## **Spintrap Design and Characterization**

(a, b) We designed and custom-made a closed centrifugation system, called Spintrap, for large volume, high throughput, and thin-layer preparation of CTCs. Due to its low fluidic resistance, the design allowed inline use with CTC-iChip with a processing time of 15 minutes for up to a fluid volume of 23 mL. The Spintrap consistently prevented air-drying and thus conserved native cell morphology. Using the Spintrap protocol (c, steps 88-92), and LBX1 cell line with cytoplasmic GFP label, we characterized cell attachment strength, yield, and resulting cellular morphology. (d) We performed automated fluorescence microscopy for quantifying efficiency of immobilization, and found the cell yield to be >95% at optimized conditions. (e, f) Confocal and epifluorescence microscopy revealed native cellular morphology at a relative centrifugal force of 50 g. A slight increase in cellular radius at 500 g and significant pancaking at 2,000 g was found, while cell circularity remained similar to a non-centrifuged control. We found centrifugation for 5 minutes at 50 g to be optimal for cell immobilization with high yield and without morphological disturbance. Cells collected with the Spintrap method showed diagnostic quality on cytological evaluation. Using Spintrap, we characterized CTCs from cancer patients isolated using CTC-iChip<sup>58</sup>.

