PUMA Köhler Illuminator
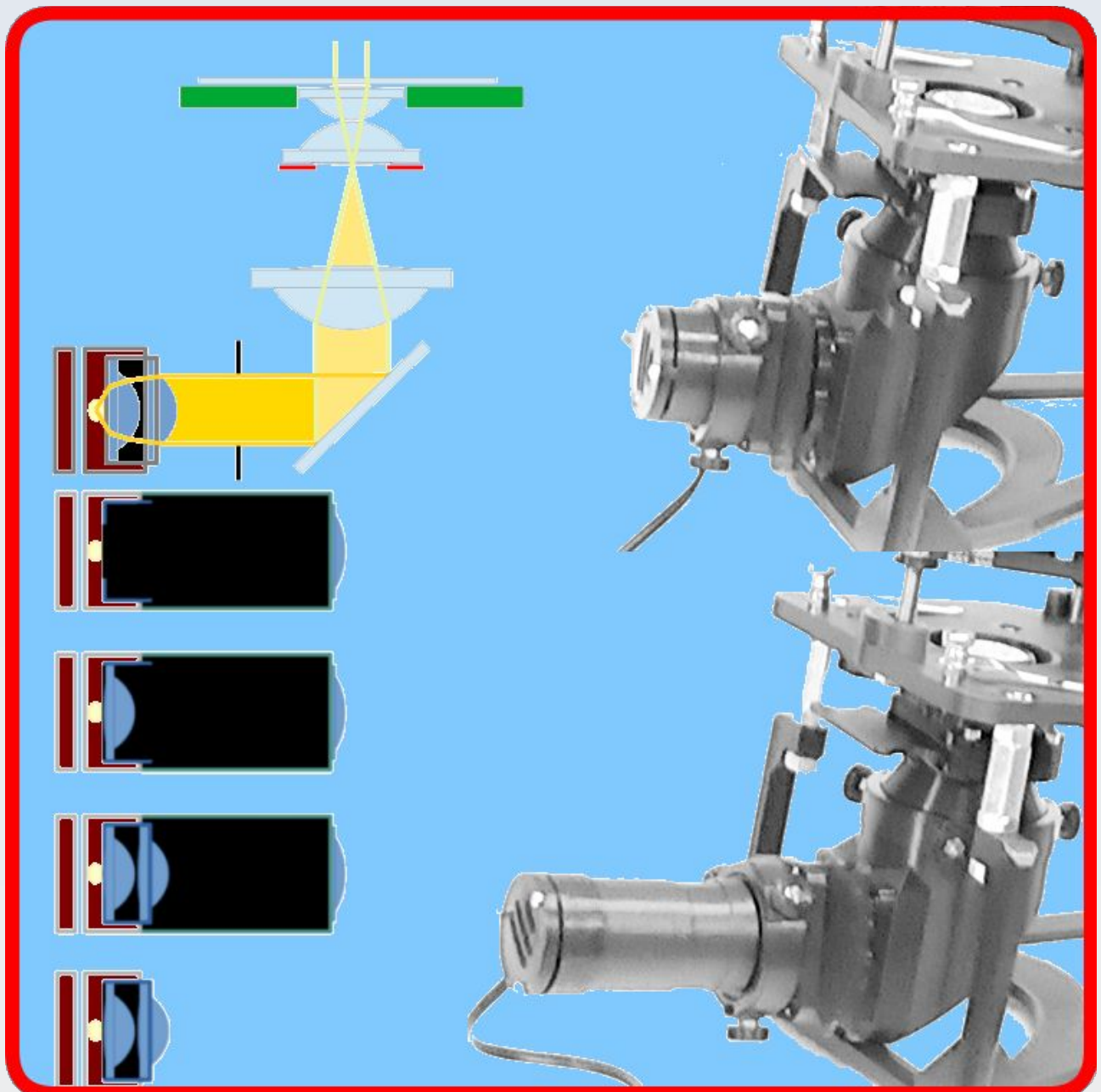
Specs and Supplementary Information

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Contents in Detail

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Abbreviations

Some common abbreviations used in this documentation

KI = Köhler illumination

NA = Numerical Aperture

BFP = Back Focal Plane

CFP = the condenser's lower (front) focal plane

IAD = Illuminating Aperture Diaphragm of the condenser

IFD = Illuminated Field Diaphragm (Field stop, Köhler diaphragm)

LPC = low power collector

LC = 2x23 mm lower collector

Introduction

The PUMA Köhler Illuminator is described in detail in this YouTube video:

https://youtu.be/XEE-el7vC5k

This document gives some supplementary information and specs to assist in deciding the best usage for your project.

This document is under constant revision and should not be considered complete and final.

System Configuration Options

One of the key features of KI is the use of a collector system to project an image of the light source into the front focal plane of the condenser (its 'lower' focal plane in the upright configuration – also known as the Fourier plane and it is a conjugate focal plane of the back focal plane, the BFP, of the objective lens).

The ideal condition – for standard bright field viewing – exists when the condenser's focal plane (CFP) is filled with a uniform patch of light up to the maximum numerical aperture (NA) of the objective lens in use.

Due to the limitations of the lenses used, there is a trade off between the size of the uniform area of illumination at the specimen plane and the NA of the fanned out beam produced by the condenser. The former is needed to completely illuminate the field of view and the latter is needed to make maximum use of the objectives resolving abilities. It is often the case that these two features are inversely related – the greater the NA the smaller is the illuminated field patch and vice versa. This relationship is not catastrophic however because higher NA is needed for higher magnification lenses which also have a small field of view. It does mean, however, that configurations which give the highest NA of illumination will not fill the field of view of lower magnification lenses and so there are some optional configurations of the lens system for the PUMA KI system that maximise / optimise viewing for either low magnification or high magnification lenses. These options include the number, type and spacing of lenses of the lower collector and Abbe condenser and whether or not to use the 5 mm spacer and / or a diffuser in the LED lamp housing.

Consequently I have made measurements on the size and distribution of the patch of light produced by the PUMA KI system in the CFP and the NA of this projection so as to assess which configurations will work best for various objectives and uses. This guide documents those measurements.

Condenser Focal Plane Projections

With the the empty (lensless) Abbe condenser in place, the size and distribution of light projected on the CFP was measured by inserting a sheet of drafting paper (tracing paper) into the IAD slot to act as a back-projection screen and photographing the resulting projections from directly above. Figure 1 shows the lensless condenser with the drafting paper inserted. These measurements were made for all the configurations of the lower collector specified below.

Figure 1. A back-projection screen is inserted into the IAD slot of the lensless condenser to make measurements of the light projected there by the collector system of the Köhler illuminator.

Numerical Aperture Measurements by the Horsfall Method

With the full Abbe condenser in place and no restrictions on either the IAD or the IFD, an Olympus SPlan x40 objective was used with a blank slide in place on the stage (the scope was first focussed on a specimen slide then the specimen was removed and the blank slide inserted). See figure 2.

The eyepiece was removed from the optical tube and the camera looked directly down the optical tube and was focussed on the BFP of the objective. Images were taken for all the configurations of the lower collector specified below.

The Olympus x40 objective has a stated NA of 0.7. To get a control image of the full NA of illumination I used an identical setup but with the KI system and Abbe condenser removed and with a sheet of paper placed on the stage, extensive enough to more than exceed the NA of the objective. This paper was illuminated from below by a desk lamp and the standard mirror illuminator (see figure 3).
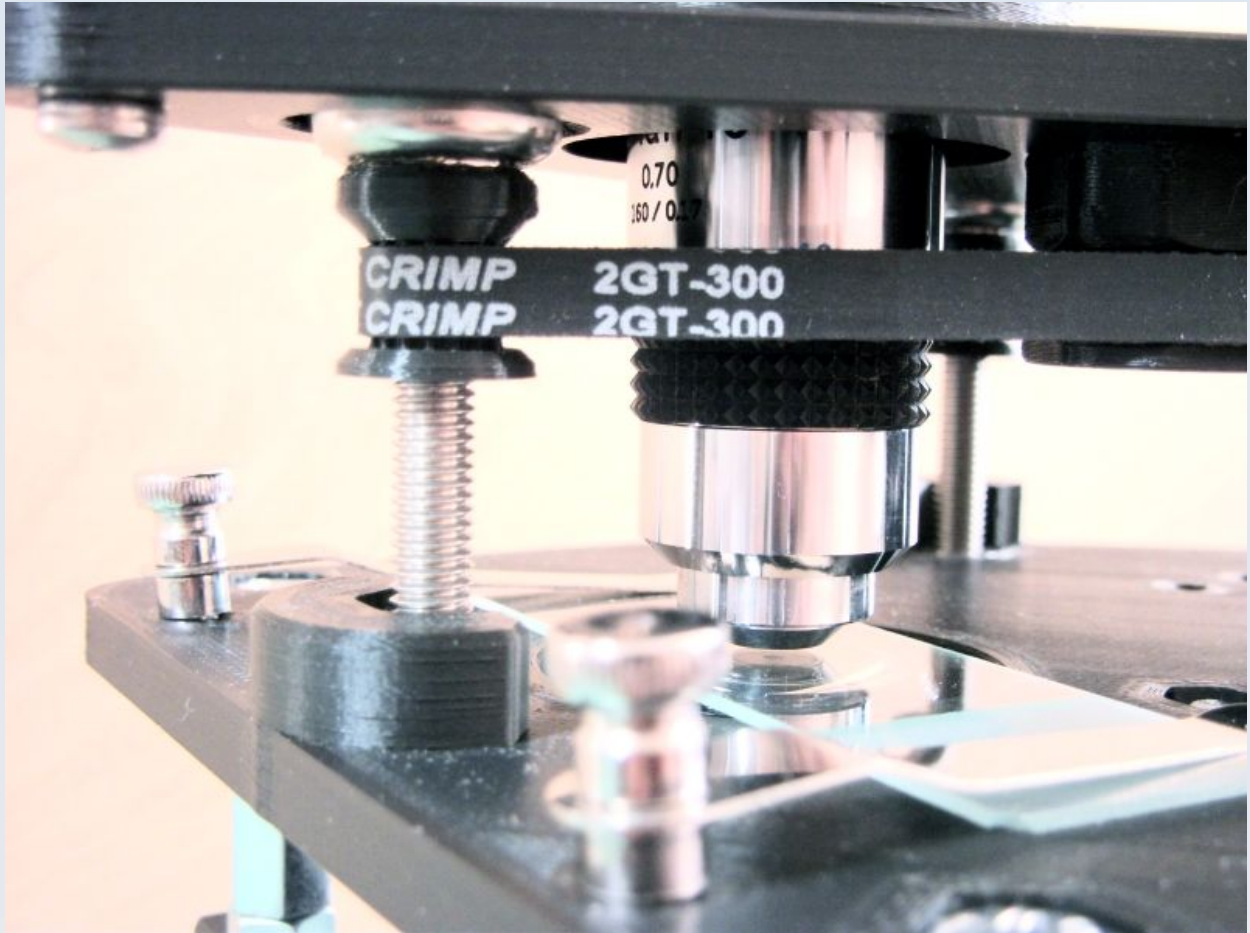
Figure 2. Stage and objective setup for measuring the NA of the illumination system with an Olympus SPlan x40 objective of stated NA = 0.7.
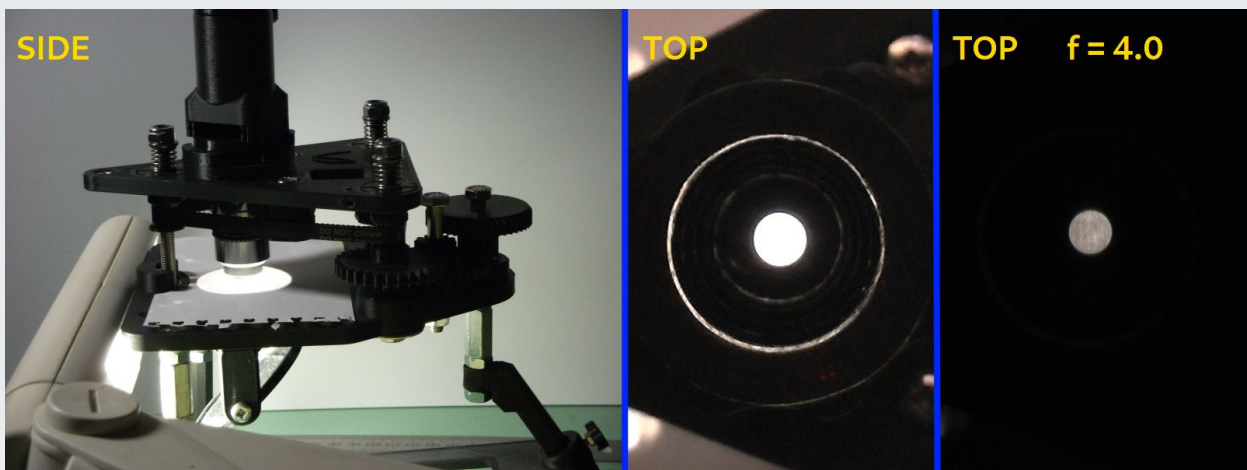
Figure 3. Setup for measuring the full NA of the objective for a comparison control. A desk lamp fluorescent strip is placed on its side and the mirror reflects the extensive light up to illuminate a piece of paper on the stage (without any condenser present). The view from the top is seen with the camera iris open (middle) to show that the camera is looking directly down the optical axis. The camera iris is stoppered down to f = 4.0 to remove saturation and make all the subsequent measurements (right).

Lower Collector Configurations

The following variations of the lower collector were used for make all measurements. In addition, for each configuration the measurement was made with and without the 5 mm spacer between the LED and the first collector lens and also with and without a membrane diffuser behind the first collector lens.

A. The LPC without a proximal lens.

B. The LPC with one proximal lens (this is the usual way to deploy the LPC)

C. The LPC with two proximal lenses. This is the 'usual' LPC but with the single proximal lens replaced by the 2x23 mm lower collector (LC)

D. The LC on its own

The configuration letters A to D above are illustrated in figure 4.

Configuration C does not give full KI because the projection is shifted slightly towards critical illumination and it is no use for low power objectives. However it can give useful flat field illumination for higher magnification objectives e.g. x10 and above so is convenient if you want to use the LPC setup for x4, you can leave the LPC tube in the IFD tray and simply swap out the proximal lens for the LC to view higher magnifications. This is useful also because precise alignment of the lamp may differ between using the LPC and the LC when each is directly coupled to the IFD via the same adjust ring.

Results: Condenser Plane Projections

Figures 4 and 5 show the condenser plane projections. The camera was kept at constant manual settings for all measurements so the brightness can be compared between configurations.

A membrane ND filter was used for all measurements. When a diffuser is inserted this is always inserted in front of the ND filter.

The camera used is a Panasonic HC-X800 3-chip camcorder on manual mode with iris set to 4=4.0 and shutter set to 1/4000.

Each square in the matrix shown in figures 4 and 5 have a physical size of 20 mm x 20 mm.

Figure 4. Results of condenser plane projection for the various configurations of the PUMA KI system. The capital letters down the side refer to the lower collector lens variant used as described in the text and also illustrated in the diagrams far left. These images show the projections in a natural linear scale of intensities. The thin dark straight lines and occasional dark specs you see are simply folds and small defects in the drafting paper screen.
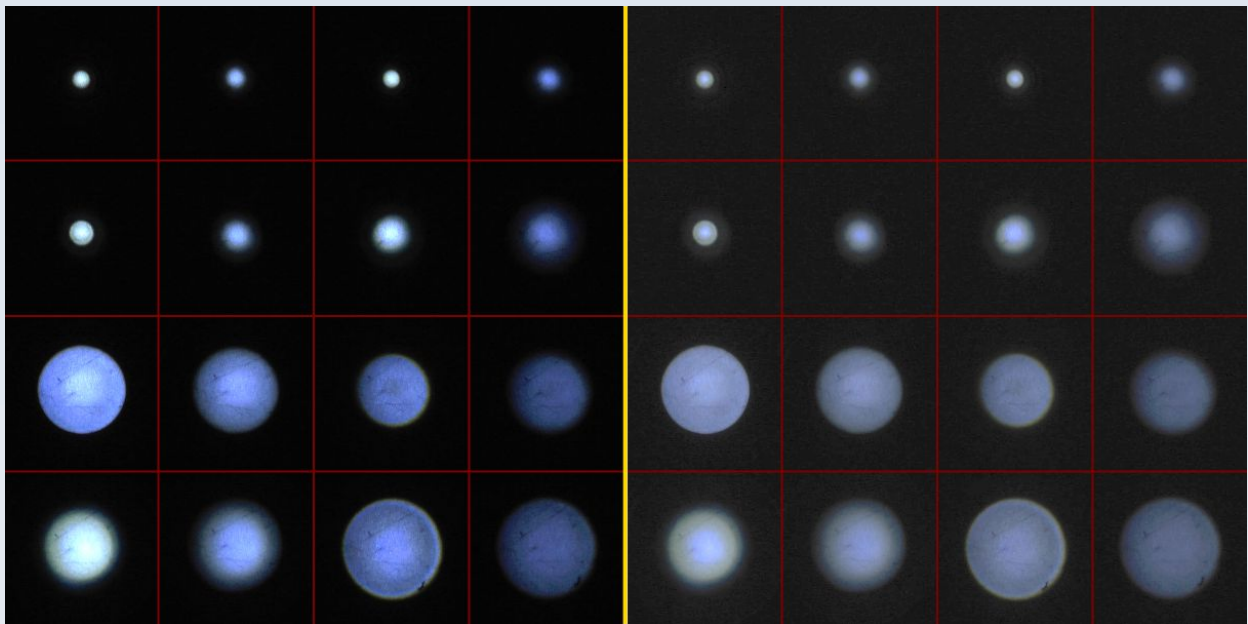
Figure 5. Condenser projections shown with intensity levels multiplied by 2 (left) and square root transformed (right) to make it easier to perceive the shape of the light distributions. The arrangement is the same as described in figure 4.

These results show that the light distribution varies in intensity in the CFP. From the perfect defocus principle this variation will not translate to the specimen plane but it does mean that resolution will vary accordingly. For the more even resolution spectrum a more uniform disc is required. The diffuser clearly helps in this regard.

Note that the smallest discs (and therefore the smallest NA of illumination) are made with the LPC lacking a proximal lens and the largest discs are made with the LC and the LPC using the LC as proximal lens.

However, the LC used with the 5 mm spacer shows the largest discs by a small margin showing that for best resolution with a x40 objective and above you should use the LC with 5 mm spacer and a diffuser will give a more even spatial resolution spectral profile.

Results: Condenser NA Measurements

Figure 6 shows the control image of the full disc of illumination of the Olympus x40 lens representing a NA of 0.7
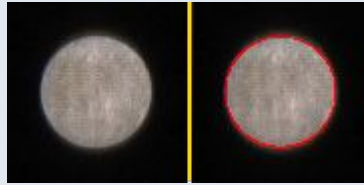

Figure 6. The full 0.7 NA of the x40 objective used in these tests. The image on the right shows a red ring drawn at the circumference for superimposing on the rest of the measurements to see how close they come to this ideal NA.

Figure 7 shows the results for the various illumination configurations used. The image matrices have the same meaning in terms of configurations as those used for condenser plane projection (see above). The camera shutter is 1/50 and the iris is set to f=4.0.
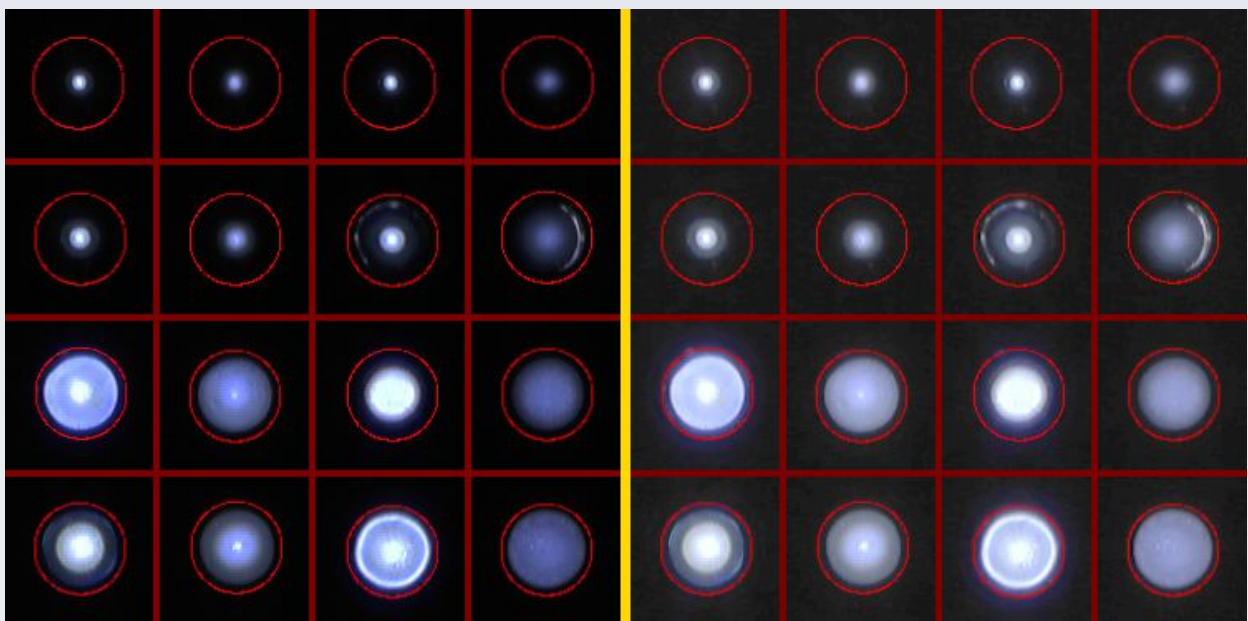

Figure 7 Illuminated numerical aperture of a x40 objective with full NA of 0.7 represented by the red rings. Left: Original linear intensity levels. Right) Square root transform of intensity levels. The meaning of the rows and columns are the same as for figures 4 and 5. The peripheral irregular thin crescents seen in images B3 and B4 are due to the ND filter that was used not being large enough to cover the whole back surface of the first collector lens.

It is interesting and important to note that, while the general shape of illuminated area is similar to the condenser plane projections as expected, the presence of an objective lens increases the contrast and exaggerates the uneven distribution in intensity of the light across the field in this conjugate Fourier plane compared to where the images of the light source was first formed (the CFP). The implication being that use of the membrane diffuser is even more helpful in evening out the spatial frequency profile of the illumination than may at first have been thought looking at the condenser projections alone.

It is also clear from these results that in order to satisfactorily illuminate a high power objective such as x40 you will need to use either the LC or the LPC with LC as proximal lens.

However, the LC alone only illuminates about half the available NA unless the 5 mm spacer is used. In this case you get enhanced high and low spatial frequencies with a relative inter-band deficiency unless a diffuser is also used. Hence, best illumination for x40 and above will be with the LC using the 5 mm spacer and diffuser.

The LPC with LC as proximal lens looks as though it will give reasonable illumination of a x40 objective with or without a spacer / diffuser. However, the extreme exaggeration of central low spatial frequencies as shown here tends to give a poor quality image in practice unless this profile is evened out with the use of a diffuser. In practice the image seems best without the spacer.

Some General Rules of Thumb

Empirical tests (backed up by the data gathered in the preceding measurements) have shown the following.

For a x2.5 and x2.0 objective you use the 2x23 mm LC with a spacer and a diffuser but use a hollow condenser.

For a X4 objective with the 2x23 mm LC this works best without spacer and without diffuser but lamp needs to be accurately centred. If using a spacer then a diffuser MUST be used (unless you are doing this for alignment purposes) and there is greater field curvature but the illumination is useable for direct vision (without a diffuser but with a spacer you get an image of the lamp – i.e. near critical illumination).

For a X10 objective with the 2x23 mm LC this works best without spacer.

For a X40 objective with the 2x23 mm LC this works best with spacer and if you use a diffuser you make the power spectrum more even (as illustrated above) so this is to be preferred but visually the difference is hard to notice with or without a diffuser.

We have seen that the LPC without a proximal 23 mm collector gives a lower NA projection cf. with a proximal lens – however this small projection still fills the back focal plane of the x4 lens so in theory and for normal bright field vision this does not matter and the illumination is uniform and good. However, I noticed that dark field microscopy seemed subjectively more even with a single 23 mm proximal lens compared to this lensless cconfig.

The LPC can be used with the 2x23mm collector without a spacer but with a diffuser to give even illumination of x10 and x40. This usage is not true Köhler because the image of the LED source is not projected onto the lower focal plane of the condenser however it gives nice even illumination so that aspect of Köhler is not necessary (provided a diffuser and no spacer is used) and both diaphragms can still be used as with standard Köhler.

In general, when doing Fourier filtration at the CFP (for example, dark field microscopy) a diffuser should be used otherwise the yellow and blue/white colours of the projected image of the LED lamp will get 'unmixed' by the filter (such as the patch stop) and the images will have a partial 'Rheinbergian' look to them.

Work Still to Do

1. Experiments with an oil immersion NA=1.25 objective
2. Experiments with the lensless condenser with an objective.