Introduction to spinning disk confocal imaging

Visualizing motion in live cells and soft matter by fluorescence imaging

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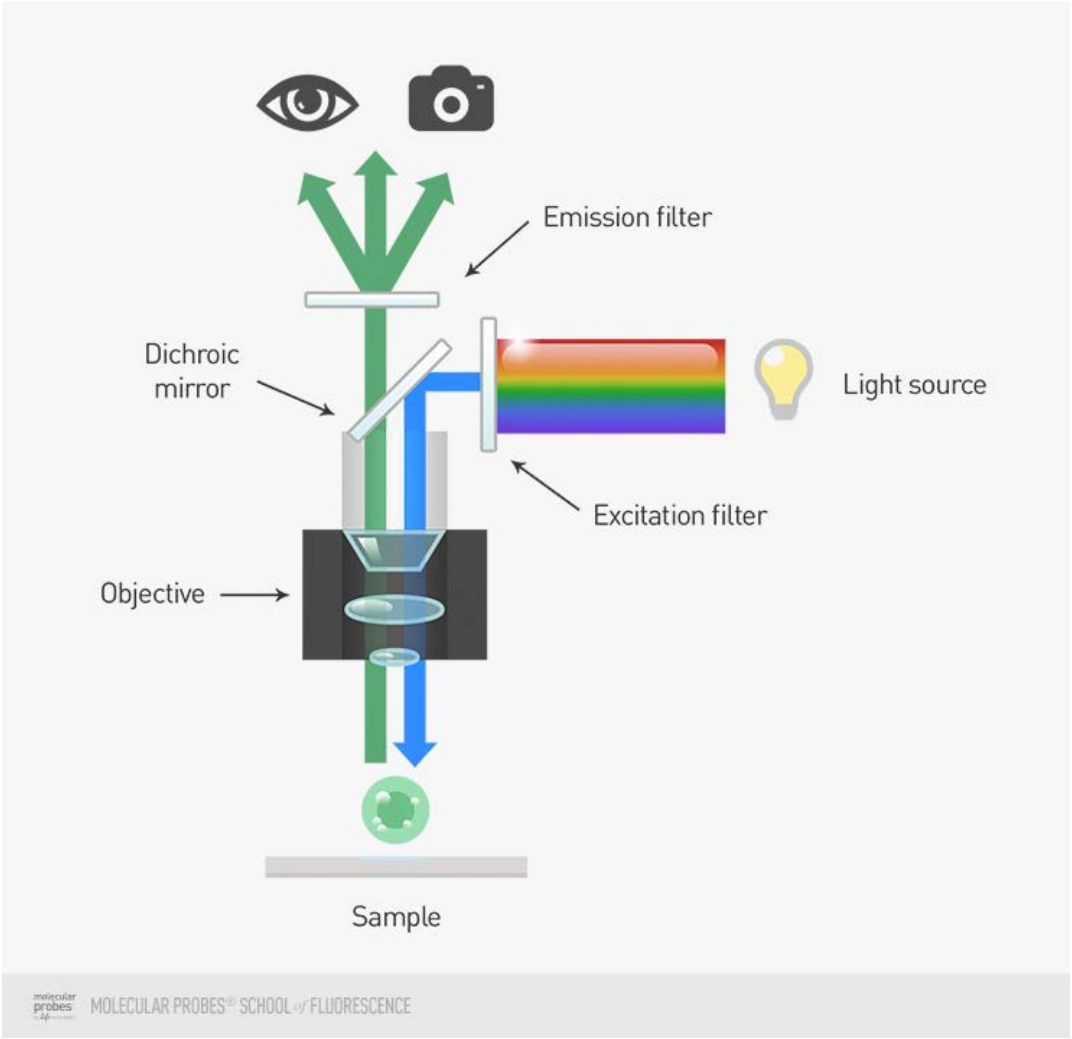
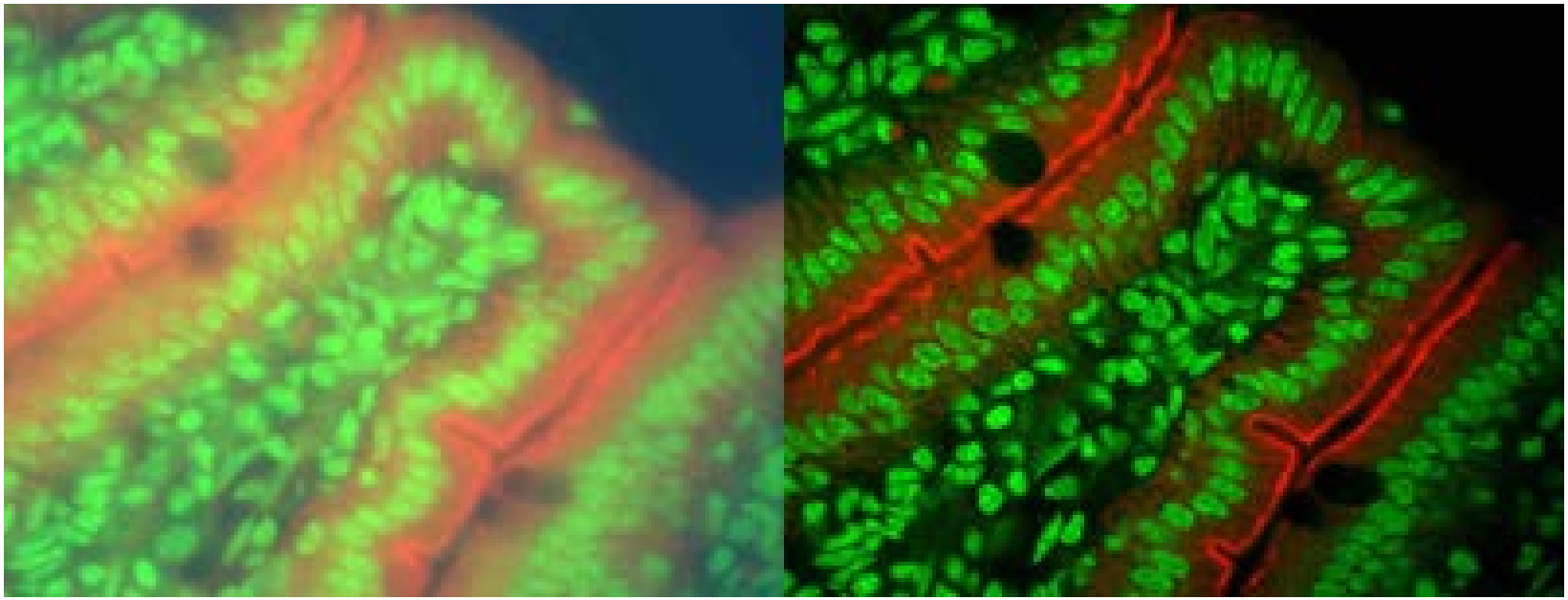
Fluorescence imaging

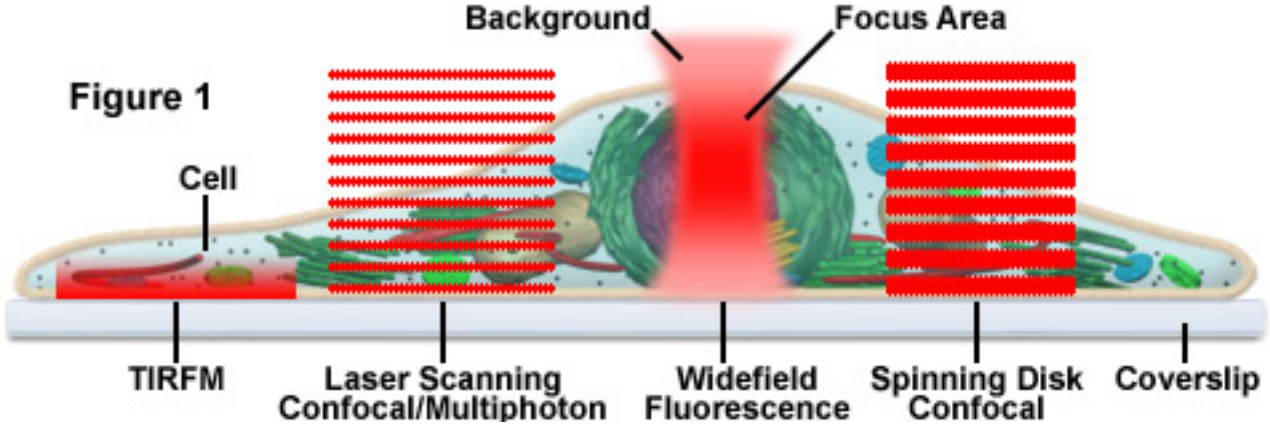


Fluorescence microscope

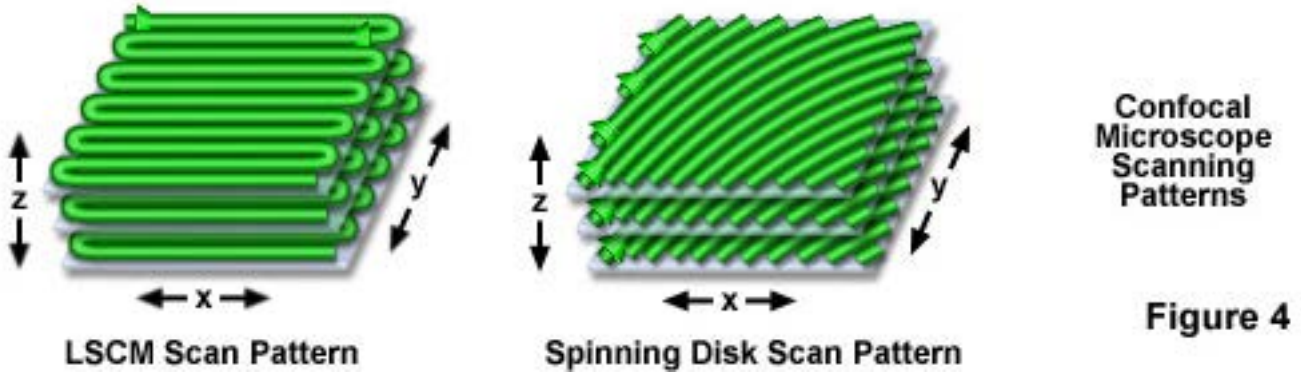
1) Create light of one color, e.g. laser.
2) Direct it to the sample.
3) Observe fluorescence of a different color.

Difference widefield and confocal imaging

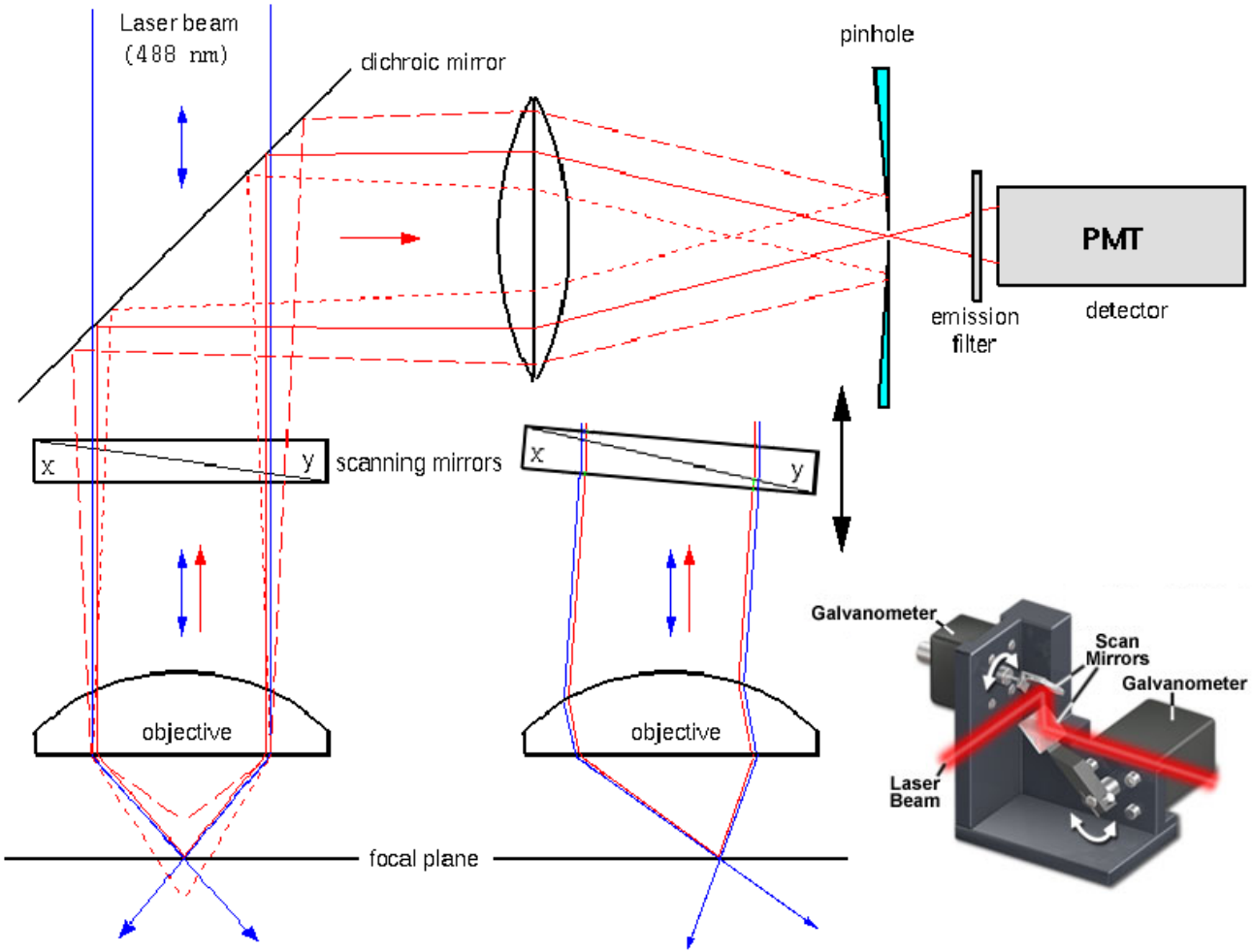




Scanning patterns



Pinhole in confocal microscopy



Intrinsically slow: 100 ms – 1 sec per confocal plane.

Multiplexing: spinning disk – array of confocal spots

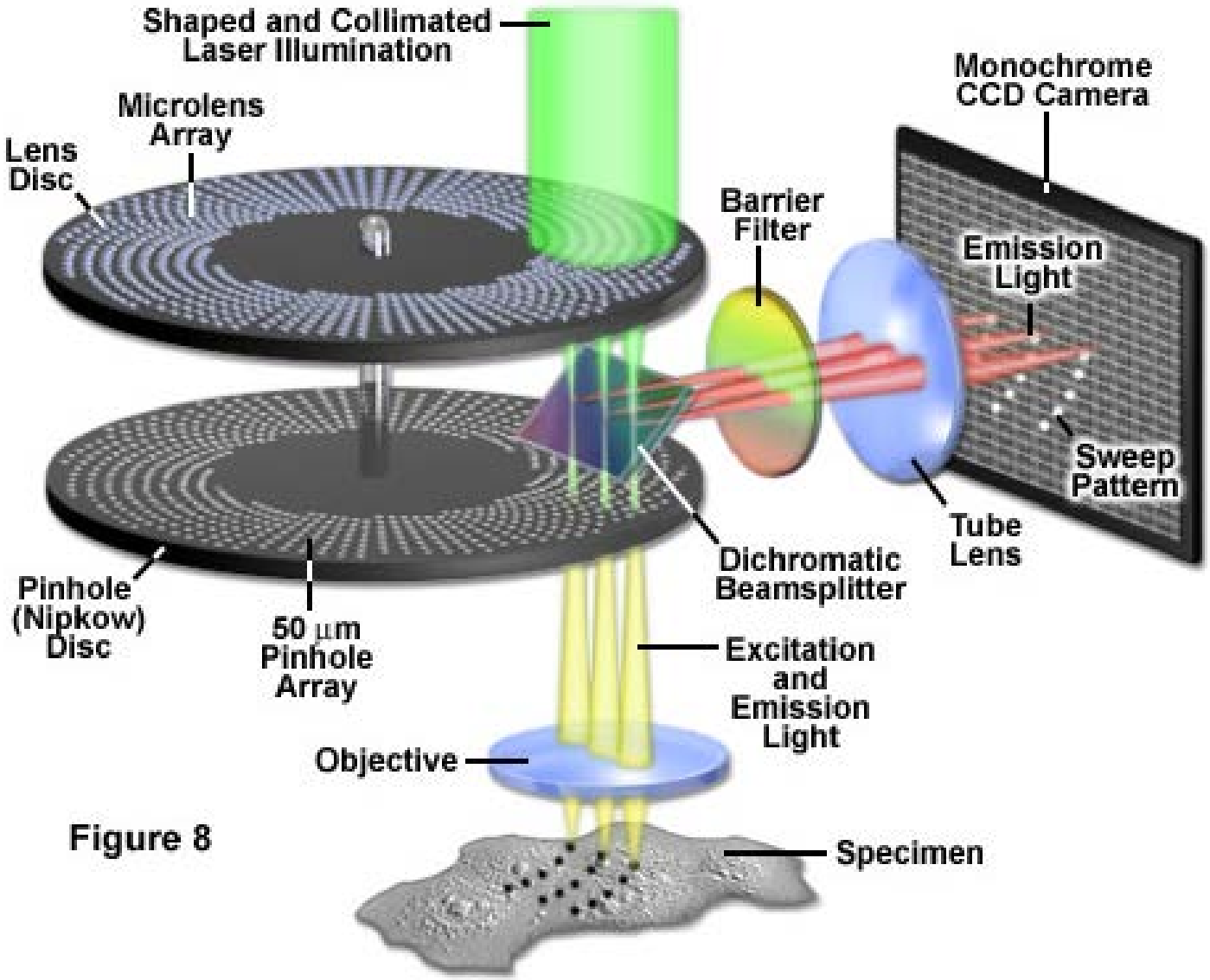


Figure 8

Multiplexing: spinning disk – array of confocal spots

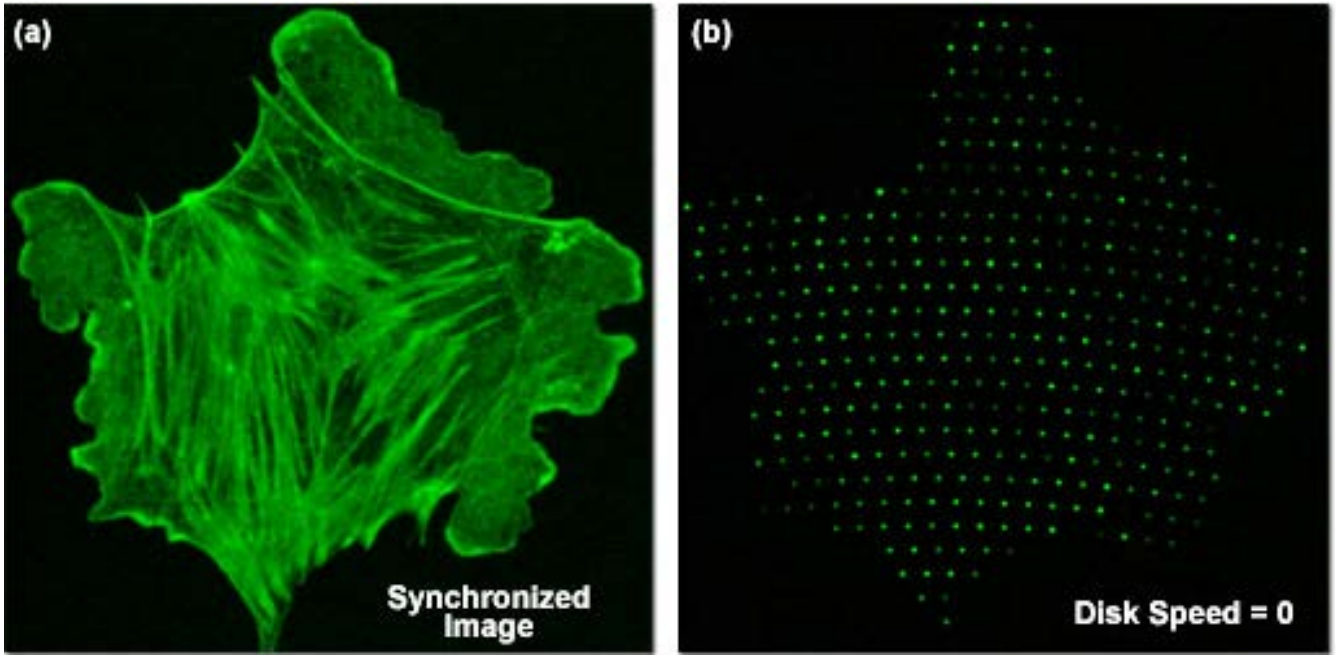


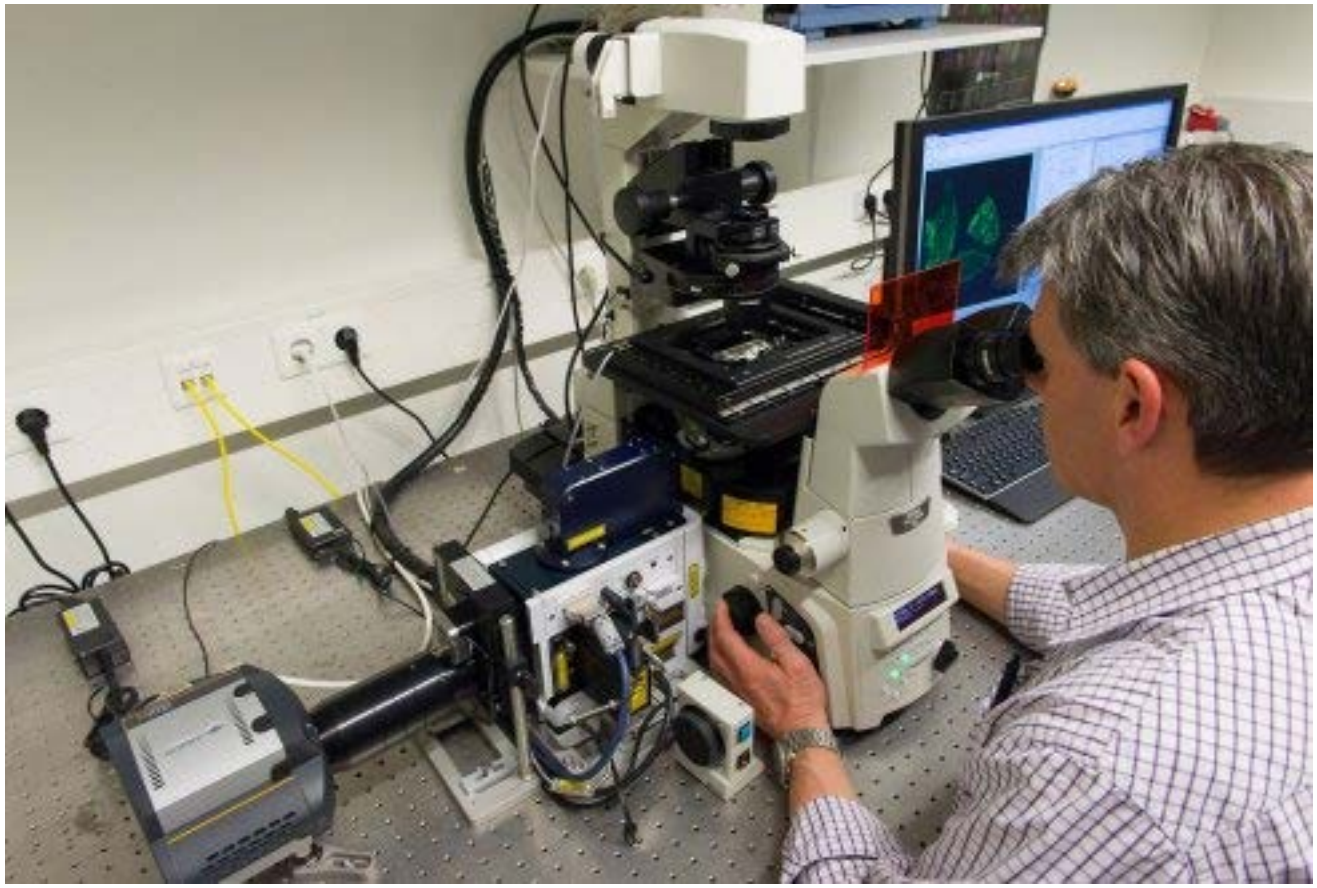
Image sensor acquires light as the disk rotates.

Each 30 degree rotation of the disk ‘writes’ a full image onto the camera. 12 images / rotation. 1000 images a second. Fast processes.

In practice: integrate multiple passes. Camera exposure time 100 ms. Total exposure time of each spot much longer compared to CLSM. Lower peak excitation intensities. Less photobleaching. Live cell imaging.

Example: Cell division.

Spinning disk microscope



Spinning disk microscope

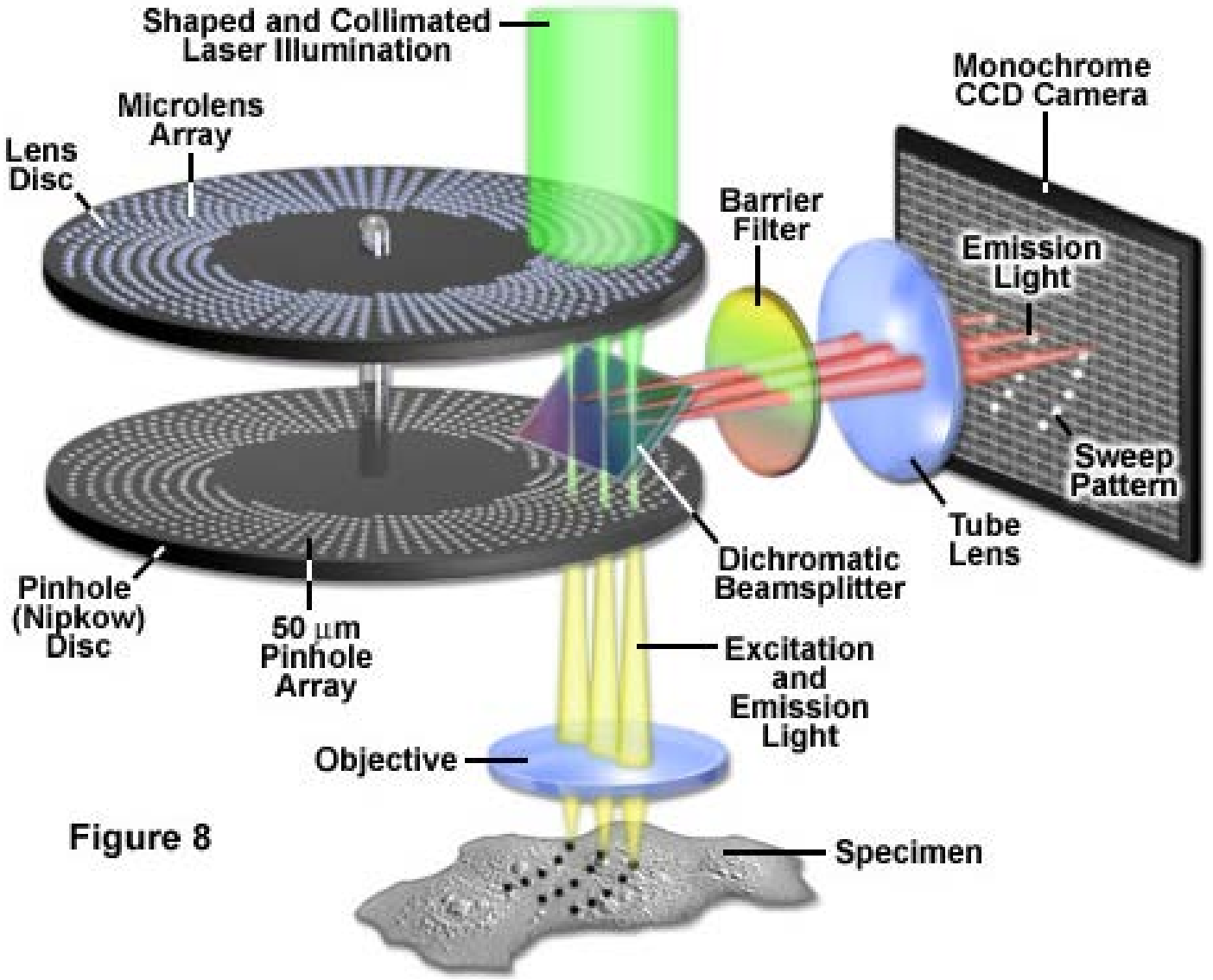
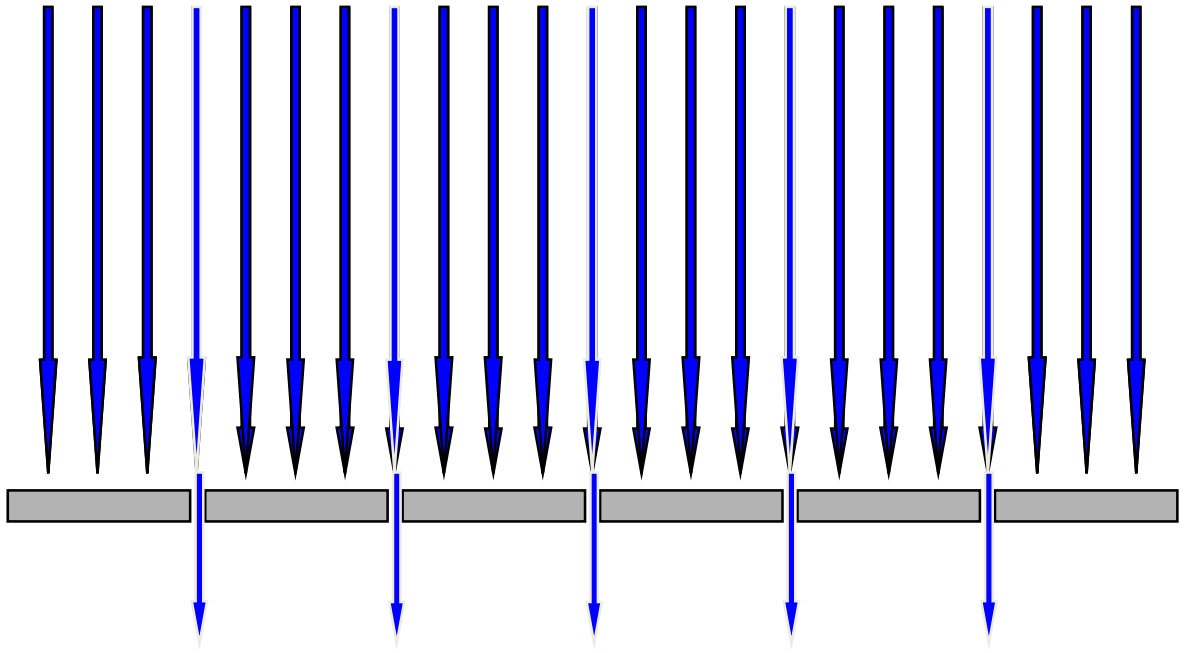


Figure 8

Increasing light throughput

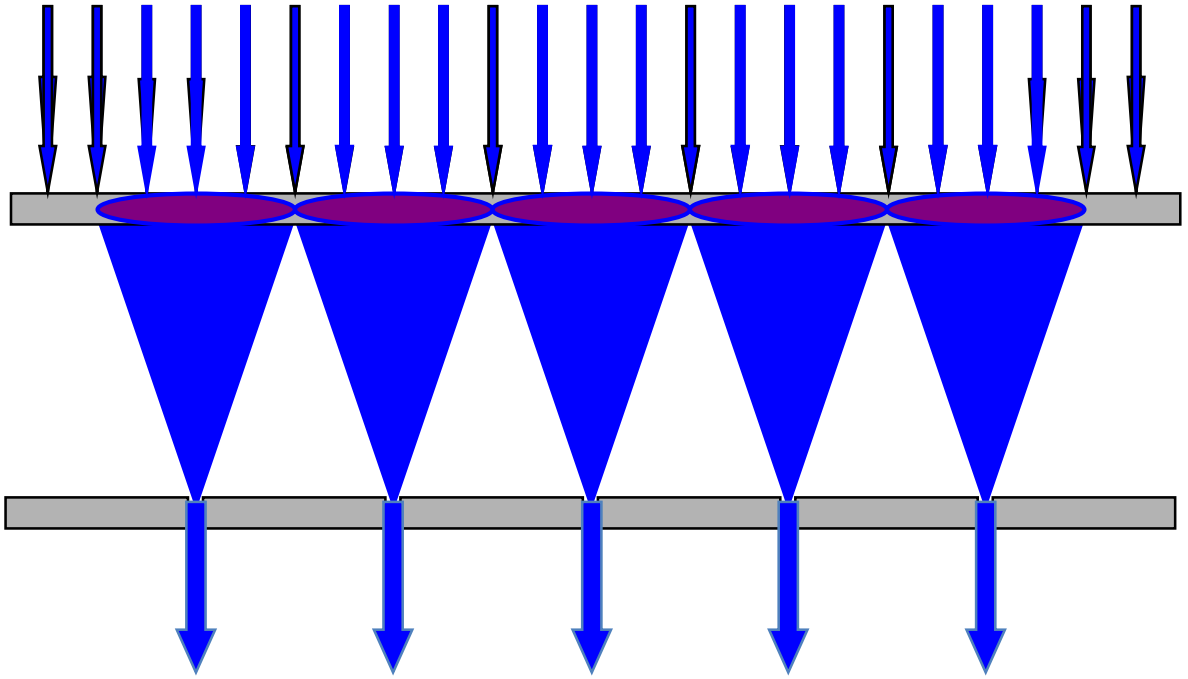


Nipkow disc with pinholes

1-2%

Increasing light throughput

Collector disc with microlenses



Nipkow disc with pinholes

70%

Spinning disk microscope

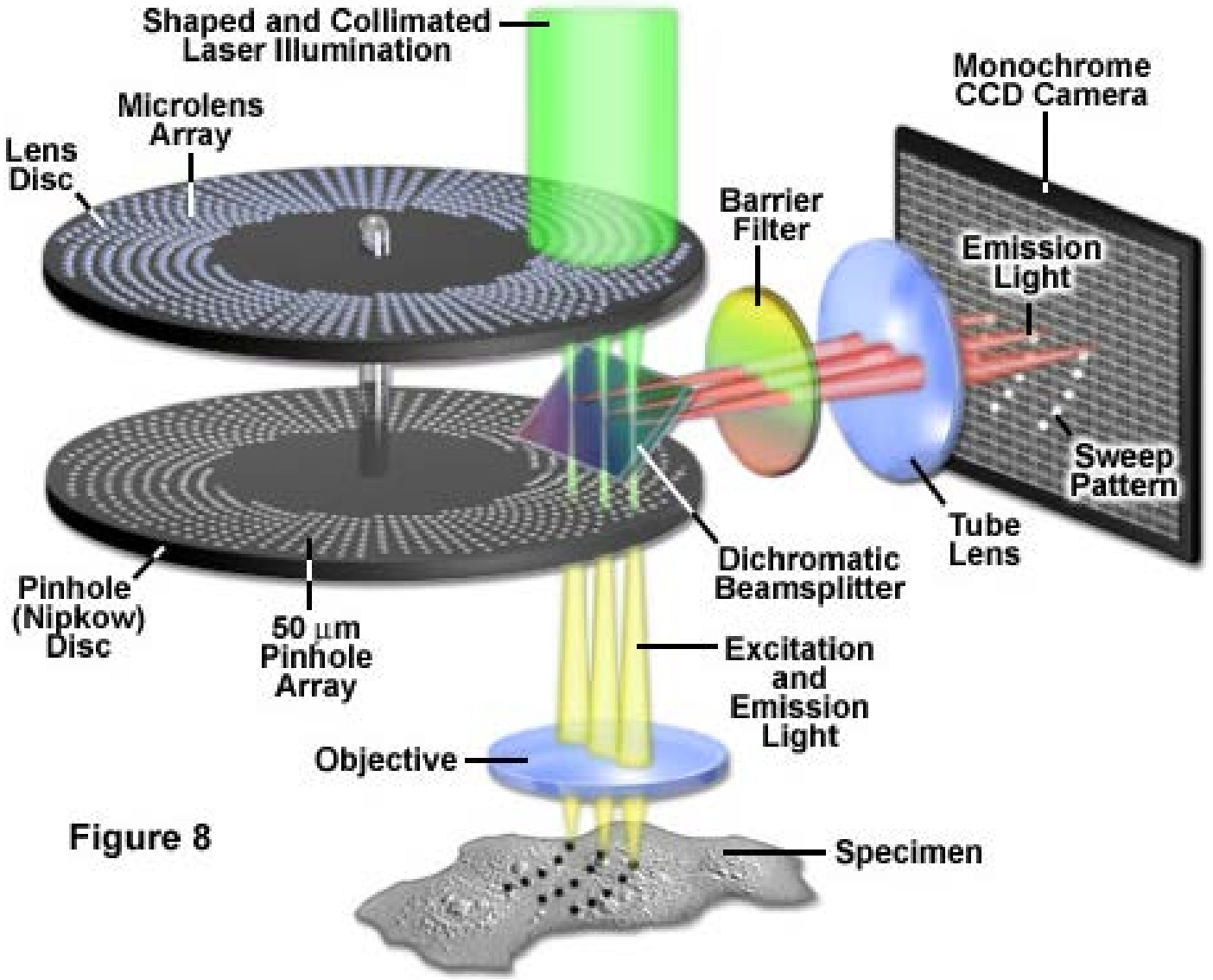
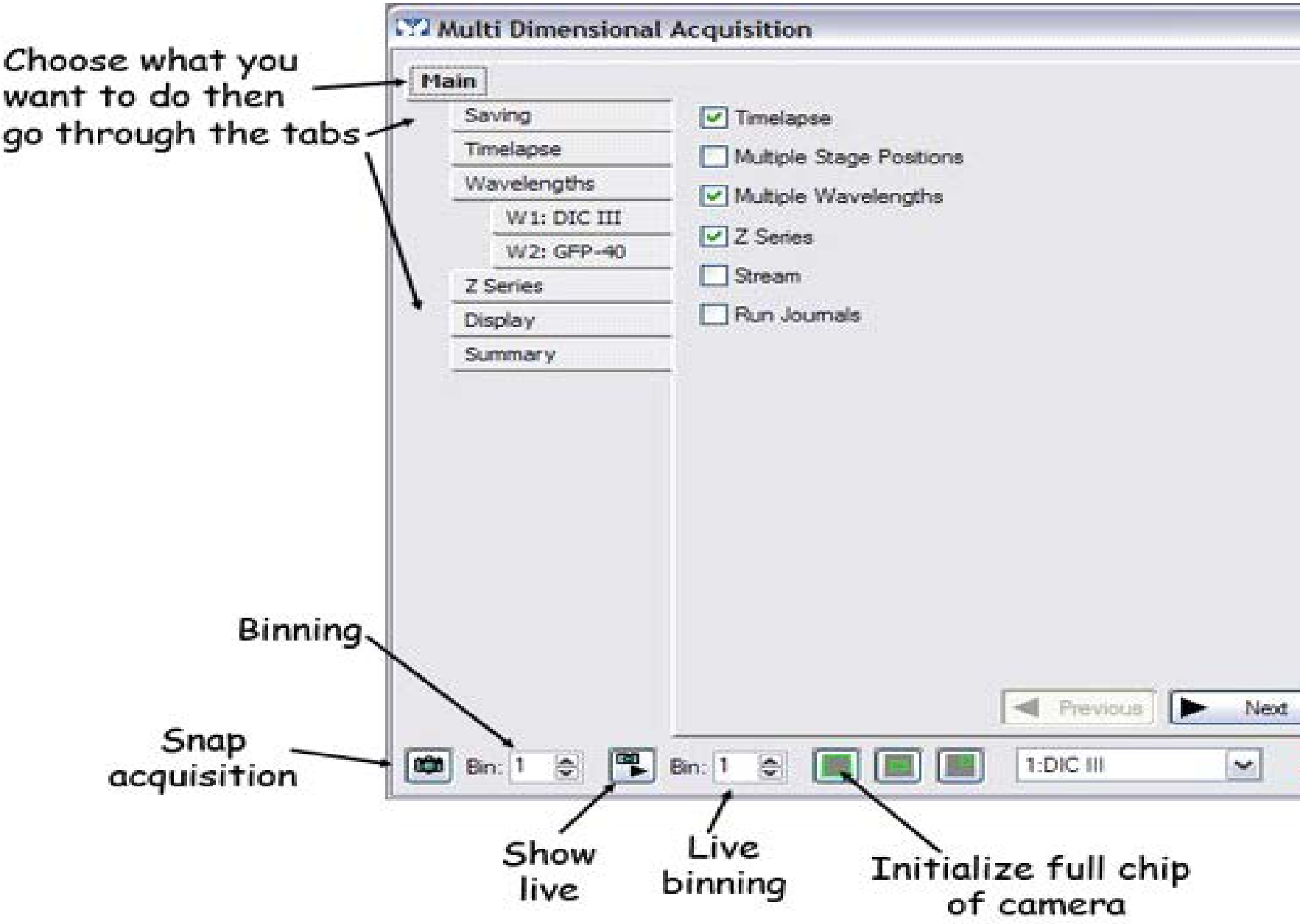


Figure 8

Overview

- Lasers: **405 nm** **488 nm** **561 nm** **633 nm**
- Camera: QE 90%, cooled -80, low read noise.
- Emission filters for various applications

Workflow


Choose what you want to do then go through the tabs
Multi Dimensional Acquisition
Main
Saving
Timelapse
Wavelengths
W1: DIC III
W2: GFP-40
Z Series
Display
Summary
Timelapse
Multiple Stage Positions
Multiple Wavelengths
Z Series
Stream
Run Journals
Previous
Next
Binning
Snap acquisition
Bin: 1
Bin: 1
1:DIC III
Show live
Live binning
Initialize full chip of camera

Overview

- Lasers: **405 nm** **488 nm** **561 nm** **633 nm**
- Camera: QE 90%, cooled -80, low read noise.
- Emission filters for various applications
- CLSM can be combined with spectral analysis and life time measurements.
- Design in SD-microscope is more rigid. e.g. fixed pinhole size.
- CO2 + temperature controller
- contact person: **Norbert de Ruijter** (Cell biology / WLMC / Radix building)
- When to use: fast processes and limited by photobleaching
- We do have the ability to perform FRAP, photoactivation and photoablation.

Fluorescence Recovery After (local) Photobleaching

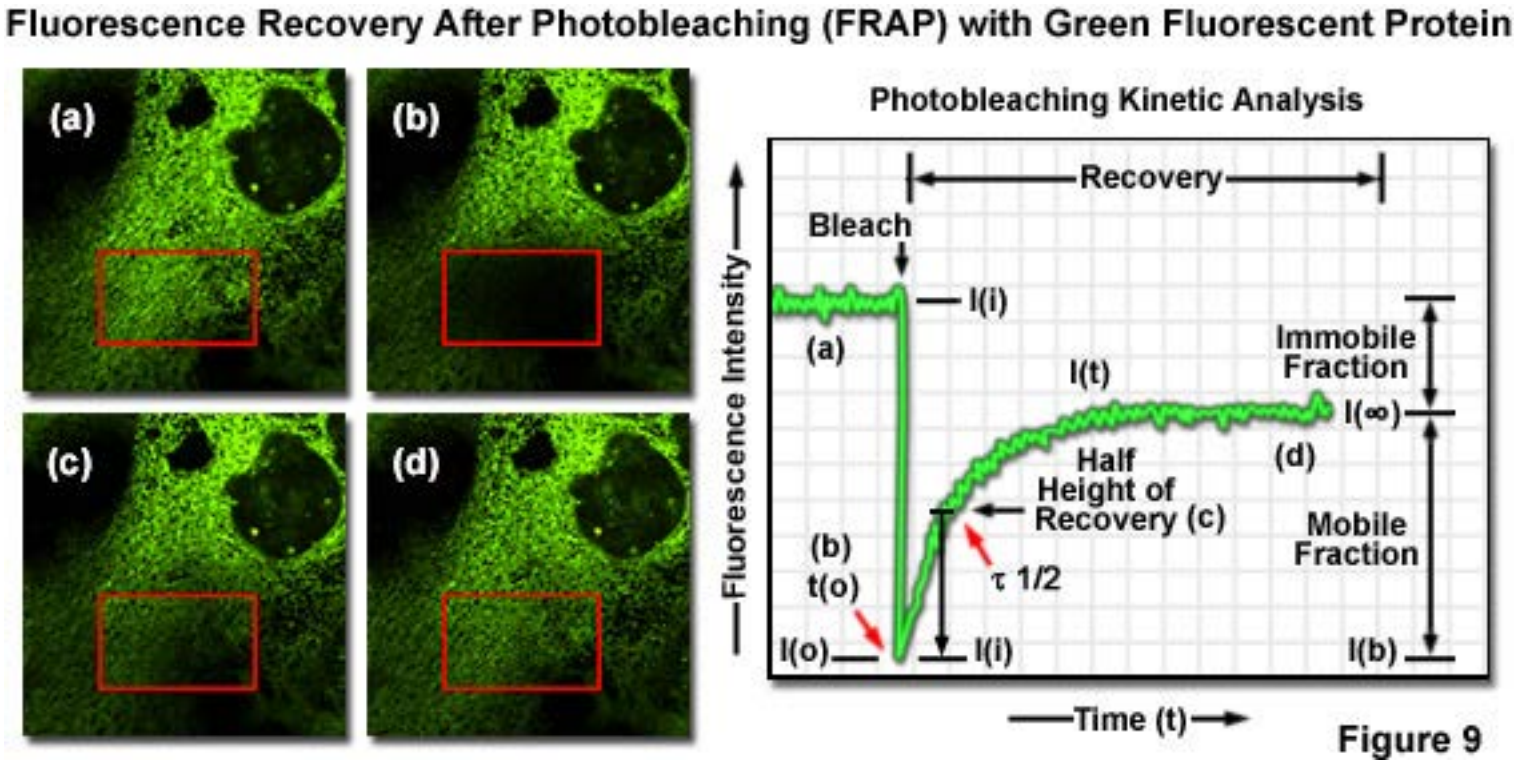
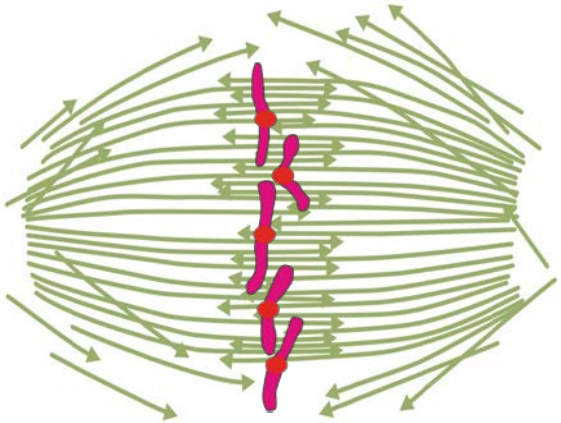
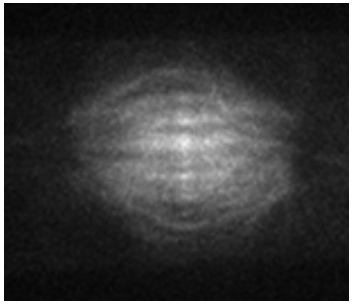


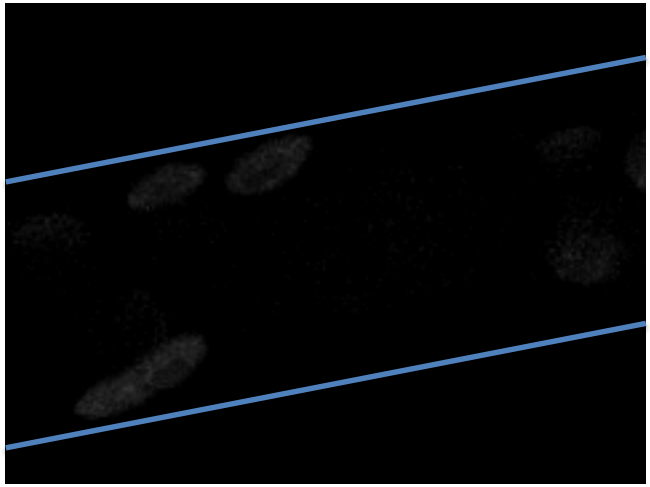
Figure 9

Photoactivation and photoconversion (405 nm laser)





mcherry-tubulin.



PA-cherry-tubulin.

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