Optical spectroscopy and biosensors for investigation of biomolecules and their interactions

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Fluorescence Spectroscopy II

Content

- Fluorescence emission at dielectric interface: super critical fluorescence (SAF)
- Fluorescence emission at metallic interface: quenching vs. enhancement
- Surface plasmon field-enhanced fluorescence spectroscopy (SPFS)
- Metallic nanostructure-enhanced fluorescence
- Bloch surface wave-enhanced fluorescence
- Two-photon fluorescence excitation
- Fluorescence lifetime readout of assays

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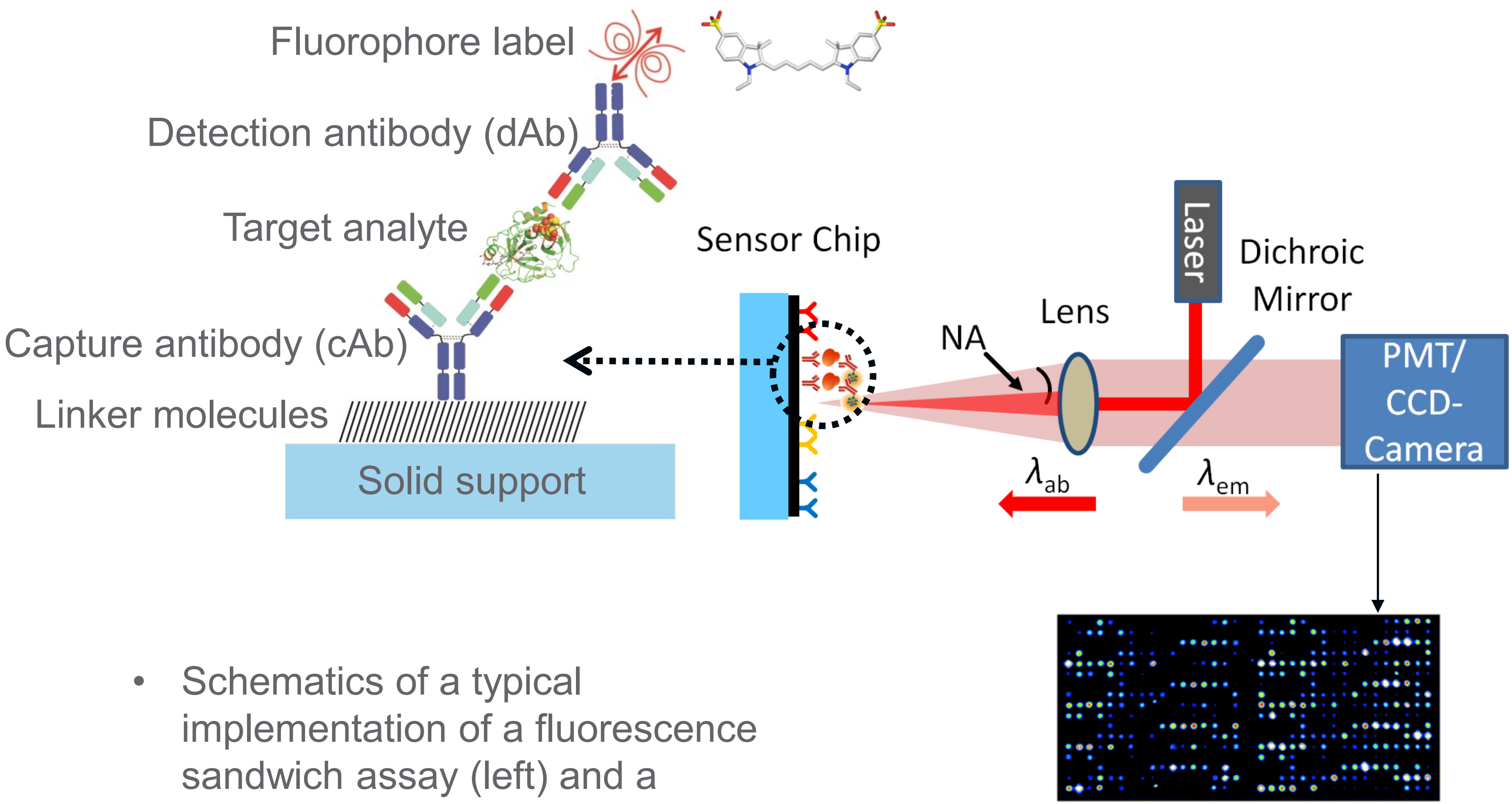
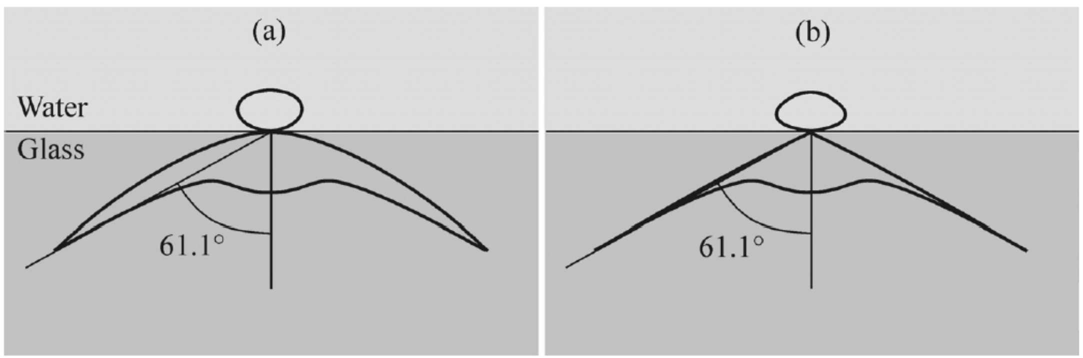
Fluorescence Immunoassays



- Schematics of a typical implementation of a fluorescence sandwich assay (left) and a respective reader (right).

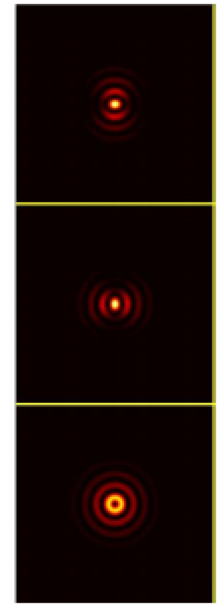
Emission at a Dielectric Interface (λ_{em})

https://doi.org/10.1364/OPEX.12.004246



Polar plots of the emission direction of isotropically oriented fluorophores with (a) a surface distance of zero and (b) a surface distance equal to a third of the emission wavelength in vacuum

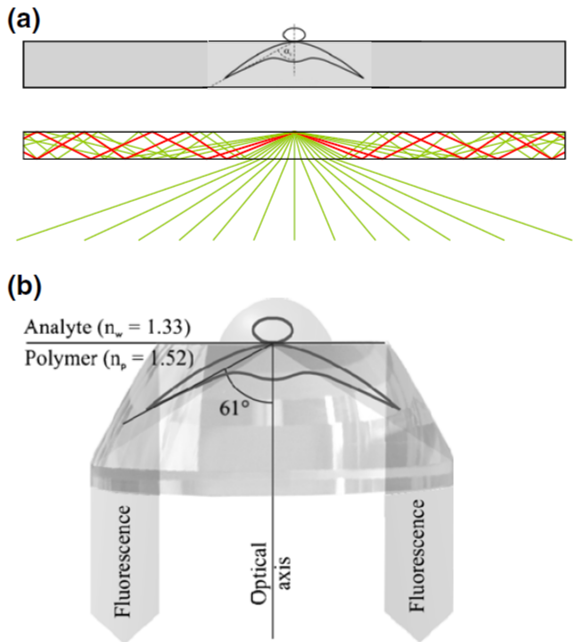
•https://doi.org/10.1364/OE.19.008011

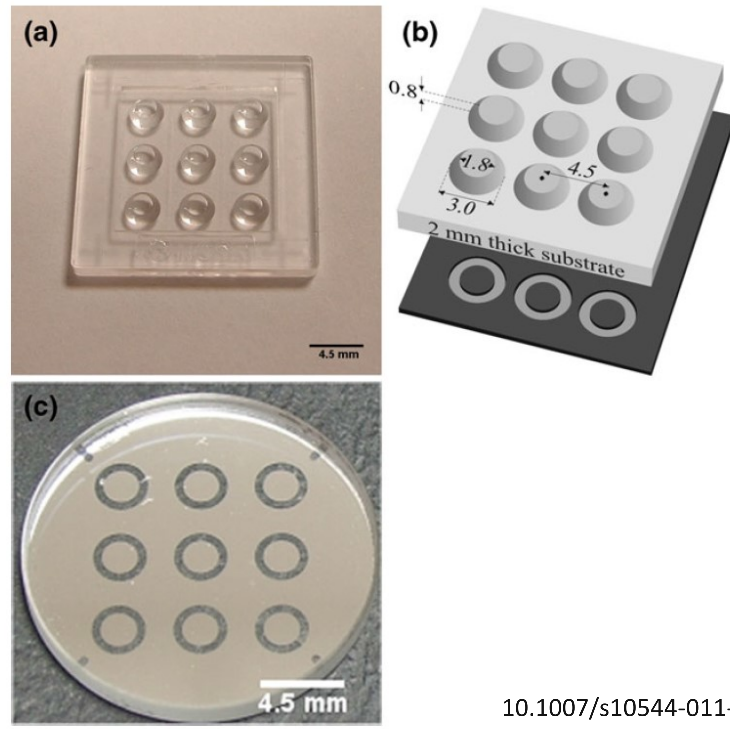


Emission pattern collected for individual emitter

- At a glass substrate, the majority of emitted fluorescence light travels in the substrate above the critical angle and thus is trapped.

Supercritical Angle Fluorescence (λ_{em})



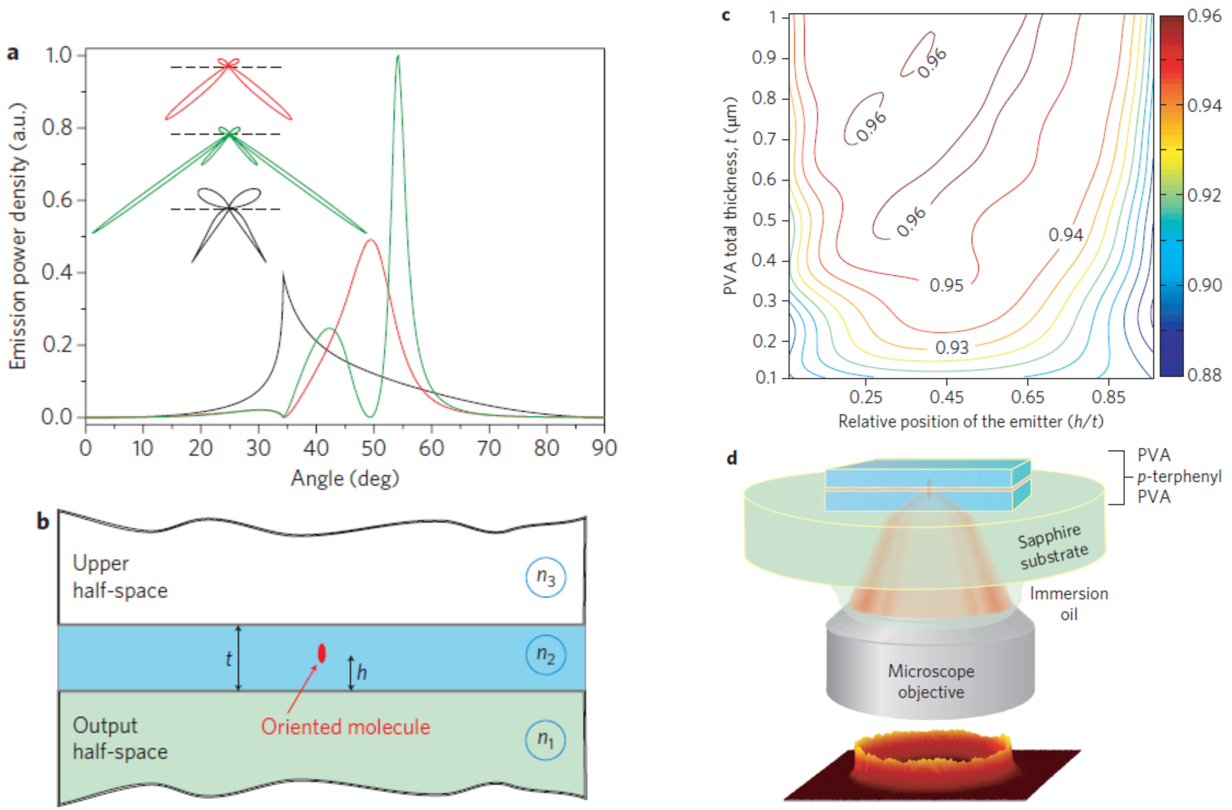


10.1007/s10544-011-9546-2

- An implementation of arrays of parabolic mirrors embossed to a plastic chip for enhanced extraction of the fluorescence light (MacCraigth group).

High Efficiency Extraction with Dielectric Structures (λ_{em})

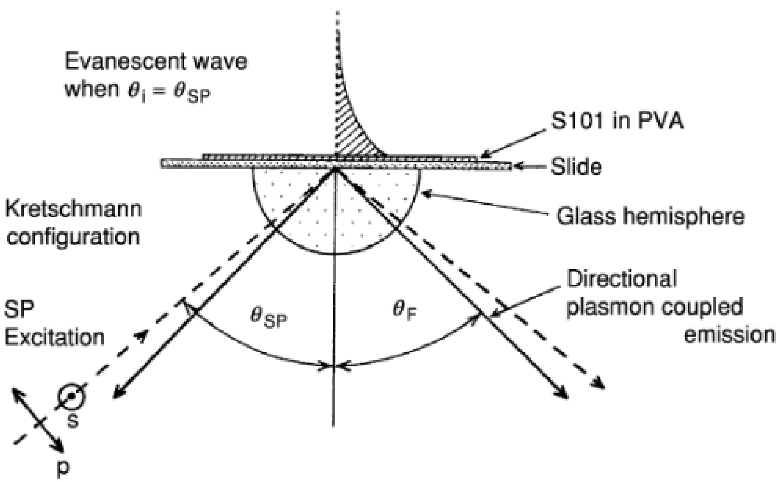
10.1038/NPHOTON.2010.312

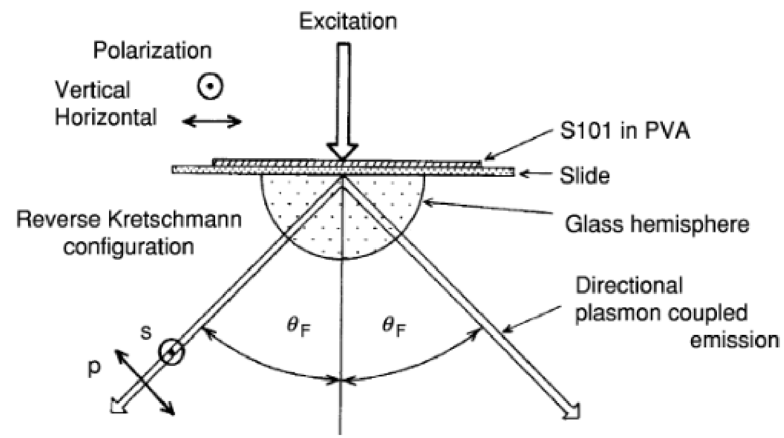


- 96% collection efficiency demonstrated by using TIR with emitters embedded in dielectric layer (Sandoghar group).

Surface Plasmon-Coupled Emission (λ_{em})

https://doi.org/10.1016/S0091-679X(04)75004-9

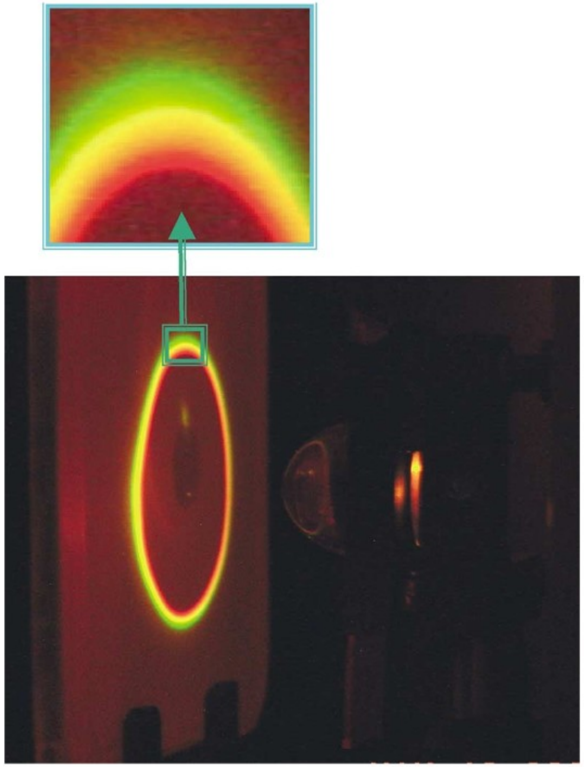




- 60% collection efficiency can be achieved by using reverse Kretschamnn configuration for surface plasmons on gold in the red part of spectrum. Investigated by Lakowicz group.

Surface Plasmon-Coupled Emission (λ_{em})

https://doi.org/10.1016/S0091-679X(04)75004-9



Photography of surface-plasmon–coupled emission cone for multifluorophore sample

- Surface plasmon-coupled emission is manifested as a cone generated by a hemispherical prims / lens.

Surface Plasmon-Enhanced Fluorescence (λ_{ex})

ELSEVIER

Colloids and Surfaces
A: Physicochemical and Engineering Aspects 171 (2000) 115–130

COLLOIDS AND SURFACES A

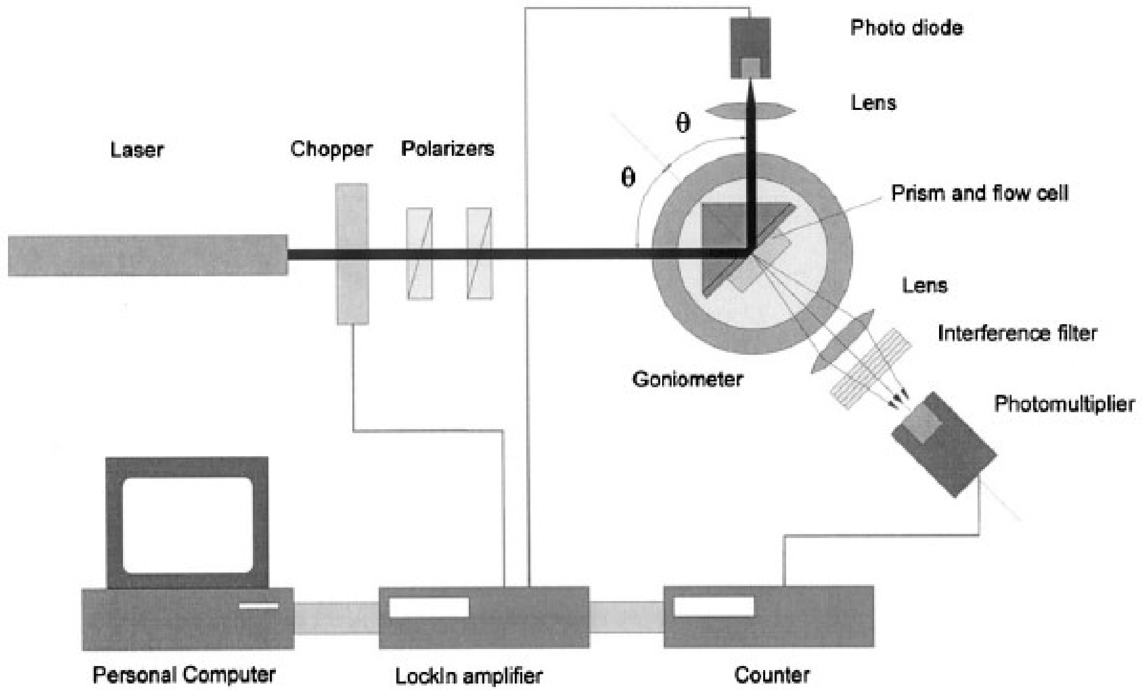
www.elsevier.nl/locate/colsurfa

Surface-plasmon field-enhanced fluorescence spectroscopy

Thorsten Liebermann *, Wolfgang Knoll

Max-Planck-Institut für Polymerforschung, Ackermannweg 10, D-55128 Mainz, Germany

Surface Plasmon-Enhanced Fluorescence (λ_{ex})



- Combination of surface plasmon resonance (SPR) and surface plasmon field enhanced fluorescence excitation (SPFS).

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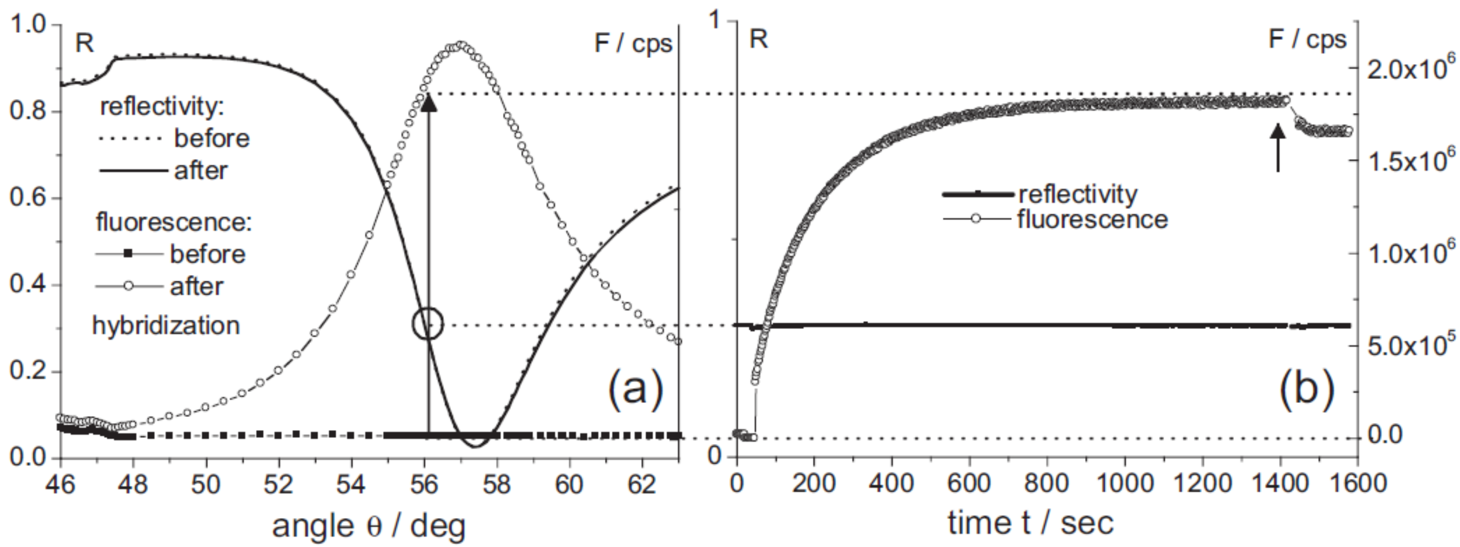
Surface Plasmon-Enhanced Fluorescence (λ_{ex})



- Comparison of SPR and SPFS for investigation of short oligonucleotide hybridization.

Surface Plasmon-Enhanced Fluorescence (λ_{ex}, λ_{em})

Sensitivity enhancement of optical immunosensors by the use of a surface plasmon resonance fluoroimmunoassay

J. W. Attridge, P. B. Daniels, J. K. Deacon, G. A. Robinson & G. P. Davidson

Serono Diagnostics Ltd., Unit 21, Woking Business Park, Albert Drive, Woking, Surrey GU21 5JY, UK

(Received 4 May 1990; revised version received 7 August 1990; accepted 9 August 1990)

Biosensors & Bioelectronics **6** (1991) 201–214

Surface Plasmon-Enhanced Fluorescence ($\lambda_{ex,}$ λ_{em})

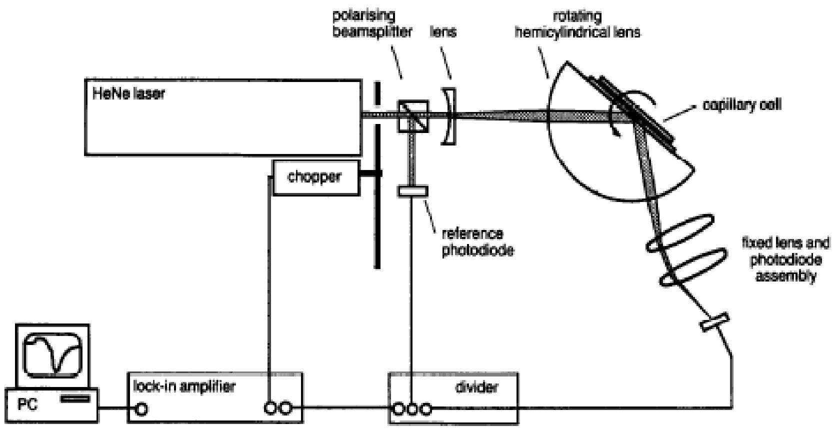
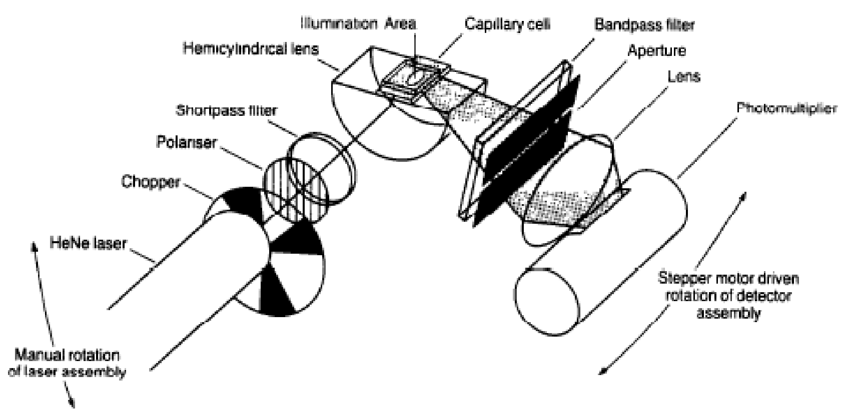
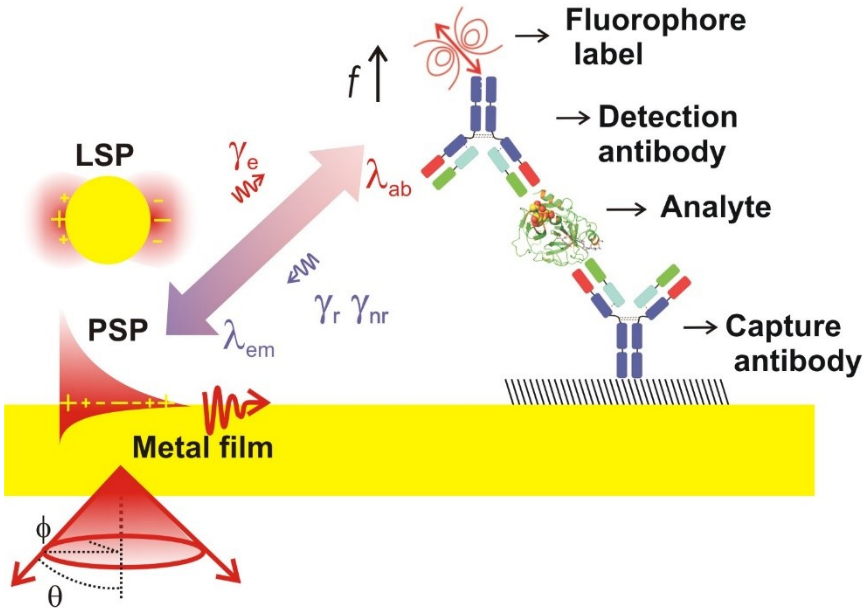


Fig. 4. Experimental apparatus used for SPR reflectance measurements.



Plasmon-Enhanced Fluorescence: on the Contributions



$$EF \propto \frac{\gamma_e}{\gamma_e^0} \times \frac{\eta}{\eta^0} \times f_d$$

Enhanced excitation rate at λ_{ab}

Increasing quantum efficiency

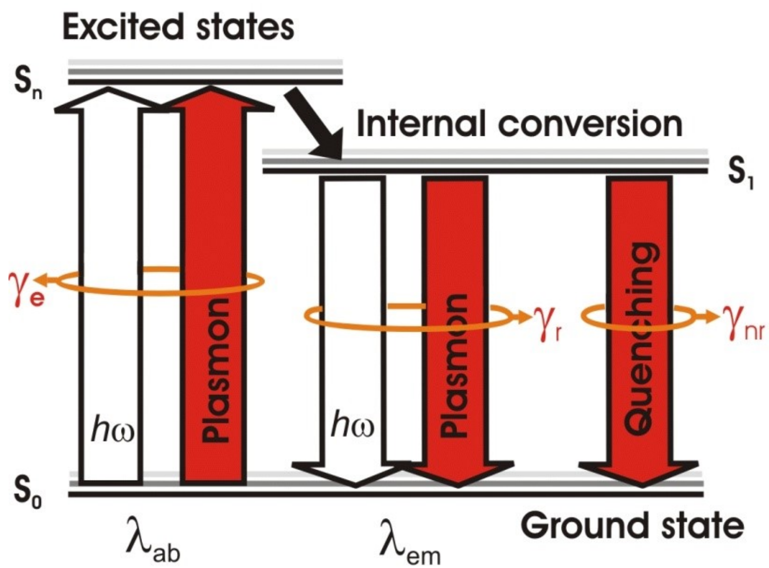
Extraction yield by directional emission at λ_{em}

Confined field of surface plasmons at λ_{ab} and λ_{em} can amplify fluorescence signal from emitters

- **Coupling at λ_{ab} by enhanced field intensity $|E/E_0|^2$ – locally increasing excitation rate γ_e.**
- **Coupling at λ_{em}, surface plasmon-coupled emission – directional emission for extracting of fluorescence light from the surface.**
- **Enhancement fluorescence signal intensity by a factor $EF > 10^2\text{-}10^3$**

M. Bauch, K. Toma, M. Toma, Q. Zhang, J. Dostalek, Surface plasmon-enhanced fluorescence biosensors: a review, Plasmonics (2014), 9 (4), 781-799.

Plasmon-Enhanced Fluorescence



Modified Jablonski diagram with transitions facilitated by surface plasmons:

- **excitation rate γ_e**
- **radiative emission rate γ_r**
- **non-radiative emission rate γ_{nr}.**

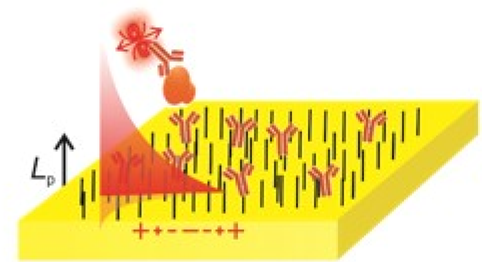
M. Bauch, K. Toma, M. Toma, Q. Zhang, J. Dostalek, Surface plasmon-enhanced fluorescence biosensors: a review, Plasmonics (2014), 9 (4), 781-799.

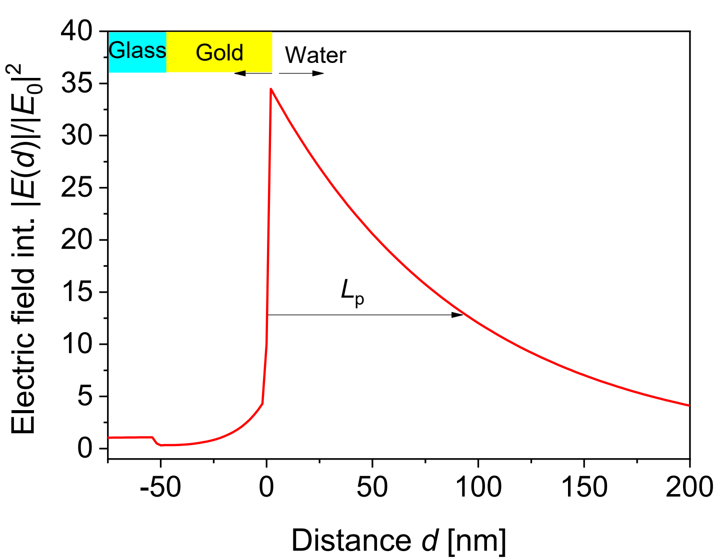
Plasmon-Enhanced Fluorescence Excitation

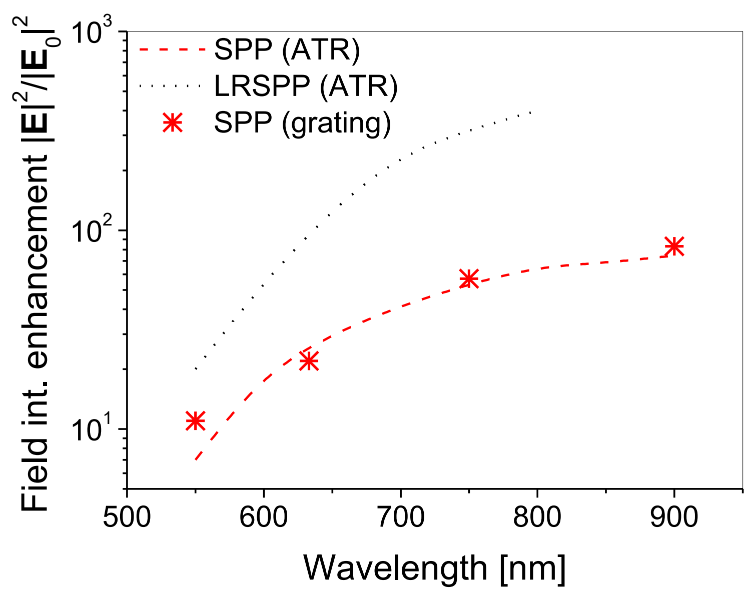
$$\gamma_{\mathrm{e}} \propto \left|\boldsymbol{E}\cdot\boldsymbol{\mu}_{\mathrm{ab}}\right|^2$$

Electromagnetic field strength

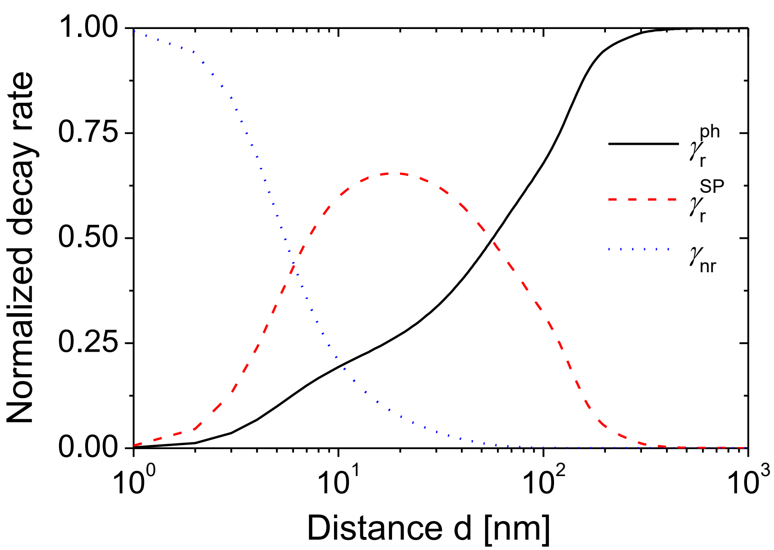
Excitation dipole moment of the fluorophore

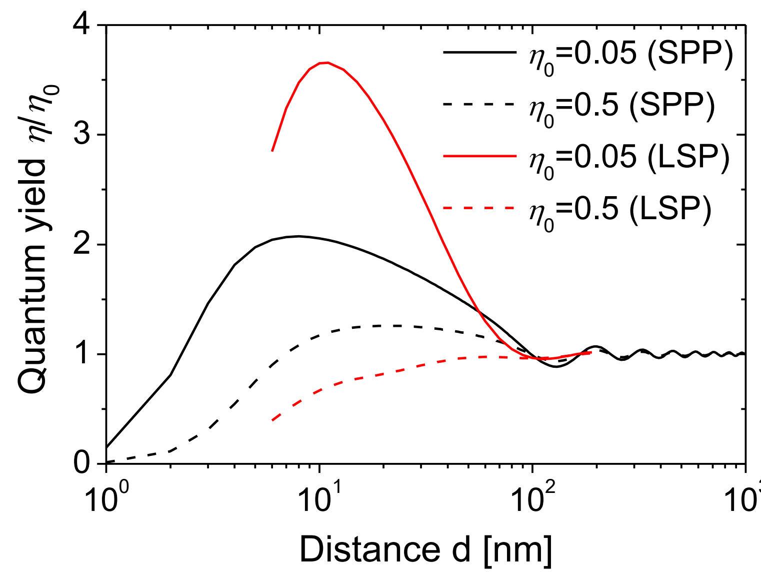






Plasmon-Enhanced Quantum Yield



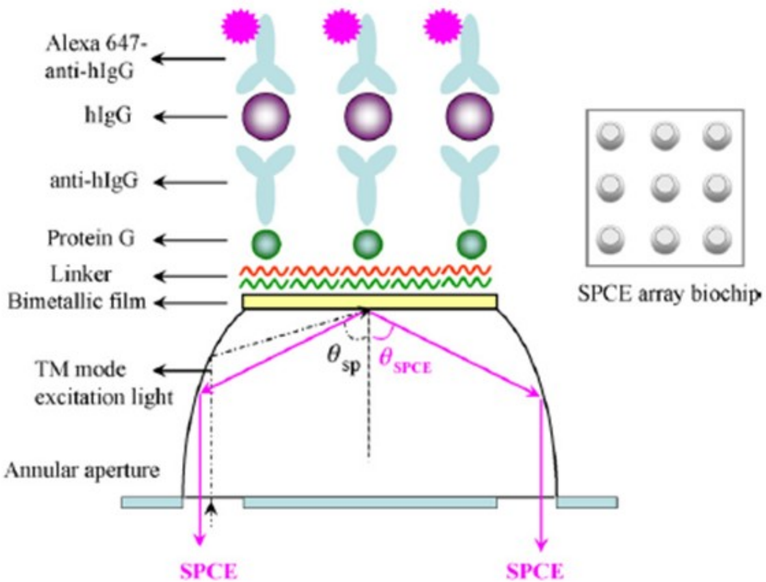


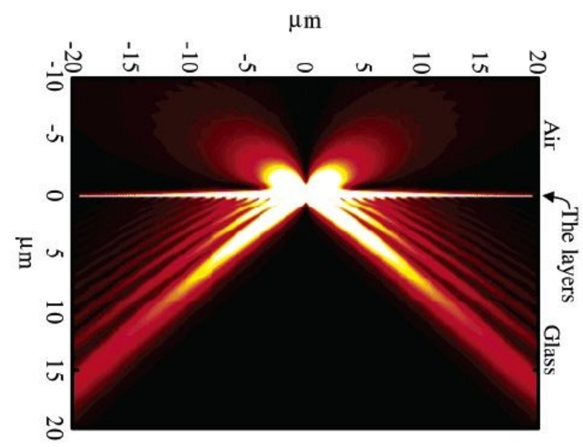
$$\eta = \frac{\gamma_r / \gamma_r^0}{\gamma_r / \gamma_r^0 + \gamma_{abs} / \gamma_r^0 + \left(1 - \eta^0\right) / \eta^0}$$

Quantum yield of a free molecule η_0 is amended by competing plasmon-enhanced radiative and non-radiative transitions

M. Bauch, K. Toma, M. Toma, Q. Zhang, J. Dostalek, Surface plasmon-enhanced fluorescence biosensors: a review, Plasmonics (2014), 9 (4), 781-799.

Plasmon-Enhanced Fluorescence Extraction Yield



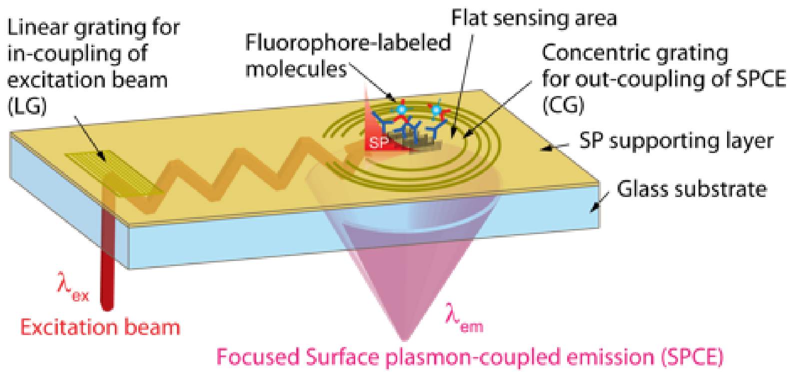


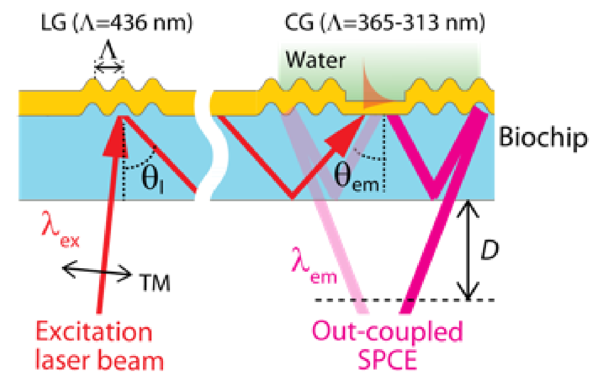
N. Calander, Anal. Chem. **76**, 2168 2004

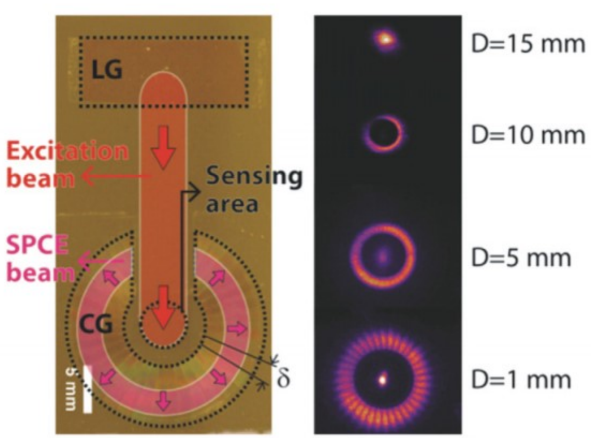
- Exploiting directional surface plasmon-coupled emission allows to collect up to 60 % of emitted light and suppressing background.

Yuk JS, Trnavsky M, McDonagh C, MacCraith BD (2010) Surface plasmon-coupled emission (SPCE)-based immunoassay using a novel paraboloid array biochip. Biosens Bioelectron 25(6):1344– 1349

Plasmonic Amplification for Compact Readers (λ_{ex}, λ_{em})





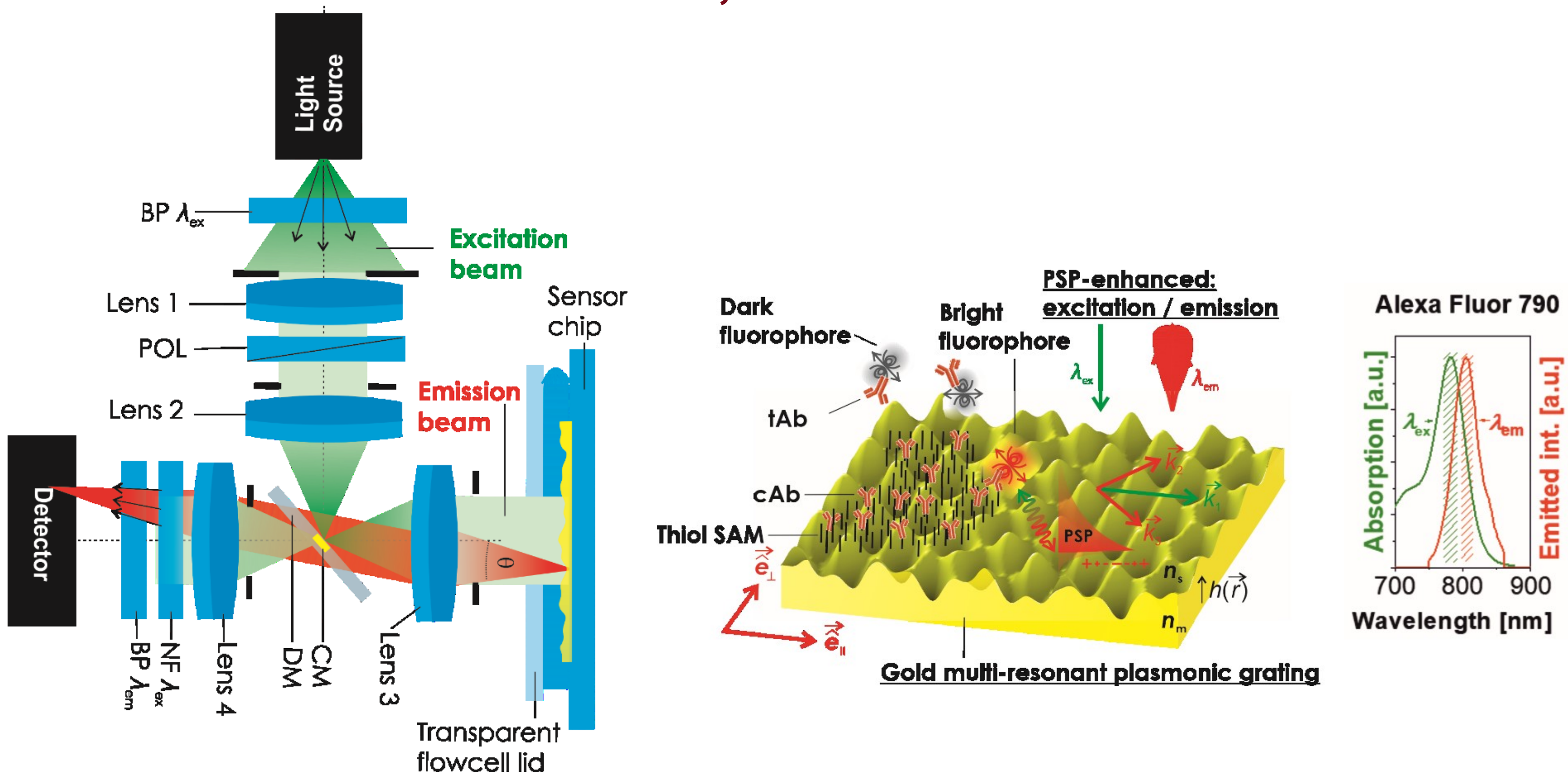


- Combined enhancement by SP excitation at λ_{ex} ($|E/E_0|^2$~|20) and collecting by SPs at λ_{em} (up to 60% efficiency)
- Diffractive optical elements DOE – compatible with NIL, hot embossing
- Simplified setup (integration by DEO, possibly filters are not needed)

K. Toma, Dostalek, Compact surface plasmon-enhanced fluorescence biochip, Optics Express (2013), 21(8), 10121-10132.

M. Toma, K. Toma, P. Adam, J. Homola, W. Knoll, J. Dostalek, Surface plasmon-coupled emission on plasmonic Bragg gratings, Optics Express, (2012), 20(13), 14042.

Plasmonic Fluorescence Amplification for Microarrays ($\lambda_{ex}, \lambda_{em}$)

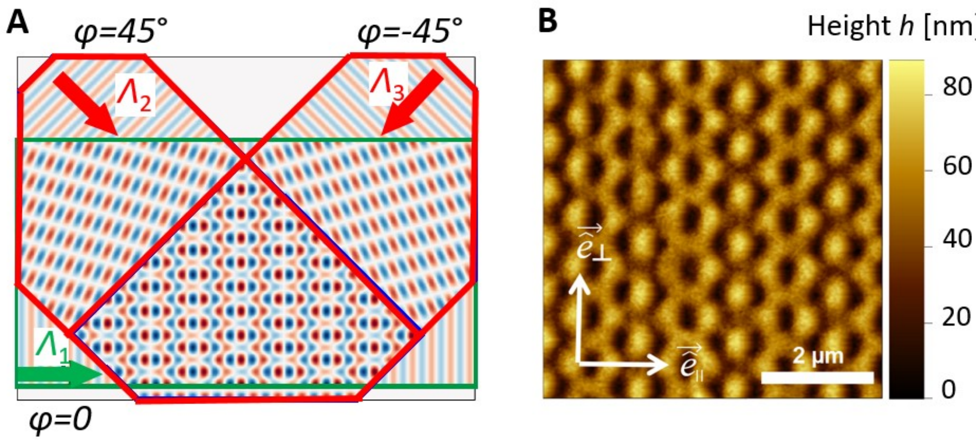


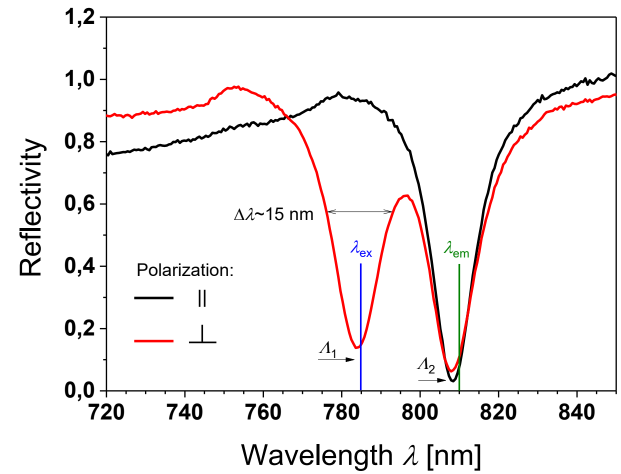
- Tailored plasmonic resonances by using **multiperiod plasmonic gratings** for fluorescence enhancement by coupling at fluorophore label at λ_{ex} and λ_{em} for imaging of arrays of spots.

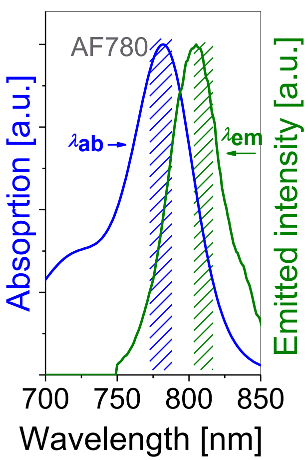
S. Fossati, S. Hagender, S. Menad, E. Maillart, J. Dostalek, Multi-resonant plasmonic nanostructure for ultrasensitive fluorescence biosensing, 2020, Nanophotonics, in press.

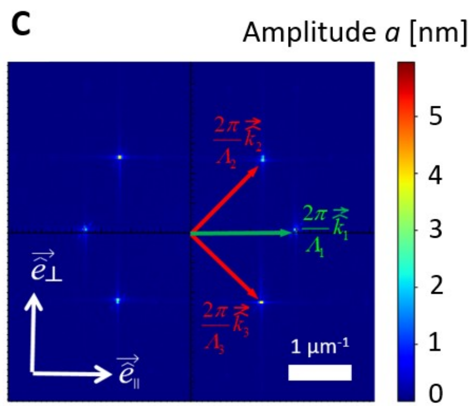
J. Dostalek, et al., Plasmon-enhanced fluorescence spectroscopy imaging by multi-resonant nanostructures, European Patent Application No. 19164960.7

Two-Resonant Plasmonic Nanostructure for PEF







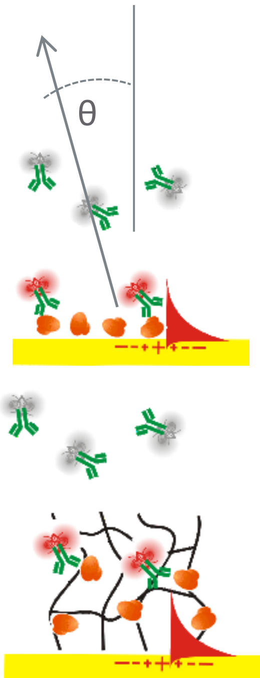


- Multi-diffractive coupling to SPPs engineered to exhibit two-resonance feature.
- Period Λ_1 serves for enhancing the excitation rate (λ_{ex}) and Λ_2 for the directional SPP-coupled emission (λ_{em}).

Stefan Fossati, Simone Hagender, Samia Menad, Emmanuel Maillart, Jakub Dostalek, Multi-resonant plasmonic nanostructure for ultrasensitive fluorescence biosensing, 2020, Nanophotonics, in press.

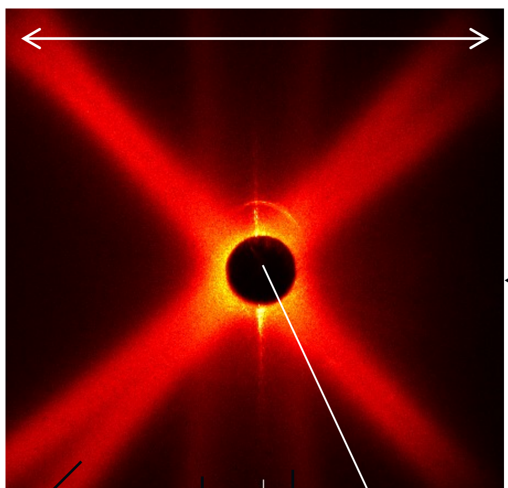
J. Dostalek, W. Knoll, S. Fossati, S. Hageneder,V. Jungbluth, Plasmon-enhanced fluorescence spectroscopy imaging by multi-resonant nanostructures, European Patent Application No. 19164960.7

Surface Plasmon-Coupled Emission

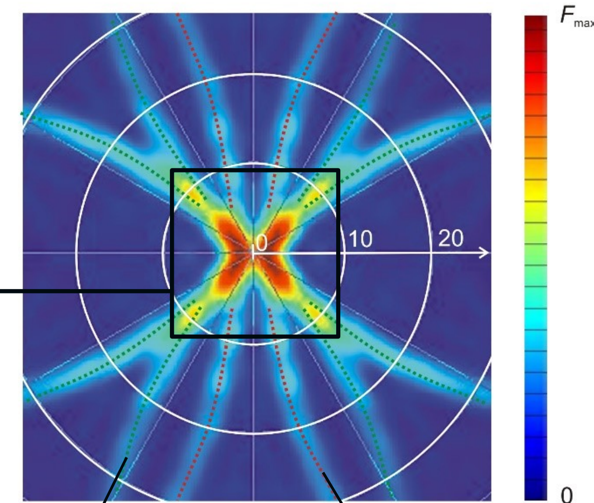


Measured distribution of emitted light in Fourier plane:

Δθ=20.4 deg (NA=0.18)



Simulated angular distribution of emitted light at λ_{em}:



Emission extracted by Λ_2

Emission extracted by Λ_1

Miniature mirror blocking excitation beam λ_{ex}

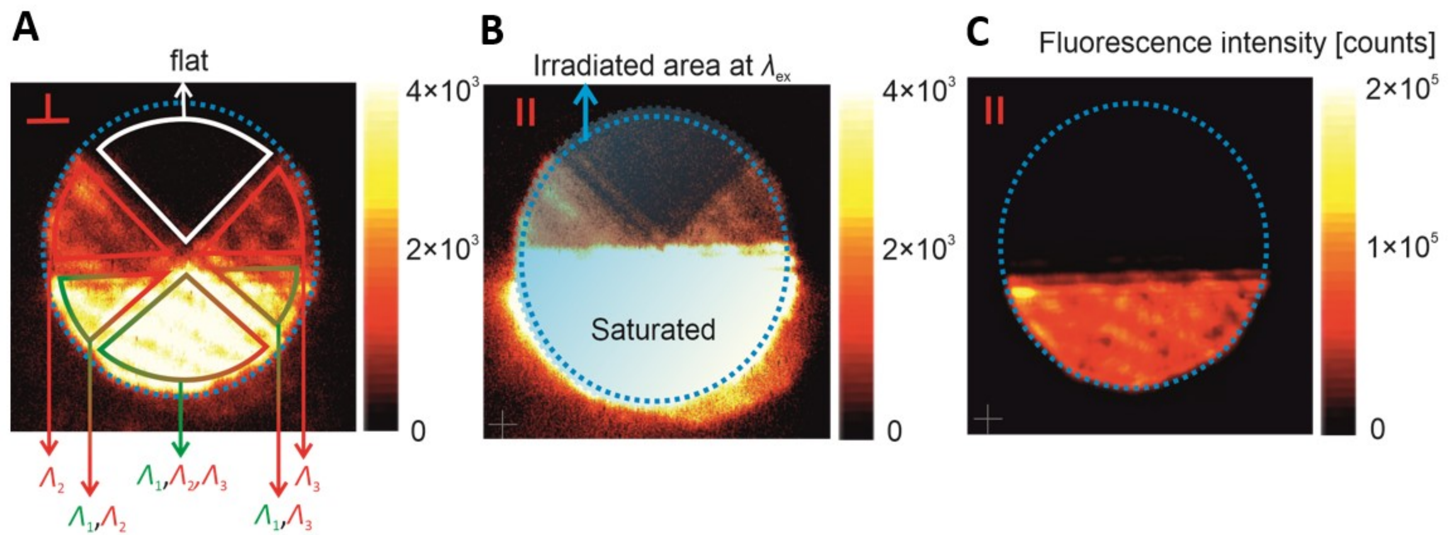
1st order out-coupling at Λ_2

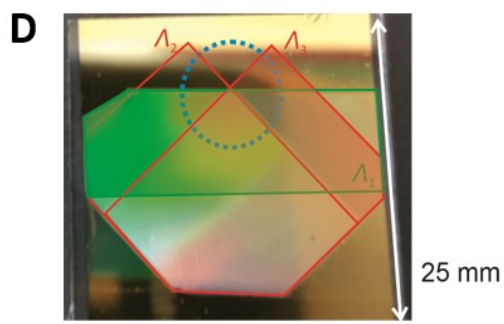
1st order out-coupling at Λ_1

Stefan Fossati, Simone Hagender, Samia Menad, Emmanuel Maillart, Jakub Dostalek, Multi-resonant plasmonic nanostructure for ultrasensitive fluorescence biosensing, 2020, Nanophotonics, in press.

J. Dostalek, W. Knoll, S. Fossati, S. Hageneder,V. Jungbluth, Plasmon-enhanced fluorescence spectroscopy Imaging by multi-resonant nanostructures, European Patent Application No. 19164960.7

Plasmonic Enhancement of Affinity Binding Response





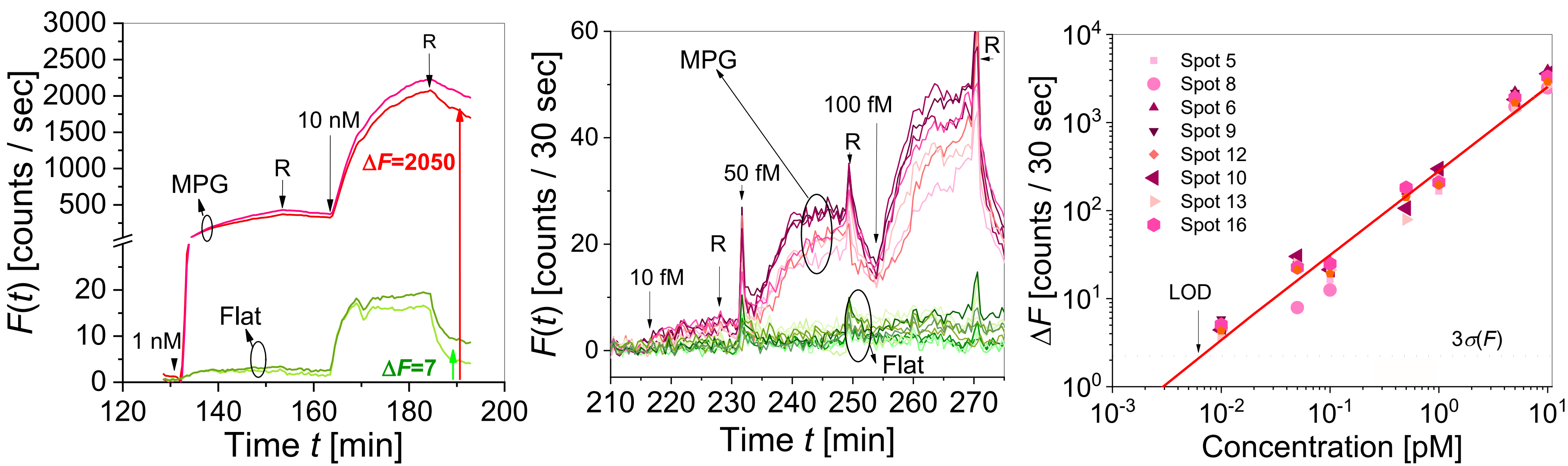
E **Enhancement factor (*EF*):**

Polarization	||	⊥
Λ_2 or Λ_3	3.7 ×	3.7 ×
$\Lambda_{1,2}$ or $\Lambda_{1,3}$	248 ×	17 ×
$\Lambda_{1,2,3}$	300 ×	25 ×

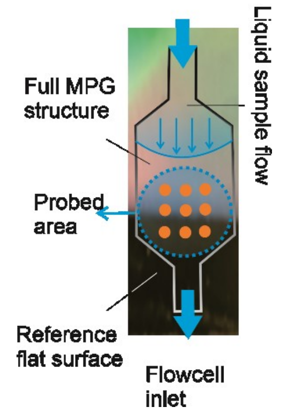
Stefan Fossati, Simone Hagender, Samia Menad, Emmanuel Maillart, Jakub Dostalek, Multi-resonant plasmonic nanostructure for ultrasensitive fluorescence biosensing, 2020, Nanophotonics, in press.

J. Dostalek, W. Knoll, S. Fossati, S. Hageneder,V. Jungbluth, Plasmon-enhanced fluorescence spectroscopy Imaging by multi-resonant nanostructures, European Patent Application No. 19164960.7

Plasmonic Enhancement of Affinity Binding Response at λ_{ex} and λ_{em}



- **Fluorescence intensity enhancement of *EF*=300** was observed for structure engineered to couple with emitters at both λ_{ab} and λ_{em}.
- **LOD = 6 fM** was achieved for a model immunoassay with about 50 spots on chip.
- Capability of **kinetics measurements** of affinity interactions (background due to bulk is suppressed).



Stefan Fossati, Simone Hagender, Samia Menad, Emmanuel Maillart, Jakub Dostalek, Multi-resonant plasmonic nanostructure for ultrasensitive fluorescence biosensing, 2020, Nanophotonics, in press.

J. Dostalek, W. Knoll, S. Fossati, S. Hageneder,V. Jungbluth, Plasmon-enhanced fluorescence spectroscopy Imaging by multi-resonant nanostructures, European Patent Application No. 19164960.7

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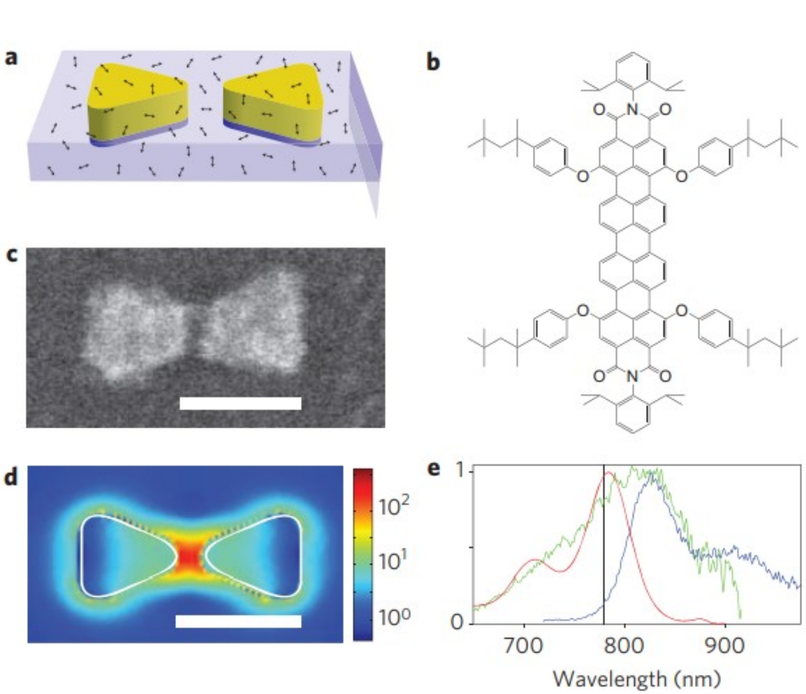
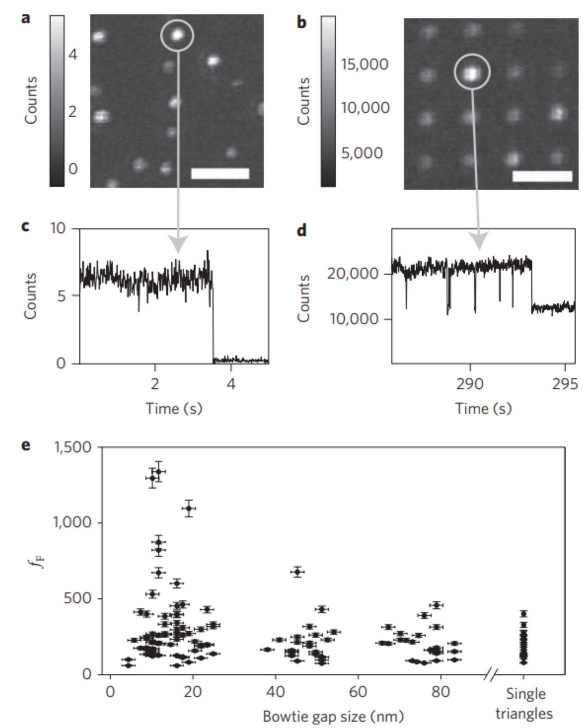
LETTERS
PUBLISHED ONLINE: 18 OCTOBER 2009 | DOI: 10.1038/NPHOTON.2009.187
nature photonics

Large single-molecule fluorescence enhancements produced by a bowtie nanoantenna

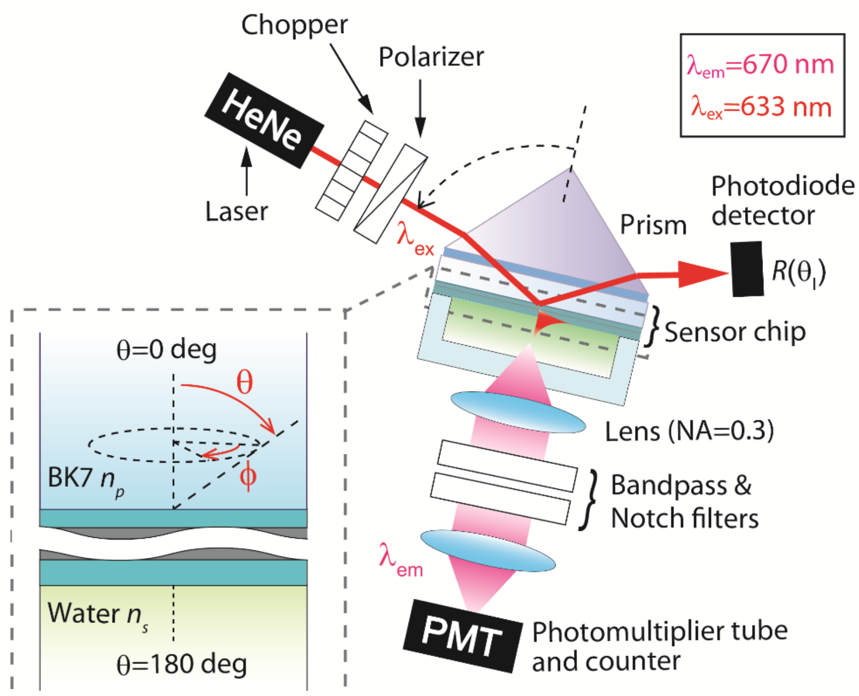
Anika Kinkhabwala[1], Zongfu Yu[2], Shanhui Fan[2], Yuri Avlasevich[3], Klaus Müllen[3] and W. E. Moerner[1]*

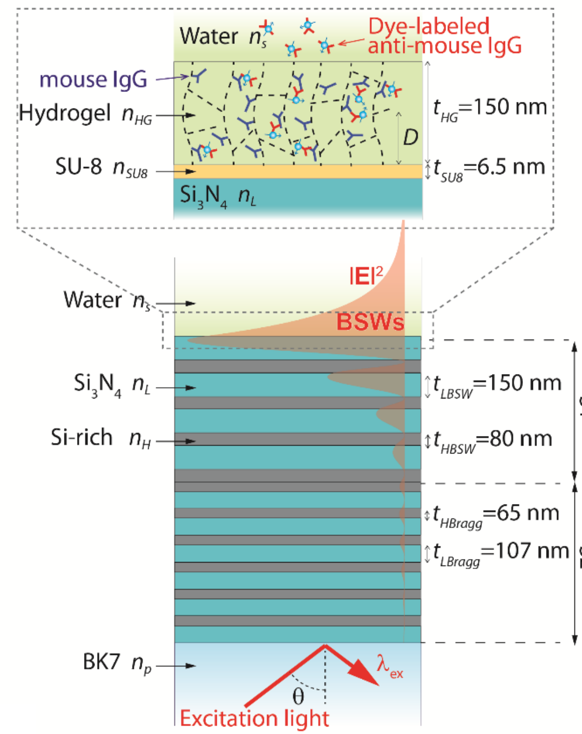




- Fluorescence intensity enhancement of *EF*>10^3 was demonstrated for individual emitters coupled to strongly confined field of localized surface plasmons.

Bloch Surface Waves

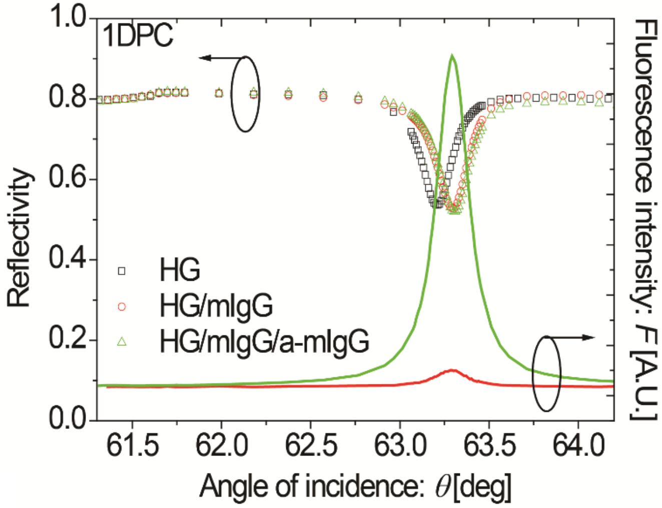


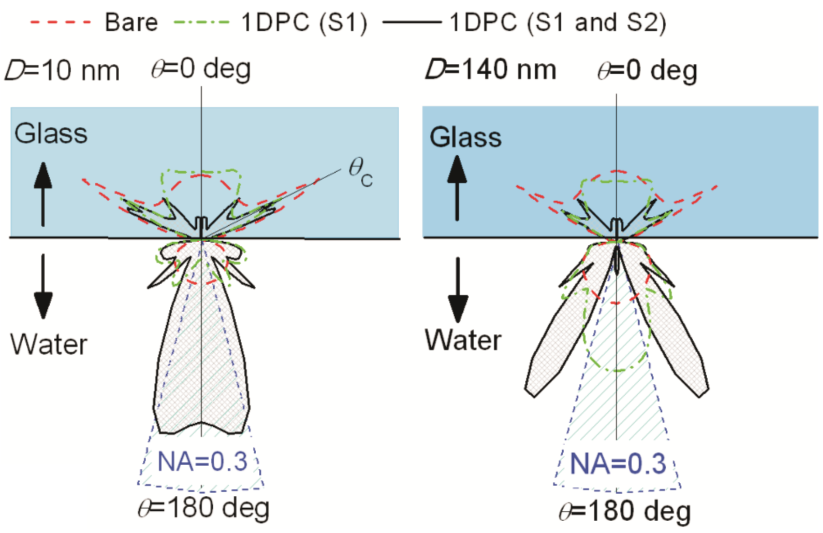


- 1D Photonic crystals (1DPC) can be design to support Bloch surface waves (BSW) and exhibit a bandgap at specific wavelengths.
- BSWs are similar to surface plasmons, but offer the advantage of less damping as the supporting multilayer structure is all dielectric.

K. Toma, E. Descrovi, M. Toma, M. Ballarini, P. Mandracci, F.Giorgis, A.Mateescu, U. Jonas, W. Knoll, J. Dostalek, Bloch Surface Wave-Enhanced Fluorescence Spectroscopy Biosensor, Biosensors and Bioelectronics (2013), 43, 108-114.

Bloch Surface Wave – Enhanced Fluorescence

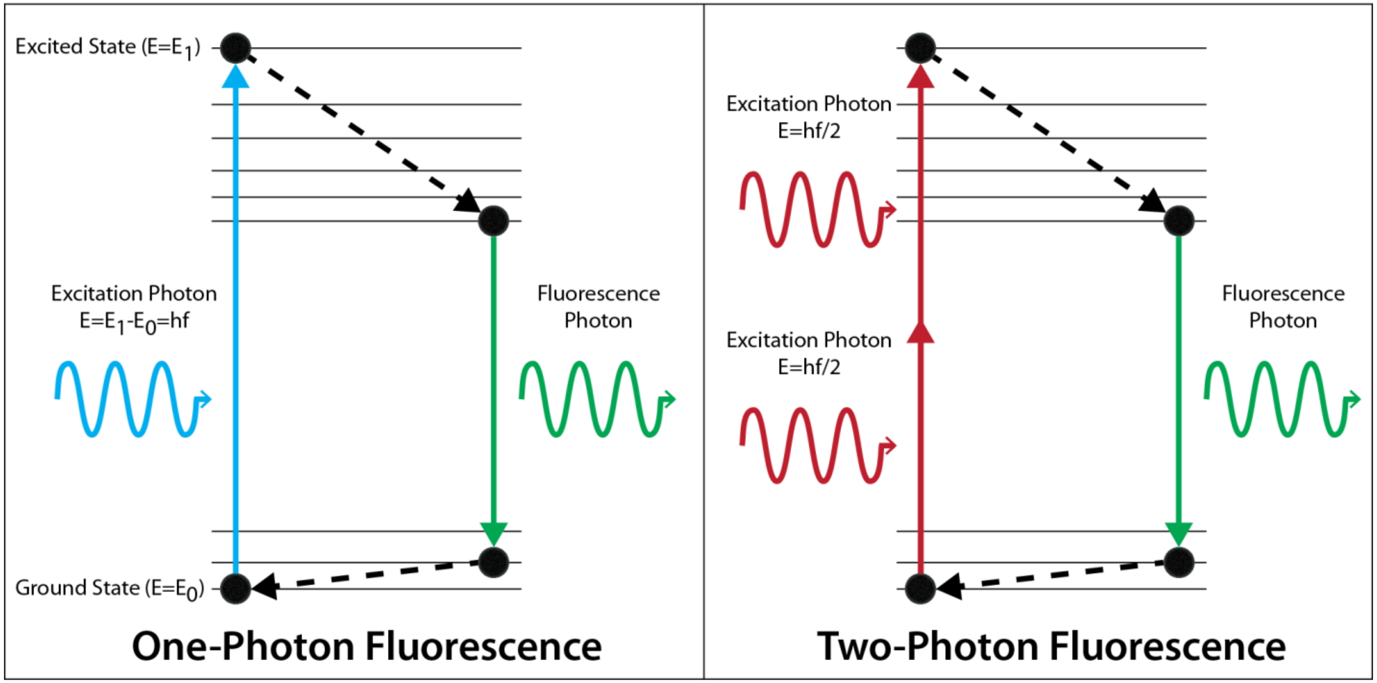




- Example of combined BSW-enhanced excitation (left) and directional 1DPC – controlled emission angular fluorescence distribution (right).

K. Toma, E. Descrovi, M. Toma, M. Ballarini, P. Mandracci, F.Giorgis, A.Mateescu, U. Jonas, W. Knoll, J. Dostalek, Bloch Surface Wave-Enhanced Fluorescence Spectroscopy Biosensor, Biosensors and Bioelectronics (2013), 43, 108-114.

Multiphoton-Photon Fluorescence

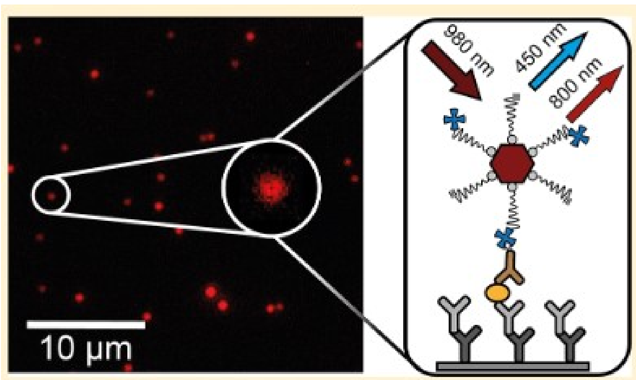


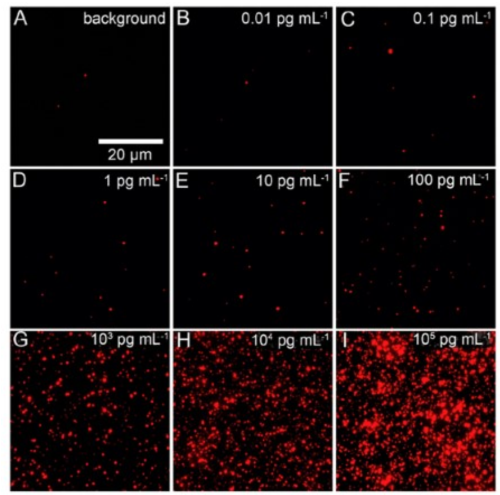
https://sites.middlebury.edu/durst/research/

- Requires using of strong excitation field is this is non-linear optical process, enables squeezing the excitation volume (excitation ~ E^4).
- Emission occurs at shorter wavelength λ_{ex} than the excitation one λ_{em}.

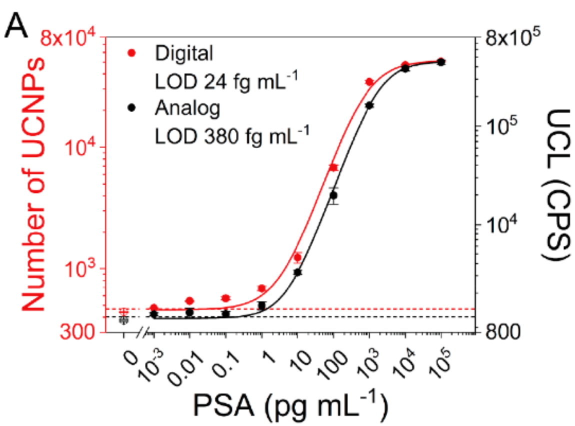
EUROPEAN UNION
European Structural and Investment Funds
Operational Programme Research, Development and Education

Photon Up-Conversion for Fluorescence Assays





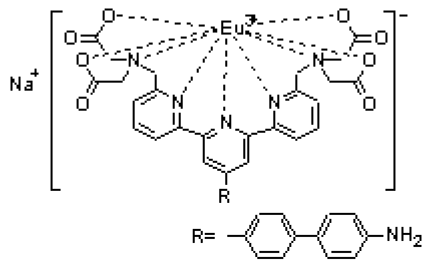
Example of digital readout of sandwich assay enabling improving the LOD by a factor of ~ 16.



- Emission at shorter wavelength λ_{ex} allows more efficient subtracting background (elimination of the autofluorescence, large difference between λ_{ex} and λ_{em}.

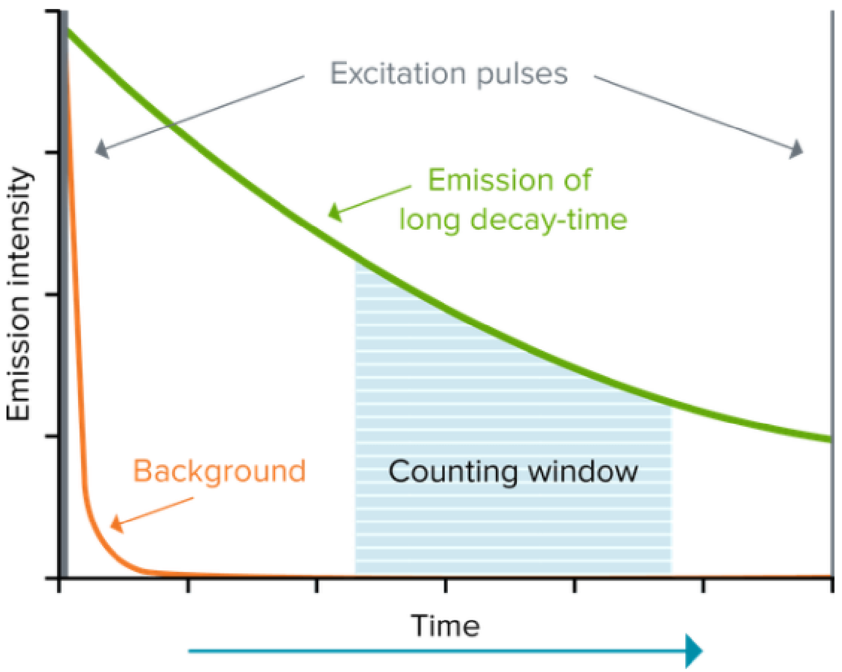
10.1021/acs.analchem.9b02872

Time-Resolved Fluorescence



A2083 ATBTA-Eu^{3+}

Europium chelate – based labels are excited with UV light and exhibit luminescence at ms time scale.



https://www.moleculardevices.com/technology/time-resolved-fluorescence-trf-tr-fret-htrf#gref

- The virtual background-free measurement provided by filtering (fast) autofluorescence based on time-delayed luminescence signal collection.