X-Cite®
Fluorescence Illumination • In Control

Using the X-Cite® XLED1 along with the X-Cite® Power Meter to calibrate Photo-Uncaging in an ATR-FTIR Spectrometer

Challenge

1) Obtaining sufficient power from an LED to efficiently uncage molecules in an FTIR spectrometer

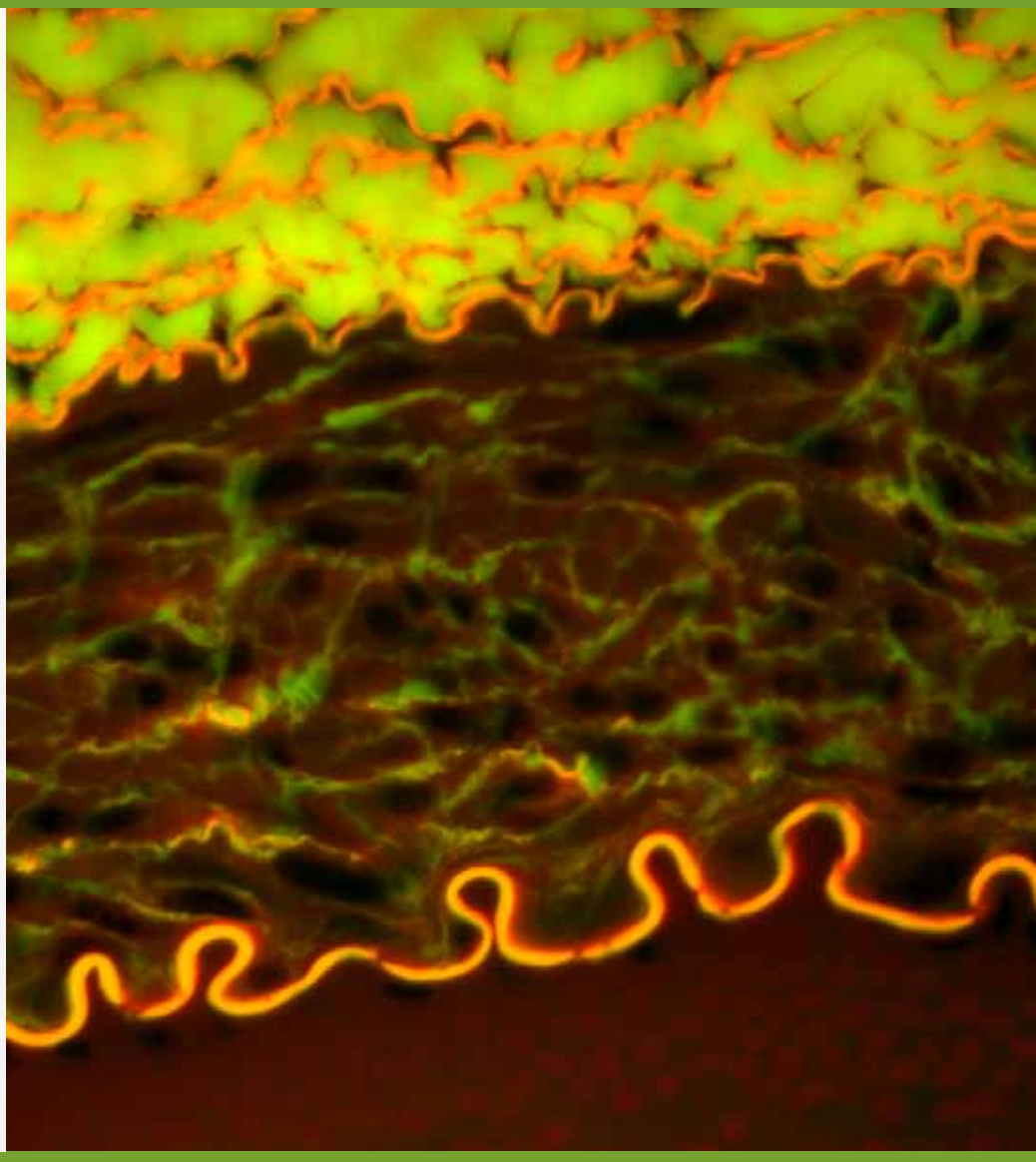
2) Measuring the intensity of the light at the sample to be uncaged

Solution

Using the X-Cite® XLED1 high power UV module to uncage a molecule and the X-Cite® Power Meter to measure light intensity

Benefit

Efficient uncaging experiments with knowledge of the actual power of light used to uncage the molecule of interest



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Introduction

Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy is a powerful technique for tracking the interactions between proteins and membranes *in situ*, including membrane-induced protein refolding and other membrane-protein interactions. Details of a protein's secondary structure is often extracted through careful examination of the C=O and N-H stretching and bending modes associated with the backbone amide bond.

INTRODUCTION (CONT'D)

For example, α-helices typically will exhibit strong absorbances at ~1650 cm^{-1} (Amide I) and ~1550 cm^{-1} (Amide II) whereas absorbances at ~1620 cm^{-1} (Amide I) and ~1680 cm^{-1} (Amide II) are more characteristic of β-pleated sheets. A particularly powerful application of IR spectroscopy is the use of difference spectra to assess changes in a protein's conformation or association state upon ligand binding, nucleotide hydrolysis, or co-factor addition. In order to characterize these events accurately as they are occurring, triggering these changes without physically disturbing the sample is a particularly powerful strategy. For example, in situ photolysis of caged compounds, by which substrates and co-factors such as nucleotides or Ca^{2+}, are released upon irradiation, has been shown to be a useful strategy for studying conformational changes in proteins (1). This is typically performed using a UV source such as a flash lamp, laser, or high power LED. A particular challenge lies in measuring the optical power at the sample itself.

METHODS

While photolysis experiments are typically performed in a transmission configuration, we are particularly interested in protein-membrane interactions and specifically, membrane-associated GTPases. Thus, we elected to employ ATR-IR spectroscopy using a ThermoNicolet Nexus 670 FT-IR spectrometer outfitted with a SmartOrbit ATR-IR accessory, a single-bounce diamond internal reflection element and a custom-built flow-through fluid cell comprising a CaF_2 window in a poly(methyl methyacrylate) housing. Experiments were conducted with protein bound to biologically relevant lipid bilayers formed on an ATR-FTIR diamond substrate.

For these studies, it was important to acquire IR spectra before and after the introduction of GTP in order to characterize conformational changes associated GTPase activity. However, disturbing the sample by physically injecting GTP can change the baseline of spectra, making it difficult to reliably compare data before and after the addition. In order to track these changes without disturbing the sample, we use a NPE-caged GTP compound (JenaBioscience, Jena, Germany) that releases free GTP upon UV irradiation. For GTP uncaging, the X-Cite® XLED1 385nm LED module was used. To determine the excitation power at the surface of the diamond element

(and by extension the membrane surface), and account for absorbance by the fluid cell itself, the X-Cite® XP750 Objective Plane Power Sensor connected to a the X-Cite® XR2100 Power Meter (collectively known as the X-Cite® Power Meter), was placed within our fluid cell to measure the power of the UV excitation light nominally at the membrane surface.

Figure 1

Figure 1: The X-Cite® Power Meter is placed under an in-house designed fluid cell to measure the amount of UV light impinging on the sample.

RESULTS

As can be seen in the following table, there was significant absorbance of UV light by the fluid cell.

Source	Directly on the sensor surface	Through the fluid cell
X-Cite® XLED1 385nm source	312mW	100mW

The XLED1 system was equipped with a dedicated power supply, light guide, coupling adaptor and an intuitive programmable touch screen controller to automate light output.

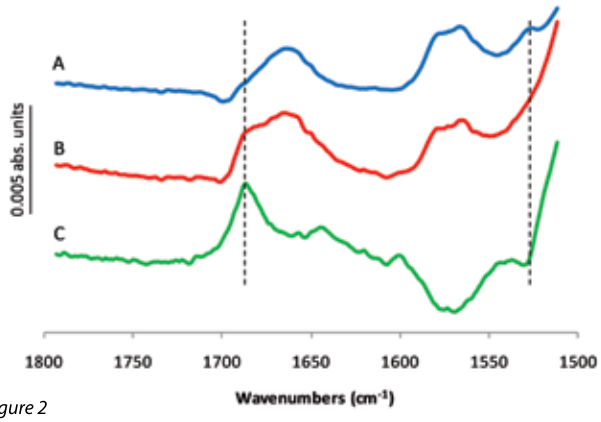

Figure 2

Figure 2: ATR-IR spectra obtained in fluid using a single-bounce diamond ATR element. 5 mM NPE-GTP in buffer solution (A) Before irradiation; (B) After irradiation with the XLED1 light source (C) Subtraction spectrum of B-A (x 2.2). The XLED1 light source provided ample power for efficient uncaging, as can be seen in the ATR-IR difference spectra. The peak at 1688 cm^{-1} and trough at 1528 cm^{-1} (dotted lines) are indicative of the uncaged NPE-GTP. Spectra are offset for display purposes and the subtraction spectrum was multiplied by a factor of 2.2 for clarity.

DISCUSSION AND CONCLUSION

The XLED1 system provided an easy-to-use, high power UV source for our uncaging experiments. The ease of tuning the power output in a stable and repeatable fashion was beneficial for replicate experiments. Furthermore, the X-Cite® Power Meter offered a simple and efficient method of calibrating the photolysis reaction. It enabled us to record precisely the amount of UV light at the desired uncaging wavelength impinging on the sample surface. From the power density and exposure time, we could then determine the approximate flux associated with the uncaging process.

As such, the X-Cite® Power Meter allows for ready characterization of photolysis reactions, allowing for precise control of experimental conditions.

REFERENCES

1. Cepus, V., Scheidig, A.J., Goody, S., and Gerwert, K., Biochemistry, 1998, 37(28):10263-10271

For more information on the X-Cite® XLED1, please visit www.LDGI-XCite.com/products-xcite-xled1.php. If you would like to share your research involving the use of an X-Cite® system in a publication of this type, please contact Dr. Kavita Aswani at Kavita.Aswani@LDGI.com. For more information on X-Cite® Products and their Applications, please visit www.LDGI-XCite.com

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