Use of caged fluorescent dyes for the study of turbulent passive scalar mixing

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Abstract The non-intrusive initialization of a flow field with distinct and spatially segregated scalar components represents a significant experimental difficulty. Here a new technique is described which makes possible the non-intrusive initialization of a spatially binary passive scalar field in a laminar or turbulent flow field. This technique uses photoactivatable (caged) fluorescent dyes dissolved in the flow medium. The scalar field within the flow field is tagged or initialized by "uncaging" the appropriate regions with an ultraviolet excimer laser. Mixing between the tagged and untagged regions is quantified using standard laser induced fluorescence techniques. The method is currently being used to study mixing in a turbulent pipe flow.

1 Introduction
The use of dyes to quantitatively study turbulent passive scalar mixing began about three decades ago (Lee and Brodkey 1964). At that time the state of the art technique involved introducing dye into a primary flow field by means of a secondary injection system. In the study of Lee and Brodkey, the dye concentration was determined by passing light through the field of interest and correlating the absorption of that light with the dye concentration using Beer's law. More recently, fluorescent dyes have been used in a similar manner. This approach, known as Laser Induced Fluorescence (LIF) (e.g. Koochesfahani and Dimotakis 1985) involves introducing a fluorescent dye into the flow field and determining local concentrations by measuring the amount light emitted by the dye following absorption of higher energy photons. Phosphorescent dyes have also been used to study mixing fronts, (Koochesfahani et al. 1993). One can excite fluid containing the phosphorescent dye and study the mixing with adjacent fluid that is not excited. These dyes have the limitation that they only remain excited for about 5 ms, thus the time scale over which they can be used is small.

The unique feature of the approach described here is the use of Photo-Activated Fluorophores (PAFs) or "caged" dyes. The basic idea of this approach is to start with a fluid containing a homogeneously mixed caged dye, which is non fluorescent. The fluorescent capabilities of the dye can be recovered ("uncaged") by photolysis with a UV photon. The scalar field can thus be precisely tagged (initialized), in any number of configurations, without affecting the velocity field.

Two key features are achieved using this technique. First, the problem of intrusive initialization is replaced by the use of UV light to initialize the flow. Second, the method yields unprecedented spatial and temporal control over the initialization of the scalar field. Subsequent comparison with the results of computational methods and/or modeling study by Kerstein and McMurtry (1994) of atmospheric chemistry modeling work. One possible explanation for the differences between the Nye/Brodkey experiments and the Kerstein/McMurtry modeling was attributed to differences in the way the marked scalar field was initialized between the two cases. Furthermore, it was hypothesized that Nye and Brodkey were not able to make measurements far enough downstream to observe the region of power-law variance decay. Experimental verification of the recent theory and comparisons with the earlier work of Nye and Brodkey are key motivations behind the development of the technique described herein.

Received: 22 December 1995/Accepted: 9 April 1996

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We would like to thank Dr. Douglas Christensen for his assistance in designing the optical and electronic equipment for this experiment. This work was supported by the National Science Foundation under grant # CTS 9258445 and by the Advanced Combustion Engineering Research Center at the University of Utah and Brigham Young University.
Fig. 1. Photolysis of an o-nitrobenzyl derivative. Upon photolysis in the UV, this molecule is split into an "uncaged" leaving group and an o-nitrosobenzoyl derivative.

Fig. 2. Photolysis of "caged" fluorescein. Photolysis cleaves off the water solubilizing 5-carboxymethoxy groups, regenerating fluorescein.

2 Photo-activated fluorophores
The novelty of the PAF is the heart of this technique and is what separates it from previous experimental studies of mixing. Fluorescent molecules (fluorophores) are aromatic compounds which absorb photons, then emit photons in an allowed transition from the first singlet excited state in the form of less energetic light as the molecule relaxes to the ground state. The fluorescence emission lifetime generally is in the range of nanoseconds.

The concept of photoactivatable (i.e., "caged") fluorophores is rooted in the covalent modification of the fluorophore such that the fluorescence property is diminished or eliminated altogether. Photolysis subsequently removes the caging group, regenerating the fluorophore. The "uncaging" photolysis is performed in the ultraviolet, completely separate from the normal excitation band of the uncaged fluorophore, which usually occurs in the green to red portion of the visible light spectrum.

Since the introduction of caged fluorophores (Krafft et al. 1988), they have been used most often in the study of cell cytoskeleton dynamics. For example, covalent modification of actin filaments with caged resorufin (Cramer and Mitchison 1993; Theriot and Mitchison 1992; 1991) and of tubulin with caged fluorescein (Rodinov et al. 1994; Okabe and Hirokawa 1993; Mitchison 1989) are excellent demonstrations of how fluorescence can be "turned on" with light, with precise spatial and temporal control in dynamic systems. In the studies referenced above, the dynamic systems are the moving cell contents. More recently, caged fluorophores have been used as chemical actinometers for fluence determination in biologically relevant tissue samples (Lilge et al. 1993), and also for use in the study of turbulent hydrodynamic flows, both for velocimetry (Lempert et al. 1995) and now for scalar mixing studies, which is the topic of this paper.

The most often used caging groups are derivatives of the o-nitrobenzyl system. It has long been known that o-nitrobenzyl derivatives, upon photolysis in the UV, undergo an internal redox reaction in which one of the nitro group oxygen atoms is transferred to the benzylic carbon atom. The original substituent at the benzylic carbon atom is split off in the process. The photoproducts, then, are the "uncaged" leaving group and an o-nitrosobenzoyl derivative (Fig. 1).

The time scale over which uncaging occurs is in the range of microseconds to seconds, depending upon the leaving group, the other benzylic substituent, and substituents on the aromatic ring of the caging group. The strategy of caged fluorophores involves the uncaged fluorophore being the leaving group. For fluorescein, a commonly used fluorophore excited maximally at 490 nm, caging requires covalent attachment of o-nitrobenzyl groups at the 3' and 6' hydroxyl groups. Such covalent modification of fluorescein renders the molecule non-fluorescent, a result of breaking conjugation between the two upper aromatic rings. Photolysis in the UV cleaves off the o-nitrobenzyl groups, regenerating fluorescein (Fig. 2). In the present case the o-nitrobenzyl groups are substituted with water solubilizing 5-carboxymethoxy groups, similar to a strategy previously used when attaching a similar caged fluorescein to a dextran (polysaccharide) for lineage tracing in developing embryos (Vincent and O'Farrell 1992).

The water used in the present experiments contained a 10⁻⁶ M concentration of caged fluorescein as well as enough sodium hydroxide to raise the pH to about 10. Increasing the pH gives substantially higher fluorescence intensity (after uncaging) that does a solution in pure water. So strong is the dependence of fluorescence intensity on pH that it has been used as a "switch" in previous LIF experiments that studied the mixing between acids and bases (Koochesfahani and Dimotakis 1986).

3 Experimental configuration
The flow facility consists of a 9.75 m long test section consisting of eight 1.22 m sections of 25 mm I.D. quartz pipe, for a total length to diameter ratio of about 390. The quartz sections are connected by brass couplers each of which is fitted with a pressure tap and constructed so as to give a smooth transition. A reservoir, pump, return section and flowmeter complete the flow facility as shown in Fig. 3. Not shown in the figure are the manometers connected to each pressure tap. These have been used to verify the linearity of the pressure drop along the pipe. Total fluid volume of the facility is about 35 l.

The use of the caged dyes was motivated by the desire to obtain a highly idealized initialization of the scalar field in the pipe. In particular, it was desired to initialize the field with