

High-throughput screening to advance *in vitro* toxicology

Anton Simeonov, Ph.D.

Scientific Director, National Center for Advancing Translational Sciences (NCATS), National Institutes of Health (NIH)

Challenges in Public Health Protection in the 21st Century: New Methods, Omics and Novel Concepts in Toxicology
November 15, 2021



The Tox21 Screening Project



Collection of diverse chemicals

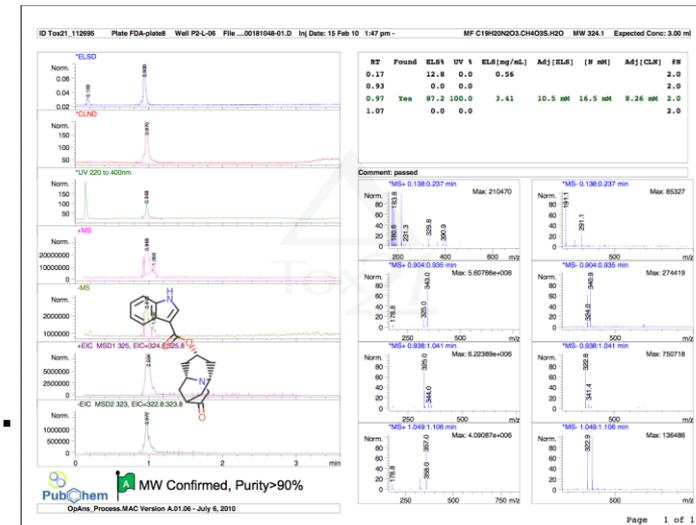
In vitro test methods, screening

High quality bioactivity data

Predictive models
(of bioactivity of a new chemical *in vitro* and, one day, *in vivo*)

>70 screening campaigns of the 10K Collection

Tox21 10K Chemical Collection: ~10,000 chemicals (nominated and procured by EPA, NIEHS, and NCATS) comprising approved drugs, failed drugs, pesticides, industrial chemicals, etc. Extensive Quality Control →



- Deposition into the public domain of the largest-ever toxicology dataset (100M datapoints), >100 publications.
- Using crowdsourcing to move from data to knowledge.



Tox21 10K Compound Library

EPA

- ToxCast I and II compounds
- Antimicrobial Registration Program
- Endocrine Disruptor Screening Program
- OECD Molecular Screening Working Group
- FDA Drug Induced Liver Injury Project
- Failed Drugs

NTP

- NTP-studied compounds
- NTP nominations and related compounds
- NICEATM/ICCVAM reference compounds for regulatory tests
- External collaborators (e.g., Silent Spring Institute, U.S. Army Public Health Command)
- Formulated mixtures

NCATS

- Approved Drugs
- Investigational Drugs
- *88 internal standards.*
- *Three library replicates.*
- *Each sample arrayed in 15 doses.*

The Tox21 10K Compound Library: Collaborative Chemistry Advancing Toxicology

Ann M. Richard,* Ruili Huang, Suramya Waidyanatha, Paul Shinn, Bradley J. Collins, Inthirany Thillainadarajah, Christopher M. Grulke, Antony J. Williams, Ryan R. Lougee, Richard S. Judson, Keith A. Houck, Mahmoud Shobair, Chihae Yang, James F. Rathman, Adam Yasgar, Suzanne C. Fitzpatrick, Anton Simeonov, Russell S. Thomas, Kevin M. Crofton, Richard S. Paules, John R. Bucher, Christopher P. Austin, Robert J. Kavlock, and Raymond R. Tice



Cite This: *Chem. Res. Toxicol.* 2021, 34, 189–216



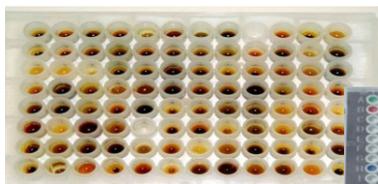
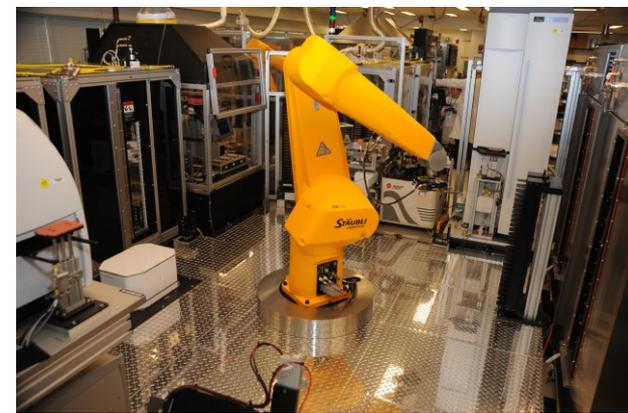
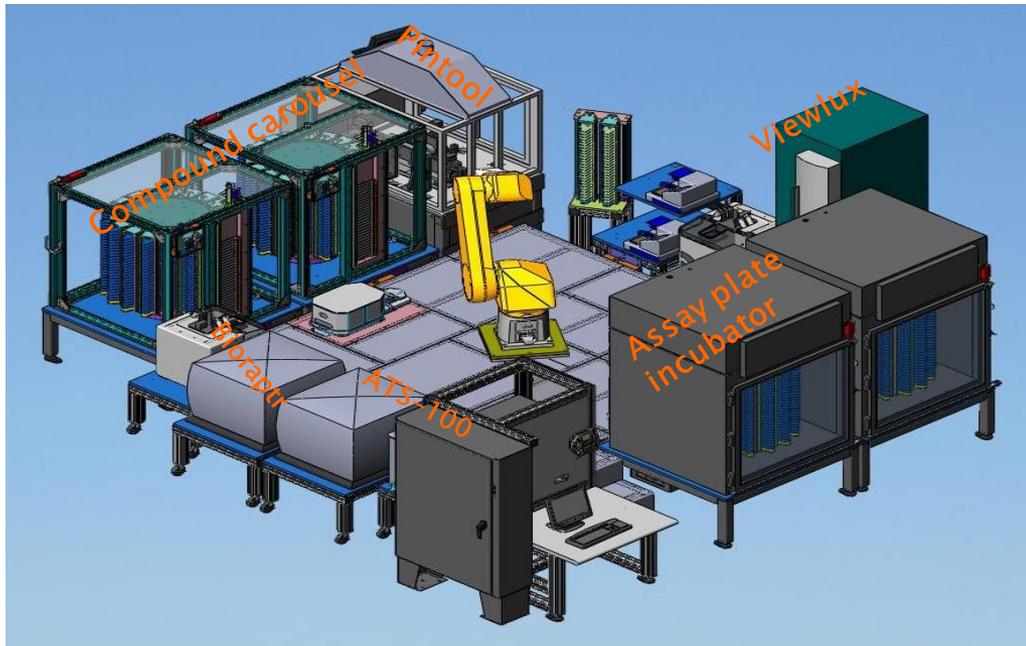
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Chem Res Toxicol 2021 34(2):189-216



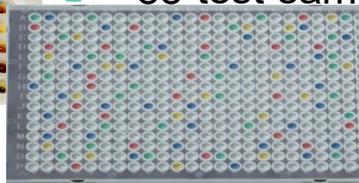
NIH National Center
for Advancing
Translational Sciences

Tox21 Robot Platform



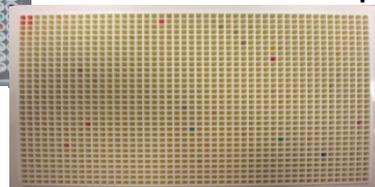
96-well plate

- 8 rows x 12 columns
- 88 test samples



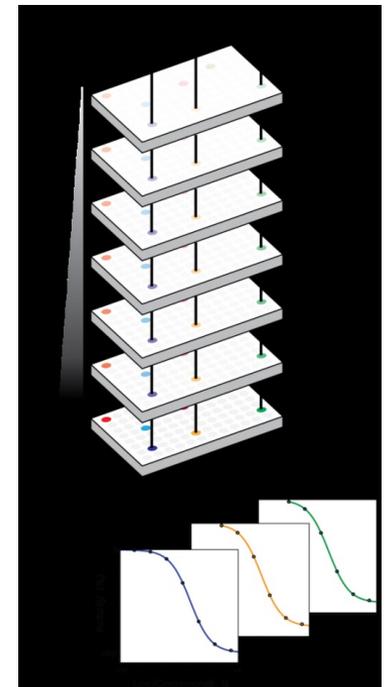
384-well plate
4 x 96-well plates

- 16 rows x 32 columns
- 352 test samples



1536-well plate
16 x 96-well plates

- 32 rows x 48 columns
- 1,408 test samples

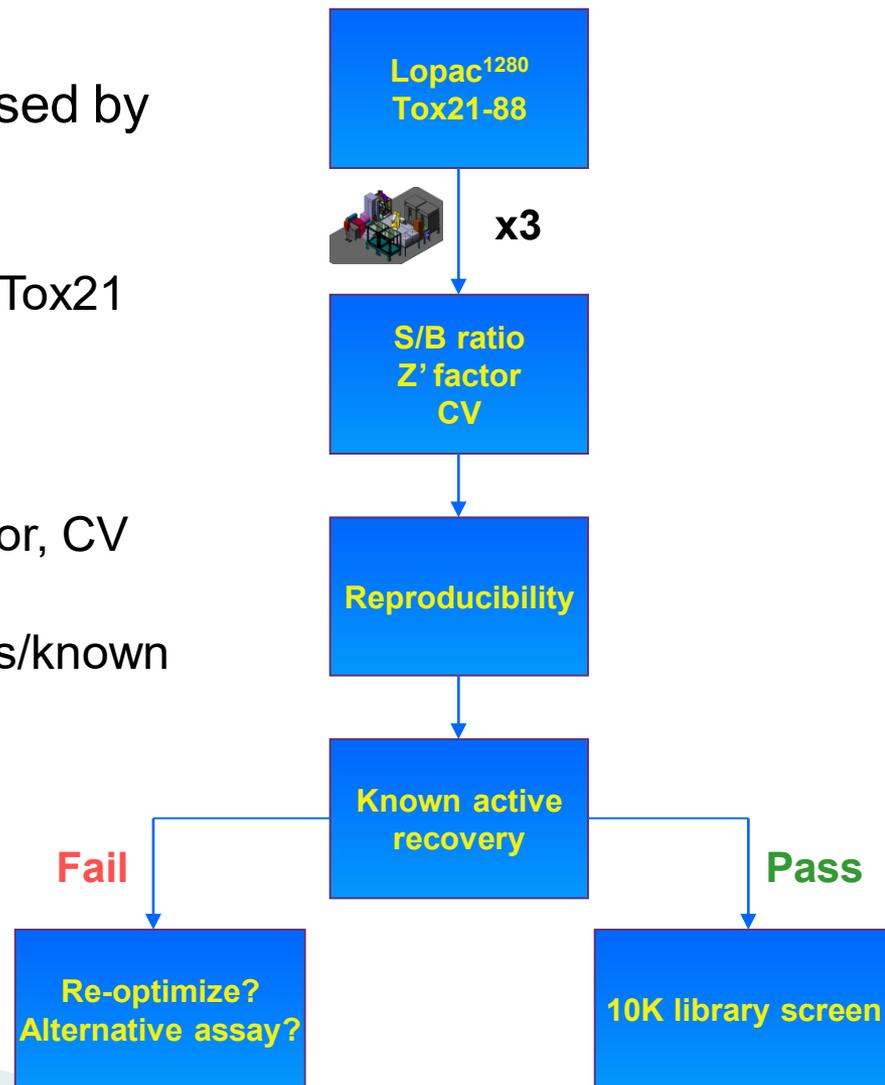


*Dose-response-based screening
Proc Natl Acad Sci 103:11473*

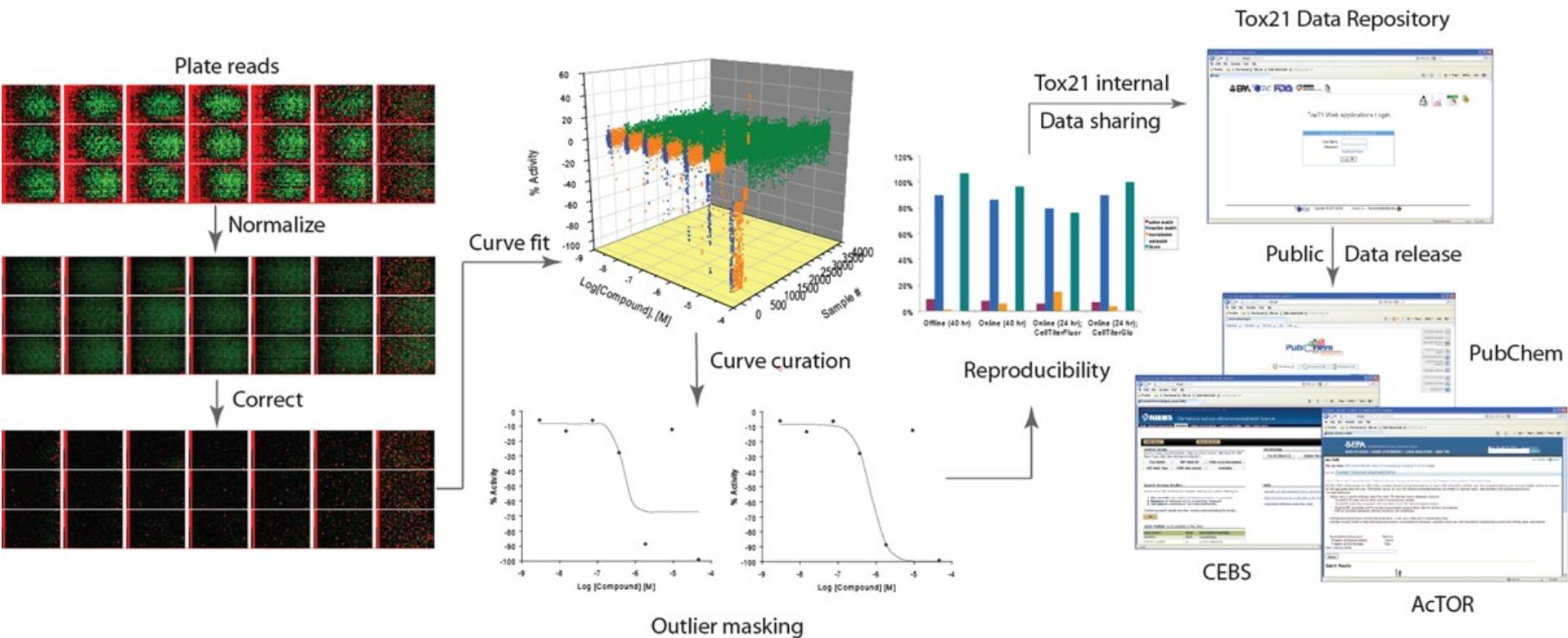


Assay Nomination and Validation Process

- Screening assay proposed and discussed by Assays and Pathways WG.
- Online validation on Tox21 Robot
 - Tox21 validation plate: Lopac¹²⁸⁰ + 88 Tox21 replicates
 - Triplicate runs
- Acceptance criteria
 - Performance metrics: S/B ratio, Z' factor, CV
 - Reproducibility
 - Ability to recover reference compounds/known actives
- Pass
 - Proceed to 10K library screening
- Fail
 - Go back to optimization?
 - Select alternative assay?



Informatics Analysis Process



Tox21 Applications Gateway

<http://tripod.nih.gov/tox/>

The screenshot displays the Tox21 Applications Gateway interface, which is divided into several functional areas:

- Search Results:** A table at the top lists search results with columns for Protocol Name, Sample Data Type, Sample ID, Sample Name, CAS, Curve Class, AC50, Efficacy, Z-score, Inhibitory Activity, HSCoef, R2, pval, and PubchemSID. The search criteria include Chemical Name: Bisphenol A and Target: Cancer.
- Assay Tracking:** A section titled "Assay Tracking" shows details for the assay "toxi1-ache-p1". It includes fields for Assay Name, Protocol Name, Sample Data Type, Treatment Incubation Time, Cell Line, Doubling Time, Assay Volume, Pos Control CAS No, Stimulator Name, Cell Passage, Channel Description, Measure Time, Cell Recovery Incubation Time, Temperature, Plate Type, Instrument, Pos Control Conc, Stimulator CAS No, Cell Culture, Medium, Z Factor, Plate First In Service Time, Cell Density, Date Run, Modes, Pos Control Name, Wavelength, Stimulator Conc, Performer, and Assay SD.
- Data Browser:** A section titled "Data Browser" features a graph showing "Response %" on the y-axis (ranging from -20 to 80) and "CAS" on the x-axis (ranging from -10 to 4). The graph displays a sigmoidal curve with data points and error bars.
- Target and Species Analysis:** Two pie charts are present. The "Target Category" chart shows the distribution of targets across various categories like Develop, Counter Screen, Cytotoxic, Gene Tar, GPCR, SR, and others. The "Species / Tissue Type" chart shows the distribution across Liver, Kidney, and other tissues.
- Table of Used in Tox21 Challenge:** A table at the bottom lists protocols used in the challenge, including columns for Protocol Name, Assay Target, Target Category, Cell Line, and Cell Type.

Predictive modeling with Tox21 data: collaborations with the scientific community

Chemical Research in Toxicology

pubs.acs.org/crt
<https://pubs.acs.org/crt?ref=pdf>

Trade-off Predictivity and Explainability for Machine-Learning Powered Predictive Toxicology: An in-Depth Investigation with Tox21 Data Sets

Leihong Wu, Ruili Huang, Igor V. Tetko, Zhonghua Xia, Joshua X

Cite This: *Chem. Res. Toxicol.* 2021, 34, 541–549

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ABSTRACT: Selecting a model in predictive toxicology often involves a trade-off between prediction performance and explainability: should we sacrifice the model performance for explainability?

JCIM JOURNAL OF CHEMICAL INFORMATION AND MODELING

Cite This: *J. Chem. Inf. Model.* 2019, 59, 4613

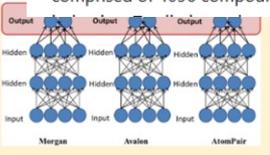
Novel Consensus Architecture To Improve Scale Multitask Deep Learning QSAR Model

Alexey V. Zakharov,* Tongan Zhao, Dac-Trung Nguyen, Tyler Pe Ruili Huang, Noel Southall, and Anton Simeonov

National Center for Advancing Translational Sciences (NCATS), National Institute of Health, Rockville, Maryland 20850, United States

Supporting Information

ABSTRACT: Advances in the development of high-throughput screening and automated chemistry have rapidly accelerated the production of chemical and biological data, much of them freely accessible through literature aggregator services such as ChEMBL and PubChem. Here, we explore how to use this comprehensive mapping of chemical biology space to support the development of large-scale quantitative structure–activity relationship (QSAR) models. We propose a new deep learning consensus architecture (DLCA) that combines consensus and multitask deep learning approaches together to generate large-scale QSAR models. This method improves knowledge transfer across different target/assays while also integrating contributions from models based on different descriptors. The proposed approach was validated and compared with proteochemometrics, multitask deep learning, and Random Forest methods paired with various



Article

> *Arch Toxicol.* 2017 Dec;91(12):3885–3895. doi: 10.1007/s00204-017-1995-9. Epub 2017 May 27.

Why are most phospholipidosis inducers also hERG blockers?

Svetoslav Slavov¹, Iva Stoyanova¹, Menghang Xia², Richard Beger¹

Affiliations + expand
 PMID: 28551711 DOI: 10.1007/

Full text links

Abstract

Recent reports have noted that a gene (hERG) ion channel also in most PLD inducers are also hERG comprised of 4096 compounds a



Chemical Research in Toxicology

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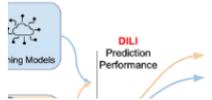
Article

Deep Graph Learning with Property Augmentation for Predicting Drug-Induced Liver Injury

in, Ruili Huang, and Junzhou Huang*

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Recommendations | Supporting Information



A Section 508 conformant HTML version of this article is available at <https://doi.org/10.1289/EHP5580>.

for Androgen Receptor Activity

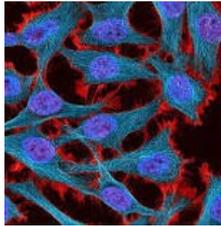
menico Albergo,⁶ Vinicius M. Alves,^{2,8} Patrik L. Andersson,⁹ Emilio Benfenati,¹⁴ Barun Bhatnagar,¹⁵ Scott Boyer,¹⁶ Alfonso T. Garcia-Sosa,¹⁹ Paola Gramatica,¹⁵ vrath,²¹ Xin Hu,²² Ruili Huang,²² Nina Jeliazkova,²³ Giuseppe F. Mangiatordi,^{6,24} Uko Maran,¹⁹ yen,²² Orazio Nicolotti,⁶ Nikolai G. Nikolov,¹³ Pogodin,²⁶ Vladimir Porokov,²⁶ Xianliang Qiao,¹⁷ Rupakheti,^{24,28} Sugunadevi Sakthiah,²⁰ vj,²⁰ Imran Shah,¹ Sulev Sild,¹⁹ Lixia Sun,²⁹ hini,¹² Weida Tong,²⁰ Daniela Trisciuzzi,⁶ ck,²¹ Zhongyu Wang,¹⁷ Eva B. Wedebye,¹³ eng,⁹ and Richard S. Judson¹

nt, U.S. Environmental Protection Agency (U.S. EPA), Research Triangle

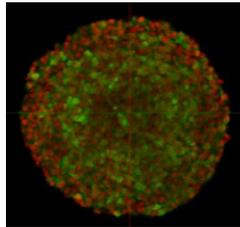
Triangle Park, North Carolina, USA
 s, Inc., Morrisville, North Carolina, USA
 Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), National Institute of Environmental Health Sciences, University of Bari, Bari, Italy
 deling and Drug Design, Faculty of Pharmacy, Federal University of Goiás, Goiânia, Brazil
 deling, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA
 University, Umeå, Sweden

Increasing the predictivity of *in vitro* assays: a continuum of 3D models of healthy and diseased tissues

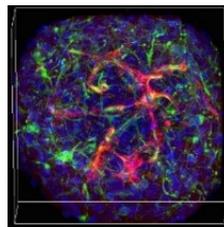
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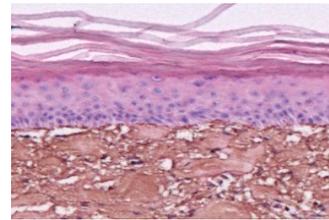
Spheroids



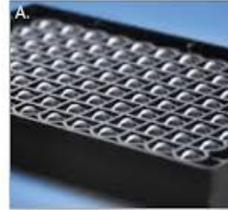
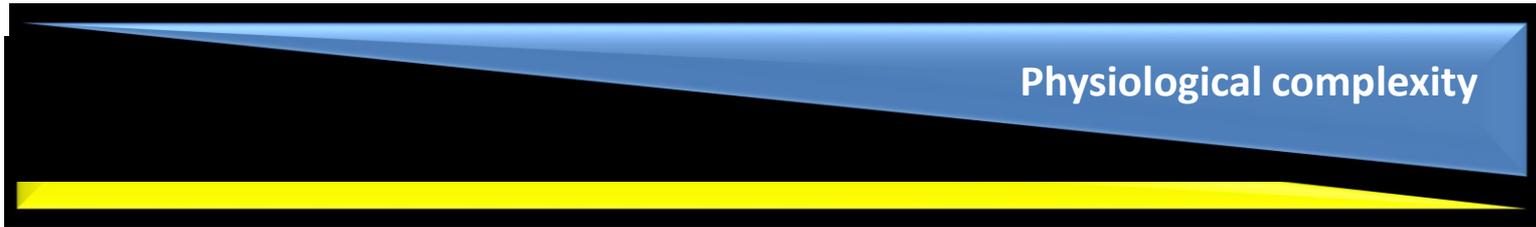
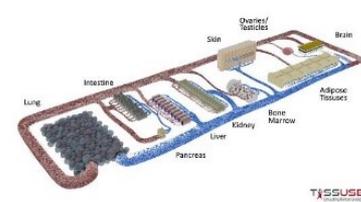
Organoids



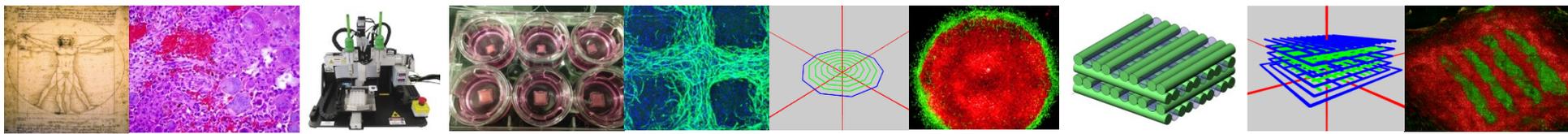
Printed Tissues



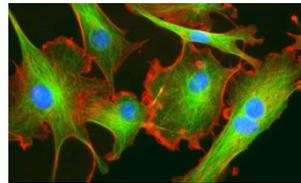
Organ-on-a-chip



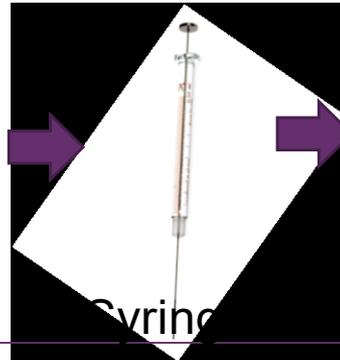
3D Tissue Bioprinting



Gel



Cells



Syringe

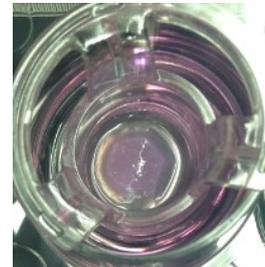


Printer

Hydrogel polymer is mixed with cells and loaded into syringe.



Printed construct



1 day



1 week

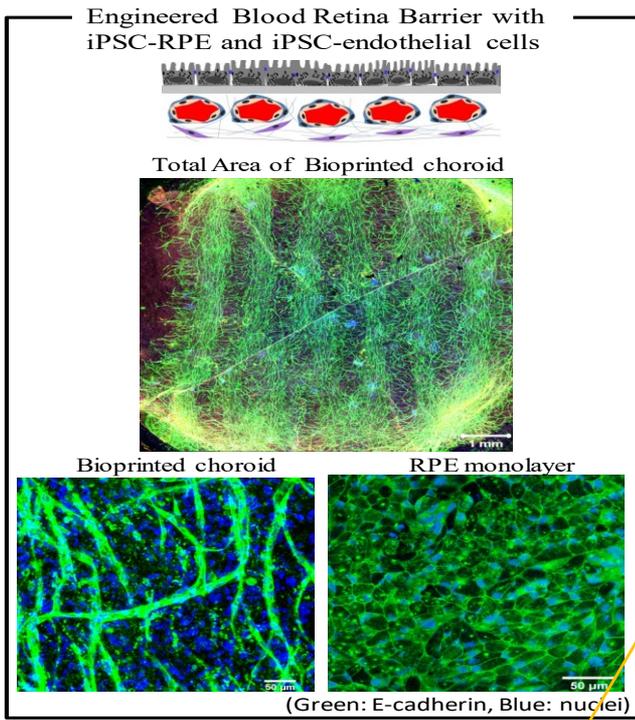


2 weeks

The printer “3D prints” the cell/gel mixture in a layer by layer approach.

The printed construct is incubated to allow the cells to form a tissue, and to enable proper cell differentiation.

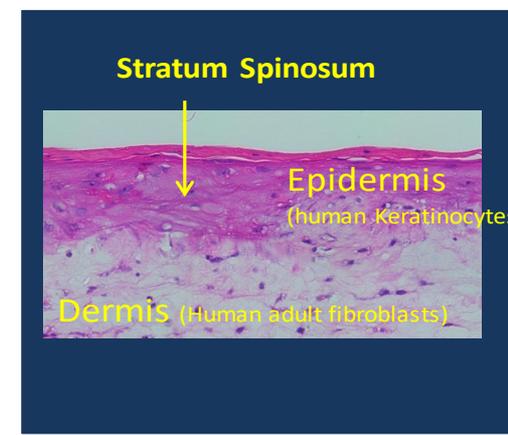
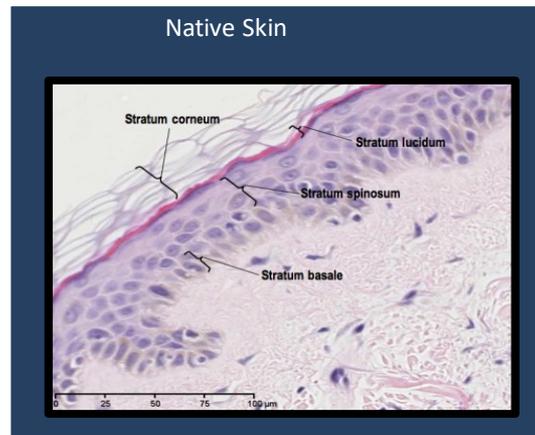
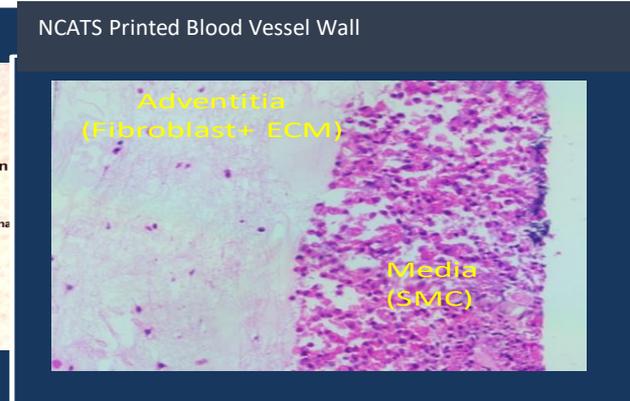
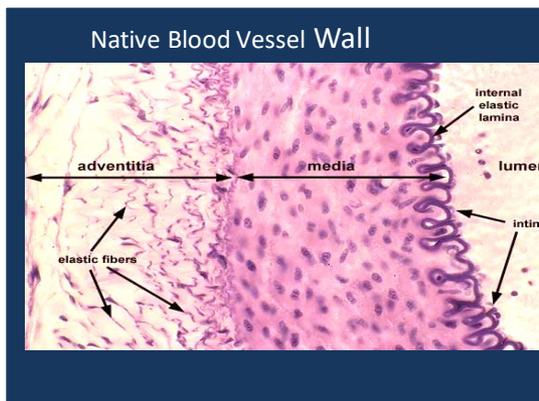
Examples of 3D Bioprinting Projects



Retina

Blood vessel wall

Skin

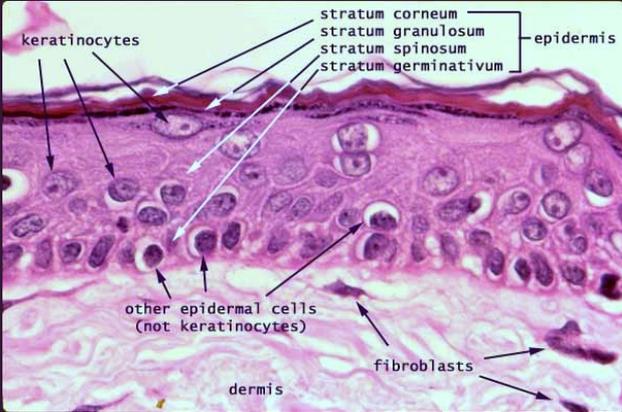


Recently initiated: lung and neuronal models for COVID-19 and pain, respectively.

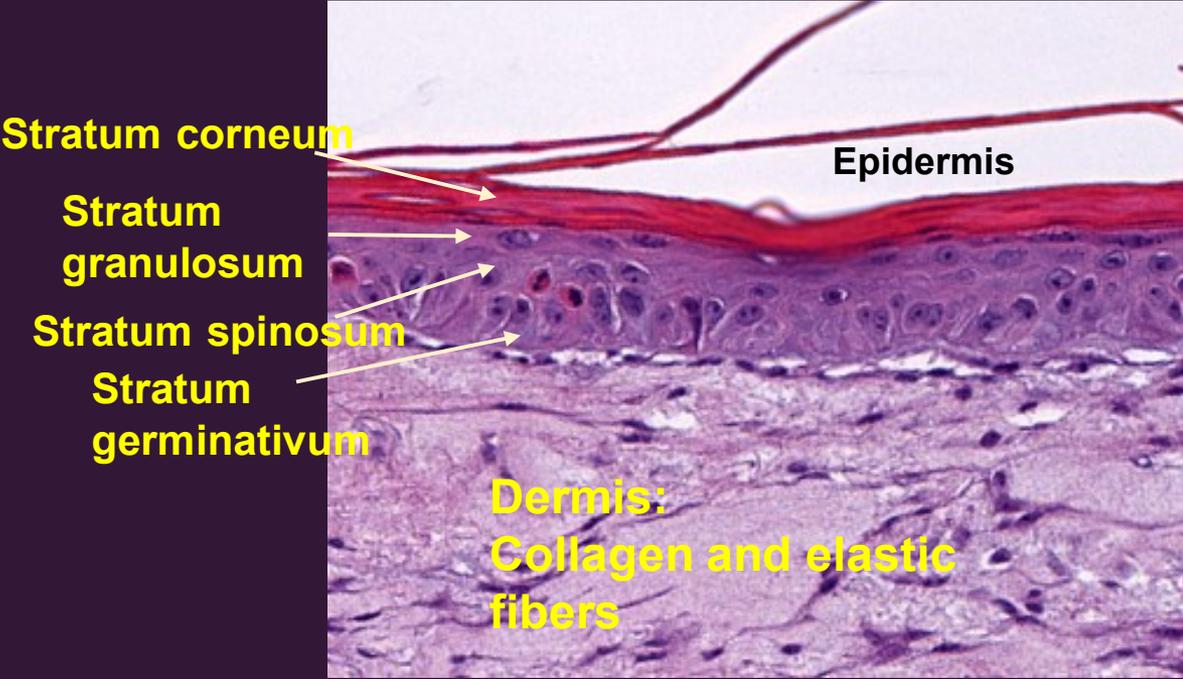


Skin biofabrication

Native Skin



3D-Bioprinted Skin

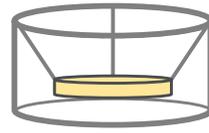


<http://www.siumed.edu/~dking2/intro/IN005b.htm>

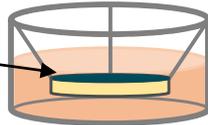
Generation of bioprinted skin tissues to test for irritants and sensitizers

Reconstructed human epidermis (RhE)

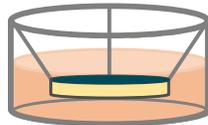
1. Coat the 96-well transwell insert membrane with collagen



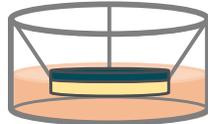
2. Add keratinocytes



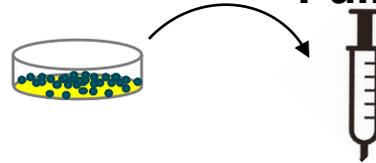
3. Submerge culture for 3 days



4. Air-liquid interface culture for 8 days



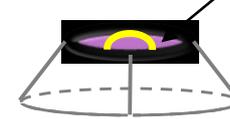
Full thickness skin tissue (FTS)



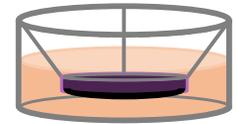
1. Suspend fibroblasts in bioprinting gel



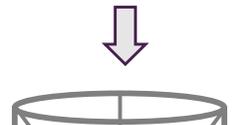
2. Bioprint fibroblast bioink to a 3-layer U shape on bottom side of 96-well transwell insert membrane



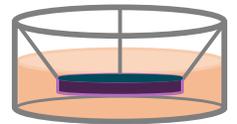
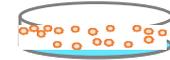
3. Add bioprinting gel to cover the U shape



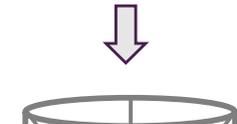
4. Submerge bioprinted tissue in medium for 7 days



5. Add keratinocytes and submerge culture for 3 days

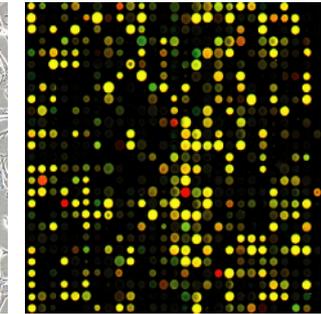
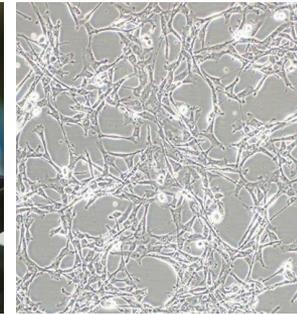
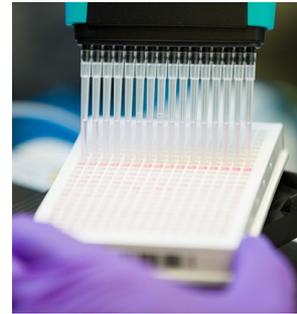
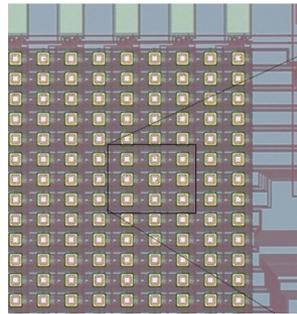
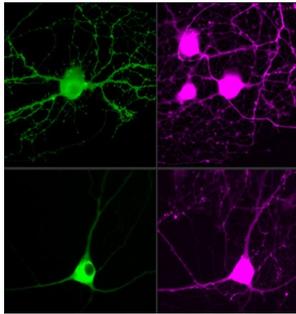
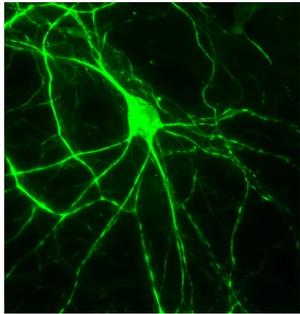


6. Air-liquid interface culture for 8 days



Enabling advanced 3D models through stem cell technologies

NCATS Stem Cell Translation Laboratory:



Access to relevant human cell types

Advanced imaging technologies for functional cell characterization

High-throughput electrophysiology methods

Measurement of signaling pathways, metabolism & specific targets

Longitudinal tracking of cell behavior

Combined single-cell transcriptomic & proteomic analyses

Sensory neurons (nociceptors) and other neuronal subtypes, hepatocytes, etc.

High-content confocal, calcium imaging, optogenetics

High-density multi-electrode arrays 26,400 electrodes/well

Cyclic AMP, PKA activity, CREB phosphorylation, energy metabolism

Multiple measurements over days, weeks or months

Drug response in individual cells

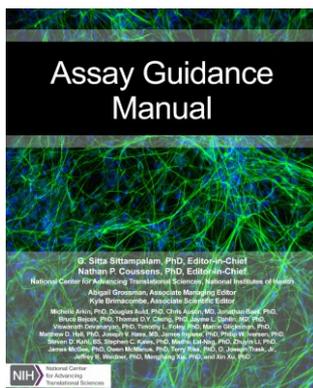
Example: a cheap 4-molecule cocktail that dramatically enhances survival of hPSCs and facilitates single-cell cloning, cell thawing and passaging, and CRISPR applications. Chen, *et al*, *Nature Methods* (2021)



**Where do I go for more
information about assay
development and screening?**



Sharing internal know-how: Assay Guidance Manual (47 chapters/ 1,338 printed pages)



<https://ncats.nih.gov/agm-video>

August 7th Videos

1. Austin, CP: **Welcome to the Assay Guidance Manual (AGM) Workshop**
2. Coussens, NP: **Strategies for Assay Selection & Robust Biochemical Assays**
3. Riss, T: **Treating Cells as Reagents to Design Reproducible Screening Assays**
4. Trask, OJ: **Assay Development Considerations for High Content Imaging**
5. Auld, DS: **Studies in Mechanisms and Methods in Assay Interferences**
6. Dahlin, JL: **Assay Interference by Chemical Reactivity**
7. Chung, TDY: **Basic Assay Statistics, Data Analysis & Rules of Thumb**
8. Devanarayan, V: **Reproducibility & Differentiability of Potency Results**
9. Sittampalam, GS: **Avoiding Artifacts & Interferences in Assay Operations**

March 26-27th Videos

1. Austin, CP: **Welcome to the Assay Guidance Manual (AGM) Workshop**
2. Coussens, NP: **Robust Assays Define Success in Preclinical Research**
3. Lal-Nag, M: **Target Identification & Validation in Translational Discovery**
4. Foley, TL: **Development & Validation of Cell-Based and Biochemical Assays**
5. Riss, T: **Treating Cells as Reagents to Design Reproducible Screening Assays**
6. Trask, OJ: **Assay Development for HCS & Best Practices for 3D HCS**
7. Roth, KD: **Mass Spectrometry for Drug Screening and Lead Optimization**
8. Dahlin, JL: **Bioassay Interference by Aggregation and Chemical Reactivity**
9. Patnaik, S: **Lead Selection and Optimization by Medicinal Chemistry**
10. Xia, M: ***In Vitro* Toxicological Testing Using a qHTS Platform**
11. Xu, X: ***In Vitro* Assessment of ADME Properties of Lead Compounds**
12. Kahl, SD: **Statistical Design of Experiments for Assay Development**
13. Guha, R: **Pharos Application to Target Evaluation and Drug Discovery**
14. Weidner, JR: **Assay Operations: Keeping Assays Robust and Reproducible**

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Assay Validation, Operations and Quality Control	5 Chapters
Assay Technologies	2 Chapters
Instrumentation	2 Chapters
Pharmacokinetics and Drug Metabolism	1 Chapter
Glossary of Quantitative Biology Terms	1 Chapter

Website: <https://ncats.nih.gov/expertise/preclinical/agm>

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Translational Sciences