

Spatially Aware Plate Layouts

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Multi-well plate experiments are fundamental to modern biochemistry, pharmacology, and molecular biology

They enabling **high-throughput screening** and systematic investigation of multiple experimental interventions

Challenge: Spatial Bias

Measurements are often systematically affected by spatial bias across the plate

Potential sources

- Evaporation
- Cell or reagent degradation
- Pipette malfunctioning
- Variation in incubation time
- Temporal drift during measurement
- Thermal gradients (incubation or readout)
- Lighting or optical non-uniformity in plate readers

How Bias Manifests

- Over- or under-estimation of true signals
- Characteristic patterns:
 - Bowl-shaped distortions
 - Linear gradients

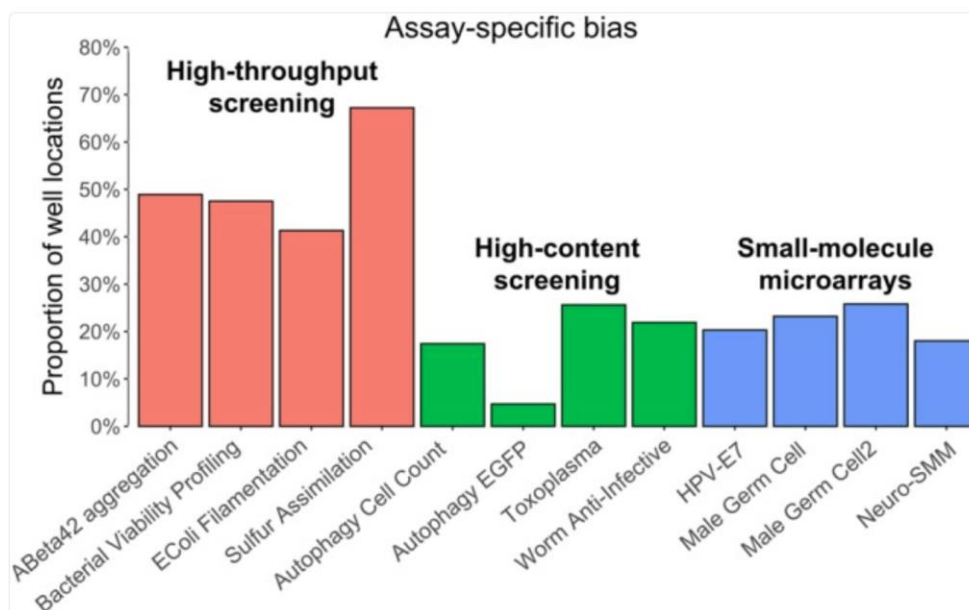
Consequence

- Increased false positive and false negative rates



Most screening technologies are prone to spatial bias

Mazoure et al. 2017



Setting

- 12 experimental assays from the ChemBank
- All wells same experiment
- Compare each well location with the remaining plate
- Report proportion of wells significantly different

Results

- All screening technologies are prone to spatial bias
- Particularly strong in HTS assays
- Use additive or multiplicative model to correct for the bias

Spatial balance improves accuracy & reduces errors

Rodríguez 2023

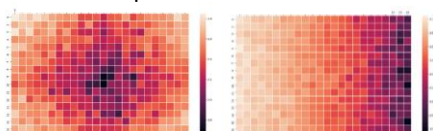
Simulation study:

- 20 negative controls
- 384 well plate

Plate effects

Bowl shaped

Linear

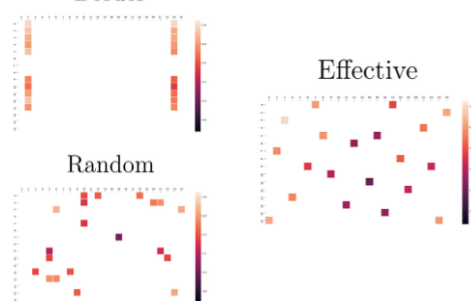


Placement of negative control

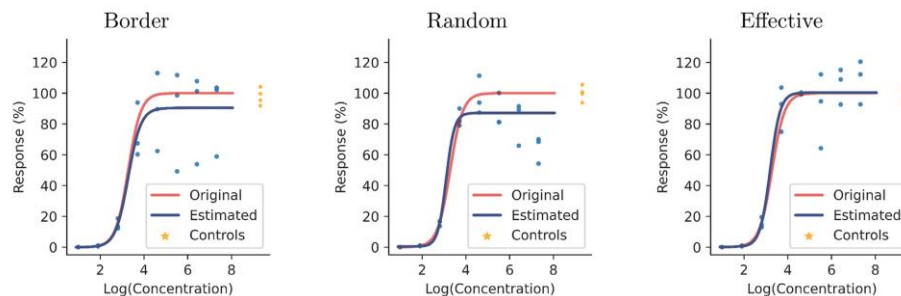
Border

Effective

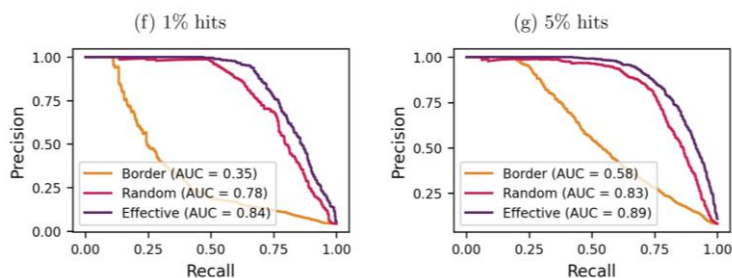
Random



More accurate dose-response curves



For drug screening: increased precision



Solution for spatial bias

Downstream correction (standard practice)

- Normalisation methods (B-score, LOESS, robust Z-scores, additive or multiplicative models)
- Plate corrections (min–max scaling, fold change)
- Key assumption: balanced plate layout

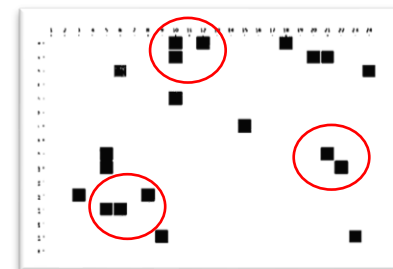
Experimental design (statistical best practice)

- Randomisation of controls and treatments [1–3]
- Spatial balance improves accuracy & reduces errors [4]

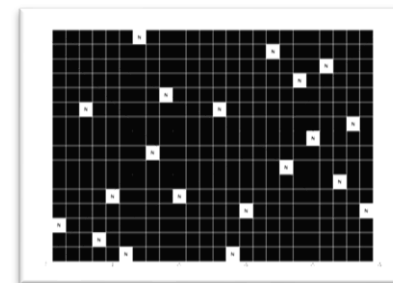
Practical limitations

- Naïve randomisation → local clustering → poor spatial coverage
- Existing approaches incompatible with many lab constraints
 - Cannot exclude edge wells
 - Do not support column-wise treatments

Naïve Random



SAPL



- 1: Normandeau, Frédéric, et al. "Spatial bias in antibody microarrays may be an underappreciated source of variability." *ACS sensors* 6.5 (2021): 1796-1806.
- 2: Mazouze, Bogdan, Robert Nadon, and Vladimir Makarenkov. "Identification and correction of spatial bias are essential for obtaining quality data in high-throughput screening technologies." *Scientific reports* 7.1 (2017): 11921.
- 3: Malo, Nathalie, et al. "Statistical practice in high-throughput screening data analysis." *Nature biotechnology* 24.2 (2006): 167-175.
- 4: Rodríguez, María Andreína Francisco, Jordi Carreras Puigvert, and Ola Spjuth. "Designing microplate layouts using artificial intelligence." *Artificial Intelligence in the Life Sciences* 3 (2023): 100073.

Method

- Understand the experimental needs
- Formalise as design constraints
- Integrate into randomisation

Generate layouts satisfying

- Randomised
- Spatial balance
- Feasible in practice

Relationship to existing methods

- Same optimality criterion as Rodríguez (2023)
- Reduces repeats in rows, columns and 3 x 3 areas
- Much broader range of experimental constraints



Spatially Aware Plate Layouts

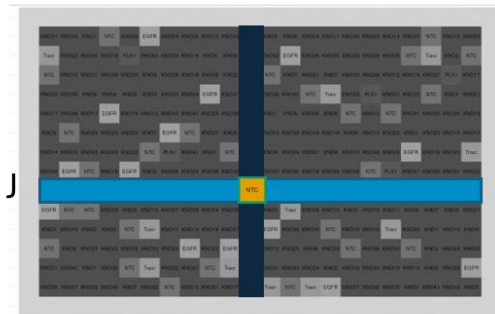
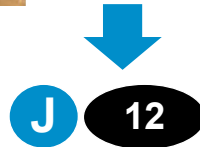
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1. Reducing repetition in rows and columns



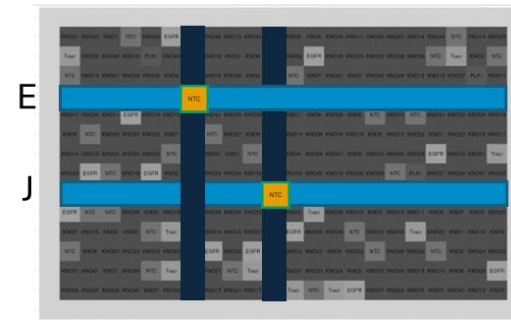
Draw at
random
without
replacement



12



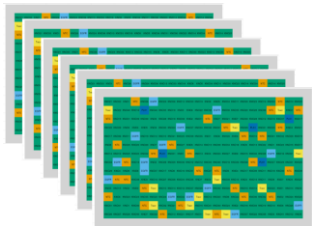
Draw at
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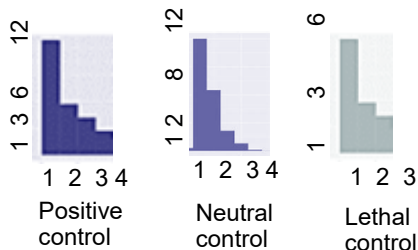
8 12

2. Reducing repetitions in local 3x3 areas

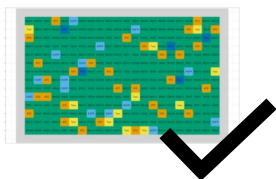
Create 100 plate maps



Count repetitions in 3x3 areas for all control types



Choose the plate map with lowest spatial variance



Compute spatial variance

For every local 3x3 area starting at well (p, q) , how many wells are filled with control type C_i for the plate map f :

$$\phi_i(p, q) = \sum_{u=p}^{p+2} \sum_{v=q}^{q+2} \mathbf{1}_{f(u,v)=C_i}$$

Compute the variance of these counts for each control type:

$$\sigma_i^2 = \text{Var}(\{\phi_i(p, q) : (p, q) \in \mathcal{P}\})$$

Define the spatial variance as the weighted sum of variances, including a penalty term for the highest count:

$$J(f) = \sum_{i=1}^k w_i \sigma_i^2 + \lambda \max_{i,p,q} \phi_i(p, q)$$

Key features

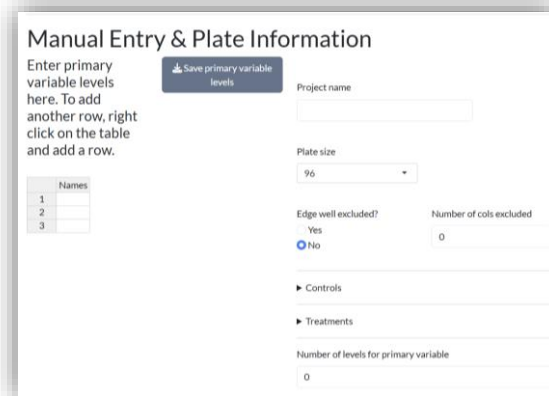
- **R shiny app** (no coding expertise required)
- Reduces generation time from **1 day to 2 min** (for 384 well plate)
- **Validation:** Extensively beta-tested with functional genomics scientists at AstraZeneca and Functional Genomics Screening Lab

Input

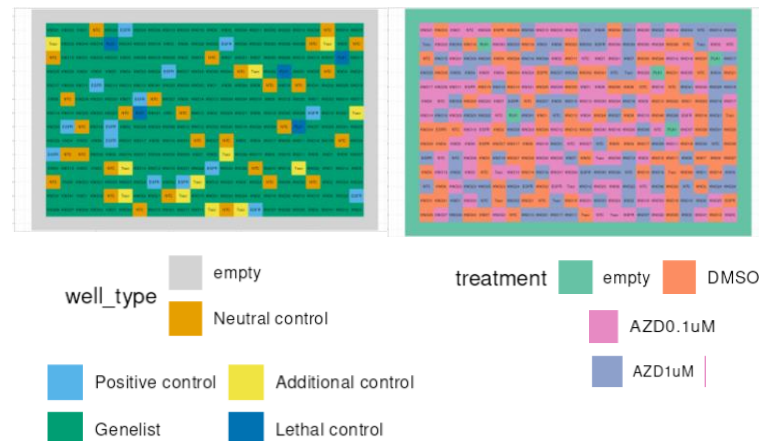
- Plate formats: **24-**, **96-**, and **384-well**
- **Flexible** inclusion of multiple positive, neutral, and lethal controls
- Accommodates **multiple treatments**, assigned freely or by column
- Generates **multiple plates** with shared or varying design features
- Customisation options: Exclude outer rows/columns, neutral controls in corners, pair neutral and positive controls

Output

- **Quality control and visual feedback**
- **Multiple export options:** E.g. exports echo pick list as an input and generates a plate map file compatible with echo dispensing system
- **Reproducibility:** Downloadable and reloadable input configurations



Visualisation



Accessing the tool & acknowledgements



The tool

- Tool (public): <https://sapl.cs.bath.ac.uk>
- For questions, advice, or feedback: be323@bath.ac.uk
- Training videos: <https://sapl.cs.bath.ac.uk/video-guides>
- Company internal version: email be323@bath.ac.uk

Collaborators

- Functional Genomics Screening Lab
- AstraZeneca

Funding

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