

An Autosynchronous Optical Chopper (ASOC) for time-gated luminescence microscopy

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Time-gated luminescence detection is a valuable technique for the suppression of autofluorescence. Most fluorophores have fluorescence lifetimes less than 100 ns whereas the luminescence from some lanthanide chelates can persist for up to a millisecond or more. It is this property that can be exploited to suppress prompt fluorescence through pulsed excitation and gated detection. Conventionally the pulsed excitation is performed with a flashlamp, LED or mechanical chopper and the gated detection achieved through an electronically gated camera or optical switch (liquid crystal or mechanical chopper).

This is the first report of an entirely novel optical chopper that combines both the excitation and emission gating into a single device. The design relies upon an angled chopper blade that diverts an excitation beam onto the sample whilst simultaneously blocking prompt emission from the observer's view. As the blade rotates the excitation pulse is terminated and any persistent luminescence is now visible to the observer. Using a prototype device fitted to an Olympus BX51 microscope, intense autofluorescence was suppressed more than 25-fold in relation to signal intensity from a 1 μm europium polymer microspheres (FluoSpheres F-20882 Molecular Probes). The image shown below was captured using an Andor DV885 electron multiplying CCD camera with gain set to 2000 and a single exposure of 50 ms. Persistent emission from the FluoSpheres was intense and visible to the eye with the ASOC fitted to the microscope, images were easily captured at gain settings of zero.

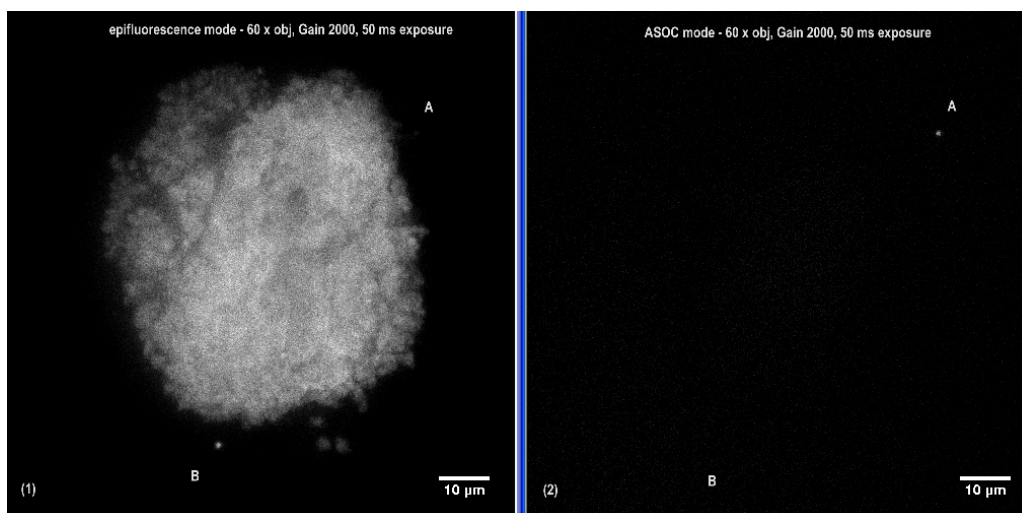


Fig. 1. The sample was comprised of a mixture of prompt fluorophores excitable at 365 nm together with 1 μm europium polymer FluoSpheres. Two (separate) high-power UV LEDs (365 nm) were used for excitation in both modes. The leftmost image was captured in epifluorescence mode and shows a fragment of intensely fluorescent dye particles in the centre with a small blue bead adjacent to the letter 'B'. The FluoSphere is barely visible with 1/6 the intensity of the central fragment. The rightmost plate was captured with the ASOC in operation (19,000 RPM) and a show the excellent suppression achieved, the FluoSphere is now clearly visible with a 25-fold increase in contrast.