Characterization of Irradiance in Light Microscopes that use the Villuminator™ Module

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Acknowledgements

- We are grateful to Nathalie Gaudreault* and Laurent Gelman** for their kind invitation
- Thanks also to Stanley Schwartz‡ for introducing us to QUAREP-LiMi

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** Head of [Facility for Advanced Imaging and Microscopy]
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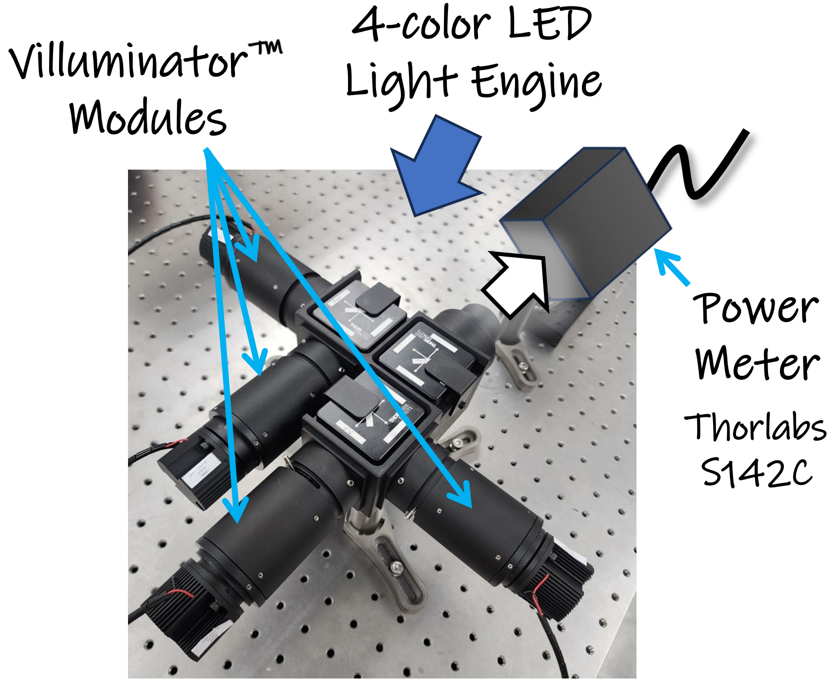
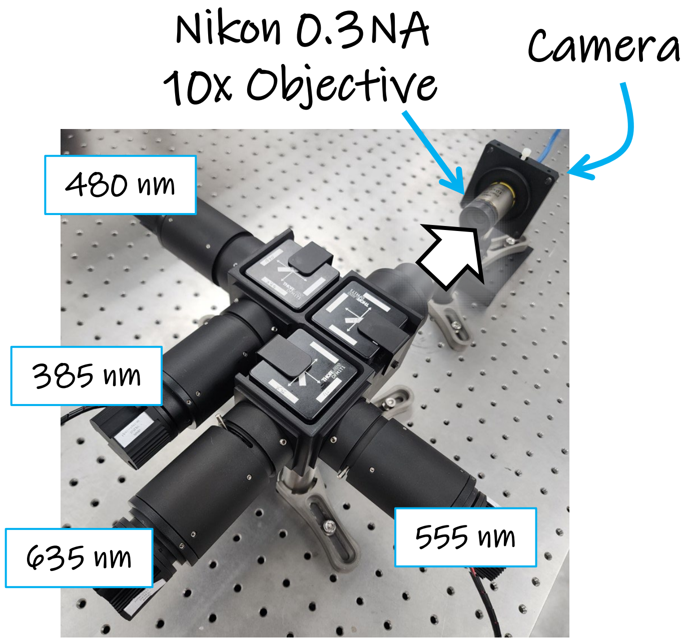
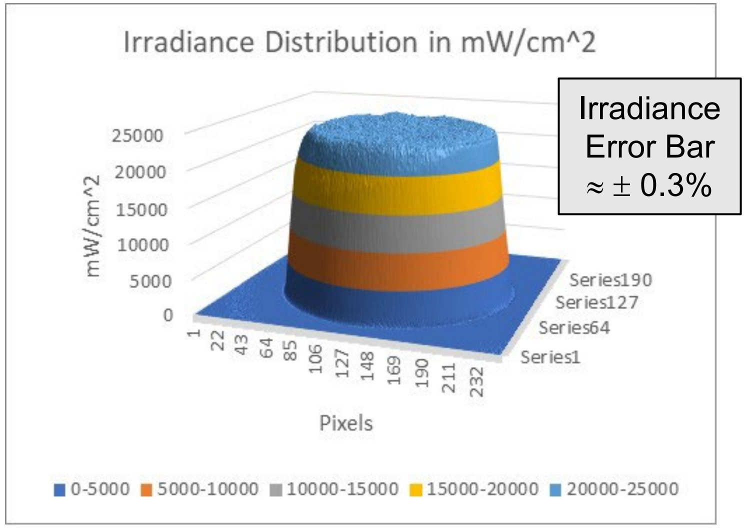
Irradiance measurement at the specimen plane of an objective (example)





This is for the 480 nm illumination (surface plot is across 250 x 250 pixels, each pixel is 2.4 µm square)



1 Measure total flux at the specimen plane using power meter

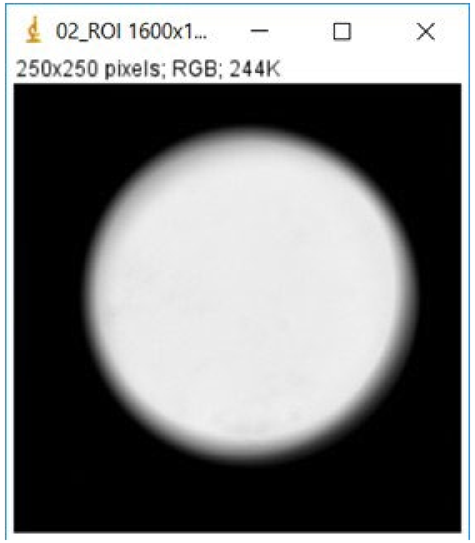
2 Capture beam profile at specimen plane using an image sensor (focus by first reducing iris at the field stop)

3 Scale pixel digital intensities using Matlab, Excel, etc.

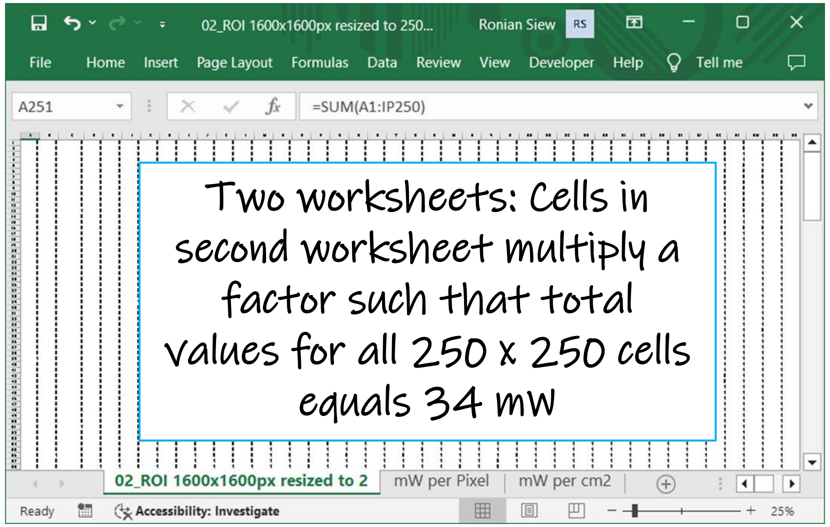
(Here, Excel was used)

Scaling of pixel digital intensities (method 1)

Resized raw image into 250 x 250 pixels (easier to handle in Excel)

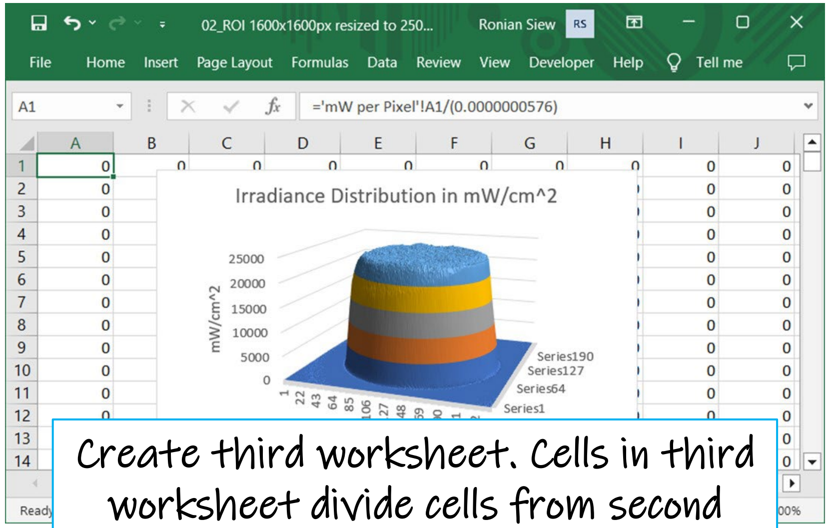


MS Excel® Spreadsheet



Two worksheets: Cells in second worksheet multiply a factor such that total values for all 250 x 250 cells equals 34 mW

MS Excel® Spreadsheet



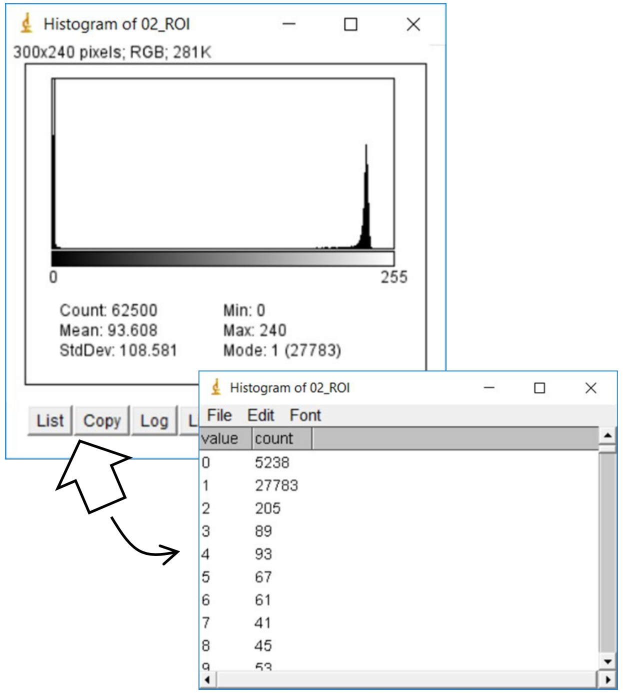
Create third worksheet. Cells in third worksheet divide cells from second worksheet by pixel size in cm^2

1 Save image file from ImageJ into .CSV text (power meter measured total flux ≈ 34 mW ± 0.3%)

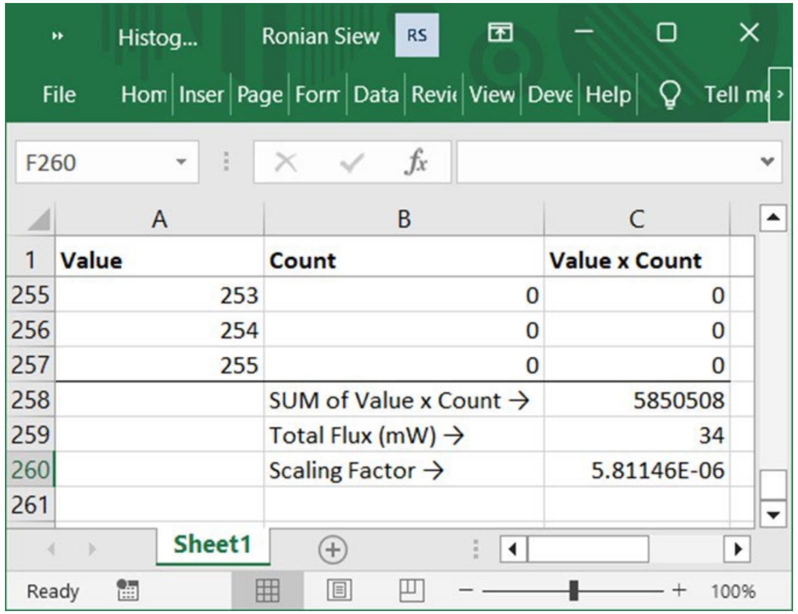
2 Open text file with Excel, scale cell values to flux values (total pixel shot noise << ± 0.3%)

3 Divide flux values by pixel area to yield flux per unit area

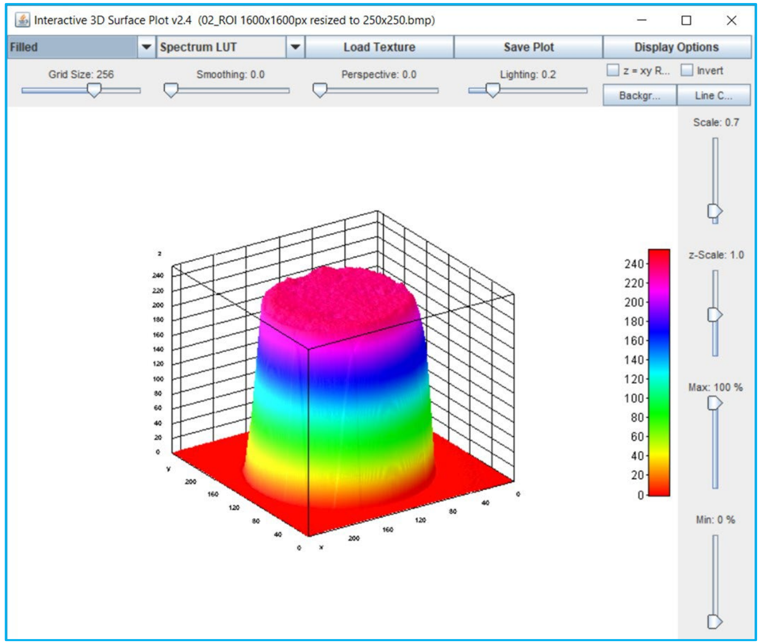
Scaling of pixel digital intensities (method 2)



MS Excel® Spreadsheet



ImageJ 3D Plot



1 Display histogram of pixel digital intensities in ImageJ, click “List” to output histogram data

2 Column C = Value x Count; Sum all, then divide flux by sum to yield scaling factor

3 Digital intensity values in 3D plot must multiply scaling factor, yielding irradiance in mW/pixel

Caveat 1

Field number divided by magnification

This is a relative term

Flux in the FOV

We assume that the signal to background ratio is “high”

Mean flux from light scatter that’s been integrated by power meter and image sensor

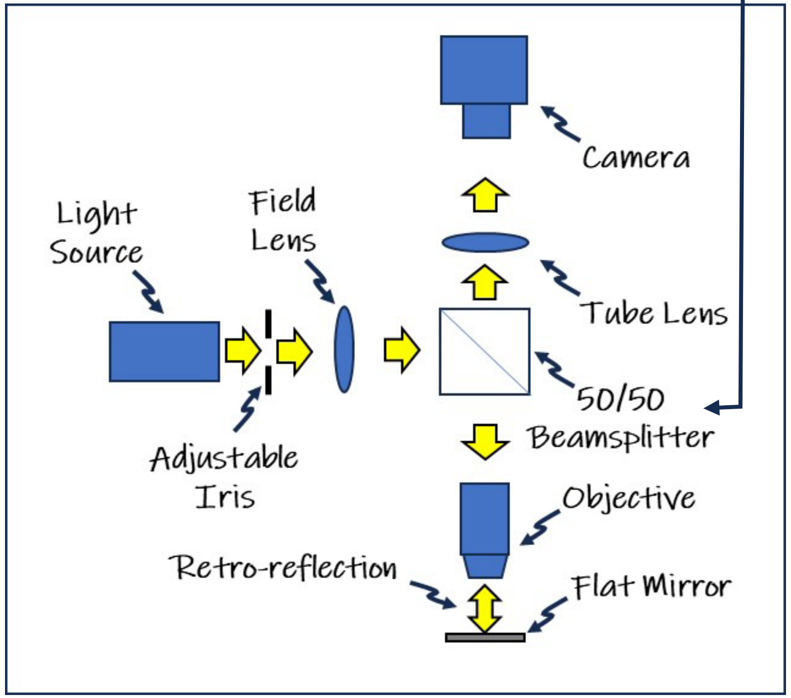
Caveat 2

What if you don't have space to mount a camera at the specimen plane?

In this case, one option is to try this experimental setup

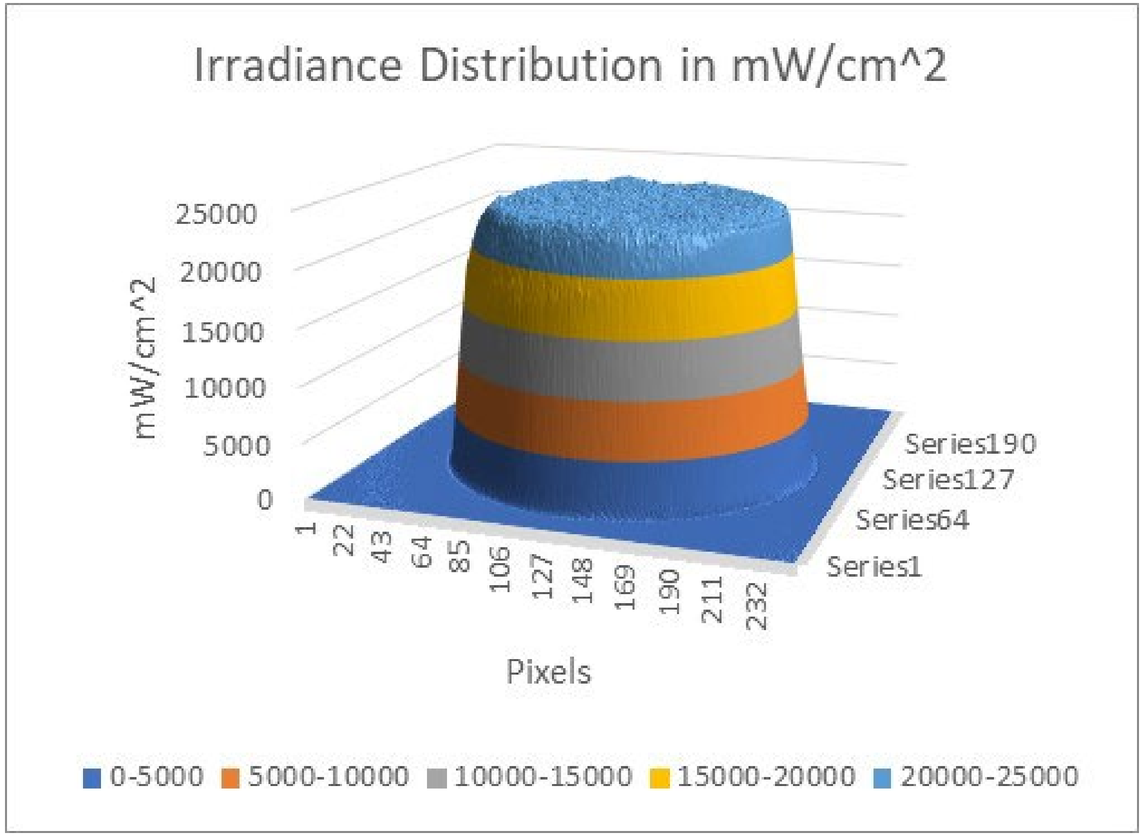
The flat mirror can be any high quality 'first surface' mirror. To focus onto the flat mirror, close the iris but leave sufficient opening so as to see the iris's inner rim, then focus until the rim appears sharp. Let the camera record the irradiance distribution. Note that the irradiance profile at the camera may not necessarily be the same as the irradiance at the mirror. In this case, perhaps you can 'baseline' your measurement by first mounting a Villuminator module as the light source to obtain a top hat irradiance profile with > 95% uniformity at the mirror plane. Then, let $V(x,y)$ be the irradiance at the mirror from the Villuminator, and $V'(x,y)$ be its image in the camera. If $T(x,y)$ is the 'transmittance function' of the imaging system, then $V'(x,y) = V(x,y)T(x,y)$. So, if $I'(x,y)$ is the image of your original irradiance profile and $I(x,y)$ is your original irradiance at the mirror, then $I'(x,y) = T(x,y)I(x,y)$. Thus, your original irradiance at the mirror is $I(x,y) = [V'(x,y)/V(x,y)]I'(x,y)$. But since $V(x,y)$ is a top hat, it is spatially constant (within 5% error). Therefore, $I(x,y) \approx V'(x,y)I'(x,y)$.

SNR* is independent of the beamsplitter's %R/%T split ratio



*SNR ≡ Signal to noise ratio

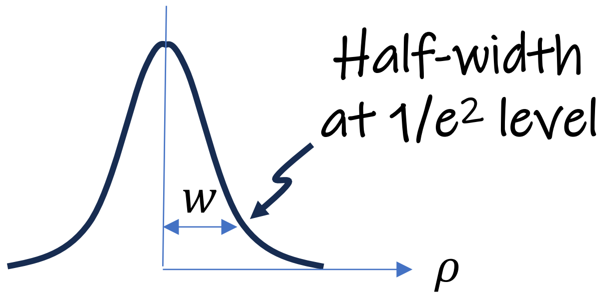
The Villuminator™ modules produce very flat irradiance distributions



So, it is of interest to characterize the irradiance profile in terms of analytic formulas

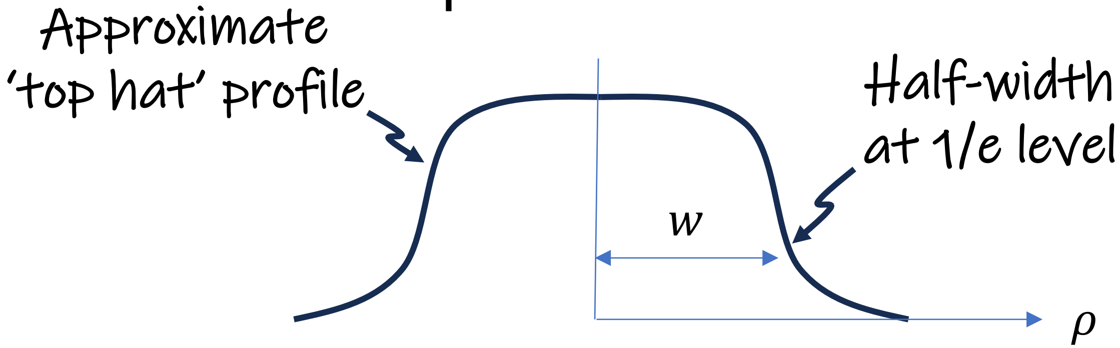
Analytic expressions relating irradiance distribution with total flux and ‘width’

Rotationally Symmetric Gaussian



$$E(\rho) = \frac{2P}{\pi w^2} \exp\left[-2\left(\frac{\rho^2}{w^2}\right)\right]$$

Rotationally Symmetric ‘Super-Gaussian’

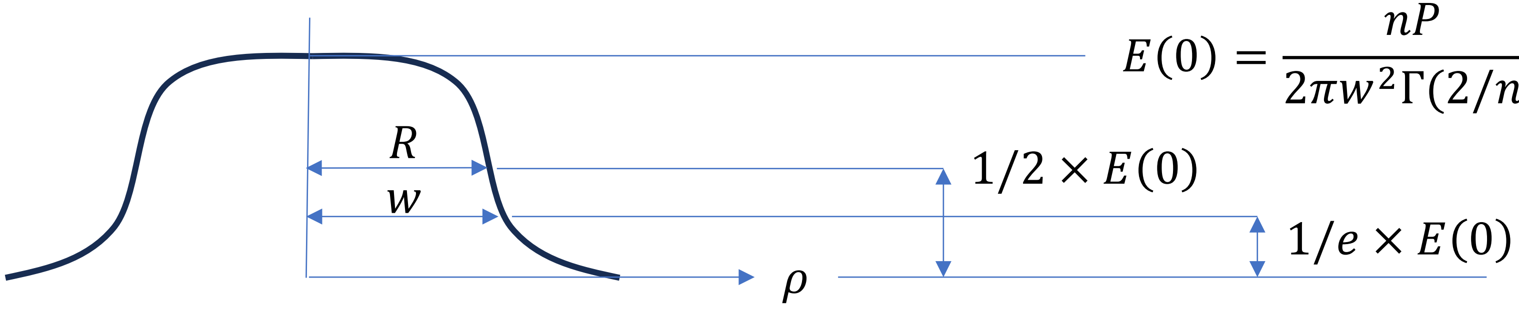


$$E(\rho) = \frac{nP}{2\pi w^2 \Gamma(2/n)} \exp\left[-\left(\frac{\rho}{w}\right)^n\right]$$

Gamma function

In both formulas, P is the total flux in the distribution

Properties of the 'super-Gaussian top-hat'

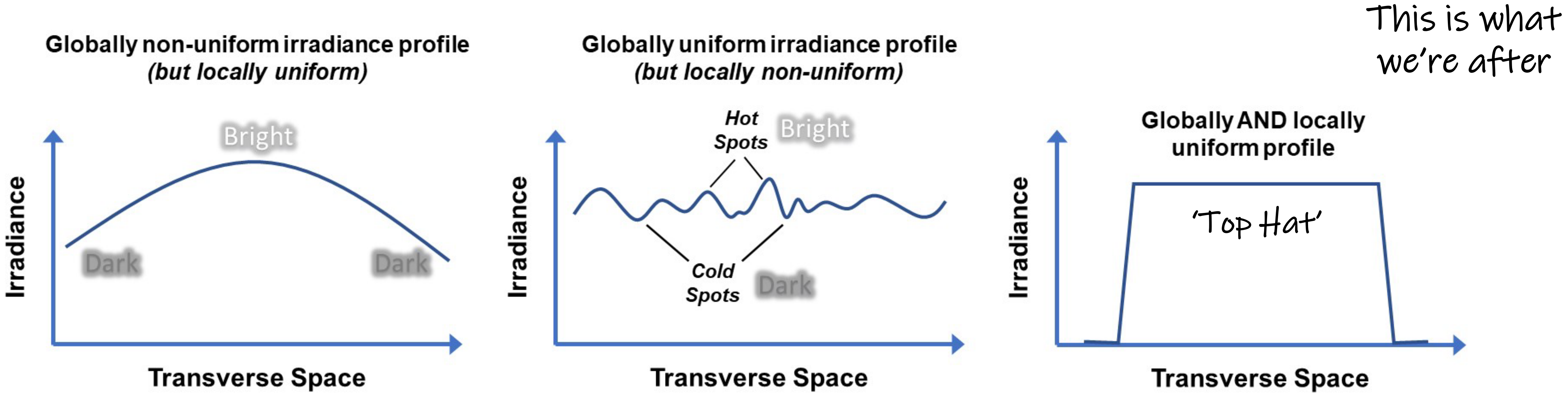


$$E(0) = \frac{nP}{2\pi w^2 \Gamma(2/n)}$$ Peak irradiance

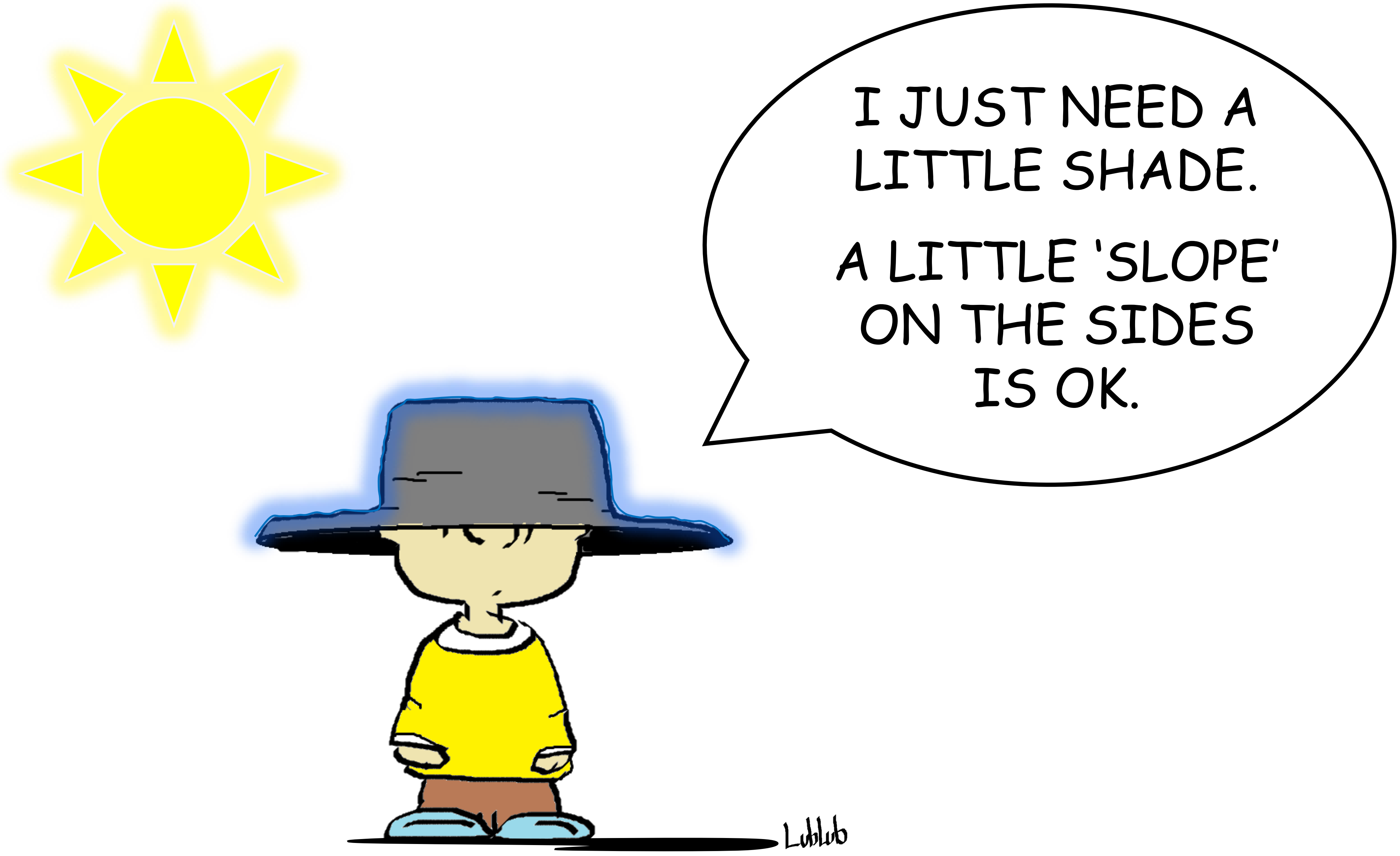
- Average irradiance in a circle of area $\pi\rho^2$ is $= \frac{2\pi}{\pi\rho^2}\int_0^{\rho} E(\rho)\rho \mathrm{d}\rho = \frac{2}{\rho^2}\int_0^{\rho} \frac{nP}{2\pi w^2\Gamma(2/n)} \exp\left[-\left(\frac{\rho}{w}\right)^n\right]\rho \mathrm{d}\rho$
- In the limit that $n \to \infty$, the distribution approaches a top hat
- $\lim_{n\to\infty} E(0) = \frac{P}{\pi w^2} = \frac{P}{\pi R^2}$ There is no distinction between R and w when the distribution is an ideal top hat

Why irradiance matters to us

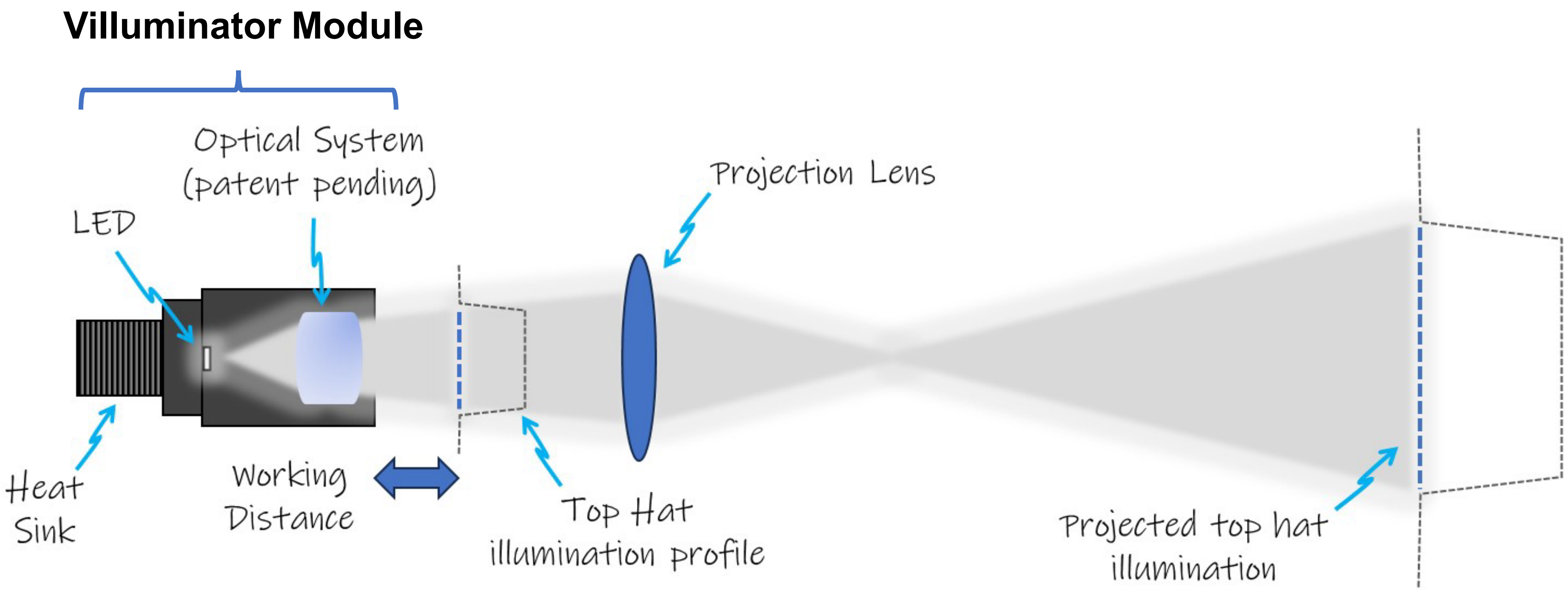
- A source's 'brightness' is proportional to the irradiance* in the image of that source
- Fluorescence emission is proportional to the irradiance from the illumination
- Given a spatial irradiance distribution $E(x, y)$, the integral $\iint_{\text{Area}} E(x, y)\mathrm{d}x\mathrm{d}y$ yields flux in the 'Area'
- The flux delivered to an area is at least partially absorbed by matter in that area
- If a distribution is 'top hat', then the irradiance times any area yields flux in that any area ✓



*R. Siew, *Eur. J. Phys.* **29**, 1105 (2008); R. Siew, *Eur. J. Phys.* **43**, 035304 (2022)

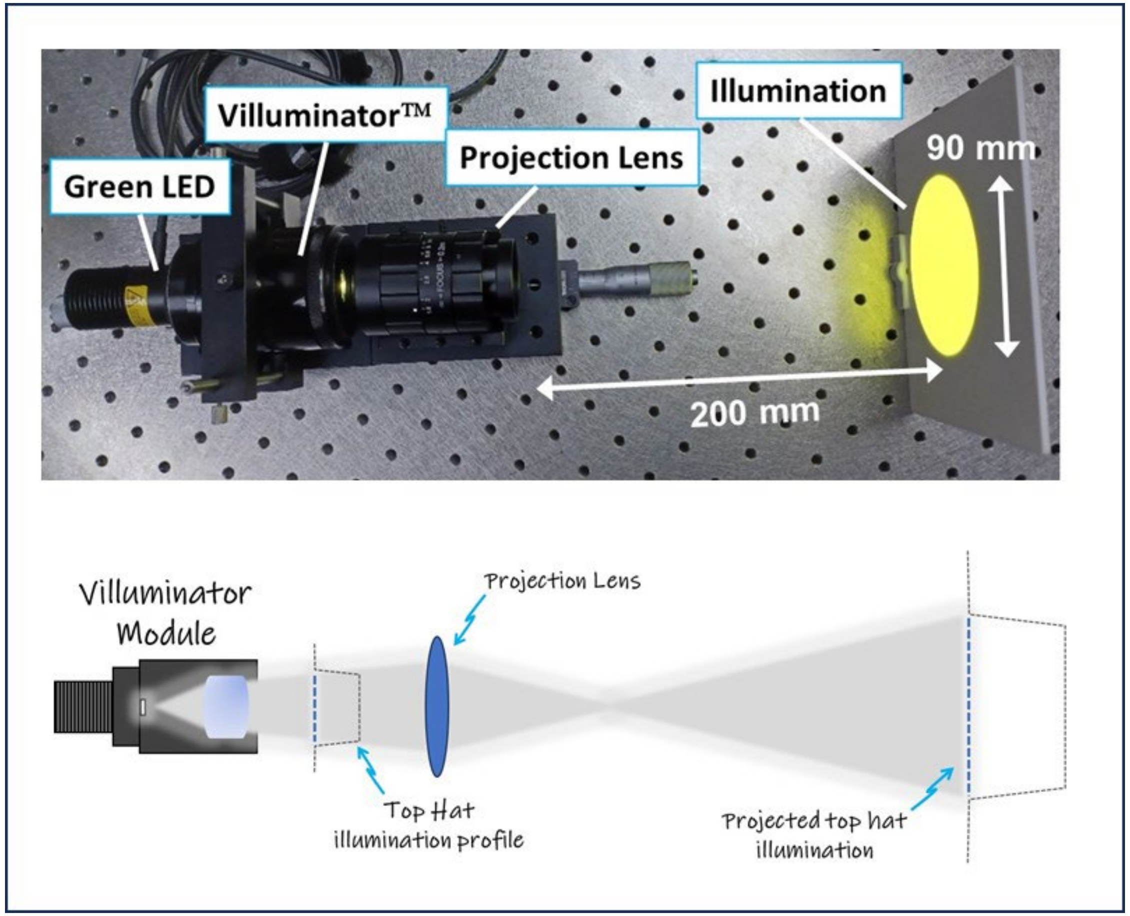

I JUST NEED A LITTLE SHADE.
A LITTLE 'SLOPE' ON THE SIDES IS OK.
Lublub

Producing top hat illumination using the Villuminator™ module: principle of operation

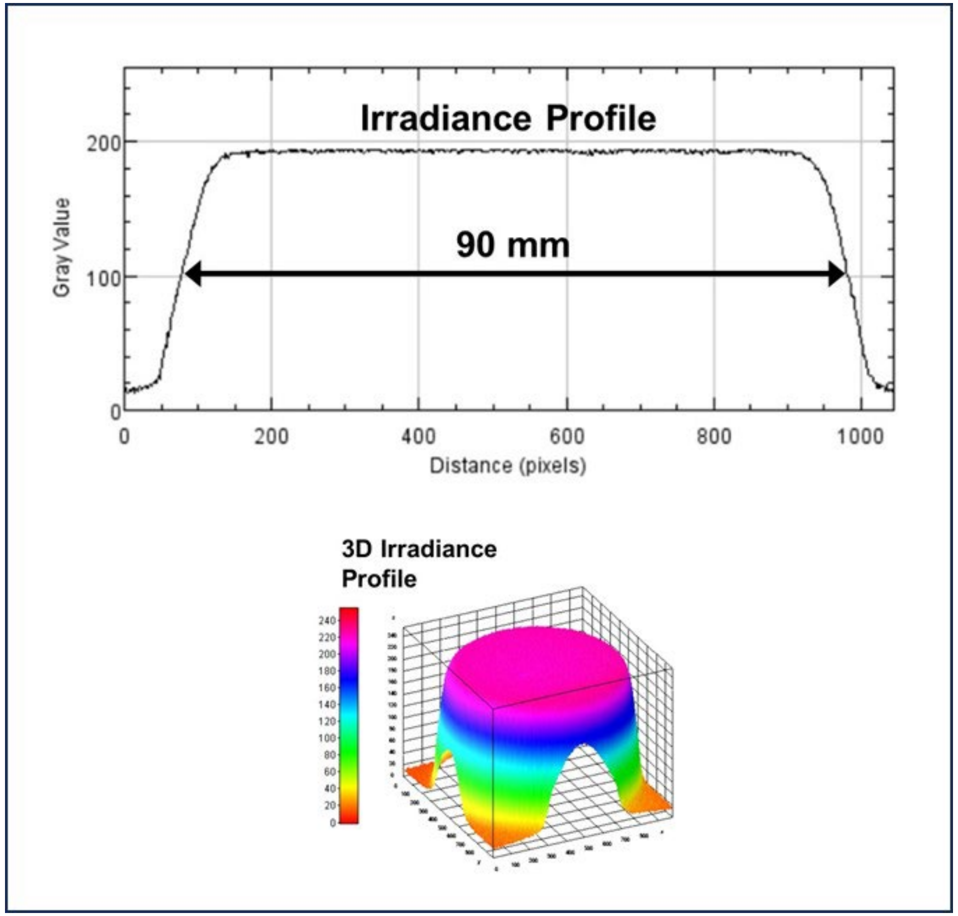


Example

Experimental Setup

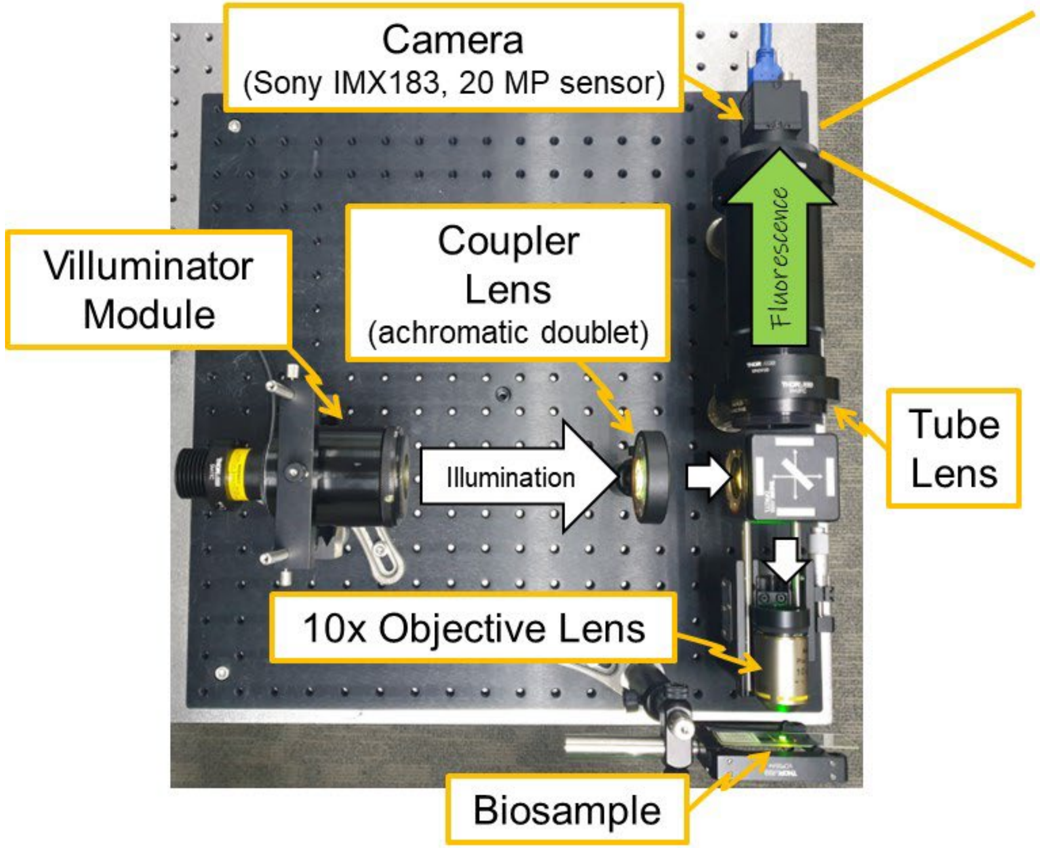


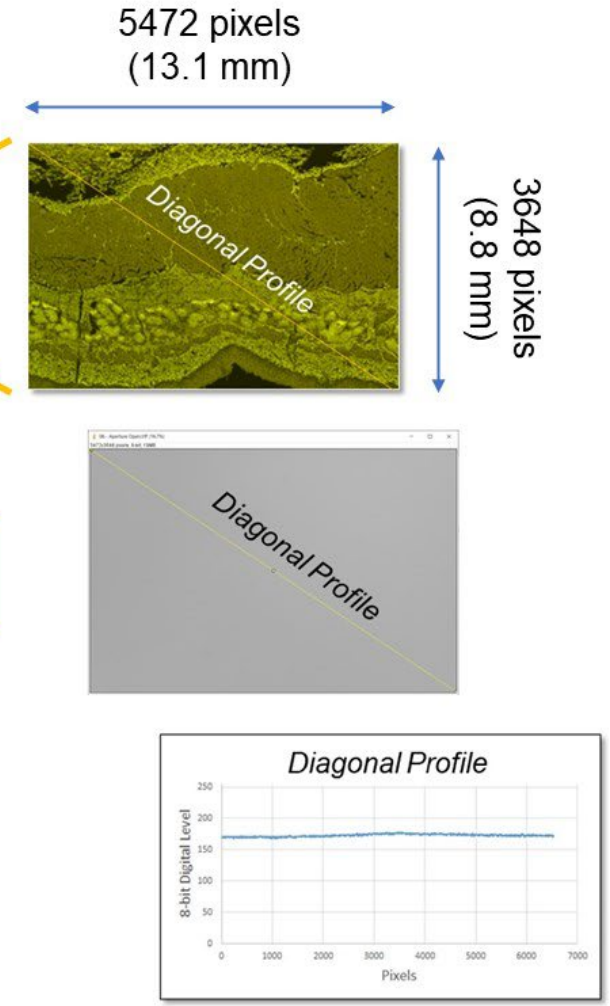
Measured Profile



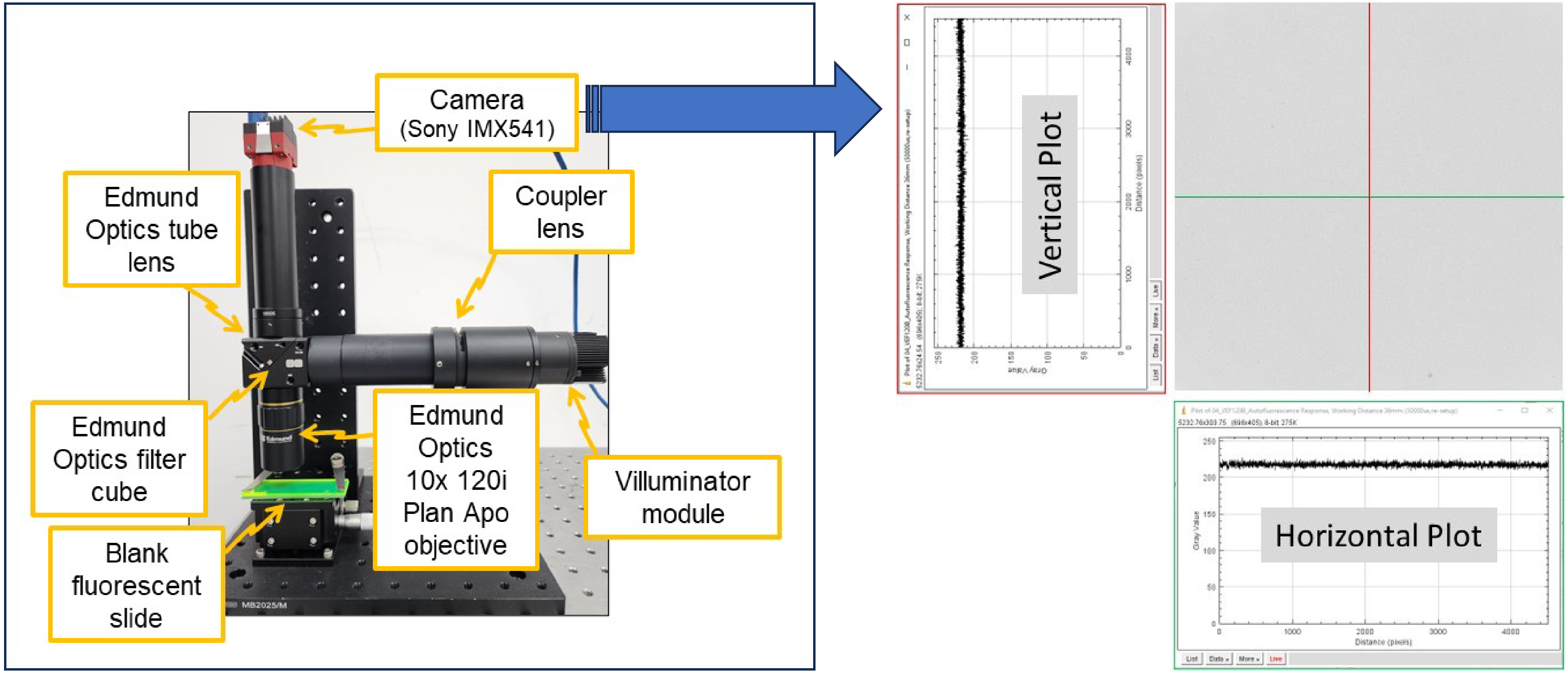
Fluorescence Microscope Setup

(Nikon 10x Plan Fluorite, 0.3 NA)

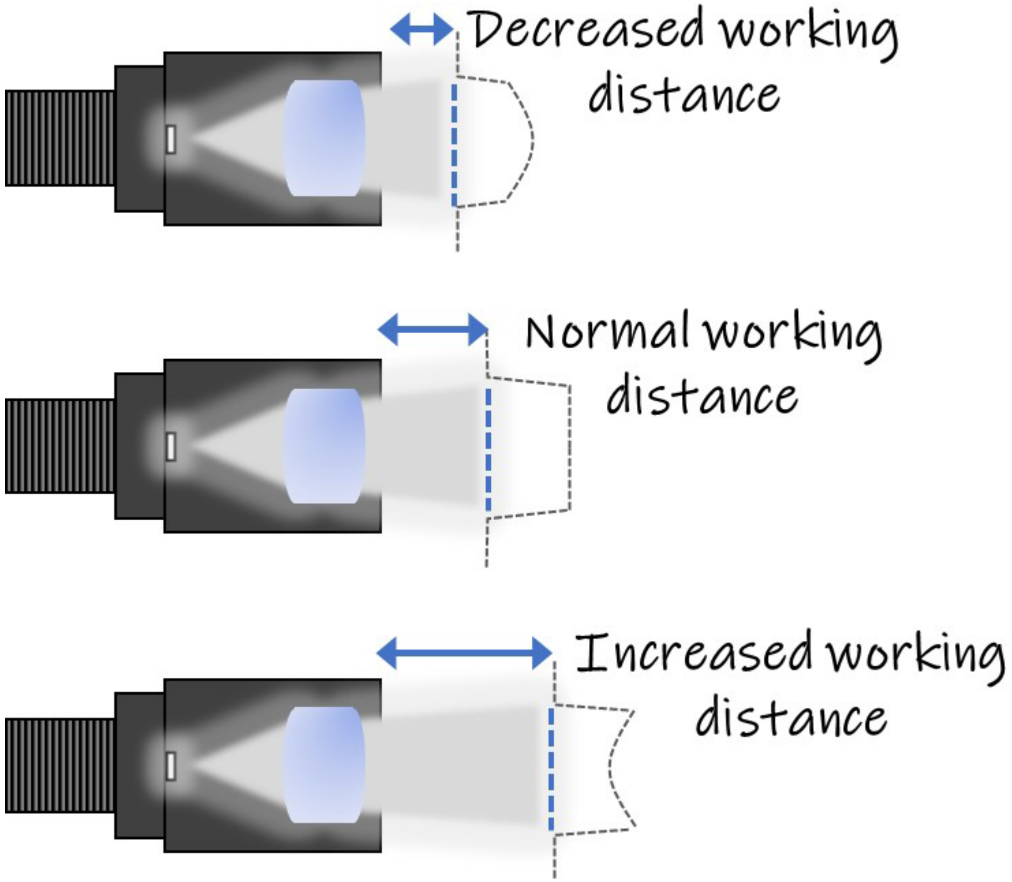




Fluorescence Microscope Setup
(Edmund Optics 10x 120i Plan Apo, 0.28 NA)



Tunability of the irradiance distribution



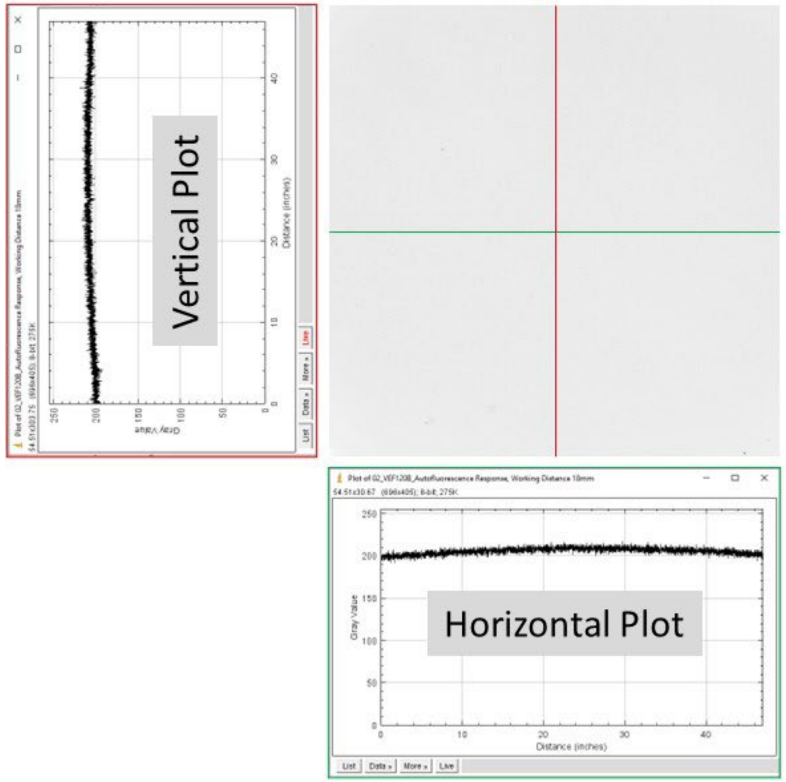
By varying the working distance, the irradiance profile can be made to change from a dome to a top hat and to an inverted-dome.

This means that the FINAL profile at the camera can be made flat even if the 'response' of a fluorescent sample (or the transverse transmittance function of the imaging system) has either a dome-shaped or inverted-dome shaped profile.

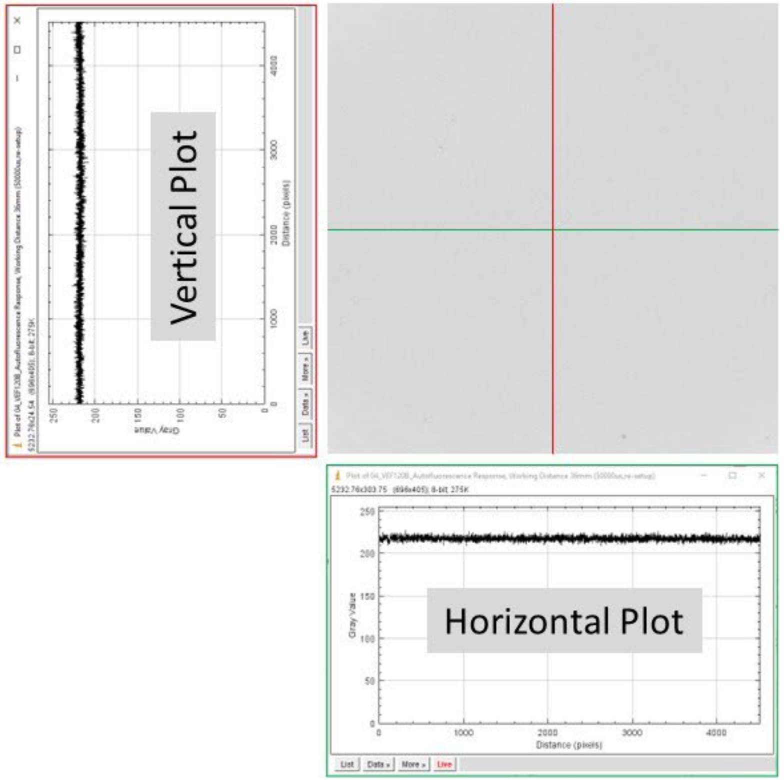
Example of tuning the profile

(Villuminator with Edmund Optics 10x 120i Plan Apo, 0.28 NA)

Working Distance = 18 mm



Working Distance = 36 mm



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V-BMB Precision Control Solutions Made Simple

WHITE PAPER
Dec 2023, Rev. 2

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.

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.

Figure 1. Compact standard Villuminator modules.

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.

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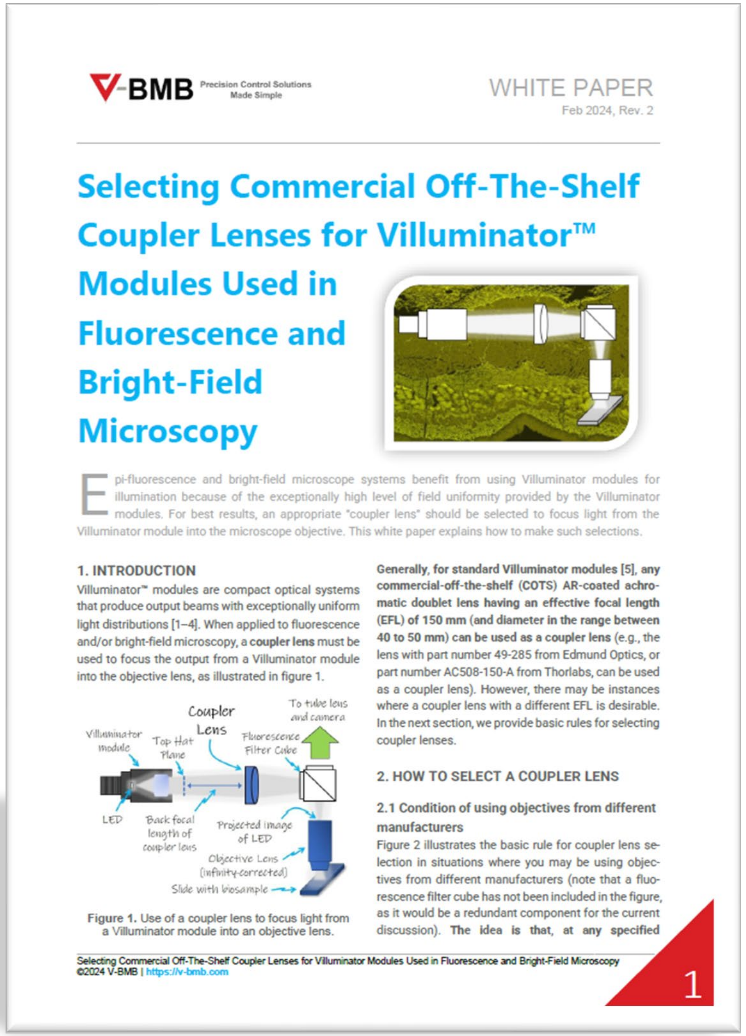
V-BMB Precision Control Solutions Made Simple

WHITE PAPER
Feb 2024, Rev. 2

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**

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Article in Biophotonics (Jan ‘2023)

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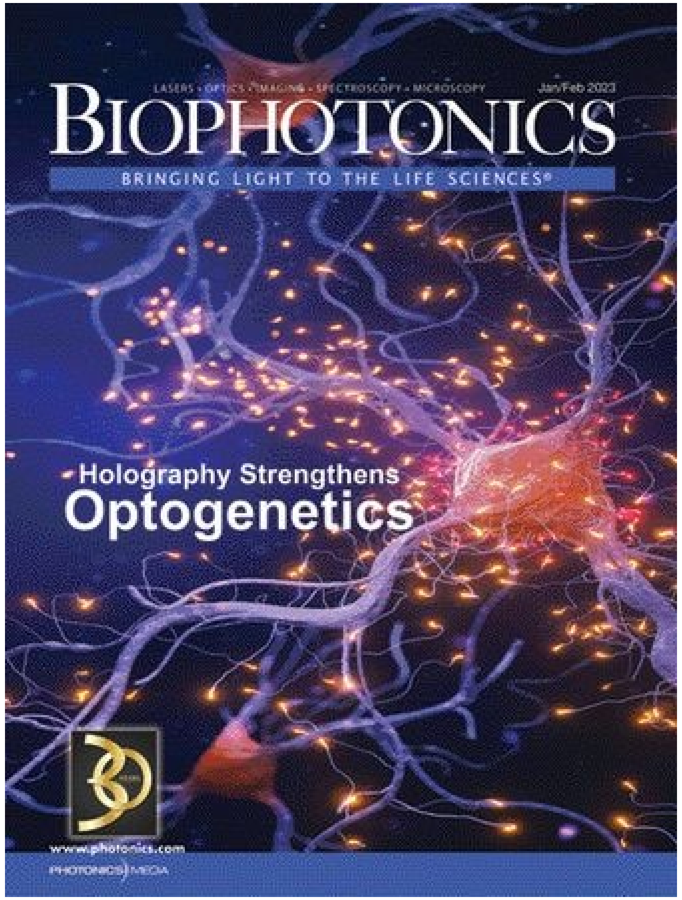

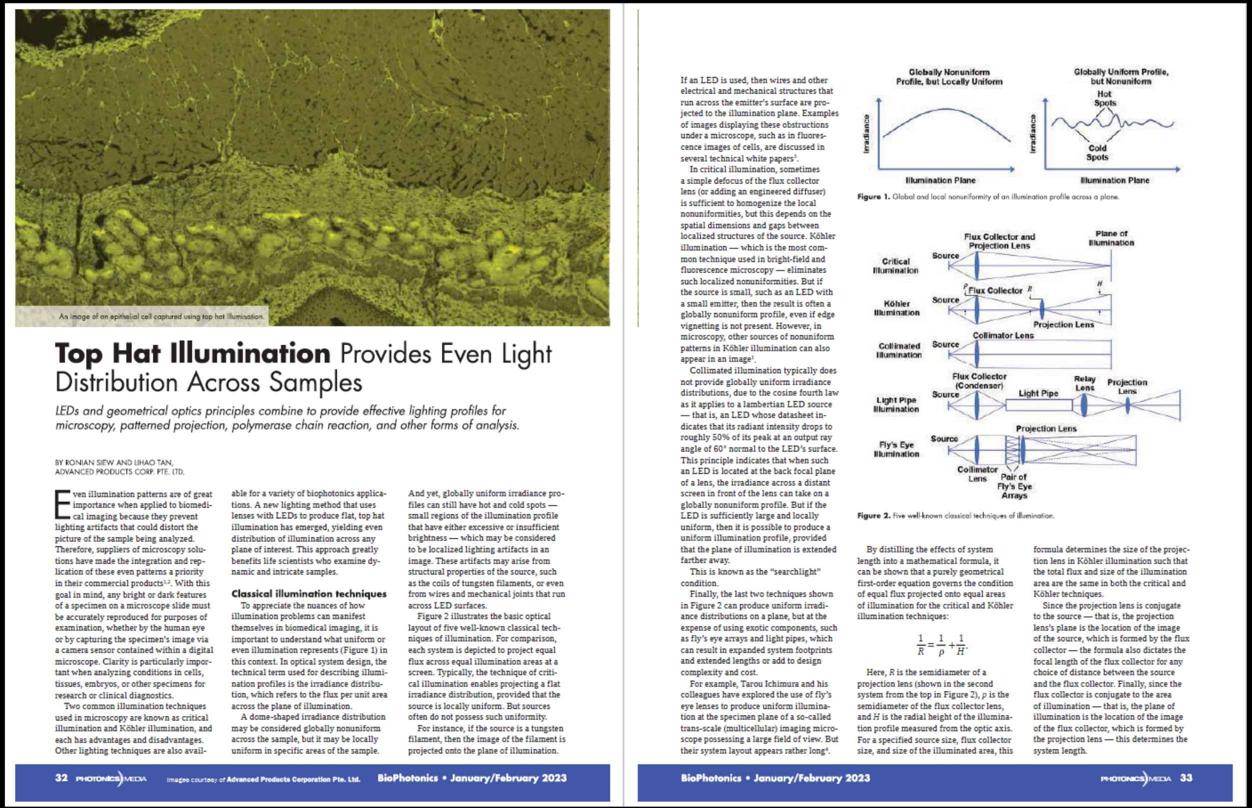

An image of an epithelial cell captured using top hat illumination.

Top Hat Illumination Provides Even Light Distribution Across Samples

LEDs and geometrical optics principles combine to provide effective lighting profiles for microscopy, patterned projection, polymerase chain reaction, and other forms of analysis.

BY RONIAN SIEW AND LIHAO TAN, ADVANCED PRODUCTS CORP. PTE. LTD.

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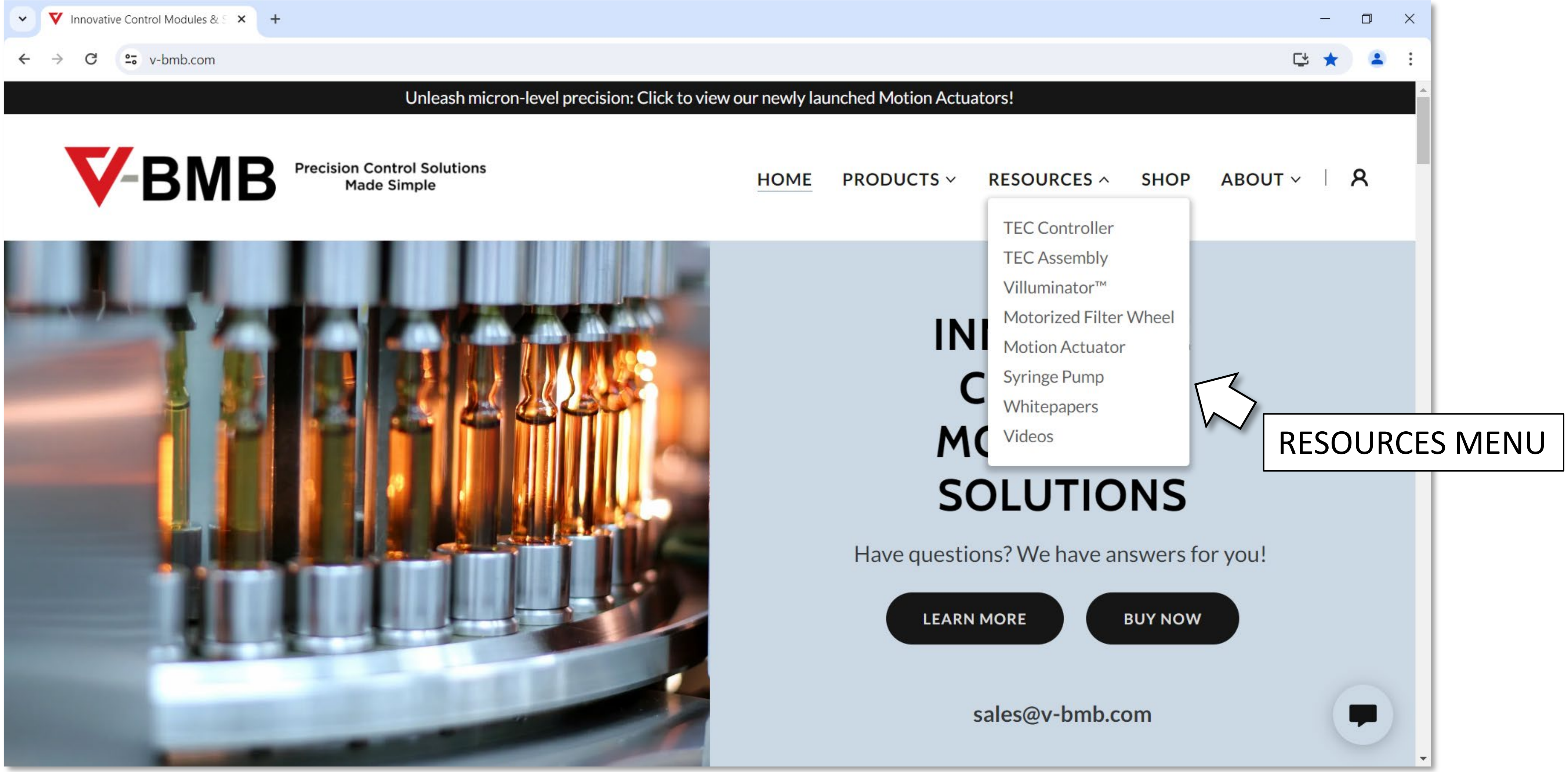

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Thank You!

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