

TIE-36 Fluorescence of optical glass

Introduction

Fluorescence is a phenomenon in which a substance absorbs light of a certain wavelength and almost instantaneously radiates light at longer wavelength (lower energy). These processes are known as excitation and emission. Many substances, organic as well as inorganic, can exhibit fluorescence. In life science applications like microscopes, fluorescence plays a major role in the optical design. The “primary fluorescence”, or “auto-fluorescence” of an optical material in a lens system leads to a significant problem in the application. In fluorescence microscopy it is desirable to have a very low background fluorescence. In such applications, fluorescing optical materials may decrease the contrast of the image. Especially in applications, where the fluorescence of fluorophores or dyes is detected, the fluorescence of optical components is generally not desirable, as it falsifies the detected signal (fluorescence of optical components as major source of unwanted background light in an image).

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1. Origin of fluorescence in glass

Excitation of an optical material at a given wavelength will primarily result in partial absorption and transmittance of the light. Most part of the absorbed light is transformed into vibrational energy, generating heat, but a part of it can be re-emitted as light of different wavelength (luminescence processes). Fluorescence is a luminescence process in which part of the absorbed light is re-emitted after ns to μs at longer wavelength (lower energy) and is known as Stokes shift. Many substances, organic as well as inorganic, can exhibit fluorescence. Fluorescence can be distinguished from phosphorescence by the lifetime or decay of the excited states, which is e.g., typically in the ms to s range for phosphorescence [1].

The efficiency of energy transfer of the absorption light, known as quantum efficiency, strongly depends on the excitation wavelength or wavelength spectrum. It has a maximum close to the absorption bands and high photon energy respectively.

The fluorescence is in contrast to the non-radiative absorption where the incident light is transferred to a higher energy level and the down conversion to lower energy levels without light

emission. This energy is absorbed as phonons (solid state vibrations), as heat or occurring as long living phosphorescence emission as mentioned earlier.

Fluorescence in optical glass is for instance generated by point defects like color centers, which are known to be originated by rare earth elements, and certain impurities [2][3][4]. The observed UV excited fluorescence correlates strongly with the content of UV absorbing ions in the material like rare earth elements (REE), Iron, Zinc or Vanadium [5] and also Pb²⁺. Most of these elements absorb and emit nearly independent (especially the REE) from the glass matrix.

Optical transitions from the ns²-levels (n=1,2,3,..), related to structural units of the glass matrix, like e.g. SbO₃, AsO₃ or ZnO, behave in a different way. Here the glass matrix and the processing (oxidation and reduction) have a strong influence on the absorption and emission characteristics.



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The fluorescence behavior of a glass can be influenced by either process technology or use of higher purified raw materials. In general the fluorescence of optical glass is very low in the spectral range of the application because of the very high transmittance and therefore low absorption of optical glasses in the visible spectrum. This is in contrast to filter glasses that have a desired blocking characteristic in certain ranges of the visible spectrum, here the absorption can lead to fluorescence [6].

Standard fluorescence measurement procedure at SCHOTT is the relative integral fluorescence measurement that generates a %- value fluorescence in comparison to the integral fluorescence of the reference optical glass SF1 containing a very high amount of Pb²⁺ as fluorophore.

2. Methods of characterization

The measurement procedure shown in figure 1 works as follows: On the excitation side a 450 Watt high pressure Xenon lamp delivers a broad band radiation spectrum from 200 to 1500 nm with a maximum intensity between 250 and 650 nm. This is the wavelength range that is mainly used for excitation. The radiation is spectrally selected and guided to the sample box by a Czerny Turner double monochromator which allows a high noise reduction in order to enable a signal to noise ratio better than 4000. The spectral linewidth of the excitation is 5–7 nm FWHM (full width half maximum) having an intensity of approximately 1 $\mu\text{W}/\text{cm}^2$. On request LED or Laser lights with higher intensities are possible as excitation source with linewidths of 20–30 and 2–10 nm FWHM respectively. The modular setup of this equipment allows an easy change of the excitation source.

The fluorescence light emits in all directions, but is either measured in scattering arrangement in reflection at an angle of 28° to the incident light (14° to the normal of the plane of the sample) to avoid the incident (excitation) light in the fluorescence measurement signal. The spectral fluorescence intensity is evaluated using a second Czerny Turner double monochromator and a photo multiplier as UV/VIS detector. Alternatively, pulsed excitation and a corresponding detection are also possible. Besides the intensity of the excitation source, the optimization of the detection efficiency depends on the proper choice of geometry and cut-off filter settings, which are required to have no intrinsic fluorescence at the applied wavelength.

The Fluorolog-3 system is part of SCHOTT's chemical and physical testing laboratories that are accredited within D-PL-14645-01-00 according to DIN ISO IEC/ 17025:2019. The typical emission spectral range is 300 nm to 850 nm.

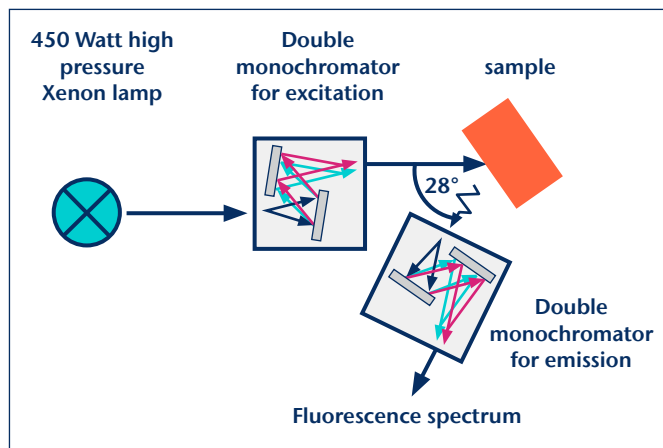


Fig. 1: Setup of the fluorescence spectrometer Jobin Yvon Fluorolog 3. The arrangement allows flexibility in geometry and excitation source. Due to the double monochromator concept no filters are required.

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3. Accuracy of method

For quantitative measurements the grating efficiency has to be evaluated using different types of calibrated standards from Starna (Starna Company (Essex, UK) and BAM (Bundesanstalt für Materialforschung und -prüfung [Berlin, Germany])). The grating consists of 1200 lines/mm and has maximum reflexion efficiency at 420 nm due to blazing of the grooves (fig. 2). For further measurements the spectrometer software allows us to correct the original spectrum with a flat line spectrum simultaneously. One has to consider that the numerical correction is most accurate in the range of the maximum sensitivity and decrease with the loss in sensitivity. The sensitivity range defines the detectable wavelength range. This means that emission between 300 and ~850 nm can be investigated.

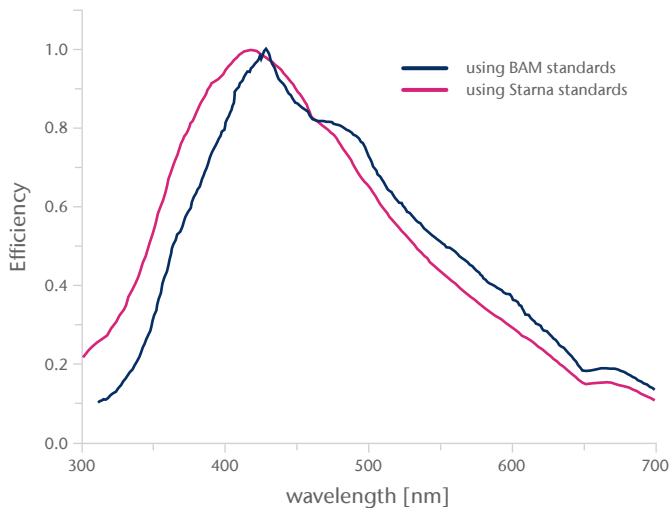


Fig. 2: Efficiency for 1200 lines/nm grating and the PM detector. The maximum efficiency is observed for appr. 420 nm.

With this setup emission spectra, as well as excitation spectra, are obtainable. The emission spectra are recorded by keeping the excitation wavelength constant, whereas the excitation spectra are recorded by keeping the emission wavelength constant.

For determination of the wavelength accuracy the emission, excitation and absorption spectra of doped samples have been investigated. In figure 3 these spectra are visualized using about 485 ppm TmF_3 as dopand in CaF_2 crystal. Doped CaF_2 is used because it exhibits a higher light yield compared to optical glass and can therefore be used even for less sensitive fluorescence spectrometers.

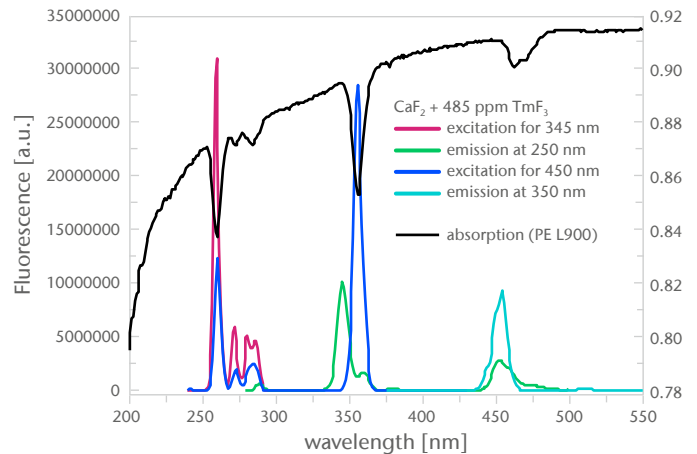


Fig. 3: Absorption, excitation and emission spectra of TmF_3 doped CaF_2 .

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Results have been compared using theoretical and analytical work and data from literature. The absorption, excitation and emission spectra for this sample have been obtained. It is observed that the peak of absorption and excitation spectra is exactly on the same position and therefore the consistency of absorption and excitation spectrum becomes visible. This means that the accuracy is close to 0.1 nm (resolution limit of the spectrometer) and well below the common step width of 1 nm. The emission peaks at 350 and 450 nm are situated on the same position as expected from the literature and known from optical glass when excited with 250 and 350 nm respectively.

The accuracy of the spectrometer has been evaluated using certified standards BAM-F01 to BAM-F05 [8][9] In figure 4 the emission spectra of Quinine Sulphate certified by NIST is shown to underline the correctness of the emission profile.

A relative accuracy or reproducibility better than two percent for the FLUOROLOG 3 spectrometer is observed. The absolute uncertainty – considering the uncertainty due to the calibration of 5% – is therefore given to 7% over the full emission range between 300 and 850 nm.

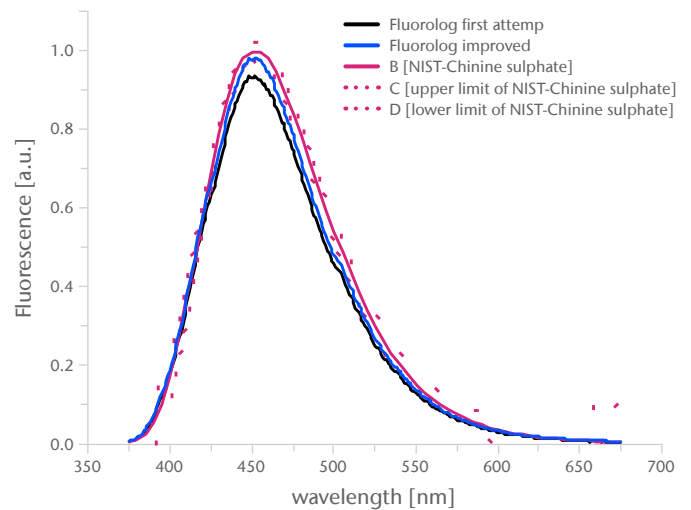


Fig. 4: Emission spectrum of NIST certificates fluorophore Quinine Sulphate.

4. Evaluation of the integral fluorescence

Typical fluorescence measurement results on N-FK58 XLD are shown in Figure 5 for 365 nm, 488 nm, and 532 nm excitation. N-FK58 XLD is an eXtremely Low Dispersion glass that is used in optical design for apochromatic color correction.

For each single excitation wavelength two fluorescence spectra are shown: The fluorescence signal of the N-FK58 XLD sample is compared to the spectrum of SF1, which inhabits a high fluorescence level and acts as a reference sample in all further evaluation. The dashed lines represent always the excitation wavelength (not to scale) and the dashed-dotted curves represent the spectrum of the SF1 reference at that excitation

wavelength. Analog to the publication from Reichel et. al on fluorescence measurements on optical filter [6], the fluorescence of optical glasses can be characterized by a single parameter that is called the Relative Integral Fluorescence (*RIF*). For 365 nm excitation the *RIF* is defined by:

$$(1) \quad RIF_{365} := \frac{\int_{370}^{700} I_{sample}(\lambda) d\lambda}{\int_{370}^{700} I_{SF1}(\lambda) d\lambda}$$

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Where $I_{sample}(\lambda)$ is the measured fluorescence spectrum (intensity) of the sample (N-FK58 XLD in Figure 5) and $I_{SF1}(\lambda)$ is the reference fluorescence spectrum of SF1 as a function of the wavelength. The subscript 365 in RIF_{365} refers to the excitation wavelength at 365 nm. According Eq. (1) the RIF_{365} of SF1 is 100%. The RIF_{365} of N-FK58 XLD was measured to be $RIF_{365} = 0.5\%$ of the integral fluorescence spectrum of optical glass SF1.

Similar to Eq. (1), the relative integral fluorescence for 488 nm excitation (RIF_{488} – Eq. (2)) or 532 nm excitation (RIF_{532} – Eq. (3)) is defined by:

$$(2) \quad RIF_{488} := \frac{\int_{535}^{800} I_{sample}(\lambda) d\lambda}{\int_{535}^{800} I_{SF1}(\lambda) d\lambda}$$

$$(3) \quad RIF_{532} := \frac{\int_{580}^{850} I_{sample}(\lambda) d\lambda}{\int_{580}^{850} I_{SF1}(\lambda) d\lambda}$$

The RIF at 405 nm and 642 nm excitation is defined accordingly. Note the different integration ranges (wavelength ranges) of the 3 definitions, Eq. (1), Eq. (2), and Eq. (3).

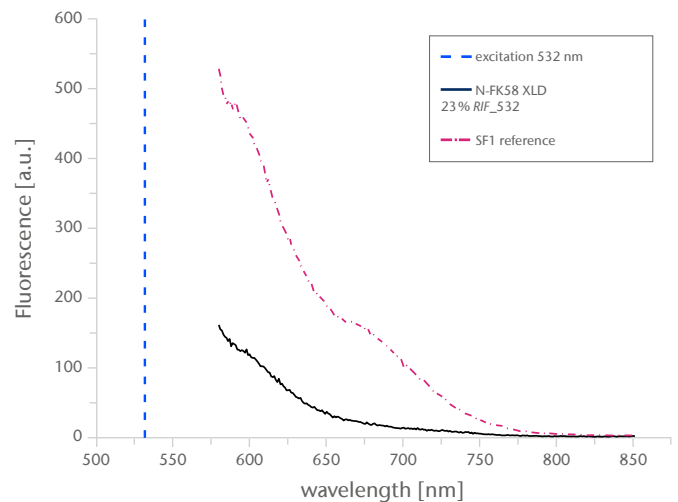
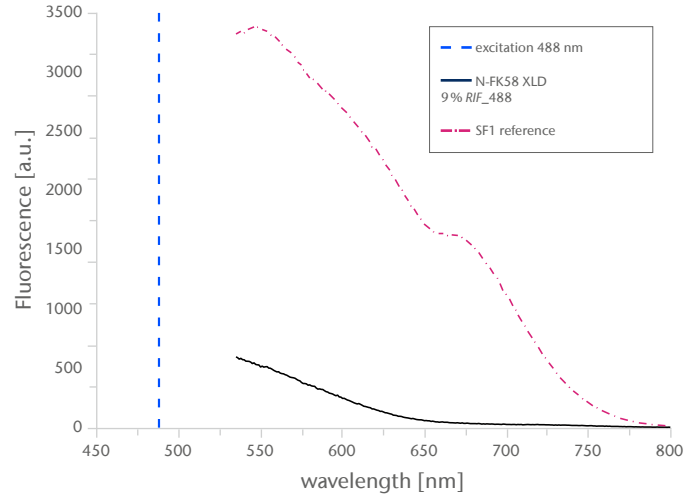
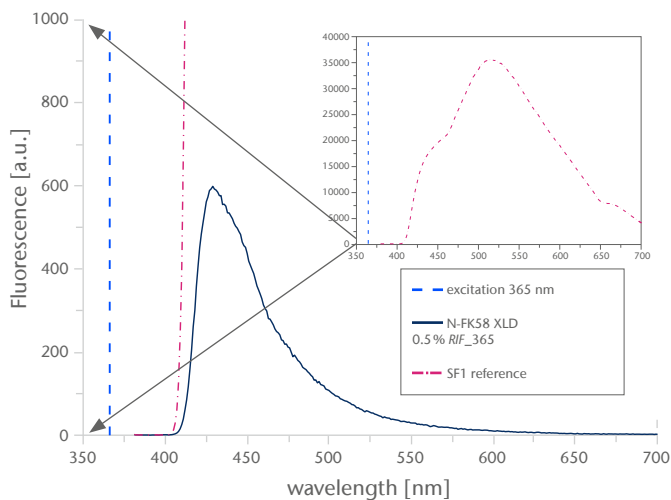


Fig. 5: Fluorescence spectrum of N-FK58 XLD with 4.97 mm sample thickness and SF1 (5.0 mm thickness) which serves as fluorescence standard. Top: measurement result for 365 nm excitation. Middle: measurement result for 488 nm excitation. Bottom: measurement result for 532 nm excitation. Note the different scale of the y-axis.

For N-FK58 XLD the relative integral fluorescence for an excitation at 488 nm was measured to be $RIF_{488} = 9\%$ whereas for an excitation at 532 nm it was measured to be $RIF_{532} = 23\%$. Again RIF_{488} of SF1 is equal to 100% as well as the RIF_{532} of SF1 is equal to 100%.

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5. Fluorescence results for optical glass

Figure 6 display emission spectra at an excitation wavelength of 365 nm measured for a variety of optical glasses with identical measurement setup parameters (slit widths, integration time, detection regime). The integral fluorescence decreases from SF57HTUltra to N-KZFS8 according to the sequence of the graphs legend.

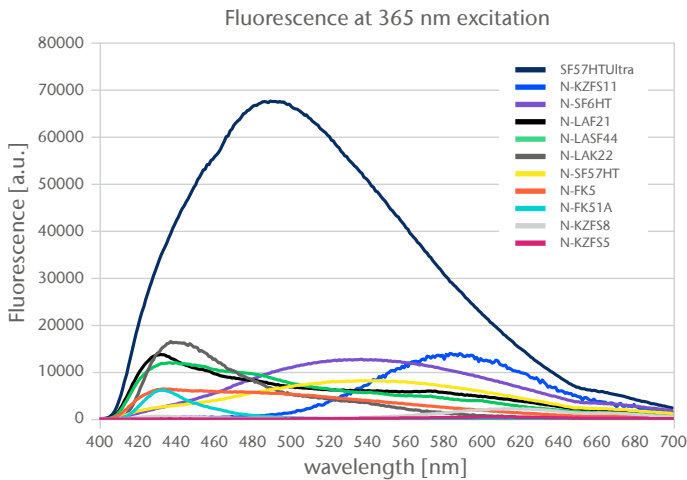


Fig. 6: Emission spectra of low fluorescent optical glasses using 365 nm excitation wavelength and a cut off filter (KV 418)

Data on the relative integral fluorescence (RIF) of optical glass at 365 nm excitation is shown in Figure 7. The data shows typical values for the glass type.

The SF1 reference sample is included in the diagram with a value of 100%. The second SF1 data column reflects the progress in melt technology over the years. The fluorescence of SF1 glass from recent production runs shows much lower RIF values than the much older SF1 reference glass.

In general, the N-type glasses show lower RIF values than the non N-type glasses. The environmental friendly N glasses have been designed to be free of lead, arsenic and antimony. Especially lead shows strong fluorescence at 365 nm excitation.

As has been shown above N-FK58 XLD and all the other fluoro-phosphates glasses are among the glasses with the lowest RIF values around 1%.

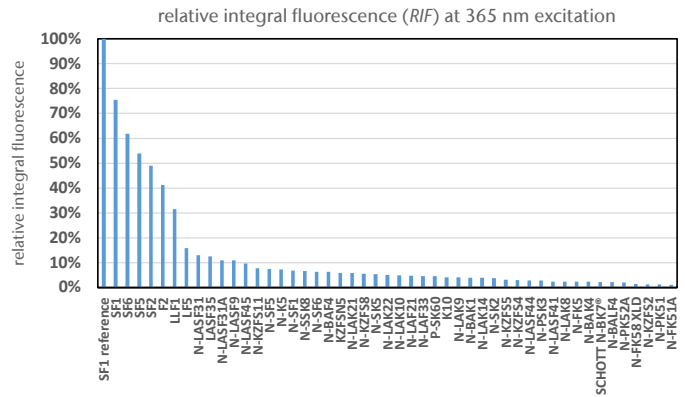


Fig. 7: Relative integral fluorescence of optical glasses at 365 nm excitation. The RIF of the SF1 reference is set to 100%.

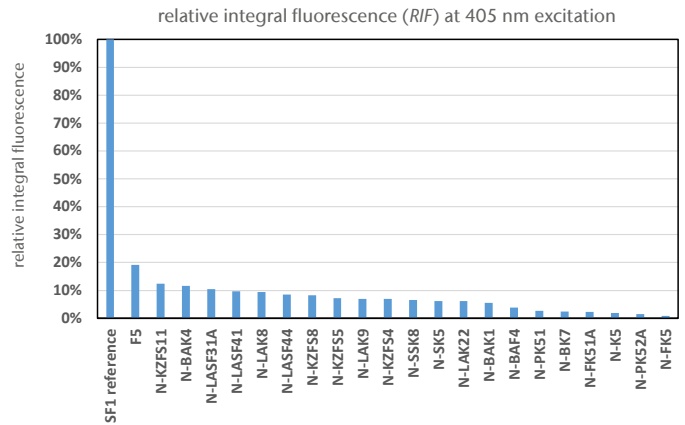


Fig. 8: Relative integral fluorescence of optical glasses at 405 nm excitation. The RIF of the SF1 reference is set to 100%.

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Figure 8 shows the relative integral fluorescence at excitation at 405 nm wavelength. The SF1 reference again marks the *RIF* 100% limit. A smaller total number of glasses have been investigated at this wavelength. The glass with the lowest *RIF* value is N-FK5 within the range of tested glasses.

Figure 9 shows the relative integral fluorescence at excitation at 488 nm wavelength. The SF1 reference again marks the *RIF* 100% limit. The SF1 from current production runs shows much lower *RIF* values (less than 60%) than the older reference sample. N-SSK8 shows a higher *RIF* than the SF1 reference. The differences between the fluorescence of N-type glasses to non N-type glasses is not so clear. The non N-type glasses are mixed in the *RIF* ranking. The glass with the lowest *RIF* value is N-FK58 and the non N-type glass with the lowest value is LLF1. One can conclude that excitation at 488 nm is less effective than at 365 nm and the origin of fluorescence is different compared to 365 nm excitation.

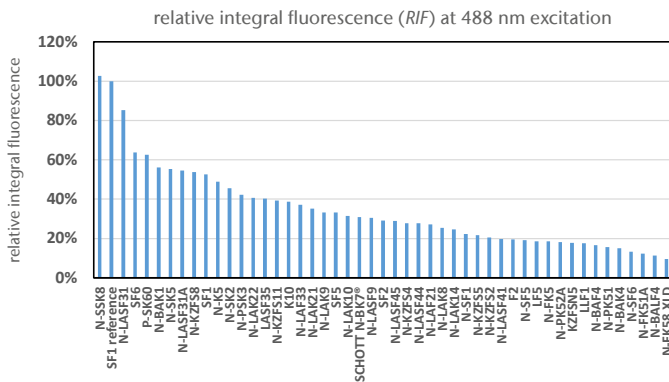


Fig. 9: Relative integral fluorescence of optical glasses at 488 nm excitation. The *RIF* of the SF1 reference is set to 100%.

It has to be pointed out there is no correlation between the 365 nm and the excitation larger than 400 nm. The reason is that the UV excitation/absorption depends mainly on impurities whereas for larger excitation wavelengths respectively lower photon energies, where merely absorption bands are observed, the glass composition is the driver for the fluorescence features.

Figure 10 shows the relative integral fluorescence at excitation at 532 nm wavelength. The SF1 reference marks the *RIF* 100%. This time many glasses show a higher *RIF* compared to the *RIF* 100% of the SF1 reference. N-BAK1 shows *RIF* value way above 600%. N-FK58 is still among the glasses with the lowest fluorescence.

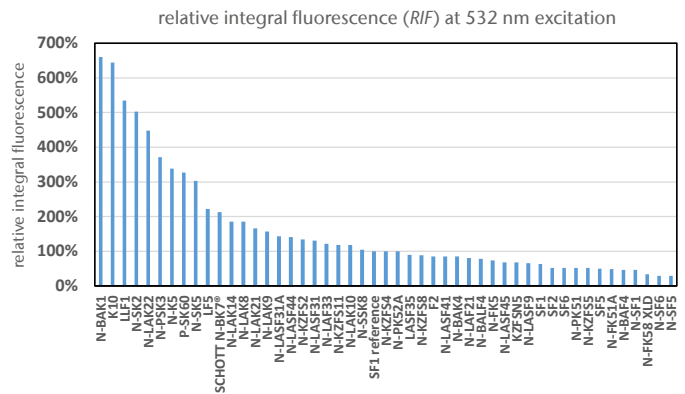


Fig. 10: Relative integral fluorescence of optical glasses at 532 nm excitation. The *RIF* of the SF1 reference is set to 100%.

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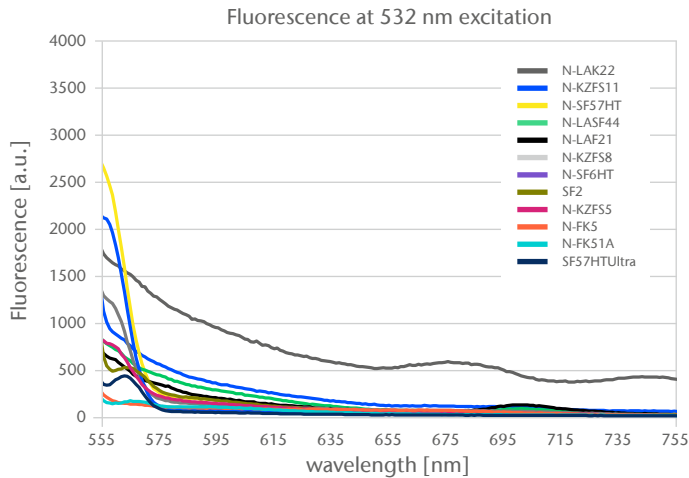


Fig. 11: Emission spectra of optical glasses using 532 nm excitation wavelength and without cut off filter.

In Figure 11 typical emission spectra using 532 nm excitation wavelength are shown for several optical glasses. Using the identical experimental setup the shape of the emission profiles are similar, but the measured intensities in are different and overall much lower. The *RIF* decreases from N-LAK22 to SF57HTUltra (according to the sequence of the graphs legend)

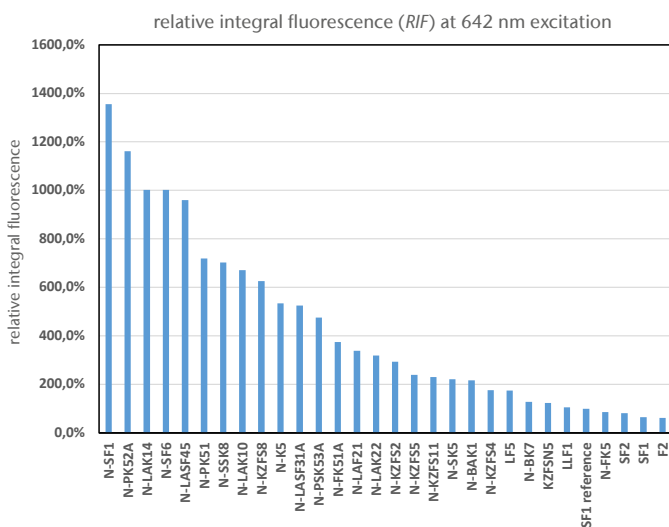


Fig. 12: Relative integral fluorescence of optical glasses at 642 nm excitation. The *RIF* of the SF1 reference is set to 100%.

Figure 12 shows the relative integral fluorescence at excitation at 642 nm wavelength. The SF1 reference marks the *RIF* 100%. Again many glasses show a higher *RIF* compared to the *RIF* 100% of the SF1 reference. N-SF1 and N-PK52A show a factor of 100 higher fluorescence compared to SF1. SF1 is among the glasses with the lowest fluorescence at 642 nm excitation.

The diagrams Figure 7, Figure 8, Figure 9, Figure 10 and Figure 12 are irritating in a way that they only show the relative tendency to produce fluorescence light between the different glass types at the given excitation wavelength.

Figure 13 shows the SF1 reference sample spectra for 365 nm, 405 nm, 488 nm, 532 nm and 642 nm excitation. With increasing excitation wavelength the maximum and area of the spectrum decreases. Setting the area below the SF1 reference spectrum at 365 nm to 100% the *RIF*'s for SF1 reference from 405 nm to 642 nm can be calculated as shown in the legend of the diagram. The *RIF* of SF1 at 642 nm excitation is only 0.04% of that at 365 nm excitation.

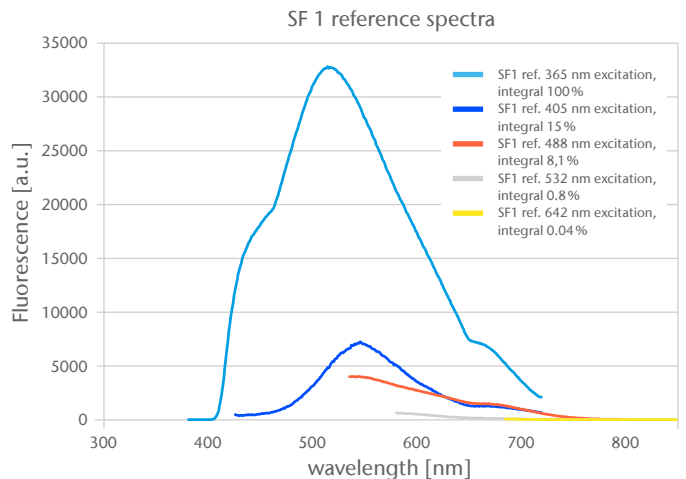


Fig. 13: SF1 reference sample spectrum for 365 nm, 405 nm, 488 nm, 532 nm and 642 nm excitation.

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The relative fluorescence of SF1 between the different excitation wavelengths can be used to compare *RIF*'s of a glass at different wavelengths. The results shown in Figure 7, Figure 8, Figure 9, Figure 10 and Figure 12 can be scaled (multiplied) with 100% (365 nm), ~15% (405 nm), ~8% (488 nm), ~0.8% (532 nm) and ~0.04% (642 nm) to take into account the much lower absolute fluorescence in arbitrary units [a.u.], this is what we call the “weighted *RIF*”. Figure 14 shows the weighted relative integral fluorescence *RIF* for several glass types in comparison.

The diagrams do not give any information regarding the quantum efficiency of the process at different wavelength. In general the quantum efficiency for fluorescence decreases with increasing wavelength and optical glasses in general show very low quantum efficiency values. This will be shown in the next chapters.

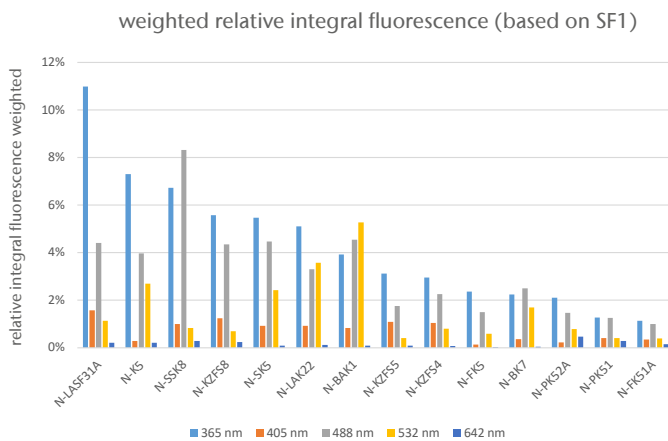


Fig. 14: Weighted relative integral fluorescence based on SF1 to compare the glasses at different excitation wavelengths.

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6. Fluorescence quantum efficiency measurement set-up [1]

The measurement of RIF has the advantage of relatively high precision, but it is a relative measurement. Thus the absolute value of “how many of the incident photons are emitted as fluorescence photons” is not given by this parameter. In order to get an estimation of the absolute fluorescence level, the (external) fluorescence quantum efficiency (FQE), is measured for N-FK58 XLD and given by:

$$(4) \quad FQE = \frac{\int I_{fluor}(\lambda) d\lambda}{\int I_{in}(\lambda) d\lambda},$$

where I_{fluor} is the fluorescent light spectrum (emitted by the probe) and I_{in} is the incident light spectrum (integrated over the whole wavelength spectrum).

FQE is measured using the instrument C9920 from the company Hamamatsu. The basic set-up is shown in Figure 15.

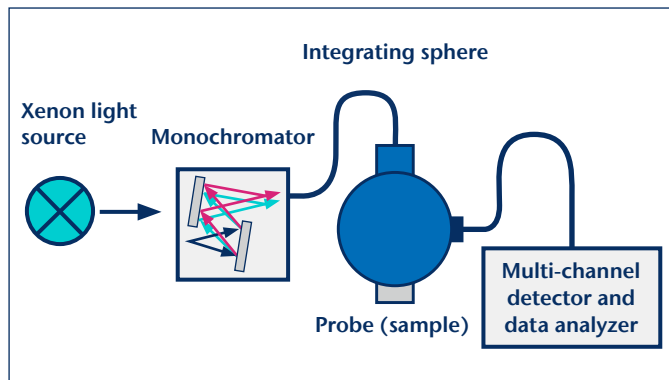


Fig. 15: Basic set-up for measuring the (external) absolute fluorescence quantum efficiency. By using a calibrated Xenon light source and a monochromator the excitation wavelength can be chosen. An integrating sphere measures the whole fluorescent light by a multichannel detector. All components have been calibrated by Hamamatsu.

The C9920 uses a Xenon light source and a monochromator to precisely adjust the excitation wavelength with a resolution better than 5 nm. The excitation light is guided into an integrating sphere. The sample is mounted inside the integrating sphere under a small angle well below 10° to suppress front- and backside reflection of the probe. The multi-channel detector is mounted at a 90° angle relative to both the excitation light and the probe and measures the complete fluorescent light avoiding any impact of the fluorescence emission angle characteristics. A data analyzer on a computer calculates the FQE .

The C9920 can estimate the self-absorption using with the same device. The self-absorption corrected effective FQE (Absorption * FQE) is the best value to describe the absolute amount of fluorescence light generated from a given illumination intensity. In a comparative experiment together with BAM (Federal Institute for Materials Research and Testing, Berlin, Germany) using a sample with perspective to act as a standard, we achieved an accuracy of $\pm 2\%$ and a re-productibility of better than $\pm 0.5\%$ for the un-corrected FQE . The required sample size is 5 – 18 mm in diameter and a thickness between 0.5 mm and 5 mm.

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7. Fluorescence quantum efficiency measurement results [1]

The *FQE* was measured for a variety of optical glasses including the SF1 reference at an excitation of 365 nm and 488 nm. The results can be found in Table 1.

	365 nm excitation			488 nm excitation		
	<i>FQE</i> [%]	Absorb. [%]	eff. <i>FQE</i> [%]	<i>FQE</i> [%]	Absorb. [%]	eff. <i>FQE</i> [%]
SF1	3.5	41.7	1.46	4.7	1.4	0.07
SF6	0.7	76.0	0.53	n.m.	16.4	n.m.
N-KZFS8	1.1	42.6	0.47	0.15	8.5	0.01
SF57	0.5	89.3	0.45	0.8	12.7	0.10
N-SF6	0.4	93.0	0.37	n.m.	19.9	n.m.
N-LASF45	0.5	76.0	0.34	0.2	22.7	0
N-LAK9	0.8	36.0	0.29	n.m.	17.3	n.m.
N-BAK4	0.9	28.0	0.25	n.m.	10.2	n.m.
SCHOTT N-BK7®	0.4	9.5	0.04	0.1	4	0.00
N-FK58 XLD	1.3	3.1	0.04	5	0.8	0.04
OG590 [5]	1.7	71	1.2	1.8	83	0.05

Tab. 1: Effective *FQE* of several optical glasses and OG590 filter glass in comparison. "n.m." means "not measurable"

Among the optical glasses SF1 shows the highest *FQE* of 3.5%. The effective *FQE* is about 1.5% with an absorption measured of 41.7% with the C9920. The lowest effective *FQE* at 365 nm excitation was found in N-FK58 and SCHOTT N-BK7® (in both cases the effective *FQE* is less than 0.05%). The effective *FQE* of SF57 and N-KZFS8 is a factor of 10 higher. The values at 488 nm excitation are in general smaller for all optical glasses due to the lower absorption, except for N-FK58 that exhibits an effective *FQE* of 0.04% at 488 nm excitation, identical to the effective *FQE* at excitation at 365 nm. For many optical glasses the fluorescence signal was too low in the C9920 at 488 nm excitation. These results are indicated with "n.m.: not measurable" at 488 nm excitation. To judge the reliability of the measurements it is important to look at the measured spectral intensity distribution.

Figure 16 shows the spectral response of the multi-channel detector during measurement for excitation at 365 nm of SF1, N-KZFS8, SCHOTT N-BK7® and N-FK58 XLD. The excitation peak at 365 nm is clearly visible. The intensity at the excitation peak in comparison to the spectrum in air reflects the absorption of the glass. At longer wavelengths most of the measurements are on the same level as the spectrum in air. No significant fluorescence can be detected. The only exception is SF1, which is significantly above the other signals. A similar behaviour can be observed in Figure 17 for excitation at 488 nm. Even the fluorescence of SF1 is not dominant anymore. The signal is on noise/air level.

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Therefore, the absolute results in Table 1 should be interpreted with care. At least it is obvious that the internal fluorescence level of most of the optical glasses is below 1% at 365 nm and decreases almost by an order of magnitude at 488 nm excitation. The C9920 is not sensitive enough to

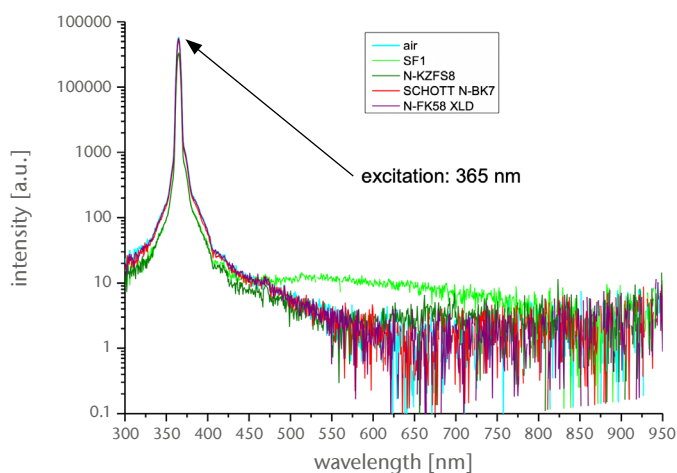


Fig. 16: FQE measurement spectrum of different optical glasses at excitation of 365 nm.

generate reliable fluorescence quantum efficiency values for optical glasses. The sensitivity must be an order of magnitude higher at least. The C9920 is optimized for substances with fluorescence levels significantly above 1%.

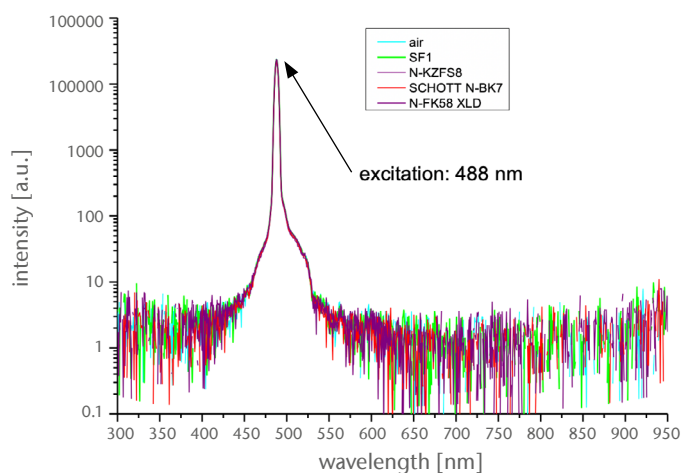


Fig. 17: FQE measurement of optical glasses at excitation of 488 nm.

8. Literature

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