Electronic Supplementary Information (ESI):

"Time-of-flight magnetic flow cytometry in whole blood with integrated sample preparation"

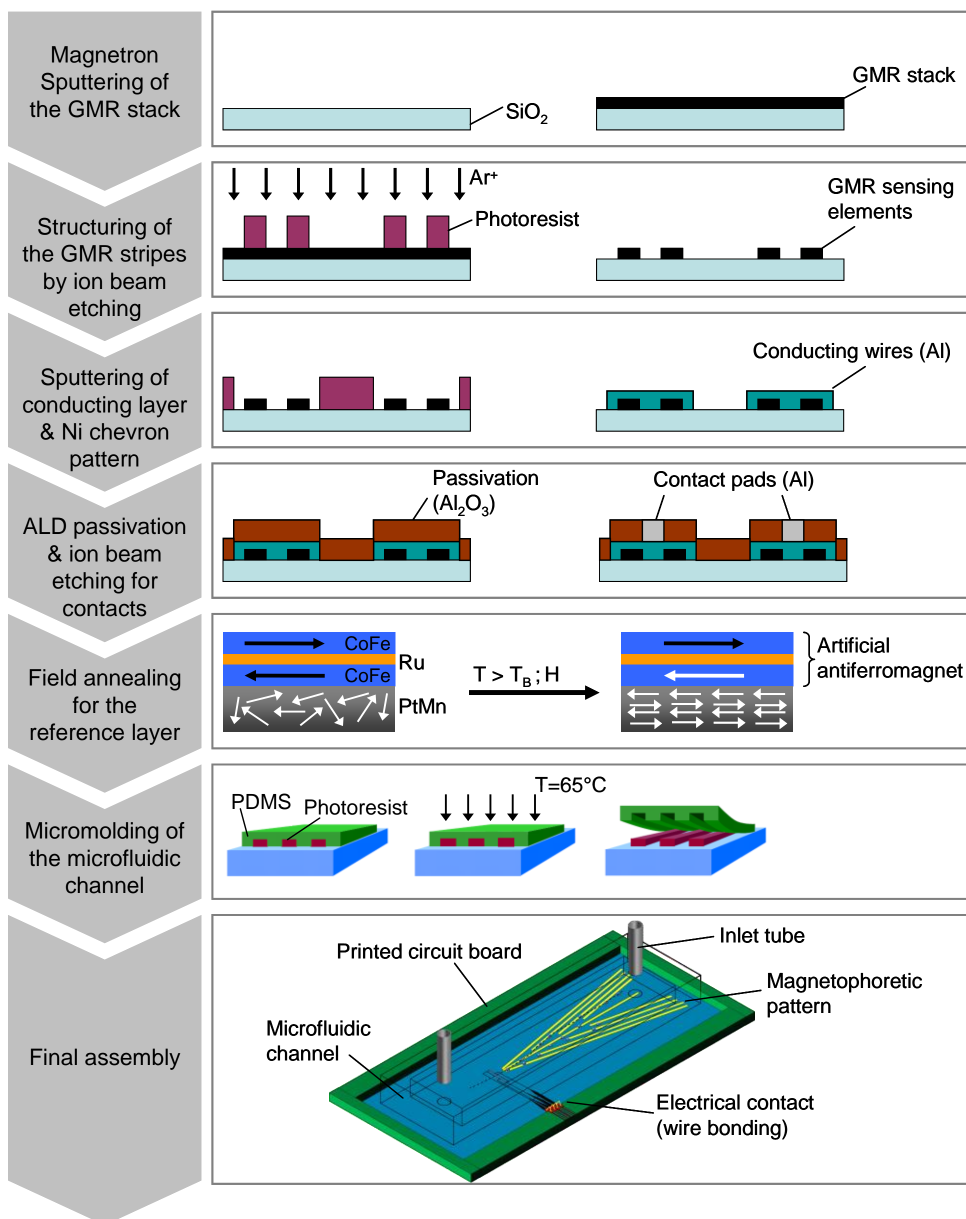

Fig. S1 Fabrication process of the magnetic flow cytometer. The GMR sensing elements, the Ni chevron pattern and the Al conducting layer are microstructured by optical lithography using sputter processes and ion beam etching on a Si wafer substrate with a 200 nm silicon oxide layer. The sensors are passivated with 70 nm Al_2O_3 by atomic layer deposition. Field annealing at 270°C and 400 kA m^{-1} is performed to pin the magnetization of the magnetically hard subsystem (artificial antiferromagnet) and thus ensure a high ridigity. The microfluidic channel is fabricated using a PDMS micromold technique from a photoresist master and bonded to the microfabricated dies.

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Vid. S2 Magnetophoretic guiding mechanism with 12 µm magnetic beads. The video shows a 100 µm wide section at at the end of the 200 nm thick Ni pattern, leading to a parallel Wheatstone bridge sensor. At a flow rate of 0.6 µL s^{-1} and an external field of 120 mT the beads are guided to the center of the microfluidic channel (700 µm x 200 µm) towards the 30 µm GMR stripes. Raw data stream and filtered stream show a strong overlapping of the characersitic bead signals illustrating the throughput limitations of the parallel sensor layout.

Vid. S3 Magnetophoretic cell enrichment of superparamagnetically labeled FaDu tumor cells. The analytes are guided to a diagonal GMR bridge at 0.4 µL s^{-1}, 150 mT external field and a Ni thickness of 300 nm.

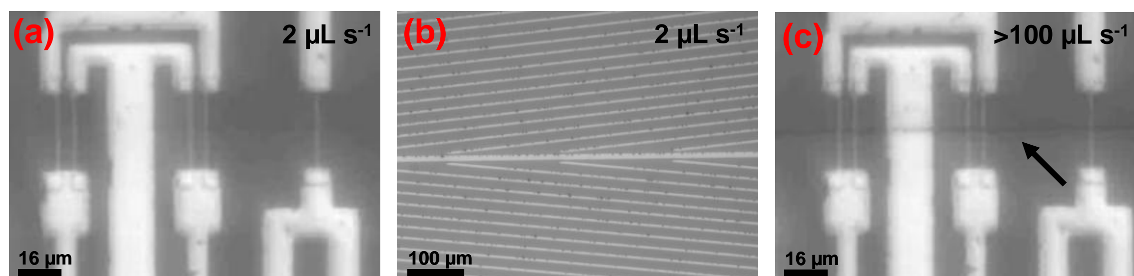

Fig. S4 In-situ background elimination. The mechanism to filter superparamagnetic particles is demonstrated with a high concentration of 200 nm labels (Stemcell Technologies, Germany) in PBS. (a) and (b) show microscopy images of the GMR sensor bridge and a ROI of the magnetophoresis area acting as a filter at a flow rate of 2 µL s^{-1}. The ferromagnetic Ni stripes retain the nanoparticles enabling a background free magnetic measurement. At flow rates >100 µL s^{-1} the fluidic drag force exceeds the retention force of the Ni stripes. The arrow in (c) indicates the position of enriched nanoparticles leaving the ferromagnetic Ni pattern flowing over the center of the GMR resistors.

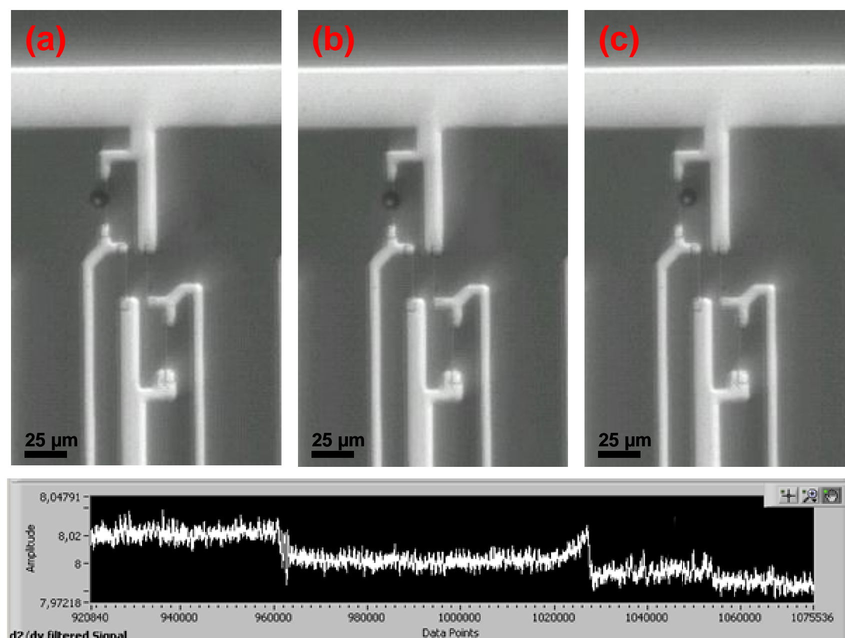

Fig. S5 Correlation between sensor response and bead position on a single GMR resistor. The magnetic bead was positioned over a single stripe of a diagonal GMR bridge. (a) Magnetic bead in front of the stripe results in an increase of the resistance caused by antiparallel orientation of the magnetic layers. (b) No signal response is observed with the bead centered on the GMR stripe. (c) A decrease of the resistance is measured with the bead opposite to position (a) with parallel orientation of the magnetic layers.

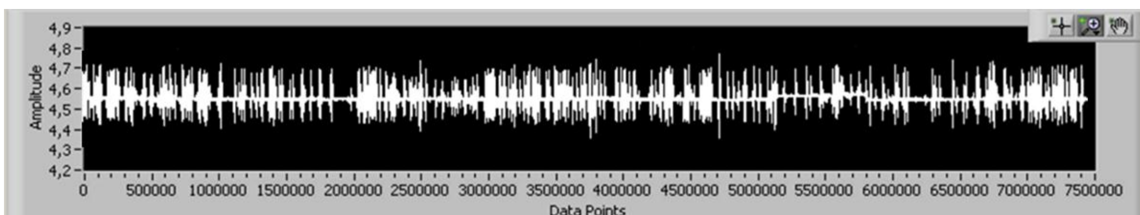

Fig. S6 Continous measurement stream (>12 minutes). Drift-free results are obtained with a diagonal GMR sensor design and 12 µm magnetic beads (Micromod) suspended in PBS.

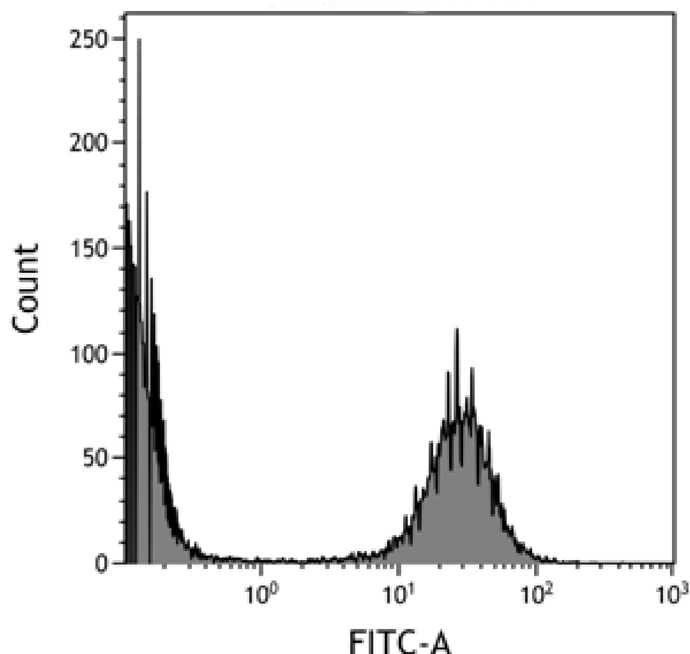

Fig. S7 Confirmation of Epcam expression on $EpCAM^+$ FaDu cells by standard fluorescent flow cytometry.
FaDu cancer cells were magnetically labeled using the EasySep® Human EpCAM Positive Selection Kit (Stemcell Technologies, Köln, Germany) according to the manufacturer's instructions. Subsequently, an aliquot of the labeled FaDu cells were analysed using magnetic flow cytometry as described in this paper. A parallel aliquot of the labeled FaDu tumor cells were stained with goat-anti-mouse IgG-FITC fluorescent monoclonal antibodies (Dianova, Hamburg, Germany). In parallel healthy donor EDTA whole blood samples were hemolyzed using a prepared lysis buffer (EasySep® Lysis Buffer, Stemcell Technologies, Köln, Germany). Next, the hemolyzed blood was further 1:1 diluted with PBS. Labeled FaDu cells were mixed in at a final concentration of $50x10^3$/ml hemolysed blood. Finally, the blood sample was analysed using standard fluorescent flow cytometry (FACS Canto, BD Biosciences, Heidelberg, Germany). The sample was acquired at 10.000 events and data analysed by standard software. Depicted in figure S7 is a representative histogram of FITC-$EpCAM^+$ FaDu cells in hemolyzed whole blood.

Materials and methods

The GMR stack Ta 4/ NiFe 3/PtMn 20/CoFe 2.5/Ru 0.8/CoFe 2.5/Cu 2.1/CoFe 0.8/NiFe 5/TaN 10 (thickness in nm), a spin valve system with artificial antiferromagnet, is deposited by magnetron sputtering. The GMR sensing elements are microstructered into stripes of 2 µm width and 30 µm length by optical lithography using ion beam etching and arranged as Wheatstone bridge configuration to avoid offset errors and temperature drifts.[1] To generate the unidirectional exchange bias field for the reference layer, the system is field-annealed at 270°C for 100 min in a magnetic field of 400 kA m^{-1} applied perpendicular to the long axis of the stripes. Under lower field conditions at 40 kA m^{-1} and a temperature of 220°C a crossed anisotropy is induced in the free layer along the stripe axis, which is essential for the sensing mechanism. The sensors are passivated with pinhole-free 70 nm Al_2O_3 deposited by atomic

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layer deposition to withstand highly corrosive conditions in whole blood and to ensure highest GMR sensitivity with a minimum sensor to analyte distance. The fabrication process flow is shown in the ESI, Fig. S1.

In order to measure the magnetic stray-field of labeled cells, a lock-in amplifier (Femto LIA-MVD-200-H) is used with a maximum gain of 90 dB. The sensor response is recorded at a sample rate of 10 kHz by an in-house LabVIEW software including a digital low-pass filter. Depending on H_z, the magnetic labeling of the cells and the thickness of the Ni guiding pattern, cells are detected with velocities up to 3 mm s^{-1}.

Cell cultivation and characterization of FaDu head and neck cancer cell line was performed as described in Stauber *et al*.[2] Expression of the EpCAM (epithelial cell adhesion molecule) cell surface protein was confirmed employing immunoblot and immunofluorescence microscopy.[2] The FaDu cells were incubated with superparamagnetic iron oxide nanoparticles (SPION), armed with antibodies directed against the EpCAM protein (Stemcell Technologies, Germany), for 30 min at 4°C in phosphate buffer (pH 7.4; PBS). Subsequently, EDTA blood provided by the University Hospital Erlangen and the Medical University Mainz, was spiked with magnetically labeled cells.

For reference measurements monodisperse superparamagnetic beads (Micromod Partikeltechnologie GmbH, Germany), are suspended in PBS. From alternating gradient magnetometer measurements the magnetic moment was determined to be ~1.8×10^{-9} emu for a single bead. Magnetic bead saturation was experimentally observed at an external field of 25 mT leading to a sensitivity of 1.7 µV mT^{-1} with a GMR effect of 6.6%.

References

1. S. Ganzer et al., *J. Appl. Phys.*, 2003, **93**, 6867-6869.
2. R. H. Stauber et al., *Oncotarget*, 2012, **3**, 31-43.