Optimized Experimental and Analytical Tools for Reproducible Drug-Response Studies

Caitlin Mills & Kartik Subramanian
Department of Systems Biology
& Laboratory of Systems Pharmacology
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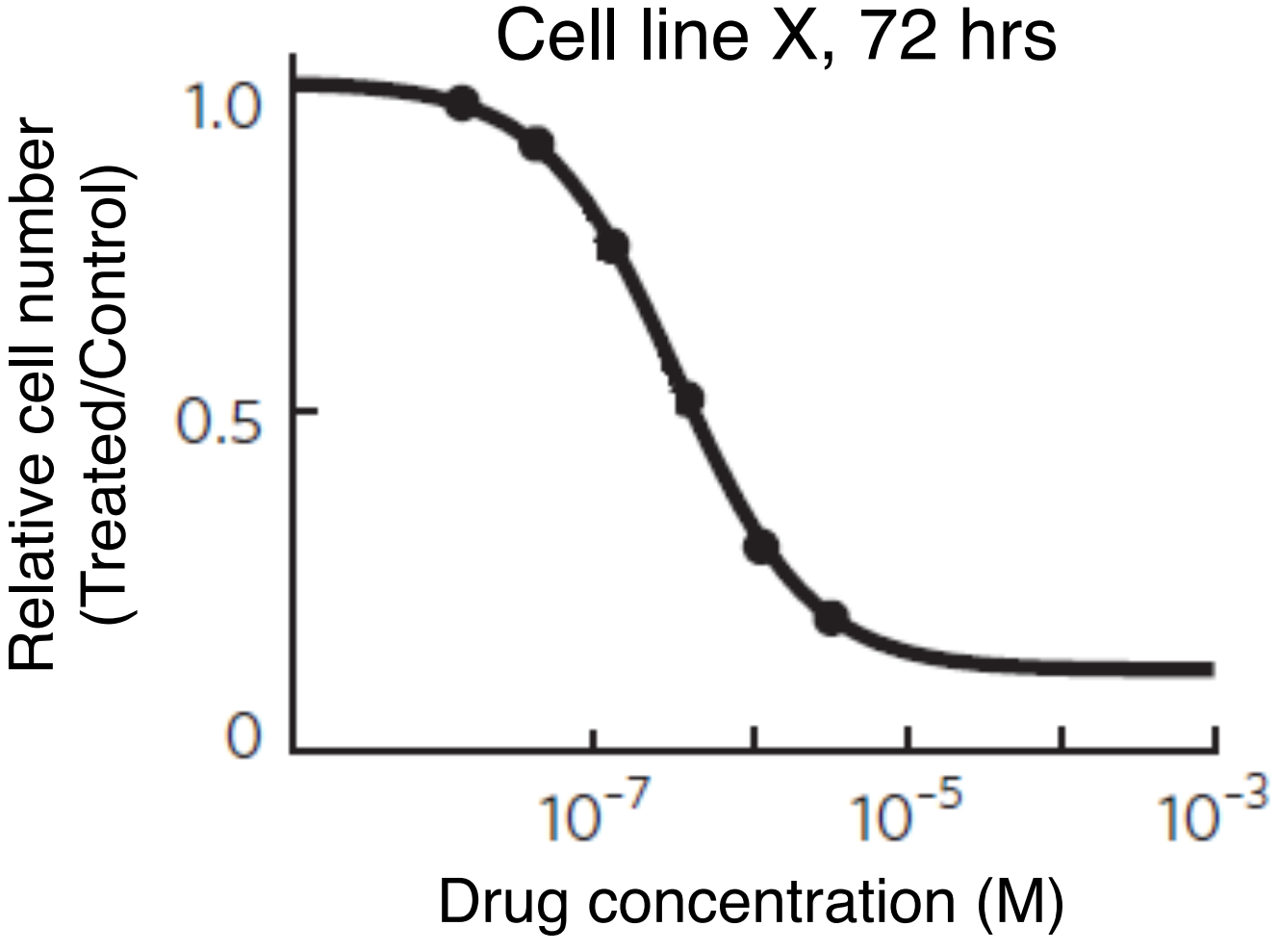
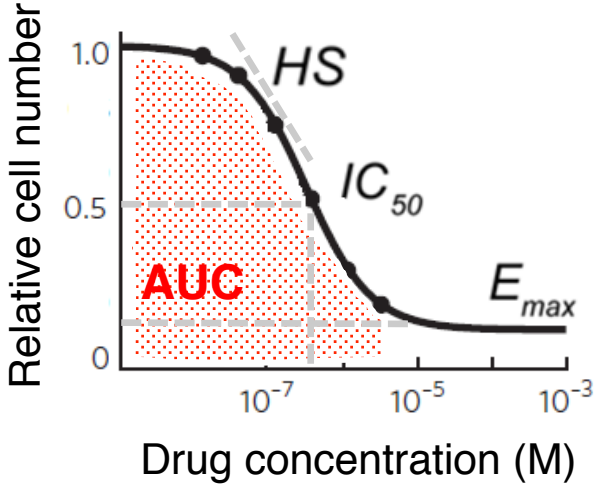
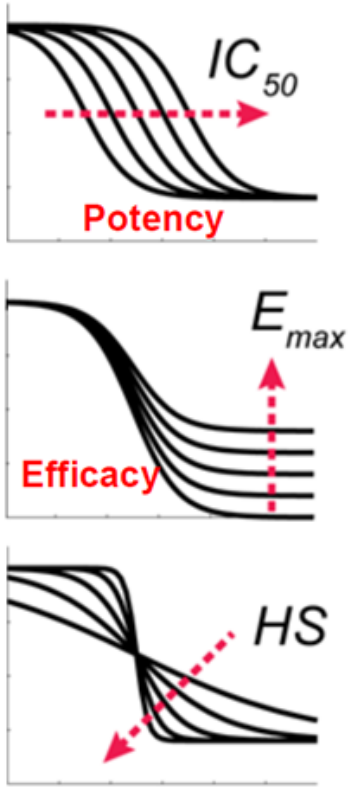
In-vitro drug-response studies in cancer are often based on relative cell number quantifications



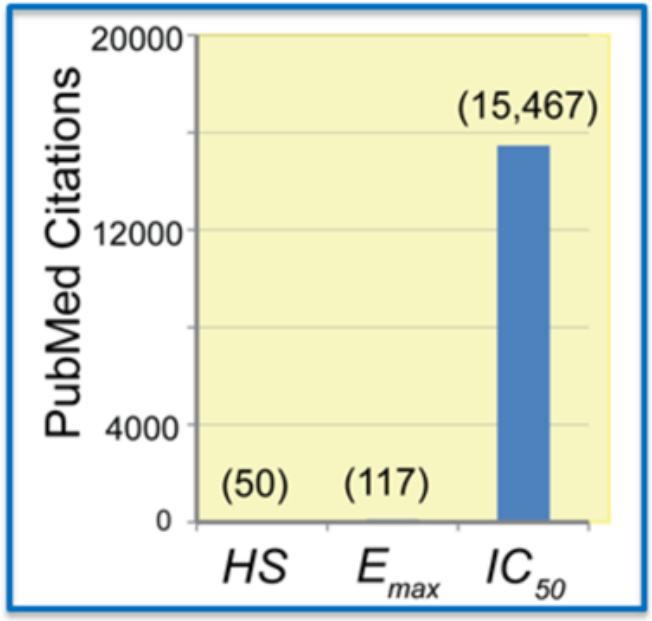
Drug-response in cancer research and conventional metrics based on relative cell number

Theory





Literature



NATURE CHEMICAL BIOLOGY DOI: 10.1038/NCHEMBIO.1337 ARTICLE

Metrics other than potency reveal systematic variation in responses to cancer drugs

Mohammad Fallahi-Sichani[1], Saman Honarnejad[1], Laura M Heiser[2], Joe W Gray[2] & Peter K Sorger[1]*

Irreproducible pharmacogenomics and other drug-dose response based studies

Inconsistency in large pharmacogenomic studies

Benjamin Haibe-Kains, Nehme El-Hachem, Nicolai Juul Birkbak, Andrew C. Jin, Andrew H. Beck, Hugo J. W. L. Aerts & John Quackenbush

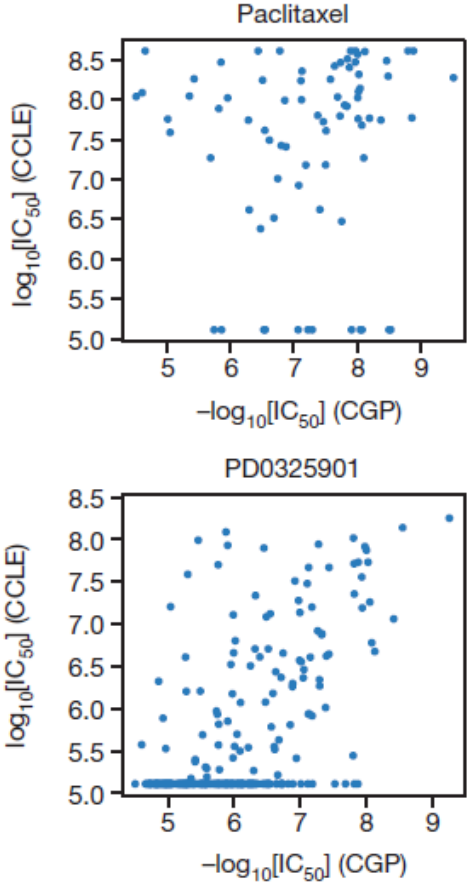
Affiliations | **Contributions** | **Corresponding author**

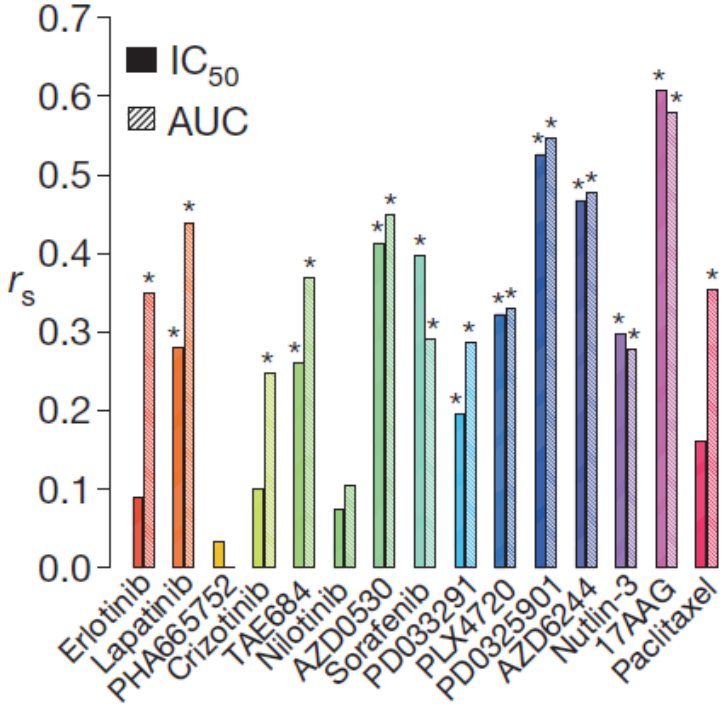
Nature **504**, 389–393 (19 December 2013) | doi:10.1038/nature12831

1. CCLE & GDC, Nature, Dec 2015
2. Haverty et al., Nature, May 2016
3. Bouhaddou et al. Nature, Dec 2016
4. Mpindi et al., Nature, Dec 2016
5. Safikhani et al., Nature, Dec 2016
6. Geeleher et al., Nature, Dec 2016

Irreproducible pharmacogenomics due to irreproducibile IC50 and other metrics

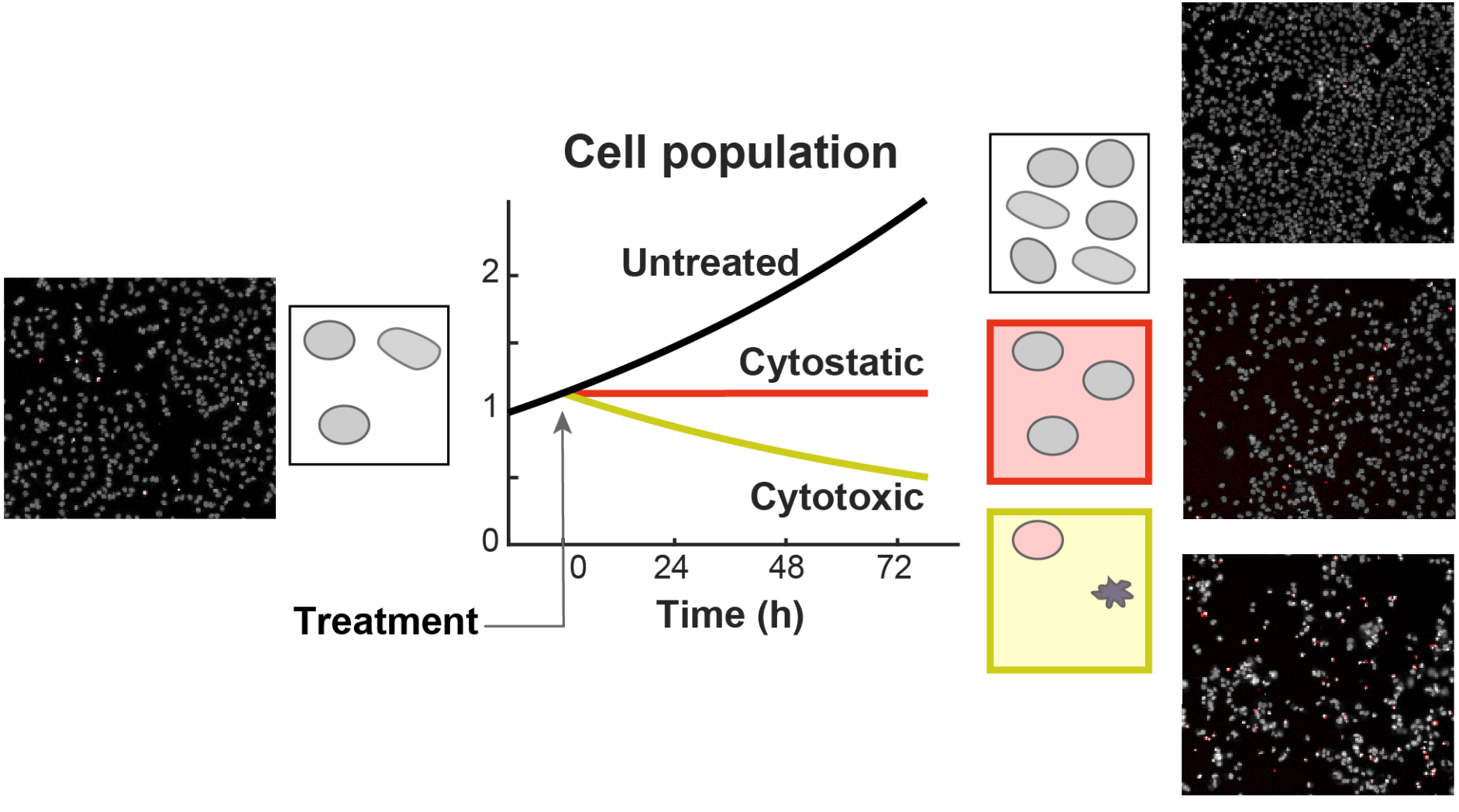
Drug-dose response correlation between Cancer Genome Project(CGP) and Cancer Cell Line Encyclopedia(CCLE):



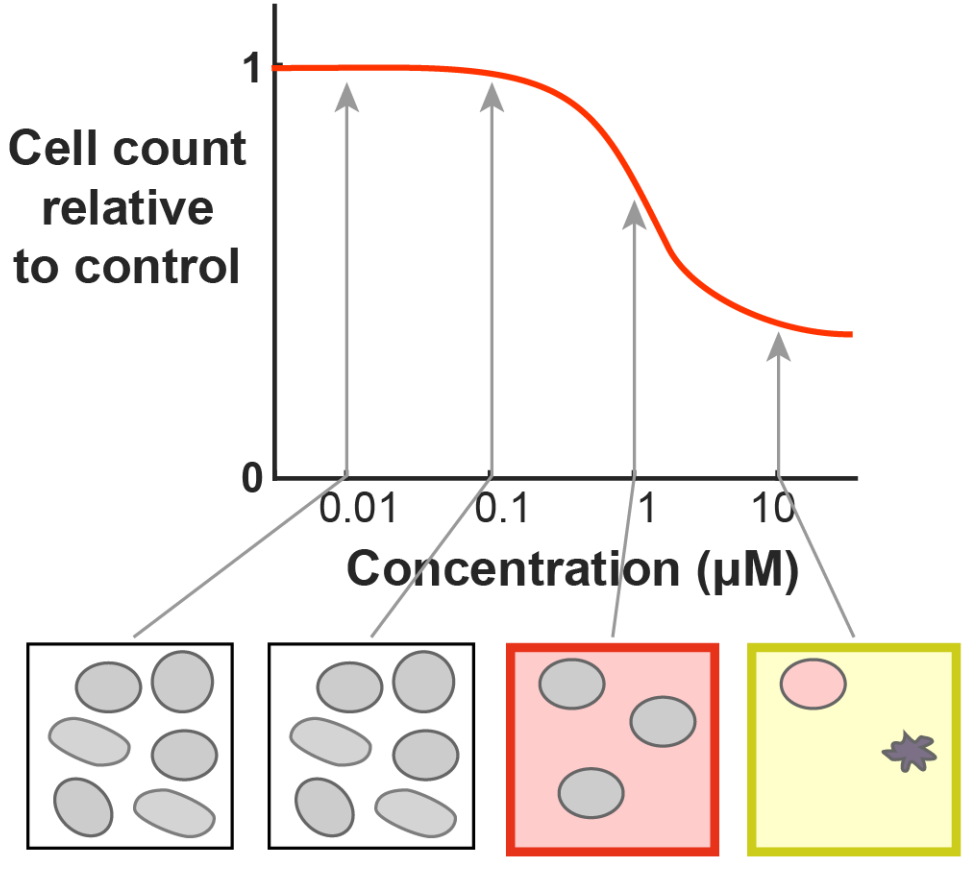


Inconsistency in large pharmacogenomics studies, Haibe-Kains et al, Nature, 2013

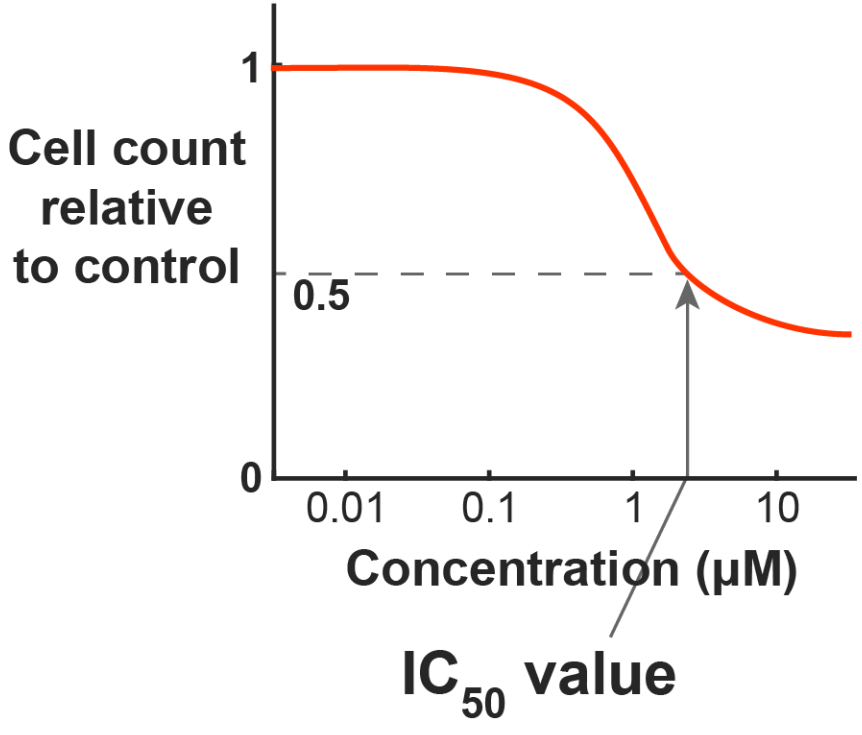
Measuring drug response is essential in pharmacology



Drug response is assayed at multiple doses

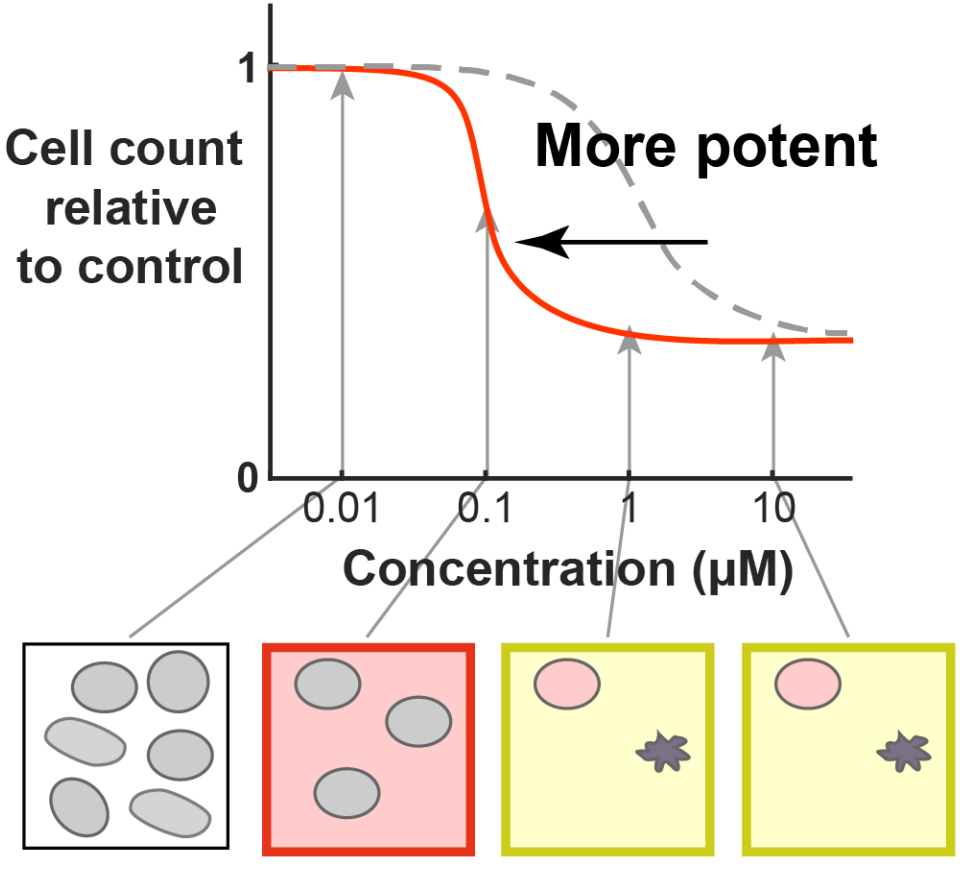


Drug response is assayed at multiple doses

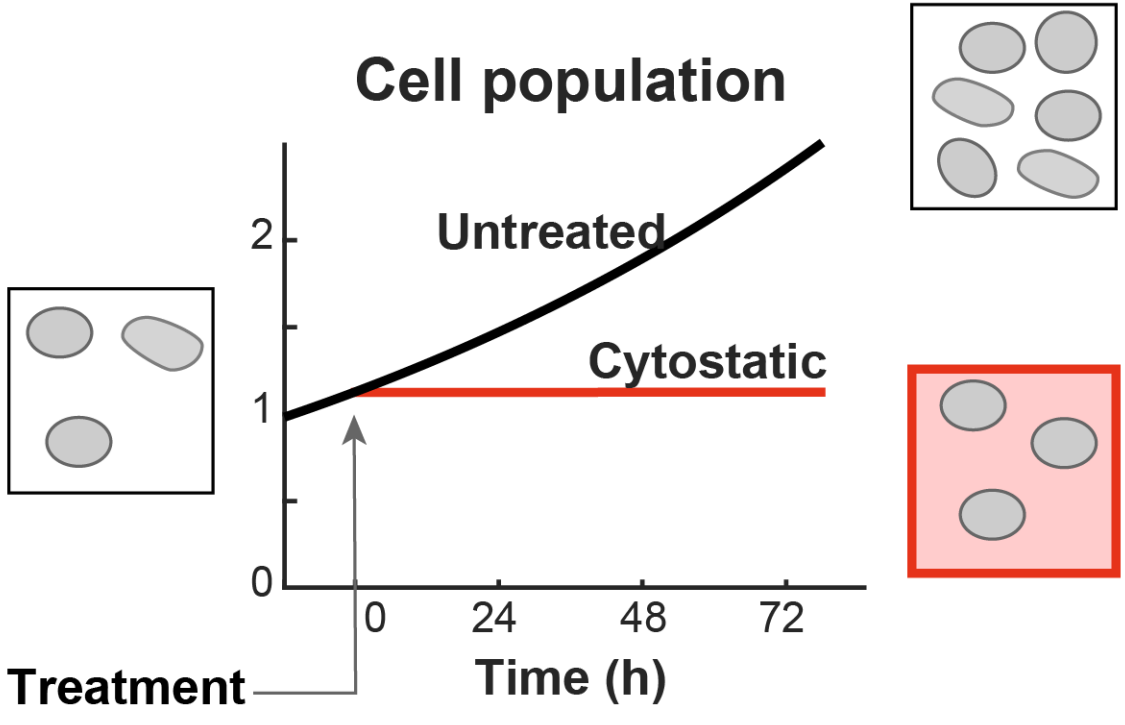


IC_{50} value is the concentration at which the relative cell count is 0.5.

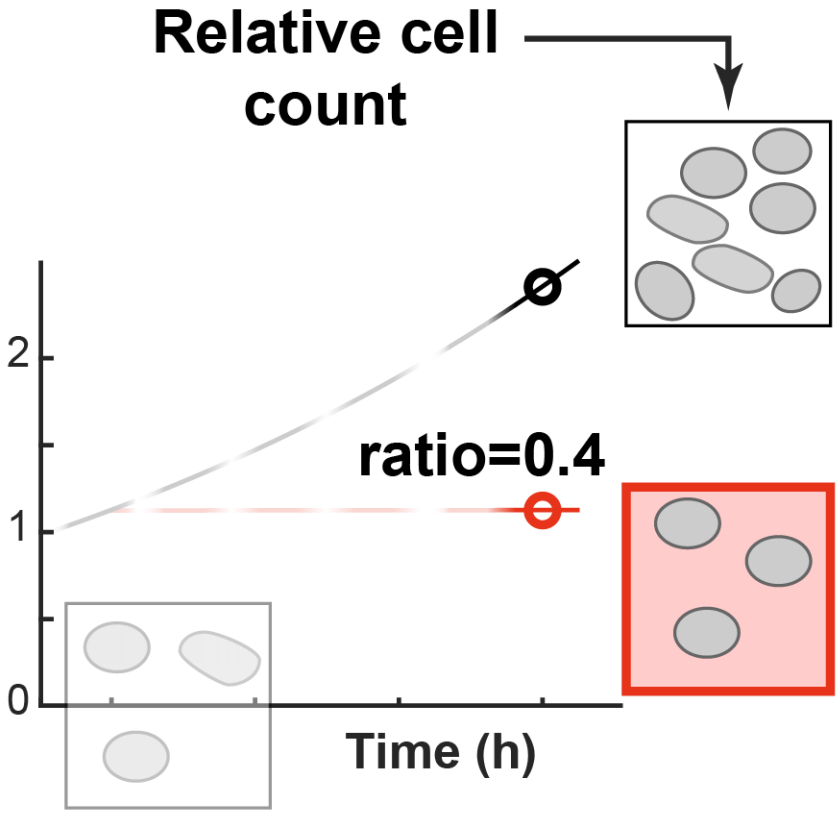
Dose response curves vary across cell lines

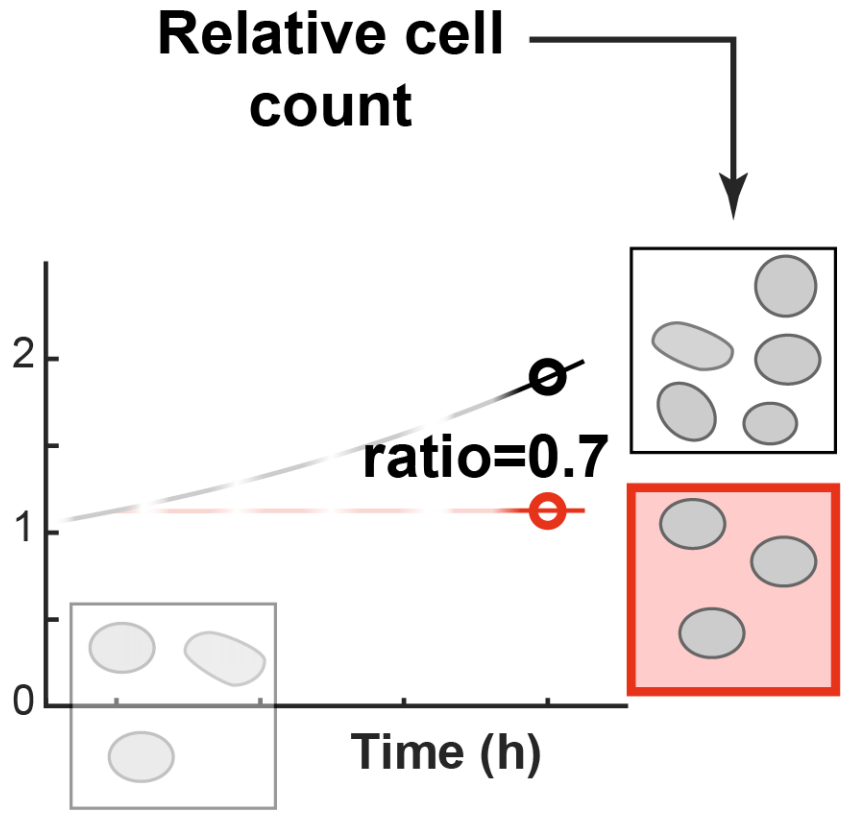


Assays that have a growing population

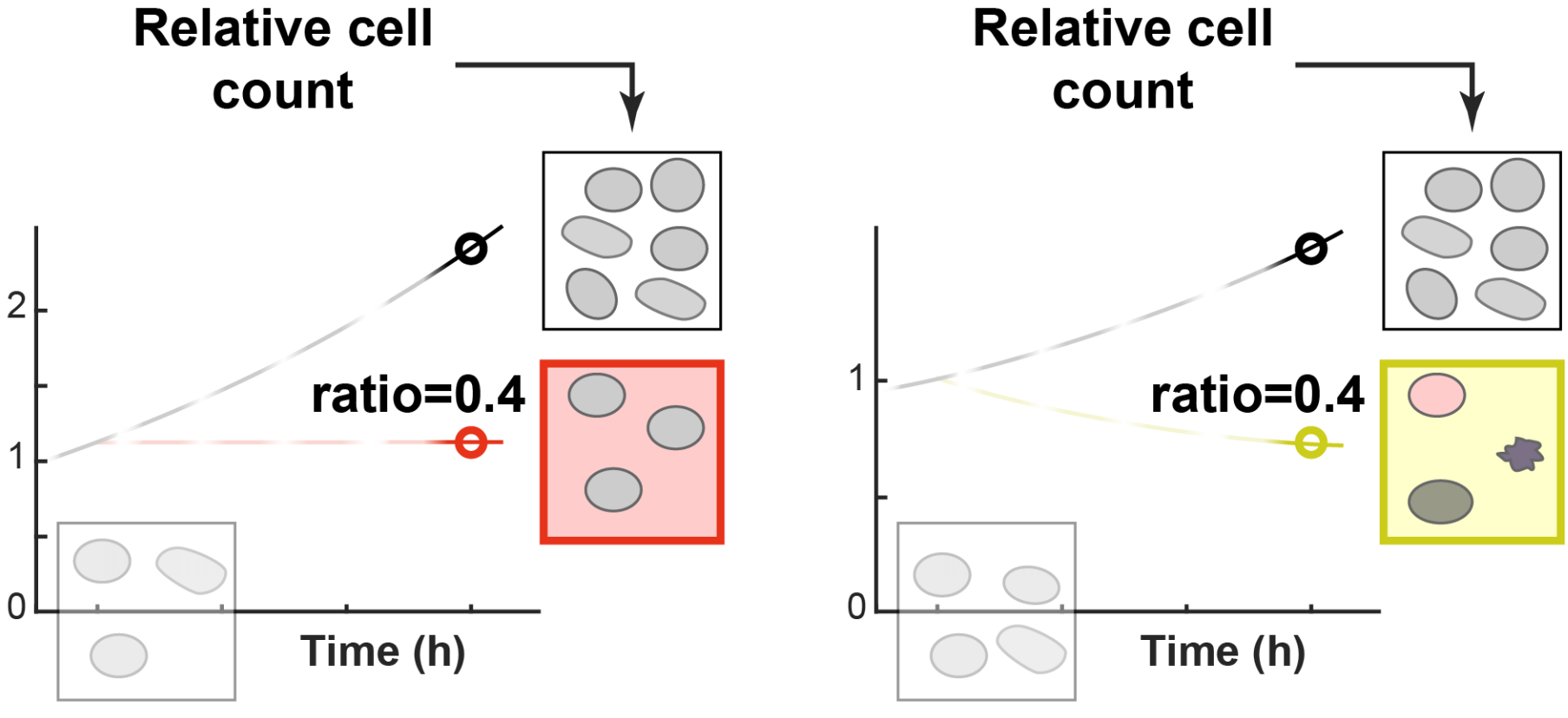


Relative cell count is biased by division rate

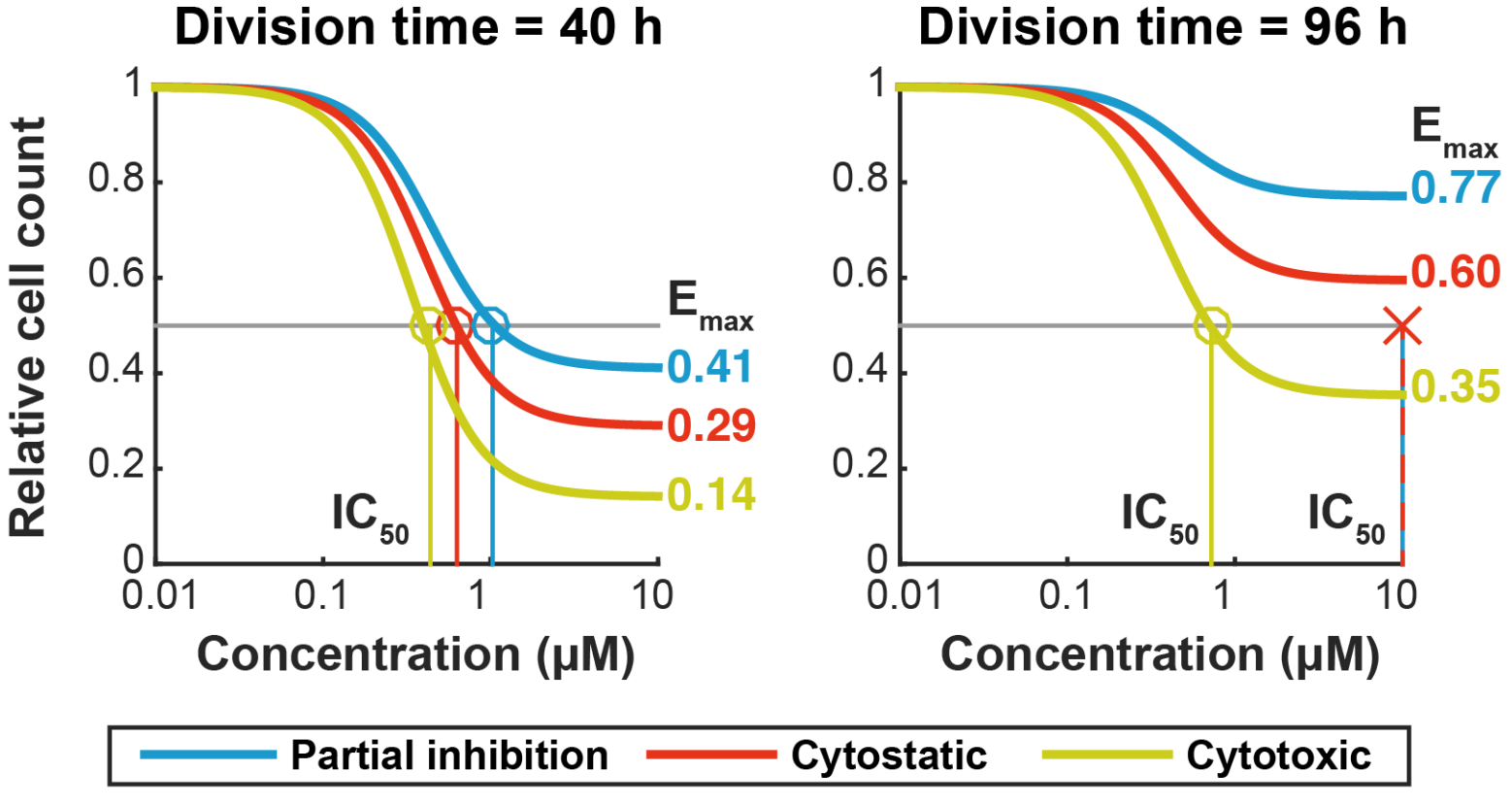




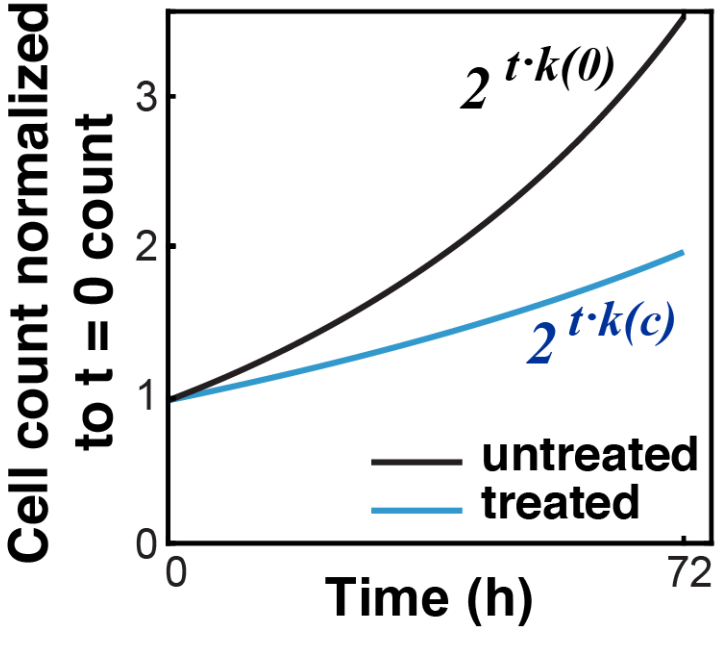
Relative cell count does not distinguish underlying phenotypes



Slow growing cell lines look systematically more resistant when using relative cell count



Normalized growth rate inhibition (GR) value



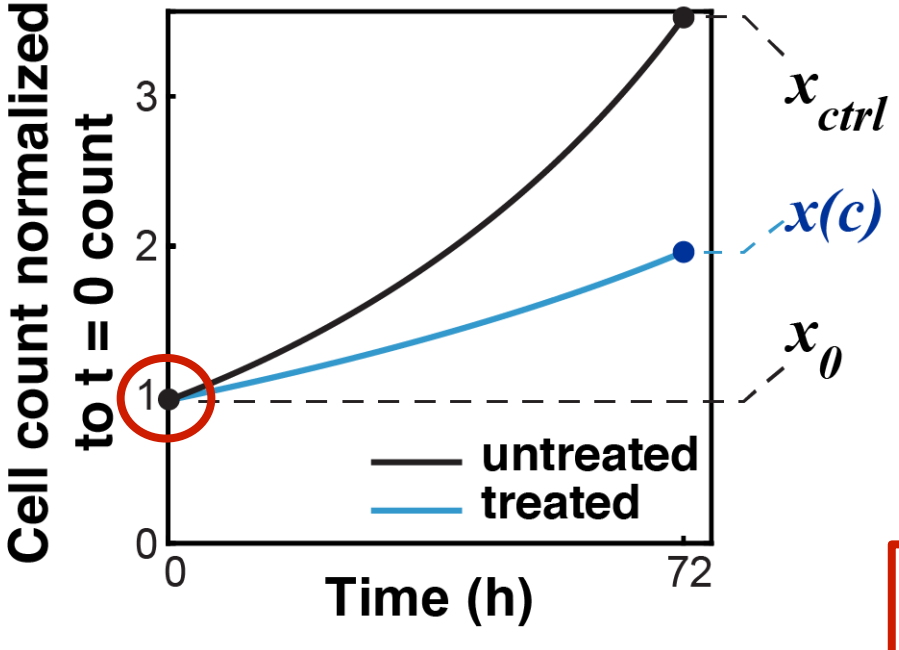
$$GR(c) = 2^{k(c)/k(0)} - 1$$

k(c) is the treated growth rate

k(0) is the control growth rate

Marc Hafner

GR values rely on three measures of cell count



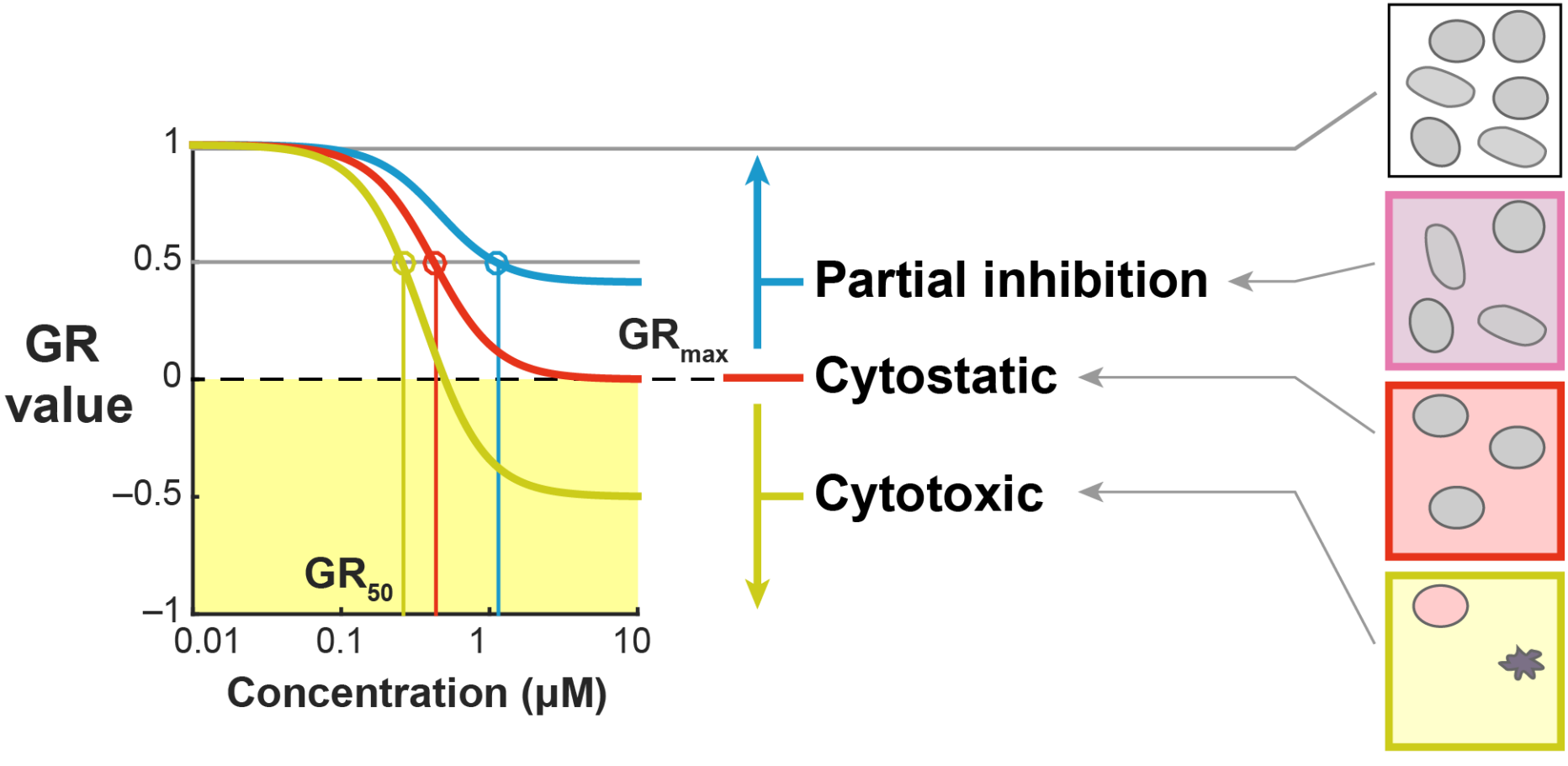
$$GR(c) = 2^{\frac{\log_2(x(c)/x_0)}{\log_2(x_{ctrl}/x_0)}} - 1$$

$x(c)$ is the treated cell count

x_{ctrl} is the control cell count

x_0 is the cell count at the time of treatment

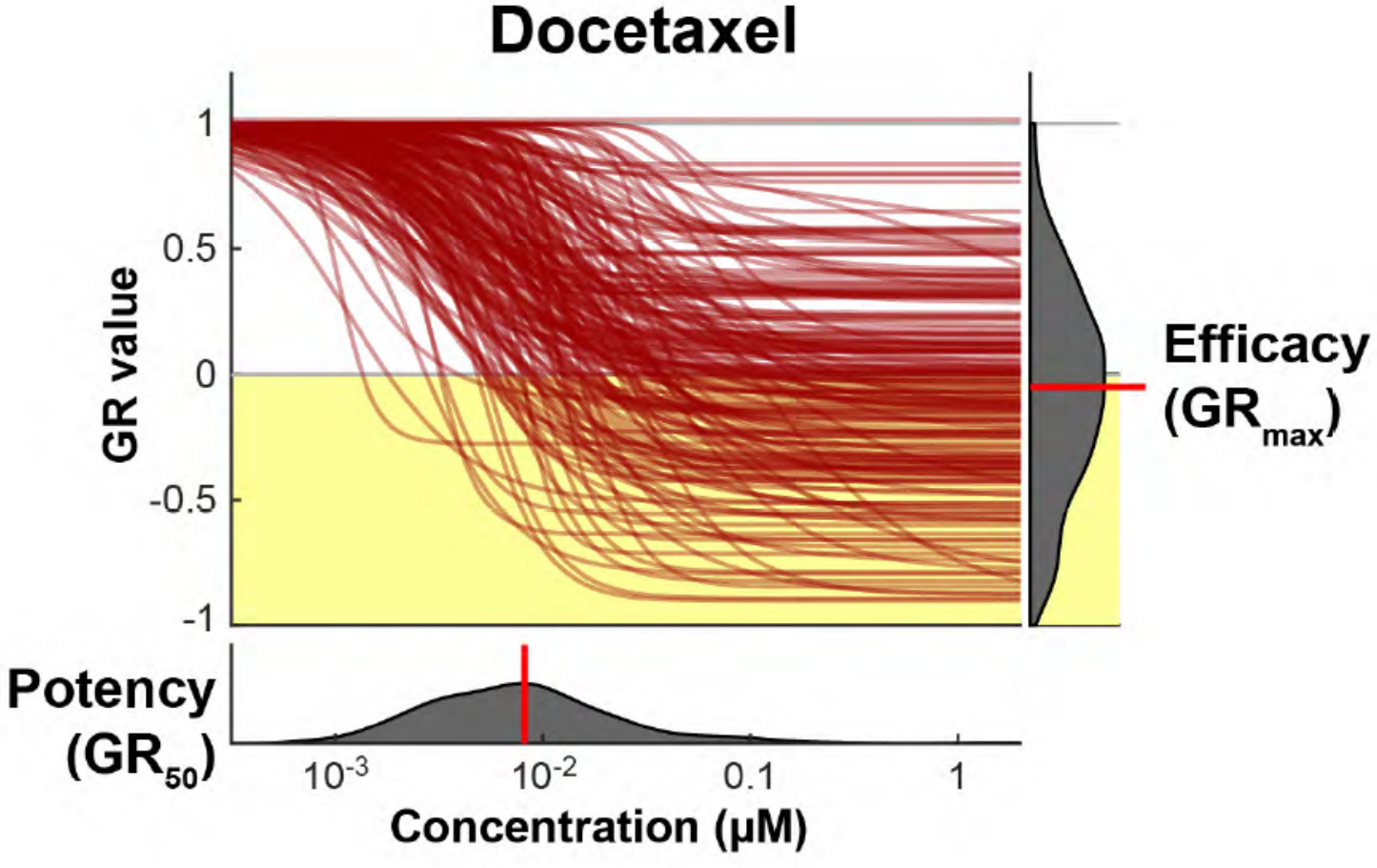
GR values are independent of the division rate and directly relate to the phenotype



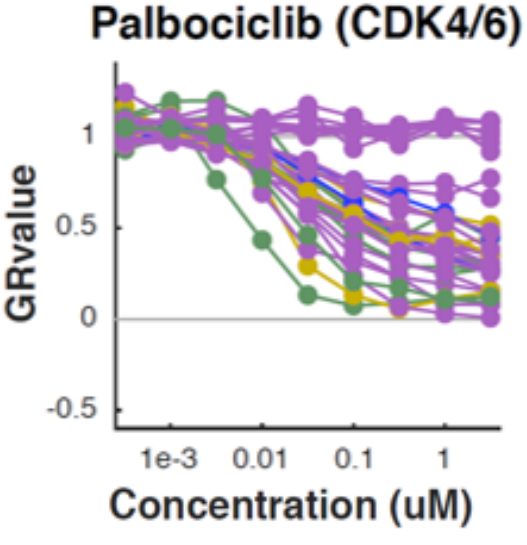
GR metrics:

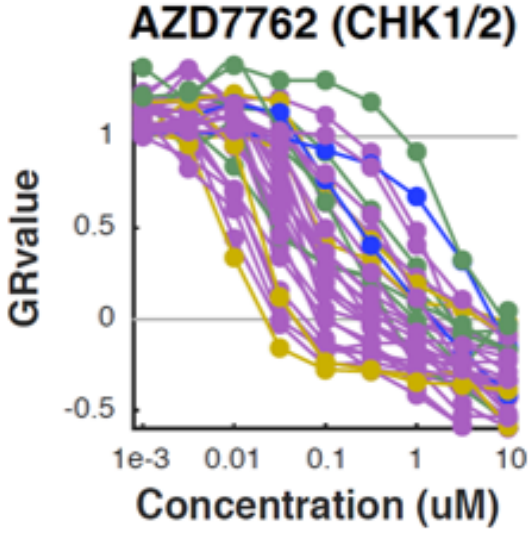
GR_{50}, GR_{max} and GR_{AOC} substitute for IC_{50}, E_{max} and AUC

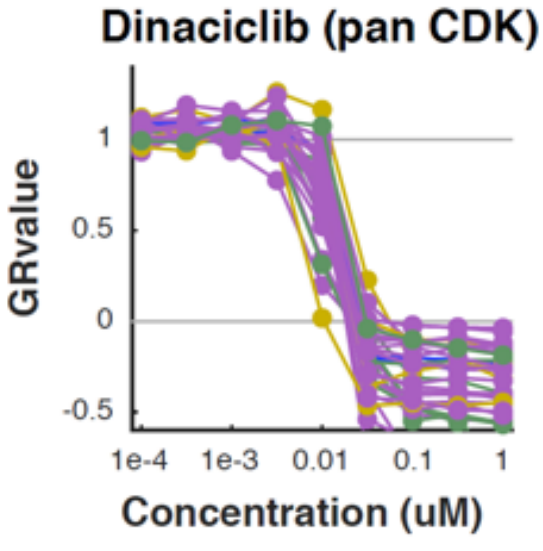
GR metric allows for an intuitive assessment of phenotypic effects across cell lines and drugs



GR metric allows for an intuitive assessment of phenotypic effects across cell lines and drugs





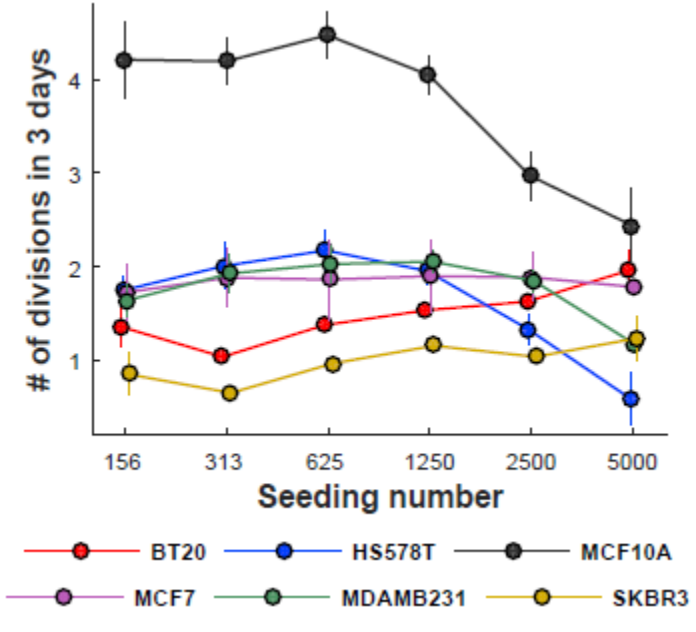


34 breast cancer cell lines

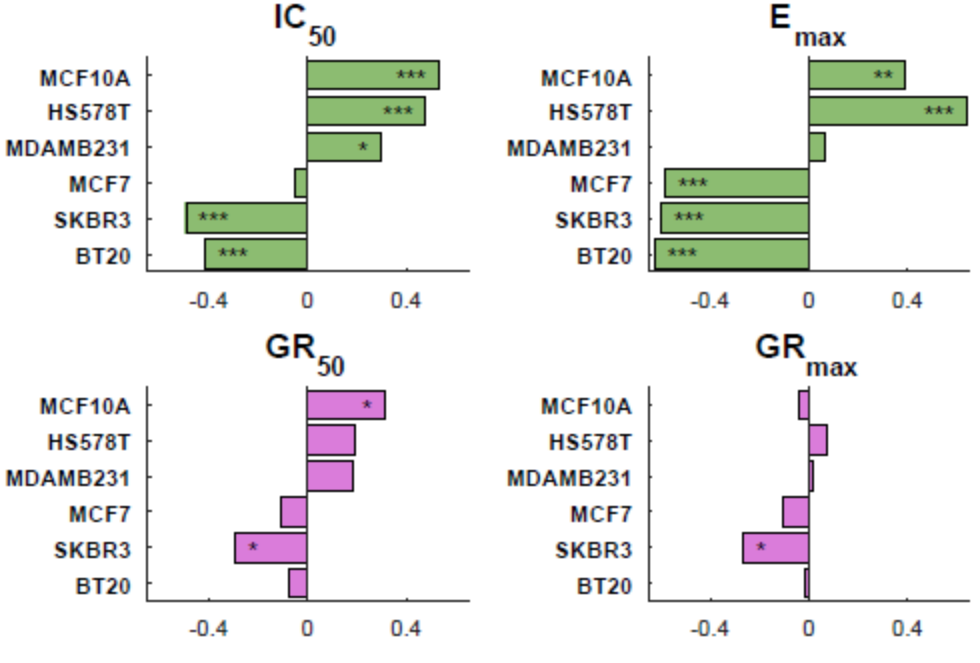
Cell seeding affects division time which biases traditional sensitivity metrics

Seeding density affects the number of divisions.

→ IC_{50} and E_{max} are correlated with density.

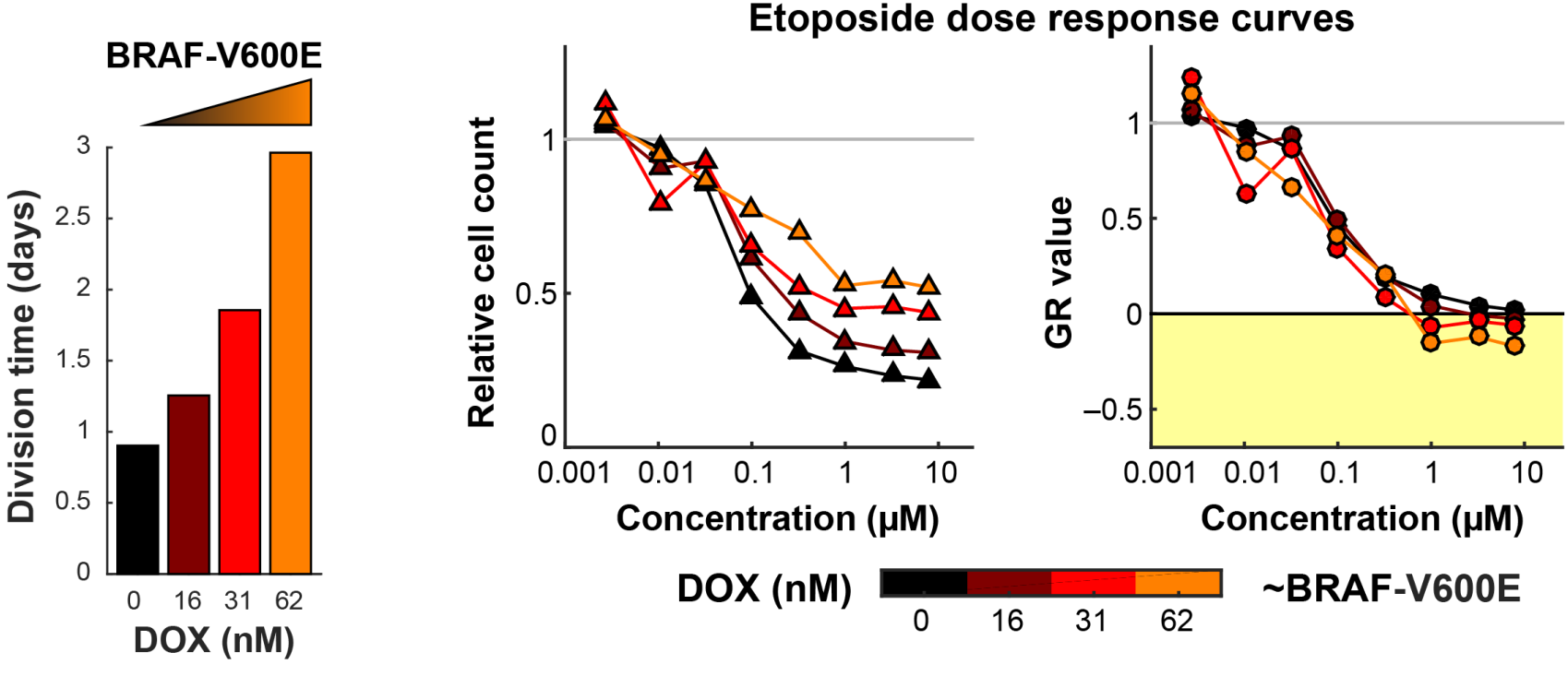


Spearman's correlation with seeding number (11 drugs)



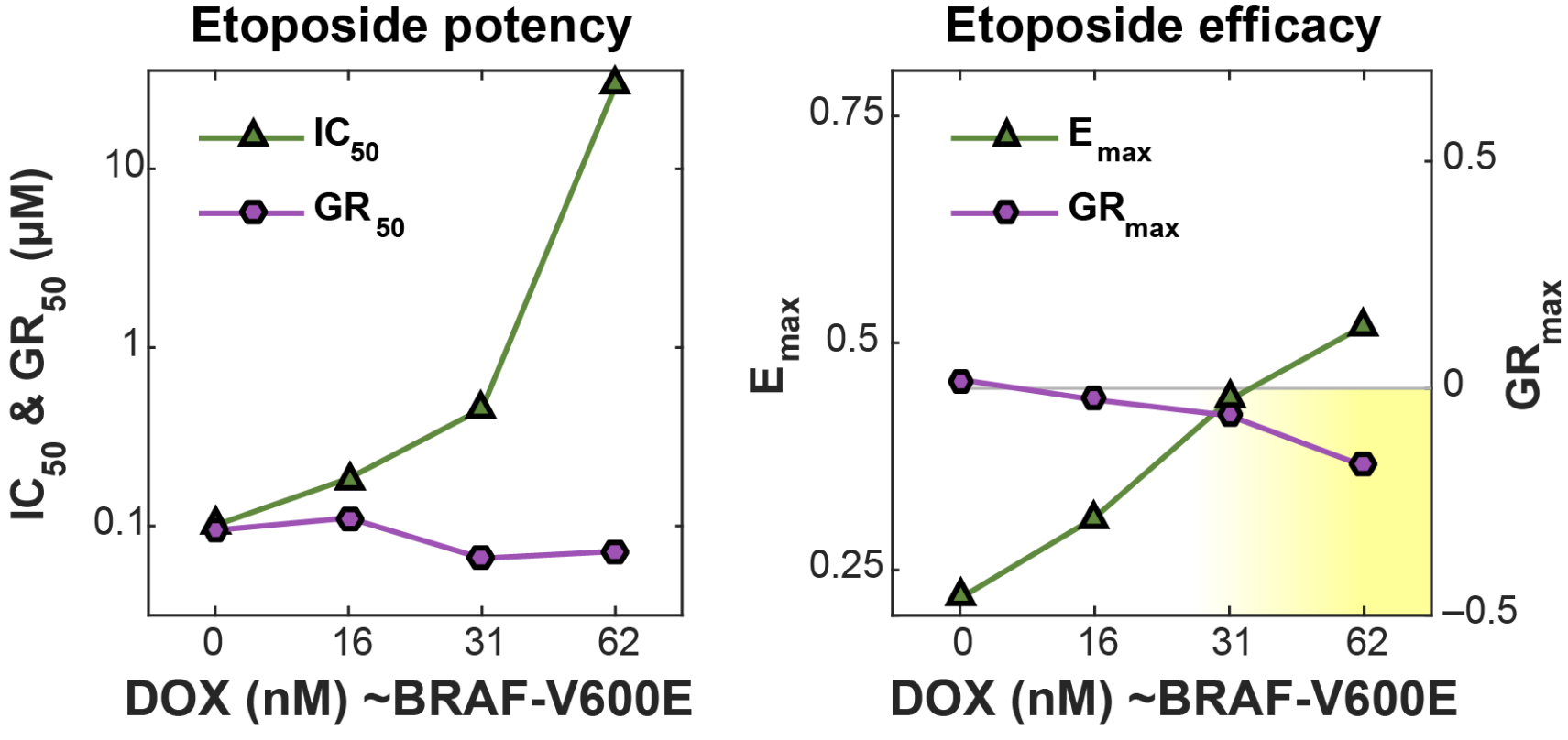
Genetic alterations affect division time which biases traditional sensitivity metrics

Etoposide sensitivity in HME RPE-1 cells with inducible $BRAF^{V600E}$ expression.



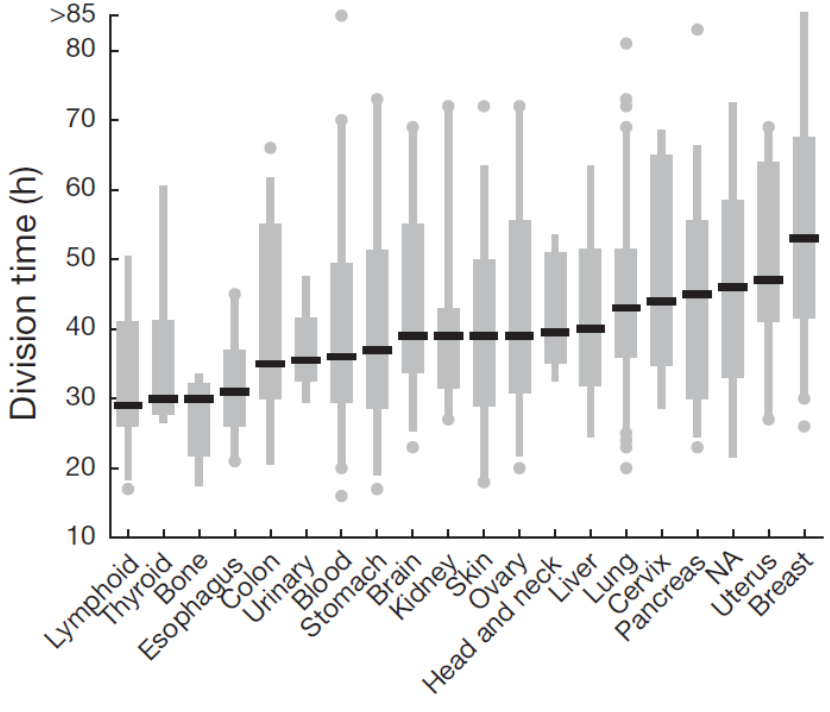
Thanks to Jia-Yun Chen for the cell line

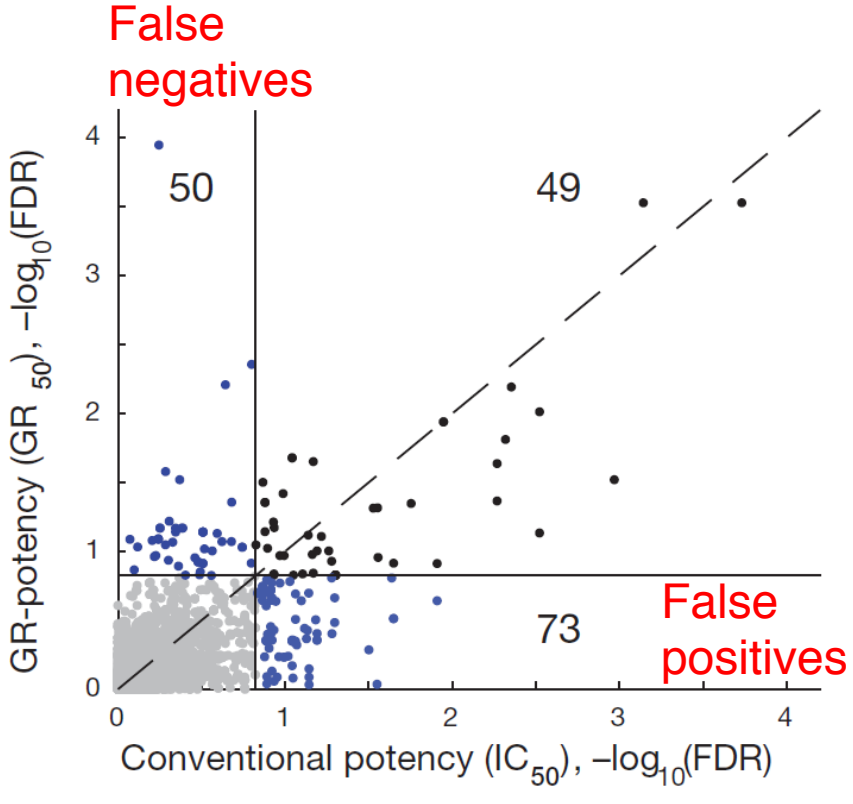
Genetic alterations affect division time which biases traditional sensitivity metrics



GR metrics correct growth rate confounders in pharmacogenomics and reveal true associations

Re-Analysis using GR metric of Genentech Cell Line Screening Initiative (409 cell lines and 16 drugs):





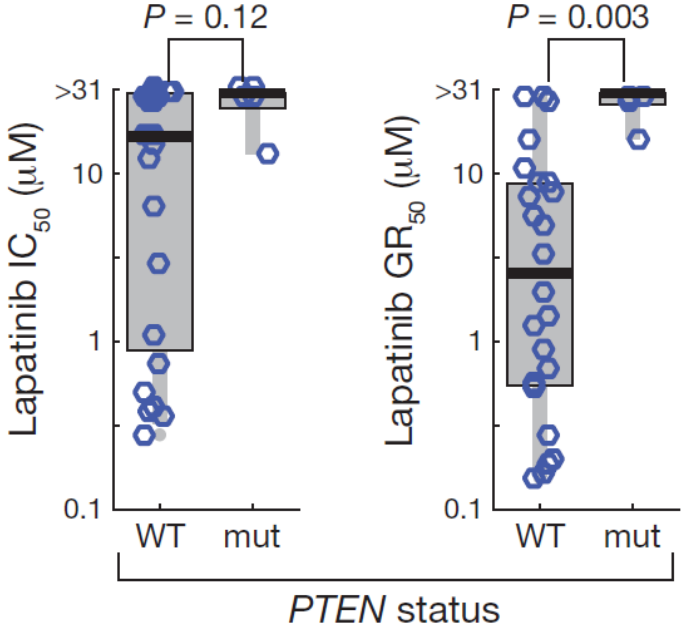
VOLUME 35 NUMBER 6 JUNE 2017 NATURE BIOTECHNOLOGY

Alternative drug sensitivity metrics improve preclinical cancer pharmacogenomics

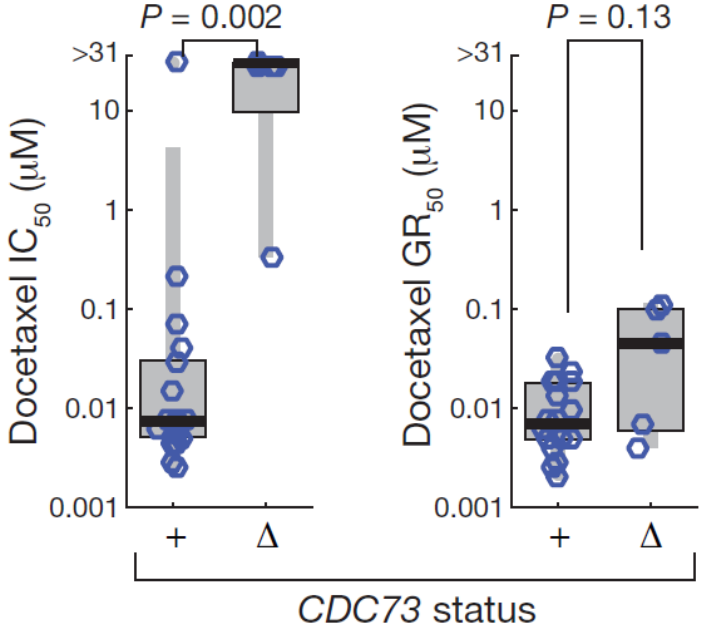
Marc Hafner, Mario Niepel & Peter K Sorger

GR metrics correct growth rate confounders in pharmacogenomics and reveal true associations

False negative example:
PTEN mutants ARE insensitive to Lapatinib



False positive example:
ΔCDC73 are NOT sensitive Docetaxel



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Alternative drug sensitivity metrics improve preclinical cancer pharmacogenomics

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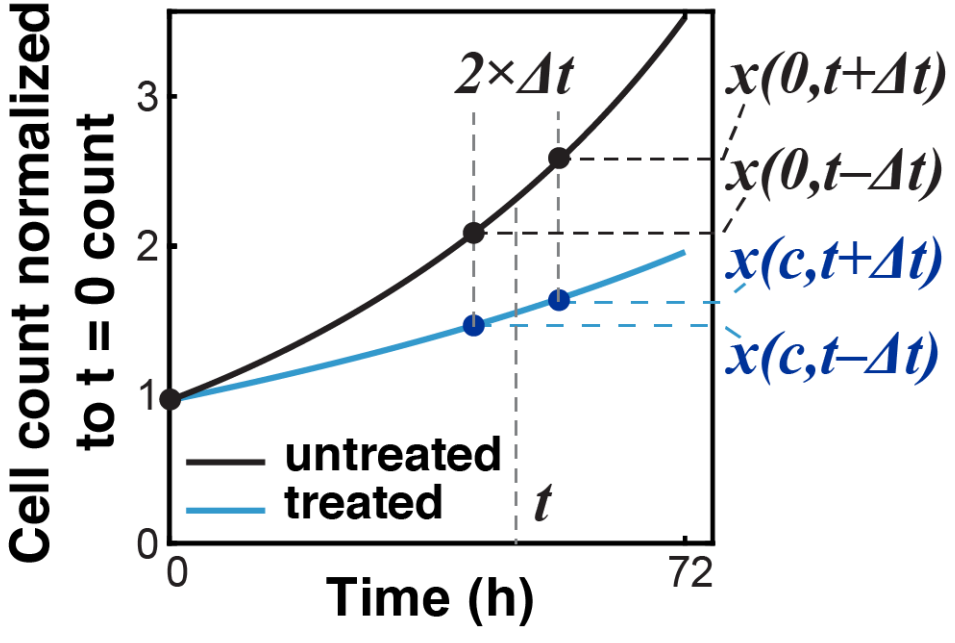
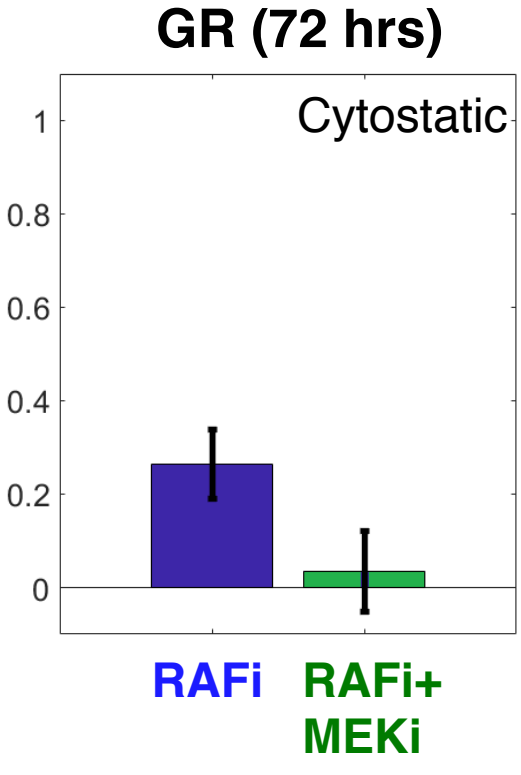
Time-dependent GR metrics

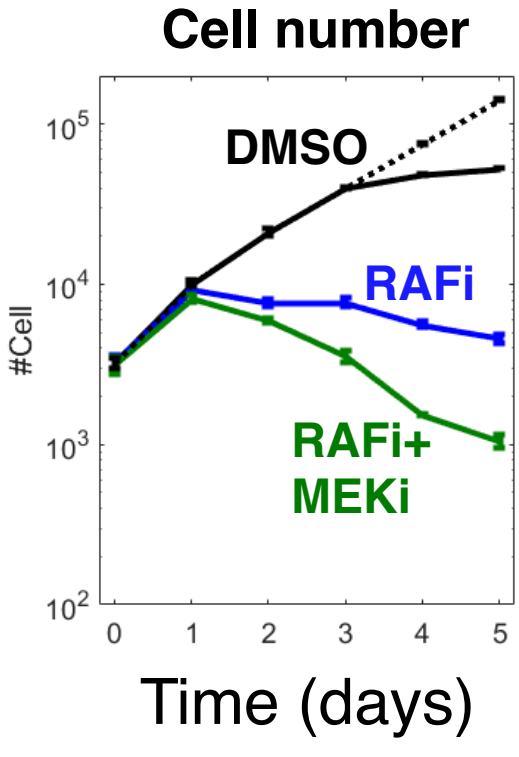
$$GR(c,t) = 2^{\frac{\log_2(x(c,t+\Delta t/2)/x(c,t-\Delta t/2))}{\log_2(x(0,t+\Delta t/2)/x(0,t-\Delta t/2))}} - 1$$

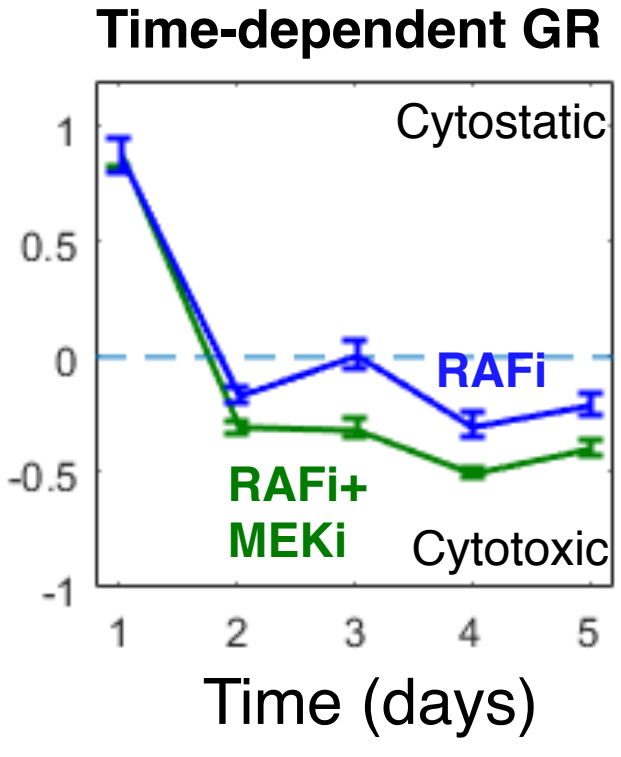


For evaluating $GR_{50}(t)$ and $GR_{max}(t)$ and quantifying adaptive response or late drug action.

Time-dependent GR can reveals dynamic changes in drug-response effects

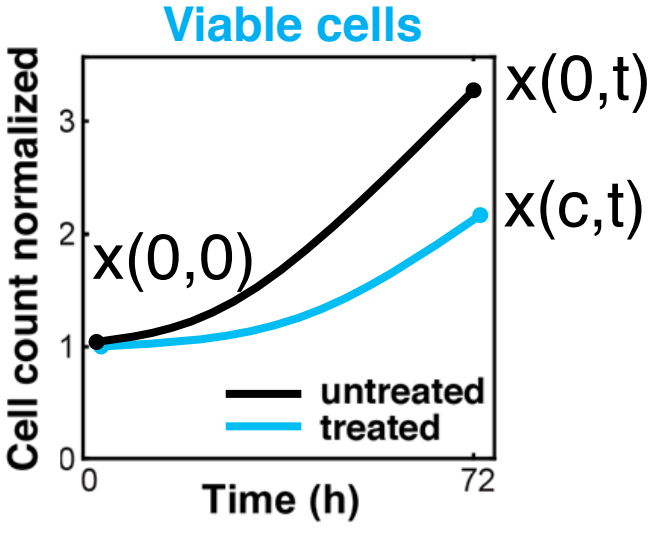


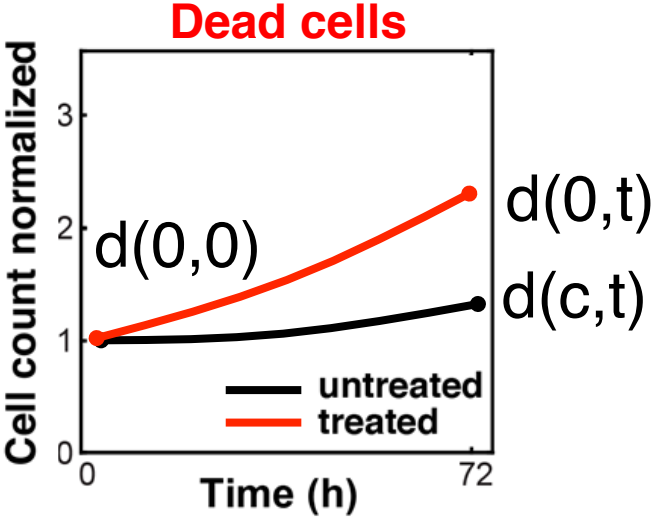




$BRAF^{V600E}$ melanoma cell line A375

Decoupling cytostatic and cytotoxic drug-responses by GR metrics





Normalized growth rate:

$$\frac{k_S(c)}{k_S(0)} = \left(1 + \frac{d(c,t) - d_0}{x(c,t) - x_0}\right) \cdot \ln\left(\frac{x(c,t)}{x(0,0)}\right) \Big/ \ln\left(\frac{x(0,t)}{x(0,0)}\right)$$

Normalized death rate:

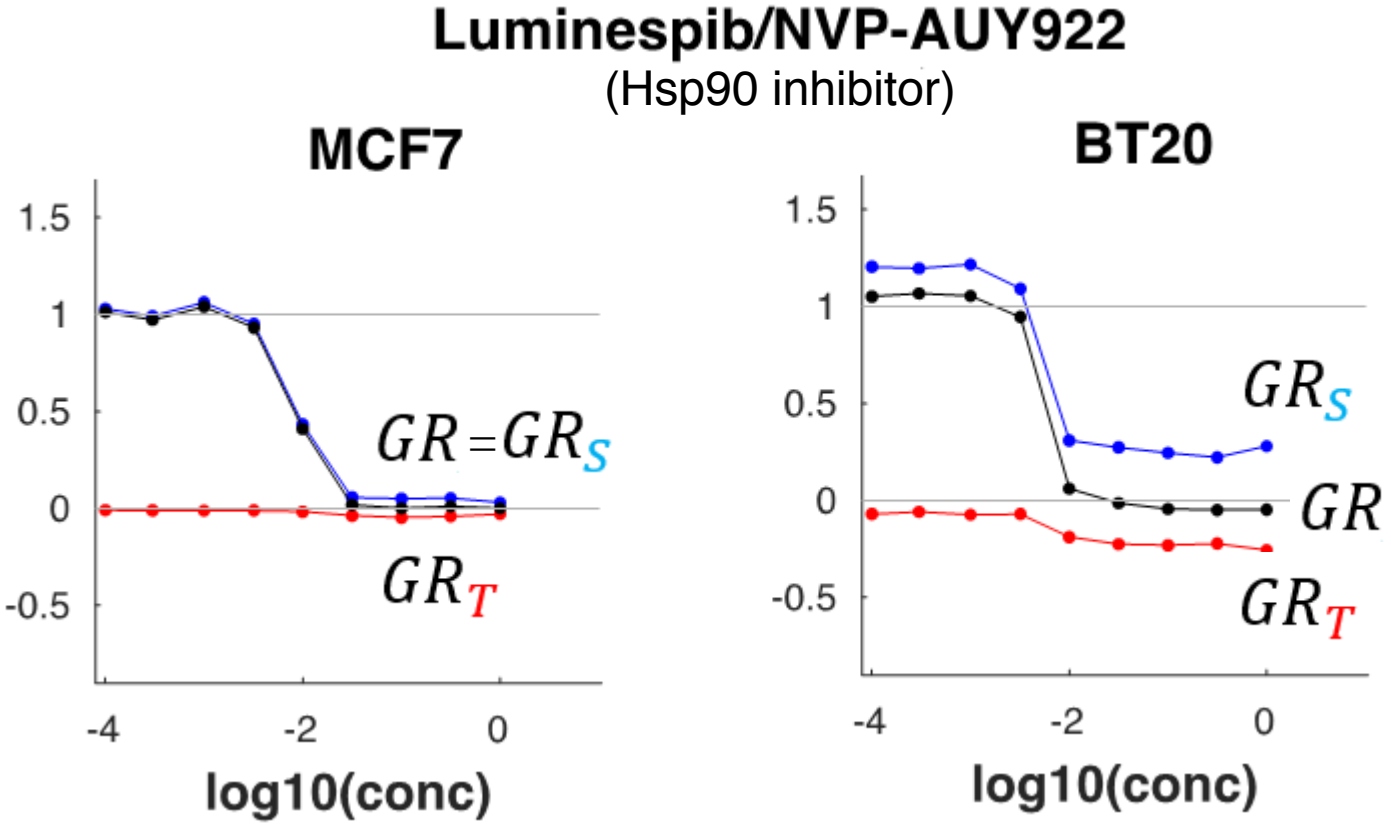
$$\frac{k_T(c)}{k_S(0)} = -\left(\frac{d(c,t) - d_0}{x(c,t) - x_0}\right) \cdot \ln\left(\frac{x(c,t)}{x(0,0)}\right) \Big/ \ln\left(\frac{x(0,t)}{x(0,0)}\right)$$

Decoupled GR metric:

$$GR_S = 2^{\frac{k_S(c)}{k_S(0)}} - 1 \qquad GR_T = 2^{\frac{k_T(c)}{k_S(0)}} - 1$$

$$GR = GR_S + GR_T + GR_S \cdot GR_T$$

Similar drug responses can be due to different combinations of cell growth and death



Conclusions on GR metrics as analytical tools for reproducible drug-dose responses

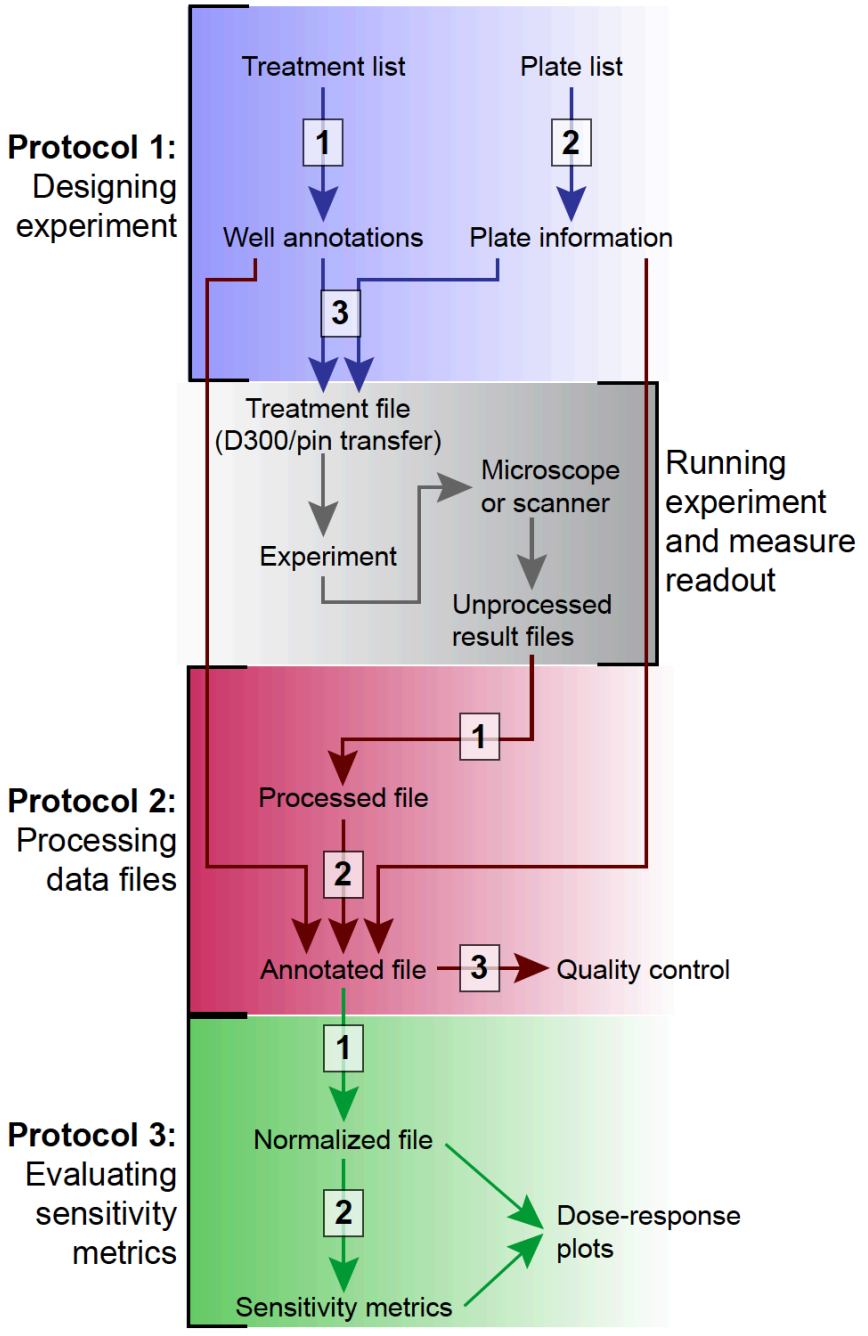
GR metrics...

- ... eliminate confounders that act by cell division bias (cell seeding, genetic background, etc...)
- ... can be extended to quantify time-dependent drug-response and to decouple cytostatic and cytotoxic effects
- .. improve reproducibility in studies that rely on measuring growth inhibition, such as in pharmacogenomics

Optimized Experimental and Analytical Tools for Reproducible Drug-Response Studies

Experimental pipeline

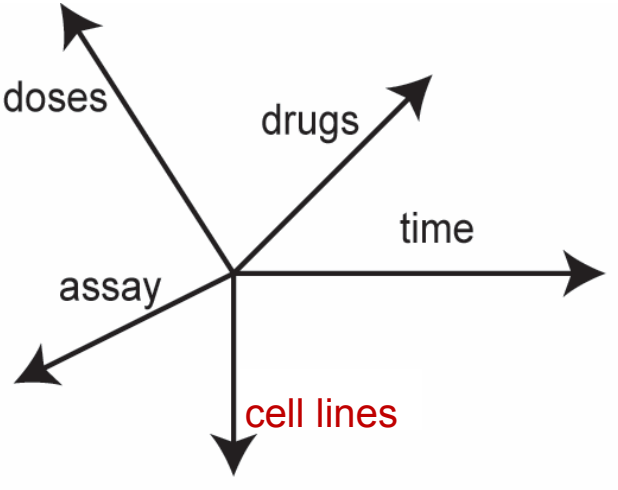
1. Designing experiment
2. Running experiment
3. Processing data files
4. Evaluating sensitivity metrics



Hafner*, Niepel*, Subramanian*, Sorger
Curr Protoc Chem Biol, 2018

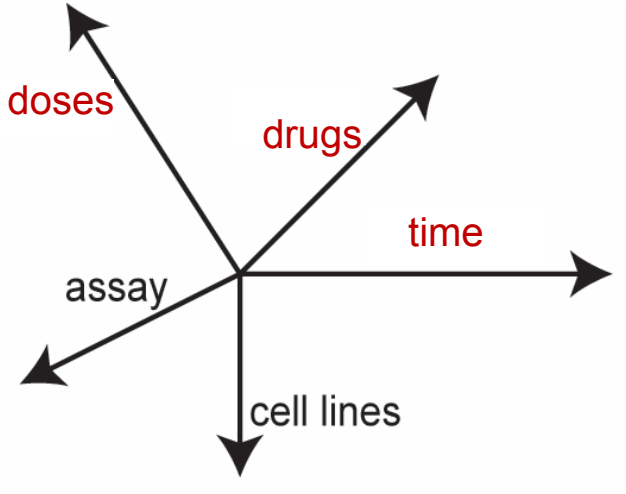
To consider *before* you start: cell lines

- How many cell lines do I want to test?
- Are they amenable to imaging?
- Are they adherent?
- Do they grow in a monolayer?
- How densely should they be seeded?

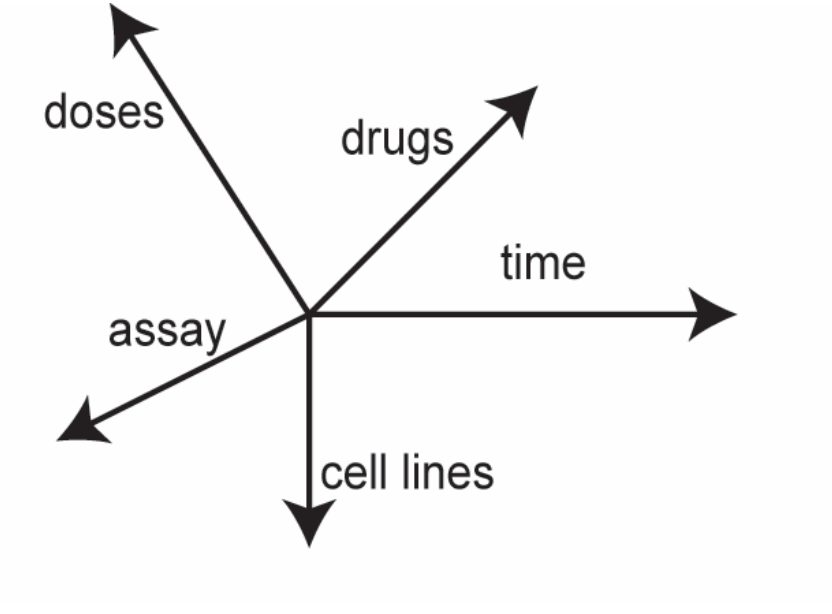


To consider *before* you start: drugs

- How many drugs do I want to collect dose response data for?
- Are they DMSO soluble?
- How many dose points do I need?
- What's an appropriate dose range?
- How many time points do I want to test?
- How long should the assay run?
- What are the expected effects of drug treatment?



To consider *before* you start



96 or 384 well plates

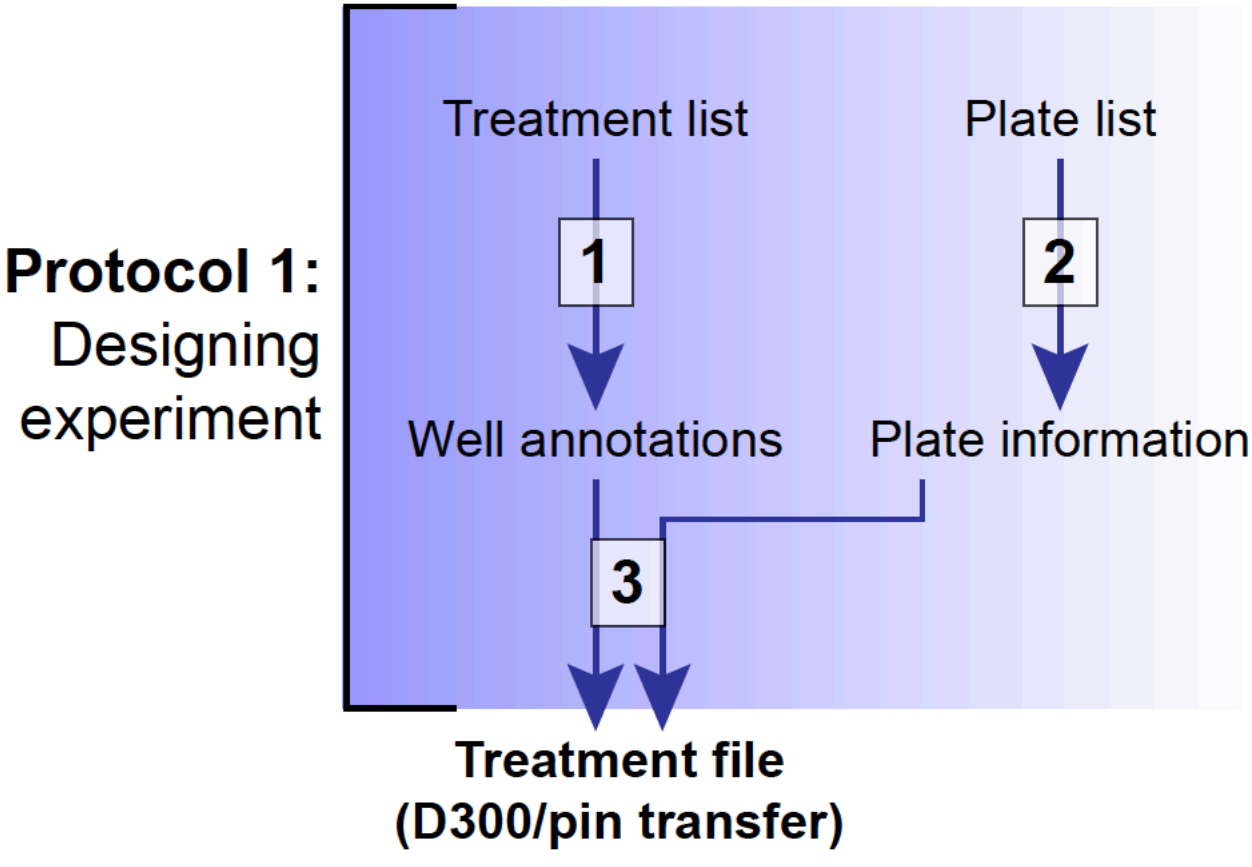
Do I need to use the GR approach?

It depends on how you answered the previous questions.

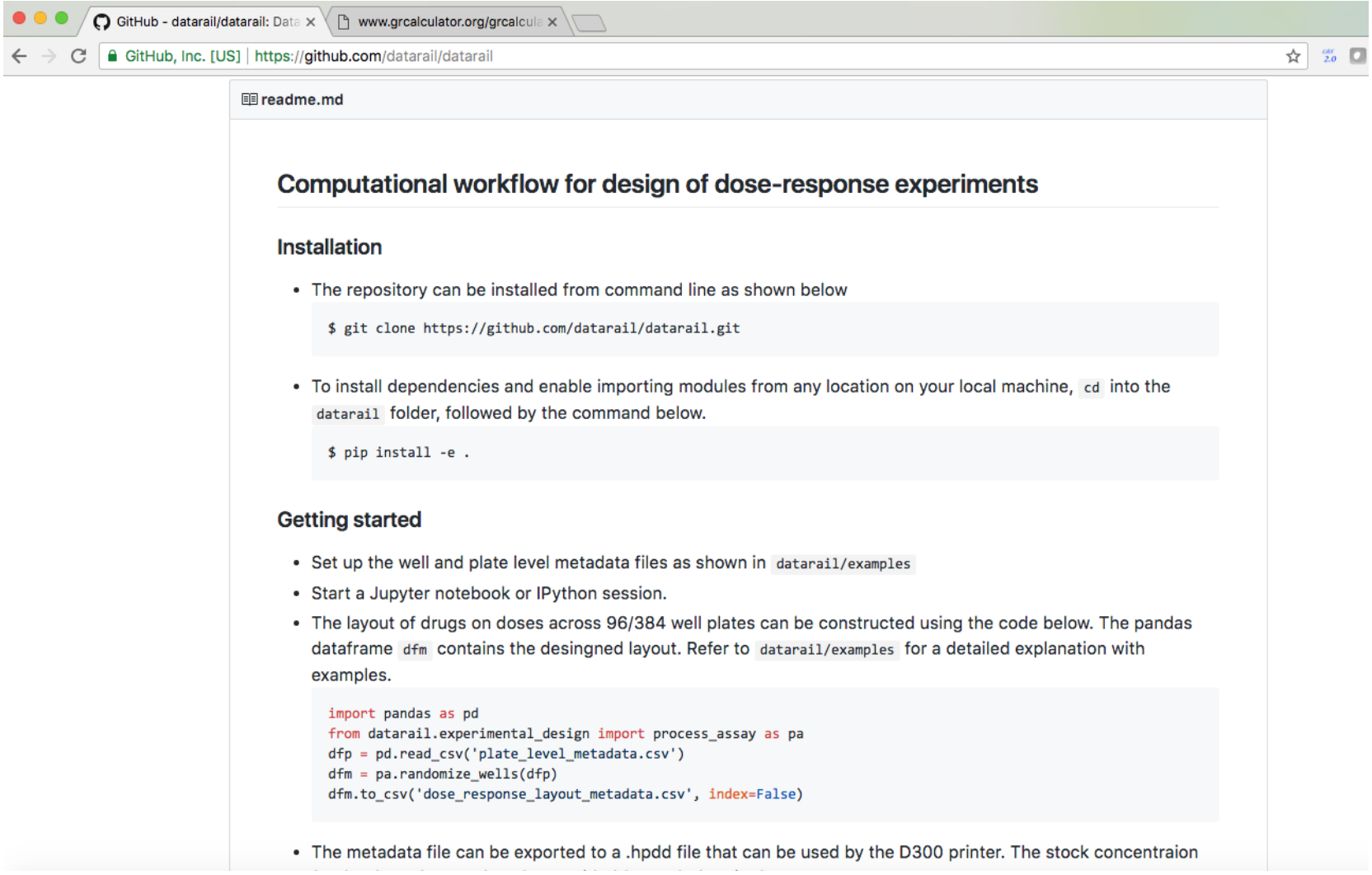
Relative cell counts are valid when the untreated controls do not change:

- Phenotype is not related to cell growth
- Untreated cells do not grow
- Short assays during which growth is negligible

Experimental design



Design scripts available at github.com/datarail/datarail



smkartik included relevant lines of code and documentation to export design to... d928a53 4 days ago

1 contributor

The example script below demonstrates how a randomized plate layout can be generated from an initial description (see input_file.csv) of the experiment

In [ ]:
```
import pandas as pd
from datarail.experimental_design import process_assay as pa
from datarail.experimental_design import plot_plate_layout as ppl
from datarail.experimental_design import hpdd_utils as hu
%matplotlib tk
```

The input file (see **`input_file.csv`**) should be broad description of the drugs and their concentrations and should contain the following columns

> **agent** : lists the names of drugs. Combinations are to specified as comma seperated strings. For example - 'agent1, agent2'
> **max_dose__um** : lists the highest dose for each agent.
> **num_doses** : lists the number of doses for each agent.
> **role** : lists the intended role for each agent **`'treatment', or 'positive_control'`**
> **num_replicates** : lists number of times the dosing schme of a drug (or a combination) is replicated on the same plate.
> **equivalent** : 0 if the combination should comprise of the full cartesian product. 1 if only equivalent doses make up the combination

The plate level file (see **`plate_id.csv`**) should provide a description of plate level metadata. It should contain the following columns

> **barcode**: list of barcodes (plate identifiers)
> **cell_line**: name of cell lines in each plate (comma seperated names)
> **timepoint**: time point corresponding to each plate. Set to **`time0_ctrl`** for plate that should be used as

Branch: master ▾ datarail / datarail / examples / create_plate_layout.ipynb Find file Copy path

smkartik included relevant lines of code and documentation to export design to... d928a53 4 days ago

1 contributor

145 lines (144 sloc) 4.53 KB Raw Blame History

In [ ]:
```
## Read in the plate level metadata file
dfp = pd.read_csv('plate_id.csv')
```

In [ ]:
```
# Construct and design file
dfr = pa.randomize_wells(dfp, exclude_outer=2)
dfr.to_csv('single_agent_design_layout.csv', index=False)
```

Metadata table

In [ ]:
```
## Plot and visualize layout of plates
ppl.plot_summary(dfr, 'single_agent_design_layout.pdf')
```

Treatment layout

In [ ]:
```
# Load file containing information on stock concentration for each agent
# Note that column names for the file should be 'name' and 'stock_concentration'.
# Stock concentration should be provided in micromolar.
dfs = pd.read_csv('stock_concentration.csv')
```

In [ ]:
```
# Export design specs to hpdd format
hu.export_hpdd(dfr, dfs, 'single_agent_design_layout.hpdd')
```

Treatment file

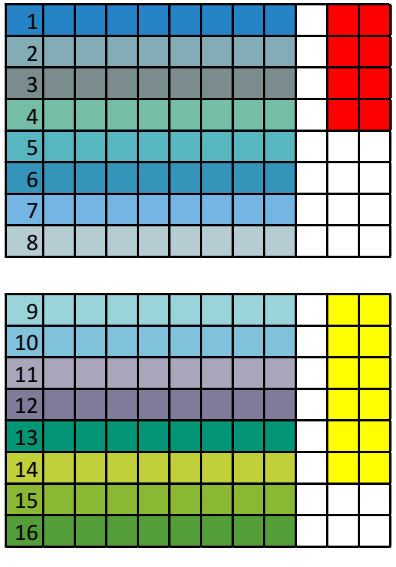
equivalent : 0 if the combination should comprise of the full cartesian product. 1 if only equivalent doses make up the combination

The plate level file (see `plate_id.csv`) should provide a description of plate level metadata. It should contain the following columns

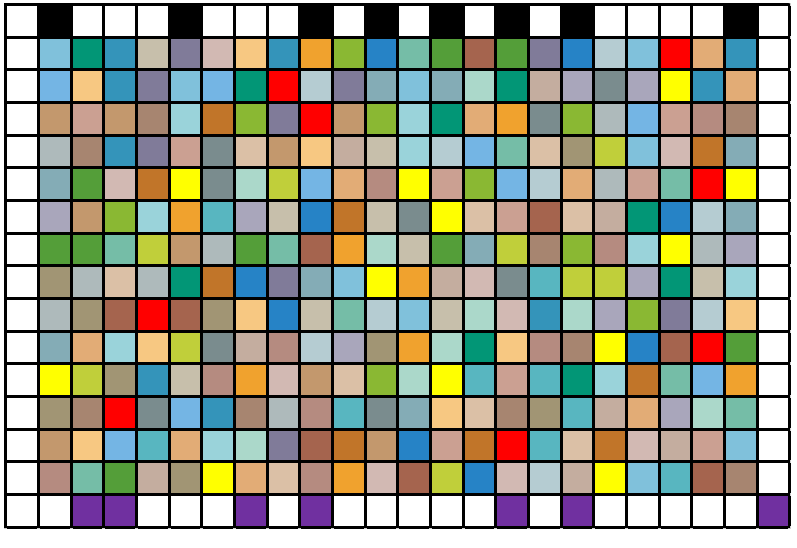
barcode: list of barcodes (plate identifiers)
cell_line: name of cell lines in each plate (comma seperated names)
timepoint: time point corresponding to each plate. Set to `time0_ctrl` for plate that should be used as

Set up library plates for pin transfer for large scale experiments

Manual layout of drugs on source plates

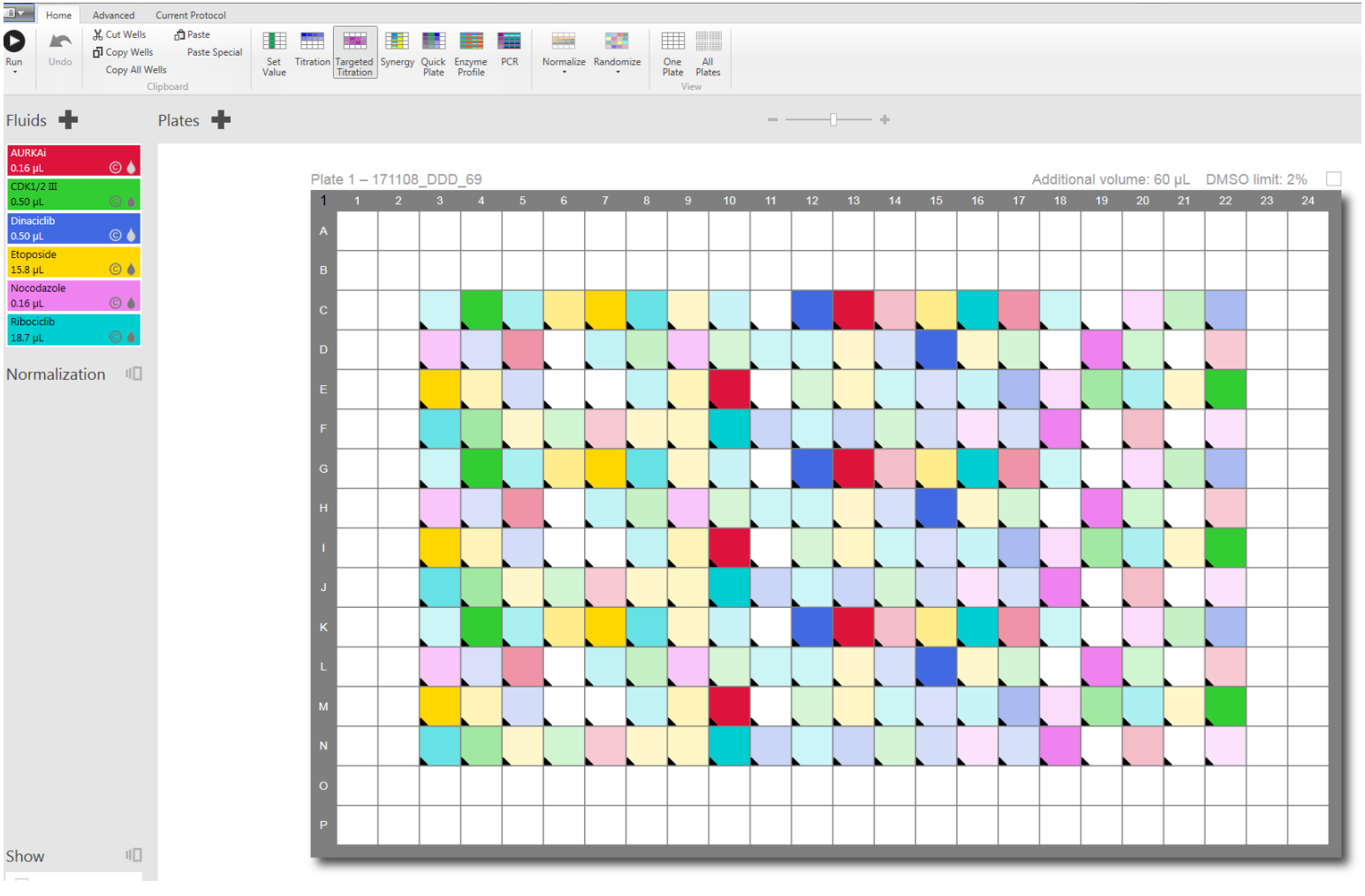


Randomized Library Plates



Use controls to ‘barcode’ library plates.

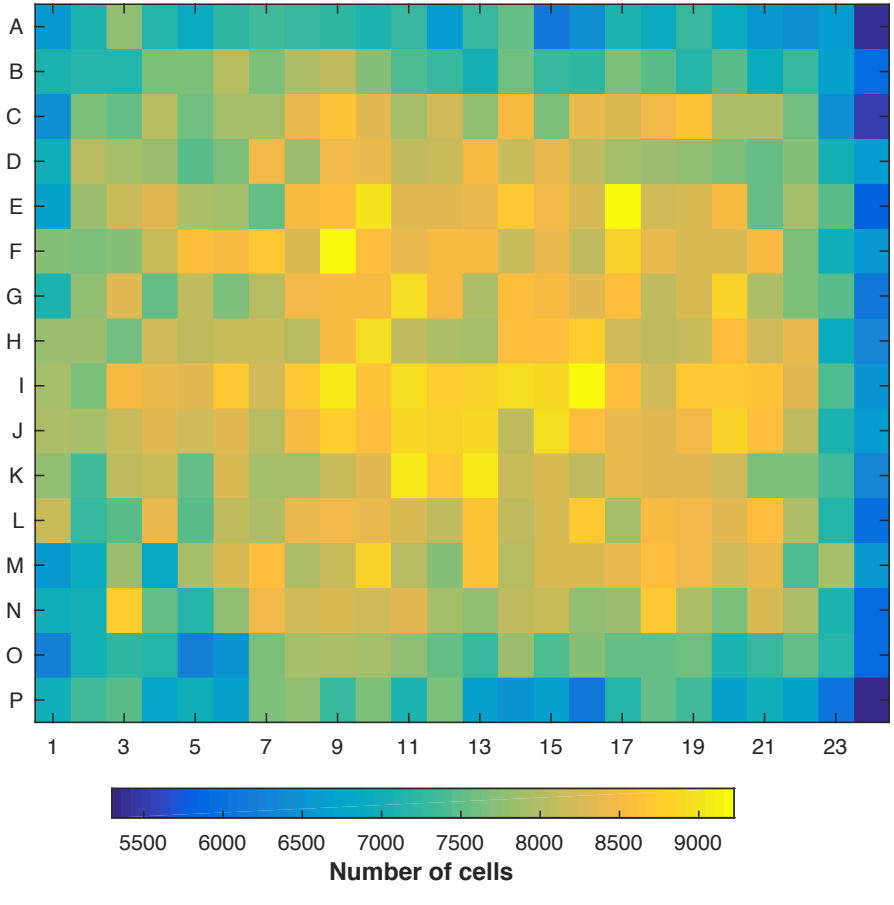
Use other automation for pilot, follow-up and smaller experiments

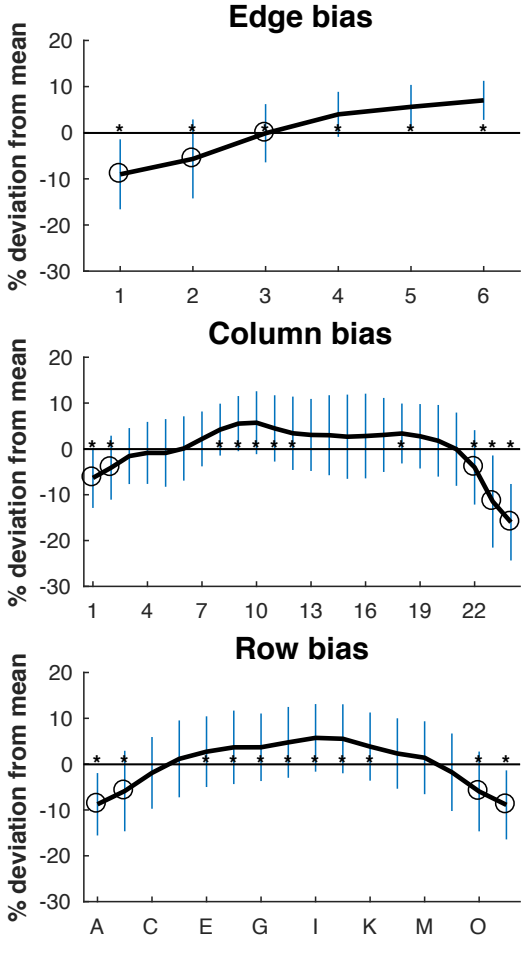


Design steps to improve reproducibility

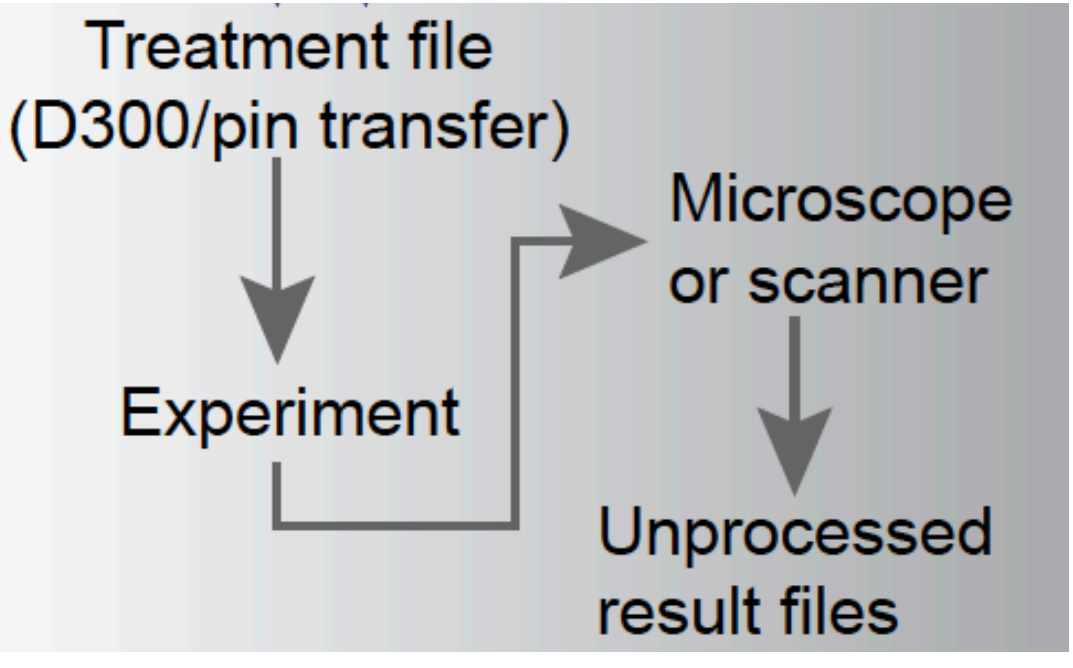
- Randomization of the treatments across multiple technical replicates
- Standardize nomenclature, barcode plates
- Control for plate bias (across day 0 plate; positive & negative controls across treatment plates)
- Robotic treatments with the D300 or pin transfer
- Exclude edge wells whenever possible

Randomization can mitigate edge effects





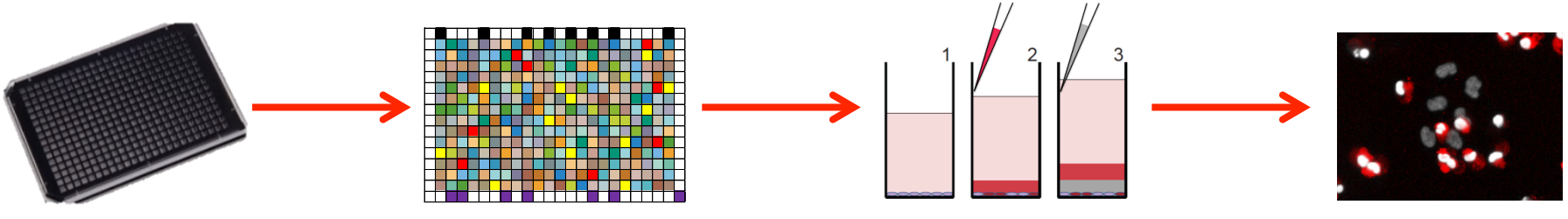
Running the experiment



Running experiment and measure readout

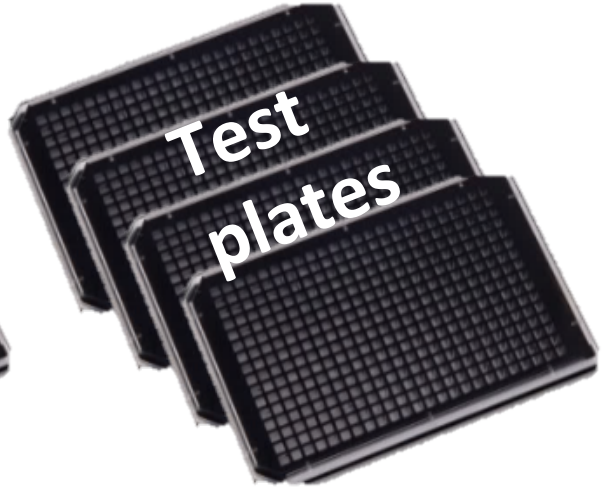
Experimental design is complete. Now what?

- Grow (happy) cells
- Seed cells at appropriate densities in multi-well plates
- Deliver drugs to multi-well plates
- Stain and fix cells
- Image cells
- Extract quantitative data from images

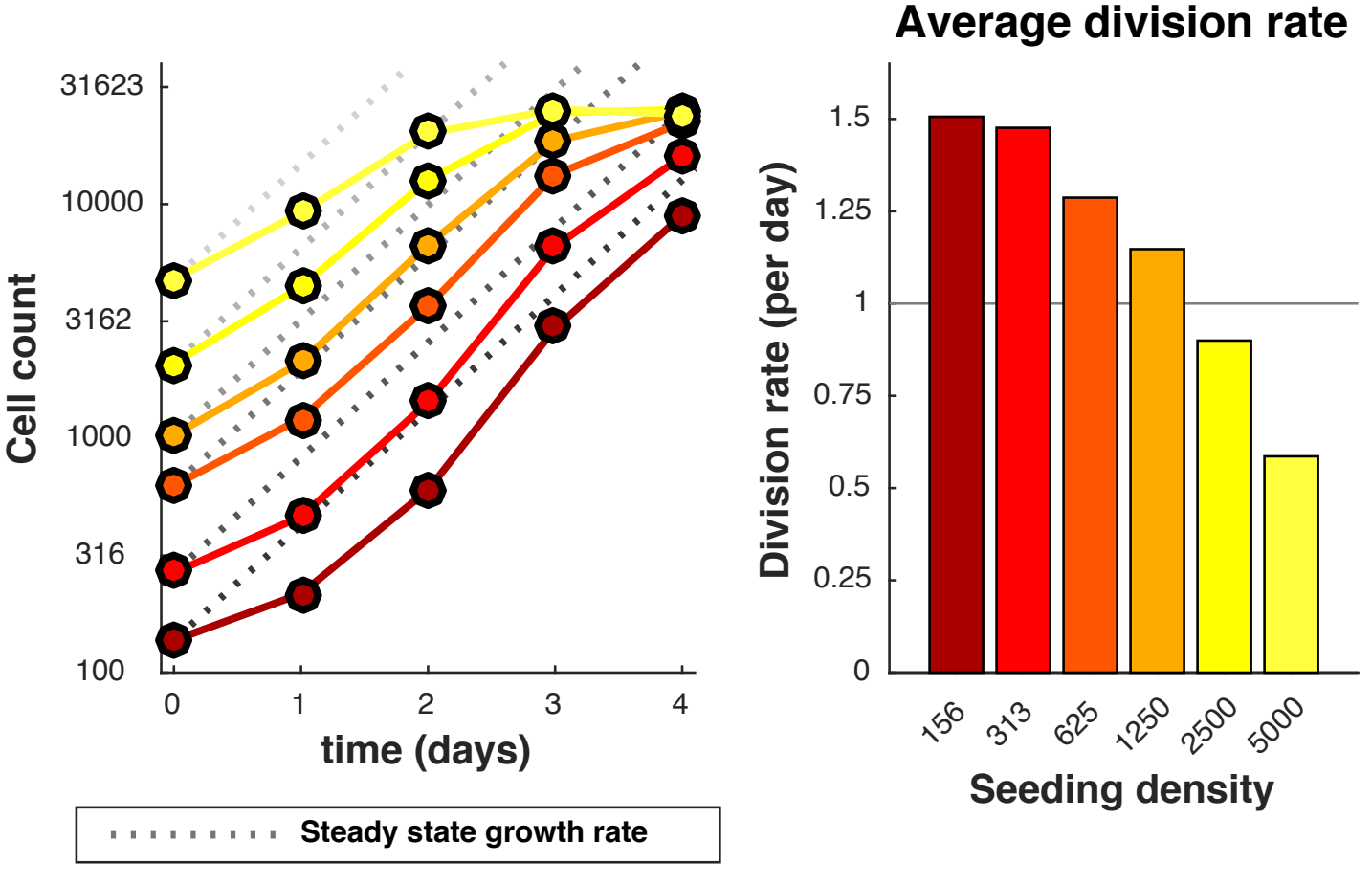


Cell seeding

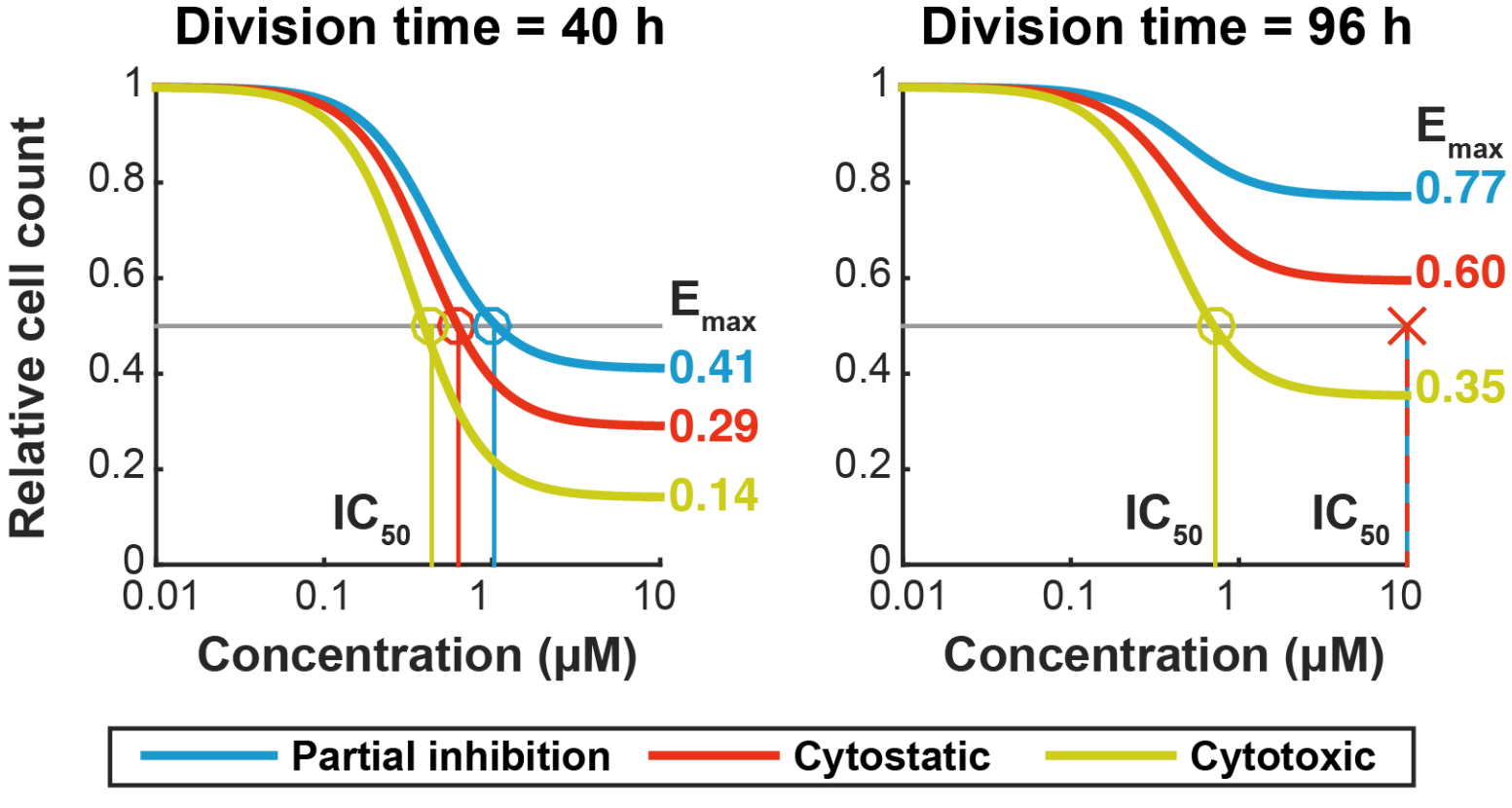
- Seed plates at an appropriate density from parent plates in log-phase growth
- Use automation if possible
- Barcode plates to keep track of them



Cell seeding density influences growth rate...

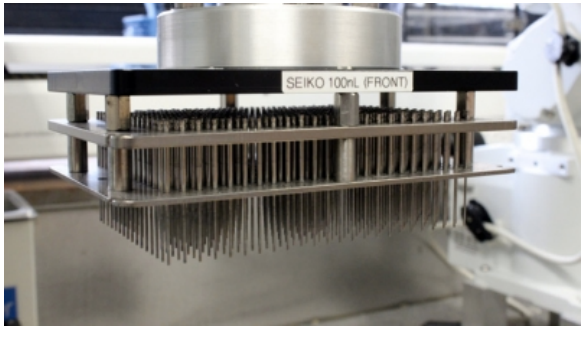


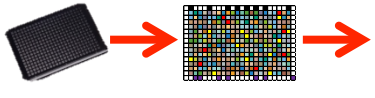
...which influences the dose response



Drug delivery via pin transfer

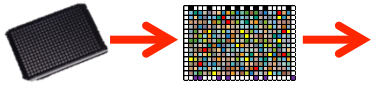
- For simultaneous delivery of many drugs
- For large scale experiments (many cell lines, conditions)
- Facilitates reproducibility



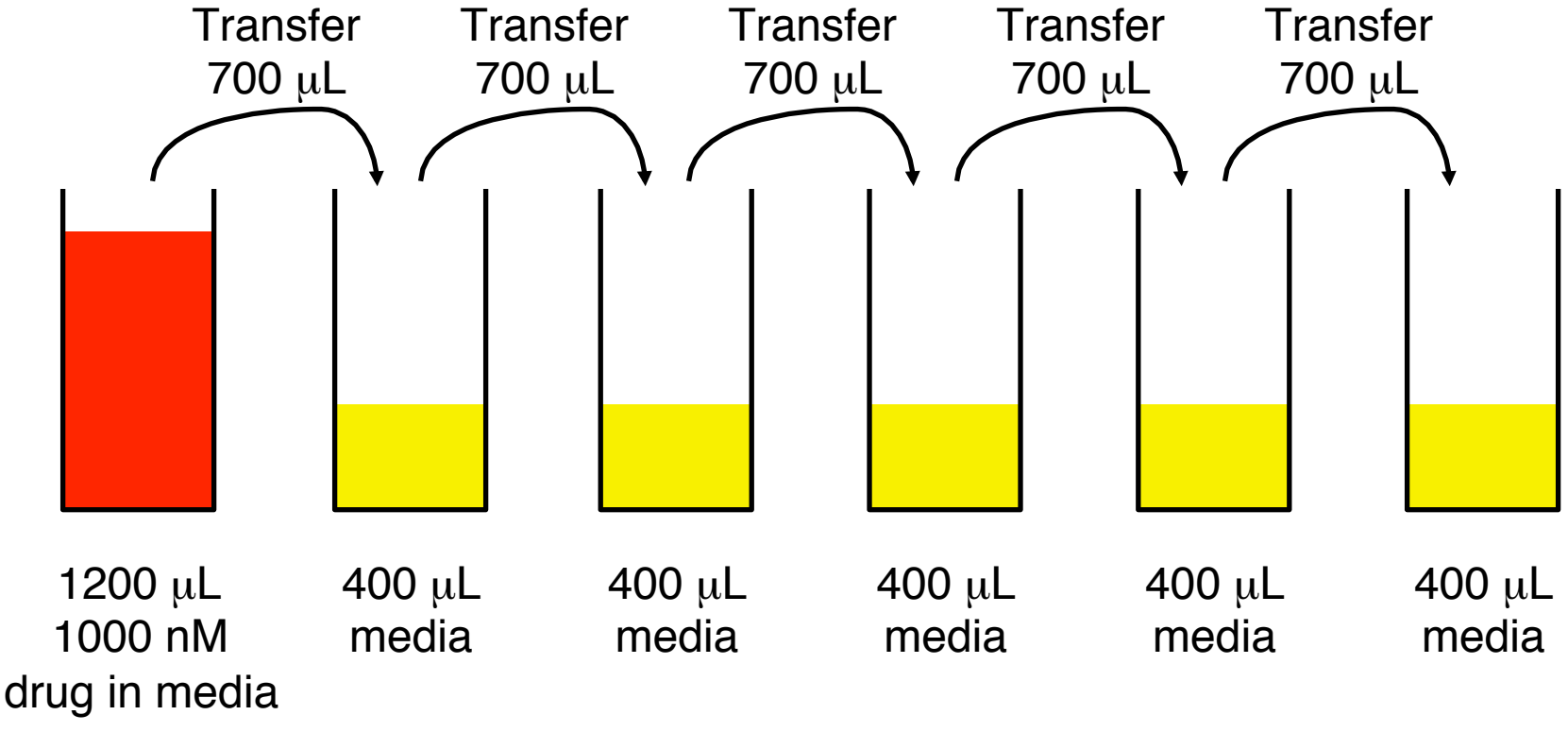


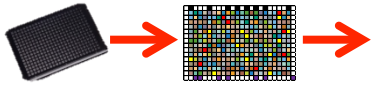
Drug delivery via digital drug dispenser

- For accurate delivery of a few drugs
- Pilot experiments- to identify appropriate doses
- Follow-up experiments, ‘hit’ validation
- Drugs that cannot be prepared in DMSO

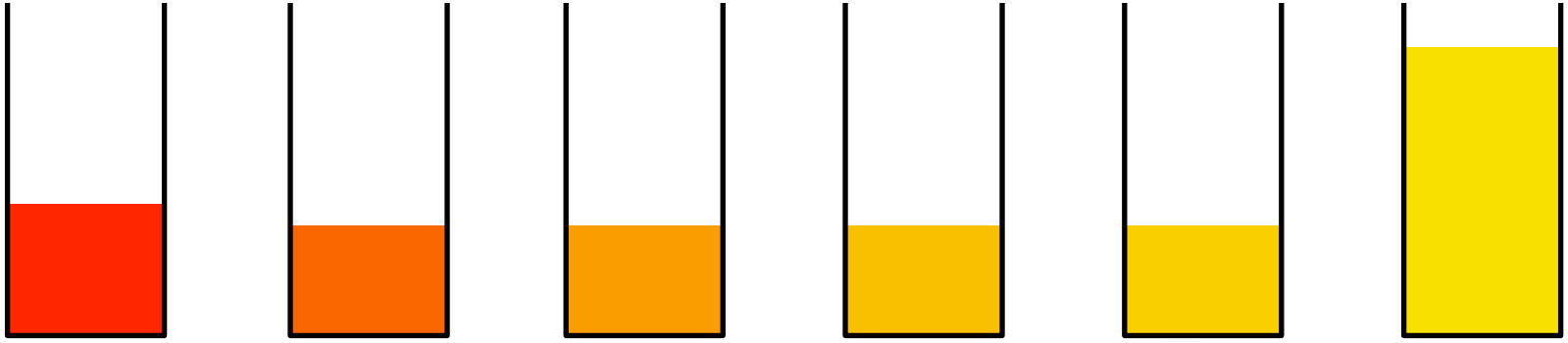


No automation? Use serial dilutions

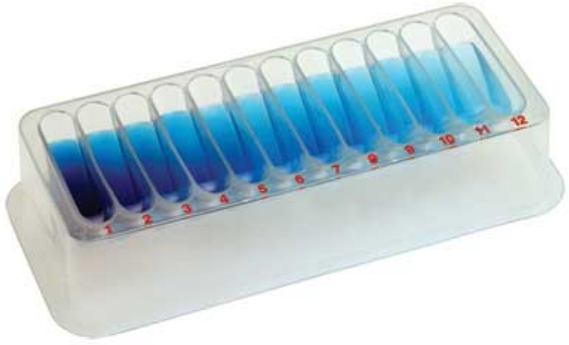




and multichannel pipettes

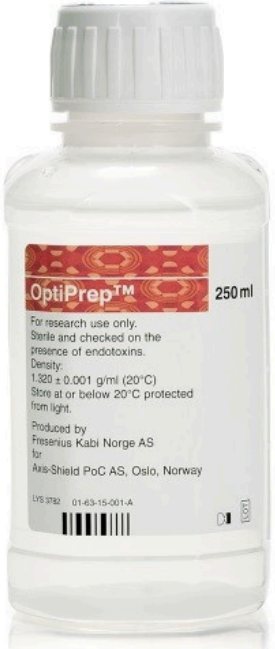


1000 nM drug in media | 636 nM drug | 405 nM drug | 258 nM drug | 164 nM drug | 104 nM drug



Dye-drop assay reagents

- Minimally-disruptive, reagent-sparing cell staining and fixation protocol



Dye-drop assay protocol

- Stain: Hoechst + LDR in 10% optiprep in PBS
- Fix: 4% formaldehyde in 20% optiprep in PBS

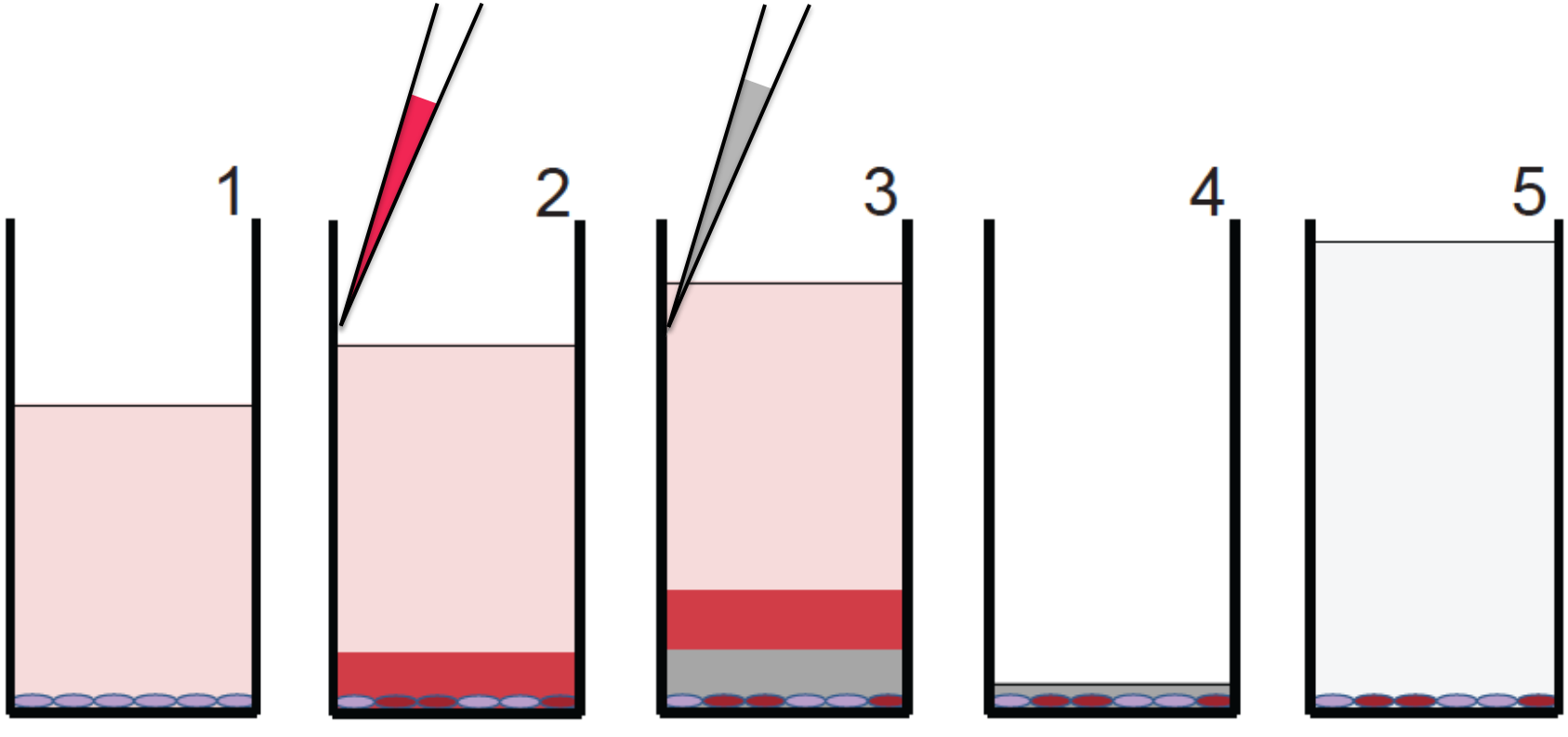


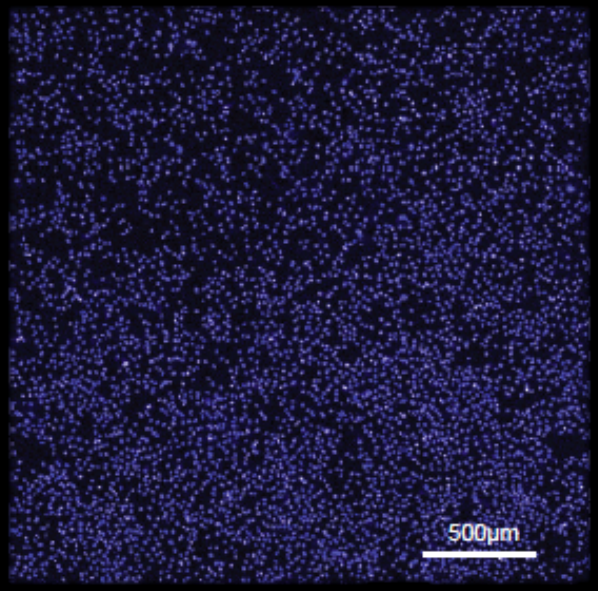
Plate washer

- Uniform and controlled aspiration and liquid dispensing

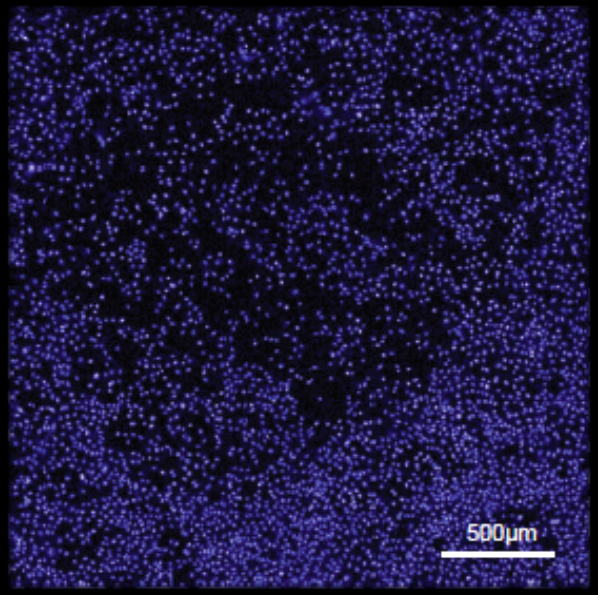
- Is repeat washing really that bad?

Repeat washing can result in cell loss…

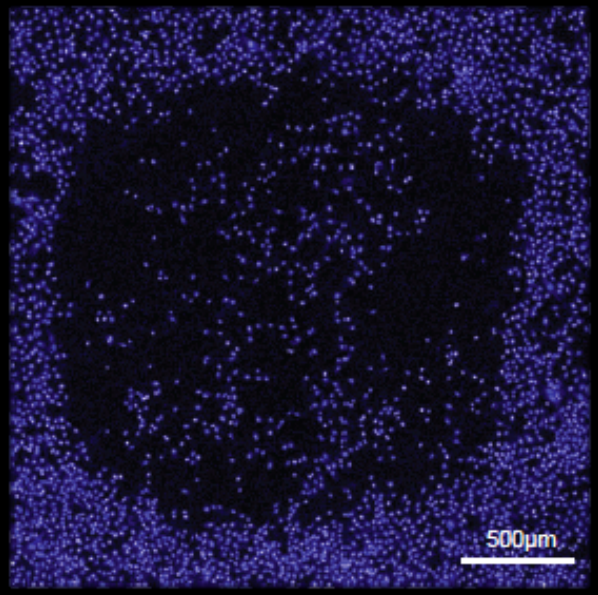
No wash



PBS wash x 1



PBS wash x 2



…that can bias your results

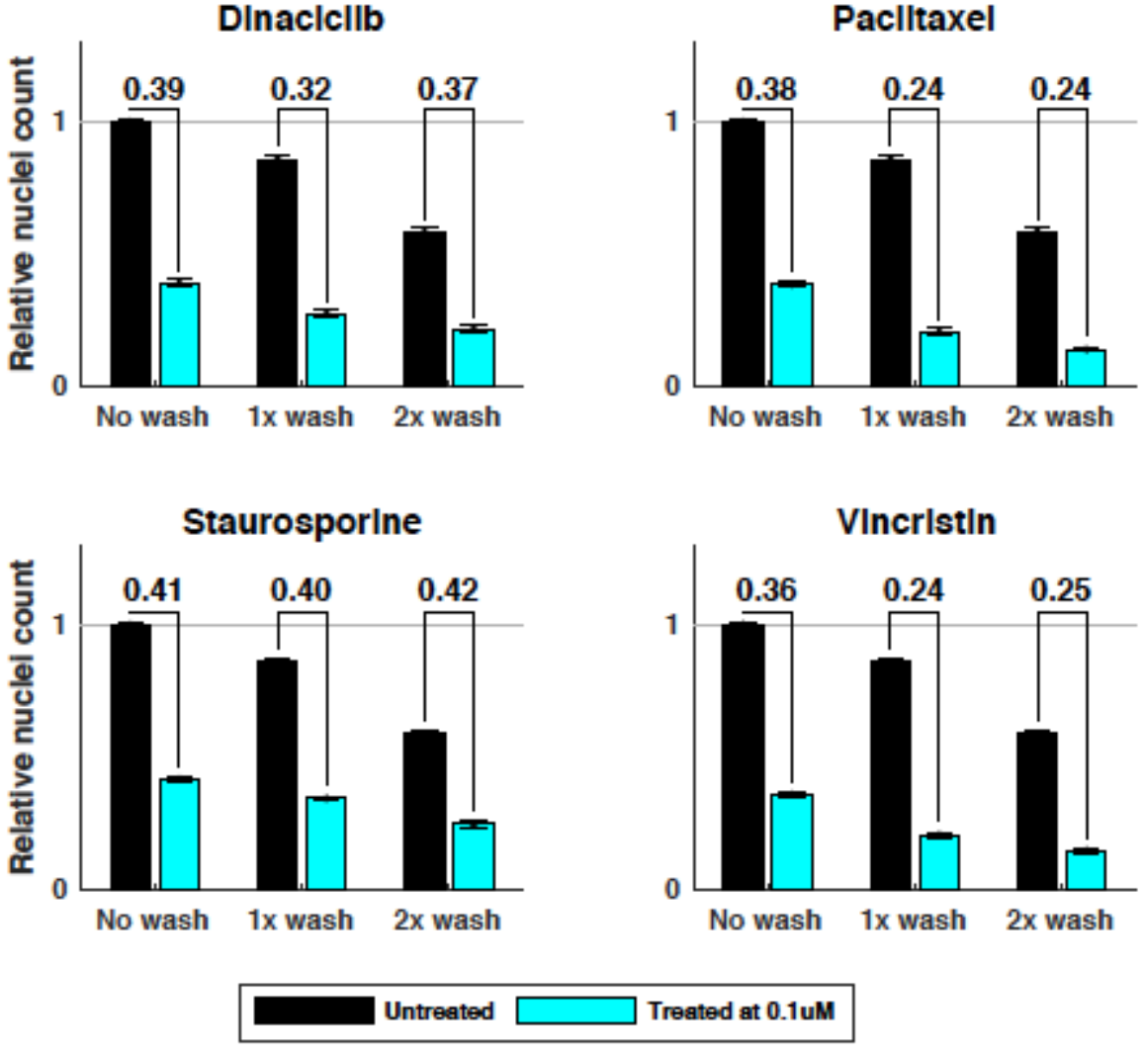


Image acquisition

- Operetta microscope with plate hotel, barcode reader & robot or similar
  - Automated data collection for 40+ plates

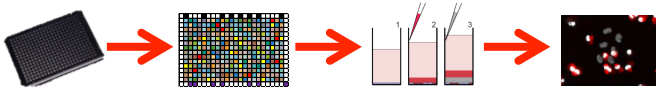
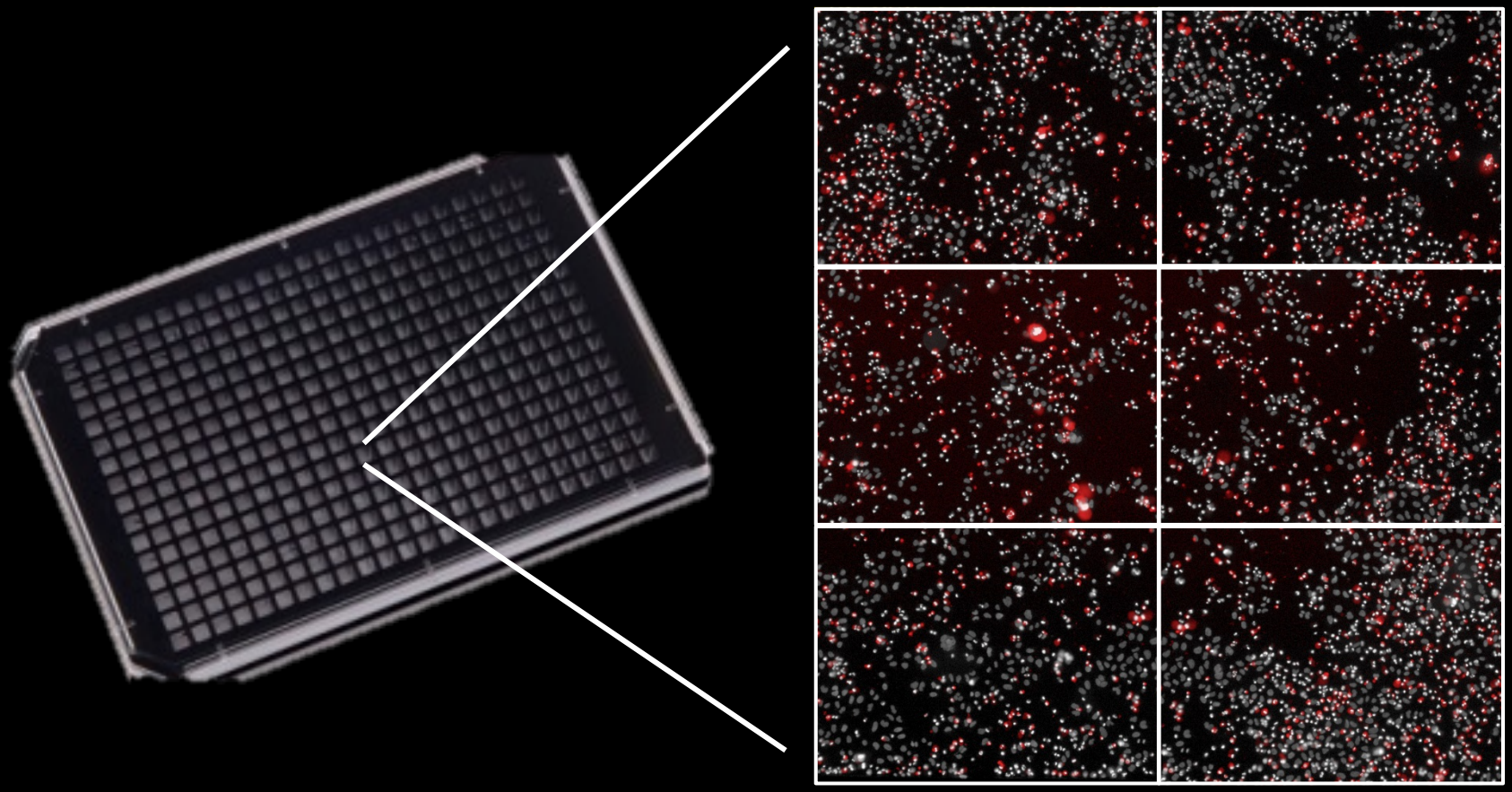
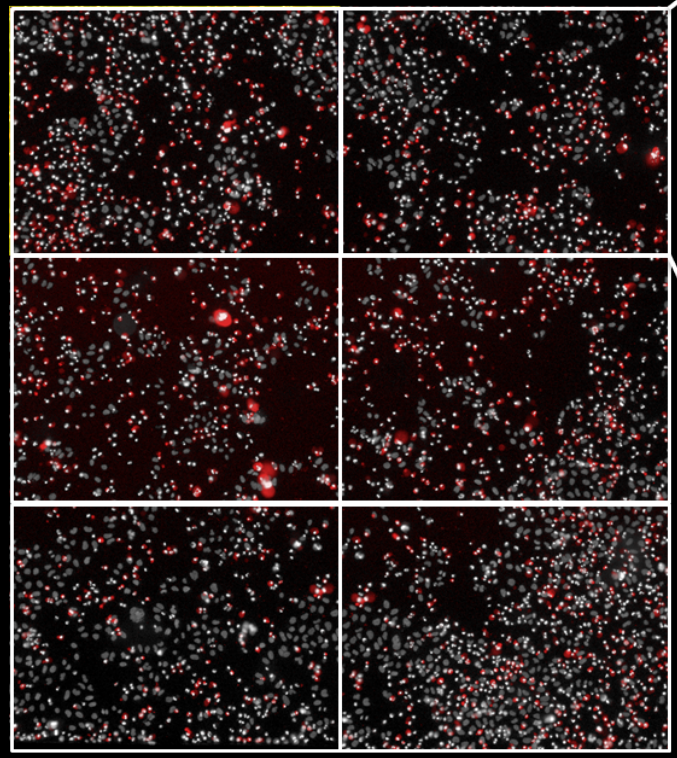


Image acquisition



Imaging 6 fields of view @ 10x
captures *almost* the entire well

Image acquisition



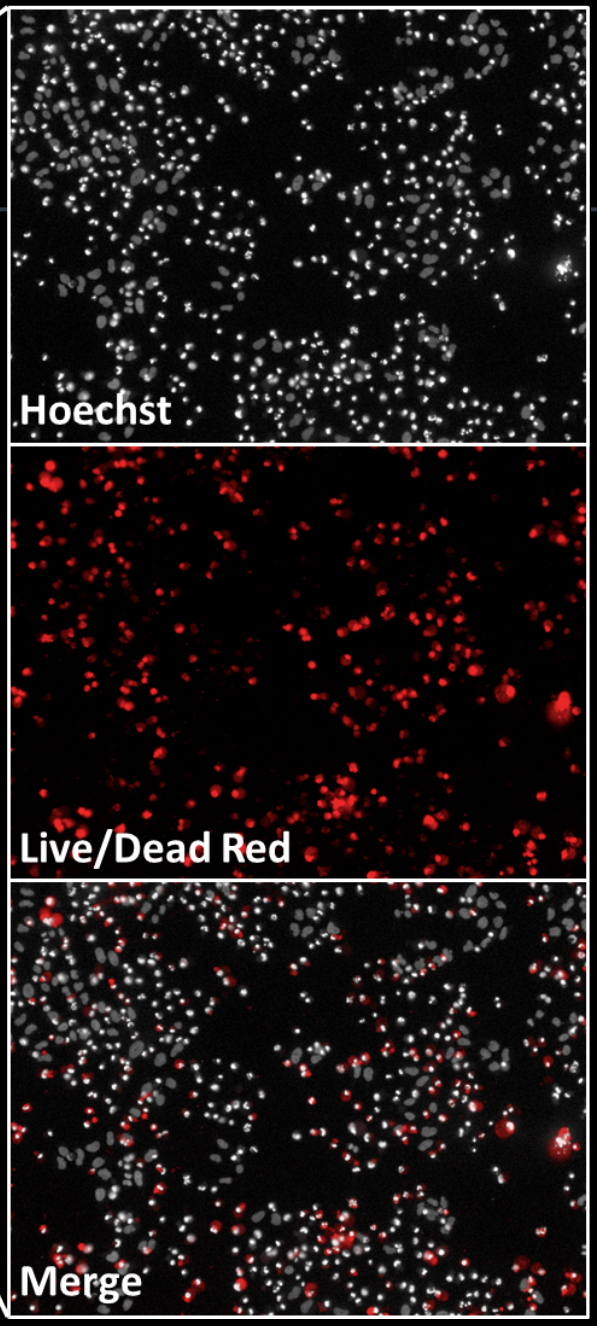
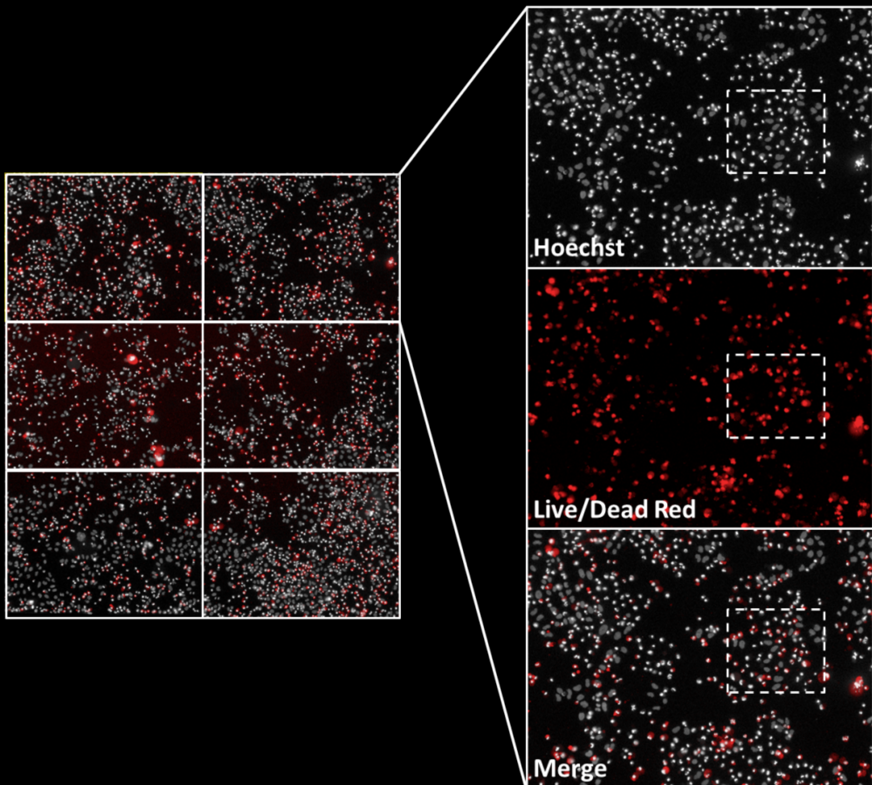
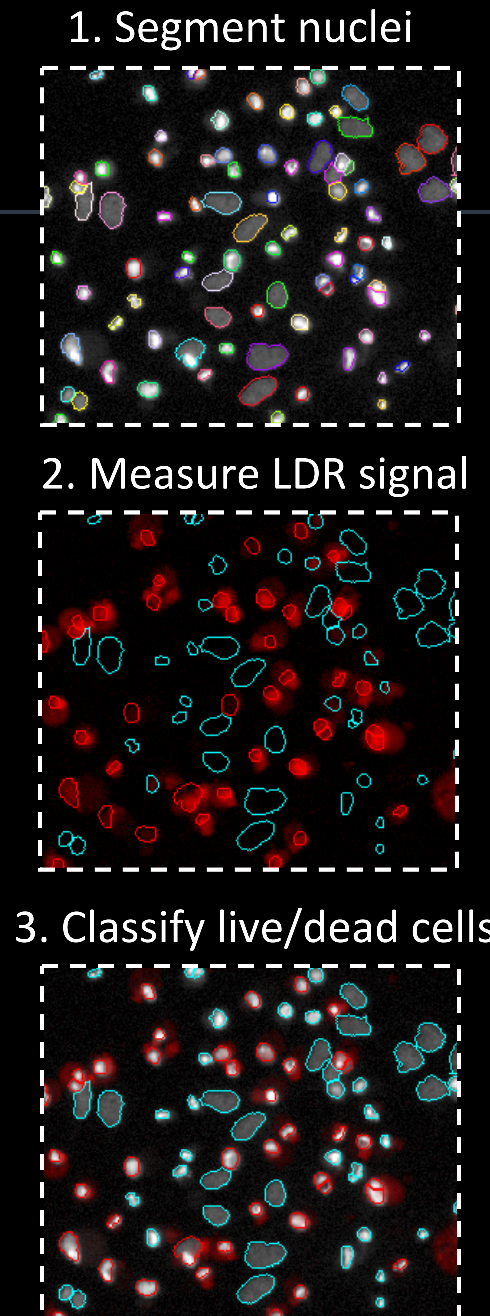


Image analysis





Well	Row	Column	Cell Line	Time point	Treatment	Dose (uM)	Cell count	Dead cell count	Cell count t=0
C2	3	2	MCF10A	72	Staurosporine	1	5091	1833	1954
C3	3	3	MCF10A	72	Staurosporine	1	5929	2137	1954
C4	3	4	MCF10A	72	Staurosporine	1	5663	2021	1954
C5	3	5	MCF10A	72	DMSO	0	8000	297	1954
C6	3	6	MCF10A	72	Staurosporine	0.316	6613	1142	1954
C7	3	7	MCF10A	72	DMSO	0	7732	329	1954
C8	3	8	MCF10A	72	Staurosporine	1	5463	2473	1954
D2	4	2	MCF10A	72	DMSO	0	8746	88	1954
D3	4	3	MCF10A	72	Staurosporine	0.316	6168	1496	1954
D4	4	4	MCF10A	72	Staurosporine	0.1	7941	636	1954
D5	4	5	MCF10A	72	DMSO	0	8529	360	1954
D6	4	6	MCF10A	72	Staurosporine	0.316	6994	1157	1954
D7	4	7	MCF10A	72	DMSO	0	8872	160	1954
D8	4	8	MCF10A	72	DMSO	0	9166	73	1954
C2	3	2	MCF10A	72	Staurosporine	1	5091	1833	1954
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C5	3	5	MCF10A	72	DMSO	0	8000	297	1954
C6	3	6	MCF10A	72	Staurosporine	0.316	6613	1142	1954
C7	3	7	MCF10A	72	DMSO	0	7732	329	1954
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C5	3	5	MCF10A	72	DMSO	0	8000	297	1954
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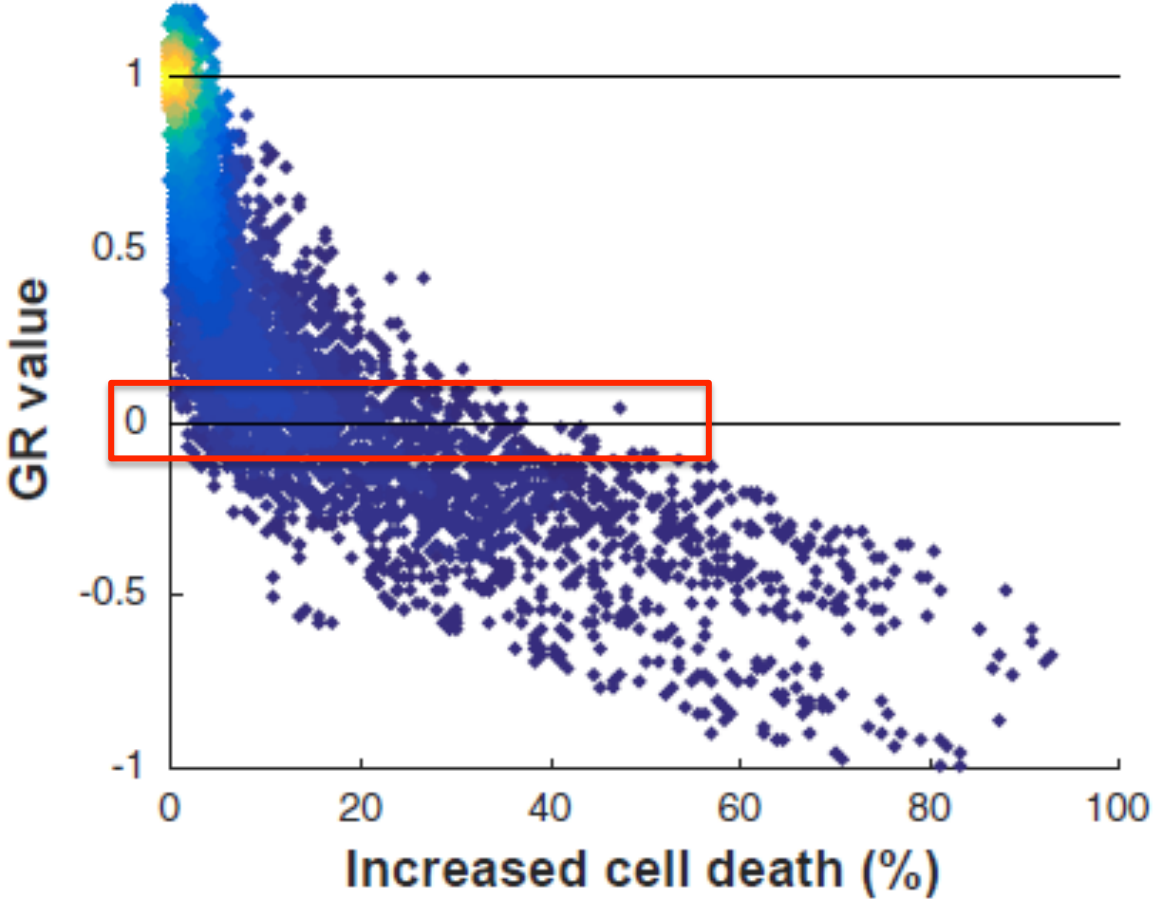
D6	4	6	MCF10A	72	Staurosporine	0.316	6994	1157	1954
D7	4	7	MCF10A	72	DMSO	0	8872	160	1954
D8	4	8	MCF10A	72	DMSO	0	9166	73	1954

Can I just count live cells?



Strengths and limitations of the dye-drop assay

- Imaging based
  - Best suited for adherent cells that grow in monolayer culture
- Image analysis can be time consuming
- Can go back and visually inspect imaging data
- Potential for multiplexing, immunofluorescence
- Reagent sparing
- Distinction between cytotoxic and cytostatic effects
- Fate of live cells unknown

Can we learn more about the live cells?
Deep dye-drop protocol

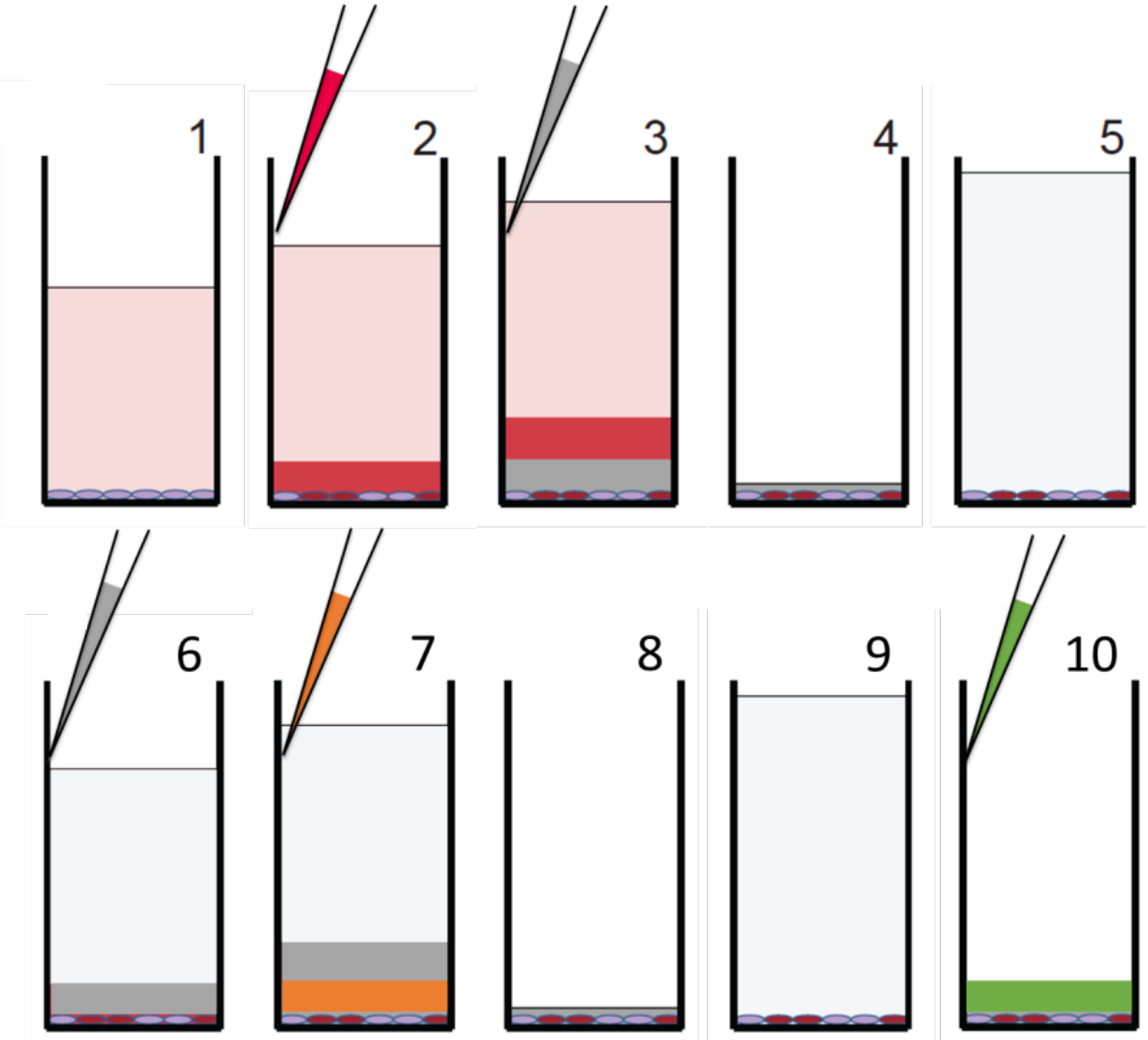


Image acquisition is more time consuming

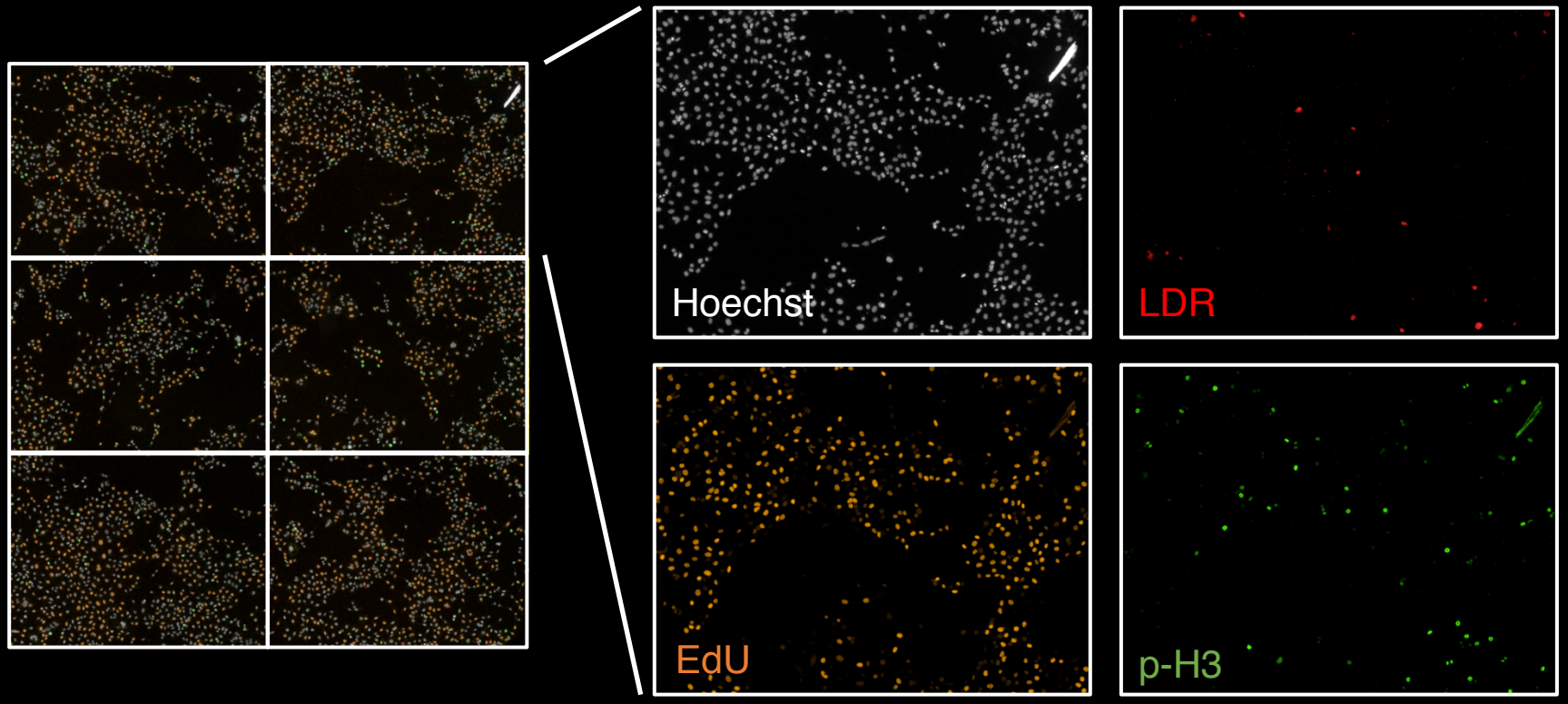
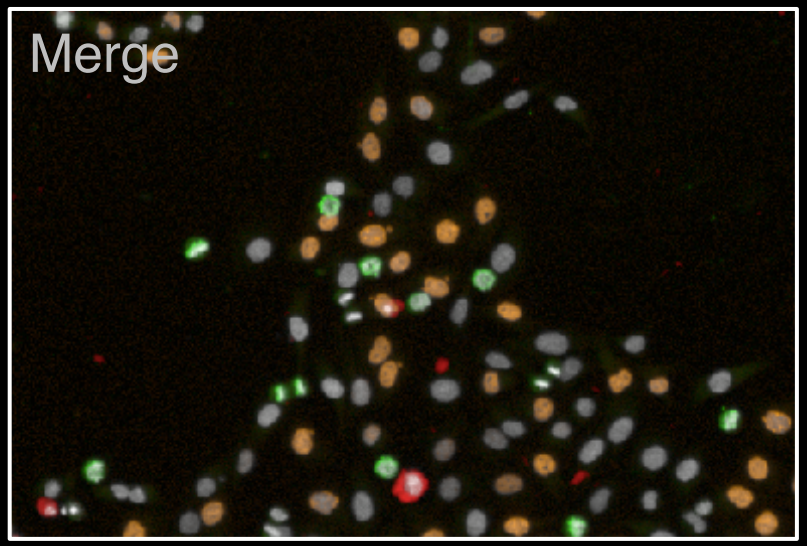
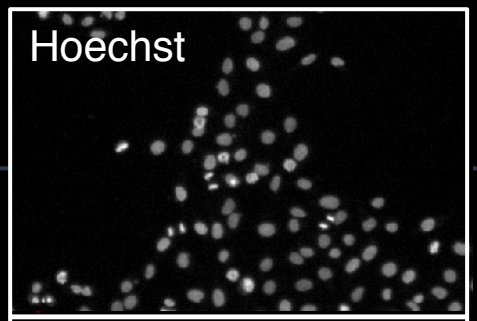
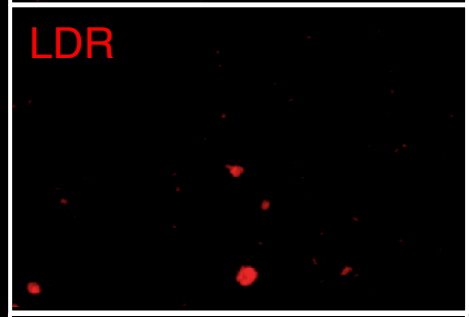


Image acquisition

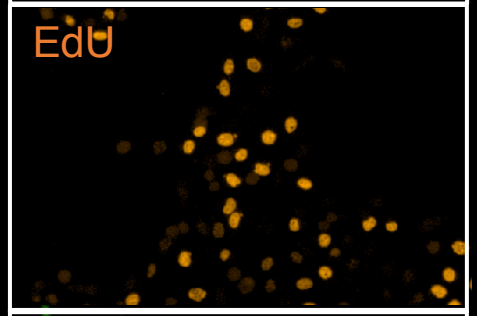




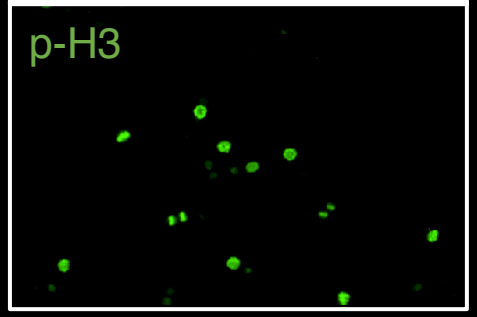
→ Segmentation
Cell count
DNA content



→ Dead cells

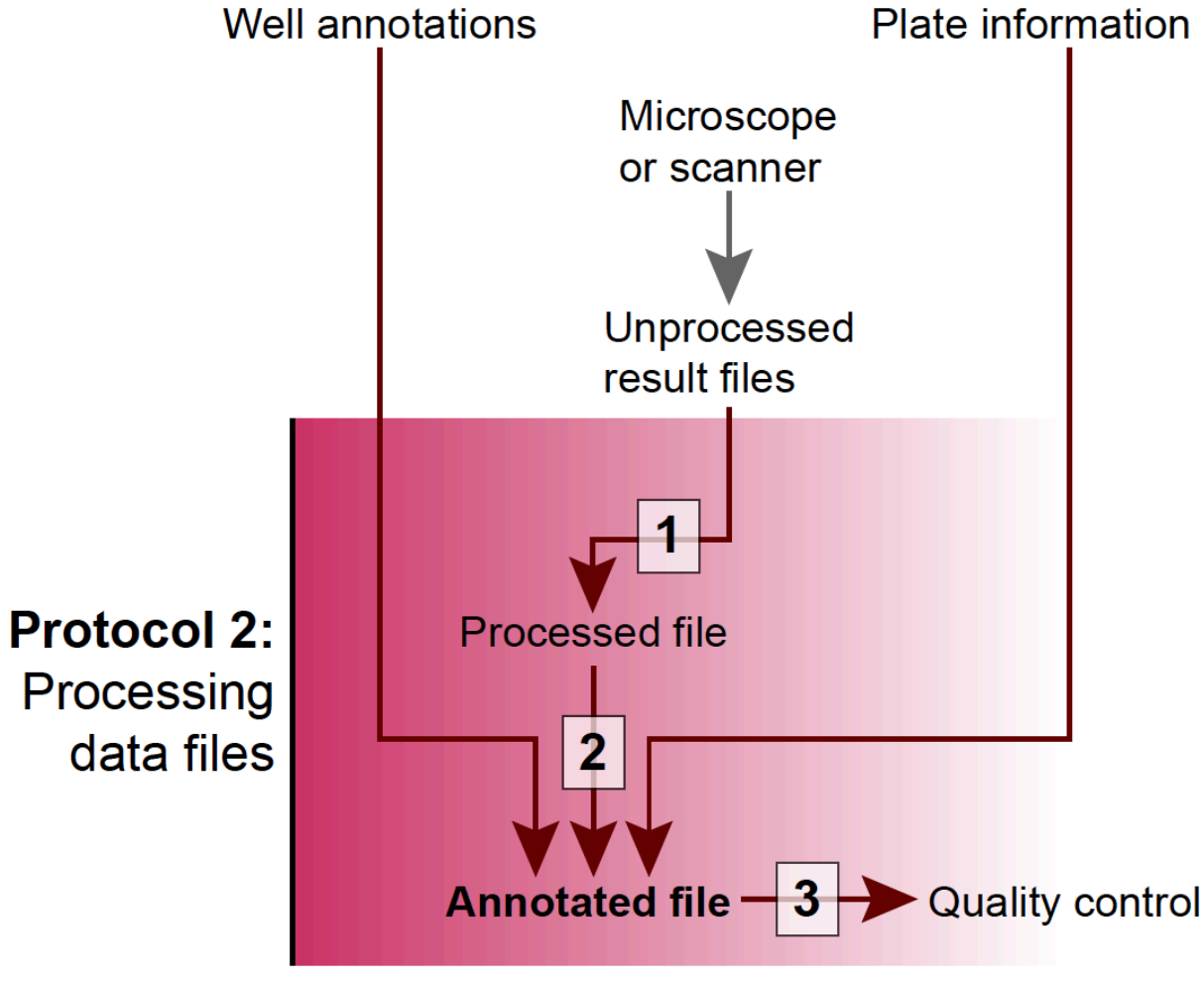


→ S phase cells

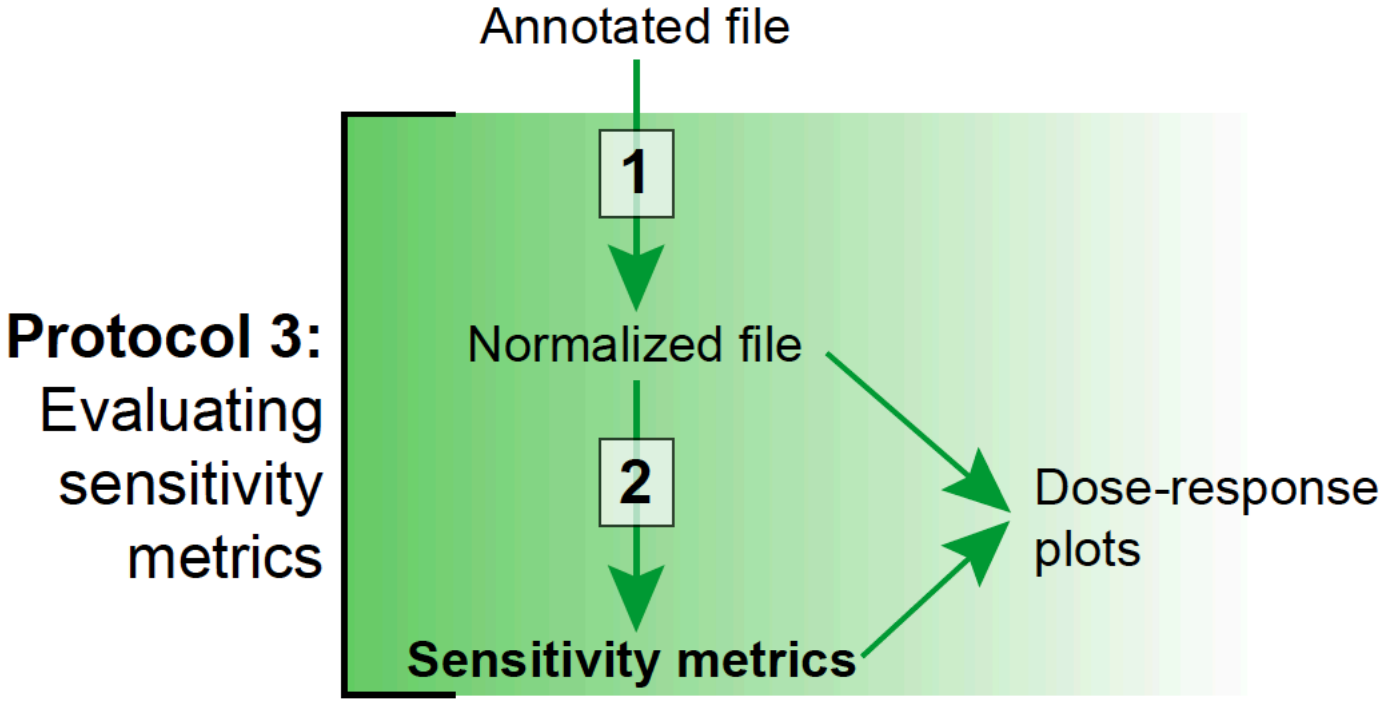


→ M phase cells

Data processing



Data analysis



smkartik updated merging data and plotting gr metrics script. abf1bc8 4 days ago

1 contributor

577 lines (576 sloc) | 16.5 KB

In [1]:
```
from datarail.experimental_design import merge_data_metadata as mdm
import pandas as pd
import gr50
```

Load columbus output, metadata and plate info files. Ensure that column heading are as shown in the below example input files

In [2]:
```
dfo = pd.read_table('columbus_output.tsv')
dfo.head()
```

Raw quantified image data

Out[2]:

	barcode	date	Row	Column	well	cell_count__total	corpse_count	cell_count__dead	cell_count
0	MH1_01	2016-06-06 12:34:56	3	3	C03	511.0	32.0	12.0	499.0
1	MH1_01	2016-06-06 12:34:56	3	4	C04	511.0	30.0	12.0	499.0
2	MH1_01	2016-06-06 12:34:56	3	5	C05	526.0	32.0	12.0	514.0
3	MH1_01	2016-06-06 12:34:56	3	6	C06	494.0	38.0	15.0	479.0
4	MH1_01	2016-06-06 12:34:56	3	7	C07	507.0	29.0	13.0	494.0

In [3]:
```
dfm = pd.read_csv('example_metadata.csv')
dfm.head()
```

Metadata from design

Out[3]:

	agent	concentration	role	well	randomization_scheme	timepoint	barcode	cell_line
0	NaN	NaN	NaN	C03	0	time0_ctrl	MH1_01	CL_1
1	NaN	NaN	NaN	C04	0	time0_ctrl	MH1_01	CL_1
2	NaN	NaN	NaN	C05	0	time0_ctrl	MH1_01	CL_1

datarail / datarail / examples / Merge columbus output with plate and well level metadata files.ipynb

smkartik updated merging data and plotting gr metrics script. abf1bc8 4 days ago

1 contributor

Generate counts file in the format required by the GR calculator → Normalized count table

In [7]:
```
df_counts = mdm.generate_GRinput(dfcw)
```

Compute GRvalue → GR values

In [8]:
```
df_grv = gr50.compute_gr(df_counts)
```

Compute summary GR metrics → Summary GR metrics

In [9]:
```
df_grmetrics = gr50.gr_metrics(df_grv)
```

In [10]:
```
from datarail.experimental_design import plot_gr_dose_response as plot
```

In [11]:
```
plot.plot_dose_response(df_grv, df_grmetrics)
```
→ Summary plots

In [ ]:

In [3]:
```
dfm = pd.read_csv('example_metadata.csv')
dfm.head()
```

Out[3]:

	agent	concentration	role	well	randomization_scheme	timepoint	barcode	cell_line
0	NaN	NaN	NaN	C03	0	time0_ctrl	MH1_01	CL_1
1	NaN	NaN	NaN	C04	0	time0_ctrl	MH1_01	CL_1
2	NaN	NaN	NaN	C05	0	time0_ctrl	MH1_01	CL_1

Analysis output files (Dye Drop)

cell_line	treatment	concentratio	cell_count	cell_count__ctrl	cell_count__time0	time
Hs578T	AZD1775	0.001	3161	3837.666667	875.2694805	72
Hs578T	AZD1775	0.001	3398	3837.666667	875.2694805	72
Hs578T	AZD1775	0.001	3493	3837.666667	875.2694805	72
Hs578T	AZD1775	0.00316228	2768	3837.666667	875.2694805	72
Hs578T	AZD1775	0.00316228	2742	3837.666667	875.2694805	72
Hs578T	AZD1775	0.003			875.2694805	72
Hs578T	AZD1775				875.2694805	72
Hs578T	AZD1775	0.01	2108	3837.666667	875.2694805	72
Hs578T	AZD1775	0.01	2268	3837.666667	875.2694805	72
Hs578T	AZD1775	0.03162278	1595	3837.666667	875.2694805	72
Hs578T	AZD1775	0.03162278	1742	3837.666667	875.2694805	72
Hs578T	AZD1775	0.03162278	1727	3837.666667	875.2694805	72
Hs578T	AZD1775	0.1	1527	3837.666667	875.2694805	72
Hs578T	AZD1775	0.1	1339	3837.666667	875.2694805	72

Normalized count table

cell_line	treatment	concentratio	GRvalue
Hs578T	AZD1775	0.001	0.87627773
Hs578T	AZD1775	0.00316228	0.71315548
Hs578T	AZD1775	0.01	0.54911891
Hs578T	AZD1775	0.03162278	0.36042471
Hs578T	AZD1775	0.1	0.28988683
Hs578T	AZD1775	0.31622777	0.15239405
Hs578T	AZD		0.06211543
Hs578T	AZD		0.01136504
Hs578T	AZD1775	10	-0.2126464
Hs578T	AZD2014	0.001	0.73724513
Hs578T	AZD2014	0.00316228	0.72666777
Hs578T	AZD2014	0.01	0.66564344
Hs578T	AZD2014	0.03162278	0.55891961
Hs578T	AZD2014	0.1	0.49154648
Hs578T	AZD2014	0.31622777	0.33929164
Hs578T	AZD2014	1	0.32070364
Hs578T	AZD2014	3.16227766	0.2845481

GR values

cell_line	treatment	GR50	GRmax	GR_AOC	GEC50	GRinf	h_GR	r2_GR	pval_GR
Hs578T	AZD1775	0.01893073	-0.2126464	0.69121549	0.04681442	-0.2378845	0.42983997	0.97294605	3.26E-06
Hs578T	AZD2014	0.06225072	0.27113099	0.51356141	0.020339	0.16159799	0.34897477	0.97356694	3.00E-06
Hs578T	AZD5363	4.66264451	0.34057138	0	4173	-1	0.25043016	0.91027375	0.00021638
Hs578T	AZD6738	5.70071745	0.15862042	0	3152	-0.3241112	1.87755981	0.983338	5.97E-07
Hs578T	BMS-265246	0.03694372	0.06418563	0.59001375	5.81104037	-0.8002119	0.18893869	0.98567468	3.52E-07
Hs578T	BVD523	1.70247689	0.27840528	0.29313938	5.64209574	-0.257788	0.3470251	0.96605461	7.21E-06
Hs578T	CFI-400945	0.00326927	0.13959787	0.71503751	0.00284445	0.1912934	3.46427746	0.97596165	2.15E-06
Hs578T	Flavopiridol	0.15093505	-0.1758999	0.24300872	0.19656651	-0.2662708	1.61622383	0.99714964	1.24E-09
Hs578T	GSK2334470	16.8060155	0.53065211	0.17491698	744.800682	-1	0.28976583	0.9734299	3.06E-06
Hs578T	LEE011/Ribo	3.98380188	0.39670684	0.33089144	1000	-0.7108281	0.16006672	0.92942059	9.34E-05

GR metrics

Analysis output files (Deep Dye Drop)

cell_line	treatment	concentratio	cell_count	cell_count__ctrl	cell_count__time0	time
Hs578T	AZD1775	0.001	3161	3837.666667	875.2694805	72
Hs578T	AZD1775	0.001	3398	3837.666667	875.2694805	72
Hs578T	AZD1775	0.001	3493	3837.666667	875.2694805	72
Hs578T	AZD1775	0.00316228	2768	3837.666667	875.2694805	72
Hs578T	AZD1775	0.00316228	2742	3837.666667	875.2694805	72
Hs578T	AZD1775	0.003			875.2694805	72
Hs578T	AZD1775				875.2694805	72
Hs578T	AZD1775	0.01	2108	3837.666667	875.2694805	72
Hs578T	AZD1775	0.01	2268	3837.666667	875.2694805	72
Hs578T	AZD1775	0.03162278	1595	3837.666667	875.2694805	72

Normalized count table

cell_line	treatment	concentratio	GRvalue
Hs578T	AZD1775	0.001	0.87627773
Hs578T	AZD1775	0.00316228	0.71315548
Hs578T	AZD1775	0.01	0.54911891
Hs578T	AZD1775	0.03162278	0.36042471
Hs578T	AZD1775	0.1	0.28988683
Hs578T	AZD		0.15239405
Hs578T	AZD		0.06211543
Hs578T	AZD1775	3.16227766	0.01136504
Hs578T	AZD1775	10	-0.2126464
Hs578T	AZD2014	0.001	0.73724513
Hs578T	AZD2014	0.00316228	0.72666777
		0.01	0.66564344
		0.03162278	0.55891961
		0.1	0.49154648
		0.31622777	0.33929164
		1	0.32070364
		3.16227766	0.2845481
		10	0.27113099
		0.001	0.91594716
		0.00316228	0.92218033
		0.01	0.80929989

GR values

reatment	GR50	GRmax	GR_AOC	GEC50	GRinf	h_GR	r2_GR	pval_GR
AZD1775	0.01893073	-0.2126464	0.69121549	0.04681442	-0.2378845	0.42983997	0.97294605	3.26E-06
AZD2014	0.06225072	0.27113099	0.51356141	0.020339	0.16159799	0.34897477	0.97356694	3.00E-06
AZD5363	4.66264451	0.34057138	0.258236		-1	0.25043016	0.91027375	0.00021638
AZD6738	5.70071745	0.15862042	0.060244		3241112	1.87755981	0.983338	5.97E-07
BMS-265246	0.03694372	0.06418563	0.59001375	5.81104037	-0.8002119	0.18893869	0.98567468	3.52E-07
BVD523	1.70247689	0.27840528	0.29313938	5.64209574	-0.257788	0.3470251	0.96605461	7.21E-06
CFI-400945	0.00326927	0.13959787	0.71503751	0.00284445	0.1912934	3.46427746	0.97596165	2.15E-06
Flavopiridol	0.15093505	-0.1758999	0.2					
GSK2334470	16.8060155	0.53065211	0.1					
LEE011/Ribo	3.98380188	0.39670684	0.3					

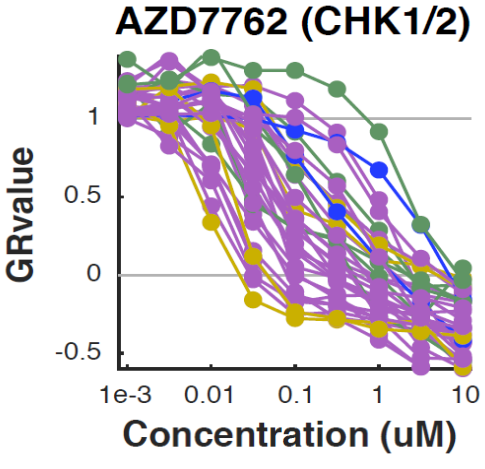
GR metrics

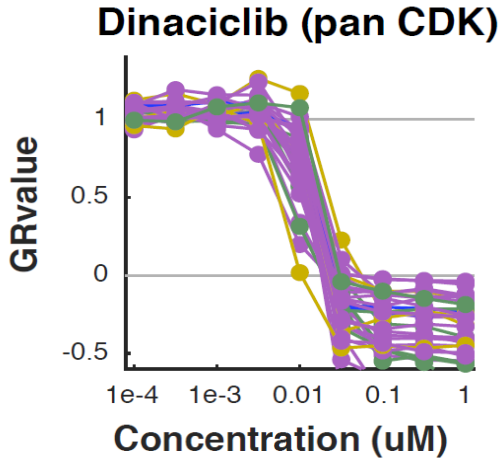
Hs578T	AZD1775	3.16227766	
Hs578T	AZD1775	3.16227766	
Hs578T	AZD1775	10	
Hs578T	AZD1775	10	
Hs578T	AZD1775	10	
Hs578T	AZD2014	0.001	
Hs578T	AZD2014	0.001	

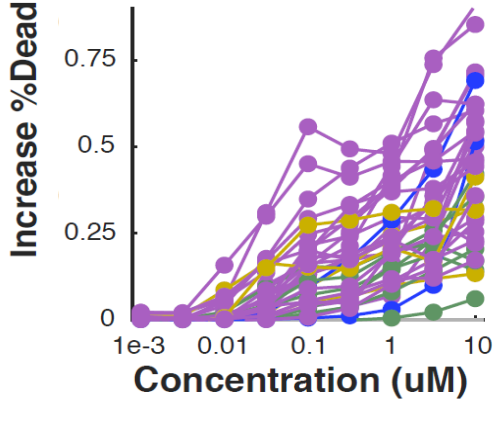
cell_line	agent	concentration	G1	S	G2	M	S_dropout	subG1	b
Hs578T	AZD1775	0.001	0.529885	0.167032	0.105082	0.018915	0.048951	0.082977	
Hs578T	AZD1775	0.003162278	0.432832	0.213221	0.112379	0.03742	0.052383	0.115278	
Hs578T	AZD1775	0.01	0.371868	0.244846	0.127247	0.060692	0.028412	0.15265	
Hs578T	AZD1775	0.031622777	0.271965	0.361385	0.106999	0.078917	0.02496	0.132671	
Hs578T	AZD1775	0.1	0.284876			52	0.059406	0.086156	
Hs578T	AZD1775	0.316227766	0.23569			86	0.051305	0.055347	
Hs578T	AZD1775	1	0.23887	0.368224	0.056187	0.180239	0.053833	0.076745	
Hs578T	AZD1775	3.16227766	0.355012	0.153131	0.065907	0.201169	0.078298	0.120687	
Hs578T	AZD1775	10	0.303839	0.008885	0.117044	0.335917	0.088932	0.127568	
Hs578T	AZD2014	0.001	0.547617	0.160385	0.117951	0.018336	0.032511	0.099066	

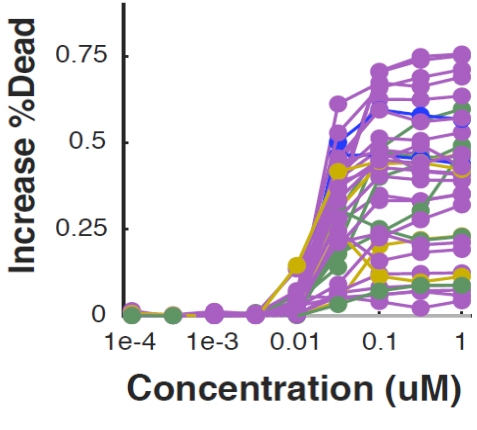
Cell cycle fractions

Results output: GR and cell death

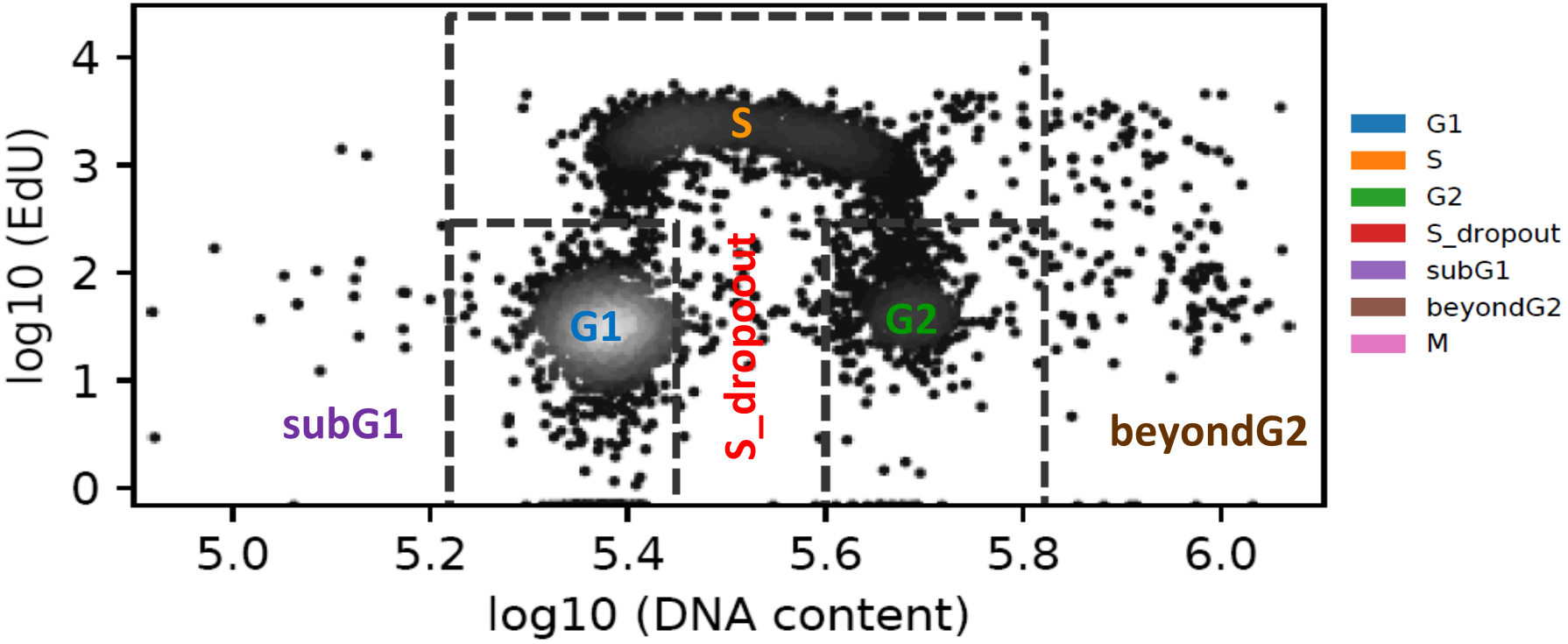




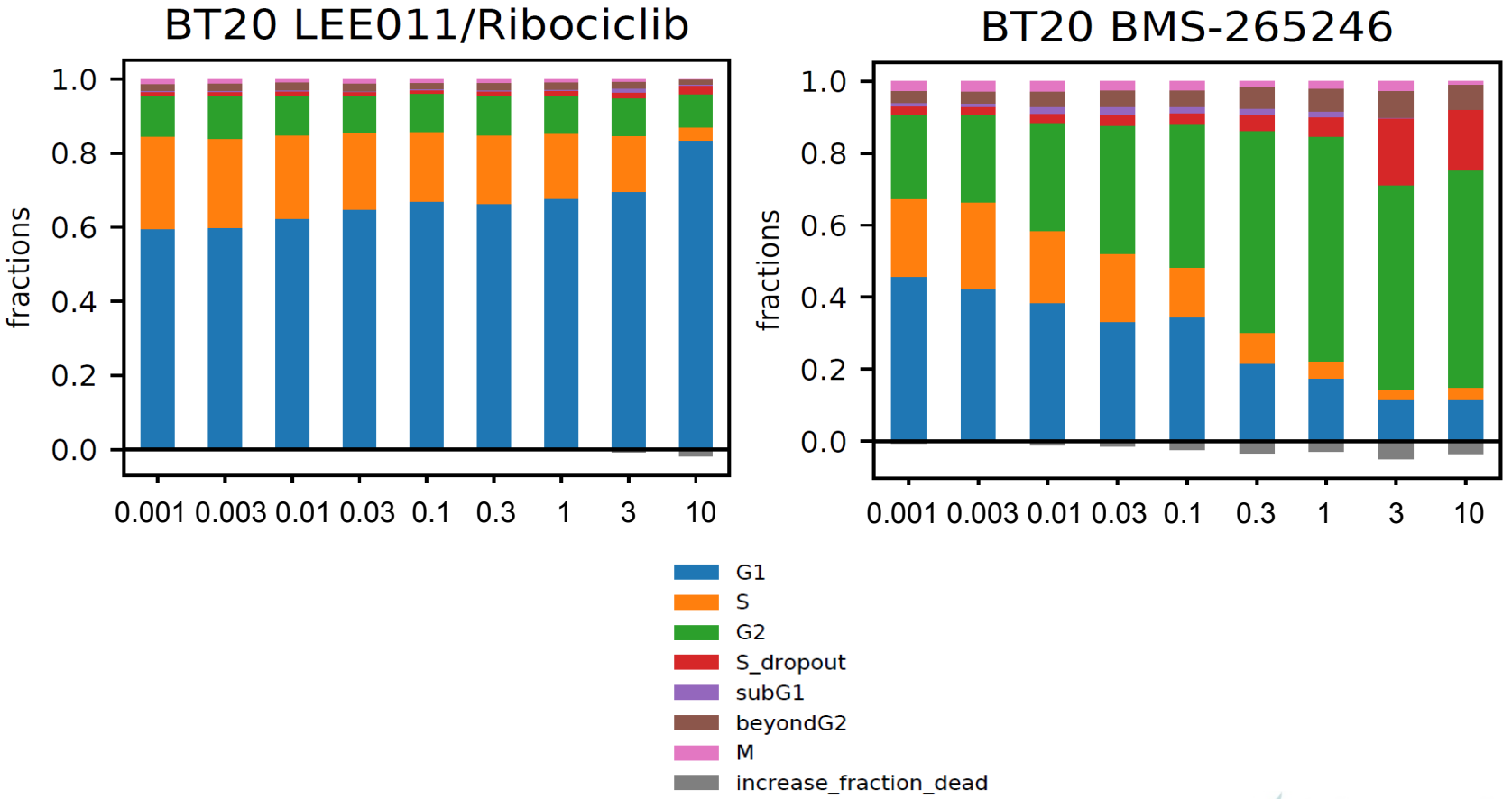




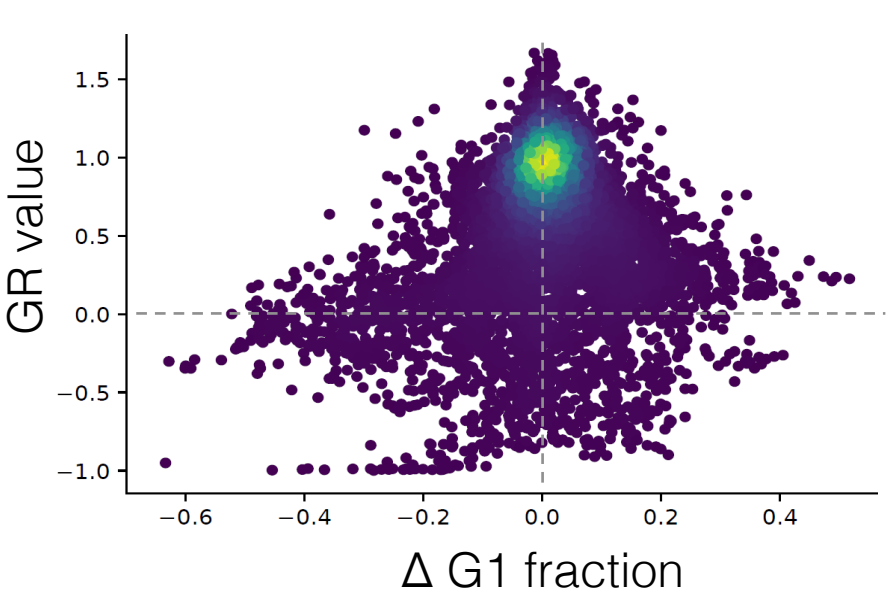
Results output: Cell cycle distribution

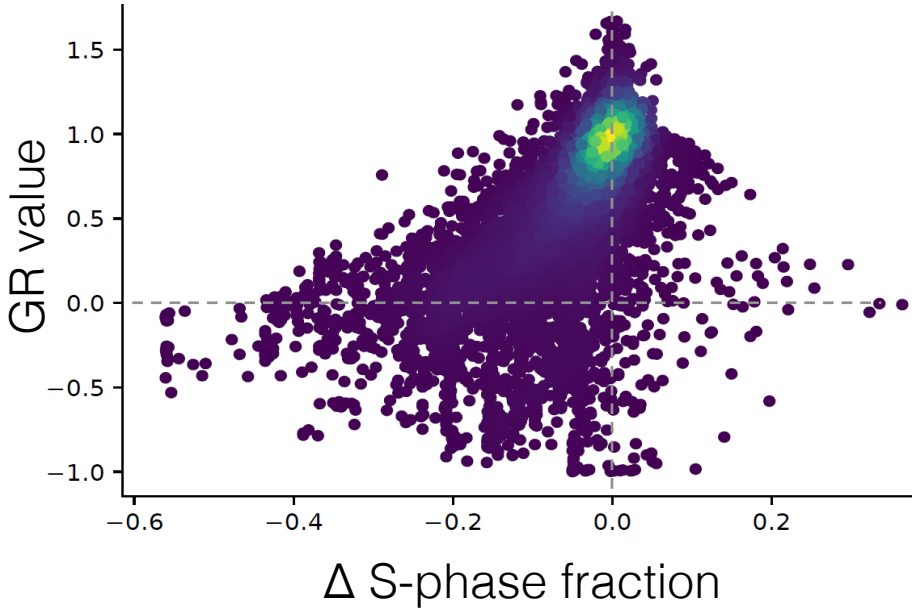


Results output: cell cycle summary

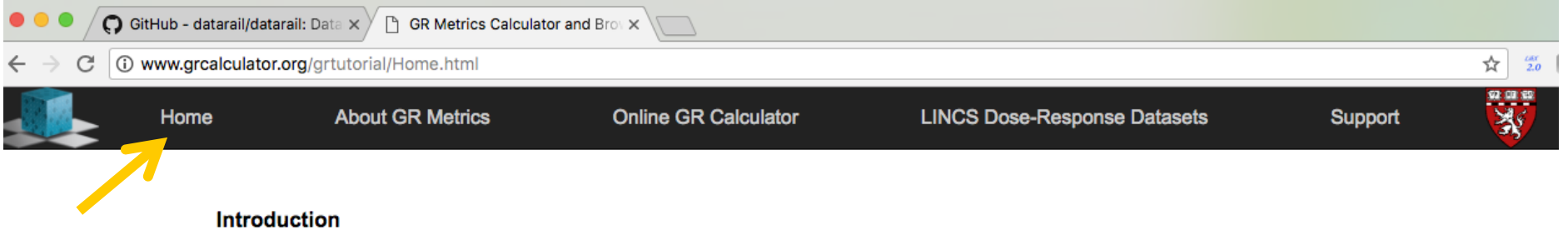


Variable effects on cell cycle distribution result in the same GR value





Online GR tool: www.grcalculator.org



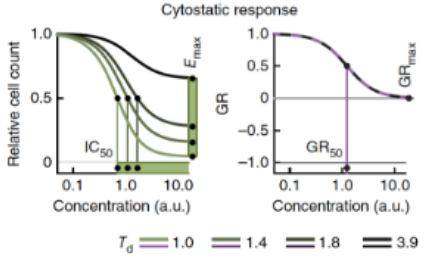
Introduction

Drug-response studies play an important role in both preclinical and clinical research, but such studies are complicated by differences in cell growth rates across samples and conditions. To improve the value and reliability of such studies, new metrics for parameterizing drug response were developed and published in Nature Methods by Marc Hafner, Mario Niepel, and Peter Sorger of the Harvard Medical School (HMS) LINCS Center. These new metrics, such as GR50 and GRmax, are derived from normalized growth rate inhibition (GR) values which are based on the ratio of growth rates in the presence and absence of perturbagen. Largely independent of cell division rate and assay duration, GR metrics are more robust than IC50 and Emax for assessing cellular response to drugs, RNAi, and other perturbations in which control cells divide over the course of the assay.

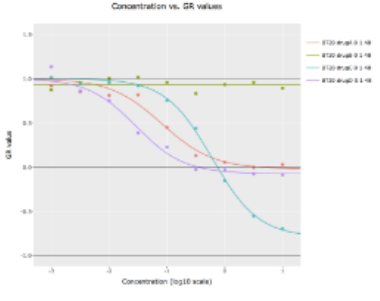
Future Updates and Improvements

We plan to continue adding features and improvements to the GRbrowser, GRcalculator, and the GRmetrics R package in the coming months. We welcome comments and suggestions at gr.calculator@gmail.com. You can find a preliminary outline of our plans here. We will be adding a more detailed roadmap of additions/improvements in the near future.

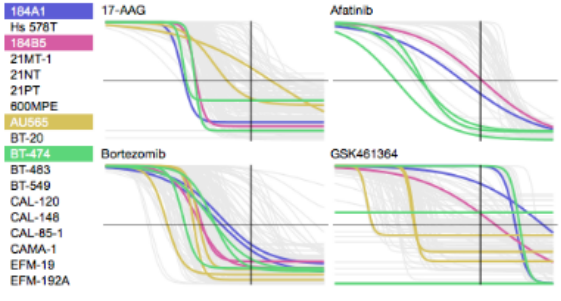
Learn More about GR metrics



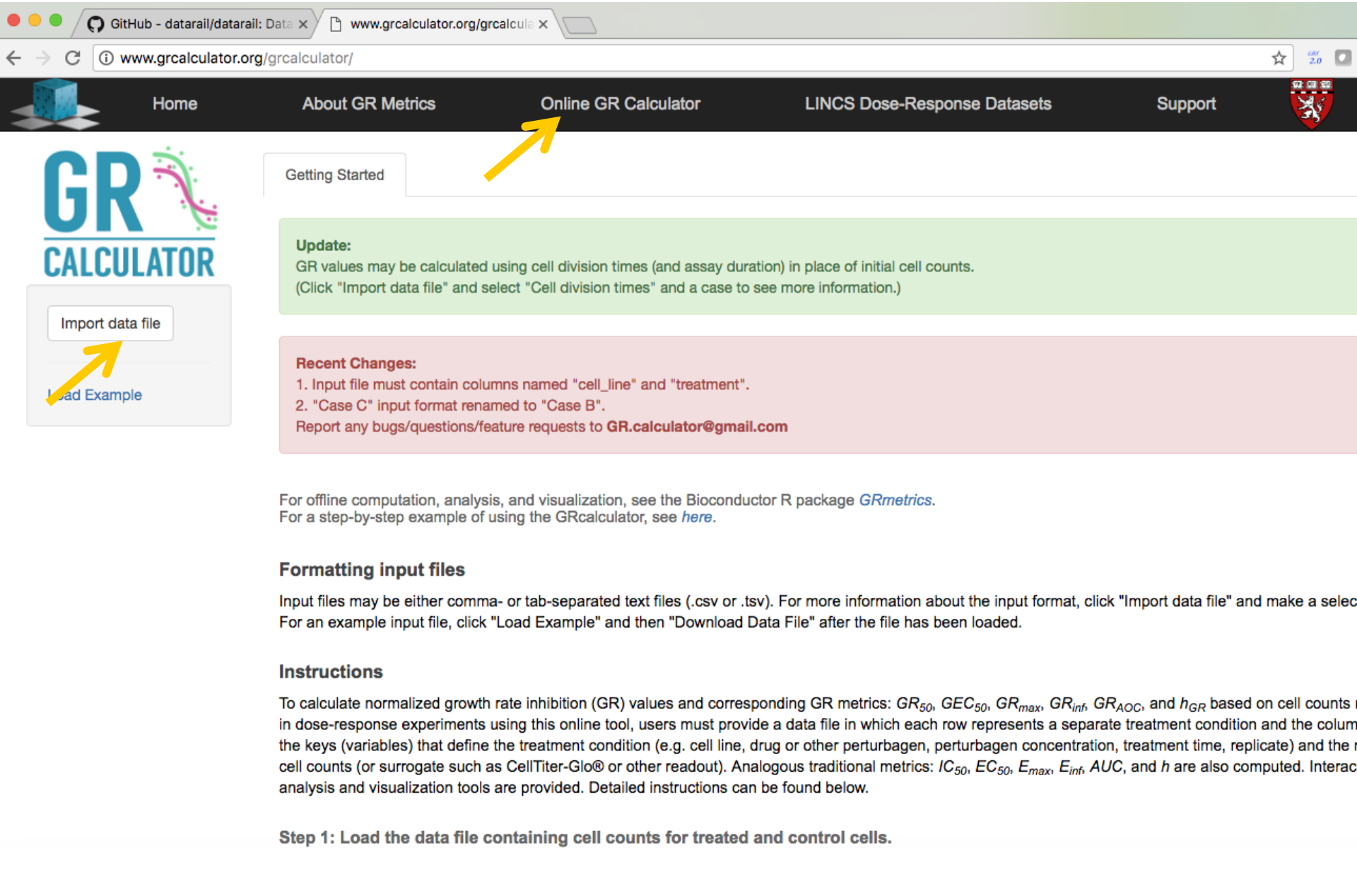
Upload and analyze your own data



Browse datasets analyzed using GR metrics



For offline computation, analysis, and visualization, see the Bioconductor R package *GRmetrics*.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Home
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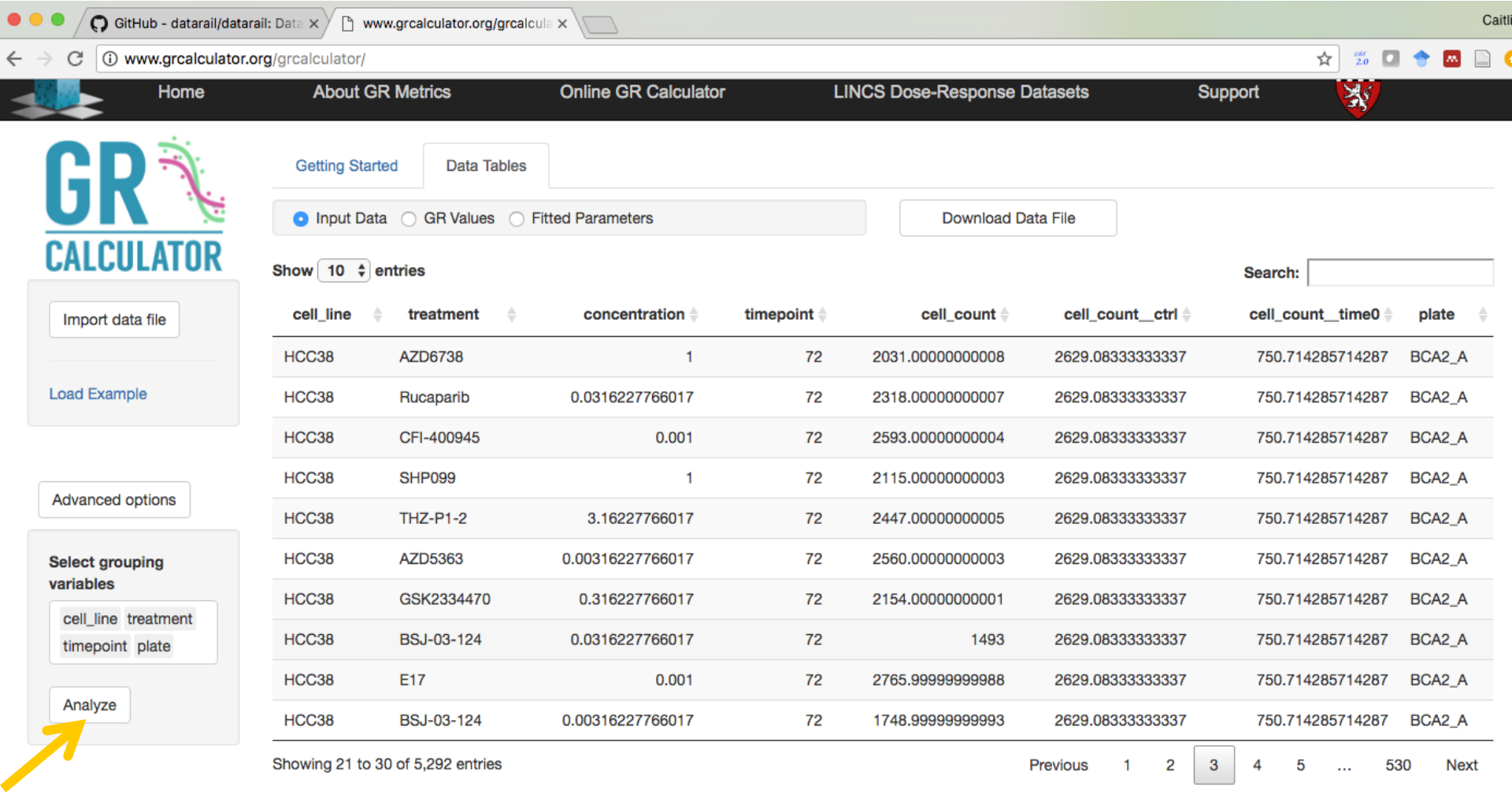
LINCS Dose-Response Datasets
Support
GR CALCULATOR
Import data file
Load Example
Getting Started
Update:
GR values may be calculated using cell division times (and assay duration) in place of initial cell counts.
(Click "Import data file" and select "Cell division times" and a case to see more information.)
Recent Changes:
1. Input file must contain columns named "cell_line" and "treatment".
2. "Case C" input format renamed to "Case B".
Report any bugs/questions/feature requests to GR.calculator@gmail.com
For offline computation, analysis, and visualization, see the Bioconductor R package GRmetrics.
For a step-by-step example of using the GRcalculator, see here.
Formatting input files
Input files may be either comma- or tab-separated text files (.csv or .tsv). For more information about the input format, click "Import data file" and make a selec
For an example input file, click "Load Example" and then "Download Data File" after the file has been loaded.
Instructions
To calculate normalized growth rate inhibition (GR) values and corresponding GR metrics: GR_{50}, GEC_{50}, GR_{max}, GR_{inf}, GR_{AOC}, and h_{GR} based on cell counts
in dose-response experiments using this online tool, users must provide a data file in which each row represents a separate treatment condition and the colum
the keys (variables) that define the treatment condition (e.g. cell line, drug or other perturbagen, perturbagen concentration, treatment time, replicate) and the
cell counts (or surrogate such as CellTiter-Glo® or other readout). Analogous traditional metrics: IC_{50}, EC_{50}, E_{max}, E_{inf}, AUC, and h are also computed. Interac
analysis and visualization tools are provided. Detailed instructions can be found below.
Step 1: Load the data file containing cell counts for treated and control cells.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Home
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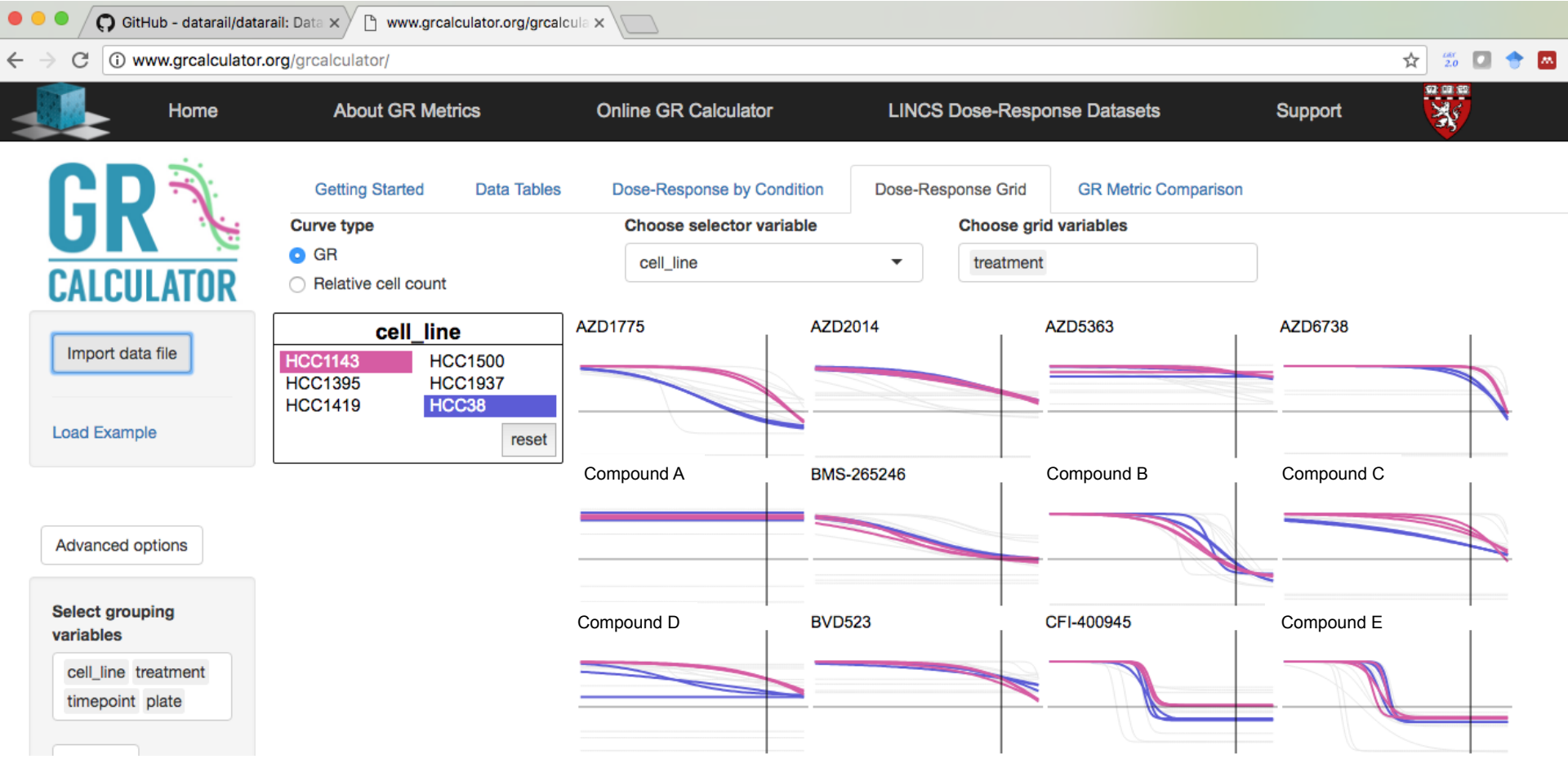
Online GR Calculator
LINCS Dose-Response Datasets
Support
GR CALCULATOR
Import data file
Load Example
Advanced options
Select grouping variables
cell_line treatment timepoint plate
Analyze
Getting Started
Data Tables
Input Data
GR Values
Fitted Parameters
Download Data File
Show 10 entries
Search:
cell_line treatment concentration timepoint cell_count cell_count__ctrl cell_count__time0 plate
HCC38 AZD6738 1 72 2031.00000000008 2629.08333333337 750.714285714287 BCA2_A
HCC38 Rucaparib 0.0316227766017 72 2318.00000000007 2629.08333333337 750.714285714287 BCA2_A
HCC38 CFI-400945 0.001 72 2593.00000000004 2629.08333333337 750.714285714287 BCA2_A
HCC38 SHP099 1 72 2115.00000000003 2629.08333333337 750.714285714287 BCA2_A
HCC38 THZ-P1-2 3.16227766017 72 2447.00000000005 2629.08333333337 750.714285714287 BCA2_A
HCC38 AZD5363 0.00316227766017 72 2560.00000000003 2629.08333333337 750.714285714287 BCA2_A
HCC38 GSK2334470 0.316227766017 72 2154.00000000001 2629.08333333337 750.714285714287 BCA2_A
HCC38 BSJ-03-124 0.0316227766017 72 1493 2629.08333333337 750.714285714287 BCA2_A
HCC38 E17 0.001 72 2765.99999999988 2629.08333333337 750.714285714287 BCA2_A
HCC38 BSJ-03-124 0.00316227766017 72 1748.99999999993 2629.08333333337 750.714285714287 BCA2_A
Showing 21 to 30 of 5,292 entries
Previous 1 2 3 4 5 … 530 Next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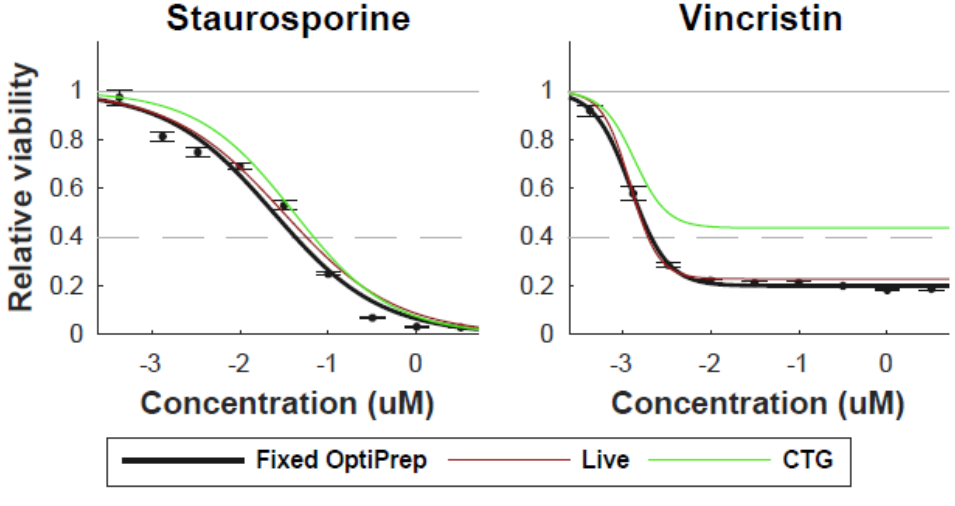
Support
GR CALCULATOR
Getting Started
Data Tables
Dose-Response by Condition
Dose-Response Grid
GR Metric Comparison
Curve type
GR
Relative cell count
Choose selector variable
cell_line
Choose grid variables
treatment
Import data file
Load Example
cell_line
HCC1143
HCC1500
HCC1395
HCC1937
HCC1419
HCC38
reset
AZD1775
AZD2014
AZD5363
AZD6738
Compound A
BMS-265246
Compound B
Compound C
Compound D
BVD523
CFI-400945
Compound E
Advanced options
Select grouping variables
cell_line
treatment
timepoint
plate

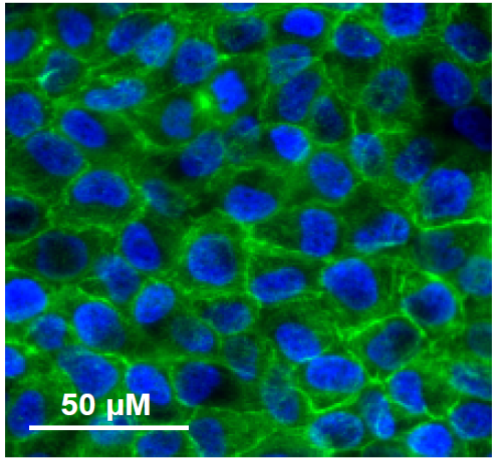
Other common dose response assays

- CellTiter-Glo etc.
  - Simple, no wash protocol
  - Luminescence read-out, simple analysis, rapid results
  - Treatment-induced changes in metabolic activity of cells can skew results
- Measurement of confluency
  - Inaccurate
  - Treatment-induced changes in morphology can skew results

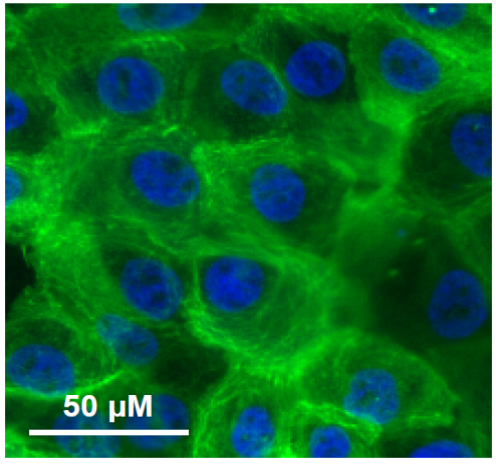


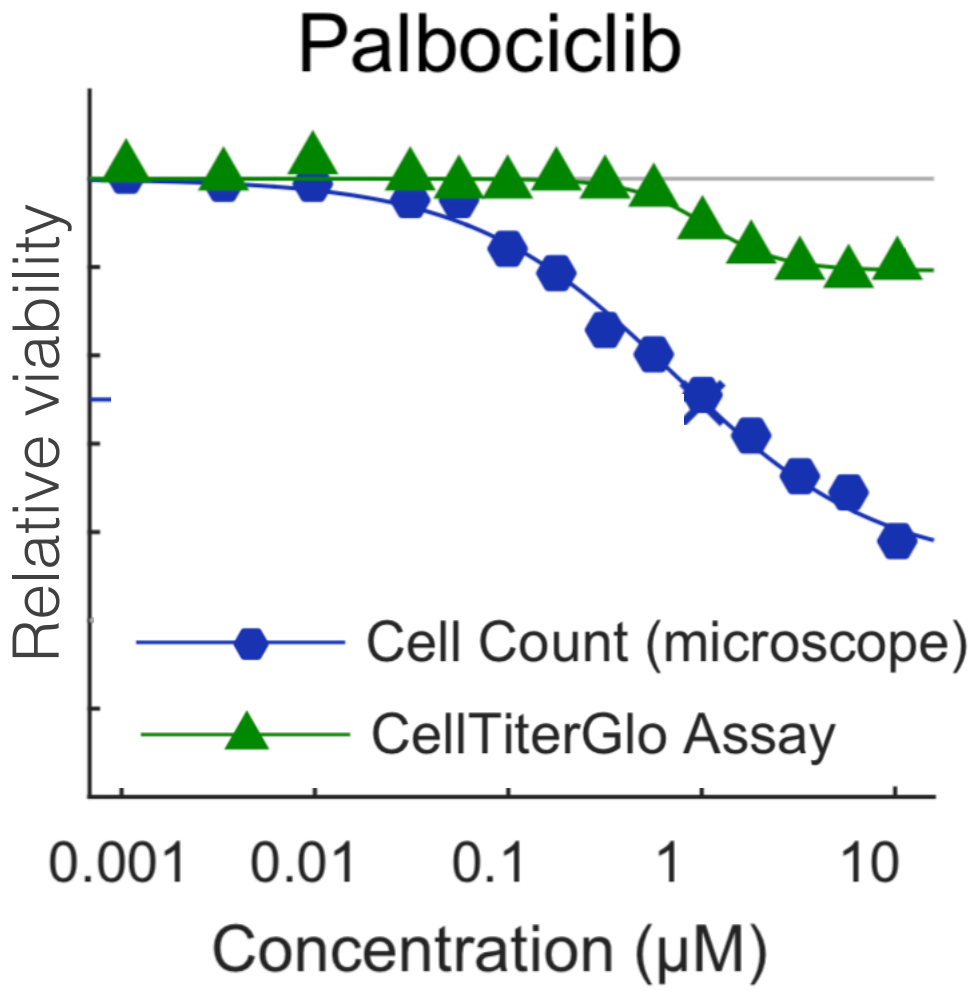
Example of artefact with a CDK4/6 inhibitor

DMSO control



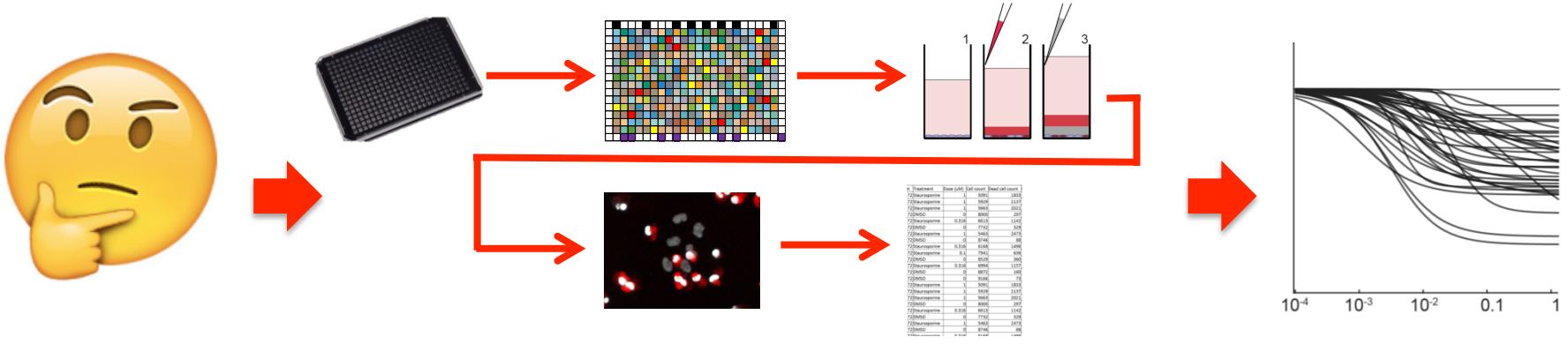
1µM Palbociclib





Conclusions

- Planning, and optimization promote reproducibility
- Automate as much as possible, know how it works
- Script the experimental design and analysis
- Use appropriate metrics for your experiment



Acknowledgements

Sorger Lab/LSP:

- Marc Hafner
- Mirra Chung
- Jeremy Muhlich
- Mario Niepel
- Luca Gerosa
- Nick Clarke
- Peter Sorger

ICCB-L:

- Stuart Rudnicki
- Caroline Shamu
- Richard Siu
- Jen Smith

Funding:

- NIH LINCS grant U54-HL127365

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- grcalculator.org
- github.com/datarail/datarail

- Hafner*, Niepel* et al. Nat Methods, 2016, 13(6):521-7
- Hafner, et al., Nat Biotech, 2017, 35(6):500-2
- Hafner*, Niepel*, Subramanian* et al., Curr Protoc Chem Biol, 2018, 9(2): 96-116
- Niepel*, Hafner* et al., Curr Protoc Chem Biol, 2018, 9(2):55-74