Lensfree Holographic Imaging of Antibody Microarrays for High-Throughput Detection of Leukocyte Numbers and Function

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Anal. Chem., 2010, *82* (9), pp 3736–3744

DOI: 10.1021/ac100142a

Publication Date (Web): April 1, 2010

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Abstract

Characterization of leukocytes is an integral part of blood analysis and blood-based diagnostics. In the present paper, we combine lensless holographic imaging with antibody microarrays for rapid and multiparametric analysis of leukocytes from human blood. Monoclonal antibodies (Abs) specific for leukocyte surface antigens (CD4 and CD8) and cytokines (TNF-α, IFN-γ, IL-2) were printed in an array so as to juxtapose cell capture and cytokine detection antibody (Ab) spots. Integration of Ab microarrays into a microfluidic flow chamber (4 μL volume) followed by incubation with human blood resulted in capture of CD4 and CD8 T-cells on specific Ab spots. On-chip mitogenic activation of these cells induced release of cytokine molecules that were subsequently captured on neighboring anticytokine Ab spots. The binding of IL-2, TNF-α, and IFN-γ molecules on their respective Ab spots was detected using horseradish peroxidase (HRP)-labeled anticytokine Abs and a visible color reagent. Lensfree holographic imaging was then used to rapidly (~4 s)

enumerate CD4 and CD8 T-lymphocytes captured on Ab spots and to quantify the cytokine signal emanating from IL-2, TNF-α, and IFN-γ spots on the same chip. To demonstrate the utility of our approach for infectious disease monitoring, blood samples of healthy volunteers and human immunodeficiency virus (HIV)-infected patients were analyzed to determine the CD4/CD8 ratio, an important HIV/AIDS diagnostic marker. The ratio obtained by lensfree on-chip imaging of CD4 and CD8 T-cells captured on Ab spots was in close agreement with conventional microscopy-based cell counting. The present paper, describing tandem use of Ab microarrays and lensfree holographic imaging, paves the way for future development of miniature cytometry devices for multiparametric blood analysis at the point of care or in a resource-limited setting.