

MAKING HOLEY FILMS

The following write—up is a step by step procedure for making holey films. A good description or explanation of holey film preparation can be found in the attached reprint.

A. Preparation of slides

1. Soak slides in chromic acid for at least 1 day (put them in the acid in the morning and then take them out the next morning). The slides are placed in a glass slide holder and the slide holder with the slides is placed in the chromic acid. Do not use a metal slide holder, Please keep hands off of slides. Always grab slides at one end and consistently handle slides at that end only. Or, wear gloves.
2. After soaking the slides in acid, rinse them thoroughly. Leave them in the holder and transfer the holder with the slides to a dish and then rinse by running distilled water over the slides.
3. After rinsing, soak slides (again still in holder) in 0.03% Amiet (water repellent). Soak for at least 1 day.
4. After the Amiet treatment, rinse the slides in distilled water as follows. Dip the slides in a dish of distilled water and take them out. Change the water and dip the slides again. Change the water again and then dip the slides but when you remove them this time pull the slide holder out slowly so that there is no trace of water on the slides except near the edges.
5. Dry the slides by air drying but keep them dust free or dry them in an oven at relatively low temperature. After drying the slides can be stored in a slide box.

B. Preparation of solutions

6. The night before making holey grid films, prepare water and ethyl acetate solution. You can make any amount but keep the ratio the same. Do not use the solution for more than a week or two. So do not prepare large quantities. The ratio is 8mls ethyl acetate for every 1ml water. I usually do the following: Take a small bottle (big enough to hold about 100mls and usable with organic solvents). Add 5mls of distilled water and then 40mls of ethyl acetate, shake and then allow solution to stand at room temperature overnight. The ethyl acetate will absorb some of the water. The next day the ethyl acetate and water will appear separated and a distinct phase boundary will be apparent.
7. Next day: Prepare Triafol' solution

- a. Triafol is trade name for cellulose acetate butyrate. Cellulose acetate butyrate is fibrous and difficult to weigh. Tweezers might be necessary to pull large chunks apart. The percentage of “Triafol” solution needed will depend on the size of the hole you want. See the reprint on making holey grids for the relation between percent solution and hole size. Reprint is attached to this procedure.
- b. The cellulose acetate butyrate will be dissolved in a solution consisting of equal amounts of ethyl acetate and hydrated ethyl acetate which is taken from the ethyl acetate layer sitting on top of the water layer. Remember the ethyl acetate and water solution you prepared the night before. The cellulose acetate butyrate is dissolved in the ethyl acetate first and then the hydrated ethyl acetate is added. I usually prepare a total volume of 40 ml (20 ml of ethyl acetate and 20 ml hydrated ethyl acetate). I would not use the solution for more than 2 or 3 days. So do not prepare large quantities. 40 ml is enough to fill a slide jar and still leaves a small area of one end of the slide free of ‘Triafol’.

After the solution is ready, store it in a slide jar with a cover and keep on ice. Solution should be on ice at least 30 to 45 minutes before making films. Do not forget to use a cover that is resistant to organic solvents. Usually, a glass Petri dish cover is suitable.

If you are planning to make more ‘Triafol’ solution, then you should replenish the ethyl acetate and water solution. Just add back what you took. If you are not going to make anymore ‘Triafol’ within the next week or so, then do not bother replenishing the solution.

C. Making holey films

8. Set-up for making the holey grid films:

The set-up is not standardized. We have tried several different ways but the set-up that seems to work for us is as follows:

- a. Find a small room that can be humidified without interruption. Humidify the room all morning (for at least 3 hours) before making the holey grid films.
 - b. Keep triafol’ solution(that you prepared and put on ice) in the room.
 - c. Take a small ice chest (styrofoam one will do) and put dry ice on bottom and cover dry ice with aluminum foil. The aluminum foil provides a clean surface on which to place slides.
9. Now you are ready to make holey grid films.

1. Take a clean, Amiet treated slide and place in ice chest. Lay it flat on the aluminum foil. Put cover on and count time. Do several slides, each at different times, usually within the range 15 s to 1 min. Once you find the time that gives the best results, keep all slides on dry ice for that time.
2. After slide is cooled, take out and wave in path of humidifier spray. Usually that part of the path farthest from the spray source is best. Wave both sides of slide. BE QUICK (a few seconds time). Sometimes, if the room is humid enough you can take a slide out of the ice chest, wait a few seconds (do not wave in path of spray) and then dip it into the 'Triafol'
3. Immediately after waving slide, quickly dip it into the 'triafol' solution. When the slide has gone as far as it can into the solution, quickly pull it out and allow to dry by propping it up on ice (you can use the ice surrounding your solution). Let slide lean against ice bucket with one end touching ice. Sometimes letting the slide dry without propping it up on ice works just as well. Try either way. Remember, you can use both sides of slide, so do not lay the slide down on one side. Always prop it up to dry.
4. Check slides on phase-contrast microscope. Holes should be Continuous across with no large empty areas between holes. Also note size. For size information see reprint about holey films. There is a problem with pseudo-holes forming. I am never sure if I have pseudo-holes but you will find out after you examine the holey grids in the electron microscope. Store good slides in slide box until ready to make holey grids. Prepare a large quantity of films and store them. The films are used up quickly.

But I have noticed (although I do not know it for sure) that the longer the films are stored, the more difficult it is to remove them from the slide.

Also note that ethyl acetate vapors are harmful. Avoid breathing too much. When you are finished for the day, store the "Triafol" solution in the bottle with a cap suitable for organic solvents. Keep at room temperature.

D. Preparing Holey Grids

Floating films off the slide

1. Prepare 0.5% Pelex solution. You can prepare 500mls and store it in the refrigerator indefinitely.
2. Fill a slide jar with the 0.5% Pelex and place 2 or 3 slides in the solution. Let slides soak in Pelex for about 3 hours. Make sure slides are not sticking to each other. Keep them separate.

3. After 3 hours take a slide out, let it air dry (only takes a few minutes). Examine slide on phase microscope to make sure entire slide is good. Sometimes only parts of the slide are usable. Take a razor blade or scalpel and cut around the areas you want. If you want the entire slide just cut along the edges of the slide (a few millimeters in on each side and at the bottom).
4. Fill a medium size crystallizing dish with water. The dish should be completely full of water such that the water forms a dome over the dish.
5. Holding the slide at an angle of 45° slowly dip it into the water. The film should float off the slide and onto the water. If it does not, put the slide back into the Pelex and soak the slide overnight. Sometimes I soak the slides 2 or 3 days before the film will come off. But if you soak the slide long enough, the film will come off in the Pelex.
6. If the film floats off, let it float on the water for about 15 minutes and then place grids on the film. Lay grids with shiny side down. After film is covered with grids take a Scintillating vial (a vial that is clean on the outside. No fingerprints) and gently press the vial to one end of the film and gently roll the vial across the film. The film is wrapped around the vial. Set the vial aside to dry overnight. Be sure the vials are kept dust free, Wrapping the film around the vial is a difficult step and if one is not careful the film with grids could end up in a worthless clump.
7. After drying overnight, pick grids off the vial with tweezers. They come off easily. No force is necessary. Put them on a clean glass slide with film side up and carbon coat them (a thick carbon layer)
8. After carbon coating, you should try to remove the plastic if you want to. The procedure used in our laboratory is not 100% effective in removing all the plastic. You may want to devise your own method. But whatever, our procedure involves ascending paper chromatography and is as follows:

Put a single layer of test tubes on the bottom of a medium size crystallizing dish. Take a piece of #1 Whatman filter paper and cut it into a rectangle. Form a small flap at one end of the longer side. Make the flap such that the paper lays flat on the test tubes and the flap touches the bottom of the dish. Prop the dish at a small angle to the benchtop. Place your grids (carbon side up) on the filter paper. Position the grids near the middle of the paper and in a single row across the paper. Put about 2ml of ethylene dichloride on the bottom of the dish where the flap is touching. Place a glass plate over the dish with just a very small area of the dish at the upper edge uncovered. The ethylene dichloride will be taken up by the paper and as it passes under the grids. It will dissolve the plastic and pull it along as it moves up the paper. After the ethylene dichloride has evaporated add 2ml more. Do this 3 or 4

times. Be sure to do this under the hood. Ethylene dichloride is a very strong organic solvent. Avoid breathing or skin contact.

After you are finished and paper is dry, remove the grids; place them on a clean glass slide with the carbon side down. Carbon coat the other side and then you can use them.

Information about the compounds used in this procedure can be found in the attached reprint. The journal from which the reference was copied is listed on the last page of the reprint.