Introduction to Using Villuminator™ Modules for Producing Uniform "Top Hat" Illumination

Villuminator modules are compact modular subassemblies that produce exceptionally uniform illumination across a surface. This property benefits many applications involving the need to illuminate planar regions of interest, such as spatial light modulators in projection systems and specimen planes being observed under a microscope. In this white paper, we explain how to use Villuminator modules for a variety of illumination applications.

1. INTRODUCTION

In the Jan/Feb 2023 issue of *Biophotonics*, Advanced Products Corporation (APC) described how biotechnology applications are benefiting from using special devices called Villuminator™ modules, which are compact optical systems that produce output beams with exceptionally uniform light distributions [1]. Soon after, the Venture Biotech Modules Business (V-BMB) was formed, offering a variety of precision control solutions, and expanding the Villuminator module product line, providing analytical instrument developers and researchers with unparalleled uniform illumination.

Figure 1. Compact standard Villuminator modules.

Villuminator modules have been offered in a variety of sizes and designs. For example, the three modules shown at the top right of this page have diameters between 50 and 60 mm, and length of roughly 130 mm (inclusive of a heat sink for the source). Their output beams are in the red, green, and blue. Other designs are smaller, such as the four modules shown in figure 1. These compact standard designs have dimensions of 40 mm (diameter) x 78 mm (length). The four modules in figure 1 produce output beam colors in green, blue, red, and white. Other colors (wavelengths) are also available, ranging from the near ultraviolet through the visible (including white) and near infrared.

2. PRIMARY COMPONENTS OF A VILLUMINATOR MODULE

Figure 2 shows the components of a Villuminator module. A module consists of a LED, heatsink, power cord, and a patent-pending optical system that projects a non-divergent beam out of the module.

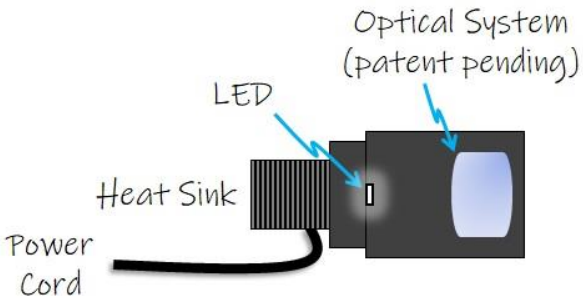

Figure 2. Components of a Villuminator module.

As figure 3 illustrates, the output beam produces a highly uniform distribution on an **intermediate plane** located a short distance from the front of the module. The distance between the front of the module and the intermediate plane is called the **working distance** of the module. Due to the exceptionally uniform illumination distribution at the intermediate plane, the profile of the illumination is referred to as a "**top hat**" distribution (i.e., the profile looks like a hat with a flat top).

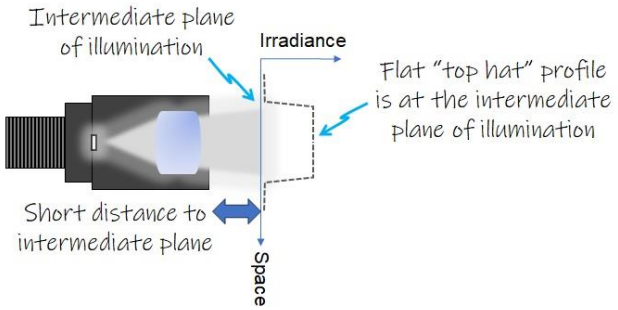

Figure 3. Uniform "top hat" illumination at the intermediate plane from a Villuminator module.

Sometimes, a top hat illumination profile is also called a "**flat field**" distribution, which is a term borrowed from observational astronomy, in which *flat-fielding* refers to a calibration process to ensure having an even response across the surface of a charge coupled device (CCD) image sensor [2]. Thus, some refer to Villuminator modules as "flat field illumination" devices. Unfortunately, the term "flat field" has a different definition in the subject of imaging optics. In particular, when a lens forms a sharply focused image over the full field of view in a camera, the image is said to have a flat field because the *field curvature* image aberration is absent, so that one has a "planar image field" at the camera sensor. Accordingly, microscope objectives that produce sharp specimen images over the entire field of view in a microscope are called "plan objectives", in which the word "plan" refers to a planar image. In order to avoid ambiguity, at V-BMB, the terms **top hat illumination** and **flat field illumination** are taken to mean the same.

3. HOW TO ILLUMINATE A DIFFERENT (SECONDARY) PLANE

Customers may wonder what is the use of a Villuminator module for illumination applications if the top hat plane is located a short distance in front of the module. In fact, while this intermediate plane can indeed serve as the final plane of illumination for some application, a different plane may be illuminated by simply projecting the image of the intermediate plane towards a secondary plane, as illustrated in figure 4. In this way, the illuminated area on the secondary plane may possess any size, depending on a suitable choice for the lens being used to perform the projection. Almost any lens with positive effective focal length (EFL) may be employed to project the illumination. For instance, as depicted in figure 4, if a simple thin double-convex lens is used, then one may apply the well-known simple lens formula given by

$$\frac{1}{f} = \frac{1}{s} + \frac{1}{s'} \qquad (1)$$

where f is the lens's EFL, s is the distance between the intermediate plane and the thin lens, and s' is the distance between the thin lens and the secondary plane to be illuminated. The magnification of the illumination is then given by the ratio s'/s. Evidently, larger illuminated areas require a condition of $s' > s$.

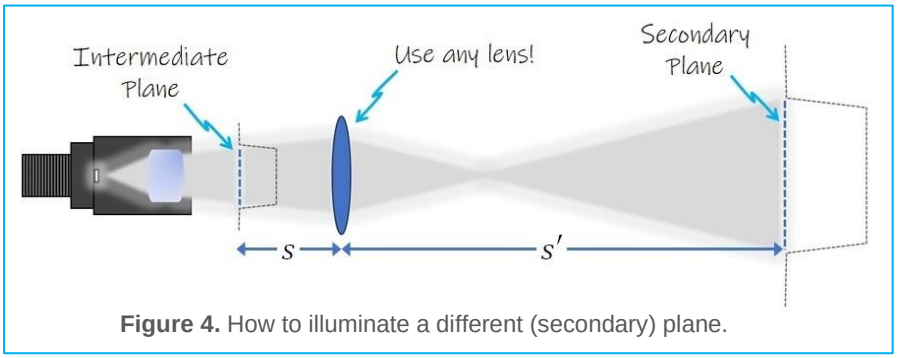

Figure 4. How to illuminate a different (secondary) plane.

A machine vision lens used in reverse can also do the job of projection, as shown in figure 5. In this figure, the lens labelled "Projection Lens" is a commercial off-the-shelf (COTS) machine vision lens whose right side is the front of the lens, and the left side (facing the Villuminator module) is the rear of the lens. In this example, rather than having an image sensor be located at

the rear focal plane of the machine vision lens, it is the intermediate plane of the Villuminator module that is located at that plane. The result is an enlarged projected image of the top hat distribution onto a screen at about 200 mm from the front of the machine vision lens. Figure 6 shows what the illumination profile looks like across, while figure 7 displays a 3D view of its full surface profile.

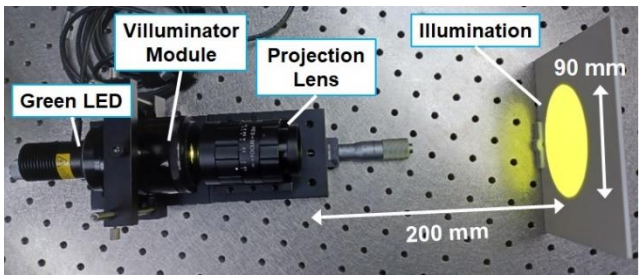

Figure 5. Example of using a machine vision lens (labelled as "Projection Lens") in reverse to project the top hat distribution from the Villuminator module's intermediate plane towards a different plane.

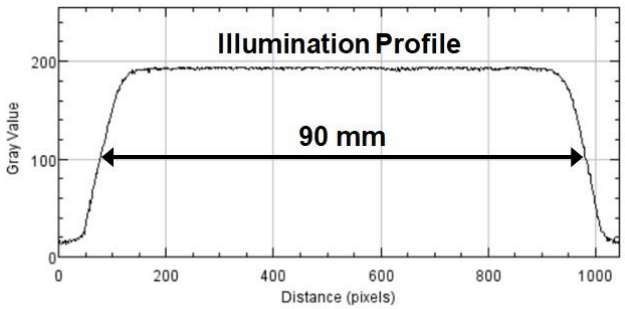

Figure 6. One-dimensional profile of the illumination at the screen of figure 5.

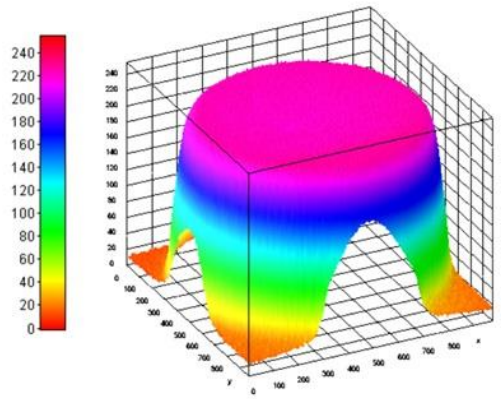

Figure 7. 3D view of the surface profile of the illumination at the screen of figure 5.

4. HOW TO CORRECT FOR NON-UNIFORM ILLUMINATION PROFILES

In some cases, the projected illumination on a screen (i.e., the secondary plane) may display some form of non-uniformity. This can often be due to three main causes: (1) The inherent imaging aberrations of the projection lens. (2) The inherent *relative illumination* properties (including some vignetting effects) of the projection lens. (3) The cosine-4th power effect of angled rays striking the screen. Any or all of these may occur despite having a top hat distribution created by the Villuminator module at the intermediate plane. However, the Villuminator module has a built-in property of being able to compensate or "correct" the final illumination profile at the secondary plane. In particular, the following rules should be applied:

Rule A: *If the illumination at the screen has a dome-shaped profile (figure 8, top), then shift the entire Villuminator module away from the projection lens until the illumination is flat (figure 8, bottom).*

Rule B: *If the illumination at the screen has an inverted-dome profile (figure 9, top), then shift the entire Villuminator module towards the projection lens until the illumination is flat (figure 9, bottom).*

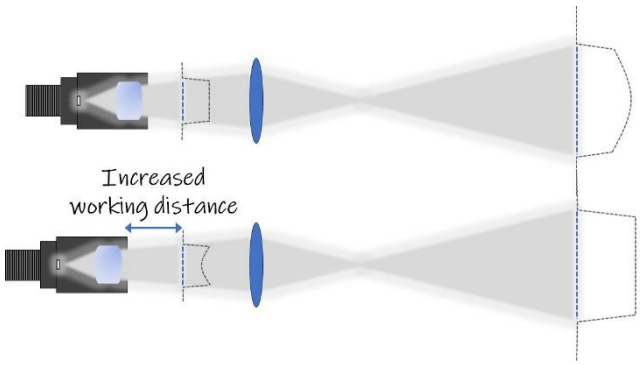

Figure 8. Correcting a dome-shaped illumination profile at the secondary plane by increasing the working distance of the Villuminator module.

Usually, it should not take more than a few millimeters of movement to perform the non-uniformity compensations described above. Therefore, an allowance of +/- a few millimeters of space should be allotted for

such corrections when integrating Villuminator modules into your final instrument or product.

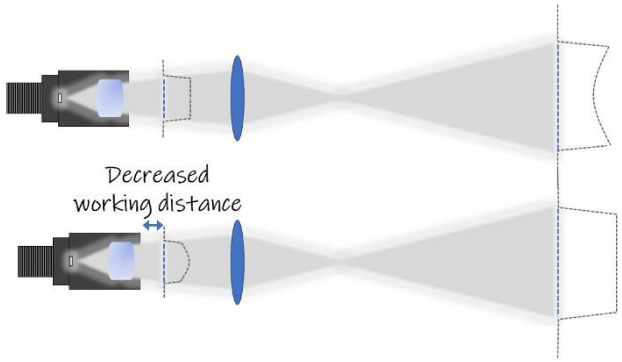

Figure 9. Correcting an inverted-dome-shaped illumination profile at the secondary plane by decreasing the working distance of the Villuminator module.

5. WHERE TO MOUNT A SPATIAL LIGHT MODULATOR FOR PROJECTION

Applications involving projection normally involve the use of a spatial light modulator (SLM), such as a liquid crystal display (LCD) panel, or a TI DLP® chip (often called a digital micromirror device or "DMD"). In the case of transparent SLMs (such as LCDs), there are two options available. First, one may mount the SLM directly at the intermediate plane of the Villuminator module (see, e.g., figure 4). Alternatively, one may mount the SLM at a secondary plane, as illustrated in figure 10. Further, an iris or aperture of any shape and size may be mounted at the intermediate plane, which is often useful for creating a boundary of illumination at the SLM and may help to prevent extraneous stray light that could overfill the SLM.

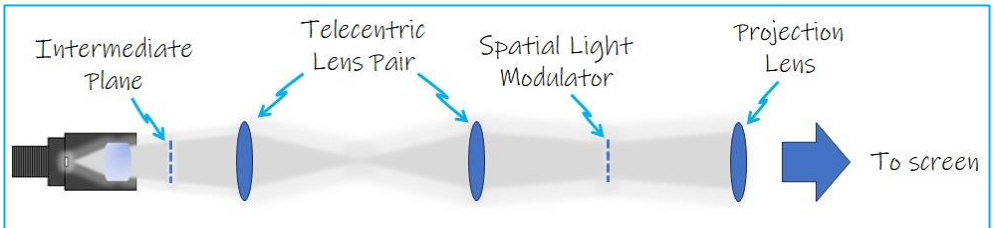

Figure 10. Alternative location for mounting a spatial light modulator.

6. USING VILLUMINATOR MODULES IN FLUORESCENCE MICROSCOPY

By setting up the type of optical layout illustrated in figure 11, Villuminator modules may be applied towards illumination in fluorescence microscopy. The "coupler" lens can be any COTS achromatic doublet having an EFL of roughly 150 mm. Its purpose is to collimate rays from the top hat plane (i.e., the intermediate plane) such that, from the point of view of the objective lens (which is assumed to be an infinity-corrected objective), the top hat plane appears as if it is located at infinity. Under this condition, **the objective projects the image of the top hat plane onto the plane of the biosample** (i.e., the focal plane of the objective). To achieve this condition, **the distance between the top hat plane and the coupler lens must be set as the back focal length of the coupler lens**.

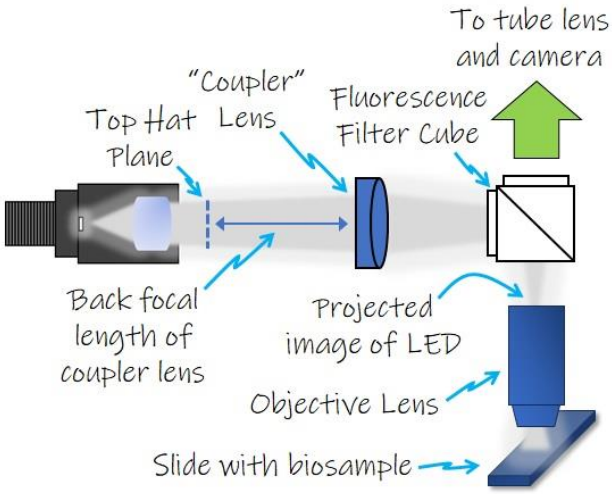

Figure 11. Schematic optical layout for a fluorescence microscope using a Villuminator module for excitation.

Actually, the orientation of the coupler lens is not very strict, so that you may either face its back or front towards the top hat plane. If facing its front towards the top hat plane, then the distance between that plane and the coupler lens is the front focal length of the coupler lens. In either orientation, the coupler lens may be thought of as being analogous to a *field lens* that is commonly used in the so-called **Köhler illumination** technique for mounting a light source in a fluorescence microscope system. And as with any microscopy setup based on Köhler illumination, the source must be projected onto the back of the objective (technically, it is the *pupil* of the

objective). This is the case in figure 11, where the beam from the Villuminator is focused by the coupler lens into the back of the objective. For proper illumination, one needs to adjust the distance between the coupler lens and the back of the objective until a projected image of the LED is seen at the back of the objective (equivalently, ensure that the beam size at the back of the objective is minimized). As a rule, the distance between the coupler lens and the back of the objective is approximately equal to the EFL of the coupler lens (begin here, then adjust accordingly).

Figure 12 shows a setup on a lab bench at V-BMB, based on the layout provided in figure 11. The LED in the Villuminator module has green output at wavelength of 550 nm. The biosample is that of an epithelial cell, whose fluorescence image is shown in figure 13a. This image displays no evidence of having any edge darkening artifacts, nor any unexpected localized "hot" (bright) and "cold" (dark) spots within the image.

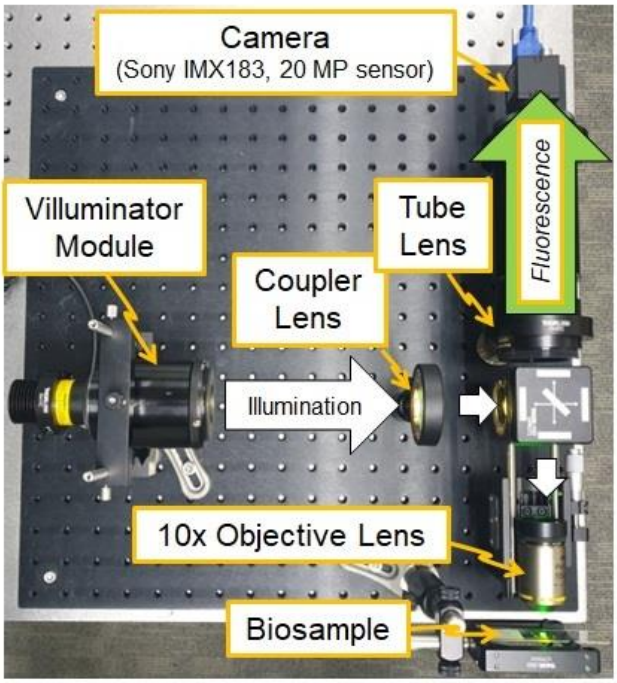

Figure 12. Optical bench setup based on the layout illustrated in figure 11.

For reference, figure 13b shows the fluorescence image of a blank fluorescent slide in place of the biosample in the setup of figure 12. The diagonal line profile in the image is shown in figure 13c, indicating a nearly flat distribution (some unevenness in the profile may occur when the blank slide possesses slight non-uniformities).

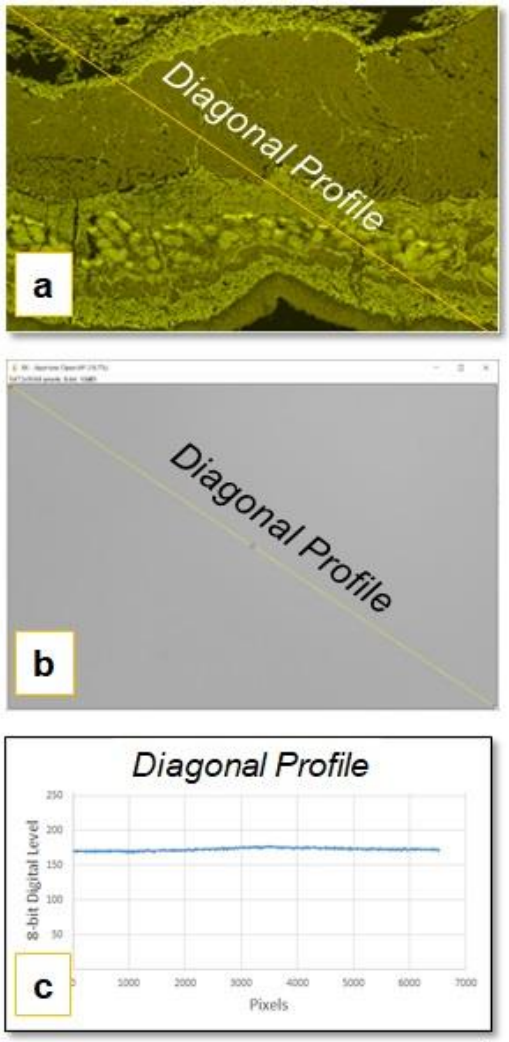

Figure 13. (a) Fluorescent image of epithelila cell used in figure 12. (b) Image of a blank fluorescent slide used in place of the epithelial cell biosample in figure 12. (c) Intensity profile of camera pixels across the diagonal indicated in figure 13b.

In microscopy applications, the use of Villuminator modules is not limited to fluorescence imaging. For example, figure 14 displays a **Villuminator Condenser** design for transmission brightfield illumination that may be used in either an upright or inverted microscope system. Microscopists know that brightfield condensers consist of a **flux collector** (for collecting and projecting light from a source) and a **substage**

condenser (for focusing light onto the biosample). In a Villuminator Condenser system, it is the Villuminator module that takes the place of the flux collector, while a low-magnification (usually 4x or 5x) infinity-corrected objective becomes the substage condenser. This arrangement produces a top hat illumination profile at the specimen plane, which is the focal plane of the objective. A customer has benefited significantly from this design, yielding wide field microscopy images with high uniformity and minimal "stitch lines" when scanning and capturing images across multi-well cell culture plates.

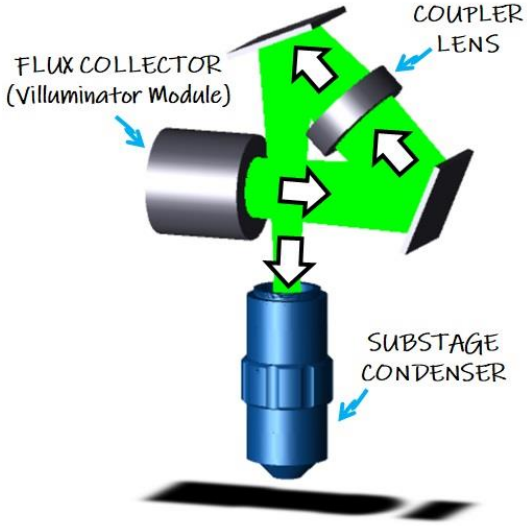

Figure 14. A microscope condenser assembly for transmission brightfield illumination by way of assembling a Villuminator module with a coupler lens and a low magnification (wide field) objective.

Villuminator modules listed on the V-BMB website (and e-store) are standard modules, which have been optimized primarily for microscopy. If you do not see a module that suits your needs, V-BMB can provide customization.

7. DEFINING ILLUMINATION UNIFORMITY

A thorough description of how illumination uniformity is defined and quantified requires a lengthier discussion with high technical content. In the current white paper, we shall touch on just the basics. Figure 15 depicts a non-uniform illumination profile in the form of a smooth curve with maximum **irradiance** at the center and minimum irradiance at the edge. The term "irradiance" is defined as the flux per unit area or *flux density* of the light distribution on the plane of illumination. It is a term taken from the subject of optical radiometry (a standard field of study in optics). In reality, the illumination must be captured on a digital camera (such as a CCD or CMOS image sensor) in order to be quantified. Thus, the illumination would be incident on a diffuser used as a screen, and the image on the screen is captured from behind using a high-performance imaging lens and digital image sensor. Consequently, the illumination profile at the sensor may possess noise, as depicted in figure 16.

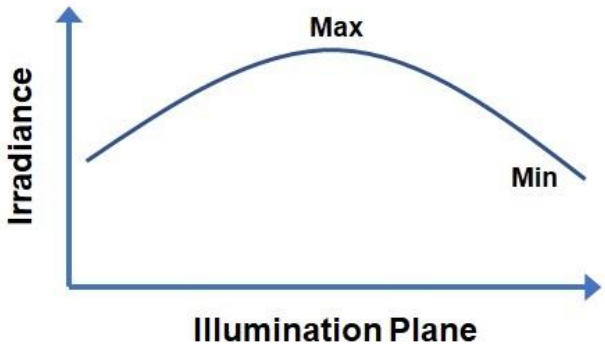

Figure 15. Illustration of a non-uniform illumination profile on a plane or screen.

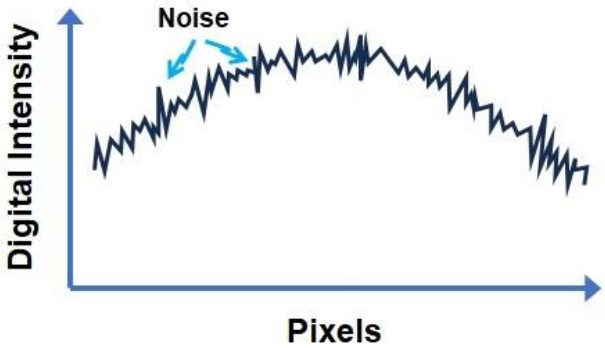

Figure 16. The illumination from figure 15 captured on a digital image sensor with noise in the pixels.

The noise that is observed in the pixels arises from a combination of the shot noise from the light signal, the dark current in the pixels, and any other electronic noise of a random nature in the camera. Due to the noise, it becomes near impossible to determine the actual value of the max and the min irradiance levels in

the illumination, which are the quantities that are commonly used for computing the uniformity. For instance, uniformity may be defined by taking the ratio given by max/min of the irradiance, or the min/max, so that in either case, perfect uniformity is a value of 1. At other times, one may prefer to define the non-uniformity of illumination by way of computing (max – min)/max. In this way, perfect uniformity occurs when the non-uniformity given by taking max – min yields zero. But when noise is present, the max and min values fluctuate about their actual readings, hence, preventing one from accurately determining the uniformity or non-uniformity in the profile.

Fortunately, the problem is not intractable. The solution is realized by first considering that the illumination profile captured on the image sensor is ideally a smooth function $f(x)$, where x is the position across the sensor. By way of the *mean value theorem* of calculus [3], the average value of a smooth (continuous) curve $f(x)$ is given by

$$\bar{f} = \frac{1}{b-a}\int_a^b f(x)\mathrm{d}x \qquad (2)$$

where $\bar{f}$ is the average value. We can extend this concept to compute the standard deviation (std dev) of the smooth curve $f(x)$, which may be expressed as

$$\sigma_\mathrm{f} = \sqrt{\frac{1}{b-a}\int_a^b \left[f(x) - \bar{f}\right]^2 \mathrm{d}x} \qquad (3)$$

where σ_f is the std dev, and $\bar{f}$ in equation (3) is given by equation (2). Now, it turns out that for a suitable multiplier M, the max value of $f(x)$ can be determined to be $\bar{f} + M\sigma_\mathrm{f}$, and the min value of $f(x)$ can be determined to be $\bar{f} - M\sigma_\mathrm{f}$. When noise is present, then the total std dev of the curve may be expressed as

$$\sigma_\mathrm{tot} = \sqrt{{\sigma_\mathrm{f}}^2 + {\sigma_\mathrm{n}}^2 + {\sigma_\mathrm{fn}}^2} \qquad (4)$$

where σ_n is the std dev due to the noise, and σ_fn is a *covariance* term [4], which exists because $f(x)$ is a smooth curve being modulated by random and independent perturbations of the noise. However, the covariance term becomes negligible when σ_f is small (i.e., when $f(x)$ is fairly uniform). When a Villuminator module is assembled and tested at V-BMB, an adjustment is made to minimize σ_f, thereby yielding an essentially zero contribution from σ_fn. As a result, the total std dev in equation (4) is given solely by the sum of the first two terms, ${\sigma_\mathrm{f}}^2$ and ${\sigma_\mathrm{n}}^2$. Furthermore, at the V-BMB lab, a means has been established to determine the noise term, σ_n. Therefore, the noise term can always be subtracted from the total std dev σ_tot, yielding a reasonably accurate estimate of σ_f. The uniformity for all Villuminator modules is then defined by

$$\mathrm{Uniformity} = \frac{\mathrm{Min}}{\mathrm{Max}} \times 100\% \qquad (5)$$

$$= \frac{\bar{f} - M\sigma_\mathrm{f}}{\bar{f} + M\sigma_\mathrm{f}} \times 100\% \qquad (6)$$

for a suitable value of M. Now, since irradiance profiles on an image sensor are actually surface profiles (e.g., figure 7) rather than 1-dimensional curves (e.g., figures 15 and 16), the mathematical formalism given by equations (2) and (3) are computed as double-integrals. When this is done, it is found that the most suitable value for the multiplier is $M = 2$ (this shall be explained in a future advanced technical note).

8. CONCLUSION

Villuminator modules are effective at producing top hat illumination profiles at projected planes and at the specimen planes of microscope objective lenses. These modules are offered in a variety of sizes, designs, and are also customizable. They are useful in many practical applications that require exceptionally uniform illumination, such as digital projection, patterned illumination, fluorescence and brightfield imaging in microscopy, qPCR, dPCR, and more.

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ABBREVIATIONS

APC, Advanced Products Corporation Pte. Ltd.
CCD, charge coupled device
CMOS, complementary metal oxide semiconductor
COTS, commercial off-the-shelf
dPCR, digital polymerase chain reaction
DLP®, digital light processing
DMD, digital micromirror device
EFL, effective focal length
LCD, liquid crystal display
LED, light emitting diode
qPCR, quantitative polymerase chain reaction
std dev, standard deviation
SLM, spatial light modulator
TI, Texas Instruments
V-BMB, Venture Biotech Modules Business Pte. Ltd.

ACKNOWLEDGEMENTS

The author thanks his colleagues at V-BMB for their support in all aspects of Villuminator development.

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ABOUT V-BMB

V-BMB specializes in advanced precision control technology solutions ranging from ready-to-use, plug-and-play modules to bespoke, customized modules in precision thermal control, optical systems and fluidic controls. V-BMB also offers customized consumable solutions and liquid handling modules for the Life Science and Medtech industries.

As a wholly-owned subsidiary of Venture Corporation Limited, a leading global provider of technology services, products and solutions, V-BMB leverages on Venture's decades of proven design experience, technical expertise and a global R&D and manufacturing footprint that spans Southeast Asia, China, the U.S. and Europe. V-BMB was created to harness the deep knowledge and expertise that Venture has amassed over the years in developing advanced instrumentation in the Life Science domain and other industrial applications.

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