a

Projektarbeit / Student Project

Dual Channel Single Molecule Localisation Microscopy

concluded at the

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advised by

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of

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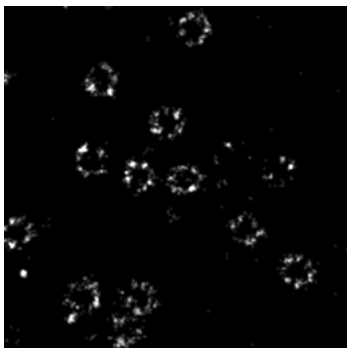

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https://github.com/imrahilias/biophysics

Contents

Chapter 1

Introduction

we want two color dyed SMLM data in sync, how to get there?

Single Molecule Localisation Microscopy (SMLM) is a technique of fitting a full Point Spread Functions (PSF) to a stack of images containing reasonably spaced fluorescence signals.

To be able to accurately estimate the 3 dimensional location of the fluorescent molecule, the PSF must be known quite well. Simply put: If one knows the shape of a point source in varying degrees of defocus, one can guess the defocus and thus the z coordinate of the fluorescence molecule.

1.1 Flouro

Flouro

Chapter 2

Methods

2.1 SMLM

SMLM

2.2 TIRF

TIRF

2.3 dSTORM

dSTORM

2.3.1 Gloxy Buffer

Single channel SMLM may employ the same buffer for both excitation wavelengths, in our case at 645 nm (red) respective at 488 nm (blue). After its central ingredients glucose and oxidase, the buffer we used throughout this paper for all single channel measurements unless noted otherwise is called Gloxy buffer:

Gloxy buffer concentration

- 50 mmol β-MercaptoEthylamine hydrochloride (MEA, Sigma-Aldrich).
- 10 vol% of a 250 g L^{-1} solution of glucose.
- 0.5 mg ml^{-1} glucose oxidase.
- 40 mg ml^{-1} catalase (Sigma-Aldrich).
- in PBS, pH 7.6 .

2.3.2 OxEA Buffer

Dual channel Fluorescence microscopy poses novel challenges to find a proper dSTORM buffer, that works for both excitation wavelengths—thus two distinct fluorophores AF647 and AF488. The buffer composition we used is based on cit, and called OxEA after its main ingredients OxyFlour and (β-Mercapto)Ethylamine:

OxEA buffer concentration

- 50 mmol β-MercaptoEthylamine hydrochloride (MEA, Sigma-Aldrich).
- 3 vol% OxyFlourTM (Oxyrase Inc., Mansfield, Ohio, U.S.A.).
- 20 vol% of 60% sodium DL-lactate solution (L1375, Sigma-Aldrich).
- in PBS, pH adjusted to 8–8.5 with NaOH.

OxEA buffer protocol

For about 1 mL of OxEA buffer we used the amounts shown in Table 2.1, to obtain above listed concentrations.

pH

The pH of the OxEA buffer is checked using both broad range pH testing strips, and a digital pH meter, to be between pH 7 and pH 8.

Table 2.1: Ingredients used for preparation of OxEA buffer for dual channel dSTORM buffer.

Order	Ingredient	Store	Vol / µL
1	Ultra pure H_2O		600
2	10 M NaOH		20
3	10× PBS		100
4	60% DL-lactate	fridge	200
5	1 M MEA	freezer	50
6	OxyFluor	freezer	30

2.4 PSF

PSF

2.5 Zernike

zern

2.6 NPC Analysis

analysis

2.7 Dual Channel Transforms

transform

Chapter 3

Results

3.1 Flouro

sample image

3.1.1 dSTORM

2d npc images

3.2 Single Molecule Localisation

It is possible to use different excitations to simultaneously measure different fluorescence markers, but for that the Point Spread Functions (PSF) has to known for each wavelength. So the PSFs are estimated both for red and blue laser light, with the Phase Retrieval program by [Jesacher et al 21?]; each on a stack of 50000 STORM images of 100 nm 100nm? beads stained with fluorescence dyes for both red (645 nm) and blue (488 nm) laser light.

3.2.1 Correction Collar

To further complicate things, the new Olympus 1.5 NA objective comes with a Correction Collar to compensate for aberrations—which naturally vary slightly for both color channels. In order to find the best Correction Collar setting of the Olympus 1.5 NA objective for SMLM, the Point Point Spread Functions (PSF) is computed for each of the three settings of the correction collar $\{0.13, 0.17, 0.19\}$; using the program by [Jesacher et al 21?].

3.2.2 Zernike Modes

The Zernike modes of the PSF are shown for each of the three correction collar settings $\{0.13, 0.17, 0.19\}$, each for blue channel respective red channel in Figure 3.1 respective Figure 3.2.

For convenience the same results are additionally shown grouped by the the three correction collar settings $\{0.13, 0.17, 0.19\}$, now for both red and blue channel in Figure 3.3, Figure 3.4 and Figure 3.5.

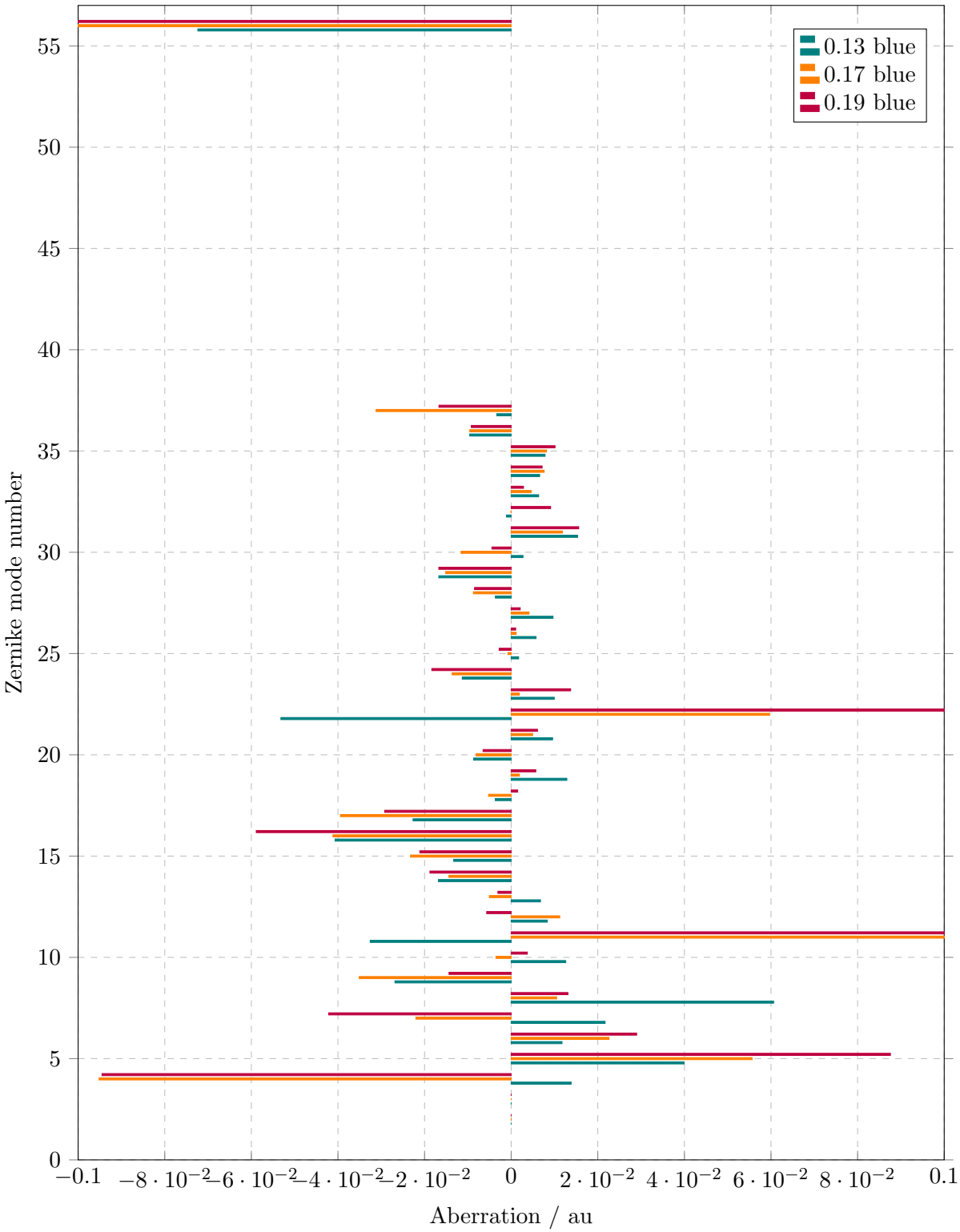

Figure 3.1: Blue channel Zernike modes $\{1 \dots 37, 56\}$ *versus aberrations of PSF model via phase retrieval, for all three correction collar settings (0.13, 0.17, 0.19).*

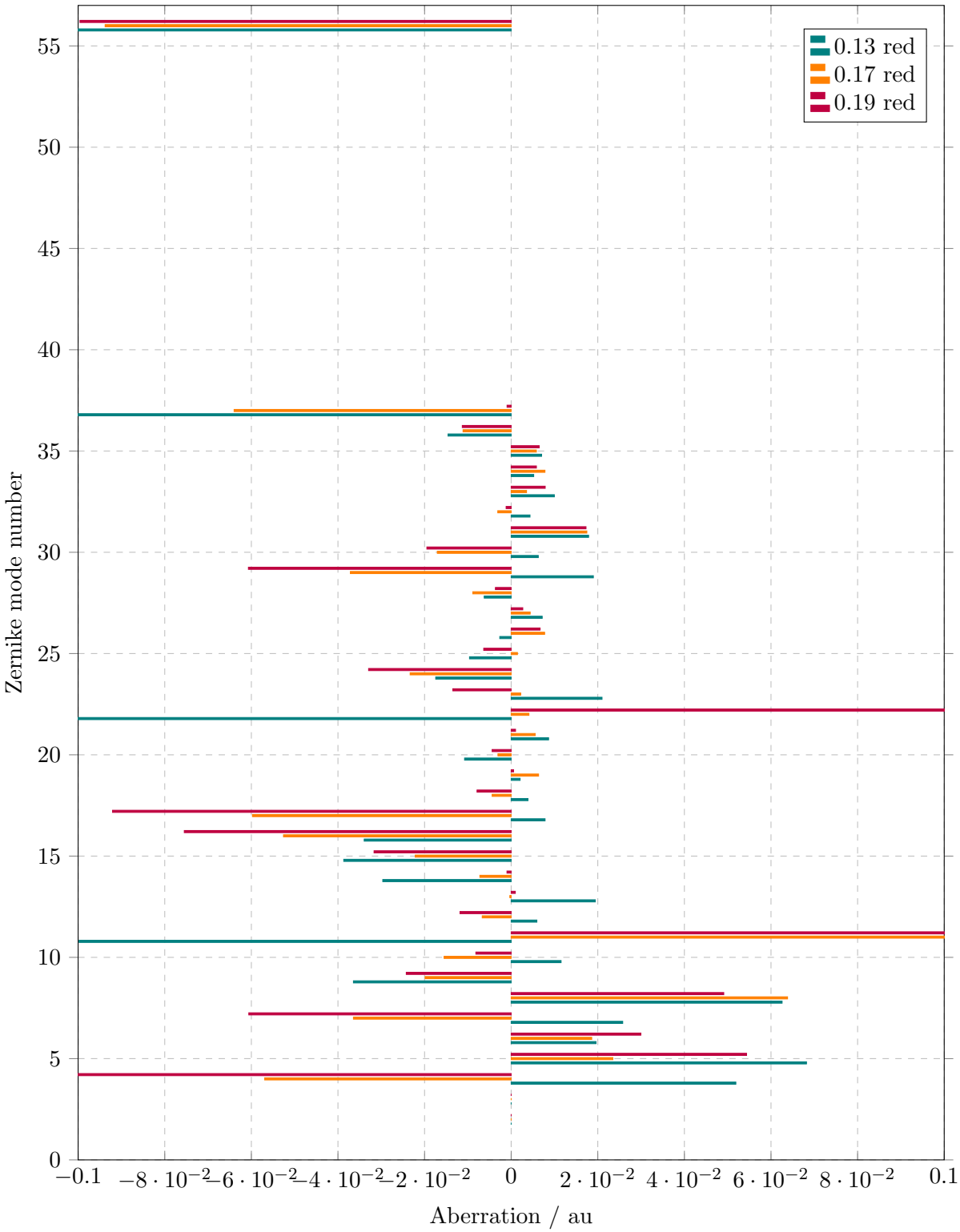

Figure 3.2: Red channel Zernike modes $\{1 \dots 37, 56\}$ *versus aberrations of PSF model via phase retrieval, for all three correction collar settings (0.13, 0.17, 0.19).*

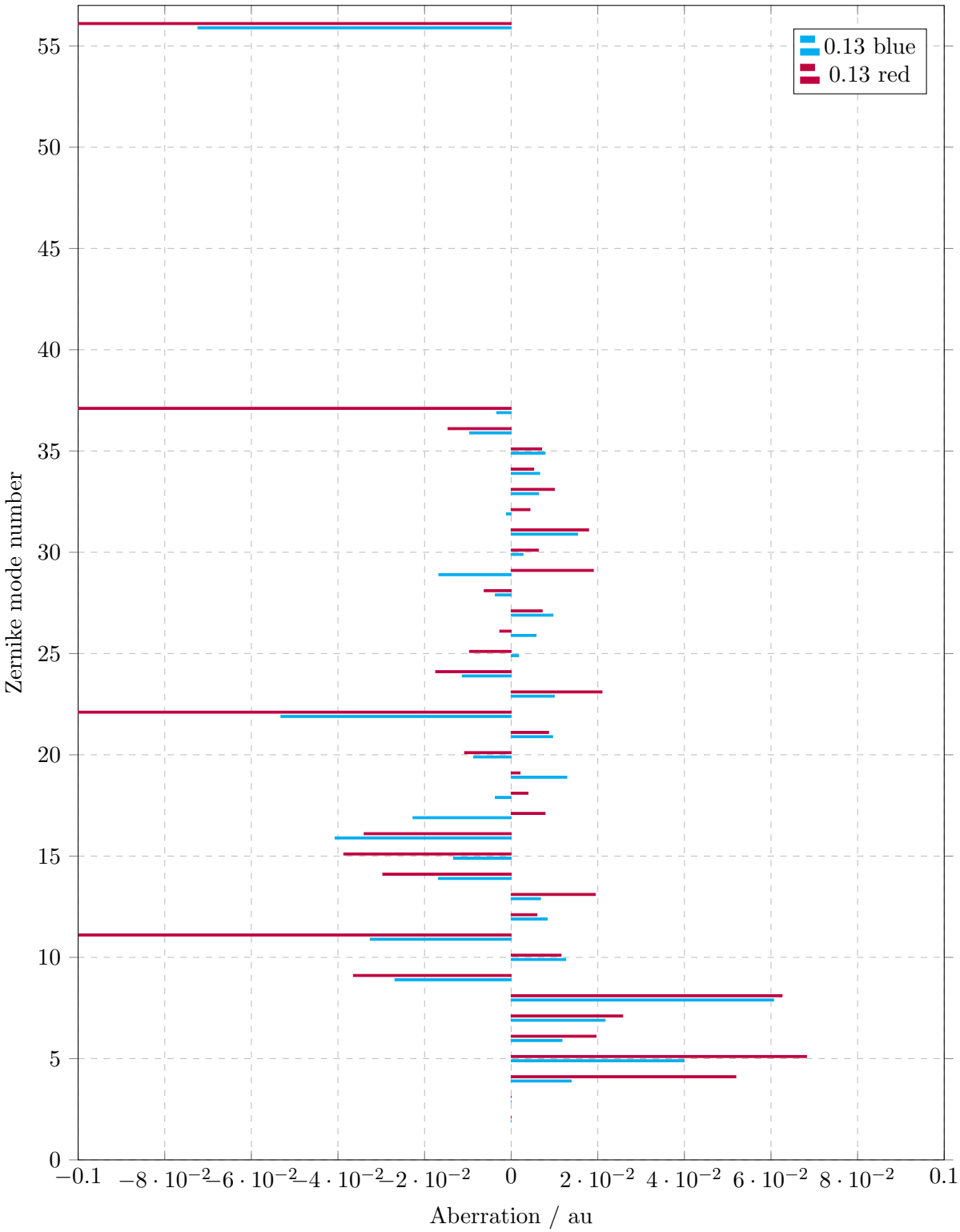

Figure 3.3: Red and blue channel Zernike modes $\{1 \dots 37, 56\}$ *versus aberrations of PSF model via phase retrieval, for correction collar setting of 0.13.*

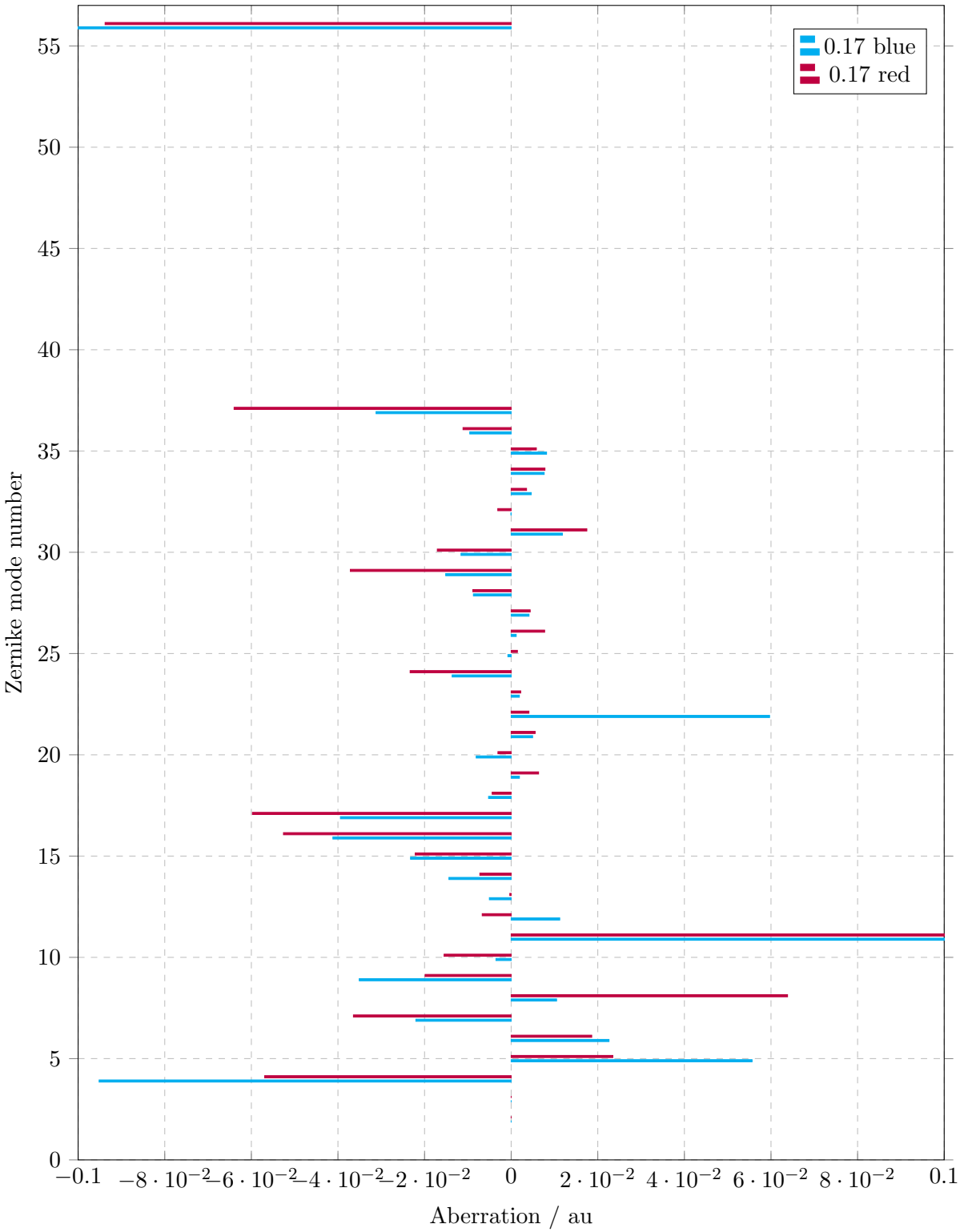

Figure 3.4: Red and blue channel Zernike modes $\{1 \ldots 37, 56\}$ versus aberrations of PSF model via phase retrieval, for correction collar setting of 0.17.

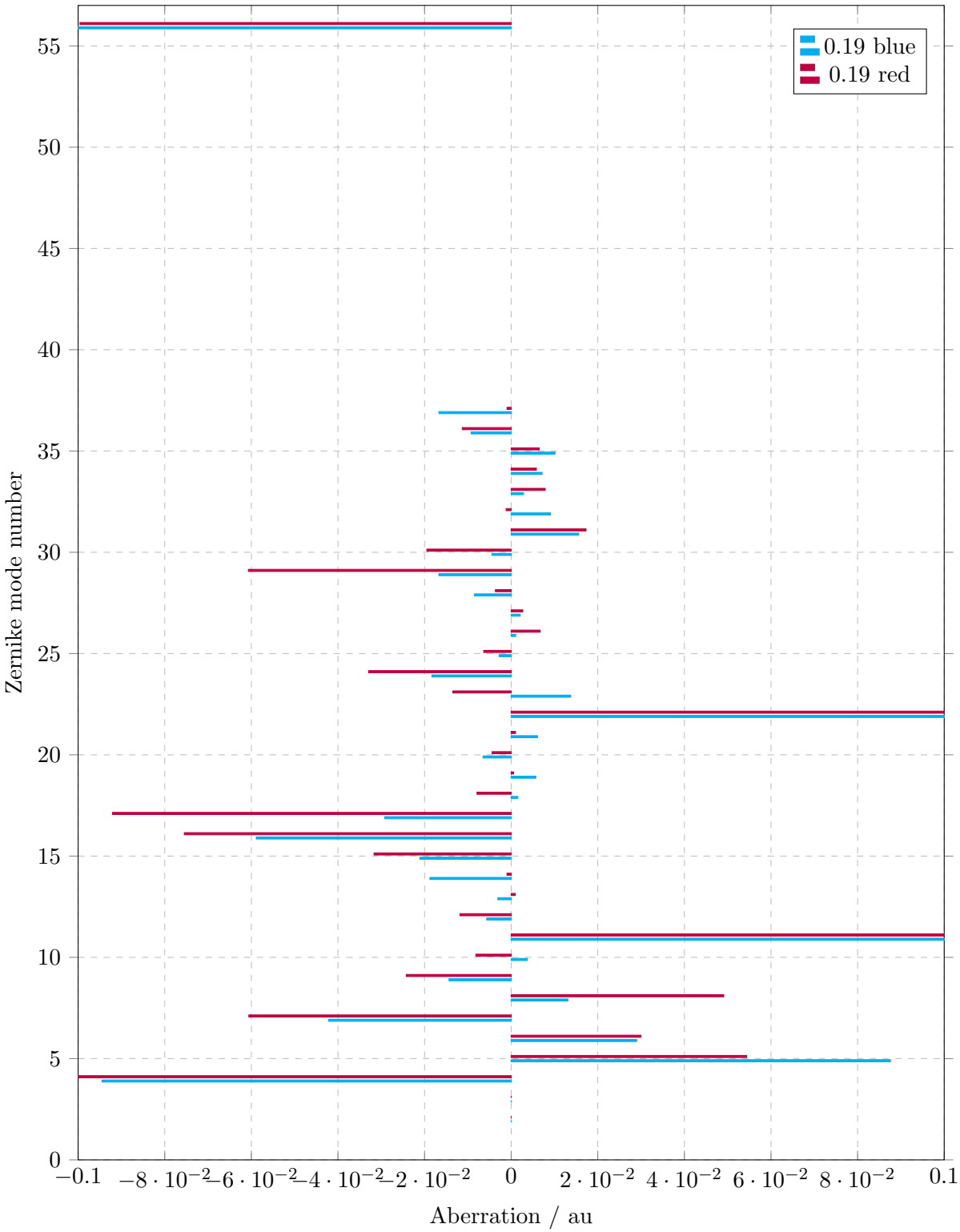

Figure 3.5: Red and blue channel Zernike modes $\{1 \dots 37, 56\}$ *versus aberrations of PSF model via phase retrieval, for correction collar setting of 0.19.*

3.2.3 Cramer Rao Lower Bound

In order to find the best Correction Collar setting of the Olympus 1.5 NA objective for SMLM, the Cramer Rao Lower Bound (CRLB) is computed using the program by [Jesacher et al 21?], with prior estimated Point Spread Functions (PSF) via phase retrieval [Jesacher et al 21?].

All CRLBs are computed at a defocus position of -500 nm, in steps of 5 nm from 0 to 500 nm. For all Estimations we assume identical arbitrary signal strength (2500) respective background (100).

The Estimated Cramer Rao Lower Bound for different correction Collar settings (variing linestyles for 0.13, 0.17, 0.19) of the Olympus 1.5 NA objective; for X, Y and Z axis (top, middle, and bottom); for both red and blue channel (colors) is shown in Figure 3.6.

Additionally plots grouped by X, Y and Z axis are shown in Figure 3.7, as well as grouped by different correction Collar settings in Figure 3.8.

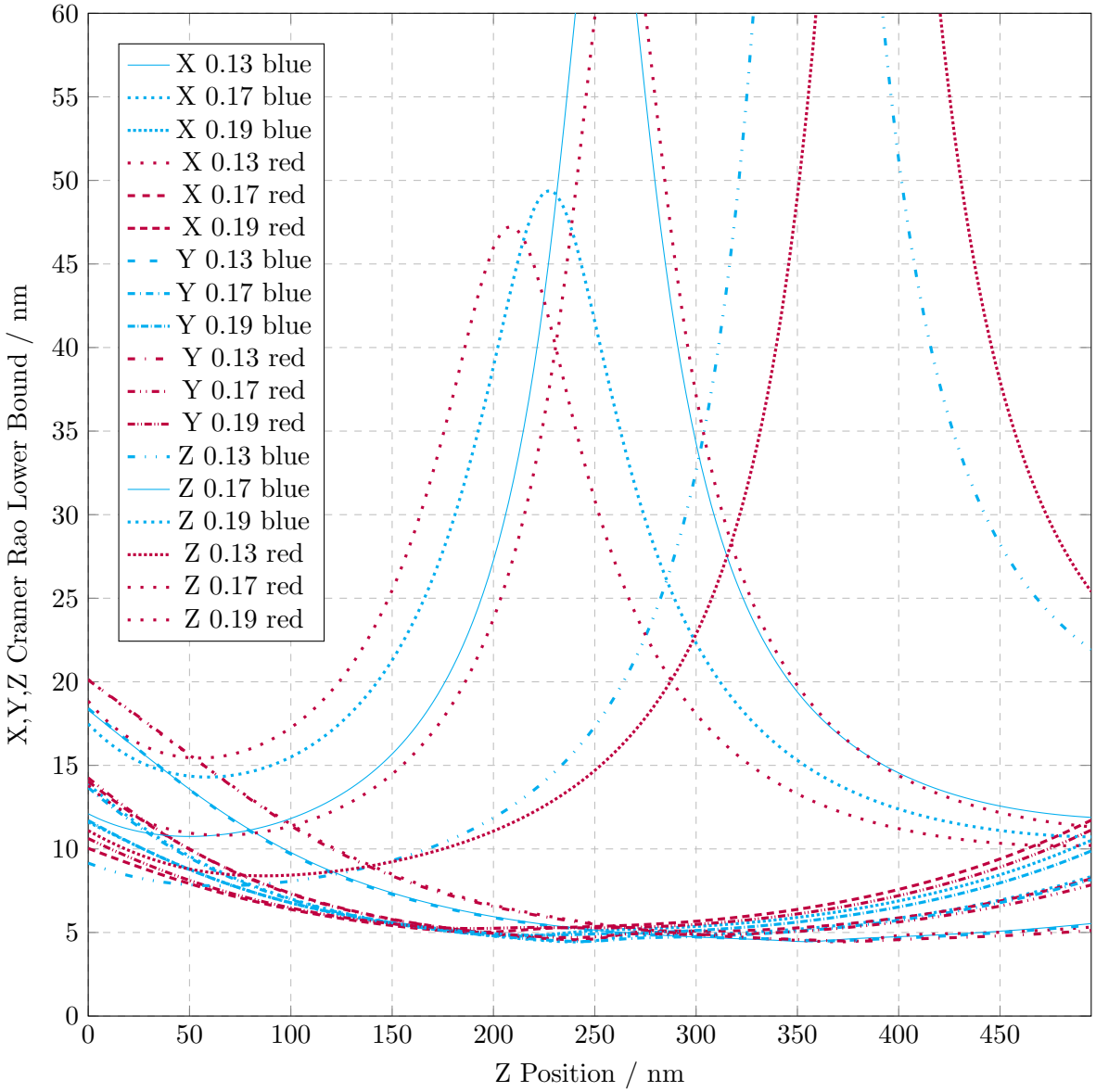

Figure 3.6: Estimated Cramer Rao Lower Bound for different correction Collar settings (variing linestyles for 0.13, 0.17, 0.19) of the Olympus 1.5 NA objective; for X, Y and Z axis (top, middle, and bottom); for both red and blue channel (colors).

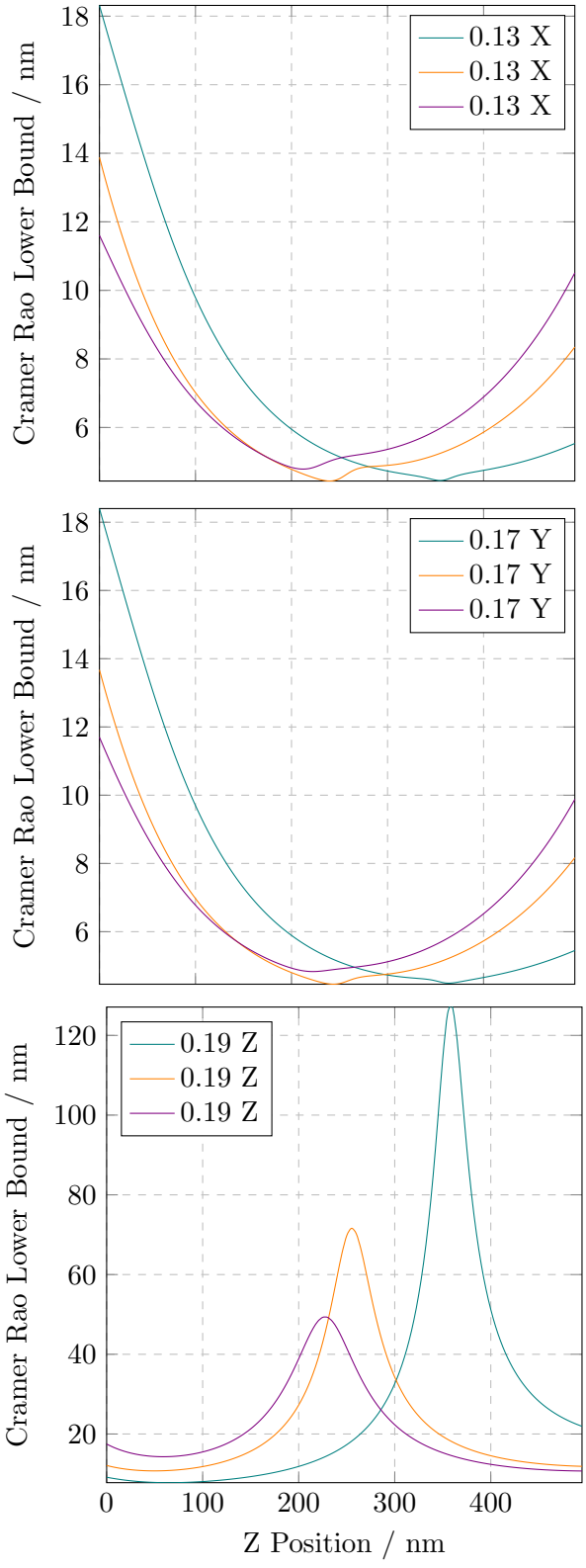

Figure 3.7: Estimated Cramer Rao Lower Bound for different correction Collar settings (teal: 0.13, orange: 0.17, purple: 0.19) of the Olympus 1.5 NA objective; grouped by X, Y and Z axis (top, middle, and bottom).

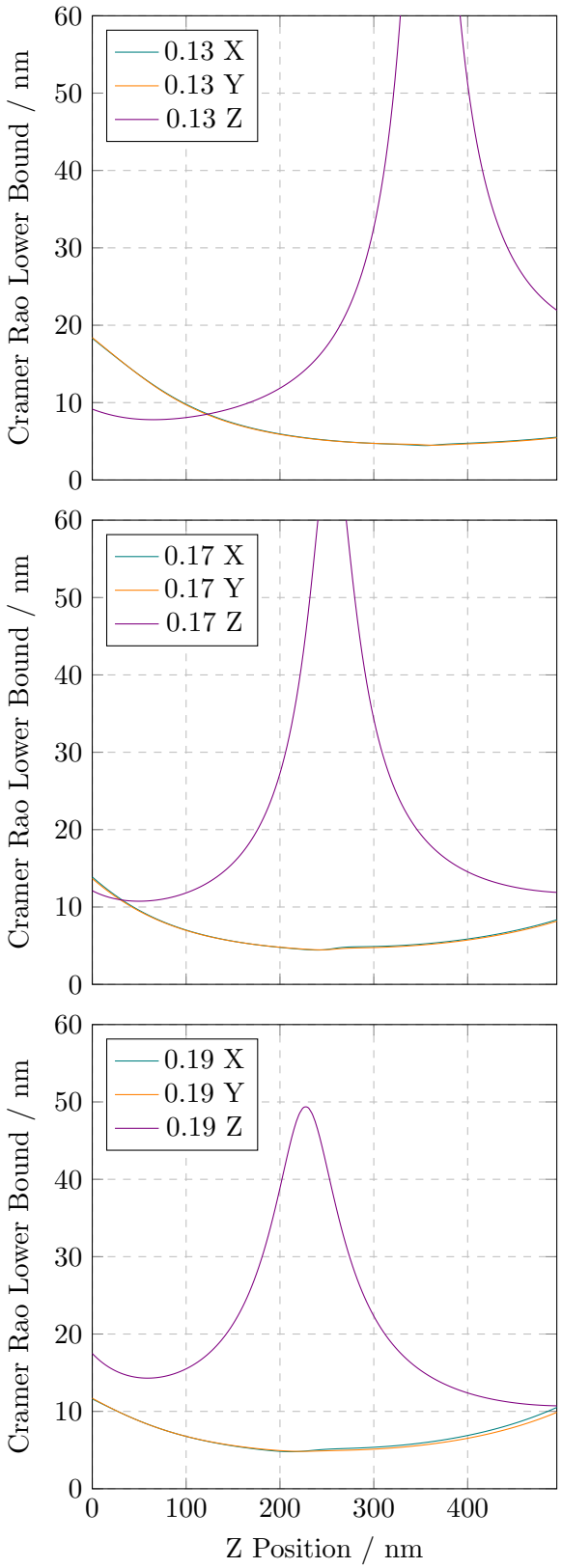

Figure 3.8: Estimated Cramer Rao Lower Bound for X, Y and Z axis (teal, orange and purple lines); grouped by different correction Collar settings (top: 0.13, middle: 0.17, bottom: 0.19) of the Olympus 1.5 NA objective.

3.3 Dual Channel Simulations

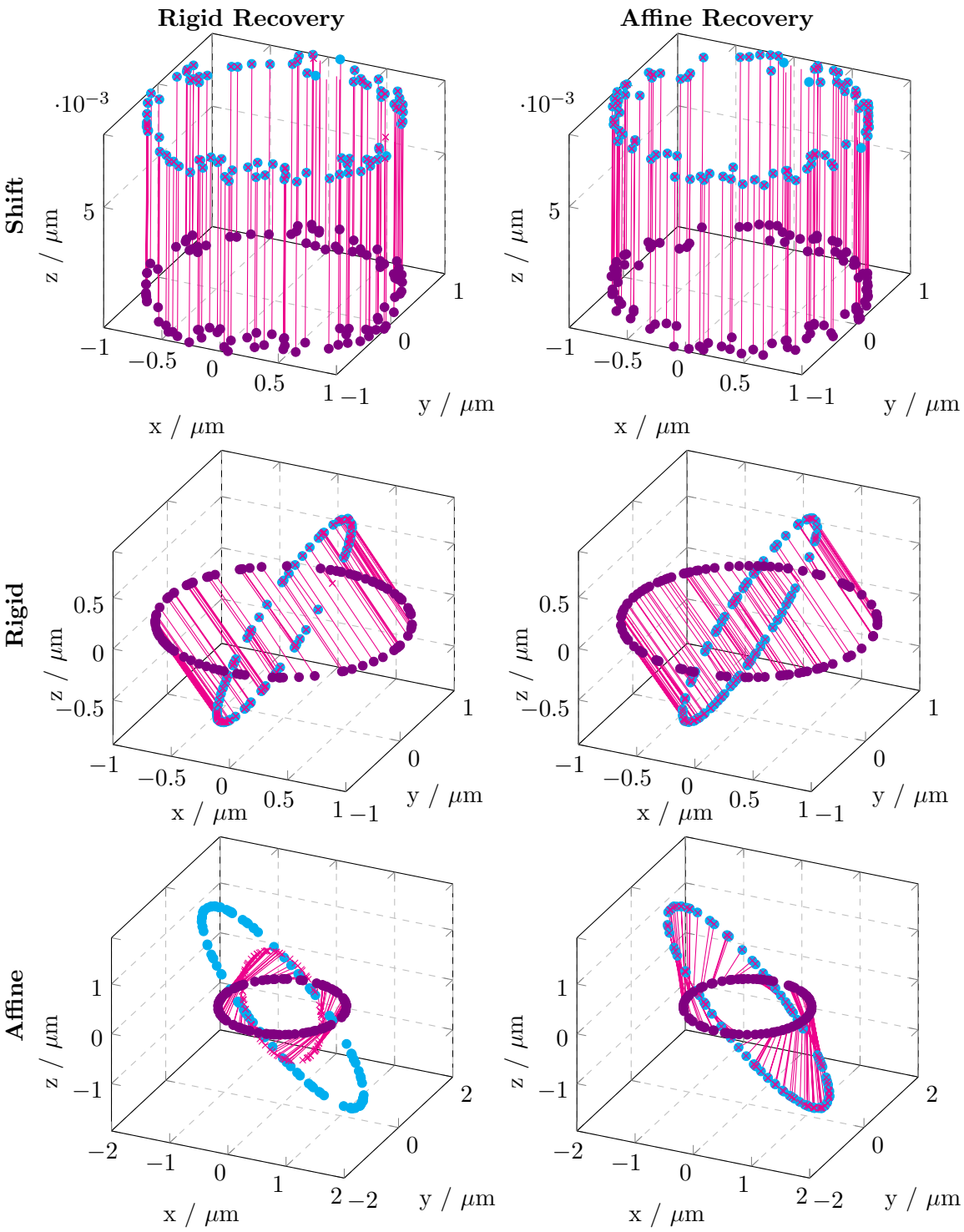

Figure 3.9: Demonstration of the recovery of rigid (left) respective affine (right) transformations, via recovering localisations of simulated two channel SMLM data; transformation (magenta) from red channel (violet) to blue channel (cyan); for the use cases of translation (top), rigid transformation (middle) and affine transformation (bottom). Obviously a rigid transformation may not correctly reconstruct an affine transformed data set (bottom left).

3.4 Dual Channel: NPC

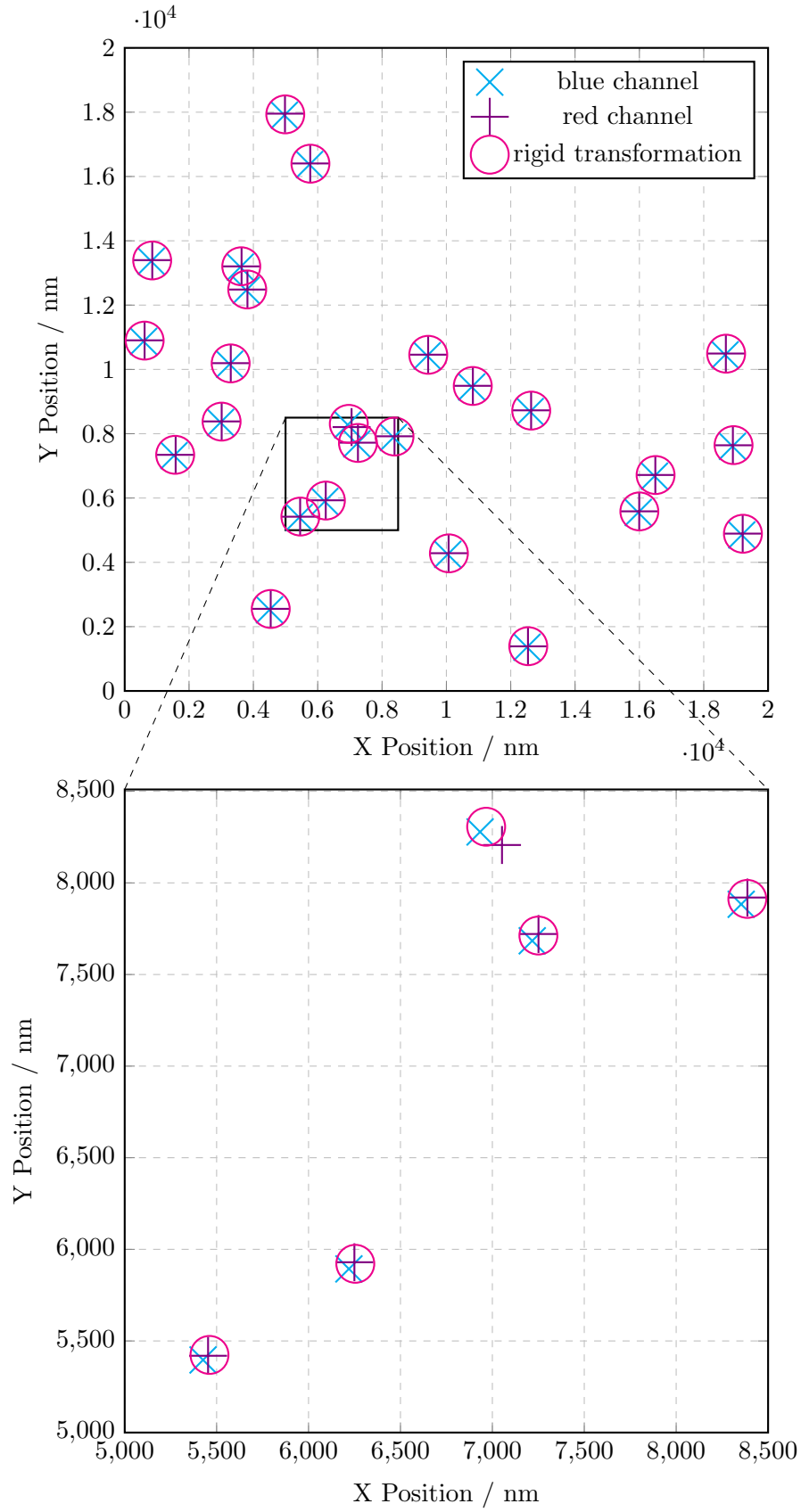

Figure 3.10: Demonstration of a rigid transformation of the localisations (magenta ∘) from blue channel (blue ×) to red channel (violet +); the transformed blue channel localisations mostly align well with the red channel localisations.

Chapter 4

Discussion

4.1 Phase Retrieval

The image stacks look ok, even if not all are perfectly symmetrical–some errors are to be expected.

The estimated Zernike modes of the PSF are mostly plausible; based on the modes and the order of magnitude.

This is further backed by the comparison of the results grouped by the three correction collar settings $\{0.13, 0.17, 0.19\}$, in Figure 3.3, Figure 3.4 and Figure 3.5. It is quite obvious, that the results for both red and blue channel are in the same order of magnitude—if not quite similar—for most of the Zernike modes, as suspected by theory zern.

Yet the phase Retrieval program gave the error ıhigh residual error for both the red and the blue channel when using the correction collar settings $\{0.17, 0.19\}$, is this a serious problem?

how can we avoid it?

4.2 Correction Collar

Based on the Figures 3.7 and Figure 3.8, and considering the fact—that we are interested in moderately defocusing (up to say 250 nm)—one might conclude, that the most preferable setting for the correction collar is 0.13.

Yet the recommended setting for our microscope setup is 0.17! Which is backed by Lukas, based on the PSF stacks: One would have guessed the 0.17 is more sensitive to z since it changes much more with different z positions than 0.13.

Which is the one we should use?

Chapter 5

Conclusion

5.1 Phase Retrieval

The Zernike modes for both red and blue channel are estimated via phase retrieval of in-focus measurements of beads at various depths. This yields a full PSF model to be used for de-focus 3d SMLM.

5.2 Correction Collar

The best setting of the Correction Collar for the Olympus 1.5 NA objective is shown to be 0.17. Here we find the preferable compromise between x,y precision and z precision, in the regime between about 0 and 250 µm.

5.3 dSTORTM Buffer

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