**ABSTRACT:** The discovery and rise of graphene were historically enabled by its ~10% optical contrast on specialized substrates like oxide-capped silicon. However, substantially lower contrast is obtained on transparent substrates. Moreover, it remains difficult to visualize nanoscale defects in graphene, including voids, cracks, wrinkles, and multilayers, on most device substrates. We report the use of interference reflection microscopy (IRM), a facile, label-free optical microscopy method originated in cell biology, to directly visualize graphene on transparent inorganic and polymer substrates at 30−40% image contrast per graphene layer. Our noninvasive approach overcomes typical challenges associated with transparent substrates, including insulating and rough surfaces, enables unambiguous identification of local graphene layer numbers and reveals nanoscale structures and defects with outstanding contrast and throughput. We thus demonstrate in situ monitoring of nanoscale defects in graphene, including the generation of nanocracks under uniaxial strain, at up to 4× video rate.

**KEYWORDS:** Graphene, sample characterization, nanoscale defects, transparent substrates, optical microscopy, optical contrast

IRM is a facile, label-free optical microscopy method originated in cell biology. A collimated beam of filtered lamp light passes through the substrate and is reflected off interfaces between the substrate, culture medium, and cell membrane: the resultant reflection interference provides outstanding contrast for cell adhesion sites (Figure S1). While offering a unique means to study nanoscale cell−substrate interactions, quantitative interpretation of results has been difficult due to complex cell geometries.

By repurposing IRM for imaging graphene, we experimentally achieved excellent contrast for graphene of different layers and provided quantitative explanation to our results. A standard inverted fluorescence microscope with oil- and water-immersion objective lenses was configured with a 50/50 beam splitter and a 532/10 nm bandpass filter (Figure 1a). The field diaphragm was closed down to slightly smaller than the recording frame size to reject stray light. We first examined copper-grown graphene that was wet-transferred onto glass, with water being the top medium (Figure 1b). Highly uniform IRM signal was observed for areas of the same number of graphene layers (m), while excellent contrast were observed for m = 0−4 (Figure 1c,d). The signal contrast of each added layer, as defined by \( C_m = (I_{m-1} - I_m)/(I_{m-1} + I_m)/2 \), where \( I_m \) represents the signal intensity for graphene of m layers, was calculated to be up to 42% for monolayer graphene on transparent substrates, further achieving outstanding sensitivity for nanoscale defects, thus enabling direct, high-throughput inspection at 4× video rate.
is IRM signal intensity on \( m \)-layer graphene \((I_0 \text{ for no graphene coverage})\), was 30\%, 33\%, 35\%, and 34\%, respectively, for \( m = 1 \) to 4 (Figure 1c,d). These results are >10-fold higher than relying on light absorption \((\sim 2\%)\)\(^{5,8,9,24}\) and even 3-fold higher than that is experimentally achieved on optimized SiO\(_2\)-capped-Si substrates \((\sim 10\%)\)\(^{2,3,10}\). The experimental signal-to-noise ratio for each additional layer of graphene, defined as \( \text{SNR}_m = (I_{m-1} - I_m)/[(\sigma_{m-1} + \sigma_m)/2] \), where \( \sigma_m \) is the standard deviation of signal between pixels for the same \( m \), is found to be 34, 32, 31, and 22 for \( m = 1 \)–4, respectively. This result suggests our data should enable unambiguous identification of graphene layers down to subpixel levels. In comparison, conventional transmission light microscopy, performed on the same microscope using the same objective lens (thus the same magnification and numerical aperture) and with comparable light intensities at the same camera, achieved low contrast of \( \sim 2\% \) and SNR of 2–3 for each layer (Figure 1c,d), thus illustrating the common difficulties in characterizing graphene on transparent substrates\(^{8–15}\).

To understand the exceptional contrast we achieved, we adapted IRM theories\(^{19–21}\) but further took into account the finite absorption of graphene and the interferences between infinite times of reflection at the substrate-graphene and graphene-medium interfaces (Figure 1a). Using transfer-matrix
method we found (Methods in Supporting Information) the intensity of reflected light (and thus IRM signal) to be

\[
I = \frac{e^{i\varphi r_{12}} + e^{-i\varphi r_{23}}}{e^{i\varphi} + e^{-i\varphi r_{12}}r_{23}} I_I
\]

where \(I_I\) is the intensity of incident light, \(\varphi = 2\pi n_d\lambda/\lambda\) is the phase change across graphene, \(r_{12} = (n_1 - n_2)/(n_1 + n_2)\), and \(r_{23} = (n_2 - n_3)/(n_2 + n_3)\). Here \(n_1, n_2\) and \(n_3\) are the refractive indices of the substrate, graphene, and overlying medium, respectively, and \(d_2 = 0.335 m\) is the thickness of graphene of \(m\) layers, and \(\lambda\) is the wavelength of incident light. For \(\lambda = 532 nm\), with glass \((n_1 = 1.52)\) and water \((n_1 = 1.33)\) being the substrate and the overlying medium, respectively, and using complex refractive index of graphene \(n_2 = 2.65 - 1.27i\) we thus calculated the theoretical \(C_m\) to be 31%, 34%, 36%, and 35%, for \(m = 1 - 4\), respectively, in good agreement with our experimental results (Figure 1c,d). Equation 1 further predicts that the achieved contrast is relatively insensitive to the wavelength, and we have experimentally achieved comparable contrast without using any optical filters (Figure S2).

The outstanding contrast of IRM is powerful in revealing nanoscale structures and defects in graphene. Figure 2a shows result on a nanopatterned graphene monolayer on glass. Excellent contrast and resolution were observed. Intensity profiles yielded \(\sim 300\) nm feature widths for the finer structures, indicating that the resolution approached the diffraction limit. Figure 2b shows a predominantly monolayer sample that was subjected to mechanical disruptions that would possibly be encountered in device fabrication. Rich features are clearly revealed, e.g., local bilayers (white arrow), tears and fold-overs (magenta arrows), and nanoscale cracks (blue arrows) and wrinkles (green arrows). In comparison, in conventional transmission light microscopy (Figure 2c) the smaller tears are overwhelmed by noise (dashed magenta arrows), and none of the nanoscale cracks or wrinkles are discernible (dashed blue and green arrows).

We next cross-examined IRM with SEM and AFM (Figure 2d–f). As the glass substrate is insulating and unsuitable for SEM, we relied on the conductivity of graphene itself.27 The SEM images (Figures 2e, S3, S4) are in agreement with IRM results but afford significantly lower contrast and SNR. Whereas bilayers and the more prominent wrinkles and cracks are visible (solid arrows), the thinner wrinkles and cracks, which are clearly resolved in IRM, are hardly observable in SEM (dashed green and blue arrows). Intensity profiles indicate that the more prominent wrinkles visualized by SEM achieve SNR \(\sim 2\) (Figure 2e inset and Figure S4): the same wrinkles achieve SNR \(>30\) in IRM (Figure 2d inset and Figure S4).

AFM also yielded structural information consistent with IRM but at much reduced contrast (Figure 2f and Figure S4). Due to the relatively rough surface of glass (RMS roughness \(\sim 0.5\) nm), features \(<\sim 1\) nm in height were difficult to identify. Consequently, bilayers are barely visible (white arrows), and for wrinkles, only the most prominent ones (\(>\sim 1.0\) nm in height) are discernible (green arrows). SNR \(<\sim 2\) was observed for the same wrinkles that achieve SNR \(>30\) in IRM (Figure 2d–f and Figure S4).

In addition to structures and defects in graphene, we note that IRM also provides excellent visualization of nanoscale contaminants, including speckle-like debris and thread-like polymer residuals that match well with SEM and AFM results (Figure 2d–f and Figure S5).

We emphasize that, besides outstanding contrast, IRM is further characterized by exceptional throughput, low invasiveness, and ease of operation. Wide-field images were captured in...
snapshots in ∼10 ms, only limited by the camera framerate. This is ∼1000-times and >10 000-times faster than SEM and AFM, respectively. Moreover, IRM does not require vacuum and avoids possible sample damage due to a scanning tip or electron beam (Figure 3k,l below). Real-time inspection of nanoscale defects is thus readily achieved over large areas at up to 4× video-rate (Videos S1 and S2).

We next examined graphene on other transparent substrates (Figure 3). Excellent IRM contrast of 42% and 39% (Figure 3a,b,h) is respectively observed for monolayers on quartz and CaF₂ substrates, which for their superior optical properties have been often employed for graphene physics and device applications. For flexible substrates, we achieved contrast of 23%–37% for monolayer graphene on five common polymer films, namely, polychlorotrifluoroethylene (Aclar), polycarbonate (PC), polyethylene terephthalate (PET), cellulose acetate (CA), and polyvinyl chloride (PVC) (Figure 3c–f). Nanoscale graphene structures/defects of different types were clearly visualized on all substrates. We note that, except Aclar, in our hands the other polymer films were noticeably attacked by solvents used to dissolve the PMMA protection layer in graphene wet-transfer. For the heat-stable PC substrate, we thus instead transferred graphene using thermal release tapes. Although a high yield was achieved, microscopic cracks were often found in the transferred graphene (Figure 3d). Transfer using Scotch tape led to very low yield and larger cracks (Figure 3e for PET). Wet transfer without PMMA protection led to low yields and frequent cracks (Figure 3f,g for CA and PVC). See Figures S6–S13 for additional data on each substrate under different preparations. By consistently achieving high contrast, IRM thus provides a way to directly characterize and compare defect levels as graphene is transferred to different potential device substrates via different procedures.

We note that these characterizations are difficult to achieve with alternative techniques. Due to the very large surface roughness of commercial-grade polymer films (>10 nm), AFM often does not provide useful contrast (Figure 3i,j). Meanwhile, SEM provides poor contrast and causes major structural changes of the sample (Figure 3k,l and Figure S14) due to electron beam. IRM thus uniquely provides nanoscale structural details for graphene on these substrates.

As a key demonstration of the enabling power of our technique, we next report in situ monitoring of the microscopic failure mode of graphene under strain, an important performance parameter for flexible electronics. Monolayer graphene on Aclar films was subjected to uniaxial stretching, during which process concurrent IRM and electrical characterizations were performed (Figure 4 and Video S3). IRM captured the very onset of graphene failure at ∼0.9% strain, where the observed emergence of nanocracks (Figure 4f and Video S3) coincided with a sudden rise in electrical resistance (Figure 4g and inset). Previous bulk Raman spectroscopy studies on monolayer graphene and carbon fibers indicate mechanical failure at similar strain levels (∼1.0%), although electrical measurements on multilayer devices have reported little resistance change up to 6% strain. Crack propagation ensued upon further stretching, and numerous nanocracks became readily visible in IRM at ∼1.5% strain (Figure 4b and Video S3). The rapid increase in crack density slowed down at ∼4% strain, where nanocracks distributed roughly evenly across graphene at a density of ∼180 mm⁻² (Figure 4c). Crack widening persisted throughout stretching (Figure 4e,f,i); the measured average crack width, as determined from the integrated light intensity of the diffraction-limited IRM images, increased from the initial ∼25 nm to ∼140 nm at 9.4% strain (Figure 4f). Resistance increased monotonically as cracks developed and widened (Figure 4g). These observations bear general similarities to that reported for thin metal oxide films under strain. However, for graphene the traditional SEM approaches are unsuitable (Figure 3k,l).

Our IRM results reveal that for graphene, strain-induced nanocracks are largely, but not strictly (as found in metal oxide films), perpendicular to the stretching direction. Frequent changes in crack orientation and position are observed, and wrinkles parallel to the stretching direction often block crack propagation and lead to discontinued cracks (Figure 4d,h). In contrast, wrinkles perpendicular to the stretching direction are often first flattened out during initial stretching but then evolve into cracks upon further stretching (arrows in Figure 4a–e and Video S3), suggesting mechanical instability. These results exemplify the extreme contrast and throughput of IRM. We thus expect IRM to transform how graphene and other 2D materials are characterized for both research and industrial applications.
REFERENCES