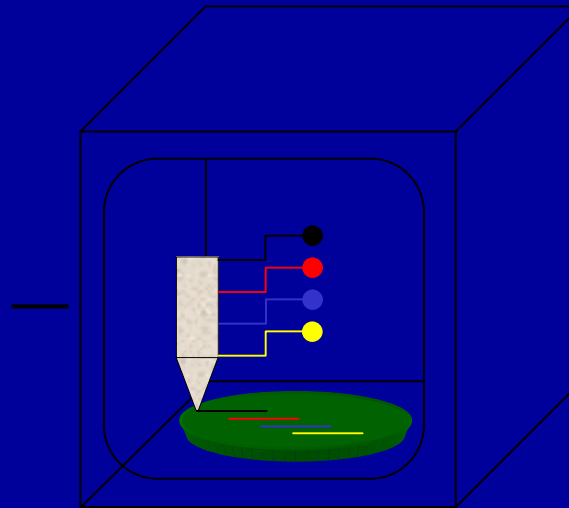
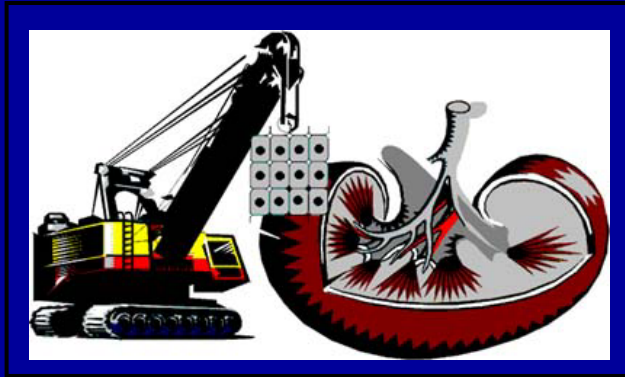


Rapid Prototyping of Living Biological Systems by Direct Write

From Designs on a Computer to Building Life



Douglas B. Chrisey, chrisey@nrl.navy.mil

Naval Research Laboratory, Washington, D.C.

12 August, 2004

NSF/WTEC Workshop on Micro-Manufacturing



Template Slides

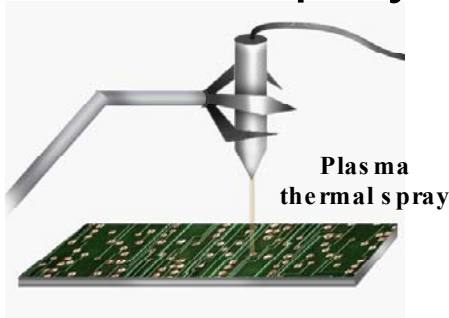
- Naval Research Laboratory, www.nrl.navy.mil
- Navy's Corporate R&D Laboratory
- State-of-the-Art: Presentation, Competitors Similar, But Different Areas and Materials
- Scientific/Technological Barriers: None, True 3-D
- Commercial Barriers: Visibility and Demonstration of Capabilities
- Site and Issues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

Goals of This Presentation

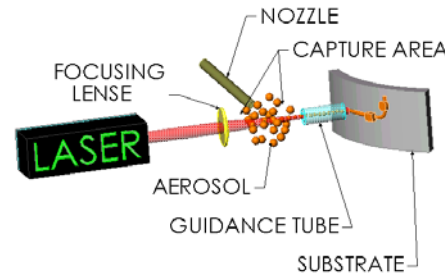
- *Direct Writing* is a Novel Approach to Fabricate Biological Micro-Manufacturing Systems
 - Use Evolutionary Genetic Machinery
- New Approach to Process Biomaterials – MAPLE DW
 - Proteins, Antibodies
 - Prokaryotic and Eukaryotic Cells
 - Extra Cellular Matrix
 - Scaffolding
 - Growth and Recruitment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

Different Direct Write Methods

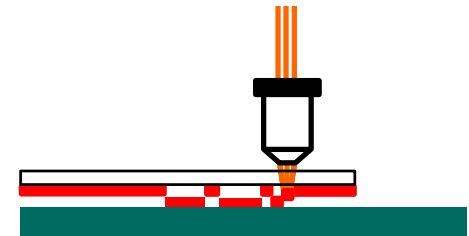
Plasma Spray



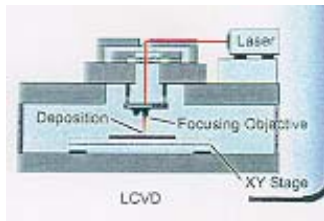
Laser Particle Guidance



MAPLE DW



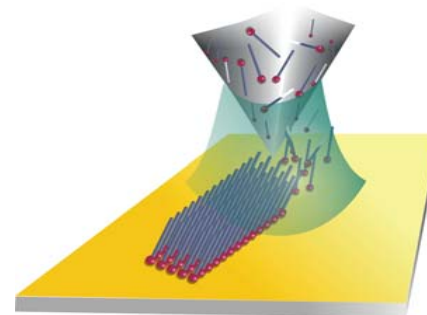
Laser CVD



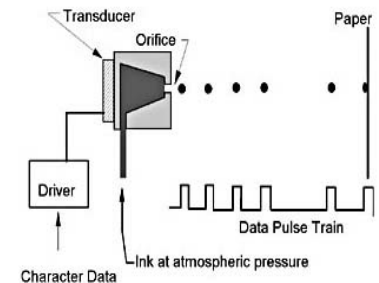
MicroPen



Dip-Pen

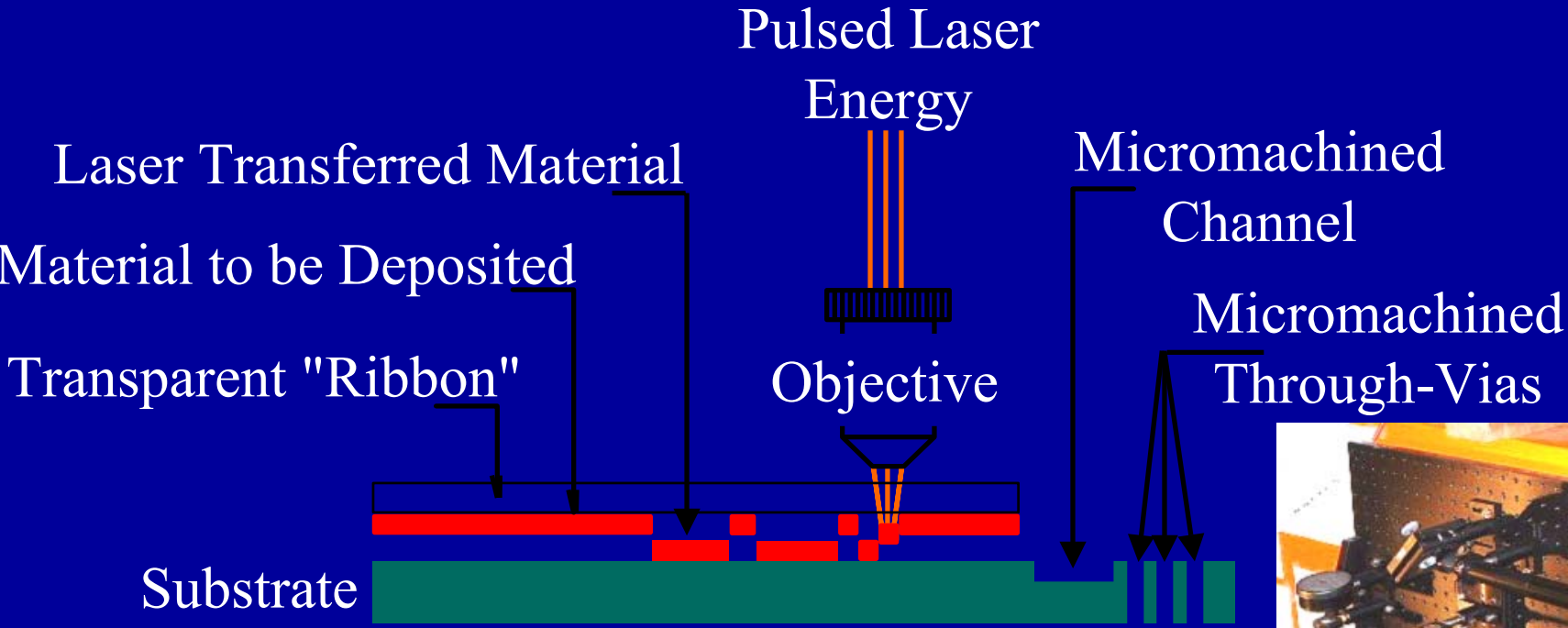


Ink Jet

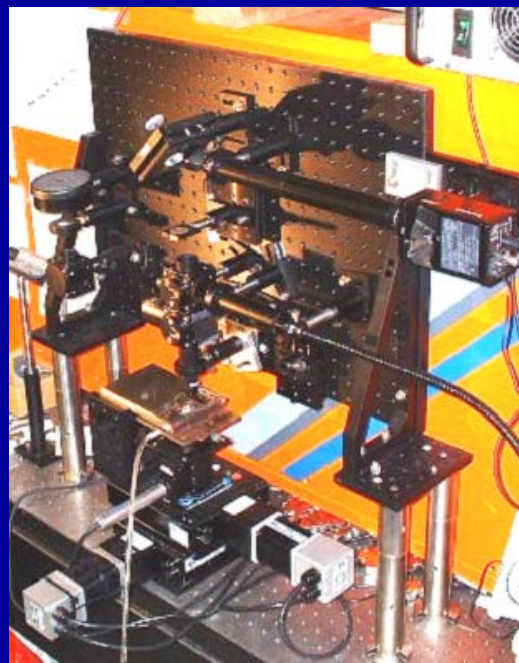


***Many Different Approaches Exist to DW Material Patterns.
Each Technique Has its Own Merits and Shortcomings.***

Matrix Assisted Pulsed Laser Evaporation Direct Write (MAPLE DW)



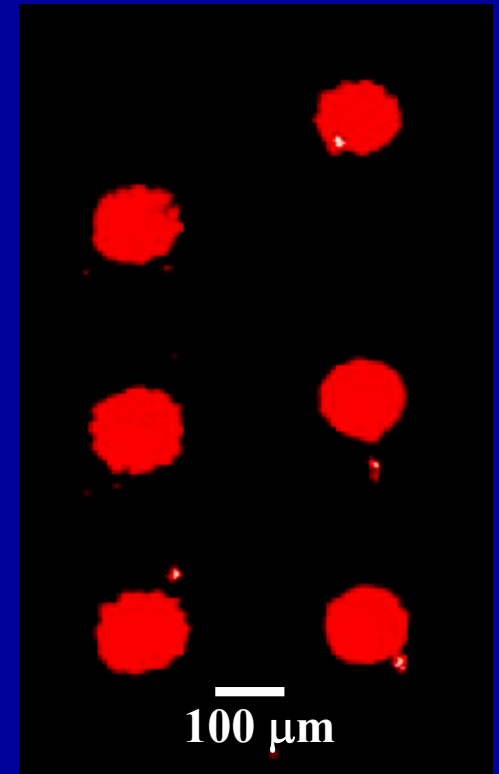
Done Under Ambient Conditions!



MAPLE DW of Active Proteins

Advantages Over Conventional Techniques

- Works With ANY Starting Material (Viscosity, Powders, Varying Conditions)
- Reproducibility
- CAD/CAM
- Reduced Spot Size $<10 \mu\text{m}$
- 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



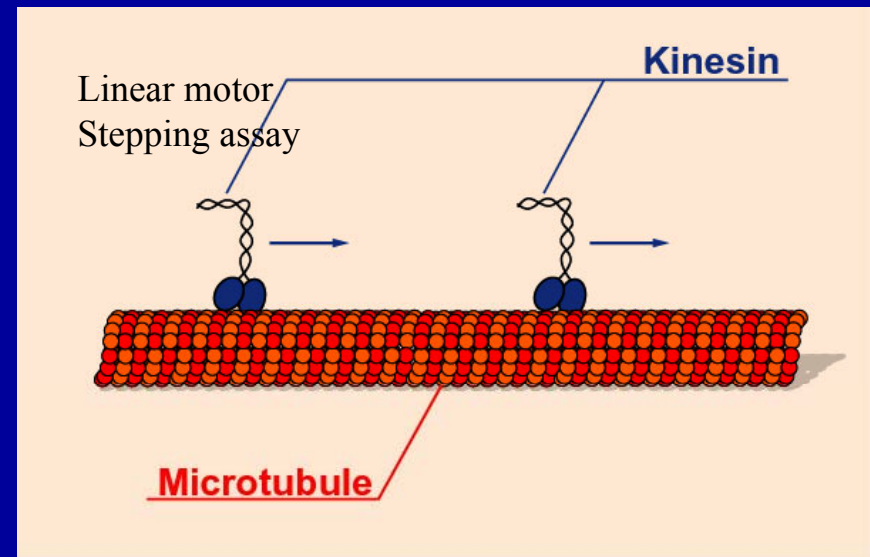
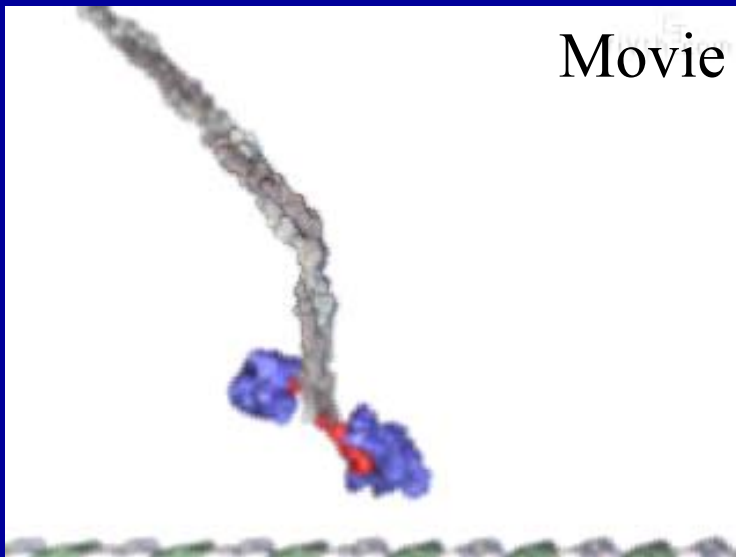
MAPLE DW
Streptavidin
(10 μm Spots)

- 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

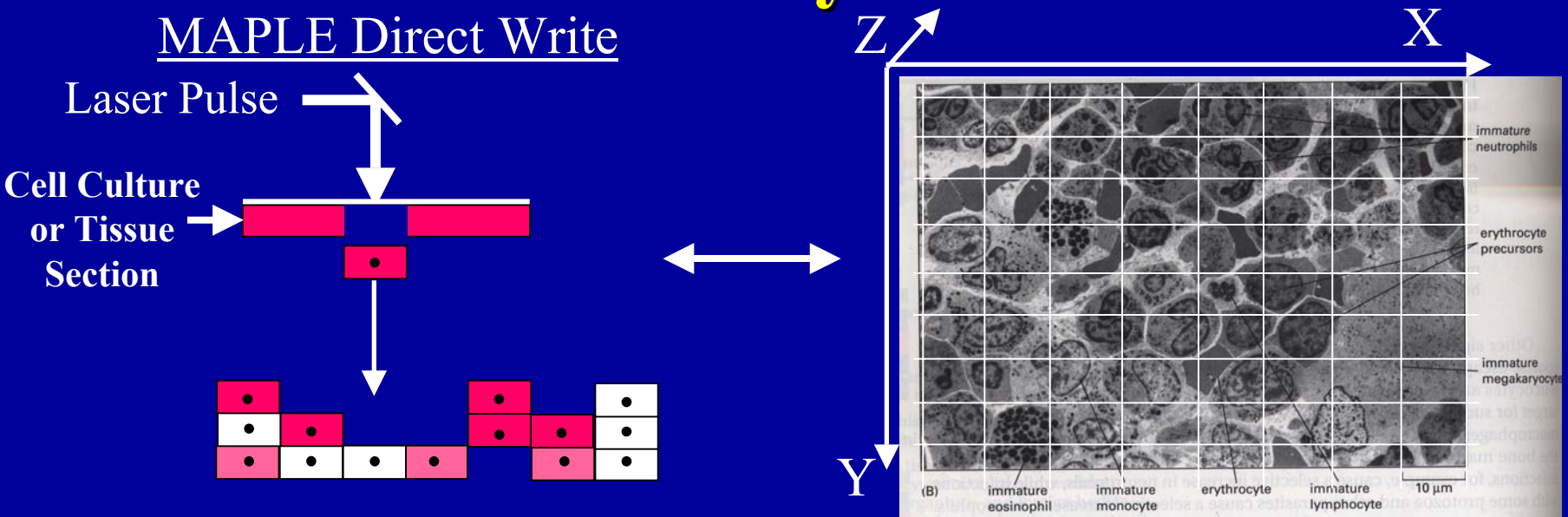
*Paradigm Shift: Mesoscopic Fabrication Yields
Nanoscale Manufacturing*

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)

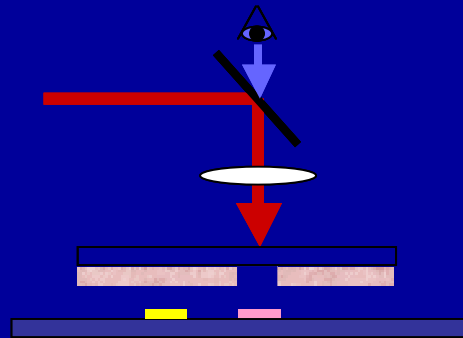
Paradigm Shift: To Go From Growing Tissues to Building Tissues

Cell Transfer by MAPLE-DW



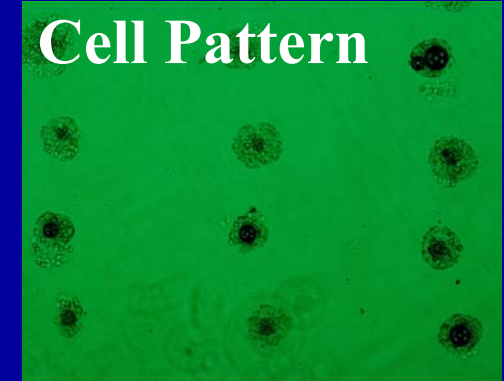
Preparation

Culture



Transfer

< 1 msec

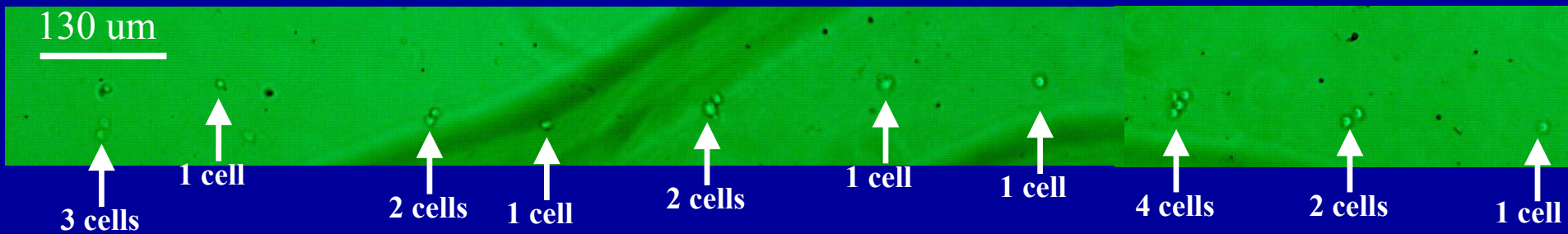


Analysis

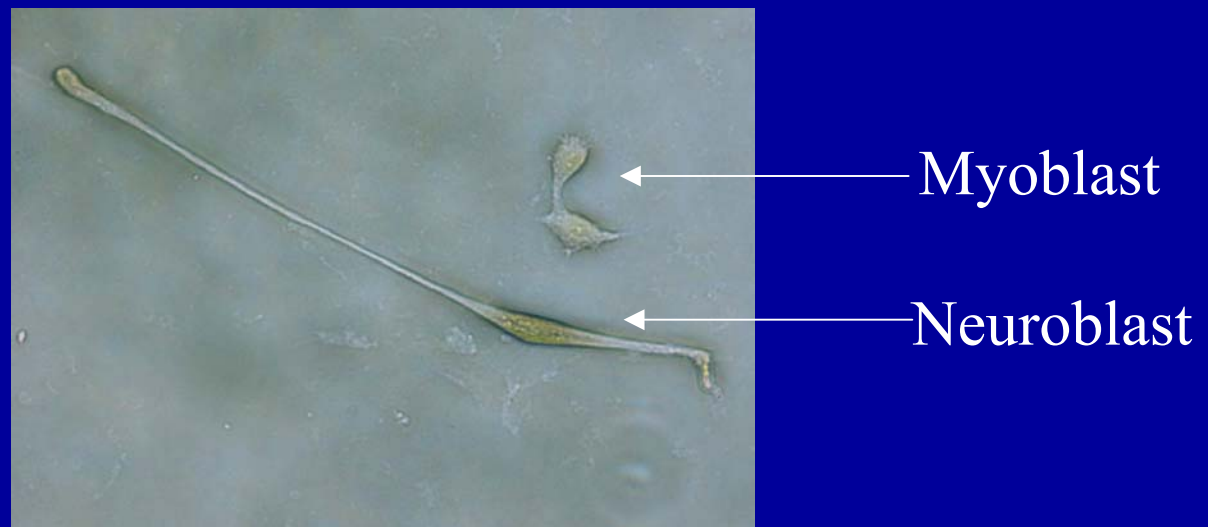
Culture



Single-Cell Resolution of Osteoblasts



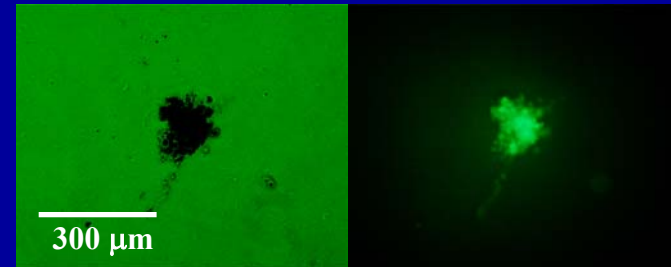
Single-Cell Resolution: Myo- and Neuroblasts



Single Shots and Multiple Shots of Rat Cardiac Cells



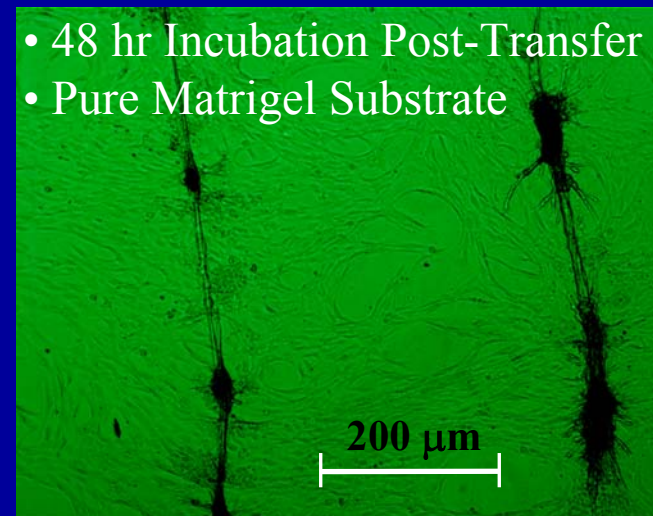
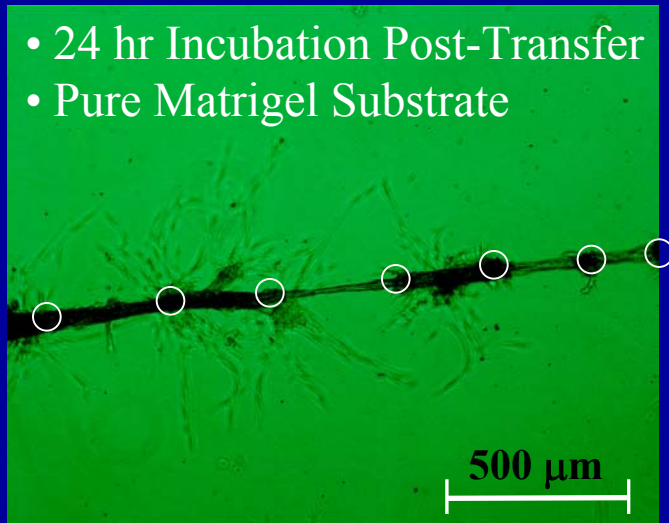
Single Shot; After 4 Days Culture
Spread Over 700 Microns; 200
Micron Original Spot Size



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!*

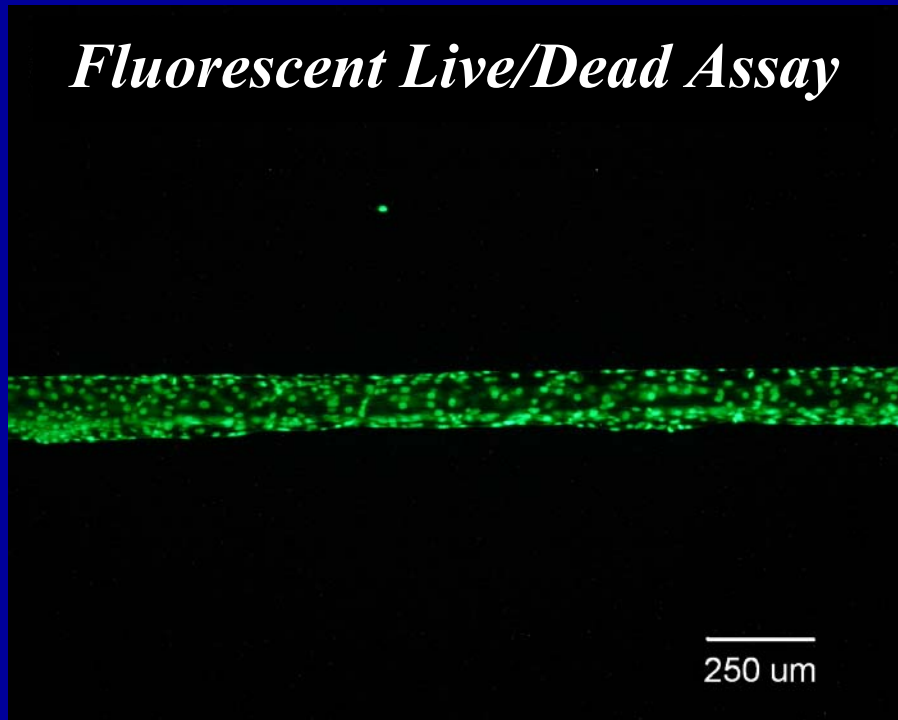
Forming Muscle-Like Structures Using MAPLE DW: Lines of Mouse Myoblasts



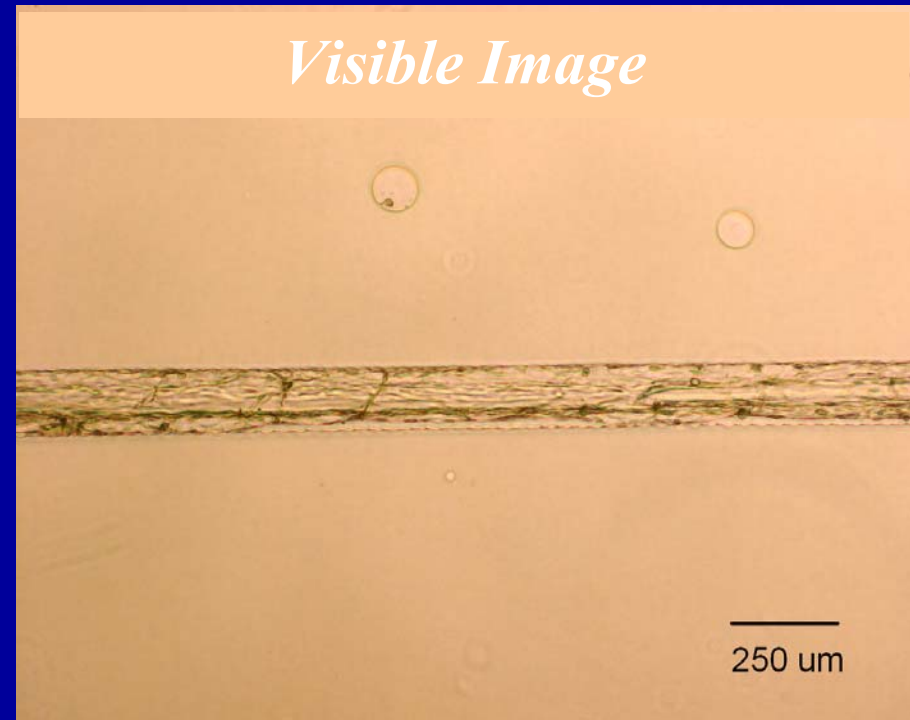
Mouse Myoblast Cell Spots Self-Formed Into 3-D Structures!

Laser Fabricated Myoid

Fluorescent Live/Dead Assay



Visible Image



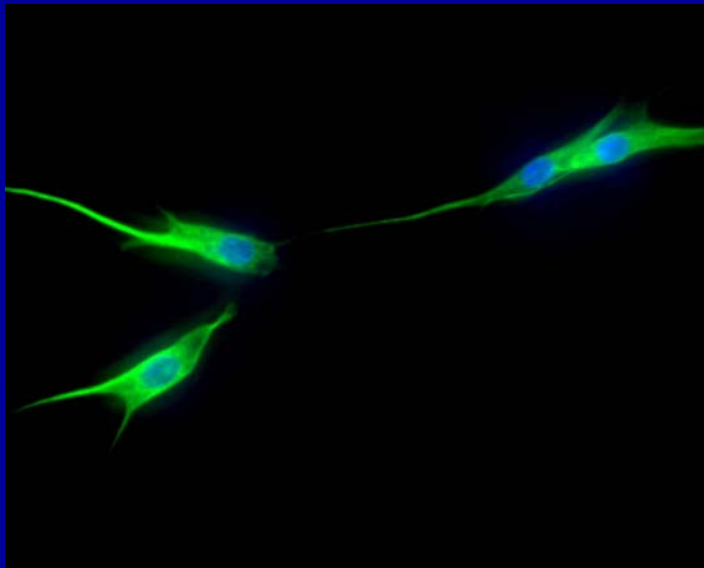
Living Myoid By Laser Fabrication, ~100 μm x 1 cm Dimensions!

Laser Fabricated Circular Myoid

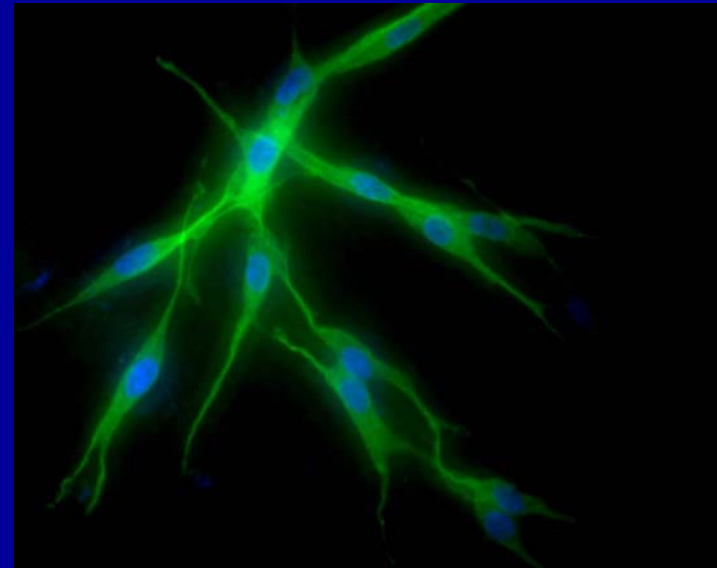


Circular Myoid, By Laser Fabrication, ~100 μm x 1 cm Diameter!

2 ½ Dimensional MAPLE DW Neural Deposition and Growth



2 Days

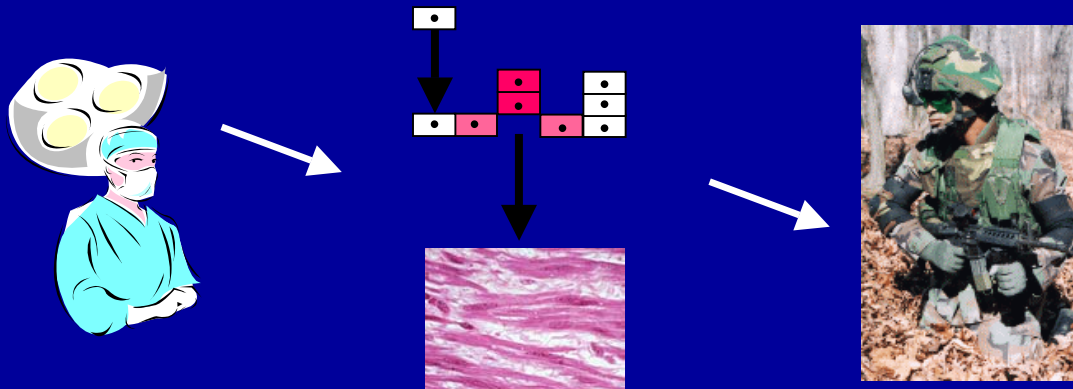


3 Days

*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 Reconstruction), 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