University of Wisconsin-Stevens Point

Hidden Details: Photon Correlations from Fluorescent Molecules

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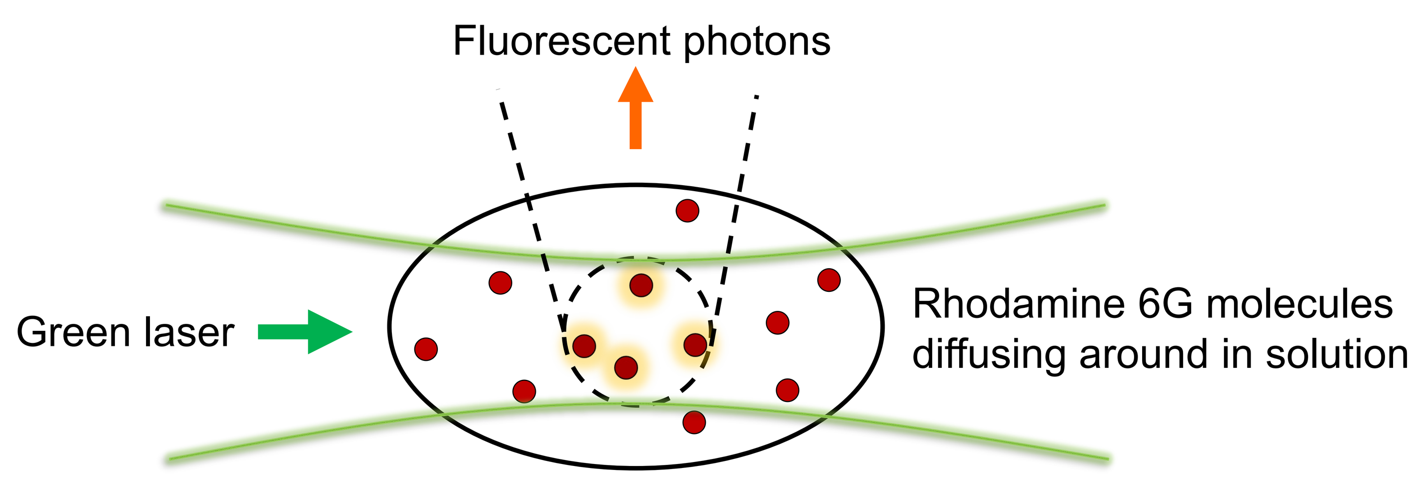
1. Introduction

- Fluorescent molecules emit a burst of photons when illuminated by a laser pulse.
- Although these photons arrive randomly, hidden statistical correlations amongst them contain information about the local molecular environment.

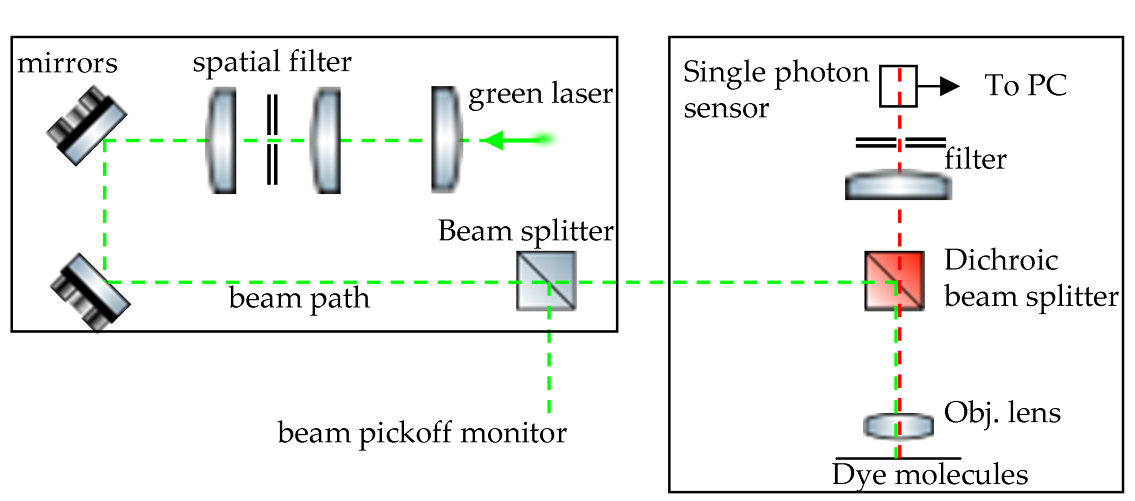


- We describe the construction and operation of an experiment designed to capture these photon bursts and measure their arrival times.
- We also describe how to compute the statistical correlations from the arrival times.
- Finally, we present some initial results from 100 nM solutions of rhodamine 6G.

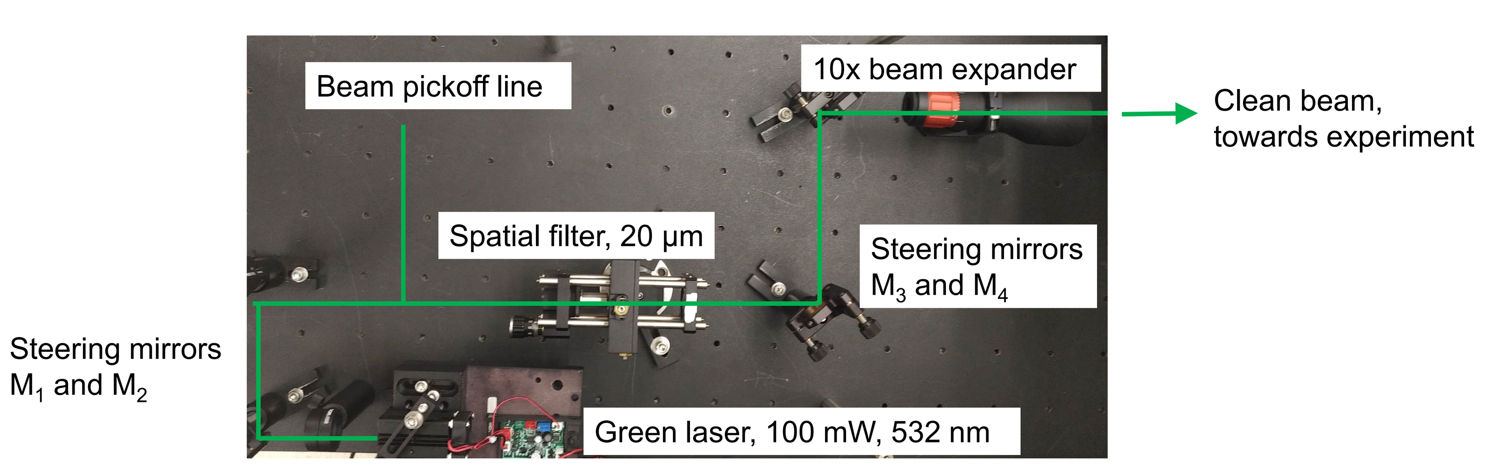
2. Experiment layout

Our experiment consists of the following:

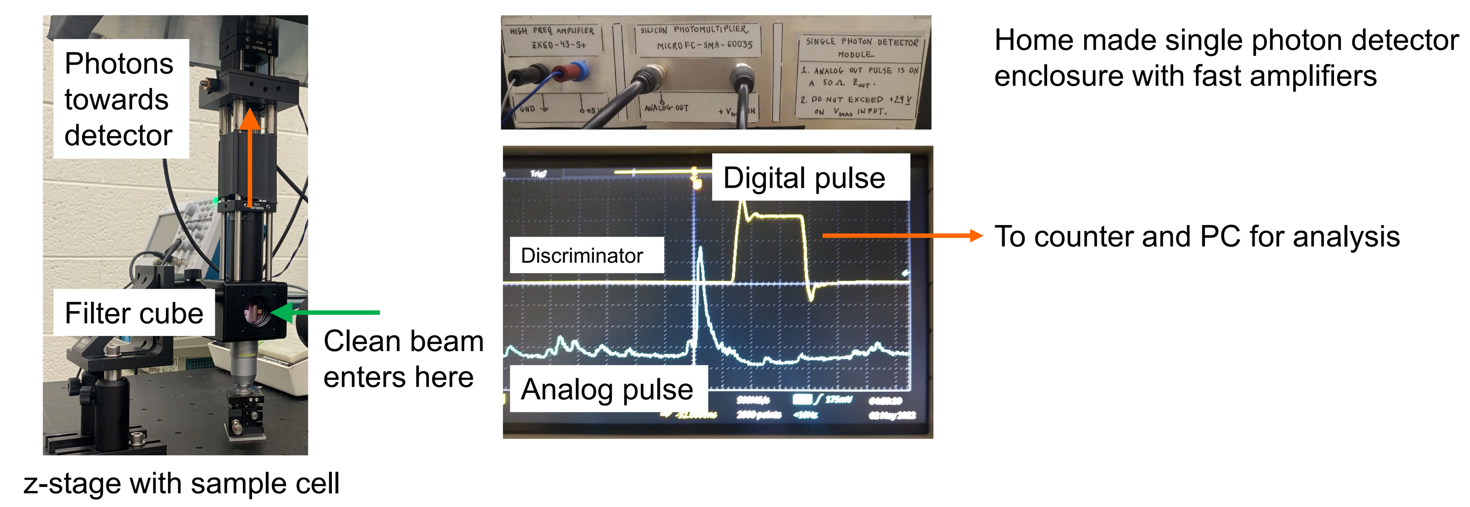
- A green laser, beam steering mirrors, optical filters, and a high magnification objective.
- A single photon detector, fast electronics, and a pulse counter that records the arrival time of each photon using a 10 MHz reference clock.



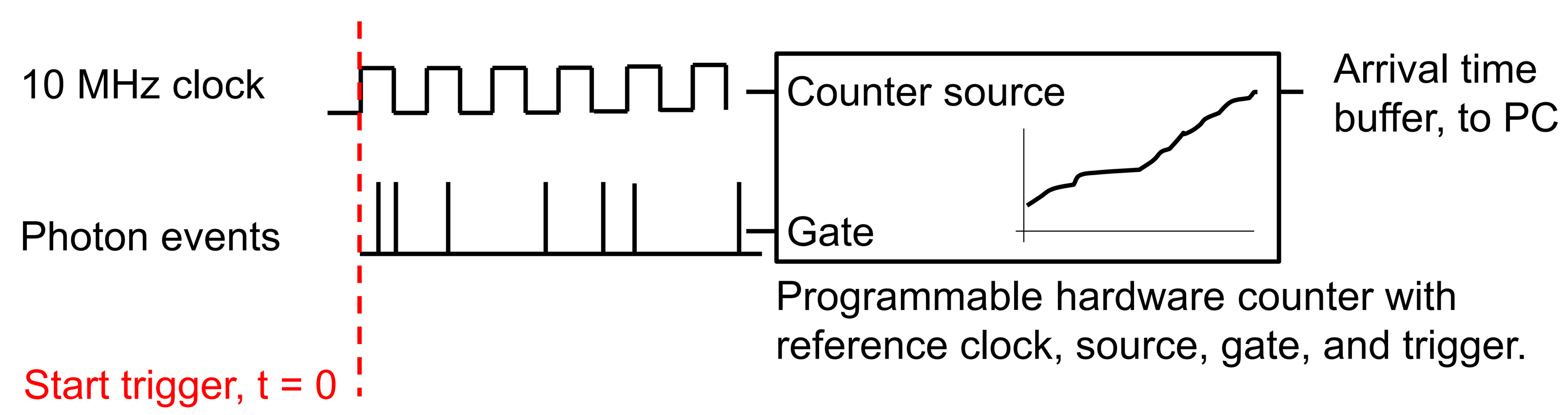
3. Hardware: the input beam module



4. Hardware: the emission beam module

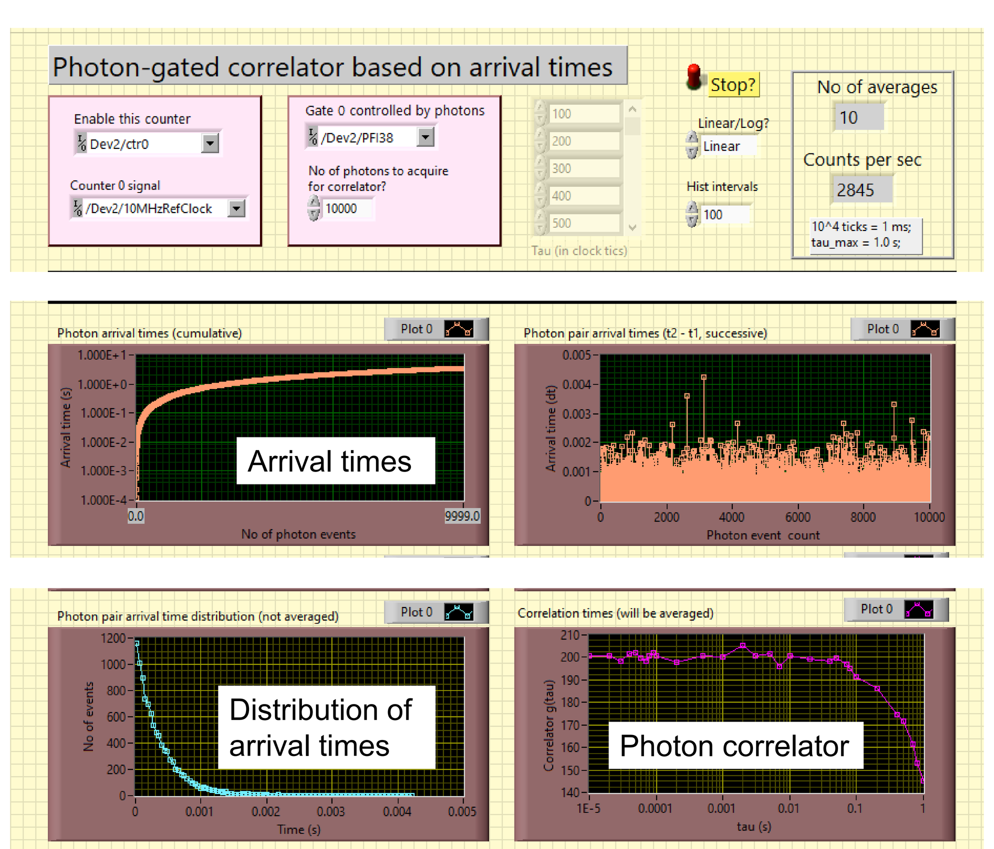


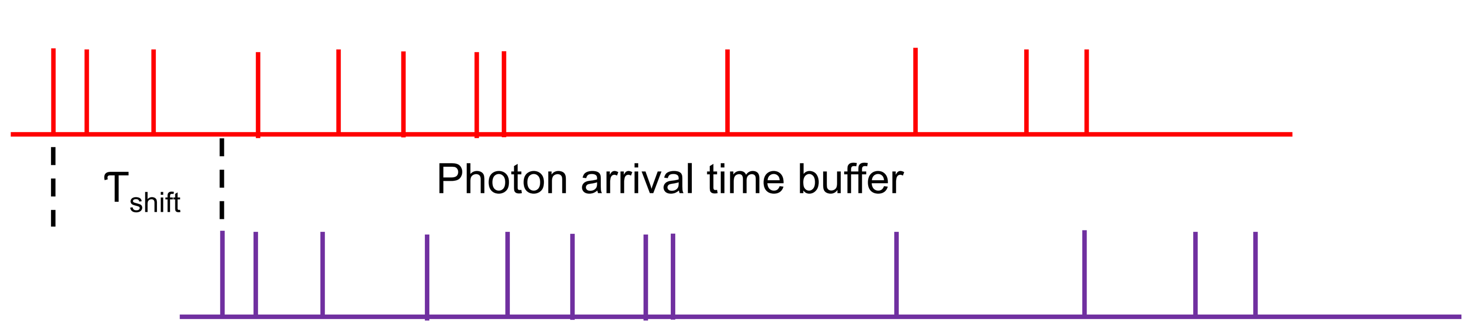
- Each fluorescent photon is converted into a 500 ns wide digital pulse.
- We count these digital pulses and measure their arrival times using a 10 MHz reference clock and a programmable counter.
- Each photon arrival opens the gate and the counter records the clock time since t = 0.



5. Experiment control

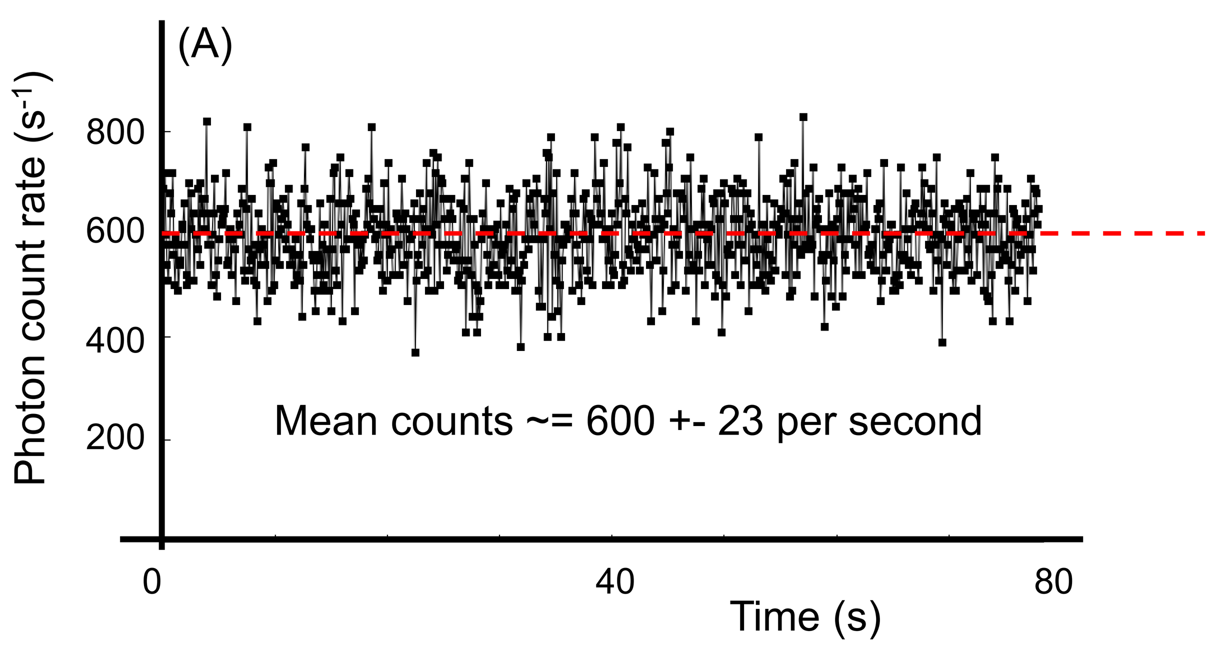
- The photon acquisition is controlled by our own custom software which periodically downloads the arrival time buffer from the counter, computes the mean count rate and the distribution of arrival times.
- The mean count rate helps with the initial alignment; the distribution of arrival times helps confirm we are dealing with random events.
- The photon correlator is computed by comparing the arrival time buffer with its copy.
- At T = 0, correlator is = 1/N, local molecular concentration!
- At T → ∞ correlator goes to zero; the system loses its memory – diffusion time!



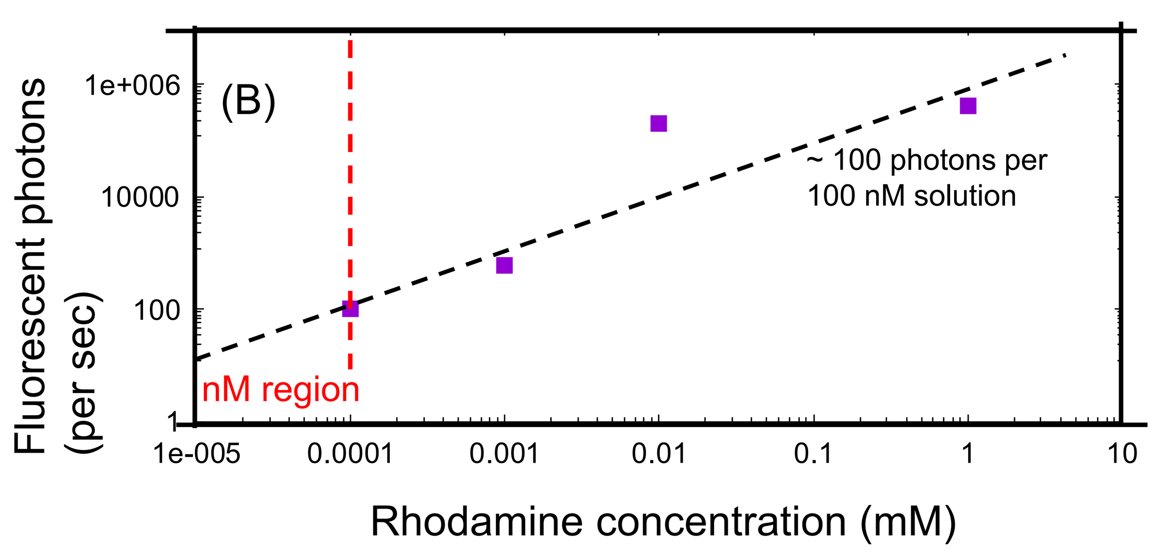


6. Results

- The experiment was aligned by measuring the fluorescent photons emitted from a 1 μM rhodamine 6G solution.



- If the solution concentration is increased, we detect more photons.



- Starting work on the nM region but need better alignment of the detector.
- Install aperture to define a small confocal volume and track diffusion of rhodamine molecules within this small volume.
- Our estimates show the diffraction limited confocal volume is about 1 femtoliter and contains only 10 molecules with a 10 nM solution.

- Our instrument prototype works! – and now gives us a chance to study systems at the few molecule level.
- Are you interested in joining this interdisciplinary project? Talk to one of us!

7. Acknowledgements

- Dr. Riha from the Dept of Chemistry for expert help and advice with preparing nanomolar solutions of rhodamine 6G.
- A Dept of Physics & Astronomy Physics 388 student research fund for help with supplies.
- A UPDC grant to construct the experiment and demonstrate a working prototype.