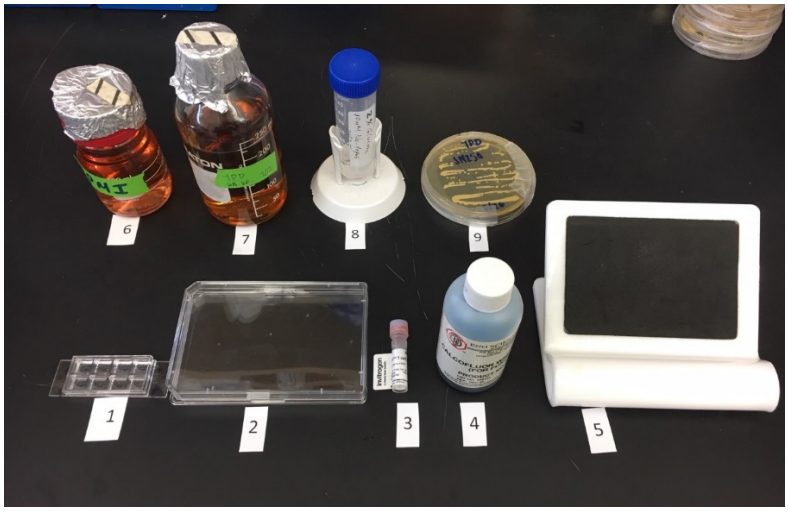
Candida albicans biofilm Fun-1 and CalcoFluor White Fluorescence staining (Hiram Sanchez Andes lab)

Materials (Catalog numbers from Fisher Scientific) See figure 1

1- IBIDI wells Cat. # 80826
2- One well plate. Cat. # 670180
3- Fun-1 Cat. # F7030
4- CalcoFluor white stain (CW) Cat. # ES6726
5- Plate stand at 30 degree angle Cat. # 50-589-590
6- 100 ml of RPMI media Cat. # 1640
7- 100ml of YPD broth (1% yeast extract, 2% peptone, 2 % Dextrose) sterilized by autoclaving.
8- Glucose Buffer: 50 ml of a 2% glucose solution containing 10mM Na-HEPES pH 7.5 (Filter sterilized)
9- Candida albicans plate.

Figure 1



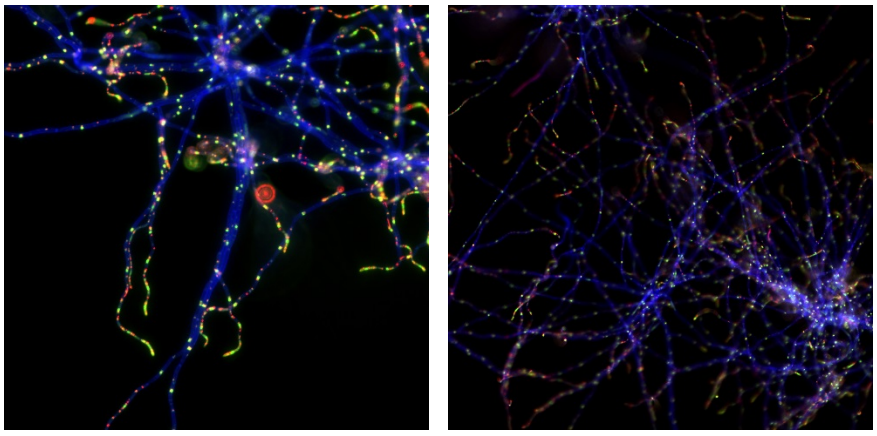
Method

1- Day 1- Inoculate 5 ml of YPD broth with a colony of Candida albicans and incubate overnight at 30C° with gentle shaking.
2- Day 2- Using the overnight culture prepare 2 ml of RPMI containing 1 X 10^6 cells/ml. (for cell counting using hemocytometer see addendum).
3- Place a folded tissue paper into a One well plate, add 5 ml of water to make the tissue wet, decant the excess water. Place the One well plate on the plate stand.
4- Remove the cover of an IBIDI plate and place it into the One well plate.
5- Add 150 µl of the 1 X 10^6 cells/ml from step 2 above into each well of the IBIDI plate, place back the cover of both plates and incubate at 37C° overnight.
6- Day-3 Prepare 2 ml of staining solution; 1:30 dilution of CW in Glucose buffer, 0.62 µl of 10mm FUN-1 /ml, mix and keep in the dark until ready to use.

7- Remove IBIDI plate from incubator, gently remove media and rinse with 150 µl of glucose buffer. Add 150 µl of the CW and Fun-1 staining solution (step 6 above) and incubate in the dark for 45 min. at 37C°, then remove the staining solution and rinse 4 X with 150 µl of glucose buffer, do not remove the last rinse.
8- View IBIDI slide under the fluorescence microscope, using DAPI, FITC & Red filters.
9- If using the 30X fluorescence microscope objective, take photos at these settings; the Bright field at 10mSec, DAPI at 40mSec, FITC at 100mSec and Texas Red at 100mSec. Fun-1 will fluoresce in both FITC & Texas Red channels.
Adjust LUTs (Look Up Table) settings within the central tool bar, LUTs is a useful tool for image color and brightness modifications.
You can use LUTs to enhance images for observation purposes so the color modifications will be non-destructive to the image data. LUTs settings are saved along with the image file. After adjusting the LUTs for each picture you can prepare a composite. See Figure 2.

10- The Fun-1 viability stains exploit the normal endogenous biochemical processing mechanisms that appear to be well conserved among different species of yeast and other fungi. The conversion of FUN-1 cell stains from a diffusedly distributed pool of green fluorescent intracellular stain to a compact form consisting of orange—red or yellow–orange fluorescent intravacuolar structures that requires both plasma membrane integrity and metabolic capability. Only metabolically active cells are marked clearly with fluorescence intravacuolar structures, while dead cells exhibit extremely bright diffuse, green-yellow fluorescence . [1]

Figure 2 60X 30X



Reference

1- Molecular Probes Inc. P.O. Bo22010, Eugene, OR 87402
Product information probes for yeast viability FUN-1. Product information # MP 07009 published 22-January-2001.

Addendum

Cell counting with hemocytometer

Prepare 5 ml of a 1:100 dilution of your overnight culture (50 µl of overnight culture into 4950 µl of RPMI Media) mix well.

1- Take 10 µl of the cell dilution and place it in the hematocytometer.
2- Count the number of cells in 4 large squares (you can also count the cells in 4 medium and 4 small size squares but all you need is 4 of the same size, you decide what to use) see Fig. 3
3- Average the number of cells in all of the four squares and multiply the average by 1000 µl.
4- Then divide that number by one of these three;
    a. If large squares were used divide by 0.00625 mm^3
    b. If medium squares divide by 0.004 mm^3
    c. If small squares divide by 0.00025 mm^3

For example:

Dilution was 1:100 and you counted the cells in four large squares, in each individual square you counted 15,19,18 & 17, when added and divided by 4, you end up with 69/4 = 17.25

17.25 is multiply by 1000 µl = 17,250. Since you counted the cells in the large square you need to divide the 17,250 by 0.00625. 17,250/0.00625 = 2760000 which is the same as 2.76 x 10^6 cell/ ml in your 1:100 dilution. The total number of cells in your original overnight culture is 2.76 x 10^6/ ml X 100 = 2.76 x 10^8 cells/ml.

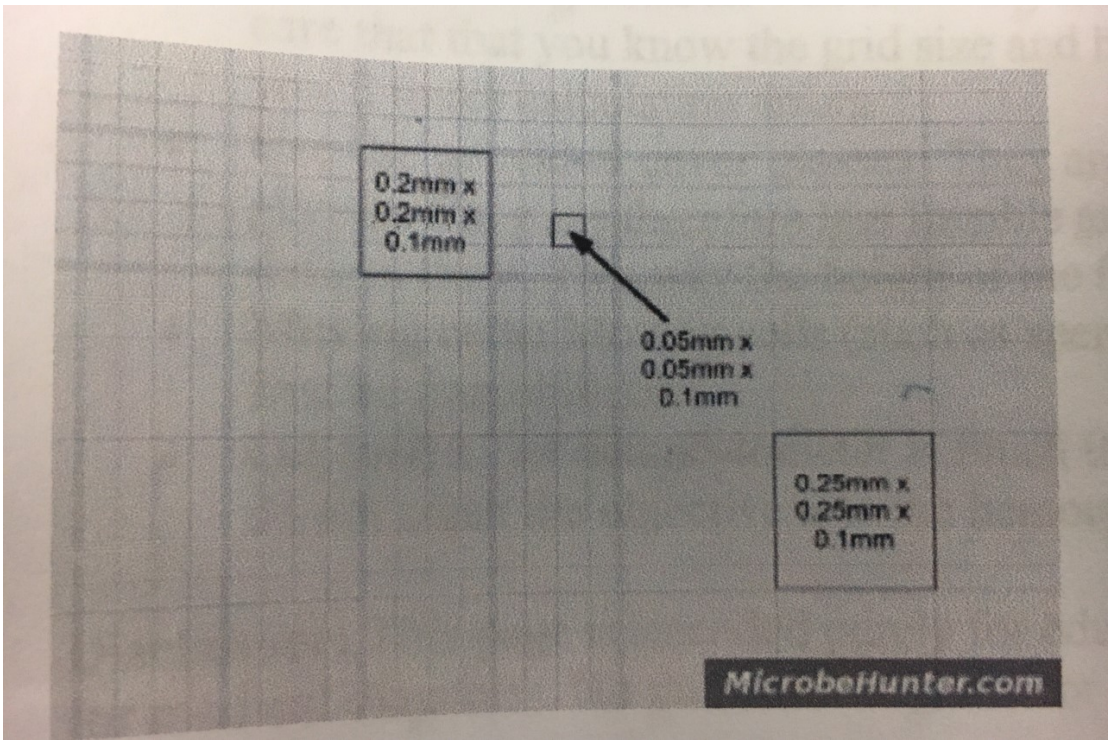
So for your experiment you need 1 X10^6 cell/ml, so follow this;

<u>(Number of cell you want) x (the volume in ml you need)</u>
Total number of cells you have in your (1:100 dilution)

= (1 X10^6) x (5 ml) / 2.76 x 10^6/ ml = 1.81 ml

Add 1.81 ml of your 1:100 dilution to 3.18 ml of media to make 5 ml at 1 X10^6 cell/ml.

Fig 3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MicrobeHunter.com