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# Microfluidic extraction and digital quantification of circulating cell-free DNA from serum

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## ABSTRACT

Miniaturized devices for the extraction of DNA have been used for assessing genetic material in biological, forensic and environmental samples. However, the ability to isolate trace amounts of highly fragmented DNA from biological fluids remains a challenge. The current work reports a microfluidic approach that combines on line a dynamic magnetic extraction procedure with droplet-based digital PCR (ddPCR). This strategy maximizes the surface area for DNA binding within the chip, in order to capture short DNA fragments, with the possibility of recovering the purified samples into picoliter volumes for high sensitivity mutation detection. The application of this technology to capture circulating cell-free DNA (ccfDNA) from serum samples of cancer patients is demonstrated herein, with efficiencies comparable to standard column-based DNA extraction methods. This technology uses lesser amounts of required material and reagents, and has a higher potential for automation and multiplex DNA analysis. Furthermore, this approach can also be extended for the detection of other circulating biomarkers, such as nucleic acid sequences with aberrant methylation patterns or miRNA.

## 1. Introduction

The study of circulating tumor DNA (ctDNA) released in the peripheral blood during tumor cell death has emerged as a promising alternative to invasive tissue biopsies [1]. It provides genetic and epigenetic information similar to that of the primary tumor [2], and its non-invasive nature, via a simple venal puncture, allows real-time monitoring of tumor burden and treatment response [3–6]. Microfluidic-based technologies such as targeted next generation sequencing (NGS) and droplet-based digital PCR (ddPCR) are currently being developed for the highly sensitive detection and quantification of ctDNA [7–10]. However, their performance is still limited by pre-analytical steps performed prior to DNA analysis (e.g., sample collection and DNA

extraction procedures) [3,11,12]. Indeed, it remains challenging to isolate efficiently ctDNA from blood, due to the very low concentration (typically few ng/mL), and high fragmentation (in the 50–1000 bp size range) of these molecules, in particular at early stages of cancer [3,11]. More efficient isolation methods are thus needed for the use of ctDNA as a non-invasive cancer biomarker.

Sample pre-treatment usually involves time consuming procedures with manual pipetting and numerous centrifugation steps that could increase the potential sources of error, DNA loss and sample contamination [13]. Developing miniaturized procedures for DNA isolation can help simplifying the process and interfacing with downstream analysis modules. Reducing the volumes of samples and reagents required per test can also be beneficial when only low amounts of samples

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are available (e.g. fine needle aspirates). A variety of microfluidic devices has been developed for the solid-phase extraction of nucleic acids [13,14], but only few studies have focused on the purification of cell-free DNA from biological samples [15–17]. Microdevices employing silica chemistries (based on previous developments for standard spin column procedures) have been used for the isolation of DNA from pre-purified samples or concentrated genomic DNA from blood samples ( $> 1 \text{ ng}/\mu\text{L}$ ), showing extraction efficiencies ranging from 45 to 70% [18–21]. However, silica-based technologies have been limited by the need of chaotropic salts for efficient DNA binding to the silica surface, and the use of several alcohol washing steps that can inhibit PCR reactions. In contrast, electrophoretic [15] or electrostatic-based approaches (e.g. those employing surface chemical treatments with a pH-induced DNA binding effect) [22–24] have the capability to operate in an all-aqueous environment, and would thus facilitate the downstream analysis of the extracted molecules. To increase the surface area for DNA interaction, paramagnetic microparticles have been implemented as solid supports for the capture of nucleic acids in microfluidic devices [21,25–27], allowing for the *in situ* manipulation of particles (suspension and collection) via external magnets [28,29]. Their surface can also be functionalized with charge switchable amine-terminated groups to capture negatively charged DNA by electrostatic interaction [30,31].

We have previously reported the use of a miniaturized fluidized bed with functionalized paramagnetic particles for the detection of trace amounts of bacteria from complex samples [32]. We standardized the microfluidic geometry to control the recirculation of microparticles inside a diamond-shaped microfluidic chamber, and maximize the binding affinity to the solid support [33]. Using an external magnetic source and a flow control module we can actively adjust the porosity of the bed of beads, with a global balance between hydrodynamic drag and magnetic forces (Fig. 1). This approach allowed processing important volumes of samples in reduced times ( $> 1 \mu\text{L}/\text{min}$ ) while avoiding clogging artifacts, and providing low backflow pressure, as well as an important surface area for analyte interaction [34].

Here, we developed a microfluidic Magnetic ExTRactiOn procedure (METRO) for efficient DNA isolation using the fluidized bed, allowing to process raw biological samples, such as undiluted serum, and deliver concentrated and inhibitor-free eluted DNA samples for downstream analysis. Our strategy relies on the combination of a first module for the selective capture of all DNA sequences present in the sample (mutant and wild type) with a second emulsification module for the compartmentalization of extracted molecules into millions of picoliter droplets

for high-resolution genetic testing by ddPCR. This digital approach provides high sensitivity and specificity to distinguish the proportion of mutant ctDNA in circulating cell-free DNA (ccfDNA, naturally present in the blood). During the first phase of this study, we used synthetic and genomic DNA samples for the characterization of the different steps of METRO. We then demonstrated the compatibility of our system with DNA amplification for the detection of low concentrated target molecules in serum, using a commercial ddPCR system. We applied these developments to the extraction and detection of circulating DNA from the blood of breast and colon cancer patients, and confronted our results with conventional column-based extraction procedures in a blind protocol. Finally, we demonstrated the direct coupling of the miniaturized DNA extraction procedure with ddPCR on chip.

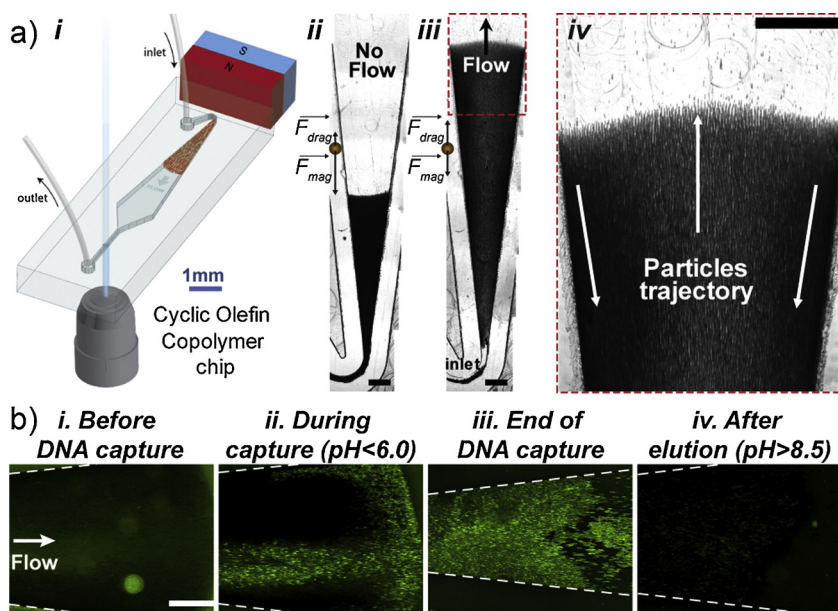
## 2. Materials and methods

### 2.1. Microfluidic device fabrication and experimental setup

The Cyclic olefin copolymer (COC, Topas<sup>®</sup> 8007, Topas Advanced Polymers, Extrusion Lab, Frankfurt, Germany) microfluidic devices were fabricated by hot embossing and sealed using a solvent assisted bonding method, as described earlier [35,36]. Channel walls were treated with Pluronic F127 (1 wt% in water, Sigma Aldrich, St. Louis, MO, USA) to prevent nonspecific adsorption of particles or DNA molecules [35]. An inverted microscope (Nikon Ti Eclipse) equipped with a cooled CCD camera (CoolSNAP HQ2, Photometrics, Roper scientific-Princeton instruments) was used for optical inspection and fluorescence analysis. Pressure and flow rate were controlled using a pressure controller and a flow sensor (Fluigent, France).

### 2.2. DNA and serum samples

200 bp dsDNA PCR products were produced using fluorescently labeled Forward and Reverse primers (EuroFins, Ebersberg, Germany, Appendix A Supplementary material Section 1.1, Fig. S1 and Table S1). DNA from the HT29 cancer cell line (ATCC, Manassas, VA, US) was fragmented with S220 sonicator (Covaris, Woburn, MA, USA) (Fig. S2). DNA fragment sizes were measured using the LabChip<sup>®</sup> GX/GXII Microfluidic system (Perkin-Elmer, MA, USA) with the DNA assay 5 K reagent kit (Perkin-Elmer) and using the 2100 Bioanalyser (Agilent, Santa Clara, CA, USA) with the DNA 12,000 Kit (Agilent). A Qubit 2.0 fluorometer (Invitrogen, ThermoFisher Scientific) with the dsDNA HS



**Fig. 1.** Schematic of the setup for METRO using the microfluidic fluidized bed (a-i). Micrographs of the fluidized bed before (a-ii) and during (a-iii) sample perfusion on chip. Magnified view showing the recirculation of beads. Fluorescence micrographs showing DNA-beads interaction inside the fluidized bed, before (b-i), during (b-ii), at the end of DNA capture step (b-iii), and after DNA elution (b-iv). Scale bars: 500  $\mu\text{m}$ .

Assay (Invitrogen) was used to measure DNA concentrations. Serum samples from 10 healthy male donors (Biological Specialty Corporation, Colmar, US), were pooled, centrifuged at 3000 rpm, and aliquoted for storage at  $-80^{\circ}\text{C}$ . A new aliquot was thawed and centrifuged at 5000 rpm for 15 min prior to each experiment. 7 archived serum samples from cancer patients, were retrospectively chosen based solely upon the identification of a potential target mutation in a biopsy from the primary tumor.

### 2.3. DNA extraction

The procedure for batch extraction of DNA from serum samples was adapted from the protocol provided by the manufacturer (ChargeSwitch<sup>®</sup> gDNA Mini Tissue Kit, Life Technologies, Carlsbad, CA), using the minimal volumes and number of particles that preserved the extraction efficiency and reproducibility of the protocol. We miniaturized the batch extraction procedure to implement the METRO protocol on chip (Appendix A Supplementary material Section 1.2 and Table S2). Briefly, we injected binding buffer into the COC device and used a NdFeB12 permanent magnet ( $30 \times 20 \times 20$  mm, N50, ChenYang Technologies, Germany) for loading and manipulating magnetic microparticles inside the capture chamber ( $25 \mu\text{g}$  of particles,  $850\text{--}900$  nm in diameter equivalent to a surface area of  $0.1\text{--}0.2\text{ mm}^2$ ), as described earlier [32]. The porosity of the bed was tuned, from highly packed to disperse particles, by simply controlling the flow rate inside the chip (up to  $1 \mu\text{L}/\text{min}$ ), while the position of the external magnetic source remained constant (Fig. 1a-i,ii,iii). Samples were incubated with lysis buffer (with RNase and Proteinase K) for 1 h at room temperature, prior to injection. Following the addition of low pH binding buffer ( $\text{pH} < 6$ ), samples were perfused through the expanded fluidized bed for DNA capture ( $30\text{--}60 \mu\text{L}$  of sample at  $0.4\text{--}0.5 \mu\text{L}/\text{min}$ ). After a washing step ( $15\text{--}30 \mu\text{L}$  of wash buffer at  $0.5\text{--}1 \mu\text{L}/\text{min}$ ), DNA was eluted ( $5\text{--}15 \mu\text{L}$  of elution buffer at  $0.2\text{--}1 \mu\text{L}/\text{min}$ ) and collected for DNA quantification ( $\text{pH} > 8.5$ ).

### 2.4. Picoliter ddPCR analysis

The extracted DNA was quantified using the RainDrop Digital PCR System (Raindance Technologies, Billerica, US) for DNA emulsification and droplet fluorescence read-out [37], with ddPCR assays developed earlier [38]. Emulsions were thermal cycled on a C1000 Touch Thermal Cycler (Bio-rad, Hercules, CA, USA). For the integrated extraction and emulsification procedure on chip, we measured the end-point fluorescence signal from each droplet using the RainDrop dPCR Sense after DNA amplification. Data were analyzed using the RainDrop Analyst software as described by the manufacturer.

## 3. Results and discussion

### 3.1. DNA extraction capabilities on chip

#### 3.1.1. Miniaturized DNA extraction procedure

Extraction using ChargeSwitch<sup>®</sup> beads follows three main steps: A) binding of negatively charged nucleic acids to the positively charged surface of the beads, B) washing to remove non-specifically bound molecules, and C) elution of DNA from the beads. We first qualitatively assessed the behavior of the fluidized bed during these steps using fluorescently labeled dsDNA (Fig. 1b). Prior to sample perfusion, the bed of beads was fluidized by flowing binding buffer to equilibrate the surface charge of particles, and homogenize their density in the diamond-shaped chamber (Fig. 1b-i). During step A, the DNA sample was injected, resulting in a rapid increase in the fluorescence intensity at the surface of the beads, verifying that fluorescent DNA molecules were captured. The fluorescence signal was higher in the center of the fluidized bed at the beginning of the capture step, and all particles were covered with fluorescent DNA molecules by the end of the process

(Fig. 1b-ii,iii). This redistribution of the fluorescent signal across the chamber was driven by the sideward recirculation of magnetic particles during the fluidization regime (Fig. 1a-iv, Video S1) [33], and was crucial for maximizing the DNA capture capacity of the device. During step B, a continuous recirculation of the bed of beads with no loss of particles was observed, enabling efficient removal of proteins and other inhibitors from the fluidized bed. More importantly, the fluorescence intensity on the beads did not decrease significantly during this step, indicating no loss of captured material. During step C, an immediate decrease in the fluorescence signal at the surface of the particles was observed, to a level comparable to the initial low auto-fluorescence from the beads (Fig. 1b-iv), demonstrating the effective flow-through elution of DNA without the need for an incubation step. We could thus expect that the amount of DNA remaining on the surface of the particles at the end of the procedure was very low (at least not detectable by fluorescence measurements).

We then evaluated the selectivity of our system regarding DNA size, by capturing and eluting fragments with varying lengths ( $100\text{ bp}\text{--}3000\text{ bp}$ ). We observed through microchip gel electrophoresis analysis that the injected and eluted samples presented the same size distribution with the same relative peak areas (Appendix A Supplementary material Section 1.3, Table S3 and Fig. S3), showing the possibility to isolate fragmented DNA molecules with no preferential selection of a particular size.

#### 3.1.2. DNA binding capacity using model DNA samples

To quantify the capture efficiency of METRO, we perfused different volumes of sample containing fluorescently labeled dsDNA in PBS buffer ( $200\text{ bp}$  in length,  $\text{MW} = 129,000\text{ g/mol}$ , and  $[\text{C}] = 11\text{ ng}/\mu\text{L}$ ). To evaluate the amount of captured DNA in real time, we assessed the level of fluorescence intensity from the sample in the outlet channel (Fig. 1a-i): 1) before injecting the beads, and 2) during the DNA capture process (Appendix A Supplementary material, Section 1.4). We measured a DNA capture rate higher than 97% for samples with up to  $200\text{ ng}$  of DNA, with a slight decrease in capture rate for  $300\text{ ng}$  of DNA ( $89 \pm 8\%$ ), probably due to saturation effects and steric repulsion from DNA molecules captured on the surface of the beads (Fig. 2a). We observed a linear capture rate for DNA inputs in the  $0\text{--}250\text{ ng}$  range (Fig. S4), demonstrating the capabilities of our system for the efficient extraction of dsDNA fragments in ideal conditions (i.e. using an aqueous matrix). We estimated the DNA binding capacity of our system to be at least in the order of  $10 \mu\text{g}$  of DNA/mg of beads; placing our system in the higher end of the  $5\text{--}10 \mu\text{g}$  of DNA/mg range expected from the supplier, using the batch procedure recommended for the ChargeSwitch<sup>®</sup> particles, and well above the capacity for other commercially

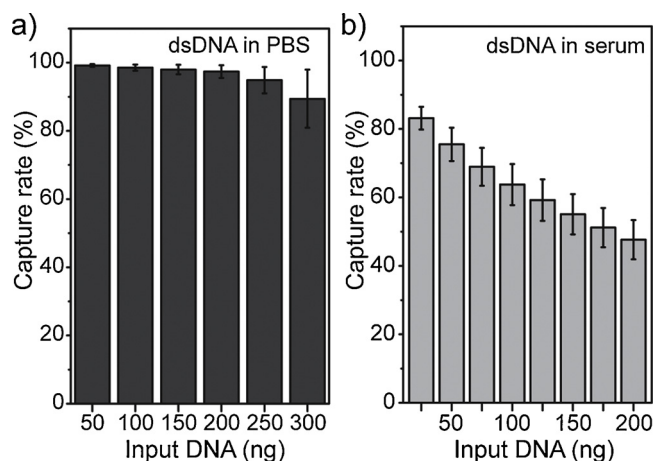
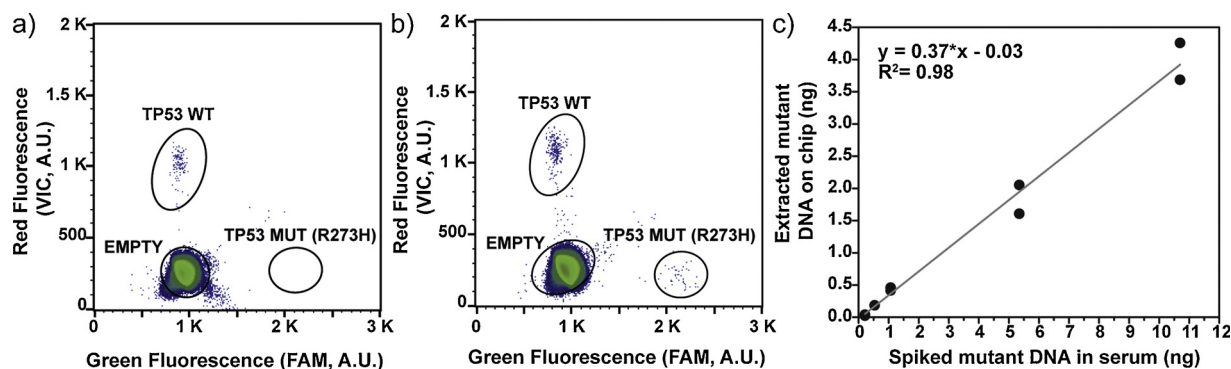


Fig. 2. Capture rates for METRO measured for different DNA inputs using fluorescently labeled dsDNA spiked in (a) PBS and (b) human serum samples from healthy donors.



**Fig. 3.** Detection of R273H TP53 mutation in serum samples spiked with mutant HT29 cell line DNA. (a-b) Two dimensional histograms of the digital PCR assay for the analysis of circulating DNA extracted from healthy donors (a) and from spiked samples (b). Calibration curve (c): Extracted R273H TP53 mutant DNA digitally quantified for varying spike in DNA amounts (0.0033 ng/genome copies). MUT probes: FAM, 6- carboxyfluorescein; WT probes: VIC, a proprietary dye of Life Technologies; A.U., arbitrary units.

available particles [22].

### 3.1.3. DNA extraction from human serum

To evaluate the effect of the sample matrix on DNA binding affinity, we processed human serum samples from healthy donors, spiked with different amounts of fluorescent dsDNA. We measured a capture rate higher than 63% for samples containing up to 100 ng of DNA and a stabilization of the capture efficiency around 50% for higher amounts of DNA ( $48 \pm 6\%$  for 200 ng) (Fig. 2b). As expected, we observed a lower capture rate when using undiluted serum as a matrix, as compared to DNA samples in PBS, which could be related to non-specific adsorption of serum proteins (e.g., albumin) or other nucleic acids (e.g., ccfDNA or miRNA from serum) to the beads surface through electrostatic interactions [11,39]. The high molecular content of complex matrices such as human serum or plasma has indeed limited the implementation of microfluidic DNA isolation procedures. One approach to avoid potential screening effects or clogging artifacts during the isolation process has been to dilute the samples prior to their injection on chip (10–100-fold) [18,22]. However, such approach is not suitable for the analysis of ctDNA, as it would radically limit sample processing capabilities, as well as the sensitive detection of rare events. In this work, the capture rate for DNA samples in serum is still particularly high considering the use of undiluted human serum, the small size of extracted DNA molecules, and the electrostatic-based (thus non-specific) nature of the DNA binding strategy.

To estimate the global extraction efficiency (including elution) for the spiked serum samples on chip, we collected the eluted DNA for off-chip quantification (Qubit<sup>®</sup> assay, Fig. S5). For an input of 200 ng of DNA using METRO, we obtained an average extraction efficiency of  $64 \pm 9\%$  ( $n = 5$ ), in the same range as the capture rate for 200 ng measured above using continuous fluorescence measurements on chip. We thus expect that all DNA captured inside the fluidized bed was efficiently eluted (i.e. no detectable DNA remaining at the surface of the beads). In comparison to the batch ChargeSwitch<sup>®</sup> procedure, the elution volume on chip was reduced by at least 15-fold (the volume of the microfluidic chamber is 0.65  $\mu$ L), while maintaining a similar extraction efficiency (Fig. S5).

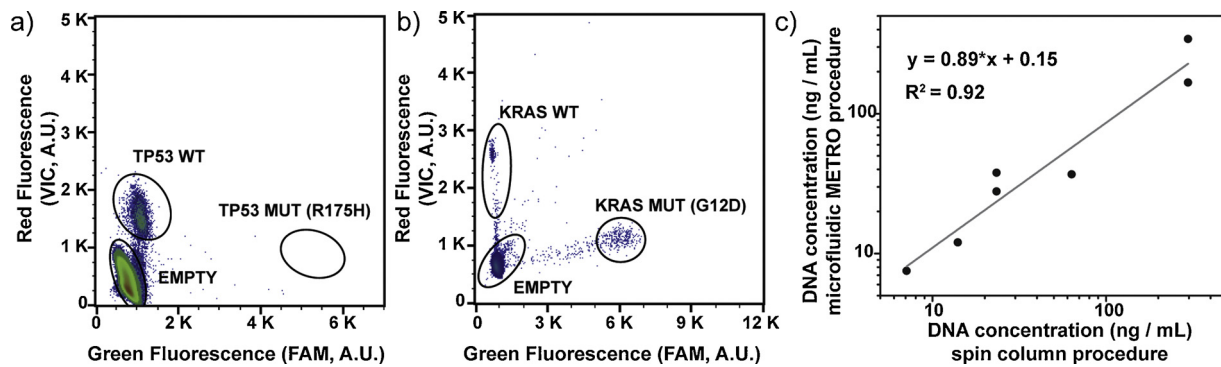
Other groups have demonstrated the interest of electrostatic-based approaches for DNA isolation on chip. Nakagawa et al. used an amino silane-coated microchip to obtain 60% capture and 45% elution rates for  $\lambda$ -DNA (2 ng/ $\mu$ L) [23]. However, their recovery ratio decreased to 27–40% when extracting human genomic DNA from whole blood, due to the competitive binding of negatively charged proteins, as demonstrated here. More recently, Gan et al. developed an original approach combining a chitosan-modified filter paper inside a microfluidic device for DNA extraction [24]. Although they obtained a 62–89% capture efficiency using  $\lambda$ -DNA (with concentrations from 0.05 to 1 ng/ $\mu$ L),

only 8.9–11.5% of the captured DNA could be eluted, thus limiting the possibility of interfacing with downstream analysis modules. Finally, non-commercial chitosan-coated magnetic particles have also been used for efficient DNA isolation, providing high stability, pH tunability and yield (52  $\mu$ g from 3 mL human saliva), but their implementation on chip has not been demonstrated [31]. Our technology provides similar or better DNA extraction efficiencies to those reported in the literature with the added advantages of a higher binding capacity, as well as a higher potential for scalability and integration with DNA detection and quantification techniques, such as droplet-based digital PCR, as shown in the following sections.

## 3.2. Processing biological samples

### 3.2.1. Quantification of DNA extraction efficiency by ddPCR

To evaluate the efficiency of METRO using more realistic samples and demonstrate its compatibility with ddPCR analysis (RainDrop Digital PCR System), we processed undiluted serum from healthy donors spiked with cancer cell line DNA, with fragment size lengths and concentrations relevant to ctDNA analysis (from 50 to 1,100 bp and  $[C] = 0$ –357 ng/mL, Fig. S2) [40]. To differentiate between spiked mutant DNA (MUT) and non-mutated ccfDNA (wild type, WT, initially present in serum samples), we used a mutation specific assay containing allele-specific hydrolysis probes (TaqMan<sup>™</sup> probes targeting the R273H mutation in the *TP53* gene). We first evaluated the levels of WT DNA for non-spiked samples, and detected an average concentration of  $23 \pm 11$  ng/mL (equivalent to  $7045 \pm 3315$  copies in 1 mL of serum,  $n = 11$ ), in line with concentrations of ccfDNA previously described for healthy donors [11]. We observed no false positives or MUT events in the healthy test samples (Fig. 3a). We then extracted the different spiked samples, and used the digital quantification to compare the number of expected vs. extracted MUT and WT genomes for each sample (Fig. 3b). We established a calibration curve for the MUT genomes (Fig. 3c) and observed a linear capture/elution relationship for the different input mutant DNA with an overall 37% extraction and detection efficiency. In parallel, we performed the extraction of the same spiked samples using the ChargeSwitch<sup>®</sup> procedure in batch. However, the analysis of eluted DNA from the bench-top protocol did not generate consistent ddPCR results, probably due to the presence of inhibiting reagents or molecules still present in the eluent (Fig. S6). METRO allowed for a more efficient washing process and buffer exchange inside the microfluidic device, thus delivering inhibitor free samples compatible with ddPCR analysis. These results demonstrated that our system allows analyzing MUT DNA fragments in concentrations as low as 3 ng/mL of serum in a background of WT DNA (leading to a mutational ratio of about 10% when considering the amount of WT ccfDNA in serum samples), using a minute amount of sample (60  $\mu$ L).



**Fig. 4.** Detection of cfDNA in the serum of breast and colon cancer patients. (a-b) Two dimensional histograms of the digital PCR assay for the analysis of circulating DNA extracted on chip from two patients bearing TP53 (a) and KRAS mutations (b). (c) Digital quantification for total cfDNA concentration (MUT + WT) following extraction using the microfluidic METRO procedure and standard column-base protocol. MUT probes: FAM, 6- carboxyfluorescein; WT probes: VIC, a proprietary dye of Life Technologies; A.U., arbitrary units.

### 3.2.2. Analysis of serum samples from cancer patients

As a proof of concept, we implemented METRO for the detection of DNA in serum samples from breast ( $n = 5$ ) and colon ( $n = 2$ ) cancer patients with a known somatic variant in tumor tissue (Table S4). Specifically, we extracted 60  $\mu\text{L}$  of serum from each of these patients on chip and quantified the number of MUT and WT genomes, using ddPCR assays specific to each patient [2,38,41]. We performed two extractions in parallel for each patient sample, using two microfluidic devices with the same operating conditions. The quantification results showed concentrations of total cfDNA (WT + MUT) ranging from 0 to 342 ng/mL of serum (Table S4), in line with previously reported concentrations for cfDNA in the serum of cancer patients [11]. We detected the somatic variant from patient #6 in the serum samples extracted on chip, with a concentration of 15.45 ng/mL of MUT ctDNA (equivalent of a 47.5% mutational ratio for the G12D mutation in the *KRAS* gene). We considered this sample as positive for ctDNA, since the concentration was higher than the limit of detection determined above for spiked serum samples (Fig. 4b,c and Table S4). We also noticed that patient #1 presented a high concentration of WT DNA but no detectable MUT ctDNA in serum (Fig. 4a,c and Table S4), which could be related to an important background of DNA from non-tumor cells (e.g., leukocytes). We observed a linear relationship when comparing the total cfDNA concentrations obtained on chip and through a standard column-based protocol, for values above the limit of detection of the microfluidic METRO procedure (Fig. 4c, Table S5, Appendix A Supplementary material, Section 1.5).

### 3.3. Integrated DNA extraction and detection procedure on chip

Finally, we developed an integrated device combining METRO, with a microfluidic module enabling the direct emulsification of the eluted DNA, together with PCR reagents, on chip (Fig. 5a,b). The generated droplets were collected into 6 individual tubes and submitted to thermal cycling for DNA amplification (Appendix A Supplementary material, Section 1.6). We evaluated the number of positive events (droplets containing amplified DNA) on each elution fraction through fluorescence detection on chip (Fig. 5c), and estimated an overall DNA extraction efficiency of 64%. We observed that most eluted DNA was contained in elution fractions from 2 to 4 (with occupation ratios ranging from 0.3 to 1.5%). Although the amount of DNA in elution fractions 1 and 6 was very low ( $< 250$  pg), it was readily preserved and amplified using this integrated procedure. Considering the operating conditions, the complete DNA extraction and emulsification of 30  $\mu\text{L}$  of sample was performed in less than 2 h (Table S2).

Although a DNA isolation and amplification procedure using continuous flow microfluidics and microchambers for sample compartmentalization was demonstrated earlier [42], we present in this work

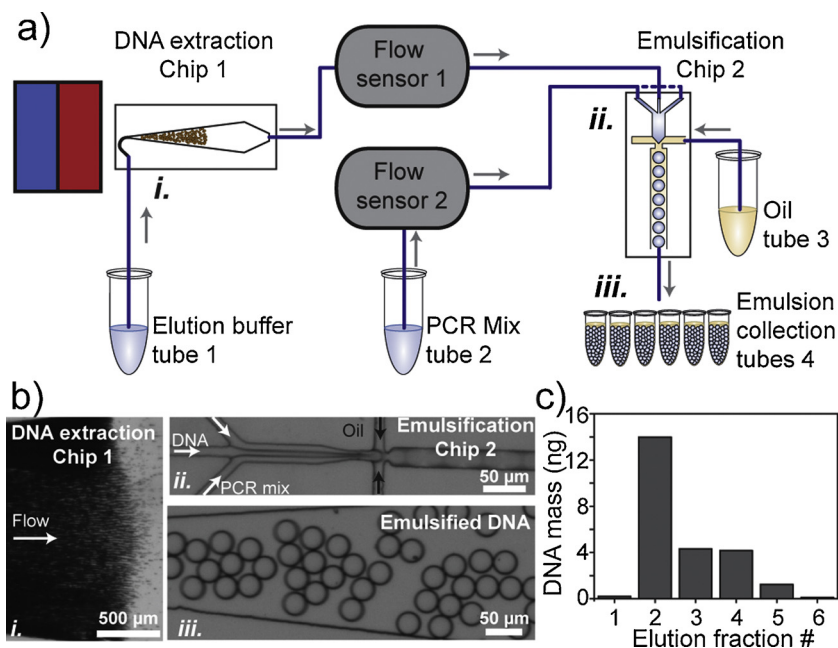
the first direct eluent emulsification method for picoliter droplet-based dPCR. This approach enables the generation of millions of individual compartments for single molecule detection. It also allows for the efficient elution and amplification of all DNA captured at the beads surface and offers the possibility to rationally modify the PCR amplification environment for each elution fraction (e.g. emulsification with a variety of primers and probes or using different thermal cycling conditions), thus opening the route for multiplex analyses.

## 4. Conclusion

We have combined on-chip, for the first time, a miniaturized DNA extraction platform with ddPCR. We applied this method for the detection and quantification of ctDNA from biological samples. Specifically, we isolated fragmented DNA in low concentrations directly from serum samples from cancer patients and demonstrated the compatibility of our system with sensitive DNA amplification methods, such as ddPCR. As compared to conventional column-based methods, this approach has the ability to perform both sample preparation and DNA detection in a single device, providing comparable results but using only a few microliters of sample, which could be of great interest for the implementation of minimally invasive cancer diagnostic procedures. Furthermore, the METRO procedure offers a high degree of flexibility in terms of size, number and surface functionalities of the magnetic particles that can be implemented (e.g. to selectively capture mRNA, microRNA, methylated DNA sequences, as well as bacterial or viral DNA).

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**Fig. 5.** a) Schematic of the integrated procedure for DNA extraction and emulsification for ddPCR analysis. b) Micrograph images from the fluidized bed (bi) and the droplet generator module (bii-iii) during elution and encapsulation of DNA. c) Amount of extracted and amplified DNA by ddPCR for the different elution fractions, collected in PCR tubes (emulsion collection tubes in Fig. 5a-iii).

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.snb.2019.01.159>.

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