**Introduction**

- Droplet microfluidics can be used to encapsulate cells for biochemical applications.
- However, the concentration of nutrients and growth factors decreases over time, while the concentration of catabolic byproducts increases, which make droplet hard for long-term cell culture.
- We developed a droplet acousticfluidic chip that can exchange cell medium in droplets by the combination of the pico-injection and the droplet split with acoustophoresis. After running droplets through this chip, cell medium in droplets got exchanged and cell growth in droplets was extended.

**Theory**

- We have previously shown that acoustophoresis can be used in droplet microfluidics. In this work, the encapsulated cells are focused in a bulk acoustic half-wavelength standing wavefield. There is a pressure node along the centre line of the channel and pressure antinodes along the channel sidewalls.
- The primary acoustic radiation force \( F = m \alpha \ddot{u} \) can focus particles on the pressure node or the pressure antinodes. Particles with a positive acoustic contrast factor are focused on the pressure node (i.e. the centre of the channel in a \( \lambda/2 \) standing wavefield), while particles with a negative acoustic contrast factor are focused on the pressure antinodes (i.e. the channel sidewalls in a \( \lambda/2 \) standing wavefield). Generally, particles and cells have a positive acoustic contrast factor in water and are thus focused on the centre of the microfluidic channel by the primary acoustic radiation force.

**Result and discussion**

- In the first case, we observed yeast cell growth in a droplet without using pico-injection. We can observe that after 8 hours there is no cell growth. In absence of fresh cell media, yeast cells do not grow after a certain time due to a lack of nutrients.
- We injected fresh cell media into the droplet after 6 hours of incubation using pico-injection in the second case. Contrary to the first case, yeast cells in a droplet continue to grow for 16 to 18 hours.
- In the third case, we injected fresh cell media into the droplet after 8 hours. Similar to the second case, we observe yeast cells continue to grow for up to 20 hours (Figure 3c).
- In Figure 3d, we plotted the average per hour yeast cell growth of collected droplets against time for all three cases. Here average per hour yeast cell growth was calculated by averaging the cell growth per hour for every droplet. Injecting fresh media with nutrients using pico-injection can allow yeast cells to grow for a longer period.

**Device Design and Operation**

- Yeast cells suspended in a cell medium are encapsulated in droplets by a PDMS chip with a T-junction. The channels were 65 \( \mu \)m wide with a height of 85 \( \mu \)m and fabricated following a standard protocol of soft lithography (Figure 1a).
- A silicon chip was used to exchange the cell medium in the droplets (Figure 1b). This chip contains droplet reposition, pico-injection and droplet split with acoustophoresis. This silicon chip was fabricated by dry etching.
- The channel width of the silicon chip was 150 \( \mu \)m which matches the acoustic frequency of 4.5 MHz to form a \( \lambda/2 \) standing wavefield.

**Conclusion**

- We demonstrate the combination of pico-injection, acoustic waves, and passive droplet splitting for exchanging the nutrient-depleted cell media with nutrient-rich cell media for yeast cell growth for a longer time.
- The microfluidic chip shows the capability to allow the yeast cell to grow for a longer period of time.

**References**