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Integrated MicroHall Magnetometer to Measure Magnetic Properties of Nanoparticles

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Abstract

Magnetic nanoparticles (MNPs) are widely used in biomedical and clinical applications, including medical imaging, therapeutics, and biological sample processing. Rapid characterization of MNPs, notably their magnetic moments, should facilitate optimization of particle synthesis and accelerate assay development. Here, we report a compact and low-cost magnetometer for fast, on-site MNP characterization. Termed integrated microHall magnetometer (μ HM), our device was fabricated using standard semiconductor processes: an array of Hall sensors, transistor switches, and amplifiers were integrated into a single chip, thus improving detection sensitivity and facilitating chip operation. By applying the μ HM, we demonstrate versatile magnetic assays. We measured magnetic susceptibility and moments of MNPs using small sample amounts (~ 10 pL), identified different MNP compositions in mixtures, and detected MNP-labeled single cells.

Graphical Abstract

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CONTRIBUTION

C.M., J.P., H.S.L., R.W., C.M.C., and H.L. designed the study, analyzed the data, and prepared the figures. C.M. and J.P. prepared the samples and performed the magnetic measurements. C.M. designed the semiconductor Hall device, set up the measurement, and fabricated microfluidic chip. C.M., J.K.M., J.W.L., D.M. K., and H.K. A. fabricated the semiconductor Hall device. Y. L., T.-H. S., and J.C. synthesized magnetic nanoparticles. C.M., J.P., and J.M. performed immunofluorescence imaging. All authors wrote and reviewed the manuscript.

We developed an integrated Hall magnetometer that can measure magnetic properties of small volumes of samples.

INTRODUCTION

The biological and clinical use of magnetic nanoparticles (MNPs) continues to increase with their use in cell sorting^{1–4}, DNA sequencing⁵, biomedical imaging^{6–8}, and therapeutics^{9,10}. MNPs generate excellent contrast within inherently non-magnetic biological systems, which enables highly selective, interference-free actuation or detection and separation^{1,2,11–14}. The biomedical usefulness of different MNPs is generally determined by their surface properties and magnetic moment^{15–22}. The latter in particular requires frequent monitoring during particle synthesis, process optimization and quality-control thereafter. Conventional magnetometers (e.g., superconducting quantum interference device, vibrating sample magnetometer) are generally used for such measurements, but their availability is often limited to specialized laboratories given their cost. Perhaps more importantly, bench-top analytical equipment often requires large amounts of MNPs (e.g., milligram amounts often in a powder form); producing these amounts is often impractical in the particle developmental phase^{23,24}.

We reasoned that Hall-effect magnetometers can be adopted for point-of-use MNP characterization. Hall magnetometry offers many practical advantages. It assumes a linear response across many orders of magnetic field strength²⁵. Large polarizing magnetic fields (>1.0 T) that effectively magnetize MNPs, can therefore be applied without saturating the sensors²⁶. Hall sensors can thus be used to measure entire magnetic responses of MNPs (e.g., saturation magnetization). Sensor fabrication is also compatible with standard semiconductor technology^{13,25,27,28}. Sensor arrays as well as signal processing electronics can be integrated into the same chip to achieve high signal-to-noise ratio (SNR) and throughput. The entire detection system can thus be realized as a self-contained, cost-effective magnetometer platform. As a biosensing technique, Hall detection can achieve high signal contrast due to the negligible intrinsic magnetic moments of biological objects. Measurements can be performed with minimal sample preparation, which reduces sample loss and simplifies assay procedures.

We herein report an integrated microHall (μ HM) system developed for micro-magnetometry. The μ HM consists of an integrated chip containing an array of Hall sensors and auxiliary circuits. Each Hall sensor attains micrometer-length scale and is connected to an on-chip amplifier, thus improving detection sensitivity for small sample volumes. We operated the chip in alternating current (AC) mode, thereby selectively measuring signals from MNPs while suppressing background. Importantly, we devised a new detection scheme that allowed us to: i) estimate MNPs' average saturation magnetic moment (μ_p), and ii) differentiate MNP types within a mixture. We applied the μ HM to measure the magnetic moment of various types of MNPs; the measurement only required ~ 1 pL of MNP samples to produce μ_p values. We further applied the system to profile single cancer cells labeled with MNPs. The developed μ HM could thus be positioned as an affordable and accessible characterization tool for MNP development and biosensing platform.

RESULT

Integrated microHall chip

We implemented an integrated microHall chip (Fig. 1a, left) containing Hall elements, switches, and amplifiers (Fig. S1). As proof of concept, sixteen Hall elements were arranged into a 4×4 array to minimize the number of output ports. The sensing area of each Hall element is $7 \mu\text{m} \times 7 \mu\text{m}$; the effective sensing volume was estimated $\sim 2100 \mu\text{m}^3$ (see SI notes). For each column in the array, four Hall elements shared a current bias line, and the output from a single Hall element was selected by transistor switches (Fig. 1a, right). With this design, the number of required ports to external devices could be reduced by four times (from 64 to 16).

To guide sample loading above each Hall element, we created an array of fluidic wells and placed them on top of the Hall chip (Fig. 1b). Each well had a lateral dimension of $20 \mu\text{m} \times 20 \mu\text{m}$, and a depth of $25 \mu\text{m}$. This volume ($10000 \mu\text{m}^3$) was larger than the effective sensing volume of a Hall element, which maximized the sensor filling factor (~ 1). The assembled sensor was mounted on a custom-designed carrier. AC magnetic field was generated by a coil attached underneath the sensor, and an adjustable direct-current (DC) magnetic field was applied by an electromagnet.

Hall magnetometry

We set up the μ HM system to measure volume magnetic susceptibility (χ_v) of MNPs (Fig. 1c). Specifically, MNPs placed on top of the μ HM, were magnetized by a static DC magnetic field (H_{DC}). Directly measuring MNP magnetization, however, was technically challenging, because the Hall generated much larger signal in response to H_{DC} . To extract signal from MNPs, we thus applied lock-in detection^{29,30}; a probing alternating current (AC) field was superimposed to H_{DC} , and Hall voltage (V_H) was measured at the AC field frequency. The resulting V_H is proportional to the slope of MNP magnetization (M) curve at the given H_{DC} ($V_H \sim dM/dH \sim \chi_v$; Fig. 1d). By sweeping H_{DC} , the overall magnetic susceptibility could be measured to construct the magnetization curve (see SI note for details).

Hall magnetometry of various magnetic particles

We next applied the μ HM system to characterize different MNP types. Ferrite MNPs of different sizes and composition (e.g., transition metal-doped ferrite) were synthesized to vary magnetic properties (Fig. 2a, see Methods). We also used various commercial magnetic beads embedded with maghemite (γ -Fe₂O₃) MNPs. The particle solution was directly loaded over the μ HM. Different Hall elements in the array were used for different particle types, thus speeding up measurements (Fig. S2). The Hall voltage was measured using the lock-in detection scheme. The DC magnetic field was swept from -200 to 200 kA/m.

Figure 2b shows normalized χ_v curves for different magnetic particles. For superparamagnetic particles, the normalized χ_v curve can be approximated as (see SI note for details)

$$\chi_v = 3\chi_{v0} \cdot \left[-\text{csch}^2 \left(\frac{\mu_0 \mu_p H_{DC}}{kT} \right) + \left(\frac{\mu_0 \mu_p H_{DC}}{kT} \right)^{-2} \right], \quad (1)$$

where χ_{v0} is the susceptibility at $H_{DC} = 0$, μ_0 is the vacuum permeability, k is Boltzmann constant, T is temperature, and μ_p is the average magnetic moment of individual MNPs^{30,31}. By fitting measured data to Eq. (1), we could construct a magnetization curve M for each particle type (Fig. 2c). Magnetic beads containing maghemite (γ -Fe₂O₃) MNPs were quickly saturated at lower H_{DC} , presumably due to their low magnetic anisotropy³². Conversely, CoFe₂O₄ MNPs, whose material has high magnetic anisotropy, showed slower magnetization response.

Characterization of magnetic particles

We further noted that the normalized response curve (χ/χ_{v0}) is a function of MNPs' magnetic moment (μ_p) only. Particularly, the full-width at half maximum (FWHM) of χ_v is inversely proportional to μ_p with

$$H_{FWHM} = 4.16 \cdot kT \cdot (\mu_0 \mu_p)^{-1}. \quad (2)$$

We reasoned that this metric can be used to estimate μ_p of MNPs regardless of sample type or loading volume. To test the hypothesis, we measured two types of particles: free γ -Fe₂O₃ MNPs and polystyrene microbeads labeled with γ -Fe₂O₃ MNPs. Indeed, the χ_v curves from the μ Hall detection were nearly identical (Fig. 3a), and extracted μ_p values were comparable to each other: 0.41×10^{-18} A·m² (MNPs only); 0.39×10^{-18} A·m² (MNPs on microbeads). We extended the measurements to other MNPs and commercial magnetic beads embedded with γ -Fe₂O₃ MNPs. Overall, γ -Fe₂O₃ MNPs in commercial magnetic beads showed higher μ_p values than Zn_{0.6}Fe_{2.6}O₄ and CoFe₂O₄ MNPs, presumably due to their large crystal size (~ 22 nm).

Measurement on mixture of magnetic particles

We next extended the $\mathcal{H}M$ measurement for multiplexed MNP detection. The $\mathcal{H}M$ measures a single parameter (i.e., magnetic susceptibility), which makes it difficult to perform multiplexed detection. We reasoned that such limitations could be overcome by employing i) MNPs with different magnetic susceptibilities, analogous to a distinct color in optical measurements, and ii) acquiring magnetic responses over sweeping external magnetic fields. When different MNP type (i) of known magnetization (μ_i) are mixed, the total magnetization M is written as

$$M = \sum_i f_i \mu_i L(\mu_i, H_{DC}), \quad (3)$$

where f_i is the number fraction of the particle type i ($\sum f_i = 1$) in a given sample volume, and L is Langevin function. We approximated M as a single functional form with an effective magnetic moment μ_{est}

$$M \simeq \mu_{est} L(\mu_{est}, H_{DC}). \quad (4)$$

Note that μ_{est} value can be obtained from the width of χ_v peak measured by $\mathcal{H}M$. With known μ_i and μ_{est} , the fraction of each MNP type (f_i) can be estimated by solving a set of equations, $\mu_{est} L(\mu_{est}, H_{DC}) = \sum f_i \mu_i L(\mu_i, H_{DC})$ measured at different H_{DC} . For the mixture of two particle types ($i = 1, 2$), the process can be further simplified to solving f_1 from a single linear equation $\mu_{est} \approx f_1 \mu_1 + (1 - f_1) \mu_2$. Note that the relative error in f_1 is equal to f_1/f_1 , as the condition number of the matrix is 1.

We applied the model to the mixture of two particle types: $Zn_{0.4}Fe_{2.6}O_4$ ($i = 1$), $\gamma\text{-Fe}_2O_3$ ($i = 2$). We first investigated the accuracy of the model. We measured the χ_v curve for each particle type alone, and obtained μ_i values for $Zn_{0.4}Fe_{2.6}O_4$ and $\gamma\text{-Fe}_2O_3$ MNPs. We then mixed the particles, and obtained μ_{est} from the χ_v curve (Fig. 4a). The measured μ_{est} matched well with the expected magnetic moments (μ_{avg}) of two particle mixture, $\mu_{avg} = f_1 \mu_1 + (1 - f_1) \mu_2$, over varying f_1 ranges (Fig. 4b); this result supports the use of a single functional form (Eq. 4) to approximate the magnetization of a MNP mixture. Consequently, the number fraction of MNPs in a mixture could be estimated from the measured μ_{est} (Fig. 4c).

Biological applications

As translational proof-of-concept, we applied the $\mathcal{H}M$ system to detect mammalian cancer cells. We first calibrated the sensor output by measuring Hall signals (at $H_{DC} = 0$ kA/m) at different MNP concentrations (Fig. 5a). The Hall voltage level was linearly proportional to MNP numbers, which enabled quantitative comparison on levels of targeted molecules. We next profiled single cancer cells by targeting the epidermal growth factor receptor (EGFR) and epithelial cell adhesion molecule (EpCAM). Ovarian cancer cells (OVCA420) were incubated with biotinylated antibodies and subsequently labeled with streptavidin-coated magnetic particles. After removing excess particles, we loaded cells to the $\mathcal{H}M$ device

through the microfluidic well structure. Figure 5b shows single-cell magnetization curves for different markers. Higher slope at zero DC magnetic field and saturation magnetization indicated higher number of MNPs present in the cell. We also used samples incubated with control IgG to account for non-specific binding. The expression levels of corresponding target molecules (EGFR, EpCAM) were quantified by subtracting baseline output hall voltage from output hall voltage at zero DC magnetic field (Fig. 5c). To validate the results, we also imaged cells labeled with fluorescent antibodies (Fig. S3). The observed fluorescent intensities from the imaging matched well with the μ HM results.

CONCLUSIONS

We developed the μ HM system for rapid, multiplexed, small-volume magnetometry. The detection chip was implemented with standard semiconductor technology, integrating an array of microHall elements and other circuits (i.e., on-chip switches, amplifiers) for chip control. We operated the device in frequency lock-in mode with AC magnetic field superimposed to strong DC magnetizing field. This strategy enabled us to measure the average magnetic moments of individual MNPs directly from a small volume (~ 1 pL) of MNP solution. By deconvoluting magnetic responses over a range of external magnetic fields, we could identify MNP types and their fractional ratios in a mixture. This capacity could be used, for instance, to profile the same cells for different biomarkers, or to detect multiple biomarkers in a single parent sample. By tagging MNPs with existing or emerging cancer markers, μ HM is poised for facile molecular profiling of single cancer cells to expand the breadth and depth of cancer diagnostics available for preclinical and clinical research.

We expect several technical improvements to further advance μ HM technology. First, implementing a monolithically integrated chip, containing Hall elements, coils, amplifiers, and signal processing units, would improve detection sensitivity, SNR, and assay throughput. Since Hall sensor has a differential output, load balancing of analog amplifiers is an important factor to determine SNR. Thus, higher SNR by the system could be achieved by reducing parasitic components from wire connections and electromagnetic interference. Integrating a large number of Hall sensors in a single chip could increase assay throughput and efficiency of single cell analyses. Second, the capacity of magnetic multiplexing could be expanded. We note that μ HM can measure both magnetic susceptibility and saturation magnetic moment. Leveraging these properties through MNP size and composition control, it is conceivable to create a panel of MNPs with distinct magnetic signatures. These particles could be used, for example, to simultaneously profile different molecular markers in individual cells. With such improvements, the μ HM could serve as a convenient tool for both MNP characterization and point-of-care biosensing.

EXPERIMENTAL SECTION

Semiconductor wafer

High mobility wafers were custom-grown (Electronics and Telecommunications Research Institute, Korea) via molecular beam epitaxy on a semi-insulating GaAs substrate³³. Following layers were formed: 5000 Å GaAs buffer, 30 periods of the AlGaAs/GaAs superlattices, an undoped Al_{0.23}Ga_{0.77}As buffer, silicon planar doping (1×10^{12} cm⁻²), a 20

Å Al_{0.23}Ga_{0.77}As spacer, a 120 Å In_{0.2}Ga_{0.8}As channel, a 35 Å Al_{0.23}Ga_{0.77}As spacer, silicon planar doping ($4.5 \times 10^{12} \text{ cm}^{-2}$), and a 250 Å Al_{0.23}Ga_{0.77}As Schottky contact layer. Finally, a 400 Å thick undoped GaAs cap layer was grown for protection against oxidation. The sheet carrier density of two-dimensional electron gas and the electron mobility measured at room temperature were $3.4 \times 10^{12} \text{ cm}^{-2}$ and $5750 \text{ cm}^2/\text{V}\cdot\text{s}$, respectively.

Hall chip fabrication

The structure for Hall elements was defined by conventional photolithography, followed by wet etching ($\text{H}_3\text{PO}_4/\text{H}_2\text{O}_2/\text{H}_2\text{O} = 4/1/50$). Metal layers (AuGe/Ni/Au, 1000/300/1200 Å) were patterned, and ohmic contacts to Hall elements were made via rapid thermal annealing in N₂ atmosphere (320 °C, 20 sec; 430 °C, 20 sec). We formed three separate metal layers. For the first metal layer including transistor gate electrode and interconnection line, Ti/Pt/Au layers (400/200/3500 Å) were patterned, and insulated with a SiN_x layer (500 Å) grown by plasma-enhanced chemical vapor deposition (PECVD). The second metal layer had Ti/Au (400/4000 Å) and PECVD-grown Si₃N₄/SiO₂ layers (500 Å/1000 Å). The top metal had Ti/Au (400/10000 Å) layers, and was covered with PECVD-grown Si₃N₄ (1000 Å). The final protection layer (SiO₂, 2000 Å) was deposited via atomic layer deposition. Metal contact areas were opened via wet etching with a buffered oxide etchant (90 sec). Al wires were bonded to connect Hall sensors to printed circuit boards.

Fluidic structure

We used a standard soft lithography method to make the microfluidic multi-well structure. A negative fluidic mold was patterned on a Si wafer. In brief, an epoxy-based resist (SU-8 2025, Microchem) was spin-coated (3,500 r.p.m, 30 sec), and fluidic patterns were defined lithographically. To make the fluidic device, the fabricated mold was chemically treated with trichlorosilane vapor (30 min). Polydimethylsiloxane (PDMS), mixed with a curing agent with a 10:1 weight ratio and degassed, was poured on the mold and cured on a hotplate at 60 °C (4 hours).

Hall magnetometry setup

We used an electromagnet (3470, GMW) to generate an adjustable DC magnetic field (-0.3 T to 0.3 T). The current to the electromagnet was provided by a bipolar power supply (BOP 50-8ML, KEPCO). We used a custom-designed routine in MATLAB (MathWorks) for control. For the AC field generation, we made a coil (35 turns; diameter, 17 mm) and placed it under the iMH chip. The coil was excited with a sinusoidal input (1 kHz, 2.425 V peak-to-peak). The bias current (500 μA) to the Hall sensor was supplied by a DC current source (2400, Keithley), and the sensor output was fed to a voltage amplifier (5113, Signal Recovery), and then the amplified signal was demodulated by lock-in amplifier (1 kHz; integration time, 2 sec). We set our integration time to 2 sec, as it achieved a voltage resolution ($= 0.1 \text{ μV}$) larger than the noise level. All data were automatically recorded with MATLAB.

MNPs syntheses

Zinc-doped ferrite MNPs ($\text{Zn}_{0.4}\text{Fe}_{2.6}\text{O}_4$) and cobalt-doped ferrite MNPs (CoFe_2O_4) were synthesized as previously reported methods^{34,35}. For carboxymethyl (CM) dextran coating, MNPs (5 mg_(metal)) were first mixed with tetramethylammonium hydroxide (TMAOH) butanol solution (2 M, 2 mL) and sonicated for 1 hour. Then, the MNPs were collected by centrifugation ($1500 \times g$, 5 min), washed with excess amount of acetone and hexane, dispersed in CM dextran aqueous solution (70 mg/mL, 5 mL), and heated for overnight at 70 °C. The resulting CM dextran-coated MNPs were purified by centrifugal filter. To further conjugate streptavidin to CM dextran-coated MNPs, 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, 50 mM), N-hydroxysulfosuccinimide (sulfo-NHS, 5 mM) and streptavidin (1 mg) were added and incubated for 2 hours at room temperature. The final product was purified by MACS column. TMAOH, streptavidin (Sigma-Aldrich); EDC, sulfo-NHS (Thermo Fisher Scientific); CM dextran (10 kDa, PK chemicals A/S); MACS column (Miltenyi Biotec); Amicon Ultra centrifugal filters (100 kDa molecular weight cut-off/MWCO, Merck Millipore) were used as received.

Biotinylation of labeling antibodies

Sulfo-NHS-biotin (10 mM, Pierce) solution in phosphate buffer saline (PBS) was incubated with antibodies for 2 hours at room temperature. Unreacted sulfo-NHS-biotin was removed using Zeba spin desalting column, 7K MWCO (Thermo Scientific). Antibodies were kept at 4 °C until use.

Cell labeling

OVCA420 ovarian cancer cell line was maintained in RPMI 1640 medium with 10% fetal bovine serum (Atlas Biologicals, S12450), supplemented with L-glutamine $1 \times$ (Corning Mediatech, 25-005-CI). Cells were cultured in a standard humidified incubator at 37°C in a 5% CO₂ atmosphere. Cells were detached from culture dish by trypsin with 0.05% EDTA (Corning Mediatech, 25-052-CI) before labeling with MNPs. Cancer cells were first washed with PBS, and then fixed with a 3:1 mixture of PBS with a paraformaldehyde for 20 mins at room temperature. The fixed cells were washed and blocked with a blocking buffer (1% bovine serum albumin/BSA with PBS) for 30 min at room temperature. The washed cells were incubated with antibody-biotin conjugates (5 µg/mL, 1% BSA with PBS) for 30 min, and excess antibodies were removed via centrifugation ($350 \times g$, 5 min). Finally, cells were incubated with magnetic particles (30 min, 20 °C), and triple washed via centrifugation ($350 \times g$, 5 min). All labeling experiments and measurements were performed at least three times.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Loureiro J, Andrade PZ, Cardoso S, da Silva CL, Cabral JM, Freitas PP. Magnetoresistive chip cytometer. *Lab Chip*. 2011; 11:2255–2261. [PubMed: 21562656]
2. Adams JD, Kim U, Soh HT. Multitarget magnetic activated cell sorter. *Proc. Natl. Acad. Sci. U. S. A.* 2008; 105:18165–18170. [PubMed: 19015523]
3. Issadore D, Chung J, Shao H, Liong M, Ghazani AA, Castro CM, Weissleder R, Lee H. Ultrasensitive clinical enumeration of rare cells ex vivo using a micro-hall detector. *Sci. Transl. Med.* 2012; 4:141ra92.
4. Issadore D, Chung HJ, Chung J, Budin G, Weissleder R, Lee H. μ Hall chip for sensitive detection of bacteria. *Adv. Healthc. Mater.* 2013; 2:1224–1228. [PubMed: 23495188]
5. Ding F, Manosas M, Spiering MM, Benkovic SJ, Bensimon D, Allemand JF, Croquette V. Single-molecule mechanical identification and sequencing. *Nat. Methods*. 2012; 9:367–372. [PubMed: 22406857]
6. Tassa C, Shaw SY, Weissleder R. Dextran-coated iron oxide nanoparticles: a versatile platform for targeted molecular imaging, molecular diagnostics, and therapy. *Acc. Chem. Res.* 2011; 44:842–852. [PubMed: 21661727]
7. Weissleder R, Elizondo G, Wittenberg J, Lee AS, Josephson L, Brady TJ. Ultrasmall superparamagnetic iron oxide: an intravenous contrast agent for assessing lymph nodes with MR imaging. *Radiology*. 1990; 175:494–498. [PubMed: 2326475]
8. Harisinghani MG, Barentsz J, Hahn PF, Deserno WM, Tabatabaei S, van de Kaa CH, de la Rosette J, Weissleder R. Noninvasive detection of clinically occult lymph-node metastases in prostate cancer. *N. Engl. J. Med.* 2003; 348:2491–2499. [PubMed: 12815134]
9. Derfus A, von Maltzahn G, Harris T, Duza T, Vecchio K, Ruoslahti E, Bhatia S. Remotely Triggered Release from Magnetic Nanoparticles. *Adv. Mater.* 2007; 19:3932–3936.
10. Park JH, von Maltzahn G, Zhang L, Schwartz MP, Ruoslahti E, Bhatia SN, Sailor MJ. Magnetic Iron Oxide Nanoworms for Tumor Targeting and Imaging. *Adv. Mater.* 2008; 20:1630–1635. [PubMed: 21687830]
11. Lee H, Sun E, Ham D, Weissleder R. Chip-NMR biosensor for detection and molecular analysis of cells. *Nat. Med.* 2008; 14:869–874. [PubMed: 18607350]
12. Gaster RS, Hall DA, Nielsen CH, Osterfeld SJ, Yu H, Mach KE, Wilson RJ, Murmann B, Liao JC, Gambhir SS, Wang SX. Matrix-insensitive protein assays push the limits of biosensors in medicine. *Nat. Med.* 2009; 15:1327–1332. [PubMed: 19820717]
13. Liu P, Skucha K, Megens M, Boser B. A CMOS Hall-Effect Sensor for the Characterization and Detection of Magnetic Nanoparticles for Biomedical Applications. *IEEE Trans. Magn.* 2011; 47:3449–3451. [PubMed: 25308989]
14. Liu PP, Skucha K, Duan Y, Megens M, Kim J, Izyumin II, Gambini S, Boser B. Magnetic Relaxation Detector for Microbead Labels. *IEEE J. Solid-State Circuits*. 2012; 47:1056–1064. [PubMed: 25308988]
15. Lee H, Shin TH, Cheon J, Weissleder R. Recent Developments in Magnetic Diagnostic Systems. *Chem. Rev.* 2015; 115:10690–10724. [PubMed: 26258867]
16. Issadore D, Park YI, Shao H, Min C, Lee K, Liong M, Weissleder R, Lee H. Magnetic sensing technology for molecular analyses. *Lab Chip*. 2014; 14:2385–2397. [PubMed: 24887807]
17. Shao H, Min C, Issadore D, Liong M, Yoon TJ, Weissleder R, Lee H. Magnetic Nanoparticles and microNMR for Diagnostic Applications. *Theranostics*. 2012; 2:55–65. [PubMed: 22272219]
18. Min C, Shao H, Liong M, Yoon TJ, Weissleder R, Lee H. Mechanism of magnetic relaxation switching sensing. *ACS Nano*. 2012; 6:6821–6828. [PubMed: 22762250]
19. Min C, Shao H, Issadore D, Liong M, Weissleder R, Lee H. Diagnostic Magnetic Resonance Technology. *Point-of-Care Diagnostics on a Chip*. 2013:197–222.
20. Srinivasan B, Li Y, Jing Y, Xu Y, Yao X, Xing C, Wang JP. A detection system based on giant magnetoresistive sensors and high-moment magnetic nanoparticles demonstrates zeptomole

- sensitivity: potential for personalized medicine. *Angew. Chem. Int. Ed. Engl.* 2009; 48:2764–2767. [PubMed: 19288507]
21. Srinivasan B, Li Y, Jing Y, Xing C, Slaton J, Wang JP. A three-layer competition-based giant magnetoresistive assay for direct quantification of endoglin from human urine. *Anal. Chem.* 2011; 83:2996–3002. [PubMed: 21417448]
 22. Issadore D, Min C, Liong M, Chung J, Weissleder R, Lee H. Miniature magnetic resonance system for point-of-care diagnostics. *Lab Chip.* 2011; 11:2282–2287. [PubMed: 21547317]
 23. Sinha B, Anandakumar S, Oh S, Kim C. Micro-magnetometry for susceptibility measurement of superparamagnetic single bead. *Sens. Actuators, A.* 2012; 182:34–40.
 24. Kim KW, Reddy V, Torati SR, Hu XH, Sandhu A, Kim CG. On-chip magnetometer for characterization of superparamagnetic nanoparticles. *Lab Chip.* 2015; 15:696–703. [PubMed: 25474348]
 25. Popovic, RS. *Hall Effect Devices*. CRC Press; 2004.
 26. Shevkoplyas SS, Siegel AC, Westervelt RM, Prentiss MG, Whitesides GM. The force acting on a superparamagnetic bead due to an applied magnetic field. *Lab Chip.* 2007; 7:1294–1302. [PubMed: 17896013]
 27. Kim J, Jensen EC, Megens M, Boser B, Mathies RA. Integrated microfluidic bioprocessor for solid phase capture immunoassays. *Lab Chip.* 2011; 11:3106–3112. [PubMed: 21804972]
 28. Gambini S, Skucha K, Liu PP, Kim J, Krigel R. A 10 kPixel CMOS Hall Sensor Array With Baseline Suppression and Parallel Readout for Immunoassays. *IEEE J. Solid-State Circuits.* 2013; 48:302–317.
 29. Boero G, Besse P-A, Popovic R. Hall detection of magnetic resonance. *Appl. Phys. Lett.* 2001; 79:1498–1500.
 30. Besse PA, Boero G, Demierre M, Pott V, Popovic R. Detection of a single magnetic microbead using a miniaturized silicon Hall sensor. *Appl. Phys. Lett.* 2002; 80:4199–4201.
 31. Rado, G. *Magnetism VI*. Academic Press; 1963.
 32. Fonnum G, Johansson C, Molteberg A, Mørup S, Aksnes E. Characterisation of Dynabeads® by magnetization measurements and Mössbauer spectroscopy. *J. Magn. Magn. Mater.* 2005; 293:41–47.
 33. Lim J, Ahn H, Ji H, Chang W, Mun J, Kim H, Cho K. A Comparative Study of a Dielectric-Defined Process on AlGaAs/InGaAs/GaAs PHEMTs. *ETRI J.* 2005; 27:304–311.
 34. Jang JT, Nah H, Lee JH, Moon SH, Kim MG, Cheon J. Critical enhancements of MRI contrast and hyperthermic effects by dopant-controlled magnetic nanoparticles. *Angew. Chem. Int. Ed. Engl.* 2009; 48:1234–1238. [PubMed: 19137514]
 35. Lee JH, Jang JT, Choi JS, Moon SH, Noh SH, Kim JW, Kim JG, Kim IS, Park KI, Cheon J. Exchange-coupled magnetic nanoparticles for efficient heat induction. *Nat. Nanotechnol.* 2011; 6:418–422. [PubMed: 21706024]

Figure 1. Integrated Hall magnetometer (iHM) chip

(a) Microscope image of the sensor chip. Sixteen μ Hall elements were fabricated into 4×4 array (left). The output ports of individual Hall elements were selected by two transistor switches, thus reducing the chip area and the number of required ports (from 64 to 16) to external device (right). (b) A microfluidic device, embedding sixteen microwells ($20 \times 20 \times 25 \mu\text{m}^3$) was placed on top of the iHM chip to confine samples. (c) Measurement setup. MNPs were magnetized by an excitation coil underneath the sensor chip. Differential output was amplified by pre-amplifier (differential input to single output) and demodulated by lock-in amplifier. (d) Magnetic susceptibility measurement scheme. Alternating current magnetic field (H_{AC}), superimposed to a static direct current magnetic field (H_{DC}), was applied perpendicular to the iHM chip. The resulting magnetization, induced from MNPs at the same frequency of the applied H_{AC} , was proportional to the slope of MNP magnetization curve at the given H_{DC} . The overall magnetic susceptibility curve was constructed by sweeping H_{DC} .

Figure 2. *i*HM measurements

(a) Transmission electron microscopy images of MNPs ($\text{Zn}_{0.4}\text{Fe}_{2.6}\text{O}_4$, left; CoFe_2O_4 , middle) and magnetic beads (right). (b) Magnetic susceptibility (χ_v) curves of different magnetic particles were measured by sweeping the external field from -200 to 200 kA/m. The measured χ_v curve was normalized to χ_v at $H_{DC} = 0$, and fitted to the first derivative of Langevin function. (c) Normalized magnetization curves were constructed from χ_v curves in (b). Magnetic beads with $\gamma\text{-Fe}_2\text{O}_3$ MNPs were saturated faster than other MNP types.

Figure 3. Characterization of MNPs

(a) Two samples, each containing free $\gamma\text{-Fe}_2\text{O}_3$ MNPs and polystyrene bead ($6.8 \mu\text{m}$) labeled with $\gamma\text{-Fe}_2\text{O}_3$ MNPs, were measured by the $\mathcal{H}\text{M}$ system. The normalized χ_v curves were nearly identical and independent of the sample volume or MNP configurations (i.e., bead-bound or free floating). (b) $\mathcal{H}\text{M}$ was used to measure magnetic moments (μ_p) of free floating MNPs ($\text{Zn}_{0.4}\text{Fe}_{2.6}\text{O}_4$ and CoFe_2O_4) and bead-bound $\gamma\text{-Fe}_2\text{O}_3$ MNPs. Due to their larger size, $\gamma\text{-Fe}_2\text{O}_3$ MNPs in magnetic beads showed higher μ_p than $\text{Zn}_{0.6}\text{Fe}_{2.6}\text{O}_4$ and CoFe_2O_4 . Bead type: (1) Spherotech SVM-025-5H; (2) Oceananotech MV0502; (3) MyOne 65602; (4) Dynabead M-280 11205D.

Figure 4. Measurement of MNP mixtures

(a) Magnetic susceptibility (χ_v) curves of single type MNPs and their mixture were measured. The average magnetic moment of a sample was estimated from full-width at half maximum (H_{FWHM}). (b) Samples containing $Zn_{0.4}Fe_{2.6}O_4$ and $\gamma-Fe_2O_3$ MNPs at different ratios were prepared. From the susceptibility measurements, the average magnetic moment (μ_{est}) of each mixture was estimated. The obtained value (block dots) showed a good match with μ_{avg} (red dotted line) which is a number-weighted average of comprising MNPs' magnetic moments. (c) The number fractions of $Zn_{0.4}Fe_{2.6}O_4$ and $\gamma-Fe_2O_3$ MNPs was estimated from the measured μ_{est} (block dots). The dotted line indicates the expected values.

Figure 5. Application of iHM system

(a) The iHM system was calibrated by measuring the Hall voltage outputs of serially diluted $\text{Zn}_{0.4}\text{Fe}_{2.6}\text{O}_4$ MNP samples. The Hall signal was linearly proportional to MNPs concentrations. (b) Ovarian cancer cells were labeled with MNPs specific to EGFR or EpCAM. The χ_v curves of a single cell were measured and quantified by the iHM system. The Hall voltage at $H_{ext} = 0$ is proportional to marker expressions. a.u., arbitrary unit. (c) Molecular profiling. The expression levels of target markers (EGFR, EpCAM) were compared for OVCA420 cells. The control sample was OVCA420 cells incubated with isotype-matched IgG antibody and MNPs.