MicroDish Flow Cell

1.0 Product Description

The MicroDish (MD) Flow Cell (*patent pending*) is a device designed for the continuous flow and supply of an aqueous solution (e.g. nutrients or drugs) underneath a MD porous aluminium oxide (PAO) slide.

The MD Flow Cell is suitable for various applications and studies such as in environmental and medical microbiology, microbial physiology and ecology studies. It can be used for bacterial growth studies or the enrichment and recovery of microorganisms present in environmental samples that are otherwise hard or not to cultivate.

The Flow Cell (Figure 1) consists of a simple metal holder with a disposable culture platform inside. This consists of a lower part (Figure 1, right: B) containing the flow channel and an opening for the PAO/glass insert and the upper part (Figure 1, right: A) with an open area for the inoculation of material (e.g. cells) and screws to tighten the two parts.

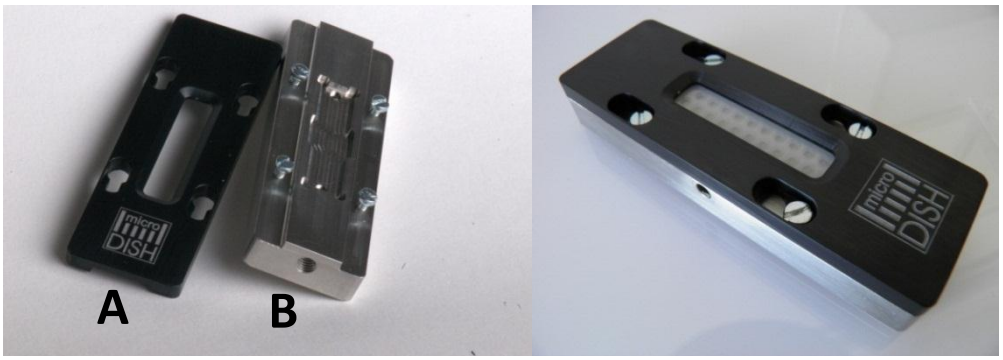


Figure 1. MD Flow cell. A: upper part; B: lower part. Photo: *left* A. van der Kant; *right* H. Dietrich.

A particular feature of the MD Flow Cell is the central PAO/glass insert, the disposable item that allows microbial cultivation (Figure 2). It is a sandwich construction consisting of a bottom glass slide with integrated flow channel bonded to the MD PAO which is again bonded to the glass slide containing the cultivation/incubation compartments. The presented insert has 20 wells with a top diameter of 1.5 mm. In principle, various well numbers and sizes are possible. Please contact us for further information.

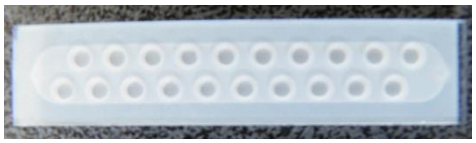


Figure 2. MD Flow Cell insert: consisting of that is a sandwich construction build-up of glass-PAO-glass. The insert shown has 20 wells with a top diameter of 2 mm. Photo: H. Dietrich.

Microorganisms can be recovered for further analysis, e.g. PCR, genomics, proteomics or can be directly imaged by fluorescence microscopy or other optical microscope techniques.

2.0 Why use the MD Flow Cell?

The MD Flow Cell can be used for many different applications and purposes (1.0 Product Description). One of its outstanding characteristic is the possibility to perform microbial physiology and ecology studies as the medium or liquid of interested can be exchanged for other solutions any time without disturbing the microorganisms growing on top of the PAO inside the wells. Waste products are also removed. So for slow growing organisms the device can be considered to approximate to a low cost, miniaturized chemostat. The Flow Cell can also be used for the study of the effect of antibiotics on bacteria. Figure 3 shows the principle of the MD Flow Cell.

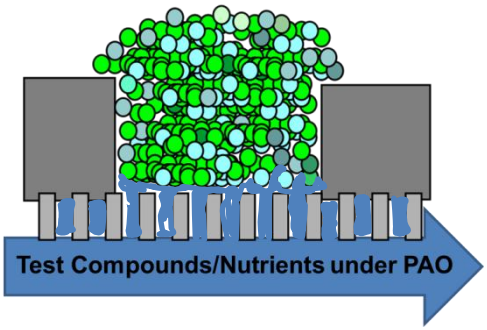


Figure 3. Illustration of the MD Flow Cell principle. One compartment (= well) is shown (grey large bars). The test fluid flows underneath the PAO and is sucked by capillary forces upwards inside each well. Microorganisms are depicted as blue/green circles.

Additionally, the MD Flow Cell is suitable to enrich and recover microorganisms from environmental samples.

Currently, most of the microorganisms present on the earth cannot be cultivated using conventional methodology as they are often based on agar containing media which contains phenolic and other compounds harmful to specific microorganisms. Using the MD Flow Cell, agar can be omitted and a medium based on a nutrient composition similar to the environmental source can be used.

3.0 Sterility and Cleaning

Generally

Cleaning can be done by conventional lab detergents. The Flow Cell can be sterilized by appropriate agents, such as ethanol (70%) and individual surfaces can be sterilized by UV or irradiation.

Sterilization, if required, is best performed with the Flow Cell disassembled. Autoclaving (15 min at 110°C) and dry heat (200°C, 2 h) are also possible.

Prior usage:

1. The disassembled Flow Cell can be cleaned using appropriate disinfecting agents, such as 70% ethanol.
2. The assembled Flow Cell plus insert should be sterilized by injecting (= flushing) 70% ethanol for at least 30 minutes and subsequent washing/flushing with demineralized water (18 MΩ) prior application of biological samples.

The Flow Cell inserts are disposables and should be new for each experiments.

4.0 Set Up and Loading

1. Insert tubing connectors and tubing on part B. Possible connectors are from IDEX Health & Sciences. The diameter of the tubing should be chosen according to personal reference (e.g. Supelco).
2. Place the two black sealing rings inside part B on top of the two little openings. The rings have to be placed as precise as possible to avoid leakage or breakage of the glass insert.
3. Put the glass insert on the bigger opening on part B.
4. Now place the top part (A) on the assembled part B/C and D and carefully tighten the screws.

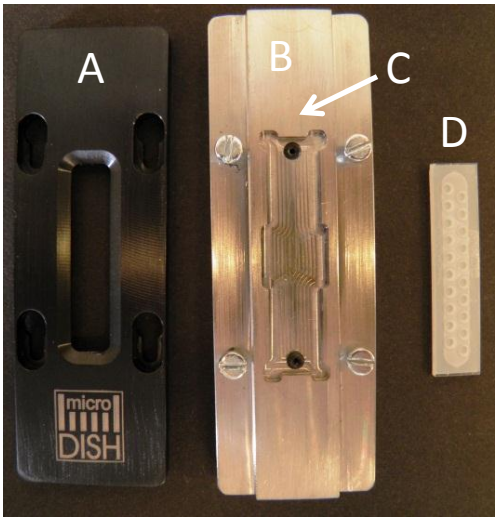


Figure 4. Disassembled Flow Cell; A. Top part (= lid); B. Bottom part with the liquid channel and space for the insert; C. Sealing rubber; D. Porous aluminium oxide and glass insert. Photo: H. Dietrich.

5.0 How to Recover or Image Samples

Sample Recovery

Microorganisms inside the wells can be recovered by increasing the liquid flow forcing the liquid through the porous aluminium oxide inside the wells.

The liquid can now be aspirated by using a pipette. These steps can be repeated several times if required.

The recovered liquid contains the recovered material. The solution may be designed for nucleic acids preparation or RNA stabilization/preservation, proteomics or organism recovery.

Optical Imaging

Microorganisms and biofilms can be imaged by fluorescence microscopy or other optical imaging techniques, such as phase contrast microscopy. Illumination must be from above.

Conventional dyes can be used to stain present microorganisms. Therefore, apply several µl of dye inside the wells and stain according to manufacturer's manual.

6.0 Which surrounding technical materials can be used

Tubing: TFE Teflon Tubing (1.58 mm OD x 0.8 mm ID), e.g. Supelco

Tubing connectors: e.g. Upchurch (VacuTight Headless)

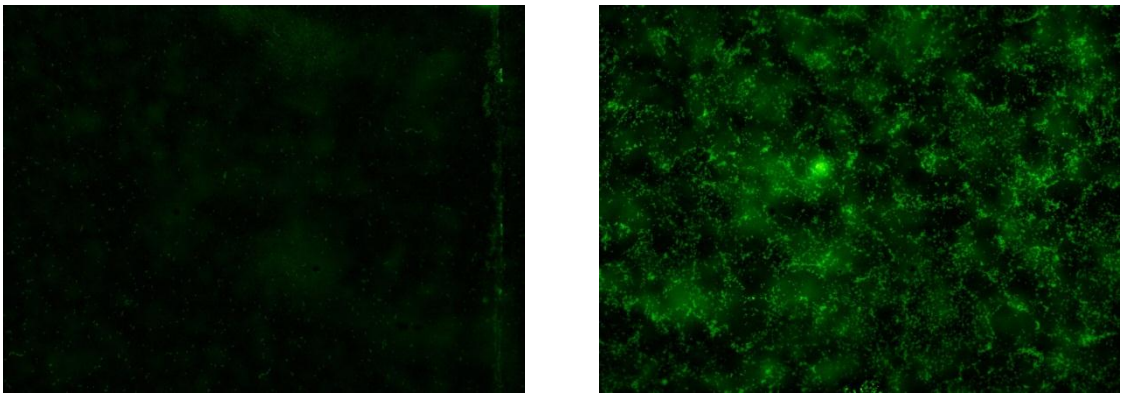
Syringe connectors: e.g. Upchurch (VacTht Fttg 1/4-28 – 1/16)

Liquid circulation: e.g. a syringe pump

7.0 Example of Use

The MD Flow Cell was used to study the growth of biofilm producing bacteria under very low nutrient conditions.

Shows *Aeromonas hydrophila* stained with Syto9. The left image depicts the bacteria at time point zero. The right image shows *A. hydrophila* grown for 6 days.



Growth of *A. hydrophila*. l*eft*: stained immediately after incubation; *right*: stained after 6 days; Syto9 was used in both cases. Images were taken using an epi-fluorescent microscope, 100x magnification.

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