



High-harmonic generation microscopy of nanostructured bulk, thin-film and 2D semiconductor materials

TANYA VAN HOREN,^{1,4}  KEVIN MURZYN,¹  REYNOLDS DZIOBEK-GARRETT,^{1,2} FRANCESCO CORAZZA,¹  LEO GUERY,¹ JORIK VAN DE GROEP,²  LYUBOV V. AMITONOVA,^{1,3} AND PETER M. KRAUS^{1,3,5} 

¹Advanced Research Center for Nanolithography (ARCNL), Science Park 110, 1098 XG Amsterdam, The Netherlands

²Van der Waals-Zeeman Institute, Institute of Physics, University of Amsterdam, Amsterdam, The Netherlands

³Department of Physics and Astronomy, and LaserLaB, Vrije Universiteit Amsterdam, De Boelelaan 1100, 1081 HZ Amsterdam, The Netherlands

⁴T.vhoren@arcnl.nl

⁵kraus@arcnl.nl

Abstract: We demonstrate point-scanning high-harmonic generation (HHG) microscopy of nanostructured solid samples up to the fifth harmonic order, extending harmonic microscopy beyond established second- and third-harmonic techniques. Using tailored silicon nanostructures, we directly measure harmonic point spread functions and quantify the achievable imaging resolution. Although higher harmonic orders narrow the point spread function, practical resolution is constrained by signal- and sample-dependent contrast. We apply HHG microscopy to exfoliated MoS₂, distinguishing mono- and bilayer regions and reconstructing crystal orientations via polarization-resolved fourth-harmonic microscopy. These results establish HHG microscopy as a viable approach for nonlinear imaging of nanostructured semiconductor materials.

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1. Introduction

Harmonic generation (HG) microscopy enables label-free mapping of electronic structure and symmetry. Second- (SHG) and third-harmonic generation (THG) microscopy are well established in applications ranging from histopathology to solid-state materials [1–4]. Opting for optical imaging provides superior speed and non-invasiveness compared to established electron microscopy techniques. Additionally, as HG is generated from ultrashort pulses, sub-femtosecond timescales are accessible with pump-probe schemes. In particular, SHG microscopy has become a key tool for characterizing 2D semiconductors, where layer number, relative layer orientation, and stacking order determine local inversion symmetry and can be mapped with polarization-resolved measurements [5–10]. Among these 2D semiconductors, transition metal dichalcogenides (TMDs) have been established as particularly promising candidates for future (opto)electronics [11].

For functional nanostructures and emerging 2D semiconductor devices, a higher spatial resolution is required than harmonic generation microscopy currently provides. In point-scanning HG microscopy, resolution depends on excitation wavelength, acquisition scheme, sample properties, and signal-to-noise ratio (SNR) [7,10,12–14]. In addition, HG microscopy is a coherent imaging modality: phase information of the excitation field is transferred to the harmonic signal, so interference and phase contrast affect how an object is translated into an intensity image and therefore also influence the attainable resolution [14–17].

Higher harmonic orders (m) are attractive from both a contrast and resolution perspective. They can probe higher-energy electronic structure and more extended real-space interactions [18]. At the same time, if the harmonic yield follows a perturbative power-law scaling with effective nonlinear order m_{eff} , the point spread function (PSF) is expected to narrow as $1/\sqrt{m_{\text{eff}}}$ [19–22]. Additionally, higher nonlinear orders are attractive for super-resolution schemes in nonlinear microscopy [23–26].

In wide-field operation, high-harmonic generation (HHG) microscopy has already provided insight into generation mechanisms, emission control, and semiconductor metrology [27–29]. By contrast, point-scanning HHG microscopy of nanostructured solids remains largely unexplored because efficient high-harmonic generation requires intense fields that readily induce damage [7]. Its implementation further demands short pulses, sensitive detection, and preferably long excitation wavelengths that improve high-harmonic generation efficiency and mitigate ionization-driven damage [30,31]. Recent work has spatially mapped harmonics up to the twelfth order in WS_2 , but with loose focusing that does not access nanostructures [18]. Nonlinear knife-edge measurements of the second, third, and fifth harmonic confirmed sharper response at higher harmonic order, but did not demonstrate nanostructure-resolved imaging [32,33]. Thus, point-scanning HHG microscopy of nanostructured solid samples has not yet been established, and its practical resolution as a function of excitation intensity remains unclear.

Here, we demonstrate point-scanning HHG microscopy up to the fifth harmonic order in nanofabricated solid samples using a home-built microscope driven at $2\ \mu\text{m}$. We directly image the third- and fifth-harmonic PSFs using a 500 nm silicon pillar and study their evolution with excitation intensity. We then examine practical imaging resolution in a phase-sensitive case by recording third- and fifth-harmonic images of an etched bulk silicon resolution target. Finally, we apply third-, fourth-, and fifth-harmonic microscopy to inspect exfoliated 2D MoS_2 , which belongs to the TMD material class. In practice, this enables mapping of the (effective) armchair crystallographic axis in monolayer and bilayer regions by polarization-resolved fourth-harmonic generation.

Fourth-harmonic generation microscopy probes the same inversion-symmetry breaking as SHG, but at higher photon energies. In general, detecting higher harmonics such as the fourth can have distinctive advantages. Past experiments have shown that accessing higher photon energies is relevant for providing access to distinct electronic transitions and complementary contrast mechanisms [34]: for instance, for harmonics above 3.5 eV in MoS_2 , a distinctive change of the angular dependence of higher harmonics has been observed and linked to the involvement of multiple conduction bands. Similarly, encoding of sub-cycle dynamics in harmonic emission requires the involvement of high frequencies [35–37] and thus higher harmonics. Consequently, studying sub-cycle dynamics on the nanoscale will benefit from the ability to detect higher harmonics in a scanning-optical microscopy setup.

Our work adds scanning-optical-microscopy functionality to high-harmonic emission from semiconductor and nanostructured samples, which thus far have only been imaged by wide-field emission [27–29]. While both scanning and wide-field microscopy have their own advantages, scanning microscopy is particularly advantageous for working with lower average powers and the possible application of super-resolution functionality such as harmonic deactivation microscopy [26]. While microscopy of the nanostructures in this work revealed sub-micron resolution, true nanoscale resolution in the tens- to single-digit nanometer range remains a future goal for super-resolution HHG microscopy [26,38] in this specific point-scanning implementation.

2. Materials and methods

2.1. Implementation of the HHG microscope

Our home-built HHG microscope is schematically shown in Fig. 1. A Ytterbium laser amplifier (CARBIDE, LIGHT CONVERSION, 80 W) pumps an optical parametric amplifier (ORPHEUS,

LIGHT CONVERSION), which produces NIR laser pulses at a central wavelength of $2\ \mu\text{m}$ (11 W) with a pulse duration of 40 fs. These pulses form our excitation beam, from which harmonics are generated in solid samples. We operate the system at a 200 kHz repetition rate.

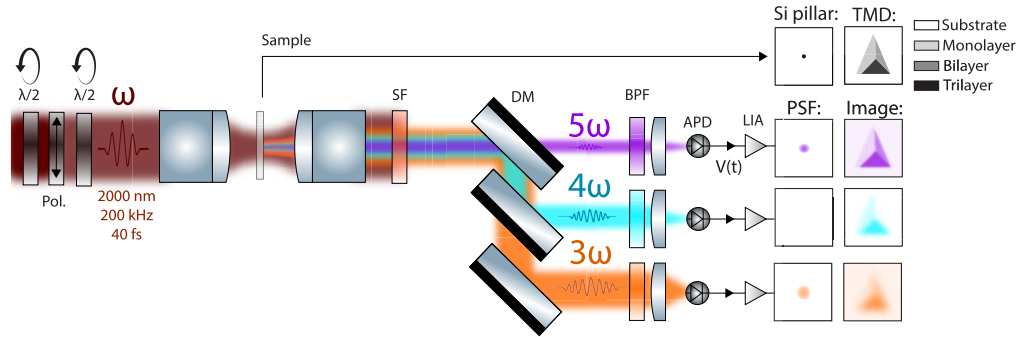


Fig. 1. Schematic of the HHG microscope. A half-waveplate ($\lambda/2$)/polarizer combination serves as a variable attenuator. NIR excitation pulses are focused with a reflective objective, and harmonics are collected with a refractive objective. A SCHOTT filter (SF) absorbs NIR light in the detection path. A set of dichroic mirrors (DM) separates harmonics, and bandpass filters (BPF) isolate individual harmonics. Harmonics are collected in parallel with a set of avalanche photodiodes (APD) followed by lock-in amplification (LIA). Polarization-resolved HHG microscopy is performed with an additional half-waveplate before the sample. The schematically indicated sub-diffraction-sized silicon pillar is inversion symmetric and samples the PSF of odd harmonics. In 2D TMD samples, inversion symmetry can be broken (depending on layer number, stacking, and orientation), allowing even harmonic generation. PSF narrowing and resolution enhancement are expected at higher harmonic orders.

In this work, we probe different harmonic generation regimes by performing NIR intensity scans. To this purpose, we rotate an automated half-waveplate followed by a fixed wire grid polarizer. The polarizer is calibrated to achieve a horizontal polarization in the sample plane. For the 2D sample, we perform polarization-resolved measurements by introducing and scanning an automated half-waveplate, located between the attenuator and focusing objective. We focus a sufficiently expanded beam with a Thorlabs LMM40X-UVV 0.5 NA or Pike 0.78 NA reflective objective. The use of a reflective objective limits the amount of overall group delay dispersion and, importantly, its achromatic focusing ensures that dispersion across the pupil of the focusing objective is homogeneous and can be compensated for [14]. We can thus obtain short pulses with high peak fields at the sample.

For the measurements in this work, we operate our setup in a transmission geometry. The NIR light and harmonics are collected with a Nikon CFI Plan NCG 100x 0.9 NA refractive objective. After collection, a SCHOTT KG3 shortpass filter absorbs the NIR light, and a set of dichroic mirrors directs the third, fourth, and fifth harmonics to different detectors. Harmonics are further isolated by bandpass filters and are collected simultaneously by a set of silicon avalanche photodiodes, followed by pre-amplification (Stanford Research Systems, SR445a) and lock-in amplification (Liquid Instruments, Moku:Lab). The lock-in amplifier uses a local oscillator set at 200 kHz and mixes it with the high-frequency AC harmonic signal. Low-pass filtering removes background noise beyond a frequency band around 200 kHz. Afterwards, the harmonic signal can be sampled easily in the DC band. Amplification and filtering are particularly important for high harmonics, which are generated at low efficiencies. The sample is raster-scanned through the focus with a piezoelectric stage to reconstruct HHG images.

To establish the NIR focus spot size and geometry, we can collect NIR light in a similar detection scheme. We reflect the NIR light before the Schott filter and detect it with a biased InGaAs detector.

2.2. Samples

In this section, we describe the samples that we fabricated and imaged. For each sample, we specify the material, the harmonic orders captured, the structure geometry, and its nanofabrication method.

A hole-in-gold thin-film sample was made to image the NIR excitation focus spot. On the basis of such a measurement, the focus size and symmetry can be determined. The sample consists of a 50 nm gold layer on a quartz substrate. A 5 nm chromium adhesion layer prevents easy detachment of the gold layer. In the gold and chromium layer, sub-diffraction-sized holes with a 500 nm diameter act as tiny pinholes to sample the NIR focus. The chromium and gold layers were subsequently deposited on the quartz substrate using a Leica DC sputter coater. Focused ion beam milling was used to create holes. Scanning electron and atomic force microscopy (AFM) images of the sample are shown in Figure S1 of the [Supplement 1](#).

A silicon nanopillar sample was made to image harmonic PSFs. The sample consists of a 150 nm thick layer of amorphous silicon on a quartz substrate. The choice of silicon on a quartz substrate results in a strong contrast between silicon (bright due to its relatively small bandgap and efficient harmonic generation) and quartz (dark). Silicon is inversion symmetric, and thus, we can generate only the odd third and fifth harmonics from it. Nanopillars with a 500 nm diameter were made to act as point emitters that sample the PSFs of the odd harmonics. After a base piranha washing, the amorphous silicon was deposited onto a quartz substrate with inductively coupled plasma chemical vapour deposition. After a 2-minute oxygen descum, the sample was sequentially spincoated (CEE Apogee) with a HMDS adhesion layer, a 200 nm CSAR positive e-beam resist layer, and an Elektra 92 conductive layer. E-beam Lithography (EBL, Raith Voyager) was used to expose the sample. To develop the sample, we sequentially put it in pentyl acetate, o-xylene, and MIBK:IPA baths. A dry-etch (Oxford PlasmaPro100 Cobra) removed the exposed amorphous silicon. Finally, an acetone bath followed by an oxygen plasma ashing step was performed to remove the remaining resist. Optical microscopy and scanning electron microscopy images of the sample are shown in Figure S2 of the [Supplement 1](#).

An etched bulk silicon resolution target was used to quantify practical resolution limits in the presence of phase contrast. The resolution target contains many structures, among which Siemens stars with varying numbers of spokes. The sample consists of a 700 μm thick single-crystalline (100) silicon wafer, and its inversion symmetry supports only third- and fifth-harmonic generation. The etched resolution targets are 80 nm deep, as confirmed by atomic force microscopy measurements (Figure S3, [Supplement 1](#)). The smallest spoke separation is 100 nm for the 16-spoke stars on the sample, and 50 nm for the 31-spoke stars. We image with the structures at the back of the sample, since it is opaque to visible light. The sample was fabricated with EBL.

Finally, we inspected a 2D MoS_2 sample with polarization-resolved HHG microscopy. The sample consists of monolayer MoS_2 flakes on a quartz substrate. The sample was fabricated through gold-assisted mechanical exfoliation [39] from a 2H-phase bulk crystal (HQ Graphene). During the exfoliation and transfer process, small bilayer regions, small tears and folds, and gold residues form in some areas, which we identify with bright-field, white-light optical microscopy. In monolayer regions, fourth-harmonic generation can be efficient provided excitation along the armchair direction, whereas in the bilayer areas, the generation efficiency additionally depends on the twist angles between the layers [6]. Third and fifth harmonic orders are generated regardless of the polarization direction. Bright-field optical microscopy images of the sample can be found in Figure S4 of the [Supplement 1](#).

3. Results

3.1. Point spread function imaging

We used our nanoscale pinhole and point emitter samples to measure the NIR excitation focus and harmonic PSFs, respectively (for the 0.5 NA objective). Imaging of a single point object yields identical results regardless of the coherence of signal conversion [14,15,17], thus our measurements reflect the intensity point spread function.

Mapping the NIR excitation focus was achieved by imaging a single hole in the gold thin-film sample. The resulting diffraction-limited focus spot is visible in Fig. 2(A). A 2D Airy disk function was fitted to find the horizontal and vertical distances from the maximum to the first minimum of the Airy disk pattern. These distances correspond to the Rayleigh limit and can be used to determine the system's effective NA. The extracted NA values were slightly higher than the 0.5 NA specified by the supplier. Slight elongation was present along the polarization (horizontal) direction. This asymmetry of tightly focused, linearly polarized beams follows from vectorial Debye theory and has been experimentally demonstrated previously [40,41].

A single silicon pillar imaged the harmonic PSF. The resulting third- ($m = 3$) and fifth- ($m = 5$) harmonic images are shown in Fig. 2(B) for different NIR excitation intensities. The upper limit in the scanned intensity range is imposed by sample damage. We stop before seeing PSF deformation, which is followed by an overall loss of harmonic signal. The fifth harmonic requires a minimal excitation intensity to reach a sufficient SNR, and thus, we show only images where a PSF can be discerned. We appreciate that the silicon pillar indeed emits harmonics brightly compared to the quartz substrate. We measure the PSF for multiple NIR peak intensities to see the evolution from perturbative to non-perturbative excitation. The harmonic PSFs can be represented by a nonlinear scaling of the NIR Airy disk focus spot, and thus approach a Gaussian function. This allows for a quantification of each PSF by its FWHM from a 2D Gaussian fit, reflecting the Abbe resolution limit [12].

In Fig. 2(C), we show the FWHM evolution of the third- and fifth-harmonic PSFs as a function of peak NIR excitation intensity. At low intensities, the harmonic PSFs approach the Abbe limit divided by the square root of the harmonic order, which matches our expectation for perturbative excitation (Figure S5 of the Supplement 1 shows a perturbative power-law measurement). As a consequence, the smallest PSF achieved corresponds to the fifth harmonic. At increased intensities, the PSF broadens, and the FWHMs correspond to a lower effective nonlinear order. This is in line with commonly observed saturation of harmonic generation at increased excitation intensity [38,42,43].

3.2. Practical resolution limits

Before imaging the silicon resolution target, atomic force microscopy was performed to quantify the sample's etch depth. The AFM image (Figure S3, Supplement 1) covered the central $30 \times 30 \mu\text{m}^2$ of a 31-spoke Siemens star. The etch depth d_{etch} will introduce a phase shift $\Delta\phi$ between the harmonics (wavelength $\lambda_m = \lambda_{\text{NIR}}/m$) traveling through the pristine and etched silicon [44]:

$$\Delta\phi = [n_{\text{Si}}(\lambda_m) - n_{\text{air}}] \cdot \frac{2\pi}{\lambda_m} \cdot d_{\text{etch}}. \quad (1)$$

Here, n_{air} is the refractive index of air, which is 1.0. $n_{\text{Si}}(\lambda_m)$ is the wavelength- and thus harmonic order-dependent refractive index of silicon, which is 3.8 for 667 nm and 5.6 for 400 nm [45]. Figure 3(A) shows simulated $\Delta\phi$ maps for $m = 3$ and $m = 5$, illustrating that for the third harmonic, phase contrast should be close to $2\pi/3$, whereas for the fifth harmonic it is close to the 2π phase wrap point. The distinct phase contrast will affect the resolution of our coherent imaging modality. For example, in a simple two-point emitter system, a $\pi/2$ phase difference would correspond to a separability equivalent to that of the incoherent Rayleigh limit, whereas

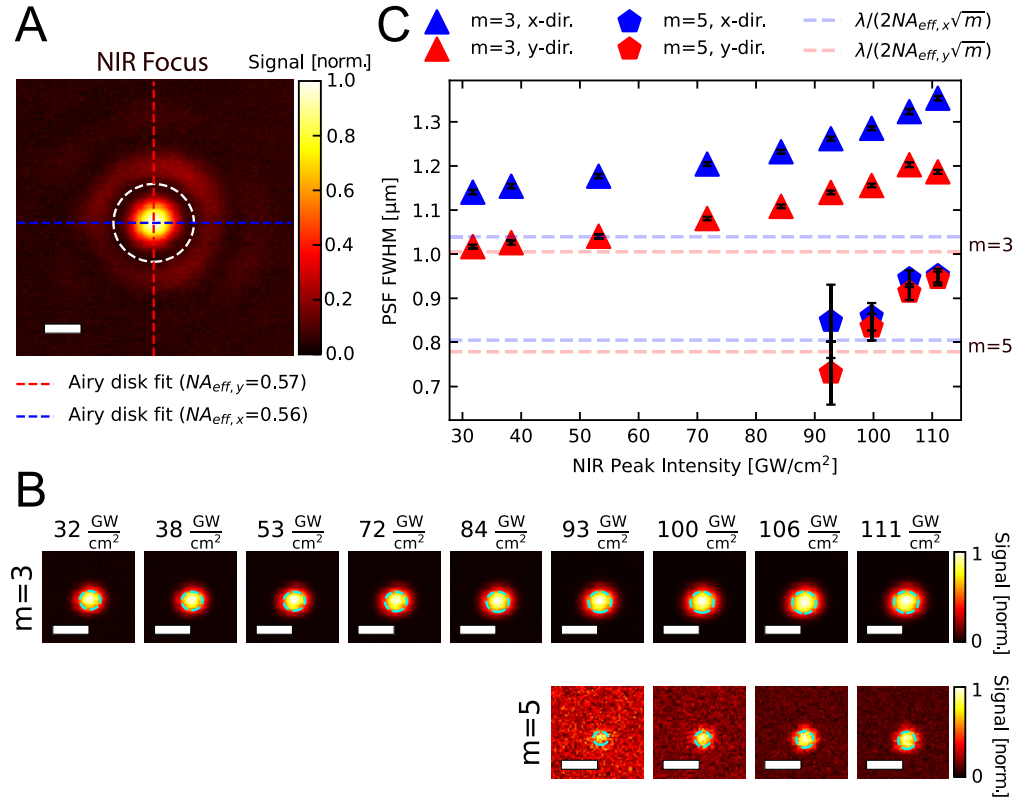


Fig. 2. Third- and fifth-harmonic PSF imaging at different NIR peak intensities. The scale bar is 2 μm in all images. (A) NIR microscopy image of the excitation beam in focus, as imaged with the hole-in-gold thin-film sample. A diffraction-limited spot is visible. We define an effective NA in the x and y direction by fitting the Rayleigh limit. The dashed white line indicates the first zero of the Airy pattern, as obtained from the fit. (B) Harmonic PSFs as imaged with the silicon nanopillar sample. We fit a 2D Gaussian to each PSF and extract its FWHM, which reflects the Abbe resolution limit. The dashed cyan lines represent the FWHM. (C) PSF FWHM as a function of NIR excitation peak intensity. Error bars indicate fit errors at 2σ . The PSF scales close to the Abbe limit of the NIR spot divided by the square root of the harmonic order for low-intensity perturbative excitation. At increased IR intensity, the PSF broadens, corresponding to a decrease in the effective nonlinear order.

zero phase difference would result in a 34% increase of the minimum resolvable distance [17]. Accordingly, for this specific sample (etch depth), we expect that the phase contrast is particularly unfavorable for the resolution of the fifth-harmonic images.

Figure 3(B) shows third- and fifth-harmonic microscopy images of a 16-spoke Siemens star ($n_{spokes}=16$) acquired with the 0.5 NA objective. Again, the maximum intensity sampled is restricted by sample damage, and at the lowest NIR intensity, we do not detect any fifth-harmonic generation. Noticeably, for both harmonic orders, the edges of the spokes are visible as regions with less signal. This is a result of HG microscopy being a coherent imaging technique; when harmonics are emitted from both sides of the spoke, they will destructively interfere due to the phase difference illustrated in Fig. 3(A). We observe no visible contrast difference between etched spokes and pristine silicon, in line with the etch depth being small compared to the few- μm Rayleigh range of our NIR excitation.

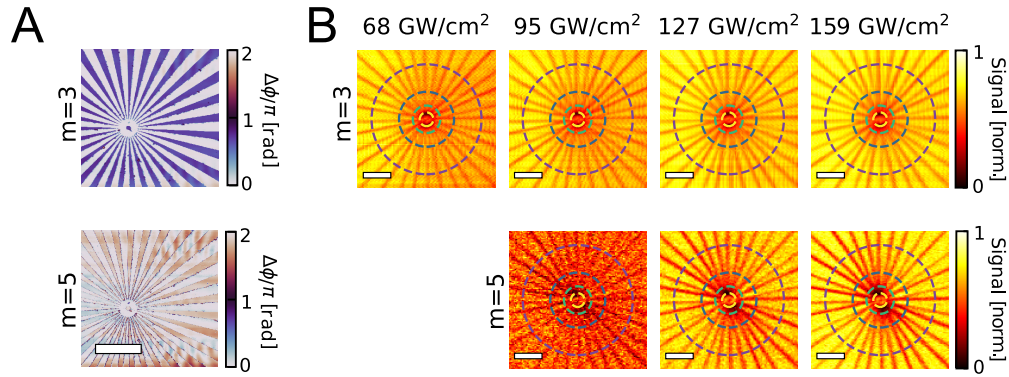


Fig. 3. Third- and fifth-HG microscopy of the etched bulk silicon resolution target. All scale bars are 10 μm . (A) An AFM etch depth map (Figure S3, Supplement 1) of the sample can be scaled by Eq. (1) to estimate the phase contrast for each harmonic, which affects the image contrast. (B) HG microscopy images of a 16-spoke Siemens star at varying NIR excitation intensity. Lineouts are indicated, which are used for resolution analysis, as presented in Fig. 4.

Typically, the resolution of such Siemens star images can be determined by taking lineouts at different radii and defining the radius down to which the spokes can be discerned. In this case, we consider the radius down to which the *edges* of the spokes can be discerned, as they exhibit the key contrast in our images. The radius R can be converted to the edge separation Δr_{edge} as $\Delta r_{\text{edge}} = R\pi/n_{\text{spokes}}$.

The dashed lines in the harmonic images indicate lineouts at different radii. In Fig. 4(A), we plotted the intensity profile along these lineouts for the highest NIR excitation intensity image. The lines were shifted relative to each other to get a visible separation of the lineouts. The spoke edges show up at a frequency of 32 cycles per revolution (2 times the number of spokes, which is 16 here), and noticeably, this frequency component disappears as we approach the center of the Siemens star.

To systematically assess spatial resolution, we analyse lineouts over a densely sampled set of radii. We again illustrate this for the highest NIR excitation intensity image. Figure 4(B) combines the Fourier transform of each lineout, normalized to the overall maximum. We can see that the 32 cycles per revolution frequency component drops off with decreasing radius/edge separation. We choose the 21.6 to 26.4 cycles per revolution range to serve as a noise-only reference. In Fig. 4(C), we plot the maximum signal at 32 cycles per revolution as a function of the edge separation for each NIR intensity. A rolling average window smoothed the curves to avoid small-scale fluctuations that would affect the determined resolution. The dashed lines indicate a threshold set at the 99th percentile of the noise. The intersection of the signal with the threshold determines the assessed resolution, which is indicated with stars in both Figs. 4(B) and 4(C).

For the third harmonic, we can appreciate that the best resolution is indeed achieved at the lowest NIR excitation intensity, in line with the PSF measurements in Section 3.1. The best-assessed resolution is close to 1.44 μm , which is larger than the incoherent lateral Rayleigh resolution limit of $\sim 1.0 \mu\text{m}$, as measured with the silicon nanopillar sample (Fig. 2). As the SNR was still sufficient at the lowest NIR excitation intensity, better resolutions could likely be achieved by further reducing the intensity.

For the fifth harmonic, however, resolution improved with increasing NIR excitation intensity. The effective resolution was hindered by the dominance of noise in the fifth-harmonic images, especially at low excitation intensities. In this particular case, fifth-harmonic generation

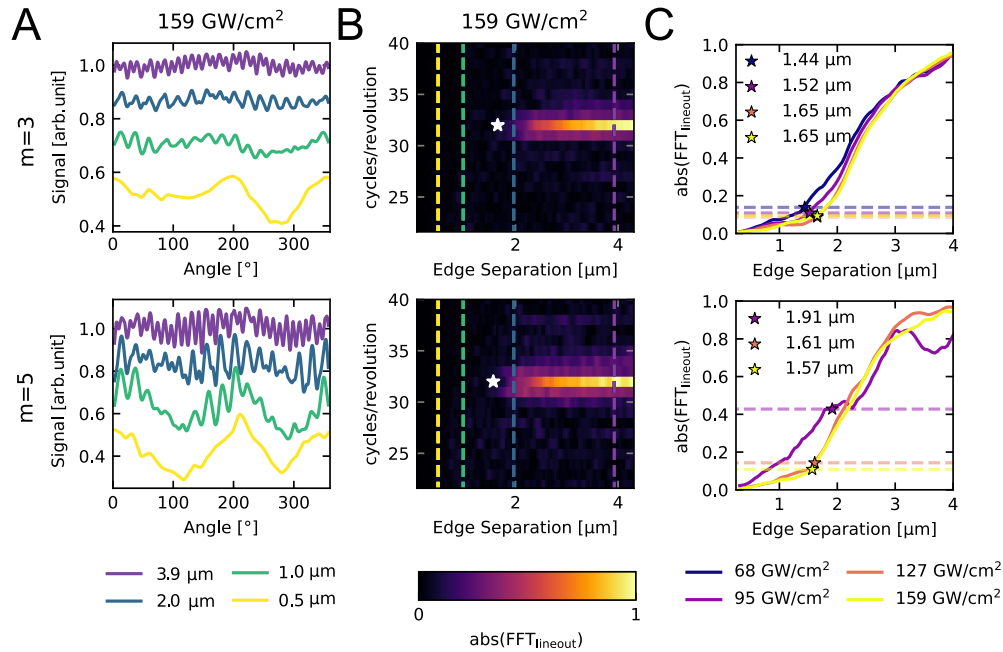


Fig. 4. Resolution analysis for third- and fifth-HG microscopy of the etched bulk silicon resolution target. Both lineouts (A for a single intensity, shifted for visible separation) and a Fourier analysis (B for a single intensity, summary in C) of the phase-contrast images show that there is a critical edge separation (lineout radius) below which individual edges are not resolved. The edge separations for the lineouts are indicated in the legend below panel A and correspond to radii of 20, 10, 5, and 2.5 μm . The critical edge separation is indicated by stars in both B and C. Whereas for the third harmonic, an increase in excitation intensity degrades image resolution, this is not true for the SNR-limited fifth harmonic. In this case, practical fifth-harmonic resolutions do not improve on those achieved with third-harmonic microscopy.

microscopy did not result in a better resolution than low-intensity third-harmonic generation microscopy. Increasing averaging and signal integration could alleviate SNR restrictions and improve the resolution. Given sufficient signal integration, the disadvantageous phase contrast will still affect the achievable resolution. Combining our findings on PSF imaging (Fig. 2(C)) and phase contrast for this particular sample (Fig. 3(A)), we would expect that the fifth harmonic resolution can best reach 1.1 μm for this sample. Given the coherent nature of HHG microscopy, resolution differences of up to 34% can be expected for any sample, depending on the phase contrast imposed by its material and geometry.

Afterwards, we imaged a 31-spoke Siemens star with the 0.78 NA objective. The NIR focal volume scales as NA^{-4} , thereby reducing to about 20% of the 0.5 NA focal volume. In this configuration, we did not detect enough fifth harmonic for a resolution analysis, likely due to the mentioned focal-volume reduction. Figure 5(A) shows the third-harmonic images at varying NIR excitation intensity, while Fig. 5(B) shows the result of the resolution analysis that was outlined before. We reduced the intensity sufficiently for the third-harmonic resolution to converge to approximately 620 nm, which is well-matched to the minimum third-harmonic PSF measured in Fig. 2, scaled by the improvement in NA from 0.5 to 0.78.

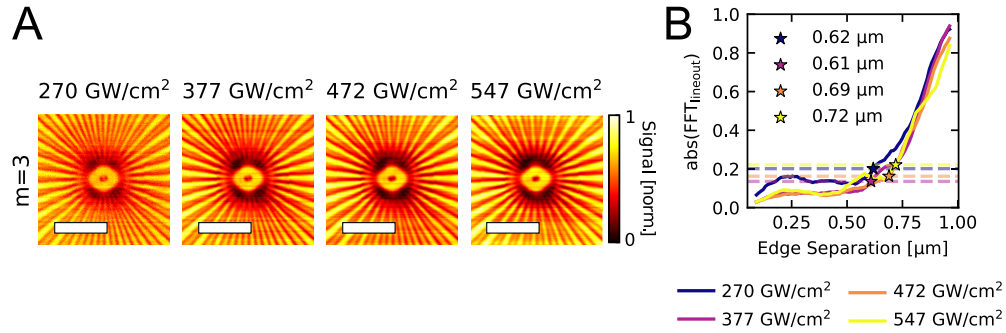


Fig. 5. Third-HG microscopy of the etched bulk silicon resolution target at 0.78 NA. (A) Third-HG microscopy images of a 31-spoke Siemens star. (B) Results of the resolution analysis. The NIR excitation intensity was sufficiently reduced for the resolution to converge at low intensities.

3.3. HHG microscopy of few-layer MoS₂

Finally, we demonstrate that our HHG microscope can be applied to study layer configurations and orientations in the 2D MoS₂ sample. With the bright-field microscopy image in Fig. 6(A), we located different materials and layer numbers to compare to our harmonic microscopy images. Each patch of unique material and/or layer number is defined as a region-of-interest (ROI) and is highlighted by a colored box.

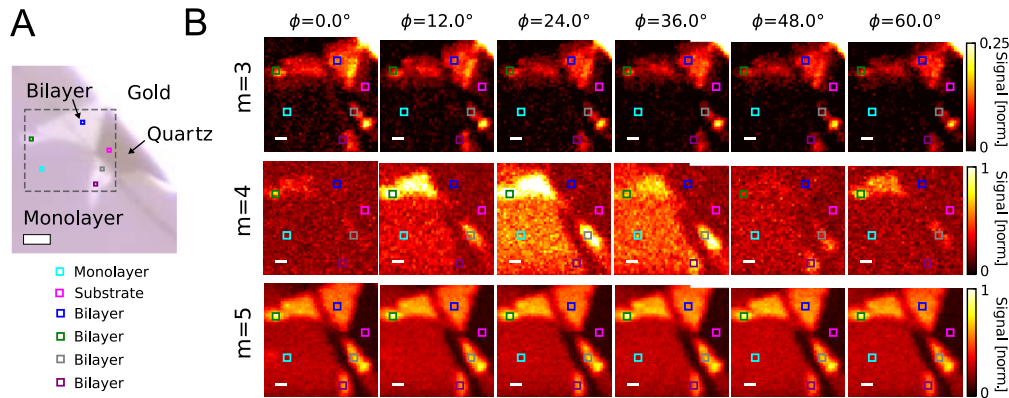


Fig. 6. Third-, fourth-, and fifth-HG microscopy of the exfoliated MoS₂ sample. All scale bars are 5 μm . (A) The bright-field microscopy structural reference, with which monolayer (dark purple) and bilayer (light purple) areas could be distinguished. Some gold residue from exfoliation and the quartz substrate were also identified. ROIs were selected and are indicated as colored boxes, and dashed lines indicate the field of view of the HHG microscopy measurements. (B) HG microscopy images at a subset of excitation polarization angles (ϕ), with indicated ROIs. The color mapping of the THG images runs up to a quarter of the maximum THG signal.

Figure 6(B) shows a subset of polarization-resolved third-, fourth-, and fifth-harmonic microscopy images of a small area. Notably, the bilayer areas (blue, green, gray, purple ROIs) of the sample emit more odd harmonics than the monolayer area (cyan ROI). This can be simply attributed to a quadratic scaling of the harmonic signal with the number of layers [18]. The fifth-harmonic microscopy images yield excellent contrast between the different materials and suffer less from strong harmonic signal near gold residues compared to the third-harmonic

microscopy images. In general, the micro-sized gold residues could confine the NIR field and enhance the overall harmonic generation efficiency in their vicinity [46]. Since the extinction coefficient of gold increases considerably below the third-harmonic wavelength (667 nm), the fifth harmonic at 400 nm is likely much more strongly reabsorbed [47]. The even fourth harmonic depends strongly on the NIR polarization angle, as it probes inversion symmetry, similar to the typically measured second harmonic [6]. In areas where inversion symmetry is broken, we can measure a nonzero fourth harmonic; its polarization signature reflects the orientation of the layers.

Figures 7(A) and 7(B) show the total fourth-harmonic signal $I^{4\omega}$ within each ROI as a function of the excitation polarization angle ϕ . In areas of MoS₂, we observe a characteristic six-fold symmetry pattern, which can be fitted to determine the (effective) armchair direction (θ_0) in monolayer and bilayer MoS₂ [6]:

$$I^{4\omega} \propto \cos^2 3(\phi - \theta_0). \quad (2)$$

In monolayer MoS₂, θ_0 simply represents the armchair direction, while in bilayer MoS₂, it is the average (effective) armchair direction of the two layers that constitute it. The θ_0 value determined from the fit is shown for each ROI. The green-, grey-, and purple-labeled bilayer MoS₂ ROIs exhibit differently rotated 6-fold symmetry patterns. As these bilayer patches likely resulted from a small tear in the monolayer, it is credible that the bilayer twist angles will be different between the patches, resulting in various rotations of the polarization signatures and overall different fourth-harmonic signal amplitudes. In the blue-labeled bilayer MoS₂ ROI, there is no fourth-harmonic signal. This specific case indicates a 60° twist angle; this specific angle will result in the two layers being mirror images, restoring inversion symmetry and thus resulting in full destructive interference of the fourth-harmonic signal [6].

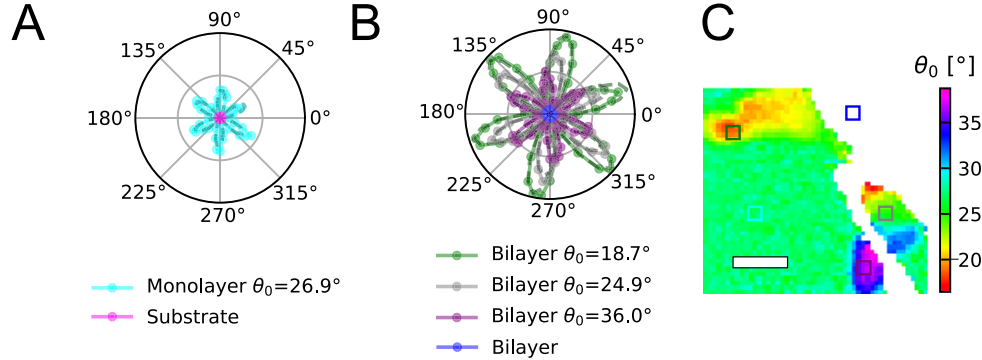


Fig. 7. Fourth-HG microscopy polarization signatures in different ROIs of the exfoliated MoS₂ sample. MoS₂ ROIs contain unique layer configurations, and can be characterized by θ_0 (Eq. (2)) [6]. (A) Polar plots of the fourth-harmonic signal in the monolayer ROIs as a function of excitation polarization, where θ_0 is the armchair orientation. (B) Polar plots of the fourth-harmonic signal in the bilayer ROIs as a function of excitation polarization, where θ_0 is the effective armchair orientation. (C) Mapping of θ_0 on a pixel-to-pixel basis. The scale bar is 5 μm .

The SNR in our fourth-harmonic images is sufficient to also map θ_0 on a pixel-by-pixel basis. Figure 7(C) shows θ_0 for each pixel in the image. Pixels for which the fitted θ_0 had a standard deviation larger than 0.2° are transparent. This threshold was chosen empirically and ensures that quartz and gold, which otherwise appear noisy because they lack a symmetry signature, are not falsely assigned a θ_0 value. In the bilayer patch that contains the grey ROI, there is a notable change in bilayer twist angle across the patch.

4. Discussion

In this work, we have shown that higher harmonic generation point-scanning microscopy of nanostructured solid samples captures the fourth and fifth harmonics along with the more conventional third harmonic. Individual harmonics result in a unique image contrast, dictated by the electronic and structural properties of the material under investigation. The signal-to-noise ratio of our images allowed for the differentiation of mono- and bilayer MoS₂, along with a reconstruction of crystal symmetries in the sample. Fifth-harmonic microscopy resulted in a sharp distinction between materials (notably monolayer and substrate) and was less affected by the proximity of gold residues than third-harmonic microscopy.

Furthermore, thin-film and bulk silicon resolution targets gave direct insights into the potential and practical resolutions of HHG microscopy. Our point spread function measurements showed that at a given intensity, sampling a higher harmonic order will give rise to a smaller intensity PSF. In line with perturbative power-law scalings of the harmonic yield as a function of excitation intensity, the harmonic PSF scales close to $1/\sqrt{m}$ at low NIR intensities. At 0.5 NA, this translates to resolutions down to 1 μm for the third harmonic, and 800 nm for the fifth harmonic. For commercially available 0.8 NA reflective objectives, fifth-harmonic microscopy could thus reach resolutions down to 500 nm.

In this work, the limited SNR was the main practical bottleneck for fifth-harmonic microscopy at high NA. The current use of a 200 kHz repetition rate with sample scanning, however, leaves room for SNR improvement. Detecting the fifth or even higher harmonics more efficiently and rapidly could be enabled by the use of a MHz-repetition-rate laser system with galvo scanning, which would allow increased signal integration within reasonable acquisition times.

Next to PSF reduction and thus potential resolution gains proportional to $1/\sqrt{m_{\text{eff}}}$, super-resolution techniques in conjunction with higher harmonic orders are very promising. Harmonic deactivation microscopy (HADES) allows sub-diffraction harmonic generation microscopy by introducing a second, donut-shaped deactivation beam that spatially confines HG [26]. In the HADES scheme, the resolution gain increases with increasing deactivation fluence, while sample damage at high fluences sets a practical limit [38]. Given this limit, it would be advantageous to move to higher harmonic orders, for which spectroscopy measurements have predicted sharper deactivation onsets [42]. Substantial resolution gains can be expected at higher harmonic orders, which will improve on the practical resolutions in this work, paving the way to sub-100 nm resolution for fifth-harmonic microscopy [26].

The premise of nanoscale resolutions is particularly interesting in the context of structured TMDs. Commercial nanofabrication of 2D semiconductor devices has attracted considerable interest in the past decade [48]. TMDs are a major candidate for future transistors, and based on recent advances in wafer-scale growth and subsequent etching, the IEEE International Roadmap for Devices and Systems predicts that the first 2D channel transistors will enter high-volume manufacturing by 2040 [11]. Recently, optical elements, such as blazed gratings and zone plate lenses, have been successfully fabricated from TMDs by e-beam lithography [49,50]. To continue advancing fabrication, suitable characterization techniques must also be available. Typical feature sizes on these devices are on the nanoscale, and thus, conventional second- and third-harmonic generation microscopy cannot be used for inspection. HHG microscopy at nanoscale resolutions would thus be a valuable tool.

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Supplemental document. See [Supplement 1](#) for supporting content.

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