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# Uniform Angular Illumination in Optical Microscopes

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**Abstract:** Angular illumination asymmetry (ANILAS) at the sample plane depends on illumination wavelength, objective type and the location of aperture stop. To extract consistent and accurate quantitative values, all the three parameters must be aligned.
**OCIS codes:** (170.2945) Illumination design; (170.0110) Imaging systems

## 1. Introduction

Non-uniform angular illumination aberration across the field-of-view is one of the often-overlooked optical aberrations that has significant effect on quantitative imaging, despite having the uniform spatial intensity with Koehler illumination design. The non-uniform angular illumination results in a loss of imaging precision and accuracy leading to less reliable quantitative measurements. Most of the commercially available optical microscopes are generally well designed and aligned. However, these microscopes have potential to readily lose their optimal illumination by misalignment of either the illumination source or the aperture diaphragm. In this paper, we mainly study the effect of misalignment of the aperture diaphragm.

In the referenced work [2-3], we proposed a simple and fast method to measure the angular illumination asymmetry (ANILAS) at the sample plane. By iteratively evaluating the ANILAS maps with careful alignment of the optical elements, it is possible to achieve the lowest distortion in the angular illumination for a given objective. However, as we reveal here that the set of optimized conditions is only good for that particular objective and illumination used. There is a good chance that simply selecting a different objective or illumination wavelength could lead to a sub-optimal illumination conditions, even if all the other conditions remain the same. Here we demonstrate that nearly every objective requires its own optimal alignment condition that helps to enhance optical imaging precision and hence obtain consistent quantitative values.

## 2. Results

A commercially available, research-grade optical microscope was used for the experiments [3] with different magnification and numerical aperture (NA) objective. We used three LED illumination sources (along with band-pass filters) to produce a narrow-band illumination centered around 405 nm ± 5 nm, 520 nm ± 5 nm and 633 nm ± 2 nm. ANILAS maps were evaluated using the dense grating method as described in an earlier publication [3] using an array of trenches in $SiO_2$ with a nominal width of 100 nm having a pitch of 1000 nm over a Si substrate as the grating target.

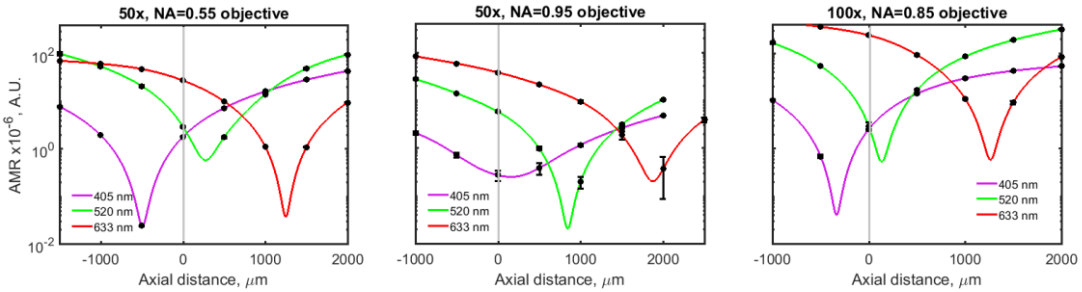


Fig. 1 Plots of ANILAS magnitude ranges (AMR) with the aperture diaphragm axial location for the three objectives studied (a) 50x, NA=0.55, (b) 50x, NA=0.95 and (c) 100x, NA=0.85. The wavelengths of illumination are shown in the legend. Data points (filled circles) were fitted with cubic spline curves. Grey vertical line represents the axial location of the original aperture diaphragm.

The axial location where the minimum AMR occurs in Fig. 1 represents the best axial location of the aperture diaphragm that results in the lowest illumination distortion for that objective, i.e. nearly uniform and symmetric

angular illumination over the entire FOV. From this point any axial deviation of the aperture diaphragm on either side results in increased degree of illumination non-uniformity in the FOV.

Based on this explanation, a few important observations can be made from Fig. 1. The first one is that the axial aperture diaphragm location where the lowest distorted illumination occurs varies with the objective type used even if all the other conditions are the same. In other words, every objective has its own optimum axial location for its lowest illumination distortions. This implies that the one fixed axial aperture diaphragm location provided in most of the optical microscopes cannot physically match with the optimum axial illumination locations for all the objectives.

A second observation is that the optimum axial location not only depends on the type of objective, but also on the illumination wavelength. For the objectives tested, the minimum AMR location moves away from the field aperture (toward the positive axial distance in the convention used here) with increasing illumination wavelength.

Minimum AMR distance data shown in Fig. 1 when presented as a function of the wavelengths as shown in Fig. 2 reveals some additional useful information. If the original aperture diaphragm axial location cannot be moved axially for illumination optimization (which is the case for most of the optical microscopes), this figure enables to determine the wavelength at which a given objective produces the lowest illumination distortions. From Fig.2 we can determine that the objectives 1, 4 and 6 have the best illumination if used with approximately 480 nm, 365 nm and 500 nm illumination wavelengths, respectively. Conversely, if the original aperture diaphragm location can be moved axially for illumination optimization, this figure enables to determine the axial location of the aperture diaphragm where a given objective produces the lowest distorted illumination. For example, if the objective number 4 is desired to be used in combination with an illumination wavelength of 600 nm, then we can determine that the aperture stop axial location of approximately 1.5 mm provides the best illumination condition (red dashed arrows in Fig. 4). It also shows that for the visible spectrum and the objectives used in the current optical microscope, it requires an aperture diaphragm axial alignment range of over 3 mm to achieve the best illumination condition.

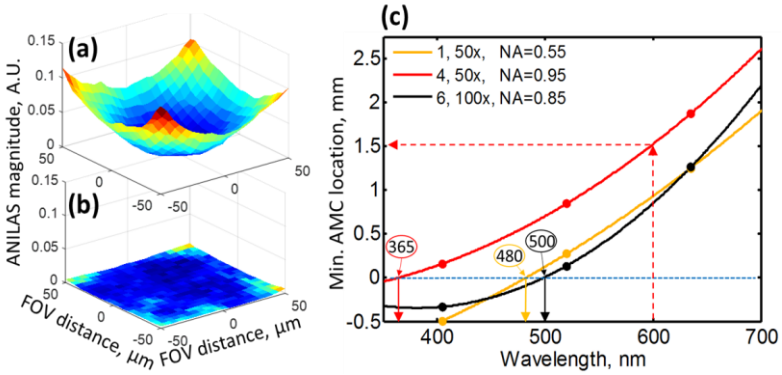


Fig. 2 Typical ANILAS maps for (a) poor, and (b) good angular illuminations. (c) A plot of the minimum AMR axial distances as a function of the illumination wavelengths for the three objectives. Horizontal blue dashed line shows the axial location of the original aperture diaphragm. The solid arrows pointing down from the blue dashed line represent the wavelengths at which the minimum AMR coincides with the original aperture axial location. The objective number, magnification and NA are shown in the legend, in that order.

## 3. Conclusion

ANILAS maps provide a convenient way to measure and visualize the quality of illumination at the sample plane. The axial location of the aperture stop corresponding to the lowest distorted illumination depends upon the objective lens type and the illumination wavelength, with a spread of about 2.36 mm in the current study conditions. Optical images for precision, quantitative imaging, and for metrology applications, this illumination aberration must be minimized by aligning the aperture diaphragm. Hence, we propose to microscope manufacturers that they provide sufficient axial alignment capability for aperture stops (in addition to lateral alignment capability).

## 4. References

[1] This summary is based on the following article: E. Agocs and R. K. Attota, "Enhancing Optical Microscopy Illumination for Quantitative Imaging,", Scientific Reports, under review.
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