

Dark-field microscopy

"Explain the technique of just one of either 'Schlieren photography' or 'dark-field microscopy' or 'phase-contrast microscopy' to a well-educated non-physicist."

Bright-field

1. Light from a plane-wave source is focused through an object by a condenser.
2. Some light is blocked (absorbed) by opaque parts of the object, or reflected away at boundaries between components/materials of different refractive indices.
3. The remainder passes through the objective lens, to the observer.
4. This produces a bright background, with object details appearing darker in the image than their surroundings. The brightness of the brightest parts of the image is determined by the source brightness and block size, while the darkest parts depend on the object.
5. This results in poorer contrast compared to dark-field, as the dark areas are generally grey rather than black.

Dark-field

1. An opaque disc is put between the source and the condenser, blocking out the middle of the beam. The size of the disc is such that it casts a "shadow" on the objective. With no sample, no light reaches the objective. See Figure 1.
2. The condenser focuses the beam onto the sample (as is done in bright-field, but with a "hollow" beam). In the focal plane, the entire sample is illuminated, despite the donut-shape of the beam before prior to being focused. This is due to far-field diffraction: the far-field image of a large, thin annulus ($r_{\text{inner}} \approx r_{\text{outer}}$) is a radial cosine, which may be approximated as a flat, constant intensity field if the annulus radius is much greater than the object's details of interest. See Figure 3 and Figure 4.
3. No light enters the objective directly from the source. Light from the beam is scattered by the sample – some scattered into the objective.
4. Only light scattered by the object enters the objective. This produces a dark background, with sample details appearing brighter than surroundings. The brightness of the brightest parts of the image is determined by the amount of light scattered by the object, while how much unscattered light "leaks" into the objective determines the darkness of the darkest parts.
5. This results in superior contrast to bright-field, as dark areas may be completely black, while increasing the brightness of the light source brightens the bright areas. See Figure 2 for a visual example.

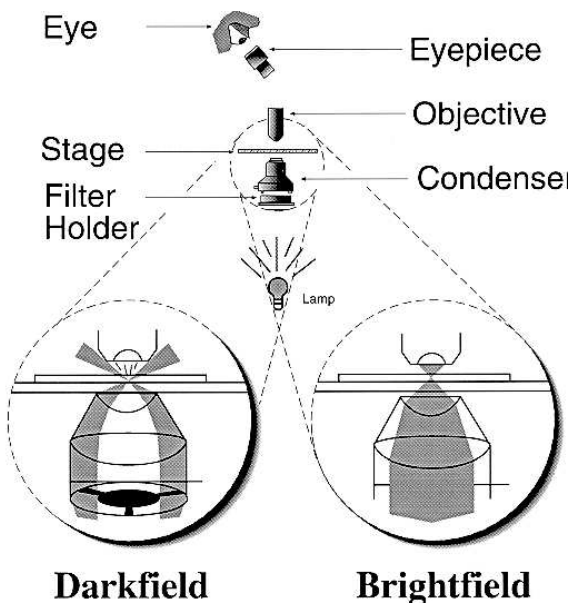


Figure 1: Comparison between dark-field and bright-field: Notice the opaque disc in front of the source in dark-field. As shown, this results in no light entering the objective, in the absence of a scattering object.

Image: <http://www.wsu.edu/~omoto/papers/Fig1.html> (10-May-2011)

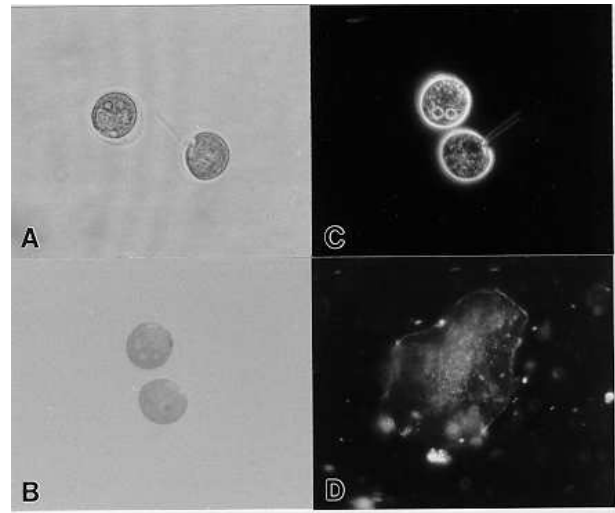


Figure 2: Algae in bright-field microscope (A/B) and dark-field (C). Notice the improved visibility of details in the dark-field images, due to the better contrast of dark-field microscopy.

Image: <http://www.wsu.edu/~omoto/papers/Fig2.html> (10-May-2011)

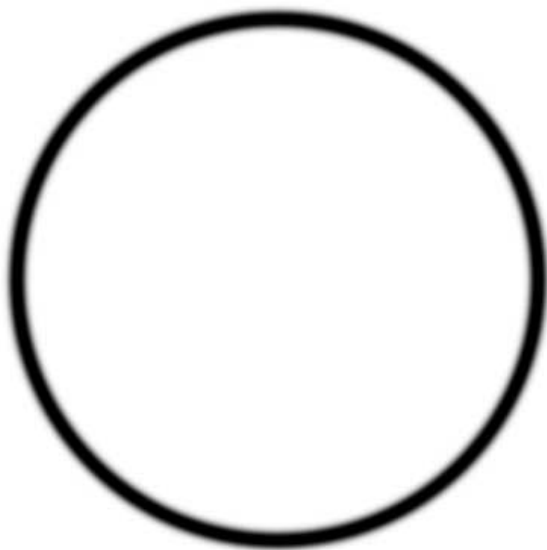


Figure 3: An annulus-shaped beam, after being partially blocked by a disc. Without an object in the optical path, this beam profile would result in no light entering the objective, if the minor radius of the beam at the objective was larger than the objective's radius.

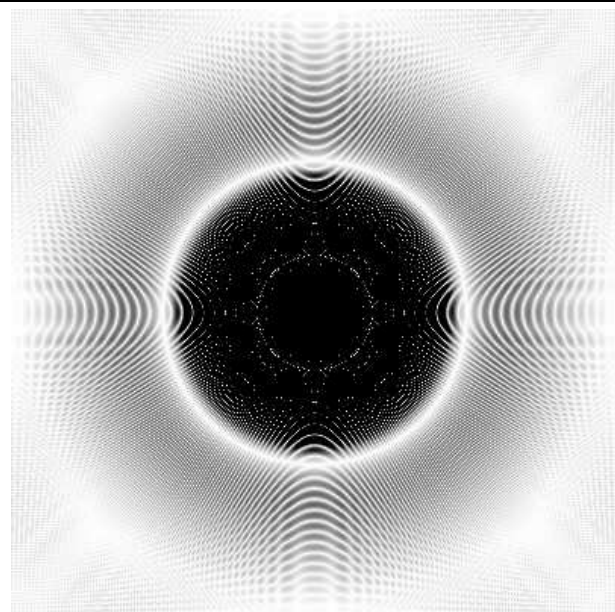


Figure 4: The beam profile in the focal plane (at the object) – this is the intensity profile of the Fourier transform of the annulus beam. The centre of this may be assumed to be flat – providing an isotropic illumination of the object. Light-scattering features of the object may “knock” light off-course, and towards the objective, allowing such features to be detected as bright spots in the image.