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Progress on the Studies on Visual Detection and Surface Modification Testing of Glass Microfiber Filter Based Biosensor

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Summary of Previous Work

► Surface of glass microfiber paper was modified with 3-aminopropyltriethoxysilane (APTES) and was suggested to be used as a biosensor in this surface-modified form.

► As an application, visual detection of the surface-immobilized yeast cells was achieved by Gram staining and shown to be increasing by modification with APTES. (*This'll not be presented here*.)

► DNA detection was performed by using unmodified and APTES-modified surface as well and visualized with YOYO-1 fluorescence upon 200 nM double stranded DNA binding.

► Surface adsorption tendency of YOYO-1 was offered as a testing method of proper surface coverage, for sensors with glass-based active surfaces.

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Materials & Methods

► Fluorescent microscope images were captured at 200 ISO, with the GFP filter. Brightness of the images was increased 64 %, for clarity.

► Invalid regions were removed from the original images.

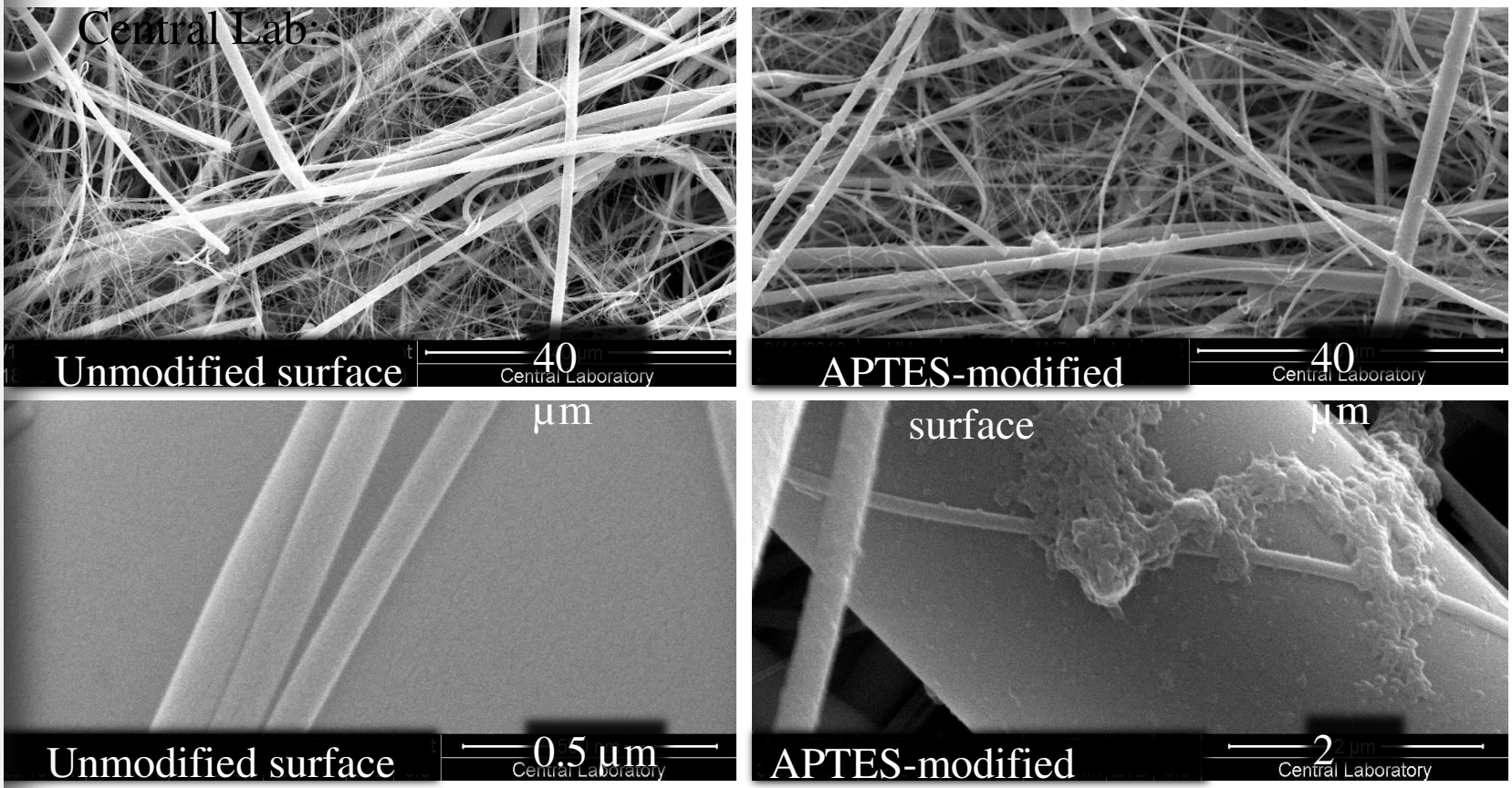
► YOYO-1 concentration in the controls were 3 folds more, as a result of experimental procedure. Brightness of the relevant images were reduced 66 %, to equilibrate YOYO-1 intensities.

► RGB profiles were plotted on a descending diagonal line that was drawn on the images, with the plugin tools of the ImageJ 1.47v.

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Results
– APTES Modification of the Surface –

Scanning electron microscopic (SEM) characterization by QUANTA 400F Field Emission SEM of the Middle East Technical University, Central Lab:



SEM images of unmodified and APTES-modified glass microfiber paper surfaces. Samples were coated with gold-palladium.

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Results

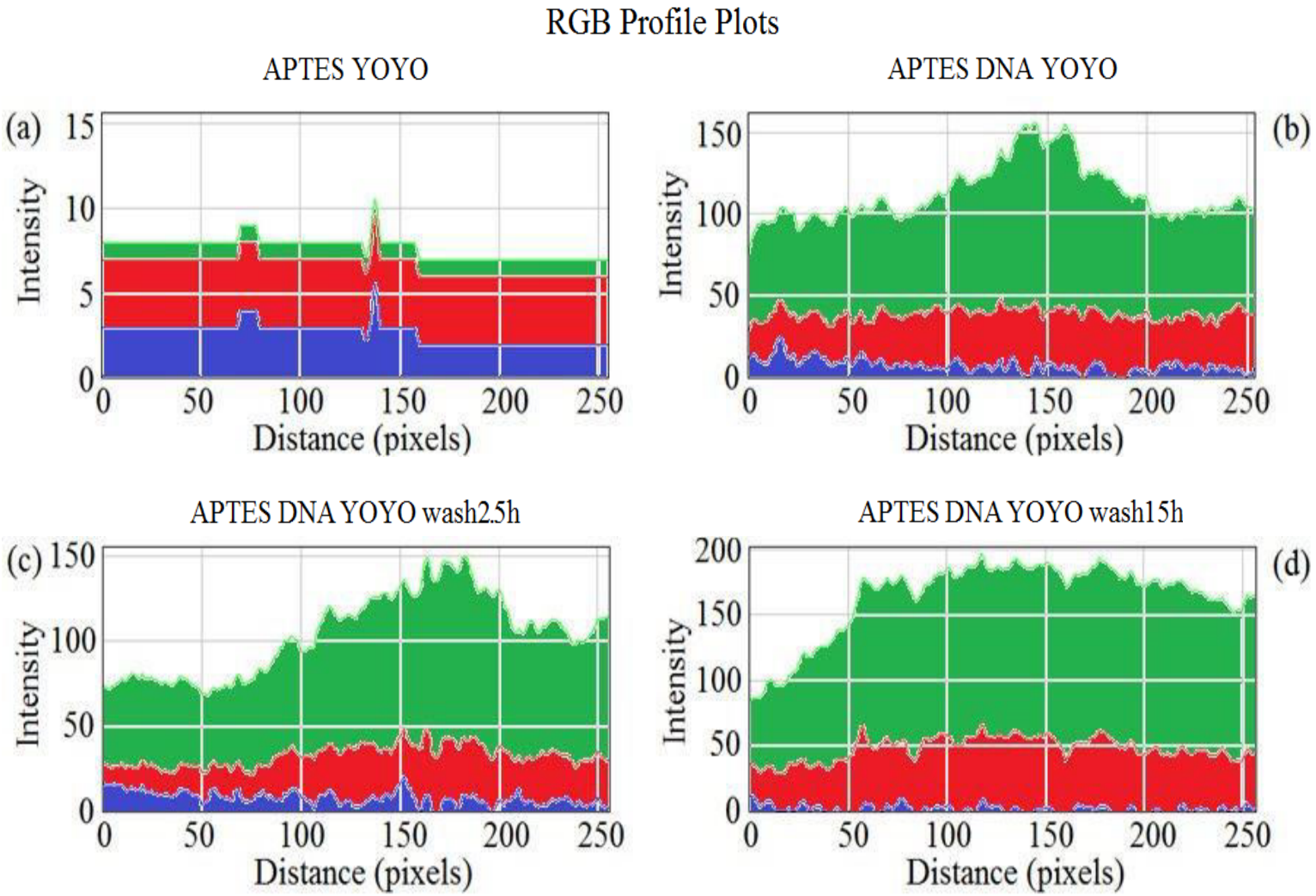
Fluorescent Microscope Images of the Samples Treated Differently*

* "Different treatment" stands for: Presence or absence of APTES-modification; or application of YOYO-1 preliminary to DNA; or changing incubation durations after washing, following treatments.

Background Image: Exemplary image for YOYO-1 interaction with DNA on glass microfiber filter paper.

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(Figure 1) RGB profile plots of fluorescent microscope images of APTES-modified samples that were treated with YOYO-1 only, or YOYO-1 and DNA, in the given order, followed by washing and incubation.

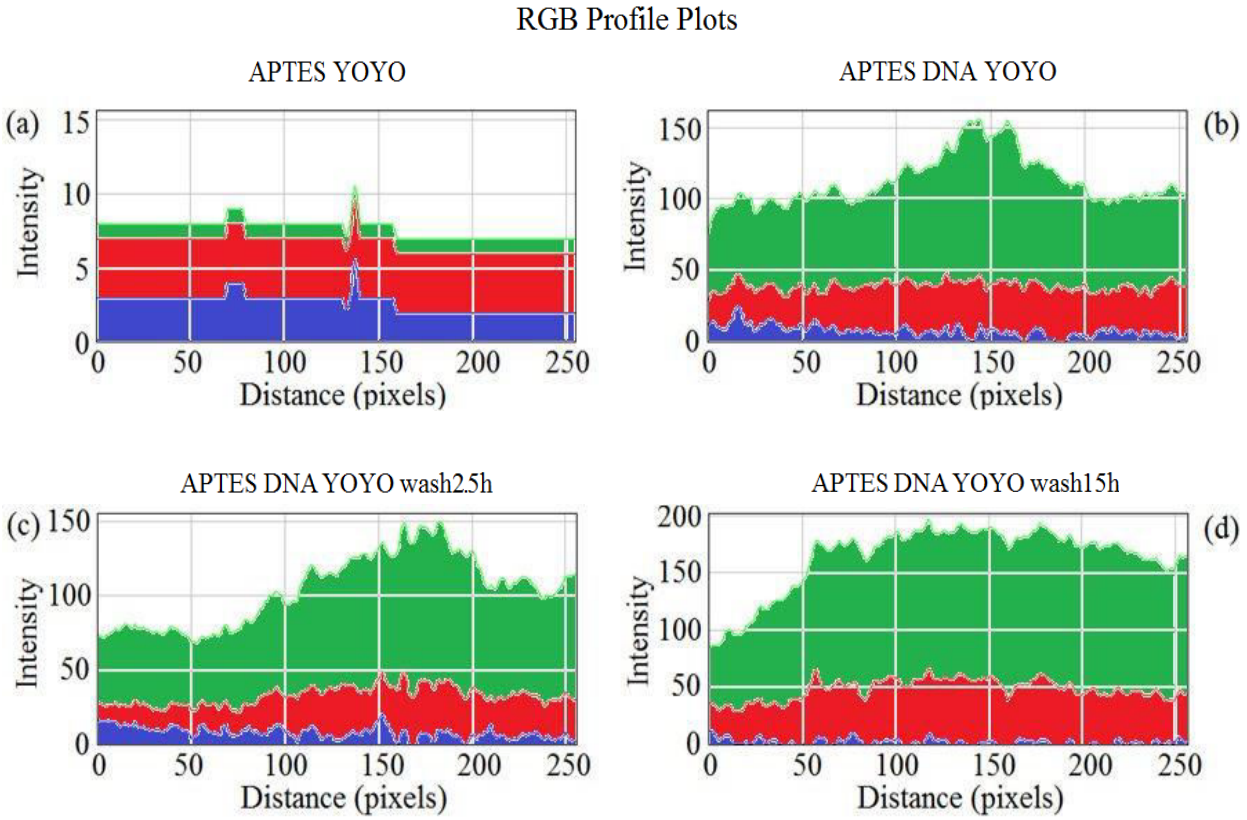


RGB profile plots of the data, published in Y. Adiguzel and H. Kulah, (2014) *Biosens. Bioel.* 54:27.

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Interpretation of Figure 1

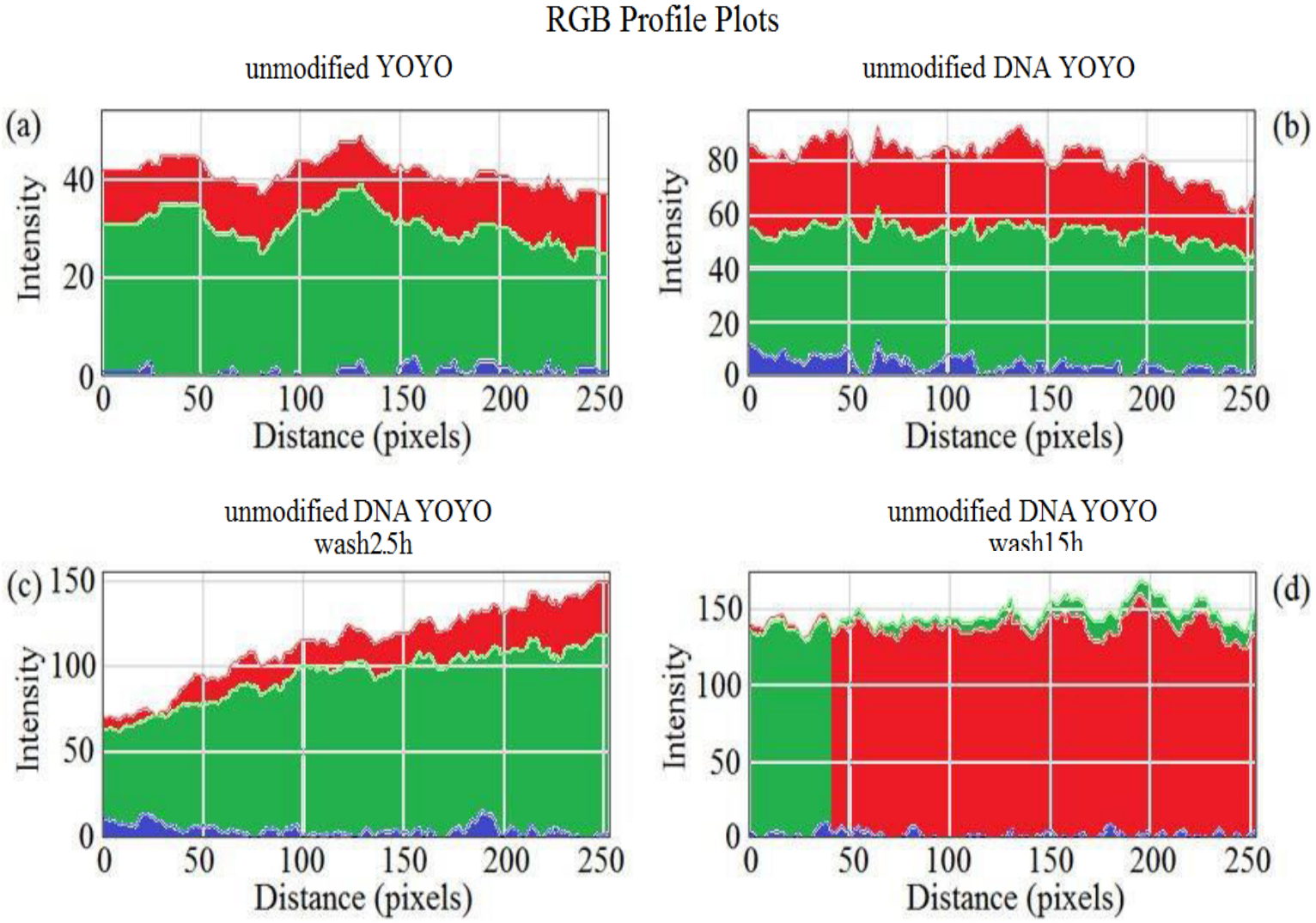
► YOYO-1 leads to a characteristic green fluorescence, when intercalated into the double-stranded DNA, as can be seen through the green channel values in (a) compared to the rest.



RGB profile plots of the data, published in Y. Adiguzel and H. Kulah, (2014) *Biosens. Bioel.* 54:27.

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(Figure 2) RGB profile plots of fluorescent microscope images of unmodified samples that were treated with YOYO-1 only, or YOYO-1 and DNA, in the given order, followed by washing and incubation.

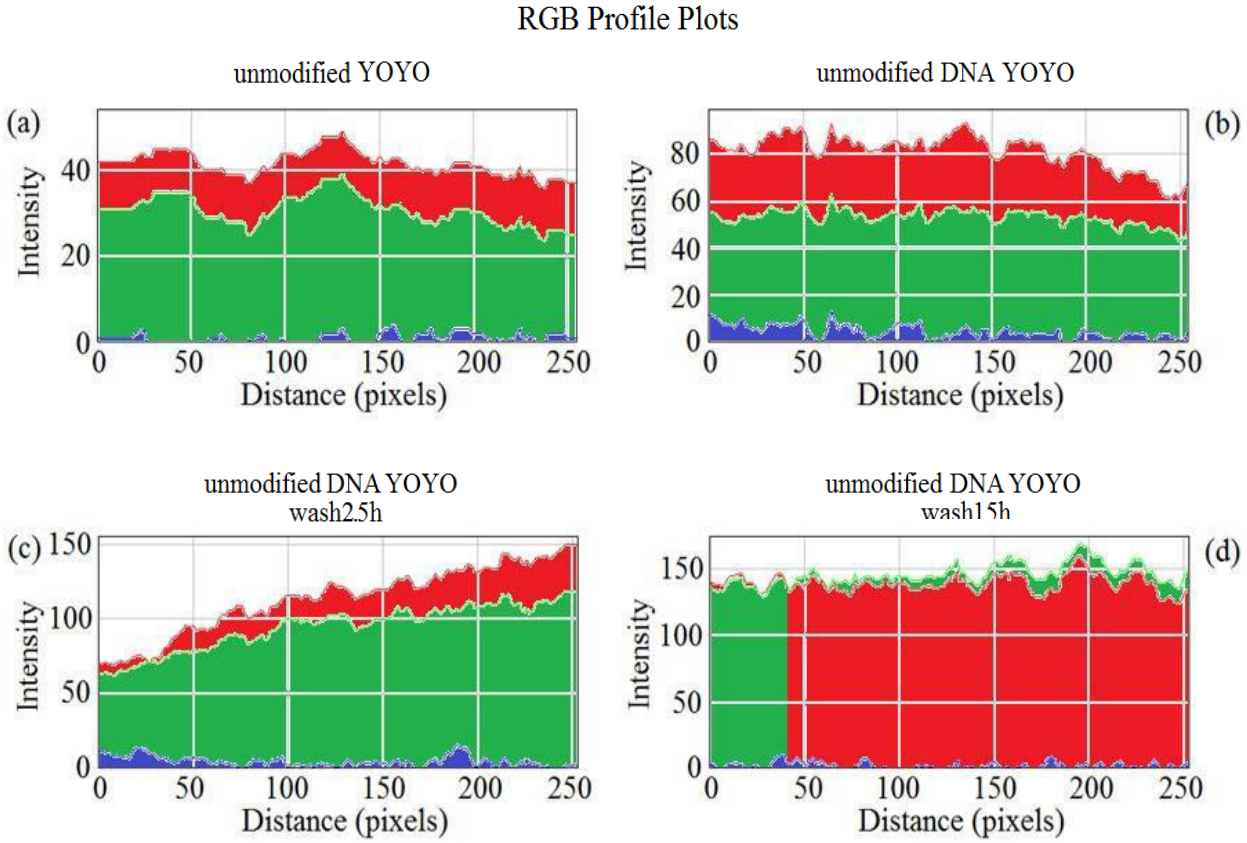


RGB profile plots of the data, published in Y. Adiguzel and H. Kulah, (2014) *Biosens. Bioel.* 54:27.

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Interpretation of Figure 2

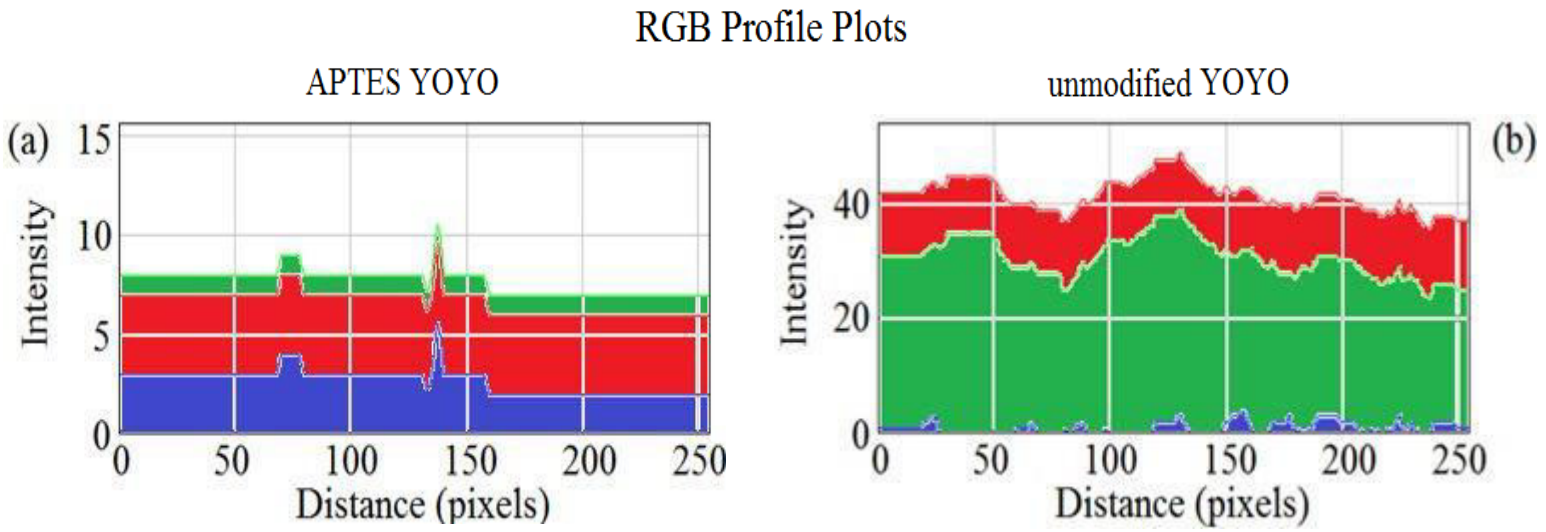
► Green YOYO-1 fluorescence was accompanied and even dominated by red fluorescence, when surface was unmodified. Intensity of these fluorescence signals rose with DNA addition.



RGB profile plots of the data, published in Y. Adiguzel and H. Kulah, (2014) *Biosens. Bioel.* 54:27.

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(Figure 3) RGB profile plots of fluorescent microscope images of YOYO-1 on APTES-modified *versus* unmodified samples.



Interpretation of Figure 3

► Green YOYO-1 fluorescence was accompanied by red fluorescence, when surface was unmodified, due to adsorption of YOYO-1 directly onto the glass surface.

► Apt coverage of the glass surfaces is important for surface modifications and following interaction kinetics.

► So, YOYO-1 can be used to test proper surface coverage.

RGB profile plots of the data, published in Y Adiguzel and H. Kulah, (2014) *Biosens. Bioel.* 54:27

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Conclusions of the Previous Work

► YOYO-1 leads to green fluorescence, when intercalated into double-stranded DNA, on APTES-modified glass surface.

► Sensitivity was found to be 2 nM (*Data was not presented here*.)

► YOYO-1 sourced emission remained with little intensity loss, after sample washing and incubation for 2.5 h more. This loss was remediated till 15 h.

► YOYO-1 fluorescence was accompanied and dominated by red fluoresÐeŶÐe, wheŶ surfaÐe was uŶŵodified. It's proďadle that this was sourced by YOYO-1 adsorption on surface.

► YOYO-1 sourced emission did not undergo any intensity loss, after sample washing and incubation for 2.5 h more.

► Apt coverage of the glass surfaces is important for surface modifications and following interaction kinetics.

► YOYO-1 can be used to test proper surface coverage.

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Further Analysis: Analysis of R/G Mean Ratios

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Further Analysis: Analysis of R/G Mean Ratios – Materials & Methods –

► Fluorescent microscope images were captured at 1600 ISO and 200 ISO, with the GFP filter.

► Invalid regions were removed from the original images.

► YOYO-1 concentration in the controls were 3 folds more, due experimental procedure. Brightness of relevant images were reduced 66 %, to equilibrate YOYO-1 intensities.

► RGB profiles were plotted on a descending diagonal line that was drawn on the images, with the plugin tools of the ImageJ 1.47v.

► R/G mean ratios were calculated from the RGB mean values of the color histogram results, which was obtained by using the color histogram plugin of ImageJ 1.47v.

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Changes in R/G Mean Ratios by Exposure

YOYO-1 on APTES-modified samples	**Change in the R/G mean ratio of images, when measured with 200iso, after the 1600iso measurements**				
	0 min incubation	1 min incubation	1 h incubation	4 h incubation	12 h incubation
Before washing (1st test)	⇧ from 1.46 to 1.53	⇧ from 1.00 to 1.23	⇧ from 1.05 to 1.25	⇧ from 1.04 to 1.11	⇩ from 0.93 to 0.91
Before washing (2nd test)	⇧ from 1.02 to 1.54	⇧ from 1.00 to 1.23	⇧ from 1.04 to 1.23	⇧ from 0.99 to 1.02	⇩ from 0.93 to 0.92
After washing (1st test)	⇧ from 1.02 to 1.55	⇧ from 1.09 to 1.22	⇧ from 1.04 to 1.22	⇧ from 0.77 to 0.78	⇩ from 0.75 to 0.69
After washing (2nd test)	⇧ from 1.02 to 1.54	⇧ from 1.00 to 1.22	⇧ from 1.06 to 1.17	⇧ from 0.68 to 0.70	⇩ from 0.74 to 0.70

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Discussion for Further Analysis

► During imaging, changing the ISO from 1600 to 200, decreases the exposure 8 folds. This change is normally not expected to influence R/G mean ratios of the RGB values.

► When imaging YOYO-1 fluorescence on the APTES-modified samples, decreasing the exposure level 8 folds increased the R/G mean ratios, for the measurements including and until the one at 4 h of incubation.

► Conversely, decreasing the exposure level 8 folds lead to diminished R/G mean ratios, in case of 12 h incubation.

► This latter observation could have implications in YOYO-1 interaction with the surfaces and DNA, such as differences in the fluorescent emission gains of molecules, or varying emission gains of the same molecule at diverse wavelengths.

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Paper-Based Microfluidics
– Previous Studies –

► Oyama et al. [(2012) *Lab Chip* 12:5155)] previously reported electroosmotic lateral flow immunoassay, with glass fiber sheets.

► In relation to this work, they confirmed the linear relationship between the concentration of analyte and the resulting fluorescence intensity from the immunoassay of C-reactive protein and insulin.

► Fang et al. [(2014) *Lab Chip* 14:911)] reported paper-based microfluidics with high resolution cut on a glass fiber membrane for bioassays.

► Microchannels were produced with a common cutter.

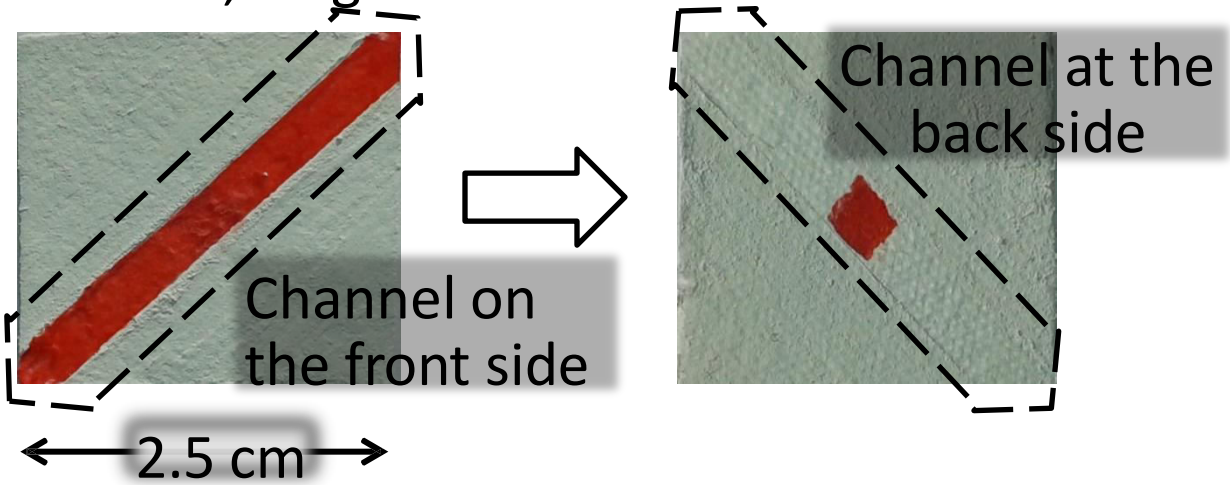
► By this means, they created a star micro-array format of multiplexed urine tests.

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Paper-Based Microfluidics
– Current Approach –

► It is possible to realize glass microfiber filter paper-based microfluidics approaches, utilizing both sides of the filter paper to built interconnected fluid channels.

► Accordingly, water-based dye, applied form a macro channel on the front side of the glass microfiber paper, is transmitted to the back side, only at the cross-section of another channel, diagonal to the one on the front:



► MEMS-based coating is likely to overcome this limitation.

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Summary

► Progress on the Studies on Visual Detection and Surface Modification Testing of Glass Microfiber Filter Based Biosensor is presented through:

1-) Data of the previous work for DNA detection by YOYO-1 fluorescence on glass microfiber paper [Y. Adiguzel and H. Kulah, (2014) *Biosens. Bioel.* 54:27], which was presented & discussed through the re-drawn RGB profile plots of the data.

2-) Performing further analysis of the data, by calculating the R/G mean ratios. R/G mean ratios were changing, along with the ISO level that resulted in 8 folds less exposure. This outcome can have relevance to some physical phenomena, and YOYO-1 interaction kinetics with DNA.

3-) Initial and early paper-based microfluidics approaches that are utilizing glass microfiber papers were presented.

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► Istanbul Kemerburgaz University, for financial support.

► BioMEMS group of METU-MEMS.

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