A STUDY ON THE IDEAL TIP GEOMETRY FOR AN APERTURELESS NEAR-FIELD SCANNING OPTICAL MICROSCOPE

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Introduction

With the first publications by Binning and Rohrer (1) on the scanning tunneling microscope (STM) a whole new field in experimental science emerged. The concept of a scanning probe microscope (SPM) has been used since for many different applications, ranging from surface science in solid state physics to protein research in biology.

One of the applications of the technique that has been suggested early on is the Near-field Scanning Optical Microscopy (NSOM) (2,3). Near-field means that the illumination source (the probe) and the sample are close to one another. In this near-field limit, the illumination field can no longer be regarded as a plane wave (as in normal widefield microscopy) but the field near the tip has to be described 3-dimensionally. In this particular application of a scanning probe microscope, the probe is used to alter the electromagnetic field of the microscopes light source. This altered electromagnetic field should push the optical resolution, normally limited to ~$\lambda/2$, to the size of the probe, ~10nm, giving optical and topographic resolution on a nm scale.

There are two distinct types of NSOM microscopes, that differ in there sample illumination scheme and the probe that is used. The aperture based NSOM and the apertureless NSOM (ANSOM). The first type has been most successfully used in experimental applications up to now. In this set-up an optical fiber is used to illuminate the sample and serve as the scanning probe at the same time. The end of the fiber is modified to decrease the radius. This makes the probe sharper, thereby leading to a higher resolution. As a result of that the opening will be smaller than the wavelength of the light that is used for illumination, creating an electromagnetic field that decreases exponentially with the distance between the sample and the probe. Because the rate of decay is determined by the size of the opening there is a natural limit to this size without losing the ability to use the fiber as an excitation source for the sample, this limit is around ~100nm (13). In this study we would like to investigate the possibilities of an apertureless NSOM. In this type of NSOM a metallic probe is used as an antenna to locally modify the electromagnetic field resulting in confinement and enhancement of the field of the laser. In this way it should be possible to get an optical resolution in the order of ~10 nm. The magnitude of the enhancement is determined by both the material properties of the tip and its geometry. Theoretical studies have indicated an ideal tip geometry and material to generate maximum enhancement of the optical signal due to near-field effects on the electromagnetic field (4).

In this project we looked at a number of different commercially available and homemade tips, focussing on different types of nanotube tips, to investigate the practical implications of the theoretical findings in building a working ANSOM.
As said in the introduction, the aim of this study is to identify the tip characteristics necessary to induce maximal tip enhancement.

In figure 1 the set-up is schematically drawn (fig 1A.) and figure 1B shows the illumination of the sample and probe (tip) in more detail. In this setup we use the electromagnetic field of the laser to get optical signal from the sample. In this way we can get two different types of information. If the sample itself has no light emitting properties (in the visible range) the light scattering from the probe is the signal from the sample. A problem in this type of experiments is the fact that the elastically scattered light has the same wavelength as the light source and therefore the signal is hard to distinguish from the illumination background. If the sample on the other hand is able to fluoresce, the light emitted has a different wavelength than the illumination source and the signal is more easily distinguishable from the excitation light. Therefore in our experiments we use fluorescent beads as a sample. Next to this we use two-photon excitation to generate the fluorescent signal which makes the difference in wavelength between the excitation and emission light even bigger.

Tip enhancement is an alteration of the (EM field) from the laser induced by the presence of the atomic force microscope (AFM) tip. To be able to use the tip in this way the EM field should have a component in the direction of the tip (fig. 1B). We can realize this by making sure the EM field has a component in the z-direction or we can place the tip under an angle with the z-axis. Due to our high NA objective the EM field already has a z-component, therefore placing the tip under an angle is not essential.
If the lasersource used to produce the electromagnetic field has the appropriate frequency for the material of the tip it is possible to transfer the energy from the laser field to plasmons in the material of the tip by exciting them at there resonant frequency. If the tip now has the right geometry the energy can be returned to the sample very locally as electromagnetic radiation, if enough energy of the original radiation is conserved during this process the locally created field will be enhanced with respect to the original field coming from the laser source (7).

Using the spatially localized and enhanced electric field to induce fluorescence in the sample will result is a superposition of the normal fluorescence (induced by the excitation due to the laser) that is confined to the confocal volume of the microscope and the spatially localized enhanced field from the tip (8). (It is this last part that will give the high resolution beyond the limit of $\lambda/2$). This superposition will result in a completely different shape of the fluorescent signal; the normal gaussian profile of the fluorescence is quenched when the tip approaches the bead and will give a more confined gaussian profile when the tip is just above the bead (fig 2)(8).

**Figure 1**  Schematic representation of the combined confocal and AFM setup (A) and a close-up of the illumination of the sample

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Figure 2  Measured relative intensity as a function of distance between the tip and a fluorescent object.
*The pattern is roughly symmetric around zero distance between object and tip*

The ratio between the peak intensity at the bead and the average “normal” fluorescence may range from 0.2 up to 1.2. This ratio will depend on the enhancement that can be obtained and on the degree of localization of the signal. It is this ratio that will determine the eventual resolution of the new image. The higher the ratio the easier to distinguish the two superimposed fluorescent signals and the higher the eventual resolution.

Because two-photon excitation is a non-linear process where the signal dependence is quadratic in the excitation intensity it takes extra advantage of the enhancement of the electric field. Using two photon excitation it should therefore be easier to distinguish the enhanced signal from the confocal illumination. This will make it easier to reach the level of enhancement necessary to increase the resolution and will improve the highest possible resolution with optimal enhancement (7).

An important parameter determining the magnitude of the enhancement and the volume to which the modified field is confined, as said, is the geometry of the tip. Theoretical calculations were performed to find general rules to estimate the enhancement expected from certain geometry (4,6). Doing approximation calculations it was found that there are a number of features that are important to induce a high field enhancement. These features are the opening angle $\alpha$ of the tip (fig. 3) the material of the tip and the base (not necessarily the same) and the shape of the base. The most optimal form for the tip (4) is a finite cone (fig.3A). This form is somewhat better than a quasi-infinite cone (fig.3B) but harder to realize in practice. The tips that we used for this study can best be approximated as an quasi-infinite cone, where the tip opening angle and length depend on the specific tip and is different in each case.
The material that is used determines the amount of energy that can be transferred from the excitation source to the tip and subsequently to the sample. To transfer the energy as efficiently as possible the plasmon frequency of the tip material should be close to the frequency of the illumination source otherwise the metal will just reflect the electromagnetic field (9). Because we want to detect the signal as fluorescence in the visible range of the spectrum, metals with a plasmon frequency in the near-infrared (two photon excitation) are required. To achieve this the real part of the dielectric constant ($\varepsilon$) must be negative at the excitation wavelength (6). In the near infrared region of the optical spectrum a metal like gold has the highest expected enhancement factors. Next to using gold we also want to get an idea about the ability to induce enhancement effects using multiwalled nanotubes with metallic properties (10,11). In this study we will use different gold coated tips and multiwalled nanotubes of different geometries to investigate their capabilities to be used as a probe in an apertureless NSOM. In addition to this we want to investigate the possibility of generating enhancement effects with nanotube tips. These nanotubes have excellent scanning abilities and are therefore interesting to use also in an ANSOM.

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Experimental Set-up

The combination of a confocal microscope with an AFM scanhead we use, asks for adjustments of both parts of the set-up. Because the presence of a tip and cantilever from the AFM prevents collection of light from that side, the illumination and collection of the light has to be performed from the same side of the sample (fig. 4 Appendix). For this reason we used an inverted microscope to illuminate and collect light through the same objective. To be able to scan the the same area of the sample not only topographically but also optically we have two options: scanning the sample with the scanhead and simultaneously scanning with the objective, or scanning the sample and putting the tip and the focus of the objective on top of each other. The latter was chosen in this setup.

Our instrument (fig. 5 Appendix Claudiu) is based on a commercial tip-scanning AFM (Bioscope™, Veeco, Santa Clara, USA). The scanning head (range 90μm x 90μm x 6 μm, resolution 0.1 nm in z, 1 nm in x,y) and its original frame are mounted on an inverted optical microscope (Zeiss Axiocounter S100TV) using a custom-designed microscope stage. This stage also accommodates a closed-loop XY piezo scanner (Physik Instrumente, P-517.2 CL, range 200μm x 200 μm, resolution 2 nm) and three screws for coarse alignment of the AFM probe to the optical axis of the microscope. The sample is cast on a glass coverslip. This coverslip is mounted on a small microtranslation stage, which is in turn fixed by screws to the PI scanner, and kept in place by springclamps.

Both the tip-focus alignment and the sample scanning were integrated into the Nanoscope data acquisition software. For this purpose, we tapped the low–voltage XY signals out of the Nanoscope Controller in order to generate externally high-voltage piezodrive signals identical to those generated by the internal amplification. The low XY piezo voltages were first fed into a Digital Sample & Hold module, which consists of a 16-bit AD/DA converter operating at a clock frequency of 10 kHz. As long as the clock is ON, the output voltage matches the input; when the clock is switched OFF the digitizing stops and the last value read in the buffer of the DA converter is constantly used as output irrespective of the changes of the input. Then an offset voltage, which is manually tunable over the entire range of the input, is added by an offset amplifier to these signals before they are fed into the high-voltage amplifier.

Due to this hardware we are able to drive the tip scanner both with the internal and externally generated signals. Only when the clock is stopped using the externally generated signals we can use these signals to drive the PI stage control to scan the sample. The scanning is controlled by the original Nanoscope software, for which the new configuration (XY scan by the PI stage and Z tracking by the Bioscope head) is seen only as another Nanoscope XYZ scanner, with a different calibration file.

We use the epi configuration for confocal fluorescence microscopy, with the sample being scanned while illuminated through a high numerical aperture objective (1.4/100x, Zeiss Apochromat). The substrate is two-photon excited with near-infrared femtosecond pulses from a mode-locked Ti-sapphire laser (Tsunami, Spectra Physics,
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USA) The sample, fluorescent beads (Molecular probes (table 1 appendix)) are excited at either 870 or 920 nm. The emission is collected through the same objective, separated from the excitation beam and the light (673nm) emitted by the laser diode (used for controlling the cantilever deflection) using an appropriate filter combination (short-pass dichroic filter (725) a BG 39 and a BP 525/75). The emission beam is focused on a single photon counting avalanche photodiode module (SPCM-AQR-14, Perkin Elmer Optoelectronics), whose active surface acts like an effective pinhole for confocal detection. A dual channel pulse counter built-in the Nanoscope Controller can independently record TTL signals from 2 photon counting detectors. These counters are synchronized with the digitization of the “classical” AFM signals, thus enabling optical data to be acquired at exactly the same time as, for example, height data.
Results

The tip geometry plays an important role in the enhancement of the electromagnetic field we therefore first want to investigate the scanning properties of the different used tips in order to be able to look for a relation between tip parameters and field enhancement. In table 2 the different tips that were used in this study are listed together with the parameters that can be used to define their scanning abilities. Two important parameters are the slope, which is defined as the angle (with respect to the glass) between the base of the object and the point at full width at half maximum (FWHM) with the glass substrate, and the ratio between the measured height of an object and the FWHM. A high slope angle and a ratio close to 1 are both indications of a sharp tip with a small opening angle and tip radius. For a sharper tip we expect a better localization of the altered electromagnetic field, and therefore a greater enhancement factor.

Table 2  List of tip parameters specifying the scanning abilities of the tip*

<table>
<thead>
<tr>
<th>Probe (fig #)</th>
<th>Commercial&lt;sup&gt;*&lt;/sup&gt;</th>
<th>Home-made&lt;sup&gt;*&lt;/sup&gt;</th>
<th>Spring constant (N/m)</th>
<th>Resonant frequency (kHz)</th>
<th>Slope</th>
<th>Height (nm)</th>
<th>FWHM (nm)</th>
<th>Ratio Height/FWHM</th>
<th>Q factor</th>
</tr>
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<tbody>
<tr>
<td>Nanoprobe SPM tips&lt;sup&gt;*&lt;/sup&gt;</td>
<td></td>
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<td>256</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nanotools High Dense Carbon&lt;sup&gt;*&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>30</td>
<td>279</td>
<td>74.4°</td>
<td>39</td>
<td>54</td>
<td>0.72</td>
<td>267</td>
</tr>
<tr>
<td>Novascan NSC12 E&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.11</td>
<td>21</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Au wire on Nanoprobe&lt;sup&gt;*&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>65.2°</td>
<td>42</td>
<td>61</td>
<td>0.69</td>
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<tr>
<td>Au coated MWNT&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.34</td>
<td>66</td>
<td>75°</td>
<td>57</td>
<td>62</td>
<td>0.92</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Long MWNT&lt;sup&gt;*&lt;/sup&gt;</td>
<td>24</td>
<td>260</td>
<td>39°</td>
<td>58</td>
<td>140</td>
<td>0.41</td>
<td>297</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MWNT on Nanoprobe&lt;sup&gt;*&lt;/sup&gt; #29</td>
<td>26</td>
<td>264</td>
<td>68.6°</td>
<td>72</td>
<td>82</td>
<td>0.88</td>
<td>289</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MWNT on Nanoprobe&lt;sup&gt;*&lt;/sup&gt; #30</td>
<td>24</td>
<td>257</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MWNT on Nanoprobe&lt;sup&gt;*&lt;/sup&gt; #31</td>
<td>25</td>
<td>262</td>
<td>79°</td>
<td>82</td>
<td>80</td>
<td>1.025</td>
<td>277</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Explanation of the different parameters used to describe the different tips:
Slope: Angle (with respect to the glass surface) between the base of the imaged feature and the point at FWHM
Height: Maximum height of imaged feature
Ratio height/FWHM: indication of imaging quality. Since the beads are spheres the height and FWHM should be equal if the tip radius is small enough to image without the known artifacts induced by a large tip radius (12)
We expect good enhancement from sharp tips. It is clear that because of the sharpness of the nanotube we are interested in these sort of tips (sharp meaning high slope angle and a ratio height/FWHM close to one). In general the nanotubes that we used in this study have excellent imaging properties. The only exception is the long multiwalled nanotube (MWNT) that was custom made in Denmark. There is a strong indication that the nanotube was no longer attached to the normal tip on the cantilever, which is supported with the absence of buckling features in the forcecurves that we took after imaging (5). What also can be seen from the data in table 2 is that the nanotubes, although they have a good height FWHM ratio, the actual size of the bead is too large. This could be caused by the fact that the nanotube is too long and therefore not stiff enough to follow the bead correctly. Coating the nanotube with gold is apparently enough to make the tube stiff enough and improve the scanning in terms of size.

### Results

<table>
<thead>
<tr>
<th>Pair</th>
<th>Horizontal Dist. (µm)</th>
<th>Vertical Dist. (nm)</th>
<th>Surface Dist. (µm)</th>
<th>Angle (°)</th>
<th>R_max (nm)</th>
<th>R_z (nm)</th>
<th>R_2 Count</th>
<th>R_m (nm)</th>
<th>R_g (Peak cutoff) (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.111</td>
<td>-74.338</td>
<td>0.167</td>
<td>63.873</td>
<td>89.964</td>
<td>0.000</td>
<td>0.000</td>
<td>35.736</td>
<td>57.263</td>
</tr>
<tr>
<td>1</td>
<td>0.104</td>
<td>14.071</td>
<td>0.136</td>
<td>7.722</td>
<td>45.160</td>
<td>0.000</td>
<td>0.000</td>
<td>18.741</td>
<td>50.897</td>
</tr>
<tr>
<td>2</td>
<td>0.104</td>
<td>-60.391</td>
<td>0.146</td>
<td>30.193</td>
<td>76.185</td>
<td>0.000</td>
<td>0.000</td>
<td>29.241</td>
<td>47.715</td>
</tr>
</tbody>
</table>
Figure 6

Topographic (A) and optical (B) image of the same sample area.

Top right corner shows sections of different lines in the sample.

Table at the bottom states FWHM (first column) The topographic image shows a FWHM of ~100nm and the optical image a FWHM of ~400nm

Figure 6 compares a fluorescence and topographic image of the same sample area in the absence of any near-field effects. In the fluorescence image the size of the beads is determined by the diffraction limit (~400 nm in the case of 870 nm excitation) and the fluorescent profile is gaussian and if we compare this with the topographic image we see that in this image the size of the bead is close to the 40nm that is specified by the supplier. From the comparison of the two images the advantages of the AFM as an imaging system for known samples become clear, it is only when the wavelength of the fluorescence can give information about the nature of the sample that a normal diffraction limited imaging system becomes useful. As explained in the theory, we want to improve the optical resolution by introducing the AFM tip in the focus. The excitation field should be enhanced and this will improve the resolution because the signal becomes more localized and stronger. On top of this localized field there is also still the normal confocal excitation. In this study we never managed to see this increase in resolution, however we saw some other near-field effects of the tip.

The effect of the tip on the optical signal can be tested using the interleave mode of the Digital Instrument software. In this mode the sample will first be scanned in contact (trace and retrace) and then the tip will be lifted a controllable distance above the service to again perform a trace and retrace scan. In this case we looked at the optical signal generated by the High Dense Carbon tip on a single line. We are in particular interested in the relation between tip-sample distance ($d$) and the optical

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signal. Assuming a flat scatterer and a point source looking at (fig. 7) it is clear that the relation of the tip-sample distance to the radius of the detection cone is linear, resulting in a quadratic relation between tip-sample distance and detection area. Therefore we expect a $1/x^2$ decay of the countrate as a function of $d$. If there are any near-field effects the relation would be an exponential decay with a characteristic length in the order of 20 nm. If we would model the system as to interfering dipoles (8) we would expect the signal to increase due to constructive interference around 400 nm. Our data shows none of these expected forms (fig. 8). We see a exponential decay with a characteristic length of 250 nm (black datapoints, red fit) and 320 nm (red datapoints, blue fit). This result can not be explained by the models mentioned above.

![Graph showing exponential decrease of countrate with characteristic lengths of 250nm and 320nm](image)

**Figure 8** Tip-sample distance vs. countrate.  
*Graph shows an exponential decrease of the countrate with a characteristic length of 250nm (red fit) and 320nm (blue fit). Both data are taken with a High Density Carbon tip.*

Although in this investigation we never saw effects on the optical resolution we did see effects on the countrate when the tip is positioned in the focus of the laser. This effect is not limited to the points where the tip is actually on top of the bead but can be seen at every point of a scanline. One way of explaining this large “background” (it is actually a signal but not the signal we are interested in) is that the highly enhanced and localized field at the end of the tip generates a white light signal in the glass substrate of the sample. If this is the case this would indicate that although we are not able to increase our resolution, the principle of generating an enhanced electromagnetic field at the end of a metal tip is working. If the background is originating from white-light we expect that that the signal depends more than quadratically on the power. This means that plotting the power against the countrate on a log log scale gives a linear relationship with a slope higher than two.

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Figure 9  Power vs. Count rate on a log log scale.
For a High Density Carbon (HDC) tip (A) and a Multi Walled Nanotube (MWNT) tip (B). The data for the HDC tip are taken right after each other under the same conditions. Both tips show a signal that originates from a non-linear process other than two-photon excitation (table 3).

Table 3  Relation between excitation power and count rate for a single line when the SPM tip is placed in the optical focus.

<table>
<thead>
<tr>
<th>Tip</th>
<th>Offset (kHz)</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Density Carbon (red)</td>
<td>1.42</td>
<td>2.57</td>
</tr>
<tr>
<td>Fig 9A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Density Carbon (blue)</td>
<td>0.986</td>
<td>3.13</td>
</tr>
<tr>
<td>Fig 9A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long MWNT (red)</td>
<td>-0.03</td>
<td>3.42</td>
</tr>
<tr>
<td>Fig 9B</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

We see for both types of tips a slope larger than two. This means that both tips are optically active. For the MWNT this is important information because it is still not clear whether all MWNT should be metallic (10). This data shows that at least some MWNT have metallic properties. The fact that two consequetive measurements on the same tip yield different values for this exponent might indicate that our system is not sensetive enough to do this type of measurements quantitatively.
The aim of this research project was to identify tip properties that are essential for inducing enhancement of an electromagnetic field but unfortunately this project did not last long enough to achieve this.

A major problem with this set-up is that it is rather hard to operate in a controlled manner. It therefore takes a long time to get familiar with the machine. And even than getting to the point where all the scanning properties can be extracted from topographic data and getting to the step of putting the tip exactly in the focus can take up to 1.5 days for a single tip. So if we are ever able to increase the resolution with this set-up it is until now much to difficult to handle to do fast controlled measurements on biological systems with it. But the combination of an AFM and a two-photon confocal in itself opens up the possibility for many interesting research. Because it enables the user to get optical and topographic data simultaneously on the same sample.

Although we might have seen some examples of near-field or enhancement effects, these were not the effects that will ultimately lead to an increase of the optical resolution. For this it is important to be able to control the white-light generation in the sample. Samples in which the excitation and emission occur at two spatially separated places might adress this problem because in that case the collection of the emission signal takes place at a different position than where the white-light is generated.

Another problem seems to be the effect that all the steps in getting the alignment have on the tip. In three cases we lost the tip while aligning the tip in the focus. This not only costs time but also unique and sometimes expensive tips. There are at least two possible reasons for losing the tip. One can be the force of the laser on the tip. If we use the spring constant and the displacement of the trace(laser off) and retrace (laser on) we can estimate the force on the tip generated by the laser. For the long MWNT tip we find a spring constant of 23.7N/m, combining this with a height difference between trace and retrace line of 25 nm we get a force on the cantilever of 0.6 μN. The other can be the generation of heat in the focus of the laser (9). According to the paper of Kawata for the materials and powers we use this heat generation should be in the order of a few degrees celcius during a single pulse.

Looking at these two numbers heating does not seem to be a likely reason for losing the tip. To melt the materials our tips are made of you would need temperatures at least three orders of magnitude higher. Also the force on the tip is not that high, therefore I assume that there is another reason for losing the tip, like the translational force on the tip when moving it into the focus.

In my opinion this technique still needs a lot of further development before it can compete aperturebased NSOM, which has successfully been used by other groups (13) both on grounds of resolution and useability. Nevertheless it is interesting to see it theory and practice will eventually meet each other leading to an optical resolution in the order of a few nm.
References


2. Wessel, J (1985) *Optical Society of America* 2:1538-1540


14 References
Appendix

Table 1  Properties of used fluorescent beads

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emission maximum</td>
<td>$515 \pm 5$ nm</td>
</tr>
<tr>
<td>Diameter</td>
<td>$0.043 \pm 0.006$ μm</td>
</tr>
<tr>
<td>Fluorescein equivalents per microsphere</td>
<td>$3.5 \cdot 10^2$</td>
</tr>
<tr>
<td>Relative quantum yield (relative to methanol solution of the dye)</td>
<td>0.47</td>
</tr>
</tbody>
</table>

The specific filterset (short-pass dichroic filter (725) a BG 39 and a BP 525/75), objective (Zeiss Plan-Apochromate, N.A. 1.4 oil immersion) and APD used gives for an excitation wavelength of 870 nm a detection efficiency in the order of 6%.