Details of the calculation of the dipolar field

As the GMR sensor is not a point-like object, the dipole field generated by a magnetic object flowing in the channel with a velocity vector along Oy must be integrated over the entire surface of the sensor located in the (xy) plane. Moreover, the anisotropic yoke shape of the sensor with a length L along x much greater than the width l along y, makes it insensitive to field variations along x or z.

Therefore, a magnetic object located at point (X_B,Y_B,Z_B) in the channel, with a moment perfectly μ_{obj} aligned in the direction Oz of the external magnetic field (the two polar and azimuthal angles θ and ϕ of the moment μ are zero) , creates a dipole field along Oy sensed by both top and bottom sensors. It can be expressed in a simplified way with respect to the original equation [Li et al]. Only the z-value, corresponding to the height of the object in the channel, differs in the equation.
For the bottom sensor, the height of the object in the channel is written:

$$h_{Bottom} = z_B + Z_{spacer\ Bottom}$$

where $Z_{spacer\ Bottom}$ is the thickness of Su8-2002 that separates the channel from the bottom sensors.

For the top sensor

$$h_{Top} = \mathrm{h} - z_B + Z_{spacer\ Top}$$

where h is the height of the channel, and $Z_{spacer\ Top}$ the thickness of optical glue NOA81 used to glue the top sensors to the channel.

Therefore, the relevant integrated signal due to a magnetic object, detected respectively by the "bottom sensor" and the "top sensor" can be expressed as follows:

$$H_{y(bottom)} = \frac{\mu_{obj}}{l \times L}\left(\frac{h_{bottom}}{q_2^2}\left(\frac{x_l}{r_1} - \frac{x_r}{r_2}\right) + \frac{h_{Bottom}}{q_4^2}\left(\frac{x_r}{r_3} - \frac{x_l}{r_4}\right)\right) \text{ (2a)}$$

(2a)

$$H_{y(top)} = \frac{\mu_{obj}}{l \times L}\left(\frac{h_{top}}{q_2^2}\left(\frac{x_l}{r_1} - \frac{x_r}{r_2}\right) + \frac{h_{top}}{q_4^2}\left(\frac{x_r}{r_3} - \frac{x_l}{r_4}\right)\right) \text{ (2b)}$$

$$x_r = \frac{L}{2} - x_B$$

$$x_l = -\frac{L}{2} - x_B$$

$$y_r = \frac{l}{2} - y_B$$

$$y_l = -\frac{l}{2} - y_B$$

$$r_1 = \sqrt{x_l^2 + y_l^2 + h_{sensor}^2}$$

$$r_2 = \sqrt{x_r^2 + y_l^2 + h_{sensor}^2}$$

$$r_3 = \sqrt{x_r^2 + y_r^2 + h_{sensor}^2}$$

$$r_4 = \sqrt{x_l^2 + y_r^2 + h_{sensor}^2}$$

$$q_2 = \sqrt{y_l^2 + h_{sensor}^2}$$

$$q_4 = \sqrt{y_r^2 + h_{sensor}^2}$$

Where h_{sensor}= h_{top} for the top sensor and $h_{Bottom}$for the bottom sensor

The calculations based on these equations of the passage height and the number of beads labeling a detected magnetic object are detailed in [Deroo et al., 2022]

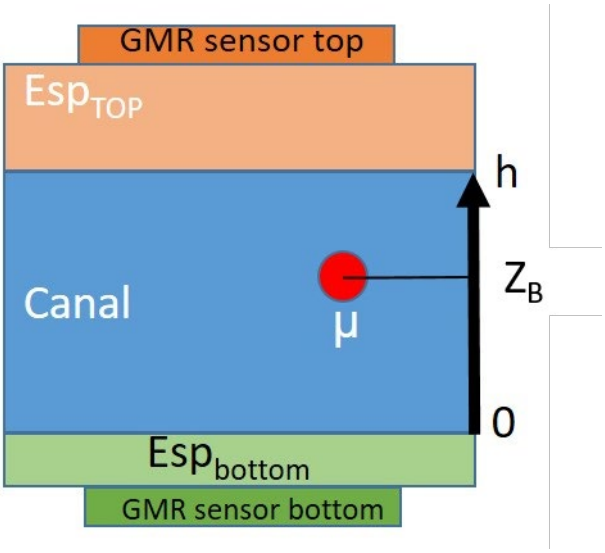

Figure S1: Scheme showing a magnetic object with a μ_{obj} moment in the microfluidic channel. $Z_{spacer\ Bottom}$(thickness of Su8-2002) and $Z_{spacer\ Top}$ (thickness of optical glue NOA81) are also shown.

The channel, whose height varies between 20 and 26 microns depending on the experiment, is separated from the top and bottom sensors respectively by around 3 µm of NOA81 and 2 µm of SU8-2002.

Details of the calculations of the calculation of the number of beads and of the magnetic moment:

Figure 6A of the previous paper [Lab Chip, 2022,22, 2753-2765] and Figure S2 simulations have shown that the signal produced by an biological object containing N superparamagnetic beads, of individual moment µ, randomly distributed on its surface, is the same as that of a point magnetic object whose magnetic moment μ_{obj} is N × µ. Magnetically labelled biological objects are therefore considered as point magnetic objects with a magnetic moment proportional to the number of beads that label them. The individual magnetic moment of the bead is given by the supplier but has also been obtained by vibrating sample magnetometer magnetization measurements.

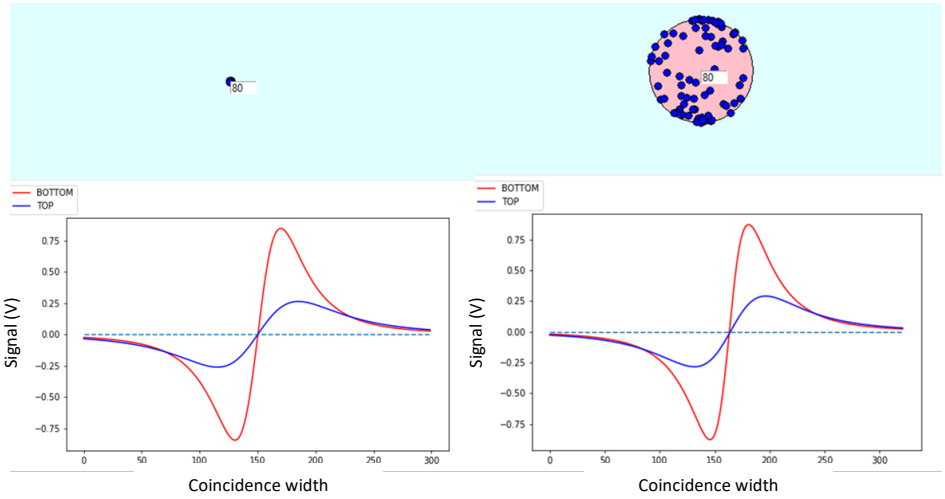

Figure S2: Simulations performed for the detection of a magnetic core containing 80 beads and a biological object 10 µm in diameter labelled by the same number of magnetic beads on the right. It can be seen that the signals emitted in these two cases are almost identical. This proves that biological objects can be considered as punctual objects

The total amplitude of each signal H_y(bottom) and H_y (top) (equation 2a and 2b) is proportional to μ_{obj} and then to the number N of magnetic beads contained in the object. The ratio of the top and bottom amplitudes eliminates μ_{obj}, leaving only the variable z_B. This ratio is numerically calculated as a function of z_B for 1000 values of z_B in the channel interval and shown in figure S3 of the previous paper

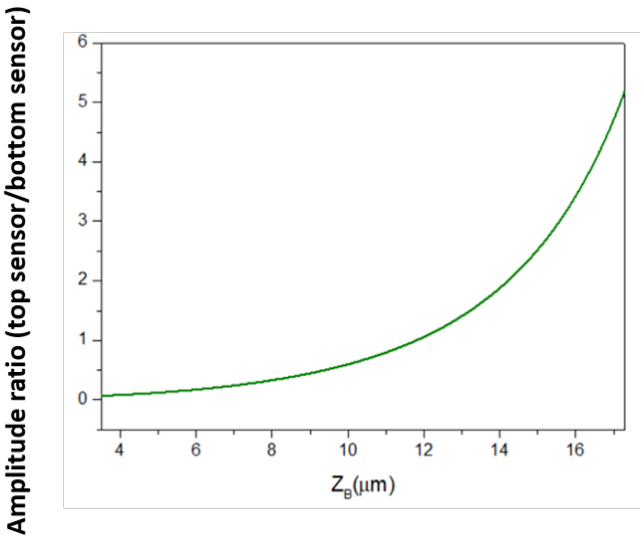

Figure S3 : Ratio between the amplitude of the high sensor signal and the amplitude of the low sensor signal for 1000 values of z_B in a channel of 20 µm. For each experimental ratio calculated, a flow height can therefore be extrapolated. The radius of the biological object is taken into account.

Thus, for each coincidence, this ratio allows to determine the height z_B in the channel of the object detected. The theoretical amplitude of the top signal (or bottom signal) of one bead of moment µ for this height z_B is therefore calculated. The ratio of the experimental top signal (or bottom signal) and of the theoretical top signal of one bead gives the number of beads N contained in the detected object. It is therefore possible to obtain the magnetic moment μ_{obj} equal to N×µ.

Simulations for different configurations in the channel of the cells labelled magnetically

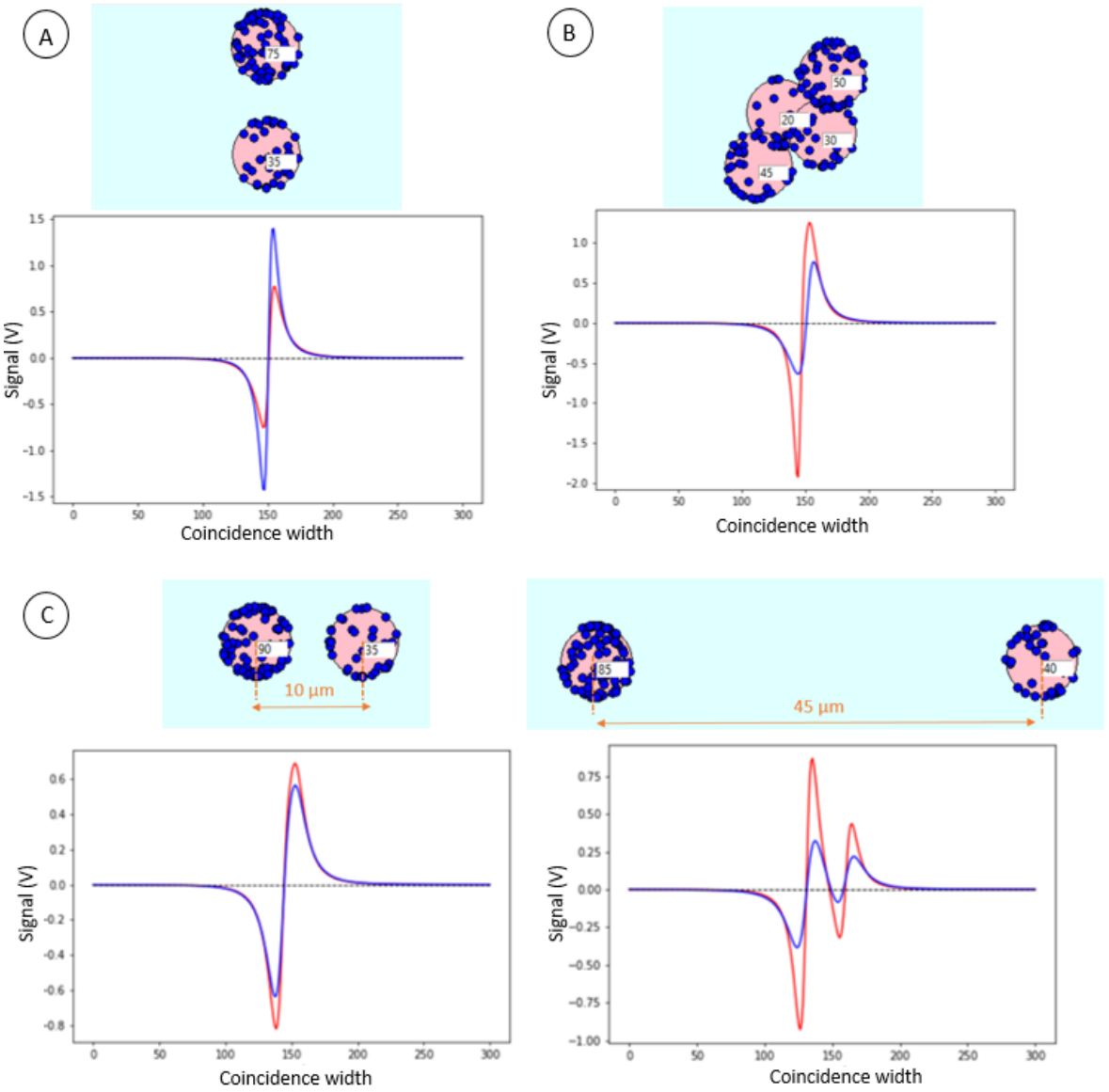

Figure S4 : A : Simulation representing two 7 µm-diameter cells labeled with a random number of magnetic beads passing one above the other in the microfluidic channel between a pair of sensors. The resulting signals are shown in blue for the upper GMR sensor and in red for the lower one. B : Simulation representing a cluster of four cells, each 7 µm in diameter, labeled with a number of random magnetic beads passing through the microfluidic channel between a pair of sensors. C : Simulation showing two cells, each 7 µm in diameter, labeled with a number of random magnetic beads spaced by 10 µm on the left and 45 µm on the right, passing through the microfluidic channel between a pair of sensors. Simulations A C D are also reported with different parameters in figure 6 of ref [21]

Labelling in various complex matrices

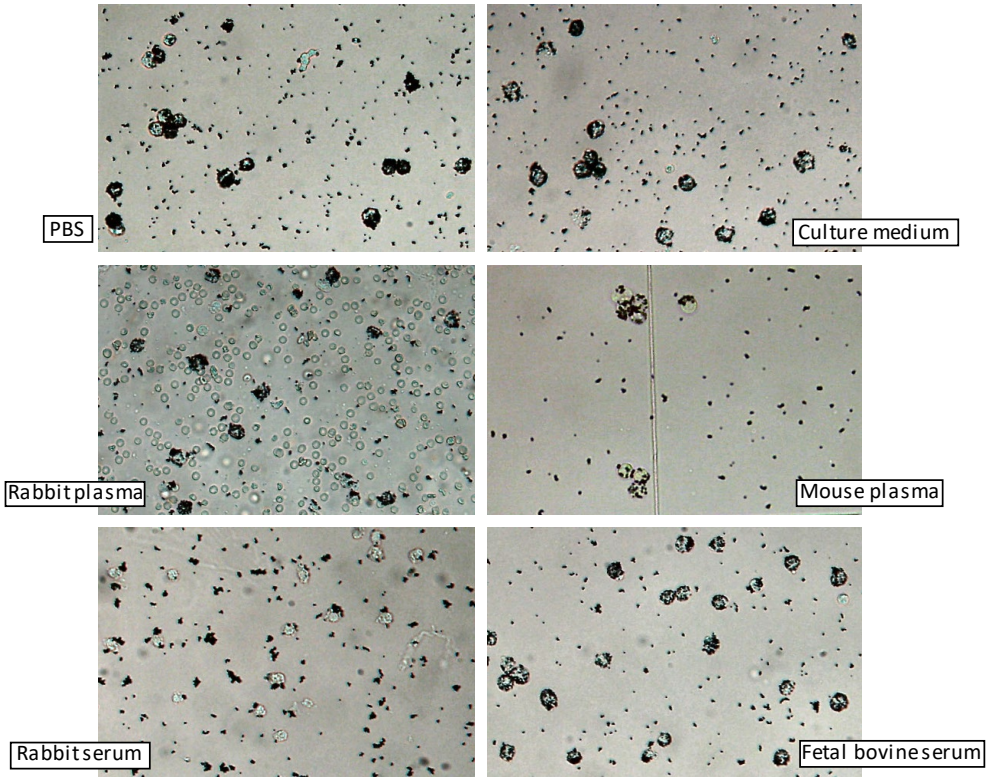

Figure S5: Pictures of NS1 cells after two hours in 1 mL of the indicated buffers with 1.5×10^7 magnetic beads Dynabead MyOne (1µm diameter) functionalized with the anti-CD138 antibody.

For all these matrices, except mouse plasma, a suspension of $5\ 10^4$ NS1 and $1.7\ 10^6$ Dynabead MyOne -anti-CD138 was mixed for 2 hours at 20°C in 1 mL of buffer, then a 100 µL sample was left to sediment for 15 minutes before being photographed 4 times for visual analysis. For mouse plasma, only 30 µL of solution was prepared using the same proportions. In culture medium, calf serum, mouse plasma and rabbit plasma, the magnetic beads immunocaptured the cells within two hours and the number and size of aggregates were comparable to the control suspension in PBS. In rabbit serum, on the other hand, few magnetic beads labelled their target and numerous aggregates have been formed. This preliminary experiment highlights the importance of investigating various complex matrices used in clinical studies.