Improvement of the resolution in optical imaging systems by interference of against each other inverted image fields

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By adding an image inversion system into one arm of an interferometer, it is possible to increase the lateral resolution of optical imaging systems. We show the functionality of such a system using the example of a microscope.

1 Introduction

There are types of microscopes which have a higher resolution than light microscopes e.g. electron microscopes. But usually it is not possible to observe processes in living cells or analyze samples which are destroyed by high energy radiation. Therefore it is important to increase the resolution of light microscopes. Confocal fluorescence microscopes are the preferred tools to observe biological and medical samples. The resolution of this microscopes depends on the effective PSF which is the result of the multiplication of the illumination PSF and the detection PSF. There were different efforts to minimize the illumination PSF but our work is based on a theoretical paper from Wicker and Heintzmann [1] which suggested a method the reduce the size of the detection PSF with the help of an interferometer.

2 Principles

Using a Mach-Zehnder-interferometer including an image inversion system Wicker and Heintzmann proposed to increase the lateral resolution of confocal fluorescence microscopes. Instead of the suggested diffractive telescope, we used a refractive one to invert the amplitude distribution in one arm of the interferometer. Therefore dispersion effects could be avoided. Fig. 1 shows the used setup.

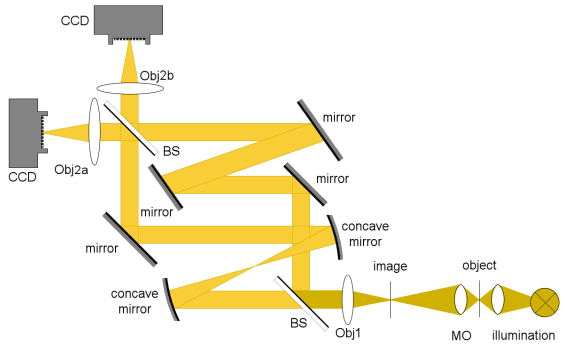

Fig. 1 *Experimental setup: Mach-Zehnder-Interferometer including a refractive telescope*

The image created by the micro objective (MO) is imaged through the image inversion interferometer (III) onto two CCD-cameras. To demonstrate the functionality of this system, we used a micro objective with a low numerical aperture (NA = 0.25). Because of the inversion in one of the both arms there is a superposition of the common amplitude distribution and the one inverted by the telescope at the outputs. If the observed sample consists of uncorrelated point sources like fluorescence molecules or the sample is incoherent illuminated, then only points near the optical axis are able to interfere. So one can observe a higher intensity around the optical axis at the constructive output and a lower one at the destructive output. By subtracting the destructive from the constructive output we are able to obtain the interference structure around the optical axis.
To make sure that the object plane is spatial incoherent illuminated we image the thermal light source into the object plane.

3 The interferometric point spread function

If there is only a point like source at the position $\vec{d_0}$ in the objective plane of the microscope imaged through the III there is not only the image of this point at the position $-\vec{d}$ in the image plane. Because of the image inversion there is also a second point at the other side of the optical axis at the position $\vec{d}$. Since the two point images are coherent, there are not only the two Airy-Disks, but also an interference structure between them around the optical axis (Equ. 1).

$$\begin{aligned} I_{\pm}(\vec{r},\vec{d}) &= \left|\frac{J_1(\vec{r}-\vec{d})}{|\vec{r}-\vec{d}|}\right|^2 + \left|\frac{J_1(\vec{r}+\vec{d})}{|\vec{r}+\vec{d}|}\right|^2 \\ &\pm 2\cdot\frac{J_1(\vec{r}-\vec{d})}{|\vec{r}-\vec{d}|}\cdot\frac{J_1(\vec{r}+\vec{d})}{|\vec{r}+\vec{d}|} \end{aligned} \quad (1)$$

If we integrate over the intensity of the whole difference image, we get a Signal S which depends only on the position $\vec{d}$ in the object plane (Equ. 2).

$$S(\vec{d}) = \int_{-\infty}^{\infty}\left(I_+(\vec{r},\vec{d}) - I_-(\vec{r},\vec{d})\right)d\vec{r} = 2\frac{J_1(2\vec{d})}{|2\vec{d}|} \quad (2)$$

In contrast to the Airy-Disk, this function depends on the doubled argument and there is also no square. Because of that, the full width at half maximum (FWHM) compared to the Airy-Disk is reduced.

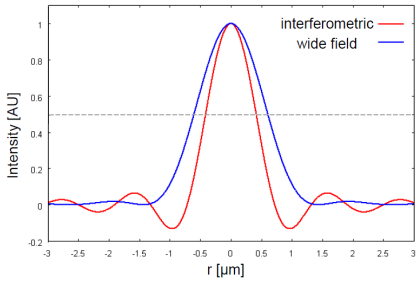

Fig. 2 *Theoretical plots of the conventional wide field PSF (blue) and the interferometric PSF (red) [λ = 589nm]*

If we integrate over the projected position $\vec{d}$ of the point like source instead of the image space the structure of the result stays the same (Equ. 3). But now it depends on the coordinates of the image space ($\vec{r}$). Therefore one can see the new interferometric point spread function in the image plane without scanning. Integrating over the position of the point like source is equivalent to a homogeneous incoherent illuminated object space. We used this method to measure the new interferometric PSF.

$$S(\vec{r}) = \int_{-\infty}^{\infty} \left(I_+(\vec{r},\vec{d}) - I_-(\vec{r},\vec{d}) \right) d\vec{d} = 2\frac{J_1(2\vec{r})}{|2\vec{r}|} \quad (3)$$

4 Results

The first results we obtained by using a sodium vapor lamp as the light source. We used this lamp, because, as a thermal source, it is spatial incoherent but there is a coherence length of above one millimeter. So the setup was easier to handle.

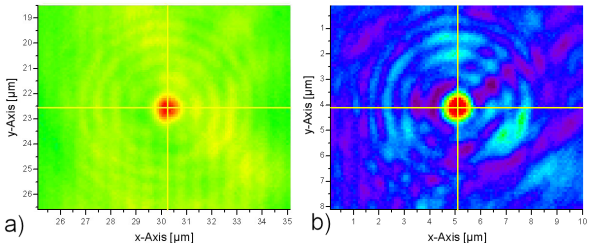

Fig. 3 *Sodium vapor lamp: a) constructive output b) difference image*

In Fig. 3 a) the constructive output of the III can be seen. There is a peak at the optical axis and side lobes around it. These are the predicted side lobes of the Bessel-Sinc function. The destructive image is quite similar but there is a dip at the optical axis. Fig. 3 b) shows the difference image. Around the optical axis one can see a high intensity and far from the optical axis there is an intensity of nearly zero. Compared to theoretical calculations, the measured PSF fits very well near optical axis. But away from the optical axis there are some differences. This difference is most likely caused by the structure of the lamp. As seen in Fig. 3 a), the illumination is not totally homogeneous and possibly there are some phase front deformations in the different arms of the interferometer.

We measured a FWHM of about 0.83 μm (λ = 589 nm). This is an improvement of about 32% and fits in very well with the theory. The measured distance between the two first minima is about 1.89 μm. That is an impromement of 34% compared to the Airy-Disk.

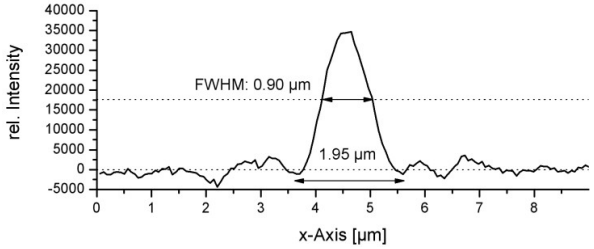

Fig. 4 *White light source: cross section through the difference image*

Because of the fact, that fluorescence light has a wide spectrum, we also used a white light source for illumination. In this case it was an ordinary light bulb. Of course, the images are similar to the images taken with the sodium vapor lamp. In Fig. 4 a cross section through the difference image is illustrated. There is also a high intensity peak at the optical axis but the side lobes are much smoother. We measured a FWHM of about 0.90 μm. If we assume an average wave length of 600 nm this fits quite well with the theory.

5 Conclusion

We were able to realize a Mach-Zehnder-Interferometer including a refractive image inversion system. By illuminating the objective plane homogeneous and spatial incoherent, we could measure the new interferometric detection PSF. Therefore only one picture on each output of the interferometer had to be recorded. The measured results fit very well with the theory. At a wave length of 589 nm a FWHM of 0.83 μm could be measured. Compared to the theoretical FWHM of the Airy-Disk, this is an improvement of 32%. Based on these results one can expect to achieve the predicted improved resolution of this system.

References

[1] K. Wicker and R. Heintzmann, “Interferometric resolution improvement for confocal microscopes,” *OPTICS EXPRESS* ***15***, 12206 – 12216 (2007)