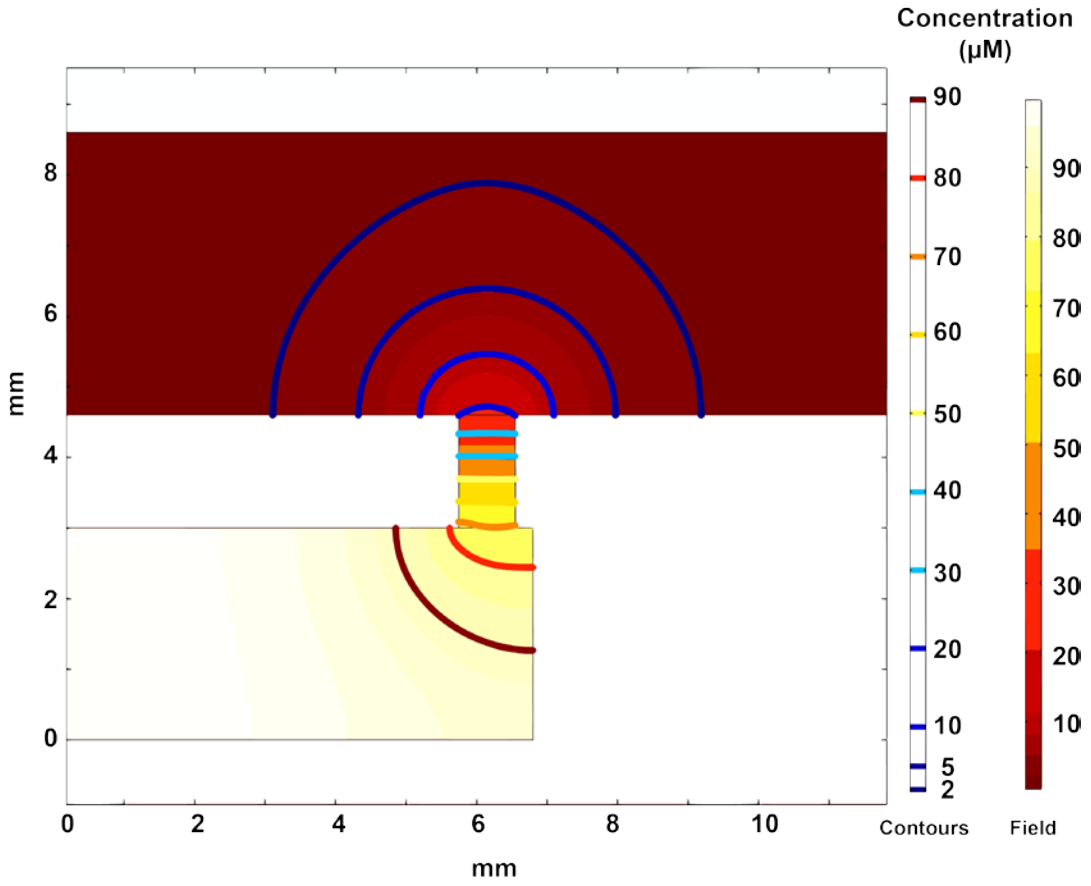
# THE IN SITU CHEMOTAXIS ASSAY

Microbial interactions influence the productivity and biogeochemistry of the ocean, yet they are often overlooked when sampling is carried out via traditional oceanographic techniques. To investigate the behaviors of motile marine microorganisms at spatially relevant scales, we have engineered an *in situ* chemotaxis assay (ISCA) based on microfluidic technology. The ISCA is designed for the field, allowing users to examine the response of aquatic natural communities to multiple compounds in a single deployment. After deployment, the device's well contents can be analyzed using a variety of techniques, depending on the scientific question at hand. Integrating ISCAs into a sampling scheme provides unparalleled insight into the role of microbial behavior within a given aquatic ecosystem and allows researchers to interrogate fundamental interactions occurring between microorganisms. A description of the ISCA was published in Nature Microbiology (2017) which includes details on the testing and validation as well as an example application.

This page is meant to provide a central location for all information related to building and deploying ISCAs, although frequently we'll link to other pages with much greater detail present. In particular, the protocols.io page for assembling and deploying the ISCA may be a useful resource.

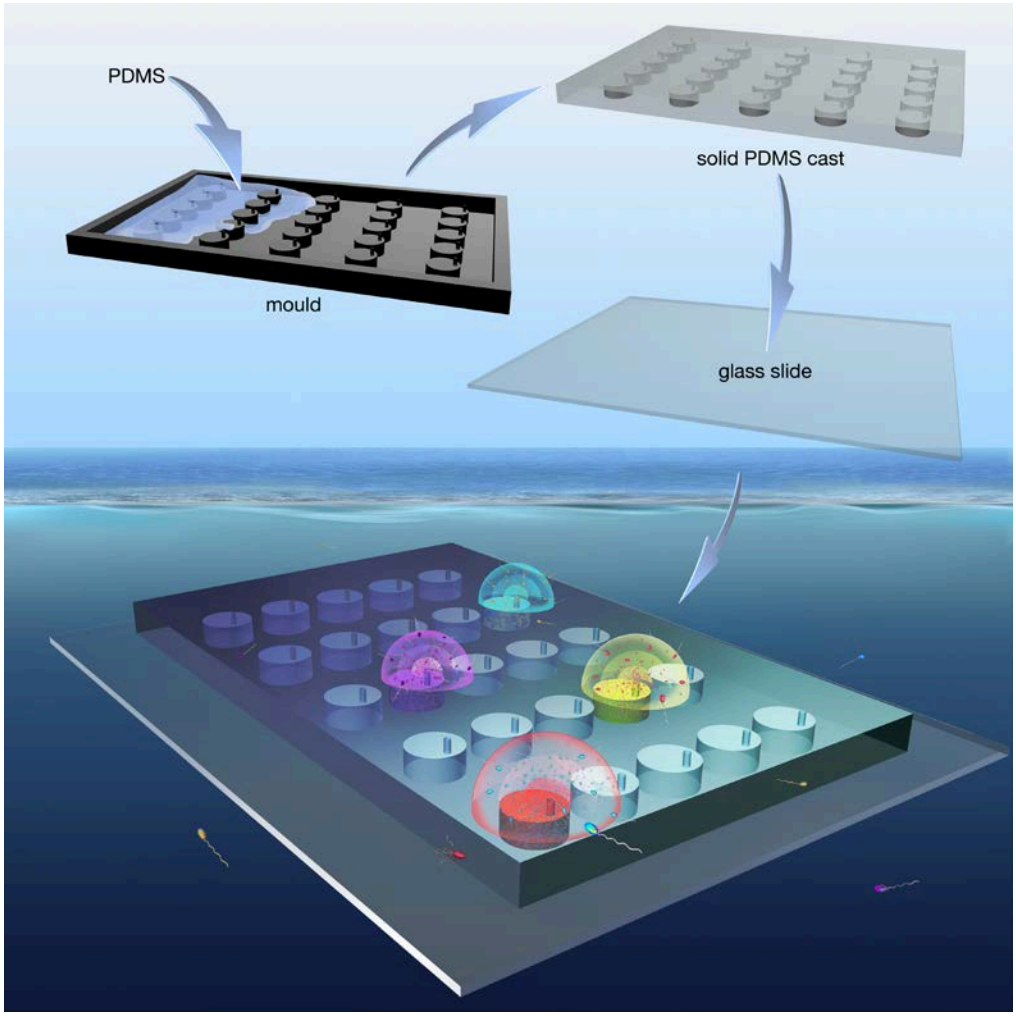
## *How it works*

The ISCA is an array of wells that are initially filled with user-specified fluids (such as seawater with an added chemical attractant). Each well has a single port serving as a link between the chemical attractant and the bulk fluid of the environment. Upon deployment the chemical attractants diffuse out of the port, creating chemical microplumes that microbes can respond to via chemotaxis. Once inside the device, the microbes have a low probability of exiting and are retained within the well.




**The chemical field extending from an ISCA well after 1 hr under quiescent conditions. Left color bar corresponds to contour concentrations and right to field concentrations of a diffusive compound. Initially, the concentration in the well is 100% and outside is 0%. Under quiescent conditions the plume extends ~3.5 mm**

In the current design iteration (outlined here: http://go.nature.com/2wxGhzg) the device consists of 25 wells in a 5x5 array.




**Conceptual process flow of fabrication and deployment of an ISCA**

When deploying the device, the 5 wells within a single row serve as technical replicates. This means with each deployment you can assay 4 treatments and a control. In principle, you could expand the device to contain more wells, and accordingly, more treatments, but we've found that there are practical limits on the number of wells we can prepare for deployment before other issues (ie. evaporation) enter the mix. We pool technical replicates in order to get enough material for downstream molecular analyses. In order to have sufficient biological replication it's generally a good idea to deploy n=3-5 ISCAs in parallel.

## *What type of data is possible?*

A lot depends on the specific system that you are working with. It's good to run some pilot studies to quantify the number of cells responding to your treatments. The biomass you obtain largely controls what you can do next. Generally the first metric we use is cell count derived from flow cytometry. Cell counts allow you to generate quantitative information about the strength of chemotaxis towards your compound of interest. Beyond cell counts, the ISCA well contents are amenable to a variety of different manipulations. It is important to keep in mind that the resulting samples are considered "low-input" by the standards of most conventional methods and some adjustment is likely required. We have explored the following options with promising initial results:

*i) Targeted isolation.*
This can be done with either liquid enrichment cultures or through plating.

*ii) Community composition.*
In oceanic or freshwater systems there should be enough material to carry out analysis of 16S/18S.

*iii) Metagenomics.*
We are in the process of developing an improved extraction and analysis pipeline to obtain both community and functional profiles from samples.

## *Building and deploying ISCAs*

A detailed protocol for fabrication and deployment (along with pictures) can be found at protocols.io (*hyperlink*).

In the supplementary information of the paper describing the ISCA (http://go.nature.com/2wxGhzg) you can find the .stl file necessary for 3D printing the device mould. You can 3D print the mould yourself, but we recommend hiring a service to carry out the print for you. The surface finish of the mould is very important, as that determines how well the ISCA structure bonds to the glass substrate and that in turn ensures there's no cross-contamination between wells.

Before heading to the field, it's important to build the flow-damping enclosure. In the Nature Microbiology paper we provide the .ai file, which can be used to laser-cut the pieces needed to build the enclosure. Again this can be done in house, but you can also outsource.

**If you'd like to discuss deployment logistics or feasibility of experiments, don't hesitate to contact me at lambertb@ethz.ch.**

**Additionally, if you find a tip or trick that helps in the process please comment on the protocols.io page.**

## ISCA PUBLICATIONS

1. Bennett S. Lambert*, Jean-Baptiste Raina*, Vicente I. Fernandez, Christian Rinke, Nachshon Siboni, Francesco Rubino, Philip Hugenholtz, Gene W. Tyson, Justin R. Seymour & Roman Stocker. (2017) A microfluidics-based in situ chemotaxis assay to study the behaviour of aquatic microbial communities. ***Nature Microbiology*** **2**, 1344–1349 doi:10.1038/s41564-017-0010-9.
(* Authors contributed equally)

*Data availability: Sequencing data (http://microscaleocean.org/data/category/12-in-situ-chemotaxis-assay); Image/video data (available upon request – massive files); Design files (available online in supplementary information)*