

Bioprinting at the Naval Research Laboratory

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What is bioprinting?

- Bioprinting is the controlled deposition of any living or non-living biological (DNA, protein, bacteria, mammalian cells, viruses) material into a computer-aided design or pattern
- Pattern resolution (spot size) can vary from a continuous sheet (macroscale, e.g. skin) to 10's of microns (microscale, e.g. microcapillaries & vessels)
- Deposition speeds can exceed 1000 droplets per second (high throughput)
- Deposition volumes range from 10^{-3} mL to 10^{-10} mL per printed droplet
- 2D (single layer) and 3D (multi-layer) applications

Potential for Bioprinting to have Broad and Far-Reaching Effect Across Biotechnology Sectors

○ Healthcare

- Transplants and regenerative medicine applications (pancreatitis, type I diabetes, parathyroid transplant, hepatic tissue transplantation, renal transplants, lung transplants, heart transplants, skin)

- Infectious disease modelling *in vitro*

○ Pharmaceutical

- Drug testing/screening (toxicity, efficacy)
- Biomarker discovery (diagnostics, vaccine and therapeutics)
- Oncology (tumor models, efficacy, biomarker testing/screening)
- Replace and/or supplement (pre-screen) animal models

○ Medical Devices

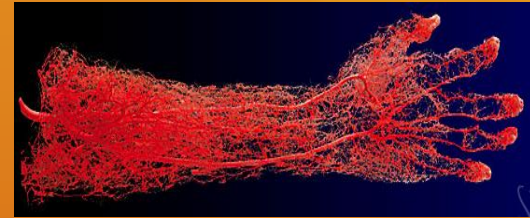
- Hybrid/artificial heart
- Prosthetics/implants

○ Defense and Homeland Security

- Bio-artificial limbs
- Chem/Bio assessment *in vitro* tissue models (human clinical trials impossible)
- Agnostic chemical threat sensors (based on 3D bioprinted cellular systems)

○ Cell Sourcing

- Automate and scale up cell production for *in vitro*/*in vivo* tissue/organ applications



MRS BULLETIN OCTOBER 2013 ISSUE

TECHNICAL FEATURE

CELL AND ORGAN PRINTING TURNS 15: DIVERSE RESEARCH TO COMMERCIAL TRANSITIONS

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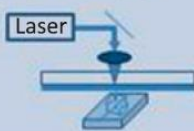















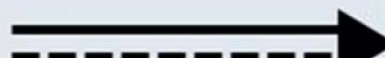
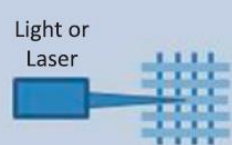

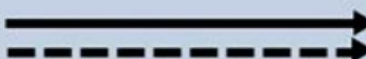
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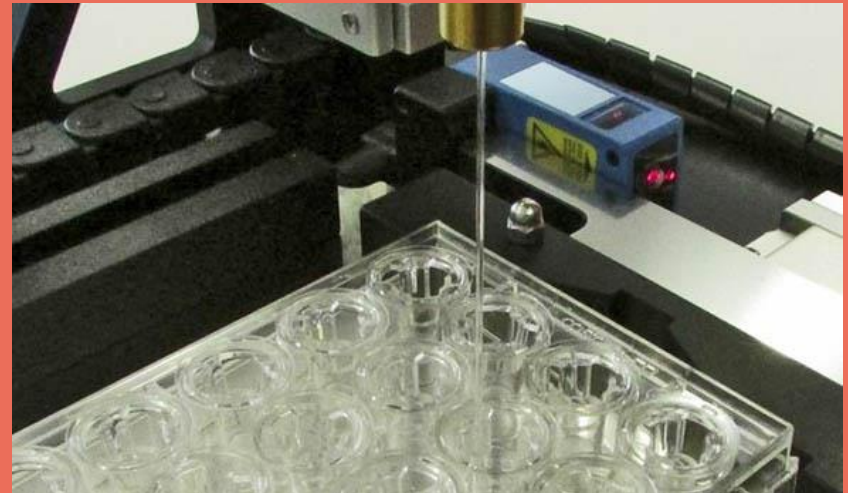
^{A8} TULANE UNIVERSITY; DOUGLASBCHRISEY@GMAIL.COM

MRS Bulletin, **38**, pp. 834-843 (2013)

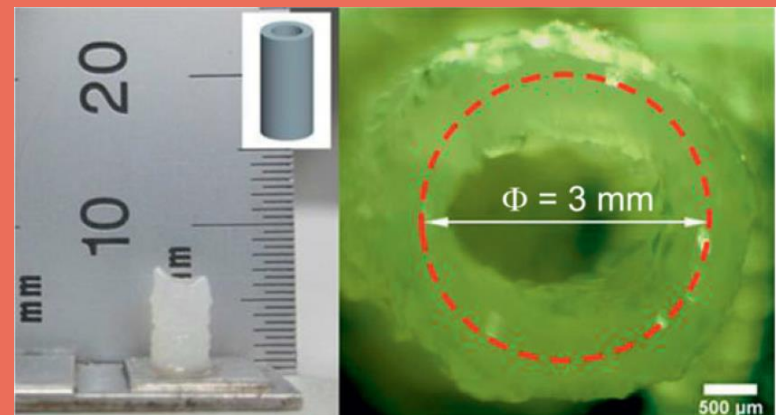
Table I. Comparison between different cell printing tools.

Printing Tool	Capabilities
Laser-Induced Forward Transfer 	 Layer-by-Layer  Scaffold + Cells 
Ink Jet 	 Layer-by-Layer  Scaffold + Cells  Cell Aggregates 
Electrospray 	 Layer-by-Layer  Scaffold + Cells 
Extrusion Pen 	 Cell Aggregates  Scaffold + Cells 
Photo-Polymerization 	 Scaffold Only 

The NovoGen Bioprinter™ fabricating tissue into a 24-well plate (organovo.com)

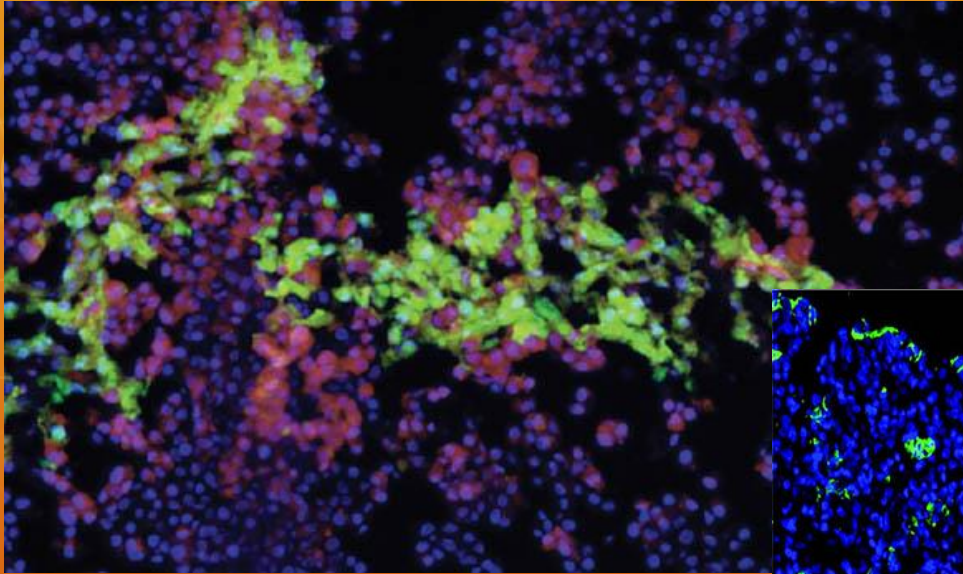


Laser Printed 3D Vessel Scaffold (MRS Bulletin, 38, pp. 834-843 (2013))

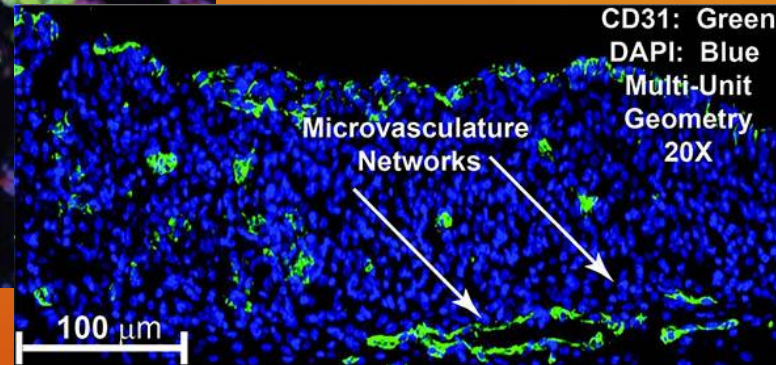


Examples of Commercial 3D Bioprinting

○ Organovo



3D "Liver" Tissue showing limited heterogeneity and vasculature



<http://www.organovo.com/tissues-services/3d-human-tissue-models-services-research/tissue-models/3d-human-liver-tissue-model>

○ Aspect Biosystems



<http://3dprint.com/16267/aspect-biosystems-3d-bioprint/>

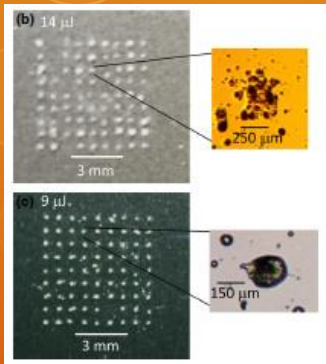


NRL's Bioprinting Tool- BioLP

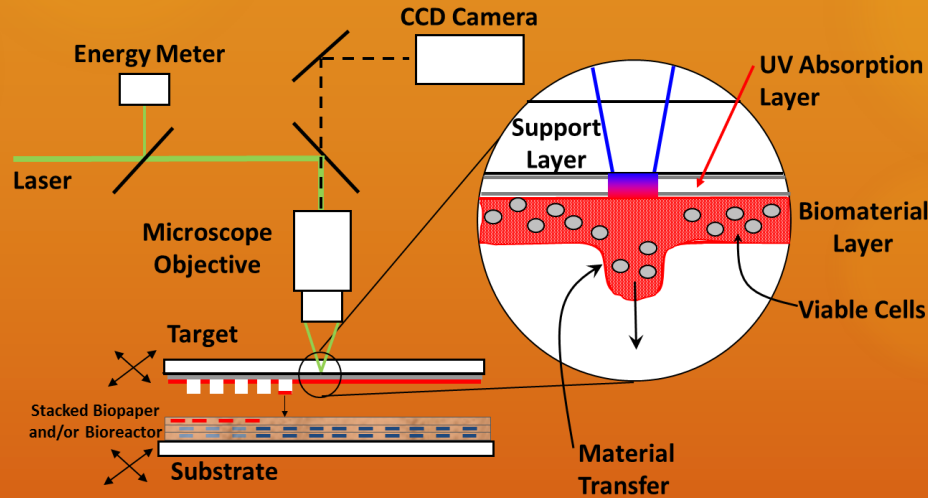


Biological Laser Printing, or BioLP, is NRL's patented laser direct write tool for creating 2D and 3D patterns of almost any biomaterial including living cells (bacteria, mammalian), soil/sediment, hydrogels and biomolecules

World's First Printed Soil Microarray



Ringeisen, et al. *Methods in Ecology and Evolution* **6** (2), 209-217 (2015)



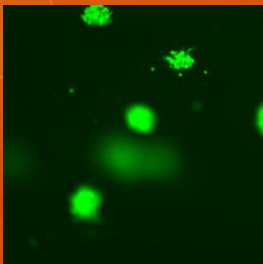
US Pat. #7,294,367; #7,381,440; #7,875,324, #8,669,086

World's First Printed Viable Bacterial Colony



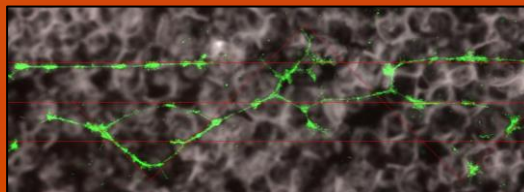
Ringeisen, Jones-Meehan, Spargo. *Biomaterials* **23**, 161-166 (2002)

Printed 3D Mammalian Cell Patterns



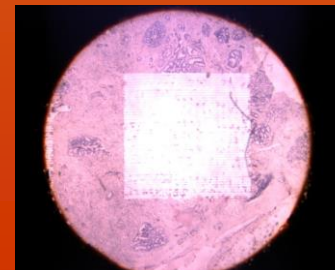
Barron, et al. *Biomed Microdevices* **6**, 139-147 (2004)

Printed Microvasculature on Polymer/Gel Biopaper



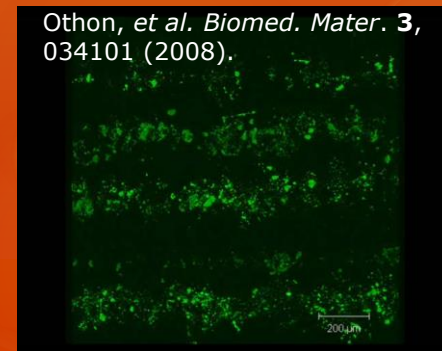
Pirlo, et al. *Biotechnol. Bioeng.* **109**, 262-273 (2012)

Licensed for Tissue Microdissection



Hood, et al. *Molec. Cell. Proteomics*, **4**, 1741-1753 (2005).

Printed 3D Nerve Conduits

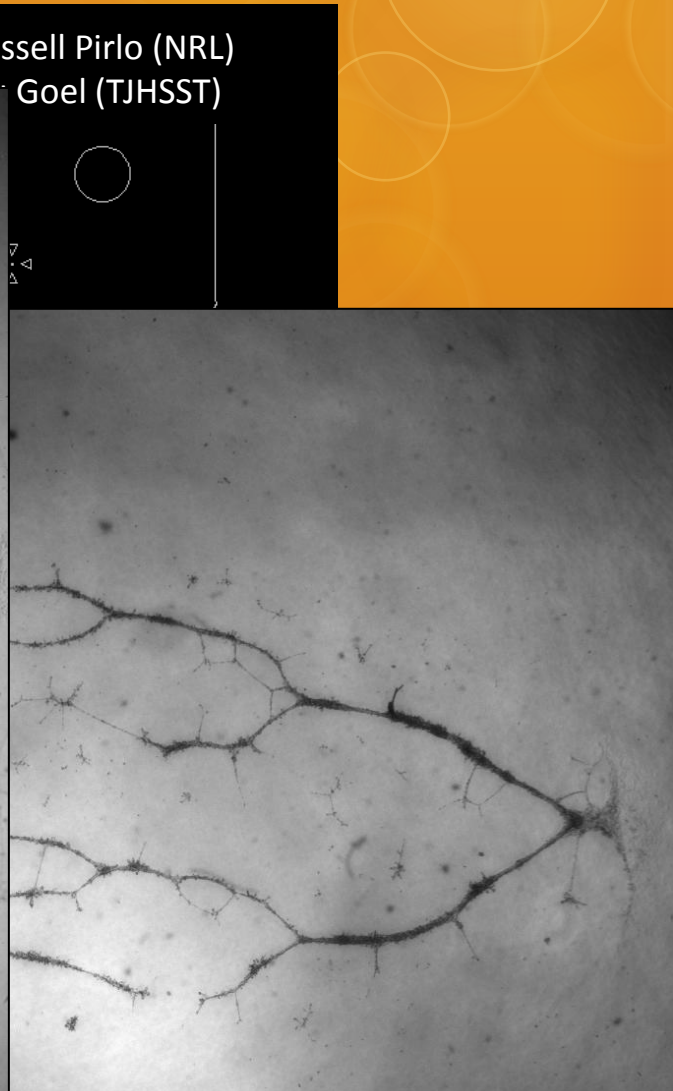
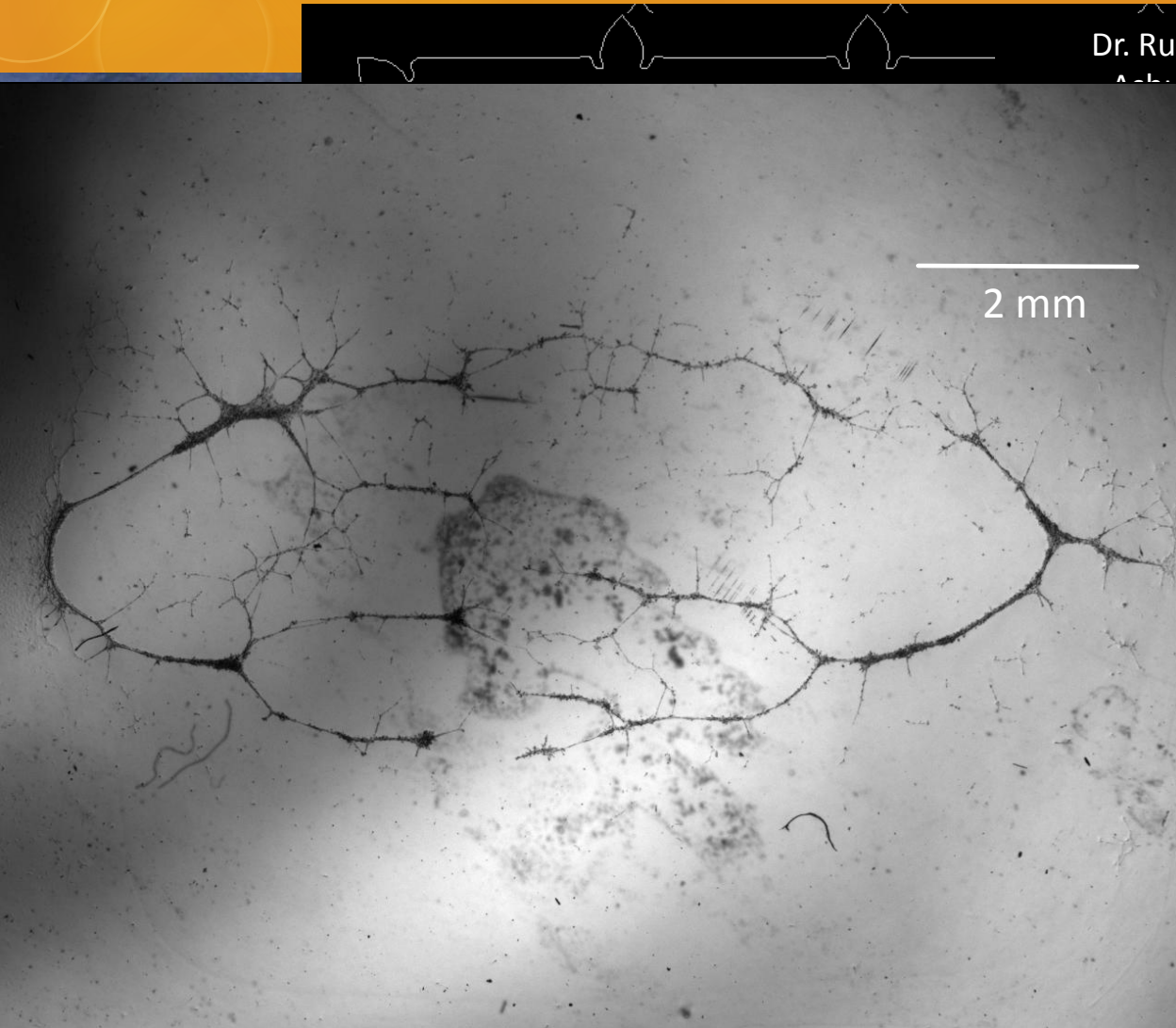


Othon, et al. *Biomed. Mater.* **3**, 034101 (2008).

Recent Advances: Printing to Biopaper and Creating Complex Vascular Designs



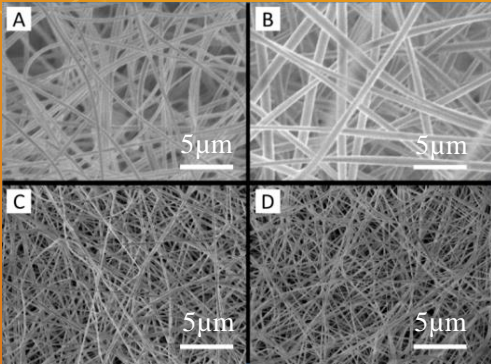
Dr. Russell Pirlo (NRL)
Ashu Goel (TJHSST)



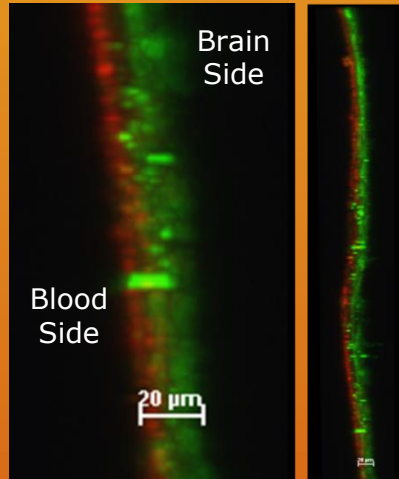


Recent Advances: Blood-Brain-Barrier (BBB) Tissue Model and Infection

NRL Human BBB Tissue Model

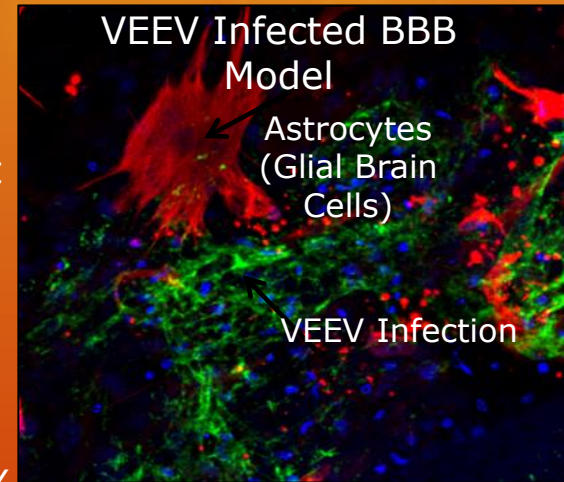


NRL Gelatin Biopaper
U.S. Patent #8,669,086



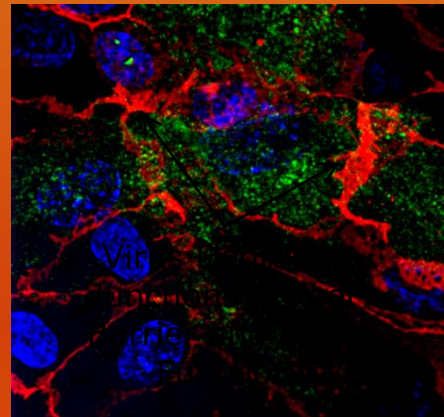
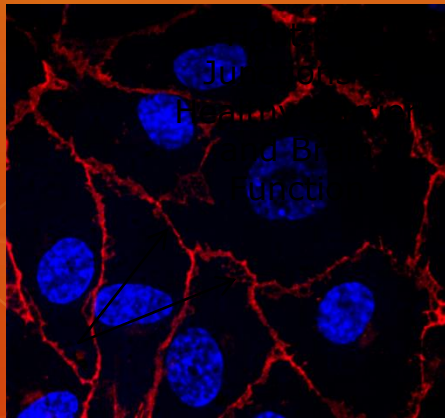
NRL
Bioreactors
Enable
Transport to
Ft. Detrick,
MD

→
Venezuelan Equine
Encephalitis Virus
(VEEV) Infection at
USAMRIID



←
Blood-Brain-Barrier integrity degrades after 24 hours post-VEEV infection

Dr. Russell "Kirk" Pirlo (NRL)
Dr. Lauren Bischel (NRL)
Dr. Connie Schmaljohn (USAMRIID)
Dr. Shannon Taylor (USAMRIID)



L.L. Bischel, P.N. Coneski, J.G. Lundin, P.K. Wu, C.B. Giller, J. Wynne, B.R. Ringeisen, R.K. Pirlo. *Journal of Biomedical Materials Research - Part A*, in press.

For Official Use Only





NRL's 3D Bioprinting Center

A collaborative DoD facility that uses a multi-disciplinary approach to advance fundamental science and derive solutions in energy, diagnostics and medical countermeasures

Soil and Sediment Printing



Core Soil Sample

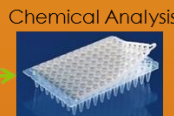


Soil Microniche

Soil Printing



Culture



Chemical Analysis



Next Gen Sequencing

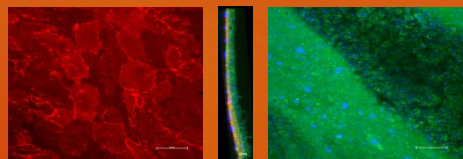
- Culturing the unculturables
- Bioprospecting natural products for energy and medical countermeasure applications

High Throughput Analysis and Culture

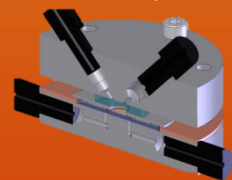


In vitro Tissue to Study Bio-Agent Infection

Engineered Air/Lung Tissue Interface



Bioreactor for Aerosol Exposure



- Lung and blood-brain-barrier tissue models
- Studying viral and bacterial bio-agent infections
- Faster and more accurate agent detection, diagnosis and treatment



3D Bioprinting Consortium

- Multi-user facility
- Focal point for inter-DoD collaborations
- Hearing loss, TBI, chem/bio agent defense, radiation exposure, trauma-induced arthritis
- Validation of DoD-funded bioprinting programs

