

Advantages of the TILL Photonics real time imaging system concept

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This article describes how the TILL Photonics real time imaging system concept allows to maximize the time resolution of your experiment and at the same time minimizes bleaching and phototoxic effects.

What is real time imaging?

In our definition real time imaging not only means that images acquired can be seen almost instantaneously with a high frame rate on the computer monitor but also that the interplay of all devices involved in the acquisition of these images is optimized and under precise timing control.

What is the benefit from real time imaging?

Less bleaching and higher frame rates. This keeps your cells happy and increases the time resolution of your experiment. Let us explain this in more detail:

A very basic imaging system

Let's say we plan to acquire 20 images with an exposure time of 10 ms at a rate of 2 images per second. The simplest way for a fluorescence imaging experiment would be to switch on the light, acquire the images and switch off the light again. It is evident that with this protocol the ratio between illumination time required for exposure of the camera ($20 \cdot 10\text{ms}$) and the effective illumination time (10.000 ms) is very low, leading to unnecessary bleaching and photo toxicity. While hopefully no imaging system on the market will use a protocol like this, it is actually how fluorescence microscopy is performed if the oculars of the microscope are used for viewing or pre-viewing.

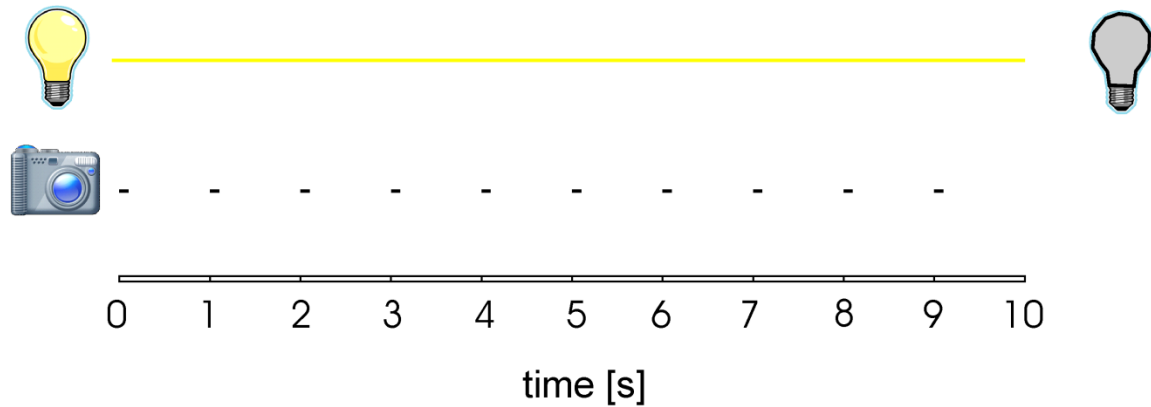


Fig1: Most simple form of light source control as described above. The ratio of required illumination time for exposure to effective illumination time is very low causing strong photo bleaching.

A standard system

A more advanced system switches off the light in-between camera exposures but if a standard PC operating software is used for control the timing is subject to some variable latency due to other tasks running on the machine. In order to account for this PC-based latency there must be a larger overlap of illumination and exposure in order to make sure that the light is switched on before exposure of the camera starts. Also the light is switched off with a delay after exposure has been finished.

An alternative approach is to send commands to a device and wait until the receipt/execution of the command has been confirmed by the device. Both methods require additional time, hence increase bleaching and reduce the maximum frame rate that can be achieved.

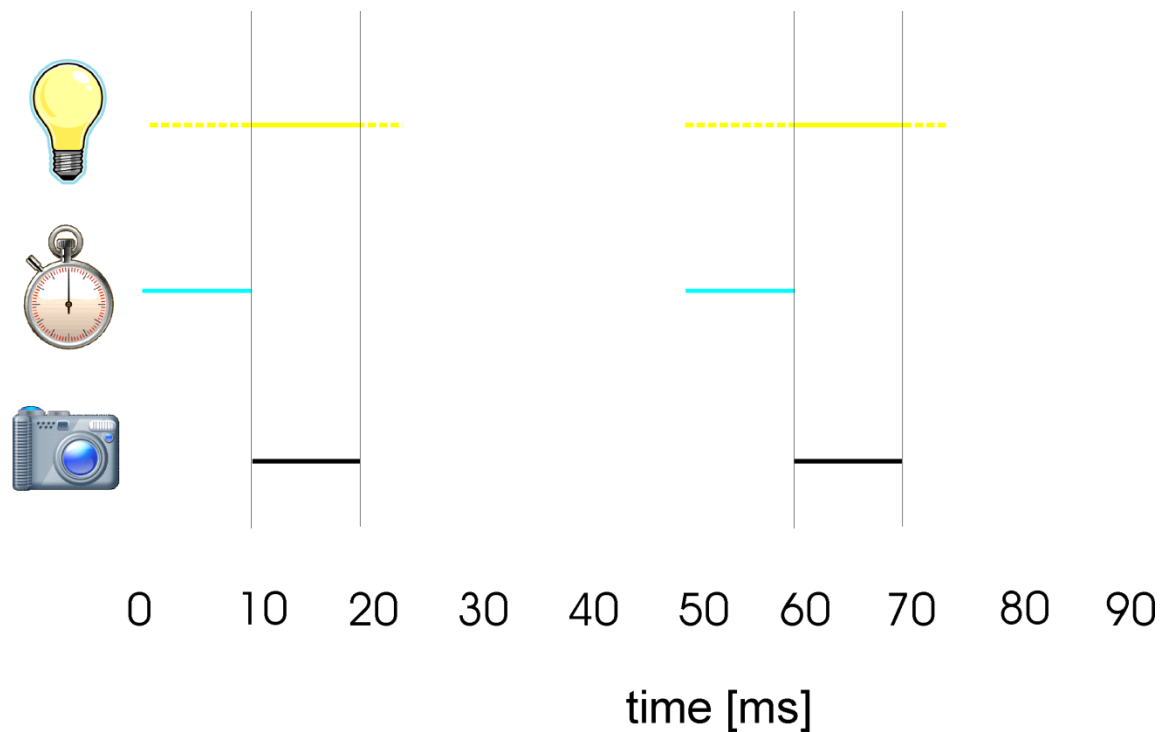


Figure 2: Imaging system realized with PC control. In order to make sure that the light source is definitely on when exposure starts, the system first switches on the light and either waits for a fixed time or until it receives a confirmation from the light source before it starts exposure of the camera. In both cases there is a delay which increases illumination time of the specimen and reduces frame rate. If exposure time is reduced the situation becomes even more disadvantageous since the ratio of illumination time required for exposure to effective illumination time decreases further.

The TILL Photonics concept

To circumvent these limitations the TILL Photonics imaging system uses a different approach.

The protocol for the light source, the camera and other devices involved is created using software running on a standard PC with a graphical user interface. This software compiles as set of instructions which are sent to a digital signal processor (DSP) in the central control unit of the system and to the other devices. The DSP in the control unit is not subject to the latency problems of a PC and controls the devices by trigger signals with a time resolution of 34 microseconds. The exact time point of the trigger signals is calculated by a timing model which is available for each supported device. As a consequence the ratio of illumination time required for exposure to effective

illumination time is minimized. Thus bleaching and photo toxicity are reduced and higher frame rates are possible.

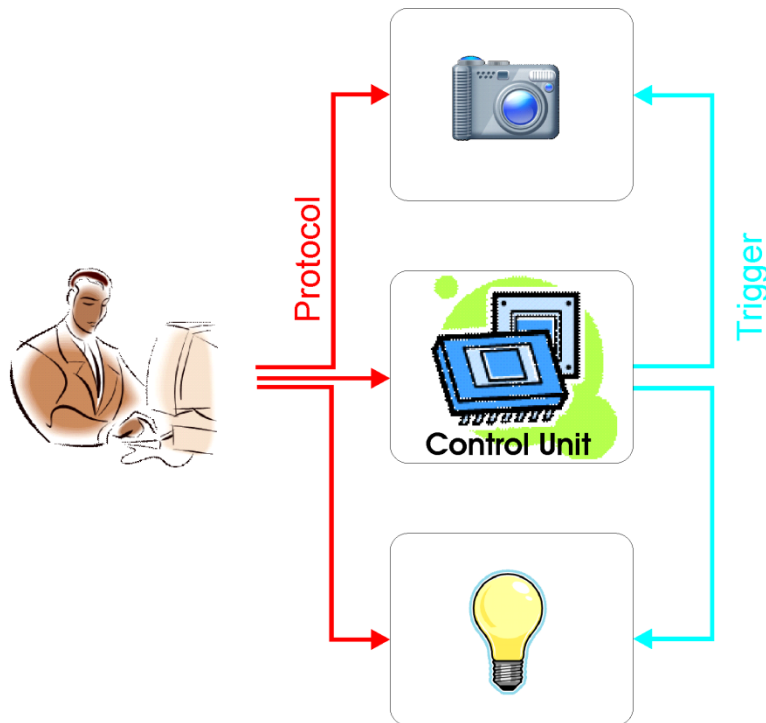


Figure 3: Concept of the TILL Photonics real time imaging system. Camera and light source receive instructions from the PC but are controlled by trigger signals from the control unit which is not subject to PC-typical latencies. Other devices like digital I/O, laser scanners, etc. are controlled by the same way.

Other methods to further increase the frame rate

So far the situation has been simplified since the light source has been treated as it could switch on infinitely fast (only delays caused by the computer have been covered). The real world situation is of course different.

Fast light sources

Several time constants have to be accounted for, in addition to switching on and off e.g. for changing intensity or setting wavelength or filter position. It is obvious that the duration of these events has a direct influence on the number of images that can be acquired per time. Therefore the TILL Photonics Polychrome or Oligochrome light

sources have been designed to change wavelength or filter position as fast as possible to allow for multi-wavelength protocols.

Intelligent camera control

The camera is another bottle neck, if the frame rate is to be maximized. It is clear that the readout frequency and the number of pixels of the CCD chip are important parameters that influence the frame rate. Less well known is the fact that the way how illumination and camera readout are combined are important for frame rate and the duty cycle of illumination and exposure. Figure 4 compares two different methods of illumination- camera interplay. Images are acquired with an exposure time of 20ms and a camera readout time of 30 ms. In the upper part of the image exposure (and illumination) follows readout of the preceding image. It takes about 500 ms to acquire ten images in this mode. The lower part shows the acquisition if readout and exposure overlap. Here it takes 320 ms to acquire and readout ten images. If the camera allows such an operation mode TILL Photonics imaging software uses overlapping readout and exposure. If you compare imaging system specifications make sure that the frame rate listed in the data sheet is not given for an exposure time of 0ms because this mode is obviously useless for an experiment and frequently indicates that the software is not capable of overlapping readout and exposure.

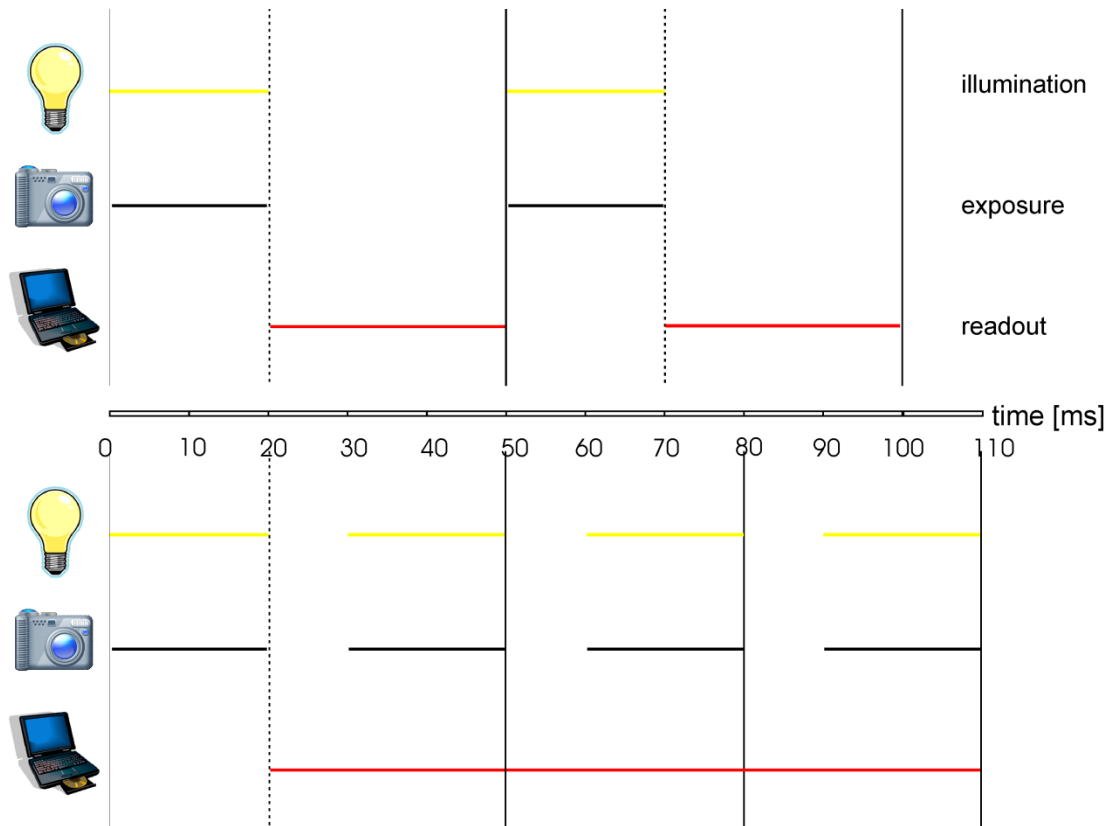


Figure 4: Overlapping readout and exposure increases the temporal resolution of your experiment. Compared is sequential and overlapping exposure and readout of the camera. Exposure time is 20 ms, readout time is 30 ms. Solid vertical lines indicate full readout of an image. If readout and exposure are sequential (upper traces) every image takes 50 ms, if exposure and readout overlap only the first image takes 50ms and all consecutive images take only 30 ms.

Specific applications that benefit from the TILL Photonics imaging concept

All fluorescence imaging applications benefit from reduced photobleaching.

Calcium Imaging

Neuroscience

Physiology

Calcium transients can be fast and have to be followed on a millisecond time scale

Vesicle/particle tracking

In order to be able to track (special software functionality) moving particles a high frame rate is required.

Electrophysiology

precise synchronization between imaging and electrophysiology setup required for integration of imaging and electrophysiology data

3D-microscopy

Applications where acquisition of z-stacks is required benefit from faster image acquisition, e.g.

Deconvolution

3D reconstruction

Synchronize Complex setups

Examples:

Measure fluorescence after liberation of caged compounds by a flash. Short delay required.

Change of solutions by valve control systems or drug application systems during fluorescence measurements.

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