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# Yeast Sample Preparation for Fluorescence Live Cell Imaging V.1

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**Protocol status:** Working
**We use this protocol and it's working**

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## Abstract

This protocol describes the preparation of yeast cells for fluorescence single molecule tracking live cell imaging. This protocol utilizes linked protocols for specifics on media preparation. Special attention is given to creating single layer yeast suspensions on the cover glass.

## Materials

**Concanavalin A (ConA) from Canavalia ensiformis:**
Sigma-Aldrich
Cat#: C2272-10MG
Lot#: 129H0322

**UltraPure Distilled Water**

**Cover slides ⌀ 25mm, No. 1.5 or 1.5H**

**Attofluor Cell Chamber**
Thermo Fisher Scientific
Cat#: A7816

**Equipment:**

10 ml centrifugation tube
10 ml pipette

Spectrometer: Molecular Devices SpectraMax m5
Plasma cleaner: Quorum Emitech K100X glow discharger
mixer
bench top centrifuge

## Stock sample preparation

2d 12h

1 Plate the yeast strain of interest onto pre-imaging solid agar plate.

Pre-Imaging Solid Growth Medium - Yeast protocol:
https://dx.doi.org/10.17504/protocols.io.8epv5r9wjg1b/v1

1.1 Incubate at 25 °C until colonies are visible. (~2-3 days 60:00:00 ). 2d 12h

Note

Temperature and duration depending on the specific yeast strain.

1.2 The agar plate with grown yeast can be stored, sealed in 4 °C fridge, and can be used as stock for 2-3 months.

## Preparation for imaging the next day

12h

2 Pick a colony from your stock sample and dilute it in 5 mL liquid pre-imaging medium in a test tube.

Note

Choose the pre-imaging medium according to your labeling system. If it includes labeled pp7-stem loop (pp7-SL), its expression can be suppressed through higher Methionine concentrations in the medium [2]. A recipe for such a medium provides the protocol below that note.
For otherwise labeled yeast it is recommended to use media with higher concentrations of Adenine to reduce autofluorescence [1]. You can follow the "Pre-Imaging Liquid Growth Medium":
https://dx.doi.org/10.17504/protocols.io.81wgbzxe1gpk/v1

Pre-Imaging Liquid Growth Medium for Methionine promoted Labeling Systems - Yeast protocol:
https://dx.doi.org/10.17504/protocols.io.kqdg329yqv25/v1

2.1 Incubate at 200 rpm, 25°C Overnight . 12h

Note

The incubation temperature depends on the yeast strain!

## Yeast conditioning

4h 5m

3 Dilute the cultured yeast to $OD_{600}$ = 0.25 - 0.3 and incubate 200 rpm, 25°C, 03:00:00 - 04:00:00 . Let the sample grow to an $OD_{600}$ of 0.8 -1.0 to establish exponential growth phase.

4h

## Sample preparation

4h 5m

4 Pipette 1.5 mL of the incubated yeast into an Eppendorf-tube.

4.1 Centrifuge at 560 x g, 23°C, 00:05:00 60

5m

4.2 Remove supernatant and re-dilute the pellet in 750 µL pre-imaging medium.

4.3 Plate the re-diluted cells into a cell chamber with a ConA prepared cover slide.

Protocol for the ConA cover slide preparation:
https://dx.doi.org/10.17504/protocols.io.kxygxy3rzl8j/v1

4.4 Cover the cell chamber with Kin wipe and let the cells settle in an incubator at 25 °C for 00:10:00 .

10m

Note

Incubator temperature depends on the Yeast strain and can differ in respect to the experiment.

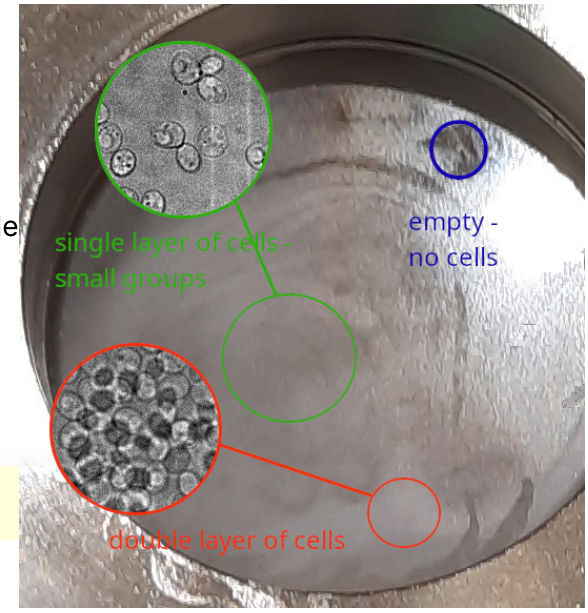
## Sample

10m

5 Remove the supernatant with a 1 ml pipette.

5.1 Tilt the chamber slightly and add 1 mL of medium from the top corner. Remove the medium at the bottom corner to remove loose cells.

5.2 Repeat the last step to remove the second layer of cells. Continue washing the sample as described, until you expect a single layer of cells in the central area of microscope slide.




Note

The pressure to remove the cells from the cover slide respectively the second layer of cells differs between yeast strains and the age (~use cycles) of the ConA.

Note

The removal of the second layer of cells reduces background fluorescence and increases the probability to have the nuclei in the same plan.
Space around the cell groups allows the yeast to grow in xy direction and less into z direction creating a new second layer, which allows for longer observation duration.

6 Cautiously add 1 mL refractive index adjusted imaging medium (RI=1.41).

Refractive index adjusted imaging medium: Iodixanol (RI ~ 1.41 and 1.42) - Yeast:

https://dx.doi.org/10.17504/protocols.io.5qpvok369l4o/v1

Note

The ideal refractive index for the imaging medium depends on your experimental setup, especially on the immersion medium.
The RI of the medium can be adjusted by varying the RI gradient substance (here Iodixanol). Examples:

- RI = 1.4 https://dx.doi.org/10.17504/protocols.io.dm6gpz3kjlzp/v1
- Ri > 1.42 https://dx.doi.org/10.17504/protocols.io.3byl4933ogo5/v1

7 Place the the chamber in the stage incubator. Wait until the temperature reached equilibrium before you start imaging.

## Protocol references

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[2] Lari, Azra, et al. "Live-Cell Imaging of mRNP–NPC Interactions in Budding Yeast." Imaging Gene Expression: Methods and Protocols (2019): 131-150.
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