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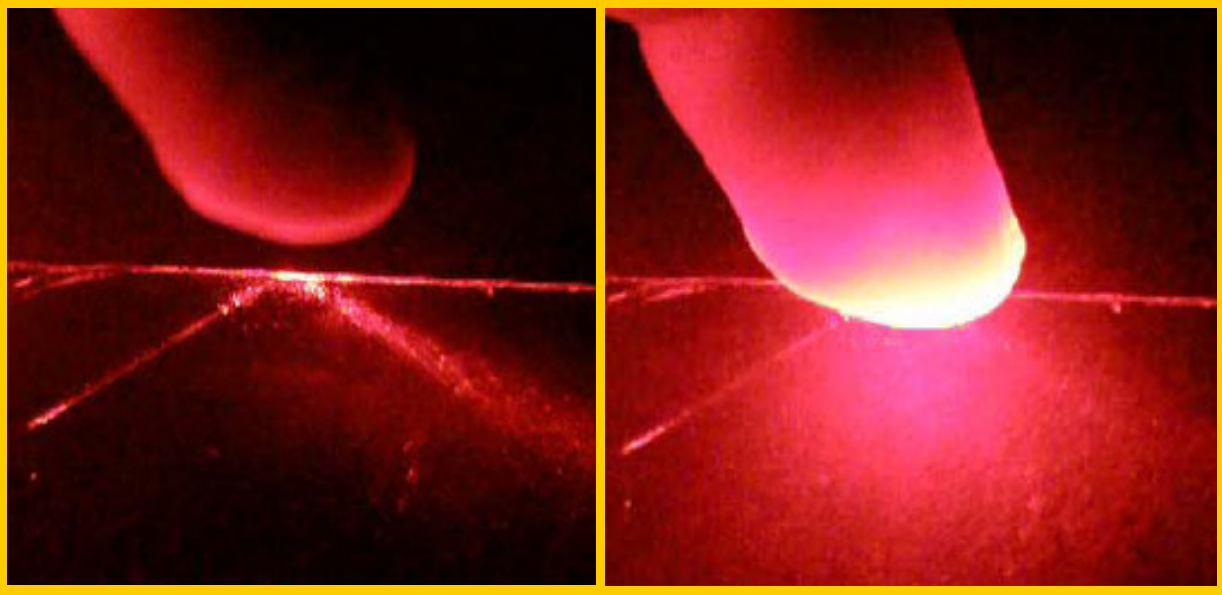

Bio-optofluidics and biophotonics at the cellular level

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Abstract *We present ongoing research and development activities for constructing a compact next generation BioPhotonics Workstation and a Bio-optofluidic Cell Sorter (cell-BOCS) for all-optical micro-manipulation platforms utilizing low numerical aperture beam geometries. Unlike conventional high NA optical tweezers, the BioPhotonics workstation is e.g. capable of long range 3D manipulation. This enables a variety of biological studies such as manipulation of intricate microfabricated assemblies or for automated and parallel optofluidic cell sorting. To further reduce its overhead, we propose ways of making the BioPhotonics Workstation platform more photon efficient by studying the 3D distribution of the counter propagating beams and utilizing the Generalized Phase Contrast (GPC) method for illuminating the applied spatial light modulators. Moving ahead, we envision optimal designs for the manipulated microstructures through the use of advanced topology optimization.*

Introduction

The ability to manipulate microscopic structures in three dimensions using only light as "invisible hands" gives rise to new and until recently unachievable micro-biological applications [1, 2]. Microscopic scaffoldings and tools [3] that can be reassembled with multiple traps can simulate biological microenvironments [4] and can potentially pave the way for advanced research in stem cell development and cancerous tissue on the cellular level. Using contemporary micro-fabrication processes provided by two-photon femtosecond photopolymerization acting as 3D scanning microprinters, specially designed microscopic tools can be fabricated and subsequently driven around biological samples for probing or sending stimulus. Similar to freely movable hand tools, 3D controllable micro-tools can be used to trigger biological, chemical or mechanical reactions in a localized and controlled manner.

For many years, manipulation of microscopic particles has been carried out using laser traps created from a single strongly focused beam using a high numerical aperture objective. Such single-beam traps rely on light intensity gradients and are usually referred to as optical tweezers. The advent of computer addressable spatial light modulators has enabled means of creating a plurality of reconfigurable traps capable of translating simple bead like structures and rotating structures with multiple optical handles. Since standard optical tweezers are based on traps that rely on high intensity regions and high numerical aperture objective lenses, the range of motion in the axial direction is inherently very limited. We overcome this limitation using a counter-propagating beam geometry on our BioPhotonics Workstation [5].

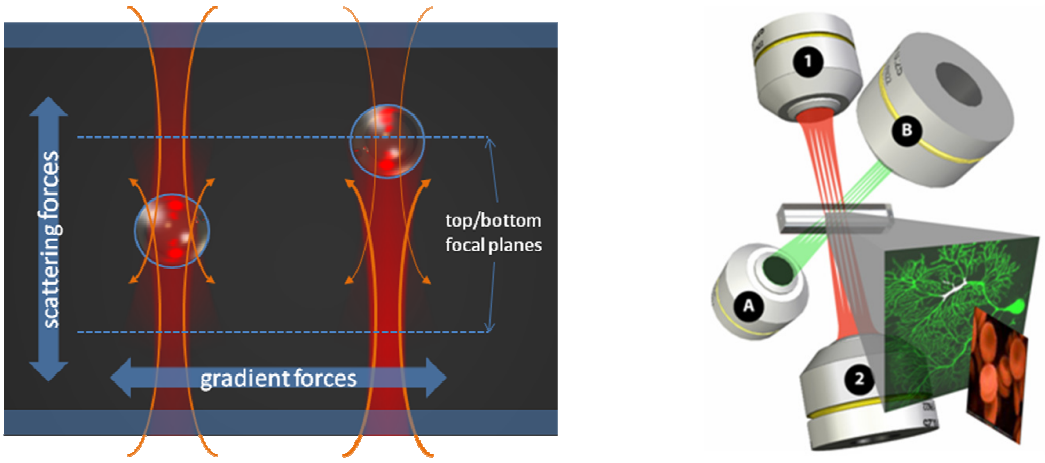

Fig. 1. Counter-propagating traps move objects axially by balancing the scattering forces from the top and bottom beams. Lateral manipulation is achieved through gradient forces. These traps (provided by objectives 1 and 2) provide a substantially extended axial trapping range compared to standard optical tweezers in addition to the possibility of using auxiliary optics (objectives A and B) along the side geometry for enhanced microscopic imaging modalities.

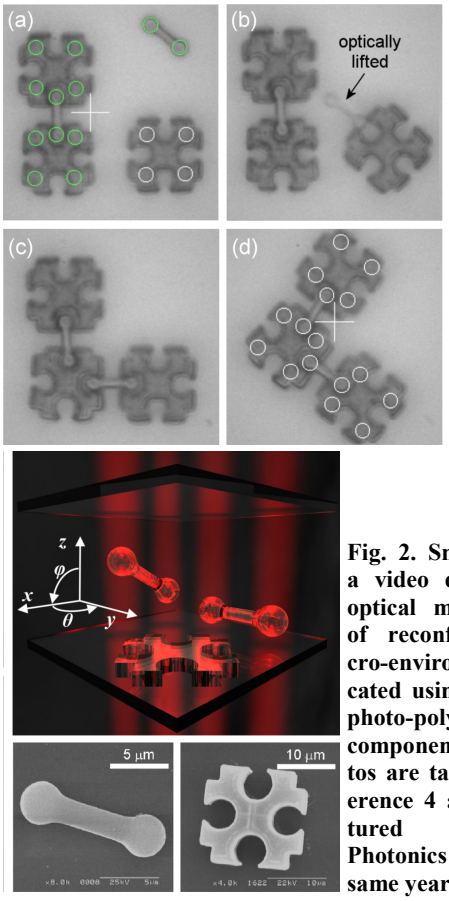

Fig. 2. Snapshots from a video demonstrating optical micro-assembly of reconfigurable micro-environments fabricated using two-photon photo-polymerized components. The photos are taken from reference 4 and were featured by Nature Photonics later the same year.

Multiple Counter-propagating beam trap

The extended axial manipulation range on the BioPhotonics Workstation is obtained by using a low NA counter-propagating beam geometry. Counter-propagating (CP) traps take advantage of the long working distance wherein the light intensity does not deviate as much compared to the high NA focusing. Trapped particles are translated axially by varying the relative intensities of the counter-propagating beams (Fig 1). This axial degree of freedom enables more interactive manipulation such as flipping of planar microstructures and lifting puzzle pieces for reconfigurable micro-environments. Therefore, with the concerted transverse and axial manipulation provided by gradient forces and scattering forces, respectively, 3D optical manipulation is achieved while overcoming the short range limitations of high NA optical tweezer based trapping.

Long range 3D optical manipulation enables a host of novel applications, especially in microscopic biological studies. For example, microscopic scaffoldings fabricated with two-photon polymerization (2PP) can be reassembled with multiple traps, simulating biological microenvironments that can influence the behavior of cells (Fig 2). With micro- or nano-fabrication processes such as 2PP or chemical micro-assembly, specially designed microscopic tools can be driven around biological samples for probing or sending stimulus. Similar to freely movable hand tools, 3D controllable micro-tools can be used to trigger biological, chemical or mechanical reactions in a localized and controlled manner.

Layout of the BioPhotonics Workstation

The schematics of the BioPhotonics Workstation is illustrated in Fig. 3. Its configuration and optical modules has been dealt with extensively so we outline only the pertinent features here. Two independently addressable regions of a spatial beam modulator are optically mapped and relayed as a plurality of reconfigurable counter-propagating beams in the sample.

The scaling between the spatially light modulating pixels and the sample plane is determined by the focal lengths of the 4f imaging lenses. The user can independently control the number, size, shape, intensity and spatial position of each CP-beamlet through a LabVIEW interface or a Microsoft .NET application supporting multiple joystick input. Each CP-beamlet can independently trap and manipulate a microscopic object in parallel with the other trapping CP-beamlets. Computational overhead is minimized because the

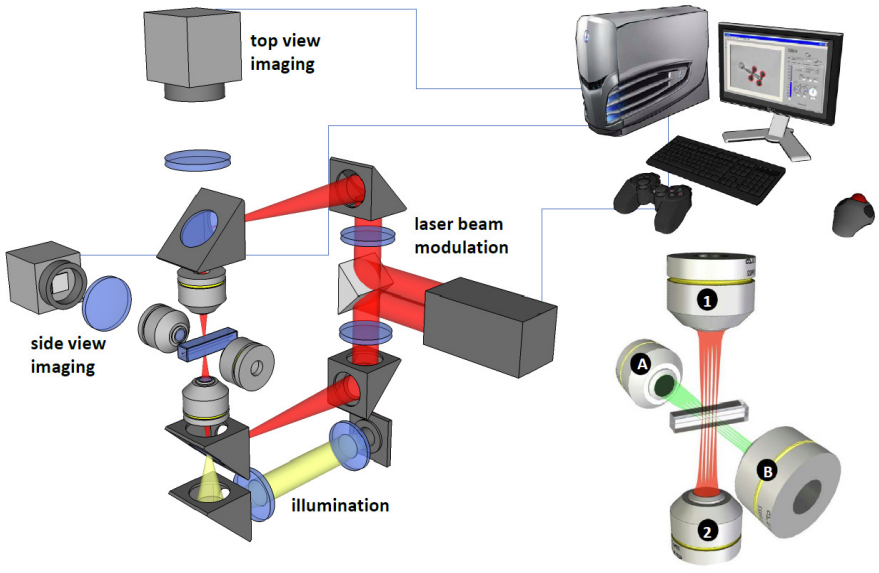

Fig. 3. Schematics of the BioPhotonics workstation. The long working distance allows an extra microscope to be mounted perpendicularly for side view or for an independent optical setup. The laser source is modulated and shaped by a spatial light modulator; the upper and lower parts of the beam are separated and projected into the sample from opposite directions. A periscopic design, with two mirrors in each arm, simplifies the beam alignment.

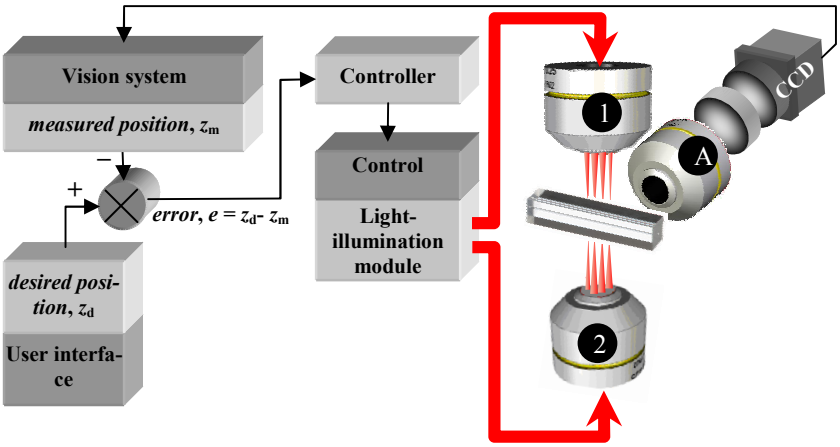

Fig. 4. Active trap stabilization on the BioPhotonics Workstation using vision feedback [8]. An array of actively regulated traps are relayed through well-separated objectives (1 and 2) that provides ample space for side-view microscopy (objective lens A, zoom lens and CCD camera). Computer vision provides real-time position feedback for regulating the traps.

imaging geometry allows intensity patterns defining the optical traps to be directly mapped into an addressable light shaping module. Hence, these counter-propagating beam traps are reconfigurable in real-time. Another important advantage of using low NA objectives is the increased workspace that gives more freedom on the sample containers or other contraptions for performing other biological studies. For example, advanced micro-spectroscopic or multi-photon characterization methods [6] can be implemented independently along the side-geometry. Cells can be subjected to stress and then characterized using different health indicators while trapped using a laser whose effects may already be known, or could be the subject being studied [7].

Machine-vision-based position feedback

Recently we demonstrated dynamic axial stabilization of counter-propagating beam traps by tracking the trajectory of particles through the side view imaging system [8]. Additional stabilization is necessary as we axially stretch the trapping region because regions with less intense light are less stable in holding particles in place. Computer vision tracks axial positions of multiple particles for use in a feedback algorithm that adjusts the respective counter-propagating beam pair intensities as needed (Fig 4). This allows particles to be moved-to or held at user-defined axial positions within the 250 μm height of a micro-fluidic channel (Hellma).

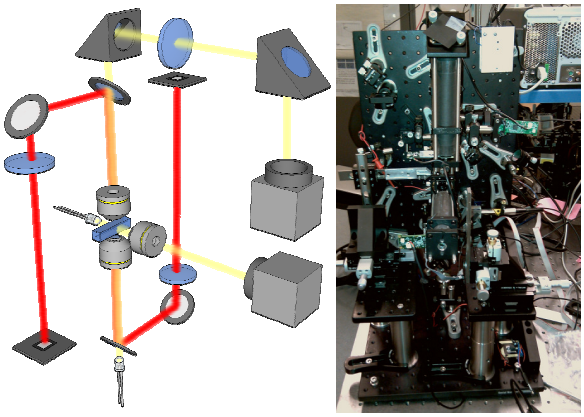

Fig. 5. A miniaturised and cost-effective BioPhotonics Workstation. By using compact opto-mechanical components, and tweaking the trapping geometry, a smaller table-top BioPhotonics Workstation has been implemented.

Miniaturized biophotonics work station

Up to this point, the BWS units in use are developed mostly with standard optical components and without requiring them to be compact or portable. The lack of such constraints allows the use of high-power optical traps, high-quality imaging and an easily extensible hardware platform. To meet different end-user demands, however, we may trade off some practical functionalities that are provided by a fully featured BWS. Developing a platform with a smaller footprint, for example, is a crucial step to make it more accessible for other intended users. This requires tweaking the BWS geometry and using more compact opto-mechanical components. Right now we are developing a miniature prototype BWS using standard and off-the-shelf components.

Bio-Optofluidic Cell Sorter (cell-BOCS)

The BioPhotonics workstation can be further trimmed down and become even more compact to adapt to specialized applications. Such is the case in our Bio-Optofluidic Cell Sorter (BOCS), wherein cells in a flow chamber only need to be pushed to a defined height after being detected. The same microscope objective used for catapulting cells is also used for imaging and machine vision. Cells or particles that are discriminated according to user-defined criteria are propelled to a height where the laminar flow has a different direction. Since only the upward beam is used, half of a full featured BWS is trimmed off and only half of the laser power is needed to get the same BWS responsiveness.

Increasing photon efficiency

In principle, opto-mechanical components can be made as small as desired using customized manufacturing techniques. The challenge in making compact optical systems lies with the laser sources since laser

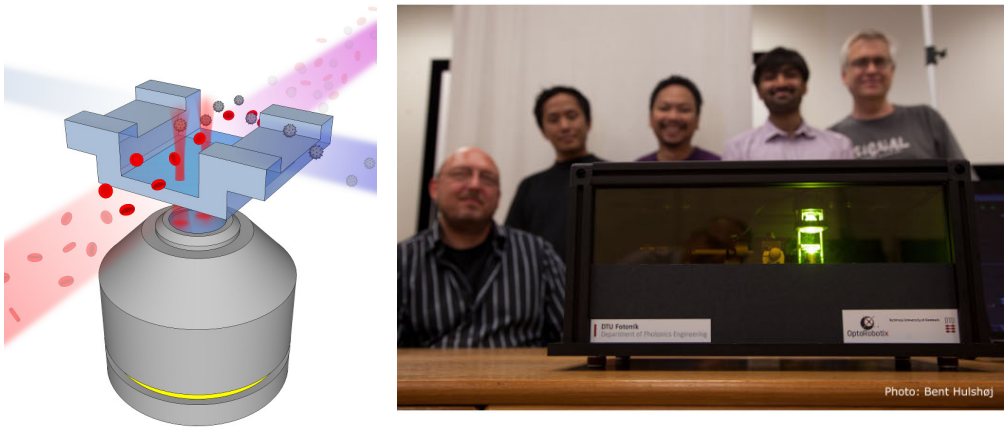

Fig. 6. The cell-BOCS sorting mechanism depicting two laminar streams displaced in height. The lower stream (purple/red) contains a mixture of cells. Detected cells are pushed up to the other stream. The table top cell-BOCS has a base that is roughly A3 in size (45cm x 30cm).

output power is correlated with its construction size. Hence, an important step for miniaturization is to efficiently use the trapping lasers, allowing smaller power lasers to be used. The conventional CP geometry requires the trapped particle to be away from the focal planes of the opposing objectives. Such a region however, has less intense light, and hence less trapping stiffness. One way of dealing with this without increasing the input laser power is to use a dynamic software feedback approach as mentioned in the previous section [8]. Another approach is to identify alternate regimes of stable trapping that exploit the higher intensities as the particle moves toward opposite image planes [9, 10]. Simulations also suggest that further stability can be done by modifying the 3D light distribution of the counter-propagating traps.

Gauss GPC: Getting more light in the first place

It is often ignored that pixel based devices such as SLMs and laser or light sources have dissimilar shapes, the former being rectangular and the latter being circular. Hence, either losses are introduced when the input laser is magnified to fill the whole SLM surface or the whole SLM is not utilized when the laser only illuminates a portion of it. Furthermore, laser sources typically have a Gaussian profile hence an SLM is not evenly illuminated. GPC can efficiently convert a Gaussian amplitude distribution into a rectangle with a more uniform intensity optimally illuminating an SLM [11-14]. Experimentally, we have demonstrated the conversion from a Gaussian to a rectangular distribution with 75% efficiency [15].

Topology optimization of optically manipulated microstructures

So far the 2PP microstructures that we had been manipulating with the BWS are based on intuitive designs that had not gone through extensive quantitative analysis [3,4]. Whereas spherical structures can be used for optical handles, and even focusing, many more dynamics can arise depending on the microstructure's geometry. Propellers or wing like structures inspired from fluid or aerodynamics had been demonstrated for example [16, 17]. The geometrical possibilities achiev-

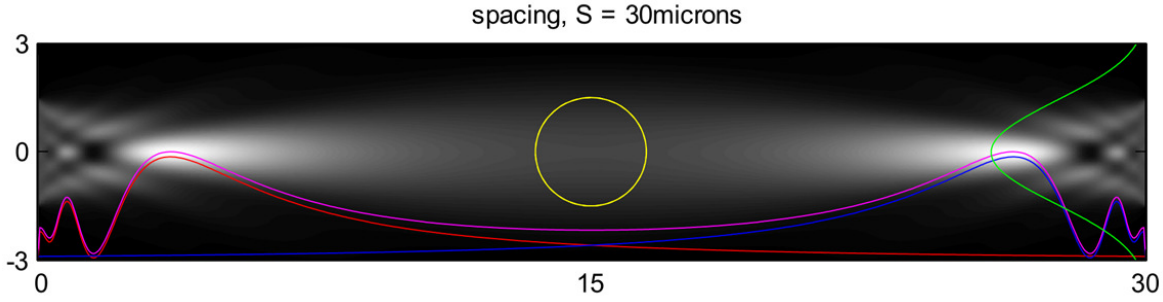

Fig. 7. The analysis of the counter-propagating light distribution and forces reveals alternate regions for stable optical trapping near the image planes where light is more intense [9]. (Top) 3D view of counter-propagating disc-shaped beams projected through opposite microscope objectives onto two image planes separated by distance, *S*, to create a stable optical trap between the image planes. An axial slice through the simulated volume intensity between two 3micron diameter light discs; overlays show the expected stable trapping position for a microsphere, together with plots of intensity linescans (red: axial intensity of right-directed beam; blue: axial intensity of the left-directed beam; magenta: total axial intensity; green: transverse intensity linescan halfway between the discs)

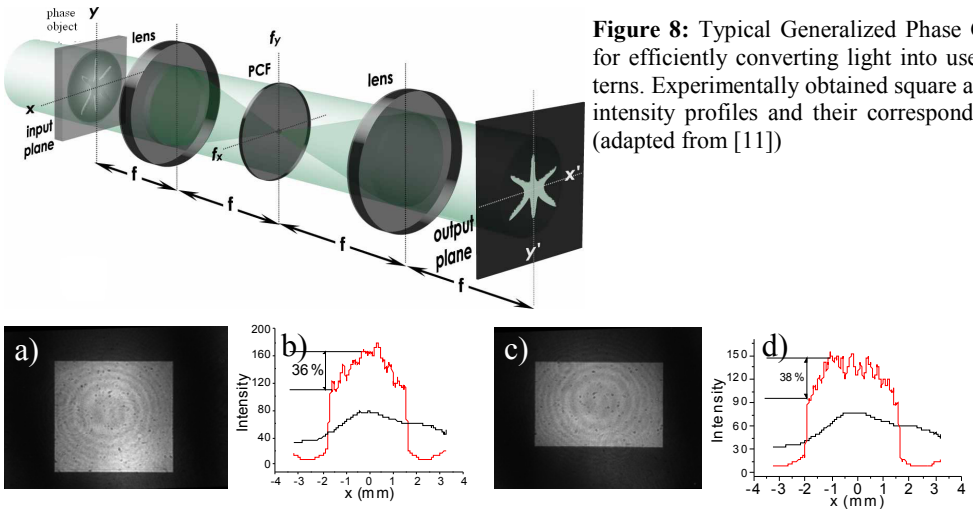

Figure 8: Typical Generalized Phase Contrast setup for efficiently converting light into user-defined patterns. Experimentally obtained square and rectangular intensity profiles and their corresponding line scans (adapted from [11])

able by 2PP can benefit from computational tools such as topology optimization. Topology optimization is a computational method using sets of iterated differential equations to gradually determine the optimal topology for a given engineering task or design. It has already been successfully applied in a plurality of engineering disciplines by e.g. optimizing the distribution of material for a wide span of situations from the load-bearing structure of an airplane wing and down to the design of tiny microrobotic grippers. The method is usually based on repeated computer calculations and the results often lead to surprisingly new designs of known and existing structures. Recently, a substantial amount of work has been focused on extending topology optimization to applications involving fluid flows and wave-propagation but has apparently not yet been applied to optimizing sculpted light-matter interaction. A first step for this undertaking would be to define the external boundaries of the design one has in mind. This should then be followed by fully 3D computer-based optimization procedure to improve the initial design in a step-wise manner. After a plurality of iterations the process should gradually arrive at the optimal way to shape the object given a particular light-matter interaction. We anticipate that scientists and nano-engineers can utilize these emerging 3D computational and engineering schemes to design and sculpt the next generation of top-tuned light–driven micromachines [17].

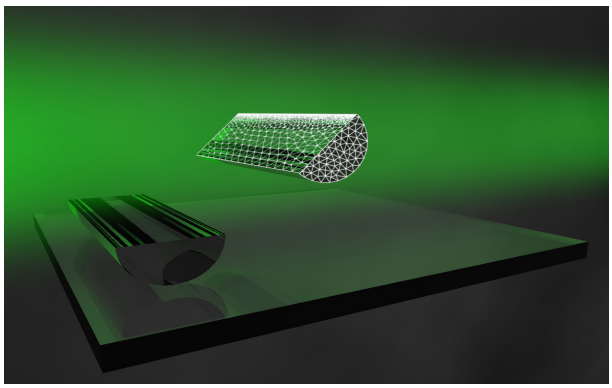

Fig. 9. The conceptualized **use of topology optimization for optimizing light matter interaction as envisioned in ref. 17.**

Conclusions

Our recently developed and miniatuarized BioPhotonics Workstation enables long range lateral and axial optical manipulation and extensible functionality through its side view imaging objectives. This is achieved by using a counter-propagating geometry coupled with long working distance optics. Direct mapping of the trap intensities to the sample allows real time manipulation of a plurality of independent traps, facilitating precise control and a rapid response in optical manipulation. Using extensions through the side view imaging, biological applications requiring different imaging requirements can be performed. Side view information has also been used to develop a stabilization scheme based on tracking the trapped particles' motions. A trimmed down version of the BWS was customized as a compact cell sorter. Ongoing research aims at developing a Next Generation BioPhotonics Workstation that is even more compact and efficient in using laser power.

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