Title

Phasor approach FLIM as an indicator for NADPH oxidase during exposure to secondary organic aerosols

Journal

Biophysical Journal, 121(3)

ISSN

0006-3495

Authors

Huang, Yukai
Fang, Ting
Wei, Jinlai
et al.

Publication Date

2022-02-01

DOI

10.1016/j.bpj.2021.11.694

regarding the quantification of pH and buffer capacity in nanoliter volumes due to interactions of the sample with the oil will be discussed. The appearance of parasites directly in droplets harvested from infected mosquitoes and their continued viability throughout these manipulations may propel future work aimed at unraveling infection-ready parasite biology. More generally, the workflow for measuring pH in nanoliter volumes by microscopy can be used for any fluid.

2009-Pos

Characterization of a large gated SPAD array for widefield NIR fluorescence lifetime imaging in vitro and in vivo

Jason T. Smith[1], Alena Rudkouskaya[2], Shan Gao[1], Arin Ulku[3], Claudio Bruschini[3], Edoardo Charbon[3], Shimon Weiss[4], Margarida M.R. Barroso[2], Xavier Intes[1], Xavier Michalet[4].
[1]Department of Biomedical Engineering, Rensselaer Polytechnic Institute, Troy, NY, USA, [2]Department of Molecular and Cellular Physiology, Albany Medical College, Albany, NY, USA, [3]AQUA Lab, Ecole Polytechnique Fédérale de Lausanne, Neuchâtel, Switzerland, [4]Department of Chemistry and Biochemistry, University of California Los Angeles, Los Angeles, CA, USA.

Optical imaging (OI) has become the most used alternative imaging tool for pre-clinical studies. Among all molecular imaging modalities, fluorescence optical imaging is central thanks to its high sensitivity, the numerous molecular probes available (either endogenous or exogenous) and its ability to simultaneously image multiple biomarkers or biological processes at various spatio-temporal scales. Especially, fluorescence lifetime imaging (FLI) has become an increasingly popular method, as it provides unique insights into the cellular micro-environment by non-invasively examining numerous intracellular parameters such as metabolic status, reactive oxygen species and intracellular pH. Moreover, FLI's exploitation of native fluorescent signatures has been extensively investigated for enhanced diagnostic of numerous pathologies. However, to perform such measurements in intact, live specimen, it is required to use near-infrared probes that exhibit very short lifetimes. Moreover, current detector technologies provide limited collection efficiency in this spectral range. To meet the need for an affordable widefield time-resolved detector with single-photon sensitivity, SwissSPAD2 (SS2), a 512x512 gated SPAD array was developed. This detection module has been characterized for FLI imaging in various scenarios, but mainly for applications *in vitro* using fluorophores emitting in the visible (i.e., ∼350-700nm wavelength) spectral range. Herein, we report, for the first time, on its ability to quantify accurately, as benchmarked against a gated ICCD, short NIR lifetimes and monitor FRET occurrence both *in vitro* and *in vivo.* Our results demonstrate that the SwissSPAD2, while coupled with phasor analysis, is capable of quantifying very short lifetimes (∼300ps) despite using large gates (∼10ns). Moreover, we demonstrate its ability to monitor target-drug engagement of Trastuzumab, a clinically relevant anti-HER2 drug, in live preclinical model bearing tumor xenografts.

2010-Pos

An optical microfluidic calorimeter for high-throughput drug screening

Ignacio Lopez-Peña[1], Jacob Chamoun[1], Joerg Martini[1], Patrick Y. Maeda[1], Daniel Cohen[1], Ilia Pavlovetc[1], Frank Torres[2], **Anne Plochowietz**[1].
[1]Hardware Research and Technology Lab, Palo Alto Research Center, Palo Alto, CA, USA, [2]Intelligent Systems Lab, Palo Alto Research Center, Palo Alto, CA, USA.

High-throughput screening (HTS) is crucial to the drug discovery process enabling rapid development of drug leads and reducing costs associated with optimization of drug activity. Calorimetry is a label-free, biophysical technique used for determination of important parameters associated with drug-protein interactions, such as binding affinity and thermodynamics. However, conventional isothermal titration calorimetry (ITC) is limited by low throughput, high sample consumption (milliliters), and long measurement time (tens of minutes).
We developed a high-throughput microfluidic calorimetry platform with an optical readout scheme to measure binding and enzymatic reaction enthalpies in sub-nanoliter droplets. Thermochromic liquid crystals (TLC) are introduced into aqueous droplets and act as optical transducers of temperature changes due to molecular reactions by temperature dependent shifts in their reflectance spectrum. We probe the reflectance spectrum of TLC in two spectral regions (450-468 nm and 516-540 nm) with multiple detection points along the droplet travel direction in the microfluidic channel, probing the droplet temperature over time. In order to perform rapid calorimetric measurements at various sample concentrations, we studied Taylor-Aris dispersion in microfluidic tubing to generate concentration gradients using a fluorescent tracer dye and implemented automated fluidic sample handling from a 96-well plate. Each 5 µl sample generates a concentration pulse that passes through the microfluidics in minutes and generates thousands of droplets, each one containing a defined reactant concentration. We validated the calorimetry platform by measuring temperature changes induced in droplets by the exothermic binding of EDTA to Ca^{2+} showing good agreement with a thermal multiphysics model. Our platform's current temperature resolution of 2.4 mK is on the same order as commercial ITCs and 10-fold better than most nanocalorimeters. This label-free microfluidic calorimeter with scalable optical read-out has the potential to accelerate the process of drug discovery in HTS campaigns.

2011-Pos

Sensing cellular metabolism in turbid media using spectral phasor analysis on UV-excited autofluorescence

Paul Urayama[1], Nick Majer[1], Thomas A. Finn[1], Taylor Phillips[2], Karthik Vishwanath[1].
[1]Department of Physics, Miami University, Oxford, OH, USA, [2]Physiology, Loyola University Chicago, Maywood, IL, USA.

Reduced nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH) are metabolic cofactors playing significant, distinct roles in cellular metabolism primarily involving cellular respiration and maintaining antioxidant defenses, respectively. Signals from NADH and NADPH are a significant component of cellular autofluorescence and are useful for metabolic sensing. For *in vivo* sensing of tissues, challenges include the presence of non-specific background fluorescence as well as perturbations of intrinsic NADH/NADPH fluorescence due to scattering and absorption. Here, we assess an approach for cellular metabolic sensing in turbid media based on a spectral phasor analysis of UV-excited autofluorescence. Recent studies of autofluorescence emission during chemically induced metabolic response in cell-only environments showed that two-component spectral behavior, i.e., spectral change acting as a superposition of two spectra, depended on whether one or multiple metabolic pathways were affected. In this way, the spectral response to chemicals affecting NADH and NADPH pathways, e.g., in response to cyanide and hydrogen peroxide, could be distinguished. Here, we demonstrate pathway-level sensing in turbid media by monitoring the autofluorescence response of yeast cells embedded in tissue-like environments containing background emission, scattering, and absorption. Additionally, we discuss the potential for sensing cellular metabolism in tissue.

2012-Pos

Phasor approach FLIM as an indicator for NADPH oxidase during exposure to secondary organic aerosols

Yukai Huang[1], Ting Fang[2], Jinlai Wei[2], Jessica E.M. Mena[3], Pascale S.J. Lakey[2], Michael T. Kleinman[3], Manabu Shiraiwa[2], Michelle A. Digman[1].
[1]Biomedical Engineering, University of California Irvine, Irvine, CA, USA, [2]Chemistry, University of California Irvine, Irvine, CA, USA, [3]Community and Environmental Medicine, University of California Irvine, Irvine, CA, USA.

Nicotinamide adenine dinucleotide phosphate oxidase (NOX) is a multi-subunit enzyme which is responsible for host defense and inflammation signaling against either bacterial infection or pollutants. During activation, free oxygen is turned into superoxide anions which further leads to cascade formation of reactive oxygen species (ROS). However, once accumulation of ROS exceeds cell capacity, it damages components including proteins, lipids, and DNA known as oxidative stress which contributes to various lethal impacts such as lipid peroxidation and cancer. Therefore, it gives rise to the importance of monitoring NOX activity to avoid ROS accumulation. While most measurements use fluorescent probes to detect ROS, it lacks the ability to differentiate readings between cellular and extracellular which does not directly relate to NOX activity only. Here we aim to detect the impact of secondary organic aerosols (SOAs) on macrophage metabolism using fluorescence lifetime imaging (FLIM) and correlate the changes in fluorescence lifetime of NAD(P)H with NOX activation. We first measured the superoxide production upon NADPH oxidase activation through different SOAs exposed to RAW 264.7 macrophages using Diogenes chemiluminescence method as the NOX activity detection. The results show a time dependent superoxide production rate which suggests the period of rapid defense against xenobiotics. Similar time dependent findings were also shown in the FLIM NAD(P)H lifetime where we show that abundant enzyme-bound NADPH was turned to NADP+ which is not fluorescent, hence, causing the imbalance of free to bound NAD(P)H ratio more to the free state transiently. Our experiments demonstrate the capability of the phasor approach FLIM to directly and non-invasively measure intracellular changes of NAD(P)H to investigate NOX activities. The impact of this work provides a FLIM-based monitoring system to evaluate potential threatening pollutant exposure in living cells.