Supplementary Information

On Chip Analysis of CNS Lymphoma in Cerebrospinal Fluid

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Design of microfluidic platform: The fluidic system has a single-layer structure that is composed of a capture site region, a fluidic channel, and a debris filter at the inlet. Injected fluids (e.g. cells, buffers, antibodies) first pass through the microfilter array (200 μm in diameter) in order to filter large aggregates and debris. The fluids then pass through the capture site region (12000 μm in width; 5800 μm in length). Figure S1 (top) shows the detailed dimensions of the single-cell capture sites, which were designed to capture lymphocytes ~10μm in diameter. There are two capture regions with different gap sizes (W_1 = 30 μm and 16 μm; L_2 = 40 μm and 25 μm) to enhance the capture rate. Figure S1(bottom) shows the schematic of a column filter at the sample inset, that removes large debris and cell aggregates. The height of the fluidic channel is 25 μm.

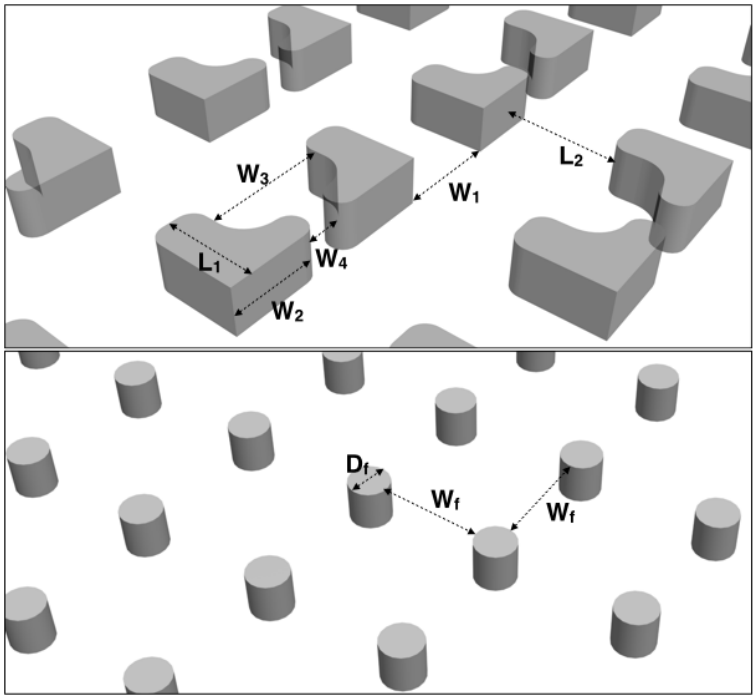

Figure S1. Fluidic structures in microfluidic chip. (Top) Design parameters for the capture sites: W_1 = 30 μm and 16 μm; W_2 = 10 μm; W_3 = 14 μm; W_4 = 4 μm; L_1 = 15 μm; L_2 = 40 μm and 25 μm. (Bottom) Structure of the on-chip column filter. D_f = 200 μm and W_f = 800 μm.

Table S1. Cell Counts in CSF

	Normal[1]	CNS lymphoma[2,3]	Inflammation[4]
B cells (per mL)	0-30	10-500,000	200-43,000
T cells (per mL)	150-2,000	250-180,000+ (max 97.2%)	9,000-460,000
Monocytes (per mL)	80-1,100	—	—
Granulocytes (per mL)	20-430	—	—
NK cells (per mL)	0-50	max 7.4%	1,500-50,000

1 de Graaf MT, Smitt PA, Luitwieler RL, van Velzen C, van den Broek PD, Kraan J, Gratama JW. Central Memory Cd4+ T Cells Dominate the Normal Cerebrospinal Fluid. *Cytometry B Clin Cytom* **2011**; 80: 43-50.

2 Quijano S, Lopez A, Manuel Sancho J, Panizo C, Deben G, Castilla C, Antonio Garcia-Vela J, Salar A, Alonso-Vence N, Gonzalez-Barca E, et al. Identification of Leptomeningeal Disease in Aggressive B-cell Non-hodgkin's Lymphoma: Improved Sensitivity of Flow Cytometry. *J Clin Oncol* **2009**; 27: 1462-1469.

3 Schroers R, Baraniskin A, Heute C, Vorgerd M, Brunn A, Kuhnhenn J, Kowoll A, Alekseyev A, Schmiegel W, Schlegel U, et al. Diagnosis of Leptomeningeal Disease in Diffuse Large B-cell Lymphomas of the Central Nervous System By Flow Cytometry and Cytopathology. *Eur J Haematol* **2010**; 85: 520-528.

4 Kleine TO, Albrecht J, Zofel P. Flow Cytometry of Cerebrospinal Fluid (csf) Lymphocytes: Alterations of Blood/csf Ratios of Lymphocyte Subsets in Inflammation Disorders of Human Central Nervous System (cns). *Clin Chem Lab Med* **1999**; 37: 231-241.

Table S2. Antibodies used in the current report.

Antigen	Clone	Provider	Fluorochrome (on chip)
CD10	HI10a	BioLegend	—
CD19	HIB19	BioLegend	R-PE
CD20	2H7	BioLegend	R-PE
CD45	HI30	BioLegend	—
Ki-67	B56	BD Biosciences	Alexa Fluor 488
κ light chain	MHK-49	BioLegend	Brilliant Violet 421
λ light chain	JDC-12	BD Biosciences	Alexa Fluor 647