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Analytical Biochemistry 344 (2005) 161–167
ANALYTICAL BIOCHEMISTRY
www.elsevier.com/locate/yabio

Directional surface plasmon-coupled emission: Application for an immunoassay in whole blood

Evgenia G. Matveeva [a,*], Zygmunt Gryczynski [a], Joanna Malicka [a], Joanna Lukomska [a], Slawomir Makowiec [a], Klaus W. Berndt [b], Joseph R. Lakowicz [a], Ignacy Gryczynski [a]

[a] *Center for Fluorescence Spectroscopy, Department of Biochemistry and Molecular Biology, School of Medicine, University of Maryland at Baltimore, Baltimore, MD 21201, USA*
[b] *Becton Dickinson Diagnostic Systems, Sparks, MD 21152, USA*

Received 15 December 2004
Available online 25 July 2005

Abstract

We present a new approach for performing fluorescence immunoassay in whole blood using fluorescently labeled anti-rabbit immunoglobulin G (IgG) on a silver surface. This approach, which is based on surface plasmon-coupled emission (SPCE), provides increased sensitivity and substantial background reduction due to exclusive selection of the signal from the fluorophores located near a bioaffinity surface. This article describes the effect of an optically dense sample matrix, namely human whole blood and serum, on the intensity of the SPCE. An antigen (rabbit IgG) was adsorbed to a slide covered with a thin silver metal layer, and the SPCE signal from the fluorophore-labeled anti-rabbit antibody, binding to the immobilized antigen, was detected. The effect of the sample matrix (buffer, human serum, or human whole blood) on the end-point immunoassay SPCE signal was studied. It was demonstrated that the kinetics of binding could be monitored directly in whole blood or serum. The results showed that human serum and human whole blood attenuate the SPCE end-point signal and the immunoassay kinetic signal only approximately two- and three fold, respectively, as compared with buffer, resulting in signals that are easily detectable even in whole blood. The high optical absorption of the hemoglobin can be tolerated because only fluorophores within a couple of hundred nanometers from the metallic film contribute to SPCE. Excited fluorophores outside the 200-nm layer do not contribute to SPCE, and their free space emission is not transmitted through the opaque metallic film into the glass substrate. We believe that SPCE has the potential of becoming a powerful approach for performing immunoassays based on surface-bound analytes or antibodies for many biomarkers directly in dense samples such as whole blood with no need for washing steps.

Keywords: Fluorescence immunoassay; Surface plasmon-coupled emission; Silver film; Whole blood; Background suppression

Fluorescence-based immunoassays are used extensively in medical diagnostics [1–5], particularly due to the fact that they are among the most sensitive assays known. Unfortunately, high background fluorescence often strongly interferes with the fluorescence signal of interest. Several approaches have been suggested to minimize the background signal caused by the sample matrix (e.g., serum or whole blood in clinical diagnostics), including fluorescence/polarization kinetics detection [6], time-gated detection based on long-lived lanthanide emission [7–9], and two-photon excitation [10,11]. Because of the high fluorescence background and optical density, immunoassays are rarely carried out in whole blood, and in cases when they are the procedure normally includes at least one washing step before the output signal is measured [9,12–15]. The development of methods for the direct analysis of whole blood samples

* Corresponding author.
E-mail address: eva@cfs.umbi.umd.edu (E.G. Matveeva).

0003-2697/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.ab.2005.07.005