SAMPLE PREPARATION

An overview of methods and protocols to prepare samples for nanoindentation with Optics11 Life Nanoindenters

OPTICS11 life

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SAMPLE HOLDERS

Different types of dishes and samples can be used with the Optics11 Life Nanoindenters. Which holder is suitable depends on the Nanoindenter and the probe used. This section will discuss dishes, well plates, perfusion chambers, and microscope slides.

Optics11 Life has three types of probes, which are schematically represented in **Figure 1**. The height of the probe determines which maximum dish height can be used. For well plates the 20mm probe has to be used while for most standard dishes the 7 mm probe is sufficient. The 14 mm probes are referred to as double ferrule probes and can be used in dishes that have higher sidewalls. For more information, visit our webshop to see the probes available.

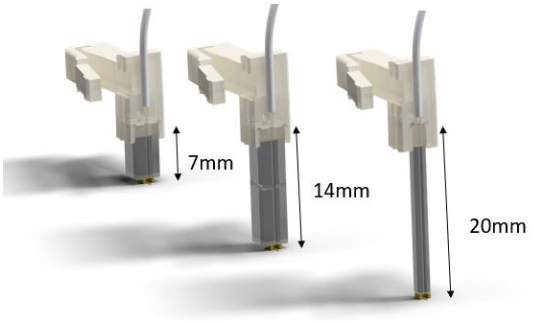

Figure 1: Schematic representation of the different probe types manufactured by Optics11 Life.

Piuma

Most glass and plastic dishes will fit on the stage as well as microscope slides. The stages can move 12 x 12 mm. With the well plate adaptor for Piuma it is possible to measure well plates in combination with the 20 mm probes, you can ask a sales engineer for a quotation.

Chiaro

Because of the flexible nature of the Chiaro, more options are available: standard dishes, microplates, and microscope slides in combination with the right microscope sample holder. Plates with 96 wells are the smallest size possible to be measured with 20 mm probes with Chiaro. Be careful not to crash the probe on the side of the well plate.

Pavone

The Pavone is designed specifically for use with well plates and 20 mm probes, therefore all well plates up to 384 can be used. Different dishes and microscope slides can also be used.

GENERAL INSTRUCTIONS

1. Samples should be mounted in a well plate (you can check in Pavone whether your well plate fits inside. There are a few brands that have non-standard dimensions).
2. Samples should be hydrated in an appropriate media, let them hydrate for a few hours (they might swell) or overnight if they were kept dry.
3. Media should be clean, no large particles floating.
4. Samples should not float i.e. well attached to the bottom, stable, attachment should be firm so that only sample is being deformed during indentation.

AGAR FOR TISSUES AND BIOMATERIALS

Agar is a gel-like substance obtained from algae, it is a simple and non-destructive method of securing samples for nanoindentation.

The agar gel is prepared by mixing the agar powder at the desired concentration with water. The mixture should be heated and stirred for optimal results. The higher the concentration of agar, the stiffer the gel will be. The mixture can be stored in a closed bottle at room temperature.

For use, reheat the agar mixture in the microwave until liquid, pipet a thin layer at the bottom of a dry and clean petri dish and place the sample on top. Add additional agar around the edges of the sample if necessary and wait for the agar to solidify. Inspect that the sample is properly attached to the agar, for instance by gently shaking the dish. Finally, add the desired medium for the measurement.

Important: The agar mixture should be stiffer than the sample to give it support during indentation. If the agar is softer than the sample, the measurement results will be inaccurate. The agar will deform with the sample during nanoindentation and a combined stiffness will be measured.

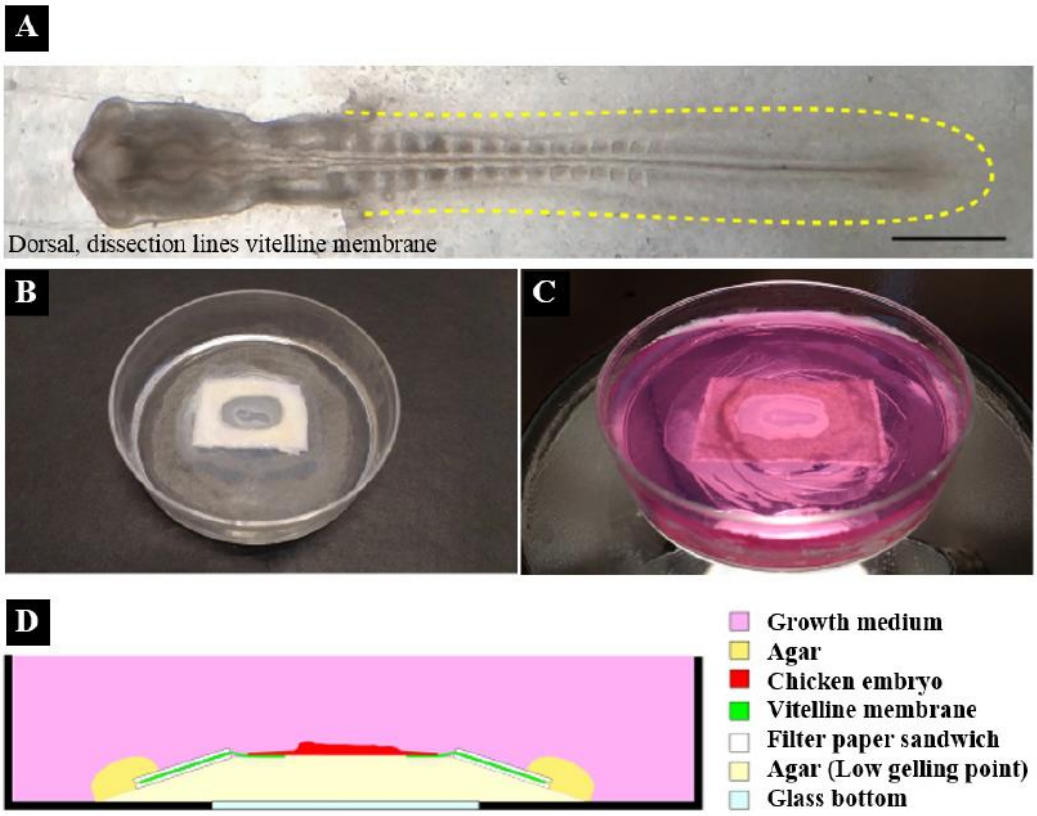

Figure 2: Example of embryo mounted on top of agar [1].

TISSUE SLICES (300 – 500 µM THICKNESS)

For these tissue slices, a perfusion chamber can be used with a glass slide at the bottom and a harp or slice anchor on top. The process is the same without a profusion chamber Important is that the slices are flat, so ideally made with a vibratome or similar.

To ensure adhesion of the glass slide with the tissue slice a 0.1 – 1 % concentration of Polyethylenimine (PEI) solution can be used. Dissolve the PEI, apply it in a thin layer, and leave it overnight to dry out the glass slide. A thin white layer should be visible afterward. Place the sample over the area with the coating, remove access liquid to make sure that the slice gets in contact with the glass. Mount the harp without pressing too much on the slice. Slowly add the desired medium and inspect if the slice is not floating in the medium.

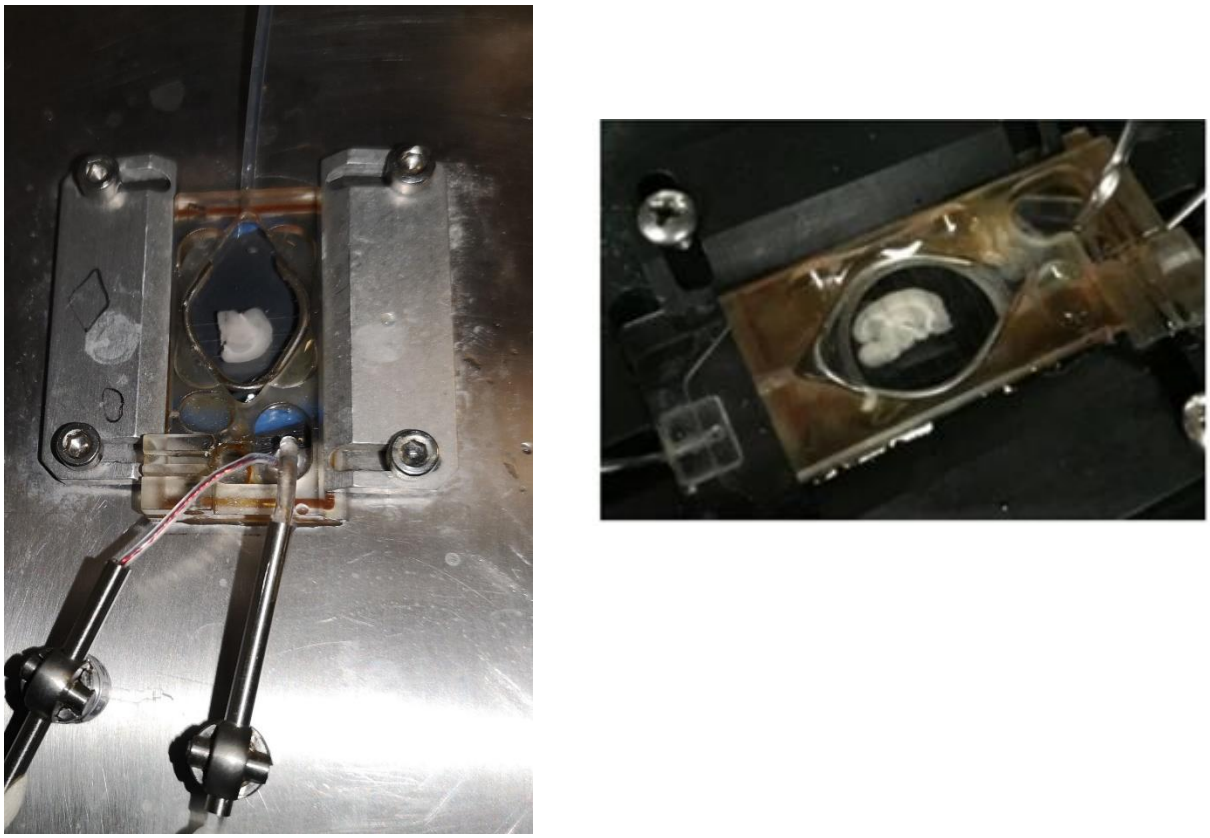

Figure 3: Example of a mouse brain tissue slice secured in a perfusion chamber [2].

CELLS IN SUSPENSION/SLICES (5 – 300 µM)

To adhere cell suspensions or thin slices of material Cell-Tak by Corning can be used. Apply a thin coat at the bottom of the petri dish and place the sample gently in the dish according to the manufacturer's instructions.

As a cost-effective alternative, transglutaminase can also be used. The following protocol was developed by Sahai et al. [3]:

1. Mixing enzyme and deionized water in a 1:4 (w/v) ratio.
2. Let the slurry set for 10 min on the dish.

Following is an adjusted protocol for spheroids by Ilaria Gregorio:

1. First prepare a 1% solution of MooGloo in water, filter it through 0.45 µm filters, make some aliquots to store in the -20 °C freezer.
2. Thaw the aliquots, and filter again through 0.22 µm filters (if sterile solution is needed).
3. First, treat the plates with gelatin (0.1% solution) and let it set for 1 hour in the incubator at 37 °C, then after removing the gelatin, add the filtered 1% transglutaminase solution (circa 100 µL in a well of a 24 well plate) and let it set for 10 min at room temperature.
4. Lay down the 3D cultures and put the plate in the incubator at 37 °C for 30 min. After that, remove the excess of transglutaminase and gently fill the well with cell medium. Put the plate back in the incubator.

Cryosections can be prepared by using Tissue-Tek.

Histoacryl Tissue glue is another alternative that can be used with the protocol by Eberle et al. [4].

MEASURING IN A DROP

This method is suitable for thin tissue slices mounted on microscope slides (cryosections) or other small samples that cannot be stabilized in another way. Draw a circle around the sample with a PAP pen to create a hydrophobic boundary around the sample, and let it dry completely. Fill the middle of the circle with the desired medium to create a semi-sphere with sufficient height for the probe to be fully submerged in the medium. Keep in mind that a dry slice will swell when submerged in liquid. Furthermore, it is important to note that the noise level of measurements in a drop will be increased and liquid will be evaporating which would change the concentration of the media. Alternatively, one can think of gluing a ring on top of the glass slide that can be filled with liquid or use chambered glass slides.

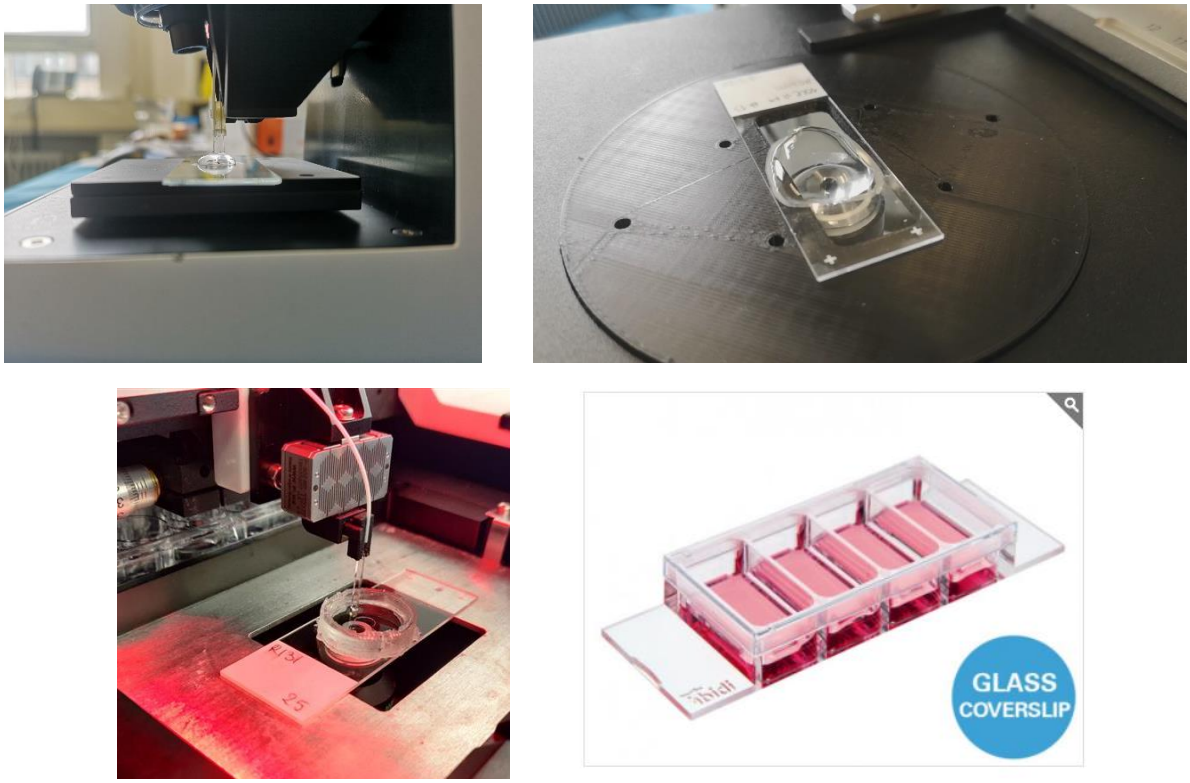

Figure 4: Top – calibrating or measuring in a drop of liquid. Bottom – ring glued on top of the glass slide and filled with liquid or chambers available from ibidi.

LARGE TISSUES

For larger tissue samples, agar mounting is still the preferred option. If the tissue sample is sufficiently large and/or heavy, then mounting or securing might not be necessary. A larger sample can be weighed down by using a metal ring or similar method.

If non-destructiveness is not of concern then super-glue can be used, the kind that solidifies when it comes in contact with water is best used. Wound glue, nail polish can also be used. This can then be used both dry and when measuring in the medium. We recommend measuring in liquid to make sure that tissue doesn't dry out. Spurr low viscosity embedding kit can also be used to secure tissue samples.

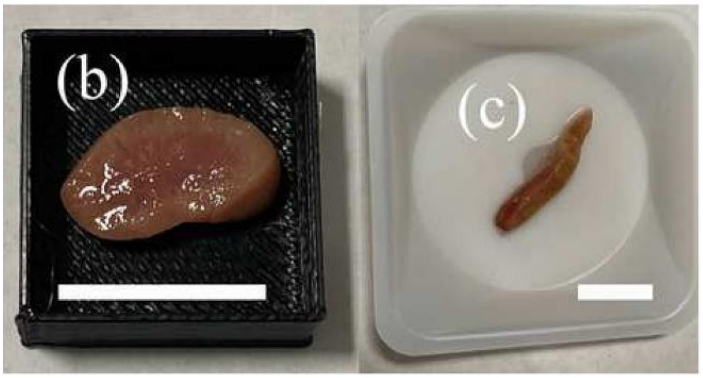

Figure 5: Example of mouse liver and kidney samples glued to dishes, scalebar is 10 mm [5].

SPHEROIDS, ORGANOIDS, OOCYTES, MICROSPHERES, ETC.

Several methods exist to indent on these samples successfully, a few methods will be outlined below.

1. Use U or V shaped well plates. Keep in mind that there are limitations to measurements in such plates due to geometry of probes. Only larger samples which are centered are reachable with Optics11 probes. Larger spheres allow to reach lower in the well.

2. Embed in a thin layer of agar or Matrigel. Be again mindful that the embedding material should be significantly stiffer than the organoid/spheroid and shouldn't be higher than the sample, you need to indent the sample directly rather than through the gel.

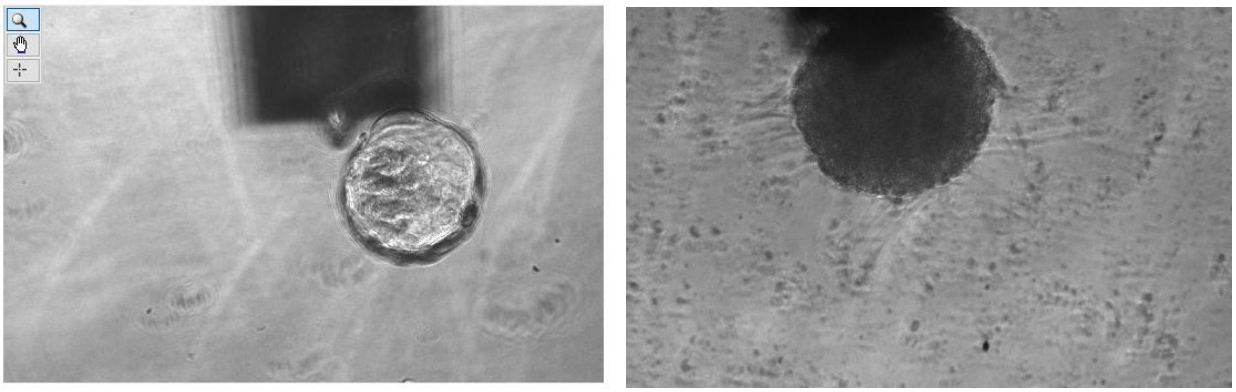

Figure 6: Left: Spheroid embedded in Matrigel, Right: Spheroid embedded in collagen gel, you can see it form attachments.

3. Coat the bottom of the dish with ECM protein to make it adhere to the sample, e.g. collagen or laminin. The exact protein will depend on the sample.

4. Coat the bottom of the dish with a very thin layer of PEI solution and let it dry completely, then add the medium. Add the spheroids and let them settle for a few minutes. This will weakly attach the spheroids to the bottom of the dish.

5. Method for spheroids from Heuer et al. [6]:
    1) Coat a flat culture plate of sufficient size to be used with the Nanoindenter with rabbit plasma mixed with enough thrombin to cause coagulation (amount may vary based on source).
    2) Place the culture plate in an incubator at 37°C for one hour.
    3) Transfer spheroids to the culture plate and add enough medium to cover.
    4) Place the plate back in the incubator and allow spheroids to adhere for 4-6 hours,
    5) Once spheroids have adhered, proceed with indentation.

6. Use a nylon mesh to trap spheroids onto a substrate. Select the mesh size to match the spheroid size and glue to mesh to a substrate, then the medium and spheroids can be transferred and measured.

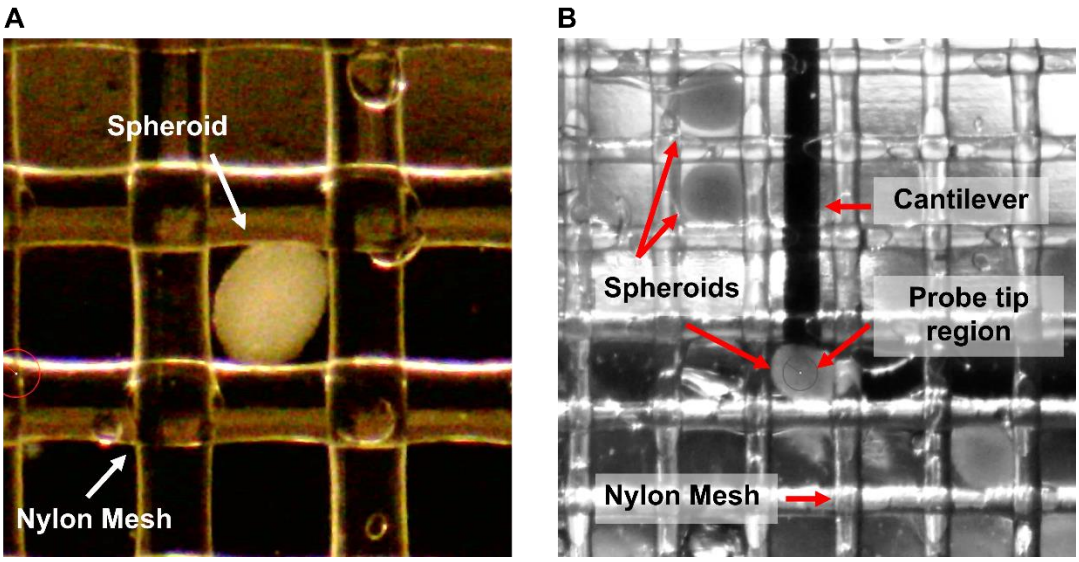

Figure 7: Spheroids in a nylon mesh, from [7].

DECREASING ADHESION OF SAMPLES

There are several methods for decreasing the adhesion of the sample with the probe. The simplest of which is by measuring in a medium rather than in air. Measuring in a medium will significantly reduce the adhesion of the sample with the probe. If the sample still shows high adhesion forces the sample can be kept in a lens cleaning solution (e.g. Opti-Free) to make it hydrophobic and then measured in PBS or water. Measuring in lens solution is not recommended as it might become too viscous and tends to form a "salt bridge" between fiber and the cantilever.

Another option is to coat the sample in 5% bovine serum albumin (BSA) solution for one hour. This works for hydrogels and elastomers [8].

It is possible to coat the tip in 1% Pluronic solution, the protocol by Shen et al. [9] is recommended which is outlined in short below:

1. Prepare the solution or buy a ready-made product.
2. Submerge the probe for 15 minutes.
3. Rinse the probe sufficiently in water.
4. Dry the probe before use.

MISCELLANEOUS PROTOCOLS

Below is a list of papers and protocols where different types of samples were mechanically measured. These can be tried if any of the protocols described in this document are not sufficient or do not apply to your sample.

- Protocol on Tissue Preparation and Measurement of Tumor Stiffness in Primary and Metastatic Colorectal Cancer samples with an Atomic Force Microscope [9].
- Kidney, Liver, Spleen, and Uterus tissue samples were measured in 4 cm Petri dishes and leveled using Shellac (Sigma) [10].
- Immobilization of embryos in Petri dishes using Sylgard 184 using bent 0.2 mm pins [11].
- Bonding of stiff (>1 MPa) membranes to plastic Petri dishes using double-sided tape and filled with medium [12].

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