Removing Halftone Patterns From Scanner Images

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ated with edges of the printed dots). In this case the mid-point of the cutoff was set to the spacing of the half-tone dots in the original image.

Figure 6 shows the final version of the power spectrum, with the periodic spots removed and the high frequencies attenuated but not eliminated. It is generally a useful learning tool to examine the power spectrum as you apply various masks and filters. Modifying the image in Fourier space makes it quite easy to remove periodic noise (or in some cases to keep periodic information and remove random noise) and to tailor the frequency response of the resulting image in ways that are difficult or impossible when working with the original pixel array.

Figure 7 shows the result of applying both filters. Now in addition to the removal of the periodic noise, the random pixel variations are also reduced, the image sharpness and contrast have been restored, and all of the details that are present in the image can be clearly seen. Note that even the cat's whiskers which are barely discernible in the original image can be clearly seen. The use of a smoothing or blur function would have erased these fine lines long before the halftone dots were smoothed together.

For a color image, in which the various halftone patterns for each ink have different orientations, the procedure would be to separate the image into color planes or channels corresponding to each color (CMYK for printed images, RGB for scanner or camera artifacts). Each image is then treated separately using the same operations as above, after which the processed color planes are recombined to produce an enhanced color image. ■

Microwave Immunohistochemistry on Mohs Cryostat Sections

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With the advent of Mohs surgery in the dermatology clinics, making sure the margins are clear of any cancer is of the utmost importance to both patient and dermatopathologist. While the patient is still in the surgery suite, immunohistochemstry staining can be done in the laboratory microwave (Pelco microwave with water load cooler, wattage controller and temperature probe). The immunohistochemistry procedure takes only 25-30 minutes as compared to the more conventional methods that take 2 hours or more.

The microwave needs to be checked for hot spots using the Pelco #36140 microwave bulb array. Make sure the area in which you are going to put your slides has no hot spots, indicated by no illuminating bulbs. Using a Sigma Diagnostics #H6644 Humid Chamber, place a paper towel in the bottom of the chamber and fill with 1/4 inch of water. This is in addition to the water loads used to eliminate hot spots. Cut a very small hole into the top of the humid chamber—just large enough for the probe to fit into—at the exact point that the probe will be immersed in the reagent that is covering the tissue section. The temperature probe will measure the reagent temperature on the slide, maintaining a constant temperature of 40°C. You can run up to six slides at once in a single humid chamber.

Vaporization is very important to this procedure. The vapor is contained in the humid chamber and enhances the reaction. Reagents used are the Vector Universal Elite Kit, Vector DAB kit

and the DAKO HMB45 primary antibody for Melanoma.

Microwave Procedure for Immunohistochemistry

1. Cut frozen section. Air-dry for 1 minute. Fix in microwave for 3 seconds (place slide on top of weigh boat containing crushed ice, microwave on 100%).
2. Fix for 30 seconds on 95% alcohol.
3. Put slides in PBS (in coplin jar) for 1 minute.
4. Put slides in humid chamber. Apply normal serum. Set microwave at 40°C at 350 watts for 2 minutes. Make sure that temperature probe is in the reagent on the side, then set for another 2 minutes in the hold mode. (This setting does not emit microwaves).
5. Blot off excess reagent.
6. Apply primary antibody at 40°C at 350 watts for 2 minutes. Set for another 2 minutes in the hold mode.
7. Rinse in PBS for 1 minute, change to new PBS and rinse for another minute.
8. Apply secondary antibody, 2 minutes at 40° C at 350 watts. Set for another 2 minutes in hold mode.
9. Rinse as listed in step (7).
10. ABC reagent at 40°C at 350 watts for 2 minutes. Set for another 2 minutes in hold mode.
11. Rinse as listed in step (7).
12. DAB reagent, develop to stainer's satisfaction. This can be done in the microwave at 40°C at 350 watts, starting at 10-30 seconds (or at RT).
13. Counterstain in dilute hematoxylin, if desired, for 30 seconds.
14. Dehydrate, clear and mount.

References:

OV Slayden, Tekehiki Koji and Robert Brenner. Microwave stabilization enhances Immucytochemical detection in frozen sections. Endocrinology 136:4012-4021.

Boon and Kok. Microwave Cookbook for Pathology, Second Edition.

Login GR, Dvorak AM 1988. Microwave fixation provides excellent preservation of tissue, cells and antigens for light and electron microscopy. Histochem J 20:373- 387.

The scientific theory I like best is that the rings of Saturn are composed entirely of lost airline luggage.
. . . . . Mark Russell