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Journal of Magnetic Resonance 171 (2004) 171–175
JMR Journal of Magnetic Resonance
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Uniform illumination of optically dense NMR samples

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Received 9 June 2004
Available online 17 September 2004

Abstract

We demonstrate a simple, inexpensive method for in situ laser illumination of NMR samples using a stepwise tapered optical fibre to deliver light uniformly along the axis of a 5 mm NMR tube. The optical path length of the incident light inside the sample is about 3 mm, allowing efficient illumination of optically dense samples. The degradation in spectral resolution and the reduction in filling factor are both minimal. Probe modifications are not required.

Keywords: NMR sample illumination; Optically dense sample; CIDNP

1. Introduction

A variety of photochemical NMR experiments require illumination of the sample inside the NMR probe, including but not limited to: photo-CIDNP (chemically induced dynamic nuclear polarization) [1–3], metal ion release from photolabile cage compounds [4,5], photochemical kinetics [6–8], and studies of photoactive proteins [9–12].

One of the biggest technical challenges faced in such experiments is to deliver light efficiently into the active region of the NMR sample. Ideally, the entire sample volume should be uniformly illuminated to maximise sensitivity and to avoid concentration and temperature gradients, which might distort the observed kinetics. Such problems are likely to be most severe for optically dense samples. It is highly desirable that uniform illumination is achieved without extensive probe modifications, which may compromise the NMR performance, and in a manner that allows facile transfer from one spectrometer to another. Several techniques have been devised in recent years, including illumination from above, below or the side, some of which are shown schematically in Figs. 1A–E.

Bringing light in from the side, through the radiofrequency (RF) coil, via a quartz light guide and a prism or a mirror (Fig. 1A), has the disadvantage of non-uniform irradiation of the sensitive region even if the sample has low optical density. Such an arrangement is incompatible with certain RF coil designs and can conflict with the presence of field gradient coils. Irradiation from below using a flat-bottomed NMR tube (Fig. 1B) is somewhat less demanding technically, but would still normally require the re-location of electronic components and variable temperature equipment inside the probe body. It also suffers from inhomogeneous illumination for optically dense samples, a problem that can be reduced by using a bespoke NMR sample tube with a tapered (“V-cone”) interior [13] to extend illumination into the active region (Fig. 1C) at the expense of both spectral resolution and sensitivity (the filling factor is reduced by ~20%). The attraction of these three approaches is that they are readily compatible with short, intense visible or ultraviolet pulses from YAG or excimer lasers as well as continuous wave (CW) light sources.

To circumvent the requirement for probe modification, various methods have been suggested for illumination

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doi:10.1016/j.jmr.2004.08.017

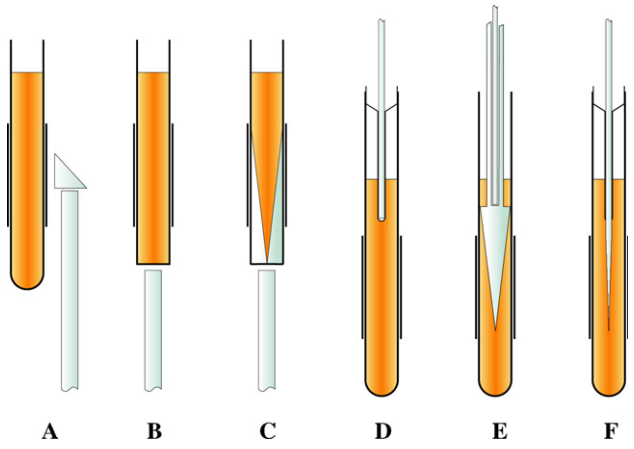

Fig. 1. Schematic drawings of NMR sample illumination methods. (A) Illumination from the side through the receiver coil via a cylindrical quartz rod installed inside the probe body surmounted by a prism or mirror. (B) Illumination from below with a quartz light guide and a flat-bottomed NMR tube. (C) A variant of (B) using a "V-cone" NMR tube to permit more homogeneous irradiation of optically dense samples. (D) Illumination from above using an optical fibre held inside a coaxial glass insert. (E) A variant of (D) in which light is distributed by means of a "pencil tip" insert. (F) The arrangement demonstrated here with a stepwise tapered optical fibre.

from above. Almost 20 years ago, Berliner and colleagues [14] demonstrated the use of an optical fibre held inside the NMR tube by means of a coaxial capillary insert (Fig. 1D), an arrangement we have used extensively for photo-CIDNP studies of proteins [15–18]. In our experience, the end of the insert must be positioned at least 2 mm above the NMR coil to avoid undue lineshape distortion. This approach is convenient, technically straightforward and particularly suitable for CW lasers. Pulsed lasers may be used only insofar as the optical power density is compatible with the fibre. Improved uniformity of illumination is afforded by combining the optical fibre with a "pencil tip" insert as demonstrated by Schwalbe et al. [4,5] (Fig. 1E). While retaining the simplicity of the Berliner method, this design, like the V-cone, sacrifices some sensitivity and resolution.

In the present paper we propose a straightforward and inexpensive method of illumination from above in which the light is distributed along the axis of the NMR tube by means of a tapered optical fibre (Fig. 1F). While illumination from above the coil (Fig. 1D) gives rise to an exponential fall in light intensity from the top of the sensitive region to the bottom (optical path length ~20 mm), the tapered tip (with a path length of ~3 mm) gives almost uniform illumination. This approach leads to minimal degradation of spectral resolution, less than a 5% loss of filling factor and requires no probe modifications. The amount of light reaching the NMR sample can be conveniently monitored via the ^{19}F photo-CIDNP enhancement of 4-fluorophenol sensitised by flavin mononucleotide (FMN). For light flashes between 1 and 100 ms, the photo-induced ^{19}F magnetisation is a direct measure of the local light intensity [18] allowing the use of NMR imaging techniques to reveal the spatial distribution of light within the sample tube.

2. Materials and methods

The end of a Newport F-MBE optical fibre (1 mm core diameter) was tapered using the following procedure. A 10 cm length of the plastic cladding was mechanically stripped from the end of the fibre, the final 2 cm of which was then immersed for 1 min in a mixture containing 30% HF, 20% H_2SO_4, and 50% H_2O at 60 °C to detach the fibre sheath from the core. The exposed core was rinsed with water and extruded stepwise (typically 1.5 mm every 30 min) into the same acid mixture from a plastic pipette tip, again at 60 °C (Fig. 2A). After about 5 h the tip was removed, washed with water, and dried. The resulting cone tapers in typically ~11 steps down to ~50 μm over a length of ~20 mm (Fig. 2B). Smaller and more frequent adjustments of the fibre in the etching medium result in a smother cone. It should be noted that the hydrofluoric acid solution is extremely toxic and volatile when hot.

^{19}F photo-CIDNP spectra were recorded on a Varian Inova 600 MHz (14.1 T) NMR spectrometer equipped with a 5 mm ^{19}F{^{1}H} z-gradient probe. The light source was a Spectra Physics BeamLok 2080 argon ion laser, operating in multi-line mode at 10 W output power, principally at 488 and 514 nm. A mechanical shutter

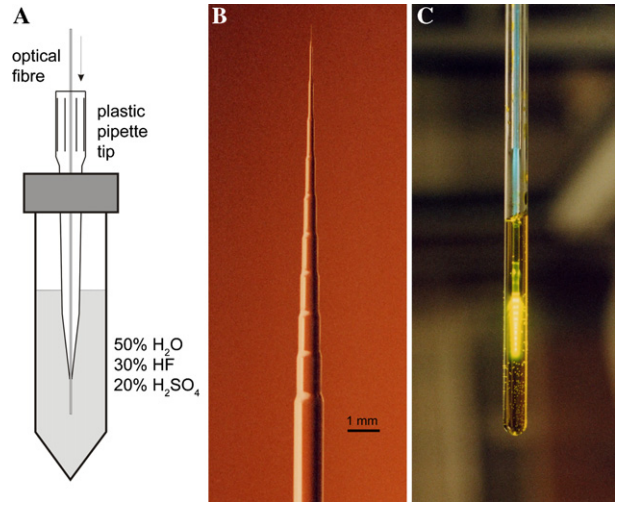

Fig. 2. (A) The production of the tapered fibre tip; (B) the resulting tip; and (C) an assembled NMR sample irradiated principally at 488 and 514 nm. The yellow colour is FMN fluorescence excited by the 488 nm laser line. The terminal diameter of the fibre cone is less than 50 μm. In (C), the optical fibre appears much thicker than it actually is because of the lensing effect of the solution.

(NM Laser Products LS200) controlled by the spectrometer was used to produce 100 ms light pulses. The light was focused into a 6 m length of optical fibre (Newport F-MBE), using a Newport M-5× objective lens, the other end of which was attached (via Newport SMA connectors) to a 2 m section of the same fibre whose tapered tip was held inside a 5 mm NMR tube by a truncated Wilmad WGS 5BL coaxial insert (Fig. 1F).

A 4 mM solution of 4-fluorophenol (Lancaster) in D_2O containing 0.2–3.2 mM FMN (Sigma) at pH 5.0 (uncorrected for deuterium isotope effect) was used in all experiments. Samples were purged with argon for 20 min prior to use. Profiles of the CIDNP intensity along the tube were recorded by applying a constant z-gradient during detection of the free induction decay in a one-scan flash-pulse-acquire experiment.

3. Results and discussion

The shape of a fibre tip resulting from the treatment described above is shown in Fig. 2B. This particular specimen contains 11 steps; in different experiments, cones with 7–15 steps were produced. The best results were obtained for 10–15 steps of 1–1.5 mm. In the absence of scratches the tips are quite robust, the most frequent damage being to the thinnest sections, caused by inaccurate insertion into an NMR tube. Such breakages are easily repaired using the etching procedure described above to produce a new tip by tapering the last few sections and at the same time creating a few new steps further up the fibre.

The light intensity distribution depends very strongly on the length of the fibre cone and the step size. The shorter cones, which have steeper shoulders, tend to emit light predominantly from the shoulders leading to a non-uniform illumination pattern. At the other extreme, very long cones with a large number of small steps and shoulders transmit light by total internal reflection to the very end of the tip, whence almost all of the light is emitted. The fairly uniform light emission shown in Fig. 3 is a result of experimental optimisation of both the length of the cone and the size of the steps. Fig. 3 also shows that the emission of light from an optimum cone occurs at an angle of ~45° to the fibre axis, predominantly from the vertical sections of the fibre between the shoulders, suggesting that the light paths are similar to those shown in Fig. 3C. There is also some emission at the steeper shoulders.

Light intensity distributions along the axis of the sample, $L(z)$, measured using ^{19}F photo-CIDNP of 4-fluorophenol, are shown in Fig. 4. Illumination from above using the arrangement in Fig. 1D yields the expected Beer–Lambert dependence (Fig. 4A):

$$L(z) = A + Be^{-Cz}, \quad (1)$$

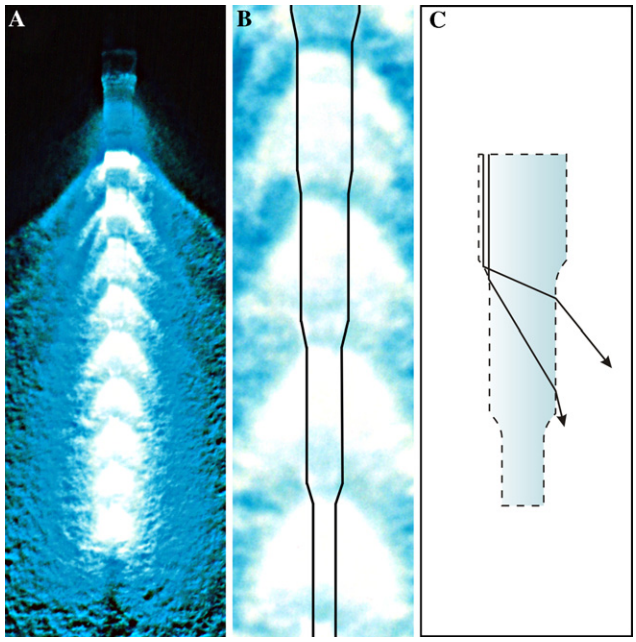

Fig. 3. (A) Emission of argon laser light from the tapered fibre tip placed on a white surface. (B) The central part of (A) magnified with the fibre outlined for clarity. (C) Likely paths of light rays in the fibre cone.

where z is distance, B and C depend on the sample concentration and the tip position, while the constant contribution A comes from the equilibrium nuclear magnetisation, from laser light reflections from the inner parts of the probe and from the 514 nm output of the Ar^+ laser which is absorbed by FMN to a far lesser extent than that at 488 nm. In the case of the conical fibre tip the light intensity profile is much more even (Fig. 4B). The light distribution image is somewhat narrower in the case of the tapered fibre, because the tip employed in this experiment is 2 mm shorter than the receiver coil. At high resolution, the images in Fig. 4B have a low amplitude sinusoidal modulation superimposed on the top of the rectangular distributions, with the number of maxima matching the number of steps in the cone (not shown).

As expected, the overall ^{19}F CIDNP signal intensity is attenuated at high optical densities (Fig. 5). This is the result of both light absorbance and attenuation of the CIDNP intensity at high photosensitizer concentrations (e.g., due to triplet quenching by ground state FMN). For the most strongly coloured solutions, irradiation from above with a square cut fibre results in very little light reaching the sensitive region of the sample inside the receiver coil (Fig. 5A), whereas the light escaping from the conical tip always illuminates the active region (Fig. 5B).

Somewhat surprisingly, the presence of the fibre cone in the centre of the NMR sample does not lead to a significant deterioration in spectral resolution (Fig. 6). After appropriate shimming, the width at half-height

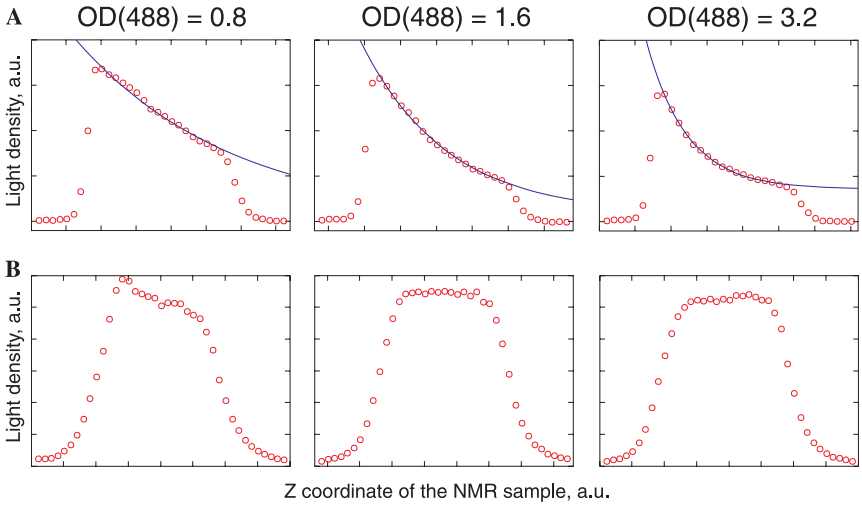

Fig. 4. The light intensity profiles along the z-axis of the sample measured using ^{19}F photo-CIDNP of 4-fluorophenol ([FMN] = 0.2, 0.4, and 0.8 mM) for (A) illumination from above using the arrangement shown in Fig. 1D and (B) for a tapered fibre (Fig. 1F).

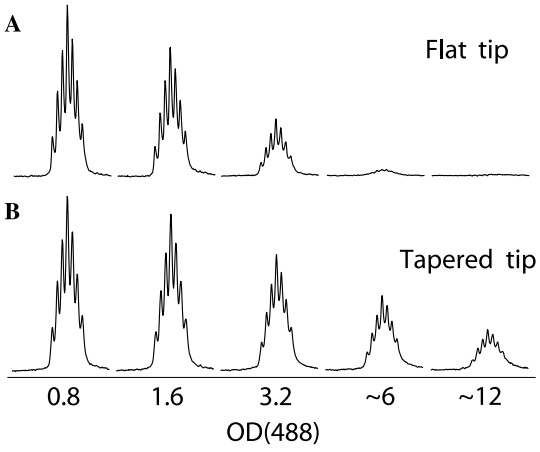

Fig. 5. ^{19}F photo-CIDNP signal amplitude as a function of photosensitizer concentration ([FMN] = 0.2–3.2 mM) for (A) illumination from above using the arrangement shown in Fig. 1D and (B) for a tapered fibre (Fig. 1F).

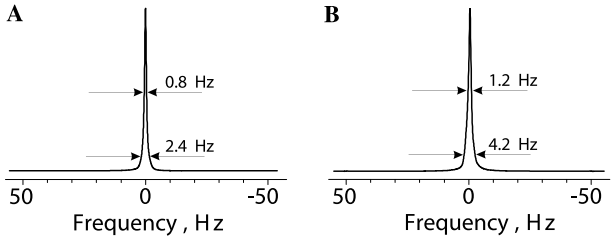

Fig. 6. ^{1}H NMR lineshapes of the residual HDO signal in a sample of D_2O, (A) without and (B) with the fibre present inside the NMR tube. The field was reshimmed after the insertion of the fibre.

of the HDO in D_2O increases from 0.8 to 1.2 Hz and there is some deviation of the lineshape from Lorentzian form. However, as this broadening should be the same for all lines in the spectrum, mild reference deconvolution [19] would, if necessary, restore the normal lineshape and width. For the linewidths commonly encountered in macromolecular NMR, the presence of the fibre should have a negligible effect on the resolution. In our experience, the simplex-based shimming algorithms supplied with Varian NMR software usually yield a sufficiently homogeneous field in less than 10 min.

The volume of the sample displaced by the cone is remarkably small. In Fig. 2C, the fibre appears thicker than it actually is because the surrounding liquid acts as a magnifying lens. Once the sheath has been removed, the untreated core of the fibre has a diameter of about 0.9 mm: simple arithmetic shows that the volume of the cone is less than 5% of the total sample volume. This may be one of the reasons why shimming is relatively straightforward. Another is that the discontinuities in magnetic susceptibility produced by introducing the fibre are almost exclusively perpendicular to the axis of the tube [20].

An important feature of the cone is its large light emission area: around 25 mm^2 as opposed to 0.8 mm^2

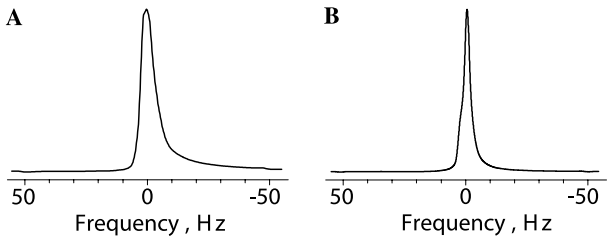

Fig. 7. The lineshape of the HDO signal in a sample of D_2O containing 0.2 mM FMN after 1 s of high-power (10 W) illumination for (A) illumination from above using the arrangement shown in Fig. 1D and (B) for a tapered fibre (Fig. 1F).

in the case of the square cut tip. As a consequence, the sample heating arising from high-power illumination is much more homogeneous. For the square cut fibre, the water signal has a long tail (Fig. 7A) indicating the presence of localised hot regions ($\Delta T = 6$ K for a 40 Hz shift). The thin layer between the end of the fibre and the top of the coil (see Fig. 1D) is probably even hotter. Supplying the same amount of energy to the sample via the conical fibre tip results in a much more uniform heating as evidenced by a more symmetrical lineshape, narrower at the base (Fig. 7B).

It should be noted that the steps on the fibre cone are essential. Our experience with a smooth cone (produced by slow continuous extrusion of the fibre into the etching medium) was that most light comes out of the very tip (diameter ~50 μm). When such a fibre was immersed into an absorptive fluid, the solution at the tip boiled and the end of the fibre disintegrated. It thus seems that the additional light reflections provided by the shoulders in a stepped tip are essential for efficient distribution of light to the sample.

Acknowledgments

This work was supported by the BBSRC, the EU (RTD Project HPRI-1999-CT-50006), and by INTAS (Project 01-2126). We thank Ken Hun Mok and Iain Day for helpful discussions. I.K. is grateful to the Scatcherd European Foundation and the Hill Foundation for a Ph.D. studentship.

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