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Slanted channel microfluidic chip for 3D fluorescence imaging of cells in flow

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Abstract: Three-dimensional cellular imaging techniques have become indispensable tools in biological research and medical diagnostics. Conventional 3D imaging approaches employ focal stack collection to image different planes of the cell. In this work, we present the design and fabrication of a slanted channel microfluidic chip for 3D fluorescence imaging of cells in flow. The approach employs slanted microfluidic channels fabricated in glass using ultrafast laser inscription. The slanted nature of the microfluidic channels ensures that samples come into and go out of focus, as they pass through the microscope imaging field of view. This novel approach enables the collection of focal stacks in a straightforward and automated manner, even with off-the-shelf microscopes that are not equipped with any motorized translation/rotation sample stages. The presented approach not only simplifies conventional focal stack collection, but also enhances the capabilities of a regular widefield fluorescence microscope to match the features of a sophisticated confocal microscope. We demonstrate the retrieval of sectioned slices of microspheres and cells, with the use of computational algorithms to enhance the signal-to-noise ratio (SNR) in the collected raw images. The retrieved sectioned images have been used to visualize fluorescent microspheres and bovine sperm cell nucleus in 3D while using a regular widefield fluorescence microscope. We have been able to achieve sectioning of approximately 200 slices per cell, which corresponds to a spatial translation of ∼15 nm per slice along the optical axis of the microscope.

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References and links
1. Introduction

Cells, the fundamental building blocks of life constitute all living organisms from simple single celled organisms such as bacteria to vastly complex multicellular organisms like humans and whales [1]. The extreme finesse of cellular functioning continues to elude scientists working in different fields related to biology and medicine, though our understanding is steadily increasing. Nature’s designs are all the more interesting, when it comes to the relationship between physical structure and the function of different cellular components. For example, the flagellum of a sperm cell enables it to efficiently propel the cell through the female reproductive tract [2]. The red blood cell is another example of design marvel, wherein the membrane deformability enables passage through the thinnest of capillaries to transport oxygen and carbon dioxide. Understanding design principles embedded in natural biology [3], is enabling the creation of synthetic cellular architectures, which are finding a wide range of applications in the fields of biotechnology and medicine [4]. From a medical perspective, understanding the structure-function relationship is leading to insights into disease mechanisms. These insights have enabled the creation of effective diagnostics and therapeutics for diseases like cancer [5].

Our understanding of cellular morphological architectures has co-progressed with the developments in 3D optical imaging techniques [6]. Most of the current commercially available instruments offer several methods for acquiring 3D spatio-morphological features of live cells. One of the most widely used methods for 3D imaging is optical sectioning using confocal microscopy. A good example is that of ascertaining the chromatin distribution in nuclei at a single-cell level [7].

At the core of a confocal imaging experiment lies the process of focal stack collection [8, 9]. Focal stack collection involves acquiring images of the sample, while different parts of the specimen/cell are in the focal plane/depth of field (DOF) of the microscope objective. In other words, it is the process of scanning the sample along the optical axis of the microscope. Owing to the limited DOF of the microscope, different parts/sections of the biological tissue along the depth come into focus as the sample is scanned. The light from out-of-focus planes also contributes to the image formation; giving rise to defocus blur. This problem is mitigated by positioning a pin-hole in front of the camera/detector. The pin-hole rejects/filters out light from the out-of-focus planes, thereby enhancing resolution. Whereas, in a conventional widefield microscope, the defocus blur is computationally removed. The acquired focal stack of images is processed to reconstruct the 3D distribution of the fluorescence over the sample. This enables the assessment of fluorescence in different planes of the sample. Of late, several other methods for 3D fluorescence imaging have been developed, which include light sheet microscopy [10].
and structured illumination microscopy [6, 11]. These methods rely on illuminating the sample with a sheet of light [10] or by patterning the illumination along with computational decoding [11] to extract slices/sections of the biological cells. The process of mechanical translation to acquire the focal stacks remains inherent even while employing these sophisticated techniques.

Apart from confocal fluorescence microscopy, some quantitative phase imaging methods also employ focal stacking. In these methods, the brightfield focal stack images are used to solve the transport of intensity equation (TIE) [12, 13] to generate a quantitative phase map of the biological cell. The volumetric or thickness information of the cell can be extracted from the quantitative phase map. These techniques enable live-cell imaging, as they do not require usage of chemical agents like stains; which tend to affect normal cell physiology.

In general, the most common approach to acquire a focal stack is the use of high-precision piezo-translation stage. Some of these translation stages tend to have a minimum incremental motion of about 50 nm (AG-LS25, Newport Inc.). Most of the current high-end commercial microscopes have similar translation stages and enable automated focal stack acquisition.

On the other hand, some optofluidic imaging techniques known as Imaging Flow Cytometry / Microfluidic Microscopy [14–20] leverage fluidic motion and its alignment with imaging optics to enable novel imaging modalities. Some of these techniques have been shown to be capable of 3D imaging. These approaches essentially involve aligning the microfluidic device with the optical axis at a predefined tilt [19, 20]. As the cells pass through the straight microfluidic channel, the tilted placement of the whole device with respect to the imaging axis, ensures that the cells pass through the focal plane of the objective. These approaches have been shown to be capable of 3D imaging of cells in brightfield [19], fluorescence [21] and quantitative phase imaging [20]. Although simplified, the regular usage of microfluidic focal stack collection in conjunction with conventional microscopy systems still requires some expertise with optics. This is due to the fact that, positioning an element at a precise tilt would require operational knowledge of opto-mechanical components. Further, the integration of additional rotational degrees of freedom into conventional microscope systems is not straightforward and can be prohibitively expensive. Most of the off-the-shelf microscopes may not provide enough gap between the objective and the sample holder to incorporate/ add an additional opto-mechanical component.

A more elegant approach would be to fabricate a small microfluidic device, consisting of channels with an ‘out-of-plane’ tilt. This would allow for the device to be flat-mounted onto any of the existing microscope stages, yet at the same time have the required slant for fluidic focal stack collection. However, the incorporation of a fine oblique angle slope in the microfluidic channel is quite challenging with conventional layer-by-layer type techniques like soft lithography [22, 23]. In these techniques, the 3D structure is brought about by modifying different layers of the master mould. As the material modification happens in a layer-by-layer manner, fabricating a fine slope within the channel using these techniques would be quite challenging.

In contrast to the conventional techniques, ultra-fast laser inscription (ULI) [24–26] enables material modification with inherent 3D capabilities. In ULI, the focal spot of an ultrafast laser is scanned through the volume of the material to modify properties of irradiated areas and thereby enable creation of arbitrary 3D structures. The power of ULI can be leveraged to implement complicated microfluidic architectures [27–32]; thereby drastically simplifying conventional experiments.

In this work, we demonstrate for the first time use of ULI for fabrication of a focal stack collecting microfluidic device in glass. The device incorporates microfluidic channels with a fine ‘out-of-plane’ slant/slope. The presence of this fine slant within the microfluidic channel enables collection of focal stacks of cells passing through them. In essence, the cells traverse
through the focal plane of the objective, enabling acquisition of cell images at different depths/focus in successive frames. This approach of slanted channel acquisition enables automated focal stack collection, without the need for high precision translation stages and very high-speed cameras. Conventional optical sectioning methods, like confocal microscopy are inherently slow due to the point-based scanning approach. Whereas the presented widefield approach enables 3D imaging of cells, while they are in flow and so is amenable for high-throughput measurements. The acquired focal stacks have been processed using computational algorithms. We demonstrate the use of computational photon reassignment algorithms to restore sectional images of cells passing through the microfluidic device. As all the planes of the cell contribute to image formation in the case of each slice image, information from each slice is recorded multiple times. This has been leveraged to significantly enhance the signal-to-noise (SNR), by computationally reassigning the photons. Thus, even while recording the image at a lower exposure time, we demonstrate an increase in the effective exposure time of the images. The retrieved slice images were used to reconstruct 3D morphology of the cells. The presented device allows for seamless integration of the fluidic focal stacking and subsequent 3D imaging modality into conventional widefield microscopy systems, which are not equipped with expensive precision automated translation stages. Moreover, the recent past has witnessed the early stage evolution of inherently slow conventional microscopic imaging towards a high-throughput flow-based imaging modality. As opposed to the now conventional smear based imaging approaches wherein cells are stationary, flow-based high-throughput imaging modalities will eventually become the norm. This is evident from some of the recently demonstrated applications of imaging flow cytometry [33, 34]. The inherent slow speed of mechanical translation in comparison to particle motion speed and acquisition rate make focal stack acquisition a challenging task in flow-based scenarios, even while using expensive, high precision translation stages. The solution proposed here forms a key element in furthering the development of flow based 3D imaging modalities which rely on focal stack acquisition.

2. Universal fluidic focal stack collection

2.1. Device design

As mentioned earlier, the process of focal stacking involves leveraging fluidic motion to move the sample into and out of the depth-of-field of an imaging system. The schematic of the presented approach is shown in Fig. 1(a). As can be seen in the schematic, the surface of the microfluidic device is flat and is parallel to the nose of the microscope objective. Whereas, the subsurface microfluidic channel present within the device is non-parallel and is tilted with respect to the focal plane of the objective. The flat geometry of the overall device enables mounting onto any microscope sample stage, quite similar to positioning a microscope slide. This channel geometry presents an elegant solution to the problem of focal stacking and enables implementation of computational imaging approaches even with off-the-shelf microscopes, without the need for modifying its opto-mechanical architecture. ULI has been employed in this work for fabrication of such a structure with great ease. The angle of tilt is an important design parameter, as it determines the pitch of focal stack for a given flow rate and acquisition frequency or frame rate. The angle has to be chosen appropriately for a given size of the cell and the length across which the sample traverses within the microscope field-of-view (FOV).

In order to collect the focal stack of a given cell, it is essential that the vertical motion it undergoes be more than sufficient to fully scan it along the depth dimension. The schematic representation of the motion of the sample is shown in Fig. 1(b). For a given tilt ($\theta$) and FOV width ($l$) along the direction of flow, the amount of vertical motion ($d$) that the cell/specimen would undergo with respect to the objective’s focal plane is given by

$$d = l \times \tan(\theta)$$ (1)
The FOV width ($l$) of a given microscope is fixed, depending upon the magnification employed and also the size of the image sensor. The user has to choose the tilt as per the size of the sample, being investigated. For a given cell size ($s$), the vertical motion should be at least more than the size of the cell. In general, it is ideal to choose the depth of the microfluidic channel to be at least twice the size of the cell. This ensures that the cells pass through without any obstruction or clogging. In the absence of a flow focusing mechanism (in the depth direction), the cell can be present anywhere within the depth of the channel. Hence, we suggest that the design incorporates vertical motion, which is more than twice the size of the cell; giving rise to relation (2).

$$\theta \geq \arctan\left(2 \times \frac{s}{l}\right)$$

This would ensure that the complete depth of the cell would be scanned, while it traverses the FOV. For example, in order to acquire the focal stack of cell, which is 20 $\mu$m in diameter - over a FOV which has a width of 300 $\mu$m, the tilt should be ideally close to 7.5°, so that the entire depth dimension of the cell is covered.

### 2.2. Device fabrication

As per the schematic shown in Fig. 1, microfluidic devices were fabricated in fused silica. The ULI technique employs focused ultrashort pulses to locally modify material properties. As the modification happens at the focal spot of a laser which can be located inside a transparent material positioned on a translation stage, the technique enables a superior degree of design freedom, than that offered by techniques such as PDMS lithography. When an ultrafast laser pulse is focused into a tight spot, the material absorbs through a nonlinear process. This non-
linear process results in the formation of nanogratings which enhance the etch rate of the material when placed in hydrofluoric acid (HF). The selective enhancement of etch rate at the irradiated locations allows for the formation of structures within the sample.

In these experiments, an IMRA μ-Jewel femtosecond laser set to a repetition rate of 500 kHz, pulse length of 360 fs and power of 350 mW was focused onto the samples mounted on an Aerotech XYZ stage, through a 0.4 NA objective. Following irradiation, the samples were etched for approximately 13 hours in 10% HF. Tubing from Upchurch Scientific of outer and inner diameter of 360 μm and 100 μm respectively was bonded to the inlets using UV curing glue (Thorlabs MIL-A-3920).

The microfluidic devices used in these experiments consist of a series of channels with an ‘out of plane’ tilt with a width of ~20 μm and height of ~31 μm. The inlets and outlets were created on the sides of the device to facilitate ease of experimenting with a conventional microscope. The imaging region is fabricated beneath the surface close to the device boundary, so as to ensure compatibility with low working-distance objectives. The inlet and the imaging region are connected via a tilted channel as shown in Fig. 2(b).

At the imaging region, the channel bifurcates into eight channels, each fabricated with a fine slant. To showcase various possibilities, we have fabricated microfluidic devices with different tilts. Side views for 1° and 5° tilted device have been shown in Fig. 2(b) and (c) respectively. As it is evident from the images, the slant is very fine and is not noticeable for 1° tilted device; whereas the slant is noticeable with the 5° tilted device. The design ensures that as the cells pass through these channels, the cells come into and go out-of-focus of the imaging system. This allows for acquisition of focal stacks with great ease. Fig. 2(d) and (e) show images of
the microfluidic device, after insertion of tubing. The device has a lateral dimension of about 4.5 mm and is only 2 mm thick. The extremely small form factor of the microfluidic devices ensures incorporation into conventional microscopes with great ease.

3. Characterization of focal stack acquisition with fluorescent microspheres

The proposed approach for focal stack collection was first characterized by collecting focal stacks of fluorescent microspheres. In these experiments, we have used fluorescence isothiocyanate (FITC) labelled microspheres of diameter - 4 μm (Thermofisher, F-8859). The fluorescent dye (FITC) is distributed uniformly over the complete volume of these microspheres. When such a bead is optically sectioned, it is expected that slices, which have a circular disc like shape are observed. When a sphere is sliced at different locations perpendicular to the axis, the diameter of the slice would vary with respect to its distance from the center. The central slice would have the highest diameter, and the diameter would decrease as we move away from the center of the sphere. Further, as the dye is uniformly distributed it is expected that all the slices would have uniform intensity.

The glass microfluidic device was flat mounted onto the stage of a conventional widefield fluorescence microscope as shown in Fig. 3. As shown in the figure, the experimental setup does not require anything else apart from a regular scientific digital microscope. In our experiments, we have employed the Nikon Eclipse Ci for acquiring focal stacks of fluorescent microspheres and cells. A suspension of fluorescence isothiocyanate (FITC) labeled microspheres of diameter - 4 μm (Thermofisher, F-8859) was pumped through the microfluidic device. The microfluidic device is flat mounted on a conventional widefield fluorescence microscope and viewed under 40X magnification (Nikon Plan Fluor, NA = 0.75). As the material (glass) of the microfluidic device does not significantly impact the imaging quality, it is expected that the system would enable a resolution of about 0.37 μm in the lateral direction. As per the Rayleigh criterion, the depth resolution is about 1.9 μm. The flow of the suspension was facilitated by a syringe pump (NE-1000, New Era Pump Systems). Videos of flow were acquired using Andor Zyla 5.5 camera mounted onto the microscope. The exposure time was set to 500 μs and the frame rate was set to 200 frames per second (fps).

The acquired videos were processed in MATLAB to extract the focal stacks corresponding to individual beads. Each given frame of the video would consist of single image of the bead, focused at a particular depth. As the bead traverses from one end to the other, it is imaged at different depths. When the bead first enters the field of view, it is focused close to the edge.
When it reaches the central portion of the FOV, the center of the bead comes into focus. The extracted raw focal stack for a given bead is shown in Fig. 4(a). These raw focal stack of images were processed using deconvolution to computationally restore sectional images of the beads. In order to deconvolve the acquired raw focal stacks, the point-spread function (PSF) of the system has to be known. In general, there are three approaches to determine the PSF: Experimental, Analytical and Computational [9]. Experimental determination would be quite challenging owing to the difficulty in recording the PSF using point-like objects in the flow-based scenario at hand. Several analytical models for calculating the PSF are available [35]. The available models have been developed for conventional slide based imaging and do not take into account the curvature of the microfluidic channel, which is relevant to the current scenario. Hence, we chose the computational approach to determine the PSF. The computational approach involves the use of blind deconvolution algorithms to estimate the PSF of the system from the acquired images. Blind deconvolution algorithms iteratively estimate both the blur-free image and system PSF simultaneously. Most Blind deconvolution algorithms work by removing the out-of-focus light, rather than reassigning the light into the proper planes as in the case of restoration algorithms like Wiener deconvolution [36]. Thus, the results are expected to be better when, PSF estimated (using blind deconvolution or otherwise) is used for photon reassignment with restoration algorithms.

As mentioned earlier, the diameter of the section is expected to increase as we move from one end of the bead to its center. However, due to the presence of out-of-focus blur in the images, the variation in diameter is not evident in the raw images of the focal stack.

To restore, the sectional images of the microsphere, we have first estimated the system PSF using Lucy-Richardson maximum likelihood blind deconvolution algorithm [9]. Following which, the estimated PSF was used to process the raw focal stack with Wiener deconvolution [36]. The deconvolved images have been shown in Fig. 4(b). The reduction/change in the diameter of the slice as we move from one plane to the next is quite evident in the case of deconvolved images. The variation in the diameter of the slices can be observed from the intensity profiles across a given slice. The deconvolved images display wave-like artifacts/circular ring, seen around the slice commonly referred to as ringing artifacts. Ringing artifacts occur due to Gibbs phenomenon, wherein the Fourier sum overshoots at discontinuities. These effects are more pronounced, when blind deconvolution algorithms are used to estimate the PSF. For the image stacks acquired using our system, the optimal number of iterations to estimate the PSF was 5 [37]. The deconvolved images were post-processed to minimize artifacts. The post-processed images have been shown in Fig. 4(c). Comparison between the intensity profiles for two different slices: Edge Slice which corresponds to the slice with the least diameter and the center Slice, corresponding to highest diameter have been shown in Fig. 5.

The loss of details like sharp boundary of the bead and uniform intensity, in the raw focal stack (Fig. 4(a)) is due to the presence of out-of-focus light overlaying on the in-focus light in the same image. In other words, when a particular plane of bead is in the DOF, the light from the planes below and above the in-focus plane is also acquired by the imaging system. Deconvolution restores the sectional images by computationally reassigning the out-of-focus
Fig. 5. Intensity Profiles drawn across the surface of two different slices of the fluorescent bead. (a) Image of the edge slice, the intensity profile shown is over the line across image. (b) Image of the Center slice, the intensity profile shown is over the line across image.

The enhancement in SNR is thus dependent on the number of slices/stacks acquired for a given specimen/cell. This is due to the fact that collecting more slices would enable reassignment of more light, thereby increasing the SNR. Conversely, not considering a selected number of slices from the originally recorded focal stack would result in a reduction in intensity of the deconvolved image(s). This is due to the reduction in light that would have been reassigned to the proper plane. In order to analyze the photon reassignment capability and the SNR enhancement, the deconvolution was performed taking only a few of the recorded slices into account. The intensity of a given restored section/slice would vary as a function of number of slices used for deconvolution. Fig. 6 shows a given sectional image, obtained by using different numbers of recorded slices for deconvolution. As evident from the figure, the intensity across the line profile along a given slice of bead is highest when the complete stack is used. The intensity reduces for subsequent reduction in the number of slices used for reconstruction. As noticed, the SNR enhancement is dependent on the number
Fig. 6. Comparison of photon reassignment, when different number of slices are used for deconvolution. The intensity profiles across the restored images when (a) Full stack (44 slices), (b) 22 slices, (c) 15 slices were used for reconstruction. The (d) raw image of the same plane and it’s corresponding intensity profile have been shown. Length of scale bar is 10 μm.

of slices acquired per cell. Further, it follows that specimen with weaker fluorescence emission would require higher enhancement in SNR. Whereas, larger specimen with higher fluorescence emission would require a lesser SNR enhancement and thereby would require lesser number of focal stack images (per cell) to be acquired. The proposed fine slant channel geometry offers great flexibility in terms of acquiring the required number of focus stack images. The slicing pitch can be very easily varied by changing the flow speed of the cell (flow rate) and the frame rate of acquisition. Subsequently, the acquired slices can be used for SNR enhancement with the implementation of a suitable photon-reassignment algorithm.

In conventional optical sectioning approaches, the out-of-focus light is physically rejected by placing a pinhole in front of the detector. Whereas, in the presented approach, light from multiple planes is collected multiple times so as to enable computational reassignment and increases the effective exposure time; thereby, making an intelligent use of the out-of-focus light present in each of the raw images in the acquired stack. Conventional confocal microscopy
Fig. 7. 3D visualization of the 4 μm fluorescent microsphere.

Involves scanning in a point-by-point manner. This makes for an inherently slow technique. In contrast, the proposed approach is a widefield technique, wherein cells are imaged while they are in flow. Thus, the aspects of speed and automation of the presented technique potentially enables high-throughput 3D imaging of cells like in imaging flow cytometry.

Further, the added advantage of the proposed method is the ability to visualize samples/cells in 3D. The restored (deconvolved) focal stacks can be used to reconstruct a 3D image of the fluorescing regions. The 3D reconstruction of the fluorescent microsphere from the acquired data (Fig. 4(a)) is shown in Fig. 7. The contours along the sphere represent the edges/borders.
of the circular regions present in each slice/plane of the bead (Fig. 4(c)). A surface is drawn through these contours to obtain the 3D structure of the object. As expected, the structure of the reconstructed object is similar to that of a 4 μm diameter sphere. In order to ascertain the fidelity of shapes in the reconstructed images, we have estimated the sizes of fluorescent microspheres. The histogram plot shown in Fig. 8 corresponds to sizes measured for about 64 beads. As per the datasheet of the manufacturer, the nominal size of the bead is 4.2 ± 0.2 μm. The size of the microspheres as measured by our system is about 4.1 ± 0.2 μm. Thus, the presented system enables fairly accurate measurement of morphological sizes of microscale specimen.

4. 3D fluorescence imaging flow cytometry

Fig. 9. (a) Raw focal stack of Bovine sperm cell nucleus. (b) Deconvolved focal stack of Bovine sperm cell nucleus. (c) Slices of bovine sperm cell nucleus, after removal of artifacts. Length of scale bar is 10 μm.

Following the characterization of the proposed approach with microspheres / fluorescent-beads, we employed the microfluidic devices to perform three dimensional imaging flow cytometry. Using the devices, we have investigated the 3D morphology of bovine sperm cell nuclei. The nuclei of cells present in a suspension were stained using DAPI (4′,6-diamidino-2-phenylindole). As detailed in the previous section, the videos of the flow stream were acquired using a widefield digital fluorescence microscope. The flow rate was set to 30 μlhr⁻¹ and the frame rate was increased to 600 fps. The low flow rate together with the small channel dimensions ensures that the flow remains in the laminar regime. This prevents the cell from rotating, which may otherwise impact the image acquisition. Also, the flow rate was optimized to ensure that the motion-blur in the acquired images is minimal. The individual frames of the video were extracted and processed using Wiener deconvolution. The complete stack for a single bovine sperm cell nucleus consisted of 226 slices; of which a few representative slices have been shown in Fig. 9. The deconvolved stacks have been used to visualize the 3D morphology of the sperm cell nucleus (shown in Fig. 10). The increase in the number of slices acquired per cell is evident from the higher density of contour lines in the case of sperm cell reconstruction.

Due to their relevance in animal and human healthcare, sperm cell analytical techniques have gained significant research interest over the last few years. Morphology is one of the most critical parameters used to assess the health of the sperm cell [38, 39]. Recent research efforts are being directed towards development of automated sperm morphological analysis techniques. The automation aspect plays a critical role in eliminating variation in testing due to environmental conditions and subjectiveness of the technician [40]. Several digital holography based techniques for sperm cell 3D morphological assessment have been demonstrated [40–43]. Although well proven, these techniques require implementation of optical interferometric setups with a high degree of sophistication. In contrast, the presented approach enables 3D morphological assessment, with a regular widefield fluorescence microscope. It does so by automatting the process of focal stack collection in an elegant manner and subsequent computational reconstruction. The presented microfluidic devices can also be used for acquiring brightfield focal stacks. These brightfield focal stacks can be processed using computational methods like transport of intensity equation, to generate a quantitative phase map [20].
Quantitative phase imaging enables retrieval of volumetric or thickness information without the need for any labels thereby enabling live-cell imaging and assessment. Thus, the proposed automated approach for focal stacking is equally applicable for label-free assessment of live cells in flow.

5. Conclusion

In this article, we have demonstrated an automated, elegant approach for focal stack collection based 3D fluorescence imaging using a generic widefield microscope. The presented approach involves the use of a small glass microfluidic device with slanted microfluidic channels fabricated in it using ULI. The slanted nature of the channel forces the cell to move perpendicularly to the focal plane of the objective as it traverses the FOV. This enables the acquisition of focal stacks, without the need for an expensive precision automated translation stage.

The proposed scheme enables acquisition of light from multiple planes, multiple times. This facilitates the implementation of computational photon reassignment to make use of the out-of-focus light to enhance SNR, as well as retrieve sectional images of microscopic specimen. The retrieved sections/slices have been used for 3D visualization of cells and fluorescent microspheres. The approach has been shown to be capable of acquiring 200 slices per cell (Bovine sperm cell nucleus), amounting to imparting an equivalent mechanical translation.

Fig. 10. 3D visualization of Bovine Sperm Cell nucleus.
of 15 nm (per slice) along the optical axis. As demonstrated, the technique operates in a widefield, flow-based manner, which would enable higher 3D imaging throughputs as compared to inherently slow, conventional methods. The presented architecture for the slanted channel microfluidic chip is a critical step towards realizing a functional prototype, which can enable 3D fluorescence imaging of cells in flow using deconvolution microscopy. The generic nature of the approach enables usage of this technique by biologists and non-optics experts as well, without the need for setting up custom optical systems. We believe that the presented innovation would democratize 3D imaging flow cytometry for usage in any biological lab, equipped with a generic widefield fluorescence microscope. Also, the proposed design of slanted channel microfluidic devices are aptly suitable for use with selective plane illumination microscopy (SPIM) systems for 3D fluorescence imaging. Usage of the proposed device with SPIM eliminates the need for computational reconstruction.

Further, the advent of portable optofluidic microscopy is enabling the implementation of imaging based healthcare tools at the point-of-care [44,45]. The integration of conventional focal stacking approaches in these portable systems is a significant challenge, owing to the requirement of additional mechanical components for imparting translation. Whereas with the use of the present scheme of slanted glass microfluidic channels, focal stacking based 3D imaging can be implemented in these point-of-care diagnostic devices as well. The presented approach has the potential to add another dimensionality in classification/analysis of cells in the case of small-form factor devices, which have been otherwise limited to 2D morphology. We envision that such implementations would revolutionize 3D morphology based cellular diagnostics in remote settings/point-of-care diagnostics.

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