Beam Shaping in Microscopy

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The application of lasers in microscopy is very popular, e.g. for excitation of molecules and cells. These lasers often have a Gaussian intensity distribution. However, the characteristic drop in energy at the edge of the Gaussian beam can also have a negative effect on the measurement. Especially, if the achievable performance and resolution of the measurement method is directly connected with the illumination conditions, a uniform illumination of the sample plane has many advantages. Thus, it will be shown in this work how a collimated Gaussian beam can be transformed into a collimated beam with homogeneous intensity distribution and which improvements for the evaluation of microscopic images could be achieved.

The afocal refractive beam shaping system, which was published first by Frieden and Kreuzer [1, 2] consists of two aspheres positioned in a certain distance to each other. The first asphere re-distributes the rays of the incoming Gaussian beam and the task of the second asphere is the re-collimation of the so-called Top-Hat beam. Due to its modular concept, the beam shaping system can be combined either with a collimated beam or a fiber-coupled source to ensure flexible adaption and perfect integration into individual set-ups [3].

In cooperation with the CREOL, different samples were examined with quantitative fluorescence imaging [4]. It was investigated to what extent a homogeneous illumination (FFI) of the samples has advantages over a Gaussian illumination and which improvements in the achievable resolution are possible for two different methods (TIRF and epi). A further investigation was to combine several images by stitching. In Fig. 1 selected results of this investigation are shown. As can be seen, a significant improved and borderless stitching image could be generated for the epi and TIRF method when using a homogeneous illumination.

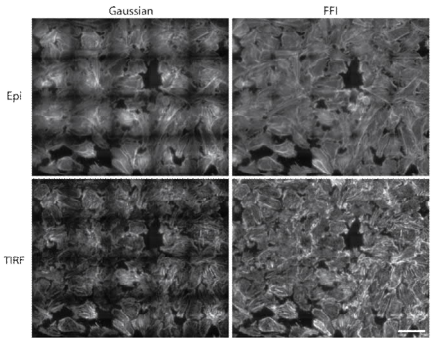

Figure 1: Stitched epi and TIRF images of actin labeled with phalloidin AF647 in U2OS cells using Gaussian illumination and FFI. Image overlap, 5% [4]

So, a homogeneous intensity distribution of the laser beam and a uniform illumination, respectively, can be very helpful in microscopy. It could be shown that a homogeneous illumination has significant advantages concerning the achievable resolution as well as the quality and efficiency of (stitched) images.

REFERENCES

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