The Future of Engineering Education
2024 Annual Conference & Exposition
Oregon Convention Center
Portland, OR . June 23 - 26, 2024
ASEE
Paper ID #41278

Versatile Recognition of Graphene Layers from Optical Images Under Controlled Illumination Through Green-Channel Correlation Method

Prof. Saquib Ahmed, The State University of New York Buffalo State University

Dr. Ahmed uses DFT, MD, and various Data Analytics tools such as ML and Neural Networks to probe atomistic, molecular, and device level phenomena within photovoltaics, battery and supercapacitors, 2D and quantum materials, and semiconductors.

©American Society for Engineering Education, 2024

Recognition of graphene layers from optical images in varied lighting conditions using the green channel correlation method for versatile identification.

Miah Abdullah Sahriar[1†], Mohd. Rakibul Hasan Abed[1†], Ratchanok Somphonsane[2], Houk Jang[3], Chang-Yong Nam[3], Saquib Ahmed[5,6*]

[1]Department of Materials and Metallurgical Engineering (MME), Bangladesh University of Engineering and Technology (BUET), East Campus, Dhaka-1000, Bangladesh

[2]Department of Physics, School of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand

[3]Center for Functional Nanomaterials, Brookhaven National Laboratory, Upton, New York 11973, USA

[5]Department of Mechanical Engineering Technology, SUNY – Buffalo State University, 1300 Elmwood Avenue, Buffalo, NY 14222, USA

[6]Center for Integrated Studies in Nanoscience and Nanotechnology, SUNY – Buffalo State University, 1300 Elmwood Avenue, Buffalo, NY 14222, USA

*Corresponding author
Email: ahmedsm@buffalostate.edu, Phone: 1.716.878.6006 Fax: 1.716.878.3033

Abstract:

The proposed method for identifying the number of exfoliated graphene layers on an oxide substrate from optical images is both simple and versatile. It involves using a limited number of input images for training, paired with a larger set of well-published Github images for testing and prediction.

This study has employed a linear regression-based method in executing its thresholding process. This method leverages the red, green, and blue color channels of image pixels and establishes a correlation between the green channel of the background and the green channel of various graphene layers. The method is positioned as an alternative to both deep learning-based graphene recognition and traditional microscopic analysis.

Notably, the proposed methodology performs well under conditions where the influence of surrounding light on the graphene-on-oxide sample is minimal. It enables the rapid identification of various graphene layers, showcasing its feasibility for non-destructive identification. The study also addresses the functionality of the methodology under nonhomogeneous lighting conditions, demonstrating successful predictions of graphene layers even in lower-quality images compared to those typically published in literature.

In summary, the proposed methodology offers a quick, inexpensive, and effective means of non-destructively identifying graphene layers from optical images. Its versatility and performance under varying conditions make it a promising approach for practical applications in graphene research. Additionally, and critically: the methods highlighted in this research can be utilized across a multitude of disciplines (from bioengineering to electrical, materials, nanoengineering, etc.) for one of the most fundamental areas of experimental research in STEM at the undergraduate level: accurately identifying multiple systems from optical images. A broad, relevant, and timely curriculum can be built around data analytics and application to solving STEM problems – including components such as data mining, cleaning, wrangling, and analysis, and critically, tying in these processes in solving real experimental challenges in a laboratory setting.

1. Introduction and Background

The realm of 2D material detection has experienced notable progress in recent times. However, certain practical challenges persist. Deep-learning techniques enable precise image recognition by utilizing high-dimensional hierarchical visual characteristics. For example, a deep-learning-driven image segmentation has been previously devised by researchers, employing an algorithm capable of real-time detection of 2D materials through optical microscope images [1]. The neural network underwent training on annotated images encompassing various 2D materials like graphene, hBN, MoS2, and WTe2, utilizing the Mask-RCNN algorithm. Nonetheless, these algorithms demand a substantial amount of time for handling extensive datasets, data preparation, training, and evaluation [2]. Their computational expense, especially on smaller datasets, can be prohibitive. The algorithm is optimized for high-end graphics processing units

(GPUs) like the NVIDIA Tesla V100, resulting in an inference time of 200 Ms. Processing without a GPU significantly prolongs the time, posing challenges in applications where real-time detection is critical. Moreover, the computational cost hinders the practicality of this approach for tasks requiring swift analysis of large datasets.

An alternative method has also been previously developed for accurate counting of graphene flakes on transparent bulk substrates using optical reflection microscopy measurements [3]. The universal optical conductance model matches reflection data for graphene flakes up to nine layers thick. However, achieving maximum sensitivity at the desired wavelength necessitates precise control over oxide thickness and oxide index of refraction. Another proposed method utilizes transmission or reflection optical microscopy to determine the number of graphene monolayers on various substrates [4]. Image modification through software analysis yields a 3D model of few layers of graphene on any substrate. However, this method relies on classical, time-consuming techniques like AFM and Raman spectroscopy for calibration. Additionally, obtaining a large volume of high-quality optical image datasets for these methods remains a challenge.

As an alternative approach, this study demonstrates the identification of graphene layer numbers using contrast in optical images. RGB (red, green, and blue) is a color model in digital images, where each pixel is represented by a combination of red, green, and blue colors in different intensities. The proposed method offers several advantages over traditional machine learning approaches for image recognition. It requires fewer data, works well with adequate lighting, and is computationally inexpensive. Instead of employing deep learning, mono/bi/few layers of graphene are recognized by extracting only the green channel pixel value (G-value) from collected graphene images [5]. The current method is flexible and suitable for controlled environments. It does not necessitate a large dataset or extensive preparation, enabling efficient and rapid analysis of images. The versatility of the approach allows for adaptation to varying conditions by tuning the threshold green channel values between different layers, achieved through a simple process utilizing only a few images for calibration.

Critically, this methodology can be applied across multiple disciplines in working with a fundamental characterization tool in any experimental STEM area: the optical microscope. Not only can the entire suite of RGB techniques be taught to undergraduate students through an interdisciplinary curriculum, from invoking how to collect and analyze data through the eyes of data analytics all the way to the final goal of utilizing these robust scripts (akin but alternative to traditional machine learning) in deciphering various systems captured in an optical image. The non-destructive nature of this methodology in achieving this final goal is an added plus.

2. Materials and Methods

2.1 Graphene Flake Sample Preparation & Deposition

The graphene samples employed in this study were produced through the mechanical exfoliation of graphite on a SiO2 substrate. A 300-nm thermal oxide Si/SiO2 wafer (NOVA Electronic Materials, LLC.) was cleaved into approximately 10 mm × 10 mm samples. These samples underwent a cleaning process, involving a 1-minute ultrasonication in acetone, followed by an IPA rinse, and finally, were dried using nitrogen gas. Subsequent to the cleaning procedure, standard scotch tape exfoliation of KISH graphite (Graphene Supermarket) was utilized to deposit the multiple graphene flakes that were analyzed in this research.

2.2 Image Preparation

The dataset was captured under controlled lighting conditions within a fully enclosed dark box with one open side for the light source. Optical microscopy, a well-established non-destructive method for imaging graphene, was utilized as the standard imaging technique. The microscope used for this set of images was obtained from Sunny Optical Technology Company Limited (Model CX-40M), operating in reflection mode with a magnification of 100X. The intensity of the microscope's LED Kohler illumination source was set to 0-5W.

2.3 Threshold Development

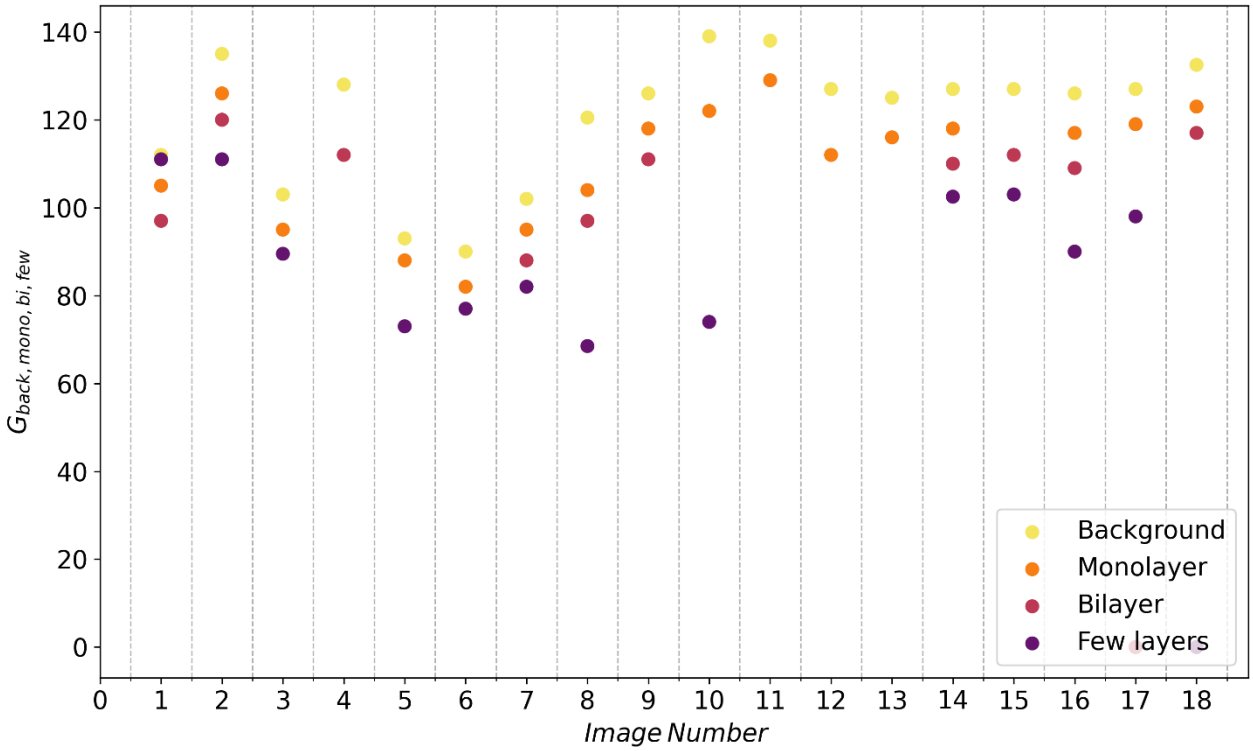

Fig. 1 Scatter plot showing similar order for G values of various layers of each image.

Different layers of graphene were captured in each image. The layers are classified as: "monolayer" (1 layer), "bilayer" (2 layers), and "few layers" (more than 2 layers). An example image is shown in **Fig. 2**

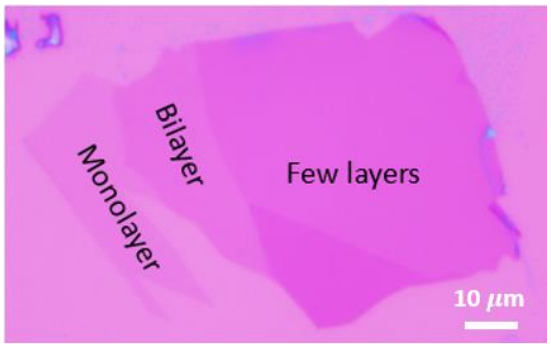

Fig. 2 Different types of graphene layers

A trained analyst manually identified and annotated the layers, and their identification was further validated using Raman spectroscopy. Pixel values were extracted from multiple points on each layer across all images. Specifically, green channel values were isolated from these extracted pixel values, and the median green channel value was determined for each layer based on multiple data points. In **Fig. 1,** a scatter plot illustrates the median green channel values for each layer in all images within the dataset. It's worth noting that certain samples lack specific layers, leading to the omission of corresponding data points in the plot.

The scatter plot (**Fig. 1**) reveals a consistent pattern where, for any given image, the median green channel value of the background consistently surpasses that of the monolayer. Additionally, the median green channel value of the monolayer is higher than that of the bilayer, and this trend continues for higher layer numbers. As the number of graphene layers increases, the corresponding zone appears darker, resulting in a reduction in intensity. So, from **Fig. 1**, **Equation (1)** becomes apparent-

$$G_{back} > G_{mono} > G_{bi} > G_{few} \dots\dots\dots\dots\dots (1)$$

The median green channel values of background (G_{back}), monolayer (G_{mono}), bilayer (G_{bi}), and few layers (G_{few}) graphene are later referred to as G_{layer} throughout the paper. **Fig. 1** suggests that there might be a relationship between G_{back} and G_{layer}. So, the $\frac{G_{mono}}{G_{back}}, \frac{G_{bi}}{G_{back}}, \frac{G_{few}}{G_{back}}$ ratios are

calculated for each individual image from the dataset. The values are succinctly summarized in **Table 1**, from which thresholds were derived using the mean values of the ratio G_{layer}/G_{back} with upper and lower limits defined as $\mu \pm 2\sigma$. This is mathematically shown in **Equation (2)**, where R_L and R_u is respectively lower and upper threshold value of the corresponding layers.

Table 1 Median green channel ratio of different layers to background

Parameter	Value		
	G_{mono}/G_{back}	G_{bi}/G_{back}	G_{few}/G_{back}
Maximum	0.946	0.889	0.869
Minimum	0.863	0.805	0.532
Mean (μ)	0.920	0.867	0.758
Standard Deviation (σ)	0.024	0.023	0.106
Variance (σ^2)	0.0005	0.0005	0.01

$$R_L = \mu - 2\sigma \; and \; R_U = \mu + 2\sigma \ldots \ldots \ldots (2)$$

Table 2 Threshold Values for Identifying Mono, Bi and Few Layers in Graphene

Range	Mono layer	Bilayer	Few layers
R_L	0.873	0.822	0.547
R_U	0.968	0.913	< 0.822

The graphical representation of thresholds is depicted in **Fig. 3**. By comparing the G_{layer}/G_{back} ratio of a given pixel with the established thresholds, it is possible to determine the corresponding layer. However, it is important to note that there exists a degree of overlap between the thresholds of the monolayer and bilayer, introducing uncertainty in the classification of pixels within this overlapping range.

To address this, for the sake of simplicity in the proposed method, ratios falling within the overlapped region will be identified as bilayer, considering the slight margin of error. As a result, segmentation can be performed using the static thresholds developed from the G_{layer}/G_{back} ratio and its standard deviation.

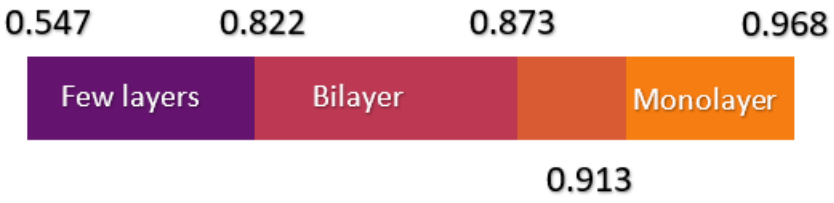

Fig. 3 Visual Representation of Thresholds for Monolayer, Bilayer and Few Layers Identification in Graphene Samples for the dataset

Once again, **Fig. 4** illustrates a linear relationship between G_{back} and G_{layer} for the image dataset. The equation of the best-fitted curve for this relationship is detailed in **Table 4,** where the tuning constant "k" is defined based on the mean deviation of data points from the best-fitted curve. It's crucial to acknowledge that fluctuations in illumination levels lead to variations in the median background green channel value, G_{back}, as well as in the median green channel value of the graphene layer, G_{layer}. However, the ratio of G_{layer} to G_{back} remains nearly constant.

The thresholds for segmenting different graphene layers are established through a linear regression approach, considering this constant ratio. It's important to note that although the linear regression approach used in this research is influenced by lighting conditions, it maintains its intrinsic dependencies and trends even with changes in lighting. Specifically, the linear relationship between G_{back} and G_{layer} remains consistent despite variations in background illumination. Changes in the background median green channel value result in corresponding changes in the median green channel value of the monolayer or bilayer.

However, when dealing with "few layers," as the number of layers in this category can be substantial, establishing a reliable linear relationship becomes challenging. Therefore, it is recommended to classify anything other than monolayer or bilayer as "few layers."

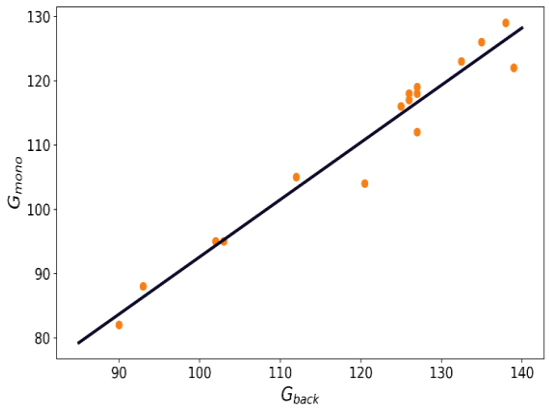

fig (a)

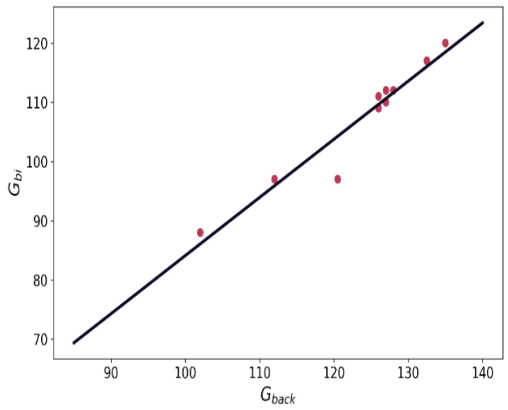

fig (b)

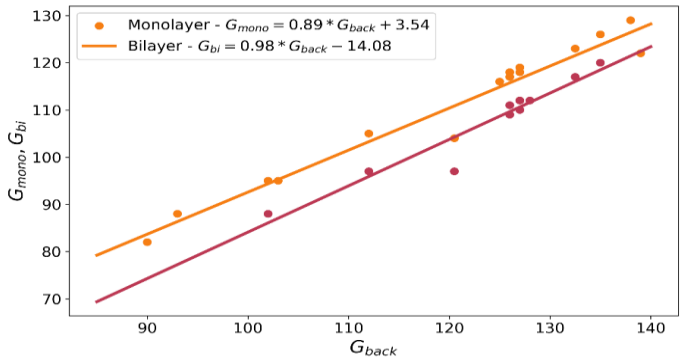

fig (c)

Fig. 4 Linear relation between (a) G_{back} and G_{mono}, (b) G_{back} and G_{bi} (c) parallel relationship between G_{mono} and G_{bi}

From the above developments, we developed the linear regression-based approach (for setting the threshold criteria) which produces a dynamic threshold.

The initial step in this approach involves the acquisition of the median green channel values for the background, monolayer, bilayer, and few layers in each image. A scatter plot of G_{layer} vs G_{back} was then generated, and the best-fitted linear curve was determined. This curve allowed us to predict the median green channel value of the monolayer and bilayer considering variations in G_{back}.

To establish upper and lower thresholds for the median green channel value of each layer, the average negative and positive deviation of the actual data points from the best-fitted curve was calculated and added to the predicted value. This calculation resulted in a dynamic threshold for

the monolayer or bilayer, enabling the segmentation of graphene layers even when the median green channel value of the background is subject to variations.

Table 3 Best-Fitted Linear Regression Equations for Thresholding Different Layers of Graphene

Range	Mono	Bi	Few
Lower (R_L)	$(0.8905 * G_{back} + 3.542) - 1.69$	$(0.982 * G_{back} - 14.08) - 1.23$	-
Upper (R_U)	$(0.8905 * G_{back} + 3.542) + 4.26$	$(0.982 * G_{back} + 14.08) + 4.635$	< R_U(Bi)
General Form	$R = (m * G_{back} + c) + k$		

3. Analysis and Results

The linear regression-based threshold was employed to segment images within the dataset, and the results, as depicted in **Fig. 5**, demonstrate convincing outcomes. In the original images, regions highlighted in yellow represent the monolayer, those outlined in green indicate the bilayer, and those marked in red indicate a few layers. The implementation of this segmentation was facilitated by a MATLAB script, and additional details can be accessed through the provided GitHub link ().

Color code for segmented images	
	Monolayer
	Bilayer
	Few layers

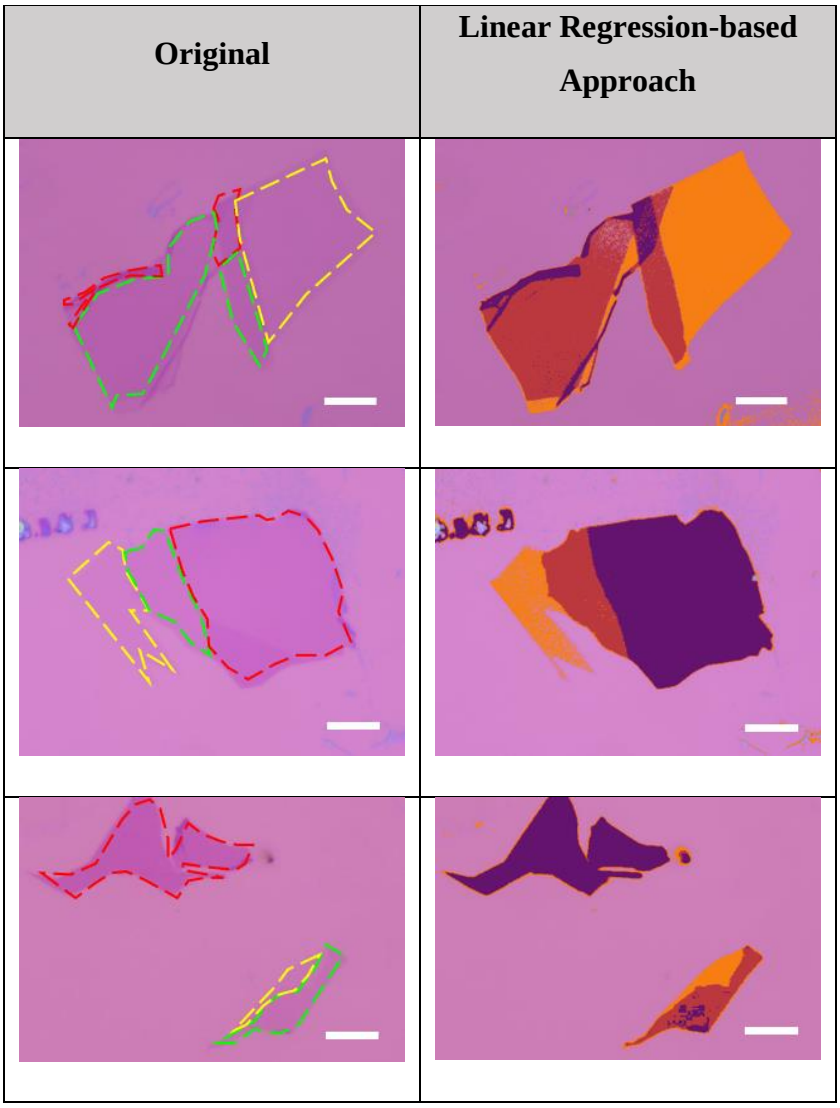

Fig. 5 Comparison of Linear regression approach vs original image of the dataset. The scale bars correspond to 10 µm.

4. Discussion

The accuracy of the linear regression has been evaluated utilizing two methods. The first approach is named 'Manual Cross-Checking'. In order to evaluate the accuracy of the segmentation, the segmented images were manually cross-checked with the annotated images. The segmented images were reviewed and compared them to the ground truth annotations. Each segmented layer was evaluated for its accuracy in terms of identifying monolayer, bilayer, or few layers of graphene. If the segmented regions exhibited a degree of similarity greater than 50% with the annotated images, they were considered to be accurately segmented. It is important to

note that in the ‘manual cross-checking’ process, the segmented images obtained through the linear regression-based approach are visually matched with reference images to calculate the detection accuracy. Due to its reliance on visualization, there may exist certain discrepancies within this evaluation technique. Nevertheless, it offers a rapid means of approximating the accuracy of the process. When comparing its outcomes to a more precise evaluation technique such as the pixel-accuracy ratio (described below), the observed accuracy shows similar trends.

The second approach is referred to as the "Pixel Accuracy Ratio". This metric quantifies the ratio of overlapping pixels between the correctly identified layers and the total number of pixels in the annotated layer. For each segmented layer, the number of pixels correctly classified as monolayer, bilayer, or few layers of graphene was divided by the total number of pixels within that layer. The resulting ratio represents the accuracy of the segmentation process in correctly identifying the number of layers for each pixel. The overall accuracy of the segmentation was determined by calculating the average of the pixel accuracy ratios for all segmented layers. The evaluation of the approaches is shown in **Table 5**.

Table 5 Comparison of Accuracy of the Linear Regression-based Approach for the given image dataset.

Layer	Accuracy (Manual Cross Checking)	Accuracy (Pixel Accuracy Ratio)
Mono layer	83.33%	75.21%
Bilayer	91.67%	78.69%
Few layers	88.24%	89.98%

It is the general observation of the authors that the accuracy of the results might be improved by considering certain factors, such as capturing images in a well-lit central zone with high magnification and in a dark room to avoid interference from surrounding light.

5. Development of Interdisciplinary Curriculum:

As a critical by-product of the current project, the methods highlighted can be utilized across a multitude of disciplines (from bioengineering to electrical, materials, nanoengineering, etc.) for one of the most fundamental areas of experimental research in STEM at the undergraduate level: accurately identifying multiple systems from optical images. A broad, relevant, and timely curriculum can be built around data analytics and application to solving STEM problems – including components such as data mining, cleaning, wrangling, and analysis, and critically, tying in these processes in solving real experimental challenges (like the highlighted optical image analyses) in a laboratory setting. The codes and datasets from this research are made public and available, so they can be utilized as desired.

6. Conclusion

The primary objective of this study was to devise a cost-effective and efficient method for determining the number of graphene layers in optical microscopic images. The linear regression-based approach explores the linear relationship between the background green channel value and the green channel values of graphene layers. A best-fitted curve is obtained, and thresholds are established by observing the constant ratio between different layers and the background. Results demonstrate that the linear regression approach, with its dynamic threshold influenced by the background green channel value, accurately identifies the number of graphene layers under consistent lighting conditions. In conclusion, this proposed methodology provides a non-destructive and cost-effective alternative to traditional graphene layer identification methods. It facilitates swift analysis of optical images with reduced data requirements and computational resources compared to deep learning-based approaches. Particularly beneficial in stable microscopic setups requiring quick results, this approach stands as a valuable tool for graphene layer identification. Additionally, and critically: the methods highlighted in this research can be utilized across a multitude of disciplines (from bioengineering to electrical, materials, nanoengineering, etc.) for one of the most fundamental areas of experimental research in STEM at the undergraduate level: accurately identifying multiple systems from optical images. A broad, relevant, and timely curriculum can be built around data analytics and application to solving STEM problems – including components such as data mining, cleaning, wrangling, and analysis, and critically, tying in these processes in solving real experimental challenges in a laboratory

setting. Further details and the implemented MATLAB script can be found in the provided GitHub link (https://github.com/saquibahmed1981/Image-Processing---first-project).

References:

1. Masubuchi S, Watanabe E, Seo Y, et al (2020) Deep-learning-based image segmentation integrated with optical microscopy for automatically searching for two-dimensional materials. npj 2D Mater Appl 4:3. https://doi.org/10.1038/s41699-020-0137-z
2. Rashid M, Singh H, Goyal V (2020) The use of machine learning and deep learning algorithms in functional magnetic resonance imaging—A systematic review. Expert Syst 37:. https://doi.org/10.1111/exsy.12644
3. Gaskell PE, Skulason HS, Rodenchuk C, Szkopek T (2009) Counting graphene layers on glass via optical reflection microscopy. Appl Phys Lett 94:143101. https://doi.org/10.1063/1.3115026
4. Obelenis F, Champi A (2014) Determination of the Number of Graphene Layers on Different Substrates by Optical Microscopy Technique. Brazilian J Phys 44:682–686. https://doi.org/10.1007/s13538-014-0260-4
5. Palus H (1998) Representations of colour images in different colour spaces. In: The Colour Image Processing Handbook. Springer US, Boston, MA, pp 67–90