
Fluorescence Imaging of Single Spins in Nitrogen-Vacancy Centers Using a Confocal Microscope

Fortgeschrittenenpraktikum I/II

September 17, 2018

Abstract

The main goal of this experiment is to understand the basic working principle of a confocal microscope. In addition we will use the confocal microscope to discover some of the exciting properties of Nitrogen-Vacancy centers. The experiment is designed such that experimental work, e.g. aligning the microscope, as well as understanding physical phenomena are balanced.

Contents

1	Introduction	3
2	The Confocal Microscope	4
3	The Nitrogen-Vacancy Center	5
4	Notes on Experimental Procedure	6
4.1	Safety and General Information	6
4.2	Short Introduction to the Software	8
4.2.1	GUI 1: Experiment Control	10
4.2.2	GUI 2: Starting a Measurement	11
4.2.3	GUIs 3 and 4: Plotting Data	11
4.2.4	GUI 5: Saving and Commenting your Data	11
4.3	Experimental Setup	12
4.3.1	Overview	12
4.3.2	Fine alignment	13
5	Tasks and Data Analysis	17
5.1	Homework	17
5.2	Fine Alignment of Confocal Microscope	17
5.3	Calibration of Sample Stage	18
5.4	Influence of Pinhole on Resolution	19
5.5	Saturation Curves	19

1 Introduction

The main goal of this experiment is to understand the basic working principle of a confocal microscope. In addition we will use the confocal microscope to discover some of the exciting properties of Nitrogen-Vacancy centers. The experiment is designed such that experimental work, e.g. aligning the microscope, as well as understanding physical phenomena are balanced.

The first two chapters this short manual give a short overview about the two key ingredients of this experiment, NV centers in diamond and the general structure of a confocal microscope, respectively. Each chapter finishes with several questions, that are mandatory to answer before the experiment can be done. Some bibliographical references are given as well, but students are encouraged to look for literature of their own choice.

The thirds chapter contains information about the experiment itself. Please read this part of the manual very carefully. It contains safety information, a short introduction to the measurement software as well as a short description of the experimental setup itself.

The tasks of this experiment are given in chapter five. Please stick to the proposed order and do not jump from one experiment to another.

2 The Confocal Microscope

In a conventional wide-field fluorescence microscope, light coming from a light source illuminates large areas of the specimen. Consequently, all parts of the specimen in the optical path are excited at the same time and the emitted fluorescence is detected all at once. This approach gives an intense signal on the cost of a rather high background signal and low spatial resolution.

In a confocal microscope only very small areas of the specimen are illuminated at the same time, as an objective with a high numerical aperture is used, with light coming from a coherent light source. Additionally, a pinhole in front of the detector eliminates signal coming from other regions in the sample than the region close to the microscope's focal plane. Compared to conventional wide-field microscopy, confocal imaging comes with higher lateral and axial resolution, but with lower signal intensities.

The experiment at hand uses a standard confocal microscope setup. Please answer the following questions carefully to make yourself comfortable with the general structure of a confocal microscope:

1. Explain the general structure of a confocal microscope on the basis of a suitable schematic. Draw the optical path and explain why a pinhole in front of the detector significantly increases resolution.
2. Compare the structure of a confocal microscope to the structure of a conventional wide-field microscope. What are the advantages and disadvantages of the two microscopes?
3. Derive equations for the lateral resolution Δr_{\parallel} and the axial resolution Δz of a confocal microscope. How can the resolution be improved? How would changing the pinhole's diameter effect lateral and axial resolutions?
4. One way to determine the resolution of a confocal microscope is to measure the width of the Gaussian beam after the objective. This can be done using the so-called knife edge method. Make yourself familiar with this method and explain how you would extract information about the beam waist of the confocal microscope.

References:

- <http://www.olympusmicro.com/primer/techniques/confocal/index.html>
- L. Novotny and B. Hecht, *Principles of Nano-Optics: Second Edition*, Cambridge University Press, 2012.

- M. Minsky, *Memoir on Inventing the Confocal Scanning Microscope*, Scanning **10**, 128 (1988).
Go on Marvin Minsky's website to have access to the full text (<http://web.media.mit.edu/~minsky/papers/ConfocalMemoir.html>).
- http://massey.dur.ac.uk/resources/grad_skills/KnifeEdge.pdf

3 The Nitrogen-Vacancy Center

The Nitrogen-Vacancy (NV) center is a point lattice defect in diamond and has attracted great interest within the last decades due to its long list of outstanding physical properties. The NV center's electronic spin can for example be manipulated coherently using magnetic fields. It is also worth noting that such experiments can be performed at room temperature (as it is done here in this lab course) as coherence times reach, even at such high temperatures, the ms range. Besides that, the NV center also serves as a single photon source and is thus suited for photonic experiments.

For the experiment at hand, it is sufficient to understand how NV centers react on the illumination with green light. Please go through the following questions and answer them carefully:

1. Describe the structure of a NV center (use a schematic). Do they occur naturally or do NV centers need to be created?
2. As the NV center can be seen as a quasi atom, it can be assigned a ground state and an excited state. Describe the electronic structure of a NV center (use a schematic of an NV⁻'s energy diagram).
3. How does a NV center react upon green light illumination?
4. In the context of the previous question, describe what a fluorescence map is and how it usually is recorded.
5. Make yourself familiar with the concept of a *saturation curve*. Why is this measurement performed and what characteristic parameters can be extracted? What function is used for fitting (see e.g. Riedel *et al.*, Physical Review Applied **2**, 064011 (2014)).

References:

- Rondin *et al.*, *Magnetometry with nitrogen-vacancy defects in diamond*, Reports on Progress in Physics **77**, 056503 (2014).
- Childress *et al.*, *Atom-like crystal defects: From quantum computers to biological sensors*, Physics Today **67**, 38-43 (2014).
- Riedel *et al.*, *Low-Loss Broadband Antenna for Efficient Photon Collection from a Coherent Spin in Diamond*, Physical Review Applied **2**, 064011 (2014).

4 Notes on Experimental Procedure

4.1 Safety and General Information

- **Laser safety**

The laser used in the experiment is a class 3 laser and therefore dangerous for the human eye if the eye is exposed directly to the beam. Diffuse reflections, e.g. reflections from paper or other matte surfaces, are however not harmful. A class 3 laser requires to wear adequate protective eyewear, such as laser safety goggles.

To avoid any directed reflections to hit your eye, do not wear watches, rings or other items that can reflect light.

Never move around with your head on the same height as the laser beam. In case you have to bend down (for example in order to pick something up), close or shield your eyes.

In case you have to place another optical element, e.g. a mirror or a lens, block the beam. If that is not possible, for example if you need to use the beam reflections for alignment, please put the optical element in the beam such that the reflected light is directed away from you or other people in the room.

The laser itself has to be switched on before the experiment can be started (just turn the key at the controller to the “on”-position, see Fig. 1). After you are done with the experiment, please switch it off again.

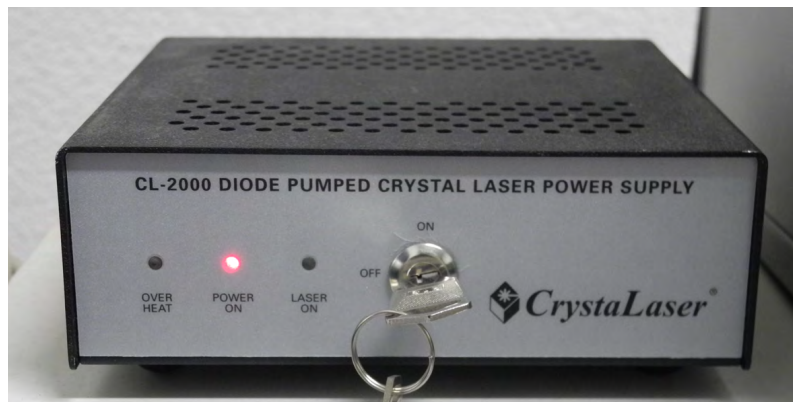


Figure 1: Laser controller to switch the laser on or off.

- **How to handle optical elements**

Optical elements, e.g. pellicle beamsplitters, can be very fragile and **reflecting regions must never be touched!** If necessary, dust can be removed by blowing it off with pressurized nitrogen gas. In case optical elements need to be cleaned and/or replaced, talk to the experiment’s supervisor first.

- **Working with the Avalanche Photodiode**

An Avalanche Photodiode (APD) is a highly sensitive electronic device. It makes use of the photoelectric effect to detect single photons and is used in the experiment to detect the NV center's fluorescence. High sensitivities are reached by exploiting avalanche multiplication, where the photocurrent of a single photon is significantly enhanced. **APDs are therefore extremely sensitive to photons and might break if they are powered and exposed to room light..** To avoid this scenario, the APD in the experimental setup is equipped with a safety box (see Fig. 2). During the experiment, the safety box has to be powered and the APD gate has to be switched on (to flip the lever, you have to pull at it and move it up or down). Note that switching the gate of the APD on does not directly supply power to the APD, i.e. the APD can be exposed to light without being damaged (however, this should be avoided in general - gating the APD is just an additional safety feature). The measurement software will apply short voltage pulses to the APD whenever fluorescence should be detected.

The second device that can be controlled with the safety box is the shutter. The shutter is placed directly in front of the laser and can block the laser beam. In *Remote* mode, the shutter is controlled by the software and is opened automatically when a measurement is in progress. The options *Open* and *Close* open and close the shutter, regardless of what the software is doing.

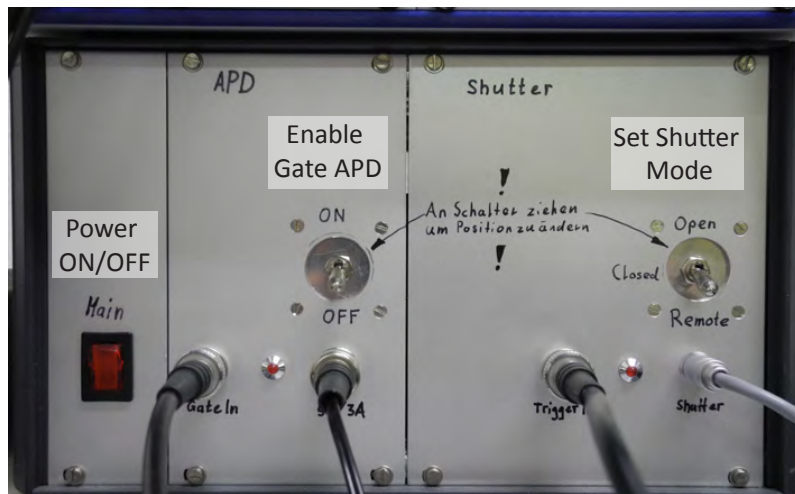


Figure 2: Safety box to control shutter and APD. Shutter mode and APD Gate mode can be set by flipping the corresponding levers.

- **Working with the piezoelectric translation stage**

The sample is mounted on a two-dimensional, piezoelectric translation stage. The controller (see Fig. 3) has to be powered (power switch is located at the backside) and control over the two axes has to be enabled by switching *SERVO 1* and *SERVO 2* on.

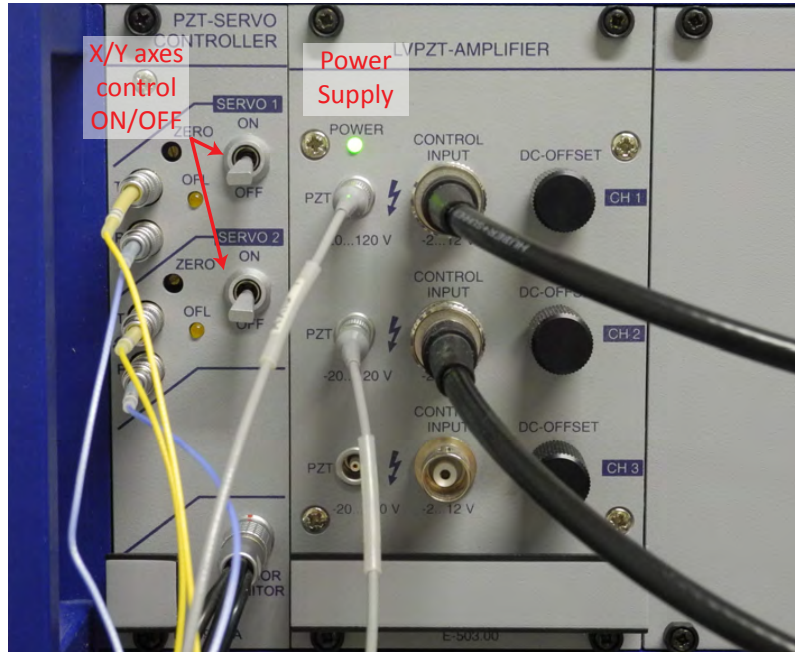


Figure 3: Controller of the piezoelectric translation stage. Switch *SERVO 1* and *SERVO 2* on to enable translation of the sample along the x- and y-directions. The main power switch of the controller is located at its backside.

4.2 Short Introduction to the Software

The software is programmed using Labview and, as it was used in a real lab, possesses many features that are not needed for the experiment here. To guide your attention towards the important features, the software will be explained quickly here. To start the software, run *SxOMv8* and the software's graphical user interface (GUI) shown in Fig. 4 will appear. You will notice that the GUI actually consist of five different subGUIs, whose functions will now be explained.

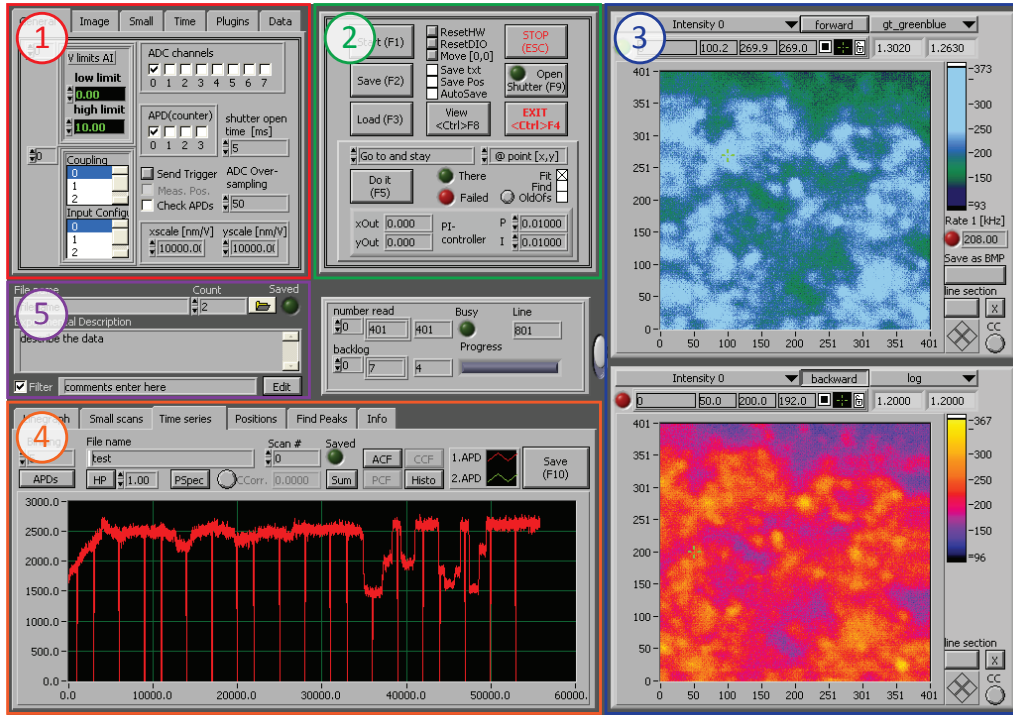


Figure 4: Graphical User Interface of the software used in the experiment. It consists of five smaller subGUIs, marked with colored boxes. The functions of each subGUIs are explained below.

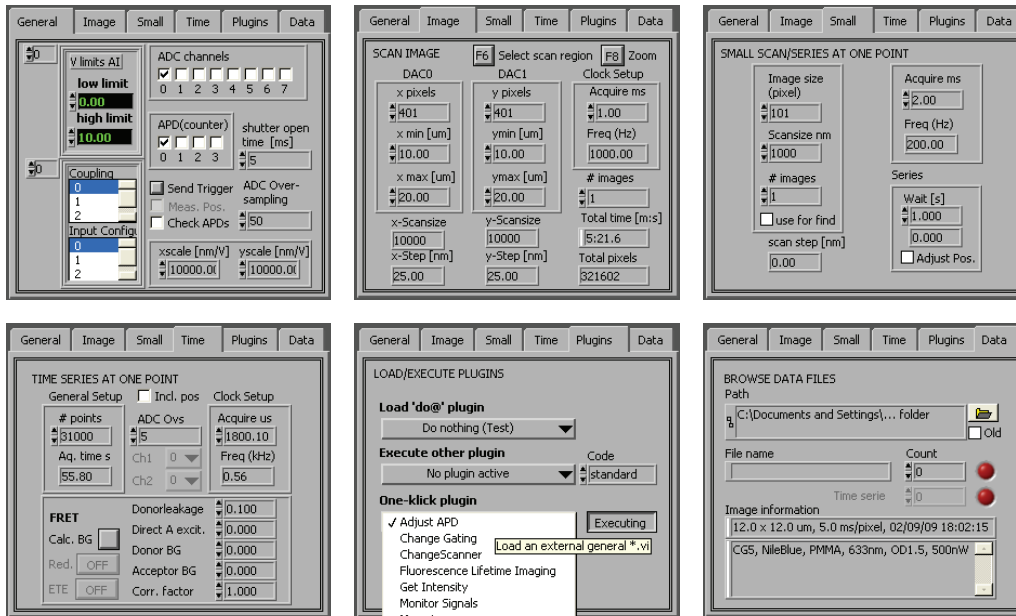


Figure 5: GUI 1 offers six different tabs (shown here) to change settings of the experiment. Note that all of these tabs can only be used to adjust the settings, but not to start a measurement. For a successful measurement, the tabs *General*, *Image* and *Plugins* are most important.

4.2.1 GUI 1: Experiment Control

The first GUI (red box, Fig. 4) controls the experiment. It allows for setting general preferences as well as preferences concerning two-dimensional scans and time series. In addition, several plugins can be loaded and executed and one can browse through data files. Available options of GUI 1 are explained in detail in the following.

General In the *General* tab (left picture of the top row in Fig. 5) you can change general settings concerning the APD (e.g. which connector is used by the computer to read out the counts registered by the APD) and the piezoelectric sample stage. Do not change any settings here. If something is not working properly and you think it is related to any general setting, talk to the supervisor.

Image The *Image* tab (middle picture of the top row in Fig. 5) gives control over two-dimensional fluorescence scans. You can set the resolution along x- and y-directions by changing the number of pixels, define the area to be scanned and set the acquisition time (i.e. how long counts are collected per point of the scan). **Note that the acquisition time must not be too long in order to not exceed the maximum number of counts per second (about 2 Mcps) the APD can handle.**

Small You can use the tab *Small* (right picture of the top row in Fig. 5) if you want to start a very well defined scan around a certain point (x,y) and do not want to enter the scan range by hand. Again, be aware of the acquisition time.

Time The tab *Time* (left picture of the bottom row in Fig. 5) gives you control over the settings of a time series. You can set the number of points and the acquisition time.

Plugins In the tab *Plugins* (middle picture of the bottom row in Fig. 5) one can start “one-klick” plugins. The only relevant plugin here is the *Adjust APD* plugin. It is very useful if you want to align the setup. A plugin can be executed by pressing the button *Execute*.

Data The last tab is called *Data* (middle picture of the bottom row in Fig. 5). It basically allows to browse through the data files you have taken so far and read the image information you have entered.

4.2.2 GUI 2: Starting a Measurement

While the first subGUI can only change measurement settings, the second subGUI (green box in Fig. 4) is used to start measurements. Note that GUI 2 is divided into two parts. Its upper half deals with two-dimensional scans only and thus requires correct settings made in the tab *Image* of GUI 1. In contrast to the upper half, the lower half deals with all other measurements for which you can change settings in the GUI 1 (i.e. small scans and time traces, see description above). You can choose which experiment you want to perform and where you want to perform it by selecting the corresponding entry of the two drop-down menus. The other buttons are labeled in a self-explanatory way. **Please do not forget to check the buttons *Save txt* and *Save pos*, as this saves both the position and the corresponding counts of your fluorescence in a text-file.**

4.2.3 GUIs 3 and 4: Plotting Data

As explained above, GUI 1 sets up the measurement and GUI 2 is used to start the desired experiment. The corresponding data is shown in GUI 3 (two-dimensional scans; blue box in Fig. 4) and GUI 4 (all other experiments; orange box in Fig. 4). GUI 3 is again divided into two plots, with the upper (lower) showing the signal of the forward (backward) scan. Again, buttons are labeled according to their functions. A handy tool is the cursor. You can position it by entering the desired coordinates (in pixels) in the second and third field from the left, right below the drop-down menu where the plotted signal is chosen. Another way to set the cursor is by just using the mouse and drag the cursor to its final position. Being able to move the cursor around is quite useful for alignment. You can, by using the *Go to and stay* command, move the focus of the confocal microscope to a certain position and stay there. Besides that, GUI 4 has several tabs that are used to plot the signal acquired during small scans and time series.

4.2.4 GUI 5: Saving and Commenting your Data

The last subGUI is GUI 5 (purple box in Fig. 4). It is used to change the names of the saved data sets and also allows for commenting what the data actually includes. Make use of this opportunity as it helps a lot during data analysis. You can set the path where your data files are saved to by click on the folder icon.

4.3 Experimental Setup

4.3.1 Overview

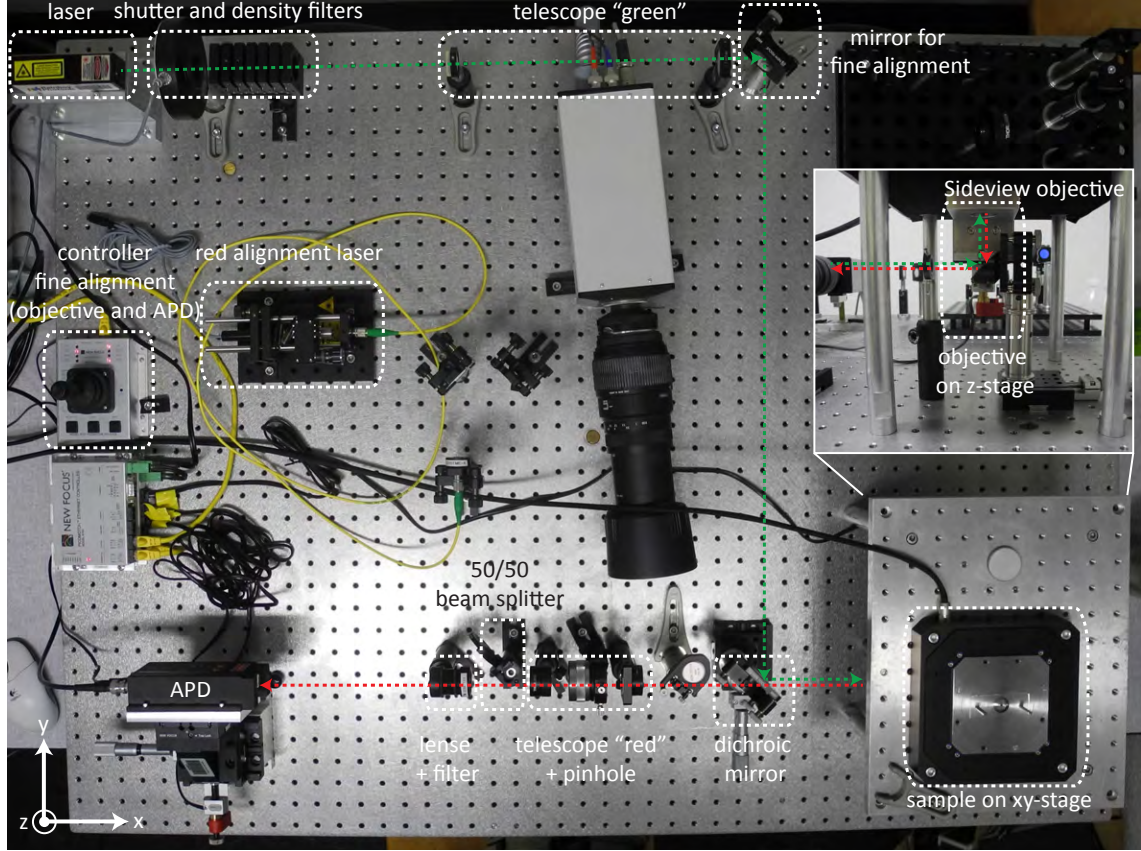


Figure 6: Topview of the experimental setup. The green light (dashed green line) passes a telescope and is then guided to an oil-immersion objective (see inset), which focuses it onto the diamond sample. The NV center’s red emission (dashed red line) is collected by the same objective, passes a second telescope with a pinhole and is finally focused onto a photodetector.

Fig.6 shows an overview of the experimental setup. The setup mainly consists of a standard confocal microscope with a green excitation arm and a red detection arm. Before the green light (dashed green line) is focused onto the sample by an oil-immersion objective, it passes a shutter, several density filters as well as a telescope. The NV center’s red emission (dashed red line) is collected by the same oil-immersion objective and, after passing a second telescope with a pinhole of fixed size (the pinhole can be changed), is focused onto an APD.

Several parts of the experiment are mounted on translation stages for better experimental control. The sample is mounted on a xy-translation stage, such that one can scan over a large sample area. The oil-immersion objective sits on a motorized z-translation stage to ensure that single NV centers can be positioned in the confocal microscope’s focus. Finally, the APD is attached to a xyz-stage, where the

x- and y-directions (z-direction) are (is) controlled by motorized adjusting screws (a manual adjusting screw) to maximize the detected signal. Both, the objective's z-stage as well as the APD's xy-stage have to be controlled by using an external controller with a joystick (see explanation below). The xy-stage, where the sample is mounted on, is controlled by the measurement software.

Another detail to mention is the *red alignment laser*. It can be coupled into the optical path of the confocal microscope by flipping up the *50/50 beamsplitter*. The alignment laser is useful if you are adding a pinhole to the setup and want to do a rough alignment step (see below). **Note however that, if you are using the alignment laser, you need to shield the APD from the rest of the setup. Otherwise the red laser will also illuminate the chip of the APD and this can damage it, even if the APD is not powered.**

4.3.2 Fine alignment

The setup is aligned to a degree where only fine adjustments are necessary for sufficient measurement quality. There are in total four elements that need to be optimized to receive sufficient spatial resolution as well as an optimized count rate at the APD. A description of the four elements and the corresponding alignment procedure is given below. To align the setup, it is most convenient to use the *Adjust APD* plugin (see section 4.2). Please note that the subsequently explained alignment procedures have to be iterated several times to ensure proper alignment.



Figure 7: Left: During rough alignment, the green beam is positioned on top of the red beam. Right: Please use this mirror only for fine adjustment of the beams' overlap.

Motor	X axis	Y axis
A	APD(y axis)	APD(z axis)
B	APD(y axis)	not used
C	Objective(z axis)	APD(y axis)

Table 1: Assignment of motorized adjusting screws to controller motors. Use motor A to control the adjusting screws attached to the APD and motor C to position the objective.

Overlap detection and excitation arm A confocal microscope works properly if the focus of the green excitation arm coincides with the focus of the red detection arm. During the rough alignment of the setup this is ensured by positioning the green beam on top of the red beam. For the final measurement this procedure is however not precise enough. Once a diamond sample is mounted and the setup is roughly aligned, one needs to optimize the overlap of both arms by changing the path of the green arm slightly and maximize the count rate of the detected red fluorescence. To change the green beam’s position, please use the designated mirror only (*mirror for fine alignment*, Fig. 6).

Position of NV center After maximizing the overlap of both arms, one also needs to position the NV center of interest in the focus of the confocal microscope. For this, choose an appropriate position in x and y (use the software’s *Go to and stay* command) and change the z-position of the oil-immersion objective. This can be done with the controller that controls the motorized adjusting screws (see Fig. 6, *controller fine alignment*). The joystick can be moved along two axes, where each axes controls one adjusting screw. Hence, only two screws can be controlled at the same time. Therefore, as we have three motorized screws, one needs to choose the *Motor* that is used to drive the intended screws - see Tab. 1 for more information.

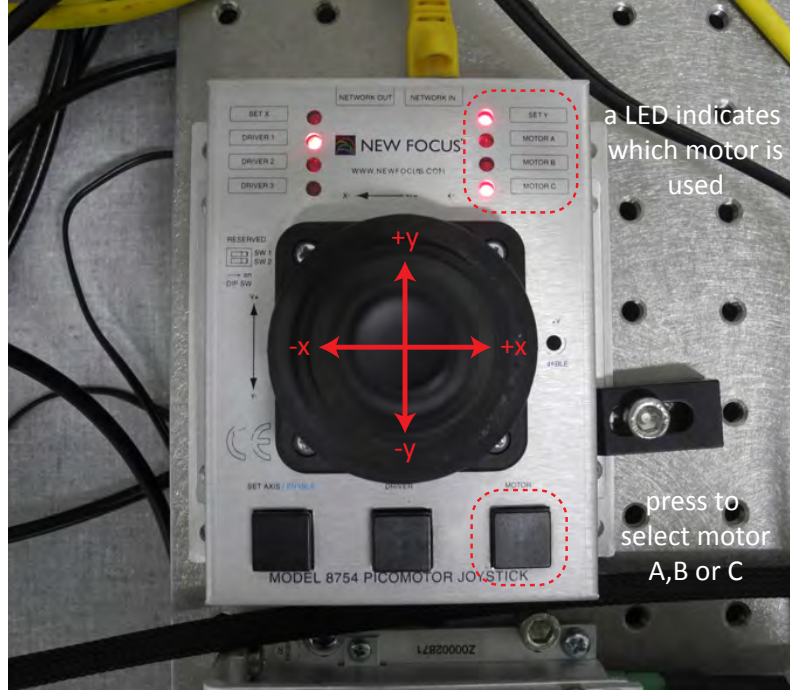


Figure 8: Controller to steer motorized adjusting screws. One can choose between motors A, B and C by pressing the button in the lower right corner. The active motor is indicated by a red LED in the top right corner. Note that the LED of motor B is broken. By moving the joystick along x or y the corresponding adjusting screw is moved (see Tab 1 for the assignment of adjusting screws to controller motors).

Position APD Once the confocal microscope is focused on a single NV center, the APD, more precisely its active area, needs to be positioned such that all of the detected fluorescence is detected. Therefore, the APD needs to be moved along all spatial directions until the count rate is maximized. As described above, use the motorized adjusting screws to control the APD's position along y and z. A manual adjusting screw moves the APD along x, i.e. the direction of the fluorescence beam.



Figure 9: The pinhole is mounted in the silver cylinder, that can be rotated to adjust the pinhole's position along the optical path of the confocal microscope (x axis). The silver cylinder is fixed on a small yz -stage to allow for proper positioning of the pinhole in the plane perpendicular to the optical axis.

Position pinhole A pinhole has to be mounted in the optical path in order to ensure confocal operation (without the pinhole, our confocal microscope would simply be a scanning fluorescence microscope). To do this, attach the silver cylinder to the small yz -stage (see Fig.9). The yz -stage allows for properly positioning of the pinhole in the plane perpendicular to the optical axis of the confocal microscope. The silver cylinder can be rotated and serves as a x -stage. By turning cylinder the pinhole can be placed directly in the focus of the telescope.

5 Tasks and Data Analysis

The goal of this experiment is to understand the basic principles of a confocal microscope and to use it to examine optical properties of Nitrogen-Vacancy (NV) centers.

5.1 Homework

To be able to understand the following it is essential to be able to answer the questions regarding theoretic principles of confocal microscopy (see chapter 2) and NV centers (see chapter 3).

From the experimental point of view, make yourself accustomed with the experimental setup and how it works. For this read section 4.3 carefully and answer the following questions:

- Compare the standard structure of a confocal microscope to the experimental setup and identify similarities and differences.
- Explain the purpose of the two telescopes (labeled with *green telescope* and *red telescope* in Fig. 6).
- Fig. 6 shows that a filter is mounted on the lense that is used to focus the red light onto the APD. Can you think of any reason that motivates the existence of this filter?

5.2 Fine Alignment of Confocal Microscope

The setup is roughly aligned but the alignment needs to be improved for the following measurements. Fine alignment of the setup is achieved by performing the four alignment procedures (see section 4.3) while looking at the red fluorescence of single NV centers. Once the setup is aligned well, one can clearly distinguish between single NV centers.

Mount *Sample A* (a polycrystalline piece of diamond glued on a glass slide) on the xy-translation sample stage and fix it there by placing the two permanent magnets on top of the glass slide. Make sure there is no pinhole mounted in the *red telescope*, as this would make fine alignment unnecessarily complicated. Switch on the green laser, enable APD gating and align the setup by maximizing the detected count rate.

Hints

- Use the *Align APD* plugin of the software.

- As already stated, the four alignment steps need to be repeated in an alternated way. In the beginning, it is very likely that you will measure a rather weak signal. This might be caused by bad alignment or simply because you are measuring sample background. It is therefore advisable to perform a two-dimensional fluorescence scan to check whether you are focused on a single NV center or not.
- The room light needs to be switched off whenever you are measuring to protect the APD from any damage.

5.3 Calibration of Sample Stage

A major goal of this experiment is to characterize the influence of a pinhole on the spatial resolution of a confocal microscope (see section 5.4). Analyzing spatial resolution quantitatively however requires detailed knowledge about the piezoelectric translation stage where the sample is mounted on. More precisely, we need to know the figure of merit nm/V , i.e. what distance the piezoelectric stage travels when a specific bias signal is applied. Sample stage calibration is done by using *Sample B*, which consists of a patterned palladium (Pd) layer on a thin layer of silicon dioxide (SiO_2). The dimensions of the Pd structures are known (see Fig. XXX). Under illumination with green light, Pd will appear bright in the confocal microscope whereas the (SiO_2) remains dark. This contrast in intensity can be used to determine a value for nm/V .

Problem 1:

Mount *Sample B* and bring it into the focus of the confocal microscope (for example by using the *Align APD* plugin of the software). Try to find an area on the sample where you observe a pattern that looks like a chessboard. To determine the figure of merit nm/V , record fluorescence scans for 5000nm/V, 7500nm/V, 10000nm/V, 15000nm/V and 20000nm/V (the value for nm/V can be set in GUI 1, see Fig. 5). Each square of the pattern is $4\mu m$ by $4\mu m$ large. Determine a correct value for nm/V and explain your approach.

Problem 2:

So far, you have been working with a confocal setup where the APD's chip served as the pinhole (the size of the chip is about $180\mu m$). The setup however also gives you the opportunity to use smaller pinholes. What do you expect to see in your experiment in terms of spatial resolution when using smaller pinholes? Compare fluorescence scans taken with pinhole sizes of $100\mu m$, $50\mu m$ and $25\mu m$ and describe your observation.

Problem 3:

Compare the experiment you have done to the knife-edge approach, which is used to determine the waist of a Gaussian beam. Are there similarities? Use the knife-edge approach to determine the beam waist of the confocal microscope and explain your approach. Compare the beam waist for different pinhole sizes.

Note

A pinhole needs to be positioned in the beam rather precisely such that you can detect some signal. Whenever you add a pinhole to the setup or want to change it, you should start with a rough alignment, where you employ the red alignment laser and the beam splitter on the left of the *red telescope*. The red alignment laser will be sent backwards through the pinhole and the goal of the rough alignment procedure is to maximize its transmission. The transmission can be quantified by using a power meter. **As explained above, you have to shield the APD from the red alignment laser, i.e. by blocking the optical path towards the APD.** Once the transmission is maximized, you can continue with optimizing the counts coming from a single NV center (this time of course without the red alignment laser and without the beam splitter in the optical path).

5.4 Influence of Pinhole on Resolution

The spatial resolution of a confocal microscope strongly depends on the size of its pinhole, as already observed in the previous experiment. Perform a two-dimensional scan of a single NV center without a pinhole and for pinholes of size $100\ \mu\text{m}$, $50\ \mu\text{m}$ and $25\ \mu\text{m}$. Determine the spatial resolution of the confocal microscope by fitting the fluorescence scan data. Compare your findings and explain what you observe.

5.5 Saturation Curves

The optical properties of NV centers can be characterized in different ways. One of the easier ways is to perform so-called *saturation curves*, where one can extract the saturation count rate of NV centers, the corresponding saturation power of green excitation as well as the strength of background fluorescence. During a saturation curve measurement, the excitation power, i.e. the power of the green laser, is de- or increased and the change of fluorescence counts is monitored. Perform this experiment for at least two different pinhole sizes on two different NV centers.

Extract the relevant parameters by fitting the taken data and compare the resulting curves. Can you observe significant differences? Explain where these differences come from. Which power would you choose if you wanted to perform a $g^{(2)}$ -measurement, where you want to detect single photon counting events and have an optimal signal-to-background ration?

Note

Use a power meter to determine the power of green excitation (the power should be measured after the dichroic mirror, i.e. right before the oil-immersion objective). The density filters can be flipped in and out of the green path. In addition, a density filter wheel allows for finer adjustment of excitation power.