

Introduction

Optical phase contrast microscopy is a popular technique for imaging low contrast, transparent samples such as cells in fluid. This technique is now standard on the **MFP-3D-BIO™** Atomic Force Microscope, enabling simultaneous AFM and phase contrast imaging.

Advanced Optics

The phase contrast illuminator feature consists of several enhancements to the MFP-3D-BIO. First, a condenser lens is integrated into the MFP-3D head. An optics module illuminates this condenser to create a hollow cone of light that converges on the sample. The angle of the hollow cone is critical; it must match the phase ring in the objective. A slider in the optics module allows the researcher to select the appropriate annulus, or remove it altogether for Köhler brightfield illumination.

The system supports microscope objectives Ph1, Ph2, and PhL/PhO/PhC objectives from Nikon, Olympus, and Zeiss. (Suitable phase contrast objectives must be specified with your optical microscope purchase.)

Optimized Design for AFM Performance

To preserve high AFM performance, the optics module is mechanically decoupled from the MFP-3D head. It mounts on a custom pillar that attaches to the optical microscope stand. The pillar includes an XYZ translator for centering and focusing the aperture plane – critical steps for establishing good phase contrast. Centering and focusing the illuminator in the field plane is carried out by adjustment screws on the MFP-3D head.



Phase contrast optical micrograph of epithelial cell and AC240 cantilever. Imaged with an Olympus LCAch 20x / 0.40 PhC objective.

A 'cold mirror', incorporated in the MFP-3D head, separates the phase contrast illumination optical path (visible light) from the cantilever measurement path (near infrared). This design allows optical access while preserving the exceptionally high resolution of the cantilever deflection signal.

Finally, the entire phase contrast illuminator is supplied with light by a six foot fiberoptic light guide. This places the lamp and fan outside the acoustic isolation, minimizing noise and thermal gradients.

Applications

The primary application for simultaneous phase contrast and AFM imaging is to visualize native cells in preparation for AFM experiments. The AFM tip can be positioned with submicron accuracy over a cell or other features of interest.

Researchers can then apply the powerful capabilities of the MFP-3D to measure their sample's topography, adhesion forces, and elasticity with nanometer and piconewton resolution.

In addition to biological samples, Phase contrast microscopy can be used for any transparent samples with micron-scale features such as diffractive optical elements, phase masks, and microfluidic structures.

MFP-3D – The Only Choice for AFM and Optical Measurements

The MFP-3D is the tool of choice for simultaneous AFM and phase contrast optical imaging, as well as epi-fluorescence, TIRF, FRET, or confocal measurements. Its unique design allows these measurements to be made easily and precisely. Researchers can now perform these measurements simultaneously and broaden their experiments to a countless number of bioscience applications.



Closeup of the Phase Contrast Illumination module and MFP-3D head.

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