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A sub-micron step closer to spatially resolving the magnetosensitivity of photochemically induced electron transfer reactions of flavins

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[Introduction] Phenomena arising as a result of the radical pair mechanism (RPM) have been extensively studied over the last 40 years. In recent times the RPM has seen renewed interest after being proposed as being a prime candidate as the physical interaction mechanism at the heart of animal magnetoreception.¹ Blue-light receptor proteins called cryptochromes and in particular the photochemistry of the flavin adenine dinucleotide (FAD) and the tryptophan triad components of cryptochromes have gained much interest in research in uncovering this mystery.² Moreover, the photochemistry involved takes place in a cellular environment and therefore one would like to examine the mechanisms taking place in a spatially resolved approach.

Here we present the newly developed transient optical absorption detection (TOAD) imaging microscope with high sensitivity and sub-micrometre spatial resolution, which allows direct imaging of photochemically generated radicals. We also demonstrate, the magnetic intensity modulation (MIM) imaging microscope that allows direct spatial imaging of magnetic field effects (MFEs), which can selectively image regions containing magnetically sensitive radical pairs (RPs). Both techniques have the potential of directly measuring flavin photochemistry and magnetosensitivity in cells and tissues, such as cryptochromes.

We display the capability of the TOAD/MIM microscope by imaging the short-lived radicals generated in the photoexcitation of flavin adenine dinucleotide (FAD) and tryptophan (Trp) in aqueous solution at low pH, demonstrating the ability to study the magnetosensitivity of FAD + Trp derived RPs in volumes of less than 4 femtolitres and with sufficient sensitivity to observe magnetic field effects of less than 1 %.

[Experimental] Measurements were conducted on the TOAD/MIM microscope. Technical details are described elsewhere.³ FAD (200 μ M) and Trp (1.5 mM) were prepared in a pH 2.3 buffer solution. For thin sample preparation, polymer beads (2.5 – 2.9 μ m) were added to the sample solution and 1 μ L of this solution was sandwiched between glass cover slips. For thicker samples, the polymer beads were replaced with 100 μ m glass beads and the volume of solution increased to 5 μ L.

[Results and Discussion] The electron transfer from Trp to the photoexcited triplet state of FAD creates a separated biradical. The FADH^{*} + Trp^{*+} radical pair was monitored at 532 nm (with a 450 nm pump) where both species absorb comparable extinction coefficients and lifetimes of a few microseconds under the conditions of the experiment. Figure 1 shows a MARY (magnetically affected reaction yield) curve recorded at a single point of the FAD (200 μ M) + Trp (1.5 mM) sample. Only 4 min was required to record a MARY spectrum at this signal-to-noise level. The vertical scale of the MARY curve is a percentage change in the output of the lock-in signal as the field is probed.

Figure 2 shows an image of a $\sim 2.5 \mu\text{m}$ bead surrounded by FAD ($200 \mu\text{M}$) + Trp (1.5 mM) in pH 2.3 buffer under flash photolysis mode (300 ns pulse width of pump light (450 nm)). The sample thickness is bead controlled (i.e. $< 3 \mu\text{m}$) and the lasers irradiate a sample volume of $< 4 \text{ fL}$ with a beam waist of $\sim 240 \text{ nm}$. The imaging signal is a direct measure of RP concentration and is reduced to zero in the area of the bead as no photochemistry can occur in this area.

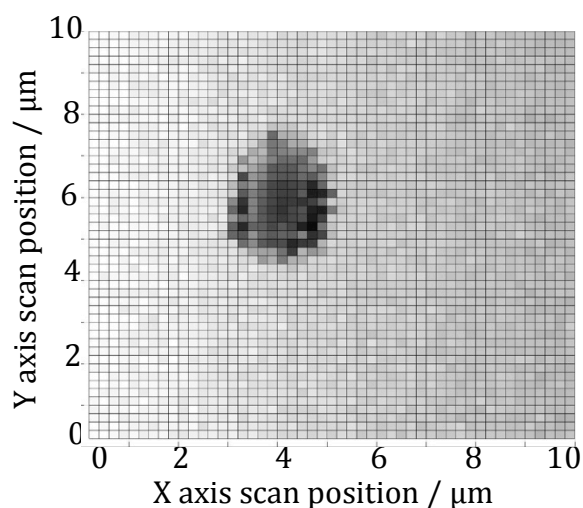


Figure 2: TOAD image of a $2.5 - 3.0 \mu\text{m}$ polymer bead surrounded by FAD ($200 \mu\text{M}$) + Trp (1.5 mM) solution at pH 2.3, $1 \mu\text{L}$ solution, $2.5 - 3.0 \mu\text{m}$ sample thickness.

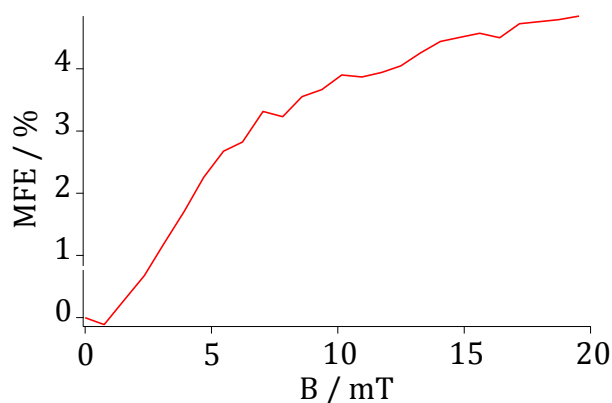


Figure 1: MARY curve for FAD ($200 \mu\text{M}$) + Trp (1.5 mM) solution at pH 2.3 with 300 ns 450 nm laser pulse, $5 \mu\text{L}$ solution, $100 \mu\text{m}$ sample thickness.

As cryptochrome magnetosensitive photochemistry involves the same species (in a protein environment), this microbead image displays the potential of the TOAD/MIM microscope to be used to image magnetically sensitive areas as photoreactions are occurring in cellular environments. The aim of unraveling the mechanisms of RPs both *in vitro* and *in vivo* will hopefully develop key connections between the fields of spin chemistry and behavioral biology.

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