

MLSCN @ CU & OME

Molecular Library Screening Center Network
at Columbia University and the Open
Microscopy Environment

Bernd Jagla, 4/22/2006

MLSCN

- This is a nationwide consortium of small molecule screening centers that has been recently funded to produce innovative chemical tools for use in biological research.
- The MLSCN performs HTS on assays provided by the research community, against a large library of small molecules maintained in a central molecule repository.
- The MLSCN has established a [collection of 100,000 chemically diverse small molecules](#).
- All of the results from the MLSCN's activities will be placed into a public database called [PubChem](#).

Columbia MLSC

- The Columbia Center in the MLSCN focuses on cell biology, high content/high resolution automated cellular imaging and image analysis, and phenotypic assay design and implementation.
- Primary screening will be done with high content assays at HTS performed in intact cells.
- Assay development will initially focus on establishing a repertoire of >50 assays providing broad coverage of signaling pathways, and associated bioinformatics tools.
- Profiling of hits and leads against this repertoire of biology will provide important information on specificity at the biological level to complement information on the compound's selectivity at the protein/target level.

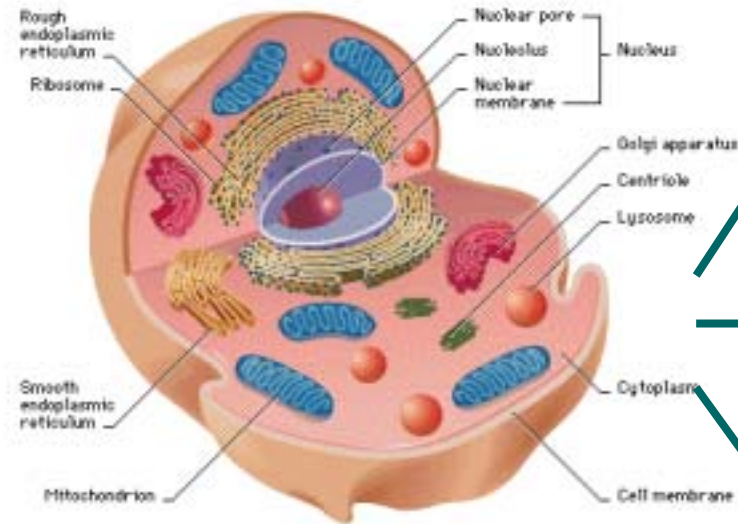
Who is involved?

- James E. Rothman (PI)
- Lars Branden (Project manager)
- Thomas Mayer (assay dev.)
- M. Beard (assay dev.)
- Deby Smith (assay dev.)
- Effie Tzilianos (assay dev.)
- Feng-Li Zhang (assay dev.)
- Mike Wyler (assay dev.)
- Nathalie Aulner (HTS)
- Udo Többen (HTS)
- Bernd Jagla (IT)
- Geoff Barger (automation)
- Martine Lecorps (secretary)
- Collaborator: Ai Yamamoto
- OME:
 - Ilya Goldberg
 - Harry Hochheiser
 - Josiah Johnston
 - Jason Swedlow
- Partek
 - DJ Meyer
 - Michael J. Venezia
- GE
 - Rick Maguire
 - Binayak Roy
 - Sarang Parnaik
 - Marcin Swiatek

Biological profiling

Assays

Cell types

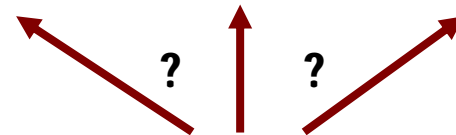


Metabolism

Gene expression levels

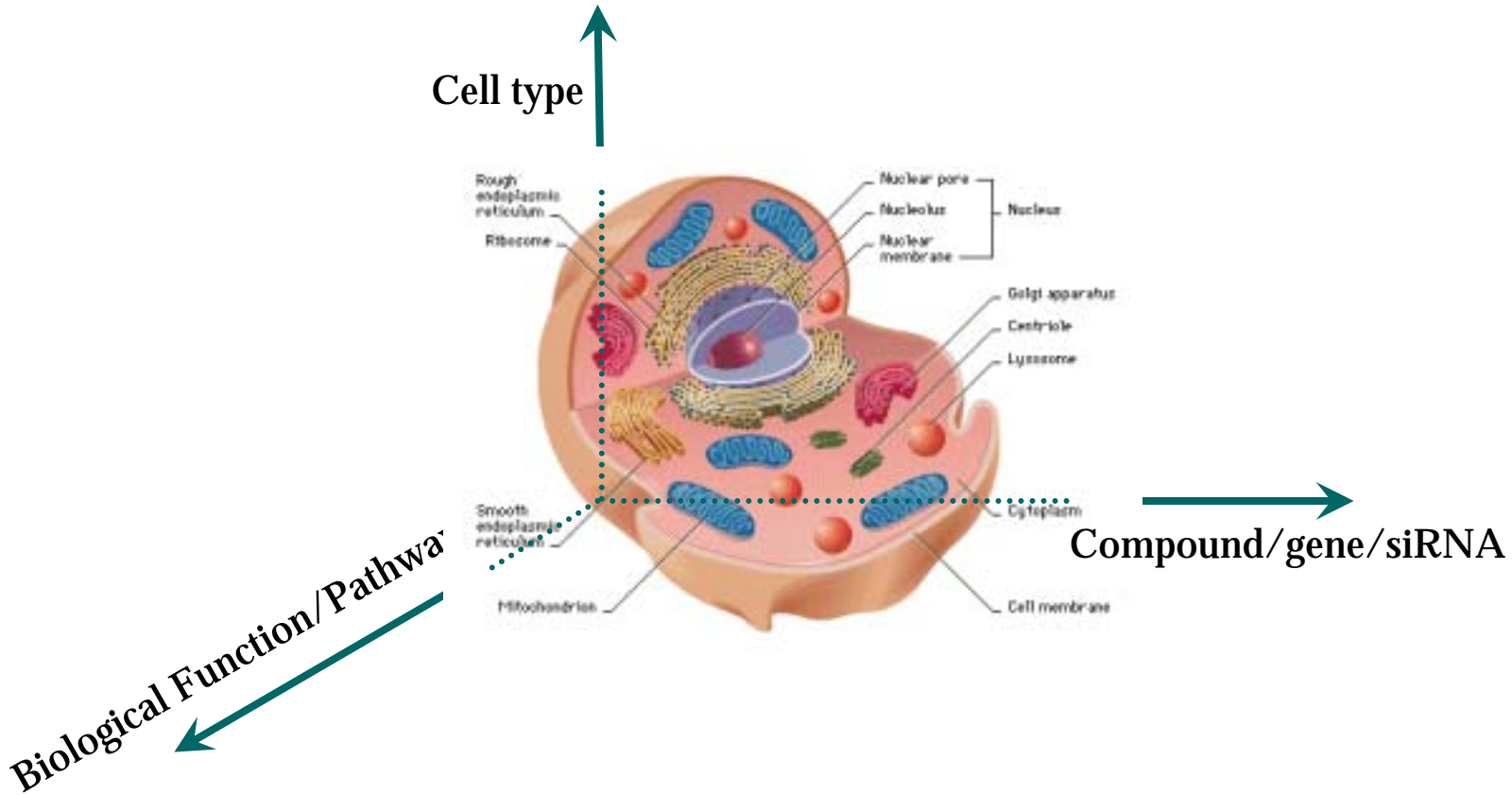
Response to stimuli

...



compound

”Three Dimensional” Biological Profiling



Cell-based “high content” functional assays



- **Automated confocal microscopy**
- **Up to 30,000 wells per day**
- Simultaneous 3 color detection
- Quantitative data analysis
- Three years' experience using prototype of GE INCell 3000 Analyzer

Biological Profiling: Specificity and Mechanism

Protein Binding Profiling

Biological Process/Pathway Profiling

Biological Processes (20)

Apoptosis
Inflammation
Cell cycle
DNA repair
Secretion
Transcription
Etc etc

Pathways (40)

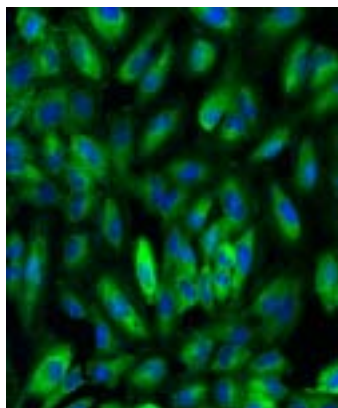
Receptor activation
Kinase activation
Transcriptional activation
Intracellular signalling
Etc etc

ADRBK1
Akt1
Akt2
Akt3
CaMKI d
CaMKII d
CaMKII
CaMKIV
CDK1/cyclin B
CDK2/cyclin A
CDK5/p35
CHEK1
CHEK2
CLK1
CSNK1D
CSNK1G2
CSNK2A1
CSNK2A2
DAPK2
IRAK4
MAP2K1
MAP2K2
MAPK3
MAPKAP-K5
PKA
PIM2
PRKD2
ROCK1
RPS6KA1
TBK1

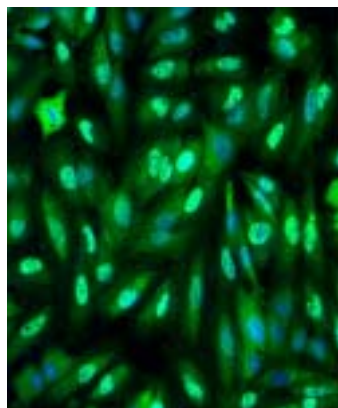
Compound X →



Gene-Plus collection (960 compounds) screen



Control



**TNF α stimulation
(positive control)**

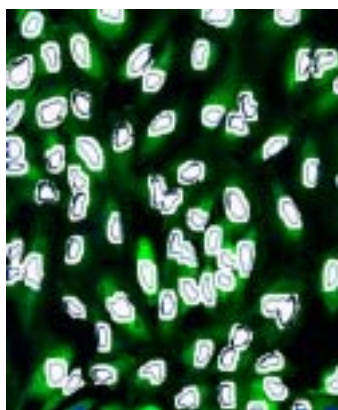
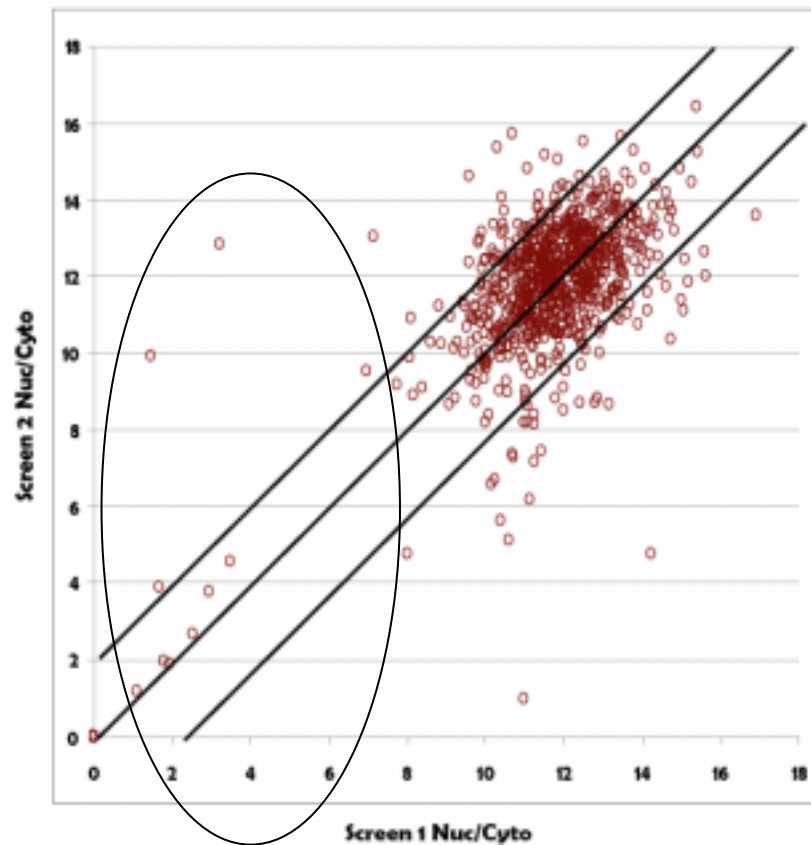
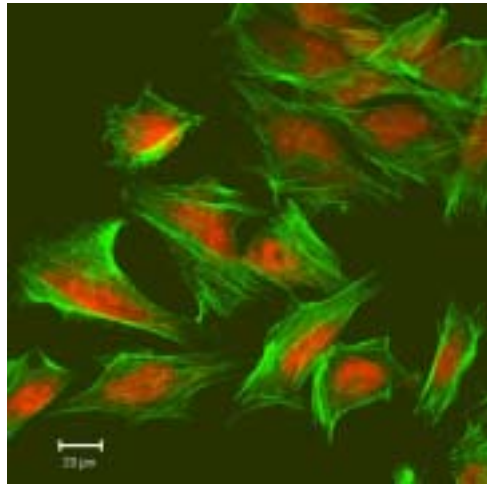
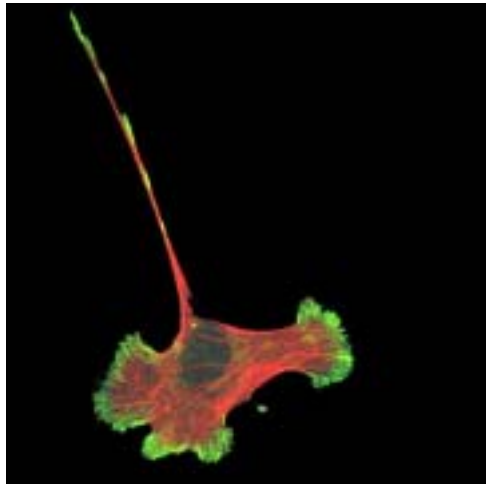


Image Analysis



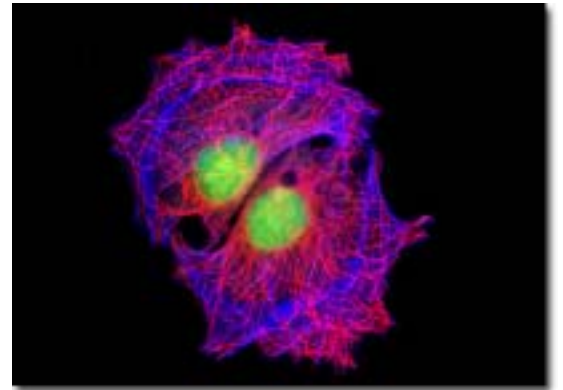
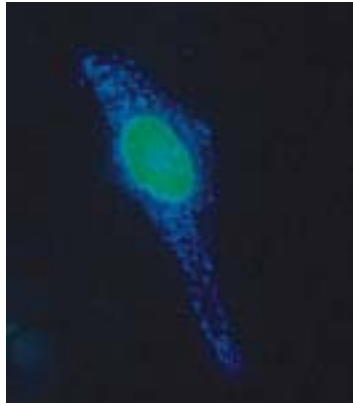


osteoblast

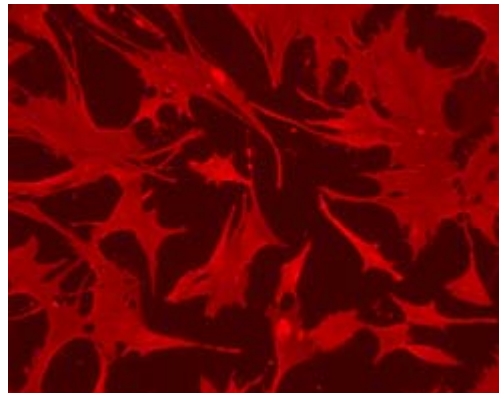
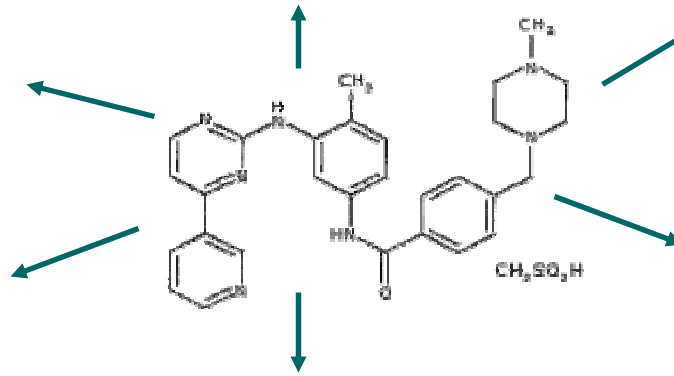


neuroblast

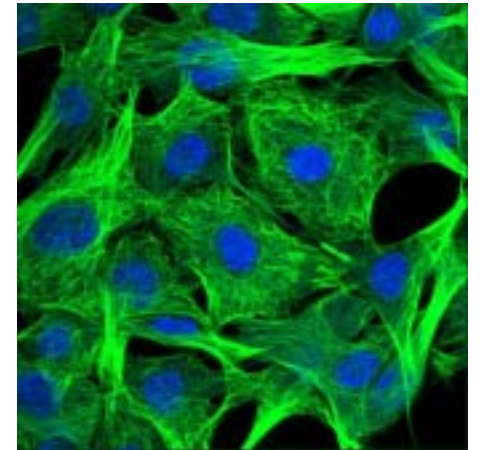
HeLa



myoblast

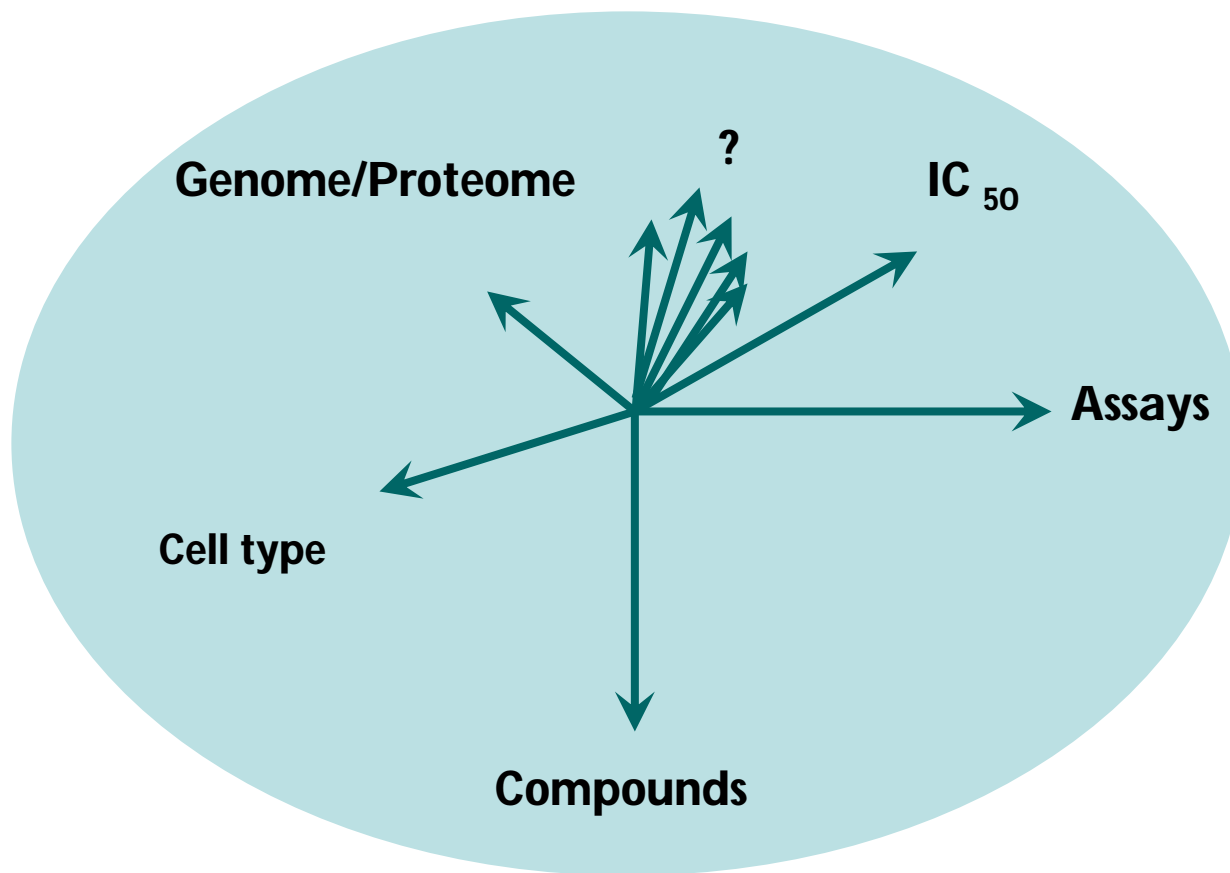


adipocyte



fibroblast

Dimensions of the profiling matrix



Known compound vs. unknown compound



New Knowledge

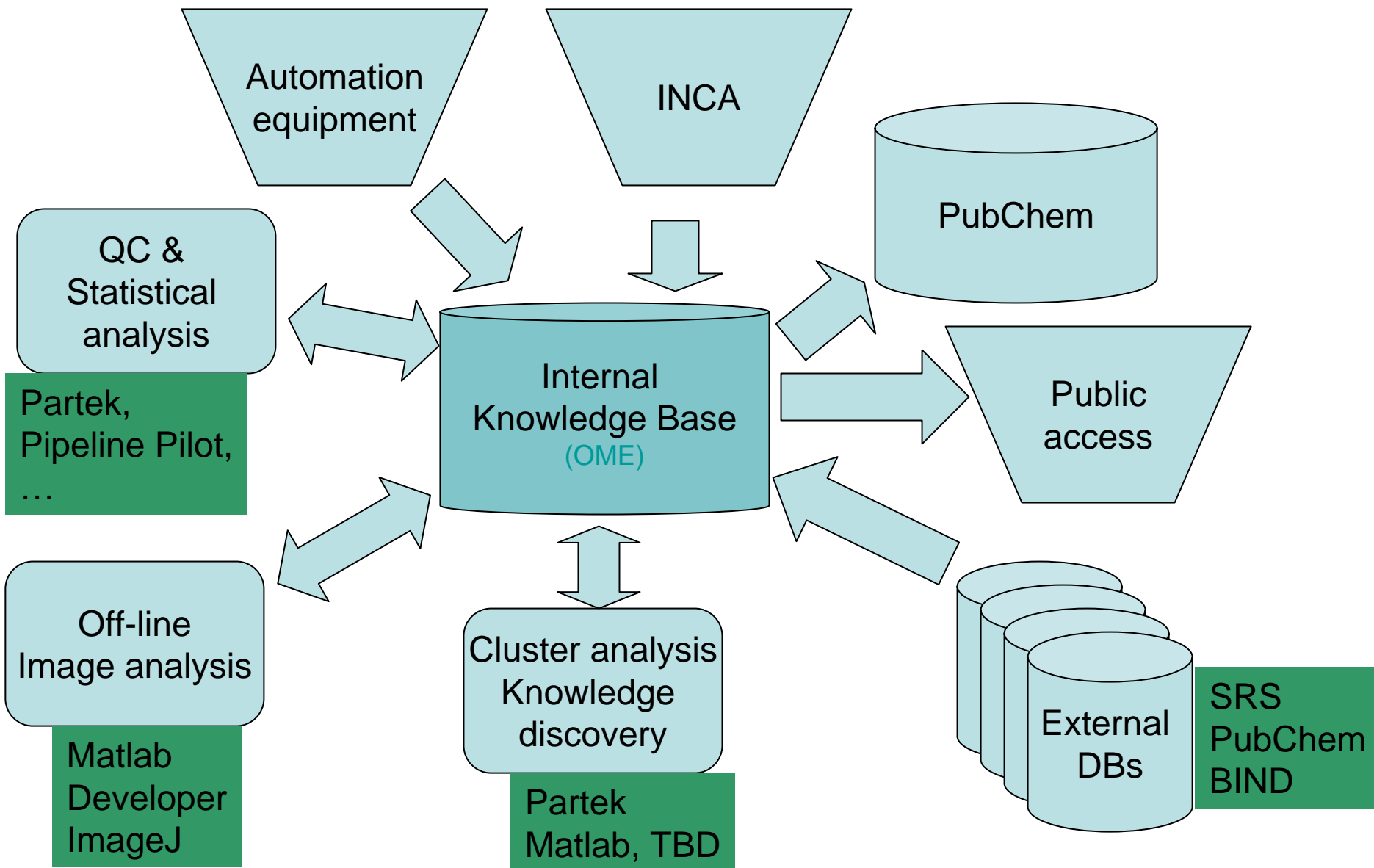


Compound ID/fingerprint



Insight into mechanism of action

Tasks modularized



Results

- Run files from INCA can be read
 - Original format can be read in and exported files (tiff, xml)
 - Plate – image relationship is transferred to OME
- Frm (image) files from INCA can be
 - Decompressed
 - Read into OME
- Analysis files
 - Mapped to corresponding runs/plates/images
 - Values are imported into OME

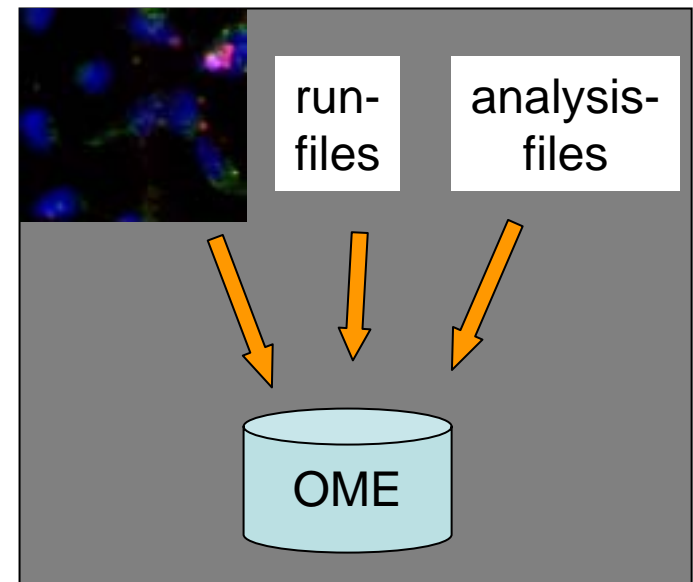


Image view

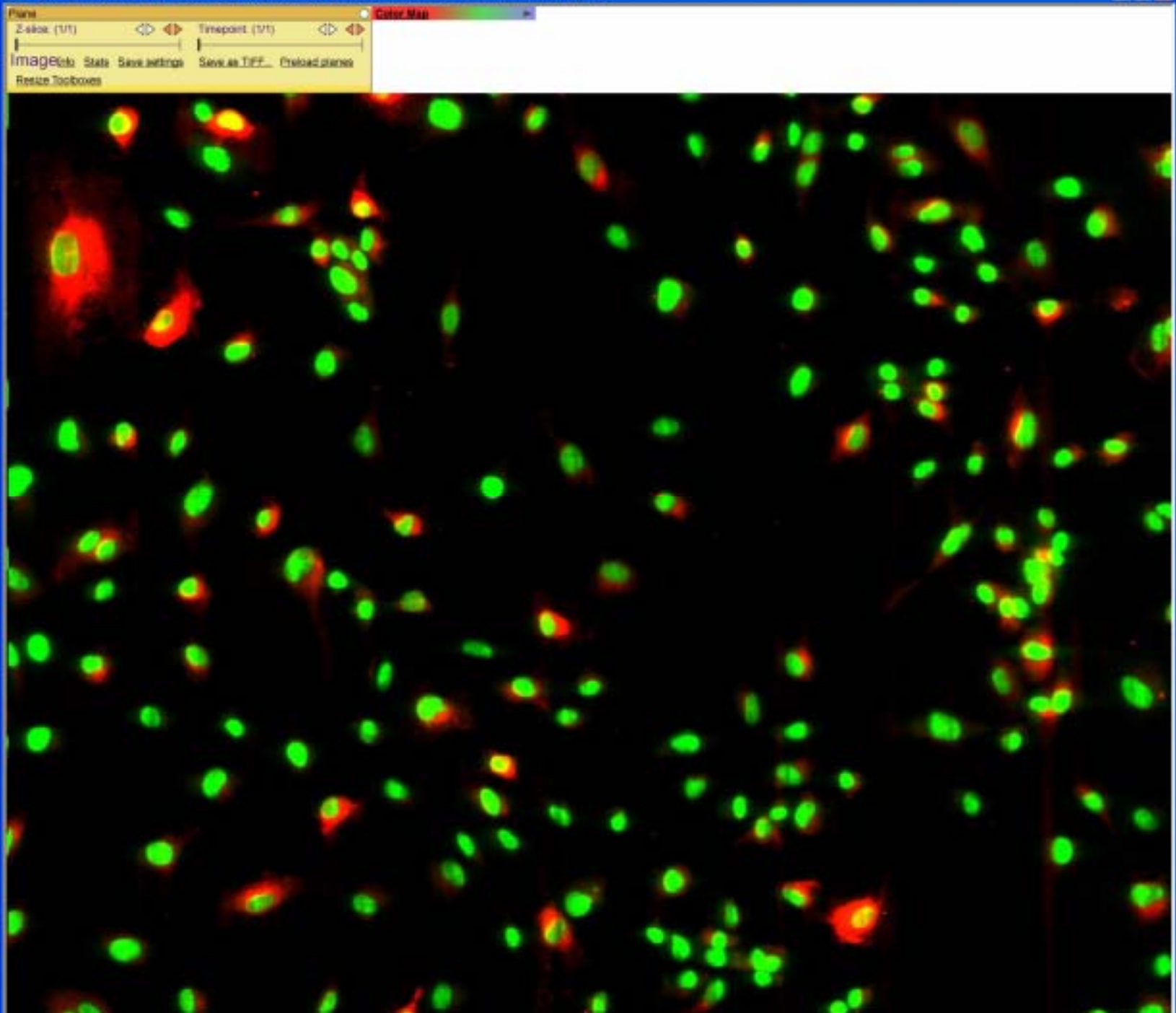
The screenshot displays the Open Microscopy Environment (OME) web interface. The browser window title is "Search for Image - Microsoft Internet Explorer". The address bar shows the URL: <http://156.146.195.172/ome/omePage/OME-Web/Search/SearchType/OME-Image>. The page title is "Open Microscopy Environment v2.4.1".

The interface includes a search bar with the text "Search" and a "From Dataset:" dropdown menu set to "Select a Dataset". Below the search bar are several filter criteria:

- That match criteria:
- Name:
- Description:
- Created:
- Inserted:
- Owner: Bernd Jägle
- GROUP: ME
- ID:

The search results are displayed in a grid format. The first row of results shows three entries, each with a thumbnail image, the name "Bernd Jägle OME", and a timestamp. Below each thumbnail is a dropdown menu labeled "ModuleExecution(s)".
















Thumbnail	Name	Timestamp
	Bernd Jägle OME	2005-03-24 18:07:40
	Bernd Jägle OME	2005-03-24 18:07:09
	Bernd Jägle OME	2005-03-24 18:07:20
	Bernd Jägle OME	2005-03-24 18:07:31
	Bernd Jägle OME	2005-03-24 18:07:41
	Bernd Jägle OME	2005-03-24 18:07:50
	Bernd Jägle OME	2005-03-24 18:07:59
	Bernd Jägle OME	2005-03-24 18:07:00
	Bernd Jägle OME	2005-03-24 18:07:11
	Bernd Jägle OME	2005-03-24 18:07:22
	Bernd Jägle OME	2005-03-24 18:07:32
	Bernd Jägle OME	2005-03-24 18:07:42
	Bernd Jägle OME	2005-03-24 18:07:51
	Bernd Jägle OME	2005-03-24 18:08:00
	Bernd Jägle OME	2005-03-24 18:07:01
	Bernd Jägle OME	2005-03-24 18:07:12
	Bernd Jägle OME	2005-03-24 18:07:23
	Bernd Jägle OME	2005-03-24 18:07:33
	Bernd Jägle OME	2005-03-24 18:07:43
	Bernd Jägle OME	2005-03-24 18:07:52
	Bernd Jägle OME	2005-03-24 18:08:01



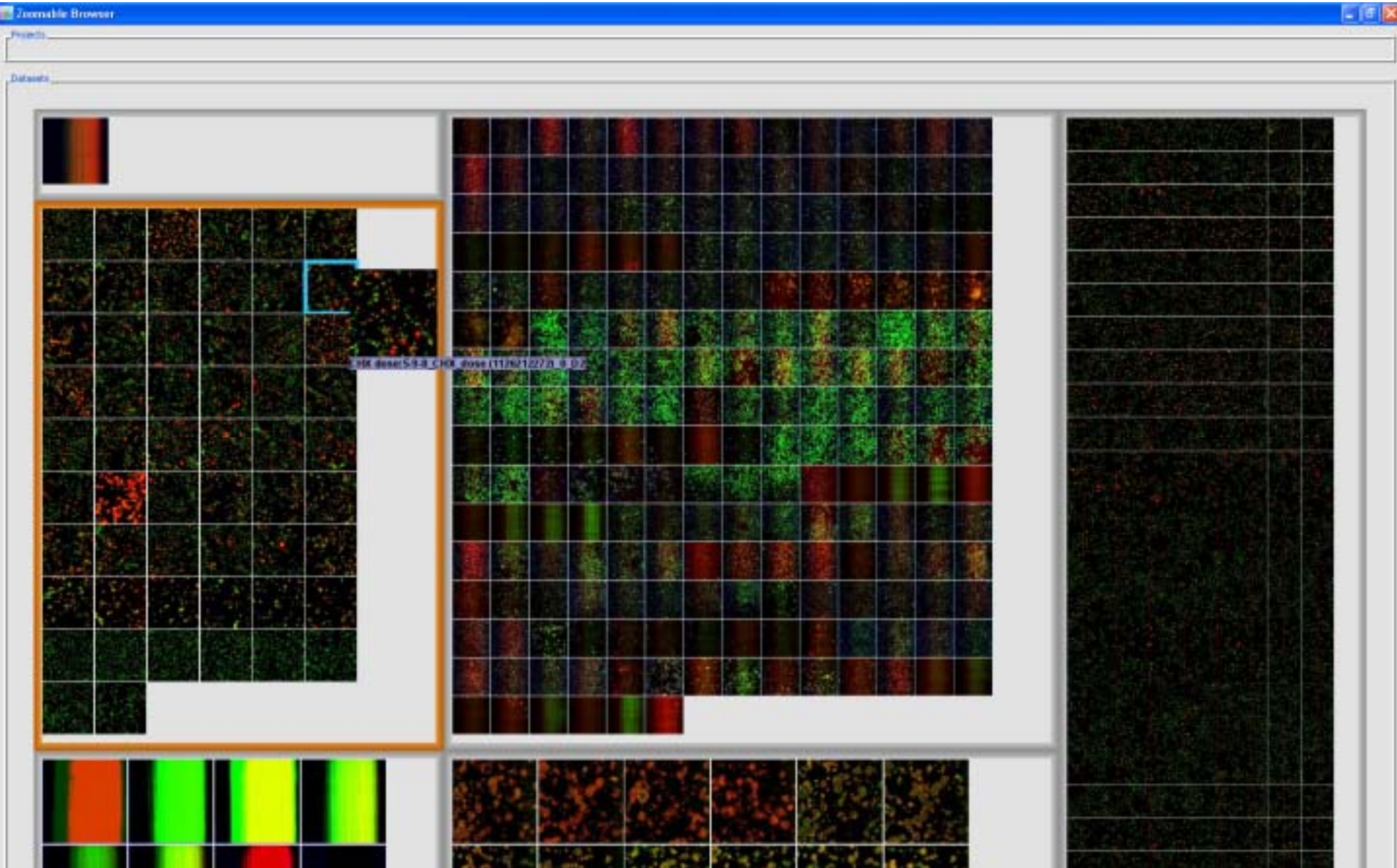
989	9/8/2005	NA	200.00	None	0	Y	C siders above bkgrnd	[9; 250]	Off	15	Y	Blue	Green	S-G- S_CHK_dose	None	4:44:32 PM	matt
-----	----------	----	--------	------	---	---	-----------------------	----------	-----	----	---	------	-------	--------------------	------	------------	------

ObjectIntensity_0_values

[Download as bit 1 of 3 >>](#)

ID	Cycle	Hist Pl	Ineg	Ipos	Meg	Mneg	Mpass	Npos	Ntot	Parameters	Flate	SDneg	SDpos	Well	neg	pos	Image
1045	0	94	0	150.56	0	0	95	95	101	989	CHX dose 0	0	58.08	H8	0	100	
1044	0	93	0	164.97	0	0	271	271	284	989	CHX dose 0	0	101.95	E5	0	100	
1043	0	93	0	140.99	0	0	376	376	397	989	CHX dose 0	0	63.44	C3	0	100	
1042	0	91	0	138.83	0	0	460	460	474	989	CHX dose 0	0	57.37	B3	0	100	
1041	0	92	0	158.71	0	0	384	384	397	989	CHX dose 0	0	63.05	F5	0	100	
1040	0	88	0	144.81	0	0	119	119	124	989	CHX dose 0	0	75.4	F6	0	100	
1039	0	92	0	87.58	0	0	525	525	576	989	CHX dose 0	0	5.75	D7	0	100	
1038	0	98	0	149.33	0	0	315	315	332	989	CHX dose 0	0	83.66	H4	0	100	
1037	0	96	0	103.04	0	0	529	529	648	989	CHX dose 0	0	16.06	B7	0	100	
1036	0	96	0	154.43	0	0	325	325	345	989	CHX dose 0	0	82.33	G4	0	100	
1035	0	94	0	157.71	0	0	418	418	437	989	CHX dose 0	0	92.74	D4	0	100	
1034	0	92	0	132.12	0	0	468	468	483	989	CHX dose 0	0	61.11	A4	0	100	
1033	0	96	0	128.93	0	0	407	407	421	989	CHX dose 0	0	43.68	D1	0	100	
1032	0	91	0	98.16	0	0	469	469	538	989	CHX dose 0	0	17.39	C7	0	100	
1031	0	89	0	134.98	0	0	403	403	418	989	CHX dose 0	0	57.87	A5	0	100	

shoola

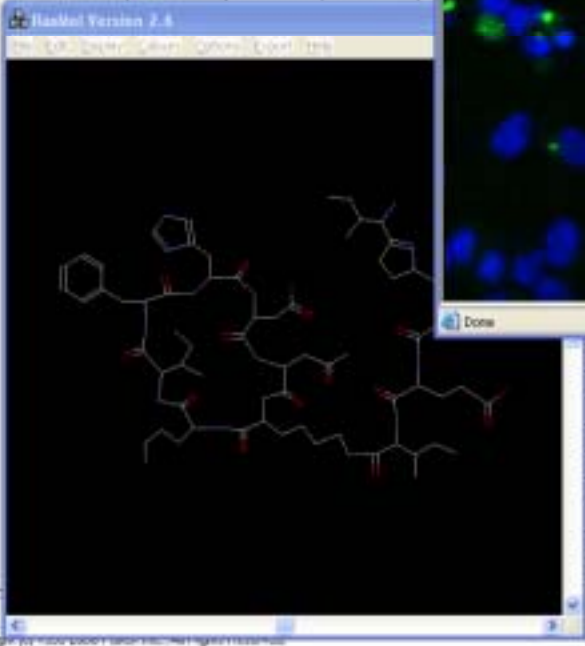


Statistical analysis of screening results

- Partek – Screeners solution
 - Statistics software for analyzing one screen
- Pipeline Pilot
 - Workflow management system for
 - statistical analysis
 - integration with chemical compound analysis products
 - Standardized analysis workflows

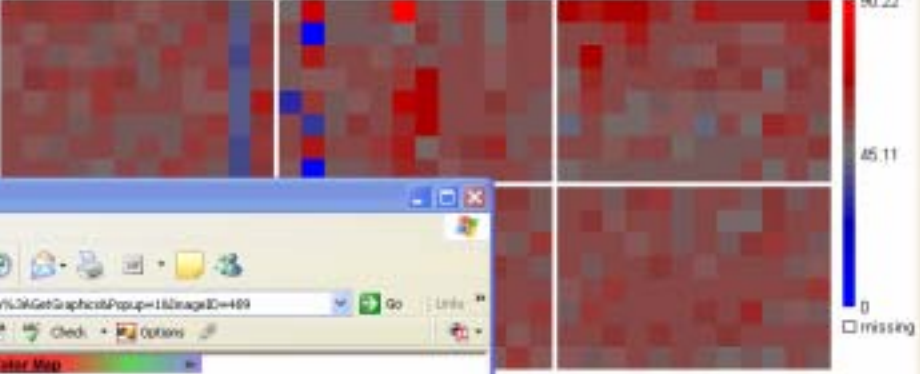

```
HasMol Command Line  
HasMol Molecular Reader  
Roger Bayle, August 1995  
Version 2.6  
  
Number of Groups ..... 1  
Number of Atoms ..... 100  
Number of Bonds ..... 103  
HasMol3
```

259	917	51.5	43
260	915	45.11	36
261	921	48.76	34
262	923	45.96	33
263	925	50.25	34
264	927	51.64	32



173.93	0.85	0.58	74
170.30	0.75	0.58	71
148.94	0.69	0.47	78
173.31	0.72	0.53	70
215.24	0.76	0.68	59
189.49	0.72	0.58	36
187.74	0.8	0.64	81
173.72	0.68	0.52	70

HTS Navigator: ol.sdl
Plate Grid



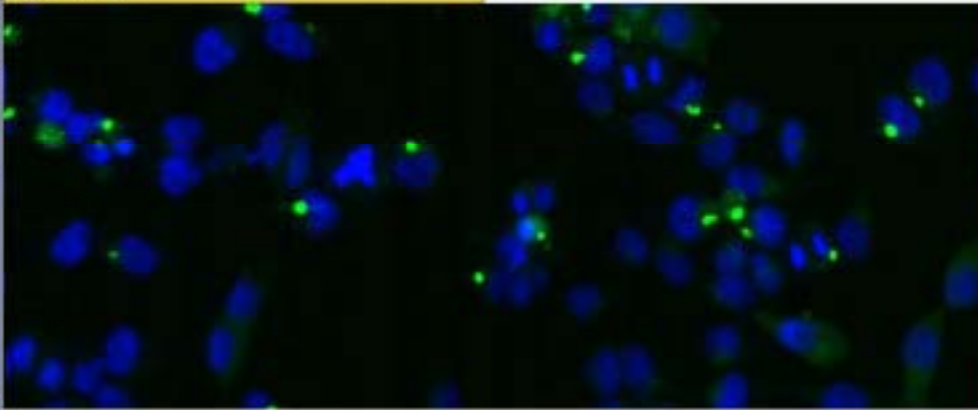
Plate

Z-slice (1/1) Timepoint (1/1)

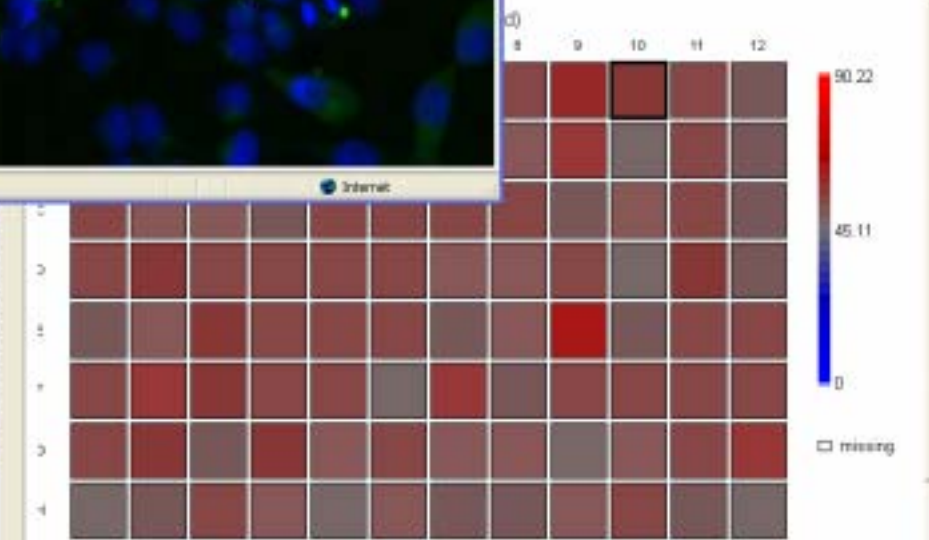
Color Map

Imagefile Save settings Save as TIFF... Pressed planes

Resize Toolboxes



Done



How is the link established

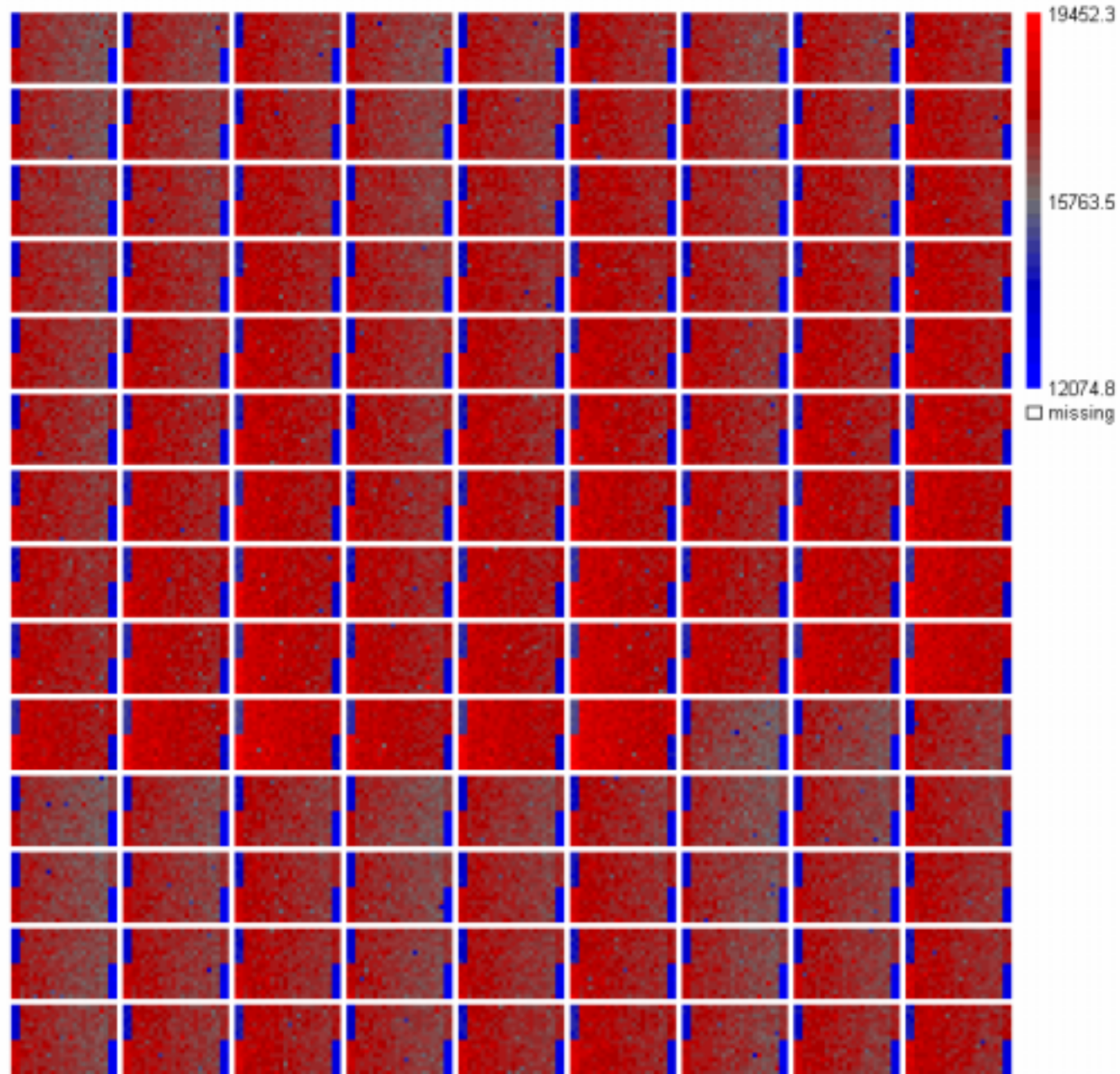
- set url

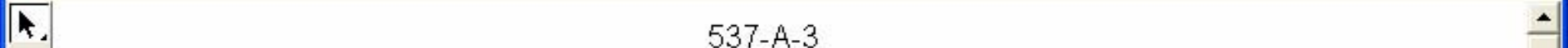
`http://156.145.195.172/perl2/serve.pl?Page=OME%3A%3AWeb%3A%3AGetGraphics&Popup=1&ImageID=${cid}`

Partek Screeners solution

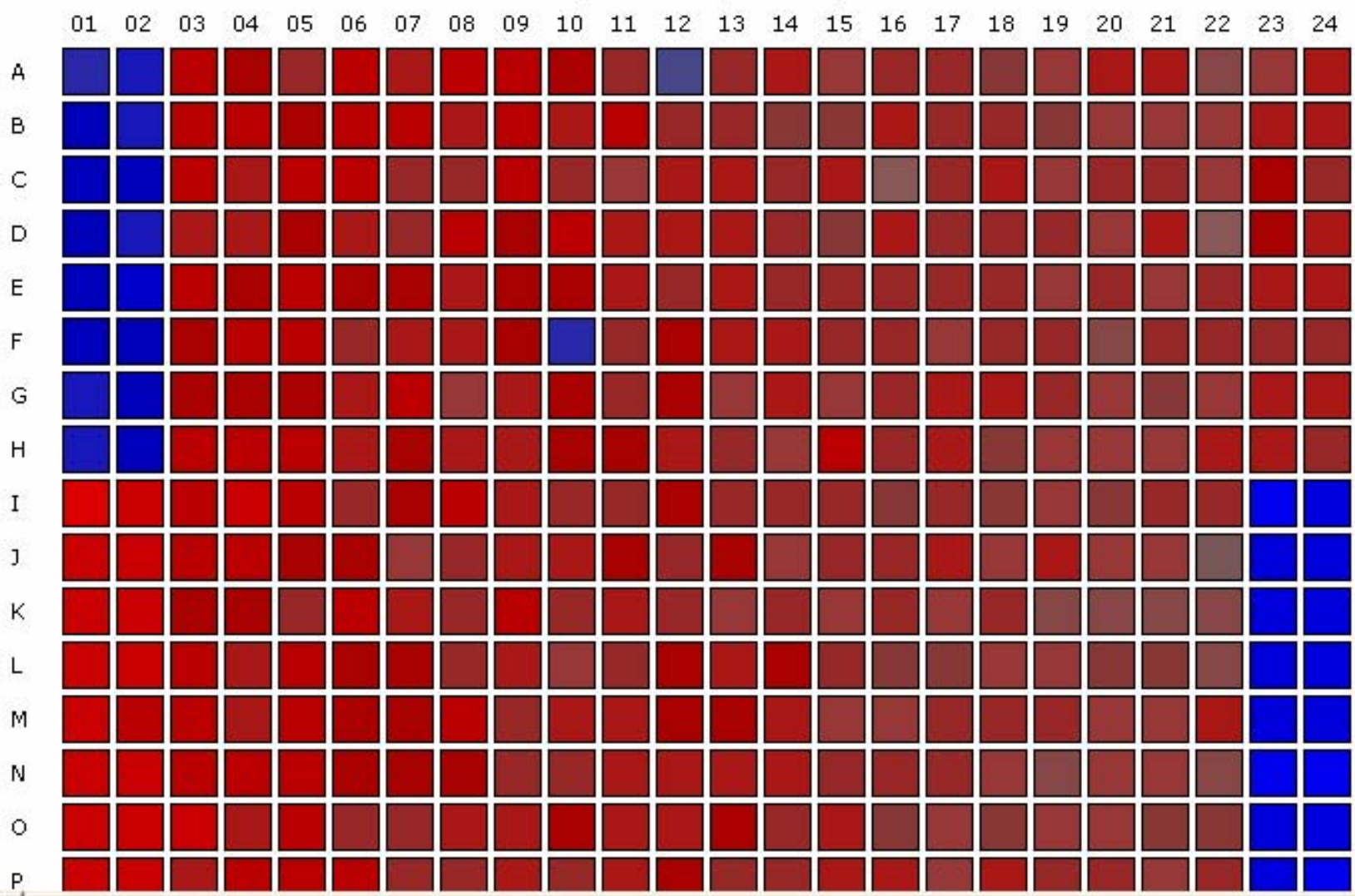
- Check for edge effects
- Z'
- Normalization tools
- Cluster of results
- Principal component analysis
- Clustering tools
- Chemical compound visualisation

Plate Grid

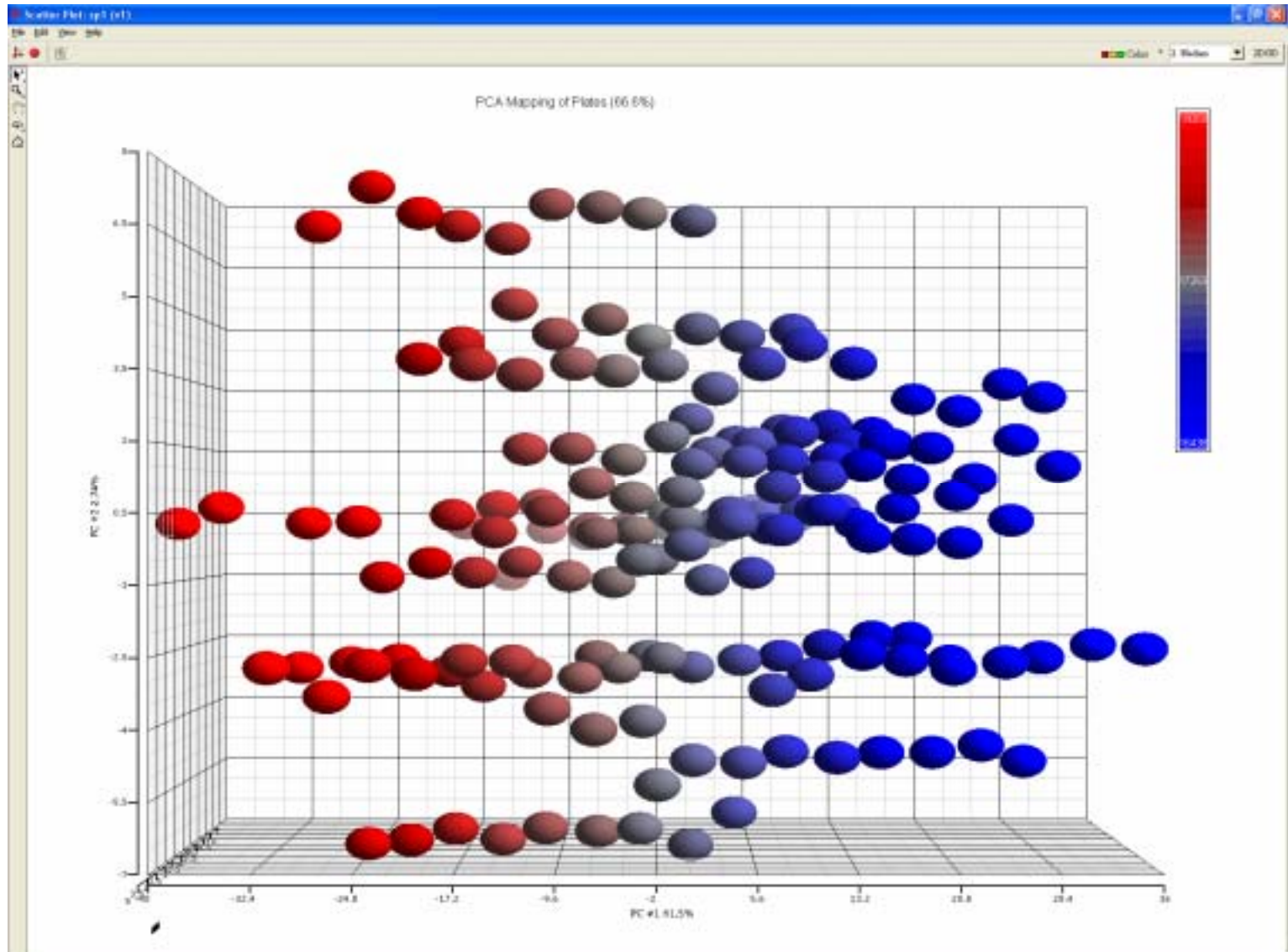




537-A-3
(Colored by Result)



PCA view



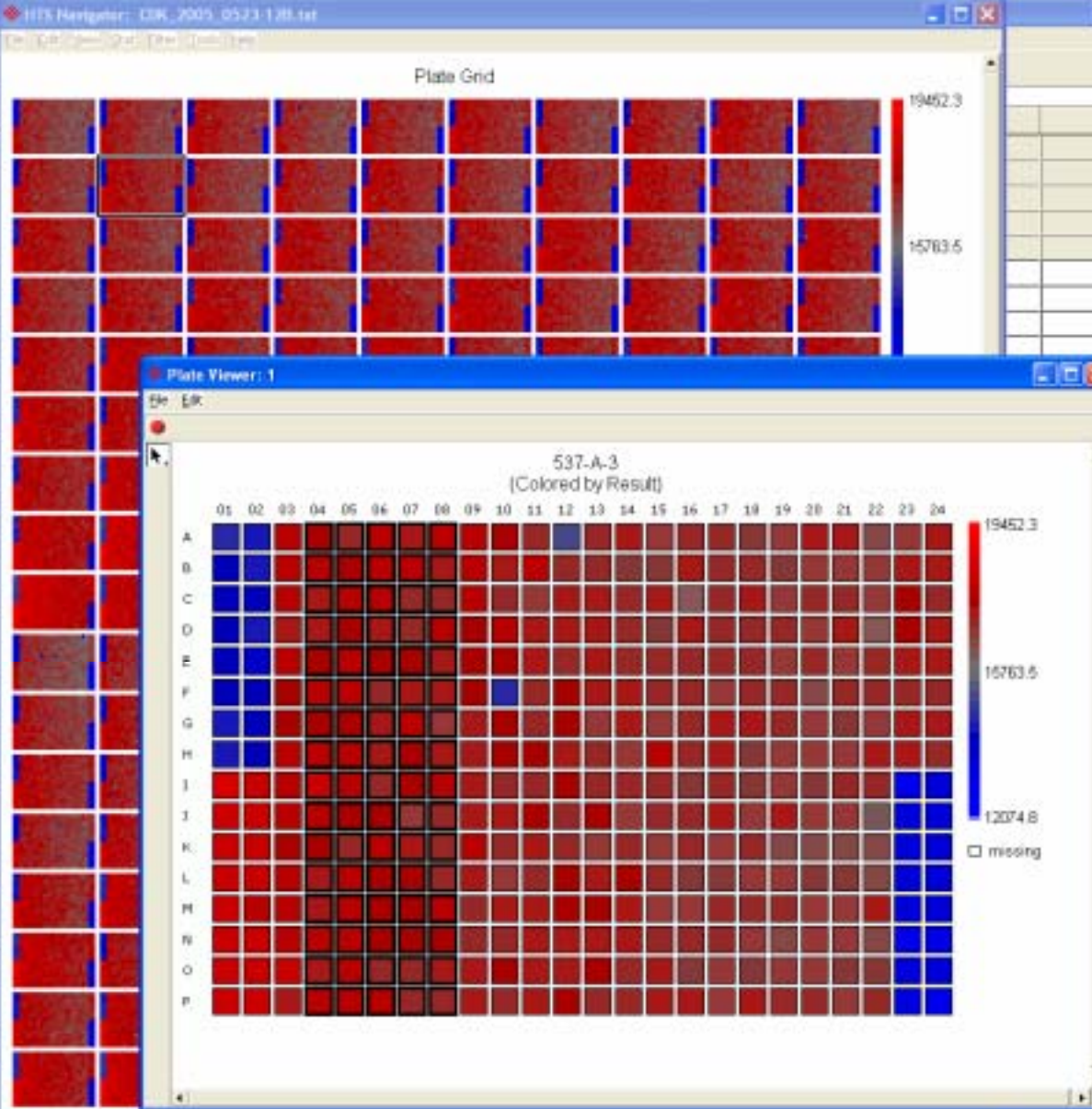
Parascreen's Solution - 1 (CDK_2005_0523_126.tst)

File Edit Database View Tools File Colors Help

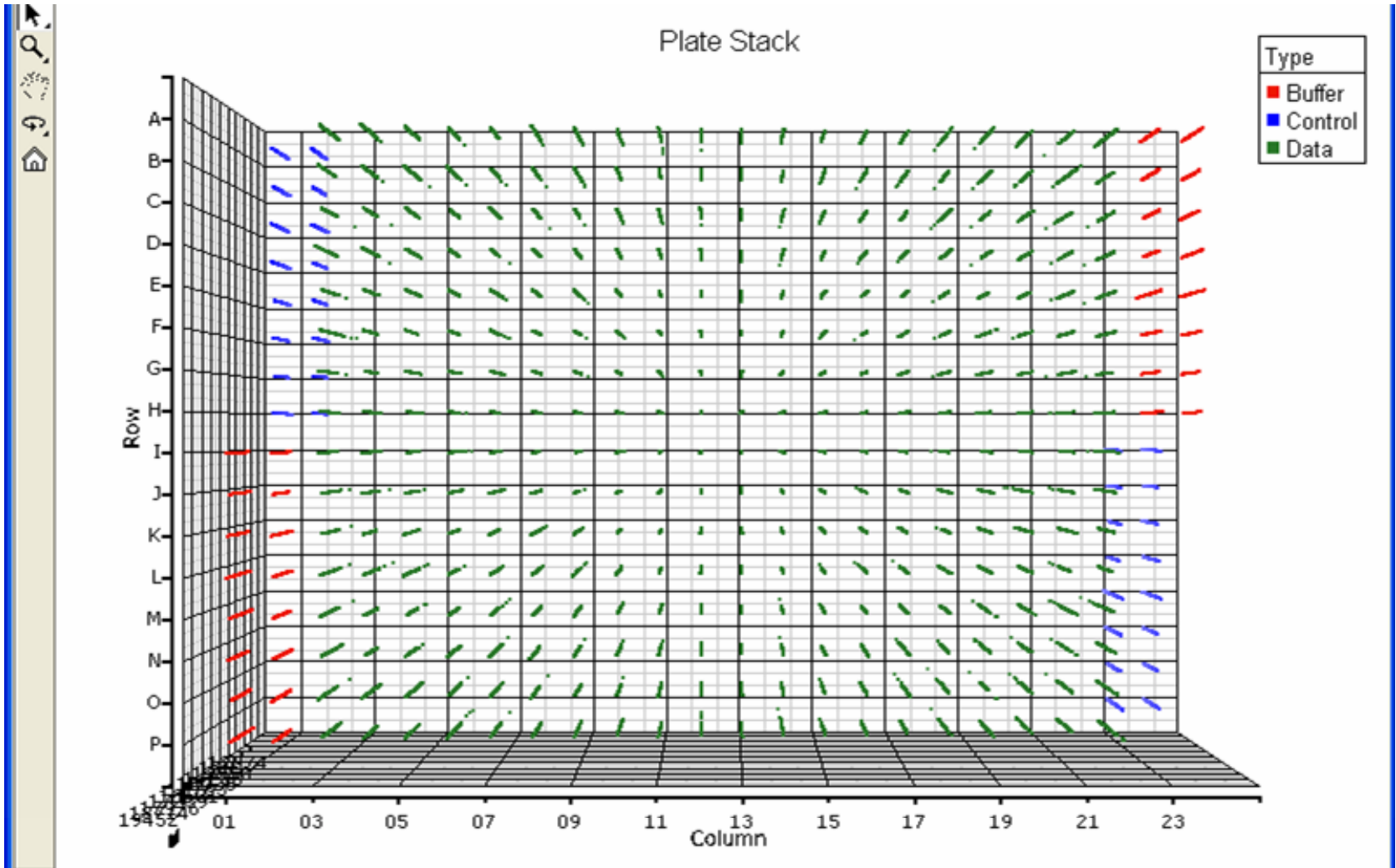
1:CDK_2005_0523_126.tst Current Selection: 537A-38-23

	1. Unique ID	2.Plate	3.Row	4.Column	5.Type	6.Result
4228	537A-34-04	537A-3	A	04	Date	306
4229	537A-34-05	537A-3	A	05	Date	308
4230	537A-34-06	537A-3	A	06	Date	304
4231	537A-34-07	537A-3	A	07	Date	288
4232	537A-34-08	537A-3	A	08	Date	292
4233	537A-34-09	537A-3	A	09	Date	258
4234	537A-34-10	537A-3	A	10	Date	240
4235	537A-34-11	537A-3	A	11	Date	224
4236	537A-34-12	537A-3	A	12	Date	208
4237	537A-34-13	537A-3	A	13	Date	192
4238	537A-34-14	537A-3	A	14	Date	176
4239	537A-34-15	537A-3	A	15	Date	160
4240	537A-34-16	537A-3	A	16	Date	144
4241	537A-34-17	537A-3	A	17	Date	128
4242	537A-34-18	537A-3	A	18	Date	112
4243	537A-34-19	537A-3	A	19	Date	96
4244	537A-34-20	537A-3	A	20	Date	80
4245	537A-34-21	537A-3	A	21	Date	64
4246	537A-34-22	537A-3	A	22	Date	48
4247	537A-34-23	537A-3	A	23	Buffer	32
4248	537A-34-24	537A-3	A	24	Buffer	16
4249	537A-38-01	537A-3	B	01	Control	360
4250	537A-38-02	537A-3	B	02	Control	367
4251	537A-38-03	537A-3	B	03	Date	351
4252	537A-38-04	537A-3	B	04	Date	335
4253	537A-38-05	537A-3	B	05	Date	319
4254	537A-38-06	537A-3	B	06	Date	303
4255	537A-38-07	537A-3	B	07	Date	287
4256	537A-38-08	537A-3	B	08	Date	271
4257	537A-38-09	537A-3	B	09	Date	255
4258	537A-38-10	537A-3	B	10	Date	239
4259	537A-38-11	537A-3	B	11	Date	223
4260	537A-38-12	537A-3	B	12	Date	207
4261	537A-38-13	537A-3	B	13	Date	191
4262	537A-38-14	537A-3	B	14	Date	175
4263	537A-38-15	537A-3	B	15	Date	159
4264	537A-38-16	537A-3	B	16	Date	143
4265	537A-38-17	537A-3	B	17	Date	127
4266	537A-38-18	537A-3	B	18	Date	111
4267	537A-38-19	537A-3	B	19	Date	95
4268	537A-38-20	537A-3	B	20	Date	79
4269	537A-38-21	537A-3	B	21	Date	63
4270	537A-38-22	537A-3	B	22	Date	47
4271	537A-38-23	537A-3	B	23	Buffer	31

Row: 64512 Col: 8



Compound view



Edge effects

Row Effect on Result in CDK_2005_0523-12B.txt

Row	N	Mean	Std. Dev.	Std. Err.	Minimum	Maximum
A**	4032	17008.385855**	952.721384	15.003951	13508.908780	18859.982579
B	4032	17002.861979	950.744700	14.972822	13453.703540	18910.109857
C	4032	16976.129457	949.139649	14.947544	13351.936082	18659.802287
D	4032	16982.392165	947.812605	14.926645	13425.857000	19164.577546
E	4032	16969.129733	951.581159	14.985995	13382.473094	18671.552071
F	4032	16978.732403	952.926337	15.007179	13316.972142	18762.027165
G	4032	16966.322348	967.088109	15.230206	13491.011643	18812.174705
H	4032	16973.755606	950.754805	14.972981	13224.462682	18781.418374
I	4032	16980.114425	1266.709488	19.948799	12335.452461	19452.331299
J	4032	16958.295326	1283.762089	20.217353	12426.390767	19155.462848
K	4032	16975.580536	1273.223120	20.051379	12309.357032	19358.999191
L	4032	16950.061808	1270.761199	20.012608	12357.286023	19427.048768
M	4032	16970.115544	1274.358901	20.069266	12074.821169	19144.761504
N	4032	16948.031374	1273.829003	20.060921	12347.809253	19276.677213
O*	4032	16931.949904*	1276.129969	20.097158	12386.094982	19211.724266
P	4032	16963.965335	1262.880281	19.888495	12420.256675	19146.690616

Summary:

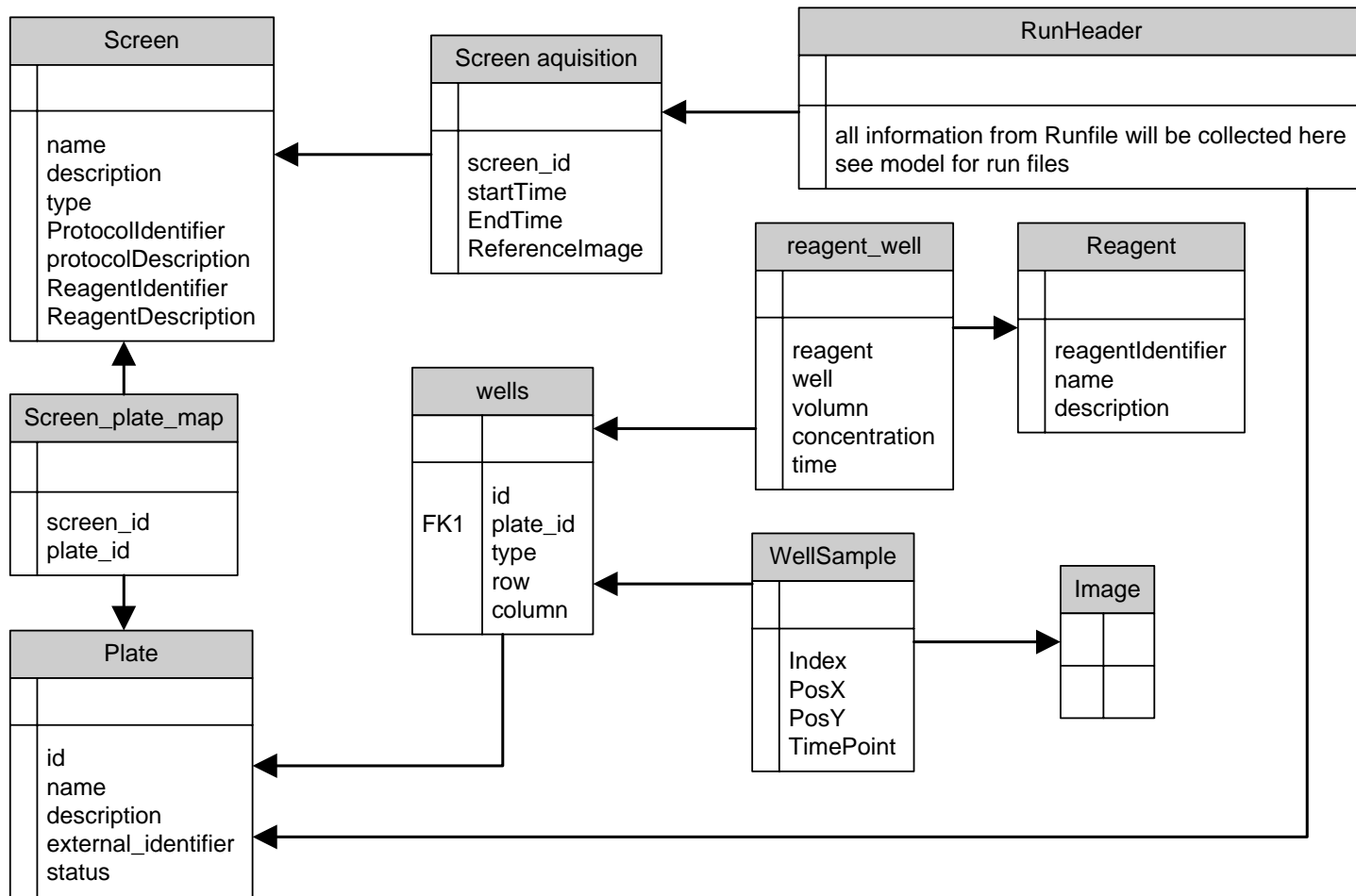
* Row O has the smallest mean (16931.949904)

** Row A has the largest mean (17008.385855)

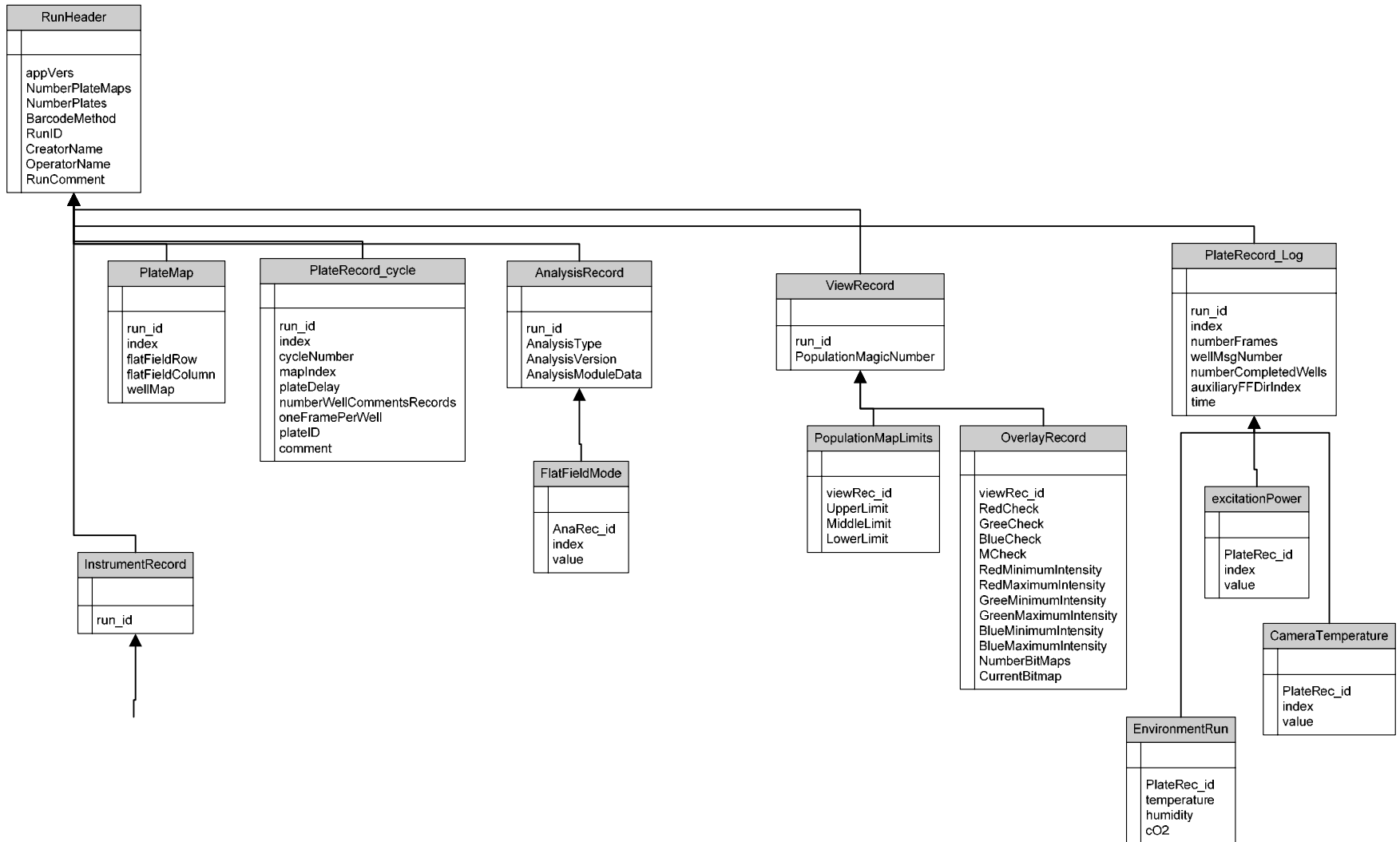
Row A is 0.45% larger than Row O

p-value: 0.214377 -- No significant edge effect has been detected

Screening model



INCell Information



Instrument Annotations

ExcitationRecord	
	Instr_id index excitationID pass exitationName NDName

ImageFormatRecord	
	Instr_id binning horizontalPixels verticalPixels scanWidth scanLength

CameraRecord	
	Instr_id index status acquisitionFFMode pass channel filterName

ScanRecord	
	Instr_id firstFrameChannel nextFrameChannel numberPassesFirstFrame numberPassesNextFrame integrationTime frameType maximumFramesPerWell cellCountCheck plateType columnsPerGroup interWellDelay cellCountThreshold interFrameDelay

EnvironmentalRecord	
	Instr_id temperature humidity cO2

DispenserRecord	
	Instr_id status spitOrder volume flowRate atFrame delay

bufferToCameraMap	
	scanRec_id index value

TransmissionRecord	
	Instr_id status cameraIndex

ConfocalRecord	
	Instr_id slitWidth Objective

AutofocusRecord	
	Instr_id autofocusOffset stackZ0 stackDeltaZ

