

## Fluorescence Lifetime Imaging Microscopy of Intracellular Glucose Dynamics

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### Abstract

#### Background:

One of the major hurdles in studying diabetes pathophysiology is the lack of adequate methodology that allows for direct and real-time determination of glucose transport and metabolism in cells and tissues. In this article, we present a new methodology that adopts frequency-domain fluorescence lifetime imaging microscopy (FD-FLIM) to visualize and quantify the dynamics of intracellular glucose within living cells using a biosensor protein based on fluorescence resonance energy transfer (FRET).

#### Method:

The biosensor protein was developed by fusing a FRET pair, an AcGFP1 donor and a mCherry acceptor to N- and C- termini of a mutant glucose-binding protein (GBP), respectively. The probe was expressed and biosynthesized inside the cells, offering continuous monitoring of glucose dynamics in real time through fluorescence lifetime imaging microscopy (FLIM) measurement.

#### Results:

We transfected the deoxyribonucleic acid of the AcGFP1-GBP-mCherry sensor into murine myoblast cells, C2C12, and continuously monitored the changes in intracellular glucose concentrations in response to the variation in extracellular glucose, from which we determined glucose uptake and clearance rates. The distribution of intracellular glucose concentration was also characterized. We detected a high glucose concentration in a region close to the cell membrane and a low glucose concentration in a region close to the nucleus. The monoexponential decay of AcGFP1 was distinguished using FD-FLIM.

#### Conclusions:

This work enables continuous glucose monitoring (CGM) within living cells using FD-FLIM and a biosensor protein. The sensor protein developed offers a new means for quantitatively analyzing glucose homeostasis at the cellular level. Data accumulated from these studies will help increase our understanding of the pathology of diabetes.

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**Abbreviations:** (AcGFP1) *Aequorea coerulescens* green fluorescent protein, (a.u.) arbitrary unit, (CCD) charge-coupled device, (CGM) continuous glucose monitoring, continuous glucose monitoring, continuous glucose monitoring, (DNA) deoxyribonucleic acid, (ECFP) enhanced cyan fluorescent protein, (EYFP) enhanced yellow fluorescent protein, (FD-FLIM) frequency-domain fluorescence lifetime imaging microscopy, (FLIM) fluorescence lifetime imaging microscopy, (FRET) fluorescence resonance energy transfer, (GBP) glucose-binding protein, (GIP) glucose-indicator protein, (LED) light-emitting diode, (MCP) multichannel plate, (NMDG) N-methyl-D-glucamine, (ns) nanosecond, (ROI) region of interest, (TD-FLIM) time-domain fluorescence lifetime imaging microscopy, (Tris) tris(hydroxymethyl)aminomethane

**Keywords:** biosensor, continuous lifetime imaging, diabetes, FLIM, frequency domain, FRET, GBP, glucose sensor

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