FDA

1. Get some FDA stock (0.5% FDA in acetone) in the Hamilton syringe. The stock is stored in the same carton as the AO/EB.

2. Suspend the cells at a normal counting concentration (about 0.5-2 x '106/m1).

3. Add FDA to cell suspension at about 1:50 using the Hamilton syringe.

4. Add a sample of this mixture to a hemocytometer slide.

5. On the American Optical microscope turn on the tungsten-halogen lamp to 9V and the normal white light to 4.5V.

6. Place the slide on the microscope stage and use the white light to find the counting grid and to focus. The white light is control1ed by the two small handles in the lamp housing.

7. Using the 20X objective, turn down the white light until both the grid and the cell fluorescence are visible. If no fluorescence is visible, check the hand1e above the microscope objective area; it should be in the "RIGHT" position and blue light should be coming from the objective.

8. You should see:

live cells	- green
dead cells	- unstained
erythrocytes	- unstained
non-nucleated cell culture blebs	- unstained or green depending on whether or not they have intact membranes

Count the types of-interest. (If more fluorescence brightness is needed, turn up the lamp to 12V. This should not be done unnecessarily since bulb life is about 10 times longer at 9V.) .

9. Turn off the tungsten-halogen lamp and white light unless someone wants to use the microscope immediately.

NOTE: As far as I know FDA is not hazardous, and cells stained with it can be run on the FACS without special precautions except extra flushing of the tubing, before switching to non-FDA samples.

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