FLUIGENT

Zurich Instruments

IMPEDANCE SPECTROSCOPY FOR CHARACTERIZATION AND COUNTING

INTRODUCTION

Microfluidics allows for the precise monitoring and control of chemical or biological events at the microscale level. At this scale, comparable to the dimensions of the biological cell, microfluidic detection and analysis of single cells is of great interest, allowing lab-on-a-chip and point-of-care applications[1]. Cell detection is generally performed using optical methods such as FACS (Fluorescent Activated Cell Sorting), where cells pass through a laser beam and the light scattered is characteristic to the cells and their components. However, these methods require additional and usually time-consuming labelling steps. Electrical impedance spectroscopy (EIS) is a label-free technique that enables real-time, high-throughput measurements, and eases the process of data extraction and processing[2]. Continuous flow microfluidic devices with embedded microelectrodes for electrical measurements can be employed for detecting and classifying single cells or particles (e.g. beads or droplets) in a high throughput manner[1]. Further, since the dielectric properties of a biological cell are defined by its cellular characteristics such as cell volume, composition and architecture[1], impedance spectroscopy can be used to differentiate between cell types. When using impedance spectroscopy, the frequency response of the cell can be measured and fitted to an equivalent circuit model. This allows quantitative measurements related to the different properties of the cell to be extracted, such as membrane thickness and cytoplasm conductivity. Moreover it is also possible to perform precise droplet analysis using EIS (droplet size, counting, cell-in-droplet quantification)[1,3].

There are several key advantages of this technique in the microfluidic environment including:

- Fast throughput: ~1000 particles/s
- Multiple parameter analysis
- Probe impedance of the analyte at multiple frequencies simultaneously
- Integrates well with other analysis methods (e.g. optical detection)
- Label free analysis method

We present in this application note our Electrical Impedance Spectroscopy Platform (or EISP) consisting of microfluidic flow controllers from Fluigent to maintain precise flow control, a chip from Micronit Microtechnologies B. V to localize impedance measurements, and a lock-in amplifier from Zurich Instruments to perform impedance measurements. We demonstrate the system efficiency by determining the size of micrometer beads and by measuring the generation rate water-in-oil droplets.

MATERIALS AND METHODS

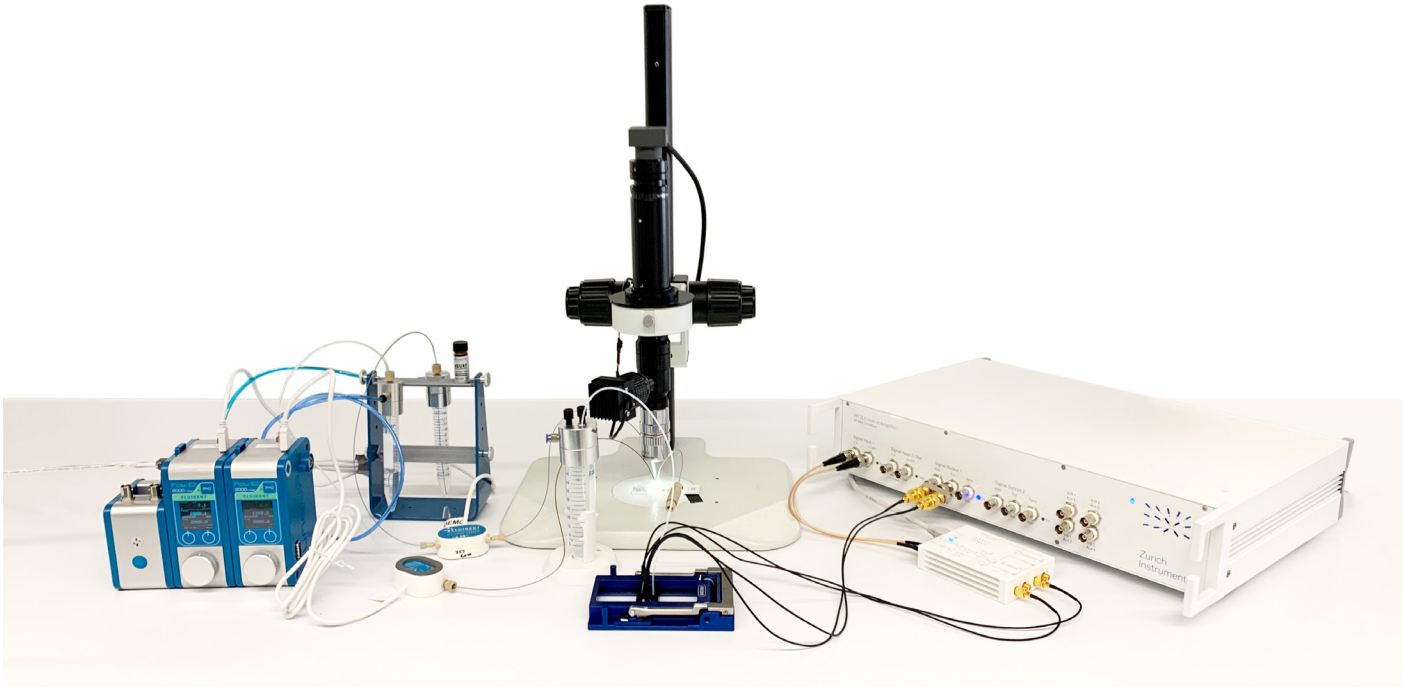


Figure 1: System setup for EISP. Two Fluigent Flow EZ flow controllers are connected to the EZ Drop chip for droplet generation. The tubing passes through Flow Units to monitor and regulate flow. The droplets generated flow into the Micronit Electrical Impedance Spectroscopy (EIS) chip. The Zurich Instruments HF2LI Lock-in amplifier with HF2TA transimpedance current amplifier is connected to the Micronit EIS-chip to measure electrical signals for characterisation and monitoring. Visualization of the EZ Drop chip channels is performed using an optical microscope.

An external pressure source is connected to two Fluigent Flow EZ flow controllers that are connected to the Fluigent EZ Drop microfluidic chip via tubing. The tubing passes through Flow Units to allow flow rate measurements. The droplets generated flow into the Micronit Electrical Impedance Spectroscopy (EIS) chip and pass through the sensing region. The Zurich Instruments HF2LI lock-in amplifier provides voltage excitation to the differential electrode pairs on the EIS chip, and measures the returning current via the HF2TA transimpedance current amplifier. Visualization of the EZ Drop chip channels is performed using an optical microscope.

1. Materials

Microfluidic flow controller

The Flow EZ is the most advanced flow controller for pressure-based fluid control. It can be combined with a Flow Unit to control pressure or flow rate. It can be used without a PC. Two Flow EZ with 2 bar full scale are used in the setup presented here. It may be desirable to use two Flow EZ 7 bar as the EIS chip is highly resistive and higher pressures may be required to reach optimal range of flow rate for some experiments.

Flow sensor

The Flow Unit is a flow sensor that allows real time flow rate measurement. By combining a Flow Unit with the Flow EZ, it is possible to switch from pressure control to flow rate control, allowing the generation of highly monodispersed droplets over a long period of time. Flow Units M and L are used here.

Reagents

Continuous phase: 3M™ Novec™ HFE7500 containing 2% dSurf. dSURF is a biocompatible fluorosurfactant providing highly reliable droplet production and stability even under PCR amplification conditions. Fluorinated oil is used instead of mineral oil as it has overall better properties (highly biocompatible, immiscible, low viscosity).
Dispersed phase: Distilled water.

Microfluidic chips

EZ Drop chip

The microfluidic chip used for droplet generation is the Fluigent EZ Drop. The chip is made of PDMS and features three droplet generator units. The EZ Drop is designed to generate droplets over a large range of droplet sizes (20 – 100 µm diameter) and generation rate. Using the EZ Drop, one can generate highly monodispersed (CV < 2%) droplets. This chip can be used for various applications such as cell encapsulation and polymer particle formation. Droplets are generated using a flow focusing method (figure 2): the dispersed phase is introduced directly into the main channel while the continuous phase is injected by two perpendicular channels. The dispersed phase is pinched on both sides by the continuous phase and droplets are generated.

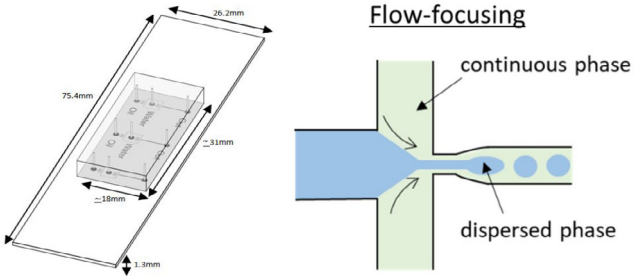


Figure 2: The EZ Drop microfluidic chip (left). Scheme illustrating droplet generation using flow focusing geometry (right)

Electrical Impedance Spectroscopy (EIS) chip

The Electrical Impedance Spectroscopy (EIS) chip is designed by Micronit Microtechnologies B. V. It is made of borosilicate glass and an inert interstitial layer of dry film resist for well-defined channels ideal for uniform flow through sensing region. The standard design for EIS-chips has a straight through channel 28 µm deep and 30 µm wide, making it suitable for complete blood count type applications. There are two sets of double electrodes, with planar separation of 20 µm and 28 µm depth separation as based on channel depth. A cross sectional schematic of the electrode layout can be seen in figure 3.

Figure 3: Electrical Impedance Spectroscopy chip from Micronit (left) Cross sectional view of electrode layout (right)

HF2LI

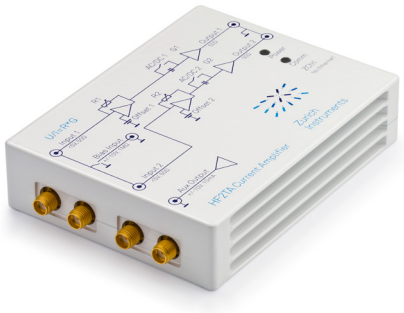
The Zurich Instruments HF2LI is a high-frequency dual channel lock-in amplifier that uses the latest hardware and software technologies to provide industry leading specification and functionality. The dynamic reserve of 120 dB sets the benchmark in the 50 MHz frequency range. In many setups a single HF2LI replaces multiple conventional instruments. The basic instrument functionality can be extended with the HF2LI-MF option to allow simultaneous measurements at up to 6 frequencies.
The HF2LI can be conveniently controlled by the LabOne software and its APIs from Zurich Instruments. The integrated toolset of LabOne includes the LabOne Plotter module and Data Acquisition (DAQ) module to visualize measurements in a temporal resolution of just 5 µs. In addition to these fast, fixed-frequency measurements, the LabOne Sweeper module also offers frequency sweep capability from 1 mHz to 50 MHz.

HF2TA

The HF2TA current amplifier converts 2 input currents to output voltages in a frequency range up to 50 MHz. This device is an active probe which can be conveniently placed close to the measurement setup. It supports most applications where a current must be converted to a voltage.

The advanced design of the HF2TA ensures stability and a smooth operation over the entire frequency range. The combination of this transimpedance current amplifier with the HF2LI enables very high performance measurements and insensitivity to background noise due to its dual channels and low noise design.

HF2LI



HF2TA

2. Impedance measurements of microbeads

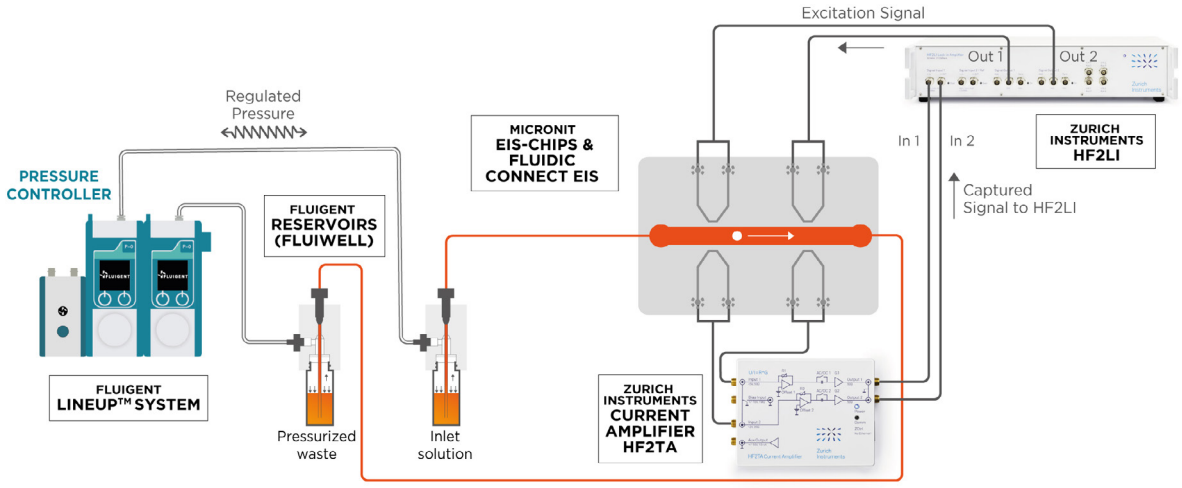


Figure 4: Schematic of the microfluidic system used for microbeads injection and impedance measurement.

A scheme of the microfluidic setup is presented in figure 4. An external pressure source is connected to the LineUp System consisting in two Flow EZ, which in turn are connected to two reservoirs to set the pressure drop between the input and output of the system. The system is thus pressurized allowing for enhanced flow control. One reservoir contains the microbead suspension to be injected (inlet solution) and the second reservoir is used to collect the solution coming out from the chip (pressurized waste). The inlet pressure is always higher than the outlet pressure to maintain a unidirectional flow from the input to the output. The reservoirs are connected to the EIS microfluidic chip using tubing. The microbeads pass the electrode pairs within the chip and impedance measurements are performed using the HF2LI lock-in amplifier coupled with the HF2TA current amplifier. The experiment is performed using beads of 3 µm and 5 µm diameter.

3. Impedance measurements of water-in-oil droplets

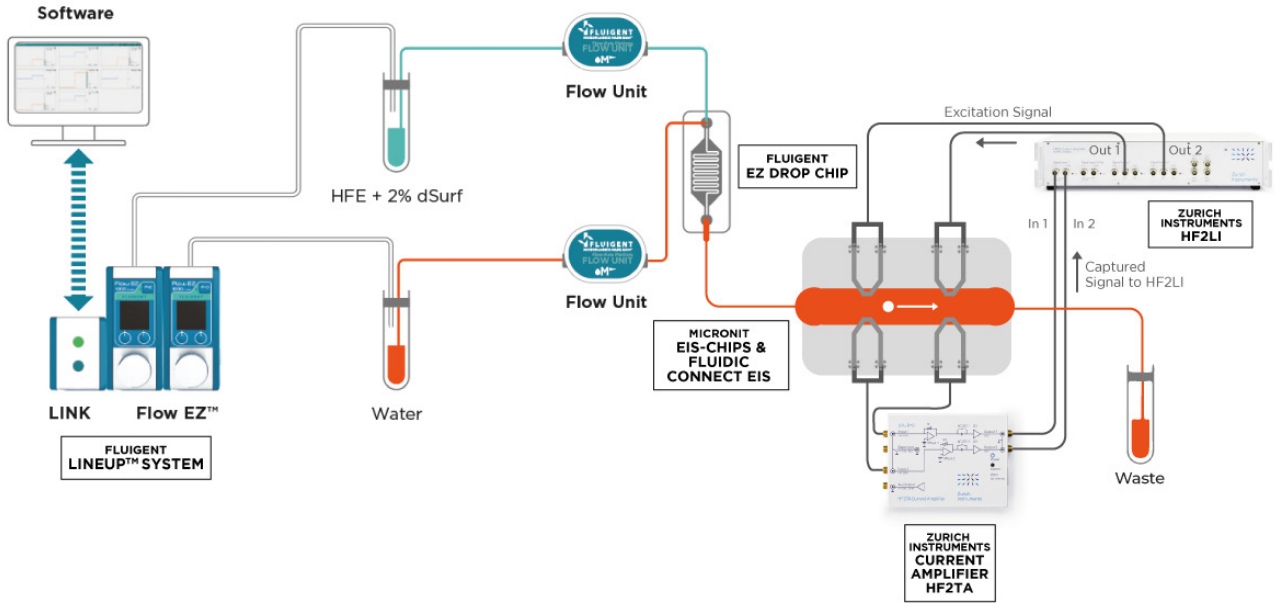


Figure 5: Schematic of the microfluidic system used for droplet generatio, injection and impedance measurement.

A scheme of the microfluidic setup is presented in figure 5. An external pressure source is connected to the LineUp System consisting in two Flow EZ, which in turn are connected to two 15 mL reservoirs containing water and 3M™ Novec™ 7500 with 2% dSurf. The reservoirs are connected to the two inlets of the EZ Drop microfluidic chip via 1/16 tubing of 250 µm and 1/32 in. PEEK tubing of 254 µm and 157 µm inner diameter. The tubing passes through Flow Units to allow flow rate measurements. Pressure is applied on the two reservoirs: water is injected in the inner channel and the oil phase is injected in the surrounding channel of the microfluidic chip. Visualization of the chip channels is performed using an optical microscope. Droplet generation within the EZ Drop channels is presented in figure 6, where we observe highly monodispersed droplet generation. Note that in this case the system was not pressurized at the outlet as the waste reservoir was not connected to a flow controller (figure 5).

Once generated, the droplets flow through the outlet tubing and are injected into the EIS microfluidic chip. The droplets pass the electrode pairs within the chip and impedance measurements are performed using the HF2LI lock-in amplifier coupled with the HF2TA current amplifier.

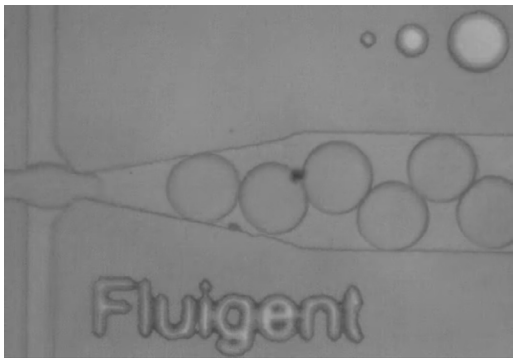


Figure 6: Production of water in oil droplets using the EZ Drop microfluidic chip

RESULTS

1. Impedance measurements of microbeads

Using the same microfluidic system presented in part 2. of the above “Materials and Method” section, 3 µm and 5 µm diameter microbead suspensions are injected into the EIS chip, where beads pass electrodes pairs surrounding the microfluidic channel allowing impedance measurements to be performed.

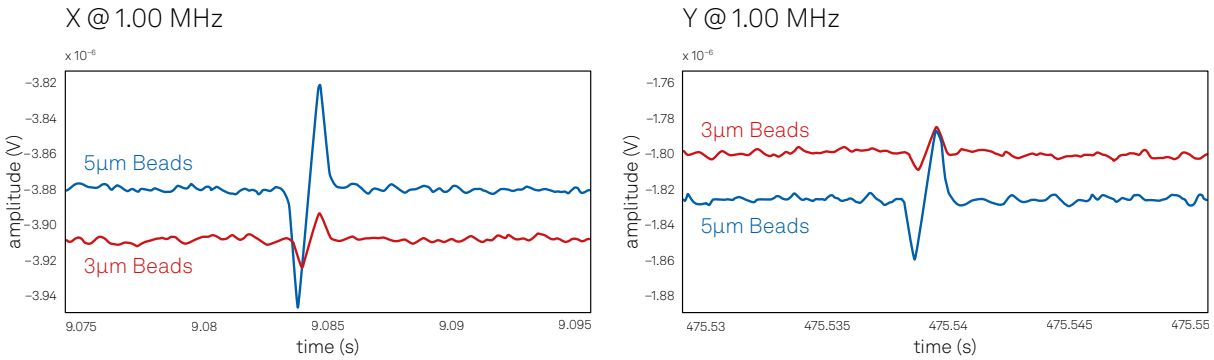
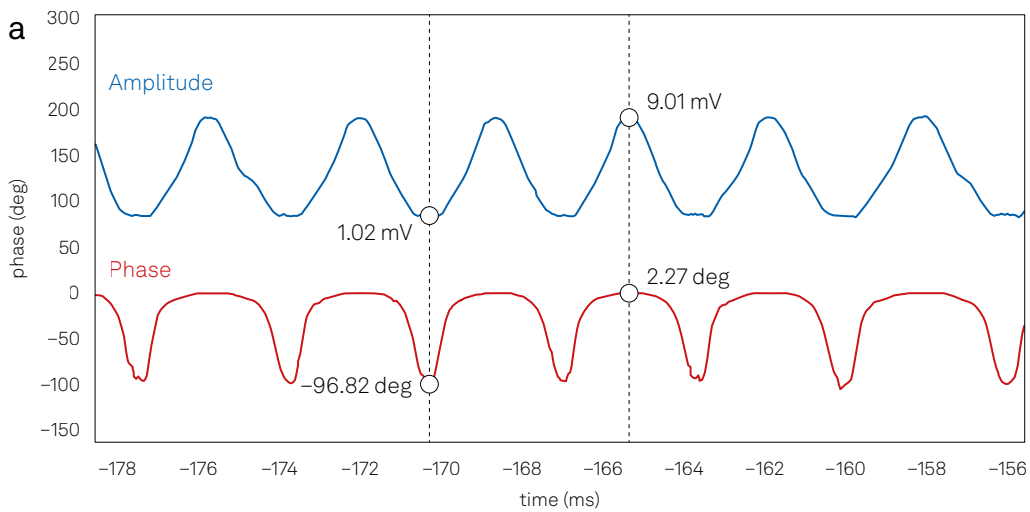


Figure 7: The (amplified) current signal of beads passing the microfluidic electrode pairs measured by the HF2LI lock-in amplifier at 1 MHz. The red trace shows the signature from a 3 µm bead and the blue trace from a 5 µm bead. X and Y stand for the real and imaginary components of the measured current, respectively.

Figure 7 shows that the signals from the 5 µm beads (blue trace) display consistently larger amplitudes than the 3 µm beads (red trace) in both X and Y. The 5 µm beads show a peak-peak amplitude change between 75 and 120 mV while the 3 µm beads between 20 and 30 mV. These results correspond nicely to the difference in volume between the two beads (a factor of 4.6). Thus, using this impedance spectroscopy signal can discriminate particles or cells according to their sizes. It is hence possible to differentiate between 3 µm and 5 µm beads using our microfluidic system.

2. Impedance measurements of water-in-oil droplets

To delve one step further, the experiment is repeated using the same microfluidic system presented in part 3. of the “Materials and Method” section. Water-in-oil droplets are injected into the EIS chip, where the droplets pass electrodes pairs surrounding the microfluidic channel allowing impedance measurements to be performed.



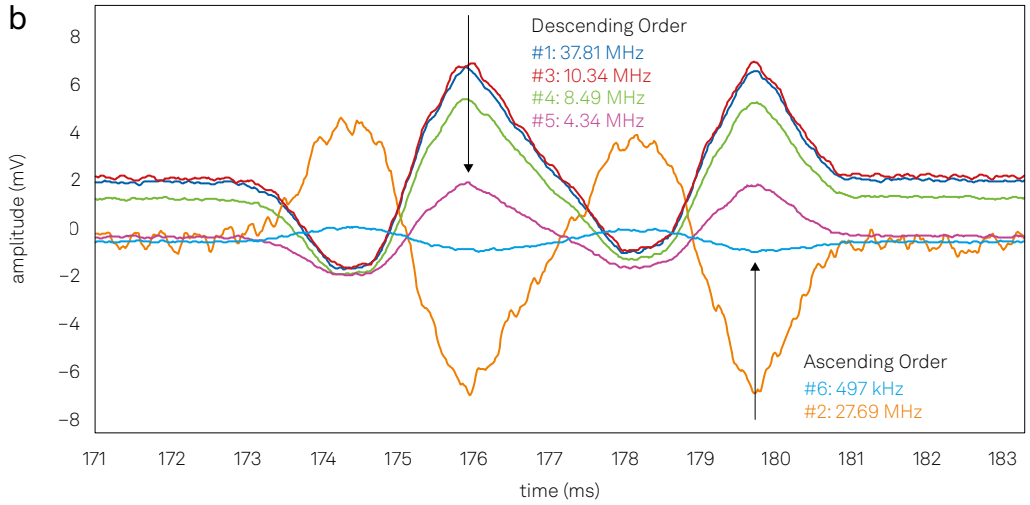


Figure 8: The (amplified) current signal of water-in-oil droplets passing the same electrode pairs described in figure 7. a. single-frequency amplitude and phase measurement at 10 MHz. b. multi-frequency measurement displaying the imaginary current signal from the droplet acquired simultaneously at six different frequencies (requires HF2-MF option).

Figure 8 shows the impedance measured at 10 MHz. Clear peaks can be seen in the current amplitude and in the phase as each droplet passes the electrode pair. The phase information indicates a clear change from resistive (fluid only) to capacitive behavior as the droplets pass the sensing region of the electrode pair. Additionally, the density of peaks in the time-domain chart gives useful information on the droplet generation rate and velocity. For instance, the 6 peaks observed over a time span of 18 ms indicates a droplet generation rate of 333 droplets per second. Considering the electrode pair spacing of 30 µm, the average velocity of the droplet is calculated as 30 mm/s. Hence this technique can be used to count even fast moving droplets, beads or cells.

Simultaneous multi-frequency measurements can be seen in the lower figure of figure 8. Here, the HF2LI equipped with the HF2-MF option measures the impedance signal (current) at six different frequencies simultaneously. The imaginary current signal varies with frequency, changing in both magnitude and phase. The inverted signal observed at both 497 kHz and 27.69 MHz is due to the frequency-dependent dielectric property of the droplets and emphasizes the need to measure at multiple frequencies. This simultaneous multi-frequency measurement offers a fuller picture of the frequency-dependent dielectric properties of the passing droplets (beads, or cells), while saving the overall measurement time by a factor of 6.

CONCLUSION

We have demonstrated the efficiency of our cost-effective EISP by determining the size of micrometer beads and by measuring the generation rate and velocity of water-in-oil droplets. Combining the LineUp system with the EIS-chip and the HF2LI lock-in amplifier enables fast detection and discrimination of individual cells or particles in flow at a speed unavailable to camera-based solutions. In addition, this label-free technique can distinguish particle sizes and cell types thanks to high sensitivity at different frequencies. Ultimately, the use of EISP on the microfluidic scale is diverse and includes applications such as:

- **Quality control in the food industry**
- **Flow cytometry for counting and sorting of cells or droplets, marker-free detection and protein engineering**
- **Blood analysis**

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