Temporal stabilization module inside MicAO 3DSR

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TECHNICAL NOTE

Summary

It is well known that the shape of thin reflective membrane deformable mirrors, and, eventually, their generated wavefront depends on the variation of the ambient temperature in the laboratory. This is not problematic when the deformable mirror is operated in a closed-loop mode because its shape is constantly adjusted using the information from the wavefront measurement. But the variation of surrounding temperature becomes much more important when the deformable mirror is operated in an open-loop mode, especially when the shape has to be kept constant to a very high precision for over 1 hour, as it is sometimes the case in single molecule localization microscopy techniques. Here we present the characterization of a modified Mirao 52e deformable mirror including athermalization for optimal temporal stability regarding temperature variations. Even when the ambient temperature in the room changed by more than four degrees, the shape generated by the modified Mirao 52e deformable mirror did not cause any visible changes of astigmatism in the PSF (less than 10nm RMS total wavefront change). The stabilization module greatly improved the temporal stability of Mirao 52e deformable mirror, thus greatly simplifying super-resolution imaging routine performed with a MicAO 3DSR adaptive optics device.

MicAO 3DSR is an adaptive optics device designed for easy implemention between any inverted-frame microscope and camera and its primary task is to optimize the point spread function (PSF) by correcting aberrations in the whole optical system. The main application of MicAO 3DSR is 3D single molecule localization microscopy (SMLM) techniques, such as PALM/STORM (Betzig et al, 2006; Rust et al, 2006), where using Mirao 52e deformable mirror inside MicAO, aberrations of the optical system are corrected and a fixed amount of astigmatism encoding depth is induced and its shape is held constant for a prolonged period of time (Izeddin et al, 2012), allowing for 3D imaging.

In common implementations of adaptive optics (AO) the deformable mirror is operating in what is called closed-loop configuration together with a wavefront sensor. In this case, the shape of the deformable mirror is constantly adjusted according to the wavefront measurement. This approach is particularly relevant in situations where the wavefront is changing over time. When considering the application of AO for SMLM, the samples are fixed therefore there is no need for temporal monitoring of the wavefront. As a consequence, inside MicAO, the deformable mirror is operating in an open-loop mode: there is no continuous feedback about the actual wavefront to the deformable mirror. The typical imaging time frame in PALM/STORM methods is about 1 hour, enough time to acquire 50k-300k frames that are used for a reconstruction of the super-resolved structure. In common laboratory environments, such a time frame is enough to benefit from a constant room temperature. But in some cases, it is necessary to image longer, therefore the intrinsic stability of the deformable mirror over the whole time period becomes a very important factor. If the deformable mirror shape changes/drifts during the image sequence acquisition, the accuracy of the reconstructed structure might be affected. **We present here an improved version of MicAO 3DSR, including high temporal stability, allowing for SMLM experiments over very long time periods.**

A deformable mirror membrane is typically made of a thin sheet of glass or polymer, and its shape is controlled by moving actuators on one side of the membrane. It is well known that the shape of the deformable mirror membrane can be affected by the variation of the ambient temperature, consequently the resulting wavefront is affected as well (Dainty et al, 1998; Vdovin et al, 2013; Reinlein et al, 2013; Xue et al, 2013). Currently on the

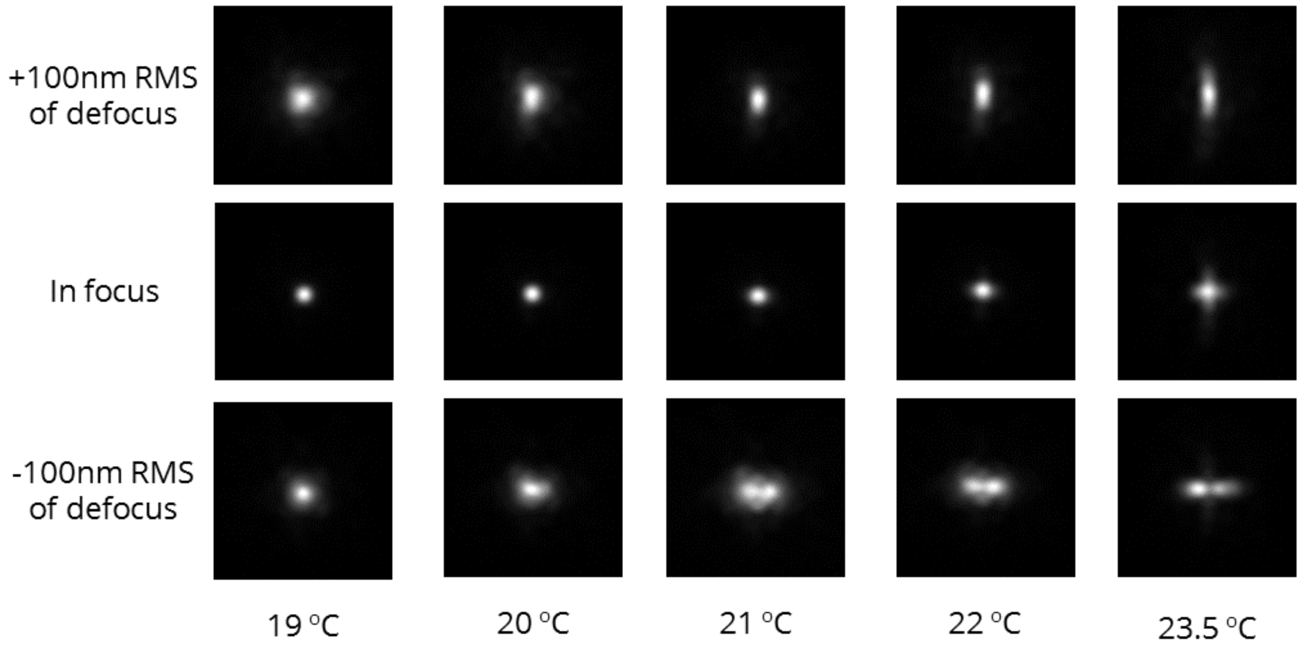

Figure 1. *Example images of the diffraction-limited fluorescent bead at different ambient temperatures without stabilization module inside MicAO. Middle row – the bead is in focus, the upper and lower rows - the bead is slightly out of focus.*

market there is a variety of available deformable mirrors, which are using very different architectures and therefore they may be affected differently by the ambient temperature variation. Temperature variation can cause changes in focus, appearance of astigmatism, spherical aberration or coma.

In the case of Mirao 52e deformable mirror, the main aberration which appears with temperature change is astigmatism. Depending on the way the membrane is assembled, the orientation of astigmatism is different and it is a generally a superposition of both astigmatism at 0° and astigmatism at 45°. In Figure 1 we show examples of the PSF of a typical Mirao 52e deformable mirror when the ambient temperature is changing. For this particular mirror the main aberration that appears is astigmatism at 0°.

Using the wavefront sensor attached to the camera port of MicAO 3DSR we measured the temperature variation of the wavefront and Figure 2 shows its decomposition into Zernike modes. The data shown in this measurement was obtained using a different Mirao 52e deformable mirror than the one used in Figure 1,

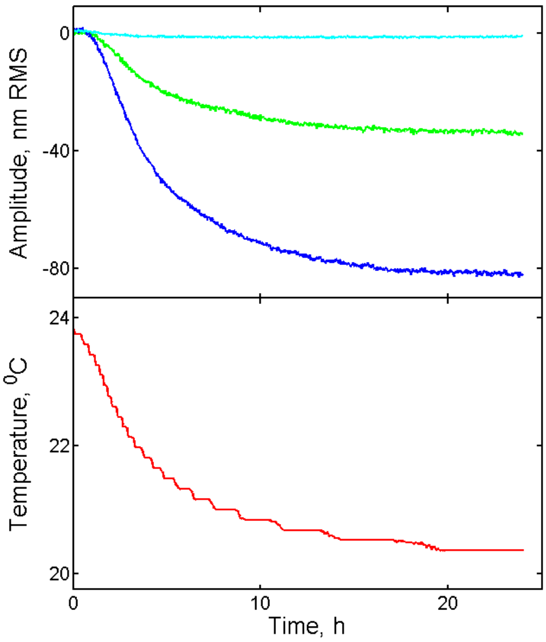

Figure 2. *Ambient temperature dependence of various Zernike modes without active control on the deformable mirror (upper panel); astigmatism 0 (blue), astigmatism 45 (green) and 3rd order spherical aberration (cyan). Temperature variation is shown in red on the lower panel.*

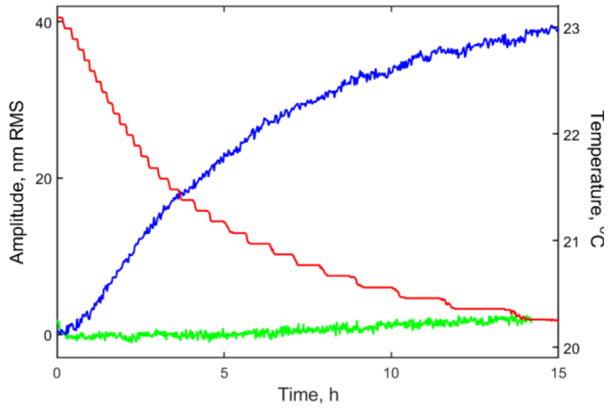

Figure 3. *Ambient temperature dependence of the wavefront without (blue) and with (green) stabilization module installed on the deformable mirror. Temperature change is shown in red.*

explaining why in this case we observe variation in both orientations of astigmatism. The variation of all other Zernike modes was negligible, therefore in Figure 2, as an example, we only show the temperature variation of the 3rd order spherical aberration.

The variation of the room temperature in the laboratory typically is not significant during 15-30 min, but for time periods exceeding 1 hour it becomes more challenging to control the room temperature within one degree precision. Larger variations in the room temperature might cause larger changes in the deformable mirror membrane and this might become visible in the PSF. That is why we implemented a stabilization module inside the MicAO 3DSR device, providing athermalization of the AO loop. This feature dramatically increases the temporal stability of the generated wavefront and stability of resulting PSF. In the example shown in Figure 3, over the course of 12 hours the room temperature cooled down by 3 degrees and the total wavefront changed by less than 5 nm RMS, therefore the MicAO stabilization module diminishes the wavefront variation by more than 10 times.

The effect of MicAO temperature stabilization module can best be seen directly on the PSF (Figure 4). According to our measurements, when the ambient temperature increased by more than 3 °C there are no visible astigmatism changes in the PSF. The Point Spread Function is very similar during the day, as well as the next morning and even the day after that, even though the ambient temperature changed by almost 4 degrees and the

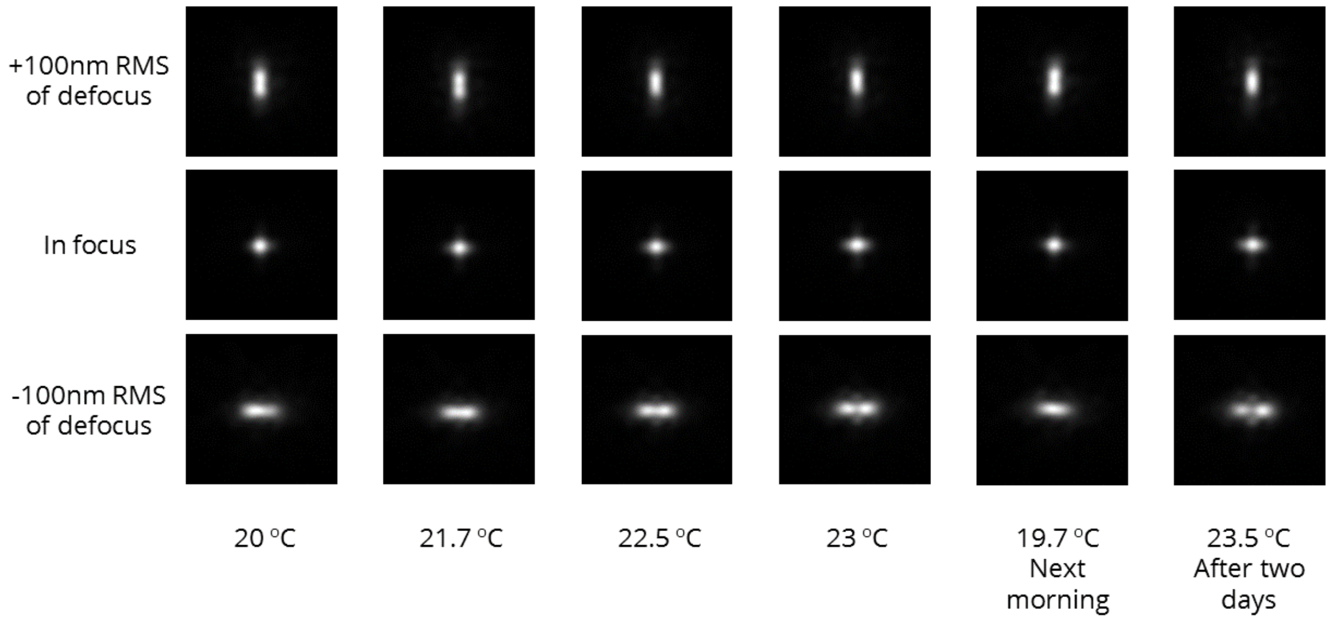

Figure 4. *Example images of the diffraction-limited fluorescent bead at different ambient temperature. The 60nm RMS of astigmatism was added using the deformable mirror. Middle row – the bead is in focus, the upper and lower rows - the bead is slightly out of focus.*

stabilization module was switched off during the night and had to re-stabilize the next morning.

The only difference that starts to be visible in the PSF due to temperature change is its splitting into two dots on one side of the focus and a smaller astigmatism extention of the PSF on the other side. These are characteristic features of small amounts of 3rd order spherical aberration. As we showed just above, the Mirao 52e deformable mirror does not induce spherical aberration due to temperature change (cyan trace in Figure 2). Most likely, this spherical aberration is due to the temperature-dependent optical components inside the microscope.

Conclusions

Here we characterized the temperature stabilization module inside MicAO 3DSR. The main advantages brought by this accessory are:

- The deformable mirror can now hold it's shape and the Point Spread Function can stay optimized for hours. The observed residual wavefront variation over the course of 12 hours is less than 10 nm RMS. This wavefront variation would have minimal effect on the localization precision and the reconstruction accuracy in SMLM methods over very long experiments.
- MicAO 3DSR device can now be used for long-term imaging sequences which increases the versatility and simplicity of the MicAO 3DSR usage.
- The PSF is now reproducible on the daily basis, regardless of the temperature variation in the room. Only very small adjustments of the PSF might be needed when placing a new biological sample on the microscope's stage.
- The improved system stability simplifies the daily use of the MicAO 3DSR device because there is no need to strictly control the temperature in the laboratory.

References

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