SUPPLEMENTARY MATERIALS

Optical properties of Rhodamine 6G

Figure S1. (a) Absorption coefficient of different concentrations of R6G in ethanol normalized to the value. Inset shows the normalized absorption coefficient. The flattening of the absorption peak around 530 nm with higher concentration is due to absorption saturation effects, since transmission is close to zero [1]. (b) Normalized PL intensity of different concentrations of R6G in ethanol under 480 nm excitation. The dashed line shows the PL spectrum of a 1.6 μM concentration sample, measured outside of an IS, for which re-absorption effects are negligible. (c) Time-resolved PL intensity excited at 485 nm for a 120 μM (red) and 6.4 μM concentration of R6G in ethanol. Inset: lifetimes for different concentrations under 440 nm (black) and 485 nm (red) wavelength excitation. The lifetimes are obtained from a mono-exponential fit yielding ~3.8 ns.

Absorption, photoluminescence (PL) spectra and decay are shown in Fig. S1. The time-resolved PL was measured using an inverted confocal microscope (Olympus, FV1000) equipped with a TCSPC module (Picoquant, Picoharp). For excitation, we used a pulsed 440 or 485 nm wavelength laser diode (Picoquant, LDH-P-C-440B or LDH-P-C-485) operated at 20 MHz (~2.7μW or 0.4μW) focused to diffraction limited spot by a 60x water immersion objective (Olympus, UPLS Apo, NA=1.2). Light is collected by the same objective lens, filtered through a 562/40 band-pass filter and detected using an avalanche photon detector (MPD, PDM). For the measurements the R6G solutions were contained in a 96-well plate.

The maximum QY that we find experimentally for R6G in Fig. 2 (in the main text) and Fig. S2 is ~86%, which is underestimated with respect to the literature value of 95% [2]. In contrast, under indirect excitation conditions we measure a QY which approaches 95% as shown in Fig. S2. To understand this difference, we simulate the QY measurements under direct (F=1) and indirect (F=0) excitation conditions for the IS geometry as described in the materials section and shown in Figs. 1 and S4. The ray tracing simulation (RTS) approach (described in detail in the next
section) shows that in our specific geometry the QY is underestimated by a factor of ~0.91 under direct excitation, whereas under indirect excitation a QY estimate close to the real value is obtained (Fig. S2b). These simulated values are in good agreement with the experimental QY values obtained for direct and indirect excitation (Figure S2b). The underestimation is related to the specific geometry of the IS setup 1, which contains home-built aluminum parts that serve as holders for the cuvette and the fibers. Indeed, when we replace the aluminum in our RTS approach with a Lambertian scatterer, both under direct and indirect excitation we obtain the correct QY value (black points in Fig. S2b). We note here that aluminium parts are firmly excluded as a possible source of the artifact or absorption shift, evidenced by the RTS results. The ability to deal with a non-uniform IS geometry shows the strength of RTS for modeling of the IS optics, which is not possible with the AM since it assumes a uniform IS geometry. Despite the fact that our geometry leads to slightly underestimated QY value (86% instead of 95% for high absorption), we note that our simulations (solid lines Fig. S2b) reveal no dependence of the QY on the absorption. Furthermore, measurements carried out in a completely different IS setup, shown in Fig. 2g-i (in the main text) and S3, again show an underestimation of the QY for weakly absorbing samples. Hence we conclude that the absorption-dependence of the QY does not arise from the specific geometry of our IS setup, but results from the QY methodology itself (as discussed in more detail in the main text).

![Figure S2](image)

**Figure S2.** (a) QY versus excitation wavelength of R6G in ethanol (~14 μM) with the sample under direct (blue) and indirect (red) illumination conditions. The solid line shows the single-pass absorption of the sample. (b) QY plotted against the single-pass absorption. The solid lines show the QY values simulated by RT under direct (blue) and indirect (red) excitation for a real QY of 95%. For these simulations we assume an IS geometry similar to that used to obtain the experimental QY values, which includes aluminum parts to hold the optical fibers and cuvette. Black points show QY values obtained by RTS simulations (circle, F=1; square, F=0) where we artificially replaced these aluminum parts by diffuse reflective parts.
Figure S3. QY of ~60μM (black) and ~6 μM (gray) solution of R6G in ethanol under direct excitation plotted against the excitation wavelength (a) and single-pass absorption (b). Measurements were carried out in QY setup 2 described in Materials and Methods in the main text.

Ray-tracing simulations
For the ray tracing simulation (RTS) we model the geometry of our experimental setup as shown in Fig. S4. The sphere is modeled as a well subdivided icosahedron with approximately 8·10³ triangles. The light distribution inside the IS is described by the radiative transfer equation [3] that can be solved by means of Monte-Carlo simulations. Multiple paradigms how to do such simulations exist [4-6]; due to the combination of materials present in our setup, here we restrict our model to tracing light particles (“photons”) from the input port (contains the excitation source) to the output port (contains the sensor). By “photon” we denote a simulation particle with associated weight which represents the differential flux carried by the particle [5]. For simplicity we model neither polarization, nor reabsorption events and we only keep notion of two types of photons characterized by two respective wavelengths: excitation and emission photons (\(\lambda_{\text{exc}}\) and \(\lambda_{\text{em}}\)). Excitation photons colliding with the active part of the sample can be transformed into emission photons.
Figure S4. Schematic of the integrating sphere used for quantum yield measurements for setup 1: Side view (a), top view (b) and zoom in of cuvette. (c) Different materials are indicated by colors; Lambertian diffuse surfaces with high reflectance (blue), quartz glass materials (red) and brushed aluminum surfaces (gray). Numbers indicate the dimensions in mm.

Figure S5. Possible paths in the IS for excitation photons (green) which are converted to emission photons (red).
Photons interacting with any surface or absorbing volume are attenuated and scattered according to an appropriate phenomenological model:

- The IS surface, baffle and the cuvette’s cap are modeled by a Lambertian model with reflectance at both wavelengths set to 0.97.
- Scattering on glass and aluminum parts is modeled according to Fresnel equations for unpolarized light.
- Scattering/absorption in the volume of the active sample is modeled according to the radiative transfer equation where we set the absorption coefficient in the range 0.02 cm\(^{-1}\) to 2 cm\(^{-1}\) to study single-pass absorption values between 2% and 87%, respectively.
An example of the path of an excitation photon is depicted in Fig. S5. A fraction of the initial photon flux that enters the sphere via the input port can, after a series of scattering events on various surfaces, end up in the active volume of the sample. There we sample the possibility of a photon being absorbed along its way across the volume (according to Lambert-Beer law) or leaving the volume unaffected. If absorption occurs, we sample the possibility that the photon is (re-)emitted with a probability given by the PL QY. Emission photons are emitted in a random direction uniformly distributed from the point of absorption. We do not consider re-absorption, which has been discussed in detail elsewhere [7], by setting $A(\lambda_{em}) = 0$. Photons of both kinds (i.e. excitation and emission) scatter around in the sphere, but also can be absorbed by loss channels such as the input port (modelled as Lambertian surfaces with 0 reflectance) or by the IS coating. Paths are terminated when they hit such a loss channel. Moreover to prevent photon paths of infinite lengths, we use the so called Russian roulette technique [4,6] to stochastically terminate the simulation of photons with some probability after each interaction. In case the photon’s path is not terminated its energy weight is proportionally increased in order to compensate for terminated photons in such a way that the expected result of the simulation stays unaffected.

**Figure S6.** Input (a) and output (b) port geometries. The basic unit is derived from the size of an optical fiber with a diameter of 1mm. The loss channels are modeled as a small rectangular geometry of zero reflectance. The input geometry (a) is capable of rotation around Y-axis while angle of emission is fixed and set to 3.4 degrees. The output geometry (b) cannot be rotated and acceptance angle is set to 20 degrees.

The input and output port geometries containing the excitation source and sensor are depicted in Fig. S6. Since in reality, light can exit the sphere via these ports, we insert in our model loss channels behind the excitation source and sensor so that photons hitting these are also removed from the IS. The loss channels are modeled as small rectangular geometries with zero reflectance sitting on aluminum beds. The excitation source in the input port is represented as a small
rectangular region in space without actual visible geometry associated. Each point of that region sends out photons into a cone with angle of 3.4 degrees. The sensor in the output port is modeled as a pinhole camera without any associated geometry either, with a field of view equal to 20 degrees, which approximates the optical fiber with NA = 0.22 used in our experiments. Within the cone set by the NA, the sensor measures the flux impinging on it from different directions. This cone is divided into a disjunct set of cells (“pixels”) each of which accumulates weights of excitation and emission photons coming from the associated directions. The resulting accumulated values in all cells are regarded as realizations of the same underlying random variable for which we estimate the mean and variance. They represent the incoming flux on the sensor, which is directly analogous to the measured photons count in the real experiment. For estimating the QY we follow the methodology of the physical experiment described in Eq. 2 (in the main text) using a test and a reference sample, where the latter does not absorb. The cuvette in the setup is modeled according to a typical commercially available cuvette (Hellma Analytics). There are several material interfaces (Air/Glass, Glass/Air, Glass/Liquid, Air/Liquid) which are accounted for in order to achieve appropriate accuracy of our model (depicted in Fig. S4c). The cap is modeled as Lambertian diffuser with the reflectance of 0.9. The cuvette holder is made of aluminum and serves also as a baffle preventing light going from cuvette directly towards output port. Reflectance of aluminum at $\lambda_{\text{exc}}$ and $\lambda_{\text{em}}$ wavelengths is computed from Fresnel equations.
Analytical model

Figure S7. Schematic of a generalized IS setup. Lines represent the different pathways for excitation (blue) and emission (green) light between different objects inside the IS: wall (w), loss channel (l), detector (d) and sample (s); $p_{xy}$ represents the probability of going from x to y. Light paths shown by dashed lines are prevented by baffles.

In this approach we simplify the setup illustrated in Figure S4 by assuming a general IS geometry as shown in Fig. S7 and described in detail in Ref. 8. Within this geometry we simulate the detected and absorbed intensities after each consecutive reflection of the excitation and emission light, where we separate the first reflection ($n=1$) from the consecutive ones ($n>1$). For $n>1$, we assume that light reflected from the different areas of the integrating sphere wall has the same probability of hitting an object in the IS, which we set to the relative area of the object to the area of IS interior. Furthermore we assume that the reference sample does not absorb, that the IS is coated with an ideal diffuse light scatterer with a reflectivity of 0.97 and 0.99 at the excitation and emission wavelength respectively and that light can be detected only after the
second reflection only due to the presence of baffles. For detailed equations, we refer to Ref 8. Simulations were carried out for an IS with a diameter of 10 cm, a spherical sample with a diameter of 1 cm and in- and output ports with a diameter of 4 and 1 mm respectively. To account for measurement uncertainty we add noise in to our simulations by describing the measured photon intensities predicted by the AM [8] by a normal distribution. By drawing semi-randomly from this distribution we obtain the probability distribution of the simulated QY. The mode of this distribution is taken to be the most-likely measured QY. For more details we refer to Ref. 8.

**Signal offsets**

In Fig. S8 we show non-linearity of the detected signal for low absorptances for emission and absorption photon counts for all three samples. While R6G (panel a) and Si QDs (panel c) exhibit signal offset only in absorption signal (1.7% for R6G and 2.2% for SiQDs), CdSe QDs also exhibit signal offset in emission signal (panel b), adding to the decrease in QY. Interestingly enough, QY of CdSe QDs after correction (Fig. 6 in the main text) still shows decrease in QY, which appears to be related to a real effect, possibly ligand instabilities, often reported for such systems.

![Figure S8](image)

**Figure S8.** Fraction of emitted (green) and absorbed (gray) photons plotted as a function of the single-pass absorptance for (a) R6G, (b) CdSe QDs and (c) Si QDs. Solid black lines are fit using analytical model (AM) approach.

**Statistical nature of the signal**

In Fig. S9 we show experimental data for the repeated measurement of the transmission signal for sample and reference $N_S$ and $N_{ref}$. This is done for two samples with different concentration and for different excitation wavelengths, to achieve single pass absorptances of 20%, 2%, 0.8% and 0.6%. Assuming normal distribution of the photon counts, we evaluate the experimental uncertainty to be $\sim$0.3%, indicating very precise measurement.
Figure S9 – Histograms of measured photon counts $N_S$ and $N_{ref}$ of Rhodamine 6G in ethanol for different values of the single-pass absorptance (a) 20%, (b) 0.6%, (c) 2% and (d) 0.8%, achieved by varying sample’s concentration and excitation wavelengths. The histograms are assumed to follow normal distribution, from which the experimental uncertainty of $\sim 0.3\%$ is evaluated from the standard deviation.

References
