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Zveme všechny zájemce – studenty i kolegy – k účasti na serii přednášek o fluorescenčních spektroskopiích a jejich aplikacích

Fluorescence Spectroscopy: Methods and Application Course

Czech Technical University in Prague, Spring 2002

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Fluorescence spectroscopy is now entering a new era as one of the most powerful scientific techniques available. Only two decades ago we would have added "…available to the physical chemist", but the unique combination of properties of molecular fluorescence are opening up new opportunities, which span biotechnology, clinical medicine, environmental and industrial applications. In this short introductory course the fundamental properties of molecular fluorescence in condensed media are outlined and the principle techniques and instrumentation are covered with an emphasis on time-resolved studies. A flavour of the versatility of fluorescence spectroscopy is then conveyed in terms of three lectures on very different applications, selected and treated in order to reinforce the fundamental principles and techniques. Finally a lecture is presented on multiphoton excitation to illustrate how advances in technology are helping to present new spectroscopic opportunities. The course requires no prior knowledge of fluorescence spectroscopy and only a basic knowledge of physical chemistry, quantum theory and optical instrumentation. A list of further reading is provided.

The course has been developed out of the lecture series, which I first gave at the University of Madras in 1999 as the Raman Visiting Professor, and founded on my 30 years experience in fluorescence spectroscopy as a university researcher, teacher and company director.

Tuesday, March 26, 3.30 p.m. KFE Troja Room 112
Lecture 1. Fundamentals

The origins and examples of fluorescence and other intra-molecular processes are outlined in terms of the Jablonski diagram for π electrons. Simple rate parameter descriptions of fluorescence quantum yield and lifetime are developed towards descriptions of Stern-Volmer quenching, fluorescence resonance energy transfer (FRET) and anisotropy for use later in the course.

Friday, March 29, 1.30 p.m. KFE Troja Room 112
Lecture 2. Fluorescence lifetime techniques and instrumentation

This lecture focuses on the time-correlated single-photon timing technique as the principle method of measuring fluorescence lifetimes. Components such as sources and detectors are described as well as the system operation, data acquisition and analysis of fluorescence decays.

Tuesday, April 2, 3.30 p.m. KFE Troja Room 112
Lecture 3. Industrial applications: the production of silica gel

Silica gel finds diverse uses spanning consumer and science markets. For example in beverage fining, as a surface coating agent in paper, as a building material, as an abrasive agent in cleaning fluids, in chromatography and in photonics. The surface area of silica gel (> 100 m^2/g) is the crucial parameter in determining performance and this depends on the size of nm silica particles growing in a sol. Fluorescence anisotropy has recently been shown to provide a more realistic method of on-line monitoring of silica gel production than small angle neutron or x-ray scattering used previously.

Friday, April 5, 1.30 p.m. KFE Troja Room 112
Lecture 4. Uses in environmental monitoring: metal ion sensing

Trace metal assay in the environment is increasingly on the international agenda. For example, the World Health Organisation specifies a maximum of 0.05 mg of lead per litre of drinking water (~ 5 ppb). FRET offers opportunities for a new generation of sensors of metal ions in water with high specificity and remote capabilities. FRET direct to transition metal ions and via a chelate will be discussed and results presented for detection limits down to 0.1 ppb.

Tuesday, April 9, 3.30 p.m. KFE Troja Room 112
Lecture 5. Medical opportunities: glucose sensing for diabetes

The non-invasive monitoring of metabolites is one of the global strategic goals of medical research. In no disease is this requirement more acute that in the *in-vivo* detection of glucose for diabetes management. The disease is reaching epidemic proportions with 220 million diabetics worldwide predicted by 2010. Fluorescence lifetimes, in the near-infrared for compatibility with the optical window in tissue, provide one of the most promising approaches. Methodologies and techniques using a competitive binding assay will be discussed over the physiological range *in-vitro* and in plasma.

Friday, April 12, 1.30 p.m. KFE Troja Room 112
Lecture 6. The new fluorescence: multiphoton excitation

Progress in photonics continues to provide new opportunities for spectroscopy in general and is opening up new molecular sources of fluorescence. This will be illustrated for the fluorescence of σ electrons using multiphoton excitation with femtosecond lasers; fluorescence which was hitherto only accessible using the inconvenience of vacuum ultra-violet excitation.

Doc. RNDr. Vlastimil Fidler, CSc.
garant kursu

Prof. Ing. Miroslava Vrbová, CSc.
vedoucí KFE FJFI ČVUT

V Praze dne 13. března 2002

Further Reading

Lecture 1
Photophysics of Aromatic Molecules, J B Birks, Wiley, 1970. Book.

Topics in Fluorescence Spectroscopy Vol.2: Principles, Ed. J R Lakowicz, Plenum, New York. 1991. Reviews.

*Principles of Fluorescence Spectroscopy,*2nd Edition, J R Lakowicz, Plenum, New York, 1999. Book.

MolecularFluorescence: Principles and Applications, B Valeur, Wiley-VCH, 2002. Book.

Lecture 2
Time-correlated single-photon counting.
D V O'Connor and D Phillips, Academic Press, 1984. Book.

Time-domain fluorescence spectroscopy using time-correlated single-photon counting.
D J S Birch and R E Imhof in "Topics in Fluorescence Spectroscopy Vol.1: Techniques", Ed. J R Lakowicz, Plenum, New York. Ch. 1, 1-95, 1991. Review.

Instrumentation for red/near infra-red fluorescence.
D J S Birch and G Hungerford in "Topics in Fluorescence Spectroscopy. Vol. 4: Probe Design and Chemical Sensing", Ed. J R Lakowicz, Ch. 12, 377-416, Plenum, 1994. Review.

Single-photon timing detectors for fluorescence lifetime spectroscopy.
G Hungerford and D J S Birch, Meas. Sci. Technol. 7, 121-35, 1996. Review.

Lecture 3
Sol-gel Science, The Physics and Chemstry of Sol-Gel Processing, C. J. Brinker, Academic, San Diego, 1989. Book.

Fluorescence nanometrology in sol-gels.
D J S Birch, C D Geddes, J Karolin, R Leishman and O J Rolinski. Spinger Series on Fluorescence Methods and Applications, Vol. 2. Review. In press.

Nanometre resolution of silica hydrogel formation using time-resolved fluorescence anisotropy.
C D Geddes and D J S Birch. J. Non-Crys. Solids. 270, 191-204, 2000.

Sol-gel particle growth studied using fluorescence anisotropy: An alternative to scattering techniques.
D J S Birch and C D Geddes. Phys. Rev. E. 62, 2977-2980, 2000.

Nanoparticle metrology in sol-gels using multiphoton excited fluorescence.
J Karolin, C D Geddes, K Wynne and D J S Birch. Meas. Sci. Technol. 13, 21-7, 2002.

1 and 2-photon fluorescence anisotropy decay in silicon alkoxide sol-gels: Interpretation in terms of self-assembled nanoparticles.
C D Geddes, J Karolin and D J S Birch, J. Phys. Chem. B. In press.

Lecture 4
Topics in Fluorescence Spectroscopy Vol.4: Probe design and chemical sensing, Ed. J R Lakowicz, Plenum, New York. 1994. Reviews.

Fluorescence resonance energy transfer sensors.
D J S Birch and O J Rolinski, Research in Chemical Intermediates. 27, 425-46, 2001. Review.

Intelligent sensor for metal ions based on fluorescence resonance energy transfer.
D J S Birch, A S Holmes and M Darbyshire, Meas. Sci. Technol. 6, 243-7, 1995.

Fluorescence lifetime sensor of copper ions in water.
D J S Birch, O J Rolinski and D Hatrick, Rev. Sci. Instrum. 67, 2732-7, 1996.

A fluorescence lifetime sensor for Cu(I) ions.
O J Rolinski and D J S Birch, Meas. Sci. Technol. 10, 127-36, 1999.

MHz LED source for nanosecond fluorescence sensing.
W J O'Hagan, M McKenna, D C Sherrington, O J Rolinski, and D J S Birch. Meas. Sci. Technol. 13, 84-91, 2002.

Lecture 5

In vivo glucose sensing for diabetes management: progress towards non-invasive monitoring.
J Pickup, L McCartney, O Rolinski, D Birch, British Medical Journal. 319, 1289-, 1999. Review.

Spectroscopic and clinical aspects of noninvasive glucose monitoring.
O S Khalil, Clinical Chem. 45, 165-177, 1999. Review.

A time-resolved near-infra-red fluorescence assay for glucose: opportunities for trans dermal sensing.
O J Rolinski, D J S Birch, L J McCartney and J C Pickup. J. Photochem. and Photobiol. B.54, 26-34, 2000.

Determination of acceptor distribution from fluorescence resonance energy transfer: Theory and simulation.
O J Rolinski and D J S Birch. J.Chem. Phys. 112, 8923-8933, 2000.

Sensing metabolites using donor-acceptor nano-distributions in fluorescence resonance energy transfer.
O J Rolinski, D J S Birch, L J McCartney and J C Pickup. Appl. Phys. Letts. 78, 2796-2798, 2001.

Near-infrared fluorescence lifetime assay for serum glucose based on allophycocyanin-labelled concanavalin A..
L J McCartney, J C Pickup, O J Rolinski, D J S Birch. Anal. Biochem. 292, 216-221, 2001.

Molecular distribution sensing in fluorescence resonance energy transfer based affinity assays for glucose.
O J Rolinski, D J S Birch, L J McCartney and J C Pickup. Spectrochimica Acta Part A 57, 2245-2254, 2001.

Fluorescence nanotomography using resonance energy transfer: demonstration with a protein-sugar complex.
O J Rolinski, D J S Birch, L J McCartney and J C Pickup. Phys. Med. Biol. 46, 221-6, 2001.

Lecture 6

Topics in Fluorescence Spectroscopy Vol.5: Nonlinear and two-photon induced fluorescence, Ed. J R Lakowicz, Plenum, New York. 1997. Reviews.

Multiphoton excited fluorescence spectroscopy of biomolecular systems.
D J S Birch. Spectrochimica Acta 57, 2313-2336, 2001. Review.

Femtosecond three-photon excitation and single-photon timing detection of a-NPO fluorescence.
A Volkmer, D A Hatrick, Y Bai and D J S Birch, Chem. Phys. Lett. 268, 439-48, 1997.

Time-resolved non-linear fluorescence spectroscopy using femtosecond multi-photon excitation and single-photon timing detection.
A Volkmer, D A Hatrick and D J S Birch, Meas. Sci. Technol. 8, 1339-49, 1997.

Near-infrared excitation of alkane ultraviolet fluorescence.
A Volkmer, K Wynne and D J S Birch, Chem.Phys.Letts. 299, 395-402, 1999.

Femtosecond two-photon excited fluorescence of melanin.
K Teuchner, W Freyer, D Leupold, A Volkmer, D J S Birch, P Altmeyer, M Stuker and K Hoffmann, Photochem. and Photobiol. 70, 146-151,1999.

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A Volkmer, V Subramaniam, D J S Birch and T M Jovin. Biophys. J.78, 1589-1598, 2000.