Artificial Intelligence Enables Structural Toxicity Testing for Enpoint and Multiple Timepoint Assays

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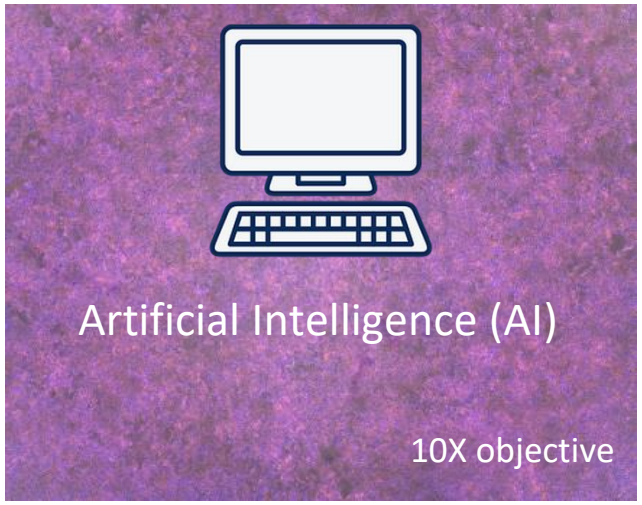
Maddah M et al. J Pharmacol Toxicol Methods. 2020

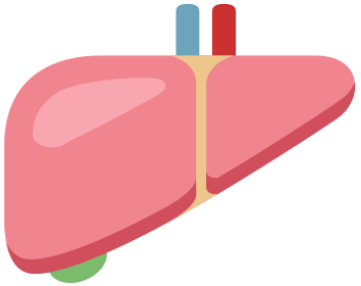
Disclaimer: The opinions expressed in this presentation are mine and do not necessarily reflect the official views of my present and past employers

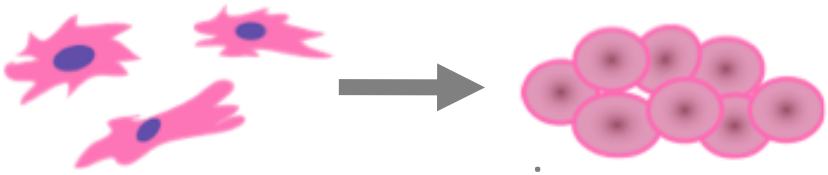
Scientific Innovation

Regulatory Application



Screen hepatic and cardiac toxicity of drugs





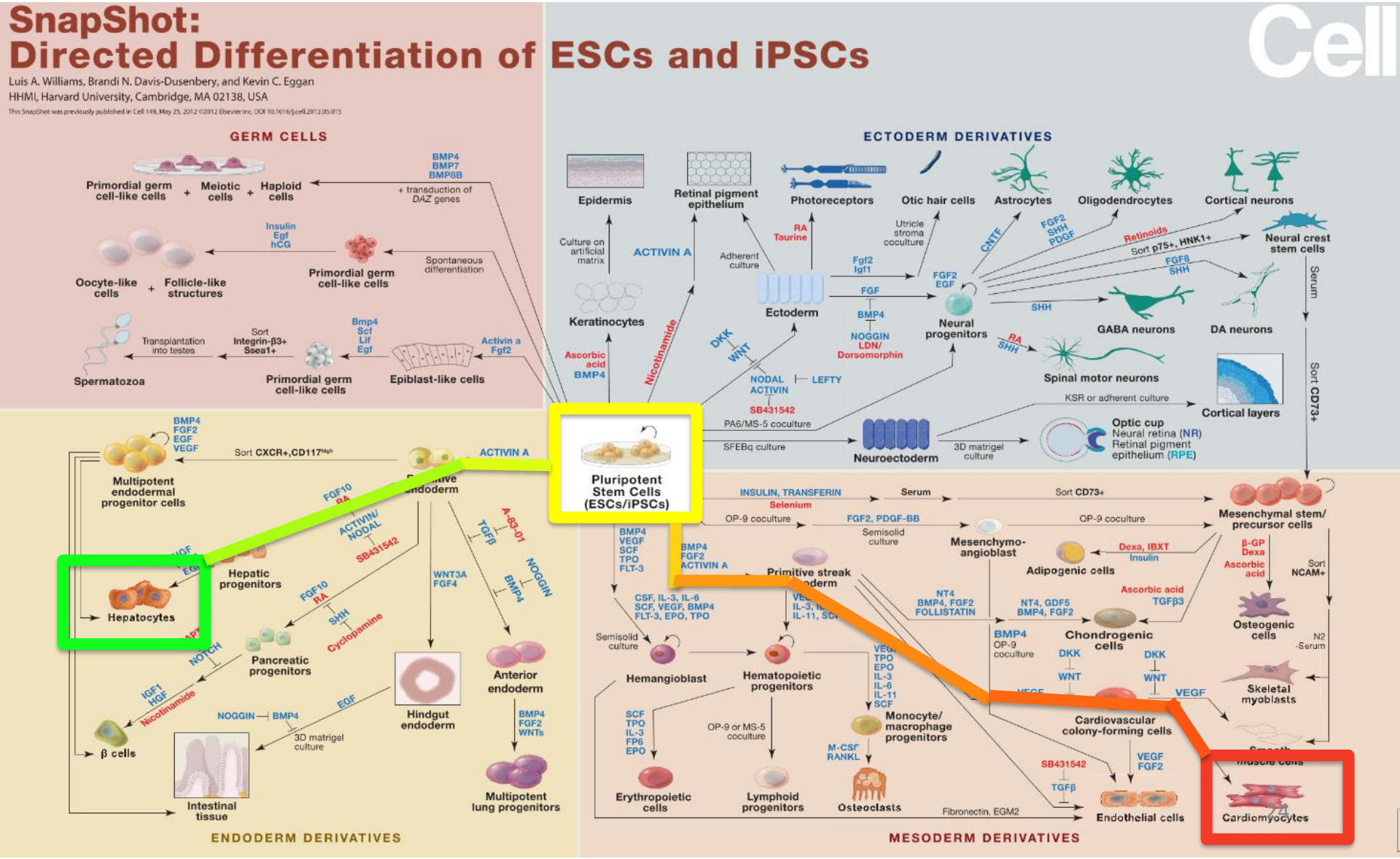
blood cells
skin cells
fat cells

reprogramming factors

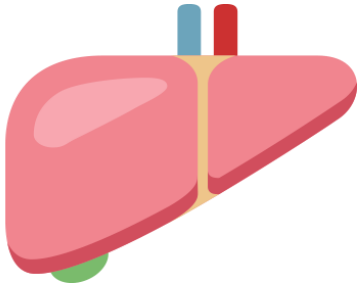
induced pluripotent stem cells

in vitro

iPSCs can be differentiated into a variety of cell fates: cardiac and hepatic



Adverse hepatic and cardiac effects are the main causes for drug attrition



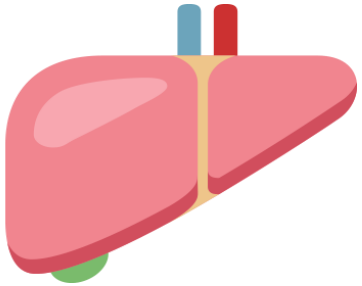
- Account >75% safety-related withdrawals: Weaver & Valentin (2019) Toxicol Sci, 167(2)
- Liabilities often detected late in drug development: Laverty, Benson et al. (2011) Br J Pharmacol, 163(4)
- Pressing need for more predictive ***in vitro* tools**
  - Development of hepatic and cardiac toxicity models is a major focus in safety pharmacology: Fermini, Coyne, & Coyne (2018) SLAS Discov, 23(8)

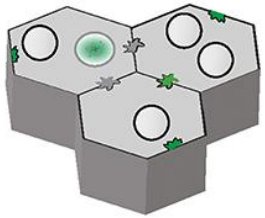
  - *"10% improvement in predicting failure before the initiation of expensive and time-consuming clinical trials could save upwards of $100 million in the costs associated with drug development"*: Allen, Jeffrey, Williams, & Ratcliffe (2010) Drugs of the future, 35(1)

Cellular systems can model functional tissue properties and enable mechanistic studies

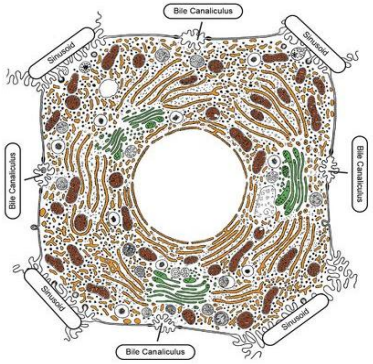
functional cell types

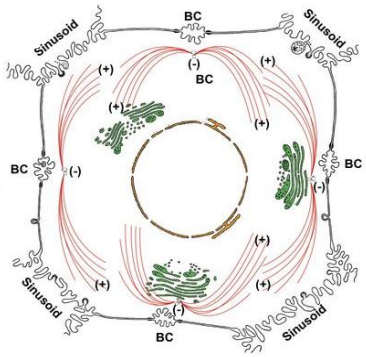
intracellular structure relates to function

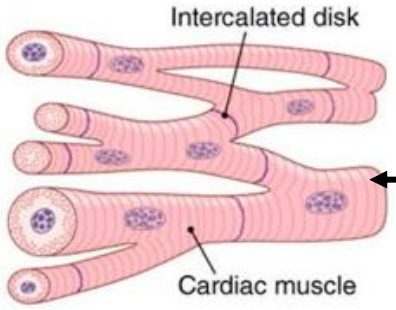




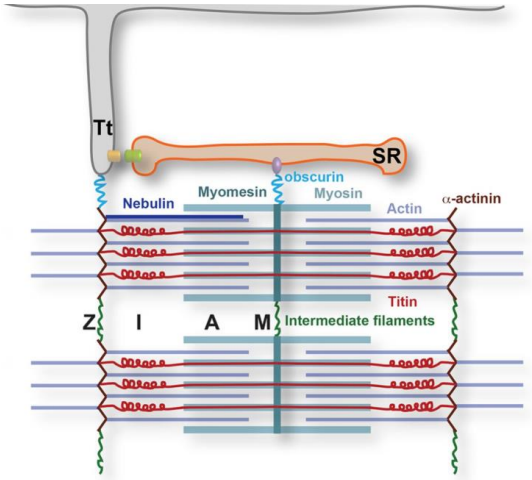
hepatocytes







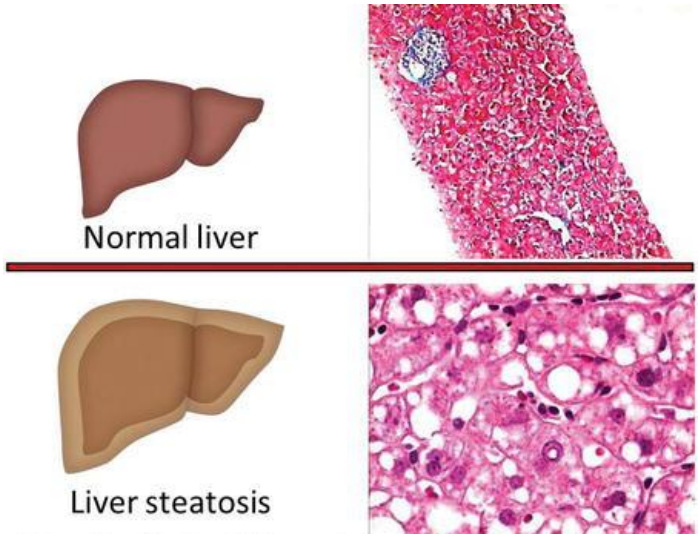
cardiomyocytes

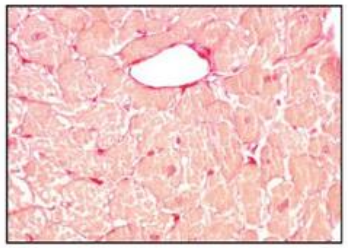


Balasubramanian et al. (2020) Front Cell Infect Microbiol 9:451
Orr-Weaver (2015) Trends Genet 31(6)
Pandit et al. (2015) Trends Cell Biol 23(11)

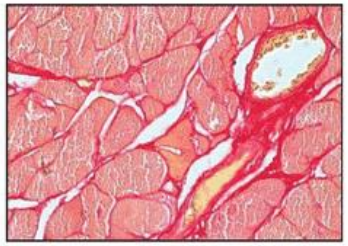
Gautel & Djinović-Carugo (2016) J Exp Biol 219(Pt 2)
Schulze et al. (2019) JCB 218(7)

Tissue disfunction involves intracellular structural changes





Normal heart



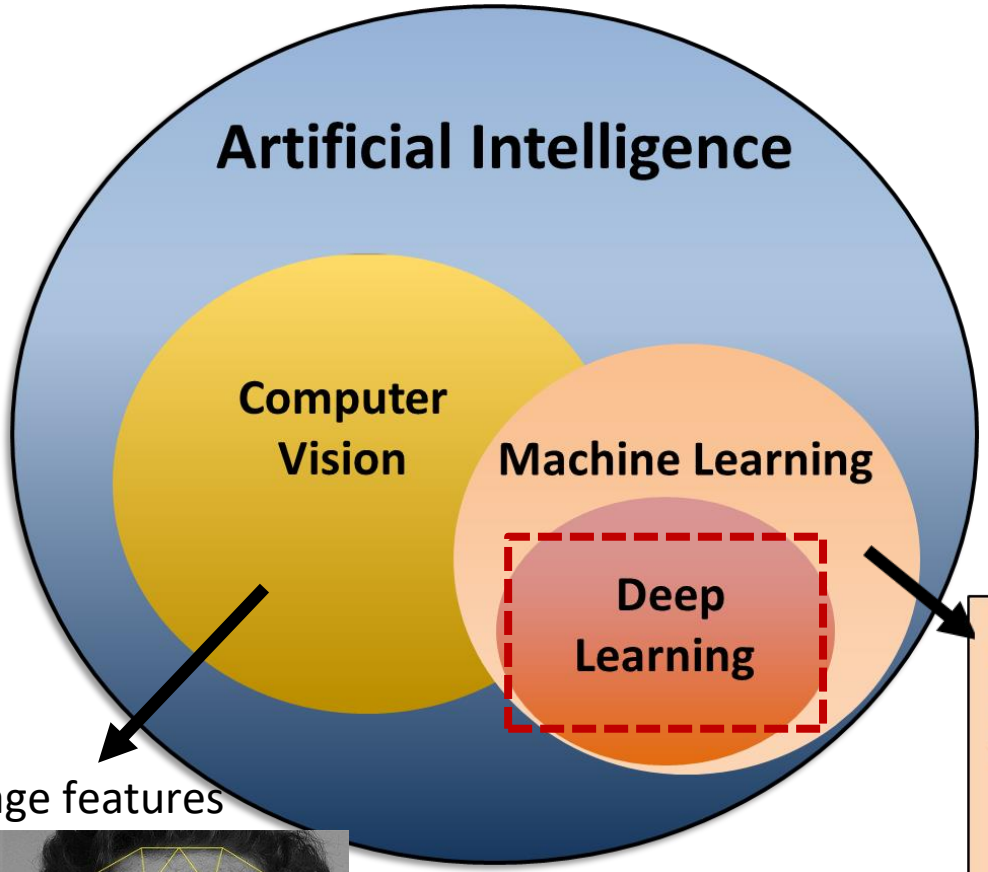
Fibrotic and hypertrophic aging heart

AI method was developed to quantify structural toxicity without bias and with high sensitivity

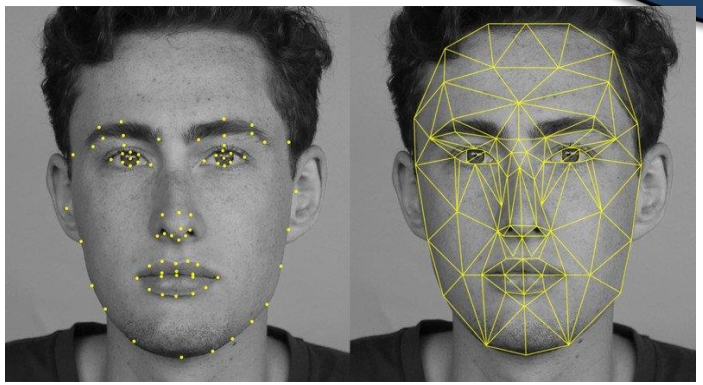
- No user input on how data is classified
- All characteristics of images can be analyzed
- Reproducibility of results

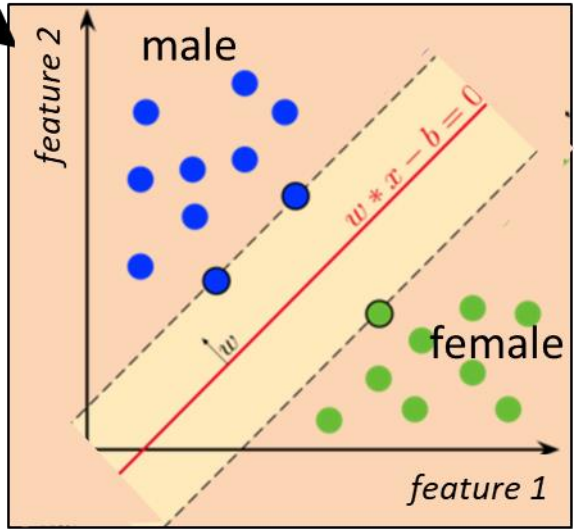
Mezale et al. (2017) IntechOpen
Masson et al. (2007) Fibrogenesis: Cellular and Molecular Basis

AI: “The science and engineering of creating intelligent machines that can achieve goals like humans do” (J. McCarthy, computer scientist, **1955**).

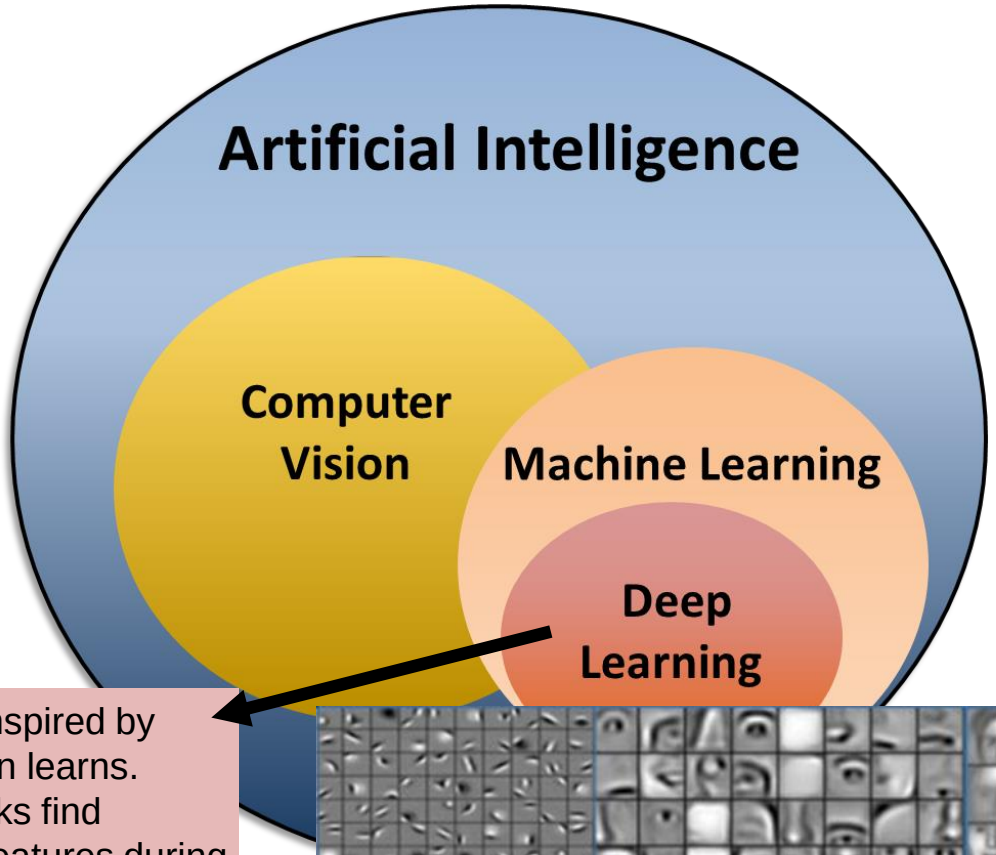


Hand-crafted image features





Training on features to learn separation between classes

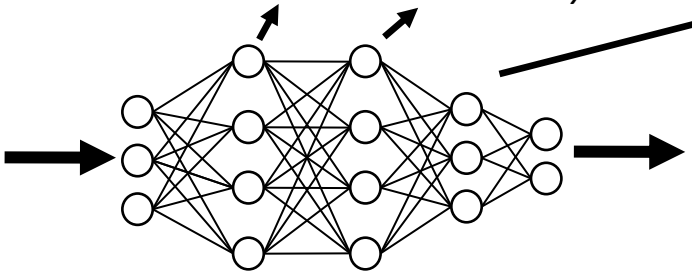


Deep Learning is inspired by how the human brain learns. Deep neural networks find patterns and learn features during the training mode.

Hidden layer 1 *Hidden layer 2* *Hidden layer 3*

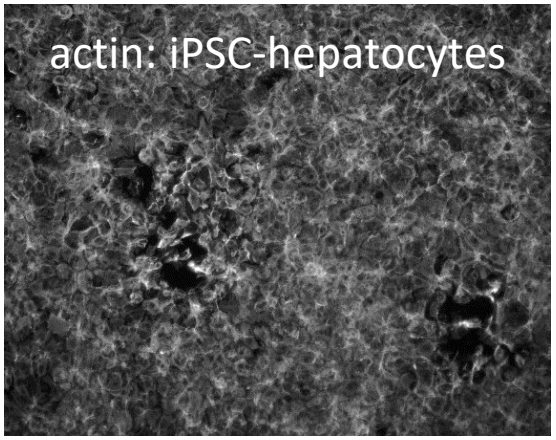
Images of female face

Images of male face

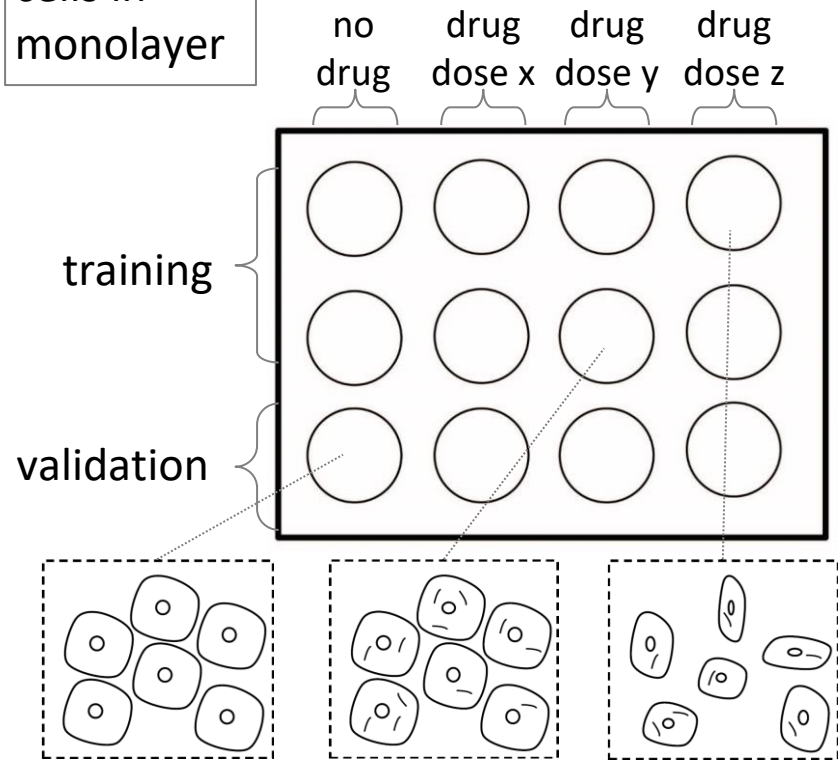


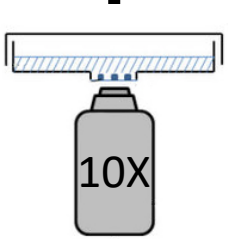
A model which has learned to classify face images to male/female

INPUT



cells in monolayer

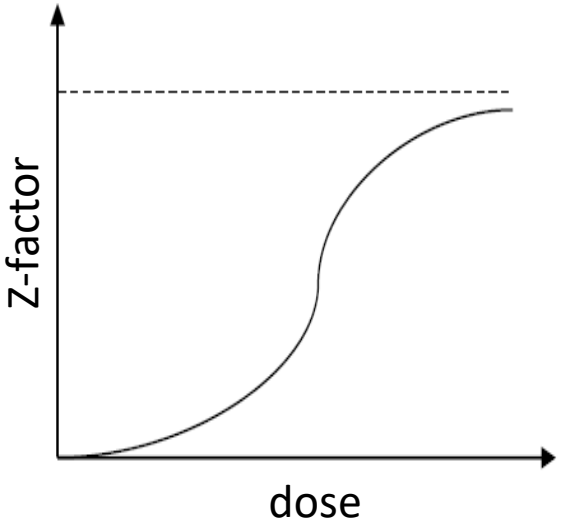




microscopy data

Deep Learning

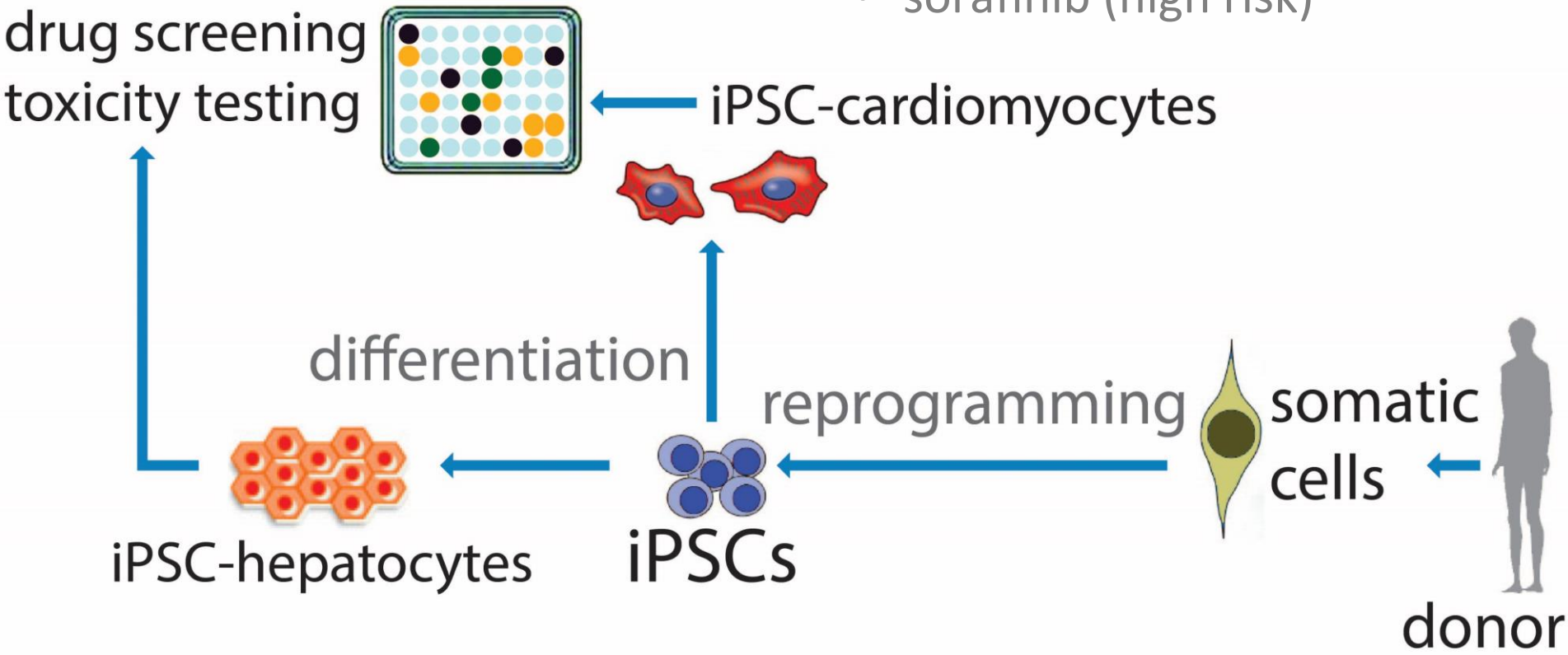
level of structural change



data on drug-induced structural toxicity

OUTPUT

- doxorubicin
- erlotinib (low risk)
- sorafinib (high risk)

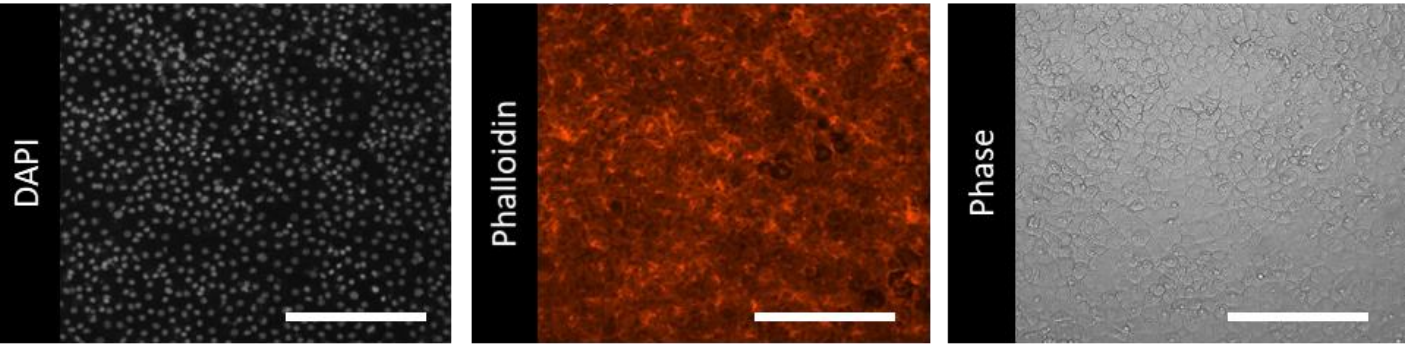


- tamoxifen
- doxorubicin

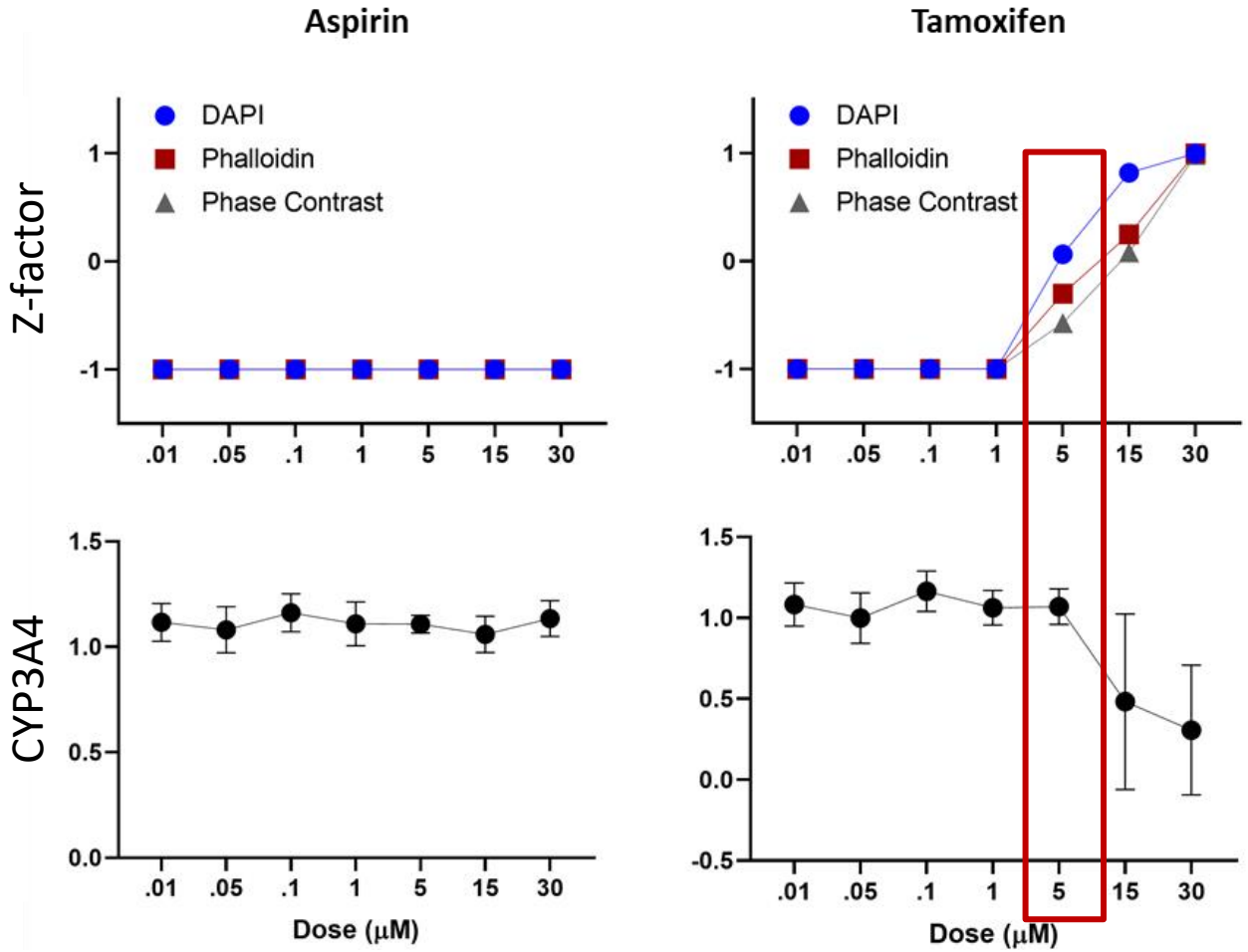
compared deep learning performance with:

cytochrome P450 (CYP3A4) + caspase 3/7 – hepatocytes
contractility + confluency - cardiomyocytes

Wang et al. (2019)
Masson et al. (2007) Fibrogenesis: Cellular and Molecular Basis



iPSC-hepatocytes



In detecting toxicity:

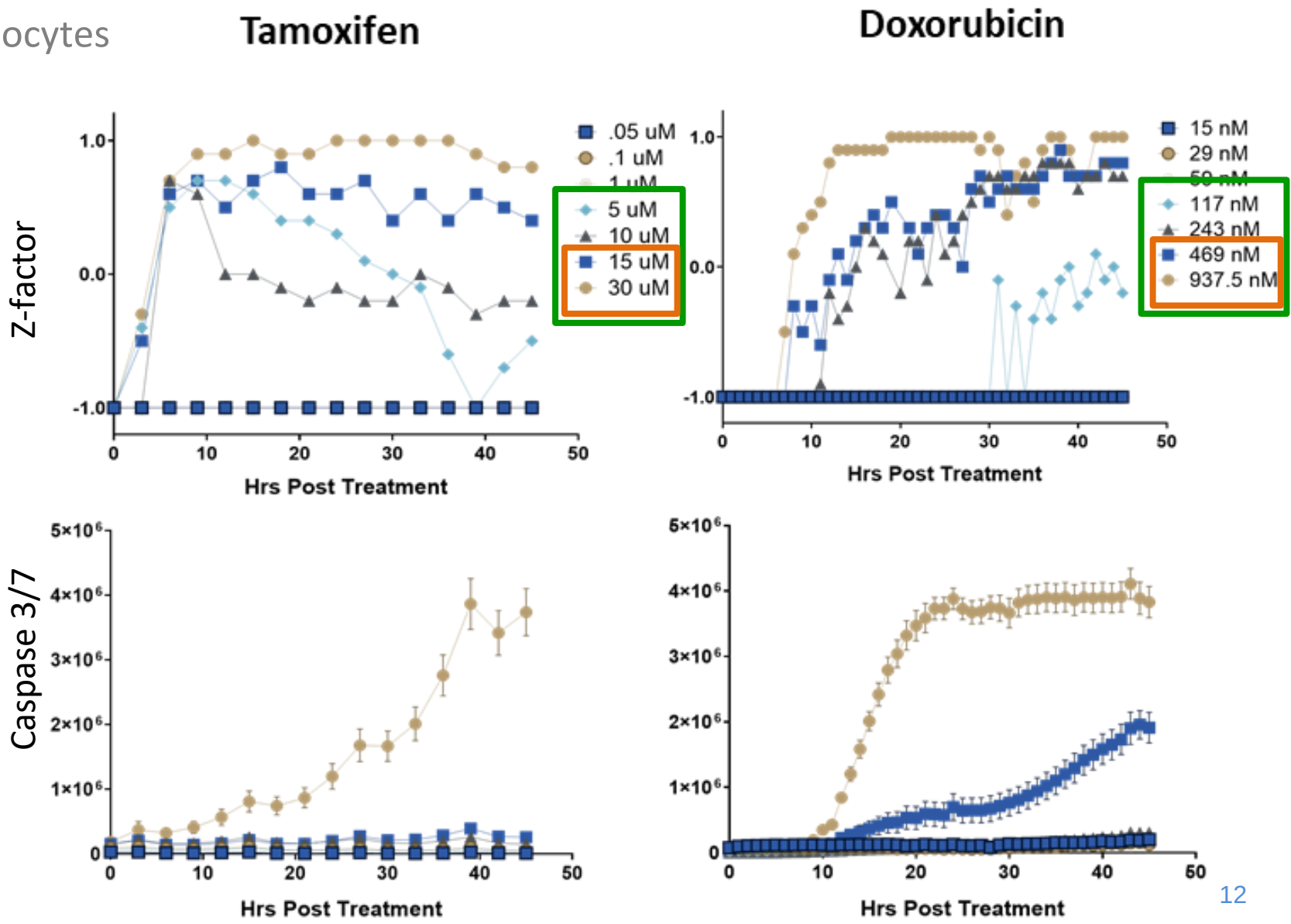
- deep learning was more sensitive
- images of nuclei showed more severe structural changes

Obtained kinetic profiles with live cells

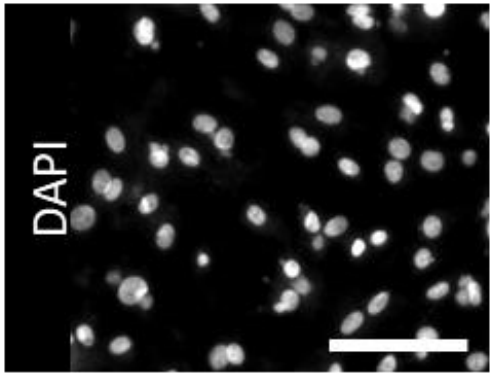
iPSC-hepatocytes

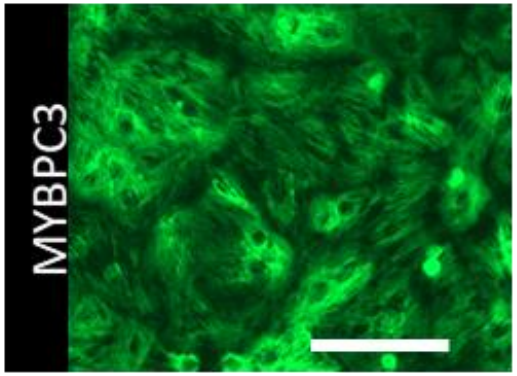
more sensitive

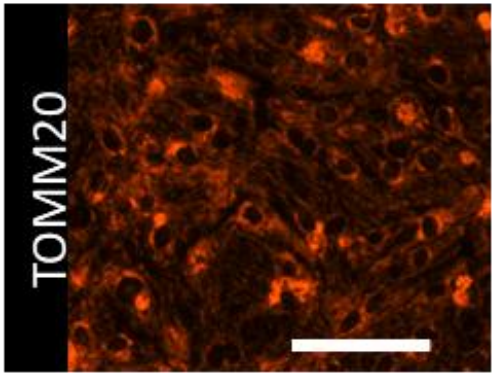
less sensitive

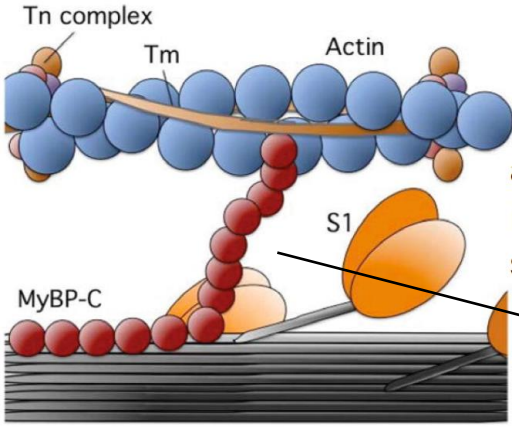


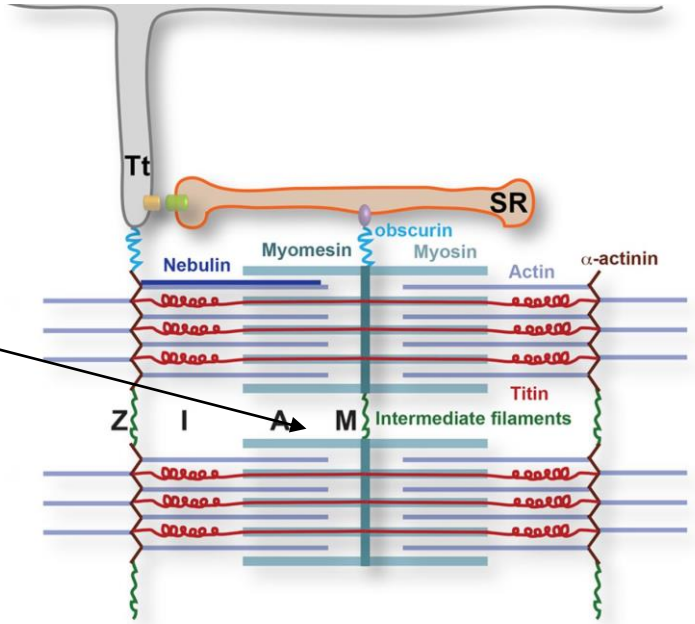
We labeled myosin binding protein C (MYBPC3) and mitochondria (TOMM20) in iPSC-cardiomyocytes



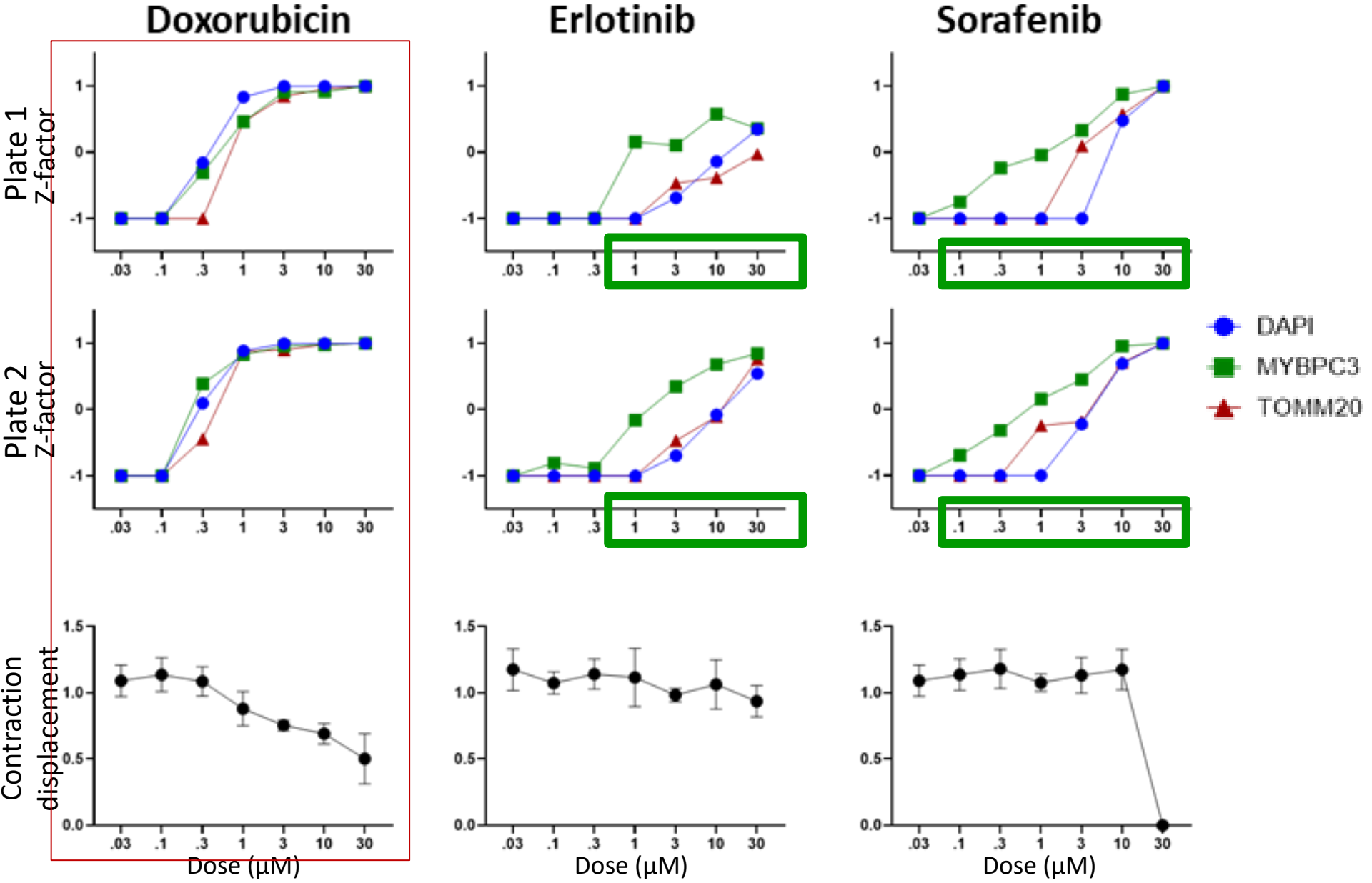








Gautel M & Djinović-Carugo (2016) J Exp Biol 219(Pt 2)
Spudich JA (2015) Biochem Soc Trans 43(1)

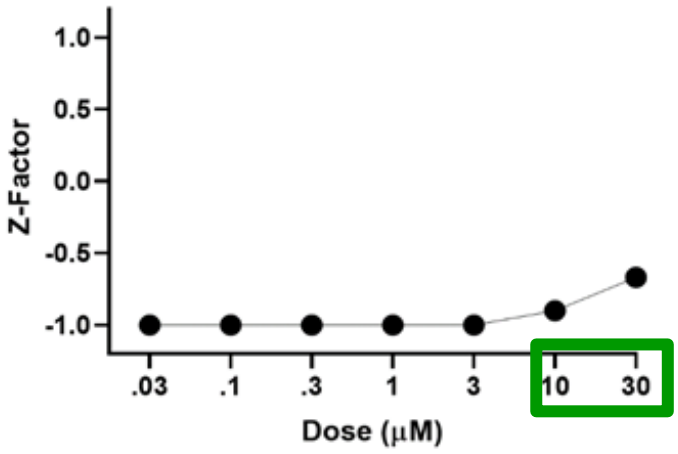


- Reproducibility between plates
- Similar trends between contractility and structural changes with doxorubicin
- Sorafenib showed a higher level of (MYBPC3) structural changes

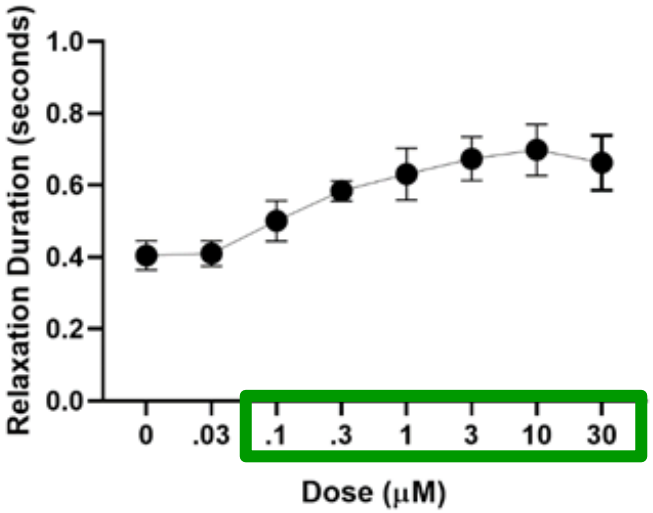
AI method specific to analyzing structural effects

iPSC-cardiomyocytes

Structural variations



Relaxation duration



- **Quinidine** prolongs action potential duration
- No structural effects

Key takeaways of AI method testing

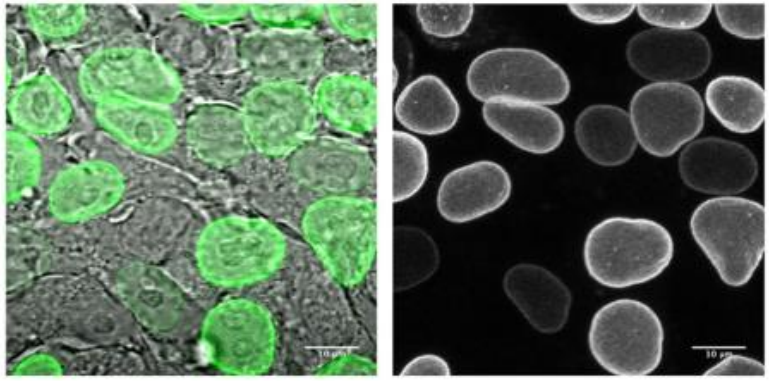
- Toxic drug effects can lead to detected structural changes
  - dependent on toxicity mechanism (must involve labeled proteins/ organelles)
  - observed reproducibility between experiments

- Improved or equivalent sensitivity in comparison with other methods
  - iPSC-hepatocytes: CYP3A4 activity, caspase 3/7
  - iPSC-cardiomyocytes: contractility

- Differential sensitivity in detecting different drug types when processing images depicting distinct structures
  - iPSC-hepatocytes: tamoxifen vs doxorubicin
  - iPSC-cardiomyocytes: TKIs vs doxorubicin

Next step with iPSCs: opportunity of fluorescently tagging proteins

Live WTC11 iPSCs with GFP-Lamin B



Roberts, B et al. Mol Cell Biol. 2017

Paxillin	RAS-associated protein RAB5A (RAB5A)
Sec61 beta	RAS-associated protein RAB5A (RAB5A)
Tom20	ATPase (ATP2A2)
Alpha-tubulin	Titin (TTN)
Lamin B1	Myosin light chain 7 (MYL7)
Fibrillarin	Gap junction (GJA1)
Beta-actin	Safe harbor locus (AAVS1)
Desmoplakin	Nucleophosmin 1 (NPM1)
LAMP-1	Catenin beta 1 (CTNNB1)
Tight junction ZO-1	**Lamin B1 (LMNB1)**
Non-muscle myosin heavy chain IIB	Myosin light chain 2 (MYL2)

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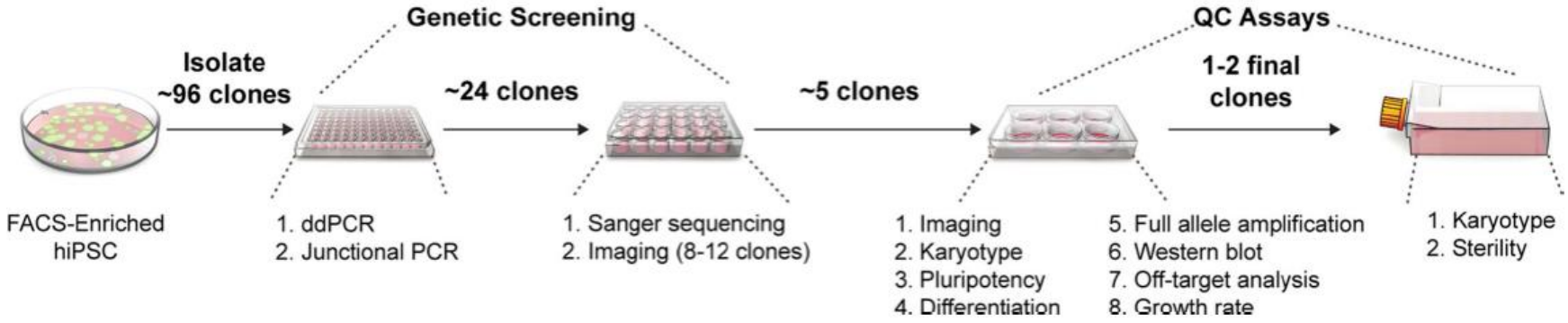

Additional cell types, tissues and involvement of drug development stakeholders

analytical validation

building confidence

translational qualification

Key Enablers

- replicate biology
- demonstrate pharmacology and toxicology
- test for analytical reproducibility
- comparative studies
- evolution of use
- learn to make decisions
- clinical outcomes
- tincture of time/experience

Find Opportunities

- investigate critical technology for drug development
- complement work done by other researchers
- collaborate with different stakeholders

Provided by Brian Berridge (National Toxicology Program and National Institute of Environmental Health Sciences)

Expanded use of approach to cardiac drug toxicity and efficacy

eLife

Deep learning detects cardiotoxicity in a high-content screen with induced pluripotent stem cell-derived cardiomyocytes

Francis Grafton[1], Jaclyn Ho[1], Sara Ranjbarvaziri[2], Farshad Farshidfar[1], Anastasiia Budan[1], Stephanie Steltzer[1], Mahnaz Maddah[3], Kevin E Loewke[3], Kristina Green[1], Snahel Patel[1], Tim Hoey[1], Mohammad Ali Mandegar[1]*

Phenotypic screening with deep learning identifies HDAC6 inhibitors as cardioprotective in a BAG3 mouse model of dilated cardiomyopathy

JIN YANG, FRANCIS GRAFTON, SARA RANJBARVAZIRI, ANA BUDAN, FARSHAD FARSHIDFAR, MARIE CHO, EMMA XU, JACLYN HO, MAHNAZ MADDAH, KEVIN E. LOEWKE, JULIO MEDINA, DAVID SPERANDIO, SNAHEL PATEL, TIM HOEY, AND MOHAMMAD A. MANDEGAR fewer Authors Info &

Science Translational Medicine

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  - Norman Stockbridge
  - Xi Yang
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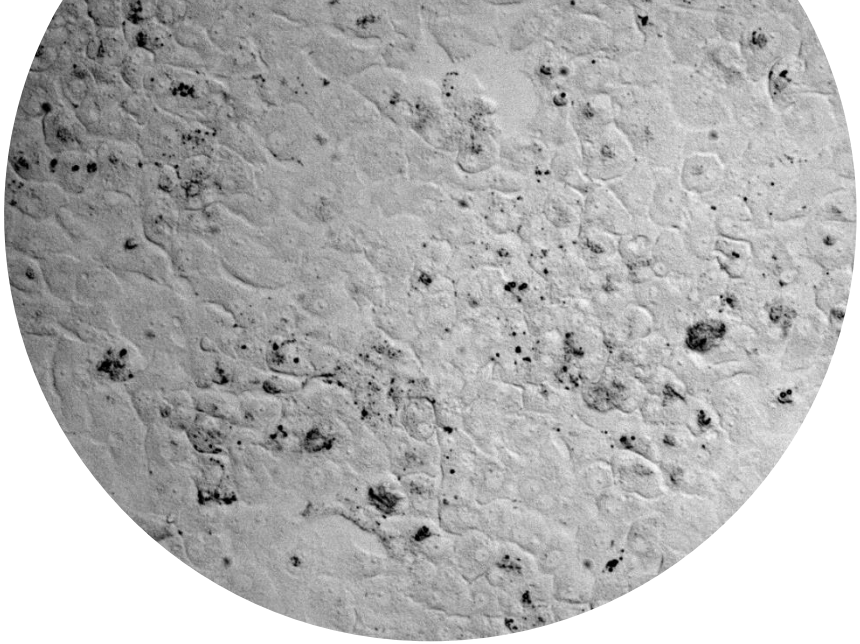

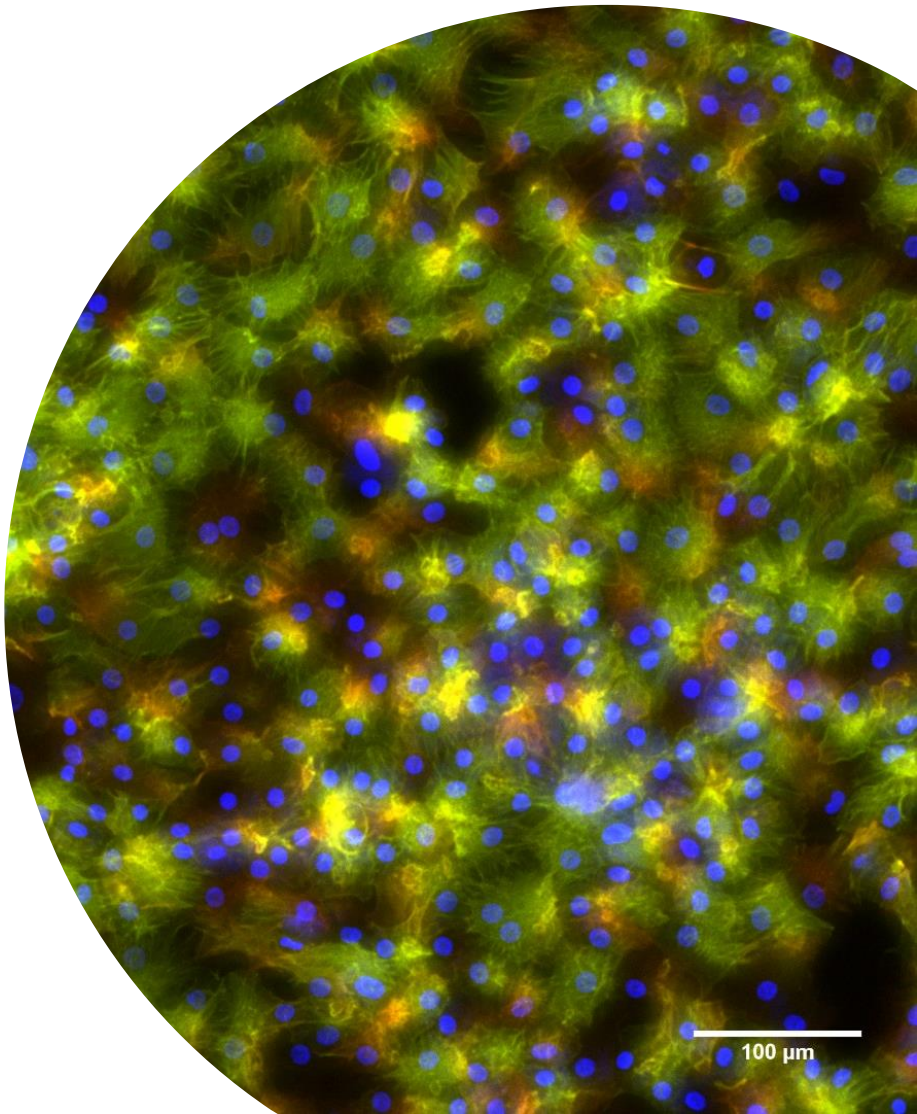

questions/ discussion