Multi-pulse based approach on superresolution microscopy

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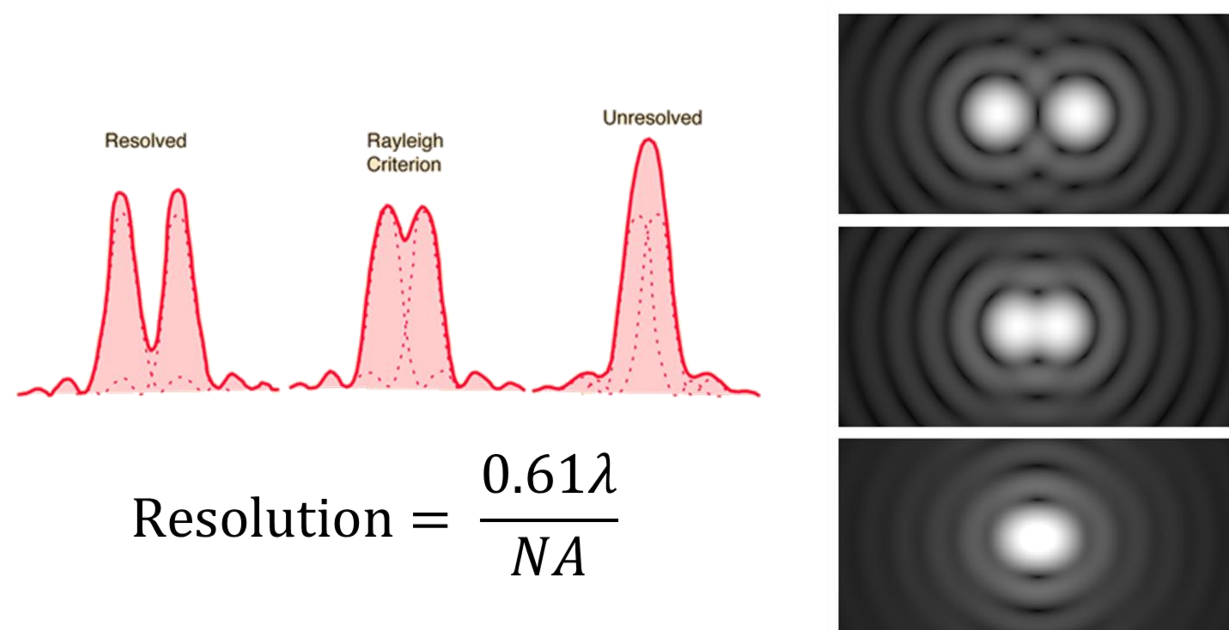
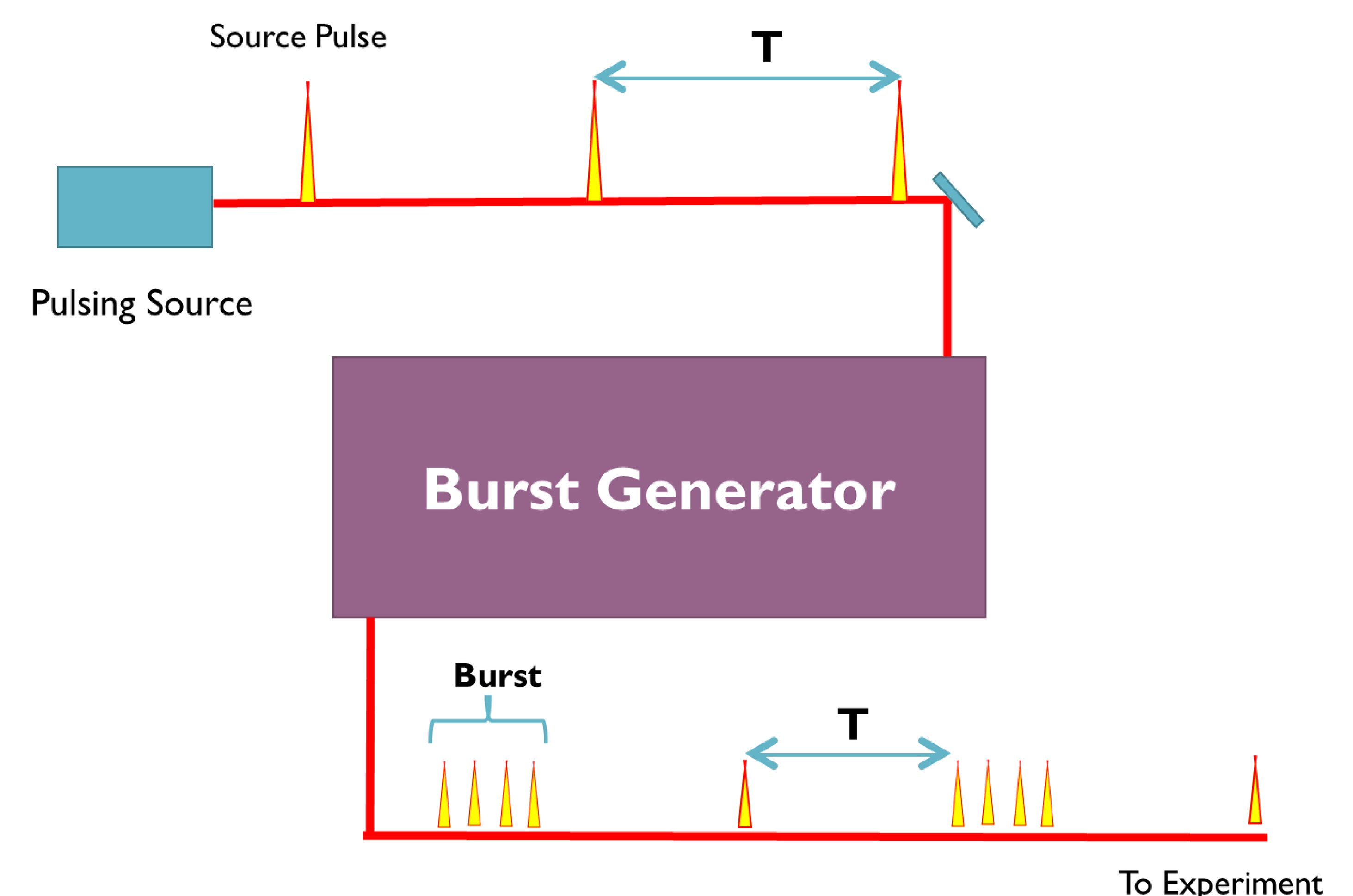
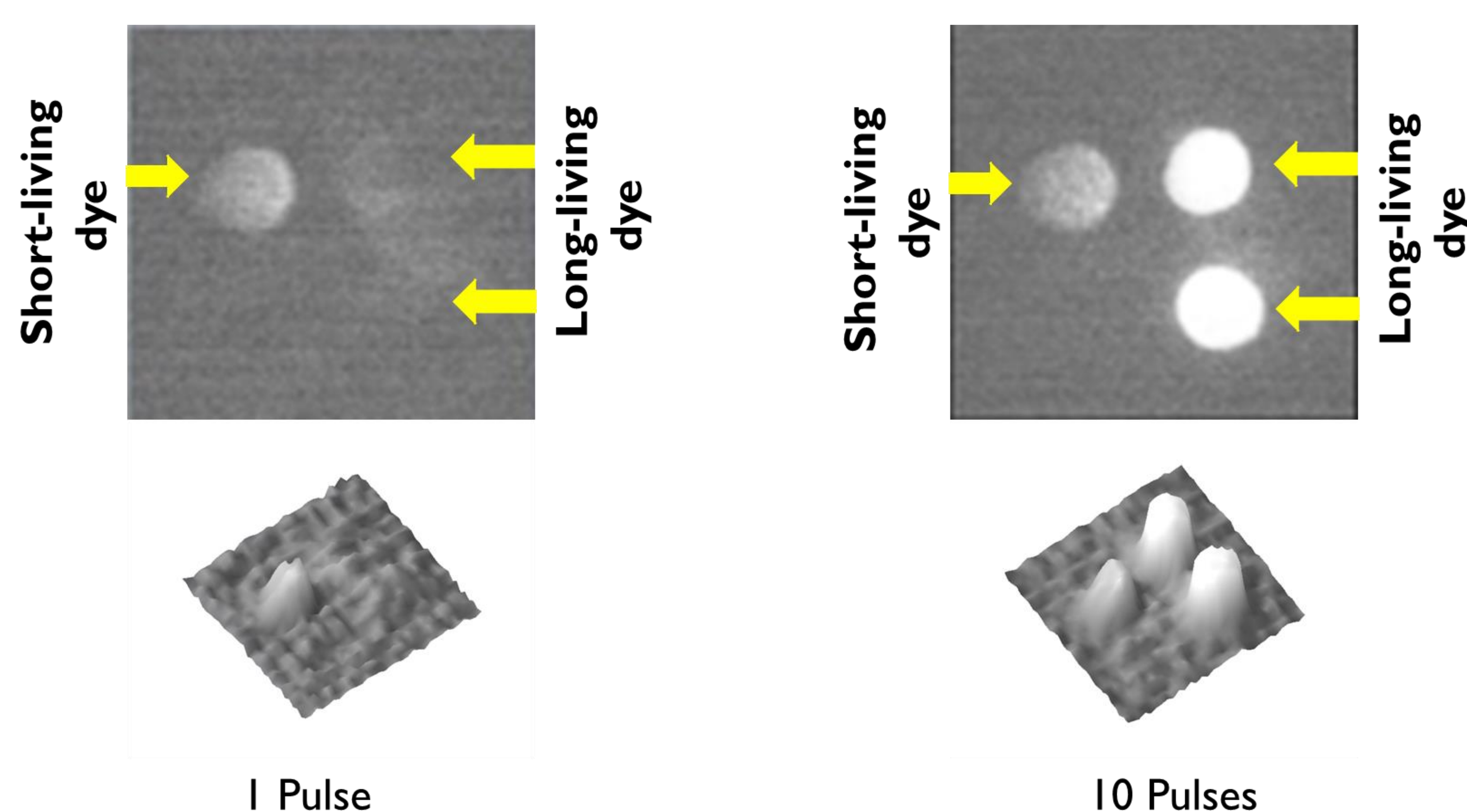
Abstract. Since the invention of on optical microscope various biological structures have been observed. Today we have a need to study subcellular structures and their dynamics. Here we encounter diffraction limit – two objects located closer than the half of the wavelength cannot be resolved as two distinct objects. Superresolution techniques have been developed to overcome this limit. They can be divided into two types: stochastic and deterministic. Stochastic ones (STORM, PALM) utilize natural ability of fluorescent molecules to blink. These methods require sparse labeling and significant amount of some time to acquire image. Deterministic ones (STED) utilize an additional pulsed light source to de-excite populated state. These methods require advanced technology. Our method is similar to deterministic superresolution techniques. We utilize long-living fluorescent dyes whose excited state population can be significantly enhanced by bursts of pulses. Enhancement occurs only when time delay between pulses within burst is shorter than the lifetime of the dye. By varying bursts and single pulses one may observe varying intensity of a dye, hence, achieve superresolution. Regular labeling methods become an advantage in this case, and such an experimental setup is not very different from conventional microscopy methods.



Rayleigh criterion.
Two single emitters located closer than ~half of wavelength they are emitting cannot be resolved as two distinct objects. Superresolution techniques allow to overcome this fundamental limit.

Burst generator concept.
Conventional pulsed light source with constant time delay between pulses is
converted into series of bursts and single pulses. Time delay between pulses within burst is shorter than time delay between burst and single pulse. This can be achieved in multiple ways: using a system of beam splitters, using a set of optical fibers with different lengths, using laser diode array.





1 Pulse

10 Pulses

Enhancement of signal with bursts.
Signal from a short living dye hasn't enhanced at all no matter how many pulses we have sent to them (time-gated detection is used to start measurements from the last pulse). But in case of long-living dyes signal have been significantly enhanced which proves that bursts can significantly vary the signal of a probe.

Conclusions : Bursts of pulses allow one to enhance the fluorescence signal form a probe. Varying single pulses and bursts allows one to quickly localize the labelling dye and achieve superresolution.