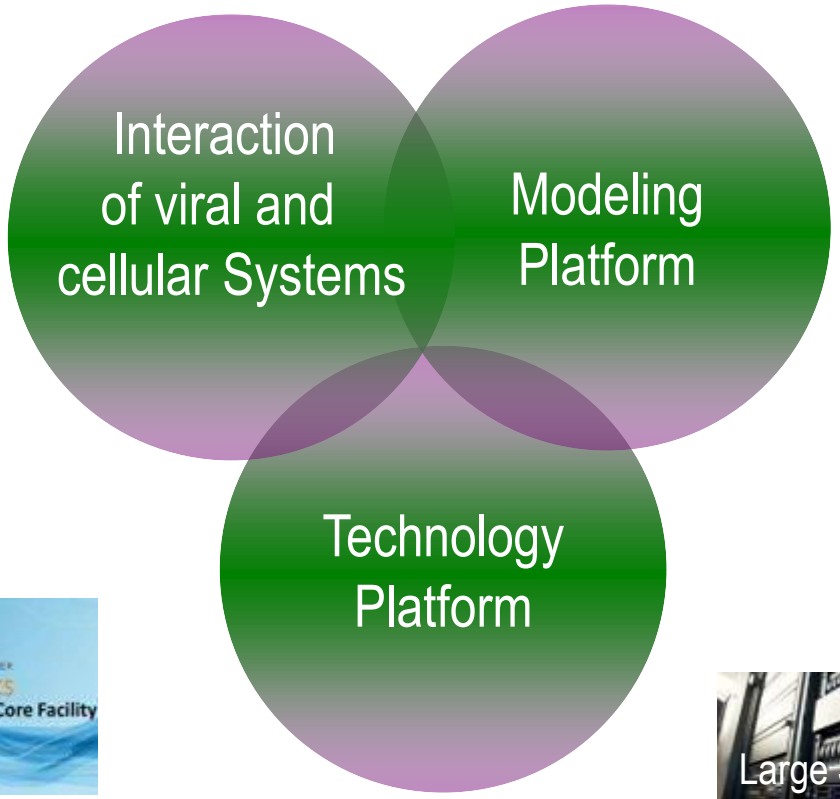


- Scientific Infrastructure
- Automated Data Pipelines
- Intelligent Automated Imaging

BioQuant – Center for Systems Biology

Quantitative Analysis of Molecular and Cellular Biosystems





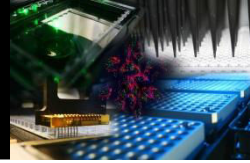
CellNetworks Deep Sequencing Core Facility

ViroQuant-CellNetworks RNAi Screening Facility

TIGA TISSUE IMAGING & ANALYSIS CENTER

NIKON IMAGING CENTER

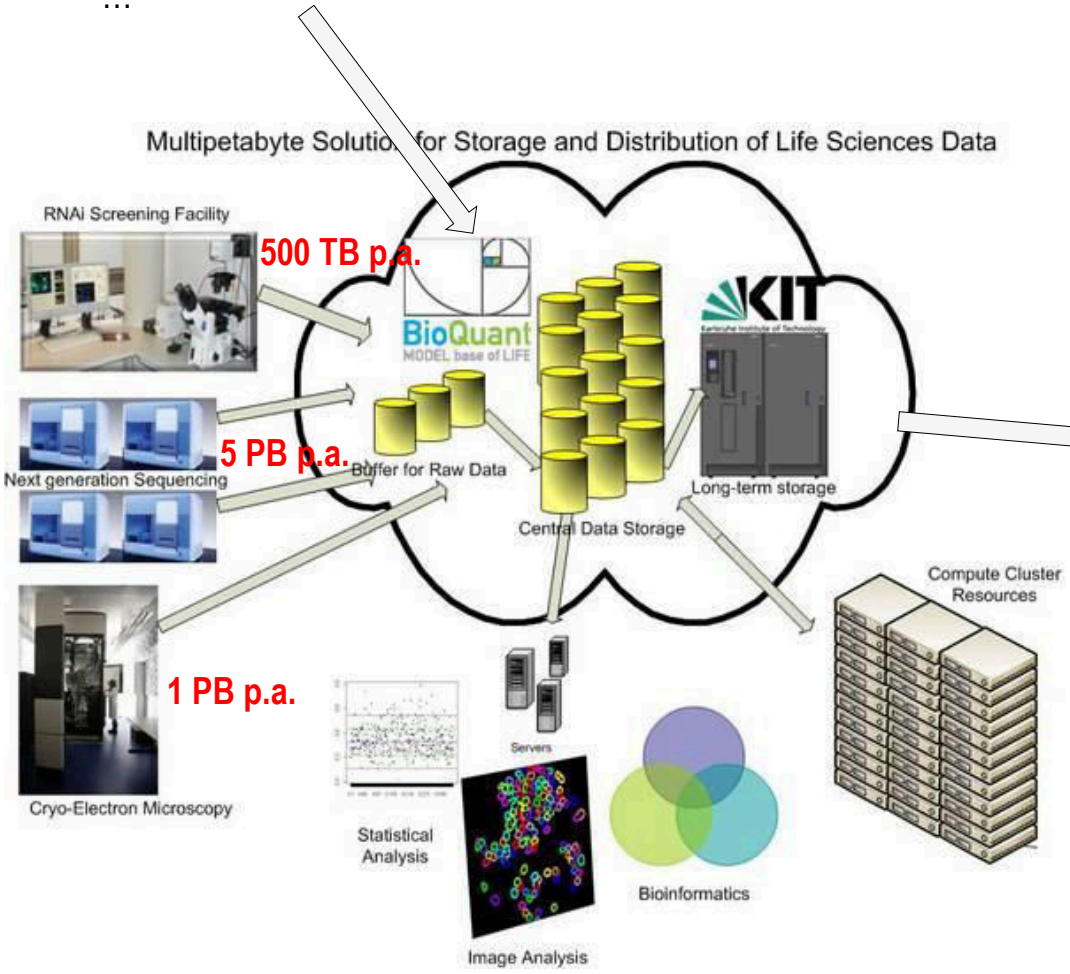
Data Analytics Infrastructure



- Super resolution microscopy (**>100 TB p.a.**)
- Light sheet microscopy (**>100 TB p.a.**)
- …

3.300 hard drives
6 PB total capacity

M. Hemberger



iCHIP integration Center for HIgh-throughPut experiments

J. Eils, C. Lawerenz

RNAi Screening Facility

Screening Core Facility

Assay automation | Automated microscopy

Data Analysis

Automated data processing pipelines

→ Support

Research & Development

Novel technologies for HCS

RNAi → Solid Phase Reverse Transfection

Screening Core Facility

Assay automation | Automated microscopy

siRNA | miRNA | cDNA
complexed with tranfection solution

↓

Gene specific degradation of target mRNA in the cells

↓

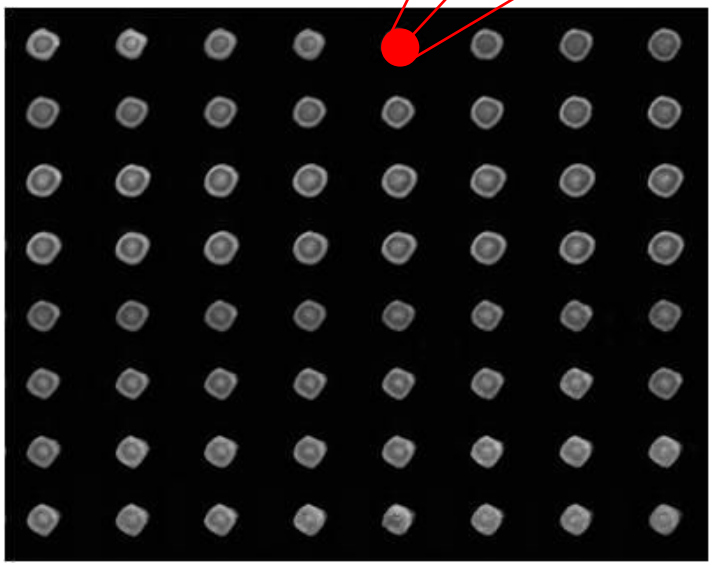
Loss of function phenotype

↓

INCENP -phenotype

siRNA spot distribution on cell array

Spot diameter: 400µm

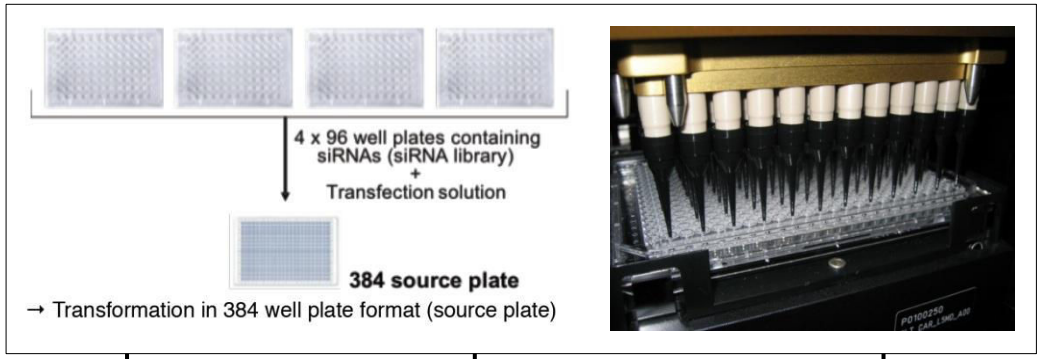


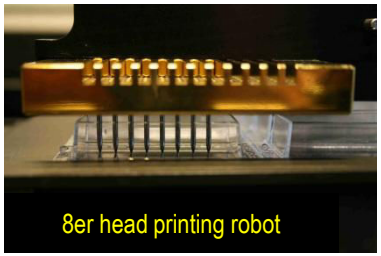
Ziauddin J. and Sabatini D.M., Nature 411, (2001)

Assay Automation

Screening Core Facility

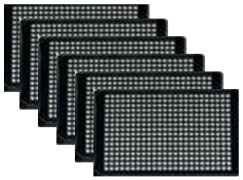
Assay automation | Automated microscopy





8er head printing robot

384er head printing robot

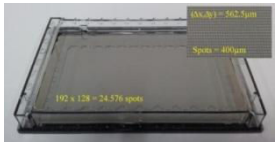


384er well plates

Erfle et al., JBS, 2008

LabTeks
(384er cell arrays)

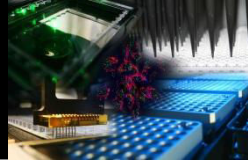
Erfle et al., Nat. Prot. (2), 2007



Cell arrays
(*whole genome*)

Reymann et al., BioTech, 2009

Object Scanning



Wide field [Olympus IX81 *ScanR*]

► **2D low resolution: Fully automated [10x/20x objective lens]**

1 scan field:	**1-3s**	(1 channel | auto-focusing)
1000 scan fields:	**1 hour**	(3 channels | auto-focusing | stage moving)

Confocal [Leica TCS SP5]

► **3D high resolution: Fully automated [60x/63x objective lens]**

1 scan field:	**30s**	(1 channel | 30 optical sections | auto-focusing)
1000 scan fields:	**1 day**	(3 channels | auto-focusing | stage moving)

Confocal [Leica TCS SP5]
Integrated: **Wide field** | **Super resolution**

► **2D super resolution: Semi-automated [63x/100x objective lens]**

1 scan field:	**3-10min**	(1 channel)
1000 scan fields:	**-**	

Screening Workflow & Timescales

Screening Core Facility

Assay automation | Automated microscopy

Assay Development

Optimisation → multiple cycles

Sample preparation
- siRNA Library
- Protocols
- Automation

Microscopy
- Resolution
- Dynamic range
- Automation

Image processing
- Sample quality
- Signal intensity

Assay

Data Acquisition

Microscopy
- Screening
  - High throughput
  - High content

Data storage
- Data management

Screen raw data

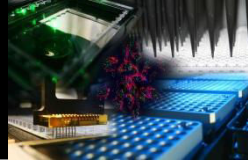
Data Analysis

Image processing
- Automation
- Quality control
- Features

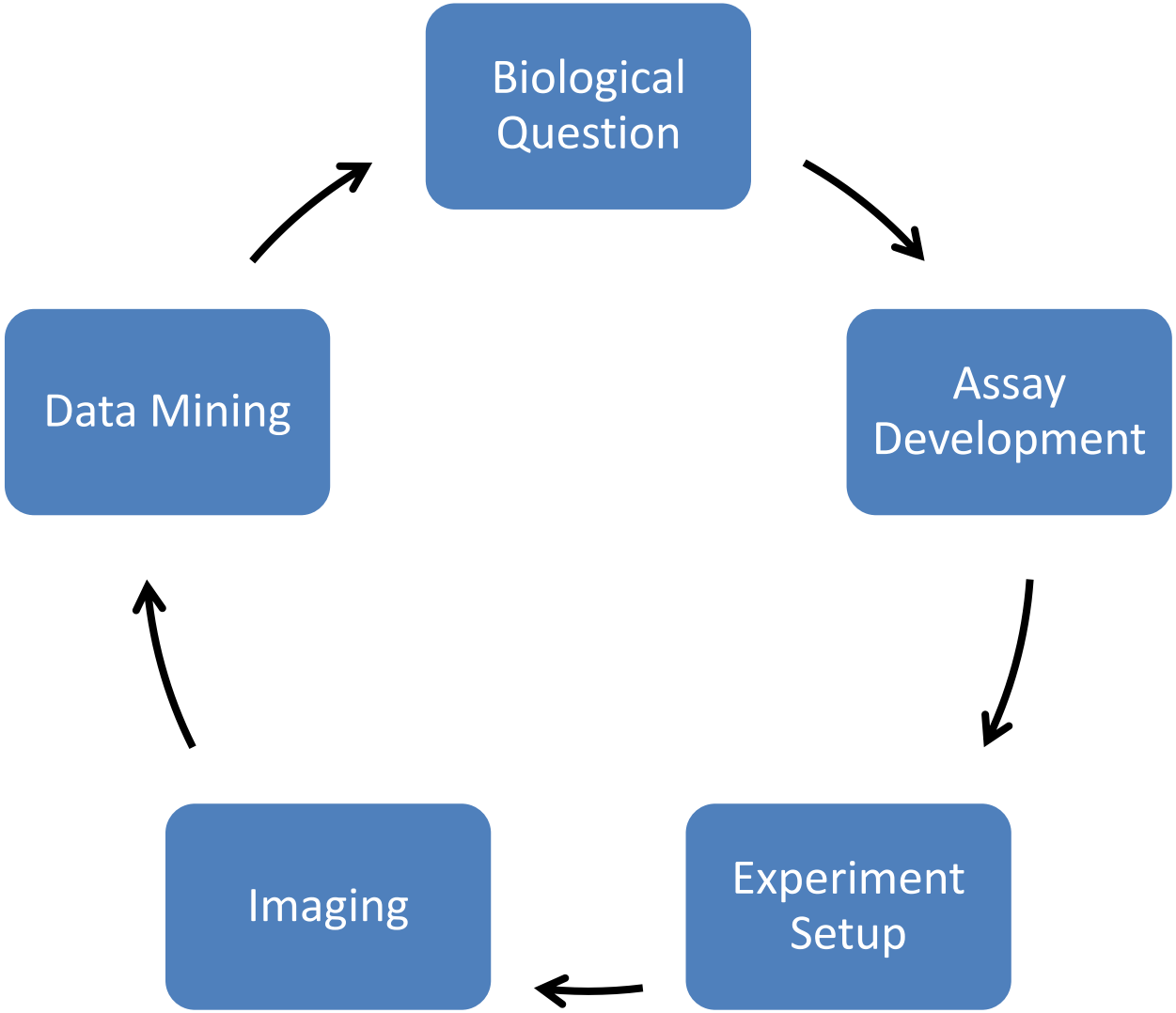
Statistics
- Normalisation
- Correlations

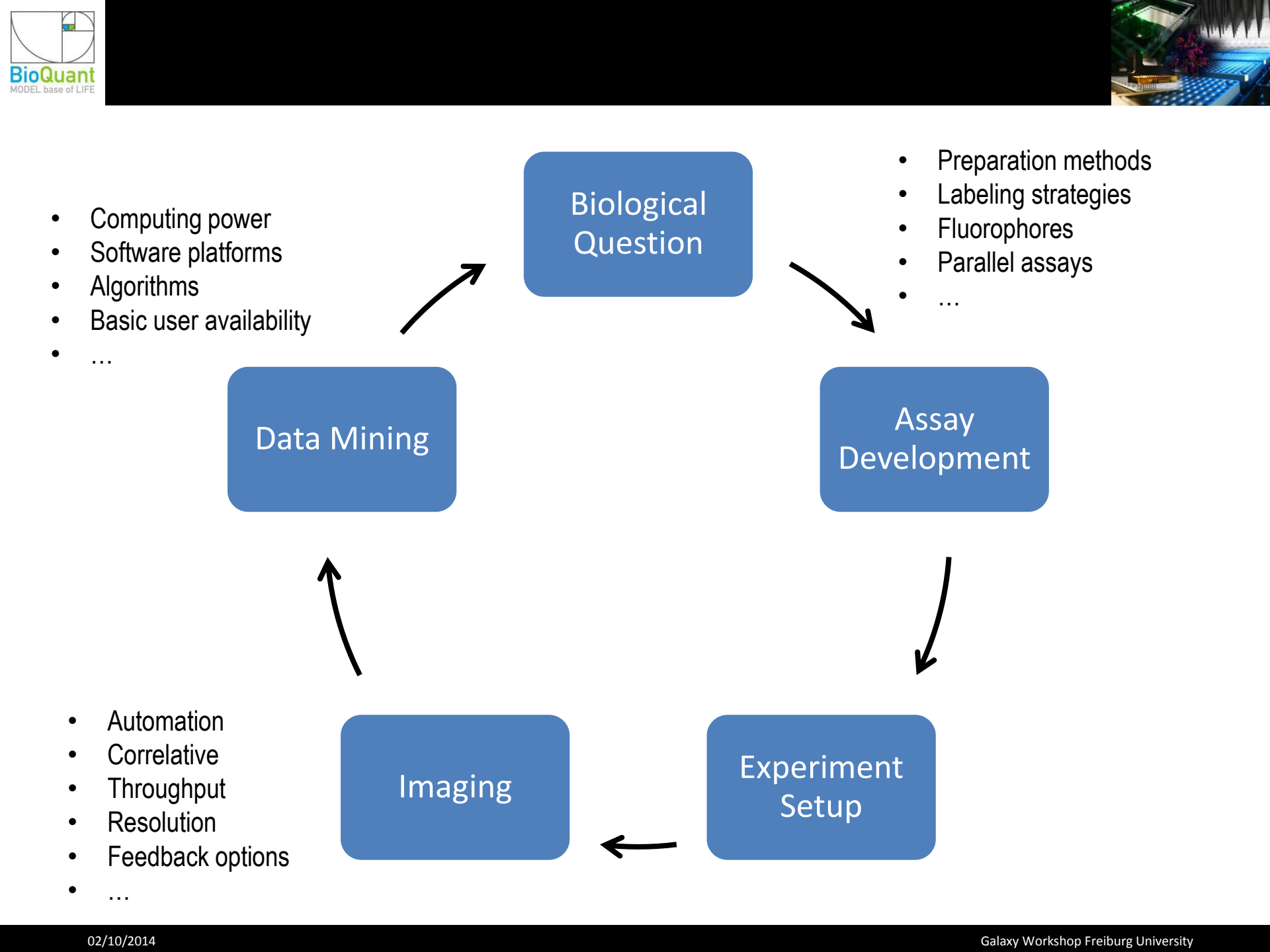
Hit calling

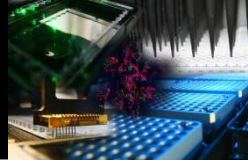
1 – 6 months → 1 – 4 weeks → 1 – 4 weeks



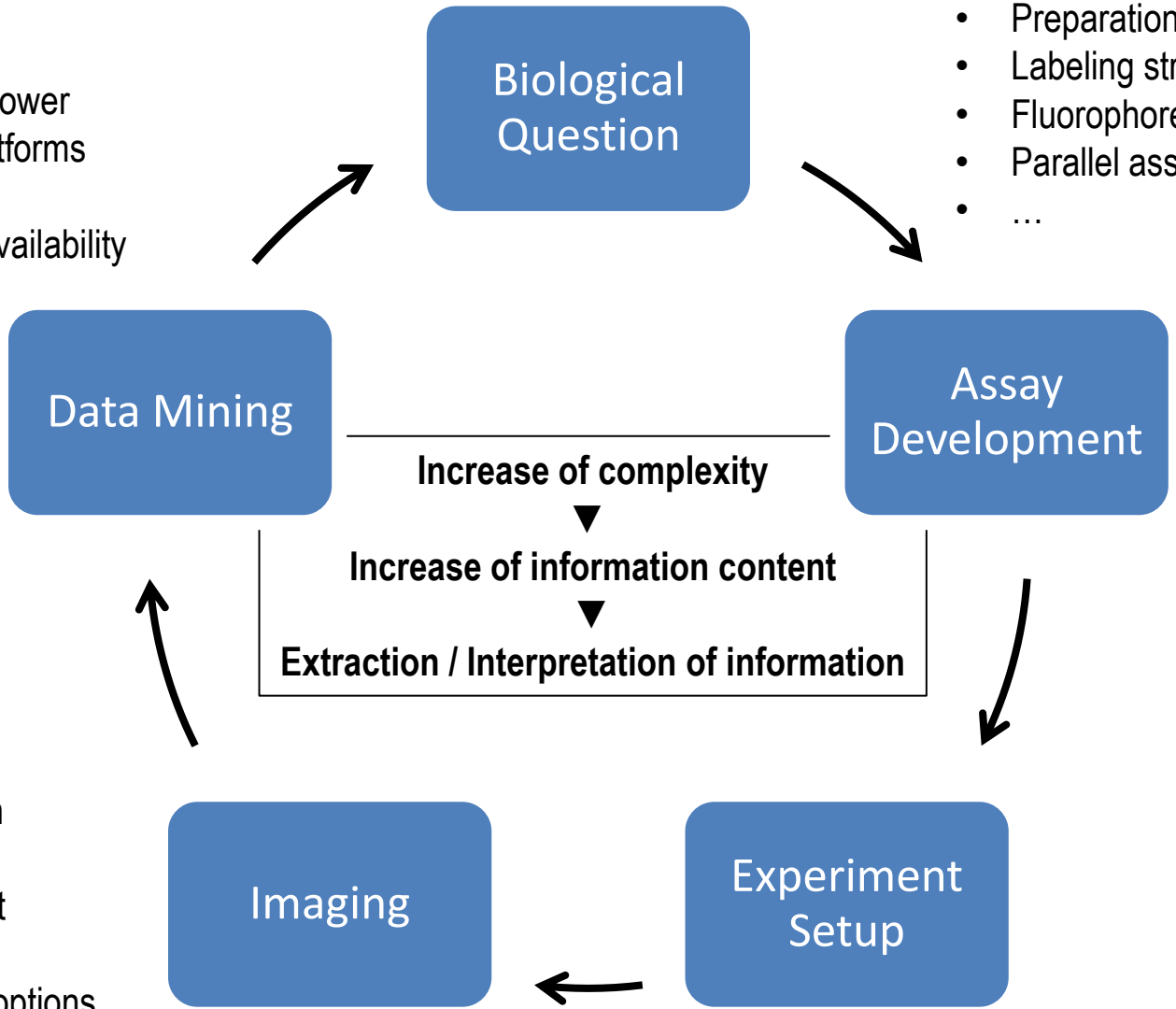
- Scientific Infrastructure
- Automated Data Pipelines
- Intelligent Automated Imaging


Biological Question
Assay Development
Experiment Setup
Imaging
Data Mining


Biological Question
Preparation methods
Labeling strategies
Fluorophores
Parallel assays
…
Assay Development
Experiment Setup
Imaging
Automation
Correlative
Throughput
Resolution
Feedback options
…
Data Mining
Computing power
Software platforms
Algorithms
Basic user availability
…

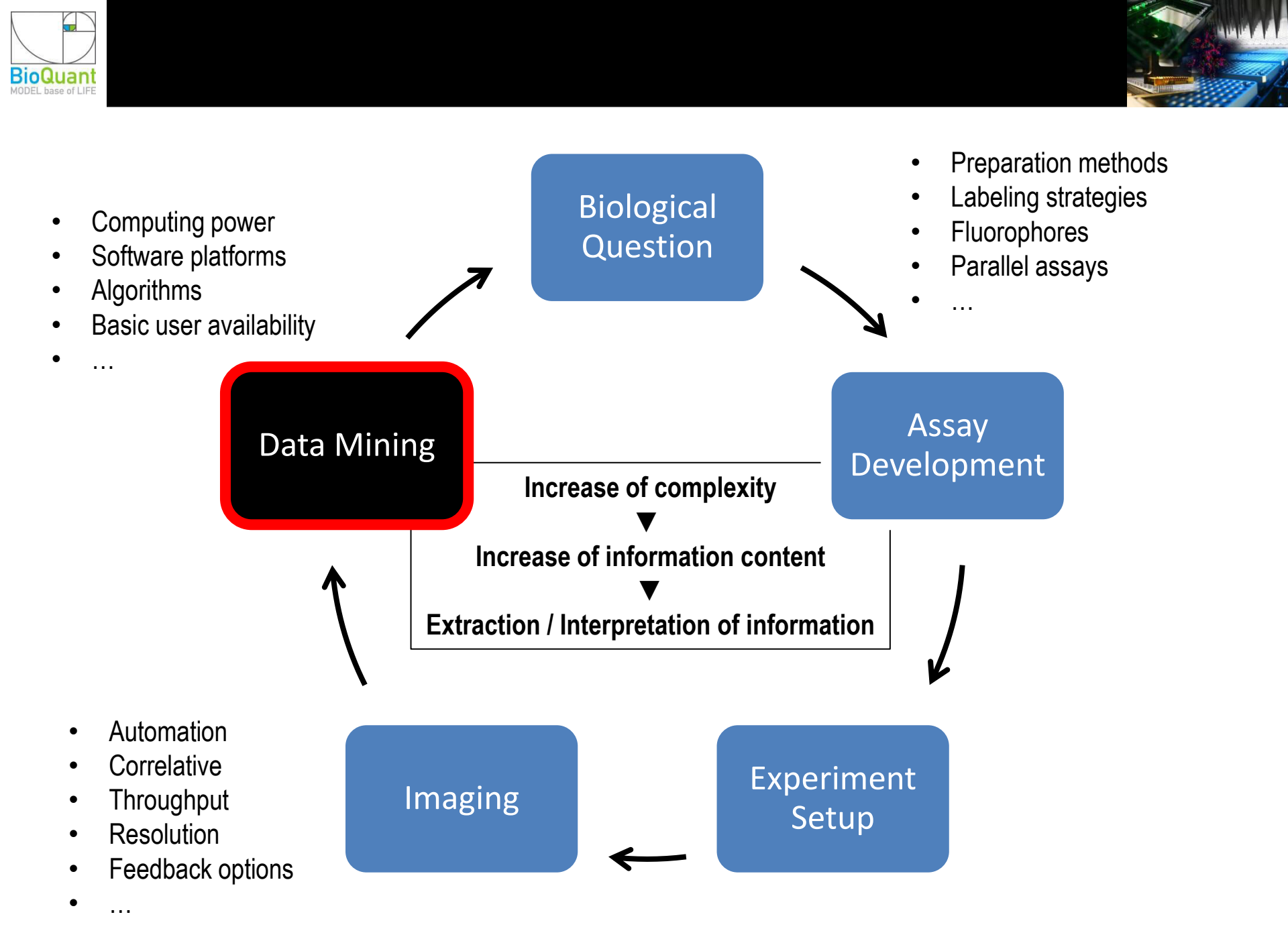


- Computing power
- Software platforms
- Algorithms
- Basic user availability
- …



- Preparation methods
- Labeling strategies
- Fluorophores
- Parallel assays
- …

- Automation
- Correlative
- Throughput
- Resolution
- Feedback options
- …

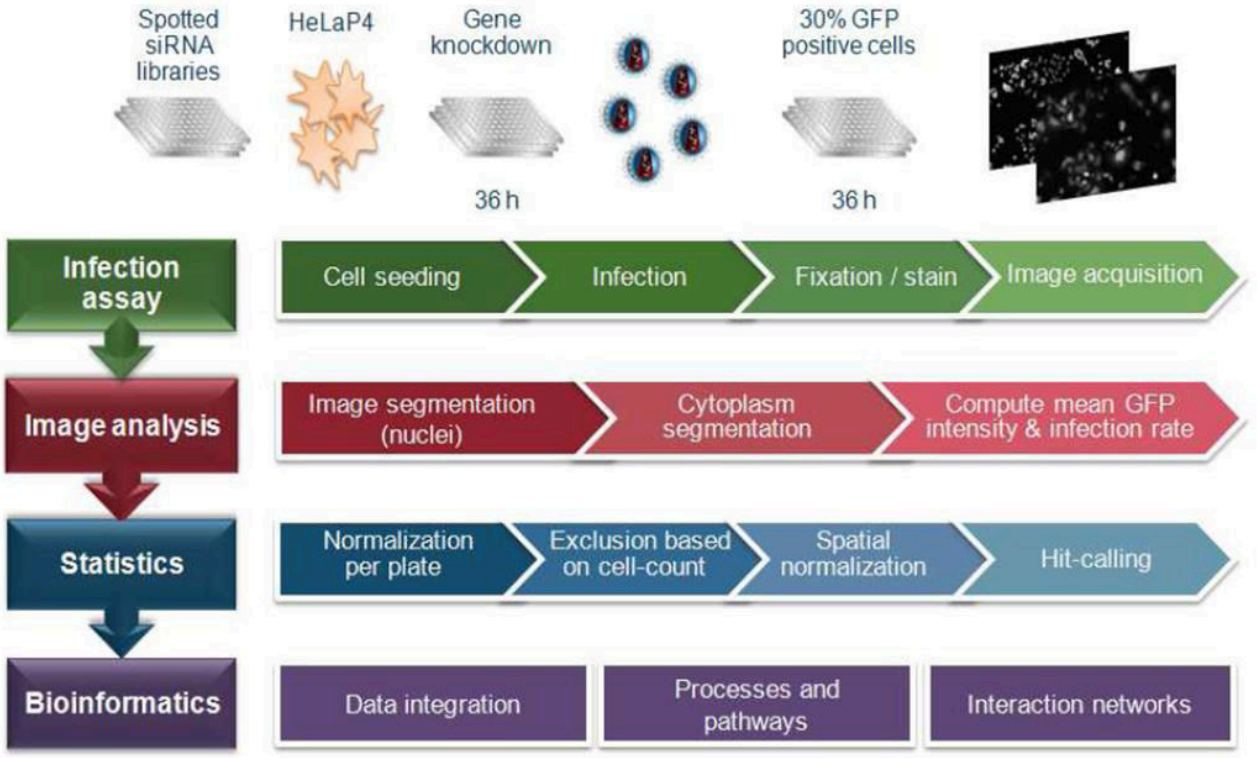

Biological Question
Assay Development
Experiment Setup
Imaging
Data Mining
Increase of complexity
Increase of information content
Extraction / Interpretation of information
• Preparation methods
• Labeling strategies
• Fluorophores
• Parallel assays
• …
• Computing power
• Software platforms
• Algorithms
• Basic user availability
• …
• Automation
• Correlative
• Throughput
• Resolution
• Feedback options
• …

Screening Workflow :: Biological Assay

Screening Core Facility

Assay automation | Automated microscopy

Genome wide RNAi screening platform to identify host factors involved in HIV-1 (HCV, DV, AAV, PV) replication



Boerner K. et al Biotechnol. J, 2010

Data Pipelines

Information Workflow :: Data Mining

Data Analysis

Automated data processing pipelines

Modular structure

Usability for **basic user**

Visualisation of raw data, meta data, intermediate and ultimate results

High-throughput capable, i.e. handling of large data sets, parallel computing, server-based functionalities

	Sample preparation	Data acquisition	Image Processing	Statistics	
siRNA library	automation	High-throughput	Quality control	Normalisation *(per plate/assay)*	Pathways
	Plate layouts	High-content	Normalisation	Spatial normalisation	Interaction networks
		Correlative	Segmentation	Hit calling	
		Data storage	Features		

Scree… … data

Image raw data
Image meta data

Analysis results

Analysis results

Extensive integrations → Libraries, format handling, data manipulation, …

Possibility to link to external **data bases**

[**Visualisation** (plate viewer, image viewer, plots) | **Tables** | **Meta**]

Data Base

Usability / Easy **Integration of established algorithms**

Data Analysis

Automated data processing pipelines

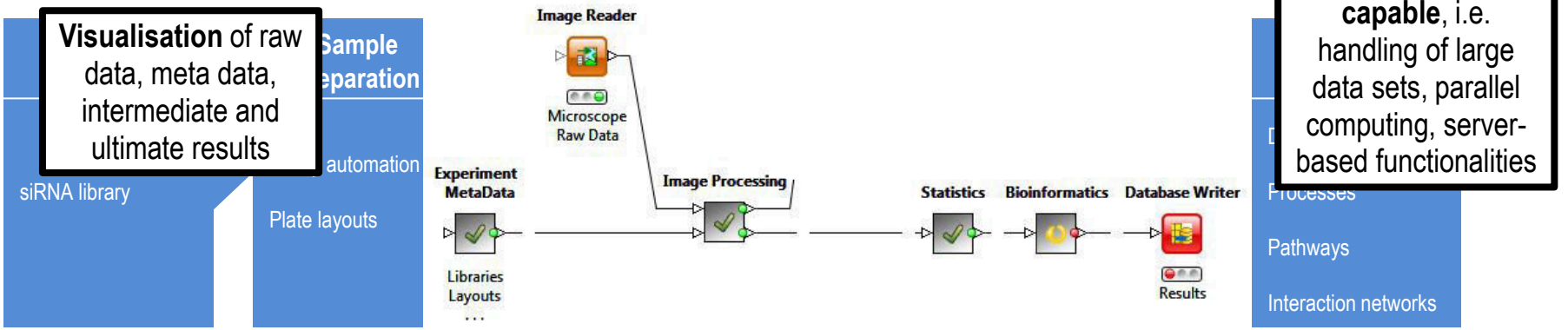
KNIME [http://www.knime.org]

Modular structure

Usability for **basic user**

Visualisation of raw data, meta data, intermediate and ultimate results

High-throughput capable, i.e. handling of large data sets, parallel computing, server-based functionalities



Extensive integrations → Libraries, format handling, data manipulation, …

Image raw data
Image meta data

Analysis results

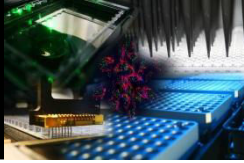
Analysis results

Possibility to link to external **data bases**

[**Visualisation** (plate viewer, image viewer, plots) | **Tables** | **Meta**

Usability / Easy **Integration of established algorithms**

Data Base



Data Analysis

Automated data processing pipelines

KNIME [http://www.knime.org]

Modular structure

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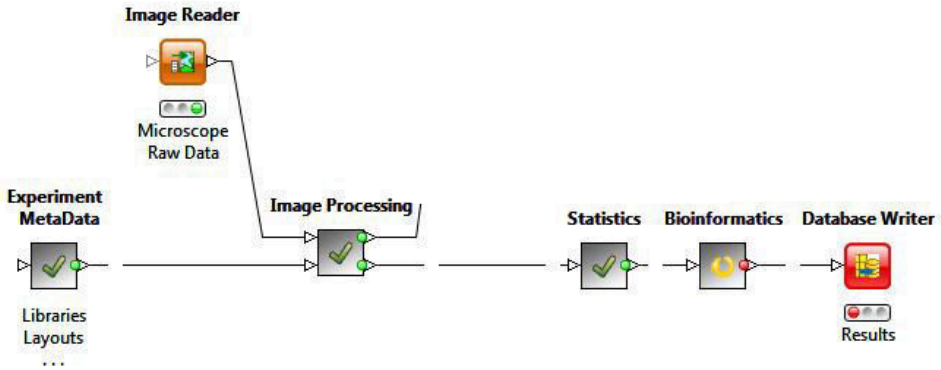
Visualisation of raw data, meta data, intermediate and ultimate results



High-throughput capable, i.e. handling of large data sets, parallel computing, server-based functionalities

Open-source / Platform independant

Extensive integrations → Libraries, format handling, data manipulation, …

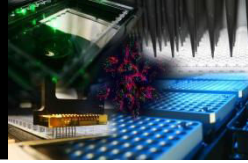
Sustainability → Supported by a large (developer) community / Usage of universal standards

Usability / Easy **Integration of established algorithms**

Possibility to link to external **data bases**

Data Pipelines

Information Workflow :: Data Mining

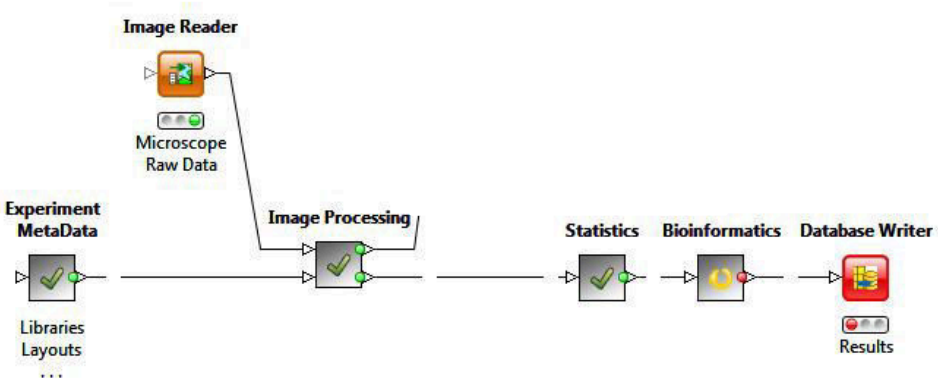


Integrations / Libraries

- ImageJ / ImageJ2
- ImgLib2 (Fiji)
- WEKA
- R
- SVM's
- Clustering
- Classifiers
- Network Mining
- Normalisations
- …

Microscope Control

- Target identification ▶ FeedBack



Data Base

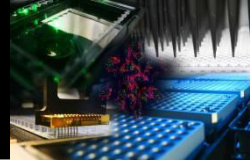
- OMERO

Data Handling / Data Views

- File reader / writer
- BioFormats (*image data*)
- OME-XML (*image data*)
- XML support
- Histograms / Plots
- Image / Table / Plate viewer
- Data manipulation
- Loop control
- …

Data Pipelines

Information Workflow :: Data Mining



File Edit View Node Help

100%

Workflow Repository

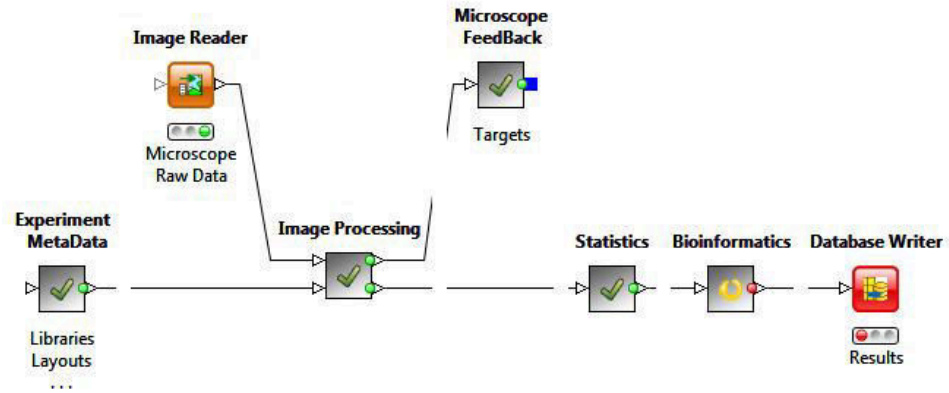
- EXAMPLES (guest@publicserver.knime.org:47037)
- LOCAL (Local Workspace)
  - _MISC_
  - _PIPELINES_
  - _PROJECTS_
  - EXPERIMENT-MetaData
  - IMAGE-PROCESSING
    - Co-localisationConfocal_point-like_CLUSTERING
    - Co-localisationConfocal_point-like_ROBUST
    - CountNuclei
    - CytoplasmIntensity
    - SpotsFinder
    - SpotsFinder_STATISTICS
  - MICROSCOPE-FeedBack
  - PHENOTYPIC-CLASSIFICATION
  - STATISTICS
  - tmp

Node Repository

- BioQuant
- IO
- Database
- Data Manipulation
- Data Views
- Statistics
- Mining
- Meta
- Flow Control
- Misc
- Community Nodes
  - KNIME Image Processing
    - IO
    - Image
    - Labeling
    - Features
    - Views
    - ImageJ2
    - ImageJ1
    - Tracking
    - Tess4J
  - HCS Tools
  - ImageBee neuro plugin
  - Matlab Scripting
  - NGS
  - R Scripting
- KNIME Labs
- Time Series
- Quick Form
- R
- Reporting
- Weka
- XML
- Nauru

Workflow Configuration

0: tmp



Outline

Console

KNIME Console

```
************************************************************************
***  Welcome to KNIME v2.9.1.0041089 - the Konstanz Information Miner  ***
***    Copyright, 2003 - 2013, Uni Konstanz and KNIME GmbH, Germany    ***
************************************************************************
Log file is located at: D:\Programming\KNIME\Workflows_2.9.x\.metadata\knime\knime.log
```

Node Description

Reads images from various file formats supported by the Bio-Formats library and imports them to the KNIME-internal image format. A list of the supported formats can be found here: http://loci.wisc.edu/bio-formats/formats

Dialog Options

Options

File browser

Choose your image files here and add them to the list of selected files. An image preview is available which will also help you to determine, whether an image format is supported or not. The list of the core meta data gives you some more information about the image object.

Additional Options

Append additional OME-XML-metadata column

You can add a column containing the metadata formated as an OME-XML-String (more details on http://www.ome-xml.org/).

Image Factory

Defines the way how the images are created and therewith kept in memory: Array Image Factory (stored as ONE array; pros: fast pixel access; cons: limited number of pixels per image), Planar Image Factory (each plane is stored in an array; pros: images are read faster; cons: limited number of pixels in an XY-plane, slower pixel access), and Cell Image Factory (multiple arrays of fixed sizes are used, pros: unlimited number of pixels per images; cons: very slow pixel reading and pixel access))

Use complete file path as row key

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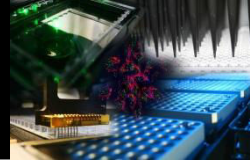
Check file format for each file

Data Pipelines

Information Workflow :: Data Mining



File Edit View Node Help

KNIME Explorer

Filter

EXAMPLES (guest@publicserver.knime.org:47037)
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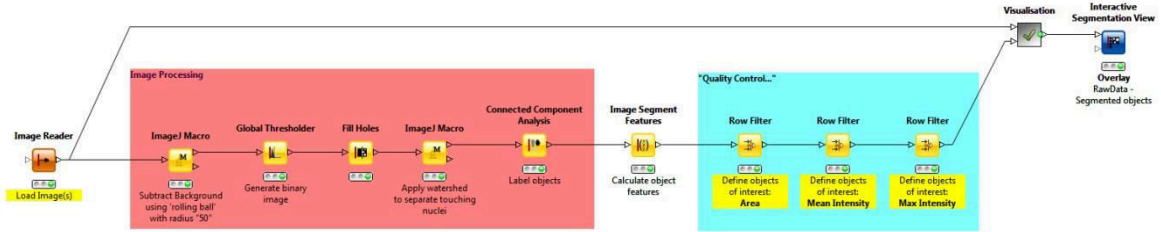
Features
Views
ImageJ2
ImageJ1
Tracking
Tess4J
HCS Tools
ImageBee neuro plugin
Matlab Scripting
NGS
R Scripting
KNIME Labs
Time Series
Quick Form
R
Reporting
Weka
XML
Nauru

0: tmp

Workflow Configuration

Nuclei registration



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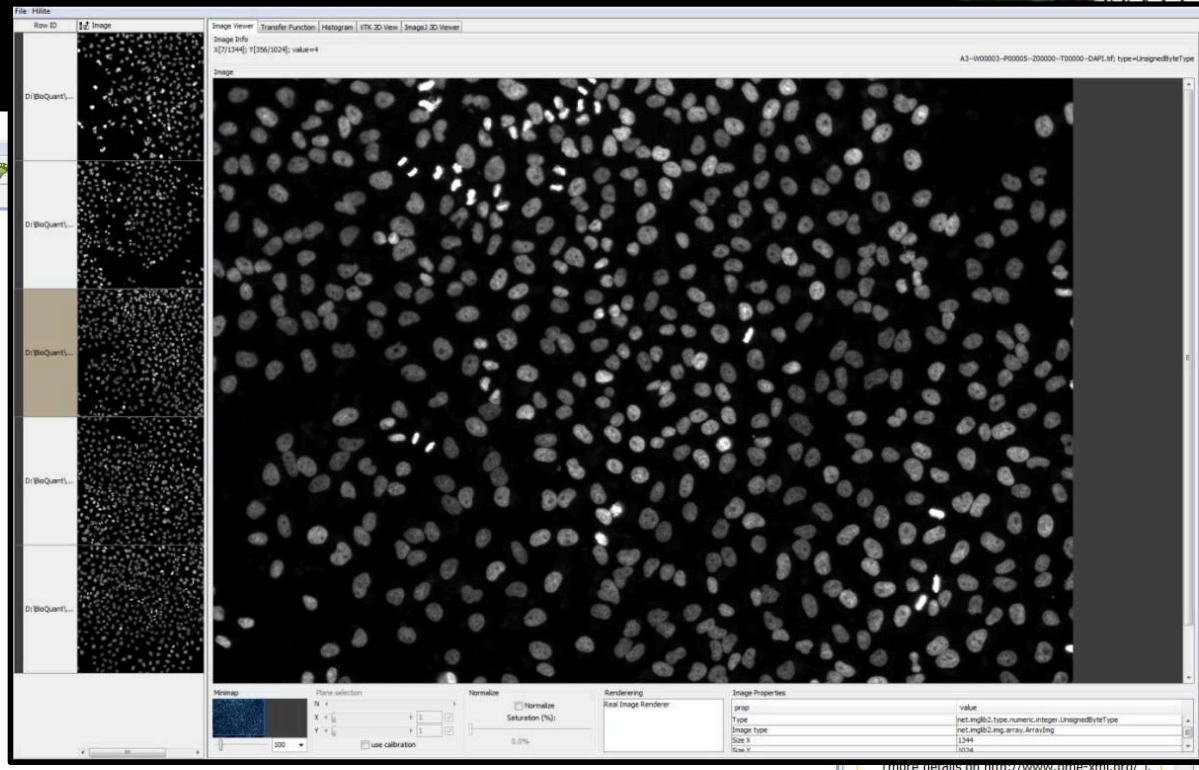
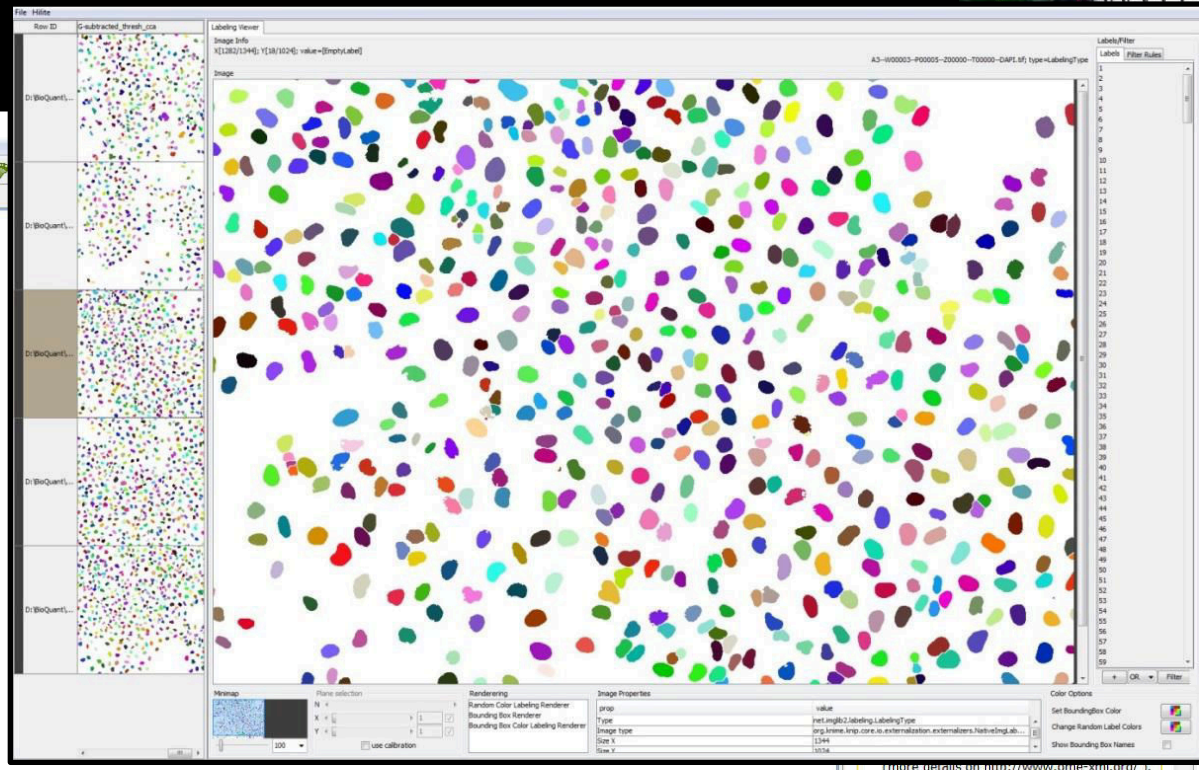
Data Pipelines

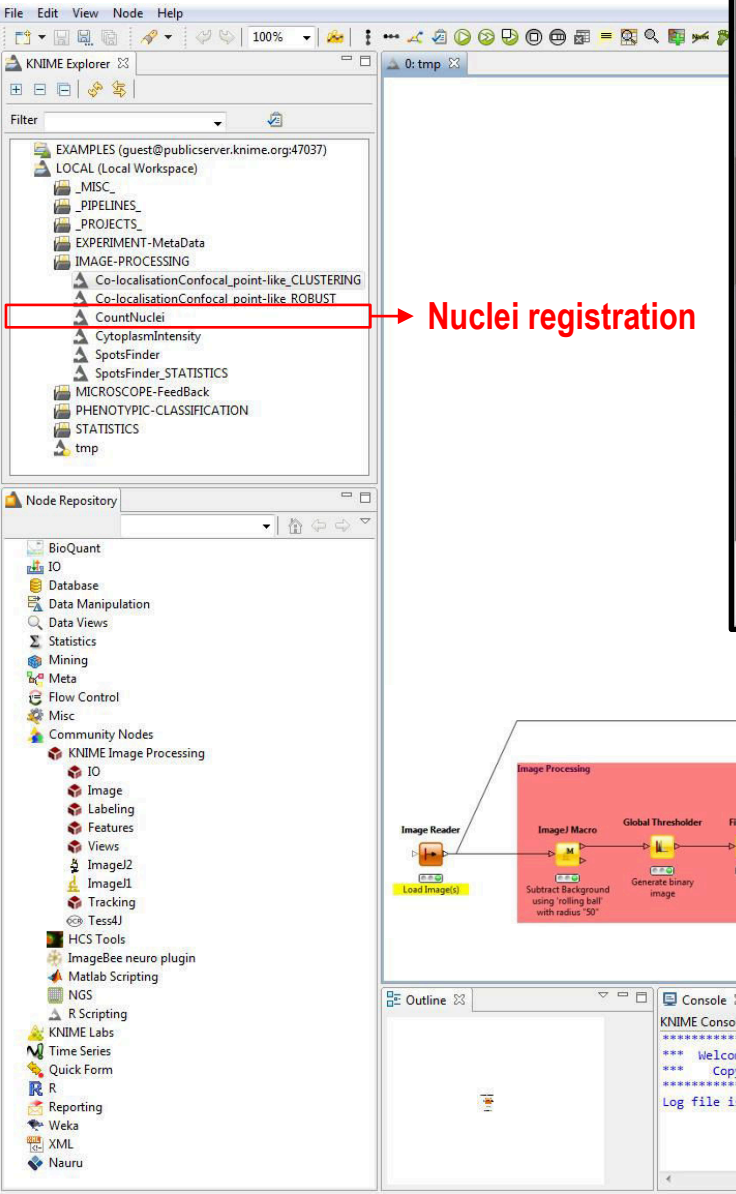


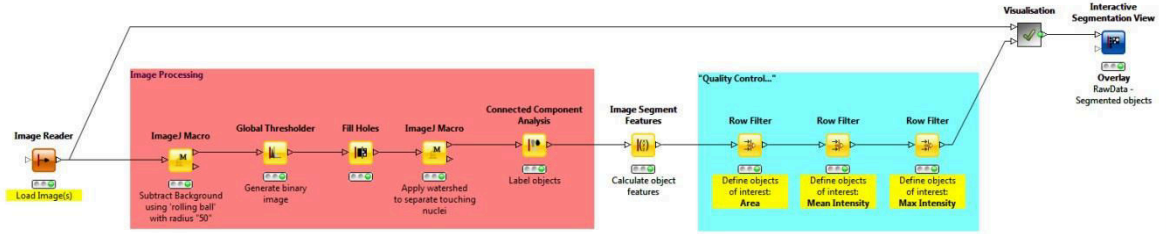
Nuclei registration

Data Pipelines



Nuclei registration





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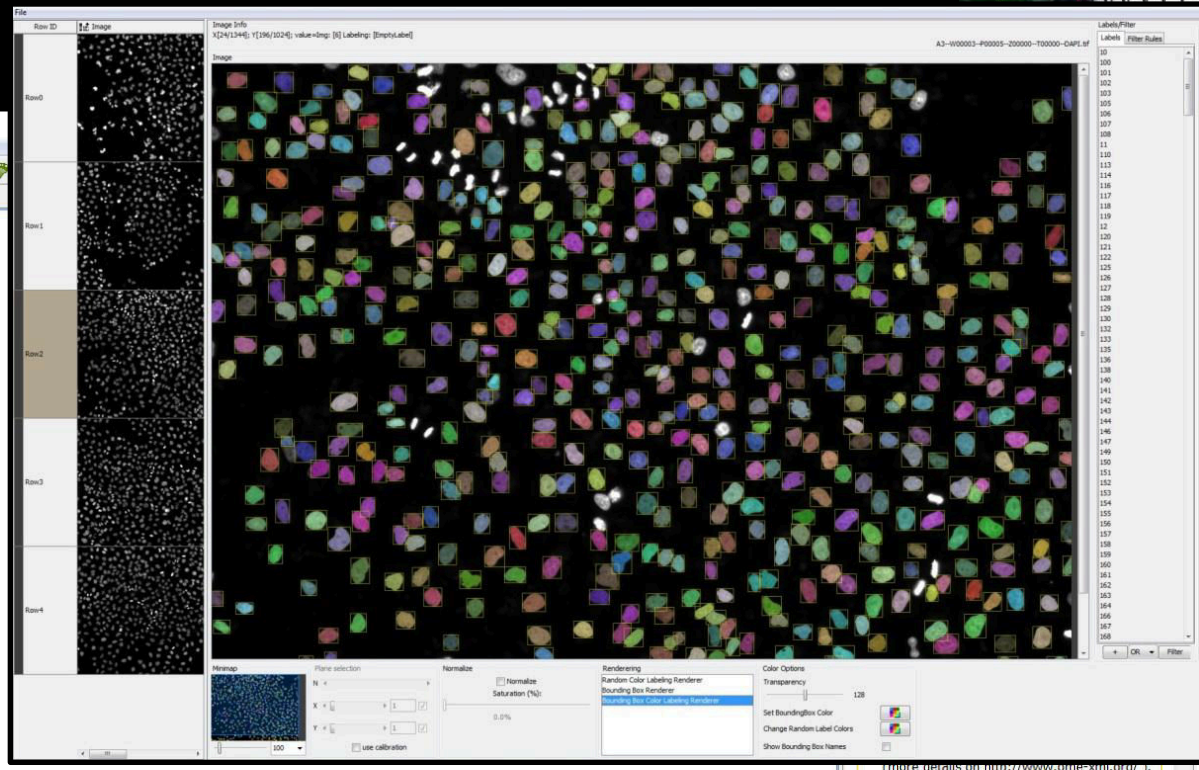
```

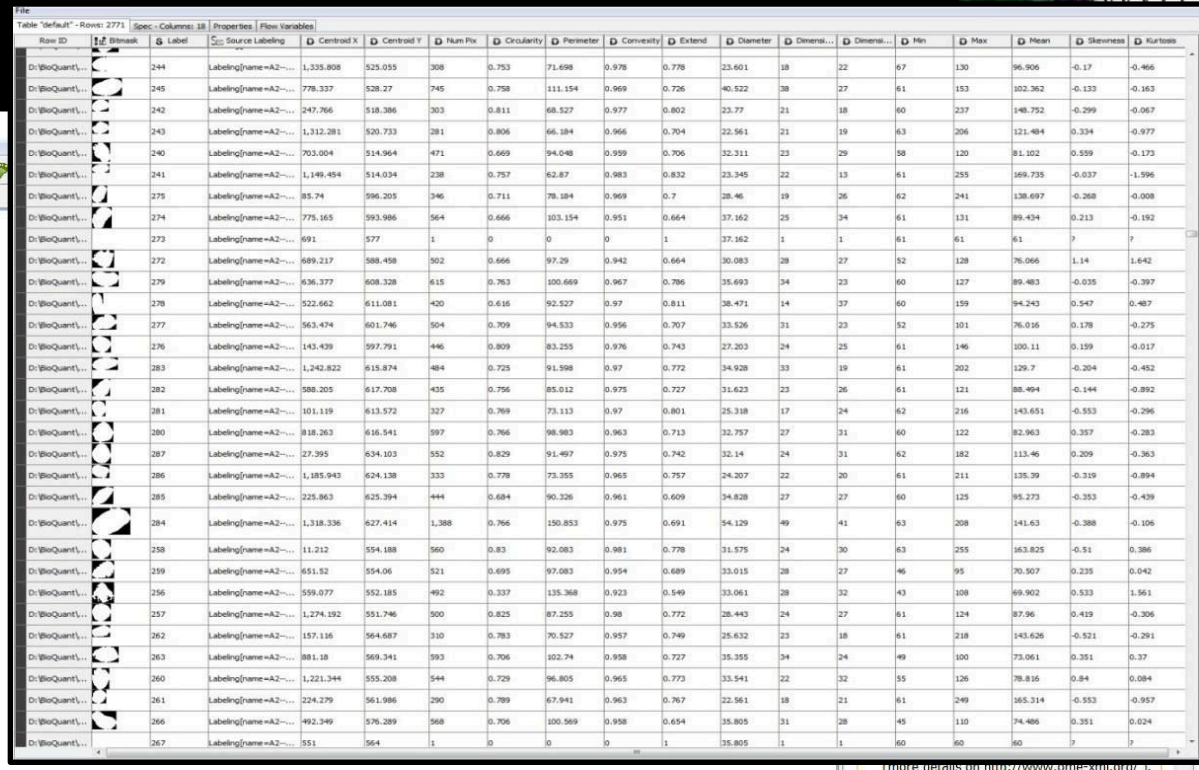
Data Pipelines

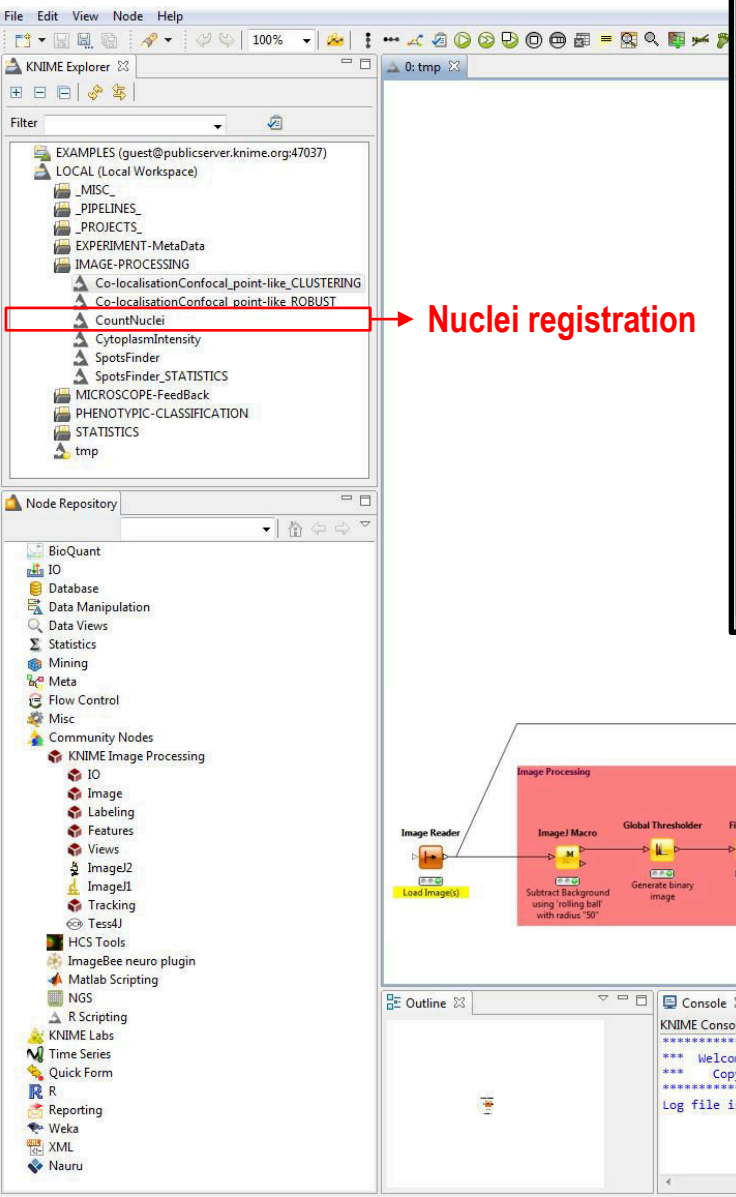


Nuclei registration

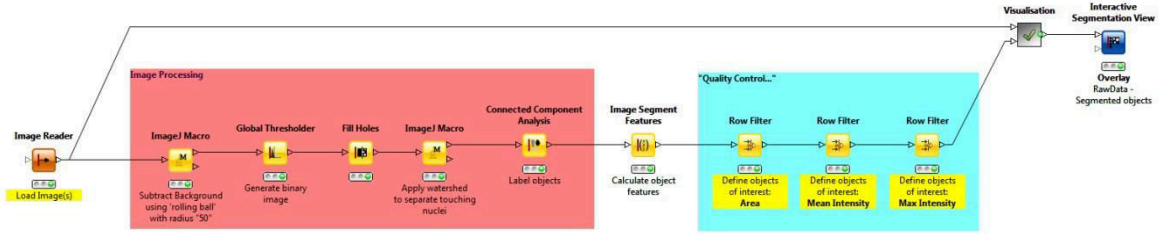
Data Pipelines

BioQuant
MODEL base of LIFE





Nuclei registration



(more details on http://www.ome-xml.org/).

Image Factory

Defines the way how the images are created and therewith kept in memory: Array Image Factory (stored as ONE array; pros: fast pixel access; cons: limited number of pixels per image), Planar Image Factory (each plane is stored in an array; pros: images are read faster; cons: limited number of pixels in an XY-plane, slower pixel access), and Cell Image Factory (multiple arrays of fixed sizes are used, pros: unlimited number of pixels per images; cons: very slow pixel reading and pixel access))

Use complete file path as row key

If checked, the complete file path will be used as row key, else only the file name.

Check file format for each file

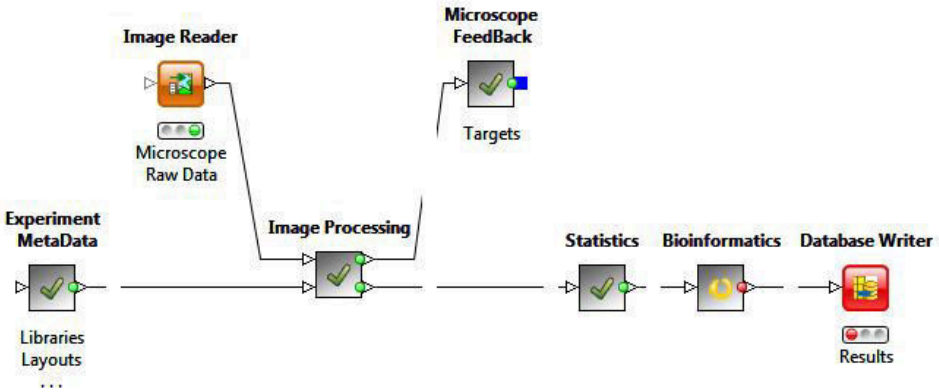
Outline

Console

KNIME Console

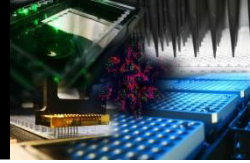
```
*** Welcome to KNIME v2.9.1.0041089 - the Konstanz Information Miner ***
***    Copyright, 2003 - 2013, Uni Konstanz and KNIME GmbH, Germany    ***
Log file is located at: D:\Programming\KNIME\Workflows_2.9.x\.metadata\knime\knime.log
```

Data Pipelines

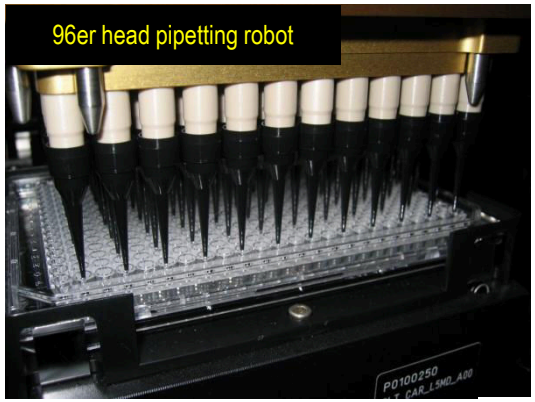


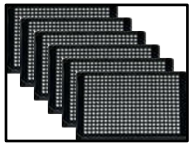
Data Pipelines

Experiment Meta Data



96er head pipetting robot





Well plates

Joined table - 0:717:681 - Joiner(Library)

File

Table "default" - Rows: 384 | Spec - Columns: 10 | Properties | Flow Variables

Row ID	Plate ID	96er Plate	Well (on 96er Plate)	Well (on 384er Plate)	Gene	Gene ID	siRNA ID	Ref Seq	siRNA Seq	Control
Row0_Row0	Plate I ExtDrg	Plate 1	A1	A1	INCENP	3619	28431	NM_020238	GGAGAAGAAGAAGCAGAU...	?
Row96_Row1	Plate I ExtDrg	Plate 2	A1	A2	CDH17	1015	14164	NM_004063	GGGACUUCUGUAUUACAA...	?
Row1_Row2	Plate I ExtDrg	Plate 1	A2	A3	C1QBP	708	9564	NM_001212	GGAAAGAAAAAUUCAGAA...	?
Row97_Row3	Plate I ExtDrg	Plate 2	A2	A4	MCP	4179	40648	NM_172356	GGAUACUUCUAUAUACCU...	down
Row2_Row4	Plate I ExtDrg	Plate 1	A3	A5	GFP	?	215041	?	CAAGCUGACCCUGAAGUU...	?
Row98_Row5	Plate I ExtDrg	Plate 2	A3	A6	CD74	972	14500	NM_001025...	GGAAGUGGCCAAAAGCUA...	?
Row3_Row6	Plate I ExtDrg	Plate 1	A4	A7	BIN3	55909	27292	NM_018688	GGCCAAGGUGGAGAAGUA...	?
Row99_Row7	Plate I ExtDrg	Plate 2	A4	A8	CCL8	6355	16649	NM_005623	GGGAUUCCAUGAAGCAUC...	?
Row4_Row8	Plate I ExtDrg	Plate 1	A5	A9	BAG3	9531	137538	NM_004281	GCCAUUGAUGUCCCAGGU...	?
Row100_Row9	Plate I ExtDrg	Plate 2	A5	A10	CCL2	6347	44845	NM_002982	GCCAGAUGCAAUCAAUGC...	?
Row5_Row10	Plate I ExtDrg	Plate 1	A6	A11	ARID5A	10865	216172	NM_212481	GCAGAACGGAAUCCAGAA...	?
Row101_Row11	Plate I ExtDrg	Plate 2	A6	A12	CLPX	10845	105146	NM_006660	GGUAAAACUCUGCUGGCA...	?
Row6_Row12	Plate I ExtDrg	Plate 1	A7	A13	AP1M1	8907	34372	NM_032493	GGUGUUUUCCGAGUACUU...	?
Row102_Row13	Plate I ExtDrg	Plate 2	A7	A14	CLDN23	137075	128552	NM_194284	GGUCCUAAUGCUGUACUA...	down
Row7_Row14	Plate I ExtDrg	Plate 1	A8	A15	DV-E	?	292261	n/a	ACACCAGAAUUGAAUCAC...	?
Row103_Row15	Plate I ExtDrg	Plate 2	A8	A16	CLDN3	1365	9801	NM_001306	GGACUACGUCUAAGGGAC...	?
Row8_Row16	Plate I ExtDrg	Plate 1	A9	A17	CBFB	865	6569	NM_022845	GGGAGAACAGCGACAAAC...	?
Row104_Row17	Plate I ExtDrg	Plate 2	A9	A18	CLDN4	1364	9892	NM_001305	GGUGCUGUAAACAGGUUU...	?
Row9_Row18	Plate I ExtDrg	Plate 1	A10	A19	CCAR1	55749	148709	NM_018237	GCAAACCCUCUUAACACA...	?
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Row10_Row20	Plate I ExtDrg	Plate 1	A11	A21	TIP120A	55832	27001	NM_018448	GGAAGAUGUCUCUGUUCA...	?
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Row11_Row22	Plate I ExtDrg	Plate 1	A12	A23	CADPS2	93664	25868	NM_017954	GGAGAUUUCACCACCACC...	?
Row107_Row23	Plate I ExtDrg	Plate 2	A12	A24	CLDN7	1366	9802	NM_001307	GGAGUAUGUGUGACCUGG...	?
Row192_Row24	Plate I ExtDrg	Plate 3	A1	B1	INCENP	3619	28431	NM_020238	GGAGAAGAAGAAGCAGAU...	?
Row288_Row25	Plate I ExtDrg	Plate 4	A1	B2	MYLK2	85366	103539	NM_033118	GGUGGUGAAUUAUGACCA...	?
Row193_Row26	Plate I ExtDrg	Plate 3	A2	B3	DSC2	1824	30869	NM_004949	GGUCCUAAAGAAAAGACA...	?
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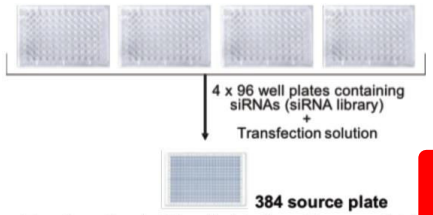
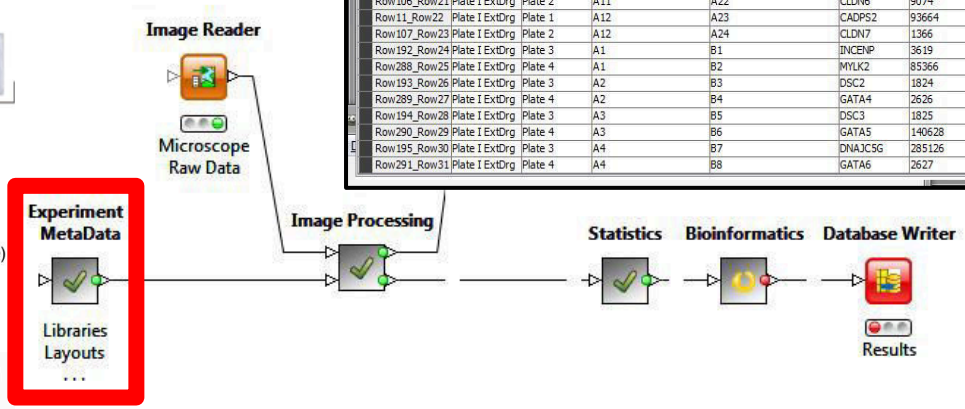
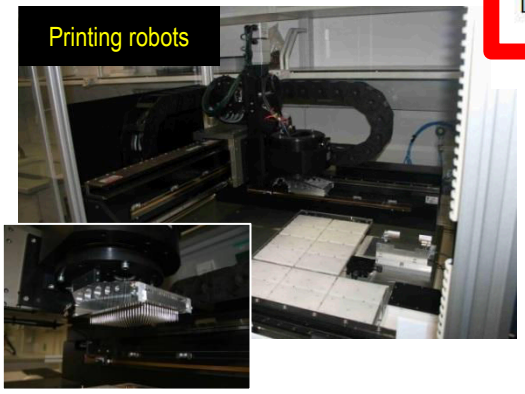
Row194_Row28	Plate I ExtDrg	Plate 3	A3	B5	DSC3	1825	10876	NM_024423	GGCAGACAAAAUAAUUGG...	up
Row290_Row29	Plate I ExtDrg	Plate 4	A3	B6	GATA5	140628	6857	NM_080473	GGGAAAGUGAAAUAAAGC...	?
Row195_Row30	Plate I ExtDrg	Plate 3	A4	B7	DNAJC5G	285126	41208	NM_173650	GGUCAGGAGCCAAAUGUG...	?
Row291_Row31	Plate I ExtDrg	Plate 4	A4	B8	GATA6	2627	3223	NM_005257	GGAAACGAAAACCUAAGA...	?

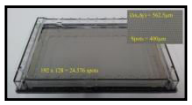




Printing robots



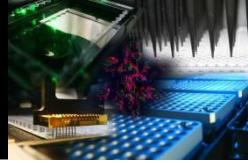
384 cell arrays (LabTeks)



Whole genome cell arrays

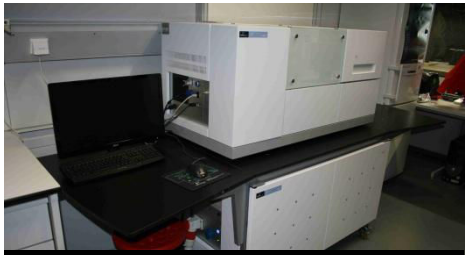
Data Pipelines

Image Raw Data

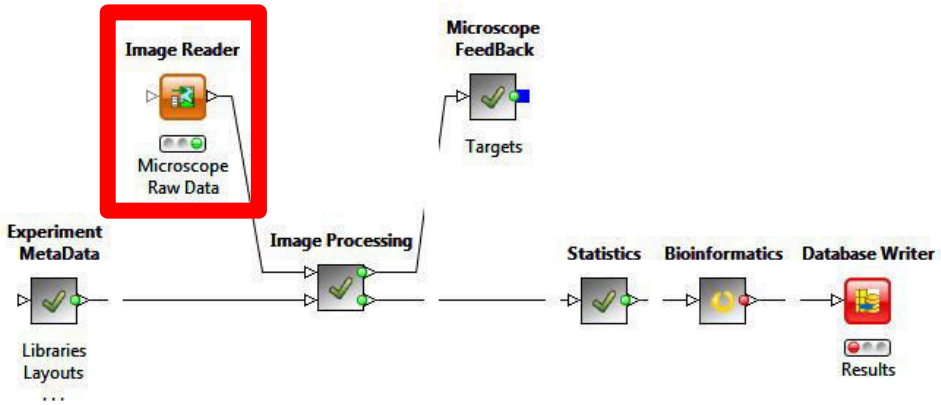


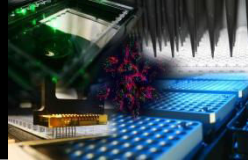
Wide field [3x Olympus IX81 *ScanR*]

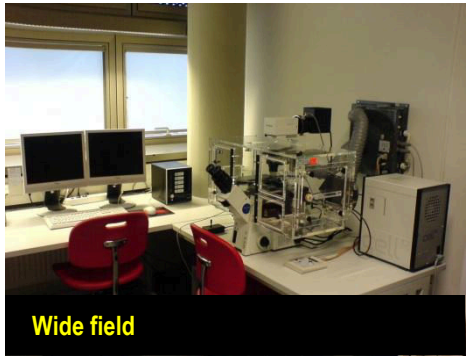
Confocal [2x (exp.) Leica TCS SP5]
Integrated: **Wide field** | **Super resolution**



Spinning disc [Perkin Elmer *Opera LX*]

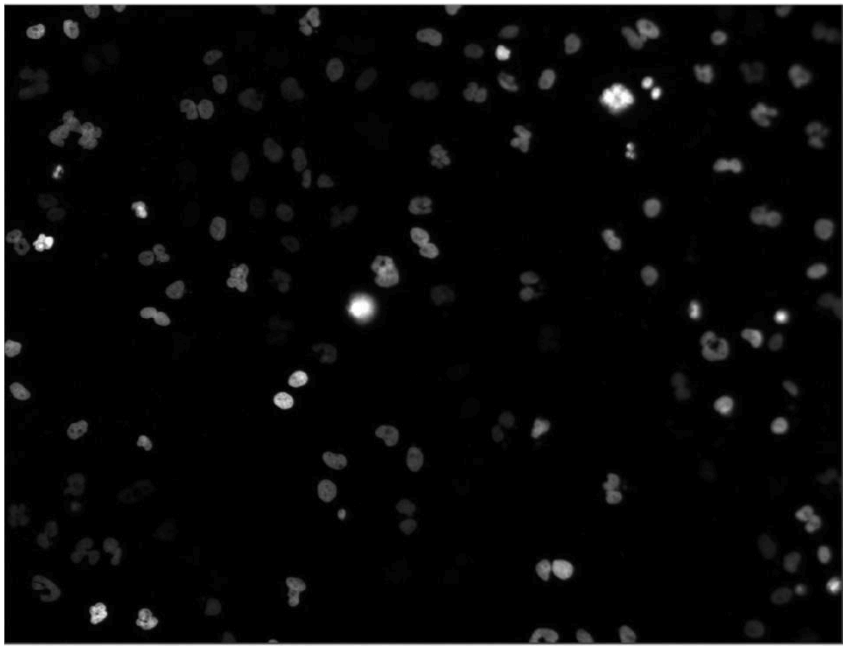


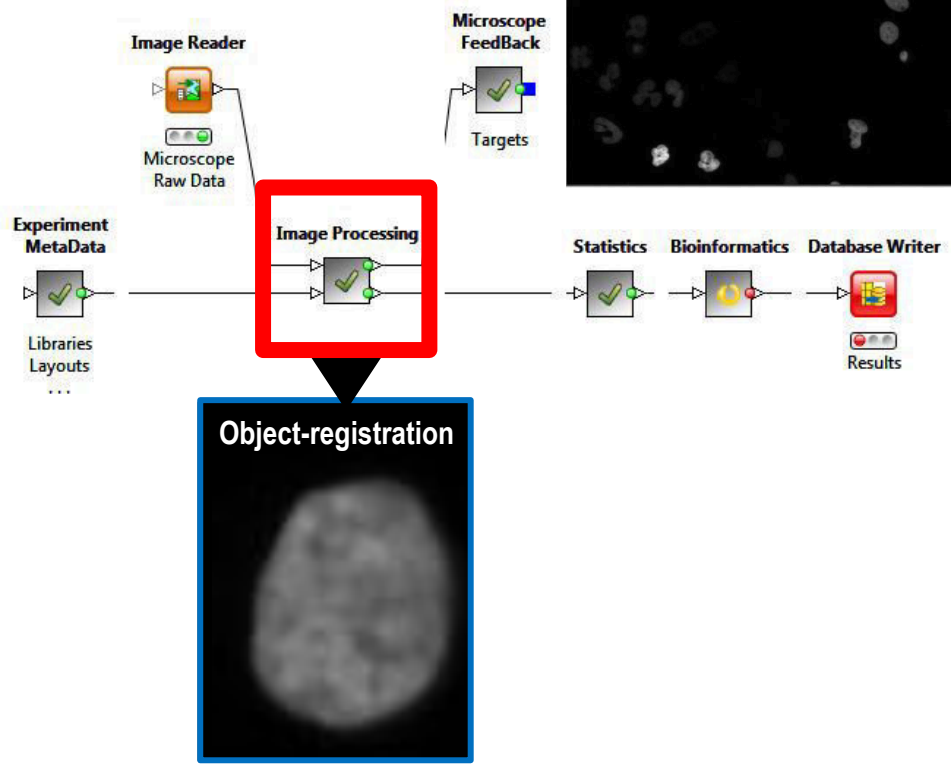




2D high-throughput images

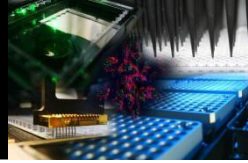
- Nuclei - NO phenotypic penetration

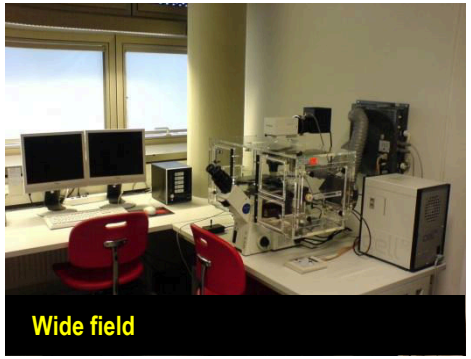




Data Pipelines

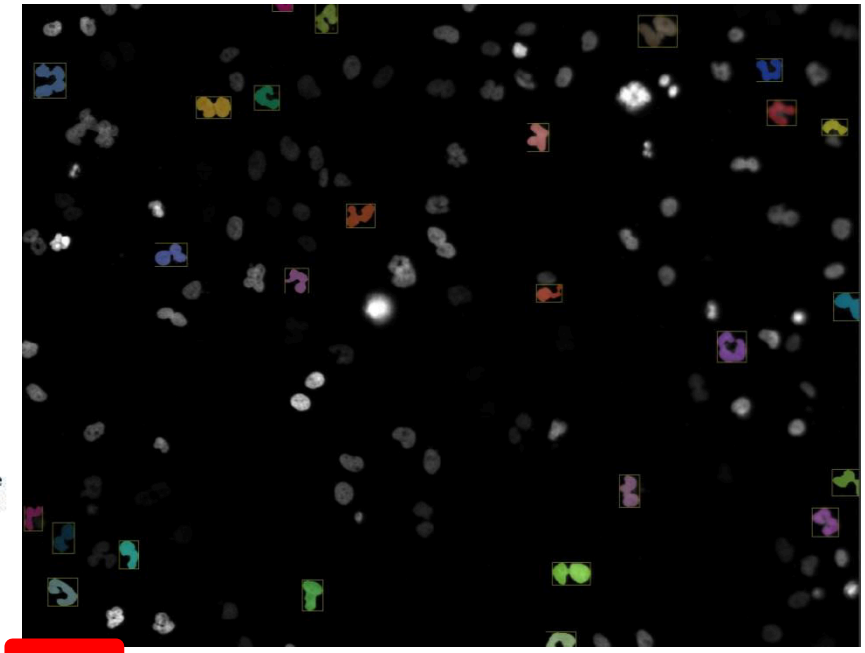
Statistics

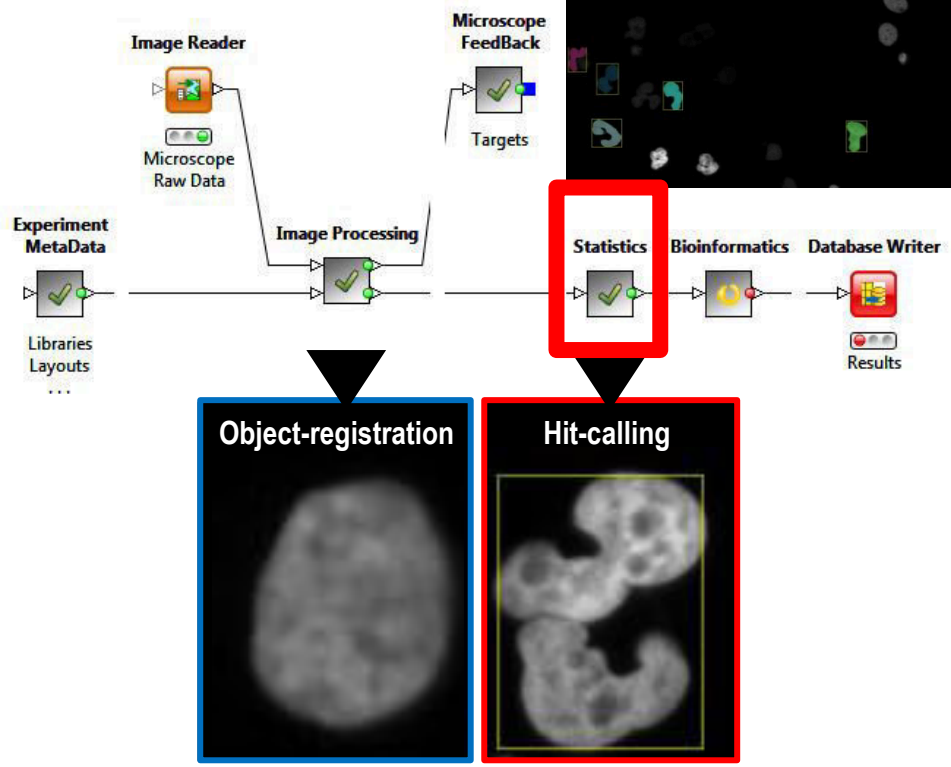


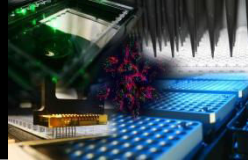


2D high-throughput images

- Nuclei - NO phenotypic penetration
- siRNA *INCENP* phenotypes

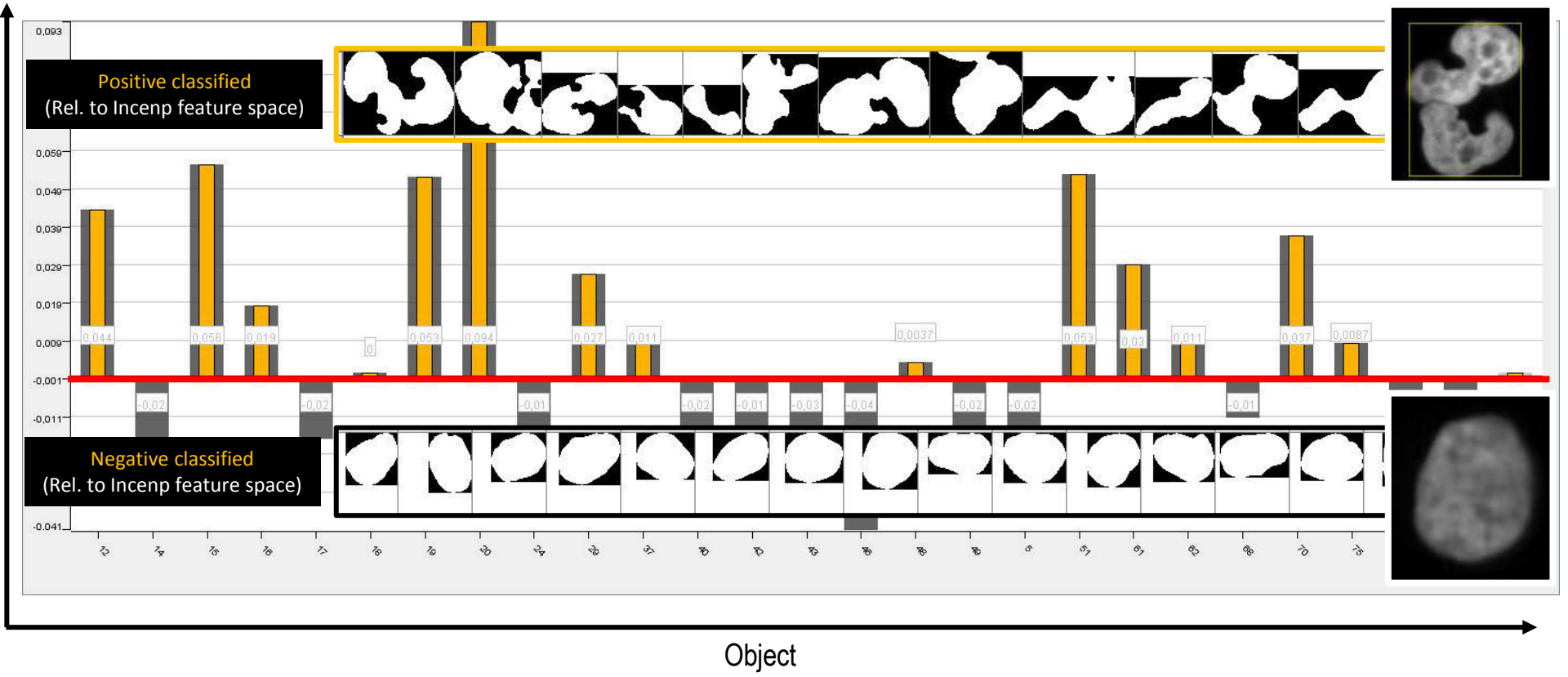


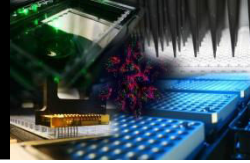




n = 8

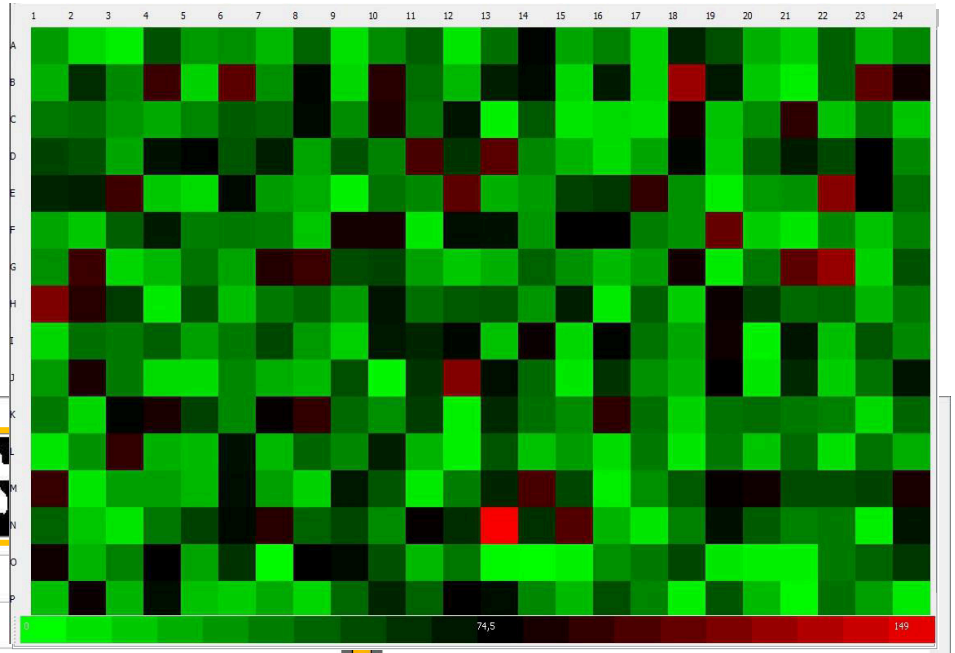
Rel. distance of object to *INCENP* feature space

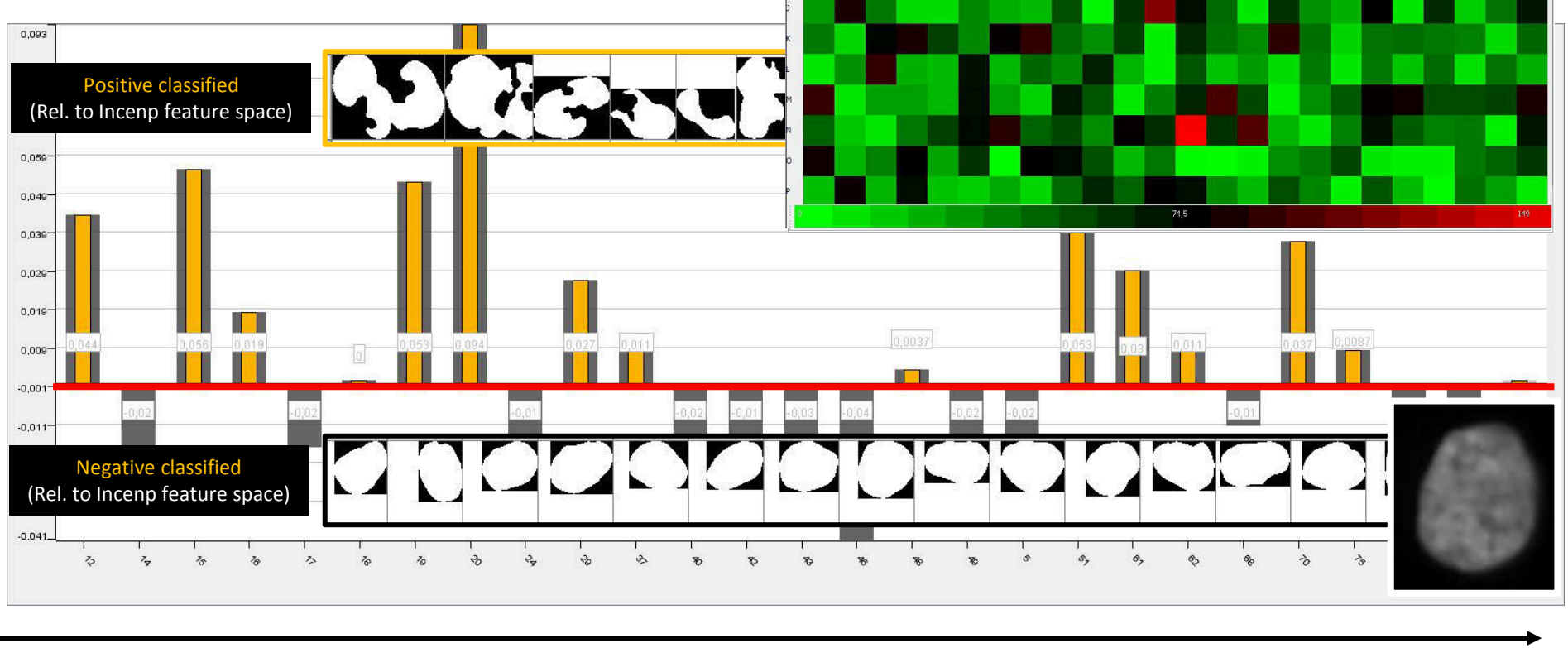




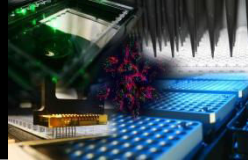
n = 8

Rel. distance of object to *INCENP* feature space





Object



UNIVERSITY OF HEIDELBERG

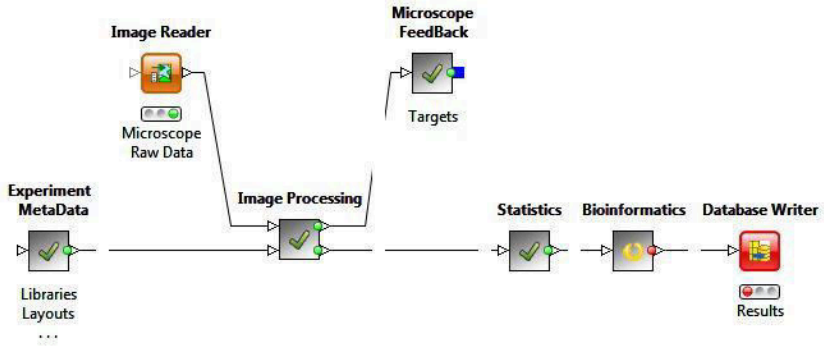
BioQuant
MODEL base of LIFE

- Home
- Screening Core Facility
- Data Analysis
  - **Workflow Repository**
    - Experiment MetaData
    - Image Processing
    - Microscope FeedBack
    - Statistics
- Research & Development
- Publications
- Contact

Contact | Search | Sitemap

University of Heidelberg > Research Institutions > BIOQUANT > Technology Platforms > VIROQUANT-CellNetworks RNAi Screening Facility > Data Analysis > Workflow Repository

Workflow Repository



» For non-executed nodes (red dot), no workflow solutions are provided so far.

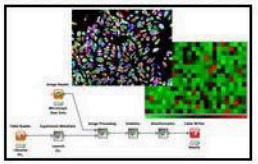
» Contact / Further information: juergen.reymann (at) bioquant.uni-heidelberg.de

Contact: E-Mail (Last update: 19/02/2014)

SEARCH & FIND

advanced search | Sitemap

KNIME



Getting started (ReadMe)

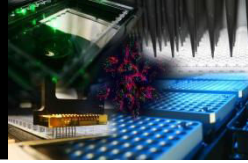
- Installation
- KNIME :: *Quickstart*

Installation files

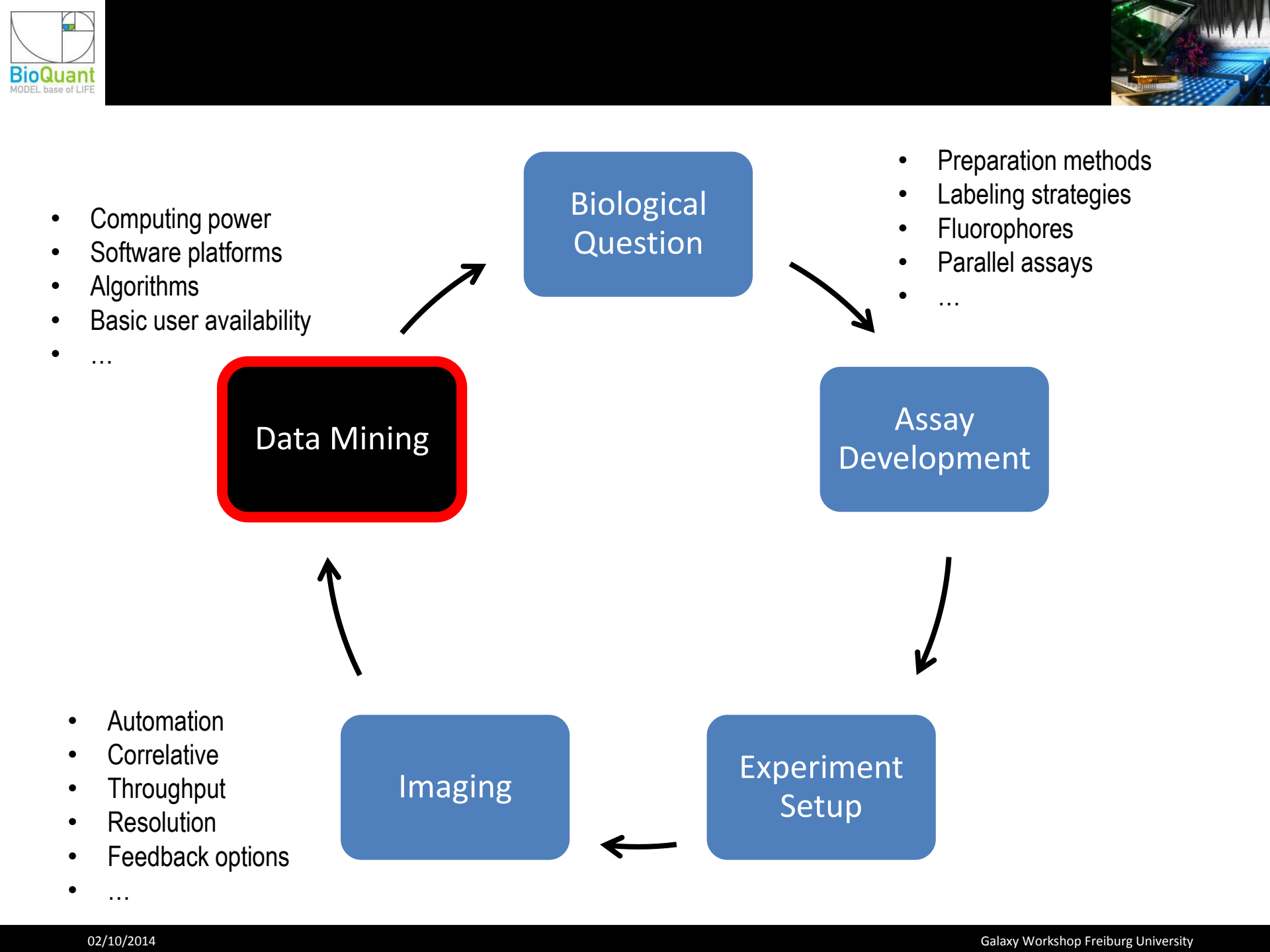
- KNIME -Desktop

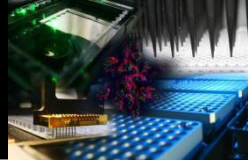
Internal plugins

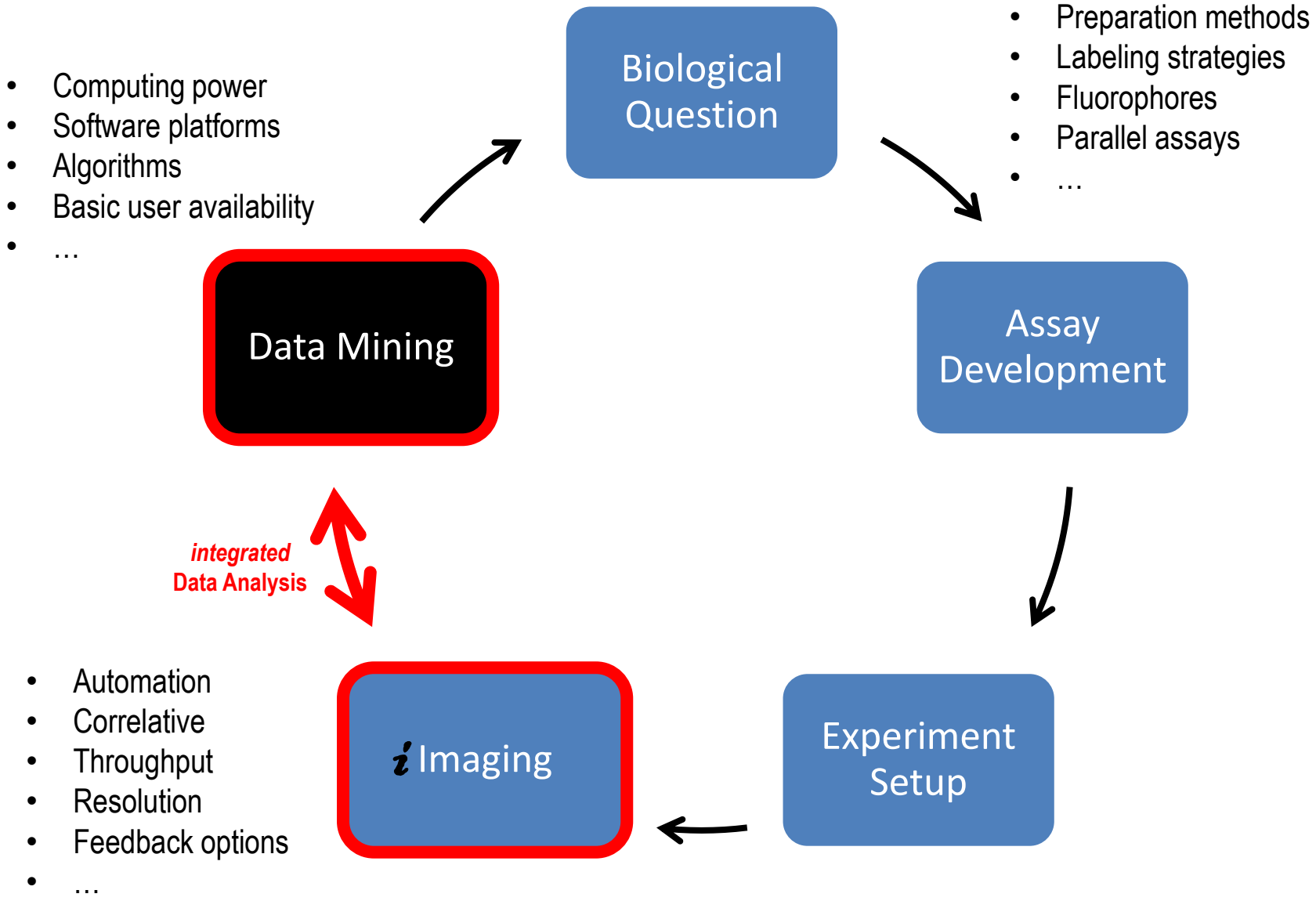
- RNAi Screening Facility: Plugin 1 (source)
- RNAi Screening Facility: Plugin 2
- RNAi Screening Facility: CellProfiler [beta]



- Scientific Infrastructure
- Automated Data Pipelines
- Intelligent Automated Imaging


Biological Question
Preparation methods
Labeling strategies
Fluorophores
Parallel assays
…
Assay Development
Experiment Setup
Imaging
Automation
Correlative
Throughput
Resolution
Feedback options
…
Data Mining
Computing power
Software platforms
Algorithms
Basic user availability
…




Biological Question
Preparation methods
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…
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Computing power
Software platforms
Algorithms
Basic user availability
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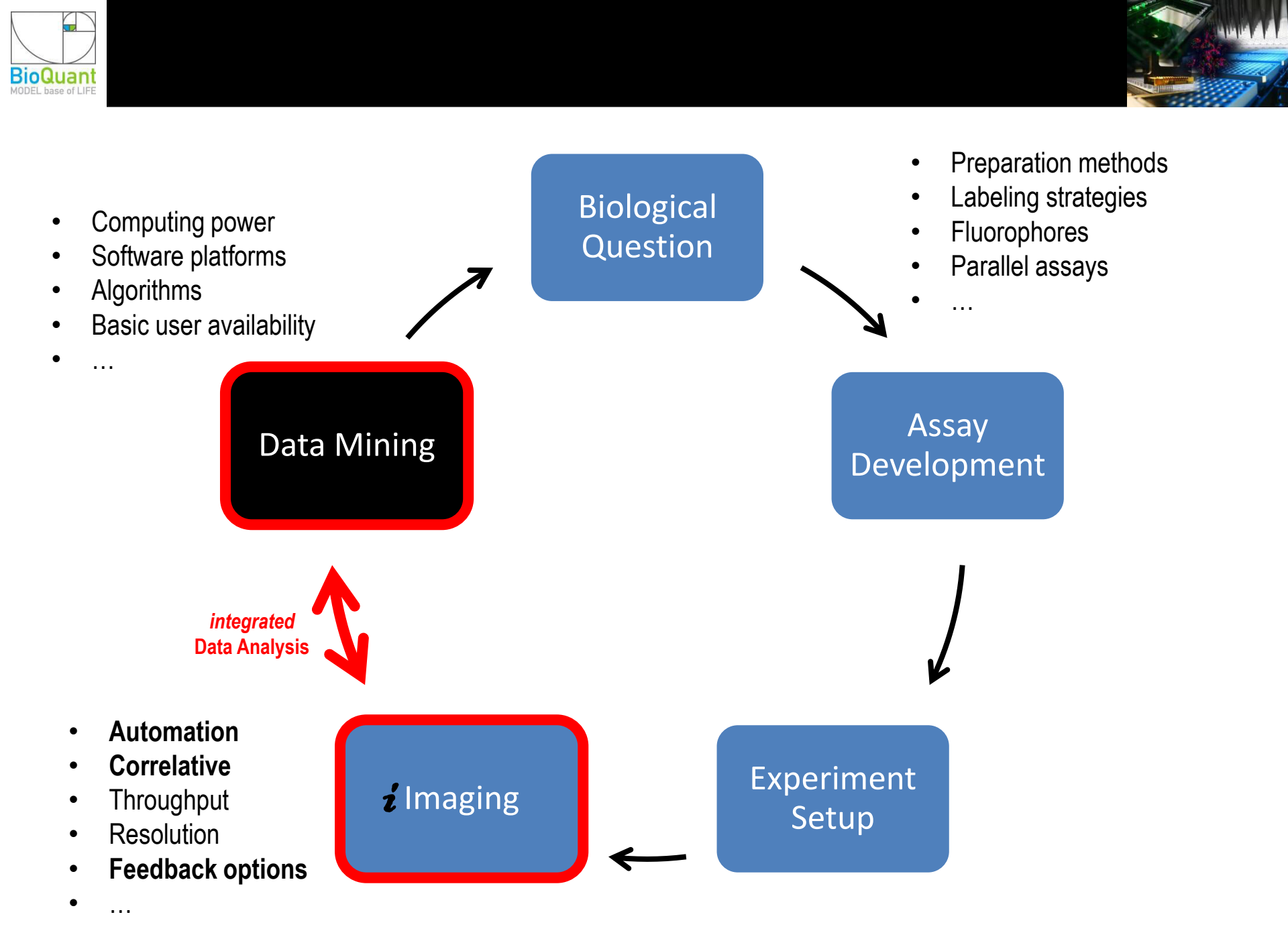
Biological Question
Preparation methods
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Feedback options
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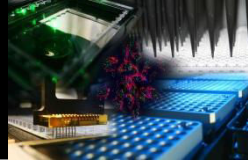


Screening

Large scale experiment number (up to genome wide)

↓

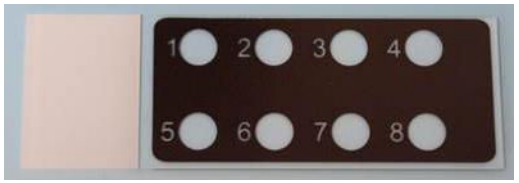
e.g.: 20.273 human genes *3 siRNAs = 60.819 experiments

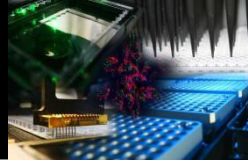
Automated DAQ *in general*

Automation NOT necessarily restricted to screening procedures

↓

- **Biological variability**
  - → Increase amount of data / # Objects to be scanned
  - → Statistical relevance
  - → Reproducibility
- **Increase image quality** (e.g. quality controls)
- **Increase information content** (e.g. correlative microscopy / time lapse)
- Simplify data acquisition procedures
- …





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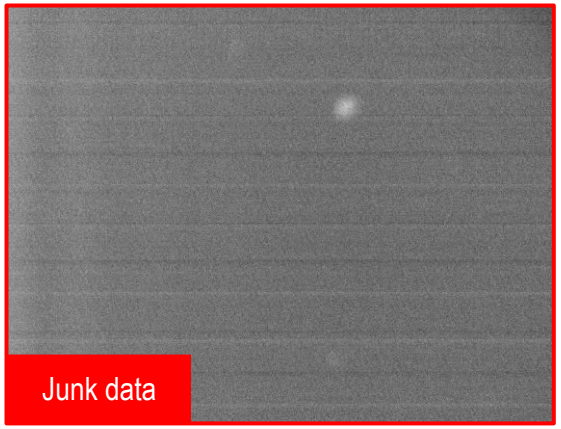
↓

e.g.: 20.273 human genes *3 siRNAs = 60.819 experiments

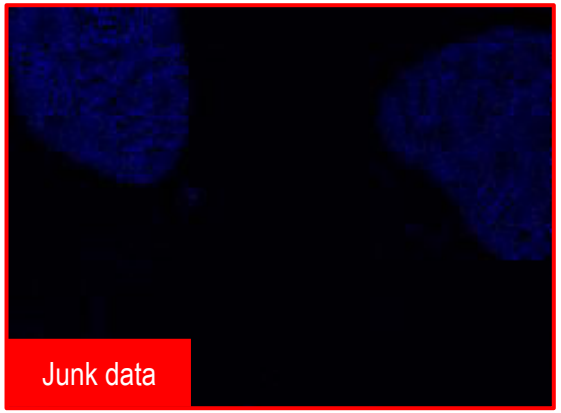


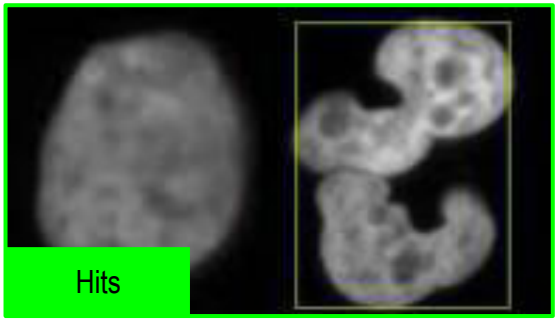
Automated DAQ *in general*

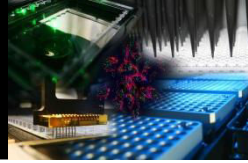
Automation NOT necessarily restricted to screening procedures

↓

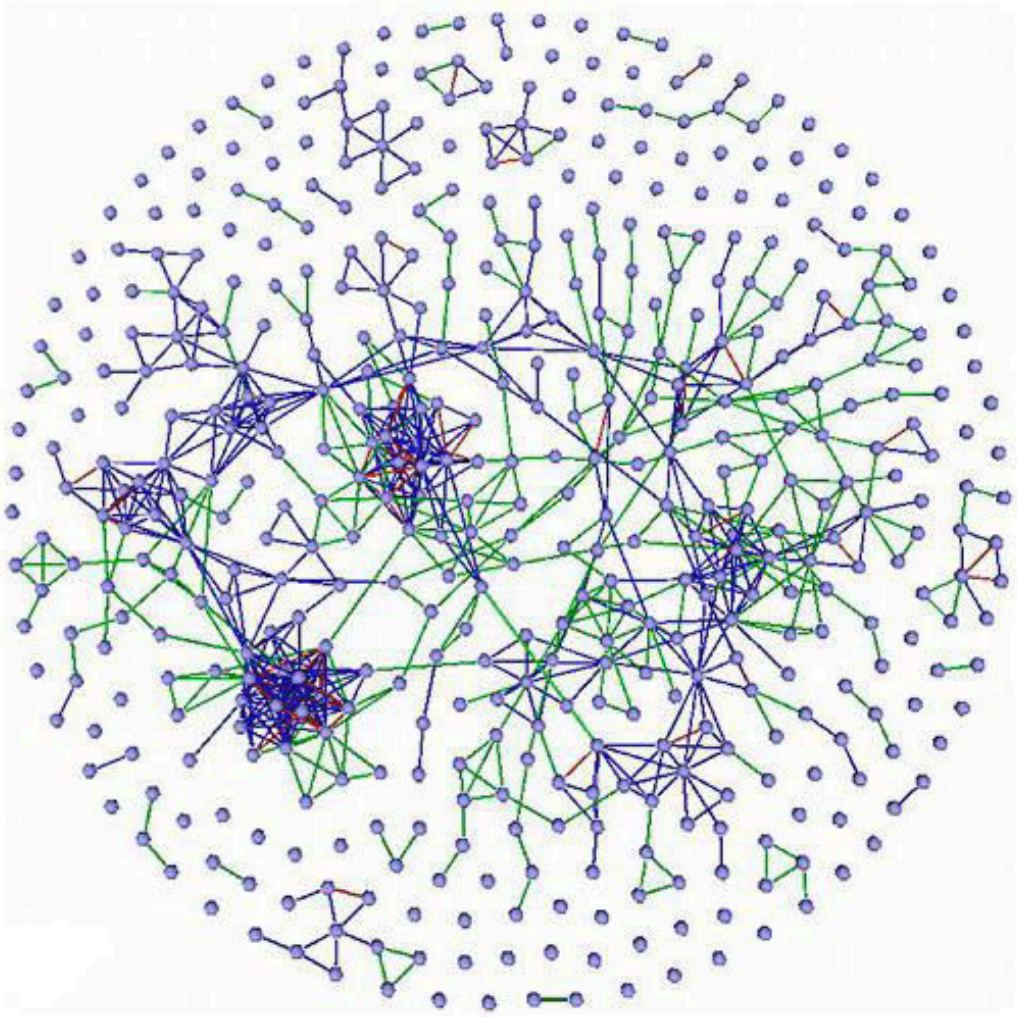
- **Biological variability**
  - → Increase amount of data / # Objects to be scanned
  - → Statistical relevance
  - → Reproducibility
- **Increase image quality** (e.g. quality controls)
- **Increase information content** (e.g. correlative microscopy / time lapse)
- Simplify data acquisition procedures
- …



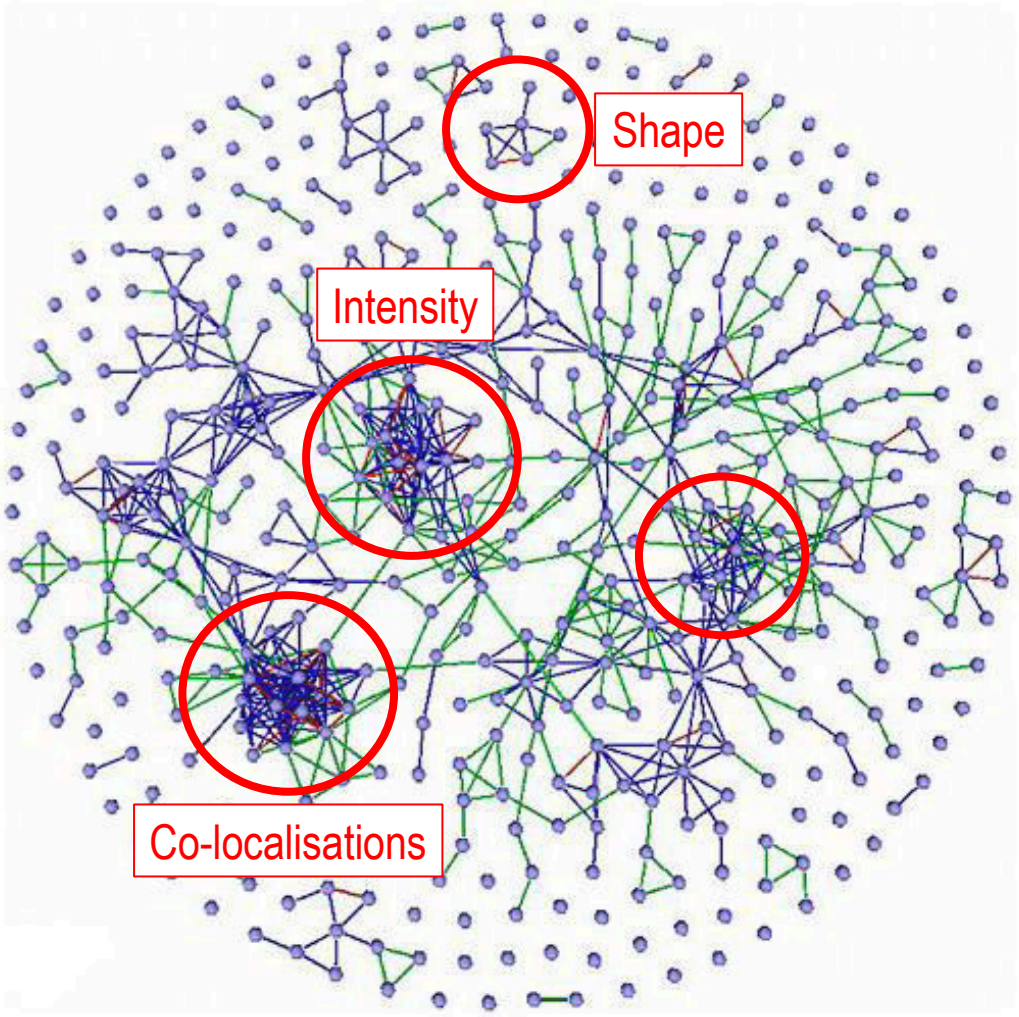


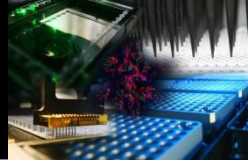


n-dim feature space of observables

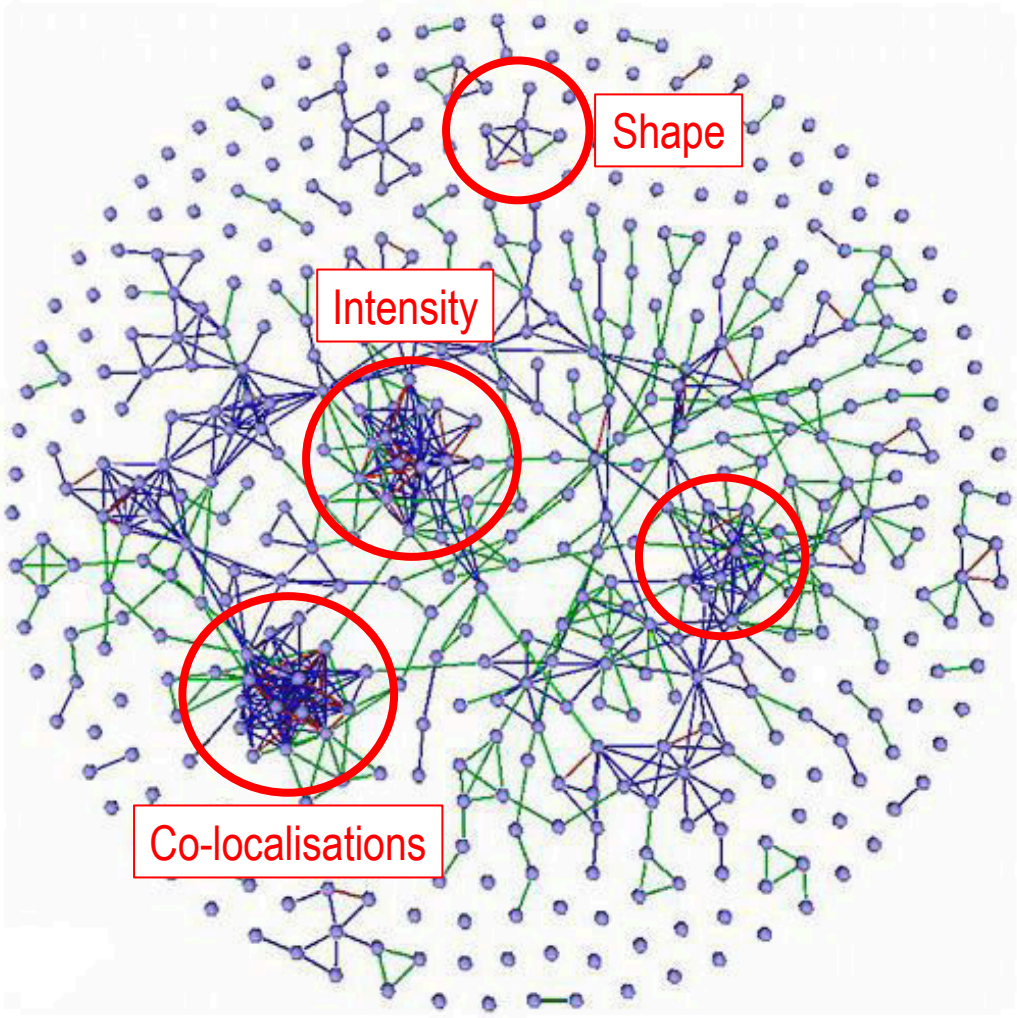


n-dim feature space of observables [cells]



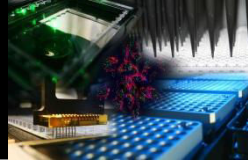


n-dim feature space of observables [cells]

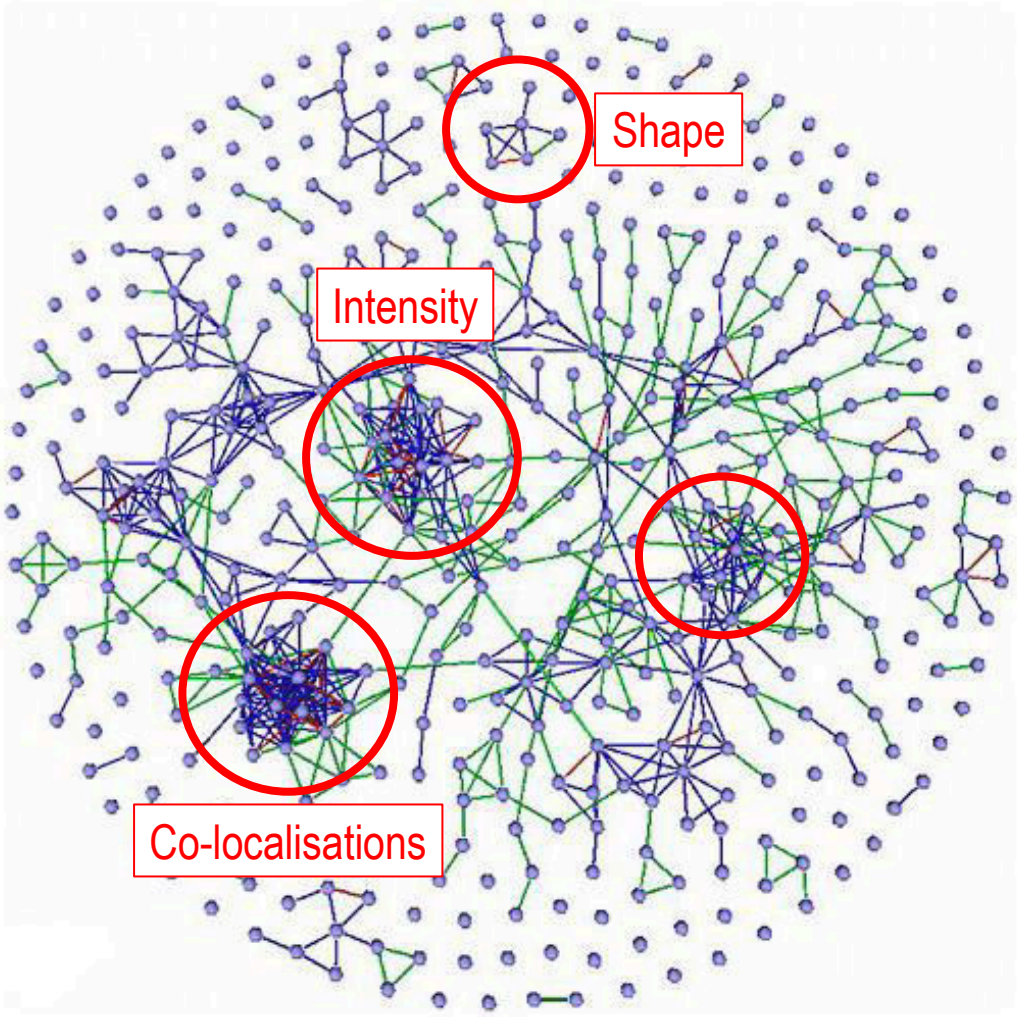


PROBLEM: General measurement provides cloud of data points containing every feature!

BUT: Every measurement aims at collecting the information content of specified features.



n-dim feature space of observables [cells]



PROBLEM: General measurement provides cloud of data points containing every feature!

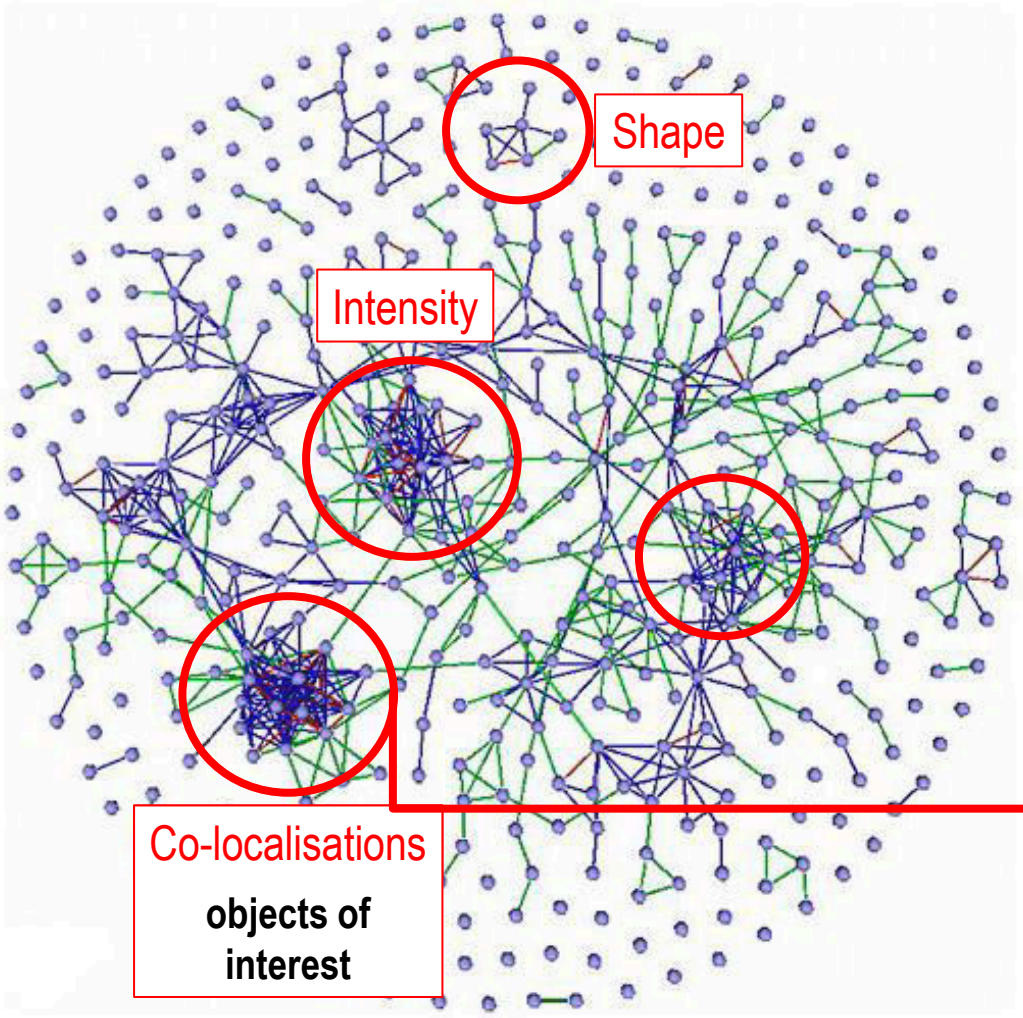
BUT: Every measurement aims at collecting the information content of specified features.

- ***Pre*-Processing**
  Identify objects of interest
- FeedBack to measuring system
- **Acquire only objects of interest**

General Considerations

FeedBac

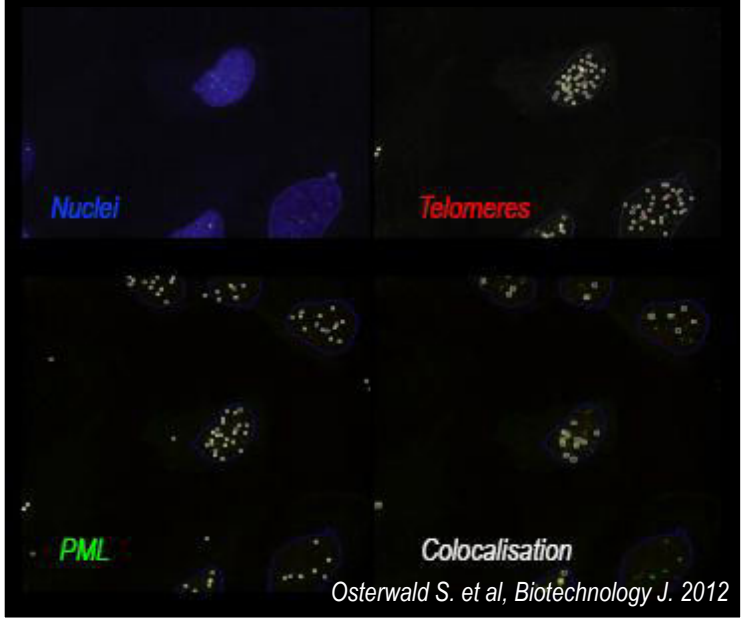
n-dim feature space of observables [cells]



High-resolution 3D colocalisation RNA interference screen

→	20	cell arrays
→	38.400	3D data stacks
→	~2 Mio	images

Pure screening time: 6 months
Junk data: ~40%



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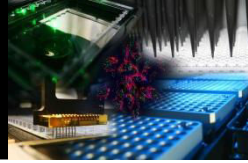
Automated Imaging

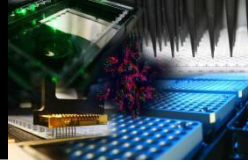


Combine/Link DAQ ↔ IP

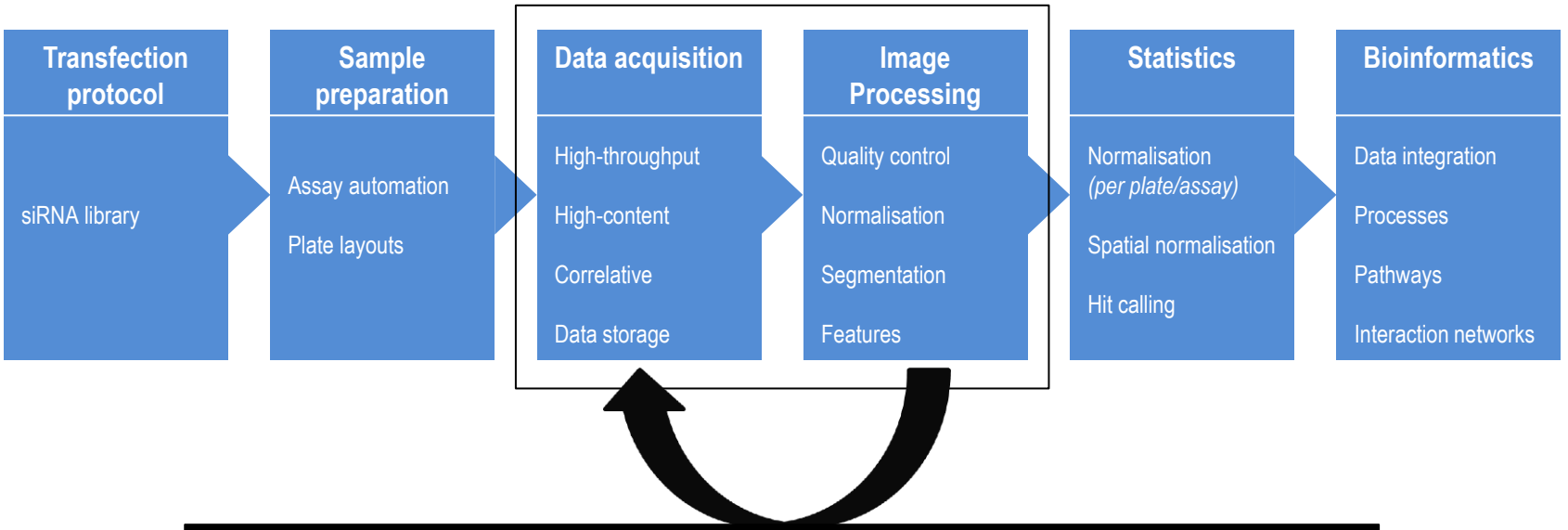
Transfection protocol	Sample preparation	Data acquisition	Image Processing	Statistics	Bioinformatics
siRNA library	Assay automation Plate layouts	High-throughput High-content Correlative Data storage	Quality control Normalisation Segmentation Features	Normalisation *(per plate/assay)* Spatial normalisation Hit calling	Data integration Processes Pathways Interaction networks

Use information from Image *Pre*- Processing in order to optimise / upgrade DAQ

Automated Imaging



Combine/Link DAQ ↔ IP



Use information from Image *Pre*- Processing in order to optimise / upgrade DAQ

Junk data | Costs | Feasibility of experiments | Bleaching

- Trigger (time lapse experiments)
- Pick only objects of interest ► Quality / Information content of image raw data
  Screening time
- Pick representative cells for high/super -resolution
- …

Automated Imaging

Data acquisition

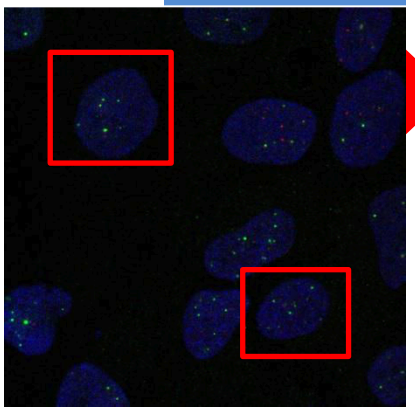


Image *Pre*-Processing

Targets | Trigger

Hit calling

Image Processing

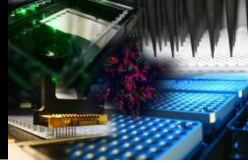
Quality control

Normalisation

Segmentation

Features

Automated Imaging



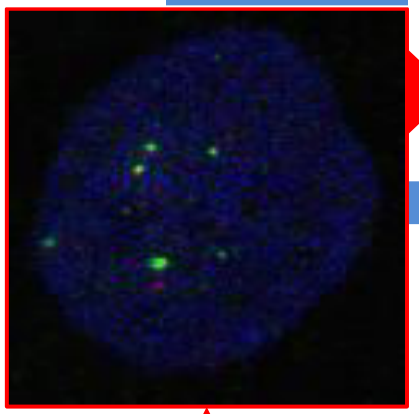
Data acquisition

Image *Pre*-Processing

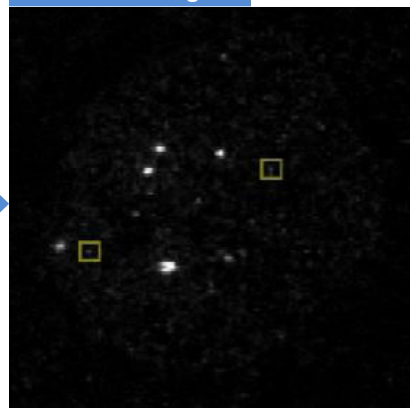
Targets | Trigger

Hit calling

Image Processing



Objects of interest



Microscope FeedBack

Targets

Image Reader

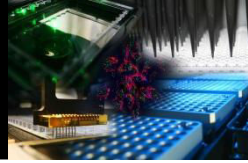
Microscope Raw Data

Experiment MetaData

Libraries Layouts

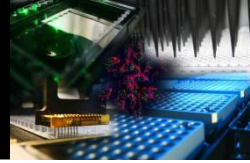
...

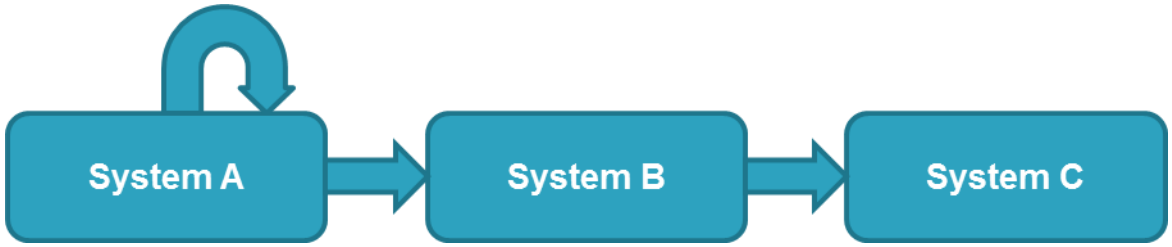
Image Processing



- Scientific Infrastructure
- Data Pipelines & Data Analysis
- Cross-Platform Solution

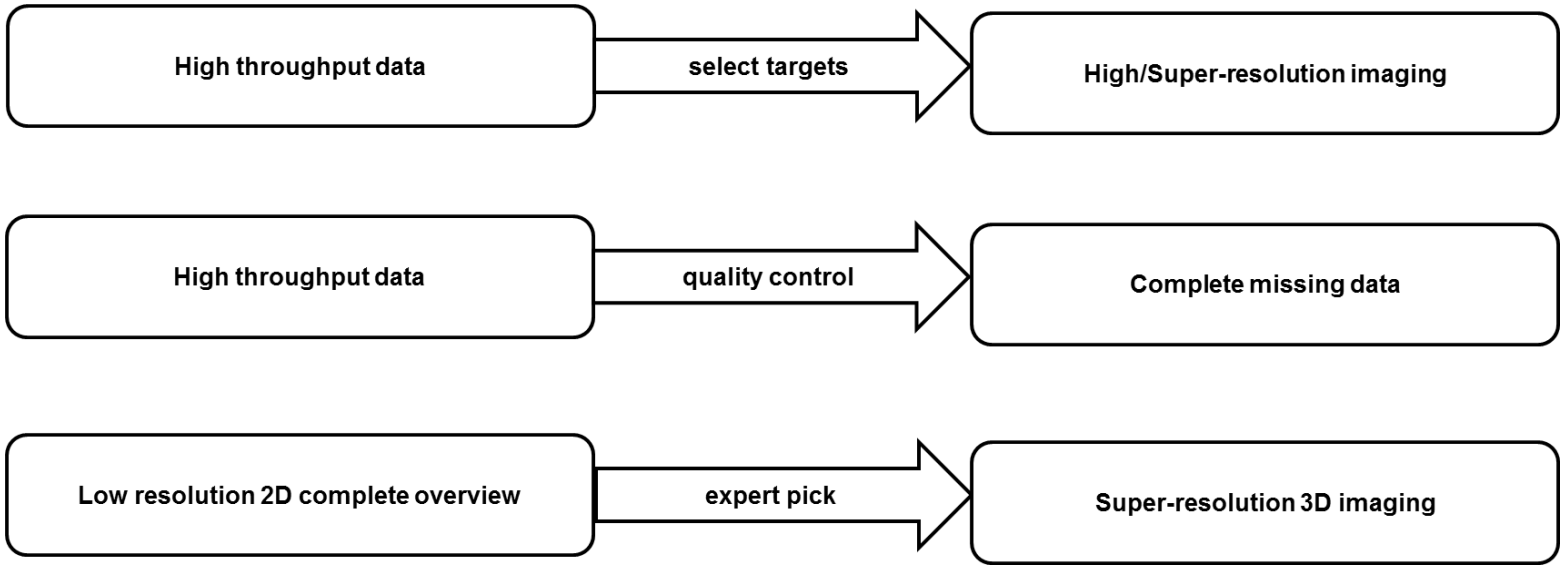
Cross-Platform Solution





Idea: Acquire images of the same sample/target at multiple systems, in order to combine the advantages of different microscopic techniques.

Examples:

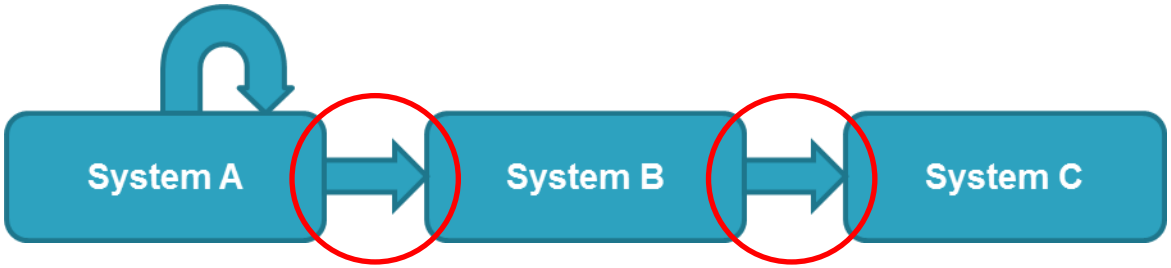


Cross-Platform Solution

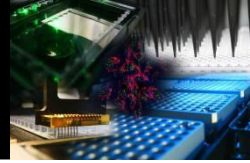
Problem: Transfer System

Coordinate transfer by

1.) Reference markers
2.) (*SIFT*) image registration



Cross-Platform Solution



Problem: Transfer System

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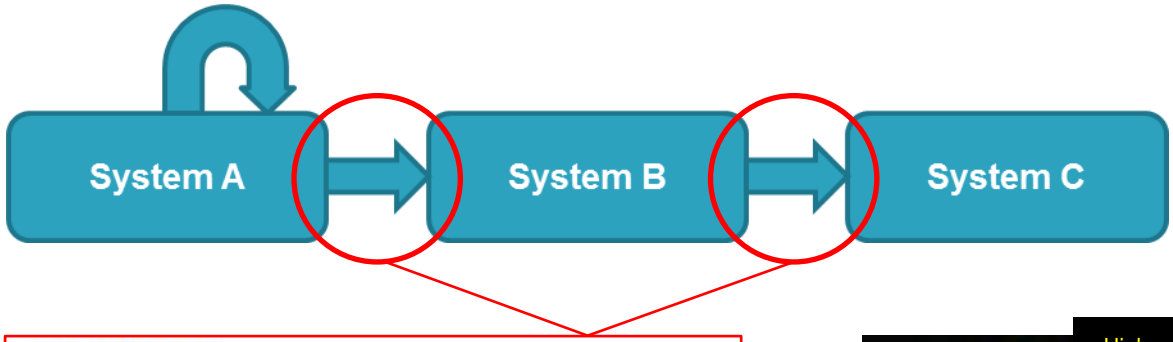
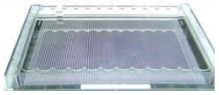
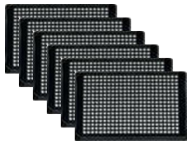
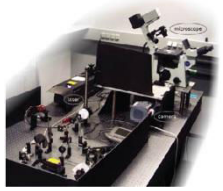
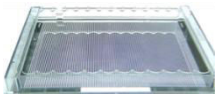
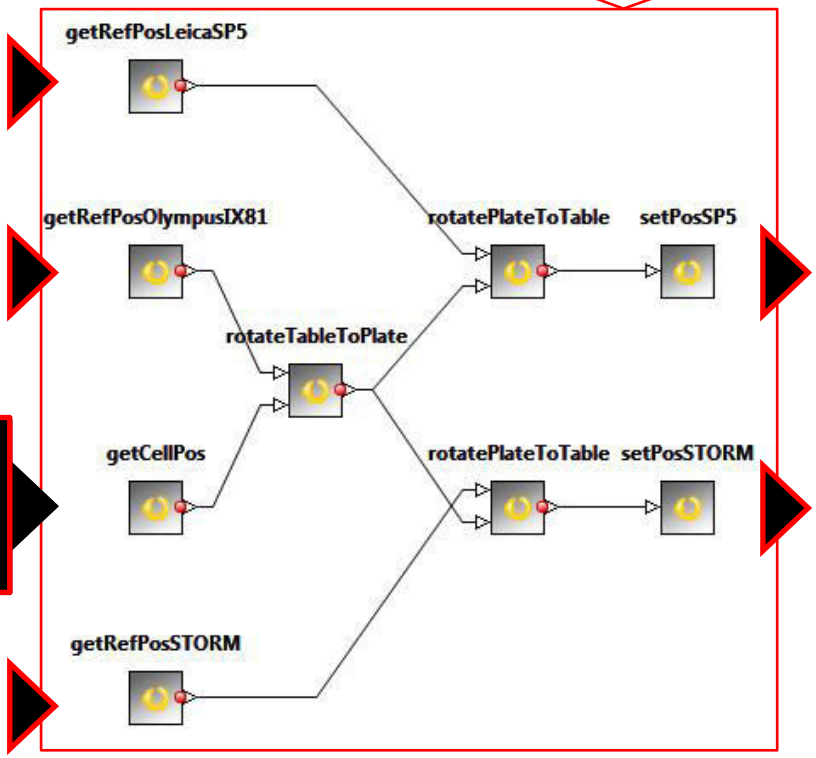


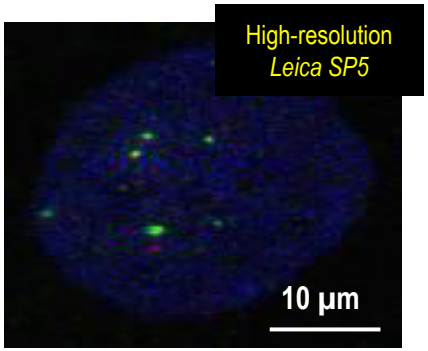


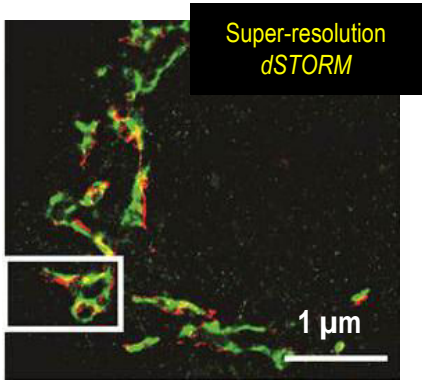








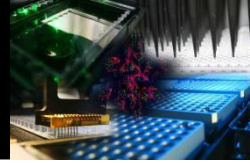




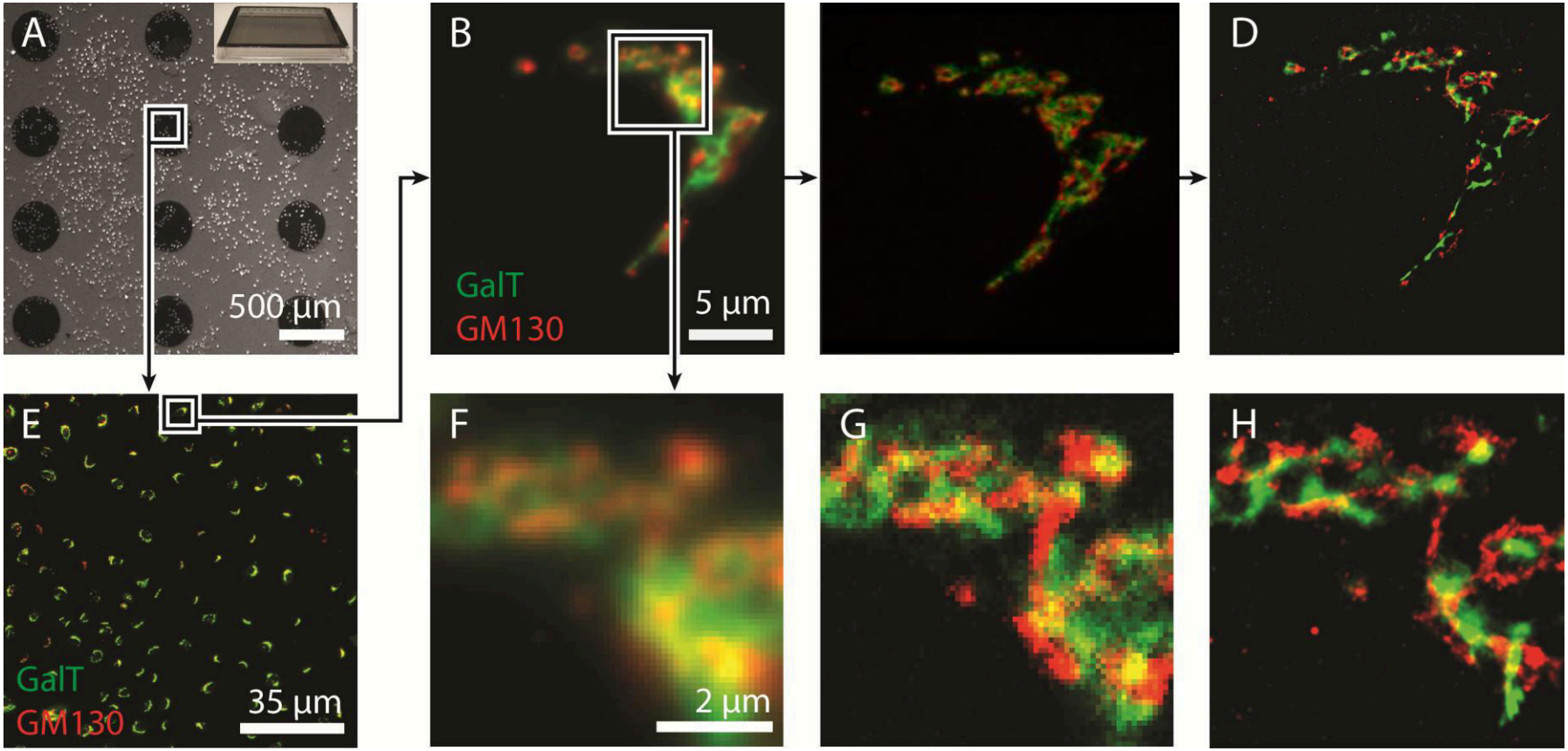
dSTORM setup: Mike Heilemann

Gunkel et al., Histochem & Cell Biol 2014

Cross-Platform Solution — Integrated Correlative Microscopy

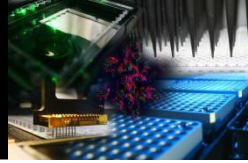


High-throughput screening [→ Target identification] **High-resolution screening** **Super-resolution screening**



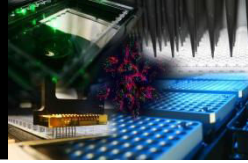
Collab.: Mike Heilemann, Fred Hamprecht, Vytaute Starkuviene

Flottmann et al., Biotechniques 2013

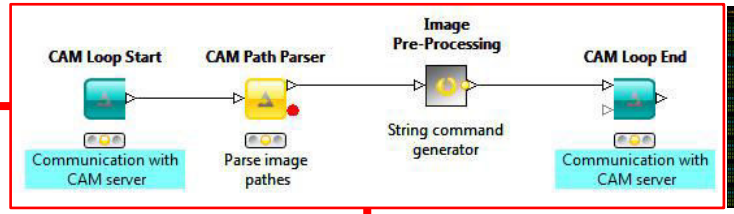


- Scientific Infrastructure
- Data Pipelines & Data Analysis
- Automated Microscopy

Automated Microscopy

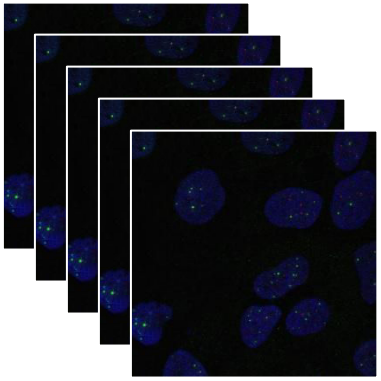


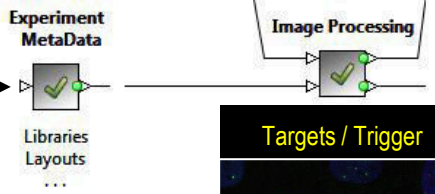
Leica Microsystems



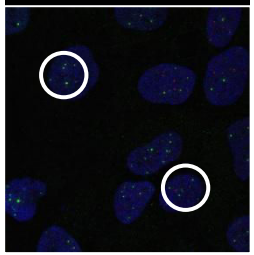
Data storage

(Pre-Screen)
Image raw data

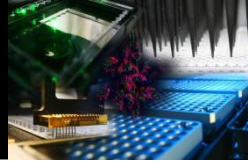


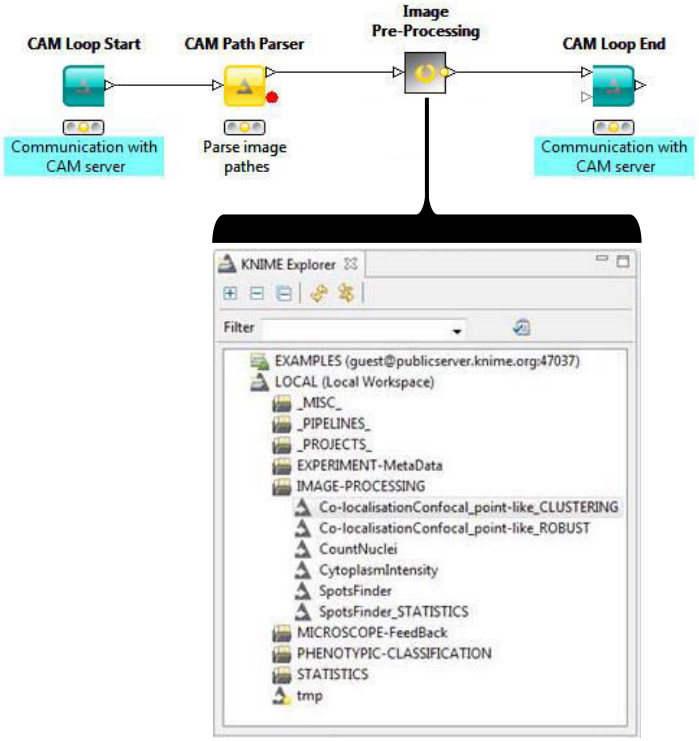


Targets / Trigger

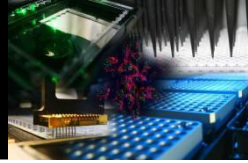


Collab.: KNIME Leica MICROSYSTEMS





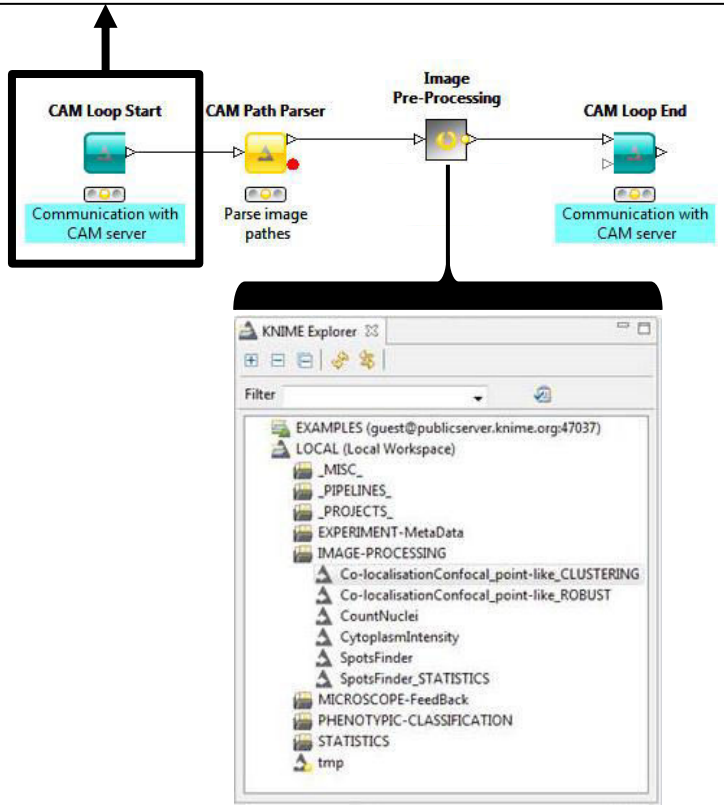
Collab.: KNIME Leica MICROSYSTEMS



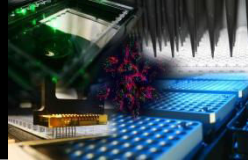
Pre-Screen

Configuration 1

▶ **2D low resolution imaging** of full sample



Collab.: KNIME Leica MICROSYSTEMS



Pre-Screen

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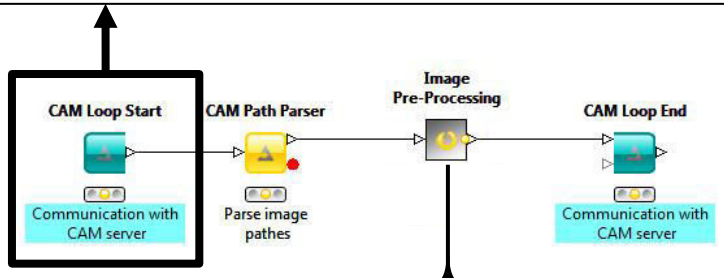
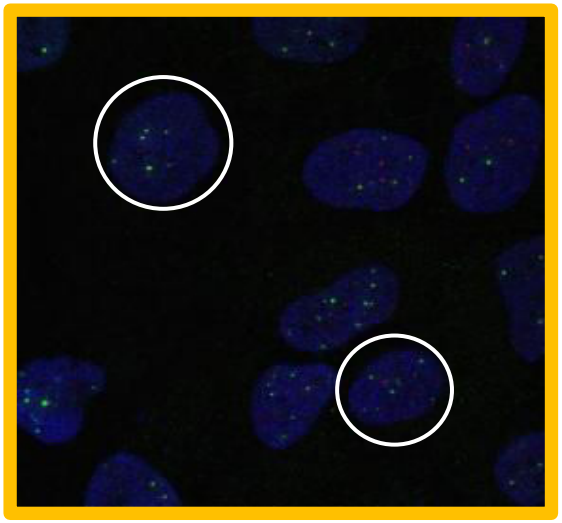




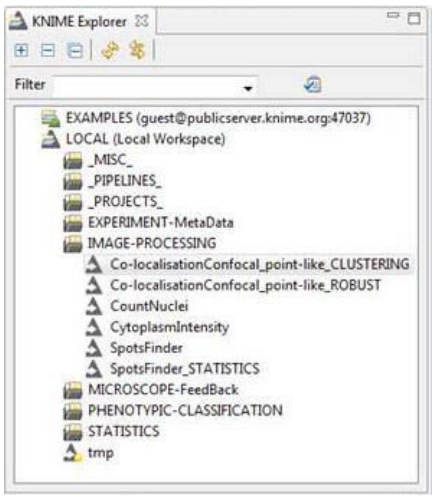
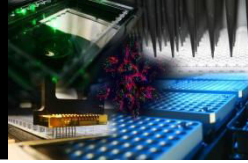


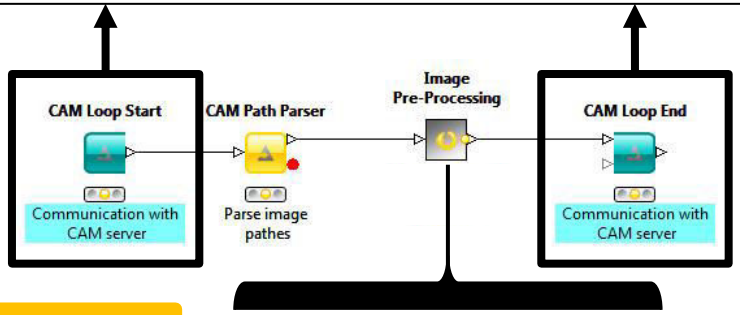
Image Pre-Processing ▶ Identify objects of interest (candidates)

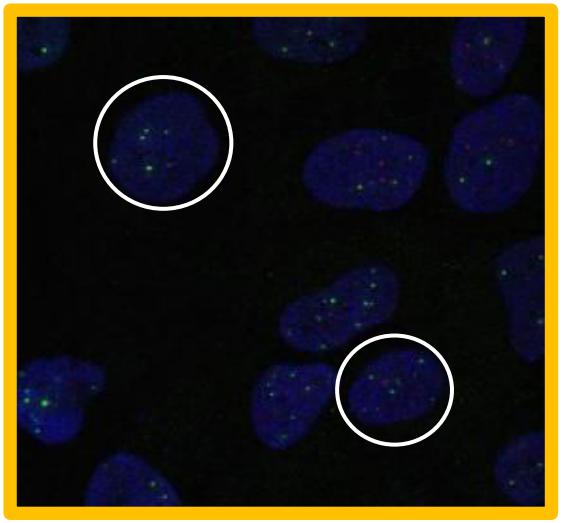
Collab.: KNIME Leica MICROSYSTEMS



Pre-Screen

Configuration 1 ▶ **2D low resolution imaging** of full sample





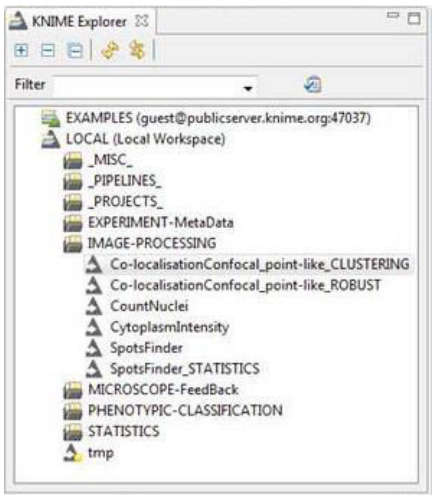
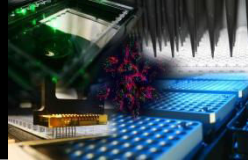


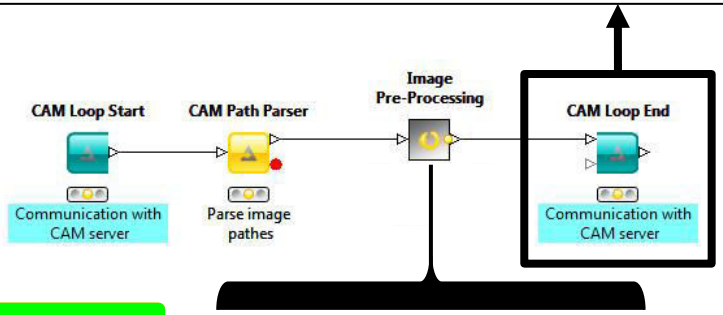
Image Pre-Processing ▶ Identify objects of interest (candidates)

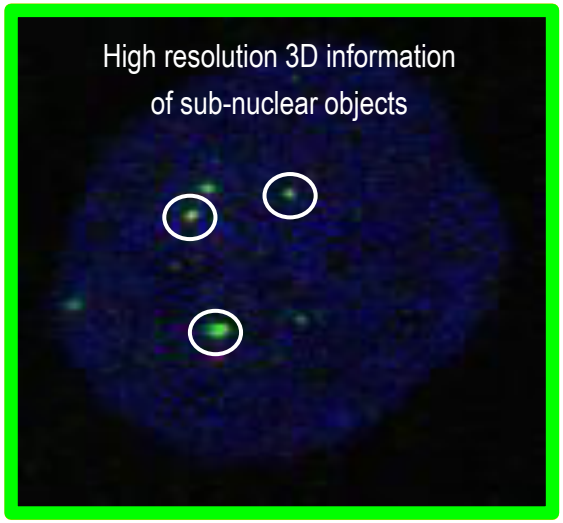
Collab.: KNIME Leica MICROSYSTEMS

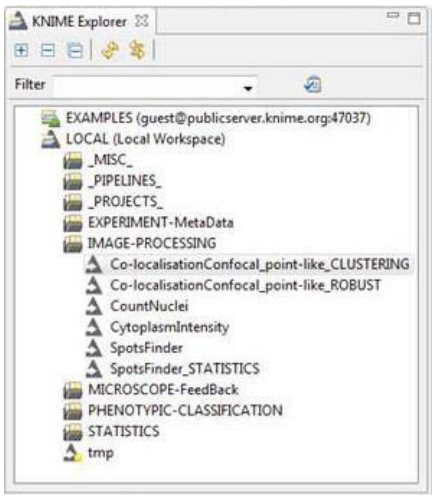


Target Screen

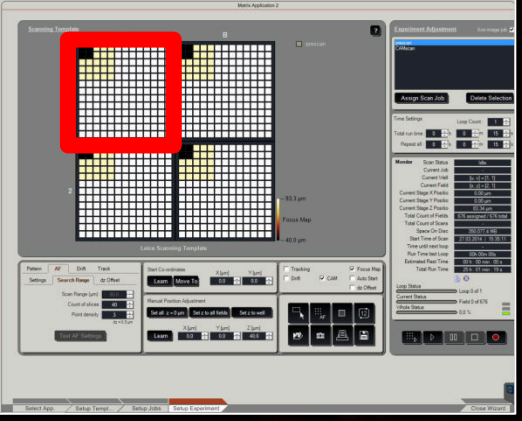
Configuration 2 ▶ **3D high resolution imaging** of candidates



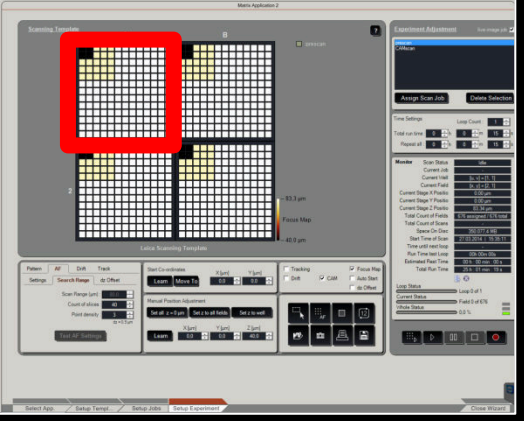


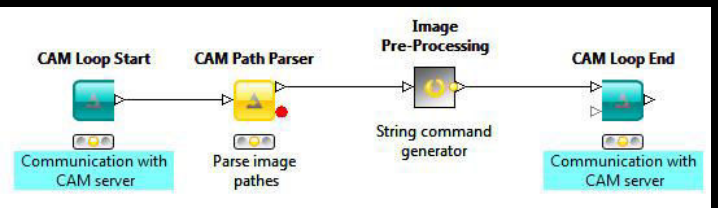


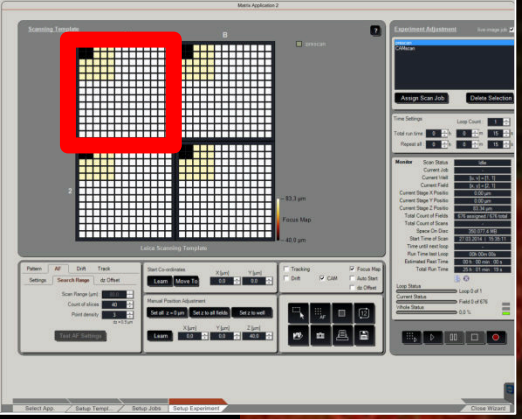
Collab.: KNIME Leica MICROSYSTEMS

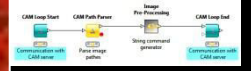

Matrix Application 2
Scanning Template
Experiment Adjustment
Assign Scan Job
Delete Selection
Time Settings
Monitor
Start Co-ordinates
Learn
Move To
Manual Position Adjustment
Set all z = 0 µm
Set z to all fields
Set z to well
Test AF Settings
Tracking
Drift
Focus Map
Auto Start
Z.AF
dz Offset
Focus Map
Leica Scanning Template
Select App
Setup Tenet
Setup Jobs
Setup Experiment
Close Wizard

25 µm

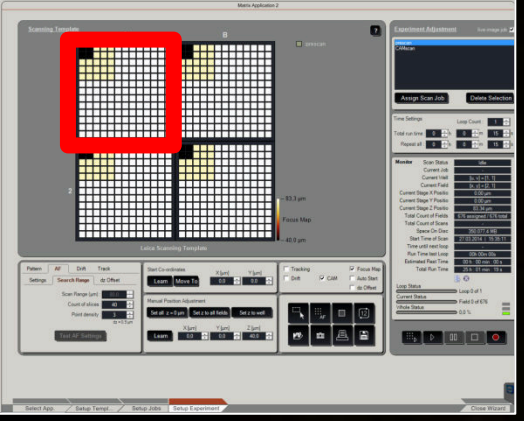


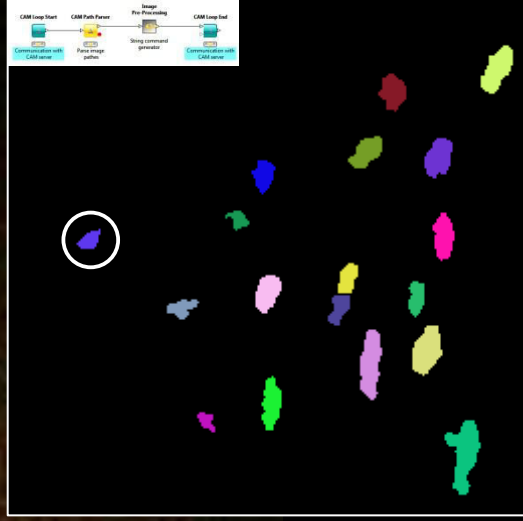

CAM Loop Start
CAM Path Parser
Image Pre-Processing
CAM Loop End
Communication with CAM server
Parse image pathes
String command generator
Communication with CAM server


Matrix Application 2
Scanning Template
Experiment Adjustment
Assign Scan Job
Delete Selection
Time Settings
Monitor
Focus Map
Lattice Scanning Template
Select Apps
Setup Tenet
Setup Jobs
Setup Experiment
Close Wizard

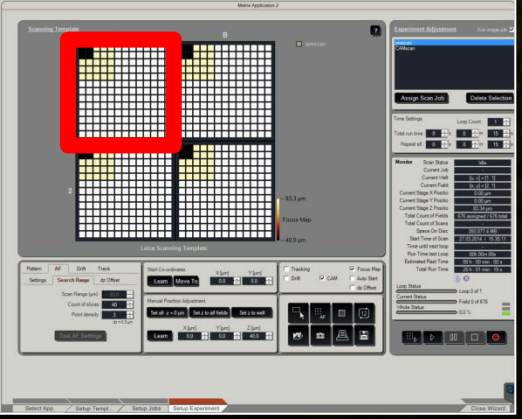

CAM Loop Start
CAM Path Parser
Image Pre-Processing
CAM Loop End
Communication with CAM server
Parse image paths
Shimg command generator
Communication with CAM server

5 µm


Matrix Application 2
Scanning Template
Experiment Adjustment
Assign Scan Job
Delete Selection
Focus Map
Leica Scanning Template
Learn
Move To
Tracking
Drift
CAM
Auto Start
Select Apps
Setup Tiered
Setup Jobs
Setup Experiment
Close Wizard


CAM Loop Start
CAM Path Parser
Image Pre-Processing
CAM Loop End
Communication with CAM server
Parse image paths
Shiny command generator
Communication with CAM server

5 µm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Experiment Adjustment
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Focus Map
Leica Scanning Template
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Manual Position Adjustment
Monitor
Select Apps
Setup Tiered
Setup Jobs
Setup Experiment
Close Wizard

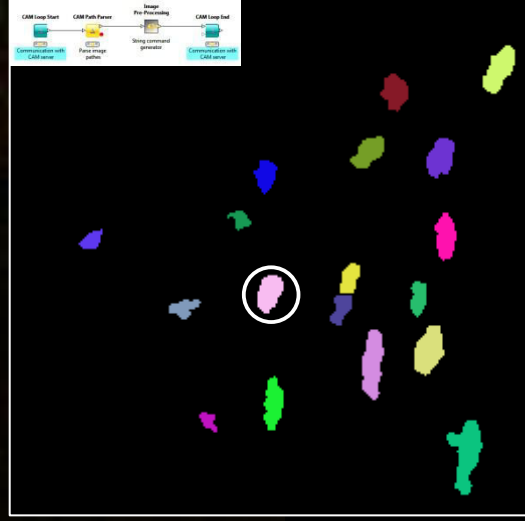
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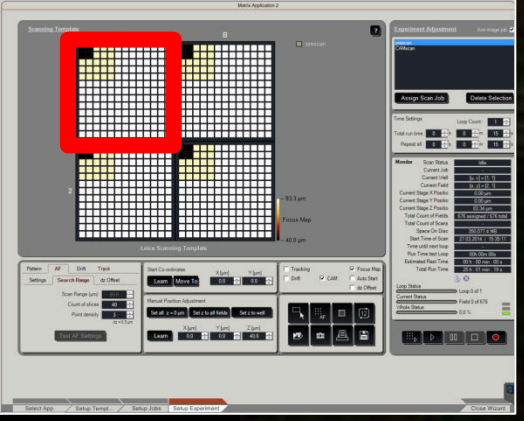
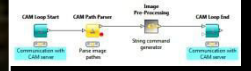
Image Pre-Processing
CAM Loop End
Communication with CAM server
Parse image paths
String command generator
Communication with CAM server

5 µm


Matrix Application 2
Scanning Template
Experiment Adjustment
Assign Scan Job
Delete Selection
Time Settings
Loop Count
Monitor
Scan Status
Current Job
Current Well
Current Field
Current Stage X Position
Current Stage Y Position
Current Stage Z Position
Total Count of Fields
Total Count of Scans
Space On Disc
Start Time of Scan
Time until next loop
Run Time last Loop
Estimated Run Time
Total Run Time
Loop Status
Current Status
Whole Status
Pattern
AF
Drift
Track
Settings
Search Range
Start Co-ordinates
Learn
Move To
Manual Position Adjustment
Tracking
Drift
CAM
Focus Map
Auto Start
dz Offset
Test AF Settings
Select Apps
Setup Tetrel
Setup Jobs
Setup Experiment
Close Wizard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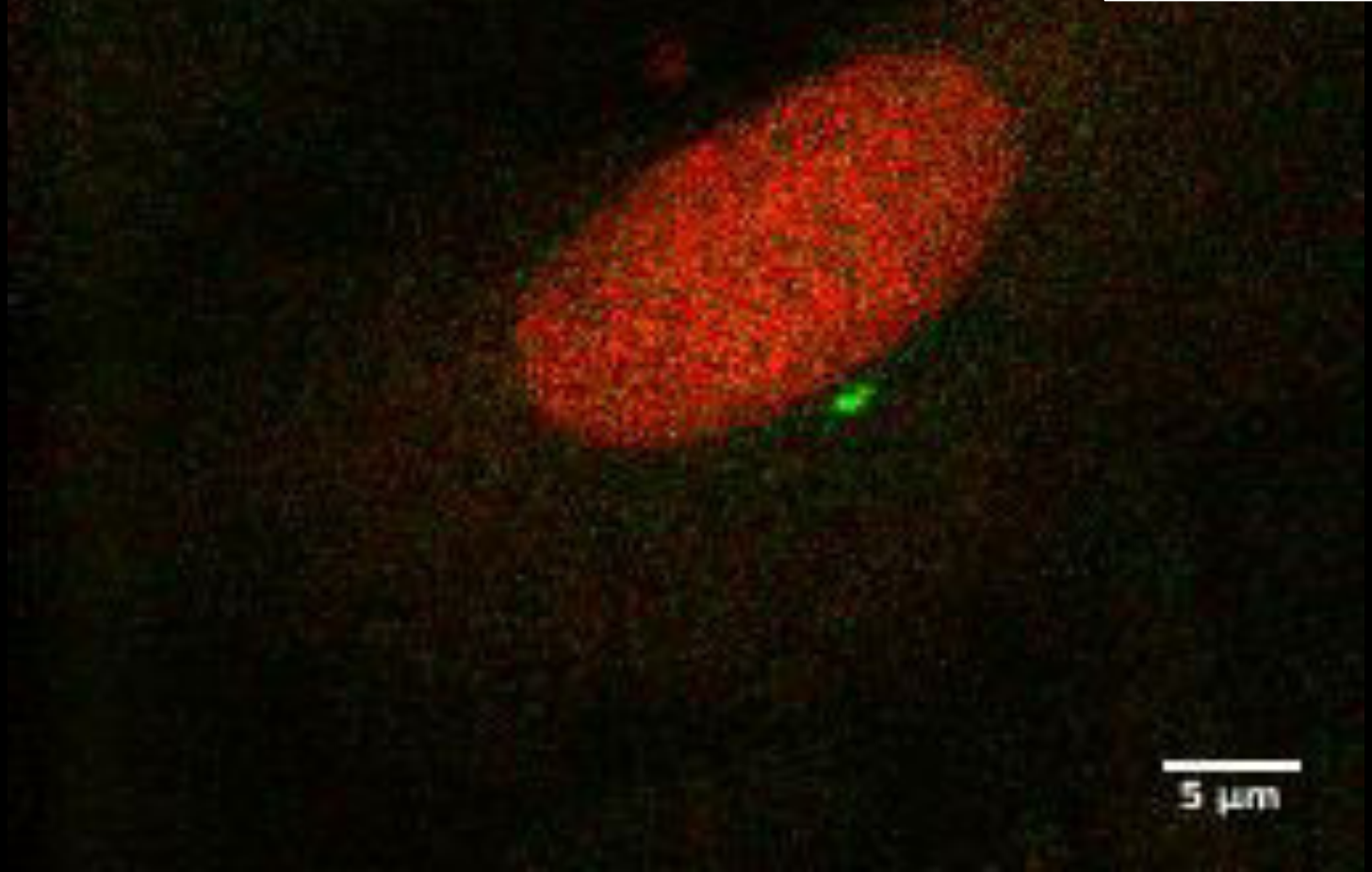
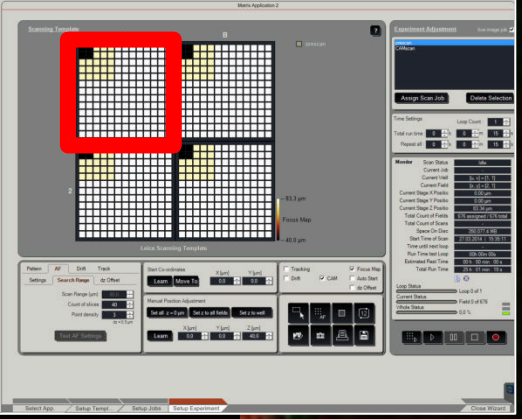
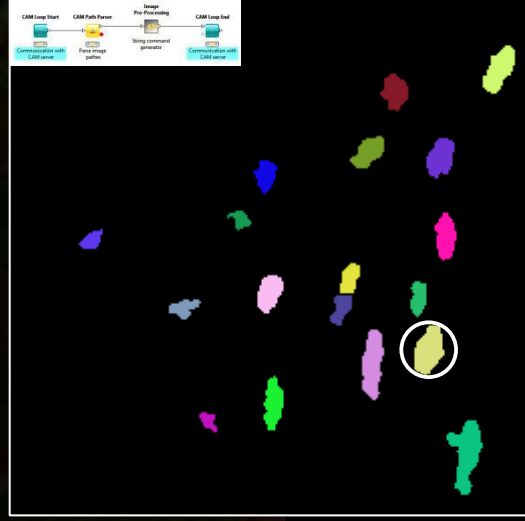
CAM Path Parser
Image Pre-Processing
CAM Loop End
Communication with CAM server
Parse image paths
String command generator
Communication with CAM server


5 µm


Matrix Application 2
Scanning Template
Experiment Adjustment
Assign Scan Job
Delete Selection
Time Settings
Monitor
Start Co-ordinates
Learn
Move To
Manual Position Adjustment
Tracking
Drift
CAM
Focus Map
Auto Start
Loop Status
Whole Status
Select App
Setup Tetrel
Setup Jobs
Setup Experiment
Close Wizard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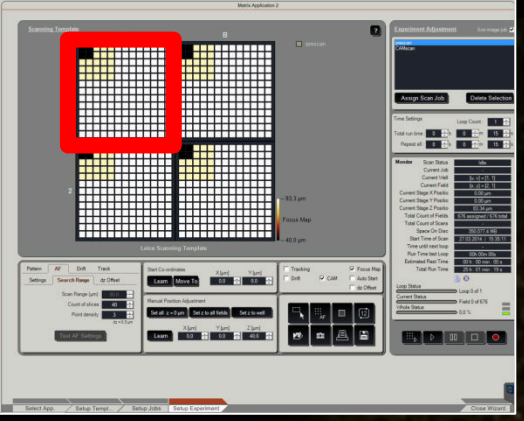
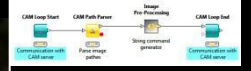
CAM Path Parser
Image Pre-Processing
CAM Loop End
Communication with CAM server
Parse image paths
String command generator
Communication with CAM server

5 µm


Matrix Application 2
Scanning Template
Experiment Adjustment
Assign Scan Job
Delete Selection
Focus Map
Leica Scanning Template
Learn
Move To
Manual Position Adjustment
Select Apps
Setup Tiered
Setup Jobs
Setup Experiment
Close Wizard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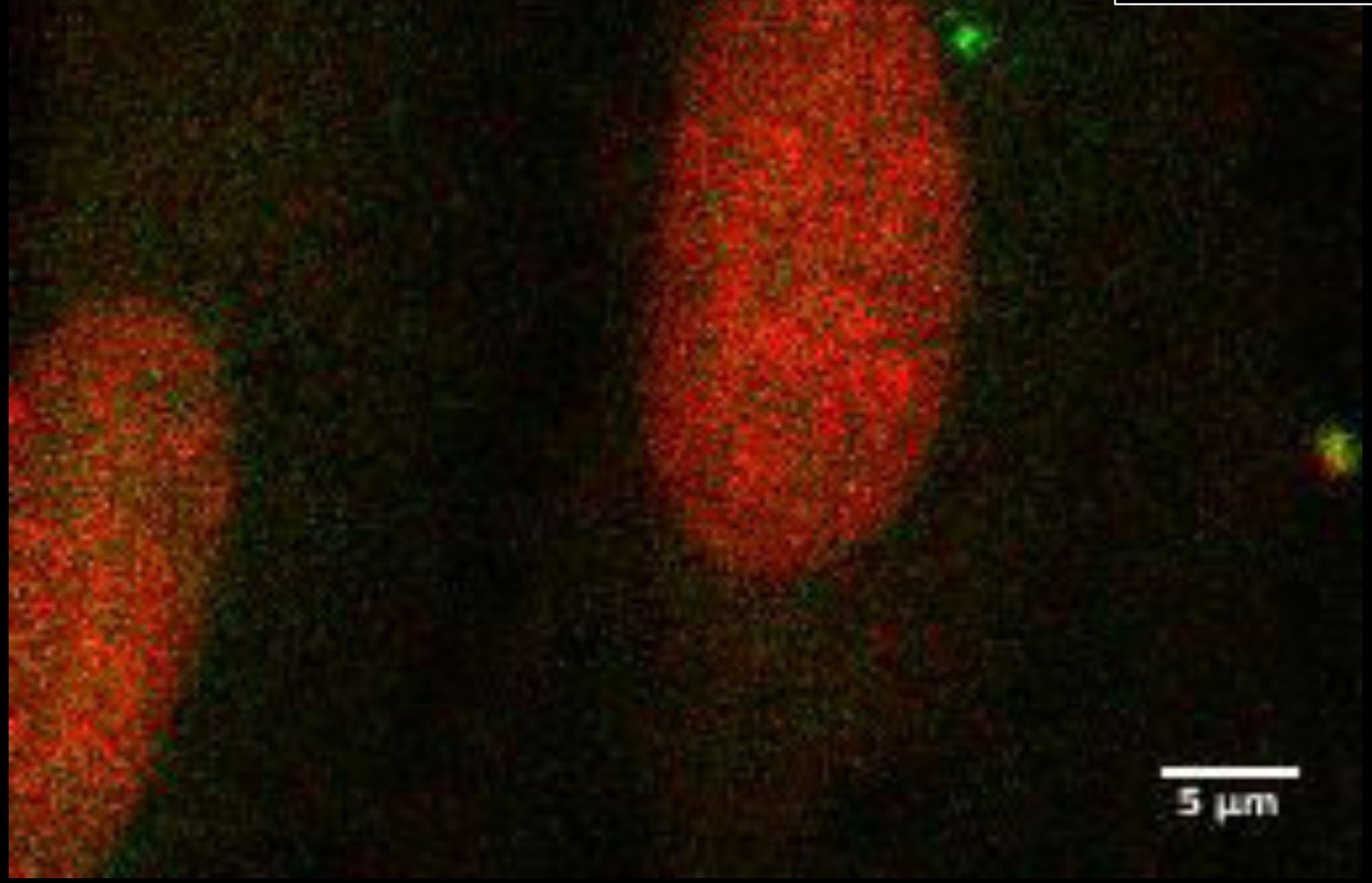
CAM Path Parser
Image Pre-Processing
CAM Loop End
Communication with CAM server
Parse image paths
String command generator
Communication with CAM server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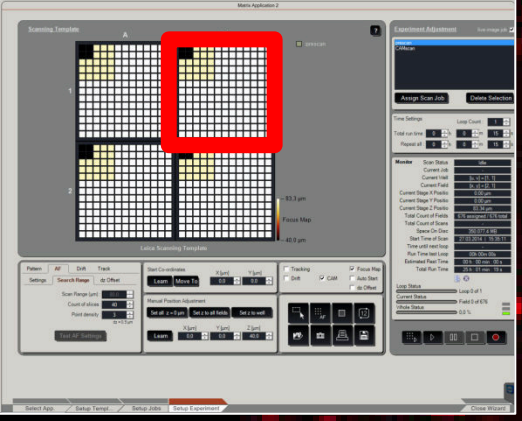
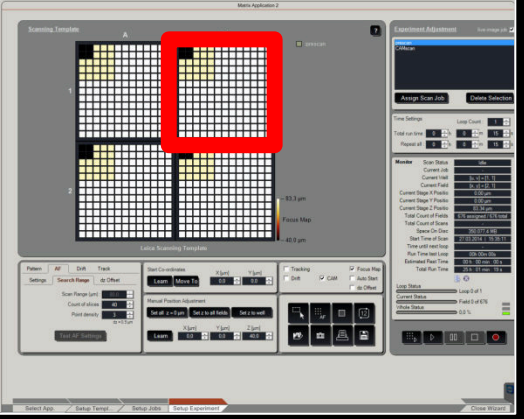
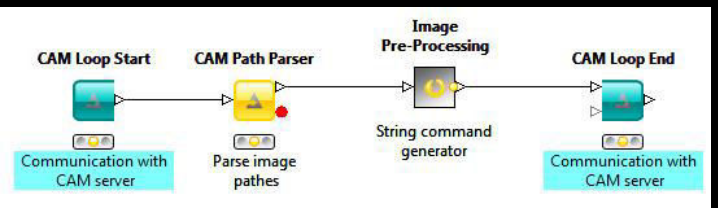

Scanning Template
Experiment Adjustment
Assign Scan Job
Delete Selection
Monitor
Focus Map
Select App
Setup Tenet
Setup Jobs
Setup Experiment
Close Wizard

25 µm




CAM Loop Start
CAM Path Parser
Image Pre-Processing
CAM Loop End
Communication with CAM server
Parse image pathes
String command generator
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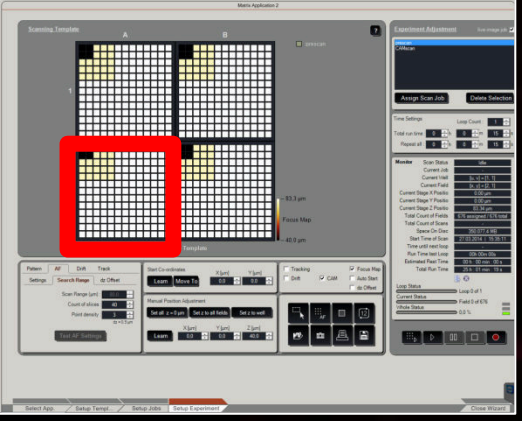
Matrix Application 2
Scanning Template
A
B
Experiment Adjustment
Assign Scan Job
Delete Selection
Time Settings
Monitor
Scan Status
Current Job
Current Well
Current Field
Current Stage X Position
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Total Count of Fields
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Whole Status
Start Co-ordinates
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Move To
Manual Position Adjustment
Set all -z- = 0 µm
Set z to all fields
Set z to well
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Focus Map
Auto Start
Select Apps
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Setup Jobs
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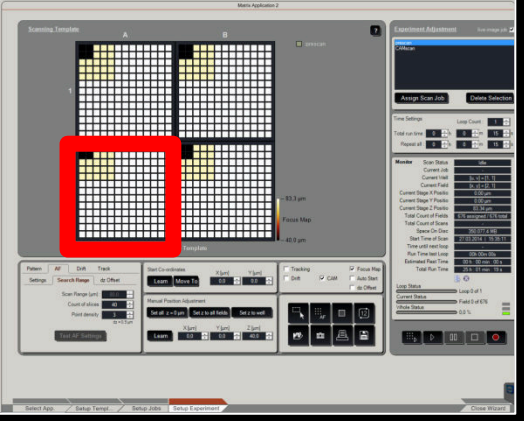
25 µm



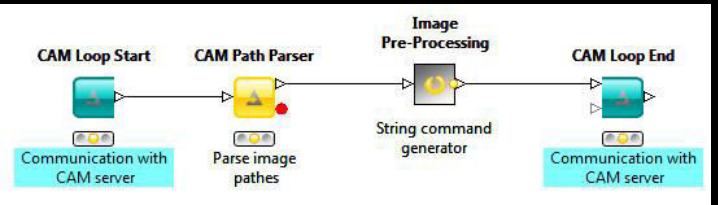

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Image Pre-Processing
CAM Loop End
Communication with CAM server
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String command generator
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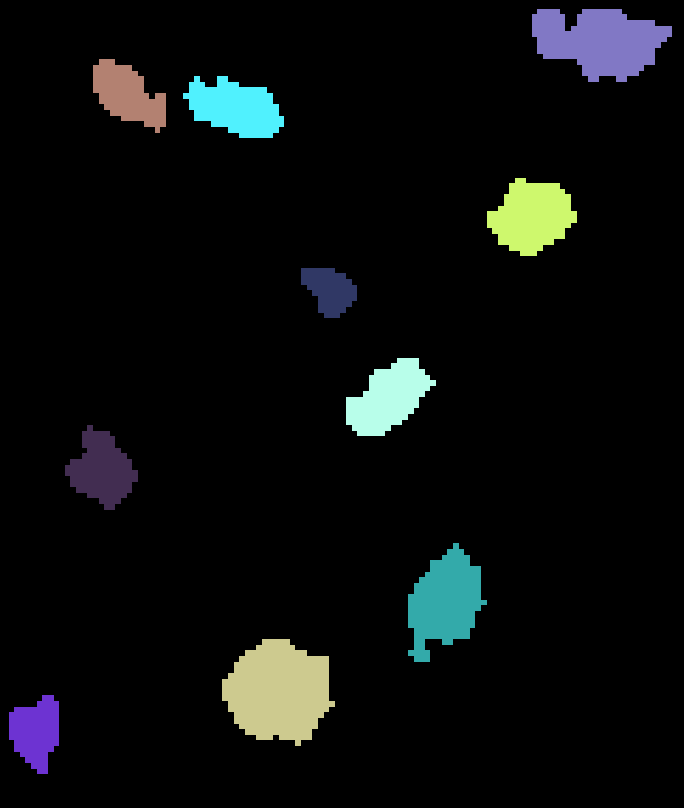
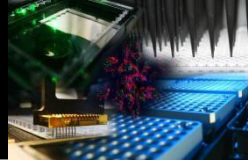
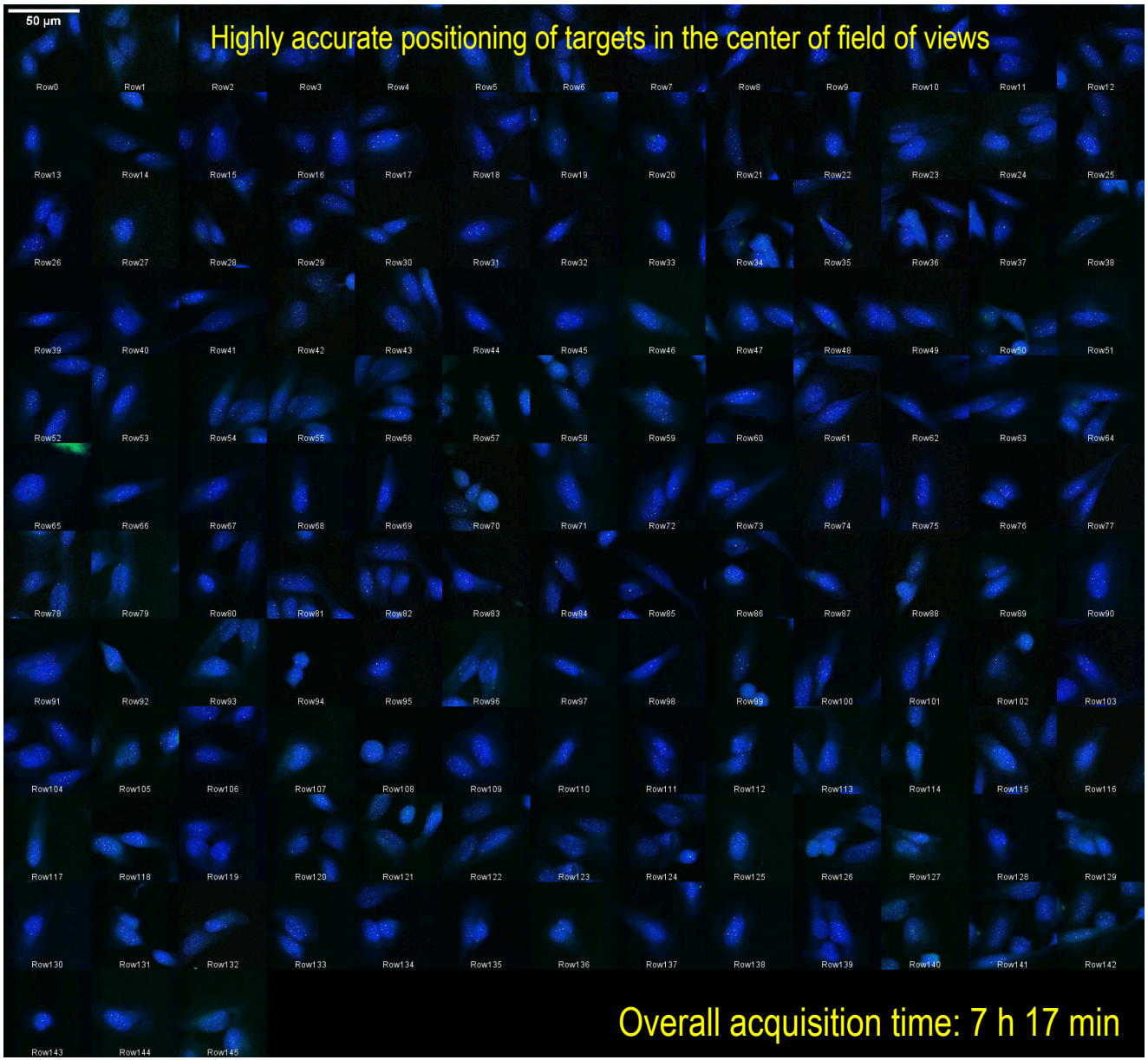


Automated Microscopy





Experiment setup

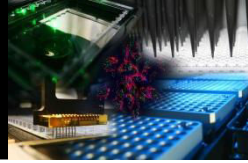
Overall sample size:
8 * 12 = 96 scan fields

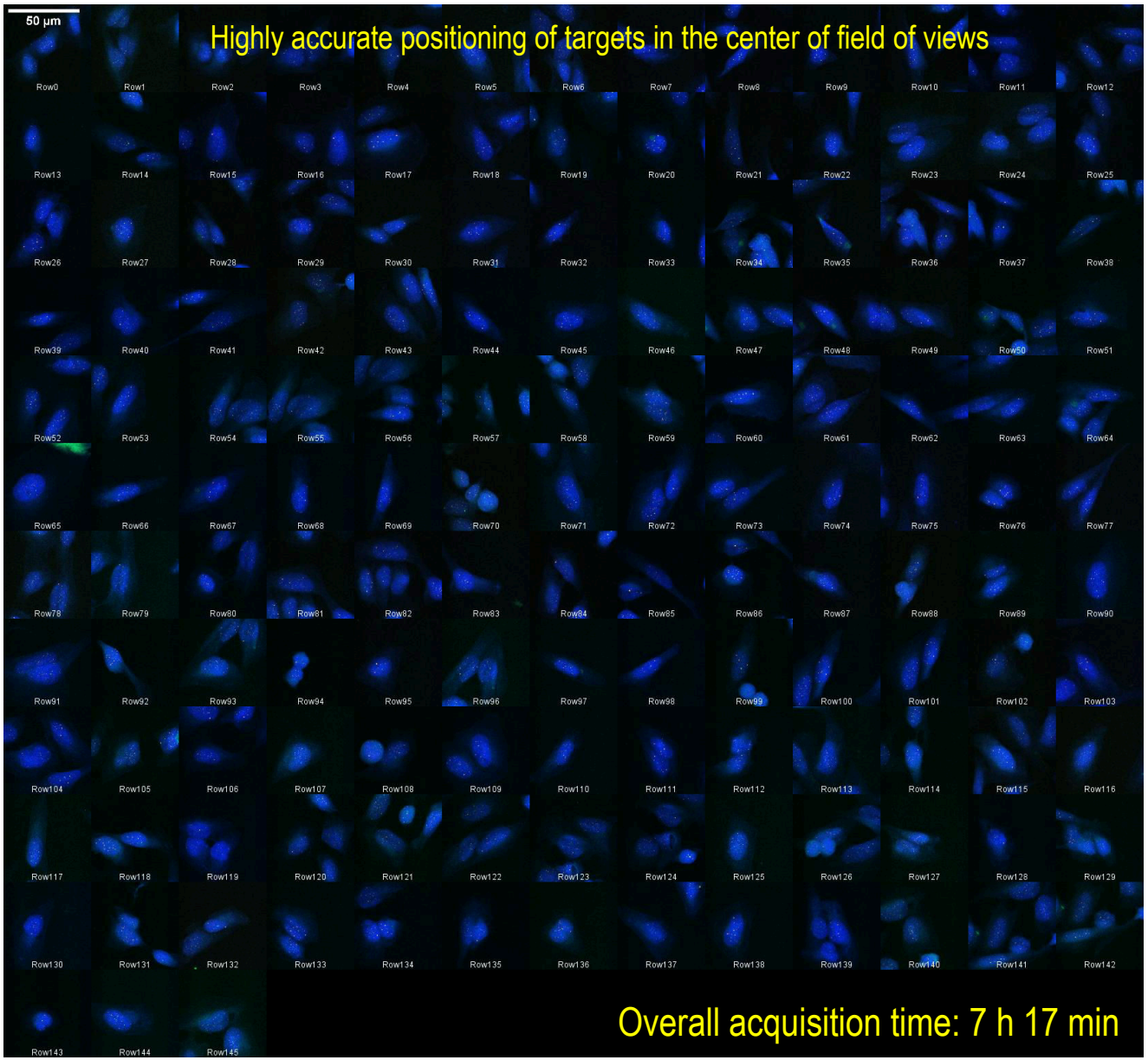
Standard DAQ *Config 2*

» Screening time: ~70h

» junk data / less targets (NOT centered !)

Automated Microscopy





Experiment setup

Overall sample size:
8 * 12 = 96 scan fields

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» Screening time: ~70h

» junk data / less targets (NOT centered !)

Automated Imaging

Pre-Screen *Config 1*

Target Screen *Config 2*

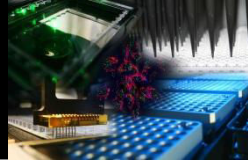
» Screening time: ~7h

» 146 target regions (in center of field of view !)

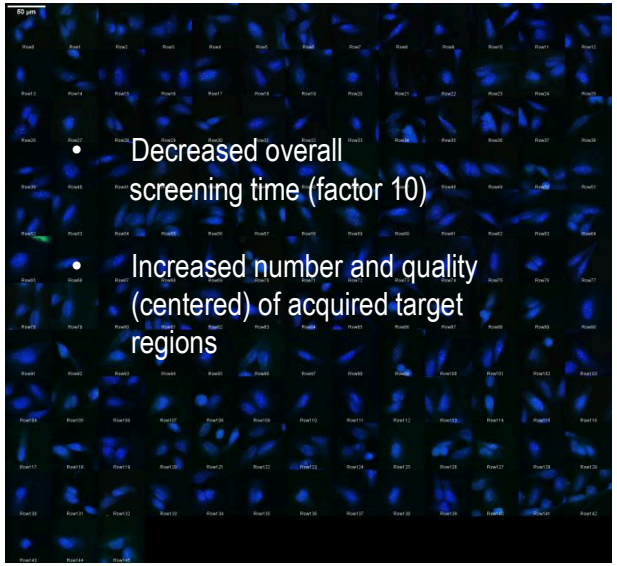
- Decreased overall screening time (factor 10)
- Increased number and quality (centered) of acquired target regions

Automated Microscopy

Some Numbers…



Costs confocal microscopy: 40€ per hour



- Decreased overall screening time (factor 10)
- Increased number and quality (centered) of acquired target regions

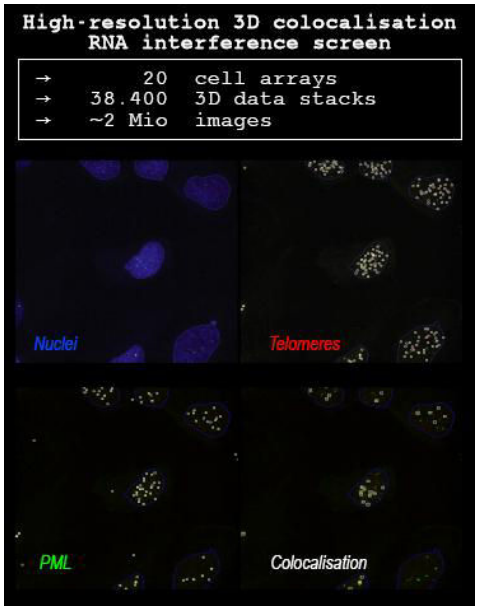
Overall sample size:
96 positions

Standard DAQ *job2*
» Screening time: ~70h

2800 €

Automated Imaging
Pre-Screen ***job1***
Target Screen ***job2***
» Screening time: ~7h

280 €



Overall sample size:
38.400 positions

Standard DAQ ***job2***
» Screening time: 6 months
» ~40% junk data

170.000 €

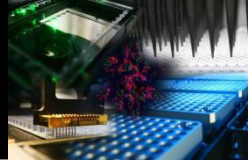
Automated Imaging
Pre-Screen *job1*
Target Screen *job2*
» Screening time: 3,6 months

104.000 €

~66.000 €
junk data

Perspectives…

Usability for **basic user**



Data Analysis

- Image Processing
- Statistics
- Bioinformatics

Data Management

- Data storage
- File access
- File formats

Visualisation

- (Image) Raw data
- (Intermediate) Results
- Hists / Plots / Viewer

Integrated Data Analysis

- Quality control
- Hit/Event identification
- Optical configuration

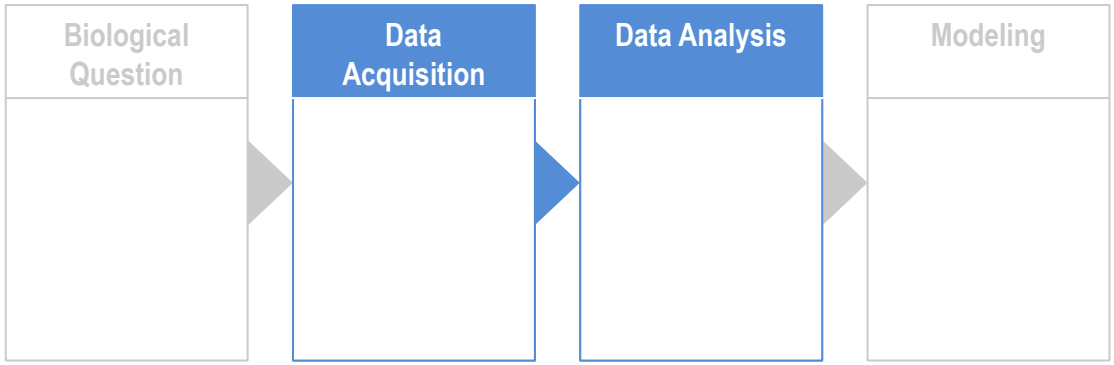
Meta Data

- Experiment
- Data Analysis
- Annotations

Data Bases

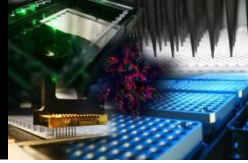
- Internal access
- External access
- Correlations

- Design automated workflow solutions
- Workflow repository



Perspectives…

Usability for **basic user**



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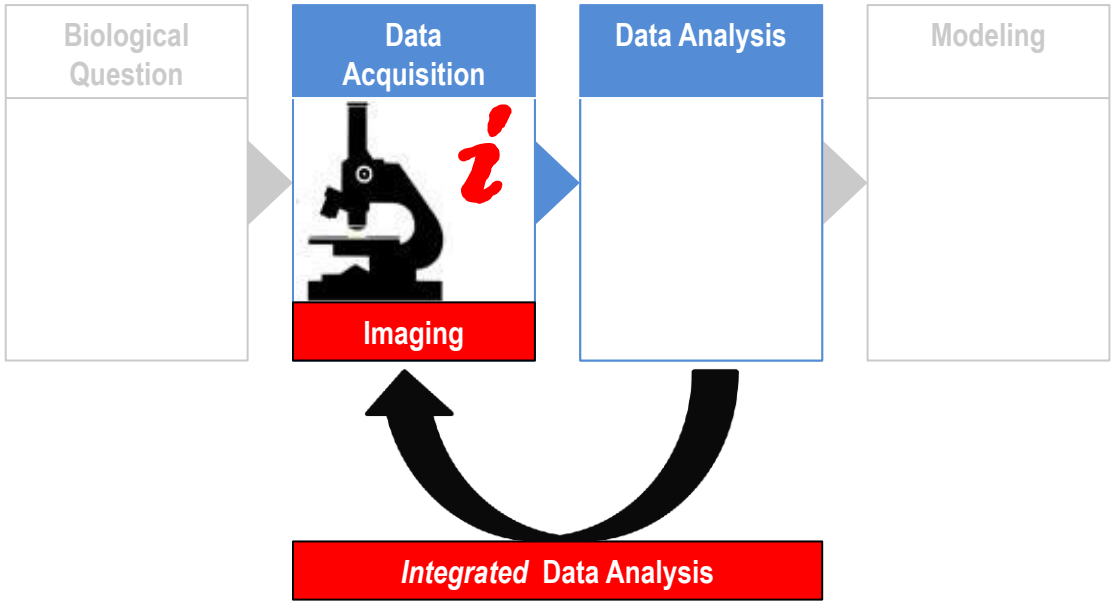
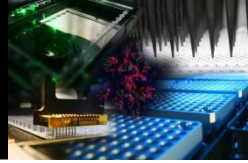


Acknowledgements



Holger Erfle
Nina Beil
Jürgen Beneke
Ruben Bulkescher
Manuel Gunkel
Tautvydas Lisauskas
Bastian Schumacher

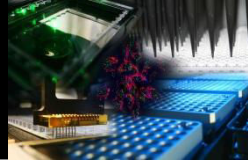
Michael Berthold
Christian Dietz
Thomas Gabriel
Martin Horn
Michael Zinsmeier

Frank Sieckmann
Constantin Kappel
Werner Knebel

Mike Heilemann
Benjamin Flottmann

Perspectives…

Bioinformatics



Joined table - 0:717:681 - Joiner(Library)

File

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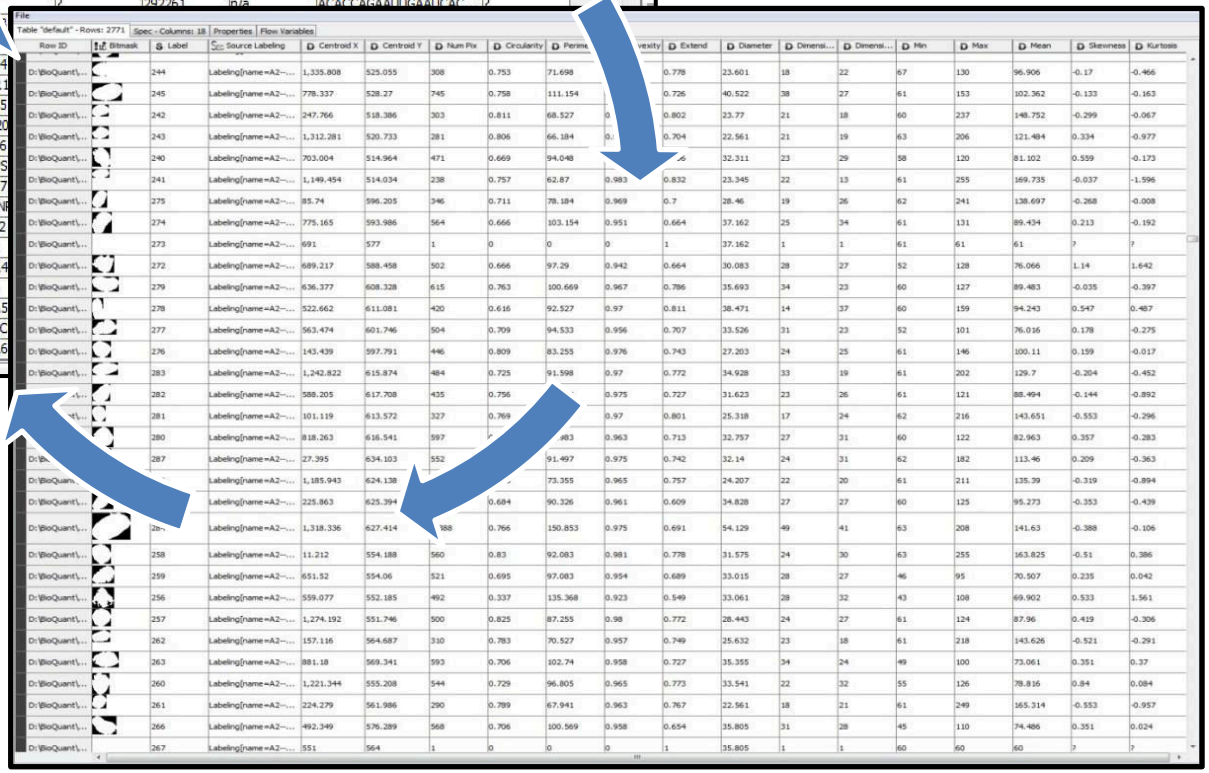
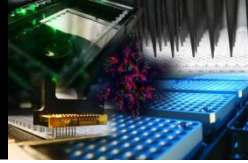
Row96_Row1	Plate I ExtDrg	Plate 2	A1	A2	CDH17	1015	14164	NM_004063	GGGACUUCUGUAUUACAA…	?
Row1_Row2	Plate I ExtDrg	Plate 1	A2	A3	C1QBP	708	9564	NM_001212	GGAAAGAAAAAUUCAGAA…	?
Row97_Row3	Plate I ExtDrg	Plate 2	A2	A4	MCP	4179	40648	NM_172356	GGAUACUUCUAUAUACCU…	down
Row2_Row4	Plate I ExtDrg	Plate 1	A3	A5	GFP	?	215041	?	CAAGCU…	?
Row98_Row5	Plate I ExtDrg	Plate 2	A3	A6	CD74	972	14500			?
Row3_Row6	Plate I ExtDrg	Plate 1	A4	A7	BIN3	55909	27292			?
Row99_Row7	Plate I ExtDrg	Plate 2	A4	A8	CCL8	6355	16649			?
Row4_Row8	Plate I ExtDrg	Plate 1	A5	A9	BAG3	9531	137538	NM_004281	GCC…UGAUGUCCCAGGU…	?
Row100_Row9	Plate I ExtDrg	Plate 2	A5	A10	CCL2	6347	44845	NM_002982	GCCAGAUGCAAUCAAUGC…	?
Row5_Row10	Plate I ExtDrg	Plate 1	A6	A11	ARID5A	10865	216172	NM_212481	GCAGAACGGAAUCCAGAA…	?
Row101_Row11	Plate I ExtDrg	Plate 2	A6	A12	CLPX	10845	105146	NM_006660	GGUAAAACUCUGCUGGCA…	?
Row6_Row12	Plate I ExtDrg	Plate 1	A7	A13	AP1M1	8907	34372	NM_032493	GGUGUUUUCCGAGUACUU…	?
Row102_Row13	Plate I ExtDrg	Plate 2	A7	A14	CLDN23	137075	128552	NM_194284	GGUCCUAAUGCUGUACUA…	down
Row7_Row14	Plate I ExtDrg	Plate 1	A8	A15		?	292261	n/a	ACACCAGAAUUGAAUCAC…	?
Row103_Row15	Plate I ExtDrg	Plate 2	A8	A16						
Row8_Row16	Plate I ExtDrg	Plate 1	A9	A17						
Row104_Row17	Plate I ExtDrg	Plate 2	A9	A18						
Row9_Row18	Plate I ExtDrg	Plate 1	A10	A19						
Row105_Row19	Plate I ExtDrg	Plate 2	A10	A20						
Row10_Row20	Plate I ExtDrg	Plate 1	A11	A21						
Row106_Row21	Plate I ExtDrg	Plate 2	A11	A22						
Row11_Row22	Plate I ExtDrg	Plate 1	A12	A23						
Row107_Row23	Plate I ExtDrg	Plate 2	A12	A24						
Row192_Row24	Plate I ExtDrg	Plate 3	A1	B1						
Row288_Row25	Plate I ExtDrg	Plate 4	A1	B2						
Row193_Row26	Plate I ExtDrg	Plate 3	A2	B3						
Row289_Row27	Plate I ExtDrg	Plate 4	A2	B4						
Row194_Row28	Plate I ExtDrg	Plate 3	A3	B5						
Row290_Row29	Plate I ExtDrg	Plate 4	A3	B6						
Row195_Row30	Plate I ExtDrg	Plate 3	A4	B7						
Row291_Row31	Plate I ExtDrg	Plate 4	A4	B8						



Perspectives…

Bioinformatics



Joined table - 0:717:681 - Joiner(Library)

File

Table "default" - Rows: 384 | Spec - Columns: 10 | Properties | Flow Variables

Row ID	Plate ID	96er Plate	Well (on 96er Plate)	Well (on 384er Plate)	Gene	Gene ID	siRNA ID	Ref Seq	siRNA Seq	Control
Row0_Row0	Plate I ExtDrg	Plate 1	A1	A1	INCENP	3619	28431	NM_020238	GGAGAAGAAGAAGCAGAU…	?
Row96_Row1	Plate I ExtDrg	Plate 2	A1	A2	CDH17	1015	14164	NM_004063	GGGACUUCUGUAUUACAA…	?
Row1_Row2	Plate I ExtDrg	Plate 1	A2	A3	C1QBP	708	9564	NM_001212	GGAAAGAAAAAUUCAGAA…	?
Row97_Row3	Plate I ExtDrg	Plate 2	A2	A4	MCP	4179	40648	NM_172356	GGAUACUUCUAUAUACCU…	down
Row2_Row4	Plate I ExtDrg	Plate 1	A3	A5	GFP	?	215041	?	CAAGCU…	?
Row98_Row5	Plate I ExtDrg	Plate 2	A3	A6	CD74	972	14500			?
Row3_Row6	Plate I ExtDrg	Plate 1	A4	A7	BIN3	55909	27292			?
Row99_Row7	Plate I ExtDrg	Plate 2	A4	A8	CCL8	6355	16649			?
Row4_Row8	Plate I ExtDrg	Plate 1	A5	A9	BAG3	9531	137538	NM_004281	GCC…UGAUGUCCCAGGU…	?
Row100_Row9	Plate I ExtDrg	Plate 2	A5	A10	CCL2	6347	44845	NM_002982	GCCAGAUGCAAUCAAUGC…	?
Row5_Row10	Plate I ExtDrg	Plate 1	A6	A11	ARID5A	10865	216172	NM_212481	GCAGAACGGAAUCCAGAA…	?
Row101_Row11	Plate I ExtDrg	Plate 2	A6	A12	CLPX	10845	105146	NM_006660	GGUAAAACUCUGCUGGCA…	?
Row6_Row12	Plate I ExtDrg	Plate 1	A7	A13	AP1M1	8907	34372	NM_032493	GGUGUUUUCCGAGUACUU…	?
Row102_Row13	Plate I ExtDrg	Plate 2	A7	A14	CLDN23	137075	128552	NM_194284	GGUCCUAAUGCUGUACUA…	down
Row7_Row14	Plate I ExtDrg	Plate 1	A8	A15		?	292261	n/a	ACACCAGAAUUGAAUCAC…	?
Row103_Row15	Plate I ExtDrg	Plate 2	A8	A16		1365	9801	NM_001306	GGACUACGUCUAAGGGAC…	?
Row8_Row16	Plate I ExtDrg	Plate 1	A9	A17		865	6569	NM_022845	GGGAGAACAGCGACAAAC…	?
Row104_Row17	Plate I ExtDrg	Plate 2	A9	A18	…N4	1364	9892	NM_001305	GGUGCUGUAAACAGGUUU…	?
Row9_Row18	Plate I ExtDrg	Plate 1	A10	A19	…AR1	55749	148709	NM_018237	GCAAACCCUCUUAACACA…	?
Row105_Row19	Plate I ExtDrg	Plate 2	A10	A20	…LDN5	7122	12866	NM_003277	GGCUAAGAAUCUGCUUAG…	?
Row10_Row20	Plate I ExtDrg	Plate 1	A11	A21	…TIP120A					
Row106_Row21	Plate I ExtDrg	Plate 2	A11	A22	CLDN6					
Row11_Row22	Plate I ExtDrg	Plate 1	A12	A23	CADPS2					
Row107_Row23	Plate I ExtDrg	Plate 2	A12	A24	CLDN7					
Row192_Row24	Plate I ExtDrg	Plate 3	A1	B1	INCENP					
Row288_Row25	Plate I ExtDrg	Plate 4	A1	B2	MYLK2					
Row193_Row26	Plate I ExtDrg	Plate 3	A2	B3	DSC2					
Row289_Row27	Plate I ExtDrg	Plate 4	A2	B4	GATA4					
Row194_Row28	Plate I ExtDrg	Plate 3	A3	B5	DSC3	1825	10876	NM_024423	GGCAGACAAAAUAAUGGG…	up
Row290_Row29	Plate I ExtDrg	Plate 4	A3	B6	GATA5	140628	6857	NM_080473	GGGAAAGUGAAAUAAAGC…	?
Row195_Row30	Plate I ExtDrg	Plate 3	A4	B7	DNAJC5G	285126	41208	NM_173650	GGUCAGGAGCCAAAUGUG…	?
Row291_Row31	Plate I ExtDrg	Plate 4	A4	B8	GATA6	2627	3223	NM_005257	GGAAACGAAAACCUAAGA…	?

Nur Bewegung

How could Galaxy contribute ?