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# Transient optical absorption and magnetic intensity modulation based microscopy of photochemical reaction intermediates

(University of Tokyo) ○Jonathan R. Woodward,
Joshua P. Beardmore and Lewis M. Antill

**[Introduction]** Although the study of magnetic field effects on chemical reactions has a long and fascinating history, very little attention has been paid to spatially resolving magnetic field dependent photochemistry. As spin chemical studies have turned to more and more sophisticated systems, particularly involving biological processes and solid state electronic devices, the need to add a spatial dimension to magnetic field sensitive measurements has increased. In particular, a key recent question in spin chemistry concerns the possibility of radical pair reactions as the source of the ability of many animals to sense the earth's magnetic field and use it for navigation. A class of blue-light photoreceptors, the cryptochromes, have been proposed as likely candidates for this magnetically sensitive photochemistry[1] and growing evidence from behavioural biological, genetic and spin chemical measurements[2] have provided growing support for this hypothesis. In this context, a long term goal has been to directly measure cryptochrome photochemistry in living cells and look for magnetosensitive responses in these processes. To this end, we have developed a new kind of microscope capable of two kinds of spatially resolved measurement: Transient Optical Absorption Detected (TOAD) imaging microscopy and Magnetic Intensity Modulation (MIM) imaging microscopy. These techniques were designed with an initial goal of studying the magnetosensitivity of flavin based photochemistry at sub-micron spatial resolution in biological and other structures and a longer term goal of using them to study a broader range of magnetic sensitive reactions in other environments, for example in solid state electronic devices.

In this lecture, the new microscopic techniques are introduced and their capabilities discussed along with recent measurements in their application to study the photochemistry of various different flavin containing systems.

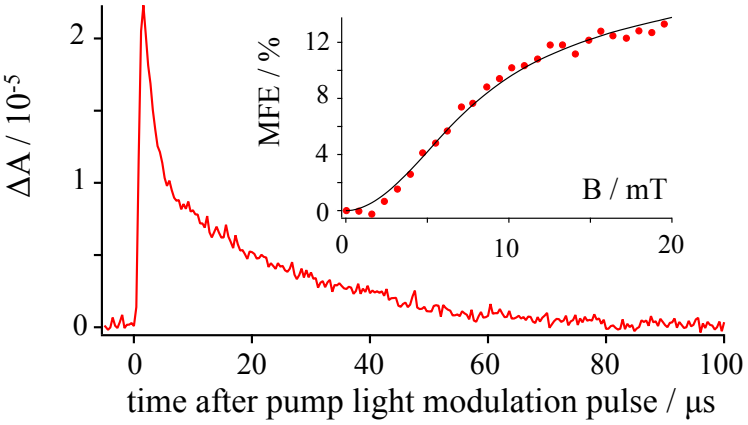


**Figure 1. Time resolved optical absorption signal recorded at 532 nm from photoexcitation of a 0.2 mM solution of FAD at pH 2.3 after application of a 300 ns 450 nm laser pulse from a volume of solution of <4 fL. The inset shows the MARY curve recorded for the same region of sample.**

**[Experimental]** The microscope employs a pair of identical high numerical aperture super apochromatic objective lenses arranged symmetrically either side of a sample contained between cover slips. Pump (450 nm) and probe (532 nm) laser beams are combined and cleaned with a single mode optical fiber before being brought to mutual focus at the sample by the first objective lens. Transmitted light is captured from the focal point by the second objective and the pump light is filtered from the beam before detection using an autobalanced detector. In TOAD measurements, the pump light can be modulated at variable duty cycles and laser powers to study flavin photochemistry in response to a short photoexcitation pulse or under pseudo-continuous illumination. Induced changes in the green light intensity are detected with very high sensitivity using either a digital storage oscilloscope or a phase sensitive detector locked to the modulation frequency. Magnetic fields of up to 30 mT can then be applied and or the sample position can be scanned in steps as small as 1 nm using a piezoelectric translation stage. In MIM measurements a double modulation scheme is applied. The pump light is applied as a short pulse at high (kHz) repetition rates and the green light response is phase sensitively detected. A DC magnetic field is applied with an AC component of typically 100 Hz provides a second modulation and the result of the second phase sensitive detection is monitored as either the DC magnetic field (modulated MARY spectrum) or the sample position (imaging) is scanned.

**[Results and Discussion]** Fig 1. shows the time resolved optical absorption signal observed for a sample of 0.2mM flavin adenine dinucleotide, demonstrating the high sensitivity and spatial resolution of the instrument. Fig 2. shows a MIM image of a 2.5 - 3.0 μm polymer microbead surrounded by 2 mM FAD at pH 2.3. In this figure, the light areas of the image correspond only to magnetic field sensitive regions of the sample and so can potentially be used to image only the regions of a cell where magnetically sensitive photoreactions are taking place.

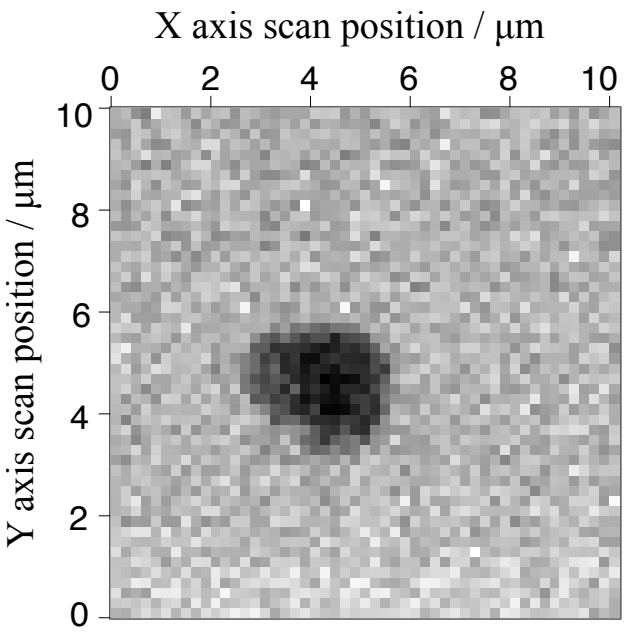


**Figure 2. MIM image of a 2.5 – 3 μm polymer microbead surrounded by 2mM FAD at pH 2.3.**

[1] Ritz T, Adem S, Schulten K, *Biophys. J.* **2000**, 78,7 07 - 718.
[2] H. Mouritsen, P. J. Hore, Curr. Opin. *Neurobiol*. **2012**, 22, 343 – 352.