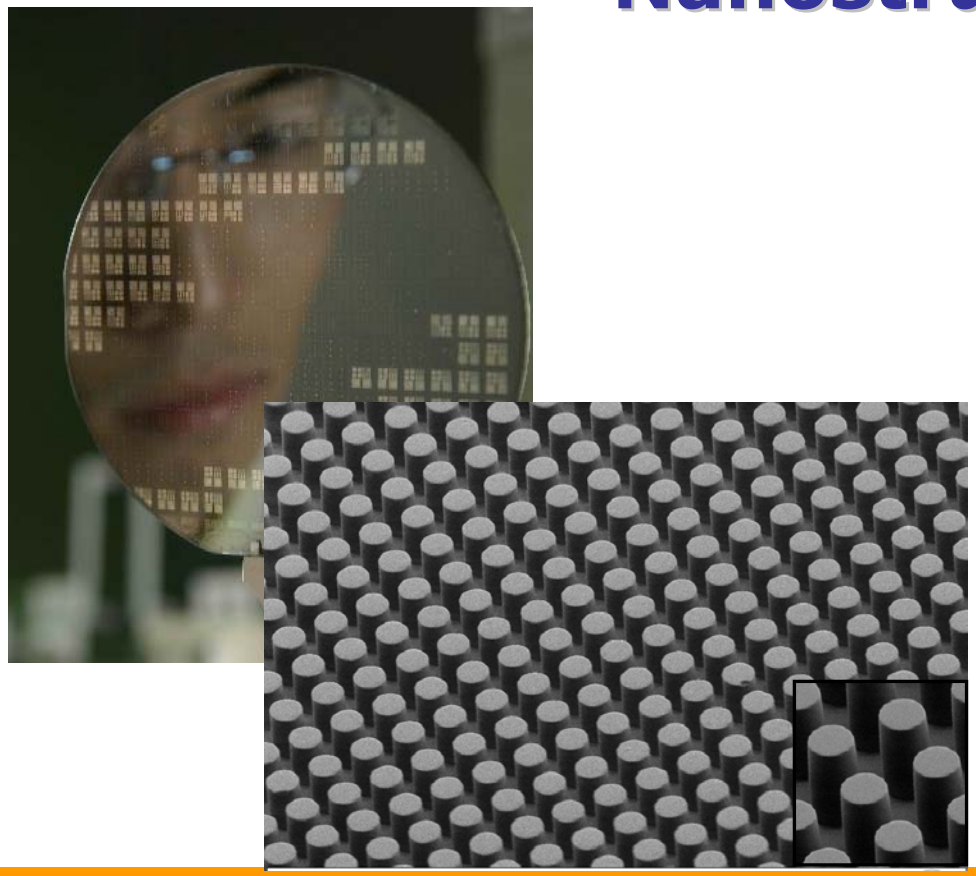
Spying on Cells: Cellular and Subcellular Analysis using Novel Polymeric Micro- and Nanostructures



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Boston University

US-Korea Nano Forum
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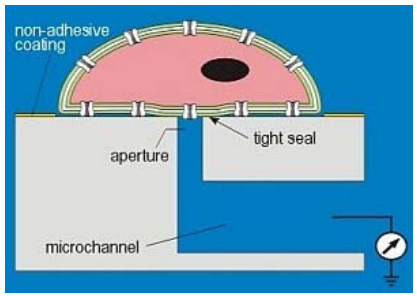
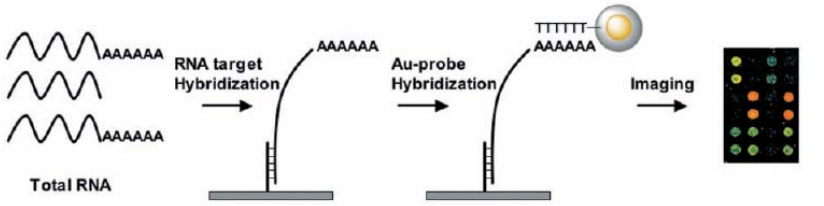
Road Map of Nanobio-sensors

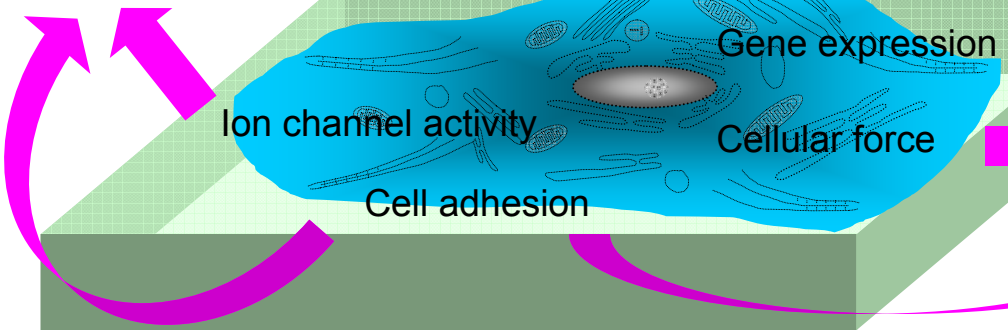
- How can we best monitor living cells in-situ and continuously to understand, characterize, and model functional behavior at the cellular levels so as to explore biosensor specificity and flexibility for distinct responses to different combinations of stimuli?
- Many key problems in biochemical sensing can be solved by converting biological or chemical response to an electrical, optical, or mechanical signal using micro/nanosystems.
- The use of living cells as sensor elements provides the opportunity for high sensitivity in a broad range of biologically active substances and physical stimuli that affect cell responses.

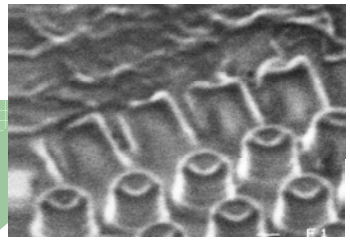


Nanoelectrical sensors



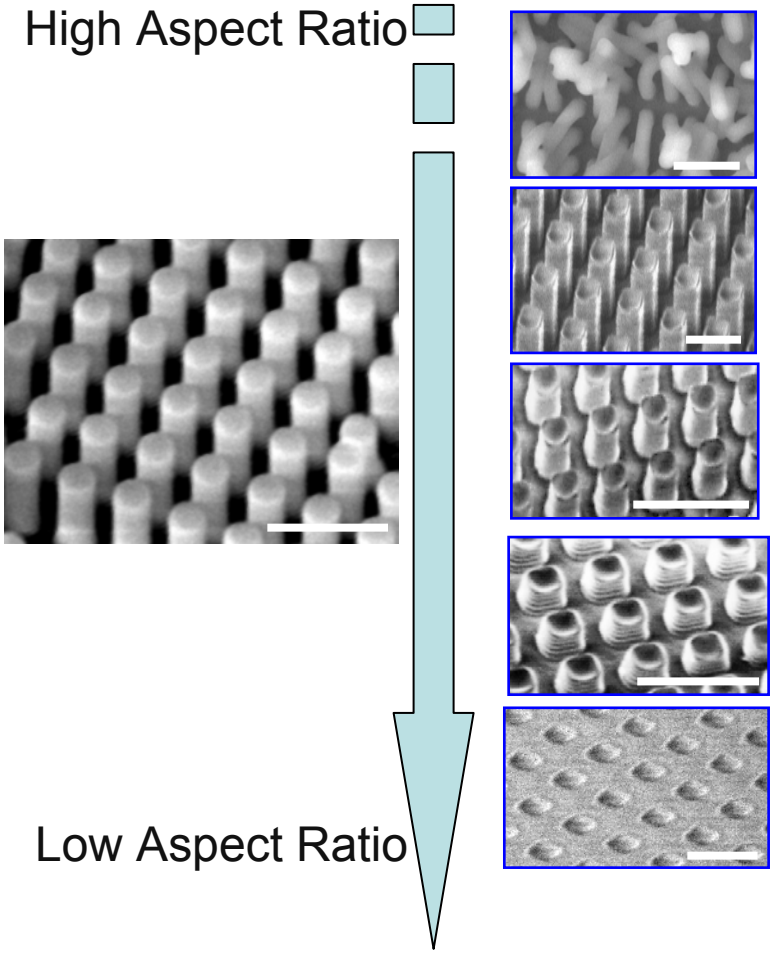
Nano-optical sensors

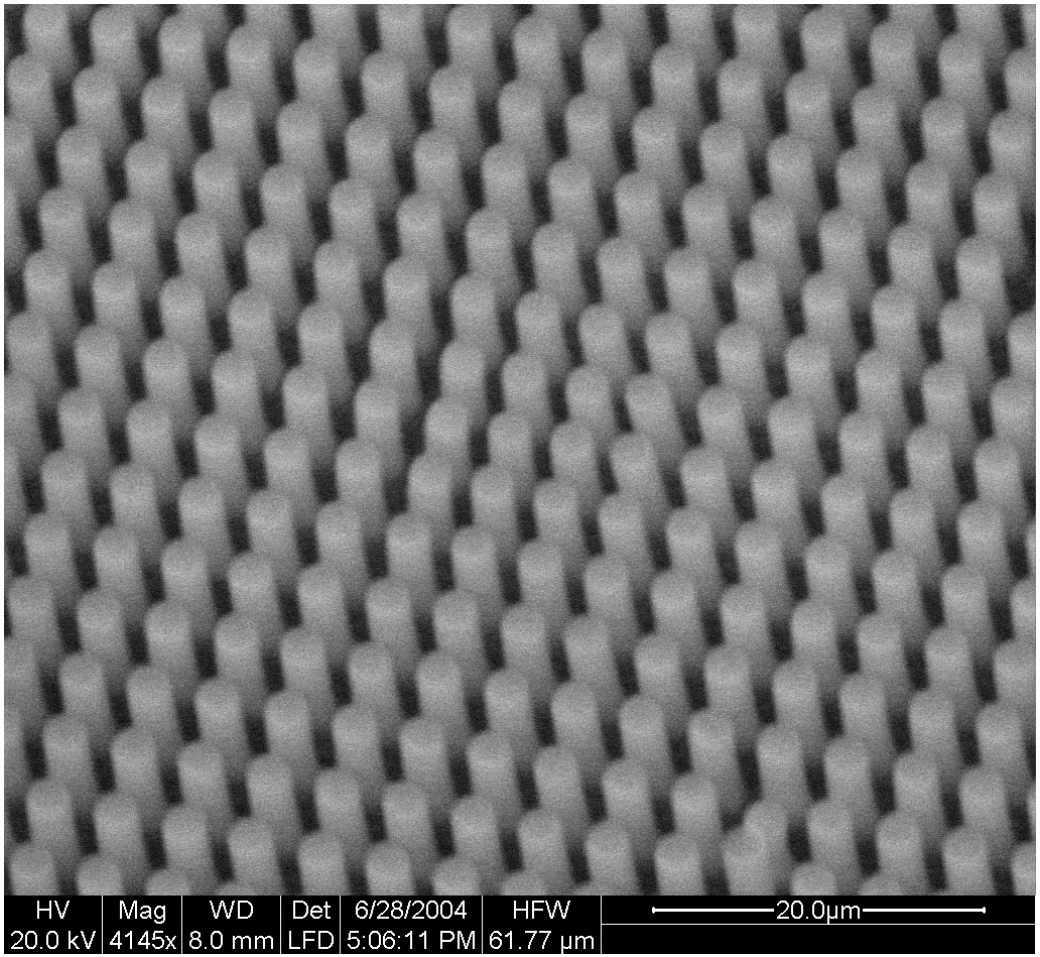




Nanomechanical sensors

Polymer Pillar Array

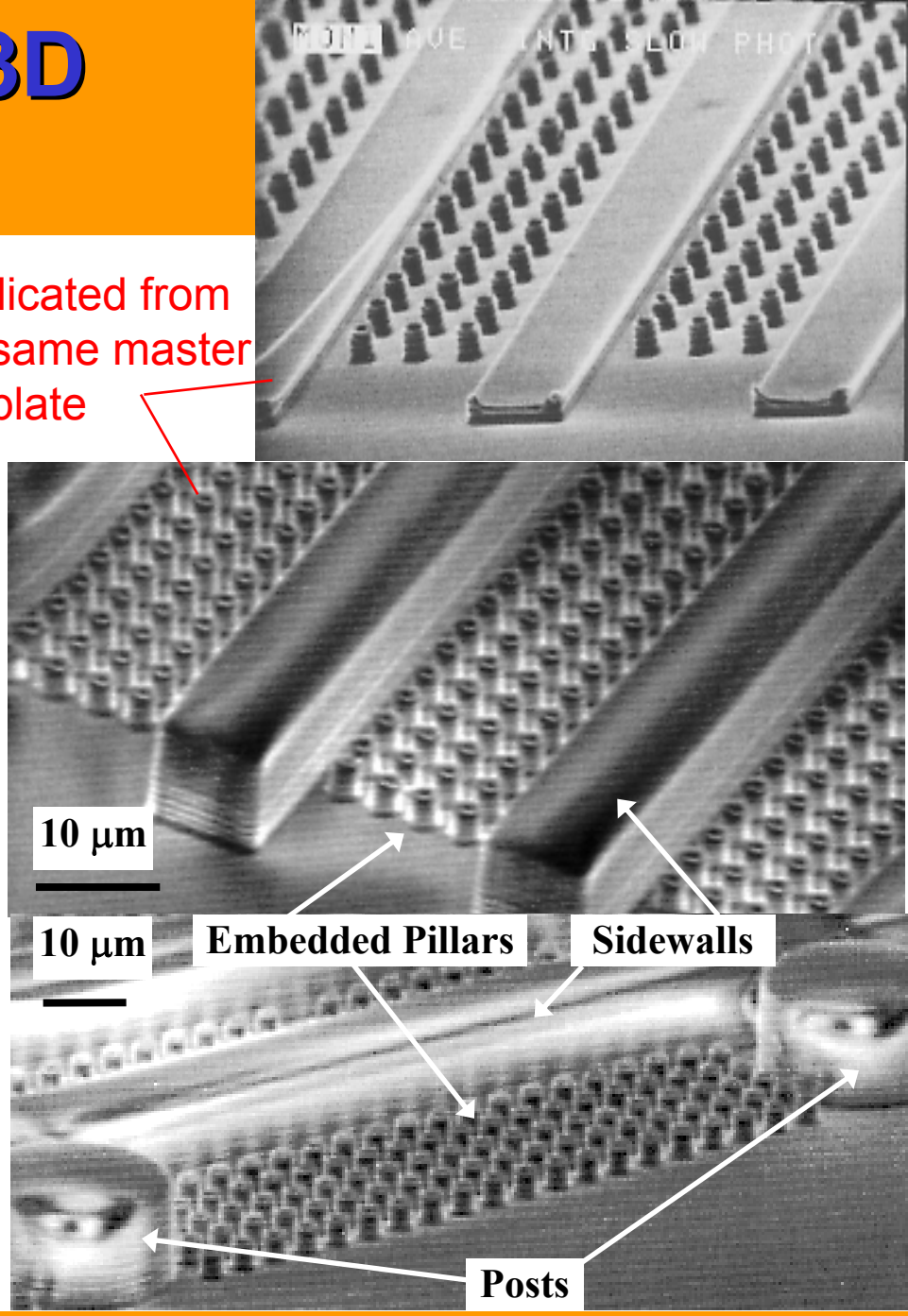




Realization of 3D Structures

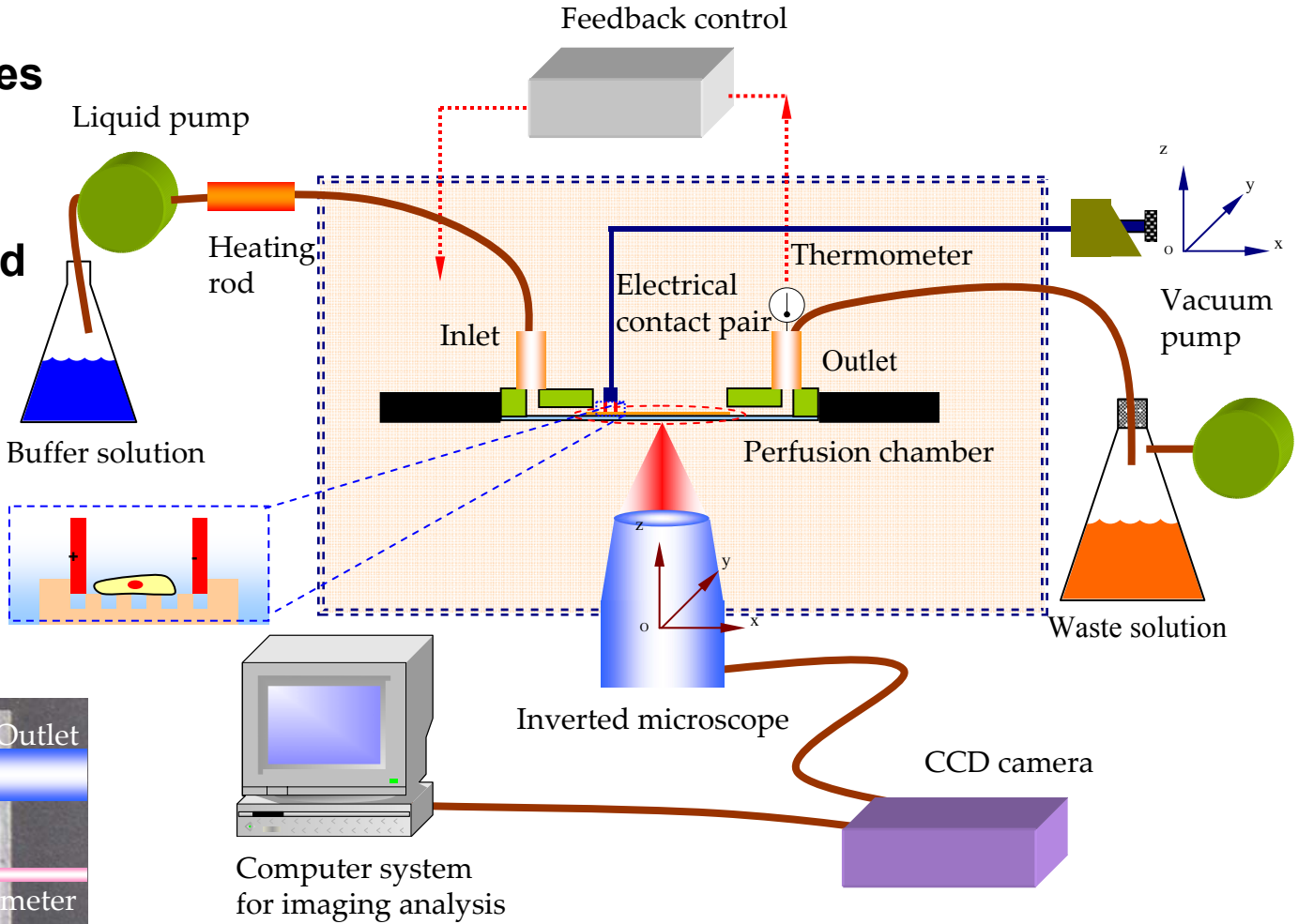
- Utilizing the micromolding process, complex structures, varying in both lateral dimension and height, are fabricated.
- Elevated sidewalls are to provide vertical surfaces for cell attachment.
  - This may avoid the artificial polarization of cells induced by conventional dishes, thus allowing a more in-vivo-like cellular morphology.
- Polymeric posts placed between the sidewalls are to further enhance cell attachment.

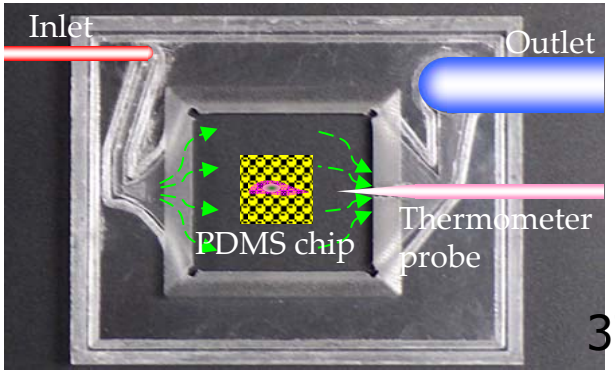
Replicated from the same master template



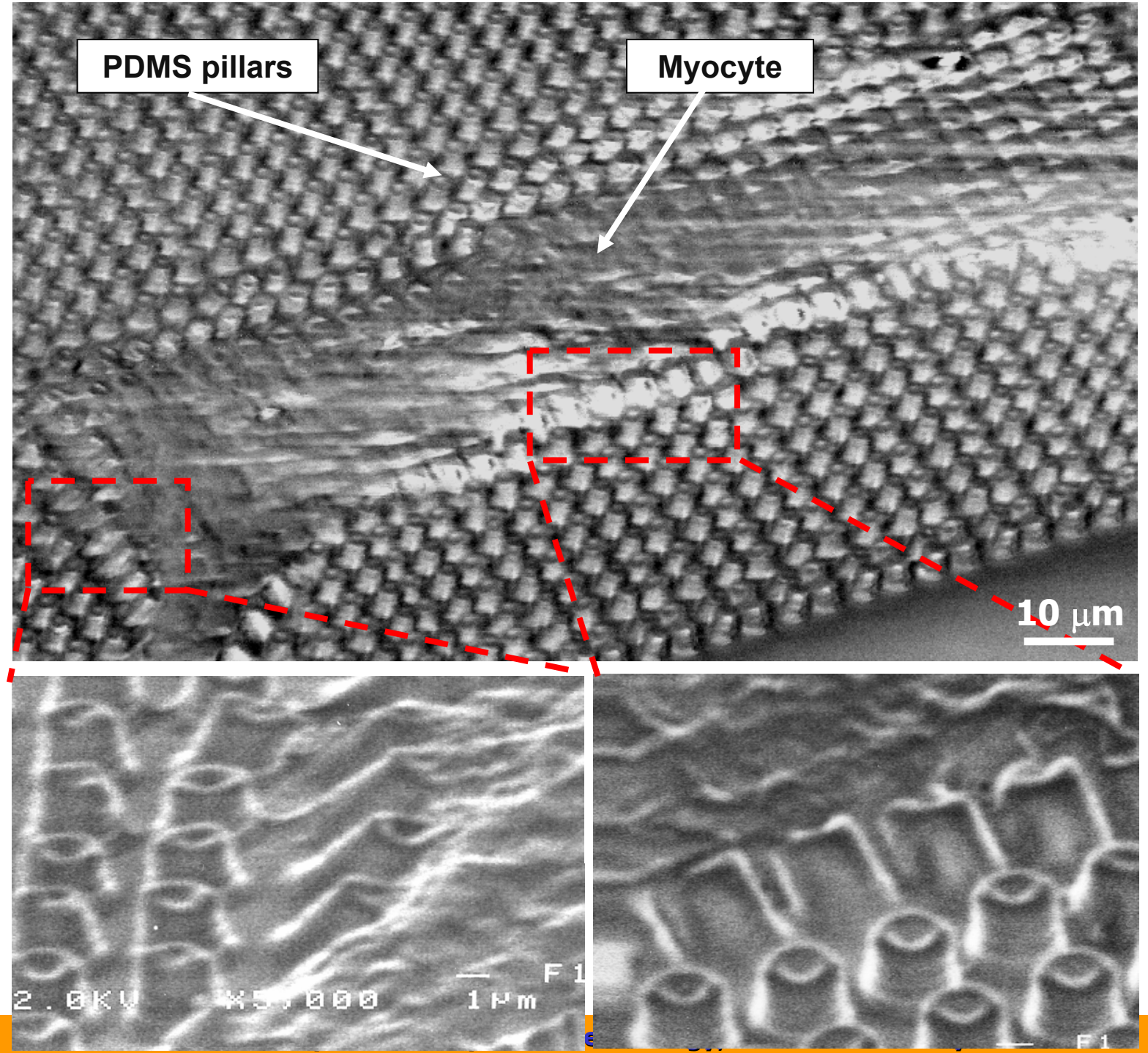
Experimental Setup for Cellular Force Measurement

- **The cardiac myocytes were isolated from Wistar rats**
- **The cells were plated on the fabricated structures**
  - ✓ Fluidic Connection
  - ✓ Electrical Connection
  - ✓ Inverted Microscope

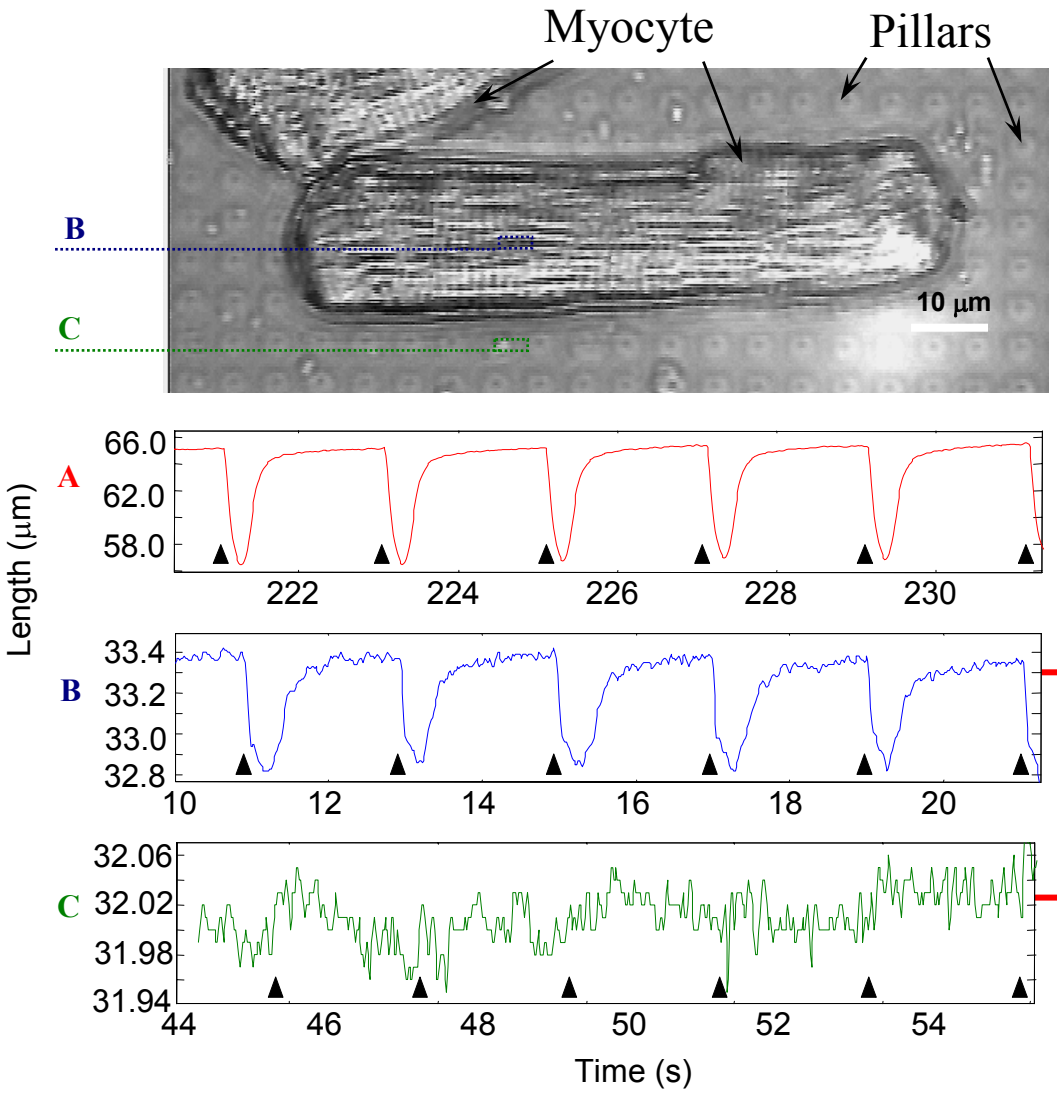




37° C; Real time; Live cell; CO_2 preferable gas concentration


PDMS pillars
Myocyte
10 μm
2.0KV X5,000 1μm F1
F1

Deformation Isolation between Cells and the Base Substrate



Conditions

- The isolated myocytes were plated on a PDMS substrate with pillars of aspect ratio 2:1, allowing 24 hours for adhesion.
- The myocytes were stimulated by a digital pacer with a periodical voltage pulse (DC 20V at 0.5 Hz), which provided an additional electrical potential besides the action potential of the myocytes to activate the contractile proteins.

The underlying pillar has periodical displacement with the cell contraction

The pillar away from the cell does not represent a obvious periodicity. The displacement is on the noise level.

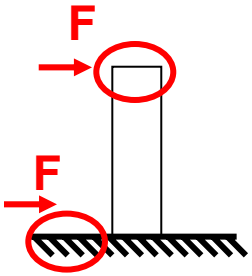
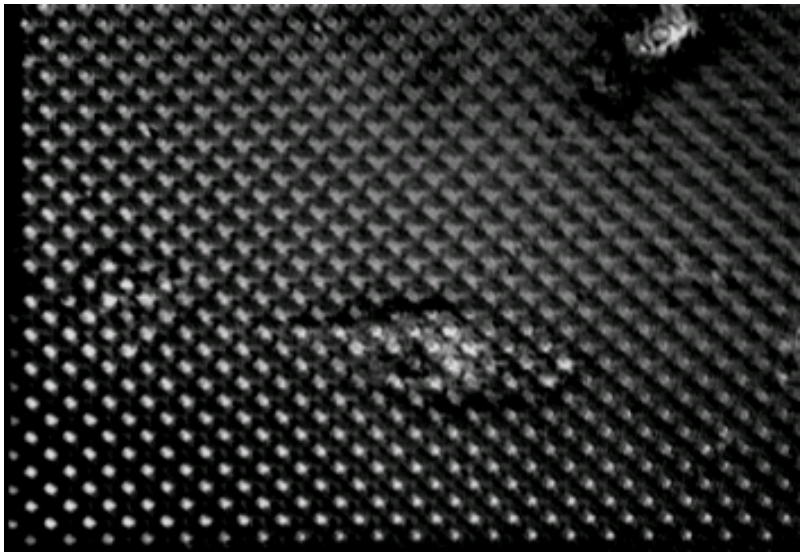
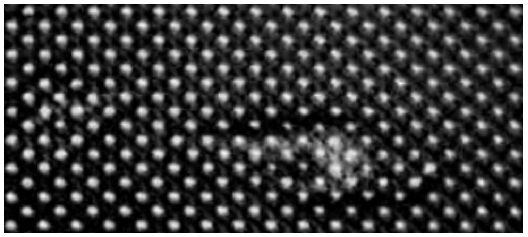
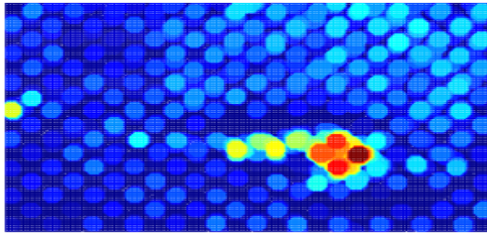


Image Processing for Cellular Force Measurement

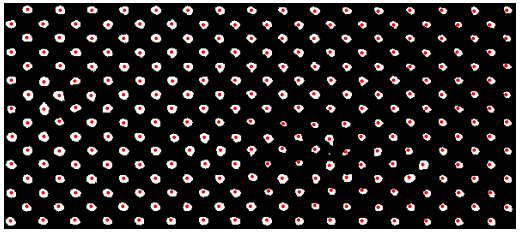




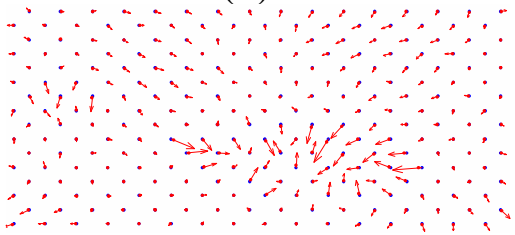
(a)



(b)



(c)

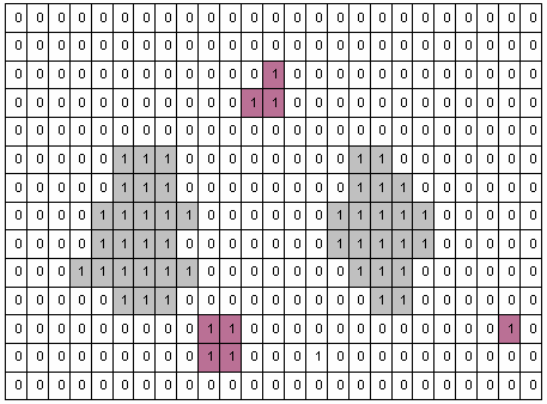


(d)

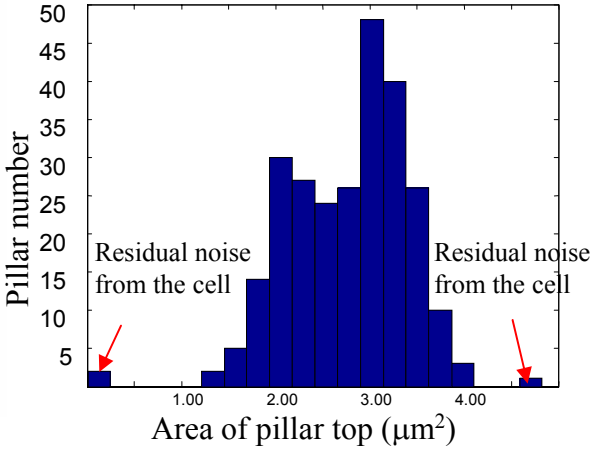
Image Processing

- Extract and remove background nonuniformity
- Applying thresholding to the image
- Locate individual pillars
- Compare derived pillars array with a reference
- Derive the deformation map and force map

Binary array



Histogram

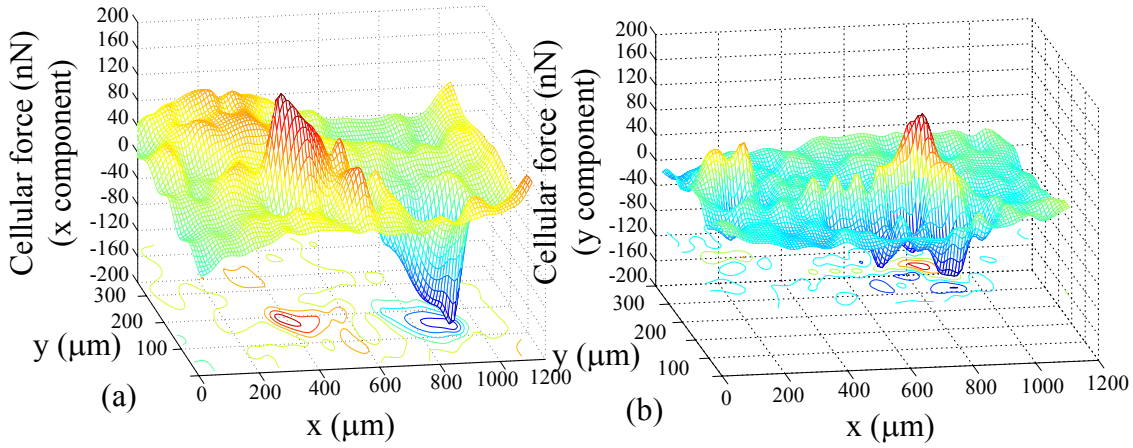


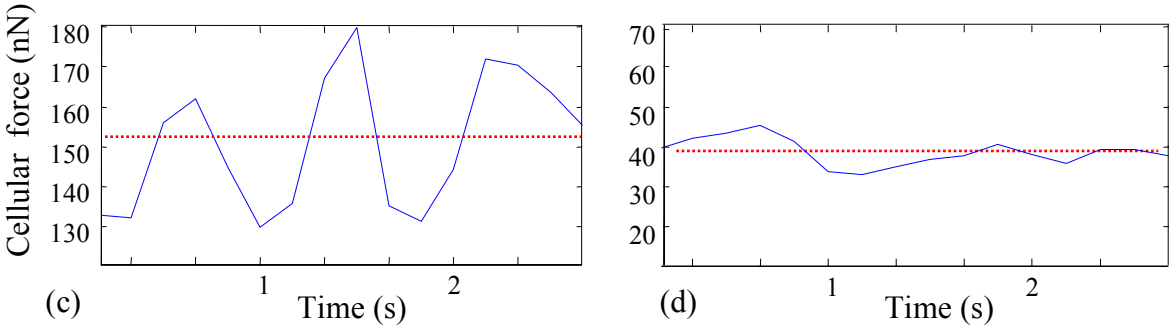
Contraction Force Analysis

Force measurement with subcellular resolution

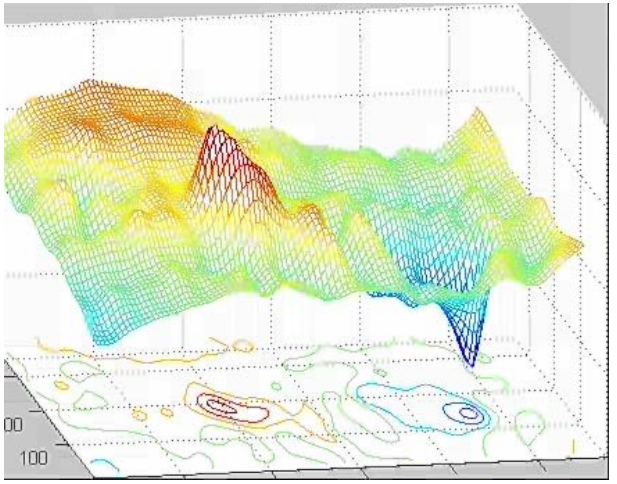
Force component along contraction axis

Force component along transverse axis

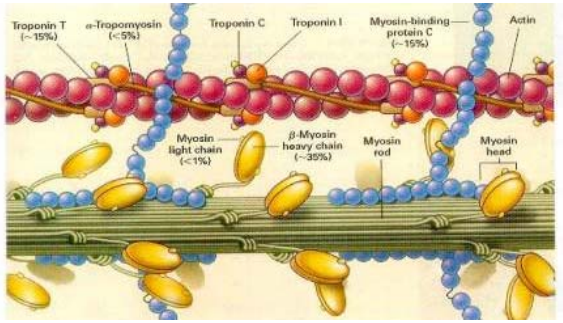




Force evolution measured in real time



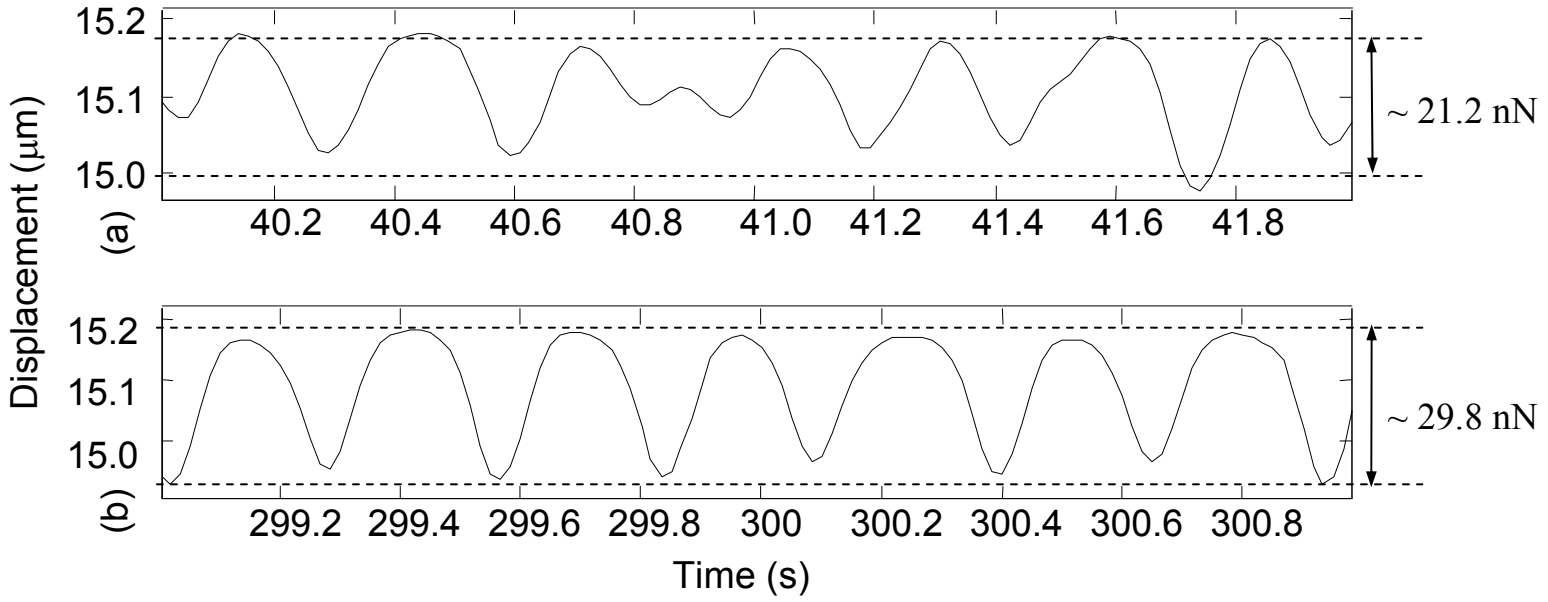
The force evolution reveals the alignment of motile units in cardiac myocyte, which conforms to the physiologic fact.



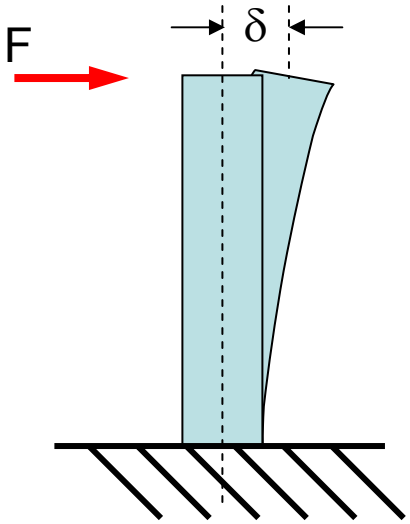
Force Evolution during Chemical Perfusion

- **Chemical Sensing**
  - Drug evaluation
  - Cell mechanics study
  - Pathology investigation
  - etc.

- Validation of the inotropic effect of the cardiac myocytes in response to the β-adrenergic stimulation
- Currently validated by an increase of inward calcium current, a greater rate of release of calcium ions from the sarcoplasmic reticulum (SR), and an accelerated reuptake of calcium into the SR



Nanoscale Biomechanosensor

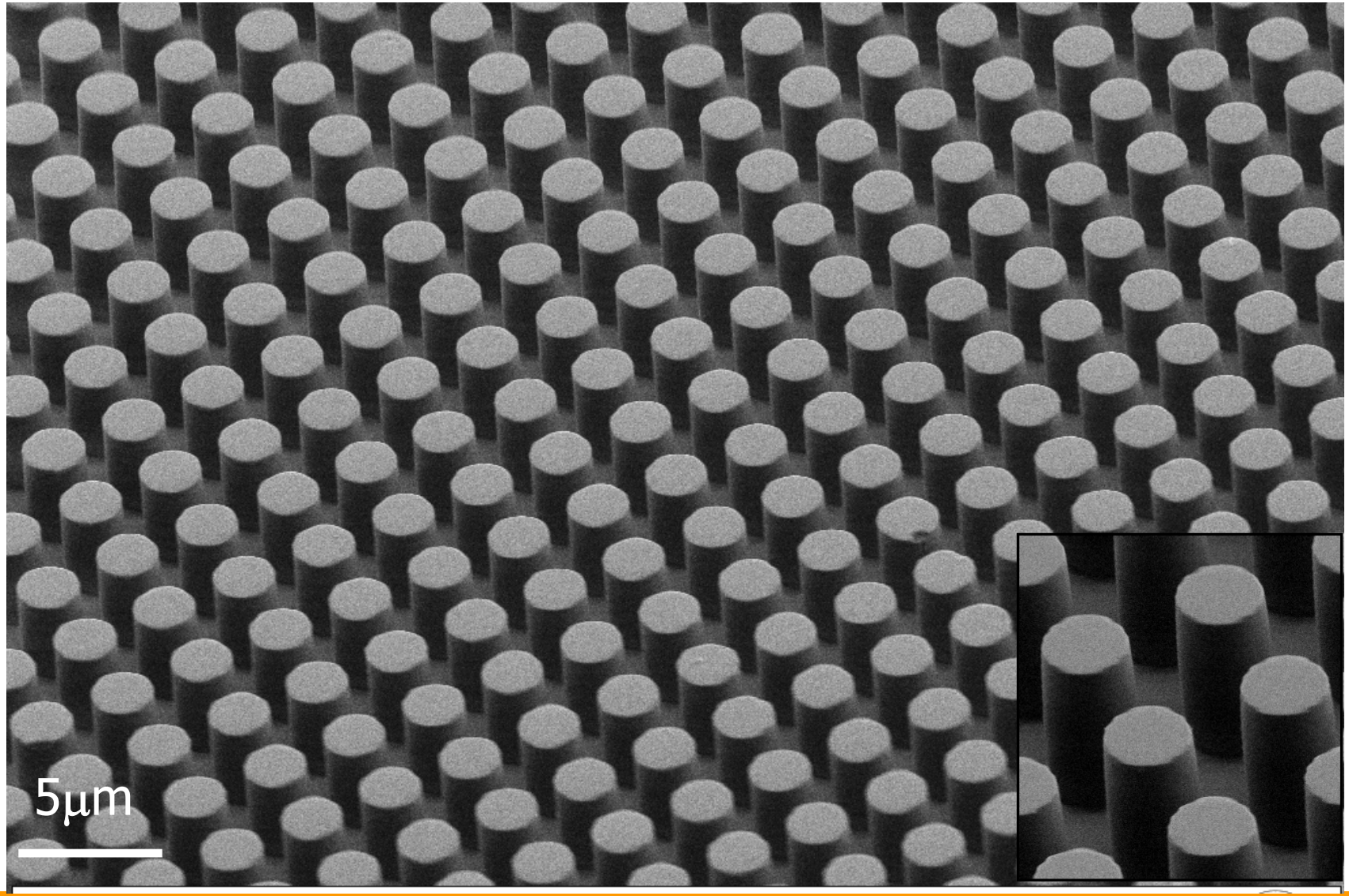


- **It is sensible to downsize the microfabricated structures to nanoscale:**
  - To enhance the probing sensitivity
  - To enhance the spatial resolution
  - To improve the material compatibility
- Direct optical measurement is no longer appropriate

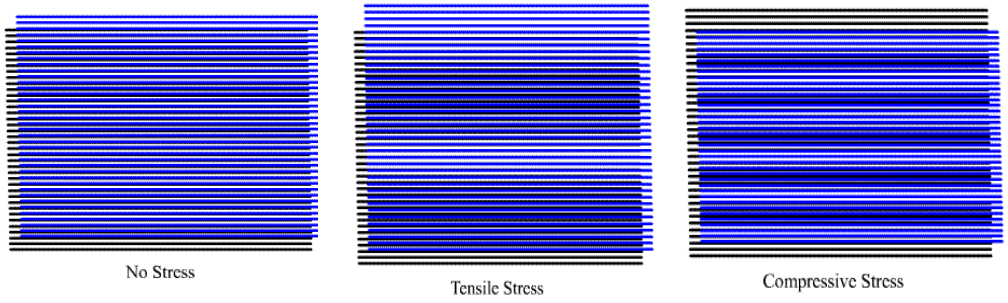
$$\lambda / 2n\sin(\alpha)$$

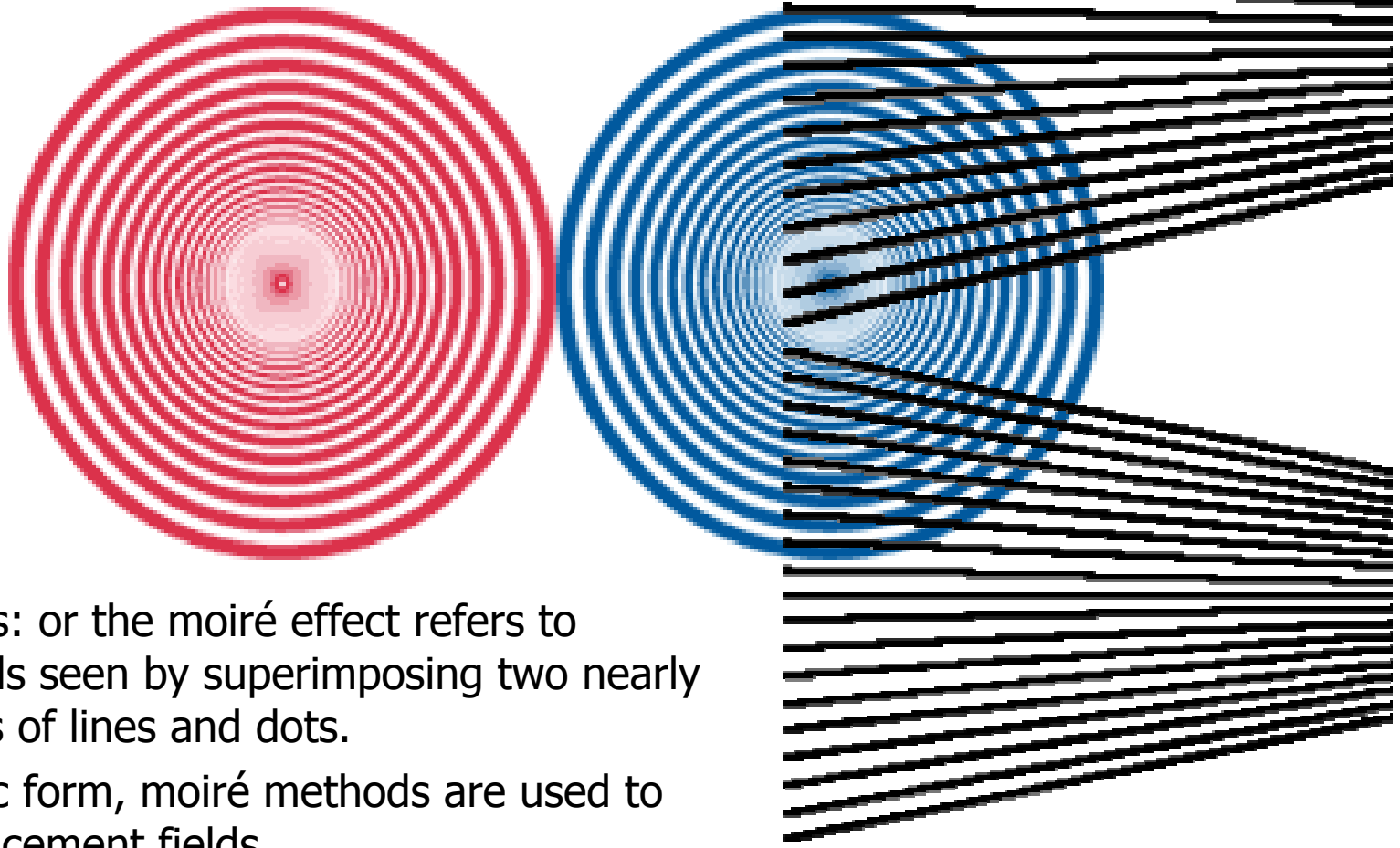
? How to measure the deformation in nanostructures?

SEM of fabricated equally spaced polymeric periodic substrate (PPS)



Imaging Interface: Optical Moiré technique

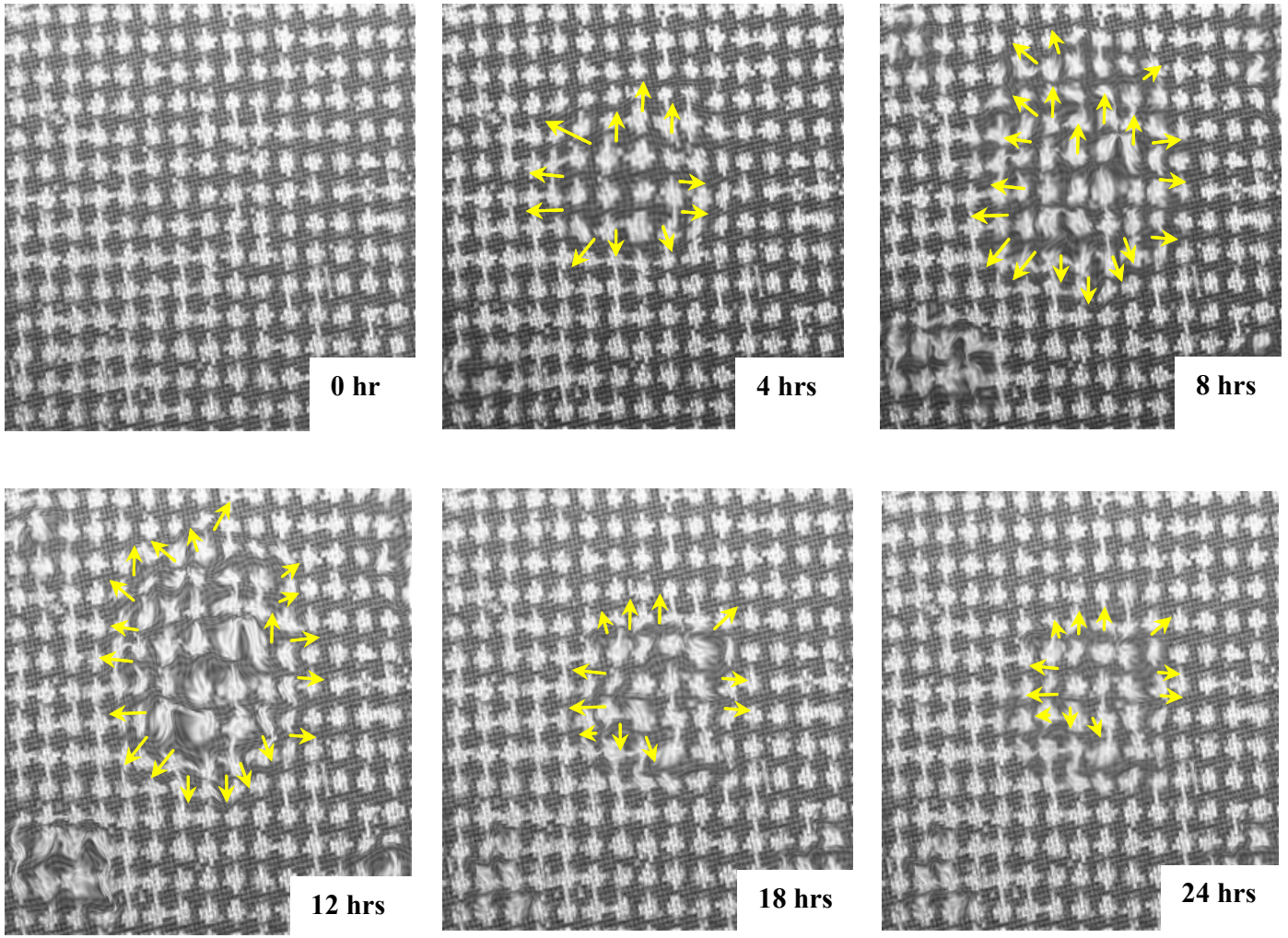




* Moiré Fringes: or the moiré effect refers to light/dark bands seen by superimposing two nearly identical arrays of lines and dots.

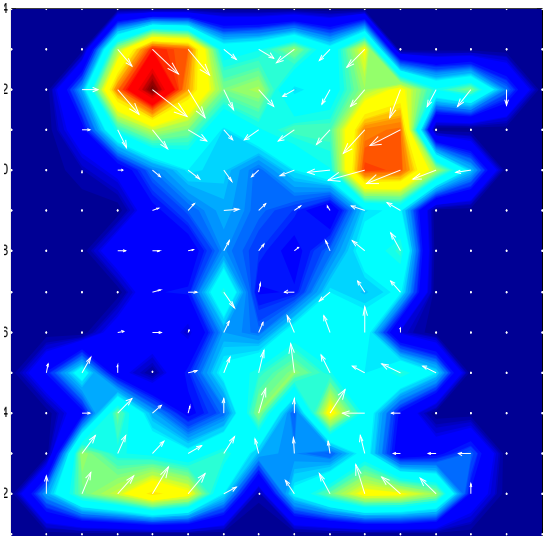
* In most basic form, moiré methods are used to measure displacement fields.

Force Mapping in Vascular Smooth Muscle Cells



As the VSMCs spread out in DMEM media with serum , the moiré patterns changed from regularly distributed to locally distorted, and further resembled a natural centrifugal pattern, revealing the concentric profile of the traction forces developed on the substrate.

Corresponding Force Map Derived from Moiré

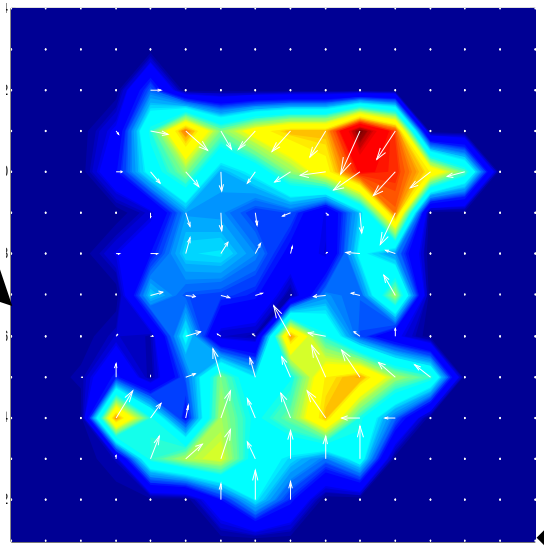


A

12 hrs

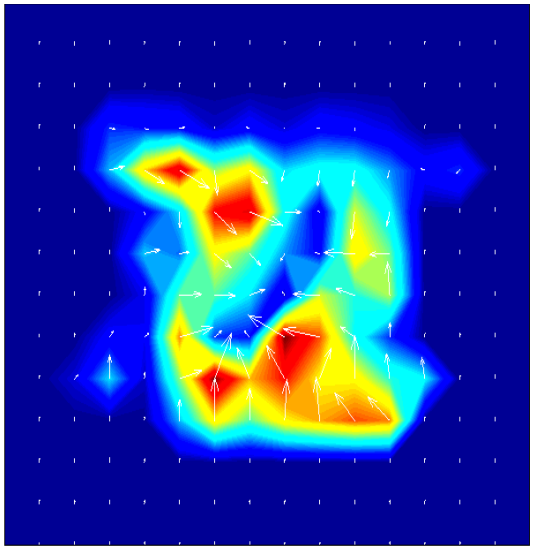
Derived cellular traction force mapping

* Length and direction of the arrows: the direction and magnitude of the forces derived from the map
* Colors: the magnitude of the displacements



B

18 hrs

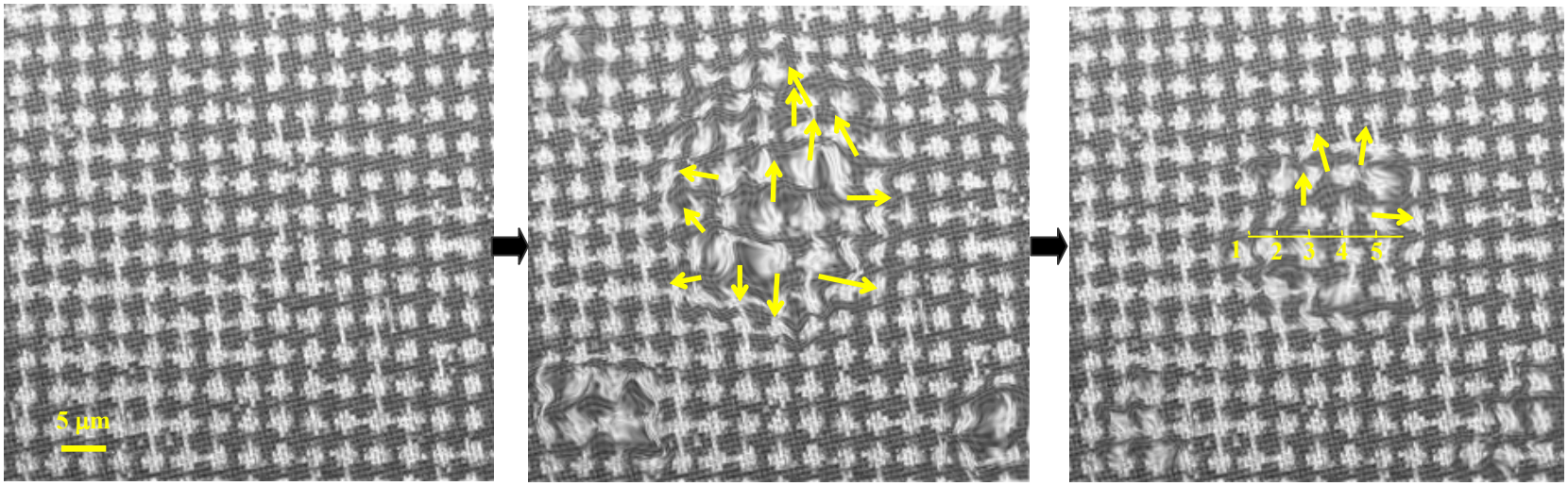


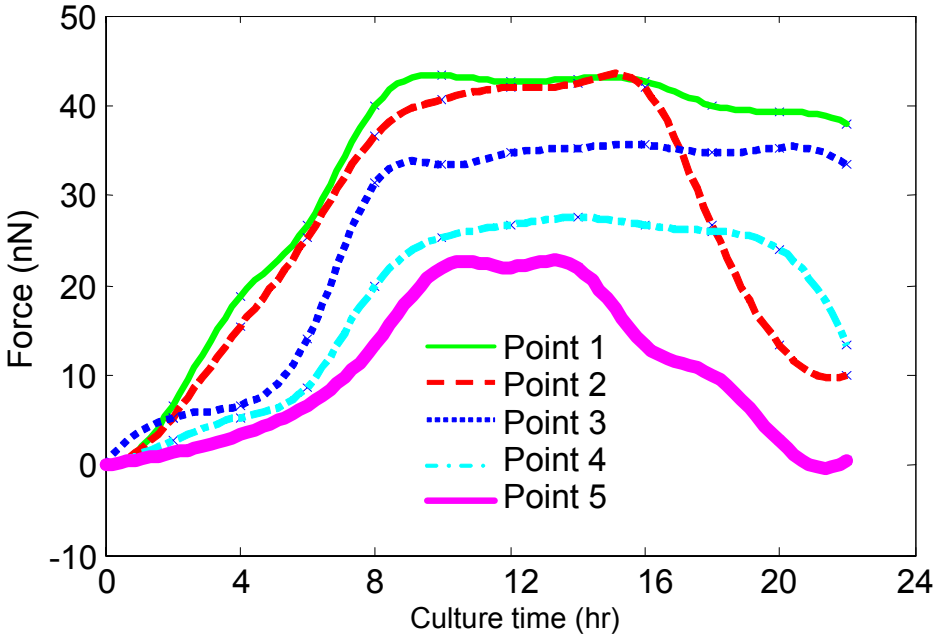
C

24 hrs

* Decrease of traction force with decreasing spreading area
* Concentrated at the boarder of the cells, pointing to the nuclei directions
* Least traction forces concentrated at the central region of the cells

Determining Force Evolutions from Moiré Patterns





To determine the magnitude of the contraction force developed on adhesion areas from the moiré patterns:

* Derived the traction force on five locations

* Mapped the evolution of the traction forces over time

Conclusions

- Fabrication
  - Polymer micro- and nanostructures with various aspect ratios
- Characterization
  - Deep beam model
  - Moiré techniques
- Measurement and Analysis
  - Micro/nanofabricated polymer based system provides in-vitro cell traction force mapping in the sub-cellullar level
  - Optical moiré approach provides robust and real-time imaging of in-plane cell traction force mapping
    - Such optical moiré system can be readily employed to study migration, morphology, motility and many other cell-substrate mechanical interactions on patterned polymer substrates.
    - Since our approach requires neither the tracking/monitoring nor the visibility of each individual pillar, we anticipate that this method will increasingly find more applications in micro and nano patterned substrates for a variety of mechanics studies in living cells.

Acknowledgements

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