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Optofluidic multi-measurement system for the online monitoring of lubricant oil

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Abstract

We show a detection system that simultaneously allows absorbance (ABS), laser-induced fluorescence (LIF) and scattering detection excited by two different laser sources at 405 nm and 450 nm. The heart of the system consists of a mass manufacturable polymer optofluidic chip. The chip is mounted in an optical detection assembly that aligns the chip to the rest of the system, seals the chip from leakage, fixes the position and connects the channels to the rest of the fluidic system. The fluidics exhibit a reduced susceptibility to perturbations caused by air bubbles, this is accomplished by making use of a serpentine channel layout. For coumarin 480, detection limits of 100 nM and 10 pM are observed for ABS and LIF respectively. An effective detection range of 4000 down to 1 nephelometric turbidity units is shown for the detection of scattered light. The viscous behaviour of the sample is analysed by a secondary FFT processing step of which the result is further processed by multivariate data analysis. This allows the identification of samples and prediction of their quality parameters. We apply this system for the monitoring of lubricant oil, demonstrating its ability to compete with spectroscopic detection techniques. The low-cost approach and multi-measurement architecture shown in this paper pave the way for miniaturized on-line monitoring of liquids in an industrial environment.

Keywords: laser-induced fluorescence, absorption, scattering, optofluidics, lubricant oil, PLS regression, multi-measurement

(Some figures may appear in colour only in the online journal)

1. Introduction

Lubricant oil is a key element in the proper functioning of industrial machinery such as turbines, compressors and presses. The condition of the oil irreversibly degrades over time due to machine operation [1]. As the world is coming to face a shortage of crude oil, increasing fossil fuels’ cost and environmental issues imply that lubricants should be utilized efficiently [2]. On-line monitoring of lubricant oil quality avoids structural damage to the machinery while optimizing the oil’s lifetime. Thus the cost of unnecessary preventive lubricant changes and the cost of their disposal is avoided [3]. Furthermore the impact lubricants have on the environment, such as pollution and CO\textsubscript{2} emissions, are reduced [2]. Many on-chip flow cytometers and optofluidic sensors can be found in literature [4–6]. In this paper we will demonstrate how multi-measurement laser-induced optical analysis can be used for the on-line monitoring of lubricant oil quality.

The work of Mignani et al [1] demonstrates that using absorption and fluorescence spectroscopy can be combined with multivariate data analysis to identify lubricant oil samples based on their appearance. A liquid’s appearance is determined...
by its color (which in turn is related to its absorption spectrum) and turbidity (preferably supplemented with fluorescent properties) and can provide information such as geographic origins, presence of contaminants and production method [7]. By definition, turbidity is an ‘optical property that causes light to be scattered and absorbed rather than transmitted in straight lines through the sample’ [8, 9]. This interaction makes the sample appear cloudy or less transparent. It is possible to quantify turbidity by measuring the amount of scattered light (typically at an angle of 90 degrees with respect to the flow direction), or the amount of light that is absorbed [8]. In Mignani’s work, data about the sample turbidity is collected by the same photo spectrometer used to collect the sample’s fluorescence spectrum, positioned at a 90 degree observation angle. Due to the use of broadband excitation, it is difficult to distinguish between scattering and fluorescence as the response spectra of both phenomena will most certainly overlap. Nevertheless, the turbidity data is present and through multivariate data analysis, the significant parts of the combined data set containing absorption and fluorescence/scattering data, samples can be identified. We can determine that the differences between lubricant oil samples are most significantly noticeable for excitation wavelengths around 405 nm and 450 nm. We have deduced this from the fact that around these wavelengths, the absorbance spectra exhibit the most significant differences [1, 10].

In this paper we show a multi-measurement system that incorporates a robust optofluidic chip that is compatible with low-cost replication. This system is capable of simultaneous absorbance (ABS) and laser-induced fluorescence analysis using time-multiplexed 405 nm and 450 nm excitation lasers. In addition a simultaneous scattering measurement at 405 nm is performed so that information about a sample’s turbidity can be acquired. We also show a plug & play fluidic interconnection and a method to avoid tedious chip bonding procedures.

Using this system we set up a LIF-ABS calibration curve for coumarin 480, allowing direct comparison with our previous multi-measurement system [11, 12]. We also set up a turbidity calibration curve using microsphere standards to demonstrate the system’s ability to detect various amounts of scattered light and the correlation to sample turbidity. Finally, the applicability of this system is shown in a context of industrial systems with the on-line monitoring of lubricant oil quality parameters. The latter is achieved through multivariate data analysis. We here with demonstrate our system’s ability to compete with spectroscopic detection techniques.

2. Optofluidic chip design

In figure 1 we show a schematic representation of the system architecture. On the left hand side we condition the output of two laser diodes to achieve two collimated excitation beams that can be aligned to the chip. These collimated beams are then combined to a collinear dual wavelength excitation beam using a right angle and a dichroic mirror. This beam is then sent into the optofluidic chip through its input facet. The amount of excitation light that reaches the channels through free-space coupling can be significantly higher when compared to fiber coupling assuming that the channels are sufficiently large. More specifically this means that the dimensions of the channels should be at least of the same order of magnitude as the beam-waist. The excitation light will interact with the sample that is contained inside the chip’s fluidic channel. The excitation light that is not absorbed leaves the chip through its output facet. The non-absorbed light then travels through a 50 : 50 beamsplitter towards detectors 1 and 2 that are equipped with bandpass filters of which the wavelength is centered on the wavelength of lasers 1 and 2 respectively. Detectors 3 and 4 and their associated filters are placed on the top- and bottom-side of the chip respectively to capture scattered light or fluorescence that is excited in the sample. The chip is clamped between two sturdy aluminum plates. Leakage is negated by exerting sufficient force on the two substrates out of which the chip consists. This way tedious bonding procedures are avoided. In the same action of clamping the chip into place, so are the fluidic channels connected to the rest of the fluidic system through the bottom-side of the chip. To demonstrate our proof-of-concept we choose a 40 x 40 mm² chip size. These dimensions can be reduced in future work.

For the design of this chip we set forth four design goals:

(i) The chip needs to be robust. Chips that are fabricated in substrates with a limited thickness (250 μm, 500 μm) can withstand pressures up to 100 bar if bonded properly [13]. However, even though they can withstand such pressure, they are fragile and should be handled with care. We want our optofluidic chip to be very robust and easy to handle. To achieve this we decide to fabricate the chip in a substrate that is 5 millimeters thick.

(ii) The chip should be insensitive to misalignment with respect to the rest of the system. Personnel with limited training should be able to replace the chip quickly and easily. We assume that the excitation beam going through the chip is fixed with respect to the optical detectors. Misalignment of the chip should hence not deviate the excitation beam.

(iii) The system presented in our previous work [11, 12] was highly sensitive to presence of air bubbles. In case one or more air bubbles were present, the measurement had to be repeated. For the current system, we want to reduce the influence of air bubbles so that they no longer can degrade individual measurements.

(iv) We want this system to improve the level of sensitivity that was shown previously [11]. We will investigate the limit of detection for coumarin 480 which was previously also used as a figure of merit for the sensitivity of the onboard ABS and LIF analysis. This allows direct comparison with our previous work.

The main factor through which the limit of detection (LOD) can be improved is by increasing the ‘interaction length’. The interaction length is the physical distance over which the excitation light interacts with the sample. An increase in interaction length improves the LOD for both ABS and LIF detection. The increase in sensitivity for ABS can be directly observed in the Beer–Lambert equation [14]. For LIF the improvement in sensitivity is caused by the increase of the excited sample
volume and thus of the fluorescent emitting volume. For small sample concentrations the fluorescence power per volume is nearly constant along the interaction length and the total fluorescent optical power is thus linearly proportional to the interaction length. Most LIF detection systems have a limited interaction length and only excite a small sample volume, and so their optical systems can be based on the assumption that the fluorescent light source can be modeled as a point emitter. The collection of fluorescence is challenging because of its omnidirectional emission. Typically, fluorescence collection efficiencies are rather low and solutions that do have good performance require costly and bulky optical systems such as microscopes. When the interaction length is increased, the assumption that the fluorescence can be modeled as a point emitter, no longer holds. The classical solution to this problem is to increase the size of the collection optics proportionally to the dimensions of the emitting object, rendering the optical system even bulkier. We aim at designing a compact but efficient system for the collection of fluorescent light that does not use such a classical optical system. Our solution is based on placing a photodetector as close as possible to the fluorescent object. From non-sequential ray-tracing simulations we conclude that in this case this is a valid approach as the diameter of the photomultiplier tube’s (PMT) sensitive surface is of the same order of magnitude (1–8 mm) as the length of the fluorescent volume (1–13 mm). This approach is also valid for the detection of scattered light.

Without using any optics we will focus on increasing the chip’s interaction length to achieve a sufficient sensitivity. The design of the chip’s fluidic channels and their layout will thus be the subject of this exercise. A classical U-channel layout is sensitive to the presence of air bubbles. Indeed an air bubble will disturb an ongoing measurement as long as one or more bubbles are present within the interaction length. Such a perturbation can cause experiments to fail due to unrecoverable distortion of the envelope of the experiments signal response. The elimination of air bubbles is possible using degassers or bubble traps. Bubble traps can be integrated on-chip, some are passive [15], others are active and require the application of a vacuum [16]. However, these integrated bubble traps operate at flow rates that are much lower than the injection speed of 30 μL s⁻¹ used in our application. Contrary to bubble traps, debubblers are suited for the aforementioned injection rate, but such devices are typically bulky and expensive [17] when compared to a mass manufactured polymer optofluidic chip. In summary, having a higher flow rate as requirement may prohibit the removal of air bubbles due to either the cost of external debubbler and vacuum pump or the potentially inadequate performance of integrated bubble traps.

To counter the effects of air bubbles, we propose to use a ‘serpentine’ or W-shape channel. Such a channel is a single channel that is folded back on itself for a given number of times. The serpentine channel’s geometry is such that the excitation beam perpendicularly crosses the channels as can be seen in figure 2. This way, air bubbles will only shortly cross the excitation beam while propagating through the channel. Thus, their influence on the signal response is shortened in time. Because of this, the envelope of the signal response will exhibit short spikes rather than the complete destruction of the envelope. Such spikes can be removed by post-processing filters. This geometry, combined with 8 mm wide entry facets makes that translating the chip will not redirect the course of the excitation beam. Rotation of the chip might cause the excitation beam to deviate. However, both rotation and translation are limited by the optical detection assembly in which the chip is to be mounted. We select channels with a depth of 900 μm and a width of 1 mm with at least 1 mm of separation between neighbouring channels to ensure successful fabrication through traditional micro-milling. In addition, given that the channel is 1 mm wide, every extra fold increases the chip’s interaction length by 1 mm. The channel height also guarantees that the complete beam waist of the excitation beam can be coupled in. The entry and exit facets are also given the same height for the same purpose.

As we are aiming to outperform our previous work [11, 12] we should select a chip design in which the interaction length exceeds 3 mm to achieve higher sensitivity for ABS and LIF. On the other hand, a chip with a longer interaction length will also exhibit a larger internal volume. The larger the internal volume, the longer it takes to rinse the chip after a
measurement. Even though a higher internal volume means a better optical performance, it also decreases the overall system performance by increasing the time needed for a single measurement. Nonetheless, given that our chip is compatible with low-cost production, it could be used as a disposable chip and as such completely eliminating the need for rinsing. It should be noted that a serpentine-shaped channel will always have a larger internal volume than a U-channel. As we are targeting lubricant oil monitoring as an application, this is acceptable as there are typically large amounts of the sample available and furthermore the actual value of the sample itself is rather limited. The sensitivity for ABS should not necessarily be increased too much as we expect the oil samples in the later experiments to have moderate to high absorption. We deduce this from the fact that many of the samples appear opaque in the small vials in which they are contained. Since these vials have an internal diameter of 7–8 millimeters it is almost certain that for an interaction length longer than this internal diameter, the ABS measurement will saturate due to all excitation light being absorbed by a strongly absorbing sample. This might make it impossible to distinguish between differing samples that are opaque. All factors taken into account we compromise by fabricating a chip with an interaction length of 5 millimeters.

3. Demonstrator setup

We use two excitation laser diodes with respectively 405 nm and 450 nm wavelengths as these show good compatibility with coumarin 480 [11, 12] and lubricant oils [1, 10]. We show the properties of these laser diodes in table 1. The strong astigmatism of both beams (i.e. a large difference between their parallel and perpendicular divergence angles) tells us that when we collimate these laser diodes, their laser spots will exhibit significant ellipticity. Due to the chip’s 8 mm wide entry facet, there is no requirement to reduce the excitation beam’s ellipticity, given that we can rotate the laser diode so that its perpendicular divergence plane is aligned with the width of the entry facet.

We use two identical compact laser conditioning assemblies, consisting of an aspheric lens mounted on a high-precision translation stage and a rotating mount, to collimate and rotate the output of each respective laser diode. The collimated output beam is then deflected by a kinematic mirror that allows for compensating any off-axis angle exhibited by the laser beam. The output beams of the respective laser conditioners are combined using a Thorlabs CM1-E02 dielectric right angle mirror and a Thorlabs DMLP425R dichroic mirror (with 425 nm cut-off wavelength). When we measure the output beams of the laser conditioners in free space at the location of the optical detection assembly, we observe output powers of 24.48 mW and 57.7 mW. The beam waists have a minimal width (FWHM) of 38.4 μm and 45.9 μm for the 405 nm and 450 nm lasers respectively. No ellipticity larger than 2.2 and opening angles less than 2.3 degrees are observed.

![Figure 2. Exploded drawing of the optical detection assembly that surrounds the optofluidic chip.](image-url)
In figure 2 we discuss from bottom to top the buildup of the optical detection assembly. The base plate features two cage guide trenches on its bottom side that allow it to be accurately mounted with respect to the laser diode assembly. The base plate’s top side contains 4 spring plungers. These spring plungers internally consist of a steel ball, partially protruding from the metal casing, suspended by a spring. When mounting the bottom assembly onto the base plate, these spring plungers will act as a counterforce to the weight of the assembly that lies above and to the down force exerted by turning the bolts. This allows for tipping and tilting and up to 2 mm of vertical translation (in case the tip/tilt angles equal 0 degrees) of the chip. The amount of vertical translation can be mechanically offset by adding offset spacers (1 mm, 2.5 mm, 5 mm) to the bottom offset assembly. The bottom assembly consists of a bottom plate and a top plate. In the bottom plate an interchangeable port cartridge is placed, containing threads for the selected microfluidic ports. This allows easy reconfiguration in case of using different fluidic ports in future chip designs. This port cartridge is clamped between the bottom and top plates of the bottom assembly. The top plate provides drains to guide any leaking fluid away from the fluidic ports towards the side of the assembly, avoiding contact with the bottom PMT. The latter is mounted using two clamps that are screwed to the underside of the bottom assembly by the bottom fluorescence filter rests on top of the bottom PMT and is positioned such that the edges of the fluorescence filter are at the same level as the top plate of the bottom assembly. Four flangeless fittings (Upchurch Scientific) are screwed into the port cartridge such that they protrude from the bottom assembly’s top plate. They serve both as fluidic interconnect and as alignment elements onto which the chip is plugged when mounted. Any sideways slipping of the chip is countermanded by the anti-slip pads provided on top of the bottom assembly.

The top plate is then mounted onto the intermix assembly by two M6 bolts. The bolts allow the top plate to exert pressure onto the chip, clamping it between the top plate and the bottom assembly. Both together immobilize the chip and ensure a leakage-free connection between the chip and the fluidics system. This fluidic connection was inspired by the work of Ocvirk [18]. This system is able to exert enough force such that bonding the layers of the chip becomes unnecessary and remains leakage free for pressures up to 20 bar. In a practical system a small amount of the excitation light is able to reach the contact surface between the two layers of the chip causing some scattering. Due to this non-permanent bonding, cleaning the inside of the chip becomes possible and can be done quickly and easily. The top plate is also equipped with an offset assembly that is slightly lower than the height of the chip. The top offset assembly differs from the bottom assembly by account of an aperture being present, allowing the excitation beam to reach the chip’s entry facet. The bottom assembly’s spring plungers are in contact with the top offset assembly and exert a counterforce to the down-force used to clamp the chip. This counterforce pushes the top plate back when the force of the clamping bolts is reduced. Into the top plate’s cavity we insert a filter tray containing the top fluorescence filter. The top PMT is mounted on top of its fluorescent filter, in a similar way as the bottom PMT, using two clamps that are screwed onto the top plate. The different parts of the optical detection assembly are manufactured out of 10 mm thick slabs of aluminium using a Datron M7 CNC milling machine.

4. Chip fabrication

We fabricate a brass master mold for the purpose of replication through hot embossing. The basic features of the brass mold are machined using a Datron M7 CNC machine. Additionally, the edges of the mold are beveled with a ball-ended tapered tool with a 6° angle and a 0.5 mm diameter for better demolding at the end of the hot embossing process. This also reduces the amount of stress in the replicated chip. The centers of the channels and facets that were tapered in the previous step are milled back to 90 degree orientation. This is done to ensure that the excitation beam propagates in a straight line through the chip and is not deviated by oblique PMMA interfaces. At this point in the process the surface roughness $R_s$ of the milled surfaces lies around 1 $\mu m$, measured using a non-contact surface profiler over an area of 600 $\mu m$ by 450 $\mu m$. In the final milling step, the top surface was pocketed with a 90% overlap value, resulting in a surface roughness $R_w$ with a value equal to 100 nm measured using a non-contact surface profiler over an area of 50 $\mu m$ by 50 $\mu m$. The higher surface roughness of the milled surfaces compared to the bottom and top surfaces causes the replicated chip to have frosted vertical surfaces (fluidic channel walls, input facet and output facet) and a transparent top and bottom. Suppose that the chip’s channels are filled with a fluid sample. The amount of scattering at the input and output facets will be much larger than at the individual walls of the fluidic channel. The reason for this is that difference between the indices of refraction of PMMA ($n = 1.505$ at 405 nm) and air ($n = 1$) is larger than that of PMMA and a fluid. To reduce the amount of scattering at the input and output facets, we use a Moore Nanotech 350FG ultra-precision diamond tooling machine to significantly lower their surface roughness, namely $R_s$ down to 15 nm (measured using a non-contact surface profiler over an area of 44 $\mu m$ by 58 $\mu m$). Even though scattering is less pronounced at the walls of the channel, multi-time reflections and scattering at the channel walls will increase the detection background. To further improve the performance of this chip in the future, the surface roughness of these channel walls may also be enhanced using ultra-precision diamond tooling to reduce scattering and lower the detection background. The fabricated master mold is shown in figure 3(a). Finally, the master mold is placed into a Jenoptik HEX04 hot embossing machine. The mold is heated to a temperature of 145 degrees celsius and then pressed into a 40 mm by 40 mm piece of PMMA with a thickness of 5 mm for 9 min. The embossed chip has a thickness of 4 mm and is shown in figure 3(b). By ensuring that the chip can be replicated by means of hot embossing we ensure that in a later stage low-cost mass manufacturability is guaranteed and that the chip can be fabricated in a wide variety of thermoplastic polymers such as cyclic olefin (co)polymers [19].
of this excitation light can be picked up by the four photodetectors present in this system (two for LIF, two for ABS) each with an individual optical filter fitting to its task. The output voltage of each detector is sent to a 4-channel programmable gain amplifier (PGA) whose gain factor can be controlled by the Labview program. The PGA is used to map the dynamic range of the detectors onto that of the CompactDAQ. The respective output voltages of the PGA are then sampled by the CompactDAQ device for further processing in Labview. The processed data will be displayed and logged onto the PC. This logged data will then be post-processed using MATLAB. From the same Labview program we are also able to completely control the fluidics system, enabling full automation of measurements. We use a CompactDAQ chassis in which we mount three modules: An analog output module, an analog input module and a relay module. Both the NI 9263 analog output module and the NI 9215 AI module have 4 channels, each of which have a maximum sampling rate of 100 kSamples/s/ch at a resolution of 16 bits over a voltage range between $-10V$ and $+10V$. The addition of the NI 9482 relay module allows us to control the injection valve (Rheodyne mx7900). The modules communicate through the chassis over universal serial bus (USB) to the PC. The two syringe pumps that drive the fluidic system are controlled over a universal synchronous receive/transmit (UART) connection. Additionally, this system is more cost-efficient to expand with more lasers and detectors due to the modular nature of NI’s CompactDAQ interfaces and the low cost of the other peripherals.

Now we will discuss how the data is organized in the Labview program’s memory. This will give the reader a better understanding of how the data is handled and processed. We generate an excitation array $W_E$ that will contain the time-multiplexed excitation signals for the laser drivers. Each row of $W_E$ corresponds to the physical input signal of a laser driver. Along its length the array can be split into segments, each segment corresponding to one multiplexing timeslot. Our system has two excitation lasers, so $W_E$ is a 2 by 2N array of doubles, with N being the length of each multiplexed segment expressed in number of samples. To achieve time multiplexing, only one row per segment is non-zero and contains the modulation signal of that segment’s corresponding laser driver. The excitation array $W_E$ is in this case segmented in two segments $S_{E1}$ and $S_{E2}$, each having its first and second rows respectively non-zero and containing the modulation signal of the first and second laser respectively.

$$W_E = \begin{bmatrix} S_{E1} & S_{E2} \\ E_{1,1} \cdots E_{1,N} & 0 \cdots 0 \\ 0 \cdots 0 & E_{2,N+1} \cdots E_{2,2N} \end{bmatrix}$$ (1)

With the non-zero samples $E_{j,n}$ equal to

$$E_{j,n} = \left[ \frac{A_j^{\max} - A_j^{\min}}{2} \cdot \sin \left( \frac{2\pi n \cdot f_m}{f_s} \right) \right] + \frac{A_j^{\max} + A_j^{\min}}{2}$$ (2)

with $j$ equal to the segment/laser number, $f_m$ the modulation frequency, $f_s$ the sampling frequency and $A_j^{\max}$ and $A_j^{\min}$
respective the maximum and minimum amplitude values for that segment’s sinusoidal modulation signal. For our system N equals 10,000 samples per segment, the modulation frequency \( f_m \) is equal to 1 kHz and the CompactDAQ’s sampling frequency is equal to 100,000 kHz. Thus, a single segment has a period of 100 \( \mu \)s.

The NI 9215 and NI 9263 analog in/out modules were selected because of their ability to work synchronously. This means that while we output each row of \( W_E \) to its respective analog output we are able to capture the measurement array \( W_M \) with the same sample clock. The measurement array \( W_M \) is similar to its excitation counterpart \( W_E \) in that it will have the same amount of segments with the same length N. However, as we are measuring the response of four analog inputs, one for each of the system’s photodetectors, \( W_M \) will have four rows, one for every photodetector in the system.

\[
W_M = \begin{bmatrix} \mathbf{S}_{M1} & \mathbf{S}_{M2} \\
M_{1,1} \cdots M_{1,N} & M_{1,N+1} \cdots M_{1,2N}
\end{bmatrix}
\]

\[
= \begin{bmatrix}
M_{1,1} & M_{1,2} \\
M_{2,1} & M_{2,2} \\
M_{3,1} & M_{3,2} \\
M_{4,1} & M_{4,2}
\end{bmatrix}
\] (3)

The first segment, \( \mathbf{S}_{M1} \), contains the measured responses to the first laser’s excitation, each row corresponding to the signal of one of four respective photodetectors. The second segment’s content is similar and contains the photodetector responses excited by the second laser. Thus \( W_M \) contains 8 timeseries \( M_{i,j} \), each with a length of N samples where i and j represent respectively the corresponding analog input \{1, 2, 3, 4\} and excitation laser \{1, 2\}.

At this point, the timeseries \( M_{i,j} \) have not been filtered to eliminate the influence of noise. We will eliminate this noise by performing a fast-Fourier transform (FFT) on each timeseries and retain the amplitude value calculated for the modulation frequency \( f_m \).

Typically, when performing the FFT of a sampled signal, the lack of synchronization between the sampling clock and the signal fundamental frequency is the main factor responsible for errors in the determination of the Fourier series’ components. In particular these errors are caused by the so-called ‘leakage error’ due to the spectral sidelobes of the windowing function that is applied when sampling. The total elimination of these errors can be attained if the sampling rate is synchronized to the signal fundamental frequency (i.e. so-called synchronous or coherent sampling) \[20, 21\]. The latter is the case in our system since our analog input and output modules share the same clock. For N equal to 10,000, \( f_m \) equal to 1 kHz and \( f_s \) equal to 10,000 kHz, a bandpass filter with a bandwidth of 10 Hz is obtained. Furthermore each segment contains 100 cycles of its excitation sinusoid, further improving the results of the FFT by averaging out the effect of random noise \[22\].

During the injection protocol, the FFT transform \( M_{i,j} \) is calculated and the amplitude at the modulation frequency \( f_m \) is stored as a timeseries. At the end of the injection protocol eight such timeseries are saved to a comma-separated-value (CSV) file for further processing in MATLAB.

6. Calibration

To determine how well our system works we perform two calibration experiments. The first experiment involves setting up a calibration curve for the detection of Coumarin 480 dissolved in ethanol. This allows direct comparison of our current system’s LIF/ABS performance to that of our previous work \[11, 12\]. The second experiment investigates the system’s sensitivity to turbidity. A calibration curve for turbidity
standards is setup and the correct operation of the scattering measurement is verified. The signal responses of both experiments were filtered using a Savitzky-Golay filter [23] and background rectification was applied [11, 12].

6.1. Coumarin 480 calibration curve

We measure the response of a series of samples of coumarin 480 (Exciton) dissolved in ethanol (Technisolv 99%). We start of by making 50 mL of stock solution with a concentration of 1 mM. From this stock solution we mix every subsequent sample, diluting by a factor of ten. As such we obtain 10 solutions with concentrations ranging from 1 mM down to 1 pM. These dilutions were executed in new glassware that was thoroughly cleaned using concentrated sodiumhydroxide + pentasodium triphosphate.

For this series of samples we will setup a calibration curve for ABS and LIF detection using 405 nm excitation. For each sample, the same injection protocol is being used, namely injecting a sample volume of 160 μL at a flow speed of 9 μL s⁻¹. This is 60 times faster than the flow speed used by Van Overmeire et al [24]. When compared to our previous work, this is about 4 times slower, but on the other hand also 2.5 times less sample volume is consumed [11, 12]. The response of coumarin 480–450 nm excitation is very limited and so we will only be using the 405 nm excitation laser for this experiment. For this purpose the top PMT and bottom PMT are both fitted with a 500 nm longpass filter and the photodetector for the absorbance detector is fitted with a 405 nm bandpass filter.

The function to which we will fit the obtained calibration curves is obtained from the Beer–Lambert law:

\[ A = -\log_{10}\left(\frac{S_b - S_x}{S_b}\right) = \varepsilon l C \quad (4) \]

with \( A \) the absorbance, \( S_b \) the background signal level when there is no absorption, \( S_x \) the signal amplitude, \( \varepsilon \) the molar absorptivity, \( l \) the interaction length and \( C \) the sample concentration. One can deduce that \( S_b = \varepsilon / (1 - \varepsilon / C) \). We can thus assume that it is possible to fit a calibration curve to the function \( y = a \left(1 - 10^{-bx}\right) \), assuming that the optical detector has a linear response curve. Also the LIF response can be fitted to the same function \( y \) as the fluorescence is proportional to the absorbance.

In the first experiment we configure the gain voltages of the PMTs such that the system can observe the signal response without overdriving their respective programmable gain amplifier (PGA) set to unity. The goal of this low-gain experiment is to see how well the system can measure small concentrations while still being able to measure large concentrations (1 mM) as well. In a second experiment, the gain voltage of the top PMT is increased to improve the performance of the detection of small concentrations. The goal of this high-gain experiment is to determine the empirical limit of detection. All measurements were executed using the previously described protocol in a temperature-controlled lab at 20 °C.

In figure 5 we have plotted the results of the low and high gain experiments. Every calibration curve also shows the signal level equal to 3.3 times the background noise \( \sigma_B \) as a horizontal line in the plot. When applying a fit to the calibration curves, it is the intersection with the aforementioned detection level of \( 3.3 \times \sigma_B \) that determines at which sample concentration lies the theoretical limit of detection. The background noise has a different value for each respective measurement channel.

The results of the low gain experiment are shown in the three rightmost curves and their respective fitted functions in figure 5. For each individual calibration curve a good fit to \( y = a \left(1 - 10^{-bx}\right) \) is obtained with \( R^2 \) values greater than 0.998. For the absorbance analysis we find that the empirical and theoretical LOD coincide at a value of 100 nM For the top and bottom PMT theoretical limits of detection of respectively 32.6 pM and 63.4 pM are found. The difference in performance between both PMTs is due to the bottom PMT being 1 mm farther removed from the fluidic channel. This is a consequence of the design of the optical detection assembly.

For the high gain experiment, with the top PMT having an increased gain voltage, we observe some unexpected behaviour in the obtained calibration curve. For concentrations lower than 1 nM we observe that the signal response level saturates towards a value of around 0.14 mV. This would suggest that the samples are somehow contaminated. However, a blank sample’s (only ethanol) response being around 35 μV, slightly over the detection limit of \( 3.3 \times \sigma_B = 27.5 \mu V \), rules out contamination of the ethanol in which the C480 is dissolved and contamination of the fluidic system outside of the chip. All samples were prepared in new glassware that was rinsed in a strong caustic solution. Measurements of the blank sample exhibit an average SNR that is larger than 3.3 and a relative standard deviation over 40%. This means that the
measurement itself is reliable and that we are observing small concentration fluctuations in the sample itself. Extrapolating this calibration curve yields values of 27 pM or 57 fM for the theoretical LOD, depending on which part of the curve that the extrapolation is performed. Concentrations larger than 10 pM can be reliably observed, so we define the empirical LOD to be equal to 10 pM. This system shows limits of detection that are 5 times smaller than those of our previous work [11, 12]. This factor applies to both the limits of detection for the LIF and ABS analysis.

### 6.2. Turbidity calibration curve

In this experiment we will calibrate our system’s response to several samples with a known turbidity value. We elect to use microsphere calibration standards as they are readily available and their use seems to involve less health risks when compared to the formazin calibration standards that are traditionally used.

For this experiment we use both the 405 nm and 450 nm laser diodes as excitation sources. The same photodetectors are used as in the coumarin 480 calibration experiment. The top and bottom PMTs for scattering detection are equipped with Thorlabs FB405-10 and FB450-10 bandpass filters respectively. The filters in front of the amplified silicon photodetectors for absorbance detection remain unchanged and are equipped with the same bandpass filters as their counterpart scattering detectors.

The calibration samples that we use are Fluka Polymer Bead Turbidity Calibration Standards obtained from Sigma Aldrich. The studied sample range consists of 1, 5, 10, 50, 100, 500, 1000 and 4000 nephelometric turbidity units (NTU) calibration standards. For each datapoint we perform the measurement at least six times as to be able to calculate the standard deviation [25]. For each measurement, the same injection protocol is respected, injecting a sample plug of 160 µL at 30 µL s⁻¹. The sample plug is propelled into the system by ultra pure water (Merck LichroSolv water for chromatography). The latter is chosen for its low turbidity, low fluorescence and low viscosity contrast with respect to the samples.

In figure 6 we show the results of this calibration experiment. The left hand graph shows the calibration curves for the absorption measurement, the right hand graph does the same for the scattering measurement. For all curves we observe that there is a clear correlation between the observed signal level and the sample turbidity. Furthermore, this relation has a clear inverse function, with exception of the 4000 NTU scattering datapoint for 405 nm excitation. The latter is due to saturation of the PMT. Both the absorption and scattering graphs also show the LOD level for each detector equal to 3.3 times the background noise σₐ. The calibration curve is reliable as long as it remains above the LOD. We observe that for 1 NTU most curves retain a good margin with respect to the LOD level, the only exception is the absorption measurement excited by 405 nm light. When looking at these data we ascertain that scattering detection with a 405 nm excitation wavelength λ overall yields the smallest value for the relative standard deviation (RSD).

![Figure 6. Turbidity calibration curve.](image)

We can conclude that correlation between the detected signal levels and sample turbidity has been demonstrated. Furthermore the demonstrated range of measurable turbidities is adequate for most applications. Due to the margin with respect to the LOD for the lowest measured turbidity value, 1 NTU, we assume that the measurement range is even wider than was observed. Unfortunately no polymer bead standards with lower turbidity values were available to confirm this empirically. Finally, it is worth mentioning that Ciacccheri et al showed that scattering detection using two wavelengths at different angles can differentiate between suspensions with different particle sizes [26]. Given how our system observes light at 90° and 180° angles it may be able to perform a similar differentiation in the future between different particle sizes.

### 7. Proof-of-concept demonstration

So far, the conducted experiments had a small difference in the viscosity of the sample and the solvent. This good matching of viscosities between the sample and solvent results in signal envelopes that show little or no perturbations unrelated to the absorbance, fluorescence or turbidity of the sample under test. In industrial applications however, such good matching can not so readily be assumed. Either the sample is propelled by a solvent with mismatching viscosity or either, in the case of a continuous measurement, inhomogeneities within the sample will cause gradients in the viscosity. This means that also gradients in the refractive index will be present as the viscosity of liquids is linearly related to the refractive index [27, 28]. The viscosity contrast between the sample plug and the solvent will have viscous fingering as a consequence [29].

Viscous fingering depends on the size of the channel and the flow speed. In an explorative experiment we study this
Figure 7. Explorative experiment with ethanol and sewing machine oil aimed to observe the influence of injection flow speed on the presence of viscous fingering. Signal responses for ABS detection at 450 nm for different flow speeds.

behaviour by varying the flow rate with which we inject a sample plug of Kroon sewing machine oil into our system, using ethanol as a solvent. Sewing machine oil has a higher viscosity than ethanol, so viscous fingering is likely to occur at some point. In figure 7 we show the signal responses for ABS detection at 450 nm for different flow speeds (0.30 μL s⁻¹, 0.75 μL s⁻¹, 1.5 μL s⁻¹ and 3.0 μL s⁻¹). For slower flow speeds (0.30 μL s⁻¹ and 0.75 μL s⁻¹), the sample plug retains a clean interface with the solvent. The absorbance response exhibits two times five steps in its envelope. This is a result of five folds being present in the chip’s fluidic channel. Each step is neighboured by positive spikes that occur when the oil-ethanol interface crosses the excitation beam. When an viscous finger (or an air bubble) is present within the channel its interface with the solvent will in most cases not be perfectly perpendicular and often curved. When this interface crosses the excitation beam as it is pushed through the chip the excitation beam is refracted as a result of the non-perpendicular sample-solvent interface. This affects the excitation beam’s direction and divergence. Any change in the direction and divergence will have an effect on the intensity that is observed at the ABS detectors. The amount with which this happens depends on the refractive indices of the air bubble or viscous finger, the fluid surrounding it and the PMMA of the substrate. Furthermore, the position of a viscous finger with respect to the excitation beam will determine how strongly the direction of the excitation beam is affected. As the flow speed is increased, the presence of viscous fingering increases up until the point that the sample plug will exist out of nothing else but viscous fingers. In this case, the sample-solvent interfaces of the many viscous fingers in the fluidic channel will cause many spikes in the signal response, making it noisy. Despite the breakup of the sample plug by viscous fingering we do observe that the experiment’s behaviour is repeatable. The fluorescence and scattering measurements are also affected by viscous fingering as its effects perturb the stable excitation beam on which they depend.

We thus conclude that in general a sample’s signal response will not only depend on the sample’s absorptive, fluorescent and scattering properties, but also on its refractive index and its viscosity. It is very interesting that information on a samples’ refractive index and viscosity can be gained through our optofluidic chip. Unfortunately, as a consequence the shape of the signal response has become more complicated. This makes the detection of the response’s amplitude more challenging and possibly also less significant. Measuring samples at a low flow rate may prove a solution to viscous fingering. However, this flow speed is unacceptably low for industrial applications. In the next section we will present how we deal with the undesired effect that viscous fingering has on the signal envelope at higher flow rates.

7.1. Predicting lubricant oil quality parameters

We studied 10 lubricant oils from the same collection that was previously used by Mignani et al [1]. These lubricants were sampled from regular gas turbines (TG), aeronautic gas turbines (TGAD) and steam turbines (TV). Lubricant oils for aeronautic gas turbines are of the polyester-synthetic kind, whereas those for regular gas turbines and steam turbines are mineral-paraffin oils. The samples belong to various brands, such as Mobil, AGIP, Chevron, Shell, and Castrol. All oils have been previously analyzed by the MECOIL laboratory using conventional techniques [1]. For each measurement the same injection protocol is respected, injecting a sample plug of 70 μL at 9 μL s⁻¹. The samples are propelled by analytical grade ethanol (Merck TechniSolv Ethanol 99.9%). The system’s top PMT is equipped with a 500 nm longpass filter (Thorlabs FEL0500) for fluorescence. The bottom PMT is equipped with a FB405-10 to collect scattered light. The absorbance detectors are preceded by Thorlabs FB405-10 and FB450-10 bandpass filters respectively.

To counter the effects of viscous fingering on the signal envelope, we will no longer study the envelope’s amplitude but rather look at its frequency spectrum. Indeed, we calculate a fast Fourier transform (FFT) for each signal response. Next we combine these FFT’s of each measurement channel into a single dataset associated with an individual measurement of the given sample.

As a next step, we now predict the values of quality parameters that are specific to each kind of sample. These quality parameters have been obtained from external measurements using classical methods. To perform such a prediction we create several predictive models using partial least squares
Table 2. Overview of PLS regression models obtained for lubricant oil samples (♣: no samples removed from set, ♦: 4 outliers removed from set, ♣: samples with zero Y-value removed from set. ♠: TGAD oils removed from set).

<table>
<thead>
<tr>
<th>Y-Parameter</th>
<th>Range</th>
<th>RMSE</th>
<th>$R^2$</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Calcium</td>
<td>0–12</td>
<td>0.716</td>
<td>0.959</td>
<td>5</td>
</tr>
<tr>
<td>(2) Calcium</td>
<td>0–12</td>
<td>0.421</td>
<td>0.990</td>
<td>4</td>
</tr>
<tr>
<td>(3) Gravimetry</td>
<td>0.2–4.6</td>
<td>0.904</td>
<td>0.686</td>
<td>7</td>
</tr>
<tr>
<td>(4) ISO 4406 R₁</td>
<td>14–19</td>
<td>0.954</td>
<td>0.688</td>
<td>7</td>
</tr>
<tr>
<td>(5) ISO 4406 R₆</td>
<td>13–18</td>
<td>0.954</td>
<td>0.688</td>
<td>7</td>
</tr>
<tr>
<td>(6) ISO 4406 R₁₄</td>
<td>10–15</td>
<td>0.866</td>
<td>0.732</td>
<td>7</td>
</tr>
<tr>
<td>(7) JOAP</td>
<td>8–110</td>
<td>6.565</td>
<td>0.962</td>
<td>4</td>
</tr>
<tr>
<td>(8) JOAP</td>
<td>8–13</td>
<td>0.367</td>
<td>0.923</td>
<td>4</td>
</tr>
<tr>
<td>(9) NAS 1638</td>
<td>5–10</td>
<td>0.854</td>
<td>0.722</td>
<td>7</td>
</tr>
<tr>
<td>(10) Oxidation</td>
<td>0–0.18</td>
<td>0.013</td>
<td>0.933</td>
<td>5</td>
</tr>
<tr>
<td>(11) Oxidation</td>
<td>0.05–0.18</td>
<td>0.006</td>
<td>0.978</td>
<td>5</td>
</tr>
<tr>
<td>(12) Phosphorus</td>
<td>0–5274</td>
<td>350</td>
<td>0.958</td>
<td>4</td>
</tr>
<tr>
<td>(13) Phosphorus</td>
<td>0–142</td>
<td>12.4</td>
<td>0.942</td>
<td>5</td>
</tr>
<tr>
<td>(14) Phosphorus</td>
<td>1–142</td>
<td>6.69</td>
<td>0.987</td>
<td>3</td>
</tr>
<tr>
<td>(15) TAN</td>
<td>0.14–0.68</td>
<td>0.0626</td>
<td>0.852</td>
<td>7</td>
</tr>
<tr>
<td>(16) Viscosity at 40 °C</td>
<td>26–45</td>
<td>2.52</td>
<td>0.870</td>
<td>4</td>
</tr>
<tr>
<td>(17) Water</td>
<td>20–282</td>
<td>19.8</td>
<td>0.942</td>
<td>4</td>
</tr>
</tbody>
</table>

(PLS) regression. In this regression, the experimental FFT data will serve as X parameters and the quality parameters serve as Y parameters. Full cross validation is performed for every model to verify the model’s robustness.

For lubricant oils, we will predict several different quality parameters [1, 30–32]. In table 2 we show the range, root mean square error (RMSE), the coefficient of determination $R^2$ and number of factors $F$ of every predictive model obtained through PLS regression. Every model is labeled with a number (1–17) which we will use to refer to the respective model during the analysis of the results.

In the following discussion we judge the obtained models based on their $R^2$, RMSE (with respect to the model’s range) and number of factors. We divide the obtained models into three categories: good, acceptable and poor. Good models are usable for predicting the quality parameters of a sample. Acceptable models can also be used for the prediction of quality parameters, however, the accuracy of their predictions will be less than those of good models. Poor models have insufficient accuracy for any decent prediction, they can however still be used to discern between ‘high’ and ‘low’ values.

We have identified four clear outliers in the sample set. These have a significant influence on the model while having no calibration value. These outliers have been removed from the sample set to the benefit of correct calibration of our regression models. The PLS models concerning contaminants and anti-wear additives (calcium, phosphorus and water) show good fits. TGAD synthetic oils typically show a high amount of phosphorus content whereas TG and MG mineral oils do not. In the first phosphorus model (12) we see an interpolation between these two groups. We remove the TGAD oils from the model and confirm that a good fit is maintained (13). Some samples exhibit values equal to zero for calcium and phosphorus. This means that they are not detectable by conventional assays. However, these samples may differ due to other properties. In this case the calibration point with an Y-value of zero will degenerate the quality of the regression model. This is confirmed by the increase in the goodness of the fit when these samples are removed from the model (2, 14). Models that relate to undissolved particles present in the sample, such as NAS 1638 (9), ISO 4406 (4, 5, 6) and gravimetry (3), exhibit poor fits and can only be used for high-low differentiation. This poor performance can probably be explained by the non-linearity of the codes by which they are classified [30]. Models for physico-chemical properties such as JOAP anti-wear index (7), oxidation (10), Total Acidity Number (TAN, 15) and viscosity (16) show acceptable to good fits. For JOAP the difference between synthetic and mineral oil is investigated as it was for phosphorus. The model’s goodness of fit slightly decreases but remains good as TGAD oils are removed (8). For oxidation, we remove the zero samples to the benefit of the model (11).

We have thus assessed the quality different models for prediction of ten quality parameters of lubricant oils, some with a few different submodels available. For these ten quality parameters, five obtain good results, two obtain acceptable results and three exhibit poor results. When we compare our work to that of Mignani et al [1] we conclude that, in addition to predicting water content, JOAP, TAN and phosphorus content we are able to predict viscosity, oxidation and calcium content. Also, high-low differentiation for undissolved particle content can be achieved through the models for NAS, ISO4406 and gravimetry. The models for JOAP, TAN and phosphorus we obtain similar results as those of Mignani et al. For the prediction of water content our model performs clearly better. However, we have only studied a set of 10 lubricant oil samples whereas Mignani et al studied a larger collection of 29 lubricant oil samples.

8. Conclusion

We have shown a detection system that is capable of simultaneous ABS, LIF and scattering measurements excited by two time-multiplexed laser sources at 405 nm and 450 nm. The system consists out of a polymer chip that is mass manufacturable through a master mold that is machined in brass by milling and ultra-precision diamond tooling and replicated by hot-embossing. The chip is mounted in an optical detection assembly that, at the same time, aligns the chip to the rest of the system, seals the chip from leakage, fixes the chip’s position and connects the onboard channels to the rest of the fluidic system. This plug and play fluidic connection in combination with a mechanical system for sealing the chip remains leakage free up to 20 bar. For other applications, such as the monitoring of brake-line fluids, that require a chip to withstand pressures up to 100 bar, a wide range of bonding techniques and fixed fluidic interconnects are available to accommodate such pressure levels [13] in case plug and play functionality is not sufficient. The ability to clean the chip internally can prolong the life of prototypes.
The chip’s serpentine channel layout results in measurements with a reduced susceptibility to perturbations caused by air bubbles in the channels. The non-absorbed light is detected by two amplified silicon photodetectors, one for each excitation wavelength. Fluorescent light and scattering are detected through orthogonally mounted photomultiplier tubes placed behind an optical longpass and bandpass filter respectively. It is shown that the laser excitation wavelengths and optical filters are selected based on an earlier spectroscopic study. Besides filters, no optical elements are used. We demonstrate that in the case of an interaction length of 5 mm, an opticless approach is valid. This approach may also be scaled to smaller optofluidic devices and detectors. A low-cost and easy to expand approach for data acquisition and FFT-based noise reduction is demonstrated. Low-cost basic laser drivers and programmable gain amplifiers are used. We demonstrate that state-of-the-art results can be achieved using basic peripherals.

For coumarin 480, detection limits of 100 nM and 10 pM are observed for ABS and LIF respectively. These limits of detection are five times smaller than the state-of-the-art [11, 12] while 2.5 times less sample volume is used. The flow speeds for these measurements are 4 times slower, but still 60 times faster than in the work of Van Overmeire et al. Furthermore the chip’s internal volume has been decreased from 250 μL to ca. 120 μL. For the detection of turbidity/scattered light we show an effective detection range of 4000 NTU down to 1 NTU. We demonstrate the applicability of this system in an industrial context with the prediction of lubricant oil quality parameters. In this application, viscous fingering can cause significant perturbation of the measurement envelope. This is countered by a secondary FFT processing step, turning the effects of viscous fingering into a source of information. The FFT data are then used for as X-values for PLS regression. We demonstrate 17 PLS regression models for the prediction of 10 quality parameters specific to lubricant oils.

We should remark that before applying this system, a preliminary spectroscopic study is required to determine suitable excitation wavelengths and optical filters. Furthermore it is required that there are commercial laser diodes available near the selected wavelengths. This may not be the case for every possible application. Currently the system is demonstrated as a flow-cytometric system with automated sampling through an automated injection valve, a sample loop and two syringe pumps. In this implementation the sample under test cannot be recovered due to the solvent that is used to push the sample into the system. In the case of online monitoring, the viscosity contrasts inside the channels will be smaller by having a continuous body of sample rather than a sample plug of a known volume with a solvent surrounding it. In this respect, less information is gained on the viscosity contrast. On the other hand, if a viscosity contrast is detected in the case of online monitoring of lubricant oils, this may be an indicator of incompatible lubricants being mixed, the presence of foreign liquids such as water or the formation of emulsification. In future work it is worth investigating how our system will behave in such an in situ online measurement installment. In an application where monitoring of the viscosity is a requirement, an automated sampling system like the one in this paper can be used with new lubricant oil as a solvent. This way, performing an automatically scheduled measurement also injects some fresh lubricant as proactive maintenance.

Regardless of these drawbacks, we see direct additional applicability of this measurement system to the work that Mignani et al have performed on the detection of hogwash oil in Chinese soybean oils [33] using excitation lasers with 520 nm and 680 nm wavelengths. It is also worth investigating the identification of the production region of single-malt Scotch whiskies [34] using excitation lasers with 405 and 1450 nm wavelengths. We believe that for many applications the sensitivity of PMT’s may not be a strict requirement to achieve good performance. We have made use of PMT’s in our setup as this allows a direct comparison with the limit of detection of coumarin 480 reported in our previous work [11, 12]. To reduce the cost of the multi-measurement system presented in this paper we deem it likely that these PMT’s can be replaced by silicon photodiodes that have a photosensitive surface with a similar diameter. In the absorption path, we use a beamsplitter and two optical detectors, each preceded by an individual bandpass filter. This configuration was chose to prevent fluorescent light from reaching these detectors when measuring the non-absorbed light at the 405 nm and 450 nm excitation wavelengths. We believe that the influence of fluorescence will be minimal when the aforementioned bandpass filters are removed. To further reduce the cost, it may be investigated if the same predictive performance, shown in this paper, may be achieved by using only a single absorption detector without a preceding optical filter. This saves the cost of a beamsplitter, a photodetector and two optical filters.

We conclude that the multi-measurement system presented in this paper is able to compete with spectroscopic detection techniques with adequate sensitivity. Furthermore we have succeeded in predicting more quality parameters than in previous work [1]. When targeting specific applications such as the on-line monitoring of quality parameters of lubricant oils this system can offer direct added value. This proof-of-concept demonstrator is a combination of many small novelties that may individually or collectively help to pave the road towards the widespread use of optofluidic systems.

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