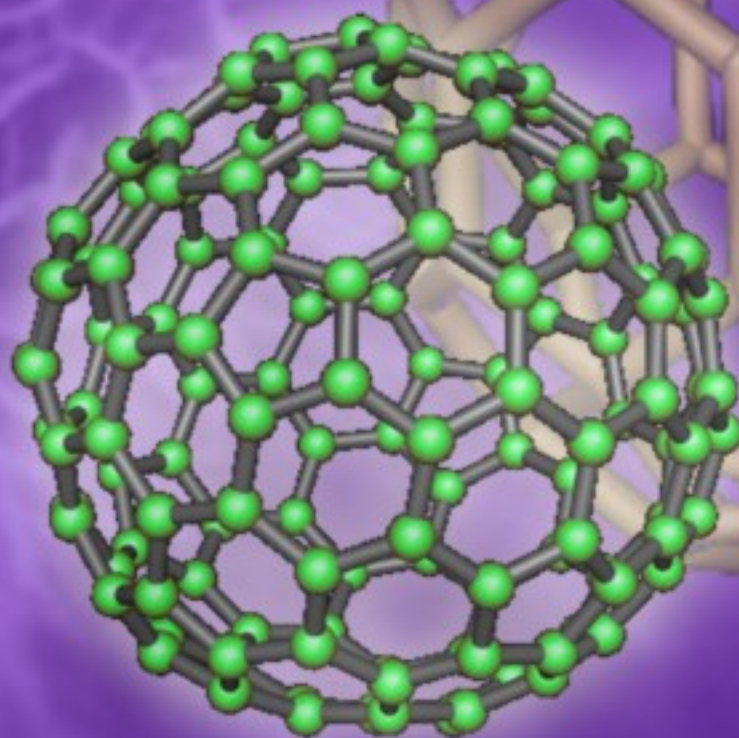
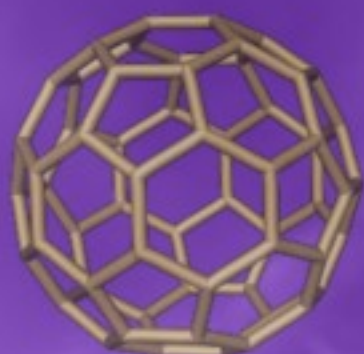


ISSN 1726-5479

SENSORS & TRANSDUCERS

11^{vol. 110}
/09



Nanosensors and Nanodevices

International Frequency Sensor Association Publishing





Editors-in-Chief: professor Sergey Y. Yurish,
Phone: +34 696067716, fax: +34 93 4011989, e-mail: editor@sensorsportal.com

Editors for Western Europe

Meijer, Gerard C.M., Delft University of Technology, The Netherlands
Ferrari, Vittorio, Università di Brescia, Italy

Editor South America

Costa-Felix, Rodrigo, Inmetro, Brazil

Editor for Eastern Europe

Sachenko, Anatoly, Ternopil State Economic University, Ukraine

Editors for North America

Datskos, Panos G., Oak Ridge National Laboratory, USA
Fabien, J. Josse, Marquette University, USA
Katz, Evgeny, Clarkson University, USA

Editor for Asia

Ohyama, Shinji, Tokyo Institute of Technology, Japan

Editor for Asia-Pacific

Mukhopadhyay, Subhas, Massey University, New Zealand

Editorial Advisory Board

- Abdul Rahim, Ruzairi**, Universiti Teknologi, Malaysia
Ahmad, Mohd Noor, Northern University of Engineering, Malaysia
Annamalai, Karthigeyan, National Institute of Advanced Industrial Science and Technology, Japan
Arcega, Francisco, University of Zaragoza, Spain
Arguel, Philippe, CNRS, France
Ahn, Jae-Pyoung, Korea Institute of Science and Technology, Korea
Arndt, Michael, Robert Bosch GmbH, Germany
Ascoli, Giorgio, George Mason University, USA
Atalay, Selcuk, Inonu University, Turkey
Atghiaee, Ahmad, University of Tehran, Iran
Augutis, Vygantas, Kaunas University of Technology, Lithuania
Avachit, Patil Lalchand, North Maharashtra University, India
Ayesh, Aladdin, De Montfort University, UK
Bahreyni, Behraad, University of Manitoba, Canada
Baliga, Shankar, B., General Monitors Transnational, USA
Baoxian, Ye, Zhengzhou University, China
Barford, Lee, Agilent Laboratories, USA
Barlingay, Ravindra, RF Arrays Systems, India
Basu, Sukumar, Jadavpur University, India
Beck, Stephen, University of Sheffield, UK
Ben Bouzid, Sihem, Institut National de Recherche Scientifique, Tunisia
Benachaiba, Chellali, Universitaire de Bechar, Algeria
Binnie, T. David, Napier University, UK
Bischoff, Gerlinde, Inst. Analytical Chemistry, Germany
Bodas, Dhananjay, IMTEK, Germany
Borges Carval, Nuno, Universidade de Aveiro, Portugal
Bousbia-Salah, Mounir, University of Annaba, Algeria
Bouvet, Marcel, CNRS – UPMC, France
Brudzewski, Kazimierz, Warsaw University of Technology, Poland
Cai, Chenxin, Nanjing Normal University, China
Cai, Qingyun, Hunan University, China
Campanella, Luigi, University La Sapienza, Italy
Carvalho, Vitor, Minho University, Portugal
Cecelja, Franjo, Brunel University, London, UK
Cerda Belmonte, Judith, Imperial College London, UK
Chakrabarty, Chandan Kumar, Universiti Tenaga Nasional, Malaysia
Chakravorty, Dipankar, Association for the Cultivation of Science, India
Changhai, Ru, Harbin Engineering University, China
Chaudhari, Gajanan, Shri Shivaji Science College, India
Chavali, Murthy, VIT University, Tamil Nadu, India
Chen, Jiming, Zhejiang University, China
Chen, Rongshun, National Tsing Hua University, Taiwan
Cheng, Kuo-Sheng, National Cheng Kung University, Taiwan
Chiang, Jeffrey (Cheng-Ta), Industrial Technol. Research Institute, Taiwan
Chiriac, Horia, National Institute of Research and Development, Romania
Chowdhuri, Arijit, University of Delhi, India
Chung, Wen-Yaw, Chung Yuan Christian University, Taiwan
Corres, Jesus, Universidad Publica de Navarra, Spain
Cortes, Camilo A., Universidad Nacional de Colombia, Colombia
Courtois, Christian, Universite de Valenciennes, France
Cusano, Andrea, University of Sannio, Italy
D'Amico, Arnaldo, Università di Tor Vergata, Italy
De Stefano, Luca, Institute for Microelectronics and Microsystem, Italy
Deshmukh, Kiran, Shri Shivaji Mahavidyalaya, Barshi, India
Dickert, Franz L., Vienna University, Austria
Dieguez, Angel, University of Barcelona, Spain
Dimitropoulos, Panos, University of Thessaly, Greece
Ding, Jianning, Jiangsu Polytechnic University, China
Djordjevic, Alexandar, City University of Hong Kong, Hong Kong
Donato, Nicola, University of Messina, Italy
Donato, Patricio, Universidad de Mar del Plata, Argentina
Dong, Feng, Tianjin University, China
Drljaca, Predrag, Instersema Sensoric SA, Switzerland
Dubey, Venketesh, Bournemouth University, UK
Enderle, Stefan, Univ. of Ulm and KTB Mechatronics GmbH, Germany
Erdem, Gursan K. Arzum, Ege University, Turkey
Erkmen, Aydan M., Middle East Technical University, Turkey
Estelle, Patrice, Insa Rennes, France
Estrada, Horacio, University of North Carolina, USA
Faiz, Adil, INSA Lyon, France
Fericean, Sorin, Balluff GmbH, Germany
Fernandes, Joana M., University of Porto, Portugal
Francioso, Luca, CNR-IMM Institute for Microelectronics and Microsystems, Italy
Francis, Laurent, University Catholique de Louvain, Belgium
Fu, Weiling, South-Western Hospital, Chongqing, China
Gaura, Elena, Coventry University, UK
Geng, Yanfeng, China University of Petroleum, China
Gole, James, Georgia Institute of Technology, USA
Gong, Hao, National University of Singapore, Singapore
Gonzalez de la Rosa, Juan Jose, University of Cadiz, Spain
Granel, Annette, Goteborg University, Sweden
Graff, Mason, The University of Texas at Arlington, USA
Guan, Shan, Eastman Kodak, USA
Guillet, Bruno, University of Caen, France
Guo, Zhen, New Jersey Institute of Technology, USA
Gupta, Narendra Kumar, Napier University, UK
Hadjiloucas, Sillas, The University of Reading, UK
Haider, Mohammad R., Sonoma State University, USA
Hashsham, Syed, Michigan State University, USA
Hasni, Abdelhafid, Bechar University, Algeria
Hernandez, Alvaro, University of Alcalá, Spain
Hernandez, Wilmar, Universidad Politecnica de Madrid, Spain
Homentcovski, Dorel, SUNY Binghamton, USA
Horstman, Tom, U.S. Automation Group, LLC, USA
Hsiai, Tzung (John), University of Southern California, USA
Huang, Jeng-Sheng, Chung Yuan Christian University, Taiwan
Huang, Star, National Tsing Hua University, Taiwan
Huang, Wei, PSG Design Center, USA
Hui, David, University of New Orleans, USA
Jaffrezic-Renault, Nicole, Ecole Centrale de Lyon, France
Jaime Calvo-Galleg, Jaime, Universidad de Salamanca, Spain
James, Daniel, Griffith University, Australia
Janting, Jakob, DELTA Danish Electronics, Denmark
Jiang, Liudi, University of Southampton, UK
Jiang, Wei, University of Virginia, USA
Jiao, Zheng, Shanghai University, China
John, Joachim, IMEC, Belgium
Kalach, Andrew, Voronezh Institute of Ministry of Interior, Russia
Kang, Moonho, Sunmoon University, Korea South
Kaniusas, Eugenijus, Vienna University of Technology, Austria
Katake, Anup, Texas A&M University, USA
Kausel, Wilfried, University of Music, Vienna, Austria
Kavasoglu, Nese, Mugla University, Turkey
Ke, Cathy, Tyndall National Institute, Ireland
Khan, Asif, Aligarh Muslim University, Aligarh, India
Sapozhnikova, Ksenia, D.I.Mendeleyev Institute for Metrology, Russia

Kim, Min Young, Kyungpook National University, Korea South
Ko, Sang Choon, Electronics and Telecommunications Research Institute, Korea South
Kockar, Hakan, Balikesir University, Turkey
Kotulska, Malgorzata, Wroclaw University of Technology, Poland
Kratz, Henrik, Uppsala University, Sweden
Kumar, Arun, University of South Florida, USA
Kumar, Subodh, National Physical Laboratory, India
Kung, Chih-Hsien, Chang-Jung Christian University, Taiwan
Lacnjevac, Caslav, University of Belgrade, Serbia
Lay-Ekuakille, Aime, University of Lecce, Italy
Lee, Jang Myung, Pusan National University, Korea South
Lee, Jun Su, Amkor Technology, Inc. South Korea
Lei, Hua, National Starch and Chemical Company, USA
Li, Genxi, Nanjing University, China
Li, Hui, Shanghai Jiaotong University, China
Li, Xian-Fang, Central South University, China
Liang, Yuanchang, University of Washington, USA
Liawruangrath, Saisunee, Chiang Mai University, Thailand
Liew, Kim Meow, City University of Hong Kong, Hong Kong
Lin, Hermann, National Kaohsiung University, Taiwan
Lin, Paul, Cleveland State University, USA
Linderholm, Pontus, EPFL - Microsystems Laboratory, Switzerland
Liu, Aihua, University of Oklahoma, USA
Liu Changgeng, Louisiana State University, USA
Liu, Cheng-Hsien, National Tsing Hua University, Taiwan
Liu, Songqin, Southeast University, China
Lodeiro, Carlos, University of Vigo, Spain
Lorenzo, Maria Encarnacio, Universidad Autonoma de Madrid, Spain
Lukaszewicz, Jerzy Pawel, Nicholas Copernicus University, Poland
Ma, Zhanfang, Northeast Normal University, China
Majstorovic, Vidosav, University of Belgrade, Serbia
Marquez, Alfredo, Centro de Investigacion en Materiales Avanzados, Mexico
Matay, Ladislav, Slovak Academy of Sciences, Slovakia
Mathur, Prafull, National Physical Laboratory, India
Maurya, D.K., Institute of Materials Research and Engineering, Singapore
Mekid, Samir, University of Manchester, UK
Melnyk, Ivan, Photon Control Inc., Canada
Mendes, Paulo, University of Minho, Portugal
Mennell, Julie, Northumbria University, UK
Mi, Bin, Boston Scientific Corporation, USA
Minas, Graca, University of Minho, Portugal
Moghavvemi, Mahmoud, University of Malaya, Malaysia
Mohammadi, Mohammad-Reza, University of Cambridge, UK
Molina Flores, Esteban, Benemérita Universidad Autónoma de Puebla, Mexico
Moradi, Majid, University of Kerman, Iran
Morello, Rosario, University "Mediterranea" of Reggio Calabria, Italy
Mounir, Ben Ali, University of Sousse, Tunisia
Mulla, Imtiaz Sirajuddin, National Chemical Laboratory, Pune, India
Neelamegam, Periasamy, Sastra Deemed University, India
Neshkova, Milka, Bulgarian Academy of Sciences, Bulgaria
Oberhammer, Joachim, Royal Institute of Technology, Sweden
Ould Lahoucine, Cherif, University of Guelma, Algeria
Pamidighanta, Sayanu, Bharat Electronics Limited (BEL), India
Pan, Jisheng, Institute of Materials Research & Engineering, Singapore
Park, Joon-Shik, Korea Electronics Technology Institute, Korea South
Penza, Michele, ENEA C.R., Italy
Pereira, Jose Miguel, Instituto Politecnico de Setebal, Portugal
Petsev, Dimiter, University of New Mexico, USA
Pogacnik, Lea, University of Ljubljana, Slovenia
Post, Michael, National Research Council, Canada
Prance, Robert, University of Sussex, UK
Prasad, Ambika, Gulbarga University, India
Prateepasen, Asa, Kingmoungut's University of Technology, Thailand
Pullini, Daniele, Centro Ricerche FIAT, Italy
Pumera, Martin, National Institute for Materials Science, Japan
Radhakrishnan, S., National Chemical Laboratory, Pune, India
Rajanna, K., Indian Institute of Science, India
Ramadan, Qasem, Institute of Microelectronics, Singapore
Rao, Basuthkar, Tata Inst. of Fundamental Research, India
Raouf, Kosai, Joseph Fourier University of Grenoble, France
Reig, Candid, University of Valencia, Spain
Restivo, Maria Teresa, University of Porto, Portugal
Robert, Michel, University Henri Poincare, France
Rezazadeh, Ghader, Urmia University, Iran
Royo, Santiago, Universitat Politècnica de Catalunya, Spain
Rodriguez, Angel, Universidad Politécnica de Catalunya, Spain
Rothberg, Steve, Loughborough University, UK
Sadana, Ajit, University of Mississippi, USA
Sadeghian Marnani, Hamed, TU Delft, The Netherlands
Sandacci, Serghei, Sensor Technology Ltd., UK
Saxena, Vibha, Bhabha Atomic Research Centre, Mumbai, India
Schneider, John K., Ultra-Scan Corporation, USA
Seif, Selemeni, Alabama A & M University, USA
Seifter, Achim, Los Alamos National Laboratory, USA
Sengupta, Deepak, Advance Bio-Photonics, India
Shearwood, Christopher, Nanyang Technological University, Singapore
Shin, Kyuho, Samsung Advanced Institute of Technology, Korea
Shmaliy, Yuriy, Kharkiv National Univ. of Radio Electronics, Ukraine
Silva Girao, Pedro, Technical University of Lisbon, Portugal
Singh, V. R., National Physical Laboratory, India
Slomovitz, Daniel, UTE, Uruguay
Smith, Martin, Open University, UK
Soleymannpour, Ahmad, Damghan Basic Science University, Iran
Somani, Prakash R., Centre for Materials for Electronics Technol., India
Srinivas, Talabattula, Indian Institute of Science, Bangalore, India
Srivastava, Arvind K., Northwestern University, USA
Stefan-van Staden, Raluca-Ioana, University of Pretoria, South Africa
Sunriddetchka, Sarun, National Electronics and Computer Technology Center, Thailand
Sun, Chengliang, Polytechnic University, Hong-Kong
Sun, Dongming, Jilin University, China
Sun, Junhua, Beijing University of Aeronautics and Astronautics, China
Sun, Zhiqiang, Central South University, China
Suri, C. Raman, Institute of Microbial Technology, India
Sysoev, Victor, Saratov State Technical University, Russia
Szewczyk, Roman, Industrial Research Inst. for Automation and Measurement, Poland
Tan, Ooi Kiang, Nanyang Technological University, Singapore,
Tang, Dianping, Southwest University, China
Tang, Jaw-Luen, National Chung Cheng University, Taiwan
Teker, Kasif, Frostburg State University, USA
Thumbavanam Pad, Kartik, Carnegie Mellon University, USA
Tian, Gui Yun, University of Newcastle, UK
Tsiantos, Vassilios, Technological Educational Institute of Kaval, Greece
Tsigara, Anna, National Hellenic Research Foundation, Greece
Twomey, Karen, University College Cork, Ireland
Valente, Antonio, University, Vila Real, - U.T.A.D., Portugal
Vaseashta, Ashok, Marshall University, USA
Vazquez, Carmen, Carlos III University in Madrid, Spain
Vieira, Manuela, Instituto Superior de Engenharia de Lisboa, Portugal
Vigna, Benedetto, STMicroelectronics, Italy
Vrba, Radimir, Brno University of Technology, Czech Republic
Wandelt, Barbara, Technical University of Lodz, Poland
Wang, Jiangping, Xi'an Shiyu University, China
Wang, Kedong, Beihang University, China
Wang, Liang, Advanced Micro Devices, USA
Wang, Mi, University of Leeds, UK
Wang, Shinn-Fwu, Ching Yun University, Taiwan
Wang, Wei-Chih, University of Washington, USA
Wang, Wensheng, University of Pennsylvania, USA
Watson, Steven, Center for NanoSpace Technologies Inc., USA
Weiping, Yan, Dalian University of Technology, China
Wells, Stephen, Southern Company Services, USA
Wolkenberg, Andrzej, Institute of Electron Technology, Poland
Woods, R. Clive, Louisiana State University, USA
Wu, DerHo, National Pingtung Univ. of Science and Technology, Taiwan
Wu, Zhaoyang, Hunan University, China
Xiu Tao, Ge, Chuzhou University, China
Xu, Lisheng, The Chinese University of Hong Kong, Hong Kong
Xu, Tao, University of California, Irvine, USA
Yang, Dongfang, National Research Council, Canada
Yang, Wuqiang, The University of Manchester, UK
Yang, Xiaoling, University of Georgia, Athens, GA, USA
Yaping Dan, Harvard University, USA
Ymeti, Aurel, University of Twente, Netherland
Yong Zhao, Northeastern University, China
Yu, Haihu, Wuhan University of Technology, China
Yuan, Yong, Massey University, New Zealand
Yufera Garcia, Alberto, Seville University, Spain
Zagnoni, Michele, University of Southampton, UK
Zamani, Cyrus, Universitat de Barcelona, Spain
Zeni, Luigi, Second University of Naples, Italy
Zhang, Minglong, Shanghai University, China
Zhang, Quintao, University of California at Berkeley, USA
Zhang, Weiping, Shanghai Jiao Tong University, China
Zhang, Wenming, Shanghai Jiao Tong University, China
Zhang, Xueji, World Precision Instruments, Inc., USA
Zhong, Haoxiang, Henan Normal University, China
Zhu, Qing, Fujifilm Dimatix, Inc., USA
Zorzano, Luis, Universidad de La Rioja, Spain
Zourob, Mohammed, University of Cambridge, UK

Contents

Volume 110
Issue 11
November 2009

www.sensorsportal.com

ISSN 1726-5479

Research Articles

Sensors Based on Nanostructured Materials: Book Review <i>Sergey Y. YURISH</i>	1
Glucose Binding Protein as a Novel Optical Glucose Nanobiosensor <i>Majed DWEIK</i>	1
Hydrogen Sensor Based on Carbon Nano-tube Fortified by Palladium <i>A. Kazemzadeh, A. F. Hessari, M. Kashani, H. Azizi and N. Jafari</i>	9
Nanostructured ZrO₂ Thick Film Resistors as H₂-Gas Sensors Operable at Room Temperature <i>K. M. Garadkar, B. S. Shirke, Y. B. Patil and D. R. Patil</i>	17
Pull-in Phenomena and Dynamic Response of a Capacitive Nano-beam Switch <i>Farid Vakili-Tahami, Hamed Mobki, Ali-asghar keyvani-janbahan, Ghader Rezazadeh</i>	26
Palladium Surface Modification of Nanocrystalline Sol-Gel derived Zinc Oxide Thin Films and its Effect on Methane Sensing <i>P. Bhattacharyya, S. Maji, S. Biswas, A. Sengupta, T. Maji, H. Saha</i>	38
Gas Sensing Properties of Indium Tin Oxide Nanofibers <i>Shiyong Xu, Yong Shi</i>	47
Design, Modeling and Optimization of a Piezoelectric Pressure Sensor based on a Thin-Film PZT Membrane Containing Nanocrystalline Powders <i>Vahid Mohammadi, Mohammad Hossein Sheikhi</i>	56
Synthesis and Properties of Thin Film Nanocomposites Sn-Y-O for Gas Sensors <i>Stanislav Rembeza, Ekaterina Rembeza, Elena Russkih, Natalia Kosheleva</i>	71
Electroanalytical Nanoparticles Electrode based on NanoTiO₂/MWCNTs Mixture <i>Ganchimeg Perenlei, Wee Tee Tan</i>	78
Structural Properties of Nanosized NiFe₂O₄ for LPG Sensor <i>N. N. Gedam, A. V. Kadu, P. R. Padole, A. B. Bodade and G. N. Chaudhari</i>	86
Low-Cost Wireless Nanotube Composite Sensor for Damage Detection of Civil Infrastructure <i>Mohamed Saafi, Lanouar Kaabi</i>	96
Cross Linking Polymers (PVA & PEG) with TiO₂ Nanoparticles for Humidity Sensing <i>Monika Joshi and R. P. Singh</i>	105
Resolution Enhancement of Thermal and Optical Nanolithography Using an Organic Dry Developing Resist and an Optimized Tip <i>Salman Noach, Michael Manevich, Naftali P. Eisenberg and Eli Flaxer</i>	112

Wireless Sensor Network: Modeling and Analysis of MEMS based Nano-Nodes <i>Rohit Pathak, Satyadhar Joshi.....</i>	120
Respiration and Heartbeat Measurement for Sleep Monitoring using a Flexible AIN Piezoelectric Film Sensor <i>Nan Bu, Naohiro Ueno and Osamu Fukuda.....</i>	131
Design Optimization of Cantilever based MEMS Micro-accelerometer for High-g Applications <i>B. D. Pant, Shelley Goel, P. J. George and S. Ahmad</i>	143

Authors are encouraged to submit article in MS Word (doc) and Acrobat (pdf) formats by e-mail: editor@sensorsportal.com
Please visit journal's webpage with preparation instructions: <http://www.sensorsportal.com/HTML/DIGEST/Submission.htm>

International Frequency Sensor Association (IFSA).

SENSORDEVICES 2010:

**The First International Conference
on Sensor Device Technologies and Applications**

July 18 - 25, 2010 - Venice, Italy



The inaugural event SENSORDEVICES 2010, The First International Conference on Sensor Device Technologies and Applications, initiates a series of events focusing on sensor devices themselves, the technology-capturing style of sensors, special technologies, signal control and interfaces, and particularly sensors-oriented applications. The evolution of the nano- and microtechnologies, nanomaterials, and the new business services make the sensor device industry and research on sensor-themselves very challenging.

Conference tracks

Sensor devices
Sensor device technologies
Sensors signal conditioning and interfacing circuits

Medical devices and sensors applications
Sensors domain-oriented devices, technologies, and applications
Sensor-based localization and tracking technologies

Important dates

Submission (full paper): February 20, 2010
Notification: March 25, 2010
Registration: April 15, 2010
Camera ready: April 20, 2010



<http://www.iaria.org/conferences2010/SENSORDEVICES10.html>



Glucose Binding Protein as a Novel Optical Glucose Nanobiosensor

Majed DWEIK

Department of Life and Physical Sciences - Corporative Research and Extension,
Lincoln University, 820 Chestnut Street Jefferson City, MO, USA

Tel.: 001-573-681-5134

E-mail: dweikm@lincolnu.edu

Received: 7 October 2009 /Accepted: 24 November 2009 /Published: 30 November 2009

Abstract: Development of an *in vivo* optical sensor requires the utilization of Near Infra Red (NIR) fluorophores due to their ability to operate within the biological tissue window. Alexa Fluor 750 (AF750) and Alexa Fluor 680 (AF680) were examined as potential NIR fluorophores for an *in vivo* fluorescence resonance energy transfer (FRET) glucose biosensor. AF680 and AF750 found to be a FRET pair and percent energy transfer was calculated. Next, the tested dye pair was utilized in a competitive binding assay in order to detect glucose. Concanavalin A (Con A) and dextran have binding affinity, but in the presence of glucose, glucose displaces dextran due to its higher affinity to Con A than dextran. Finally, the percent signal transfer through porcine skin was examined. The results showed with approximately 4.0 mm porcine skin thickness, 1.98 % of the fluorescence was transmitted and captured by the detector. *Copyright © 2009 IFSA.*

Keywords: FRET, NIR, Dextran, Concanavalin A, Glucose biosensor

1. Introduction

In vivo glucose sensing has gained more focus by scientist and researchers, but remained a difficult area due to the number of problems, such as biofouling, poor optical transmission, and poor glucose sensitive probes. There are several enzymes used to detect glucose. But a major problem with these enzymes is the byproduct produced. In the case of glucose oxidase, the hydrogen peroxide will cause major problems with the optical dyes that are pH sensitive. To overcome this problem, Glucose Binding Protein (GBP) will be used to detect glucose. In this research project the GBP structure provides a glucose binding site in a shape of a pocket. When glucose is introduced, it will bind to the glucose binding sites and will cause a conformational change.

The glucose binding protein of *Escherichia coli* (*E. coli*) is considered as an initial component for both chemotaxis towards glucose and high-affinity active transport of sugar. *E. coli* is a bacterial periplasmic binding protein. It is an essential component that serves as initial receptor for active transport systems [1]. The X-ray structures of this protein have provided a molecular view of the sugar-binding site and of the site for interacting with the Trg transmembrane signal transducer [2]. Fig. 1 provides a schematic of the geometry of the sugar-binding site. It is located in the cleft between the two lobes of the bilobate protein. It is designed for tight binding and sequestering of either the alpha or beta anomer of the D-stereoisomer of the 4-epimers galactose and glucose [3]. The diameter of glucose binding protein (GBP) is 50 Å [4].

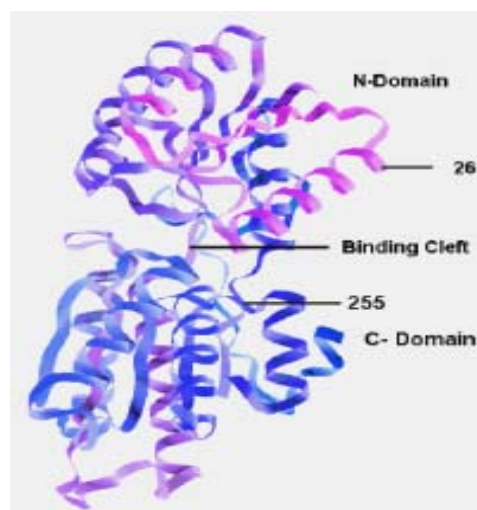


Fig. 1. Schematic of the geometry of the glucose-binding site [5]
(<http://talaga.rutgers.edu/people/images/GBP.png>).

Mature GBP consists of 309 amino acids with a molecular weight of 33,310 Da [6]. It is ellipsoidal in shape with two different but similarly folded domains connected by three different peptide segments that serve as a flexible hinge and produce a cleft between the two folded domains. The ligand-binding site is deep within this cleft. Each domain has a core of six β -sheet strands flanked by two or three helices on both sides [7]. In the absence of D-glucose, the two domains remain far apart with the cleft accessible to solvent. Glucose induces a hinge motion in GBP and the sugar becomes completely engulfed in the deep cleft between the two domains. This conformational change in GBP results in the exclusion of solvent molecules from the binding site and enables efficient hydrogen-bonding interactions between the sugar and the residues in the binding site. D-glucose binds to GBP with dissociation constants, K_d , of 0.4 mM [8]. The conformational change produced in GBP upon binding glucose constitutes the basis of FRET nanobiosensor development strategy.

AF750 and AF680 is a viable FRET pair that will be used to label the GBP. Wild-type *E. coli* GBP has multi sites of amino but no cysteine moieties. By incorporating a unique cysteine to the protein, this will permit site-specific labeling with fluorophore and the optimization of the induced fluorescence change in the presence of glucose.

Site-directed mutagenesis was performed to incorporate a single cysteine in GBP at site 175 to produce GBP mutant. This site was chosen because the crystal structure of GBP [9] shows that these residues are located near the binding cleft. The response of this labeled mutant GBP upon binding with sugar was monitored by following the changes in the fluorescence intensity of the probes. Calibration plots were then constructed by relating the changes in signal with the amount of ligand present.

2. Material and Methods

2.1. Material

Glucose binding protein from *E. Coli* that contains a unique cysteine at position 175 was purchased from Senseomics (Lexington, KY). Dithiothreitol (DTT) was purchased from Sigma Chemical Co. (St. Louis, MO). β -D-Glucose and PBS also were purchased from Sigma Chemical Co. (St. Louis, MO). Alexa Fluor 750 C5-maleimide, which was used to label the unique cysteine on the GBP, was obtained from Invitrogen. The Alexa Fluor 680 carboxylic acid, succinimidyle ester was also purchased from Invitrogen. DMSO 78.13 MW was obtained from the MU Chemical Recycling (Columbia, MO). Dialysis tubing 6000-8000 MWCO was obtained from Fisher Scientific (Fairlawn, NJ).

2.2. Instrumentation

A UV-Vis absorbance spectrometer (Beckman DU 520) was used to collect absorbance spectra. The slit size (4 nm) and scanning speed (595nm/min) were held constant throughout all the experiments. A scanning fluorescence spectrometer (FluoroMax-3 Jobin Yvon, Hobira) was used to collect fluorescence emission spectra by exciting the sample at 680 nm. The slit size and integration time were 7nm and 0.3s, respectively.

2.3. GBP Purifying

The GBP was mixed in Tris-HCl buffer. The addition of Dithiothreitol (DTT) to the GBP was carried out to reduce the disulfide bonds in the GBP. This was accomplished by making a 1 M solution of DTT and then adding GBP. Then the solution was dialyzed in PBS to eliminate the excess DTT. With this process the GBP was ready to allow the cysteine site to be conjugated. The labeling of GBP with AF750 was prepared by using the labeling kit protocol from Ivitrogen.

Bio-Rod experiment was conducted to determine the final protein concentration and the degree of labeling. The final GBP-AF750 concentration was 0.8091 mg/ml and the degree of labeling (DOL) was 1 since there was only one cysteine.

2.4. AF680 Labeling on the GBP Amine Sites

By following the protein conjugation protocol from Ivitrogen; the AF680 was conjugated on the GBP amino sites. The conjugated stock was dialyzed in PBS to remove the excess AF680. Then the final conjugated GBP concentration was determined by using a Bio-Rod kit. The final concentration was 0.7842 mg/ml. The DOL was calculated according to this formula

$$\text{DOL} = (A_{\max} \times \text{MW}) / ([\text{Protein}] \times \epsilon_{\text{dye}}),$$

where A_{\max} is the absorbance at the maximum peak of the fluorophore used in conjugation, and ϵ_{dye} is the extinction coefficient at λ_{\max} in $\text{cm}^{-1}\text{M}^{-1}$, and $[M]$ is the molarity of the agent. MW is the molecular weight of the protein. The DOL was 1.5, which means that for every mole of protein there was 1.5 moles of dye.

2.5. The existence of AF 680 and AF 750 on the GBP

Experiments were conducted in 2 ml total volume of PBS. First, 11.32 ug of GBP-AF750 was placed in the 4 ml cuvette. It was scanned for background signal of the AF750. The excitation was set at 750 nm with slit opening at 5. The increment was set at 1 nm and the integration time was at 0.3 sec. Second, 11.32 ug of GBP-AF680-AF750 was placed in the 4 ml cuvette with 2 ml PBS. The scanning fluorescence spectrometer was adjusted to conduct the experiment according to the fluorophore used. The excitation was set at 680 nm, which was the excitation wavelength for the AF680 located on the amine sites. The increment was set at 1 nm and the integration time was at 0.3 sec.

2.6. Baseline scan from the FRET pair labeled GBP

It is imperative to establish baseline scans from the FRET labeled GBP. The baselines provide background spectra prior to the addition of glucose.

2.7. Glucose Response Experiments

After 2 hr incubation of the 1.5684 μg of labeled GBP in PBS buffer probe samples were tested for glucose response. The tests consisted of six additions of glucose concentrations, 0 to 60 μM . For each glucose addition, 5 min. was allowed for incubation at room temperature. Calibration plots were obtained by relating the average fluorescence change with the concentration of glucose in the sample. The data points shown in the figures are the average of three measurements ± 1 SD.

3. Results and Discussion

3.1. GBP Labeling and Fluorescence Resonance Energy Transfer (FRET) Experiments

Fig. 2 indicates a successful labeling of the AF750 on the one cysteine site and AF680 on the amino sites. When GBP-AF680 was excited at 680 nm, an emission peak signal was detected at 702 nm. After the labeling of AF750, the labeled GBP with AF680 and AF750 was excited at 680 nm. There were two emission peaks. One peak was at 702 nm, which is an indication of the presence of AF680, and another peak was at 776 nm, which is an indication of the presence AF750.

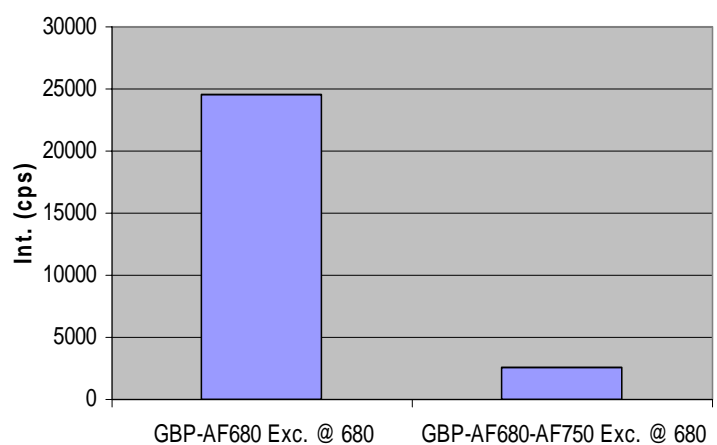


Fig. 2. Background signal of the labeled GBP-AF750-AF680.

The successful labeling of GBP with the NIR FRET dye pair will allow us to quantify FRET results after exposure to glucose. When the GBP was first labeled with the donor, AF680, a scan of GBP-AF680 in PBS was obtained. The maximum peak from the AF680 without the presence of the donor was 24480 CPS as shown in Fig. 3. The labeled GBP with AF680 and AF750 was scanned, the donor peak result was 2610. Both peaks of AF680 without the presence of the acceptor and with the presence of the acceptor was used to quantify the energy transfer that took occurred between the donor and the acceptor prior to glucose additions.

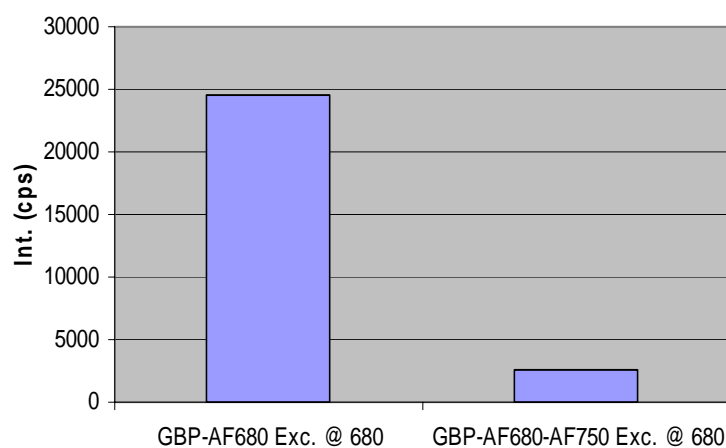


Fig. 3. Donor peaks at 702 nm with and without acceptor presence.

The Equation below was used in the quantification.

$$\% \text{ Energy transfer} = 1 - I_{DWA} / I_D,$$

where I_D is the donor's intensity without acceptor and I_{DWA} is the donor's intensity with acceptor present. The result indicated an energy transfer of 89.34 %. These results provided conclusive observations of energy transfer in terms of an increase in the acceptor peak and decrease in the donor peak prior to glucose additions.

3.2. GBP Glucose Response Experiments

Since it was determined that the AF680 and AF750 fluorophores were labeled to the GBP, the next step was to utilize the fluorophores in a FRET-based glucose biosensor. In these experiments glucose response was studied by utilizing 1.56 μg of GBP-AF750-AF680 in 2 ml PBS as a baseline and adding various concentrations of glucose.

The labeled GBP was incubated in the PBS for 2 hours in PBS. Then three different D-glucose concentrations were added: 10 μM , 40 μM , and 120 μM . The scanned data was plotted as donor/acceptor (D/A) ratio vs. glucose concentrations. As shown in Fig. 4, there was an exponential decrease to the ratio as glucose concentration was increased. This change in the signal is due to the conformational changes of the GBP. As glucose binds to its GBP binding site, the distance between the labeled sites was changed. The results of the increase in the acceptor peak and decrease of the donor peak indicated a decrease in the distance between the donor and the acceptor, resulting in an increased in energy transfer.

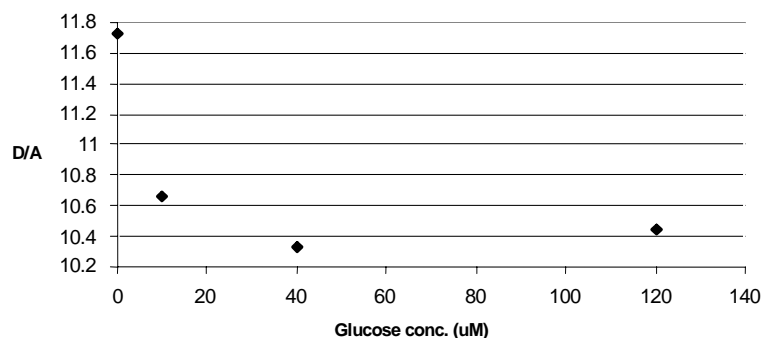


Fig. 4. D/A vs. glucose concentration (detection range 0 to 120 uM).

At 120 uM glucose, the D/A ratio was not consistent with the other two D/A ratios. That result was further investigated by looking into the detection range for the labeled GBP.

A smaller dynamic range of the glucose concentration was tested from 0 to 60 uM. Fig. 5 provided a consistent decrease in sensor response as the glucose concentration was increased.

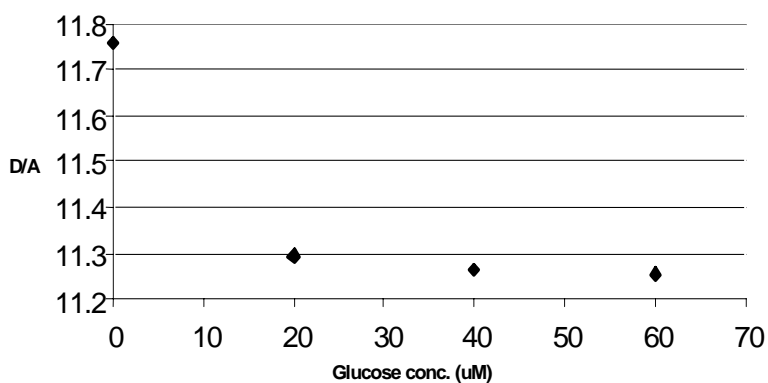


Fig. 5. D/A vs. glucose concentration (detection range 0 to 60 uM).

The final experiments were to target improvements in the signal by modifying the labeling process. In the following section, the ratio of mole dye/mole protein was about 3.6:1. We had 8.7 pmole of dye to every 2.38 pmole of protein. There were more glucose concentration additions within the same tested range of 0 to 60 uM.

Glucose response was also studied at t=0 min. and t = 5 min. At t=0 min., the scan of the sample was performed immediately after mixing. No time was allowed for incubation. The result still showed an exponential decrease to the D/A ratio as the glucose concentration increase as shown in Fig. 6.

Fig. 4.6 shows the final graph all three experiments. This result is represented by the following exponential equation was obtained.

$$Y = 10.43 e^{-0.0006 x}$$

The R² value is 0.8583

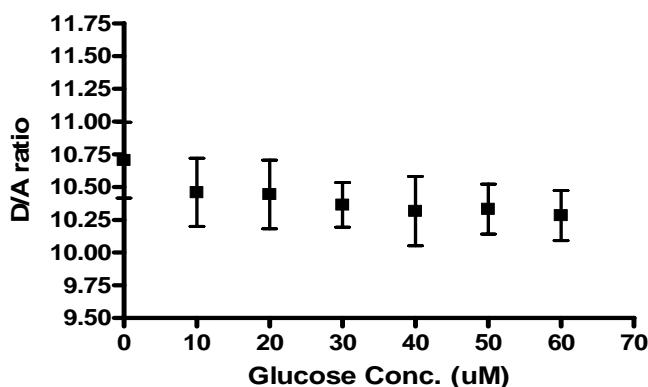


Fig. 6. Glucose response to different samples at t=0min.

At t = 5 min., the D/A ratio was more consistent in the exponential decrease as the glucose concentration was increasing as shown in Fig. 7.

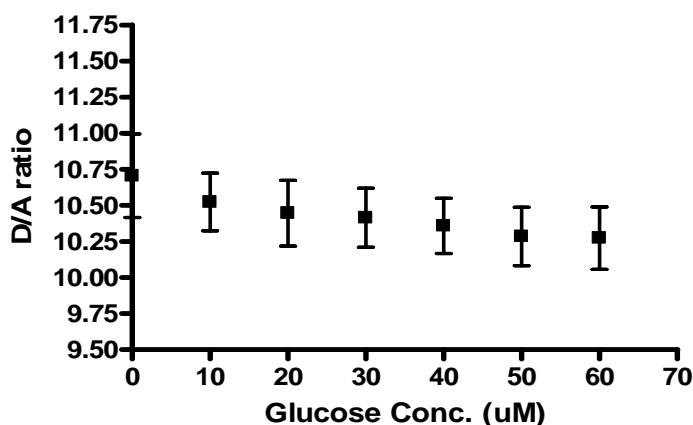


Fig. 7. Glucose response of three samples at t=5 min.

Fig. 4.7 shows the final graph all three experiments. By using Fig. 4.7, we got the following exponential equation was obtained.

$$Y = 10.391e^{-0.0005x}$$

The R^2 value is 0.7317.

It is conclusive that incubation time is very important after the addition of different glucose concentrations.

4. Conclusions

Glucose binding protein (GBP) is a monomeric periplasmic protein. It binds glucose with high affinity. The binding mechanism is based on a hinge motion due to the protein conformational change. This change was used as an optical sensing mechanism by applying Fluorescence Resonance Energy Transfer (FRET). With the introduction of a single cysteine at a specific site by site-directed

mutagenesis, a single-label attachment at specific sites with a fluorescent probe, AF750, was ensured. The other sites are amino sites, which were labeled with a donor fluorophore, AF680. Since this residue was not involved in ligand binding and since it was located at the edge of the binding cleft, it experienced a significant change in environment upon binding of glucose. The sensing system strategy was based on the fluorescence changes of the probe as the protein undergoes a structural change on binding. This structure change allowed a reduction in the distance between the donor and the acceptor, which allowed more energy transfer as glucose concentration was increasing. Also by allowing 5 min for glucose to incubate, the response from the biosensor was decreasing exponentially as glucose concentration was increasing. The detection range was determined between 0-60 μM . The dynamic range could be improved by increasing the GBP concentration.

All the above results can be used in designing more sensitive optical glucose biosensor. These sensors may eventually be deployed for *in vivo* glucose sensing with superior sensitivity and without the concern of toxicity.

Acknowledgments

The authors gratefully acknowledge funding from the Missouri F21C and the Bioprocessing and Biosensor Center.

References

- [1]. P. S. Grant, McShane, M. J., Development of Multilayer Fluorescent Thin Film Chemical Sensors Using Electrostatic Self Assembly, *IEEE Sensors Journal*, 3, 2003, p. 139146.
- [2]. Birch, D. J. S.; Rolinski, O. Res., *Chem. Intermed.* 27, 2001, p. 425.
- [3]. Wilson, D. B., and Smith, J. B., in *Bacterial Transport* (Rosen, B. P., Ed.), Dekker, New York, 1978, pp. 495–557.
- [4]. Furlong, C. E., in *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology* (Neidhardt, F. C., Ed.), *Am. Soc. Microbiol.*, Washington, D. C., 1987, pp. 768–796.
- [5]. Ames, G. F. -L., Bacterial periplasmic transport systems: Structure, mechanism, and evolution. *Annu. Rev. Biochem.*, 55, 1986, pp. 397–425.
- [6]. Quioco, F. A., Meador, W. E. & Pflugrath, J. W., *J. Mol. Biol.*, 133, 1979, pp. 181-184.
- [7]. Gilliland, G. L. & Quioco, F. A., *J. Mol. Biol.* 146, 1981, pp. 341- 362.
- [8]. Newcomer, M. E., Miller, D. M., III & Quioco, F. A., *J. Biol. Chem.*, 254, 1979, pp. 7529-7533.
- [9]. Newcomer, M. E., Gilliland, G. L. & Quioco, F. A., *J. Biol. Chem.*, 256, 1981, pp. 13213-13217.

Guide for Contributors

Aims and Scope

Sensors & Transducers Journal (ISSN 1726-5479) provides an advanced forum for the science and technology of physical, chemical sensors and biosensors. It publishes state-of-the-art reviews, regular research and application specific papers, short notes, letters to Editor and sensors related books reviews as well as academic, practical and commercial information of interest to its readership. Because it is an open access, peer review international journal, papers rapidly published in *Sensors & Transducers Journal* will receive a very high publicity. The journal is published monthly as twelve issues per annual by International Frequency Association (IFSA). In addition, some special sponsored and conference issues published annually. *Sensors & Transducers Journal* is indexed and abstracted very quickly by Chemical Abstracts, IndexCopernicus Journals Master List, Open J-Gate, Google Scholar, etc.

Topics Covered

Contributions are invited on all aspects of research, development and application of the science and technology of sensors, transducers and sensor instrumentations. Topics include, but are not restricted to:

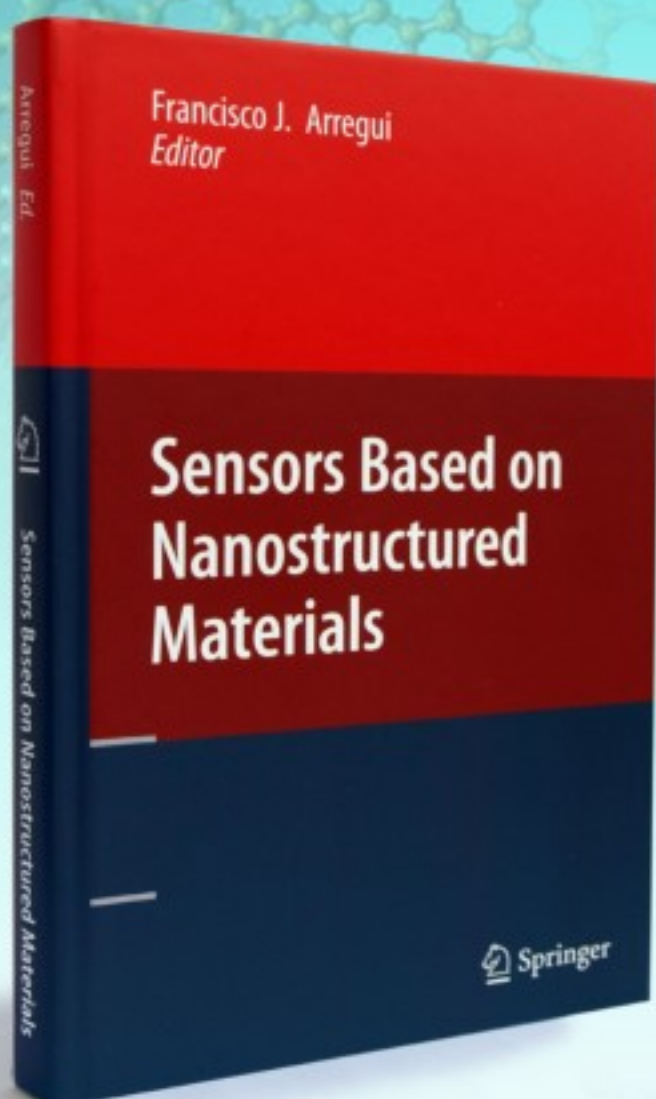
- Physical, chemical and biosensors;
- Digital, frequency, period, duty-cycle, time interval, PWM, pulse number output sensors and transducers;
- Theory, principles, effects, design, standardization and modeling;
- Smart sensors and systems;
- Sensor instrumentation;
- Virtual instruments;
- Sensors interfaces, buses and networks;
- Signal processing;
- Frequency (period, duty-cycle)-to-digital converters, ADC;
- Technologies and materials;
- Nanosensors;
- Microsystems;
- Applications.

Submission of papers

Articles should be written in English. Authors are invited to submit by e-mail editor@sensorsportal.com 8-14 pages article (including abstract, illustrations (color or grayscale), photos and references) in both: MS Word (doc) and Acrobat (pdf) formats. Detailed preparation instructions, paper example and template of manuscript are available from the journal's webpage: <http://www.sensorsportal.com/HTML/DIGEST/Submission.htm> Authors must follow the instructions strictly when submitting their manuscripts.

Advertising Information

Advertising orders and enquires may be sent to sales@sensorsportal.com Please download also our media kit: http://www.sensorsportal.com/DOWNLOADS/Media_Kit_2009.pdf



'Sensors Based on Nanostructured Materials presents the many different techniques and methods of fabricating materials on the nanometer scale and specifically, the utilization of these resources with regard to sensors. The techniques which are described here are studied from an application-oriented perspective, providing the reader with a broader view of the types of nanostructured sensors available.

Sensors Based on Nanostructures Materials is suitable for academic and industrial research scientists as well as engineers.'

"It is a valuable source for those who need to have a summary of nanosensors based on nanostructured materials fabricated with many different techniques."
(Sergey Y. Yurish, *Sensors & Transducers*, Vol.110, Issue 11, November 2009).

Order online:

http://www.sensorsportal.com/HTML/BOOKSTORE/Nanostructured_materials.htm