NANOLIVE
Looking inside life

Preparation Guide

Short sample preparation guide for the CX-A

NANOLIVE'S 96-WELL PLATE FORMAT

A. Multi well imaging with the CX-A requires Nanolive custom-made labware. Full specifications and information about Nanolive's 96-well plate is available here. In this format, a maximum of 60 wells in the centre of the plate can be used for imaging (see Image 1).

B. Nanolive's 96-well plate has a glass bottom. It is highly recommended to coat the well plates with fibronectin prior to seeding your cells.

C. Ideal cell confluency is between 50-70%. It is possible to image samples with higher or lower confluency, if the cells are located in the middle of the dish, please see Recommendation 1.1.

D. Optically transparent mounting media possessing a refractive index that is close to the values in living material (RI~1.35) (i.e. DMSO) are preferable. Medium that scatters slightly (e.g. culture medium with red phenol) can also be used.

E. The crucial step in sample preparation for the 96-well plate format is the well medium volume. The volume in each well must be between 75 µl and 90 µl and ***important*** each well must have exactly the same volume.

F. The cleanliness of the sample is crucial to ensure optimal quality of the image. It is recommended to pre-wash the cell medium with pre-heated medium 1h prior to imaging.

G. The CX-A device must be thermalized before live cell imaging. Switch it on for a minimum of 1h before image acquisition. The 96 well plate must be thermalized for an additional 10 mins prior to the image acquisition.

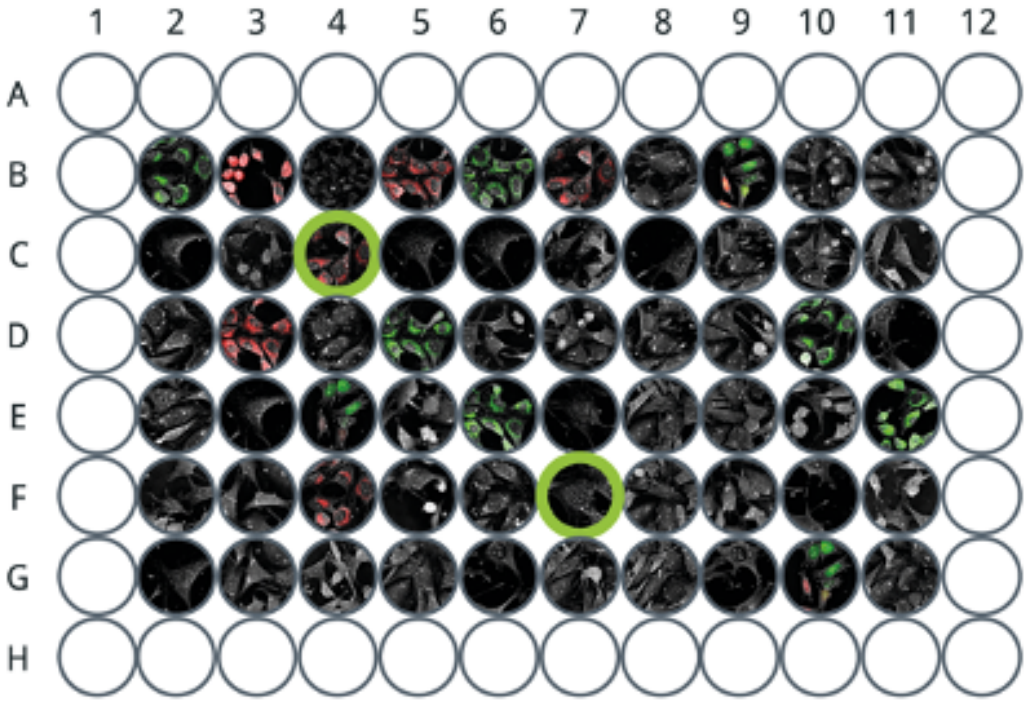

Image 1. Nanolive 96-well plate format

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4-DISH WELL PLATE FORMAT

A. When the 4-dish well plate is used, 4 low border ibidi dishes (available here) need to be placed in each well plate position. Imaging cannot be performed without all 4 dishes in place.

B. Cells need to be seeded on the low border ibidi dishes. Please note, that imaging with high border dishes is possible using the CX-A but must be completed without lids.

C. Ideal cell confluency is between 50-70%. It is possible to image samples with higher or lower confluency, if the cells are located in the middle of the dish. If the user has a limited number of cells available, please see Recommendation 1.1.

D. Optically transparent mounting media possessing a refractive index that is close to the values in living material (RI~1.35) (i.e. DMSO) are preferable. Medium that scatters slightly (e.g. culture medium with red phenol) can also be used. Please be aware of the minimum and maximum medium volume, please see Recommendation 1.2.

E. In some conditions, it is recommended to use DIC lid. Please see Recommendation 1.3.

F. The cleanliness of the sample is crucial to ensure optimal quality of the image. It is recommended to pre-wash the cell medium with pre-heated medium 1h prior to imaging.

G. The CX-A device must be thermalized before live cell imaging. Switch it on for a minimum of 1h before image acquisition. During the devices' thermalization, dish holder holes need to be closed. Once the device is thermalized, sample dishes can be placed and require an additional 10 min thermalization period within the closed chamber.

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TIPS & TRICKS

Recommendation 1.1.

To ensure cells are located in the centre of the dish, especially if the number of available cells is low, users should seed the cells in a drop of medium in the middle of the dish and leave in the incubator for 30 min to 1 h. They should then add more medium and continue the incubation until the desired cell adhesion is obtained.

Recommendation 1.2.

Labware specifications	Minimal volume (ml)	Maximal volume (ml)	Comments
Low border 35mm dishes	1	2	Short term (≤4h) imaging requires minimum volume. Long term imaging (>4h) requires maximum volume

Recommendation 1.3.

Differential Interference Contrast (DIC) lids (see here) can be used: A) to assure sample sterility and B) for very long periods of imaging (>18h) to ensure evaporation of the medium is minimal.

FAQ

Q1. I need to image only two of the four dishes. What should I do with the other 2 dishes?
Answer: We recommend adding PBS or water into the other two unused dishes.

Q2. What kind of sample can I image?
Answer: Please read the Sample preparation manual for the 3D Cell Explorer here.

Q3. The calibration failed. What can I do now?
Answer: Please read Recommendation 1.1. Calibration failure can occur if the density cells of cells in the centre of the dish is too low, or because a non-suitable sample is detected (e.g. dirty sample or a sample has a high number of dead cells floating).

Q4. Can I use coating?
Answer: Yes. Thin and transparent coatings e.g. fibronectin are suitable.

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