Microfluidic integration of photonic crystal fibers for online photochemical reaction analysis

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Liquid-filled hollow-core photonic crystal fibers (HC-PCFs) are perfect optofluidic channels, uniquely providing low loss optical guidance in a liquid medium. As a result, the overlap of the dissolved specimen and the intense light field in the micron-sized core is increased manyfold compared to conventional bioanalytical techniques, facilitating highly efficient photoactivation processes. Here we introduce a novel integrated analytical technology for photochemistry by microfluidic coupling of a HC-PCF nanoflow reactor to supplementary detection devices. Applying a continuous flow through the fiber, we deliver photochemical reaction products to a mass spectrometer in an online and hence rapid fashion, which is highly advantageous over conventional cuvette-based approaches

Microfluidics is to chemistry what photonics is to optics. Both technologies seek the miniaturization of common laboratory procedures for increased transportability, minimal material consumption and quicker results. Merging the two into the novel field of optofluidics offers enormous potential for rapid and efficient (bio)chemical analysis [1,2].

It is well-known that hollow-core photonic crystal fibers (HC-PCFs), in which both core and cladding channels are filled with liquid, can act as low-loss optofluidic waveguides [3]. HC-PCF offers several major advantages over conventional sample cells. Firstly, low waveguide losses of a few dB/m allow the use of long optical path lengths, greatly enhancing the effective light-matter interaction. Secondly, typical core diameters in the 10-20 μm range reduce the sample volume to some nL per cm of interaction length (5 orders of magnitude less than in conventional sample cells). Thirdly, HC-PCFs are easily fabricated [3] from chemically inert, high-quality silica glass with negligible scattering, absorbance and fluorescence. Finally, the propagating light is confined to the hollow-core, allowing it to interact strongly with the sample. At comparable input powers, the resulting intensity in the hollow core is 5 orders of magnitude higher than in cuvette-based approaches.

These advantages were exploited in our recent photochemistry studies on low-quantum-yield photolysis [4] and photo-induced catalysis [5] in HC-PCF 'nanoreactors'. However, this work was limited to a stationary sample, making it impossible to analyze the reaction products with additional, non-optical methods.

In this Letter we demonstrate the successful connection of a kagome-structured HC-PCF nanoreactor into a microfluidic circuit via a conventional microfluidic chip. In this way, dead volumes are kept to a minimum, which results in small transition times between consecutive process steps. We show that in a continuous-flow configuration, photochemical reaction products can be monitored online by high-resolution mass spectrometry on timescales not previously achievable with standard cuvette-based experiments.

A schematic diagram of the setup is shown in Fig. 1(a). The connection between the HC-PCF and the microfluidic chip is made such that the fiber facets remain optically accessible, see Fig. 1(b). To this end, the pre-cleaved fiber ends are mounted orthogonal into one of the 16 cuboidal channels (200 μm x 100 μm x 18 mm, V_{dead} = 360 nL) of a through-hole polymeric microfluidic chip (MFC), onto which from one side a 130 μm-thick transparent cover lid is readily attached by the manufacturer (microfluidic ChipShop). The fiber ends are introduced through the 1.37 mm deep hole from the open side, leaving a small gap between the fiber facet and the cover lid. The fiber ends are surrounded and fixed by standard PEEK tubing connectors, which are modified to be readily screwed into a custom-built fiber coupling stage mount and thereby pressed against the microfluidic chip. Teflon-tape is used for additional sealing. The fiber used in the experiments is a kagome-structured HC-PCF (see Fig. 2(a)). The typical fiber length was $L = 25$ cm, corresponding to a liquid volume of 2300 nL in the cladding holes and 85 nL in the hollow core.

The kagome HC-PCF was designed for guidance in the blue region of the spectrum when both core and cladding holes are filled with water. The mode intensity profile at the fiber output shows that the guided light is indeed well-confined in the liquid-filled core (Fig. 2(b)). Despite the presence of small surface imperfections in the etched channels of the MFC, incoupling efficiencies of order 50% (3 dB incoupling loss) were achieved into the core mode. The bulk absorption of water at this wavelength is very small: 0.07 dB/m. The waveguide loss in the filled HC-PCF at 488 nm was less than 5 dB/m (measured by cut-back), more than two orders of magnitude below that of a straight, water-filled silica capillary waveguide with same inner diameter (620 dB/m) [4].

A continuous flow through the system is established with a syringe pump. After exiting the fiber, the flow of analytes passes through a flow meter (SLG1430-150,

Sensirion) to allow sensitive flow-rate monitoring and bubble detection. The output of the device during the initial filling of the microfluidic circuitry is shown in Fig. 2(c). Subsequently, the sample enters the electrospray-ionization source of a high-resolution mass spectrometer (HR-MS) (maXis, Bruker Daltonics) [6]. The flow rate required for optimum signal in the mass spectrometer is 27.7 nL/s. This corresponds to a flow velocity in the fiber core of 1.25 cm/s and hence to an in-fiber sample residence time of ~20 seconds.

As proof-of-principle, we studied the photoaquation of cyanocobalamin (CNCbl), Fig. 3(a), to aquacobalamin (H_2OCbl) in aqueous solution [4,7] at low pH (<2). Figure 3(b) shows how the absorption peak shifts to shorter wavelength after excitation at $\lambda = 488$ nm. The optical power required to achieve full photoconversion in the flow reactor was estimated from a calibration experiment in a stationary fluid. To this end, the system was filled with sample solution in the dark and allowed to come to rest. The irradiation was started and the amount of transmitted light monitored with a photodiode. Figure 3(c) shows that at an in-core intensity of 54 W/cm^2 the transmission decreases by 30% over 0.71 s. These data are in reasonable agreement with reaction-kinetics modeling [4] without freely adjustable parameters (green, dashed line).

For subsequent continuous-flow measurements, we started the syringe pump until constant flow conditions were reached. Currently, the overall dead-time is not solely determined by the dead volume of the circuitry, but is significantly increased by intrinsic spray stabilization and ion travel times in the HR-MS used: typically, the MS-signal starts to build up after approximately 10 minutes and takes another 5 minutes to reach maximum intensity.

Figure 4 shows the mass spectra obtained. The top row refers to a reference measurement performed in a cuvette; the bottom row shows the outcome of the microfluidic HC-PCF-approach. Mass spectra acquired in the dark (left-hand side), in which the sample is either directly injected into the mass spectrometer ("cuvette") or pumped through the non-irradiated fiber circuitry ("HC-PCF"), are equivalent as expected. The series of peaks at a mass-over-charge-ratio of around 678.3 *m*/*z*, which is a result of the isotopic distribution of C, N and O in the molecules, refer to the doubly charged species $[CNCbl + 2\,H^+]^{2+}$.

On the right-hand side, samples of 100 µmol L^{-1} concentration were irradiated at 488 nm. From our reaction-kinetics model, we conclude that a moderate input power in the fiber experiment of $P_0^{HC\text{-}PCF} = 2.35$ mW is sufficient for > 99% photoconversion. Despite a doubled input power of $P_0^{cuv} = 4.5$ mW, the intensity of light in the cuvette is 5 orders of magnitude lower compared to that in the fiber ($I_0^{cuv} = 40$ mW/cm^2, $I_0^{HC\text{-}PCF} = 700$ W/cm^2). As a result the cuvette experiment took over 10 hours, in marked contrast to the fiber approach. Moreover, whereas in a cuvette ~1 mL of total sample volume is typically used, 25 µL was sufficient in the integrated case. The mass spectra from both approaches are in good agreement. In addition to the CNCbl series of peaks, new peaks are clearly apparent at 664.8 *m*/*z*, 673.8 *m*/*z* and 675.8 *m*/*z*, which were assigned as $[Cbl^+ + H^+]^{2+}$, $[H_2OCbl^+ + H^+]^{2+}$ and $[Cbl^+ + Na^+]^{2+}$. This pattern corresponds to the photoproduct aquacobalamin H_2OCbl, as was proved by direct injection of a pure aqueous H_2OCbl solution (data not shown) and therefore verifying successful photoconversion in both cases. We note that the mass spectrometric technique in general does not allow for quantitative analysis. Cyanocobalamin is still present in both the cuvette and the fiber spectra, though for different reasons. In the cuvette case, the reaction was most probably incomplete. In the fiber case, the volume flow of analyte through the dark fiber cladding holes is about 5.6 times higher than through the irradiated fiber core (calculated using Hagen-Poiseuille's law for laminar flow in a parallel circuit of tubes), which simultaneously introduces unreacted species. This feature of the HC-PCF could in future be used to act as an inherent dark reference of the otherwise non-quantitative mass spectra in a convenient way.

In this pilot study we have introduced a novel integrated technique for the analysis of photochemical reactions. The combination of a low-volume microfluidic, continuous flow circuitry with high optical intensities in the HC-PCF renders the approach some 50 × less sample- and time-consuming than the cuvette-based analytical approach.

Pushing the limits of the technique further, we are trying to detect shorter-lived reaction species that are undetectable using conventional techniques. This will play an important role in our current investigation of the photochemical decomposition pathway of new types of photoactivatable metal-based anticancer prodrugs [8].

For the future we foresee that our approach can be combined with other lab-on-a-chip devices [9,10] for sample preparation, separation and (multimodal) analysis in a straightforward fashion.

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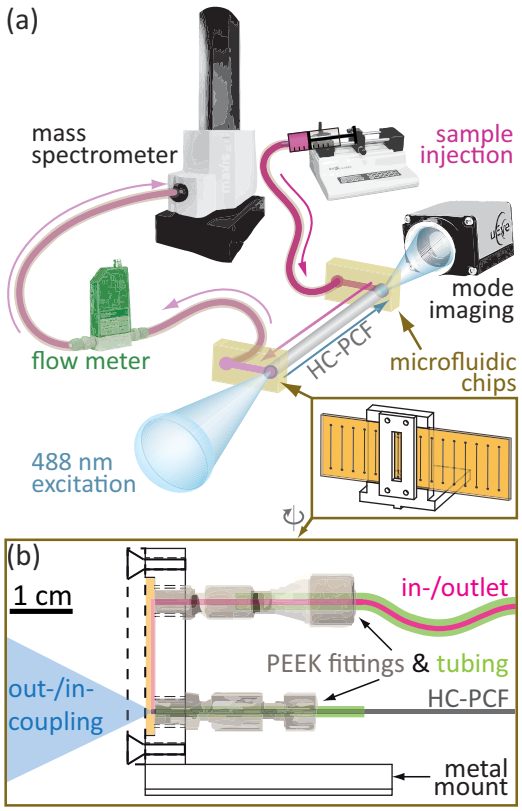

Fig. 1. (Color online) (a) Schematic of setup (not to scale). Microfluidic circuitry denoted in pink (total dead volume ~15 µL). Inset: technical drawing of MFC-to-fiber mount (b) Side view of MFC-to-fiber mount. Standard PEEK-tubing fittings are screwed into a custom-built aluminum fiber-coupling stage mount and against the MFC-channel holes to provide a leak-tight connection (sealed with teflon-tape).

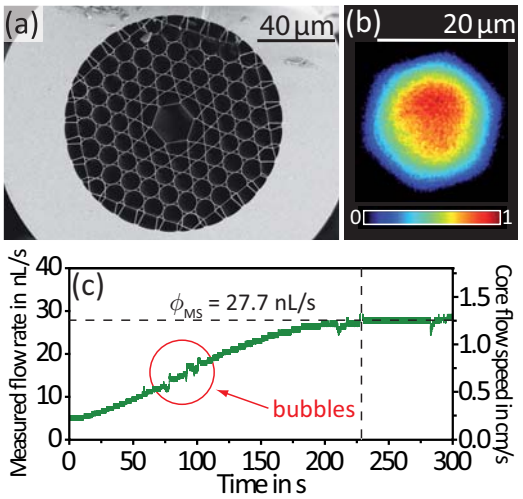

Fig. 2. (Color online) (a) Cross-sectional scanning electron micrograph of the kagome-structured HC-PCF (core diameter 19.7 µm). (b) Measured mode irradiance profile at 488 nm (fiber length is 25 cm). (c) Measured flow rate into the spectrometer. After ~ 225 s, the optimal flow rate for the mass spectrometer ϕ_{MS} = 27.7 nL/s is reached. The right-hand axis shows the corresponding flow speed in the fiber core. Flow disturbances caused by bubbles are easily detected (red circle).

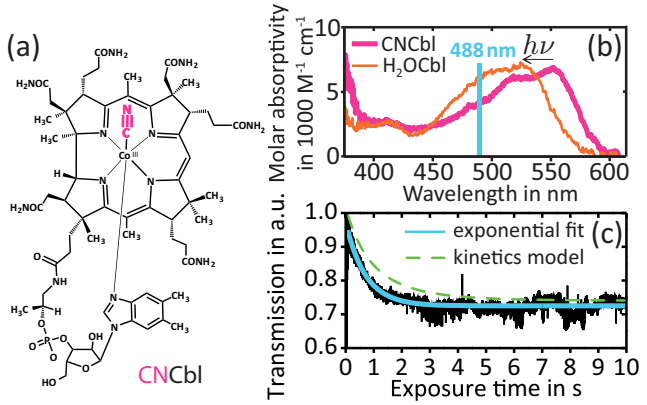

Fig. 3. (Color online) (a) Structure of cyanocobalamin (CNCbl); the cyano-group (pink) dissociates upon irradiation and is replaced by a water molecule to form aquacobalamin (H_2OCbl) (b) Absorption spectra before (CNCbl) and after (H_2OCbl) irradiation (data from [4]). (c) Fiber transmission decrease at 488 nm and zero flow due to photoconversion of a 5 µmol L^{-1} sample of CNCbl to H_2OCbl (intensity 54 W/cm^2); the blue curve corresponds to a single exponential decay fit, the green dashed curve to a reaction-kinetics model without freely adjustable parameters.

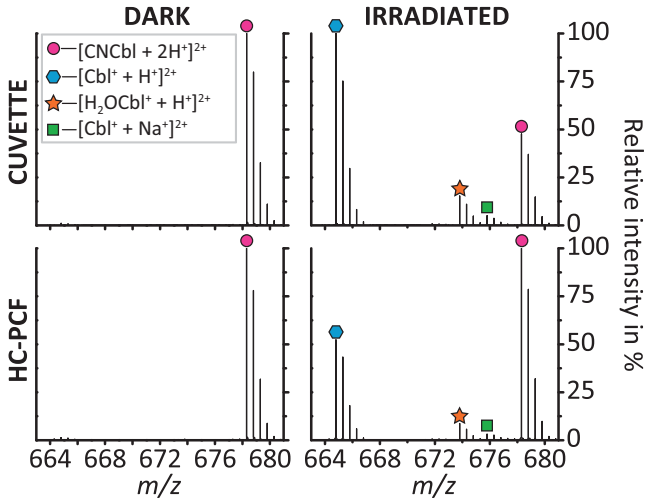

Fig. 4. (Color online) Mass spectra for cyanocobalamin (CNCbl) photoconversion. Top: sample directly injected into the mass spectrometer, not irradiated (left) and irradiated for 10 h at I_0 = 40 mW/cm^2 in a cuvette (right) Bottom: Sample introduction via the integrated HC-PCF-system, mass spectra obtained after ~ 15 min; not irradiated (left) and irradiated at I_0 = 700 W/cm^2 for 20 s (right).

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