Optimization and Application of a Multivariate Image-Based Phenotypic Profiling Assay for Screening of Environmental Chemicals in U-2 OS and MCF7 Cells

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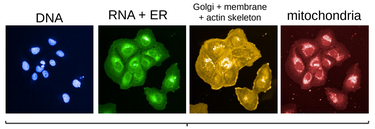
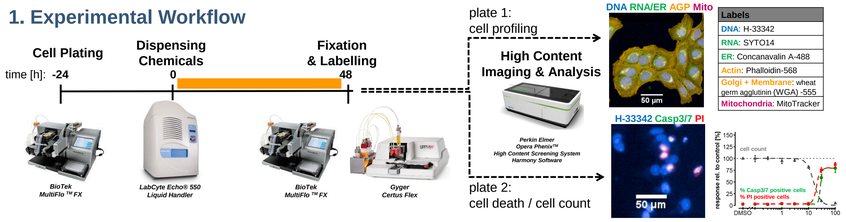
Background

- 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

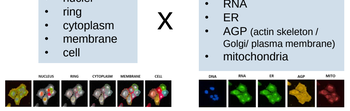
X

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

X

parameters:
- morphology
- intensity
- texture

= 1701 endpoints



3. Data Reduction

cell-level data (~500 cells/well)

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

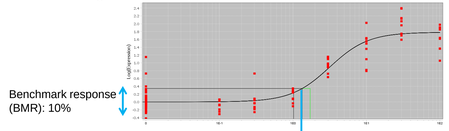
normalized cell-level data

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 (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* point-of-departures (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 more sensitive than cytotoxicity-derived PODs.**

Results

1. Observed profiles in U-2 OS cells

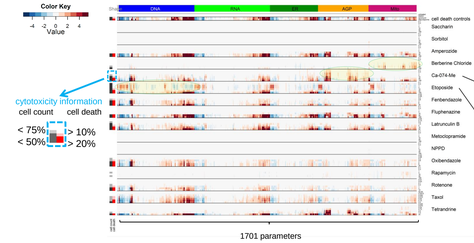


Fig 1.: MAD normalized well-level data of U-2 OS cells were averaged across 3 technical and 3 biological replicates. Endpoints are ordered according to the corresponding channel/organelle. The color key on the left indicates reductions in cell count and increases in cell death.

⇨ Treatment with different chemicals results in distinct profiles

⇨ Effects observed at non-cytotoxic concentrations

2. Observed profiles in MCF7 cells

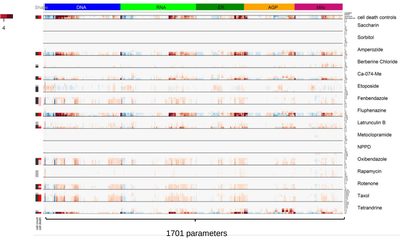
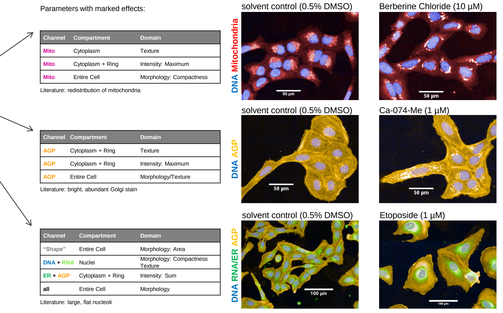


Fig 2.: MAD normalized well-level data of MCF7 cells were averaged across 3 technical and 3 biological replicates. Endpoints are ordered according to the corresponding channel/organelle. The color key on the left indicates reductions in cell count and increases in cell death.

⇨ MCF7 profiles were similar to profiles observed in U-2 OS cells

⇨ Effects are less pronounced



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

⇨ Measured differences correspond to visual phenotypes

3. Reproducibility among experiments

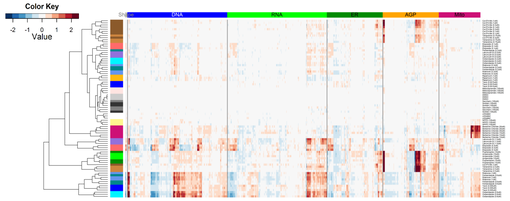


Fig 3.: MAD normalized well-level data of U-2 OS cells were averaged across 3 technical replicates. Each row represents a biological replicate of selected conditions. Conditions were filtered for effects on cell morphology in the absence of pronounced cytotoxicity. Endpoints are ordered according to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

Potential Applications

1. Biological similarity

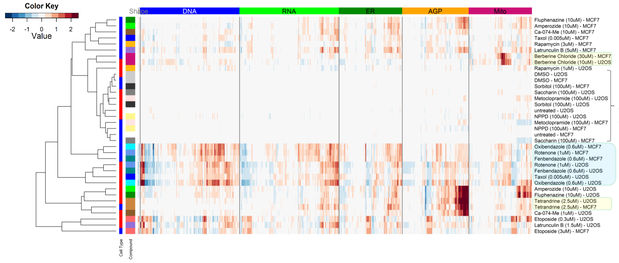


Fig 4.: MAD normalized well-level data of U-2 OS or MCF7 cells were averaged across 3 biological replicates. Each row represents a biological replicate of selected conditions. Conditions were filtered for effects on cell morphology in the absence of pronounced cytotoxicity. Endpoints are ordered according to the corresponding channel/organelle.

⇨ Treatments with strong effects cluster together across cell types

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

2. Derivation of *in vitro* point-of-departures (POD)

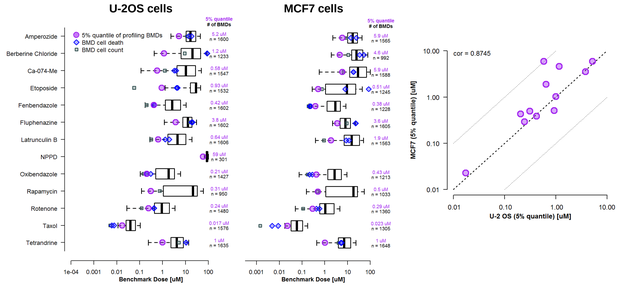


Fig 5.: MAD normalized well-level data were pooled from 3 independent experiments (9 values) to model BMDs. The boxplot displays the range of the estimated BMDs from all parameters that were changed. The black line indicates the median: whiskers are at an interquartile range of 1. The 5% quantile of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

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

⇨ Similar PODs are derived from both cell lines

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 use in screening level risk assessment.