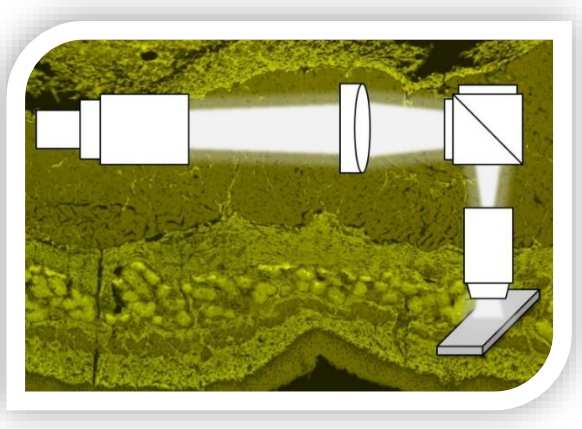
Selecting Commercial Off-The-Shelf Coupler Lenses for Villuminator™ Modules Used in Fluorescence and Bright-Field Microscopy



Epi-fluorescence and bright-field microscope systems benefit from using Villuminator modules for illumination because of the exceptionally high level of field uniformity provided by the Villuminator modules. For best results, an appropriate "coupler lens" should be selected to focus light from the Villuminator module into the microscope objective. This white paper explains how to make such selections.

1. INTRODUCTION

Villuminator™ modules are compact optical systems that produce output beams with exceptionally uniform light distributions [1–4]. When applied to fluorescence and/or bright-field microscopy, a **coupler lens** must be used to focus the output from a Villuminator module into the objective lens, as illustrated in figure 1.

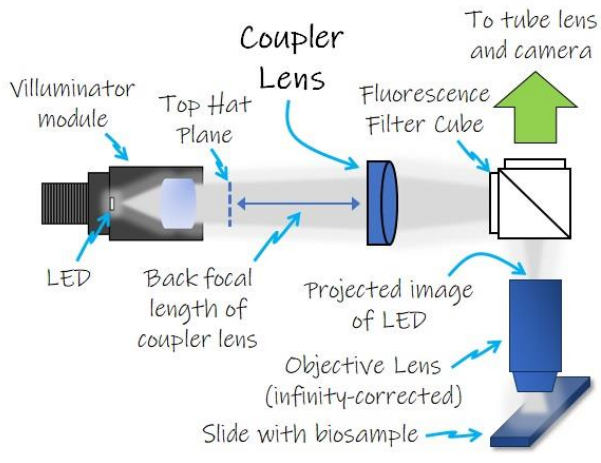


Figure 1. Use of a coupler lens to focus light from a Villuminator module into an objective lens.

Generally, for standard Villuminator modules [5], any commercial-off-the-shelf (COTS) AR-coated achromatic doublet lens having an effective focal length (EFL) of 150 mm (and diameter in the range between 40 to 50 mm) can be used as a coupler lens (e.g., the lens with part number 49-285 from Edmund Optics, or part number AC508-150-A from Thorlabs, can be used as a coupler lens). However, there may be instances where a coupler lens with a different EFL is desirable. In the next section, we provide basic rules for selecting coupler lenses.

2. HOW TO SELECT A COUPLER LENS

2.1 Condition of using objectives from different manufacturers

Figure 2 illustrates the basic rule for coupler lens selection in situations where you may be using objectives from different manufacturers (note that a fluorescence filter cube has not been included in the figure, as it would be a redundant component for the current discussion). **The idea is that, at any specified**

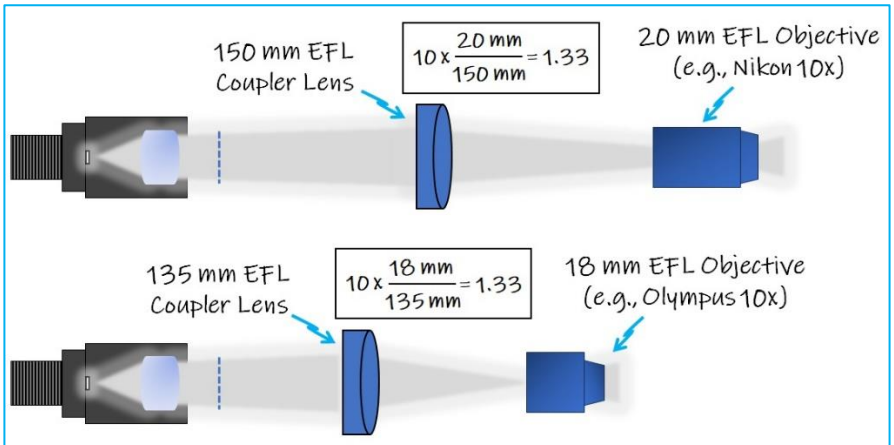


Figure 2. EFL scaling rule for coupler lens selection.

magnification, the product given by the magnification times the ratio of the EFL of the objective to the EFL of the coupler lens should be roughly 1.33:1. Thus, at 10x magnification and an objective with EFL = 20 mm, use a coupler lens with EFL equal to 10 x 20 mm divided by 1.33, which is 150 mm (figure 2, top illustration). Accordingly, if, at 10x magnification, the objective from a different manufacturer has EFL = 18 mm, then use a coupler lens whose EFL is 10 x 18 mm divided by 1.33, which is 135 mm (figure 2, bottom illustration). To determine the EFL of a manufacturer's objective lens, simply divide the EFL of the manufacturer's tube lens by the objective's specified magnification. Typical tube lens EFLs are 200 mm (for Nikon and Leica), 180 mm (for Olympus), and 164.5 mm (for Zeiss) [6]. Thus, for Nikon microscope systems, dividing 200 mm by 10x yields 20 mm, which is the EFL of a Nikon 10x objective (e.g., figure 2, top illustration). For Olympus, dividing 180 mm by 10x yields 18 mm, which is the EFL of an Olympus 10x objective (e.g., figure 2, bottom illustration).

The "EFL scaling rule" described above for coupler lens selection originates from applying the following equation (see Appendix A for a simple derivation):

$$\mathrm{FN}_{\mathrm{ILLUM}} = 21 \times M \times \frac{f_o}{f_c}, \qquad (1)$$

where $\mathrm{FN}_{\mathrm{ILLUM}}$ is the field number that can be accommodated by the illumination from standard Villuminator modules, based on choices for the quantities f_o (the EFL of the objective) and f_c (the EFL of the coupler lens). The quantity M is the magnification of the objective **as specified by the objective's manufacture**r (e.g., if a 10x Nikon objective is used, then let $M = 10$). The value "21" is the diameter (in mm) of a circular area at the top hat plane for which uniformity within a square (inscribed by the circular area) is greater than 95%. Based on equation (1), it can be seen that for a desired fixed field number for the illumination, the quantity given by $M \times (f_o/f_c)$ is a constant. Thus, at fixed magnification across different manufacturers of objectives, the coupler lens EFL must scale proportionately with the EFL of the objective lens, and vice versa. It so happens that when standard Villuminator modules were being designed, the "baseline" (reference) objective lens was assumed to possess a focal length of 20 mm and magnification of 10x. Further, in order to minimize the size (diameter) of the Villuminator module and optimize uniformity, the diameter enclosing the surface area at the top hat plane was fixed at 21 mm. It was then determined that a coupler lens focal length of 150 mm would not only be readily available as a COTS lens, but it would — based on applying equation (1) — yield $\mathrm{FN}_{\mathrm{ILLUM}} = 28$ mm, which should cover the field numbers specified by most manufacturers of microscope objectives. Accordingly, this optimized state for standard Villuminator modules for use in microscopy is characterized by the quantity $\mathrm{FN}_{\mathrm{ILLUM}}/21 = 28\ \mathrm{mm}/21\ \mathrm{mm} = M \times (f_o\ /f_c) = 1.33$.

2.2 Condition of using objectives from the same manufacturer

Under the condition of switching between objectives that are from the same manufacturer of the objectives, the rule is that **you should maintain using the same coupler lens at each magnification**. For example, if you had selected a COTS lens with 135 mm to be used as a coupler lens for a 10x Olympus objective, then you

would use the same 135 mm EFL coupler lens when Olympus objectives with different magnifications are used.

The rationale for the above rule is as follows: when objectives from the same manufacturer are used, the product given by $M \times f_o$ in equation (1) is constant. For example, Nikon infinity-corrected objectives with magnifications at 4x, 10x, and 20x have focal lengths at 50 mm, 20 mm, and 10 mm, respectively. Notice that in each case, multiplying the objective's specified magnification with its focal length yields 200. For Olympus, objectives with magnifications at 4x, 10x, and 20x have focal lengths at 45 mm, 18 mm, and 9 mm, respectively. Here, notice that the product given by $M \times f_o$ yields 180 in each case. Thus, since $M \times f_o$ is constant when switching between objectives from the same manufacturer, then by virtue of the rule that $M \times (f_o\,/f_c) = 1.33$, the coupler lens's focal length should remain fixed.

Despite what has been described above, you can, if you wish, decide to lower the desired value for $\mathrm{FN_{ILLUM}}$, because some objective lenses are specified with field numbers much lower than 28 mm. Furthermore, many commercial cost-effective CCDs and CMOS cameras that have been developed for microscopy applications have image sensor sizes that are significantly smaller than the field numbers of many objective lenses. This means that $\mathrm{FN_{ILLUM}}$ in equation (1) can be < 28 mm. When the desired value for $\mathrm{FN_{ILLUM}}$ is made variable, the value for f_c can be made variable as well, so that you may select any other COTS lens with EFL that is suitable for your needs, as long as f_c, f_o, and M satisfy equation (1).

WORKED EXAMPLE #1: Suppose that a Nikon objective with 10x magnification with specified field number of 22 mm is used in an epi-fluorescence microscope setup in the manner depicted in figure 1. What coupler lens EFL should be used? To answer this question, we first determine the EFL of a 10x Nikon objective. It was stated above that a Nikon tube lens has EFL = 200 mm. So, dividing 200 mm by 10x yields 20 mm, which is the EFL of a 10x Nikon objective. This means that $f_o = 20$ mm. Next, since the 10x Nikon objective is specified for a field number of 22 mm, we may let $\mathrm{FN_{ILLUM}} = 22$ in equation (1) and solve for the required coupler lens EFL, which is $f_c = 190.91$ mm. So, you would try to find a COTS lens with EFL = 190.91 mm. Such a lens can be a single-element plano-convex lens or an achromatic doublet (see section 4 of this white paper). However, it is difficult to find a COTS lens with that exact EFL of 190.91 mm. Moreover, even if you are able to find a COTS lens that has EFL = 190.91 mm, the resulting illumination would cover exactly the 22 mm diameter field number of the 10x Nikon objective. What we prefer is that the illumination covers more than this diameter in order to ensure good flat field illumination across that entire field number. This is achieved if we select a coupler lens with lower EFL. In particular, if we select one with EFL = 150 mm, then according to equation (1), this would result in $\mathrm{FN_{ILLUM}} = 28$ mm. This "illumination field number" of 28 mm would overfill the 22 mm field number specified by the 10x Nikon objective, hence, ensuring that the 22 mm field number of the objective possess top hat illumination. On the other hand, perhaps you may feel that this is "overkill". You may happen to have in your possession an achromatic doublet with part number AC508-180-A from Thorlabs, which has a focal length of 180 mm. If you let $f_c = 180$ mm, $f_o = 20$ mm, and $M = 10$ in equation (1), you would obtain $\mathrm{FN_{ILLUM}} = 23.33$ mm, which is just above the 22 mm field number specified for the Nikon 10x objective. This is a reasonable result, so that under the conditions in the current worked example, using a lens with EFL of 180 mm as a coupler lens with a standard Villuminator module to set up an epi-fluorescence microscope (as depicted in figure 1) is a viable approach. Now, suppose you decide to switch to a 4x Nikon objective. In this case, by way of the rule given above for the condition of using objectives from the same manufacturer, you would maintain using either the 150 mm or the 180 mm EFL coupler lens discussed above.

WORKED EXAMPLE #2: Suppose that an Olympus 10x objective is used instead of the Nikon 10x objective in the previous worked example. What coupler lens EFL should be used with this Olympus 10x objective? As stated earlier, since Olympus tube lenses have 180 mm EFL, dividing 180 mm by 10x yields 18 mm, which

is the EFL of an Olympus 10x objective. So, we have $M = 10$ and $f_o = 18$ mm in equation (1). Suppose we maintain using a coupler lens with 150 mm EFL. This means that we have $f_c = 150$ mm. Applying these values into equation (1) and solving for the field number that is accommodated by these quantities, we obtain $\mathrm{FN_{ILLUM}} = 21 \times 10 \times (18\ \mathrm{mm}/150\ \mathrm{mm}) = 25.2$ mm. This means that if the Olympus 10x objective's specified field number is either equal to or less than 25.2 mm, then a coupler lens with 150 mm can be used with this objective. If this objective's field number is precisely 25.2 mm, then we may wish to overfill the illumination area by selecting a coupler lens with smaller EFL. This can be done by applying the scaling rule provided in figure 2, which suggests that a lens with 135 mm EFL should be used as the coupler lens to go with the Olympus 10x objective. The result would be that $\mathrm{FN_{ILLUM}} = 28$ mm, which can cover the field number specified for most Olympus objectives. However, you may not be able to locate a COTS lens with the precise value of EFL = 135 mm. One way to address this is to use part number 47-739 from Edmund Optics, which is an achromatic doublet with 120 mm EFL and 40 mm diameter. Another option is to combine two "thin" lenses and apply the following equation:

$$\frac{1}{F} = \frac{1}{f_1} + \frac{1}{f_2} - \frac{d}{f_1 f_2}, \qquad (2)$$

where f_1 and f_2 are the EFLs of a first and second lenses, respectively, d is the distance separating the two lenses, and F is the EFL resulting from the two-lens combination. As a simple example, consider the two lenses given by part numbers 49-287 and 49-288 from Edmund Optics. These are achromatic doublets whose EFLs are 250 mm and 300 mm, respectively. If we mount them very close to each other so that $d \approx 0$ in equation (2), then the combined EFL is $F = [(1/250) + (1/300)]^{-1} \approx 136$ mm, which is close to the desired 135 mm focal length.

3. CONSIDERATION FOR THE OBJECTIVE'S ENTRANCE PUPIL DIAMETER

According to figures 1 and 2, notice that when light travels from the coupler lens into the objective, the light rays are made to focus and converge into the back of the objective. This is done in order to ensure that all of the light from the Villuminator module enters what is called the **entrance pupil** of the objective (when light travels in "reverse" — from the specimen plane towards the objective — then the entrance pupil is referred to as the objective's **exit pupil**). Manufacturers of microscope objectives often provide the following formula to compute the size (diameter) of the objective's entrance pupil:

$$\mathrm{ENPD} = 2 \times \mathrm{NA} \times f_o, \qquad (3)$$

where ENPD is the entrance pupil diameter, NA is the objective's numerical aperture, and f_o is the EFL of the objective (i.e., it is the same variable shown in equation (1)).

It can be seen from equation (3) that if either the EFL or NA (or both) of the objective are reduced, then the ENPD is also reduced. This can cause problems for coupling light from a Villuminator module into the objective, as the light may be clipped or "vignetted" by the objective's entrance pupil, which can result in poor illumination uniformity at the specimen plane. **Fortunately, if you follow the basic EFL scaling rule for coupler lens selection that is provided in figure 2, then you would generally not have to be concerned about the reduction in ENPD.** The reason is because the diameter of the projected rays at the back of the objective is proportional to the EFL of the coupler lens. Accordingly, the scaling rule accommodates for the size of the ENPD [7]. In cases where no COTS coupler lenses exist for your application, please contact us at V-BMB and we will do our best to support you (in some special cases, we may be able to customize the Villuminator module such that the physical size of the LED inside is reduced, thereby producing a reduced projected size of rays into the back of the objective).

It is useful to gain an intuitive feel for the impact of the objective's ENPD on the illumination uniformity at the specimen plane. Figure 3 shows what the image of the LED from the VIL151B standard Villuminator module looks like at the back of an objective when the module is used with a 150 mm EFL achromatic doublet from Edmund Optics as the coupler lens (as

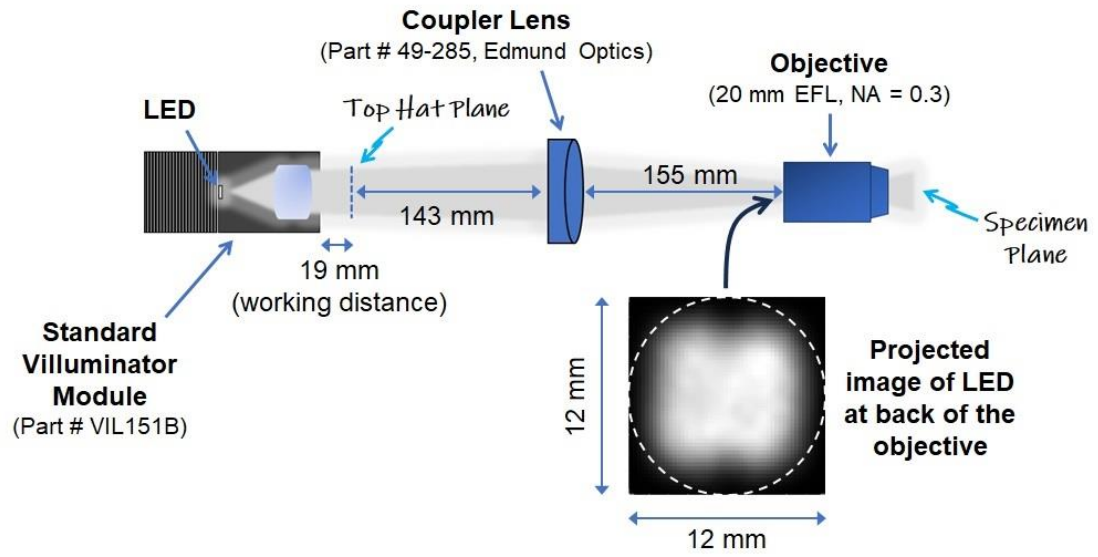


Figure 3. The light distribution at the back of an objective when using the VIL151B standard Villuminator module with part number 49-285 (from Edmund Optics) as the coupler lens.

mentioned in reference 5, the VIL151B part number refers to a standard Villuminator module that uses a white light LED). In this figure, notice that the distance between the top hat plane and the left surface of the coupler lens is 143 mm. This distance is roughly the back focal length (BFL) specified by Edmund Optics for this COTS lens (you would see it specified on their website for this lens). This means that here, we are letting the back of this achromatic doublet face towards the Villuminator module. Actually, as is explained in a separate white paper [2], the orientation of the coupler lens is not very strict, so that you may face either its front or back surface towards the Villuminator module. Reference 2 provides further guidance on the placement of the coupler lens, relative to the objective.

Now, in figure 3, we display a dotted circle with 12 mm diameter superimposed on the image of the LED. The 12-mm diameter is the ENPD resulting from using an objective having NA = 0.3 and EFL = 20 mm (see equation (3)), which are typical values for the NA and EFL of a Nikon 10x objective. By applying the data for the projected LED image shown in figure 3, it becomes possible to use an optical design program to model the illumination profiles at the specimen plane for a variety of "representative ideal objective lenses" from different manufacturers of objectives, as shown in figure 4. In this figure, the objectives have been modelled in the Ansys Zemax OpticStudio® program by way of applying the so-called "cardinal lens" optical model that has been developed by professor J. P. Wilde [8]. This special lens type models an **aplanatic lens**, which, roughly speaking, is the type of lens that satisfies equation (3). It so happens that many well-designed high NA microscope objectives tend to satisfy equation (3), so that it becomes possible to apply the cardinal lens to model idealized microscope objective lenses from different manufacturers without knowing the actual design prescriptions of those objectives.

As shown in figure 4, an idealized model of a 10x Zeiss objective with 0.3 NA has ENPD = 9.87 mm (by virtue of the fact that its EFL = 16.45 mm and by applying equation (3)), which results in clipping of some light at the objective's pupil. This has caused the illumination profile to be non-uniform at the specimen plane. To fix this, figure 5 shows what happens when a coupler lens with lower EFL is used for that objective. Applying the EFL scaling rule as before, the ideal coupler lens EFL for a 10x 0.3 NA Zeiss objective should be (10 × 16.45 mm)/1.33 ≈ 123 mm. A close approximation is part number 47-739 from Edmund Optics (40 mm diameter, 120 mm EFL, achromatic doublet).

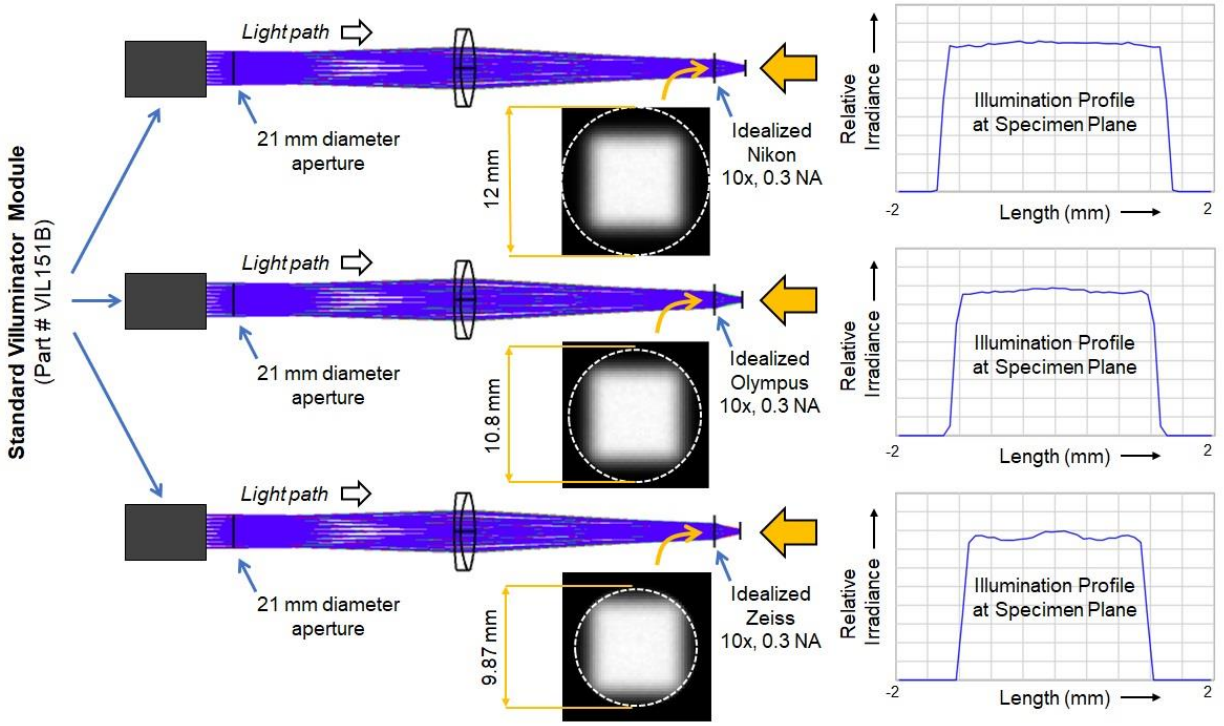


Figure 4. Simulated illumination profiles at the specimen plane when the system in figure 3 is used with idealized models of objectives specified with 10x magnification and NA = 0.3 for Nikon (top), Olympus (middle), and Zeiss (bottom). Also shown is the projected image of the LED at the back of the objectives. See figure 5 for the solution to having a small ENPD.

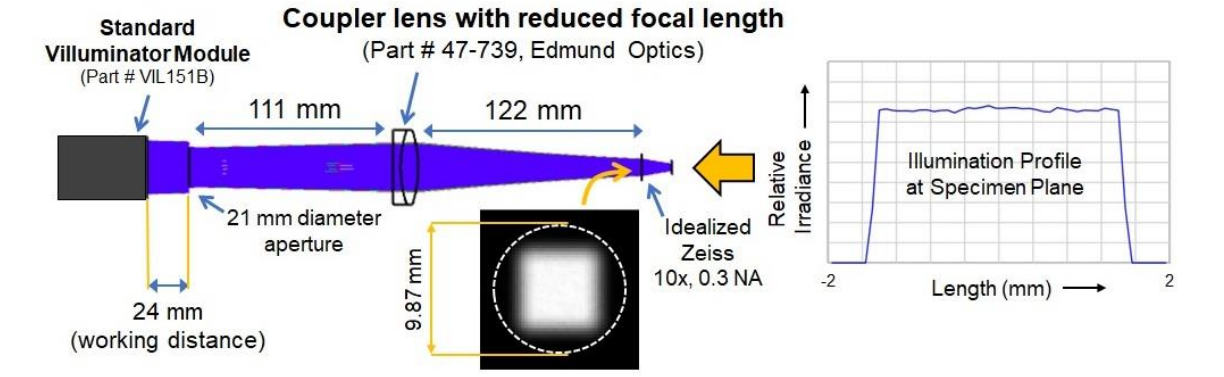


Figure 5. Improved simulated illumination profile at the specimen plane for the idealized model of the Zeiss objective from figure 4, based on using a coupler lens with lower focal length.

Applying this lens yields the improved illumination uniformity displayed in figure 5. Additionally, the **working distance** (distance between the front of a Villuminator module and the top hat plane [2]) has been adjusted. As explained in reference 2, the working distance is a variable and can be adjusted to compensate for some residual non-uniformity observed in the final plane of illumination (such as the specimen plane of a microscope objective). Of course, it should be noted that higher NAs yield larger ENPDs, which help in

minimizing the light-clipping effects. For instance, there are Zeiss objectives with 10x magnification that have NA = 0.45 or higher (see, e.g., the Zeiss Plan-Apochromat 10x/0.45 M27, item # 420640-9900-000).

4. COUPLER LENS DIAMETER AND OTHER CONSIDERATIONS

Once the EFL of a coupler lens has been decided upon, select an AR-coated achromatic doublet with diameter in the range between 40 to 50 mm. The AR coating ensures minimal "ghost" reflections from the coupler lens's surfaces (note that if you are using a Villuminator module with ultraviolet output, you may wish to select a COTS lens with extended range of wavelengths for its AR coating). The 40-50 mm diameter ensures no clipping of light by the edges of the coupler lens. An achromatic doublet is preferred over single-element lenses because this enables you to use the coupler lens for any choice of LED wavelengths (including white light LED) offered for Villuminator modules. However, if you cannot find an achromatic doublet possessing a desired EFL, then you may open up your choices of lenses to include single-element plano-convex or double-convex lenses.

5. SUMMARY

- For illumination in epi-fluorescence and bright-field microscopy systems, use compact standard Villuminator modules (see reference 5 for a description of their ordering part numbers)
- Compute the EFL of your objective by dividing the objective's pairing tube lens's EFL (specified by the objective's manufacturer) by the objective's specified magnification
- After computing the objective's EFL, apply the scaling rule illustrated in figure 2 to determine the EFL for a coupler lens (i.e., the product given by $M \times (f_o/f_c)$ should be $\approx$ 1.33:1)
- For further flexibility in determining "in-between" EFL values for coupler lens choices, you may apply equation (1) by first deciding on a sufficiently large illumination field number (i.e., the quantity $\mathrm{FN}_{\mathrm{ILLUM}}$), then calculate the resulting coupler lens EFL, based on the desired magnification M and objective focal length f_o
- If necessary, combine two COTS lenses by way of applying equation (2) in order to arrive at a coupler lens EFL value that is close to the desired f_c value (i.e., attempt to satisfy $F = f_c$, where F is given by equation (2))
- Once the EFL of the coupler lens has been determined and that it matches with that from a COTS lens, ensure that the diameter of the COTS lens is in the range 40 to 50 mm
- Try to select an available COTS achromatic doublet with the desired EFL to be used as the coupler lens (ensure that it is AR coated); Otherwise, single element plano-convex COTS lenses are also "ok"

APPENDIX A: DERIVATION OF EQUATION 1

Figure A1 illustrates the geometry for rays that are related to deriving equation (1). In this figure, the dotted black rays are traced from the rim of the field aperture (located at the top hat plane) to a focal area at the entrance pupil of the objective. They then travel pass that focal area and end up at the specimen plane, where they illuminate a circular area with diameter D_{ILLUM}. Thus, the quantity D_{ILLUM} is the diameter of the illuminated area with top hat uniformity, and it is also a projected image of the field aperture, whose diameter is D_{APER}. The quantities f_c and f_o are the EFLs of the coupler lens and the objective lens, respectively. The dotted black rays generate two triangles between the coupler lens and the specimen plane, whose trigonometric relationship is described by

$$\frac{D_{\mathrm{APER}}}{f_c} = \frac{D_{\mathrm{ILLUM}}}{f_o}. \qquad \text{(A.1)}$$

Cross-multiplying and solving for D_{ILLUM} in equation (A.1), we obtain

$$D_{\mathrm{ILLUM}} = D_{\mathrm{APER}} \times \frac{f_o}{f_c}. \qquad \text{(A.2)}$$

Now, when light from the specimen plane travels through the objective and a tube lens, which creates the image of the specimen plane onto an image

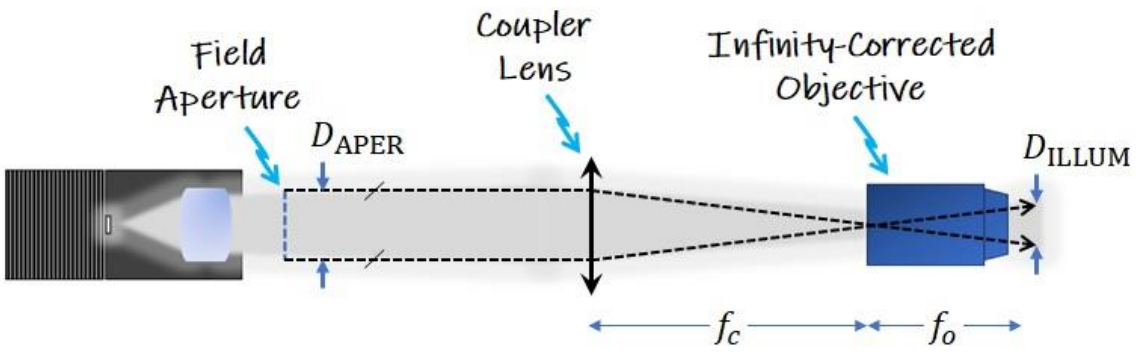


Figure A1. Geometrical construction for deriving equation (1).

sensor in a camera, the illuminated area with diameter D_{ILLUM} at the specimen plane would be magnified by a factor M at the camera. This magnification factor can possess any value given by the ratio of the focal length of the tube lens being used to the focal length of the objective. While it is sometimes possible to mix and match tube lenses and objectives from different microscope manufacturers, it is often recommended by the manufacturer to use tube lenses and objectives that have been produced by the same manufacturer. In this case, if we make the conventional assumption that the magnification M specified for an (infinity-corrected) objective is the magnification for that objective, then multiplying the right side of equation (A.2) by M yields the "illuminated field number" at the camera plane, which we denote as $\mathrm{FN}_{\mathrm{ILLUM}}$. Thus, we have the expression:

$$\mathrm{FN}_{\mathrm{ILLUM}} = D_{\mathrm{APER}} \times M \times \frac{f_o}{f_c}. \qquad (A.3)$$

Finally, since standard Villuminator modules are designed to have $D_{\mathrm{APER}} = 21$ mm, substituting this value into equation (A.3) yields equation (1).

REFERENCES & NOTES

[1] R. Siew and L. Tan, "Top Hat Illumination Provides Even Light Distribution Across Samples," *Biophotonics* **30**(1), pp 32-37.
[2] R. Siew, "Introduction to Using Villuminator™ Modules for Producing Uniform 'Top Hat' Illumination," V-BMB white paper, available at https://v-bmb.com/vil-whitepapers.
[3] How to use Villuminator™ modules to produce uniform "top hat" illumination in projection and bioimaging: VBMB Solutions Made Simple (SMS) Video Series in Optics – 01: watch on YouTube.
[4] Tuning illumination uniformity using Villuminator™ modules: VBMB Solutions Made Simple (SMS) Video Series in Optics – 02: watch on YouTube.
[5] Standard Villuminator modules (sometimes also called "compact standard Villuminator modules") are modules whose part numbers are written in the form "VIL151X", where the letter "X" identifies the wavelength choice. The most popular wavelength identifier numbers are B, E, K, N, and Q, which are, respectively, neutral white, ultraviolet (385 nm), blue (470 nm), lime (567 nm), and red (623 nm). Thus, a module with the part number VIL151B refers to a Villuminator module with a white beam output, due to the use of a white light LED in the module. The complete brochure for standard Villuminator modules is available at https://v-bmb.com/villuminator%E2%84%A2
[6] T. S. Tkaczyk, *Field Guide to Microscopy* (SPIE Press, 2010), p 35.
[7] It should be noted that the product given by $\mathrm{NA} \times f_o$ for objectives from the same manufacturer tends to be fairly constant, so that objectives with lower f_o (from the same manufacturer) tend to possess proportionately higher NA and vice versa. It is in this sense that the scaling rule provided in figure 2 accounts for the ENPD. In cases where only the NA

of an objective is of some value that further reduces the ENPD, then simply treat the reduction in the NA as being equivalent to having the f_o be reduced. For example, suppose that you have been successfully using a standard Villuminator module with a 10x Nikon objective (whose EFL is 20 mm) at 0.3 NA. Then, suppose that you would also like to use a 10x Nikon objective but with 0.25 NA. In this case, both objectives have the same EFL, but the second one has a reduced NA, which reduces the ENPD. To fix this, we first note that the reduction in NA is 0.25/0.3 ≈ 0.83. Next, multiply 0.83 by the objective's EFL to get 0.83 x 20 mm = 16.67 mm. Finally, apply the scaling rule (figure 2) by computing (10 x 16.67 mm)/1.33 ≈ 125 mm. This is the EFL of the coupler lens to be used with the 10x Nikon objective with 0.25 NA.

[8] J. P. Wilde, "Ray-tracing model of a perfect lens compliant with Fermat's principle: the Cardinal Lens," *App. Opt.* **63**(4), pp. 1110-1124 (2024).

ABBREVIATIONS

AR, anti-reflection
BFL, back focal length
CCD, charge coupled device
CMOS, complementary metal oxide semiconductor
COTS, commercial off-the-shelf
EFL, effective focal length (used synonymously with the term "focal length" in this white paper)
LED, light emitting diode
NA, numerical aperture
V-BMB, Venture Biotech Modules Business Pte. Ltd.

AUTHOR

Ronian Siew is an optical consultant at V-BMB. He has published technical books and scientific peer-refereed research papers on optical system design, and serves as an associate editor for SPIE's Spotlight series in the area of optical design and engineering.

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