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Concept and Implementation of a Compact Multi-Channel Fluorescence-Microscope Unit

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Motivation

Due to the large number of single experiments in systems biology there is a demand for accelerating image acquisition. Here the concept and the implementation of a compact multi-spectral microscope unit is presented, which is able to acquire images of four fluorophores in parallel in one field of view without moving components.

Conceptual Design

Spectral parallelization in one microscope unit

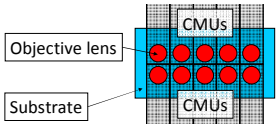
- Four excitation channels
- Four emission channels
- One standard objective lens
- One tubus lens and one camera for each emission channel

Channel	Excitation	Emission	Fluorophore
Blue	360 ± 20 nm	455 ± 25 nm	DAPI
Green	485 ± 8 nm	525 ± 25 nm	GFP
Red	570 ± 10 nm	620 ± 30 nm	RFP, MCherry
NIR	680 ± 20 nm	785 ± 15 nm	DRAQ5

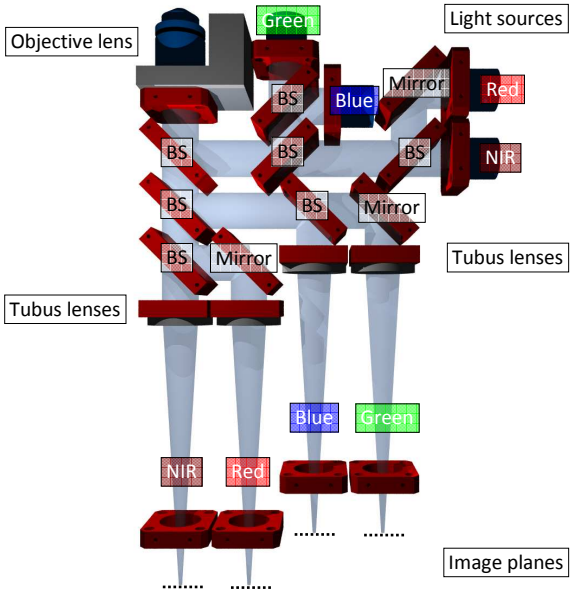
Excitation and emission bands of the four channels

Spatial parallelization

- Combining multiple microscope units



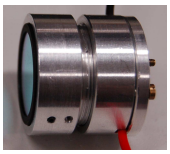
Multiple microscope units



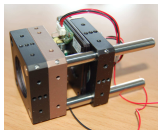
Implementation

Light sources

- **Blue channel:** UV-LED Nichia NCSU033B
  - Peak wavelength: 360 - 370 nm
  - Spectrum half width: 9 nm
  - No bandpass filter needed
- **Green channel:** Luxeon Rebel Blue
  - Peak wavelength: 475 - 480 nm
  - Spectrum half width: 20 nm
  - Bandpass filter: 485 ± 7.5 nm
- **Red channel:** Halogen bulb
  - Coupled into fiber
  - Mechanical shutter
  - Bandpass filter: 570 ± 10 nm
- **NIR channel:** Luxeon Rebel DeepRed
  - Peak wavelength: 662.5 nm
  - Spectrum half width: 20 nm
  - Bandpass filter: 680 ± 20 nm



LED holder with collimation lens and bandpass filter



Fiber-mount for halogen light source with shutter, collimation lens and bandpass filter

Imaging components

- **1 objective lens:** Zeiss Plan-Apochromat
  - Magnification: 10 x
  - NA: 0.45
- **4 tubus lenses:** Zeiss 452960
  - Focal length: 164.5 mm
- **4 cameras:** Basler scA 1400 – 30gm
  - 1392 x 1040 pixel
  - Pixel size: 6.45 µm x 6.45 µm

Components for spectral selection

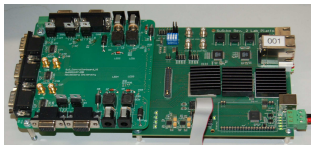
- 7 dichroic beam splitters
- 4 bandpass filters in front of the cameras

Moving axes

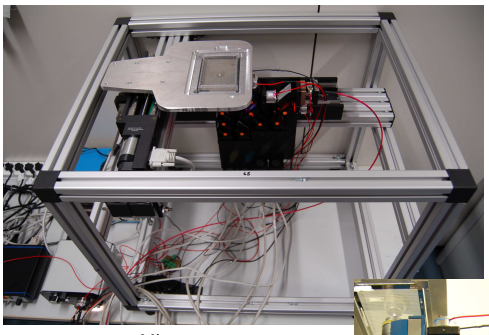
- **Substrate holder:** PI M-404 Stage
- **Microscope unit:** PI M-414 Stage
- **Focusing unit:** Piezosystem Jena PiFoc

FPGA controller board

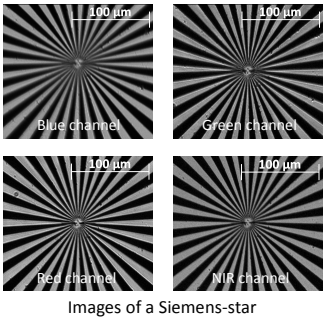
- Configure and trigger light sources
- Trigger cameras



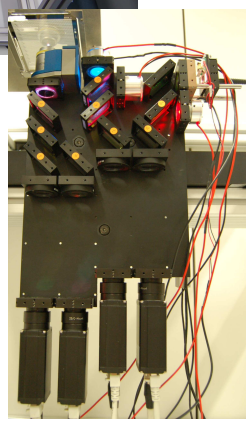
FPGA controller board



Microscope setup



Images of a Siemens-star



Compact microscope unit

Conclusion

With this microscope, it is possible to observe different cell structures, which are marked with four different fluorophores, at the exact same point in time. Compared to state-of-the-art automated fluorescence microscopes, there are no filter wheel movements needed to select the desired excitation and emission bands. The only moving parts are the two axes and the piezo focusing unit when another position on the substrate should be observed. Therefore the rate of analysis when performing experiments with living cells can be increased and only depends on the exposure time and the time needed to move to different positions.

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