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Sample Preparation Manual for the 3D Cell Explorer

November 2016

Contents

1. Important facts for the sample preparation

1. Cells need to be seeded on a glass surface as thick as a coverslip – we suggest either a glass bottom culture dish or in a coverslip on a glass slide.

2. The instrument is suitable for imaging any kind of eukaryotic or prokaryotic cells. Thin tissue slices can be imaged as well.

3. There are no limitations in the cell confluency. Cells grown in monolayer or multilayers can be observed unless it exceeds the maximum thickness of sample structure (see specification table 1 and 2).

4. Optically clear medium (i.e. transparent live cell imaging solution, PBS) are preferred. Slightly scattering medium (like culture medium with red phenol) can be used as well. Please be aware of the maximum medium volume (see specifications table 2).

5. The optical surfaces should be as clean as possible and cell holders should be carefully cleaned as to avoid that any kind of debris or remains are floating in the mounting medium.

6. For sealing the medium chamber, tape imaging spacer are preferred to nail polish in order to avoid overflow from the coverslips.

2. Specifications overview

Specification table 1

Microscopy Slide		
Conditions	**Specification for 3D Cell Explorer**	**Comments**
Coverslip thickness	170 +/- 20 µm	
Max. thickness of sample structure	30 µm	

Specification table 2

Glass bottom culture dish		
Conditions	**Specification for 3D Cell Explorer**	**Comments**
Glass bottom culture dishes	35 mm or larger	
Max. thickness of sample structure	30 µm	
Max. volume using optically clear medium	2 ml	Depending on optical properties
Max. volume using scattering medium	1 ml	List of tested media: Agar or agarose hydrogel

3. Sample preparation

The 3D Cell Explorer allows to measure the internal components of living cells offering the researcher the possibility to acquire high resolution 3D images within seconds. The instrument is suitable for any kind of eukaryotic or prokaryotic cells. Thin slices of tissues can be observed, too. Moreover, thanks to the extremely low power of the laser source and the compatibility with cell culture accessories (see section 4), the 3D Cell Explorer is suitable for long-term live cell imaging.

This manual has the aim to guide researchers step by step through the sample preparation methods.

3.1 Cell Confluency

In sample preparation, no limitations in the cell confluency are given. Cells grown in monolayer or multilayers can be observed unless the maximum thickness of sample structure is exceeded (see specification table 2).

3.2 Compatible disposables

Imaging with the 3D Cell Explorer requires clean optical conditions throughout the whole optical system, which comprises the microscope and the sample. This means that the disposable's quality is crucial for optimal imaging results.

3.2.1 Microscopic slides and coverslips

The 3D Cell Explorer is compatible with samples mounted on optically transparent slides and coverslips. These glass disposables should have a thick bottom between 130-170µm.

Recommended slides: Slide SuperFrost (e.g. 85-0811-00 BioSystems)
Recommended coverslips: Hirschmann (http://www.hirschmannlab.com/)

3.2.2. Channel Slides

The system is compatible with the following ibidi µ-Slides:

- µ-Slide 2 Well
- µ-Slide I Luer Family
- µ-Slide III 3D Perfusion
- µ-Slide III 3in1
- µ-Slide y-shaped

3.2.3 Microscopy dishes

The observation can be made through glass bottom culture dishes. The system is compatible with the following recommended ibidi µ-Dishes:

- µ-Dish 35 mm, high
- µ-Dish 35 mm, low
- µ-Dish 35 mm, low/high Grid-500
- µ-Dish 35 mm, high Grid-500 Glass Bottom
- µ-Dish 50 mm, low

Please note: The access of the 3D Cell Explorer to the µ-Slides and µ-Dishes is limited to the center of the slide/dish. Please visit "http://ibidi.com/xtproducts/en/Instruments-Accessories/Nanolive-3D-Cell-Explorer" to get more information about all these products.

If you want to use any other kind of disposables (e.g. multi-well plates, lids, etc.), please contact Nanolive's customer service for more information.

3.3 Mounting medium

As a general requirement for any type of sample and in order to have a high quality image the buffering medium should be optically clear, (i.e. no optical scattering of the laser beam). Best are clear liquids like PBS, HEPES or live cell imaging solution specifically developed for live cell imaging applications. However, solutions with red phenol showed to be suitable for the image acquisition when a minimum amount of liquid is used to fully cover the bottom of the dish.

Figure 1: Amount of mounting medium in glass bottom culture dishes

A list of compatible culture media is proposed directly on STEVE software before the 3D acquisition takes place, as shown in Figure 2.

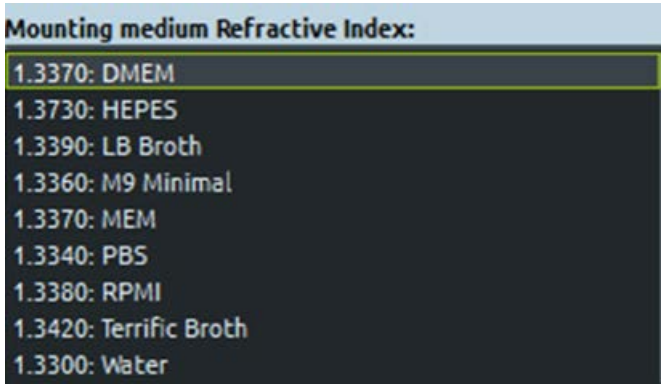


Figure 2: List of common mounting media RI values

Generally, mammalian cells are characterized by a Refractive Index (RI) value like water based solutions [1.33 – 1.44]. Therefore, the suggested mounting media are suitable for obtaining good quality acquisitions.

Few other different biological samples (e.g. vegetal cells, fungi etc.) could present RI values out of this range. To obtain high quality images for these samples, the mounting medium RI value should be as close as possible to the RI components of the biological sample.

Example: By mixing different percentages of glycerol and PBS, it is possible to obtain a clear optical mounting medium with a specific RI.
The RI value graphic is reported on the Appendix chapter 6.

Please note: These solutions discussed in this manual serve as a starting point to establish and perfect your own preparation protocols.

3.4 General preparation rules

We are going to present in this section few key steps for the three main sample categories:

- On coverslip - fixed cells
- On glass bottom culture dish – live or fixed cells
- Tissue slides

3.4.1 On coverslip - fixed cells

General preparation procedure (Room temperature):

1. Place sterile coverslips in a 35mm diameter dish.
2. Coat the petri dish and the cover slips with appropriate coating buffer if needed.
3. Seed about 100‘000 cells of interest, add the appropriate culture medium.
4. Culture cells until they reach the equivalent of monolayer confluency.
5. Wash the cells 3 times with 2ml of pre-warmed PBS.
6. Fix the cells with 2ml 1%PFA and incubate for 15 minutes at room temperature.
7. Wash the cells 3 times with 2ml PBS.
8. Take out the coverslip and place it on a microscope slide.
9. Put the imaging spacer on the coverslip, add a drop of mounting medium on top to fill the imaging spacer, close the sample with a second coverslip.
10. Store samples at 4°C.

Suggested mounting medium:

- PBS
- Ibidi 50001 [1.447 RI]
- Other specific RI clear optical mounting (e.g. glycerol/PBS)

Sealing method:

The medium chamber is sealed to avoid liquid drying or leakage. There are two ways of possible sealing:

- Recommended: tape imaging spacer (SS1X9-SecureSeal Imaging Spacer, Grace Biolabs, Product #654002)
- Nail polish (as second choice since it frequently overflows on the coverslip).

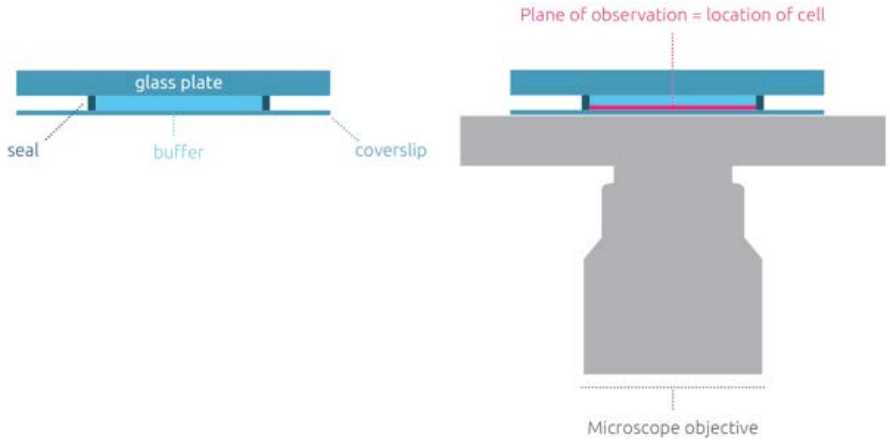


Figure 3. Schematic of observation of cells with the 3D Cell Explorer located between glass plate and coverslips

3.4.2 On glass bottom culture dish – Live or fixed cells

General preparation procedure:

for **fixed cells**

1. Coat the glass bottom culture dish with the appropriate coating buffer if needed.
2. Seed about 10’000 cells of interest, add the appropriate culture medium.
3. Culture cells until they reach the equivalent of monolayer confluency.
4. Wash cells 3 times with 2ml of pre-warmed PBS.
5. Fix cells with 2ml 1%PFA and incubate for 15 minutes at room temperature; then wash 3 times with 2ml of PBS.
6. Cover the cells with 2ml of PBS.
7. Store samples at 4°C.

for **living cells**

1. Coat the glass bottom culture dish with the appropriate coating buffer if needed.
2. Seed about 10’000 cells of interest, add the appropriate culture medium.
3. Culture cells until they reach the desired confluency.
4. Wash the cells 3 times with 2ml of pre-warmed PBS.
5. Cover the cells with 2ml pre-warmed transparent live cell imaging solution.

Suggested mounting medium:

- PBS
- Other specific RI clear optical mounting (e.g. glycerol/PBS)

The cells can be directly observed in a dish designed for observation after lid removal (unless the lid of the dish is in glass). See **Figure 4**.

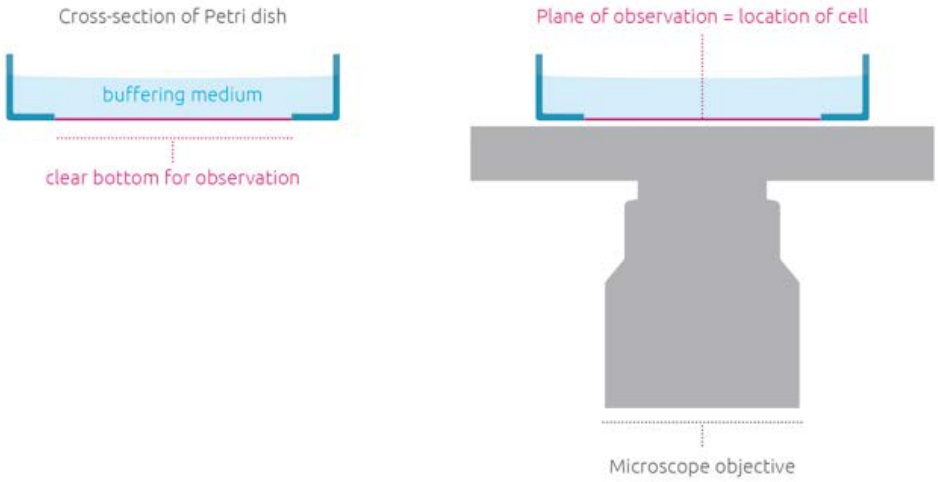


Figure 4. Schematic of observation of cells with the 3D Cell Explorer located in dish with glass bottom surface.

3.4.3 Tissue slides

The 3D Cell Explorer is also suitable for imaging slices of tissues, respecting the following conditions:

- The maximal tissue thickness should be less than the one recommended for the cells maximal thickness of sample structure (see Specification table 1); the best results could be obtained with very thin tissue sections (few micrometers).
- Since OCT/paraffin are not fully transparent and they could interfere with the acquisitions, it is necessary to remove the OCT or paraffin from the tissue sections before mounting the slide.
- Make sure that the mounting medium solution is an “aqueous mounting medium” or similar, the RI value of that should be in the range 1.33 - 1.44! This value is generally reported on the product data sheet.

We recommend to use the following mounting media solutions:

- Fluoromount-G (0100-01 SouthernBiotech) [1.40 RI]
- Ibidi 50001 [1.447 RI]

3.5 Cleanness of sample

The cleanness of the sample is crucial to insure good quality of the image.
Due to the rotating illumination system, debris (either floating or out-of-focus) can be hit by the beam. Therefore, optical surfaces must be as clean as possible and cell holders should be carefully cleaned so that dead cells or any kind of remains are not floating in the mounting medium.

Depending on the support used for the experiment, there are two procedures to follow before starting the acquisition with the 3D Cell Explorer:

- Coverslip: lens tissues (like Thorlabs™, #MC-50E) are recommended to clean the surface of the coverslip in contact with the objective. Lens tissues are ideal for removing all dust or fingerprints from the coverslip without leaving any trace of lint or fibers. To clean the coverslip, wet the tissue with few drops of Ethanol and gently rub the surface.
- Glass bottom culture dish: to remove any dead cells or cell debris wash the cells 3 times with PBS and then add the appropriate mounting medium; using the same procedure described above for the coverslip, clean the external surface of the glass bottom dish which should be in contact with the objective.

Due to the field of view of the microscope and the depth of field (for exact specifications, please check the technical specification sheet in the user's manual or on our website (http://nanolive.ch/hardware/) dimensions of the cell should be inferior to these values to be fully reconstructed.

4. Accessories

4.1. Top-stage incubator

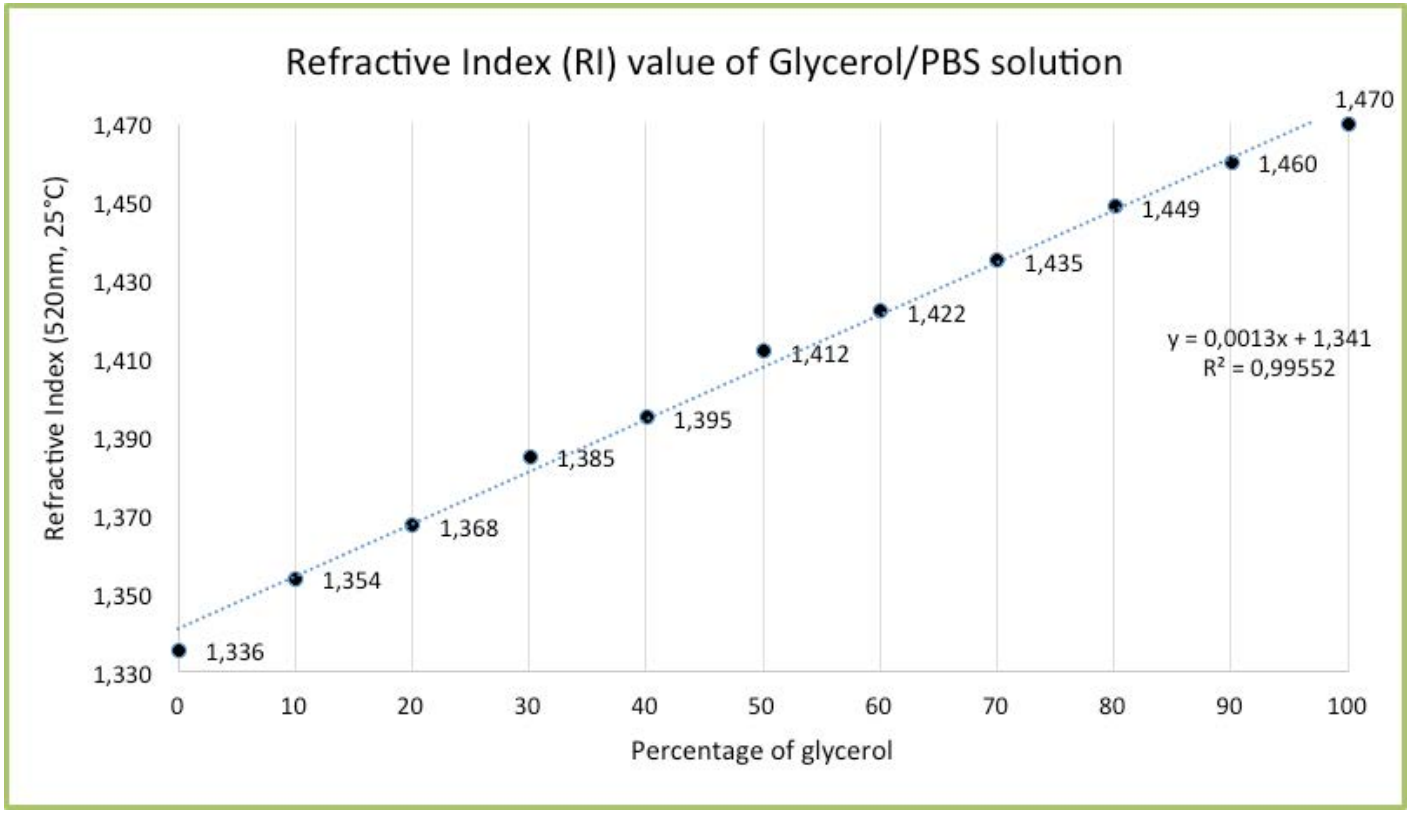
The 3D Cell Explorer, in combination with a top stage incubator, allows for non-invasive 4D long-term live cell imaging for several days in physiological environmental conditions.

The Nanolive-ibidi top-stage incubator brings all the required incubation controls directly on the stage of your 3D Cell Explorer: temperature, humidity and CO_2 can be meticulously regulated during your time-lapse cell experiments to give you the most accurate living cell results.

The Nanolive-ibidi top-stage incubator is our recommended model and it is already available on our web store. Please visit our website (http://nanolive.ch/accessories/) to get a quote and find the technical specifications.

If you want to learn more about ibidi and their product portfolio, please visit www.ibidi.com.

5. Appendix



Graph 1. Refractive Index (RI) value of Glycerol/PBS solution

Glycerol reference: G5516 – Sigma Aldrich
Instrument: AR4 Series Abbe Refractometer (KRUSS OPTRONIC)