



Sample Preparation 3D Cell Explorer

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1. Important points to keep in mind for the sample preparation

1. Cells need to be seeded on a glass surface as thick as a coverslip – we suggest either a Fluorodish (glass bottom culture dish) or in a coverslip on a glass slide.
2. The instrument is suitable for any kind of mammalian or bacterial cells. Thin slices of tissues can also be observed.
3. There are no limitations in the cell confluency. Cells grown in monolayer can be observed unless it exceeds the maximum thickness of cell structure (see specifications 1).
4. Optically clear medium (i.e. transparent live cell imaging solution, PBS) are preferred. Please be aware of the maximum mounting medium depth (see specifications 1). Slightly scattering solutions (like red phenol) can be used.
5. The optical surfaces must 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. Sample preparation specifications and data sheet

Conditions	Specification Cell Explorer (mid 2015)	Comments
Coverslip thickness	170 +/- 20um	
Glass bottom culture dishes	35 mm	
Size mammalian / bacterial cells	between ~1 and 25 μ m	
Max. thickness of cell structure	30 μ m	
Max. mounting medium depth	5 mm	Depending on optical properties
Max. depth when using scattering medium	2 mm	List of tested media: <ul style="list-style-type: none">• red phenol• AGAR

3. Sample Preparation Protocol

The 3D Cell Explorer allows measuring the inside a living cell offering the researcher the possibility to acquire high resolution 3D images of a cell within seconds:

- cells fixed on glass coverslips or FluoroDish™
- cells grown on FluoroDish™

Thanks to extremely low light exposure and compatibility with cell culture accessories (see section 2.1), the 3D Cell Explorer is suitable for long-term live cell imaging. We have designed this protocol to guide researchers through the sample preparation methods for observation.

NB: All protocols discussed in this guide serve as a starting point to establish and perfect your own protocols.

3.1 Accessories

Coverslips and recommended dishes

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 cleanness of samples is crucial for optimal imaging results.

The observation can be made either through glass bottom culture dishes (FluoroDishes™ WPI, #FD35-100) or coverslips (best standard & practice for microscope objectives).

Should you wish to use any other kind of sample conditions (e.g. multi-well plates, lids, etc.), please contact Nanolive's client service for more information.

Top-stage incubators

The 3D Cell Explorer is suitable for long-term live cell imaging with top-stage incubators (recommended models will be available on our web store soon).

3.2 Preparation Protocols

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 specially 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 shows the way cells are to be observed in a dish plate sample.



Figure 1: Amount of mounting mediums in FluoroDishes™

Confluency

No limitations in the cell confluency are given. Cells grown in monolayer can be observed unless the maximum thickness of cell structure is exceeded. Thin slices of tissues can also be observed.

3.2 Preparation Protocols

On coverslip - fixed cells

Figure 2 shows the way the glass plate sample is observed in the 3D Cell Explorer.

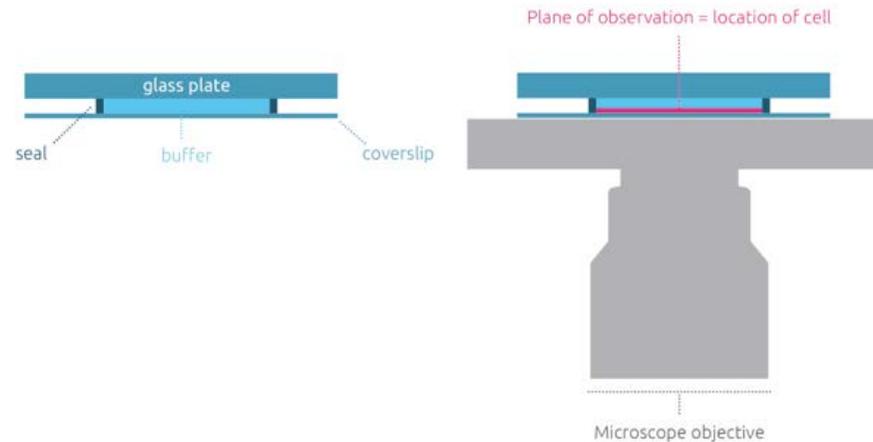


Figure 2. Schematic of observation of cells with the 3D Cell Explorer located between glass plate and coverslips

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

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

Reagents to be used:

- Coating buffer
- Culture medium
- PBS
- 1% Paraformaldehyde (PFA) in PBS

Procedure:

Please follow these steps at room temperature.

1. Place 4 sterile coverslips in a 10 cm 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 the coverslip out and place it on a microscope slide. Put the imaging spacer on the coverslip, add a drop of PBS on top to fill the imaging spacer, close the sample with a second coverslip.

Store samples at 4°C.

3.2 Preparation Protocols

On FluoroDish™ – Live or fixed cells

For living cells experiment, the cells can be directly observed in a dish designed for observation after lid removal (unless the lid of the dish is in glass) (FluoroDishes™ WPI, #FD35-100). See Figure 3.

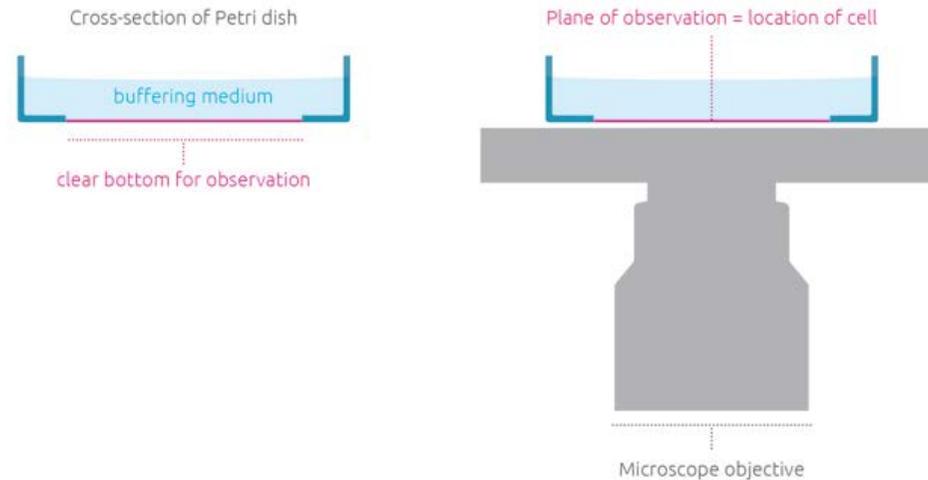


Figure 3. Schematic of observation of cells with the 3D Cell Explorer located in dish with clear bottom surface.

Reagents to be used:

- Coating buffer
- Culture medium
- PBS
- 1% Paraformaldehyde (PFA) in PBS

Procedure:

for **fixed cells**

1. Coat the FluoroDish 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.

Store samples at 4°C.

for **live cells**

1. Coat the FluoroDish™ 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 imaging buffer.

3.2 Cleanness of sample

The cleanness of the sample is crucial to insure good quality of the images.

Due to our rotating illumination system, debris (either floating or out-of-focus) can be hit by the beam while not being in the field of view. 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.
- FluoroDish™: firstly, to remove any dead cells or cell debris wash the cells 3 times with PBS and then add the appropriate mounting medium; secondly, using the same procedure described above for the coverslip, clean the external surface of the glass bottom dish which has to 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 guide manual or on our web site <http://nanolive.ch/hardware/>) dimensions of the cell should be inferior to these values to be fully reconstructed.