

Application Note

March 2025

Using the Blazerz Spotlight One for fluorescence (lifetime) measurements

This whitepaper describes the performance of the Spotlight One, a 2 Watt pulsed 775 nm laser for fluorescence (lifetime) measurements in the near-infrared wavelength spectrum.

Introduction

Blazerz's first laser, the Spotlight One, was developed specifically for widefield excitation of fluorescence in the near-infrared (NIR) wavelength spectrum. It sets itself apart from the competition by delivering several watts of picosecond-pulsed excitation power, enabling shorter acquisition times and facilitating real-time widefield fluorescence lifetime imaging.

In this paper, we evaluate the Spotlight One for fluorescence intensity and fluorescence lifetime measurements, comparing its performance against two commercial lasers from NKT Photonics (Katana HP) and PicoQuant (VisIR).

NIR fluorescence lifetime imaging requires a specialized imaging system and laser:

- Wavelength of 775 nm to efficiently excite dyes such as ICG and IRDye800CW.
- Irradiance $> 10 \text{ mW/cm}^2$ over a $10 \times 10 \text{ cm}$ area to enable short acquisition times.
- Repetition rate between 10 MHz and 80 MHz balancing high power with the ability to capture full decay curves for dyes with lifetimes of 1 ns or less.
- Pulse fall time well below 1 ns to resolve lifetimes of 1 ns or less.
- Fiber coupled to an illuminator for uniform excitation over a wide field of view.



Spotlight One performance

Figure 1 shows the illumination pattern of the Spotlight One coupled to a custom Blazerz illuminator. A uniform illumination is created over an area of 10 x 10 cm with a power of 15 mW/cm². As such, the Spotlight One boasts state-of-the-art average power illumination.

Table 1 compares the performance of the Spotlight One to the PicoQuant VisIR and NKT Photonics Katana HP. The results indicate that

the Spotlight One delivers two and four times more output power, respectively, outperforming both competing systems.

Figure 2 presents the measured output spectrum, indicating a center wavelength of 775 nm with a full-width at half-maximum (FWHM) of 4.51 nm. The shape of the output pulse is given in Figure 3, with a pulse width of 300 ps and a fall time of 150 ps.

	Blazerz <i>Spotlight One</i>	PicoQuant <i>VisIR-775</i>	NKT Photonics <i>Katana HP</i>
Measured power at 40 MHz			
At fiber coupler output	2100 mW	950 mW	500 mW
At target (1 cm ²)	15 mW	6.7 mW	3.7 mW
Center wavelength	775 nm	775 nm	775 nm
Pulse width (FWHM)	300 ps	320 ps	45 ps
Pulse fall time (80-20)	150 ps	130 ps	25 ps
Repetition rate	1 – 80 MHz	1 – 80 MHz	1 – 80 MHz

Table 1 Comparison of performance of the Blazerz Spotlight One to lasers from PicoQuant and NKT Photonics.

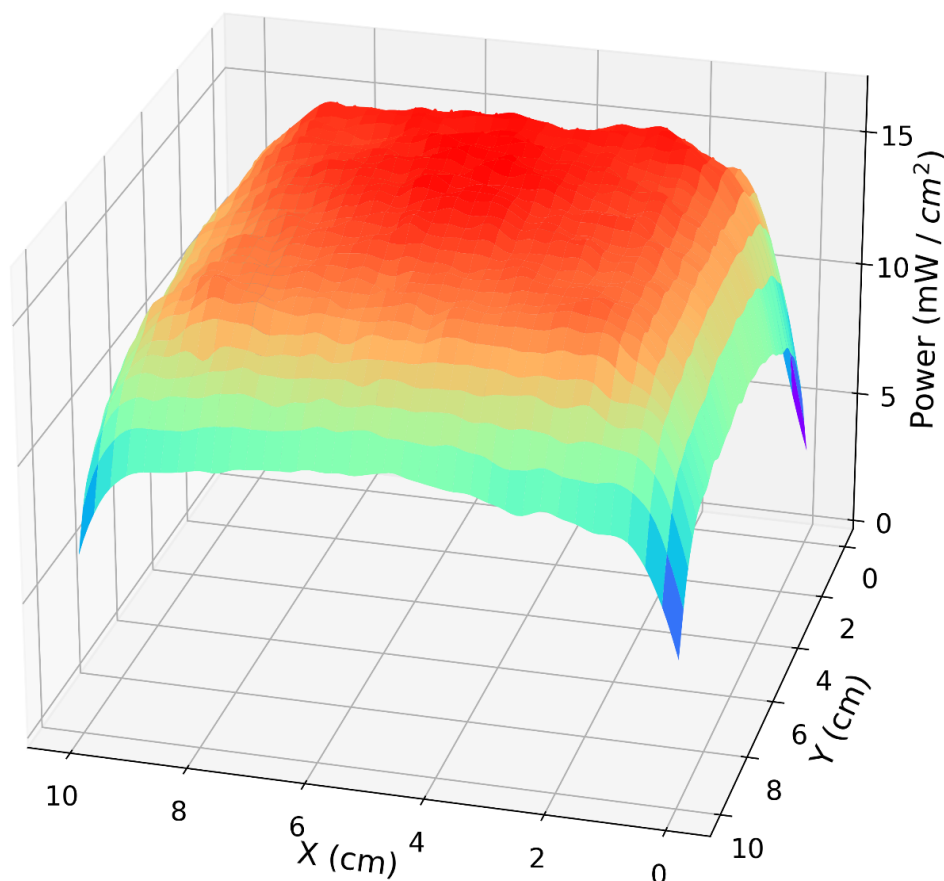


Figure 1 Illumination pattern of the Spotlight One coupled to a custom Blazerz illuminator. A uniform illumination is created over an area of 10 x 10 cm with a power of 15 mW/cm².

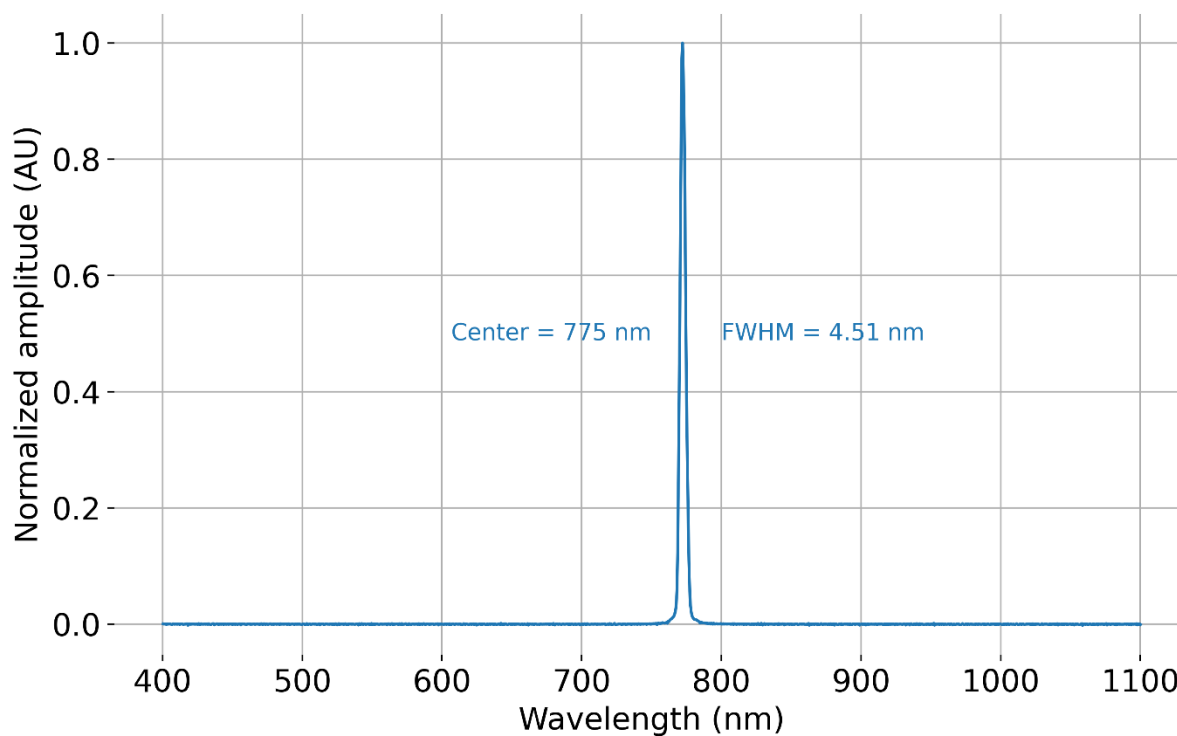


Figure 2 Measured output spectrum of the Spotlight One.

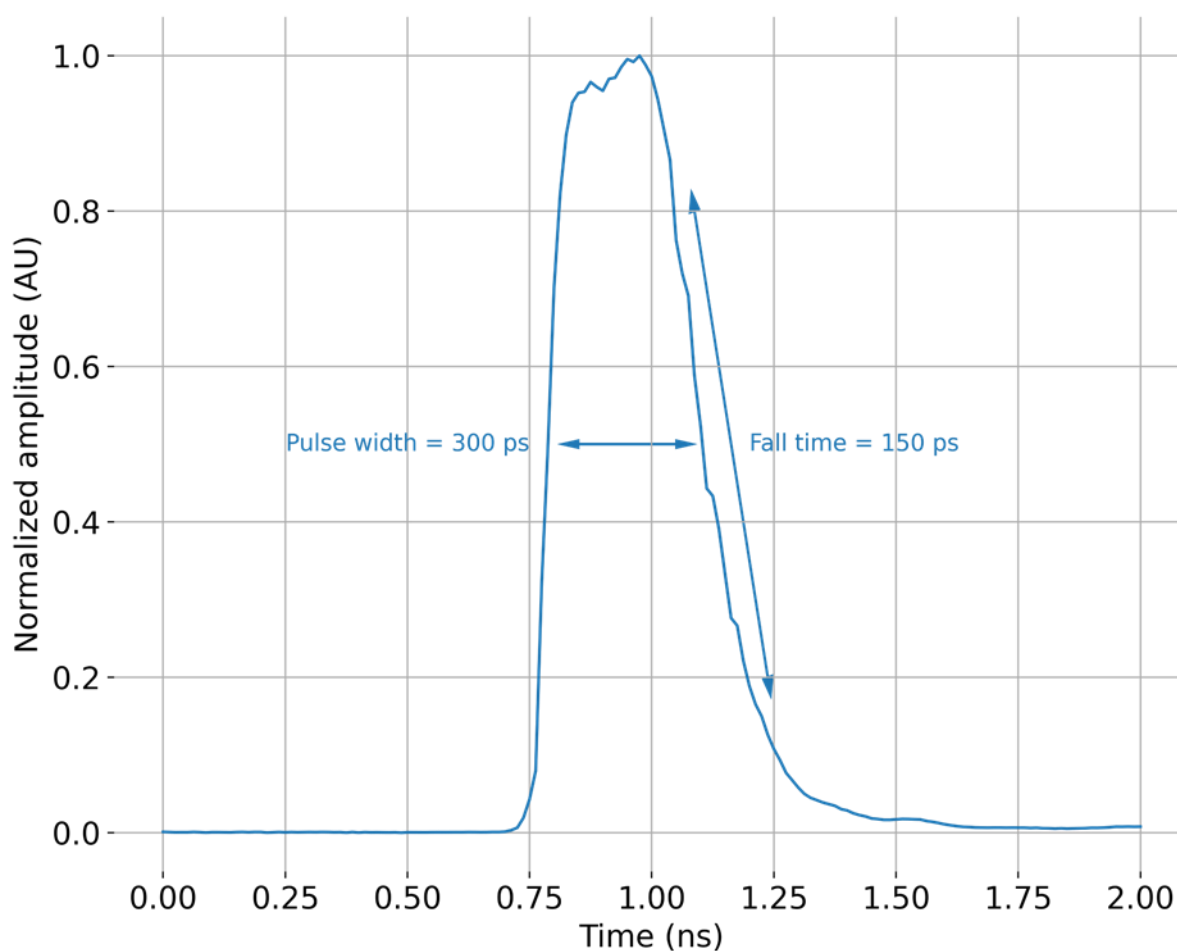


Figure 3 Measured pulse shape of the Spotlight One.

Fluorescence Intensity Imaging

Experimental setup

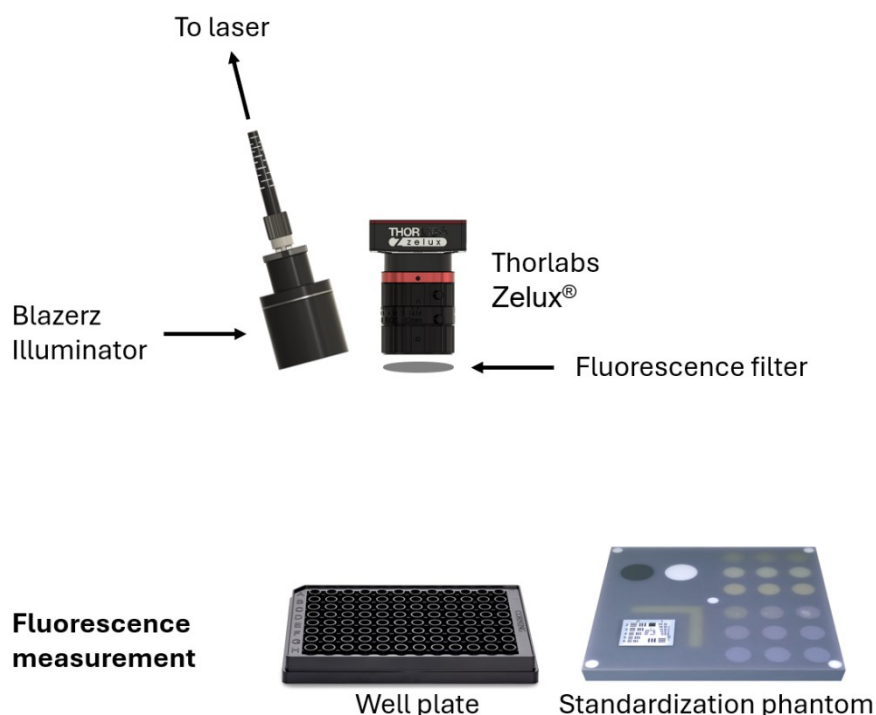


Figure 4 Fluorescence intensity imaging setup. A well plate with a dilution series of different fluorescent solutions is excited by the Spotlight One 775nm laser through a custom Blazerz illuminator, creating a 13.5 x 13.5 cm uniform excitation. A Thorlabs Zelux 1.6 MP camera with fluorescence emission filter is used to image the well plate.

The experimental setup for the fluorescence intensity measurement is shown schematically in Figure 4. A well plate or phantom is excited by the Spotlight One 775nm laser through a custom Blazerz illuminator, creating a 13.5 x 13.5 cm uniform excitation. A Thorlabs Zelux 1.6 MP camera with fluorescence emission filter (Semrock FF01-835/70-25 interference filter) is used to image the subject.

The Spotlight One is compared to two other lasers, the VisIR-775 from PicoQuant and the Katana HP from NKT photonics. Each laser output is coupled into a multimode fiber (Thorlabs 400 μm core, 0.39 NA) and illuminates the scene through the custom Blazerz illuminator. The repetition rate of each laser is set to 40 MHz.

The well plate consists of four dilution series: ICG dissolved in Phosphate Buffered Saline (PBS), Bovine Serum Albumin (BSA), ethanol, and methanol. The concentrations of the wells are chosen such that the intensity of the brightest wells are similar. Each well in the is diluted by 1/3 w.r.t. the previous well.

The same setup is used for imaging a Standardization phantom for intra-operative fluorescence molecular imaging, developed by Dimitris Gorpas et al [1]. The phantom implements different fluorescent features for assessing sensitivity versus depth, different optical properties, resolution, dynamic range, and light leakage. Most importantly, it is a large (10 x 10 cm) phantom, requiring a uniform widefield illumination of sufficient power.

Results

Fluorescence intensity imaging of the well plate and Standardization phantom was performed with the Blazerz Spotlight One, NKT Photonics Katana HP, and PicoQuant VisIR.

Figure 5 presents intensity images acquired using the three lasers under identical exposure conditions. The Spotlight One, with its higher output power, clearly produces the highest intensity, enabling visualization of even the lowest-concentration wells; a capability that is barely achieved with the VisIR and Katana HP.

Figure 6 shows the results of the experiment repeated with the Standardization phantom. Once again, the Spotlight One outperforms its competitors, revealing more structural details of the phantom. Additionally, the homogeneous intensity of the 'L'-shaped inclusion highlights the uniform illumination provided by the Blazerz illuminator.

In both experiments, a 13.5 x 13.5 cm area is illuminated, demonstrating the Spotlight One's ability to deliver uniform, widefield, and high-power illumination.

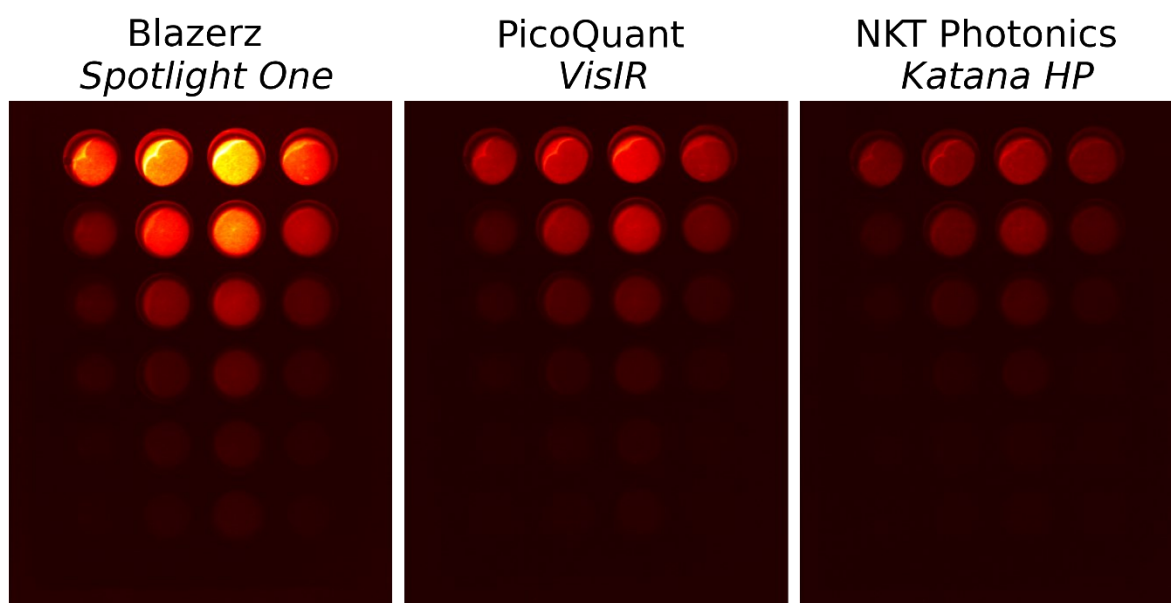


Figure 5 Fluorescence intensity imaging of a well plate with four dilution series.

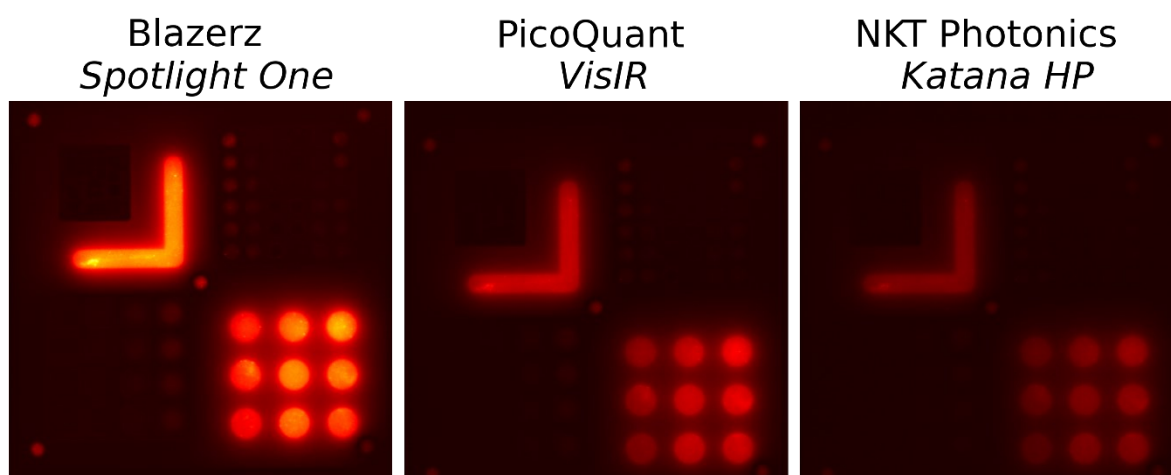


Figure 6 Fluorescence intensity imaging of a Standardization phantom.

Fluorescence Lifetime Imaging

Experimental setup

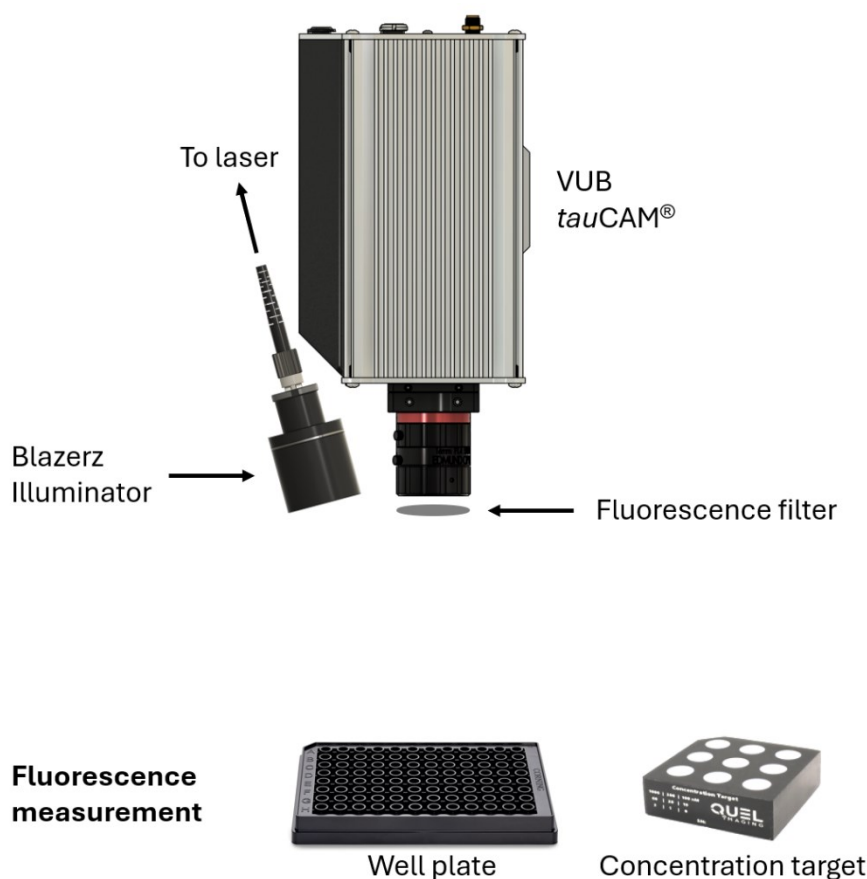


Figure 7 Fluorescence lifetime imaging setup. A well plate with a dilution series of different fluorescent solutions is excited by the Spotlight One 775nm laser through a custom Blazerz illuminator, creating a 13.5 x 13.5 cm uniform excitation. The VUB tauCAM fluorescence lifetime camera with fluorescence emission filter (Semrock FF01-835/70-25 interference filter) is used to image the well plate.

The experimental setup for the fluorescence lifetime measurement is shown schematically in Figure 7. A well plate or phantom is excited by the Spotlight One 775nm laser through a custom Blazerz illuminator, creating a 13.5 x 13.5 cm uniform excitation. The VUB tauCAM with fluorescence emission filter (Semrock FF01-835/70-25 interference filter) is used to image the subject.

Again, the Spotlight One is compared to two other lasers, the VisIR-775 from PicoQuant and the Katana HP from NKT photonics. Each laser output is coupled into a multimode fiber (Thorlabs 400 μm core, 0.39 NA) and illuminates the scene through the custom Blazerz illuminator. The repetition rate of each laser was is to 40 MHz.

The well plate consists of four dilution series: ICG dissolved in Phosphate Buffered Saline (PBS), Bovine Serum Albumin (BSA), ethanol, and methanol. The concentrations of the wells are chosen such that the intensity of the brightest wells are similar. Each well in the is diluted by 1/3 w.r.t. the previous well.

The same setup is used for imaging an ICG-equivalent concentration sensitivity target from Quel Imaging (RCS-ICG-ST01-QUEL02) [2]. It provides an ICG-equivalent fluorescent material with concentrations of 1000 nM to 3 nM with a lifetime of approximately 1 ns [3]. The first five wells (1000 nM, 300 nM, 100 nM, 30 nM, 10 nM) were imaged for comparison.

The VUB *tau*CAM is a fluorescence lifetime camera developed by the Vrije Universiteit Brussel (VUB) for imaging of fluorescence in the NIR region, specifically with sub-ns lifetimes. It has a resolution of 128 x 128 pixels, with a quantum efficiency of 40% at 780 nm and an Instrument Response Function (IRF) of only 320 ps [2, 3].

Results

For fluorescence lifetime measurements, the intrinsic decay of the falling edge of the system IRF puts a lower bound on the fluorescence lifetime decay that can be measured. For example, an imaging system with an intrinsic decay of 10 ns will not be able to resolve a fluorescence lifetime of 1 ns, as that system would be too slow to detect the fast fluorescence decay. For this reason, the VUB *tau*CAM has an IRF with an intrinsic decay of only 320 ps.

The intrinsic decay of the imaging system can be found from the system IRF, being the convolution of laser IRF with camera IRF:

For measurement of the IRF, the fluorescence filter is replaced with a neutral density (ND) filter, and the well plate/phantom with a white copy paper. The white copy paper provides diffuse reflection of the excitation light, which is attenuated by the ND filter to avoid saturating the VUB *tau*CAM.

$$IRF_{system} = IRF_{laser} * IRF_{camera}$$

Ideally, the laser IRF is significantly shorter than the camera IRF; as such, the intrinsic decay is purely limited by the camera and not hampered by the laser source.

Figure 8 shows the system IRF for the VUB *tau*CAM coupled to different lasers. As can be concluded, the IRF of each system is dominated by the camera IRF. In other words, the increased pulse width of the Spotlight One (300 ps) and VisIR (320 ps) compared to the Katana HP (30 ps) is also perfectly suited for fluorescence lifetime measurements with the VUB *tau*CAM.

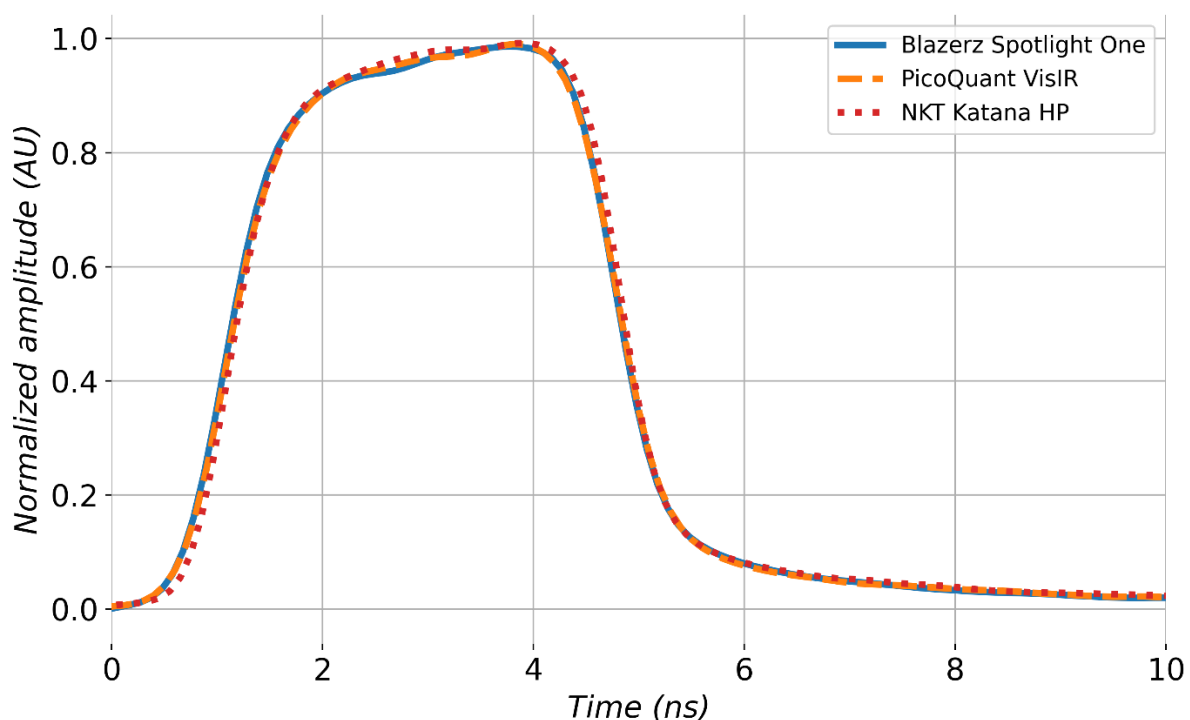


Figure 8 Comparison of the system IRF for the VUB *tau*CAM coupled to the different lasers.

The IRF is used for fluorescence lifetime processing algorithms to eliminate the system response from the lifetime calculation. The most well-known use is fitting a recorded decay to a decay model which is convolved with the system IRF, from which the lifetime is extracted. For the lifetime measurements, the experimental setup of Figure 7 was used, and all lasers were set to an excitation power of 500 mW for the fairest comparison. The lifetime was acquired by measuring 100 points in the decay, with a timestep of 100 ps and an acquisition time per frame of 40 ms. An example measurement is shown in Figure 9, where we see that all three systems measure the same decay curve.

Figure 10 shows the fluorescence lifetime of a subset of 3 x 3 wells from the well plate, with the corresponding lifetime values listed in Table 2. Each of the three measured dilution series exhibits a distinct lifetime, demonstrating the system's ability to resolve lifetime contrast. The measured values across the three laser systems show close agreement, confirming that the Spotlight One provides the necessary excitation for reliable fluorescence lifetime measurements.

Figure 11 presents the fluorescence lifetime measurements for the five brightest inclusions of the concentration sensitivity target, with the corresponding values summarized in Table 3. The average lifetimes measured with the three laser systems are in close agreement, confirming the reliability of each system. This demonstrates that the Spotlight One provides the necessary excitation for accurate fluorescence lifetime measurements. For both experiments, the field of view was reduced to 6.5 × 6.5 cm to ensure sufficient spatial resolution for the camera.

The measurement was repeated with each laser operating at maximum power. While the original acquisition time was 4 seconds, it remained unchanged for the Katana HP (already at full power), but was reduced to 2.1 seconds for the VisIR and just 0.95 seconds for the Spotlight One. Since acquisition time directly impacts the maximum achievable frame rate for fluorescence lifetime imaging, these results underscore the advantages of high-power illumination sources like the Spotlight One in enabling faster fluorescence lifetime video acquisition.

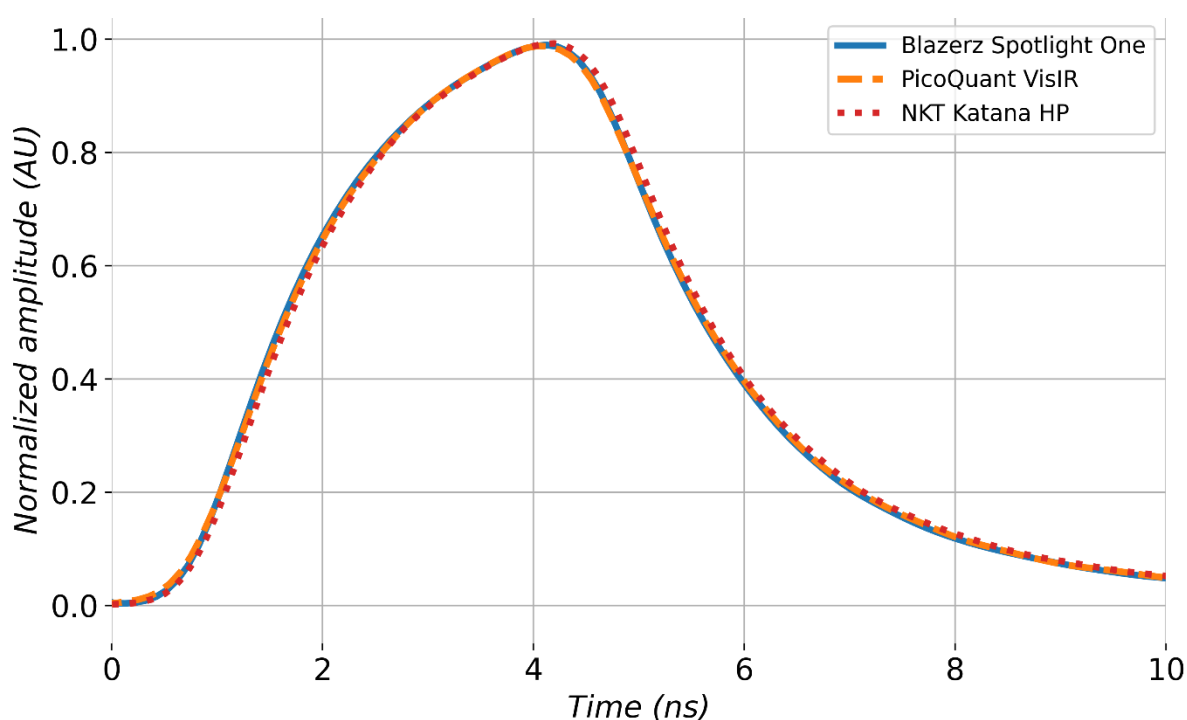


Figure 9 Comparison of the decay for one of the inclusions of the concentration sensitivity target.

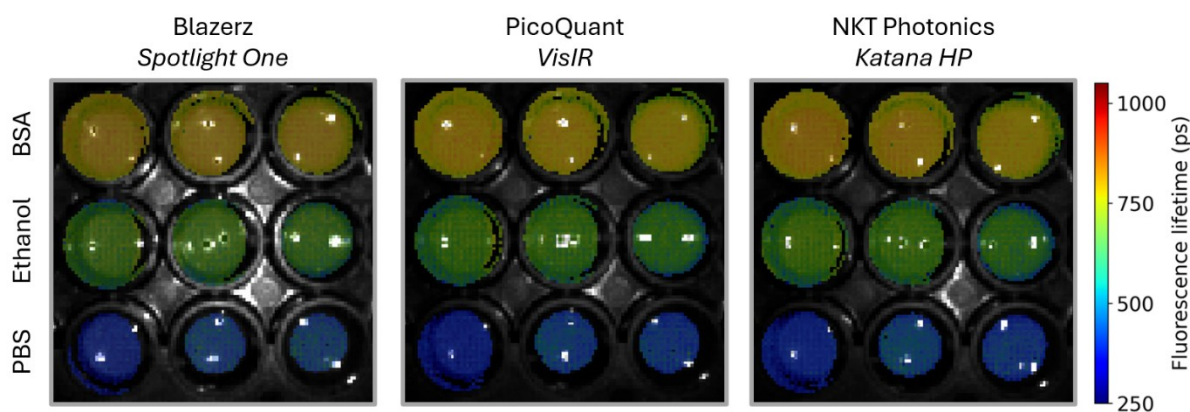


Figure 10 Fluorescence lifetime measurement of a subset of 3 x 3 wells of the well plate.

	Blazerz <i>Spotlight One</i>	PicoQuant <i>VisIR-775</i>	NKT Photonics <i>Katana HP</i>
BSA	820 ps	811 ps	828 ps
Ethanol	614 ps	610 ps	621 ps
PBS	470 ps	474 ps	486 ps

Table 2 Fluorescence lifetime values for the middle concentration of each of the dilution series, as measured by the three fluorescence lifetime systems, each with a different laser source.

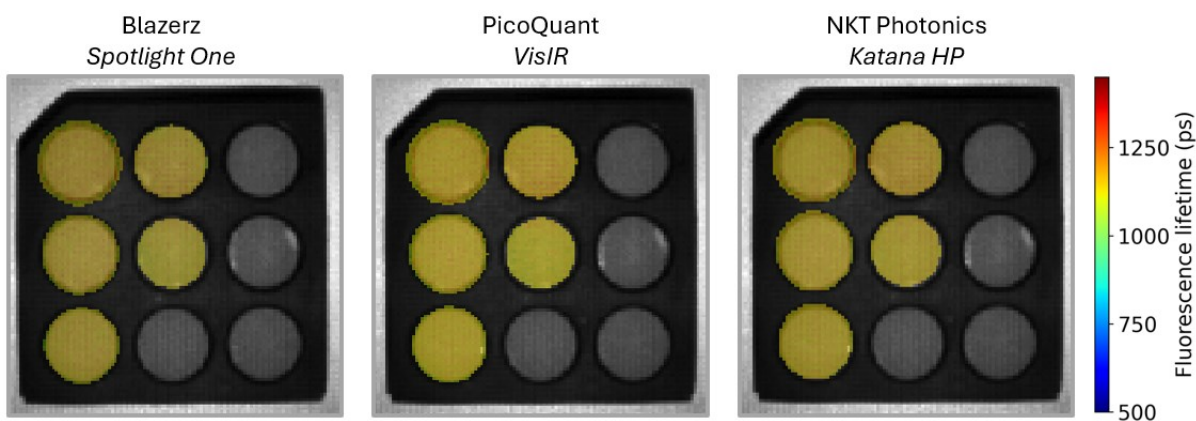


Figure 11 Fluorescence lifetime measurement of the concentration sensitivity target.
Left, top to bottom: 1000 nM, 300 nM, 100 nM. Middle, top to bottom: 30 nM, 10 nM.

	Blazerz <i>Spotlight One</i>	PicoQuant <i>VisIR-775</i>	NKT Photonics <i>Katana HP</i>
1000 nM	1177 ps	1173 ps	1170 ps
300 nM	1177 ps	1173 ps	1179 ps
100 nM	1168 ps	1156 ps	1161 ps
30 nM	1181 ps	1186 ps	1196 ps
10 nM	1133 ps	1129 ps	1156 ps
Average	1167 ps	1163 ps	1172 ps
Standard deviation	20 ps	22 ps	14 ps

Table 3 Fluorescence lifetime values for the 5 brightest inclusions of the concentration sensitivity target, as measured by the three fluorescence lifetime systems, each with a different laser source.

Conclusion

The **Spotlight One** is a high-performance laser source for fluorescence intensity and lifetime imaging, offering superior output power compared to competing systems. It delivers over **2 W at the fiber output** and achieves **15 mW/cm² at the target**, doubling the power of its closest competitor. This increased excitation power is particularly beneficial for fluorescence lifetime imaging, where multiple frames are required to reconstruct decay curves, significantly reducing acquisition time and enhancing frame rates.

For fluorescence lifetime measurements, the **short pulse width (320 ps) and fall time (150 ps)** ensure minimal influence on the system's instrument response function (IRF), making it well-suited for detecting short lifetimes. Operating at **775 nm**, the Spotlight One efficiently excites near-infrared (NIR) dyes such as **ICG and IRDye800**, while its narrow spectral output enables precise excitation without restricting emission filter selection. Experimental results confirm its ability to generate lifetime contrast and accurately resolve fluorescence lifetimes.

References

1. D. Gorpas et al., "Standardization phantom for intra-operative fluorescence molecular imaging," in *Medical Laser Applications and Laser-Tissue Interactions IX*, Vol. EB106 of SPIE Proceedings (Optica Publishing Group, 2019), paper 11079_39.
2. Quel Imaging ICG-equivalent concentration sensitivity target RCS-ICG-ST01-QUEL02: <https://shop.quelimaging.com/product/icg-equivalent-concentration-sensitivity-target/>
3. P. Iranian et al., "Fluorescence Lifetime Endoscopy with a Nanosecond Time-Gated CAPS Camera with IRF-Free Deep Learning Method, " *Sensors* 2025, 25(2): 450, Doi: 10.3390/s25020450
4. T. Van den Dries et al., "64×64 Pixel Current-Assisted Photonic Sampler Image Sensor and Camera System for Real-Time Fluorescence Lifetime Imaging," in *IEEE Sensors Journal*, vol. 24, no. 15, pp. 23729-23737, 1 Aug. 1, 2024, doi: 10.1109/JSEN.2024.3409418.
5. T. Van den Dries et al., "Compact sub-nanosecond time-gated camera based on a new 128x128 Current-Assisted Photonic Sampler (CAPS) for pre-clinical macroscale fluorescence lifetime imaging," *Proc. SPIE PC12891, Silicon Photonics XIX, PC128910G* (13 March 2024); doi: 10.1117/12.3001047.