Doubling the far-field resolution in mid-infrared microscopy

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Abstract: The spatial resolution in far-field mid-infrared (λ>2.5 µm) microscopy and microspectroscopy remains limited with the full-width at half maximum of the point-spread function ca. λ/1.3; a value that is very poor in comparison to that commonly accessible with visible and near-infrared optics. Hereafter, it is demonstrated however that polymer beads that are centre-to-centre spaced by λ/2.6 can be resolved in the mid-infrared. The more than 2-fold improvement in resolution in the far-field is achieved by exploiting a newly constructed scanning microscope built around a mid-infrared optical parametric oscillator and a central solid-immersion lens, and by enforcing the linear polarization unidirectional resolution enhancement with a novel and robust specimen error minimization based on a particle swarm optimization. The method is demonstrated with specimens immersed in air and in water, and its robustness shown by the analysis of dense and complex self-assembled bead islands.

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References and Links


1. Introduction

Table-top microscopy at mid-infrared (mid-IR) wavelengths ($\lambda>2.5$ $\mu$m) is performed with tunable or broadband laser sources [1–4]. Like synchrotron-based mid-IR microscopes [5, 6], these collimated coherent sources support diffraction-limited imaging in the far-field. However, at the current state-of-the-art, mid-IR point-spread functions (PSF) exhibit full-width at half maximum (FWHM) that are not narrower than ca. $\lambda/1.3$ with the best reflective objectives as these exhibit numerical aperture (NA) well below 1 [7, 8], and far-field mid-IR optics and methods clearly underperform compared to commonly accessible visible and near-infrared optics. Contrasts arising from the vibrationally-resonant absorption in the mid-IR spectrum are routinely exploited to image biological specimens [9–11] and there is thus a strong need for improving the mid-IR resolution for the analysis of many relevant sub-cellular features.

Central and aplanatic solid immersion lenses (c-SIL and a-SIL) are respectively hemispheres and hyper-hemispheres that facilitate high NA focusing and/or enhance collection of scattered light [12, 13]. Aplanatic SILs are attractive to improve the spatial resolution in back-side near-infrared imaging of integrated circuits (ICs) [14], and within the silicon media (refractive index $n = 3.4$) the resolution of metallic lines edge-to-edge spaced by ca. 100 nm ($\lambda/3n$) has been recently demonstrated with finely tuned confocal pinhole filtering [15]. With respect to mid-IR imaging and especially for organic specimens and biological tissues, central-SIL is preferable to a-SIL as c-SIL should not suffer from chromatic aberration and should show higher tolerance to variations in thickness, curvature, and angle [16]. c-SIL should also afford larger fields of view whilst a priori keeping the angular NA at 1 to achieve diffraction-limited far-field imaging in various media. In a first attempt, a wide-field mid-IR microscope was built around a ZnSe c-SIL [17]. Although, the microscope demonstrated a resolution improvement of ca. 40% with the c-SIL, it failed to achieve diffraction-limited imaging, with polystyrene (PS) beads of diameter 2.5 $\mu$m measured as features of ca. 3.2 $\mu$m FWHM ($\lambda/1.05$, with $\lambda = 3.4$ $\mu$m). A CaF$_2$ c-SIL was later used with a substrate of same materials to show a similar resolution enhancement. Importantly the expected reduced chromatic aberration and dispersion within the context of Fourier transform IR micro-spectroscopy were demonstrated [18]. Thus, within the framework and experimental peculiarities of mid-IR imaging, the full resolution potential of c-SIL has not been experimentally achieved.

At high NA, the focal spot is known to be elliptical when the polarization is linear [19, 20] and it has been more recently discussed that combining pairs of images recorded at crossed linear polarizations in the near-infrared with an a-SIL is required to achieve a uniform spatial resolution of ICs along both image axis [21, 22]. As a proof-of-concept, a uniaxial FWHM of ca. $\lambda/2.6n$ in silicon immersion was experimentally demonstrated with linear polarization and angular spectrum control at the silicon-dioxide/silicon interface [23]. A first method was introduced to reconstruct the specimen by simultaneously optimising pairs of cross-polarized images [21, 22]. Sets of parallel lines apart by ca. $\lambda/1.7n$ centre-to-centre were effectively retrieved, setting the effective resolution along both axes to ca. 224 nm in the silicon. Although this is yet to be demonstrated, if a c-SIL mid-IR microscope is made to work at the diffraction limit, a similar unidirectional resolution enhancement should be observed and could a priori be exploited to image organic specimens at high resolution in relevant media. One should also expect the need for a robust processing method to accommodate the typically difficult mid-IR PSFs resulting from the requirement for focussing with reflective objectives and from the large difference in refractive index between the c-SIL and the specimens.

In this manuscript, we report the construction of c-SIL based mid-IR microscope that effectively performs at the diffraction limit. Polystyrene (PS) beads of diameter 1 $\mu$m are measured with a FWHM of 1.5 $\mu$m and the microscope optically resolves in water PS beads that are 1.7 $\mu$m apart, when using a wavelength of 3.4 $\mu$m. To take full advantage of the polarization diversity, naturally stemming from the high NA achieved here, and to produce
super-resolution representations of the specimens based on the pairs of crossed-polarized images, a robust algorithm has been developed. The new algorithm is made reliable by exploiting experimentally retrieved effective PSFs and is found to handle properly the unusually strong PSF side-lobes arising from the large difference in refractive index in the imaging plane and from the central obscuration of the reflective objective. The processing involves the optimization of a specimen reconstruction by a particle swarm optimization (PSO), avoiding thus the difficulty of performing deconvolutions of the data at or beyond the diffraction limit. Overall, our experiments demonstrate mid-IR imaging of an organic specimen with a more than two-fold improvement in spatial resolution when compared to a state-of-the-art mid-IR confocal microscope [7].

2. Experimental methods

The linear-polarized mid-IR beam was generated from a synchronously-pumped optical parametric oscillator (SP-OPO) based on a rapidly tunable periodically-poled lithium neobate crystal and pumped with a fibre laser at \( \lambda = 1030 \) nm (2.9 W) with a repetition rate of 41 MHz and a pulse duration ca. 40 ps (Multitel, Belgium). The mid-IR idler was tunable from 2.2 to 4.1 \( \mu \text{m} \) and the average signal power was ca. 300 mW. For these experiments, the wavelength was kept at 3.4 \( \mu \text{m} \) and the power reduced to ca. 1 mW at the objective entrance. Key to the results presented below are the good OPO mid-IR beam profile, high power, and excellent beam pointing stability that together ensure facile beam expansion and spatial filtering for the generation of well-defined PSFs, as well as day-to-day preservation of the optical alignment.

The home-made microscope shown in Fig. 1 exploits a long working distance reflective objective (OBJ) of NA 0.4 (25 \( \times \), 16.7% central obscuration, 14.5 mm working distance, 6.3 mm entrance aperture) compatible with the c-SIL dimensions or a stand-alone reflective objective of NA 0.65 (74 \( \times \), 13.3% central obscuration, 1 mm working distance, 3.35 mm entrance aperture, Edmund Optics). The objectives were used to focus the mid-IR beam on the front or backside of a Si substrate and collect the back-scattered mid-IR, which was reflected by a pellicle beam splitter 55:45 (BS, BP145B4 Thorlabs) and focussed into a nitrogen-cooled mercury cadmium telluride (MCT1) detector (Hamamatsu), without confocal pinhole.

A reference MCT (MCT2) was used to generate a reference signal, by which the data were normalized. The sample (SPL) and reference signals were extracted by lock-in amplifiers (Signal Recovery) locked onto the frequency (500 Hz) of a mechanical chopper wheel (CH) installed in the incident beam path. A piezoelectric scanner (Physics Instruments) of maximum range 100 \( \times \) 100 \( \mu \text{m}^2 \) was used to raster scan the substrate, which effectively limited the scan range to a maximum of 29.8 \( \times \) 29.8 \( \mu \text{m}^2 \) when focusing through the silicon c-SIL, as a result of the c-SIL-induced demagnification [12]. The latter range was independently verified by imaging a calibration specimen made of Au stripes milled on a glass slide with a focussed ion beam. A GPIB-interfaced DAC (National Instruments) was used to control the scanner and record the lock-in outputs with a LABVIEW code. Typically, the dwell time is of several milliseconds per point and is limited by the chopper frequency.

The c-SIL consists of a 5.0 mm radius Si hemisphere truncated to a thickness of 4.0 mm (ISP Optics) and used with a double-side polished Si substrate (University Wafer) of 1.0 mm thickness (SPL), on which the polystyrene beads (nominal diameter 1 \( \mu \text{m} \), Polyscience) were drop casted after dilution in distilled water. As demonstrated in section 3 by the experimental realization of far-field PSFs of FWHM ca. \( \lambda/2 \) in air, the combination of the reflective objective (NA 0.4) and c-SIL achieves an overall angular aperture of ca. 1, and the specimen were imaged with the beads in air or in distilled water. It is noted that any rays beyond a NA of ca. 0.3 entering the reflective objective are unused when imaging in the c-SIL central plane (depth 5.0 mm) because of the optically unmatched interface between SIL and silicon substrate backside at a depth of 4.0 mm, where these rays undergo total internal reflection. The imaging of the specimen is thus achieved in the far-field as confirmed below.
To facilitate the sequential imaging between the cross-polarized beams, whilst avoiding mechanical perturbation of the optical alignment, the SP-OPO output was split into two paths and recombined with two 55:45 pellicle beam splitters (BS). In one of the paths a wavelength tunable half-wave plate (1/2WP, Alphalas) complemented by a linear polarizer (LP, crossed with the polarization of the second path) was introduced. A shutter (S) was used to rapidly switch the imaging between the two polarizations without any alteration of the PSFs.

Fig. 1. Mid-IR scanning microscope scheme with c-SIL. M: mirror; F1 and F2: near-IR filters; L1 and L2: beam expander/collimation; CH: mechanical chopper wheel (500 Hz); LP: linear polarizer; OBJ: reflective objective; BS: pellicle beam splitter; S: beam shutter (blocking the vertically polarized beam path as shown or used to block the horizontally polarized beam path); TP: thin plate; MCT1 and MCT2: mid-IR MCT detectors; SPL: sample with specimen side immersed in air or water; 1/2WP: wavelength tunable half-wave plate; SIL: silicon solid-immersion lens. The vertical and horizontal polarizations (versus optical bench surface) are marked in green. A close-up on the 4 mm thick SIL and 1 mm thick silicon SPL substrate is also shown, highlighting the optically unmatched interface between SIL and SPL, as well as the imaging media at the SIL central plane.

3. Results and discussion

With the c-SIL and objective of NA 0.4, single PS beads are imaged at the air/silicon interface as asymmetric depressions with FWHMs of 1.8 ± 0.1 and 1.6 ± 0.1 µm respectively along the axis matching with and normal to the direction of polarization [see Figs. 2(a) and 2(b)]. As shown in Fig. 3, the strong asymmetry is not observed in the absence of SIL and the reflective objectives of NA 0.4 and 0.65 alone reveal circular images of FWHM 2.8 ± 0.1 and 4.8 ± 0.1 µm, respectively (i.e., ca. 10% wider than \(\lambda/2\)NA). The asymmetry of the spot with c-SIL is generated by the amplification of longitudinal field in the imaging plane resulting from the large refractive index difference between SIL and imaging media [24]. Additionally, the longitudinal depth profile recorded across a single bead with the SIL confirms the far-field nature of the images [see inset in Fig. 2(b)] and, in keeping with the FWHMs measured in the specimen plane being ca. \(\lambda/2\), the optical NA of the combination of c-SIL and NA 0.4 objective is found to be approximately 1. The intense side-lobes observed with the SIL are attributed to the reflective objective central obscuration as well as to the large index difference in the central plane. The side-lobes are indeed reduced upon imaging at the water/silicon interface [see Figs. 2(c) and 2(d)]. The FWHMs are also slightly reduced with water immersion (1.7 ± 0.1 and 1.5 ± 0.1 µm respectively along the axis matching with and normal to the direction of polarization).
Fig. 2. Images and line profiles of single 1 µm PS beads recorded at the air/silicon interface with the linear polarization set along the X axis (a) and Y axis (b), with X and Y defined as the horizontal and vertical directions in the images. Same at the water/silicon interface (c,d). For all data the background is normalized to unity. Scale bars 2.0 µm, color scales in arbitrary units and identical for (a) and (b). Same for (c) and (d). The double-tipped white arrows mark the direction of polarization. The inset in (b) shows the axial image where Z is the longitudinal (depth) axis, with the scale bar at 5 µm.

Fig. 3. Images of 1 µm PS bead deposited on a glass slide and recorded (a) with the reflective objective of NA 0.65 and (b) NA 0.4 in reflection mode without SIL. (c) and (d) are line profiles extracted from the images (a) and (b), respectively. The beads are imaged as circular depressions with FWHM 2.8 ± 0.1 and 4.8 ± 0.1 µm (i.e., ca. 10% wider than λ/2NA).

Linear polarization achieves narrower point-spread functions in the direction normal to the polarization axis and thus the combination of images recorded with crossed linear polarizations is necessary to achieve the highest resolution along both imaging axes. Images recorded with a single polarization resolve indeed linear chains of closely spaced beads better when these are aligned normal to the direction of polarization. This is indeed observed in Figs. 4(a) and 4(b), where two beads roughly aligned along the Y axis and center-to-center apart by 1.9 µm are clearly resolved with X polarization and not with Y polarization. Depending on how the beads are arranged and spaced, the side-lobes may limit the ability to
resolve the specimen, as shown in Figs. 4(c) and 4(d) where beads apart by roughly 2.5 µm and approximatively aligned along the Y axis (see dashed circle) are better resolved with Y polarization as side-lobe build-up occurs with X polarization. The side-lobes are reduced at the water/silicon interface and beads apart by ca. 1.7 µm (λ/2) or more are then systematically resolved in the images where the polarization is normal to the bead alignment [see Figs. 4(e)-4(h)]. The effective optical resolution is thus λ/2 and far beyond that achieved with the high NA reflective objectives alone in confocal microscopy (ca. λ/1.3) [7] and earlier wide-field demonstration with SIL in the mid-IR (λ/1.05) [16].

The multiplicity of images of a same sample area provided by the X and Y polarizations also allows analyzing the specimen beyond the diffraction limit, and the reconstruction of optically unresolved islands of PS beads is indeed demonstrated below. The reconstruction involves first the generation of two effective PSFs defining how the PS beads are imaged with each polarization, and second the reconstruction of a specimen that will be optimized so that its convolutions with the effective PSFs show the least error simultaneously with both X and Y polarization experimental images. For each polarization, the effective PSFs were deconvolved from pairs of X and Y polarization images of single and isolated PS beads. These deconvolutions were performed with Matlab® by using standard discrete Fourier transform functions, representing the single bead as a single point scatterer, using a low-pass flat apodization in the Fourier space, and treating the imaging as incoherent [25]. It is worth noting that a thorough description of SIL microscopy may involve more comprehensive models for generating the PSFs [26, 27], however the effective PSFs as defined above proved efficient here and advantageously accounted for any deviation of the experimental PSFs from ideality as well as for the specificities of the PS bead scattering. The specimens are then reconstructed from a collection of n point scatterers, defined by two spatial coordinates in the sample plane. A third coordinate is used to scale the magnitude of the scattering for each bead. The latter value is expected to be uniform for all beads within the frame of one image but may slightly differ from unity when the effective PSFs are computed from data recorded away from the region of interest, as a result of SIL aberrations. With only (2 + 1)n parameters to optimize, the reconstruction naturally exploits the sparsity of the specimen in the sense defined by [28] and [29] with the number of parameters largely less than twice the number of...
The reconstruction is also equivalent to a weighted localization approach although it is here robust enough to handle optically unresolved scatterers. The error minimization was realized using a PSO algorithm also implemented in Matlab® and modified from the code introduced in [30]. The algorithm is presented in Fig. 5 and it is further discussed in Appendix. Typically swarms of m = 30 particles were used and several hundreds of iterations were required per optimization from random initial coordinates.

![Graphical representation of the adapted PSO iterative algorithm used for PS bead specimen reconstruction from pairs of X and Y polarization images.](image)

The efficiency, validity and reliance on X and Y polarizations of the approach are first demonstrated from the analysis of the pair of experimental images presented in Figs. 6(a) and 6(b) and recorded at the air/silicon interface. To simplify the discussion, dense islands at bottom and top left corners have been removed, and the presented data suggest a simple bead configuration with two single and two unresolved beads. A total of 4 beads is thus expected from observation of the experimental data. The optimization was performed for 8 sets of beads with the number of beads varying from 2 to 9. The spatial coordinates of the beads were left free across the entire frame (256 × 256 pixels, pixel size ca. 116 nm) and the scattering magnitude kept between 0 and 2, so that the beads that are not required can a priori fade away into the background. The fully optimized specimen reconstructions for the sets with 4 (blue) and 9 (yellow) beads are shown in Fig. 6(c), with each bead represented by a disk centered on the optimized coordinates and of diameter linearly scaling with the optimized magnitude. With the set of 9 beads, only 4 beads retain a substantial magnitude (superposed to the 4 blue beads) and the other 5 have a magnitude below 0.2 and positioned in the background. The reconstructed images for the 9 beads set are shown in Figs. 6(d) and 6(e) and agree with the experimental data. When considering the plot showing the error as a function of the number of beads in the sets [Fig. 6(f)], a clear threshold is also seen at 4 beads. The PSO-based analysis thus strongly agrees with expectation of 4 beads and cannot provide a satisfactory reconstruction if the number of beads is not ≥4. Moreover, in all these cases only 4 non-negligible beads are found and systematically positioned at same 4 locations in the frame with variation ≤2 pixels. Any bead beyond 4 is used by the algorithm to minimize deviation from background flatness in the images, which also explains the progressively diminishing error when the number of bead is increased beyond 4.
Fig. 6. Images recorded with X and Y polarizations at the air/silicon interface showing two isolated and two unresolved PS beads and with incomplete features masked for simplicity (a,b). Reconstructed specimen (9 beads, yellow circles) and reconstructed X and Y polarizations images, with X and Y polarizations simultaneously (c,d,e). The specimen reconstruction optimized with a 4 beads set is also shown in blue in (c). Reconstruction error as a function of the number of beads tested with the PSO algorithm (f). The reconstruction error with scattering magnitude limited to 0.7 is shown in inset. Reconstructed X polarization image of a fully optimized 9 bead specimen with the optimization completed with Y polarization only (g). Reconstructed Y polarization image of a fully optimized 9 bead specimen with the optimization completed with X polarization only (h). Specimen reconstruction corresponding to the 2-polarization (yellow, Error = 30.06), Y-polarization (brown, Error = 31.78), and X-polarization (purple, Error = 31.25) optimization for 9 beads (i). Scale bars 5 µm. All images and image reconstructions are at same intensity scale, given in arbitrary units.

These results are further validated by another optimization performed with the scattering magnitude kept above 0.7, in which case the smallest reconstruction error is also for 4 beads [see inset Fig. 6(f)]. Interestingly, a posteriori enforcing a super-resolving sparsity regularization such as the one introduced in [28], which would involve here the elimination of all the weaker beads, will thus systematically lead to only 4 beads surviving and to a unique satisfactory 4 bead-based reconstruction of the specimen from the pair of X and Y polarization images, in full agreement with what is expected from the observation of the data.

The PSO outcome is expected to crucially depend on the simultaneous exploitation of both the X and Y polarization experimental images and poorer reconstructions should be obtained when a single polarization is used, with the worst results to be found when the PSF narrow axis is not matched to the specimen orientation [Figs. 6(g)-6(i)]. In the example discussed in Fig. 6, the exclusive use of X polarization with the PSO is thus expected to
outperform the exclusive use of Y polarization, given that the central pairs of beads is roughly normal to the X axis. Considering the 9 beads set, it is found that when the optimization is done solely using Y polarization, the total error remains high, with the reconstruction of the X polarization image appearing indeed to be very poor [Fig. 6(g)] with respect to the original reconstruction [Fig. 6(d)]. The optimized specimen reveals then 3 non-negligible beads in the marked area where only 2 should be found [Fig. 6(i), brown circles]. Remarkably, when the optimization is done solely using X polarization, the optimized specimen is more alike the one recovered with simultaneously both polarizations and the reconstructed Y polarization image is also acceptable [Fig. 6(h)], although still of lesser quality than when both polarizations are simultaneously optimized [Fig. 6(e)].

That the optimization of the two optically unresolved beads is better with X polarization alone than with Y alone confirms the unidirectional enhancement of resolution expected from the polarization-dependent asymmetry in the PSF, and it can be generalized that the exploitation of both linear polarizations is required for specimen defined along both dimensions. Beyond the polarization-diverse, complementary and uniaxial improvement in spatial resolution, the simultaneous exploitation of the two images also enforces redundancy and lessens the impact of PSF inaccuracies and image noise, as seen in our experiments with the overall error remaining less when both polarizations are used simultaneously.

The method is further tested by analyzing the substantially more complex images of a dense island obtained at the water/silicon interface [see Figs. 7(a) and 7(b) for X and Y polarization images and see Fig. 7(c) for a combined polarization image]. Although the area (i) can be readily assessed optically and optimized with 7 beads, it is not possible to a priori propose a structure for the area (ii). However, by exploiting the pair of X and Y polarization images, the PSO reveals a clear minimum in the reconstruction error with the area (ii) best described as a dense island of 9 beads [Fig. 7(d)]. The optimized specimen leads to good reconstructed images shown in Figs. 7(e) and 7(f), and to a hexagonal bead arrangement with a center-to-center spacing of ca. 1.3 µm shown in Fig. 7(g). Remarkably, when the same sample is imaged by visible microscopy, closed-packed islands with hexagonal symmetry and bead center-to-center spacing of 1.30 ± 0.5 µm are also measured [see Fig. 7(h)], fully supporting the mid-IR super-resolved reconstruction.

Exploiting the multiplicity of images with crossed linear polarizations allows thus a substantial improvement in the optical resolution of far-field mid-IR microscopy (from PSF FWHMs of ca. $\lambda/1.3$ to PSF FWHMs of $\lambda/2.3$ with demonstrated resolution of $\lambda/2$) and is also seen to support further robust optimization strategy to analyse the specimens beyond the diffraction limit (down to resolving $\lambda/2.6$). Overall, the far-field mid-IR resolution is thus more than doubled. It remains interesting to benchmark our results with the sparsity-regularized non-quadratic optimization recently discussed by others, where an over-complete dictionary of stationary objects was setup to reconstruct a specimen of parallel metal lines etched on silicon and imaged in the near-IR [21, 22]. The PSF in that case was theoretical. It was seen that optically unresolved lines with a pitch of 224 nm were relatively well reconstructed, and that when the pitch was 256 nm the lines are just optically distinguished, so that the optimization improves the resolution in silicon to ca. $\lambda/1.7n$. With the drop-casted and random self-assembled PS beads, the PSO used here achieves thus a relatively higher resolution in the mid-IR with the organic specimens of interest in air and water ($\lambda/2.6$). A benefit of the PSO approach lies as well in the absence of a regularization coefficient which must be otherwise empirically adjusted case by case.
Fig. 7. Images recorded with X and Y polarizations at the water/silicon interface, and combined (averaged) experimental X and Y polarization images of same data (a,b,c). The frames show a readily interpreted area (i) including 7 beads (see inset of c) and a dense area (ii) where the bead arrangement cannot be a priori proposed. Reconstruction error as a function of the number of beads tested with the PSO algorithm for the area (ii) (d). Reconstructed X and Y polarization images (e,f). Optimized structure for area (ii) (5 best optimizations highlighting the variation between tests) (g). Scale bars are 5 µm, and all images are shown with same color scale given in arbitrary units. Double tipped arrow shows the direction of polarization. Wide-field visible white light microscopy (NA 0.75, 100 ×, reflection) image of PS beads (1 µm) drop-cast on silicon (h). In-plane calibration was established using a copper grid (40 µm). Scale bar: 10 µm. The beads are not systematically observed to be close-packed (iii and iv). Self-assembled and close-packed arrangements (v, vi) are however also found. The center-to-center distance between adjacent beads is then 1.30 ± 0.05 µm.

The microscope described here demonstrates diffraction-limited imaging in the mid-IR and future developments will aim at achieving far-field chemical imaging of cellular and sub-cellular features in biological specimens and tissues at high spatial resolution and high speed, specifically taking advantage of the wavelength dependant contrasts arising from resonant mid-IR absorption [9–11]. The super-resolution here further relies on a PSO algorithm that is to be compared to other approaches recently discussed in the literature where the sparsity of the specimens was exploited, for example with coherent diffraction imaging [21, 22] and spatial light modulation microscopy [31]. Remarkably, as argued in [28] and [31], it is nearly always possible to describe a specimen in a sparse basis, and within the context of biological imaging, sparsity-regulated sampling and analysis was successfully established to reduce noise and photo-bleaching in fluorescence microscopy [32], to enhance 2D and 3D super-resolution localization microscopy [33–35], and to exploit hyperspectral information [36]. The latter is found to be highly relevant to support future development of super-resolution in
the mid-IR, and should be considered when processing biological specimens here in the mid-
IR.

It is clear that challenges lie ahead when considering the processing of complex mid-IR
scenes. Yet, the PSO processing here does not assume all objects identical and it will be
readily modified to account for multiple types of object; for example, by associating different
effective PSFs or by defining different object types with different sets of coordinates, or
combinations of both. Observation of the specimens at different wavelengths and with
additional PSF patterns is also expected to reinforce redundancy and thus fidelity of the
reconstructions. The prospect of further developing our mid-IR approach is thus high and on a
par with others developed in the visible and near-infrared.

4. Conclusion

We have experimentally demonstrated a scanning mid-IR microscope and a processing
method capable of resolving features at $\lambda/2.6$, a two-fold enhancement with respect to the
state-of-the-art in mid-IR confocal microscopy [7]. The far-field development relies on an
OPO as mid-IR source and on a c-SIL for enhancing the total angular NA at the specimen in
air and water to 1. A method exploiting crossed linear polarization images and specimens
reconstruction based on PSO has also been developed and demonstrated to resolve dense
islands of particles beyond the diffraction limit. Recent theoretical works exploiting PSF
narrowing by pupil engineering with radial [37] and azimuthal polarizations [38–40] can now
be usefully translated to mid-IR imaging, and further enhancement in performances are thus
to be expected. With the support of increasingly reliable reconstruction methods and
experimental PSF engineering approaches, mid-IR imaging with c-SIL is moving towards the
observation of complex organic and biological specimens at high resolution.

Appendix: Particle swarm optimization (PSO)

PSO is an optimization algorithm developed in 1995 and inspired by bird flocks and fish
swarms [41]. In the present manuscript, the PSO algorithm is largely based on the script
presented in [30] with minor adaptations made by the authors to account for the PS bead
specimen reconstruction. Briefly, the PSO is adapted here to optimize the reconstruction of
the specimen to best match with the polarization-diverse experimental images. This is
achieved by minimizing an error criterion $\Sigma$ written as the sum of the $I_2$ norm of
reconstructed images ($I_h$ and $I_v$ for horizontal and vertical polarizations) versus measured
images ($I_{exp,h}$ and $I_{exp,v}$). In the specimen reconstruction $s_j$ each bead is defined by the
position of a single pixel in the frame, and the pairs of reconstructed images are computed by
convolution of $s_j$ with the effective point spread functions $PSF_h$ and $PSF_v$.

The swarm is made of $m$ particles moving in a $(2+1)n$ dimensional space, where $n$ is
the number of PS beads used for a given test. Two coordinates are used to position a bead
within the image plane and one coordinate is used to weight the bead scattering magnitude
(size) versus a bead of reference. In PSO [30], the optimization involves velocities $v_{j,i}$
associated to the $j^{th}$ particle coordinates $r_{j,i}$ ($i=1,(2+1)n$) to stimulate the particles
motion bounded within a predefined range of values. For example, a coordinate associated
with the position of a bead in the frame is bounded between 1 and 256 for a 256×256 pixel
square image. Velocities are also bounded to avoid too large leaps in a single iteration and
typically set at ca. 20% of the corresponding coordinate range.

At each iteration, $\Sigma$ is evaluated for each particle and individual best coordinates $p_{best,j}$
and global best coordinates $g_{best}$ are memorized, where best is taken as corresponding to
the lowest $\sum$ with respect to all the past iterations. New velocities $v'_{j,i}$ and coordinates $r'_{j,i}$ are then computed forcing each particle to be simultaneously attracted towards their personal best and towards the global best coordinates:

$$v'_{j,i} = \omega \times v_{j,i} + c_1 \epsilon_1 \times (p_{best,j} - r_{j,i}) + c_2 \epsilon_2 \times (g_{best} - r_{j,i})$$

$$r'_{j,i} = r_{j,i} + v'_{j,i}$$

(1)

In the above, $\omega$, $c_1$, and $c_2$ are coefficients defining respectively the particles inertia, and attraction strength towards personal best and global best, with values here set at 0.5, 0.5 and 0.25. $\epsilon_1$, and $\epsilon_2$ are uniformly distributed random numbers between 0 and 1. The algorithm is sketched in Fig. 5 and is terminated when $\sum$ remains unchanged with respect to a preset variation or when a preset number of iterations is completed. The last $g_{best}$ is taken as the optimum set of coordinates. The algorithm is typically initialized with random values and each optimization is repeated to verify that the global minimum is found. The algorithm was executed in Matlab® using standard functions.

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