

Figure S1. Fluorescent image of DiI-stained cells and chitosan microparticles (diameter $\approx 164 \ \mu$ m). The chitosan microparticles were loaded with 1% by weight carbon black to attenuate their intrinsic autofluorescence. The one bright microparticle contained no carbon black and emitted a strong autofluorescence that was much brighter than the microparticles encapsulating carbon black or fluorescently stained cells. This image was taken using an inverted fluorescent microscope and a TRITC filter set (Ex: 520-570; Em: 535-675) at 10× magnification.



Figure S2. Fluorescent biotin increased the fluorescent intensity of streptavidin-functionalized chitosan microparticles. The chitosan microparticles, which encapsulated 1% by weight carbon black, were covalently functionalized with streptavidin. Following exposure to fluorescent biotin in solution followed by washing, a significant increase in fluorescent intensity was observed. These images were taken using an inverted fluorescent microscope and a TRITC filter set (Ex: 520-570; Em: 535-675) at 10× magnification.



Figure S3. Attachment of small biotinylated polystyrene spheres (6-8 μ m) to streptavidin-functionalized chitosan microparticles. When chitosan microparticles were mixed with the commercially available biotinylated polystyrene spheres, significant surface attachment of the spheres to the chitosan microparticle demonstrated that covalently bound streptavidin was present at the microparticle surface.



Figure S4. Additional CTC model samples (MCF-7 cells spiked into whole blood) analyzed using the microfluidic packed bed. The total number of cancer cells captured and enumerated in the packed bed exhibited a linear response across the physiologically relevant range with a capture efficiency $\approx 21\%$. This experiment was identical to the one shown in Figure 5, except that the whole blood was collected from a different healthy donor. This system response is identical to our previous work.



Figure S5. Cumulative cells captured as a function of position in the packed bed. The top line represents data from a single capture experiment (figure 5C) where 160 MCF-7 cells were spiked into 200 μ L whole blood and run through the packed bed of chitosan beads. The bottom line shows results of the control run where 200 μ L of whole blood that did not contain any MCF-7 cells were run through the packed bed.

Enumeration of Captured Cells for Figure 5:

Dil stained cancer cells were captured using a packed bed of chitosan microspheres as described in Figure 5. To analyze the data in Figure 5, fluorescent images of the column were taken using two different filter sets. The first filter cube (a; TRITC: excitation at (520-570) nm and emission at (535-675) nm) overlapped with the excitation and emission of membrane cell stain (DiI: excitation peak \approx 549 nm; emission peak \approx 565 nm). The second filter set (b; FITC: excitation at (450-490) nm and emission at (515-565) nm) did not detect the cell stain. The chitosan microspheres exhibited a broadband autofluorescence that was detected in both filter sets. Thus, the difference between the filter sets (c) allowed facile enumeration of the cells. Overlapping images at each point along the packed beds were manually stitched together (e.g. Figure 5a) and the cells were counted (e.g. Figure 5c; Figure S4) using a simple fluorescent intensity threshold in ImageJ.

