CARS IMAGE SEGMENTATION FOR SETS OF DNA AND GRAPHENE-COVERED GLASS

Volodymyr Fedorov[1], Andrej Dementjev[2], Galyna Dovbeshko[1]

[1]Department of Physics of Biological Systems, Institute of Physics, Natl. Acad. of Sci. of Ukraine, Prospect Nauki, 46, Kiev-03028, Ukraine,
[2]Department of Molecular Compound Physics, Center for Physical Sciences and Technology, Saulėtekio al. 3, LT-10257 Vilnius, Lithuania

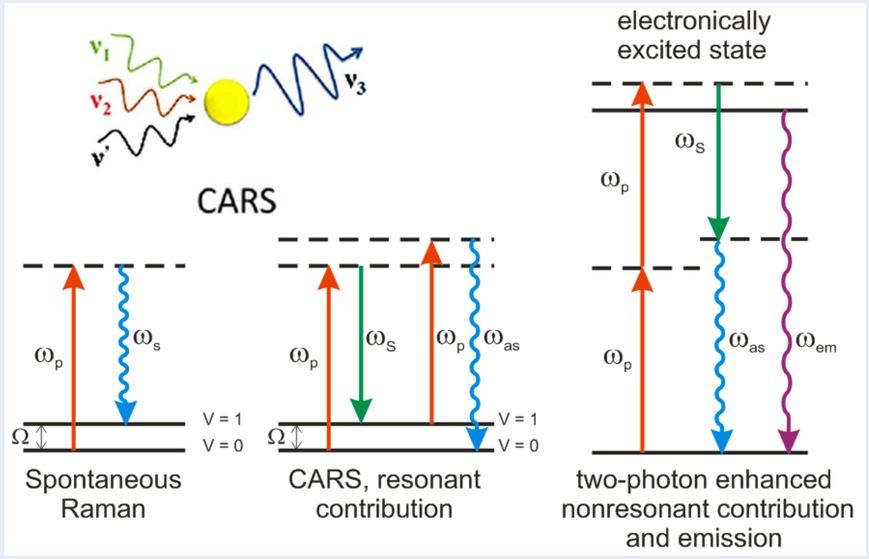
CENTER FOR PHYSICAL SCIENCES AND TECHNOLOGY

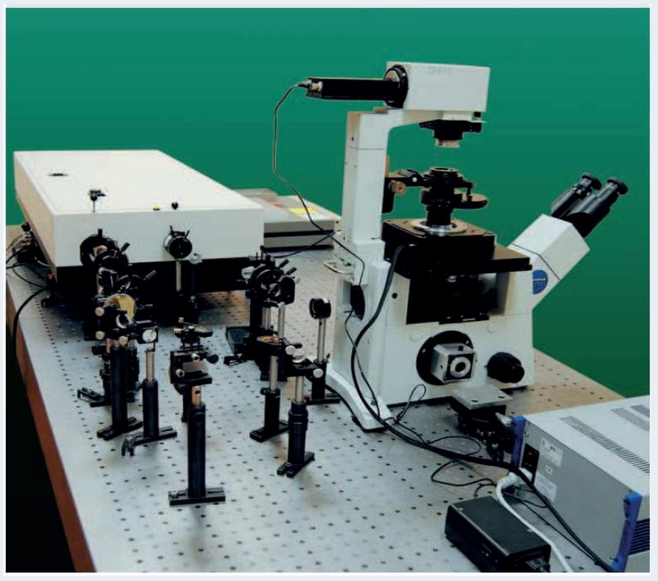
Introduction

Sobel–Feldman operator is used in image processing and computer vision, particularly within edge detection algorithms where it creates an image emphasising edges. Technically, it is a discrete differentiation operator, computing an approximation of the gradient of the image intensity function. At each point in the image, the result of the Sobel–Feldman operator is either the corresponding gradient vector or the norm of this vector.
The Sobel–Feldman operator is based on convolving the image with a small, separable, and integer-valued filter in the horizontal and vertical directions and is therefore relatively inexpensive in terms of computations. On the other hand, the gradient approximation that it produces is relatively crude, in particular for high-frequency variations in the image.

Methods and Materials

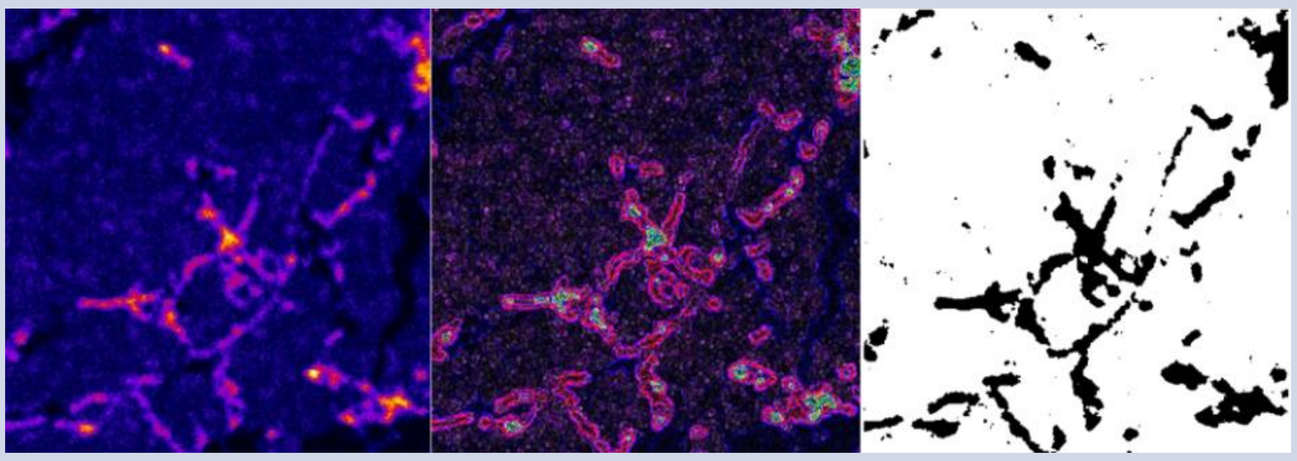
DNA was deposited on the monolayer graphene-covered glass substrate from the aqueous solution with concentration (1mg/ml). CARS images of DNA were registered using a home-built CARS microscope with a compact laser source (EKSPLA Ltd.). The pump and Stokes pulses of 6 ps at 1 MHz repetition frequency were provided by 1064 nm Nd:YVO4 laser and a travelling wave optical parametric oscillator (OPO) operating in the wavelength range from 690 to 2300 nm, respectively. For calculation of quantitative and qualitative parameters of DNA, first of all it is necessary to separate pixels of DNA image from those of monolayer graphene-covered glass. The CARS images consist of a resonance signal from DNA and a non-resonance one from the substrate.





A home-built CARS microscope (Center for Physical Sciences and Technology, Vilnius, Lithuania) with a simple and compact laser (EKSPLA Ltd.) was used. The cell images are registered in the forward and EPI mode at the 1600, 1360 and 2800 cm^{-1} region.

Result and discussion



Original CARS image, (b) – Sobel-Feldman operator image processing, (c)– separated pixels of DNA on graphene-covered glass

The method of image segmentation is based on the use of the Sobel-Feldman operator.

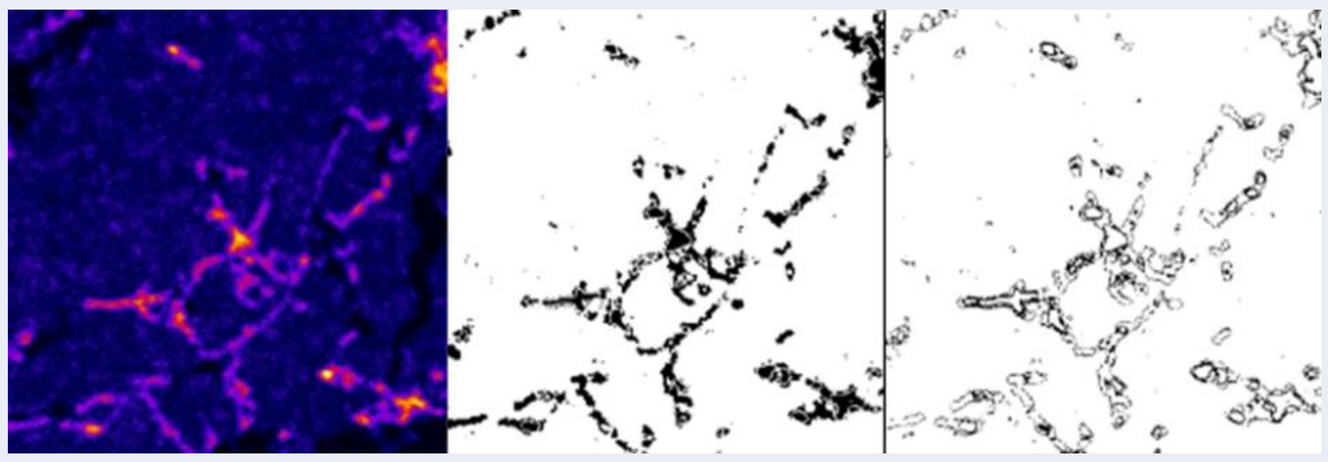
- At the first step, an array of pixels is separated that make (build) up the DNA structures. This array does not contain absolutely all the pixels comprising the DNA image, but every pixel from this set belongs to the DNA structure.
- At the second step, this set is processed and as a result, the global binarization threshold of the original image is calculated.
- At the third step, based on the calculated global binarization threshold, the entire image is divided into two sets, namely, the set A (pixels that make up DNA structure) and the set B (substrate pixels that are not a part of DNA structure).

The data from the RGB color model are converted to the HSL color model (hue, saturation, lightness (intensity)). Let a lightness L be a numerical representation of the light absorption intensity of DNA and the graphene substrate. Let's draw an L histogram for the both pixel sets and calculate a standard deviation of L arithmetic mean for the A (SA) and B (SB) pixels sets. These parameters represent fluctuations of L value for pixels from each of the sets - SA = 0.003716, SB = 0.001257.

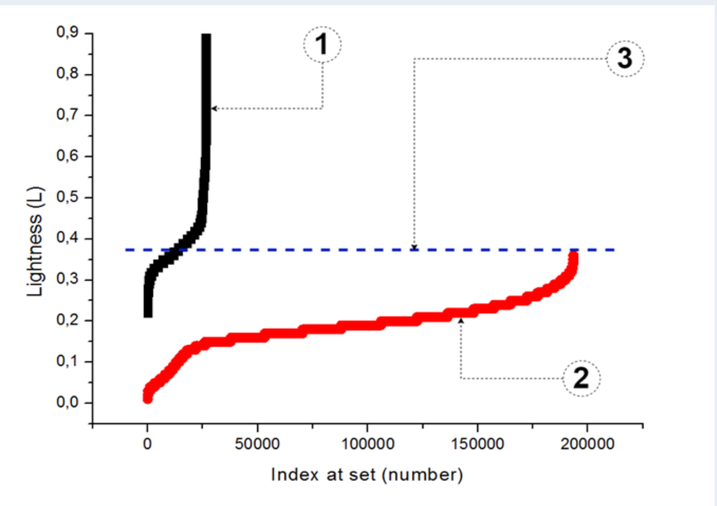
As a result, the L value fluctuations for the pixels from the set A are three times lower than those from the set B. This observation corresponds to the physical nature of CARS imaging DNA and underlying graphene substrate.

The pixels of the set A are clearly divided into three subsets with low, medium and high L value. Let subtract from the set A its intersection with the set B over the L value. As a result, the set A does completely lose its lower L value subset and includes only two subsets comprising the medium- and high-value L. The latter corresponds to Fig. 1b showing the application of the Sobel–Feldman operator to identification of two different sets of pixels in the CARS image of the DNA structures.

Fig. 2. demonstrates the partition of the set A by the maximum value LBmax of the set B into two subsets A1 and A2. A1 is a subset of pixels with L>LBmax and A2 is a subset of pixels with L< LBmax. The pixels of the subset A2 mainly lie at the boundaries between the substrate and the A1 subset pixels.



a – original CARS image, b – subset A1 - non intersection of A set with B by L value , c – subset A2 - intersection of Set A with set B by L-value.



Histogram of lightness L of pixels from original image: 1 – lightness LA of pixels from the set A (DNA), 2 – lightness LB of pixels from the set B (graphene-covered glass), 3 – threshold of intersection of LA and LB)

Conclusions and Acknowledgments

Therefore, the pixels of A2 subset are the regions of uncertainty where the original recorded analogous signal, after registration and digitization, could belong to both the DNA structure and the substrate. As a result, the A1 subset (Fig. 3b) is the required set of pixels, containing the data only from the DNA structures.