Field uniformity:

# Metrics:

Following “Evaluation of illumination system uniformity for wide-field biomedical hyperspectral imaging”, Travis W Sawyer et al 2017 J. Opt.19 045301

That’s the Mickelson contrast, used when imaging a grating (?)

Ranges from 0 to 1, 0 is perfectly uniform distribution

is the standard deviation of the distribution and is the mean value. Perfect uniform illumination is represented by the 0 value

Following CIC 2019 Kunkel presentation, the RMS contrast ratio is:

# Case 1: Before, Objective 20X NA=0.8

Light guide tip position versus collector lens adjusted to image the tip at the condenser diaphragm following the ”Proper alignment and adjustment of the light microscope” guide. The frosted glass tube holder is in place between the collector lens and the condenser.

Image acquisition at 550nm (BW 10nm) and broadband

Camera shutter time: 550 nm = 0.4 ms; BB = 0.14 ms

# Case 1: After, Objective 20X NA=0.8

Integrating sphere between the light guide tip and the collector lens. The tuning of the collector lens position obtained previously is kept. The frosted glass tube holder is in place between the collector lens and the condenser.

Image acquisition at 550nm (BW 10nm) and broadband

Camera shutter time: 550 nm = 21 ms; BB = 6 ms

BB

550 nm

Before

After

Before

After



Figure 1: 2 metrics for broadband illumination (BB) and at 550 nm. Contrast Ratio (CR), Average Deviation (AD) and RMS contrast (CRMS) are null for perfectly uniform images.

# Conclusion

Based on the contrast ratio values, the integrating sphere improves the field uniformity for the broadband illumination but not for the 550nm wavelength. Using the Average deviation values, the integrating sphere improves the field uniformity for the broadband illumination as it’s pretty much the same for the 550 nm wavelength. Same conclusion for the RMS contrast ratio. The shutter time is multiplied by 40 to 50 when adding the integrating sphere in the light path.



Figure 2: Img-min(img)

# Interference patterns at 550nm

## Objective 20X NA=0.8

With the slide in, focus adjustment and Kohler illumination adjustment. When the slide is removed, the position of the objective is the same and the condenser is risen to meet Kohler illumination condition again. In both cases, there are interference fringes, so it’s not dependent of the glass slide.



Figure 3: Img-min(img): Objective 20x NA 0.8, with and without glass slide

## Objective 10X NA=0.25



Figure 4: Img-min(img): Objective 10x NA 0.25, Glass slide

## Objective 20X NA=0.5



Figure 5: Img-min(img): Objective 20x NA 0.5, with and without glass slide

Again, it’s independent of the presence of the glass slide

## Conclusion

The interferences seem dependent of the objective, but they are not dependent of the presence of the glass slide.

# Measurement of Kodak Wratten Color Filter

The results with no integrating sphere in the illumination path are from the Biomedical Optics Express (tentative) publication, reproducibility measurements increased the uncertainties (compared to reproducibility only measurements, i.e. 10 successive measurements). are the spectra from the spectroradiometer measurements and are from the spatially averaged images acquired by the camera. The measurements with the spectroradiometer were made at speed “Fast” instead of “8XFast” (usual setting) because the signal was too weak.

Table 1: Results for the KW32 filter for both types of illuminations

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Filter | Transmittance |  |  |  |  |  |
| No Int sphere |  |  |  |  |  |  |
|  |  |  |  |  |
| Int pshere |  |  |  |  |  |  |
|  |  |  |  |  |



Figure 6: Transmittance of KW32 color filter



Figure 7: CIELAB results for KW32 color filter with the integrating sphere in the illumination path

## Conclusion

We can use the integrating sphere in the illumination light path.