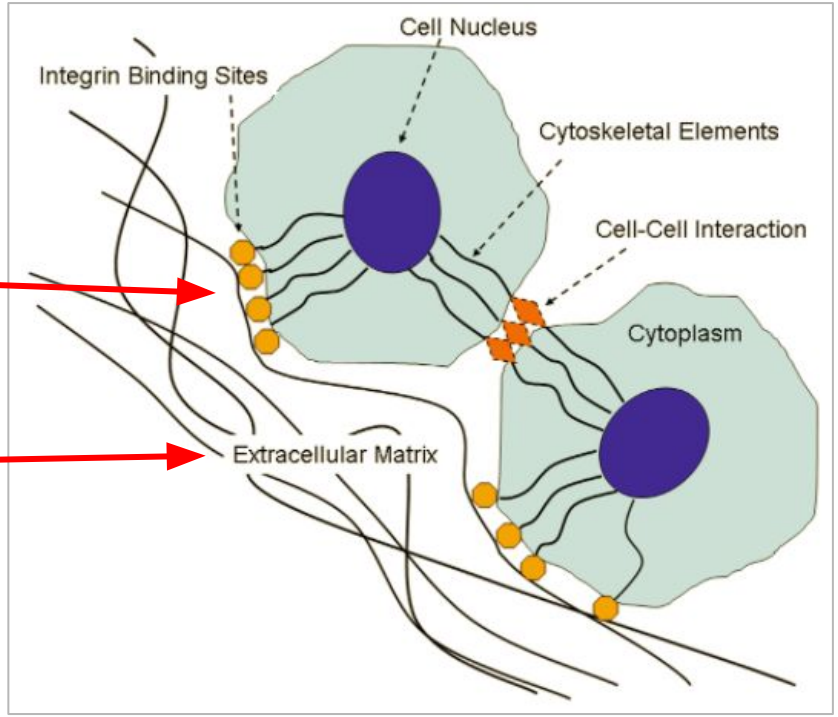
Direct Patterning of Proteins with the Alvéole PRIMO

Erica Castillo & Joy Franco, PhD Candidates, Mechanical Engineering
Swaroop Kommera, SNF Mentor
Gaspard Pardon & Leeya Engel, Post-doctoral Mentors

E241 | 5 DEC 2017

A simple, close look into a cell's environment…



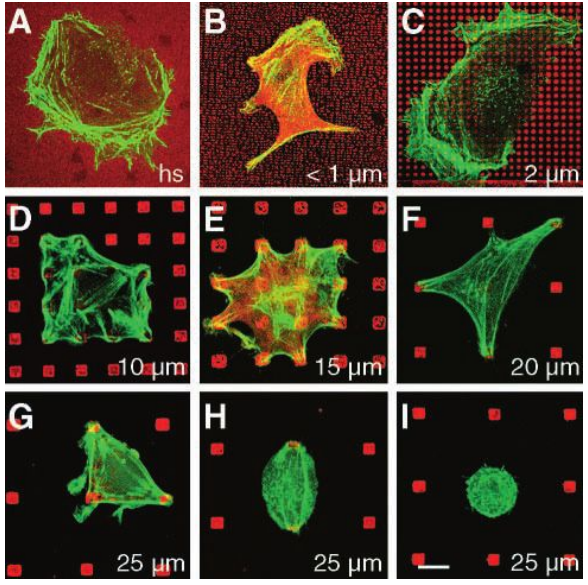
Sell, S. A., *et al.* *Polymers* **2(4),** 522-553 (2010)

Cells adhere/bind to proteins in the extracellular matrix (ECM).

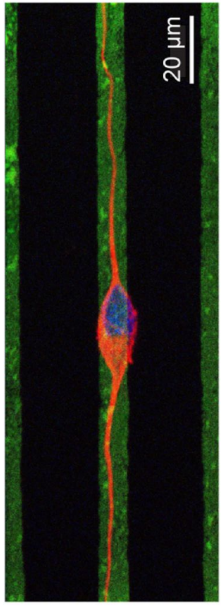
Why pattern protein? - Controlled cellular microenvironment

Def. protein patterning - “Protein immobilization within specific locations in a 2D or 3D space” (Blawas 1998)

Cellular morphology

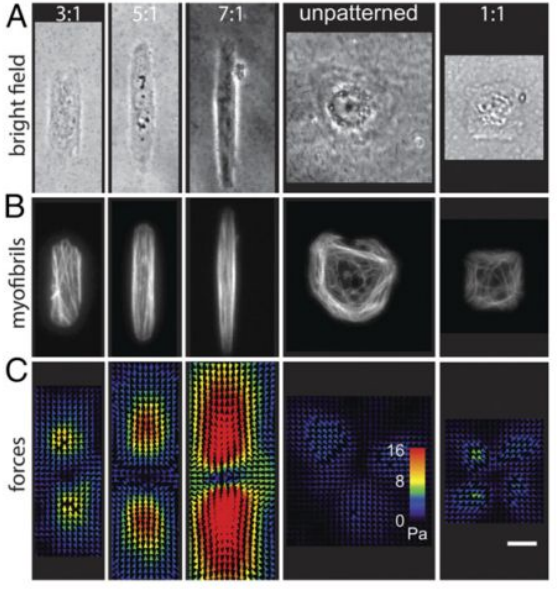


Lehnert *et al.*, J. Cell Science 2004



Grevasse *et al.*, Scientific Reports 2015

Mechanical Output

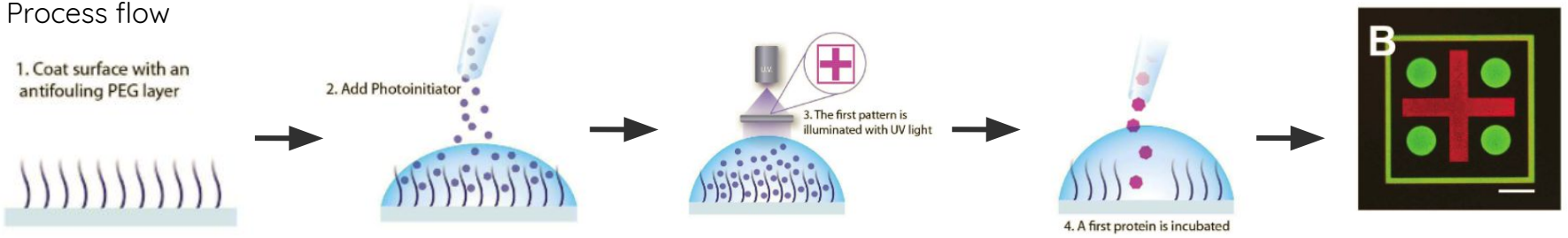


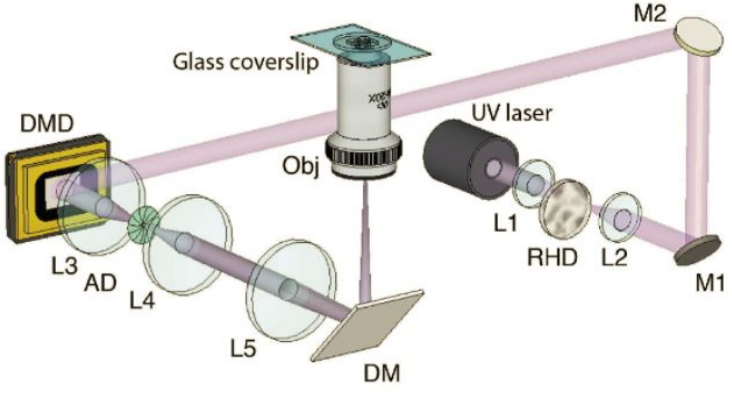
Ribeiro *et al.*, PNAS 2015

Blawas, A. S. & Reichert, W. M. *Biomaterials* **19,** 595–609 (1998)

A new approach to µ-patterning protein: Alvéole PRIMO

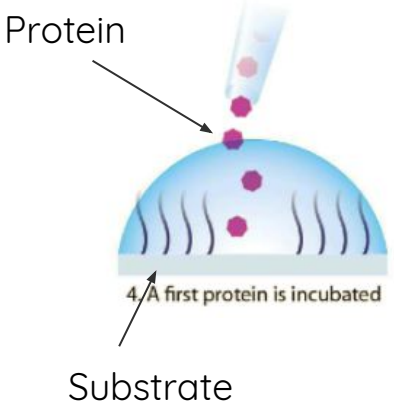
Process flow





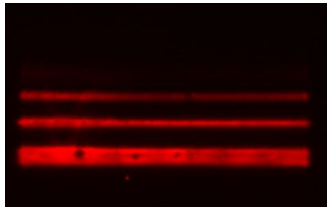
Strale, P.-O. *et al. Adv. Mater.* **28,** 2024–2029 (2016).

Characterizing ease of use // making μ-patterning accessible to all

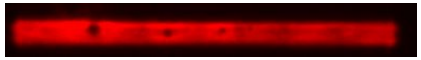
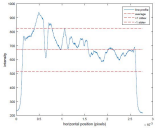
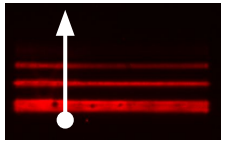
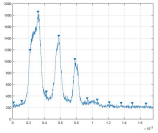
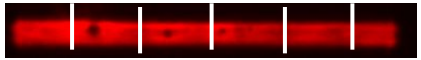
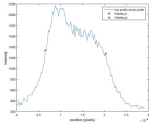


Protein | Substrate Matrix	Glass	TEM Carbon Grids
Peanut Lectin (Fluo)	❑ Study neurons ❑ Integration of new protein ❑ Resolution Study	❑ Study protein structure ❑ Integration of new substrate ❑ Feasibility
ELP-RGD (Fluo)	❑ Engineered protein ❑ Integration of new protein ❑ Feasibility	X

Image Analysis - Overview

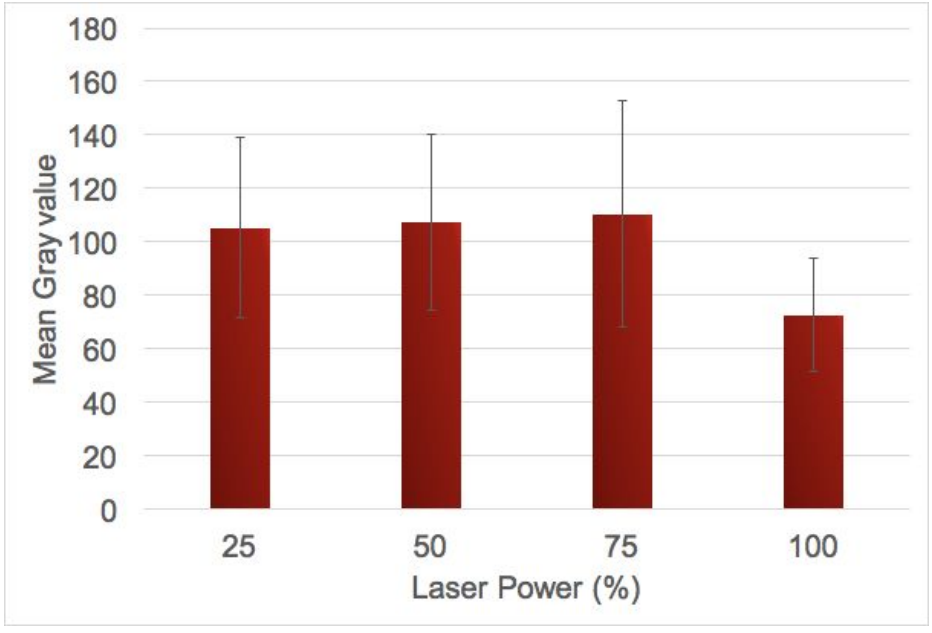


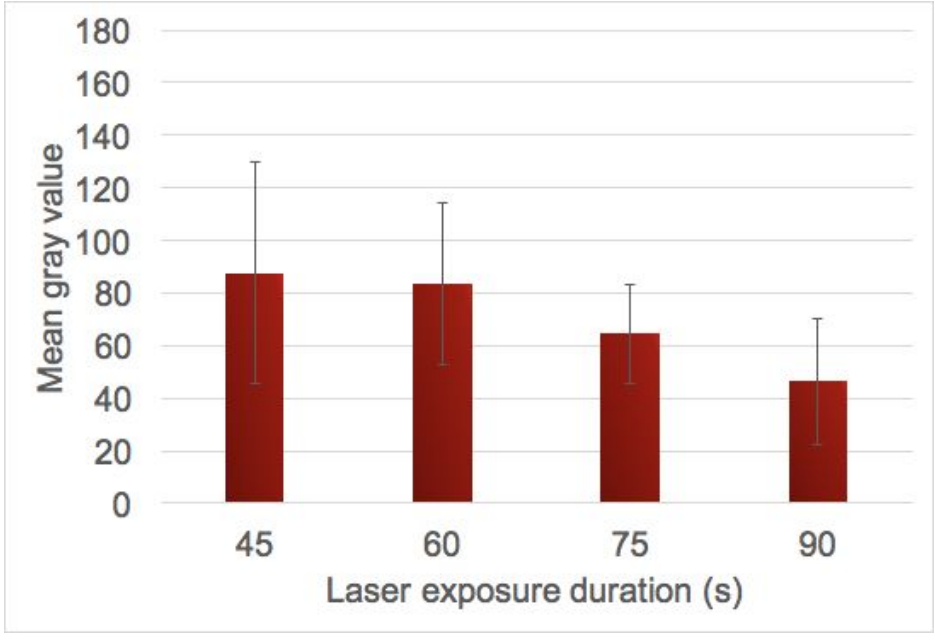
Software:
ImageJ
Matlab Scripts

Parameter	Analysis Output	Ideal Trend Desired
Area Uniformity 	Standard deviation 	**Minimize STDEV** Less spread
Resolution 	SNR = Imax/Inoise 	**Maximize SNR** obtain smallest resolution
Line Width Consistency 	Standard deviation 	**Minimize STDEV** Less spread

Strale, P.-O. *et al. Adv. Mater.* **28,** 2024–2029 (2016).
Blawas, A. S. & Reichert, W. M. *Biomaterials* **19,** 595–609 (1998)

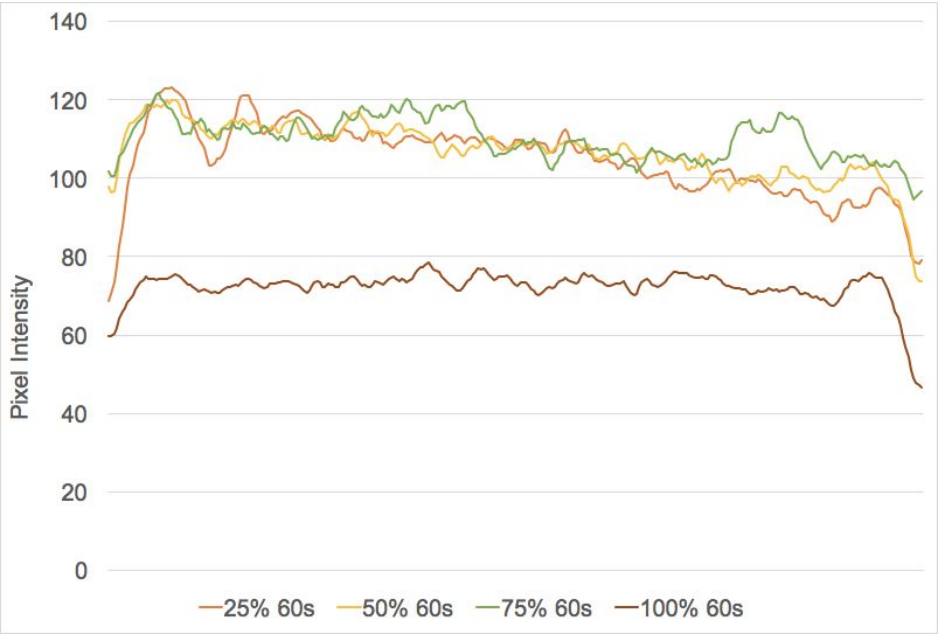
Laser power and duration do not affect average protein signal from patterns

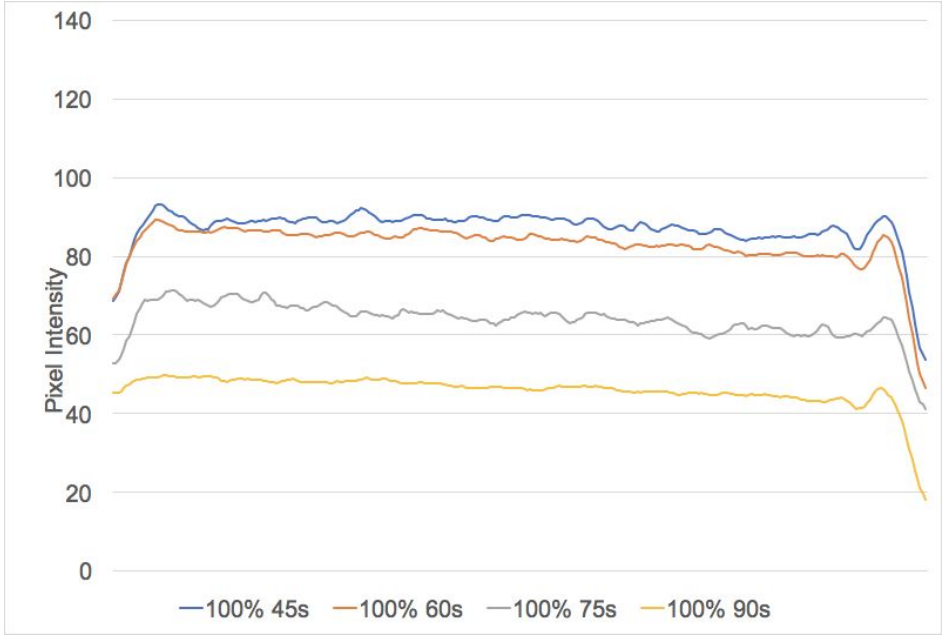


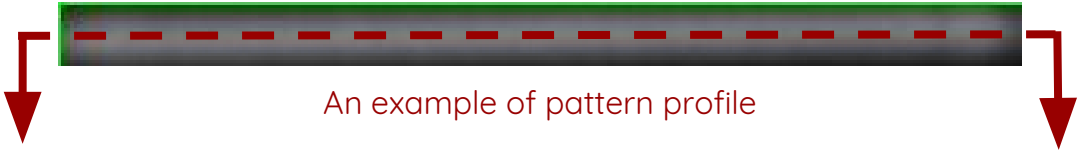


An example of pattern uniformity

Higher laser power and longer duration may improve signal uniformity

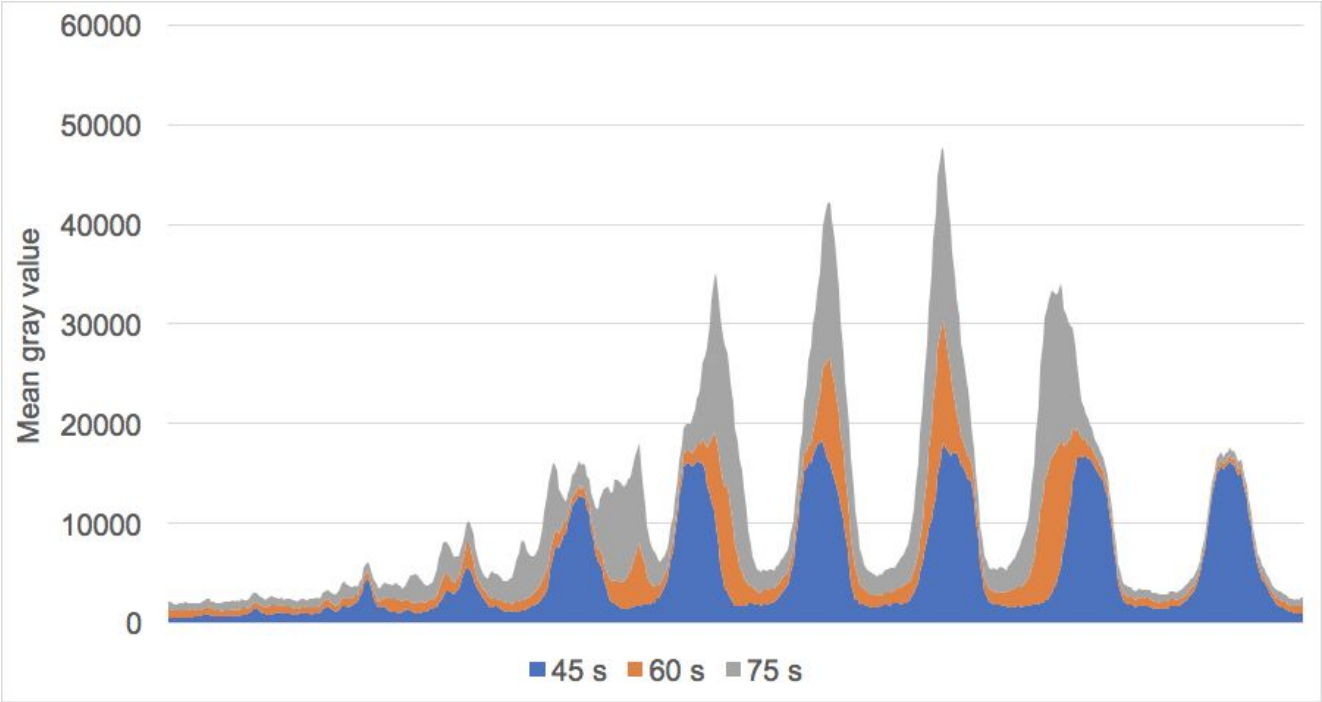


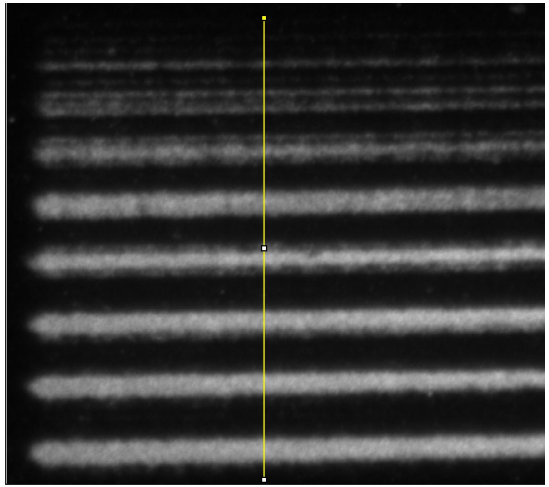




An example of pattern profile

Shorter exposure duration results in narrower lines

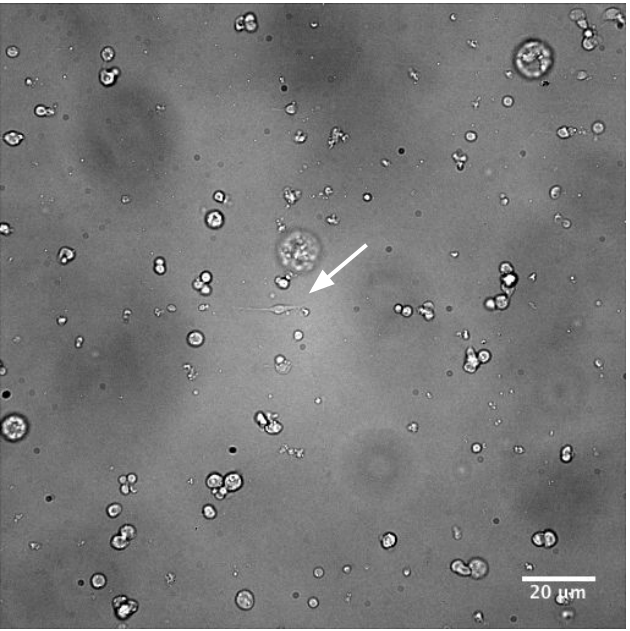




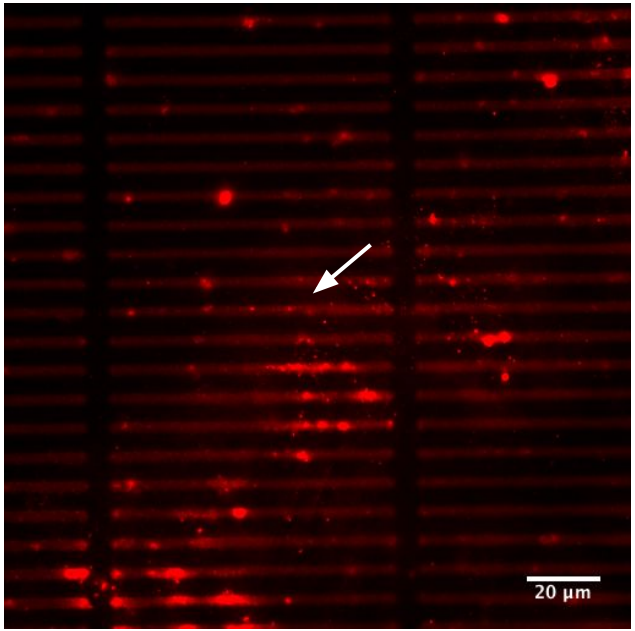
An example of acquiring a line width profile

Proof of concept: Cells can grow on patterns

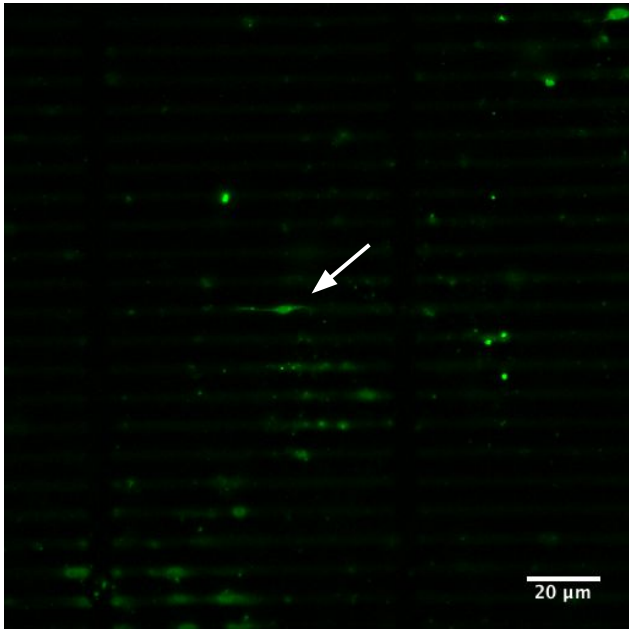
Transmitted light



Fluorescent red pass filter

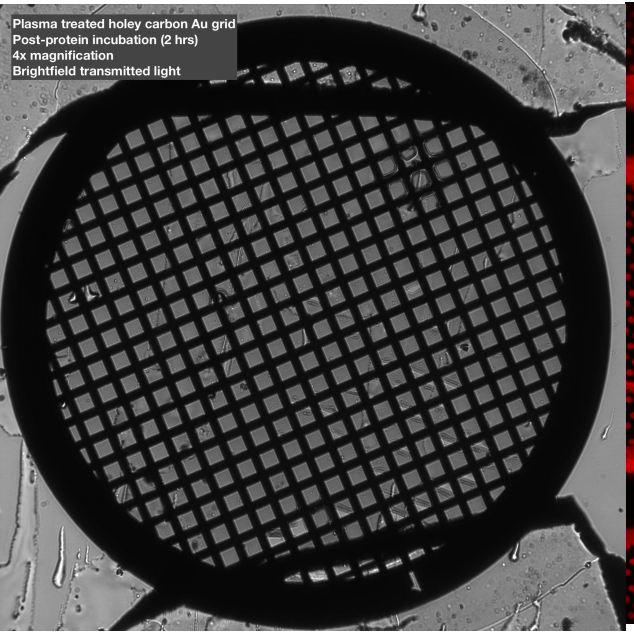


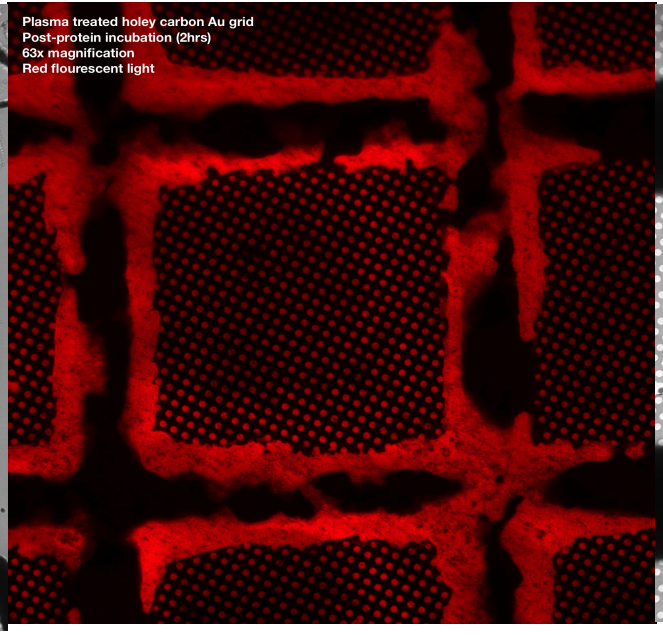
Fluorescent green pass filter

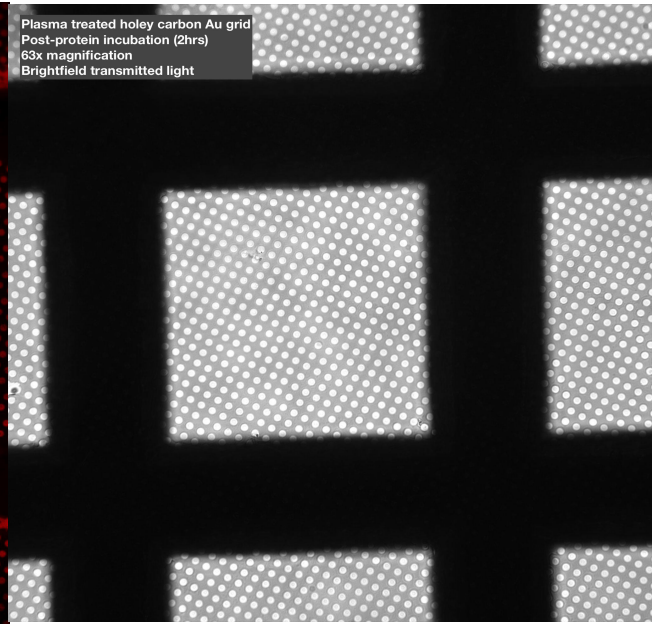


White arrow is pointing to the known location of a GFP (+) cell

Peanut Lectin | TEM Carbon Grids





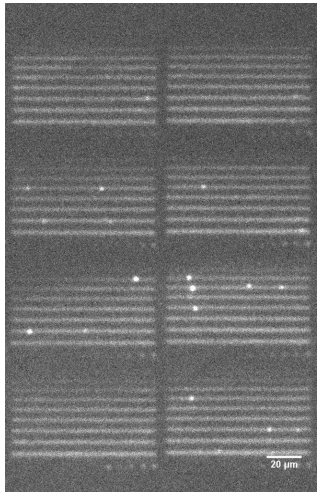


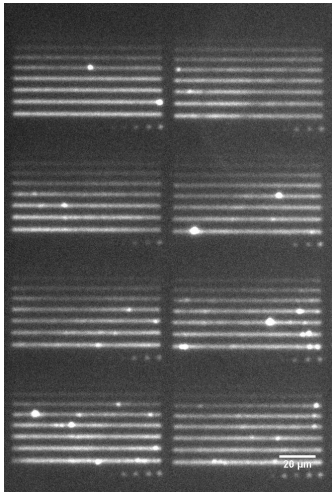
- With some handling practice, the grids (12 nm carbon on Au grid) can withstand all treatment steps
- Imaging the grids presents the greatest challenge
- For future users:
  - Adjust protein incubation time
  - Consider protein pH & salinity
  - Try first on glass coated in carbon
  - Use critical point dryer

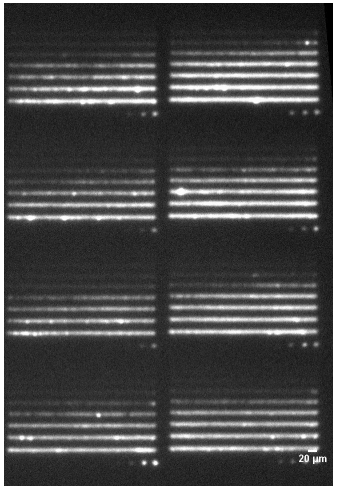
ELP-RGD | Glass

Objectives

- **Integration of new protein** - Engineered , recombinant, matrix-mimetic proteins that allow for additional tunability
- **Feasibility** - Used standard parameters (100% Power, 60sec exposure), n=3







What a novice can expect in the first two months of using Alvéole PRIMO:

- "Masks" can be made with ImageJ (open source software, popular in biology)
  - We recommend a minimum feature spacing of 15 pixels (~4.2 um)
- Glass must be very clean and particle free
- Plasma treating the glass improves the quality of patterning
- Maintaining PEG hydration throughout handling is critical
- Consideration must be given to protein solution
  - pH
  - salt concentration
  - Incubation time and temperature
- 100% power, 60 second duration is a good starting point, but individual users may want to optimize these parameters to improve
    - Uniformity
    - Resolution
- PRIMO is easier to learn and has fewer barrier to entries that lift off or microcontact printing
  - For large scale operations, Heidelberg lift-off (not direct writing) may be quicker
- Future users may want to try diluting the PLPP and increasing exposure time as an approach to improving minimum feature size

Thank you mentors!



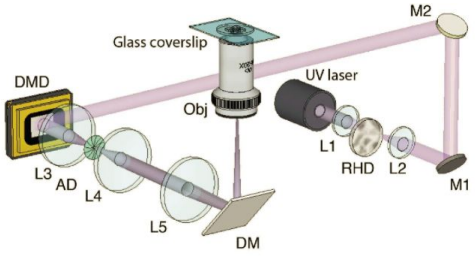




Stanford Microsystems for Mechanobiology

PI: Professor Beth Pruitt | Bioengineering | Molecular & Cellular Physiology

A new approach to µ-patterning protein: Alvéole PRIMO



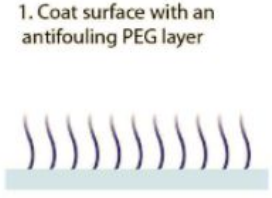
PRIMO components

UV light wavelength 375nm
DMD-based projection system

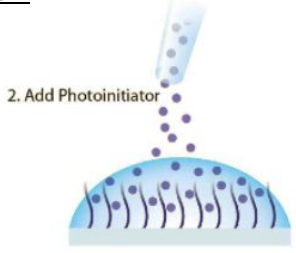
PRIMO features

Gradients (256 gray scales)
Multi-protein
1.2 um resolution
Alignment
Compatible with cells

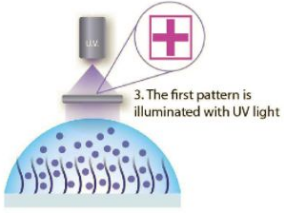
Substrate Preparation



Clean Substrate
Plasma
Incubation of PEG

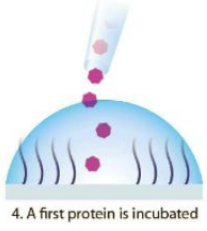


Thorough Rinsing
PLPP



UV light + PLPP =
Locally cleaves PEG

UV power
UV exposure time



Incubation of Protein

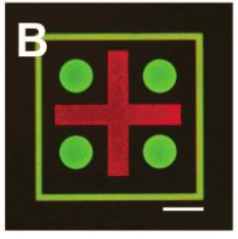


Image with
microscope

Strale, P.-O. *et al. Adv. Mater.* **28,** 2024–2029 (2016).