High Sensitive Screening of GPCR-Related Compounds using Prism-Based TIRFM System

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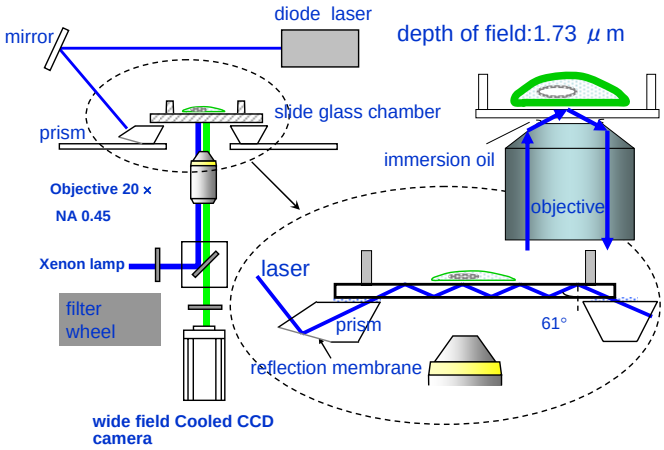
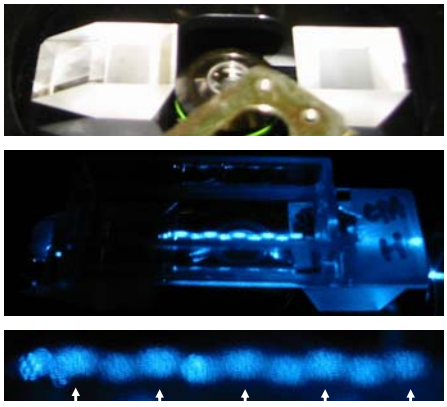
1.Abstract

A cell orchestrates second messengers to regulate a myriad of cell functions through activation of G protein coupled receptors (GPCRs) upon binding extracellular signals. Therefore, GPCRs are potential drug targets for many diseases. Fluorescence imaging is a powerful method to visualize and analyze generation of such GPCR-mediated second messengers in a living cell. Recent advances in genetic engineering and optical instrumentation have allowed us to develop a prism-based total internal reflection fluorescence (TIRF) microscopy for simultaneous measurement of multi-second messenger signals.

Configurations of prism-based TIRFM





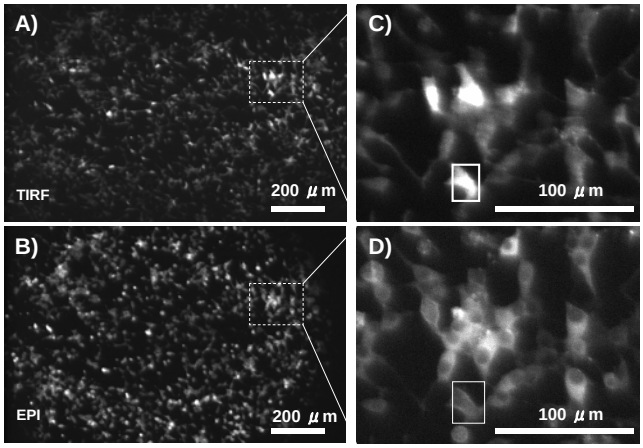
1st 2nd 3rd 4th 5th
multi-evanescent field

3.Novelty and Originality

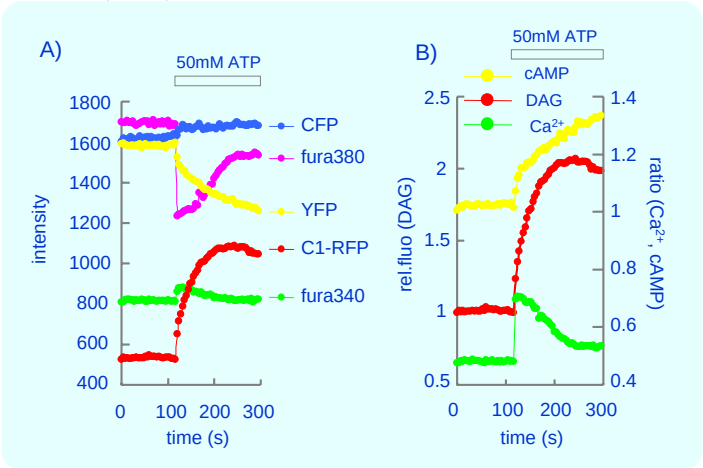
A new prism-based TIRF microscopy: entry and exit prisms are aligned on the stage of an inverted epifluorescence microscope. Incident laser light is introduced into a glass chamber slide on the prisms, through which wide multi-evanescent field (ca 1.2mm x 2mm) is generated. Our optical instrumentation enables us to simultaneously capture images under both epifluorescence (EPI) and TIRF microscopy without changing focus dial by alternatively switching on and off each shutter

2.Applied Research for collaboration

We have developed a new high-throughput cell-based assay for discovery of GPCR-related compounds, which employs prism-based TIRF microscopy with wide field CCD camera (4000x 2460 pixels) to detect multi-second messenger signals using a Ca2+ sensitive dye, fluorescent cAMP sensor and diacylglycerol (DAG) fluorescent sensor. In particular, TIRF microscopy enables us to yield high DAG signal to background ratio near the plasma membrane of cells as does not epifluorescence microscopy.



Images of depolarization-evoked translocation of PKCa-GFP captured by wide-field CCD camera with the PBTIRFM system. Images were alternately acquired using PBTIRFM **(A)** and EPI **(B)** with an ORCA-HR (4000 x 2664 pixels**)** CCD camera. PKCa-GFP-expressing INS-1 cells were depolarized by TEACl. **C)** and **D)** Expanded areas denoted by the dashed box in A and B. White box in **C** and **D** shows the same stimulus-responding cell under PBTIRFM and EPI. Scale bar 200 μm in A and B; 100 μm in C and D



*Simultaneous monitoring of three second messengers using a combination of PBTIRFM and EPI A***)** Representative results from 6 independent experiments are shown (n=127). Reciprocal change in fluorescence intensity of CFP and YFP and of fura 340 and fura 380, and increase in RFP after treatment with 50 mM ATP. **B)** Ratio of CFP to YFP (yellow, Epac1-camp as a cAMP sensor) and fura 340 to fura 380 (green, Fura-2 as a Ca^{2+} sensor), and relative fluorescence intensity of mRFP (red, $C1_2$-mRFP as a DAG sensor) in **B**.

4. Keywords: G protein coupled receptor, Second messenger, Total internal reflection fluorescence microscopy, Cell-based fluorescence assay, Drug screening

5. Patent status & Patent owner contact

- **Patent license is available.**

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