

Optical nanosensors for intracellular pH measurements

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Introduction: We present the development of fluorescent nanoscale optical sensor for intracellular pH measurements. For this purpose core-shell type silica nanospheres were synthesized using a modified Stöber method in the 50-100 nm size range. The sensing was based on the dual lifetime referencing (DLR) method [1]. Two fluorescent indicators with overlapping emission bands, one pH-sensitive, short-lived indicator and a pH-insensitive reference with a decay time in the μ s range have been chosen. N-allyl-4-piperazinyl-1,8-naphthalimide (APN) have been synthesized according to the literature [2] and have been used as fluorescent pH indicator while ruthenium(II) tris(diphenylphenanthroline) (Ru(dpp)) complex has been used as reference fluorophore.



Fig. 1. Fluorescence enhancement mechanism



Fig. 2. APN fluorescence at different pH

Experimental: The pH sensing principle of APN is shown in Fig 1, and its fluorescence properties on Fig 2. The core of the silica sphere was prepared by dissolving fluo(dpp) in the ethanol tetraethoxysilane (TEOS) and water were added. The mixture was stirred and sonicated for 30 minutes. The mixture was thermostated at 25°C. Ammonia catalyst was rapidly added and the mixture was stirred for further 24 h, then centrifuged (5000 RPM, 15 minutes), dried and annealed at 200 °C. The sensing shell of the sensor was prepared in two steps. First APTES and vinyltriethoxysilane (VTES) were dissolved in ethanol, and irradiated with UV (250nm) lamp for 1 hour. In the second step the appropriate amount of APN/VTES solution, 0.01% of APTES was added to suspension of the reference silica spheres and sonicated for 30 minutes. The ammonia catalyst was added and the mixtures were stirred for 24 hours. The resulting nanosensors was centrifuged and washed with ethanol and DI water. Finally it was dispersed and stored in DI water until further use. This way the reference indicator was encapsulated in the core of the silica beads that was covered by pH sensitive indicator containing shell. The calibrating curves were obtained both in intensity and in life-time domain. The fluorescence measurements were made with AVANTES AvaSpec 2048 spectrophotometer, by exciting the samples with LED (430 nm) through a 400 broad range IF filter, while a long pass filter with cut off wavelength of 510 nm was placed in front of detector. Phase shift measurements were performed with dual-phase lock-in amplifier (DS2P-650, Stanford Research Inc.) in a home made flow through cell. Optical system consisted of a blue led (430 nm) a 430 broad range band pass filter, bifurcated fiber bundle, and Hamamatsu (R15760-01) PMT equipped with 510 nm cut off wavelength long pass filter.

Results: SEM images of the synthesized nanospheres are shown in the Fig 3. The main size of the particles was 210 nm. The particles formed a stable suspension in water. Hydrogen ions strongly enhance the fluorescence intensity of APN, which shows no fluorescence above pH 12. The fluorescence properties of pH sensing nanospheres are presented on Fig 4. The phase shift of the modulation frequency as a function of the modulation frequency are shown in the Fig 5. It has been found that the pH sensing spheres have the largest phase shift around 10 kHz modulation frequency. Fig. 6. shows the obtained calibration curve of the sensing nanospheres in B-R buffer at 300mM ionic strength.



Fig. 3. SEM image of the pH sensing nanospheres

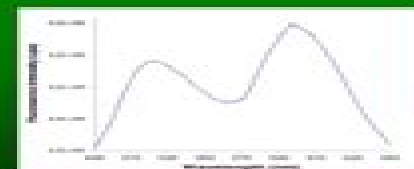


Fig. 4. Fluorescence of the pH sensing nanospheres



Fig. 5. Phase shift as a function of modulation frequency



Fig. 6. Calibration of sensing layer

Conclusion: A pH sensing nanospheres has been prepared with covalently bonded pH sensitive dye and co-immobilized reference dye. The spectral properties of the nanosensor are suitable for imaging measurements with most CMOS and CCD cameras [3].

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DOI: 10.1007/978-94-007-5026-8_1
 ISBN 978-94-007-5026-8
 DOD: 4.2.1.9-10000000-0000-0000
 ISBN 978-94-007-5026-8

The Development Of Optical Nanosensors For Biological Measurements Book

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Optochemical Nanosensors Andrea Cusano, Francisco J. Arregui, Michele Giordano, Antonello Cutolo, 2016-04-19 This book provides an overview of the state of the art in optical and chemical nanosensors for industrial environmental diagnostic security and medical applications It summarizes the various types and developments in optical and chemical sensor technology and then explains how the integration of optical chemical sensors and nanomaterials creates new opportunities The text also reviews optochemical sensors starting from the basics in optoelectronics and concluding with the principles of operation at the basis of optochemical devices The authors offer insight into future trends in this growing field and present a range of applications in the fields of medicine security and bioterrorism *Surface-Enhanced Raman Scattering* Katrin Kneipp, Martin Moskovits, Harald Kneipp, 2006-06-21 Almost 30 years after the first reports on surface enhanced Raman signals the phenomenon of surface enhanced Raman scattering SERS is now well established Yet explaining the enhancement of a spectroscopic signal by fourteen orders of magnitude continues to attract the attention of physicists and chemists alike And at the same time and rapidly growing SERS is becoming a very useful spectroscopic tool with exciting

applications in many fields SERS gained particular interest after single molecule Raman spectroscopy had been demonstrated This book summarizes and discusses present theoretical approaches that explain the phenomenon of SERS and reports on new and exciting experiments and applications of the fascinating spectroscopic effect **Book of Abstracts**, 2000 **The Development of Optical Nanosensor Technology for Single Cell Analysis**, 2004 Advances in modern biosciences and optical biosensor technology have provided exciting new insights and capabilities The integration of these fields has witnessed revolutionary advances which include the development of optical nanosensors Optical nanosensors are devices based on a direct spatial coupling between biologically active molecules and a signal transducer element interfaced to electronic equipment for signal amplification acquisition and recording Optical nanosensors consist of biorecognition molecules covalently immobilized onto the nanotips nanoscale optical fiber that serves as the transducing element By combining the specificity of biorecognition molecules and the excellent sensitivity of laser based optical detection optical nanosensors are capable of detecting and differentiating biochemical constituents of complex systems enabling the provision of sensitive and specific identification of specific molecular events inside living cells This work explores and focuses on the development and application of novel optical nanosensors for single living cell analysis In this context single cell analysis involves the application of optical nanosensor technology to observe and possibly map molecular events inside single living cells Previous studies have focused on the bulk response of cells and this largely increases the probability of missing critical underlying mechanisms specific to the single cell The ability to perform single cell analysis can dramatically improve our understanding of basic cellular processes e g signal transduction as well as improving our knowledge of the intracellular transport and the fate of therapeutic agents at the single cell level This is important not only because of the capability to perform minimally invasive analysis but also to overcome the problem of ensemble averaging This capability to overcome ensemble averaging has the potential to yield new information that is not available from population averaged cellular measurements This work involves the development and application of optical nanosensors for specific and sensitive chemical and protein analysis within single living cells The ability of these sensors to successfully perform chemical and protein analysis at the single cell level lay in their design specifications size specificity sensitivity and eliminating interferences

Journal American Chemical Society, 2003 *Development of Optical PH Nanosensors for Biological Insights Into the Intracellular Trafficking of Nanomedicines* Arpan Desai, 2014 **The British National Bibliography** Arthur James Wells, 2009 Nanosensors Teik-Cheng Lim, 2016-04-19 Nanosensors are rapidly becoming a technology of choice across diverse fields They offer effective and affordable options for detecting and measuring chemical and physical properties in difficult to reach biological and industrial systems operating at the nanoscale However with nanosensor development occurring in so many fields it has become di **Advances in Nanosensors for Biological and Environmental Analysis** Aakash Deep, Sandeep Kumar, 2019-06-14 Advances in Nanosensors for Biological and Environmental Analysis presents the

current state of art in nanosensors for biological and environmental analysis also covering commercial aspects Broadly the book provides detailed information on the emergence of different types of nanomaterials as transduction platforms used in the development of nanosensors These include carbon nanotubes graphene 2 D transition metal dichalcogenides conducting polymers and metal organic frameworks Additional topics include sections on the way nanosensors have inspired new product development in various types of biological and environmental applications that are currently available and on the horizon Features detailed information on various types of biological and environmental nanosensors Gives particular attention to the different categories of advanced functional interfaces processes for their development and application areas Includes the current state of the art in terms of commercial aspects

Optical Sensor Systems in Biotechnology Govind Rao,2009-11-03 Of all things natural light is the most sublime From the very existential belief of the origins of the universe to its role in the evolution of life on earth light has been inextricably woven into every aspect of our lives I am grateful to Springer Verlag and Thomas Scheper for this invitation to organize this volume that continues to expand the use of light to create next generation sensing applications Indeed the very act of expanding the frontiers of learning and knowledge are referred to in many languages and cultures as enlightenment Early optical instruments relied largely on simple combinations of mirrors prisms and lenses With these simple devices substantial progress was made in our understanding of the properties of light and of its interactions with matter Things got more complicated with the evolution of optical instruments in laboratory use Early systems used bulky and expensive hardware to generate light split it into the desired wavelengths and finally collect it for analysis The discovery of the laser pushed the technology further but did not do much to make its adoption more widespread as the lasers themselves were large and required substantial electrical power to operate The true revolution is just beginning Advances in microelectronics have resulted in the possibility of truly low cost using the consumer electronics industry as a parallel devices that exploit optical measurement technology

Optical Nanosensors for Chemical Analysis Inside Single Living Cells Heather Ann Clark,1999 Nanotechnology in Biology and Medicine Tuan Vo-Dinh,2017-10-03 The second edition of Nanotechnology in Biology and Medicine is intended to serve as an authoritative reference source for a broad audience involved in the research teaching learning and practice of nanotechnology in life sciences This technology which is on the scale of molecules has enabled the development of devices smaller and more efficient than anything currently available To understand complex biological nanosystems at the cellular level we urgently need to develop a next generation nanotechnology tool kit It is believed that the new advances in genetic engineering genomics proteomics medicine and biotechnology will depend on our mastering of nanotechnology in the coming decades The integration of nanotechnology material sciences molecular biology and medicine opens the possibility of detecting and manipulating atoms and molecules using nanodevices which have the potential for a wide variety of biological research topics and medical uses at the cellular level This book presents the most recent scientific and technological advances of

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Chauhan,2014 *New and Improved Nanomaterials and Approaches for Optical Bio- and Immunosensors* Boris B Dzantiev,2023-07-26 The book presents new approaches for bio and immunosensors with nanodispersed labels and their optical registration The use of nanoparticles as carriers and labels provides possibilities for simple measurements and reaching low detection limits In this way novel sensors obtain competitive advantages and good potential for implementation into diagnostic practice The collected articles demonstrate the current state of developments and their most promising directions in the creation and characterization of such optical sensors **Smart Nanosensors** Imran Uddin,2025-04-21 This book serves as a comprehensive compilation of contemporary research conducted in the domain of nanosensors The amalgamation of many elements within the emerging field contributes to the development of a useful collection specifically designed for inexperienced researchers in the domain of smart materials and nanosensor technologies An adequate range of subjects has been incorporated into the present book It includes enzyme mimetic use of smart nanomaterials for enhanced biosensing applications theranostic utilization of smart nanomaterials for targeted drug delivery sensors for pollutant detection and the utilization of smart nanomaterials in the development of biosensors for studying host microbe interactions Nanosensors have emerged as a promising avenue for various applications including sensing in the fields of medicine packaging and heavy metal ion detection Recent developments in the field of smart nanomaterials have led to significant advancements in the application of intelligent switches and sensors within the domains of agriculture food production and water treatment The primary emphasis of this book is the study of the synthesis and fabrication processes involved in the production of smart materials together with their application within the domain of sensor technology The existing body of literature has two main categories introductory textbooks that provide fundamental knowledge about the field and specialized publications that focus exclusively on certain subtopics within the domain of sensor technology The existing material of the book makes it a complete reference resource that is well suited for researchers in the area It especially caters to advanced graduate students who are seeking senior graduate MTech and MS degrees in the subject of sensor technology Additionally this publication would function as an essential resource for researchers across diverse disciplines within the area of materials science who are aiming to propel the development of smart materials

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