BioMEMS and Nanoparticles for the Detection and Treatment of Cancer

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Background and Introduction

- Richard Feynmann – There's lots of room at the bottom….(1961 APS talk)
- Several people could benefit from implantable or injectable systems for class detection and treatment
- This talk examines two types of small structures for cancer detection

Why the Focus on Cancer?

- Several people suffer from cancer – either directly or through contact with someone that has cancer
- The biggest problem is really one of early detection – existing methods of detection are limited in spatial resolution
- The other major problem is the severe effect of existing cancer treatment methods e.g. radiation and chemotherapy (localized theraputics to be addressed in the next class)
- This class presents some new ideas on cancer detection

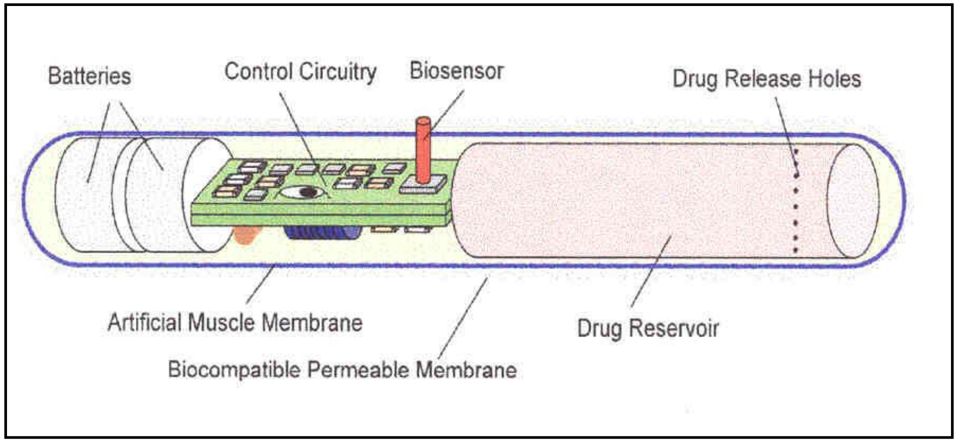
  - BioMEMS for the detection of cancer

  - Magnetic nanoparticles for cancer detection

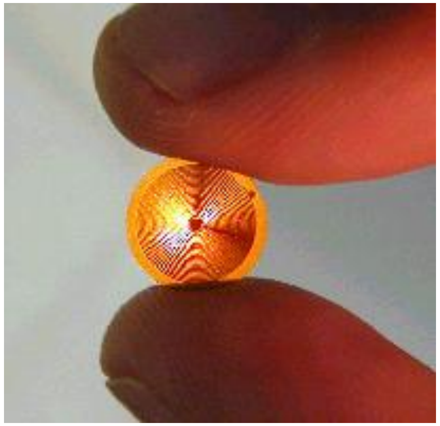
Introduction to BioMEMS Systems

- BioMEMS structures are micron-scale devices that are used in biomedical or biological applications
- At this scale, a wide range of devices are being made (e.g. pressure sensors, drug delivery systems, and cantilever detection systems)
- Explosive growth in emerging markets – civilian and military applications expected to reach multi-billion dollar levels

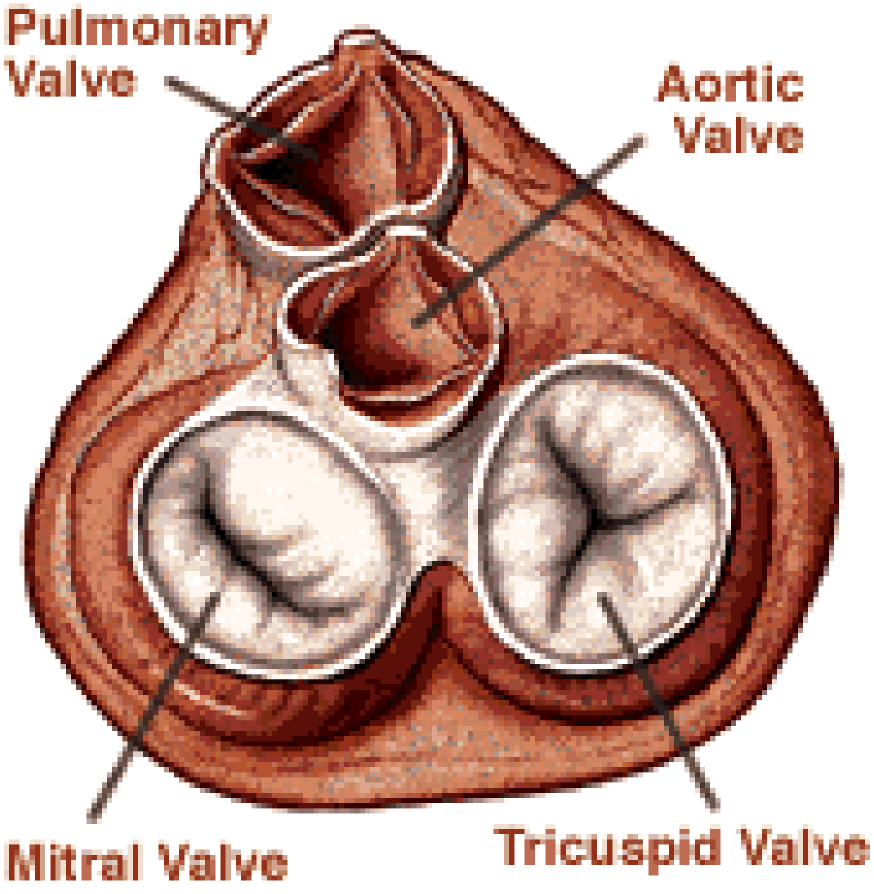
Drug Delivery System

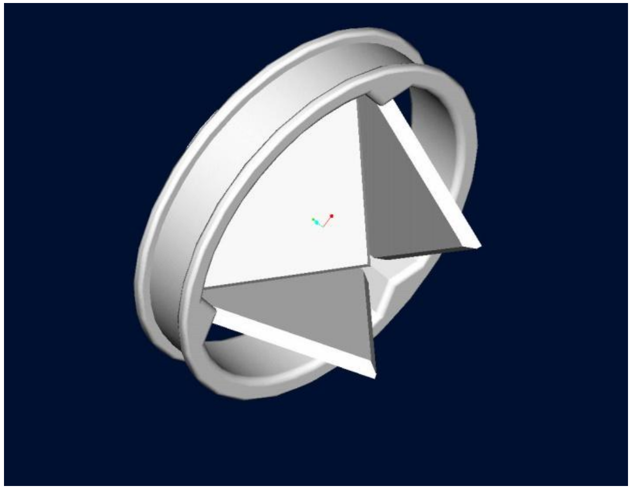


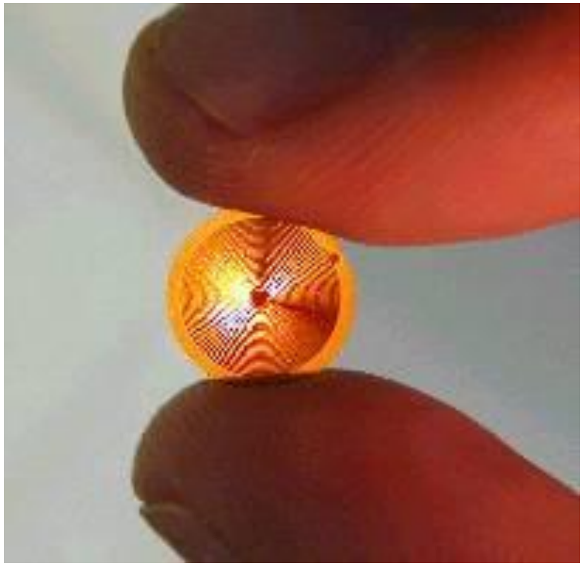
Implantable Blood Pressure Sensor



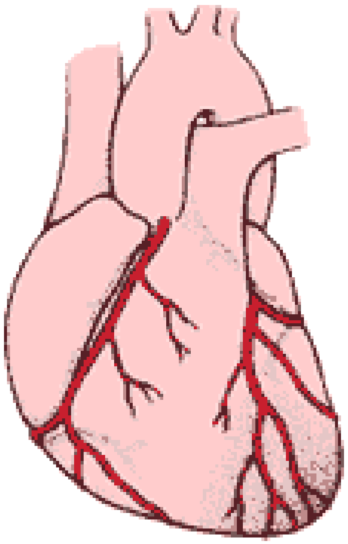
MEMS-Enhanced Trileaflet Valve



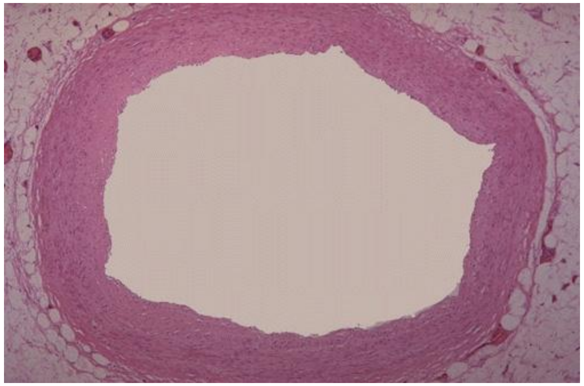


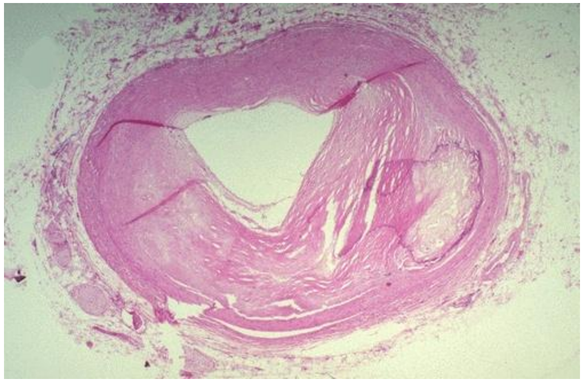


Atherosclerosis



- Atherosclerosis is the hardening and narrowing of blood vessels caused by buildup of plaque
- Plaque is made up of cholesterol, calcium, and other blood components that stick to the vessel walls
- When plaque bursts, blood tends to clot, thus creating more blockage

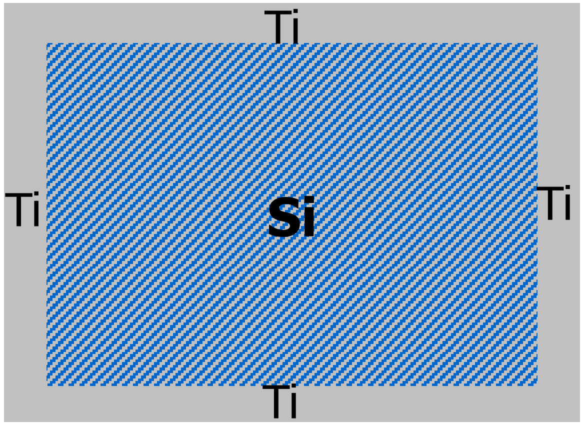




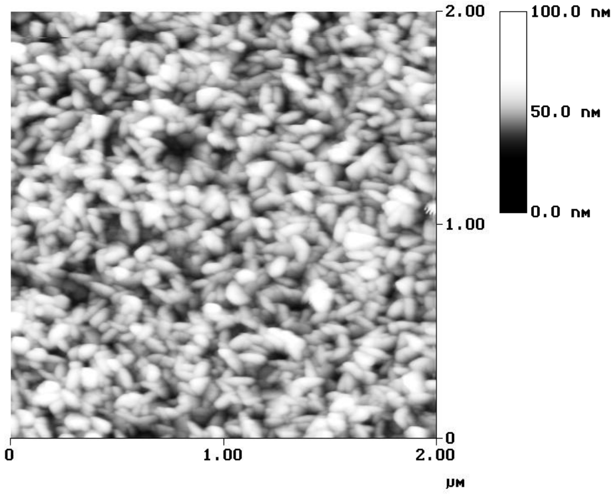
Biocompatibility of Silicon MEMS SystemS

- Si is not the most biocompatible material
- Can be made biocompatible through the use of polymeric or Ti coatings.
- Polymeric coatings used on Si drug release systems.
- Ti coating approaches are also being developed.

Coated BioMEMS Structure

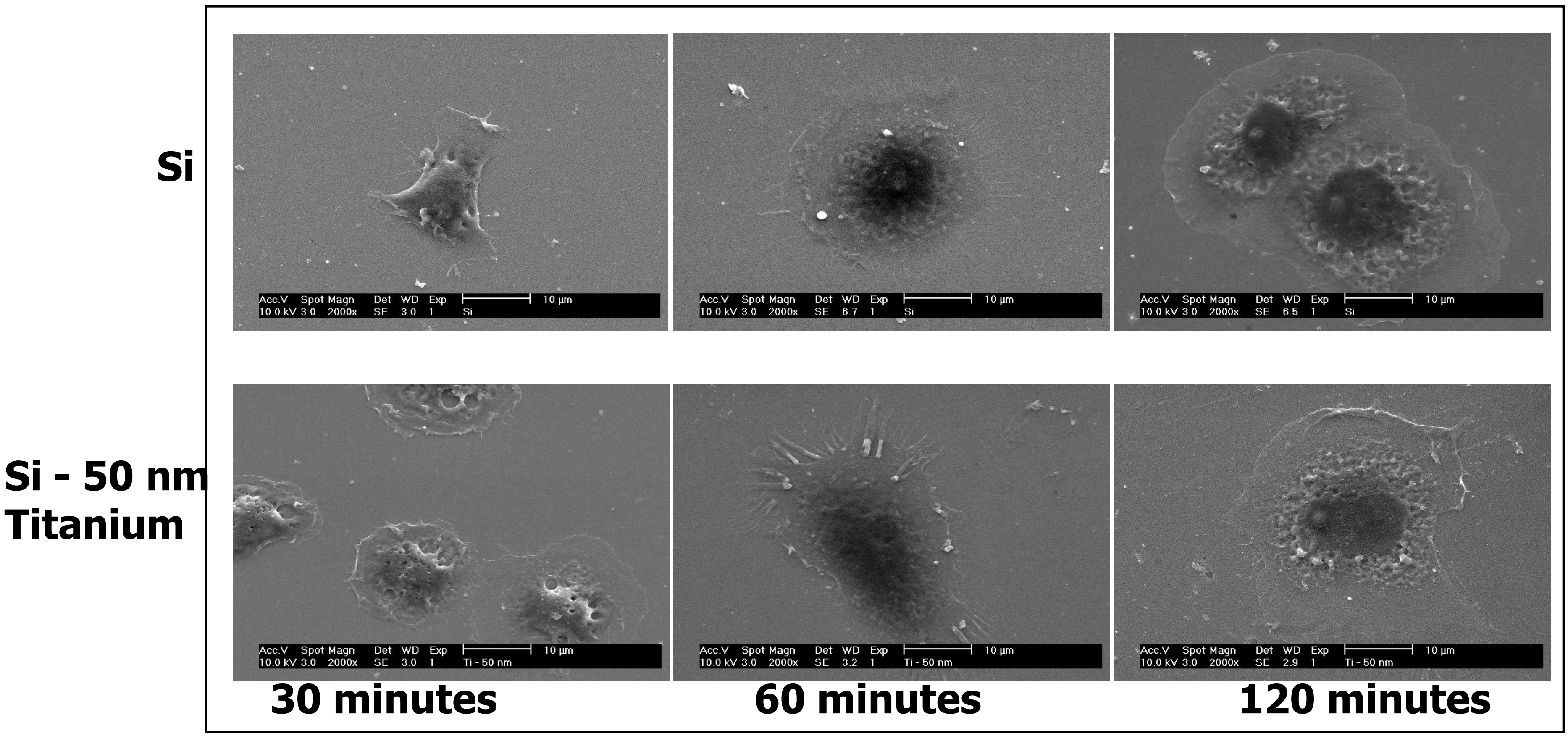


500 nm Ti Layer on Si



SURFACE CHEMISTRY – CELL SPREADING

HOS Cells



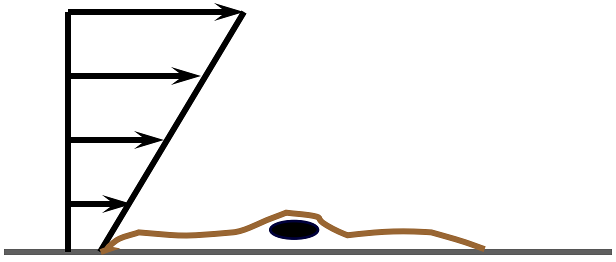
SHEAR ASSAY MEASUREMENT OF CELL ADHESION

- Shear stress for detachment is given by

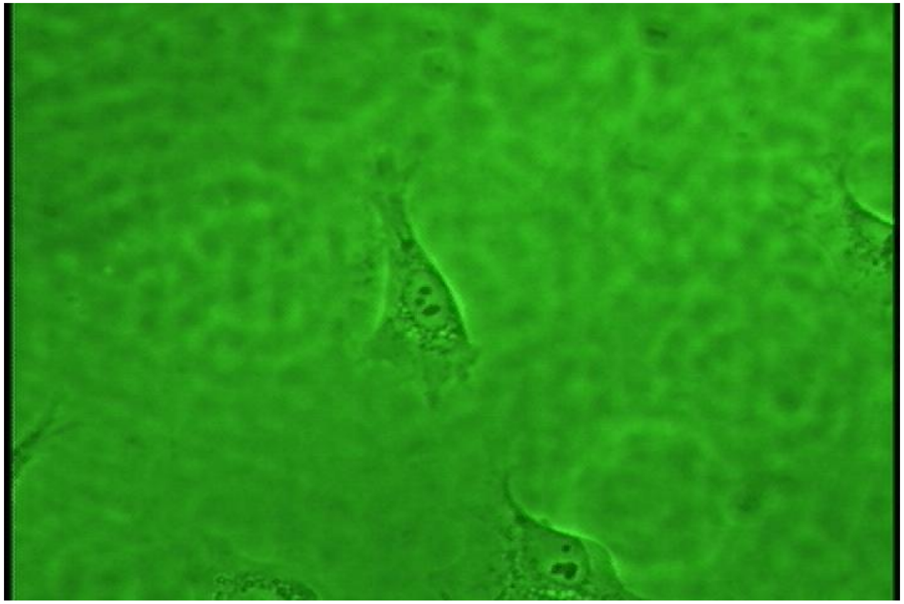
$$\tau = \frac{6Q\mu}{wh^2}$$

- Where Q - flow rate & μ -dynamic viscosity
- Considering initial onset of detachment to correspond to "adhesion" strength:
  - $\tau = 70$ Pa Polystyrene (PS)
  - $\tau = 81$ Pa Ti Coated PS

Shear Flow Schematic

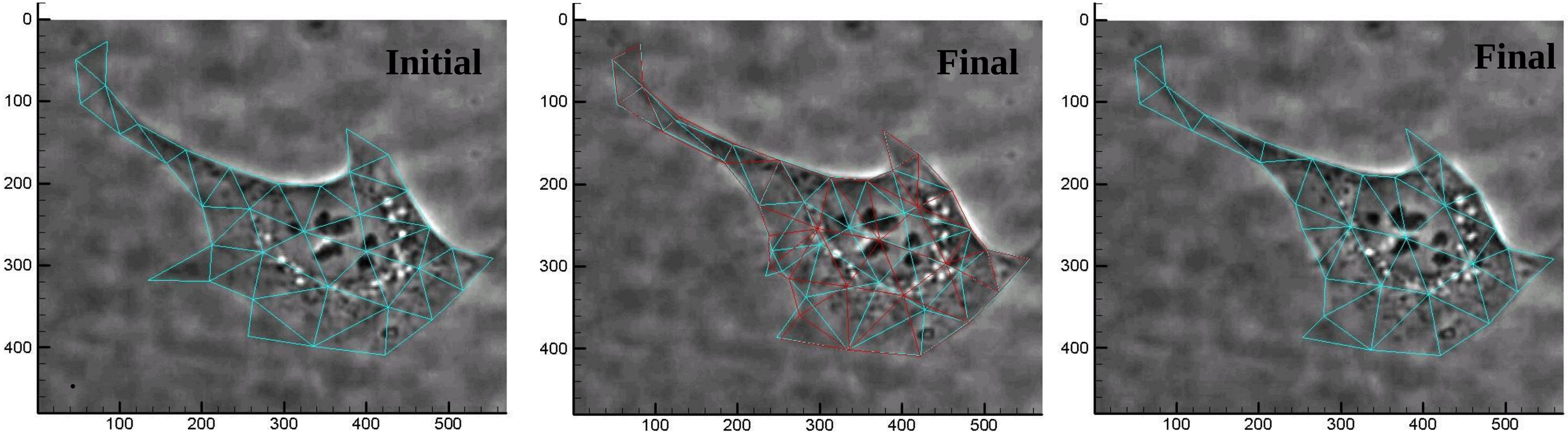


Cell Detachment

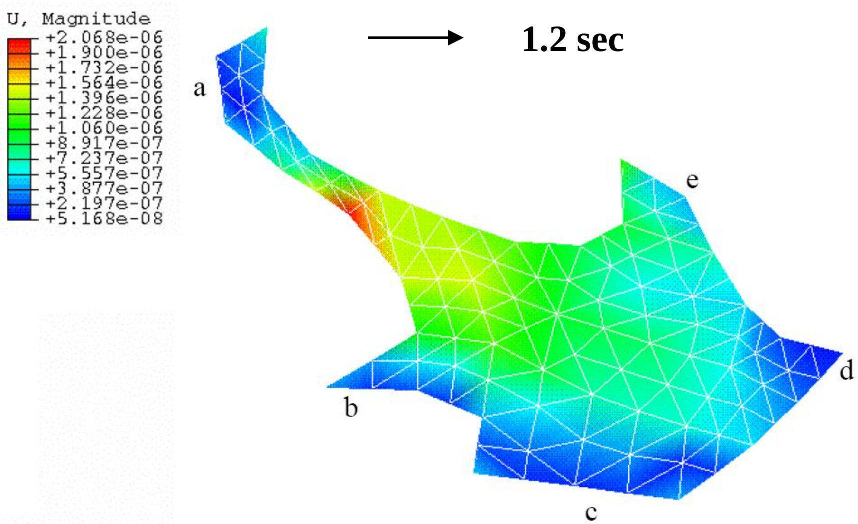


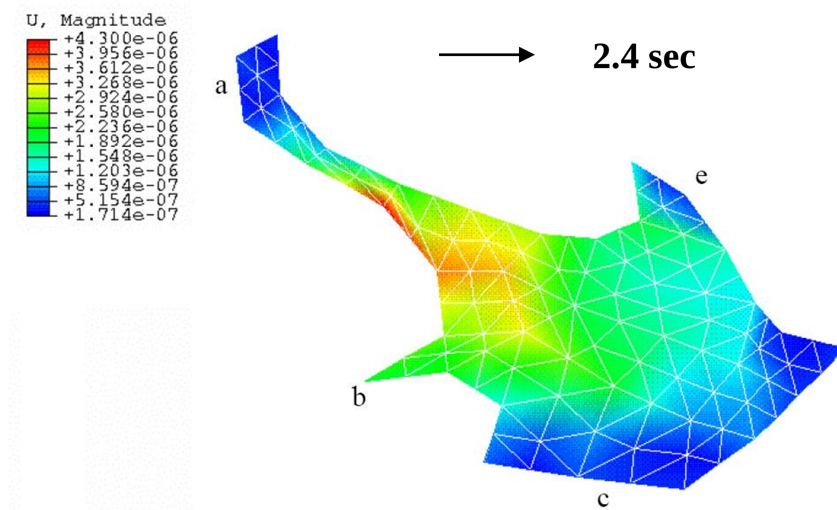
Digital Image Correlation

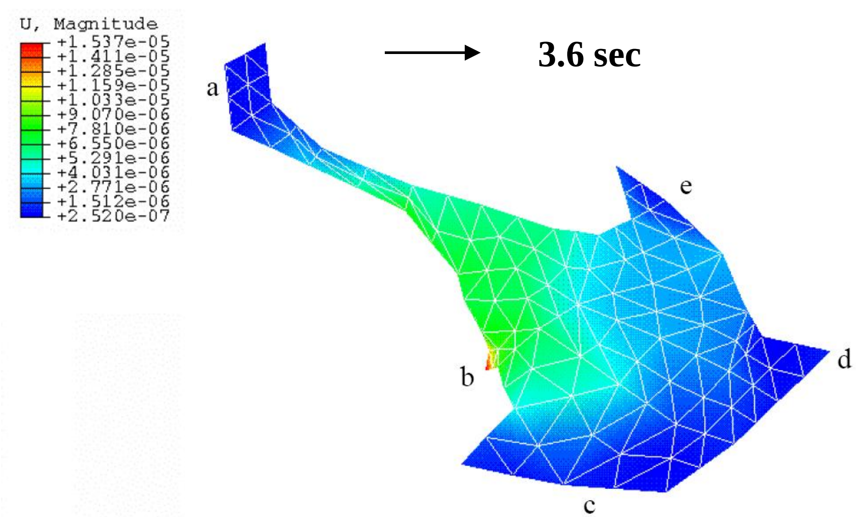
- Global Digital Image Correlation (GDIC) can be effectively utilized to characterize the cell deformation pattern by sequential correlating the images recorded during the assay shear test.
- The deformation mapping between these two images is obtained by a multi-variable minimization which conducted on a constrained system determined by the mesh
- Due to the severe deformations experienced by the cell during the assay test, a remeshing step is required to preserve the mesh quality



Cellular Displacement Subjected to Shear Flow



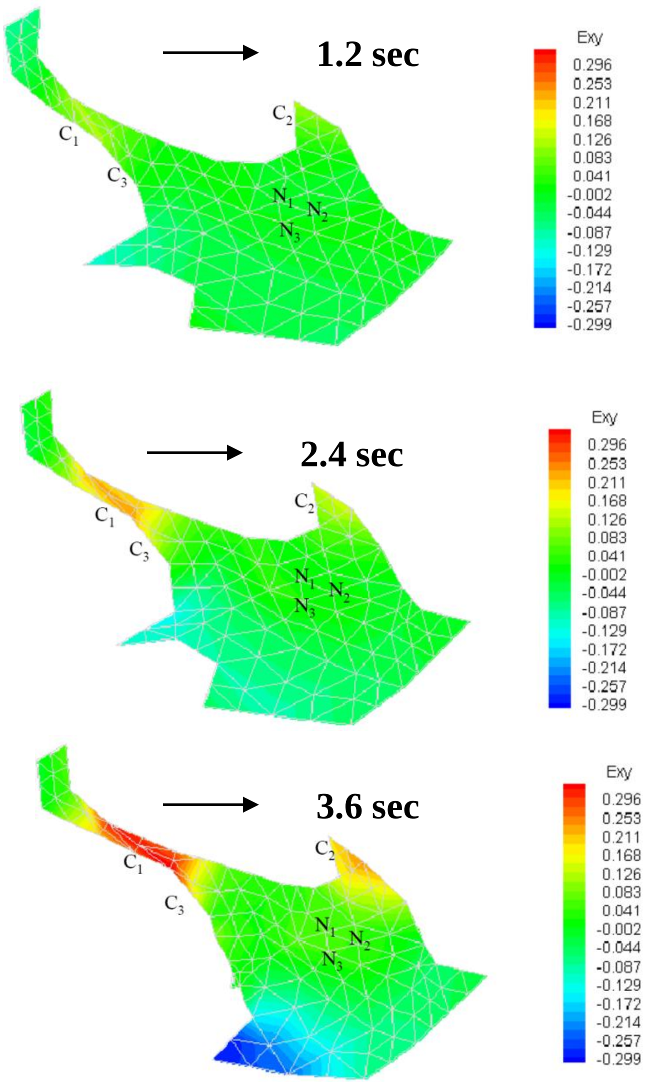


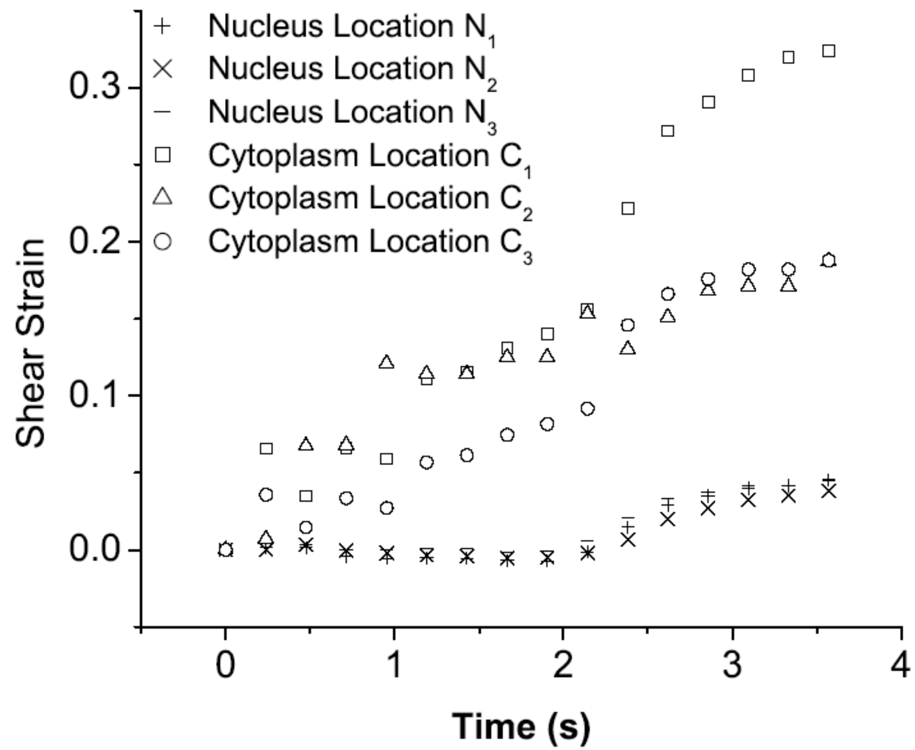


Higher mobility was observed at the rear edge (region b), compared to the front edge subjected to shear flow

displacement.avi

Cellular Strain Subjected to Shear Flow

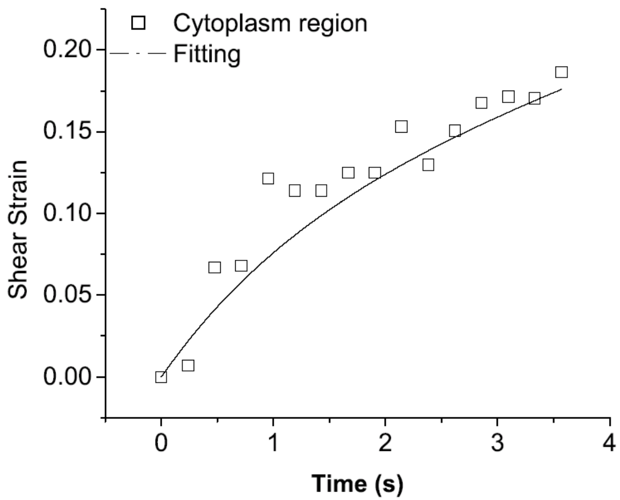


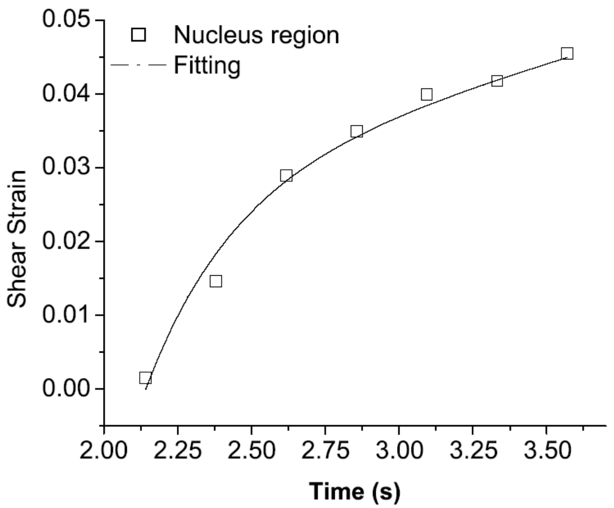


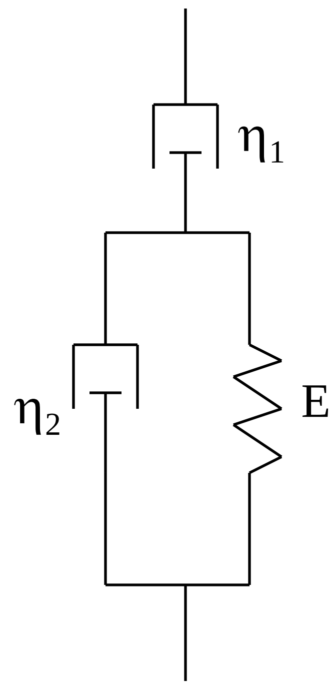
The shear strains in cytoplasm increased more significantly than those obtained in the nucleus during the shear assay experiment

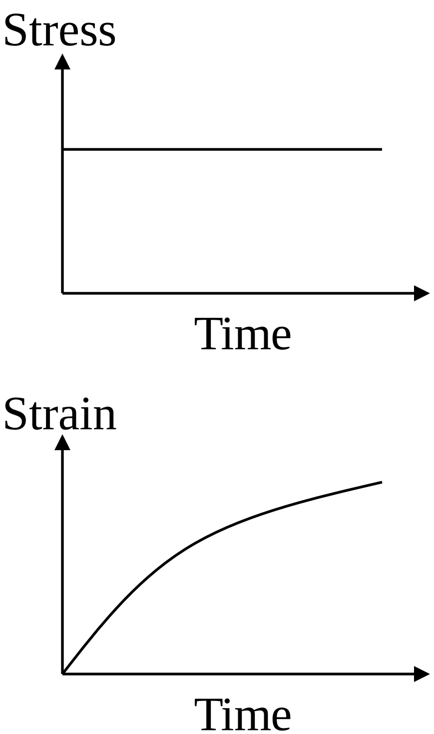
shearstrain.avi

Viscoelastic Modeling



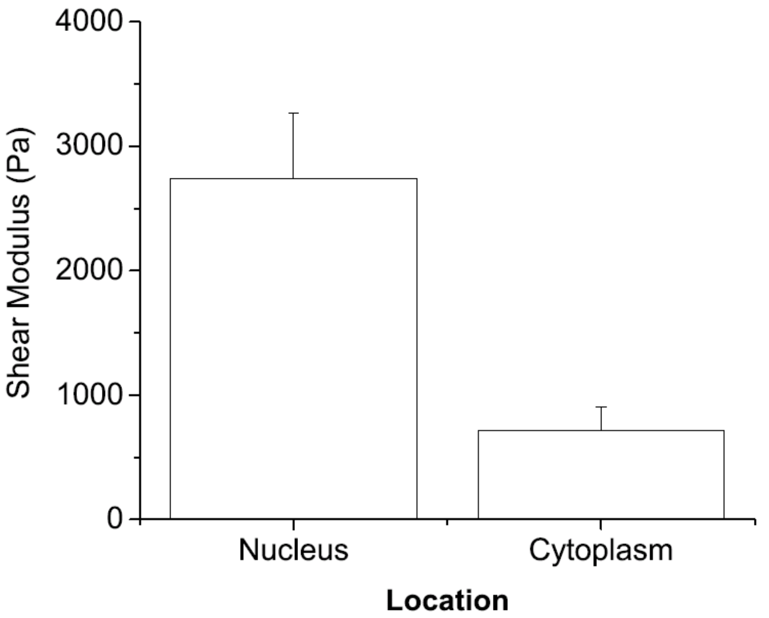


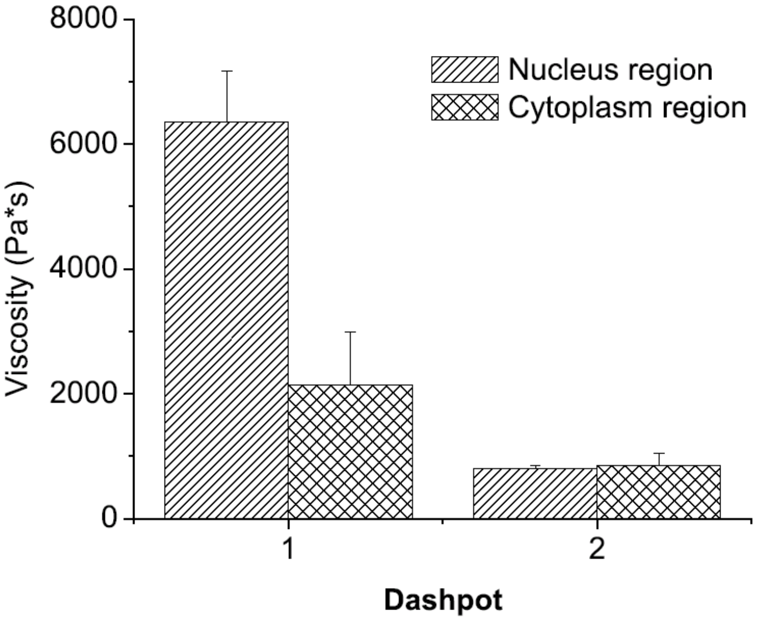




$$\epsilon = \frac{\sigma}{E}[1 - exp(\frac{-t}{\tau})] + \frac{\sigma}{\eta_1}t$$

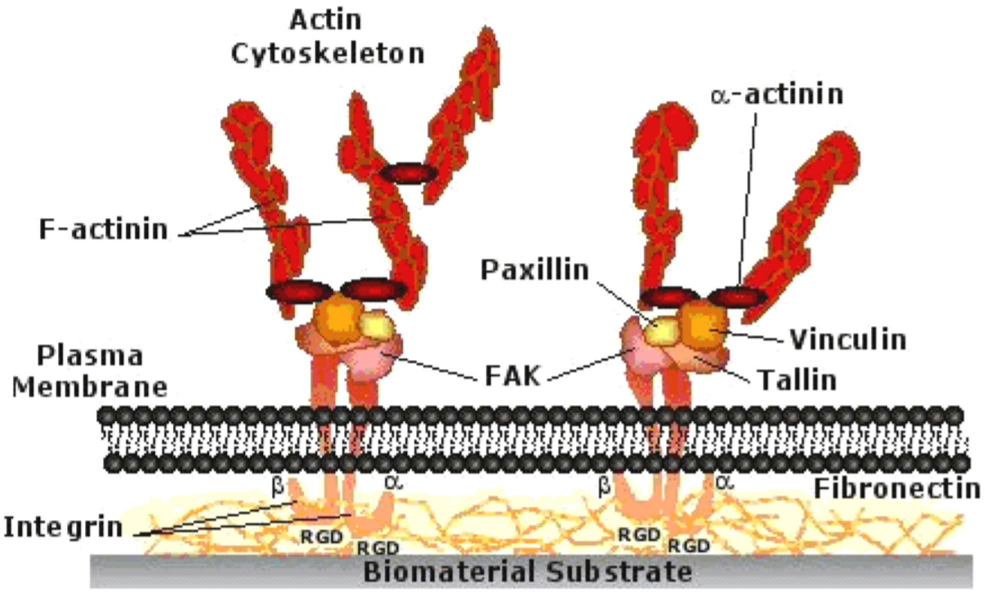
Obtained Shear Moduli and Viscosities





The fact that the nucleus is more rigid than the cytoplasm can explain why the nucleus deforms less than the cells when subjected to shear flow in the current study, or when the substrate is stretched.

Cellular Adhesion Apparatus



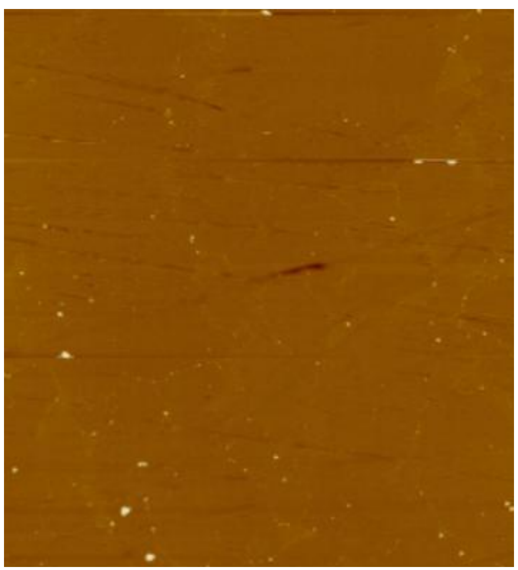
- Interaction between cell-cell and cell extracellular matrix are by specific contacts of adhesion molecules
- Cells in culture often form focal adhesion sites, a specialized and discrete region of the plasma membrane
- Cell viscoelastic deformability is determined largely by the composite shell envelope and cell cytoskeleton

Surface Preparation

- Polishing
- AFM
- RGD-Coating

Polished Surfaces

20 μm



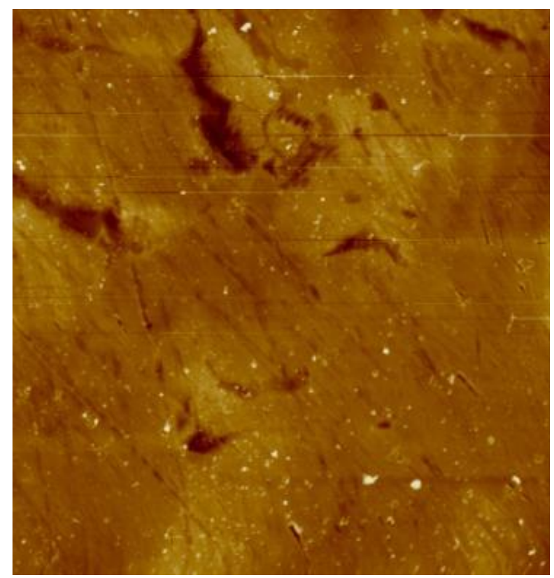
Roughness
5 nm

Control

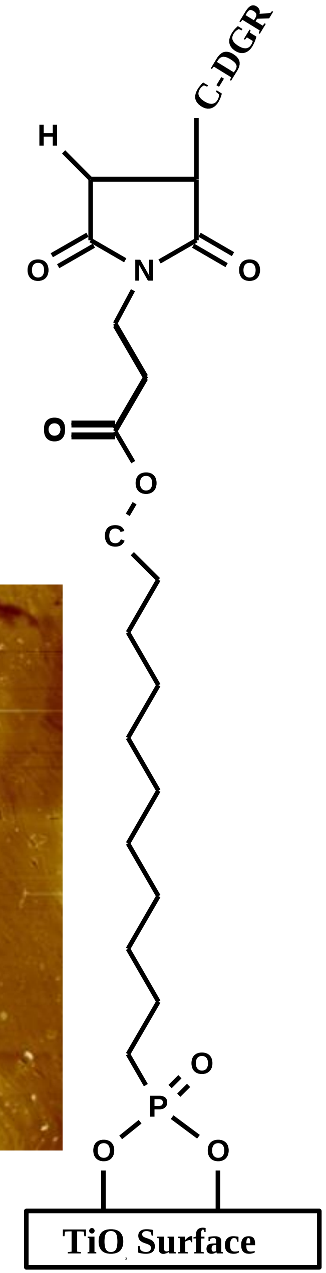
100nm

50nm

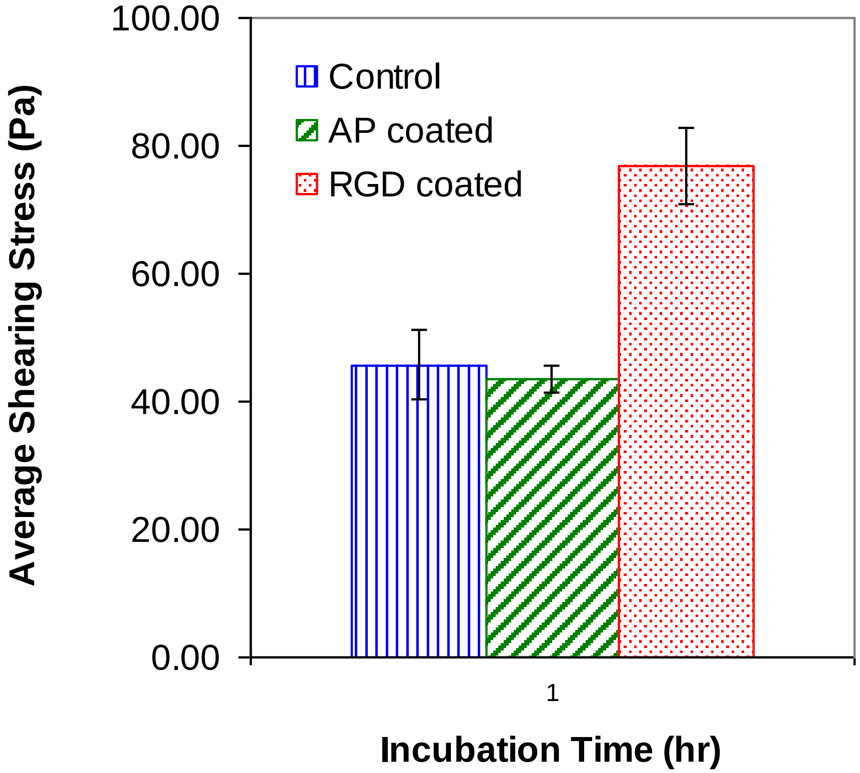
0nm

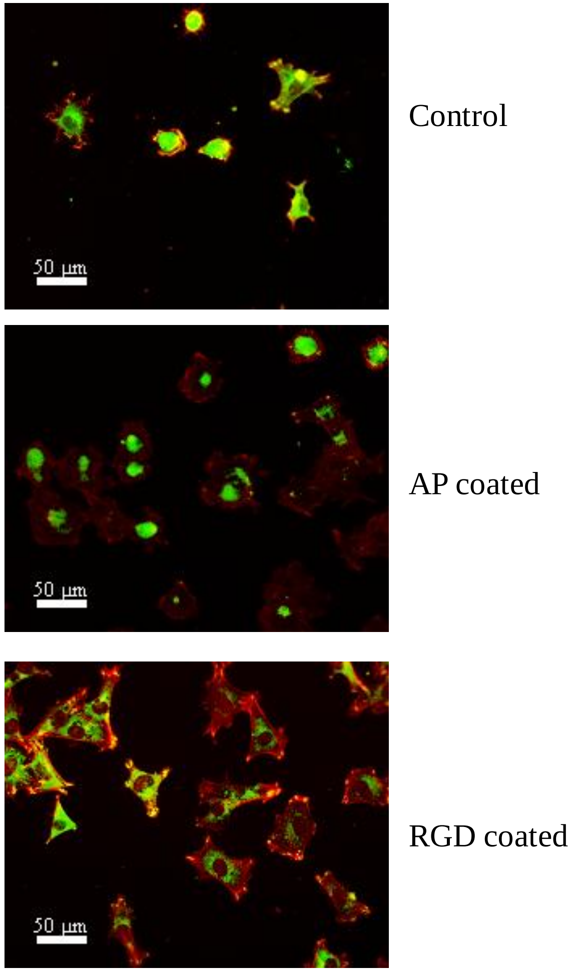


RGD coated



Interfacial Shear Strength Measurement

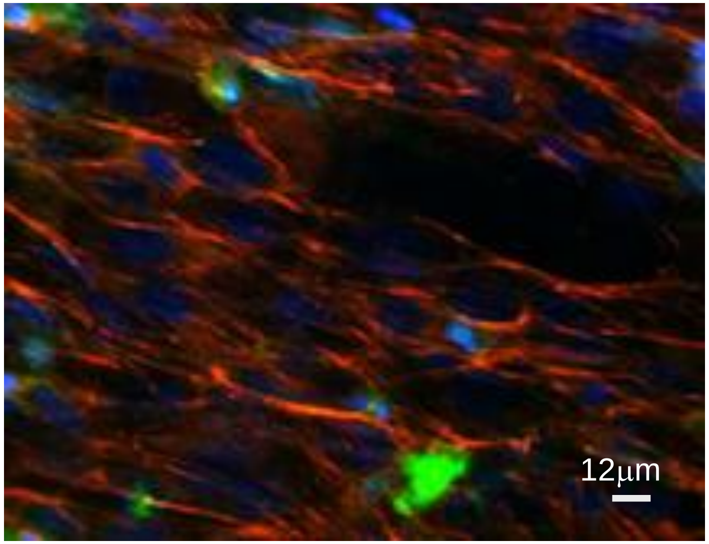




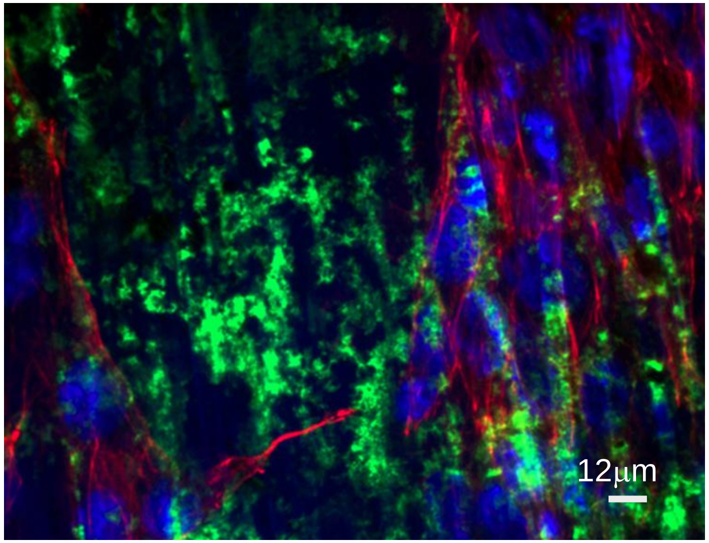
Effects of RGD Coating

- Short term effects of RGD coating:
  - Increased spreading and cellular adhesion
  - Increased protein organization of the cytoskeleton
- Previous studies indicate long term effects (mineralization):
- Clinical studies very promising

Control - 7 day



RGD - 7 day

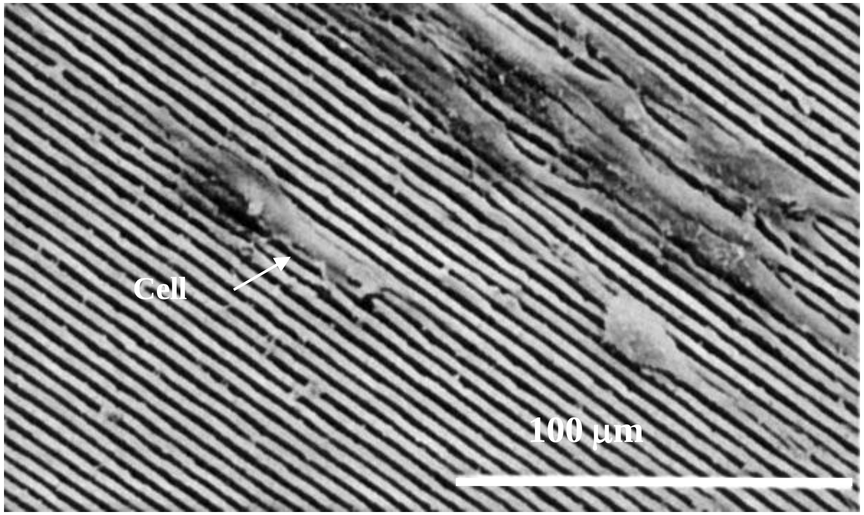


Stained for actin (red), nucleus (blue), hydroxyapatite (gree

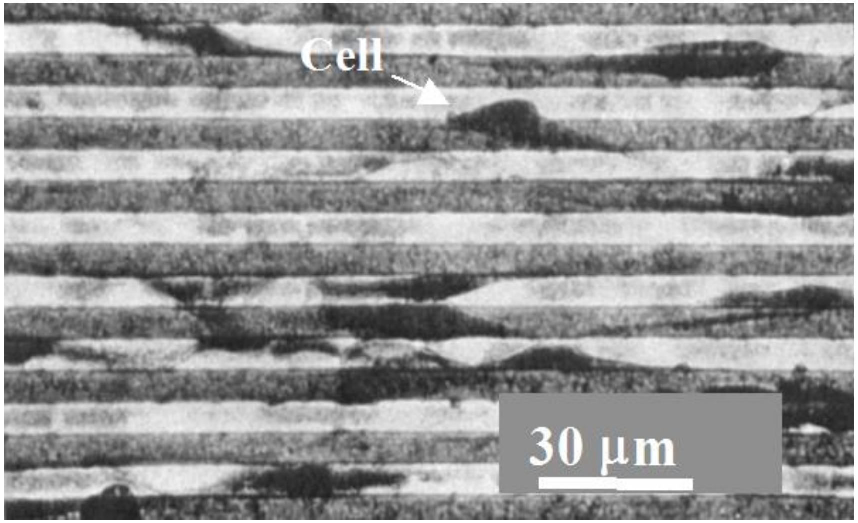
Micro-Groove Geometry and Cell/Surface Interactions

- Cells can undergo contact guidance when in contact with micro-grooved geometries
- This depends on the size of the grooves relative to the size of the cells
- Contact guidance has implications for wound healing and scar tissue formation

2 μm Micro-Grooves

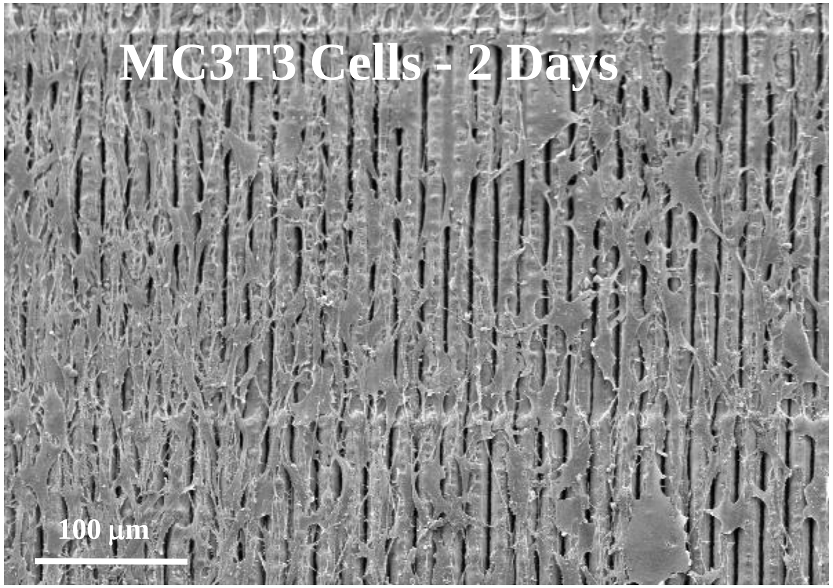


12 μm Micro-Grooves

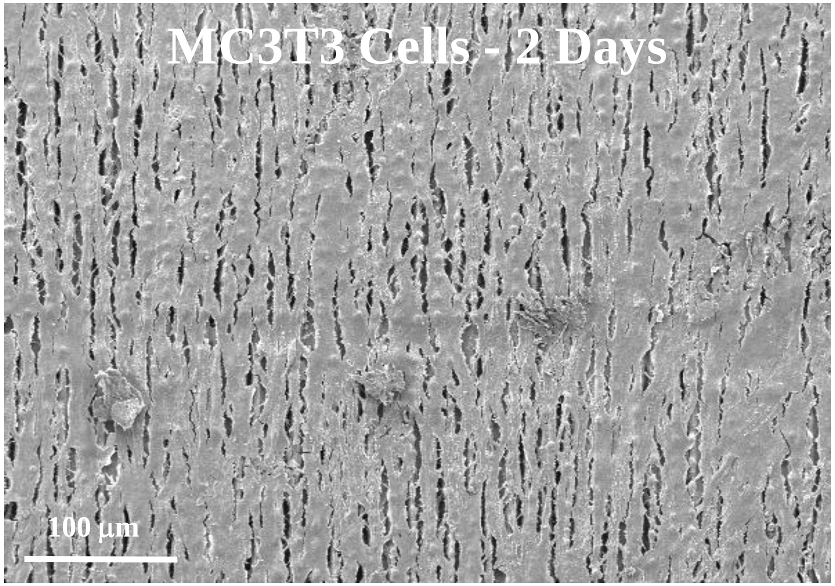


Laser Textured Surfaces - Cell/Surface Interactions

8 μm Grooves

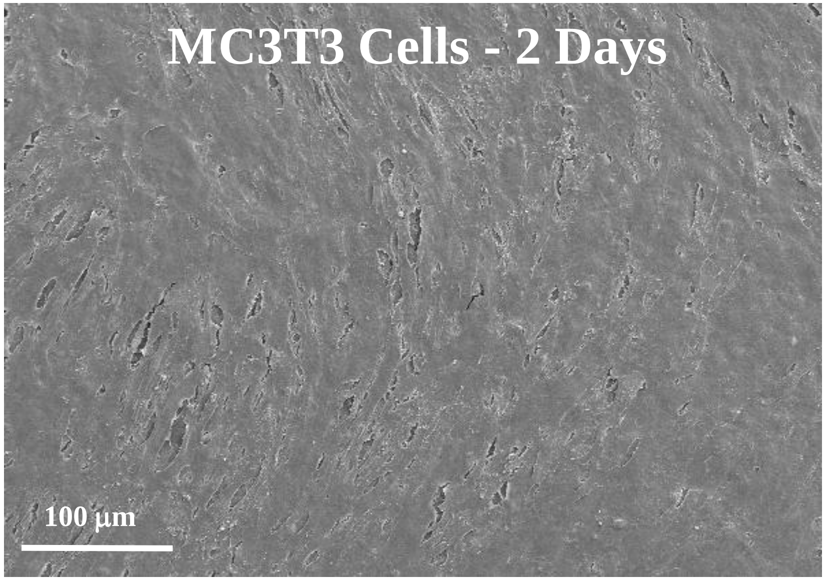


12 μm Grooves

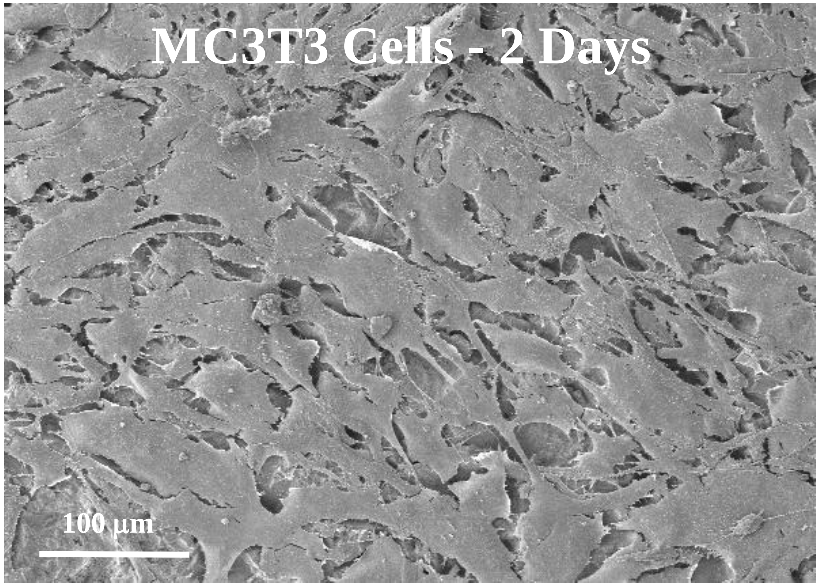


Surface Texture - Cell/Surface Interactions

Polished

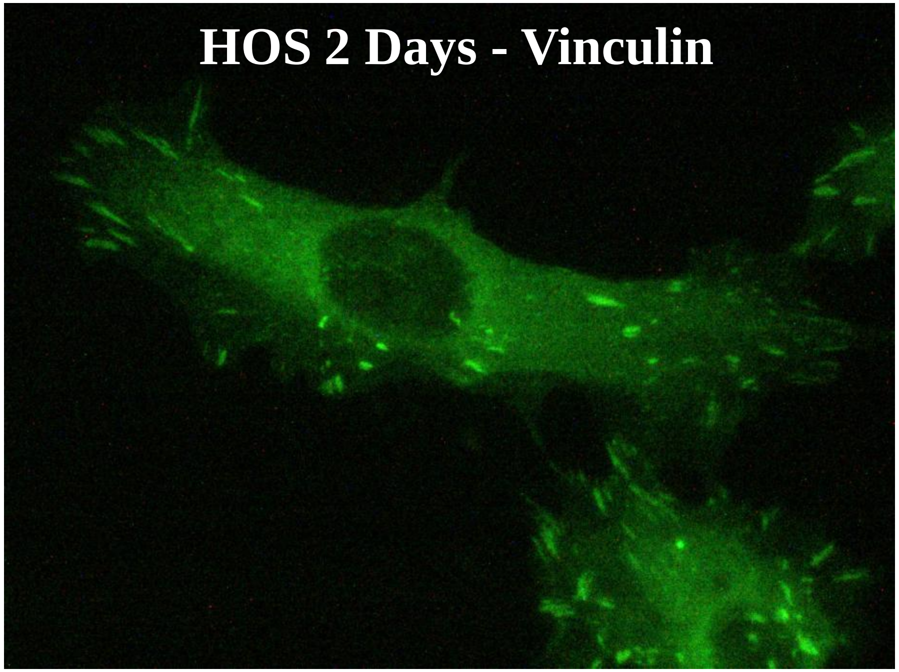


Al_2O_3 Blasted

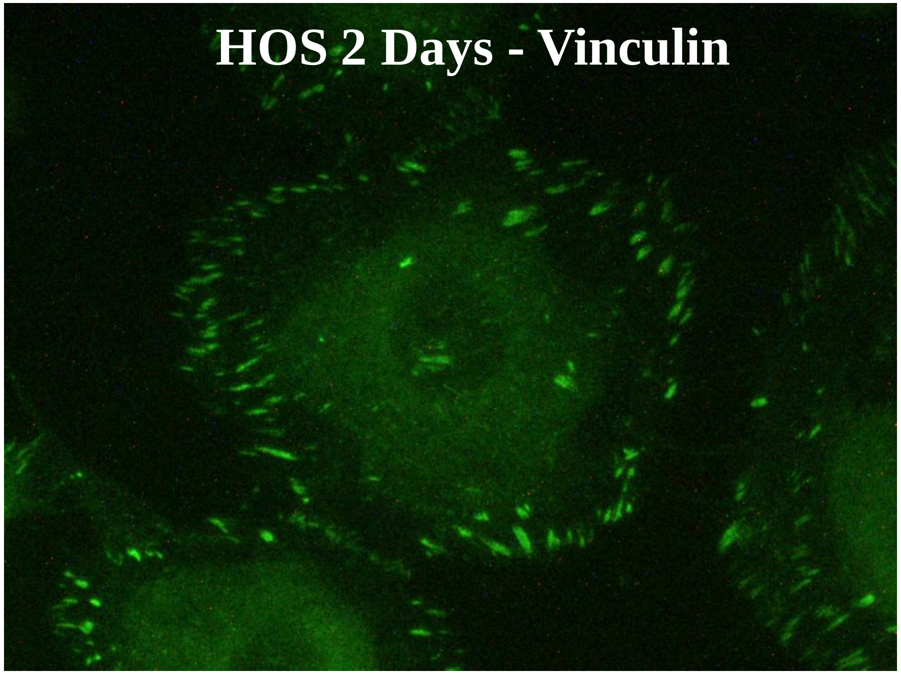


Surface Texture – UV Laser Textured Surfaces IF Staining

11 μm Laser-Grooved Surface

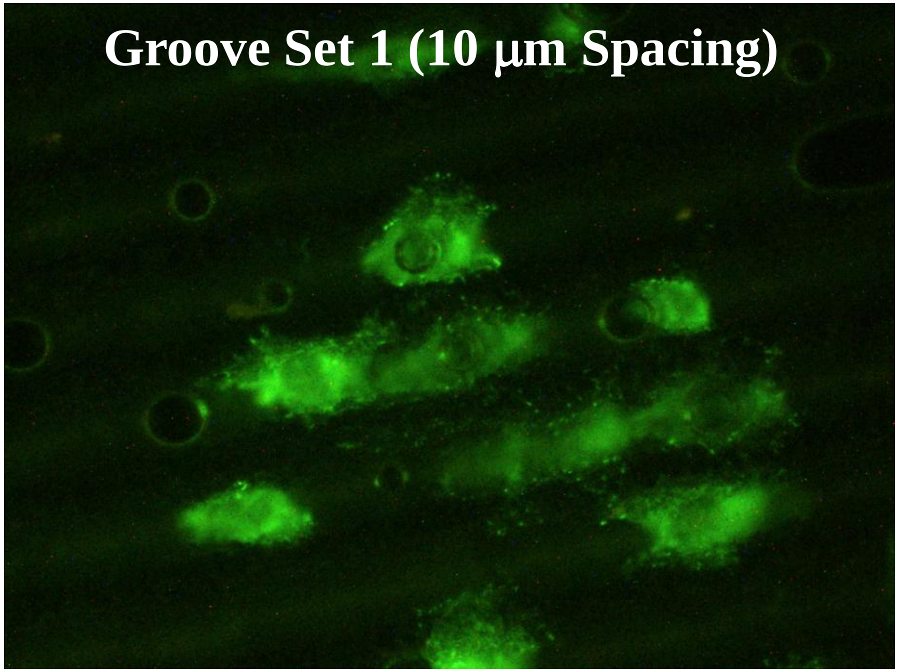


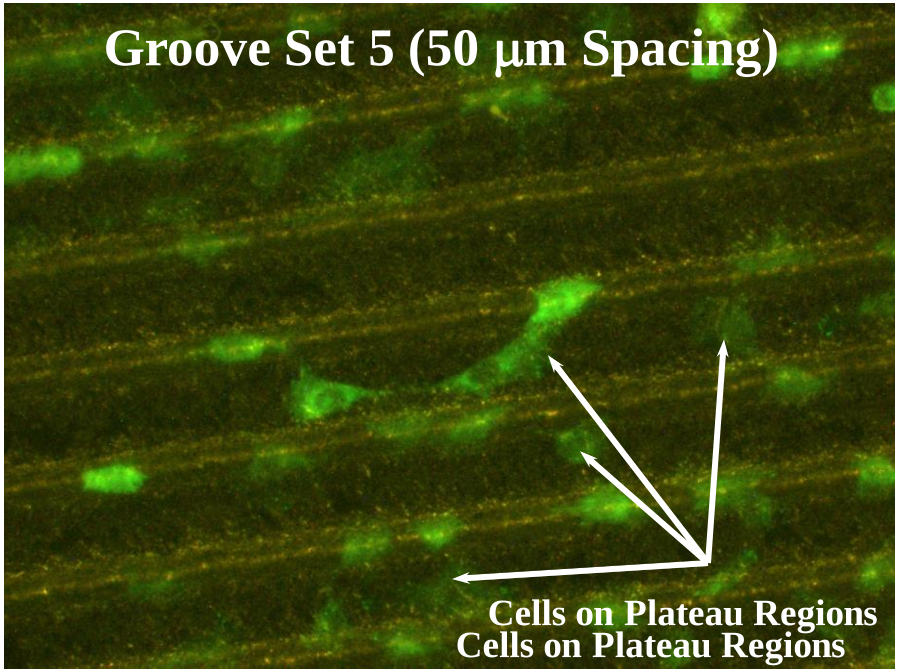
Polished Surface



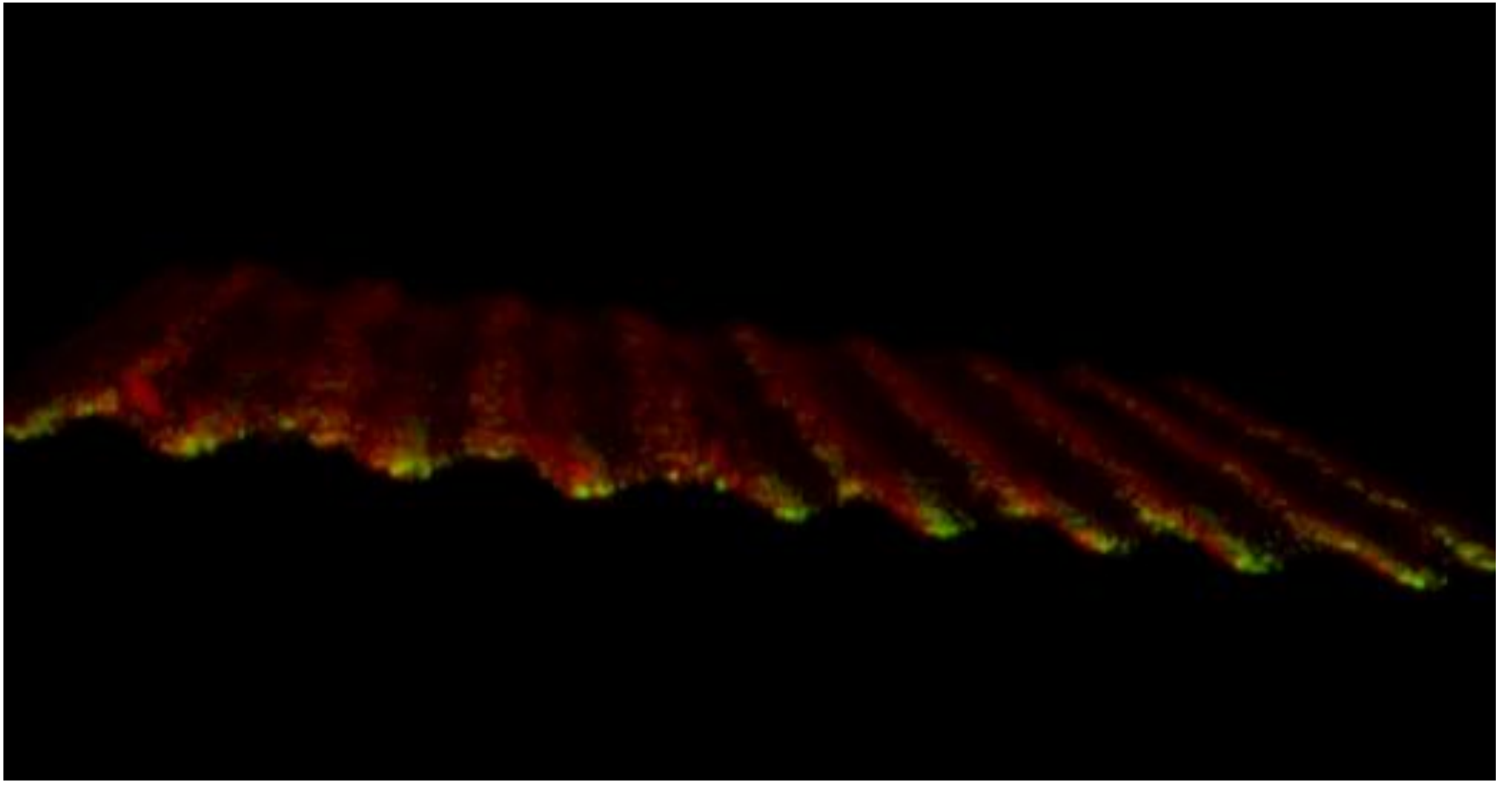
Surface Texture – UV Laser Textured Surfaces
Cell/Surface Interactions

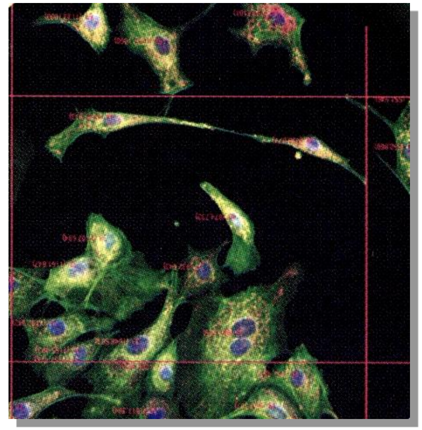
- HOS cells cultured on groove set 1 (10 μm spacing) lie within micro-grooves – high level of contact guidance.
- HOS cells cultured on groove set 5 (50 μm spacing) display a moderate level of contact guidance – some cells span across plateau regions.



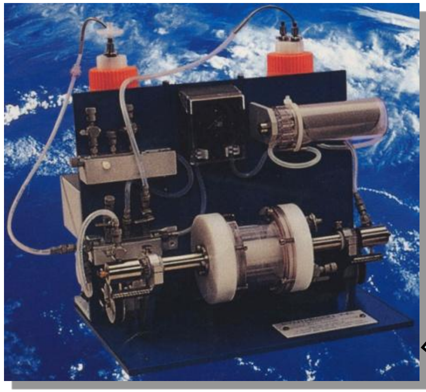


3-D View (Horizontal, 10micron)





Cells

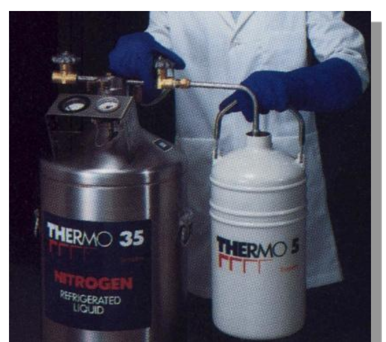


Bioreactor Design

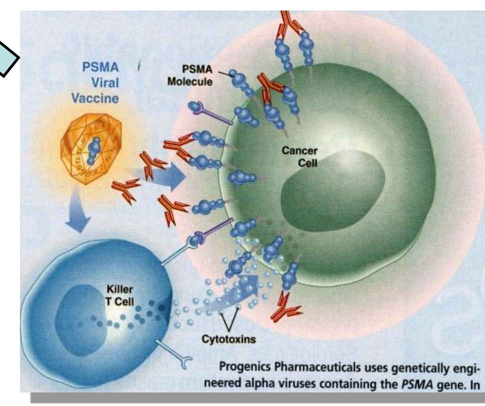
Scaffolds

Engineered Tissue

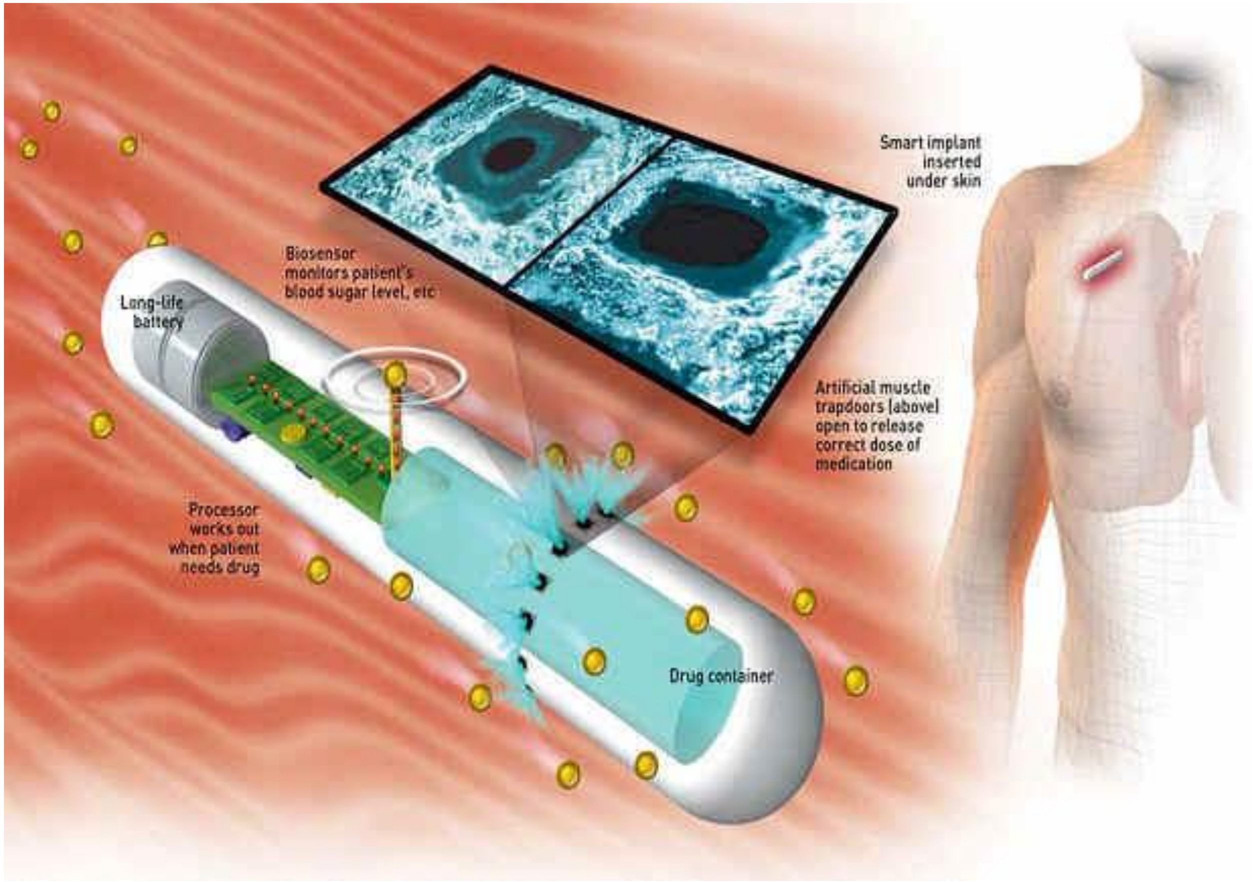
Upscaling



Preservation



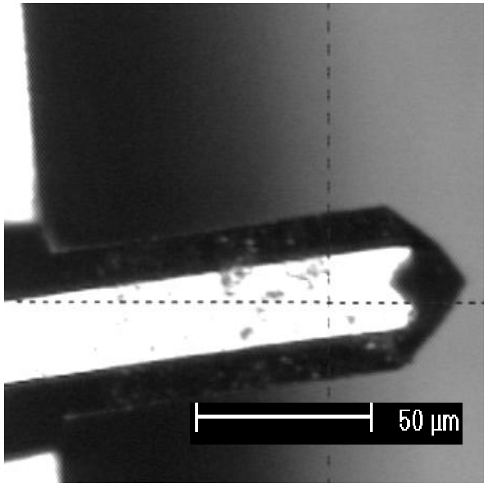
Immunoisolation/
Compatibility



Smart implant
inserted
under skin
Biosensor
monitors patient's
blood sugar level, etc
Long-life
battery
Artificial muscle
trapdoors (above)
open to release
correct dose of
medication
Processor
works out
when patient
needs drug
Drug container

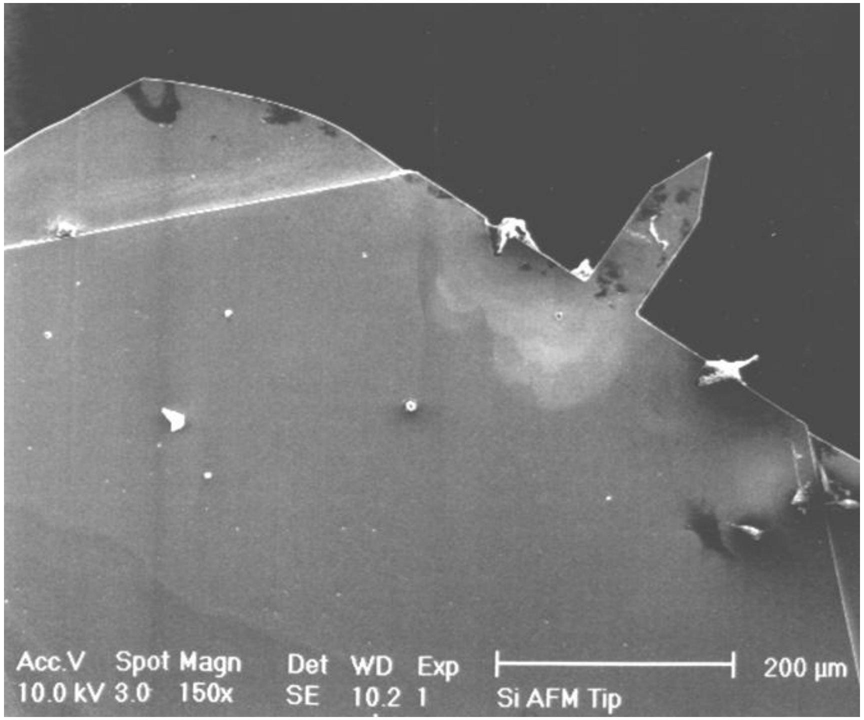
A FEW METHODS FOR DETECTING CANCER

- View under a microscope at high magnification
- Use a biochemical assay to reveal cells
- Use a bioMEMS cell detector e.g. a cantilevered MEMS structure
- External imaging system, e.g. MRI

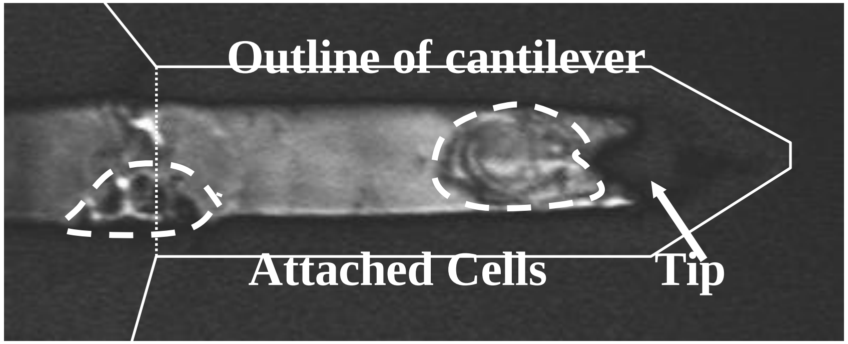


Single HOS Cell on Si Cantilever in AFM

Single cell on Si Cantilever



CELL DETECTION ON CANTILEVER



Cantilever No. 17

Initial Frequency: 263.36 KHz

Spring constant: 44.86 N/m

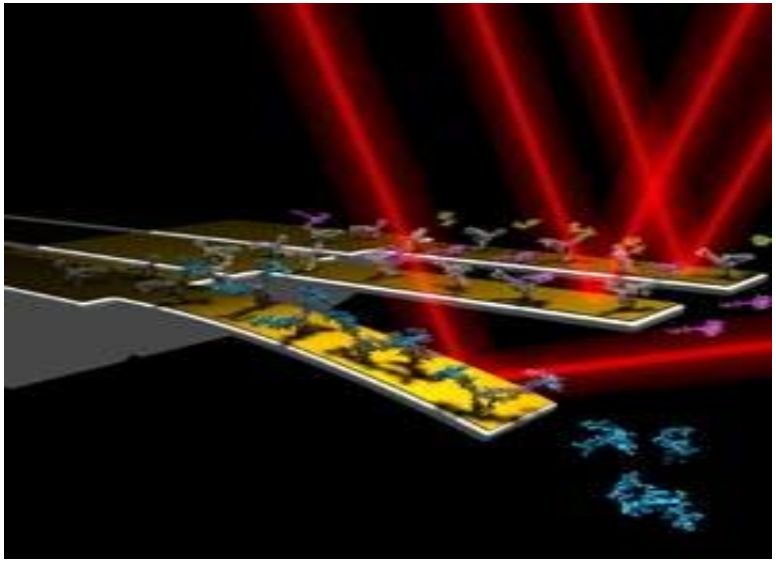
Final Frequency: 261.59 KHz

Difference: 1.77 KHz

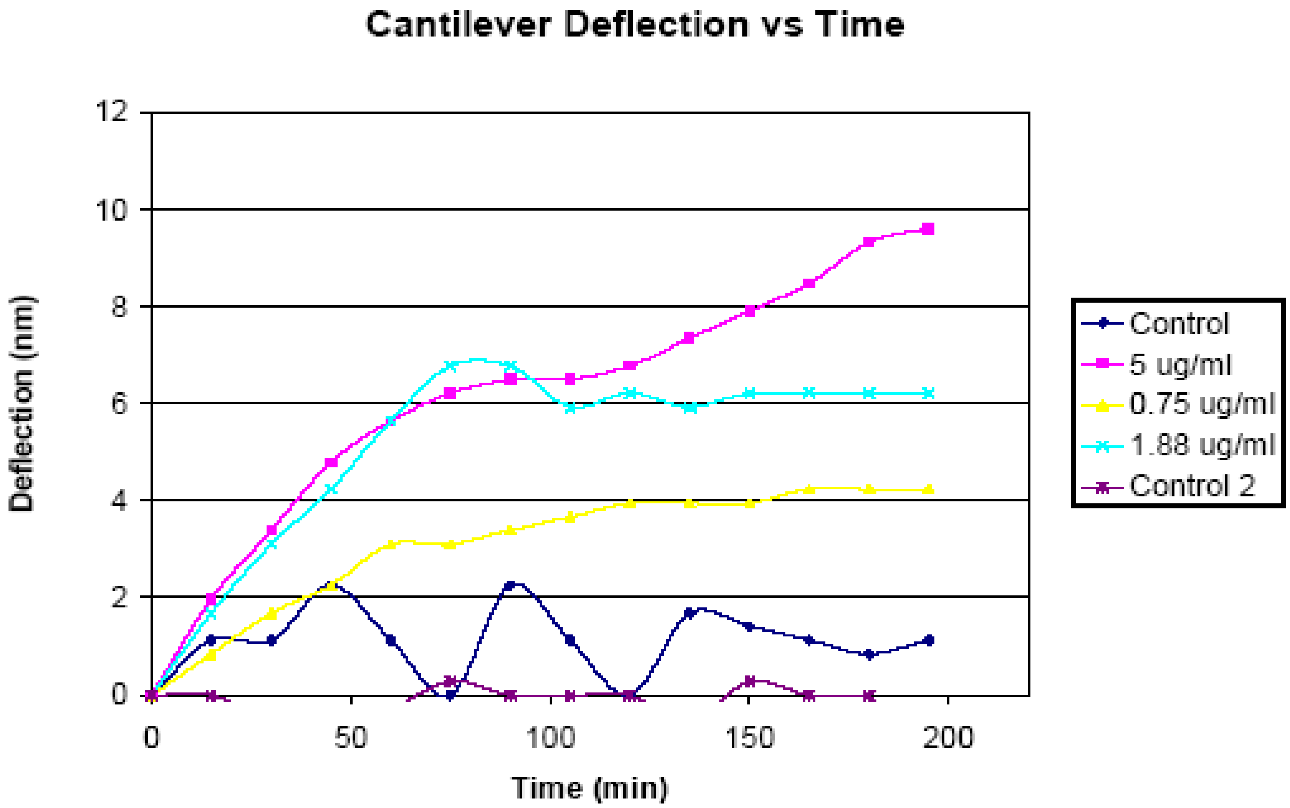
- Cantilever shows the presence of two cells
  - one attached near the tip, the other is at the base of the cantilever

Antibody/Antigen Interactions

- Antibody/antigen interactions cause surface stresses to develop
- These surface stresses are the result of new conformations of molecular structures at the surface
- Interactions between Vimentin antibodies and antigens gives rise to surface stress and cantilever deflection



Cantilever Deflection data



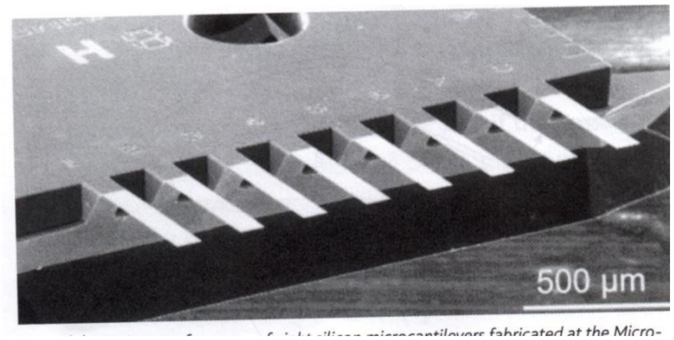
THE FUTURE OF CANTILEVERED BIOMEMS STRUCTURES – BIOMOLECULAR DETECTION

- Research will lead to future cantilevered bioMEMS structures
- Devices may be resonating devices for improved sensitivity
- However, non-resonating devices can also be used
- Multifunctional structures emerging with multiple cantilevers

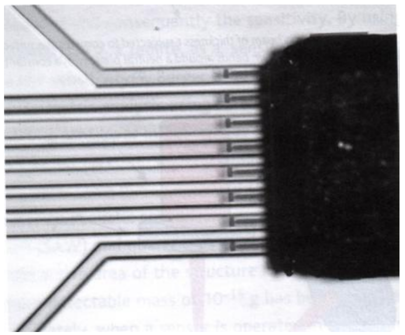
Functionalized Cantilever

- DNA
- Folic Acid
- Antibodies

Microcantilever Array

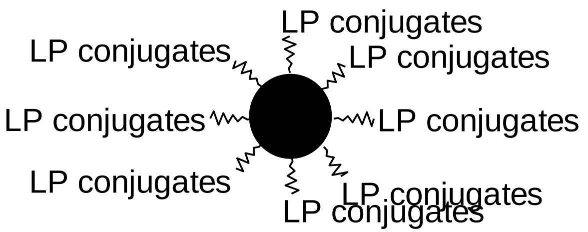


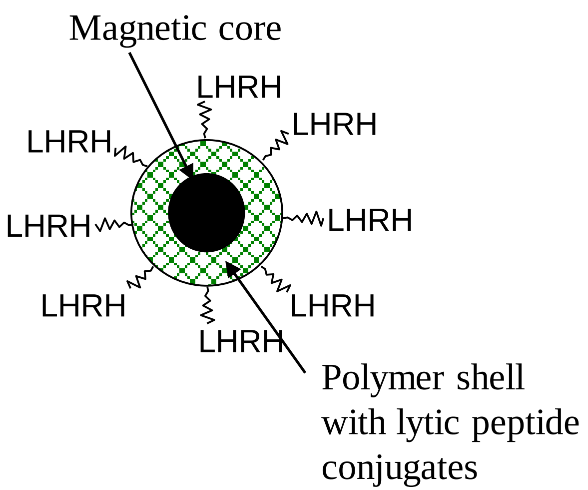
Packaging

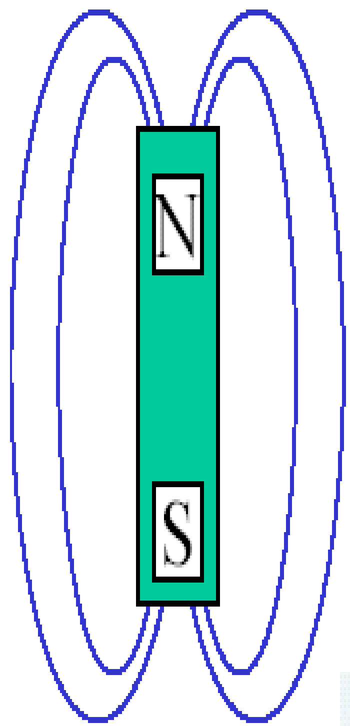


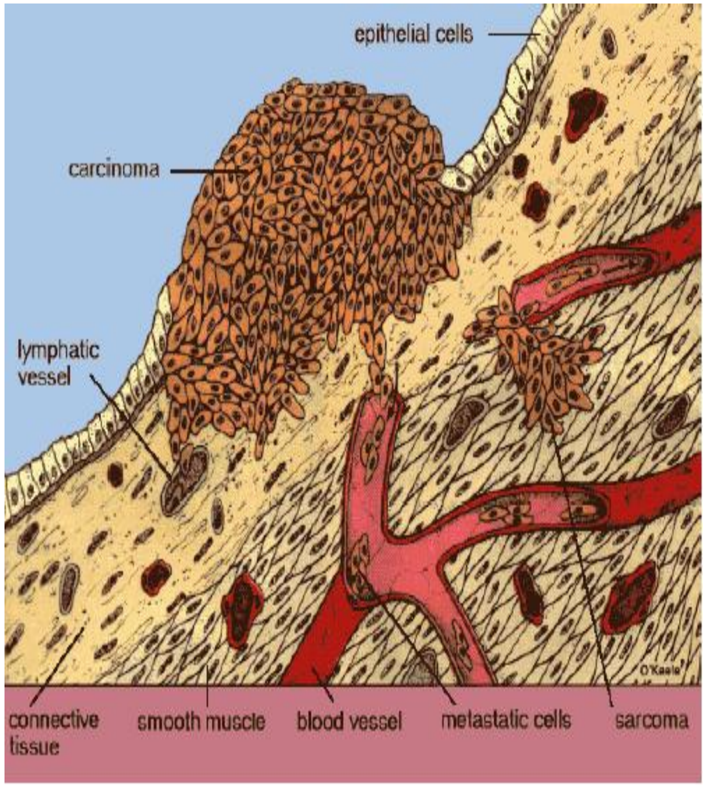
Our Approach to Early Cancer Detection and Treatment!

A novel use of magnetic fields and magnetic particles to deliver therapeutic drugs at the desired time in the correct dosage to the correct site in the human body.

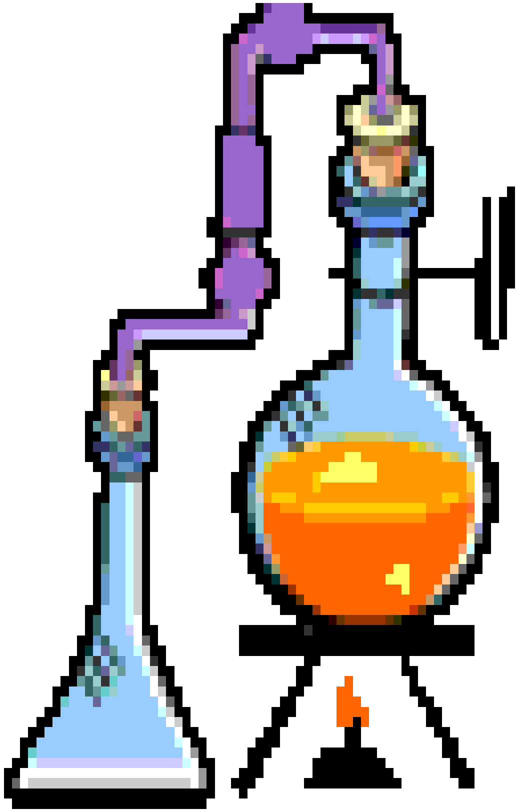








Wet Chemical Synthesis of Nano-particles

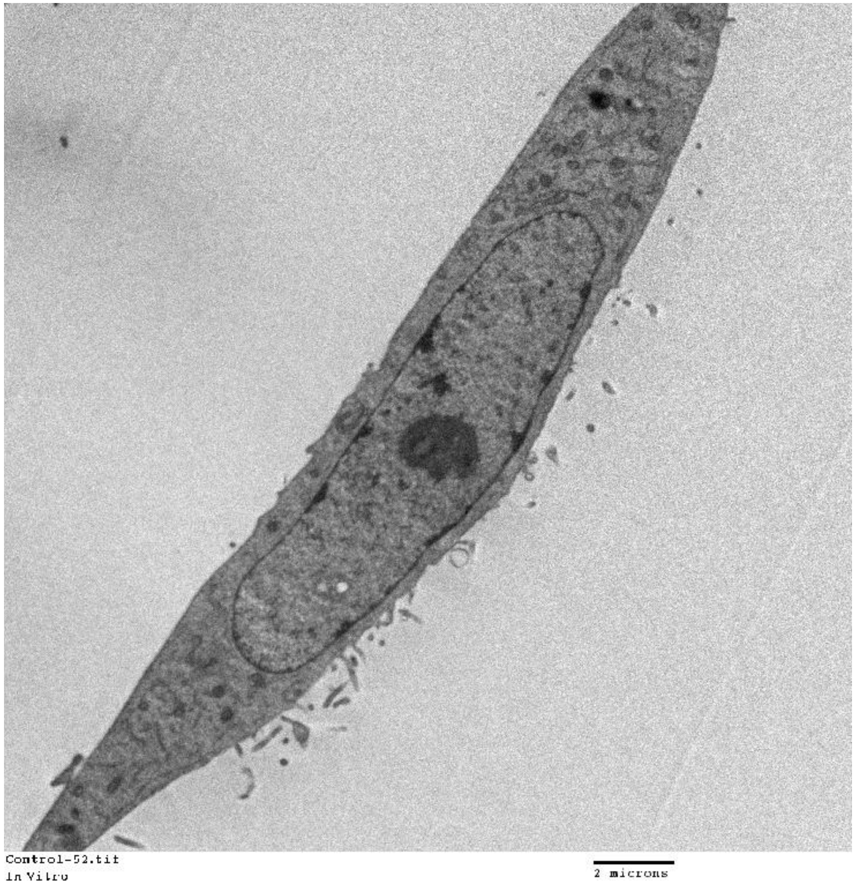


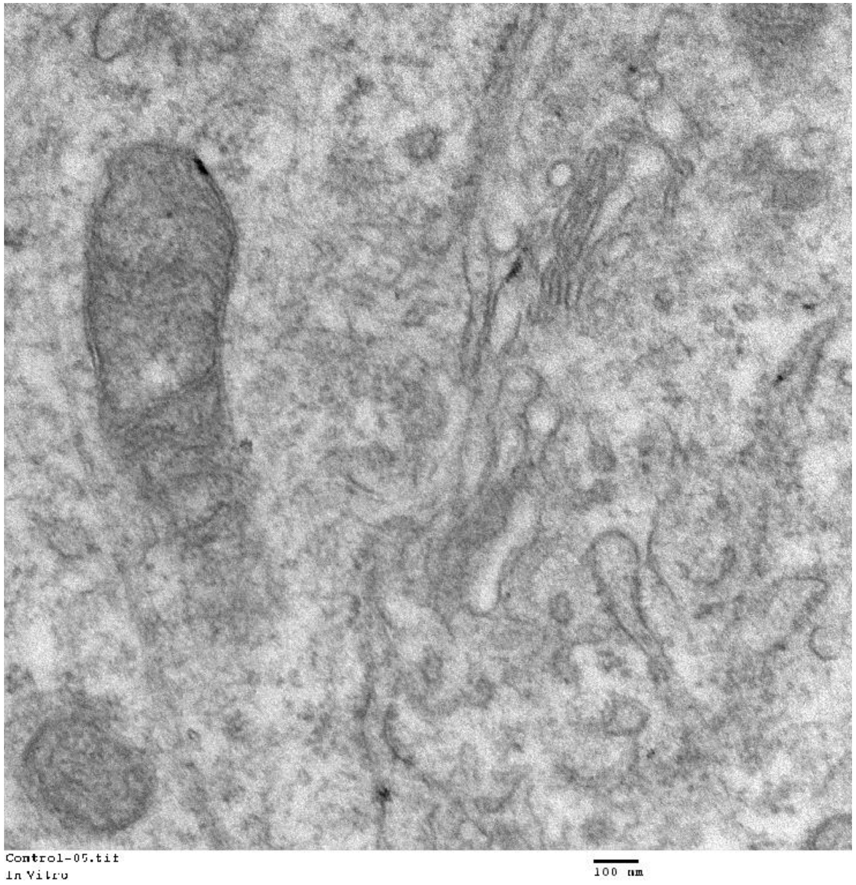
- Metallic, polymeric and metal-polymer Nano-particles using bottom-up approaches
- Novel Micro reactor technology for scale-up and controlled synthesis
- Synchrotron radiation based X-ray absorption Spectroscopic characterization
- Capability to attach bio-molecules

In-Vitro Experiments

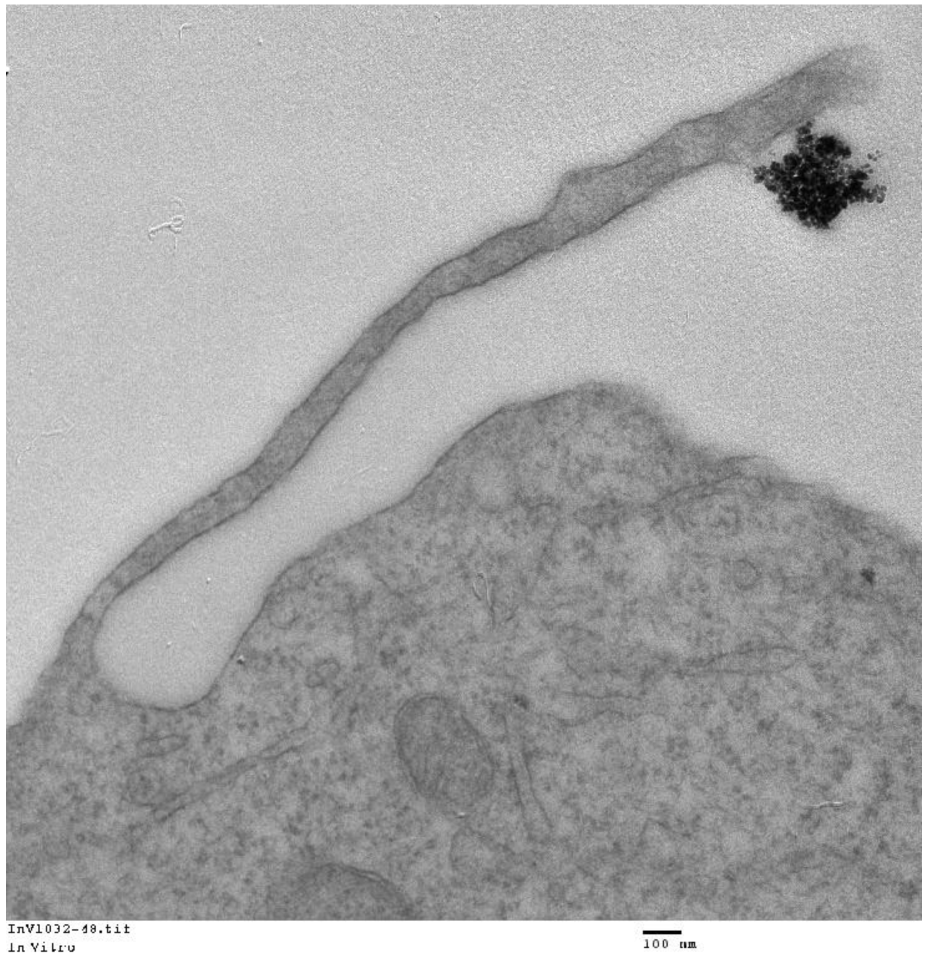
- Studied attachment of nano-particles in cell culture experiments
- Studied effects of temperature and time
- Imaging done using TEM after fixing
- Studies conducted on breast cancer cells with LHRH receptors
  - Unconjugated nanoparticles
  - LHRH-coated nanoparticles

TEM Images of Breast Cancer Cells (Control)



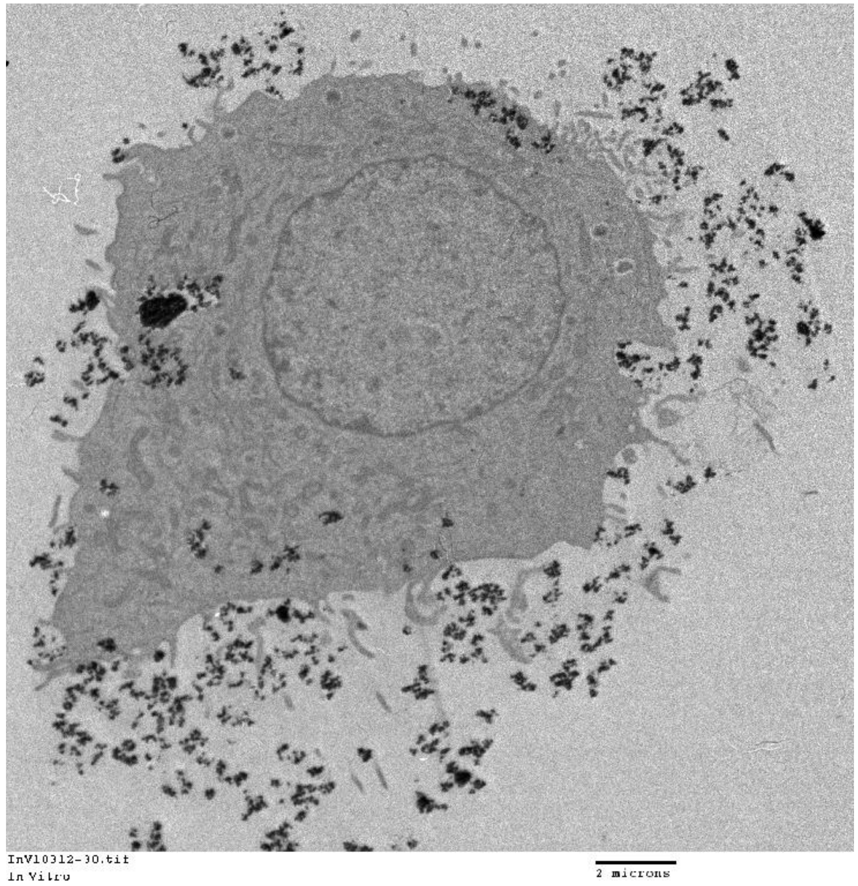


SPION Uptake - 37 C for 30 Minutes



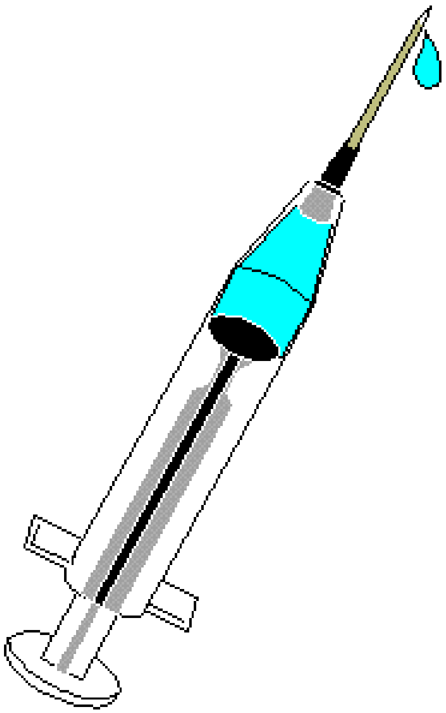
LHRH-SPION Uptake - 37 C for 3 Hours

- MNPs-LHRH, 37 C, 3 Hr
- Note encryption process by which cells attach
- Engulfed cells carried within the cell
- Excreted or egested within 30 days



In-Vivo Experiments

- Mice injected in 4 different ways:
  1. LHRH nanoparticles
  2. saline solution
  3. nanoparticles
  4. LHRH nanoparticles but with mice that do not contain breast tumor



Materials Characterization of Organs (TEM and Histology)

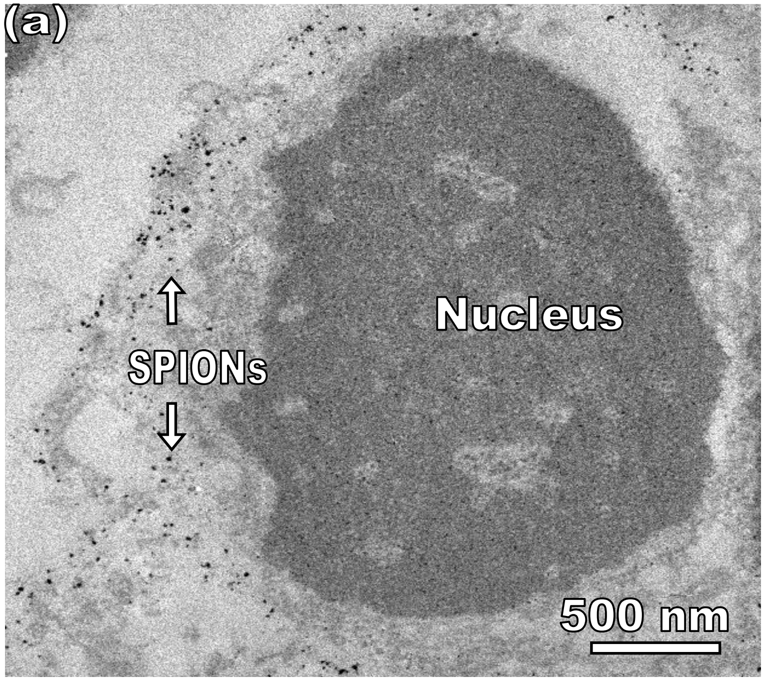
Organs obtained:

- breast or prostate tumor
- Kidney
- Lung
- Liver

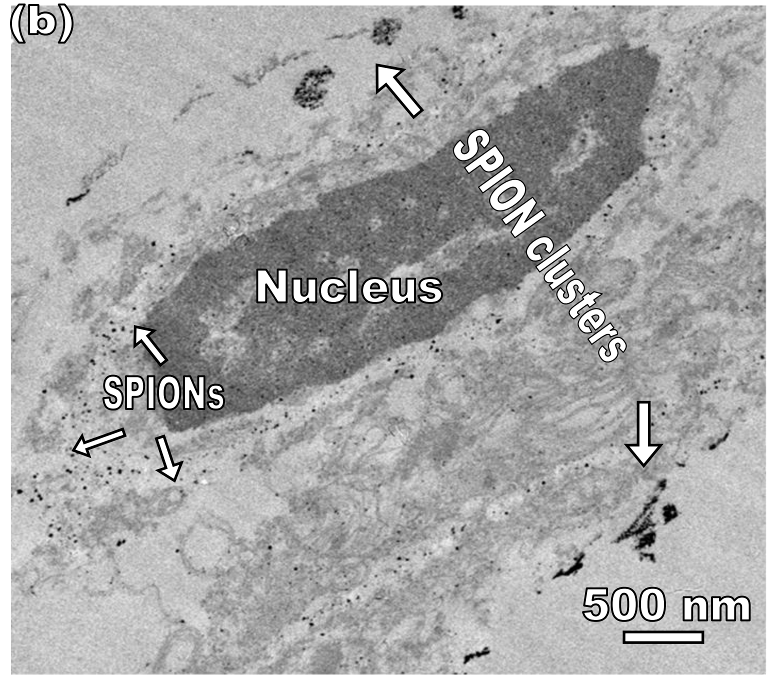
Ensure that the nanoparticles do not accumulate in other major organs.

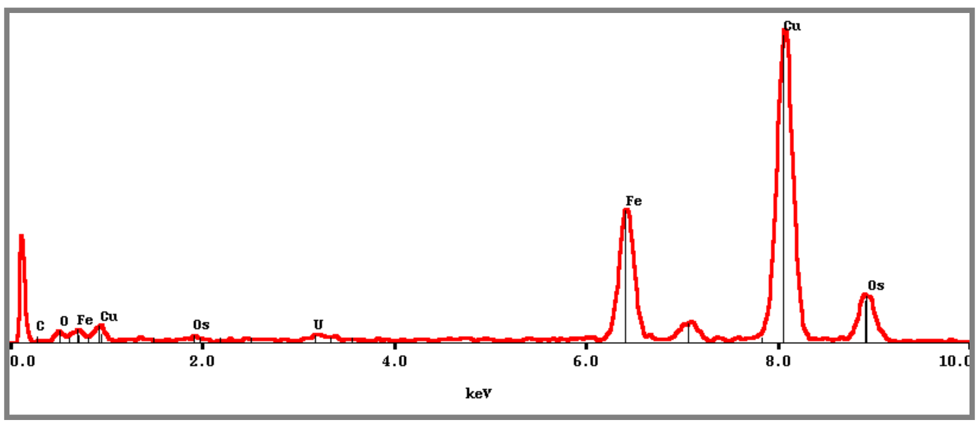
SPION/SPION-LHRH in Breast Tumor

SPION in Tumor



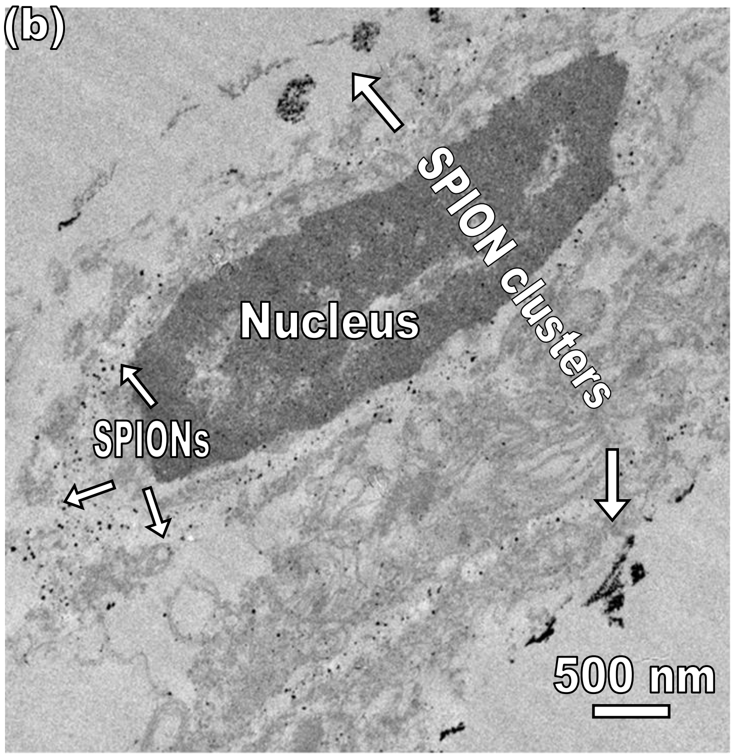
LHRH-SPION in Tumor



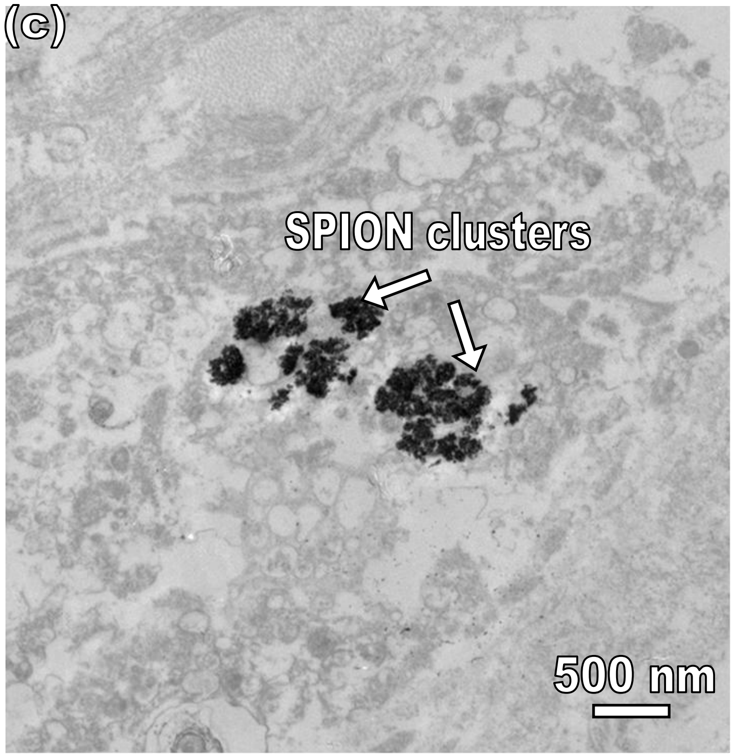


SPION/SPION-LHRH in Breast Tumor

LHRH-SPION in Tumor

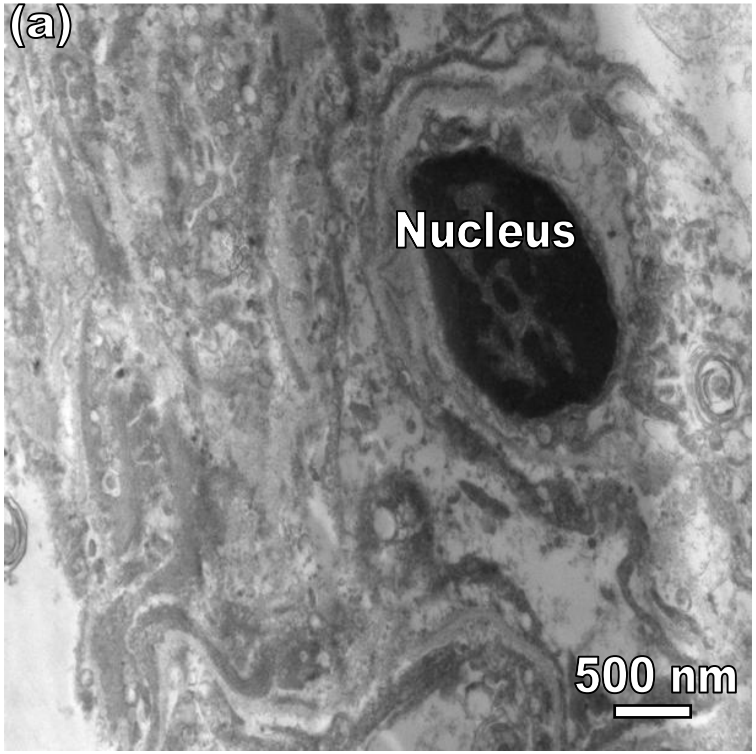


LHRH-SPION in Tumor

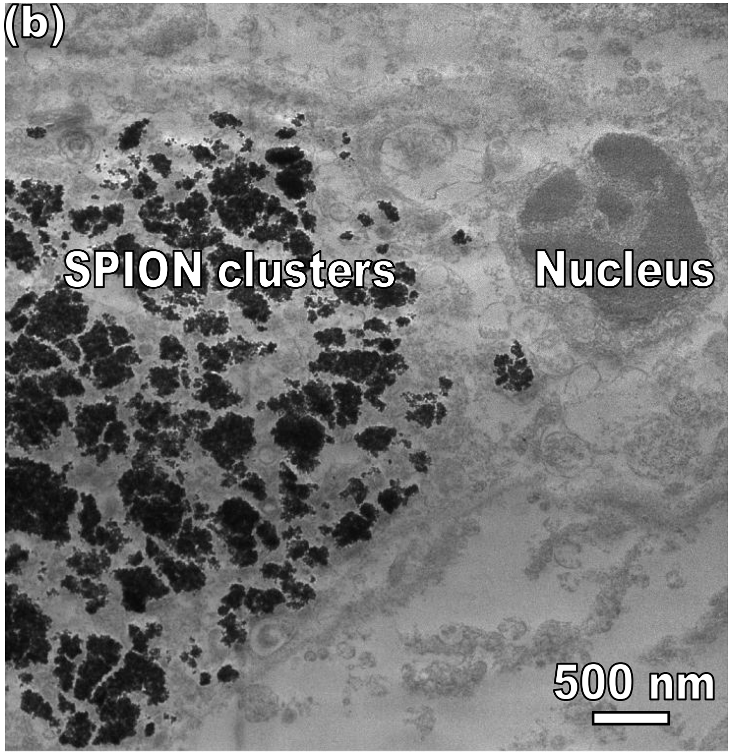


SPION in Lung

SPION in Lung

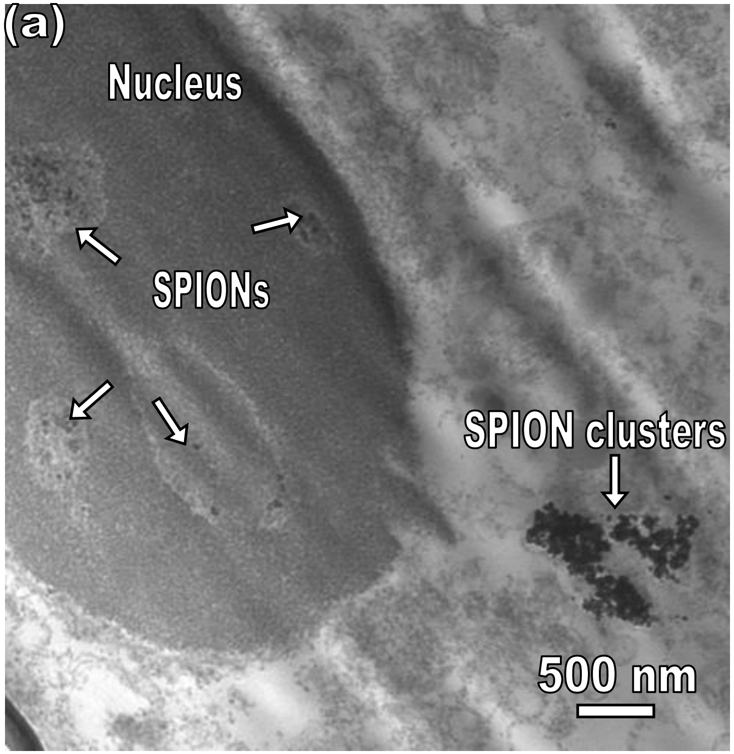


LHRH-SPION in Lung

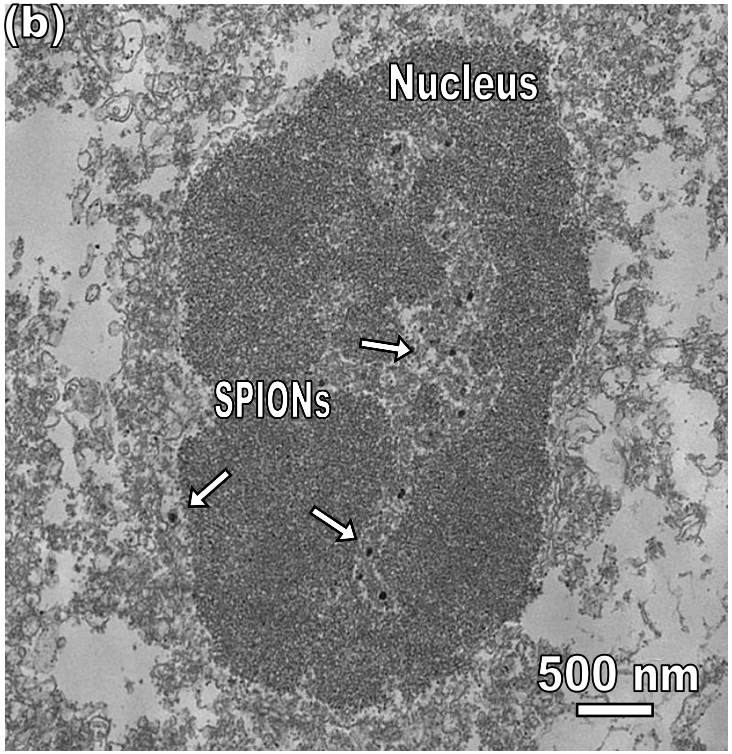


SPION/SPION-LHRH in Liver

SPION in Liver

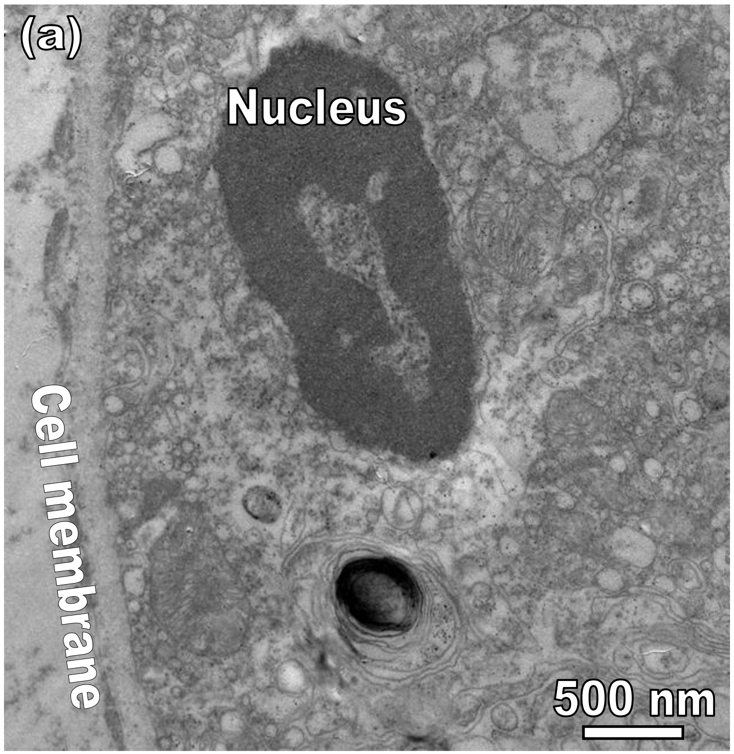


LHRH-SPION in Liver

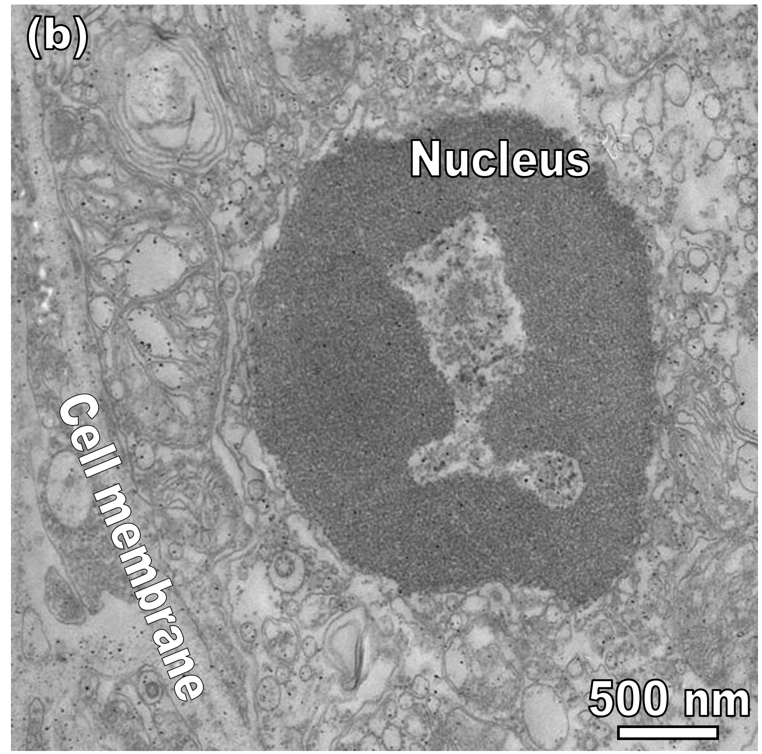


LHRH-SPION in Kidney

SPION in Kidney

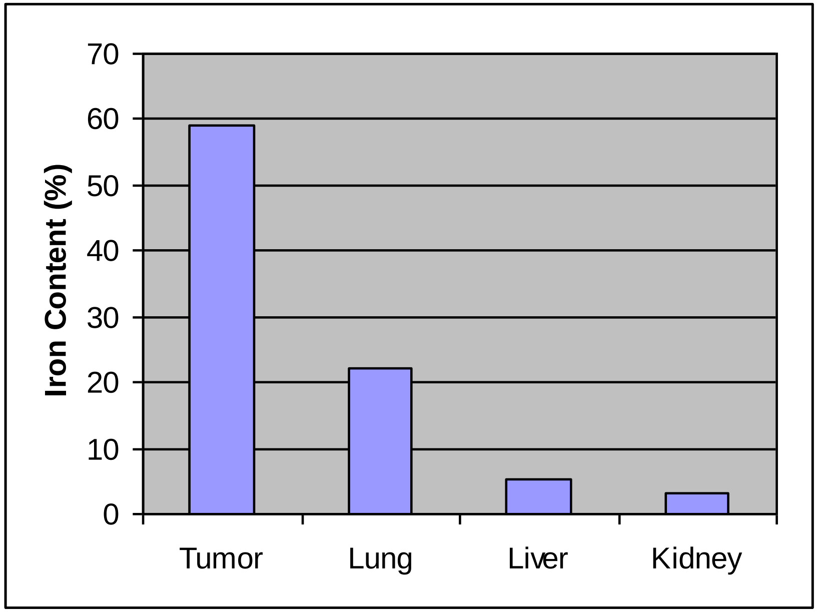


LHRH-SPION in Kidney

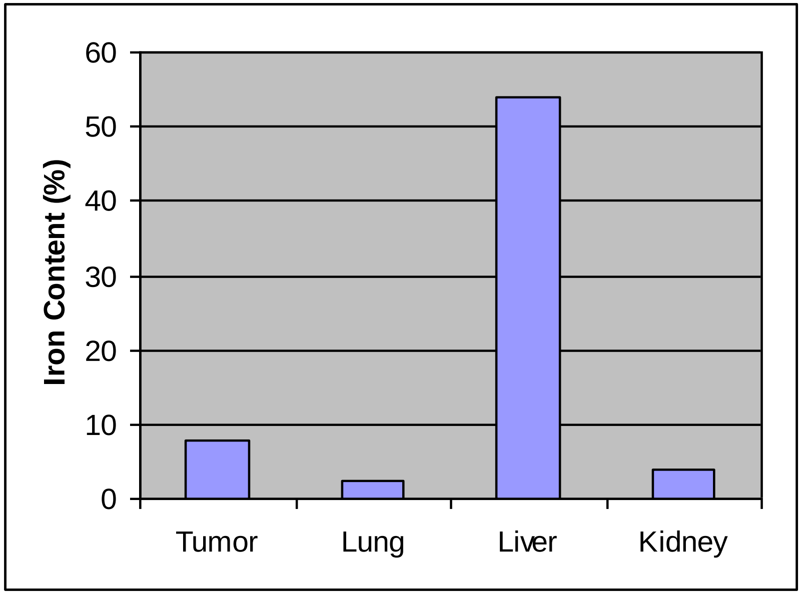


Biological Distribution of SPIONs

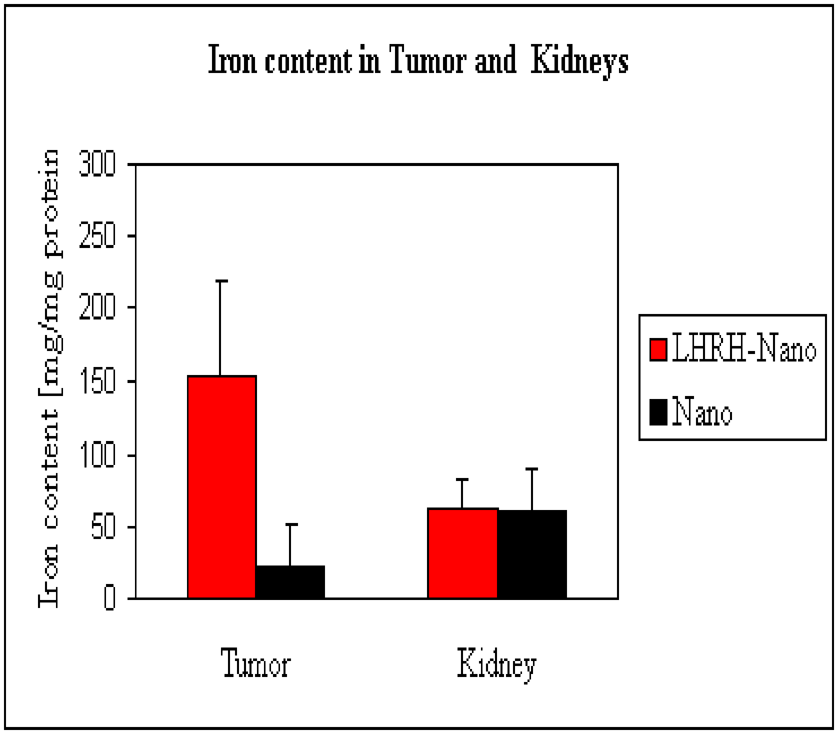
LHRH-SPION in Mouse



SPION in Mouse



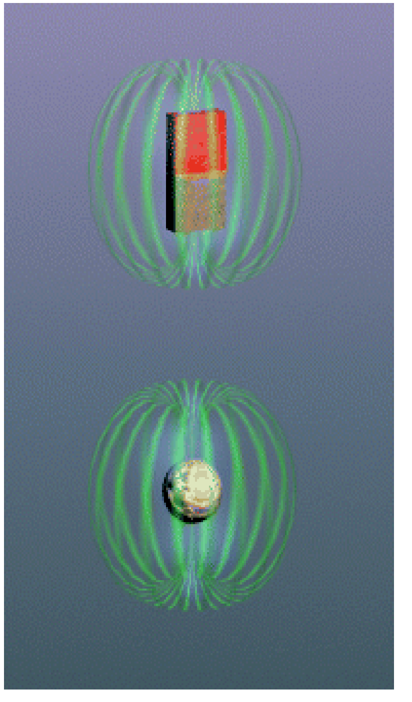
Targeted Destruction of Prostate Cancer in Balb/c athymic nude mice



- PC-3.luc Xenograft bearing male nude mice were used
- LHRH bound nanoparticles effectively bind to tumor
- Use of Nano-LHRH results in accumulation 68% of nanoparticles in tumor
- Distribution of iron in other tissues is being mapped

Fundamentals of Magnetic Resonance Imaging (MRI)

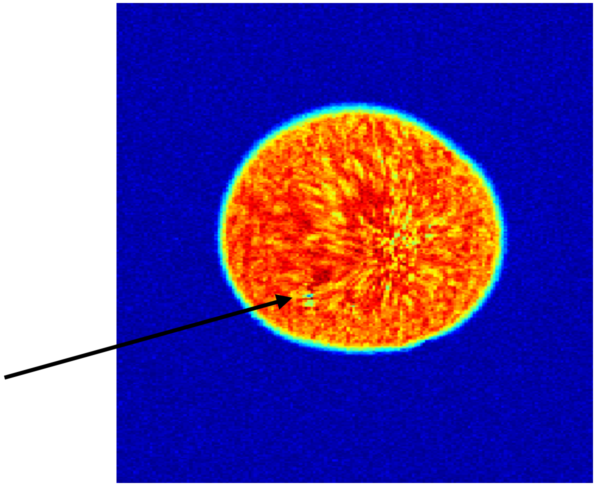
- Hydrogen atoms in water have a property called spin
- MRI generates a magnetic pulse that aligns all of the spins in a certain direction
- The magnetic resonances of the nuclei will cause differences in how they return to their normal spin state
- The MRI machine records the energy released as they realign at different times and generates an image
- A set of images are generated at certain small time intervals after the pulse sequence

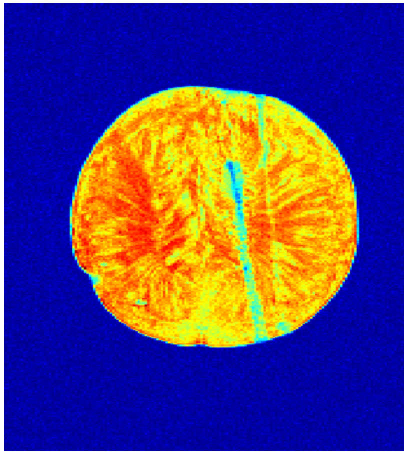


Initial MRI Experiments: Cherry Tomato and Grape

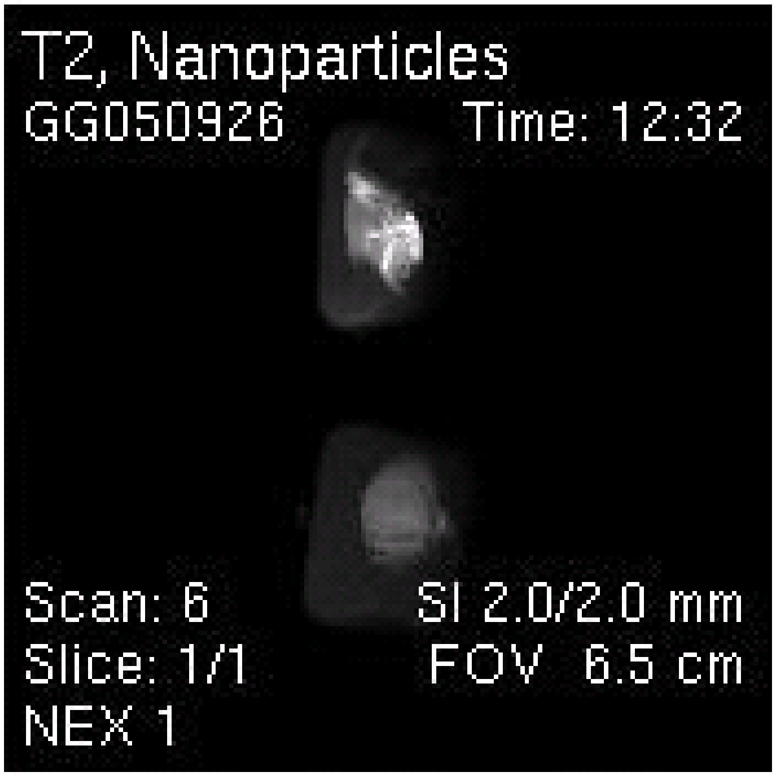
- Injected grapes with saturated saline solution of nanoparticles
- Observed contrast at the location of the injection (nanoparticles)

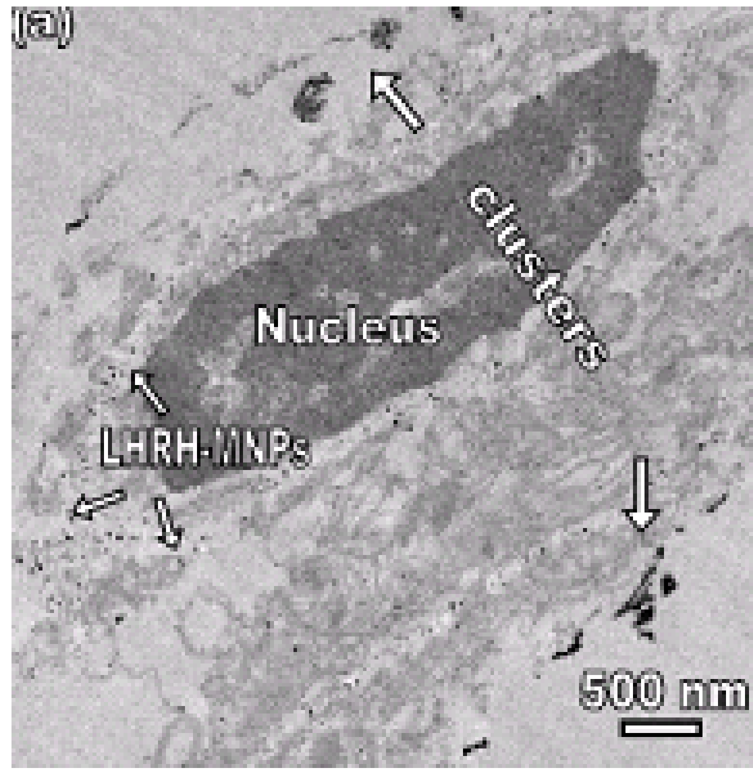
The iron creates a magnetic field in the water, thus creating a blind spot (dark) for the MRI





T2 Images of Tumors – Contrast Enhancement Due to LHRH-MNPs





Summary and Concluding Remarks

- Overview of some recent work on bioMEMS and bio-nanotechnology for disease (mostly cancer) detection and treatment
- Nanoscale biocompatible titanium coatings and micro-grooves promote adhesion and contact guidance on bioMEMS surfaces
- In-vitro and in-vivo TEM reveal stages of specific nanoparticle attachment and encryption
- LHRH-coated magnetite particles provide opportunities for early MRI detection and treatment of breast & prostate cancer
- PNIPA- Fe_3O_4 systems can be used for hyperthermia and controlled drug release (temperature controlled by MNP concentration and H)