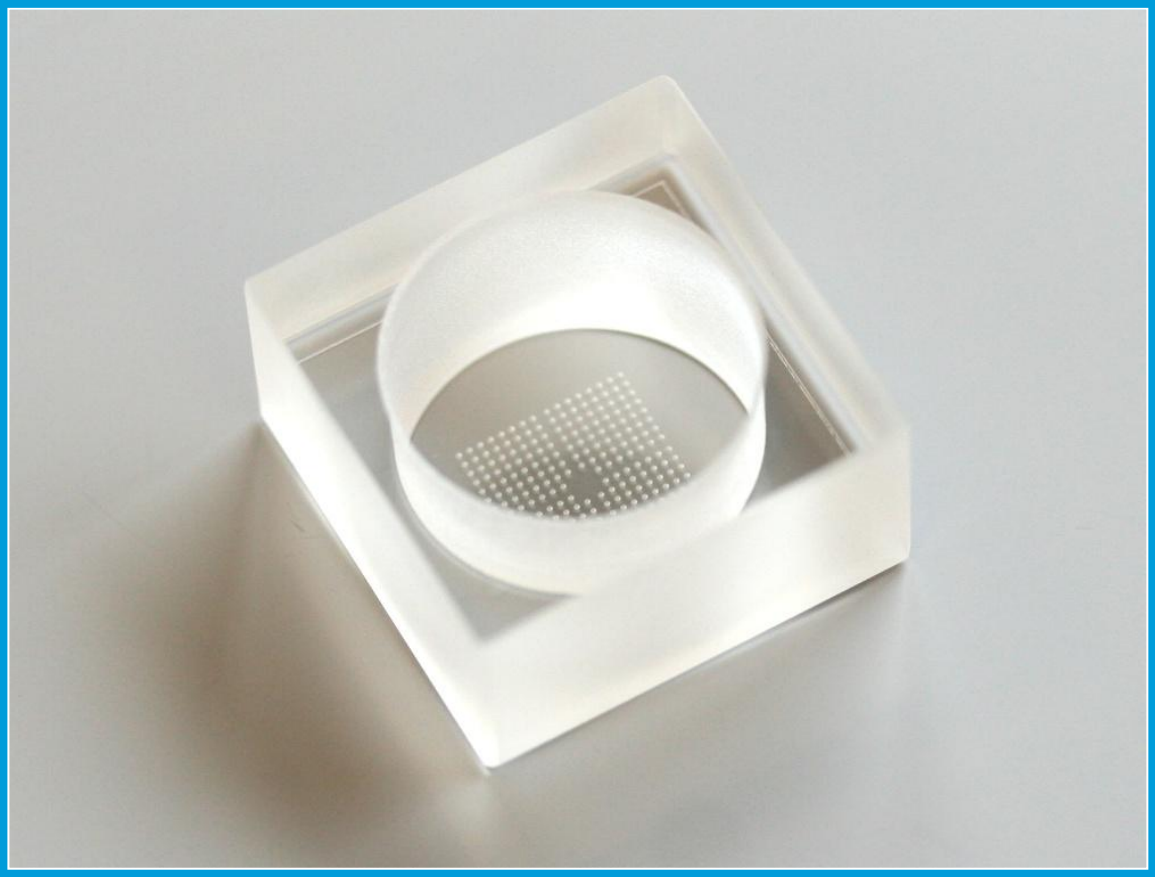


dolomite

EMBRYO IMMOBILISATION CHIP

USER INSTRUCTIONS

Contents

1. Introduction

Live imaging of a large number of small non-adherent samples for a long time and at high resolution poses several challenges in sample handling. These challenges include:

- Mounting samples onto a microscope stage as well as dismounting can result in sudden displacement, and once imaging has started temperature fluctuations within the medium lead to currents, which displace samples out of the field of image capture.
- Certain types of experiments require samples to be retrieved after imaging for further analysis. This process is not easy to achieve without the risk of mixing samples, due to movements when taking them off the microscope and carrying them to another location, for example to a binocular microscope for sample recovery.

The Embryo Immobilisation Chip addresses these challenges in a product made of glass optimized for high-resolution imaging, and thus enables high throughput experimentation.

2. Product description

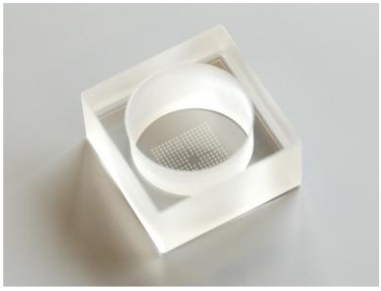
The Embryo Immobilisation Chip (Part No. 3200208) is designed for the immobilisation and imaging of small (up to 150µm diameter) non-adherent samples such as embryos or cell aggregates.

Samples can be transferred with a micro-pipette into well chambers that are open to the medium and have a bottom thickness that corresponds to a standard optical cover slip. The specific design of the well chambers keeps samples in place throughout imaging and during mounting / dismounting from the microscope.

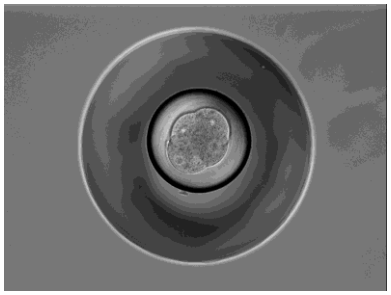
Samples can also be safely carried in the device without displacement. This allows in-vitro culture and simultaneous high-resolution imaging for several days. Samples can be easily retrieved thereafter with a micropipette from the device for further analysis, such as fixation and staining, or genotyping.

The dimensions of the currently available device are optimised for pre-implantation mouse embryos up to the blastocyst stage.

Embryo Immobilisation Chip in interface



The Embryo Immobilisation Chip enables high resolution imaging of embryos

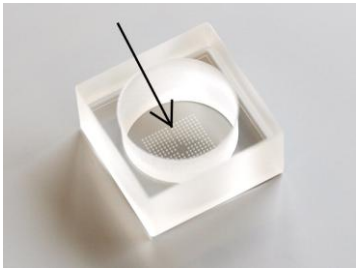
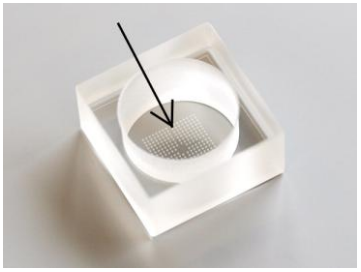
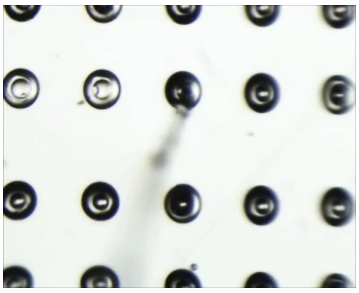
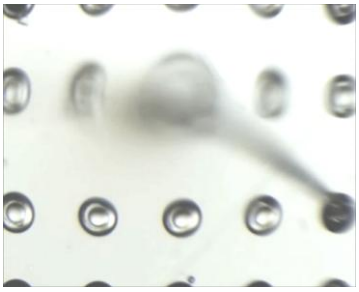


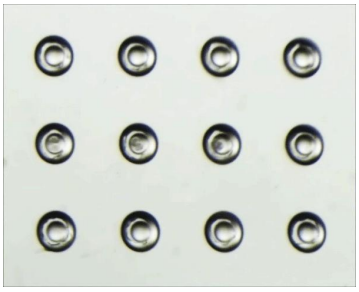
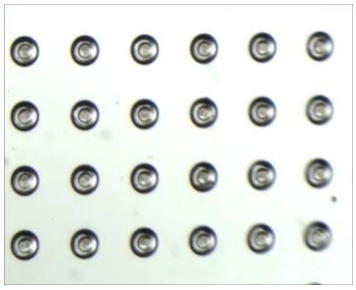
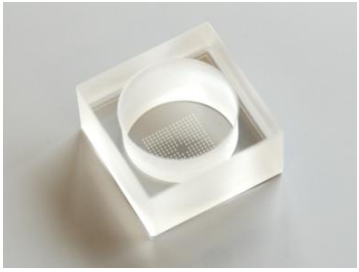
A 4-cell stage mouse embryo is shown within a well, at 40x magnification The embryo has a diameter of 100µm

3. Using the Embryo Immobilisation Chip

The Embryo Immobilisation Chip set-up process is described below. This is based on experiments with pre-implantation mouse embryos up to the blastocyst stage.

Note: Prior to use, the Embryo Immobilisation Chip should be sterilized either by dry heat or autoclaving.

	Description	
1	500µl of medium is to be filled into the reservoir of the device. Less medium can be used, but as a pronounced meniscus will form within the lowest point of the centre of the device, there is a risk that the central wells are not covered with sufficient medium for loading samples. The extent of the meniscus also varies with the type of medium used.	
2	Subsequently, the medium is covered with a layer of mineral oil to avoid evaporation. When using the device with a CO2 dependent medium at 37°, incubation for at least 3-4 hours is recommended to equilibrate pH.	
3	Due to the small size of the wells and the surface tension the aqueous media will not wet the inside surface of the wells. As a result each well chamber will contain a trapped air bubble after the reservoir has been filled.	
4	The air bubbles will need to be removed before sample loading by using a fine glass pipette drawn out to less than 150µm in diameter. Touching and suction through a mouth-pipette or rotating the pipette tip in the well chambers will release the air bubble.	

5	When using a CO2 dependent medium at 37°, we suggest incubating for an additional hour after de-bubbling the well chambers. Sample loading can then be carried out.	
6	Samples can be transferred to the Embryo Immobilisation Chip using glass pipettes, drawn out to the appropriate diameter. Once released, samples will sink into the well chambers under gravity.	
7	For mounting onto a microscope, the Embryo Immobilisation Chip is placed into a holder called Embryo Immobilisation Chip Interface (Part No. 3200209). The Chip Interface is designed to fit into microscope stage inserts for standard 35mm petri-dishes.	
8	Following experiments the Embryo Immobilisation Chip can be cleaned and reused. If mineral oil was used to cover the medium, after aspiration of oil and medium, the device can be immersed in iso-propanol for 2 hours and subsequently rinsed 3 times for 30 minutes in acetone. Subsequently the device can be heat sterilized. Dolomite also provides an ultrasonic cleaning process which will remove debris from the wells. Please contact Dolomite for information about this process.	

For more information on the device please view our Embryo Immobilisation Chip video, accessible via our website www.dolomite-microfluidics.com/downloadsmain/videos

The Embryo Immobilisation Chip has been developed in collaboration with Gurdon Institute (University of Cambridge) www.gurdon.cam.ac.uk

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