

TILL Imaging System with an Olympus BX 51 microscope

# TILL Imaging Systems -

## fastest integrated system solution for fluorescence microscopy

### General Information

Fluorescence microscopy has become an indispensable tool for the examination of living cells and cellular structures. Modern fluorescence imaging techniques provide scientists with a toolset for the study of cell morphology, fluorescent probe detection (GFP etc.), Fluorescence Resonance Energy Transfer (FRET) and the analysis of fast cellular processes such as changes in intracellular ion concentration.

TILL Imaging Systems allow gap-free acquisition of image bursts at the maximum speed of the camera. Exposure-times are as long as the experiment requires them to be. Depending on the camera settings up to 100 ratio-images per second can be acquired.

TILL Imaging Systems integrate individual components to guarantee their optimum synchronization. The result is better data which is acquired in a shorter time.

A TILL Imaging system includes the following components:

- Programmable light source
- Real-time control unit
- Scientific camera(s)
- Microscope-specific adapter
- Imaging software and workstation

### Key Benefits

- Minimized phototoxicity
- Minimized photobleaching
- More and better data in a shorter time

### Key Features

Exact timing:  $\mu$ s accuracy

- Image acquisition and transmission of control signals executed in precisely timed manner via a 1 ms raster

High frame rates

- Full frame rates of 30 Hz and up to 200 Hz (at reduced spatial resolution) through optimized image read-out technique

Flexible Image analysis

- Software for multi-dimensional image analysis including a wealth of image analysis functions and application orientated modules, for example ratio analysis or particle tracking

## Applications and Fields

All Fluorescence imaging applications benefit from reduced photobleaching. TILL's Imaging Systems are ideal for investigating dynamic processes in single cells or groups of living cells. They are commonly used with electrophysiological measurements to determine concentration changes in intracellular ions, such as Calcium or H<sup>+</sup>. Concentration changes are often the subject in fields such as (neuro-) physiology and pharmacology.

The combination with TIRF (total internal reflection fluorescence) microscopy allows observation of plasma membrane specific processes. This is particularly interesting to cell biologists and neurophysiologists.

The ease, speed and flexibility with which excitation wavelengths can be altered combined with the ability of separating multiple emission wavelengths lends the system to fluorescent protein imaging (e.g. CFP, GFP YFP etc) and FRET measurements.

### Calcium Imaging

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

### Vesicle/Particle Tracking

- Tracking of moving particles requires a high frame rate (requires special software)

### Electrophysiology

- Precise synchronization of imaging and electrophysiology setup necessary for

integration of imaging and electrophysiology data

### 3D Microscopy

- Applications requiring acquisition of z-stacks benefit from faster image acquisition, for example 3D reconstruction

### Synchronization of Complex setups

- Measuring 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

## Functional Overview

### Basic Imaging System

The simplest way of acquiring 20 images with an exposure time of 10 ms at a rate of two images / second is to switch on the light, acquire the images and switch off the light again.

With this method, also referred to as a protocol, the ratio of illumination time necessary for camera exposure (20\*10 ms) and effective illumination time (10.000 ms) is very low. This results in unnecessary bleaching and phototoxicity.

While hopefully no imaging system on the market uses such a protocol, this is actually how fluorescence microscopy is performed, when the eyepieces of the microscope are used for viewing or pre-viewing.

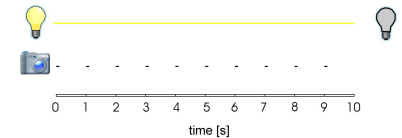


Fig. 1: Image acquisition in a very basic imaging system.

### Standard system

A more advanced system switches off the light between camera exposures. When using standard PC operating software for device control, the timing is subject to variable latency because of other tasks running on the computer at the same time. To account for PC-based latency, a larger overlap of illumination and exposure is necessary, ensuring that light is switched on before camera exposure starts. After exposure is completed, the light is switched off with a delay.

An alternative approach is to send commands to a device and wait for the confirmation of the receipt/execution of the command. Both methods use additional time, increase bleaching and reduce the maximum achievable frame rate.

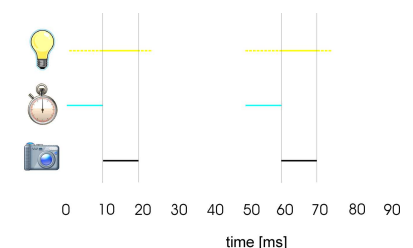


Fig. 2: Imaging system with PC control. To ensure the light source is switched on when exposure starts, the system switches on the light first and either waits for a predefined time or a confirmation from the light source is received before beginning camera exposure. The delay in both cases increases illumination time of the specimen and reduces frame rate. If exposure time is reduced, the situation becomes even more disadvantageous: The ratio of illumination time necessary for exposure to effective illumination time decreases further.

## TILL Photonics' concept

TILL Photonics approach circumvents the limitations of basic and standard imaging systems. It allows for maximum time resolution of the experiment with minimal bleaching and phototoxic effects.

The protocol for the light source, the camera and other devices is created by software running on a standard PC with a graphical user interface.

This software compiles a 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. It controls the devices by trigger signals with a high time resolution of 34 microseconds. The exact time point of the trigger signals is calculated by a timing model available for each supported device. As a consequence the ratio of illumination time to effective illumination time is minimized. Thus bleaching and phototoxicity are reduced and higher frame rates are possible.

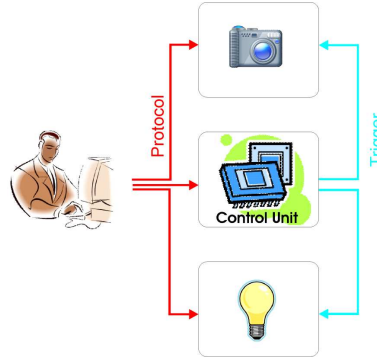


Figure 3: TILL Photonics' real time imaging system concept. Camera and light source receive instructions from the PC and are controlled by trigger signals from the control unit. The control unit is not subject to PC-typical latencies. Other devices, such as digital I/O, laser scanners, etc. are controlled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

## Other methods to further increase the frame rate

So far the situation has been simplified and the light source was treated as if it could be switched on infinitely fast (only delays caused by the computer were covered). The real world situation is of course different.

## Fast light sources

In addition to switching on and off, for example to change intensity or to select a wavelength or filter position, several other time constants have to be accounted for. The duration of these events directly influences the number of images that can be acquired per time.

TILL Photonics' Polychrome and Oligochrome light sources are designed to change wavelength or filter position as fast as possible, allowing for multi-wavelength protocols.

## Intelligent camera control

The camera is another bottle neck, if the frame rate is to be maximized. The readout frequency and the number of pixels of the CCD chip influence the frame rate. Less well known is the fact that the way in which 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 20 ms 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.

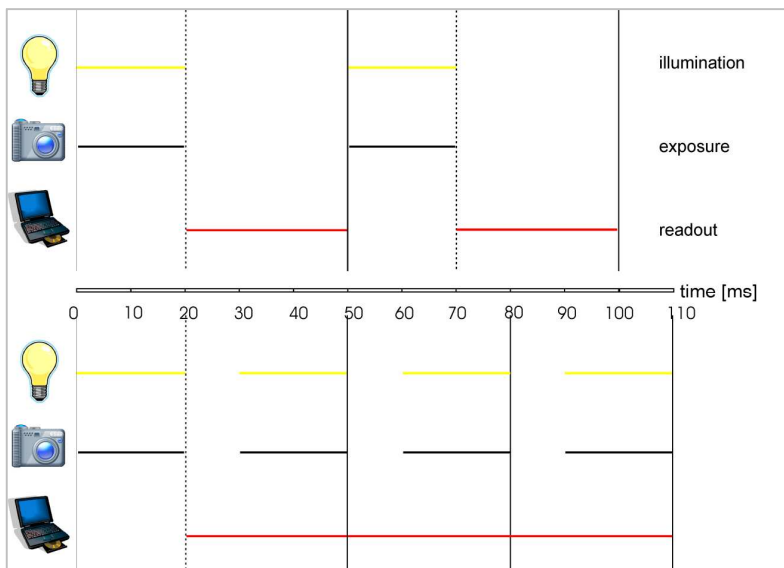


Fig 4: Overlapping readout and exposure increases the temporal resolution of your experiment. Compared is sequential and overlapping exposure and readout of the camera. Exposure time is 20 ms, readout time is 30 ms. Solid vertical lines indicate full readout of an image. If readout and exposure are sequential (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

# TILL Imaging System

## High Speed Imaging with TILL Photonics' Imaging Systems

TILL Photonics' Imaging Systems allow image acquisition on a very accurate and fast time scale.

Chip binning and selection of exposure area on the CCD sensor further speed up the acquisition process.

In addition, the systems allow exact synchronization of all functions including:

- Illumination
- Image acquisition
- Control of external devices

TILL Photonics' Imaging Systems offer the following unique features:

- Acquisition of 30 full frames/s at an exposure time of 30 ms with a 14 bit cooled CCD camera
- Acquisition of up to 200 frames/s by selecting a smaller area of interest (AOI) and switching between two wavelengths while acquiring at these rates
- Running an excitation spectrum with 1 nm increments to find the optimal excitation point for any fluorescent probe/filter set combination
- Use of a camera with a read out rate of 12.5 Mega pixels/s with a low read noise of 13 electrons/pixel and a full 12 bit dynamic range

Product specifications and descriptions in this document are subject to change without notice.

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## Fluorescence Microscopy Solutions from TILL Photonics

TILL Photonics offers fully automated, integrated and modular digital microscope solutions for live-cell applications in research and education.

For more information call +49 89 895 662-0, or contact your local TILL Distributor.



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