Optimization and Application of an Image-based Phenotypic Profiling Assay to Estimate *in vitro* Points of Departure for Chemical Bioactivity

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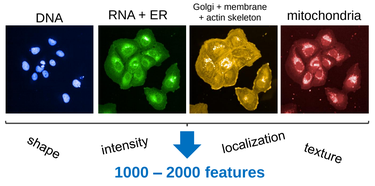
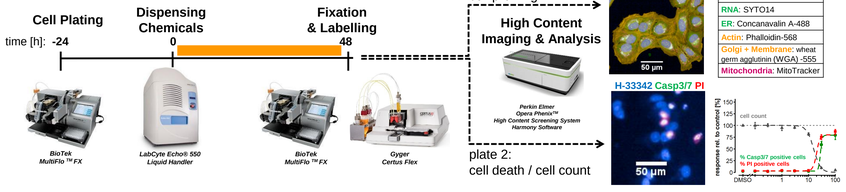
Background

- Image-based phenotypic profiling is a chemical screening method that measures a large variety of morphological features of individual cells in *in vitro* cultures.
- Successfully used for functional genomic studies and in the pharmaceutical industry for compound efficacy and toxicity screening.
- No requirement for *a priori* knowledge of molecular targets.
- **May be used as an efficient and cost-effective method for evaluating the chemical bioactivity.**



Methods

1. Experimental Workflow



Experimental Design
2 cell types: U-2 OS / MCF7
384-well plates
16 chemicals
7 concentrations (3 $\log_{10}$ units)
3 replicates / plate
3 independent experiments

2. Image Analysis Workflow (Cell Profiling)

compartments:
- nuclei
- ring
- cytoplasm
- membrane
- cell

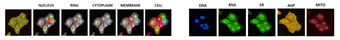
X

channels (organelles):
- DNA
- RNA
- ER
- AGP (actin skeleton / Golgi/ plasma membrane)
- mitochondria

X

parameters:
- intensity
- texture
- morphology:
  - symmetry
  - compactness
  - axial
  - radial
  - profile

= ~ 1700 endpoints



3. Data Reduction

cell-level data (~500 cells/well)

$$\frac{\text{cell value} - \text{median}_{DMSO}}{1.4826\ \text{MAD}_{DMSO}}$$

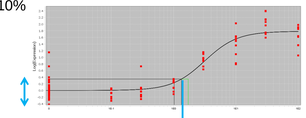
normalized cell-level data

median

well-level data

4. BMD Modelling

- Well-level data x 3 technical replicates x 3 biological replicates = 9 values
- Filtered for affected parameters using ANOVA ($p \leq 0.01$, FDR adjusted)
- BMD modelling with BMDExpress 2.0
  - 3 models: Hill, Power, Poly2
  - model selected with best logLikelihood
  - Benchmark response = 10%



Aims

1. **Miniaturize an existing assay (Bray et al. 2016) and establish a microfluidics-based laboratory workflow suitable for high-throughput screening purposes.**
2. **Test a set of 14 phenotypic reference and 2 negative compounds in two cell lines.**
3. **Evaluate the applicability of the assay for:**
   a) **grouping of chemicals with similar biological effects**
   b) **derivation of *in vitro* points of departure (POD)**

Conclusions

1. **The method was successfully miniaturized and adapted to a microfluidics-based laboratory workflow.**
2. **The method was amenable for use in multiple cell lines.**
3. **Treatment with reference compounds resulted in distinct, reproducible profiles of effects across the chemical set.**
4. **Profiling-derived PODs were often lower than cytotoxicity-derived PODs.**

Results

1. Observed profiles in U-2 OS cells

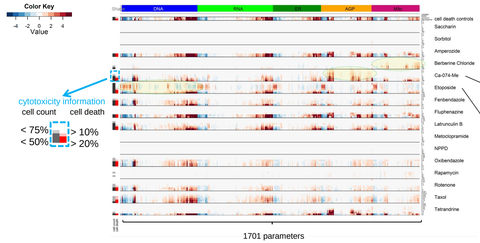
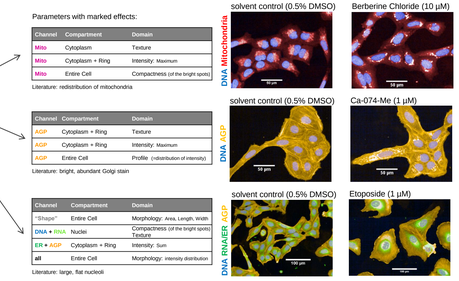


Fig 1.: MAD normalized well-level data of U-2 OS cells were averaged across 3 technical and 3 biological replicates. Endpoints are ordered according to the corresponding channel/organelle. The color key on the left indicates reductions in cell count and increases in cell death.

⇨ Treatment with different chemicals results in distinct profiles

⇨ Effects observed at non-cytotoxic concentrations



⇨ Profiles mostly consistent with literature (Gustafsdottir et al. 2013)

⇨ Measured differences correspond to visual phenotypes

2. Reproducibility among experiments

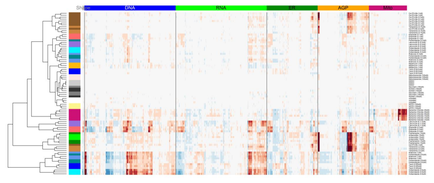


Fig 2.: MAD normalized well-level data of U-2 OS cells were averaged across 3 technical replicates. Each row represents a biological replicate of selected conditions. Conditions were filtered for effects on cell morphology in the absence of pronounced cytotoxicity. Endpoints are ordered according to the corresponding channel/organelle. Only robust endpoints are shown (e.g. their standard deviation in all DMSO control wells was < 0.25). This was the case for 1527/1701 parameters.

⇨ Biological replicates have similar profiles

⇨ Biological replicates of like treatments cluster (mostly) together

3. Chemical profiles in two cell types

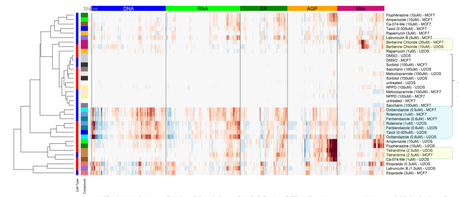


Fig 3.: MAD normalized well-level data of U-2 OS or MCF7 cells were averaged across 3 biological replicates. Each row represents a biological replicate of selected conditions. Conditions were filtered for effects on cell morphology in the absence of pronounced cytotoxicity. Endpoints are ordered according to the corresponding channel/organelle.

⇨ Treatments with similar effects cluster together across cell types

⇨ Profiles of related chemicals are more similar within cell types than across cell types

Potential Applications

1. Derivation of putative *in vitro* points of departure (POD)

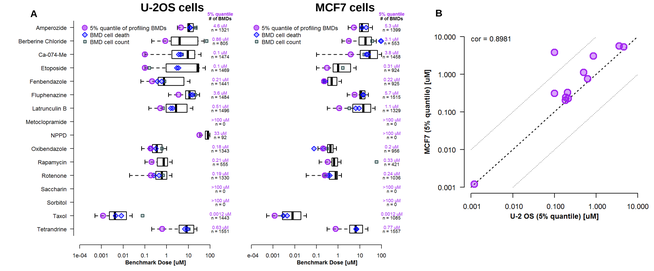


Fig 4.: MAD normalized well-level data were pooled from 3 independent experiments (9 values) to model BMDs. **(A)** The boxplot displays the range of the estimated BMDs from all parameters that were changed. The black line indicates the median: whiskers are at an interquartile range of 1. The 5% quantile of this distribution is considered the point of departure (POD) and is indicated in violet. BMDs derived from cytotoxicity and cell count measurement are indicated in blue and green for comparison. **(B)** Comparison of the PODs of the 12 active chemicals across both tested cell types.

⇨ For the majority of compounds (9/12), the profiling POD is lower than cytotoxicity-derived BMDs

⇨ Similar PODs are derived from both cell lines

2. Putative PODs of different cellular functions

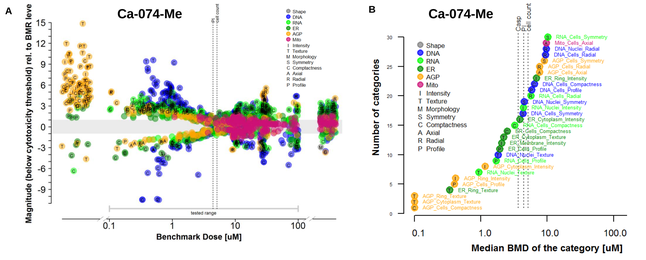


Fig 5.: MAD normalized well-level data from U-2 OS cells were pooled from 3 independent experiments (9 values) to model BMDs. Color-codes correspond the channel colors. BMDs derived from cytotoxicity and cell count measurements are indicated with dotted lines for comparison. **(A)** The derived BMDs of single endpoints are plotted against the maximal magnitude of the endpoint at non-cytotoxic concentration. The magnitude is further normalized to the benchmark response (BMR) level. Example: + 10 means 10 times above the BMR level (i.e. above noise). **(B)** The derived BMDs were grouped in categories. The accumulation plot displays the median BMD of these categories. The top 30 categories are ordered from most potent (bottom) to least potent (top).

⇨ Grouping of parameters into biological categories may inform affected cellular functions.

Future Directions

- Evaluate additional cell lines (cancer-lines and immortalized non-cancer lines)
- Test a broader set of reference compounds, and subsequently test compounds
- Investigate utility for *in vitro-in vivo* extrapolations (IVIVE) and potential applications for chemical safety decisions.