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Spatial Filtering in Microscopy: The Use of Matched Filters to See Further in Time and Space

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1958 Zernike described a set of optical experiments in microscopy which are based on modifying the optical properties of the backfocus planes of both the condenser and the objective. Since the introduction of the Phase Contrast Microscope in 1955 (Science 121: 345-349), few improvements have been made despite a plethora of technical advances in optics (e.g. coherent light). The use of matched filters in the planes of spatial filtering (= backfocus; Figs. 1 and 2) allowed us to observe biological events with an extended range in time and space (Fig. 3).

The introduction of a density gradient in the plane of the object as an additional optical element resulted in seeing hydrodynamic disturbances caused by moving objects (e.g. animals, mouthparts, swimming appendages, Fig. 4). Furthermore, we can observe chemical gradients and their temporal

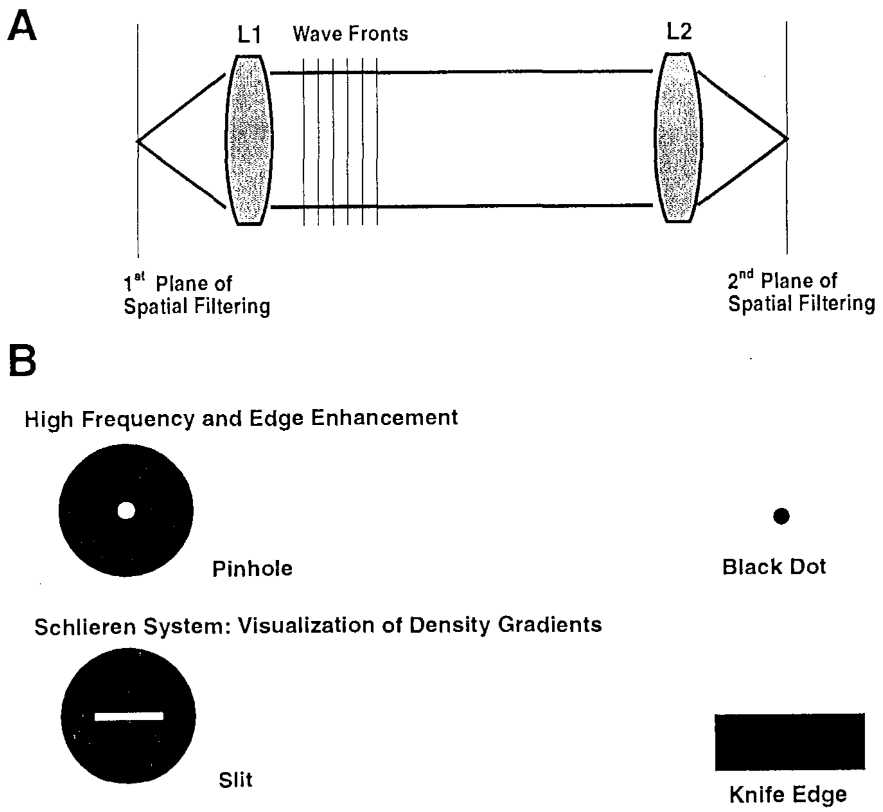

Fig. 1. Schematics of the principle of matched filters in the optical design of observational set-up. A) Production of plane wave fronts and position of spatial filters. B) Two examples of matched filters, one for high frequency and edge enhacement, the other for visualization of density gradients perpendicular to the knife edge.

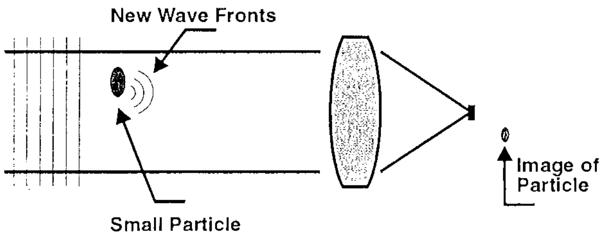

Fig. 2. Schematics of image formation in matched filter optical design of observational set-up. Small particles and focus is given because particles distort wave fronts anywhere within the collimated and expanded laser beam. Very small particles form new centers of light emission and are visible even if their sizes do not allow image formation.

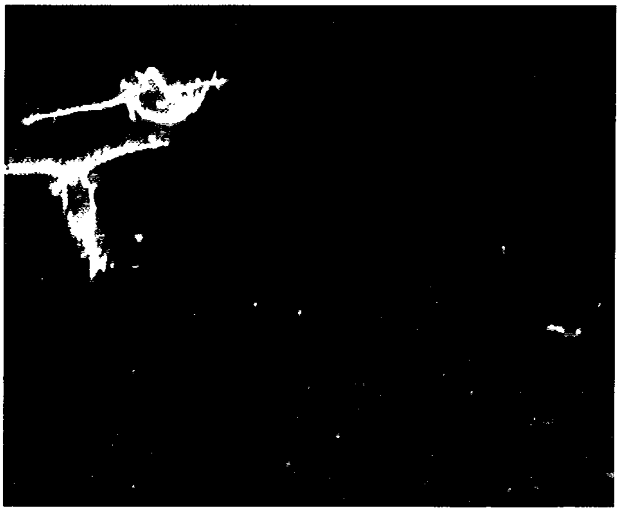

Fig. 3. One frame from a video observation of the feeding behaviors of *Diaptomus minutus*. The two animals are separated in the dimension of the optical axis by 1 cm. Upper animal is in focus. The behavior patterns of the lower animal can still be evaluated with the same temporal resolution as the ones of the upper animal. Visualization of the feeding current can only be performed with animals in optical focus. The suspended particles are algae in the size range of 5 to 10 microns.

behavior which either are created or modified by live animals. We use this optical approach to study zooplankton of a size range of 50 microns to one centimeter.

Well tuned optical systems allow us also to observe ecological interactions between aquatic

Fig. 4. One frame from a video observation of the creation of hydrodynamic disturbances by feeding and swimming calanoid copepods (*Diaptomus minutus*). Animal on the left glides capturing algae. Animal on the right entered the picture from the left, avoided encountering the animal on the left by executing three short hops and creating three vortex rings in the water. Body lengths on both animals are 1 mm. With this technique of adding a density gradient as an optical element we can observe very fast events like escapes at 500 body lengths per second because we can trace the hydrodynamic disturbances rather than film at high frame rates (over 10,000 frames per second would be necessary, at times of random escapes).

predators and their prey with a resolution of five microns in a field of view and a depth of focus of one centimeter. To date, the largest set-up allowed us to observe, in three dimensions, interactions between zooplankters (e.g. mating) within a vessel of one liter with a resolution of 50 microns. These observations were possible because we used carefully matched filters (Fig. 1A) which canceled all distortions of the wave fronts that we did not perceive as informative.

REFERENCE

Zernike, F. 1958. The wave theory of microscopic image formation. In: Concepts of classical optics (J. Strong, ed.), Appendix K, 525-536.