Compact Nanowire Sensors Probe Microdroplets

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Supporting Information

ABSTRACT: The conjunction of miniature nanosensors and droplet-based microfluidic systems conceptually opens a new route toward sensitive, optics-less analysis of biochemical processes with high throughput, where a single device can be employed for probing of thousands of independent reactors. Here we combine droplet microfluidics with the compact silicon nanowire based field effect transistor (SiNW FET) for in-flow electrical detection of aqueous droplets one by one. We chemically probe the content of numerous (∼104) droplets as independent events and resolve the pH values and ionic strengths of the encapsulated solution, resulting in a change of the source–drain current $I_{SD}$ through the nanowires. Further, we discuss the specificities of emulsion sensing using ion sensitive FETs and study the effect of droplet sizes with respect to the sensor area, as well as its role on the ability to sense the interior of the aqueous reservoir. Finally, we demonstrate the capability of the novel droplets based nanowire platform for bioassay applications and carry out a glucose oxidase (GOx) enzymatic test for glucose detection, providing also the reference readout with an integrated parallel optical detector.

KEYWORDS: Silicon nanowires FET, nanosensor, droplet-based microfluidics, point-of-care diagnostics, glucose assay

The fast evolution of high-performance automated laboratory tests,1 point-of-care (POC) approaches for medical diagnostics,2 and smart routes toward new logic for chemical information processing is to a great extent driven by the smart combination of microfluidics and nanotechnology. Droplet-based microfluidics (or digital microfluidics)—a versatile approach for operating a large amount of same size reactors in parallel3 is of particular interest. With each single droplet being an independent miniaturized laboratory for biochemical experiments, this technology allows extremely parallelized and fine-tuned measurements, surpassing the precision of conventional assays (Figure 1a).†‡–14 Although making an important contribution to classical detection and characterization laboratory assays,10,15,16 the application of droplet microfluidics in the spirit of miniaturized and lightweight devices for, e.g., POC applications remains a work in progress. The main impeding factor is related to the need for development and integration of novel miniaturized optics-less detection principle, outperforming or being complementary to the conventional approaches.17–19 In contrast, new devices make the measurement processes independent of the limitations of optical microscopy, i.e., dynamic range or use of molecular labels. Another shortcoming is related to the ability to probe the interior of each droplet with sensitive and nondestructive sensors for monitoring the biochemical processes inside. The latter will gain crucial importance for, e.g., a compact medical diagnostics platform, where conventional microscopy is not an option.

In these regards, recently proposed integrated nanosensors, i.e., ion-sensitive field effect transistors (ISFETs), offer real-time and ultrasensitive detection of disease markers or pathogens20–27 as well as monitoring of multiple physiological parameters, like glucose level29 or pH.29 Although the ISFET principle goes back to the 1970s,30,31 it found a new fascinating realization in the past decade with utilizing silicon nanowires (SiNWs)20–25,32–35 (Figure 1b) carbon nanotubes, etc., as sensor building blocks. In comparison with planar semiconducting elements, the performance of nanowire devices, namely the sensitivity,22,25,33,36,37 is significantly improved by reducing their size and increasing surface-to-volume ratio.38 Silicon nanowire-based FETs reveal great electrical characteristics with high output current and low power dissipation30,39,40 and are CMOS compatible.31,41 While being an excellent candidate for integration as transducers for pH or biomarkers detection in single liquid phase conditions,23,41 to our knowledge

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SiNW FETs have not been combined with the multiphase droplet-based approach so far. Because of the inherent amplification, the current of an ISFET is a sensitive measure for surface potential changes that suggests the potential excellent compatibility of this approach with water-in-oil emulsion. Namely, the digitalized flow of the water droplets separated by the oil phase results in significant signal changes in the sensor elements. This facilitates reliable one-by-one droplet detection and counting. Furthermore, the relatively thin oil film separating droplet contents from the sensor surface (typically a few nanometers thick) makes it possible to probe the interior of the aqueous droplets and to obtain information about, e.g., ionic strength and charged constituents, pH value, or biomolecules. The generic functionalization is an oxide surface, which allows for pH detection, but can also serve for detection of the concentration of other ions in the liquid.

Here, we realize for the first time the integration of droplet-based microfluidics with silicon nanowire field effect transistors (SiNW FET) and demonstrate in-flow electrical detection of aqueous emulsion droplets one-by-one (Figure 1c). For this
miniatuized microdevices, integrating both the silicon nanowire transistors and microfluidics network on a single chip (see Figure 1c), are fabricated. We chemically probe the content of every single droplet in a row as independent events and investigate the effect of the pH values and ionic strengths of the solution; the source drain current $I_{SD}$ of the device at constant gate voltage. Further, we discuss the underlying physical processes, which take place once the droplet and the microscopic reference electrode (liquid gate) are in close contact.

We believe that even ordinary pH and ionic concentration sensing in droplets opens a huge range of applications, since the majority of chemical processes performed in liquid environment are associated with the change of ionic composition or pH value in a medium. While ionic strength of the solution drives the synthesis and catalysis of diverse chemical species, it also tremendously influences biological processes and responses. Cytosolic pH changes, chromatin aggregation, antimicrobial peptide activity, and controlled DNA (dis)assembly into supramolecular systems as a response to changing ionic and pH surroundings are only some of the many prominent examples. On the other hand, the ionic composition and pH level serve as indicator helping to monitor the actual state of the ongoing chemical process, such as microorganisms growth or enzymatic activities. In order to support aforementioned statements we finally demonstrate the capability of the novel nanowire platform for bioassay applications and carry out a glucose oxidase (GOx) enzymatic test for glucose detection and measure related pH change in every single droplet.

Results and Discussion. The key component of the developed droplets detection platform is a large-area SiNW FET, revealing high output currents and low standby power consumption. The fabrication of ISFET devices is briefly described in the following (see Figure 2a, stages I–IV). First, a dense array of vertically aligned intrinsic SiNW with a length distribution from 2 to 20 μm and a mean diameter of about 20 nm are grown on a silicon wafer using vapor–liquid–solid (VLS) mechanism. The nanowires are then transferred onto the rigid receiver Si substrate with an isolating SiO2 top layer of 100 nm (panel I in Figure 2a). This procedure results in a large scale and dense array of horizontally aligned nanowires, which are further integrated into a conventional FET electrode geometry. Parallel arrays cover 100 × 400 μm, leading to up to $10^5$ nanowires per ISFET device (areal density 0.03 nanowires/μm$^2$). The connection of multiple nanowires in parallel helps to deliver macroscopic current outputs which greatly outperforms single nanowire FETs (generating current outputs of about 1 μA). Furthermore, devices connected by NW arrays reveal broader dynamic range and higher on/off ratio, which is beneficial for the sensing devices. Interdigitated microelectrodes (source–drain) are formed via sputtering of a 50 nm nickel (Ni) film on top of the substrate, patterned by means of optical lithography (see Methods). Thermal annealing of the sample at 450 °C was performed in order to reduce the contact resistance between Si nanowire and Ni electrode. This process causes the axial diffusion of Ni into the NW body and decreases the contact resistance by approximately factor of 2. Metal–oxide–semiconductor (MOS) geometry was realized by atomic layer deposition (ALD). At last, the chip was equipped with Ag/AgCl top gate electrodes on top of its FET structures (see Methods) (Figure 2a, panel II and d). The transfer and output characteristics of the fabricated transistors are displayed in Figure 2e and f and demonstrate the excellent ambipolar behavior, reaching the high 10$^{-4}$ on/off current ratio and a subthreshold swing of about 205 mV/dec. Due to residual negative charged traps in the interface oxide between Si and Al2O3 and the electron–hole asymmetry in barriers to the connecting electrodes in real devices, the current minimum of SiNW FETs as a function of the gate voltage is slightly shifted toward positive gate voltages (see Figure 2e).
Finally, we assembled a microfluidic channel system with a width of 300 μm and a height of 15 μm onto a chip with 20 integrated SiNW FET devices (see Figure 2, panel IV and Methods). In order to make the surfaces of the chip (PDMS and Al2O3) homogeneously hydrophobic for water droplets propagation, channels were functionalized with (3-aminopropyl)triethoxysilane (APTES) (see Figure 2a, panel III and Methods). Mineral oil (M5904, Sigma-Aldrich) with adding nonionic 2% Span 80 (S6760, Sigma-Aldrich) was used for preparing water-in-oil emulsion. The flow-focusing geometry for droplets formation results in a chain of uniform aqueous reservoirs directly guided over the sensing area of multiple FET structures (Figure 2c, insets I and II). Droplets were produced with an oil flow rate 2 μL/min (PHD2000, Harvard Apparatus). This value allowed for an exact observation of every single droplet with a bright field microscope and to provide a sufficient number of samples per droplet. The ratio of water and oil flow rates was adapted to modulate the size and rate of the droplets (Figure 2S in Supporting Information). The measurements are shown for two different volumes of 2.5 nL (flow ratio Rwater/oil = 0.15, top panel) and of 9 nL (Rwater/oil = 1, bottom panel), which correspond to 200 and 900 μm long droplets, respectively. Assuming that the sensor area, exposed to the liquid environment, is around 100 × 300 μm (see Figure 3c), the droplets detected in Figure 3d have the lengths below and above the size of single sensor device. Interestingly, the plateau in the time-dependent ISD curve was formed only for droplets that were larger than the sensor dimensions (see Figure 3d, bottom panel, and Figure 4a, bottom panel). It has to be stressed that the established plateau current increases abruptly by more than 10% up to 0.265 μA; once the oil–water interface approaches the FET sensing area, the current increases by a gradual decay of the signal to a plateau at about 0.255 μA (blue area in Figure 3c), corresponding to the case when the aqueous phase covers the FET completely; crossing the sensor area by the droplet’s tail leads to the source drain current decrease and further signal stabilization in an oil phase (blue data points in Figure 3c).

In order to gain an understanding of the device sensitivity and its dynamic range, we measured transfer characteristics of SiNW ISFET devices in aqueous conditions (exemplary solutions) as well as in organic solvents (that resemble the oil properties), as shown in Figure 3a and b, respectively (Figure 3S in Supporting Information). Measured characteristics clearly demonstrate that the ability of the gate voltage Vg to change source drain currents ISD, so-called gate coupling, is directly related to the conductivity (Table 1S in Supporting Information) of the liquid medium. While the deionized water (DI) water and ethanol, having electrical conductivities above 10−12 A/V, lead to strong gate coupling and thus, easy gating of FET devices, the nonconductive substances, e.g., toluene and hexane lead to the similarly weak gate coupling. Evaluation of the results in Figure 3a–b suggests that the water-in-oil droplets can be detected using SiNW FET in the subthreshold regime, applying gate voltage in the range between 0 and 1 V (Figure 3a–d). We therefore probe the p-type branch of transistor characteristics.

In the following we investigate the aspects crucial for probing the interior of every single droplet, i.e., interaction of the droplets interface with the liquid reference (gate) electrode, and size of droplet–reactor with respect to the sensor dimension. Figure 3c demonstrates ISD modulation in a time domain upon arrival of a single DI water in mineral oil. The red dashed line in the figure indicates marks its front appearing directly on top of FET device. Synchronization the videos of droplets flow at the sensors surfaces (see Supporting Video S1) with the FET signals, reveals that the ISD is higher for the water than for the oil phase. In the absence of the aqueous phase ISD is stable at the value of about 0.24 μA (red data points in Figure 3c); once the oil–water interface approaches the FET sensing area, current increases abruptly by more than 10% up to 0.265 μA; this increase is followed by a gradual decay of the signal to a plateau at about 0.255 μA (blue area in Figure 3c), corresponding to the case when the aqueous phase covers the FET completely; crossing the sensor area by the droplet’s tail leads to the source drain current decrease and further signal stabilization in an oil phase (blue data points in Figure 3c).

As we assume that the signal overshoot, taking place during the detection of the oil–water interfaces, is caused by the polarization of oil-dispersed DI water droplet in an external electric field of the gate electrode. Furthermore, such a signal change can also be attributed to the strong disturbance of the surface potential of the liquid gate electrode, once a water droplet interface is passing the sensing area. The further relaxation of the ISD peak into the plateau is probably due to a stabilization of surface potential at the nanowires after the passage of the charged interface. Figure 3d shows the typical electrical signal readouts recorded for multiple water-in-oil emulsion droplets (note the possibility of scanning the thousands of droplets in one run, see Figure 7S in Supporting Information). The measurements are shown for two different volumes of 2.5 nL (flow ratio Rwater/oil = 0.15, top panel) and of 9 nL (Rwater/oil = 1, bottom panel), which correspond to 200 and 900 μm long droplets, respectively. Assuming that the sensor area, exposed to the liquid environment, is around 100 × 300 μm (see Figure 3c), the droplets detected in Figure 3d have the lengths below and above the size of single sensor device. Interestingly, the plateau in the time-dependent ISD curve was formed only for droplets that were larger than the sensor dimensions (see Figure 3d, bottom panel, and Figure 4a, bottom panel). It has to be stressed that the established plateau current increases abruptly by more than 10% up to 0.265 μA; once the oil–water interface approaches the FET sensing area, current increases abruptly by more than 10% up to 0.265 μA; this increase is followed by a gradual decay of the signal to a plateau at about 0.255 μA (blue area in Figure 3c), corresponding to the case when the aqueous phase covers the FET completely; crossing the sensor area by the droplet’s tail leads to the source drain current decrease and further signal stabilization in an oil phase (blue data points in Figure 3c).
in the $I_{SD}$ current profile indicates the stabilization of the surface potential, which is convenient for probing the interior of the aqueous phase. The possibility to detect droplets at different frequencies, i.e., 3, 5, and 10 Hz, is summarized in Figure 8S in Supporting Information. In contrast, the $I_{SD}$ current profile for small droplets measured by NW FET in Figure 4a (top panel) resembles a sharp peak with the heights, comparable to those detected for the oil–water interface in larger droplet (see the red area in Figure 3c). This statement is investigated in details by analyzing the ratio of source–drain currents corresponding to peak and plateau values of droplets with different sizes. Figure 4b suggest that three different regimes in droplet detection can be considered, depending on their length: smallest droplets are characterized by the highest peak-to-plateau ratio (assuming that the background oil signal serves as a reference, see Figure 4a, top panel); the middle droplet size regime is characterized by the evolution of plateau for droplets comparable or slightly larger than the sensor size (Figure 4a, middle panel); droplets larger than the sensor area are characterized by a stabilized signal of peak-to-plateau ratio (Figure 4a, bottom panel). Although the presence of the peaks makes a strong influence onto $SD$ current during detection of DI water droplets, it can be substantially reduced by increasing ionic strength of the solution. In particular, higher concentrations of ions lead to the increased charge screening at the nanowire surface and at the water–oil interfaces inside droplet, better gate coupling, and more efficient stabilization of the surface potential, which finally lowers peak values, caused by droplets interfaces. This statement is supported by detecting the droplets of phosphate buffered saline (PBS, 10 mM of $PO_4^{3−}$) which raises the ionic strength of the solution. The blue data points and a curve in Figure 4b show that the peak-to-plateau ratio stays practically unchanged for PBS droplets of different sizes.

The observations above enable us to conclude that the droplets shorter than the NW sensor linear dimension are less suitable for probing their inner environment (due to the strong influence of the oil–water interfaces) and, e.g., using them for monitoring the biochemical processes inside. However, this configuration might be of higher interest for monitoring the reactions happening at the water–oil interface, i.e., structural reorganization of proteins and liposome formation. Thus, further experiments for probing the pH and ionic concentrations inside single reactors are realized employing the droplet plugs with lengths of about 400 μm and $R_{water/oil} = 0.25$ that slightly exceeds the sensor size. In the following, the ratio of $I_{SD}$ currents for the plateau values and the oil signals as a background are used for the data analysis (Figure 6S in Supporting Information).

Sensing the pH and Ionic Strength in Droplets. Finally, we perform the detection of pH values and ionic strengths of solution inside of the aqueous droplets using SiNW FETs. Figure 5 summarizes the obtained results, represented by recording source drain currents versus time upon altering pH and ionic concentrations (in Figure 5a and c), and the corresponding quantitative analysis of pH and ionic sensitivities, respectively (in Figure 5b and d). For pH analysis the prepared PBS solutions (isosomotic solution for multiple biochemical species, i.e., bacteria, cells) were mixed with 1 M HCl and 0.1 M NaOH to tune pH value from acidic pH = 4 to alkaline one pH = 8 (for details see Methods and Figure 7S in Supporting Information). Figure 5a and b shows the increase of the source–drain current once the pH values in droplets become higher. Whereas the raw data depicting the readout of every single droplet is represented in panel a, the averaged current change versus pH [average over 30 measurements per data point (Figure 6S in Supporting Information)] is represented in panel b, respectively. Such a trend can be briefly explained in terms of energy band diagram shift, summarized below. Since the applied gate voltage is slight positive, the current is driven by holes through valence band, which is clearly seen from the left subthreshold regime at low...
VG (see Figure 3b). This results in the horizontal shift of the transfer characteristics due to the inherent surface charges. The lower pH protonates the APTES surface; thus, it works as local positive gate suppressing the hole current (see inset of Figure 5a). Note that this tendency is in agreement with the results demonstrating the pH sensing with SiNW FETs in a single phase aqueous medium, previously reported by multiple groups.22,64,65 In addition to the analysis of absolute current values, we calculated the ratio of $I_{SD}$ for each droplet, via separate determination of current levels for both phases (water and oil). This thereby eliminates the influence of potential current drifts due to, i.e., temperature instabilities or electric field fluctuations at the reference electrode (see Figure 5b).

Further, we proceed with altering the ionic strength of the aqueous medium, realized via gradual addition of DI water to the aliquots of PBS stem solution. The results for four different concentrations of PBS, spanning from $10^{-3}X$ to $1X$ ($10^{-2}$ to 10 mM PO$_4^{3-}$), is depicted in Figure 5c and d. In contrast to the tendency, observed for pH analysis, we observe a significant $I_{SD}$ decrease as a response on higher salt concentrations (Figure 5c,d). This is due to the screening of local gate field from surface charge by accumulated ions (Figure 5c, inset). Overall, the dependence of the source drain current versus ionic strength of the solution is in qualitative agreement with investigations of, i.e., ionic concentration detection using SiNW FETs.66

Although pH values and ionic strength were detected in droplets with high signal-to-noise ratio, the demonstrated nominal sensitivity of the measurements is lower, compared to similar technology applied in aqueous medium. Such sensitivities of absolute current (about $1.4 \times 10^{-3}$, and 0.03 decades per decade of concentration change of H$^+$ and PO$_4^{3-}$ ions, respectively) could be partially caused by arbitrary device fluctuations, i.e. not proper operating the FET in the subthreshold regime but in a different controlled range in the trans-conductance regime. Generally, observed aqueous buffer-to-oil ratio unveils the weak gate coupling of the mineral oil phase (see Figure 5a,c). Therefore, in order to optimize the sensing of emulsions and to avoid the situation of thermodynamically undefined surface potential at the nanowires, the better gate coupling for oil phase has to be realized in the future platforms, by e.g. using conductive oils or organic solvents, enriched by metal salts. The proof-of-concept validation for this our assumption is introduced in the inset of Figure 5b. We used dichlorobenzene with diluted tetraethylammonium tetrafluoroborate (TBABF4, 0.1%) for making large plug-like aqueous droplets with different pH values (pH from 4 to 8) and their further detection using SiNW FET sensor. The measured profile reveals better stabilization of the continuous phase signal, compared to the mineral oil signal. Water-in-dichlorobenzene emulsion could be formed, but its stability should be improved for controllable formation of monodisperse droplets in the channel. Thus, research on the stabilizing of the aforementioned emulsion using suitable interfacial agents, as well as looking for an alternative

Figure 6. (a) Realization of label free GOx assay in multiple droplets, using SiNW FET for signal readout; (b) time-dependent change of pH in droplets, measured by SiNW sensor, reflected in a change of water-to-oil ratio in droplets. Enzymatic decomposition of glucose leads to a lowering of pH (pink region), followed by the restoring of pH in citric buffer (light pink region). (c) Optical absorbance readout measured for comparison purposes. (d) Calibration measurements, depicting the droplet to oil ratio as a function of pH (black data points); determination of differential ratio of water to oil, with respect to the baseline (water-to-oil ratio before the reaction $R_0$) (blue data points); (e) GOx assays in droplets with different concentration of glucose.
biocompatible solvents, and suitable stabilizing agents, is ongoing (see Figure 95 in Supporting Information).

Among other reasons leading to the lowering of the device sensitivity, we name the formation of the thin lubricating oil film between the sensor surface and the droplets, which can cover the sensor either partially or completely (due to the hydrophilic nature of isolating oxides) and the weak gate coupling of the oil phase. Further improvements of the system are necessary, in order to use its full power for performing and monitoring the biochemical reactions in time. For example, tuning emulsion composition to involve the conductive oils or organic solvents, the use of high-quality threshold voltage followers, and implementing the selective chemical functionalization of the channels, enabling the hydrophilic surface at the sensing area, are the crucial steps to do.

Finally we verify the capability of the novel droplets based nanowire platform for a bioassay applications and carry out a glucose oxidase (GOx) enzymatic test for glucose detection. The GOx enzyme catalyzes the oxidation of glucose into gluconolactone, which spontaneously hydrolyzes to D-gluconic acid by releasing a proton and giving rise to an acidification of the buffer (see Figure 6, panel a), measured in time.\(^7\) This time-dependent pH decrease should be detected by the FET.\(^7\) For this, the first enzymatic reaction was linked to a second one involving horseradish peroxidase (HRP). This second enzyme reduced the hydrogen peroxide (\(\text{H}_2\text{O}_2\)) produced from the initial reaction by concomitantly oxidizing a chromogenic substrate, e.g., 3,3',5,5'-tetramethylbenzidine (TMB), into TMBH\(_2\). The last oxidation step resulted in a color change from clear to blue. Thus, the success of enzymatic reaction could be confirmed by the parallel readout of the droplets using integrated optical detection setup\(^5\) (see Figure 6b,c).

In detail, 2 mM glucose in DI water was mixed in a 1:1 ratio with 2 mM TMB in 0.1 M phosphate citrate buffer (pH 7) which contained 3.3% GOx and 1.6% HRP. After mixing the solution, it was immediately guided to the microfluidic geometry, thereby creating droplets in a continuous oil phase with a final concentration of 1 mM glucose and 1 mM TMB in each droplet. Here, each droplet, passing the nanowire FET structure, allows to monitor precisely every stage of the reaction. In order to determine the change of the pH value during the reaction, the ratio of the droplet signal (droplet) to the baseline (oil) \((R_{\text{droplet/oil}})\) passing the FET was first calculated for each reservoir. Furthermore, \(\Delta R_{\text{droplet/oil}}\) was derived via subtraction the instantaneous \(R_{\text{droplet/oil}}\) from the mean value of droplet-to-oil ratios before the reaction \((R_0)\) (see Figure 6d). Here, \(R_0\) \((R_0 = 1.085, \text{at pH of about } 6)\) is exclusively valid for the observed glucose oxidation reaction and comprises negative values for acidification and positive values for alkalinization processes, as depicted by pink region in Figure 6d. By comparison of the arising ratio \(\Delta R_{\text{droplet/oil}}\) value with reference droplets (2 mM glucose—0.1 M phosphate citrate buffer 1:1) \(R_0\) (see Figure 6, panel d) we able to determine the pH changing dynamics during the reaction. Thus, Figure 6b shows an acidification process during the initial phase of the reaction with the FET. This leads to a lowering of the droplets signal (pink region). After completion of the glucose decomposition process, the FET signal was recovered. We attribute this to the pH equilibrating character of the phosphate citrate buffer, as it was later confirmed by using a pH meter to follow the dynamics of the reaction. Here, a decrease of 2 pH units was measured for the first minute, which returned later to the original value.

For the optical measurement we used a LED with a cold white spectrum. The light was focused onto the transparent FEP tubing with the emulsion inside. A fiber coupled spectrometer was used as a detector on the other side of the tubing. Here the integrated signal of the spectrometer was used for the measurement of the absorbance. When the liquid is changing its color from clear to blue, it is acting as a filter blocking the yellow wavelengths of the LED which is causing the decrease of the intensity as shown in Figure 6c.

Finally we realized GOx assay to demonstrate the decomposition of different concentrations of glucose in the range between 0 mM and 1 mM (\(c[\text{GOx}]\) is constant). Lowering of the glucose concentrations inside of the droplets leads to the weaker pH change during the reaction and results in a decrease of \(\Delta R_{\text{droplet/oil}}\) as shown in Figure 6e.

Conclusions. In conclusion, we presented a new optics-less method for high throughput detection and analysis of emulsion droplets in the microfluidic channel using compact integrated nanowire based ISFET. In particular, we investigate the effect of the droplet size with respect to the sensor area and its role on the ability to sense the inner environment of the droplet. Namely, we report that the small drops (shorter than the sensor linear dimensions) are not suitable for probing their inner environment with ISFET, due to the strong influence of the oil–water interfaces on the liquid gate potential but can rather be used to detect the interfacial charges. Note, we chemically probe the content of every single droplet in a row as independent events and resolve the pH values (H\(^+\) sensitivity) and ionic strength of the solution, resulting in a change of a source–drain current \(I_{\text{SD}}\) through the nanowires. Taking into account that the sensor is driven in electron conduction mode by a positive gate voltage, we achieve the increase of the source-drain current upon increase of the pH value in solution; the \(I_{\text{SD}}\) decrease, once the concentration of PBS in the solution becomes higher, is detected. Finally, on a proof of concept level, we demonstrate the GOx assay in droplets, measured by SiNW FET sensor and integrated optical spectrometer in parallel. Such realization of dual detection (SiNW sensor and optical luminescence) is demonstrated for the first time to the best of the authors’ knowledge.

We envision the high potential impact of the described technology for the early detection of pathogens in vitro assays, microorganisms, and drug screening or establishing the low-cost enrichment of nucleic acids\(^6\) in miniaturized format, where the change of pH or ionic strength can serve as an indicator of the ongoing biochemical process.\(^6\) In these regards, the advantage of the proposed platform is in the possibility of high throughput analysis and automatization of complex assays, where single nanowire sensor device can be employed for probing of thousands independent reactors. Finally, the discrete and pronounced signal change between oil and water phases allows for a stable counting of numerous droplets (Figure 75 in Supporting Information), allowing a repeated checking of the signal of every individual aqueous reservoir. This feature opens the plethora of applications in, e.g., chemical computing or information processing, where a single drop, detected by an integrated nanodevice can serve as an information bit (so-called droplet logic\(^1,4,7\) concept), able to trigger an avalanche of the programmed chemical processes.

Methods. Fabrication of Schottky Barrier Si-NW FETs. The sensing device fabrication is described elsewhere in detail\(^2,5\) and is shown in Figure 2. Briefly, the chip consists of a parallel array of bottom-up silicon nanowires transferred to...
the chip substrate by contact printing technique. Following the electrode design, consisting of nickel, is deposited on the substrate (I in Figure 2a). The FET geometry consists of an interdigitated electrode pair connected via silicon nanowires. By a silicidation process an intruded atom-sharp NiSi2–Si Schottky junction is formed within the intrinsic Si-nanowires. Exhibiting a Schottky-barner height of 0.45 eV for holes and 0.66 eV for electrons, the Schottky-FET shows a preferred p-type polarity. After the silicidation, the device was passivated with a 20 nm of Al2O3. The concentration of parallel nanowires can reach up to 109 per device, having a diameter of 22 nm ± 5 nm. Additionally, the chip was equipped with Ag/AgCl liquid gate electrodes at its FET structures (II in Figure 2a). The chip was spin-coated (Karl Süss RC8, Garching, Germany) with the image reversal photore sist (AZ 5214E, Micro-Chemicals GmbH, Ulm, Germany) at 4000 rpm for 50s. The electrode pattern was realized by a double UV illumination (MJB4, Süss Microtec, Garching, Germany) including a heating step for 2 min at 120 °C. Now, 100 nm pure silver with a 5 nm chrome adhesion layer were evaporated on the chip and residual resist removed in acetone (VWR International GmbH, Darmstadt, Germany) for 15 min. For the electrochemical reaction to AgCl, one electrode of the FET and a covering 0.1 M KCl solution was developed (mr600 dev, Microresist Technology GmbH, Germany) at 4000 rpm for 50 s. Now, 15 μm SU-8 were coated, prebaked at 95 °C for 3 min, and exposed to UV light (Karl Süss MJ8, Garching, Germany). After post-baking at 95 °C for 6 min, the structure was developed (mr600 dev, Microresist Technology GmbH, Germany), cleaned, and finally baked at 120 °C for 30 min.

PDM-Based Microfluidic Integration. Silicone elastomer and its curing agent (Sylgard 184 and Sylgard 184 silicone elastomer curing agent, Dow Corning, USA) were mixed well in a 1:6 ratio and vented in the vacuum oven (Vacutherm VT6025, Heraeus, Hanau, Germany) for 10 min. After curing at 100 °C for 15 min over the master structure, PDMS was treated in O2 plasma and gently brought in contact using a little weight and incubated at 70 °C in the oven and left overnight at room temperature. Finally, Teflon tubes were connected to the channel openings and additionally sealed with PDMS (IV in Figure 2a).

Measurement Setup for Droplet Detection. For droplet formation, either DI water or PBS aliquots with different pHs and molar concentrations were applied. The continuous phase consisted of a mixture of mineral oil (M5904, Sigma-Aldrich, USA) with 2% nonionic surfactant (Span80, Sigma-Aldrich, USA). Control of liquid flow rates was given by a low pressure microfluidic pump system (Nemesis, Cetoni GmbH, Germany). By connecting of the electrode pads of a single SiNW FET to the integrated conductive needles of a tip probe station (Karl Süss, Garching, Germany) to the sourcemeter allowed measuring and manipulating the conductivity of the respective SiNW FET and detection of droplets. For real-time observation the chip was placed under the built-in microscope with an integrated camera (Olympus SZX-ZB12, Hamburg, Germany). For a time-dependent droplet detection the SiNW FET was set at constant gate voltage with continuously recording of the /JG.

Solutions for pH and Ionic Measurements. Phosphate-buffered saline (PBS) tablets (Sigma-Aldrich, USA) were dissolved in 200 mL of DI water resulting in 1x PBS solution, pH 7.4 (containing 10 mM PO4 3−). In order to manipulate the pH, aliquots from the stem solution were manipulated with 1 M HCl and 0.1 M NaOH to alter the pH from 4 to 8 using a pH meter (Inolab, Germany). PBS concentration gradients (10 mM to 10 μM) were created by diluting aliquots of PBS with DI water in the desired quantity.

ASSOCIATED CONTENT

Supporting Information

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APTES functionalization of the chip substrate, droplet size analysis, conductivities of the liquids, gate coupling for different liquids, real-time measurement data of PBS droplets with pH values ranging from 4 to 8, real-time measurement data of PBS droplets with decreasing ionic concentrations, raw data ratio analysis for various pH values and ionic concentrations and long-time high throughput measurement data (PDF (AVI)

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J.S., F.Z., B.I., R.I., and S.P. conducted experiments under supervision of L.B. Simulations were realized by D.N., L.B., W.W., L.B., G.C., and T.M. wrote the proposals and oversaw the research in their groups. The manuscript was written by L.B. and J.S. with input from G.C, W.W., and T.M. All authors cowrote the paper and agree to its contents.

Notes
The authors declare no competing financial interest.

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