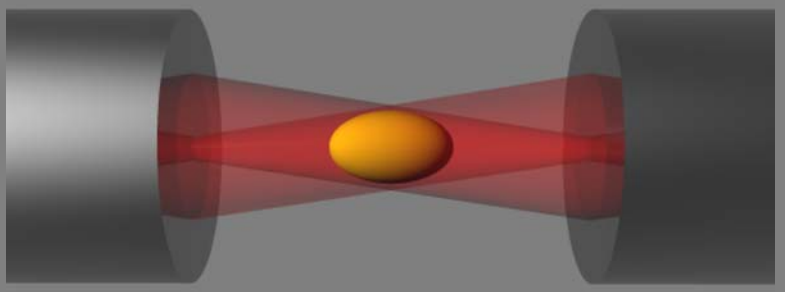
UNIVERSITÄT LEIPZIG

The Optical Stretcher

Technology and Applications



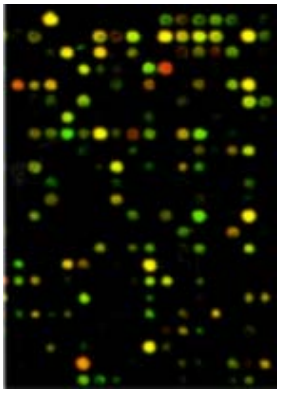
Prof. Dr. Josef Käs and Dr. Jochen Guck
Institute for Soft Matter Physics
Physics Department

UNIVERSITÄT LEIPZIG

From Genomics and Proteomics to Cellomics

- The Human Genome Project:
  -> 3 billion base pairs
  -> 20,000 -25,000 genes
  -> encoding 100,000 proteins
- Flood of molecular information cannot be related to cell function

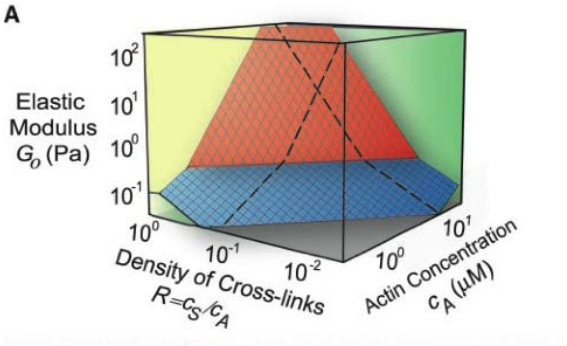
=> Cell elasticity is a powerful marker that provides integrated information of a cell's state and function



UNIVERSITÄT LEIPZIG

Cell Deformability as an Inherent Cell Marker

Small changes in concentrations of cytoskeletal proteins are nonlinearly enhanced in elasticity



$$G' \propto c_A^{2-6.7}$$

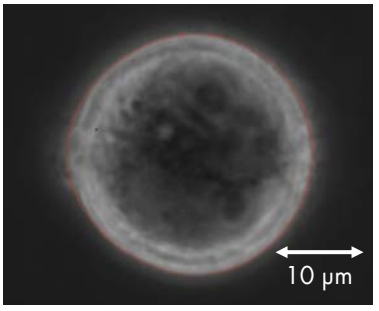
Cell deformability provides built-in, non-destructive, strong amplification of molecular changes in single living cells.

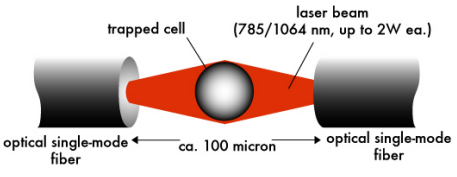
M. Gardel et al., *Science* (2004)

UNIVERSITÄT LEIPZIG

The New Paradigm: Feeling with Light

- Since Abbé and Leeuwenhoek light has been used to study biological samples – but so far only by visual inspection
- The Optical Stretcher adds the tactile world as a new dimension to biophotonics
- Instead of "looking" for changes we can "feel" for changes
- Advantage : no contact, gentle



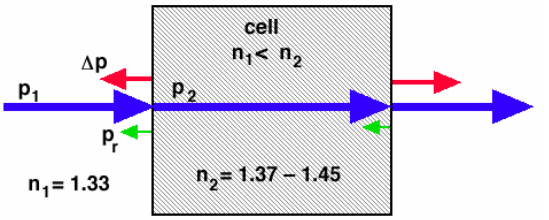


Optical Surface Forces

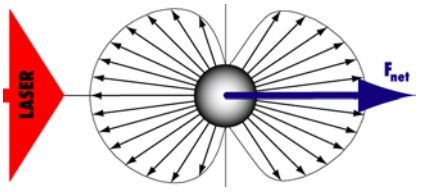
Momentum of light:

$$p = \frac{n_i \cdot E}{c}$$

Gedankenexperiment:



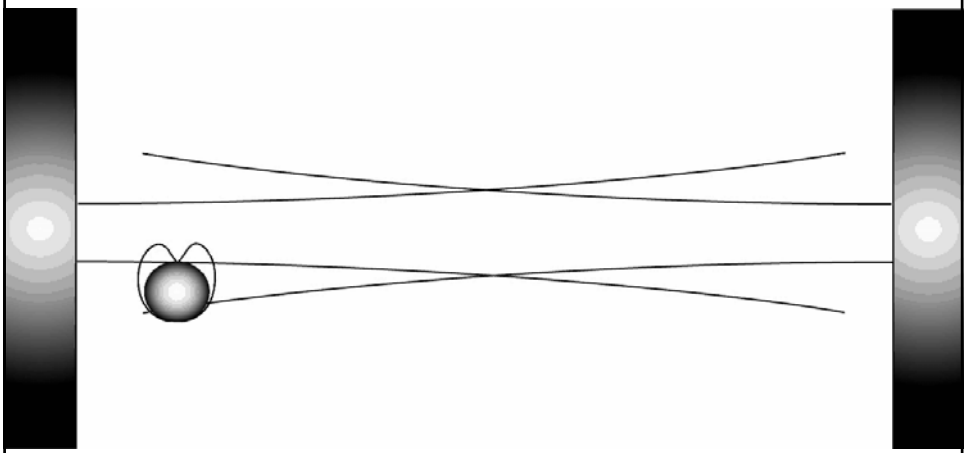
Whenever light enters or exits a dielectric medium it exerts a force *away* from the denser medium and *normal* to the interface.



Quantifiable forces without calibration!

J. Guck et al., *PRL* (2000)

The Optical Stretcher



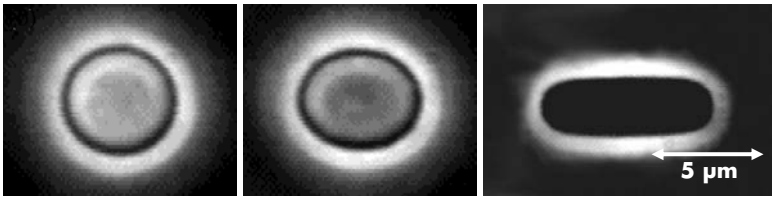
Advantage: self-centering and quantitative understanding of cell handling and cell deformation

Force Range

UNIVERSITÄT LEIPZIG

Optical Tweezers	0.1 – 10 pN	Intermolecular forces
Optical Stretcher	10 pN – 1 nN	Cell deformation
AFM	1 nN – 1 µN	Intramolecular forces

Deformation of a human erythrocyte:



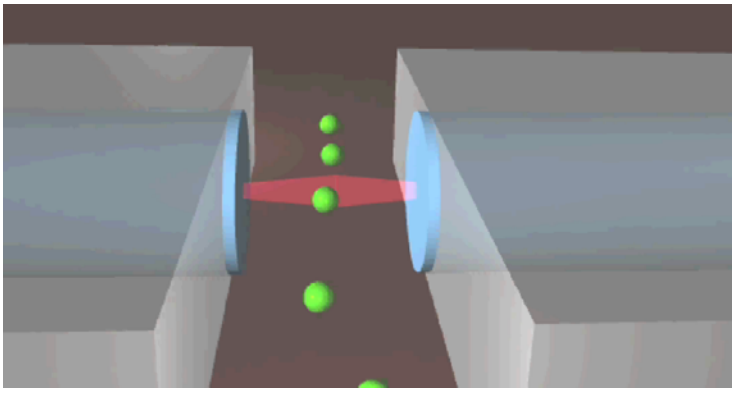
5 mW 85 mW 250 mW

Ideal force range to manipulate and deform cells

J. Guck et al., *Biophys. J.* (2001)

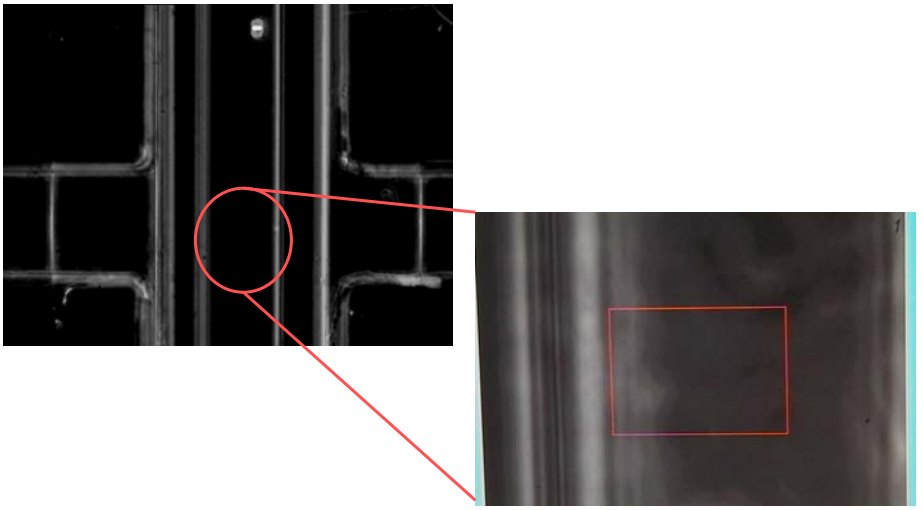
Microfluidics

UNIVERSITÄT LEIPZIG



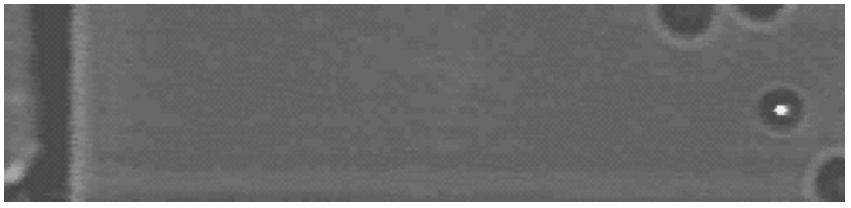
J. Guck et al., *Biophys. J.* (2005); B. Lincoln et al., *Cytometry* (2004)

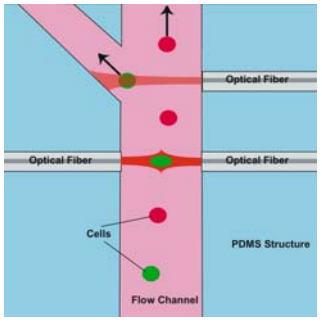
Microfluidics



J. Guck et al., *Biophys. J.* (2005); B. Lincoln et al., *Cytometry* (2004)

Cell Sorting





Cells can be sorted.

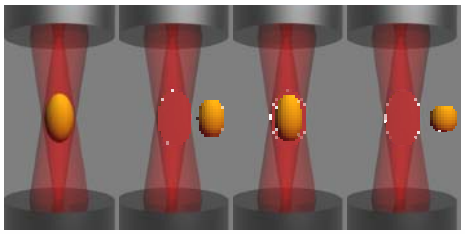
Cells stay viable for further investigation:

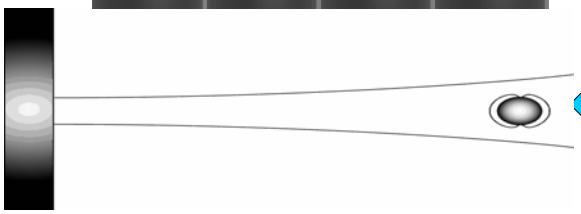
- Short term check:
  cell adheres after stretching

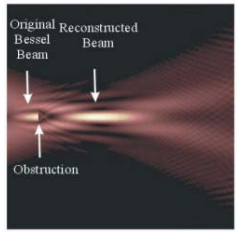
- Long term check:
  proliferation rates remain unchanged

High-Throughput Measurements

UNIVERSITÄT LEIPZIG







- Stretching in steady flow
- Several cells simultaneously in Bessel beam
- Parallelization
- Measurement rates up to 100–1000 cells/s

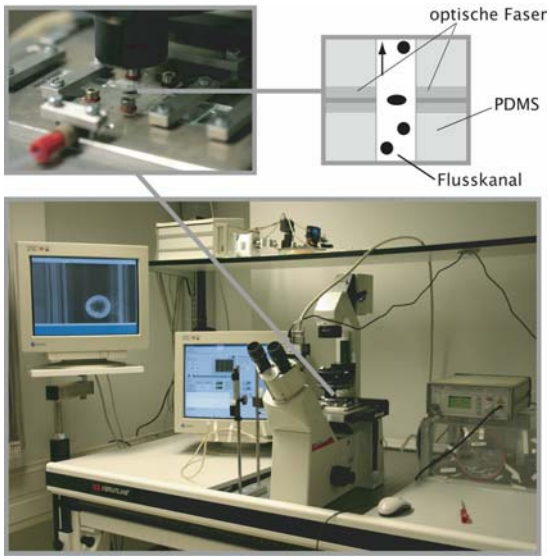
Enabling Elasticity-based Flow Cytometry

Other stretcher

B. Lincoln et al., *Cytometry* (2004)

The Instrument

UNIVERSITÄT LEIPZIG

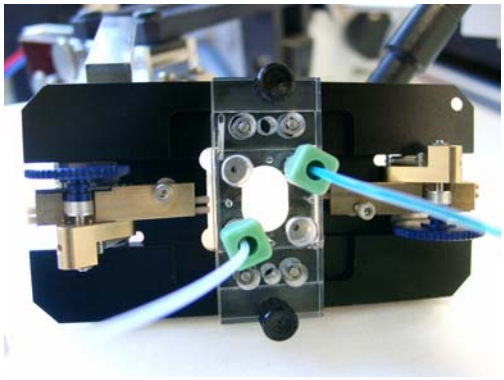


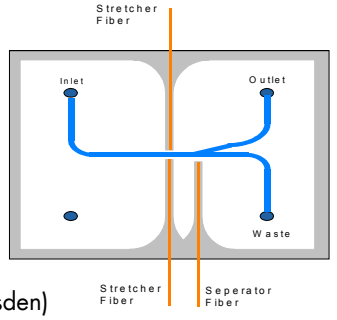
- Simple to set up
- Simple to use
- Small
- Inexpensive
- Add-on for any microscope

S. Schinkinger et al., *Laborwelt* (2005)

Microfluidic Chip

UNIVERSITÄT LEIPZIG



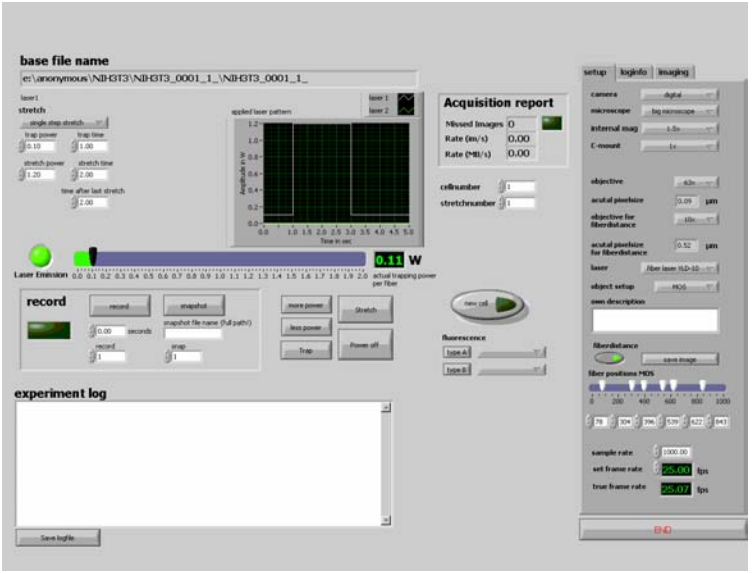


(developed in collaboration with GeSiM mbH, Dresden)

F.U. Gast et al., *Microfluid. Nanofluid.* (2005)

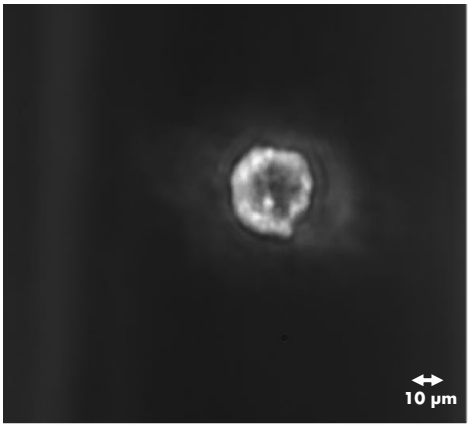
Software Interface

UNIVERSITÄT LEIPZIG



High-Content Cell Analysis I

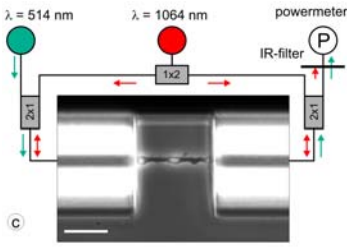
UNIVERSITÄT LEIPZIG



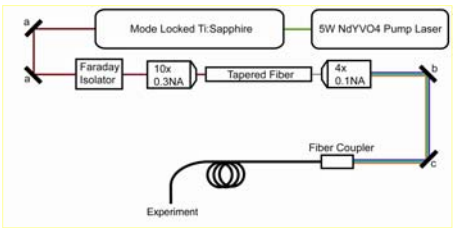
- Any kind of microscopy incl. CLSM, Multiphoton microscopy
- Tomography combined with SPIM or SIM
- Quantitative proteomics, e.g. LSC, FCS, FRET

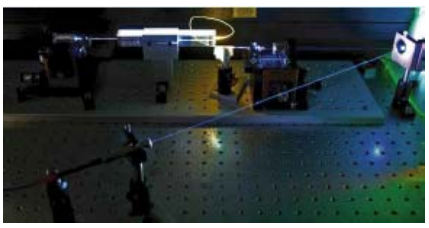
High-Content Cell Analysis II

UNIVERSITÄT LEIPZIG



- Photonic crystal fibers
- Spectroscopy in trap (Raman, CARS,…)
- Supercontinuum laser sources
- Measurement of dielectric properties

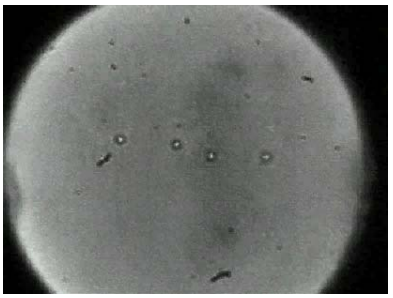


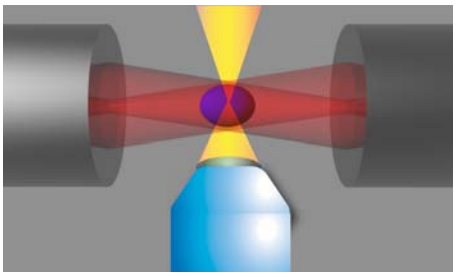


K. Franze et al., *Nature* (2005); Betz et al., *J. Biomed. Opt.* (2005)

Modular Cell Handling

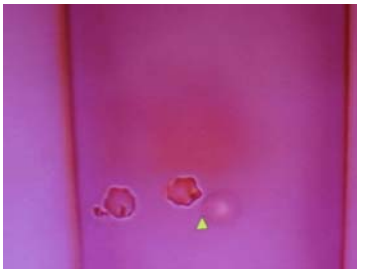
UNIVERSITÄT LEIPZIG





Combination with
PALM microbeam

- Cell fusion
- Cell-poration
- CALI
- FRAP
- …



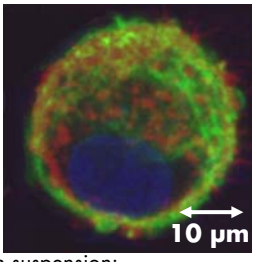
Summary I

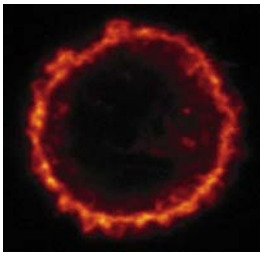
UNIVERSITÄT LEIPZIG

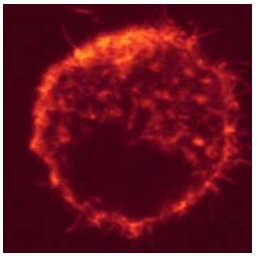
- **Built-in nonlinear amplification in single living cells**
- **Ideal force range, cells stay viable**
- **High-throughput microfluidic measurement (self-centering) and sorting (cell shooting)**
- **Small, modular, inexpensive, user-friendly**
- **High-content (can be combined with any state-of-the-art cell analysis technique)**
- **Various modes of cell handling**

The Cytoskeleton: Defining and Describing the Cellular State

UNIVERSITÄT LEIPZIG







In suspension:
well-defined and highly regular cytoskeleton.

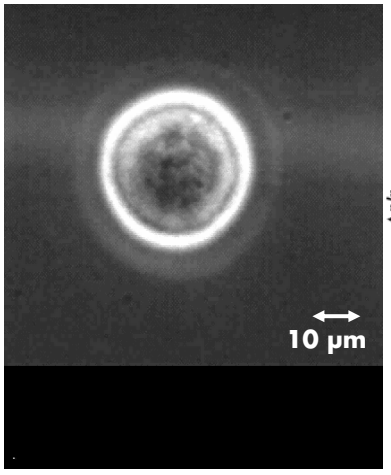
Cell differentiation increases cytoskeleton.

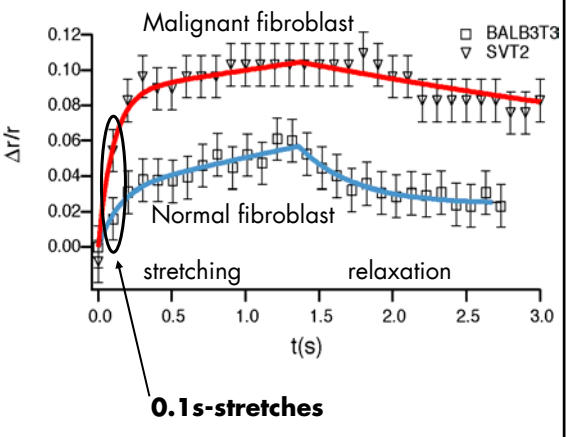
In the editorial of the January 23, 1998 issue of the journal *Science*, S.M. Hurtley stated:

"Problems with the cytoskeleton can cause disorders of the skin, the nervous system, and the muscles. Changes in the cytoskeleton are key, and even diagnostic, in the pathology of some diseases, including cancer. Understanding the basic cell biology of the cytoskeleton has contributed to our understanding of the pathology of some of these disorders and will continue to affect approaches to understanding diagnosis, and therapy for various conditions."

Optical Deformability as Cell Marker I

UNIVERSITÄT LEIPZIG



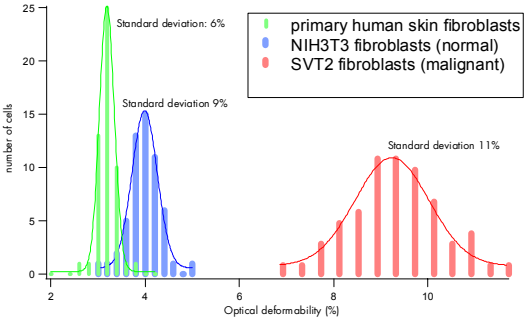


0.1s-stretches

(-> F. Wottawah et al., *Phys. Rev. Lett.* 95 (2005), F. Wottawah et al., *Acta Biomat.* (2005), S. Schinkinger et al., *J. Biomed. Opt.* (2005), F.-U. Gast et. al. *Nanofluidics and Mircofluidics*, (2005))

Optical Deformability as Cell Marker II

UNIVERSITÄT LEIPZIG



Distinguishing cells by 0.1s-stretches:

- Very narrow Gaussian distribution for a broad range of cell lines and primary cells.
- Cell populations can be distinguished with a confidence level of 99.9% with <100 cells.
- Minimal sample size: 50 - 100 cells. Projected maximal throughput: 100 cells/s
- No molecular markers!

(-> F. Wottawah et al., *Phys. Rev. Lett.* 95 (2005), F. Wottawah et al., *Acta Biomat.* (2005), S. Schinkinger et al., *J. Biomed. Opt.* (2005), F.-U. Gast et. al. *Nanofluidics and Mircofluidics*, (2005))

UNIVERSITÄT LEIPZIG

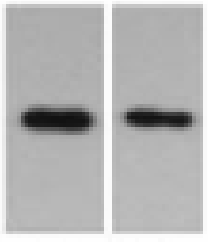
Detecting Cytoskeletal Changes

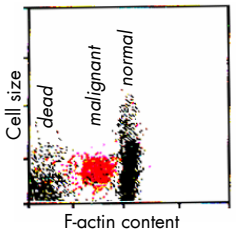
In the malignant fibroblasts the amount of actin is reduced by 30-35%!

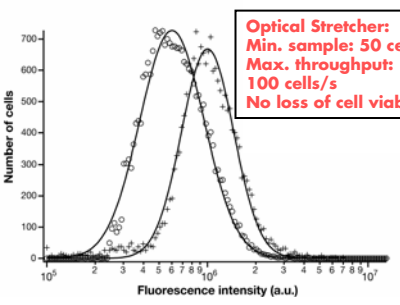
sensitivity →

Western Blot | FACS | Laser Scanning Cytometry (LSC)

Normal fibroblast | Cancerous fibroblast







Optical Stretcher:
Min. sample: 50 cells
Max. throughput: 100 cells/s
No loss of cell viability

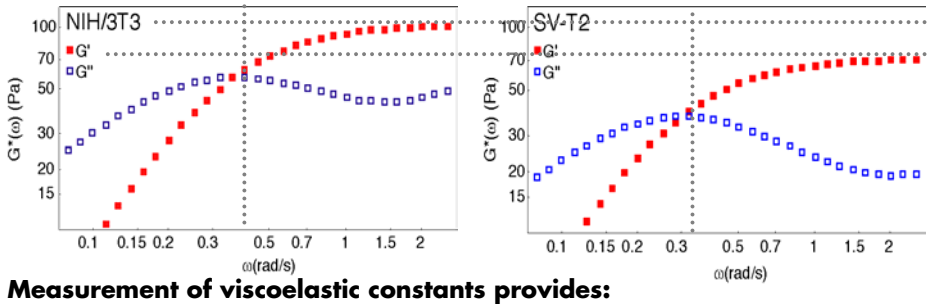
required minimal sample size →

10^6-10^7 cells	10^5-10^6 cells	10^3-10^4 cells
lysed cells	significant loss of viability	fixed cells

(-> J. Guck et al., *Biophys. J.* **88**:5 (2005))

Biomechanics and Cytoskeletal Proteomics

UNIVERSITÄT LEIPZIG



Measurement of viscoelastic constants provides:

linear response

- $c_{F\text{-}actin}$, $c_{microtubules}$
- $c_{F\text{-}actin\ cross\text{-}linker}$, $c_{microtubule\ cross\text{-}linker}$
- $k_{actin\ cross\text{-}linker}$
- $E_{coupling\ actin\text{-}microtubules}$

nonlinear response

- $c_{intermediate\ filaments}$
- $F_{crit.\ mechano\text{-}act.}$ $F_{crit.\ mechanotrans\text{-}}$
- F_{active}
- + $n_{refractive}$, R_{cell}

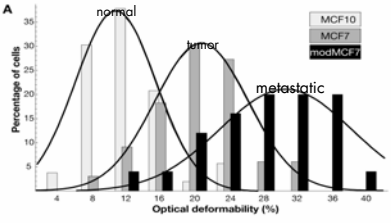
=> 12 cell parameters simultaneously

(-> F. Wottawah et al., *Phys. Rev. Lett.* 95 (2005), F. Wottawah et al., *Acta Biomat.* (2005), S. Schinkinger et al., *J. Biomed. Opt.* (2005), R. Ananthakrishnan et al., *Curr. Sci.*, 88 (2005))

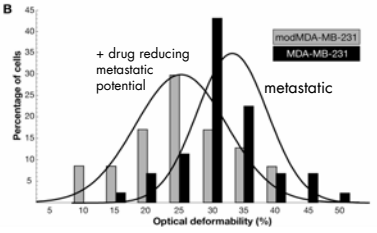
Direct Detection of Metastatic Cells

UNIVERSITÄT LEIPZIG

In case of breast cells normal, tumor, and metastatic populations become visible:



Addition of a weak drug reducing the metastatic aggressiveness can be detected:



Optical Stretcher requires minute samples that can be obtained by minimally invasive techniques such as fine needle aspiration and cytobrushes.

Ultimate goal:

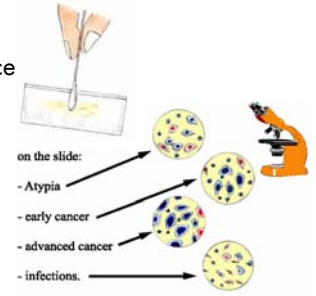
Precise staging of cancer progression from dysplasia, through neoplasm, to metastasis directly from a small tumor sample obtained by minimal invasive techniques such as fine needle aspirations or cytobrushes.

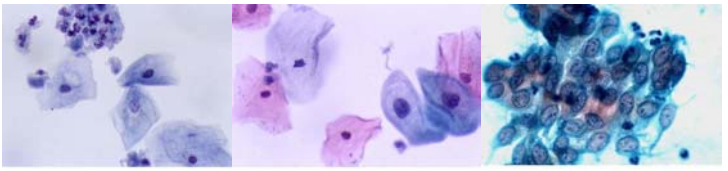
(-> J. Guck et al., *Biophys. J.* **88**:5 (2005) B. Lincoln et al., *Cytometry* **59A** (2004))

Cytobrushes and Cancer Screens

Cervix:

- PAP smears are an established screen for cervical cance
- Standard test during a women's annual gynecological examination
- Thin preps are ideal samples for the Optical Stretcher
- Current test is not quantifiable and depend on the subjective judgment
- Early dysplastic states need more refined screening techniques -> Optical Stretcher





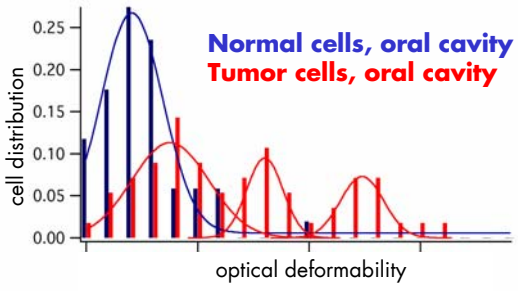
normal dysplasia advanced cancer

UNIVERSITÄT LEIPZIG

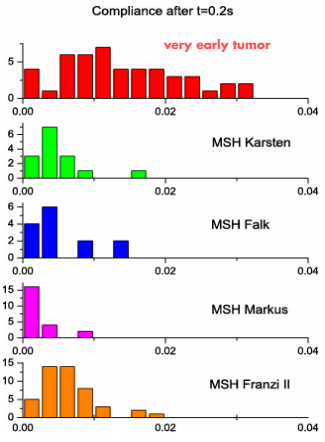
Cytobrushes and Cancer Screens

Oral cavity:

- No equivalent to PAPsmears for oral cancer at the annual dental exam
- Sample too complex for visual inspection
- Good results with Optical Stretcher:



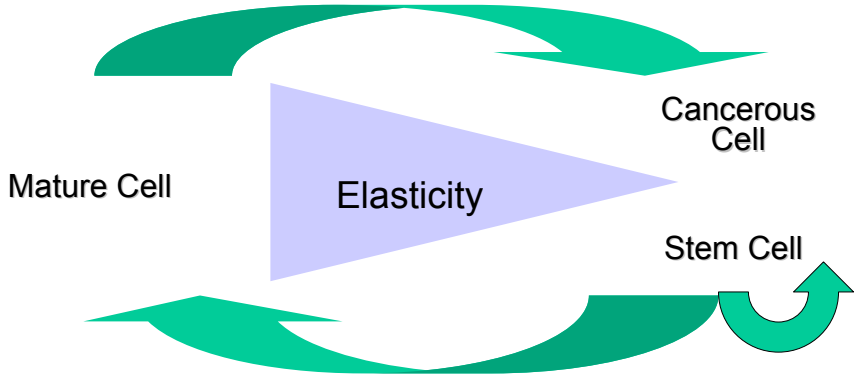
Variability in normal patient samples vs early tumor samples:



Optical Deformability, a Marker for Cell Differentiation

UNIVERSITÄT LEIPZIG

Malignant Transformation

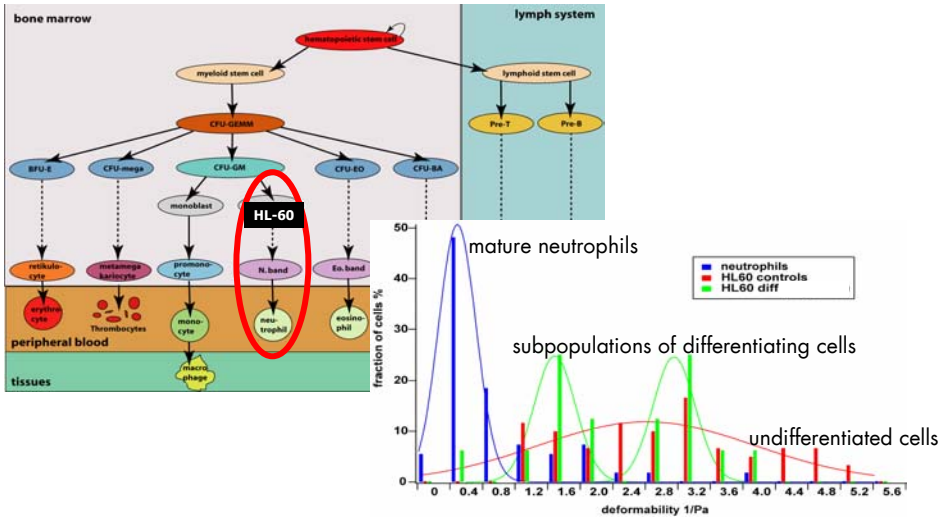


Differentiation

Molecular markers for cell differentiation stem predominately from developmental biology. It is unclear whether they detect all changes in differentiation of adult cells.

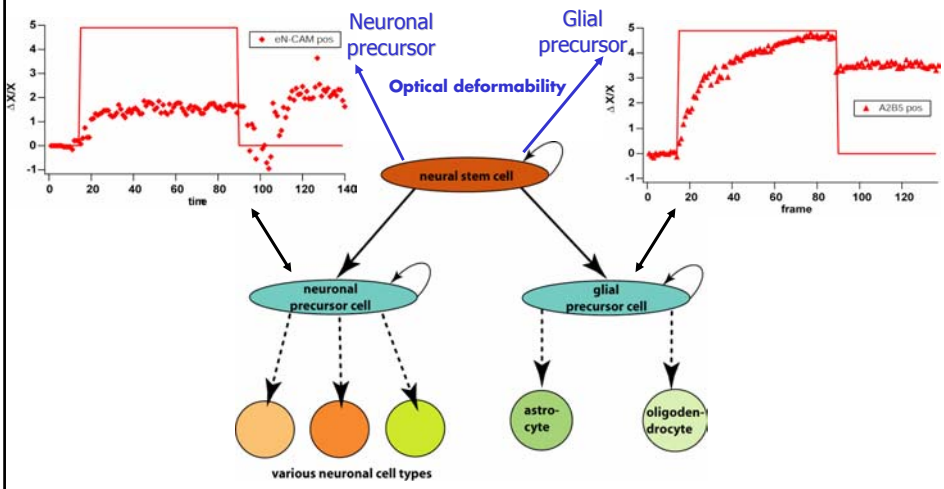
Optical Deformability, a Marker for Cell Differentiation

UNIVERSITÄT LEIPZIG



Neural Stem Cells and Parkinsons Disease

UNIVERSITÄT LEIPZIG

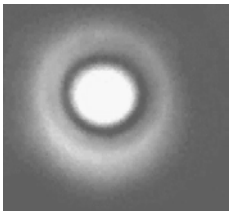


Sorting of neuronal precursor cells for the treatment of neurodegenerative diseases (Parkinson's disease)

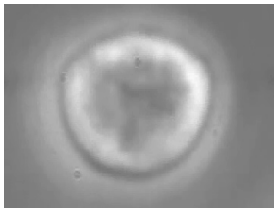
Analyzing Blood

UNIVERSITÄT LEIPZIG

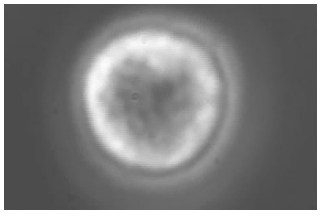
Laser power: 5 mW –> 200 mW



erythrocyte



neutrophil, leukemia



human neutrophil

- Ideal sample since all cells are inherently suspended
- Isolating embryonic stem cells from cord blood
- Detecting Malaria at early infection stages and isolate these cell for drug screening
- Isolate metastatic cells from vascular system since optical stretcher is ideally suited to target rare cells in a large amount of cells

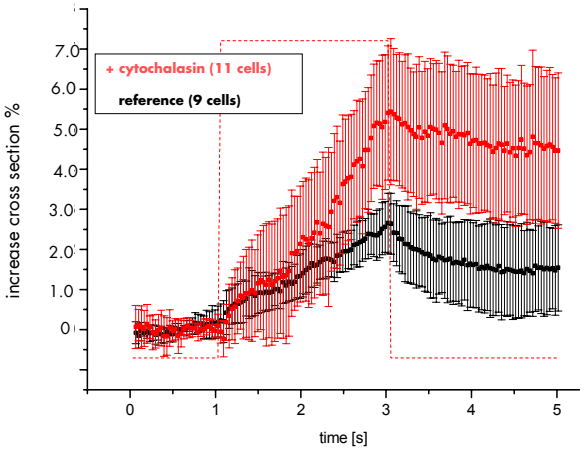
(-> J. Guck *et al.*, PRL, 2000; J. Guck *et al.*, Biophys. J, 2001; J. Guck *et al.*, Jour. of Phys.: Cond. Mat., 2002.)

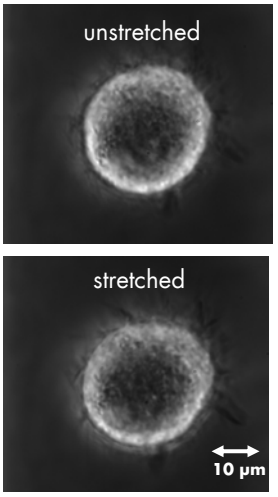
Drug-Screening I

UNIVERSITÄT LEIPZIG

Effect of cytoskeletal toxins (e.g. Botulinum toxin) on human skin fibroblasts:

Cell softening through addition of Cytochalsin





Drug-Screening II

UNIVERSITÄT LEIPZIG

Cell stiffness inversely correlates with metastatic aggressiveness:

		Cell speed (*µm/hr*)	Elasticity (kPa)
Normal cells	BALB (n=10) SE	**7.3** **4.5**	**1.35** **0.32**
Metastatic cells	SV-T2 (n=10) SE	**12.1** **4.8**	**0.63** **0.34**
	H-ras (n=10) SE	**24.7** **12.0**	**0.57** **0.37**

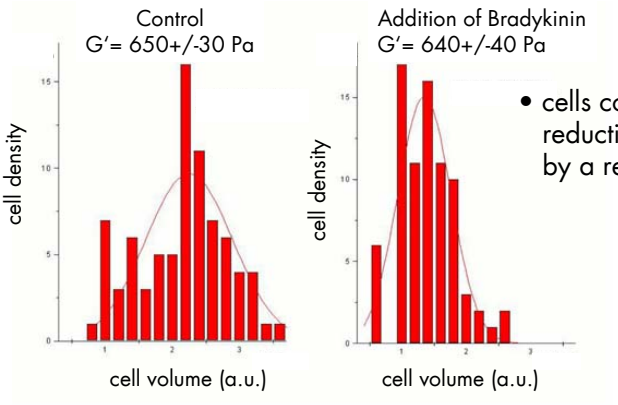
Novel chemo-therapies: Reagents that stiffen cells reduce metastasis.

(S.Park *et al.*, Biophys. J, 89 (2005))

Drug-Screening III

UNIVERSITÄT LEIPZIG

Avoiding respiratory distress syndrome, softening of lung epithelia cells:



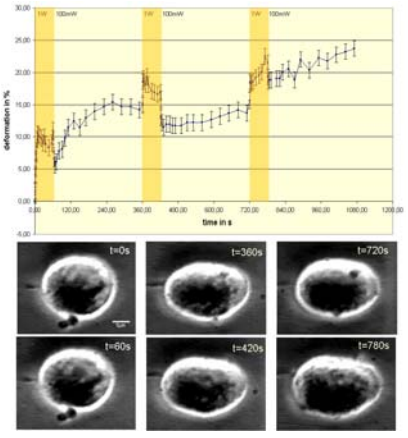
- cells compensate the reduction in actin cytoskeleton by a reduction in volume

Optical stretcher allows us to study the effect of drugs on an individual cell level.

Applying Well-defined Mechanical Stimuli

UNIVERSITÄT LEIPZIG

Mechanically activated fibroblast:



generated force:280±13 pN

- only technique that allows to apply a quantifiable, spatially variable stress to cells $\sigma_{stress}(\mathbf{r})$
- quantify characteristics of stress-induced ion channels $F_{crit.\ ion}$
- change gene expression by mechanotransduction $F_{crit.\ mechanotr.}$
- study stress-induced cytokine release $F_{crit.\ zyto.}$
- activate cells for faster growth in tissue engineering F_{active}

(-> F. Wottawah et al., *Phys. Rev. Lett.* 95 (2005))

Summary II

UNIVERSITÄT LEIPZIG

- **Cytoskeletal elasticity is a essential descriptor of cell state**
- **New cell phenotypes**
- **High precision cell marker (high sensitivity with <100 cells)!**
- **No molecular tags!**
- **Optical deformability => high content, 12 cellular variables simultaneously**
- **Direct staging of cancer including metastasis with minimally invasive biopsies**
- **Quantifiable cancer diagnosis and new cancer screens**
- **Marker free stem cell isolation, new sources of adult stem cells**
- **Ideally suited for analysis of blood (metastasis, malaria, cord blood)**
- **Ideal drug screen for individual cell studies**
- **Quantifiable mechanical stimuli to cells**
- **Fundamentally new basic technology => many more applications**

UNIVERSITÄT LEIPZIG

Acknowledgements

Current group:

• Staff: Elke Westphal, Undine Dietrich, Bernd Kohlstrunk, Claudia Honisch

• Students: Timo Betz, Bjoern Stuhrmann, Michael Goegler, Karla Müller, Claudia Brunner, Mireille Martin, Cornell Wolf, Franziska Wenzel

•Group leader, Jochen Guck: Falk Wottawah, Stefan Schinkinger, Susanne Ebert, Frank Sauer, Bryan Lincoln, Maren Romeyke, Kort Travis, Franziska Lautenschläger,

• With collaborators: Kristian Franze, Jens Gerdelmann, Yun Bi

Collaborators:

• T. Remmerbach (Leipzig), J. Schwarz (Leipzig), V. Adams (Leipzig), T. Kahn (Leipzig), H. Wirtz (Leipzig), M. Löffler (Leipzig), I. Röder (Leipzig), F. Jülicher (Dresden), S. Shorte (Paris), M. Beil (Ulm), U. Seifert (Stuttgart), E. Paluch (Dresden), A. Tarnok (Leipzig), K. van Vliet (Boston), D. Discher (Philadelphia), J. Derouard (Grenoble), F. Gallet (Paris)

Funding:

- DFG (Biophysics)
- EC, 6th Framework, STREP Active BIOMICS
- UNIVERSITÄT LEIPZIG
- Sächs. Aufbaubank
- Gesim, Evotec
- Ms. Marianne Duda
- Graduiertenkolleg Interneuro
- Neuroprogen, Euroderm, Beiersdorf
- Alexander von Humboldt Stiftung / Foundation