1. Introduction

The detection of single molecules in a solid at cryogenic temperature by absorption in 1989 [1] and by fluorescence in 1990 [2] opened the wide field of single-molecule spectroscopy. The detection of only a single molecule at a time allowed for the first time measurements of not only the mean but also the distribution of the observable of interest (e.g. orientation or line-width), which was obscured in ensemble spectroscopy. Typically, the concentration of single fluorescent guest molecules is extremely low compared to the concentration of host molecules, such that they can be considered impurities in the host matrix. The high sensitivity of the molecular zero-phonon line towards changes in the local environment of the fluorescent guest molecules can be exploited to probe the structure and low temperature dynamics of the matrix materials (for reviews see [3–8]). To probe the structure of matrix materials, the orientation of single molecules is measured as a function of spectral and spatial position. Common matrices for such studies are, e.g., Shpol'skii systems or polymers. Dynamics of matrix materials are often observed in spectral instabilities of the guest molecule in the host environment, leading to discontinuous jumps in the resonance frequency of a guest molecule. Such studies could demonstrate for the first time the existence of tunneling two-level systems.

In highly ordered materials, like single crystals, the distribution of spectroscopic parameters due to environmental influences can be minimised. As a consequence, single molecules at cryogenic temperatures behave like nearly ideal two-level quantum systems that are rigidly fixed in space. The latter feature makes them attractive model systems for quantum optical experiments (for reviews see [3, 4, 9]).

The first observation of single molecules at room temperature followed later, using aperture scanning near-field optical microscopy [10] with near-field excitation and far-field detection. The scanning near-field optical microscope was invented in 1984 [11] after the scanning tunnelling microscope in 1982 [12] and followed by the atomic force microscope in 1987 [13, 14]. The best resolution claimed with scanning near-field optical microscopy using aperture probes is on the order of ~ 10 nm [15], which is far beyond the diffraction limit, but not as good as the atomic resolution obtained in scanning tunnelling microscopy and atomic force microscopy [12, 14]. However, fundamental differences between the applicability of these techniques exist, namely, the scanning tunnelling microscope is limited to conducting samples, whereas the atomic force microscope measures the total forces between a sample and a cantilever tip. The aperture scanning near-field optical microscope on the other

hand is used to excite fluorescent molecules or particles close to (or at) the sample surface. The fluorescence or scattered light from these molecules or particles gives information on the chemical composition of the sample.

The aim to achieve highest possible optical resolution triggered further development of the scanning near-field optical microscope, leading to near-field single-molecule spectroscopy at cryogenic temperature in 1994 [16]. Unfortunately, no imaging of single molecules was achieved. Another interesting point would have been a direct comparison of near-field optical spectroscopy to confocal spectroscopy on the same spatial position of the sample to demonstrate directly the increase of resolution due to a decrease in excitation volume.

However, at low temperature, most of the experiments are still performed using far-field optical techniques. Unanswered fundamental questions that could be addressed with single-molecule spectroscopy at low temperature often don't require the use of a scanning near-field optical microscope. Besides, the use of scanning near-field optical microscopy at low temperature seems to be technically problematic. The experiments that are described in this thesis deal with saturation effects in the images of single molecules, single-molecule detection by absorption and scanning near-field optical microscopy and spectroscopy of single molecules at 1.8 K.

Chapter 2 introduces the concepts of single-molecule spectroscopy at low temperature needed to understand the experiments that are described in this thesis. The energy-level scheme, the methods to get down to the single-molecule level, the requirements for the molecule-matrix system, the absorption cross-section of a single molecule and the saturation properties are explained. It also points out the differences between fluorescence excitation and absorption spectroscopy. After this theoretical part, the most important properties of the experimental techniques used in the experiments described in this thesis, confocal microscopy and aperture scanning near-field optical microscopy, are discussed.

The experimental apparatus is presented in chapter 3. It starts out with an overview of the whole set up and is followed by more detailed information on some separate parts of the set up. The last part of this chapter summarises the sample preparation of the two different molecule-matrix systems that were used in the experiments: terrylene in *p*-terphenyl and terrylene in a stretched film of linear low-density polyethylene.

The accuracy with which positions of single molecules can be determined in three dimensions is much better than the diffraction limit, as was shown at low temperatures in Ref. [17–20]. Position determination accuracy in two dimensions is applied in many experiments at room temperature like in biophysics [21, 22]. Contrary to position determination accuracy, little attention has been paid to the spot size of a single molecule. The spot size of a single molecule is studied in chapter 4. The experiments deal with the imaged spot size of a single molecule at low temperature as a function of excitation intensity and excitation frequency, respectively. To check the validity of the results, Monte Carlo simulations were performed. The measured spot size as a function of excitation intensity sheds light on the influences of saturation on optical imaging. A strong increase in the spot size was found. The same holds for the measured spot sizes of single molecules under excitation intensities below the saturation level. It turns out that even at intensities below saturation, the effects of saturation are already visible in a series of images of the same molecule while tuning the excitation frequency through the absorption line of the molecule. This also means that by imaging a molecule in resonance and out of resonance, eventual saturation effects can be observed immediately in the difference in spot size. Even from only one image of a single molecule, it would be possible to determine the saturation intensity and maximum emission rate by fitting with the appropriate function, which takes into account the deviations from a Gaussian spot as a function of intensity.

The main experiment of the work in this thesis, single molecule detection by absorption, is described in chapter 5. It starts with the desired properties of the "ideal" sample for single molecule detection by absorption, followed by the characterisation of the new sample for low-temperature spectroscopy: terrylene in a stretched film of linear low-density polyethylene. After that, simultaneous detection of single molecules by fluorescence and absorption is presented. Absorption spectroscopy makes use of the interference between the light scattered resonantly by the molecule and a weak reflection of laser light from the sample surface. Absorption signals thus show a dispersive behaviour. The simultaneously recorded fluorescence excitation spectra on the other hand provide direct information on the spectral position of the resonance frequency and line width of a single molecule. Dynamical processes like blinking and spectral jumping of single molecules are observed in both detection channels. In particular, molecules are observed that do not emit detectable Stokes-shifted fluorescence but show a strong absorption signal. The "noisy" appearance

of the absorption spectra is caused by the dynamics of single molecules and should not be misinterpreted as system noise. Nevertheless the signal-to-background ratio in absorption spectra is rather poor, compared to the simultaneously recorded fluorescence excitation spectra. A method to improve the signal-to-noise ratio obtained in the absorption experiment could be to go to near-field excitation. However, aperture scanning near-field optical microscopy is not yet a well-established technique at temperatures of $\sim$ 2 K. Chapter 6 explores aperture scanning near-field optical microscopy and spectroscopy on single molecules at cryogenic temperature. Near-field imaging of single molecules in fluorescence at low temperature is demonstrated for the first time as well as a direct comparison between near-field and confocal fluorescence excitation spectra of single molecules. Differences between these spectra are explained in terms of orientation and position with respect to the aperture.

Finally, possible future experiments following the line of the experiments described in this thesis are presented in the outlook.

2. Fundamentals of single-molecule spectroscopy

The first part of this chapter describes the basic properties of single-molecule spectroscopy at low temperature. It explains the energy-level diagram, the methods that are used to detect single molecules, the requirements for the molecule-matrix system and the effect of saturation. It provides a background for the experiments that are described in this thesis.

The second part focuses on the experimental techniques that were used to study single molecules at cryogenic temperature: the confocal microscope and the aperture scanning near-field optical microscope. The basic principles are described with an emphasis on the differences between the techniques.

2.1 Single molecules at cryogenic temperature

2.1.1 Physical principles

The energy-level scheme of a single molecule embedded in a matrix at cryogenic temperature is depicted as a three-level system consisting of the (electronic) singlet ground state S_0, the first (electronic) singlet excited state S_1 and a triplet state T_1. Additionally, vibrational states are on top of all these levels. Various transitions are possible between these states, as is shown in the so-called Jablonski diagram in Fig. 2.1. Radiative transitions are represented by solid arrows, while dashed arrows indicate non-radiative transitions. Excitations can involve electronic, vibrational and phonon states. In most low-temperature experiments, single molecules are excited resonantly from the lowest vibrational level of the singlet ground state S_0 into the lowest vibrational level of the first singlet excited state S_1. This transition is often referred to as the 0-0 transition and is a purely electronic zero-phonon line [23]. In the case of impurity molecules in crystals at ~ 2 K, the line width of this line is close to the life-time limited value, as temperature induced processes are frozen out. Zero-phonon absorption lines are excitations not involving phonons. Absorptions involving phonons are blue-shifted with respect to the zero-phonon line and build up the broad phonon wing of the line. Zero-phonon lines also exist between the singlet ground state and the vibrational levels of the first singlet excited state. After absorption into one of the vibrational levels of the S_1 state, rapid decay (via phonons) will occur to the lowest vibrational level of the S_1 state, from which radiative decay to the ground state can take place (Kasha's rule). Typically the zero-phonon line of vibrational sidelines is three orders of magnitude broader than the zero-phonon line of the 0-0 transition.

After excitation to the S_1-state, the molecule can decay to the ground state by emission of a photon. Emission of a photon of exactly the same wavelength as the excitation is called "resonance fluorescence". Decay to a vibrational level on the electronic ground state occurs by emission of a photon with a longer wavelength (smaller energy) and is called "Stokes-shifted fluorescence". Further de-excitation then takes place via non-radiative processes, in which the excess electronic excitation is directly converted into heat by the creation of phonons. Non-radiative decay from the S_1 state is possible by the creation of a large number of phonons. This process is