ORGANOID PREPARATION

OPTICS11 life

CONTACT INFORMATION
Optics11 Life
+31 20 809 6000
info@optics11.com
support@optics11.com
www.optics11life.com

COMPANY ADDRESS
Optics11 Life
Hettenheuvelweg 37-39
1101 BM Amsterdam
The Netherlands

COMPANY INFORMATION
Optics11 B.V.
KvK/CC: 52469417
VAT: NL850459734B01
Amsterdam, NL

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SPHEROIDS, ORGANOIDS, OOCYTES, MICROSPHERES, ETC.

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

1. Use U or V-shaped well plates (see **Figure 1** below). Keep in mind that there are limitations to measurements in such plates due to the geometry of our probes. Only larger samples which are centered are reachable with Optics11 probes. Larger spheres allow reaching lower in the well. **Note**: the largest sphere that can fix inside well plates is 50um, as larger radius probes are only for Piuma and Chiaro and do not fix inside 96 well plates.

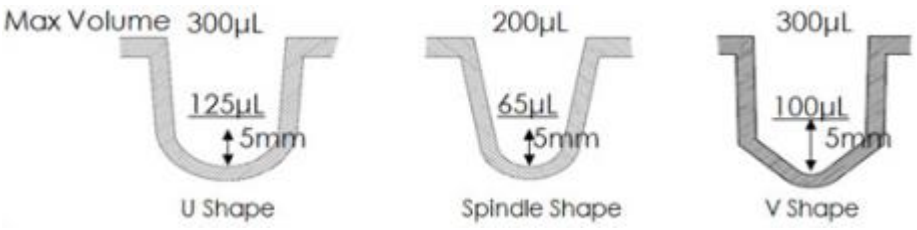

Figure 1: Well bottom shapes [1]. The U shape (left) or V shape (right) are suitable for measurements with Optics11 probes.

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. This process involves removing the spheroids from the well plate they are grown in and adding agar onto the outside. It may be difficult to recover the spheroids from this process.

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. PEI acts as an attachment promoter and will weakly attach the spheroids to the bottom of the dish.

5. Method for spheroids from Heuer et al. [2]:
    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 h.
    5) Once spheroids have adhered, proceed with indentation.

6. Use a nylon mesh to trap spheroids onto a substrate (see **Figure 2** below). Select the mesh size to match the spheroid size and glue the mesh to a substrate, then the medium and spheroids can be transferred and measured.

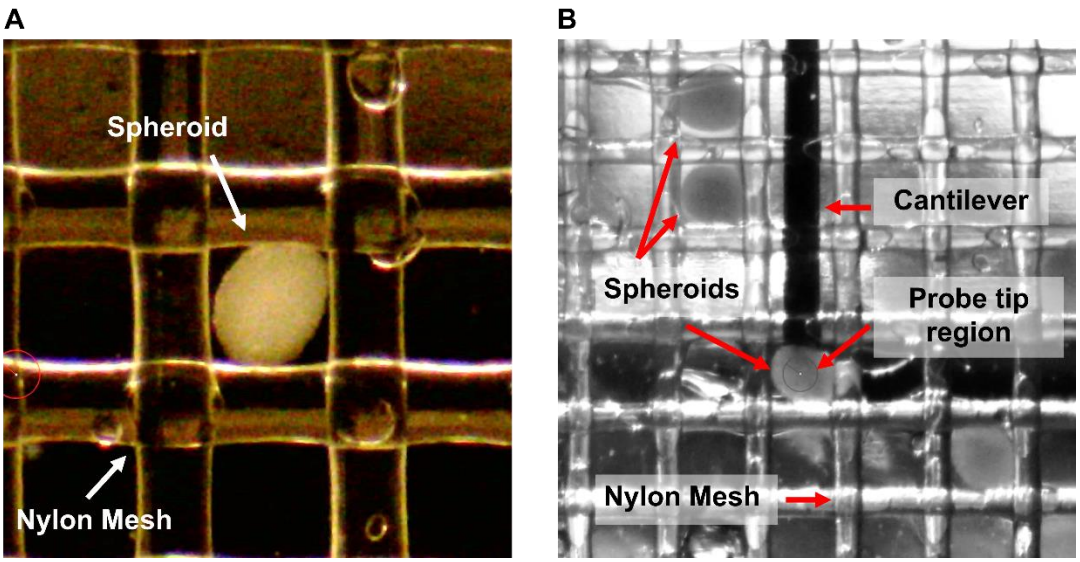

Figure 2: Spheroids trapped in nylon mesh [3].

REFERENCES

[1] "3D Culture Spheroid plates," 03 05 2023. [Online]. Available: https://labchem-wako.fujifilm.com/europe/category/01182.html.

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[3] T. Feijão, M. Neves, A. Sousa, A. Torres, S. Bidarra, I. C. D. Orge and C. Barrias, "Engineering injectable vascularized tissues from the bottom-up: Dynamics of in-gel extra-spheroid dermal tissue assembly.," *Biomaterials,* p. 121222, 2021.