

CELL ALIGNING MICROSTRUCTURES FOR IMAGE CYTOMETRY

Tycho Scholtens¹, Frederik Schreuder¹, Jan Greve¹, Arjan Tibbe², Leon Terstappen³
¹Biophysical Engineering, University of Twente, P.O. Box 217, 7500 AE Enschede, The Netherlands. ²Immunicon Europe Inc., Enschede, The Netherlands. ³Immunicon Inc.,
Huntingdon Valley, PA, USA.
E-mail: t.scholtens@utwente.nl

KEY WORDS: Image cytometry, Cell alignment, Rare cell detection, Immuno-magnetic selection, Microstructures, PDMS, Circulating tumor cells.

Introduction

For detection of rare events, such as tumor cells in blood, fast and accurate cell imaging is required [1]. For microscopic analysis cells are usually randomly distributed on the analysis surface. To reduce imaging times, we developed PDMS microstructures that align the cells in predefined areas and at the same time offer unobstructed imaging. These microstructures are produced by making PDMS imprints of etched silicon wafers (C2V and Lionix, Enschede, Netherlands). The unique properties of PDMS make it an excellent material to use for the imprints: it is optically transparent down to 300nm, can easily be glued to supporting structures, is cost effective and replicates structures with sub-micron accuracy.

Method and results

Gravitational or magnetic forces [2] are needed to align the cells in the structures and characterization of these cells is done by scanning them with 3 homogenized laser spots. Essential properties of two produced microstructures, cell alignment efficiency and image quality, were investigated using SKBR3- and white blood cells. Figure 1 gives an overview drawing of the two structures that were investigated.

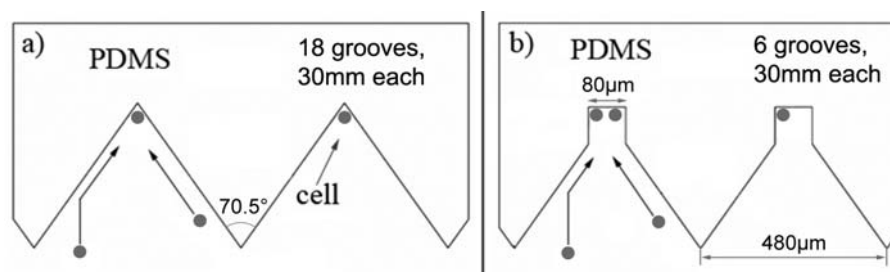


Figure 1. Schematic overview drawing of a) V-Groove- and b) Chimney microstructures

Due to the hydrophobic nature of PDMS some cells adhere to the surface and don't reach the correct position (~1-2%). The V-Groove structure guides the cells in 1 long file, like in Flow Cytometry, and is suited for basic cell counting. The Chimney structure concentrates the cells by a factor of 6 and can accommodate up to 10.000 cells. This allows for imaging at a higher magnification than before (40X instead of 10X), while increasing the scanning time only by a factor of 2.

1. M. Cristofanilli, G.T. Budd, M.J. Ellis, A. Stopeck, J. Matera, M.C. Miller, J.M. Reuben, G.V. Doyle, W.J. Allard, L.W.M.M. Terstappen, D.F. Hayes. "Circulating Tumor Cells, Disease Progression and Survival in Metastatic Breast Cancer". *NEJM*, **351**, 781-791 (2004).
2. A.G.J. Tibbe, B. de Grooth, J. Greve, P.A. Liberti, G.J. Dolan, L.W.M.M. Terstappen. "Optical tracking and detection of immunomagnetically selected and aligned cells". *Nature Biotechnology*, **17**, 1210-1213 (1999).