DLS Sample Preparation Guide

The Leica TCS SP8 DLS is an innovative concept to integrate the Light Sheet Microscopy technology into the confocal microscope. Due to its unique optical architecture samples can be mounted on standard glass bottom petri dishes and require only little adaptations when compared to conventional mounting procedures, as for confocal imaging. This quick guide was designed to give the DLS user an overview of the mounting possibilities, guidelines required for proper mounting and some concrete examples of mounting procedures.

Material:

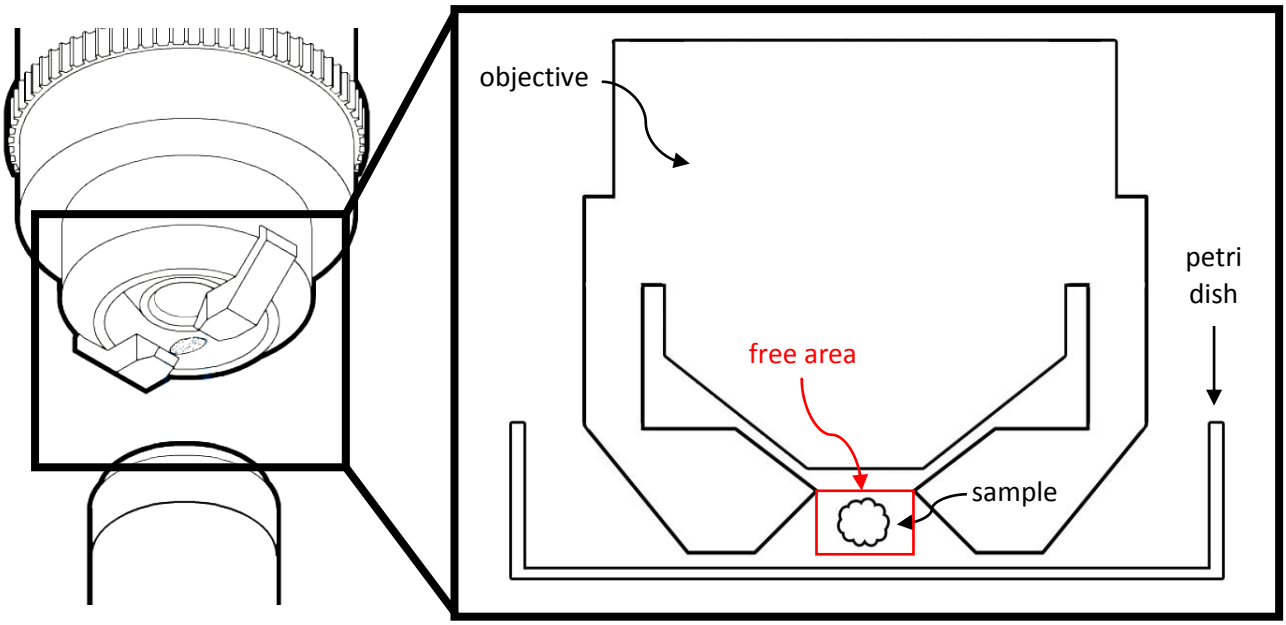
- Low melting agarose 1% (in water, buffer or medium), for creating a “bed” lifting the sample to the height of mirror caps.
- Microwave or heating plate for solving, or re-melting the agarose.
- Falcon tubes or glass bottles for agarose.
- Plastic and glass Pasteur pipettes.
- Glass bottom dishes, round, 35mm (preferably CellView from Greiner Bio-One).
- Distilled water.
- Fine forceps for preparing samples, or brushes for manipulating specimen.
- Scalpel for removal of excessive agarose.
- Stereo microscope for sample preparation.
- Paper tissue and 70% ethanol for cleaning of dishes, objectives and mirror caps.
- Parafilm for sealing .
- Optional: Twinsil for sealing and fixation of samples.
- Optional: 200µl Eppendorf pipette with tips for filling of FEP tubes.
- Optional: FEP tubes of appropriate diameter.
- Optional: Low melting agarose 0.5 %, for embedding living, growing samples like zebra fish.

Sample requirements:

Current DLS objectives are based on electro-physiology dip-in objectives and were exclusively designed for imaging of living and fixed samples in aqueous solutions. Imaging with other embedding media of higher refractive indexes have a high probability to fail, as the light sheet cannot be properly placed on the focal plane of the detection objective. Additionally, some clearing solutions

(e.g. BABB) are aggressive to objectives and might cause irreversible damage to the optical components.

The size of the samples and mounting procedures are dependent of the architecture of the DLS microscope, as depicted below:



For image acquisition, the specimen preparation has two major requirements. The sample needs to be placed into the focal plane of the detection objective, in between the two mirrors of the cap; and it needs to be slightly elevated from the petri dish bottom for the mirrors/light sheet to reach the distal part of the sample.

- Mirror cap distance:

  The mirror cap distance is dependent of the field of view of the illumination objective, which in the current solution is a 2,5x magnification. For the 2,5x illumination objectives the corresponding mirrors distance consist of 5mm. For a sample to be wholly imaged (using tile-scan), it shouldn't be significantly larger than 2mm, at least in the direction of the mirrors. Perpendicularly to the mirrors axis the sample may be larger, as there are no limitations regarding the cap.

- Cover glass offset:

  The sample needs to be placed on a slightly elevated position for the light sheet to reach its distal region. Different materials can be used for this purpose, but generally gels (like agarose gels) are chosen, since they are almost transparent, have a refractive index close to water

and are generally harmless to living samples and tissues. But also plastic FEP tubes are commonly used. Regularly, placing the sample 500µm above the cover glass should suffice. The sample shouldn't be placed too high above the cover glass either, less than 5mm are advisable.

Mounting procedures:

Gels:

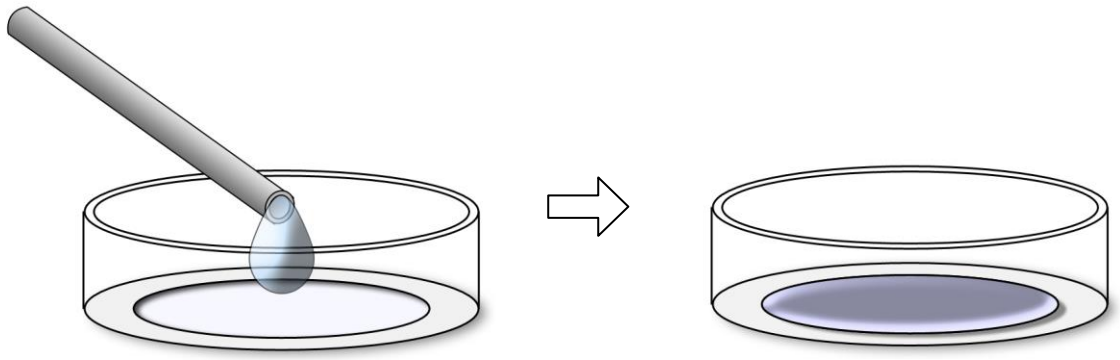
Gels, traditionally made with low melting agarose, are the most common way to mount samples for light sheet imaging. In rather low concentrations (0,5% to 1,0%) it is translucent, cheap, chemically inert and has a refractive index close to water. Agarose works well with living intact samples like zebra fish and *Drosophila* embryos. But it also can be used for fixed tissues, as for example 3D cell cultures and dissected organs.

Cultured living 2D and 3D cells often require special matrixes (Matrigel and other hydrogels, collagen, gelatin or fibronectin) and media. The usage of these gels only require to be stiff enough for the cells to be elevated 500µm over the cover glass and shaped in a way that the sample still fits between the mirrors.

Below two mounting examples for Zebrafish and *Drosophila* embryo:

Zebrafish

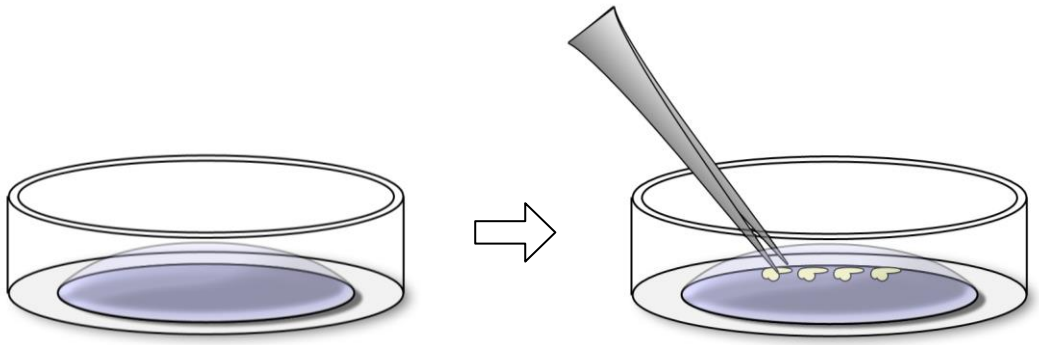
1. **Create an agarose spacer for the fish**



- Use petri dishes with glass bottom (preferably 170µm). Clean the glass bottom with ethanol as the agarose will otherwise not attach properly.
- Use 1% agarose concentration prepared with egg water (e.g. E3)

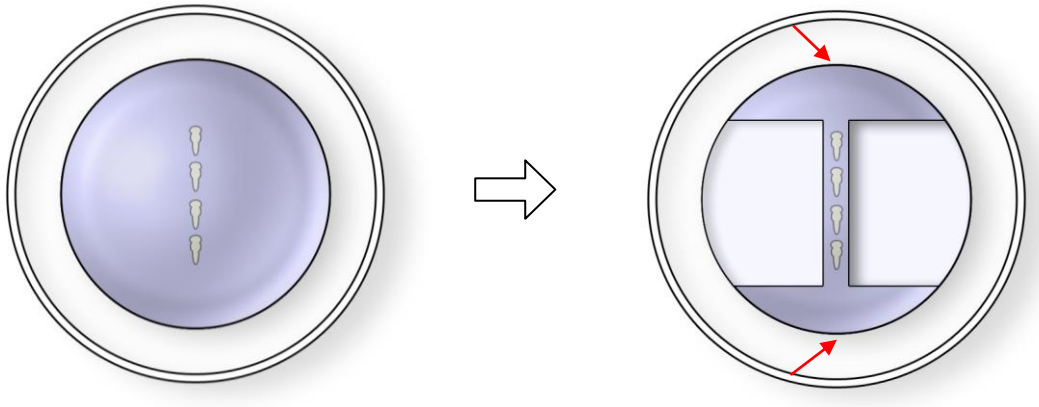
- Place a droplet of agarose on the petri dish using a pipette. Apply only enough of the gel to fill out the glass and create a bed (~1mm) to avoid the fish from sinking to the cover glass.
- Wait for the agarose to polymerize.

2. Apply second layer of agar and place fishes



- Use low concentrations of agarose for the solution (around 0.5%) for fishes to grow freely
- Keep the agarose on constant temperature, between 40°C and 50°C to avoid heat-shocking the fish.
- Place a drop of 0,5% agarose on top of the polymerized 1% agar spacer.
- Use a pipette/tweezers to place the fished in the liquid agarose and orient the sample for imaging
- Wait until the agarose is polymerized

3. Remove excessive agarose from the sides for the mirrors



- After gelling carefully remove excessive agar with a scalpel. Adjust the width of the sides to meet the requirements of the mirror cap.
- It is important not to damage the lower part of the agar droplet, which should perfectly stick to the cover glass. Otherwise the immersion solution will make the agar detach from the glass bottom and float away.

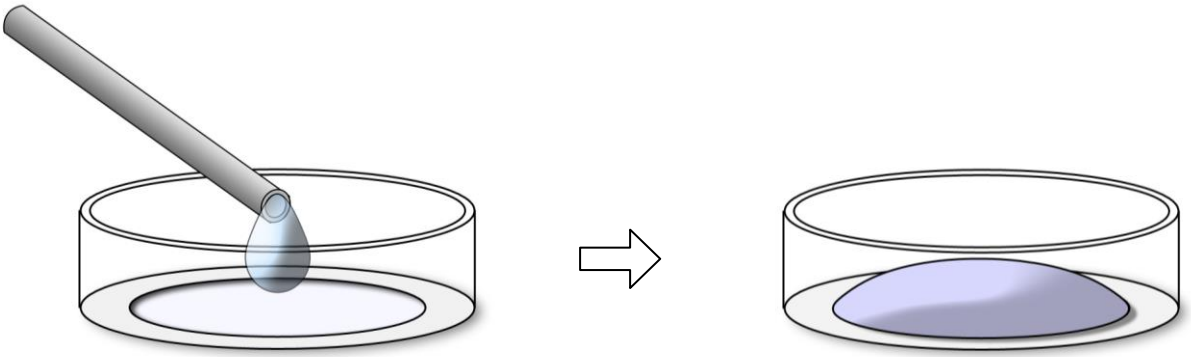
- Make clean side cuts, to avoid artifacts in the light sheet.
- Use paper tissues to remove the water/medium from the cut edges.
- Apply two very small droplets of agarose on the highlighted positions (red arrows).
- Let the agar dry for a couple of minutes to improve adhesion of agar and glass.

4. Add immersion solution

- Place the petri dish into the sample holder of the DLS microscope
- Slowly pour the immersion solution into the petri dish.
- Solutions can be exchanged during imaging procedure to alter its properties (ion concentrations, anesthetics, etc.).
- Agarose shouldn‘t be touched by the mirrors to avoid the detachment from the glass bottom when the dish is filled with liquid.

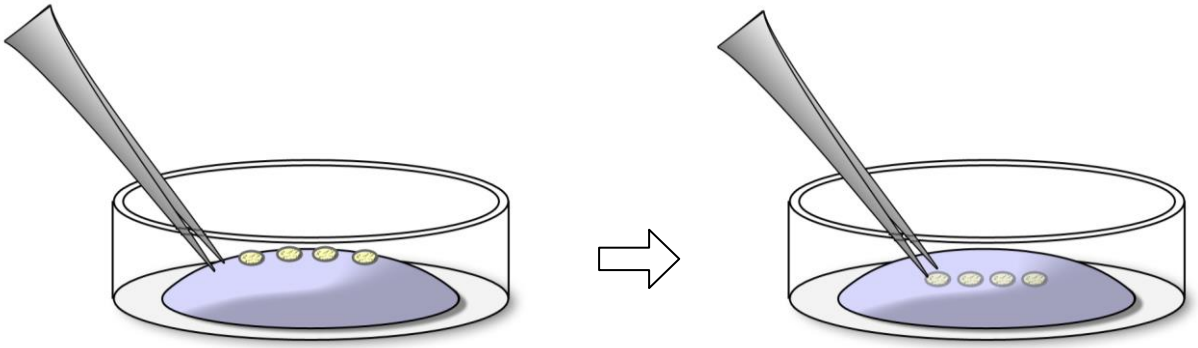
Drosophila embryo

1. Place the agar on the petri dish



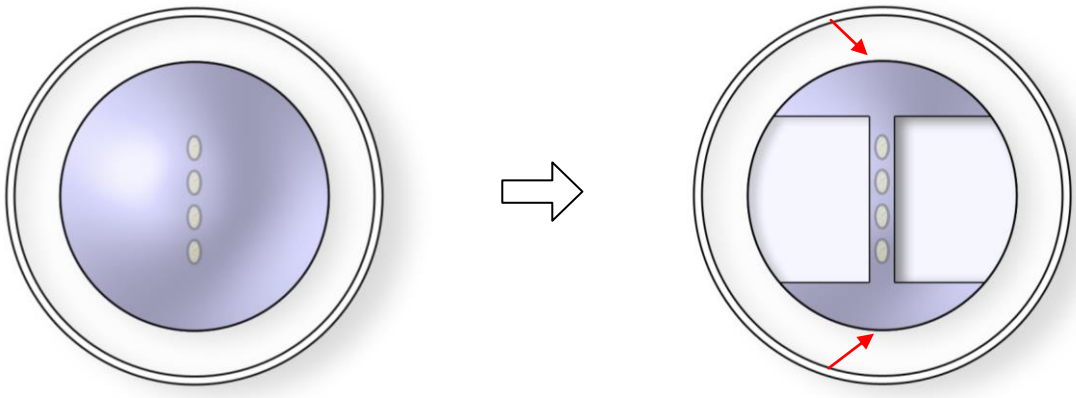
- Use petri dishes with glass bottom (preferably 170µm). Clean the glass bottom with ethanol as the agarose will otherwise not attach properly.
- Use 1% agarose concentration prepared with buffer (e.g. PBS).
- Place a droplet of agarose on the petri dish using a pipette.
- Wait for the agarose to polymerize.

2. Place samples into the agar:



- Place dechorionated embryos on top of the solidified agarose droplet using tweezers or a brush. The embryos cannot sink into the liquid agarose.
- Use the tweezers to create a small rupture into the agarose and push the embryo into the hole.
- With the tweezers poke the embryo in the agarose until it has the right orientation
- Apply a very small droplet of agarose on top of the embryos so they don't get released into the medium while imaged

3. Remove excessive agar from the sides



- After gelling carefully remove excessive agar with a scalpel. Adjust the width of the sides to meet the requirements of the mirror cap.
- It is important not to damage the lower part of the agar droplet, which should perfectly stick to the cover glass. Otherwise the immersion solution will make the agar detach from the glass bottom and float away.
- Make clean side cuts, to avoid artifacts in the light sheet.

- Use paper tissues to remove the water/medium from the cut edges.
- Apply two very small droplets of agarose on the highlighted positions (red arrows).
- Let the agar dry for a couple of minutes to improve adhesion of agar and glass.

4. Add immersion solution

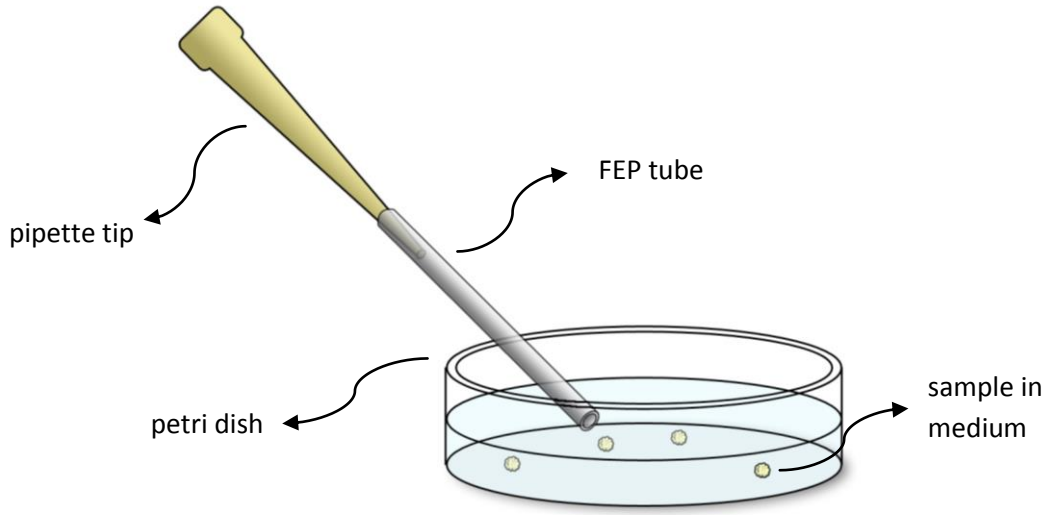
- Place the petri dish into the sample holder of the DLS microscope
- Slowly pour the immersion solution into the petri dish.
- Solutions can be exchanged during imaging procedure to alter its properties (ion concentrations, anesthetics, etc.).
- Agarose shouldn't be touched by the mirrors to avoid the detachment from the glass bottom when the dish is filled with liquid.

FEP Tube:

An alternative mounting method to gels consists in using containers, as capillaries and tubes, which are more robust than e.g. agarose attached to the cover glass, enables a hermetically sealed environment to keep specimen for longer periods of time and avoid contaminations.
Compared to gels the optical properties of these containers are in general worse, because the materials used (e.g. FEP or even glass) have a refractive index which is higher than water.

Below one method is shown of how to mount samples on FEP tubes:

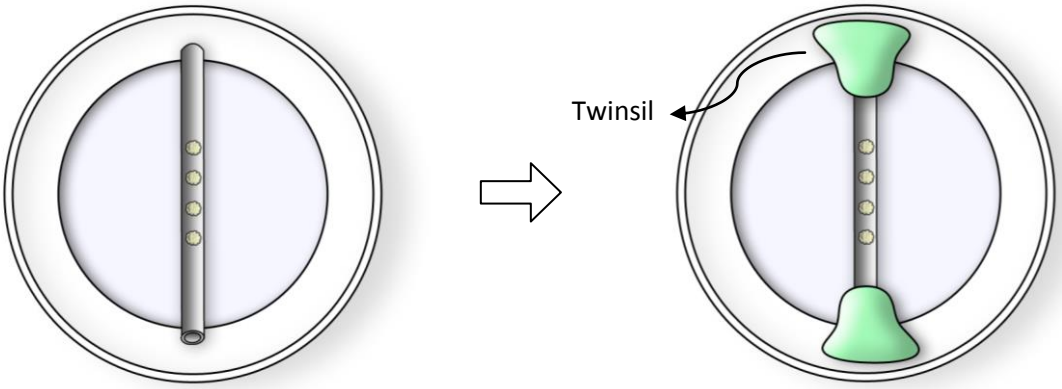
1. Load the FEP tube with the samples



- Fill a petri dish with medium/warm agarose/buffer and place the samples in the dish.

- Use an FEP tube with appropriate dimensions (e.g. Saint-Gobain, FEP, outer diameter 1,95mm, wall thickness 150µm) and cut a piece of the tube which is slightly smaller than the dimension of the petri dish.
- Take a 200/100µl micro-pipette and stick the (yellow) tip into the FEP tube.
- Suck the sample into the FEP tube by using the micro pipette. Sample position can be adjusted by pushing and pulling the pipette and rotating the capillary in the horizontal position
- Remove the pipette tip and place the sample into the glass bottom petri dish

2. Fixing the tube in the petri dish



- Place the FEP tube in the center of the petri dish.
- Prepare the two-component Twinsil silicone.
- By using a pipette tip spread the silicone mass on both endings of the FEP tube. This will fix the tube in place and seal off the inner compartment. Alternatively the tubes can be kept open to allow the exchange between media in- and outside of the tube.
- Other silicones and glues could be used as long as they don't interfere with the living samples
- Fill the petri dish with the immersion solution / medium

Stamps and molds:

In some experiments, especially concerning multiplexing and screening of several samples, a more regular and reproducible way of mounting is required. For this, application specific molds and stamps can be created, which have a regular pattern, and enable samples to automatically orient itself to a desired position. These molds are of course application specific need to be designed according to the needs of the experiment

Below an example of molds which are used for spheroid/organoid culturing. With such molds of different layouts, small hydrogel containers can be created that could be attached to the cover glass surface in and DLS microscope and immensely simplify the mounting procedure.

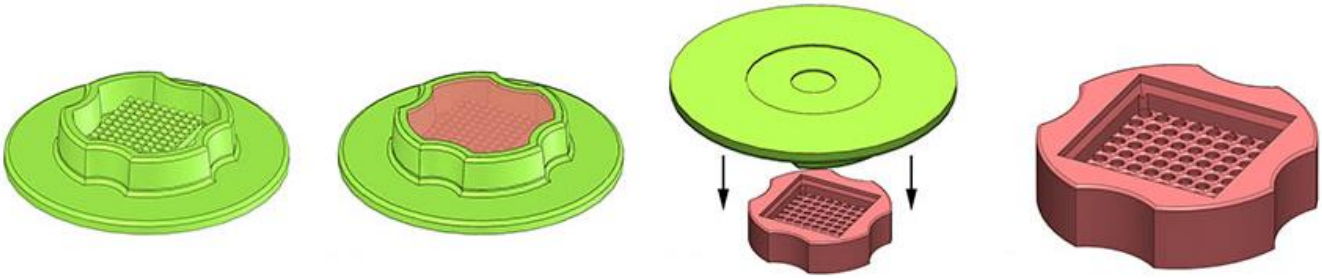
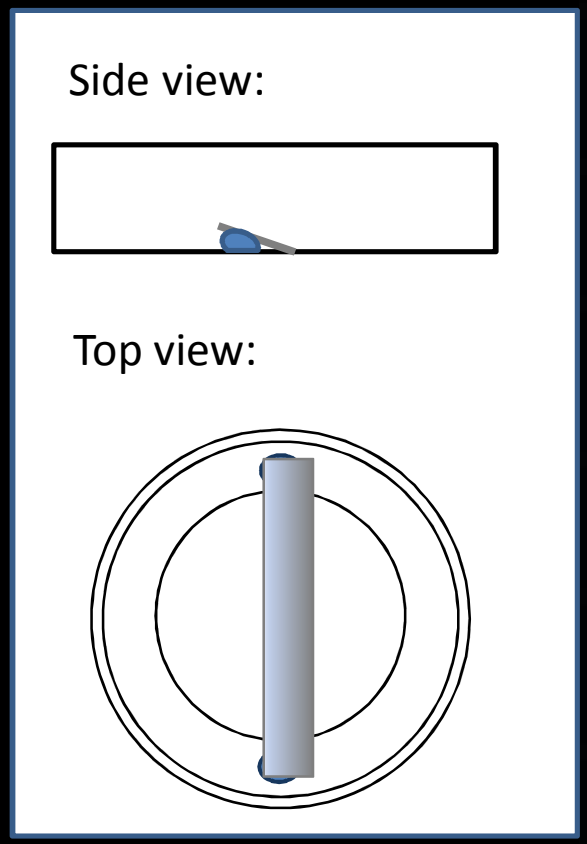
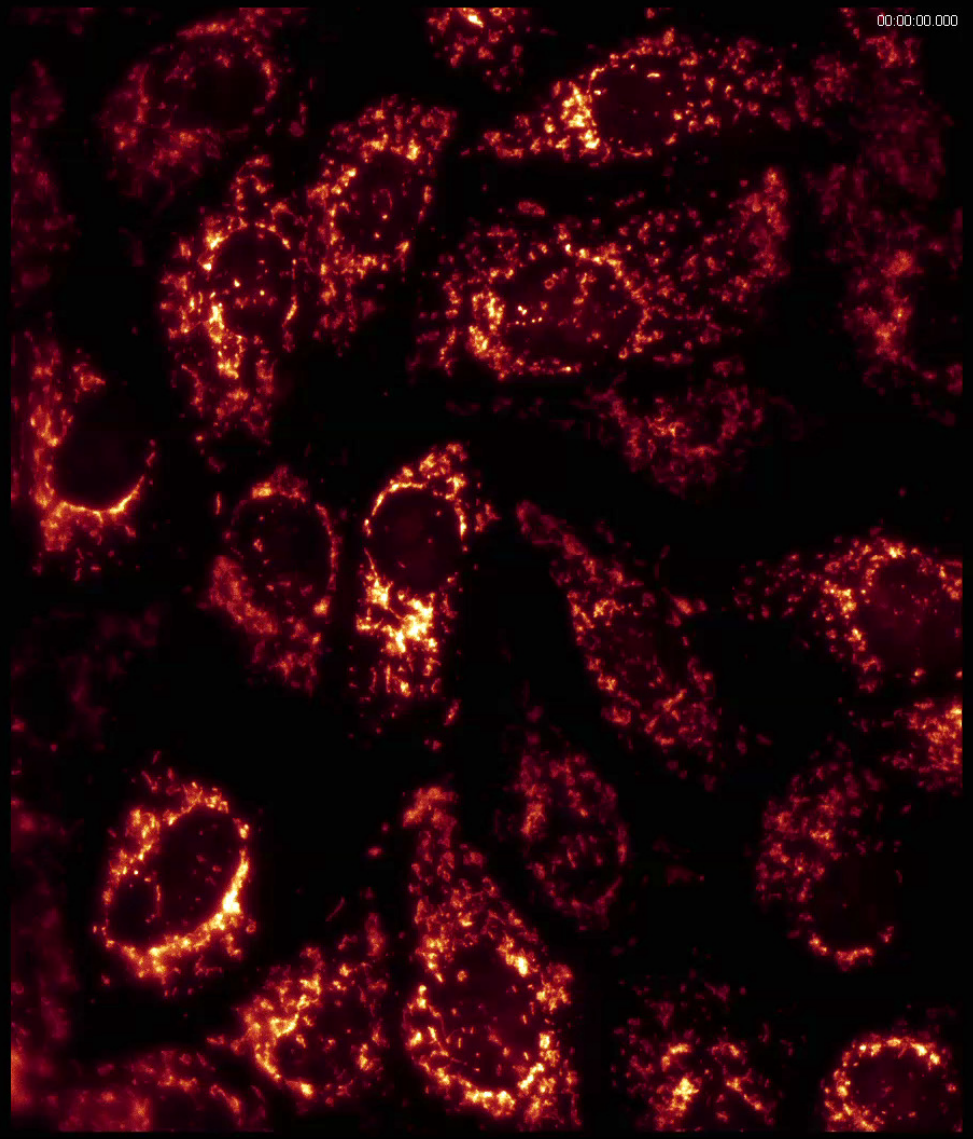


Image taken from www.microtissues.com

Imaging of adherent cells

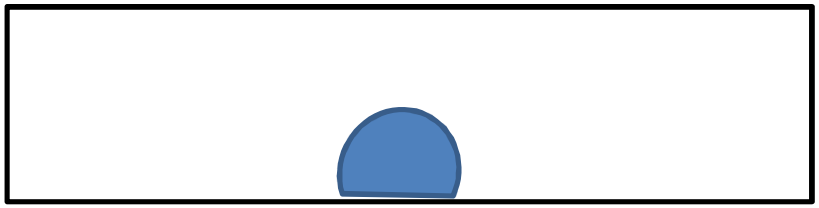
Sample: cell culture
Marker: Mitotracker



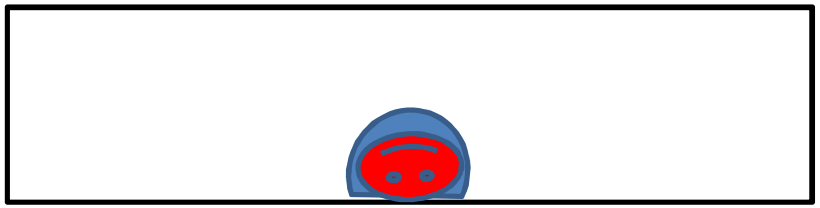


Dr. Okada, Japan

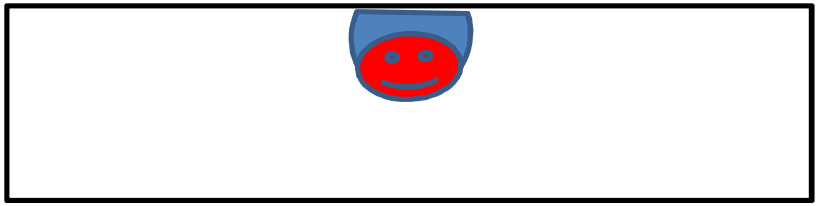
DLS Mounting in Matrigel (for e.g. Spheroids)



Add a drop (or several) of Matrigel into a glass bottom dish



Transfer a spheroid to such a drop



Turn the dish around, place it in incubator at 37℃, Matrigel will polymerize