PUMA Köhler Illuminator

Specs and Supplementary Information

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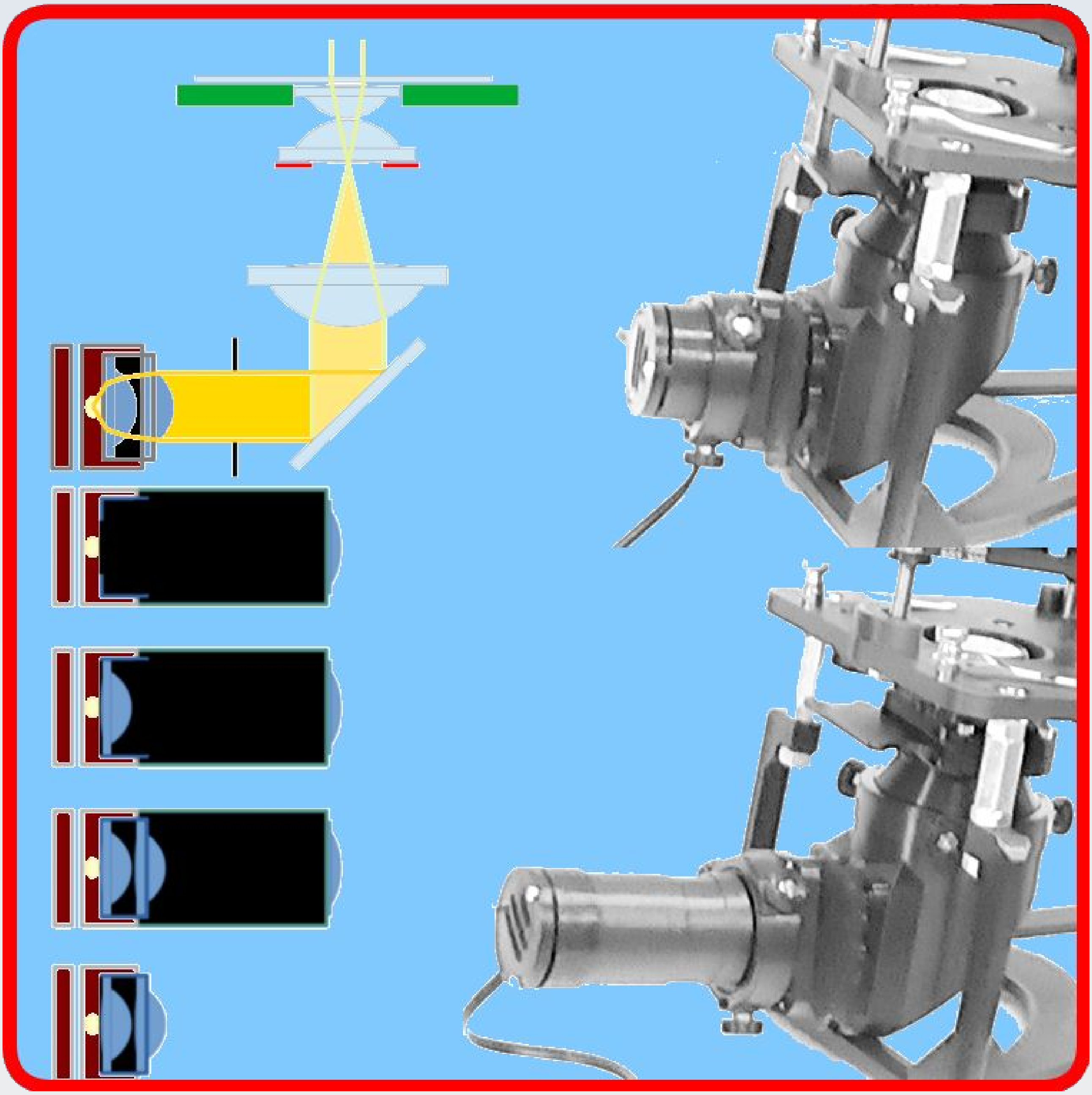

Contents

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Dr Paul J. Tadrous, TadPath and OptArc cannot accept any liability for any such loss or damages that may occur. All those who attempt to build or use any aspect of the PUMA project or derivatives thereof do so at their own risk.

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Throughout this manual please take heed of **warnings given in bold text and highlighted yellow** to avoid possible damage to equipment and/or harm to people.

PUMA microscopes and associated systems are not toys. They contain small parts which may come loose such as tiny metal screws and washers and glass components that may splinter or break or otherwise present a choking or sharp object hazard or chemical hazard (for batteries). **Please do not let babies or young children play with or use any aspect of a PUMA system without close appropriate adult supervision. Likewise keep PUMA systems away from pets**.

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

0. 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.

Here I also show results and calculations for the maximum NA of the PUMA condenser system with and without the Köhler illuminator.

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

1. Lens and Optical Path Specifications

Figure 1.1 shows the optical train of the Köhler-Abbe system with the standard 2x23LC (no spacer and no LPC). Refer to the figure legend for more detail about lens spacing.

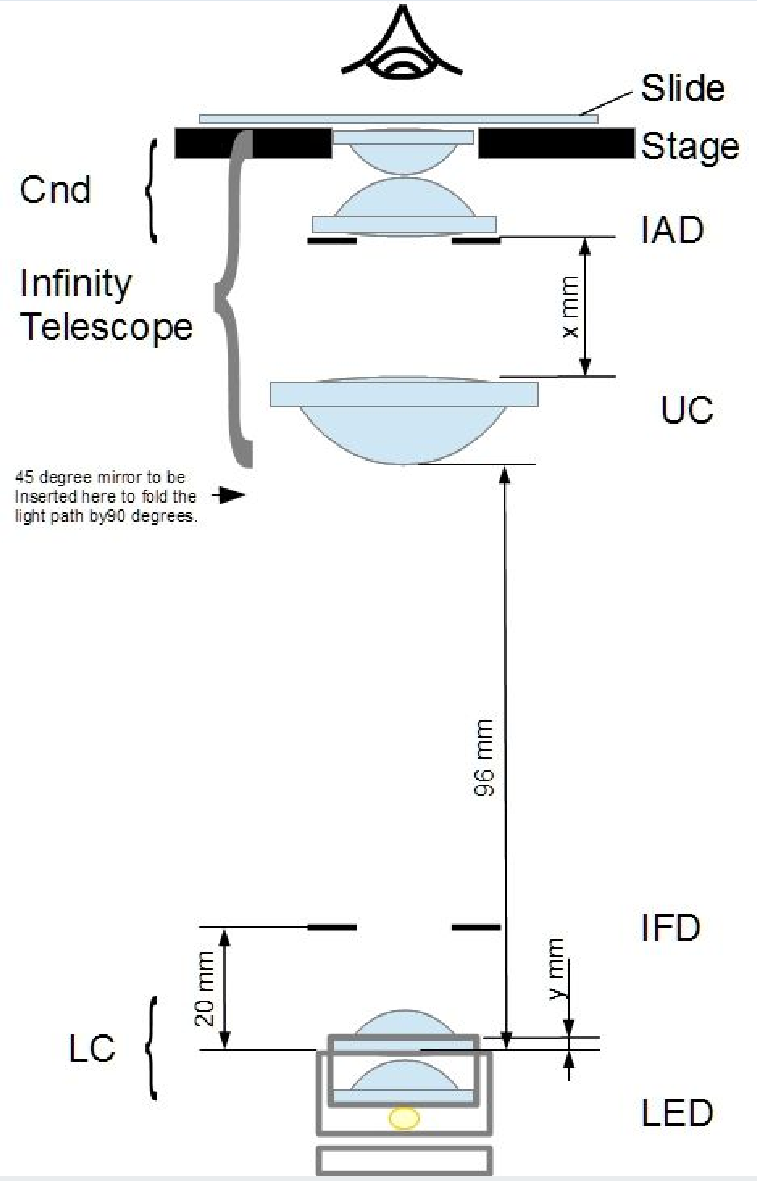

Figure 1.1. Arrangement of lenses and measurements for the Dominus Köhler illuminator using the 23/30 Abbe condenser. Cnd = condenser, the lenses being arranged at a distance apart that just allows an object placed at the flat surface of the 30 mm lens to appear in focus when looking into the distance from the top of the 23 mm lens (position of observer marked with eye symbol). The back surface of the 30 mm lens is therefore, by definition, the back focal plane (BFP) of the condenser assembly. IAD = illuminating aperture diaphragm. This is placed at the BFP of the condenser. UC = upper collector. This is positioned at such a distance (labelled as 'x' mm) from the condenser so that if an observer looks through the 23 mm lens of the condenser they will see objects at infinity in focus – the condenser/UC assembly therefore will form a telescope focussed at infinity from the point of view of the observer when x is appropriate (in practice x is about 23 mm). This implies that the front focal plane of the UC lens coincides with the BFP of the condenser. IFD = illuminated field diaphragm. This is placed at such a distance from the UC and condenser combination that an image of the IFD is brought to a focus at the level of the specimen on the slide. In this figure the distance from the lamp housing is shown (20 mm) and this translates to 96-20 = 76 mm from the lowest point of the UC 44 mm lens. LC = lower collector lens assembly and is made from 2 closely applied 23 mm lenses (they are about 8.25 mm apart, held that way by the 7 mm collar). The LC is designed to produce a collimated parallel beam of the light emitted from the LED source. However, all the lenses used in this Kohler system are not perfect and some adjustment of the distance of the LC from the LED is required for optimal illumination of the x40 lens compared to all the lower powered objectives. The distance shown as 'y' represents this adjustment. The figure illustrated the extended lampholder container and y is measured from the lip of that lampholder to the lip of the LC housing. When the LC is fully lowered the back of the second lens just touches the plastic dome case of the LED and y=3.5 mm. This is the position used for all except the x40 objective. For the x40 objective the LC assembly is brought forward (moved away from the LED) by 1.5 mm so y = 5 mm. The whole length of this Kohler illuminator is 171 mm from the bottom of the stage to the bottom of the lamp housing.

Table 1.1 gives the specifications of the lenses used in the Köhler illuminator and Abbe condenser (measurements are in mm). The symbols in the table refer to the measurements shown in the figure 1.3 and three focal lengths are provided for each lens. The 'nominal' focal length is the focal length specified in the seller's / manufacturer's web page. The other two focal lengths given are actual measurements made (by me) with a parallel collimated laser beam expanded to 5 mm in diameter approaching the lens from the side shown by the two parallel arrows as shown in figure 1.4.

Lens ID	**Uses**	**D**	**L**	**Dc**	**Te**	**Tc**	**B**	**P**	**f nominal**	**fB**	**fP**
44 mm	Köhler	44	2	40	3	19	16	0	23.00	49.00	24.00
30 mm	Abbe Cnd	30	1	28	3	15	12	0	25.00	31.50	10.50
30 sq.parab	Alternative Abbe	30	2	26	2.5	13	10.5	0		34.80	13.90
30 sq. sph	Not used	30	1	28	3	10	7	0		40.00	28.60
23 mm	LC, Abbe Cnd	23	1	21	1.25	8	6.5	0.25	18.00	28.00	17.70
LPC Lens	LPC	23	0	23	2	4	2	0	60.00	61.00	56.20

Table 1.1 Lens parameters for the Köhler illuminator and Abbe condenser (all measurements in mm). For meaning of symbols see figure 1.2 and main text. There are three 30 mm lenses in the table. The first is the original 30 mm lens used in this project. This lens may not be available from all suppliers so two alternatives are shown. The 'sq. parab' is a paraboloid shape lens with a squared off rim. This is slightly 'weaker' (i.e. has longer focal lengths) than the original. The second alternative '30 sq. sph' is much weaker and should not be used (details are given here so you know if you receive one of these lenses from a supplier). Figure 1.2 shows photos of these three lenses so you can see the difference between them in shape. The 'LPC Lens' is the thin lens used in the LPC attachment.

Figure 1.2. The three different types of 30 mm lens you may receive from online sellers. Only the two on the left may be used for making a PUMA Abbe condenser (the best performance given by the middle one with the thinner curved base rim). If you get the spherical type (shown on the right) this will be too weak to give a high numerical aperture fan beam and appropriate access to the Fourier plane so should not be used.

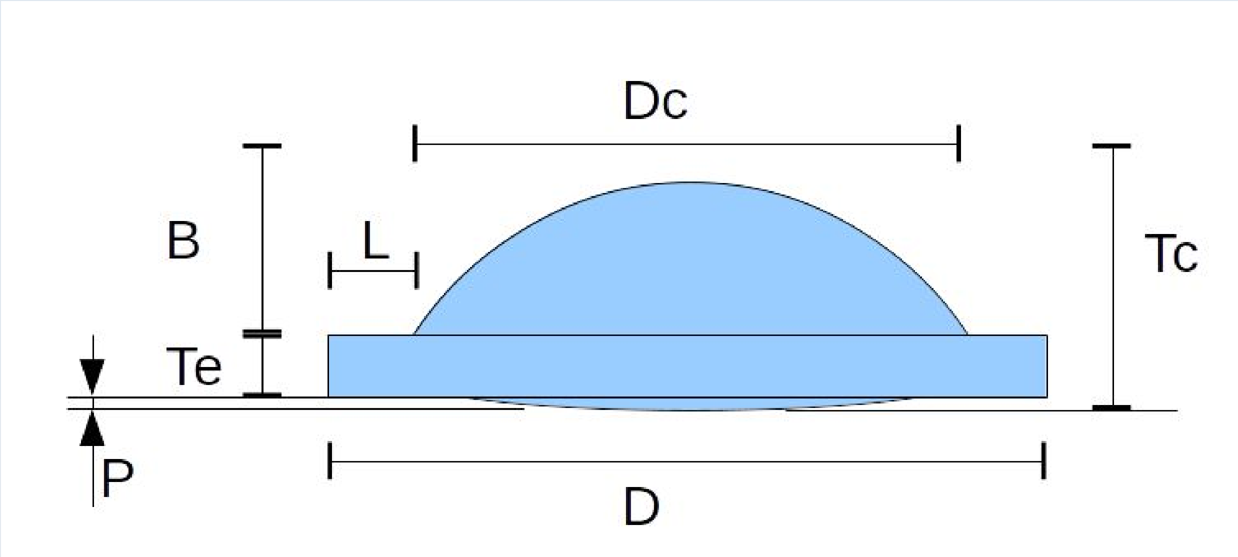

Figure 1.3 Lens dimensions. The symbols are those used in table 1.1.

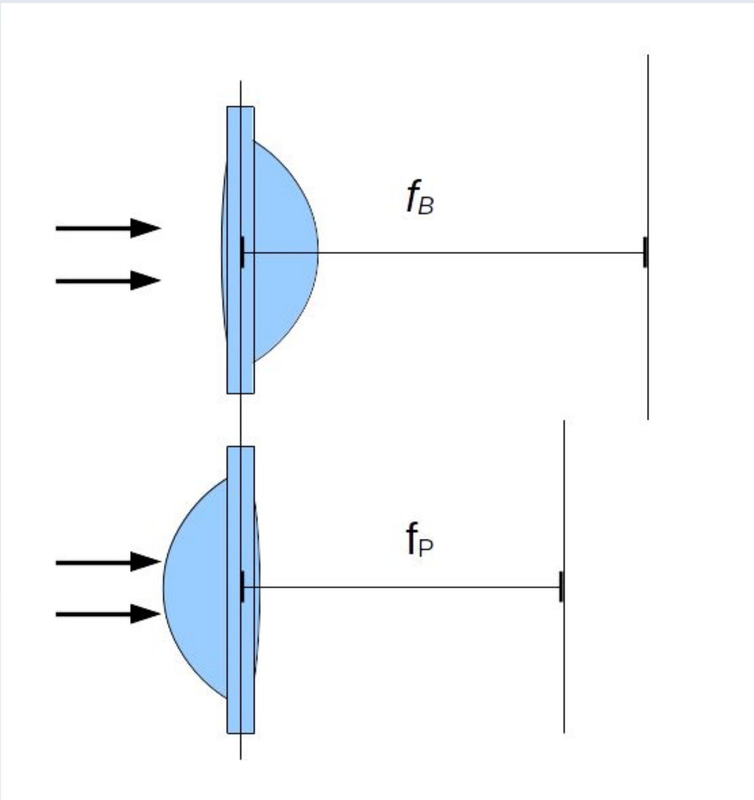

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Figure 1.4. Definition of the two focal lengths measured for the figures in table 1.1. Arrows on the left indicate the incident collimated beam expanded laser beam. Vertical line on the right indicates the plane this beam comes to a focus in.

Figure 1.5 shows the optical path through the system.

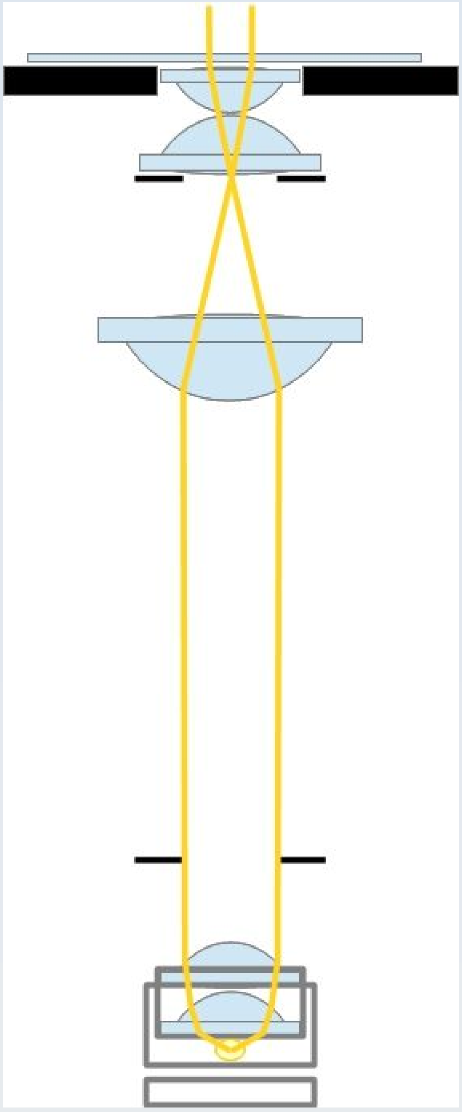

Figure 1.5. Light path from a point on the LED source for the Dominus Köhler illuminator using the 23/30 Abbe condenser.

The slightly longer focal lengths associated with the new form paraboloid 30 mm lens may be expected to give slightly lower NA illumination to the original and may also slightly offset the Fourier plane, in theory. However practical experiments have since shown that neither of the above two expectations are realised in practice – at least not in a simple way.

Regarding the Fourier plane, this is maintained with the new form 30 mm lens. Because these lenses are not planatic the whole Fourier plane is not in focus all across the field (with either 30 mm lens). The new form 30 mm lens has better focus near the central area of the Fourier plane and the original 30 mm lens has slightly better focus near the periphery of the Fourier plane. However these differences to not make one lens overall better than the other and the Fourier plane is essentially in correct focus with both lenses taking the lack of planarity into account.

For the effects of NA of illumination see table 4.1 and the discussion in section 4 below.

2. 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.

2.1 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 2.1.1 shows the lensless condenser with the drafting paper inserted. These measurements were made for all the configurations of the lower collector specified below.

Figure 2.1.1. 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.

2.2 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 2.2.1.

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 2.2.2).

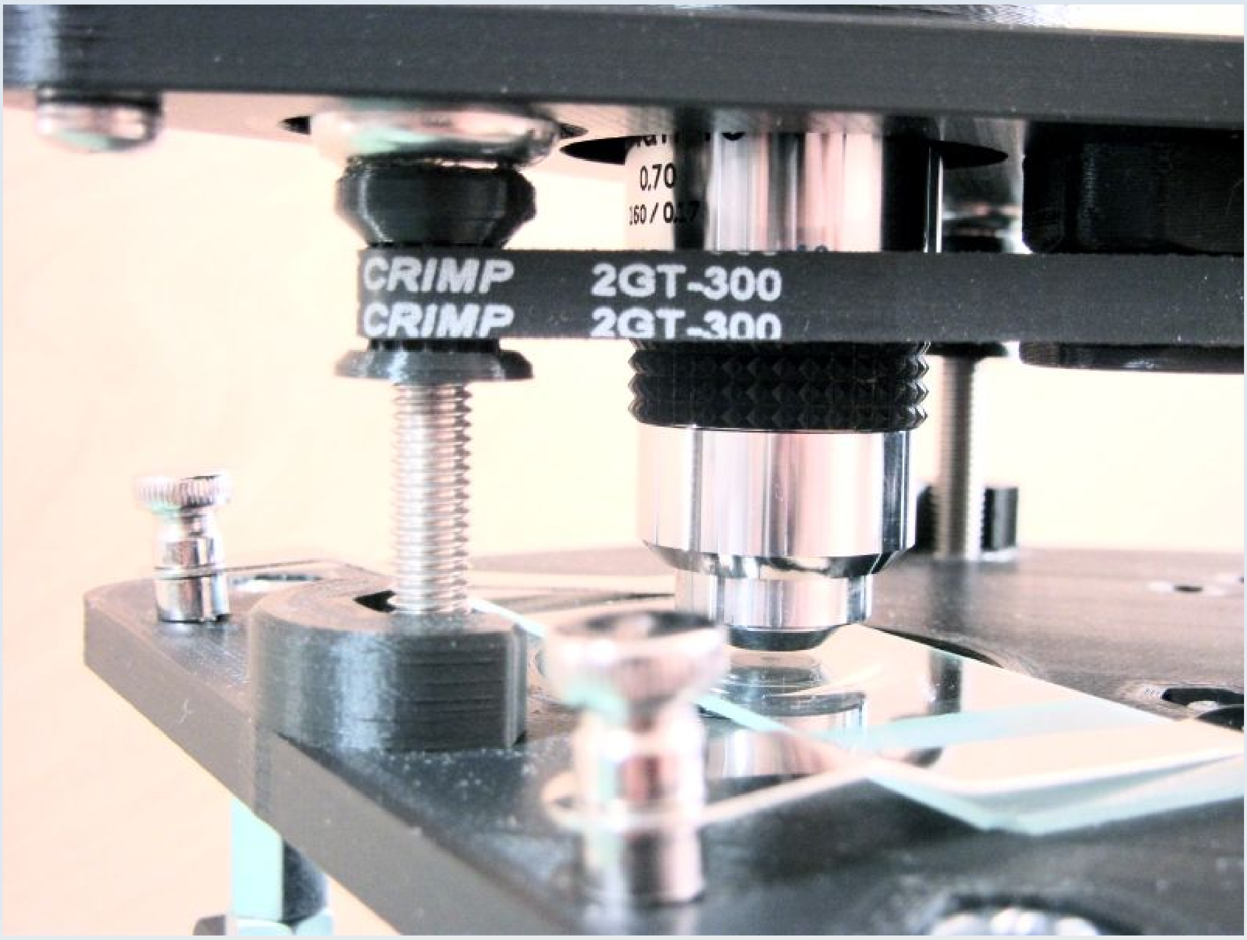

Figure 2.2.1. Stage and objective setup for measuring the NA of the illumination system with an Olympus SPlan x40 objective of stated NA = 0.7.

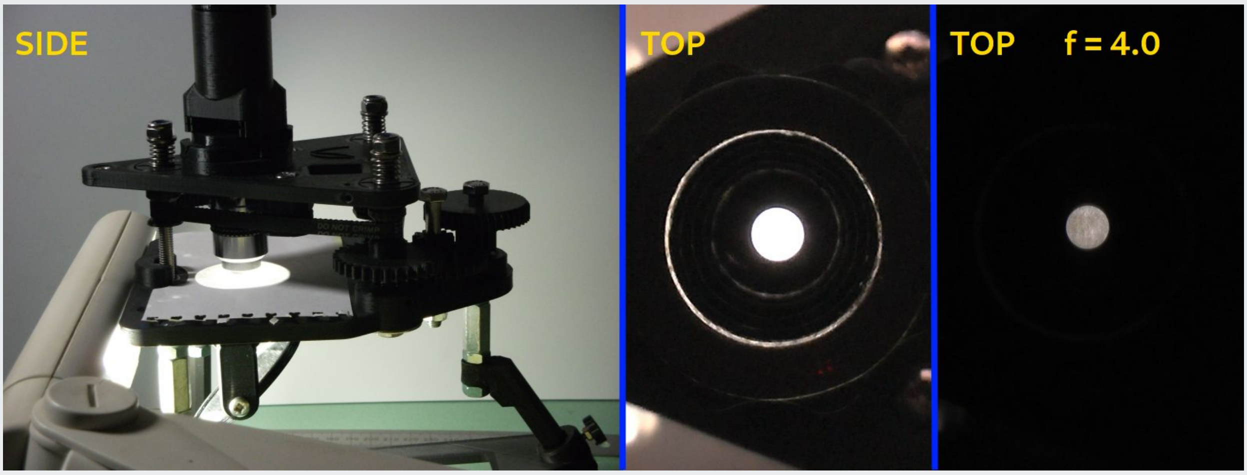

Figure 2.2.2. Setup for measuring the full NA of the objective for a comparison control. A desk lamp fluorescent strip is placed on its side and the mirror reflects the extensive light up to illuminate a piece of paper on the stage (without any condenser present). The view from the top is seen with the 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).

2.3 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.

3. Results: Condenser Plane Projections

Figures 3.1 and 3.2 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 3.1 and 3.2 have a physical size of 20 mm x 20 mm.

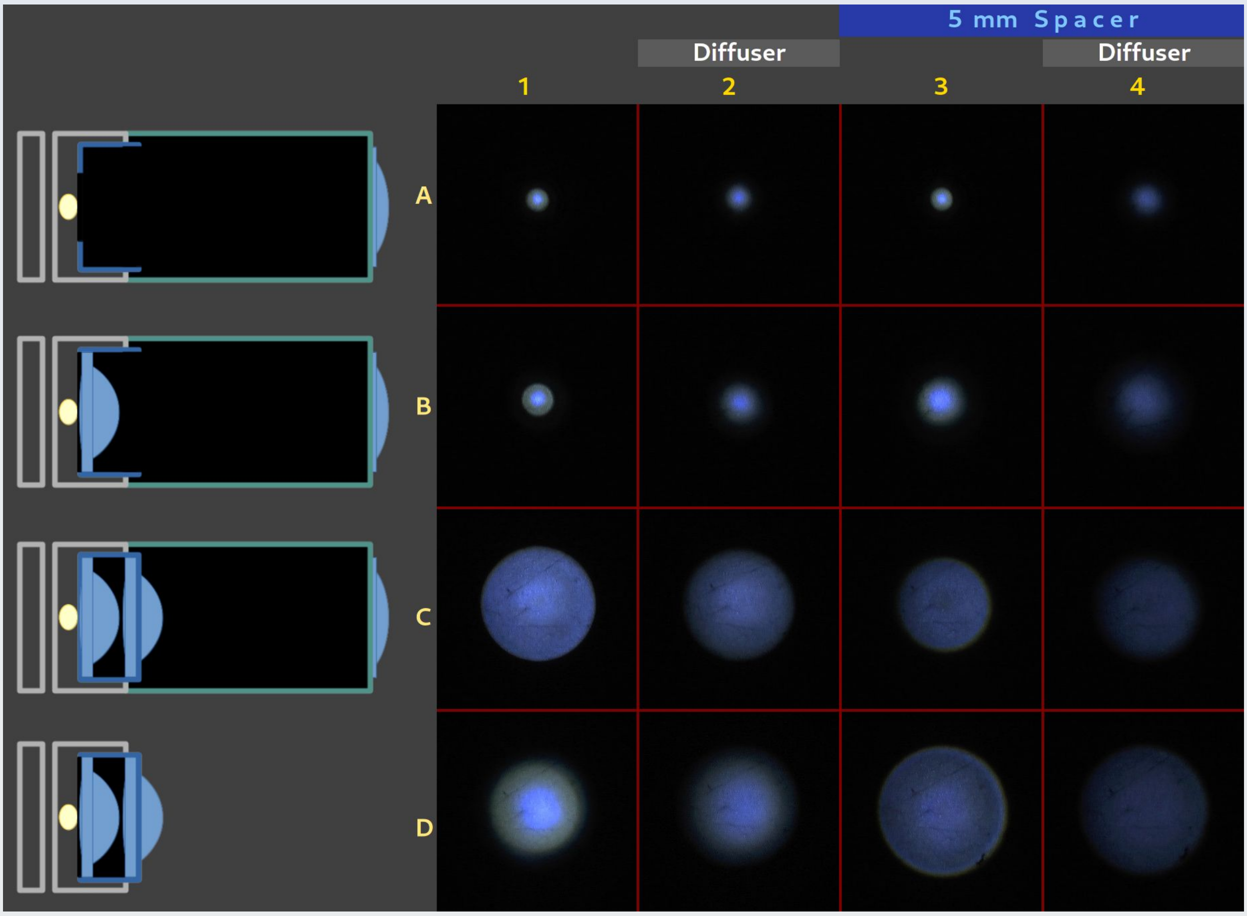

Figure 3.1. Results of condenser plane projection for the various configurations of the PUMA KI system. The capital letters down the side refer to the lower collector lens variant used as described in the text and also illustrated in the diagrams far left. These images show the projections in a natural 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.

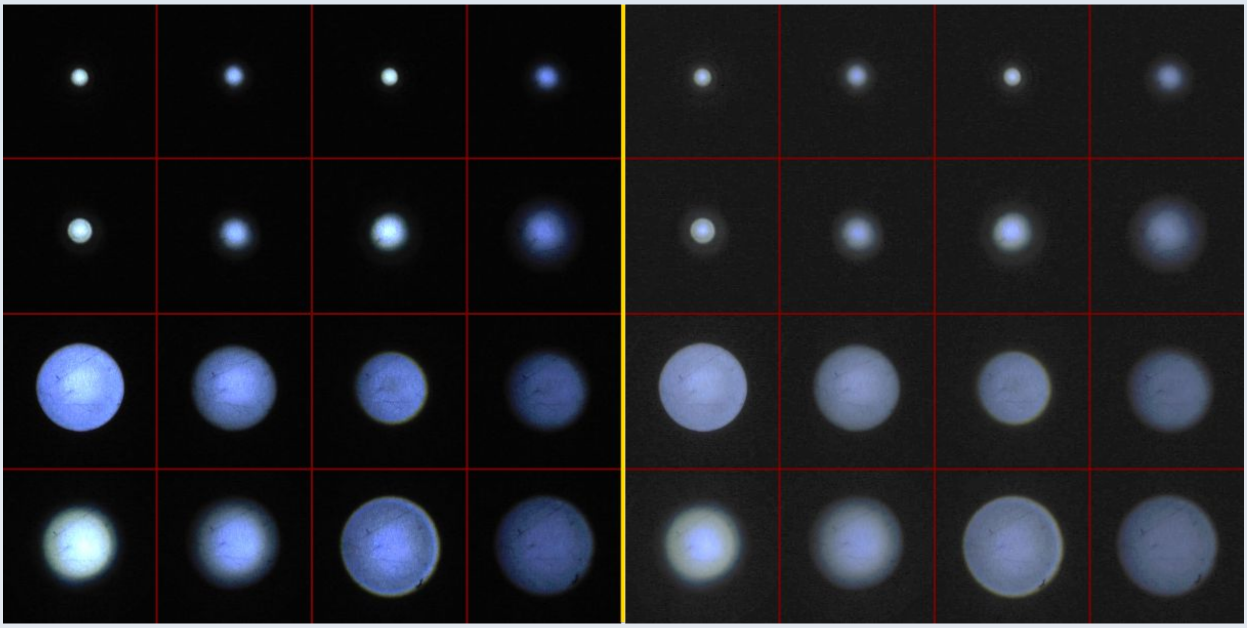

Figure 3.2. Condenser projections shown with intensity levels multiplied by 2 (left) and square root transformed (right) to make it easier to perceive the shape of the light distributions. The arrangement is the same as described in figure 3.1.

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.

4. Results: Condenser NA Measurements

Figure 4.1 shows the control image of the full disc of illumination of the Olympus x40 lens representing a NA of 0.7

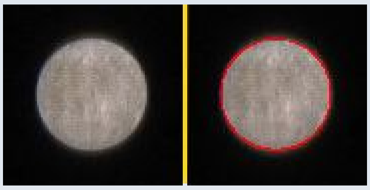

Figure 4.1. The full 0.7 NA of the x40 objective used in these tests. The image on the right shows a red ring drawn at the circumference for superimposing on the rest of the measurements to see how close they come to this ideal NA.

Figure 4.2 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.

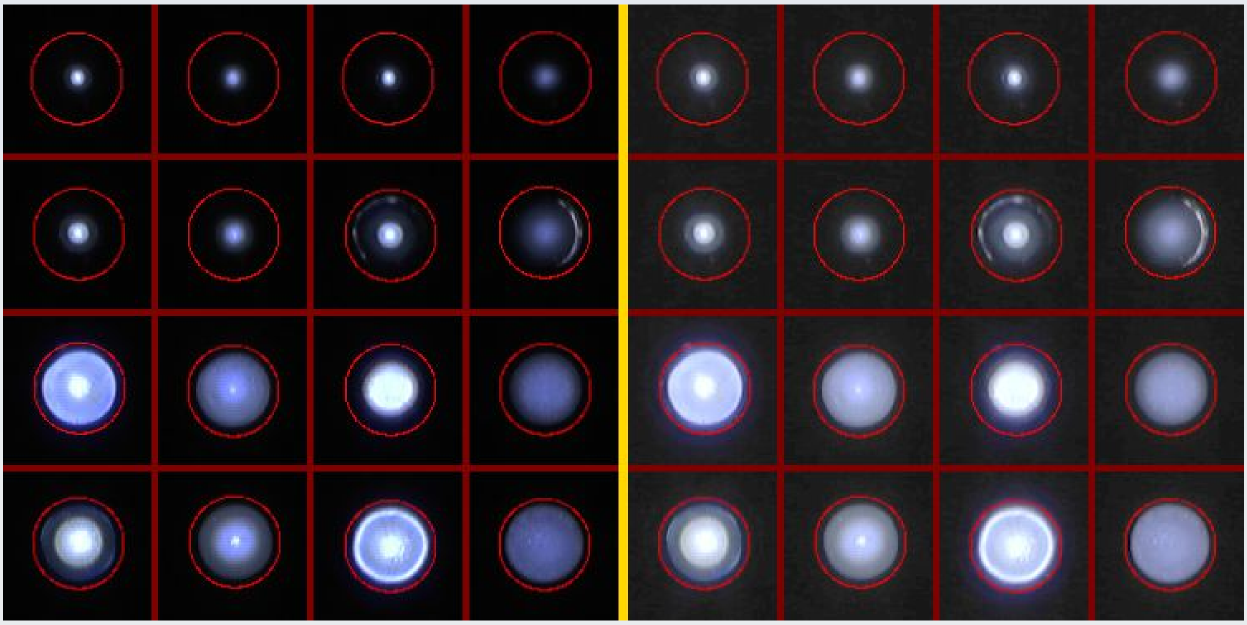

Figure 4.2 Illuminated numerical aperture of a x40 objective with full NA of 0.7 represented by the red rings. Left: Original linear intensity levels. Right) Square root transform of intensity levels. The meaning of the rows and columns are the same as for figures 3.1 and 3.2. The peripheral irregular 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.

As the more modern paraboloid 30 mm lens has a slightly longer focal length compared to the original paraboloid 30 mm lens it will refract light slightly less and provide a slightly lower NA. To see how significant this difference is in practice with a x40 objective, comparisons were made with the condenser using the original 30 mm lens and the new 30 mm lens. Results are shown in figure 4.3.

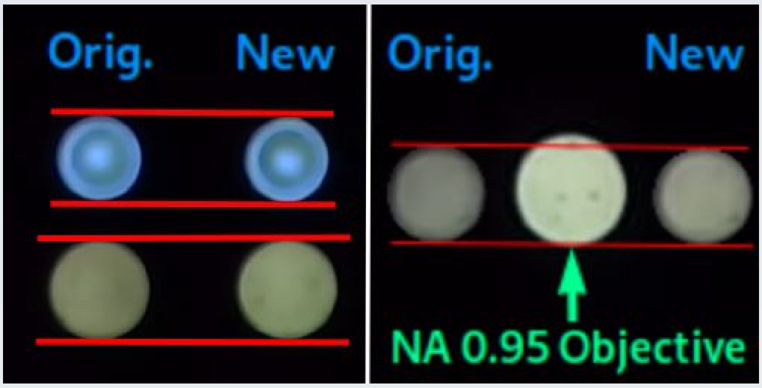

Figure 4.3 Comparison of the NA of the Abbe condenser made with the original 30 mm paraboloid lens (“Orig.”) and new paraboloid 30 mm lens (!New”). ***The left panel*** shows results with an Olympus SPlan x40 objective with NA = 0.7. The top row is with the Köhler illuminator and the bottom row without the illuminator (just using the condenser alone with diffuse illumination of its entire aperture similar to that shown in figure 4.1). ***The right panel*** shows the full NA of the diffusely lit condenser (no Köhler illuminator) with an Olympus MSPlan dry infinity corrected x100 objective with stated NA of 0.95 compared to the full NA of that same objective without a condenser. Both condensers have essentially the same maximum NA of just over 0.92 compared to this. More accurate quantitation of condenser NA is given in table 4.1

This shows that the NA of the condenser using the new 30 mm lens is essentially the same as when using the original 30 mm lens by visual inspection for these dry lenses. Both condensers fill the entire 0.7 NA of the Olympus x40 objective and both show essentially the same maximum NA (of just over 0.92) when compared to the full aperture of a 0.95 NA Olympus MSPlan x100 dry objective. For more detailed results I have performed quantiation of the NA of the condenser (without the Köhler illuminator, i.e. using full illumination of the lower aperture of the condenser with and external diffuse light source – so not restricted to the limited size disc of light projected by the Köhler projector). The results are shown in table 4.1

Objective	**Base parts**	**Condenser NA (original)**	**Condenser NA (new)**
Dry x40	On	0.703	0.705
Dry x100	On	0.921	0.923
Dry x100	Off	0.925	(not tested)
Oil x100	On	1.117	1.106
Oil x100	Off	1.126	1.143

Table 4.1. Numerical aperture measurements of the PUMA condenser using the Horsfall method. The objectives used were Olympus SPlan dry x40 (NA 0.7), Olympus MSPlan dry x100 (NA 0.95, infinity corrected objective), Zeiss Plan oil-immersion x100 (NA 1.25). The immersion oil used was Leica DIN immersion oild with NA=1.518. 'Base parts' refer to whether the IAD slot and base of the condenser were in place ('On') or removed ('Off') for the measurement. Two condensers were tested, one with the original 30 mm lens ('original') and one with the new style 30 mm lens ('new').

Because the base two 3D printed components of the condenser assembly (that make up the IAD filter slot and connection to mirror or Köhler illumination system) partially obscure the full aperture of the bottom aspect of the 30 mm lens to wide angle illumination, I tested the condenser with and without these parts. The only real significant improvement seen without these parts comes with NA over 0.92 – i.e. using oil immersion for higher NA objectives.

Simplistic expectations that the new form factor 30 mm lens might give lower NA compared to the original 30 mm lens (that has slightly shorter focal lengths) were not born out by these experiments. It turns out that the 'square base' rim of the new form factor 30 mm lens can refract and internally reflect light at the periphery in such a way as to compensate (or even over-compensate) for it slight elongation of focal lengths. This is responsible for the surprise result that the maximum possible condenser NA is actually higher (NA = 1.143) with the new form-factor lens than the original (NA = 1.126). Figure 4.4 shows the comparison between the illuminated apertures with the original and new 30 mm lenses in the oiled PUMA Abbe condenser compared to the full 1.25 NA aperture of the x100 oil lens.

Figure 4.4 Comparison of illuminated apertures in the back focal plane of a Zeiss Plan x100 oil immersion lens with full NA illuminated (centre) compared to illumination with an oiled PUMA Abbe condenser using the original 30 mm lens (left) and new form 30 mm lens (right). Note the slight improvement (increase) in NA with the new form factor lens. Although the PUMA condenser still cannot give full 1.25 illumination, the 1.143 illumination provided by the new form 30 mm lens gives a very useable illumination even with x100 oil immersion lenses provided that both the condenser and the lens are oiled. A small air bubble in the oil is seen bottom right in the left had image. This does not affect the NA measurements.

In figure 4.4, the peripheral off-centre dark rim you see inside he light disc with the new 30 mm lens condenser is the junction of the flat rim of the 30 mm lens with its parabolic curved surface. The fact that this rim can also contribute to the illuminated disc is what pushes the NA of the new form factor lens just above that of the original lens, despite the fact that the focal length of the new form factor lens is slightly longer than the original (and so would lead to a simplistic prediction of lower NA illumination).

5. 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 config.

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.

For very high NA imaging, the disc of light produced by the Köhler illumination projector is insufficiently large to fill the lower focal plane of the condenser so alternative means of illuminating the condenser must be used for best (highest resolution) results. When used in this way the PUMA Abbe condenser can provide a maximum NA of 1.143 when used with DIN immersion oil between the condenser and the slide as well as between the coverslip and an oil-immersion objective and with the lower elements of the condenser casing removed to allow maximum angle of incoming light to hit the bottom of the 30 mm lens.

Work Still to Do

1. Experiments with the lensless condenser with an objective.