DAPI staining of S. pombe cells

Fixation

Glutaraldehyde fixation (beautiful way)

Nucleus, cell wall, and septum can be detected

Culture cells in YES until OD600=0.5~1.0, @30˚C or appropriate temperature, 25ml
Get 900 ul of culture in a microfuge tube.
Add 100ul of glutaraldehyde.
Immediately vortex, for 30sec.
Place the tube on ice for 30min.
Wash cells in ice-cold 1xPBS three times
(Centrifuge, discard sup, add 1ml of 1xPBS to the pellet, vortex)
Suspend cells in 100 ul of 1 x PBS, keep tubes on ice
** Cells can be stored @4˚C (take pictures within 24hr, but the sooner the better)
Centrifuge briefly to collect cells at the bottom of tubes, keep tubes on ice
Mix 1ul of cells (from the bottom) and 1ul of DAPI (50ug/ml) on a slide glass.
** in the microscope room.
Place a cover slip (22x22) over the cells

Ethanol Fixation (dirty way)

Nucleus can be detected

10ml of cell culture in 15ml conical tube
Centrifuge, 2400rpm, 4˚C, 3min
Discard sup
Add 70% Ethanol to the cell pellet
Vortex well, incubate cells @RT for 10min
**Cells can be stored @-20˚C
Wash cells in ice-cold 1xPBS three times
(Centrifuge, discard sup, add 1ml of 1xPBS to the pellet, vortex)
Suspend cells in 100ul of 1xPBS, keep tubes on ice
** Cells can be stored @4˚C (take pictures within 24hr, but the sooner the better)
Centrifuge briefly to collect cells at the bottom of tubes, keep tubes on ice
Mix 1ul of cells (from the bottom) and 1ul of DAPI (50ug/ml) on a slide glass.
** in the microscope room.
Place a cover slip (22x22) over the cells

No Fixation (the most beautiful way)

Nucleus, cell wall, and septum can be detected

1.5ml of cell culture in a microfuge tube
Wash cells in ice-cold 1xPBS **once**
(Centrifuge, discard sup, add 1ml of 1xPBS to the pellet, vortex)
Suspend cells in 100ul of 1xPBS

Centrifuge briefly to collect cells at the bottom of tubes, keep tubes on ice

*** **Cells cannot be stored. Take all pictures within 30min.**

Mix 1ul of cells (from the bottom) and 1ul of DAPI (50ug/ml) on a slide glass.

** in the microscope room.

Place a cover slip (22x22) over the cells