Optical Trapping Techniques for the Control and Actuation of Microstructures

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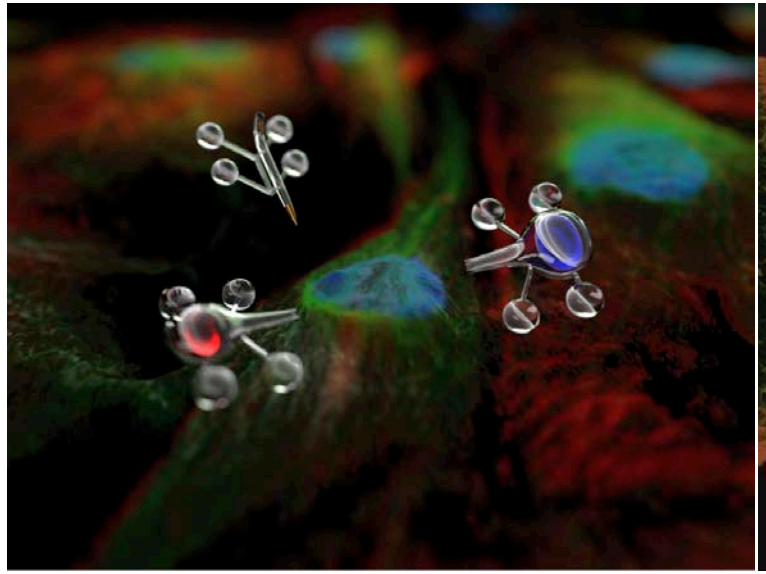

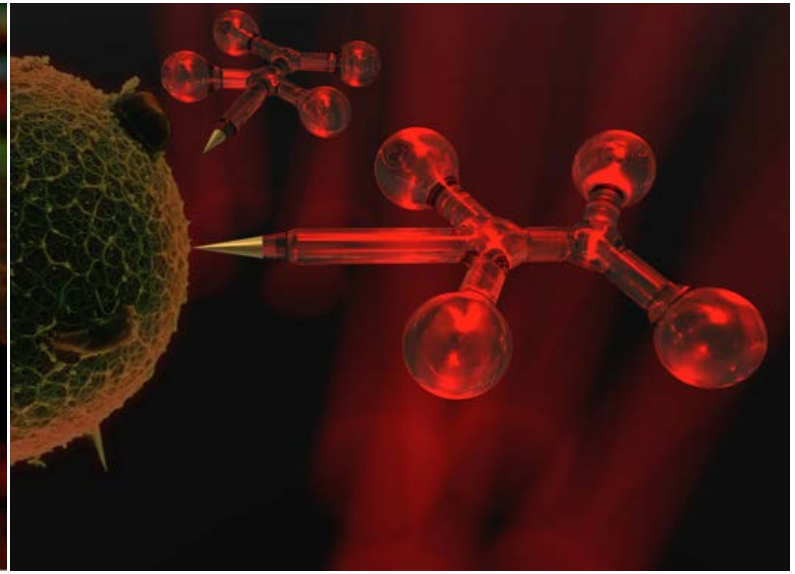

Alexandre Wetzel, Einstom Engay, Ada-Ioana Bunea, Jesper Glückstad

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What will be the topic of this presentation?

1. **Optical trapping at the microscale**
   i. **What is optical trapping at the microscale ?**
   ii. **What are the techniques employed?**
   iii. **What are the applications?**

Generalized Phase Contrast (GPC)

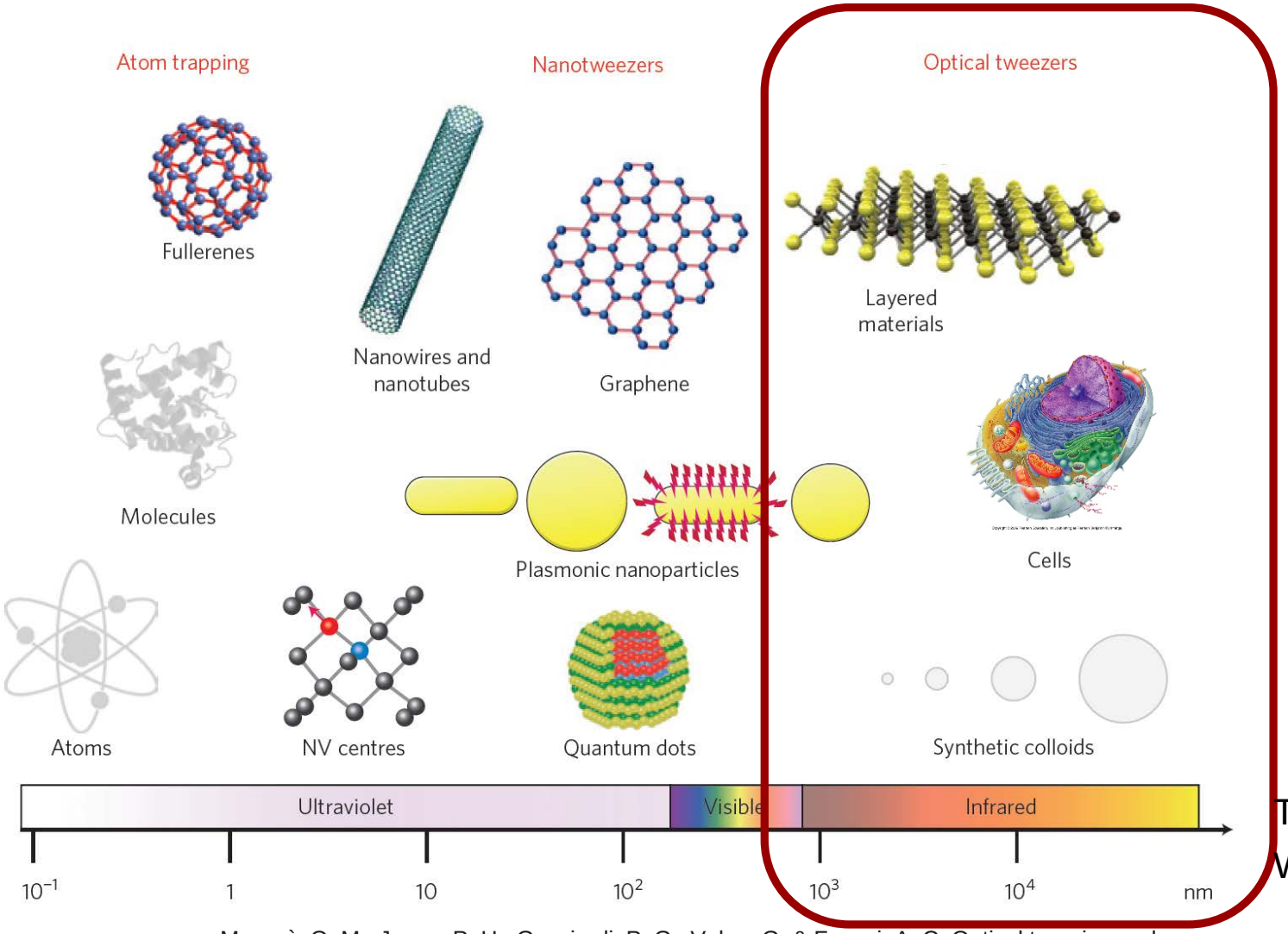
Light Robotics

2. Optical trapping in biological media
   i. Challenges for optical trapping in turbid media
   ii. Wavefront correction algorithms
   iii. Real-time implementation for optical trapping

3. Future perspectives & Conclusions

1. i. What is optical trapping at the microscale ?

Objects that can be trapped



Trapping Wavelength

Maragò, O. M., Jones, P. H., Gucciardi, P. G., Volpe, G. & Ferrari, A. C. Optical trapping and manipulation of nanostructures. Nat. Nanotechnol. 8, 807–819 (2013).

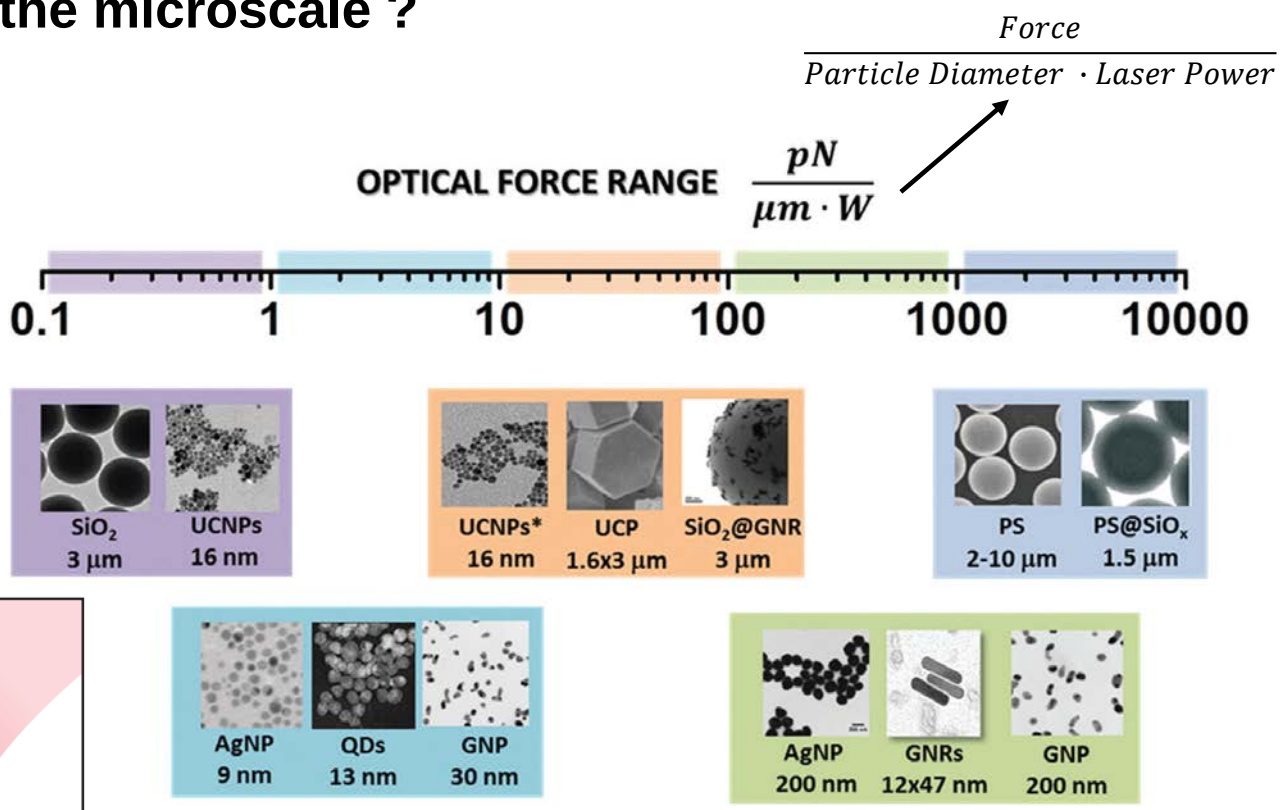
1. i. What is optical trapping at the microscale ?

Trapping force between ~0.1 pN to about ~1.5 nN

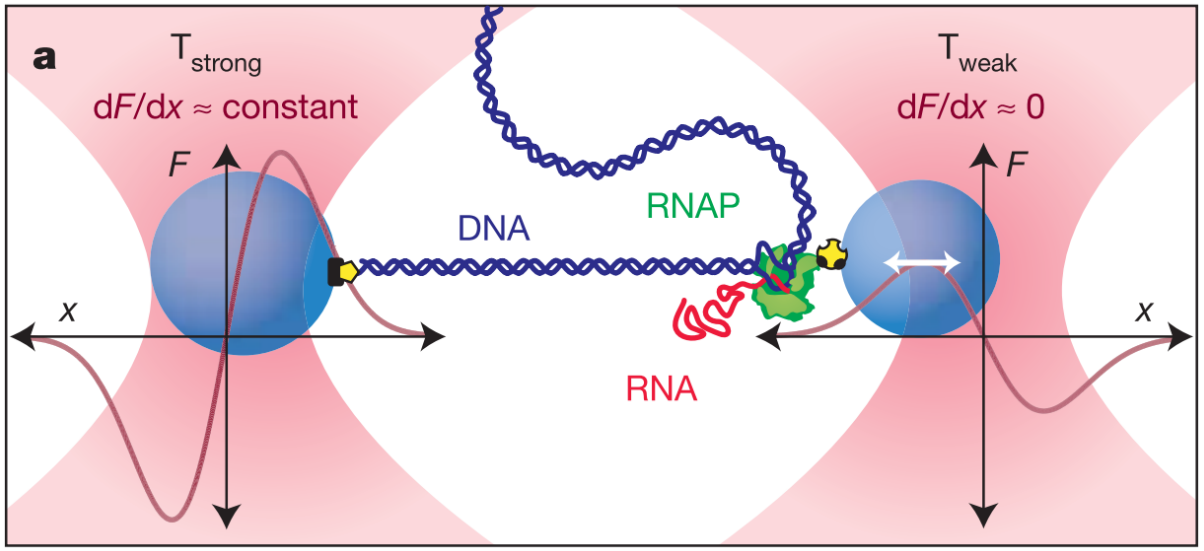
Force is tunable with laser intensity (trap stiffness)

Precise Position tracking: resolution ~ 2 - 8 nm usually (maximum achieved ~3.4 Å)

Precise Force tracking: resolution ~0.1 pN



Rodríguez-Sevilla, P., Labrador-Páez, L., Jaque, D. & Haro-Gonzáles, P. Optical trapping for biosensing: materials and applications. J. Mater. Chem. B 5, 9085–9101 (2017).

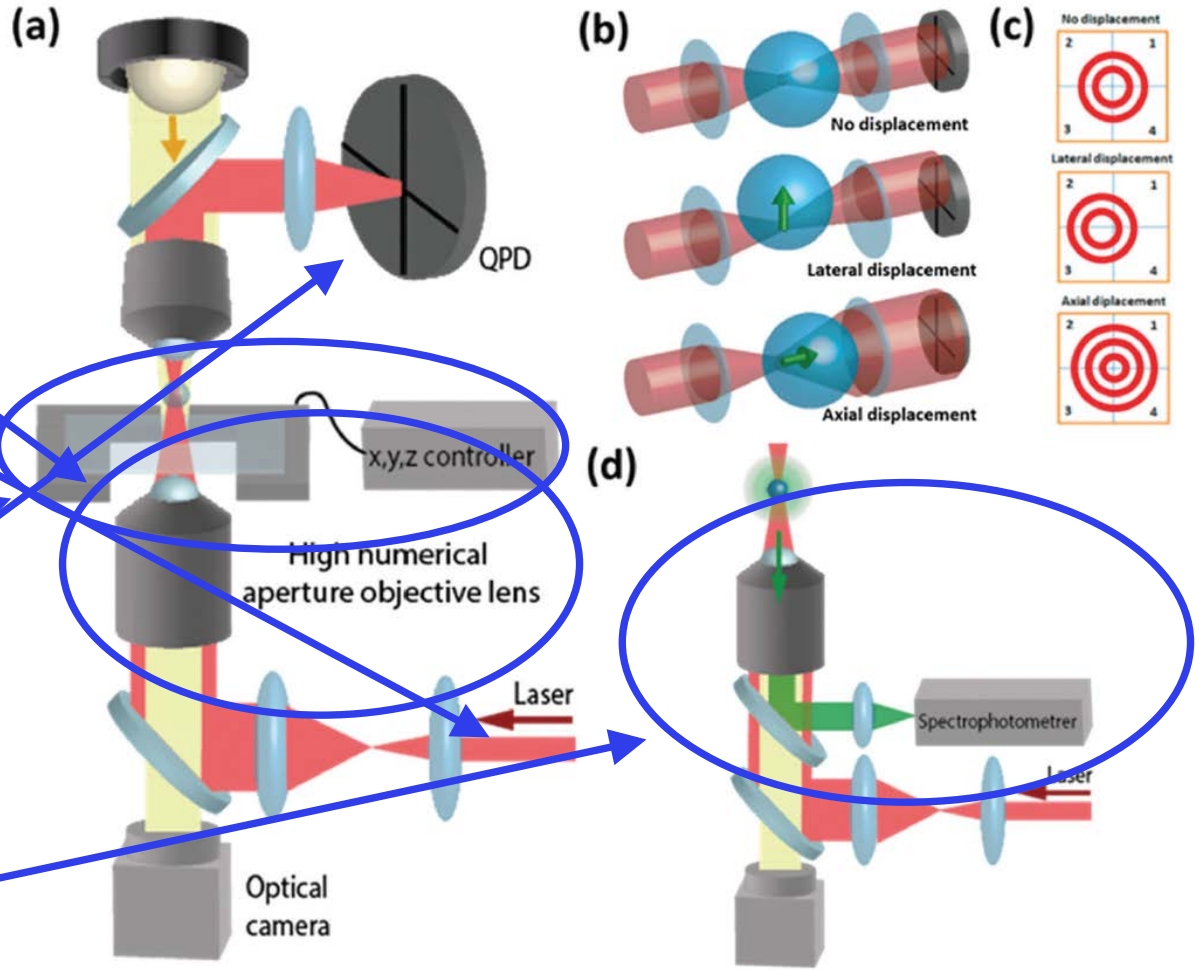


Abbondanzieri, E. A., Greenleaf, W. J., Shaevitz, J. W., Landick, R. & Block, S. M. Direct observation of base-pair stepping by RNA polymerase. Nature 438, 460–465 (2005).

1. ii. What are the techniques employed ?

Simple trapping set up (from inverted microscope):

- Microscope objective with a large NA (usually $\mathrm{NA} > 1$).
- The trapping laser is incorporated into the microscope path
- Precision translation stage (piezoelectric actuators)
- Position measurement device (QPD/Camera)
- Optional Integration of characterization techniques (fluorescence, spectroscopy,…)



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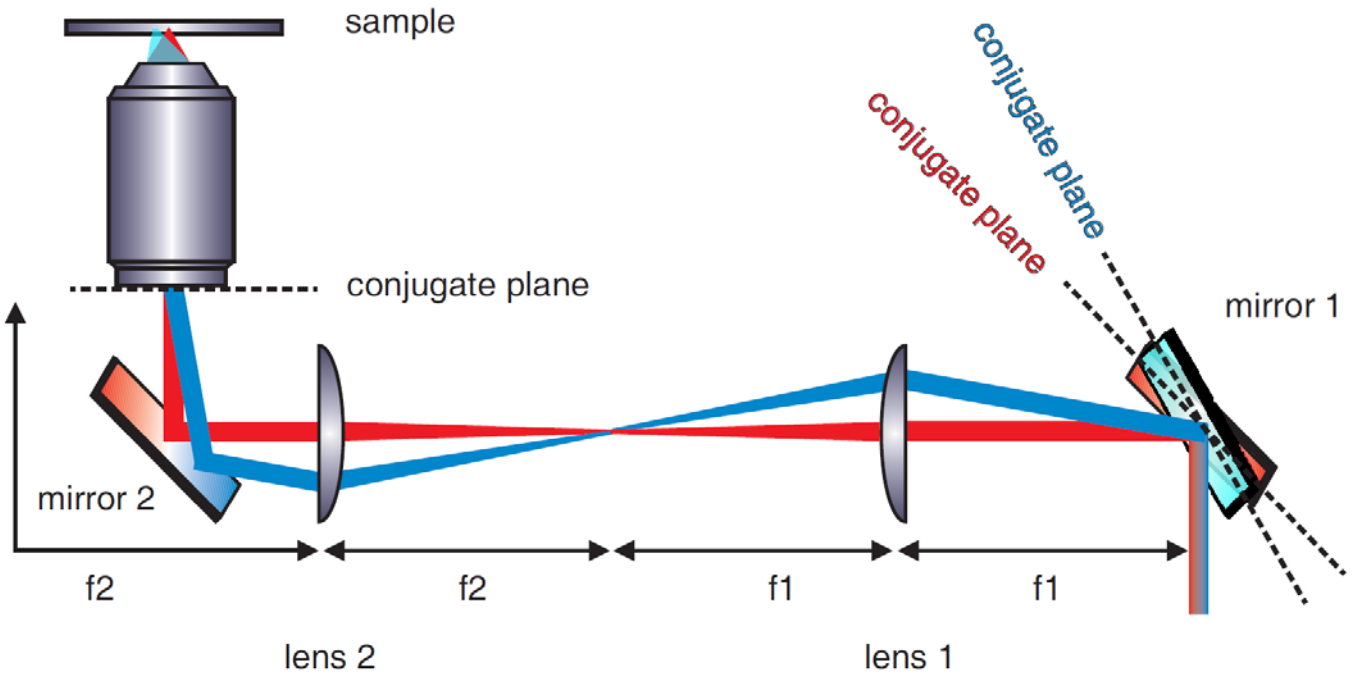
1. ii. What are the techniques employed ?

Steerable traps (1):

- **Scanning Mirrors**
  - Not that fast (response time ~100 μs)
  - Transmittance > 95%
  - Large deflection angles
- **Acousto-optic deflectors (AOD)**
  - Fast (response time ~1,5 μs/mm of laser beam diameter)
  - Transmittance ~ 60 - 85%
  - Limited deflection angle
  - Control of position and trap stiffness
- **Electro-optic deflectors (EOD)**
  - Fast (response time ~1 μs)
  - Transmittance ~ 90%
  - Limited deflection angle
  - High cost



Dienerowitz, M. Optical manipulation of nanoparticles: a review. J. Nanophotonics 2, 021875 (2008).

1. ii. What are the techniques employed ?

Steerable traps (2):
Holographic Optical Tweezers (HOTs)

Use a spatial light modulator (SLM) :

- Simultaneous multiple traps
- arbitrary shape and size of traps
- Much slower (response time ~10ms).
- 3D manipulation of traps

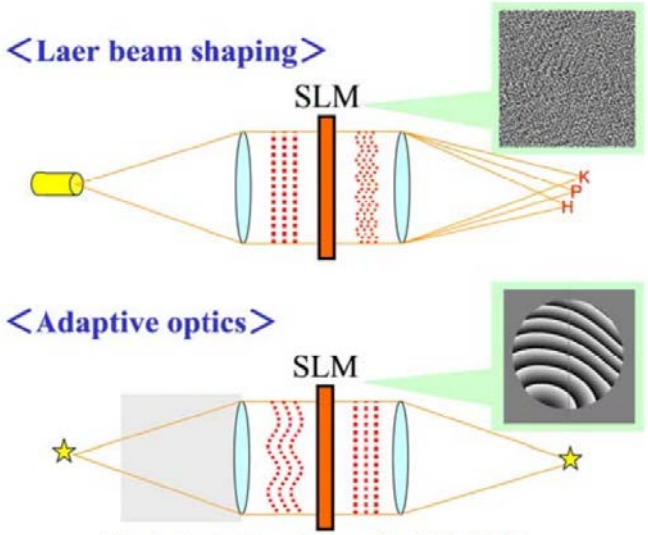
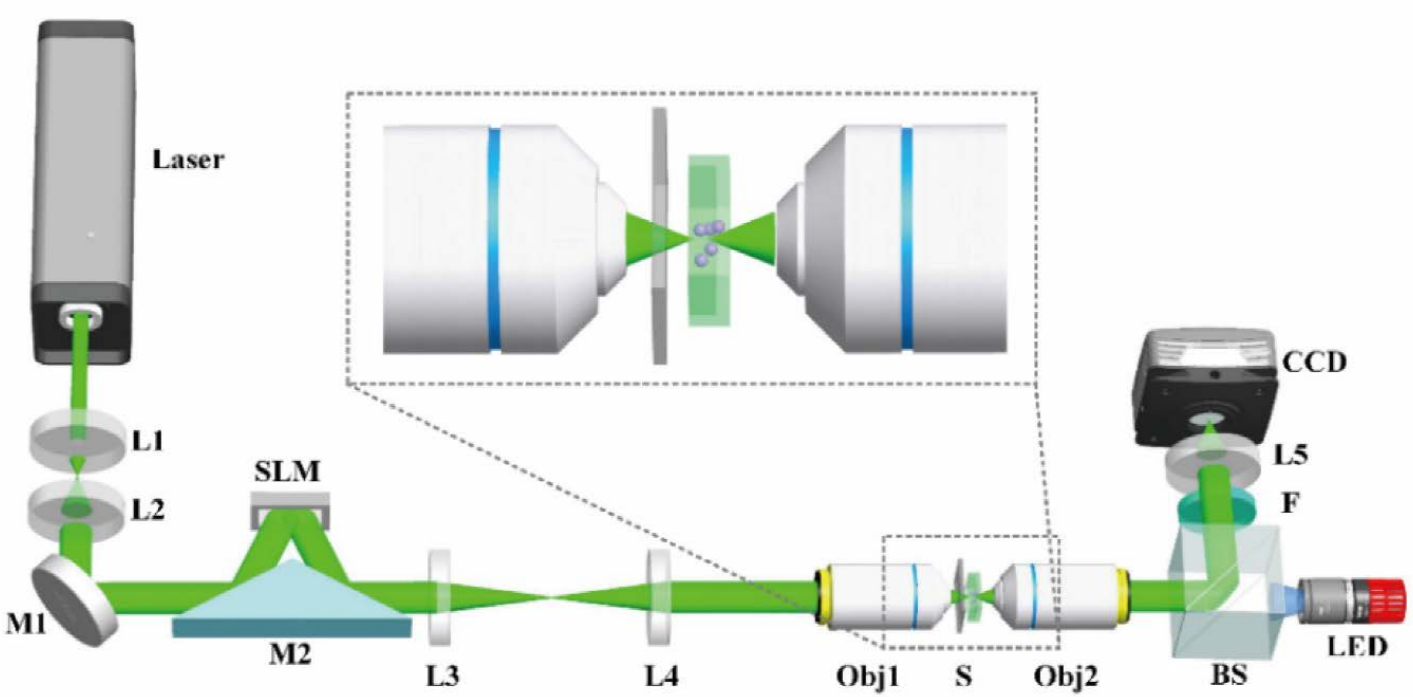


Fig.4 Basic functions of LCOS-SLM

Hara, T., Wu, M. H. & K, H. P. K. Advances in Application of Liquid Crystal on Silicon Spatial Light Modulator (LCOS-SLM) Haruyoshi Toyoda , Takashi Inoue , Naohisa Mukozaka *,. 559–562 (2014).



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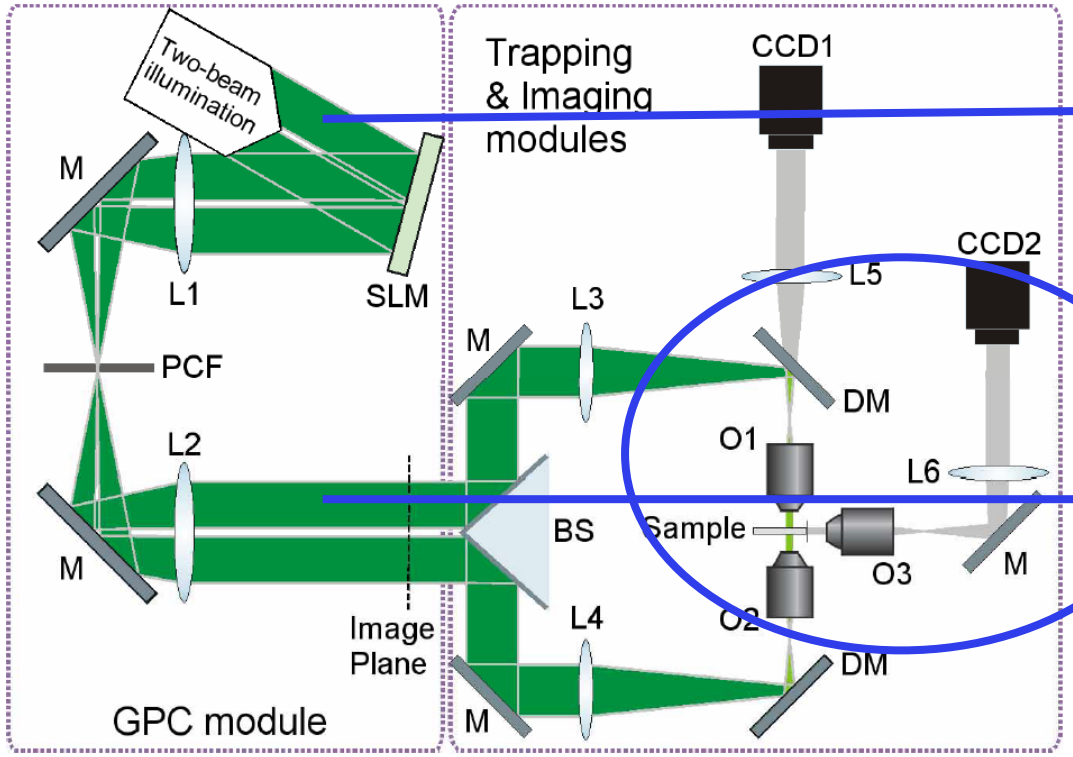
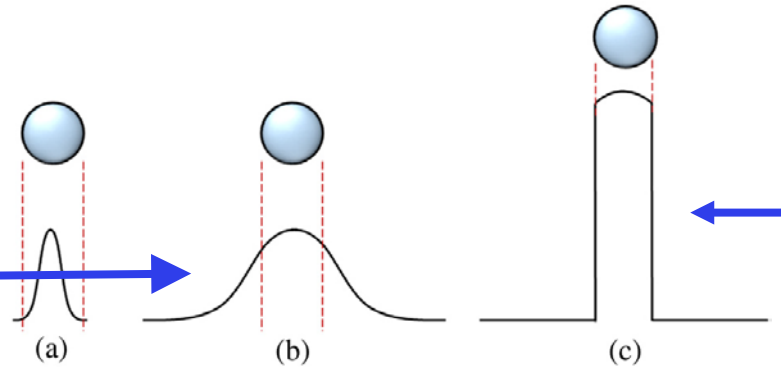
1. ii. What are the techniques employed ?

GPC: Generalized Phase Contrast



John Rodrigo, P., Perch-Nielsen, I. R., Amadeo Alonzo, C. & Glückstad, J. GPC-based optical micromanipulation in 3D real-time using a single spatial light modulator. Opt. Express 14, 13107–13112 (2006).



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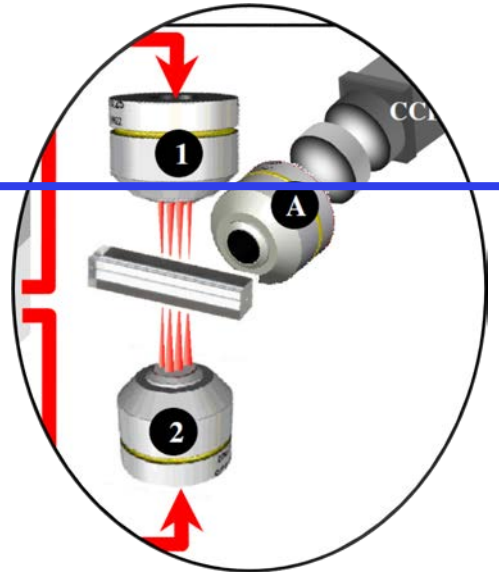
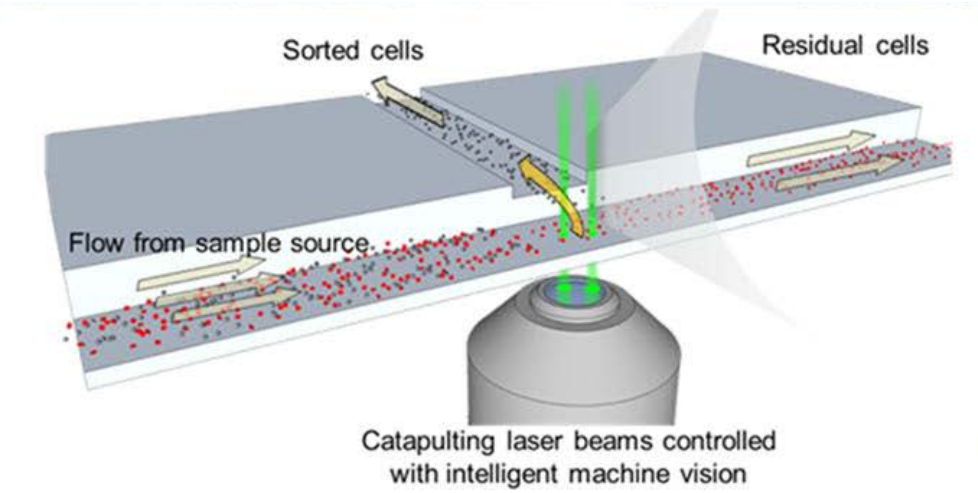


Tauro, S., Bañas, A., Palima, D. & Glückstad, J. Dynamic axial stabilization of counter-propagating beam-traps with feedback control. Opt. Express 18, 18217–18222 (2010).

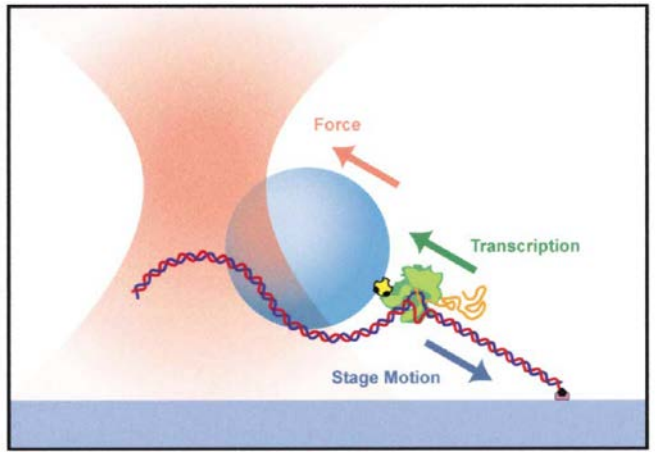
1. iii. What are the applications ?

Cell Sorting



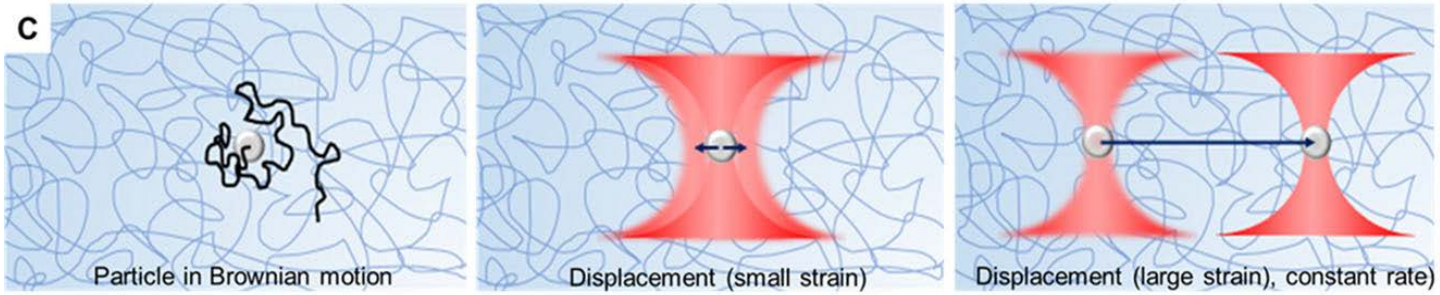
Bañas, A., Palima, D., Villangca, M. & Glückstad, J. Cell sorting using efficient light shaping approaches. in Proceedings of SPIE (2016).

Single Particle as handle: Force Sensing



Neuman, K. C., Abbondanzieri, E. A., Landick, R., Gelles, J. & Block, S. M. Ubiquitous transcriptional pausing is independent of RNA polymerase backtracking. Cell 115, 437–47 (2003).

Single Particle as tracer: Viscosity/ Temperature Sensing



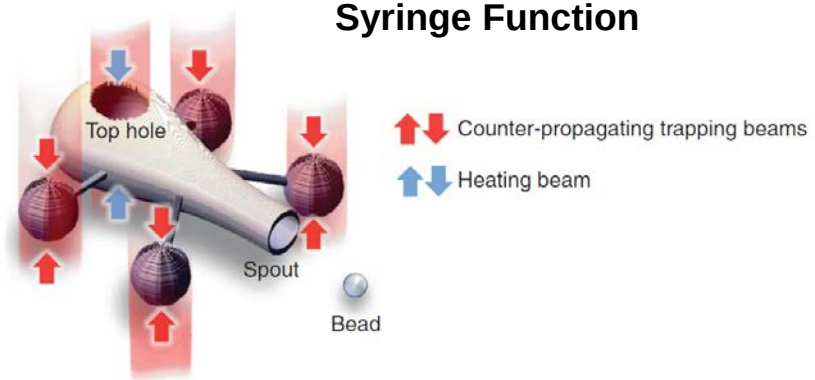
Bunea, A.-I. & Glückstad, J. Strategies for Optical Trapping in Biological Samples: Aiming at Microrobotic Surgeons. Laser Photon. Rev. 1800227, (2019).

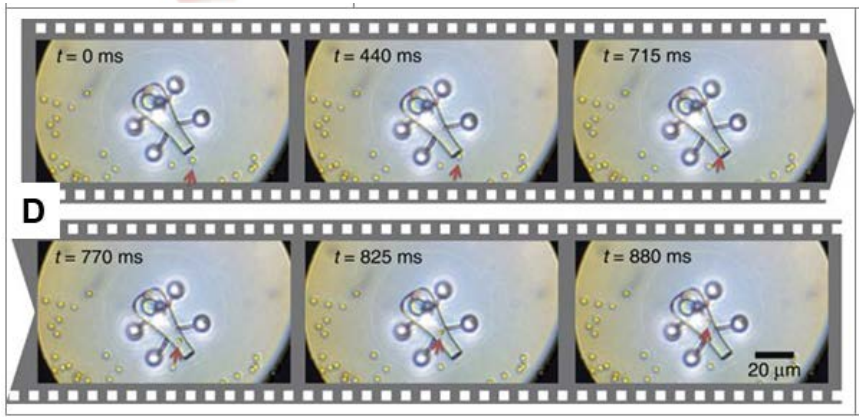
1. iii. What are the applications ?

Light Robots (microrobots):

Using multiple handles, it's possible to create structures with specific functions

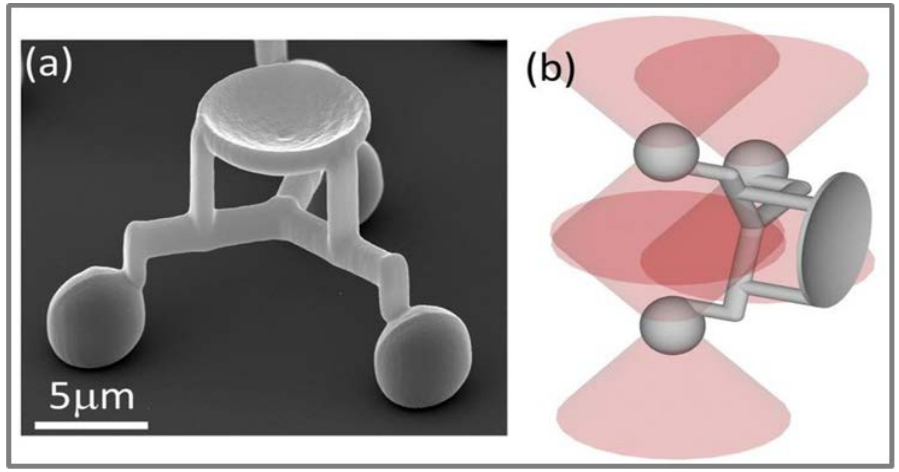
Syringe Function





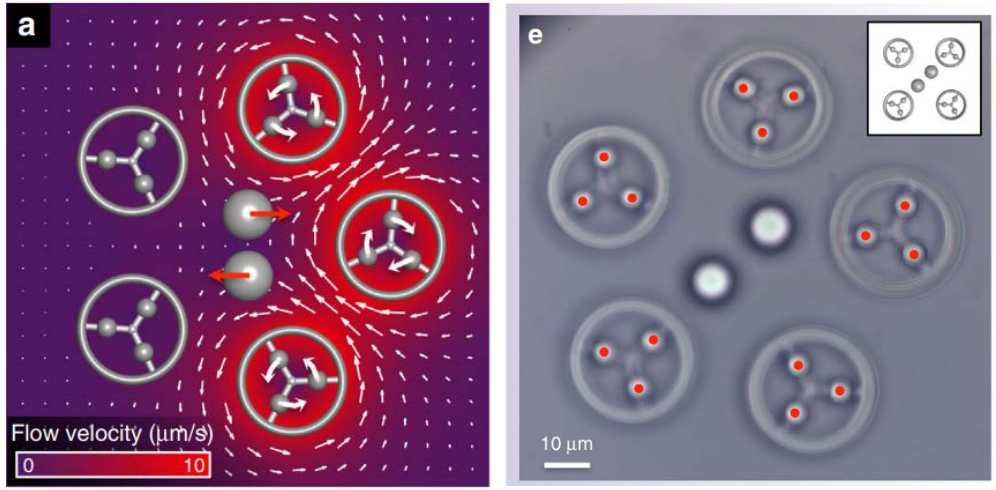
Villangca, M. J., Palima, D., Bañas, A. R. & Glückstad, J. Light-driven micro-tool equipped with a syringe function. Light Sci. Appl. 5, e16148 (2016).

Cell Manipulation Platform



Vizsnyiczai, G. et al. High accuracy indirect optical manipulation of live cells with functionalized microtools. in Proceedings of the IEEE 9922, 992216 (2016).

Micro-rotors



Būtaitė, U. G. et al. Indirect optical trapping using light driven micro-rotors for reconfigurable hydrodynamic manipulation. Nat. Commun. 10, (2019).

1. Optical trapping at the microscale
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   ii. What are the techniques employed?
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2. **Optical trapping in biological media**
   i. **Challenges for optical trapping in turbid media**
   ii. **Wavefront correction algorithms**
   iii. **Real-time implementation for optical trapping**

3. Future perspective & Conclusion

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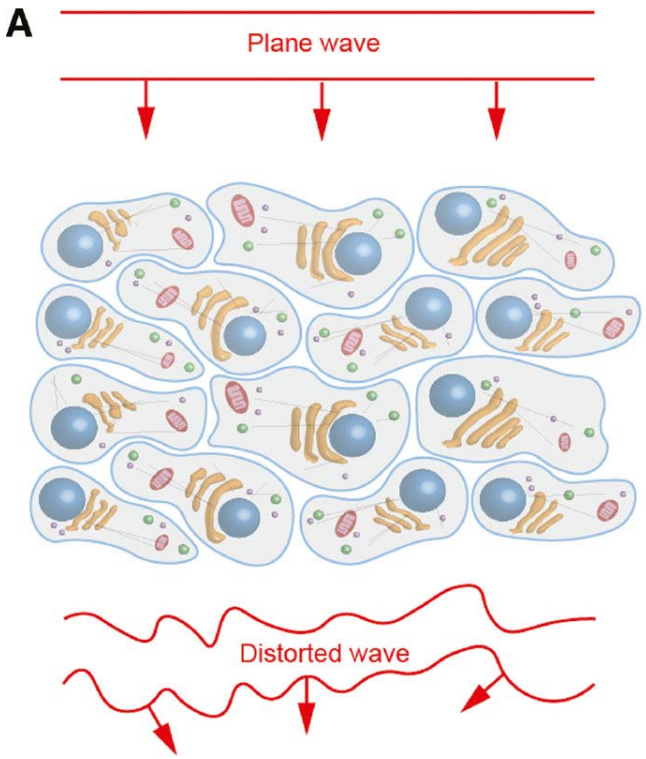
2. i. Challenges for optical trapping in turbid media

- Most Optical Trapping Experiments are done in water
- For a better understanding of biological systems:
  - perform optical trapping in biological media
- However, biological fluids induce additional scattering and absorption:
  - It will reduce the trap stiffness
- Biological media have also:
  - A different viscosity than water
  - Non uniformity
  - Sample to sample variability



Favre-bulle, I. A., Stilgoe, A. B., Scott, E. K. & Rubinsztein-dunlop, H. Review article Optical trapping in vivo : theory , practice , and applications. **8**, 1023–1040 (2019).

2. i. Challenges for optical trapping in turbid media

Random Scattering through biological media:

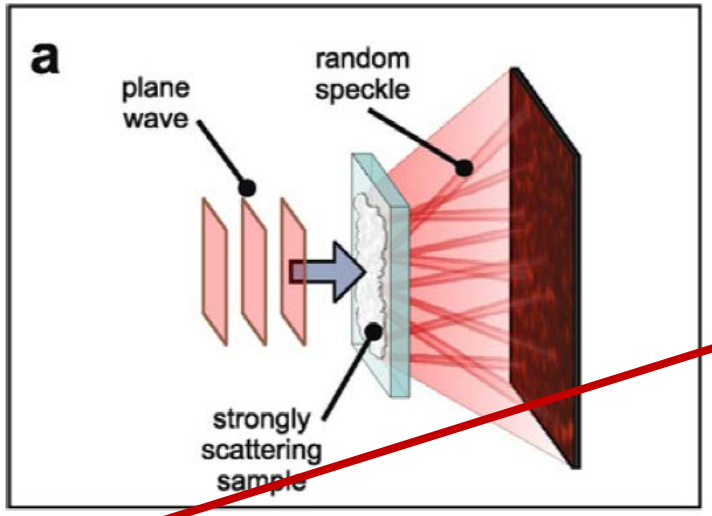
Spatial Coherence is lost.

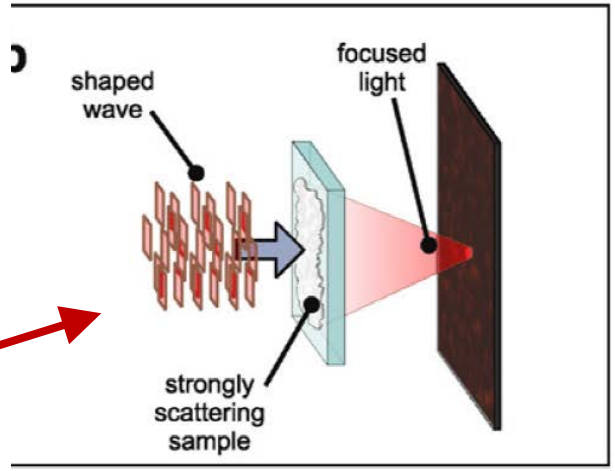
Resulting pattern: Volume speckle field, no correlation with the incoming field.

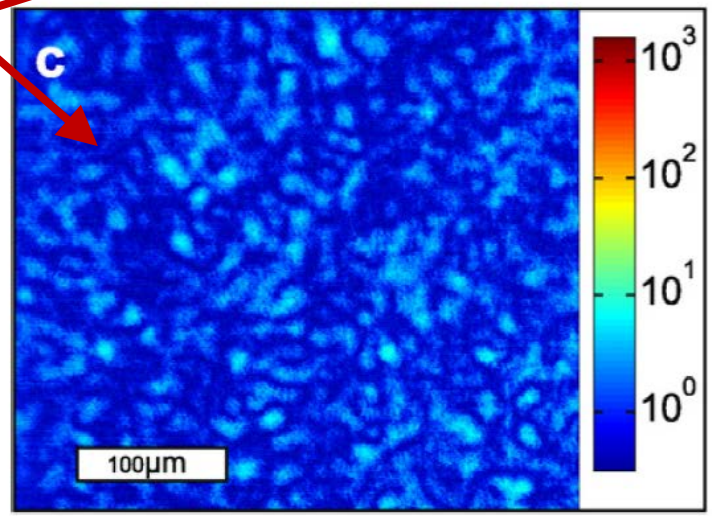
Linearity of the Scattering process:

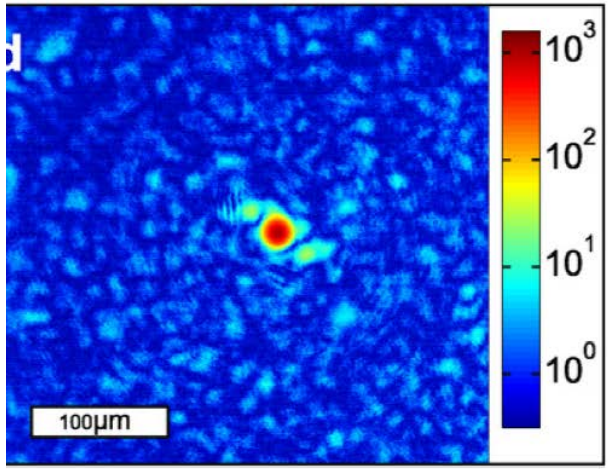
Algorithm to match the incoming wavefront by inverse diffusion.

Works only using a SLM to correct the incoming beam phase → Holographic Optical Tweezers

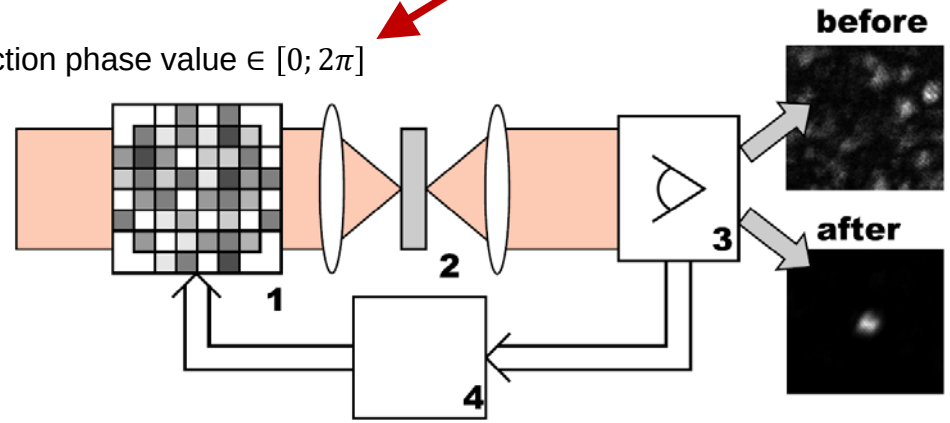








Correction phase value $\in [0; 2\pi]$

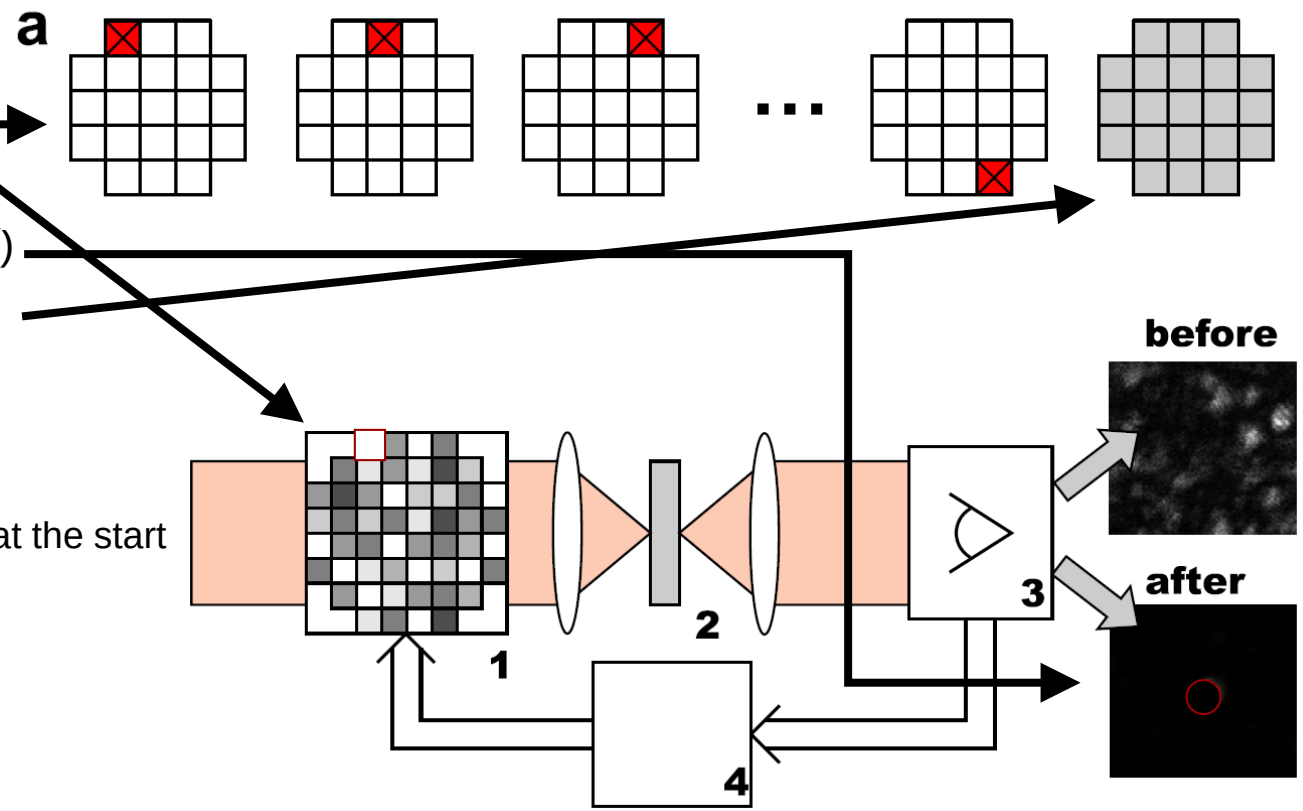


Vellekoop, I. M. & Mosk, A. P. Phase control algorithms for focusing light through turbid media. Opt. Commun. 281, 3071–3080 (2008).

2. ii. Wavefront correction algorithms

Basic Algorithm principle:

- The SLM is divided in N square segments.
- The Phase is varied from 0 to 2π
- A cost function is evaluated at the output (target)
- After evaluating all segments: Set the corrected phase value



Enhancement: $\eta \equiv \frac{I_N}{\langle I_0 \rangle}$ Intensity over target / Overall average Intensity at the start

Fluid as scattering sample: Speckle pattern will decorrelate after a time $\boldsymbol{T_p}$ (persistence time)

The time to complete one iteration of the algorithm is called $\boldsymbol{T_i}$.

If $\boldsymbol{NT_i} \gg \boldsymbol{T_p}$, the algorithm will not work.

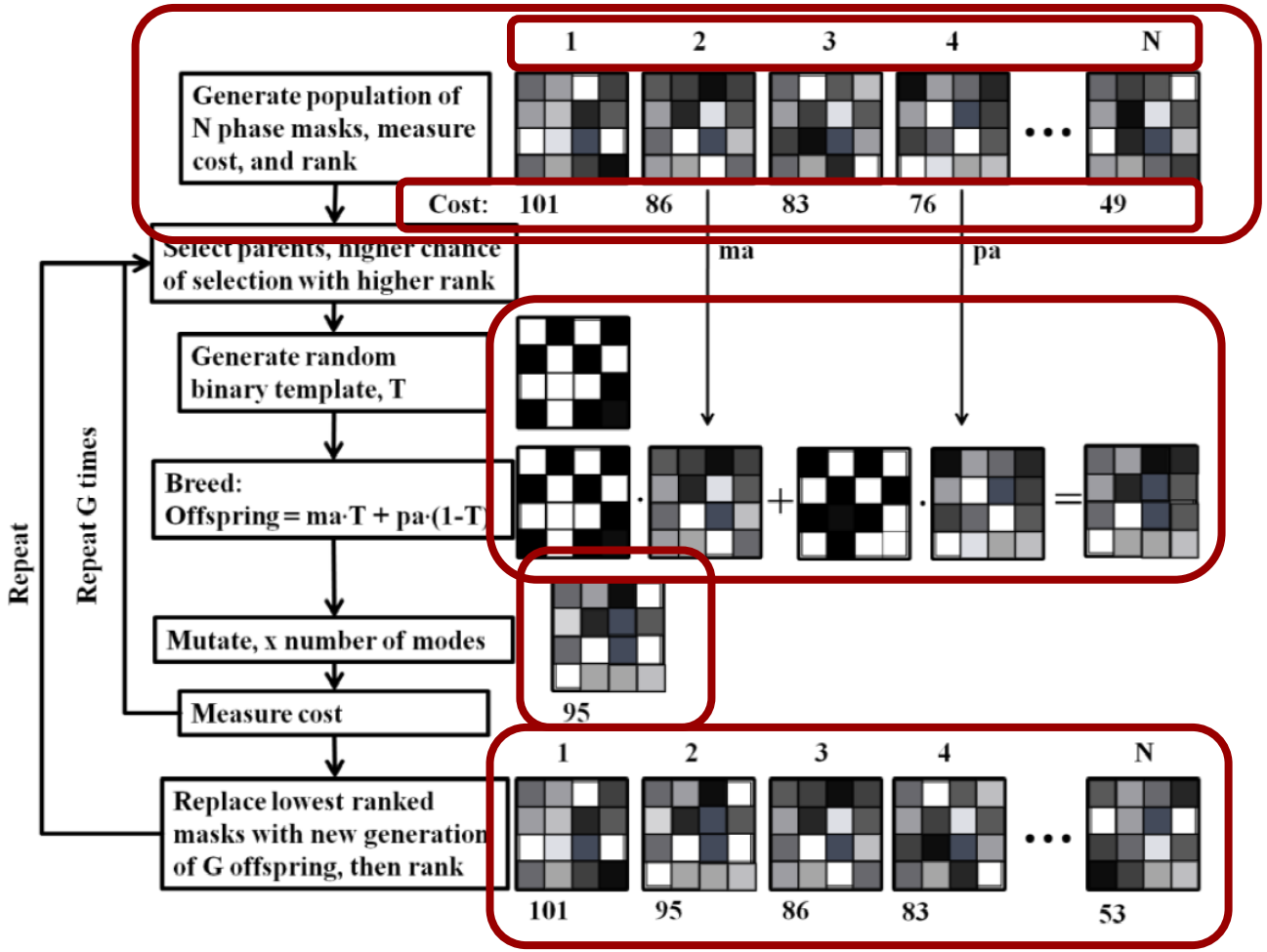
Vellekoop, I. M. & Mosk, A. P. Phase control algorithms for focusing light through turbid media. Opt. Commun. 281, 3071–3080 (2008).

2. ii. Wavefront correction algorithms

Advanced Algorithm: Genetic Algorithm (GA)

Based on species Breeding and Mutation to determine the best correction pattern.

- Generation of Random correction Pattern
- Ranking of the patterns
- Breeding of best ranked patterns
- Add Mutation
- Replace lowest ranked with new offspring



Conkey, D. B., Brown, A. N., Caravaca-Aguirre, A. M. & Piestun, R. Genetic algorithm optimization for focusing through turbid media in noisy environments. Opt. Express 20, 4840 (2012).

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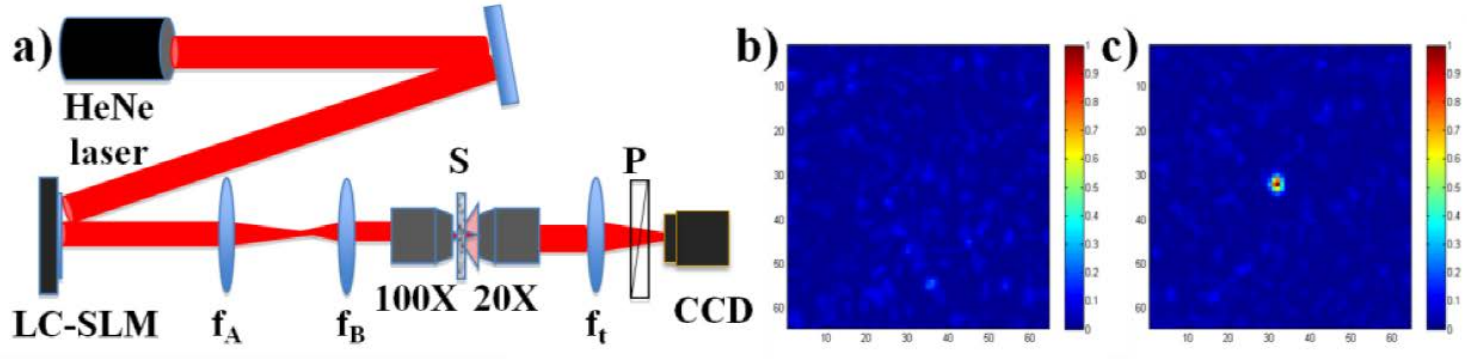


Algorithms are rated based on the enhancement vs time (or number of iteration)

The GA Algorithm performs better in noisy environments

*GA: Genetic Algorithm
**CSA: Continuous Sequential Algorithm
***TM: Transmission Matrix Focusing
****PA: Partitioning algorithm

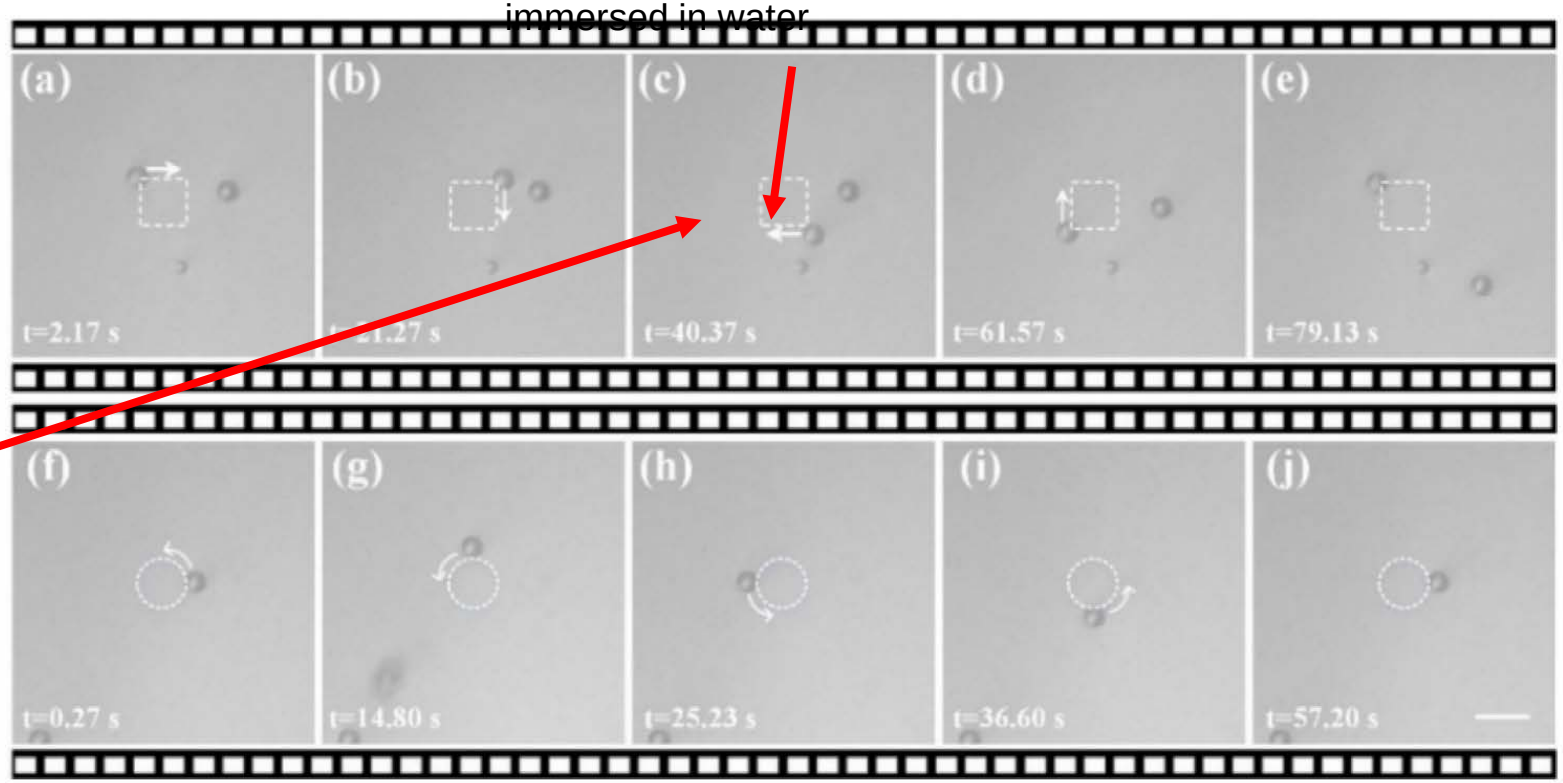
2. iii. Real-time implementation for optical trapping

Real-time optical manipulation through turbid media was achieved this year.

Peng et al. used an algorithm called Interleaved Segment Correction (ISC). It compromises between noise robustness and convergence speed.

A thin layer of milk was used as a random scattering medium.

Note that the trapped particles are still immersed in water.



Peng, T. et al. Real-time optical manipulation of particles through turbid media. Opt. Express 27, 4858–4866 (2019).

Scale bar: 10 μm

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3. **Future perspectives & Conclusions**

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3. Future Perspectives & Conclusions

- Optical Trapping is a powerful and versatile tool, especially for the study of biological systems
- Most of the optical trapping experiments are done in water (no scattering)
- Holographic optical tweezers and Specific Algorithms allowed for real-time control of structures through turbid media:
  - Enables new possibilities for the study of biological system in real environment
  - Limited in terms of manipulation speed at the moment.
  - No such structures manipulation **IN** turbid media as been demonstrated so far
- New Learning Algorithms (machine/deep Learning) might speed up the computations in the future.

Thank you

Acknowlegements

PhD Supervisors:

Rafael J. Taboryski
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PostDoc
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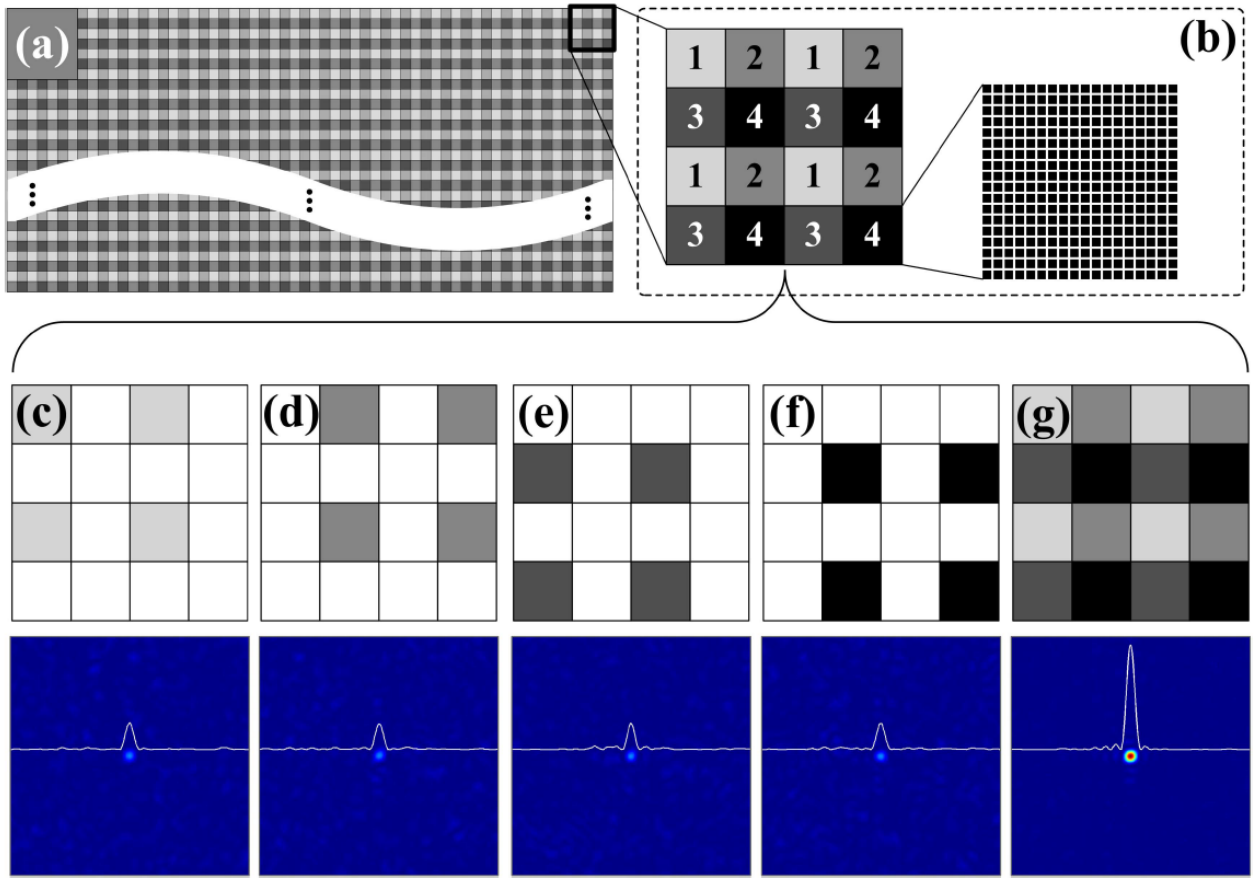
2. iii. Real-time implementation for optical trapping

Interleaved Segment Correction (ISC).



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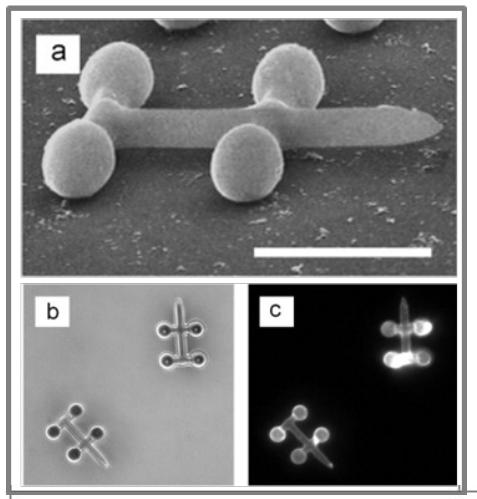

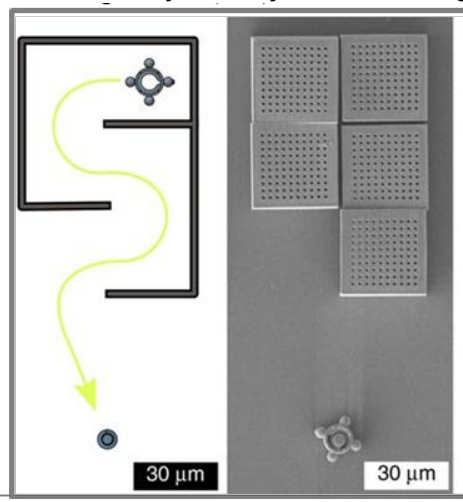

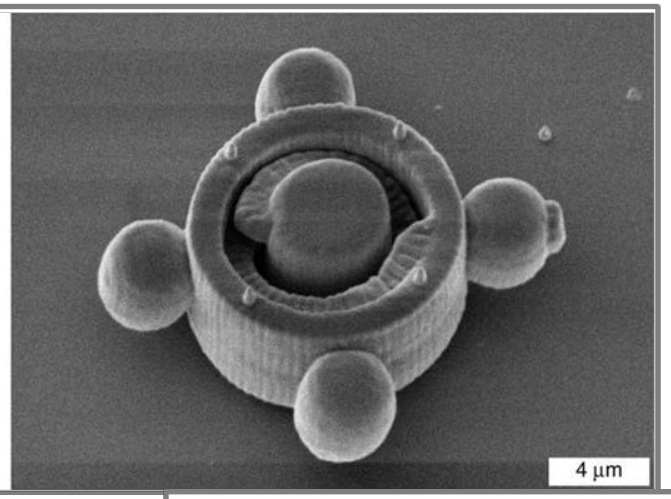

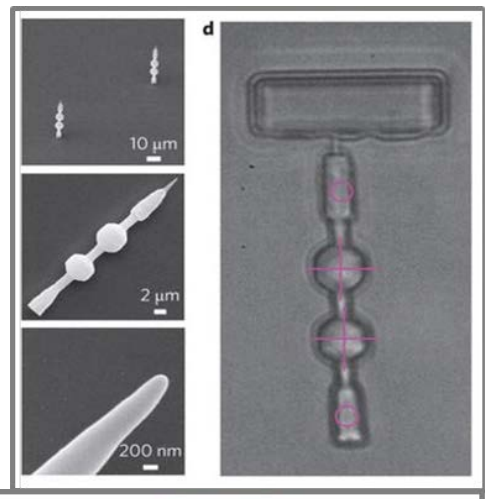

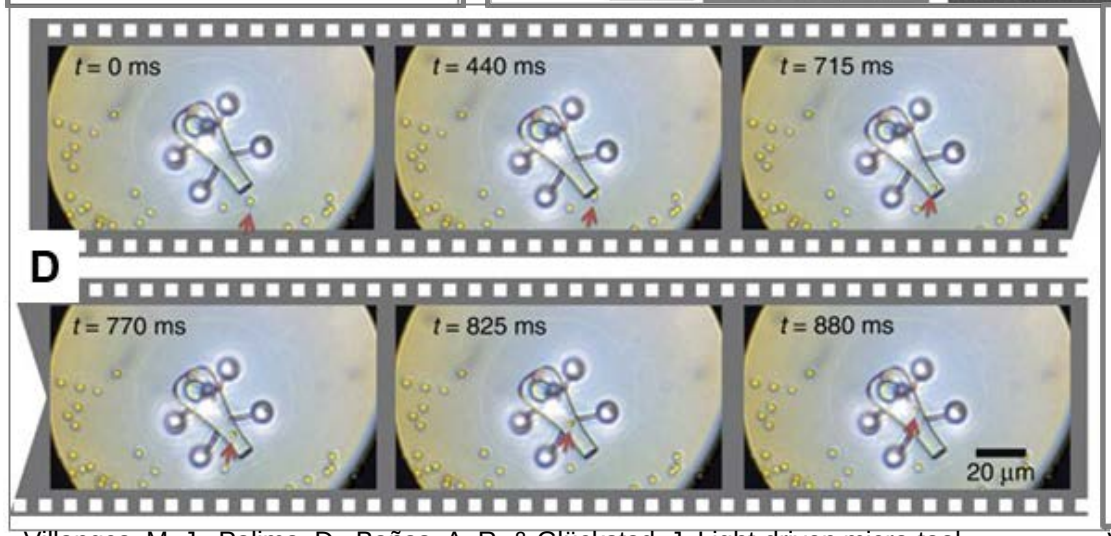

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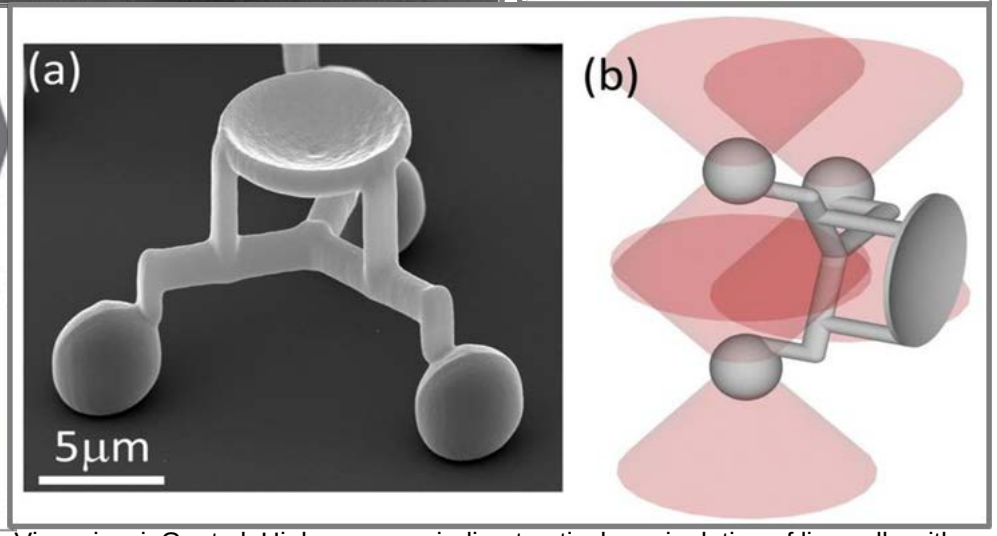


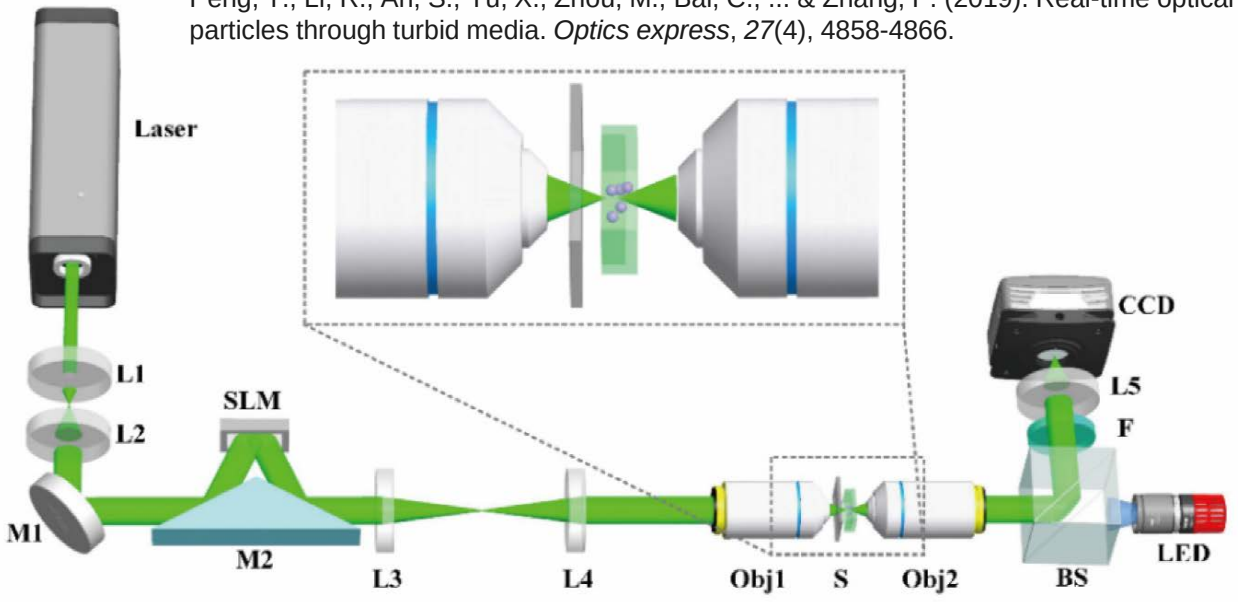
Vizsnyiczai, G. et al. High accuracy indirect optical manipulation of live cells with functionalized microtools. *Proc. SPIE* **9922**, 992216

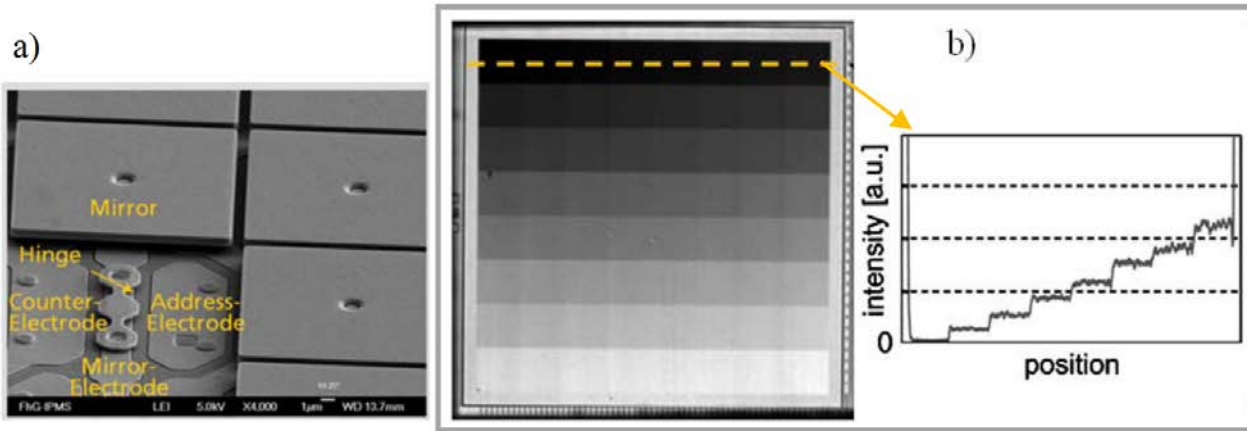
Bunea, A.-I. & Glückstad, J. Strategies for optical trapping in biological samples: Aiming at microrobotic surgeons. *Accepted for publication in Laser Photonics Rev.*

Holographic Optical Traps

- Using an SLM (spatial light modulator) :
  - Simultaneous multiple traps
  - arbitrary shape and size of traps
  - Much slower (response time ~10ms).
  - 3D manipulation of traps

Peng, T., Li, R., An, S., Yu, X., Zhou, M., Bai, C., ... & Zhang, P. (2019). Real-time optical manipulation of particles through turbid media. *Optics express*, *27*(4), 4858-4866.





Schenk, H. et al. Micro mirrors for high-speed laser deflection and patterning. Phys. Procedia 56, 7–18 (2014).

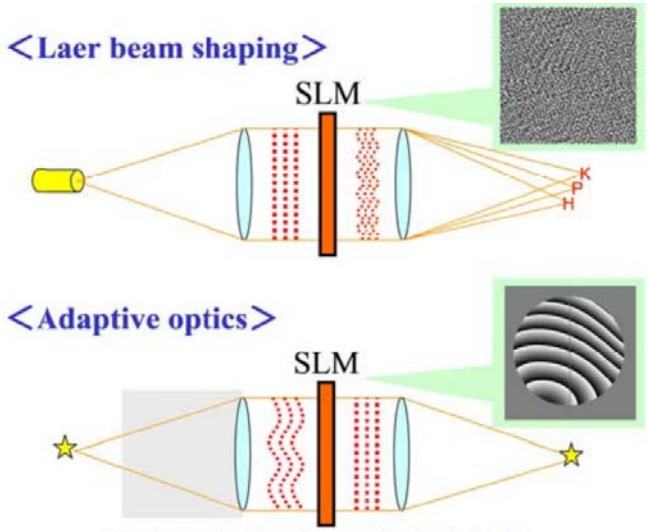


Fig.4 Basic functions of LCOS-SLM

Hara, T., Wu, M. H. & K, H. P. K. Advances in Application of Liquid Crystal on Silicon Spatial Light Modulator (LCOS-SLM) Haruyoshi Toyoda , Takashi Inoue , Naohisa Mukozaka *,. 559–562 (2014).

1. i. What is optical trapping at the microscale ?

Two components: 1) Scattering Force
2) Gradient Force

Ray Optics Regime (particle radius $a \gg \lambda$):

Direct force computation from reflection, refraction and absorption – net momentum change. If $n_p > n_m$, the net force is in the direction of the intensity gradient.

Raleigh Regime (particle radius $a \ll \lambda$):

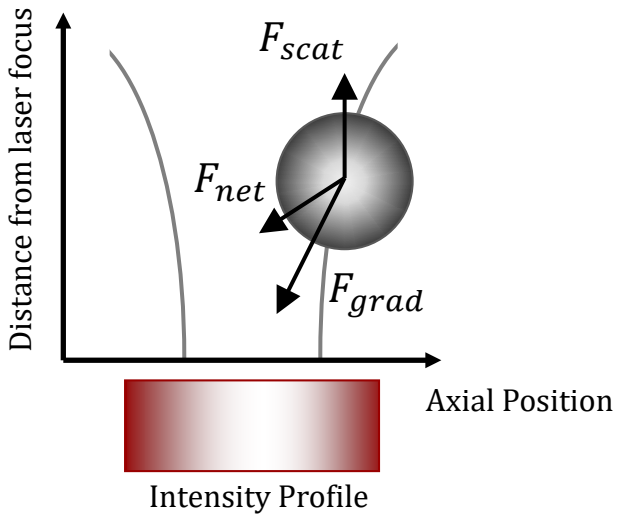
The particle can be considered as a point dipole.

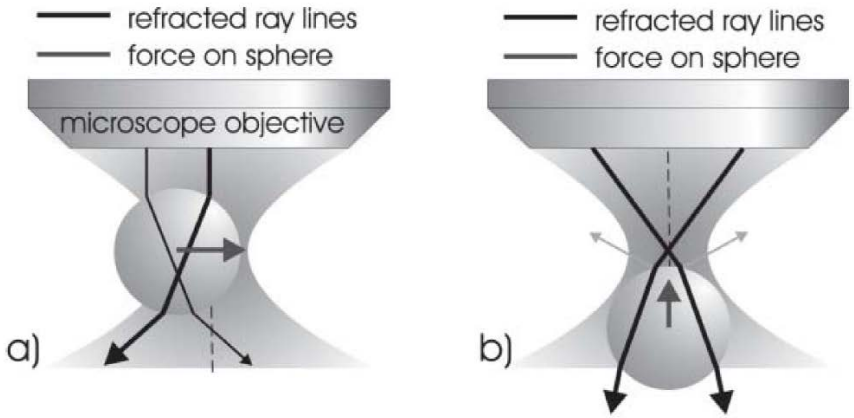
$$F_{scatt} = \frac{I_0 \sigma n_m}{c}$$

$$F_{grad} = \frac{2\pi\alpha}{c n_m^2} \nabla I_0$$

Intermediate Regime (Lorentz-Mie Regime) ($a \sim \lambda$):

More difficult to model. Most interesting applications fall under this regime.



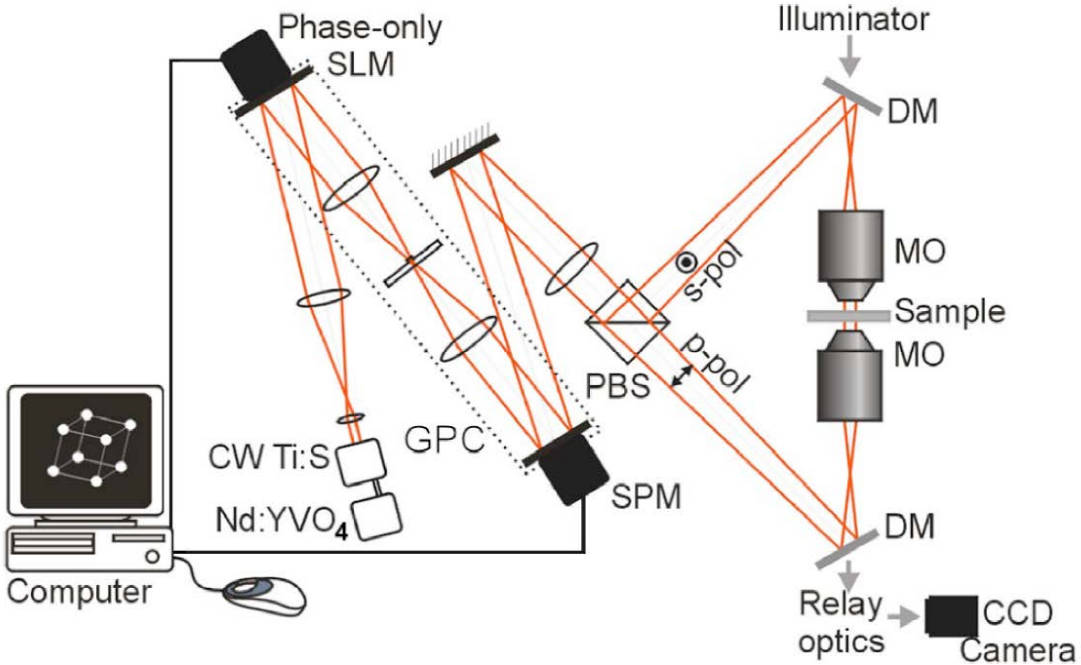


Dholakia, K., Reece, P. & Gu, M. Optical micromanipulation. Chem. Soc. Rev. 37, 42–55 (2008).

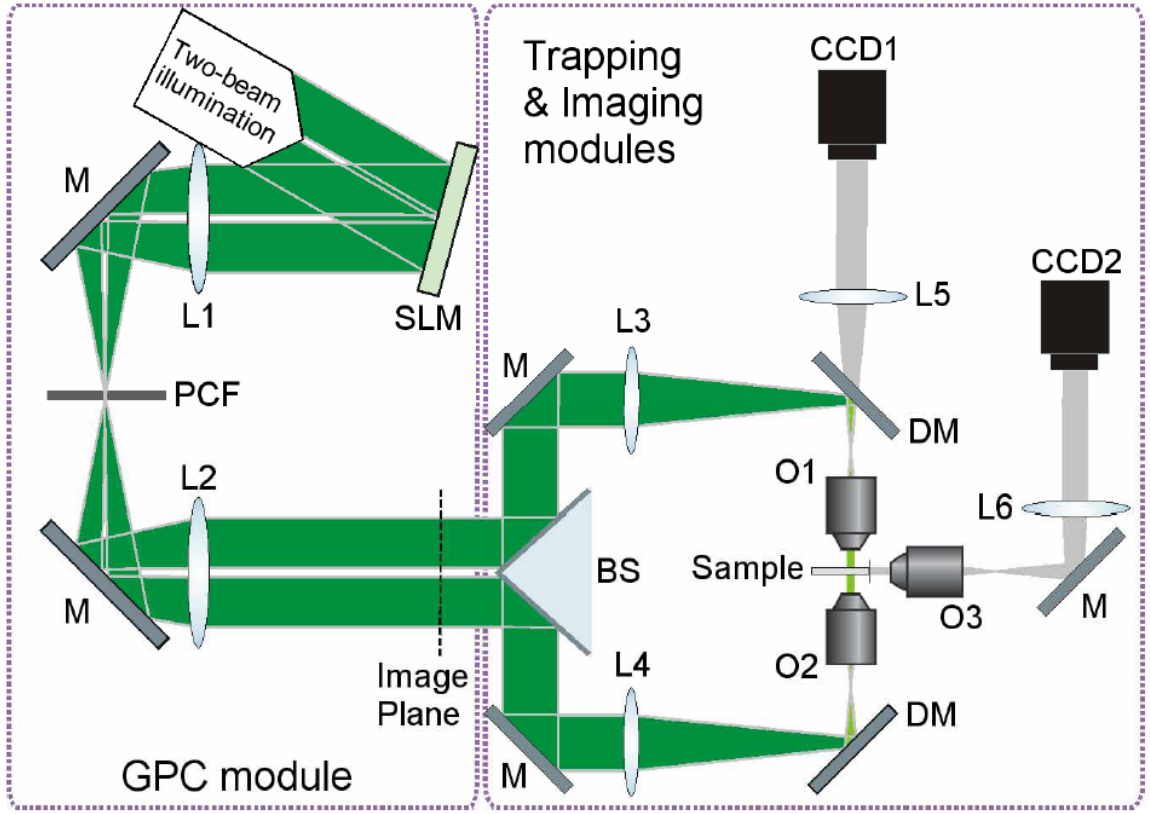
1. ii. What are the techniques employed ?

Steerable traps

GPC



Rodrigo, P. J., Daria, V. R. & Glückstad, J. Four-dimensional optical manipulation of colloidal particles. Appl. Phys. Lett. 86, 074103 (2005).



John Rodrigo, P., Perch-Nielsen, I. R., Amadeo Alonzo, C. & Glückstad, J. GPC-based optical micromanipulation in 3D real-time using a single spatial light modulator. Opt. Express 14, 13107–13112 (2006).

1. ii. What are the techniques employed ?

Scanning technology comparison:

- Galvo
- MEMS
- EOD
- AOD

Table 1. Comparison of scanning technologies for near-infrared wavelength range [14,29].

Scanning technology	Galvanometer	AOD	EOD (KTN)
Max angular deflection speed in 10^3 rad/s	0.2	20	30
Max deflection angle in rad	~0.5	0.05	0.15
Max aperture in mm	11…30	5…25	0.5…1
Transmittance	> 95 %	~ 60-85 %	~ 90%
Response time in µs	200	~1.0	~1.0

Römer, G. R. B. E. & Bechtold, P. Electro-optic and acousto-optic laser beam scanners. Phys. Procedia 56, 29–39 (2014).

1. ii. What are the techniques employed ?

Scanning technology comparison:

- Galvo
- MEMS
- EOD
- AOD

Table 1. Properties* (typical values) and comparison of laser beam scanners for a near-infrared wavelength.

Scanning technology	Aperture [mm]	Max. deflection angle θ [rad] (Number of resolvable spots N)	Max. velocity $\dot{\theta}$ [10^3 rad/s] (Rate of resolvable spots $\dot{N}$ [10^6])	Accuracy $\Delta\theta$ [μrad] (Accuracy per resolvable spot [%])	Response time τ [μs]	Efficiency or Transparency
Galvo scanner	7 ... 30	0.5 ... 1 (3000 ... 18000)	~ 0.1 (0.5 ... 1)	< 2 (< 5)	~ 10^3	> 95%
Polygon scanner	2 ... 12	0.6 ... 1 (2000 ... 7000)	1 ... 10 (5 ... 40)	~ 200 (~ 120)	$\gg 10^3$	> 90%
Piezo scanner	10 ... 25	0.01 ... 0.1 (100 ... 1000)	0.01 ... 0.1 (0.1 ... 1)	~ 1 (~ 1)	~ 10^3	> 95%
MEMS scanner – static	1 ... 2.5	~ 0.5 (200 ... 1000)	0.1 ... 1 (0.1 ... 1)	n.a.	~ 10^3	> 90%
MEMS scanner – resonant	~ 1	0.5 ... 1 (500 ... 1000)	10 ... 30 (5 ... 20)	n.a.	$\gg 10^3$	> 90%
EOD (Pockels effect)	2	~ 0.001 (2)	2 ... 20 (3 ... 30)	~ 1 (~ 0.2)	0.04 ... 1	> 85%
EOD (Kerr effect, KTN)	0.5	~ 0.2 (50)	~ 40 (15)	n.a.	~ 10	> 90%
AOD	1 ... 10	0.01 ... 0.05 (10 ... 500)	5 ... 250 (20 ... 80)	$\ll$ 0.1 ($\ll$0.1)	0.5 ... 15	60% ... 80%

*Data according to official information by Cambridge Technology Inc., Scanlab AG, Raylase AG, Piezosystem Jena GmbH, Physik Instrumente (PI) GmbH & Co. KG, Fraunhofer-Institut IPMS, Lemoptix SA, Adriatic Research Institute, Hamamatsu Photonics K.K., AA Opto-Electronic Company, Gooch & Housego, Brimrose Corp., Isomet Corp., IntraAction Corp., NTT Advanced Technology Corp. and Conoptics Inc.

Römer, G. R. B. E. & Bechtold, P. Electro-optic and acousto-optic laser beam scanners. Phys. Procedia 56, 29–39 (2014).

1. ii. What are the techniques employed ?

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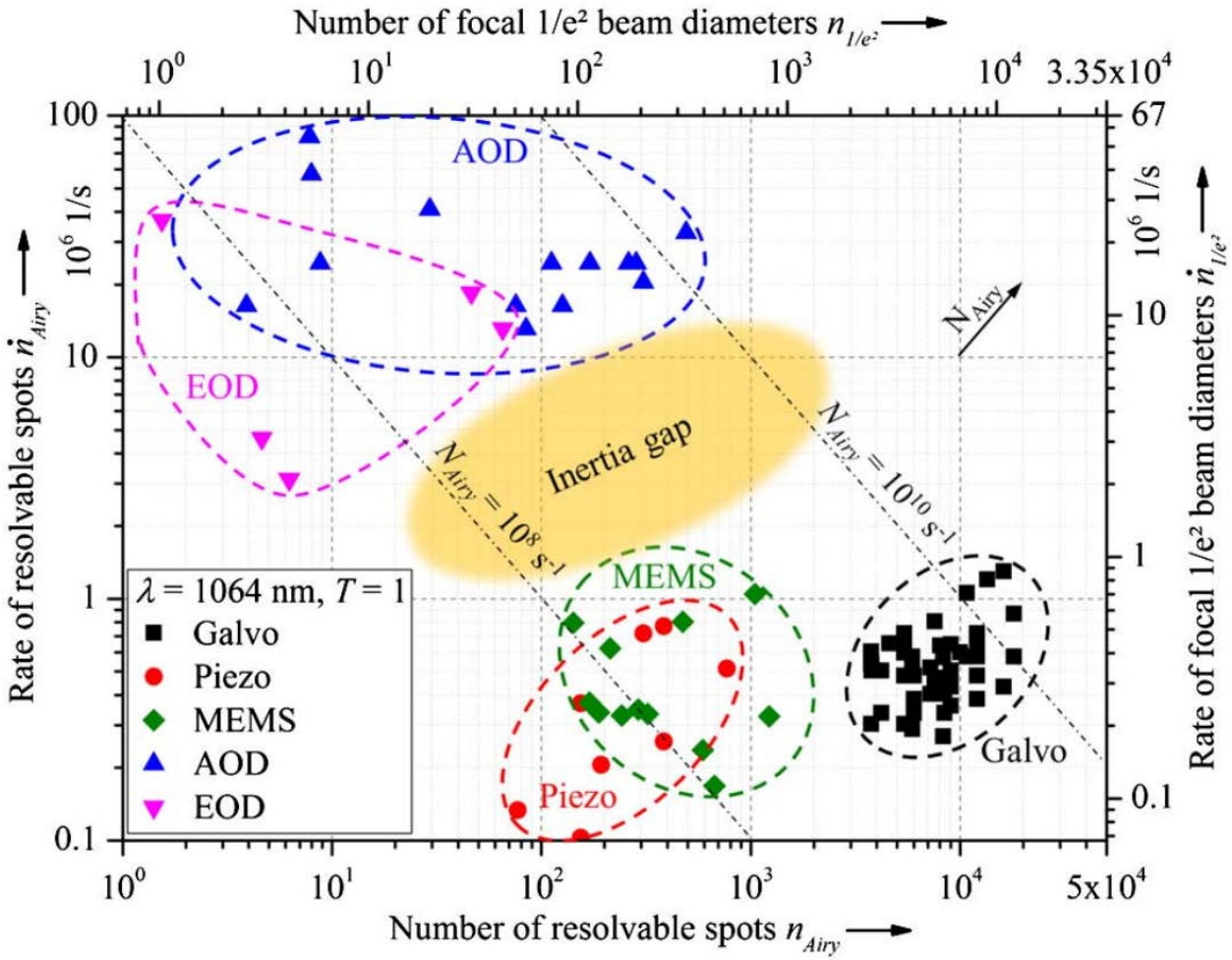


Fig. 2. Number and rate of resolvable spots and focal $1/e^2$ beam diameters for $\lambda = 1064$ nm and $T = 1$.

Römer, G. R. B. E. & Bechtold, P. Electro-optic and acousto-optic laser beam scanners. Phys. Procedia 56, 29–39 (2014).

Displacement Calibration Techniques:

1) Video-based position sensing:

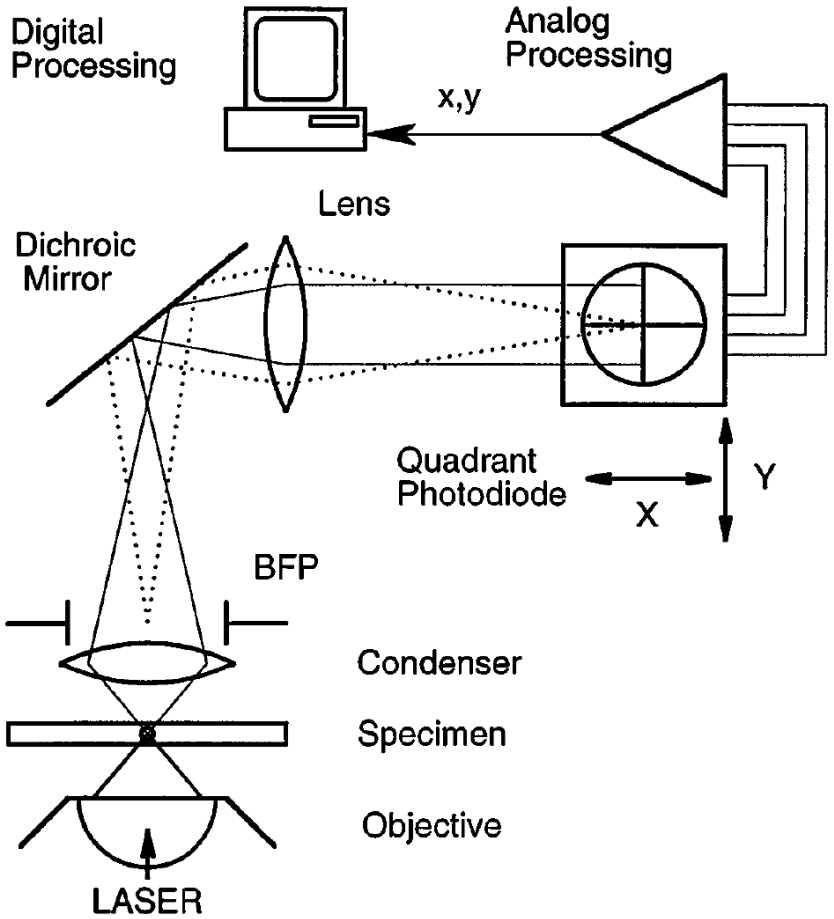
Imaging of the sample plane using a CCD camera. Algorithms for the particle centroid tracking in order to obtain subpixel accuracy.

2) QPD/CCD-base position sensing (forward scattering):

The trapped particle will scatter part of the light. We image the back focal plane of the condenser lens on the QPD/CCD where the light scattered from the particle interferes with the input beam

Two methods: Fixed particle and piezoelectric stage, or displacement of steerable trap with a know distance from particle.

Fixed particle on a coverslip in sample plane. Recording of the QPD/Camera signal for multiple steps of the piezoelectric stage.



Frederick Gittes and Christoph F. Schmidt, "Interference model for back-focal-plane displacement detection in optical tweezers," Opt. Lett. 23, 7-9 (1998)

Displacement Calibration Techniques:

2) QPD/CCD-base position sensing (forward scattering):

The signal on the QPD/Camera is given in the following manner:

$$QPD_x = \iint_{x>0} I(x,y) - \iint_{x<0} I(x,y)$$

$$QPD_y = \iint_{y>0} I(x,y) - \iint_{y<0} I(x,y)$$

$$QPD_z = \iint I(x,y)$$

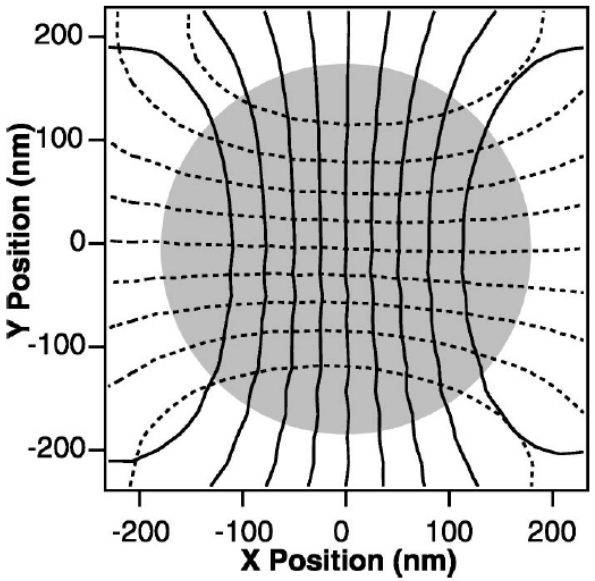


FIG. 6. Lateral two-dimensional detector calibration (adapted from Ref. 59).

Neuman, K. C., & Block, S. M. (2004). Optical trapping. *Review of scientific instruments*, *75*(9), 2787-2809.

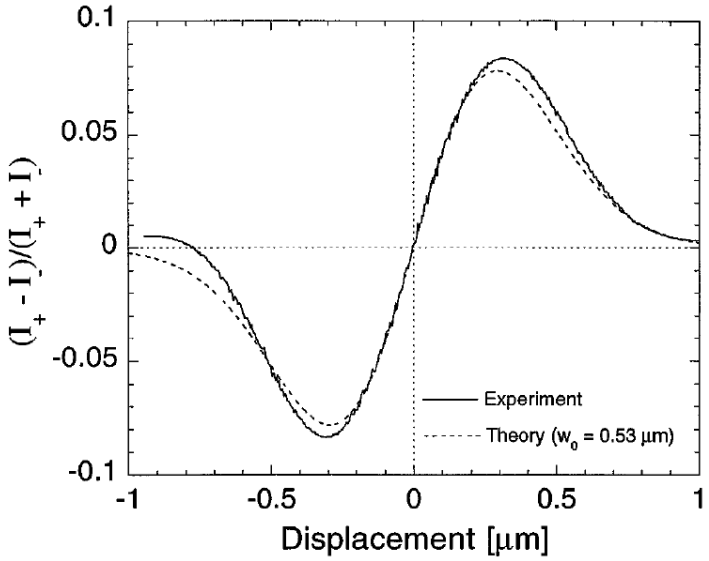


Fig. 3. Experimental calibration data compared with model response. Solid curve, a silica bead fixed upon a cover slip was moved by a piezoelectric stage through the sensitive range of the detector. Dashed curve, expression (7) with no adjustable parameters besides the nominal sphere size (0.5 μm) and a focus size ($w_0 \approx 0.53$ μm) estimated from the input beam width. The function $G(x/w_0)$ in expression (7) has a slope of $w_0{}^{-1}$ at $x = 0$ and extrema $G(x/w_0) = \pm 0.334$ at $x = \pm 0.552$ w_0.

Frederick Gittes and Christoph F. Schmidt, "Interference model for back-focal-plane displacement detection in optical tweezers," Opt. Lett. 23, 7-9 (1998)

Displacement Calibration Techniques:

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The signal on the QPD/Camera is given in the following manner:

$$QPD_x = \iint_{x>0} I(x,y) - \iint_{x<0} I(x,y)$$

$$QPD_y = \iint_{y>0} I(x,y) - \iint_{y<0} I(x,y)$$

$$QPD_z = \iint I(x,y)$$

Note that the integrals above should be replaced by finite sums in the case we use a CCD camera.

With this technique, we can also measure the axial position of the particles using a similar calibration.

K. C. Neuman and S. M. Block

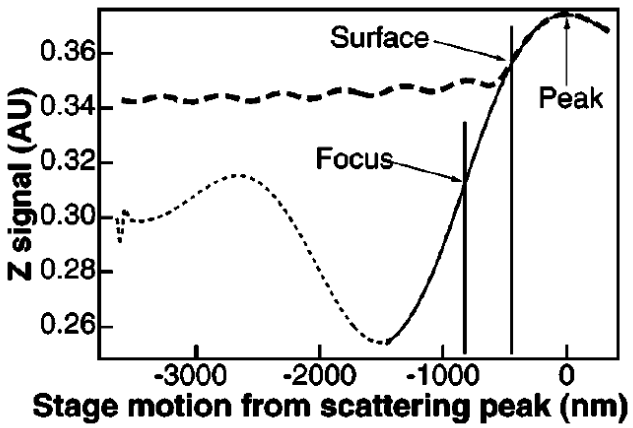

FIG. 8. Axial position signals for a free (heavy dashed line) and stuck (light dashed line) bead as the stage was scanned in the axial direction. All stage motion is relative to the scattering peak, which is indicated on the right of the figure. The positions of the surface (measured) and the focus [calculated from Eq. (5)] are indicated by vertical lines. The axial detection fit [Eq. (5)] to the stuck bead trace is shown in the region around the focus as a heavy solid line.

Neuman, K. C., & Block, S. M. (2004). Optical trapping. *Review of scientific instruments*, *75*(9), 2787-2809.

Trap Stiffness calibration:

1) Active stiffness calibration based on viscous drag force method

We can either:

- Induce known viscous forces on the particle (not fixed) using a piezoelectric stage.
- Force the particle into an oscillatory motion with a steerable trap.

In the first case, the viscous drag experienced by a spherical particle in a moving viscous medium is:

$$F_{drag} = \beta \cdot \upsilon = \frac{6\pi\eta\upsilon r}{1 - \frac{9}{16}\left(\frac{r}{h}\right) + \frac{1}{8}\left(\frac{r}{h}\right)^3 - \frac{45}{256}\left(\frac{r}{h}\right)^4 - \frac{1}{16}\left(\frac{r}{h}\right)^5}$$

Where β is the drag coefficient, r is the bead radius, υ is the fluid velocity and h is the height of the bead from the coverslip. We can then record the particle position and extract the force for small displacement using a linear fit:

$$\mathrm{F} = -k \cdot x$$

Where k is the trap stiffness. Once the force sensing is calibrated, we can measure the drag coefficient for different solutions.

Sarshar, M., Wong, W., & Anvari, B. (2014). Comparative study of methods to calibrate the stiffness of a single-beam gradient-force optical tweezers over various laser trapping powers. *Journal of biomedical optics*, *19*(11), 115001.

Trap Stiffness calibration:

1) Active stiffness calibration based on viscous drag force method

In the second case, we can force the particle into an oscillatory motion using a steerable trap. We get the following equation of motion

$$m^*\ddot{x} + m^*\beta\dot{x} + (k_{ot} + k)x = k_{ot}A\cos(\omega t)$$

Where m^* is the effective mass of the particle (includes also the inertia of the liquid around the particle), β is the drag coefficient, k_{ot} is the trap stiffness, k is the elastic modulus of the solution, A is the amplitude of the tweezers motion and ω the oscillation frequency.

Knowing β, we can obtain k_{ot} from the phase shift of the particle's motion. Then, once the trap stiffness known, we can measure different solution viscosities.

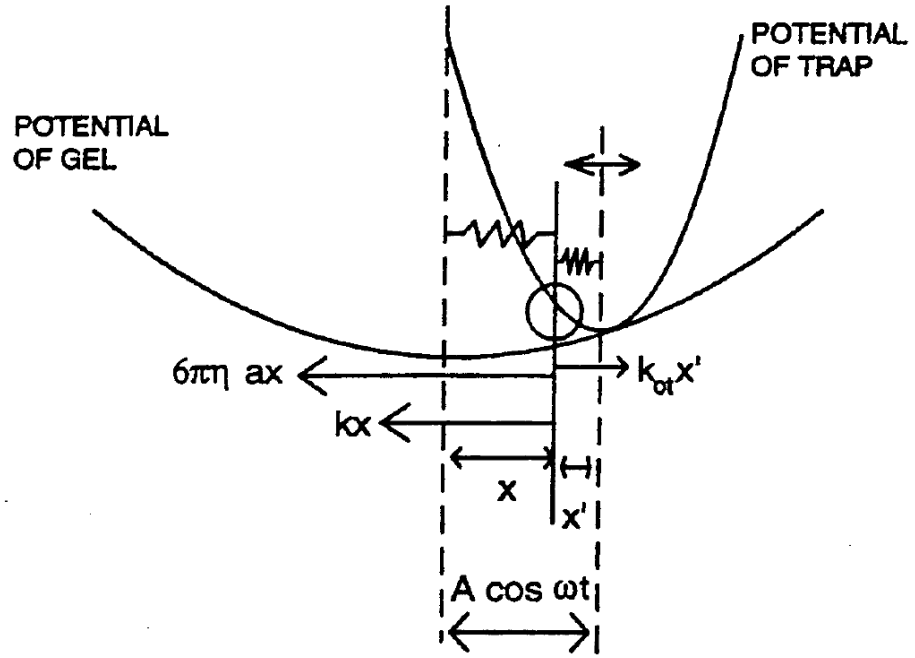

FIGURE 15.2 Forces on a single particle set in forced oscillation in a viscoelastic medium.

Ou-Yang, H. D. (1999). Design and applications of oscillating optical tweezers for direct measurements of colloidal forces. *Colloid-Polymer Interactions: From Fundamentals to Practice*, 385-405.

Trap Stiffness calibration:

2) Passive stiffness, power spectral density method

Brownian motion of particle in an optical trap can be described by the Langevin equation. For fluids with low Reynolds numbers, the power spectrum of the Brownian motion is:

$$S(f) = \frac{K_B T}{\pi^2 \beta (f^2 + f_0^2)}$$

We can measure f_0 the roll-off frequency and determine the trap stiffness using:

$$k = 2\pi\beta f_0$$

Where β is the hydrodynamic drag coefficient (must be known beforehand).

This method requires that we can acquire data at frequencies between 10 and 50 kHz in order to determine f_0. Therefore it can be achieved using a QPD but not usually with a CCD.

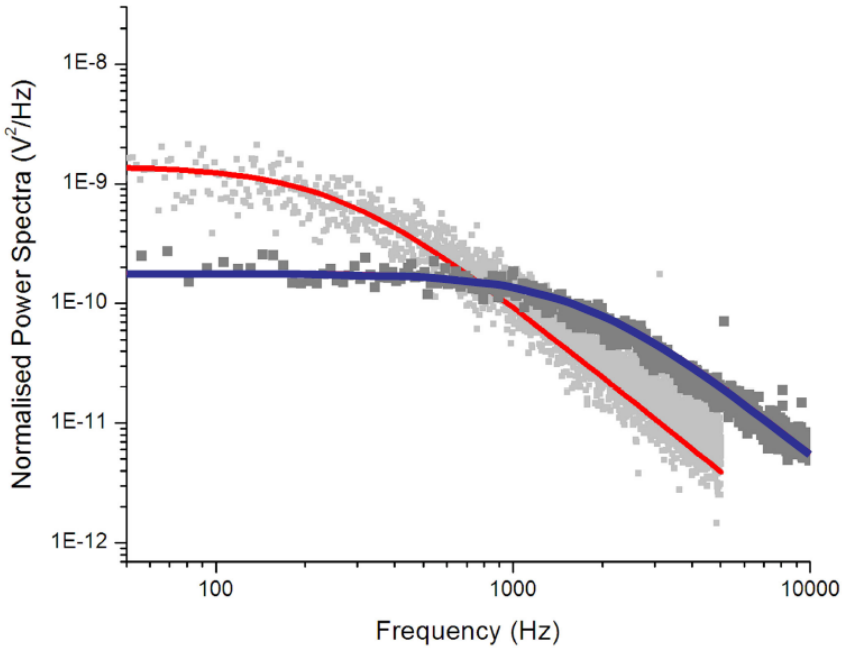

Fig. 13. The power spectrum of a trapped 100 nm gold (blue) and 100 nm polysterene (red) sphere can be fitted wit a Lorentzian curve. The curve fit provides the roll off frequency f_0 for each particle in at a given power in a specific optical trap. The roll off frequency determines the trap stiffness κ.

Dienerowitz, M., Mazilu, M., & Dholakia, K. (2008). Optical manipulation of nanoparticles: a review. *Journal of Nanophotonics*, *2*(1), 021875.

Trap Stiffness calibration:

3) Passive stiffness, equipartition theorem method

The equipartition theorem assumes $0.5K_BT$ of thermal energy for each degree of freedom. The energy associated with thermal fluctuations of a particle in an optical trap with stiffness k_x in the x direction is given by $0.5k_x\langle x^2\rangle^{-1}$, where $\langle x^2\rangle$ is the position variance of the particle in the x direction.

The trap stiffness can then be determined using:

$$k_x = K_BT\langle x^2\rangle^{-1}$$

Using this method, we don't need know the drag coefficient nor the viscosity of the medium beforehand.

Sarshar, M., Wong, W., & Anvari, B. (2014). Comparative study of methods to calibrate the stiffness of a single-beam gradient-force optical tweezers over various laser trapping powers. *Journal of biomedical optics*, *19*(11), 115001.

Trap Stiffness calibration:

4) Passive stiffness, Boltzmann statistics method

The particle position histogram is assumed to have a normal distribution resulting from a Gaussian trapping beam. Boltzmann statistics describe the probability density $p(x)$ of the particle position as a function of the optical trap potential $E(x)$:

$$p(x)dx = Ce^{\frac{E(x)}{K_B T}}$$

Where C is a normalization factor.

We can calculate then the potential $E(x)$ from the histogram and fit it with a quadratic equation to determine the trap stiffness k_x:

$$E(x) = \left(\frac{k_x}{2}\right)x^2 + c$$

This method does not require knowing the particle's shape, drag coefficient or medium viscosity.

Sarshar, M., Wong, W., & Anvari, B. (2014). Comparative study of methods to calibrate the stiffness of a single-beam gradient-force optical tweezers over various laser trapping powers. *Journal of biomedical optics*, *19*(11), 115001.