Observing live cells by fluorescence microscopy

1. Grow cells overnight in YES. Cells must be in log phase (OD600=0.5 max) before processing so if too dense dilute back to OD600=0.1 and allow to grow for 3-5 hours.
2. Concentrate cells by centrifuguation (spin 5-10mls)
3. Resuspend in 1 ml of YES containing 0.1mM N-propyl gallate (to reduce phototoxicity).
4. Spin down and remove most YES.
5. Apply 5 ul cells to slide, add 5 ul 1.2 % LMT agarose in YES/0.1mM N-propyl gallate & mix immediately (melt agarose at 65°C then cool to 37°C before using).
6. Push cover slip on top (firmly)
7. Allow to set before observing. If necessary can use VALAP to seal edges of cover slip.

YES mountant
0.12 g LMT agarose
10 ml YES
0.01 ml 100mM Npropyl gallate (dissolved in ethanol)

Dissolve agarose in microwave. Store at 4°C in dark. Before use melt at 65°C and cool to 37°C before use.

Alternative mountant – use YES/10% gelatin/0.1 mM N-propyl gallate

DNA staining
For observing location of nucleus: do steps 1, 2. Then wash cells x2 in deionized water. Resuspend in water containing 1 µg/ml Hoechst 33342 and leave at rt in dark for about 30 mins (best time may be strain & growth medium dependent – EMM grown cells seem to strain faster). Then spin down and proceed as above.

Hoechst stain H33342. Stock is 1 mg/ml in water. Keep at -20°C. Wear gloves when using!

VALAP
VALAP is wax-based sealant to quickly and easily seal aqueously mounted specimen – it is non toxic.

- Vaseline (Fluka #94830)
- Lanolin (Fluka #61440)
- Paraffin, 56° C melting point for histology (Fluka #76242)

mix 1:1:1 in a small beaker on heated plate at low temp. to prevent evaporation. Use a fine paintbrush to seal slides. VALAP does not crack at extreme temparatures and allows gaseous exchange. You can use the slides immediately. There is no need for drying as with the use of nail polish or DEPEX. The Valap mix in beaker can be reused any time you reheat it.