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.

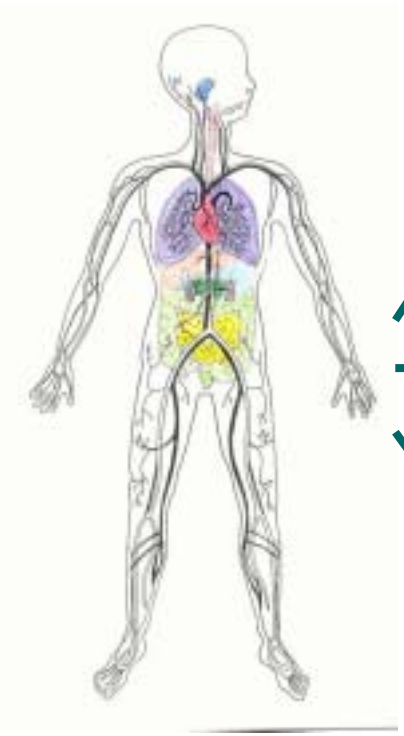
Who is involved?

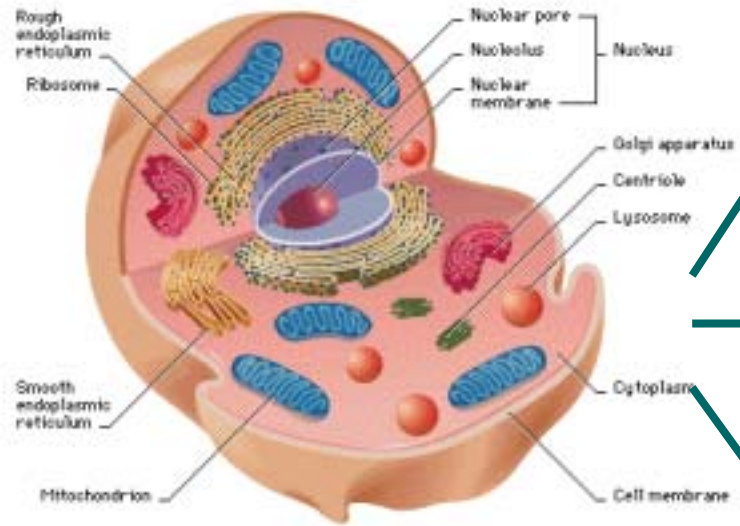
- James E. Rothman (PI)
- Lars Branden (Project manager)
- Thomas Mayer (assay dev.)
- M. Beard (assay dev.)
- Deby Smith (assay dev.)
- Effie Tzilianos (assay dev.)
- Feng-Li Zhang(assay dev.)
- Mike Wyler (assay dev.)
- Nathalie Aulner (HTS)
- Udo Többen (HTS)
- Bernd Jagla (IT)
- Geoff Barger (automation)
- 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

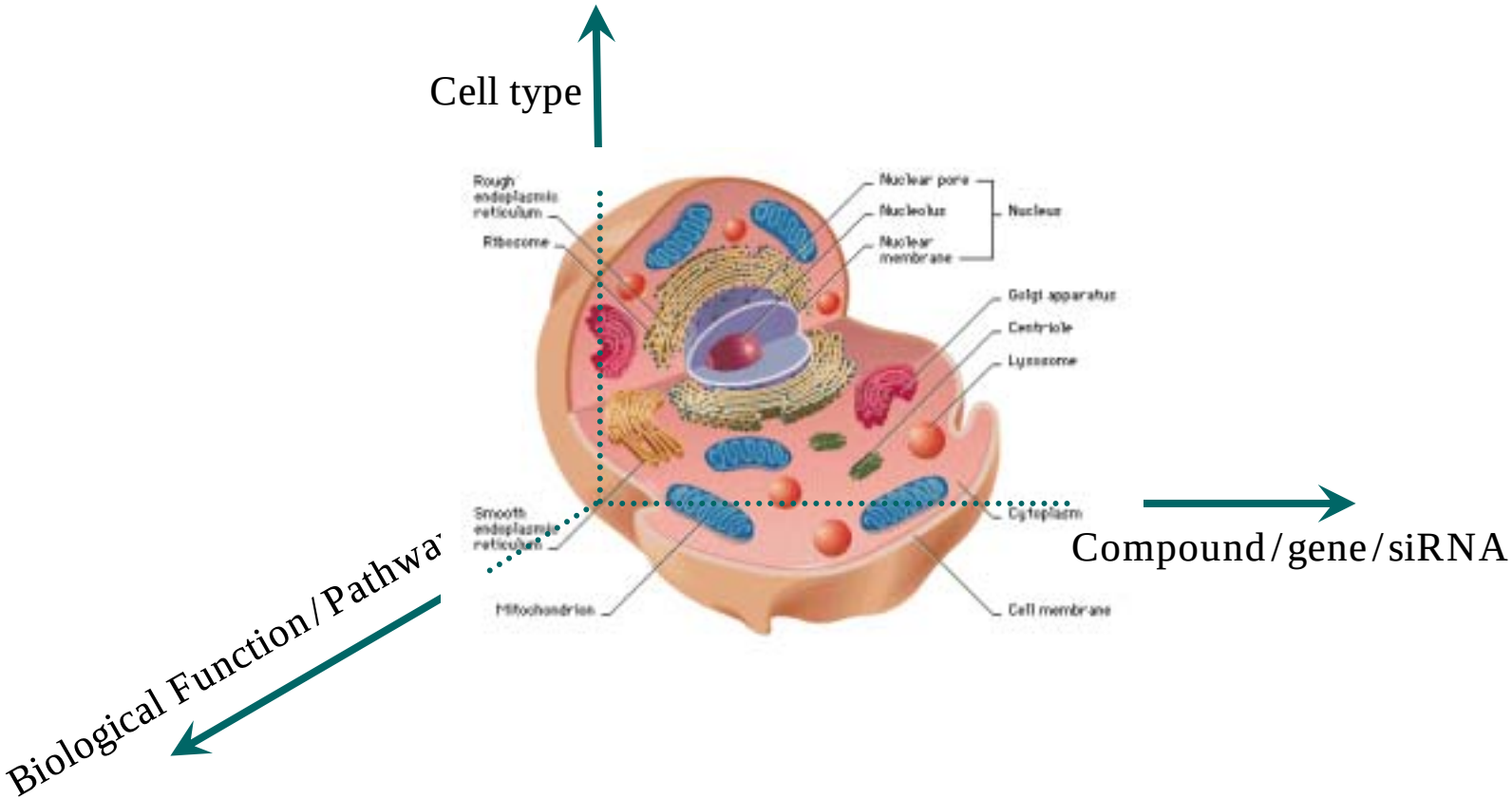
Response to stimuli

...

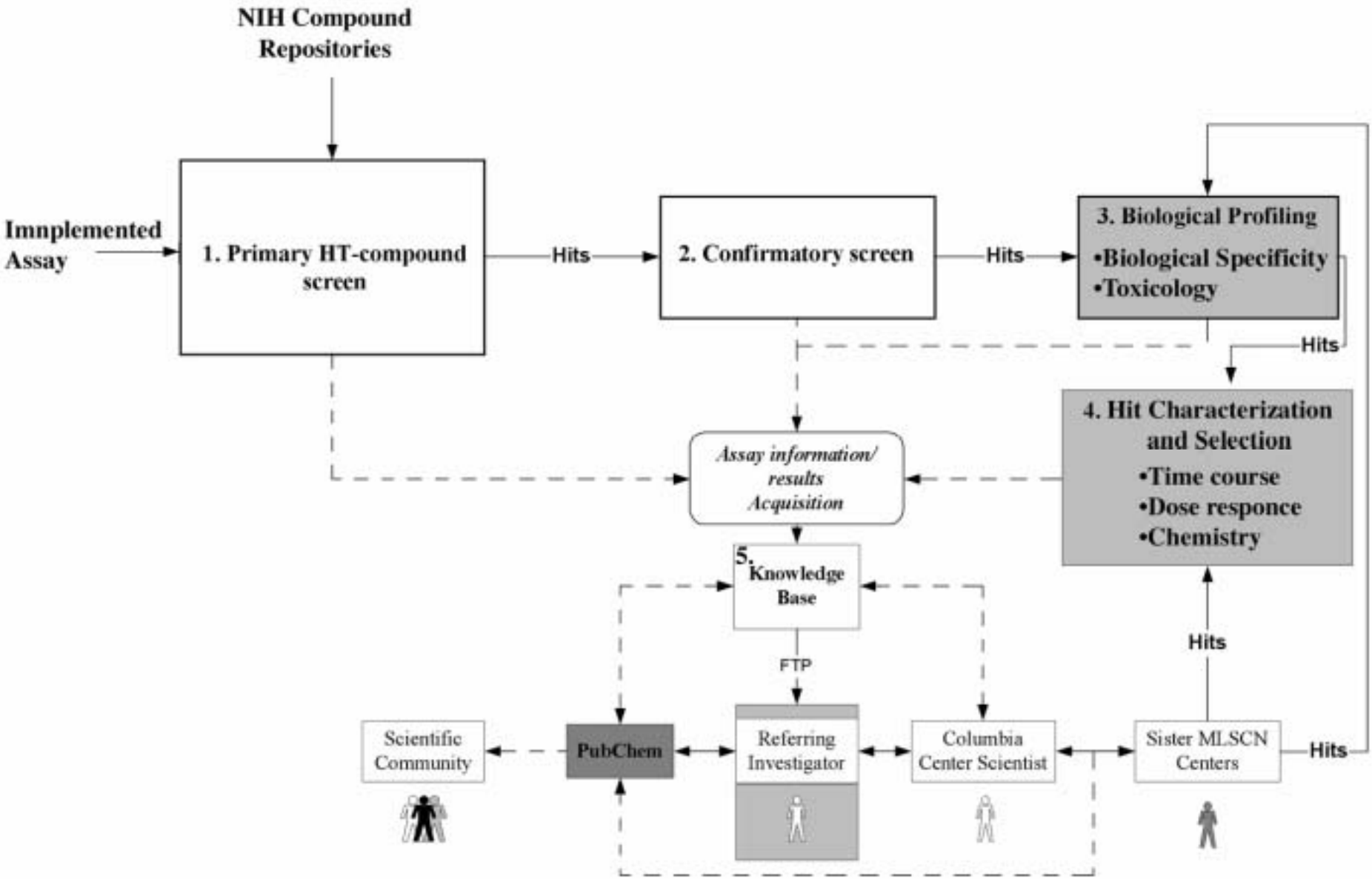
? ?

compound

”Three Dimensional” Biological Profiling



HT screening work-flow



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

Compound X →

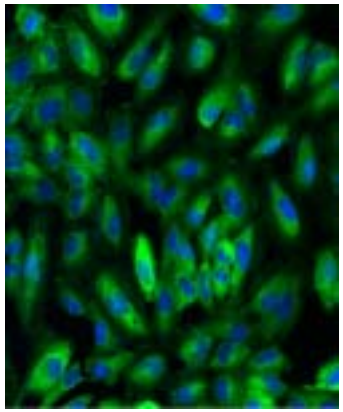
Biological Processes (20)
Apoptosis
Inflammation
Cell cycle
DNA repair
Secretion
Transcription
Etc etc

Pathways (40)
Receptor activation
Kinase activation
Transcriptional activation
Intracellular signalling
Etc etc

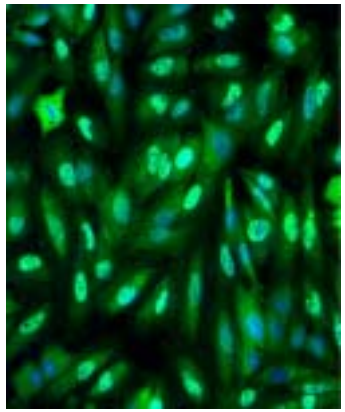
- - - - - - - - - - →

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

Gene-Plus collection (960 compounds) screen

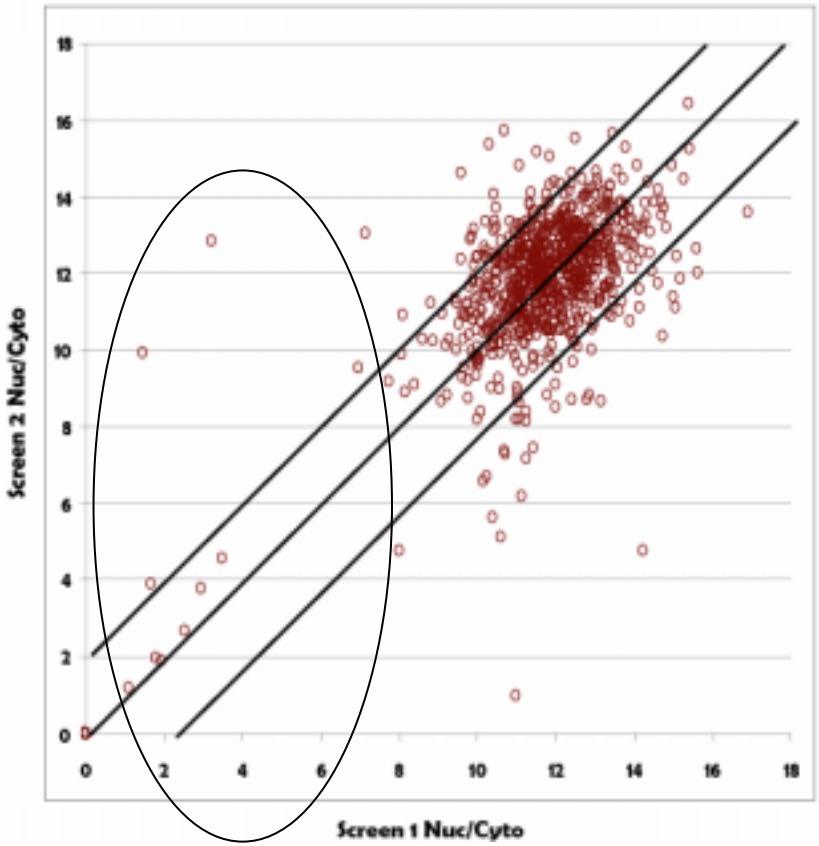


Control

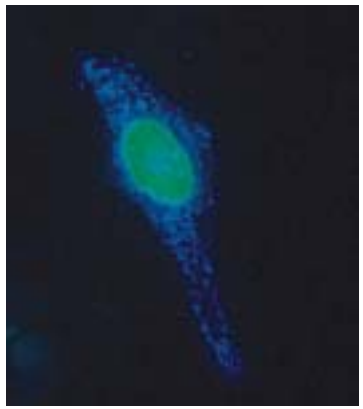


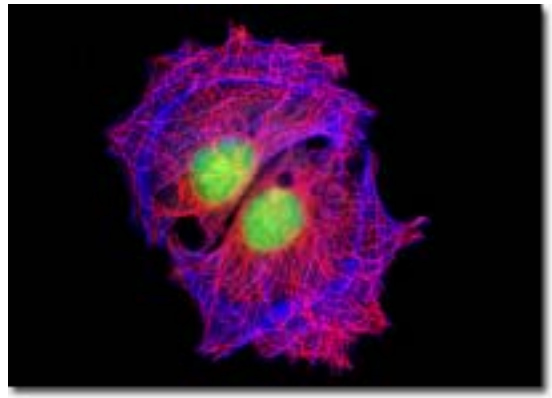
TNFα stimulation
(positive control)

Image Analysis

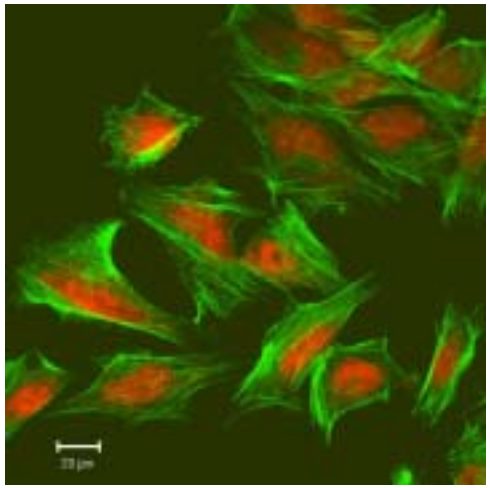


HeLa

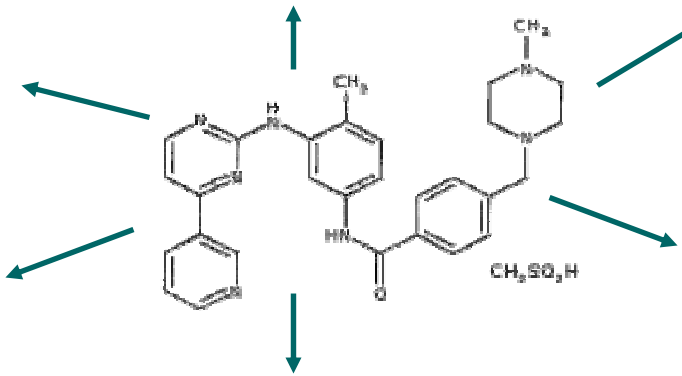


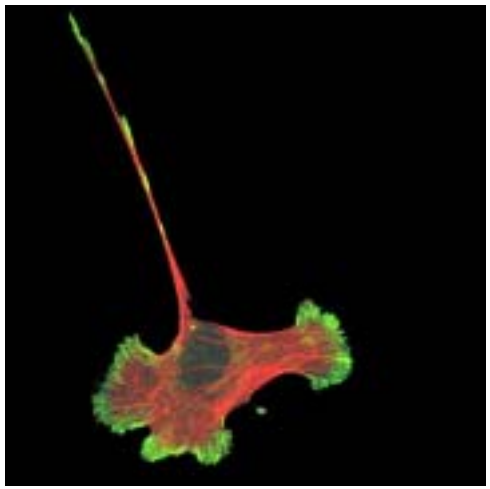


myoblast

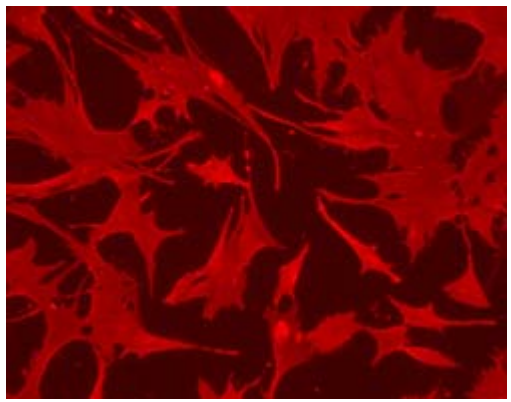


osteoblast

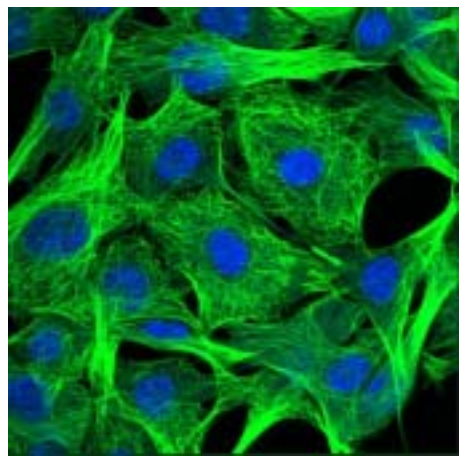




neuroblast

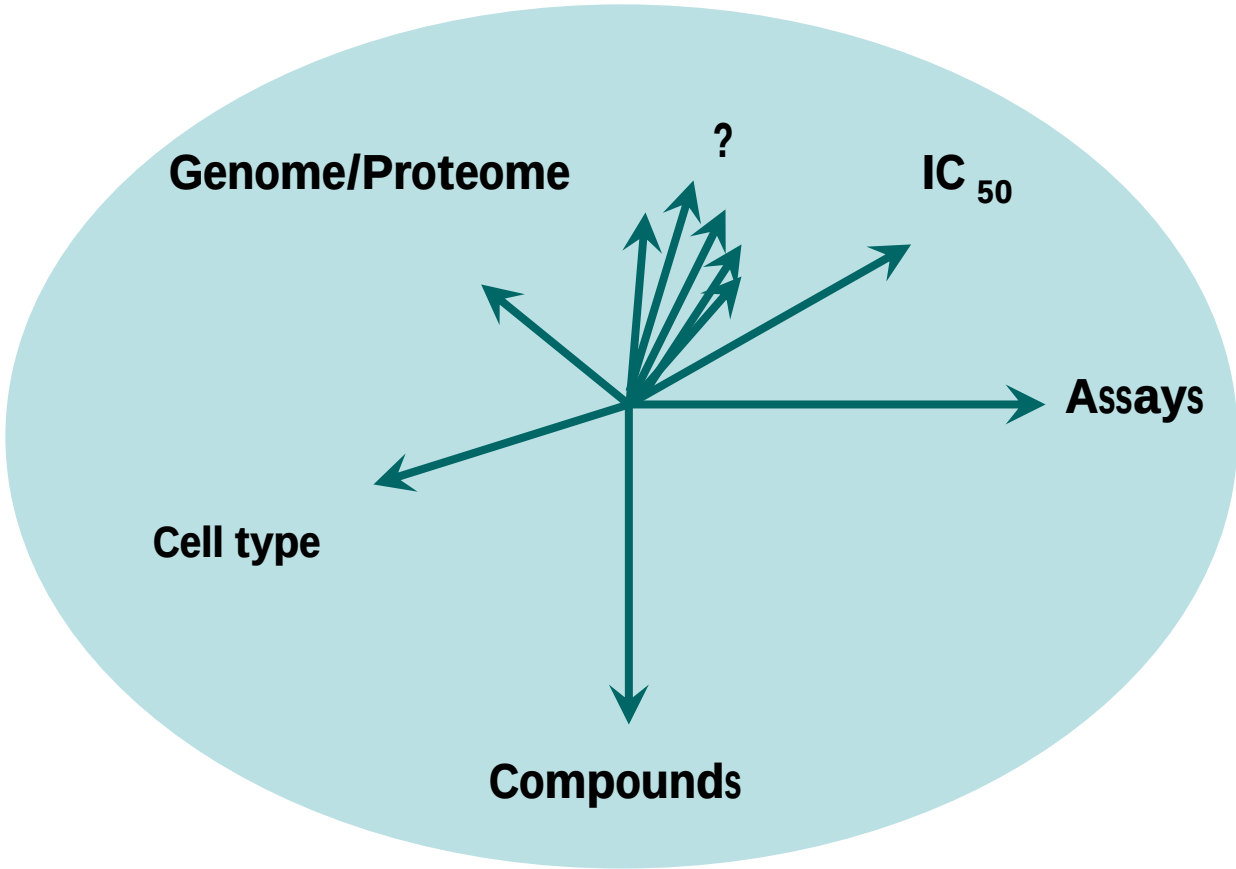


adipocyte



fibroblast

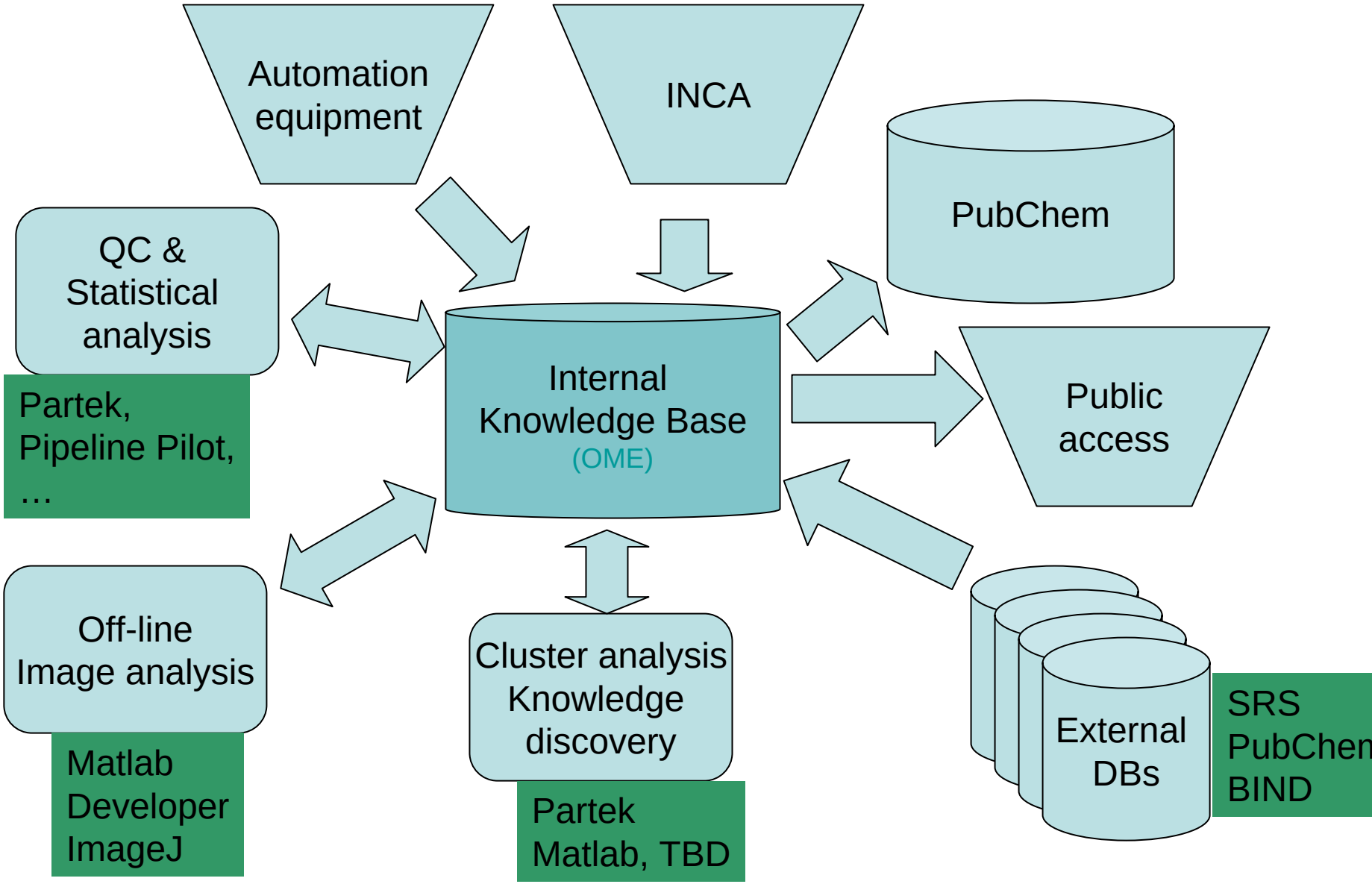
Dimensions of the profiling matrix



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

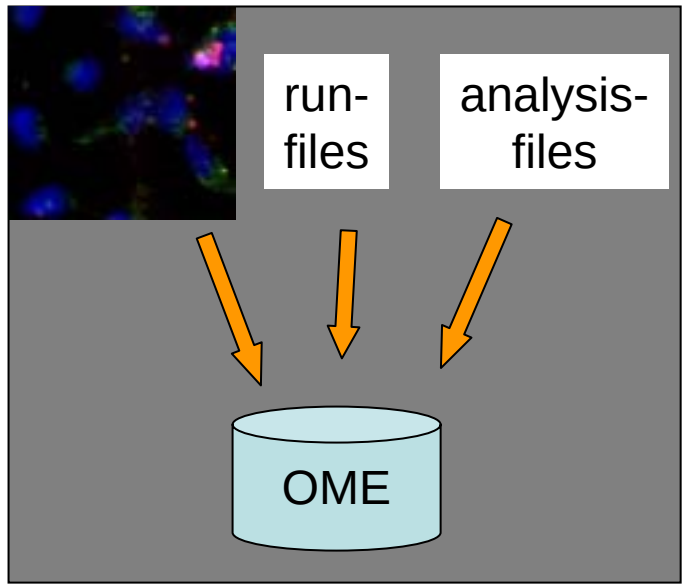
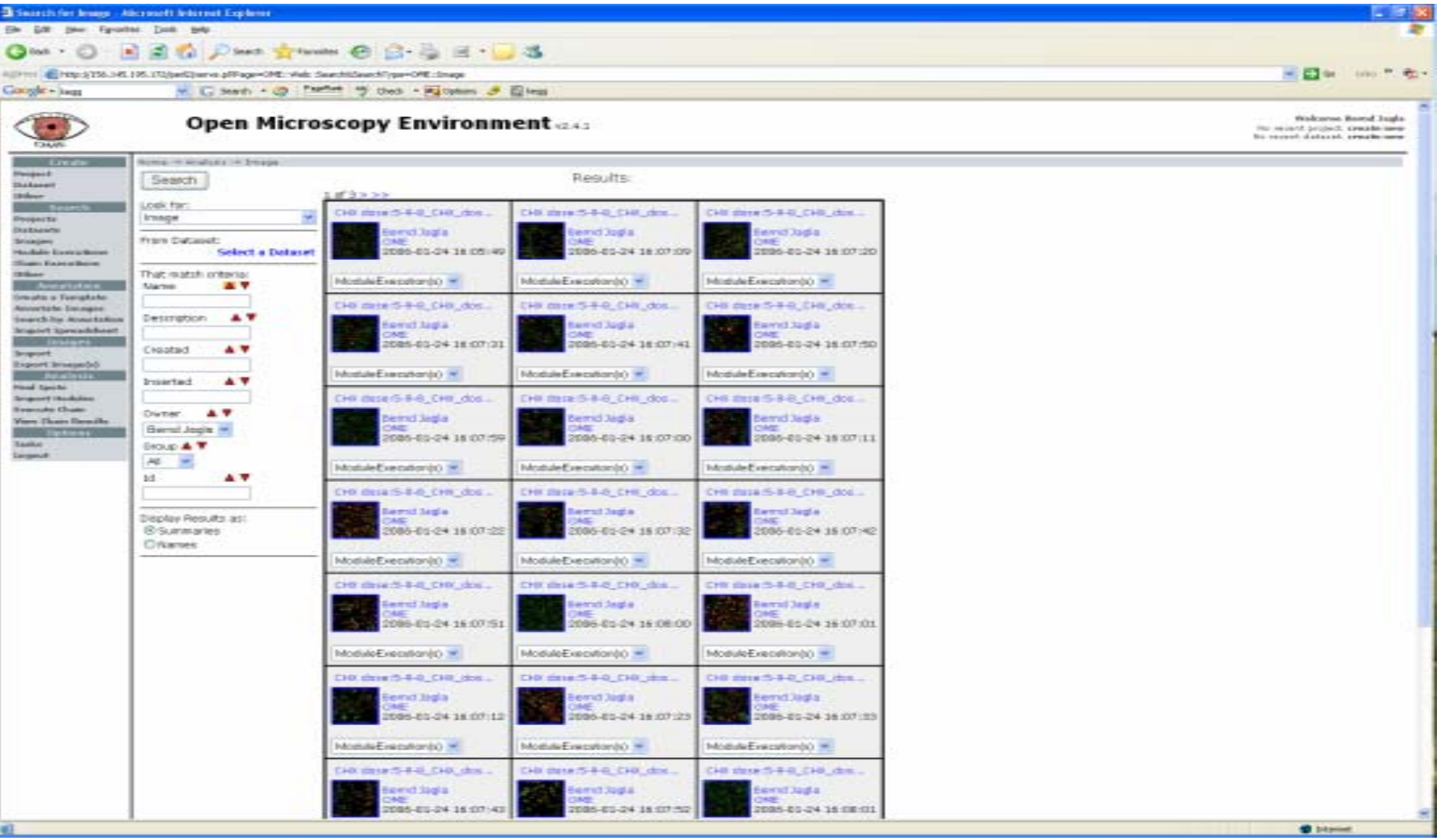
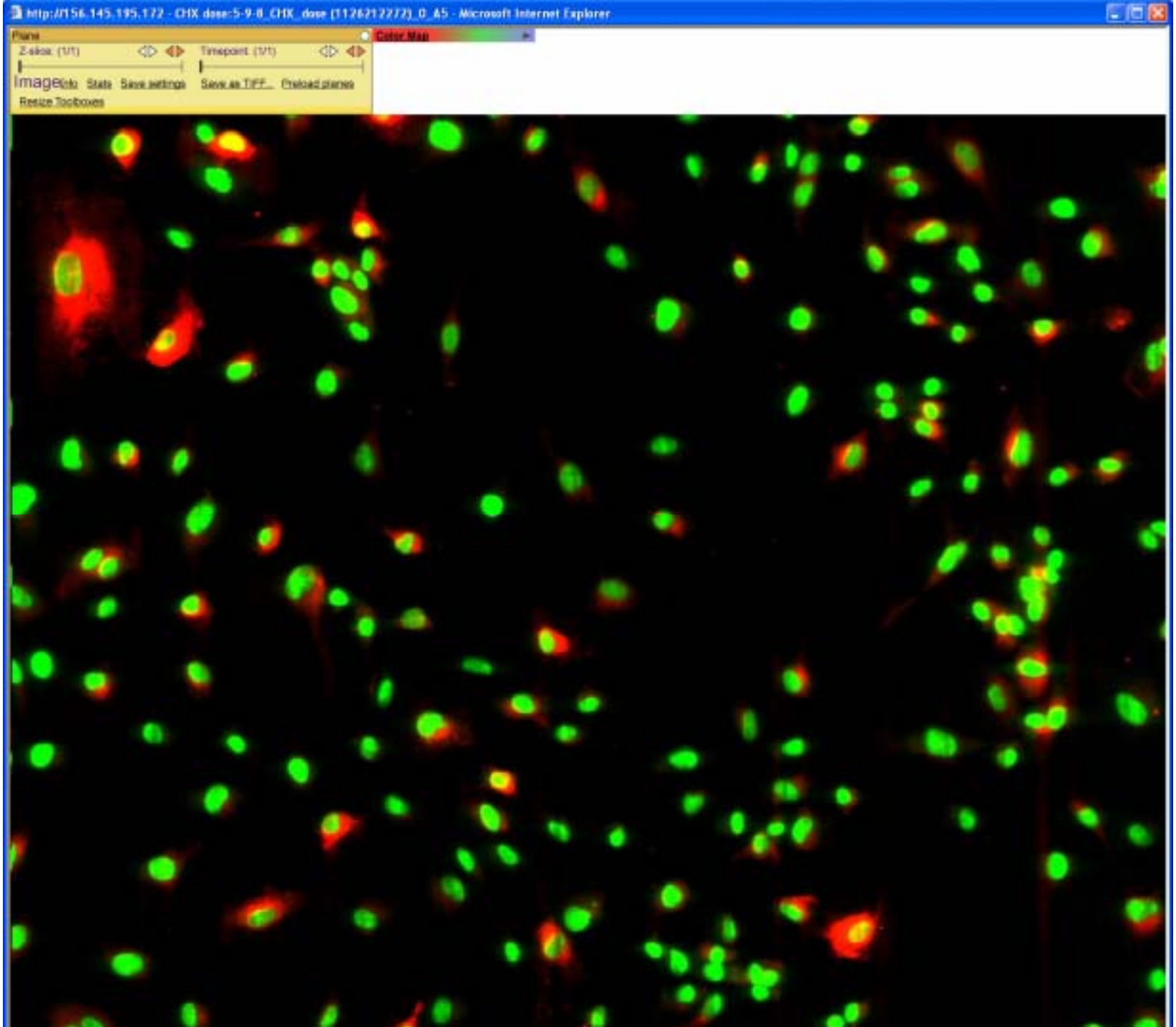


Image view




http://156.145.195.172 - CHX dose:5-9-8_CHX_dose (1126212272)_0_A5 - Microsoft Internet Explorer
Plane
Z-slice: (1/1)
Timepoint: (1/1)
Image Info State Save settings
Save as TIFF... Preload planes
Resize Toolboxes
Color Map

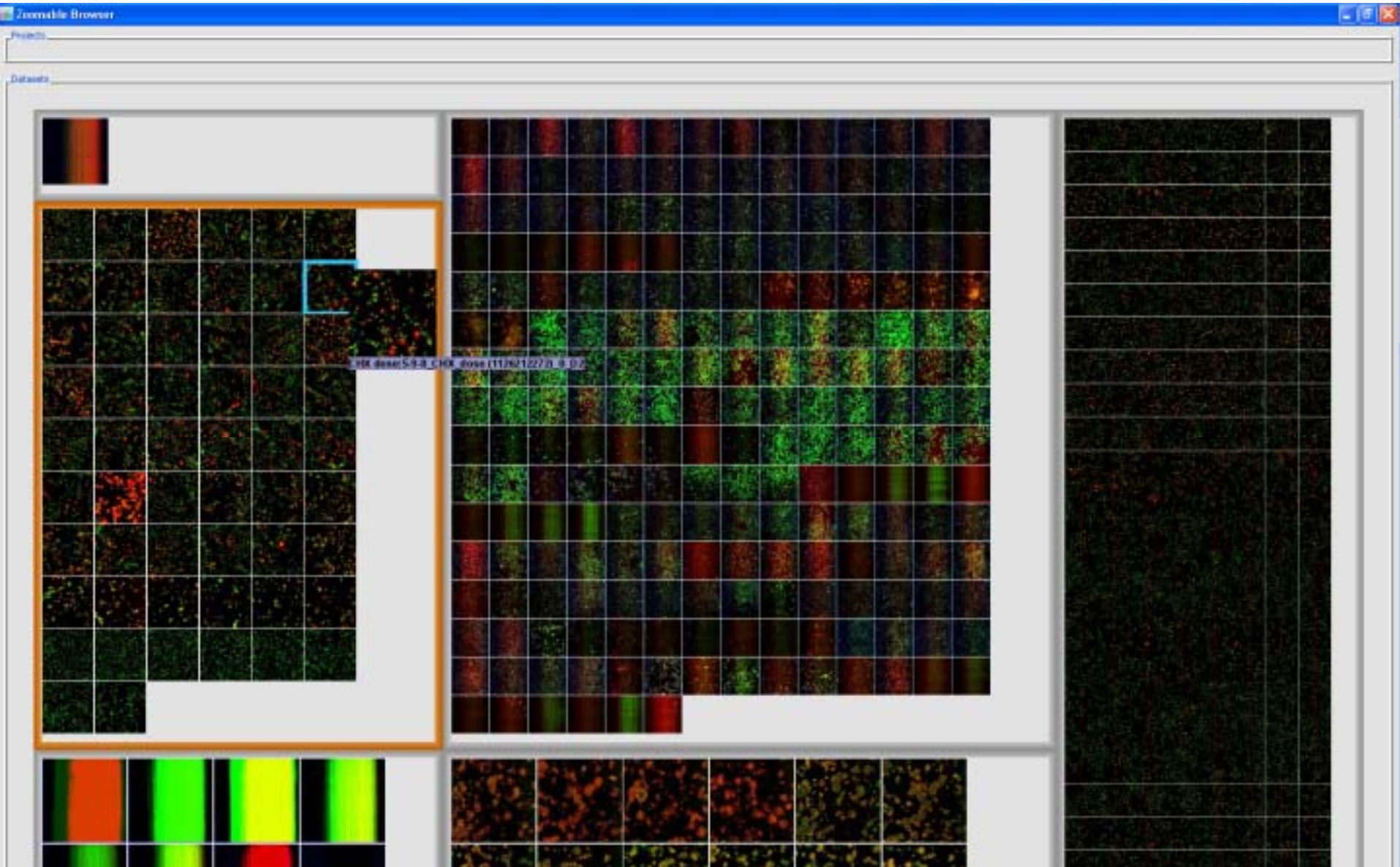
989	9/8/2005	NA	200.00	None	0	Y	C sdevs above bkgnd	[9; 250]	Off	15	Y	Blue	Green	5-9-8_CHX_dose	None	4:44:32 PM	matt

ObjectIntensity_0_values

Download as txt | 1 of 3 > >>

ID	Cycle	Hist Pk	Ineg	Ipos	Msg	Nneg	Npass	Npos	Ntot	Parameters	Plate	SDneg	SDpos	Well	neg	pos	Image
1045	0	94	0	150.56	0	0	95	95	101	989	CHX dose	0	58.08	H6	0	100	
1044	0	93	0	164.87	0	0	271	271	284	989	CHX dose	0	101.95	E5	0	100	
1043	0	93	0	140.99	0	0	376	376	397	989	CHX dose	0	63.44	C3	0	100	
1042	0	91	0	138.83	0	0	460	460	474	989	CHX dose	0	57.37	B3	0	100	
1041	0	92	0	158.71	0	0	384	384	397	989	CHX dose	0	63.05	F5	0	100	
1040	0	88	0	144.81	0	0	119	119	124	989	CHX dose	0	75.4	F6	0	100	
1039	0	92	0	97.58	0	0	525	525	576	989	CHX dose	0	5.75	D7	0	100	
1038	0	98	0	149.33	0	0	315	315	332	989	CHX dose	0	83.66	H4	0	100	
1037	0	96	0	103.04	0	0	529	529	648	989	CHX dose	0	16.06	B7	0	100	
1036	0	96	0	154.43	0	0	325	325	345	989	CHX dose	0	82.33	G4	0	100	
1035	0	94	0	157.71	0	0	418	418	437	989	CHX dose	0	92.74	D4	0	100	
1034	0	92	0	132.12	0	0	468	468	483	989	CHX dose	0	61.11	A4	0	100	
1033	0	96	0	139.93	0	0	407	407	421	989	CHX dose	0	42.68	D1	0	100	
1032	0	91	0	98.16	0	0	469	469	538	989	CHX dose	0	17.39	C7	0	100	
1031	0	89	0	134.98	0	0	403	403	418	989	CHX dose	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

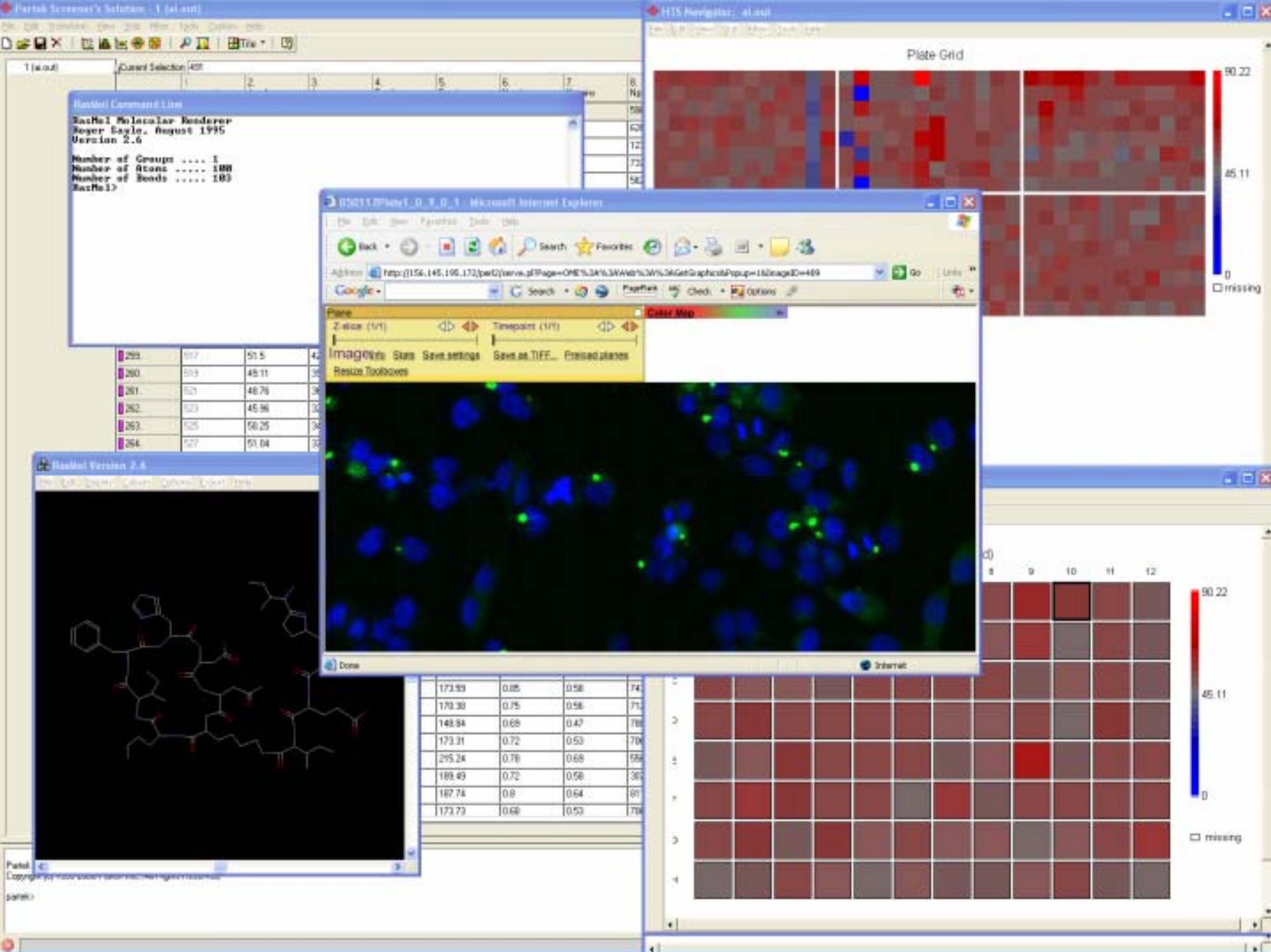


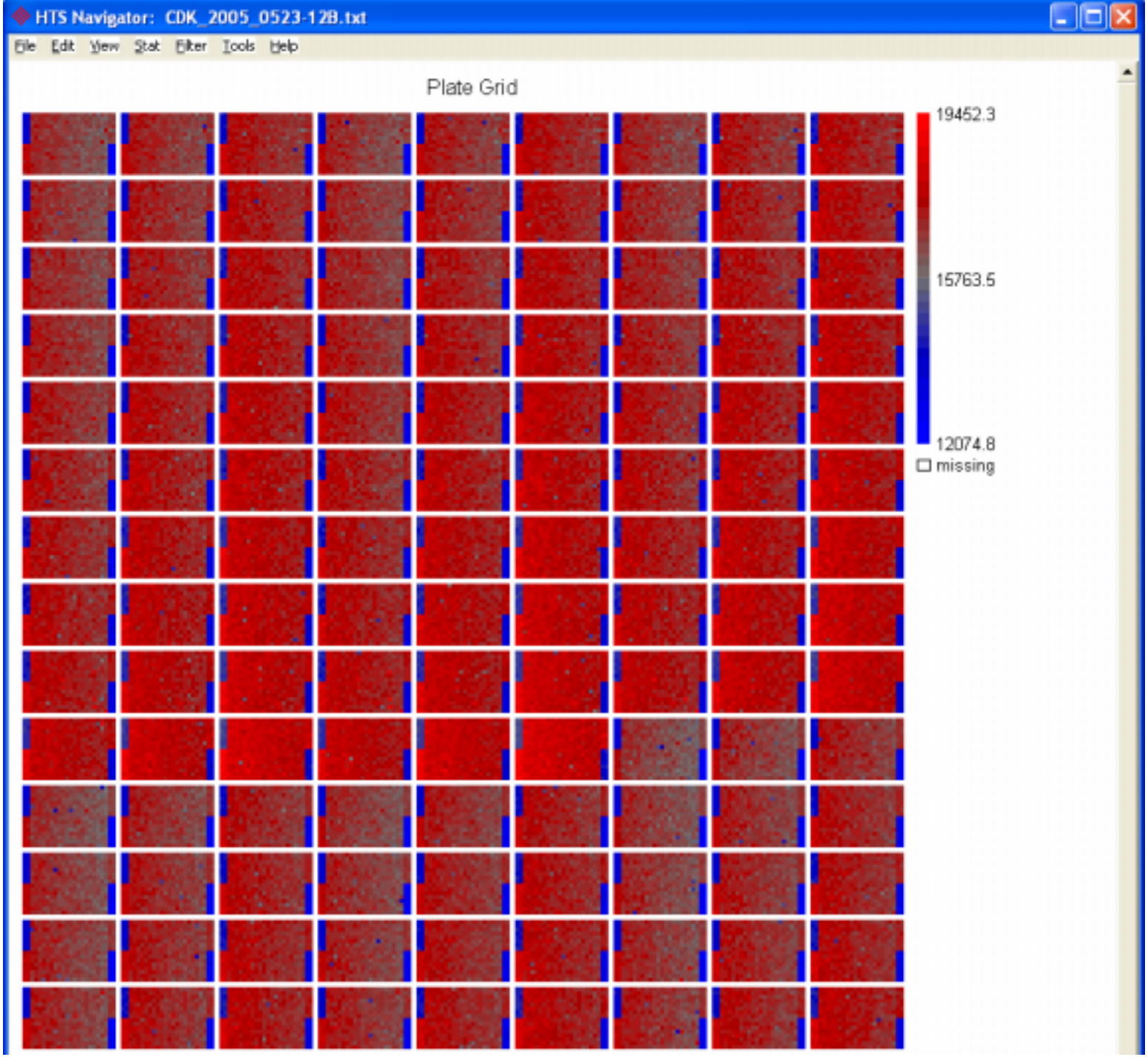
Plate Grid
90.22
45.11
0
missing
RasMol Command Line
RasMol Molecular Renderer
Roger Sayle, August 1995
Version 2.6
Number of Groups 1
Number of Atoms 188
Number of Bonds 183
RasMol>
Microsoft Internet Explorer
Back
Search
Favorites
Go
Plane
Z-slice (1/1)
Timepoint (1/1)
Color Map
Images
Stats
Save settings
Save as TIFF...
Preload planes
Resize Toolboxes
Done
Internet
RasMol Version 2.6

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


HTS Navigator: CDK_2005_0523-12B.txt
File Edit View Stat Filter Tools Help
Plate Grid
19452.3
15763.5
12074.8
missing

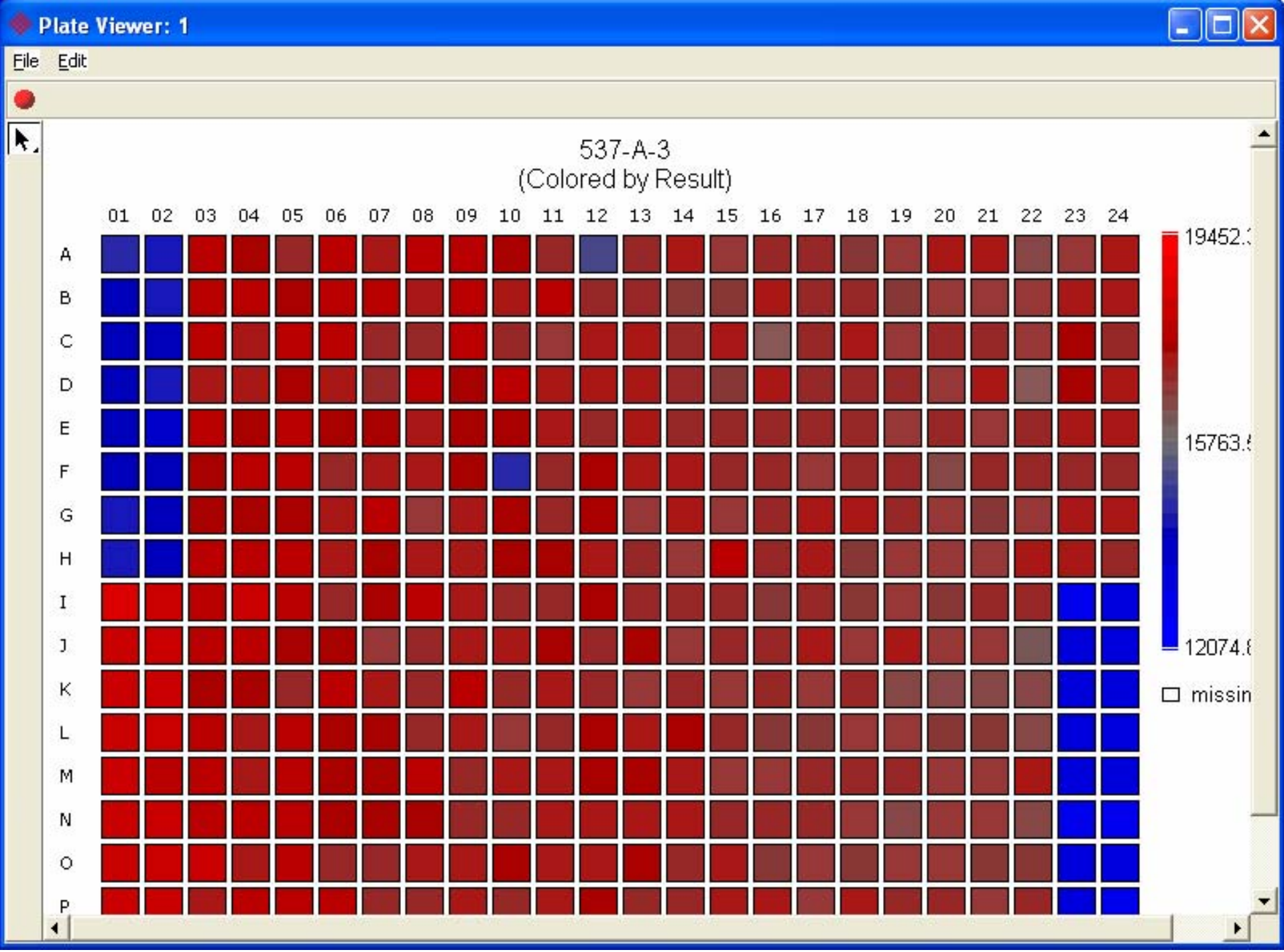
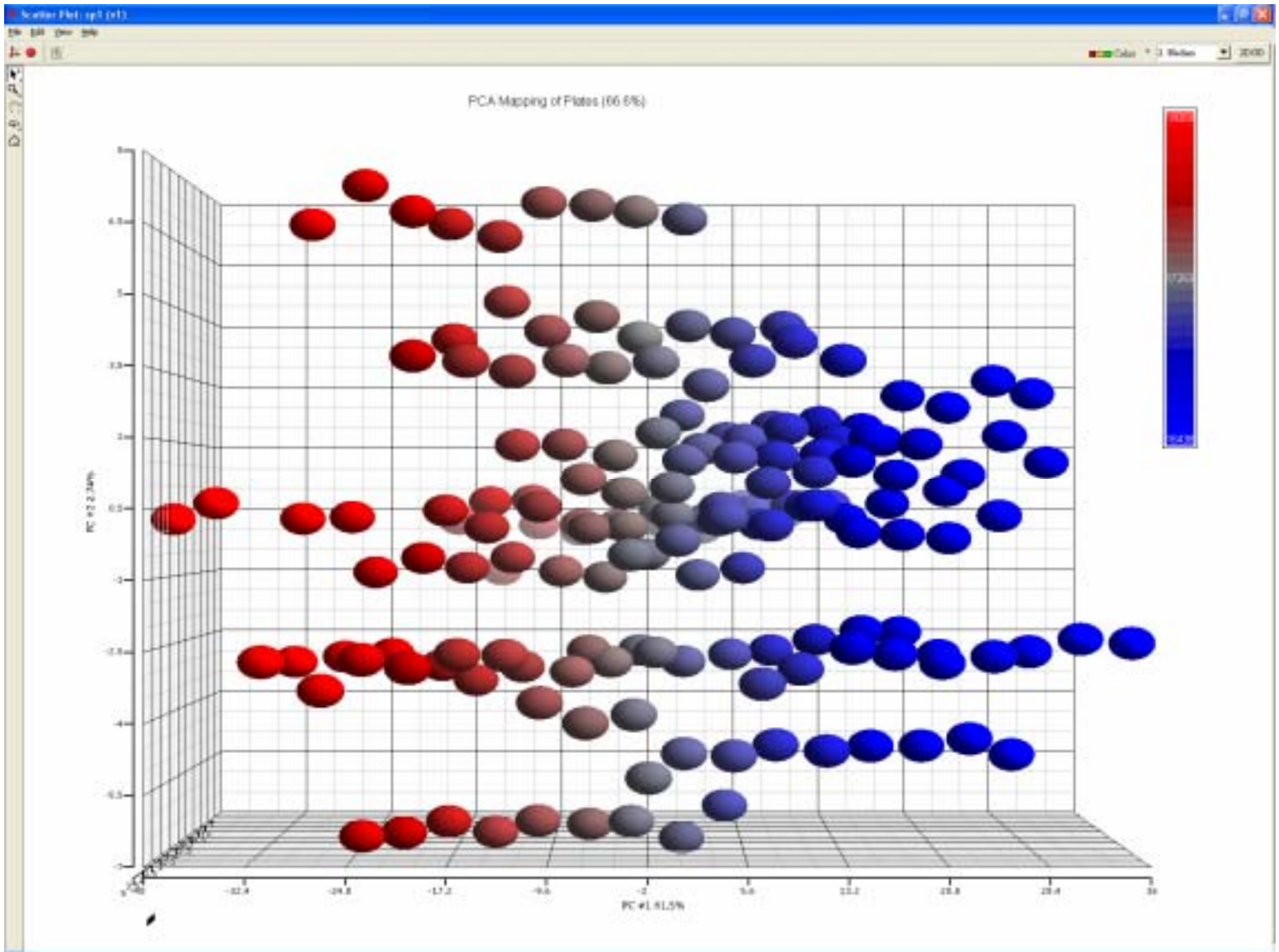


Plate Viewer: 1
File
Edit
537-A-3
(Colored by Result)
01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
19452.
15763.
12074.
missin

PCA view



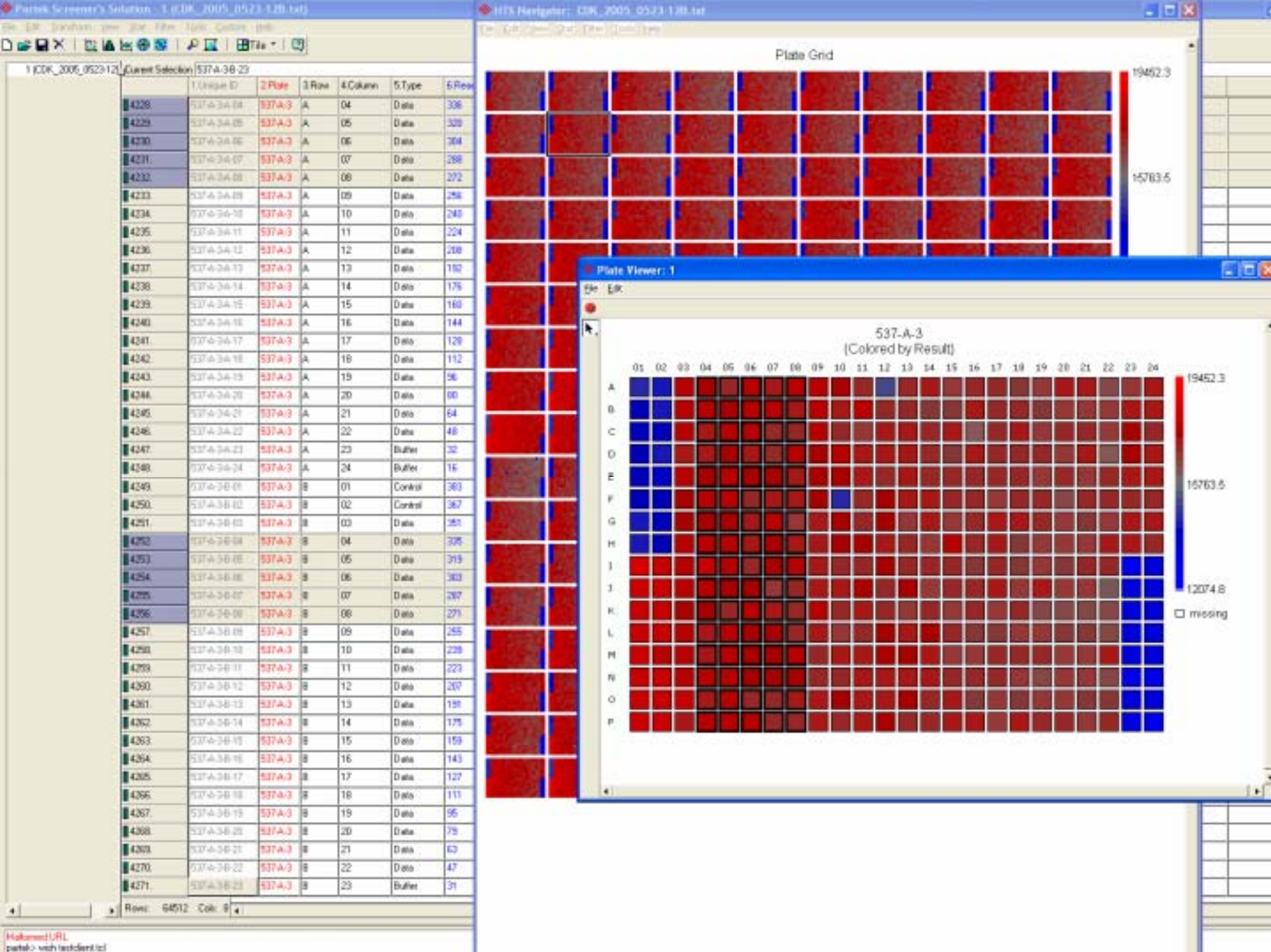
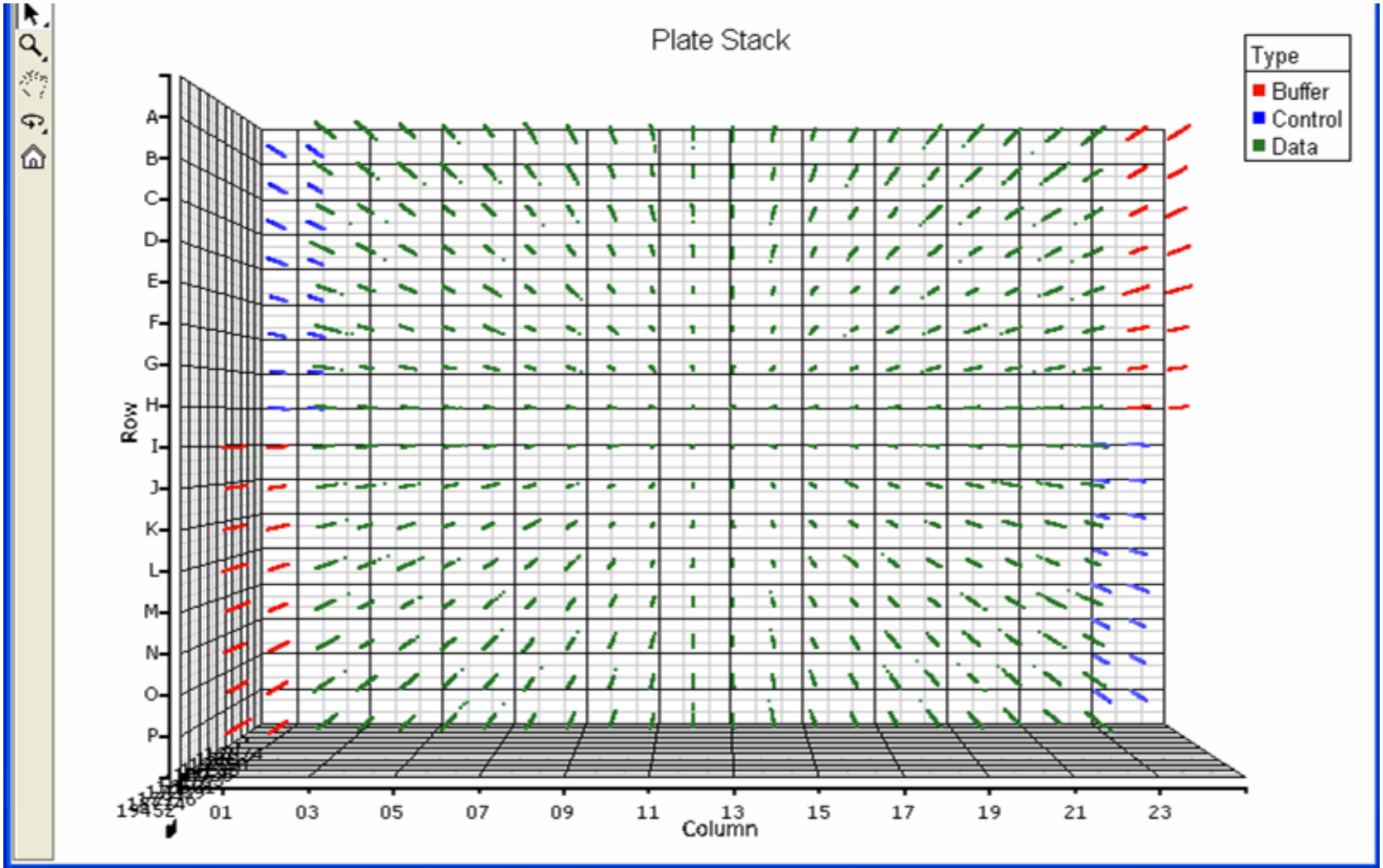


Plate Grid
19452.3
16763.6
Plate Viewer: 1
File Edit
537-A-3
(Colored by Result)
01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
A B C D E F G H I J K L M N O P
19452.3
16763.6
12074.8
missing

Compound view



Edge effects

Perkin Inc.
www.perkin.com

January 29 2006
01:52:21 PM

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:

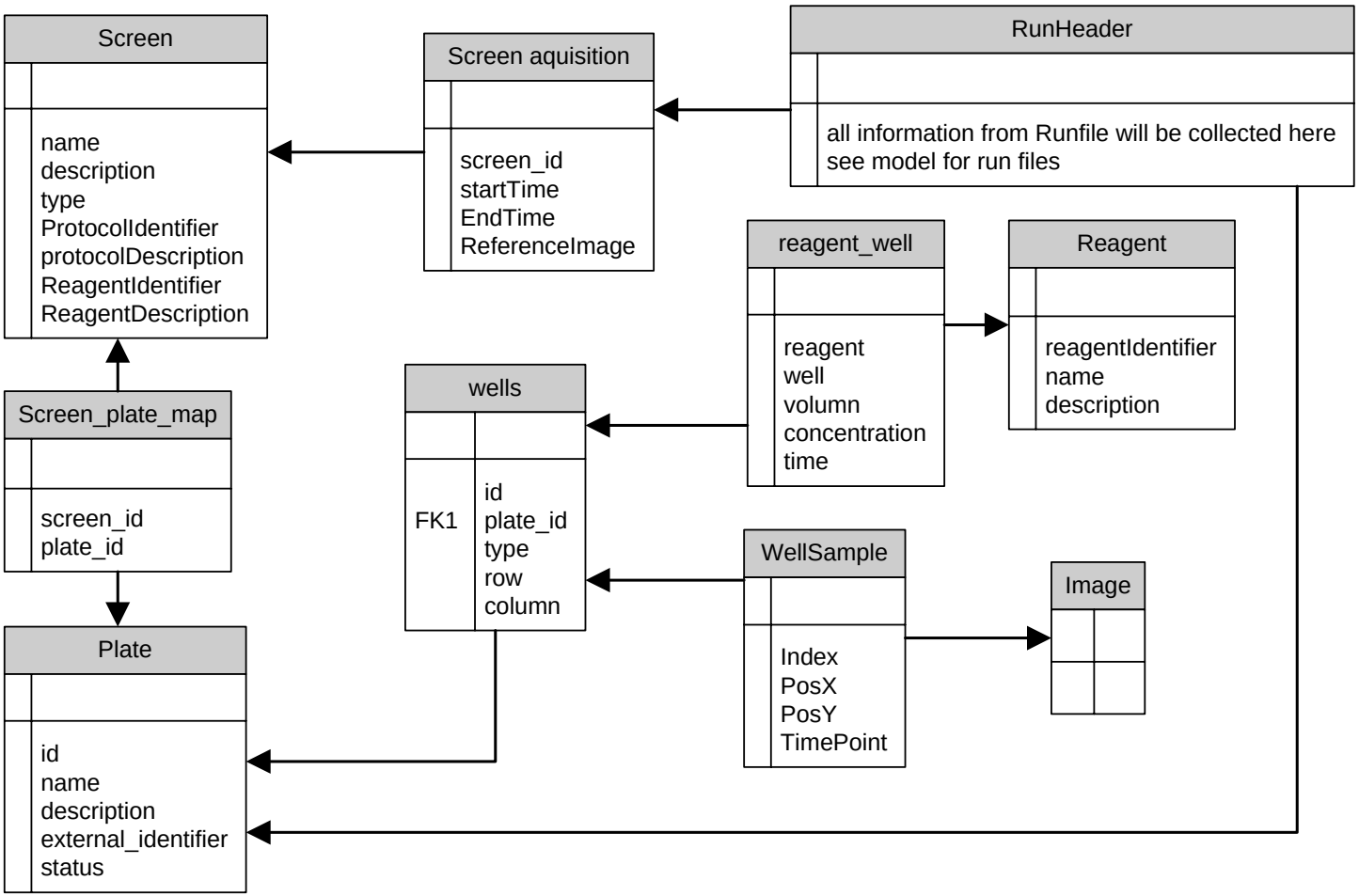
* Row O has the smallest mean (16931.949904)

** Row A has the largest mean (17008.385855)

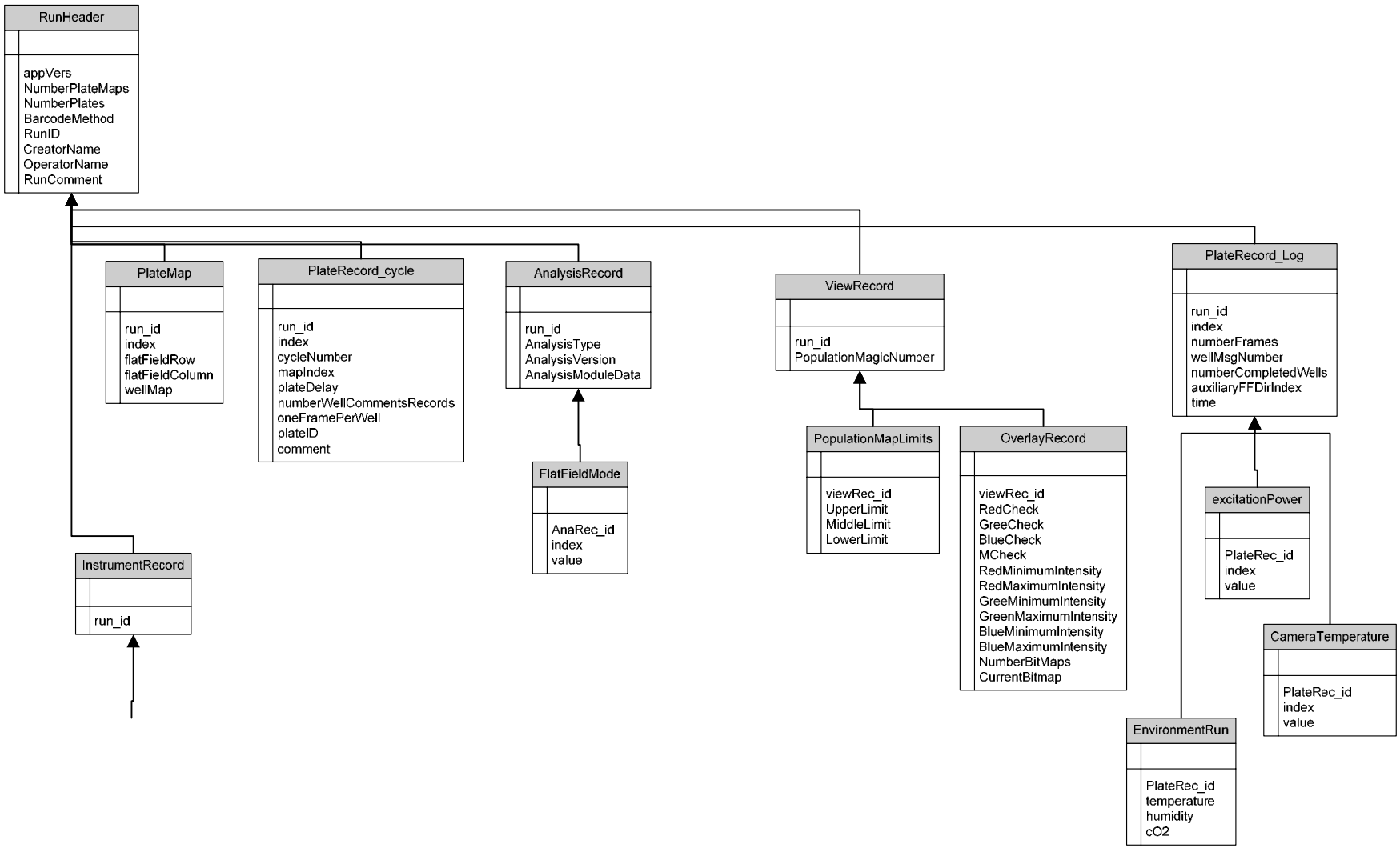
Row A is 0.45% larger than Row O

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

Screening model



INCell Information



Instrument Annotations