AI-Powered Cell Profiling Enables the Quantitative and Functional Evaluation of Therapies Targeting Muscle Disorders in Patient-Derived Myotubes

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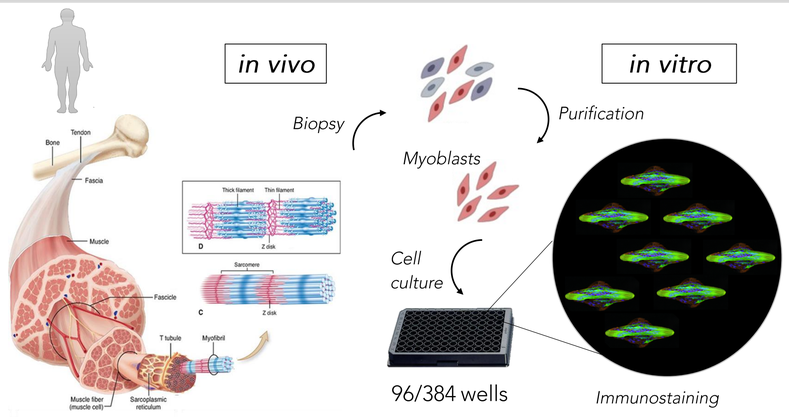
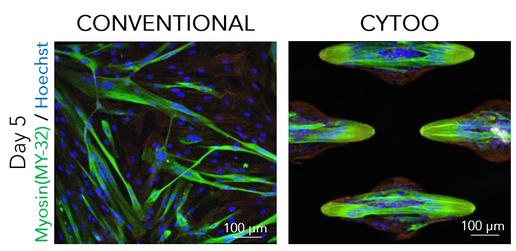
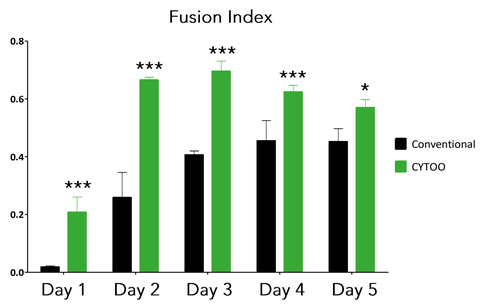
MyoScreen™ Discovery Platform: Patient-derived primary myoblasts



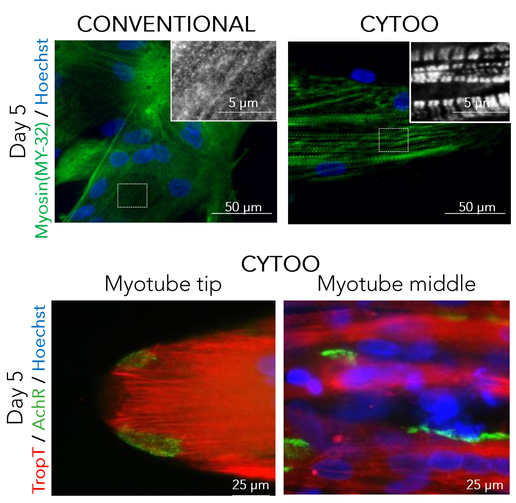
From Young et al., 2018



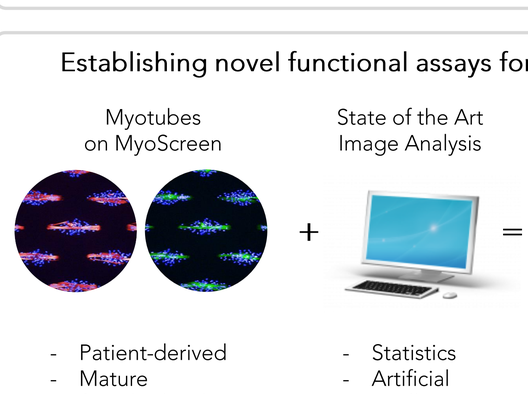
- Aligned and linear myotubes from patient-derived myoblasts
- Myotubes are standardized and can be easily quantified by High Content Analysis (100 per well/96 wells plate)



- Myotube maturation is greater and faster on MyoScreen plates compared to conventional culture



- Sarcomeric striation is increased on MyoScreen plates compared to conventional plates



- Myotubes are poised for motor neuron connections as indicated by AChR clusters typical for neuro-muscular junctions at the tip and in the middle of myotubes

Establishing novel functional assays for muscle disorders

- Patient-derived
- Mature
- Standardized
- 100 per well (96w)

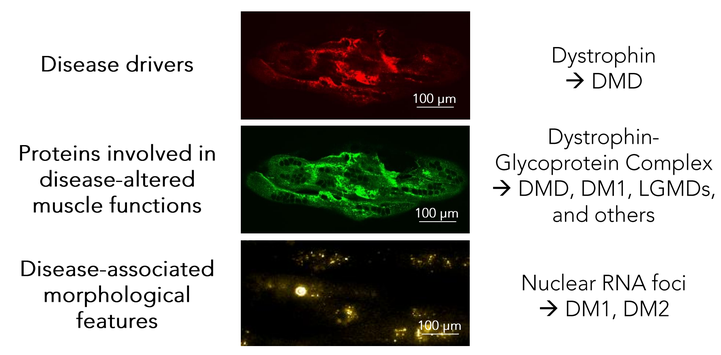
- Statistics
- Artificial Intelligence

- Colocalization
- AI-powered cell profiling

AI-Powered Cell Profiling: Monitoring disease reversal with Health-Score™

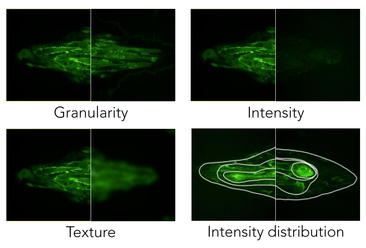
A. Identify phenotypes that distinguish healthy and disease donors

AI-powered cell profiling can be performed with diverse image markers:



B. Image segmentation and feature extraction

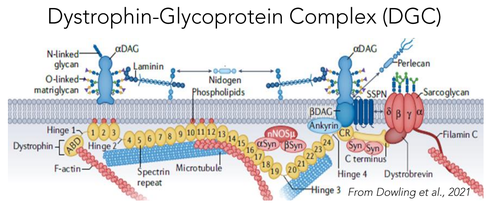
- Segmentation of myotubes using CellProfiler™
- Phenotypic image features extracted for each MyoScreen pattern
- 594 features extracted per pattern



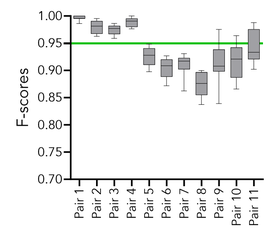
C. Optimization of cell profiling and profile generation for DMD

- Dystrophin expression and DGC assembly is impaired in DMD
- DGC assembly is critical for DMD therapy development

Dystrophin-Glycoprotein Complex (DGC)



From Dowling et al., 2021



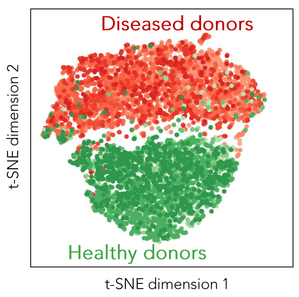
Pair	DGC 1	DGC 2
1	Utrophin	α-SG
2	Utrophin	δ-SG
3	Utrophin	α-DG
4	Utrophin	β-DG
5	α-DG	α-SG
6	β-DG	α-SG
7	α-SG	δ-SG
8	δ-SG	α-DG
9	α-SG	Syntrophin
10	α-SG	Dystrobrevin
11	Dysferlin	δ-SG

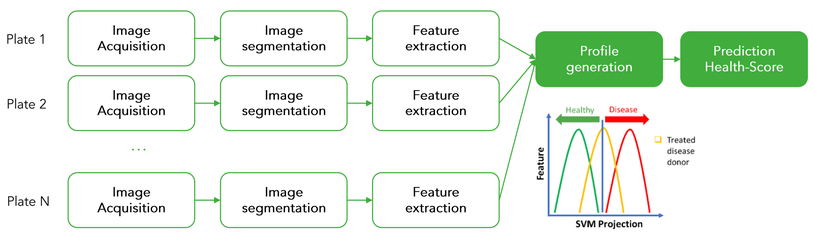
SG: Sarcoglycan
DG: Dystroglycan

- Identification of the optimal combination DGC proteins
- Pair1: Utrophin and α-Sarcoglycan fluorescent staining were chosen for cell profiling based on highest F-score

- Generalization to a variety of phenotypes and morphologies
- 15,000 replicates per phenotype
- 5 healthy donors
- 7 DMD donors with deletions in following exons:

Exon 18-44	Exon 46-55	Exon 45-52
Exon 46-52	Exon 45	Exon 45 Immortalized

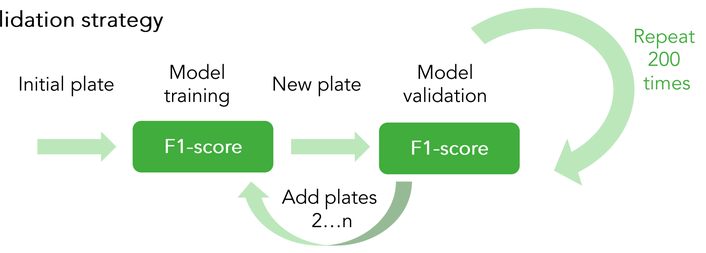




D. Effect of new plates and donors on F1-Score: Training data expansion

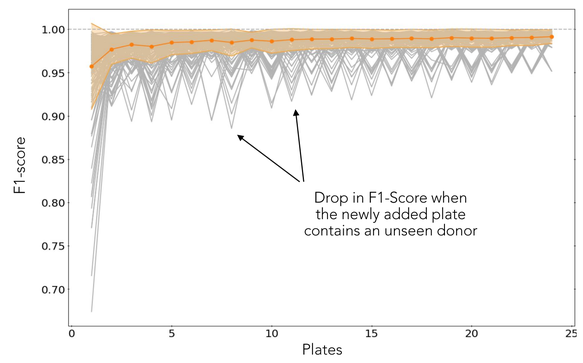
SVM model trained on a variety of phenotypes to improve robustness
Goal: F-score > 0.95

Validation strategy



Performance Improvement

- The addition of each plate to the training data consistently enhances separation on the unknown plate, steadily improving the F1-score

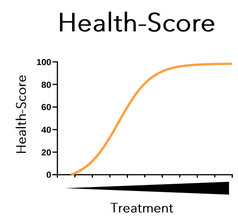


Initial Drop in Performance

- With the introduction of a new plate, there is a notable decrease in the F1-score attributed to the emergence of new donors
- Convergence of F1-score after 8 plates

E. Quantify the phenotypical response of disease donors to therapy

- Analysis of treated disease donors
- Quantify the number of myotubes that have recovered healthy-like phenotype through treatment
- Health-Score = % phenotypically healthy myotubes out of total



F. Applications for AI-powered cell profiling

- AI-powered cell profiling assays are cell-based functional assays that quantify the ability of therapies to reverse disease phenotypes in patient-derived cells using a Health-Score
- Profiling of phenotypic features does not require knowledge of disease mechanism of action
- AI-powered cell profiling assays serve all stages of drug discovery/development

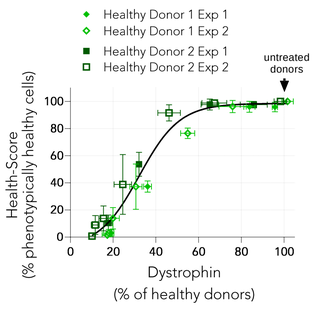
AI-Powered Cell Profiling

Target ID & Validation	Lead ID/OP	Candidate Selection	Pre-clinical Development	Clinical Development	Market
– Disease reversal	– Potency – Efficacy – Specificity		– MOA – Biomarker ID		– GMP-compliant potency assay – Patient stratification

Health-Score assessment in muscle disorders

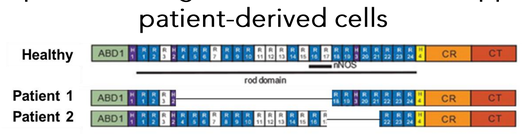
DMD

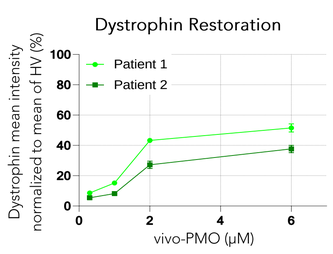
The Health-Score calibrates the response of cells to loss of dystrophin



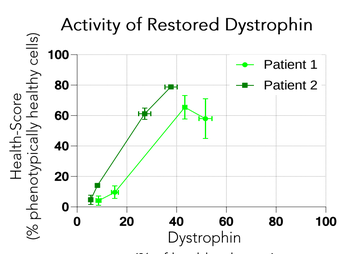
- RNAi-induced downregulation of DMD in healthy donors
- Cells with dystrophin levels <20% display a disease phenotype
- Cells with dystrophin levels >60% display a healthy phenotype
- Aligns with results from animal studies

The Health-Score assesses the activity of restored dystrophin after oligo-mediated exon 45 skipping in patient-derived cells





Patient 1 responds better to exon skipping therapy than patient 2



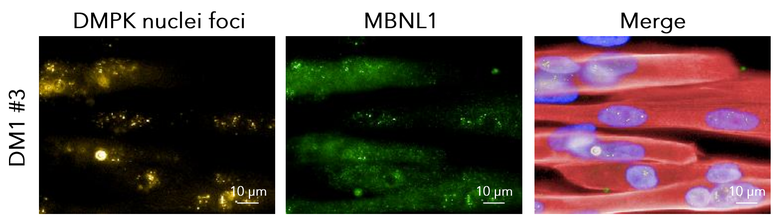
Restored dystrophin of patient 2 is more active

DM1

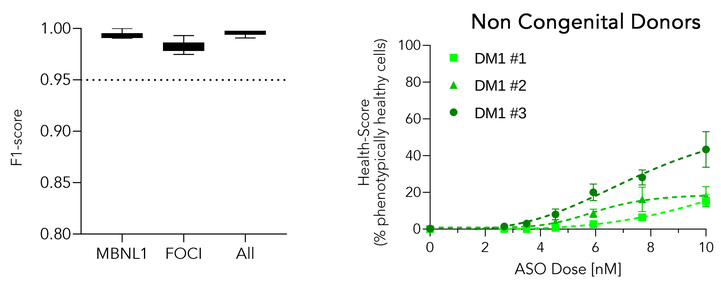
Labeling myotubes for DMPK foci/MBNL1 segregates healthy and DM1 donors

Disease	Donor	Sex	Age	Form	CTG repeat size (blood)	Muscle weakness	Myotonia
Healthy		F	20	-	5-37	-	-
DM1	DM1-1	M	27	Infantile	1300	+	++
	DM1-2	M	38	Juvenile	350	++	+++
	DM1-3	M	34	Late onset	300	None	None

+ low
++ moderate
+++ severe



ASO treatment of DM1 donors restores a phenotypically healthy phenotype



- AI-powered cell profiling of DM1 donors monitoring DMPK foci/MBNL1
- DM1 donors differ in their sensitivity to the ASO treatment

Conclusions

- MyoScreen enables the development of novel quantitative and functional cell-based assays
- In particular, the AI-powered cell profiling assays
  - are robust, versatile assays that can be applied to human primary cells or immortalized cells lines with disease-associated phenotypes
  - serve various stages from drug discovery and development, to potency assays for commercial release of gene therapy products
  - can be applied to High Content Screening and batch release testing
- AI-powered cell profiling assays can be exploited for
  - evaluating the efficacy of therapies aiming to restore the activity of a disease driver
  - analyzing the responses of different patients to a therapy
- AI-powered cell profiling will enable drug discovery for disorders with complex genetic backgrounds, multifactorial mechanisms, or unidentified mode of action, such as metabolic myopathies

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Acknowledgment

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