Achieving about billion-fold fluorescence enhancement for biosensing applications

Logeeshan Velmanickam[1], Ivan T. Lima Jr[1,2], and Dharmakeerthi Nawarathna[1,2]
[1]Department of Electrical and Computer Engineering, [2]Biomedical Engineering Program
North Dakota State University, Fargo, ND, 58102

Corresponding author; email: dharmakeerthi.nawara@ndsu.edu

Abstract: We have demonstrated that AC electric fields can be used to amplify the intensity of fluorophore molecules. AC electric fields enhance the metal-fluorophore-light interactions, which produce significant enhancements of fluorescence intensities. This concept can be applied to developing novel sensitive biosensors for basic research and clinical applications.

Low-cost, highly-sensitivity, and minimally-invasive tests for the detection and monitoring of life-threatening diseases such as cancer can reduce the worldwide disease burden. Fluorescence is commonly used as the method of detecting and quantifying the biomarker level expressions in samples [1, 2]. However, the challenge is to accurately detect the weak fluorescence intensities produced by few target biomarker molecules in samples. One potential solution to this problem is to increase the fluorescence intensity of biomarker molecules. Studies have shown that traditional gold or silver nanostructure-based plasmonic structures, called hotspots, could enhance fluorescence intensity of fluorophore molecules by a number of fundamental near-field light-metal-fluorophore interaction mechanisms [1]. Due to the unique nature of these interactions, detection methods available today can use only one or few of these near-field interactions, limiting enhancements to a few thousand-fold and resulting in detection in the sub-nM range, which is insufficient for detecting sub-pM miRNA levels in clinical samples [2]. Ideally, more than a million-fold fluorescence enhancement is needed for biomarker analysis in biological and clinical samples [2]. We have found that low frequency electric fields can be used to significantly enhance the fluoresce intensity of molecules. Moreover, we experimental results show that electric fields minimize the fluorophore quenching, increasing radiative decay rate (RDR) of fluorophore molecules, and increasing the energy harness from the excitation source by placing fluorophore molecules in the local hotspots [4]. Below, we explain how AC electric fields could enhance the fluorescence intensity and provide some experimental evidences to support our claims.

Fluorophore molecules are oscillating dipoles that can couple with surface plasmons of the metal to transfer energy. Coupling increases the fluorophore lifetime or decreases the intensity of the fluorophore (quenching). The critical metal-fluorophore distance needed for coupling in gold is about 5 nm or less [2]. When external electric fields are used, they stretch molecules (e.g., antigen), align them along the field lines, and produce electric polarizability (α) differently on biomarker and fluorophore molecules. The α values produce a dielectrophoretic (DEP) force on molecules. The largest positive α of biomarker molecules is about 100-times larger than that of fluorophore molecules, resulting in a stronger force (about 100×) on biomarker molecules. Therefore, the stronger DEP force attracts biomarker molecules closer to the gold edges of hotspots and keeps the fluorophore molecules about 1 length of biomarker molecules from the metal, preventing fluorophore quenching. This does not enhance the fluorescence, but in low-molarity biomarker detection, quenching could lead to incorrect results. Eliminating quenching helps to improve the limit of detection of sensing. We have conducted experiments using fluorescein labelled microRNA molecules and investigated effect of DEP force on quenching (Fig 1). Note that the molecules that concentrated near the hotspots using DEP force has the highest fluorescence

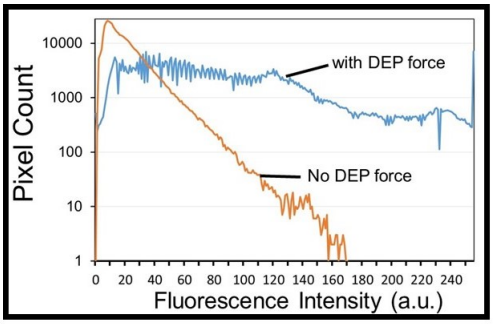

Fig 1: Use of DEP force to reduce the fluorophore quenching.

intensity. Moreover, low fluorescence intensity were recorded for the molecules that did not experience DEP force.

When fluorophore molecules interact with externally applied electric fields at the frequency where maximum α occurs, the fluorophore dipole aligns with the largest electric field (typically along the sharp edges of the hotspots) to maximize energy transfer from electric fields [1]. When a fluorophore is excited with light, surface plasmonic dipoles are also formed along the sharp edges or plasmonic axis [2, 5]. Therefore, if an external AC electric field and surface plasmons are simultaneously used, the fluorophore is aligned with plasmonic axis, resulting in significant increases in RDR and subsequently increasing fluorescence intensity [5]. The initial studies of Nitzan and Gersten produced an electromagnetic model that can predict the expected enhancement based on the size and shape of hotspots and metal-fluorophore distance [3]. Using this model, we calculated the expected enhancement to be about 50-fold. As RDR increases, fluorophores become stable. Therefore, the illumination intensity can be increased by additional 50-fold; subsequently, fluorescence is enhanced by additional 50-fold. To study this, we have measured the fluorescence lifetime values of Tetramethylrhodamine (TRIC; Table 1). TRIC was choose because it has low quantum yield value. The experimental details including and other information can be found elsewhere [4].

Experiment	Lifetime (ns)
No hotspots and electric field	2.20
With hotspots but no DEP force	0.72
With hotspots and DEP force	0.50

Table 1: Experimentally measured lifetime values with and without electric fields and DEP force.

When the wavelength of excitation light > size of the hotspots, the excitation light scatters in the hotspots and produces locally high electric fields. The DEP force produced by external AC electric fields on molecules is used to manipulate the biomarker molecules in hotspots and place them in the area where there is a large electric field from the scattered excitation light. When biomarker molecules are placed in the high local electric fields, the maximum fluorescence intensity is enhanced by a factor given by the square of the enhancement of electric field. We performed a COMSOL simulation (COMSOL Multiphysics, Burlington, MA) and calculated about a 15-fold electric field enhancement for the hotspots; this number agrees with what has published [1]. Also, we previously showed that additional 100-fold enhancement is produced by concentrating molecules using DEP force. Combining these two effects, we expect ~100 × 15^2 = 22,500-fold fluorescence enhancement. Details of experiments conducted and results were published elsewhere [4].

In conclusion, we have demonstrated that electric field can be used to significantly enhance weak fluorescence intensities produced by the lower molarities of biomarker molecules. To incorporate all mechanisms discussed above, it is needed to label biomarker molecules low quantum yield fluorophores, use electric fields to minimize the quenching, increase RDR (or quantum yield), and place the fluorophore molecules in the local hotspots and increase the fluorescence intensity. The combination of all mechanisms discussed above a total of 50×50×22500 = .6 × 10^8-fold fluorescence enhancement is possible to achieve. Enhancements of 100-fold demonstrated the detection of μM concentrations, therefore, expected enhancements could enable detection of sub-fM concentrations.

References

[1] Lakowicz, J.R., 2001. Radiative decay engineering: biophysical and biomedical applications. *Analytical biochemistry*, 298(1), pp.1-24.

[2] Velmanickam, L., Bains, M., Fondakowski, M., Dorsam, G.P. and Nawarathna, D., 2018. iLluminate-miRNA: Paradigm for high-throughput, low-cost, and sensitive miRNA detection in serum samples at point-of-care. *Journal of Physics D: Applied Physics*, 52(5), p.055401.

[3] Gersten, J. and Nitzan, A., 1981. Spectroscopic properties of molecules interacting with small dielectric particles. *The Journal of Chemical Physics*, 75(3), pp.1139-1152.

[4] Velmanickam, L., Fondakowski, M., Lima Jr, I.T. and Nawarathna, D., 2017. Integrated dielectrophoretic and surface plasmonic platform for million-fold improvement in the detection of fluorescent events. *Biomicrofluidics*, 11(4), p.044115.

[5] Velmanickam, L., Lima Jr, I.T. and Nawarathna, D., 2019, March. External low frequency electric fields maximize the fluorescence enhancement through light-metal-fluorophore interactions of target biomolecules. *In Imaging, Manipulation, and Analysis of Biomolecules, Cells, and Tissues XVII (Vol. 10881, p. 108810S). International Society for Optics and Photonics*.