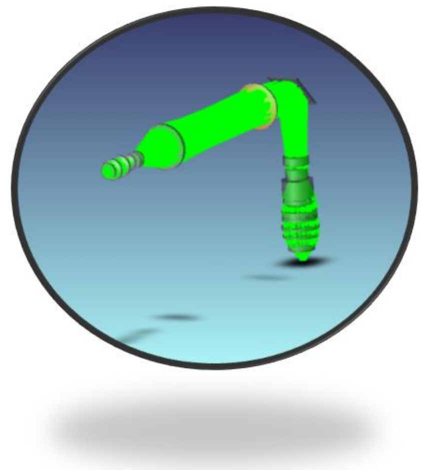
Fluorescence Microscopy with Flat Field Illumination using LEDs and Off-The-Shelf Spherical Lenses



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July 28, 2021, Rev. 1
https://doi.org/10.6084/m9.figshare.15073053

1. INTRODUCTION

In a 2018 article [1], Henry Schek wrote, "Like other applications, fluorescence microscopy wants the tantalizing flexibility of solid-state lighting. But adapting low-cost lighting to the ubiquitous compound microscope requires innovation." He is right! Today, a continuing problem requiring innovation when using solid-state lighting is the generation of uniform "top-hat" or "flat field" illumination with incoherent sources, such as light emitting diodes (LEDs). In contrast to this, the solution is relatively straightforward for coherent sources — such as lasers — where TEM_{00} Gaussian beams have zero étendue at the beam waist [2 – 10]. For the incoherent case, if the source is small relative to an optical system's entrance pupil, then the source's étendue may be considered approximately zero, and it becomes possible to generate flat irradiance distributions at a distant screen using a small LED with aspherical lens elements [11], or even with spherical elements [12 – 14]. If the LED is large, then there are many published techniques using aspherical and freeform lenses [15 – 19]. Recently, I developed a special technique using a combination of "condenser and shaper" spherical lenses for the case of large LED sizes [20]. I now briefly describe its application to producing flat field illumination for fluorescence microscopy.

2. FLAT FIELD ILLUMINATION USING OFF-THE-SHELF SPHERICAL LENSES AND A LED SOURCE FOR FLUORESCENCE MICROSCOPY

The technique that is described in my earlier study [20] uses a combination of custom-designed spherical lenses comprising a condenser group and a shaper group (Fig. 1). The result is an irradiance distribution (flux per unit area) at a plane that is uniform and also does not contain any localized structures that may be present on the source's surface, which is evident if one notices that the source's intermediate image lies inside the shaper group. Additionally, even if the source is non-circular, the shape of the perimeter of the irradiance distribution is approximately circular. This is due to the "heavy" amount of spherical aberration induced by the spherical elements. These properties seem to be just nice for applications involving illumination in the compound microscope, because a microscope's objective lens is often designed to have a circular field of view, and, as Schek [1] mentioned, there is interest in applications involving fluorescence microscopy to use LEDs as illumination sources. Moreover, it seems highly desirable to have uniform illumination (i.e., uniform "globally" in the sense of having an overall flat top distribution, and uniform "locally" in the sense of having no visible source structures) across the field of view.

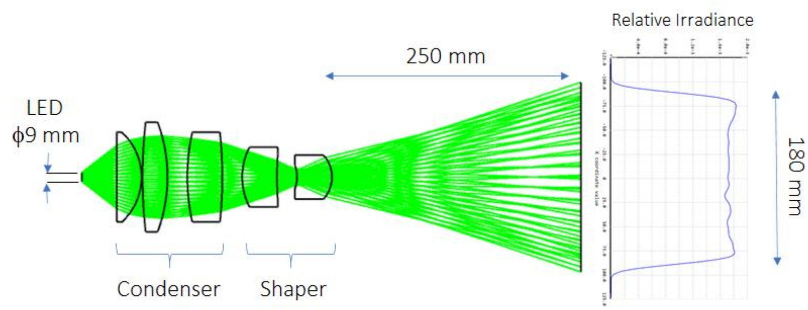

Fig. 1 All-spherical lens system comprising "condenser" and "shaper" groups and a 9-mm diameter LED, producing top hat illumination at a screen located 250 mm from the last element.

In order to apply the system depicted in Fig. 1 to illumination in fluorescence microscopy, one needs to couple light into a microscope objective. Usually, such objectives are infinity-corrected. This implies that, since the desired uniform illumination must be at the plane of focus of the objective, the plane having top hat illumination shown in Fig. 1 must be imaged onto the focal plane of the objective. This is achieved by mounting a field lens near the flat field plane to roughly collimate the rays, followed by another lens to couple light into the objective. The result is an optical system layout depicted in Fig. 2.

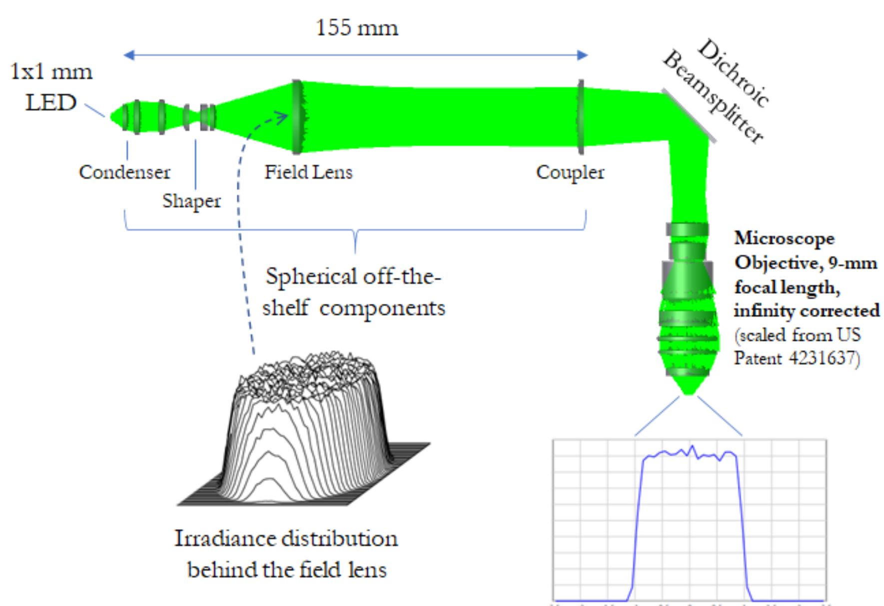

Fig. 2 Illumination system producing a uniform top hat distribution at the focus of a microscope objective. All lenses in the illumination system are spherical commercial off-the-shelf lenses.

In Fig. 2, the condenser-shaper lens groups were first scaled down in size, followed by re-optimization (using Zemax® OpticStudio®) and iterating between replacing the lenses with commercial off-the-shelf (COTS) spherical elements. For confidentiality, the part numbers and system prescription are not revealed here. The field lens and coupler lens are both also COTS lenses, and both are plano-convex. The microscope objective was adapted from US Patent 4231637 by scaling (it is provided by Zemax's Zebase lens catalog, which I used). The LED is assumed to be square (1 mm x 1 mm), with a Lambertian emission profile. Even if a LED does not have a Lambertian emission, as long as its radiant intensity (flux per unit solid angle) is rotationally symmetric, one can numerically determine the right design solution [20]. However, it should be noted that if light guides are used to channel light from a LED, the output of the light guide is potentially highly non-Lambertian and likely to have angular ray distributions that change the irradiance profile as the beam propagates from the exit of the fiber. For such systems, it is highly desirable to characterize the output from the fiber before attempting the beam shaping technique described in this white paper.

3. IT IS NOT REALLY A KÖHLER ILLUMINATION SYSTEM

Contrary to popular belief, when coupling illumination into a microscope that uses an infinity-corrected microscope objective, it is not necessary to image the source onto the back (or entrance pupil) of the objective in order to homogenize the illumination (to avoid seeing localized structures at the source). An infinity-corrected objective is a well-corrected lens, which means that pairs of rays traversing a line from infinity will meet at the focal plane of the objective. Therefore, if it is desired to have a plane of uniform irradiance be imaged onto the objective's focal plane, then that uniform plane just needs to be at infinity from the perspective of the objective. Don't worry about the source plane, because as long as it is not conjugate with the field plane, then no matter what you do, the source can't make it to the objective's focal plane, due to fundamental geometrical optics. All that is needed is that the numerical aperture of the coupling lens match the half-angle field of view of the objective (which is determined by the semi-diameter of the objective's field of view and the objective's effective focal length). This concept was applied to the system shown in Fig. 2, where no attempt was made to focus the image of the source onto the aperture stop of the objective (which lies inside the objective). Shifting the objective towards and away from the coupler lens does nothing to the irradiance distribution at the sample plane, other than vignetting. Due to the concept explained, the illumination system shown in Fig. 2 is not strictly of the Köhler type, nor is it of the Abbé or Nelson (critical) type. It is quite distinct from any of these illumination concepts (I have been calling it "Ronian Illumination" for amusement).

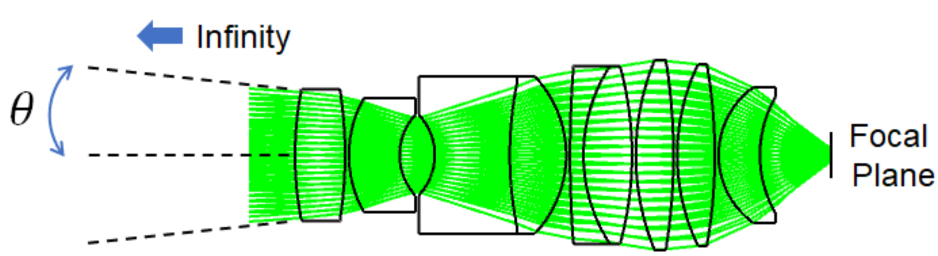

Fig. 3 The half-angle field of view θ for an infinity-corrected microscope objective determines the acceptable numerical aperture for illumination rays coupling into the objective.

4. CONCLUSION

Generation of "locally" and "globally" uniform "top hat" irradiance distributions from LED sources is possible using only spherical lenses, and, under some conditions, it is even possible to use commercial off-the-shelf spherical lenses. Of course, customized lens designs offer optimum performance, especially for a wider set of conditions (such as if using larger size LEDs, or LEDs with a non-Lambertian radiant intensity). Additionally, it is also possible to replace any of the spherical lenses with aspherics, where needed. But spherical lenses are the simplest and most cost-effective lenses for design and manufacture, and they are available in many commercial lens catalogs. Therefore, the technique and system presented in this white paper is expected to be of interest to anyone involved in fluorescence microscopy applications.

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