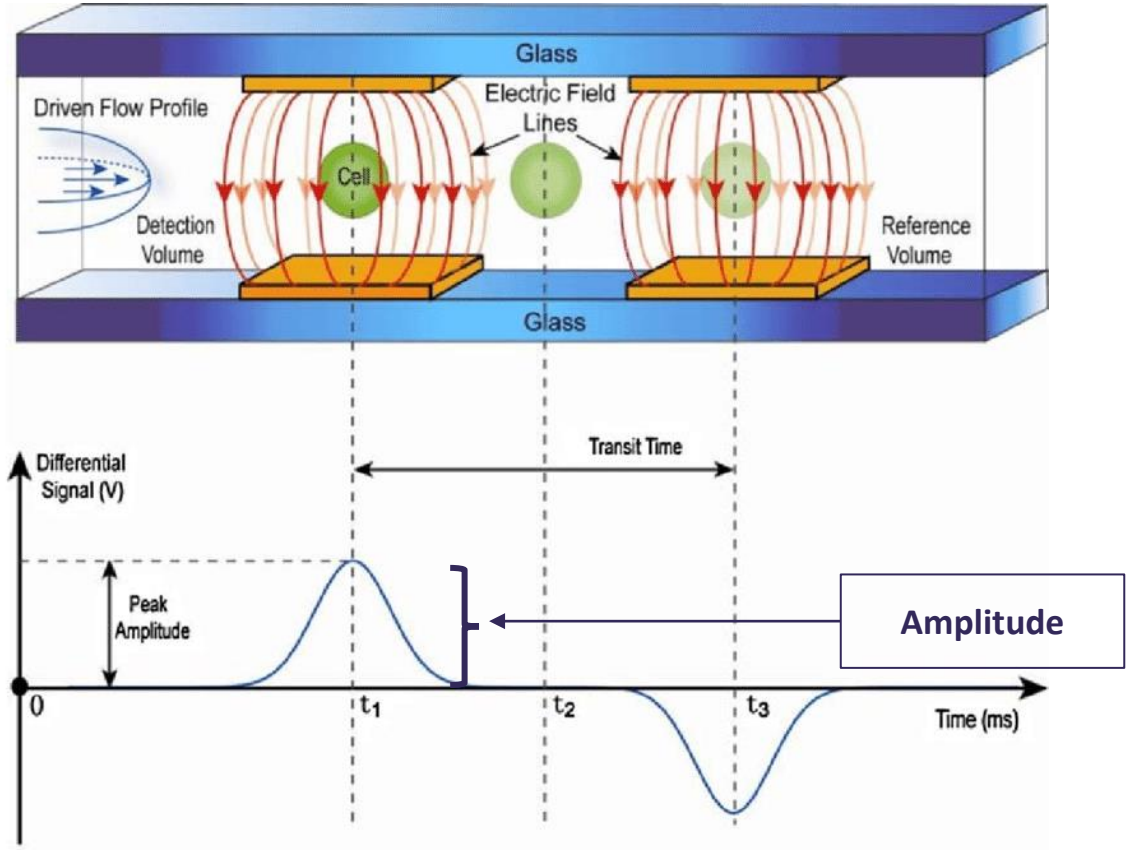
Label-free Enumeration of Bacteria by Impedance Flow Cytometry

Contents

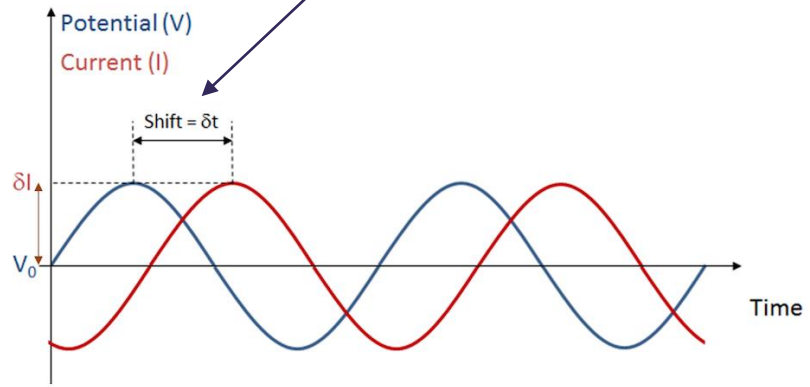
Impedance flow cytometry in a nutshell



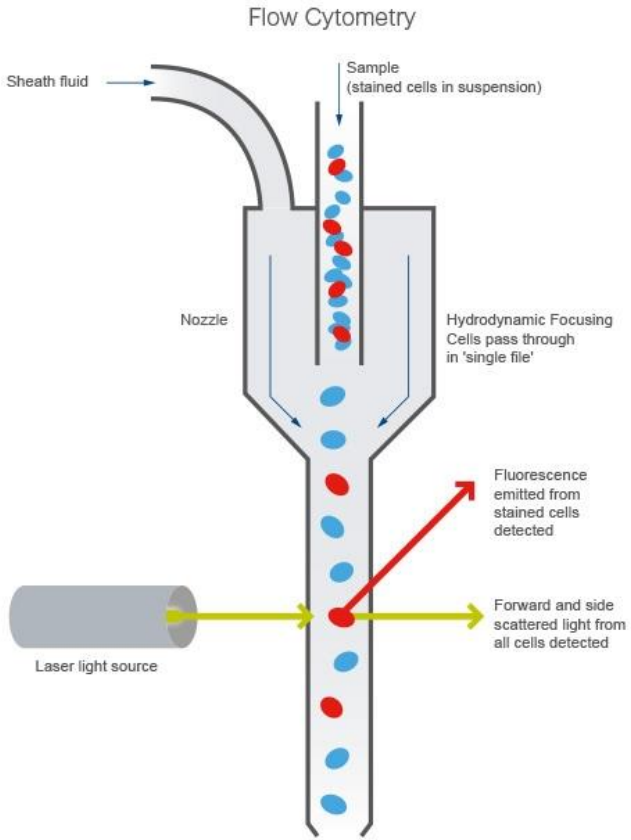
Sun, T., van Berkel, C., Green, N. G., & Morgan, H. (2009). Digital signal processing methods for impedance microfluidic cytometry. Microfluidics and Nanofluidics, 6(2), 179–187. https://doi.org/10.1007/s10404-008-0315-3

Phase shift

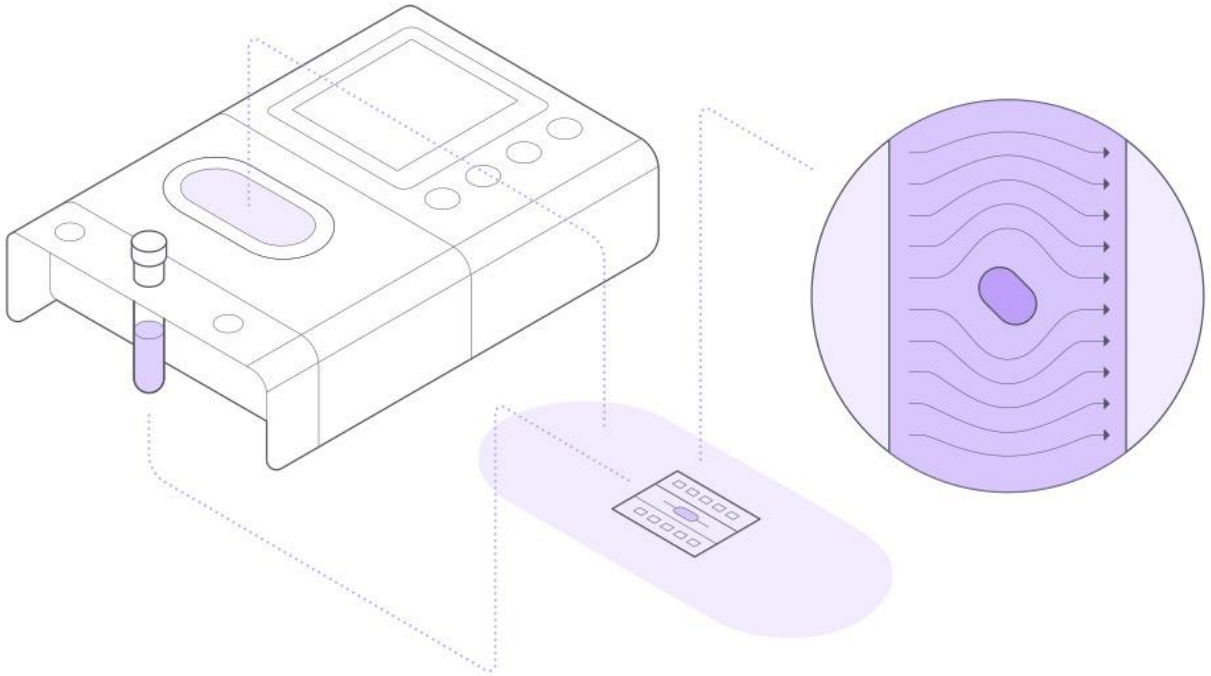
- A “delay” of electrical signal as the it passes through different objects or materials.
- The phase shift will depend on the applied electrical frequency



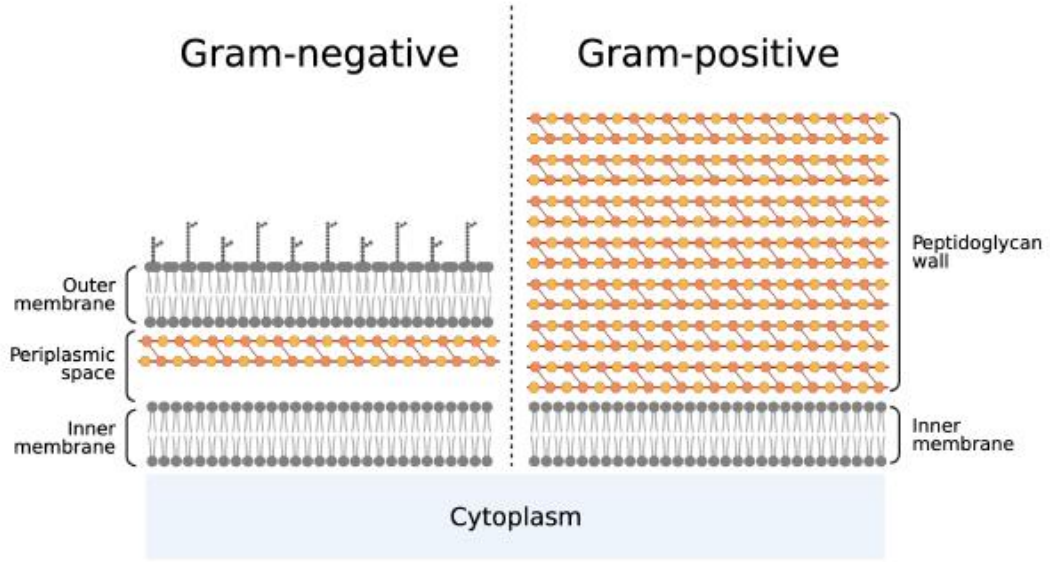
Fluorescence flow cytometry (FFC)

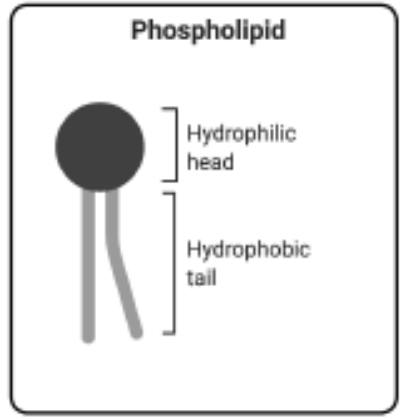


Impedance flow cytometry (IFC)

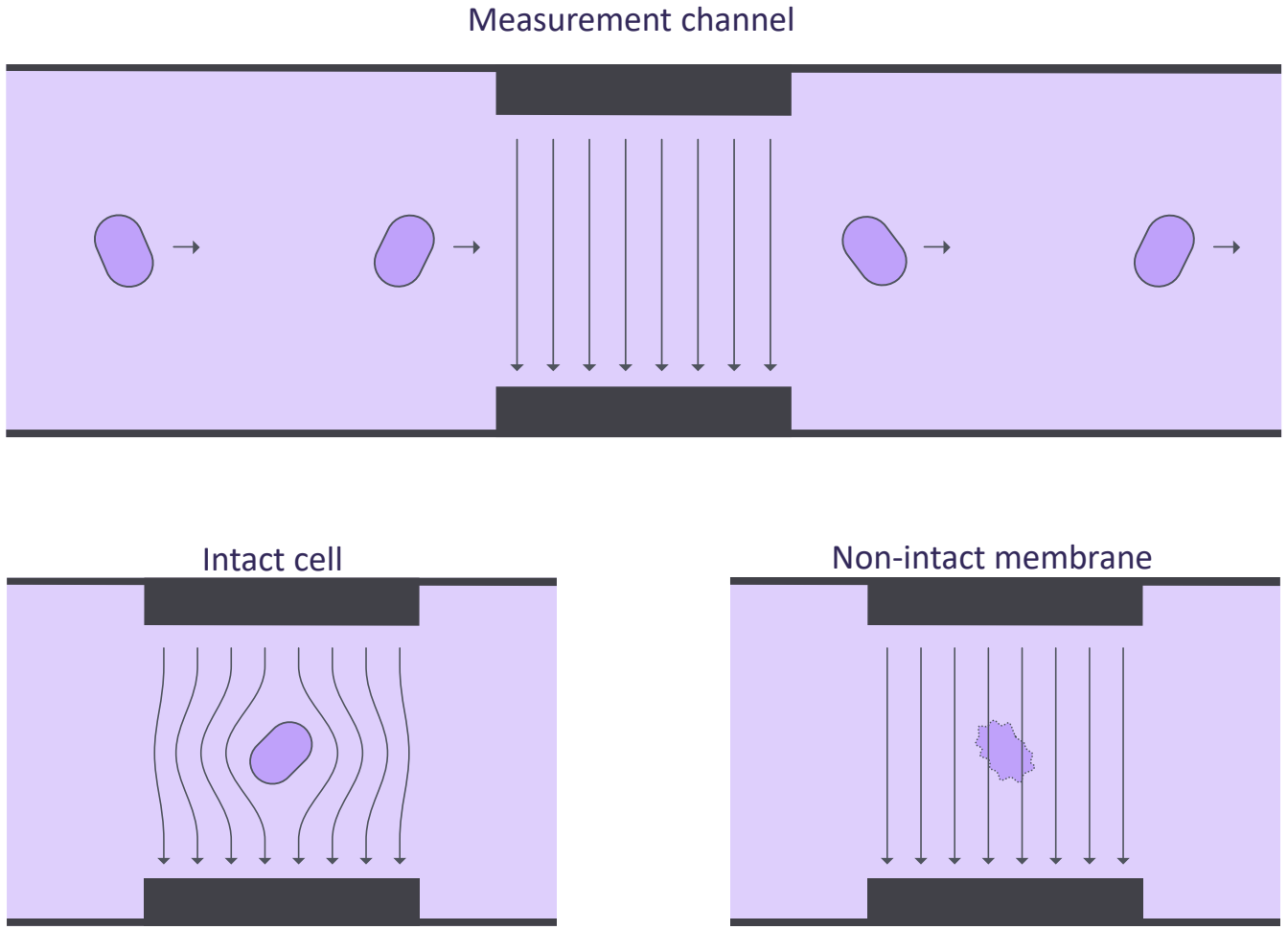


Impedance fingerprints for intact bacterial membranes

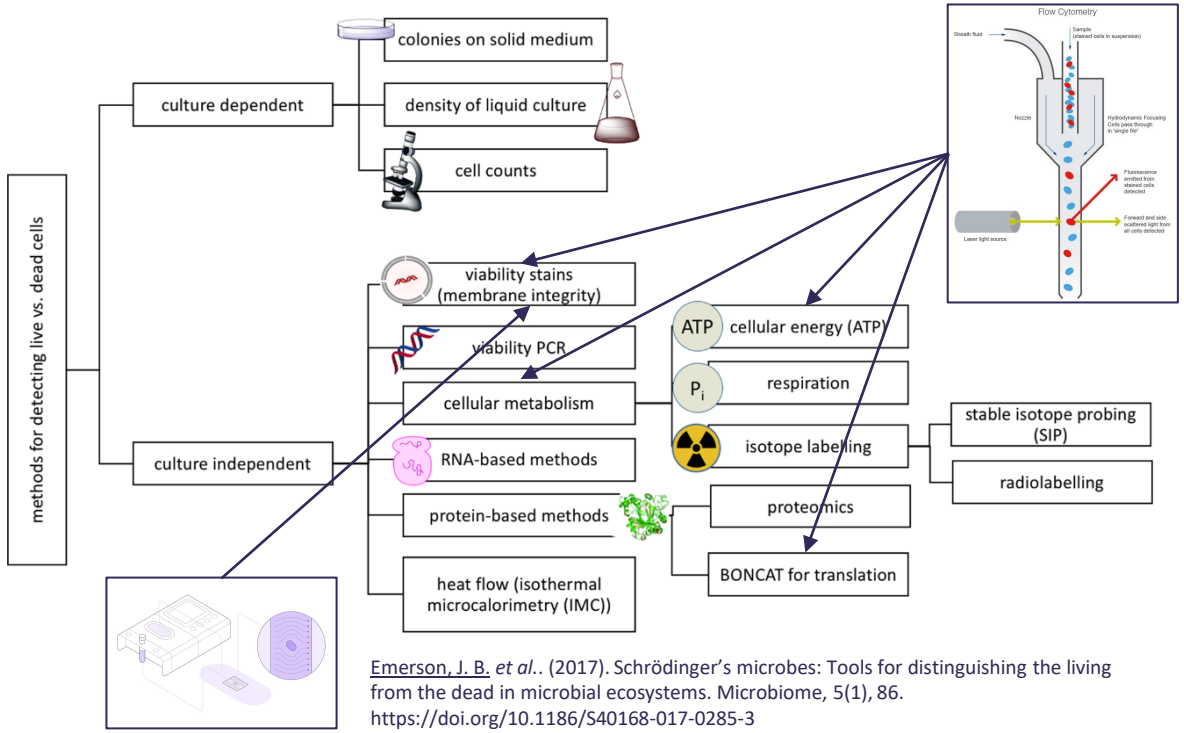




IFC detection principle and live/dead differentation



Membrane integrity as a proxy for viability



Emerson, J. B. *et al.*. (2017). Schrödinger's microbes: Tools for distinguishing the living from the dead in microbial ecosystems. Microbiome, 5(1), 86. https://doi.org/10.1186/S40168-017-0285-3

"It is generally accepted (but not a universal rule) that a cell must be **intact**, **capable of reproduction** and **metabolically active**, in order to be considered alive and different viability assessments are designed to measure one or more of these properties, either directly or by proxy"

Why use membrane integrity as proxy for viability.

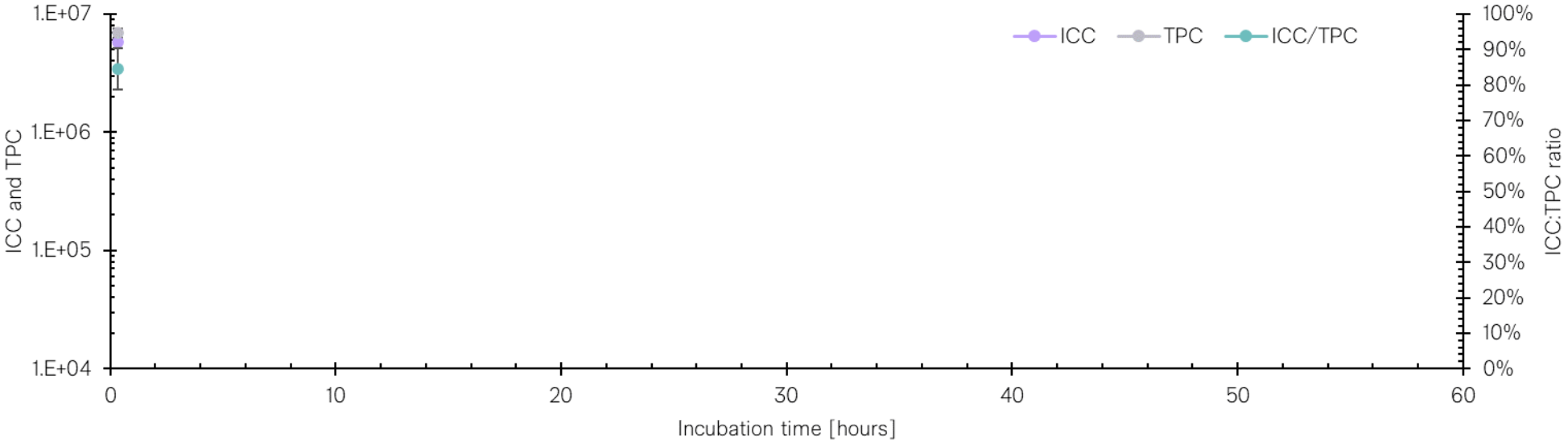
1. "The outer cell membrane is critical to all life on earth"
2. "Membrane integrity is considered to be a biomarker for viable cells because cells with compromised membranes are – or will soon be – dead"

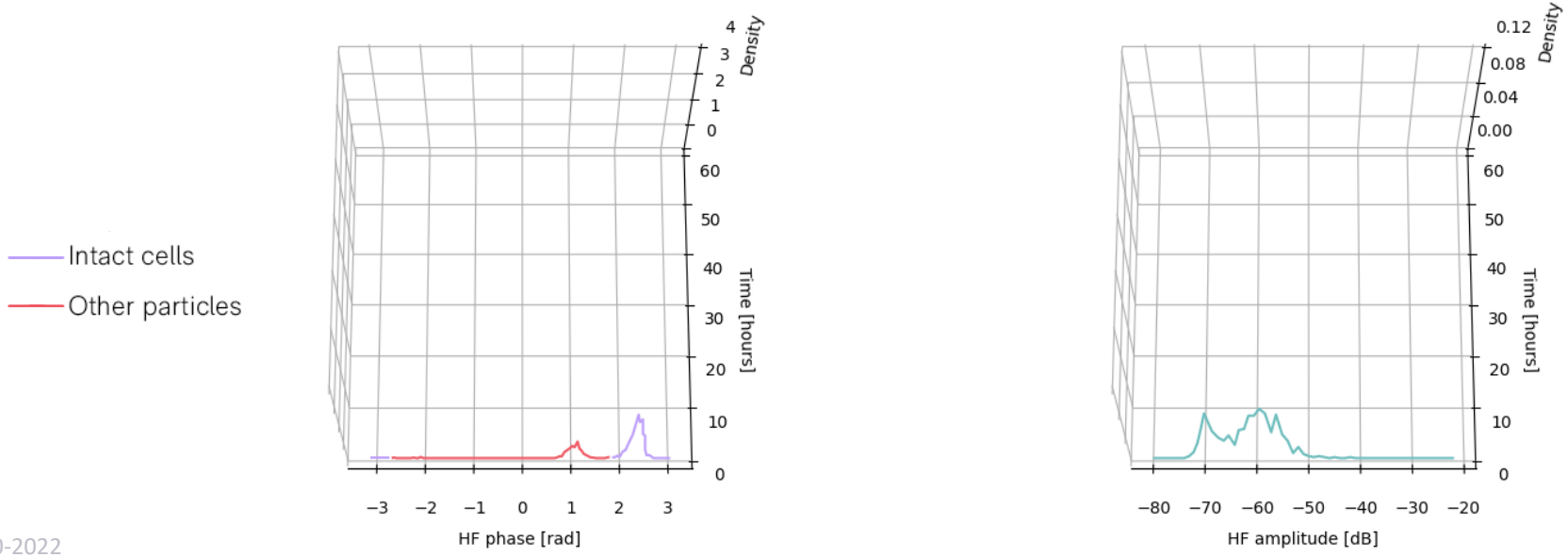
Caveats

1. "Lethal stress may not lead to immediate membrane disintegration"
2. "The dyes that are typically employed to assess membrane integrity may be ineffective against cells with a hardy membrane or cell wall such as spores"

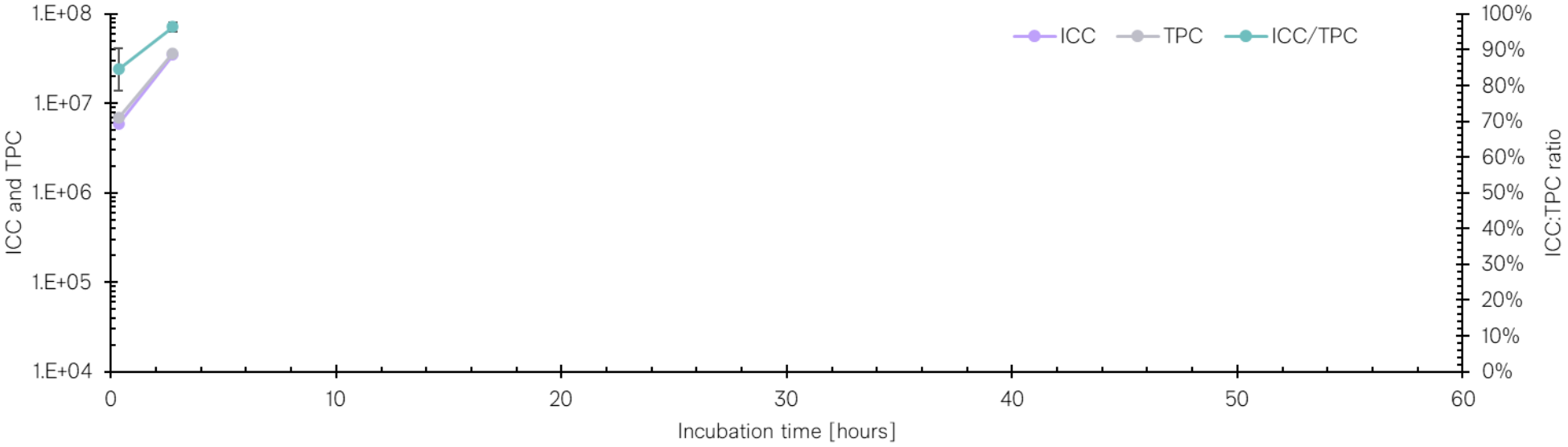
	Alive?	Dead?
Colony present	Yes	No
Colony absent	Maybe (VBNC etc.)	Maybe
Intact membrane	Maybe	Maybe
Non-intact membrane	No	Yes

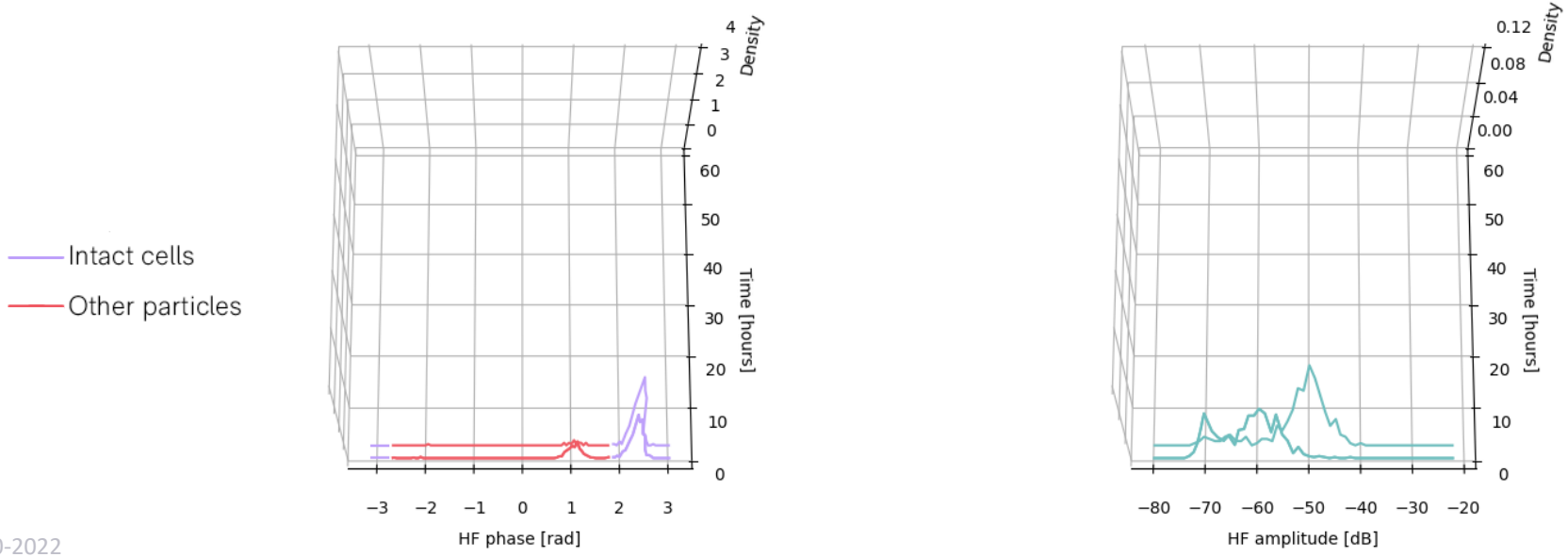
Staphyloccus epidermidis shake flask example | 0.3 hours



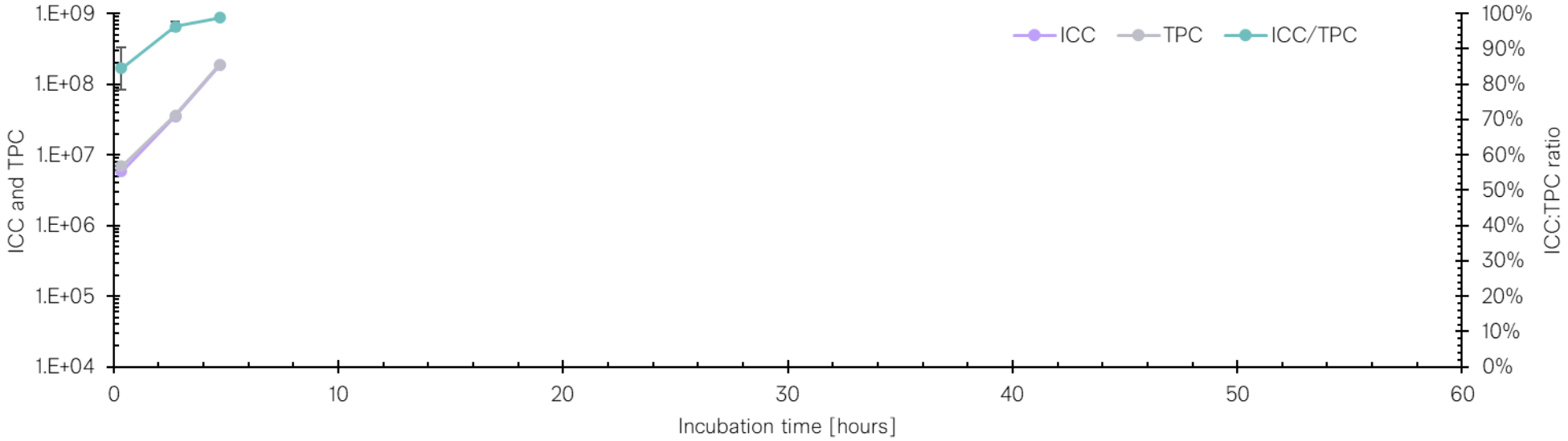


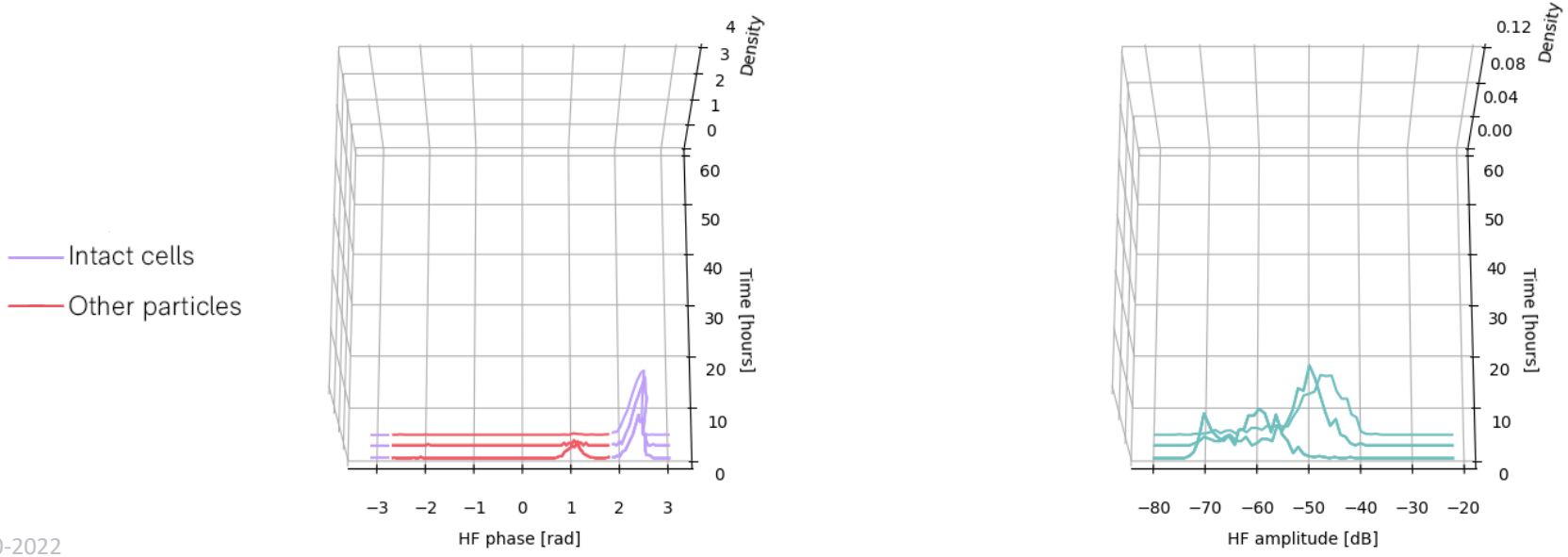
Staphyloccus epidermidis shake flask example | 2.7 hours



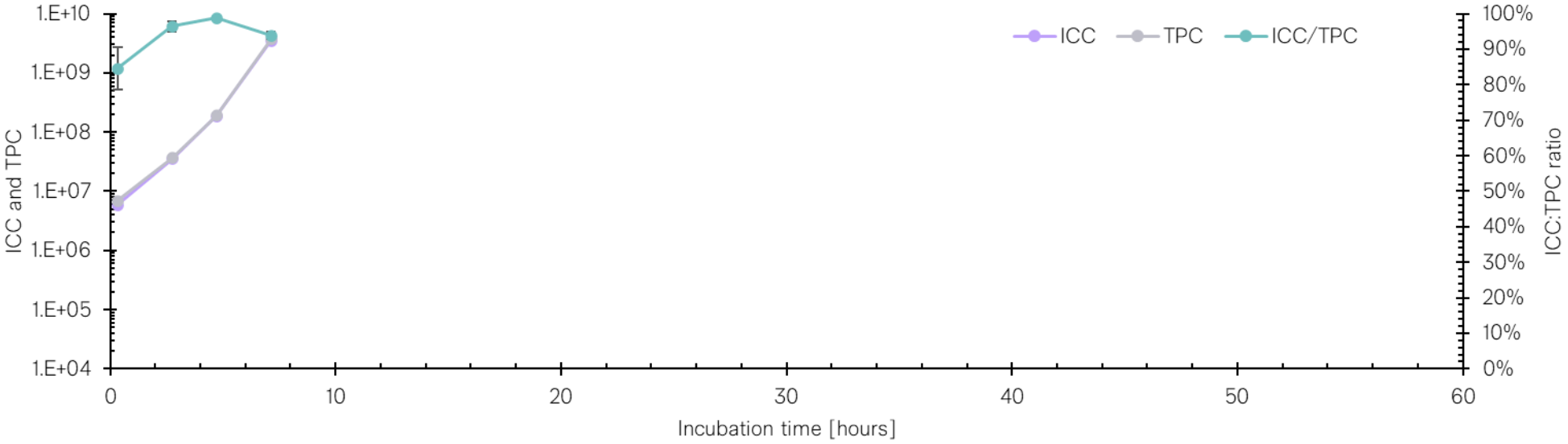


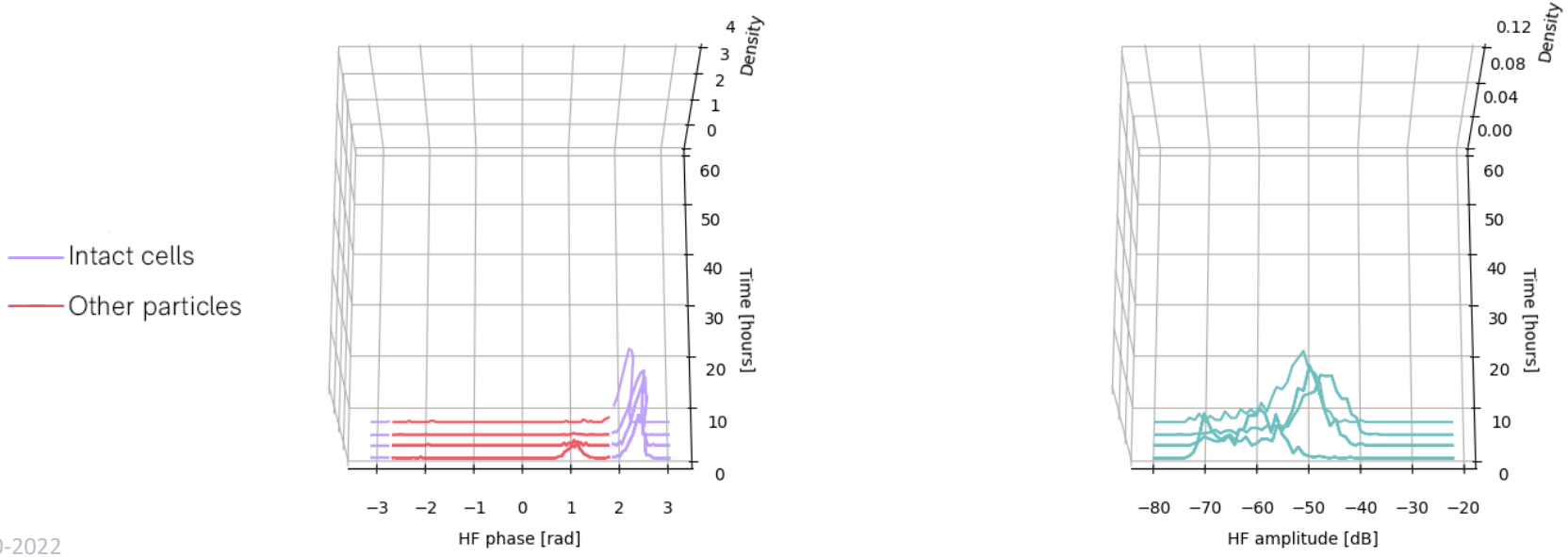
Staphyloccus epidermidis shake flask example | 4.7 hours



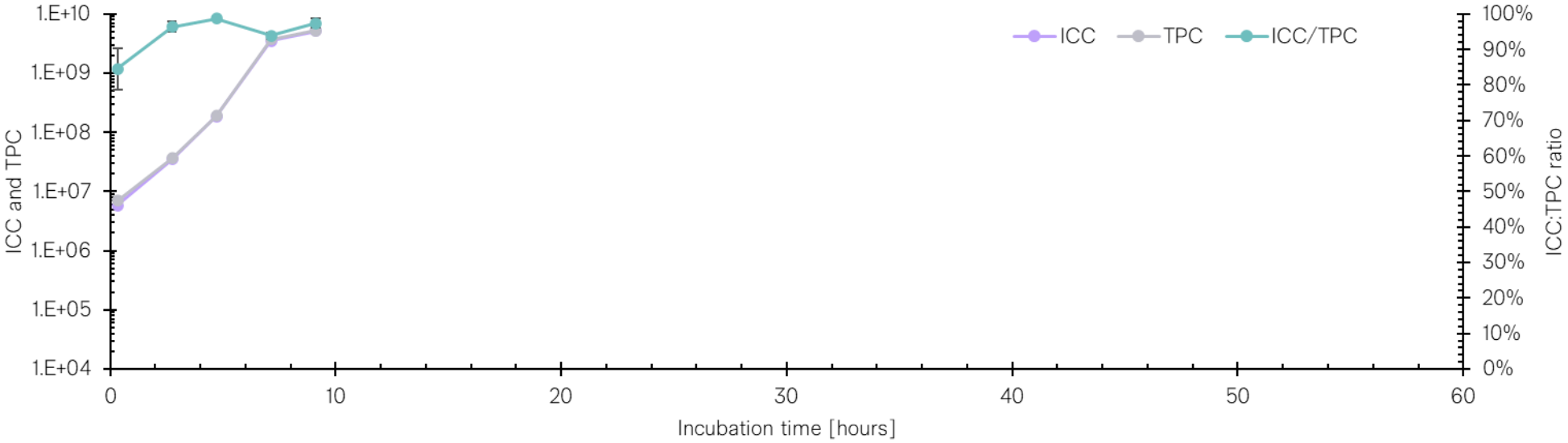


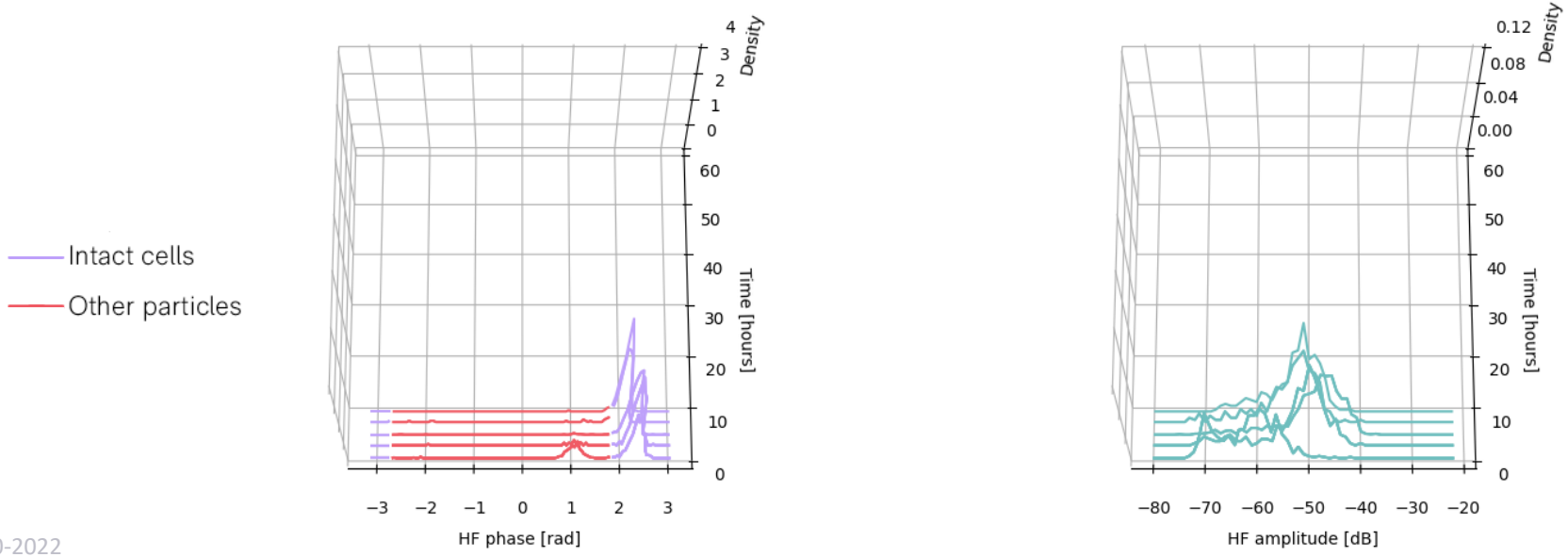
Staphyloccus epidermidis shake flask example | 7.1 hours



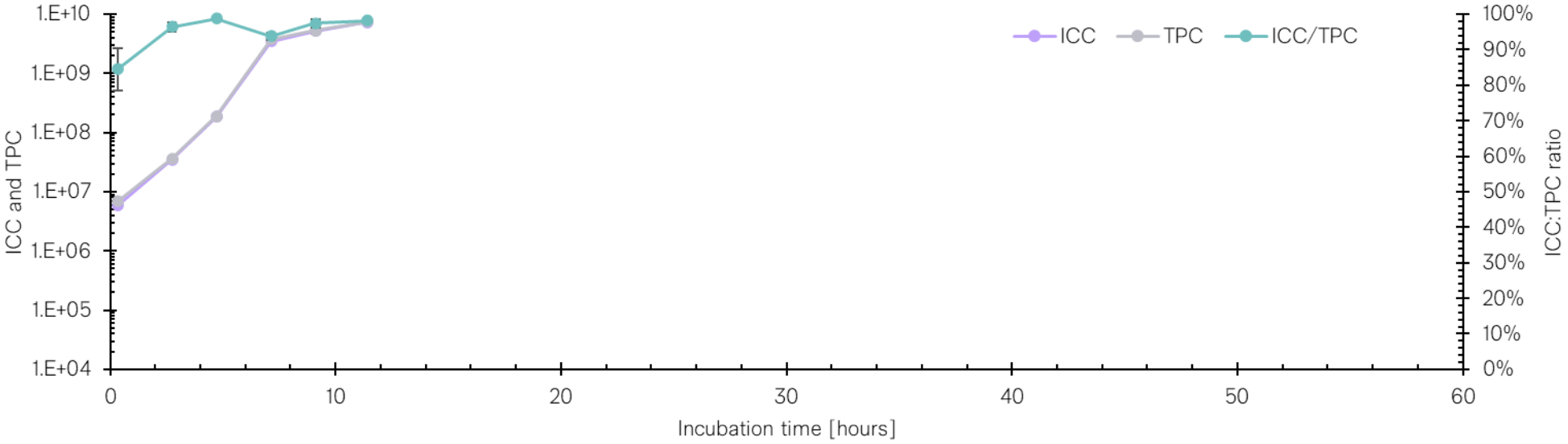


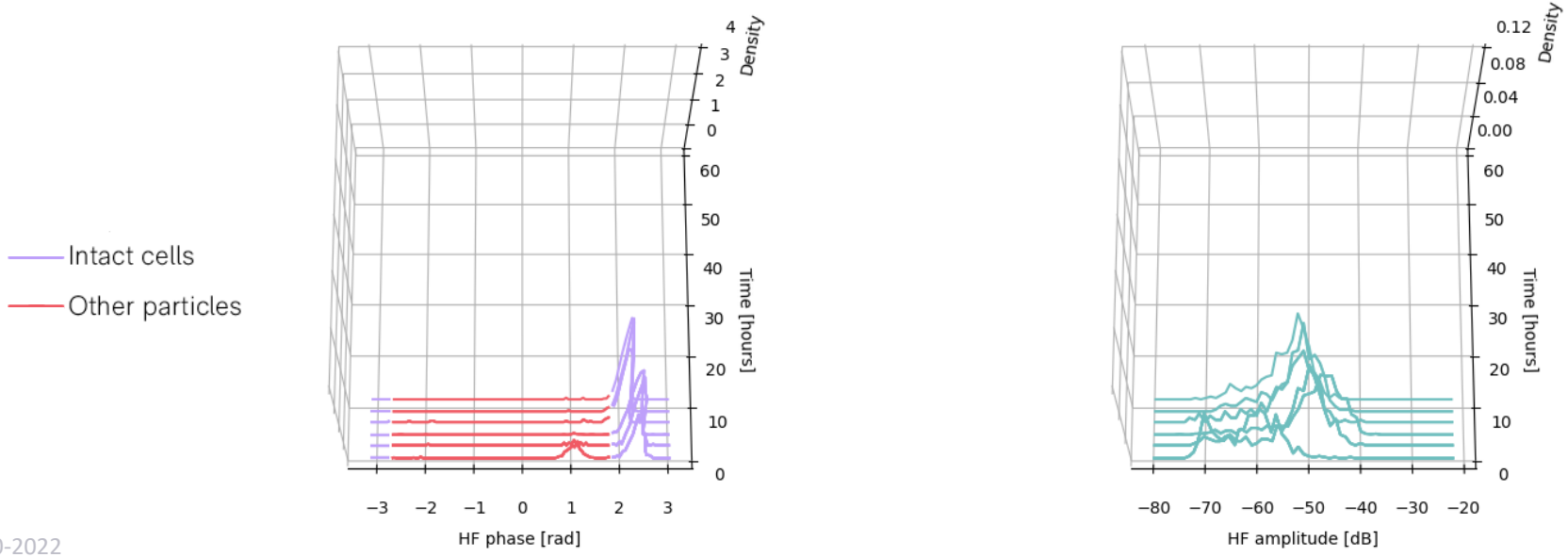
Staphyloccus epidermidis shake flask example | 9.1 hours



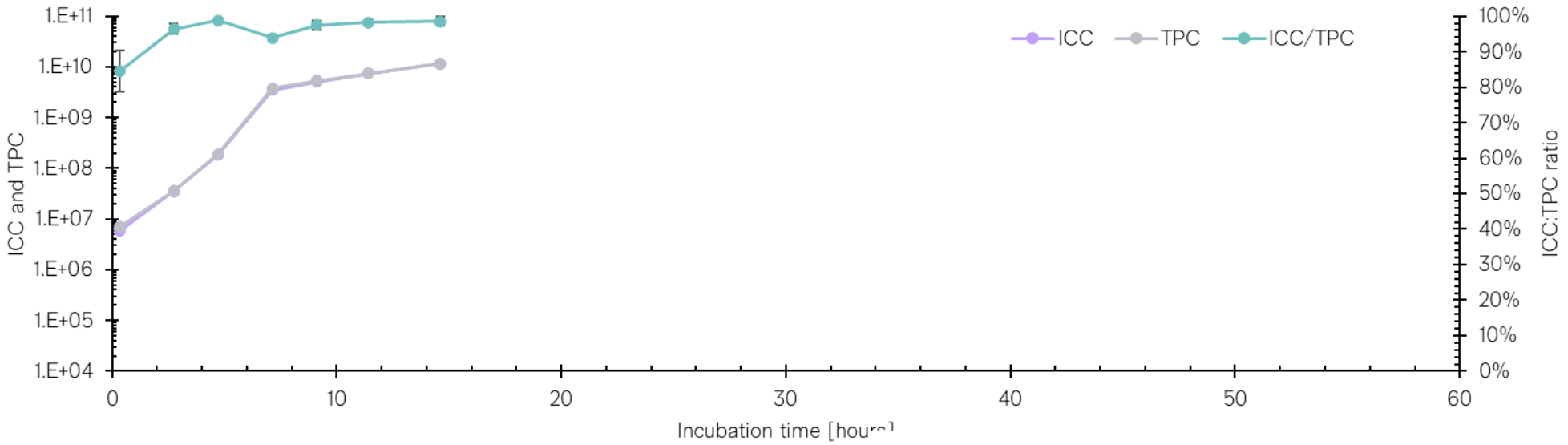


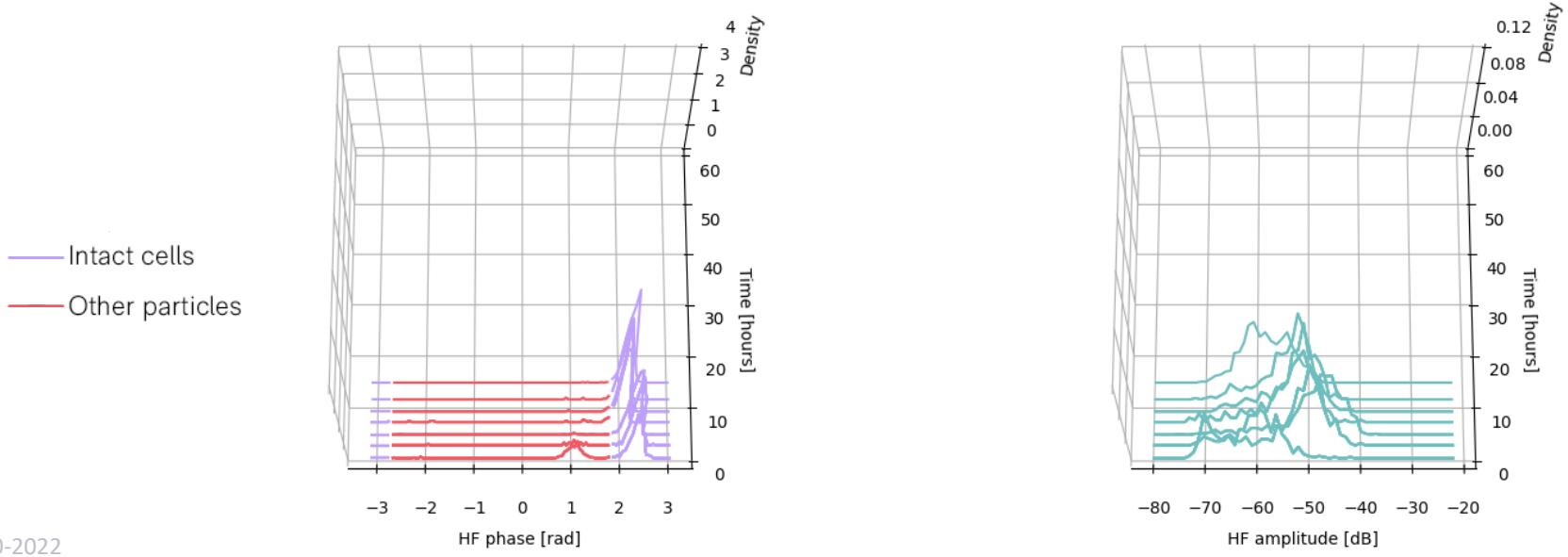
Staphyloccus epidermidis shake flask example | 11.4 hours



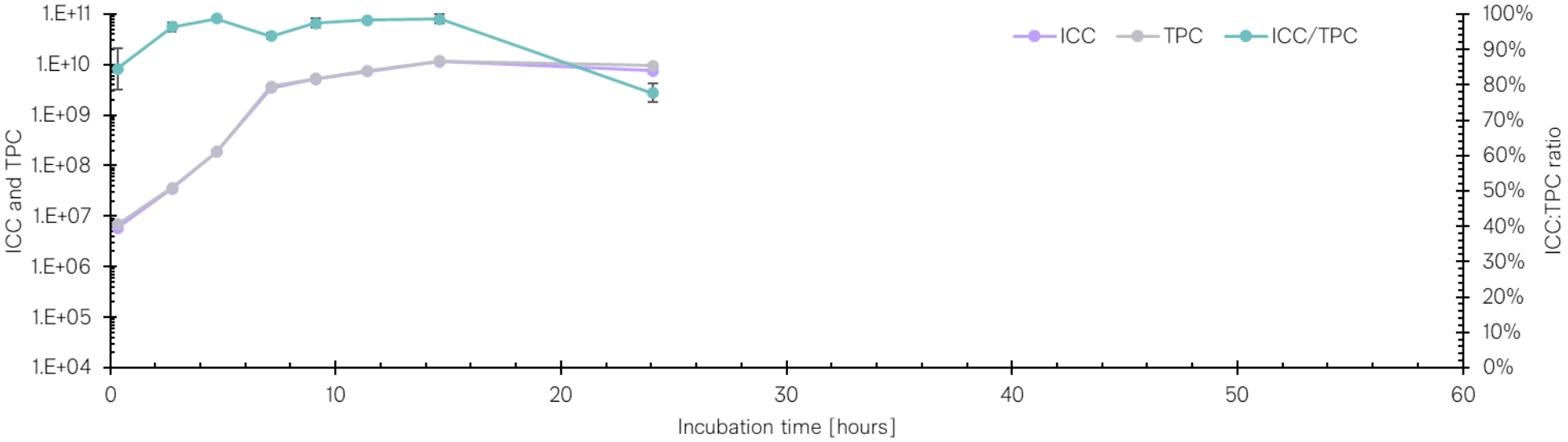


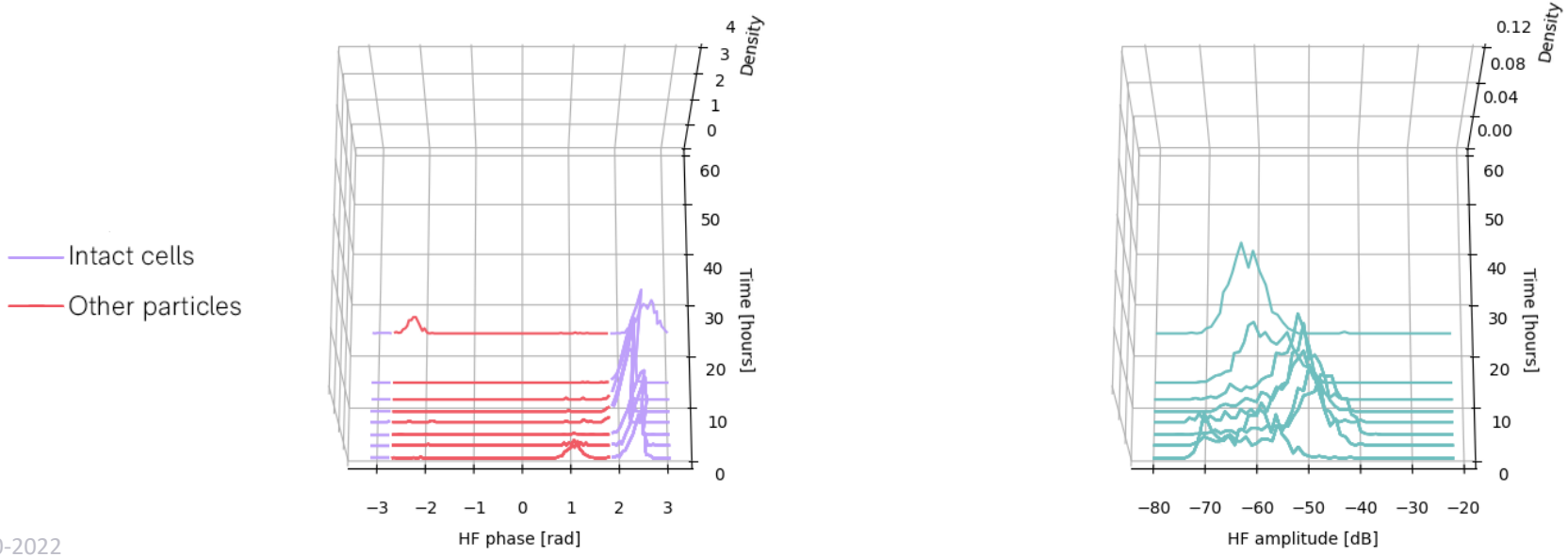
Staphyloccus epidermidis shake flask example | 14.6 hours



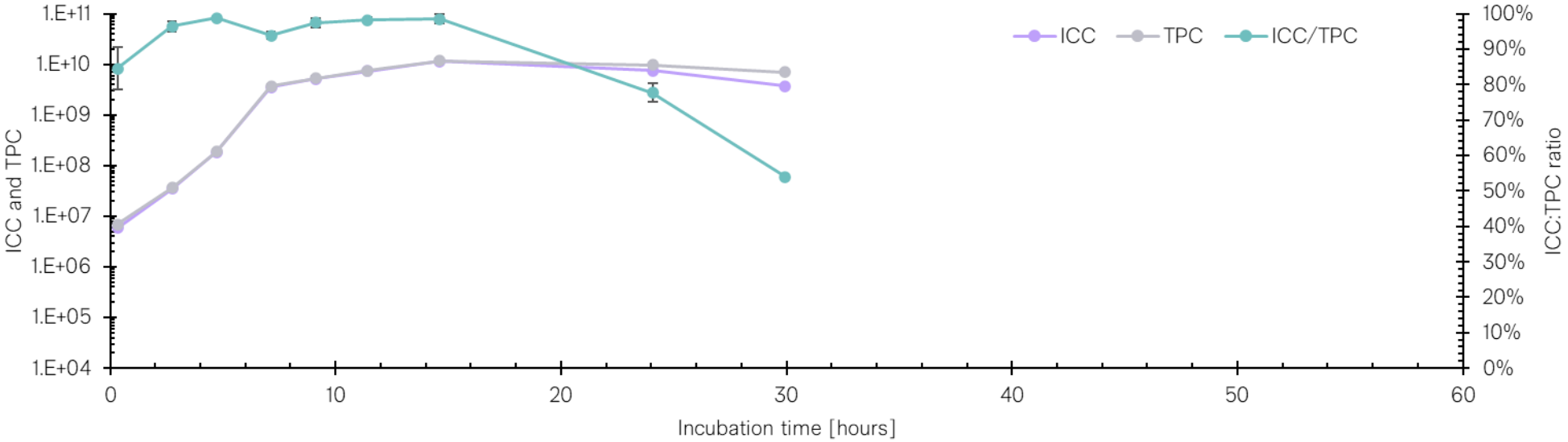


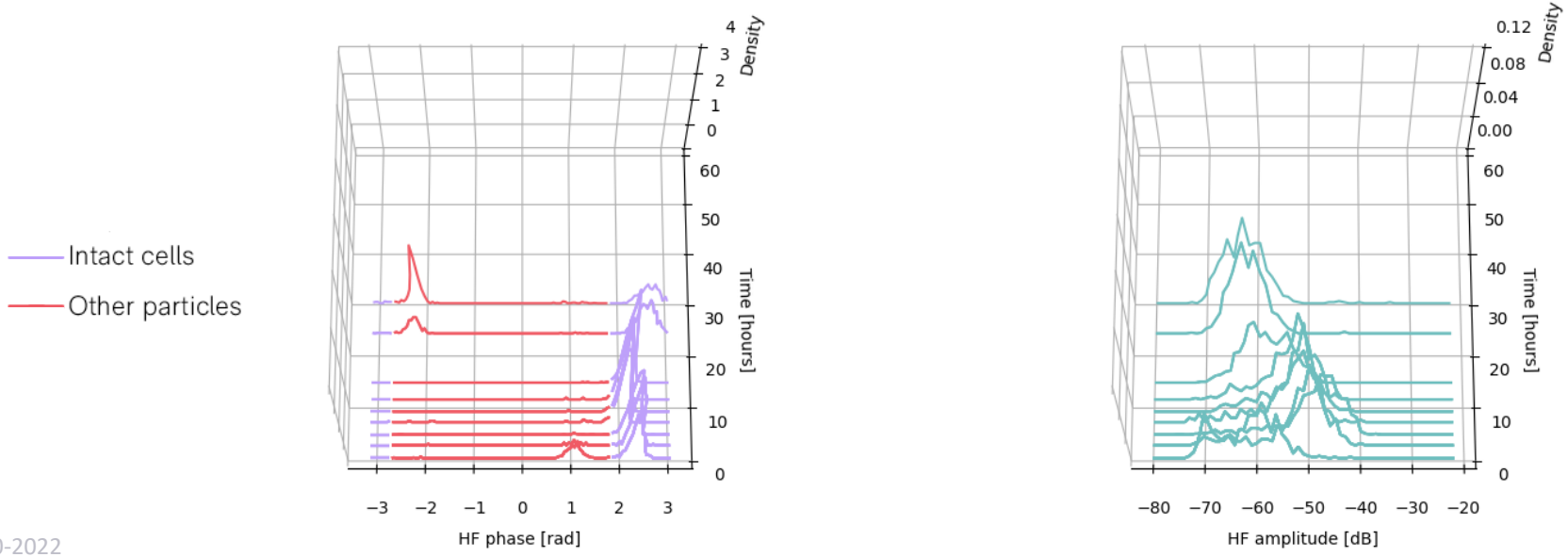
Staphyloccus epidermidis shake flask example | 24.1 hours



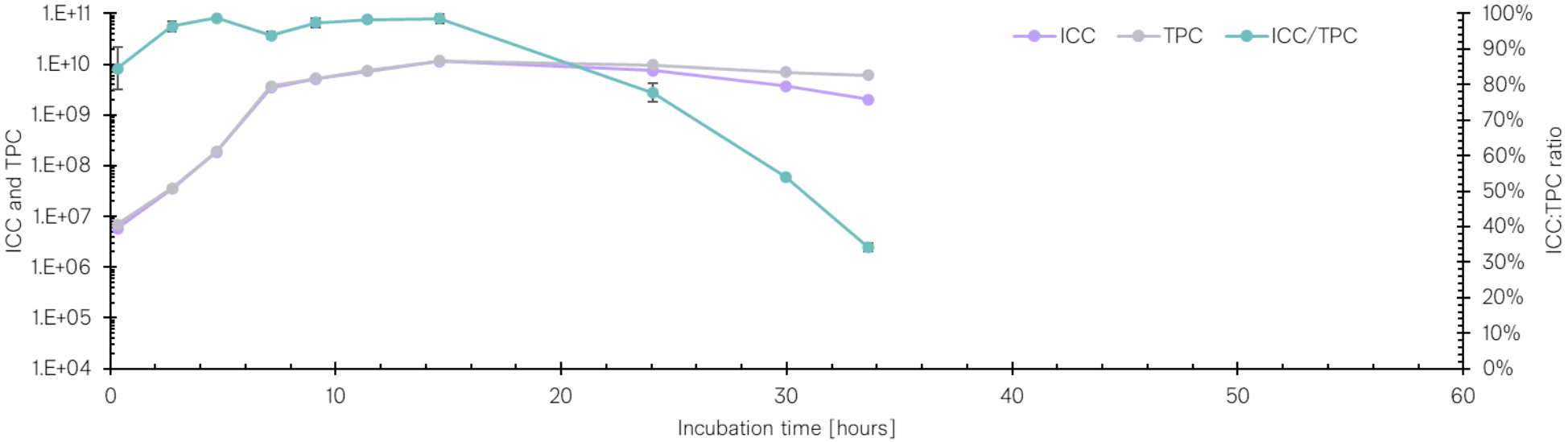


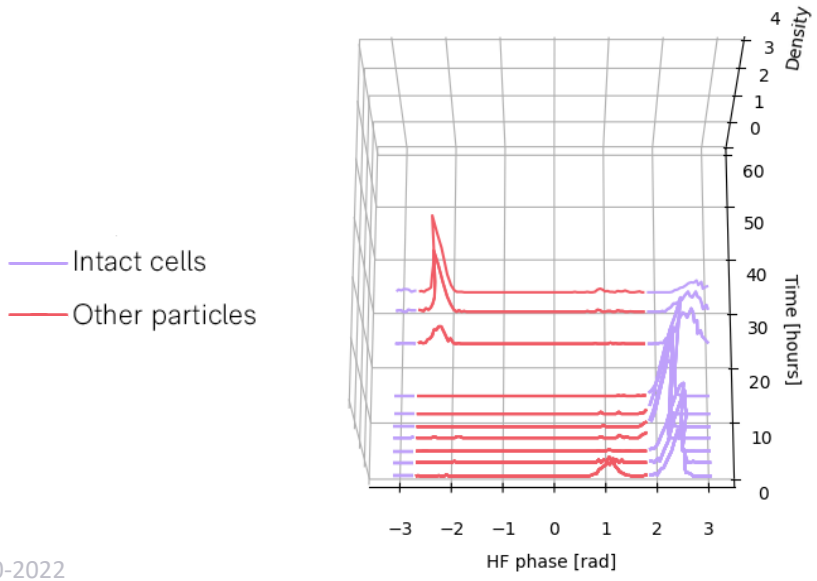
Staphyloccus epidermidis shake flask example | 30.0 hours

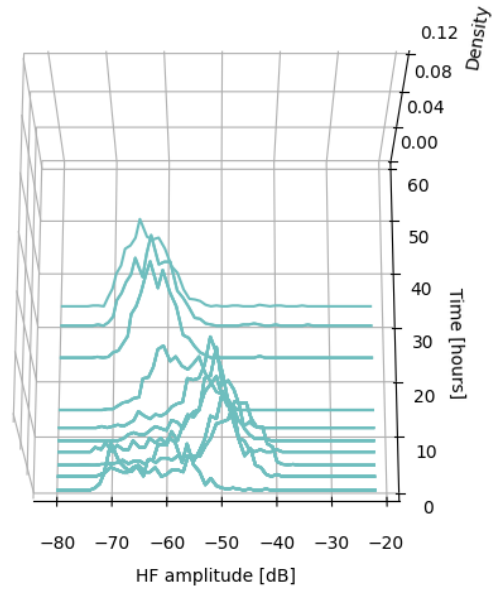




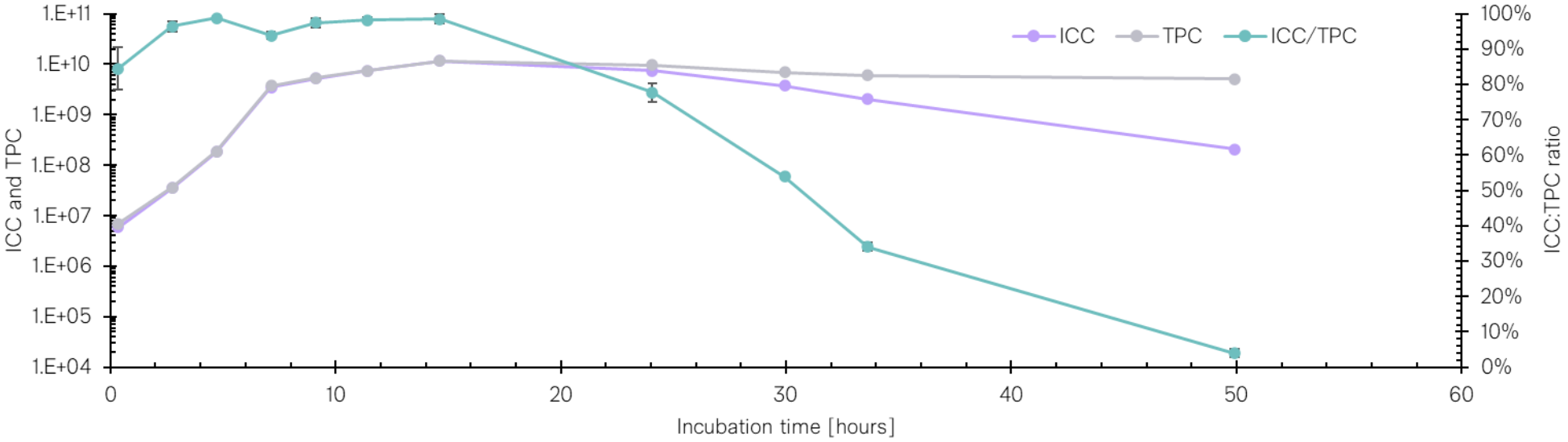
Staphyloccus epidermidis shake flask example | 33.6 hours

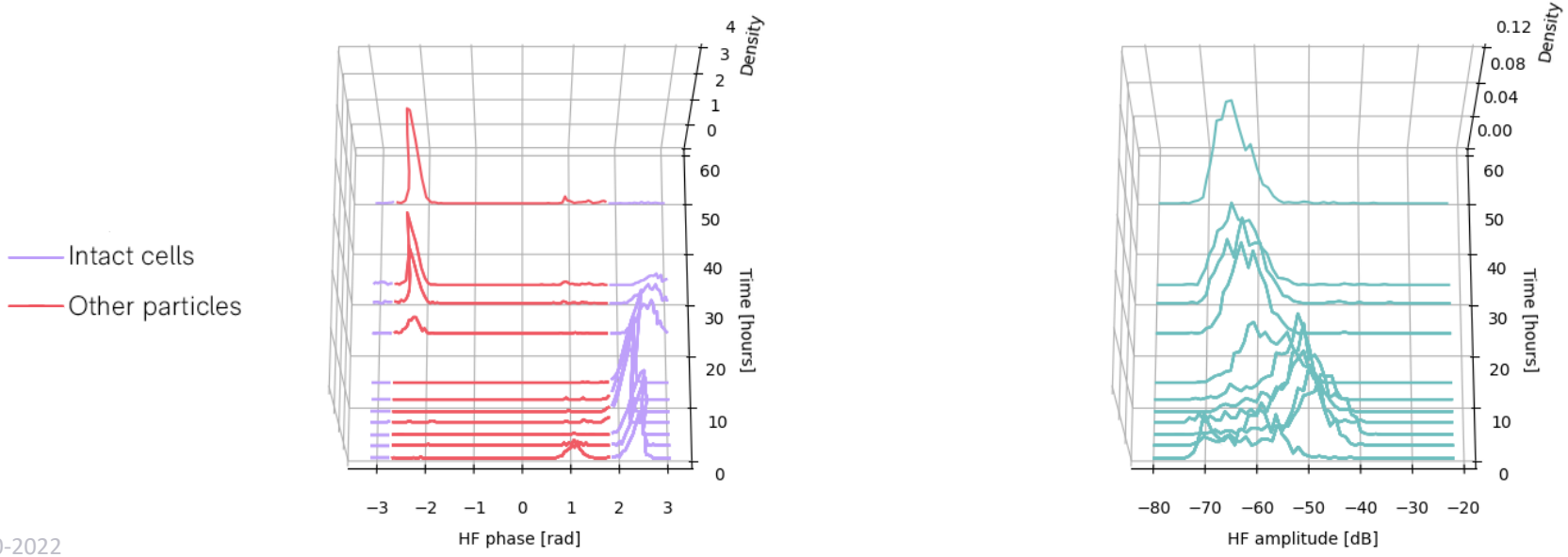




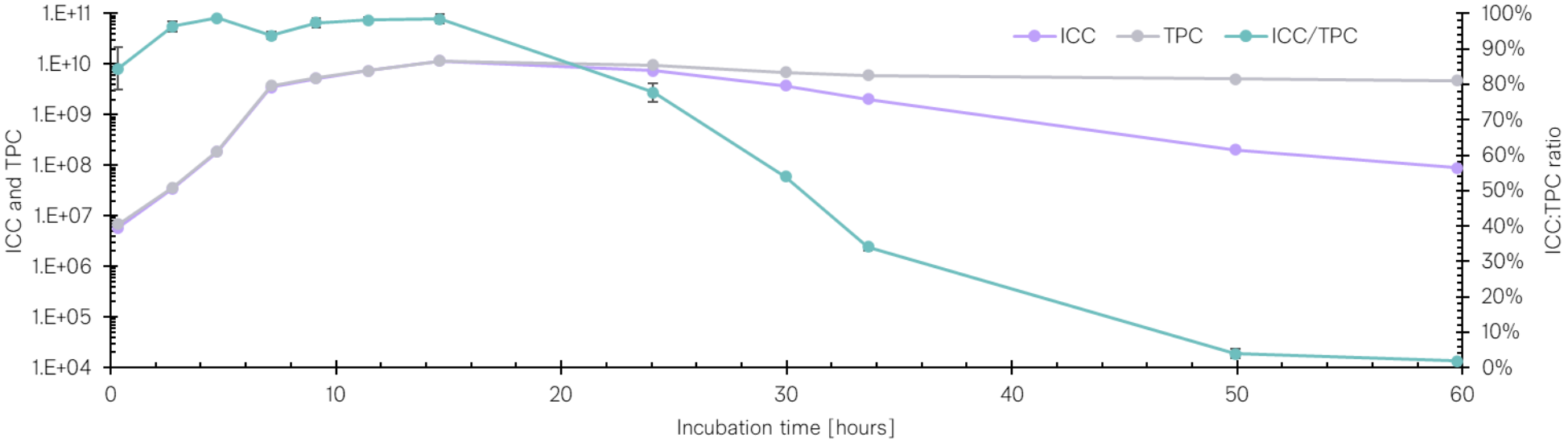


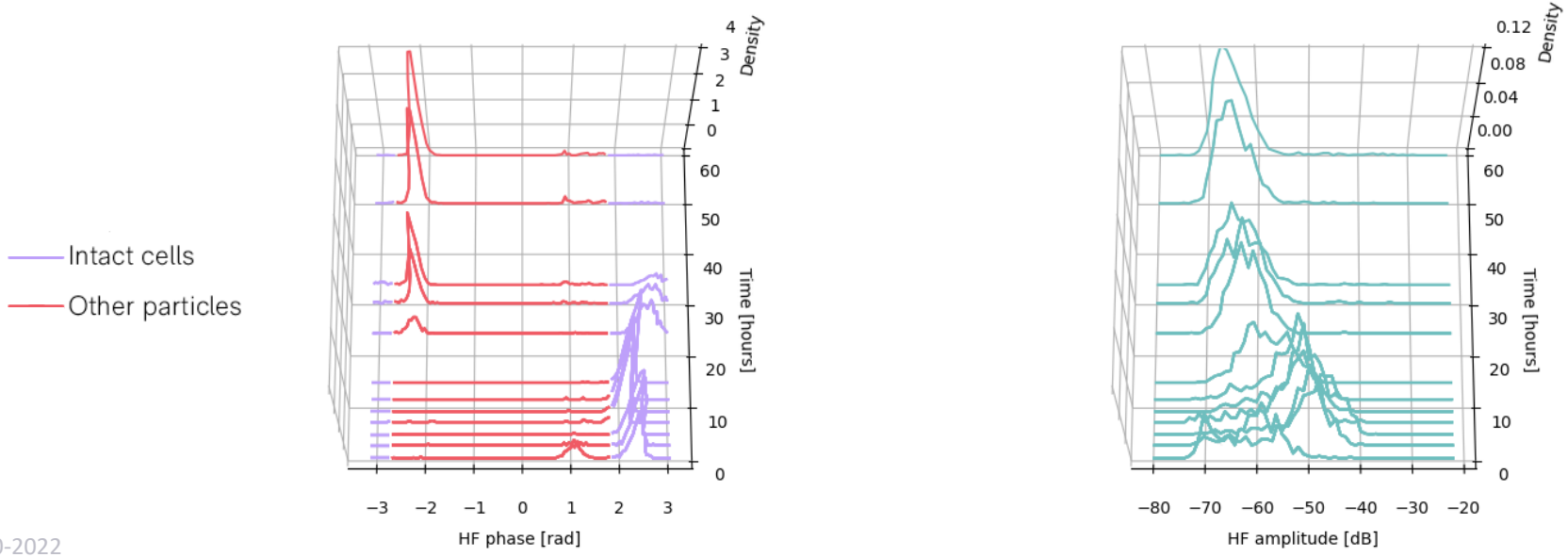
Staphyloccus epidermidis shake flask example | 49.9 hours



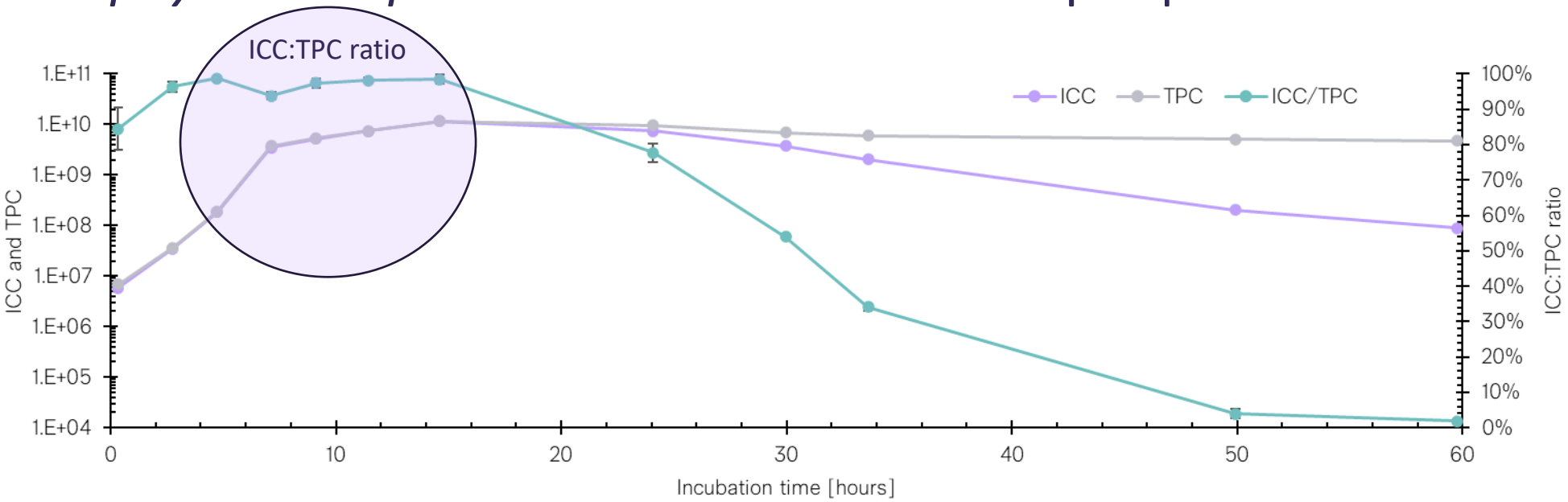


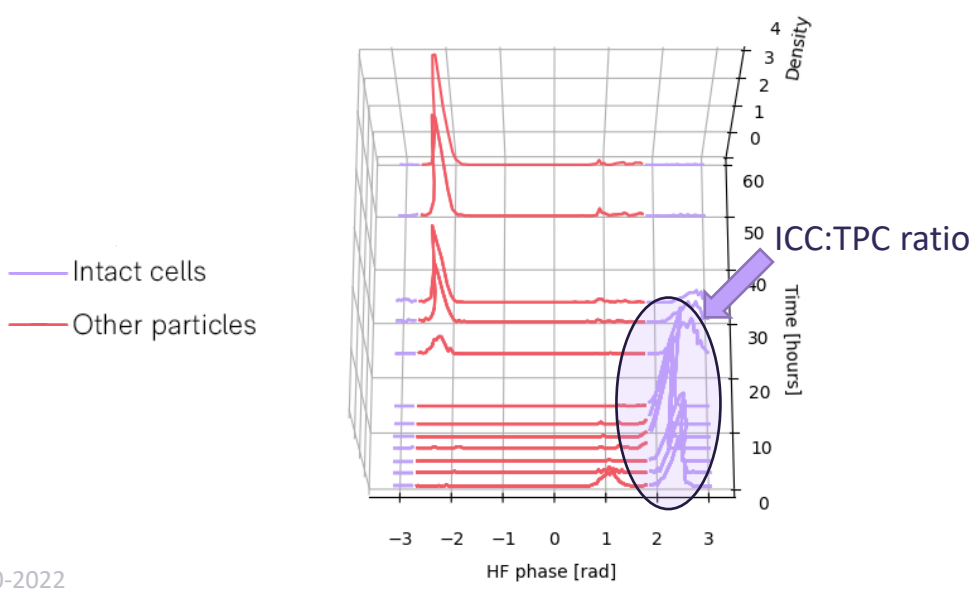
Staphyloccus epidermidis shake flask example | 59.7 hours

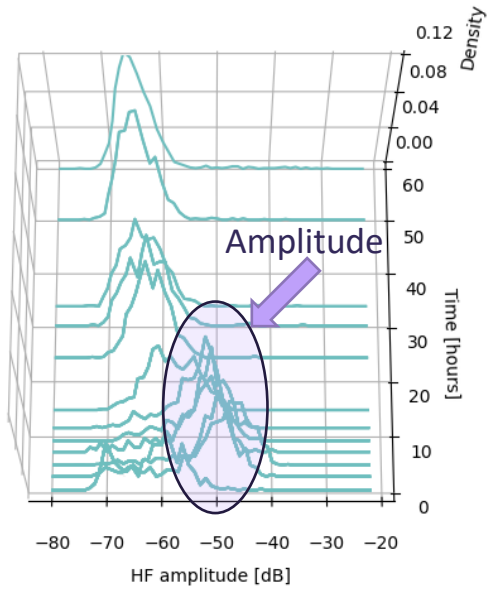




Staphyloccus epidermidis shake flask example | 59.7 hours

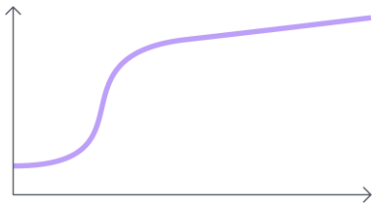






Use cases

01

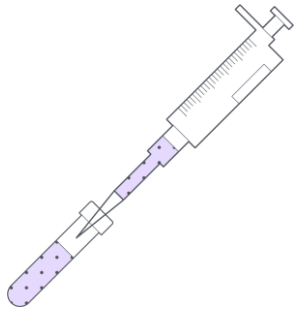


Monitor bacterial growth curves

02

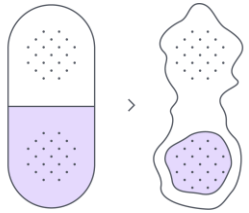
Determine potency of freeze-dried bacterial products

03



Adjust bacterial test suspensions in real time

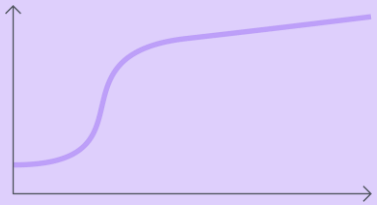
04



Enumerate bacterial endo- and exospores

Use cases

01

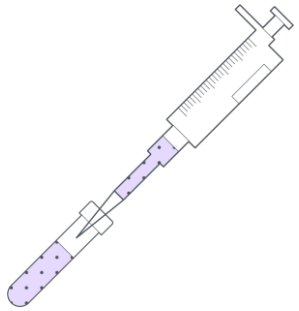


Monitor bacterial growth curves

02

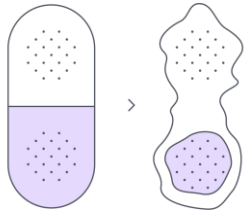
Determine potency of freeze-dried bacterial products

03



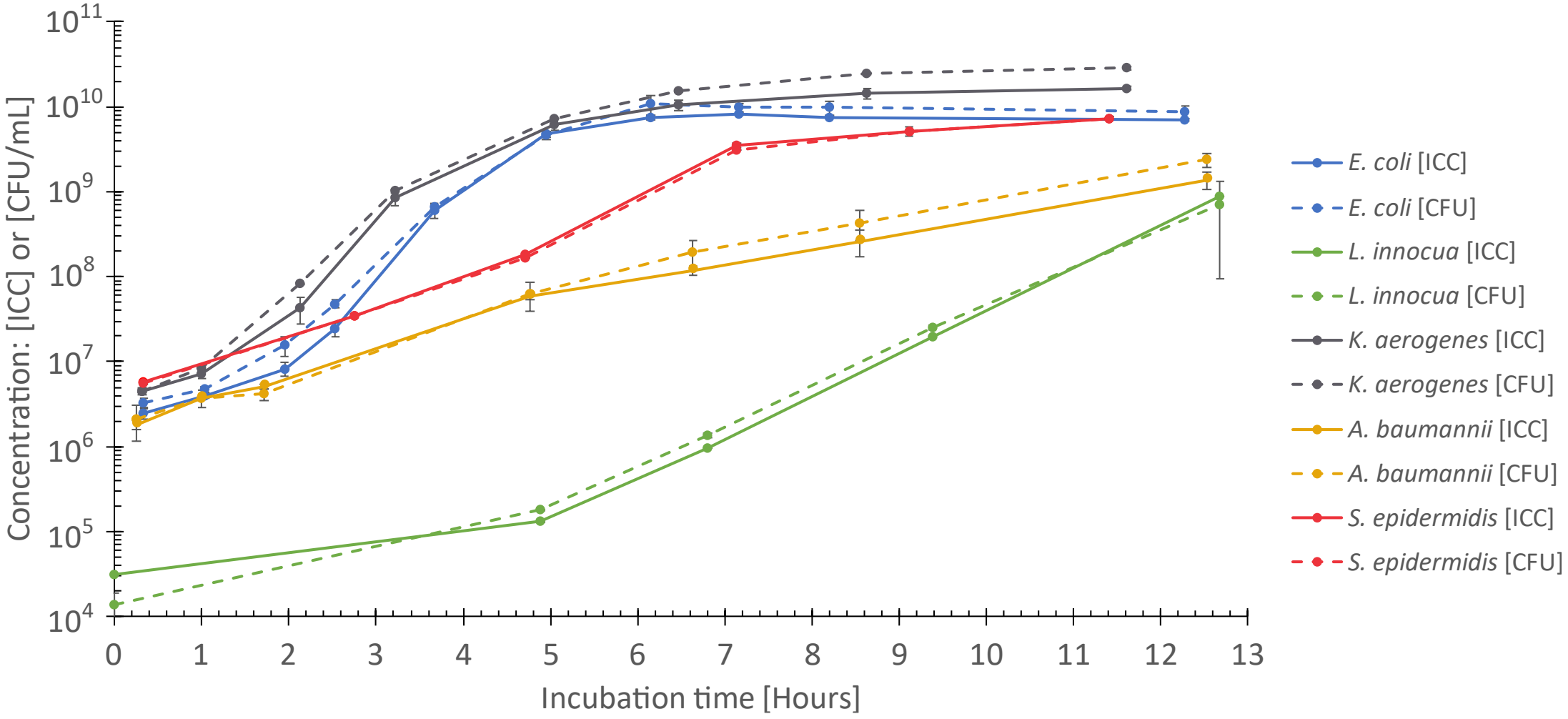
Adjust bacterial test suspensions in real time

04



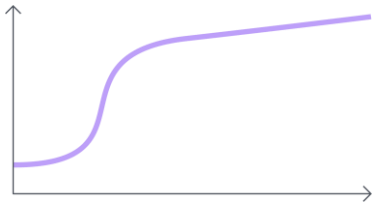
Enumerate bacterial endo- and exospores

Monitor bioprocesses in real time



Use cases

01

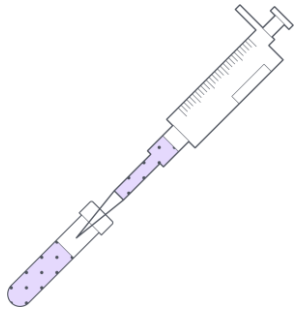


Monitor bacterial growth curves

02

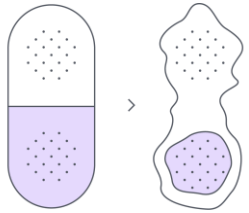
Determine potency of freeze-dried bacterial products

03



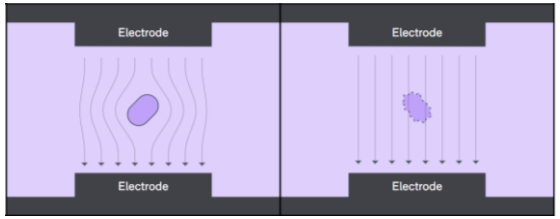
Adjust bacterial test suspensions in real time

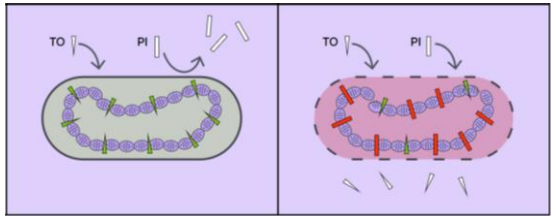
04

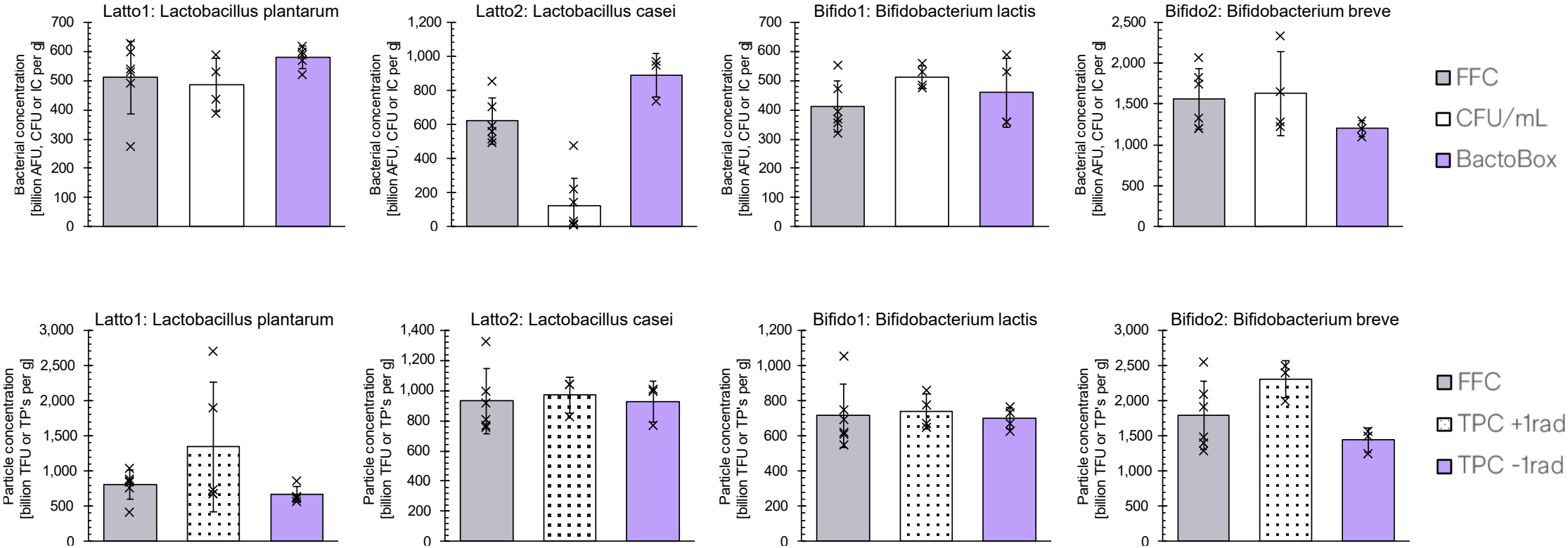


Enumerate bacterial endo- and exospores

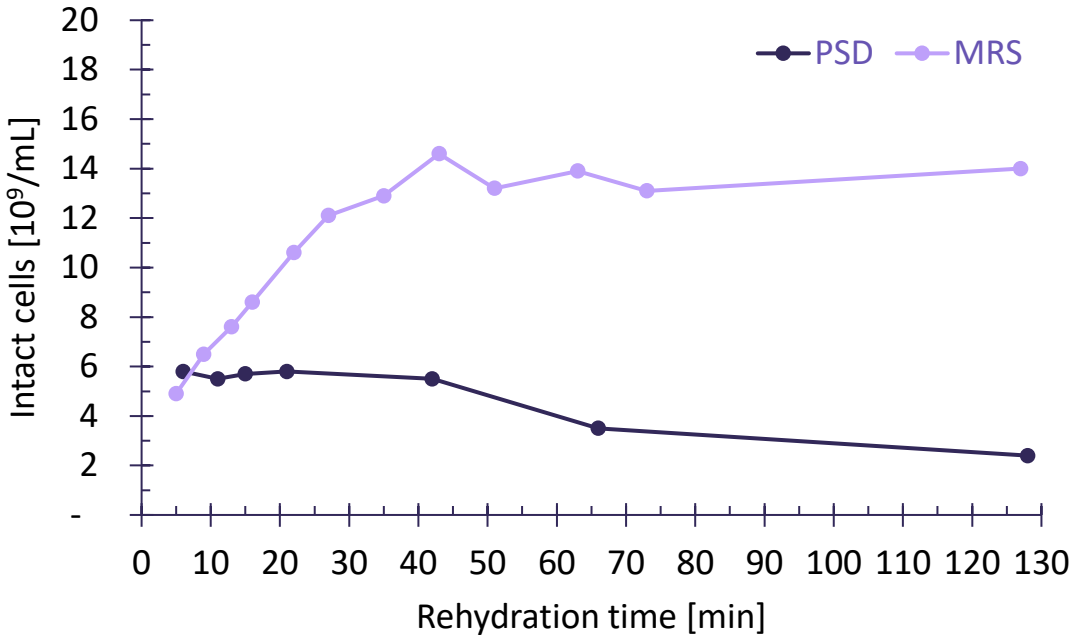
Determine potency of freeze-dried bacterial products

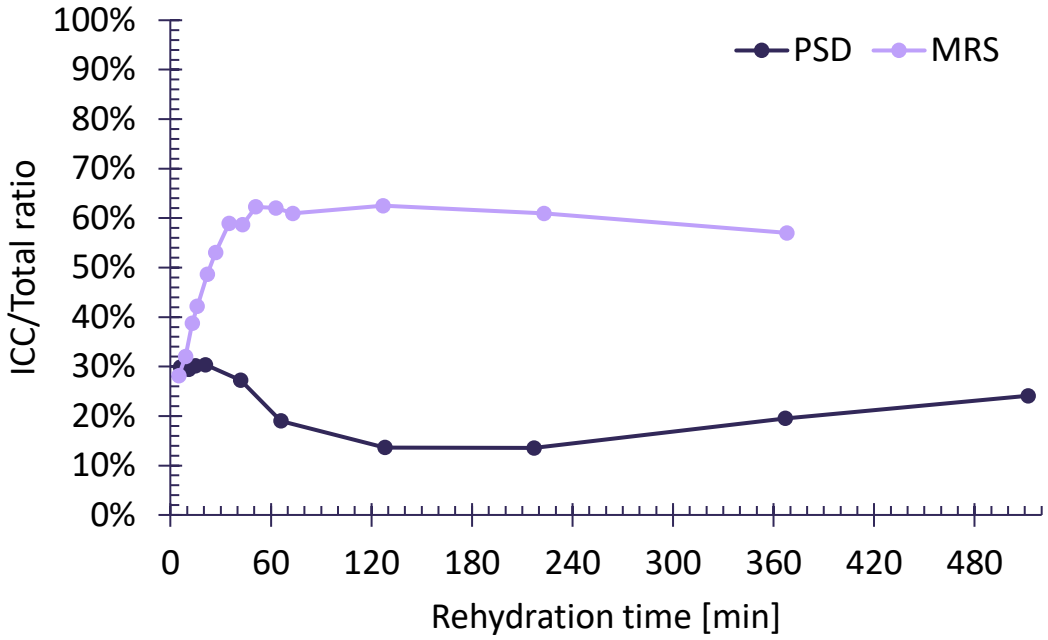


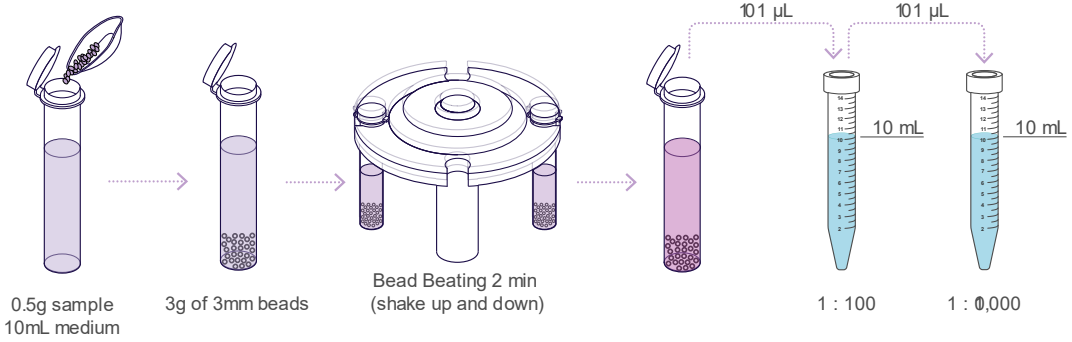




Screen for suitable resuscitation parameters

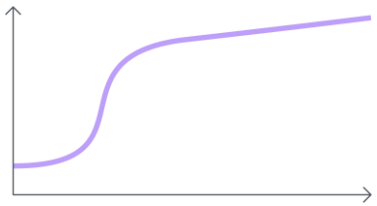






Use cases

01

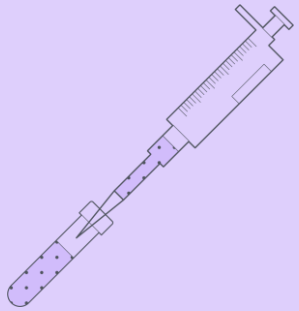


Monitor bacterial growth curves

02

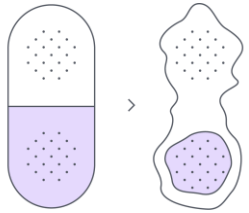
Determine potency of freeze-dried bacterial products

03



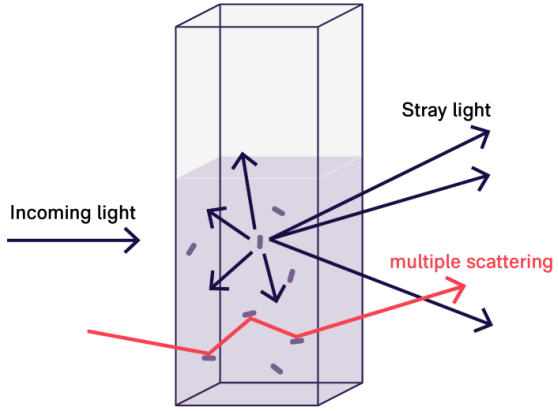
Adjust bacterial test suspensions in real time

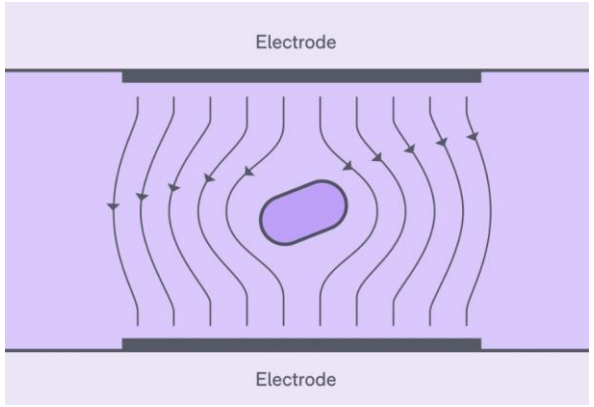
04

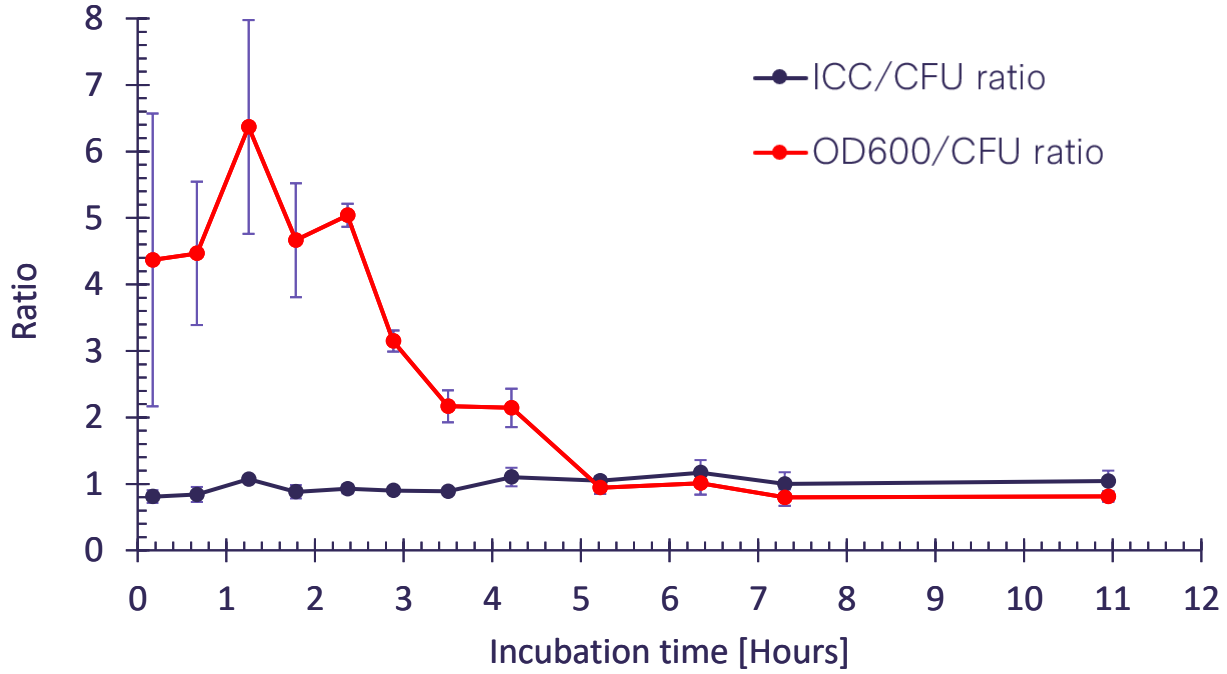


Enumerate bacterial endo- and exospores

Adjust test suspensions in real time

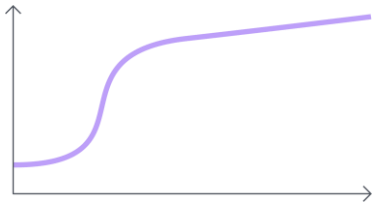






Use cases

01

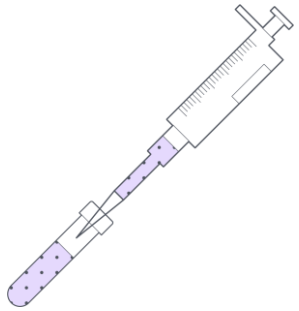


Monitor bacterial growth curves

02

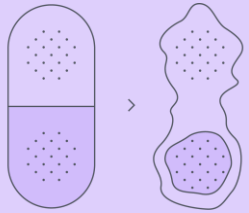
Determine potency of freeze-dried bacterial products

03



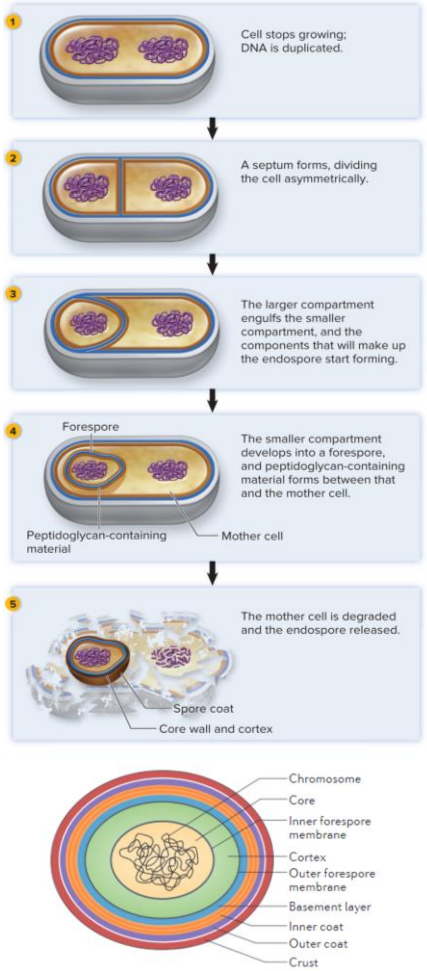
Adjust bacterial test suspensions in real time

04

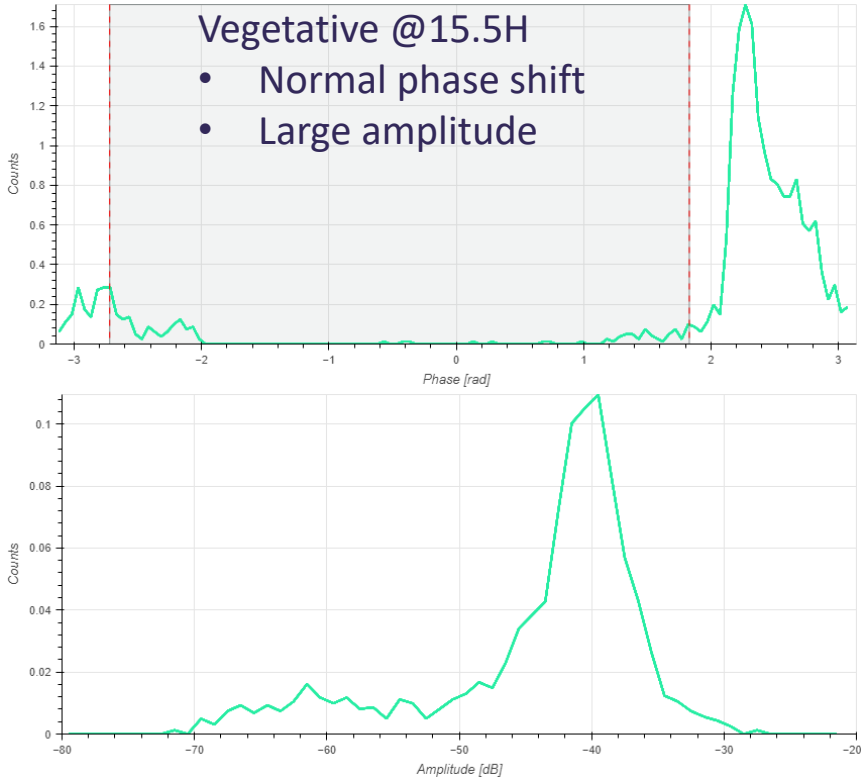


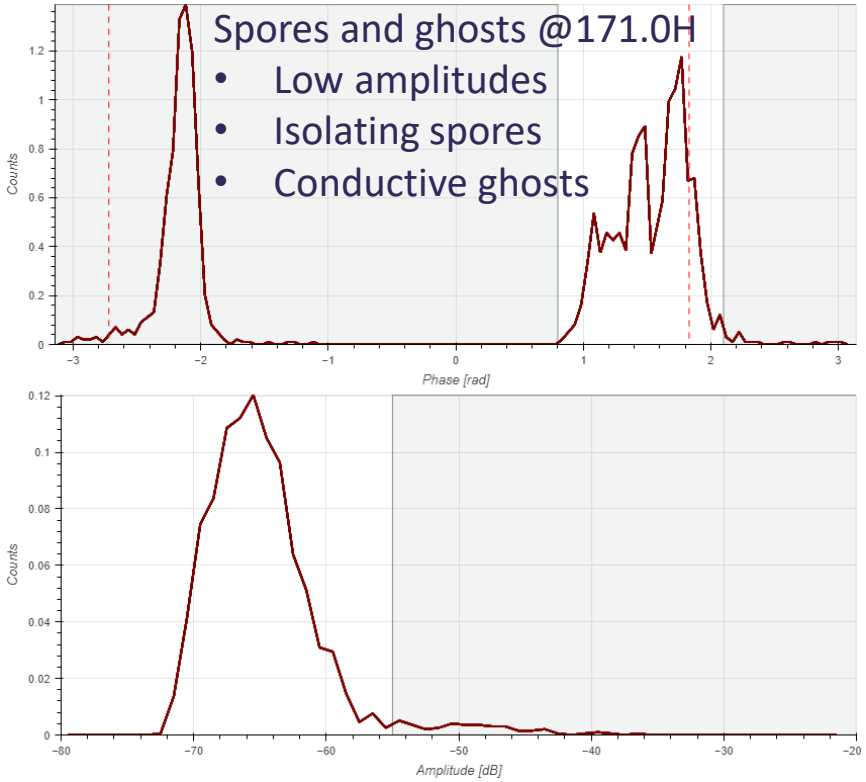
Enumerate bacterial endo- and exospores

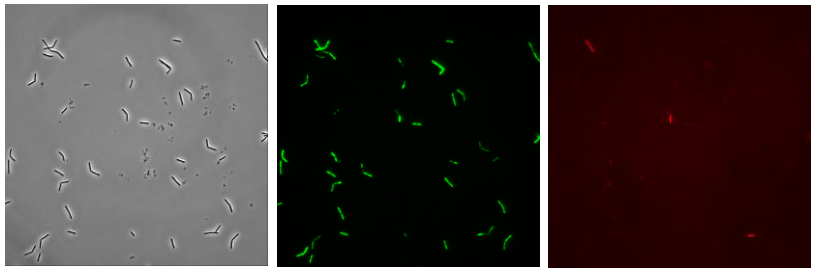
Enumeration of endospores: *Bacillus subtilis* DSM 618



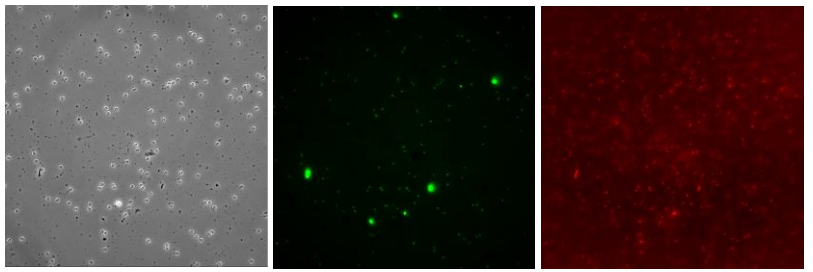
Mckenney, P. T., Driks, A., & Eichenberger, P. (2013). The Bacillus subtilis endospore: Assembly and functions of the multilayered coat. Nature Reviews Microbiology, 11(1), 33–44. https://doi.org/10.1038/nrmicro2921





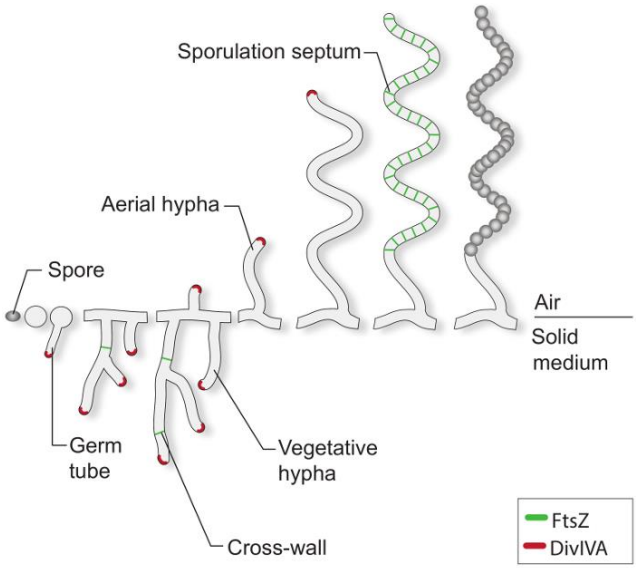


Phase contrast | SYBR-green I | TOTO-3

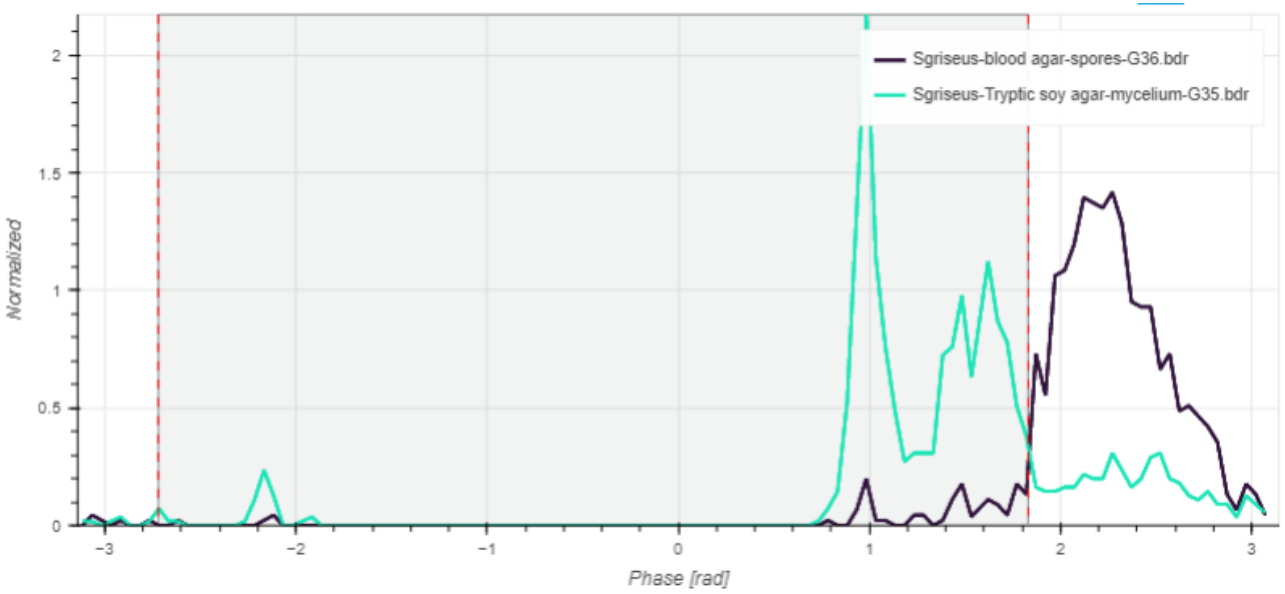


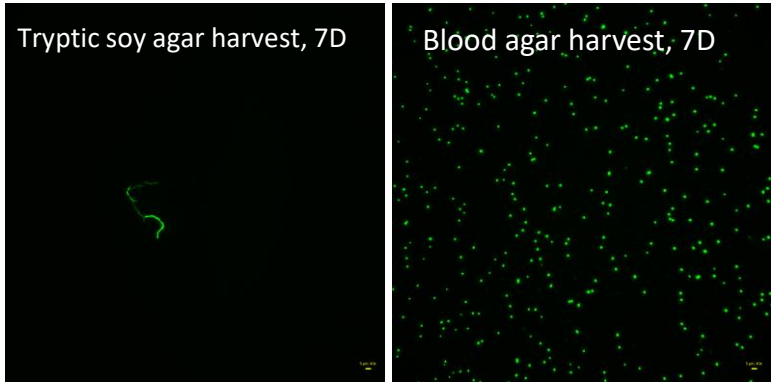
Phase contrast | SYBR-green I | TOTO-3

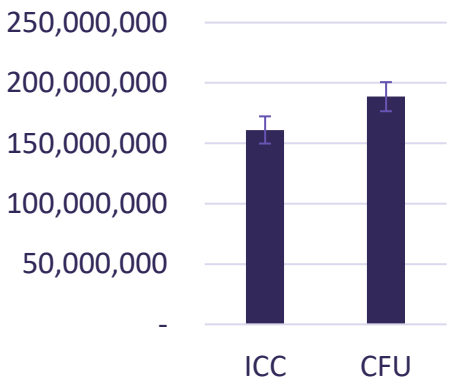
Enumeration of exospores: *Streptomyces griseus*



Schlimpert S, Flärdh K, Buttner J. J Vis Exp. 2016 Feb 28;(108):53863. doi: 10.3791/53863. Erratum in: J Vis Exp. 2016 Jul 01;(113)







Take home messages

IFC probes the presence of lipid membranes

IFC is similar to FFC, but no stains are required

Bioprocesses and potency can be monitored in real time

Bacterial test suspensions can be adjusted reliably

Custom gating enables enumeration of spore products