Microscopy with undetected photons in the mid-infrared region

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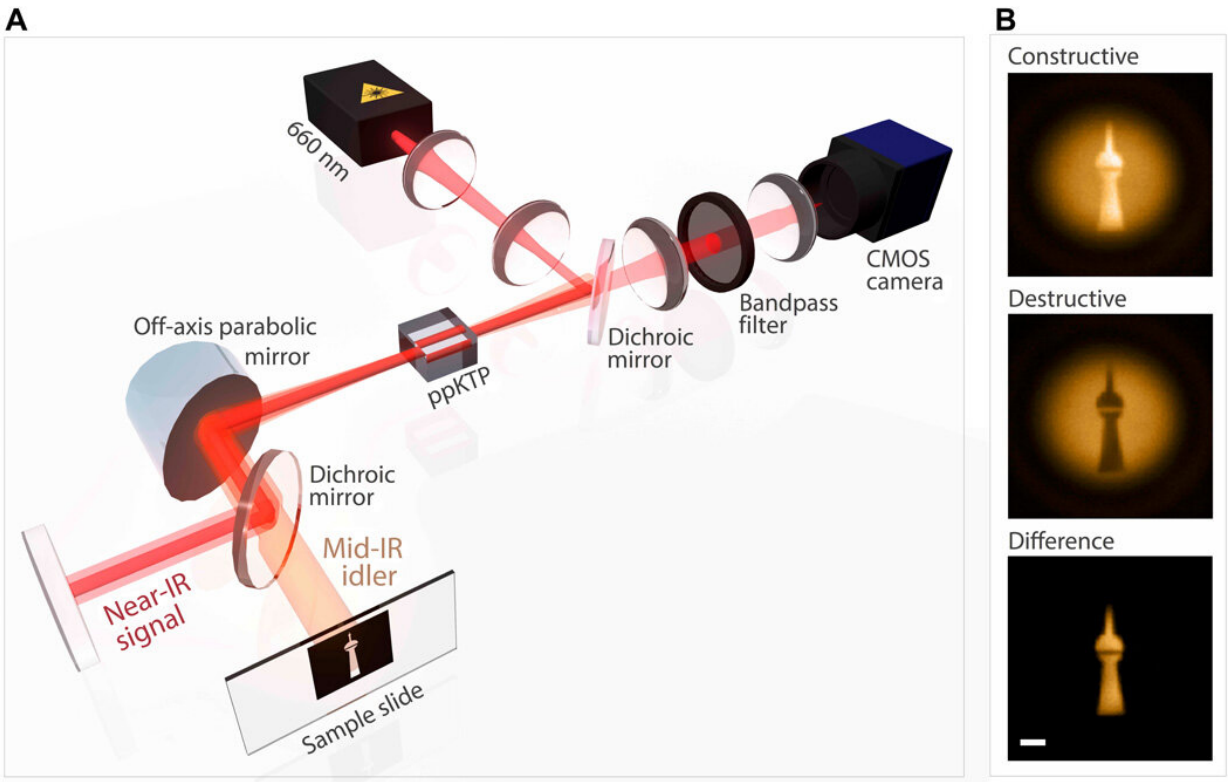


Experimental setup and interference images. (A) A 660-nm continuous-wave laser pumps a highly nondegenerate SPDC process. The signal and idler fields generated on the first pass of the 2-mm ppKTP crystal are split via a dichroic mirror (DM). The sample to be imaged is placed in the Fourier plane of the idler, which coincides with its end mirror. Both the idler and signal fields are reflected back, recombined, and backpropagated into the nonlinear crystal with the coherent pump field. The resulting signal field is imaged on a CMOS camera. (B) Constructive, destructive, and difference interference images of the signal for a cardboard cutout probed by the mid-IR idler. Scale bar, 2 mm. Credit:

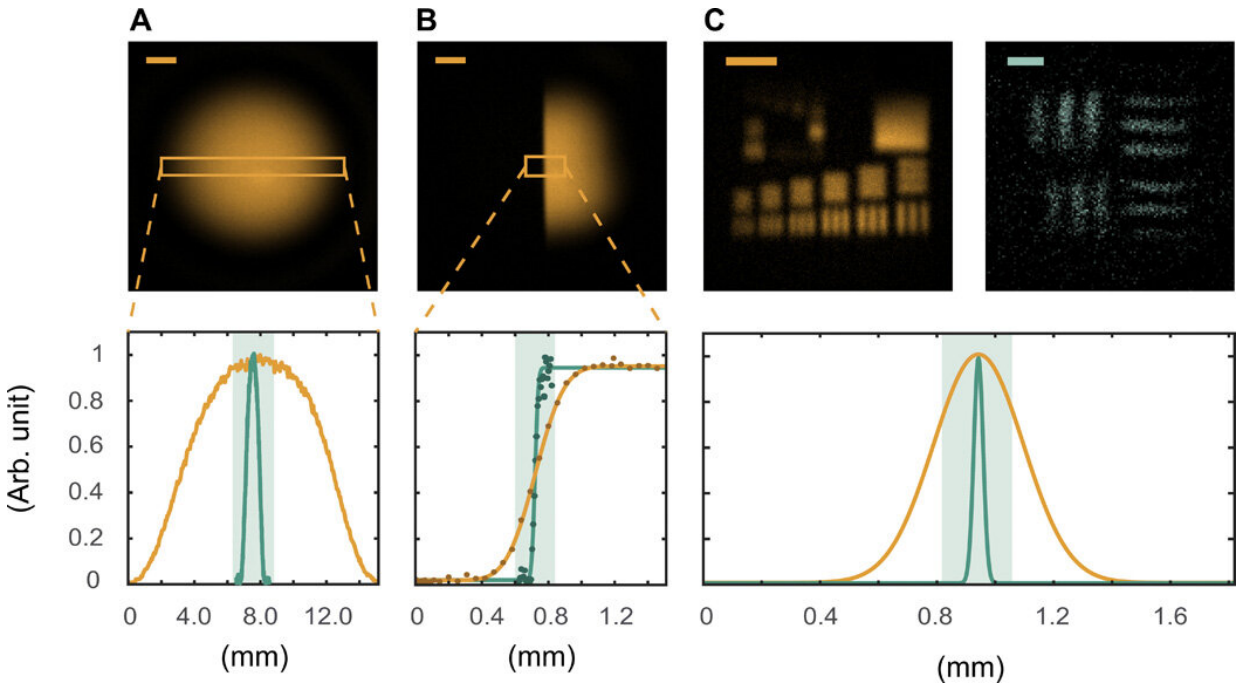
Science Advances, doi: 10.1126/sciadv.abd0264

Microscopy techniques that incorporate mid-infrared (IR) illumination holds tremendous promise across a range of biomedical and industrial applications due to its unique biochemical specificity. However, the method is primarily limited by the detection range, where existing mid-infrared (mid-IR) detection techniques often combine inferior methods that are also costly. In a new report now published on *Science Advances*, Inna Kviatkovsky and a research team in physics, experimental and clinical research, and molecular medicine in Germany, found that nonlinear interferometry with entangled light provided a powerful tool for mid-IR microscopy. The experimental setup only required near-IR detection with a silicon-based camera. They developed a proof-of-principle experiment to show wide-field imaging across a broad wavelength range covering 3.4 to 4.3 micrometers (μm). The technique is suited to acquire microscopic images of biological tissue samples at the mid-IR. This work forms an original approach with potential relevance for quantum imaging in life sciences.

Mid-IR imaging

Microscopy and mid-IR imaging have broad ranging applications across biology, medicine, environmental sciences and microfluidics. For example, researchers can use mid-IR light to sense the distinct rotational and vibrational modes of specific molecules as a "spectral fingerprint," to overcome the need for labeling. Such label-free and non-invasive techniques are important for bioimaging procedures in largely unaltered living tissues. Fourier transform IR spectroscopic imaging is a state-of-the-art mid-IR imaging technique that heavily depends on broadband IR sources and detectors. The IR detectors are, however, technically challenging, costly and sometimes require cryogenic cooling. To bypass

the need for IR detectors, researchers must develop coherent Raman and anti-Stokes scattering microscopy methods. In a markedly different approach, they used the interference of an entangled photon pair with widely different wavelengths that do not require laser sources or detectors at the imaging wavelength. In this work, Kviatkovsky et al. used highly multimodal quantum nonlinear interferometry as a powerful tool for microscopic imaging in the mid-infrared region with only a medium powered visible laser and standard custom metallic-oxide semiconductor (CMOS) camera. They derived explicit formulas for the field-of-view and resolution of wide-field imaging with highly nondegenerate photon pairs.



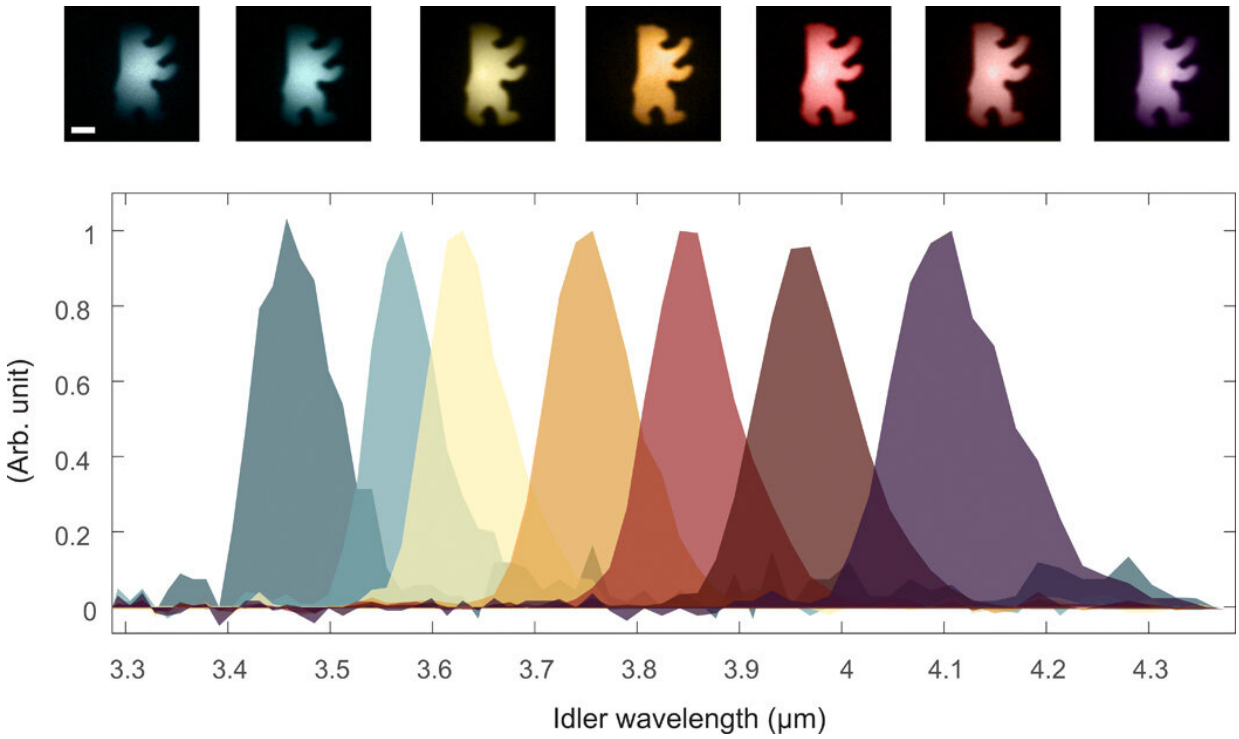
Characterization of the imaging arrangements. The images and data of the unmagnified and magnified setups are presented in orange and green, respectively. (A) Measured FoVs of the unmagnified and magnified setups are 9100 ± 82 and 819 ± 9 μm, respectively. (B) Edge response functions fitted to the data of the two imaging arrangements. (C) Measured resolutions of the unmagnified and magnified setups are 322 ± 5 and 35 ± 5 μm, respectively. The

smallest features in a resolution target that can be resolved for each arrangement are presented. The 10-fold magnification, resulting in the scaling of the resolution and FoV, is manifested in a narrower extend along the horizontal direction (accentuated by the green shaded rectangle in the plots). Orange scale bar corresponds to 2 mm, and green scale bar corresponds to 0.1 mm. Unmagnified (magnified) images were acquired with 1-s integration time and 200 (400)–mW pump power. Credit: Science Advances, doi: 10.1126/sciadv.abd0264

The experimental setup

The scientists developed a nonlinear interferometer by double passing a periodically poled potassium titanyl phosphate (ppKTP) crystal in a folded Michelson geometry (an interference pattern). The pump passed the crystal twice to generate a single pair of signal and idler photons through spontaneous parametric down-conversion (SPDC) - a non-linear optical process where a photon spontaneously splits into two other photons of lower energies in an optics lab. The SPDC method forms the foundation of many quantum optical experiments in labs at present, ranging across quantum cryptography, quantum metrology to even facilitate the testing of fundamental laws of quantum mechanics. The signal and idler modes aligned after the first pass of the crystal to propagate back for the second pass and perfectly overlap to generate biphotons. Kviatkovsky et al. measured the interference by looking at the signal photons with a CMOS camera, without including complex or cost-intensive components to realize such a setup. The team engineered the nonlinear crystal for highly nondegenerate signal and idler wavelengths and selected the idler wavelengths using broadband phase matching. In this way, the experiment allowed simultaneous retrieval of the spatially resolved phase and amplitude information of a sample and the team characterized the mid-IR imaging properties with an off-the-shelf CMOS camera to detect and acquire microscopic images of a

biological sample.



Multispectral imaging. Obtained signal transmission images for varying mid-IR illumination wavelengths. Scale bar, 2 mm. The spectra were recorded at the signal wavelength with a grating spectrometer and converted to the corresponding mid-IR wavelength. Credit: Science Advances, doi: 10.1126/sciadv.abd0264

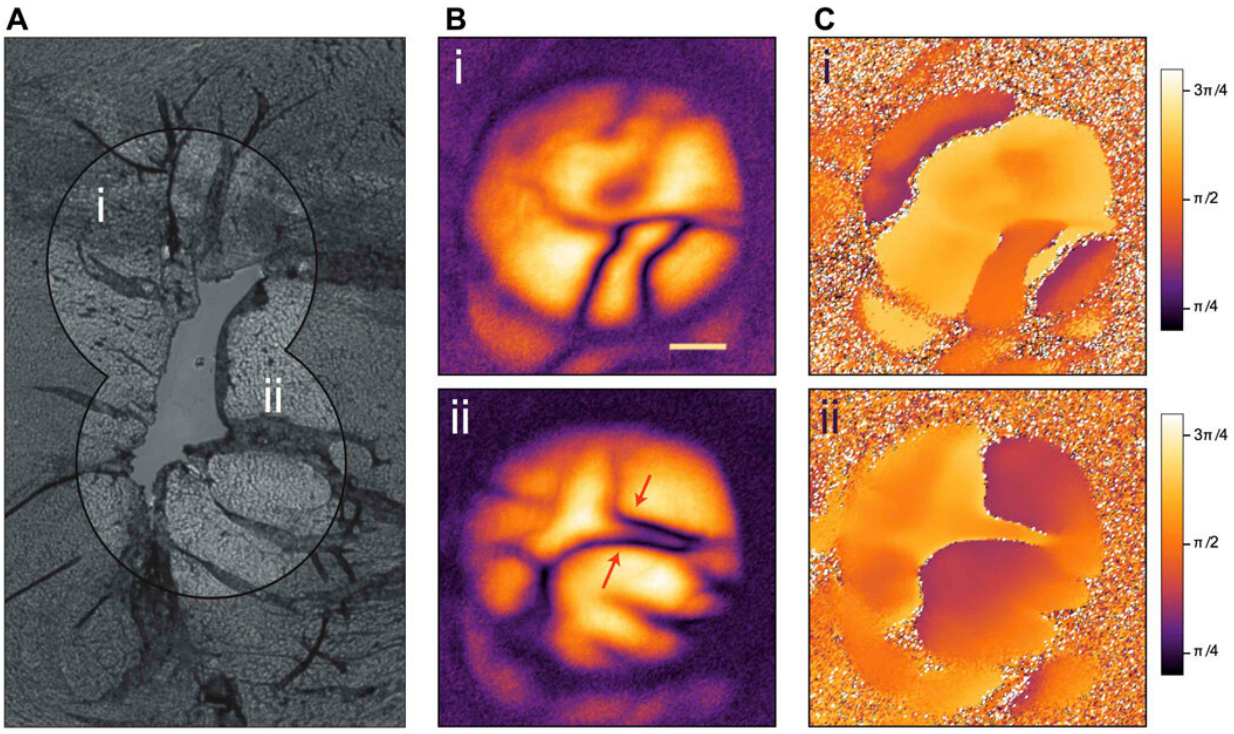
Experimental characterization and proof-of-concept

During the initial characterization of the imaging technique, Kviatkovsky et al. placed both mirrors of the interferometer at the far-field of the crystal and then placed the sample to be imaged on the idler mirror. The unmagnified configuration provided a straightforward process to characterize the imaging capacity of the system, although with

limited resolution. The scientists illuminated a U.S. Air Force (USAF) clear path resolution target, where the resulting values were consistent with a theoretical framework generalized from ghost imaging. They combined the highly broadband nature of the down-conversion source with tight energy correlations shared between the signal and idler to easily allow hyperspectral imaging. During proof-of-concept demonstrations, they used a tunable interference filter with a 3.5 nm bandwidth immediately before detection and achieved enhanced spectral resolution with narrower filtering.

Using the method for bioimaging

The team showed the potential of the method to investigate biological samples by using an unstained histology sample of a mouse heart. They obtained mid-IR images by axially scanning the interferometer displacement inside the coherence length and extracted the visibility and phase of the interference signal for each pixel. The results eliminated any ambiguity between loss and destructive interference that could arise in a single-shot measurement. The work permitted straightforward reconstruction of the wide-field phase-contrast images. The resulting images showed a portion of the endocardium, the inner-most layer lining the heart ventricles in dark purple to indicate high photon absorption. The layer separated the ventricle and the myocardium; the cardiac muscle that consists the bulk of the heart tissue. The clarity of imaging highlighted the high tolerance of the presented imaging method to overcome loss and scattering.



Bioimaging. Histology sample of a mouse heart with (A) bright-field microscopy with visible light for illustration of the part of the sample we investigated with our method. (B and C) Mid-IR microscopy of the same sample with undetected photons for absorption (B) and phase (C) imaging. Scale bar, 200 μm. Images were reconstructed by averaging 10 images at 1-s integration time for 15 axial positions within the coherence length of the biphoton. Pump power was 400 mW corresponding to a sample illumination power of less than 20 pW. Credit: Science Advances, doi: 10.1126/sciadv.abd0264

Real-world promise

In this way, Inna Kviatkovsky, and colleagues showed how mid-IR imaging with nonlinear interferometry played a significant role in real-world imaging tasks that require cost-efficient components for frugal science. The team achieved an imaging feature down to the scale of 35 microns, where extended hyperspectral imaging was uncomplicated due

to the use of a broadband spontaneous parametric down-conversion (SPDC) strategy. The team showed real-world promise of this new method through non-destructive biological sensing while imaging a wet biological sample with low sample illumination. The strategy allowed any information carried by an idler photon to be perfectly transferred to the signal photon. Although the spatial resolution of this work was still higher than that anticipated for state-of-the-art mid-IR systems, extensions to accomplish increased imaging capabilities were straightforward.

The team showed nonlinear interferometry with experimentally entangled photons to provide a powerful and cost-effective method for microscopy in the mid-IR region. The work harnessed the maturity of silicon-based near-IR detection technology for mid-IR imaging with exceptionally low-light level illumination. The work can be extended to hyperspectral imaging across the microscale. As proof of concept, the scientists imaged a biological sample using quantum light to reveal morphological features with high resolution. The results will pave way for broadband, hyperspectral mid-IR spectroscopy with wide-field imaging for varying applications in biology and biomedical engineering.

More information: Kviatkovsky I. et al. Microscopy with undetected photons in the mid-infrared advances.sciencemag.org/content/6/42/eabd0264, *Science Advances*, DOI: 10.1126/sciadv.abd0264

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