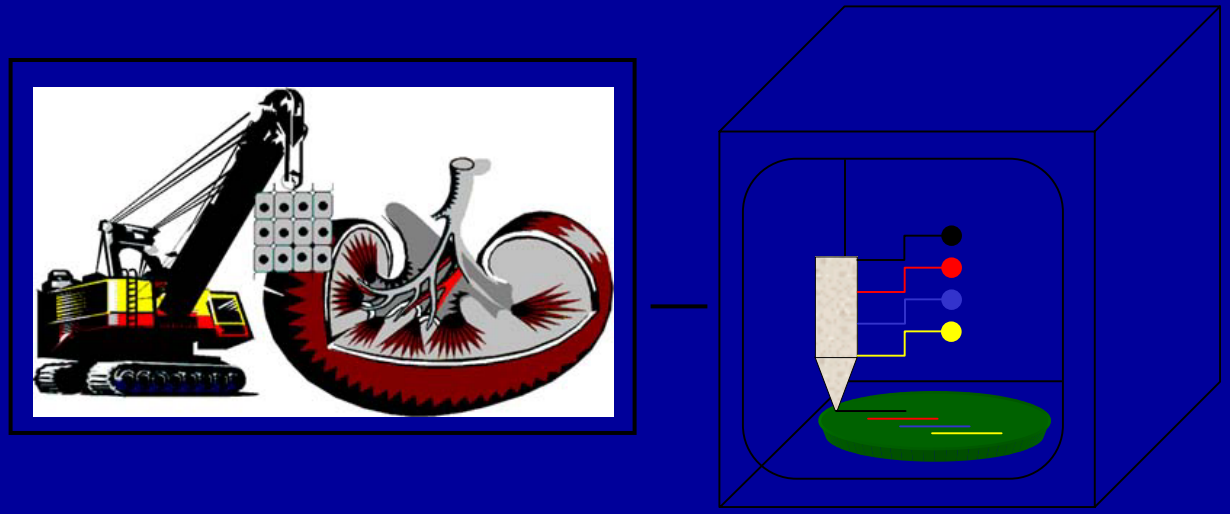
Rapid Prototyping of Living Biological Systems by Direct Write

From Designs on a Computer to Building Life



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Naval Research Laboratory, Washington, D.C.

12 August, 2004

NSF/WTEC Workshop on Micro-Manufacturing

Template Slides

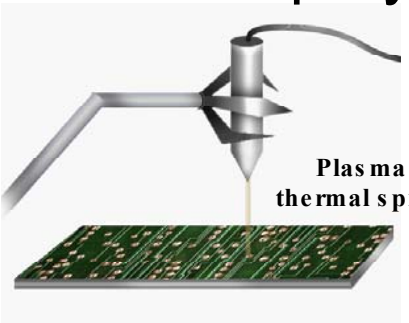
- Naval Research Laboratory, www.nrl.navy.mil
- Navy's Corporate R&D Laboratory
- State-of-the-Art: Presentation, Competitors Similar, But Different Areas and Materials
- Scientific/Technological Barriers: None, True 3-D
- Commercial Barriers: Visibility and Demonstration of Capabilities
- Site and Issues to Be Explored: Similar R&D in US and Europe

Goals of This Presentation

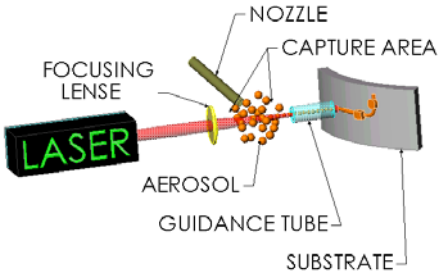
- *Direct Writing* is a Novel Approach to Fabricate Biological Micro-Manufacturing Systems
  - Use Evolutionary Genetic Machinery
- New Approach to Process Biomaterials – MAPLE DW
  - Proteins, Antibodies
  - Prokaryotic and Eukaryotic Cells
  - Extra Cellular Matrix
  - Scaffolding
  - Growth and Recruitment Factors

Different Direct Write Methods

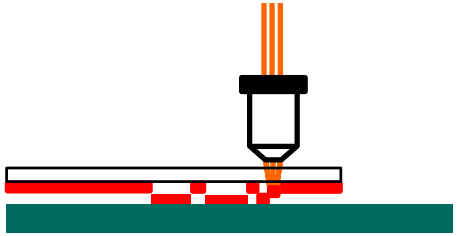
Plasma Spray



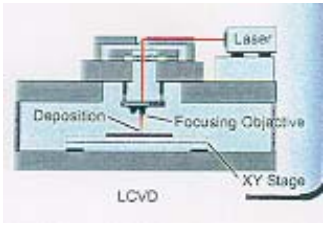
Laser Particle Guidance



MAPLE DW

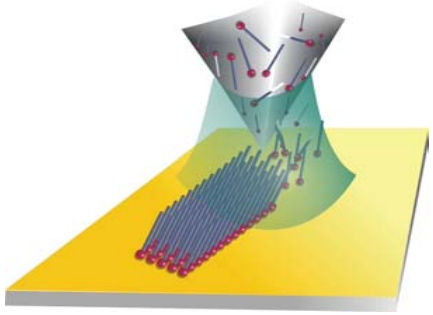


Laser CVD

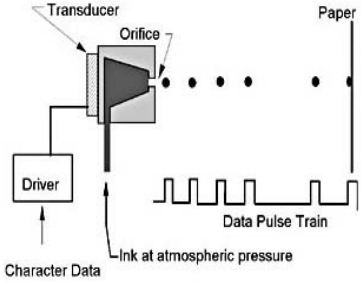


MicroPen

Dip-Pen

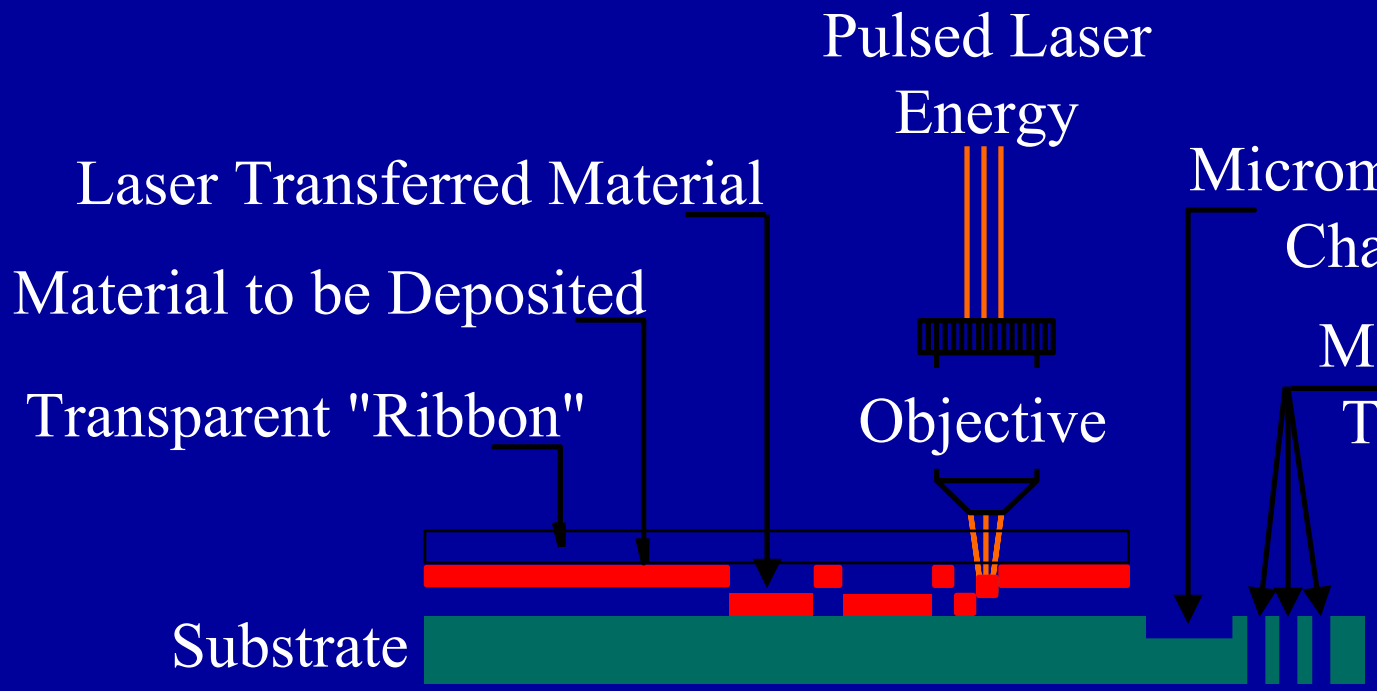


Ink Jet

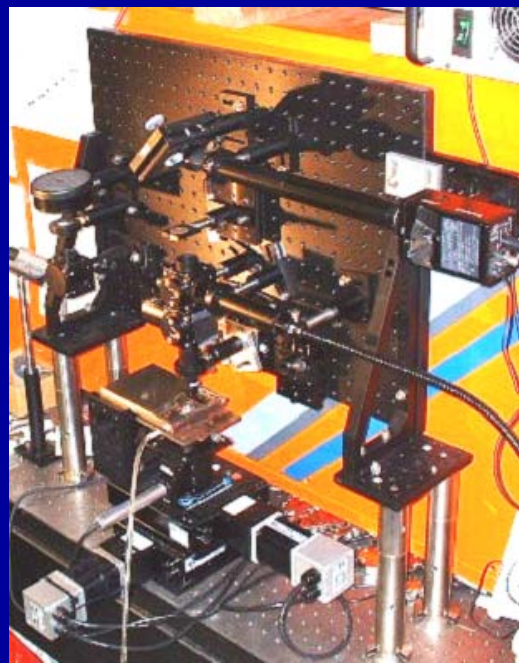


Many Different Approaches Exist to DW Material Patterns. Each Technique Has its Own Merits and Shortcomings.

Matrix Assisted Pulsed Laser Evaporation Direct Write (MAPLE DW)



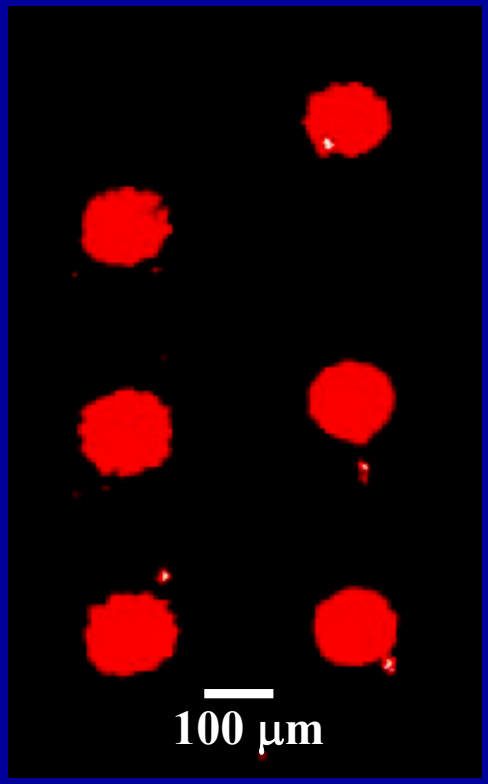
Done Under Ambient Conditions!



MAPLE DW of Active Proteins

Advantages Over Conventional Techniques

- Works With ANY Starting Material (Viscosity, Powders, Varying Conditions)
- Reproducibility
- CAD/CAM
- Reduced Spot Size <10 μm
- Increase Material Utilization/Efficiency by 10^5
- Works on Planar Substrates and Microwells
- One System Does Complete Array of Multiple Array Elements



Single Element Antibody Microarray (Anti-BSA)

DNA as Biomolecular Template: Integration into Microelectronic Molecular Arrays

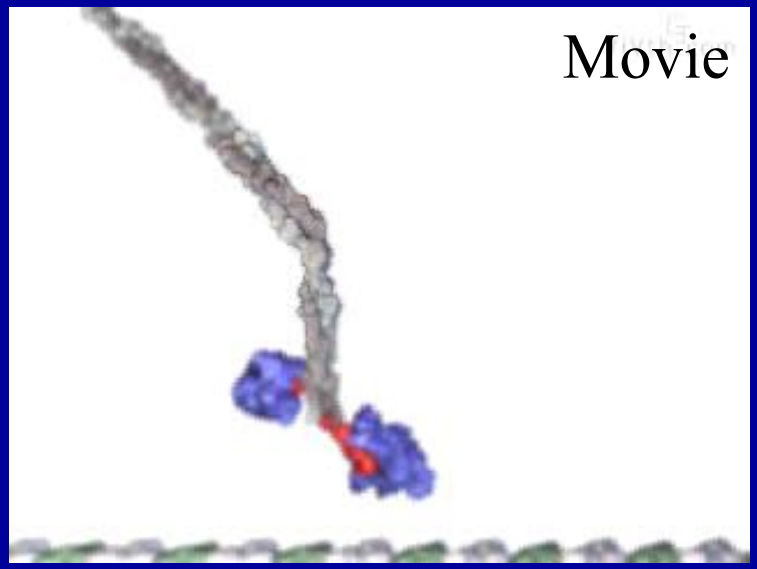
MAPLE DW Streptavidin (10 um Spots)

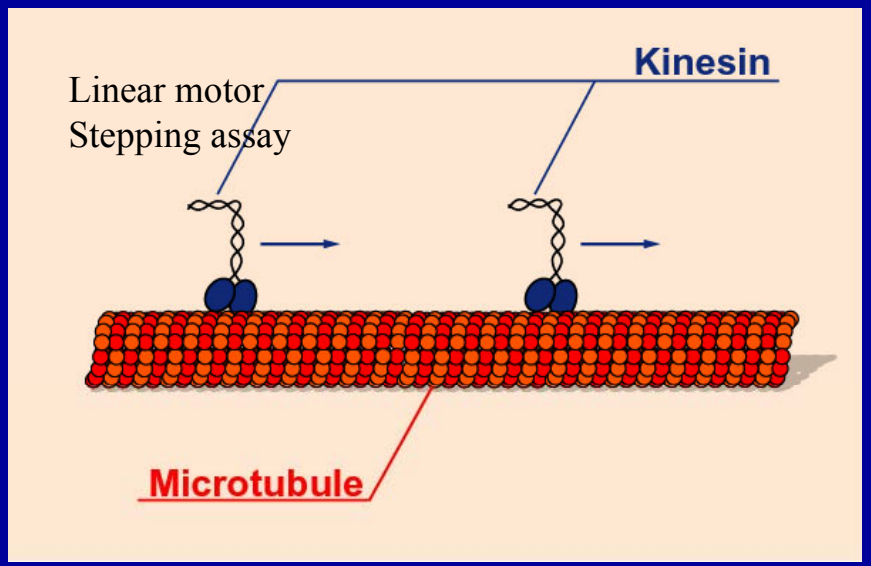
- Position DNA on Patterned Proteins, e.g., Biotin/Streptavidin
- Manipulation with Motor Proteins (or Flow Assay) and Microtubules by Bridging Adjacent Protein Patterns
- Metallization of the DNA Wire-like Geometry
- CAD/CAM Direct Writing of Template Proteins Increases the Complexity Including 3-D Sophisticated Architectures

Paradigm Shift: Mescopic Fabrication Yields Nanoscopic Manufacturing

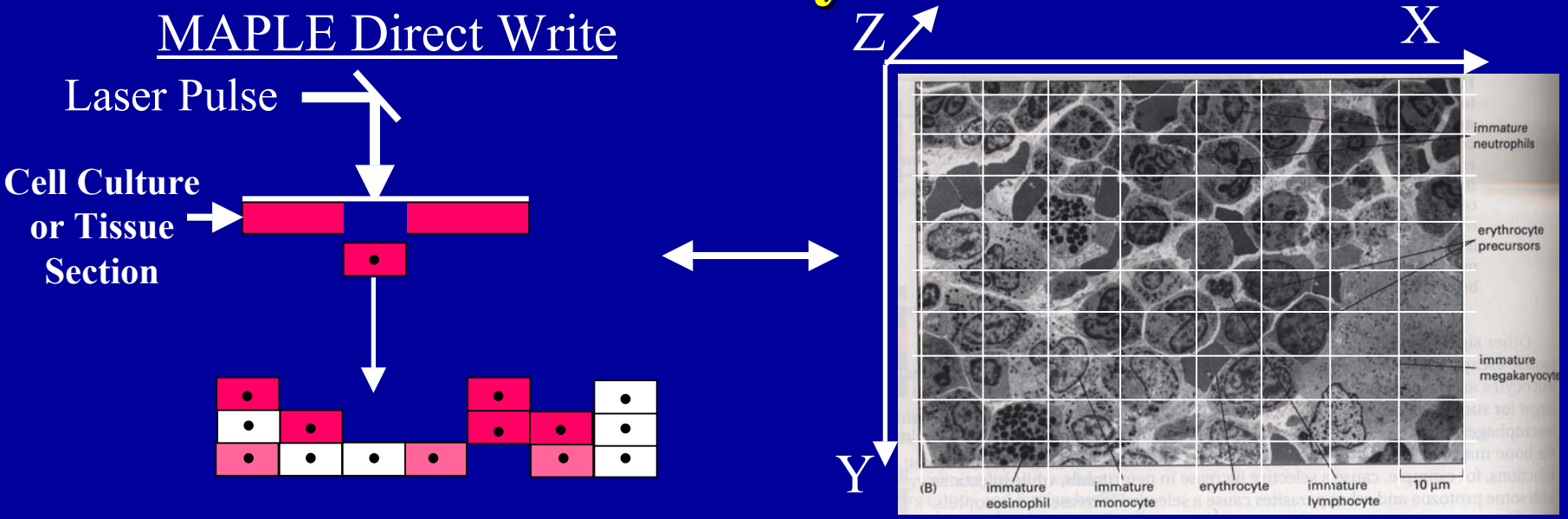
Microtubules as Active Molecular Decision Systems: Performing Positional 3-D Assembly

- Assembly, Transport, Delivery of Active Biomolecules to Predefined Pattern's Sets in a CAD/ CAM Manner by MAPLE DW in a 3D Aqueous Workspace
- Progressive Molecular Transport using the Evolutionary Machinery of the Cell (Motor Protein Kinesin and its Track, the Microtubule)





What if Tissue Could Be Constructed Cell-by-Cell?



- ***Is MAPLE DW Suited For Cell-by-Cell Tissue "Construction"?***

√ Rapid, Computer-Controlled Placement of Different Materials at 10 to 100 µm

√ Multiple Cell Types

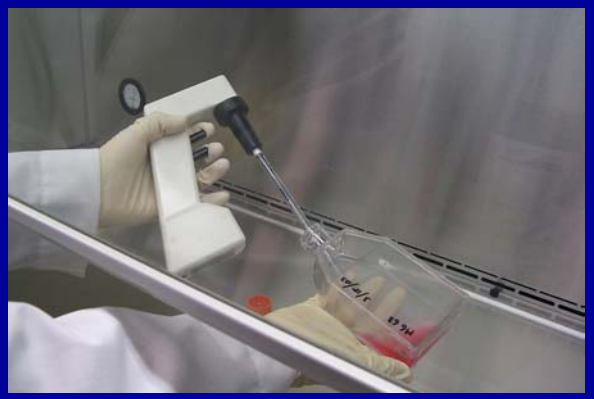
√ Molecules Like Growth Factors, Recruitment Factors, Differentiation Chemicals

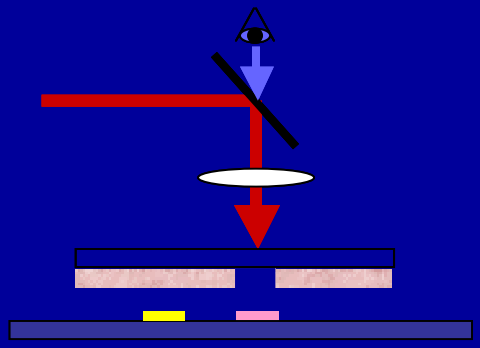
√ Novel Scaffolding Materials (Inorganic/Organic Composites)

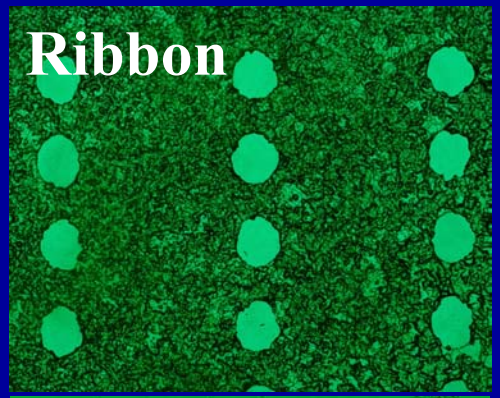
? Vascularization (Constructs >1 mm)

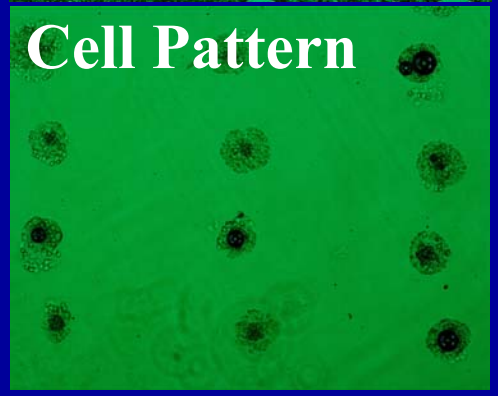
Paradigm Shift: To Go From Growing Tissues to Building Tissues

Cell Transfer by MAPLE-DW





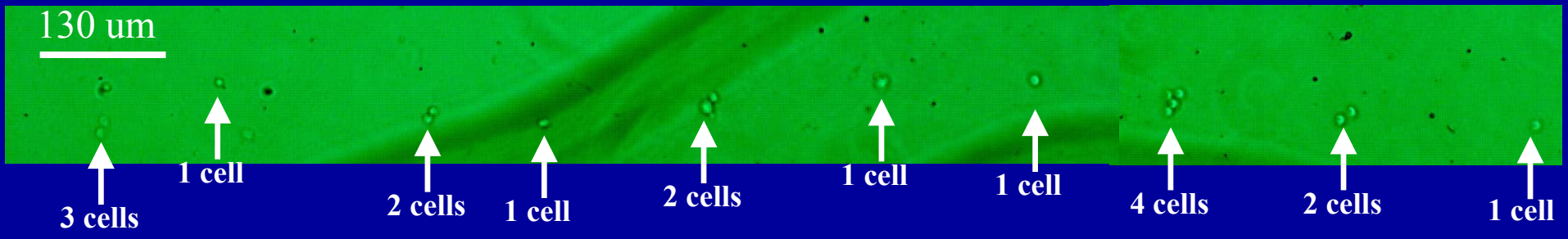




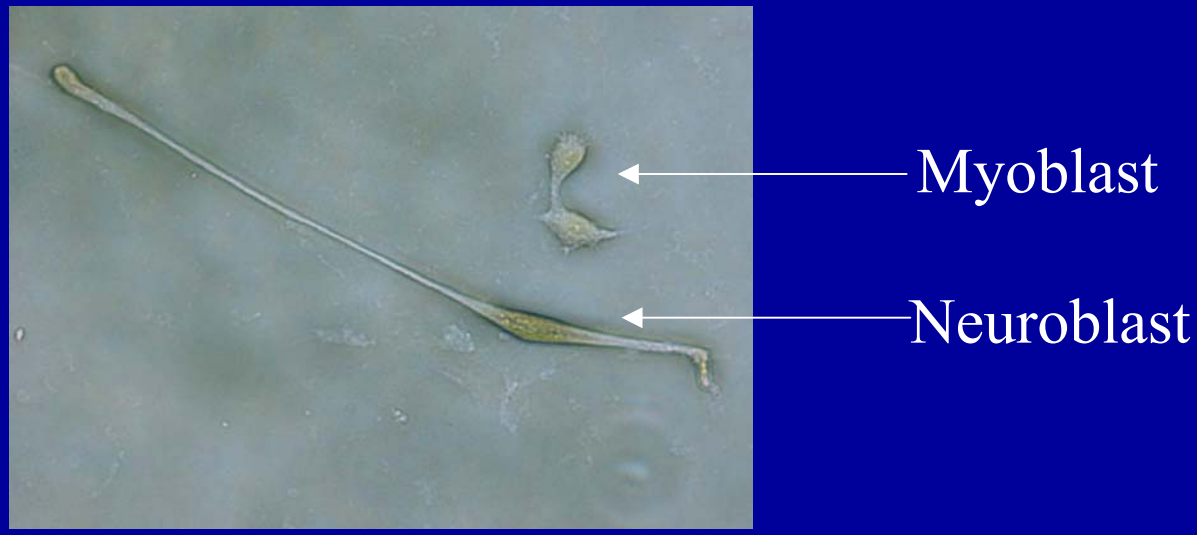
Preparation — Transfer — Analysis

Culture — < 1 msec — *Culture*

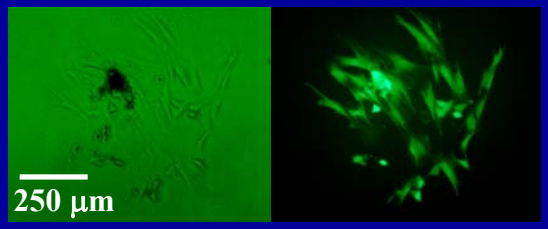
Single-Cell Resolution of Osteoblasts



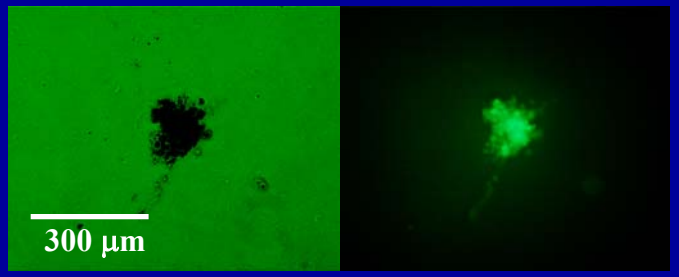
Single-Cell Resolution: Myo- and Neuroblasts



Single Shots and Multiple Shots of Rat Cardiac Cells



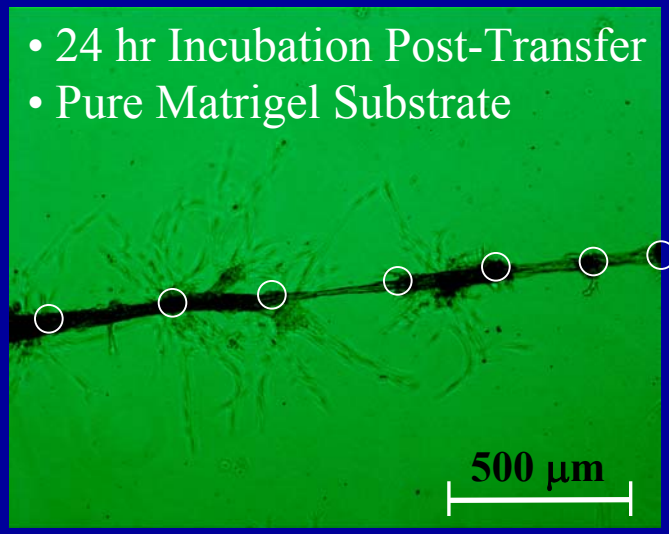
Single Shot; After 4 Days Culture Spread Over 700 Microns; 200 Micron Original Spot Size

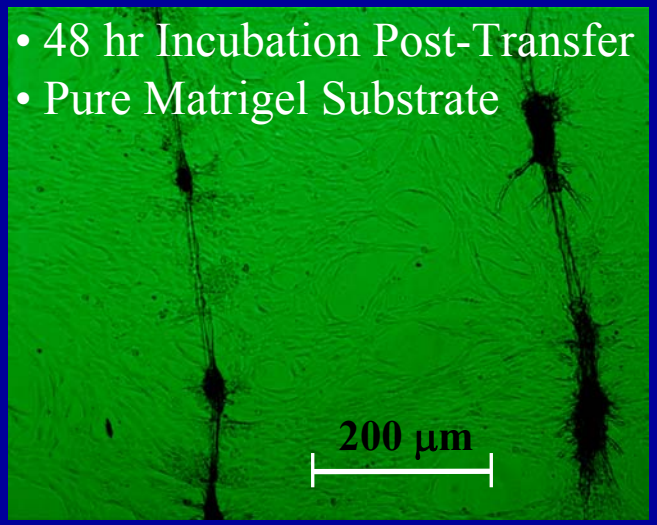


3 Shots; After 4 Days of Culture; Cells Did Not Spread; Bound in Cage/Matrix

Rat Cardiac Cells Behaved Differently Depending on the Local Environment!

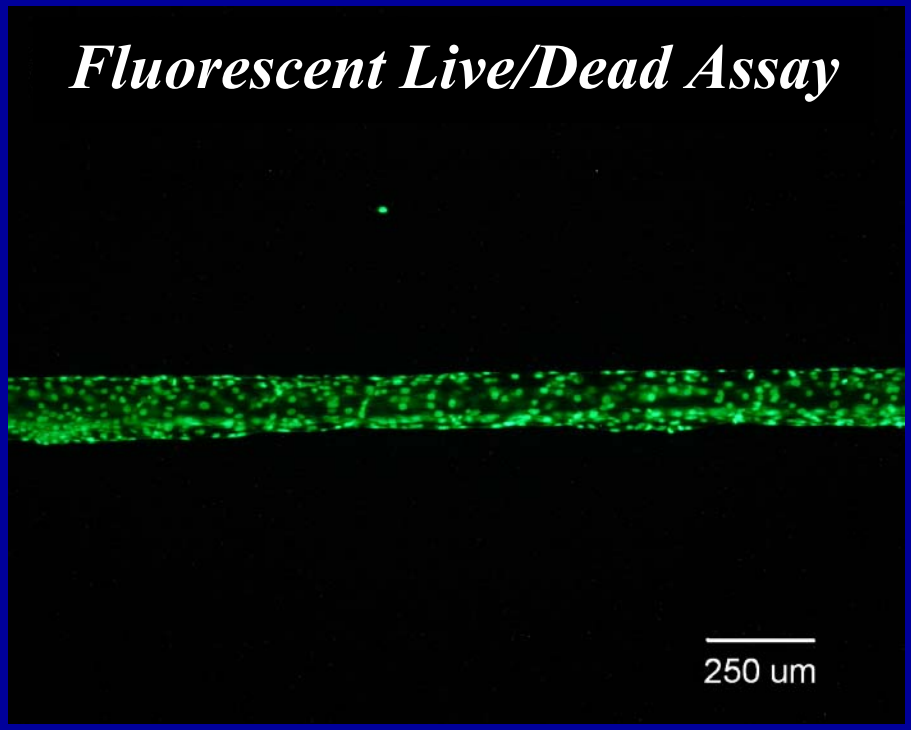
Forming Muscle-Like Structures Using MAPLE DW: Lines of Mouse Myoblasts

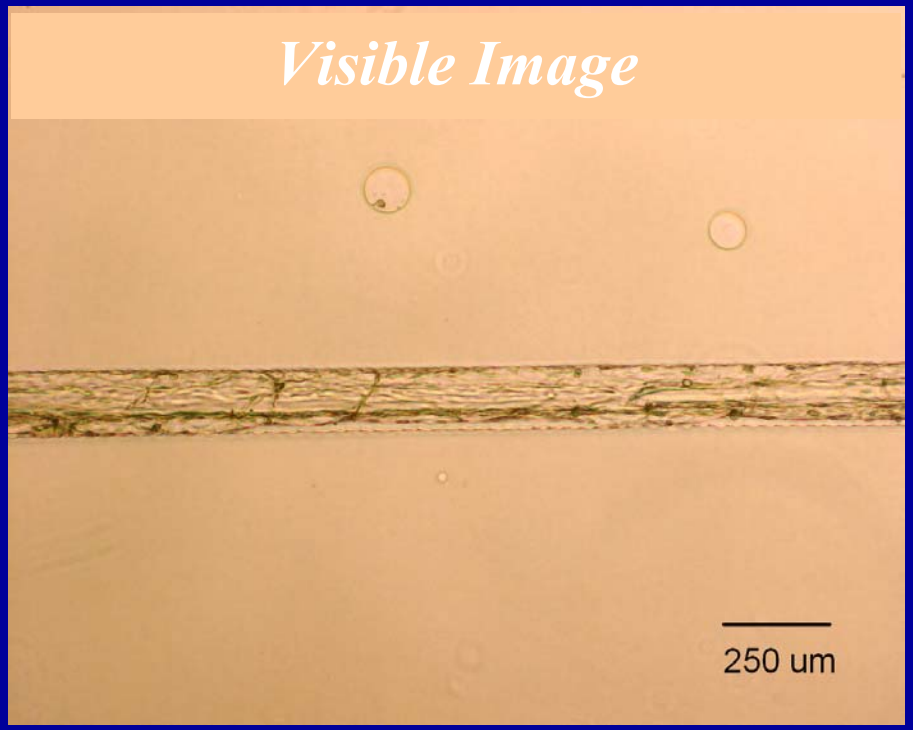




Mouse Myoblast Cell Spots Self-Formed Into 3-D Structures!

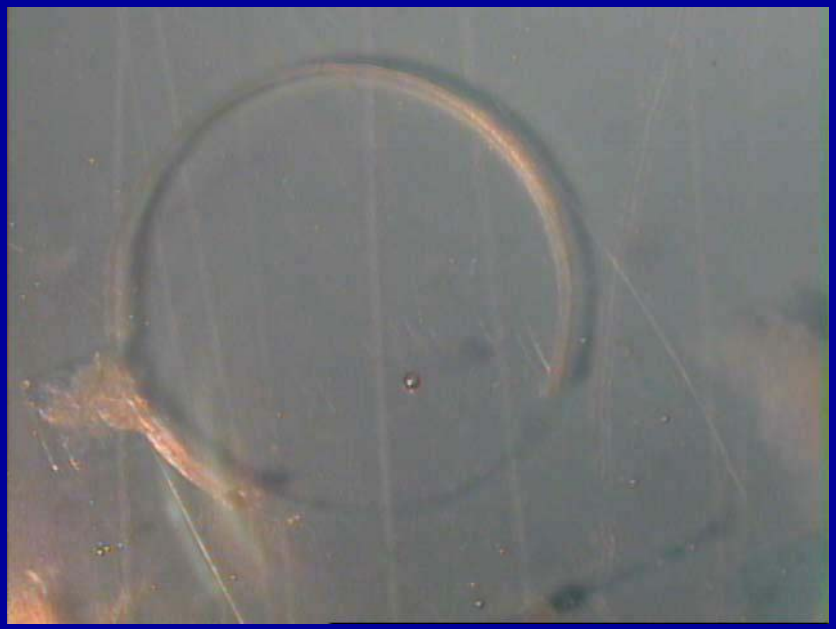
Laser Fabricated Myoid





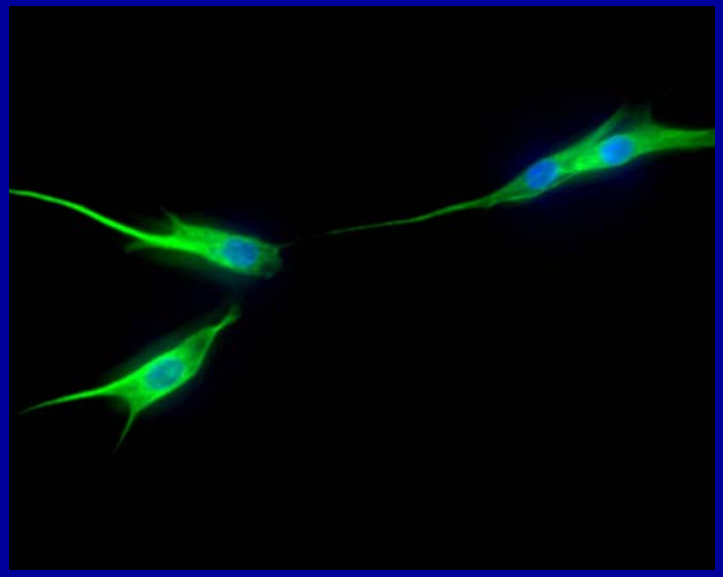
Living Myoid By Laser Fabrication, ~100 μm x 1 cm Dimensions!

Laser Fabricated Circular Myoid

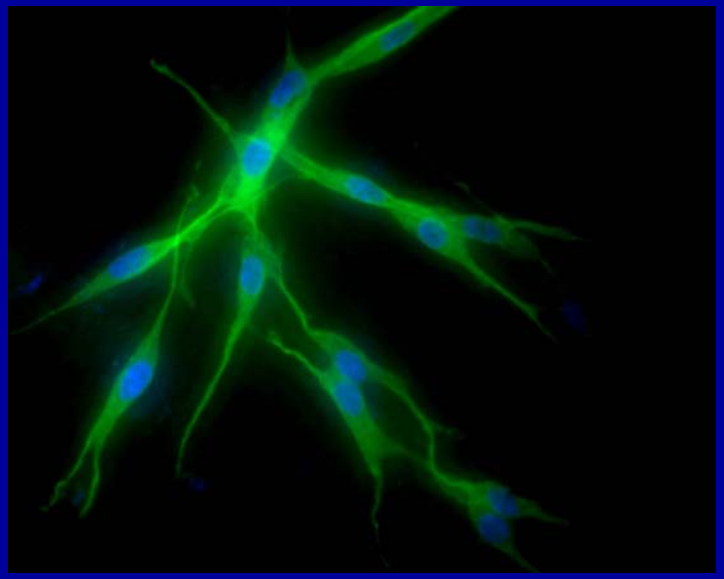


Circular Myoid, By Laser Fabrication, ~100 μm x 1 cm Diameter!

2 ½ Dimensional MAPLE DW Neural Deposition and Growth



2 Days

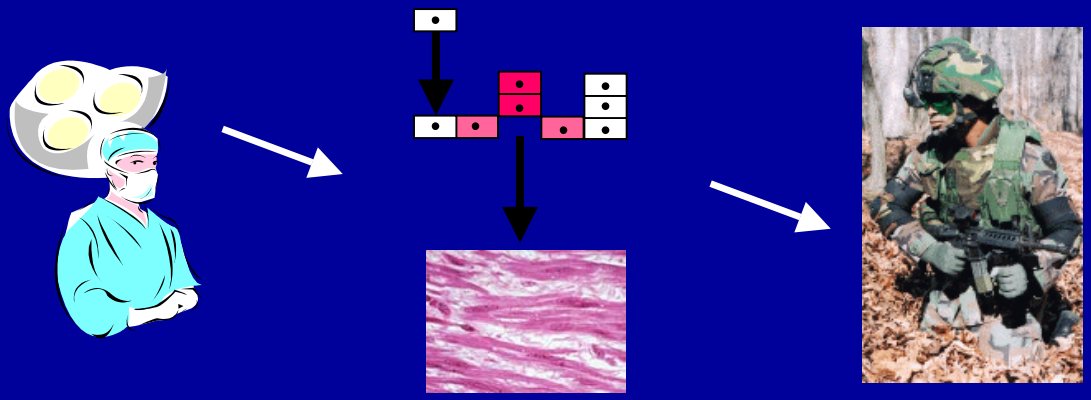


3 Days

Neural Cells Deposited at Different Depths in ECM Grow Together and Form Networks

Engineering Tissue-Based Structures and Devices Cell-By-Cell

- 3-D *Ex Vivo* Tissue Constructs (e.g., Hepatic, Immunological, Neural Living Systems)
- Tissue-Based Sensing of Warfare Agents and Environmental Toxins
- Cell Signaling Platform with Protein Identification Capability
- Cell Separation for Sub-Culturing by Living Microdissector
- Living Microfluidic Devices and Hybrid Biological Motors (Pumps, Valves, Dispenser for Microfluidic Chips)
- Battlefield Repair of Wounded Tissue (*In Situ* Tissue Re-Construction), Computer-Aided Surgeon



Rapid Prototyping Living Biological Systems

- Direct Writing is a Novel Approach to Fabricate Biological Micro-Manufacturing Systems:
  - Lasers Have Unique Attributes for the CAD/CAM Processing Biomaterials
  - Rapid Prototyping of Biological Systems
    - Protein Arrays
    - Cells

MAPLE DW Enables One to Take the Complexity of Biology and Transform It Into the Elegance of Engineering