Mid-Infrared Laser Assisted Bioprinting

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APE GmbH - Angewandte Physik & Elektronik GmbH

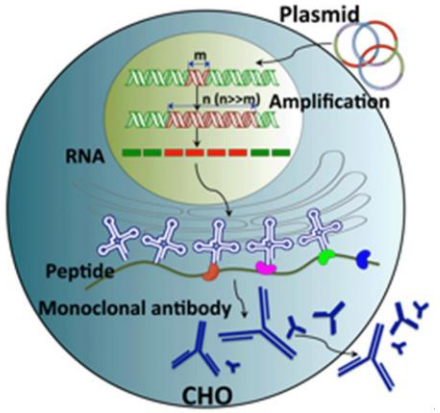
- SME located at Berlin, founded in 1992
- Manufacturer of devices for ultrafast laser technology

  for pulse characterization, modification and management
- Customized ultrafast laser systems
- APE devices are used worldwide
- Main markets:
  - Universities & research labs
  - trusted OEM supplier for industrial companies

Laser Induced Forward Transfer (LIFT) - application on single cell isolation and tissue printing

1. Application of transgenic mammalian cell lines

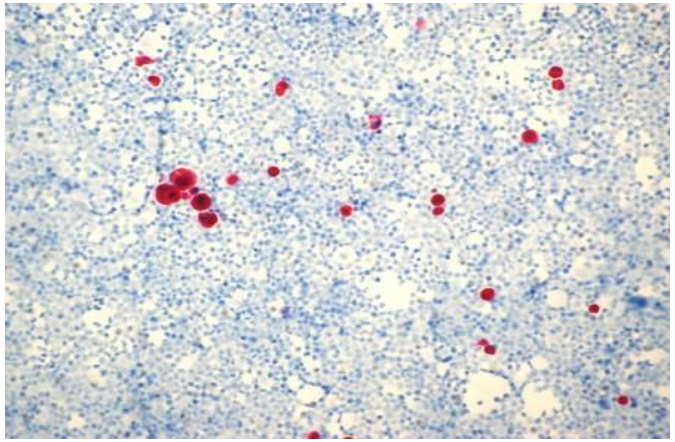
- Biologics production: Antibodies, Vaccines, Proteins
- Higher human compatibility
- **Monoclonal high-producer cell lines**



Xu, N., Ou, J., Gilani, AK.. et al. Front. Chem. Sci. Eng. (2015) 9: 376. https://doi.org/10.1007/s11705-015-1531-5

2. Isolation of rare cells

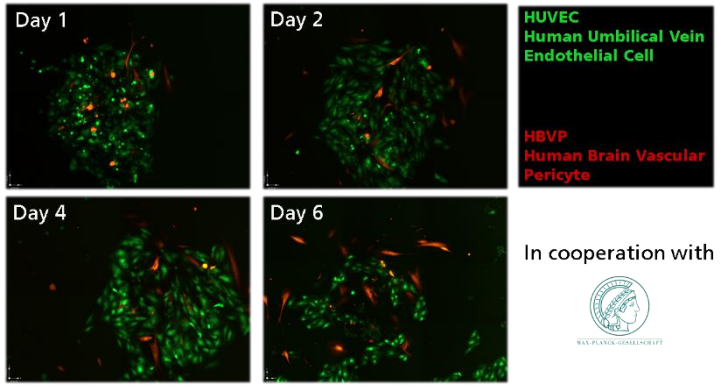
- Tailored therapies
- Identification of unknown diseases



http://www.pathogenesys.com/html/rare_cell_detection.html

3. Printing of 3D-tissue like structure

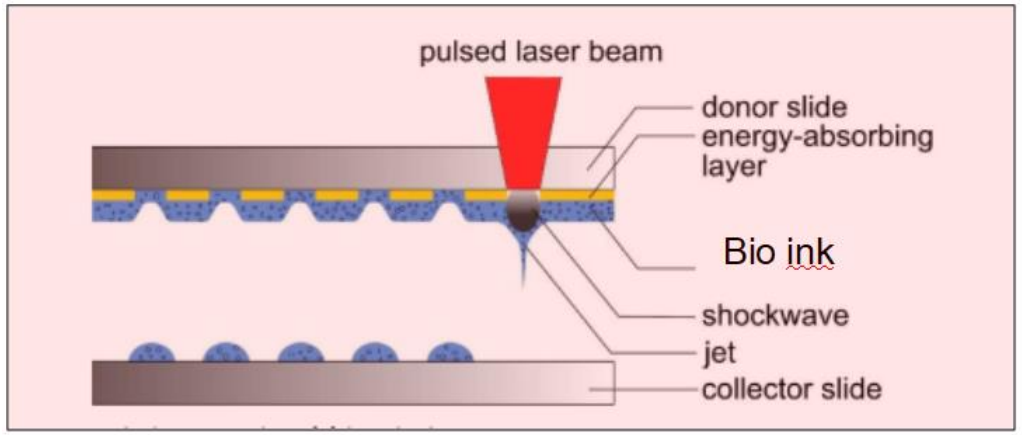
- High accuracy
- High selectivity
- Controlled cell stoichiometry



Motivation

- Tissue manufacturing - high medical needs
  - e.g. artificial skin substitutes (care of burnings, chronic or acute wounds)
  - Fast growing market; estimation in total: $6bn in 2017e / $11.5bn in 2022e
- Current tissue manufacturing methods - bottleneck impeding large-scale market adoption (1)
- Conventional LIFT scheme

typ. 1 µm ns laser



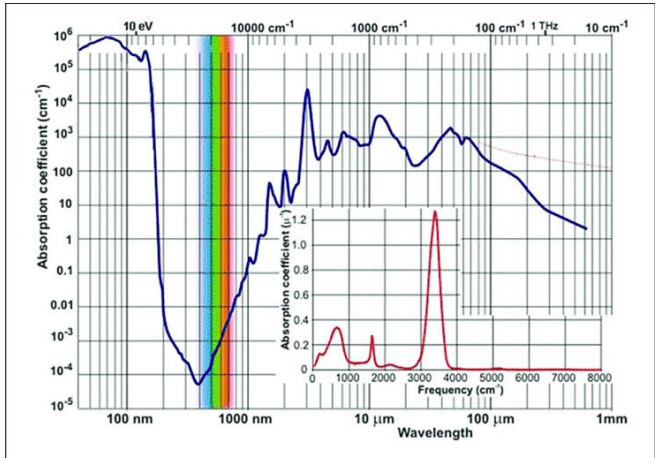
Metal sacrificial layer

- Limitations:
  - Metal contamination – difficult, *poisonouos surroundings* for living cells
  - Donor loss – exchange necessary, *discontinuous process*

Note: (1) Advanced Therapies Investment Report - Phacilitate

How to overcome this problem ?

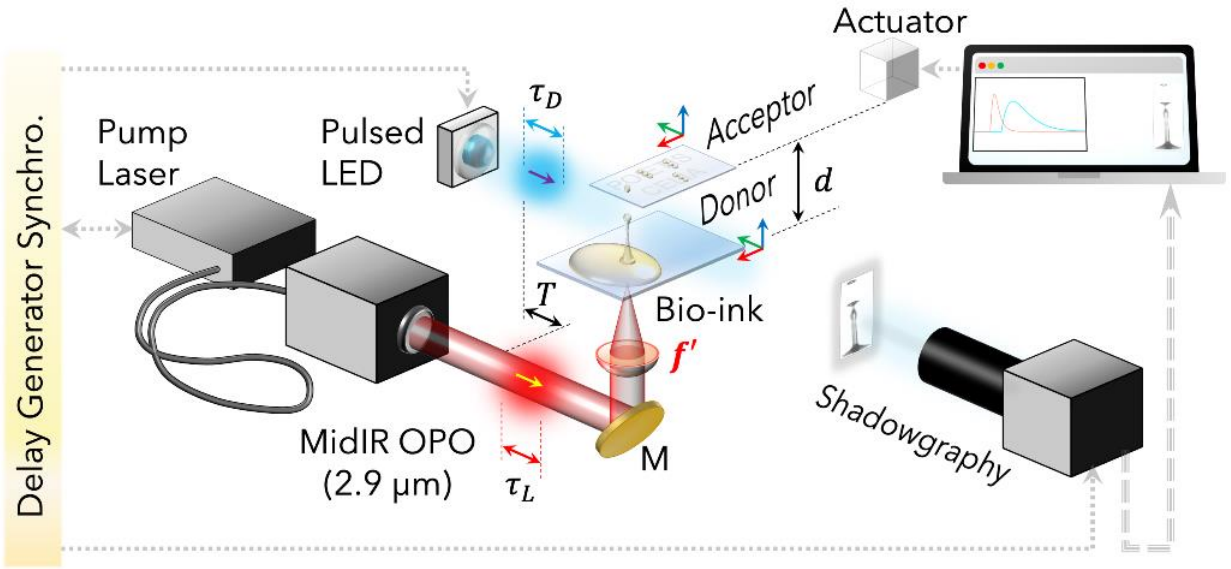
- Water is everywhere !
- Linear absorption of water in the hydrogel ink (cell matrix)
- → waiving the sacrificial donor layer
- Highest absorption at ~3 µm
  - extremely short absorption length
  - even the thinnest layer will fully absorb the laser pulse
  - no energy entry into the cell



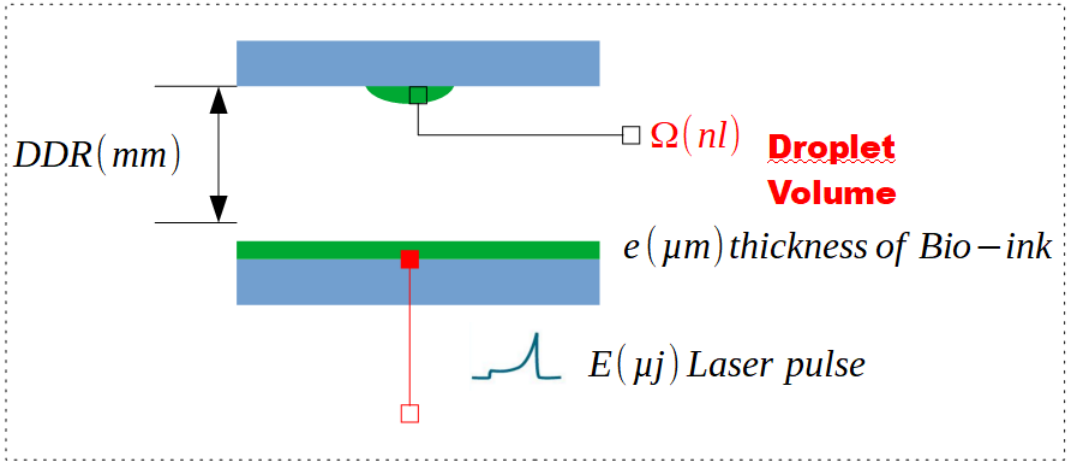
Beć, Krzysztof & Huck, Christian. (2019). Breakthrough Potential in Near-Infrared Spectroscopy: Spectra Simulation. Frontiers in Chemistry. 7. 10.3389/fchem.2019.00048

Characterisation

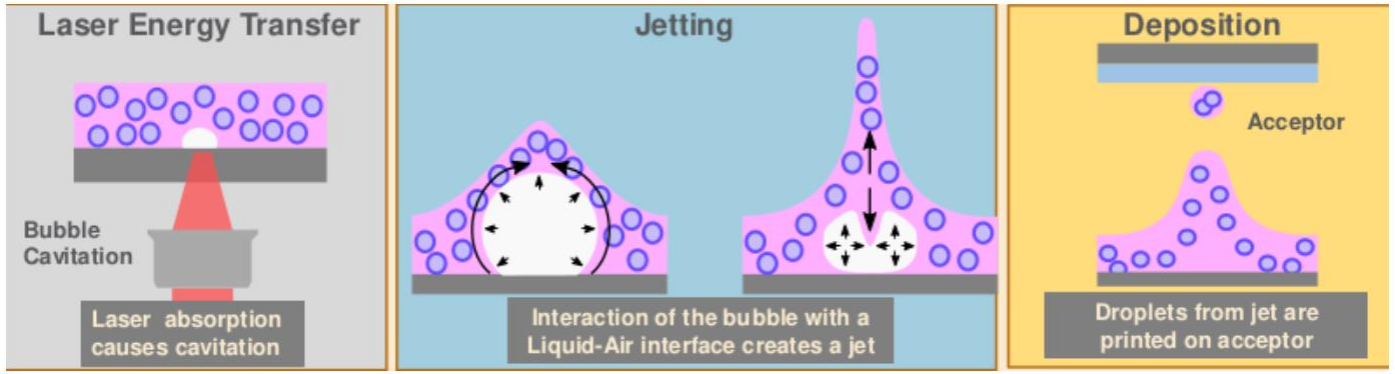
Setup



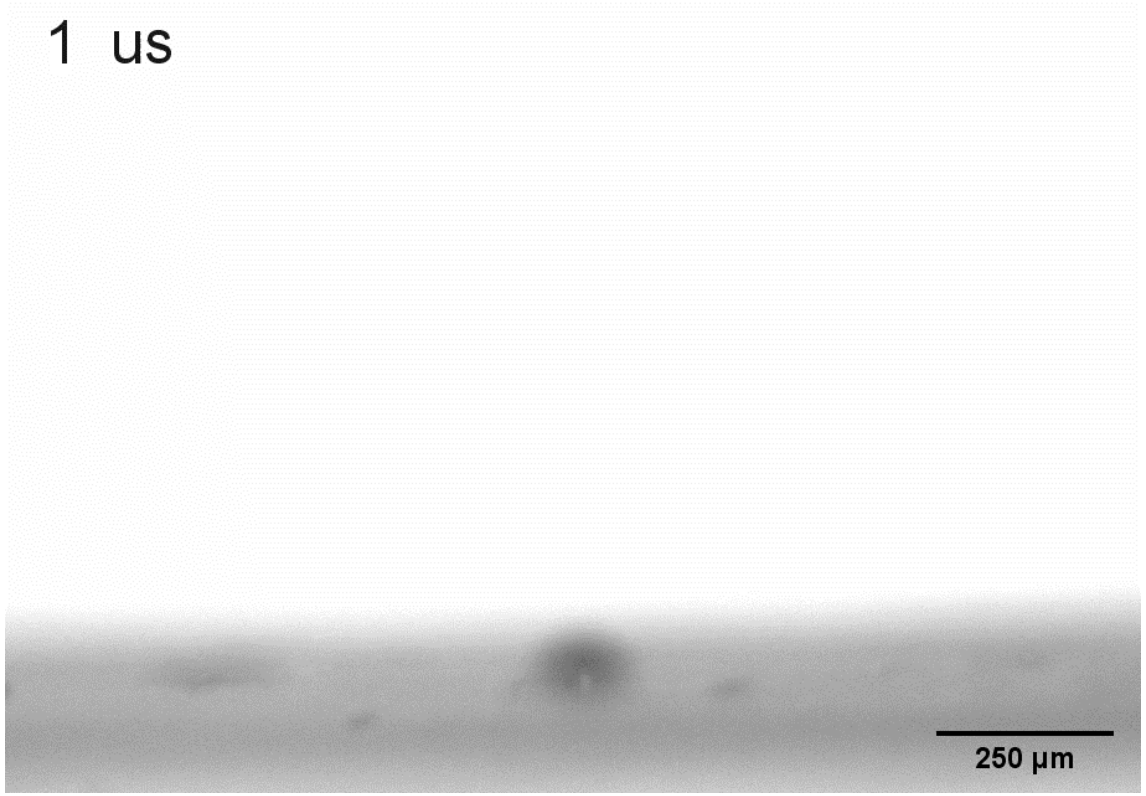
LIFT parameters

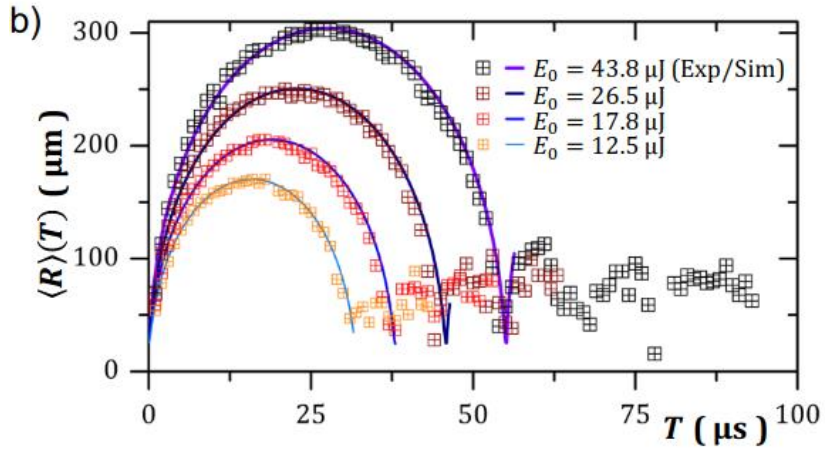


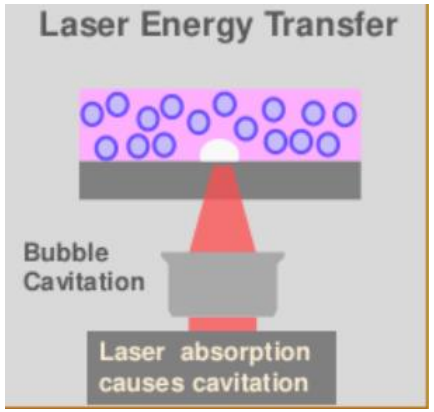
3 major steps



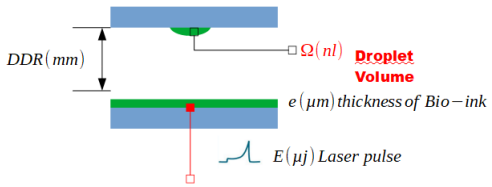
Bubble dynamics

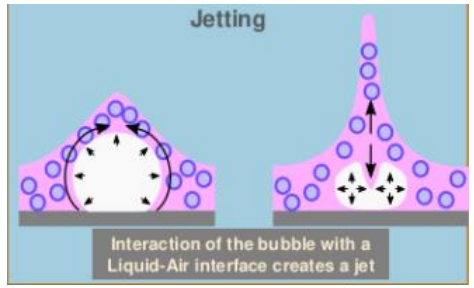


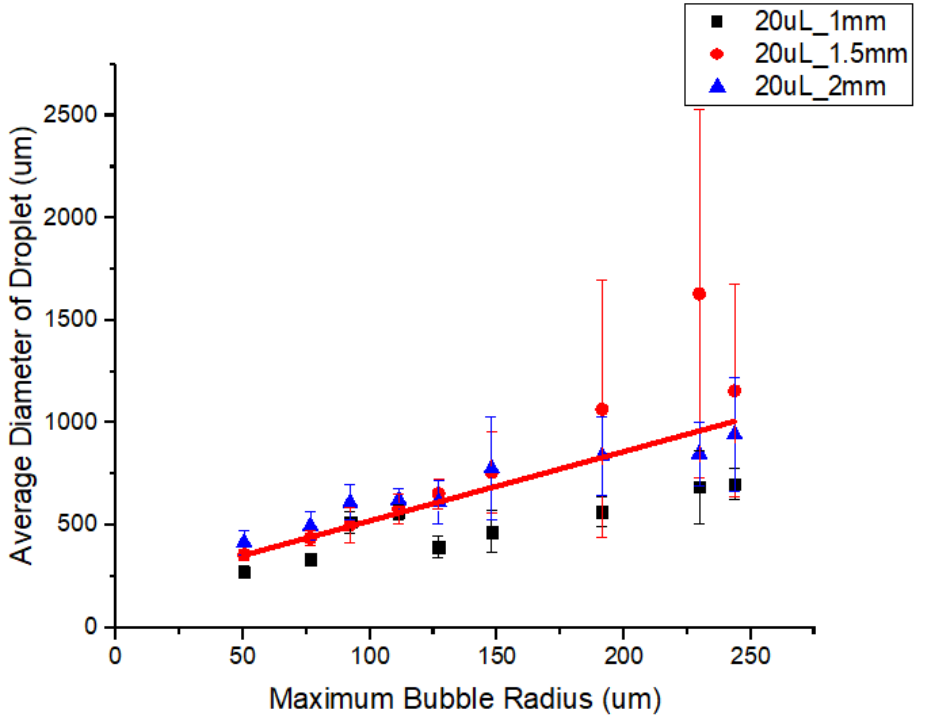




Droplets printing - numerical simulations and real tests



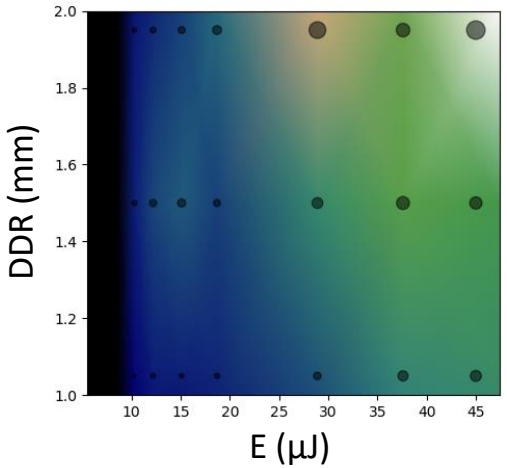




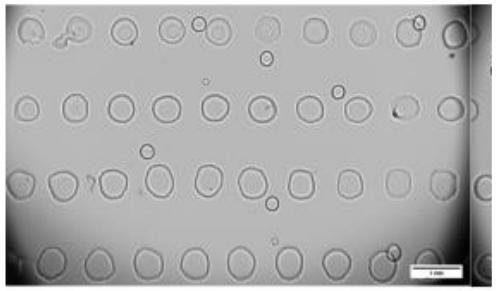
Gautier Dussuyer, Dominique Legendre
Institut de Meccanique des Fluides de Toulouse

Results

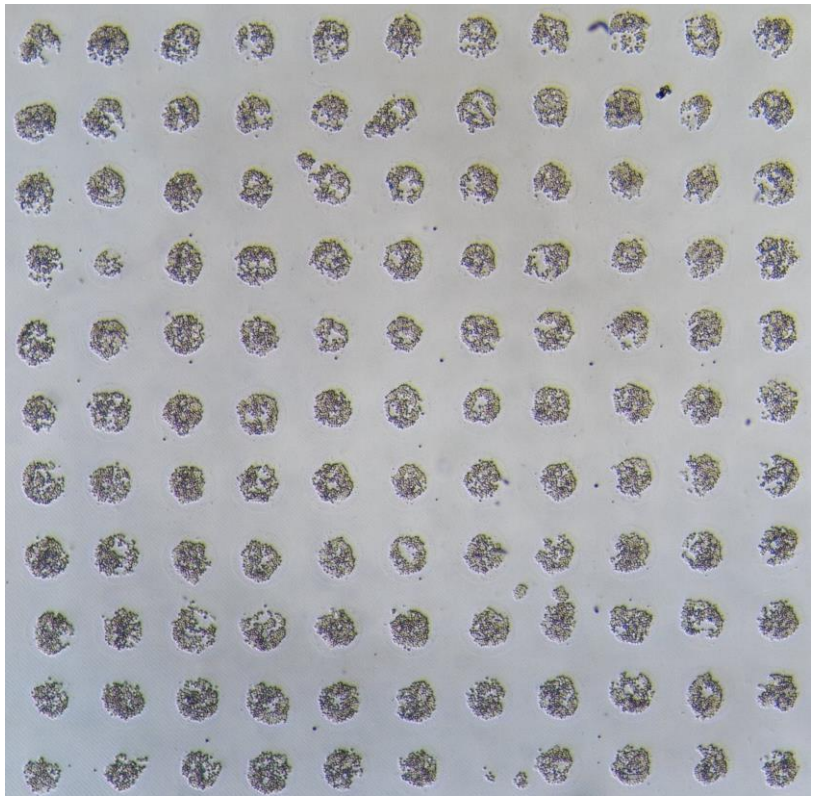
e = 75 µm

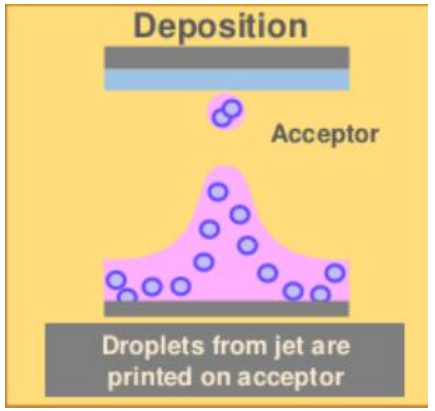


e = 150 µm DDR = 1,5 mm



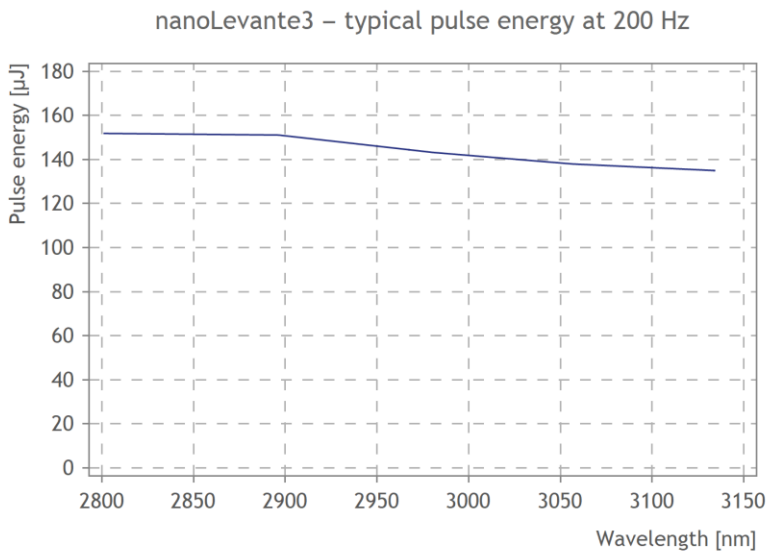
Printed Keratinocyte cells (human skin)





Laser source

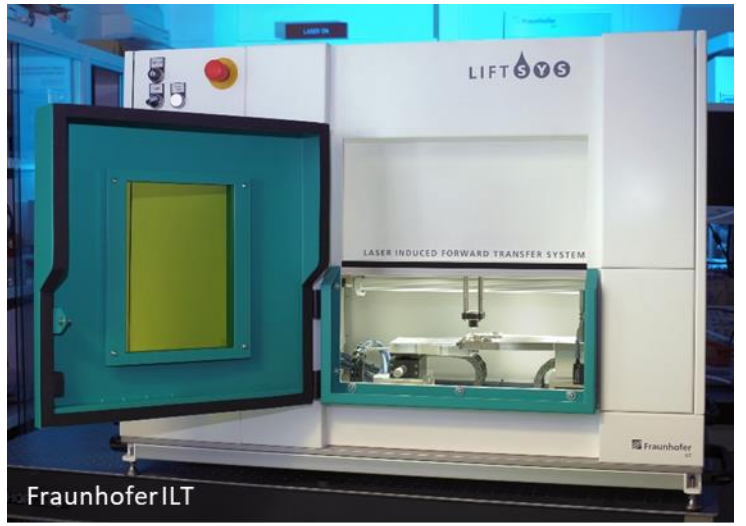
- Optimum parameter range: ~3 µm, 50 µJ, ~10 ns, a few kHz, high beam quality
- Challenge, because not available on the market
  - Laser: mJ / a few 10 Hz; OPO: a few 10 MHz / nJ or mJ / a few Hz / low beam quality
- → Dedicated laser system: Optical Parametric Oscillator in a *new parameter range*
  - Using industrial 1 µm pump laser
- Compact and robust system
- OPO → tunable 2.2 … 4 µm
  - Access to other absorption lines
  - Alternative Applications

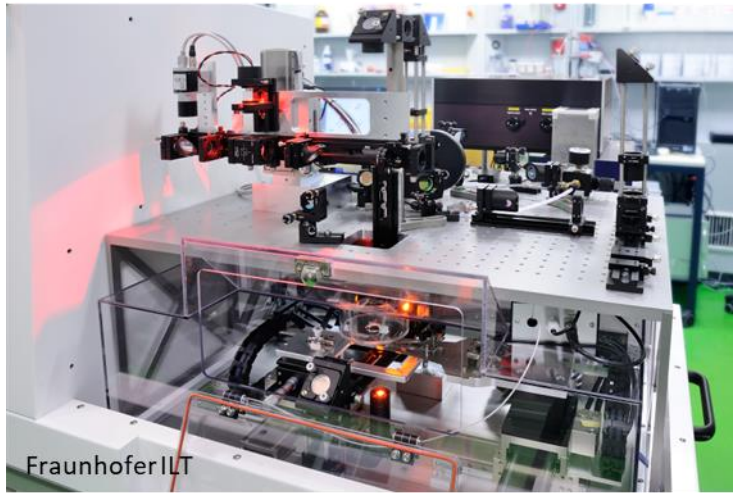


Conclusions

- LIFT directly out of cell suspension
- Transfer stability: >95 % successful transfer
- Cell viability: >80 % depending on cell type
- Advantages:
  - No metal carrier – natural, healthier surroundings for living cells
  - Continuous process - no donor exchange
  - Simplified automation of biofabrication process
  - Higher throughput cell droplets deposition
  - Compatible setup
- Cons:
  - Special optical requirements

    MIR optics and scanner, MIR-transmissve substrate





Acknowledgements

- Collaborative work by the following equipollent participants

poietis make tissues real	Antonio Iazzolino, PhD Bertrand Viellerobe
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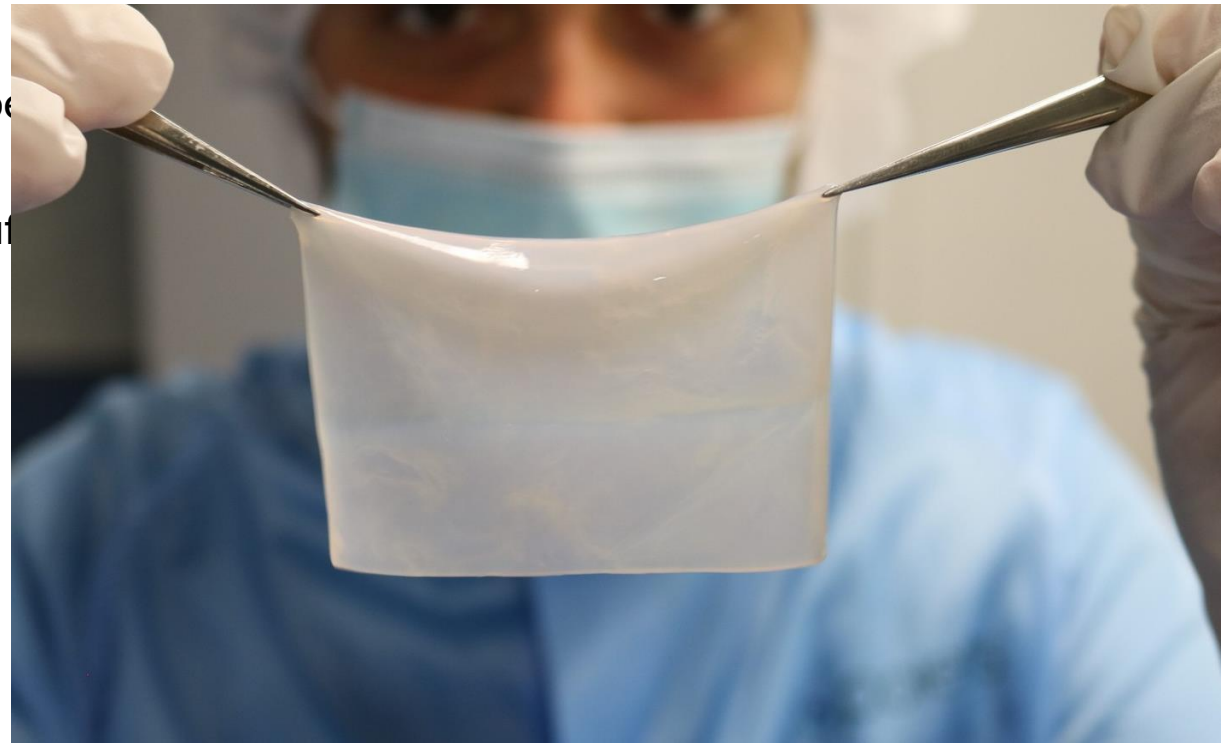
Outlook

- *What can I do for the others?*
- MIR-assisted LIFT
  - No contamination - more reliable and therefore be
    experiments
  - More efficient process for large scale tissue manuf



- *What can the others do for me?*
- Applicable for other media (absorption lines)
- Applicable to other processes, e.g.
  - IR MALDI mass spectrometry
  - Wavelength selective micro material processing