The Optical Stretcher
Technology and Applications

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
**Cell Deformability as an Inherent Cell Marker**

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

\[ G' \propto c_A^{2.67} \]

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

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**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:
\[ p = \frac{n_1 \cdot E}{c} \]

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!

The Optical Stretcher

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

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Range (pN/nN)</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optical Tweezers</td>
<td>0.1 – 10 pN</td>
<td>Intermolecular forces</td>
</tr>
<tr>
<td>Optical Stretcher</td>
<td>10 pN – 1 nN</td>
<td>Cell deformation</td>
</tr>
<tr>
<td>AFM</td>
<td>1 nN – 1 pN</td>
<td>Intramolecular forces</td>
</tr>
</tbody>
</table>

Deformation of a human erythrocyte:

Ideal force range to manipulate and deform cells

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**Microfluidics**

**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**

- Stretching in steady flow
- Several cells simultaneously in Bessel beam

**Enabling Elasticity-based Flow Cytometry**

- Parallelization
- Measurement rates up to 100–1000 cells/s

**The Instrument**

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

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S. Schinkinger et al., Laborwelt (2005)
Microfluidic Chip

(developed in collaboration with GeSiM mbH, Dresden)


Software Interface
High-Content Cell Analysis I

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

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High-Content Cell Analysis II

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

Modular Cell Handling

Combination with PALM microbeam
- Cell fusion
- Cell-poration
- CALI
- FRAP
- …

Summary I

- 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

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

10 µm

In suspension:
well-defined and highly regular cytoskeleton.

Cell differentiation increases cytoskeleton.

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Cell differentiation increases cytoskeleton.

Optical Deformability as Cell Marker I

10 µm

Normal fibroblast

Malignant fibroblast

stretching relaxation

0.1 s stretches

BALB3T3, SVF2

F. Wottawah et al., Acta Biomater. (2005)
F. Wottawah et al., Acta Biomater. (2005)
**Optical Deformability as Cell Marker II**

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!

**Detecting Cytoskeletal Changes**

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

- Western Blot
- FACS
- Laser Scanning Cytometry (LSC)

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

**required minimal sample size**

- $10^7$ cells: lysed cells
- $10^5-10^6$ cells: significant loss of viability
- $10^5-10^4$ cells: fixed cells
Biomechanics and Cytoskeletal Proteomics

Measurement of viscoelastic constants provides:
- Linear response:
  - $c_{\text{F-actin}}$, $c_{\text{microtubules}}$
  - $c_{\text{F-actin cross-linker}}$, $c_{\text{microtubule cross-linker}}$
  - $k_{\text{actin cross-linker}}$
  - $E_{\text{coupling actin-microtubules}}$
- Nonlinear response:
  - $c_{\text{intermediate filaments}}$
  - $F_{\text{crit. mechano-act}}$, $F_{\text{crit. mechanotrans}}$
  - $F_{\text{active}}$
  - $n_{\text{refractive, } R_{\text{cell}}}$

$=> 12$ cell parameters simultaneously

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Direct Detection of Metastatic Cells

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.

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Cervix:

- PAP smears are an established screen for cervical cancer
- 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

![Cervix Images](image)

Oral cavity:

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

![Oral cavity Images](image)
Molecular markers for cell differentiation stem predominately from developmental biology. It is unclear whether they detect all changes in differentiation of adult cells.
Neural Stem Cells and Parkinson’s Disease

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

Analyzing Blood

Laser power: 5 mW → 200 mW

- 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
Drug-Screening I

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

Cell softening through addition of Cytochalasin

Drug-Screening II

Cell stiffness inversely correlates with metastatic aggressiveness:

<table>
<thead>
<tr>
<th></th>
<th>Cell speed (µm/hr)</th>
<th>Elasticity (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB (n=10)</td>
<td>7.3</td>
<td>1.35</td>
</tr>
<tr>
<td></td>
<td>4.5</td>
<td>0.32</td>
</tr>
<tr>
<td>SV-T2 (n=10)</td>
<td>12.1</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td>4.8</td>
<td>0.34</td>
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<tr>
<td>H-ras (n=10)</td>
<td>24.7</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>12.0</td>
<td>0.37</td>
</tr>
</tbody>
</table>

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

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**Applying Well-defined Mechanical Stimuli**

Mechanically activated fibroblast:

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

generated force: 280±13 pN

[Ref: F. Wottawah et al., Phys. Rev. Lett. 95 (2005)]
Summary II

- Cytoskeletal elasticity is an 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

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- Group leader: Jochen Guck

Collaborators:
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