Issues for Tissue Engineering by Direct-Write Technologies

Daniel Young

Wright State University, Dayton, OH

daniel.young@wright.edu

Douglas B. Chrisey

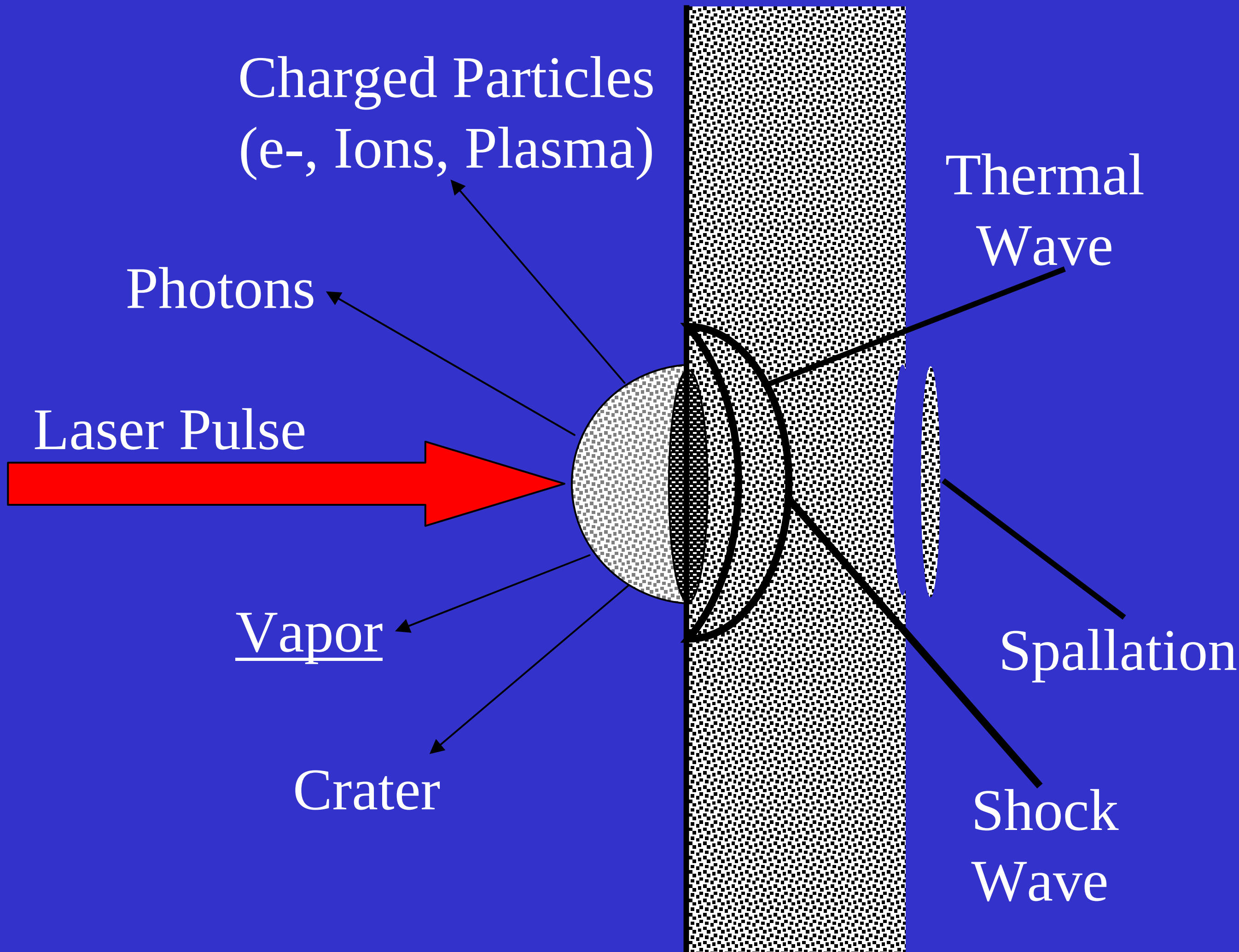
U.S. Naval Research Laboratory, Washington, DC

Douglas.Chrisey@nrl.navy.mil

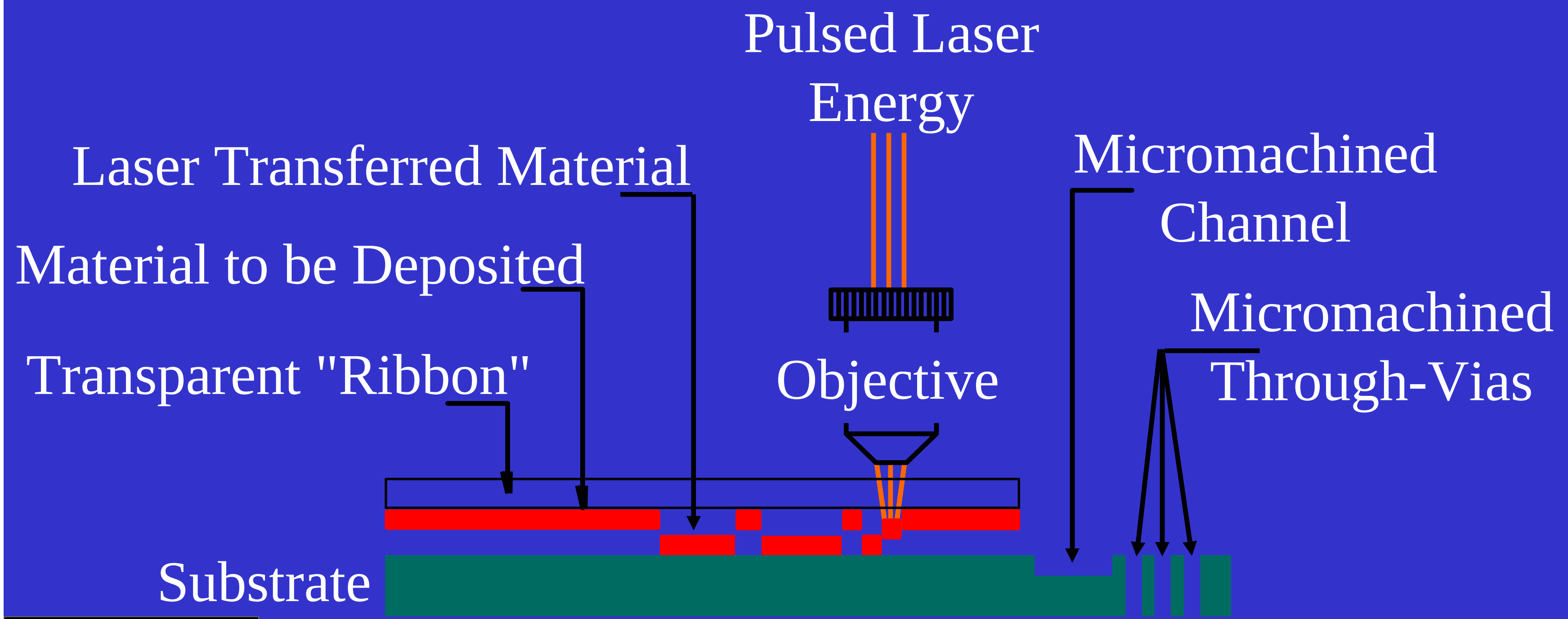
Outline of Presentation

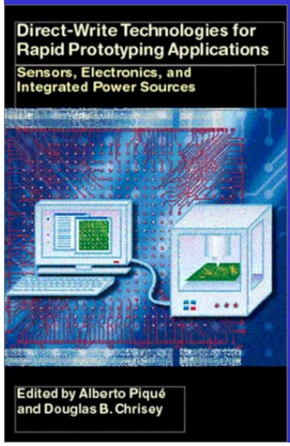
- Introduction to MAPLE Direct Write
- Direct Writing of Electronics
- Direct Writing of Biological Materials
- Conclusions

The Laser-Solid Interaction

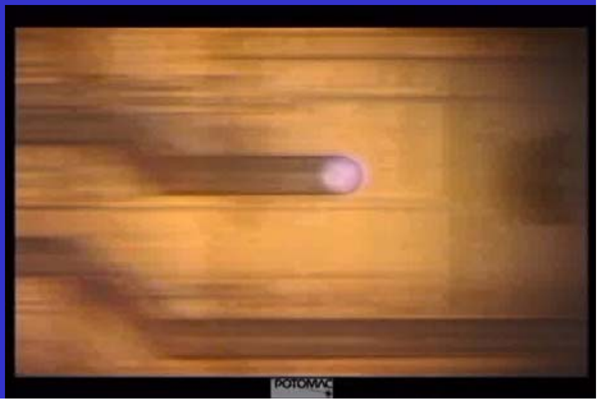


Matrix Assisted Pulsed Laser Evaporation Direct Write (MAPLE DW)



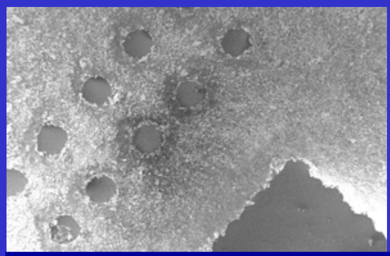


Done Under Ambient Conditions!



“Ribbons” in MAPLE DW

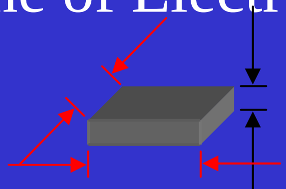
The Interaction of the Laser with the Ribbon is the Novelty in MAPLE DW. It is Both a Liability and an Asset.



Ribbon

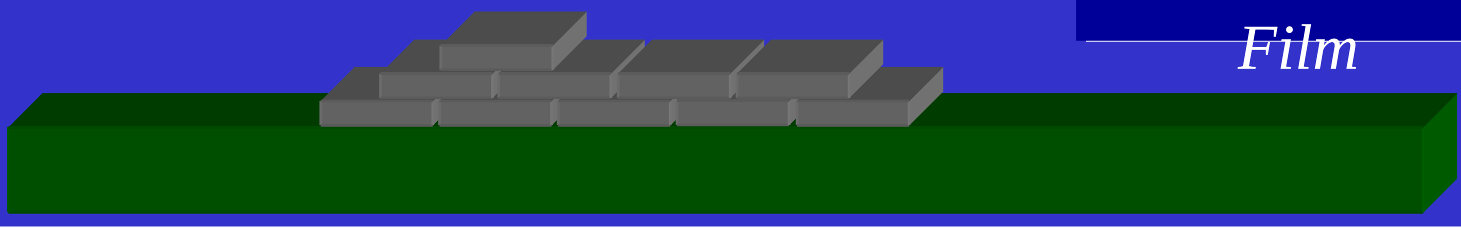
Liability: Ribbons are Difficult to Fabricate.

Asset: Ribbons Effectively “Quantize” the Material Transferred Making MAPLE DW Coatings Highly Reproducible. Each Laser Pulse Deposits an Identical Mesoscopic Volume of Electronic Material.



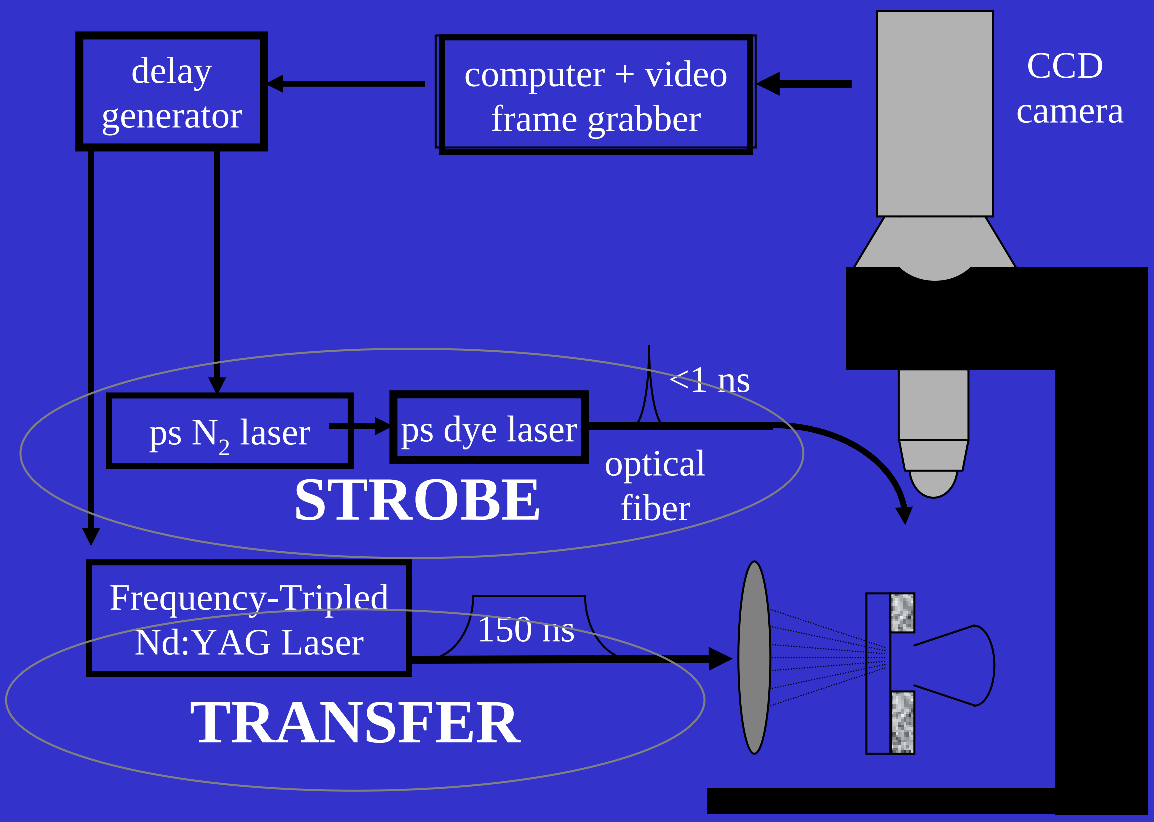
"Beam" Cross-Section X Ribbon Thickness)

Film



Ultra-High Speed Imaging Of The MAPLE-DW Transfer Process

Side Imaging

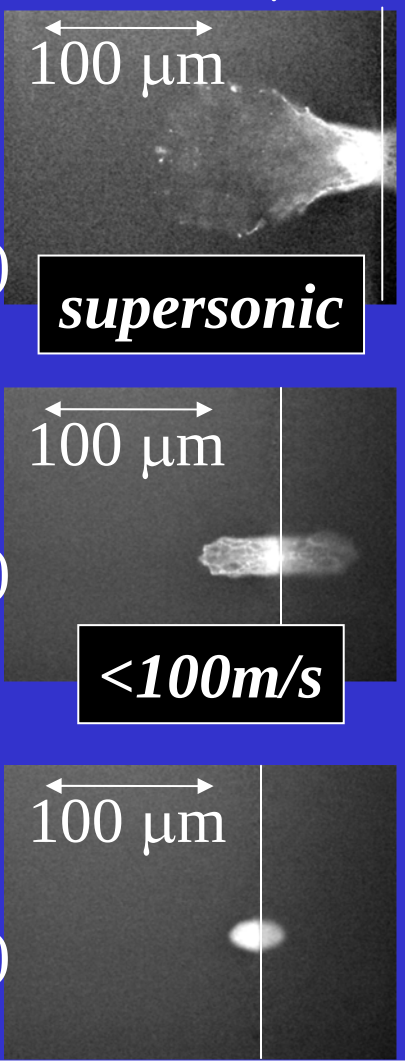


~1 µs

Divergent Plume (68 mJ/cm^2)

Jetting (26 mJ/cm^2)

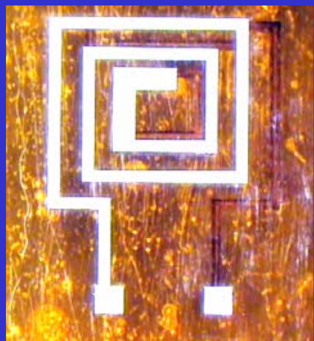
Sub-Threshold (19 mJ/cm^2)



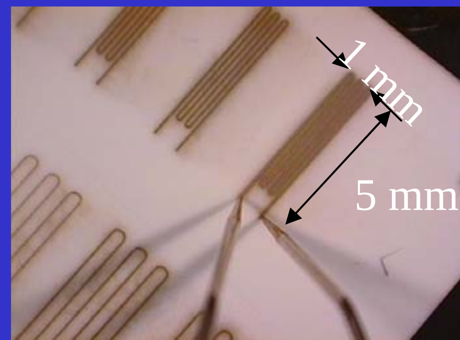
Transferred Spots Are Actually Smaller Than Laser Just Above Threshold

MAPLE DW of Passive Devices

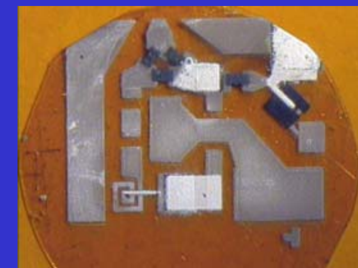
Spiral Inductor



Interconnects

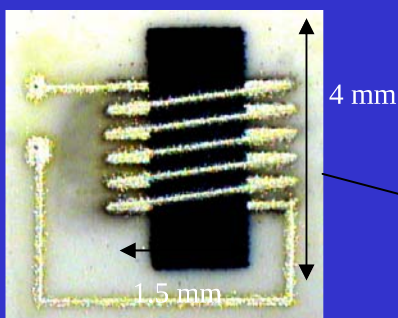


600 MHz Oscillator

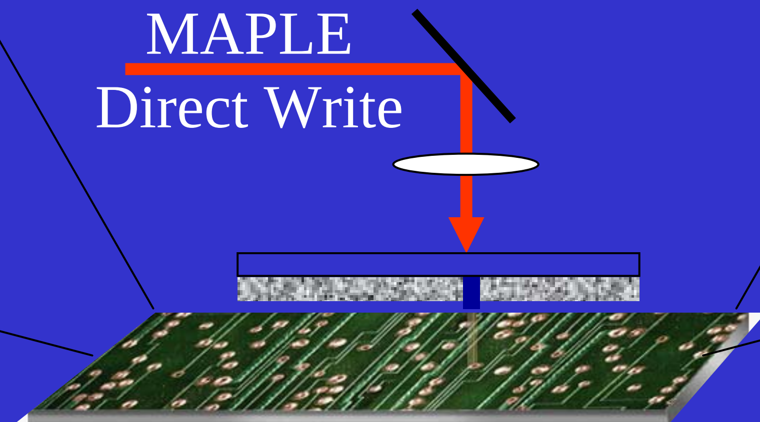


*

YIG Core Inductor

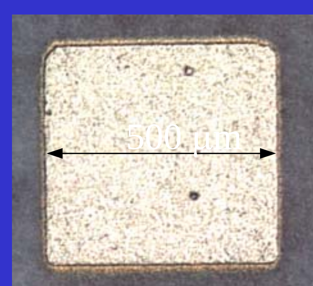


MAPLE Direct Write

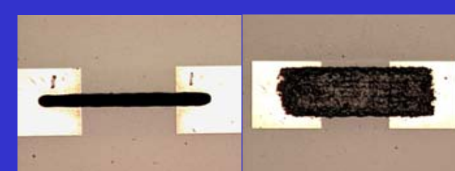


Fractal Antenna

Transmission Lines

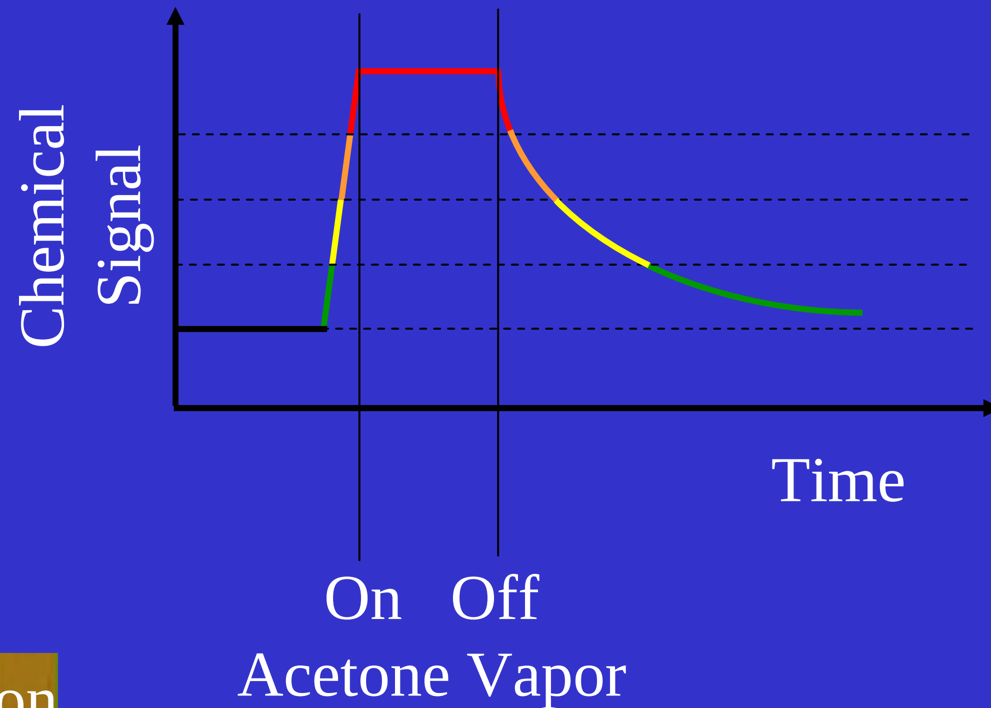


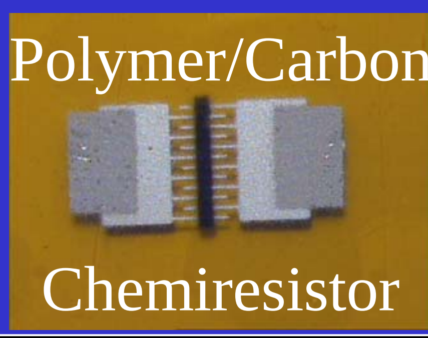
Capacitors



Resistors

Prototype Chemiresistive Sensor Subsystem

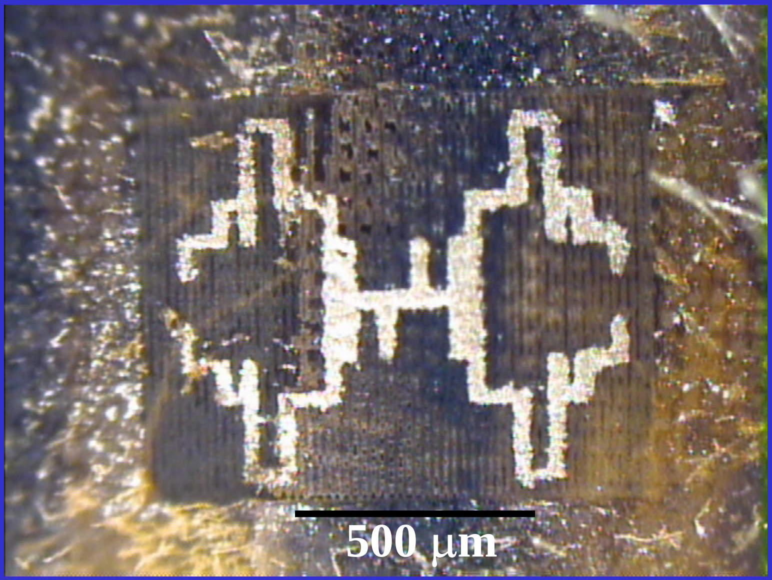




Chemical Signal is Rapidly Adsorbed to Chemoselective Polymer (Detected as Resistance Change) And More Slowly Desorbed!

Fractal Antenna on the Abdomen of a Worker Honey Bee by Laser Direct Write

Size = 1 mm x 1mm, Total Weight < 1 mg

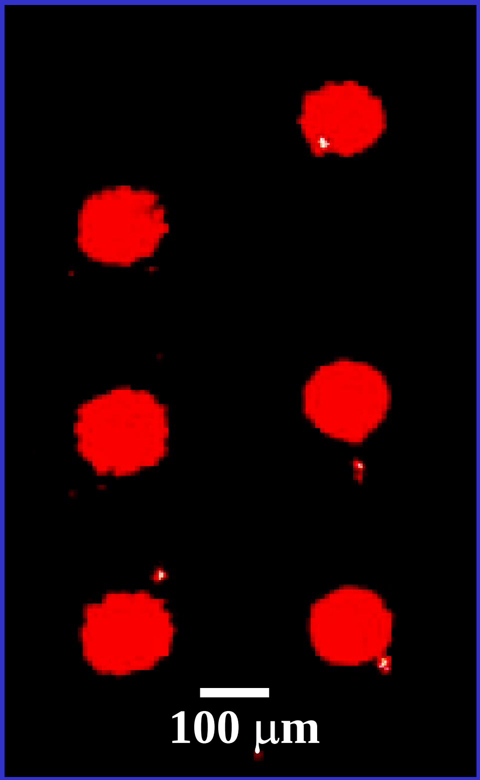


Fractal Antenna Pattern Deposited Onto the Abdomen of a Dead Honey Bee. A Rectangular Area of Hair Was First Removed by Laser Micromachining, And Then Laser Forward Transfer Was Used to Deposit the Silver Pattern. The Resonant Frequency Is Estimated to Be 54 GHz.

MAPLE DW of Active Proteins

Advantages Over Conventional Techniques

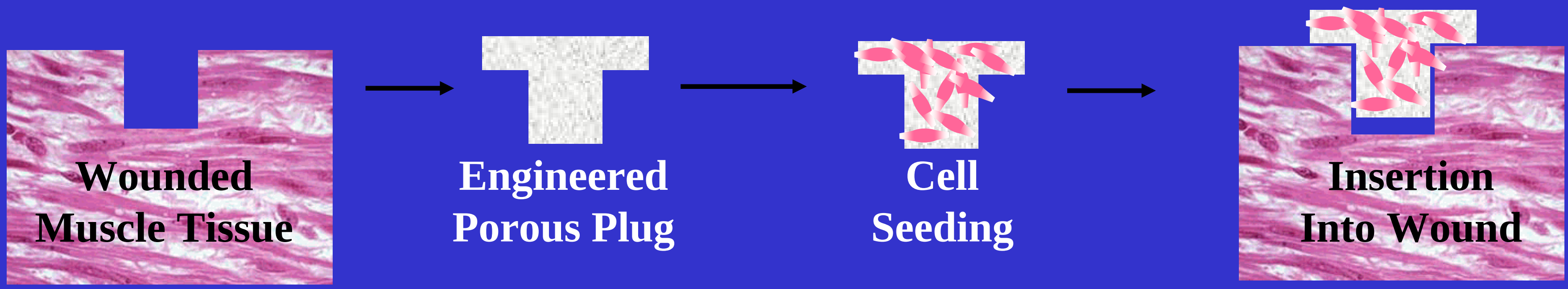
- Works With ANY Starting Material (Viscosity, Powders, Varying Conditions)
- Reproducibility
- CAD/CAM
- Reduced Spot Size <10 μm
- Increase Material Utilization/Efficiency by 10^5
- Works on Planar Substrates and Microwells
- One System Does Complete Array of Multiple Array Elements



Single Element Antibody Microarray (Anti-BSA)

The Possibility to Expand Upon Traditional Tissue Engineering

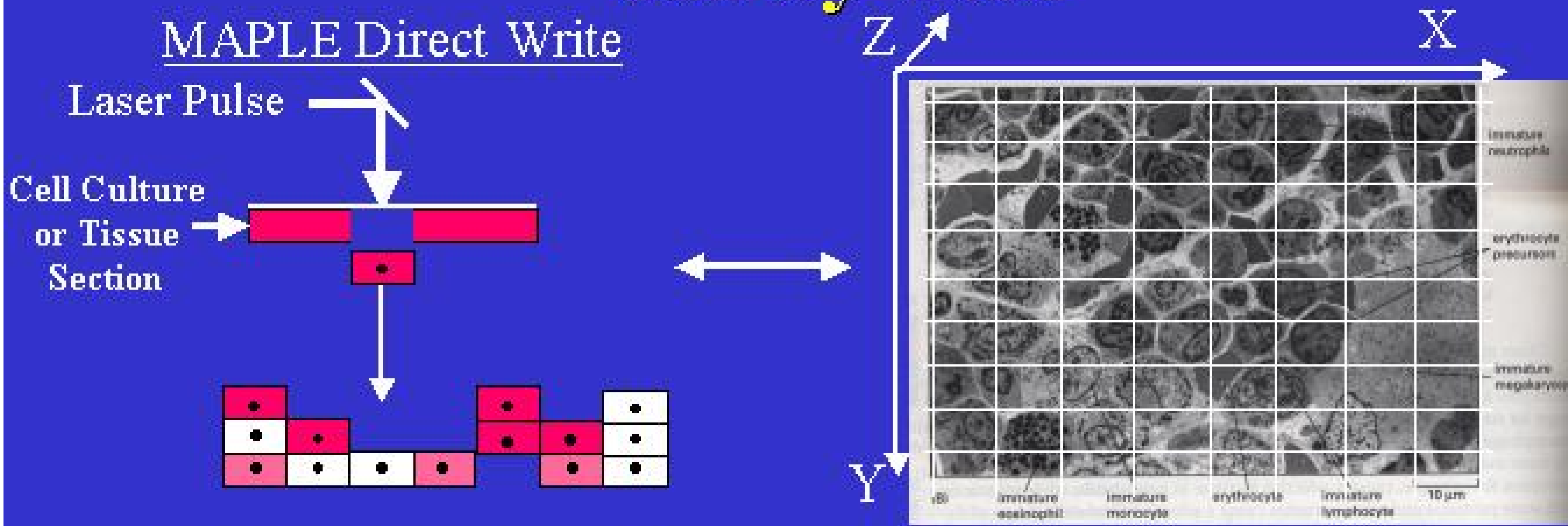
- Seed Cells Into Biocompatible, Engineered Scaffolds
  - Artificial Structure Increases Rate of Recovery and Strength of Repaired Tissue



- *Vascularization Is Needed if Construct is >1 mm Thick*
- *<u>Random</u> Cell Attachment to Macroscopic Structure*

All <u>Evolved</u> Tissue Structure is Lost

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



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

  √ Rapid, Computer-Controlled Placement of <u>Different Materials</u> at 10 to 100 μm

  √ <u>Multiple Cell Types</u>

  √ <u>Molecules</u> Like Growth Factors, Recruitment Factors, Differentiation Chemicals

  √ Novel Scaffolding Materials (Inorganic/Organic Composites)

  ? Vascularization (Constructs >200 μm), Accelerated Tissue Maturation

Paradigm Shift: To Go From <u>Growing</u> Tissues to <u>Building</u> Tissues

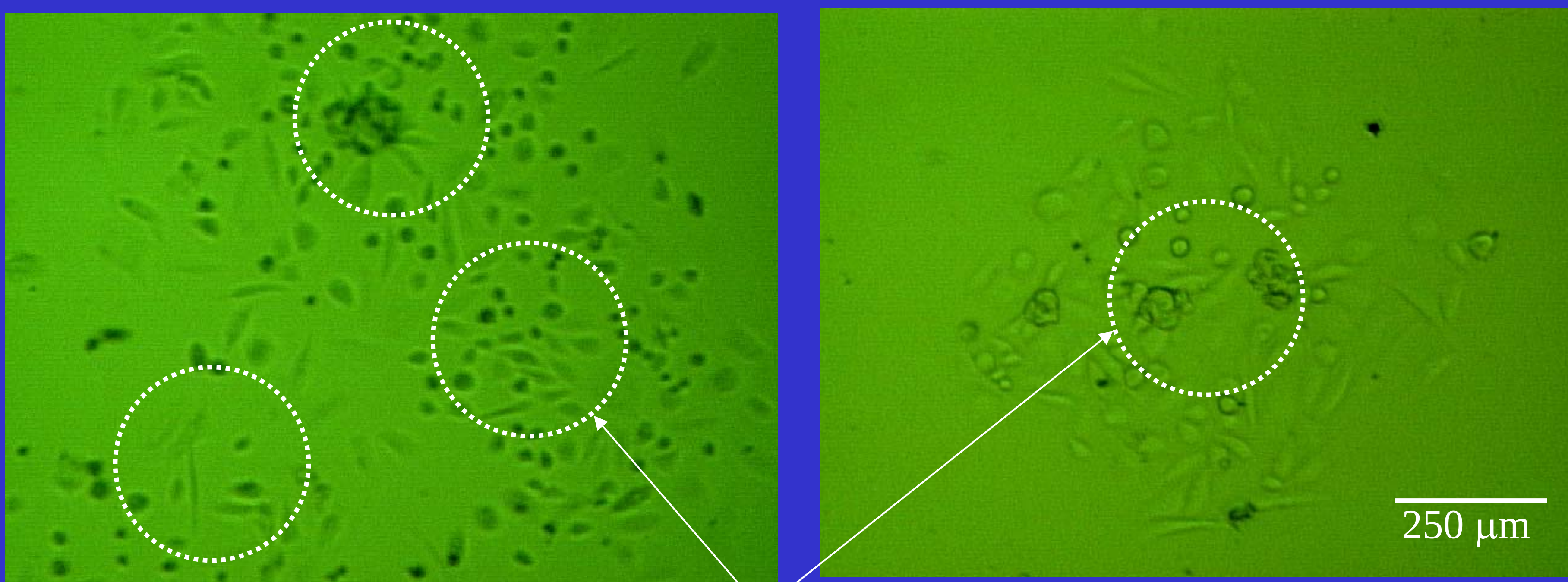
Chinese Hamster Ovaries (CHO) Transferred By MAPLE DW


CHO Cells Growing on a Hydrogel Quartz Ribbon

CHO Cells Immediately After Laser Transfer

- Accurate Transfer of Approximately 40 CHO Cells
- These <u>Eukaryotic</u> Cells Appear Similar to Pre-Transfer Culture

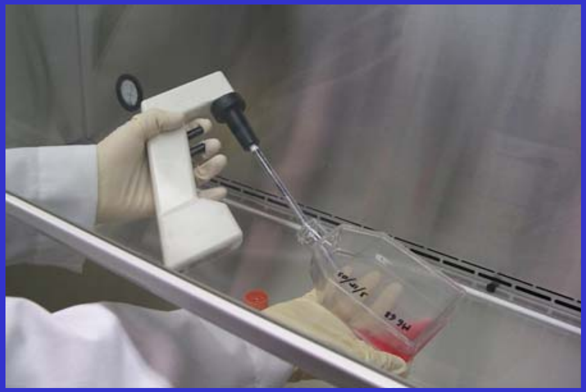
Laser Transferred CHO Cells After Culturing for Three Days

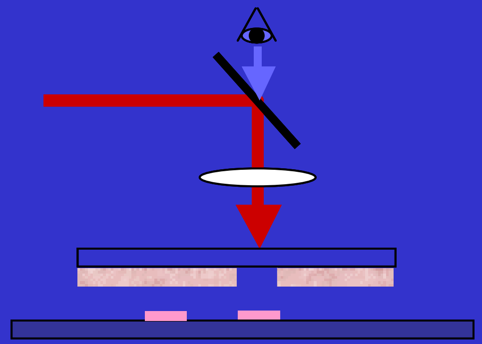


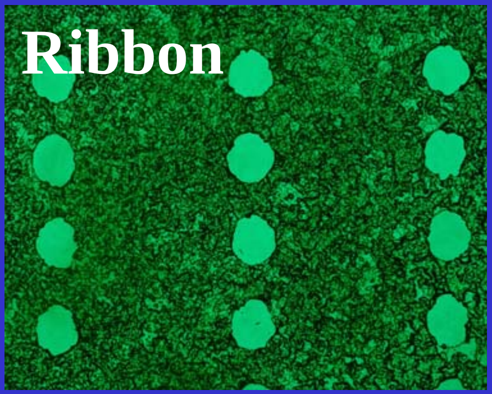
Original Spot Area

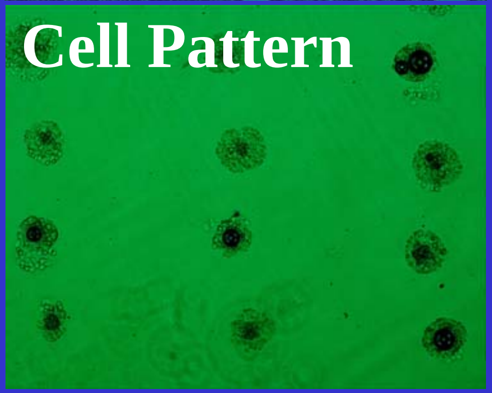
Transferred Cells Remain Alive and Have Multiplied (growth rate unaffected)

Cell Transfer by MAPLE-DW





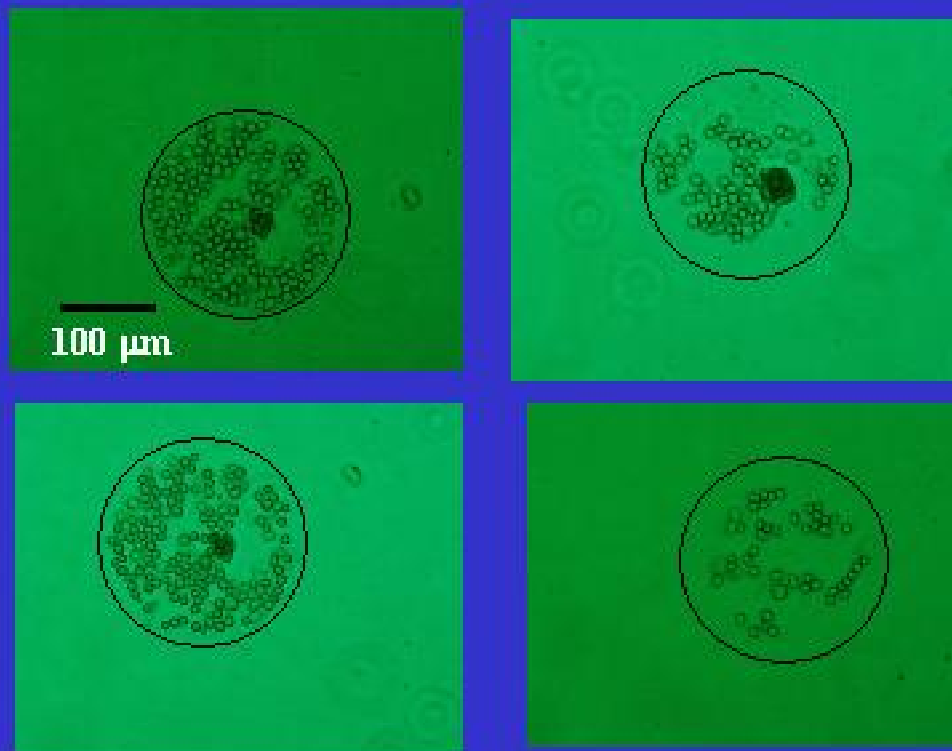




Preparation | Transfer | Analysis

Culture | 1 μsec | *Culture*

Human Osteoblast Transfers by Laser Direct Write



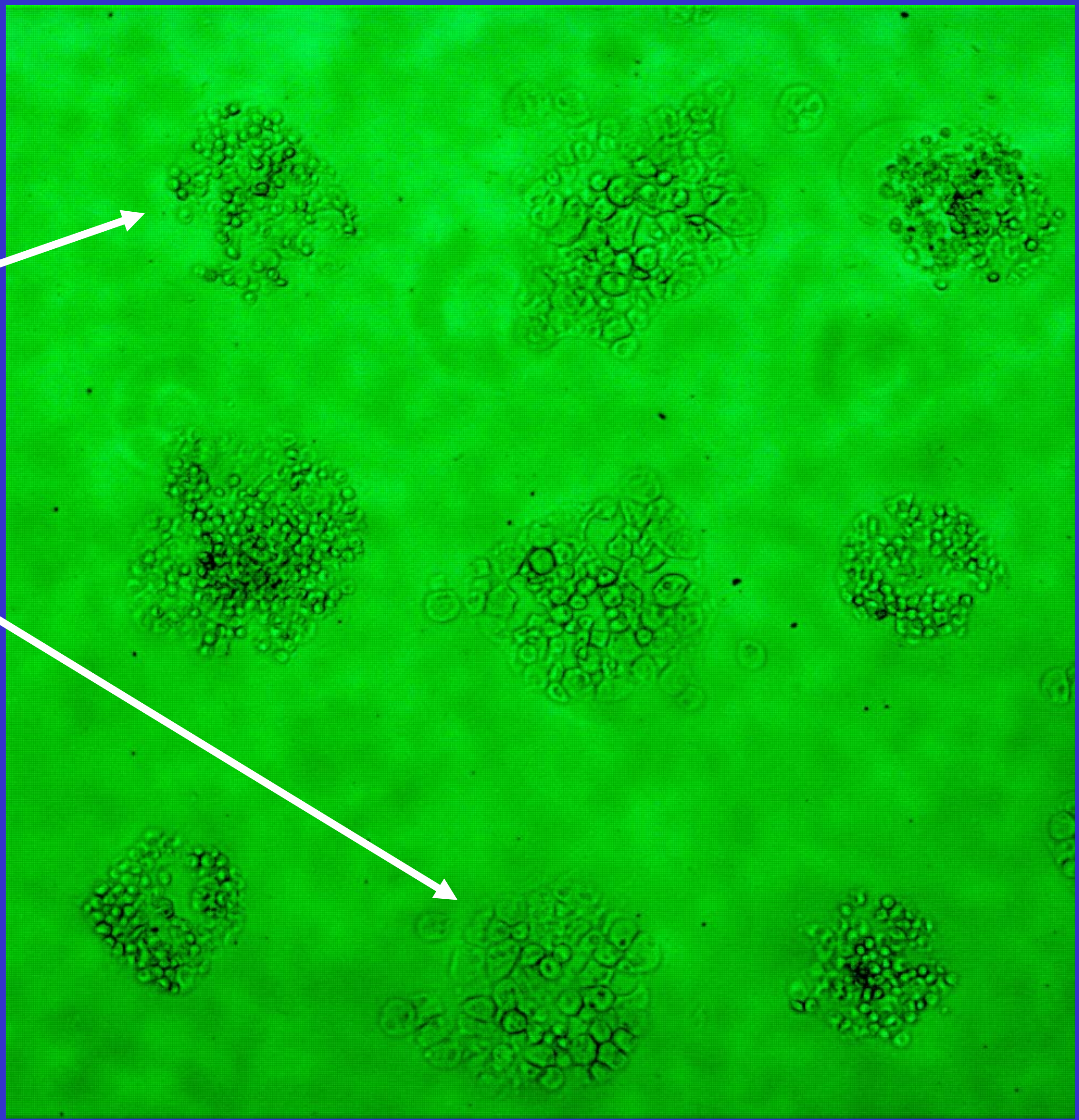
- 100 X Immediately Post Transfer

Two Cell Type Microarray

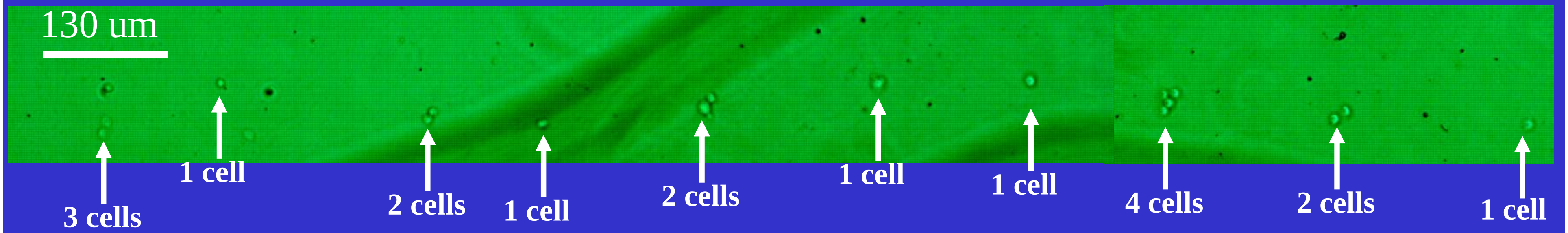
Osteoblasts

Cardiocytes

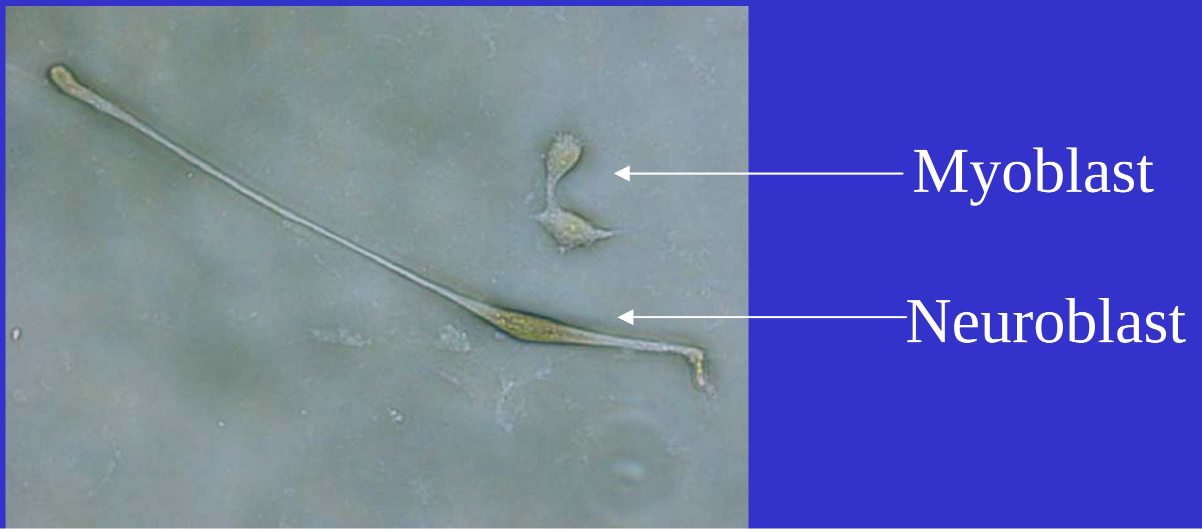
Note the Difference
In Cell Morphology



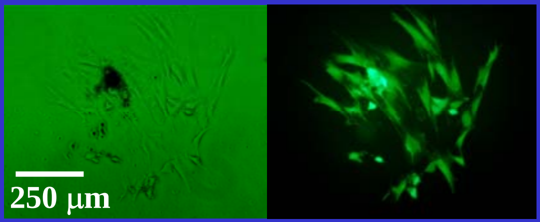
Single-Cell Resolution of Osteoblasts



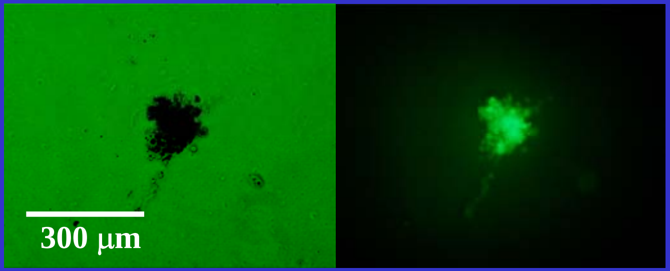
Single-Cell Resolution: Myo- and Neuroblasts



Single Shots and Multiple Shots of Rat Cardiac Cells



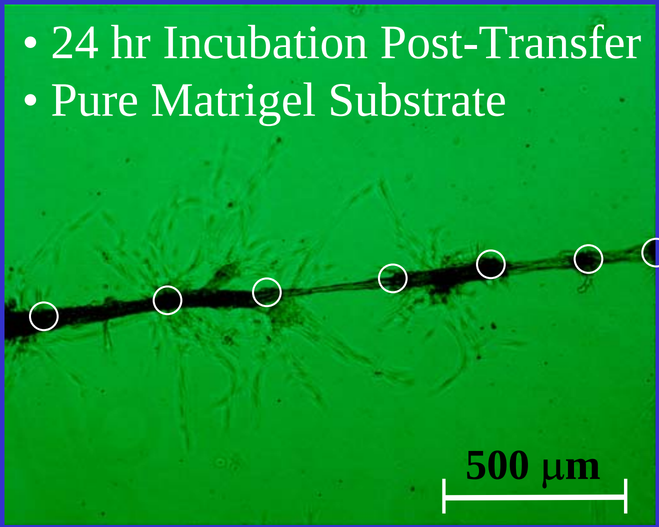
Single Shot; After 4 Days Culture Spread Over 700 Microns; 200 Micron Original Spot Size

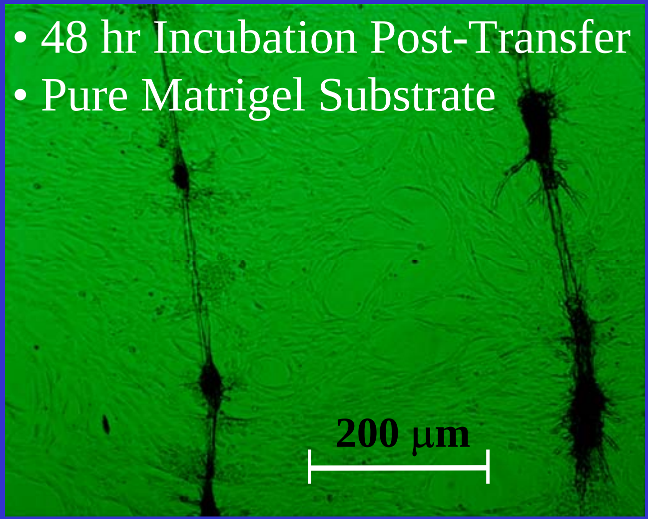


3 Shots; After 4 Days of Culture; Cells Did Not Spread; Bound in Cage/Matrix

Rat Cardiac Cells Behaved Differently Depending on the Local Environment!

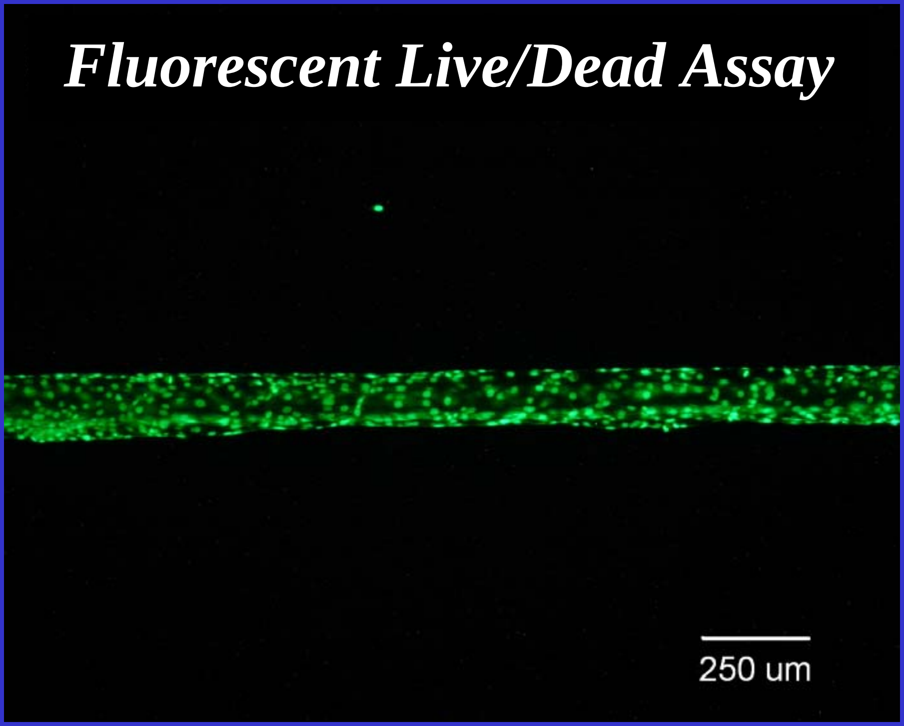
Forming Muscle-Like Structures Using MAPLE DW: Lines of Mouse Myoblasts

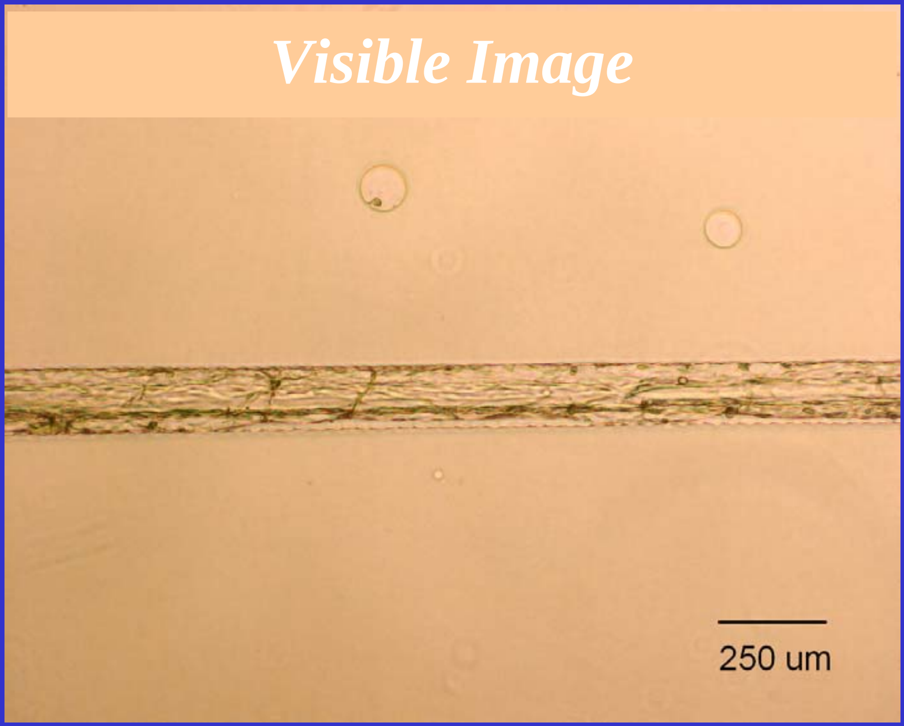




Mouse Myoblast Cell Spots Self-Formed Initially Into Organized 2-D Structures

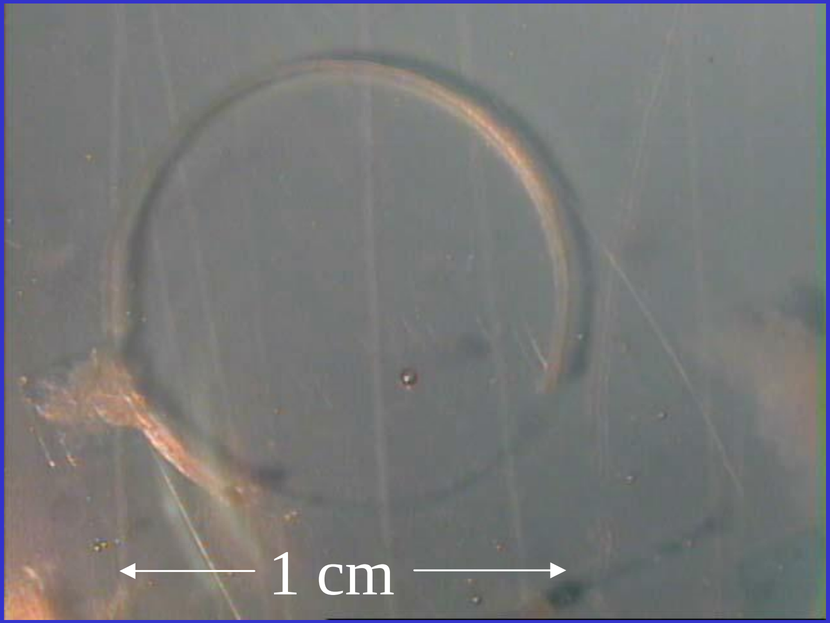
Laser Fabricated Myoid: Differential Adherence





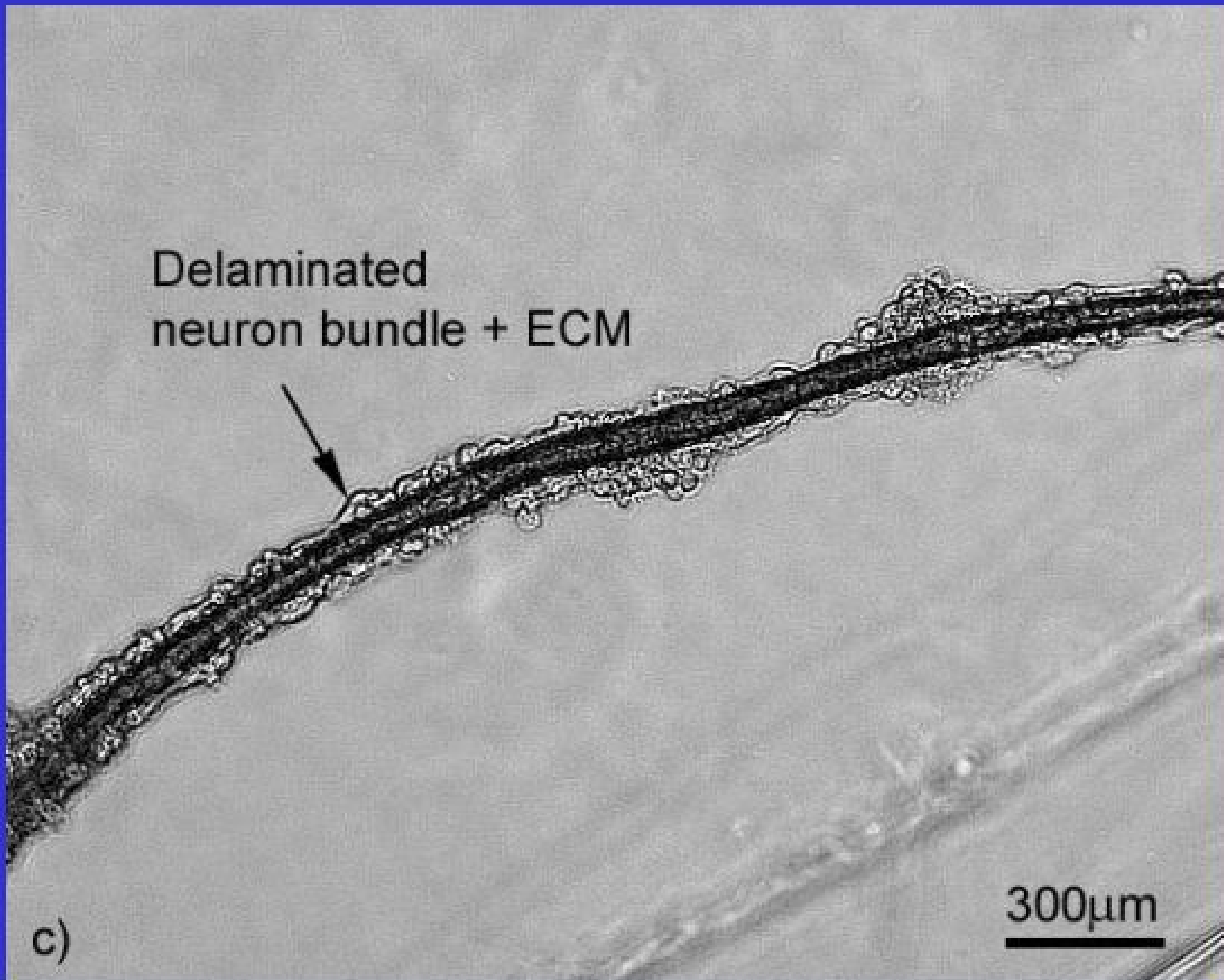
Living Myoid By Laser Fabrication, ~100 μm x 1 cm Dimensions!

Laser Fabricated Circular Myoid

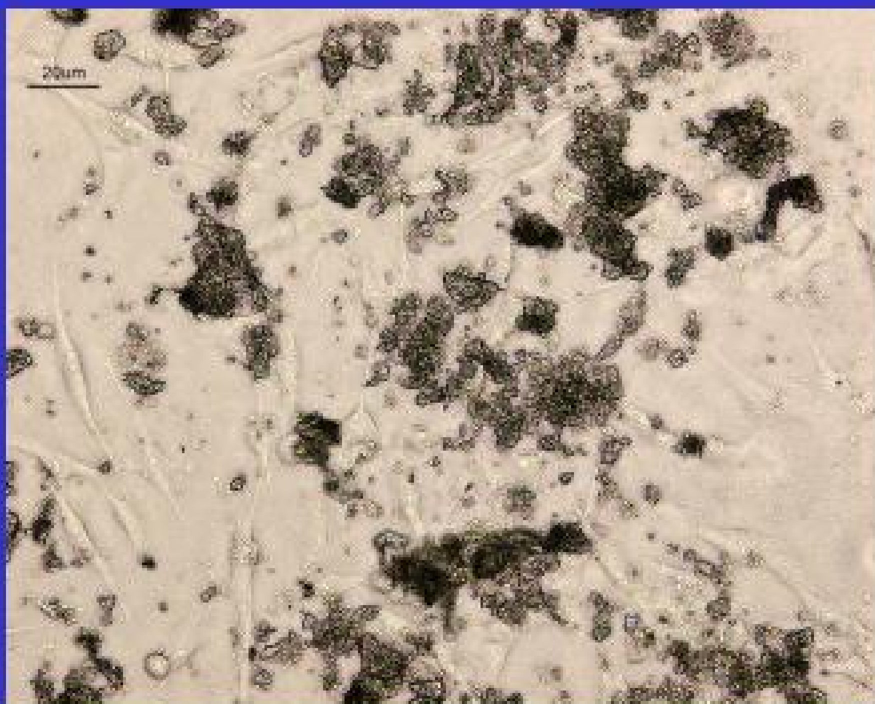


Circular Myoid, By Laser Fabrication
~100 μm x 1 cm Diameter!

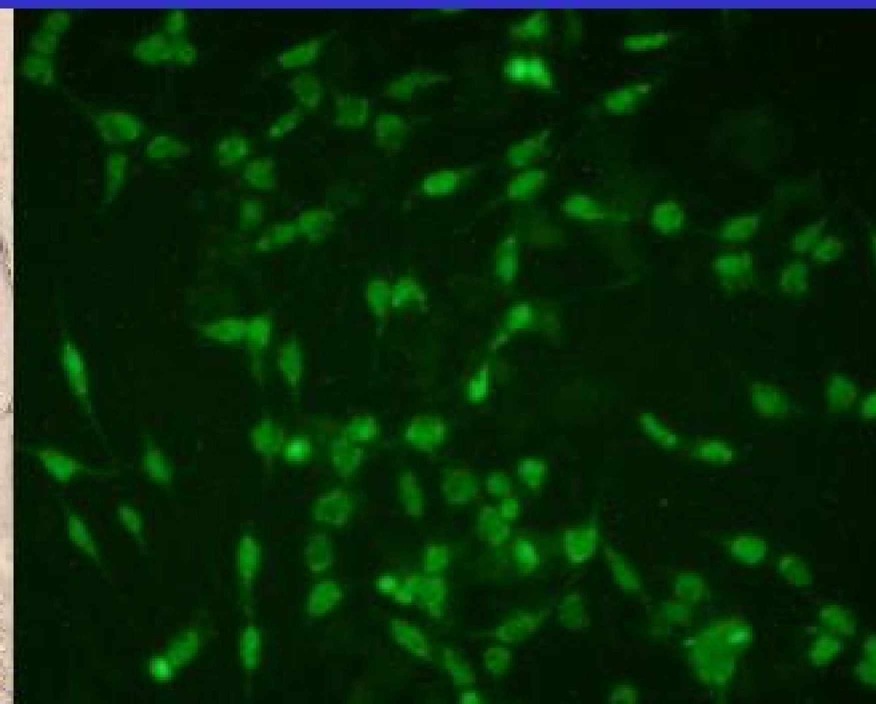
Laser Fabricated Neural Bundle



MAPLE DW of Hydroxylapatite (HA) and HA With Human Osteoblasts



Optical Image



Live/Dead Assay

Osteoblasts Deposited Alive with Ceramic HA Scaffold

MAPLE DW of Heterogeneous Tissue Constructs

- Direct Writing Techniques Presents Some Unique Capabilities and Challenges for Tissue Engineering
- Significantly Different From Other RP Methods
- Increased Heterogeneity for Direct Written Constructs (Cells, Scaffolds, and Biomolecules) Can Accelerate Tissue Development and Enhance Function
- Scaffolding/Structure Integrity Issues

Heterogeneous Biomaterials by MAPLE DW

Resolution ⟷ Type of Material

• Living	-	Non-Living	√
• Ceramic	-	Polymeric	√
• Single Cell Type	-	Multiple Cell Types	√
• Single Cell	-	Multiple Cells	√
• Pure Mat'l	-	Composites	√
• Low Viscosity	-	High Viscosity	√
• 20 μm Lateral, 40 μm Depth Resolution			√
• Pico-Liter	-	Micro-Liter	√
• Stress, Pulsatile Flow of Culture Medium			-
• Innervated and Vascularized			-
• 2-D	-	3-D	-

Three Pillars of Tissue Engineering

Organs

Cell Proliferation and Differentiation

Cells

- Determination of the Appropriate Cell Type
- Identification of Sources
- Isolation and Purification, Cultivation Techniques
- Design of Bioreactors
- Storage and Preservation Techniques

Biomolecules

- Stimulation of Growth and Differentiation
- Ways of Application
- Regulation of Activity

Scaffold

- Directed 3D-Growth
- Directed Vascularization
- Delivery of Nutrient Oxygen, Developmental Stimuli
- Disposal of Metabolic Waste
- Mimicry of ECM

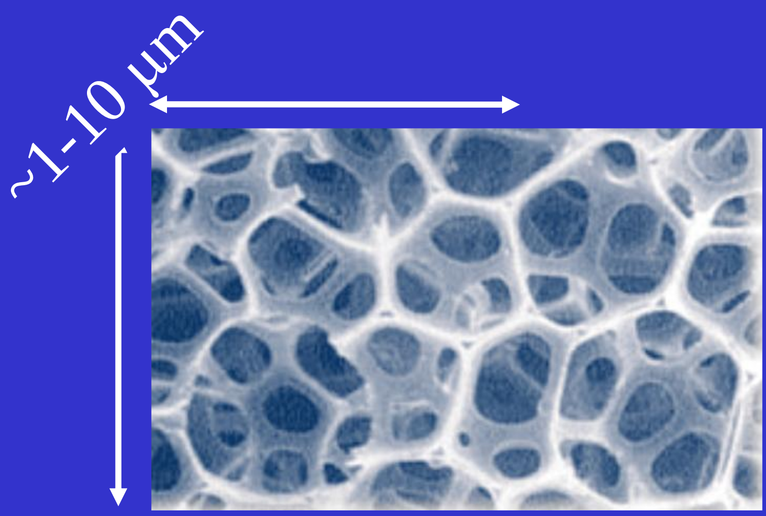
One Pillar Alone Not Enough to Support Tissue Growth, << Organs
Can One Technique Do Everything?

Conventional Tissue Engineering

Scaffold ⟶ Cell Seeding ⟶ Tissue Function

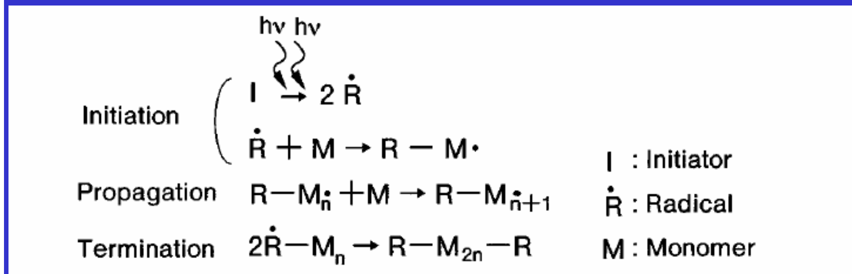
"Ideal" Tissue Scaffold

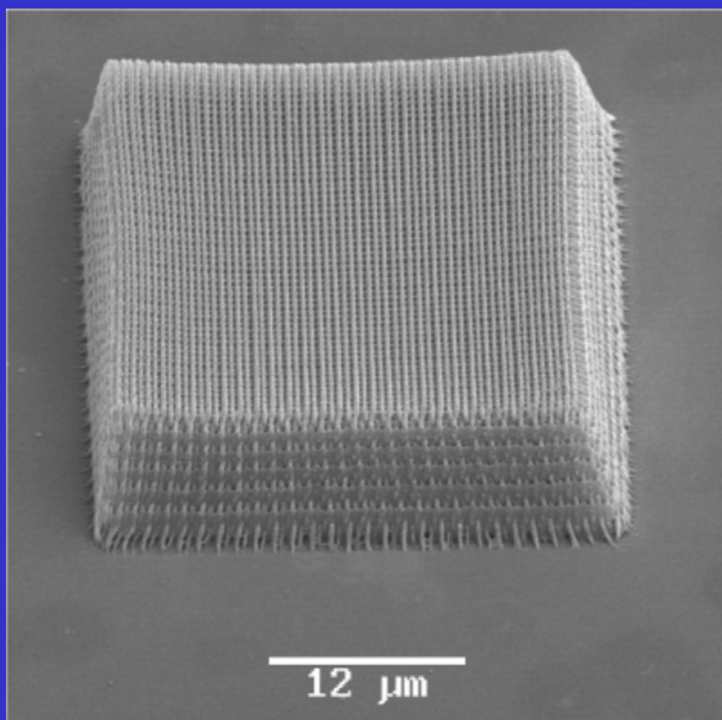
- Undefined
- 3D Matrix
  - Structural Integrity
  - Flexibility in Shape, Size, Pore Size, Overall Dimensions
  - Materials Used, Recruitment Molecules, Cellular Attachment,
  - Amenable to Vascularization and Adherent Stacking
  - Variable Bioresorbability



Can Ideal Scaffold Be Achieved?

Two Photon Induced Polymerization

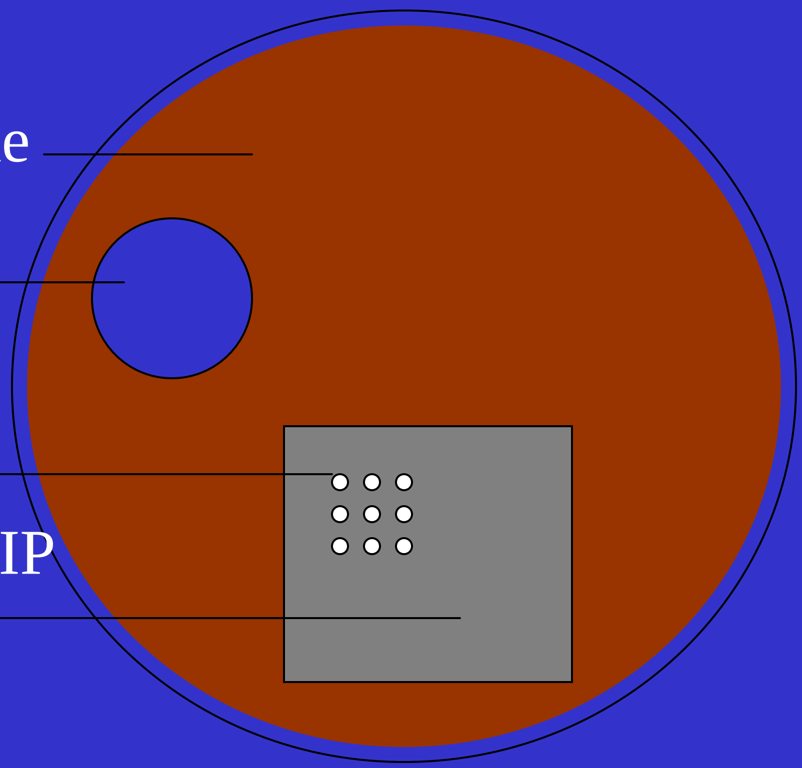




- Quadratic Dependence of Pulse Energy
- High Resolutions (100 nm), Ease of Fabrication,
- Rapid Prototyping Technique
- Applications: Optical Storage, Waveguides, Photonic Bandgaps, Microrotors, Microtweezers

Designer Scaffolds Using Chemically Designed Organic/Inorganic Hybrid Polymers

Biocompatibility Test Protocol



<u>Cell Types Used</u>

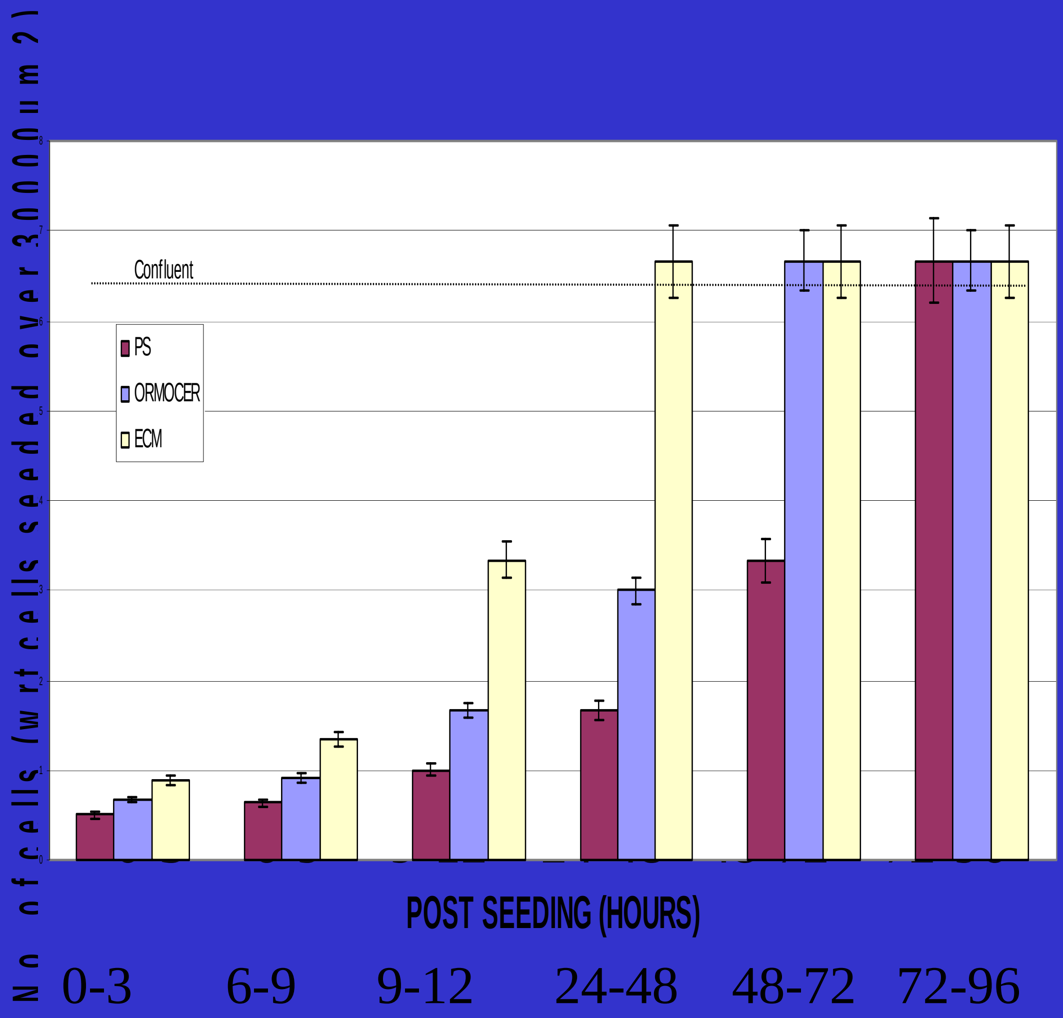
-C2C12

-B35

-HT1080

Compare Ormocer Growth to Common Substrate Materials!

Growth and Proliferation - B35



Universally Functional Ormocer Scaffold
"LEGO" Stacking Tissue Components

Cortex

Epithelial

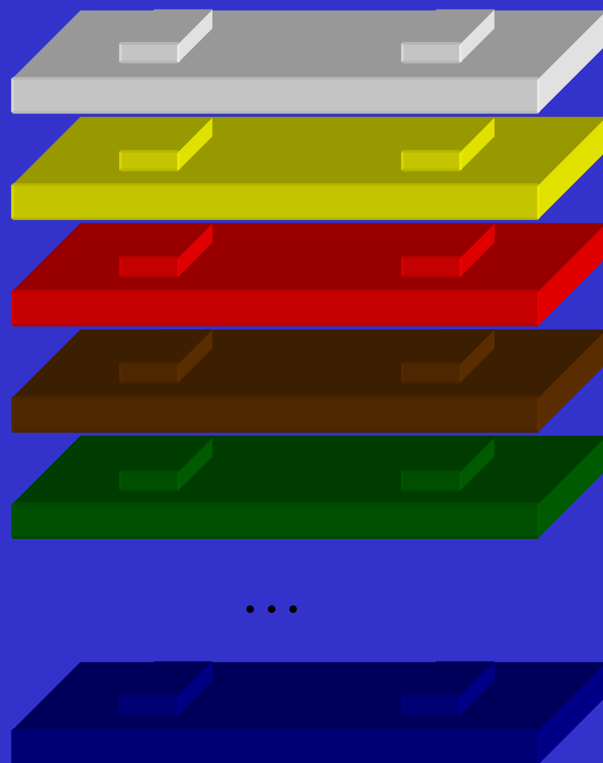
Vascular Network

Neural Communication

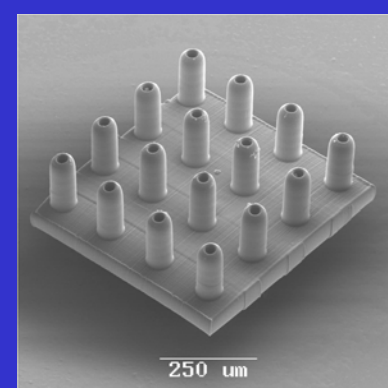
Endothelial

(Misc. Components)

Organ Specific Cells



- Common Scaffold Structures at Mesoscopic Scale
- Each LEGO Has Different Functionality
- CAD LEGO Design



Plug'n Play Tissue Components at Mesoscopic Scale

Conclusions

• Laser Processing Offers a Diverse Set of Materials Interactions

• MAPLE-DW Processing Enables Biomaterials and Living Cells to be Printed on a Micro/Mesoscopic Scale.

• Issues with Extension of MAPLE-DW to Thick Structures.

• Suggestion: Similarities Exist Between Biomanufacturing and Electronics Manufacturing/Rapid-Prototyping.

Potomac Photonics	*A. Pique*	*B. Ringeisen*
Superior Micropowders	*H.D. Wu*	*D. Young*
D. Chrisey	*R. Modhi*	*R.C.Y. Auyeung*