A MICRO MACHINED LIGHT SHEET ILLUMINATION SYSTEM

IMPLEMENTED IN A CONVENTIONAL MICROSCOPE

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ABSTRACT:
3D images reveal different and significant more information than 2D images. A good example is the dynamics of filopodia at the periphery of living macrophages, which actively grabs for particles and bacteria. This prey can be presented to the cells by optical tweezers and the change of the particles 3D motion - recorded interferometrically during contact with the filopodia - allows to extract significant information about the working principles of these fascinating cellular protrusions [1]. However, since often one or more filopodia suddenly appear in the image plane and grab for the particle, fast 3D imaging is required to correctly interpret the filopodia dynamics. Light sheet fluorescence (LSFM) microscopy is a suitable choice of microscopy technique due to better sectioning when compared to conventional wide field imaging [2]. Conventional light sheet illumination systems have a low NA objective lens placed perpendicular to the detection axis to generate the light sheet within a sample embedded in a gel cylinder. However, the presence of high NA tracking and trapping objectives with very small working distances makes it impractical to use this traditional illumination set up to generate the light sheet.

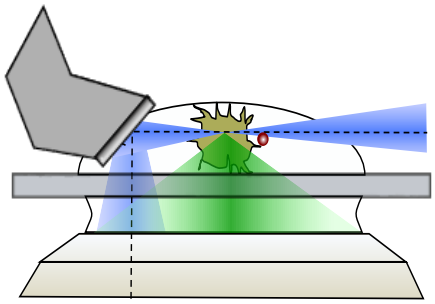

Figure 1: Schematic of the illumination set up.

We designed an illumination setup which works simultaneously with trapping and tracking. Therefore, we use the same objective for illumination and detection. A small mirror is placed at 45° to the detection axis using a precision mirror holder. A narrow illumination beam reflected by the mirror, illuminates the sample perpendicular to the detection axis. The light sheet is generated by scanning the beam perpendicular to the detection axis. Shifting of this light sheet through the sample and simultaneous refocusing using the electrically tunable lens enables fast acquisition of 3D image stacks of the cell.

Line confocal detection using the rolling shutter of the camera further enhances sectioning. Since tunable lens in detection helps to capture the 3D image, motion of the sample is not required.

REFERENCES

[1] F. Junger *et al.*, "Measuring Local Viscosities near Plasma Membranes of Living Cells with Photonic Force Microscopy," *Biophysical journal*, vol. 109, no. 5, pp. 869–882, 2015.

[2] T. Meinert, O. Tietz, K. J. Palme, and A. Rohrbach, "Separation of ballistic and diffusive fluorescence photons in confocal Light-Sheet Microscopy of Arabidopsis roots," *Scientific reports*, vol. 6, p. 30378, 2016.