

Back Focal Plane and Darkfield Video Transcript

This video follows the Köhler Illumination video and explains how to set up the color camera, also known as the back focal plane camera. This video also shows how to achieve a darkfield image with a zero-order mask.

To set up the color camera, open up the aperture iris and field iris. Connect the color camera. Open a window in the ThorCam software and adjust the exposure.

Make sure the color camera is perpendicular to the rail. Adjust the position and angle of the beamsplitter until you can see the light on the camera.

Close down the aperture iris until it is slightly open and increase the exposure if needed. Simultaneously translate and rotate the beamsplitter until the aperture iris is in focus with as little flaring as possible. Once this is achieved, secure the position of the beamsplitter.

Turn the exposure down to reduce saturation. This helps to see whether the aperture iris is in focus. Move the color camera back and forth until the edges of the aperture iris are sharp. If the image is oversaturated, you can use a neutral density filter.

Once the iris is in focus, the color camera is conjugate to the back focal plane, the aperture iris, and the lamp filament, and can image all of them simultaneously.

To achieve a darkfield image, make sure the aperture iris is open, and take the zero-order mask and attach it to the objective. A dot will appear in the color camera image. This dot will always be slightly out of focus.

Close down the aperture iris on the dot. If the dot is not centered within the iris, rotate the objective to center it.

Next, close down the BFP iris on the aperture iris. Adjust the angle of the objective so the two irises close evenly on one another.

Lastly, open the BFP iris and close the aperture iris until illumination is blocked by the zero-order mask. Turn up the exposure on the sample camera until you can see a darkfield image.