IEBMC

INTERNATIONAL EXPERIMENTAL BIOLOGY & MEDICINE CONFERENCE

REGENERATIVE MEDICINE

5 PLENARY SESSIONS FEATURING WORLD LEADERS IN REGENERATIVE MEDICINE

PLENARY SESSION 3: ORGANS ON A CHIP

Chair: John Wikswo
Co-Chair: Steve Goodman

8:45am - 9:20am
John Wikswo, Vanderbilt University
"Closing the hermeneutic circle of biology: systems biology, organs-on-chips, mass spectrometry, synthetic biology, and robot scientists"

9:20am - 9:55am
Megan McCain, University of Southern California
"Engineering Cardiac and Skeletal Muscle on a Chip Systems for Multi-Modal Physiological Analysis and Disease Modeling"

9:55am - 10:05am
BREAK

10:05am - 10:40am
Yoko Ambrosini, Washington State University
"Pathomimetic human intestinal disease-on-a-chip: From organomimicry to precision medicine"

10:40am - 11:15am
Peter Loskill, Eberhard Karls University Tübingen
"Microphysiological platforms, organoids and enabling technologies: Integrated Organ-on-Chip models recapitulating complex human tissues"

ABSTRACT PRESENTATIONS
Chair: Warren Zimmer

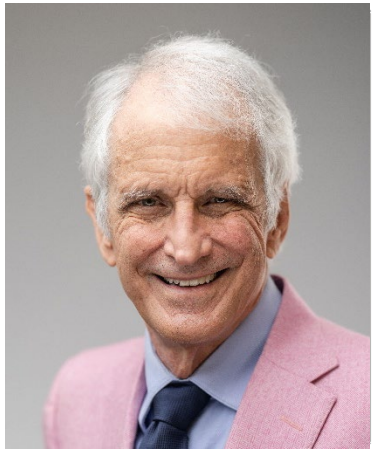
11:15am - 11:30am
Xiang Li, SUNY Upstate Medical University
"ABl1 regulates STAT3 transcriptional activity through a DNA binding mechanism"

11:30am - 11:45am
Chao Ma, New York University
"Leukemia-on-a-Chip for Modeling and Decoding Chemotherapy Resistance"

11:45am - 12:00pm
Solomon Owumi, University of Ibadan
"Unfettered antibiotics access, microbial resistance and carcinogenesis"

12:00pm - 12:15pm
Roshan Kumari, University of Tennessee Health Science Center
"Role of SMAD2/3 on Diet-Induced Obesity and Adiposity"

Plenary Session 3: Organs on Chips



John P. Wikswo, PhD

Gordon A. Cain University Professor

A. B. Learned Professor of Living State Physics

Professor of Biomedical Engineering, Molecular Physiology and Biophysics, and Physics

Founding Director, Vanderbilt Institute for Integrative Biosystems Research and Education

Vanderbilt University

“Closing the hermeneutic circle of biology: systems biology, organs-on-chips, mass spectrometry, synthetic biology, and robot scientists”

Megan McCain, PhD

Associate Professor, Department of Biomedical Engineering

Viterbi School of Engineering

University of Southern California

“Engineering Cardiac and Skeletal Muscle on a Chip Systems for Multi-Modal Physiological Analysis and Disease Modeling”

Yoko M. Ambrosini, DVM, MPVM, PhD, DACVIM (SAIM)

Assistant Professor, Department of Veterinary Clinical Sciences

College of Veterinary Medicine

Washington State University, PhD

"Pathomimetic human intestinal disease-on-a-chip: From organomimicry to precision medicine”

Peter Loskill, PhD

Professor for Organ-on-Chip-Research

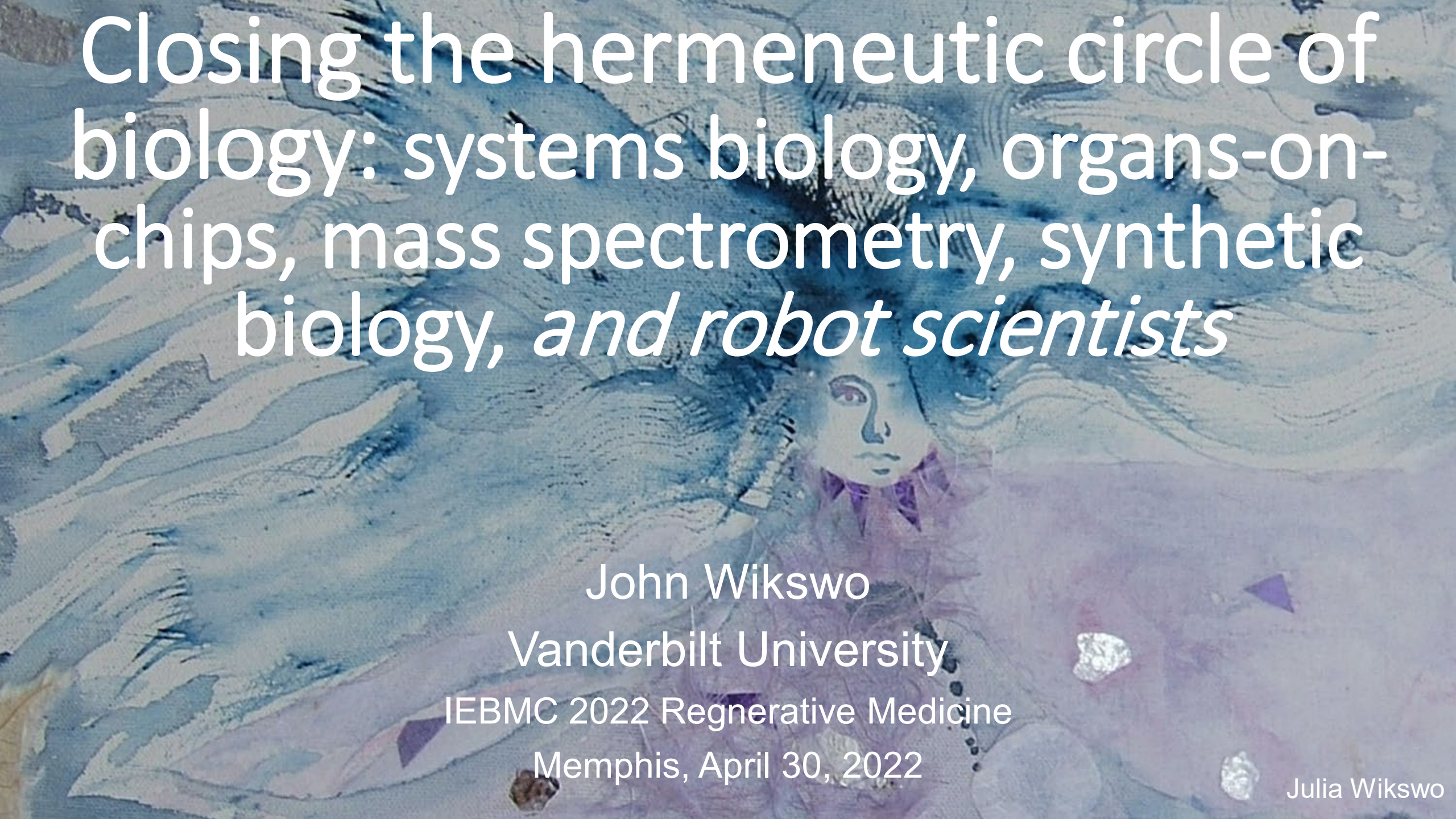
Eberhard Karls University Tübingen and the Natural and Medical Sciences Institute (NMI)

“Microphysiological platforms, organoids and enabling technologies: Integrated Organ-on-Chip models recapitulating complex human tissues”

Closing the hermeneutic circle of biology: systems biology, organs-on-chips, mass spectrometry, synthetic biology, *and robot scientists*

John Wikswo
Vanderbilt University

IEBMC 2022 Regnerative Medicine
Memphis, April 30, 2022



Julia Wikswo

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Meharry Medical College
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University of Pittsburgh
Lans Taylor, Peter Alexander
Albert Gough, Lawrence Vernetti
University of Washington
Jonathan Himmelfarb
University of Wisconsin
William Murphy

Bold = heavy lifting Blue = PI * = Vanderbilt University Medical Center *Italics = former collaborator*

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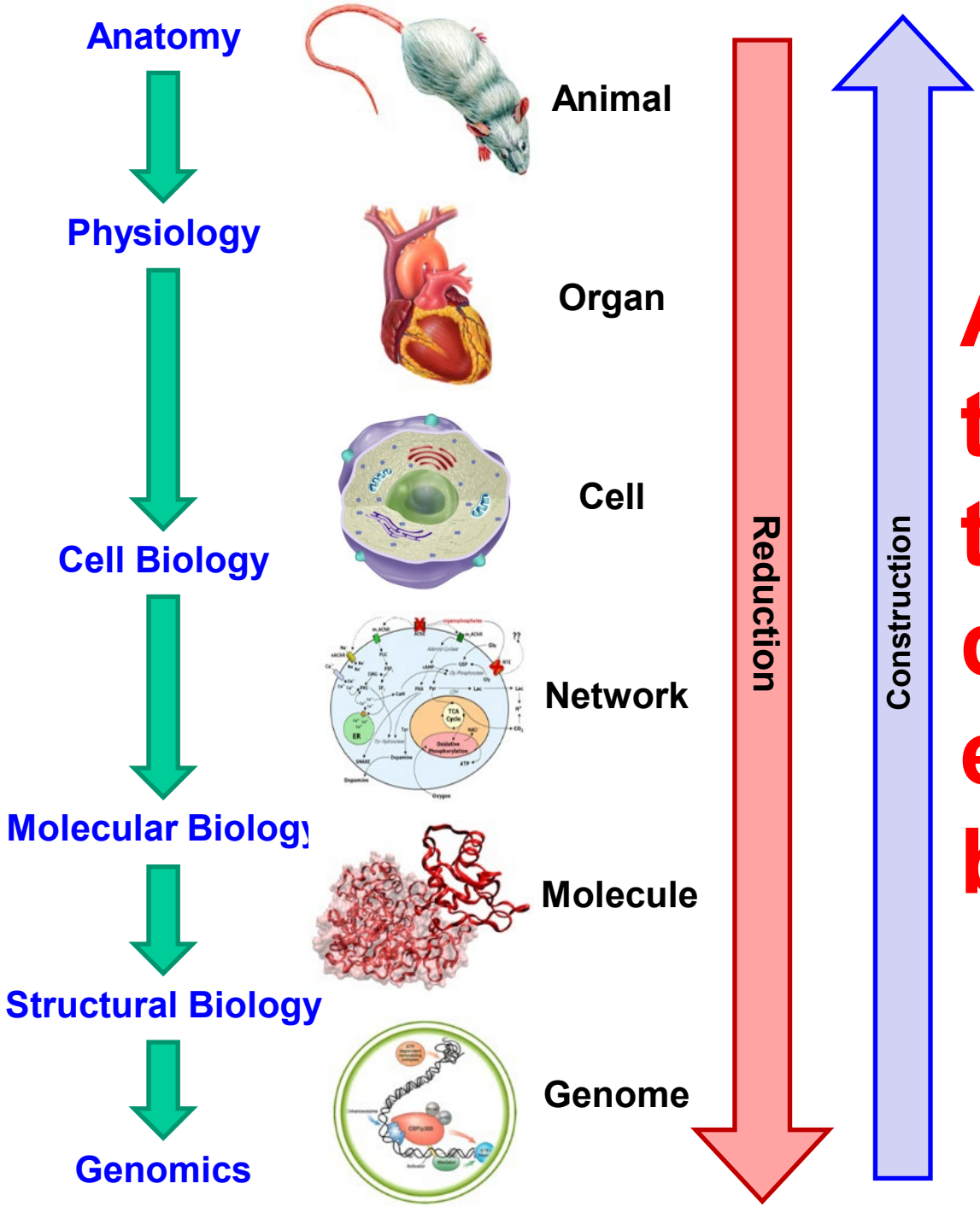
How do organ chips fit into the grand scheme of biology?

A brief history of biology



Are we using the wrong topology as we describe the exploration of biology?

JP Wikswo and AP Porter, *EBM*, 2015

Hermeneutics, *noun*

[hərməˈn(y)o͞odiks/]

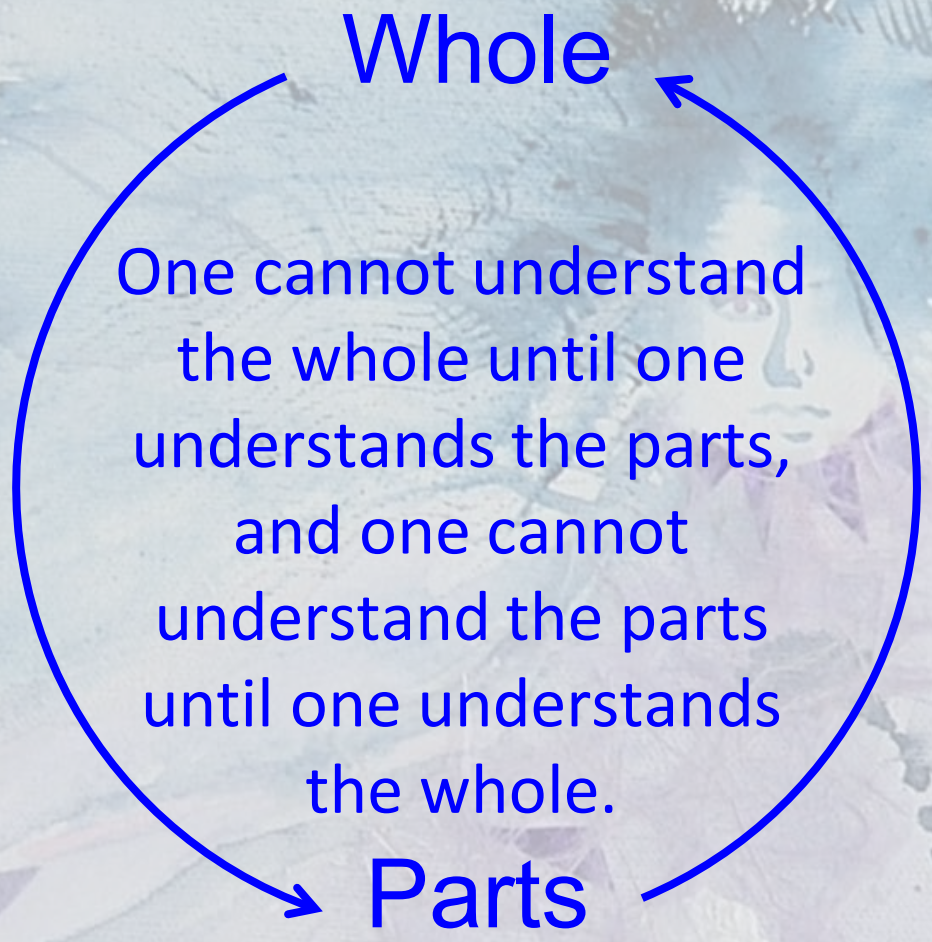
The study of the methodological principles of interpretation (as of the Bible).

http://www.merriam-webster.com/dictionary/hermeneutic

The first order art and the second order theory of understanding and interpretation of linguistic and non-linguistic expressions.

http://plato.stanford.edu/entries/hermeneutics/

Hermeneutic Circle, *noun*
[hərməˈn(y)o͞odikˈsərk(ə)l]



Gadamer,HG. Truth and Method. 2nd ed., Continuum, New York, 2000

Anatomy

Physiology

Cell Biology

Molecular Biology

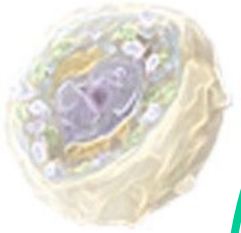
Structural Biology

Genomics

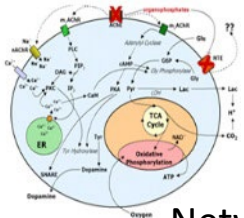
Organ

Animal

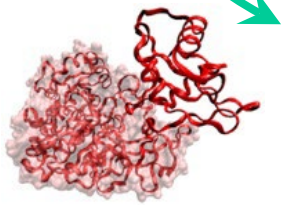
1960s



Cell



Network



Molecule

Genome

Anatomy

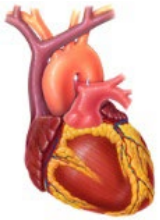
Physiology

Cell Biology

Molecular Biology

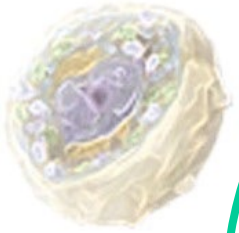
Structural Biology

Genomics



Organ

Animal

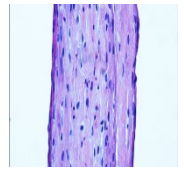


Cell

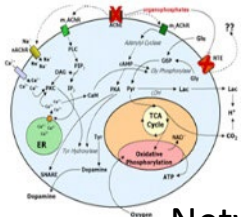
1960s

Today:
Post-Genomic,
Post-Proteomic
Biology and Tissue
Engineering

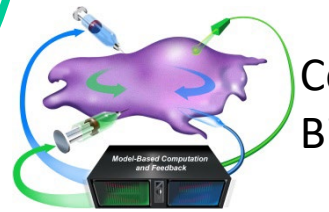
2014



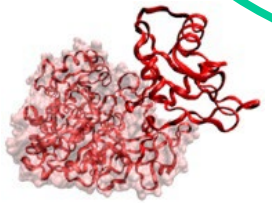
Organotypic
Constructs



Network

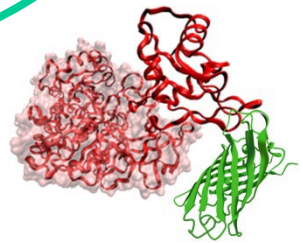


Control of
Biology



Molecule

Genome



Engineered
Molecules

Anatomy

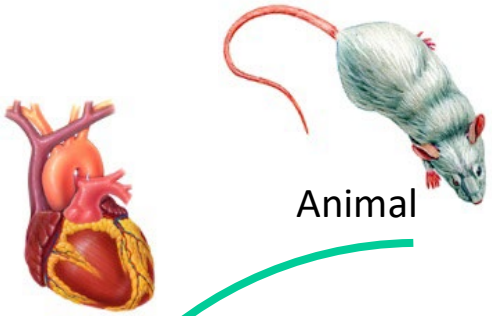
Physiology

Cell Biology

Molecular Biology

Structural Biology

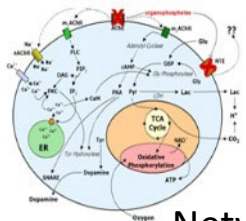
Genomics



Animal

Organ

Cell

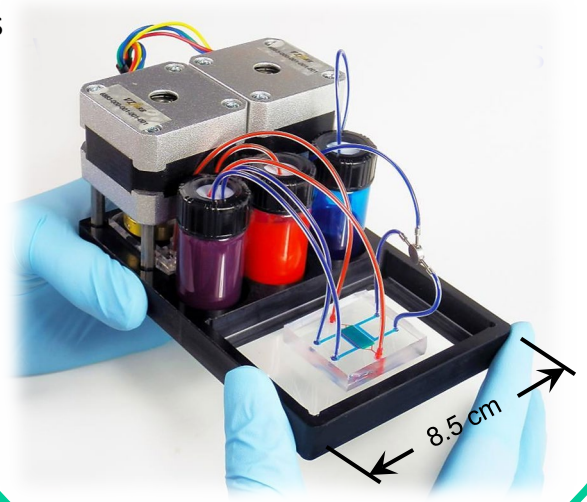


Network

Molecule

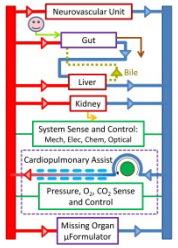
Genome

1960s



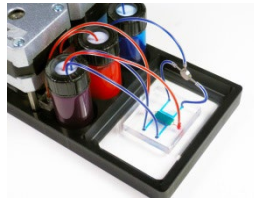
2014

Today!

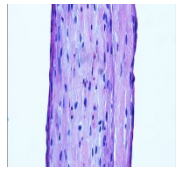


Coupled Human Microphysiological Systems

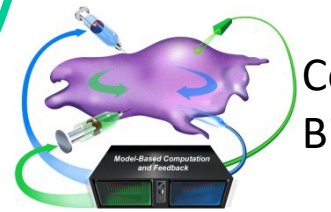
Tomorrow



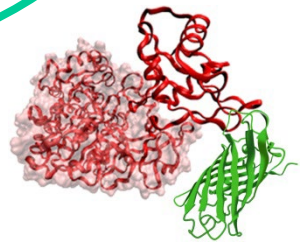
Organ Modules



Organotypic Constructs

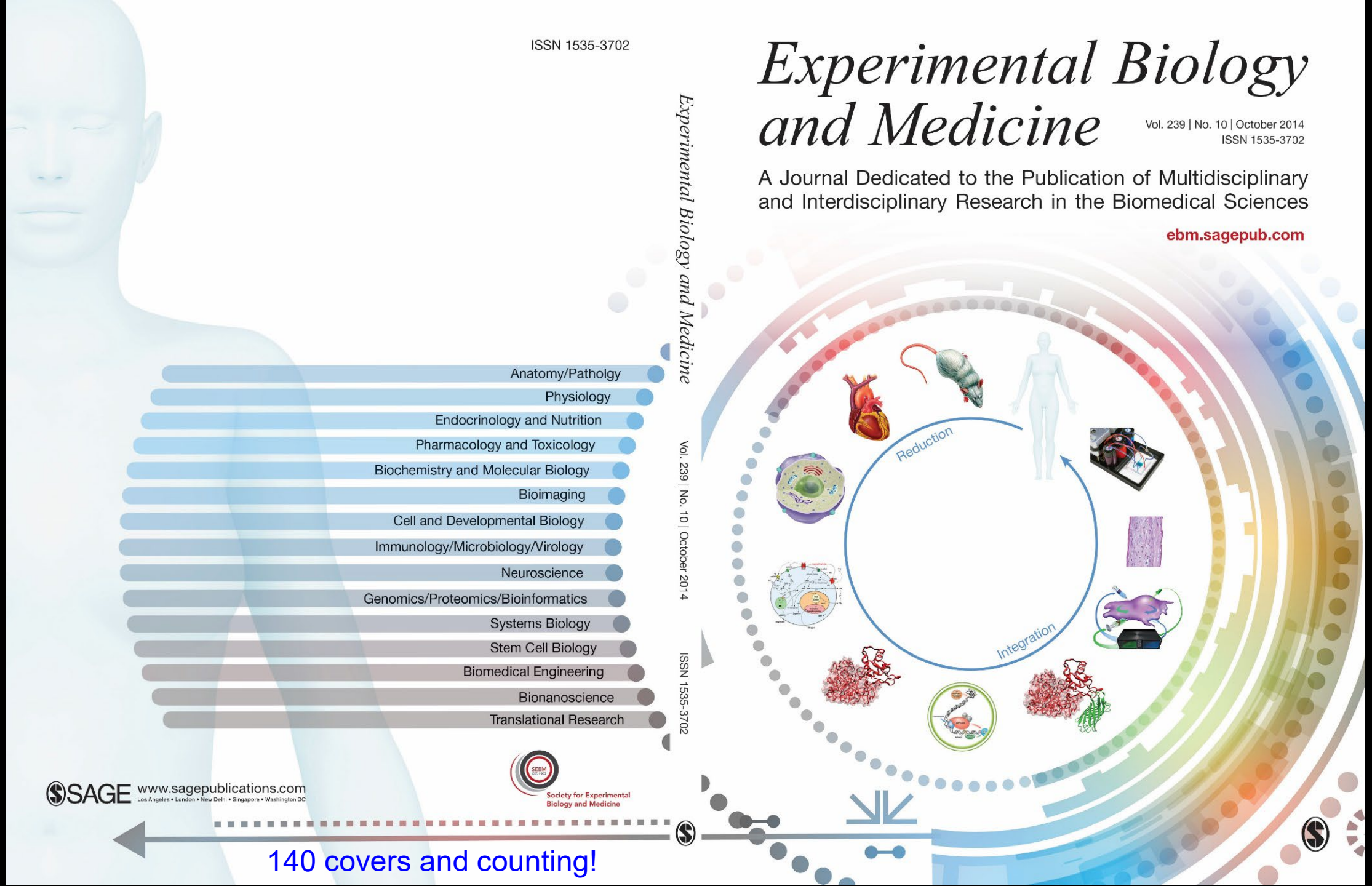


Control of Biology



Engineered Molecules

JP Wikswo. The relevance and potential roles of microphysiological systems in biology and medicine. Exp.Biol.Med. 239:1061-1072, 2014.



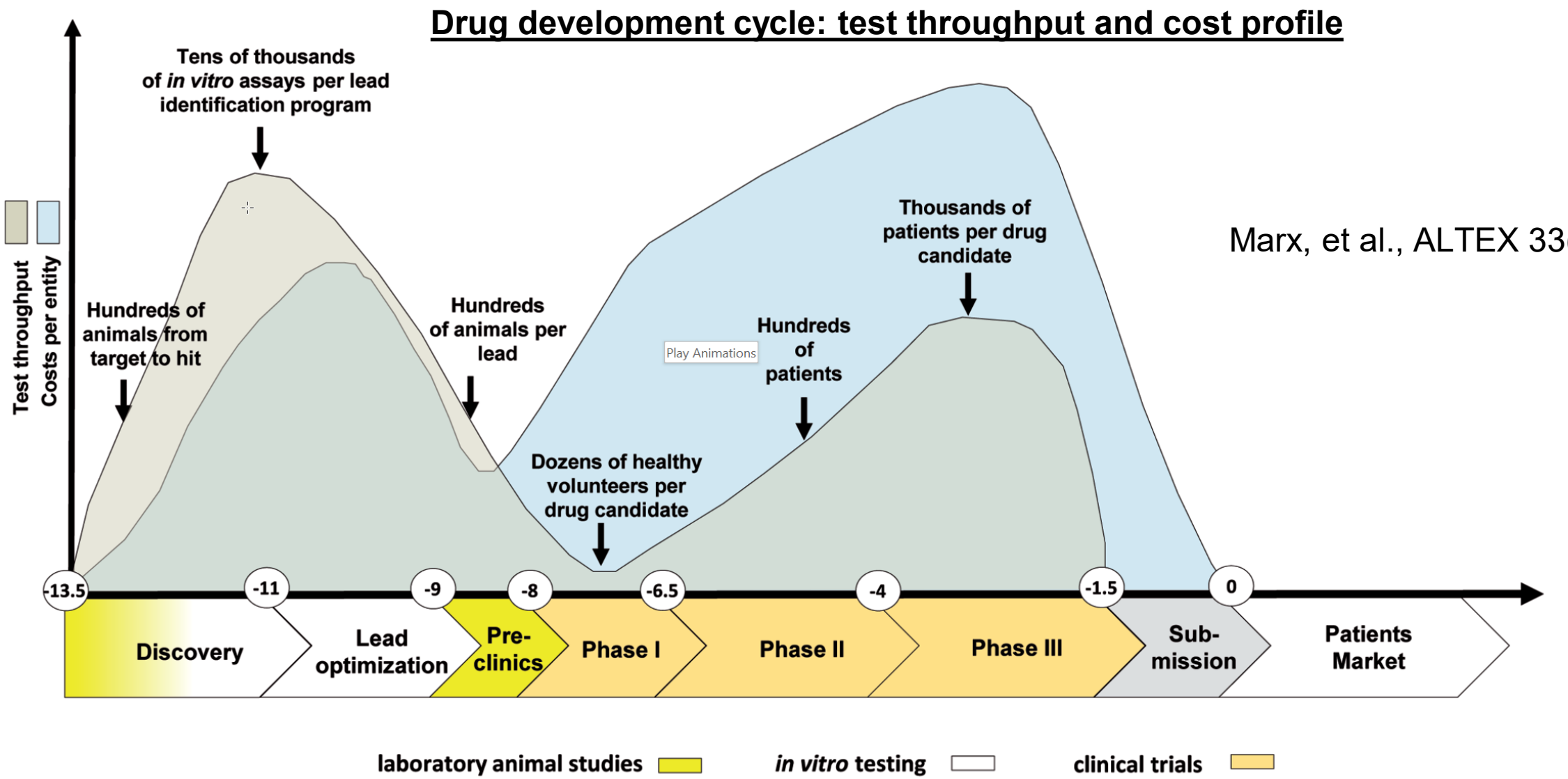
140 covers and counting!

What are microphysiological systems?

Miniature *in vitro* models of human physiological systems comprising

- Engineered organoids (EOs), or
- Single organs-on-chips (OoCs), or
- Tissue chips (TCs), or
- Multiple interconnected organ chips, or
- A human-on-a-chip.
- NOT monocultures on flat plastic

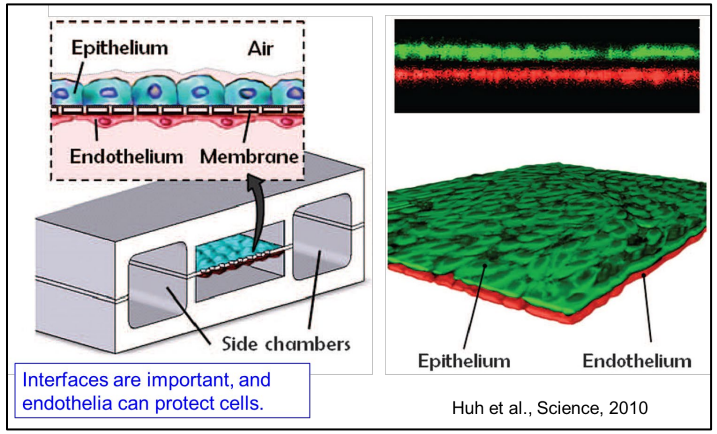
How do you test a drug or regeneration protocol?

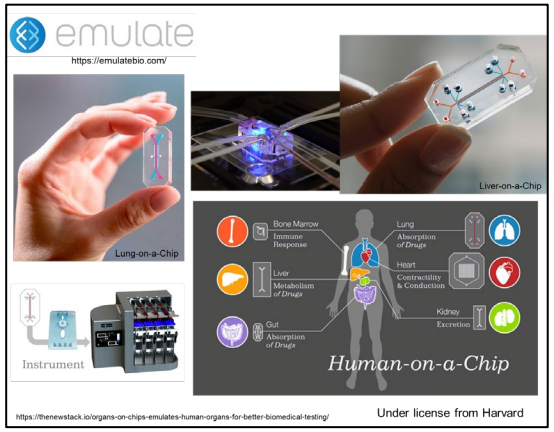


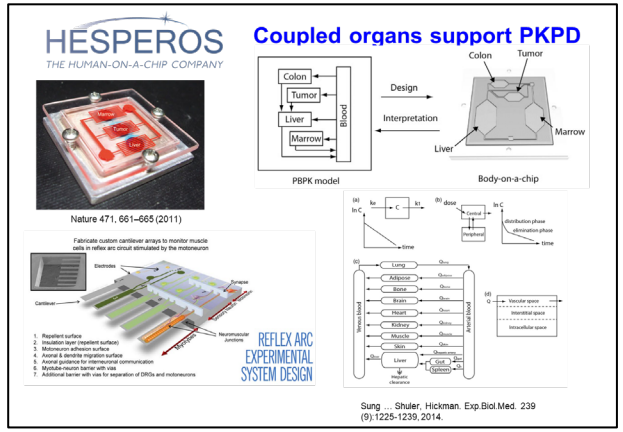
Marx, et al., ALTEX 33(3), 2016

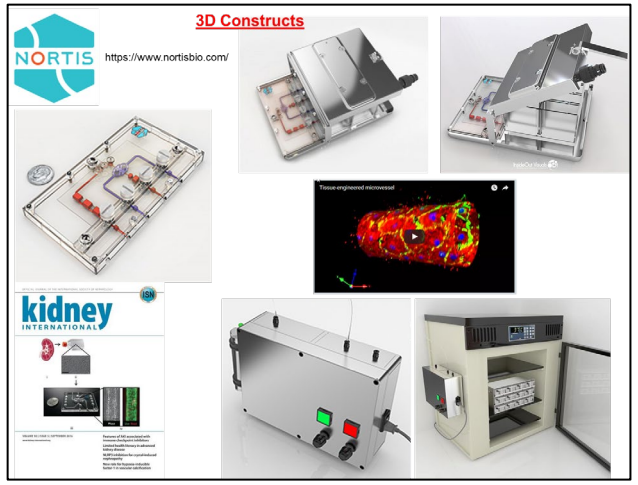
The vertical axis illustrates approximate numbers of tests performed (grey) and related spending (blue). The horizontal axis illustrates the development time in years.

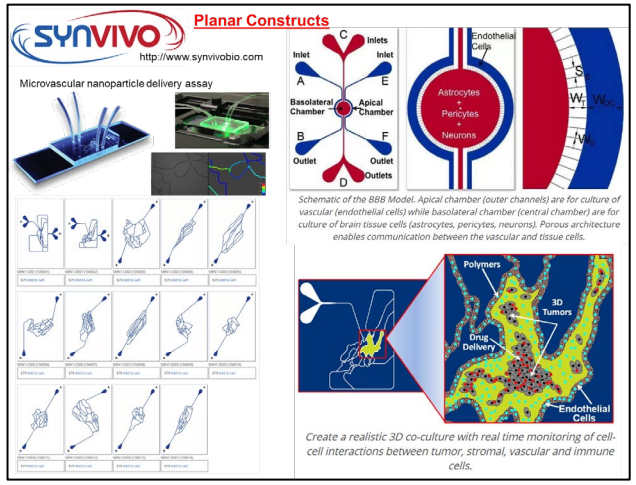
Organ-Chips, Organ Chips, Organ Chips…

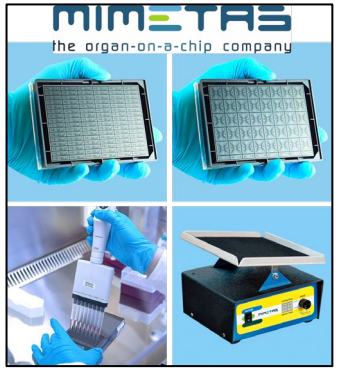












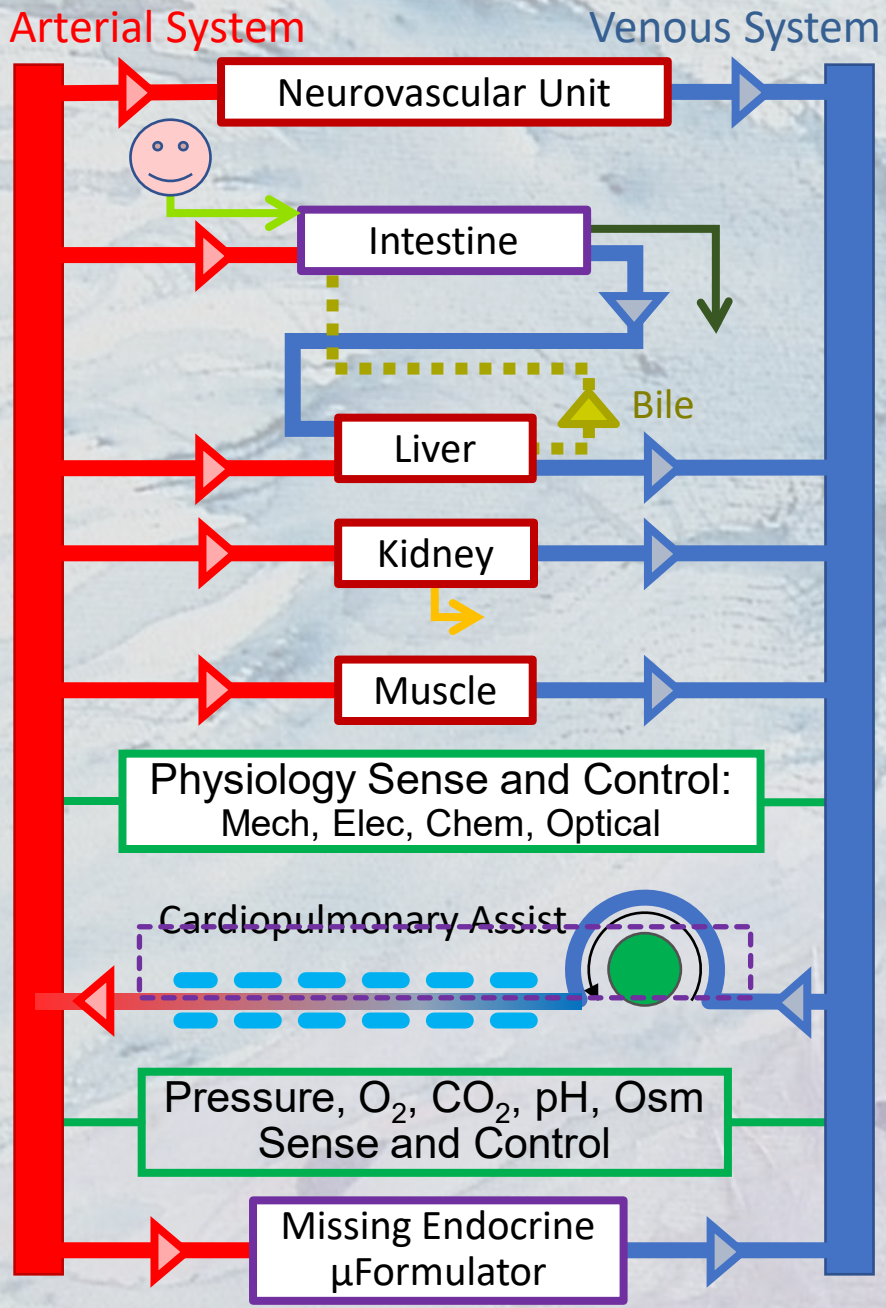
Others, and there's room to grow!

How are organs-on-chips being used?

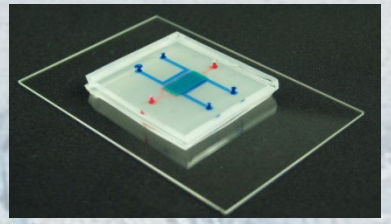
Single and coupled organ-chip studies for

- Drug and environmental toxicology
- Pharmacology (PK/PD)
- Determination of drug efficacy
- The biology of rare diseases
- Organ-organ communication, including metastasis and toxicity
- Studying phenomena for which animal models are inadequate

NIH-NCATS MPS Integration

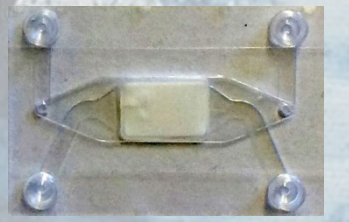


NVU



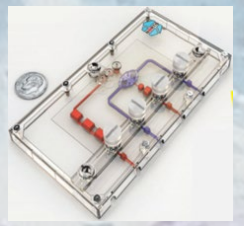
Vanderbilt

Intestine



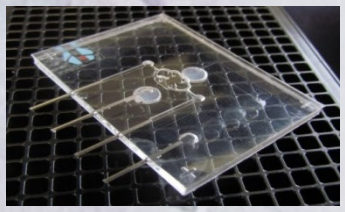
Hopkins, Baylor

Liver



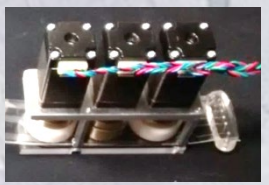
Pittsburgh

Kidney



Washington

ME-µF



Vanderbilt

Plenary Session 3: Organs on Chips

Megan McCain, PhD

Associate Professor, Department of Biomedical Engineering

Viterbi School of Engineering

University of Southern California

“Engineering Cardiac and Skeletal Muscle on a Chip Systems for Multi-Modal Physiological Analysis and Disease Modeling”

Yoko M. Ambrosini, DVM, MPVM, PhD, DACVIM (SAIM)

Assistant Professor, Department of Veterinary Clinical Sciences

College of Veterinary Medicine

Washington State University, PhD

"Pathomimetic human intestinal disease-on-a-chip: From organomimicry to precision medicine”

Peter Loskill, PhD

Professor for Organ-on-Chip-Research

Eberhard Karls University Tübingen and the Natural and Medical Sciences Institute (NMI)

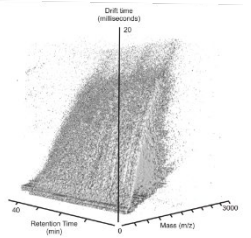
“Microphysiological platforms, organoids and enabling technologies: Integrated Organ-on-Chip models recapitulating complex human tissues”

What makes regenerative medicine hard?

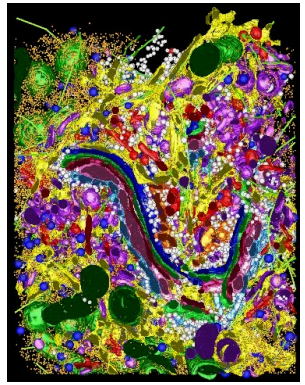
- The complexity of biology
  - Developmental sequences
  - Multiscale interactions
- The inaccessibility of the systems
  - Sensing
  - Control
- The lack of good *in vitro* models
- The lack of good systems biology models

Why is biology so complex?

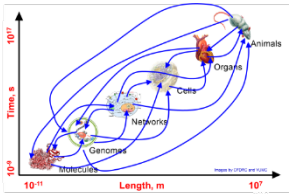
- Today, one can easily detect 100,000 chemical species in 100 µL of rat serum.
- Cells are NOT well-stirred bioreactors but have anomalous diffusion and active transport.
- 10^9 - 10^{11} interacting cells in some organs.
- Cell signaling is dynamic, non-linear, multiscale, redundant, and has positive and negative feedback.
- Metabolism may have 5000 reactions.
- All-of-biology models might need Avogadro's number of PDEs, i.e., a Leibniz of PDEs (1 L = N_A).
- We need new experimental approaches to get the optimal data to create better models.



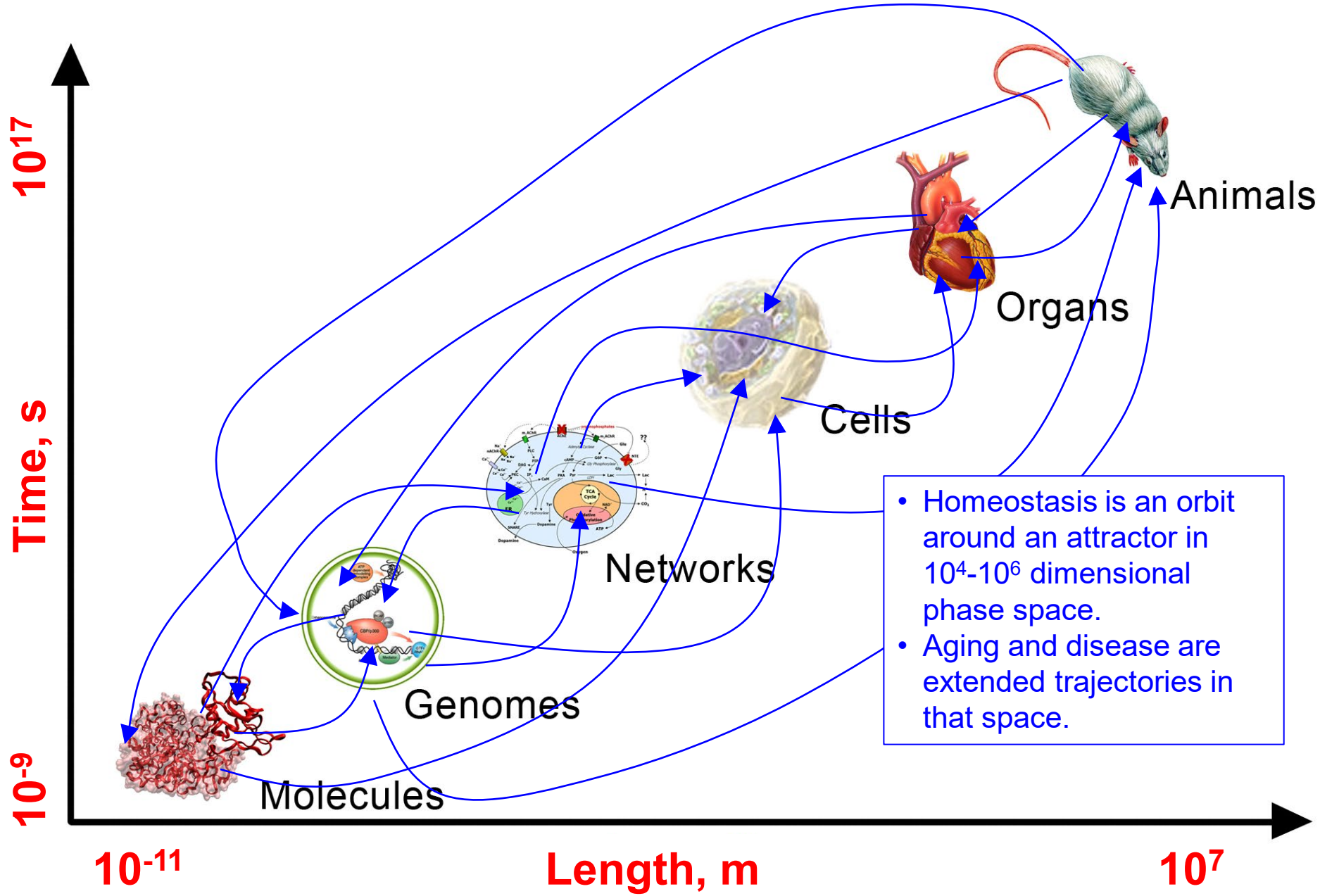
UPLC-nESI-IM-MS
John McLean



3.1 x 3.2 x 1.2 µm^3 beta cell
Brad Marsh, PNAS, 2001



Complexity from multiscale interactions



How have we been studying biology?

- People

  We are severely limited in isogenetic controls, interventions, and data when studying normal subjects and patients.

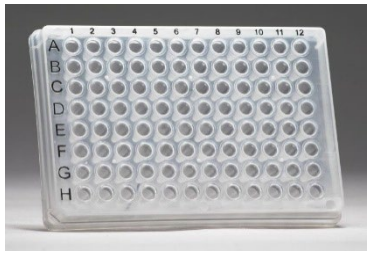
- Animals

  Animals, including non-human primates, are not people and have significant genetic and physiological differences.

- Cells *in vitro*

  2D biology on plastic: Many biological experiments are conducted on cells that
  - have cancer,
  - are inbred,
  - are diabetic,
  - are potatoes on a stiff plastic couch without exercise,
  - enjoy neither gender nor sex,
  - live almost entirely in the dark,
  - gorge themselves on sugar once a day,
  - may be slowly suffocating in an increasingly acidic environment,
  - live in their own excrement,
  - never bury their dead,
  - may take a complete or only partial bath every day or two,
  - and talk only to cells of like mind.

glasslaboratory.com/files/2245127/uploaded/GL-P100%20Petri%20Dish.jpg



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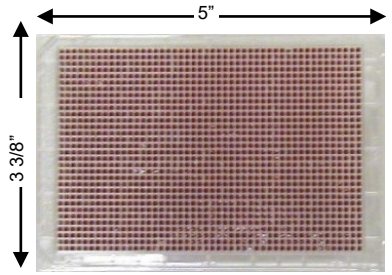
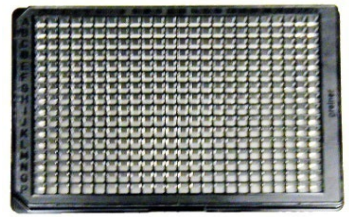
tpp.ch/page/bilder/Produkte/TC_flasks_standard/flasks_all2.jpg



1536 Well ~8 µl



384 Well ~40 µl

384 and 1536 images courtesy of David Weaver

One might get reproducible, statistically significant results, but are they relevant to human biology and disease?

Watson, Hunziker, and Wikswo, Exper. Biol. and Med., 2017

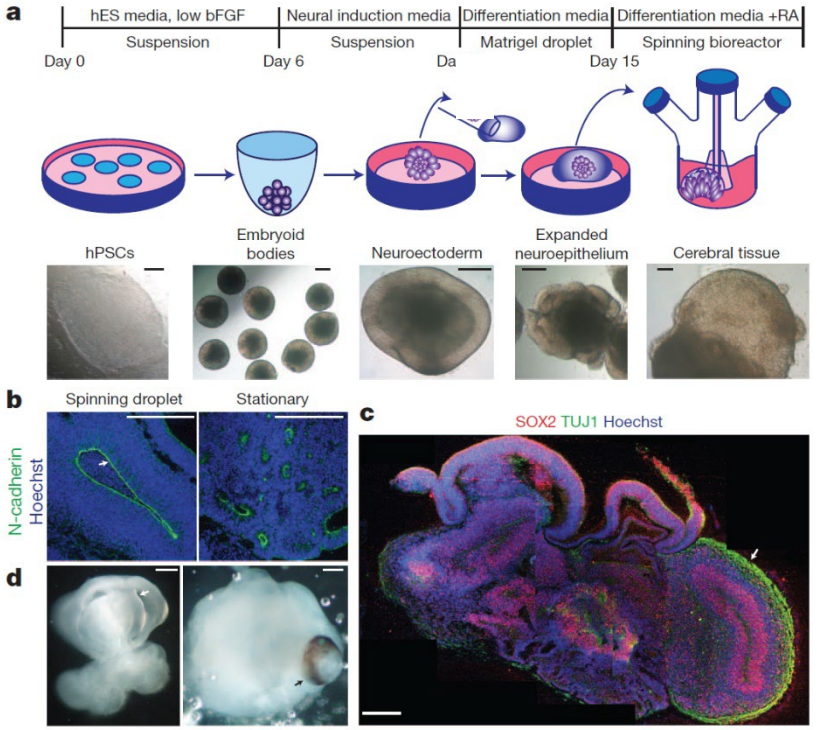
A hot, new *in vitro* model for biology

Complex 3D biology is a better model than 2D biology.

- 3D Organoids

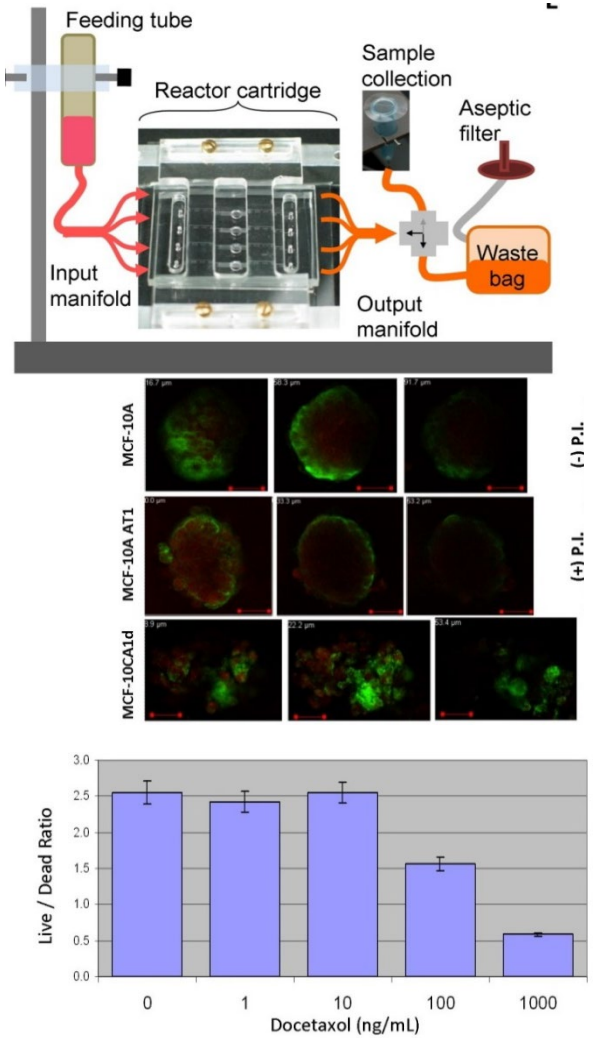
Are self-organizing models with tissue-level functions and disease phenotypes.
Demonstrate development
Excellent cell-cell coupling
Good for immunofluorescent imaging
Can be transplanted
Can be a medium-to-high throughput assay
Hard to replicate an individual organoid
Hard to perfuse or apply uniform shear stress
Poor clearance / high buildup of compounds on inner lumen
No effluent sampling from intracellular compartments
Low tissue volumes for secretome sampling
Hard to quantify barrier functions
If floating, cannot apply mechanical stresses/loading
Can be dependent on Matrigel or engineered hydrogels
Hard to visualize when living
Hard to integrate with other organ systems with proper volumes

Contributions from Kapil Bharti (NIH/NEI)



Lancaster, … , Knoblich. Cerebral organoids model human brain development and microcephaly. Nature, **2013**.

2012



Markov, … , McCawley. Thick-tissue bioreactor as a platform for long-term organotypic culture and drug delivery. Lab Chip 12:4560-4568. 2012.

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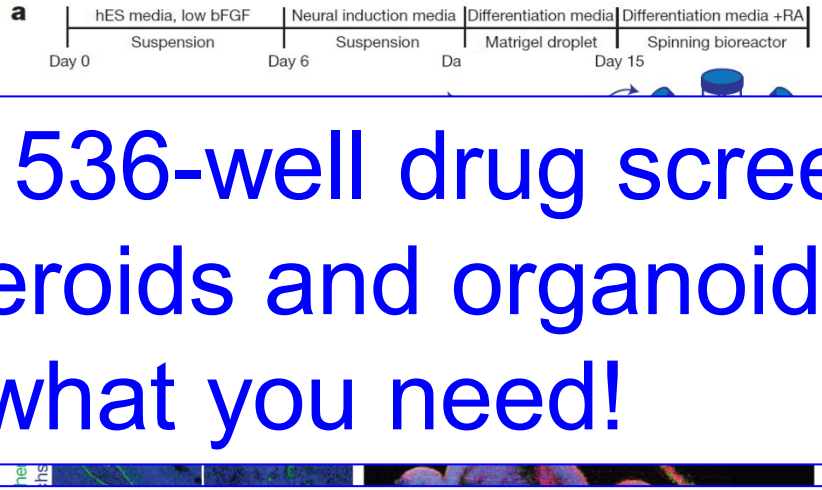
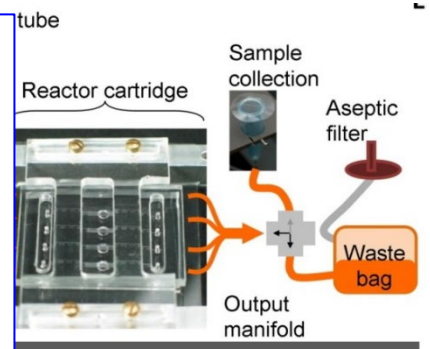
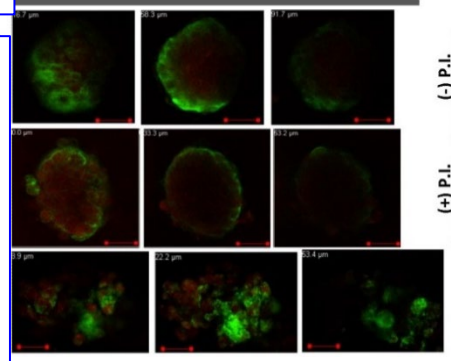
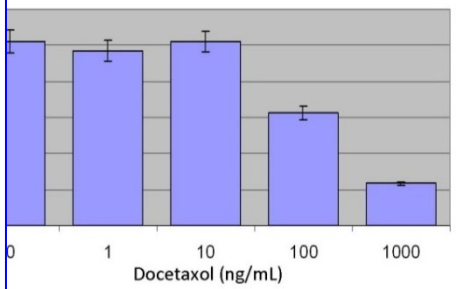


2012







If you need 1536-well drug screens, then 3D spheroids and organoids may be just what you need!

If you need more sample mass for quantitative analysis, polarized endothelial/epithelial barriers, or are worried about organ-organ interactions, then you need to think about organ chips!

Contributions from Kapil Bharti (NIH/NEI)

… , McCawley. Thick-tissue bioreactor as … for long-term organotypic culture and drug delivery. Lab Chip 12:4560-4568. 2012.

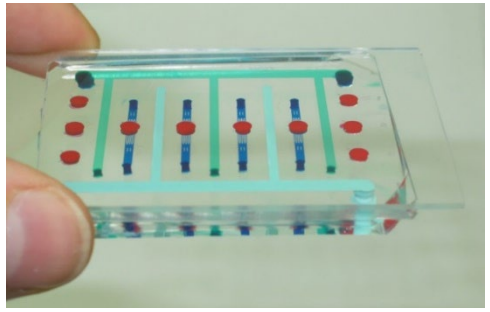
Another hot new *in vitro* model for biology

Complex 3D biology is a better model than 2D biology.

- Organ Chips

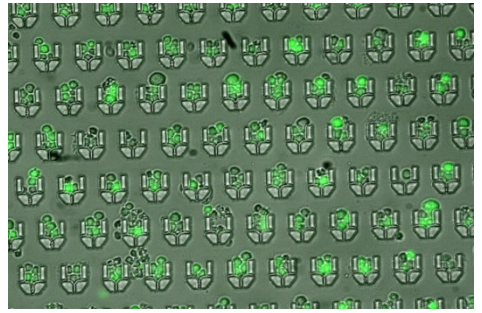
Better than 2D biology
Ideal for barrier functions
Good sampling of effluent from both apical and basal side of cell layer
Can reproduce physiological flows
Provides a thick ECM for scaffolding and drug/factor binding
Support organ-organ interactions
Sufficient tissue for multi-omics of 10's to 1000's of variables
Can use minimal media volumes
Will be vascularized soon
May ultimately reduce drug costs
Possible to build a single-patient homunculus
Could build animals-on-chips
Can require microfluidics and control
Not yet high throughput
Today it is difficult to extract cells
Are expensive today (hardware, effort, human cells, real estate)
Not fully validated vs *in vivo, e.g.,* no WGCNA yet
Can't be transplanted

Mammary gland on a chip



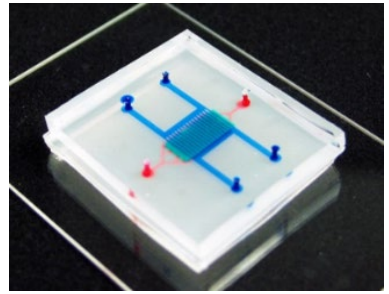
Lisa McCawley and Dmitry Markov, Vanderbilt

T cells in a lymph node on a chip



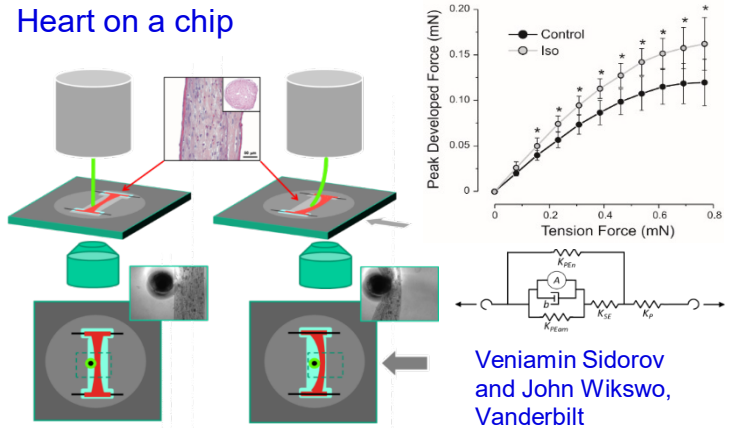
Shannon Faley, Kevin Seale and John Wikswo, Vanderbilt

Brain on a chip



Jacquelyn Brown and John Wikswo, Vanderbilt

Heart on a chip



Veniamin Sidorov and John Wikswo, Vanderbilt

The Volume Problem in MicroPhysiological Systems

The "Volume problem"

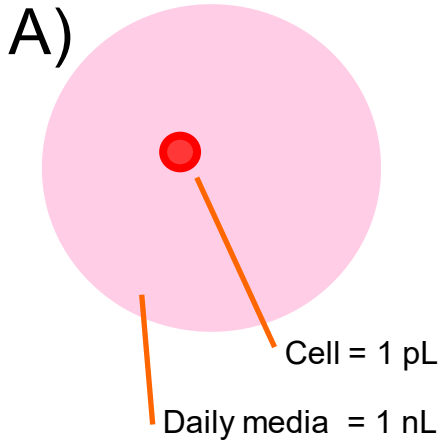
A) A pL cell requires a nL of fresh media each day.

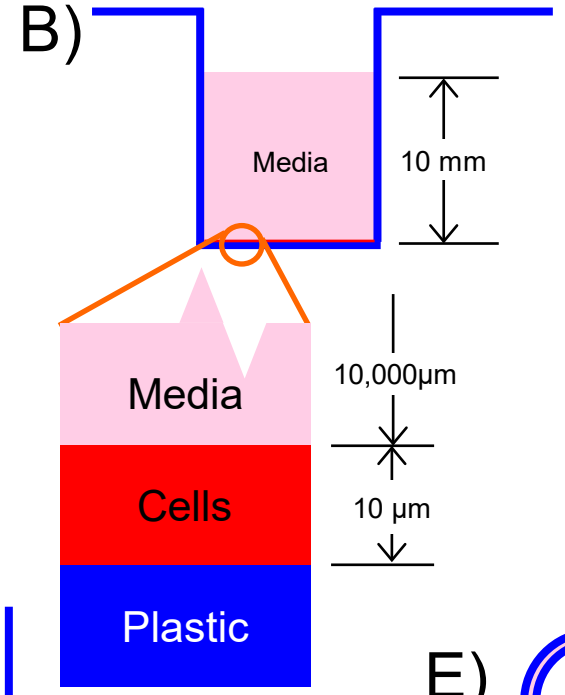
B) The "Volume problem" in dishes and wells: paracrine, autocrine and endocrine factors diluted by a factor of 1000.

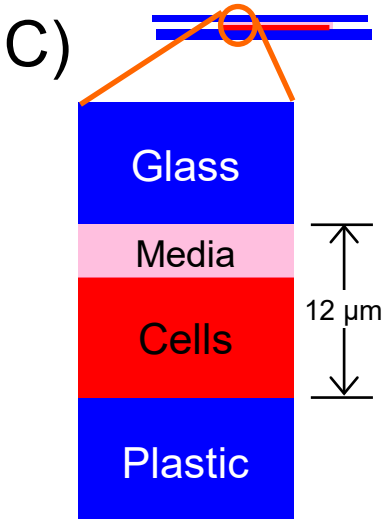
C) Microfluidics can reduce the volume of a single organ-on-chip.

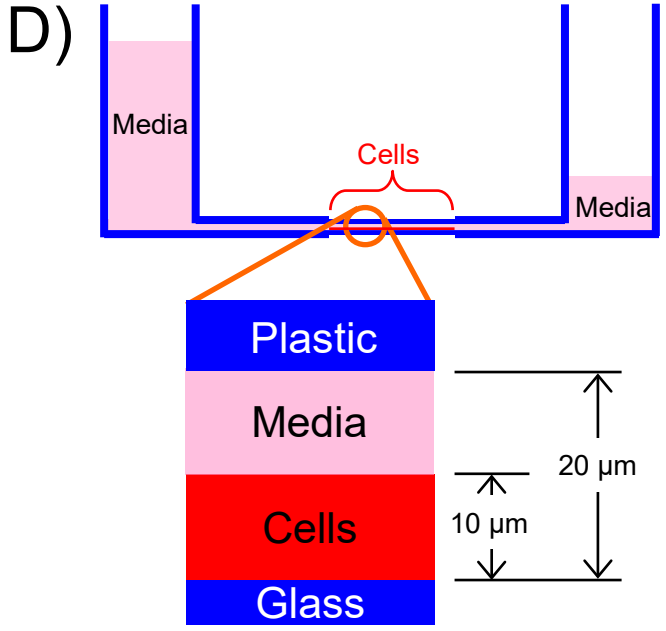
D) Pipetting between reservoirs may not solve the volume problem

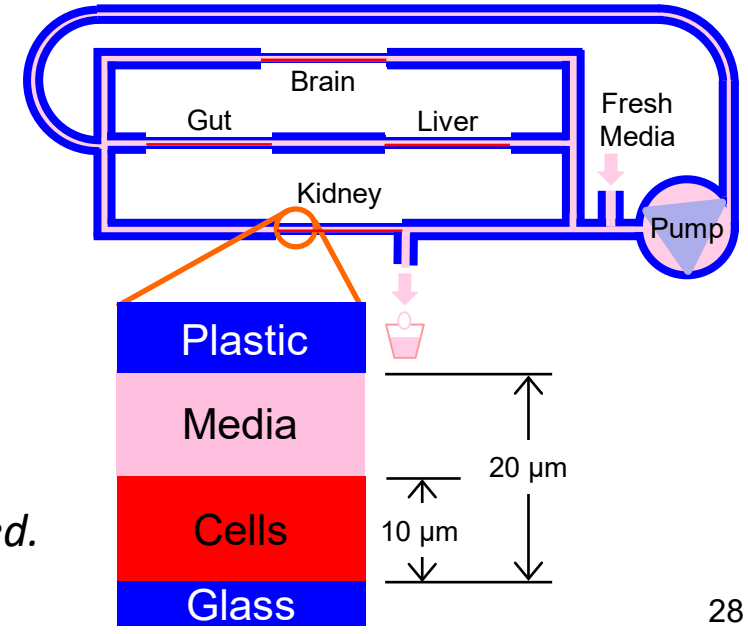
E) Integrated microfluidics should solve the coupled organ volume problem.











JP Wikswo, *Exp.Biol.Med. 239: 1061-1072, 2014.*

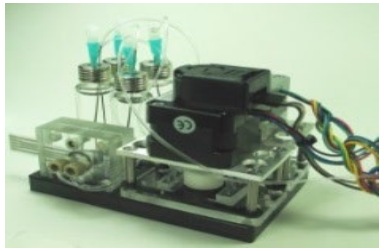
How might microphysiological systems contribute to regenerative medicine?

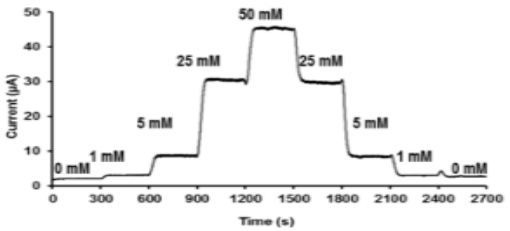
- Provide *in vitro* models that are more realistic than biology on flat plastic
- Provide *in vitro* models that are simpler than animals and easier to control
- Support measurement of cell-cell and organ-organ communication
- Support demonstration of cell-level control

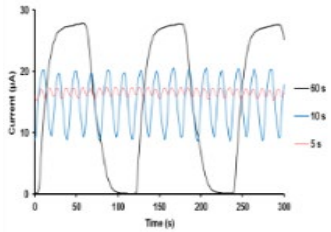
What do we need to make both regenerative medicine and microphysiological systems easier?

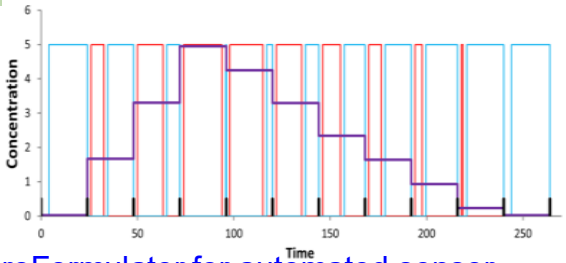
- Sourcing cells — Optimization of cell differentiation and expansion
- Assembling cells
- Perfusing cells
- Maintaining physiological scaling
- Analyzing the cells
- Controlling the cells — Better models of cell signaling and metabolism

VIIBRE'S Microformulators – Single then Multiwell

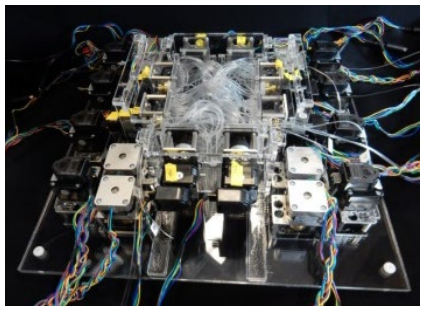


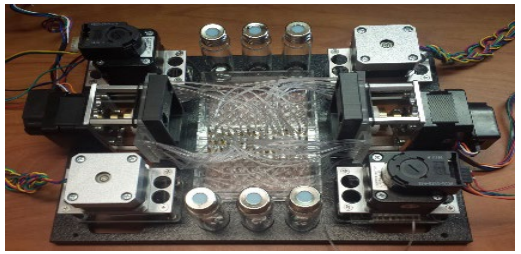






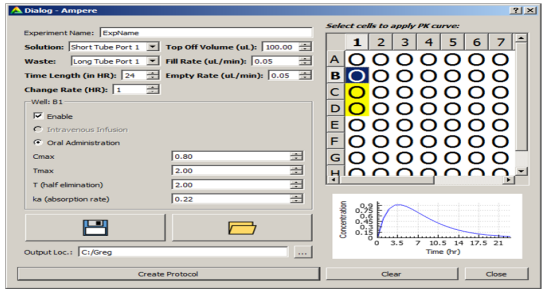
MicroClinical Analyzer/MicroFormulator for automated sensor calibration and time-division multiplexing to create toxin and drug cocktail exposures

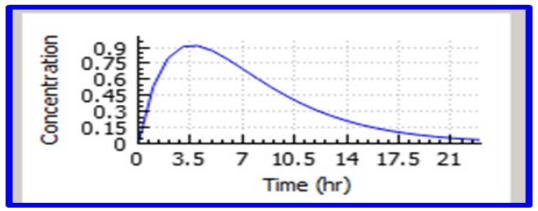


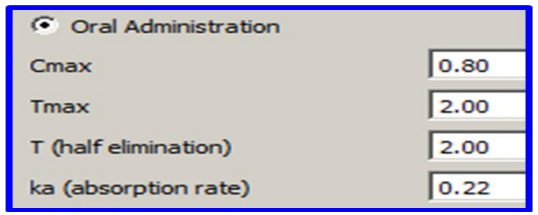


V1.0 24- and 96-Channel push-pull MultiWell MicroFormulators for *in vitro* pharmacokinetic control at AstraZeneca

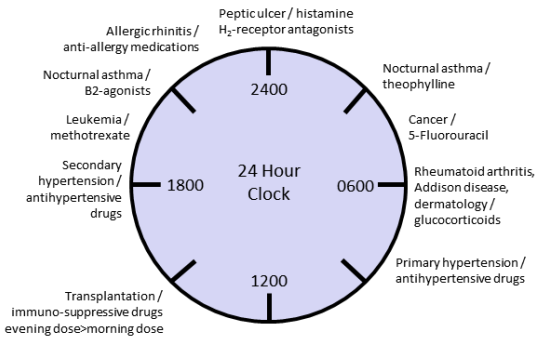
Lead engineer Ron Reiserer



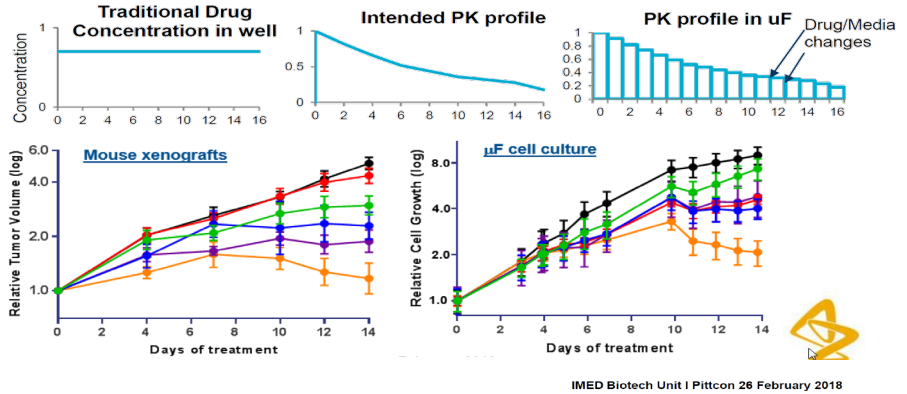




VIIBRE's Automated Multi-Pump Experiment Running Environment (AMPERE) Well Plate Tool. Lead engineers Erik Werner and Greg Gerken



Enables *in vitro* chronobiology



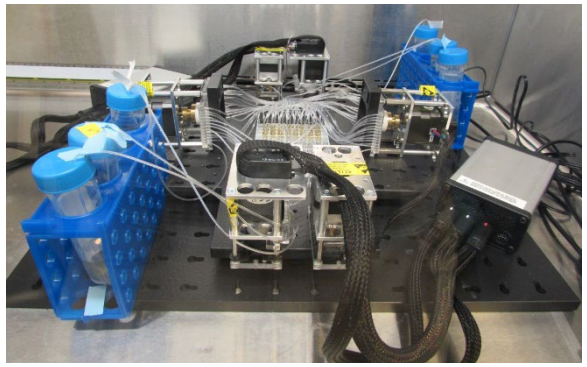
AstraZenca: *in vitro* simulation of *in vivo* toxicology

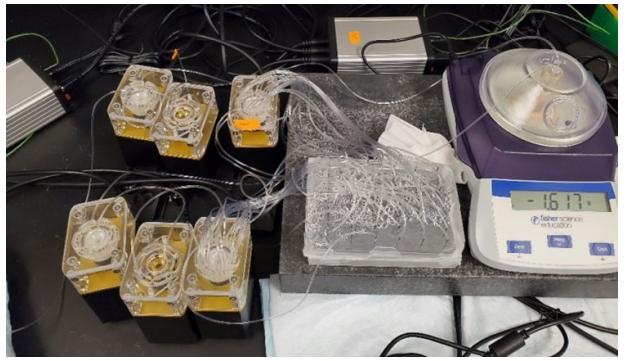
Developed by CN Bio Innovations under license from Vanderbilt University

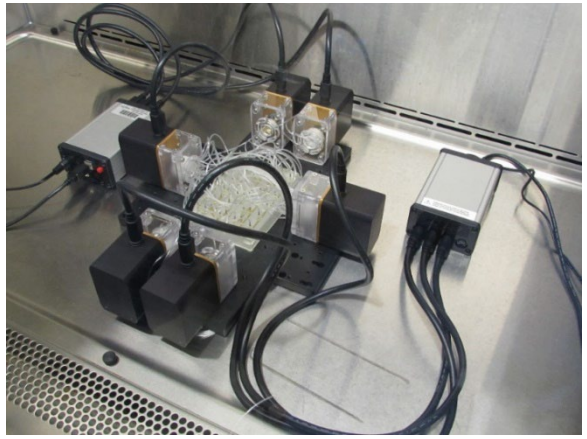
CN Bio Innovations PhysioMimix™ Exposure Response System

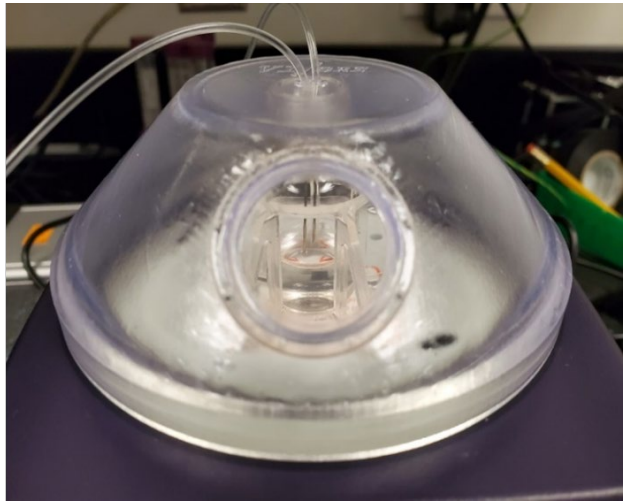
iPSC-Differentiation MicroFormulator (Lippmann & Wikswo)

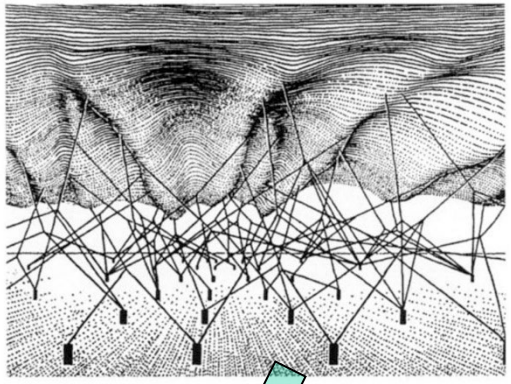
The MicroFormulator will provide long-term control of the cellular chemical environment.

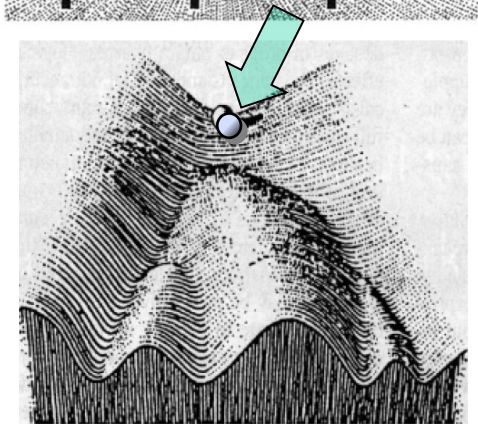




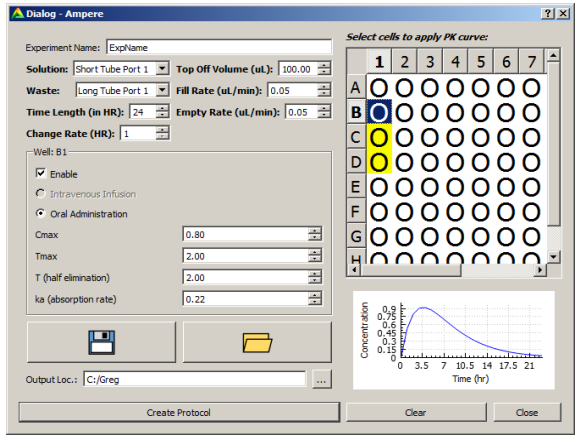


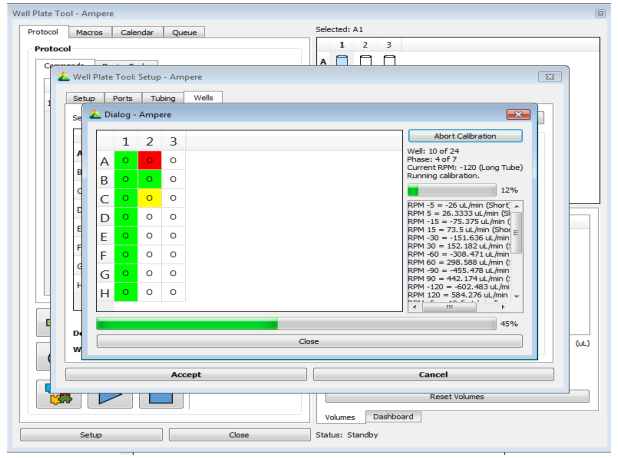




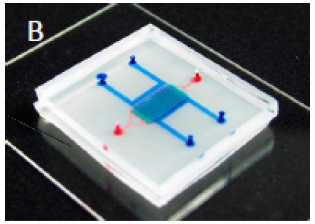


Waddington 1957



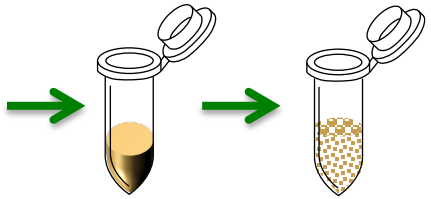


NVU/BBB UPLC-IM-MS workflow

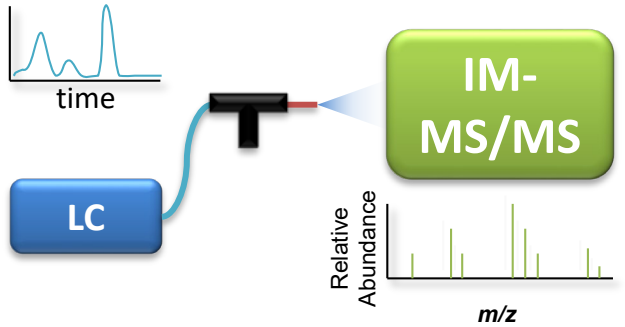


NVU

NVU exposed to cytokine cocktail

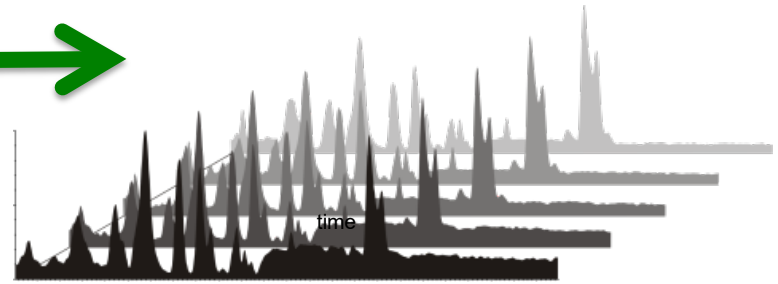


Sample preparation
metabolites extracted using ice cooled methanol:H_2O (80:20), incubated -80ºC overnight, spun down at 15,000 rpm, 15 min
dried down *in vacuo*

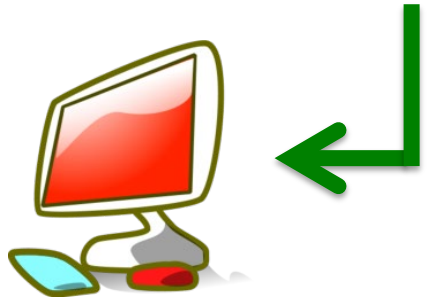


Sample Acquisition
LC IM-MS/MS of metabolite extracts

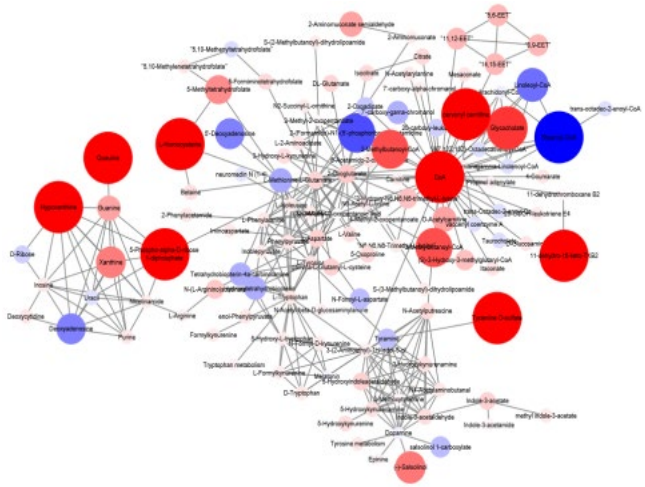
LPS or Cytokine treated samples



Data Alignment and Biostatistical Analysis
Progenesis QI



Network and Pathway analysis
Mummichog



Pathways Analysis	p-value
Vitamin E metabolism	8.00E-05
Glutathione Metabolism	1.13E-03
Prostaglandin formation from arachidonate	6.48E-03
Aspartate and asparagine metabolism	9.95E-03
Drug metabolism - cytochrome P450	9.97E-03

Network & Pathway Module Output

Brown *et al.*, J. Neuroinflammation, 2016

Metabolomic pathway analysis with high mass-accuracy MS facilitates the incorporation of untargeted metabolomics into mechanism of action studies.

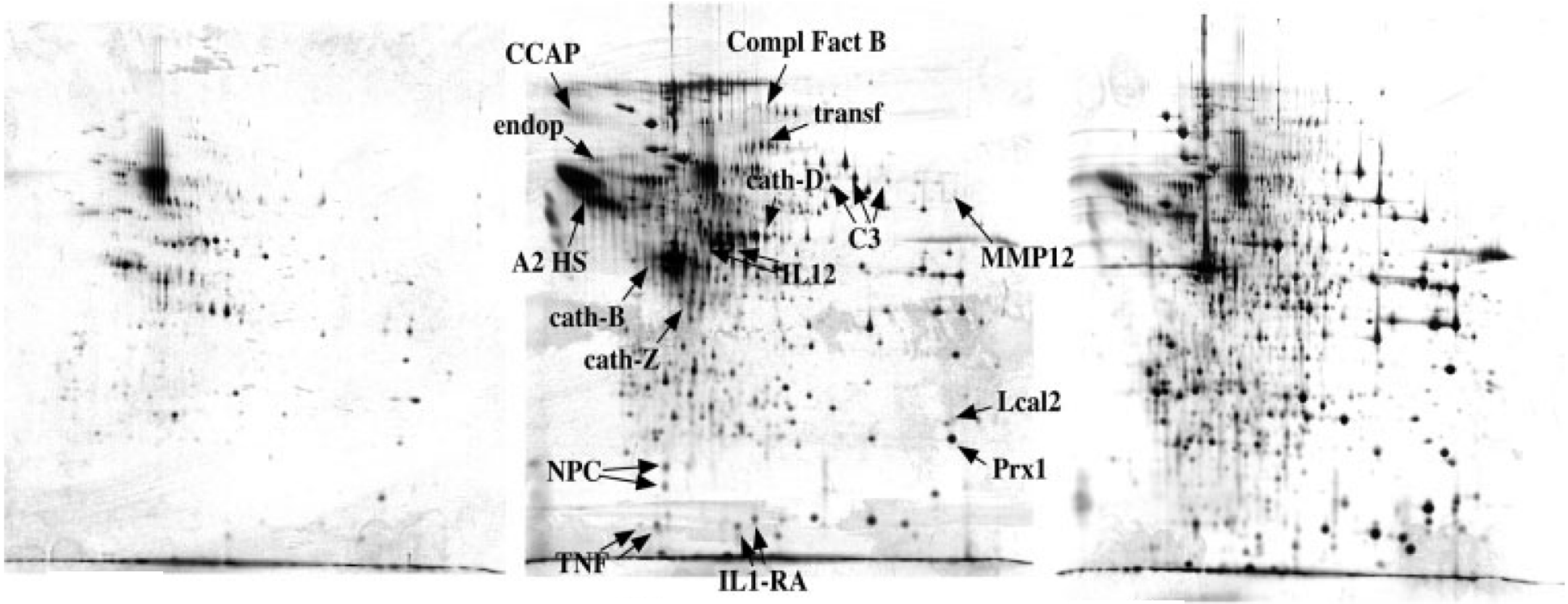
Does the metabolomic (and eventually proteomic) secretome contain sufficient information about cellular differentiation to provide a non-destructive control signal for iPSC differentiation?

Proteins in Secretome vs Cytosol

Protein Secretome of Immature Dendritic Cells

Protein Secretome of LPS-Activated Dendritic Cells

Proteins From Cell Lysate



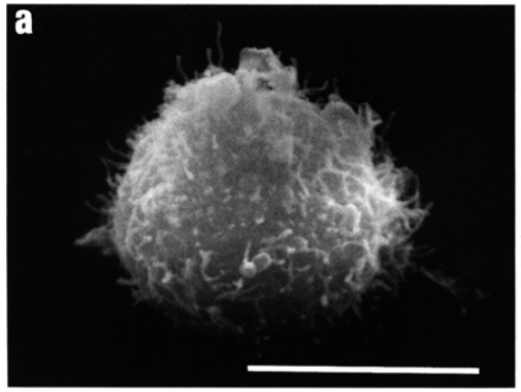
Protein Secretome

Lysate Proteins

What is the small-molecule, metabolite secretome?

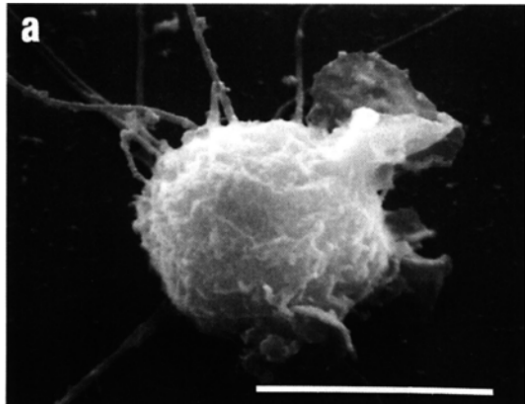
Chevallet, et al. *Proteomics.* 2007.

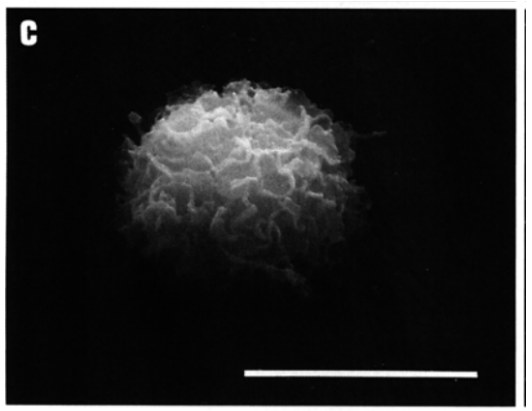
HL60 Differentiation to Neutrophil-Like Cell



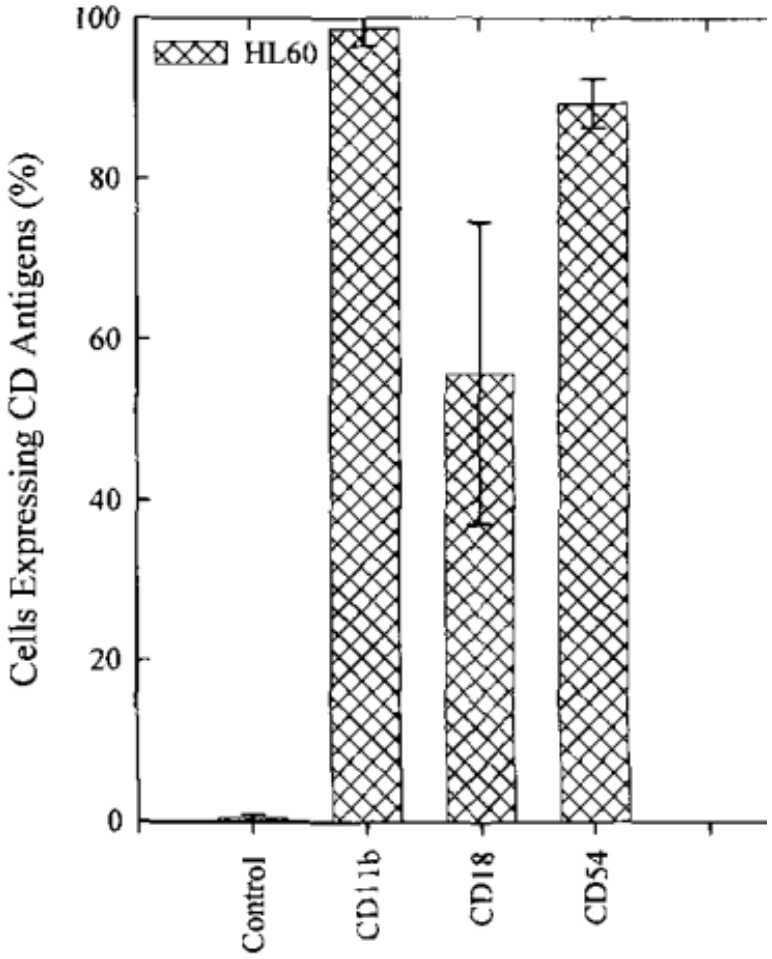
DMF

ATRA





Morphological changes evident after 3-4 days of treatment with either dimethylformamide (DMF) or all-*trans*-retinoic acid (ATRA), including changes in surface microvilli to more ruffled structures (scale bar 10 microns)



HL60 cells treated for 96 hrs show increased antigen expression consistent with neutrophil-like behaviors

Images from Fleck 2003 *In Vitro Cell Developmental Biology*

Cell Fates as High-Dimensional Attractor States of a Complex Gene Regulatory Network

Sui Huang,[1,*] Gabriel Eichler,[1] Yaneer Bar-Yam,[2] and Donald E. Ingber[1]

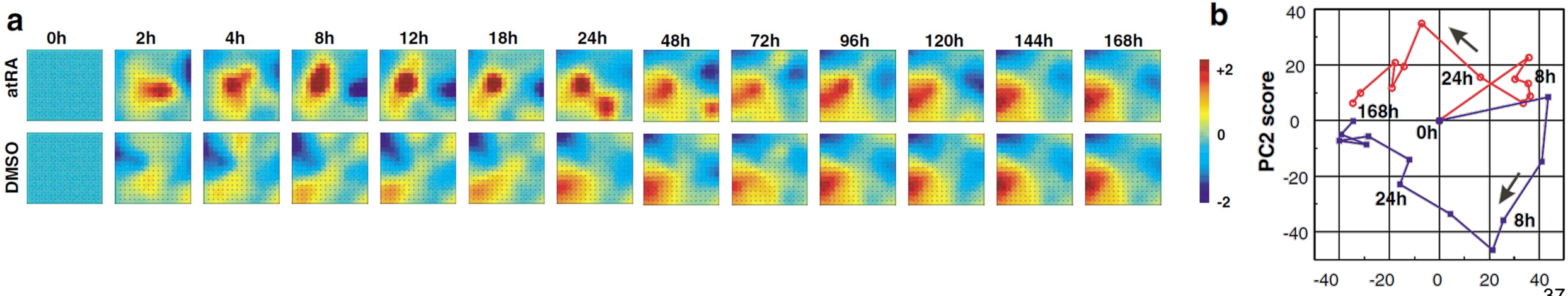
[1]*Vascular Biology Program, Departments of Pathology & Surgery, Children's Hospital and Harvard Medical School, Boston, Massachusetts 02115, USA*

[2]*New England Complex Systems Institute, Cambridge, Massachusetts 02138, USA*

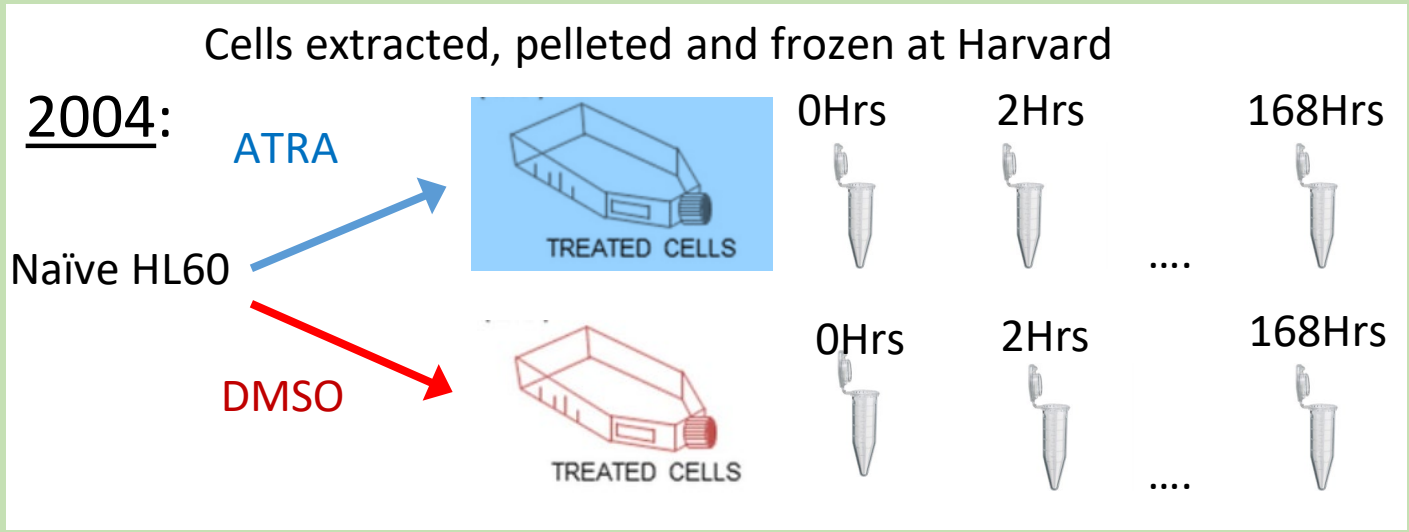
(Received 13 September 2004; published 1 April 2005)

Cells in multicellular organisms switch between distinct cell fates, such as proliferation or differentiation into specialized cell types. Genome-wide gene regulatory networks govern this behavior. Theoretical studies of complex networks suggest that they can exhibit ordered (stable) dynamics, raising the possibility that cell fates may represent high-dimensional attractor states. We used gene expression profiling to show that trajectories of neutrophil differentiation converge to a common state from different directions of a 2773-dimensional gene expression state space, providing the first experimental evidence for a high-dimensional stable attractor that represents a distinct cellular phenotype.

DOI: 10.1103/PhysRevLett.94.128701 PACS numbers: 89.75.-k, 05.45.-a, 87.16.Yc, 87.18.-h

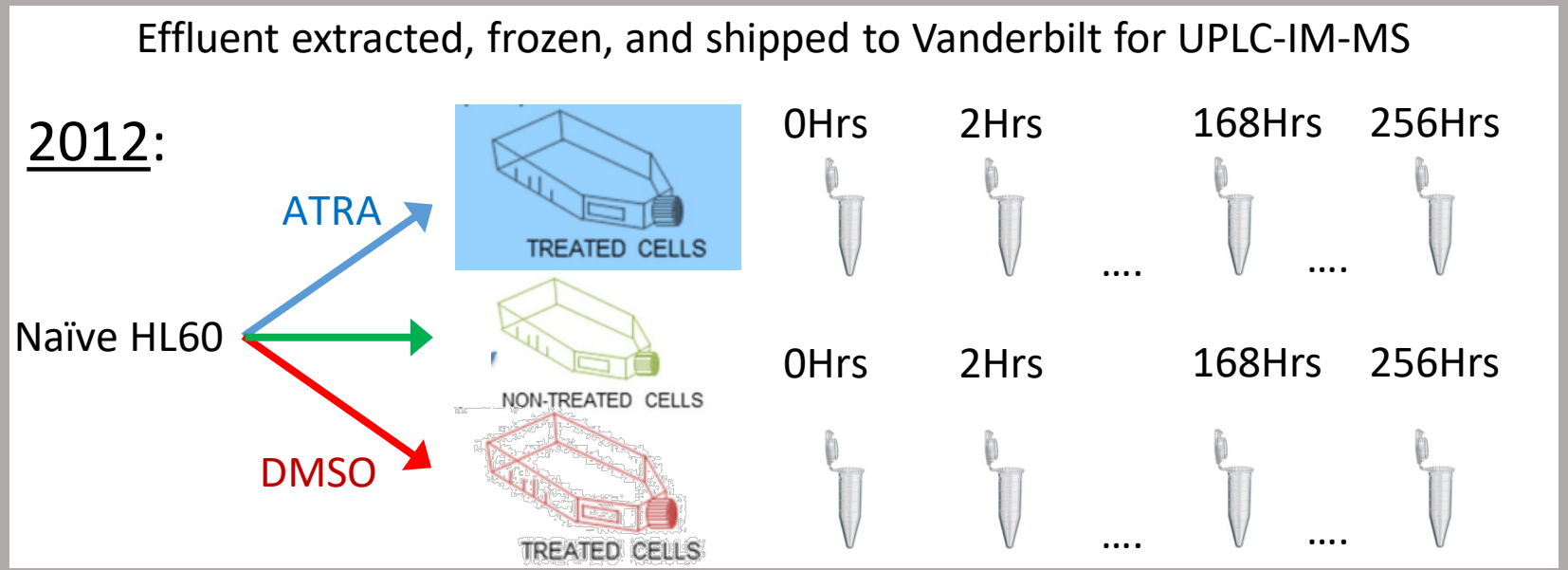


HL60 Differentiation - Data Sets:



RNA measured by Affymetrix gene expression
3,861 species

Huang, S., et al., "Cell fates as high-dimensional attractor states of a complex gene regulatory network." Physical Review Letters 94(12): 128701 (2005).

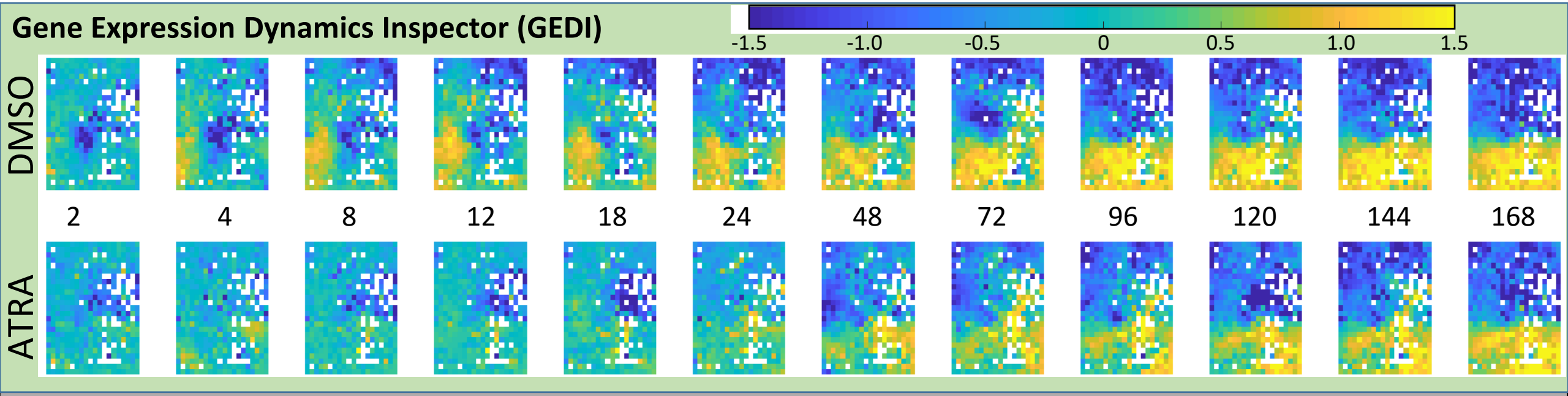


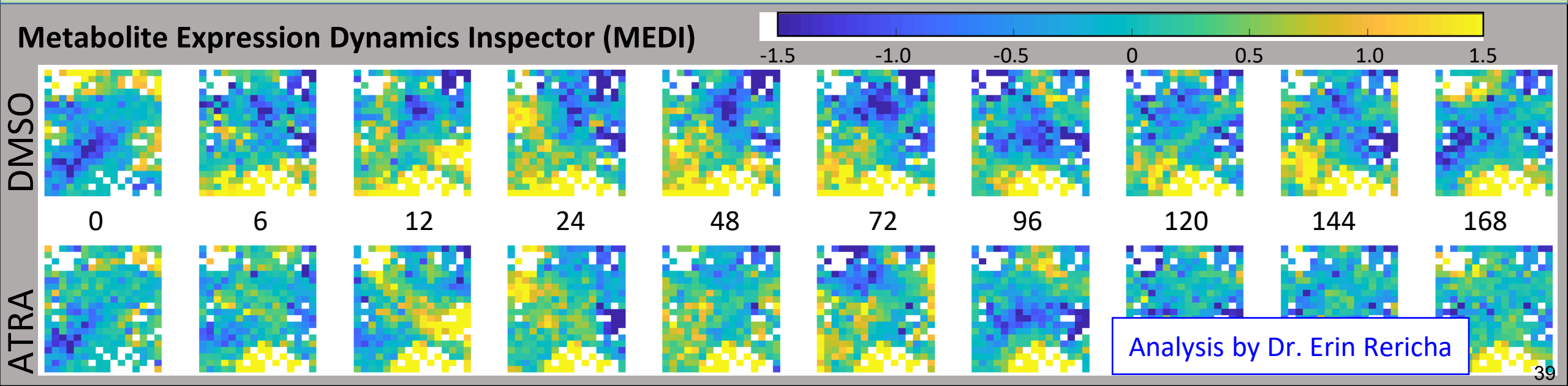
Metabolite concentration measured by UPLC-IM-MS
1649 species

Analysis by Dr. Stacy Sherrod

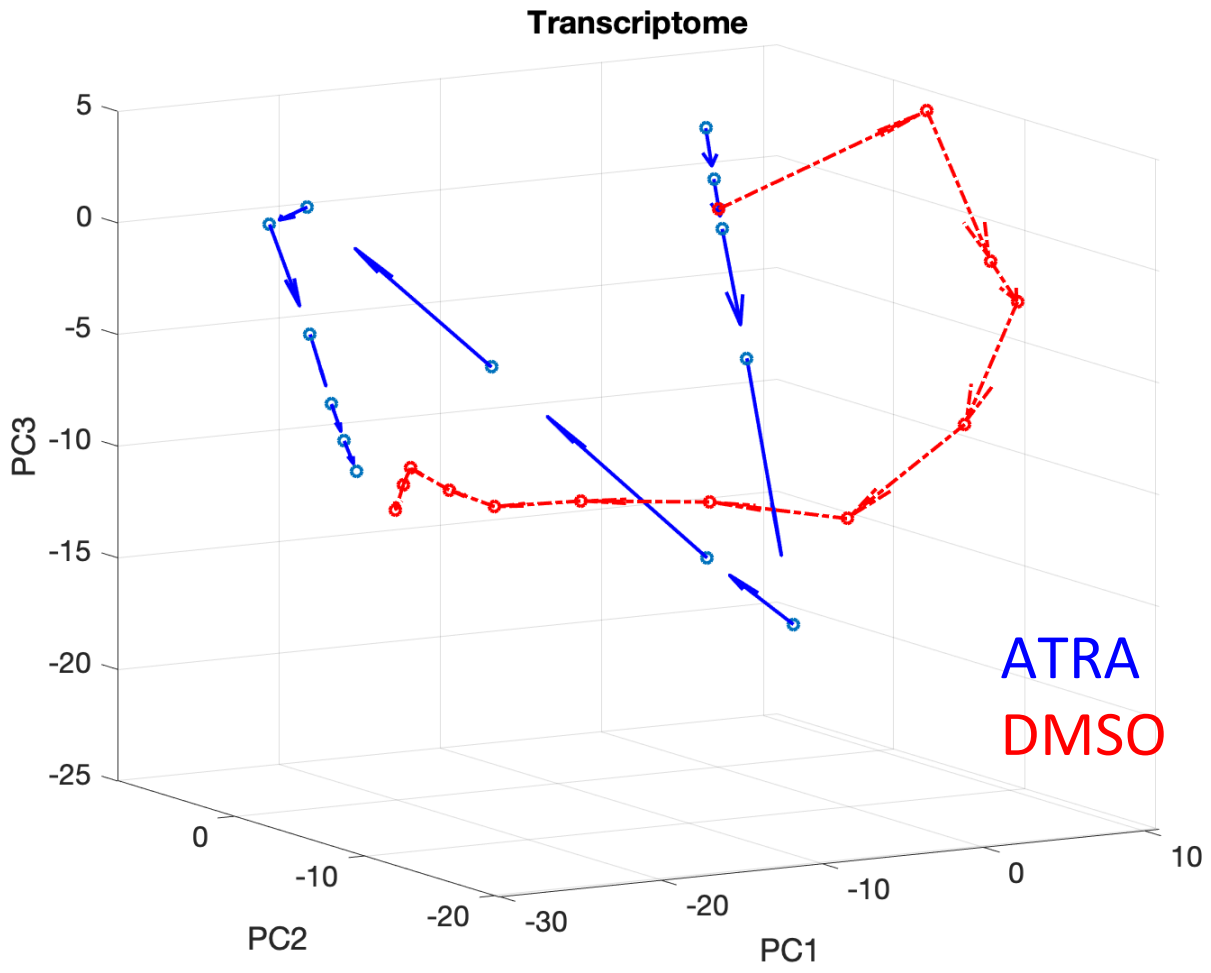
Sui Huang arranged the relative expression (to t=0) of the 3861 species into self-organizing maps (SOMs), showing treatment paths in genetic space

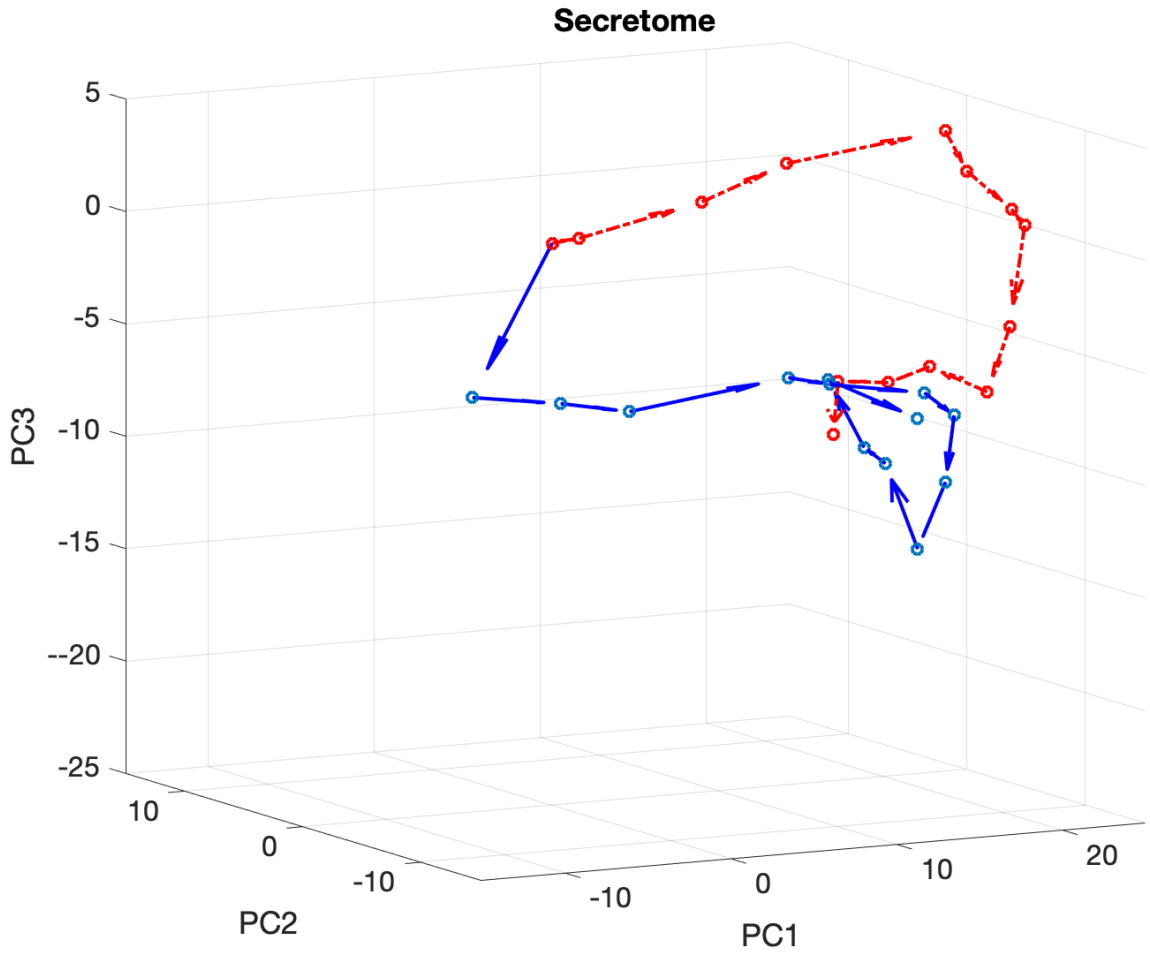
Huang, S., et al., "Cell fates as high-dimensional attractor states of a complex gene regulatory network." Physical Review Letters 94(12): 128701 (2005).





Principal Component Analysis of the Differentiation Trajectories



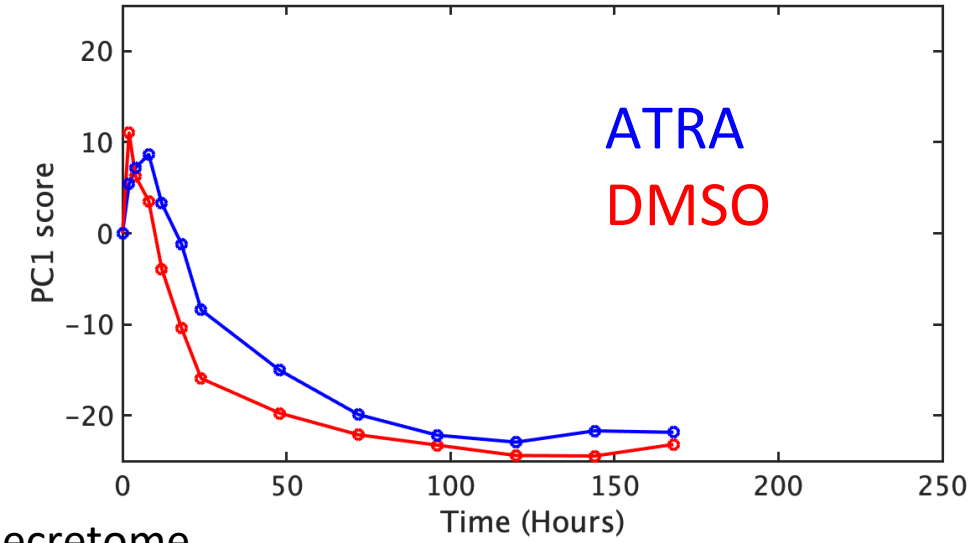


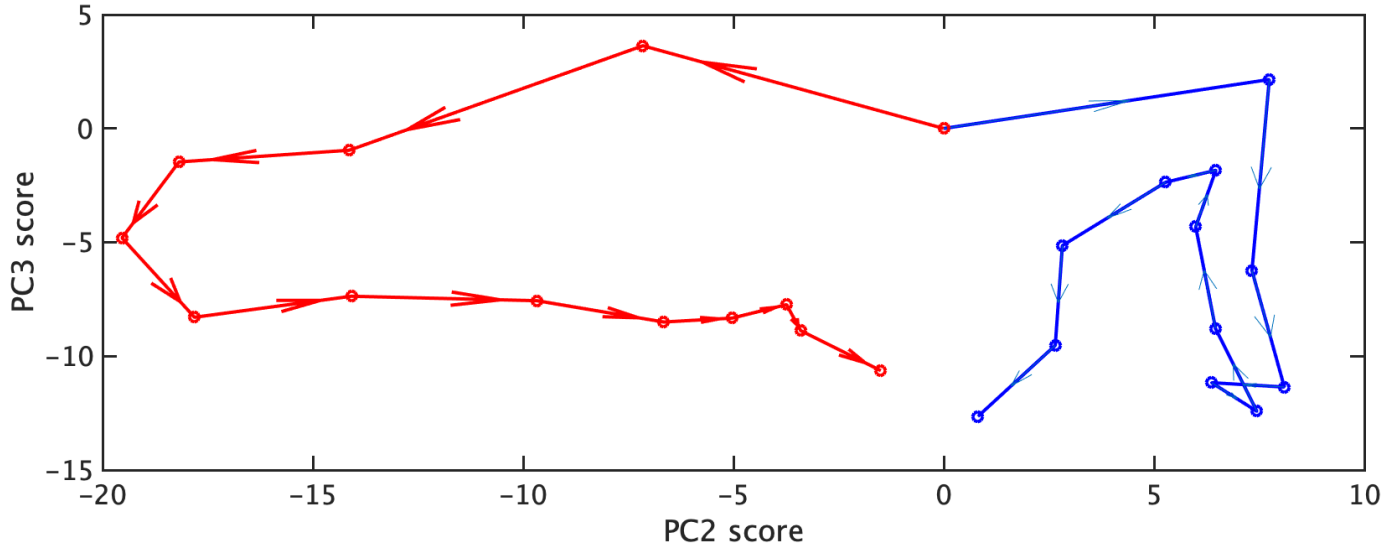
The metabolomic secretome can distinguish between the two differentiation pathways.

Analysis by Dr. Erin Rericha

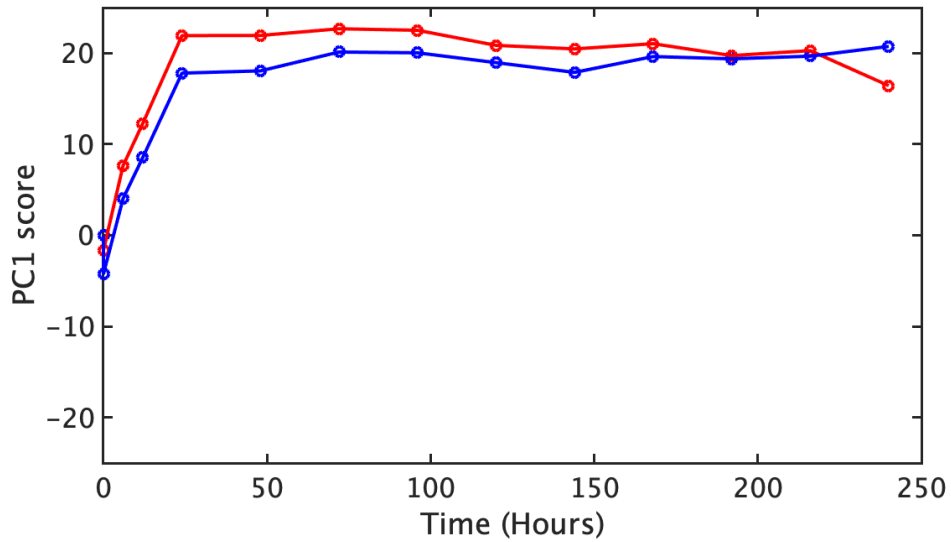
Principal Component Analysis of the Differentiation Trajectories

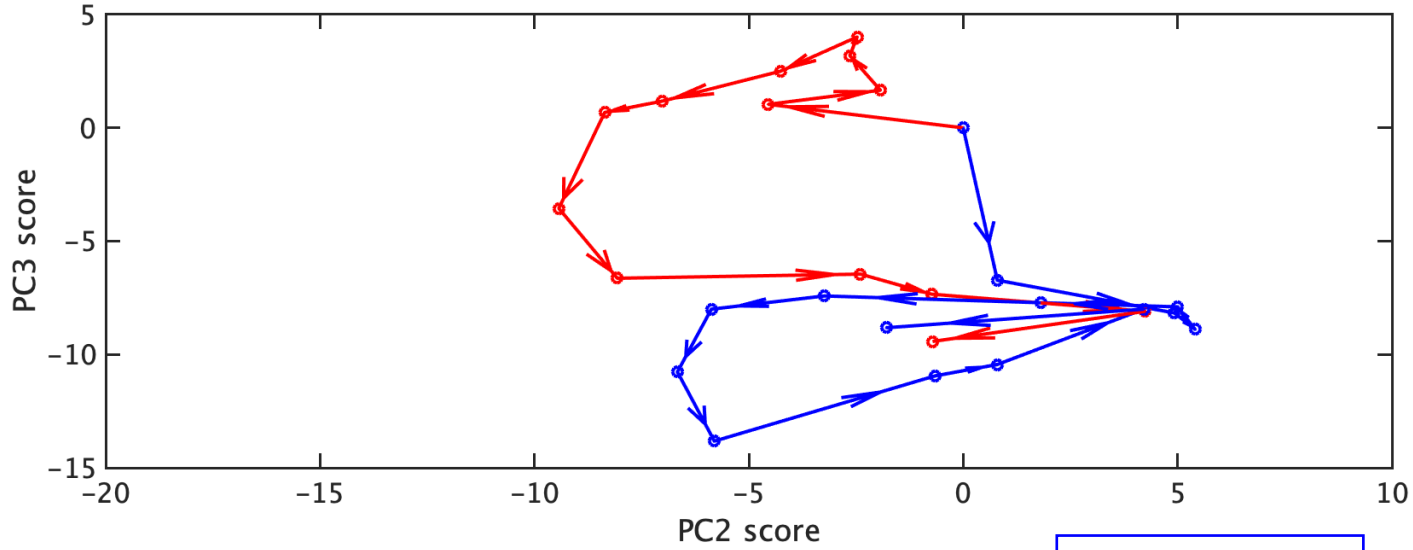
Transcriptome





Secretome





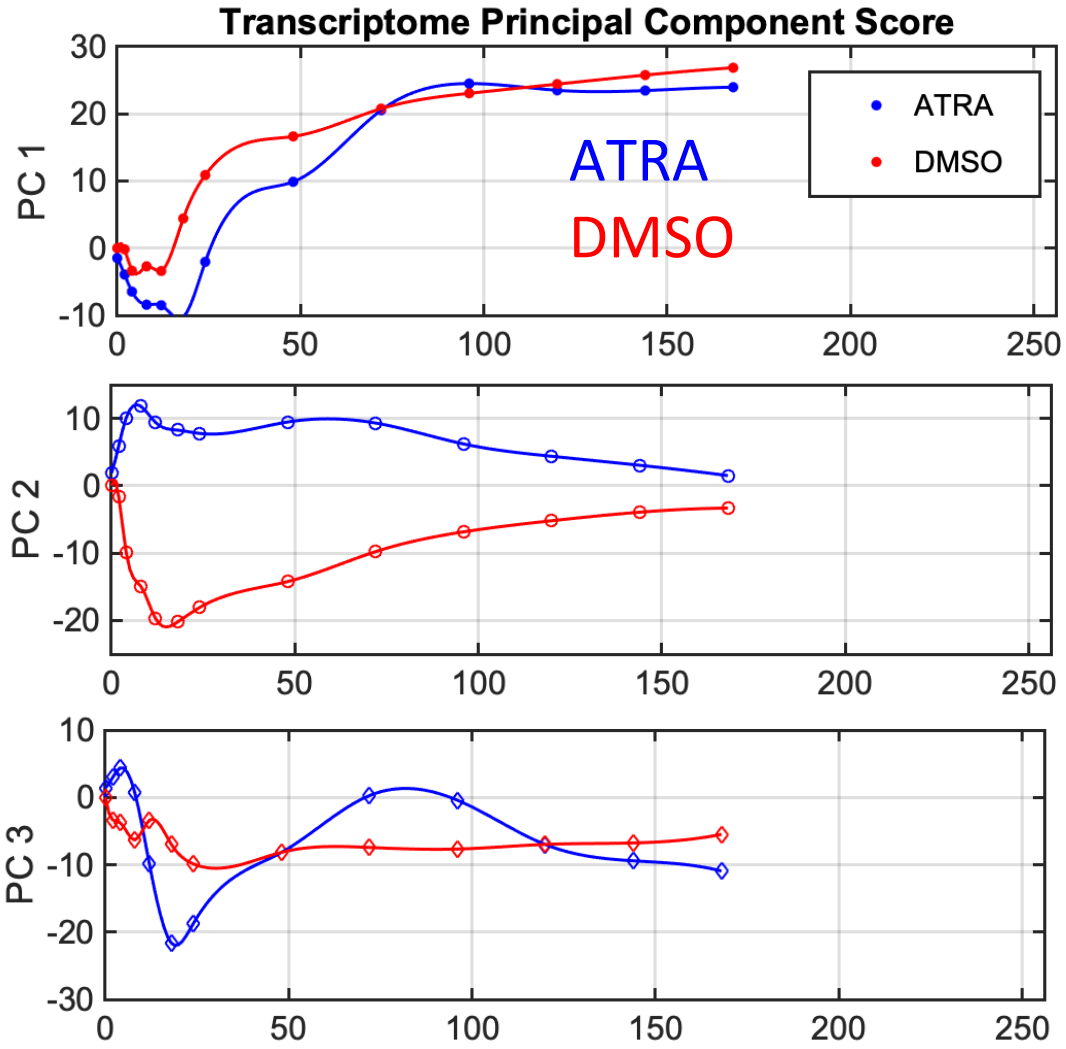
PC1 is HL-60 differentiation

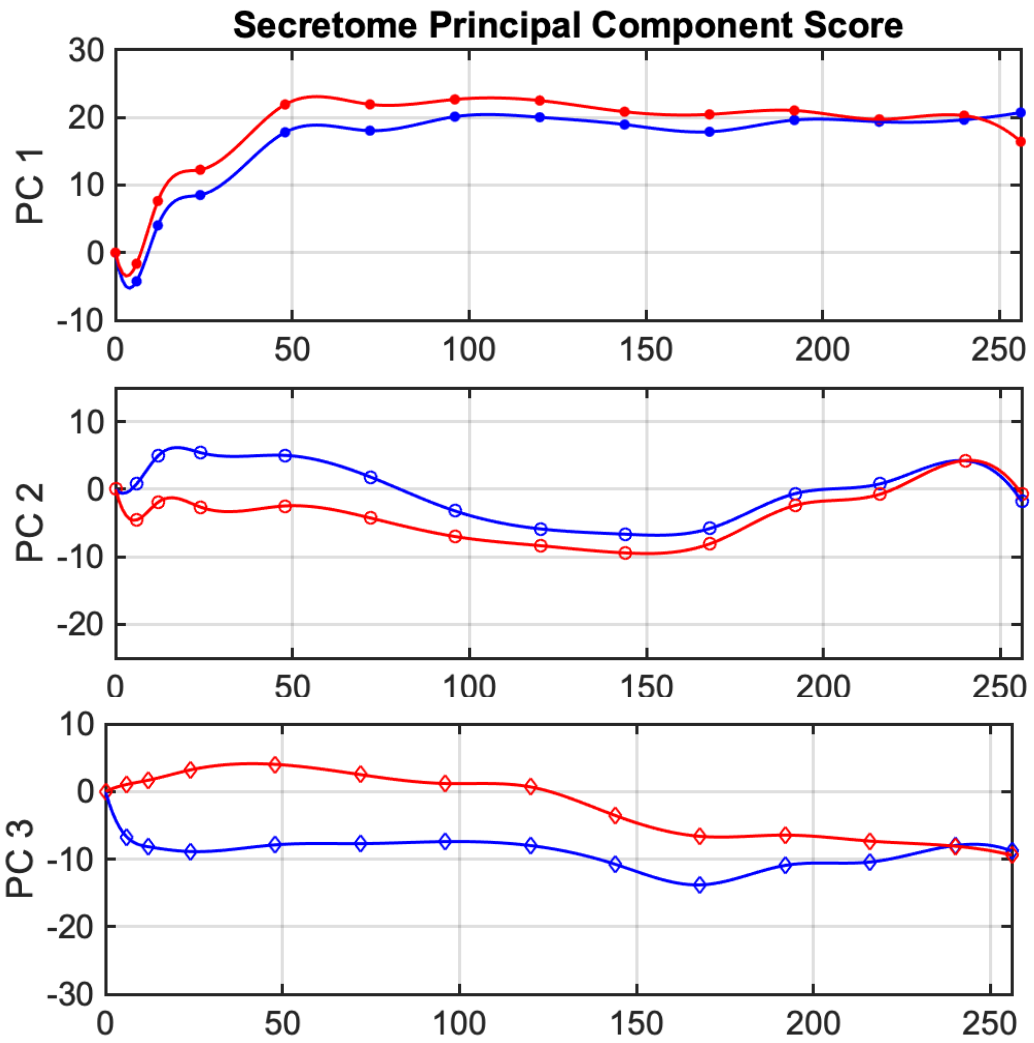
PC2 & PC3 show the pathway differences

Analysis by Dr. Erin Rericha

HL60 Differentiation to Neutrophil-Like Cell:

Matlab Principal Component Analysis



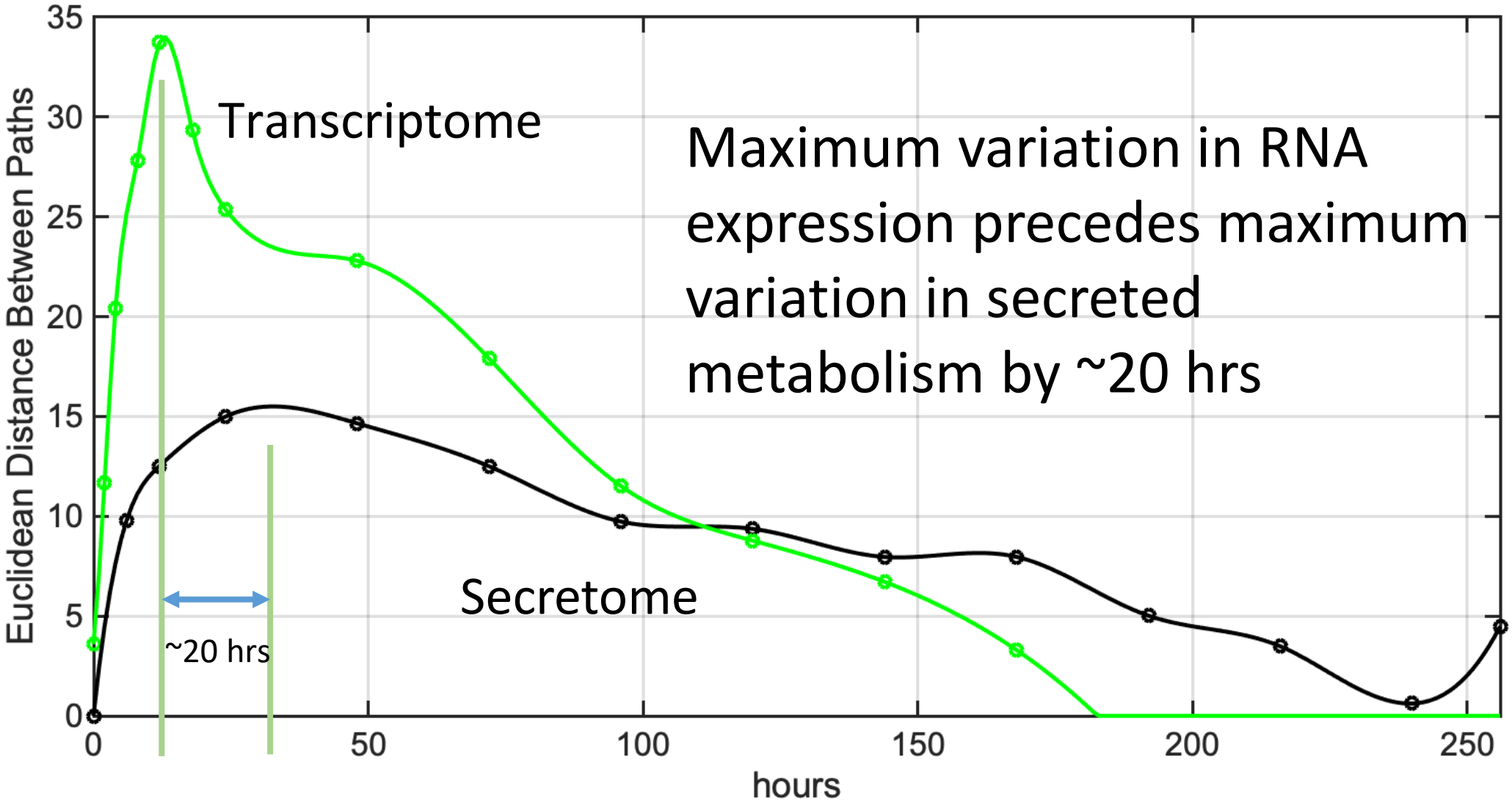


Transcriptome principal components 1, 2, & 3 explain 52% of the variance

Analysis by Dr. Erin Rericha

Secretome principal components 1, 2, & 3 explain 49% of the variance

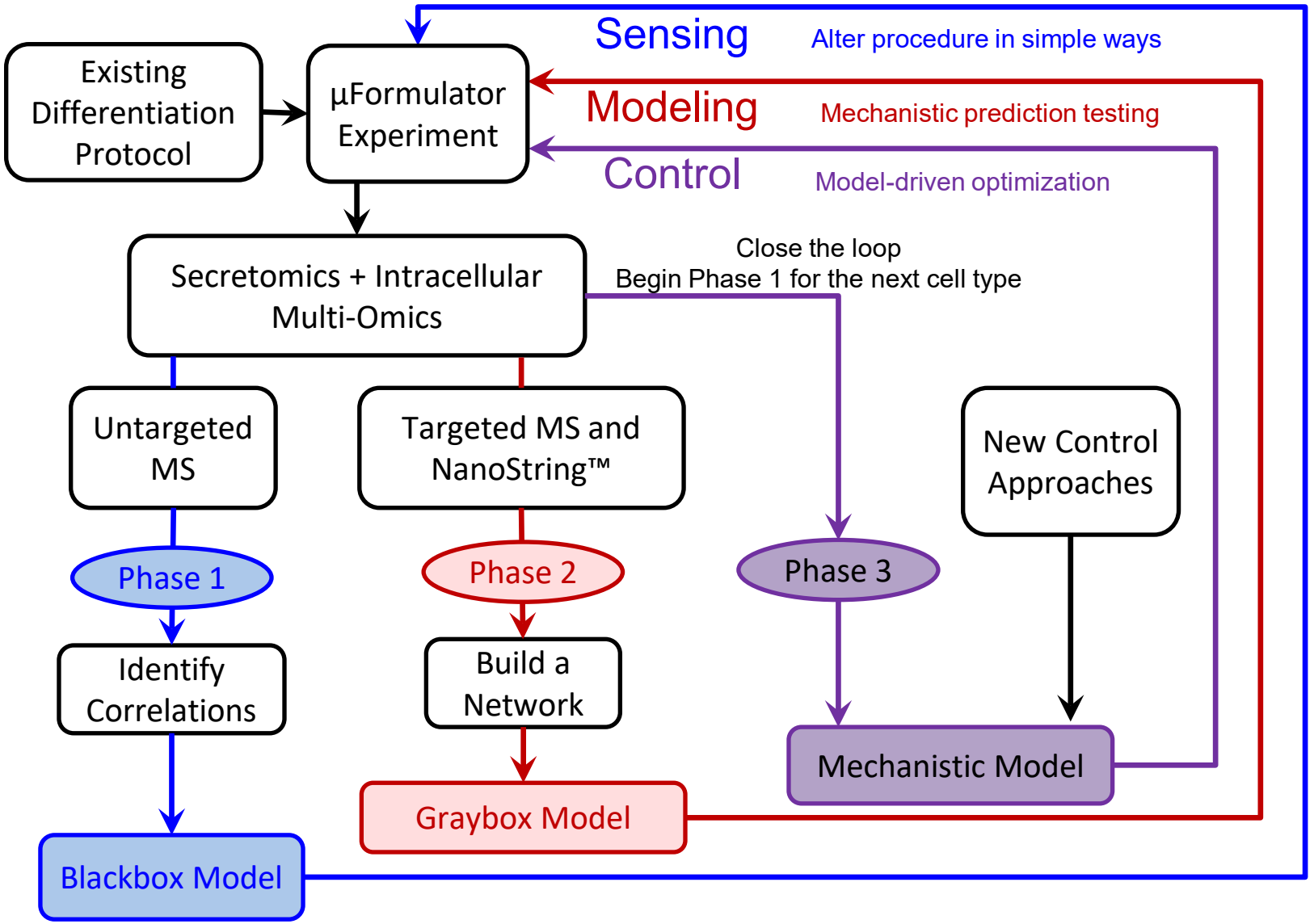
HL60 Differentiation to Neutrophil-Like Cell: Euclidean Distance



The secretome contains differentiation information, but with the expected delay and broadening.

Analysis by
Dr. Erin Rericha

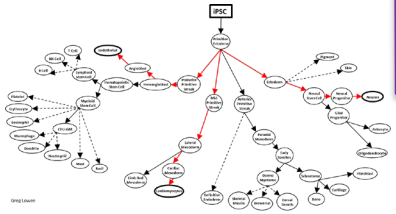
Development of closed-loop control

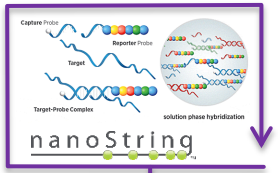


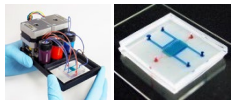
Joey Simmons

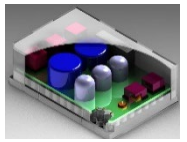
A three-phase analysis should improve control of differentiation.

Secretome and Cellular Multi-Ome for Controlling iPSC Differentiation and Bioprinted Tissue Maturation









CAPCAS SmartLid Well-Plate Feeder

iPSC Cell Culture → Cell Differentiation → Cell Characterization → Cell Maintenance → Organ Chip & Tissue Bioprinting → Tissue Maturation → Tissue Characterization

MicroFormulator Optimizations of Differentiation Protocols

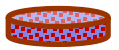
Data Integration and Modeling

Secretome Metabolomics and Proteomics

MicroFormulator Media Optimization

MALDI Mass Spectrometry Imaging

Cellular Proteomics and Histology



Tissue

Cell Pellet

Secretome

MicroFormulators

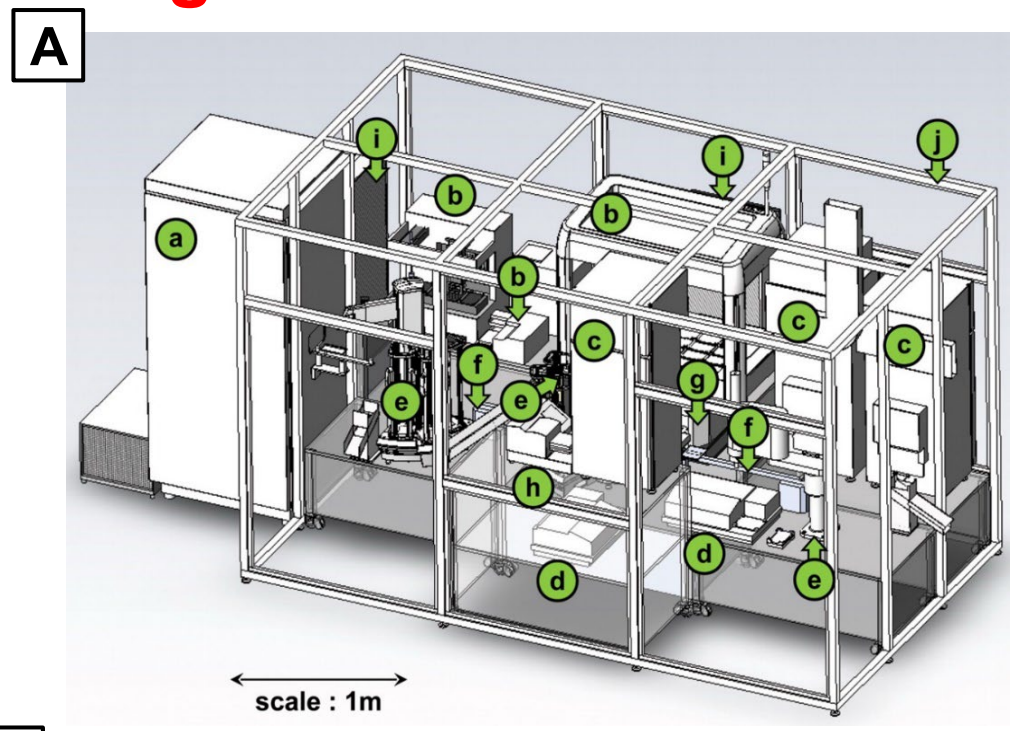
Mass Spectrometers

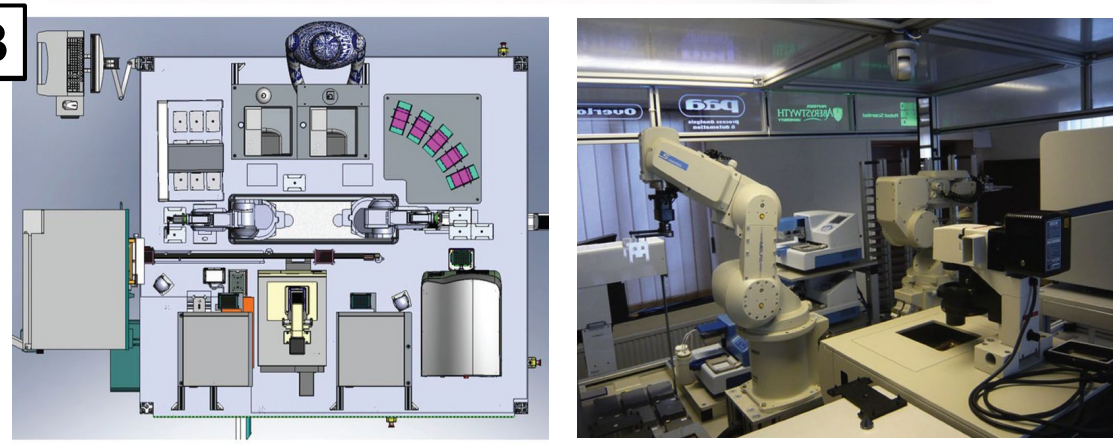
What do we need to make both regenerative medicine and microphysiological systems easier?

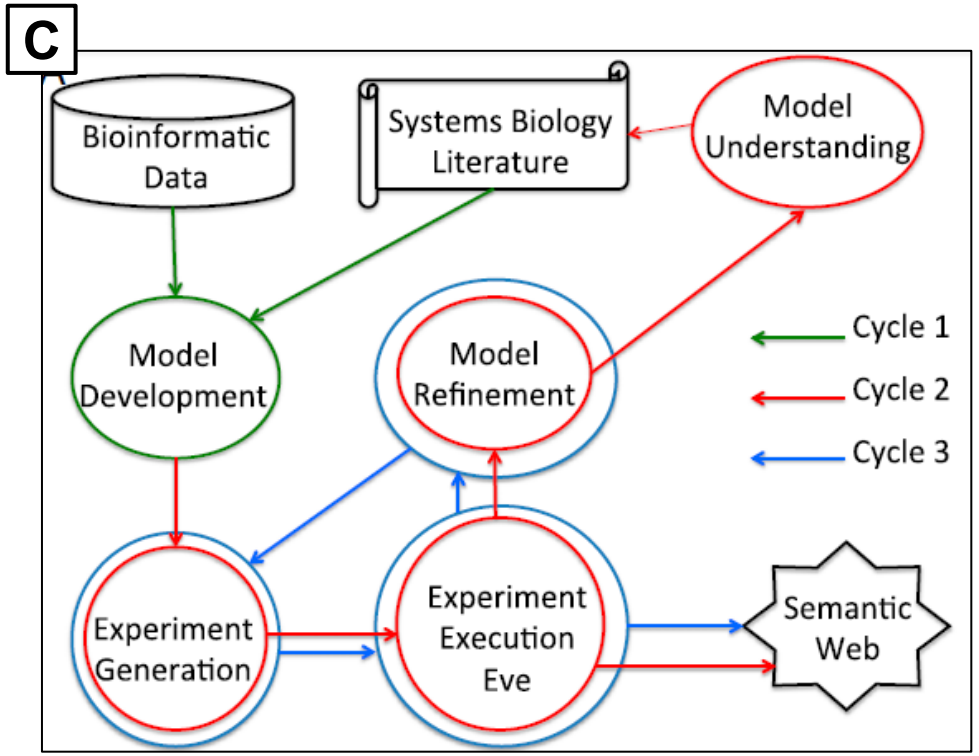
- Sourcing cells
- Assembling cells
- Perfusing cells
- Maintaining physiological scaling
- Analyzing the cells
- Controlling the cells Better models of cell signaling and metabolism

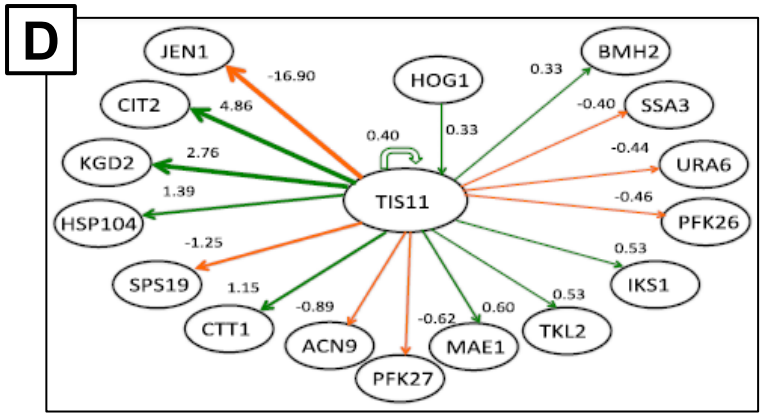
Given the complexity of biology, these models need large numbers of measurements that are optimally designed. This is best done by artificial intelligence and machine learning.

Ross King's Robot Scientists *Adam* and *Eve*



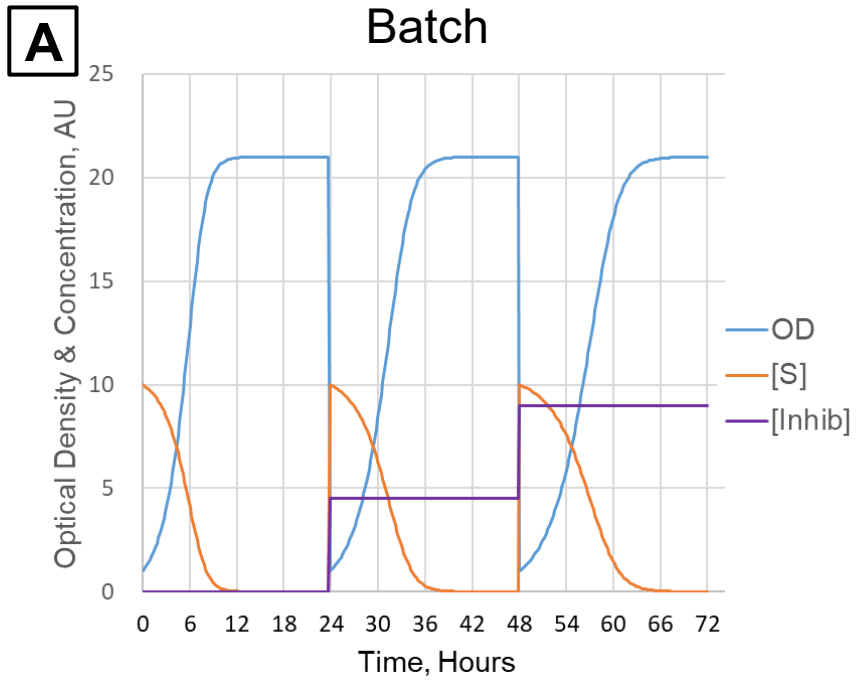




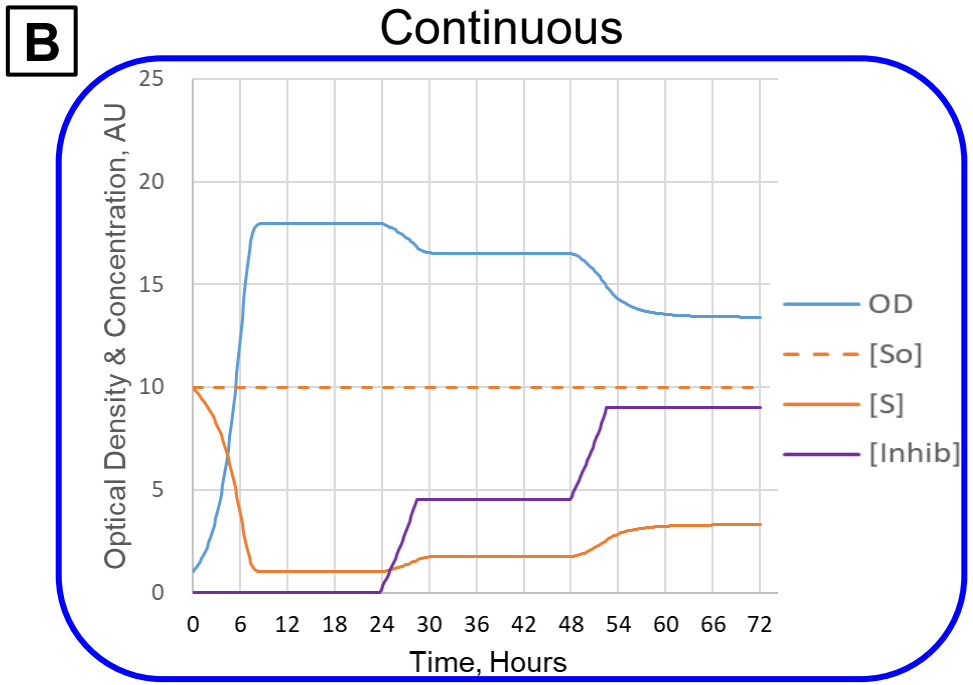


A) Adam, with an automated –20°C freezer, three liquid handlers, three automated +30°C incubators, two automated plate readers, three robot arms, two automated plate slides, an automated plate centrifuge, an automated plate washer, two high-efficiency particulate air filters, and a rigid transparent plastic enclosure. Autonomously, Adam specified and recorded 6,657,024 optical density measurements @595 nm to form 26,495 growth curves, and formulated and tested 20 hypotheses concerning genes encoding 13 orphan enzymes. B) Eve, constructed at the University of Manchester and now being reassembled at CUT, combined multiple software tools with integrated laboratory robotics to execute C) three semiautomated cycles of diauxic shift model improvement. All the experiments were formalized and communicated to Eve's cloud laboratory automation system for execution to expand the current model of the yeast diauxic shift. The final model adds a substantial amount of knowledge: 92 genes (+45%) and 1,048 interactions (+147%), illustrated in part in D). King et al., Comp. 2009;. Williams et al., J R Soc Interface, 2015; Coutant, et al., PNAS, 2019.

Microbial culture: Batch versus continuous



A) Three serial-batch measurements of yeast growth with differing inhibitor concentrations.



B) A continuous-perfusion chemostat experiment that after seeding reaches a steady state growth rate that represents a balance between inflow of media with the rate-limiting nutrient and efflux of cells and media. The inhibitor concentration can be changed without reseeding the bioreactor.

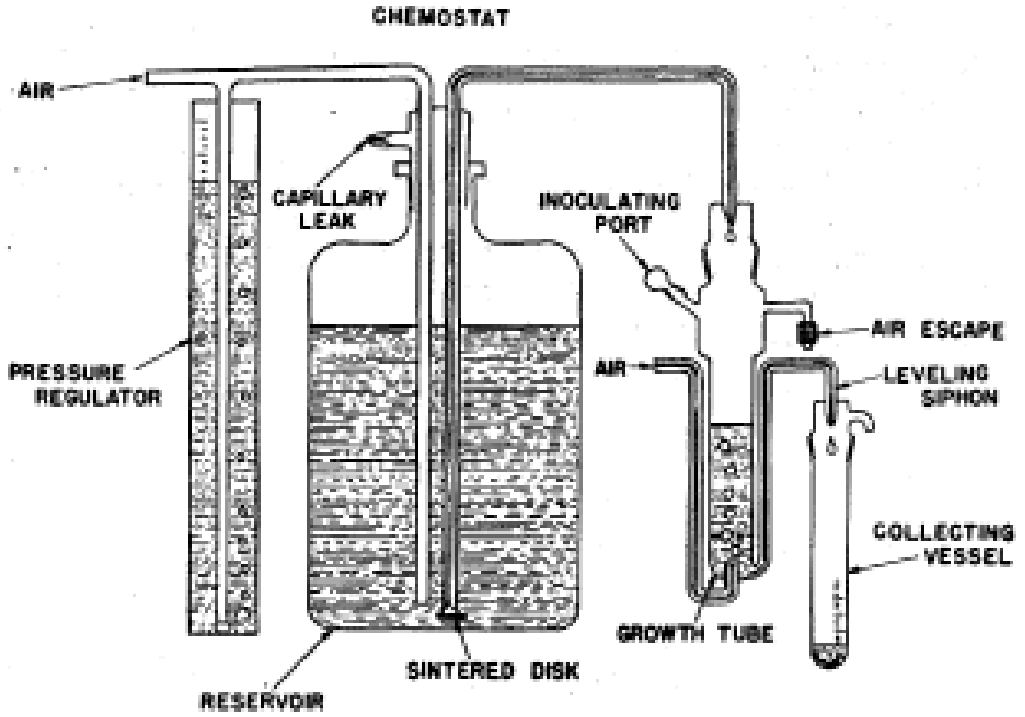
A) In batch culture, gene expression profiles change throughout the growth phase, with continuously changing levels of nutrients, metabolites, and signaling molecules. This may not matter for an end-point analysis.

B) In chemostats, gene expression profiles are relatively constant for long periods of time, which is ideal for quantitative multi-omic measurements of signaling and metabolism required for network reconstruction.

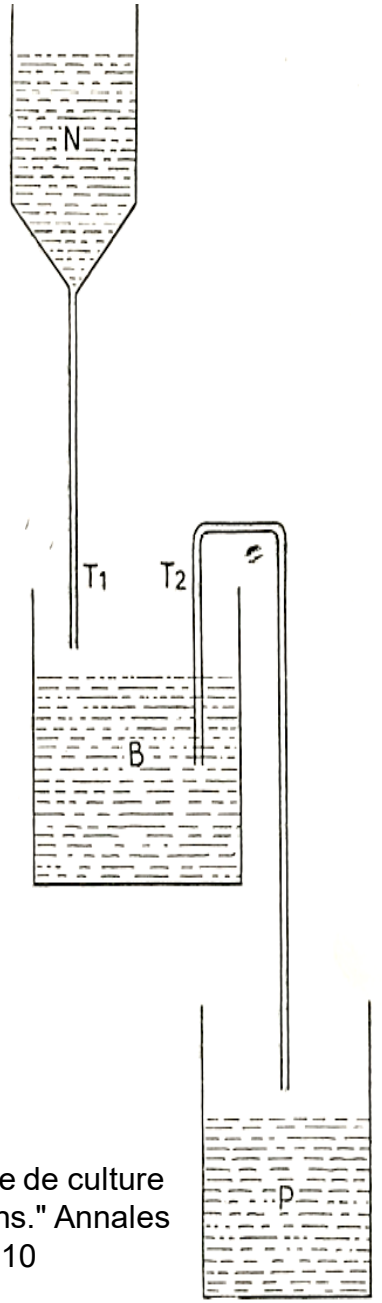
The Genesis Project

- Ross King to John Wikswo, October 2019
  - Can you build my third-generation robot scientist, Genesis, a 4,000 channel chemostat?

The Microbial Chemostat, 1950



Novick A and Szilard L. Experiments with the Chemostat on Spontaneous Mutations of Bacteria. PNAS 36 (12):708-719, 1950.
Aaron. Novick and Leo Szilard. Description of the Chemostat. Science 112 (2920):715-716, 1950.

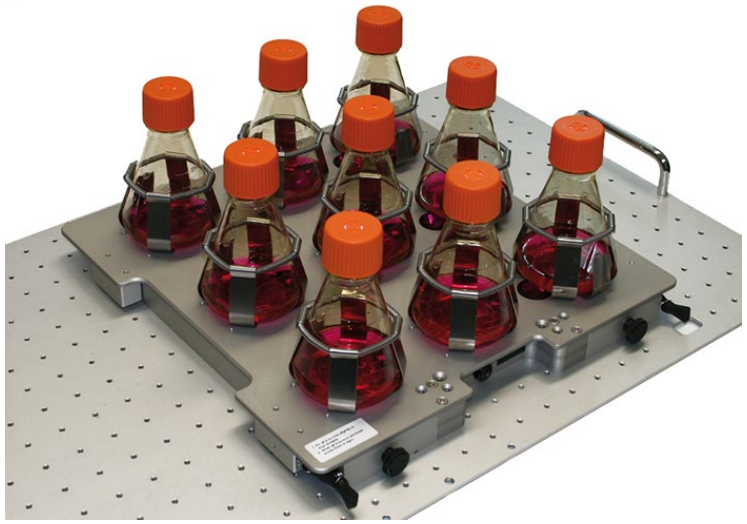


Monod, J. (1950). "La technique de culture continue théorie et applications." Annales de l'Institut Pasteur 79: 390-410

Fig. 1. — Schéma d'un appareil à culture continue.

Benchtop Bioreactors Today

The traditional benchtop bioreactor has been a 3-litre unit which offers a lot of flexibility and information to plan scale-up to production and considerable flexibility. These are available from reputable suppliers and include Mobius from Merck (Germany), BioBLU from Eppendorf (Germany), Applikon from Getinge (Sweden), Infors (Germany), Cytiva (USA/Sweden), and Pall (USA).



MERCK

PALL®

GETINGE

cytiva

Smaller volume, higher-count systems are needed!

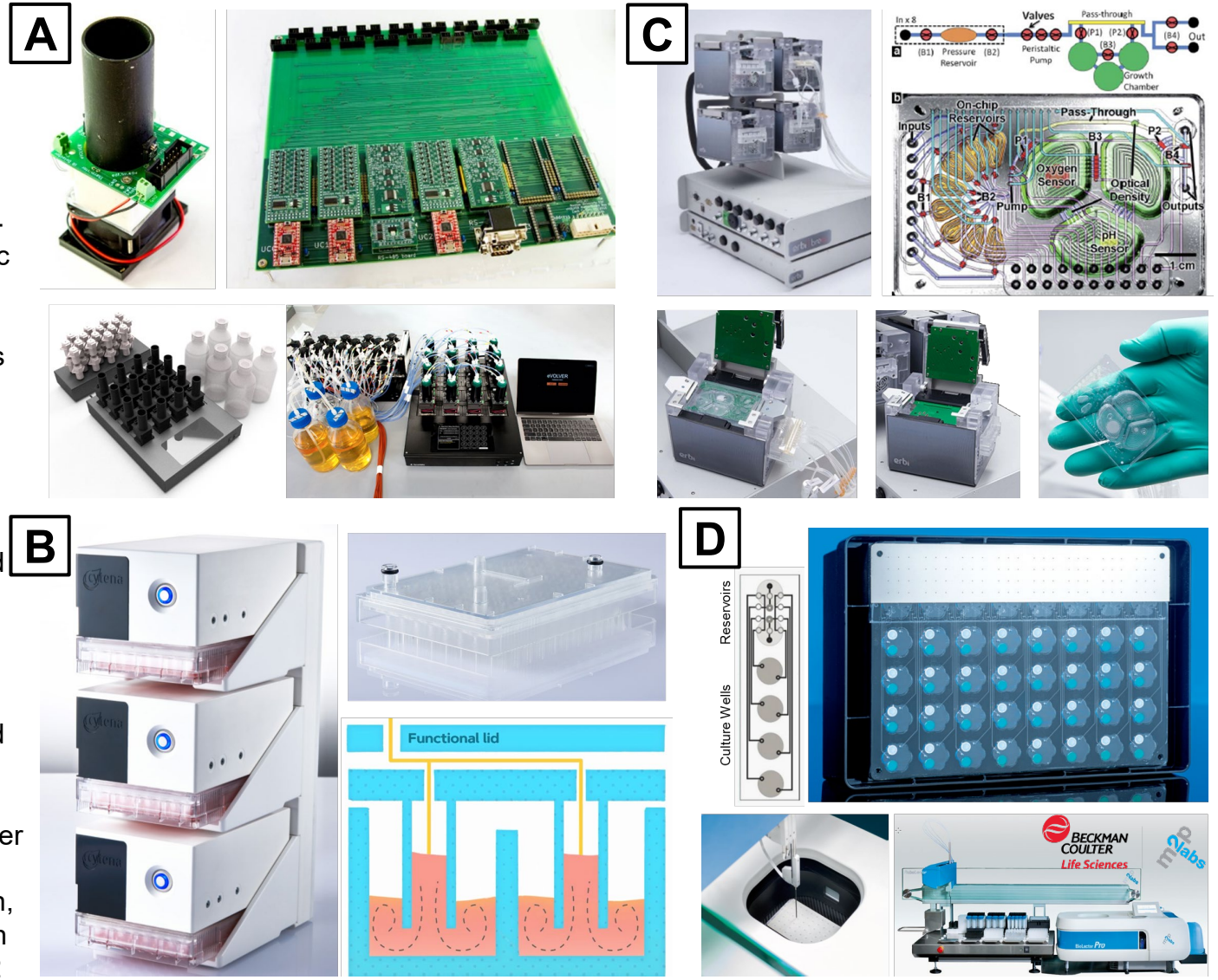
What is available for small-scale, multichannel bioreactors?

A) The open-source eVOLVER system developed at Boston University and available from Labmaker.org provides 16 10 mL bioreactors, pumps, and control electronics.

B) The Cytena c.Bird continuous-mixing modules use pneumatic actuation to increase oxygen transfer rate in a 96- and 24-well plate. It cannot operate as a chemostat.

C) The Erbi Breez™ single-use microbioreactor, developed at MIT, has a 2 mL working volume with independent measurement of pH, dissolved oxygen (DO), optical density (OD), and temperature, can input up to four fluids, and controls three gasses.

D) The m2p Labs BioLector™ and RoboLector™ automated fed-batch, pipette-loaded fermentation system uses either a single 48 flower-shaped shaken well plate or, as shown, a microfluidic enabled one with 4 banks of 8 bioreactors and 2 banks of 8 reservoirs for pH and nutrient control.



Existing multichannel chemostats, clonal expansion systems, and well plate shakers don't scale to a thousand parallel, independent systems.

51

What are the medium to large-scale systems?

A

B

1. Gas Sparge Tube
2. Sample Port with Cap
3. pH and DO Sensor Spots
4. Impeller

C

D

Ambr® 250
High Throughput

E

Biostat® B
Univessel® SU 2L

Biostat STR® 50

Biostat STR® 200

F

Biostat STR® 500

Biostat STR® 1000

Biostat STR® 2000

G

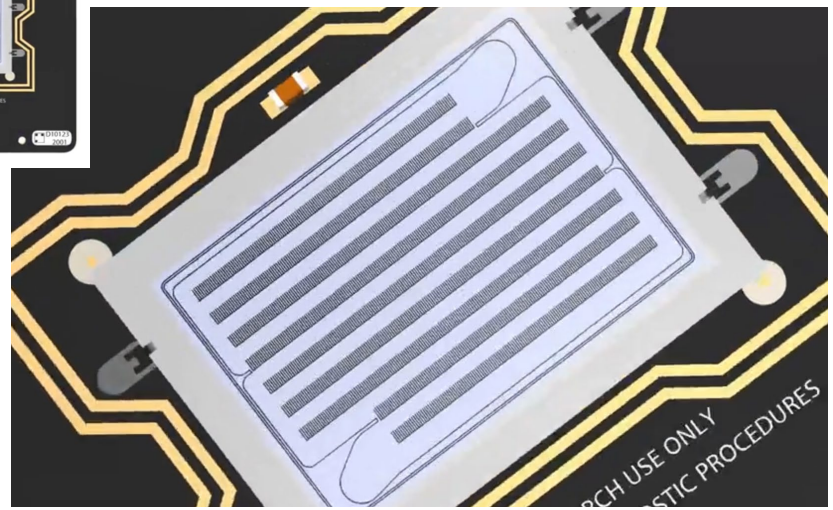
A-B) Sartorius “High throughput system” with 24 15 mL bioreactors
C-D) Sartorius “High throughput system” with 24 250 mL bioreactors
E-F) Sartorius Bioreactors scaled to production.
G) The NovaBiomedical BioProfile Flex2 Automated Cell Culture Analyzer, which can withdraw samples from up to 10 bioreactors, count cells, and perform metabolic measurements every 10 minutes.

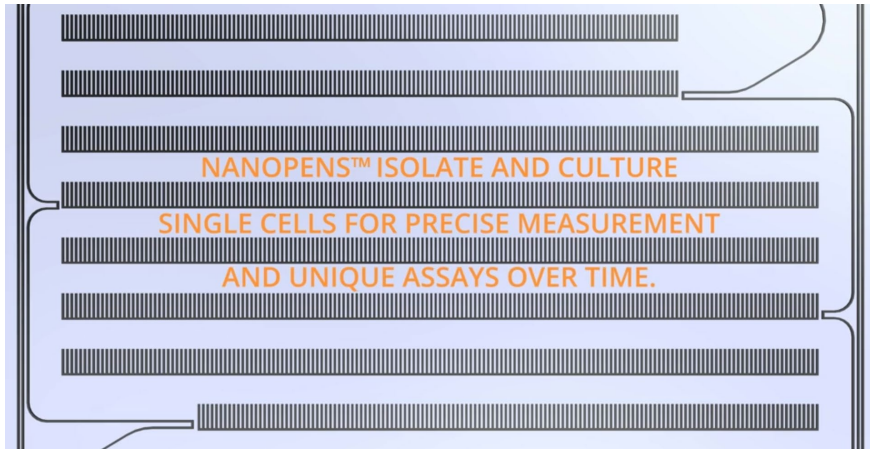
Smaller volume, higher-count systems are needed!

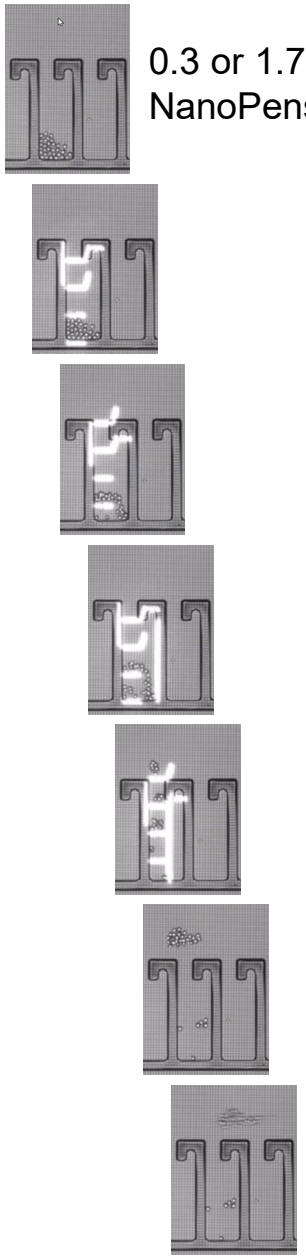
What's New?

Berkeley Lights - Beacon Optoselect

1.7nl x1750, 0.32nl x11000







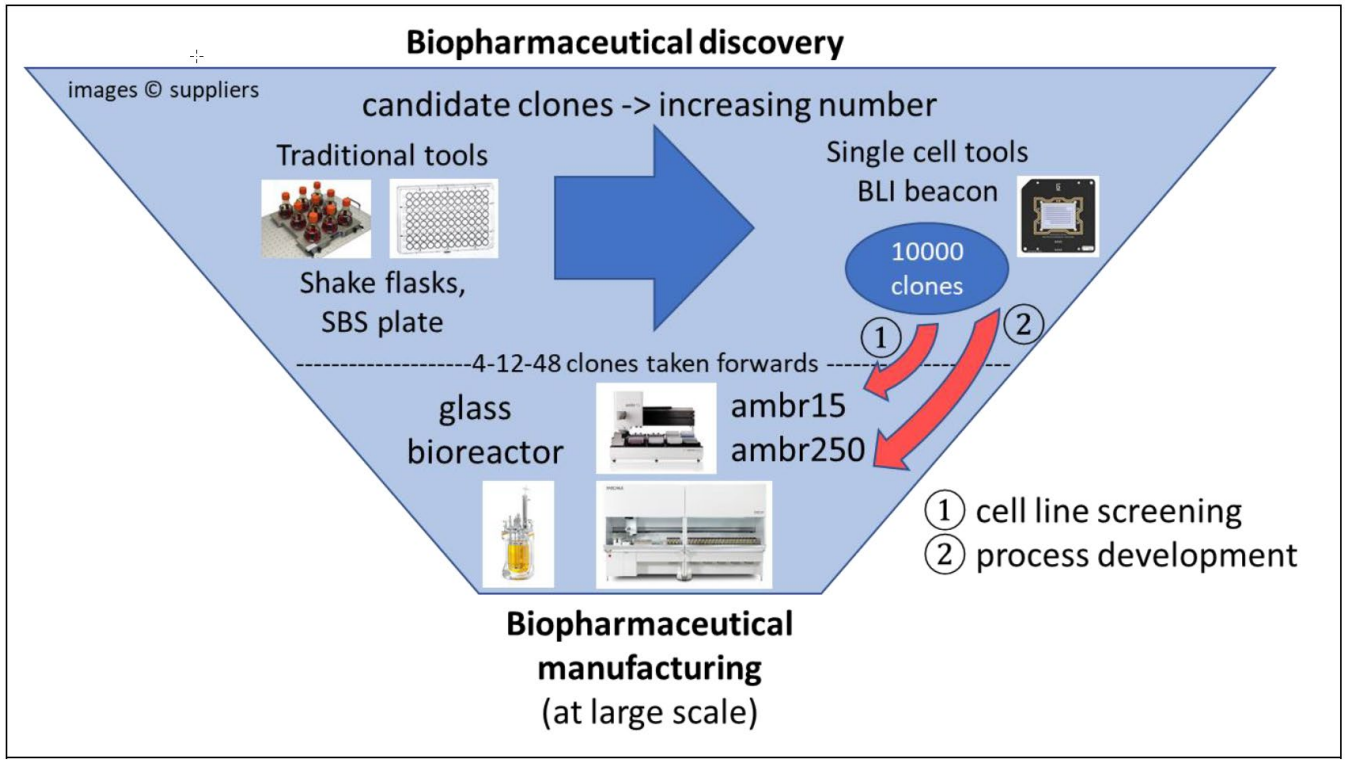
How do you best expand from thousands of single cells to a dozen cloning bioreactors?

The Market Driving the Genesis Project

- Biology is complicated
- There is an intense need to accelerate cellular sensing and control
  - Medicine
  - Pharmacology
  - Biotechnology
  - Basic research
- There is an immediate market for systems with thousands of perfused, mL-volume bioreactors, wells, or chemostats
  - Microbial chemostats
  - Continuous flow bioreactors
  - Batch-fed bioreactors
  - Organ chips
  - Well plates and TransWells
  - Zebrafish larvae

The explosive growth in biopharmaceuticals and the high attrition of candidate clones from discovery to manufacturing ensure a potential market for Genesis.

Artificial intelligence and machine learning can accelerate discovery by creating general-purpose, modular, self-driving laboratories for fully automated biology. **This is Genesis.**

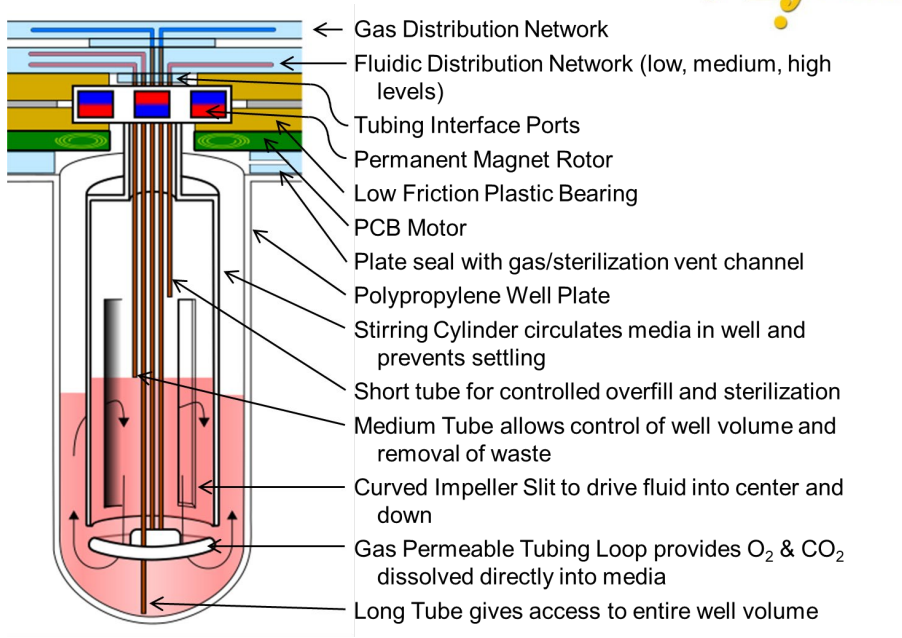


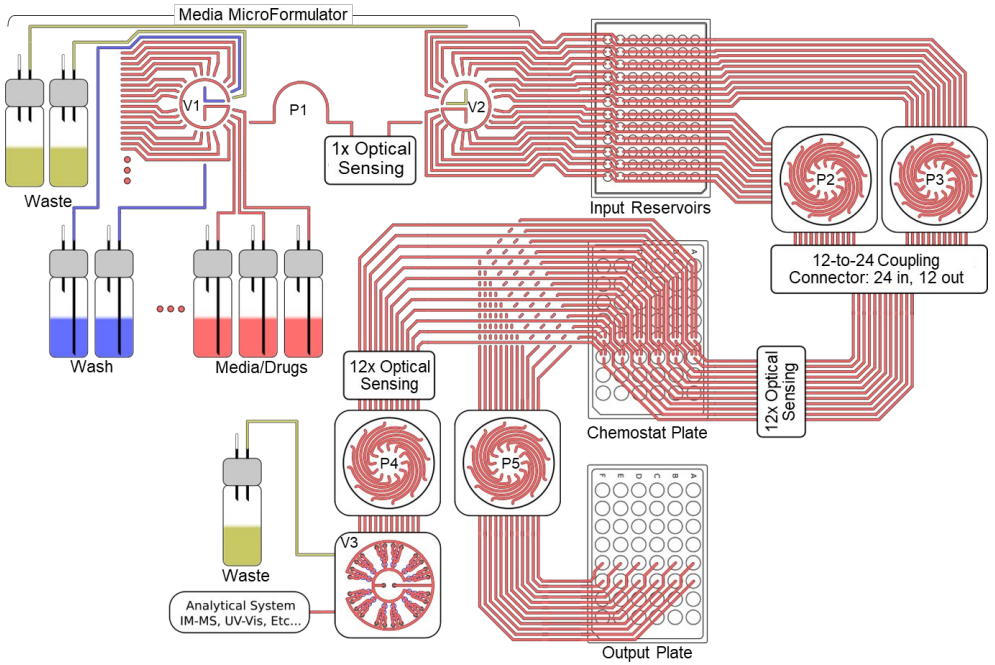
The Genesis Concept

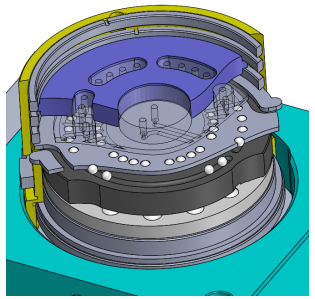
Ron Reiserer

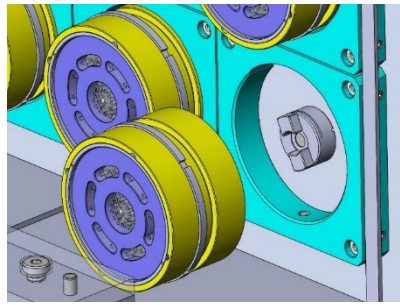
Genesis 1.0 and 2.0 Concept

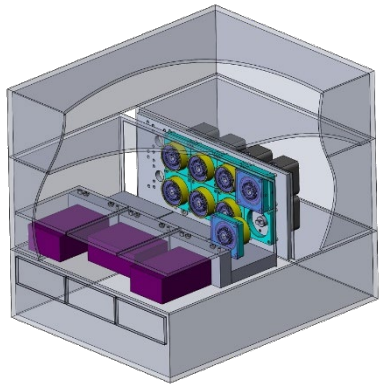
- Compact, mL chemostats operating in a deep 48-well plate
- Multichannel microfluidic pumps and valves
- An automated fluidic control system
- Self-contained sterility, thermal, and gas control

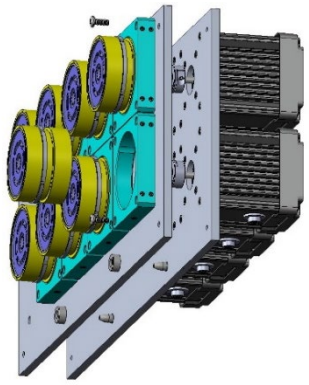






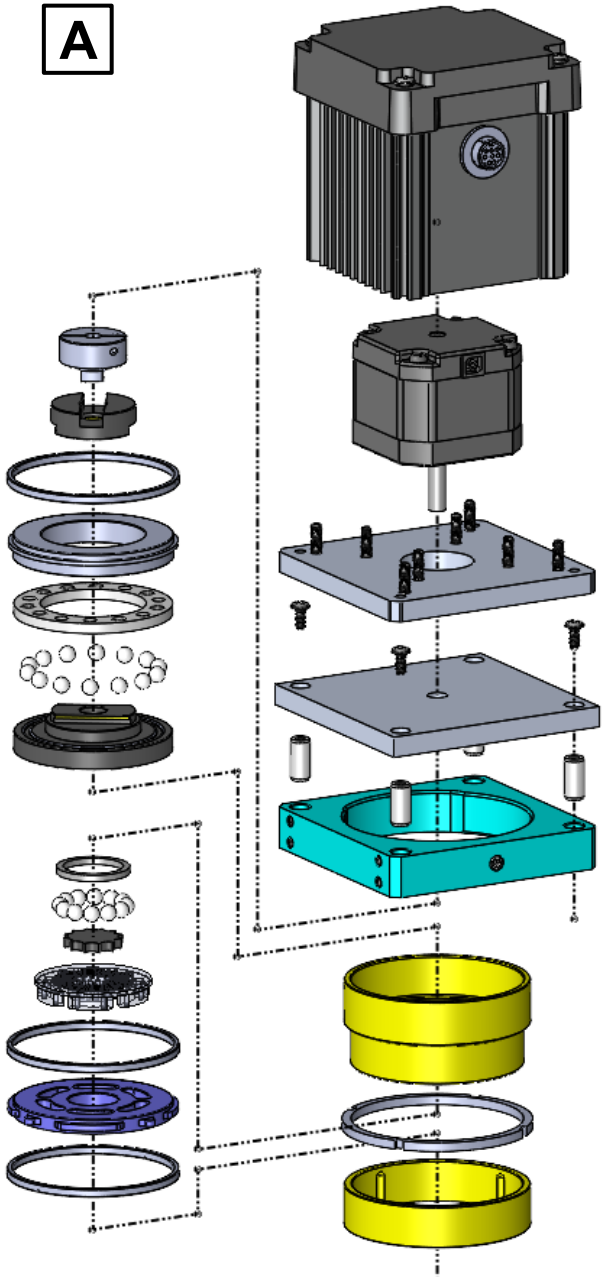






VIIBRE Version 4.0 Microfluidic Pumps and Valves

A



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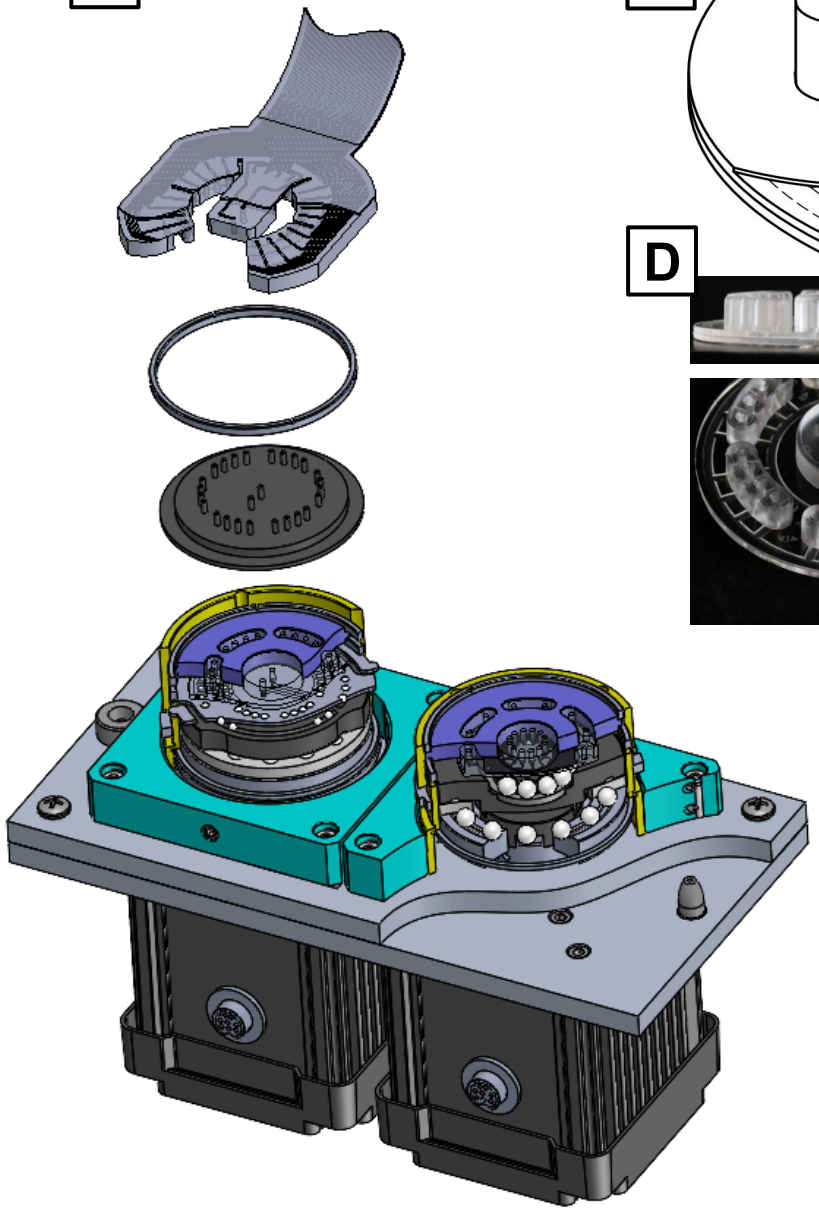

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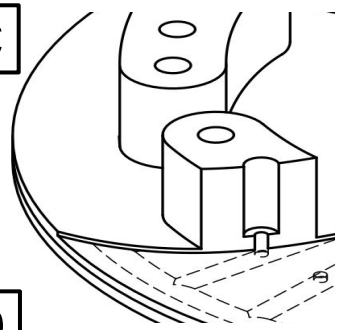

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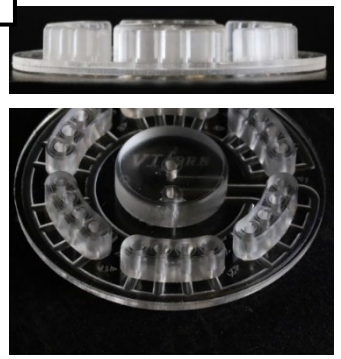

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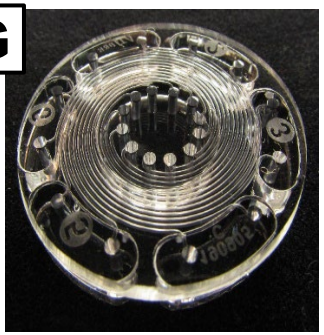

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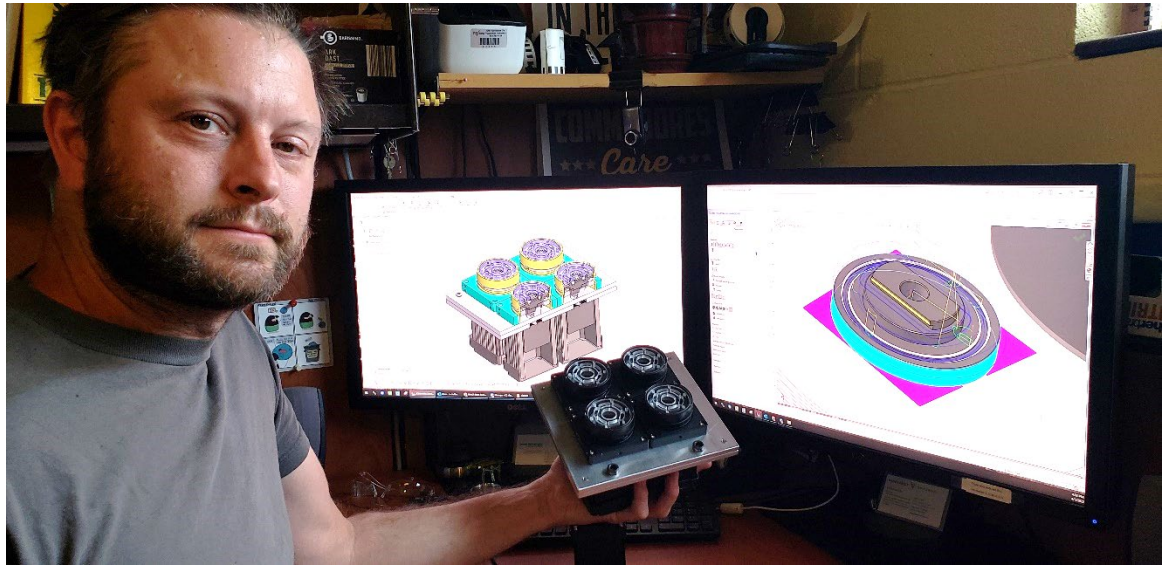
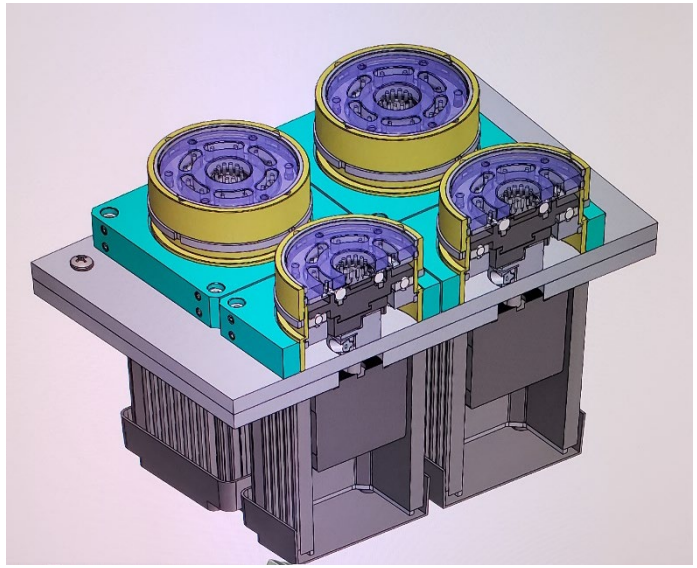
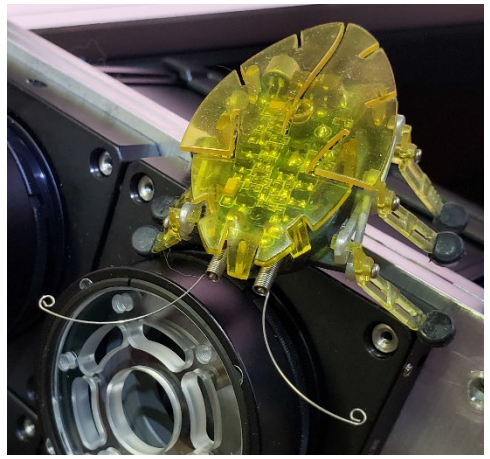
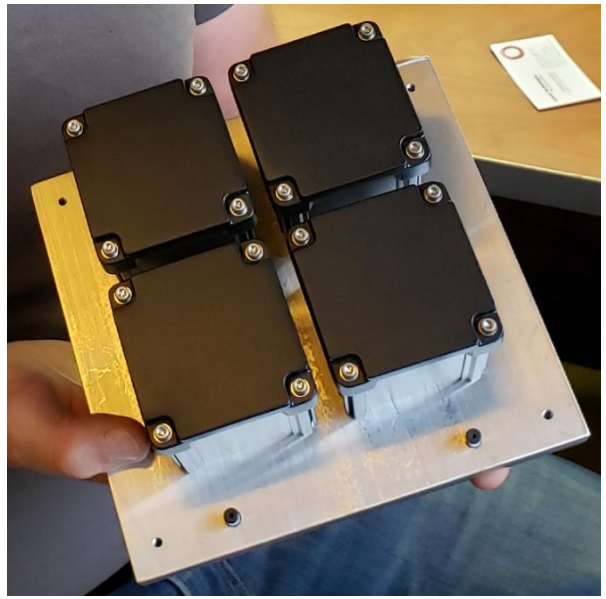
VIIBRE Version 4.0 Microfluidic Pumps and Valves



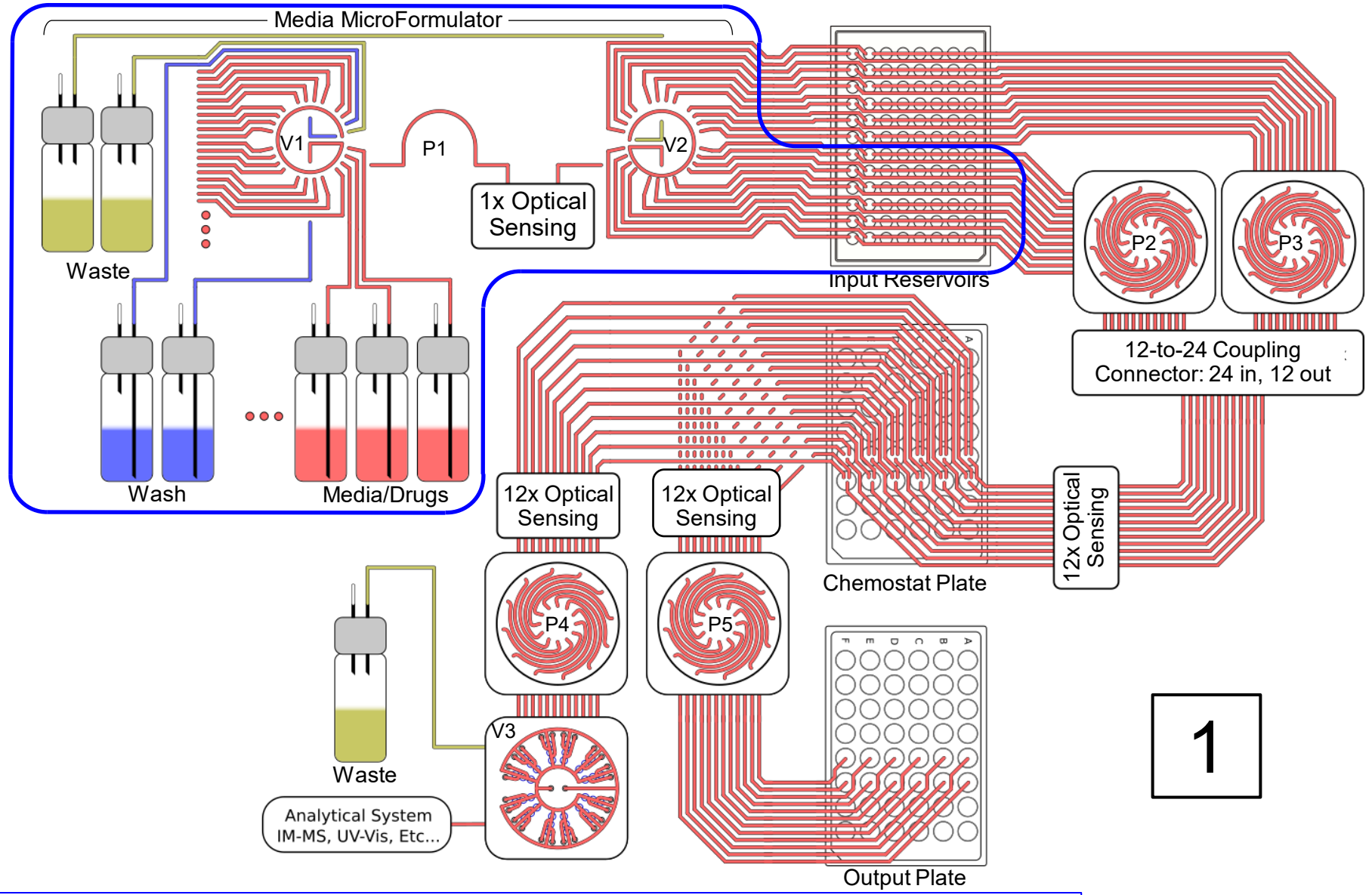






We are steadily debugging our CNC G-Code.

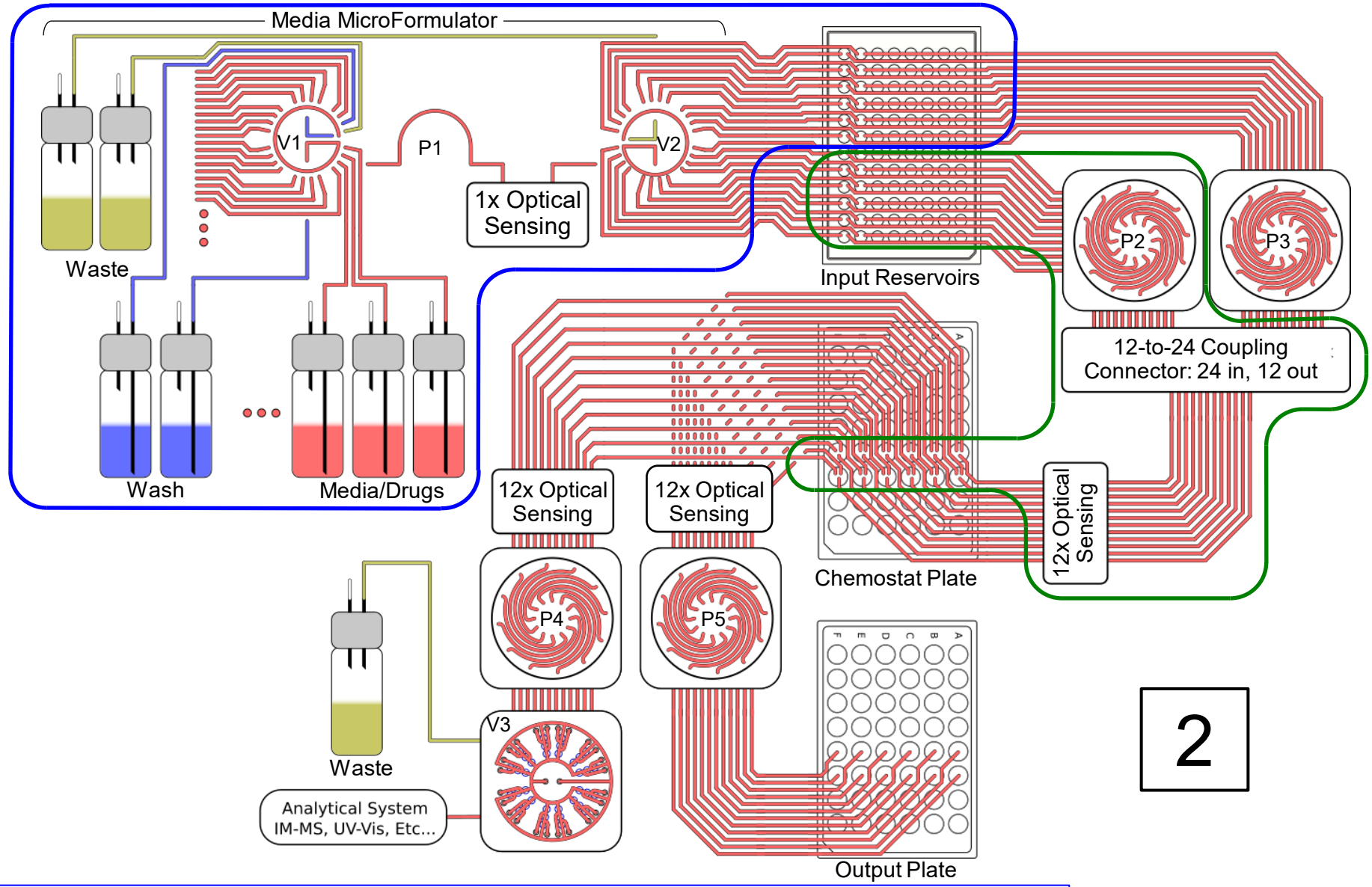
Genesis 1.0 12-Well MicroChemostat



- **Formulate the lower bank of Input Reservoirs**

Patents issued and pending

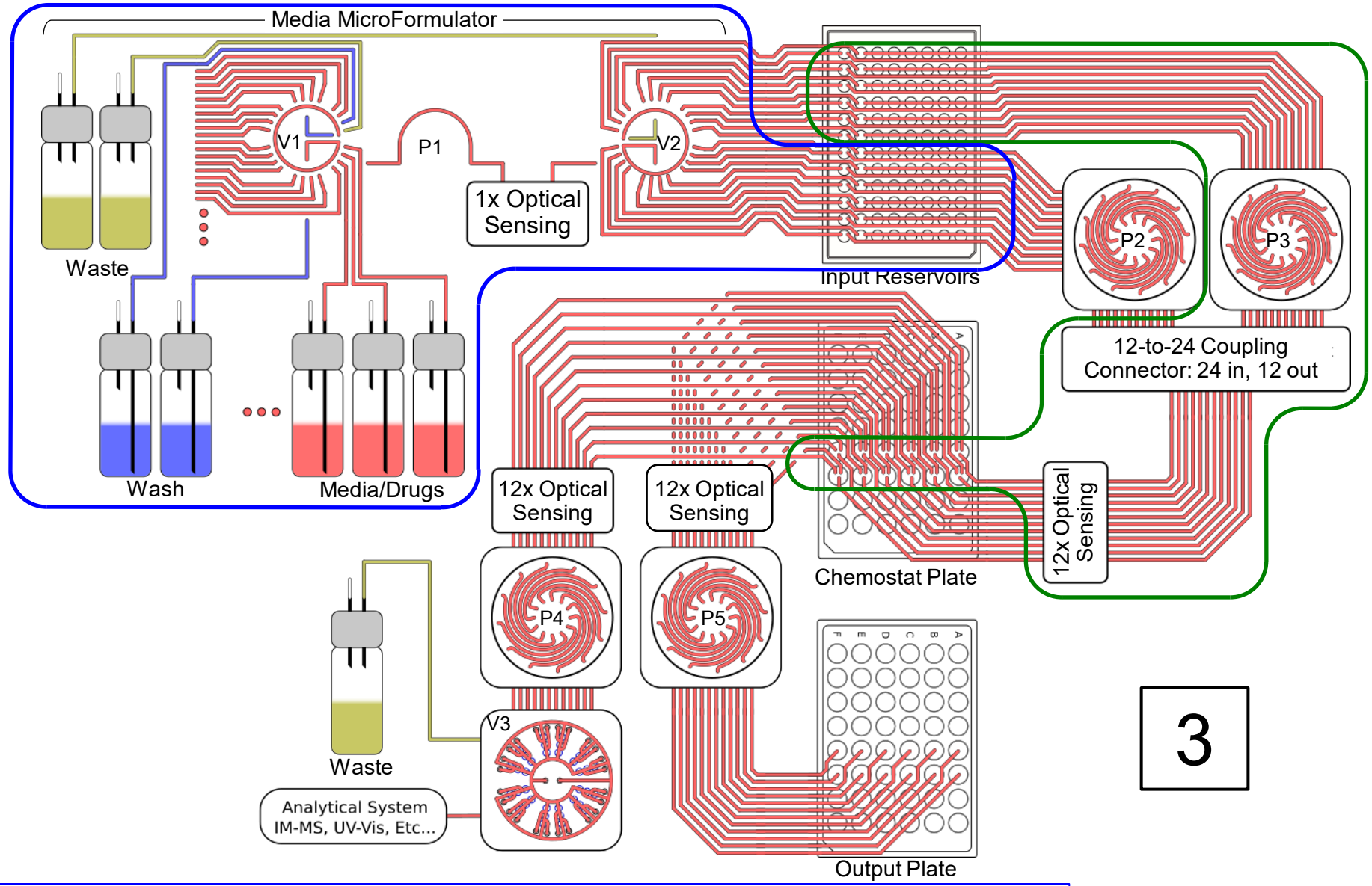
Genesis 1.0 12-Well MicroChemostat



- **Formulate the upper bank of Input Reservoirs**
- **Pump the lower bank of Input Reservoirs into the MicroChemostats**

Patents issued and pending

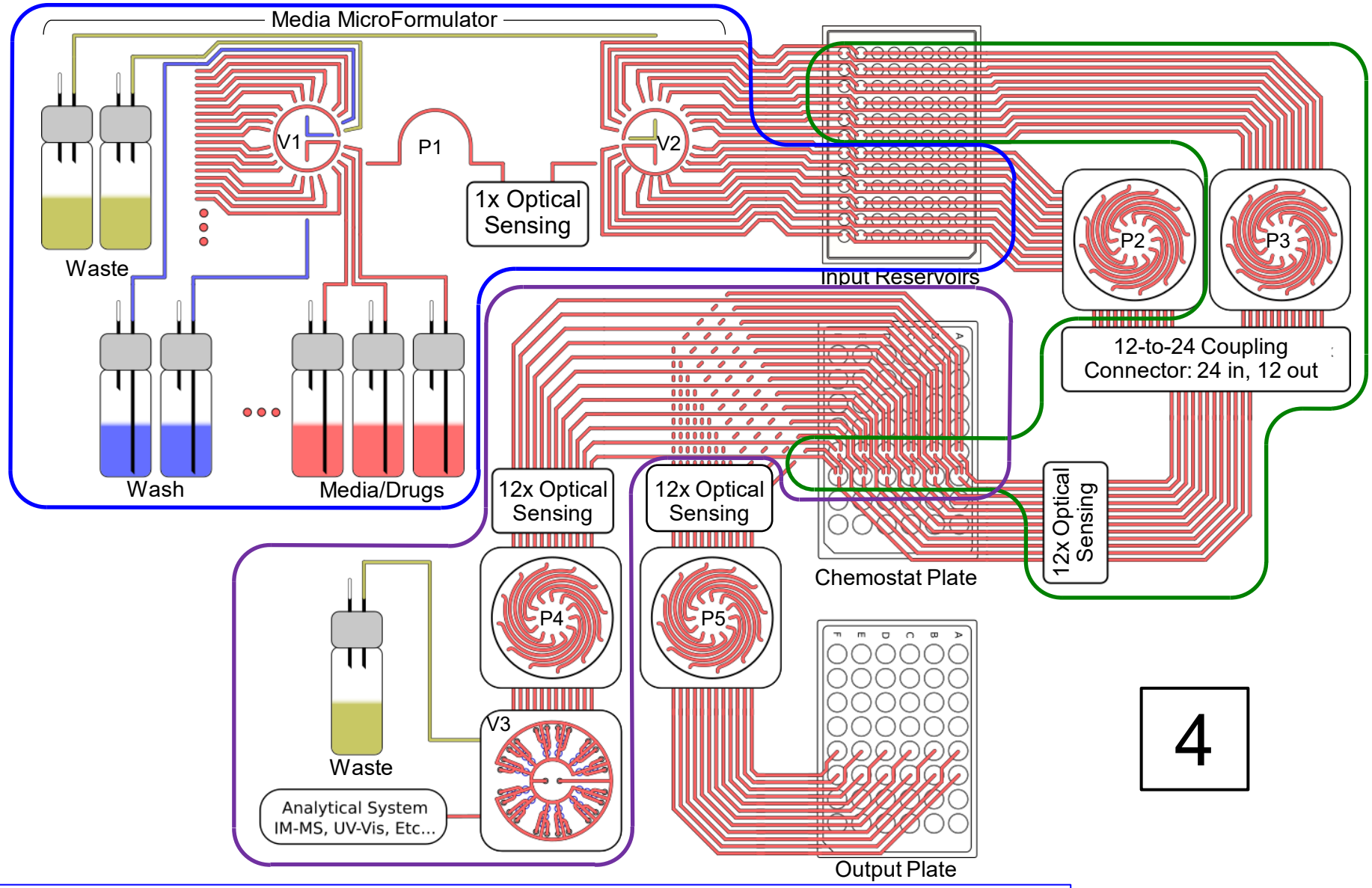
Genesis 1.0 12-Well MicroChemostat



- **Formulate the lower bank of Input Reservoirs**
- **Pump the upper bank of Input Reservoirs into the MicroChemostats**

Patents issued and pending

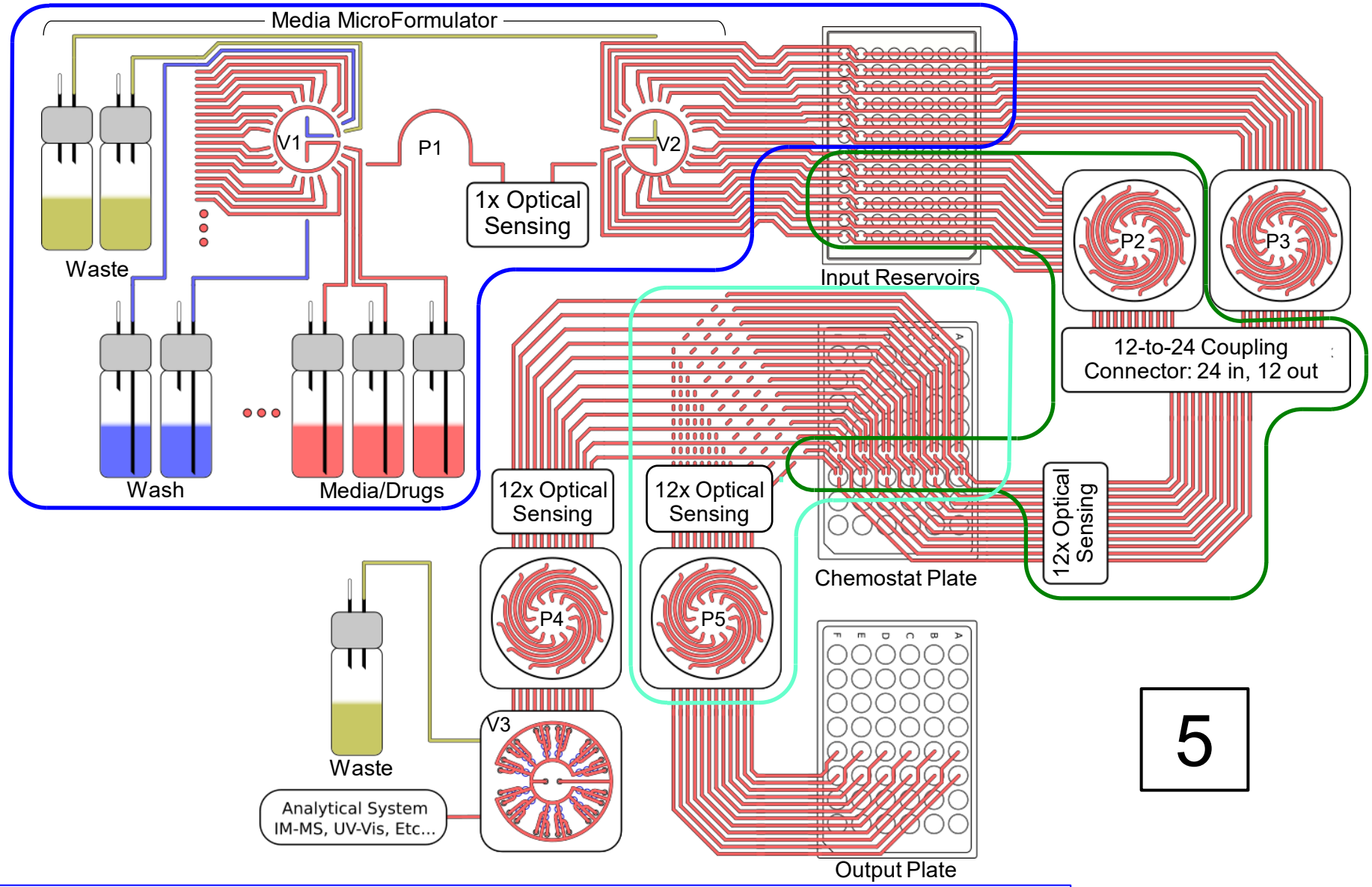
Genesis 1.0 12-Well MicroChemostat



- **Formulate the lower bank of input reservoirs**
- **Pump the upper bank of input reservoirs into the chemostats**
- **Continuously remove media from chemostats and send to analysis and waste**

Patents issued and pending

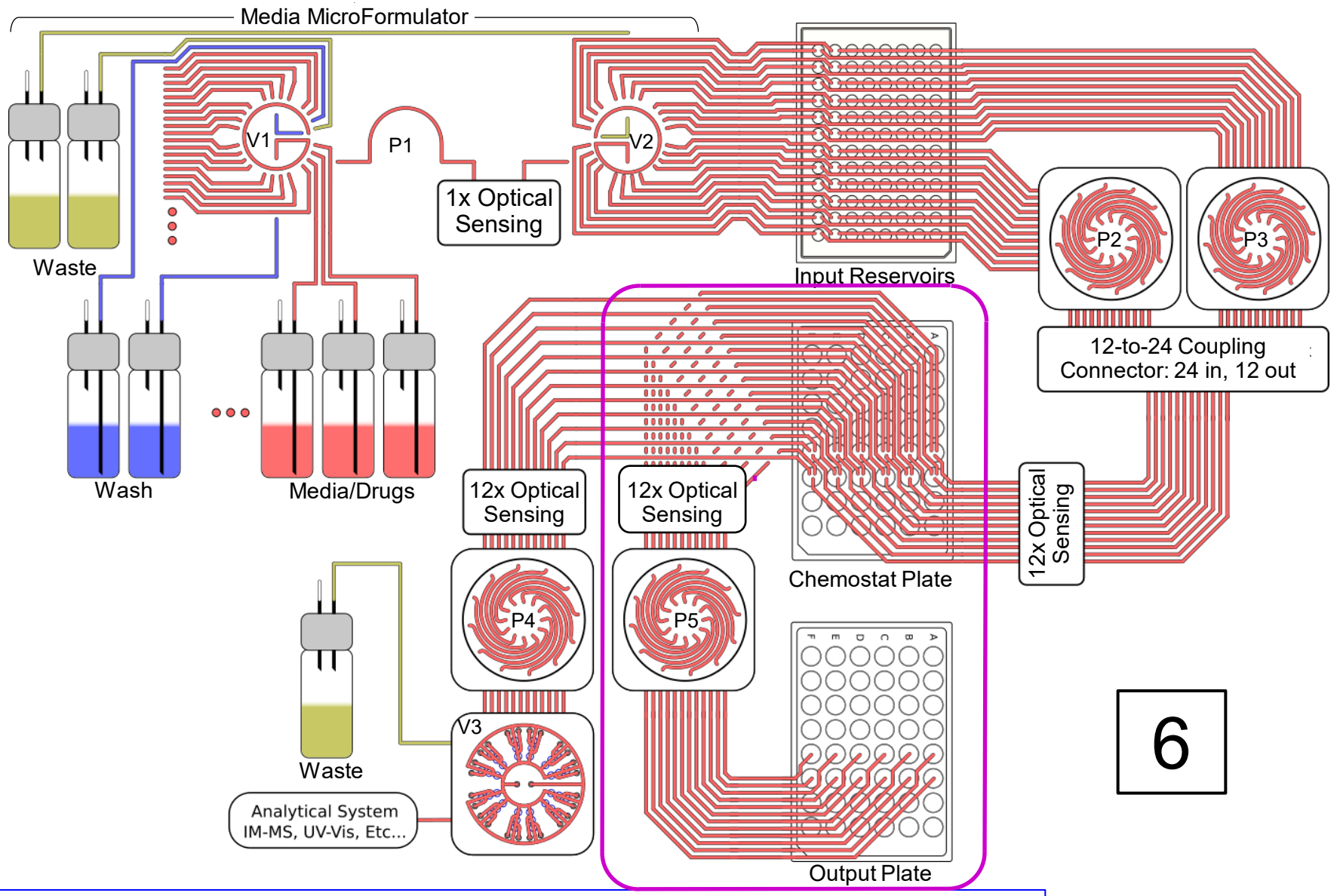
Genesis 1.0 12-Well MicroChemostat



- **Formulate the upper bank of input reservoirs**
- **Pump the lower bank of input reservoirs into the chemostats**
- **Cycle a small volume of media from chemostats and back to measure pH**

Patents issued and pending

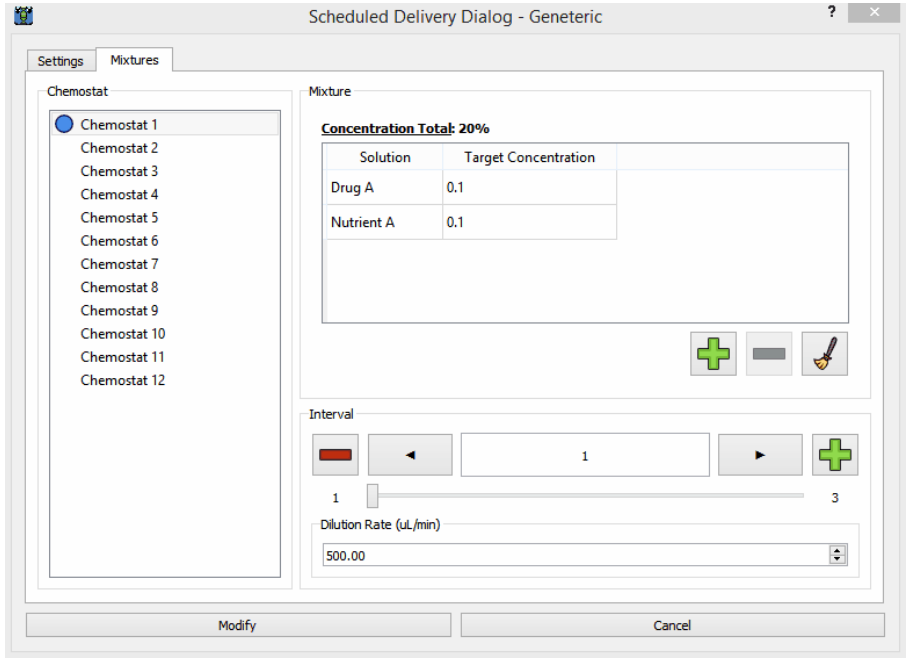
Genesis 1.0 12-Well MicroChemostat

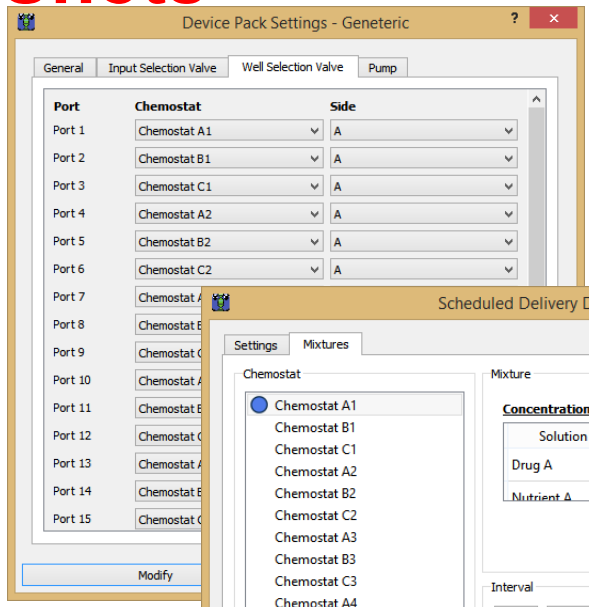


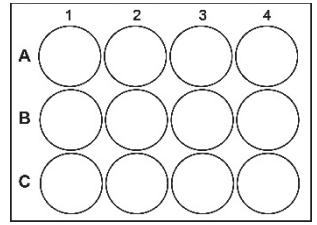
- **Stop MicroFormulator**
- **Stop chemostat pump**
- **Rapidly transfer media to Output Plate for freezing and transcriptomics, etc.**

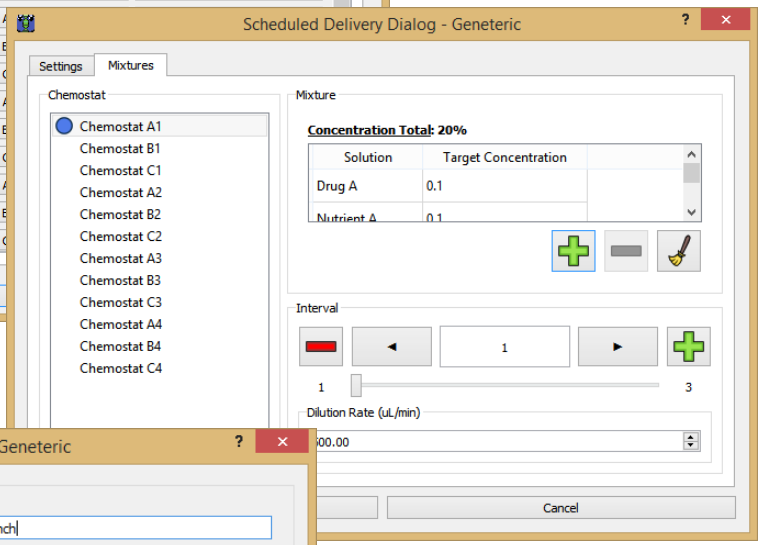
Patents issued and pending

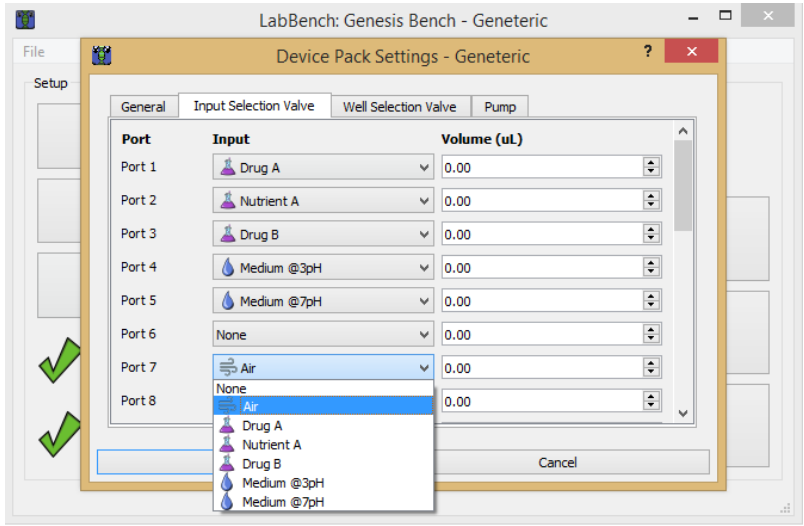
Geneteric Control Software Screen Shots

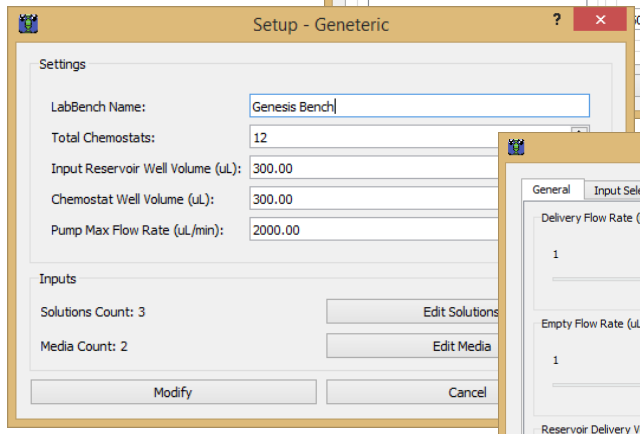


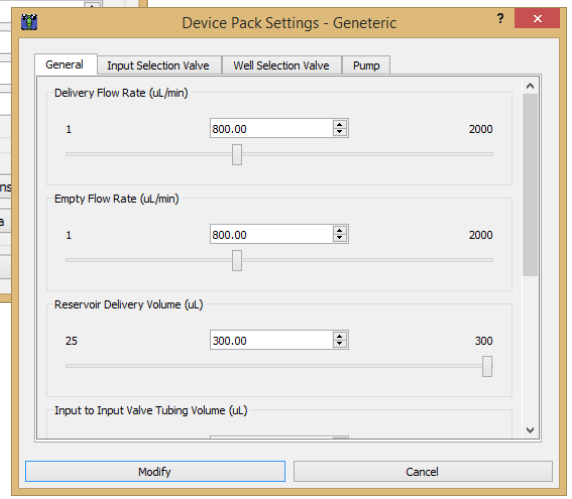








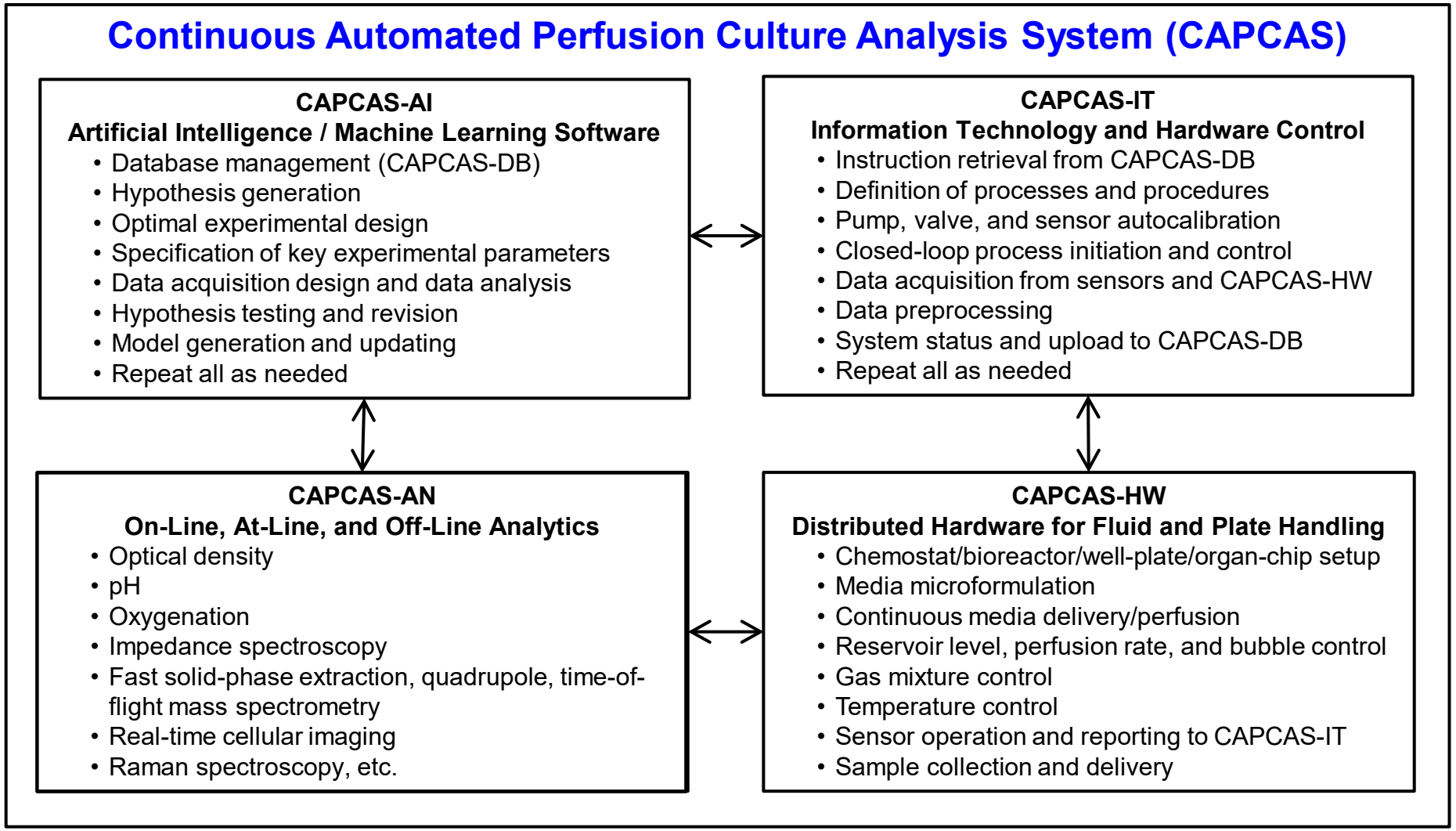




24 February 2022

GUI for planning and system debugging. CAPCAS HW will be driven by CAPCAS DB without user intervention.

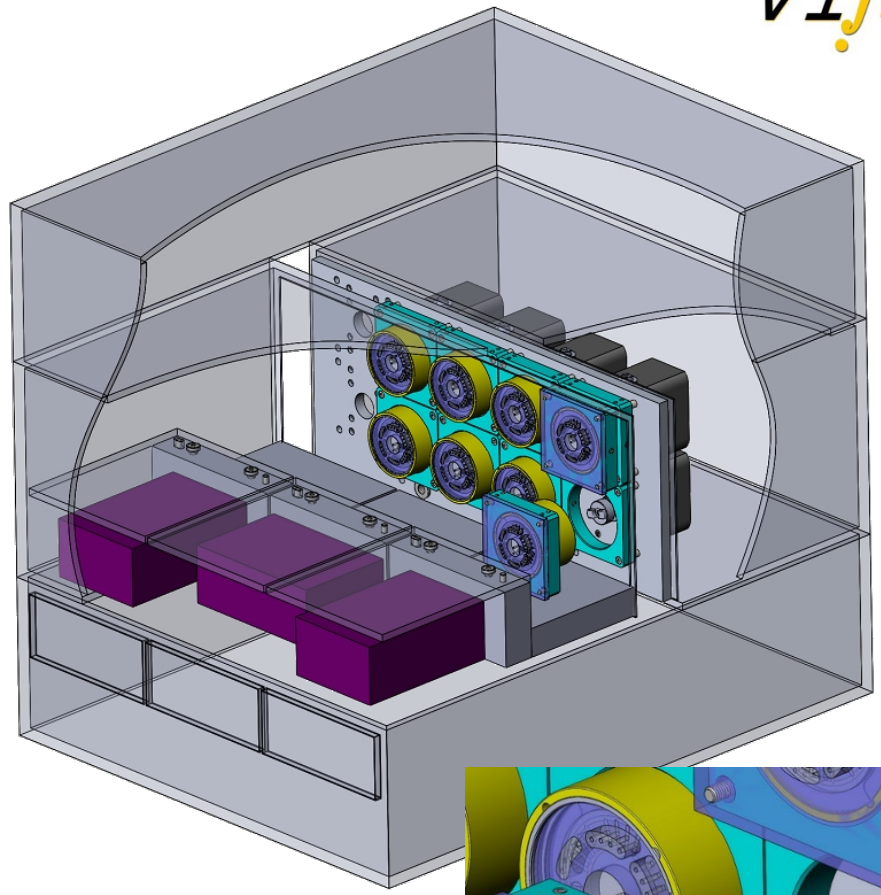
CAPCAS Subsystems

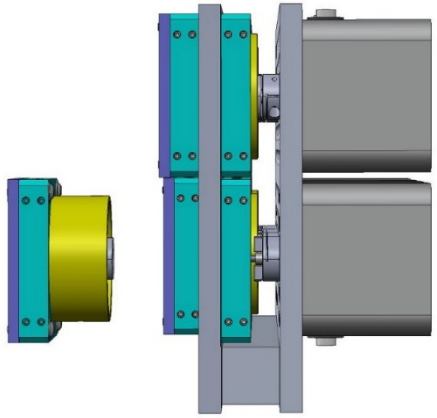


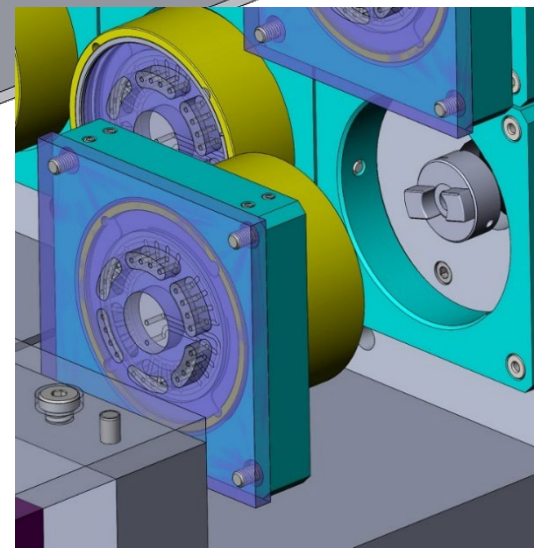
CAPCAS provides unique experimental design, control, and analysis capabilities!

Genesis 1.0 & 2.0 Chassis concept

- Self-contained sterility, thermal, and gas control
- One 96-well input reservoir plate
- One 48-well deep-well chemostat plate
- One 96-well sample-transfer well plate
- Separate motor/electronic and fluidic compartments
- Modular fluidics can be treated as a disposable cartridge
  - Input module
  - Chemostat module
  - Output module







Genesis Applications

- Chemostats
  - Yeast
  - Bacteria
  - CHO cells
  - Manufactured cellular products
- Gravity-perfusion of organs-on-chips
- Conventional 6 to 96 well plates with adherent cells
  - Optimization of stem-cell differentiation protocols
  - Imposition of multi-hormone circadian rhythms
- Continuous/circadian feeding of zebrafish in 12-well TransWell plates
- Mechanical and electrical stimulation with cardiac I-Wire
- Perfusion bioreactors with cell capture/return for antibody production, harvest of conditioned media, and protein engineering
  - Spiral-sorter
  - Tangential flow filter
  - Alternating tangential flow
- Automation in BSL-3 and BSL-4
- All capable of being driven by a Robot Scientist

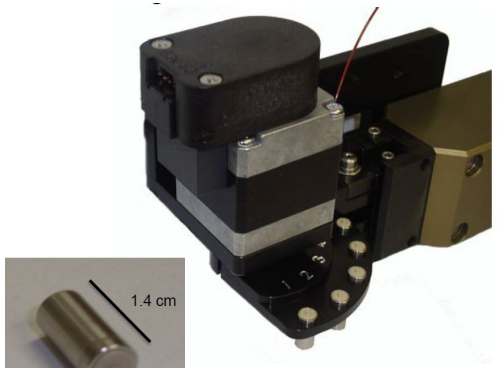
The ultimate value of Genesis is the information that pharma and biotech can obtain with it to guide the design, optimization, and control of cell lines and cell-culture processes.

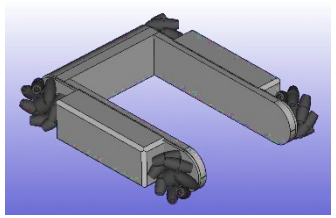
Patents issued and pending

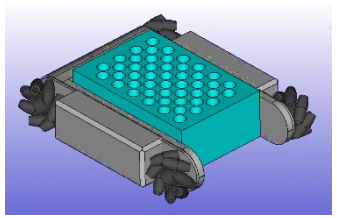
How do you go from one 48-well-plate chemostat to 200 separate plates?

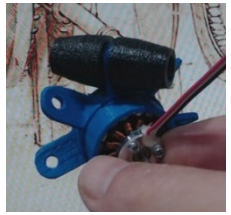
Genesis 3.0 Concept

- Self-driving laboratory with artificial intelligence/machine learning
- 10 sec/sample SPE-IM-Q-TOF mass spectrometry metabolomics
- iPlateBot holonomic robot
- Continuous Automated Perfusion Control and Analysis System (CAPCAS) enclosure for >1,000 chemostats, perfusion bioreactors, and organ-chips

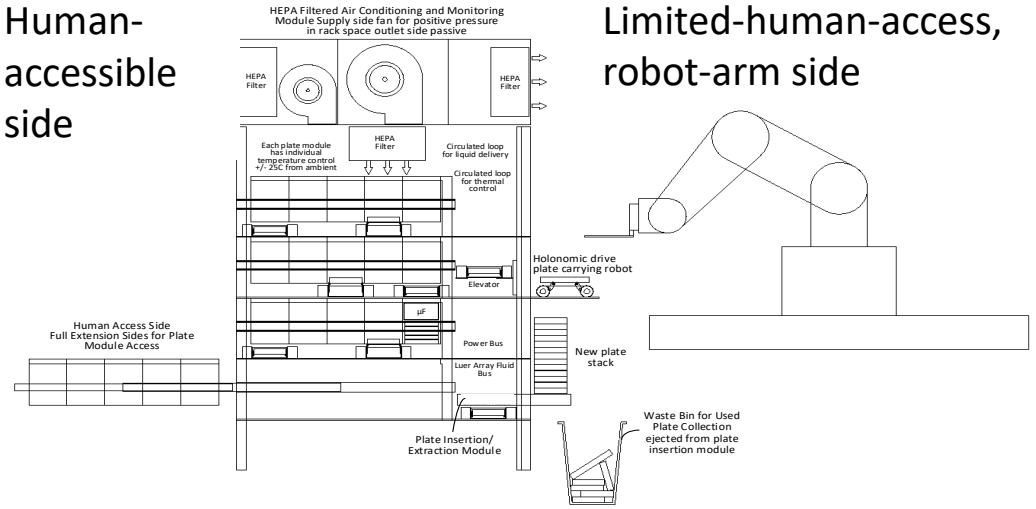




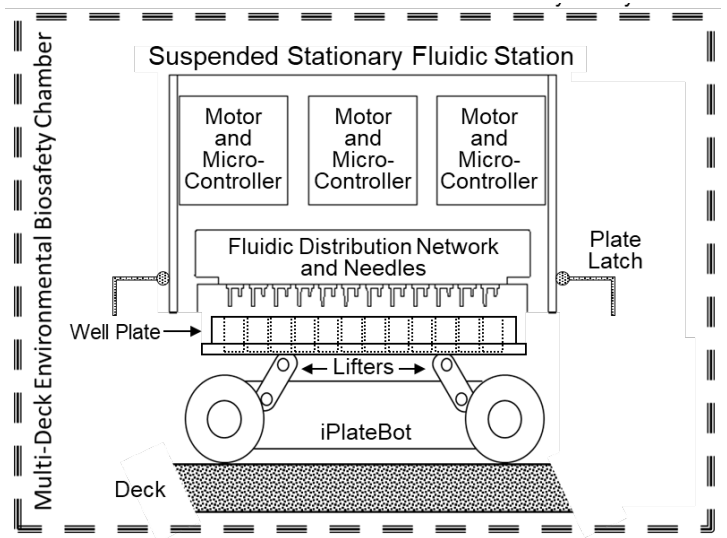




Human-accessible side

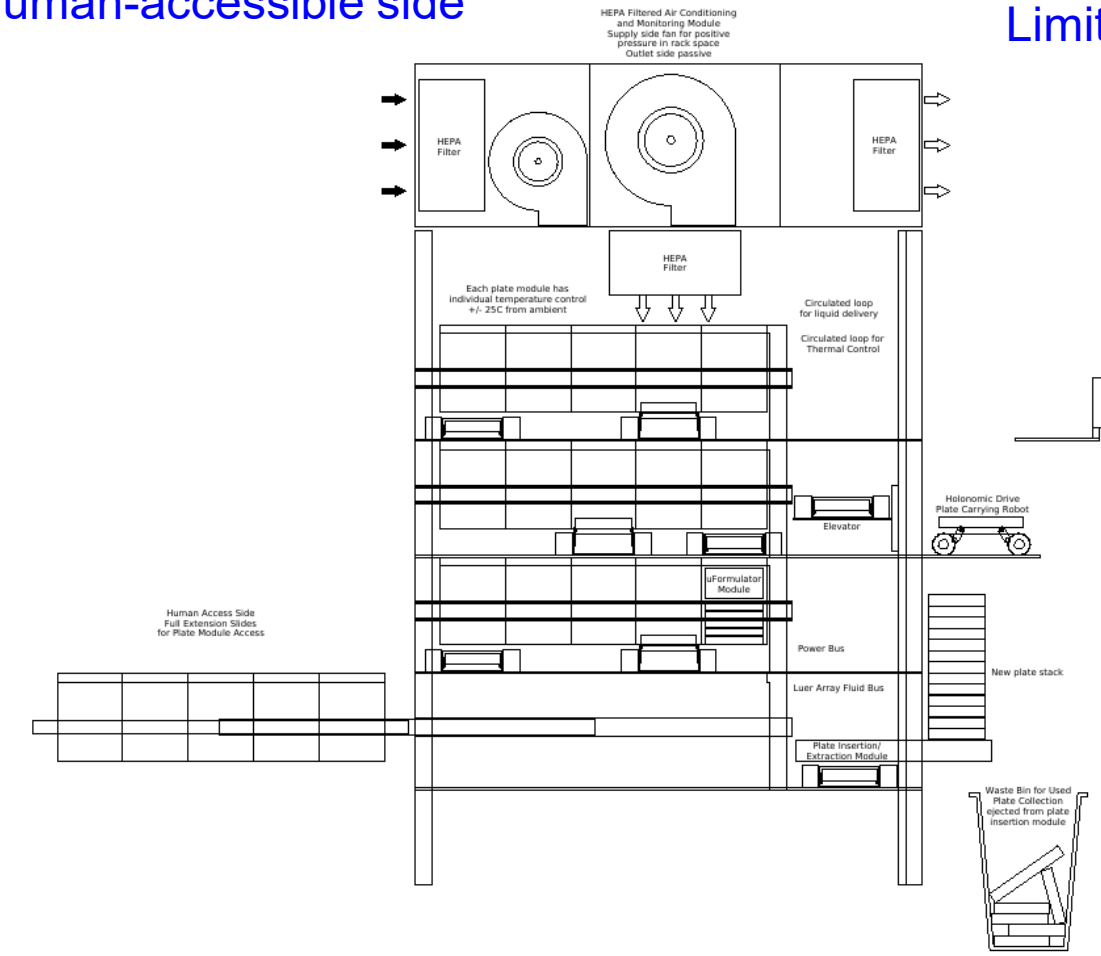


Limited-human-access, robot-arm side

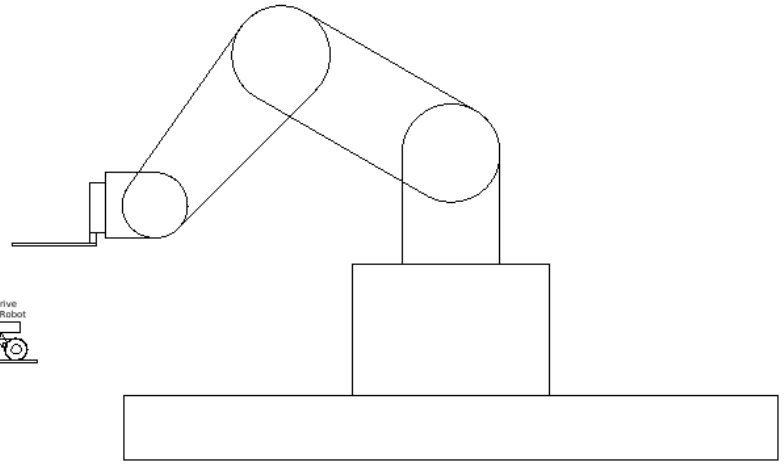


Genesis in a multi-deck environmental chamber

Human-accessible side



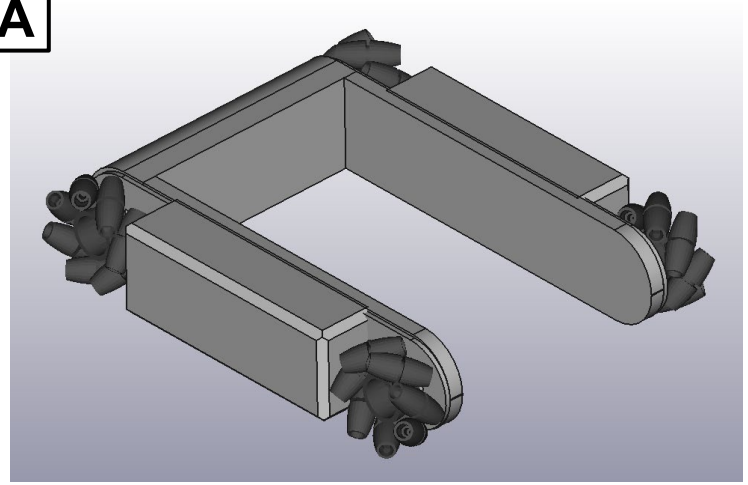
Limited-human-access, robot arm side



Patents issued and pending

Genesis holonomic iPlateBot

A



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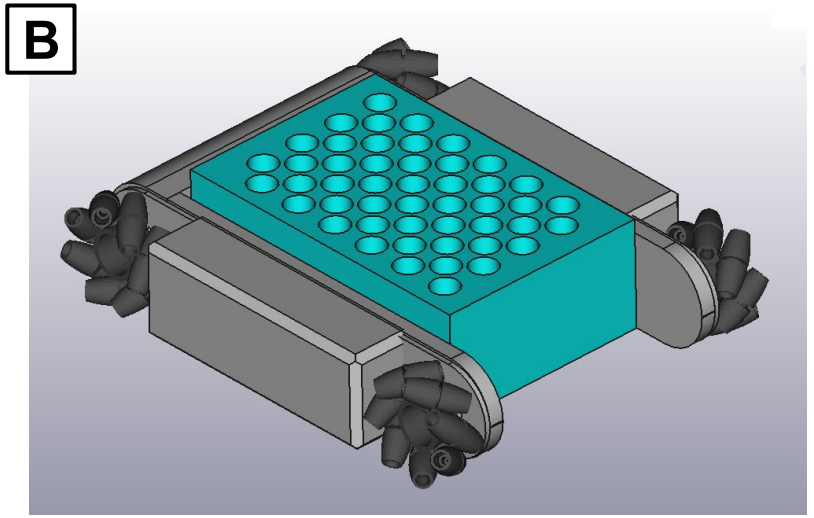

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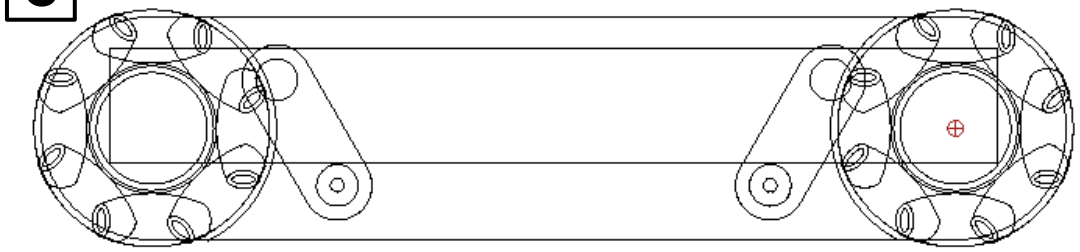

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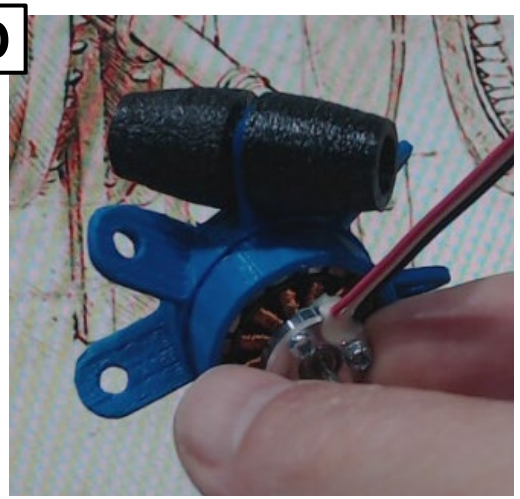
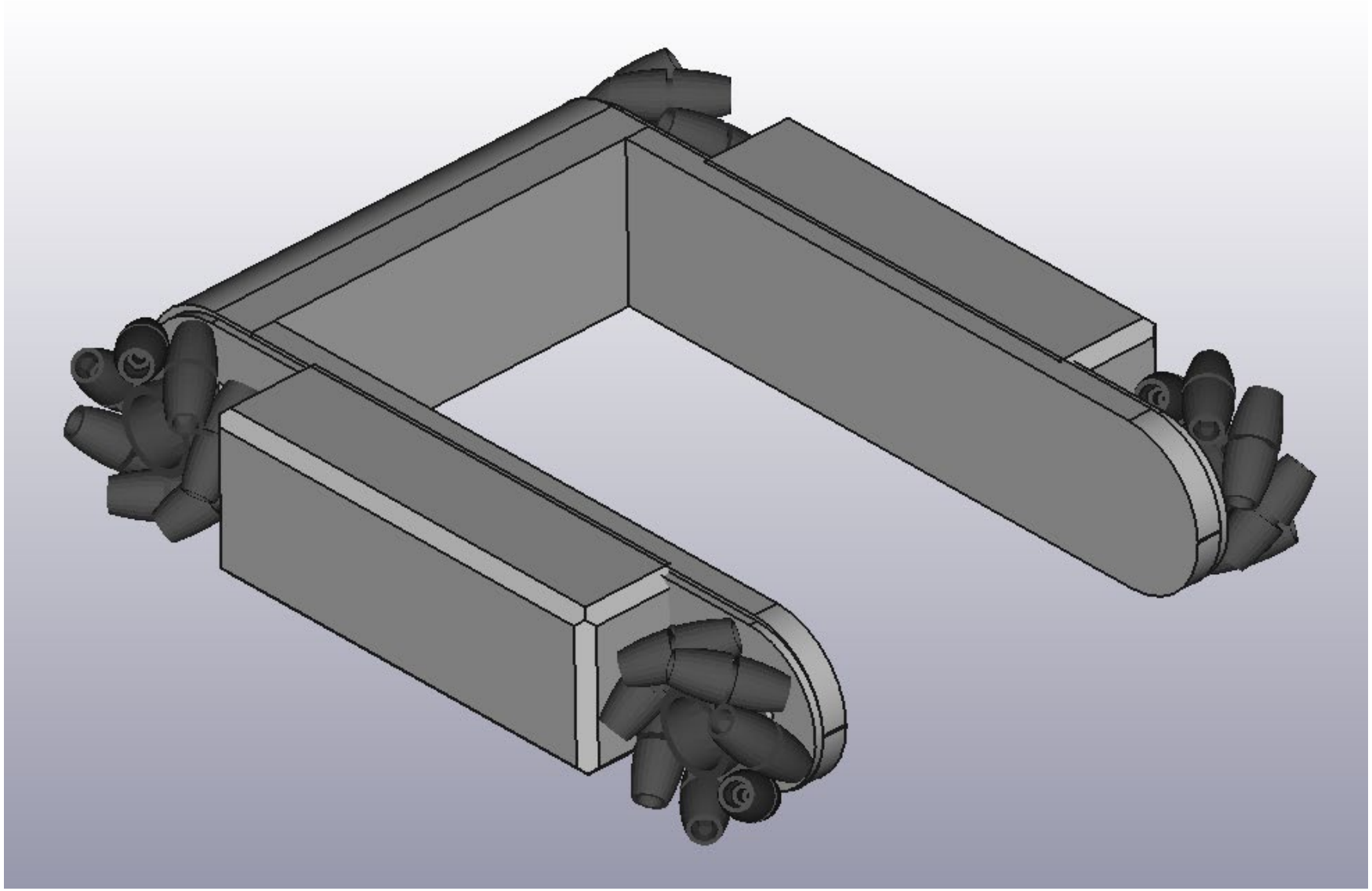


A-B) An empty iPlateBot and one carrying a deep 48 well plate.
C) Plate gripper/lifter detail.
D) First 3D print of a holonomic wheel-motor assembly.

The iPlateBots are small and light enough that they do not present a hazard to human operators. Hence these robots can operate alongside a human on a benchtop, in a cell culture hood, or at other workspaces.

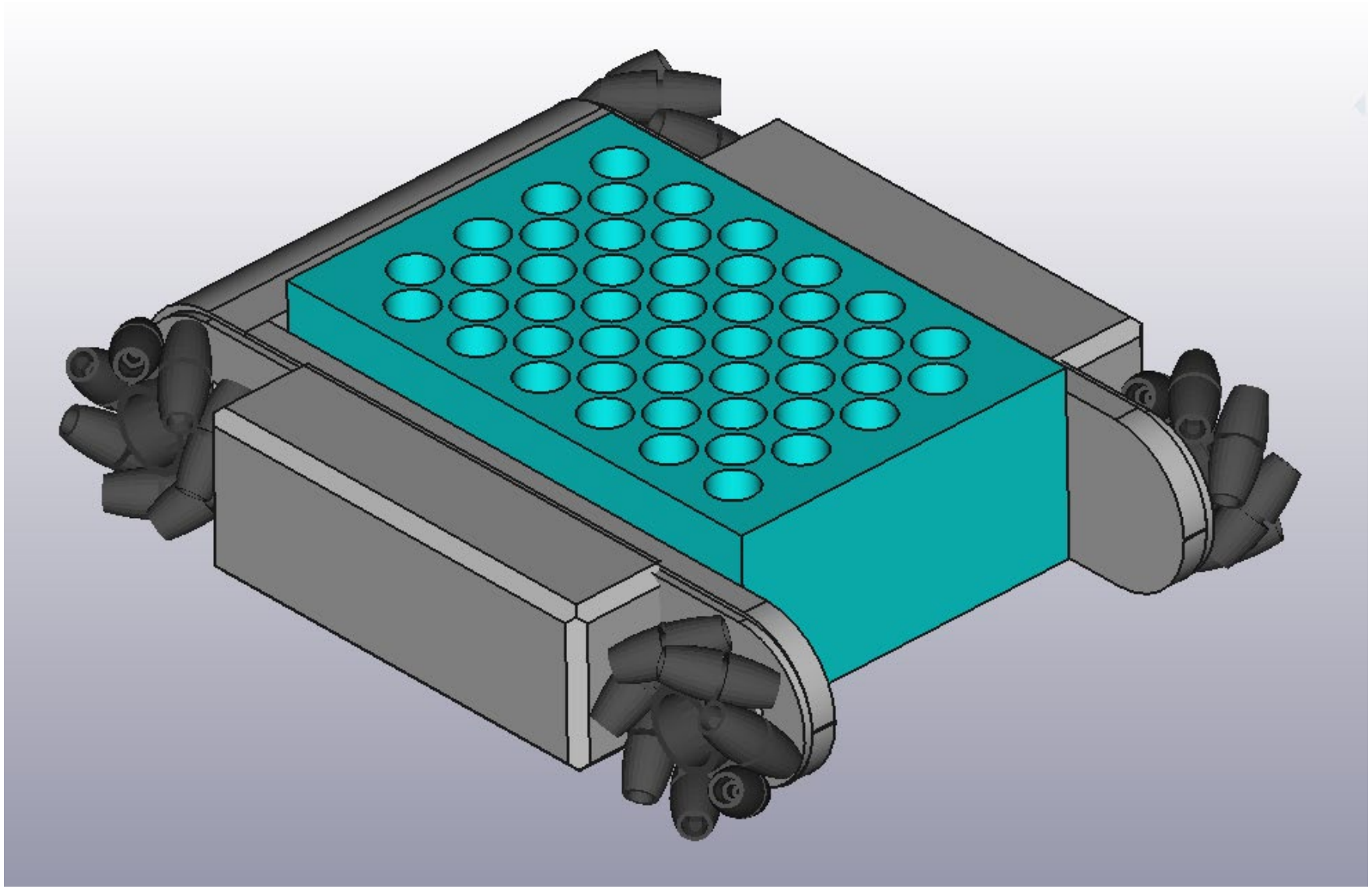
Patents issued and pending

Empty iPlateBot



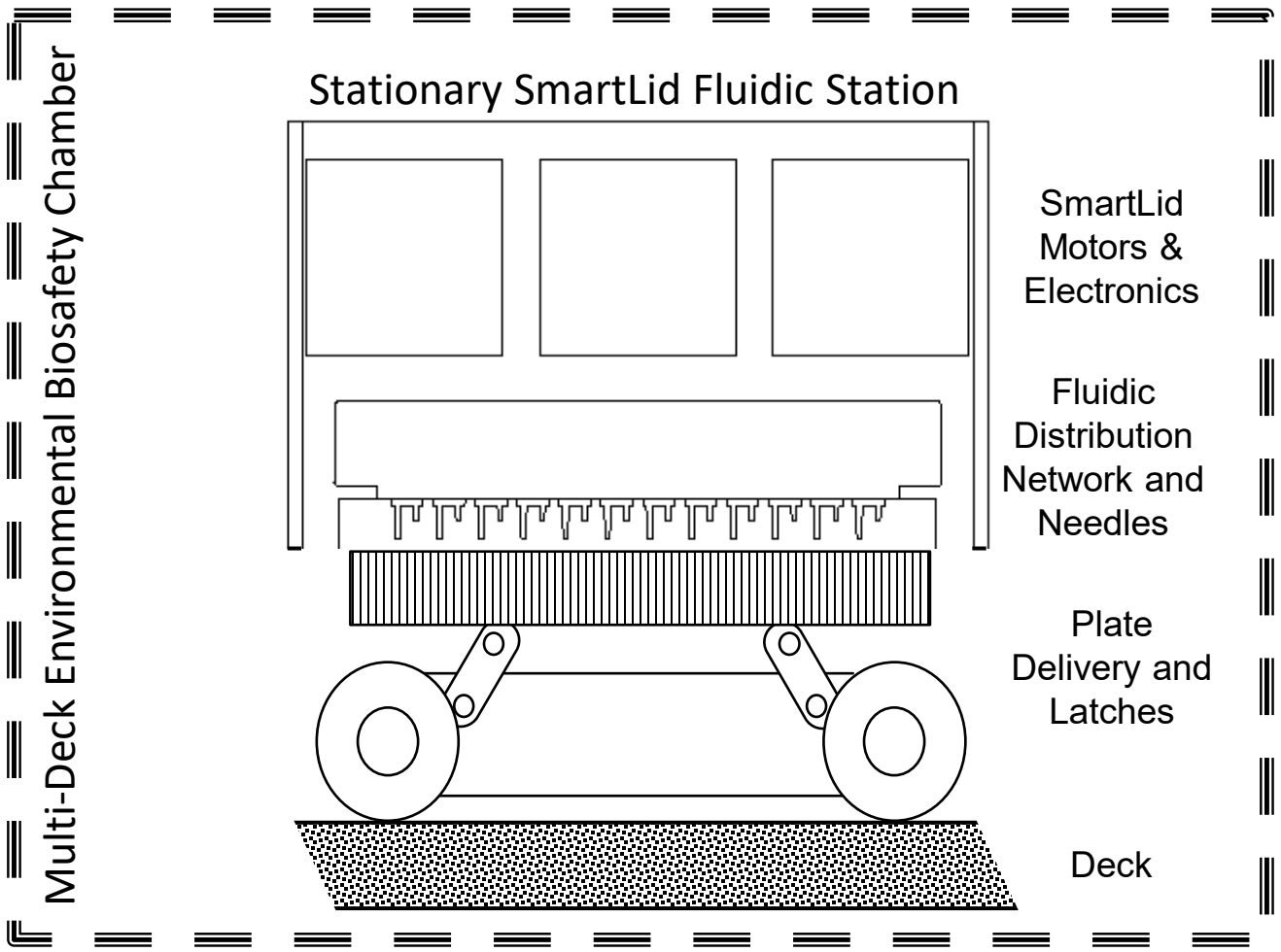
Patents issued and pending

iPlateBot carrying a deep-well plate



Patents issued and pending

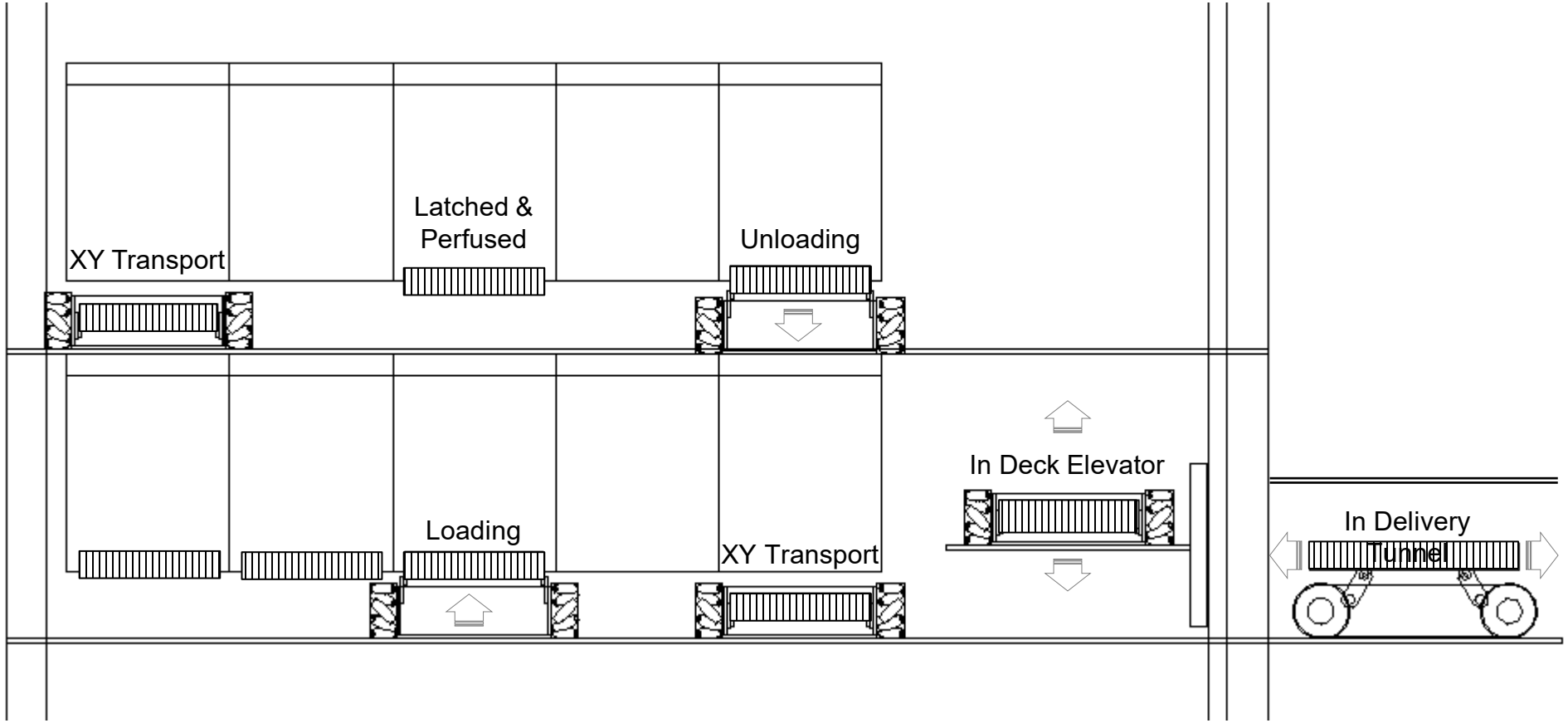
Well plate lifted into place and latched



Patents issued and pending

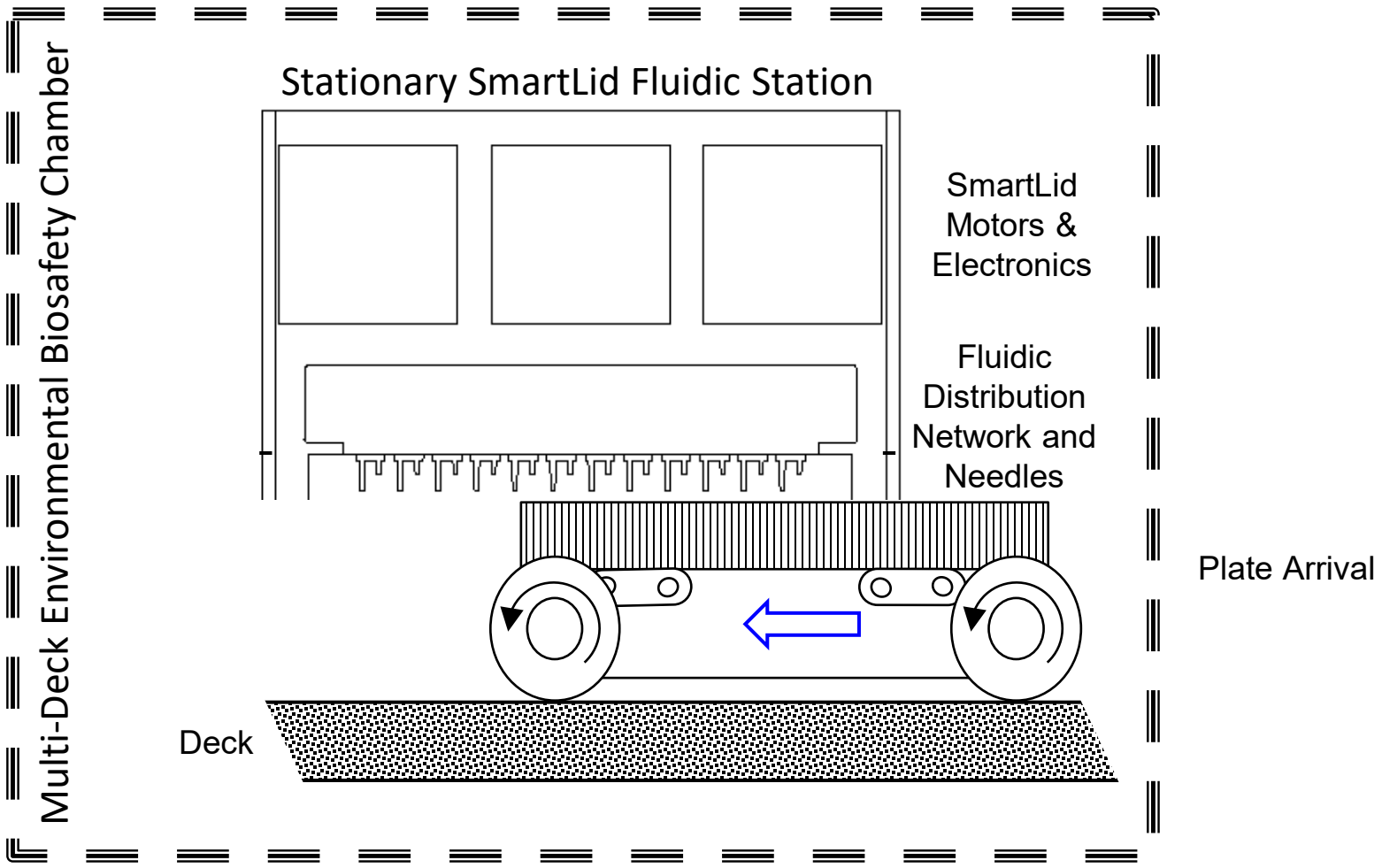
iPlateBots in an automated incubator

- The iPlateBot delivers a plate to an assigned fluidic station in the incubator.
- The plates are raised and latched into the fluidic handling station.
- The iPlateBot departs for another assignment.

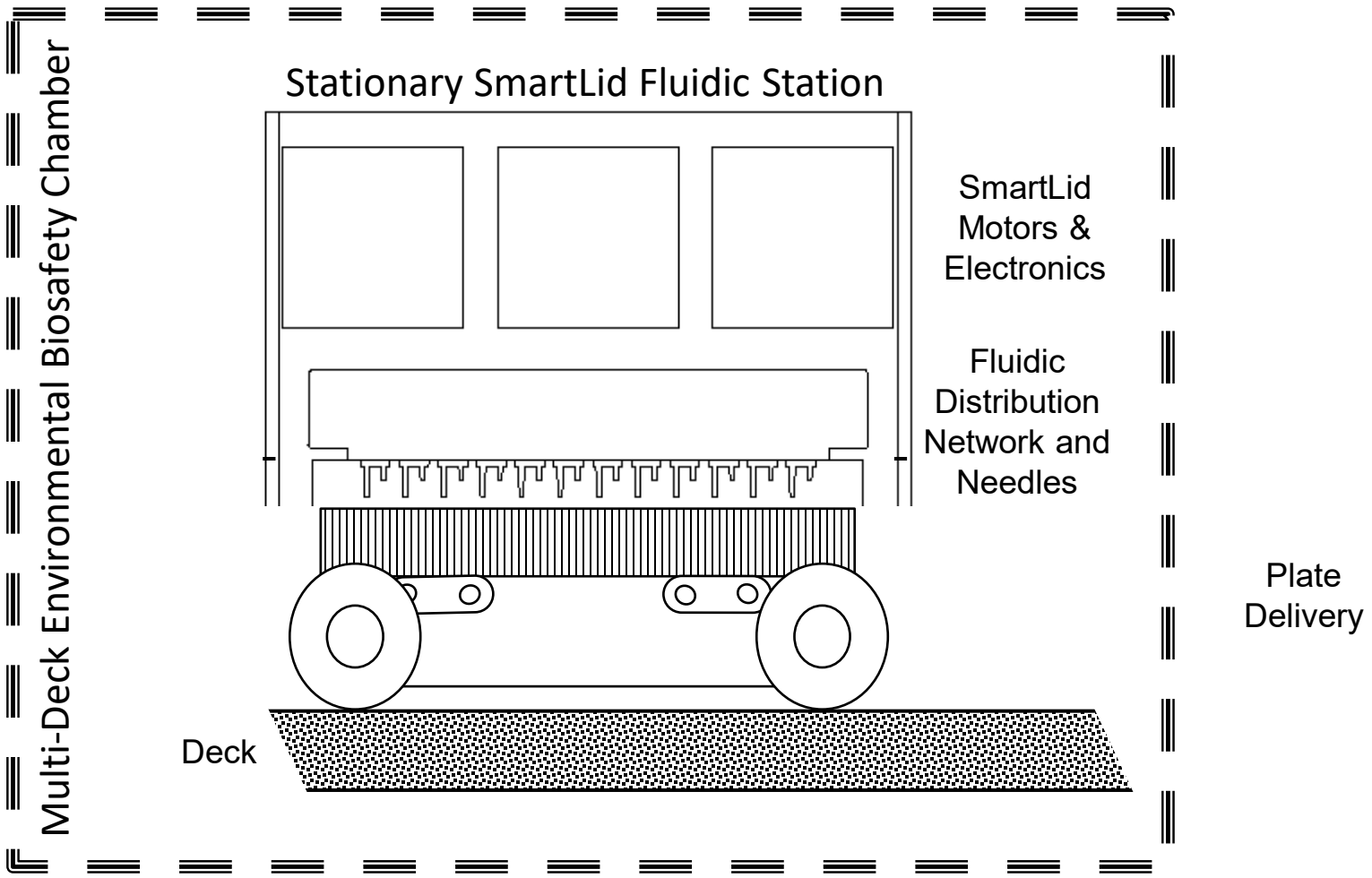


Patents issued and pending

Well plate lifted into place and latched

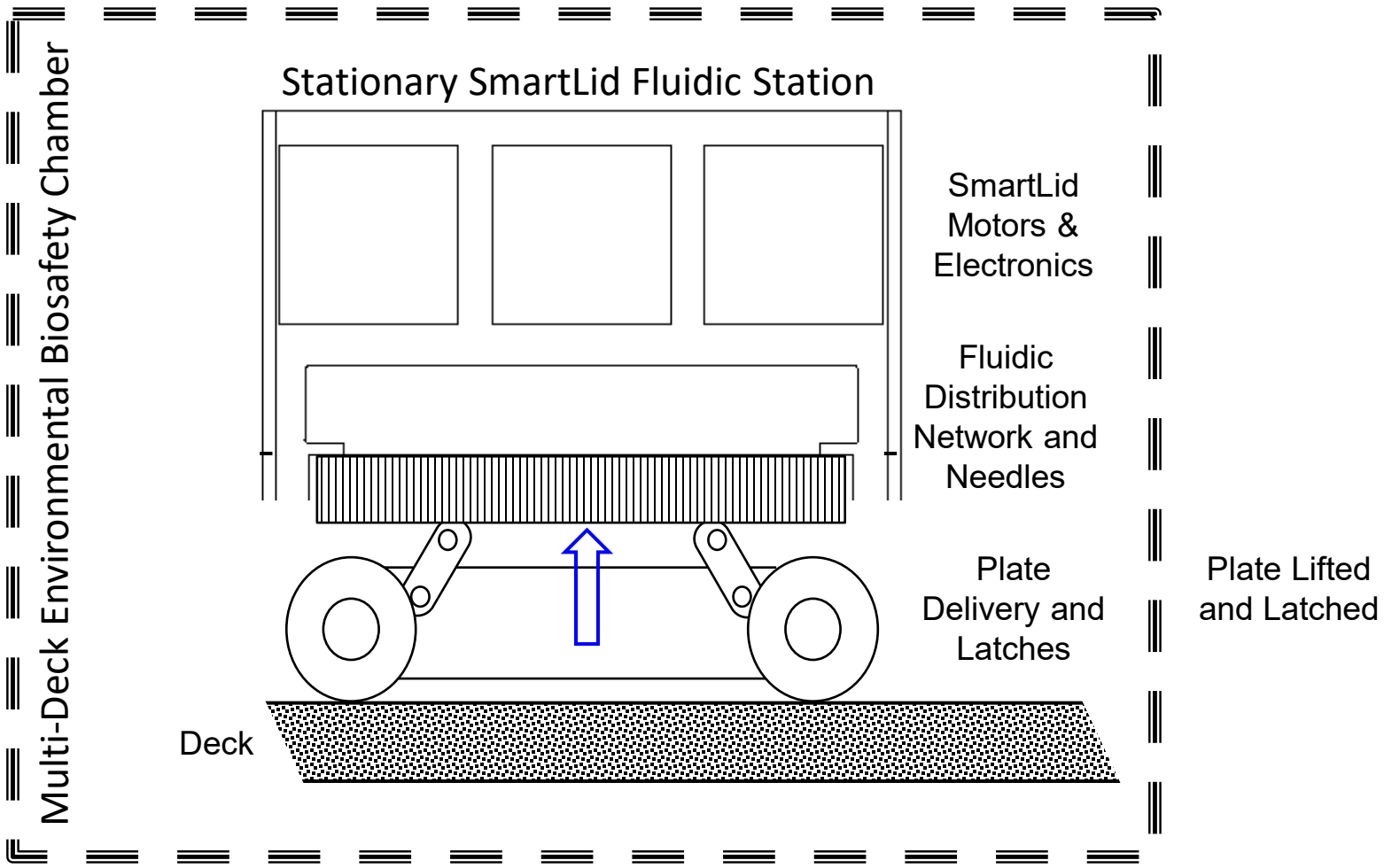


Well plate lifted into place and latched



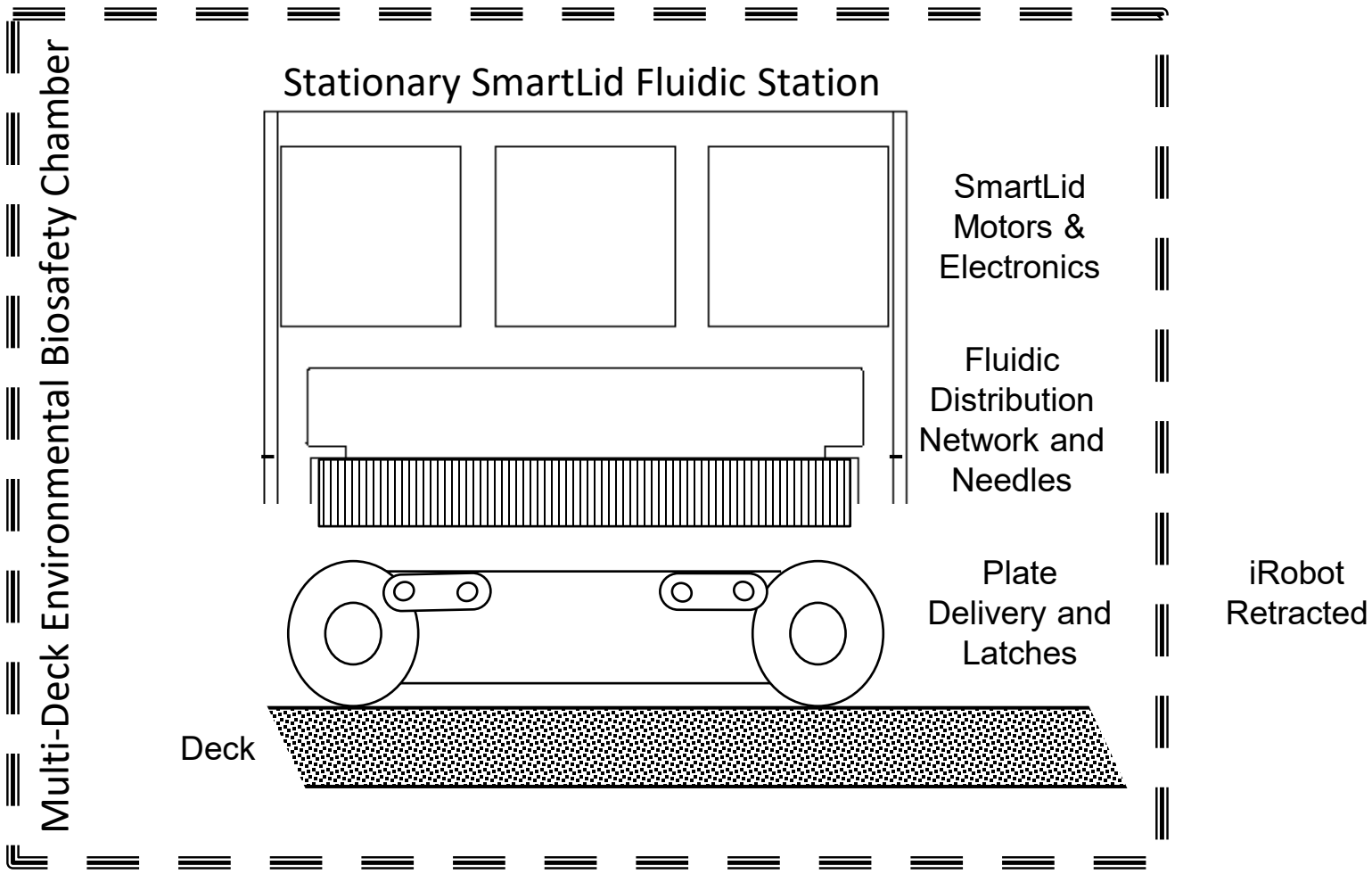
Patents issued and pending

Well plate lifted into place and latched



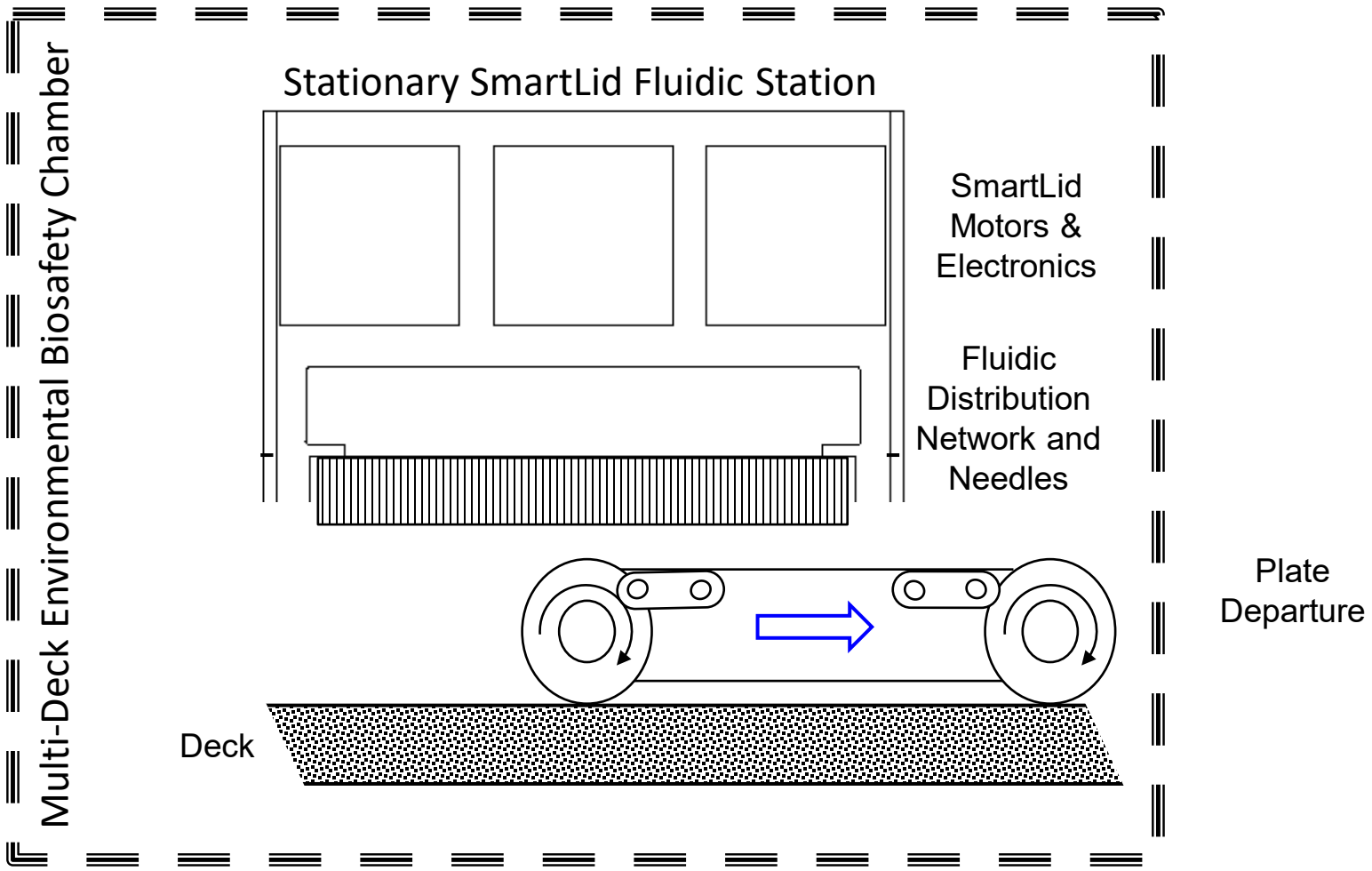
Patents issued and pending

Well plate lifted into place and latched



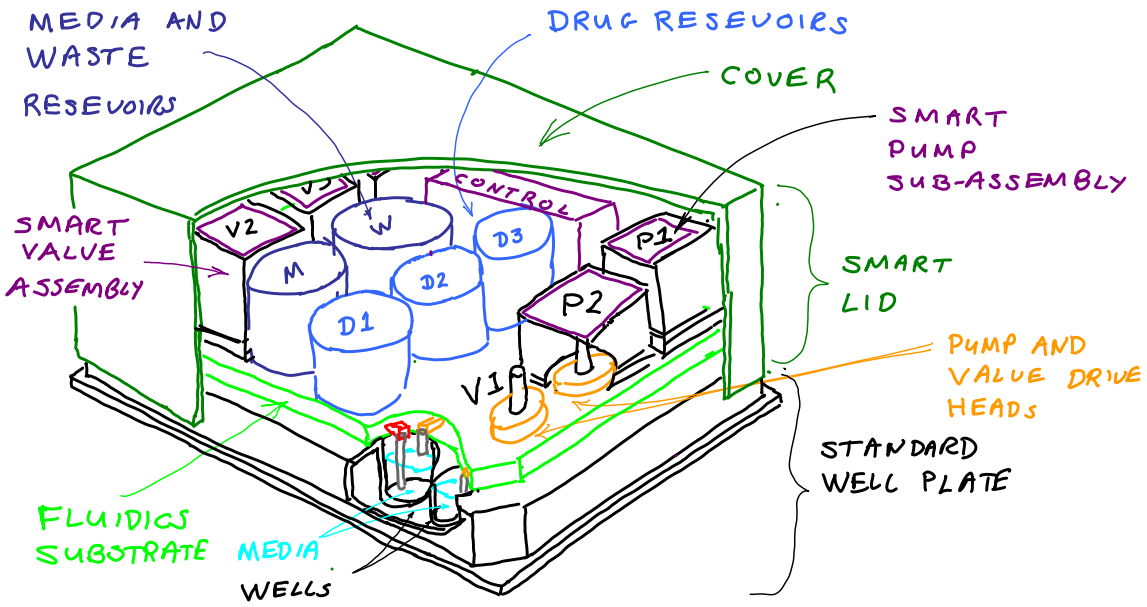
Patents issued and pending

Well plate lifted into place and latched



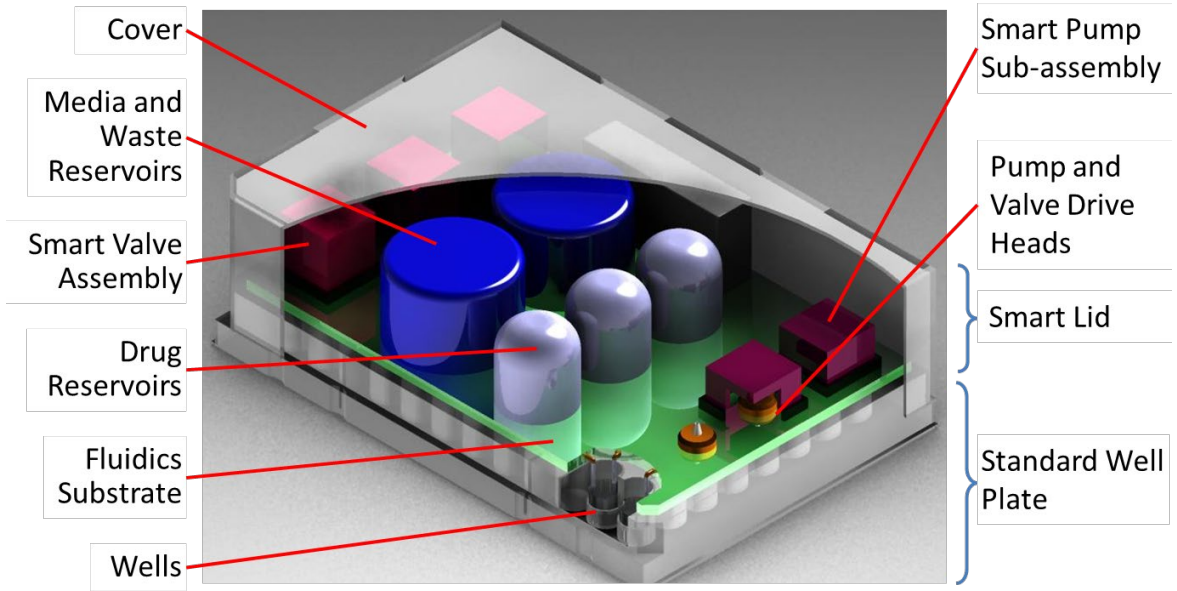
Patents issued and pending

SmartPlate Technology for Advanced Cellular Models (STAC-M)



Idea

The Smart Lid Concept - 2015



Proposal

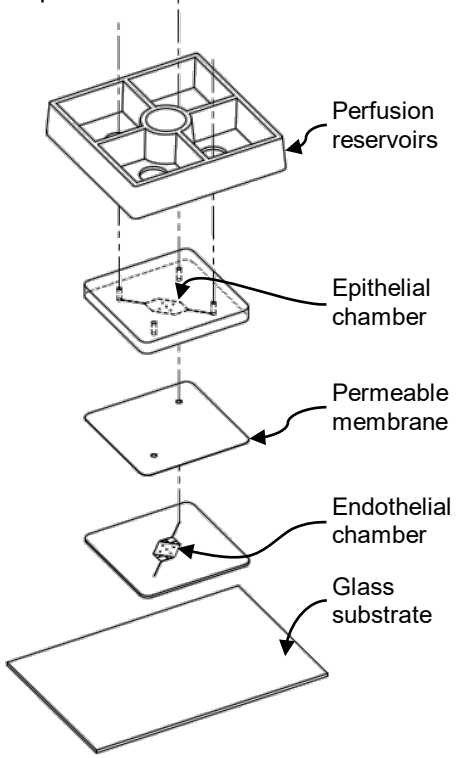
NIH/NCATS SBIR Contracts HHSN271201600009C and HHSN271201700044C to CFDRC, Kapil Pant, PI

Sam Michael, NIH/NCATS technical manager

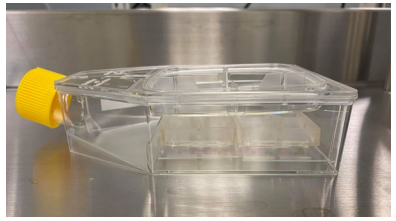
Patents issued and pending

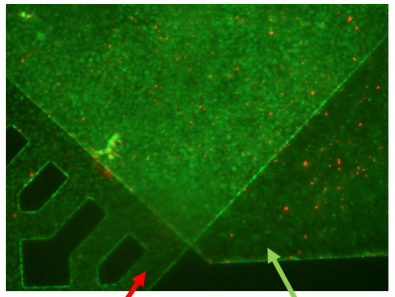
BSL-3 Airway and BBB organ-chip SARS-CoV-2 experiments are easier with gravity perfusion

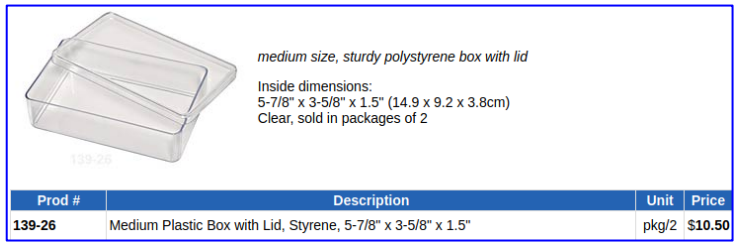
As soon as we were shut down for COVID-19, we started engineering new devices for BSL-3 that required no external hardware:

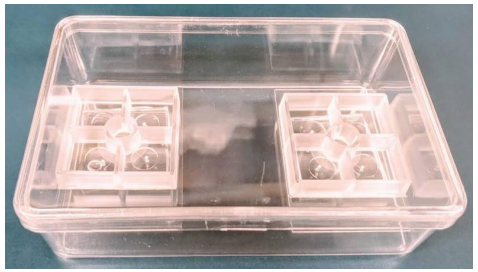


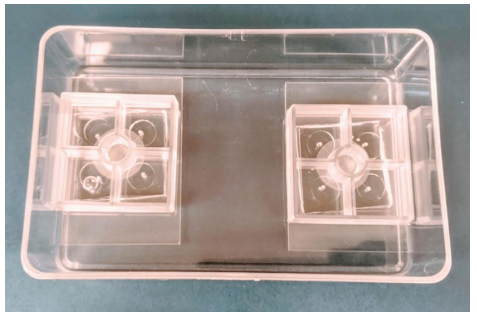
Large reservoirs = 24-hour perfusion at 1 to 5 µL/min with no bubble problems



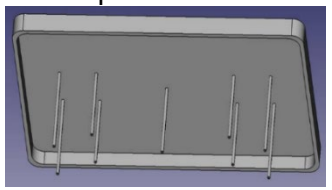


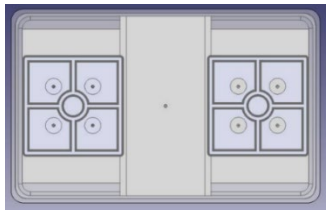


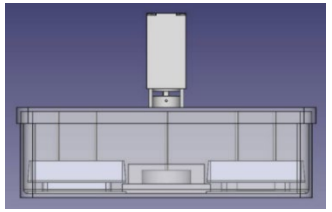




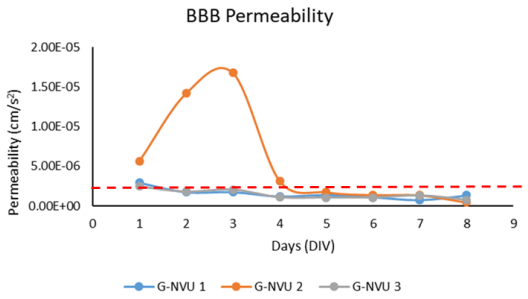
A plastic dish can provide secondary containment and an internal reservoir can ensure humidification of the chips.

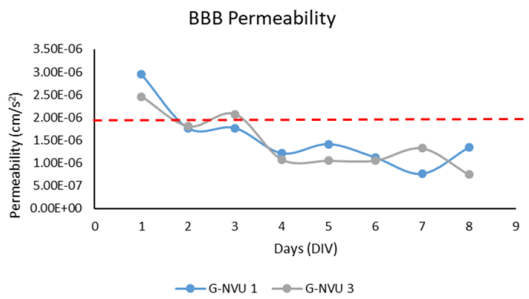






A lid with an integrated pump, fluidic channels, and needles will enable long-term recirculation.





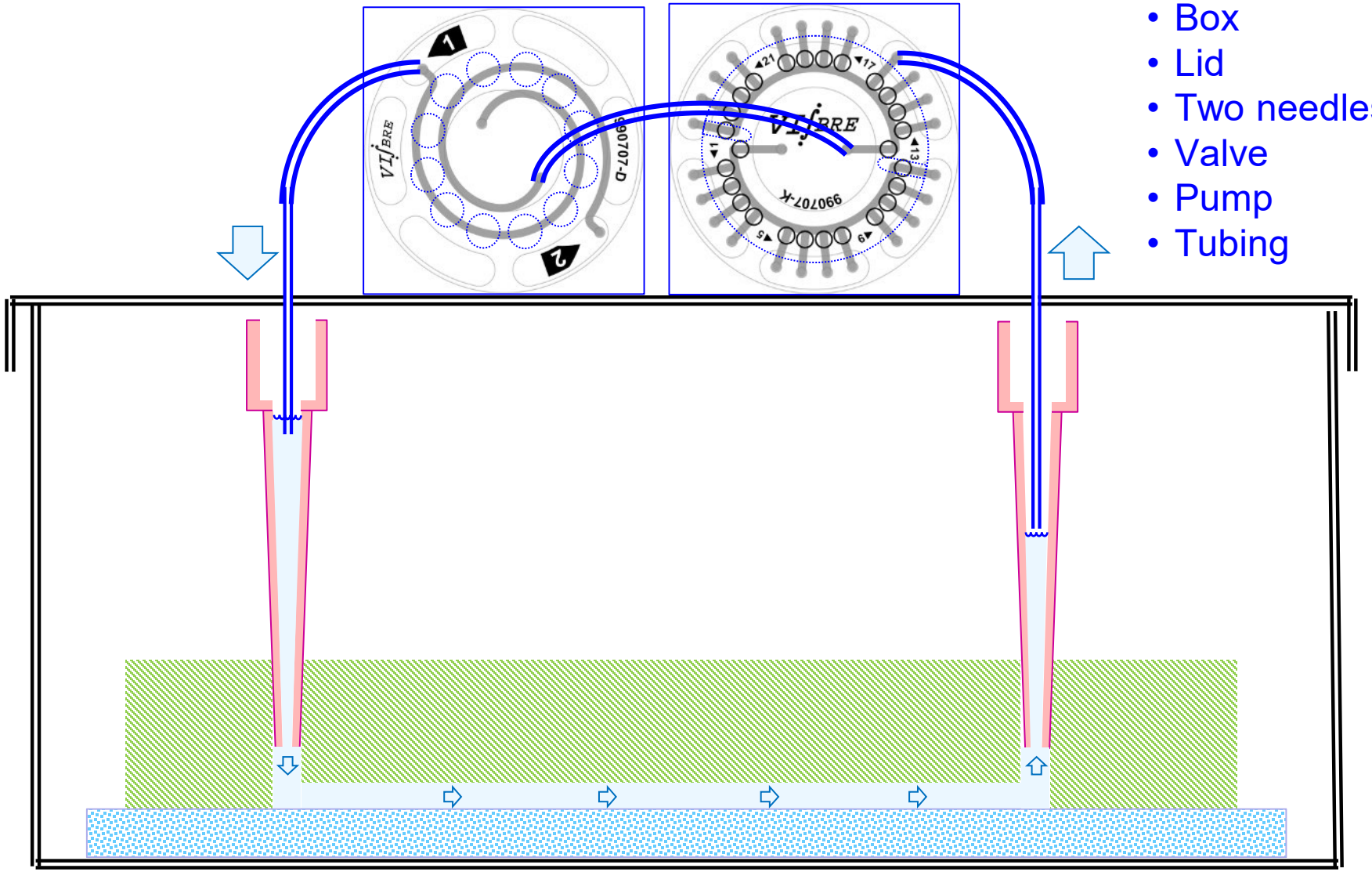
In all three devices, blood-brain barrier function was better than (below) our $2x10^{-6}$ cm/s^2 cut off!

Compatible with iPlateBot and Genesis

Patent Pending

Funded by NIH NCATS, in collaboration with Dr. Aarthi Narayanan, GMU

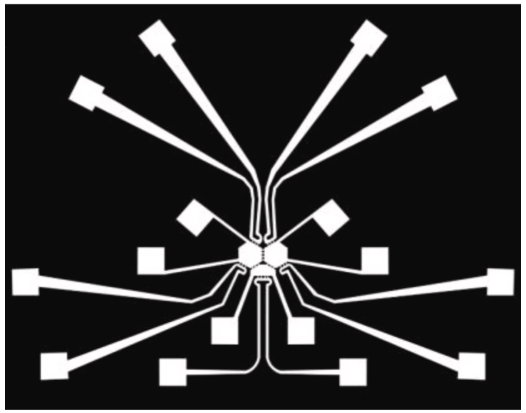
Multi-Organ Sampling System - Under development

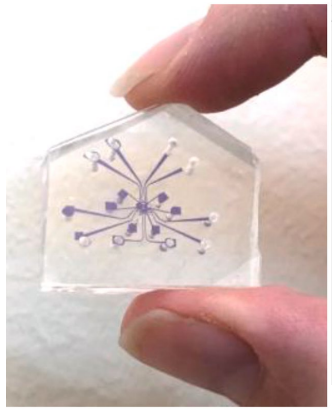


- Box
- Lid
- Two needles
- Valve
- Pump
- Tubing

The hybrid pumped/gravity feed may be the best of both worlds.

Bone Marrow Chip





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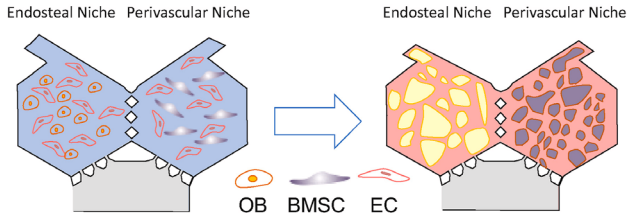
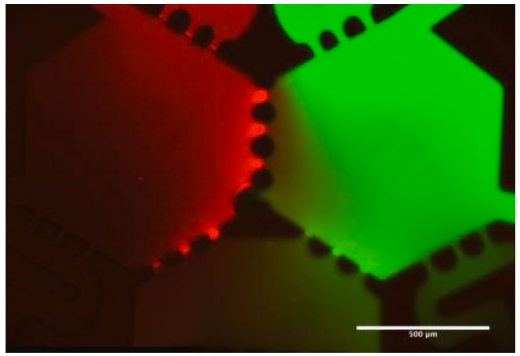
journal homepage: www.elsevier.com/locate/biomaterials

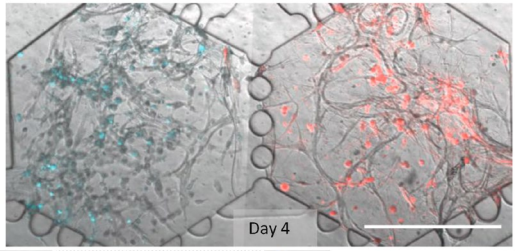
Organ-on-a-chip model of vascularized human bone marrow niches

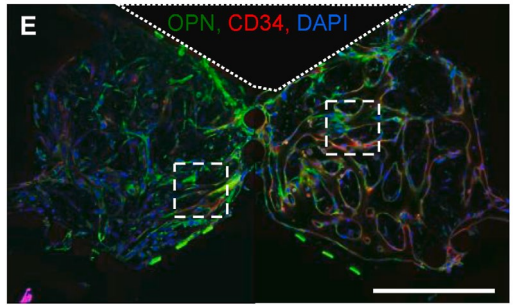
Drew E. Glaser [a], Matthew B. Curtis [a], Peter A. Sariano [a], Zachary A. Rollins [b], Bhupinder S. Shergill [a], Aravind Anand [a], Alyssa M. Deely [a], Venktesh S. Shirure [a], Leif Anderson [a], Jeremy M. Lowen [a], Natalie R. Ng [c], Katherine Weilbaecher [d], Daniel C. Link [d], Steven C. George [a,*]

UCDAVIS BIOMEDICAL ENGINEERING

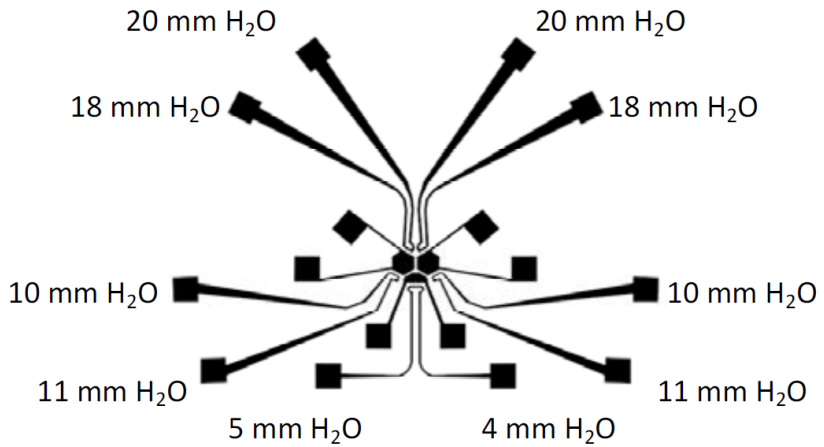


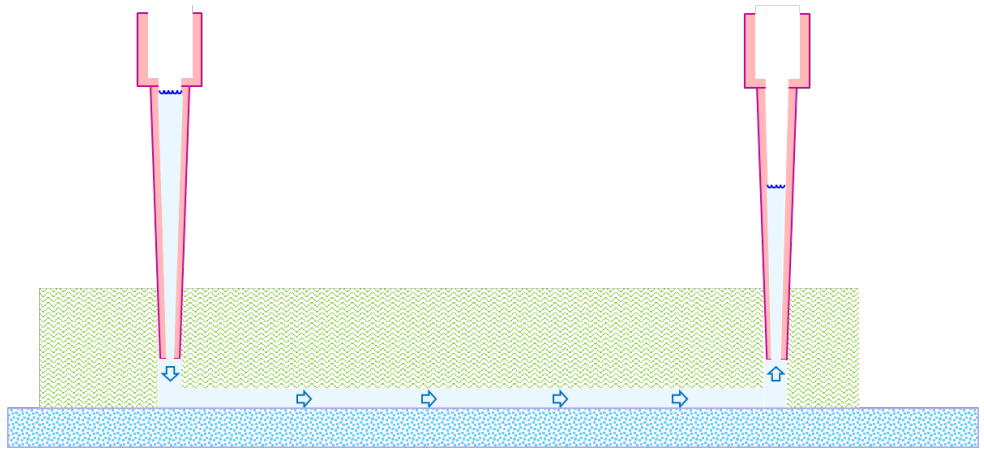






Careful pressure regulation is required to make this chip function properly.





Lots of pipettes, tight pressure ranges, but it works nicely!

- Nature self-organizes the branching, lengths, and diameters of the microvasculature to achieve proper function.
- It will take a bit of engineering to recapitulate the physiology.
- It will require even more engineering to make that massively parallel.

Steve George and Scott Simon – Proposed concept

UCDAVIS BIOMEDICAL ENGINEERING

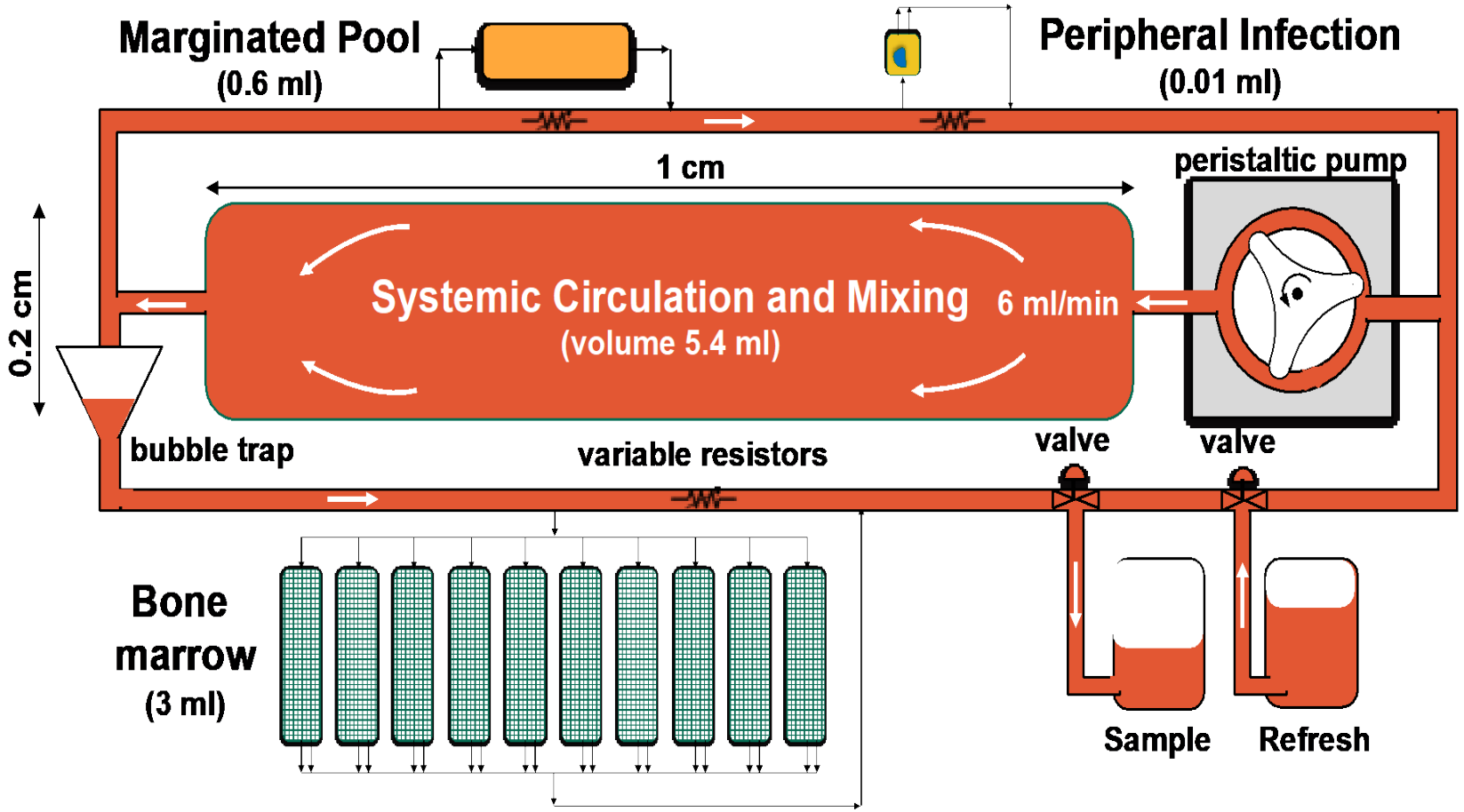


Figure 6. ***Schematic of initial design concept for peripheral infection-bone marrow microfluidic device.*** The device should achieve numerous unique and challenging features of mimicking peripheral infection-bone marrow interactions; in particular, the relative volumes and flows of the systemic circulation, the marginated pool of neutrophils, bone marrow, and site of infection (see text for details). In the initial design, the volume of bone marrow is subdivided into 10 separate parallel compartments, similar to the distribution of bone marrow *in vivo* (details below in **Fig. 7**). The systemic circulation is a larger compartment that facilitates mixing, the marginated pool of neutrophils receives mixed systemic circulation, and the peripheral infection compartment (site of infection is blue) is a separate, smaller compartment (details below in **Fig. 8**). Media/blood is recirculated using a peristaltic pump, and there are ports for sampling fluid/cells and to refresh the media/blood. A fraction of the systemic media/blood flow is diverted to the bone marrow and peripheral infections compartments using a variable resistor in the systemic line that is proportional to the volume of the tissue.

The numbers

UCDAVIS BIOMEDICAL ENGINEERING

Table 1. *Quantitative Platform Features*

Variable Description	Value/Range
Bone marrow:infection volume	2000-3000*
Bone marrow:peripheral circulation volume	0.5*
Bone marrow volume	3 ml
Peripheral infection volume (initial)	0.001 ml
Peripheral circulation volume	6 ml
Residence time in peripheral circulation	1 min $
Blood/media flow systemic circulation	6000 $\mu l \cdot min^{-1}$ §
Blood/media flow bone marrow	300 $\mu l \cdot min^{-1}$ #
Blood/media flow infection (initial)	0.3 $\mu l \cdot min^{-1}$ #

* Based on a typical human adult volume of 2.5 liters of marrow and 5 liters of blood, and the size of a typical peripheral infection (1 cm_3).

$ Adult blood volume of 5 liters and a cardiac output of 5 l/min at rest.

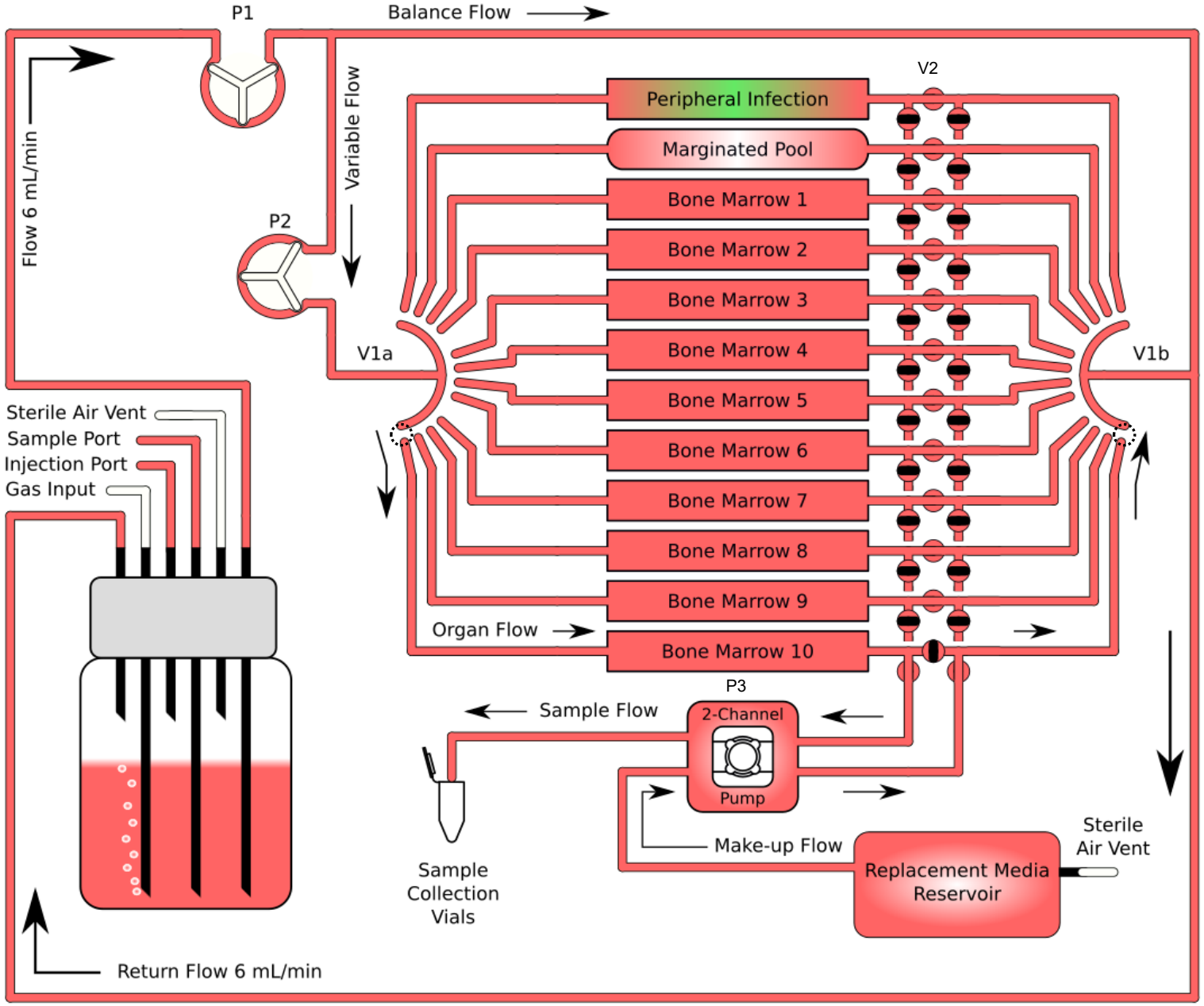
§ Based on volume and residence time of the peripheral circulation.

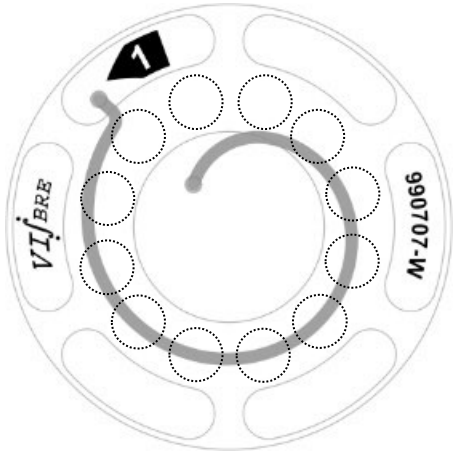
clearance rate of radioisotope in human bone marrow equivalent to resting muscle and blood flow to bone is ~ 3-5% of cardiac output; corresponds to 0.02 $ml.ml^{-1}.min^{-1}$ [3, 4]

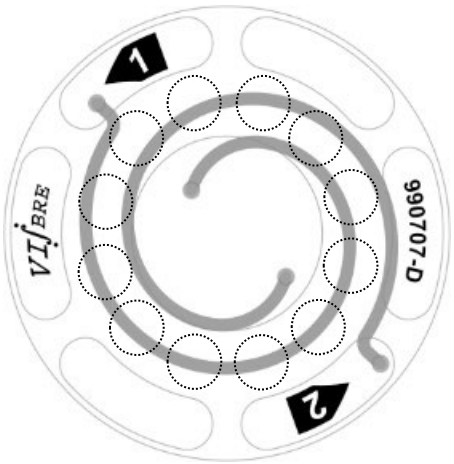
3E3 dynamic range

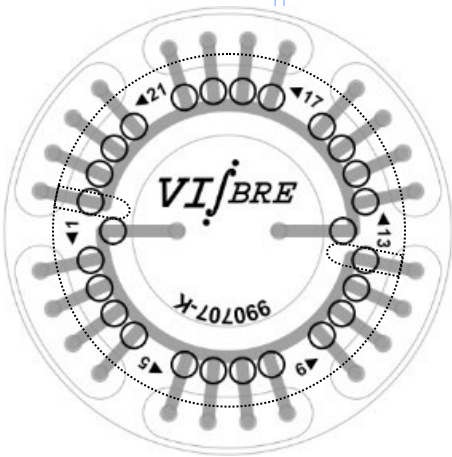
2E4 dynamic range

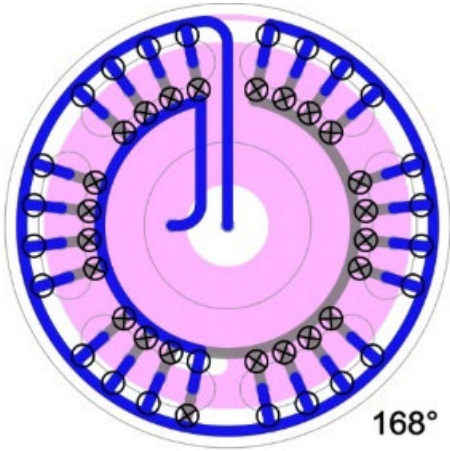
Multi-Organ Sampling System – Funded concept



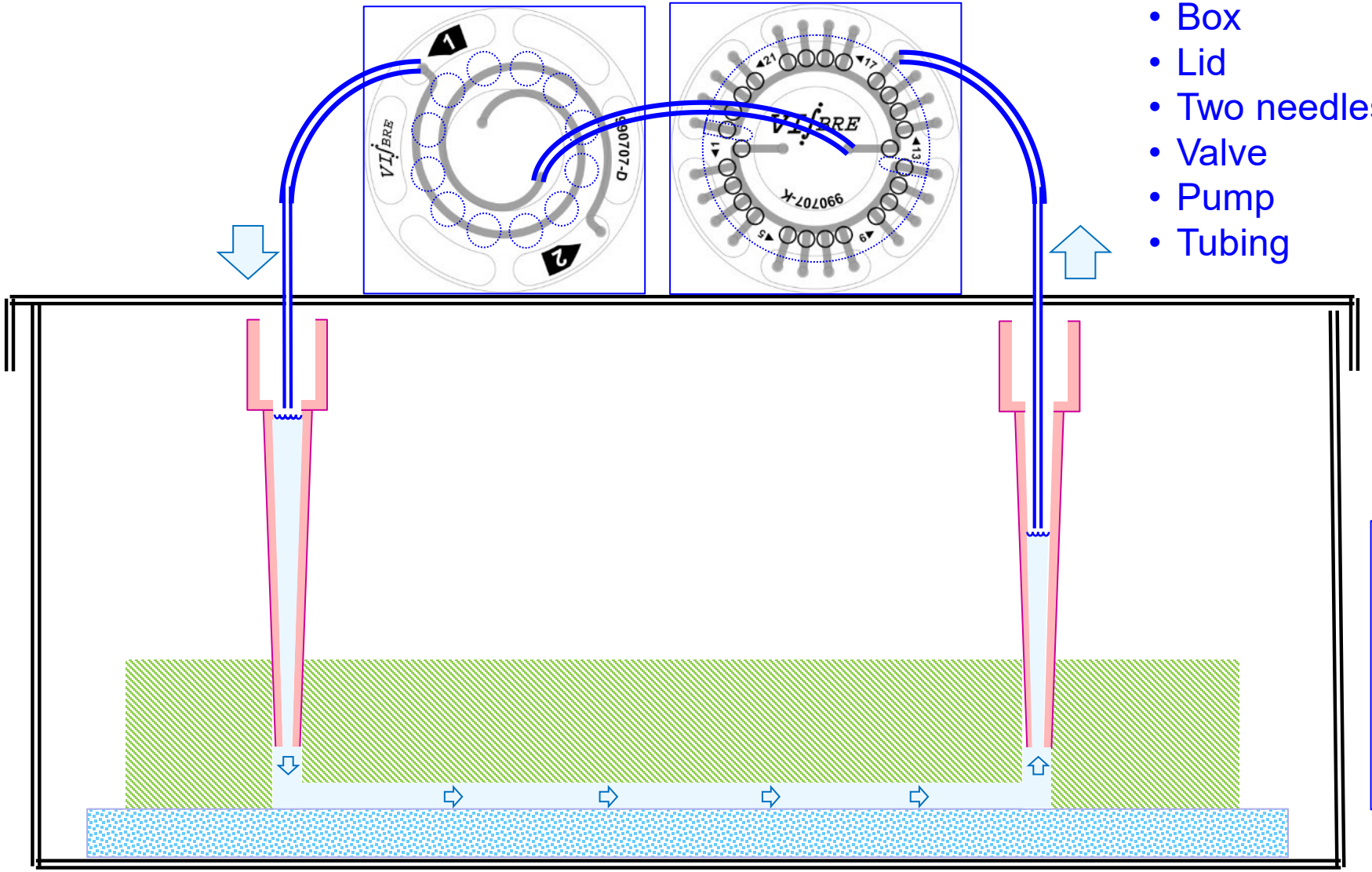








Multi-Organ Sampling System - Under development



- Box
- Lid
- Two needles
- Valve
- Pump
- Tubing

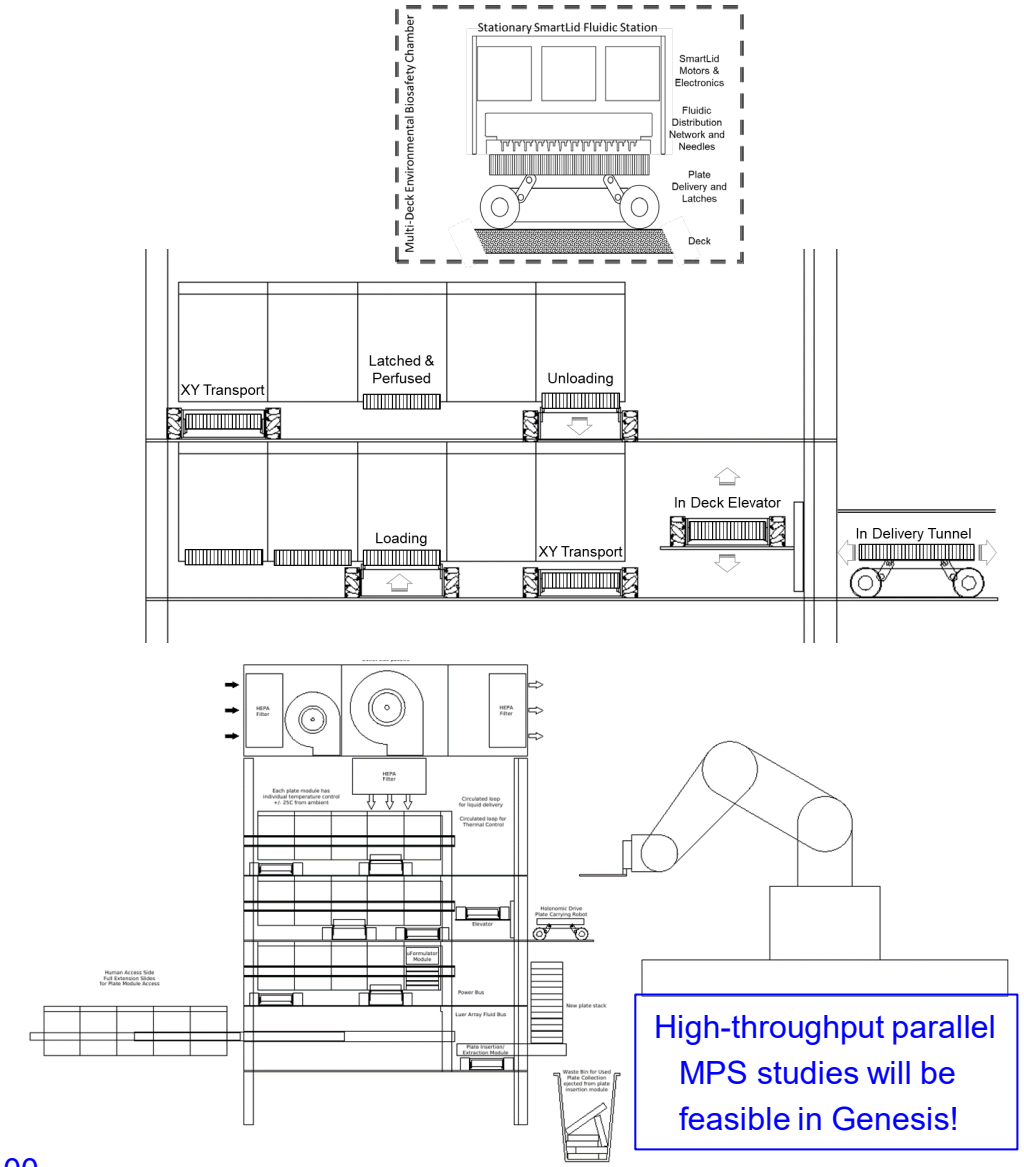
If you can do this for one chip, Genesis can do it for hundreds and even thousands, all controlled by a Robot Scientist.

The hybrid pumped/gravity feed may be the best of both worlds.

Genesis: high throughput biology for a wide variety of samples

- 42 U telecom/server rack with HEPA filtering, thermal control, and iPlateBot access to every plate station on each deck.
- 1.0 mL stirred chemostats in a 48-well plate
  - 48 chemostats per chemostat plate
  - 3 plates per module (input, chemostat, output)
  - 4 modules per deck
  - 9 decks per rack
  - 1,728 chemostats per rack, or
- Gravity perfused organ chips
  - 2 organ-chips per plate
  - 12 plates per deck
  - 9 decks per rack
  - 216 chips per rack
  - If redesign for 12 per plate for 1,296 per rack, or
- 200 µL perfused wells in a 96 well plate
  - 96 wells per plate
  - 1 plate per module
  - 12 modules per deck
  - 9 decks per rack
  - 10,368 perfused wells per rack, or
- 24-well Transwells
  - 24 wells per plate
  - 1 plate per module
  - 12 modules per deck
  - 9 decks per rack
  - 2,592 perfused Transwells per rack, or
- 12 well Transwells with zebrafish
  - 5 zebrafish per well
  - 12 wells per plate
  - 12 plates per deck
  - 9 decks per rack
  - 6,480 zebrafish per rack

Pump-pressurized lids will enable perfusion heights greater than 10 mm (>100 Pa) and will minimize endothelial/astrocyte overgrowth



iPlateBots and Genesis are ideal for BSL-3 and BSL-4

Patents issued and pending

How do we design the experiments?

What do we do with the terabytes of data?

Artificial Intelligence: The future of biology

Five types of artificial intelligence (AI) / machine learning (ML)

- Symbolic, e.g., Ross King and abductive reasoning models
- Connection, e.g., neural nets and deep learning
- Evolutionary, e.g., Hod Lipson and genetic programming
- Bayesian, e.g., probabilistic inference
- Analogy, e.g., support vector machine

Each has its strengths and weaknesses for Genesis:

- Genesis is using symbolic AI to design optimal experiments.
- It could use genetic programming to optimize protocols without models.
- As soon as Genesis has statistical data and/or prior knowledge, it can use Bayesian approaches.
- As soon as there are correlative data, it will readily use deep learning and support vector machines.

Dennis Bray understands the problem…

- “The past few decades have seen such an explosion of knowledge about the contents of living cells that we now swim in an ocean of data.”

D. Bray. Reductionism for biochemists: how to survive the protein jungle. *Trends Biochem.Sci.* 22 (9):325-326, 1997.

- “How can we come to terms intellectually with such an enormous number of interacting entities?”

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A possible failure mode

Ontological failure: The phenomenon you are interested in requires elements or laws outside of the set you have been given.

D. Bray. Reductionism for biochemists: how to survive the protein jungle. *Trends Biochem.Sci.* 22 (9):325-326, 1997.

94

The solution to ontological failure

Get more data…

95

A possible failure mode

Ontological failure: The phenomenon you are interested in requires elements or laws outside of the set you have been given.

There is a second possible failure mode

Epistemological failure: You have enough elements and the laws do apply, but you yourself cannot understand the explanation that they provide.

D. Bray. Reductionism for biochemists: how to survive the protein jungle. *Trends Biochem.Sci.* 22 (9):325-326, 1997.

96

The solution to epistemological failure

Get a smarter, bigger brain…

97

There is yet one more potential problem…

- We may not be able to understand what the computer tells us about biology.
- The next challenge is to create computers that can explain their findings to us…

- It might be as hopeless as explaining Shakespeare to a dog.

Hod Lipson, 2009

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All may not be lost

See Spot Read: Willow the Dog understands written commands

"….the dog can now sit up when a card says 'Sit Up,' [play] dead when a card reads 'Bang,' and wave a paw when a sign says 'Wave.' "

http://www.peoplepets.com/news/amazing/see-spot-read-willow-the-dog-understands-written-commands/1

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Are there risks with AI and biology?

- Machine learning can identify or design toxic chemicals
- Robot scientists can test for or optimize toxicity
- Robot scientists can screen for or evolve pathogens

Dual use of artificial-intelligence-powered drug discovery

Fabio Urbina, Filippa Lentzos, Cédric Invernizzi and Sean Ekins

Nature Machine Intelligence, 4: March 2022, pp 189–191

"An international security conference explored how artificial intelligence (AI) technologies for drug discovery could be misused for de novo design of biochemical weapons. A thought experiment evolved into a computational proof."

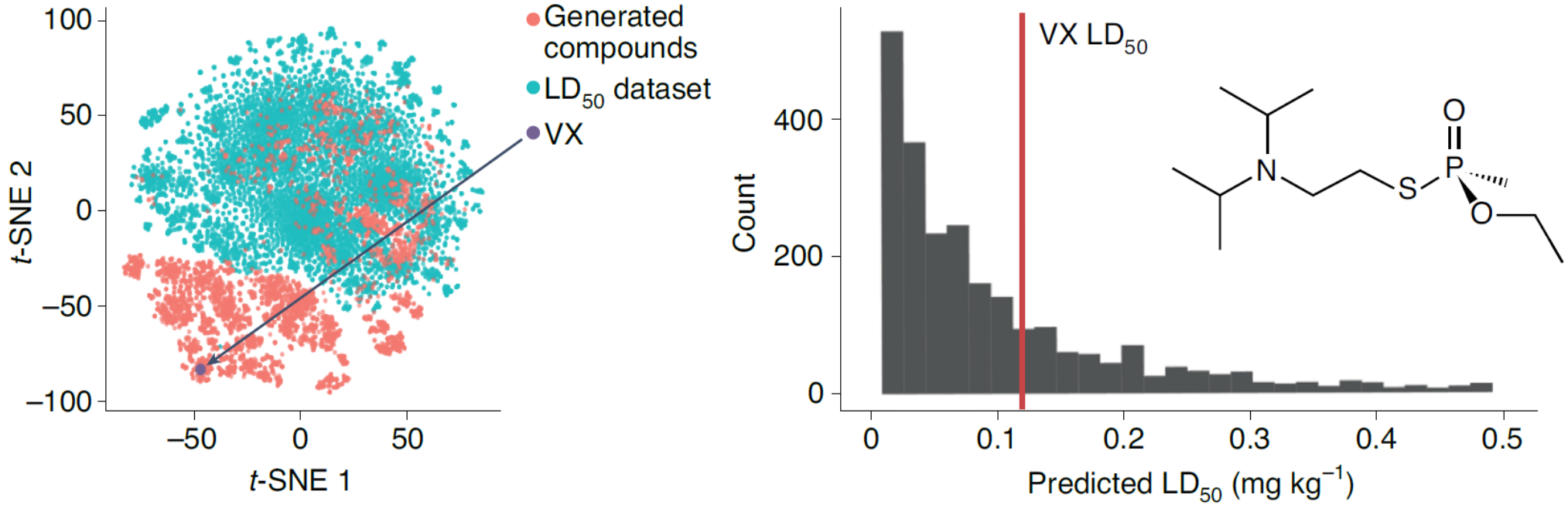
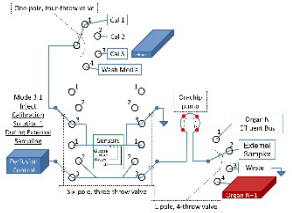


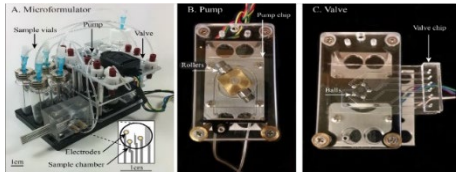
Fig. 1 | A *t*-SNE plot visualization of the LD_{50} dataset and top 2,000 MegaSyn AI-generated and predicted toxic molecules illustrating VX. Many of the molecules generated are predicted to be more toxic in vivo in the animal model than VX (histogram at right shows cut-off for VX LD_{50}). The 2D chemical structure of VX is shown on the right.

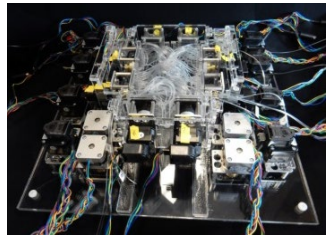
The full automation of biology will present ethical challenges.

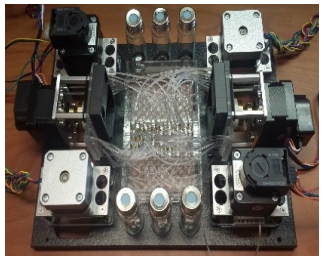
We need to address them at the outset and design in safeguards.

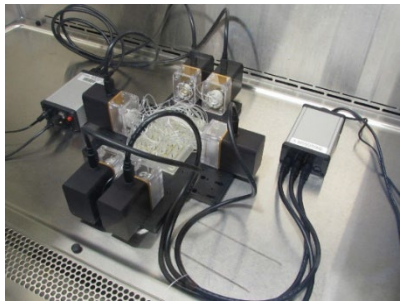
A decade of progress …



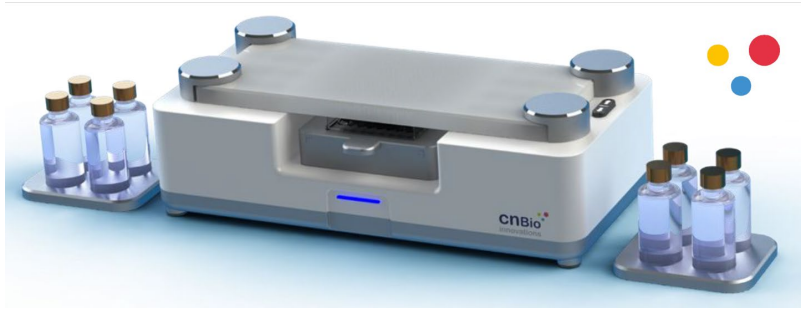


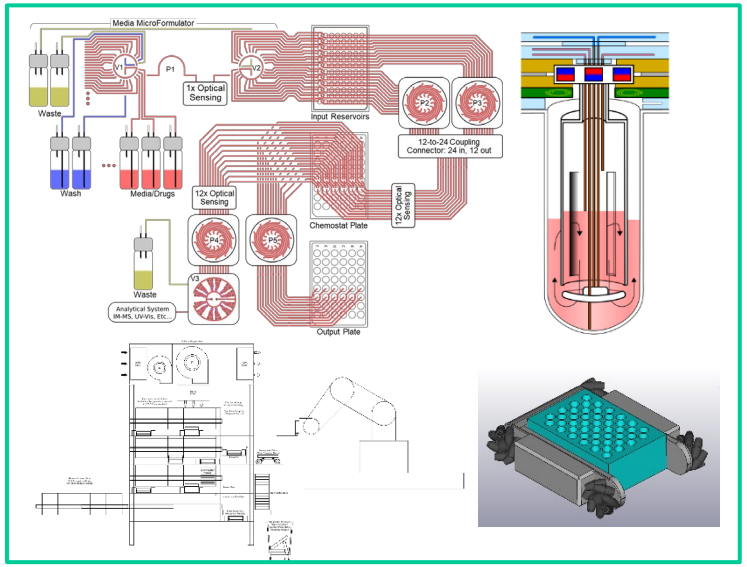






Today!





MicroPhysiological Systems need MORE engineering!

Tomorrow

Genesis: A self-contained, integrated, intra-incubator, high-throughput, fluidic experiment control system for BSL 2, 3, and 4 and robot science

An alternative to large, room-sized systems that rely on daily media changes and transport of plates or organ chips between incubator, fluid handler, and plate reader:

- Multi-channel microfluidic WiFi pumps and valves, and ribbon fluidics microformulate media for each well/chemostat/organ.
- SmartLid system provides continuous perfusion, PK and circadian profiles, metabolic sensing, and sample collection.
- Well plates, transwells, 48-well mL chemostats, perfusion microbioreactors, and gravity-perfused organ chips all have an ANSI/SBS well-plate footprint for transport and containment.
- Can connect to SPE-IM-MS metabolomics @ 10 s per well.
- SmartLid fluidic control modules are fixed above multiple decks within a vertical environmental chamber that provides biosafety containment, temperature, and humidity control.
- Holonomic iPlateBot well-plate transporter delivers a well plate or organ chip array to a stationary plate station, lifts it and latches it in place, and departs for another assignment.
- iPlateBots pose no mechanical risk to human operators.
- iPlateBot can deliver different SmartLids to a station for different configurations and well or organ layouts.
- iPlateBots can move through aseptic tunnels between workstations and enclosures.
- Refreshed/recirculated gravity perfusion of organ chips eliminates the bubble problem and enables massive parallelization.
- Bag-based perfusion fluid delivery.
- On-demand ImagingBot visits well plates as needed.
- Can have seeding/infection stations within the enclosure.
- Plate can be autoclaved at the end of the experiment without otherwise leaving the enclosure.
- Chamber can be UV and gas sterilized.
- Can be configured at user appropriate scale, from single deck with 12 plates operating in a containment hood to a self-enclosed rack with 9 decks and 10,368 perfused wells.
- Can operate as a Robot Scientist for automated design and execution of experiments.