REALM compact user manual

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1. Introduction

Thank you for reading this compact user guide for the open source REALM micro-manager plugin. REALM stands for Robust and Effective Adaptive optics in Localization Microscopy and performs aberration correction in Single Molecule Localization Microscopy (SMLM). It can however also be used for aberration correction on bead(s) or other small fiducial markers or to flatten the deformable mirror on a bead sample.

REALM provides an user-friendly interface to allow users to perform SMLM or other types of microscopy in tissue or other complex samples. It is designed for users with little background adaptive optics, but provides access to important parameters such that advanced users can tune the aberration correction algorithm.

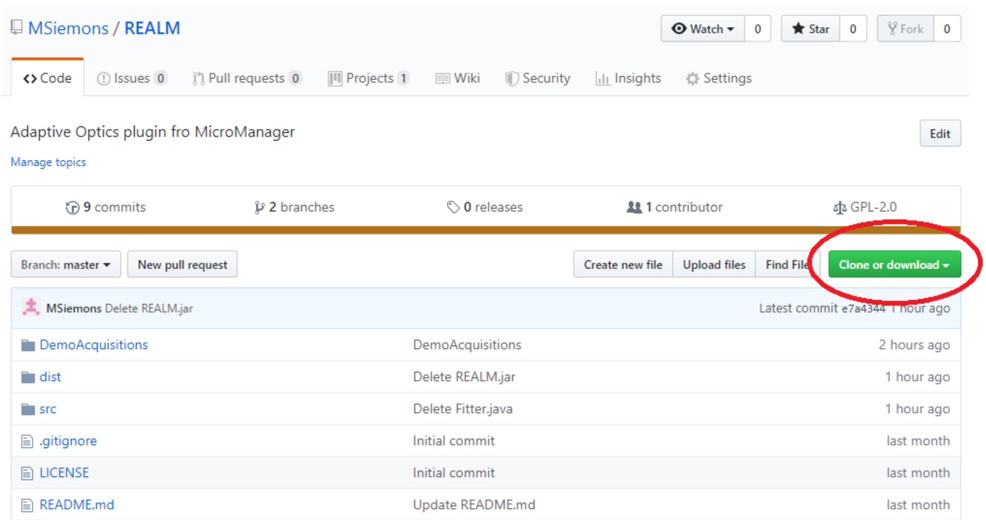
2. Deformable Mirror support

Currently REALM supports the deformable mirror MIRAO52E from Imagine Optics. MicroManager uses device adapters to communicate with devices. You can download the device adapter for MIRAO52E from https://github.com/MSiemons/MIRAO_DeviceAdapter. Here a manual is provided on how to install this device adapter.

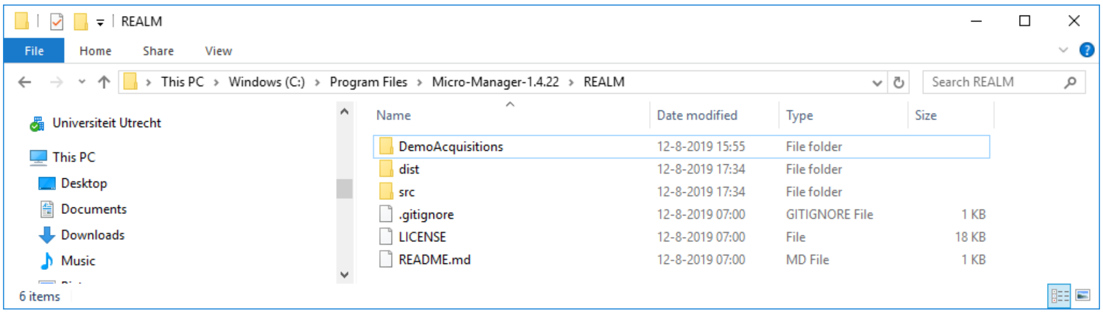
If you would like to use your own deformable mirror in combination with REALM please contact Marijn Siemons (m.e.siemons@uu.nl) on how to interface with REALM.

3. Installation

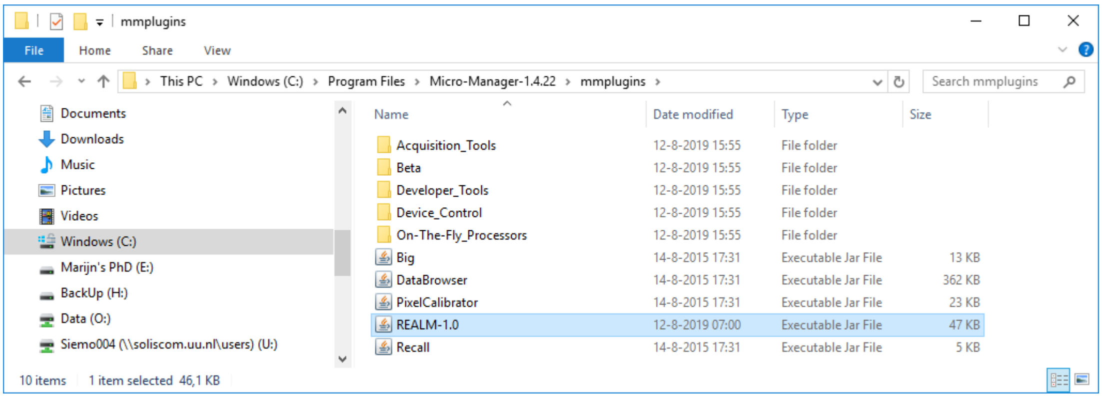
1. Download the .zip file from the repository https://github.com/MSiemons/REALM.



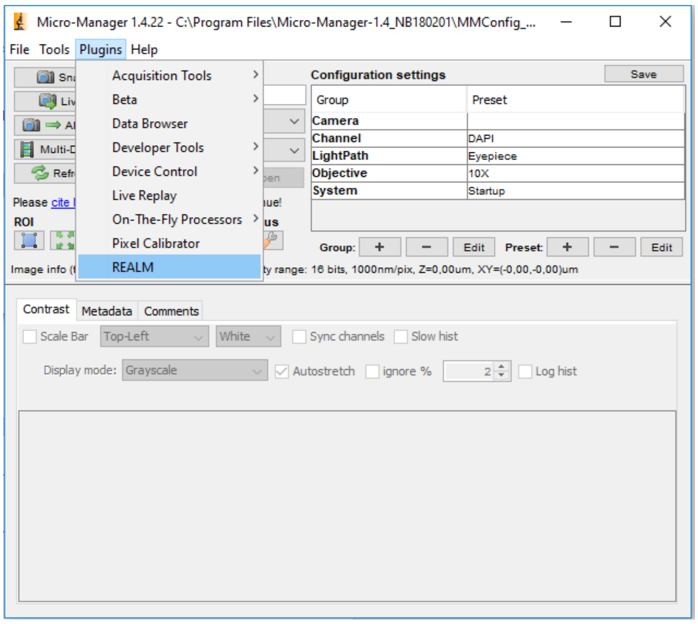
2. Create a folder name “REALM” in the Micro-Manager installation folder, extract the zip-file and copy all files to this folder.



3. Copy the latest version REALM-X.jar from the dist-folder to the mmplugins folder.



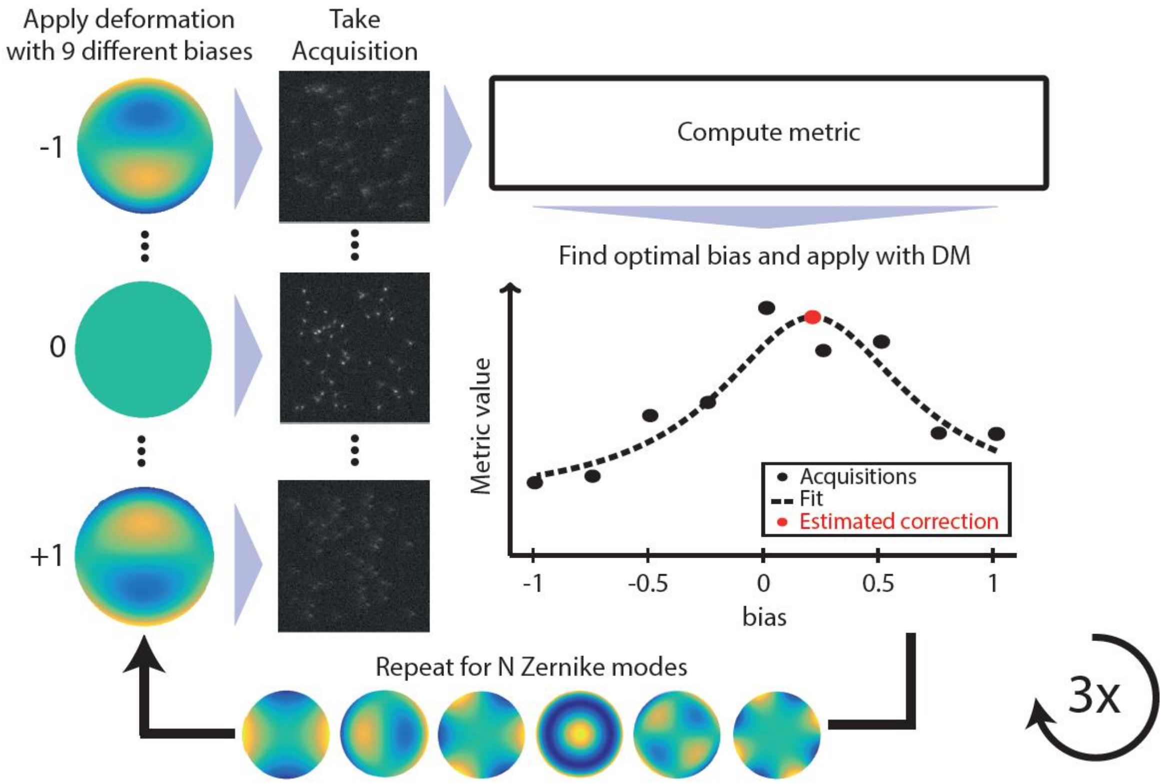
4. Start Micro-Manager and check that Micro-Manager recognizes the plugin. REALM can be found under ‘Plugins’. REALM is now installed.



4. Aberration correction with REALM

4.1 Method: Model-based optimization

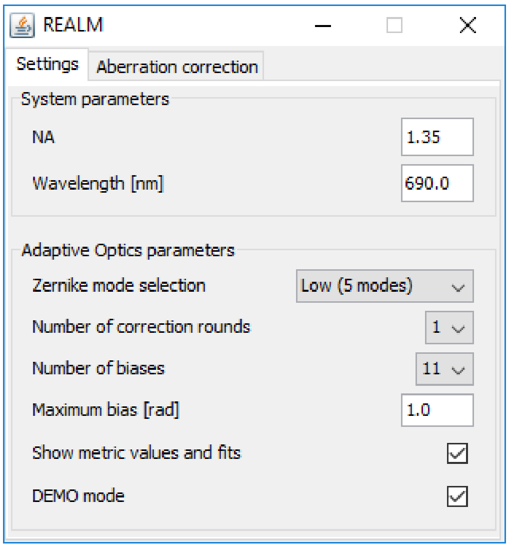
REALM uses model-based optimization to iteratively correct Zernike modes. In brief, each Zernike mode is corrected by applying a sequence of different amounts of biases of the to be corrected Zernike mode. From these acquisitions a metric is computed. Next a Gaussian is fitted through these metric values to find the optimum. This is then performed for all the to be corrected Zernike modes and is repeated for multiple correction rounds.



REALM allows you to control 4 parameters of the optimization algorithm to tune this to your needs. These parameters are: the number of biases per Zernike mode, the range of the biases (in radians), the Zernike modes you want to correct and the number of correction rounds. Default for these are 9 biases, 1 radian, 3 correction rounds and Zernike modes up to *Z42* (medium). The number of total acquisitions taken by the correction algorithm is number of biases * number of Zernike modes * number of correction rounds.

4.2 The Settings tab

The settings tab provides access to set the correct parameters of your set-up and experiment as well as advance adaptive optics parameters.

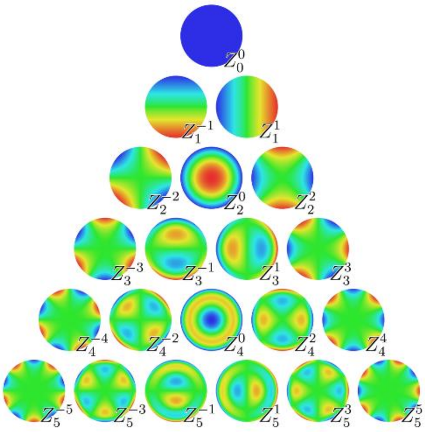


The system parameters are

- **NA**
  This is the numerical aperture of the objective you use. However, if the numerical aperture is larger than the refractive index of the mounting medium, the effective NA is lowered. In this case you should use the refractive index of the mounting medium instead of the NA.

- **Wavelength**
  The peak emission wavelength in nanometer.

The adaptive optics parameters are



- **Zernike mode selection**
  With this dropdown list you can select the Zernike modes you want correct. It features 3 sets of Zernike modes:
  1. **Low**
     Z22, Z2-2, Z31, Z3-1, Z40
  2. **Medium**
     Z22, Z2-2, Z31, Z3-1, Z33, Z3-3, Z40, Z42, Z4-2
  3. **High**
     Z22, Z2-2, Z31, Z3-1, Z33, Z3-3, Z40, Z42, Z4-2, Z44, Z4-4, Z60

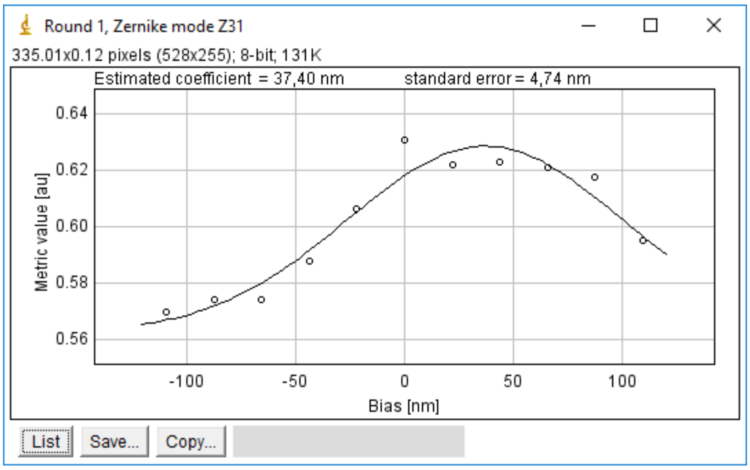
  The modes are corrected in the order they are listed here.

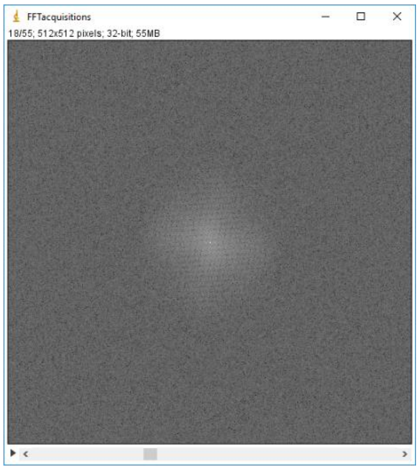
- **Number of rounds**
  The number of correction rounds as described in section 4.1.

- **Number of biases**
  The number of biases per Zernike mode as described in section 4.1. Options are: 5, 7, 9, 11 and 13.

- **Maximum bias**
  This parameters controls the bias range applied for each Zernike.

- **Show metric values and fits**
  If ticked, Micro-Manager plots all metric values and fits. It also shows the Fourier transformed acquisitions in the end. This gives you acces 'under the hood' of REALM.

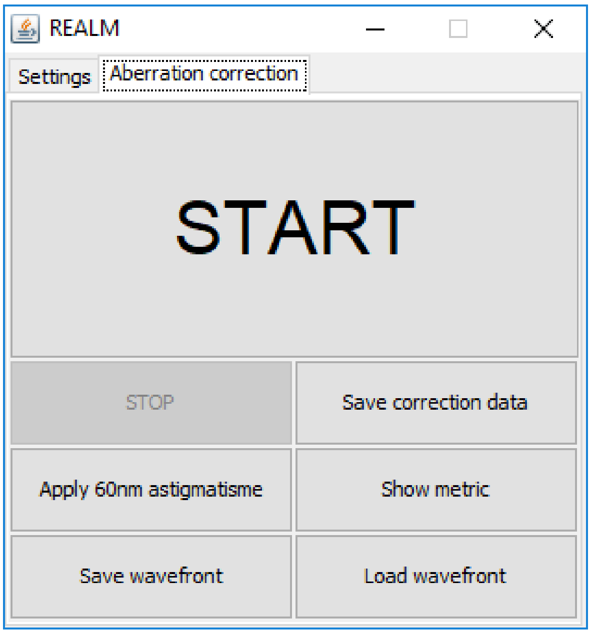




- **DEMO mode**
  If ticked, REALM goes into DEMO mode. This mode doesn't require a deformable mirror and loads simulated acquisitions. These acquisitions should be in the folder *REALM/DemoAcquisitions*.

4.3 The Aberration correction tab

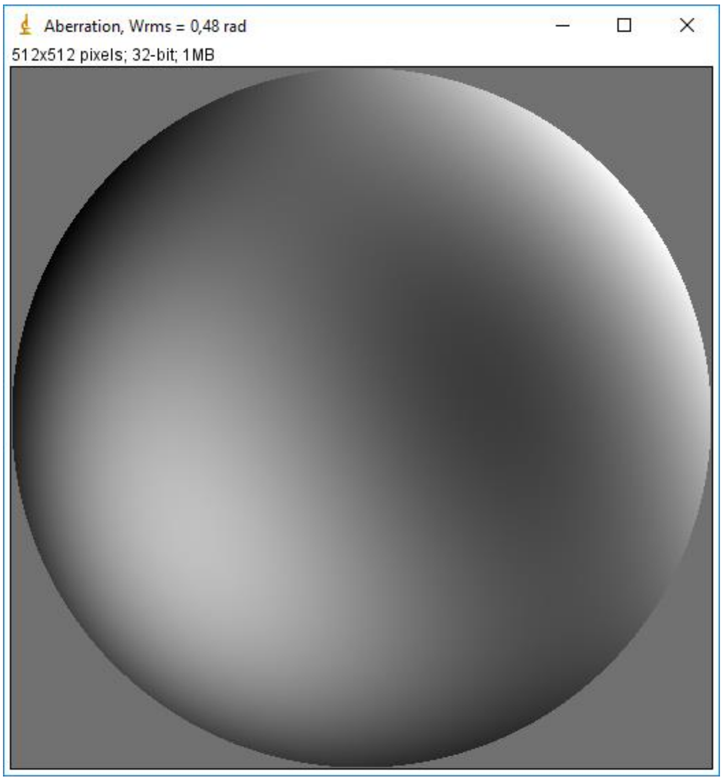
This tab contains all the action buttons and is the heart of REALM. It allows you to start and stop the aberration correction algorithm. Furthermore you can save the correction data and load and save new wavefronts. By applying 60nm of astigmatism you can mimic a cylindrical lens and do 3D imaging. The show metric button shows the numerator and denumerator in frequency space.



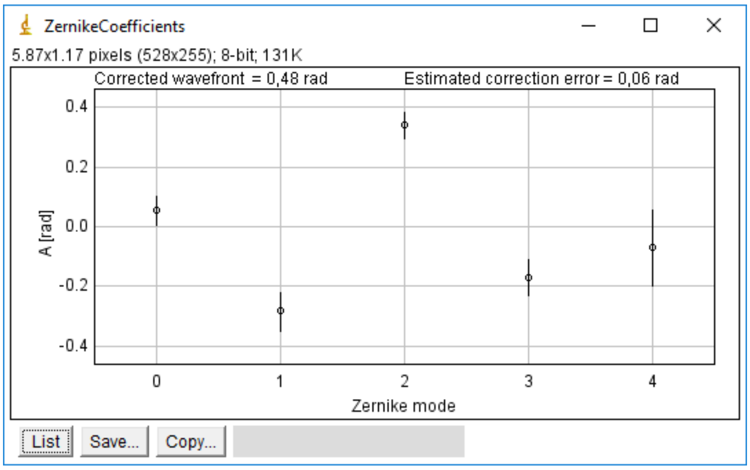
4.4 REALM output after aberration correction

When the aberration correction is finished REALM outputs the following plots:

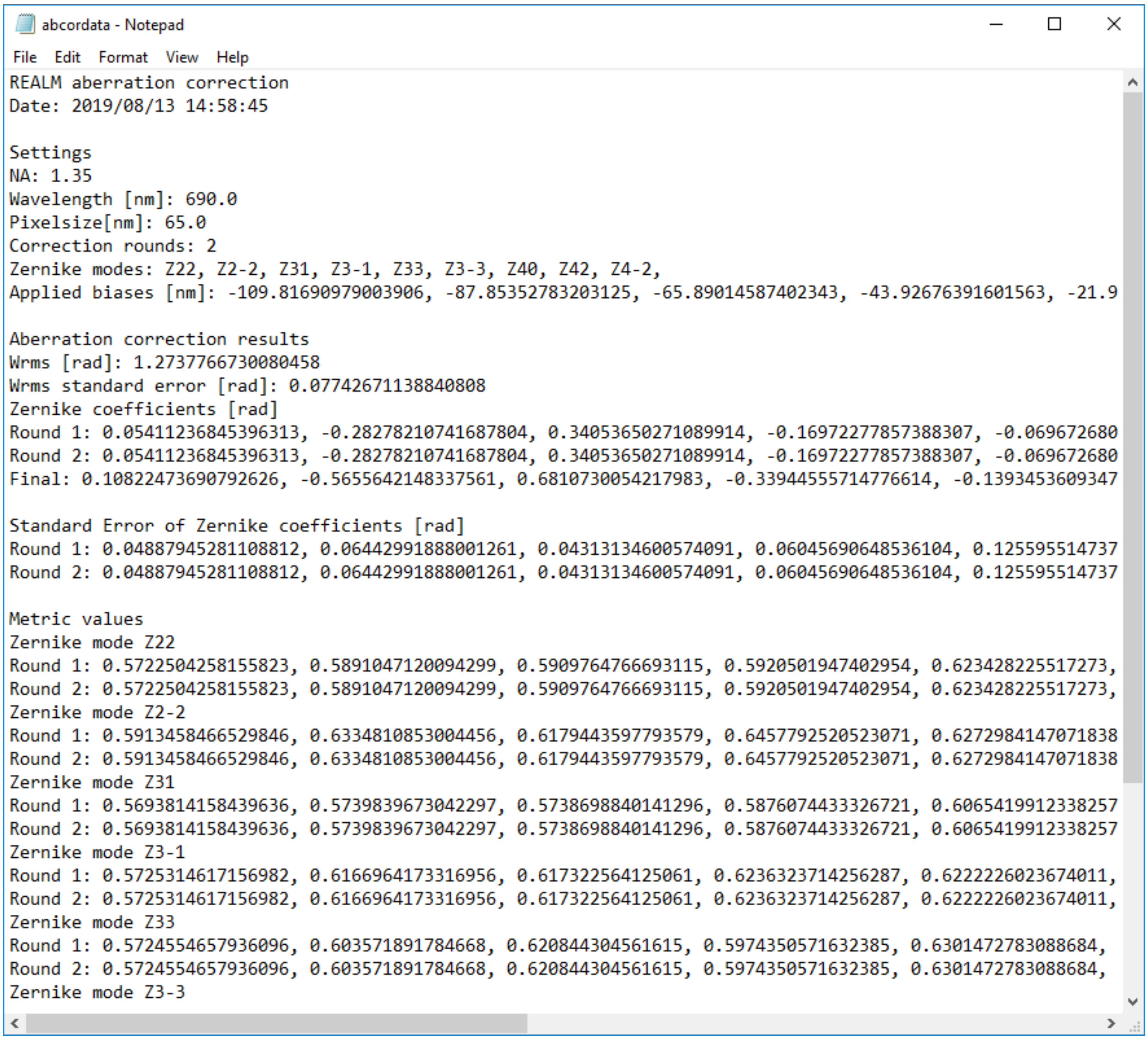
- The estimated aberration in a 512 x 512 image. The pixel values are the physical deformation of the mirror in nanometer. The title contains the rms aberration level.



- The rms normalized Zernike coefficients in radians. The error flags indicate the standard error of the coefficient. The ordering of the Zernike can be found in the aberration correction save file or above in section 4.2.



- The “Save correction data”-button allows the user to save all correction data in a text file.



abcordata - Notepad

File Edit Format View Help

```
REALM aberration correction
Date: 2019/08/13 14:58:45

Settings
NA: 1.35
Wavelength [nm]: 690.0
Pixelsize[nm]: 65.0
Correction rounds: 2
Zernike modes: Z22, Z2-2, Z31, Z3-1, Z33, Z3-3, Z40, Z42, Z4-2,
Applied biases [nm]: -109.81690979003906, -87.85352783203125, -65.89014587402343, -43.92676391601563, -21.9

Aberration correction results
Wrms [rad]: 1.2737766730080458
Wrms standard error [rad]: 0.07742671138840808
Zernike coefficients [rad]
Round 1: 0.05411236845396313, -0.28278210741687804, 0.34053650271089914, -0.16972277857388307, -0.069672680
Round 2: 0.05411236845396313, -0.28278210741687804, 0.34053650271089914, -0.16972277857388307, -0.069672680
Final: 0.10822473690792626, -0.5655642148337561, 0.6810730054217983, -0.33944555714776614, -0.1393453609347

Standard Error of Zernike coefficients [rad]
Round 1: 0.04887945281108812, 0.06442991888001261, 0.0431313460057409l, 0.06045690648536104, 0.125595514737
Round 2: 0.04887945281108812, 0.06442991888001261, 0.0431313460057409l, 0.06045690648536104, 0.125595514737

Metric values
Zernike mode Z22
Round 1: 0.5722504258155823, 0.5891047120094299, 0.5909764766693115, 0.5920501947402954, 0.623428225517273,
Round 2: 0.5722504258155823, 0.5891047120094299, 0.5909764766693115, 0.5920501947402954, 0.623428225517273,
Zernike mode Z2-2
Round 1: 0.5913458466529846, 0.6334810853004456, 0.6179443597793579, 0.6457792520523071, 0.6272984147071838
Round 2: 0.5913458466529846, 0.6334810853004456, 0.6179443597793579, 0.6457792520523071, 0.6272984147071838
Zernike mode Z31
Round 1: 0.5693814158439636, 0.5739839673042297, 0.5738698840141296, 0.5876074433326721, 0.6065419912338257
Round 2: 0.5693814158439636, 0.5739839673042297, 0.5738698840141296, 0.5876074433326721, 0.6065419912338257
Zernike mode Z3-1
Round 1: 0.5725314617156982, 0.6166964173316956, 0.617322564125061, 0.6236323714256287, 0.6222226023674011,
Round 2: 0.5725314617156982, 0.6166964173316956, 0.617322564125061, 0.6236323714256287, 0.6222226023674011,
Zernike mode Z33
Round 1: 0.5724554657936096, 0.603571891784668, 0.620844304561615, 0.5974350571632385, 0.6301472783088684,
Round 2: 0.5724554657936096, 0.603571891784668, 0.620844304561615, 0.5974350571632385, 0.6301472783088684,
Zernike mode Z3-3
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5. Example of REALM workflow

1. Initial flattening of the mirror
   - Place a bead sample on the microscope and load a recent wavefront. Focus on the beads using the stage and select an Region Of Interest (ROI) in the middle of the Field of View (FOV). Select the following adaptive optics parameters:
     - Zernike mode selection: High
     - Number of biases: 5
     - Number of correction rounds: 2
     - Maximum bias: 1 rad

     For optimal correction: focus again on the bead and re-run the correction algorithm.

2. Mount your sample, focus and select and ROI. For optimal correction the size of the ROI should be
   - Square and a power of 2 (128x128, 256x256, 512x512).
   - Contain little background or non-blinking fluorescence.

3. Select the parameters for aberration correction. We suggest:
   - Zernike mode selection: Low or Medium
   - Number of biases: 13
   - Number of correction rounds: 2
   - Maximum bias: 1 rad
   - Show metric values and fits: on

4. Initiate blinking and optimize blinking dynamics with laser power and exposure time. Aberration correction works best in the present of 50 or more emitters per frame.

5. Start aberration correction by pressing start. Watch and check if improvement occurs. REALM outputs the following things in the end:
   - A plot of the Zernike coefficients
   - An image of physical deformation of the mirror

6. Add 60 nm of astigmatism for 3D SMLM. (Optional)

7. Start your regular SMLM acquisition stream.

8. Save correction data, aberration and Zernike coefficients