Bioimage Informatics

Lecture 21, Spring 2012

Biological Applications (III)

Bioimage Informatics for High-Throughput / High-Content Screens

Empirical Performance Evaluation of Bioimage Informatics Algorithms

Outline

- Some historical perspectives on bioimage informatics
- Introduction to high-throughput / high-content screening
- Case I: a high content screen based on static cellular imaging (immunofluorescence)
- Case II: a high content screening based on dynamic cellular imaging (live cell imaging)
- Empirical performance evaluation of bioimage Informatics algorithm

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What is Bioimage Informatics?

- The main goal of bioimage informatics is to use computational methods to analyze and understand images of biological processes.

- Bioimage informatics is often considered as a branch of bioinformatics and/or computational biology.

- Key components of bioimage informatics
  - low-level image analysis (e.g. feature detection)
  - high-level information extraction (e.g. pattern recognition)
  - image data management (database)
  - image visualization (graphics)

Origination of Bioimage Informatics Techniques

- Origination of bioimage informatics is driven by the synergy of several forces (first meeting in 2005).

- Technical needs
  - Changes to how biological systems are studied
  - Changes to how drugs are developed

- Enabling techniques
  - Automated sample and reagent preparation
  - Automated liquid handling
  - Automated image collection
  - Cost effective data storage and access
  - Automated image analysis techniques

Some Historical Perspectives

- Digital image processing started to become widely applied to bioimage analysis in early 1980s.
  - Shinya Inoue pioneered the use of video devices and image processing in microscopy.

- Computational image analysis can
  → improve image quality
  → extract quantitative measurements
  → contribute to image understanding

Shinya Inoue,
Marine Biology Lab, Woods Hole, MA

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Introduction to High-Throughput Screening (I)

- The technique originated approximately 30 years ago from natural product screening for pharmaceutical R&D.

- Currently the technology is used in a broad range of applications such as:

  - Pharmaceutical R&D
  - Biotechnology R&D
  - Large scale basic biological research

http://www.eppendorfna.com

Table 1 Successful prototype identification dependent on: sample diversity and number screened

Traditional screening	*High throughput screening*
Single tube	Array format 96-well
Large assay volume ~1 ml	Small assay volume 50–100 μl
Compound used ~5–10 mg	Compound used ~1 μg
Assay components added singly	Assay components added simultaneously
Mechanical action 1 : 1	Mechanical action 1 : 96
Dry compounds—custom solution	Compound file in solution—DMSO
Assay slow and laborious	Assay fast and efficient (~1 min/step/96-well plate)
Screen 20–50 compounds/week/lab	Screen 1000–10 000 week/lab
Limited number and diversity screened	Unlimited number and diversity screened

Abbreviation: DMSO, dimethyl sulphoxide

Pereira & Williams, *British. J. Pharmacology*, 152:53-61, 2007.

96 = 8x12, 384=16X24, 1536=32x48, 3456 = 48X72

Introduction to High-Throughput Screening (II)

- The basic concept of HTS is to perform large numbers of biological tests or experiments in parallel using automation techniques to achieve very high efficiency.

http://pubs.acs.org/cen/government/87/8725gov1.html

- Core techniques
  - Automated sample and reagent preparation
  - Automated liquid and plate handling
  - Automated data collection and analysis

- HTS systems typically rely on simple readouts to achieve high efficiency.

Introduction to High-Content Screening (I)

- High-content screening shares many of the core techniques with high-throughput screening.

- Major differences

  - HCS typically get multiplex readouts from cell assays.

  - HCS typically involves live cell imaging and aims to extract spatial and temporal dynamics information.
    → Heavy dependence on imaging and image analysis

  - HCS aims to balance collecting comprehensive information with achieving high efficiency (throughput).

D. L. Taylor, High Content Screening, Humana Press, 1st ed., 2006.

Introduction to High-Content Screening (II)

- Misuses of the terms HTS and HCS are quite common.
- The boundary between HTS and HCS is often blurred.
- HTS and HCS often are used to provide a starting point for more in-depth studies.

Case Studies

[1] Perlman et al, Multidimensional drug profiling by automated microscopy, *Science*, 306:1194-1198, 2004.

[2] Neumann et al, High-throughput RNAi screening by time-lapse imaging of live human cells, *Nature Methods*, 3:385-390, 2006.

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Experiment Design

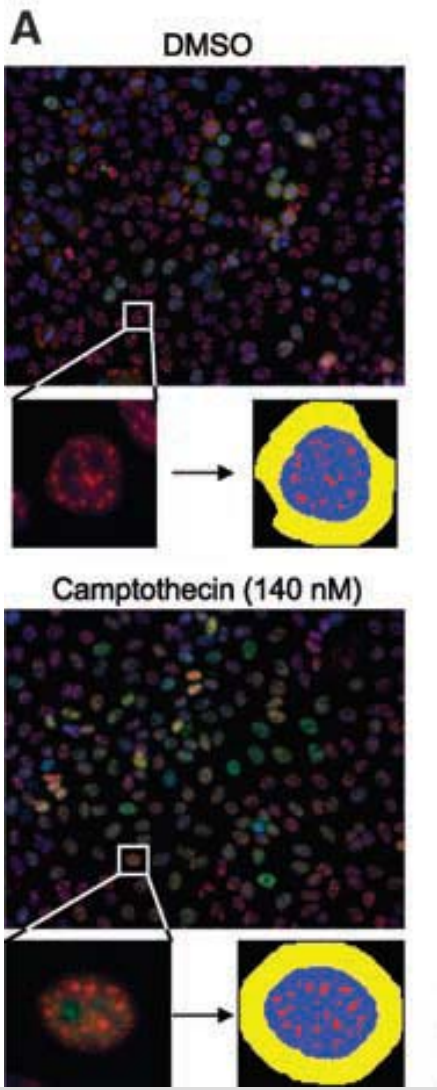
- Main goal: to characterize and predict effects of drugs at different concentrations on mammalian cells.
- 100 drug compounds were tested. Among them 10 were blindly tested.
- Each compound is tested at 13 concentrations ranging from micromolar to picomolar.
- A 384 well plate is used, with 3000 cells per well.
- 11 different probes. Two colors + cell nucleus labeling per well. Nine images per well.
- Experiments performed in duplicate.

Compound distribution:

Column	Plate 1	Plate 2	Plate 3	Plate 4	Plate 5	Plate 6
1	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO
2	1	21	41	61	81	DMSO
3	2	22	42	62	82	DMSO
4	3	23	43	63	83	DMSO
5	4	24	44	64	84	DMSO
6	5	25	45	65	85	DMSO
7	6	26	46	66	86	DMSO
8	7	27	47	67	87	DMSO
9	8	28	48	68	88	DMSO
10	9	29	49	69	89	DMSO
11	10	30	50	70	90	DMSO
12	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO
13	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO
14	11	31	51	71	91	DMSO
15	12	32	52	72	92	DMSO
16	13	33	53	73	93	DMSO
17	14	34	54	74	94	DMSO
18	15	35	55	75	95	DMSO
19	16	36	56	76	96	DMSO
20	17	37	57	77	97	DMSO
21	18	38	58	78	98	DMSO
22	19	39	59	79	99	DMSO
23	20	40	60	80	100	DMSO
24	DMSO	DMSO	DMSO	DMSO	DMSO	DMSO

Image Analysis and Quantification (I)

- Background subtraction
- Image segmentation
  - nucleus segmentation
- Definition of descriptors (total=93)
  - nucleus: area, eccentricity, perimeter, intensity,…
  - actin: intensity, area, intensity ratio,…



List of Descriptors

CaM_AnnToNucIntRatio
pERK_AnnToNucIntRatio
pCREB_AnnToNucIntRatio
anillin_AnnToNucIntRatio
p38_AnnToNucIntRatio
SC35_AnnToNucIntRatio
p53_AnnToNucIntRatio
p38_AnnulusAveIntensity
actin_AnnulusAveIntensity
SC35_AnnulusAveIntensity
cFos_AnnToNucIntRatio
actin_VarIntensity
actin_AveIntensity
p38_GrayScaleCentroidOffset
pERK_GrayScaleCentroidOffset
MT_VarIntensity
DNA_Eccentricity
DNA_VarIntensity
actin_AnnToNucIntRatio
SC35_GrayScaleCentroidOffset
DNA_AveIntensity
MT_AnnulusAveIntensity
anillin_GrayScaleCentroidOffset
MT_AveIntensity
p53_GrayScaleCentroidOffset
anillin_AnnulusAveIntensity
CaM_GrayScaleCentroidOffset
cFos_GrayScaleCentroidOffset
MT_AnnToNucIntRatio
cFos_AnnulusAveIntensity
DNA_GrayScaleCentroidOffset
actin_NucInttoDNARatio
p53_AnnulusAveIntensity
pERK_AnnulusAveIntensity
actin_GrayScaleCentroidOffset

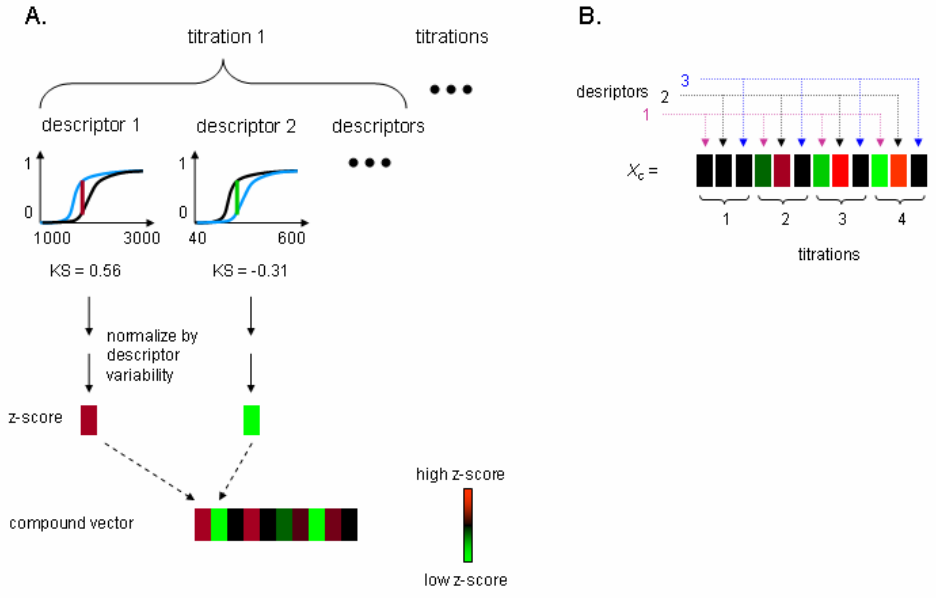
p38_VarIntensity
DNA_ShapeFactor
pCREB_AnnulusAveIntensity
MT_NucInttoDNARatio
pCREB_GrayScaleCentroidOffset
cFos_AveIntensity
MT_GrayScaleCentroidOffset
CaM_AnnulusAveIntensity
cFos_NucInttoDNARatio
p38_NucInttoDNARatio
SC35_SC35toDNARatio
p38_AveIntensity
actin_TotalIntensity
SC35_AveIntensity
SC35_VarSpeckleIntensity
SC35_VarIntensity
SC35_SpeckleCount
p53_AveIntensity
SC35_SpeckleArea
DNA_Solidity
SC35_AveSpeckleIntensity
cFos_VarIntensity
CaM_NucInttoDNARatio
p53_NucInttoDNARatio
cFos_AnnulusArea
p53_AnnulusArea
cFos_TotalIntensity
DNA_TotalIntensity
MT_TotalIntensity
p53_VarIntensity
SC35_AnnulusArea
anillin_AnnulusArea
anillin_AveIntensity
anillin_VarIntensity
pERK_NucInttoDNARatio

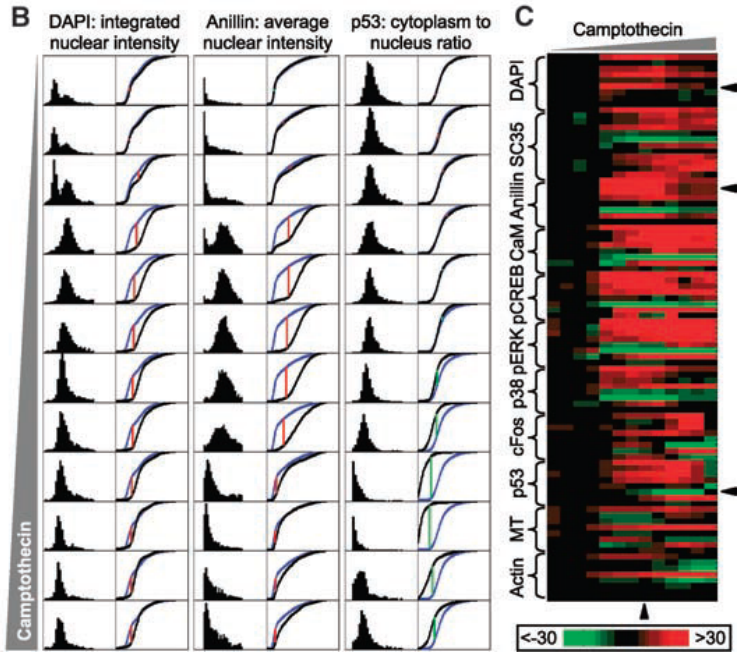
MT_AnnulusArea
actin_AnnulusArea
pCREB_VarIntensity
pCREB_NucInttoDNARatio
pCREB_AveIntensity
CaM_AnnulusArea
pCREB_AnnulusArea
pERK_AveIntensity
pERK_TotalIntensity
pCREB_TotalIntensity
CaM_TotalIntensity

A. *DNA*	1	Area	Pixel area of nuclear region
	2	Eccentricity	Ratio of axes of the best ellipse fit to nuclear region
	3	Perimeter	Area in pixels of nuclear region boundary returned by Matlab primitive `bwperim`
	4	Shape Factor	4π Area / (Perimeter)2
	5	Total Intensity	Integrated intensity in nuclear region
	6	Average Intensity	Average intensity in nuclear region
	7	Intensity Variance	Variance of intensity in nuclear region
	8	Gray Scale Centroid Offset	Distance in pixels between grayscale and binary centers of mass for nuclear region
	9	Solidity	Ratio of area of the nuclear region to the area of its convex hull

Image Analysis and Quantification (II)

- Population response
  - based on cumulative distribution





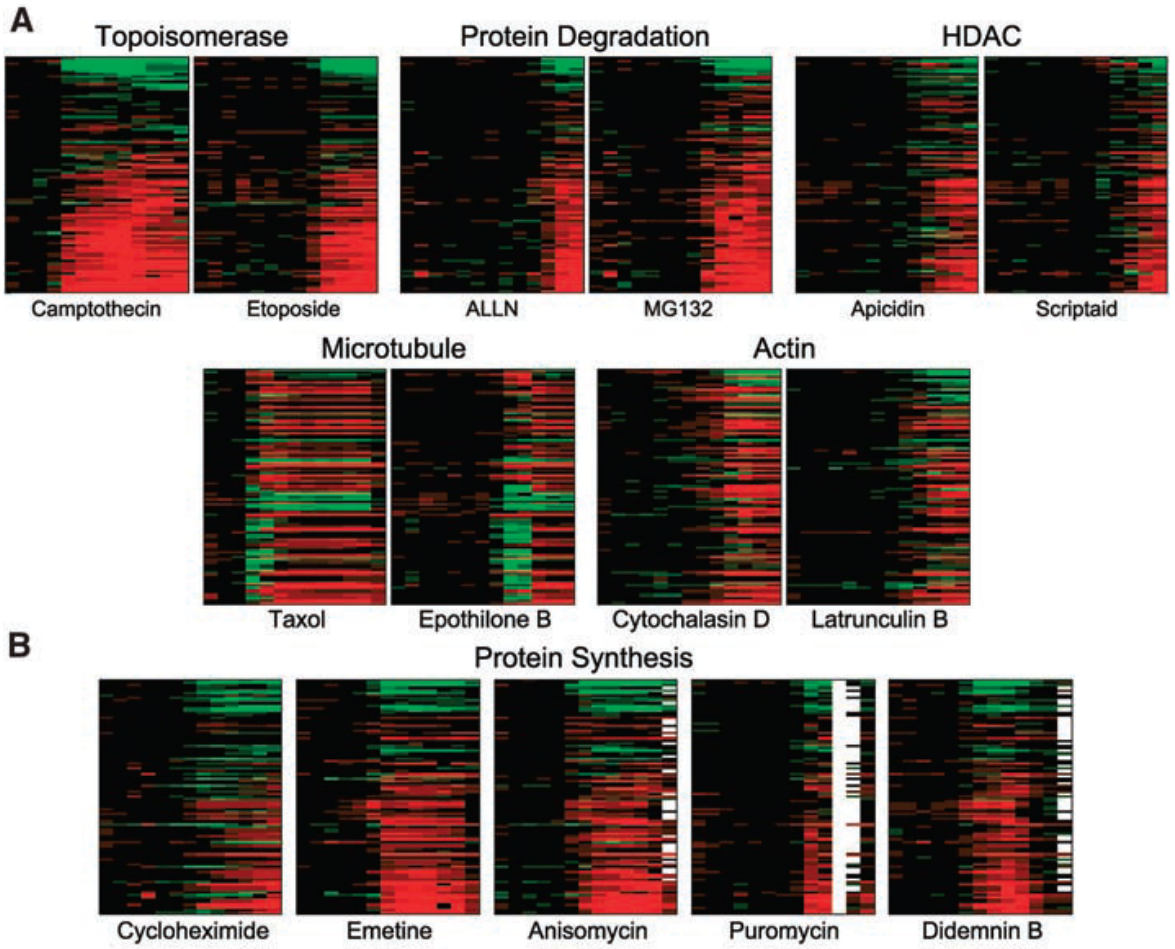
$$z_{c,d,t} = \frac{KS_{c,d,t}}{std\left(q_d\left(n\right)\right)}$$

c: compound index

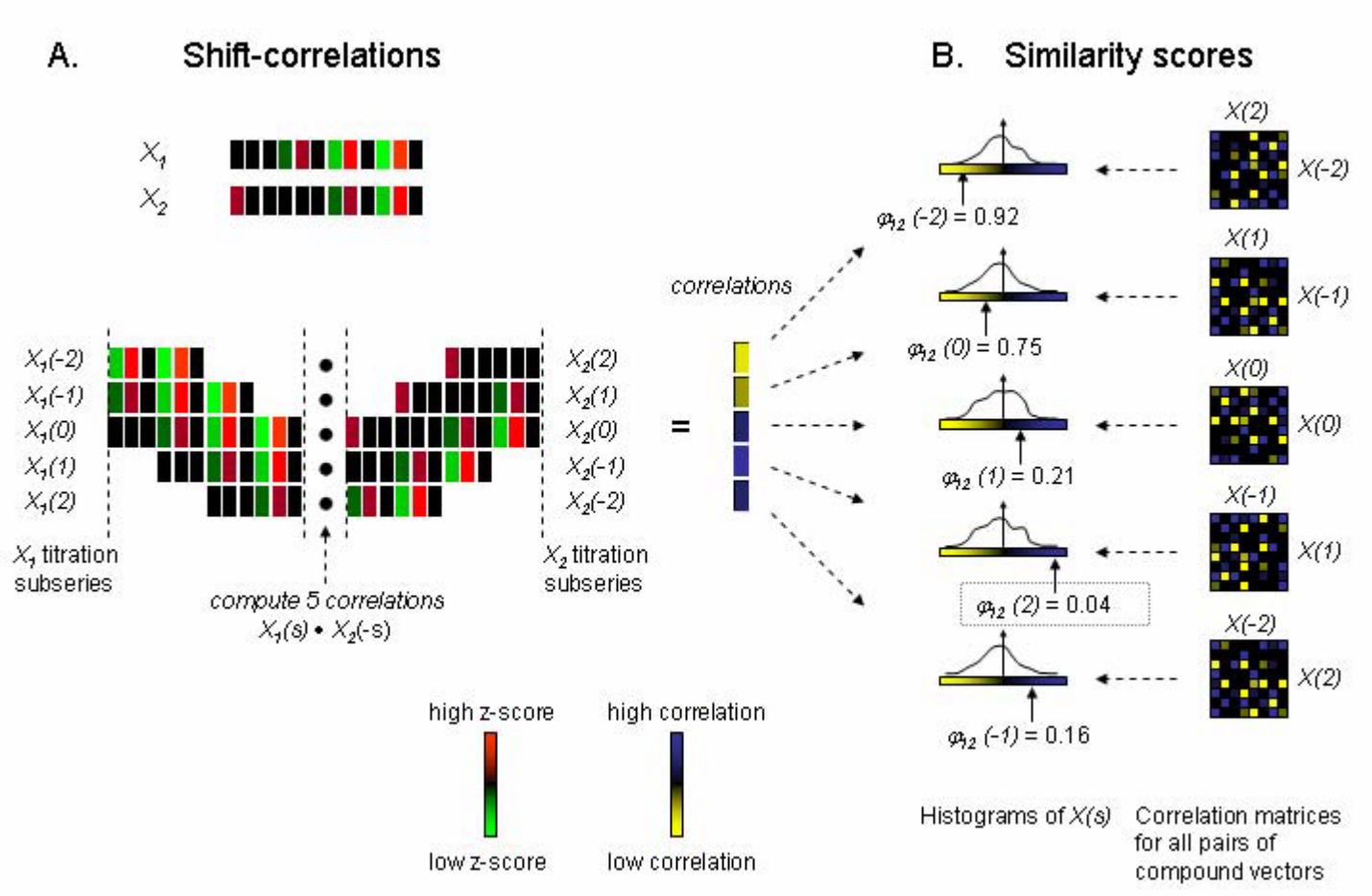
d: descriptor index

t : titration (concentration) index

Response Comparison of Different Drugs

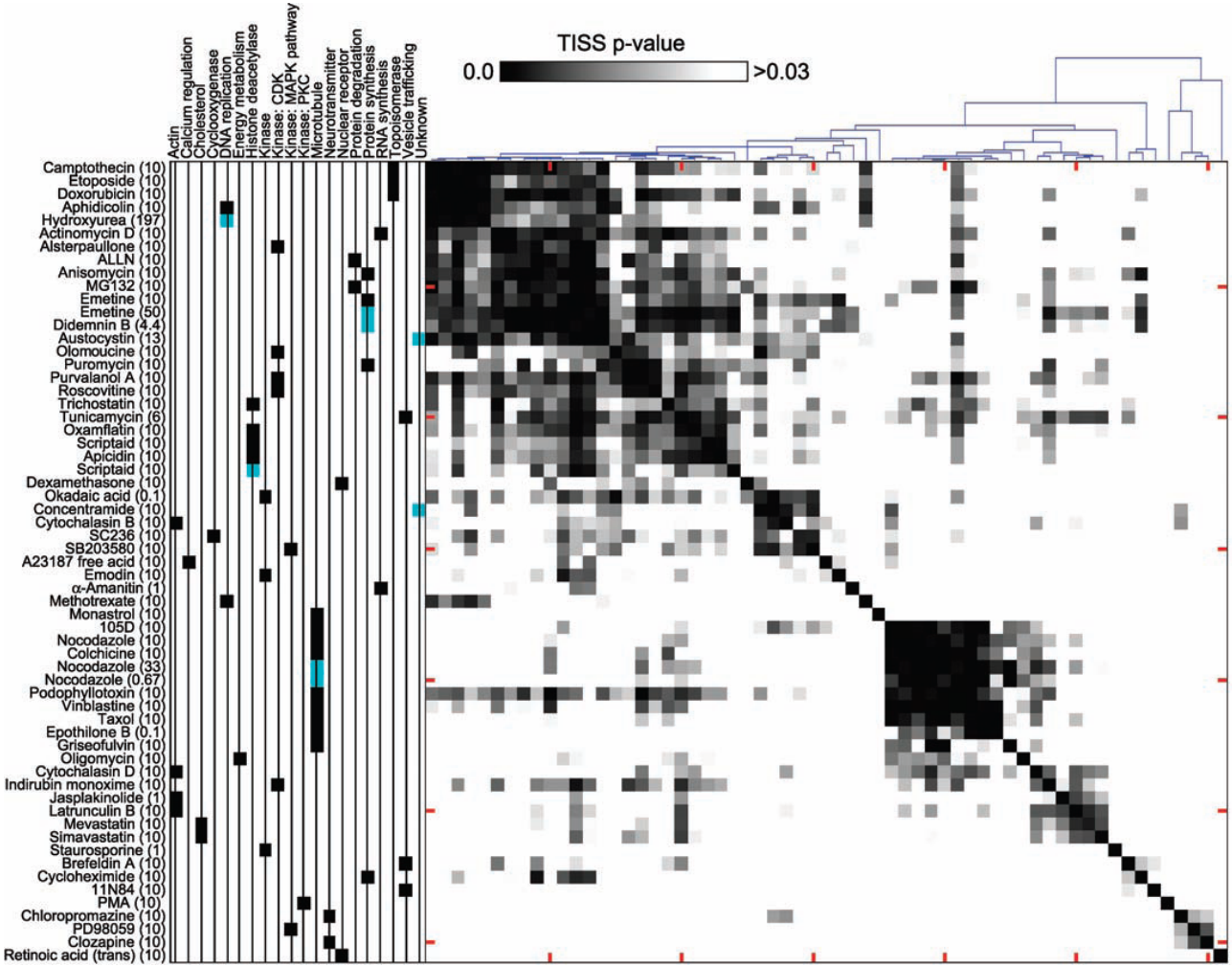


Classification based on TISS Clustering (I)



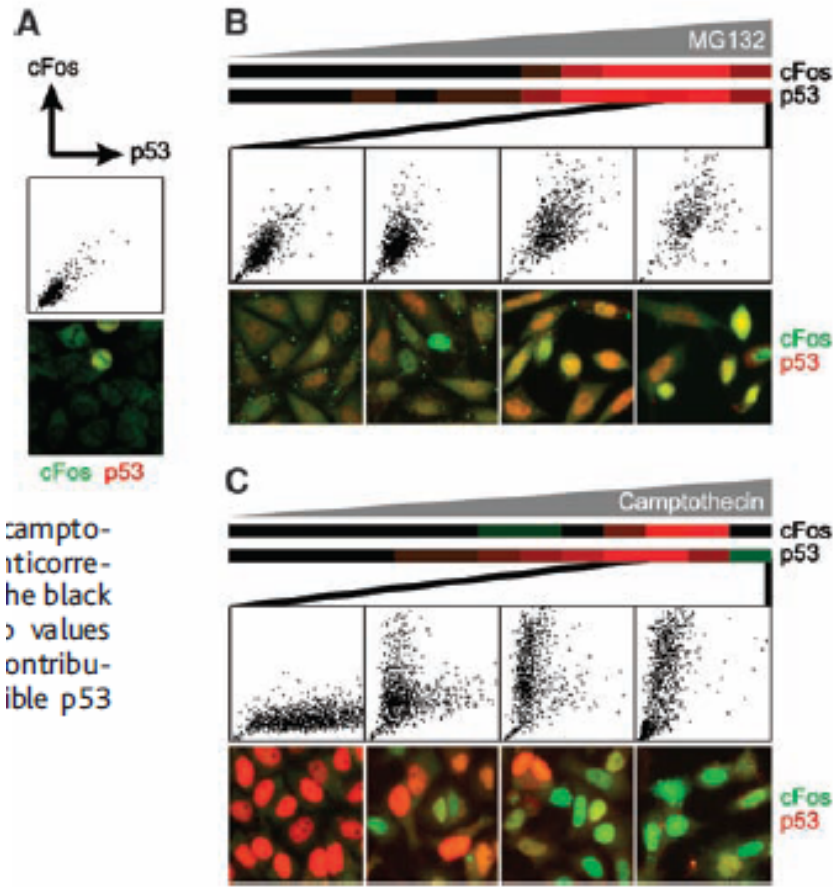
TISS: titration invariant similarity score

Classification based on TISS Clustering (II)



Heterogeneity in Drug Response

- MG132: inhibitor of protein degradation
- Camptothecin: inhibitor of transcription



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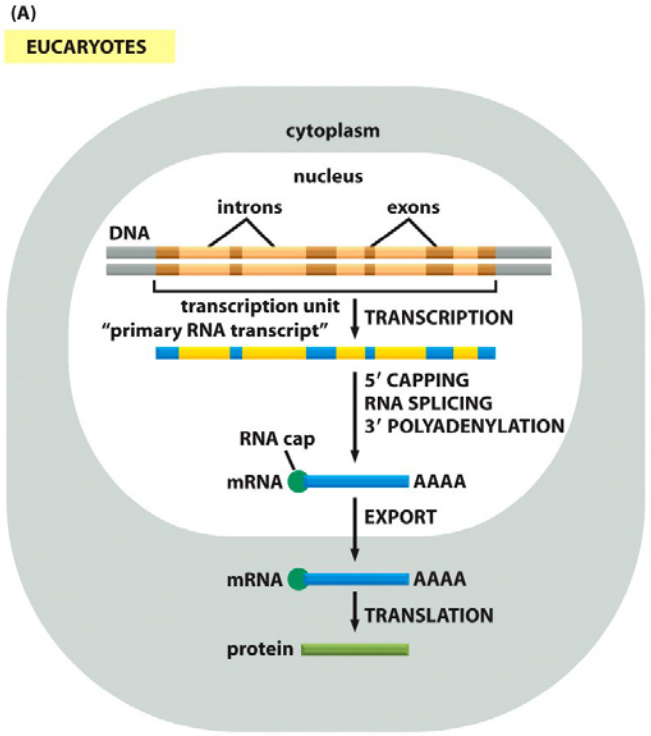
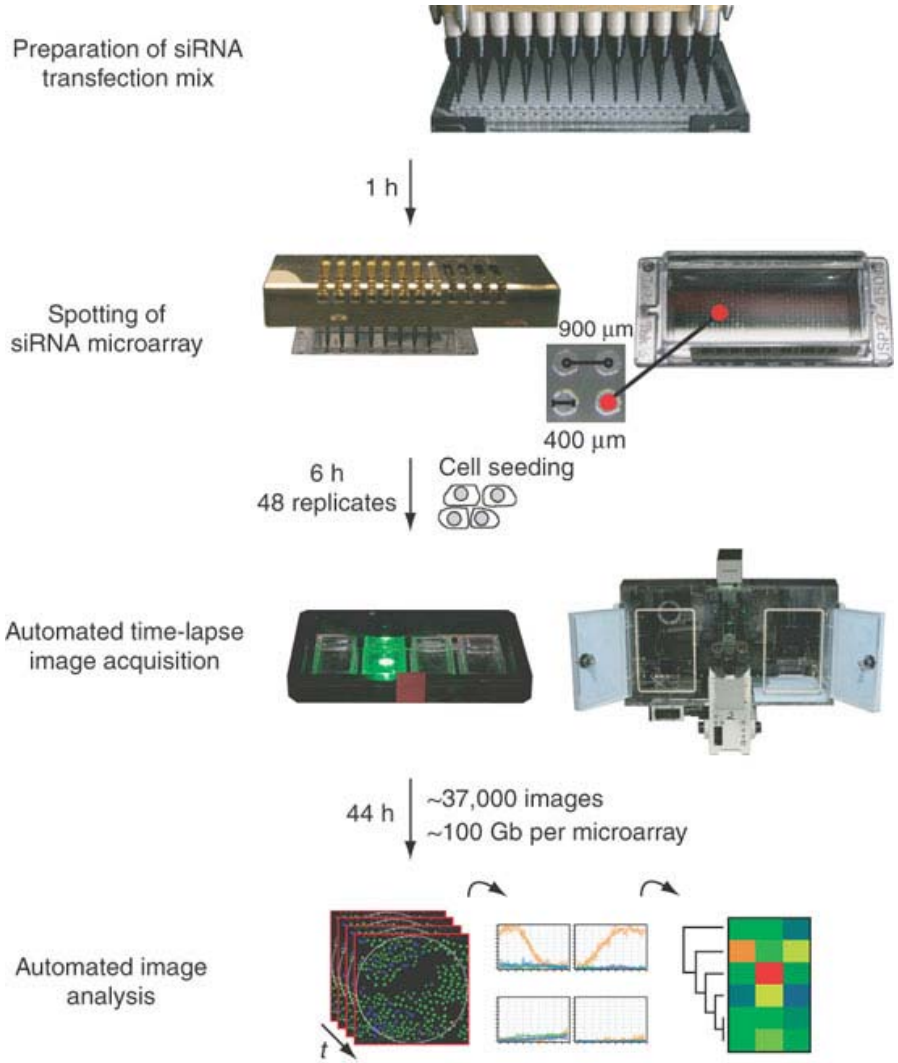
Experiment Setup

- A total of 49 targeted genes related to chromosome segregation or nuclear structure.
- Gene knockdown through RNA interference.
- Using four 384 well plates in parallel.
- Around 50 cells per well.
- 30 minutes per frame for 44 hours.

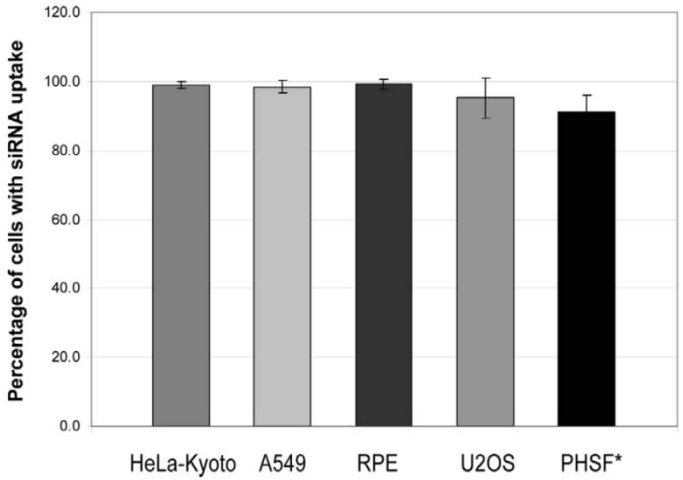


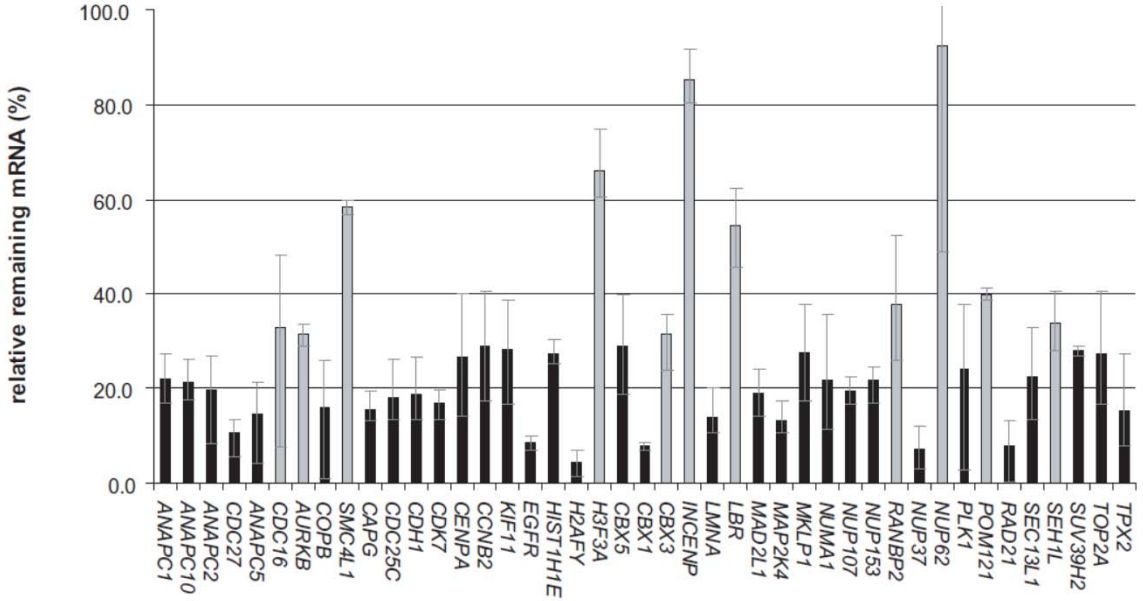
Alberts *MBoC* 5e

Case II: Workflow

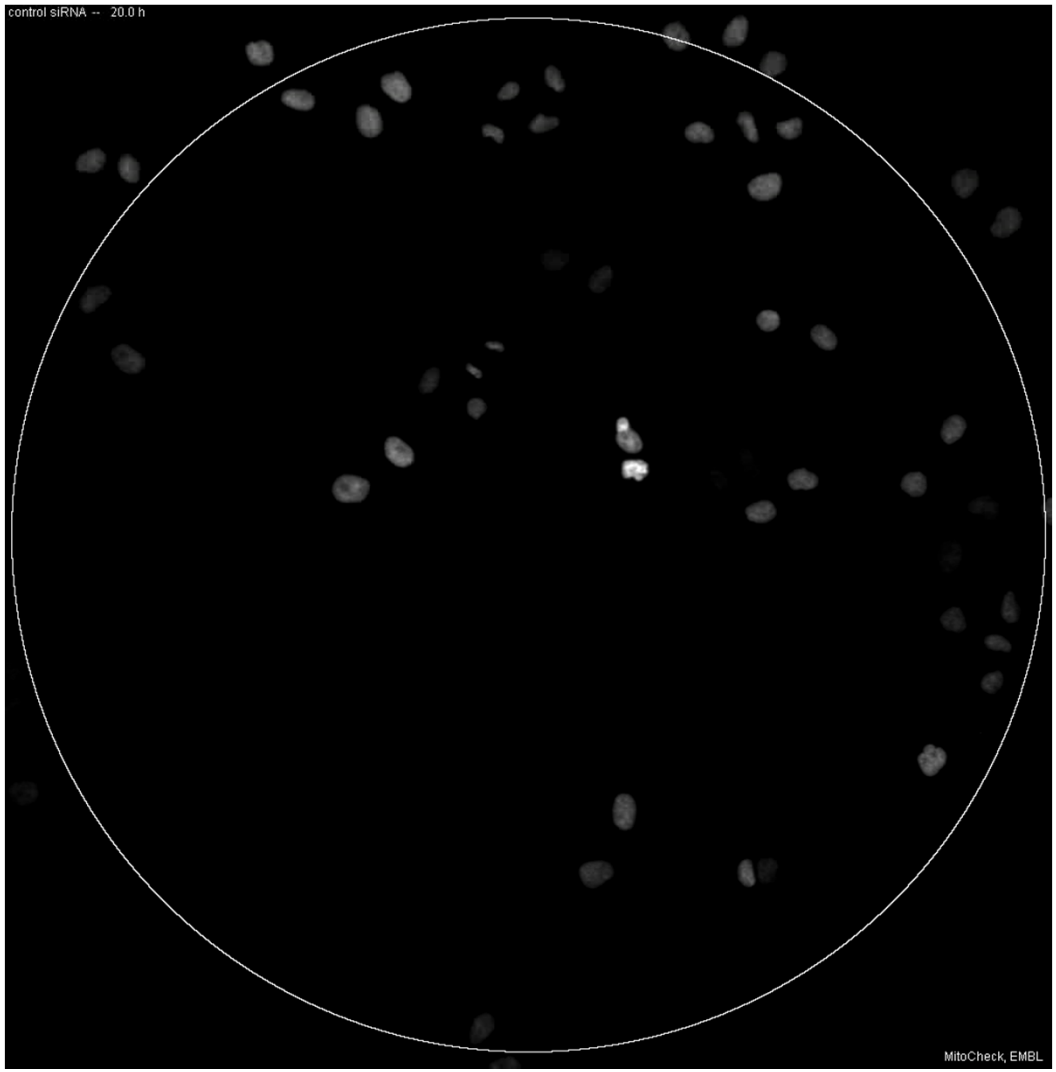


Case II: siRNA Uptake Validation



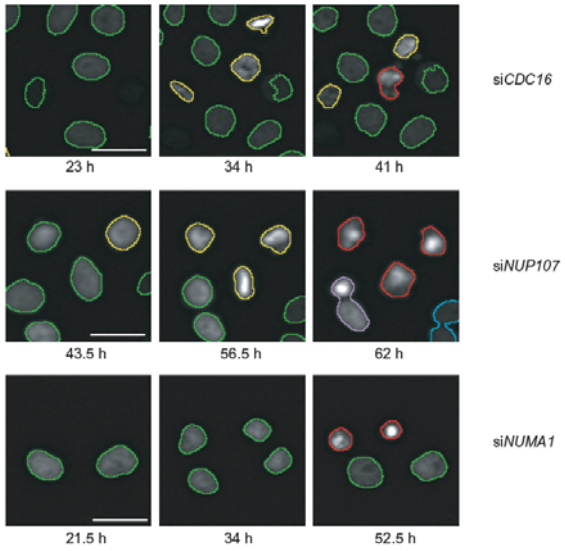


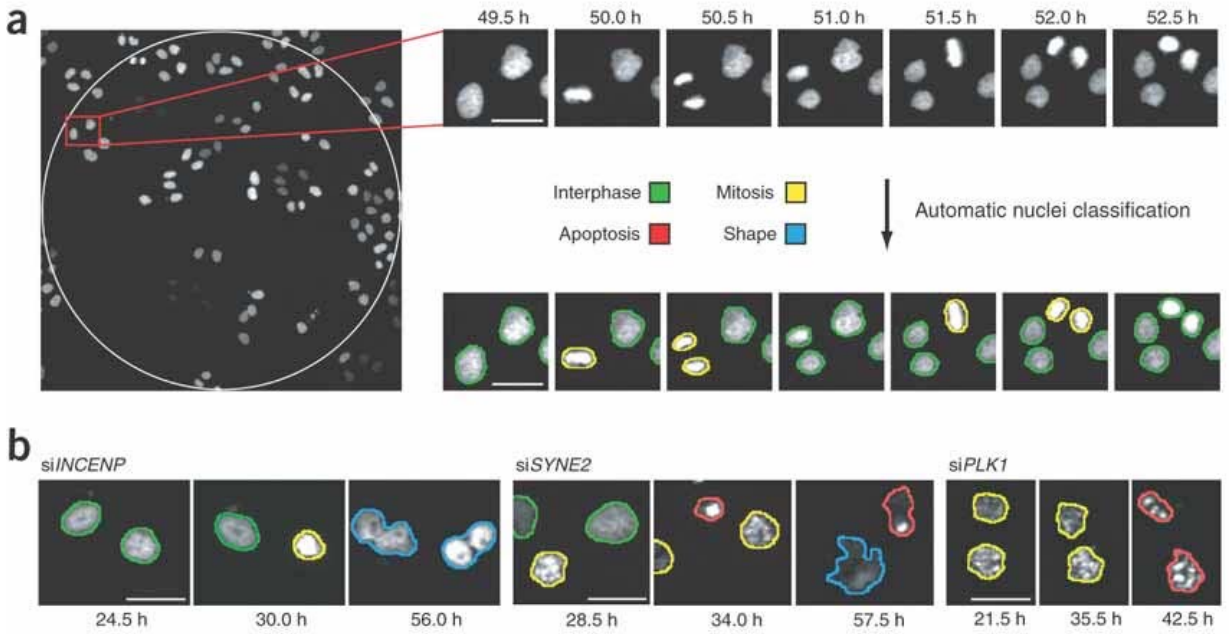
A Sample Video



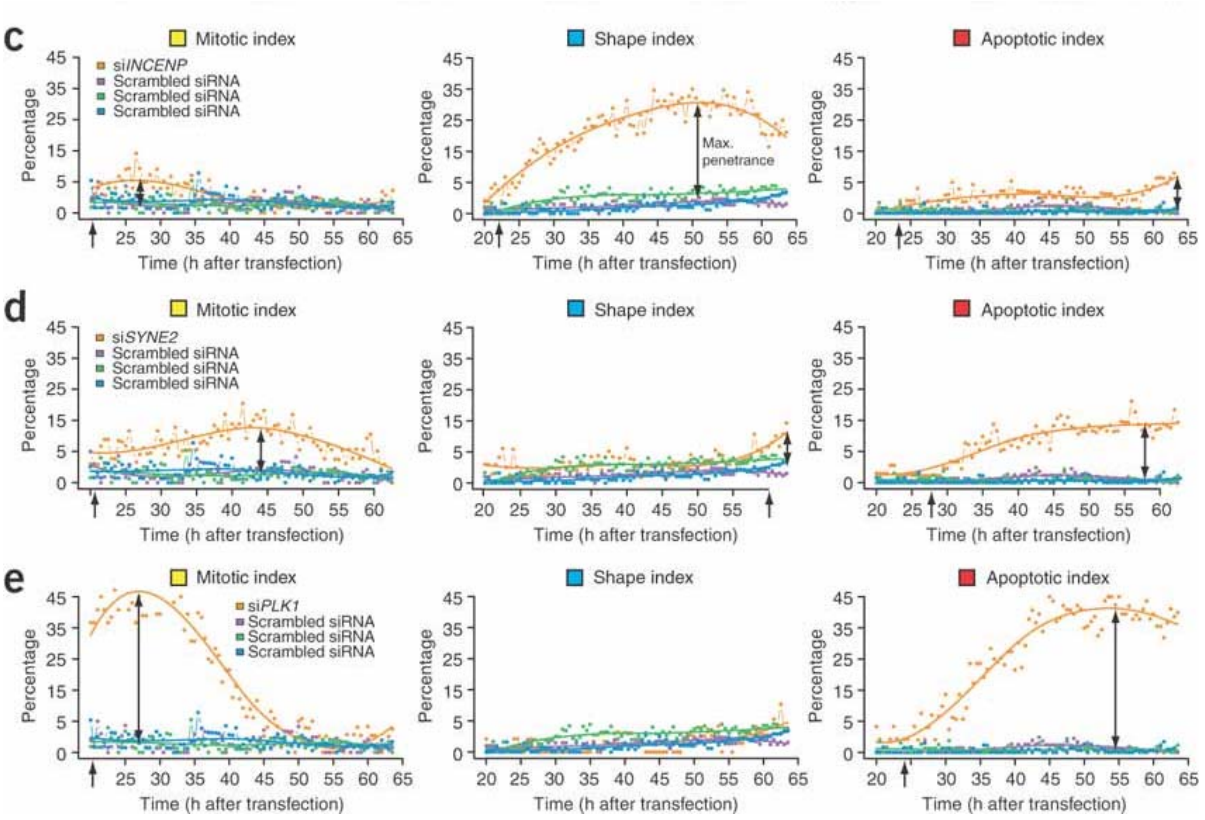
Case II: Image Analysis (I)

- Step I: image segmentation (optimized local adaptive thresholding).
- Step II: feature classification (texture + morphology).
- Step III: event detection (classification, time, additional background control).



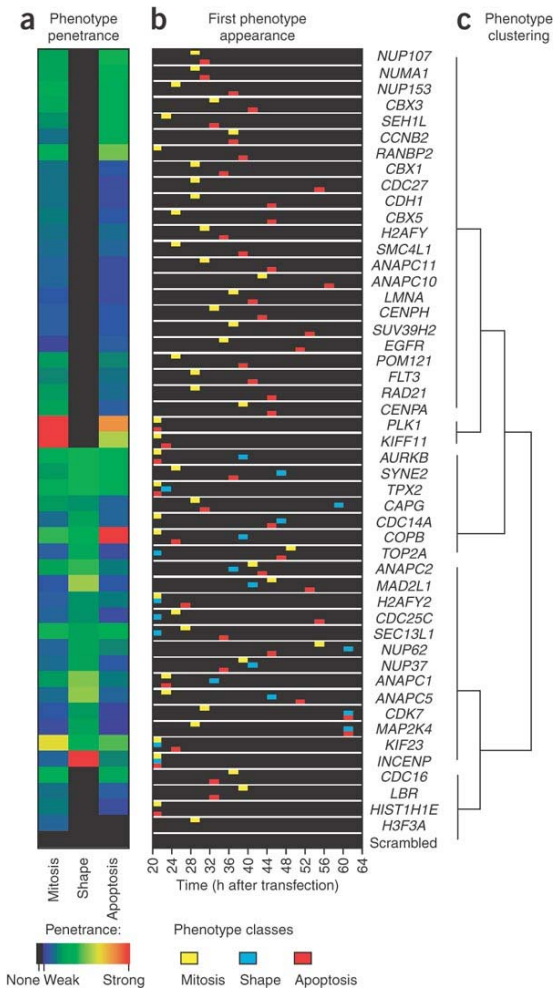


Case II: Image Analysis (II)



Case Study II: Information Mining

- Statistical clustering based on both penetration and temporal dynamics.
- Validation using results from small scale studies.



Summary: HTS & HCS

- Main properties of HTS and HCS applications
  - Large volume of image data
    →Both data and data analysis must be organized.
  - Multidimensional readout
  - High-dimension data analysis
  - Connection to biological questions
- Robust yet sensitive image analysis; Statistical data analysis.
- Data quality control to avoid artifacts.
  - Internal consistency check.
  - Validation using other techniques.
- Visualization and interpretation of data.

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Overview of Algorithm Evaluation (I)

- Two sets of data are required
  - Input data
  - Corresponding correct output (ground truth)
- Sources of input data
  - Actual/experimental data, often from experiments
  - Synthetic data, often from computer simulation
- Actual/experimental data
  - Essential for performance evaluation
  - May be costly to acquire
  - May not be representative
  - Ground truth often is unknown

Overview of Algorithm Evaluation (II)

- Simulated data

  Advantages

  - Ground truth is known
  - Usually low-cost

  Disadvantages

  - Difficult to fully represent the original data

- Realistic synthetic data

- Quality control of manual analysis

Test Protocol Development

- Implementation
  - Source codes are not always available and often are on different platforms.
- Parameter setting
  - This is one of the most challenging issues.
- Quantification of success rate

Table I
Different Types of Output of a Simple Event Test

Classification	Definition
True positive (TP, true acceptance, true match)	A positive event is correctly identified as positive
True negative (TN, true rejection, true nonmatch)	A negative event is correctly identified as negative
False negative (FN, false rejection, false nonmatch, type I error)	A positive event is incorrectly identified as negative
False positive (FP, false acceptance, false match, type II error)	A negative event is incorrectly identified as positive

Test Administration

- Comparison of algorithms

  M. Heath, S. Sarkar, T. Sanocki, and K.W. Bowyer, "A Robust Visual Method for Assessing the Relative Performance of Edge-Detection Algorithms" *IEEE Transactions on Pattern Analysis and Machine Intelligence*, Vol. 19, No. 12, pp. 1338-1359, 1997.

  Barron, J.L., Fleet, D.J., and Beauchemin, S. Performance of optical flow techniques. *International Journal of Computer Vision*, 12(1):43-77, 1994.

- Large-scale open evaluation of algorithms is often superior but costly.

Examples of Open Benchmarking Datasets

- Berkeley segmentation dataset and benchmark

  "A Database of Human Segmented Natural Images and its Application to Evaluating Segmentation Algorithms and Measuring Ecological Statistics" D. Martin, C. Fowlkes, D. Tal, J. Malik, ICCV2001.

  http://www.eecs.berkeley.edu/Research/Projects/CS/vision/bsds/

- Retrospective image registration project

  "Comparison and evaluation of retrospective intermodality image brain image registration techniques", Journal of Computer Assisted Tomography, J. West et al, vol.21, pp. 554-566, 1997

  http://www.insight-journal.org/rire/

Evaluation of Algorithm Efficiency

- Two complementary approaches

  - Theoretical computational complexity analysis
  - Empirical evaluation of efficiency

Table II
Commonly Used Time Complexity Terms

If running time of an algorithm is proportional to	Its time complexity is called	Increase in running time when N is increased by 10
1	Constant	0
$\log N$	Logarithmic	2.303
N	Linear	10
$N \log N$	$N \log N$ or linearithmic	23.03
N^3, N^3, ...	Polynomial	100, 1000, ...
2^N	Exponential	1024

References on Performance Evaluation

[1] J. F. Dorn, G. Danuser, G. Yang, Chapter 22 Computational processing and analysis of dynamic fluorescence image data, in Methods in Cell Biology, vol. 85, pp. 497-538.

[2] K. Bowyer, P. J. Phillips, Empirical evaluation techniques in computer vision, IEEE Press, 1998.

[3] H. I. Christensen, P. J. Phillips, Empirical evaluation techniques in computer vision, World Scientific, 2002.

Questions?

Quantification of Success Rates (continued)

- Rigorous approaches on feature detection can be learned from the field of signal detection theory.

- Some references on signal detection theory can be found from

  http://en.wikipedia.org/wiki/Detection_theory