

SUPPLEMENTARY MATERIAL FOR:

**Quantification of small cell numbers with a microchannel device**Nisha M. Badders<sup>1</sup>, Hongmei Yu<sup>2</sup>, Caroline M. Alexander<sup>1</sup>, and David J. Beebe<sup>2</sup><sup>1</sup>McArdle Laboratory for Cancer Research and <sup>2</sup>Department of Biomedical Engineering, University of Wisconsin, Madison, Madison, WI, USA*BioTechniques* 45:321-325 (September 2008)

The microcounter design was produced using Illustrator 9.0 (Adobe, San Jose, CA, USA) and printed onto a transparency film. The channel dimensions were 0.1 mm (*h*) × 200 mm (*l*) × 0.5 mm (*w*), while the grid pattern consisted of twenty 10 μm (*h*) × 0.5 mm (*w*) squares molded into the ceiling of each channel. Photoresist SU-8 100

(Microchem Corporation, Newton, MA, USA) was spin-coated onto a 3-inch silicon wafer, baked at 95°C for 1.5 h, overlaid with the transparency film, and exposed to 200 mJ/cm<sup>2</sup> UV. The wafer was then baked at 150°C for 3 h and developed with a SU-8 developer (Microchem Corp.). The master was then air-dried and hard baked at

95°C for 30 min. PDMS prepolymer (Sylgard 184 Silicone Elastomer kit, Dow Corning, Midland, MI, USA) was then mixed 10:1 with curing agent and poured onto the master. The polydimethylsiloxane (PDMS) microchannels were then cured at 80°C for 2 h and subsequently exposed to UV for 20 min.

**Supplementary Table S1. Statistical Data of Each Counting Device**

Hemocytometer	Mean	sd	Confidence	<i>n</i>
100,000	107,500	25,372	20,302	6
50,000	52,592	18,143	14,517	6
25,000	30,416	17,206	13,767	6
12,500	19,166	9443	7556	6
6250	5416	3323	2659	6
3125	n/d	n/d	n/d	–
1562	n/d	n/d	n/d	–
Microcounter				
100,000	90,250	15,798	12,641	6
50,000 <sup>a</sup>	58,214	6109	4888	6
25,000 <sup>a</sup>	24,583	4104	3284	6
12,500 <sup>a</sup>	10,333	2503	2003	6
6250	7500	3082	2466	6
3125	4667	2887	3267	3
1562	1000	1000	1131	3
<sup>a</sup> <i>P</i> < 0.05. The initial concentration of 293 cells was determined with the hemacytometer. The volume was then adjusted to attain a concentration of 50,000 cells/ml and serially diluted to achieve each subsequent concentration. The cells for each theoretical concentration were counted in duplicate with each device. The mean cell counts (cells/ml) were determined from six independent experiments. The standard deviation (sd) and confidence limits (95% confidence level) were determined for each mean, and statistical significance was determined by analysis of variance (ANOVA) for repeated measures and multiple comparison F-ratios.				