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micromachines MDPI

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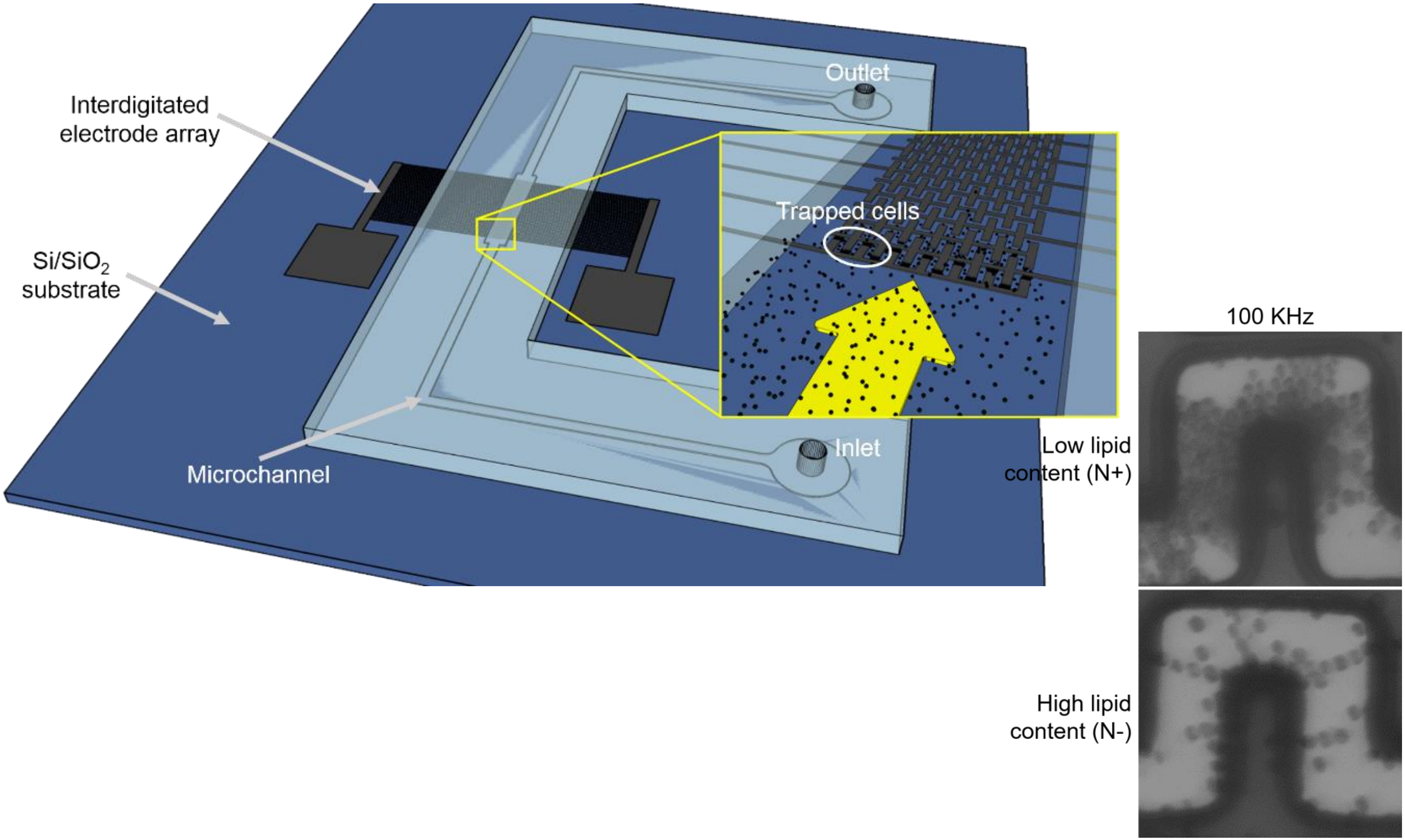
Graphical abstract



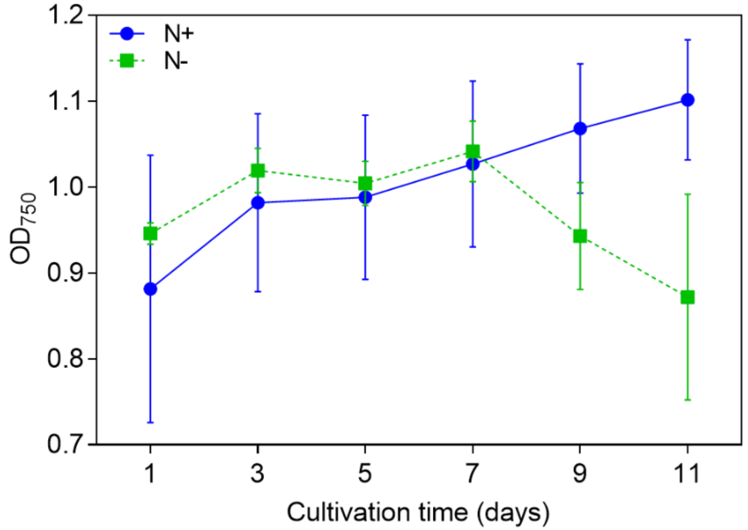
Abstract

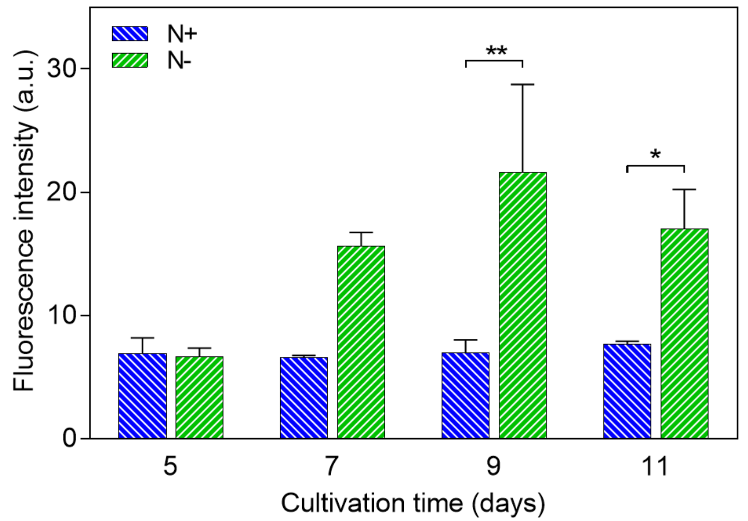
- In this study, heterogeneous cytoplasmic lipid content of Neochloris Oleoabundans was dielectrophoretically assorted in a microfluidic device using castellated carbon microelectrodes. For this, a nitrogen-replete (N+) cell suspension, and a nitrogen-deplete (N-) cell suspension were cultured to promote low and high lipid production in cells, respectively. The cell population was monitored by spectrophotometry, and the resulting lipid development among cells of the two samples was quantified by Nile red fluorescence. The microfluidic device simply comprehends a glassy carbon castellated microelectrode array bonded to a PDMS microchannel. A Finite element analysis was conducted to determine the dielectrophoretic trapping zones across the electrode gaps. Experiments were carried out on a wide frequency window (100 kHz to 30 MHz) at a fixed amplitude of 7 VPP. Experimental results showed a statistically significant difference between the dielectrophoretic behavior of N+ and N- cells at low frequencies (100-800 kHz), and a weak response for mid and high frequencies (1-30 MHz). These results suggest that low-cost glassy carbon is a reliable material for microalgae isolation between low lipid content and high lipid content cells, through a fast, reliable and straightforward mechanism, such as dielectrophoresis.

Keywords: dielectrophoresis, microalgae, biofuels, microfluidics, Carbon-MEMS.

Biofuel from microalgae

- Microalgae is an attractive biomass source for biofuel and fatty acids production. However high harvesting cost is a challenging limitation for mass production of biomass: 20-30% of total production expense. In this sense, lipid content monitoring in microalgae is crucial not only to determine the optimum harvesting time, but also to identify highly-producing strains from environmental samples.
- For this purpose, in this study, two samples of microalgae were cultured under different nitrogen conditions for different lipid content. Fig 1 shows microalgae growth after 11 culturing days.





AC dielectrophoresis

- In AC DEP, polarizable particles suspended in a fluid are manipulated by applying an spacially non-uniform AC electric field, **E**. The DEP force acting on a spherical particle, $\mathbf{F}_{DEP}$, depends upon the particle shape and size, the dielectric properties of the particle and the suspending medium, and the non-uniformity of **E**:

$$\mathbf{F}_{DEP} = 2\pi r^3 \varepsilon_m \varepsilon_0 \mathrm{Re}\left[f_{CM} \right] \nabla \mathbf{E}^2$$

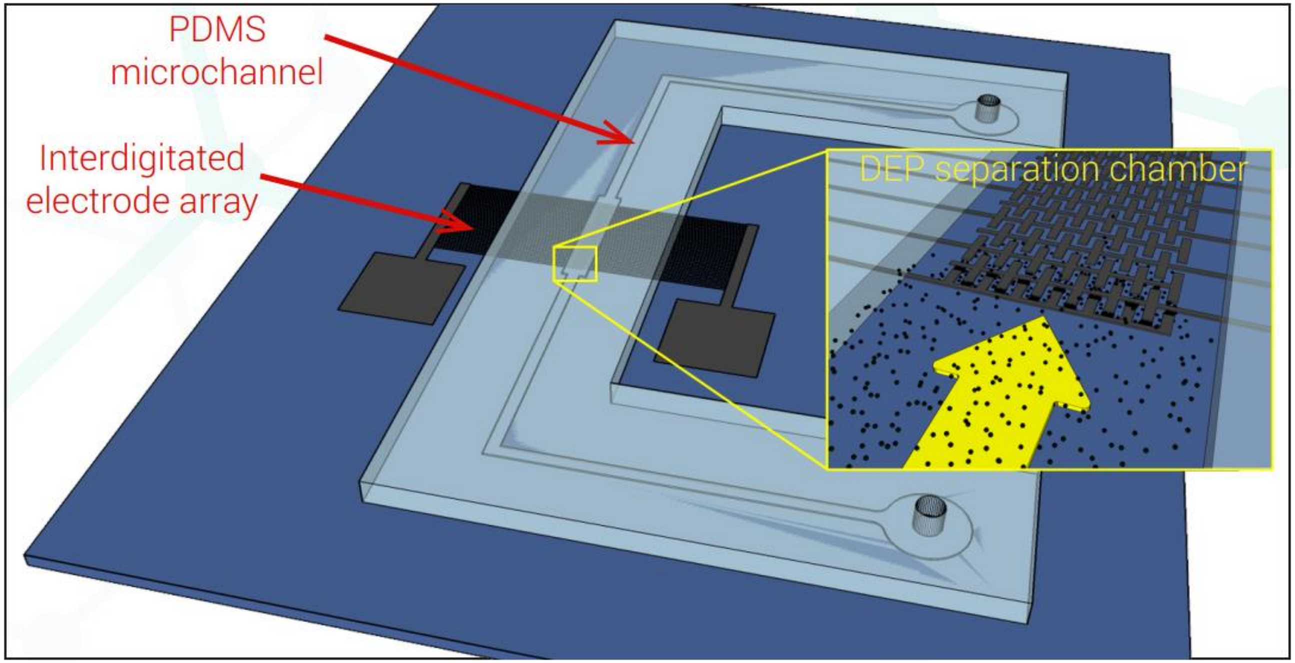
- where *r* is the particle radius, ε_m is the relative permittivity of the suspending medium, ε_0 is the dielectric constant in vacuum, and f_{CM} is the real part of the frequency-dependent Clausius-Mossotti factor.

$$f_{CM} = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*}$$

- with ε^*_m and ε^*_p representing the complex permittivity of the particle and the medium, respectively.

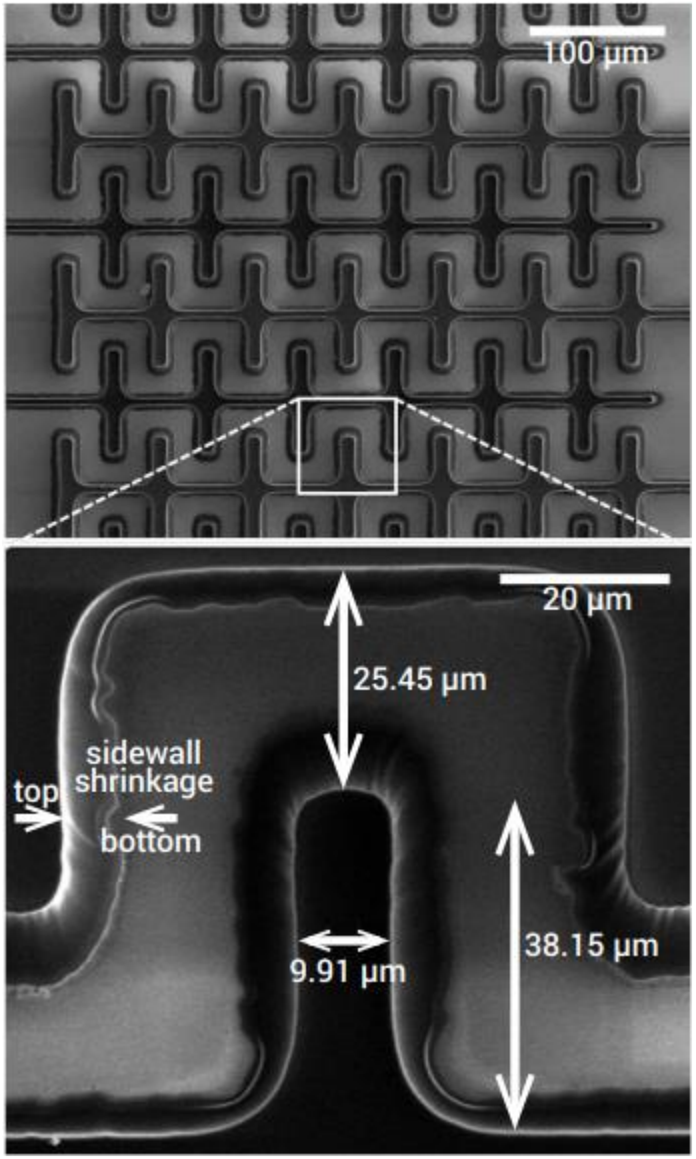
Microfluidic chip

- The microfluidic chip was designed to study the DEP behavior of microalgae with high and low lipid content. The use of castellated interdigitated electrode array (IDEA) guarantees high-magnitude $\mathbf{E}^2$ using low-amplitude AC signals and enough room to separate large amounts of cells. The IDEA is confined by a PDMS microchannel fabricated by soft-lithography.



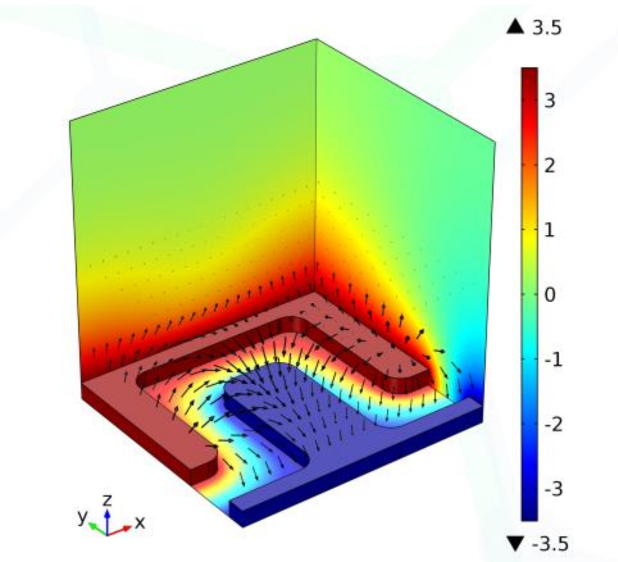
Microfluidic chip

- The IDEA was fabricated using the Carbon-MEMS process—i.e. photolitographic patterning, followed by pyrolysis. The resulting glassy carbon material has a wide electrochemical stability window that allows to work with highly conductive fluids without electrolysis or gas formation.

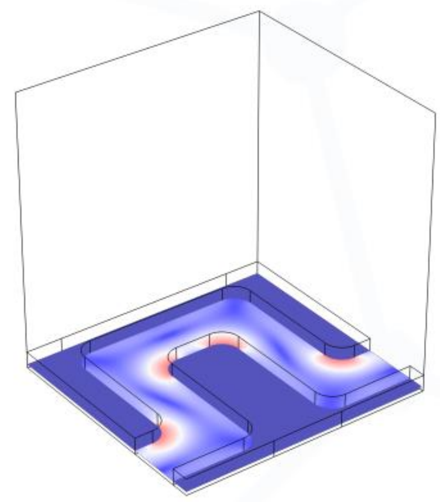


Results

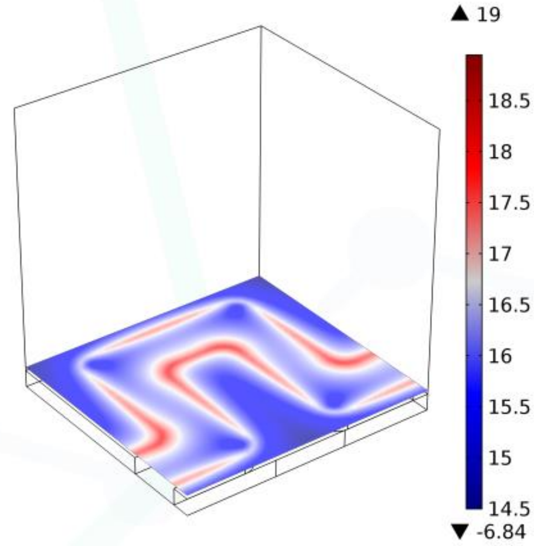
- Finite element analysis of the used electrode geometry of **E**:



Electric field distribution



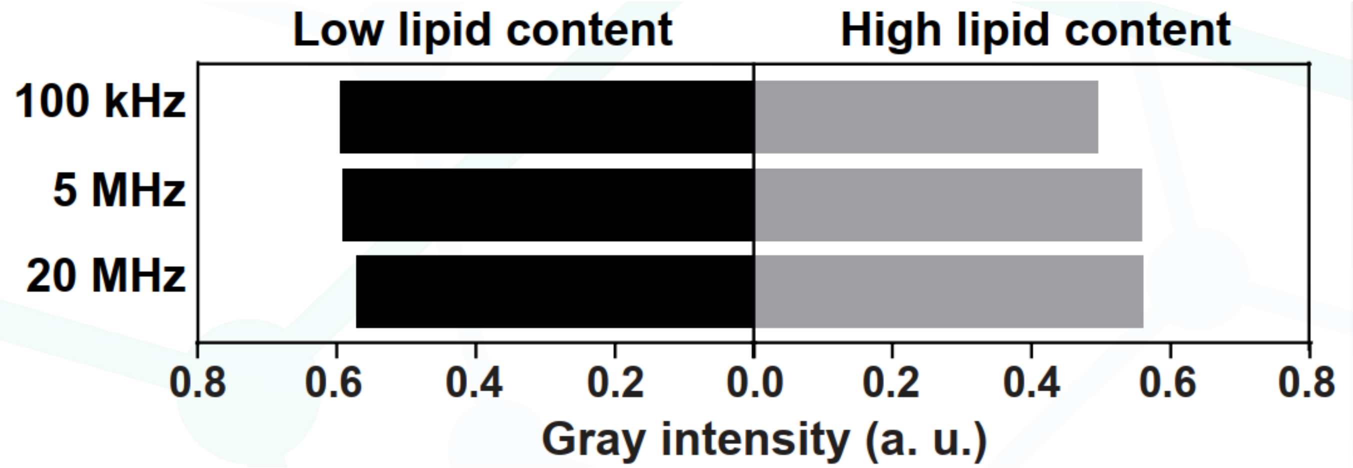
pDEP at channel floor



pDEP at top of electrodes

Results

- Experiments were carried out over three frequency ranges, comprising (a) the low frequency (100 kHz to 800 kHz), the mid frequency (1 MHz to 10 MHz), and the high frequency (15 MHz to 30 MHz). Signal amplitude was 7 V_{PP} for all experiments.



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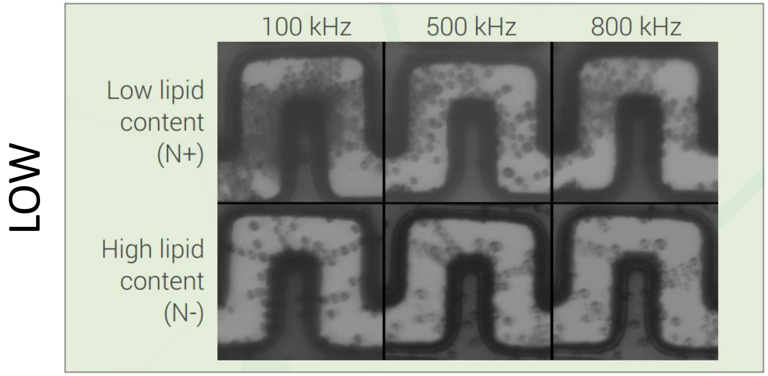
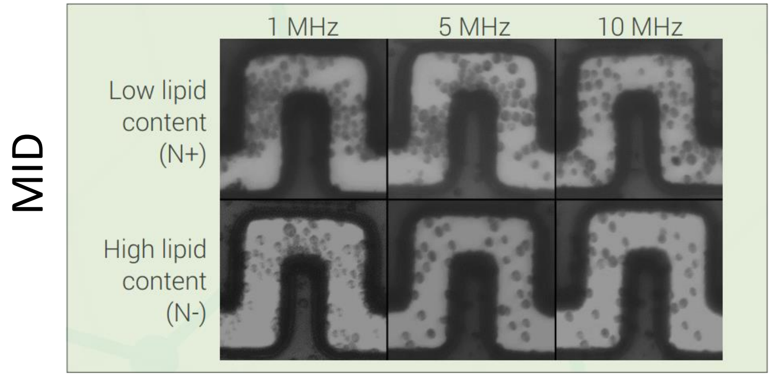
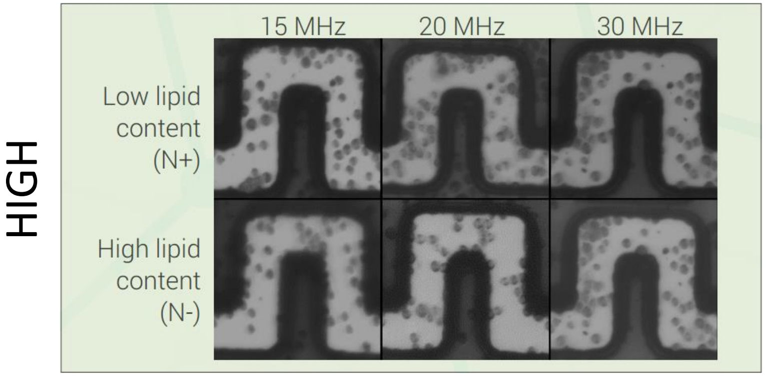
Results

- DEP characterization for the three frequency ranges for each cell type. The highest cell differentiation was found at 100 kHz







Conclusions

- In this study, Neochloris Oleoabundans microalgae with high and low lipid content were differentiated by their DEP response when stimulated with an AC amplitude of 7 VPP and frequencies ranging between 100 kHz and 30 MHz using a carbon castellated IDEA.
- With our set-up, we demonstrated promising results for the characterization of microalgae with high and low lipid content using a low-amplitude AC signal on a wide frequency range.
- This suggests that the cells possess a “dielectrophoretic fingerprint” that allows to determine the high and low lipid contents based on their DEP behavior.
- As shown in this study, Carbon-DEP devices decrease operation costs due to the reduced time required to screen lipid content in microalgae samples, which serves as an indicator of the best harvesting time, a critical step for large scale microalgae production.
- The use of the Carbon-MEMS process for electrode fabrication also represents a cost-effective alternative to conventional and costly technologies based on other materials (typically noble metals), without sacrificing performance and reliability.
- We believe that due to the simplicity of the Carbon-MEMS and soft-lithography processes, our approach can be scaled for mass production of Carbon-DEP-based microfluidic devices.
- Finally, the intrinsic chemical properties of glassy carbon electrodes, including excellent biocompatibility and a wide electrochemical stability window, suggest that this material can be used to characterize the DEP response of a wide variety of living cells in a label-free fashion.

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