

Coded wavefront sensing for video-rate quantitative phase imaging and tomography: validation with digital holographic microscopy

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Abstract: We demonstrate coded wavefront sensing (Coded WFS) for video-rate quantitative phase imaging and 3D refractive index (RI) tomography of biological specimens. To evaluate the accuracy, we implement an experimental setup that supports measurements of specimens with Coded WFS as well as with digital holographic microscopy (DHM) under identical conditions, enabling direct comparison. We image a static 3D phantom fabricated via additive manufacturing and a rotating HEK293 cell in an acoustofluidic chamber. Our results demonstrate good agreement between the two methods, with the advantage that, in contrast to DHM, Coded WFS enables simple integration with standard microscopes. Furthermore, we apply a standard tomographic reconstruction algorithm to the HEK293 cell data for comparison, which demonstrates the potential of Coded WFS in tomography.

1. Introduction

The volumetric distribution of the refractive index (RI) in cells is linked to biologically relevant structures in their interior [1]. RI tomography enables the recovery of volumetric RI distributions in inhomogeneous specimens such as biological cells, enabling a quantitative characterization of their morphology. It holds great potential for a non-invasive and quantitative study of intracellular matter as it utilizes the intrinsic phase contrast without the need for staining and genetic modifications to create fluorescent markers [2–4].

RI tomography is based on i) quantitative phase imaging (QPI) techniques that enable the label-free imaging of phase specimens and ii) a set of measurements under varying illumination and/or viewing conditions:

i) Digital holographic microscopy (DHM) [5–7] is the gold standard of QPI as it provides reliable, high-resolution access to the complex wavefields. QPI methods can be grouped into two categories: *Single-shot QPI-capable methods*, for example, those that record interferograms, such as DHM, and *computational methods* that record the intensity two or more times, usually at different focus planes, including transport of intensity-based methods [8–10], using optimization algorithms to recover the quantitative phase. The latter group is inherently challenging for snapshot QPI. Even though coded wavefront sensing (Coded WFS) belongs to this category, the reference image can be taken offline and it is, therefore, snapshot-capable. The use of QPI to measure the scattering properties of cells and tissues has been extensively reviewed in [11–13].

ii) The required actuation of the specimens can be achieved by mechanical confinement to a tip or in a capillary attached to a rotation stage [14]. Alternatively, contactless manipulation is

46 a promising research avenue, that has, e.g., been achieved by using optical tweezers to rotate
47 suspended biological cells [15]. Optical trapping for the manipulation of arbitrarily shaped
48 specimens without prior geometric information has also been successfully demonstrated [16].
49 Acoustic forces have also been used to position and rotate a suspended specimen [17–19]. An
50 alternative method is to provide angled illumination to a static specimen [20, 21]. However, this
51 setup generally suffers from the missing cone problem as the range of angles is smaller. For
52 QPI-based tomography, retrieved complex wavefields from multiple viewing directions have
53 been combined to yield volumetric RI distribution [14–17, 20–23].

54 In this paper, we propose Coded WFS [24] as a promising candidate for QPI-based 3D
55 RI tomography due to its affordability, single-shot capability, and seamless integration with
56 standard microscopes. We integrate an acoustofluidic device [19] in our setup, enabling specimen
57 rotation and contactless measurements from a full 360° range of viewing angles. This setup fully
58 leverages the benefits of QPI while simultaneously reducing the hardware overhead associated
59 with interferometric methods. In the following, we review both DHM and Coded WFS, as well
60 as RI tomography.

61 **Holographic Microscopy.** DHM is an established interferometry-based snapshot QPI method,
62 which inherently offers support for video-rate phase imaging [25, 26]. Gabor originally introduced
63 holographic imaging in an attempt to improve the resolution in electron microscopy [27, 28]. The
64 field of digital holography was only later developed, distinguishing itself from holographic imaging
65 in that it recorded the hologram electronically and not on film [29]. DHM is the application of
66 digital holography to microscopic imaging and has found widespread application in this area due
67 to its ability to measure the phase contrast of objects with high precision [25, 26, 30, 31].

68 In off-axis DHM, the interference of the object wave with a tilted plane wave ensures that
69 the amplitude and phase of the specimen can be encapsulated within a single intensity image.
70 DHM retrieves the propagated phase of the specimen in the image plane, which is then digitally
71 propagated back to the object plane [5]. The retrieved quantitative phase measures the optical
72 path difference of the specimen along a single axis.

73 Digital holography remains an active research field for microscopy, tomography, cell identifi-
74 cation, and more, as is comprehensively explored in [32]. Moreover, the high accuracy of the
75 recovered phase maps has made DHM the default choice to not only benchmark the performance
76 of other QPI methods but also to evaluate the quality of fabrication processes of sub-micrometer
77 3D phantoms [33–35].

78 However, DHM requires careful setup and dedicated hardware for its realization, including
79 damped optical tables and lasers, which makes integration with other optical systems challenging.
80 For more extended 3-dimensional objects, undoing phase wrapping, which occurs when the optical
81 path difference (OPD) exceeds the illumination wavelength, requires additional consideration.

82 **Coded Wavefront Sensing.** Another class of QPI methods includes wavefront sensors
83 operating on the same principle as the Hartmann test, which treats the specimen wavefront as
84 transverse aberrations compared to an ideal wavefront [36]. The Hartmann sensor involves an
85 array of apertures a short distance away from the image plane and tracks the motion of diffraction
86 spots relative to their ideal positions due to a non-ideal wavefront and integrates the resulting
87 vector field to retrieve the phase of the specimen.

88 The aperture array is replaced with a lenslet array to improve the light efficiency in the
89 Shack-Hartmann wavefront sensor (SHWS) [37]. The size of the lenslets has also been reduced
90 to increase the spatial resolution of the SHWS [38]. However, as a consequence, the range of
91 movement for the diffraction spots that can be tracked unambiguously, corresponding to the
92 lenslet size, also reduces. Smaller regions restrict SHWSs to the measurement of small distortions.
93 SHWSs are, therefore, limited by a fundamental trade-off.

94 It is possible to replace the lenslet arrangement with a thin diffuser or a random phase
95 mask [39–42]. This changes the reference image from a regular grid of spots to a random speckle

96 pattern and enables continuous tracking of the resulting deformation of the speckle grains. We
97 refer to these QPI methods as coded wavefront sensing [42].

98 Coded WFS requires a reference-specimen pair of intensity images, where the reference is
99 measured only once for an optical setup. The reference speckle pattern is the diffraction pattern
100 of the phase mask, recorded in the absence of a specimen. A second speckle pattern is recorded
101 after inserting the specimen in the optical system. Coded WFS leverages a useful phenomenon
102 known as the optical memory effect [43–45], which relates local tips or tilts in the wavefront
103 incident on the phase mask with local shifts in the reference. In Coded WFS, the motion of
104 the pixels between the reference-specimen pair provides information about the gradient of the
105 phase of the specimen. Optical flow-inspired [46] methods are used to track the motion, which is
106 integrated to obtain the quantitative phase of the specimen.

107 More recently, algorithmic advancements in Coded WFS have allowed the simultaneous
108 recovery of amplitude and phase of weakly absorbing phase objects [24]. Like DHM, Coded
109 WFS is a snapshot method that allows QPI at video rates. However, Coded WFS is readily
110 integrable with bright-field microscopes, leading to less stringent hardware requirements and a
111 higher potential of being implemented in standard laboratories.

112 **Refractive Index Tomography.** Given several input phase maps under different known
113 incident illumination and/or observation directions, the 3D RI distribution of a specimen may be
114 determined in a process known as optical diffraction tomography first described by Wolf [47, 48].
115 It is based on the Fourier diffraction theorem (FDT) [49, 50] which states that, in the case of
116 weakly scattering objects, the Fourier transform of the field is related to a hemispherical surface
117 in the Fourier transform of scattering potential of the specimen. First practical demonstrations of
118 RI tomography on biological samples were implemented using DHM [14] and utilized optical
119 projection tomography which can be used in conditions where the scattering angles are sufficiently
120 small to enable the use of the Fourier slice theorem (FST) instead of the FDT [21, 22]. Joint
121 absorption and RI tomography based on mechanical scanning of the specimen has been described
122 earlier in [51]. Recent developments include combinations with structured illumination [3], and
123 deconvolution-based recovery from focal stacks [4]. Improved forward models can extend the
124 range of the scattering regime beyond the Born approximation [52–55].

125 **Overview.** In Sec. 2, we briefly review the QPI techniques DHM and Coded WFS, where
126 DHM is used as a gold standard to validate Coded WFS as a technique for snapshot QPI, as well
127 as the acoustofluidic trapping device which is fundamental for our dynamic QPI experiments that
128 serve as 3D RI tomography inputs. We provide the details of the experimental setup in Sect. 3.1,
129 which enables imaging the specimen in an identical setting by both methods. We validate the QPI
130 accuracies in Sect. 3.2 by imaging a phantom with a known design OPD: a 3D-printed cluster
131 of objects resembling HeLa cells. We then showcase their video capabilities in Sect. 3.3 by
132 performing video-rate QPI on a real rotating HEK293 cell, where the sustained and periodic
133 rotation of the cell in the acoustofluidic device enables a fair comparison. In Sect. 3.4, we
134 estimate the 3D RI distribution of the HEK cell. We provide a discussion and draw conclusions
135 in Sect. 4.

136 **2. Methods**

137 *2.1. Digital Holographic Microscopy*

138 In standard off-axis digital holography, coherent plane wave illumination is split into an object
139 beam that is modulated by the specimen and a reference beam that is tilted by a mirror. The wave
140 fields are combined and measured in the sensor plane. The interference of the object beam with
141 a tilted plane wave ensures that the spectrum of the measurement contains an easily separable
142 propagated transfer function of the unknown specimen, where the separation is governed by the
143 amount of reference beam tilt. Off-axis DHM has been comprehensively developed and analyzed

144 in [6, 7, 56, 57].

145 DHM offers dependable snapshot QPI capabilities, which has also been leveraged to benchmark
146 the performance of newly developed methods [33, 34]. In this paper, we use a common-path
147 shearing interferometer inspired by [6], tailored for use with the acoustofluidic device. Instead of
148 using a pinhole to generate the reference wave, this DHM variant introduces a spatial shift on the
149 (unfiltered) reference wave, such that a flat part of the beam is overlapped with the location of the
150 object at an angle in the sensor plane. This necessitates that the sample is sparsely distributed,
151 which is fulfilled in our case due to the nodes in the acoustic trapping potential. This precludes
152 the need to consider path matching inside the microscope. The schematic of the setup is shown
153 in Fig. 1 **a**.

154 2.2. Coded Wavefront Sensing

155 In Coded WFS, a random phase mask that modulates the phase of the incident wave is placed
156 in close proximity (≈ 1 mm) to the camera sensor. In the absence of the specimen, plane wave
157 illumination generates a speckle pattern in the image plane, which serves as the reference image,
158 $I_0(r)$, for the setup. Subsequently, the unknown phase specimen, $e^{i\phi(r)}$, is inserted in the object
159 plane of the optical setup. The modified speckle pattern in the image plane is measured and is
160 referred to as the object image, $I(r)$.

161 The relation between $I_0(r)$ and $I(r)$ is estimated by leveraging the optical memory effect [43, 44],
162 using the fact that the local changes in the speckle pattern, measured by the apparent flow of the
163 pixels $u(r)$, are proportional to the gradient of the phase of the unknown specimen,

$$164 u(r) = \frac{z}{k} \nabla \phi(r), \quad (1)$$

165 where, z is the optical distance between the mask and the sensor determined in a calibration
166 step [24], $k = \frac{2\pi}{\lambda_0}$ is the wavenumber and λ_0 is the illumination wavelength in vacuum.
167 Consequently, the measurements can be written as,

$$168 I(r) = I_0(r - u(r)), \quad (2)$$

169 which simplifies wavefront sensing to an optical flow optimization problem [46] to estimate
170 $u(r)$. Either $u(r)$ can be first estimated and then integrated to retrieve the OPD [41, 58], or an
171 optimization strategy can be devised to estimate the OPD from $I(r)$ and I_0 in a single step as
172 proposed in [42].

173 In this paper, we use the formulation in [24], which allows the unwrapped phase estimation of
174 specimens with weak absorption while also providing the speckle-free bright-field amplitude
175 of the specimen. Moreover, as Coded WFS has been shown to work well with broadband (or
176 white-light) illumination [24, 41], we integrate a white-light illumination unit in the combined
177 experimental setup in Sect. 3.1.

178 2.3. Acoustofluidic Trapping Device

179 To image trapped and rotating single cells, we employ an acoustofluidic platform developed
180 in [19] shown in Fig. 1 **b**. The device is tailored for transmission imaging and mounted on an
181 inverted light microscope. Bulk acoustic waves are generated by three lithium niobate (LiNbO_3)
182 transducers coupled to a fluid-filled chamber to generate standing waves in three orthogonal
183 directions. The sample is introduced via microfluidic channels into the middle of the chamber
184 where all three acoustic waves intersect. The optically transparent vertical transducer levitates
185 the sample and grants optical access for illumination. The channels are milled in an aluminum
186 carrier and the bottom of the chamber is sealed with a cover slip acting as a reflector for the
vertical sound waves and ensuring imaging compatibility. By tuning the relative amplitudes of
the three acoustic waves we control the trapping in 3D and the sample orientation. To induce

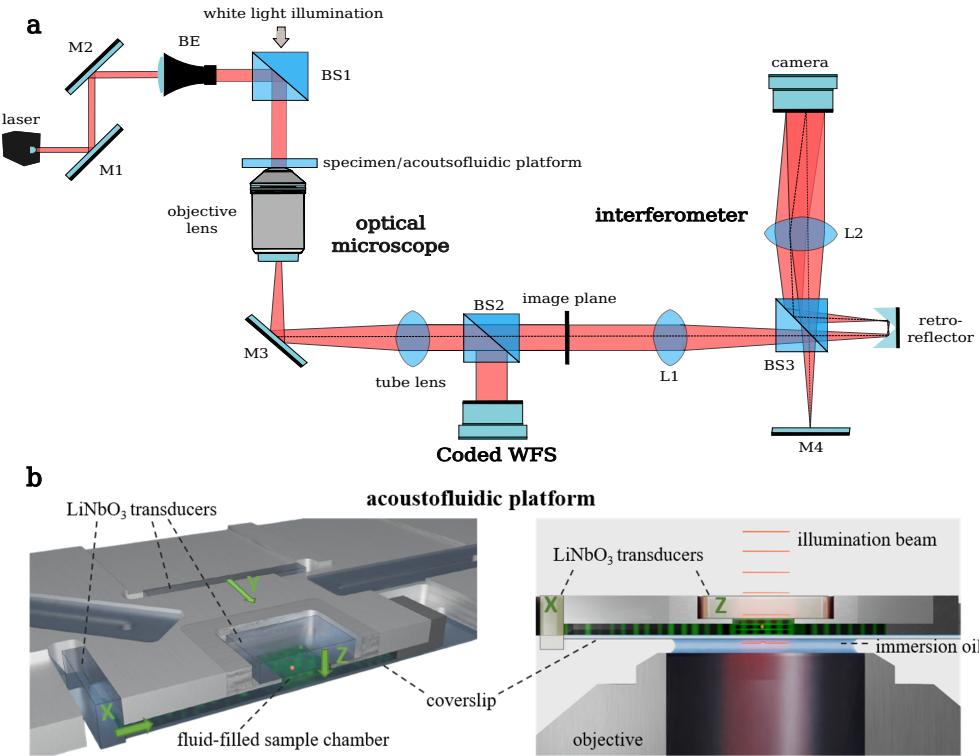


Fig. 1. **a** Experimental setup. The sample is mounted on a commercial inverted microscope (Nikon ECLIPSE Ti2-E) and separately illuminated by two sources: A fibre-coupled diode laser for DHM and a broadband LED for Coded WFS. The scattered light by the specimens is collected using a water immersion objective (1.15 NA, 40x), and two output ports of the microscope are operated sequentially for dynamic measurements. M, mirror; BE, beam expander; BS, beam splitter; L, lens. **b** Acoustofluidic platform. Schematics of the acoustofluidic platform with an angled top view and a cross-section. The propagation direction of the ultrasound from the three orthogonal LiNbO_3 transducers in x-, y- and z-direction are indicated with green arrows.

187 sustained rotations of the sample around an axis orthogonal to the imaging axis, we tune the
 188 relative phase and amplitude of the vertical and one horizontal transducer driven at the same
 189 frequency (5th harmonic frequency around 20 MHz). The rotating sample maintains rotation
 190 periodicity, ensuring comparable successive revolutions. To record the specific background, the
 191 sample is acoustically moved out of the field of view by tuning the respective frequency.

192 3. Experiments and Results

193 3.1. Combined Experimental Setup

194 Our experimental platform is schematically shown in Fig. 1 **b**. The sample chambers and the
 195 acoustic device are mounted on a commercial inverted light microscope (Nikon ECLIPSE Ti2-E).
 196 The QPI measurement systems, DHM and Coded WFS, are installed on two separate observation
 197 ports, labeled image plane in Fig. 1 **a**.

198 In the DHM setting the sample is illuminated by a collimated beam from a fibre coupled

199 diode laser (TOPTICA iBeam smart, $\lambda = 640$ nm). The light scattered by the sample is
200 collected by an objective lens (Nikon CFI Apochromat LWD Lambda-S 40 \times NA 1.15, water
201 immersion) and imaged onto either of the two output ports of the microscope. A home-built
202 common path shearing interferometer based on [6] enables phase-stable DHM measurements
203 with trans-illumination through the acoustofluidic device. We record off-axis interferograms on
204 a camera (mvBlueFOX3-2071) to obtain quantitative information about the amplitude and the
205 phase of the optical field.

206 In the Coded WFS case, we use a broadband white LED (Thorlabs MWWHL4) with a smooth
207 spectrum (color temperature 3000K) for illumination. It is mounted on the microscope after
208 tilting back the original illumination module. We used a lens to focus the LED illumination
209 onto the sample, increasing the illumination throughput. For the operation of the Coded WFS, it
210 is necessary to capture a single reference image. For static samples, we move the observation
211 region, and with the acoustofluidic chip, we acoustically move the sample out of the field of view.
212 Afterwards, we set the target observation region and capture images of static or moving objects.
213 The Coded WFS consists of a random binary phase mask, positioned 1.43 mm in front of a
214 monochromatic sensor (Thorlabs 1501M-USB-TE), replacing the protection cover glass [24] (see
215 Supplement 1, Section 1 for details).

216 In order to create phase videos, we mount the acoustofluidic trapping device on the microscope
217 stage to trap and rotate individual fixated HEK 293 cells.

218 3.2. Validation Experiments

219 To verify the QPI capabilities of our Coded WFS setup against the DHM, a 3D-printed cluster
220 of artificial ‘HeLa cells’ was imaged. The fabrication was performed using a two-photon
221 polymerization lithography system, which enables 3D printing of structures of variable height on
222 top of microscopy slides [35]. Identical models of HeLa cells, manufactured using a polymer
223 with a RI of 1.55 at 633 nm, are placed in different orientations to form a cluster, where the
224 maximum designed height of each cell is $\approx 8.4 \mu\text{m}$. The height map was converted to an OPD
225 map (in μm) by taking the product $h(r)\Delta\eta$, where h is the height and $\Delta\eta = 0.038$ is the RI
226 contrast, considering immersion of the model in Zeiss Immersol 518F (RI = 1.512 at 640 nm),
227 and is referred to as Phantom Design in Fig. 2 a.

228 The fabricated cluster is correspondingly immersed in Immersol 518F prior to imaging. Fig. 2 a
229 shows the retrieved phases of the phantom using DHM and Coded WFS. Figs. 2 b and c compare
230 the cross-sections and the pixel-wise absolute differences, respectively, of the OPDs retrieved
231 by the two techniques with the designed OPD map of the phantom. The results, in addition to
232 validating Coded WFS, demonstrate that non-planar reference waves can be used for Coded WFS
233 in this setting, providing advantages in system setup.

234 The disagreements between the measured and designed OPDs are explained partially by the
235 limited accuracy of the fabrication process. Fabrication of variable-height structures using this
236 method has been found to deviate from the ideal design maps due to anisotropic shrinkage and
237 varying energy dose of the polymerization beam. The discrepancies can occur due to the shape
238 of the structures and their positions within the printed field of view [35]. Therefore, we also
239 expect variation in the OPD profiles of different cells in the phantom. Nonetheless, printed 3D
240 phantoms replicating complex cells enable the quantitative comparison of different QPI systems
241 and serve as an intermediary step before imaging real dynamic cells.

242 To improve the quality of the acquired OPDs, the following additional steps were taken. In
243 DHM, we evaluate the complex field of the background (in the absence of the specimen), which
244 is subtracted from the complex field of the object, ensuring the removal of tilts and artifacts in
245 the background. For Coded WFS, the background is removed using a fit on the empty area of the
246 phase map. To evaluate phase delay relative to the background (immersion), the average phase
247 delay due to the immersion is subtracted.

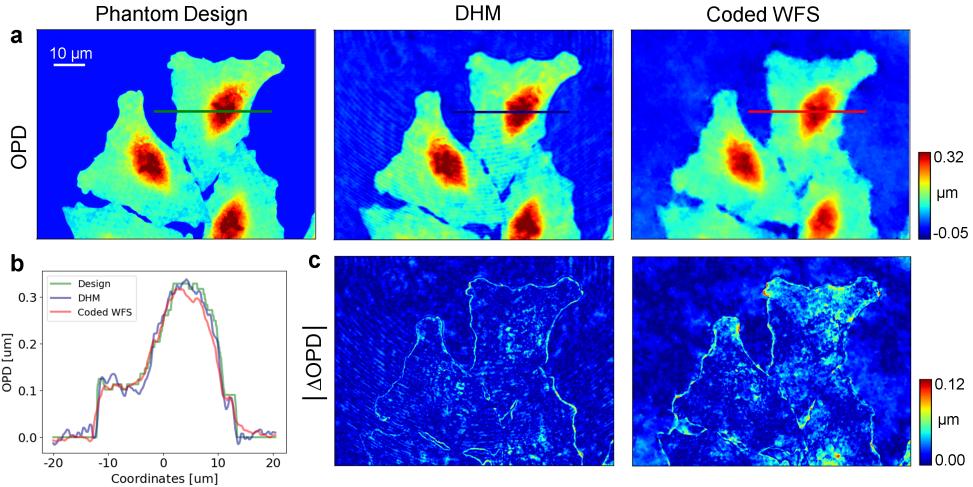


Fig. 2. Performance validation using 3D-printed cluster of artificial HeLa cells.

a Designed OPD map of the HeLa cell cluster and measured OPD maps of the fabricated phantom using DHM and Coded-WFS. **b** Cross-sections of the OPDs in **a** relative to the immersion. **c** The pixel-wise absolute difference $|\Delta\text{OPD}|$ between the designed OPD map and the OPDs retrieved using DHM (left) and Coded-WFS (right).

248 3.3. Quantitative Phase and Amplitude Imaging of Rotating Cells

249 An inherent advantage of both DHM and Coded WFS over standard TIE-based curvature sensing
 250 techniques [9] and other computational microscopy methods is that only a single image of the
 251 specimen is required to recover the complex field at the image plane. For Coded WFS, the
 252 reference image without the specimen is required only once for a specific optical setup, which
 253 therefore does not hinder snapshot wavefront reconstruction.

254 We leverage the snapshot capabilities of DHM and Coded WFS to recover both the amplitudes
 255 and phases of cells at video rates ≈ 30 fps. A single HEK cell is trapped and actuated using
 256 the acoustofluidic trapping device described in Sect. 2.3 such that the cell rotates about an
 257 axis orthogonal to the imaging axis at $\approx 0.39 \text{ rad s}^{-1}$. The exposure times for DHM and
 258 Coded WFS are 1.5 ms and 2 ms, respectively. The combined experimental setup, described
 259 in Sect. 3.1, enables video recording of the rotating cell in identical conditions for DHM and
 260 Coded WFS as it leaves the microscope, including the acoustofluidic chamber in the specimen
 261 plane, entirely unchanged except for the illumination unit. A two-observation port microscope
 262 allows for designated installation points for DHM and Coded WFS, and common-path shearing
 263 interferometry precludes changes within the microscope, ensuring separation of the two QPI
 264 systems. The same HEK cell rotating with the same periodicity [19] is therefore recorded
 265 successfully by each system in succession.

266 Fig. 3 shows agreement between the retrieved intensities and phases of selected frames of
 267 the cell at different angles of rotation using DHM and Coded WFS. Coded WFS retrieves clear
 268 brightfield intensity images while DHM suffers from diffraction artifacts. However, the spatial
 269 resolution of the DHM reconstructions is observed to be qualitatively better than Coded WFS.
 270 The differences are more perceptible in the video (see **Visualization 1**), where a side-by-side
 271 comparison of intensities and phases retrieved by both methods for one complete rotation (485
 272 frames) is provided. Note that in both methods, the measurement rate is only limited by the
 273 camera hardware and not by the methods themselves. Therefore, due to the high temporal

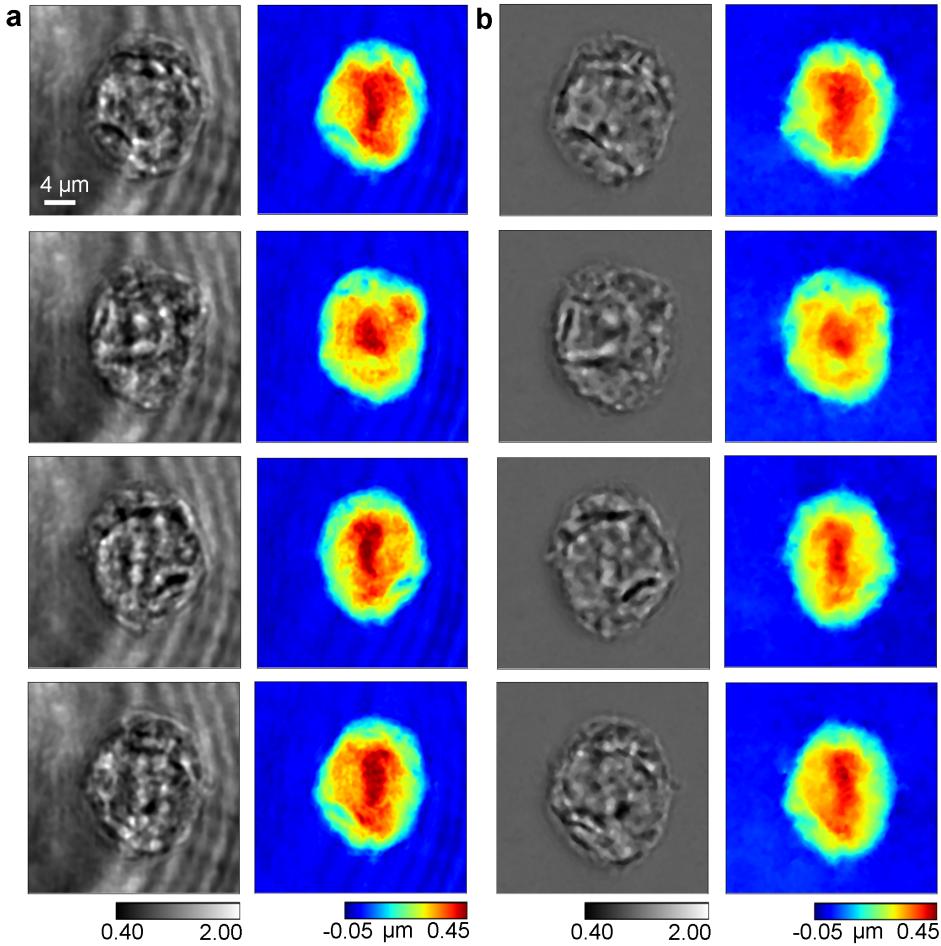


Fig. 3. Video-rate (≈ 30 fps) QPI. Quantitative reconstructions of intensity (left) and OPD (right) of corresponding frames using **a** DHM and **b** Coded WFS of a rotating HEK293 cell. The frames are chosen to reveal informative internal structures of the biological cell. Refer to the video (see **Visualization 1**) for one complete revolution of retrieved DHM and Coded WFS intensities and phases.

resolution of both DHM and Coded WFS, they are highly suitable to study dynamic specimens.

3.4. 3D Refractive Index Estimation

Traditional tomography setups use motorized mirror mounts to illuminate the specimen from different angles [21]. The finite angular extent of illumination leads to the missing cone problem, resulting in undesirable artifacts in the reconstruction. An alternative is to rotate the object by mechanical confinement and a motorized stage [14].

In comparison, the trapping and rotation of unknown objects using the acoustofluidic chamber is both contactless and allows unimpeded access to 360° of viewing angles about one (or more) object axis. However, because the object rotation is variable and unknown *a priori*, achieving an accurate tomographic reconstruction becomes more involved [23, 59].

In this work, our goal is to showcase the simplicity of the hardware setup required for

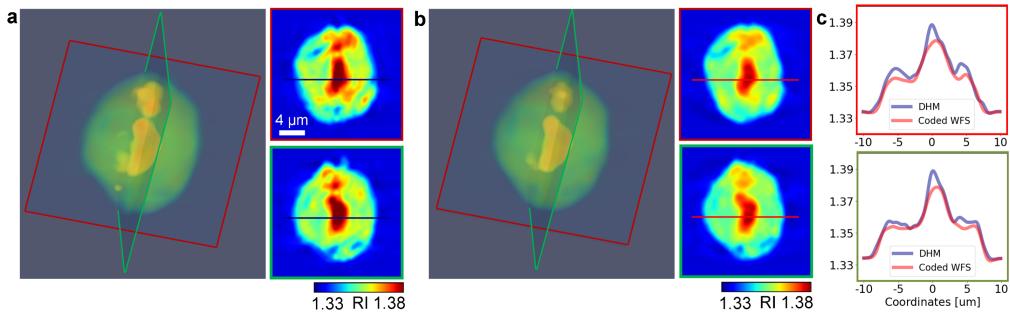


Fig. 4. 3D RI estimation. 3D RI distribution (left) and two slices (right) corresponding to the planes in the 3D visualization of a HEK293 cell estimated using 360°-view OPDs retrieved by **a** DHM and **b** Coded WFS. **c** The red and green-bordered RI line profiles from the similarly bordered RI slices in **a** and **b**.

tomography with a Coded WFS scheme and to verify the estimated RI distribution by comparing it with DHM. For this purpose, we (i) retrieve the OPDs of the rotating HEK293 cell in Sect. 3.3 frame-by-frame, (ii) treat each OPD as a projection of the 3D RI distribution corresponding to a pose (angle of rotation corresponding to a reference), and (iii) make the following approximations for simplicity: First, since the precise pose information is not known, we approximate that the poses are distributed uniformly. Second, we apply a low-pass spatial filter to (a) mitigate specimen jitter and reduce perturbations between adjacent frames and (b) limit the frequency coverage of the data, enabling the use of the Fourier slice theorem (FST) for tomography.

We use the parallel geometry setting in the TIGRE toolbox [60] to simulate orthographic projection and the classical FDK algorithm [61] to estimate the 3D RI distribution of the HEK293 cell, which is shown in the left of Figs. 4 **a** and **b**. To inspect the quantitative RI, we examine two slices corresponding to the planes highlighted in the 3D visualization, where each point on the plane is an RI. The RI profiles corresponding to the lines on these slices are plotted in Fig. 4 **c**, which demonstrate that the retrieved RIs using DHM and Coded WFS are similar up to $|RI| \lesssim 0.01$. The dissimilarity follows from Fig. 3, where the Coded WFS underestimates OPDs compared to DHM. Smaller OPDs translate to smaller RIs. **Visualization 2** showcases the 360° view of the 3D RI distribution retrieved by the two methods. Even though no ground truth is available, the recovered RI range is in agreement with expected values [1].

While the approximations enable quick and easy 3D RI estimation, they reduce the fidelity of the tomographic reconstruction. Analyzing the spectrum of the OPD projections reveals that most of the energy is concentrated in the region where the Fourier diffraction theorem (FDT) arcs are approximately linear (see Supplement 1, Section 2 for details). This allows us to apply FST, provided we filter out the remaining spectrum. However, this approach comes at the cost of losing the ability to retrieve high-frequency structures. Moreover, the pose approximation is only accurate for objects with a spherical cross-section and a homogeneous density distribution. Considering a large frequency support without optimizing the poses first may introduce artifacts into the reconstruction.

312 4. Discussion and Conclusion

313 We have proposed and validated a novel approach to 3D RI tomography for individual cells: the
314 acoustofluidic manipulation of the cell to enable the acquisition of 360 degree object poses in
315 combination with video-rate QPI implemented via Coded WFS. Our approach lends itself to an
316 implementation in an unmodified commercial microscope and, therefore has the potential to be

317 widely applicable.

318 For validation, we have developed a combined experimental setup which enabled QPI using
319 DHM and Coded WFS of phase specimens under identical experimental conditions. We have
320 designed and fabricated a phantom replicating a cluster of cells, which enabled validation of
321 phase accuracy of the Coded WFS method directly with the DHM.

322 The integration of the acoustofluidic chamber in the same setup allows for the levitation and
323 rotation of biological cells. The retrieved intensities and phases of video-rate (≈ 30 fps) data,
324 recorded sequentially for DHM and Coded-WFS, demonstrate good agreement, validating Coded
325 WFS as a suitable video-rate QPI method for our proposed technique. While DHM has been
326 previously applied to 3D RI tomography [14], this is the first time, to our knowledge, that Coded
327 WFS has been tested in this setting.

328 Our reported results rely on several restrictions and simplifying assumptions, namely i) a
329 weak scattering regime, ii) a roughly spherical shape of the specimen to assume a uniform
330 rotation over time, and iii) a limited maximum scattering angle caused by the specimen. Future
331 work will address these challenges and allow for higher 3D spatial resolutions. To address i),
332 multi-slice techniques in the forward model as e.g. in [55] would allow for stronger scattering to
333 be successfully treated. A more complex specimen shape will cause more irregular rotations since
334 the relevant forces apply varying amounts of torque at different object points. Pose estimation
335 of the specimen may solve this problem and address issue ii). Finally, improved reconstruction
336 algorithms can more fully account for the optical diffraction tomography geometry in the Fourier
337 domain, contributing to improve issue iii).

338 Summarizing, we believe that our proposed approach carries significant potential for a
339 simplified 3D RI tomography approach that may be well suited for a wide application.

340 **Funding.** This work was funded in part by the Austrian Science Fund (FWF) SFB 10.55776/F68
341 *Tomography Across the Scales*, project F6806-N36 Inverse Problems in Imaging of Trapped Particles and the
342 German Research Foundation under DFG FOR 5336 *Learning to Sense* project IH 114/2-1. For open access
343 purposes, the authors have applied a CC BY public copyright license to any author-accepted manuscript
344 version arising from this submission.

345 **Acknowledgement.** Monika Ritsch-Marte and Ivo Ihrke would like to thank the Institute for Pure and
346 Applied Mathematics (IPAM) at UCLA, where their current collaboration was initiated during the long
347 program "Computational Microscopy". We thank Judith Hagenbuchner and Michael Auerlechner (Medical
348 University Innsbruck) for providing us with the fixated HEK 293 cells. We thank Armin Sailer (Institute for
349 Experimental Physics, University of Innsbruck) for CNC-manufacturing the high-NA chip according to our
350 design.

351 **Disclosures.** The authors declare no conflicts of interest.

352 **Data availability.** Data underlying the results presented in this paper are not publicly available at this time
353 but may be obtained from the authors upon reasonable request.

354 **Supplemental document.** See Supplement 1 for supporting content.

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