Supplementary Information

ESI 1. Biochip microfabrication and encapsulation

Masters for the microfluidic chips were fabricated by standard photolithography and soft lithography [9]. For that purpose, a hard-mask was obtained by depositing Au (5)/ Cr (100) onto glass and patterning by DWL 2000 (Heidelberg, Germany), followed by Cr chemical etching. A mask aligner MA6BA6 (Suss Microtec, Germany) was used to transfer the pattern onto 50 µm thick SU8 2050 (MicroChem, USA), using an exposure of 160 mJ/cm^2.

CNC (FlexiCAM, Germany) was used to machine a 3-layered PMMA holder, with the purpose of obtaining highly reproducible replicates with a thickness of 2mm, and including inlets and outlets, defined by metal plugs [16]. 10:1 silicon elastomer (Poly(dimetylsiloxane), PDMS) was mixed, degassed, and cast over the master inside the holder, prior to curing for 1h at 65ºC. Bonding of the microchannel to the glass slide was carried out by oxygen plasma for 15 s, low power (Harrick, USA). In parallel, bonding of the microchannel to the sensor chip was obtained activating the surfaces with a UV/ozone cleaner (Jelight, USA) for 15min before manual alignment of both components took place under a stereomicroscope.

ESI 2. Measurement setup

Signal from the sensors was sampled at 100 kHz, acquired by the electronic platform, recorded in real-time by the host PC, split and saved in 314 KB files. Such electronic platform is composed of a custom build alternating current (AC) coupled amplifier (10.000x gain, 8 kHz bandwidth), a commercial data acquisition (DAQ) model DT9836OEM (Measure Computing, USA) and filters [16].

ESI 3. Cell Culture

Cell culture SW480 cells derived colon adenocarcinoma cells were obtained from American Tissue Culture Collection (ATCC, USA) and cultured in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% fetal bovine serum, and 1% Penicillin-Streptomycin both from Gibco (Thermo Scientific, USA), at 37ºC in a 5% CO2 atmosphere. When reaching confluence, cells were harvested by incubation in 0.25% Trypsin-EDTA (from Sigma Aldrich, USA) and washed with PBS 2%BSA.

ESI 4. Numerical simulations of magnetic beads

Magnetic beads are magnetized by a constant out-of-plane field of 100mT and present a total magnetic moment (m_z). This moment is modelled as a dipole moment, centered in the MB. The SV sensor will sense the x-component of the magnetic bead fringe field. This field is given by:

$$H_x(x,y,z) = m_z \frac{3xz}{(x^2+y^2+z^2)^{5/2}}$$ (Eq. S1),

where *x* and *y* are the in-plane coordinates and *z* out-of-plane distance from the center of the MB and the sensor plane position.

The model simulates a sensor with dimensions of 200µm in the y-axis and 3µm along x-axis that averages the magnetic field, $\langle H_x \rangle$, at sequential coordinates along the x-direction, simulating the bead flowing over the sensor. The sensor output, ΔV, is then given by:

$\Delta V = S.i_{bias}.\langle H_x \rangle$ (Eq. S2), where *S* is the sensitivity of the sensor (dR/dH) and i_{bias} is the current applied to sensor.

For the simulation of the signal coming from labelled cells, 1730 beads were randomly distributed on a spherical surface with diameter measured of cells with half the bead's diameter. The average magnetic field $\langle H_X \rangle$ over the sensor's area was calculated by the sum of individual magnetic fields coming from each label.

ESI 5. Video of vertical flow focusing effect using two fluorophores.

Video is composed of two videos, top and bottom, of the same microchannel flow for two fluorescent channels. SRB was loaded in the sample inlet, the lateral flow rate was flowed with PBS, and FSS loaded in the vertical inlet at Q=(40,80,120). Trajectory of the videos are along x-axis (see Figure 1 b)), from the coordinates (x,y)=(4,0) to (x',y')=(7.5,0) focused on half of the heights microchannel. The top video fluorescence of the sample sample is laterally focused along the trajectory. When reaching the vertical inlet, (x'',y'')=(5.5,0), FSS fluorescence is observed from this point forward, observed on the bottom channel.

ESI 6. Average velocity determination using SV sensors

The variation of the linear velocities of 1µm beads with increased vertical flow rates were obtained and depicted in Figure S6 for three different flow conditions (20,40,Q_V), (30,60,Q_V), (40,80,Q_V). Detected beads can flow at different heights from the sensor and thus different velocities as indicated in Fig. 5(d), thus an average of 10 beads was taken in Figure 6 (a). Linear velocity was calculated for 10 signal events. The TOF value for each condition was obtained from one sensor S1 for a total of 100 detected events. For each given flow condition a linear increase in the velocity is observed with the increase vertical flow rate as expected. This in agreement with the expected vertical focusing of the sample. The system can be operated at high-speed flow conditions, reaching up to 7cm/s in the detection of single 1µm diameter MBs as observed in Fig. S6(a).

The average TOF of a population of 100 detected beads also shows a linear drop with the increased vertical flow rate, as shown in Figure 6(b). Taking the calibration presented here, and taking the TOF average of 100 beads one can deduct the average velocity of the beads in real time, showing the potential of the system for the real-time assessment of velocity of 1µm beads based only on the TOF acquired from one sensor as depicted in Fig.6 (a). For instance, obtaining an average to of 0.8ms for 100 beads, the linear velocity is around 1cm/s and the flow conditions of Q=(20,40,0).

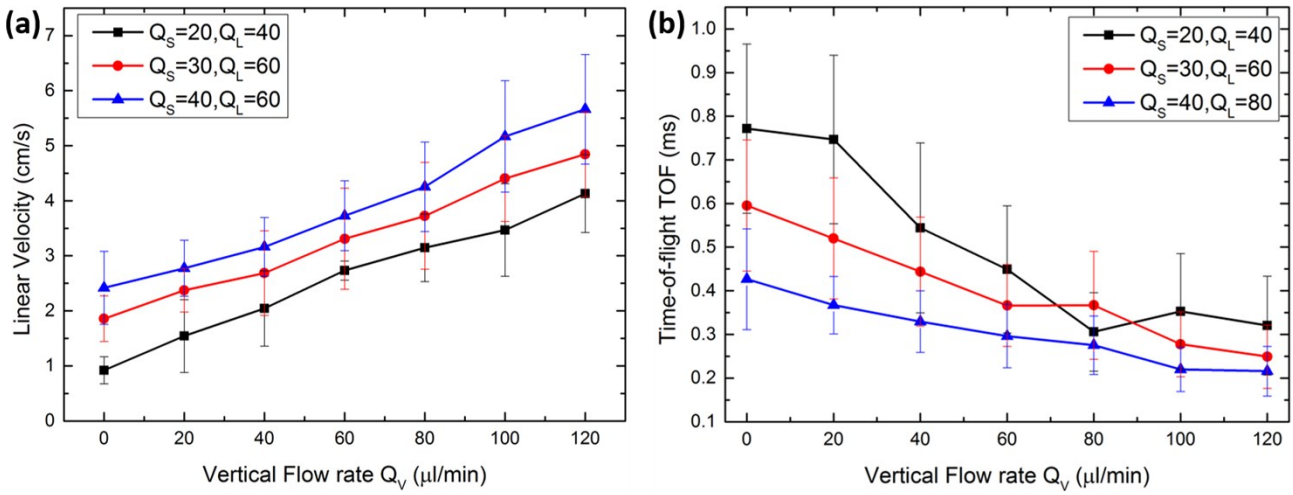

Fig. ESI 1. Experimental evaluation of linear velocity of 1µm magnetic beads. (a) Linear velocity of ten 1µm beads measured under different flow conditions and increased vertical flow rate. (b) Time-of-flight values of 100 detected events under different flow conditions and increased vertical flow rate.