The Confocal Laser Scanning Microscope

Laser
- Light source – projected into specimen
- Emission filter: adjustable via intensity control
- Motorized or piezooptics (piezo) for fine focus
- Raster scan formed by using beam tube current
- Beam diameter is decreased (by a maximum of 2x)
- Scanning mode: prevents laser interference, not suitable for
  precise focus
- Laser line can be chosen via selection device (AOI, MOTF)
  depending on fluorescent dye. Generally, the shorter
  the wavelength, the higher the resolution
- Resolution: (1) Protect specimen (reduce dye
  bleaching and photostability) by reduction of laser power.
  (2) Maximize fluorescence signal (higher S/N) by longer
  pixel dwell times or averaging

Scanning Mirrors
- Scanning unit (for wavelength selection) across
  specimen line by line
- Y- Mirror: defines frame rate (frames/second) and
  pixel time, i.e. time the specimen is illuminated
- Scan time: influences S/N of image, the longer the scan time,
  the more photons per pixel, the less noise in the picture;
  but bleaching of fluorochromes may increase
- Scan resolution: maximum resolution can be achieved if
  pixel size is set correctly (at least 4 x 4 pixels, preferably
  smaller = distance to nearest optical slice thickness)
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Z-Motor
- Focusing the specimen – acquisition of image stacks
  or z sections
- z-Axis: definition distance between two optical slices (step size)
  = 0.5 x optical slice thickness
- Optimum z step size: 2 x 0.5 x 0.5 = 0.25 pixels
- Maximum frame rate = 512 x 512 pixels
- y/z position: factory adjusted for all beam path configura-
  tions; can be modified manually (= “Maintain-Profile”)

Confoocal Pinhole
- Depth discrimination – confocal aperture to prevent
  detection of out-of-focus light (optical sectioning)
- Diameter: determines thickness of optical slice; optimum
  design: 1 Airy unit = best trade-off between depth
  discrimination capability and efficiency
- Scanning (factory adjusted) for all beam path configura-
  tions; can be modified manually (= “Maintain-Profile”)

Beam Splitter
- Fluorescence beam path – deflned by combination of
  main (HFT) and secondary (NFT) dichroic mirrors and
  emission filters
- BP: bandpass, LP: longpass, HFT: separates excitation and
  emission light
- NFT: effects spectral division of (different) fluorescence
  emissions (e.g. NFT 545: reflects light of λ > 545 nm and
  transmits light of λ < 545 nm)
- JE (LP): determines bandwidth of fluorescence
  emission for the respective channel (e.g. LP 505: λ > 505 nm – detection)

Objective Lens
- Optical image formation – determines properties of
  image quality such as resolution (y, z) and
- Numerical Aperture (N.A): determines the cap of light
  (jointly with wavelength), and substantially influences
  the optical image quality
- Depth discrimination: by combination of (different) fluores-
  cent emissions and emission filters
- Objective lens: 1 Airy unit = best trade-off between depth
  discrimination capability and efficiency
- Best confocal multi-fluoroscope images (VIS, UV)
- Use water immersion objectives with apochromatic correction (C-Apochromat)

Microscopy from Carl Zeiss

View specimen in VIS mode
Focus the specimen in epifluorescence mode using the binocular
and center the field of view; change to appropriate objective magnification
(consider use of correct immersion medium)

Load an LSM configuration
A click on “Load” opens the configuration control
and selects a predefined (by choosing the objective)
from list (Single Track).

Scan an image
Click on “Find” button (right row in window “Scan Control”)
See operating manual for scanning a stack of slices, time series etc.