AIP | Review of Scientific Instruments

Note: Small anaerobic chamber for optical spectroscopy

Adrien A. P. Chauvet, Rachna Agarwal, William A. Cramer, and Majed Chergui

Citation: Review of Scientific Instruments **86**, 106101 (2015); doi: 10.1063/1.4932183
View online: http://dx.doi.org/10.1063/1.4932183
View Table of Contents: http://scitation.aip.org/content/aip/journal/rsi/86/10?ver=pdfcov
Published by the AIP Publishing

Note: Small anaerobic chamber for optical spectroscopy

Adrien A. P. Chauvet,[1,a)] Rachna Agarwal,[2] William A. Cramer,[2] and Majed Chergui[1]
[1]*Ecole Polytechnique Fédérale de Lausanne (EPFL), Laboratoire de Spectroscopie Ultrarapide, ISIC, Faculté des Sciences de Base, Station 6, 1015 Lausanne, Switzerland*
[2]*Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907, USA*

(Received 24 June 2015; accepted 19 September 2015; published online 1 October 2015)

The study of oxygen-sensitive biological samples requires an effective control of the atmosphere in which they are housed. In this aim however, no commercial anaerobic chamber is adequate to solely enclose the sample and small enough to fit in a compact spectroscopic system with which analysis can be performed. Furthermore, spectroscopic analysis requires the probe beam to pass through the whole chamber, introducing a requirement for adequate windows. In response to these challenges, we present a 1 l anaerobic chamber that is suitable for broad-band spectroscopic analysis. This chamber has the advantage of (1) providing access, via a septum, to the sample and (2) allows the sample position to be adjusted while keeping the chamber fixed and hermetic during the experiment. © *2015 AIP Publishing LLC.* [http://dx.doi.org/10.1063/1.4932183]

The advent of pulsed lasers allowed the development of time resolved optical spectroscopy. A consequence of the increase in complexity of these analytical tools is that the required laser systems became even more cumbersome. While often taking most of the space on the optical bench, only little is allocated for the actual sample. Space thus becomes a matter of cost associated with the complexity of these tables. Furthermore, the sophistication of the analytical tools comes hand in hand with an increase in performance and ultimately with the need to control more of the sample parameters, including the atmosphere in which the sample is enclosed. Because working under vacuum is generally not an option, especially when dealing with liquid samples, the only alternative is to work under a controlled atmosphere. For this aim, one can choose either to enclose the whole setup in a chamber, which is usually neither practical nor cost effective, or to enclose only part of it. It is then a matter of compromising between practicality and feasibility. In order to accommodate both the needs, the limited space usually available, and the need to work under controlled atmosphere, we propose here a small 1 l-anaerobic chamber that encloses only the sample.

The scheme of the anaerobic chamber is presented in Figure 1. In the present case, the chamber is made suitable for pump-probe experiments on liquid samples using broadband (450-750 nm) ultra-short (40 fs) laser pulses. The pump and probe beam are passed through 100 μm thin glass windows fixed on the main container, as shown in Figure 2. Epoxy is used as a sealant. Due to their limited thickness, the windows have a negligible effect on both the intensity and dispersion of the laser pulses. It was determined that the decrease in intensity of the light passing through the chamber, in the region from 450 to 750 nm, was less than 5%.

The body of the anaerobic chamber is made out of transparent plastic for convenience. The 1 l container, originally purchased with a hermetic lid, is chosen to enclose the entire micro fluidic flow-cell,[1] which itself contains the sample. The flow-cell, which is made of a decantation chamber (home-made), a flow-through pump (Swiss Center for Electronics and Microtechnology, CSEM), and a capillary (Composite Metal Services Ltd., 0.5×0.5 mm square quartz), are fixed to the lid via the fixation poles, as illustrated in Figure 1. While the lid is attached to the optic table, the capillary through which the sample is studied is fixed on an independent pole that passes through the lid. The pole is attached to 3-dimensional axis translation and rotational stages that allow adjustment of the capillary's position while the lid is fixed. The capillary's fixation pole is hermetically linked to the lid via taped flexible plastic sheet in order to maintain flexibility. The alimentation of the pump motor is also accomplished via the lid by means of pin connectors. Epoxy is used as sealant.

Having all connections made through the lid allows one to freely adjust the sample without being confined to the chamber's restricted volume. Once the microfluidic flow-cell is set, the main container is put in place and attached to the lid via the lid's latches. The chamber is then purged, in the present case with argon (Ar), via flexible tubing that is connected to the main container. Note that the gas outlet of the chamber is on the top surface to allow for the relatively lighter oxygen to be effectively removed. On the top of the chamber is also a septum made of thin taped flexible plastic sheet that allows access to the decantation chamber of the flow-cell via syringes. To ensure that the chamber's inner atmosphere remains uncontaminated from any external agent (even after perforation of the septum), the chamber is continuously flushed with Ar. The resulting over-pressure is monitored via flexible tubing that is attached at the chamber's gas outlet and plunged into water (Figure 1): the difference between the water level inside and outside the tubing is an indicator of the pressure inside the chamber.

In order to illustrate the chamber's effectiveness, we studied the multi-heme protein cyt b_6f complex[2] by means of ultrafast transient spectroscopy as described elsewhere.[3] In

a)Author to whom correspondence should be addressed. Electronic mail: adrien.chauvet@gmail.com

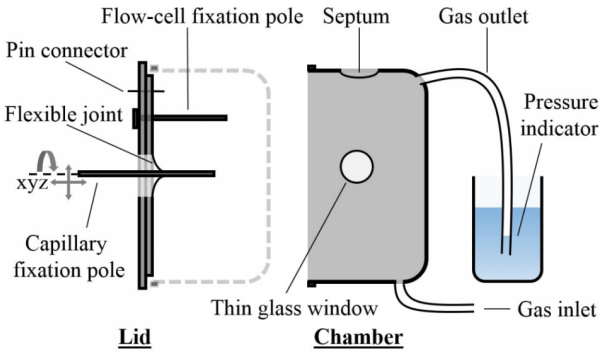

FIG. 1. Anaerobic chamber, schematic side view.

particular, the heme *b* in its ferrous state is highly susceptible to oxidation via molecular oxygen present in the air (Figure 3(b)). The heme's oxidation states were followed by monitoring the distinct α-absorbance bands via the 40 fs white light continuum probe pulses.

First, the sensitivity of the cyt b_6f's heme *b* to oxygen is illustrated. The sample is prepared in a nitrogen filled glove box and housed in the microfluidic flow-cell system. The reduction state of the energy transducing cyt b_6f protein complex is then monitored via the *b*-heme reduced α-band (Figure 3(a)) that characterizes its ferrous state. As shown in Figure 3(b), the time evolution of the wavelength difference (561–580 nm) shows that full reduction takes place within seconds after addition of dithionite. The reduction is quickly followed by a complete oxidation in hundreds of seconds. The delay between the addition and the start of the subsequent oxidation is understood as the time required for all the solvated dithionite to be degraded by oxygen. The oxidation of the ferrous heme *b* is fit as a mono-exponential with a decay-constant of about 80 s. Due to this short life time, the heme *b*'s ferrous state is therefore not suitable for any aerobic analysis by means of regular ultrafast transient spectroscopy. The short life-time also illustrates the sample's extreme sensitivity to oxygen.

We then incorporated the sample in the anaerobic chamber. As shown in Figure 3(c), the reduction state of the heme *b*, again depicted by the amplitude of the wavelength difference (561–580 nm), is now stable over an extended period of time.

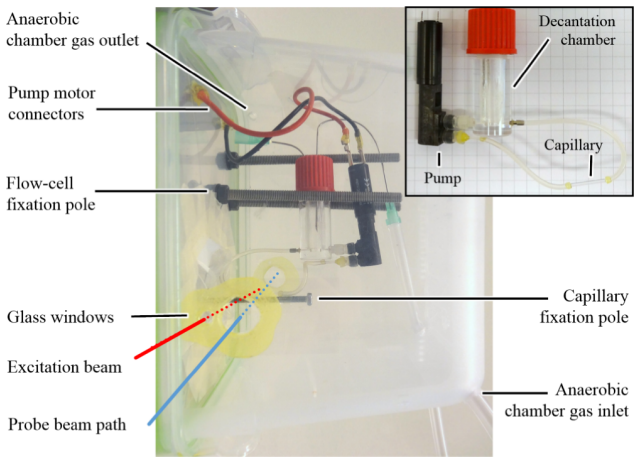

FIG. 2. Anaerobic chamber, side view, with the microfluidic flow-cell installed. Inset: microfluidic flow-cell.

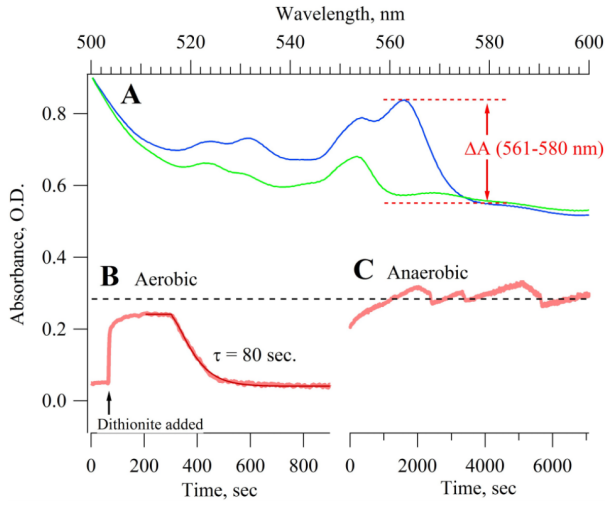

FIG. 3. (a) Spectrum of the cyt b_6f complex while the heme *b* is oxidized (green) and reduced (blue). The dynamics of the heme *b*'s reduction state are represented by the absorption difference between the wavelength 561 and 580 nm (red curves). (b) Heme dynamics in aerobic conditions. The addition of dithionite to the solution is indicated by the black arrow. The following oxidation of the heme is then fitted with a mono-exponential decay of ~80 s (smoothed red line). (c) Dynamics when the sample is housed in the anaerobic chamber. The black dashed line is added as an eye guide only.

The small amplitude fluctuations correspond to the evaporation and consequent increase of the sample's concentration due to the Ar flow within the cell. Each "kink" (Figure 3(c)) corresponds to the addition of buffer solution through the septum. Once reduced, the heme *b* retains its electron over a time course that is sufficient for an experiment to be performed. The data also confirm that the chamber's atmosphere is preserved from any oxygen contaminant even after multiple perforations of the septum (to add buffer solution) and consequently illustrates the chamber's effectiveness.

In conclusion, we have implemented a small 1 l-anaerobic chamber. Its size and functionality make it adequate for compact setups. The use of thin glass windows is appropriate to most spectral analyses as it only minimally affects the transmission of laser pulses and the broad band visible continuum probe beam. The use of flexible plastic sheets as sealing between the lid and the capillary's pole allows for fine positioning of the capillary in the beam path, while keeping the chamber fixed. The chamber's septum gives access to the sample even after the chamber is put in place. The chamber is cost-effective and easy to implement and enables the study of organic complexes and macromolecular protein complexes under a controlled atmosphere.

This project has been funded by the Swiss NSF via the NCCR:MUST, by the FP7 Marie Curie COFUND (M.C.), and by the U. S. NIH No. GM-038323 (W.A.C.). We thank particularly Dr. Euro Solari, scientist at the "Laboratoire de Chimie Supramoléculaire," UNIL, for his precious help.

[1]A. Chauvet, T. Tibiletti, S. Caffarri, and M. Chergui, Rev. Sci. Instrum. **85**, 103118 (2014).
[2]W. A. Cramer, H. Zhang, J. Yan, G. Kurisu, and J. L. Smith, Annu. Rev. Biochem. **75**, 769-790 (2006).
[3]C. Consani, M. Prémont-Schwarz, A. Elnahhas, C. Bressler, F. van Mourik, A. Cannizzo, and M. Chergui, Angew. Chem. **121**, 7320–7323 (2009).