Active management of spherical aberration in live cell microscopy

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In live cell microscopy a mismatch of refraction indices of immersion and embedding liquids often causes spherical aberration and therefore the image quality is reduced. This kind of spherical aberration can be corrected by moving simultaneously both object and image position.

1 Introduction

Usually objectives for microscopy are corrected for objects located next to the cover glass. In live cell imaging objects are in incubation vessels embedded in nutrition liquid. When the refraction indices of immersion and nutrition liquids are not equal the image quality is reduced because of an upcoming spherical aberration.

In order to overcome this kind of spherical aberration there are special correction-objectives with moveable elements. They are mechanical complicated, very sensitive and therefore expensive.

Other solutions introduce supplemental optical systems into the microscope in order to create an additional artificial spherical aberration.

2 Spherical aberration from violation of the Herschel-condition

Objectives for microscopy must follow Abbes sine-condition. Otherwise the image quality is reduced by coma. In consequence of this the Herschel-condition is violated and the image quality is perfect at only one object position. When this position is changed the image quality becomes reduced because of spherical aberration.

The idea is to introduce such a change of object position that the resulting spherical aberration compensates the one which was described in the introduction.

3 Proof of principle

An experiment with objective Plan-Apochromat 40x/0.95 and different thicknesses of cover glass was done. The object was a pinhole. The image quality is nearly perfect for the thickness 0.17 mm only. In all other cases a spherical aberration as described in the introduction was detected (see Fig.1).

After this the positions of object and image were changed according to the values of Tab. 1. With this shifts for all cover glass thicknesses the spherical aberration was reduced significantly and the image quality has become almost perfect.

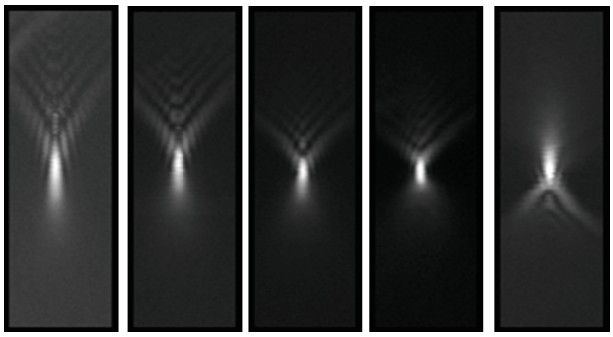

Fig. 1 *Light distribution in image space along the optical axis; Objective: Plan-Apochromat 40x/0.95, cover glass thikness: 0.14, 0.15, 0.16, 0.17, 0.18 (mm)*

cover glass thickness	0.14	0.15	0.16	0.17	0.18
object shift	26mm	17mm	8mm	0mm	-8mm
Image shift	16.3µm	10.6µm	5µm	0µm	5µm

Tab. 1 *Shifts of object and image planes*

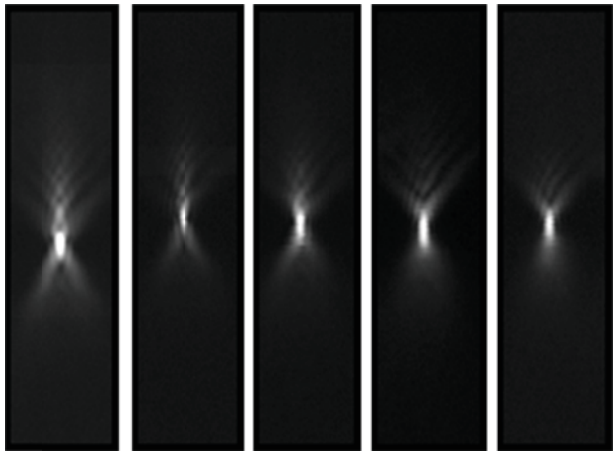

Fig. 2 *Light distribution in image space along the optical axis after shifting object and images according to Tab. 1; Objective: Plan-Apochromat 40x/0.95, cover glass thickness: 0.14, 0.15, 0.16, 0.17, 0.18 (mm)*

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4 Calculation of the shifts

In order to find a formula for calculation it is necessary to investigate to object room.

After focusing to an object deep into the of embedding liquid by moving the objective by Δs to the object the path difference between paraxial ray and the marginal ray becomes different to zero (see Fig 3). This difference δs can be eliminated by changing the radius of reference sphere.

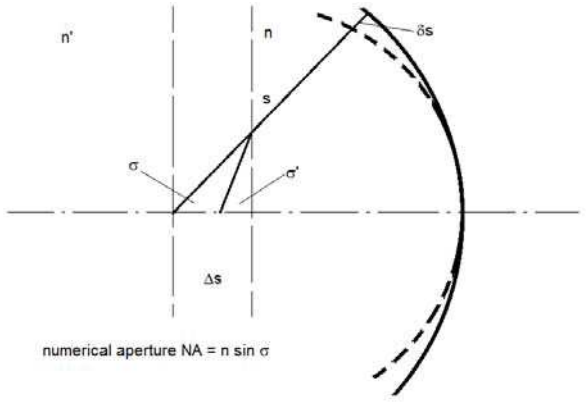

Fig. 3 *Object space after focusing Δs into the embedding liquid.*

Formula for δs is:

$$\delta s = \frac{\Delta s}{n}\left\{\frac{n'^2-n^2}{\sqrt{n^2-NA^2}} - n' \cdot \sqrt{\frac{n'^2-NA^2}{n^2-NA^2}} + n\right\} \qquad (1)$$

Δz, the change of reference sphere radius can be calculated from δs:

$$\Delta z = \frac{n \cdot \delta s}{n-\sqrt{n^2-NA^2}} \qquad (2)$$

Δz is the shift of the object plane. Therefore the shift of the image plane Δz' is:

$$\Delta z' = \frac{\Delta z}{n}\beta^2, \qquad (3)$$

where β is the magnification of the microscope. The combination of the 3 formulas gives the shift of the image plane as:

$$\Delta z' = \frac{\Delta s \cdot \beta^2}{n-\sqrt{n^2-NA^2}}\left\{\frac{n'^2-n^2}{n \cdot \sqrt{n^2-NA^2}} - \frac{n'}{n}\sqrt{\frac{n'^2-NA^2}{n^2-NA^2}} + 1\right\} \qquad (4)$$

The shift of the object plane results from the combination of (1) and (2) as:

$$\Delta z = \frac{\Delta s}{n-\sqrt{n^2-NA^2}}\left\{\frac{n'^2-n^2}{\sqrt{n^2-NA^2}} - n' \cdot \sqrt{\frac{n'^2-NA^2}{n^2-NA^2}} + n\right\} \qquad (5)$$

The last two formulas (4) and (5) allow the calculation of both shifts Δz for the object plane and Δz' for the image plane from Δs.

5. Limitations

Objectives with low magnification usually have got only small numerical apertures. Therefore the spherical aberration from mismatch of refractive indices is also small and there is no need to correct it. On the other hand for high magnifications the magnitude of Δz' becomes too big for a sensible mechanical solution (see (4)). Therefore the presented method is feasible for middle range magnifications from 20x to 63x.

According to (4) und (5) the values of shifting depend strong on n and n'. Therefore the possible penetrations of embedding liquid with corrected spherical aberration are very different. Usually the range is from 20 µm up to 650 µm.

Because of ergonomic reasons applying the method to visual applications is complicated.

6 Summary

In live cell microscopy the image quality is often reduced. The reason of this is usually a mismatch of refraction indices of immersion and embedding liquids which causes spherical aberration. A simultaneous shift of object and image plane can correct this kind of image degradation. The magnitudes of this two shifts are described by formulas (4) and (5).

References

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