A Benchmark Dataset for Phenotypic Compound Similarity in Inflammasomes Screens

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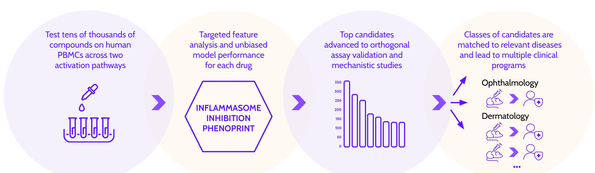
Introduction

Scientists at Spring have identified 7+ inflammasome inhibition targets that can be advanced in clinical programs whose pathologies are most relevant to the targets and their mechanisms of action.



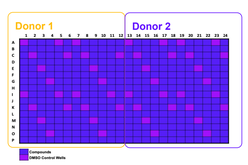
One of the key technologies that powered this effort is a method to computationally score the phenotypic similarity between compounds.

This analytical method can be applied to compound screening data and used as a filter to identify compounds that have true and consistent signals above experimental confounders. Similarity scoring from a filtered set of compounds can then be used to validate compound classes, to identify novel compounds with high similarity to known controls, or to create compound similarity classes that can be investigated for common mechanisms of action. This approach is agnostic to other biological readouts and can be used as a complementary and unbiased tool to identify and validate compound hits.

Methods

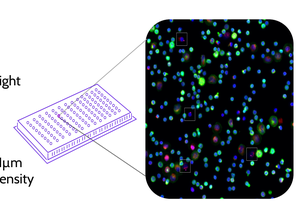
Experimental setup

SCREEN
- 16 Primary human PBMC donors
- 384 well plates
  - 2 donors per plate
  - 168 compounds per plate
  - 4 sets of compound (672 compounds total)
- Cells plated at 15k cells per well in RPMI 1640 + 10% HI FBS
- Activated 1 µg/mL Flagellin
- Incubated for 4 hrs
- 8 plates over 4 runs

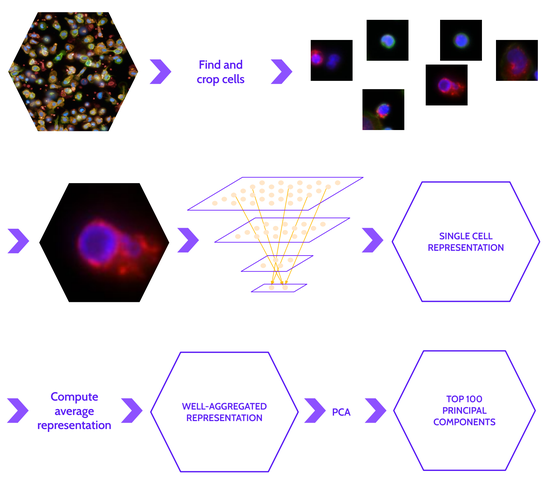


STAINING
- Hoechst: 0.25 µg/mL [Blue]
- Phalloidin: 33 nM [Green]
- Concanavalin A: 24 µM [Red]
- Straining post fix and perm overnight

IMAGING
- ImageXpress Confocal HT.ai
- 40x ELWD air objective
- 4 tiles per well, 4 image stacks at 1µm
- 2D projection using maximum intensity



Spring's well representation



Conclusions

We presented here a method to assess the impact of compound behavior in the context of inherent sources of experimental variability such as independent donors, plates, wells, and execution dates. Importantly, this kind of analysis allows us to assign a measurement to the true signal of compounds over experimental noise so we can accurately gauge compound consistency (how similar a compound is to itself across experimental variables), and compound similarity to other molecules (how similar a compound is to other compounds) in an unbiased manner.

For example, there is a subset of seven compounds in this dataset that have been previously identified to act on the same protein target. Of these seven, five are predicted from our dataset to have very close similarity to each other, while two diverge and instead are highly similar to compounds that have been reported to induce cell death. Orthogonal biological readouts validated this finding [1] where the five compounds that behave similarly have robust, non-toxic effects on cells, while the two divergent compounds result in pronounced cell death and toxicity.

The benchmarks introduced here also provide a target to refine the algorithmic choices within the compound similarity method itself. For example, we show that the dimensionality reduction via principal component analysis, and the choice of model architecture for feature extraction play an important role in boosting the capability of recognizing similar phenotypes.

Reference:

[1] Using Machine Learning to Harness the Complexities of Inflammasome Biology for Novel Drug Discovery

[slide deck]
Inflammasome Therapeutics Summit • December 1, 2022

[video presentation]
Spring Discovery • July 26, 2022

Explore The Dataset!

Access the Inflammasome Benchmark Dataset on the Spring platform:

https://www.springdiscovery.com/SLAS

Results

Compound similarity

The cosine distance between well representations is used to quantify the similarity of the corresponding compounds. The accuracy of matching wells treated with the same compound provides a benchmark for the algorithm.

Benchmark definition
- Given a query well and a search pool, rank the wells in the search pool by ascending cosine distance.
- Accuracy metrics:
  - Top 0.1%: at least one well with the same compound as the query is ranked among in the top 0.1% of the pool
  - Top 50: at least one well with the same compound as the query is ranked among in the top 50 of the pool
- Search pools:
  - All Wells: the search pool includes all the wells in the dataset
  - Not Same Plate: only wells from plates other than the query well's are included in the search pool
  - Not Same Donor: only wells from donors other than the query well's are included in the search pool

	# of Pairs Tested	Top 0.1%	Top 50
All Wells	1,259,968	14.9%	2.18%
Not Same Plate	1,218,384	14.7%	2.04%
Not Same Donor	1,186,952	14.9%	2.23%
Not Same Plate & Not Same Donor	1,163,088	14.7%	2.08%

Compound phenotypic consistency

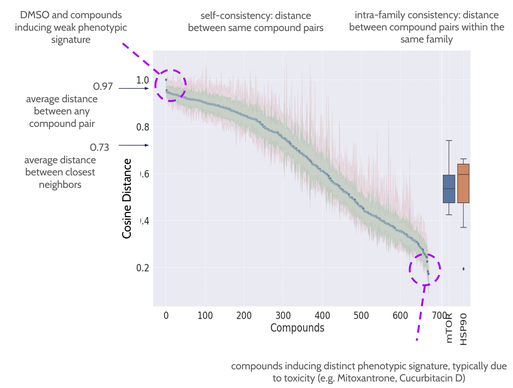
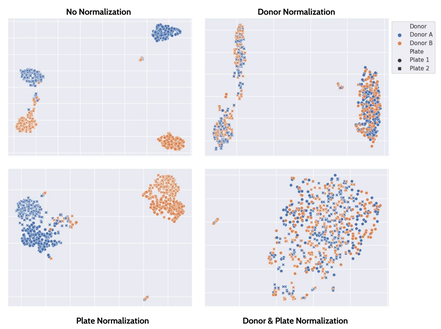
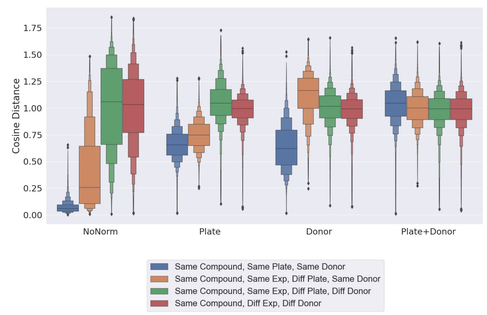


Plate and donor confounders

Normalization: center and standardize the well representation using DMSO wells as reference.
- Plate Normalization: use DMSO wells from the same plate as reference
- Donor Normalization: use DMSO wells from the same donor as reference





	No Normalization		Plate Normalization		Donor Normalization		Plate & Donor Normalization	
	Top 0.1%	Top 50	Top 0.1%	Top 50	Top 0.1%	Top 50	Top 0.1%	Top 50
All Wells	13.48	1.12	15.12	2.01	15.44	1.96	14.91	2.18
Not Same Plate	11.53	0.48	14.17	1.61	13.42	1.33	14.74	2.04
Not Same Donor	9.70	0.33	11.37	1.38	14.65	1.56	14.95	2.23
Not Same Plate & Not Same Donor	8.89	0.27	11.40	1.28	13.50	1.32	14.72	2.08

(all values represent percentage accuracies)

Impact of algorithmic choices

Visual feature extraction model

Model	All Wells	Not Same Plate	Not Same Donor	Not Same Plate & Not Same Donor
VGG	1.90	1.80	1.96	1.85
EfficientNet-V2	2.18	2.04	2.23	2.08

PCA projection and trim

	All Wells	Not Same Plate	Not Same Donor	Not Same Plate & Not Same Donor
No PCA	1.72	1.61	1.76	1.64
PCA All	1.94	1.80	2.00	1.84
PCA100	2.18	2.04	2.23	2.08

Well-aggregation strategy

	All Wells	Not Same Plate	Not Same Donor	Not Same Plate & Not Same Donor
Median	1.90	1.76	1.91	1.77
Mean	2.18	2.04	2.23	2.08

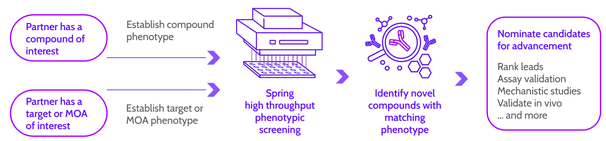
Distance metric

	All Wells	Not Same Plate	Not Same Donor	Not Same Plate & Not Same Donor
Euclidean	2.36	2.26	2.34	2.24
Cosine	2.18	2.04	2.23	2.08

(all values represent top 50 percentage accuracies)

Work With Us!

Spring models complex disease phenotypes to help partners identify novel candidates



Partner with Spring

Spring's technology is being used in many different therapeutic areas (including immunology and dermatology) with a range of partners, and we're open to more. We're excited to discuss both strategic collaborations and software licensing with biotech companies, pharma companies, academic groups and other research organizations looking for novel biological insights or tools to accelerate discovery and development.

- Automated lab and software system capable of screening >2,500 compounds per week and measuring >100 features simultaneously in a single well
- Many human donors across many tissue and primary cell types - fibroblasts, myoblasts, PBMCs, and tissue histological samples
- >12,000 small molecules readily available in our library that can be augmented with proprietary small and large molecular libraries
- Machine learning powered software MegaMap to navigate vast amounts of complex high throughput data along with customizable scoring that applies differential weights to hundreds of features to accelerate compound discovery and decision making

Acknowledgements

Disclosures:
All authors were employees of Spring Discovery at the time this work was conducted.

Link to poster