Fluorescence of surface adsorbed dyes: Investigation of a new detector for molecule interferometry

Fabienne Goldfarb, Sarayut Deachapunya, André Stefanov, Alexander Stibor, Elisabeth Reiger and Markus Arndt
Institut für Experimentalphysik, Boltzmanngasse 5, 1090 Wien, Austria
E-mail: markus.arndt@univie.ac.at

Abstract. Matter-wave interferometry with large molecules is partially limited by the inefficiency of the commonly used ionization detection schemes. Here we discuss the possibility of employing a surface imaging method in which molecular interferograms with periods in the micron range are adsorbed on a surface and subsequently read using fluorescence microscopy. Our preliminary experiments show that the detection efficiency can be significantly increased compared to electron impact ionization and quadrupole mass spectrometry. However the interpretation of fluorescent molecular patterns requires a good understanding and control of the molecular mobility, the bleaching behavior, and the coverage dependence of the fluorescence intensity.

1. Introduction
Since the first demonstration of the quantum wave nature of matter [1], matter interferometry has been performed with various particles, including neutrons [2], atoms [3], small molecules [4], cold He clusters [5], ultra-cold Bose-Einstein condensates [6] and more recently also hot and large molecules [7, 8].

Intense sources for large, slow, neutral molecules are still under development, and present-day interference experiments have to face the challenge of increasing both the particle flux and the detection efficiency in order to optimize the experimental signal-to-noise ratio.

Our macromolecule experiments up to date have been based on effusive beams in combination with either continuous wave (cw) laser ionization [9] or electron impact ionization [10]. For very stable molecules such as the fullerenes, cw-laser ionization is a highly efficient (up to a few percent) and molecule-selective technique. However, it cannot be applied to large biomolecules which would rather change their structure or even break into neutral pieces, when they absorb a sufficient number of photons. For instance, in our own experiments with the biodye tetraphenylporphyrin (TPP) no ions could be observed under irradiation with a 15 W Ar laser (multiline visible), although the optical absorption cross section is expected to be comparable to that of the fullerenes. A higher ionization energy – provided by the impact of fast (50–70 eV) electrons – overcomes this limitation and enables interferometry with TPP. But already for C_{60}F_{48}, at present the most complex and most massive particle in matter-wave experiments, a detection efficiency of the order of 10^{-4} is a severe limitation. This strongly suggests that new means have to be found if we want to explore the wave-particle duality of objects with masses beyond 10,000 amu.
Figure 1. Concept of direct surface recording of a molecular interference patterns in a Talbot-Lau setup. An adsorbing substrate is placed in the distance $L$ to the second grating, where $L$ also represents the distance between the two gratings.

Compared to earlier far-field diffraction experiments with fullerenes, improvements have been obtained along two routes: The first one is related to an interferometer concept, which accepts an uncollimated molecular beam and therefore allows a much higher throughput and signal. This Talbot-Lau (TL) interferometer, based on a suggestion by John Clauser [11] and adapted in Vienna to the needs of large molecules [12, 13] was first demonstrated for fullerenes [8]. It proved to be also the essential element for the successful observation of the wave-nature of TPP and $C_{60}F_{48}$ as well as for subsequent decoherence investigations [14, 15].

Up to now the Talbot-Lau interferometer was always composed of three identical gratings, which were separated at equal distances: the first grating prepared the required transverse coherence, the second one probed the quantum nature (caused diffraction) and the third one scanned the molecular interference pattern. As more than 1,000 grating lines (with a period of nominally 990 nm) are simultaneously illuminated by the full molecular beam, the Talbot-Lau setup offers an enormous gain. A molecular beam divergence of a few mrad is still acceptable whereas in far-field diffraction the divergence had to be limited to a few microradians. The TL-design permits to achieve a gain in transmission and count rate of the order of 10,000 compared to the far-field experiments.

Another experimental improvement is at the focus of the present article. When ionization efficiencies drop to values as small as $10^{-4}$...$10^{-5}$ a large factor may still be gained if we recur to better detection methods. One could imagine various properties and mechanisms, which scale favorably with the increasing mass, size and complexity of the interfering particles:

First, the mass itself increases and beyond a certain limit it will be interesting to explore bolometric [16] or weighing devices [17]. Second, as the size increases the detection by topographical scanning methods (STM, AFM) is facilitated. And third, we finds a richer variety of fluorescent particles or molecules that can be labeled with fluorescent markers, with growing size and functionality.
In an earlier paper [18] some of us discussed already the possibility of detecting dye molecules in free flight. Although this is in principle a valid concept, the molecular beam densities in interferometry limit the applicability of this method. One further limiting reason was the short interaction time between the exciting light and the passing molecules. For molecular beams at 200 m/s and a laser waist around 100 µm the interaction time is limited to below 1 µm, which allows only the emission of a few photons per molecule. In addition it is well known that the fluorescence of a large molecule shows a strong Stokes shift. This results in the deposition of about 0.1...0.3 eV per photon into the internal degrees of freedom of the absorbing molecule. The number of scattering events is therefore also limited by the onset of the thermal modification or even decomposition of the molecule.

A significant improvement can be obtained when the molecular beam is first adsorbed on a clean substrate. The observation time under a fluorescence microscope is then only limited by photo-induced bleaching processes. And also the energy relaxation to the surface then reduces the overheating of the molecules.

In the following we will discuss the gain, the challenges and the proposed solutions for direct surface recording of molecular density patterns. In this setup, which is shown figure 1, the third grating of the interferometer would be replaced by a surface, on which the molecules stick. We propose that under appropriate surface conditions interferograms can then be imaged by fluorescence microscopy, or later on by scanning probe microscopy (SPM) methods.

2. Velocity encoding on the surface
When operating a Talbot-Lau interferometer for the demonstration of the molecular wave-nature it is important to prove that the observed density pattern cannot be explained by a classical Moiré shadow image. Indeed, to first order the fringe visibility of a Moiré pattern does not depend on the molecular velocity – and it is still only weakly dependent even if we take into account molecule-wall interactions at the second grating. In contrast to that a genuine quantum interferogram shows a characteristic contrast modulation when the de Broglie wavelength is varied [12].

Therefore, a big advantage of our surface recording scheme is that a single acquisition simultaneously stores interferograms for all arriving molecular velocity classes [19]. In all previous detection schemes they had to be registered one after the other. Already this simple fact increases the detection efficiency by an order of magnitude compared to the integrating ionization schemes. In particular with rare materials and weak sources this advantage is expected to become important.

The molecular trajectories in the Earth’s gravitational field follow free-flight parabolae, which are each determined by three points. The first slit is the oven aperture (200 µm), the second is a 150 µm slit placed 1.2 m away from the oven, and the third one is given by the height of the detection zone on the surface another 0.8 m downstream. Remark, that the detector height can also be chosen a posteriori, when the deposition experiment has long been finished. The arriving molecules are therefore vertically sorted on the plate, with the slowest particles arriving at the lowest end as quantitatively explained in figure 2. A molecule, leaving the oven at the height \( s_0 \) with an angle \( \alpha \) with respect to the vertical, passes the second selection slit at height \( s_1 \) and distance \( L_1 \) before it is detected at height \( s_2 \) and distance \( L_2 \). We assume that the initial velocity distribution of the molecular flux is described by a Boltzman distribution [20]:

\[
\frac{dN}{dt}(\alpha, v) \propto \frac{v^3}{v_w^3} e^{-\frac{\varepsilon w_0}{kT}} \sin^2 \alpha \cdot d\sigma \cdot dv \cdot d\alpha
\]

with \( v_w = \sqrt{2kT/m} \) the most probable speed in the distribution, and \( d\sigma \) the surface element in the oven aperture. The velocity distribution is then obtained by integrating over the angle \( \alpha \),
e.g. by evaluating the quantity $\left[\alpha/2 - \sin(2\alpha)/4\right]$ between the limits determined by the width $\Delta s_i$ of the selecting slits and the detection area. These limits can be calculated by considering the parabola drawn by the molecule in the gravity field: $z = -\frac{1}{2}gt^2 + v_0\sin\alpha t + s_0$, where $v_0$ and $s_0$ are the initial velocity and vertical position of the molecule leaving the oven. We neglect the horizontal beam divergence and by adding the conditions given by the selection slit and the detection zone, we obtain:

$$\sin^2\alpha \sim \frac{(L_1^2 L_2 - L_3^2 L_1)^2}{(L_1^2 L_2 - L_3^2 L_1)^2 + (L_1^2 (s_2 - s_0) - L_3^2 (s_1 - s_0))^2},$$

(2)

and

$$v_0 \sim \sqrt{\frac{g (L_1^2 L_2 - L_3^2 L_1)^2 + (L_1^2 (s_2 - s_0) - L_3^2 (s_1 - s_0))^2}{2 (L_1^2 L_2 - L_3^2 L_1) (L_2 (s_1 - s_0) - L_1 (s_2 - s_0))}}.$$ 

(3)

The limits of the integral can be determined from these equations for each velocity. The result of this simulation is depicted in Fig. 3. Here, the oven slit was set to $z=0$ mm and the selection slit to $z=0.5$ mm. The effective detector height was set to $\Delta s_2 = 50 \mu$m. One recognizes that the slow molecules are much better velocity selected than the fast ones. A further reduction in the slit height results in an improved velocity selection but even more strongly in a loss of signal. In present experiments and for molecules at velocities around 100 m/s, a useful velocity is therefore $\Delta v/v \simeq 10\%$.

3. **Choice of the surface: molecular immobilization**

For most of the following test we used the molecule TPP as a reference. The patterns in present-day TL-interferometry have periods of the order of 1 $\mu$m and feature sizes as small as 500 nm and the expected fringe contrast is of the order of 30..50%. We are therefore looking for a surface on which the molecular mobility will not wash out the expected structure.
Figure 4. TPP deposited on graphite, mica and quartz (pictures (a), (b) and (c) respectively), covered with a mask. The small structures have a diameter of 2 µm and are supported by a 100 µm square grid.

Figure 5. TPP deposited on quartz. The picture was taken with a 1 µm grating mask over the surface. The big lines, separated by 50 µm, are the support structure.

We performed tests with different surfaces, among them graphite, mica and quartz. Graphite and mica are often used in scanning probe experiments, since they can be easily prepared in a very clean state. Mica can be cleaved using finger nails to prepare a fresh and pure surface. Highly oriented pyrolytic graphite (HOPG) can be ‘reset’ to an atomically clean state by lifting off the top surface using scotch tape. However, experiments with fullerenes on HOPG indicate that the molecular mobility on these surfaces is rather high [21].

A simple measure for the suitability of a certain molecule/surface combination for our interferometry is the possibility to discern clear edges in a shadow image of the molecular beam after passage through a tiny mesh in front of the substrate. The deposition is first done in high vacuum (10^{-7} mbar). The sample is then extracted from the chamber and studied in air using a Zeiss epi-fluorescence microscope sitting on a clean-air bench. The time between the deposition and the observation was a few hours. During that period molecules may already diffuse until they bind to edges or steps on the substrate or until they find molecular partners to which they
can bind. The details of this process depend on several factors, such as the molecular coverage of the surface, the substrate’s nano-roughness, the temperature, and various other boundary conditions.

Two different structures were used for contact imaging. A mesh for electron microscopy with $2 \mu m$ grid size was used in a first step. If this imaged molecular structure was not smeared out by diffusion, a 990 nm gold grating with 490 nm openings was employed to mimic the conditions in our Talbot-Lau interferometer. Figure 4 shows fluorescence images of tetraphenylporphyrin deposited through the $2 \mu m$ structure on (a) HOPG, (b) mica and (c) quartz. The deposition time in all three cases was, corresponding to an average coverage of about seven monolayers. This assumes that all molecules reaching the surface were also actually sticking to it.

It is obvious from the pictures, that TPP moves a lot on graphite. Large fluorescent agglomerates are covering the $2 \mu m$ mesh completely. Also Fig. 4 (b) shows a limited contrast only, as molecules have crossed the shadow regions. This can also be particularly well seen in the shadows cast by the support structure. Here, a ribbon of molecular agglomerates can be found across more than $4 \mu m$, which demonstrates that room temperature Mica is inappropriate for our proposed application.

In contrast to the previous two surfaces, quartz seems to immobilize porphyrins quite well: the rims of the support structure are black and sharp and the $2 \mu m$ mesh can be imaged with high contrast. Figure 5, the shadow image of an interference grating ($1 \mu m$ period) on quartz, shows that porphyrin molecules are trapped on a scale well below 500 nm. We observe a (classical) fringe visibility of 27% instead of the 70% as expected on the basis of the resolution function of the fluorescent microscope alone.

Silica is extensively used in studies of photochemistry of aromatic molecules [22]. On these materials molecules are frequently immobilized by siloxane and silanol bounds [23]. Moreover, several fluorescence studies with arenes on silica or quartz surfaces, underlined that these insulators may support a higher molecular fluorescence yield than metals or semiconductors [24, 25].

TPP is most efficiently excited in the blue (405...435 nm) where ordinary BK7 and related glass substrates would reduce the detection sensitivity because of a rather high auto-fluorescence. This is much suppressed in quartz and it can be further drastically reduced by a suitable chemical cleaning procedure (RCA-cleaning [26]) followed by bleaching the substrate for 30 min with a 15 W Argon ion laser expanded to a circle with a 1/e diameter of about one inch. Commercial quartz plates can also be easily obtained with a surface of $\lambda/10$ which permits high resolution optical imaging in a fluorescence microscope over a large area without the need of successive focus adjustments. Observations with atomic force microscopy in air [27] show that TPP molecules tend to form islands on the 200...400 nm scale, which do not drift any further, and thus permit still relatively sharp edges on the micron scale.

The immobilization and imaging quality for TPP on quartz is therefore sufficient for the recording of Talbot Lau interference fringes and the surface detection of molecular interference patterns is currently being investigated.

4. Photoinduced bleaching
It is well known, that molecules don’t cycle light as atoms do. After a certain number of scattering events a molecule may undergo a transition and stop fluorescing. Such a bleaching process can be reversible when the molecule falls into a dark triplet state, or irreversible when it is related to proton transfer, isomerisation, photo chemistry or photo-induced fragmentation. For the interpretation of molecular interferograms – which crucially depends on a correct assessment of ratios of fluorescence intensities – we studied bleaching curves of TPP on mica and quartz. As the results on mica showed anomalous bleaching, the same experiment was also repeated with rhodamine molecules. The deposition conditions were set to have about one molecular
monolayer arriving on the surface. For rhodamine, the vapor pressure is not known. We chose a three times longer deposition time than for TPP. The bleaching curves were recorded with a homogeneous coverage of the surface, i.e. without adding mechanical masks in front of the surface.

The two bleaching curves in Fig. 6 were obtained as follows: The mercury lamp was set to an intensity of about 4\(\mu W/\mu m^2\) at the sample surface and a time series of images was recorded collecting fluorescence from \(\sim 1400\mu m^2\).

The observed 'bleaching' behavior is obviously anomalous in that the TPP fluorescence on mica even increases in the first minute, before it then follows the expected decay. A second experiment at ten times weaker intensity shows an even more subtle phenomenon. Here the intensity first decreases significantly and linearly increase again after about 50 s, and for at least the following 1000 seconds.

This phenomenon only appears some hours after the deposition and is somewhat enhanced when the waiting time before the bleaching time is longer. This is strongly indicative of an
underlying diffusion process – which still has to be identified. The data of Fig. 6 were actually taken two days after the surface deposition.

The fluorescence decay curve of TPP on quartz and rhodamine on mica is as expected and shown in Fig. 7. The bleaching of TPP on quartz is well fitted by a biexponential decay curve. Poly-exponential bleaching curves were registered for one molecular structure in two different binding sites [24], and the presence of two different complexes on surface is known for inducing non single exponential decay laws [28]. The same experiment was repeated under several irradiation intensities and also for different molecular coverages of TPP on quartz. For relatively low coverages, the qualitative behavior remains similar, and ‘normal’ – which is important for the interpretation of fluorescence strengths for probing the relative number of molecules on a surface. When the number of molecules reaching the surface corresponds to approximately 15 monolayers, we saw a non biexponential decay, but never a fluorescence recovery such as the one on mica.

The absence of any recovery effect for rhodamine on mica or TPP on quartz supports the idea that specific molecule-surface interactions have to be considered for explaining the anomalous fluorescence of TPP on mica. One current hypothesis focuses on the presence of metals in mica (KAl$_2$(Al,Si$_3$)O$_{10}$(OH)$_2$, muscovite, SPI Inc.). The diffusive formation of complexes between the metal atoms and the molecules might be related to the observation. An additional spectroscopical investigation in comparison with the spectra of metalloporphyrins might shed more light on this phenomenon.

5. Conclusion

The direct detection of surface adsorbed molecules is promising for the parallel recording of molecular interferograms. For TPP our method will reduce the time for recording a set of velocity dependent interferograms by about an order of magnitude. With more specialized equipment – including low temperature probes and laser excitation – the detection of single molecules should be also feasible.

Even at room temperatures we can identify a surface material, which allows trapping and immobilizing porphyrin within 200–400 nm of where it hits the surface. This is sufficient for the recording of interferograms in a present-day Talbot-Lau setup.

For the imaging of even finer structures, we are currently investigating the improved binding of molecules on cryogenically cold surfaces, the preparation of reconstructed silicon surfaces such as Si 111 (7x7), or surfaces with chemically prepared nanopores.

We have seen that the molecule-surface interaction has an important influence on the proposed application: the mobility, the fluorescence yield as well as the photo-bleaching behavior depend on the surface chemistry and a detailed knowledge of these parameters is important for the interpretation of the molecular fluorescence pictures and requires further investigations.

The good detectability of porphyrin on quartz is a good start and promises even better suitability of a fluorescence method for experiments with dyes molecules, fluorescent proteins (GFP), or also fluorescent semiconductor nanocrystals. They are known, not only to have a higher mass, but also to exhibit a higher absorption cross section, better fluorescence yield and smaller photo-bleaching rates. In that sense, fluorescence detection of surface adsorbed fluorophores is scalable.

Surface adsorption of molecular patterns offers the advantage of parallel recording of many positions and velocity classes at the same time, and may image interference patterns with periods that are smaller than the period of the material grating masks. This opens interesting perspectives for molecular nanolithography. And one can imagine a variety of schemes that will allow to create more sophisticated molecular patterns on surfaces than simple lines.
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