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# Accurate unsupervised estimation of aberrations in digital holographic microscopy for improved quantitative reconstruction

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**Abstract:** In the context of digital in-line holographic microscopy, we describe an unsupervised methodology to estimate the aberrations of an optical microscopy system from a single hologram. The method is based on Inverse Problems Approach reconstructions of holograms of spherical objects. The forward model is based on a Lorenz-Mie model distorted by optical aberrations described by Zernike polynomials. This methodology is thus able to characterize most varying aberrations in the field of view in order to take them into account to improve the reconstruction of any sample. We show that this approach increases the repeatability and quantitativity of the reconstructions in both simulations and experimental data. We use the Cramér-Rao lower bounds to study the accuracy of the reconstructions. Finally, we demonstrate the efficiency of this aberration calibration with image reconstructions using a phase retrieval algorithm as well as a regularized inverse problems algorithm.

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## 1. Introduction

Optical microscopy can be used to extract several characteristics from a biological sample, such as morphological parameters, birefringence or a phase shifts introduced by an unstained sample. For quantitative measurement of these properties, an accurate optical model is required [1]. Accounting for the characteristics of the optical system is an essential component of reconstruction algorithms in optical microscopy. For example, in fluorescence microscopy, accurate modeling of the Point Spread Function (PSF) is a way to improve the deconvolution step [2–5]. It can be performed using either a dedicated calibration step (by directly measuring the PSF on “point-like” objects [2]) or by estimating the PSF directly on an image that presents aberrations [4,6,7]). In the literature, estimating aberrations or PSF have been widely addressed using various microscopy methods (fluorescence, single-molecule localization, wide-field microscopy, holography, etc.), with different measurement or reconstruction approaches and models of the PSF. These models can be very simple (*e.g.* Gaussian model), more realistic, like the Gibson-Lanni model [5,7,8], or more versatile and general, like the Zernike polynomials of the pupil function [6,9,10]. In the two latter cases, the coherent PSF is modeled as a phase error function in the exit pupil plane of the objective.

In the particular case of digital holographic microscopy, the issues of aberrations estimation and correction have been widely studied for off-axis configuration (*e.g.* [11–14]). However, it concerns essentially the wavefront mismatch between the object and the reference beams, which creates distortions of the interference fringes, thus inducing errors in the reconstruction.

In-line digital holographic microscopy requires a simpler setup involving a single beam. It

45 simply consists in recording the intensity pattern diffracted by a sample. It is less bulky and  
46 less sensitive to vibrations than off-axis holographic setups [15, 16]. Image processing makes  
47 it possible to reconstruct the optical properties of the sample including its absorption and its  
48 phase shift. These can be discriminant in a classification task and makes possible medical  
49 diagnoses [17].

50 The aberrations of an in-line holographic optical system can have different causes, such  
51 as non standard uses of the objective, tilts or collimation errors in the illumination. These  
52 aberrations are dependent on the setup, its alignment and vary in the field of view. They lead to  
53 reconstruction errors, not only in the quantitative estimation of the modulus and the phase but also  
54 in the geometrical properties of the reconstructed objects. Thus, the repeatability as well as the  
55 reproductibility of the reconstructions is affected. However, the aberrations of the optical system  
56 are usually not considered in the reconstruction step. Accounting for the aberrations in the image  
57 formation model makes it possible to reduce the bias introduced in the reconstructions. These  
58 aberrations are an important issue to overcome in applications such as medical diagnoses that  
59 require reconstructions to be as accurate as possible to make the decision as robust as possible. To  
60 our knowledge, it is only recently that the influence of optical aberrations has been studied in the  
61 context of in-line digital holographic microscopy [1, 18, 19]. These studies underlined the need  
62 for a fine estimation of aberrations in order to improve the quantitativity and the repeatability  
63 of the phase reconstructions as well as the axial positioning, by reducing the aberration-driven  
64 biases.

65 In the present paper, we first address the problem of estimating aberrations in the context of  
66 in-line digital holographic microscopy. To that end, we use calibration beads to estimate an  
67 aberrated forward model. Using an Inverse Problems Approach (IPA), we simultaneously fit  
68 Zernike coefficients and calibration beads parameters, which are parameters of the forward model,  
69 on data. Unlike many PSF estimation studies, our approach does not require axial stacks of  
70 images *i.e.* only one hologram is needed. Moreover, we made no assumption of an aberration-free  
71 PSF in the center of the field, like in Zheng's *et al.* study [10]. Finally, this model of aberration is  
72 more general than the Gibson-Lanni model [5, 7, 18]. As a forward model, we use a Lorenz-Mie  
73 model of the calibration beads that has been extended to account for the aberrations of the optical  
74 system using Zernike polynomials [9]. To jointly estimate the calibration beads and aberration  
75 parameters, we choose a parametric IPA as it is known to be accurate in estimating the parameters  
76 of simple shape objects [20–22] and of the experimental parameters required for calibration. It  
77 has already been successfully applied in the context of autofocusing [23], for the estimation of  
78 the spectral crosstalk on a Bayer sensor [24] and to estimate the parameters of an astigmatic  
79 reference wave [25].

80 Once Zernike coefficients estimated locally for each bead, they can be used to perform  
81 aberration free reconstruction of the sample. These reconstructions can be performed using  
82 regularized IPA algorithm [26,27] or Fienup algorithm [28,29]. To test the proposed methodology,  
83 we use the experimental procedure of Martin *et al.* in [18], *i.e.* the use of a water immersion  
84 microscope objective with a correction collar that causes aberrations when not set correctly.

85 In the following section, we describe the method to estimate aberration parameters (Zernike  
86 coefficients) and use them to refine the PSF model of our holographic setup in order to reconstruct  
87 aberration-free images. In the third section, we detail the setup used to validate the proposed  
88 methodology. In the fourth section, to demonstrate the robustness of the approach to reconstruct  
89 various kinds of aberrations, we first present the estimation of both aberrations and beads  
90 parameters on simulated holograms and on experimental holograms. Finally, to illustrate the  
91 relevance of our approach on experimental data, the experimental data are reconstructed with  
92 phase retrieval algorithms (Fienup and regularized IPA algorithms) that take into account the  
93 estimated aberrations.

## 94 2. Estimation of the aberration parameters and reconstruction

95 Inverse problems are a general class of problems where unknowns are linked to measurements  
 96 through a known image formation model (simulating the measurements is referred to as the  
 97 “forward problem”). In this framework, reconstructions are based on minimizing the discrepancy  
 98 between the hologram (the data)  $\mathbf{d}$  and an image formation model (forward model)  $\mathbf{m}$ . In a general  
 99 case, such phase retrieval problem is ill-posed as it has many degeneracies (more unknowns than  
 100 data, twin image, *etc.*). To solve it, it is necessary to inject some *a priori* on the solution into the  
 101 minimization problem by adding regularization terms and/or constraints. Another way to solve  
 102 this degeneracies is to use a model of the measurement that depends on only a few parameters.  
 103 The problem can be then solved using the parametric IPA framework [30]. This framework  
 104 is well suited to calibrate the aberrations using holograms of spherical objects as the image  
 105 formation model depends only on the parameters of the objects (position, diameter and refractive  
 106 index) and on the aberrations that can be modeled with a complex pupil function described  
 107 by few parameters. Once these aberrations are estimated, they can be used in a regularized  
 108 reconstruction method to reconstruct any sample without any aberration artefacts. Figure 1  
 109 shows a flowchart representing the two main steps, the calibration and the reconstruction, that are  
 110 detailed here after.

### 111 2.1. Calibration : aberration parameters estimation

112 The diffraction pattern  $\underline{\mathbf{a}}^{\text{Mie}}$  of a spherical bead is accurately modeled by the Lorenz-Mie  
 113 model [31] which depends on the set of bead parameters  $\boldsymbol{\vartheta} = \{x, y, z, r, n\}$ , where  $x, y, z$   
 114 corresponds to the 3D position,  $r$  is the radius and  $n$  is the refractive index. The Lorenz-Mie  
 115 model has been successfully used to reconstruct spherical objects from holograms by fitting  
 116 methods [20, 22] or, in a more general framework, by parametric IPA [21, 32, 33]. In the presence  
 117 of aberrations, the new image formation model of the diffraction pattern of the beads  $\mathbf{m}^{\text{P}}$  (P  
 118 stands for Parametric) also depends on the aberration parameters  $\boldsymbol{\alpha}$  of the optical system that can  
 119 be included in the model by mean of a complex pupil plane as follows:

$$120 \mathbf{m}^{\text{P}}(\boldsymbol{\vartheta}, \boldsymbol{\alpha}) = \left| \mathcal{F}^{-1} \left[ \underline{\tilde{p}}(\boldsymbol{\alpha}) \odot \underline{\tilde{\mathbf{a}}}^{\text{Mie}}(\boldsymbol{\vartheta}) \right] \right|^2 \quad (1)$$

121 where  $\mathcal{F}^{-1}$  is the inverse Fourier Transform,  $\underline{\tilde{p}}(\boldsymbol{\alpha})$  is the pupil function in Fourier domain  
 122 that depends on  $(\kappa_x, \kappa_y)$ , the spatial frequency coordinates. For the sake of compactness,  
 123 Fourier space coordinates and spatial coordinates are omitted in the equations when they are not  
 124 required.  $\boldsymbol{\alpha} = \{\alpha_n^m\}_{(m,n) \in \mathbb{Z}^2}$  is a vector of aberration parameters, that will be referred as Zernike  
 125 coefficients in this work.  $\underline{\tilde{\mathbf{a}}}^{\text{Mie}}$  is the Fourier Transform of  $\underline{\mathbf{a}}^{\text{Mie}}$ , and  $\odot$  is the Hadamard product.

126 As described in [9, 34], Zernike polynomials  $\{\mathbf{Z}_n^m\}_{m,n}$  provide a suitable basis to describe the  
 pupil function  $\underline{\tilde{p}}$  (see Appendix A for details):

$$127 \underline{\tilde{p}}(\kappa_x, \kappa_y, \boldsymbol{\alpha}) = e^{i \left[ \sum_{n,m} \alpha_n^m \mathbf{Z}_n^m(\kappa_x, \kappa_y) \right]} \quad (2)$$

128 To characterize the aberration effects of the optical system, the Zernike coefficients  $\boldsymbol{\alpha}$  have  
 129 to be estimated. Assuming a white and Gaussian noise, the maximum likelihood estimation of  
 130 model parameters  $\{\boldsymbol{\vartheta}, \boldsymbol{\alpha}\}$  of the bead and the aberrations corresponds to a least squares fitting  
 problem [20, 32]:

$$131 \left\{ \boldsymbol{\vartheta}^\dagger, \boldsymbol{\alpha}^\dagger \right\} = \underset{\boldsymbol{\vartheta} \in \mathbb{P}, \boldsymbol{\alpha} \in \mathbb{D}}{\text{argmin}} \|\mathbf{d} - \mathbf{m}^{\text{P}}(\boldsymbol{\vartheta}, \boldsymbol{\alpha})\|_2^2 \quad (3)$$

132 where  $\{\mathbb{P}, \mathbb{D}\}$  are optimization constraints and  $\|\cdot\|_2$  is the  $L_2$ -norm. Note, taking the weighted  
 133 version of the  $L_2$ -norm (*i.e.* the squared Mahalanobis distance) makes it possible to consider  
 non-stationary and correlated noise into account [32, 35, 36].



To numerically solve this optimization problem (equation 3), only the first 15 Zernike coefficients are estimated in the following. As a phase piston has no effect on the image formation model (intensity image formation model),  $\alpha_0^0$  is set to 0. As varying Zernike coefficients  $\alpha_1^{-1}$  and  $\alpha_1^1$  simply amounts to shift parameters  $x$  and  $y$ , these Zernike coefficients are also set to 0. In these conditions, seventeen parameters are studied:

$$x, y, z, r, n, \alpha_2^{-2}, \alpha_2^0, \alpha_2^2, \alpha_3^{-3}, \alpha_3^{-1}, \alpha_3^1, \alpha_3^3, \alpha_4^{-4}, \alpha_4^{-2}, \alpha_4^0, \alpha_4^2, \alpha_4^4.$$

134 A study of the correlations between the estimated parameters is presented in Appendix B.  
 135 It shows some high correlations in the correlation matrix. All the parameters  $\{\vartheta, \alpha\}$  should  
 136 therefore be estimated simultaneously. An iterative detection/local optimization scheme [21] is  
 137 used to guarantee the rapid and accurate reconstruction of a set of objects. Since the beads are  
 138 monodispersed, a narrow parameter research domain  $\mathbb{P}$  can be chosen depending on the size and  
 139 refractive index of the beads used experimentally.

140 Since the aberration can differ depending on the location of the beads in the field of view, the  
 141 aberration parameters have to be estimated for several different bead locations.

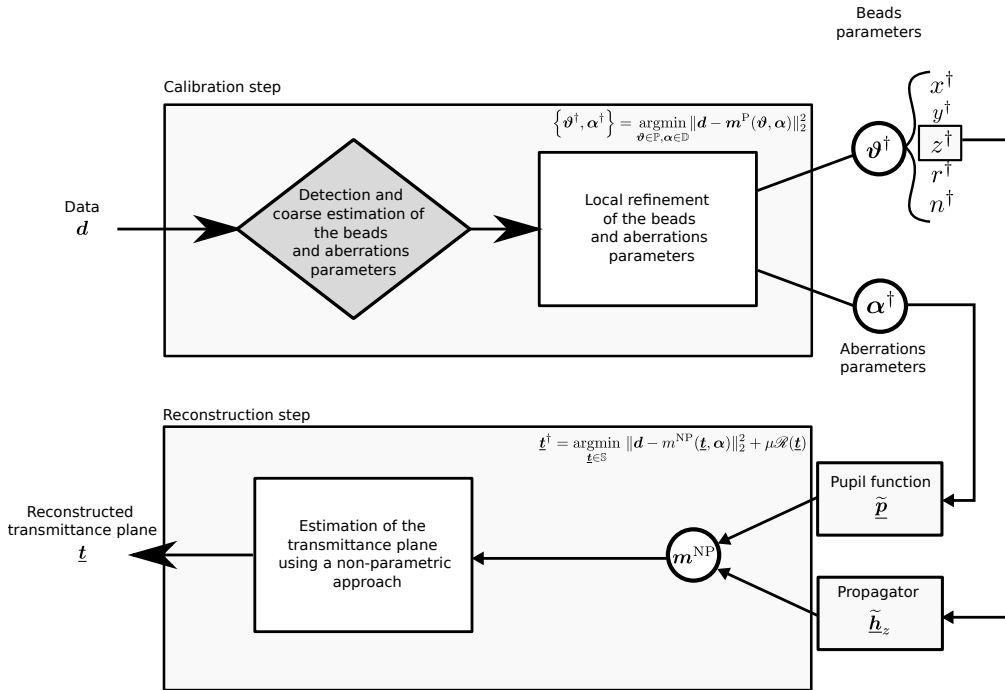


Fig. 1. Flowchart representing the two main steps of the proposed method: calibration and reconstruction.

## 142 2.2. Reconstruction: including aberration model

143 Once the aberrations are modeled, they are taken into account to better reconstruct the modulus  
 144 and the phase of the objects of interest. These samples are modeled by a 2D transmittance plane  
 145  $\underline{t}(x, y)$ . In that case, the image model will be referred as non-parametric. For an infinite aperture  
 146 and aberration free imaging system, this model is the squared modulus of the convolution between

147 the Rayleigh-Sommerfeld propagation kernel  $\underline{h}_z^{\text{RS}}$  and the transmittance plane  $\underline{t}$  where:

$$\underline{h}_z^{\text{RS}}(x, y) = \frac{z}{i\lambda} \frac{\exp\left(i\frac{2\pi}{\lambda}\sqrt{x^2 + y^2 + z^2}\right)}{x^2 + y^2 + z^2} \quad (4)$$

148 and  $\lambda$  is the wavelength of the illumination [37]. In order to account for aberrations in the  
 149 image formation model, an aberrated PSF model should be used. Assuming a shift invariance of  
 150 the pupil function with  $z$ , the Optical Transfer Function (OTF), which is equal to the Fourier  
 151 Transform of the complex-valued PSF  $\underline{h}_z$ , can be expressed as follows:

$$\widetilde{\underline{h}}_z(\alpha) = \widetilde{p}(\alpha) \odot \widetilde{\underline{h}}_z^{\text{RS}} \quad (5)$$

152 where  $\widetilde{\underline{h}}_z^{\text{RS}}$  is also called the angular spectrum.

153 In addition, the beads estimated depth provides reliable estimation of the propagation distance  $z^\dagger$   
 154 [23]. Thus, the aberration corrected non-parametric model  $\mathbf{m}^{\text{NP}}$  can be expressed as:

$$\mathbf{m}^{\text{NP}}(\underline{t}, \alpha) = |\underline{h}_z(\alpha) * \underline{t}|^2 \quad (6)$$

155 Unlike the parametric case (section 2.1), minimizing the discrepancy between data and model  
 156 is not sufficient to solve this ill-posed problem. *A priori* information about the sample must be  
 157 added in the form of constraints on the optimization space  $\mathbb{S}$  and in the form of a regularization  
 158 term  $\mathcal{R}^{\text{NP}}$  [27, 38, 39]:

$$\underline{t}^\dagger = \underset{\underline{t} \in \mathbb{S}}{\operatorname{argmin}} \|\mathbf{d} - \mathbf{m}^{\text{NP}}(\underline{t}, \alpha)\|_2^2 + \mu \mathcal{R}^{\text{NP}}(\underline{t}) \quad (7)$$

159 where  $\mu$  is an hyperparameter.

160 This reconstruction is called a regularized reconstruction. The knowledge of the propagation  
 161 distance  $z$  is crucial because the image formation model depends on it. This distance is  
 162 chosen according to the parameters of the beads previously estimated with the parametric IPA  
 163 reconstructions [23].

164 In the following, the regularization term is a hyperbolic total variation term [40]. The  
 165 hyperparameter is chosen empirically. The optimization domain is restricted to the unitary disk  
 166 corresponding to a non-emissive object hypothesis. A FISTA algorithm is used to perform this  
 167 minimization [41].

### 168 3. Experimental study

#### 169 3.1. Principle

170 High quality microscope objectives are supposed to be diffraction limited as long as they are used  
 171 in the standard conditions for which they have been optimized (coverslip thickness, refractive  
 172 indices of the immersion medium, the sample medium and the coverslip and position of the  
 173 sample relative to the coverslip) [8, 42]. Yet, in some applications, these golden rules may be  
 174 broken (wrong coverslip thickness, for instance). In inset A of Fig. 2, the refraction of the beam  
 175 in the coverslip is shown before entering the objective. This illustrates the origin of the possible  
 176 wavefront errors that may occur between the paraxial rays and the high angle rays when the  
 177 standard conditions of use are not met. This wavefront error has been described by several  
 178 authors [8, 42] in on-axis situations, but it may vary with the position in the field of view. Finally,  
 179 even when the rules are strictly applied, residual aberrations may still exist, especially out of the  
 180 optical axis, and may differ from one objective to another. To experimentally study the influence  
 181 of such aberrations, we used a water immersion objective with a coverslip correction collar. Thus,  
 182 for a given coverslip thickness, a wrong correction collar setting will give rise to aberrations.  
 183 This idea was recently proposed by Martin *et al.* [18].

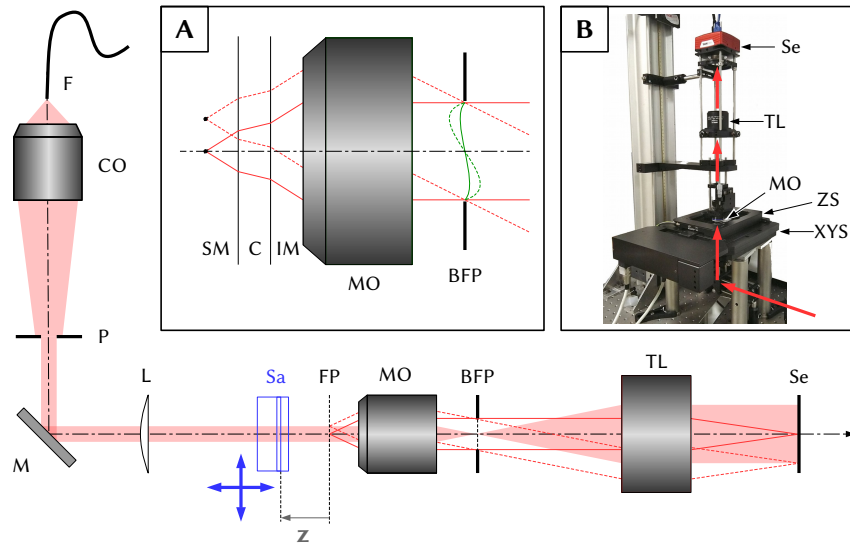


Fig. 2. Experimental setup. F: monomode fiber coupled laser source, CO: collection optics, P:  $200\mu\text{m}$ -pinhole, M: mirror, L: lens, Sa: sample that can be precisely moved in XYZ-directions, z: defocus distance of the sample from the focus plane, FP: objective focal plane, MO: microscope objective, BFP: objective back focal plane, TL: tube lens, Se: sensor. **Inset A:** zoom on the sample and the objective showing the refraction of the rays occurring through the coverslip. SM: sample medium, C: coverslip, IM: immersion medium. **Inset B:** Picture of the setup showing the imaging system and the precision piezo-stage (ZS) and the XY-translation stage (XYs).

### 184 3.2. Setup

185 Our home-made experimental setup [19] is presented in Fig. 2. The setting of a spatially coherent  
 186 illumination may be difficult in a microscopy setup as it is very sensitive to any stray reflections  
 187 or dust particles and leads to complex, sometime unstable speckle patterns. In this setup, the  
 188 coherent illumination is set by illuminating a  $200\mu\text{m}$ -pinhole (P) and a lens (L) set in a  $2f$   
 189 configuration. Thus, an airy pattern illuminates the sample, with a large enough central peak  
 190 to illuminate the whole field of view, but without inserting too much stray light in the imaging  
 191 system. This leads to moderate vignetting which is corrected by dividing the holograms by a  
 192 background intensity image.

193 In the present study, the sample was composed of  $1\mu$ -diameter polystyrene beads diluted in  
 194 glycerol. The use of transparent beads is interesting here, because we aim at reconstructing  
 195 phase objects. The diameter of the beads is chosen to mimic biological objects such as bacteria.  
 196 Usually, sub-resolution objects are used for PSF calibrations. However, in our context, with  
 197 sub-resolution beads, the contrast of holograms would be too low and Rayleigh-Sommerfeld  
 198 based models would fail to reconstruct correctly the beads [43].

199 As polystyrene beads float in glycerol and thanks to its high viscosity, the beads were located  
 200 just below the coverslip and did not move during the exposure of one hologram (typically, few  
 201 milliseconds). According to the Gibson-Lanni model of the aberrations [8] induced by wrong  
 202 coverslip thicknesses and/or refractive indices, the fact that the sample medium was glycerol  
 203 instead of water should not induce additional aberrations as the beads were just below the  
 204 coverslip (*i.e.*  $t_s = 0$  with the Gibson-Lanni notations). A coherent illumination with a laser at

205 637.6nm was used. The illumination power was sufficient to keep exposure times as short as 5ms  
 206 with our Thorlabs-S805MU1 camera. The sensor pixel size was  $5.5\mu\text{m}$ . With 22.6mm diagonal,  
 207 the sensor covers an important part of the field of view of the image (the objective field number  
 208 is 26.5mm). The microscope objective was a water immersion microscope objective (Olympus  
 209 PlanSApo, 60 $\times$ , 1.2NA) with a coverslip correction collar. The tube lens was a 200mm-focal  
 210 length apochromatic TTL200MP from Thorlabs that was used in a telecentric configuration. The  
 211 measured magnification was 66.5, and not 60, as the tube lens has a greater focal length than the  
 212 Olympus standard (180mm).

### 213 3.3. Experimental protocol

214 Five cases of aberration were tested in this experiment with the correction collar at different  
 215 settings (0.13, 0.15, 0.17, 0.19 and 0.21mm). The coverslip thickness was measured to be  
 216 0.170mm with a digital indicator (with a resolution of  $\pm 1\mu\text{m}$ ). Thus, the 0.17mm setting of  
 217 the correction collar is assumed to be the aberration free situation. A single bead was tracked  
 218 through the whole field of view in regular steps in the X and Y directions. A total of 35 images  
 219 ( $7 \times 5$ ) were acquired in order to regularly cover the whole field of view ( $273 \times 204\mu\text{m}$ ). For each  
 220 XY-position in the field, an axial stack was recorded with defocus positions ranging from  $-10\mu\text{m}$   
 221 to  $+20\mu\text{m}$  from the focus position with a step size of  $0.5\mu\text{m}$ . This stack is used for the illustration  
 222 of Fig. 3, but only one axial position will be reconstructed in the next section. It should be noted  
 223 that the sample is the only moving part, which is important for recording a background image by  
 224 calculating the median value of the 35 XY-shifted images recorded at focus.

225 A view of a typical hologram is shown in the top part of Fig. 3. XZ-views of the stack along  
 226 the vertical axis of the bead are represented at the bottom of Fig. 3. As shown in the figure, a  
 227 change in the focus position is observed as a function of the setting of the correction collar, as  
 228 well as modifications in the XY-profiles. The radial symmetry of the PSF is not always valid, as  
 229 can be seen, for example, for the 0.13mm setting of the correction collar (green). This asymmetry  
 230 is due to aberration effects that may break the radial symmetry of the holograms (*e.g.* coma,  
 231 astigmatism, etc.). All aberrations may originate from the objective, but also from the tube lens  
 232 or from misalignment of the illumination or the imaging parts. Moreover, aberrations can also  
 233 originate from inhomogeneities of the slide and the coverslip.

Wavelength	637.6nm
Magnification	66.5
Pixel pitch	83nm
Total field of view	$273 \times 204\mu\text{m}$
Beads diameter*	$(1.0 \pm 0.06)\mu\text{m}$
Beads refractive index*	1.587 (polystyrene)
Refractive index of immersion medium	1.47 (glycerol)
Coverslip thickness	$(0.170 \pm 0.001)\text{mm}$
Typical defocus	$12\mu\text{m}$

Table 1. Experimental parameters (\*from manufacturer, ThermoFisher Scientific, Inc.)

## 234 4. Results

235 In this section, we first apply the proposed method to simulated holograms to demonstrate  
 236 the robustness of our approach for several kind of aberrations, especially in cases of difficult

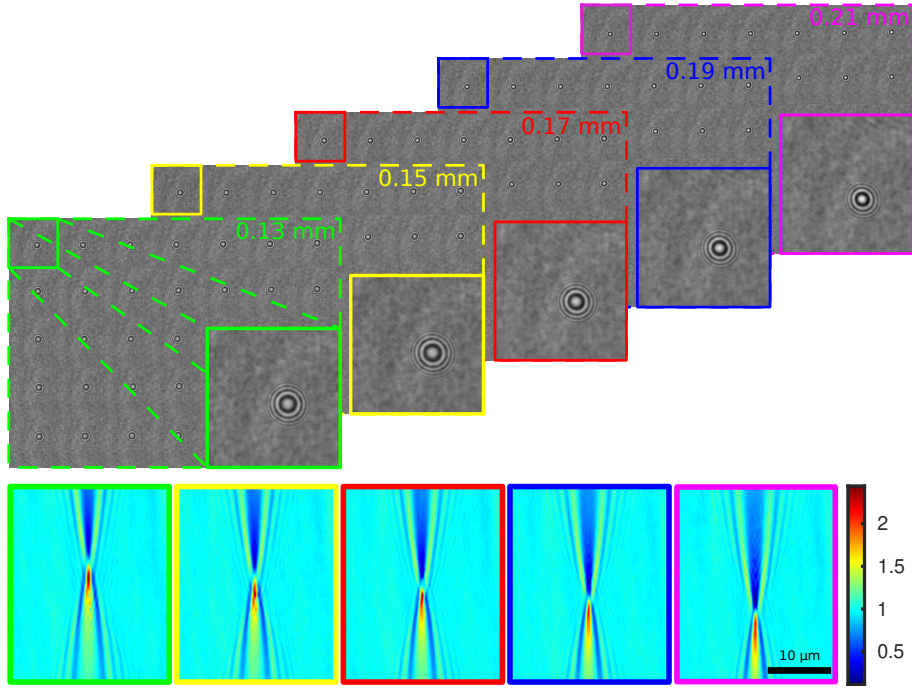


Fig. 3. Example of a mosaic of holograms (top) of  $1\mu\text{m}$ -diameter polystyrene beads in glycerol for an approximate defocus of  $12\mu\text{m}$  under 5 different settings of the correction collar (from left to right: 0.13 (green), 0.15 (yellow), 0.17 (red), 0.19 (blue) and 0.21mm (magenta)). XZ-views of the hologram stacks for the different correction collar settings (bottom).

237 optimizations, *i.e.* with highly correlated Zernike coefficients. We then apply it to experimental  
 238 holograms of beads. We compare our results with state-of-the-art parametric reconstruction  
 239 algorithms in both simulated and experimental cases and finally evaluate and discuss the effects  
 240 of aberration on regularized reconstructions.

#### 241 4.1. Reconstructions on simulated data

242 A mosaic of  $7 \times 5$  in-line holograms was simulated with aberrations varying in the field of view  
 243 (see Fig. 4). Each hologram is a  $512 \times 512$  pixels sub-image simulated with the experimental  
 244 parameters described in Table 1 and with the aberrated Lorenz-Mie model (see equation 1). The  
 245 defocus is set to  $12\mu\text{m}$ . This distance was chosen to improve the accuracy of the estimation of the  
 246 Zernike coefficients, as indicated by CRLB analysis of this parameter (see Appendix B Fig. 11).

247 To simulate a varying PSF in the field of view, the aberrated pupil function was considered  
 248 to depend on the position of the bead in the field of view. This pupil function corresponds to a  
 249 linear combination of oblique astigmatism ( $Z_2^{-2}$ ), vertical coma ( $Z_3^{-1}$ ), horizontal coma ( $Z_3^1$ ),  
 250 spherical aberration ( $Z_4^0$ ) and oblique secondary astigmatism ( $Z_4^{-2}$ ) (see Appendix A). This  
 251 linear combination is weighted by the corresponding Zernike coefficients  $\alpha$  (see Section 2.1).  
 252 We arbitrarily chose to set a linear behavior along  $y$  for  $\alpha_2^{-2}$  and  $\alpha_3^1$ , a linear behavior along  $x$  for  
 253  $\alpha_3^{-1}$  and  $\alpha_4^{-2}$ , and we set  $\alpha_4^0$  constant in the field of view. This set of coefficients was chosen to  
 254 demonstrate the performance of the proposed method in difficult cases, *i.e.* we chose Zernike  
 255 coefficients that were highly correlated in the corrected model (see Appendix B, Table 6).

256 Finally, a white Gaussian noise  $\epsilon$  was added to the simulated holograms, which led to a

257 Signal-to-Noise Ratio (SNR) of 4 in the holograms ( $\text{SNR} = \frac{\Delta m}{2\sigma_\epsilon}$ , where  $\Delta m$  is the peak-to-peak  
 258 amplitude of the model).

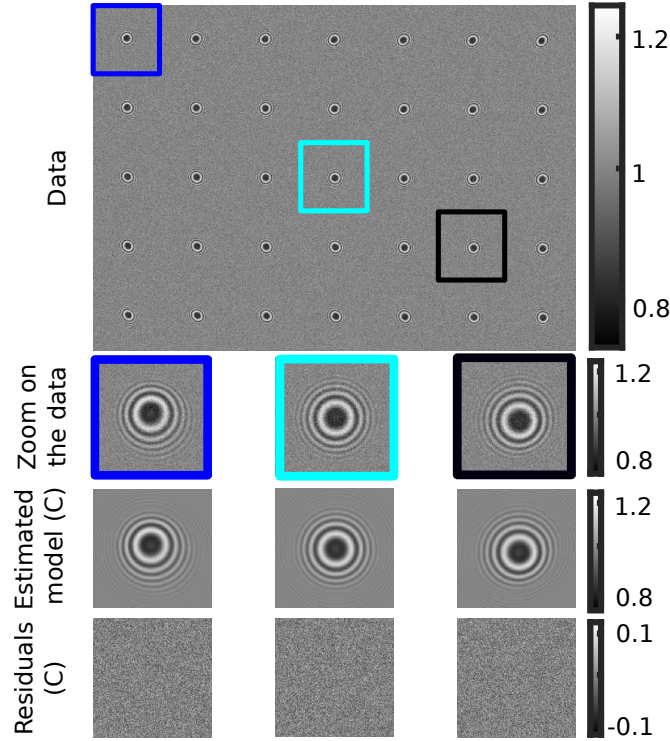


Fig. 4. **Top:** 35 holograms simulated with variable Zernike coefficients depending on the position in the field of view. **Bottom:** magnifications of 3 holograms from different areas (first line), estimated model accounting for aberrations (C)(second line), residuals i.e. difference between the first line and the second one (third line).

259 For each simulated hologram, the reconstruction was performed using parametric IPA with  
 260 or without aberration corrections in the model. The abbreviations C (standing for corrected),  
 261 and UC (standing for uncorrected) will be used in the following. The optimization algorithm we  
 262 used was the LINCOA algorithm [44]. To perform the reconstructions with the corrected model  
 263 (C), the first step implies an exhaustive search in a 17 parameters space, which can be really  
 264 demanding in terms of computational time. To reduce this exhaustive search, it can be fairly  
 265 convenient to have at least a coarse knowledge of the Zernike coefficients. As our aberrations  
 266 were quite low, we performed this step by considering no aberration, *i.e.* all Zernike coefficients  
 267 were set to zero.

268 Then, the optimization step was performed with the fully corrected model (eq.3), with the  
 269 constraints on parameters described in Table 2. The optimization domains  $\{\mathcal{P}, \mathcal{D}\}$  were chosen  
 270 quite large in order to check the robustness of the proposed method.

271 Table 3 shows the bead parameters reconstructed without (UC) or with (C) taking the aberration  
 272 into account in the model. It shows the biases introduced by geometrical aberrations. When  
 273 using an unaberrated model (UC), the reconstructions converge either on a local optimization  
 274 minimum or to the constraint domain bounds. Conversely, when using an aberrated model, the  
 275 reconstructions always converge to the global minimum with low bias and a standard deviation  
 276 close to the theoretical lower bound given by Cramér-Rao analysis.

	$z$	$r$	$n$	$\alpha_2^{-2}$	$\alpha_2^0$	$\alpha_2^2$	$\alpha_3^{-3}$	$\alpha_3^{-1}$
Lower bound	10	0.2	1.52	-10	-10	-10	-10	-10
Upper bound	14	0.7	1.63	10	10	10	10	10
	$\alpha_3^1$	$\alpha_3^3$	$\alpha_4^{-4}$	$\alpha_4^{-2}$	$\alpha_4^0$	$\alpha_4^2$	$\alpha_4^4$	
Lower bound	-10	-10	-10	-10	-10	-10	-10	
Upper bound	10	10	10	10	10	10	10	

Table 2. Optimization constraints for each estimated parameters ( $z$  and  $r$  are in micrometers)

$\vartheta_i$	$\vartheta_i^{GT}$	$\langle \hat{\vartheta}_i \rangle^{UC}$	$\langle \hat{\vartheta}_i \rangle^C$	$\sigma_{\vartheta_i}^{CRLB}$	$\sigma_{\hat{\vartheta}_i}^{UC}$	$\sigma_{\hat{\vartheta}_i}^C$
$z(\mu m)$	12	11.048	12.001	0.002	0.579	0.004
$r(\mu m)$	0.5	0.267	0.500	0.001	0.030	0.001
$n$	1.58	1.619	1.5798	0.0006	0.0311	0.0007

Table 3. Statistical results on the estimated bead parameters with aberration corrected (C) and uncorrected (UC) models: Ground Truth (GT) parameters  $\vartheta_i^{GT}$ , means of the estimated parameters  $\langle \hat{\vartheta}_i \rangle$ , lower bounds of their theoretical standard deviations  $\sigma_{\vartheta_i}^{CRLB}$  and standard deviations of their estimates  $\sigma_{\hat{\vartheta}_i}$

277 Residuals between the data and the model are very low, indicating that the model fits the data  
278 accurately (see the bottom line in Figure 4). On the upper part of Fig. 5 are presented the phase  
279 of the pupil functions that were simulated in each part of the field of view. This gives another  
280 view, in Fourier space, of the type of phase errors that aberrations may imply. On the lower part  
281 of Fig. 5 the residuals of the estimated pupil functions are presented (from the simulated ground  
282 truth). From these residuals, we see that our estimations of the Zernike coefficients are accurately  
283 describing the phase function introduced by aberrations in Fourier space.

284 In the most difficult cases (upper part and lower part of the field on Fig. 5), the residuals are  
285 not negligible for the highest spatial frequencies, close to the cutoff frequency imposed by the  
286 numerical aperture of the objective (represented by a black dashed circle). Indeed, as we did  
287 not use a sub-resolution object, the power spectrum of the object is not filling the entire pupil.  
288 In the inset of Fig. 5, the typical power spectrum of the object is presented and a white dashed  
289 circle shows the part of the spectrum including 95% of its energy. In this white dashed circle,  
290 the residuals remain low. Actually, this is an unsurprising limitation of this approach: as the  
291 object spectrum does not cover the whole aperture of the objective, the pupil phase function can  
292 not be estimated precisely for the highest frequencies. However, the pupil function is correctly  
293 estimated for the spatial frequencies corresponding to the spectrum of the object, which ensures  
294 that a similar object will be correctly reconstructed. If the aberrations are important, this effect  
295 must be considered for the choice of the calibration objects: the size of the beads chosen for  
296 aberration estimation must be at least equal or smaller than the smallest detail of interest.

#### 297 4.2. Reconstructions on experimental data

298 The experimental parameters are given in table 1 and were the same as those used in the simulations.  
299 Once again, since the accuracy of the estimated parameters is better in a specific range of defocus  $z$

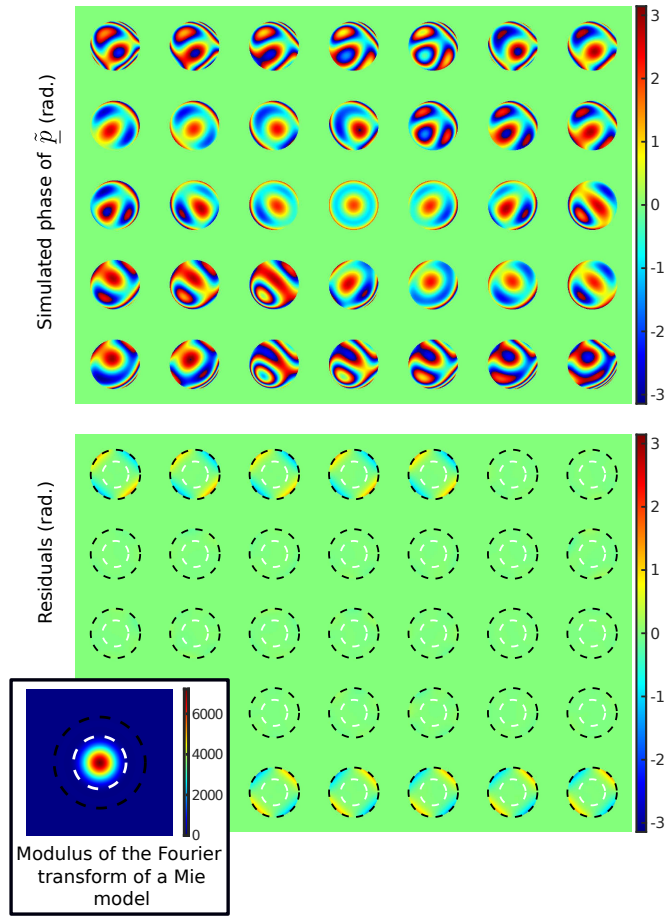


Fig. 5. Simulated phase correction in the pupil planes  $\tilde{p}(\alpha^\dagger)$  (a) and residuals of the estimated pupil functions from the ground truth (b). The white dashed circles correspond to the disk in which 95% of the energy of the power spectrum of the object (c) is contained. The black dashed circles correspond to the aperture (calculated from the numerical aperture of the objective).



300 (see Appendix B), the holograms to be reconstructed were located approximately  $12\mu\text{m}$  from the  
301 focus position, as in the simulations. They were reconstructed using parametric IPA, with the  
302 same workflow that was described in the reconstructions of the previous subsection. Again, to  
303 compare the effect of aberrations on the estimation of the beads parameters, both corrected (C)  
304 and uncorrected (UC) models are used for the reconstructions. As illustrated in Section 3, the  
305 position of the focus varied with the setting of the correction collar. Parametric IPA provides an  
306 estimation of the defocus distance  $z$  between the sample and the focal plane of the objective.

307 Table 4 presents a list of the mean values and the standard deviations of all 35 positions in  
308 the field for parameters  $z$ ,  $r$  and  $n$  and for both (UC) and (C) reconstructions. According to the  
309 comparison of standard deviations for each collar setting, the dispersion over the field was only  
310 moderately modified by the model (UC) or (C). However, the mean values changed, especially  
311 that of the estimated defocus  $\hat{z}$ . A maximum difference of  $1.68\mu\text{m}$  in the estimated defocus was  
312 found between the two models (UC) and (C).

313 Moreover, the estimated defocus highly depends on the correction collar setting, which varied  
314 from  $8.8$  to  $13.9\mu\text{m}$  (UC) and from  $10.5$  to  $12.5\mu\text{m}$  (C). Thus, this dispersion was reduced by  
315 taking the aberrations into account, indicating a correction of the bias in the evaluation of the  
316 defocus. Since regularized reconstruction algorithms rely on a precise knowledge of the image  
317 formation model (including the defocus distance), any misestimation of the axial position of the  
318 sample would bias the reconstructions. Finally, it must be noted that the remaining dependence  
319 of the estimated defocus with the correction collar setting may have a physical origin. Indeed,  
320 wrong settings of the correction collar may really change the focus position as it changes the  
321 properties of the objective.

322 For the estimated radii  $\hat{r}$  and refractive indices  $\hat{n}$ , the dispersion over the field was reduced  
323 when the aberrations were taken into account. The averages were also less dispersed, but to a  
324 lesser extent. Indeed, some biases that depend on the correction collar setting appeared to remain.

325 Fig. 6 presents the estimated bead parameters as scatter plots. This makes it possible to  
326 visualize the correlations between the estimated parameters  $z$ ,  $r$  and  $n$ .

327 Moreover, for each collar setting (one color for one collar setting), taking the aberrations into  
328 account improved the repeatability of the parameter estimation independently of the introduced  
329 aberrations. Indeed, the aberration corrections not only reduce the biases between the different  
330 collar settings (differences from one color point cloud to another) but also reduce correlations  
331 coefficients between parameter estimations (correlations within one color point cloud). This is  
332 presented quantitatively on Table 5, for both models (C) and (UC) and for the less aberrated case  
333 ( $0.17\text{mm}$ ). According to Table 5, the decorrelation is particularly important between  $r$  and  $n$ .

334 According to the manufacturer's specifications, the radius should be  $(0.5\pm 0.03)\mu\text{m}$  and the  
335 refractive index should be around  $1.587$ . The estimated parameters obtained with or without  
336 an aberration model were within the manufacturer's confidence interval ( $0.47$ - $0.53\mu\text{m}$ ). It is  
337 important to note that the fit with the Mie model is constrained by the spherical hypothesis and  
338 thus may be quite robust to errors in the model, contrary to the case of regularized reconstruction  
339 that have more degrees of freedom, and will be more sensitive to aberrations, especially to  
340 non-radially symmetric ones, as it will be seen later on regularized reconstructions.

341 With the 35 recorded holograms corresponding to 35 bead positions in the field of view, we  
342 were able to check that the Zernike coefficients vary in the field of view, following continuous  
343 evolutions similar to those described in another work [10]. The Figure 7, illustrates the evolution  
344 of the Zernike coefficients associated with oblique astigmatism, defocus, vertical coma, horizontal  
345 coma and spherical aberrations. These appeared to be the main components of the aberrated  
346 pupil function  $\tilde{p}$ . The evolution of these coefficients is continuous and, not surprisingly, increases  
347 with increasing errors in the correction collar setting. Vertical coma increases from the left  
348 to the right whereas horizontal coma increases from the top to the bottom of the field of view.  
349 Spherical aberration and defocus do not depend on the location in the field of view but change

	UC	C	UC	C
Collar	$\langle \hat{z} \rangle$	$\langle \hat{z} \rangle$	$\sigma_z$	$\sigma_z$
0.13	13.913	12.579	0.802	0.817
0.15	13.049	12.542	0.483	0.440
0.17	11.706	12.046	0.548	0.546
0.19	10.496	11.556	0.510	0.484
0.21	8.838	10.525	0.442	0.495
Collar	$\langle \hat{r} \rangle$	$\langle \hat{r} \rangle$	$\sigma_r$	$\sigma_r$
0.13	0.526	0.501	0.008	0.005
0.15	0.519	0.505	0.006	0.005
0.17	0.502	0.513	0.007	0.004
0.19	0.495	0.519	0.007	0.003
0.21	0.497	0.522	0.007	0.012
Collar	$\langle \hat{n} \rangle$	$\langle \hat{n} \rangle$	$\sigma_n$	$\sigma_n$
0.13	1.5733	1.5901	0.0041	0.0029
0.15	1.5773	1.5882	0.0027	0.0024
0.17	1.5856	1.5837	0.0044	0.0022
0.19	1.5902	1.5809	0.0036	0.0023
0.21	1.5878	1.5798	0.0032	0.0046

Table 4. For the 5 correction collar settings, averages  $\langle \hat{\vartheta}_i \rangle$  and standard deviations  $\sigma_{\hat{\vartheta}_i}$  of the estimated parameter  $\hat{z}$ ,  $\hat{r}$  and  $\hat{n}$  using (UC) uncorrected model and (C) aberration corrected model . All lengths are in micrometers.

Uncorrected (UC)				Corrected (C)			
$\vartheta_i$	$z$	$r$	$n$	$\vartheta_i$	$z$	$r$	$n$
$z$	1	0.02	0.51	$z$	1	0.03	-0.26
$r$	0.02	1	-0.56	$r$	0.03	1	0.01
$n$	0.51	-0.56	1	$n$	-0.26	0.01	1

Table 5. Correlation coefficients between the estimated parameters without aberration correction (left) and with aberration correction (right) for a correction collar setting of 0.17mm (less aberrated case)

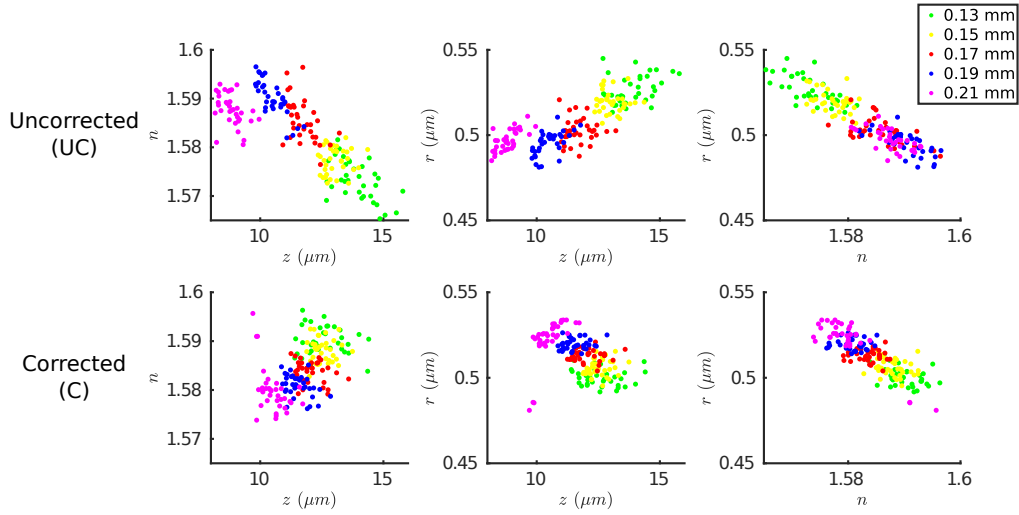


Fig. 6. Scatter plots showing the biases and correlations between the estimated defocus  $\hat{z}$ , radius  $\hat{r}$  and refractive index  $\hat{n}$  for a single bead, for the 35 positions in the field, for the 5 settings of the coverslip correction collar and with corrected models (C) and uncorrected models (UC). With correction of the aberrations, the bias and the dispersion of the estimations due to aberrations are reduced.

350 with the correction collar setting, with almost no spherical aberration and defocus for the less  
 351 aberrated case (0.17mm). This is quite logical as a coverslip thickness error is known to induce  
 352 spherical aberrations [18]. On the contrary, oblique astigmatism varies in the field of view  
 353 without depending too much on the correction collar setting.

354 Figure 8 illustrates the evolution of the phase correction for the 35 positions in the field  
 355 of view and for a correction collar of 0.17mm. For this supposedly aberration-free case, the  
 356 setup still suffer from aberrations that change in the field of view. These phase functions show  
 357 significant aberration effects but, as expected, lower than for the other correction collar settings  
 358 (not represented). This indicates the necessity of taking aberrations into account for hologram  
 359 reconstruction even when the optical system is supposed to be compensated for aberrations.  
 360 Indeed, these aberrations may come from residual aberrations of the objective, but also from  
 361 other sources, like thickness inhomogeneities of the slide and the coverslip, as well as alignment  
 362 issues.

363 From the numerical point of view, the detection of all 35 beads in the mosaic takes around  
 364 30 seconds on a 3296×2472 pixels image. The local optimization step for each bead takes  
 365 around 10 seconds when not considering aberrations while it takes 45 seconds when considering  
 366 them. These estimations have been realized using an Intel Core i9-11950H CPU 2.60GHz with  
 367 16GBytes of RAM.

### 368 4.3. Reconstructions on experimental data using regularized algorithms

369 The evaluation of aberration's effects on reconstructions is performed using beads holograms.  
 370 This allows us to compare quantitatively the reconstructed transmittance with a ground truth  
 371 (assumed to be the transmittance of the bead whose parameters are estimated by parametric  
 372 IPA). However, since the non-parametric model is very general (not limited to spherical objects),  
 373 similar results will be obtained with an aspherical sample. The reconstruction is performed with  
 374 (C) and without (UC) the previously estimated aberration pupil function  $\hat{p}$  and the  $\hat{z}$  parameters.

375 A Fienup phase retrieval algorithm [28, 29], as well as a regularized IPA (as presented in 2.2)

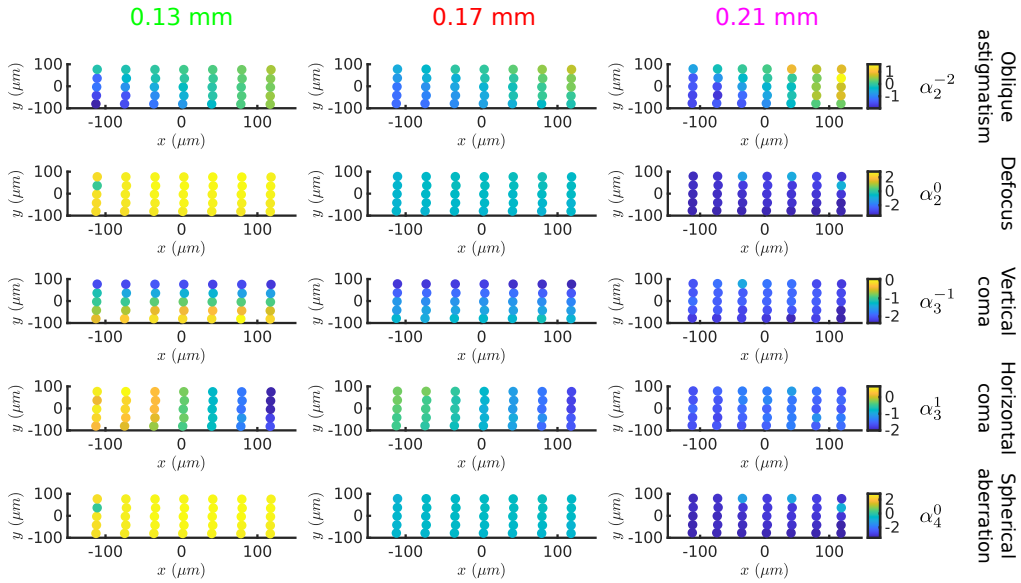


Fig. 7. Estimated Zernike coefficients  $\alpha_n^m$  as a function of the position in the field of view and for 3 settings of the coverslip correction collar (0.13 mm, 0.17 mm, 0.21 mm). The evolution of the Zernike coefficients is continuous in the field of view. The coma and astigmatism coefficients depend on the position in the field of view and on the correction collar setting whereas defocus and spherical aberration only depend on the correction collar setting.

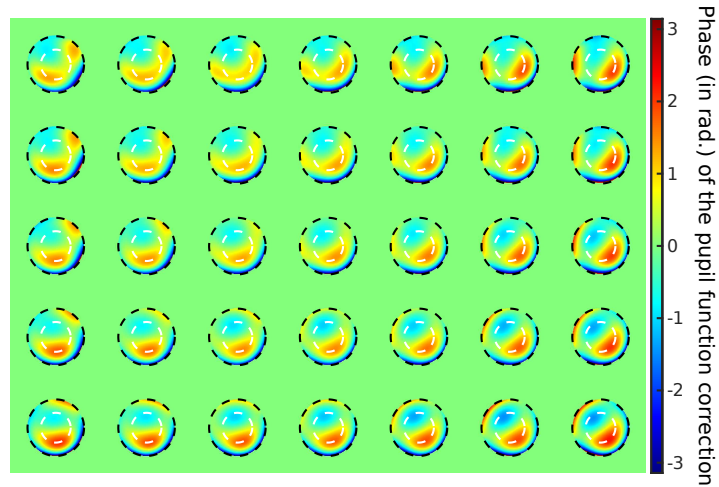


Fig. 8. Evolution of the phase (in radians) of the pupil function correction in the field of view for a setting of the coverslip correction collar of 0.17 mm and for the 7x5 positions in the field where the aberrations were estimated. The black and white dashed circles are defined on Fig.5

376 are used to reconstruct the data. These reconstructions are performed using the uncorrected  
 377 propagator  $\underline{h}_z^{\text{RS}}$  (UC) or the corrected propagator  $\underline{h}_z$  (C) in the model (eq.6). Fig. 9 illustrates  
 378 the reconstructions results for both algorithms. The estimated aberrated Mie model that fits the  
 379 data has been back propagated at the center plane of the bead (BPMie-C) and is considered as  
 380 the ground truth here because it is the most accurate model. Similarly, a back propagation of  
 381 the Mie model estimated without aberration has also been computed (BPMie-UC). Because of  
 382 the coma aberrations, the bead position  $(x, y)$  is not the same for (BPMie-C) and (BPMie-UC)  
 383 parametric inversions, as mentioned in Appendix B. For comparison purpose the beads have then  
 384 been centered in Fig. 9.

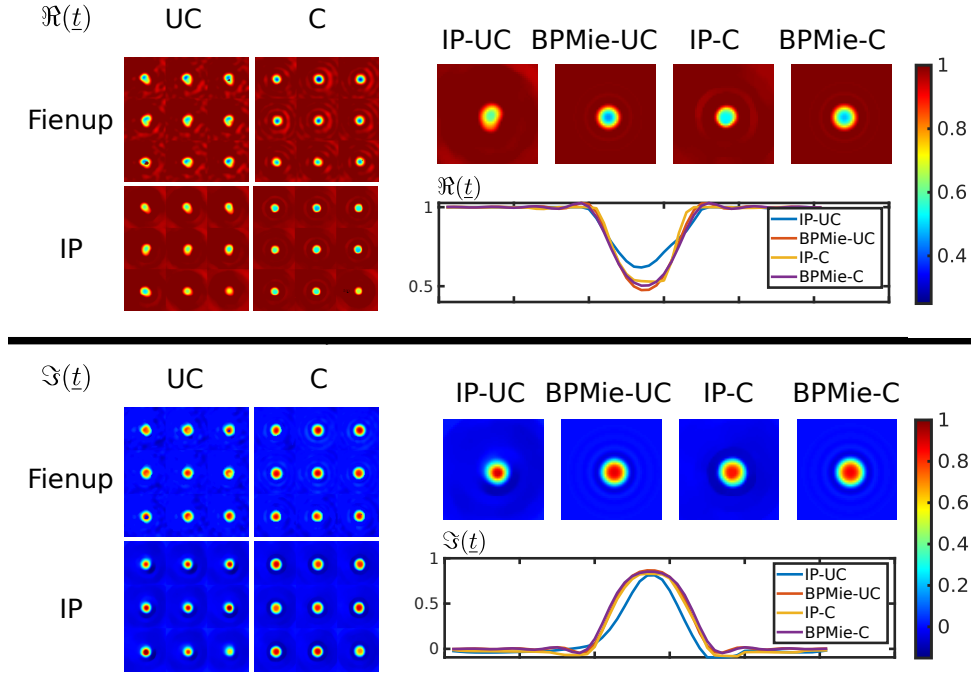


Fig. 9. Non-parametric reconstructions using regularized IPA and Fienup algorithm with (C) or without (UC) aberrations correction. The reconstructions are presented in real part an imaginary part. A reconstruction is compared with the back-propagation of the estimated Mie model without aberration estimation (BPMie-UC) and the back-propagation of the Mie model with aberration estimation (BPMie-C). Profiles of the real part and imaginary part at the center of the bead are presented.

385 When aberrations are not considered in the reconstruction model, the morphological properties  
 386 and quantity of the reconstructions are compromised. Indeed, either with Fienup or  
 387 regularized IPA, the bead does not show a circular shape. As the aberrations vary in the  
 388 field of view, the same bead does not have the same shape for each lateral position. The  
 389 back-propagation of the Mie model without aberration illustrates the model error when the  
 390 aberrations are not considered, but the radial symmetry is maintained as the Mie model is based  
 391 on a spherical model. The regularized reconstructions without aberrations do not match with  
 392 this model indicating bias in the estimation of the bead parameters. However, with aberrations  
 393 correction the reconstructions fit the corresponding back-propagated Mie model and have the  
 394 expected geometrical and quantitative properties. It demonstrates that whatever the reconstruction  
 395 algorithm, aberrations should be taken into account to restore accurately the morphological and  
 396 quantitative properties of the sample.

397 Taking into account the aberrations in regularized reconstructions has no effect on the  
 398 computational time as the aberrated forward model has the same complexity as angular spectrum  
 399 propagation. In the example of Figure 9, reconstructing a whole field of view ( $2472 \times 3296$ )  
 400 and considering the spatial evolution of the PSF takes less 10 minutes. These estimations have  
 401 been realized using an Intel Core i9-11950H CPU 2.60GHz with 16GBytes of RAM. This  
 402 computational time can be reduced using GPU.

## 403 5. Conclusion

404 In this article, we present a method to estimate the aberrations and thus reduce reconstruction  
 405 errors, by using a more accurate image formation model, in in-line holographic microscopy. This  
 406 method is based on the use of calibration beads. We show that the rigorous and highly constrained  
 407 Mie model can be used to estimate bead parameters and Zernike coefficients at the same time  
 408 with a good precision and repeatability. Moreover, this approach requires only one hologram and  
 409 does not require any assumption on the PSF evolution in the field of view. This calibration step  
 410 could be done sequentially, like standard calibrations or *in-situ* by inserting calibrated beads in  
 411 the biological sample itself. However, this may depend on the application or on the main origin  
 412 of the aberrations (from the optical setup or from the sample itself). Actually, adding calibration  
 413 beads in the sample has already proven to be useful for autofocusing [23]. In this context, with  
 414 the present method of correction of aberrations, this autofocusing would be even more accurate.

415 Once the Zernike coefficients have been estimated, it is then possible to use them in a  
 416 regularized approach framework to reconstruct any biological objects (spherical or not), as long  
 417 as the sparsity constraint required in in-line digital holography is fulfilled. This methodology of  
 418 aberration estimation was applied for the improvement of regularized reconstruction of holograms  
 419 with the in-line holographic microscopy configuration. However, it is also applicable to off-axis  
 420 holography or other coherent imaging techniques or simply used as a calibration method for  
 421 microscopy systems.

422 The method proposed here offers interesting perspectives for reconstructing more accurately  
 423 and with more quantitativity the absorption and the phase of the objects of interest, even with  
 424 poorly corrected or misaligned optical systems, non-standard optical configurations (various  
 425 sample media, variable axial position of the objects below the coverslip) and more generally, for  
 426 any non-standard microscopy configurations that may introduce aberrations.

427 In this study, we estimated aberrations parameters on a discrete grid. The next step could be to  
 428 interpolate the spatially varying PSF. This PSF can then be used in the image reconstruction step,  
 429 but with a high computational cost. Nevertheless, fast algorithms can be used [45, 46].

## 430 Appendix A : Zernike polynomials

Zernike polynomials depend on two parameters: the azimuthal angle  $\phi = \arctan\left(\frac{\kappa_y}{\kappa_x}\right)$  and the  
 normalized radial distance  $\rho = \frac{\lambda}{\text{NA}} \sqrt{\kappa_x^2 + \kappa_y^2}$  and are defined as follows :

$$Z_n^m(\rho, \phi) = \begin{cases} R_n^{|m|}(\rho) \sin(m\phi) & \text{if } m > 0 \\ R_n^{|m|}(\rho) \cos(m\phi) & \text{otherwise} \end{cases}$$

where  $n \in \mathbb{N}$ ,  $m \in \mathbb{Z}$  and  $R_n^m(\rho)$  is defined as :

$$R_n^m(\rho) = \sum_{k=0}^{\frac{n-m}{2}} \frac{(-1)^k (n-k)!}{k! \left[\frac{n+m}{2} - k\right]! \left[\frac{n-m}{2} - k\right]!} \rho^{n-2k}$$

431 with  $n \geq |m|$  and  $n - |m|$  even.

432

433 Because of the numerical aperture, the pupil function is zero out of the disk defined by  $\rho \leq 1$ .  
 434 An illustration of the polynomials is given on Fig. 10 [47].

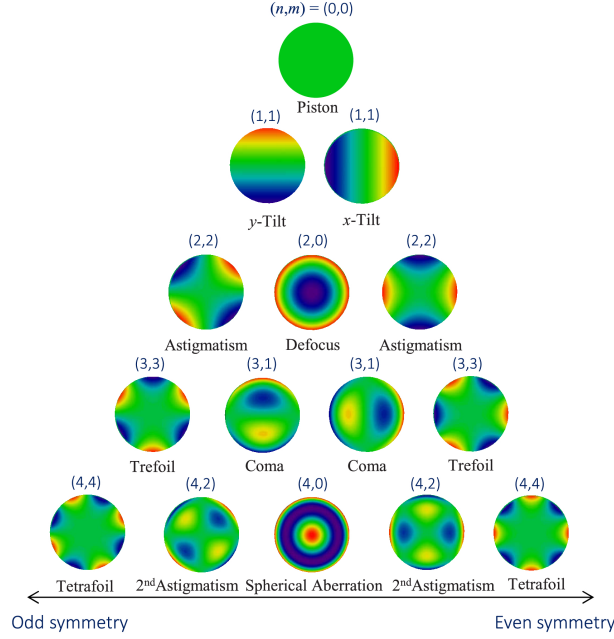


Fig. 10. Illustration of the 15 first Zernike polynomials (adapted from [47]).

### 435 Appendix B : Theoretical study of the aberration parameters accuracy

436 In this appendix, we aim at estimating the achievable precision on each estimated parameter and to  
 437 study the correlation between these parameters. For these purposes, Cramér-Rao Lower Bounds  
 438 (CRLB) and the correlation matrix are computed [48] using our aberrated model  $\mathbf{m}^P$  presented in  
 439 section 2.1 eq. (1). According to Cramér-Rao inequality, the variance of any unbiased estimator  
 440  $\hat{\zeta} = \{\zeta_i\}_i = \{\boldsymbol{\theta}, \boldsymbol{\alpha}\}$  of the unknown vector parameter  $\zeta^\dagger$  is bounded from below by i-th diagonal  
 441 coefficient of the inverse of the Fisher information matrix:

$$\text{Var}(\hat{\zeta}_i) \geq [\mathbf{I}^{-1}(\zeta^\dagger)]_{i,i} = \sigma_{\zeta_i}^{\text{CRLB}^2} \quad (8)$$

442 where  $\mathbf{I}(\zeta^\dagger)$  is the Fisher information matrix. It is linked to the curvature of the cost function in  
 443 the parameters space:

$$[\mathbf{I}(\zeta)]_{i,j} = E \left[ \left. \frac{\partial^2 \mathcal{D}^P(\mathbf{d}, \mathbf{m}^P(\cdot))}{\partial \zeta_i \partial \zeta_j} \right|_{\zeta} \right] \quad (9)$$

444 In the case of white Gaussian noise of standard deviation  $\sigma_\epsilon$ , neglecting quantization effect  
 445 and considering a centered model [49] :

$$[\mathbf{I}(\zeta)]_{i,j} = \frac{1}{\sigma_\epsilon^2} \sum_k \left( \frac{\partial \mathbf{m}^P(x_k, y_k, \zeta)}{\partial \zeta_i} \frac{\partial \mathbf{m}^P(x_k, y_k, \zeta)}{\partial \zeta_j} \right) \quad (10)$$

446 These bounds are computed for a bead at the center of the field of view and for several defocus  
 447 distances with parameters of Table 1 ( $\boldsymbol{\theta}$  ( $x = 0 \mu\text{m}$ ,  $y = 0 \mu\text{m}$ ,  $z, r = 0.5 \mu\text{m}$ ,  $n = 1.58$ )).

448 As the aberrations happen to be quite low in our case, the accuracy study has been performed  
 449 with Zernike coefficient set to zero. Thus, the accuracy on the Zernike coefficients has been  
 450 studied around a zero value.

451 Figure 11 illustrates the evolution of the CRLB with the propagation distance  $z$  (*i.e.* the lower  
 452 bound variance of each parameter versus  $z$  value).

453 These CRLB have been computed considering  $\sigma_\epsilon$  constant and using numerical derivatives.  
 454 For most parameters the best accuracy is obtained for defocus distances between 10 and 15  $\mu\text{m}$ .  
 455 In this study, the defocus distance  $z = 12\mu\text{m}$  was considered.

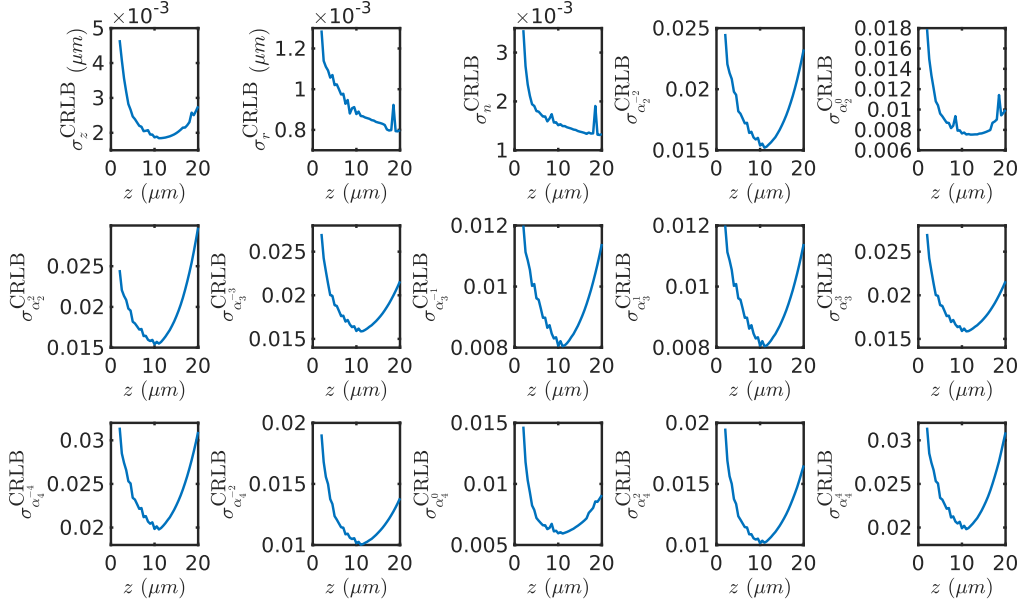


Fig. 11. Evolution of Cramér-Rao Lower Bounds on each parameter as a function of  $z$ . The experimental parameters of the model are given in Table 1. These CRLB have been computed for a hologram without aberrations.

456 The correlation matrix  $\Sigma$  is obtained by:

$$\Sigma_{i,j}(\zeta) = \frac{[\mathbf{I}^{-1}(\zeta)]_{i,j}}{\sigma_{\zeta_i}^{\text{CRLB}} \cdot \sigma_{\zeta_j}^{\text{CRLB}}} \quad (11)$$

457 Table 6 shows this correlation matrix for the selected seventeen parameters. Coefficients below  
 458 0.05 are set to zero for a better visualization.

459 The correlation matrix indicates strong correlations between several parameters. Unsurprisingly,  
 460  $r$  and  $n$  are highly correlated as the phase shift induced by an object depends on the product of  
 461 these two parameters and the phase shift has a strong effect on the propagation. It is interesting  
 462 to notice that coma coefficients represented by  $\alpha_3^{-1}$  and  $\alpha_3^1$  are highly correlated with  $x$  and  
 463  $y$ . Therefore, ignoring the coma aberration could lead to lateral shifts in the reconstructions.  
 464 Correlations between  $\alpha_2^{-4}$  and  $\alpha_2^{-2}$ ,  $\alpha_4^0$  and  $\alpha_2^0$  or  $\alpha_4^2$  and  $\alpha_2^2$ , may lead to misestimations of  
 465 these coefficients. This is studied in section 4.1 on simulation experiments. However, it would  
 466 be probably worse not to take them into account because that would systematically introduce  
 467 errors in the model. Most of the other coefficients of the correlation matrix are low or null  
 468 and the corresponding parameters can then be considered as decorrelated. Because of the high



	x	y	z	r	n	$\alpha_2^{-2}$	$\alpha_2^0$	$\alpha_2^2$	$\alpha_3^{-3}$	$\alpha_3^{-1}$	$\alpha_3^1$	$\alpha_3^3$	$\alpha_4^{-4}$	$\alpha_4^{-2}$	$\alpha_4^0$	$\alpha_4^2$	$\alpha_4^4$
x	1	0	0	0	0	0	0	0	0	-0.89	0	0	0	0	0	0	0
y	0	1	0	0	0	0	0	0	0	0	-0.89	0	0	0	0	0	0
z	0	0	1	0.24	-0.13	0	-0.49	0	0	0	0	0	0	0	-0.39	0	0
r	0	0	0.24	1	-0.85	0	-0.13	0	0	0	0	0	0	0	0	0	0
n	0	0	-0.13	-0.85	1	0	0.22	0	0	0	0	0	0	0	0.05	0	0
$\alpha_2^{-2}$	0	0	0	0	0	1	0	0	0	0	0	0	0	0.72	0	0	0
$\alpha_2^0$	0	0	-0.49	-0.13	0.22	0	1	0	0	0	0	0	0	0	0.92	0	0
$\alpha_2^2$	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0.72	0
$\alpha_3^{-3}$	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
$\alpha_3^{-1}$	-0.89	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
$\alpha_3^1$	0	-0.89	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
$\alpha_3^3$	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
$\alpha_4^{-4}$	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
$\alpha_4^{-2}$	0	0	0	0	0	0.72	0	0	0	0	0	0	0	1	0	0	0
$\alpha_4^0$	0	0	-0.39	0	-0.05	0	0.92	0	0	0	0	0	0	0	1	0	0
$\alpha_4^2$	0	0	0	0	0	0	0	0.72	0	0	0	0	0	0	0	1	0
$\alpha_4^4$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1

Table 6. Correlation matrix of the 5 beads parameters and 12 Zernike coefficients. High correlation or anti-correlations are represented in red, moderate correlation or anti-correlations in yellow, low correlations or anti-correlations in green.

469 correlation values in the correlation matrix, all parameters must be estimated at the same time to  
470 prevent estimation errors.

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473 github.io/GlobalBioIm/index.html](https://biomedical-imaging-group.github.io/GlobalBioIm/index.html)). The Zernike polynomials models have been computed  
474 using Fricker’s implementation [52] ([https://www.mathworks.com/matlabcentral/  
475 fileexchange/7687-zernike-polynomials](https://www.mathworks.com/matlabcentral/fileexchange/7687-zernike-polynomials)). LINCOA optimization strategies have been computed  
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