Advanced Imaging Regimes & Measurement Control

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Goals: Providing answers to the questions

What are the typical limitations of classical/basic fluorescence imaging regimes?

Can I use my commercial microscope to implement this fancy microscopy technique I read about?

I am building my own microscope for a new imaging technique. Do I have to write my own software?

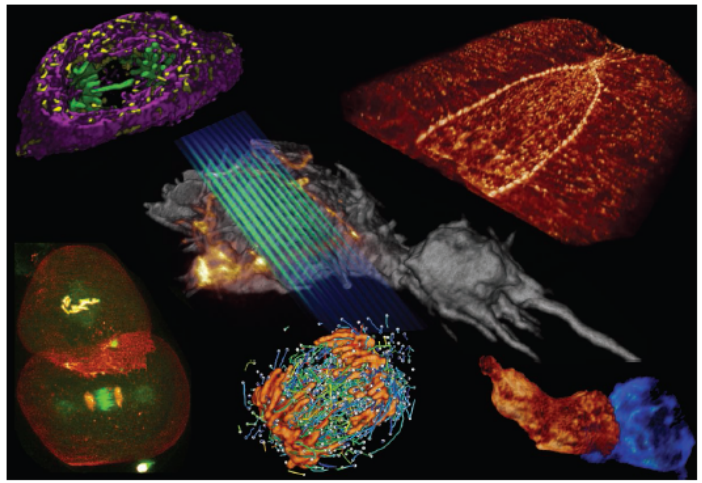
I found this fancy science paper, what the heck are they talking about?

RESEARCH ARTICLE SUMMARY

ADVANCED IMAGING

Lattice light-sheet microscopy: Imaging molecules to embryos at high spatiotemporal resolution

Bi-Chang Chen, Wesley R. Legant, Kai Wang, Lin Shao, Daniel E. Milkie, Michael W. Davidson, Chris Janetopoulos, Xufeng S. Wu, John A. Hammer III, Zhe Liu, Brian P. English, Yuko Mimori-Kiyosue, Daniel P. Romero, Alex T. Ritter, Jennifer Lippincott-Schwartz, Lillian Fritz-Laylin, R. Dyche Mullins, Diana M. Mitchell, Joshua N. Bembenek, Anne-Cecile Reymann, Ralph Böhme, Stephan W. Grill, Jennifer T. Wang, Geraldine Seydoux, U. Serdar Tulu, Daniel P. Kiehart, Eric Betzig*



Lattice light-sheet microscopy. An ultrathin structured light sheet (blue-green, center) excites fluorescence (orange) in successive planes as it sweeps through a specimen (gray) to generate a 3D image. The speed, noninvasiveness, and high spatial resolution of this approach make it a promising tool for in vivo 3D imaging of fast dynamic processes in cells and embryos, as shown here in five surrounding examples.

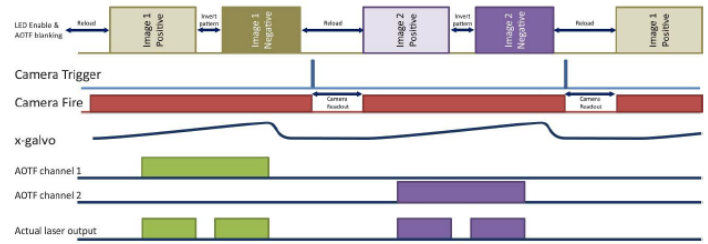


Fig. S18. FPGA control signals. Galvo and AOTF control signals as synchronized to the SLM during the acquisition of a single plane of data in two colors in the dithered mode.

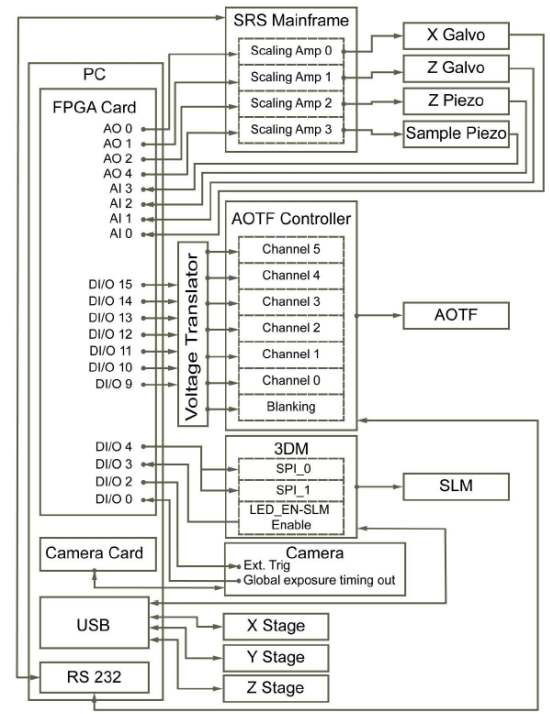
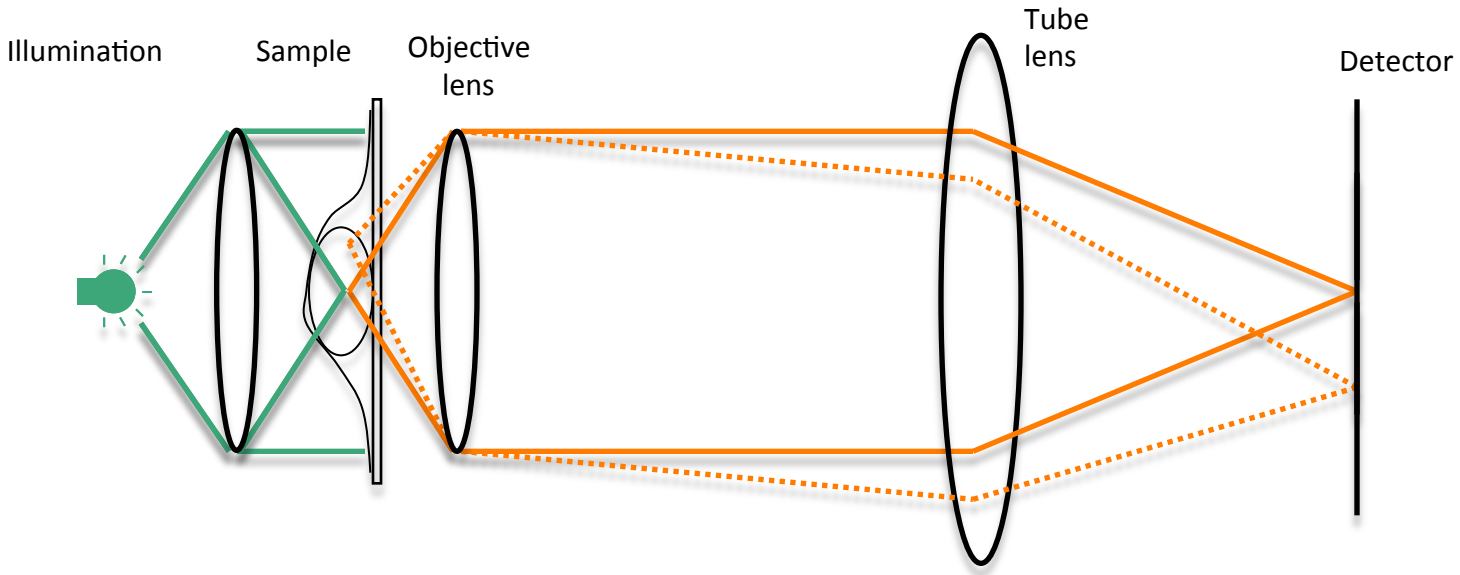


Fig. S14. Control schematic for the microscope. The SLM LED Enable output is used as the master clock source, but the FPGA provides the analog /digital outputs to control most of the other electronics critical to image acquisition. Additional boards in the computer receive the data from the sCMOS and inspection cameras, and control the sample positioning stages.

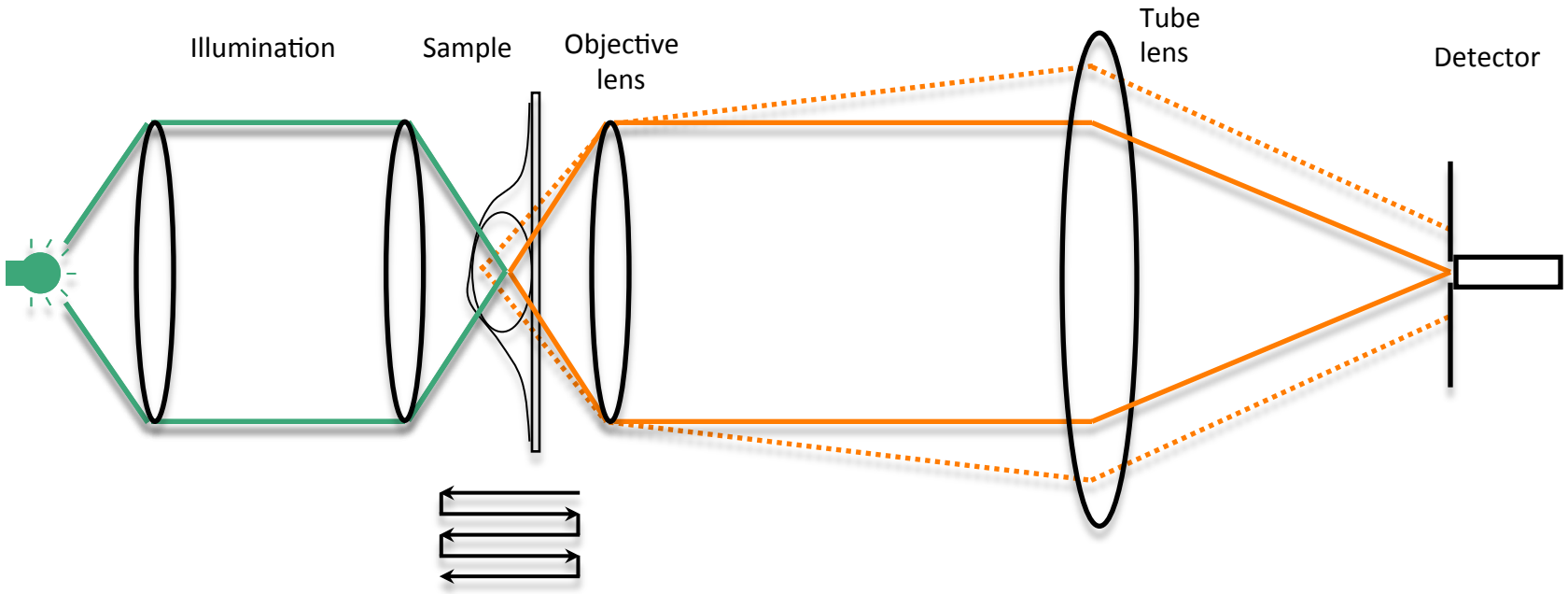
Basic Fluorescence Microscopy

Widefield

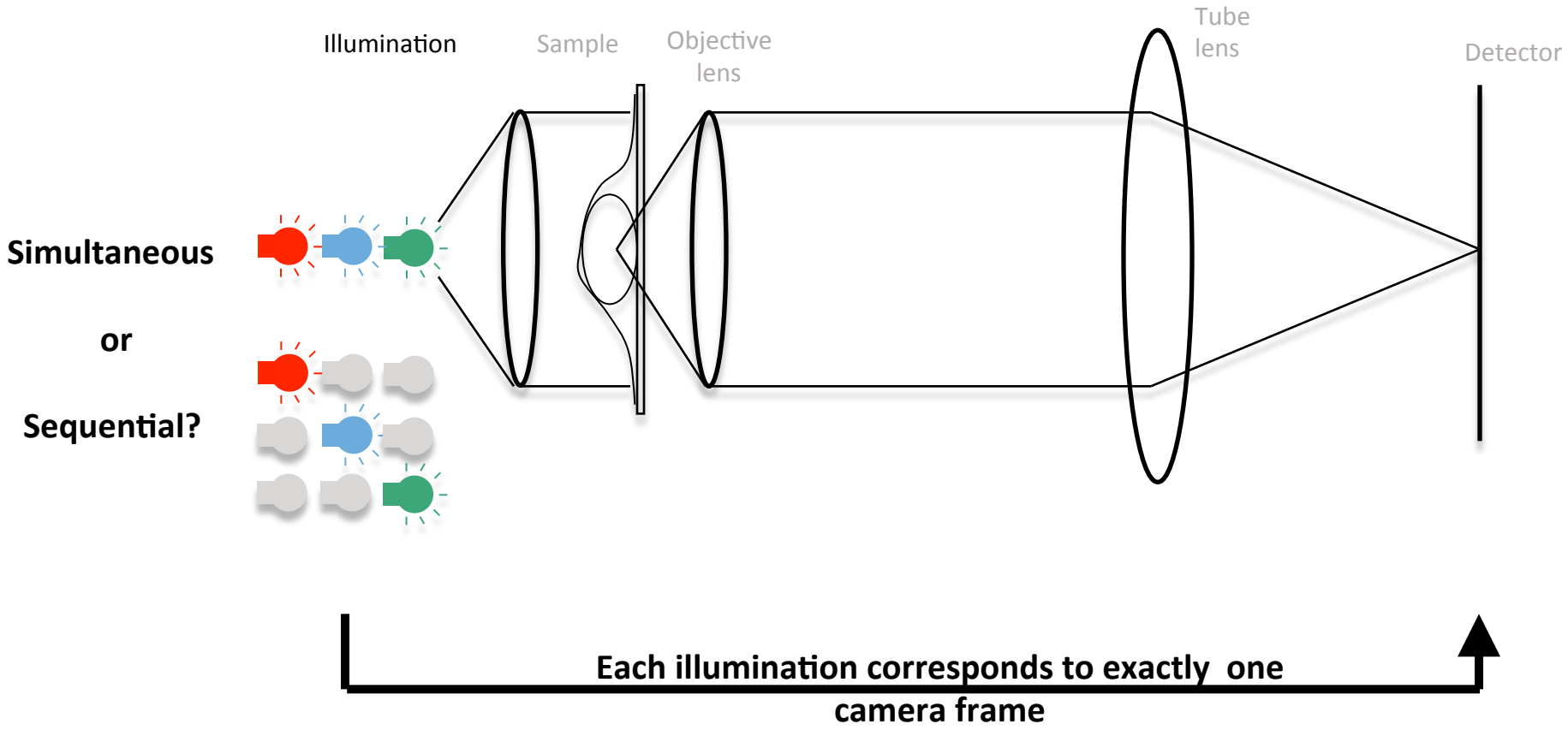


Basic Fluorescence Microscopy

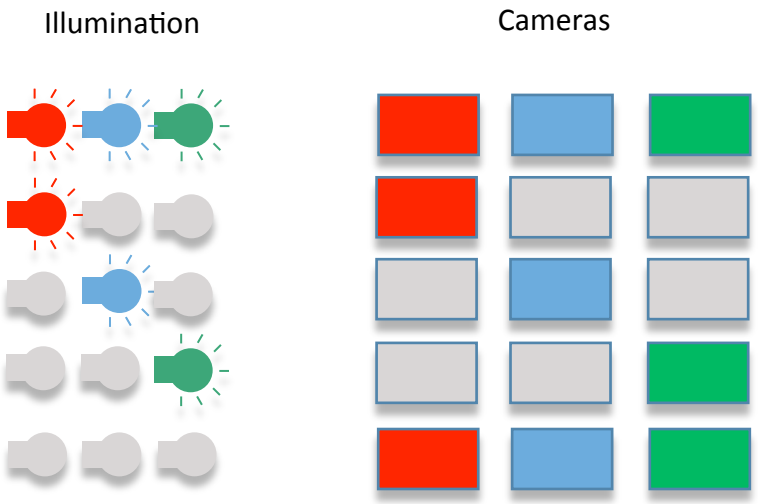
Confocal



Basic Fluorescence Microscopy

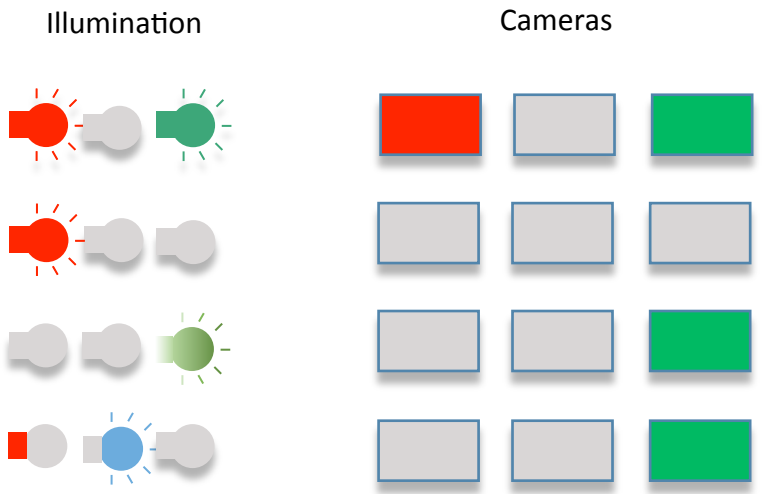


Commercial Software for Basic Imaging



Works well for certain advanced imaging methods like widefield deconvolution, PALM and STORM because these methods rely mostly on sample preparation and post-processing.

Bespoke Imaging Regimes for Advanced Imaging



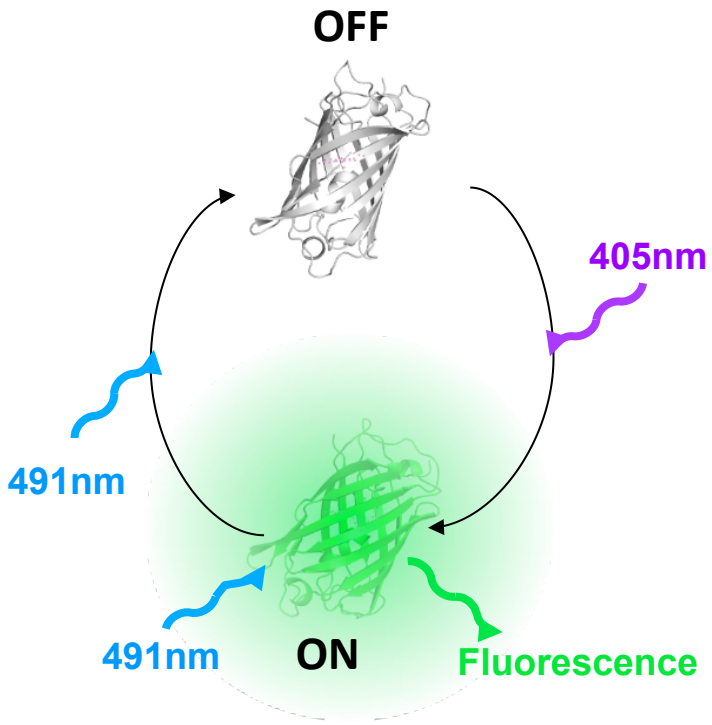
Advanced imaging modalities *may* require more flexibility!

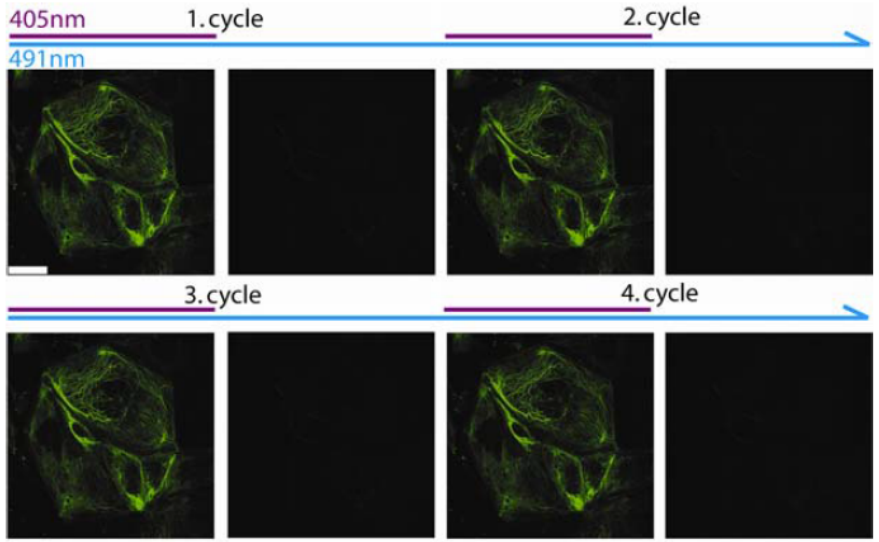
Think first about your application and then about how to best implement the measurement control.

Try to be efficient: full flexibility comes at a high cost (e.g. writing your own software)

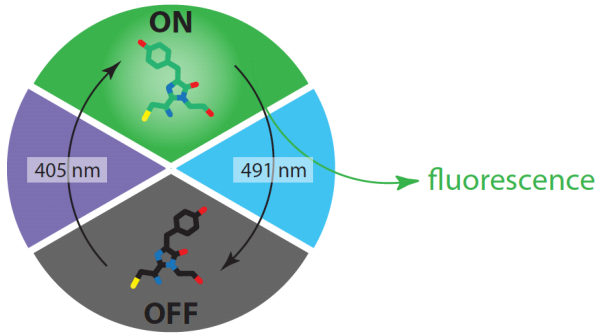
Example: RESOLFT Imaging

RESOLFT = **RE**versible **S**aturable **O**ptica**L** **F**luorescence **T**ransitions
= molecular switches





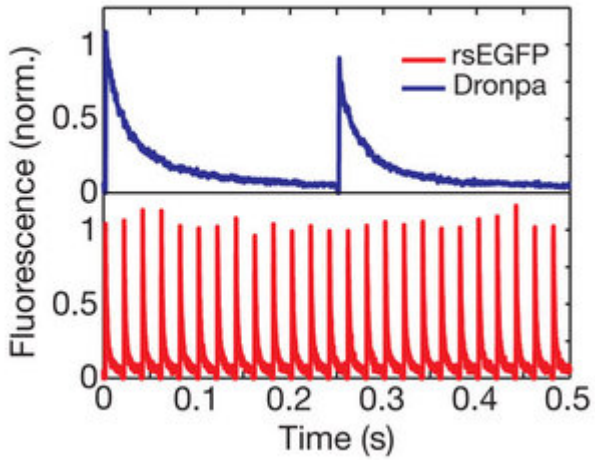
Reversibe switchable fluorescent proteins:
Switching through e.g. *cis-trans* isomerization



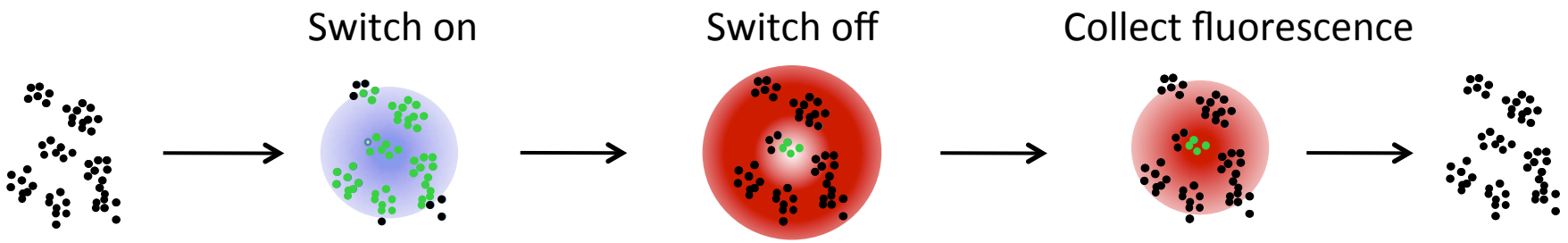
Grotjohann, Hell, et al., Nature 478, p. 204 (2011)

Example: RESOLFT Imaging

Switching kinetics: ON … 100 µs
OFF … 1-10 ms

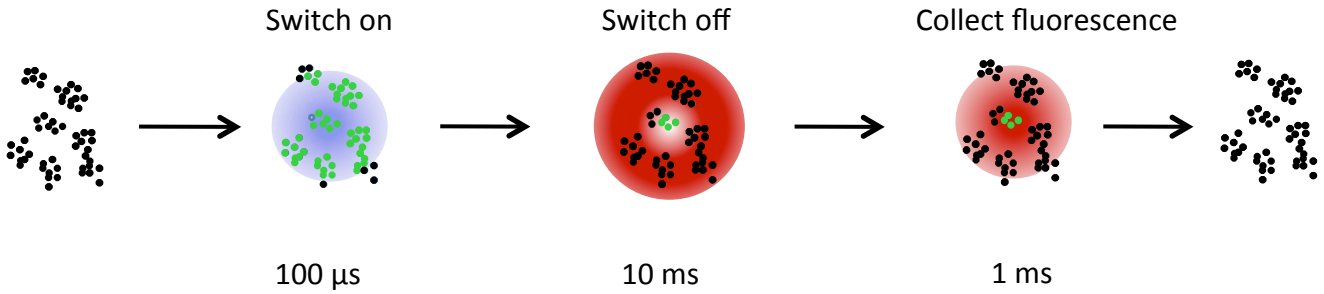


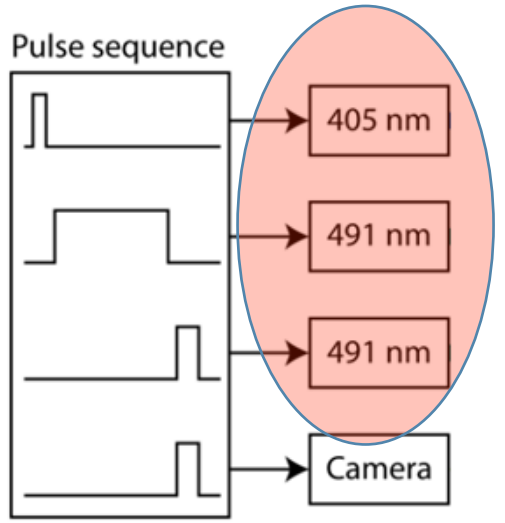
Application: Super-Resolution



Grotjohann, Hell, et al., Nature 478, p. 204 (2011)

Example: RESOLFT Imaging





How?

Identification of special imaging requirements:

1) Three sequential illumination but just one camera exposure

2) Individual durations of illuminations

3) Illumination two and three with same colour (but not necessarily same power and with the same beam profile)

Mechanical shutters and filters?
Too slow!

Directly triggering the lasers?
Depends on laser!

Acousto-optics?
Expensive but powerful!

Acousto-optical tunable filter

AOTF: Acousto-optical tunable filter

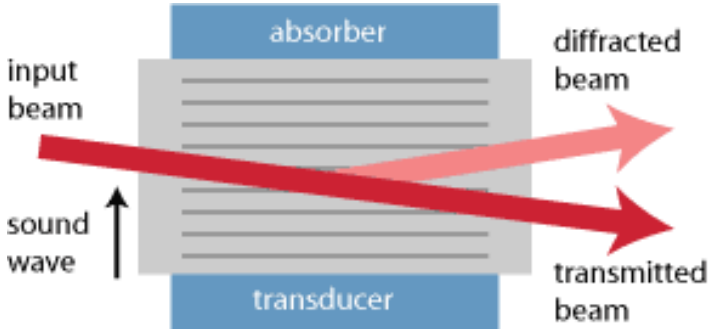
Advantages:

- Up to 8 channels/colours
- Amount of diffracted light can be set individually
- Very fast: rise time of <2µs

AOM: Acousto-optical modulator

- Only one channel but much cheaper

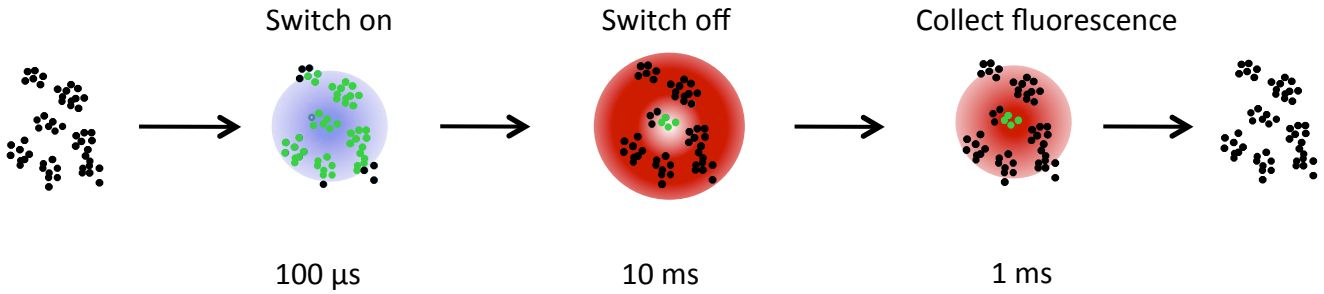
Working principle

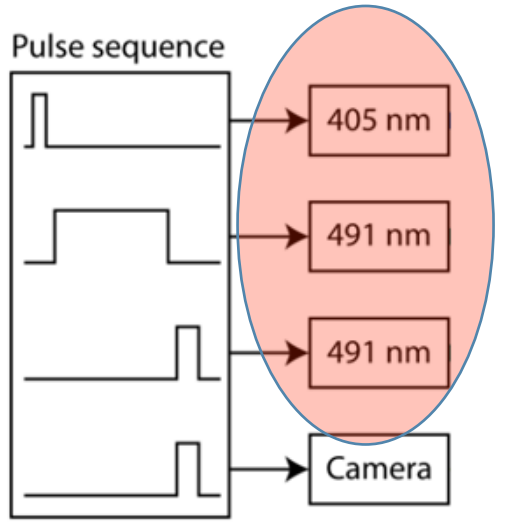


Piezoelectric transducer attached to a crystal is used to excite a sound wave with a frequency of the order of 100 MHz

Light gets diffracted at the traveling periodic refractive index grating generated by the sound wave

Example: RESOLFT Imaging





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Identification of special imaging requirements:

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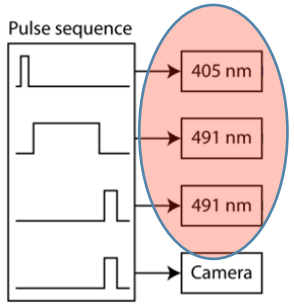
✓
✓

AOTF

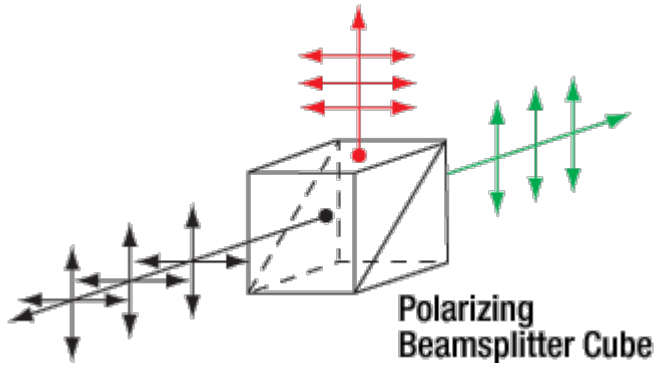
How to get two laser lines?

How to extract two beam lines out of one laser?

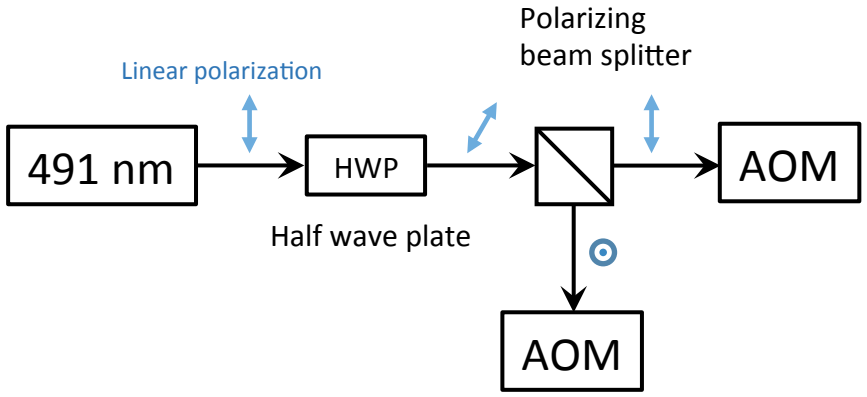


How?

Generating two 491 nm laser lines
- Buying two laser (expensive)
- Exploiting polarization



Option 1: Two acousto-optical devices

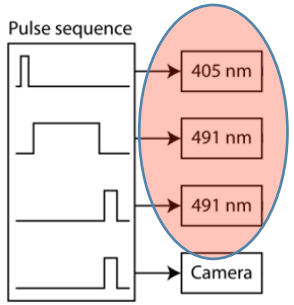


Option 2: Electro-optical modulator (EOM)

EOM = device which can be used for controlling the power, phase or polarization of a laser beam with an electrical control signal.

Principle of operation = based on linear electro-optic effect, the modification of the refractive index of a nonlinear crystal by an electric field

How to extract two beam lines out of one laser?



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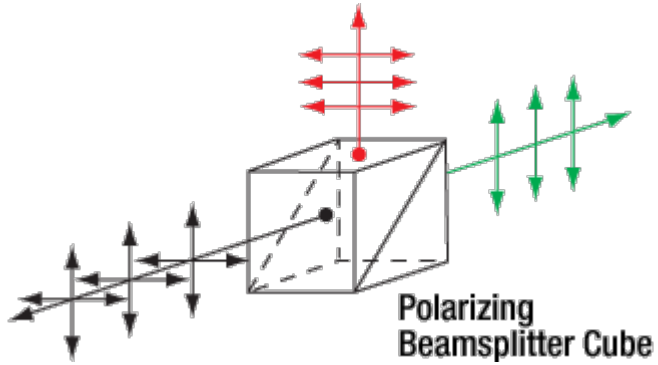

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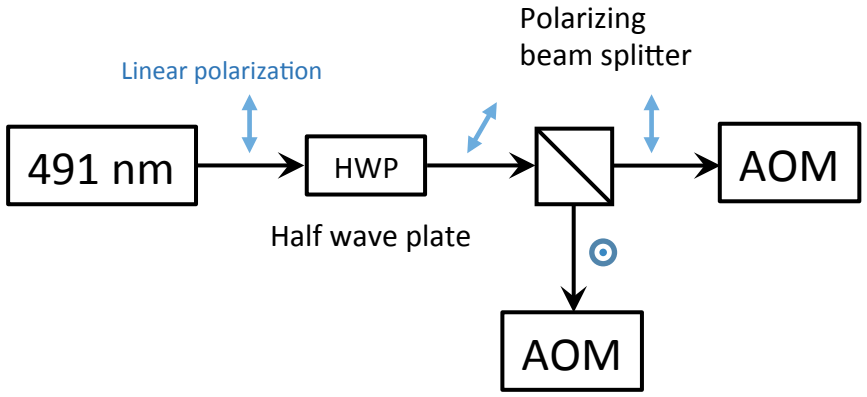

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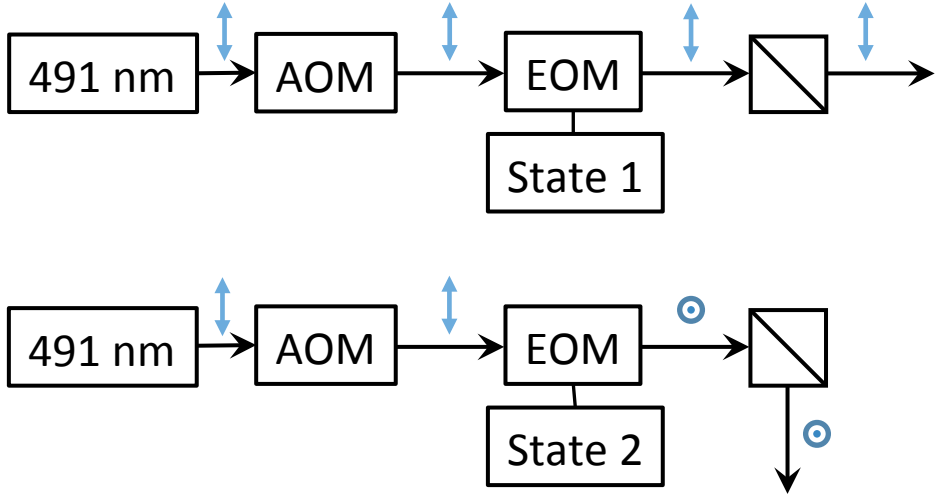
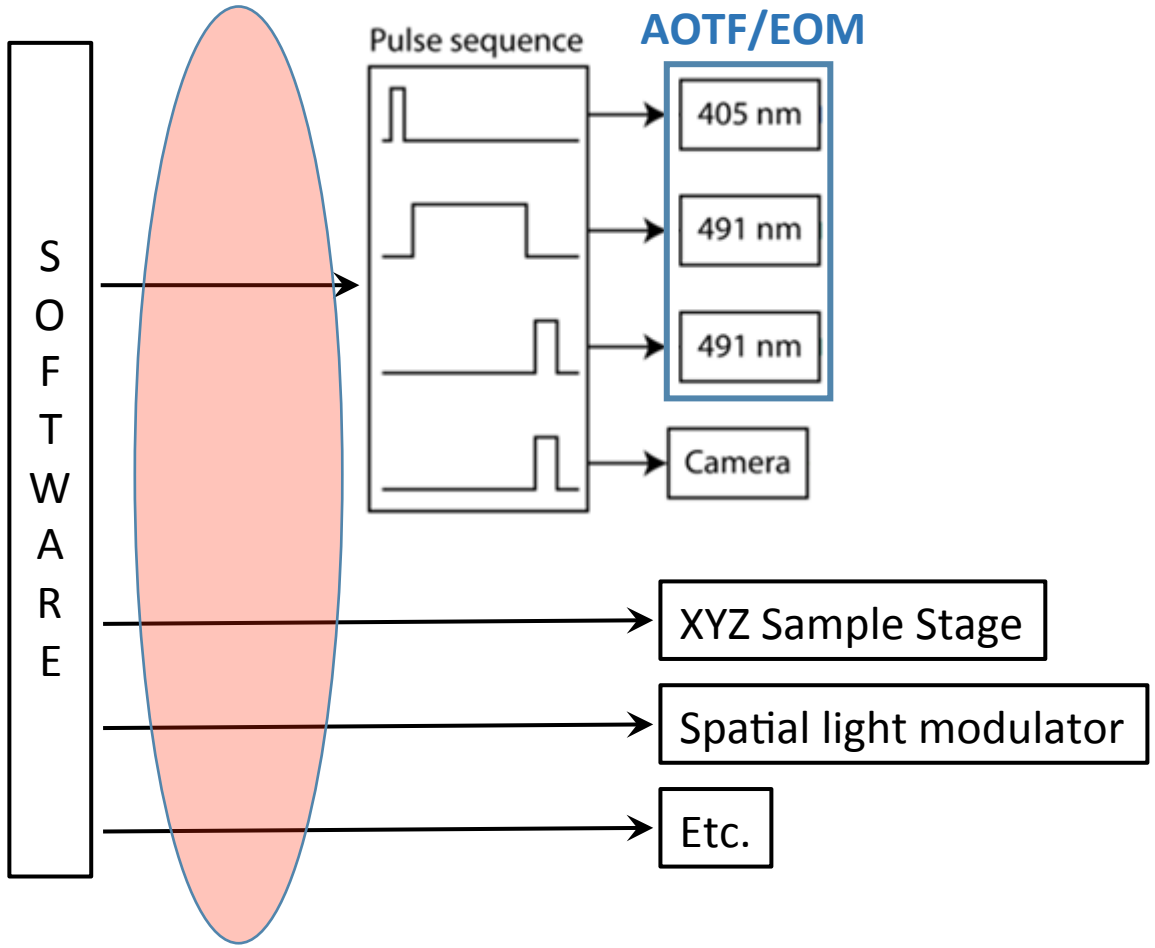


Setting up a measurement – The problem of hardware synchronization

Commercial:
Leica, Olympus,
Zeiss, API, etc

Free:
µManager,
Home-built



Computer sends commands to the devices each time a change is required.

This will cause latencies (up to 100 ms) since the application software run on a standard desktop operating system can not produce accurate timings.

Two solutions:

1) Additional hardware
2) Advanced triggering

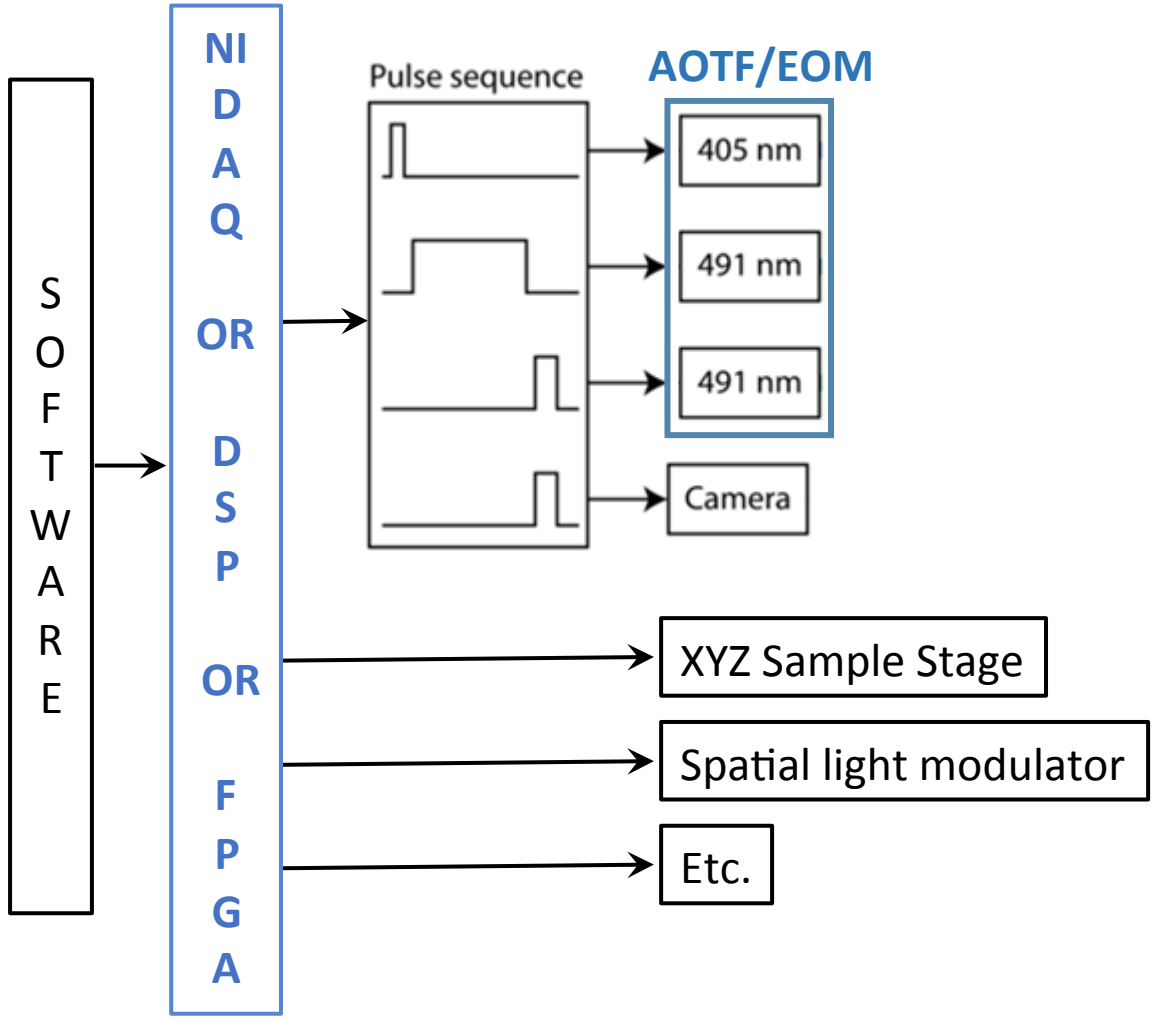
Hardware synchronization

Solution 1: Using an additional piece of hardware

Commercial:
Leica, Olympus, Zeiss, API, etc

Free:
µManager (depends),

Home-built



Very powerful, but time consuming to integrate.

National Instuments Data Acquisition (**NIDAQ**) is a low cost input, output device with analoge, digital and counters. Control through Labview.

A digital signal processor (**DSP**) is a specialized microprocessor with its architecture optimized for the operational needs of digital signal processing.

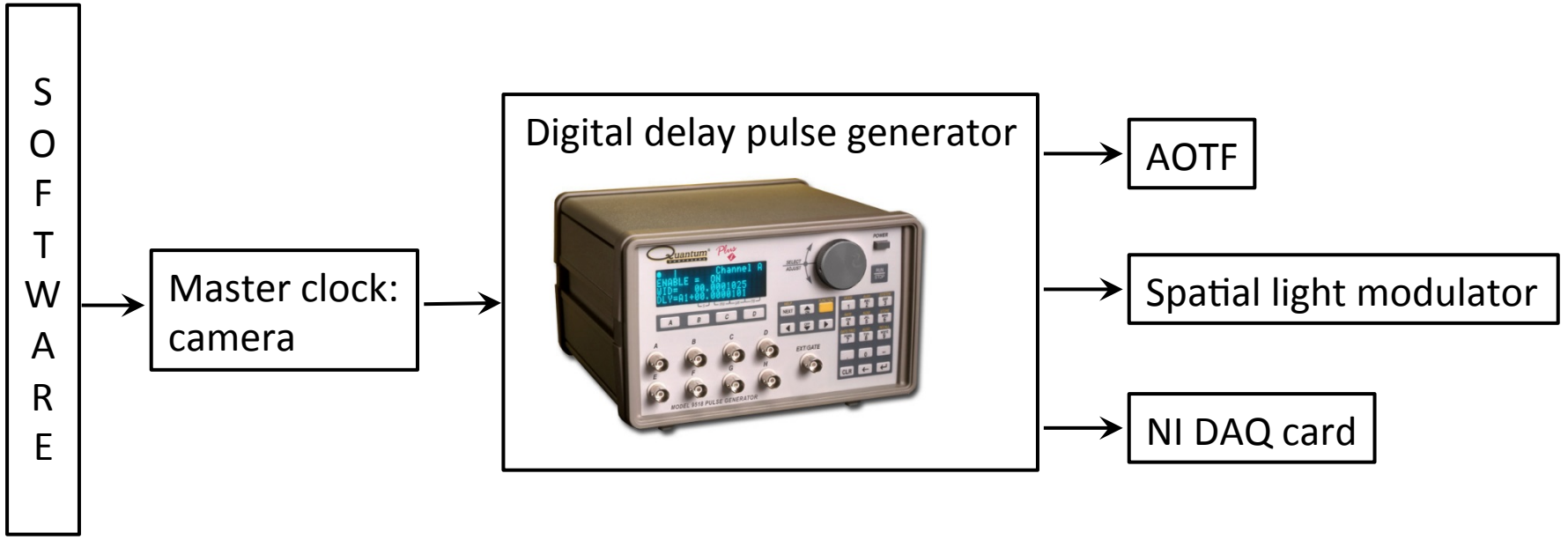
A field-programmable gate array (**FPGA**) is an integrated circuit designed to be configured by a customer or a designer after manufacturing – hence "field-programmable".

Hardware synchronization

Solution 2: Clever triggering

Commercial:
Leica, Olympus,
Zeiss, API, etc
(depends)

Free:
µManager,
Home-built

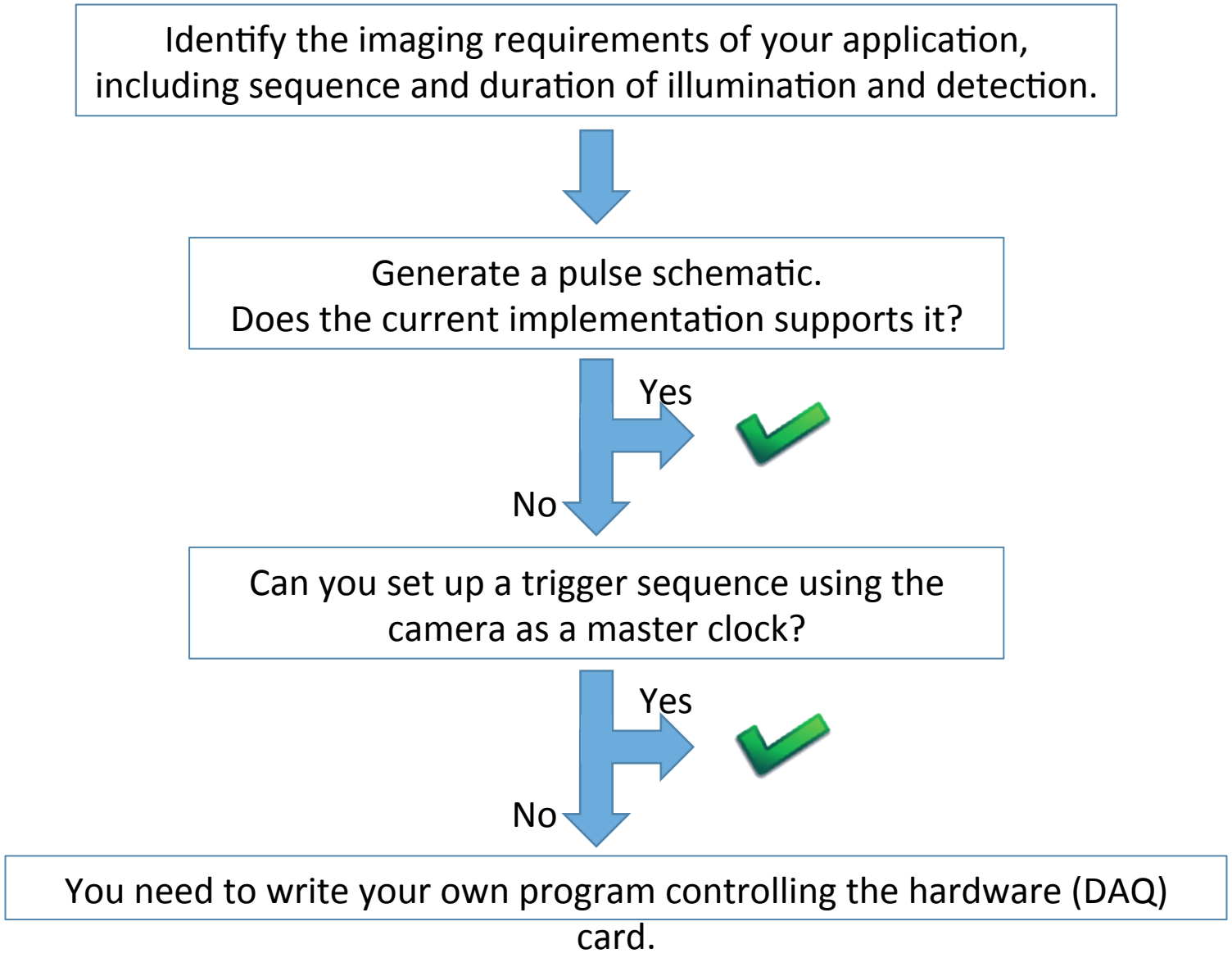


Software delegates control to camera, which provide fast and accurately timed operation

A digital delay pulse generator creates the bespoke pulse sequence for the particular imaging application

Synchronization between the devices is achieved by routing TTL pulses over signal cables (BNC)

Building your advanced imaging system



Take home messages

- Find out what your application needs.
- Clever triggering can save you a lot of work.
- If you need a very flexible illumination regime you need to write your own measurement software.

Now you are ready to

understand this.

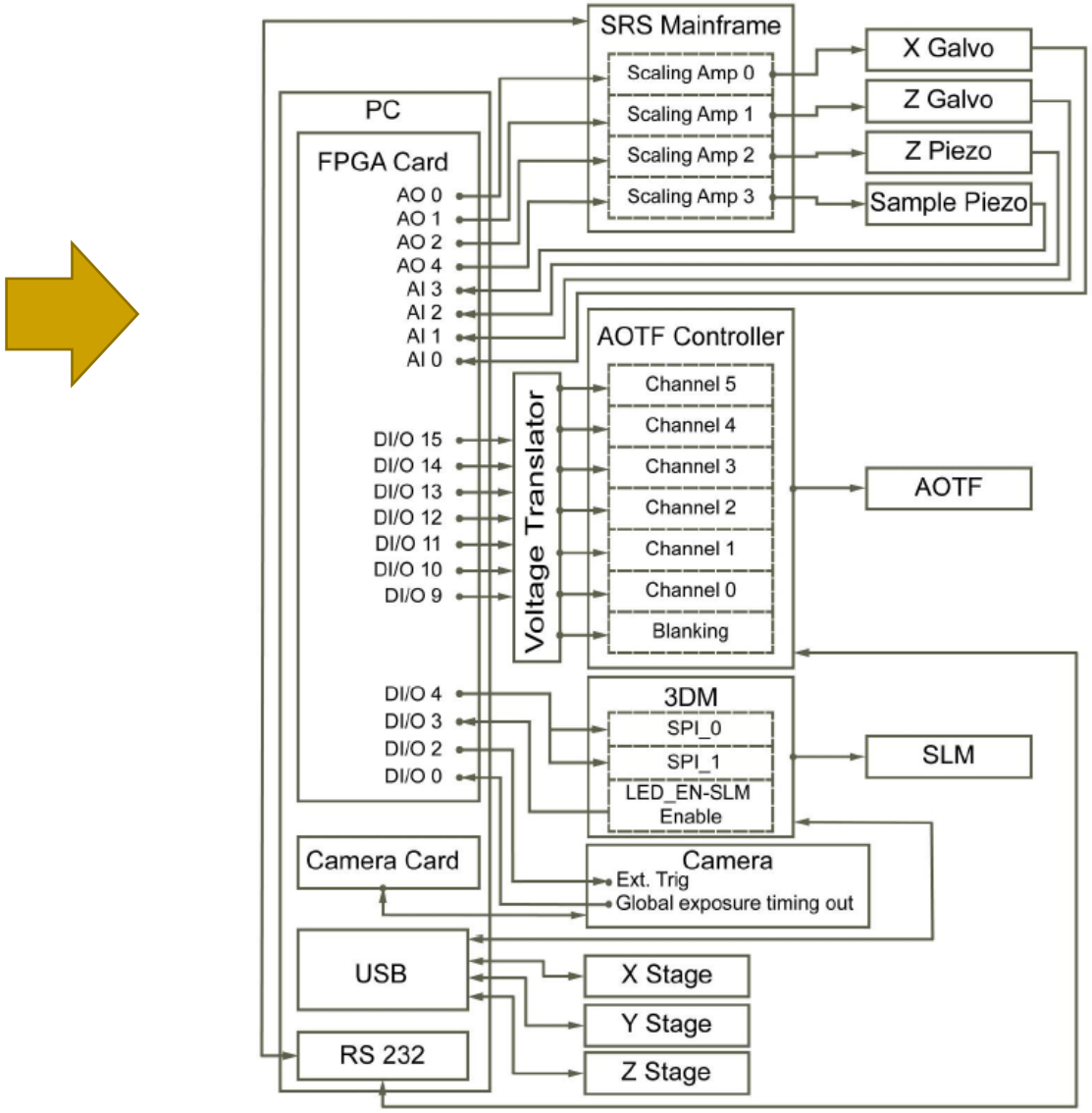


Fig. S14. Control schematic for the microscope. The SLM LED Enable output is used as the master clock source, but the FPGA provides the analog /digital outputs to control most of the other electronics critical to image acquisition. Additional boards in the computer receive the data from the sCMOS and inspection cameras, and control the sample positioning stages.