Fluorescence quenching microscopy (FQM), an optical technique originally developed for imaging graphene-based sheets on arbitrary substrates is extended by J. Huang and co-workers on page 3253 to other two-dimensional materials, such as molybdenum disulfide (MoS₂) sheets (background image). The panels in the main image are the MoS₂ flakes imaged by reflectance mode optical microscopy (left), FQM (middle), and scanning electron microscopy (right).
Seeing Two-Dimensional Sheets on Arbitrary Substrates by Fluorescence Quenching Microscopy

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Two-dimensional (2D) sheets of layered materials such as graphene-based sheets\(^1\) and transition metal dichalcogenides\(^3\)–\(^6\) have attracted great interest in recent years due to their exciting new properties and potential applications. Microscopy is usually the first-line characterization tool for these 2D materials\(^7\) and is indispensable for identifying microscopic 2D sheets on a substrate and evaluating morphological features such as the lateral size and number of layers. Microscopy is also used to examine the microstructures of thin films made of 2D sheets, and is instrumental for inspecting the overall coverage, film uniformity and how the neighboring domains interact. Being able to see these 2D layers is a critical step for studying these materials.

However, imaging these 2D single atomic/molecular sheets has been challenging. Due to their small thickness,\(^8\) direct optical microscopy (OM) imaging under transmission mode has been difficult. Under reflectance mode, graphene\(^1,9,10\) and other 2D sheets\(^11\)–\(^14\) can be visualized, which has enabled numerous device works. However, OM only works best on dielectric coated silicon wafers under optimized illuminating wavelengths, and may require further image processing. Among other common techniques for imaging flakes, scanning electron microscopy (SEM)\(^7,15\) works very well, but typically only for samples deposited on conducting substrates. Atomic force microscopy (AFM) is a general imaging technique that is particularly suitable for thin sheets.\(^7,16\) However, AFM is rather low throughput and typically only works well for atomically smooth substrates. Raman imaging\(^11\)–\(^14,17,18\) is a powerful technique for counting the layers of those thin sheets with strong layer-dependent Raman signal, but there are concerns about sample/substrate damage by laser heating, and signals may be overwhelmed if the substrate has auto fluorescence. Alleviating these constraints for imaging and characterization can boost the advancement of research on 2D materials. Therefore, a facile and high throughput imaging technique for visualizing these 2D materials with minimum constraints would be highly desirable.

Previously we developed a general technique named fluorescence quenching microscopy (FQM)\(^7,19\) for seeing graphene-based sheets, in which the carbon sheets become highly visible by quenching the fluorescence of nearby dye molecules through long range energy transfer. The dye layer is typically applied by spin-coating from solution, in which a polymer is added to improve the uniformity of the fluorescent coating (Figure 1a). FQM allows rapid, high contrast imaging of various graphene-based sheets on arbitrary substrates and even in solution. Since the resonance energy transfer between the excited state of the dye molecules and graphene could extend over tens of nanometers in range,\(^20\) the degree of fluorescence quenching would increase with increased number of layers. Therefore, FQM offers high layer counting resolution and at the same level of magnification, can produce as high quality images as those taken by SEM. Use of FQM has been extended for imaging large area graphene thin films made by chemical vapor deposition (CVD) after transferring from their metal foil catalyst onto insulating substrates,\(^21\) graphene/graphane superlattices\(^22\) and graphene nanostructures.\(^23\)

Fluorescence quenching could occur by energy transfer between the excited state of the dye molecules (acting as donor *D*) and graphene (acting as acceptor A) through two major mechanisms involving energy transfer through dipole-dipole interactions and electron/charge transfer.\(^24\) For the dipole-dipole mechanism, the interaction between *D* and A is achieved through space by the overlap of the dipolar electric fields of the two. For the charge exchange mechanism, the interaction is made through the overlap of the orbitals of *D* and A. Therefore, the charge transfer mechanism works over short distance since it requires overlap of molecular orbitals, which can only happen within van der Waals contact. In contrast, the dipole-dipole mechanism can work over much longer distance through empty or even occupied space.\(^24\) Both mechanisms can contribute to fluorescence quenching and thus the imaging contrast of graphene-based sheets against the background.\(^19,25\) However, only the long range mechanism, essentially Förster resonance energy transfer, is suitable for FQM imaging applications as it allows multiple layers of graphene-based sheets to interact with the dye molecules and cumulatively quench the fluorescence, thus...
enabling layer contrast.\textsuperscript{[19,20]} The strength of dipole-dipole interaction is determined by the transition dipole moments of D and A corresponding to their ground to excited state transition, spectral overlap of the donor emission spectrum and the acceptor absorption spectrum, the relative orientation of the two and their separating distance. These very general requirements suggest that FQM should not be limited to graphene since fluorescence quenching is generic. As long as the object quenches fluorescence differently than the substrate, it should become visible in FQM. When performed on a common optical microscope, the lateral resolution of FQM is diffraction limited. Therefore, FQM is especially well suited for imaging objects with larger, micron-scaled lateral dimensions such as the sheets of 2D materials.

A typical procedure for FQM is delineated in Figure 1a. A dye doped polymer, such as polyvinylpyrrolidone (PVP) doped with fluorescein, is spin coated onto an arbitrary substrate with pre-deposited sheets. When the sample is imaged under a fluorescence microscope, the areas where graphene-based sheets are deposited appear dark against brighter background, due to the strong fluorescence quenching effect of the sheets. Previously we have demonstrated that FQM can be used to image graphene on insulating substrates such as glass and plastics. Figure 1(b–d) shows monolayer graphene flakes grown on a copper foil by CVD\textsuperscript{[26,27]} imaged by OM, SEM and FQM. Under bright field reflectance OM (Figure 1b), the graphene sheets were barely visible on the copper substrate. However, FQM produced a high contrast image (Figure 1d) that is comparable to the SEM image (Figure 1c). Although metals themselves are strong fluorescence quenchers, in the FQM image, the monolayer graphene flakes appear even darker than the copper background. This is because both the flakes and the copper underneath them can contribute to fluorescence quenching under the long range energy transfer mechanism, making the areas covered by graphene appear darker. Figure 1d again illustrates that FQM can be performed on arbitrary substrates.

The generic nature of dipole-dipole interaction\textsuperscript{[24]} suggests that FQM should be suitable for seeing other 2D materials. In the study below, we investigate if FQM can also be performed on inorganic 2D crystals, using MoS$_2$ as an exemplar. FQM would be a timely addition to the set of tools for research in MoS$_2$, especially since recent advances in the synthesis and sample preparation have made large flakes available.\textsuperscript{[4,28,29]} We demonstrate that, as was the case for graphene-based sheets, MoS$_2$ sheets can indeed be visualized with high contrast and high throughput, even on insulating substrates such as quartz.

MoS$_2$ sheets were exfoliated from bulk crystal and deposited on SiO$_2$/Si substrates using mechanical cleavage with Scotch tape.\textsuperscript{[4]} The same area of the sample was imaged by FQM (Figure 2a), OM (Figure 2b) and SEM (Figure 2c). The majority of flakes obtained were thick stacks and only the piece shown at the center of the images is made of few layers, which was also imaged by AFM (Figure 2d). Note that using SiO$_2$/Si substrates facilitates mapping and identification of the same sheet under OM. However, the contrast of thin sheets in OM is subtle and experienced eyes are required to locate and identify thin MoS$_2$ sheets. In comparison, the image taken with FQM (Figure 2a) shows significant enhancement in contrast compared to the OM image (Figure 2b), and the contrast obtained in FQM is comparable to that obtained with SEM (Figure 2c). AFM (Figure 2d) of the flake confirms that the thinnest regions correspond to 2–3 layers of MoS$_2$. While AFM gives the most accurate information about 2D materials, including the thickness of the sheet, slow line scan
speeds (∼1 Hz) are often necessary and image acquisition can be very time-consuming. On the other hand, FQM is a technique based on optical microscopy. Thus, FQM shares many advantages as common OM including high throughput sample observation over large areas, which is especially suitable for quick sample check in developing new material synthesis. Figures 2 (e–h) show the same area of MoS₂ sheets grown by CVD [28] imaged by FQM (Figure 2e), OM (Figure 2f), SEM (Figure 2g), and AFM (Figure 2h). As in the case of mechanically exfoliated sheets, the FQM image of CVD-grown MoS₂ (Figure 2e) shows enhanced contrast over OM (Figure 2f), and is comparable to the image acquired by SEM (Figure 2g). AFM (Figure 2h) confirms that the thinnest sheets correspond to 2–3 layers of MoS₂. Therefore, FQM offers a high-contrast method for surveying a large area of sample, and therefore can be used as a technique for obtaining immediate feedback to optimize CVD process conditions.

Images in Figure 2 were taken for MoS₂ sheets deposited on SiO₂/Si substrates to compare FQM with OM and SEM. However, the greatest advantage of FQM is that it can readily visualize samples deposited on substrates that would be very difficult to image by OM and SEM. One such example is quartz, which is insulating and has been extensively used to grow MoS₂ sheets [28] to facilitate device fabrication. SEM is not a suitable imaging technique for such insulating substrates due to the charging effects induced by the electron beam. Reflective mode of OM, which has been extensively used for imaging 2D sheets on dielectric coated silicon wafers, does not work well on such transparent substrate, either, due to the lack of interference-based color contrast. Since FQM is substrate independent, it can fulfill the niche imaging need for seeing 2D sheets on technically important substrates that are non-conductive and transparent, such as glass, quartz and plastics. Figure 3a shows an
OM image of MoS₂ sheets grown on a common quartz substrate (Figure 3a, inset) using CVD. The thinnest sheets of MoS₂ are barely visible under OM due to the low overall optical absorbance. AFM imaging (Figure 3b) also turned out to be challenging since the roughness of the substrate nearly overwhelmed the topological features of the sheets. However, the FQM image (Figure 3c) of the same region provides a high-contrast image with topological details, of which the quality is comparable to SEM images of MoS₂ deposited on more conventional substrates (Figures 2c and g). Note that the bright spots seen in the FQM image (Figure 3c) correspond to the black dots seen in the OM image (Figure 3a), and presumably arise from accumulation of the dye at these exceptionally tall features during the spin-coating process. This striking variety of detail demonstrates that FQM can be performed on MoS₂ atop an arbitrary choice of substrates. Thus, FQM would be a versatile tool for the development of diverse applications including MoS₂-based transparent electronics.

The contrast in FQM originates from fluorescence quenching of the dye layer by the underlying sheets. Quenching can proceed by either short range electron transfer or long range energy transfer. For graphene, we have shown that FQM works by long range energy transfer facilitated by the exceptionally long quenching distance of graphene, which has been calculated to be up to 30 nm. Previously, we designed a “spacer experiment” showing that graphene can indeed remotely quench the fluorescence of dye molecules through a layer of 20 nm thick polystyrene.

In Figure 4, such experiment is repeated with MoS₂ sheets. A mechanically exfoliated MoS₂ flake was used in the experiment. The AFM image (Figure 4a) shows that the thinnest part of the flake has an apparent thickness of 2 nm. An approximately 15 nm thick polystyrene thin film was spin-coated onto the flake to serve as a spacer. Subsequently a dye-doped polymer, consisting of 4-(dicyanomethylene)-2-methyl-6-(4-dimethylaminostyryl)-4H-pyran (DCM) and poly(methyl methacrylate) (PMMA) was spin-coated on top of the spacer (Figure 4b). The FQM image in Figure 4c shows that fluorescence quenching of dye molecules by MoS₂ sheets could still be observed through the 15 nm spacer, implying that the underlying quenching mechanism should also be long range energy transfer.

The success of FQM in imaging MoS₂ suggests that it should, in principle, be universally applicable to other 2D materials as well. Although at this stage, large thin flakes of other 2D materials are not easily available to test FQM, simple solution fluorescence quenching studies suggest that FQM should be applicable. Figure 5a shows vials of 2D sheet dispersions of a variety of layered materials including MoS₂, WS₂, Bi₂Te₃, MoSe₂, NbSe₂, and TaS₂, exfoliated by extensive ultrasonication in N-methyl-2-pyrrolidone (NMP) as reported previously. A small aliquot of each suspension was added to solutions of three different dyes: DCM, fluorescein, and 2,5-bis(5-tert-butyl-2-benzoxazolyl)thiophene (BBOT), which emits red, green and blue fluorescence, respectively. The fluorescence spectra in Figure 5b show that all the layered materials investigated were able to quench fluorescence for all the three dyes. This implies that FQM should be applicable for seeing these 2D sheets using one of the three dyes. When methods to produce large area sheets of various 2D materials become available, FQM should offer a very powerful and general imaging tool for rapid sample evaluation.

In conclusion, we showed that FQM can be used to obtain high contrast images of MoS₂ sheets on arbitrary substrates, including transparent and non-conductive substrates with high surface roughness such as common quartz plates. Since fluorescence quenching by long range energy transfer is quite generic, FQM should be applicable to other 2D materials and systems. Solution fluorescence quenching experiments indeed suggest that FQM could be useful for a wide array of layered transition metal chalcogenide materials. As the synthesis of 2D materials advances and large sheets become more available, FQM should become a very useful imaging technique for quick sample characterization, providing rapid feedback to further improve materials synthesis and processing. Moreover, since sheets of different properties should have different quenching efficiency for the same dye molecule, FQM could also be used to differentiate sheets of different materials deposited on the same substrate or patterned sheets with domains of different properties.

Figure 4. Remote fluorescence quenching by a MoS₂ flake. (a) AFM image and height profile of a MoS₂ flake showing the thinnest region corresponds to 2–3 layers. (b) Schematic of the dye–spacer bilayer structure atop a MoS₂ flake. The spacer was measured to be ~15 nm thick. (c) FQM reveals that MoS₂ quenches the fluorescence of dye remotely through the spacer, which suggests that the quenching mechanism is long range energy transfer.
Experimental Section

Preparation of MoS₂ and Graphene Samples: SiO₂/Si wafers were cleaned by standard RCA treatment (1/1/5 = NH₄OH/H₂O₂/de-ionized water) for 20 min, followed by 5 min boiling in acetone and isopropanol. Just before usage, the wafers were cleaned by O₂-plasma for 5 min. Exfoliated MoS₂ sheets were prepared by repeated micromechanical cleavage of single crystal MoS₂ (SPI Supplies) using adhesive tape and deposited onto the freshly cleaned SiO₂/Si wafers. CVD growth of MoS₂ and graphene sheets were carried out according to techniques reported elsewhere. All chemicals were purchased from Sigma-Aldrich and used as is.

Preparation of Fluorescent Films: For FQM of CVD graphene on copper foils, PMMA (M_w = 350 000) doped with DCM was used as the fluorescent film. To prepare the DCM/PMMA solution, 5 mg of DCM was dissolved in 20 mL of anisole. Separately, 400 mg of PMMA was dissolved in 20 mL of anisole. The DCM and PMMA solutions were then mixed in a 1:1 v/v ratio, resulting in an overall concentration of 0.125 mg/mL of DCM, and 10 mg/mL of PMMA in anisole. For FQM of MoS₂ sheets on SiO₂/Si substrates, a similar procedure was followed to prepare a dye/polymer system with an overall concentration of 0.25 mg/mL of fluorescein and 5 mg/mL of PVP (M_w = 55 000) in ethanol. To form a dye/polymer film atop the graphene or MoS₂ samples, the dye/polymer was spin-coated onto the samples at 3000 rpm for 1 min. In our previous work, it was found that the fluorescein/PVP film can be rinsed away with ethanol without disrupting or contaminating the underlying sheets. All chemicals were purchased from Sigma-Aldrich and used as is.

FQM: FQM was performed on a Nikon Eclipse TE2000-U inverted fluorescence microscope, equipped with an Exfo X-cite illumination system as the light source. An ET-GFP filter cube (FITC/Cy2, Chroma Technology Corp) was used to select the appropriate excitation/emission wavelengths. Images were captured with a CCD camera (Photometrics, CoolSNAP HQ²).

Characterization by Other Microscopy Techniques: SEM images were acquired with a FEI NOVA 600 SEM microscope in secondary electron imaging mode at an acceleration voltage of 5 kV for samples on SiO₂/Si substrates and 10 kV for samples on copper foil. AFM images were acquired with a Park Systems XE-100 AFM under tapping mode. Optical microscopy was carried out with an upright microscope (Nikon Eclipse E600 POL) equipped with a CCD camera (Diagnostic Instruments Inc., SPOT Insight QE 4.2).

Liquid-phase Exfoliation and Fluorescence Spectroscopy: Liquid-phase exfoliation was carried out on MoS₂, WS₂, Bi₂Te₃, MoSe₂, NbSe₂, and TaS₂ powders sonicated in NMP (1 mg/mL) for 10 hours. Fluorescence quenching by suspensions of layered materials. (a) Photo showing MoS₂, WS₂, Bi₂Te₃, MoSe₂, NbSe₂, and TaS₂, respectively, exfoliated in NMP by ultrasonication. (b) Fluorescence spectra of (i) DCM, (ii) fluorescein, and (iii) BBOT dyes before and after adding small aliquots of the layered materials, showing apparent quenching of the dye emission.

Figure 5. Fluorescence quenching by suspensions of layered materials. (a) Photo showing MoS₂, WS₂, Bi₂Te₃, MoSe₂, NbSe₂, and TaS₂, respectively, exfoliated in NMP by ultrasonication. (b) Fluorescence spectra of (i) DCM, (ii) fluorescein, and (iii) BBOT dyes before and after adding small aliquots of the layered materials, showing apparent quenching of the dye emission.

References:
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Acknowledgements

J.H. thanks the supports from the Alfred P. Sloan Research Foundation, the National Science Foundation through a CAREER award (DMR 0955612) and the Sony Corporation through a gift donation. J.K. thanks the support from the Ryan Fellowship and the International Institute for Nanotechnology at Northwestern. A.T.L.T thanks the Defence Science and Technology Agency of Singapore for an overseas scholarship and Dr. D. J. Late for helpful discussions. L.J.L thanks the support from Academia Sinica (IAMS and Nano program) and National Science Council Taiwan (NSC-99-2112-M-001-021-MY3).


Received: January 6, 2013 Published online: April 2, 2013