# Cell biophysics & cancer

Maria Hansson

# Outline

- My coming presentations
- Cancer – An introduction
- Mechanical properties of cancer cells
- Nanomechanical analysis of cells from cancer patients

# My coming presentations

1. Mechanical properties of cancer cells
2. Regulation of cancer cells
3. Imaging
4. Diagnostics
5. Therapy

# Cancer – An introduction

- Abnormal cell that divide without control and is able to invade other tissues.

- The genetic material (DNA) becomes damaged or changed. Cells do not die when they should and new cells form when the body do not need them.

- More than 100 different types of cancer

- Main categories
    - Carcinoma: In the skin or tissues that cover the internal organs.
    - Sarcoma: In bone, cartilage, fat, muscle, blood vessels or other connective or supportive tissues.
    - Leukemia: In blood forming tissue such as bone marrow.
    - Lymphoma and myeloma: In the immune system.
    - Central nervous system cancer: Brain and spinal cord.

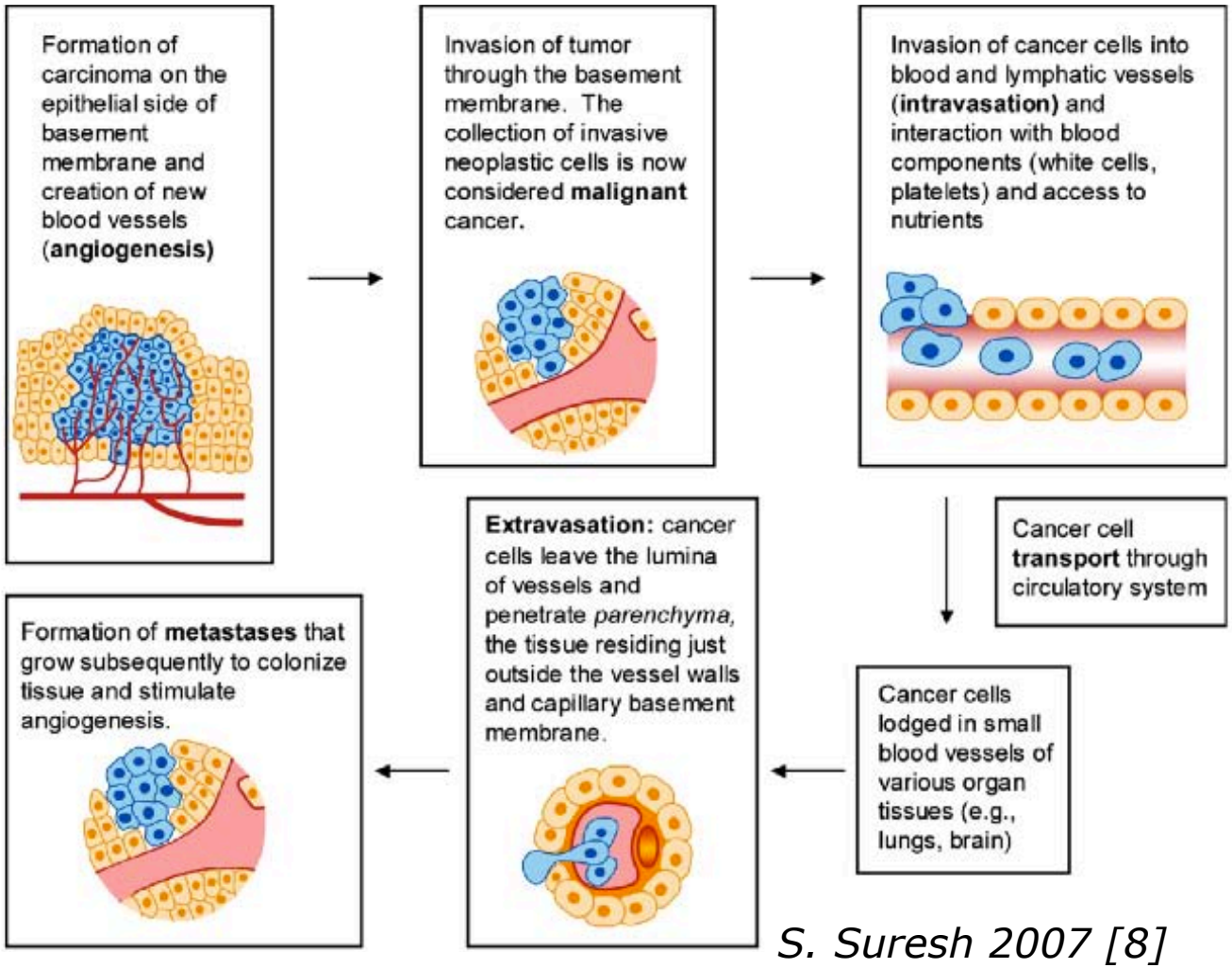
# Cancer – An introduction

• Benign tumors: Not cancerous. Can often be removed and do not come back or spread to other parts of the body.

• Malignant tumors: Cancerous and can invade nearby tissues or spread to other parts of the body. Metastasis – the spread of cancer cells.

• Oncogenes: A gene that can induce the conversion of a normal cell into a cancer cell.

# Cancer cell invasion



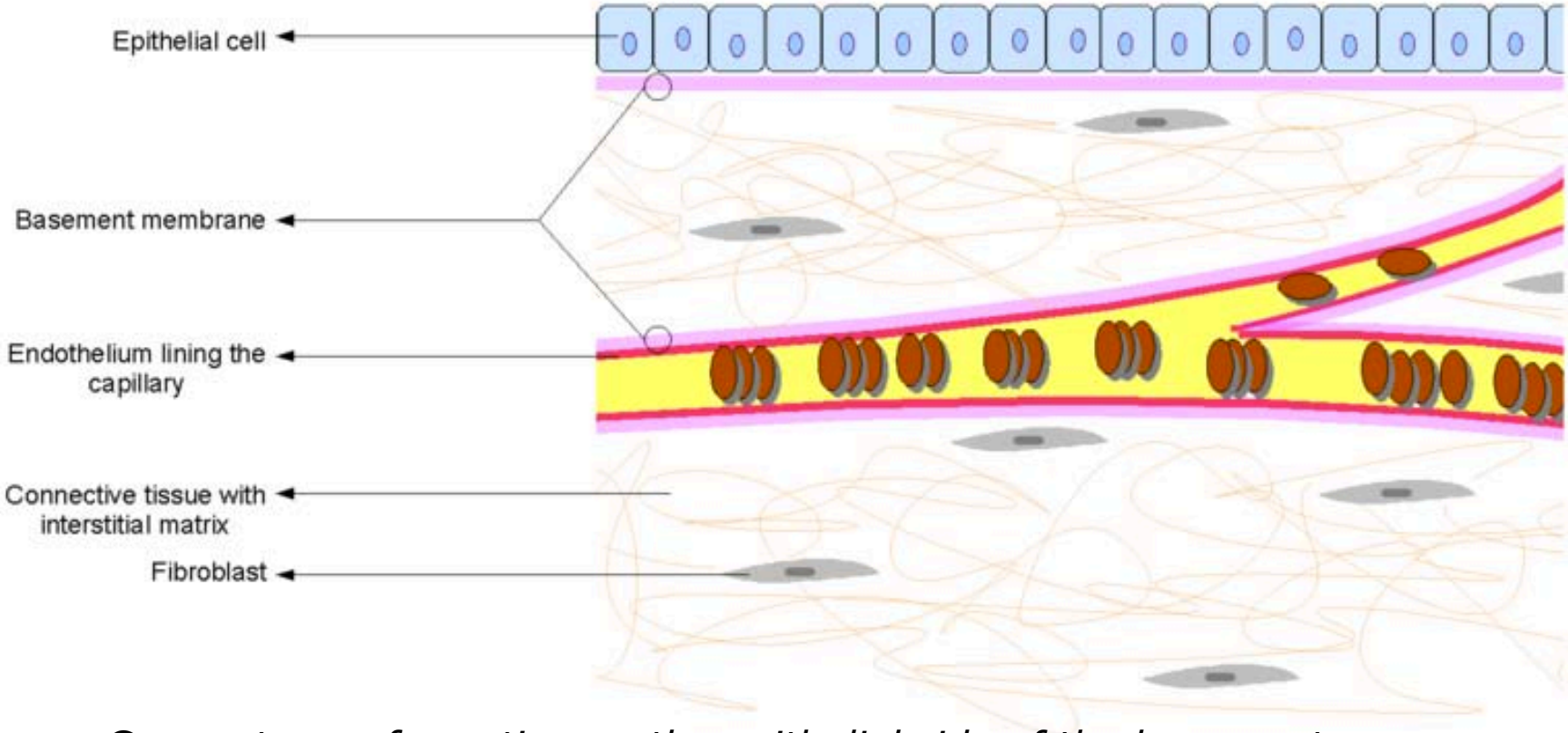

*S. Suresh 2007 [8]*

Carcinoma= invasive malignant tumor of epithelial cells.

Angiogenesis= formation of new blood vessels from already existing vessels.

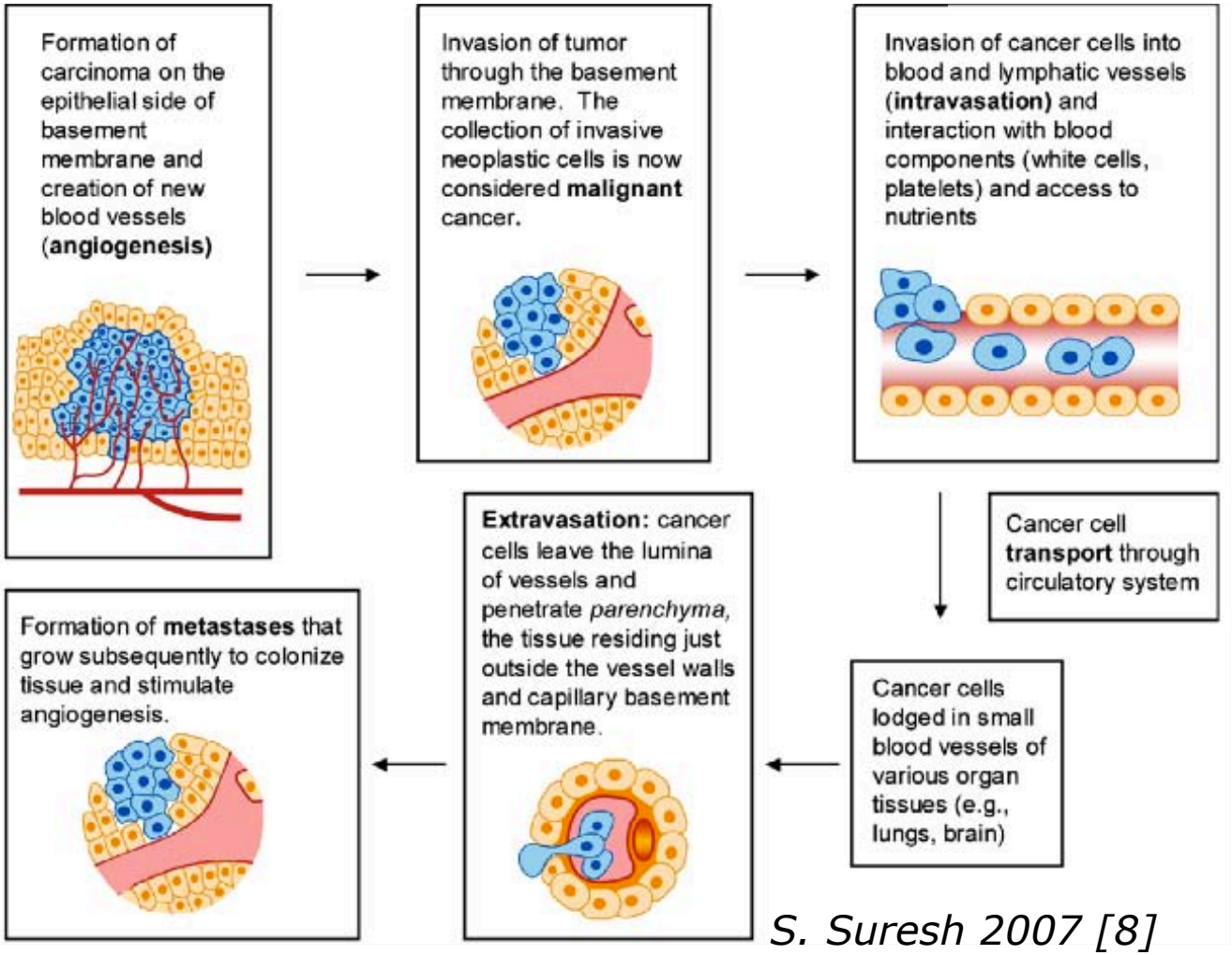
Neoplasm= Abnormal growth of cells.

# Cancer – An introduction




*Cancer tumor formation on the epithelial side of the basement membrane [II] .*

# Cancer cell invasion

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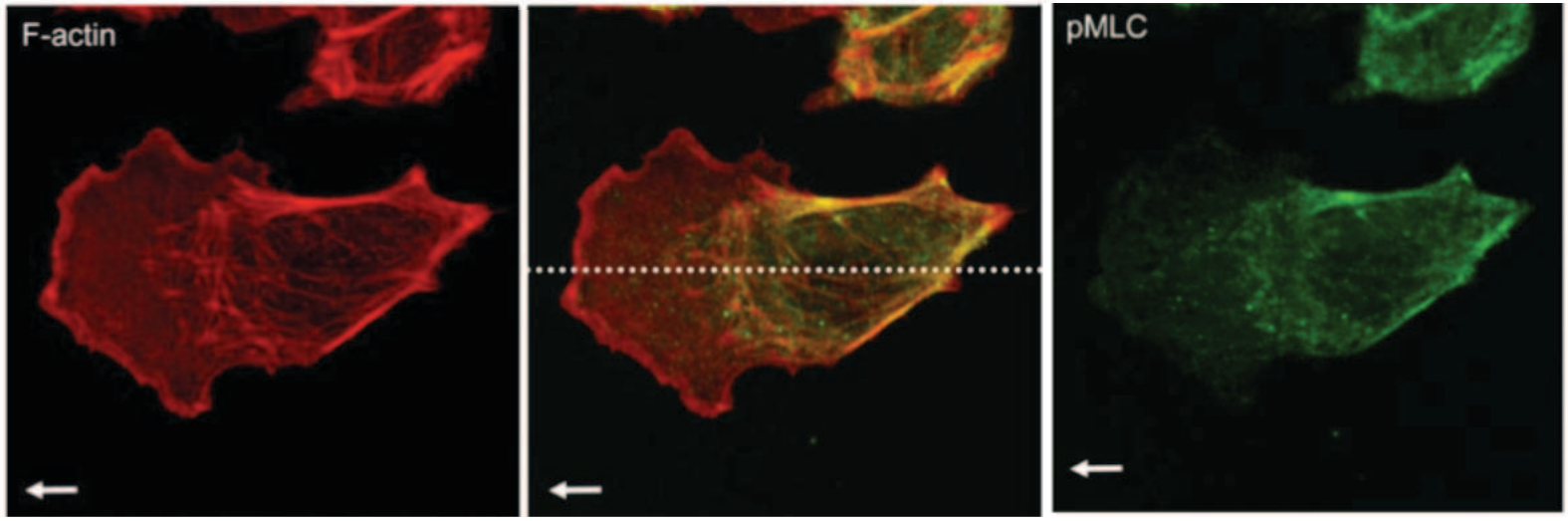
# Cancer cell migration




*Olson M.F, Sahai E (2009) [14]*

Red = F-actin
Green = Myosin

F-actin in the front of the cell is not associated with the myosin wheares F-actin in the rear is. Actin in the front extends away while the middle is pulled towards the middle by myosin (yellow).

# Cancer – Nanotechnology

• Nanoscale devices <50 nm can easily enter most cells. Nanoscale devices <20 nm can move out of blood vessels.

• Interact with surface and inside of cells

• Nanoscale delivery systems can be targeted to particular cancer cells.

• Nanoparticles have a large surface area and can be coated with functional groups for diagnostic (e.g. optical or radio isotropic) or therapeutic purposes.

# Mechanical properties of cancer cells

• The cell is a dynamic system and both the interior and the environment is rapidly changing. Therefore no fixed mechanical properties.

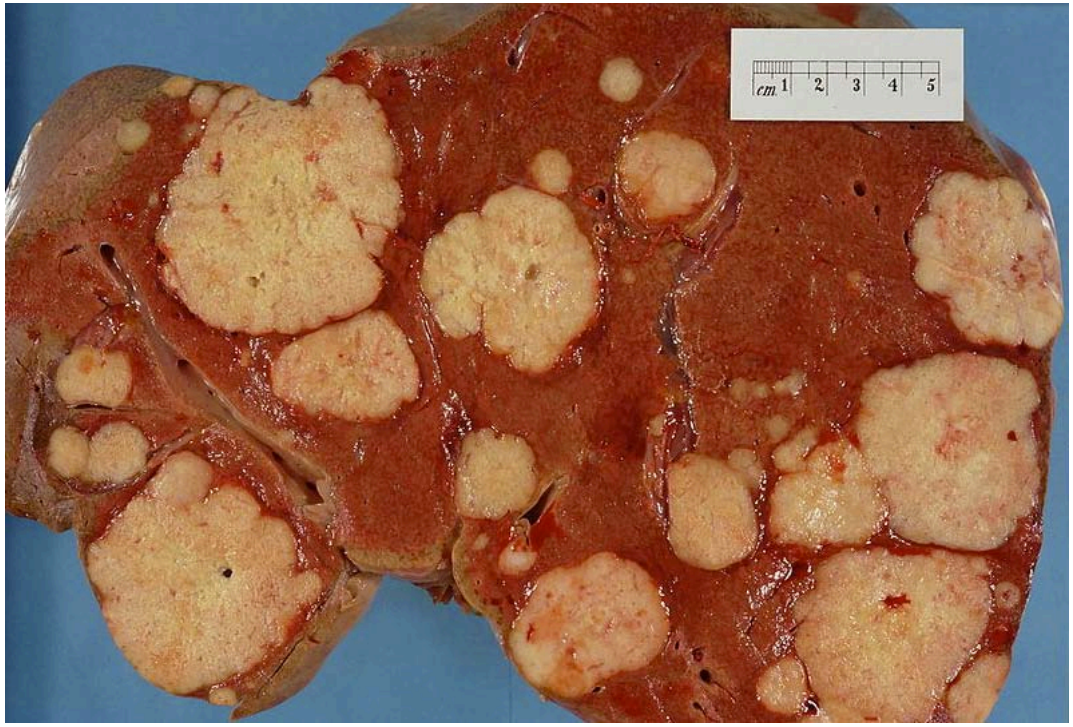
• The structure of the cell changes due to the environment and to different diseases such as cancer.

• The structure of the cytoskeleton and the extracellular matrix are altered when a cell is transformed into a cancer cell.

• An altered structure change the ability of a cancer cell to contract or stretch.

• The motility of cancer cells is different because of their different structure and because of this they can migrate through tissue and form metastasis.

# Mechanical properties of cancer cells

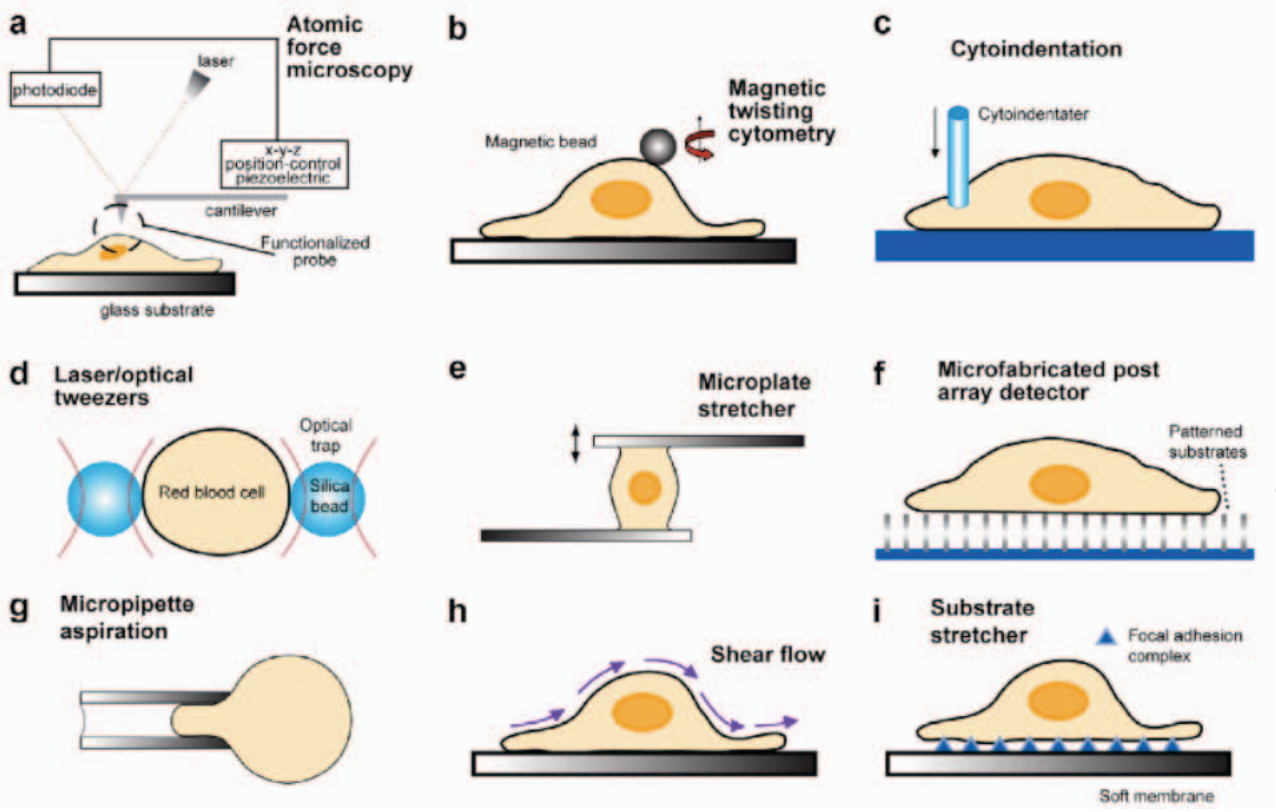



Secondary tumor deposits in the liver from a primary cancer of the pancreas [III] .

Alterations in the cell in the form of foreign organisms or disease development alter the mechanical properties.

By measuring the mechanical properties, diseases can be detected at an early stage.

# Experimental biophysical probes




*S. Suresh 2007 [8]*

Determination of mechanical properties of cancer cells.

a) AFM
b) Magnetic twisting cytometry
c) Cytoindentation
d) Laser/optical tweezers
e) Microplate stretcher
f) Microfabricated post array detector
g) Micropipette aspiration
h) Shear flow
i) Substrate stretcher

# Experimental biophysical probes

Portion of the cell surface:

- AFM
- Magnetic twisting cytometry
- Cytoindentation

Displacements smaller than 1nm and forces on the order of $10^{-12}$-$10^{-6}$ N.

The whole cell:

- Laser/optical tweezers
- Microplate stretcher
- Microfabricated post array detector
- Micropipette aspiration

Displacement of submicrometer and forces of $10^{-12}$-$10^{-7}$ N.

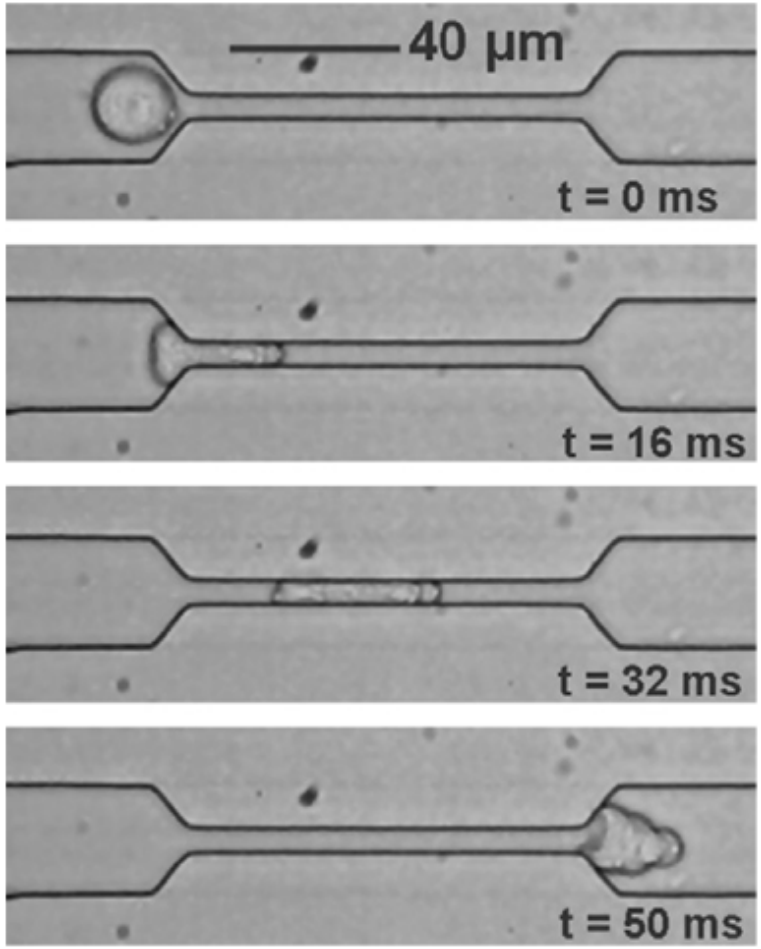
Populations of cells:

a) Shear flow

b) Substrate stretcher

Monitor adherence, deformation and mobility.

# Experimental biophysical probes



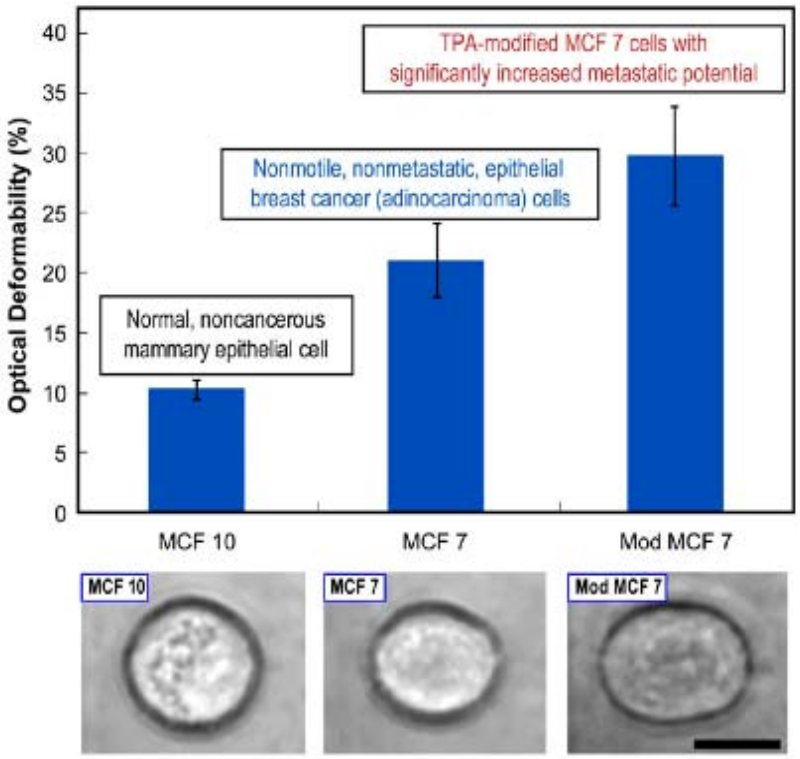

*S. Suresh 2007 [8]*

Simulation of flow of cells through blood vessels are being done with microfluidic and nanofluidic assays.

The inside of the channel is coated with for example a layer of endothelial cells to mimic the environment in vivo.

The cell in the picture is a human pancreatic cancer cell.

# Mechanical properties of cancer cells




*Guck et al. [10]*

Shows the deformability of normal breast epithelial cells and of breast cancer cells.

The deformability is measured with a microfluidic optical stretcher.

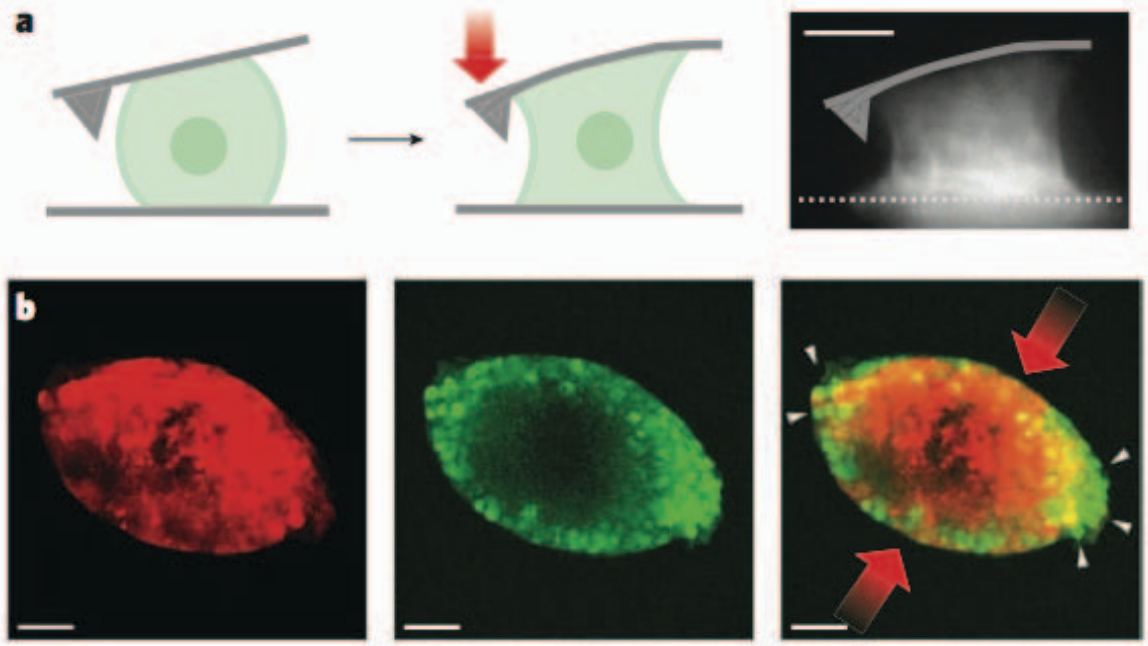
- MCF 10 = normal cells
- MCF 7 = cancer cells
- Modified MCF 7 is cancer cells that are modified to have an increased ability to form metastasis.

The scale bar is 10 µm.

Conclusion: The cancerous cells are more deformable than the normal cells.

Drawbacks:
- Direct exposure
- Smaller deformation than in vivo

# Mechanical properties of cancer cells




*Cheng et al (2009) [11]*

Osteosarcoma – aggressive bone cancer cells attached between an AFM cantilever and the surface.

a) The cells can exert contractile forces of >100 nN (red arrow). The white structure is actin filaments. Scale bar is 10 μm.

b) Mechanical stress changes the growth of a tumor. The right picture is a combination between the red (cancer cells) and green (rapidly growing cells)

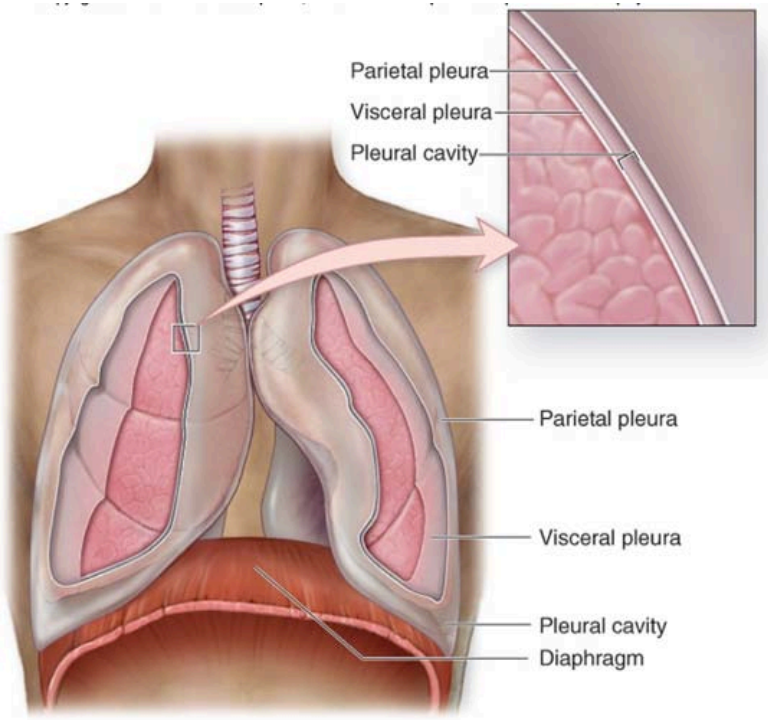
# Nanomechanical analysis of cells from cancer patients

- Using AFM to measure cell stiffness

- Earlier methods were based on imaging. Not accurate since normal cells can look like cancer cells.

- The goal of the paper is to develop a method for distinguishing between cancer cells and normal cells in fluid samples.

- Human pleural fluid samples




*The human pleural cavity [II]*

# Nanomechanical analysis of cells from cancer patients

- The pleural cavity is connected with the lymphatic system.

- Tumor cells in body fluid are all metastatic.

- Metastatic cells – cells that spread.
The cells in the metastatic tumor is of the same type as the cells in the original tumor.

- Benign cells – Harmless "normal" cells. Not cancer but can cause tumors.

- Malignant metastatic cells in the pleural cavity fluid is a sign of widespread cancer.

# Nanomechanical analysis of cells from cancer patients

- Earlier cancer cell detection was based on morphological changes.

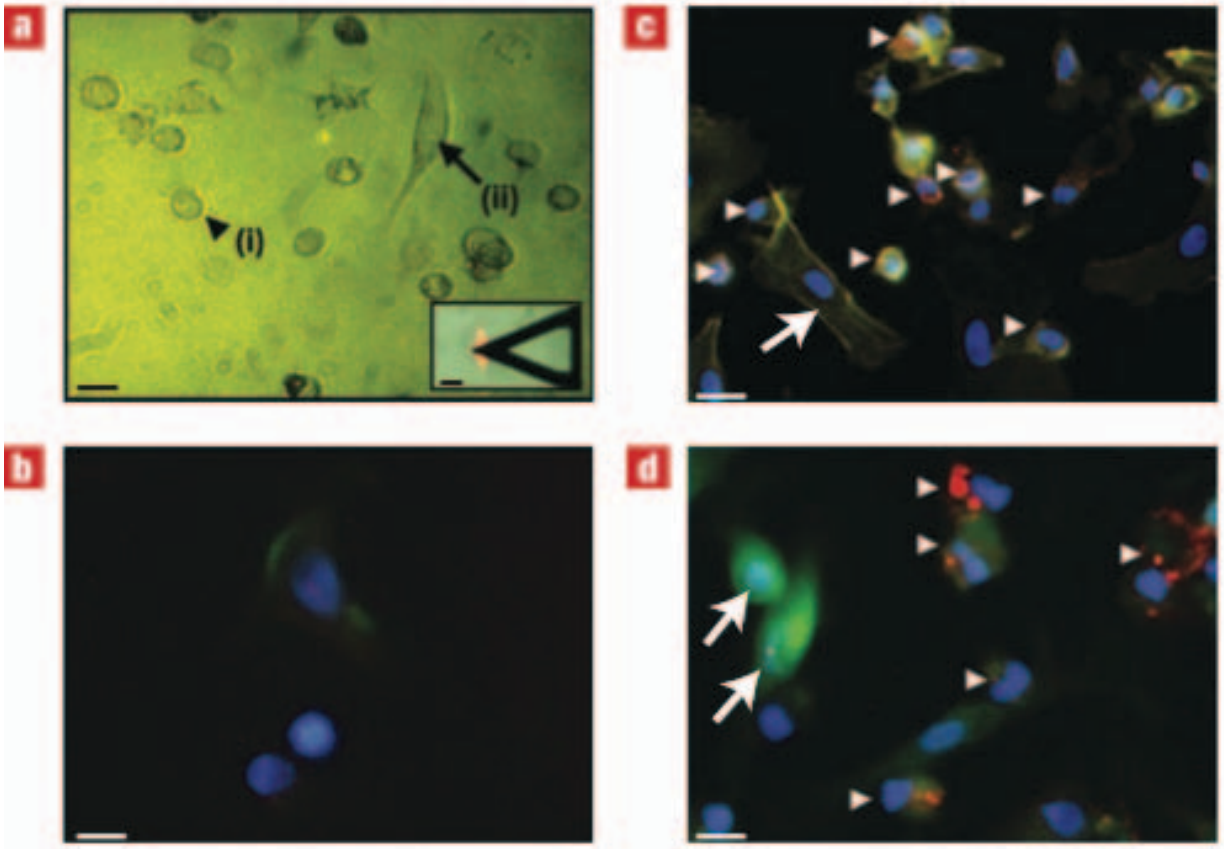
- Not an accurate method since mesothelial cells can mimic metastatic cancer cells.

- Measured the mechanical properties of metastatic cells and mesothelial cells in cavity fluid samples from patients with suspected metastatic cancer.

- Incubation gave differentiation between the cells. Tumor cells are more round whereas mesothelial cells are large and flat.

- AFM Result confirmed by immunofluorescence analysis using biomarkers specific for metastatic cancer and mesothelial cells.

# Nanomechanical analysis of cells from cancer patients




a) Round tumor cell (i) and flat mesothelial cell (ii).

b) DNA – blue
F-actin - green

c) Calretinin – green (Protein marker for some cancers)

d) Arrowheads - tumor
Arrow – mesothelial cells

Scale bar is 10 µm.

# Nanomechanical analysis of cells from cancer patients

• 8 cancer cells and 8 mesothelial cells were selected from each sample for AFM analysis.

•AFM performed separately on the two cell populations.

• Force displacement curves recorded to determine the relative cell stiffness (Young's modulus, E)

• Force – dicplacement curves of cells from the fluid sample was recorded at 1 Hz.

# Nanomechanical analysis of cells from cancer patients

• 2D mapping of the sample. The tip is scanned across the sample while recording the deflection of the AFM tip as a function of its vertical position.

• The load applied by the cantilever to the tip can be computed by Hooke's law: $F = k_c \cdot d$
F= the applied load
$k_c$=the spring constant of the cantilever (Experimentally determined to 0.02$Nm^{-1}$).
d= the displacement of the cantilever

• The force-displacement curves are now converted into force-indentation curves that is later on going to be used to calculate the elasticity (Young's modulus)

# Nanomechanical analysis of cells from cancer patients

• The indentation depth delta = the distance that the the tip pokes into the cell. The tip goes in to the cell and therefore the deflection of the cantilever is smaller than the height of the sample.

• δ=z-d
z= the height of the sample
d= the displacement of the tip

• Since the deflection of the free cantilever is not zero so d have to be corrected as ($d-d_0$) where $d_o$ is the deflection offset. The actual load is then $F = k_c \cdot (d - d_0)$

• Since the tip makes a small jump upon contact with the sample, the height z needs to be corrected as ($z-z_0$). $z_0$ is the height of the jump.

# Nanomechanical analysis of cells from cancer patients

• With the correlation due to the automatic deflection of the cantilever and the jump upon contact, the indentation depth is: $\delta=(z-z_0)-(d-d_0)$

• This gives us load vs. indentation curves.

• The next step is to extract values of Young's modulus from the force-indentation curves. We now use Hertz model.

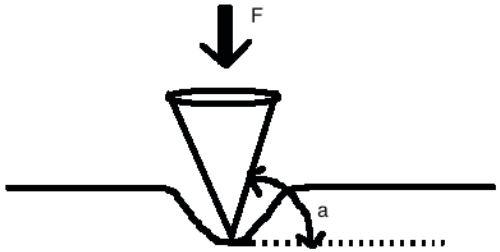
• The Hertz model describes the indentation of a non-deformable tip (AFM tip) into a extending deformable elastic half sphere (the sample). The AFM tip can be modeled as a cone or a parabolic indenter.

# Nanomechanical analysis of cells from cancer patients

- The load vs. indentation depth are given by:

(1) $$F_{cone} = \frac{2}{\pi} \cdot \tan\alpha \cdot E^* \cdot \delta^2$$

(2) $$F_{paraboloid} = \frac{4}{3} \cdot E^* \cdot R^{1/2} \cdot \delta^{3/2}$$




α= Half opening angle of cone shaped tip ($36^O$)
R= Radius of curvature of spherical or paraboloid indenter
$E^*$= Surface elastic constant of the sample

(3) $$E^* = \frac{E}{1-v^2}$$

E=Young's modulus
v=Poisson's ratio (0.5 as typical for soft biological materials).

# Nanomechanical analysis of cells from cancer patients

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# Nanomechanical analysis of cells from cancer patients

**Table 1 Patient characteristics and cytological diagnosis versus mechanical measurements (Young's modulus, *E*).**

| Case no. | Age/sex | Clinical history | Cytological diagnosis of pleural fluid* | Stiffness (kPa): 'Tumour' | Stiffness (kPa): 'Normal' |
|---|---|---|---|---|---|
| 1 | 52/Female | Non-small cell carcinoma of the lung | Positive for metastatic malignant cells | 0.56 ± 0.09 | 2.10 ± 0.79 |
| 2 | 60/Female | Non-small cell carcinoma of the lung | Positive for metastatic malignant cells | 0.52 ± 0.12 | 2.05 ± 0.87 |
| 3 | 49/Female | Breast ductal adenocarcinoma | Positive for metastatic malignant cells | 0.50 ± 0.08 | 1.93 ± 0.50 |
| 4 | 85/Male | Pancreatic adenocarcinoma | Positive for metastatic malignant cells | 0.54 ± 0.08 | 0.54 ± 0.12 |
| 5 | 40/Male | Liver cirrhosis | Negative for malignant cells | – | 1.86 ± 0.50 |
| 6 | 47/Male | Fever and hepatic failure | Negative for malignant cells | – | 1.75 ± 0.61 |
| 7 | 92/Female | Anasarca peripheral oedema | Negative for malignant cells | – | 2.09 ± 0.98 |

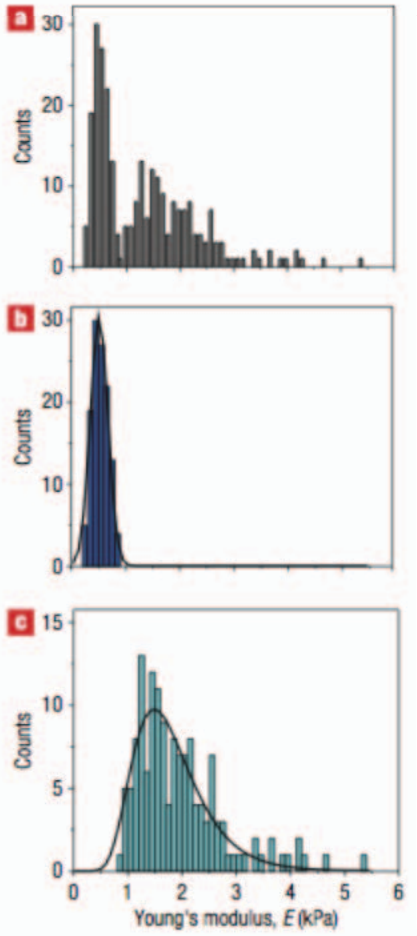
*Cytomorphological diagnosis was made based on morphological analysis combined with immunohistochemical analysis (see Supplementary Information). Stiffness values (*E*) represent mean ± s.d.

- Young's modulus, E is 0.53±0.10 kPa (tumor cells) and 1.97±0.70 kPa (benign mesothelial cells).

- Tumor cells displayed a narrow, spiked peak with little spread. Benign meothelial cells displayed a broad peak.

- The cell stiffness of metastatic cancer cells is 73±11% less stiff than normal cells.

# Nanomechanical analysis of cells from cancer patients



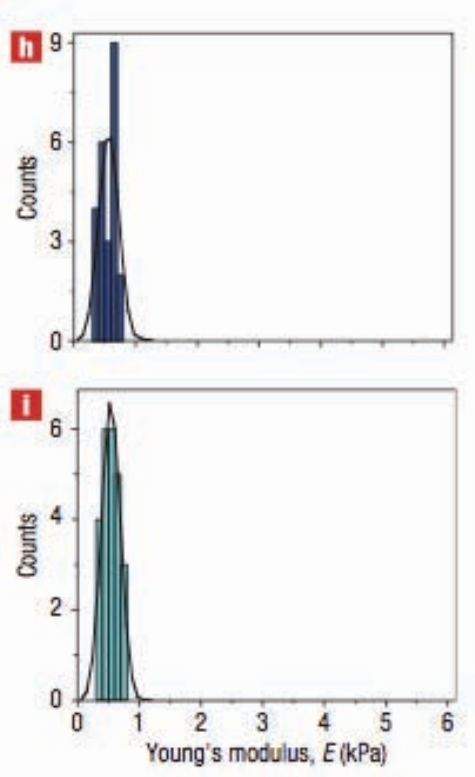

**Seven samples**

a) Histogram of E for all data from 7 samples.

b) Gaussian fit for tumor data from the 7 samples.

c) Log-normal fit of normal cells

# Nanomechanical analysis of cells from cancer patients




**One sample**

Gaussian fit of metastatic tumor cells.
Log-normal fit of normal cells.

Mechanical analysis showed that both cell population could be fitted with a Gaussian and hence all the cells were tumor cells.

# Nanomechanical analysis article - Summary

• What is new with this article is that cancer cells that were optically identified as normal cells could be confirmed to be cancerous when doing mechanical measurements.

• The conclusion from this study is that different tumor types and patients display the same modulus.

• It was also found that tumor cell stiffness could be fitted with a normal distribution whereas benign mesothelial cells was better fitted with a log normal distribution.

• There was no overlap between the measured elasticity values for normal cells and the tumor cells.

• The next step is to develop a method for diagnosis that combine optical evaluation with mechanical measurements.

# Summary

•Diseases such as cancer alters the mechanical properties in a cell and the ability to contract and stretch and the motility becomes different.

• Cancer cells constantly change their cytoskeleton to be able to migrate.

• Cell stiffness in cancer cells have been shown to be >70% lower than in normal cells.

• By measuring the mechanical properties, cancer can be detected from pleural cavity fluid samples.

• Mechanical measurements can be combined with optical determination.

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# References (Pictures)

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[II] http://academic.kellogg.edu/herbrandsonc/bio201_mckinley/Respiratory%20System.htm
[III]http://en.wikipedia.org/wiki/File:Secondary_tumor_deposits_in_the_liver_from_a_primary_cancer_of_the_pancreas.jpg