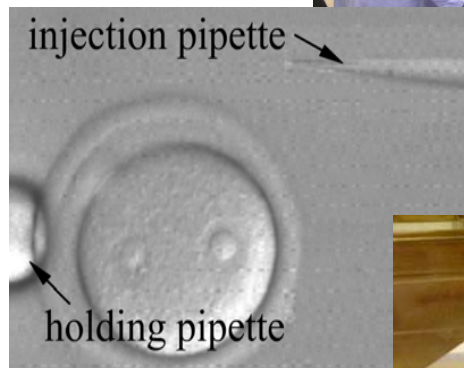
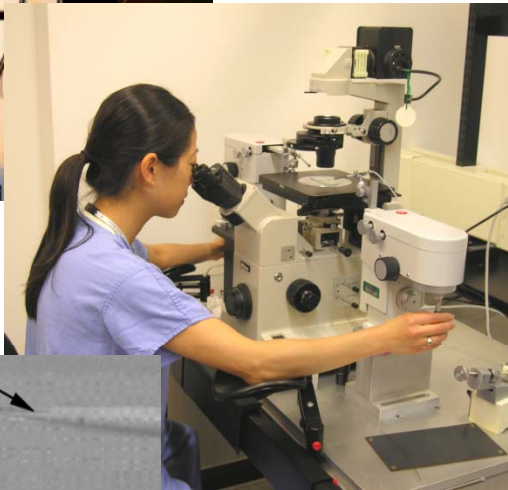


# Molecule Screen and Cell Quality Assessment – A Robotic and BioMEMS Approach

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# Microrobotic Cell Manipulation



- Slow
- Low success rate
- Low cell survival rate
- Long learning curve
- Inter-operator variability
- Skill dependent
  
- Molecule testing
- Enucleation
- Polar body biopsy
- Clinical ICSI



- **Limited speed and reproducibility**

- Search & immobilize: random
- manual switch: slow
- orient: trial-error; slow; poor controllability
- speed: 2 cells/min



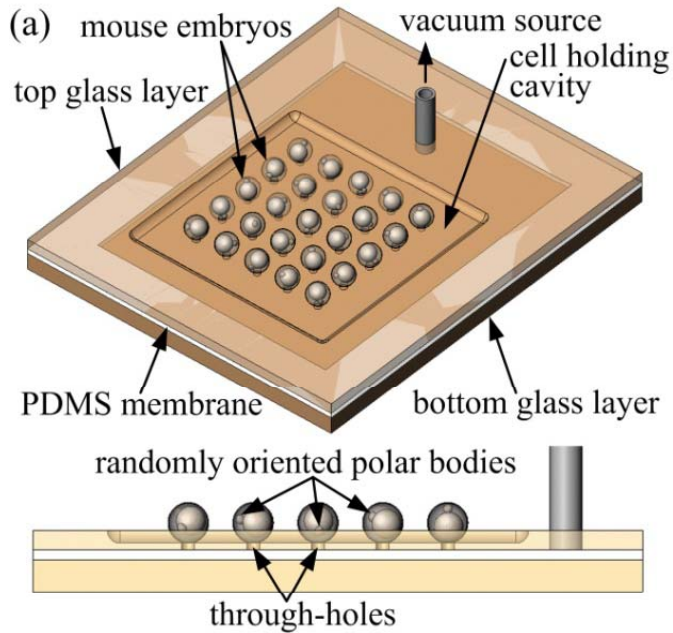
- **Targets**

- speed: 12 cells/min
- rapid cell immobilization and orientation
- high success/survival rates
- grad students can do the work

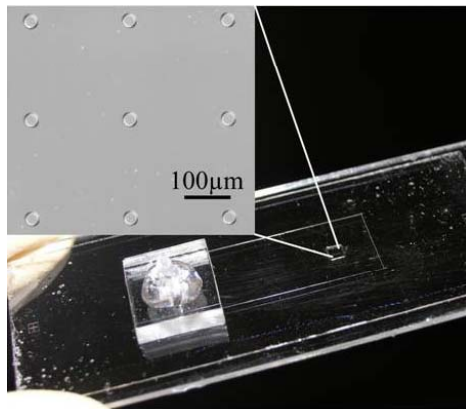


Sun and Nelson, *IJRR*, Vol. 21 (2002)

# Cell Holding Devices



*Biomed. Microdevices*, Vol. 11, 2009



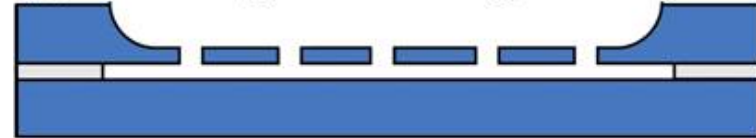
(a) Top-side HF etching.



(b) Bottom-side HF etching.



(c) PDMS-glass bonding.

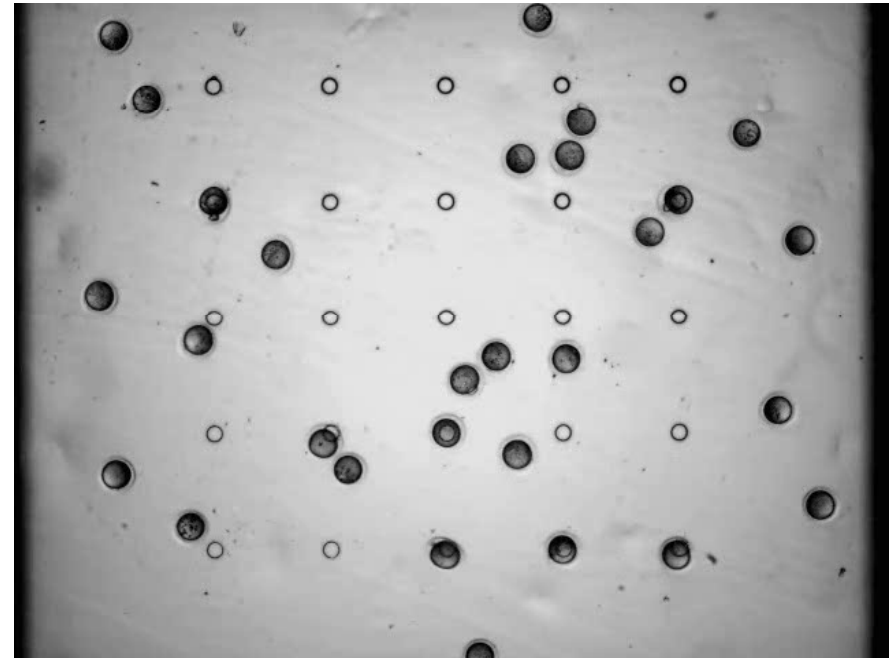
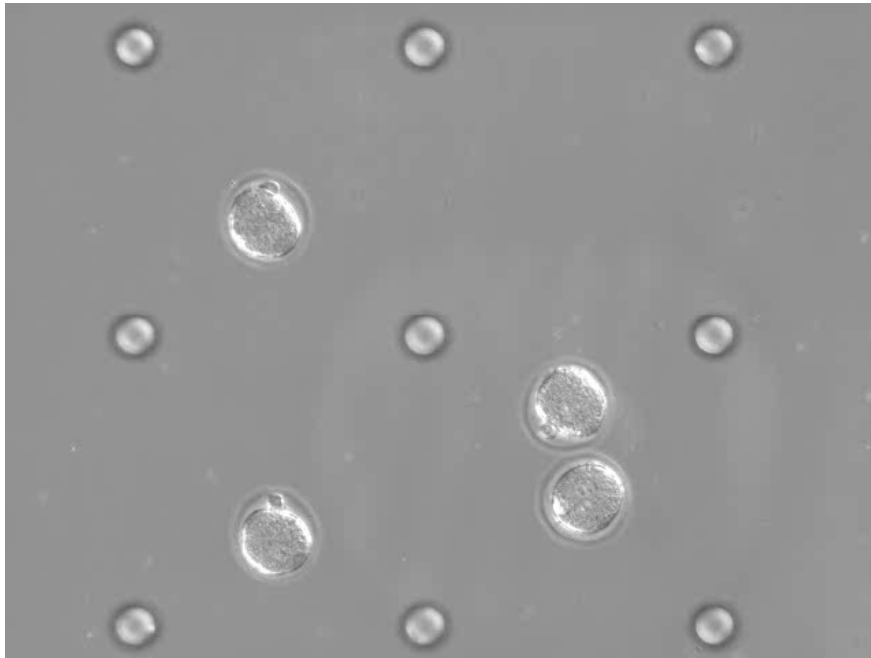


Cr/Au
  glass
  PDMS

Three devices every two days

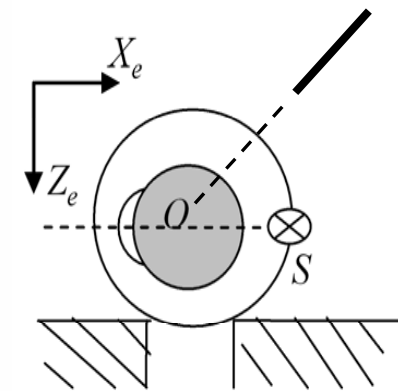
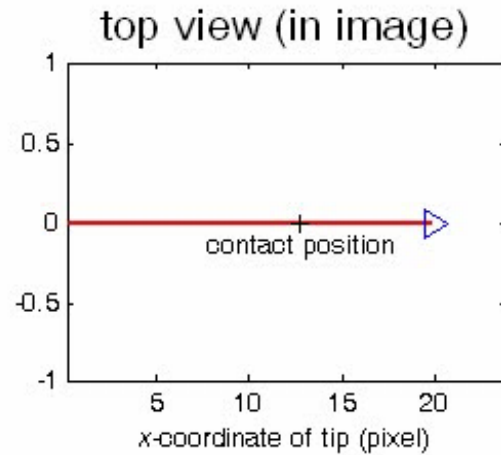
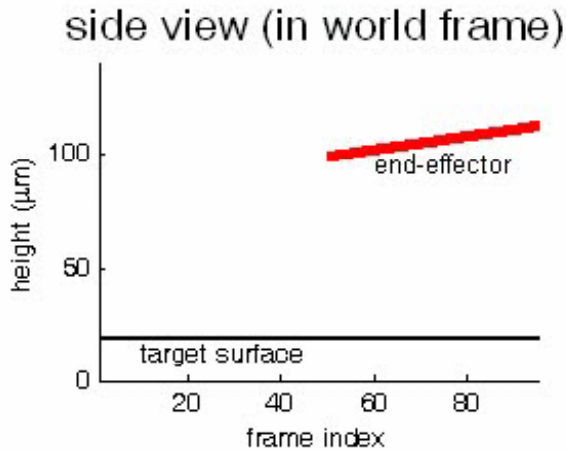


# Fast Cell Immobilization



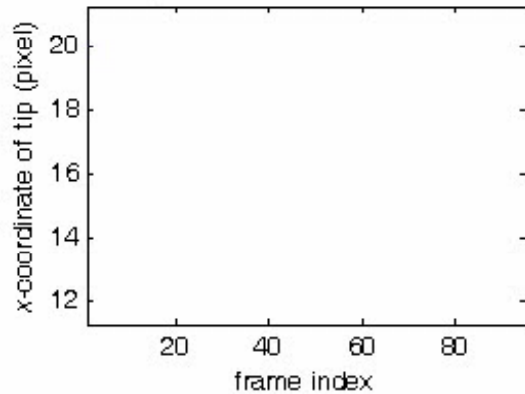
- **Pressure: 1.6~2.2kPa (mouse zygotes)**
- **Immobilization time: 10~16sec ( $5 \times 5$  array)**

# Vision-Based Contact Detection

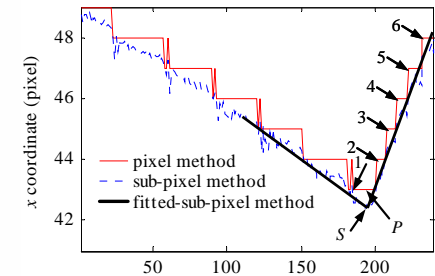
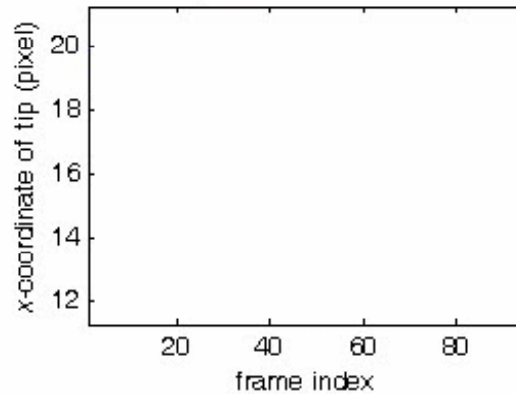


Side view

pixel-resolution

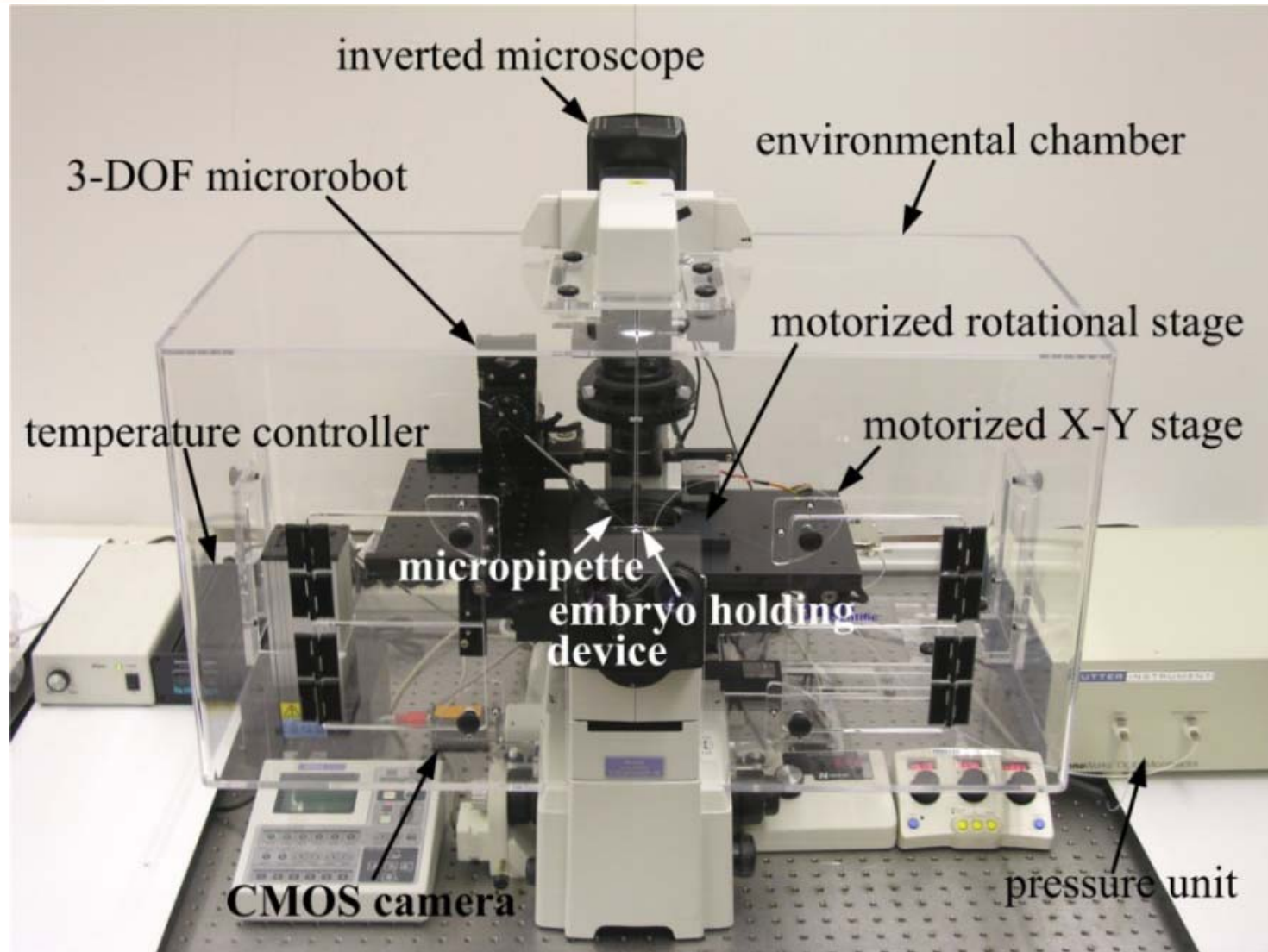


subpixel-resolution

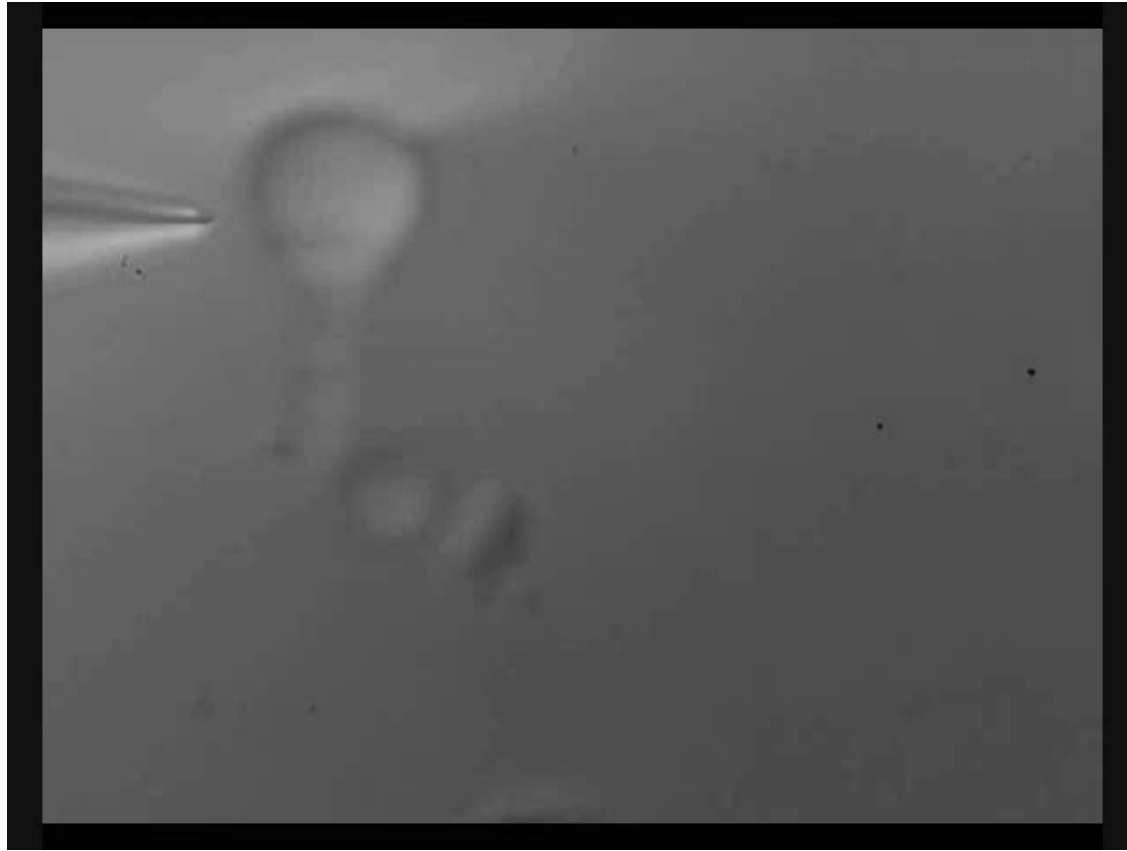


Wang, Liu, and Sun, *Int. J. Robotics Research*, Vol. 26 (2007)

# Microrobotic Cell Injection



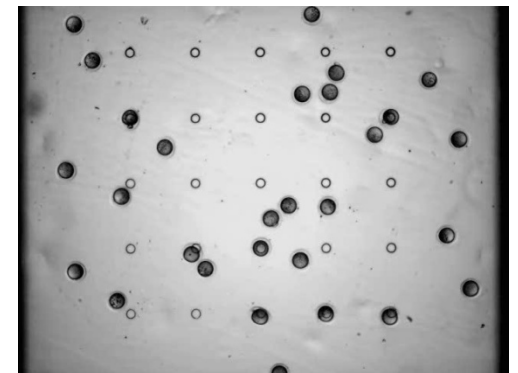
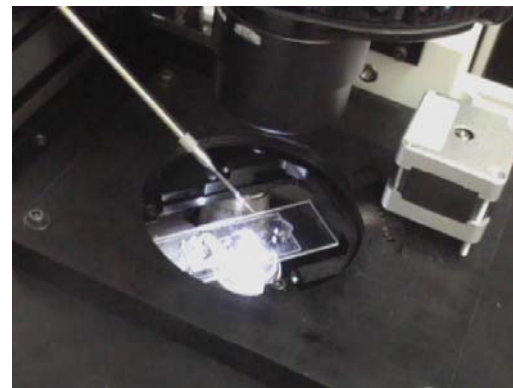
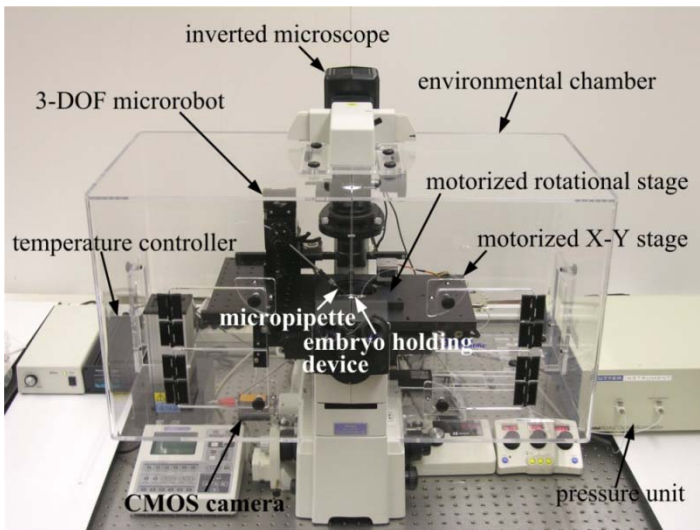
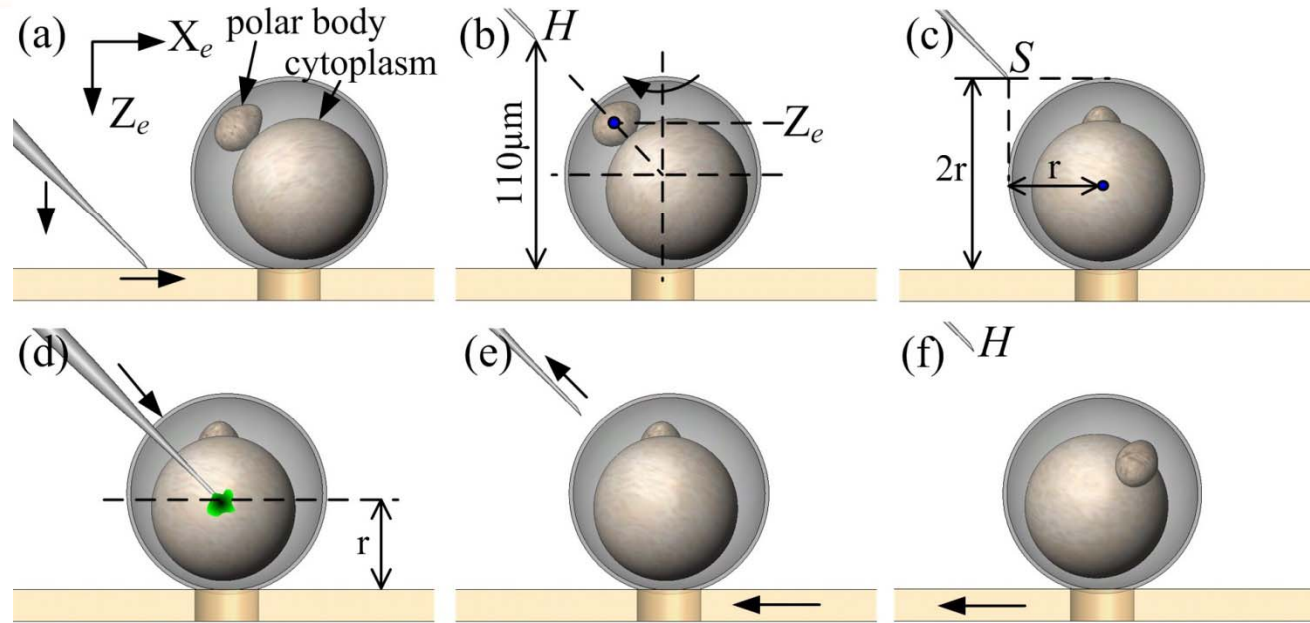
# Microrobotic Cell Injection



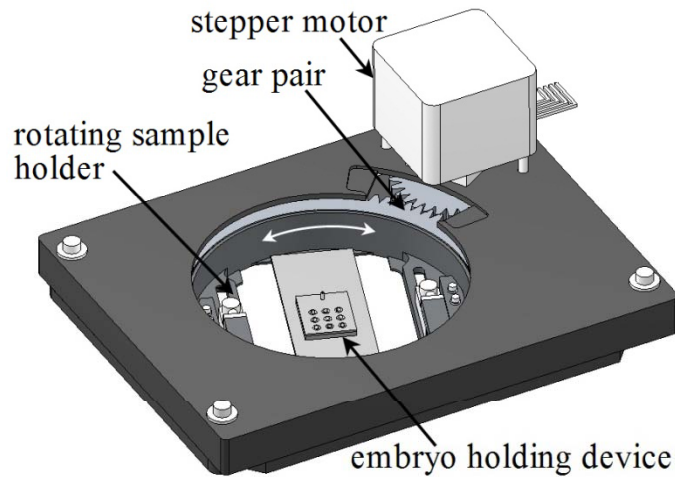
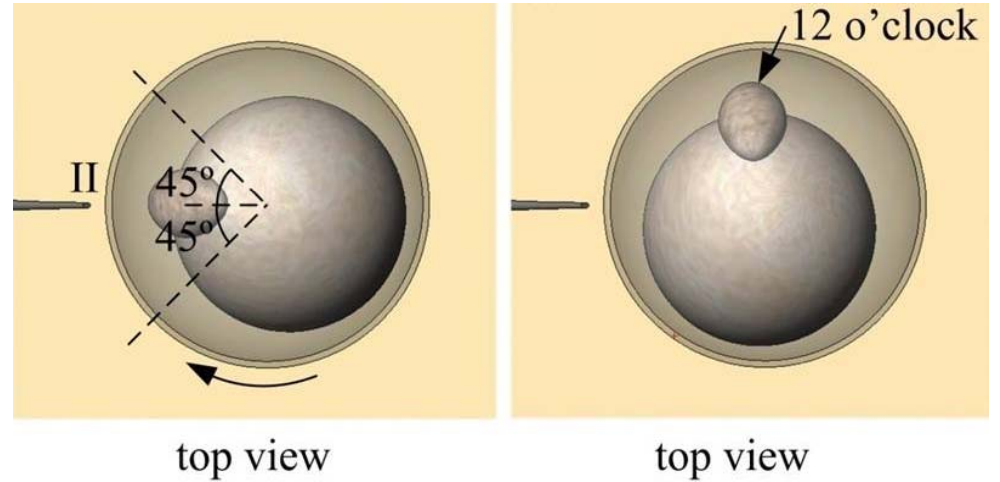
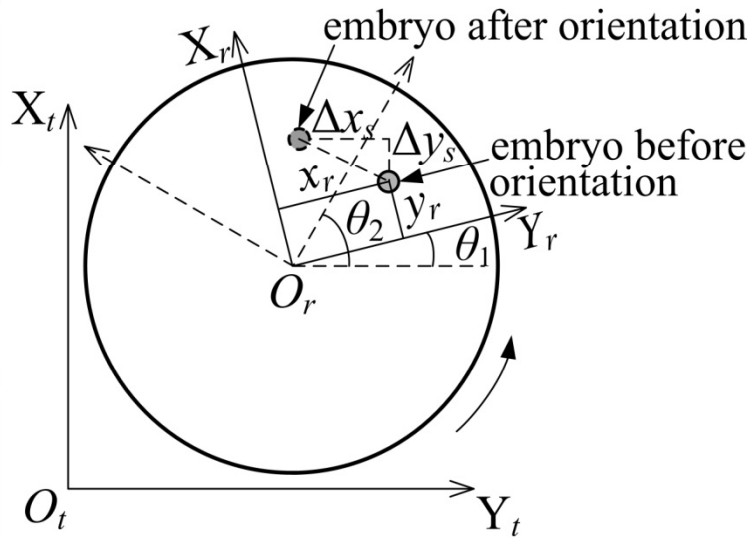
video speed: 1X



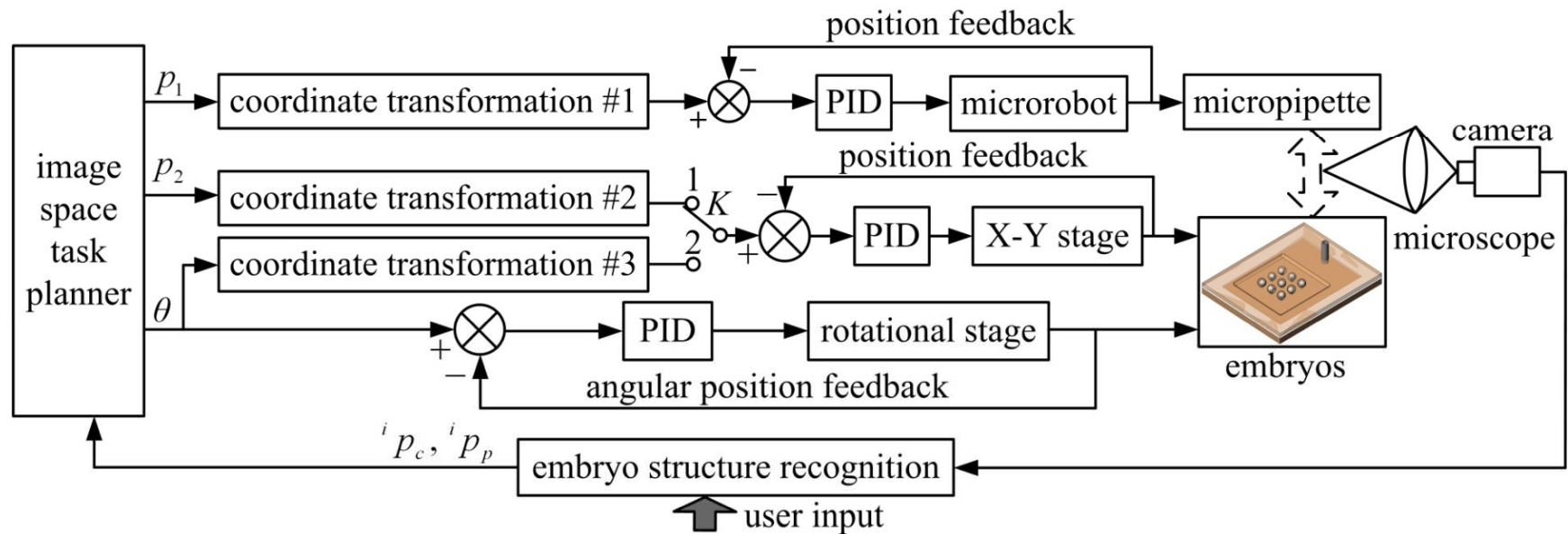
# Cell Orientation Control



# Cell Orientation Control



# System Control Architecture



- Image space task planner
- Looking then moving
- On-line coordinate transformation

# System Performance



- **Mouse embryo preparation**
  - ICR mice (8~12 weeks)
  - PSMG and hCG injection (48hr)
  - timed pregnancy (12hr)
  - embryo collection: ~20 cells/mouse
- **System optimization (n=306)**
  - injection speed: 200 $\mu$ m/sec
  - retraction speed: 500 $\mu$ m/sec
  - injection pressure: 40~45kPa
- **PBS injection (n=240)**
  - speed: 12 cells/min (2 cells/min)
  - success rate: 98.9% (90%)
  - survival rate: 89.8% (80%)



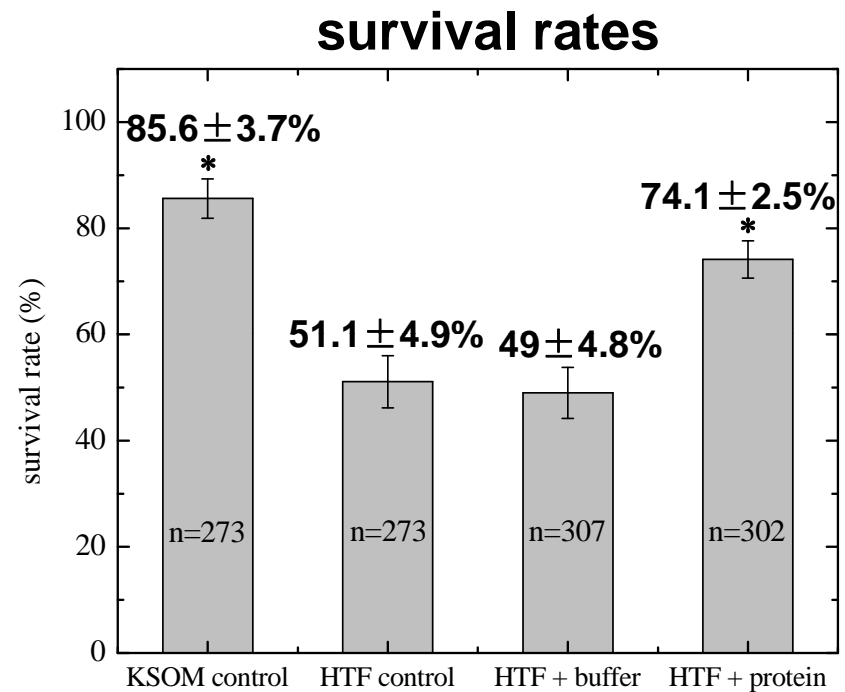
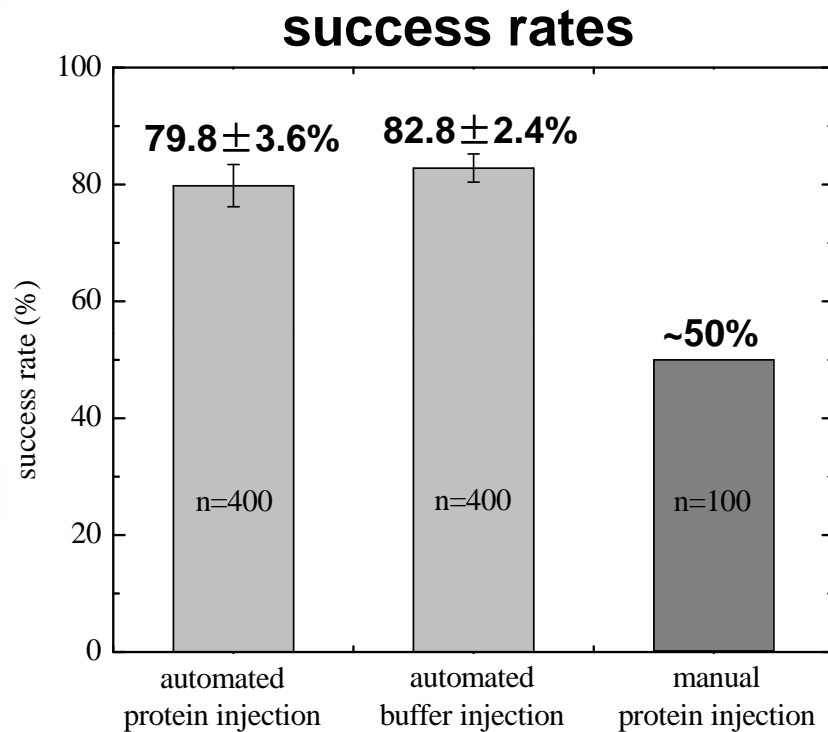
mouse dissection



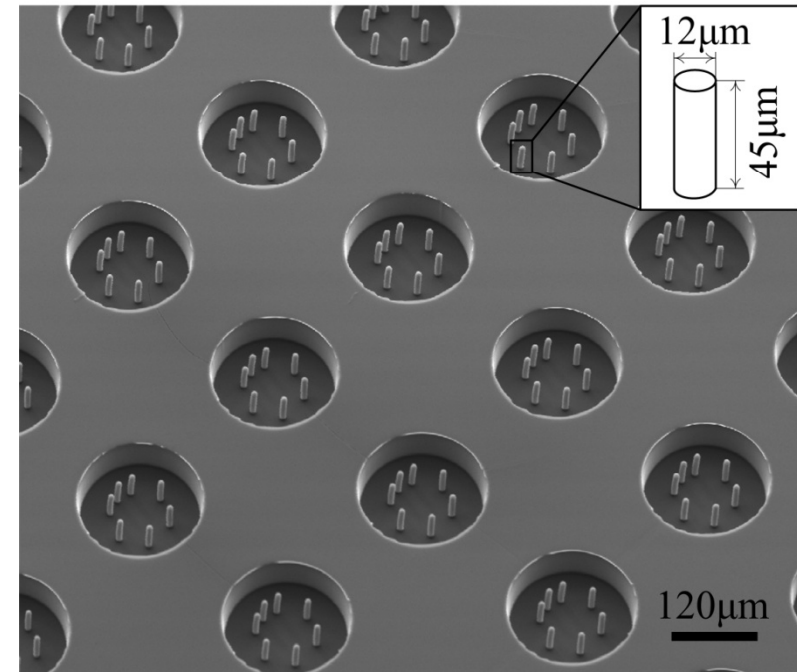
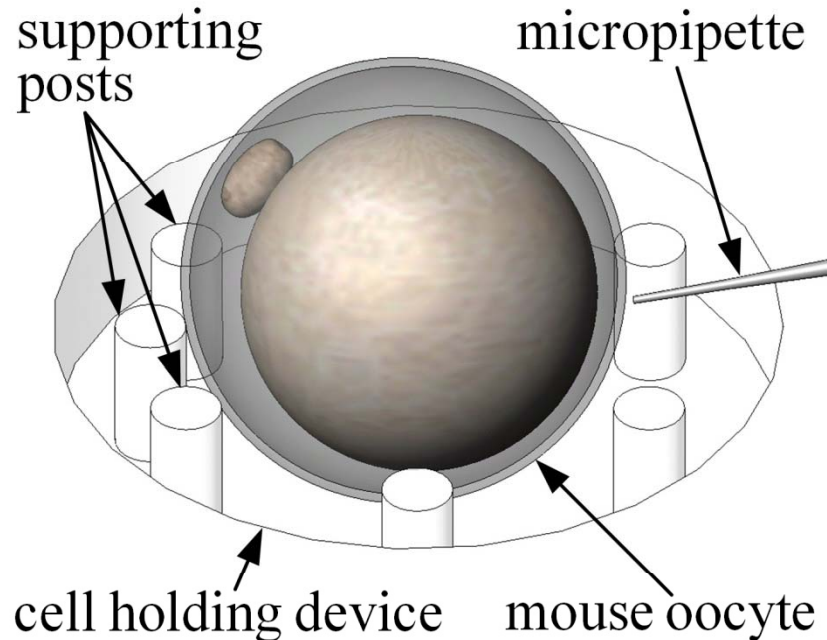
mouse blastocysts



# Results – Mitochondrial Protein Testing



# Vision-Based Force Measurement



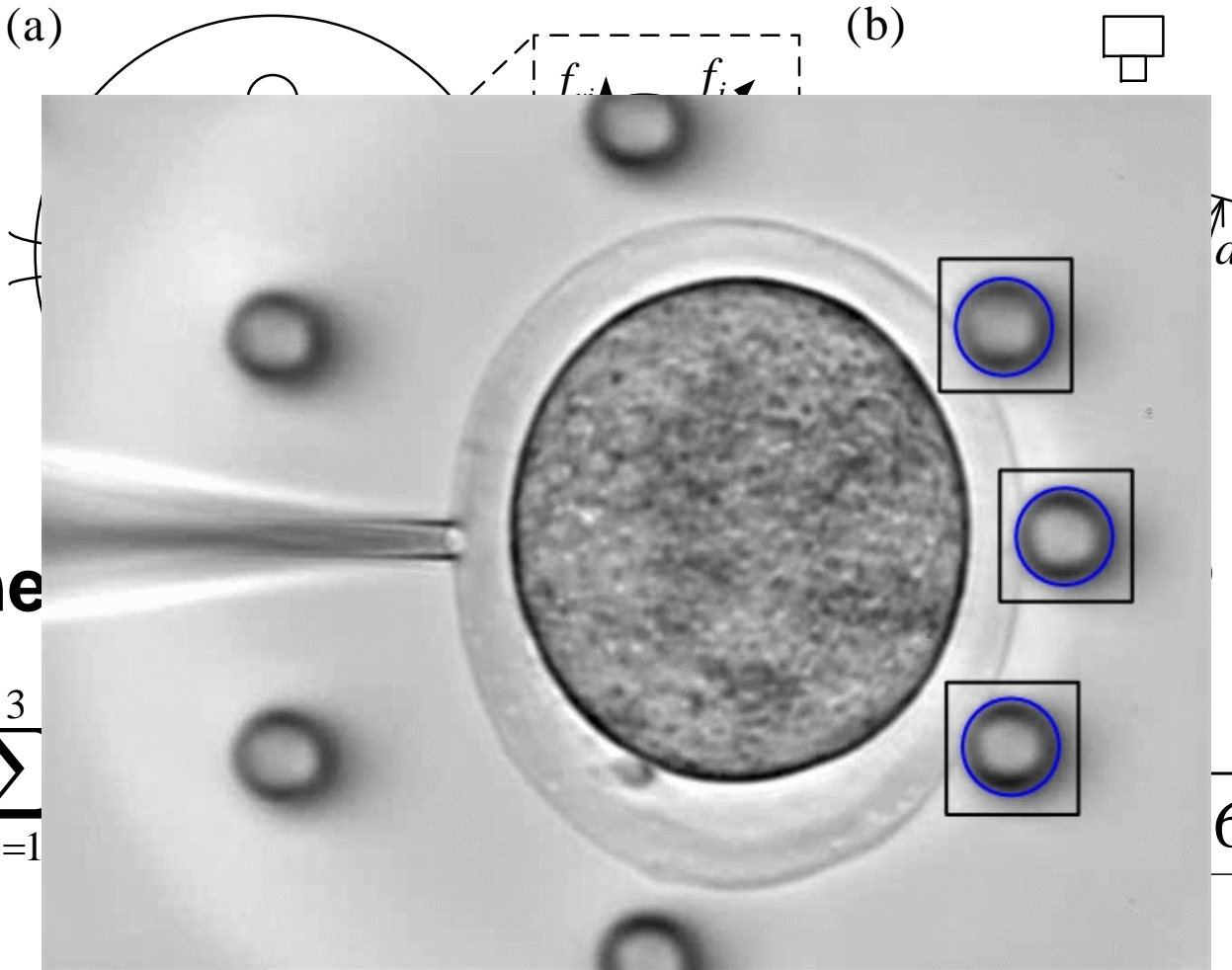
- **PDMS cell holding device and a sub-pixel visual tracking algorithm**
- **Post deflections → injection forces**

# Force Analysis



- Line

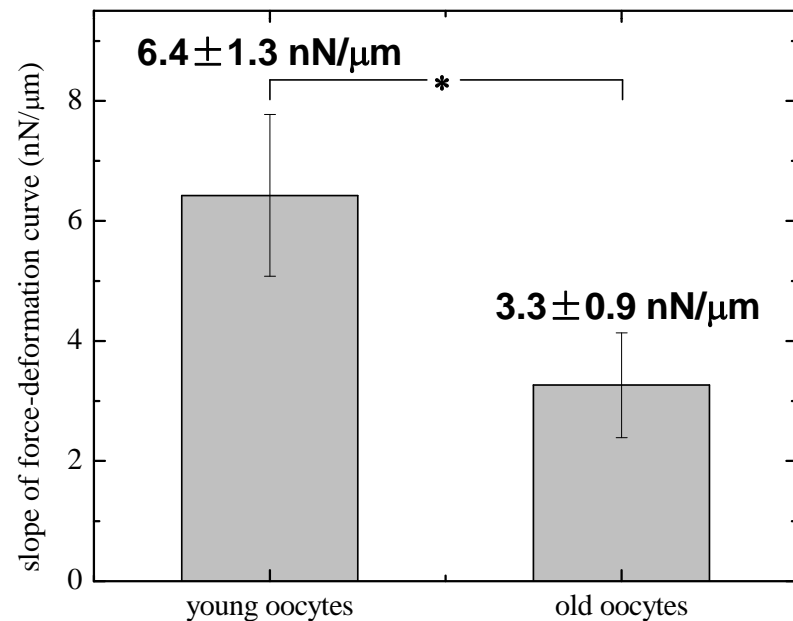
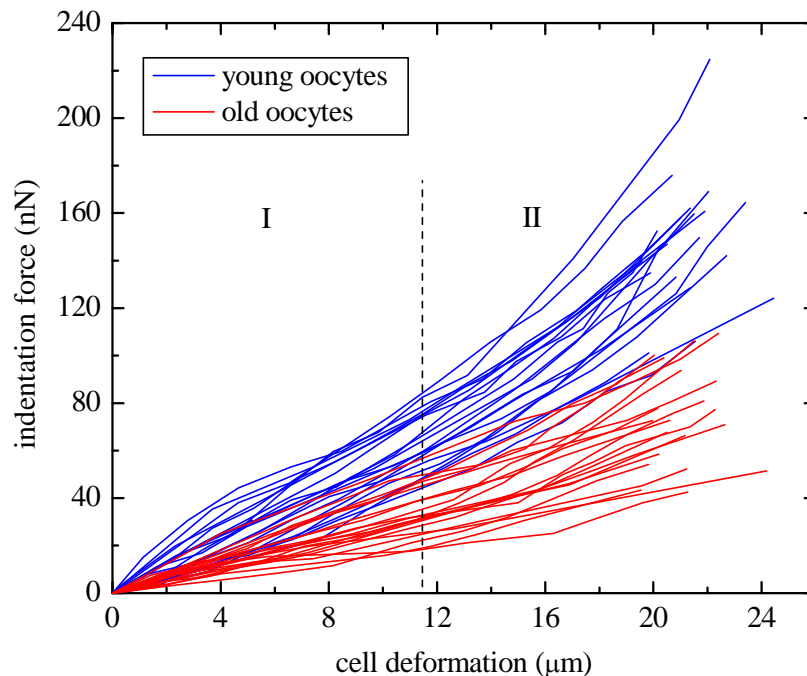
$$F = \sum_{i=1}^3$$



$$6H^2 a_i^2)$$

# Distinguish Normal/Defective Oocytes

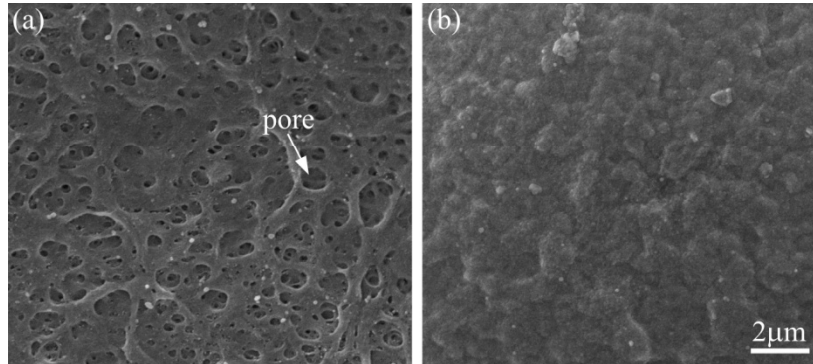
- ICR young (n=20) and old (n=20) mouse oocytes
- **Stiffness**: slope of force-deformation curve
- **Overlap**: 4.4-4.8 nN/ $\mu$ m



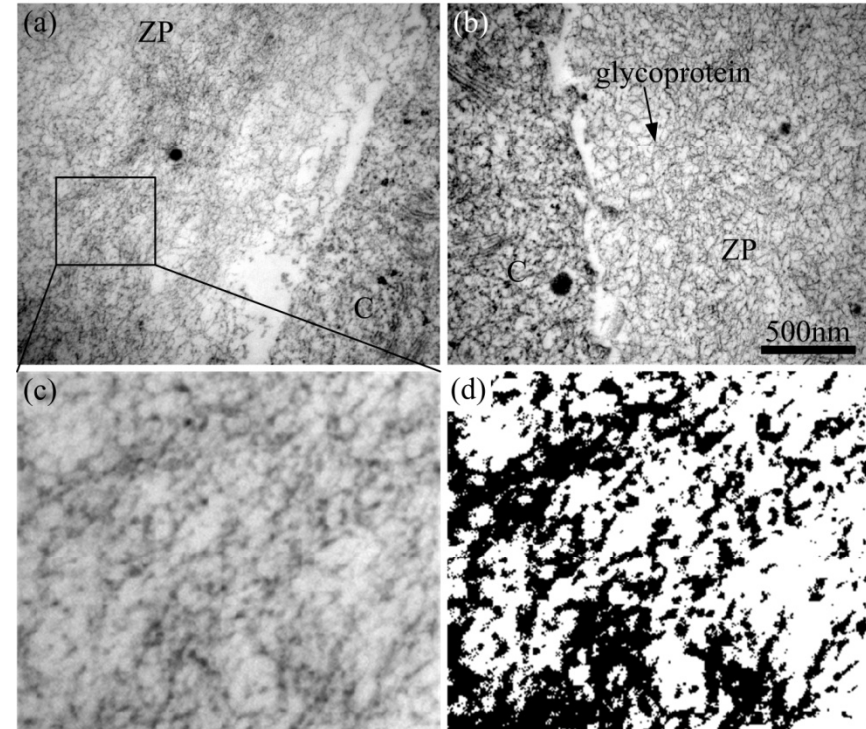


# Zona Pellucida (ZP) Structure Analysis

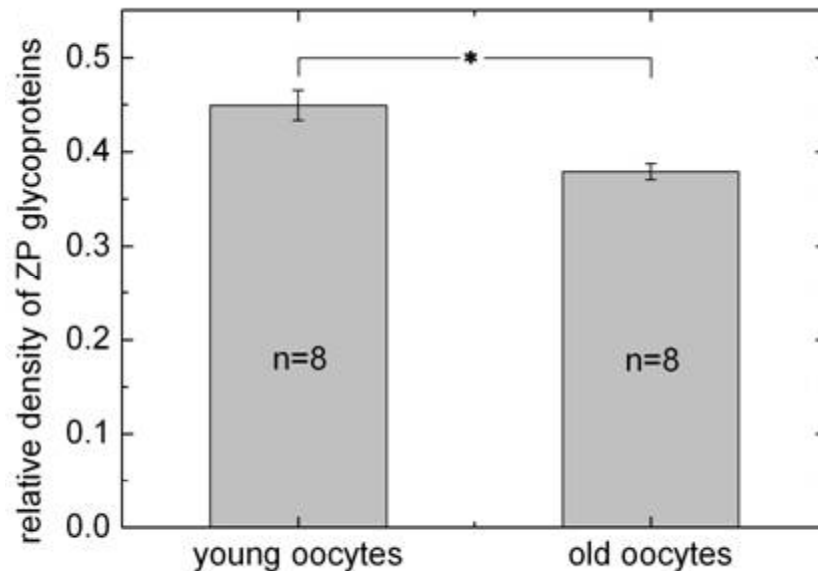
## surface morphology (SEM)



## glycoprotein density (TEM)



## glycoprotein density

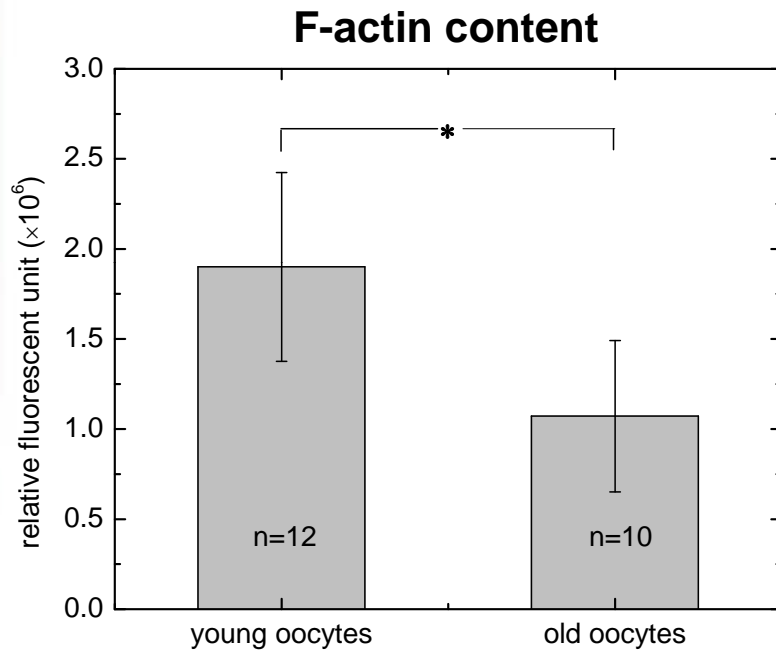


- **ZP1-3**: low expression in old oocytes (*Hum. Mol. Genet.*, 2004)

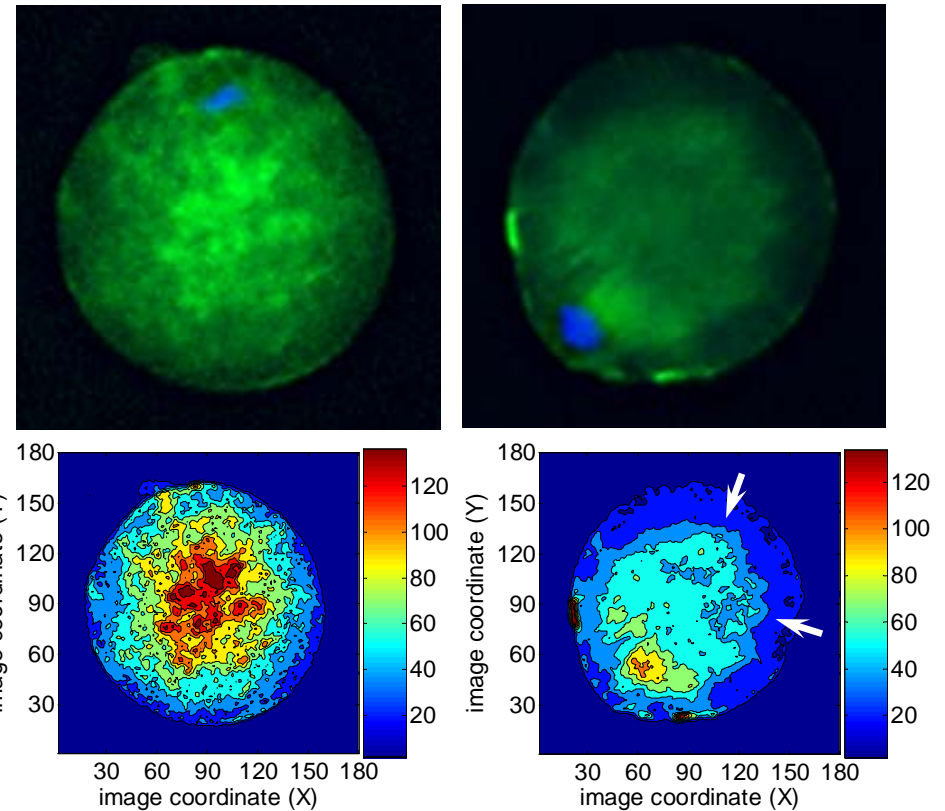
# F-Actin Content Analysis



- **Phalloidin-FITC staining of F-actin**



- **Krt8 and Myo10: low expression in old oocytes (*Hum. Mol. Genet.*, 2004)**



- Synergy: microrobotics and bioMEMS
- Microrobotic cell injection
  - Enabled molecule testing and MEL gene prioritization
- In situ distinguish healthy and aged mouse oocytes to assess cell quality for cell selection