Supporting information

Rapid purification of lung cancer cells in pleural effusion through spiral microfluidic channels for diagnosis improvement

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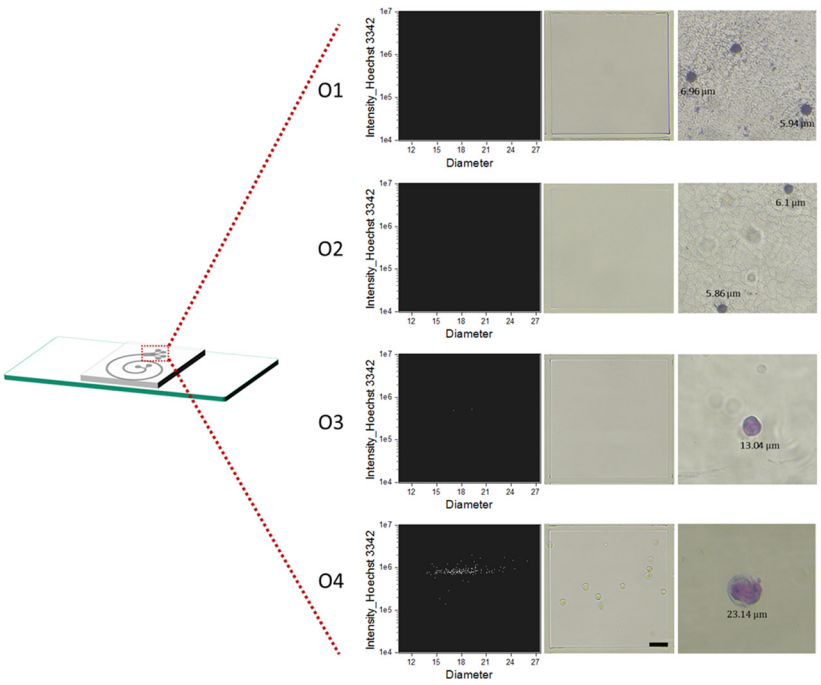


Figure S1. (A) The specimens reveal the A549 cells distribution in O1-O4. (B) The bright field images of sorting fraction of A549 cells in medium. Scale bar is 50 µm. (C) The bright field images of sorting fraction of A549 spiked in plural effusion.

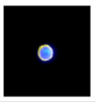
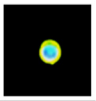
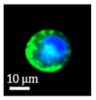
Order	Characteristic	Type	Ex.	
1	Jagged shape, dark fill, or dark outline in bright-field	Debris		Background cells
2	EpEAM-/CD45+/Hoechst 33342+	WBC		
3	EpEAM+/CD45+/Hoechst 33342+	WBC		
4	EpEAM+/CD45-/Hoechst 33342+	Cancer cell		LCs

Figure S2. Collected cells were enumerated using objective criteria based on immunofluorescence staining characteristics, as shown in the table. Each cell was assessed for each criterion in the order listed until the characteristics matched.

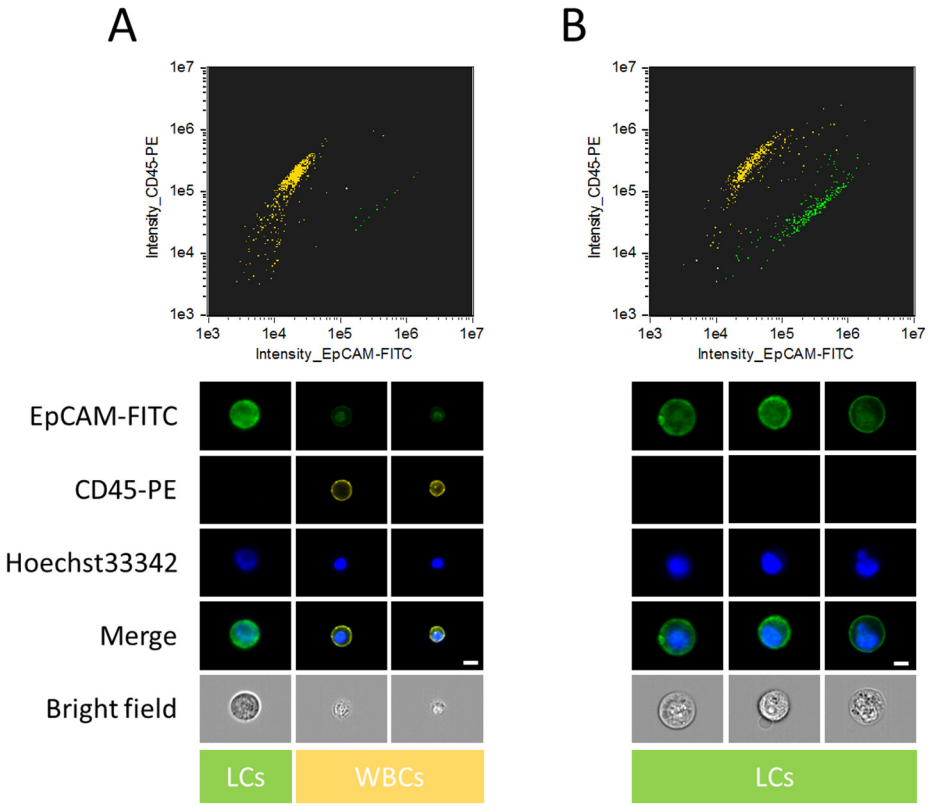


Figure S3. Enumeration criteria for pleural effusion from a notable patient. (A) The specimens without spiral microfluidic channel pretreatment, background cells (yellow) and lung cancer cells (green) were detected with imaging flow cytometry (IFC). (B) The overall purity of cancer cells (green) was increased by spiral microfluidic channel pretreatment and detection by imaging flow cytometry (IFC). A diverse set of cells in pleural effusion were collected from patients, which varied in immunofluorescence and displayed all combinations of EpCAM, CD45, and Hoechst 33342 expression. Scale bar is 10 μm.

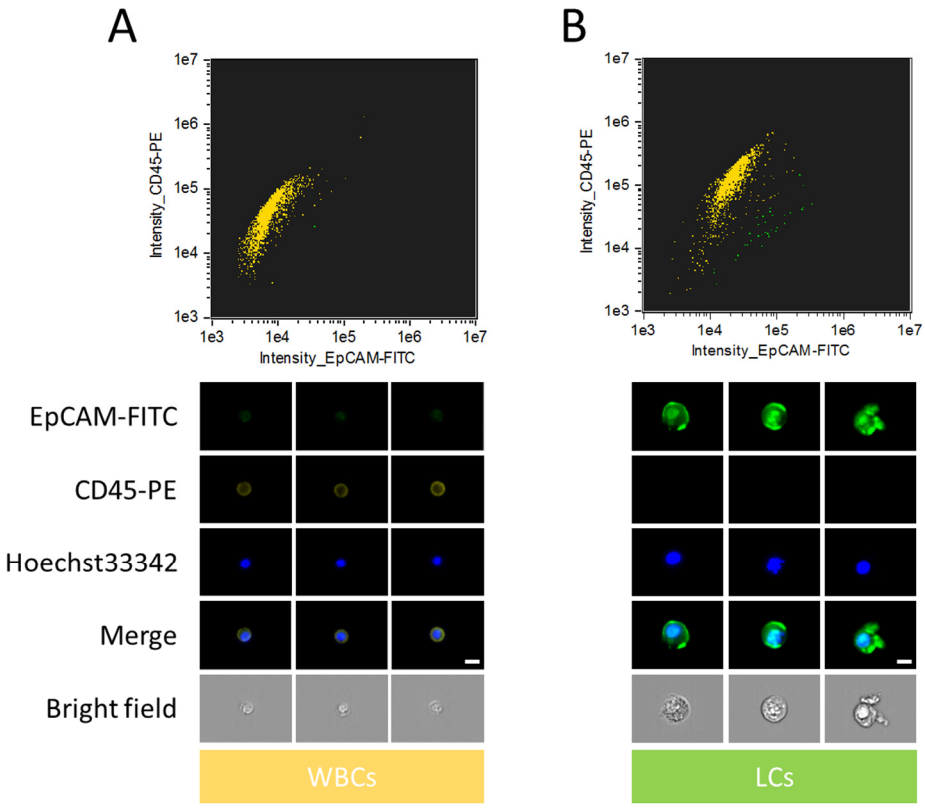


Figure S4. Enumeration criteria for pleural effusion from an insignificant patients. (A) The specimens without spiral microfluidic channel pretreatment, no lung cancer cells (green) were detected with imaging flow cytometry (IFC). (B) The overall number of cancer cells (green) was significant increased by spiral microfluidic channel pretreatment and detection by imaging flow cytometry (IFC). A diverse set of cells in pleural effusion were collected from patients, which varied in immunofluorescence and displayed all combinations of EpCAM, CD45, and Hoechst 33342 expression. Scale bar is 10 μm.