Введение в методы микроскопии в биологии. Оптическая микроскопия

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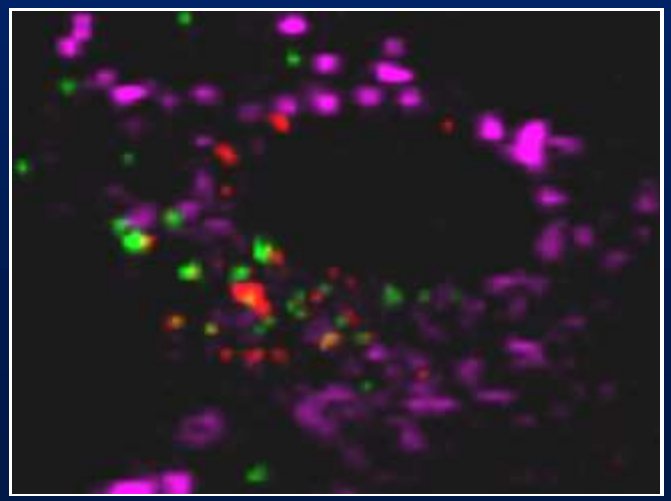
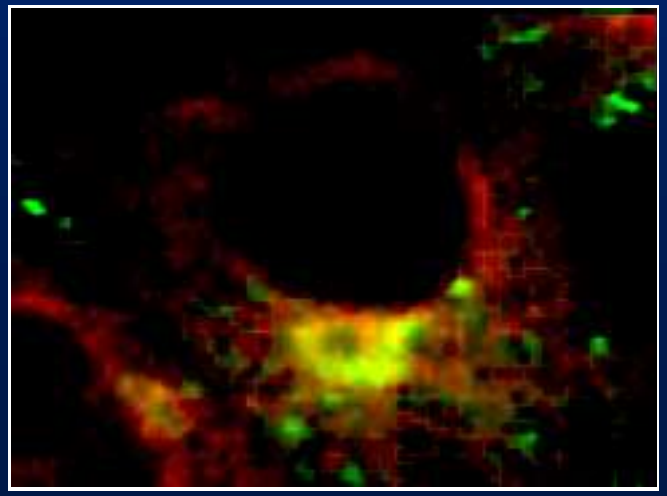
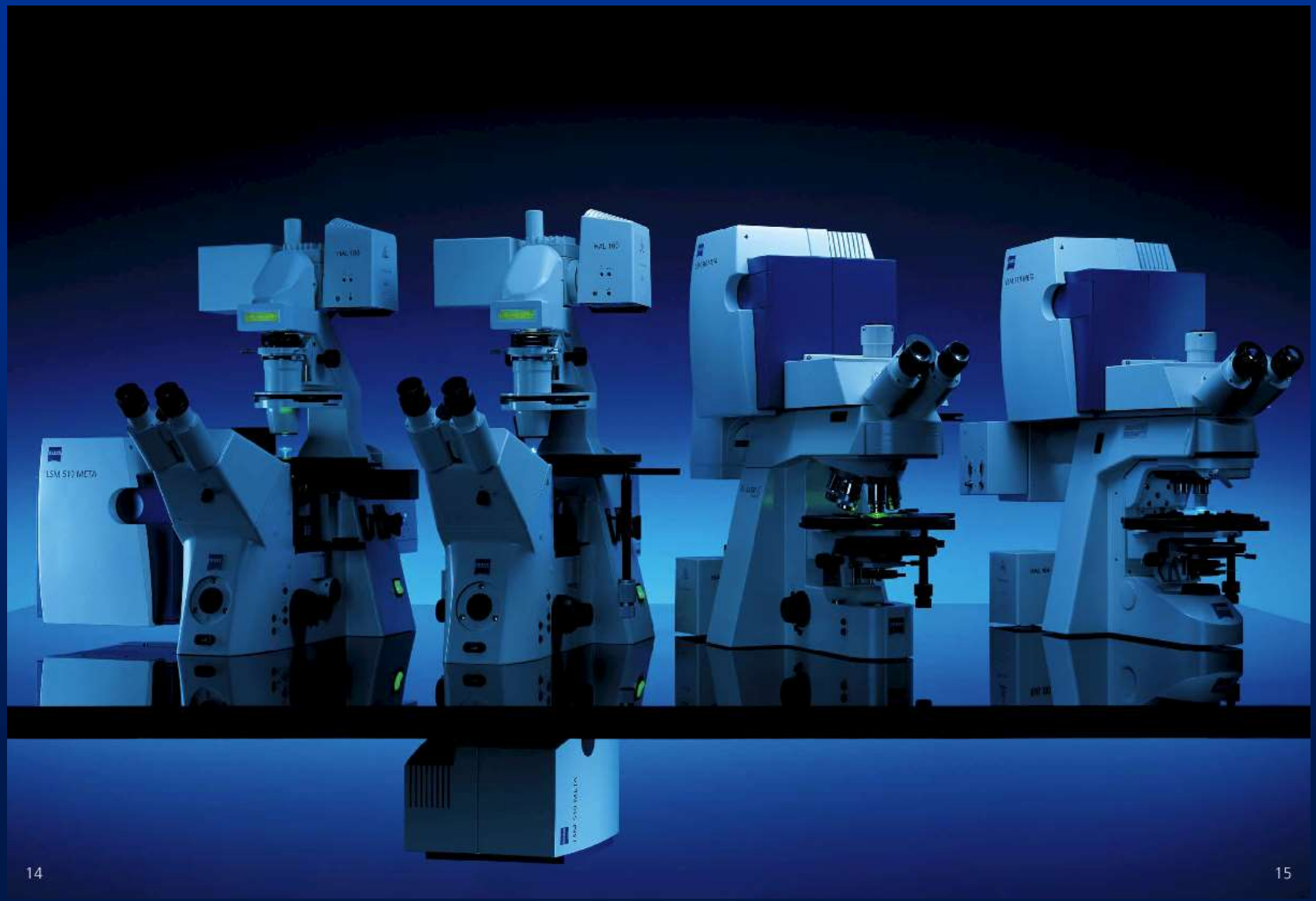
Лаборатория оптической микроскопии и спектроскопии биомолекул
ИБХ РАН

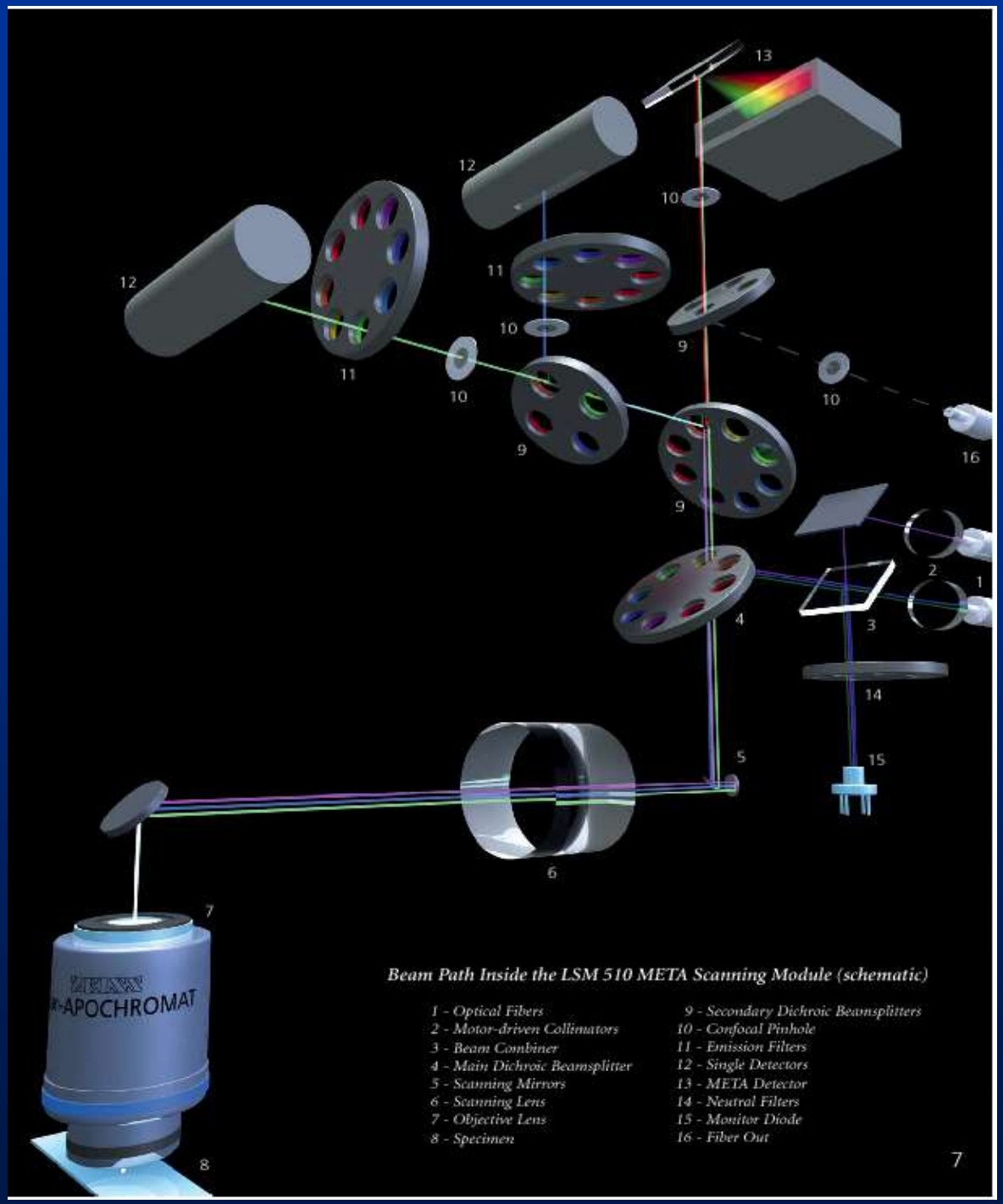




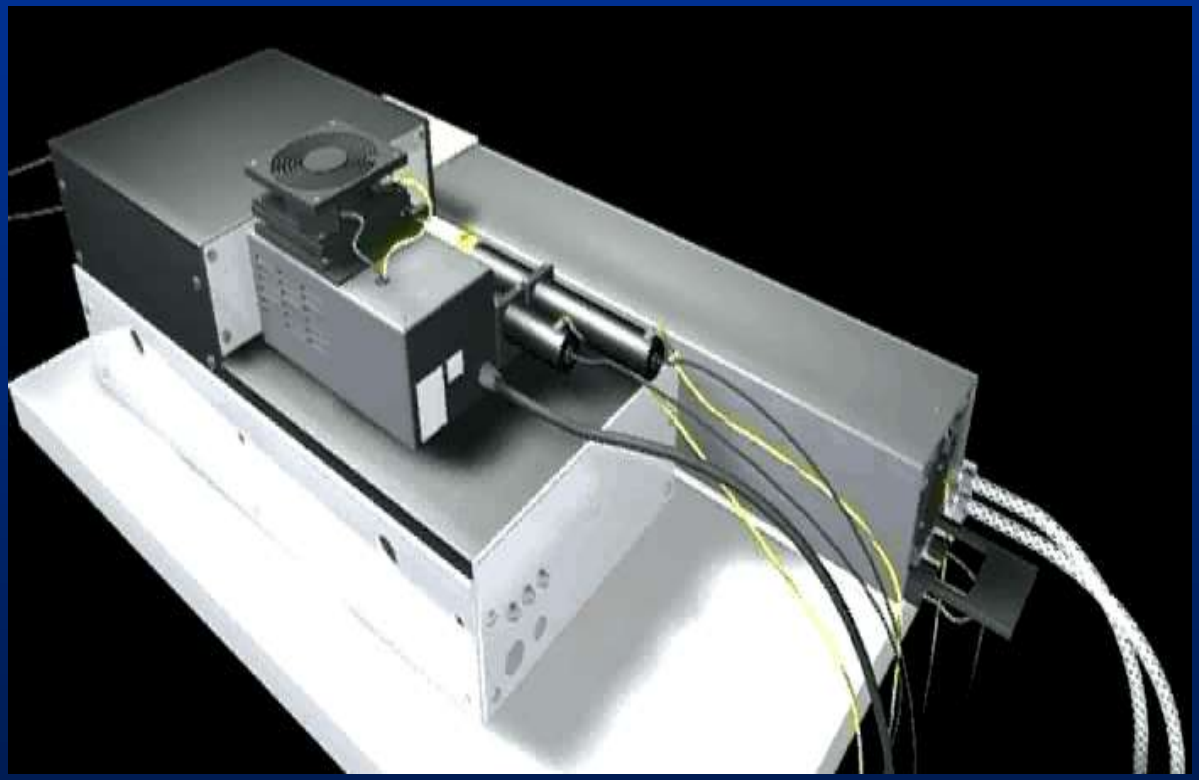
Внешний вид конфокальных микроскопов фирмы ZEISS



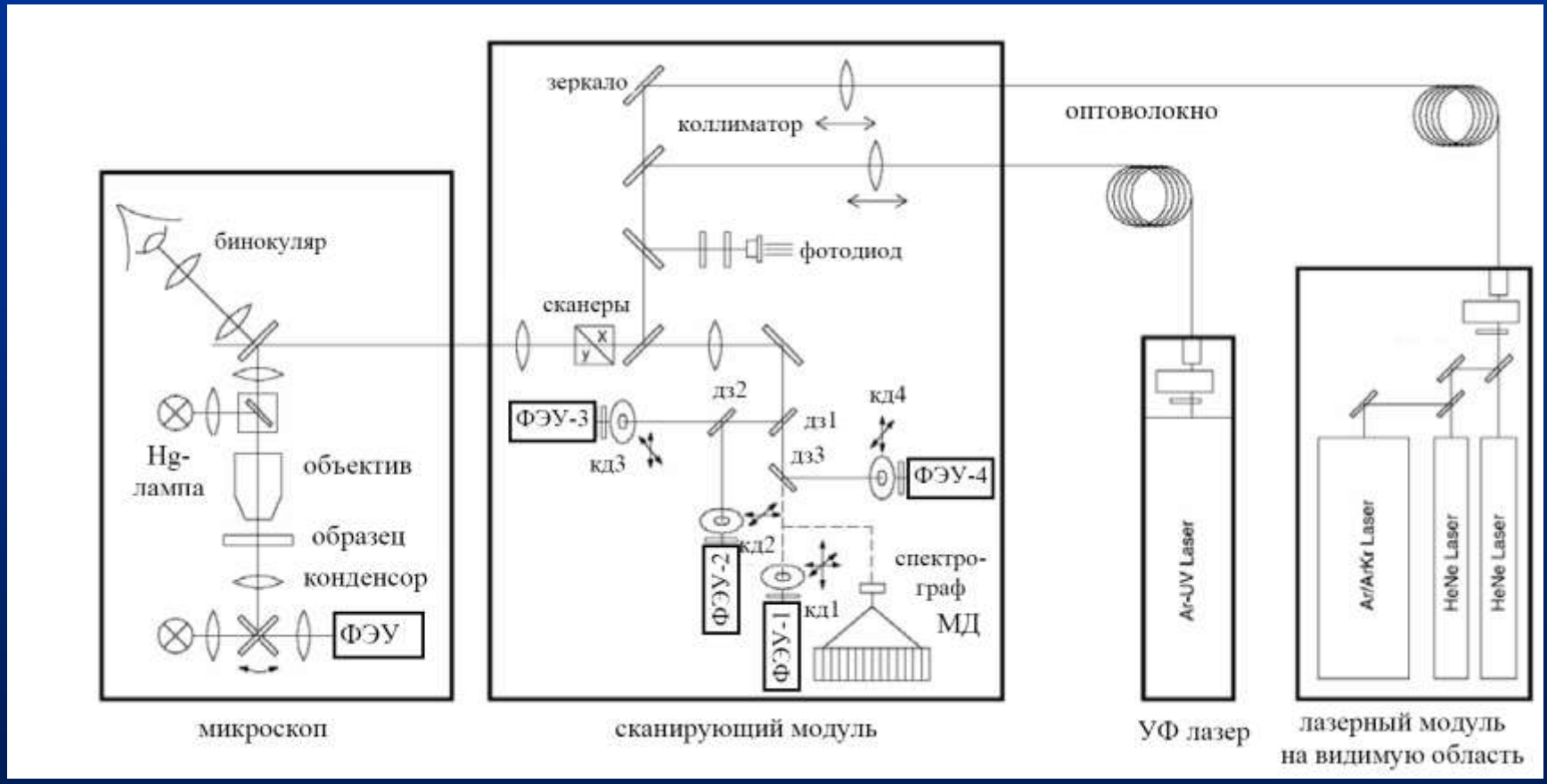
Принципиальная оптическая схема конфокального микроскопа



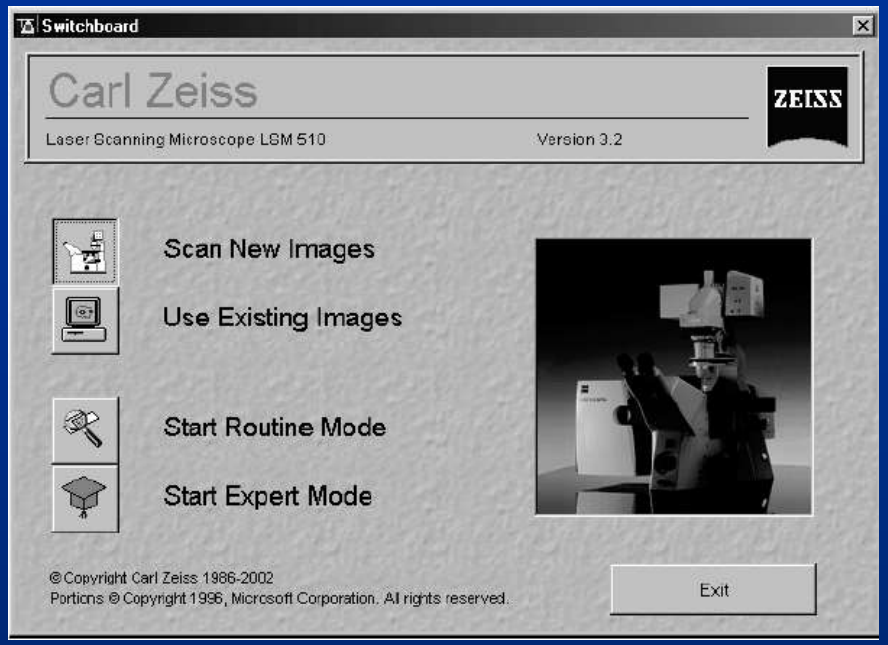
Принципиальная оптическая схема конфокального микроскопа фирмы **ZEISS LSM510-Meta**

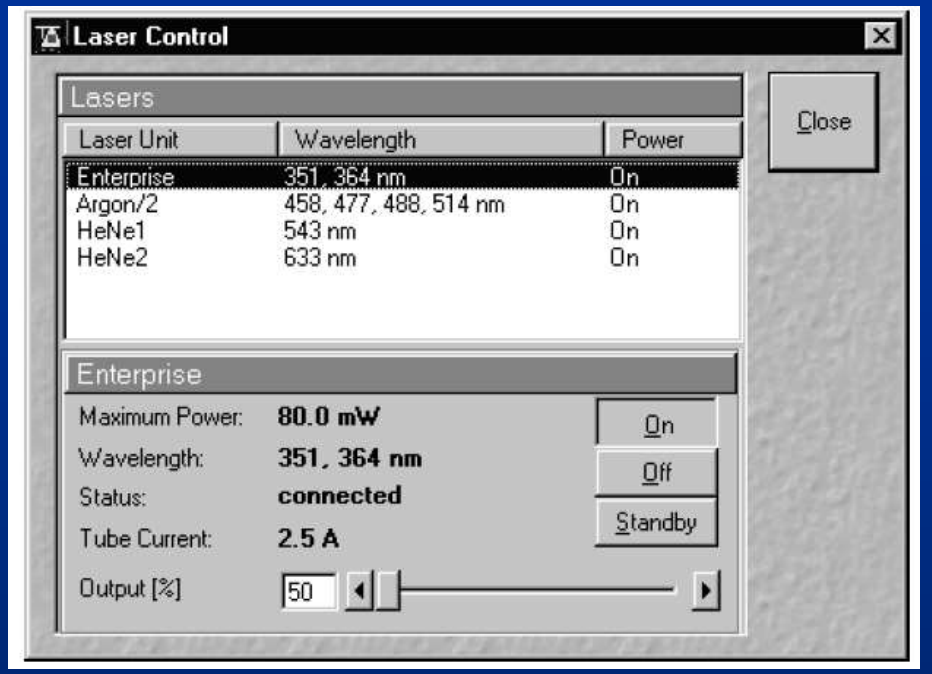


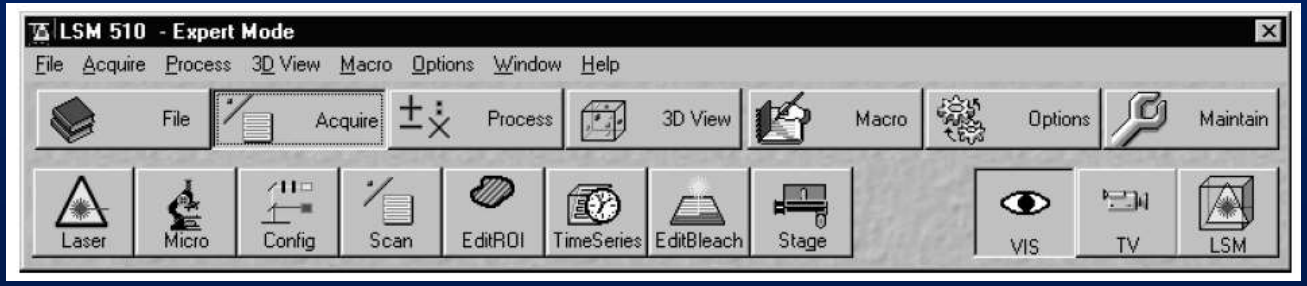
Блок-схема микроскопа «LSM510-META» фирмы Zeiss со спектральным модулем.

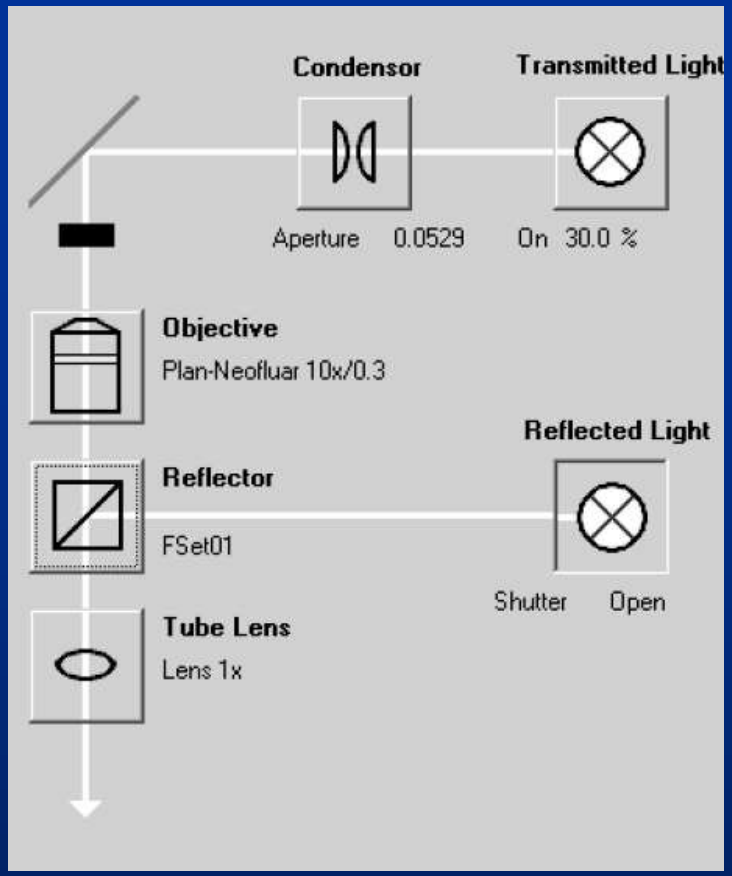


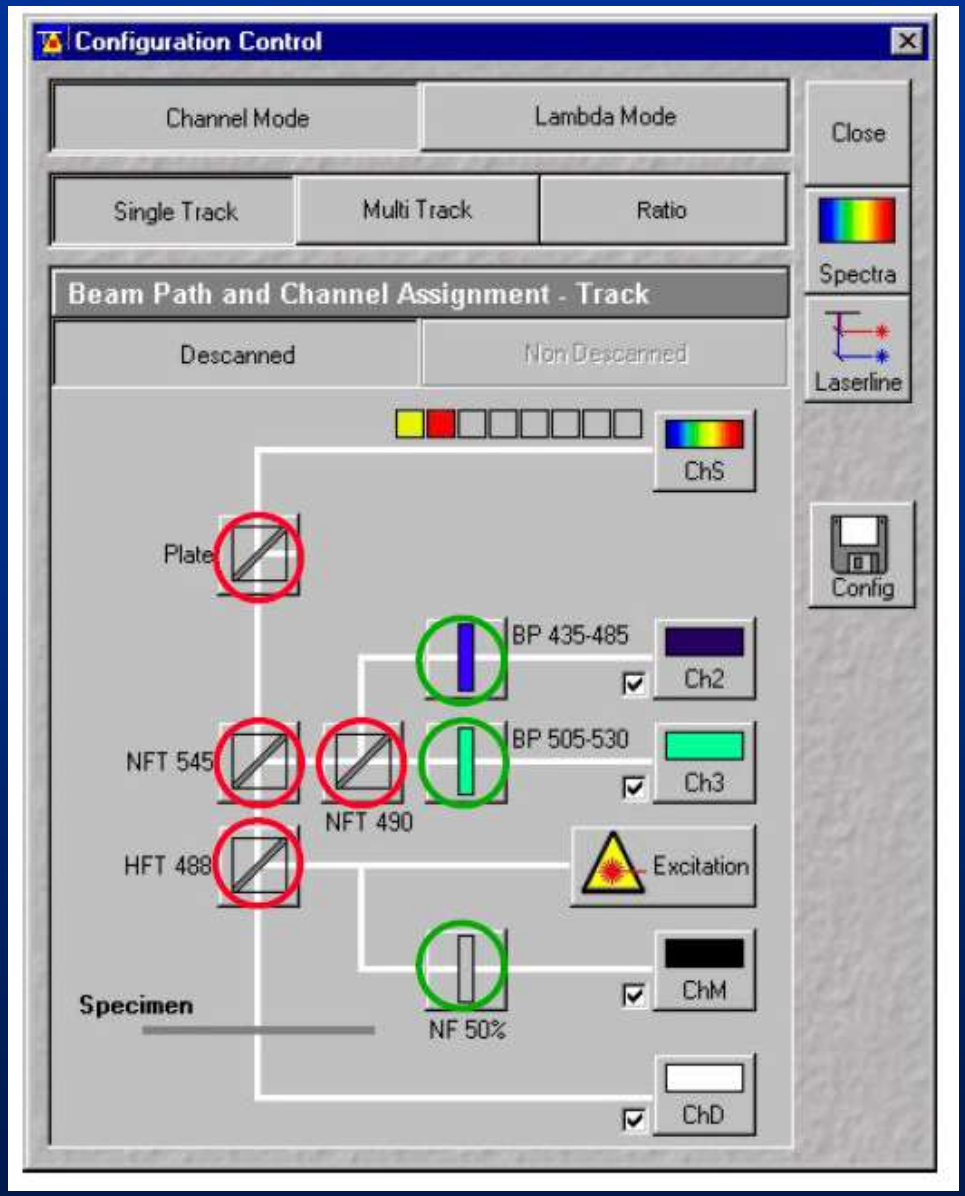
кд- конфокальная диафрагма; дз – дихроичное зеркало; ФЭУ- фотоэлектронный умножитель,
МД – многоканальный детектор

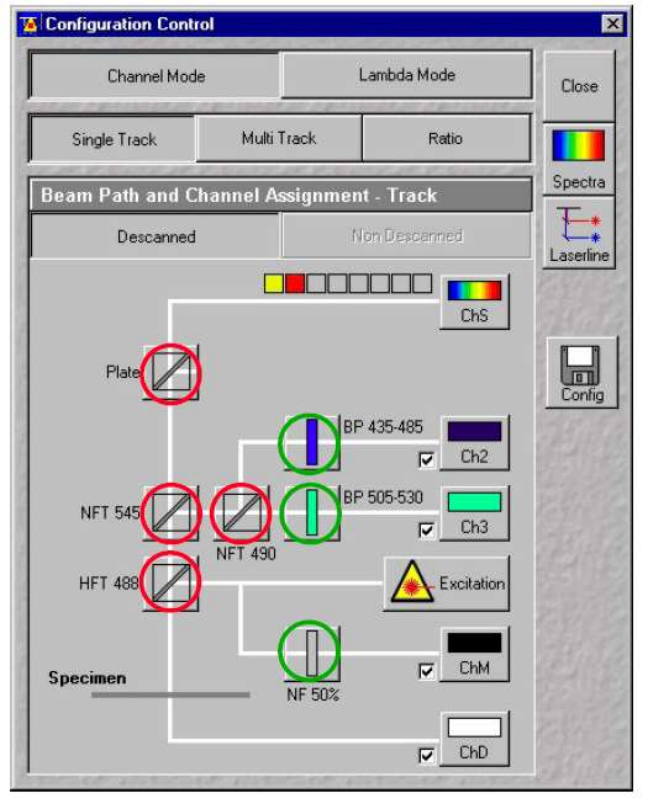

Switchboard
Carl Zeiss
Laser Scanning Microscope LSM 510
Version 3.2
ZEISS
Scan New Images
Use Existing Images
Start Routine Mode
Start Expert Mode
© Copyright Carl Zeiss 1986-2002
Portions © Copyright 1996, Microsoft Corporation. All rights reserved.
Exit

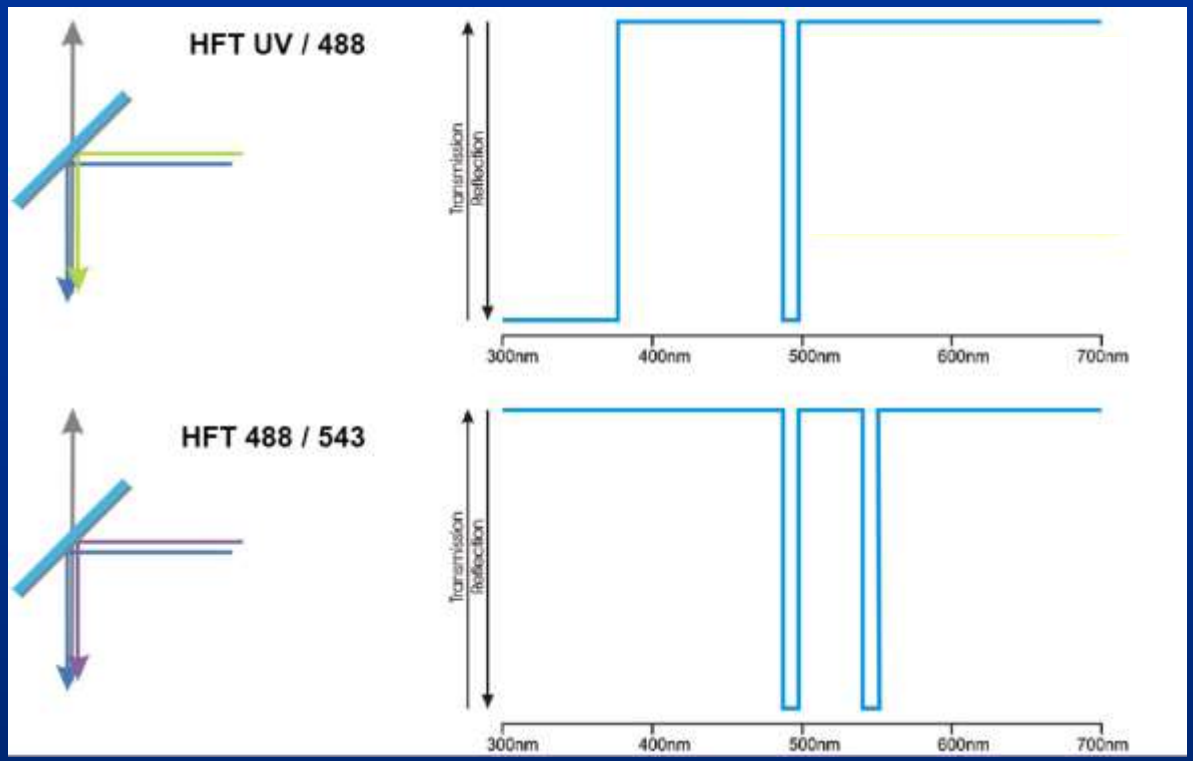

Laser Control
Lasers
Laser Unit
Wavelength
Power
Enterprise 351, 364 nm On
Argon/2 458, 477, 488, 514 nm On
HeNe1 543 nm On
HeNe2 633 nm On
Close
Enterprise
Maximum Power: 80.0 mW
Wavelength: 351, 364 nm
Status: connected
Tube Current: 2.5 A
Output [%] 50
On
Off
Standby

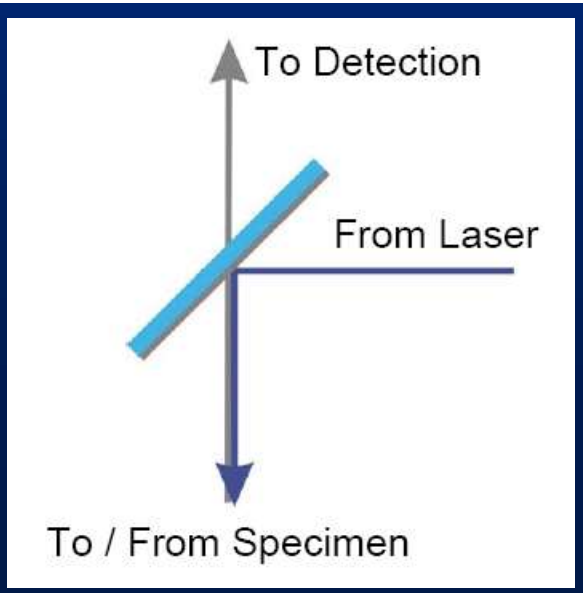

LSM 510 - Expert Mode
File Acquire Process 3D View Macro Options Window Help
File
Acquire
Process
3D View
Macro
Options
Maintain
Laser
Micro
Config
Scan
EditROI
TimeSeries
EditBleach
Stage
VIS
TV
LSM

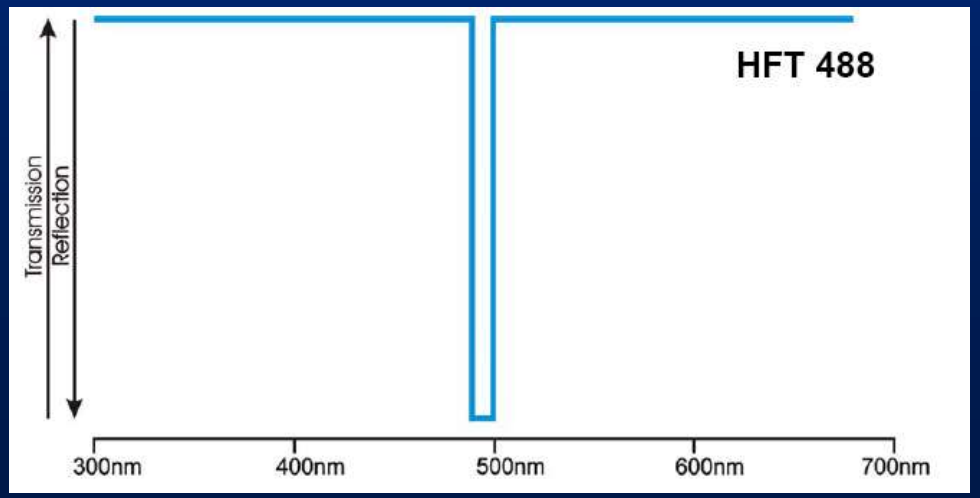

Condensor
Transmitted Light
Aperture 0.0529
On 30.0 %
Objective
Plan-Neofluar 10x/0.3
Reflected Light
Reflector
FSet01
Shutter Open
Tube Lens
Lens 1x

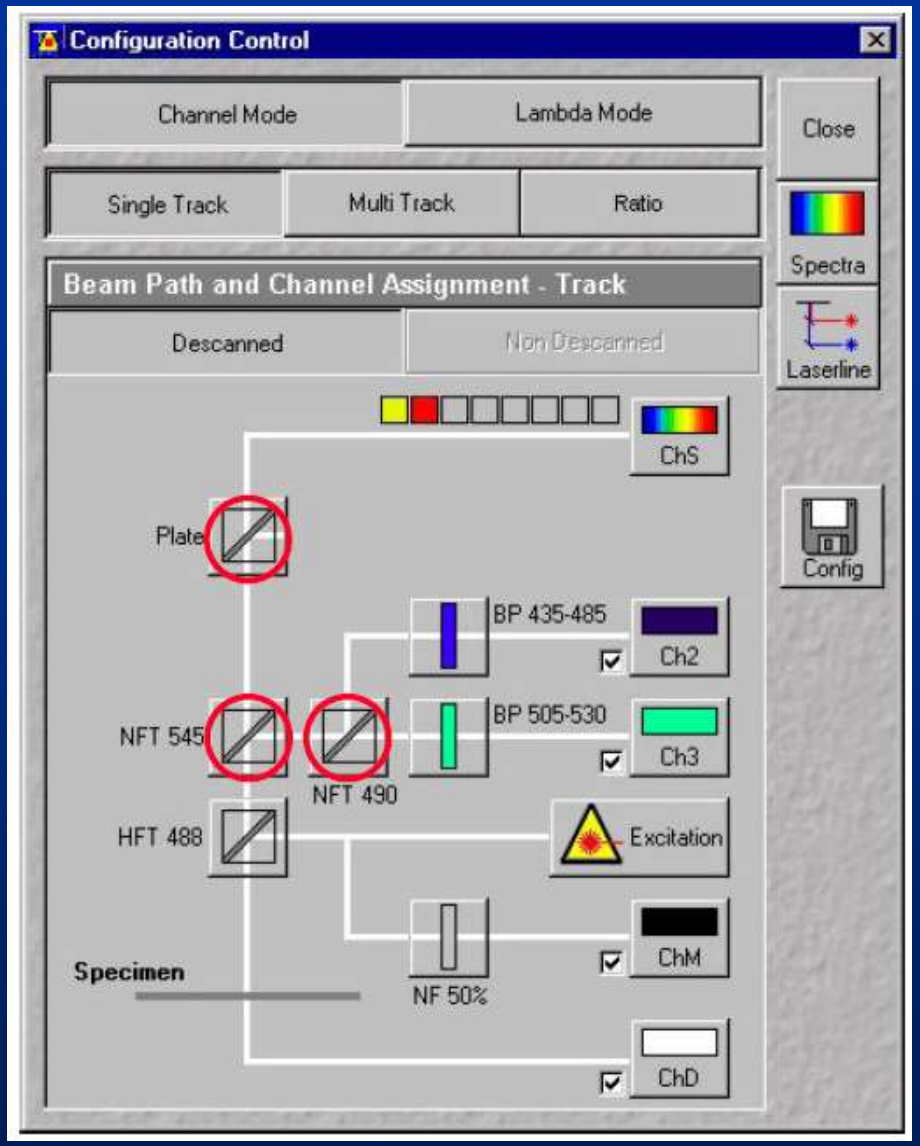

Configuration Control
Channel Mode
Lambda Mode
Close
Single Track
Multi Track
Ratio
Spectra
Beam Path and Channel Assignment - Track
Descanned
Non Descanned
Laserline
ChS
Plate
Config
BP 435-485
Ch2
NFT 545
NFT 490
BP 505-530
Ch3
HFT 488
Excitation
ChM
Specimen
NF 50%
ChD

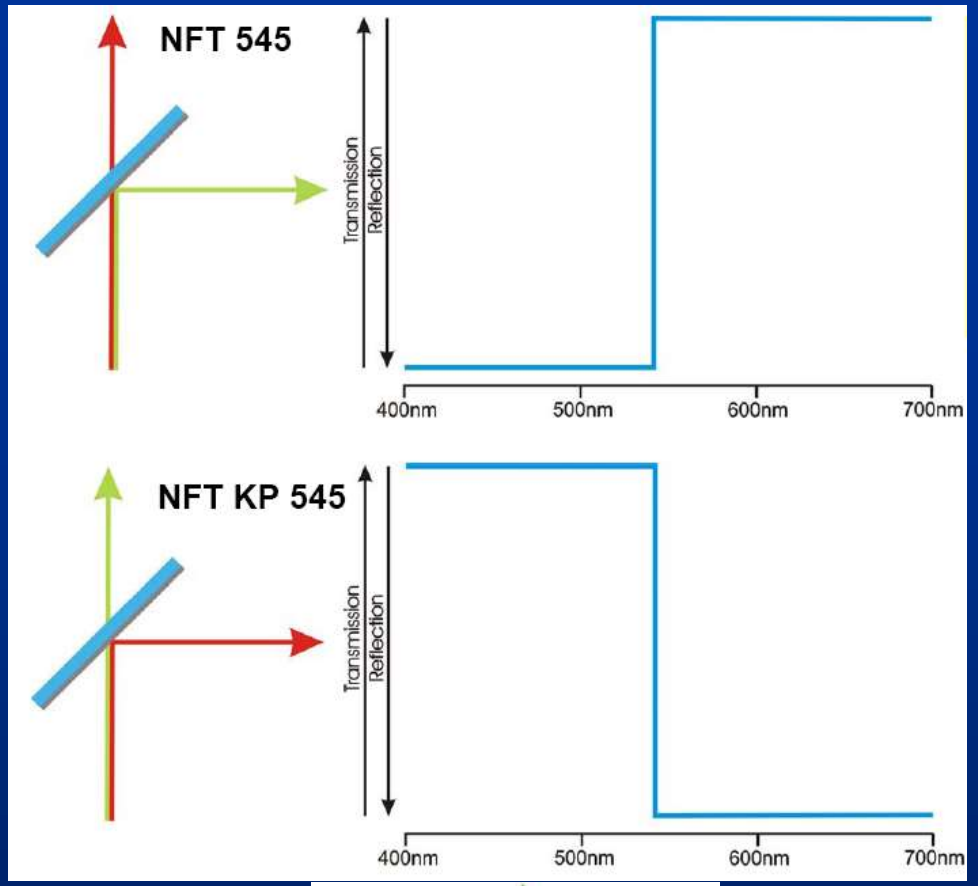

Configuration Control
Channel Mode
Lambda Mode
Close
Single Track
Multi Track
Ratio
Spectra
Beam Path and Channel Assignment - Track
Descanned
Non Descanned
Laserline
ChS
Plate
Config
BP 435-485
Ch2
NFT 545
BP 505-530
Ch3
NFT 490
HFT 488
Excitation
ChM
Specimen
NF 50%
ChD

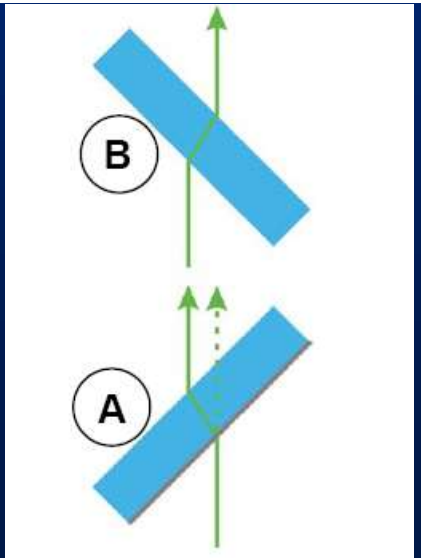

HFT UV / 488
Transmission
Reflection
300nm
400nm
500nm
600nm
700nm
HFT 488 / 543
Transmission
Reflection
300nm
400nm
500nm
600nm
700nm

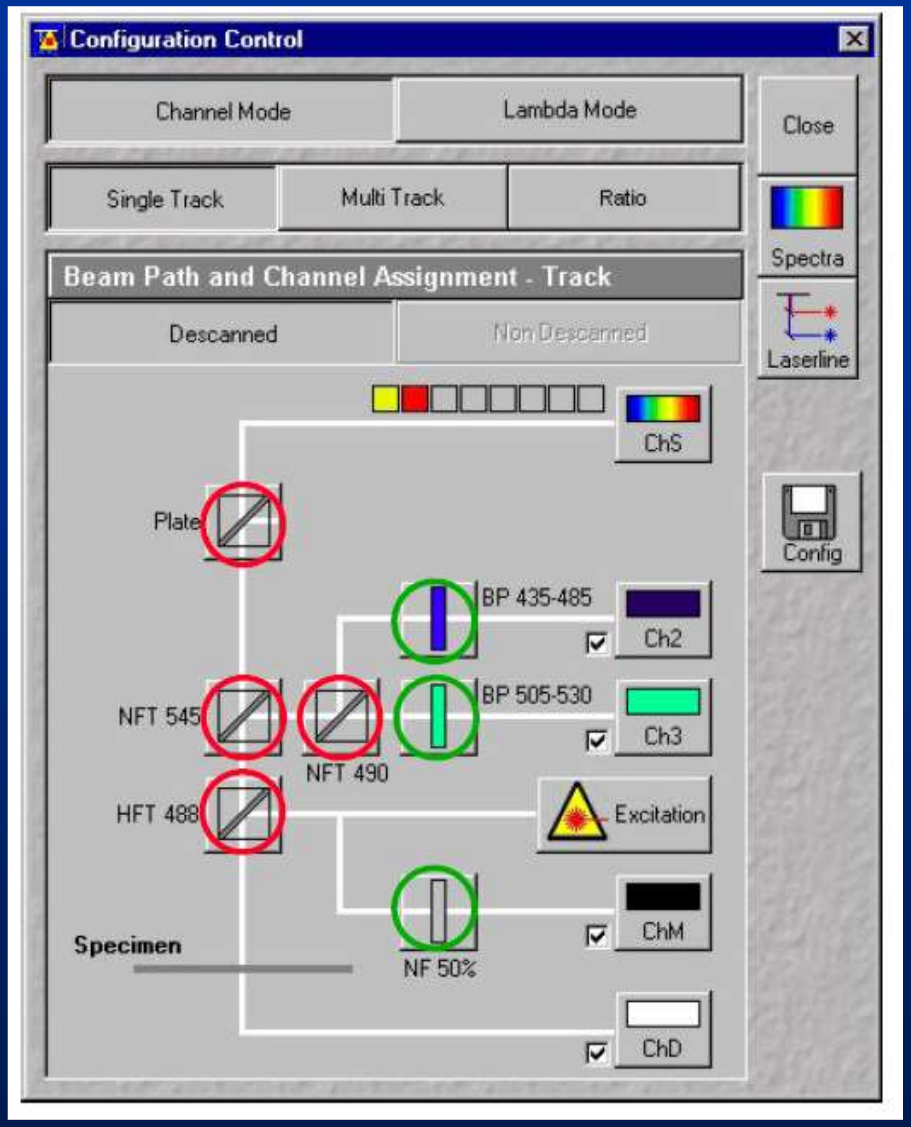

To Detection
From Laser
To / From Specimen

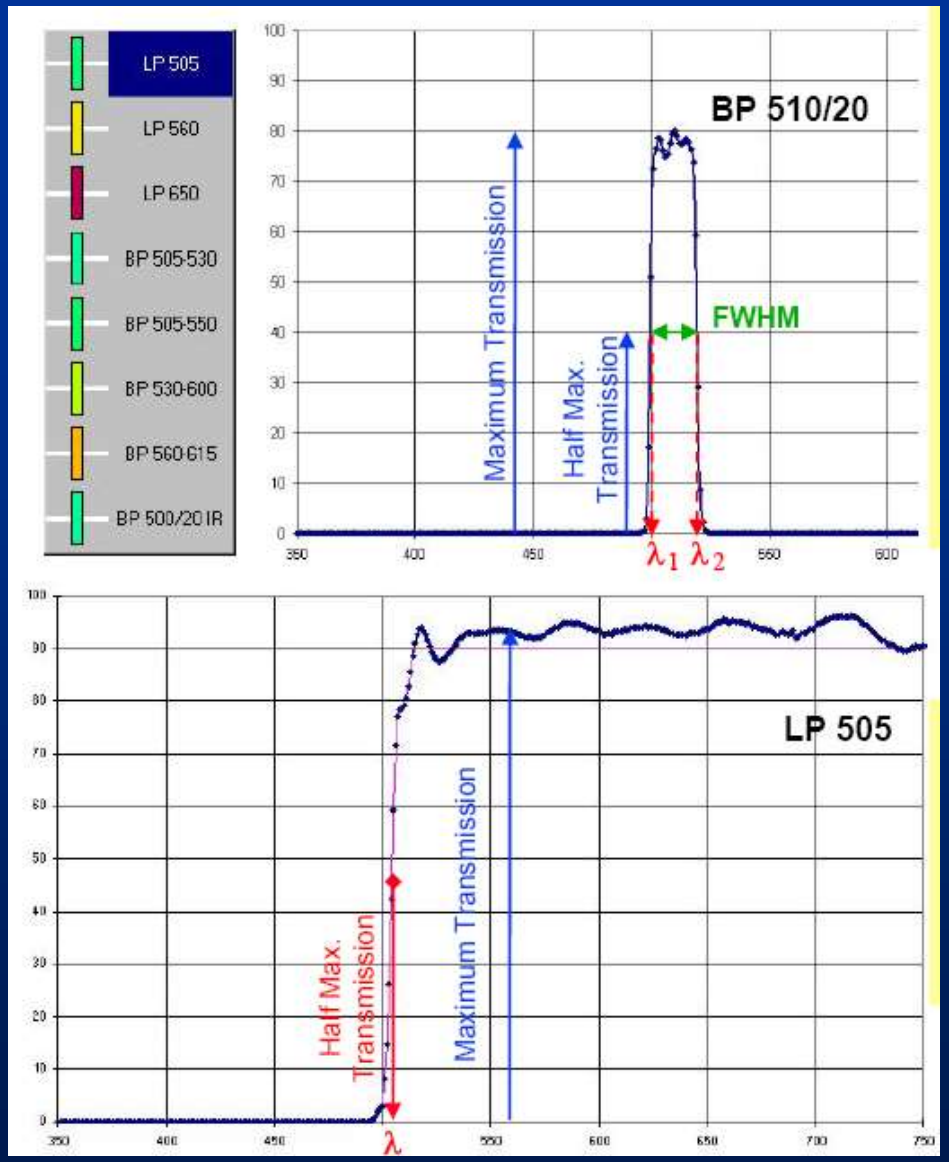

HFT 488
Transmission
Reflection
300nm
400nm
500nm
600nm
700nm

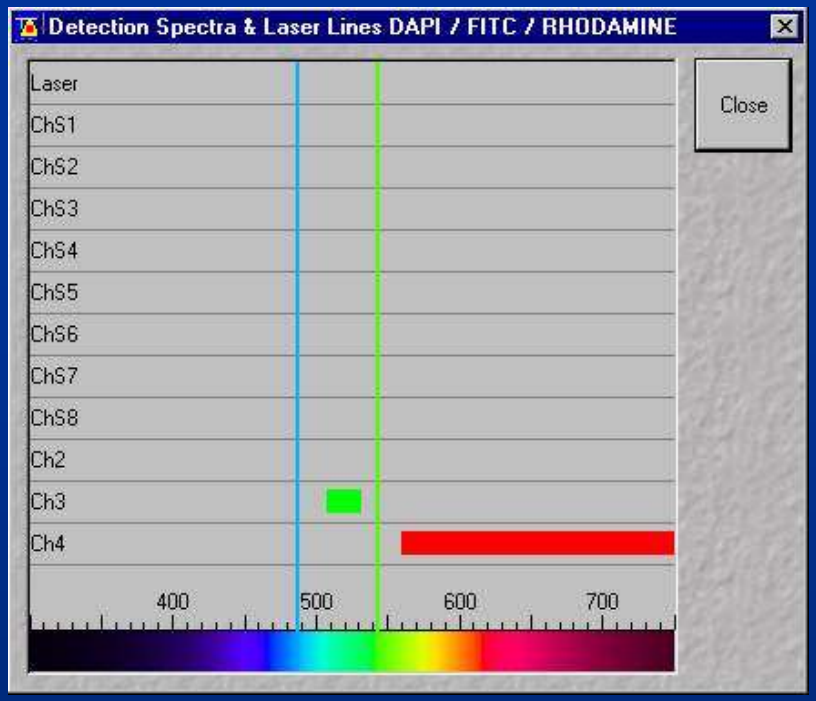

Configuration Control
Channel Mode
Lambda Mode
Close
Single Track
Multi Track
Ratio
Spectra
Beam Path and Channel Assignment - Track
Descanned
Non Descanned
Laserline
ChS
Plate
Config
BP 435-485
Ch2
NFT 545
NFT 490
BP 505-530
Ch3
HFT 488
Excitation
ChM
Specimen
NF 50%
ChD


NFT 545
Transmission
Reflection
400nm
500nm
600nm
700nm
NFT KP 545
Transmission
Reflection
400nm
500nm
600nm
700nm


B
A

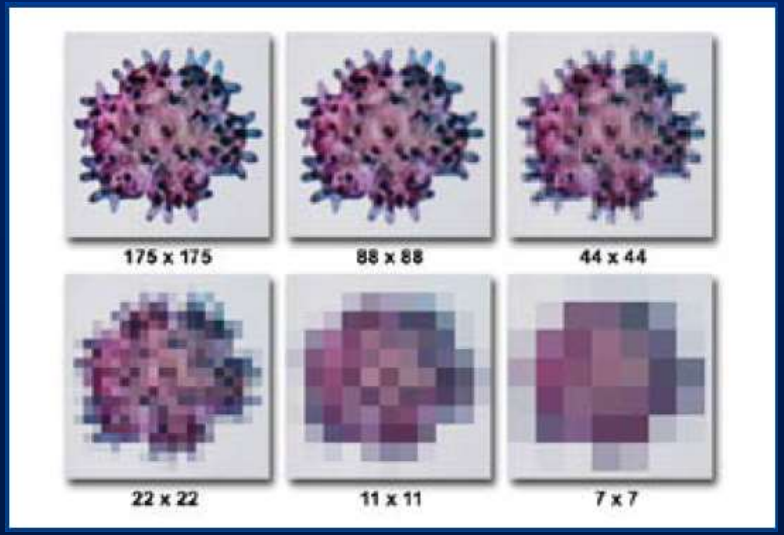

Configuration Control
Channel Mode
Lambda Mode
Close
Single Track
Multi Track
Ratio
Spectra
Beam Path and Channel Assignment - Track
Descanned
Non Descanned
Laserline
ChS
Plate
Config
BP 435-485
Ch2
NFT 545
NFT 490
BP 505-530
Ch3
HFT 488
Excitation
ChM
Specimen
NF 50%
ChD

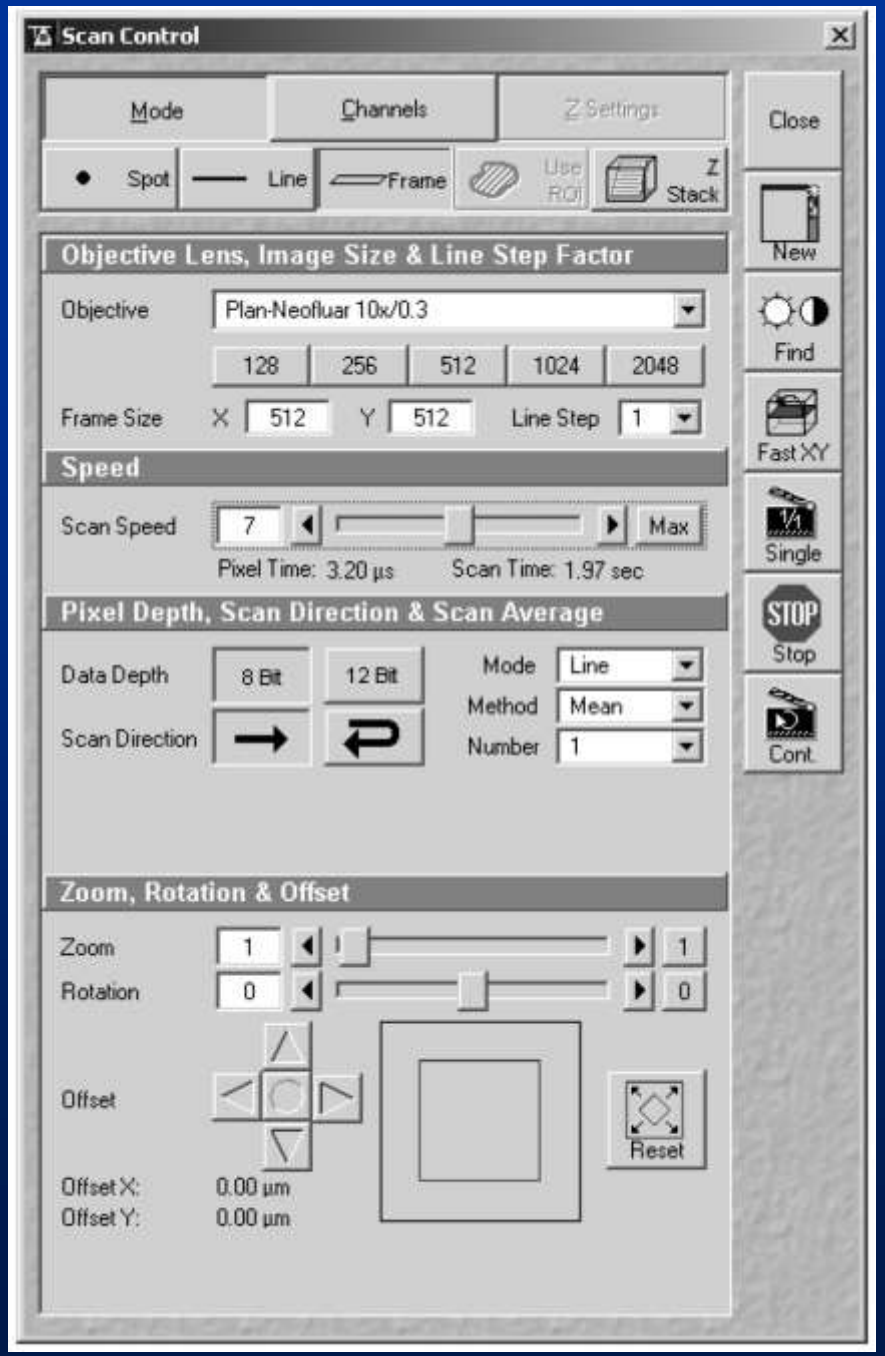

LP 505
LP 560
LP 650
BP 505-530
BP 505-550
BP 530-600
BP 560-615
BP 500/20 IR
BP 510/20
Maximum Transmission
Half Max. Transmission
FWHM
λ1 λ2
LP 505
Half Max. Transmission
Maximum Transmission
λ



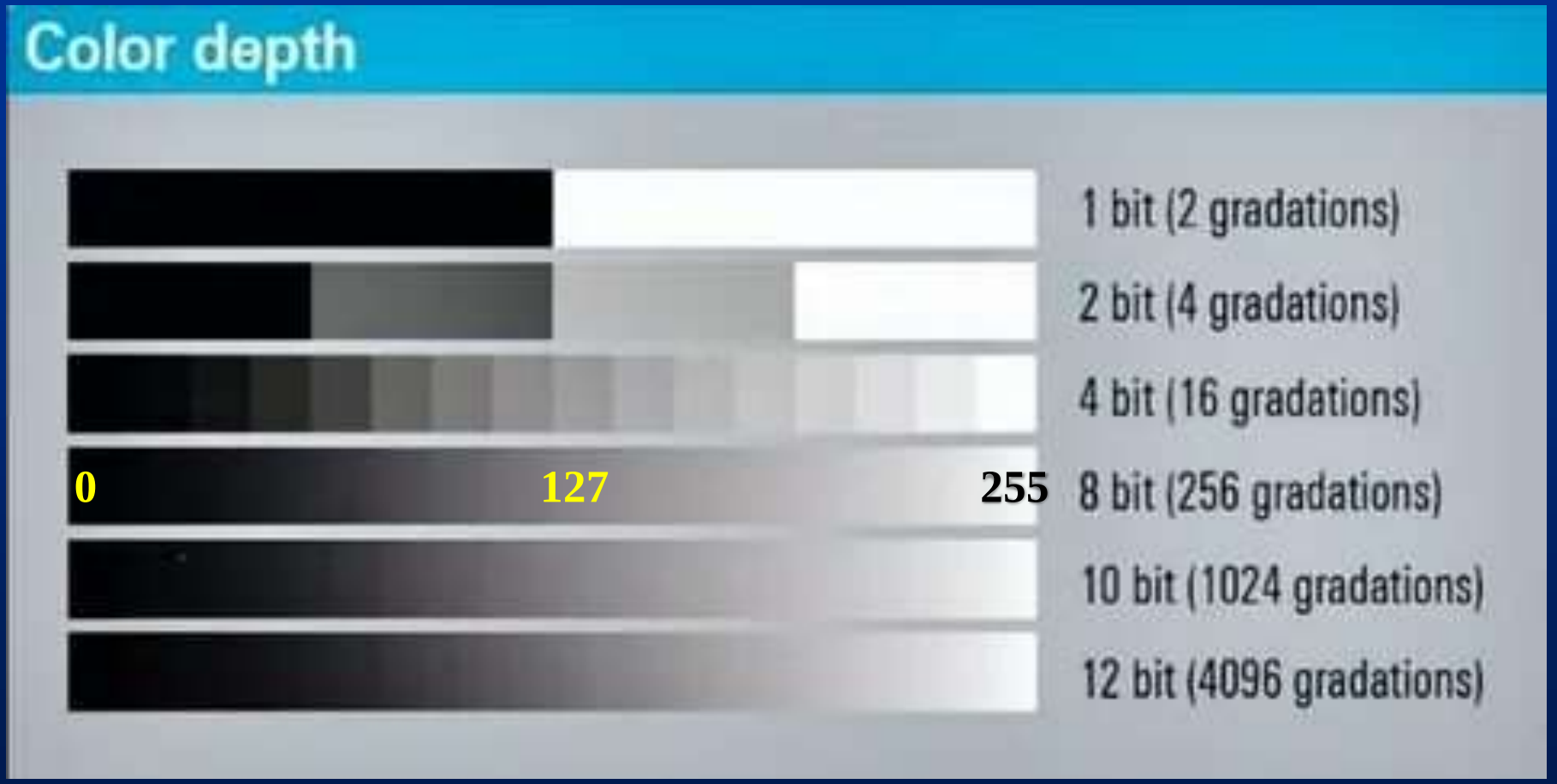
$$N_x = 2L_x/\Delta X,\ N_y = 2L_y/\Delta Y$$

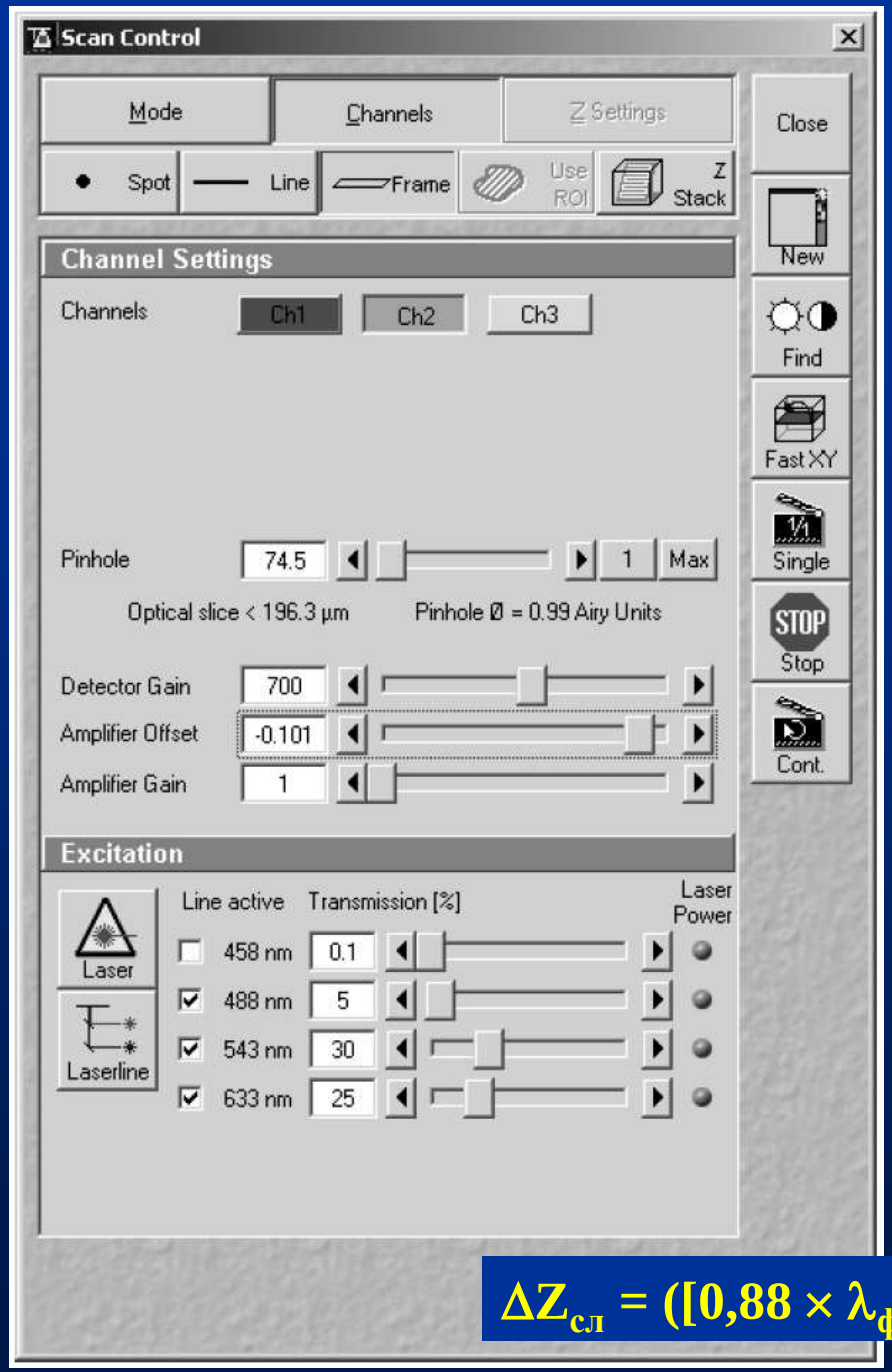
$$\Delta X = \Delta Y = 0{,}51 \times \lambda_в / A;$$

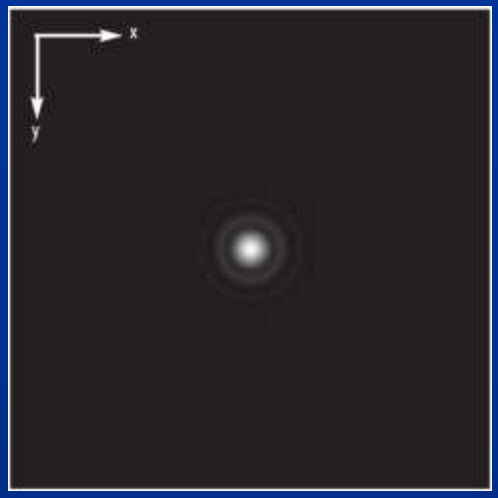


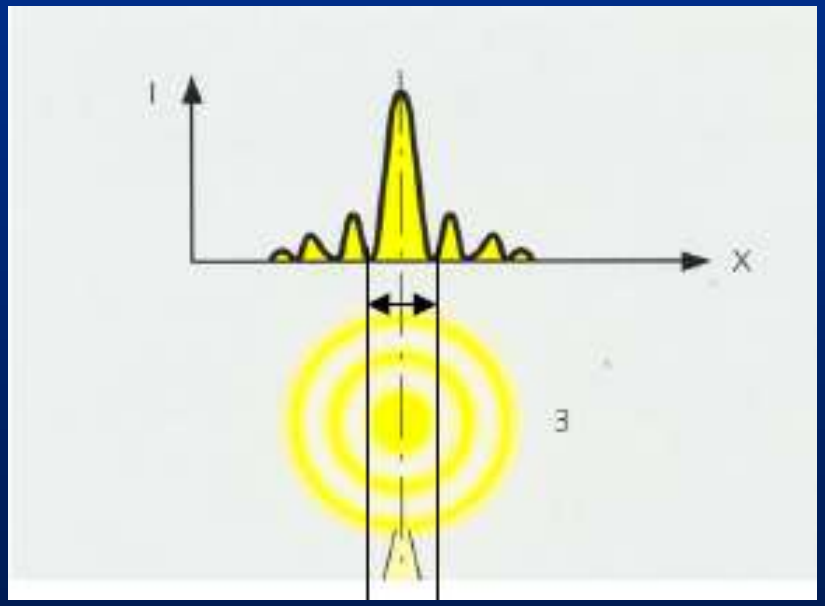


Digitization of intensity, color depth and gray scale

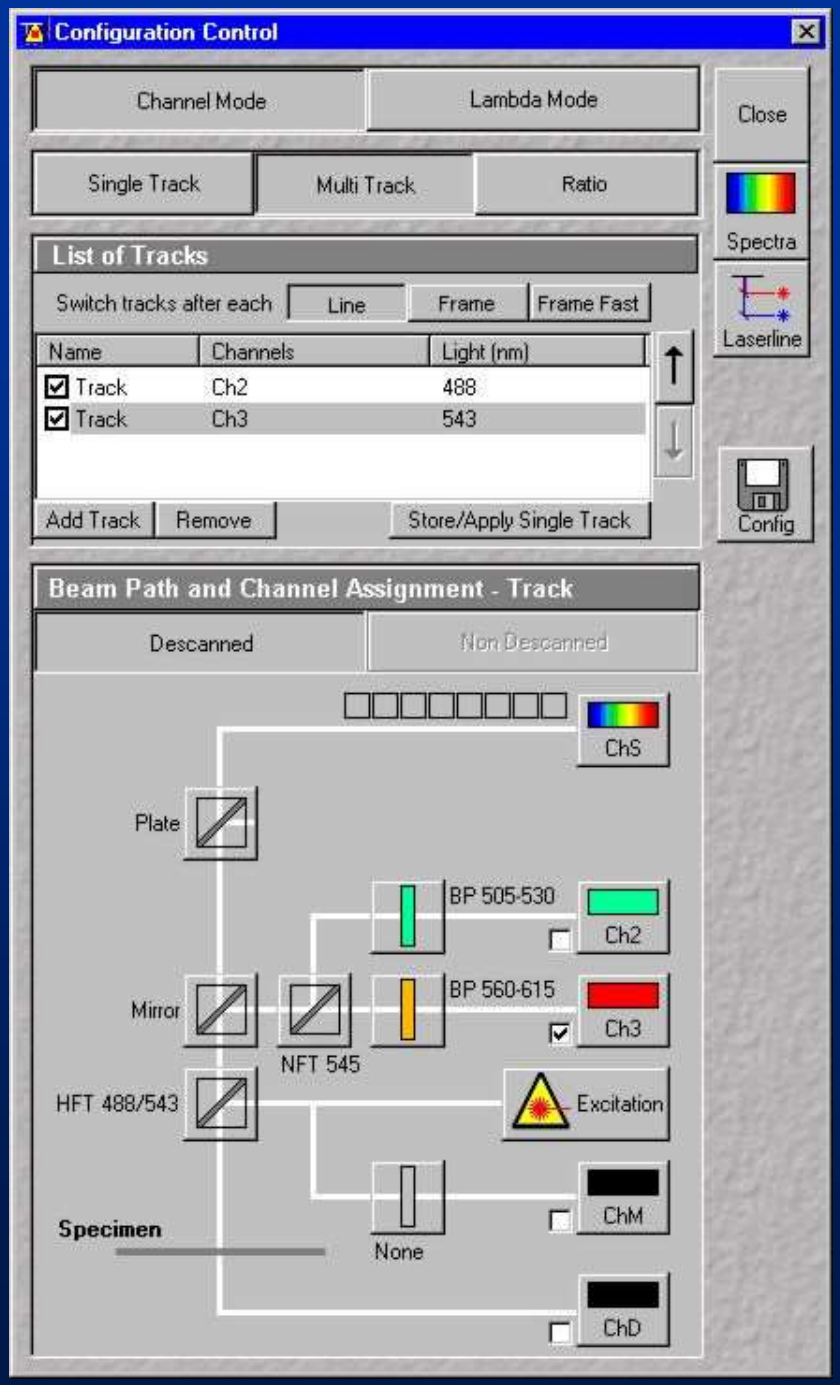


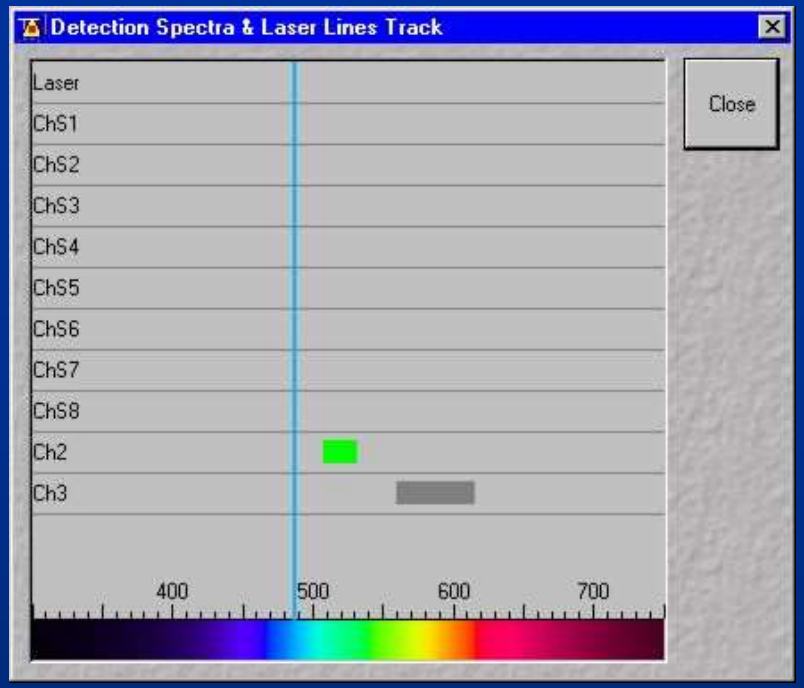


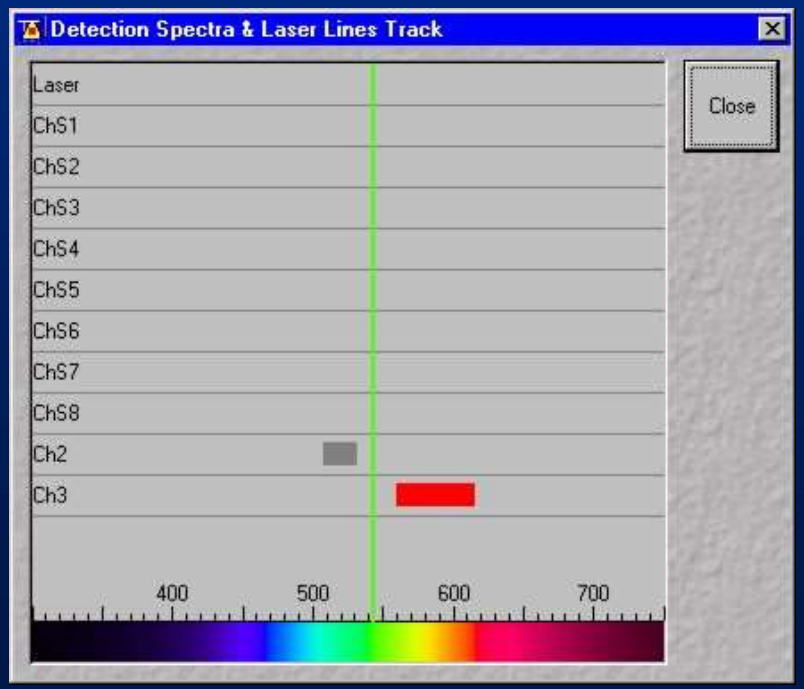


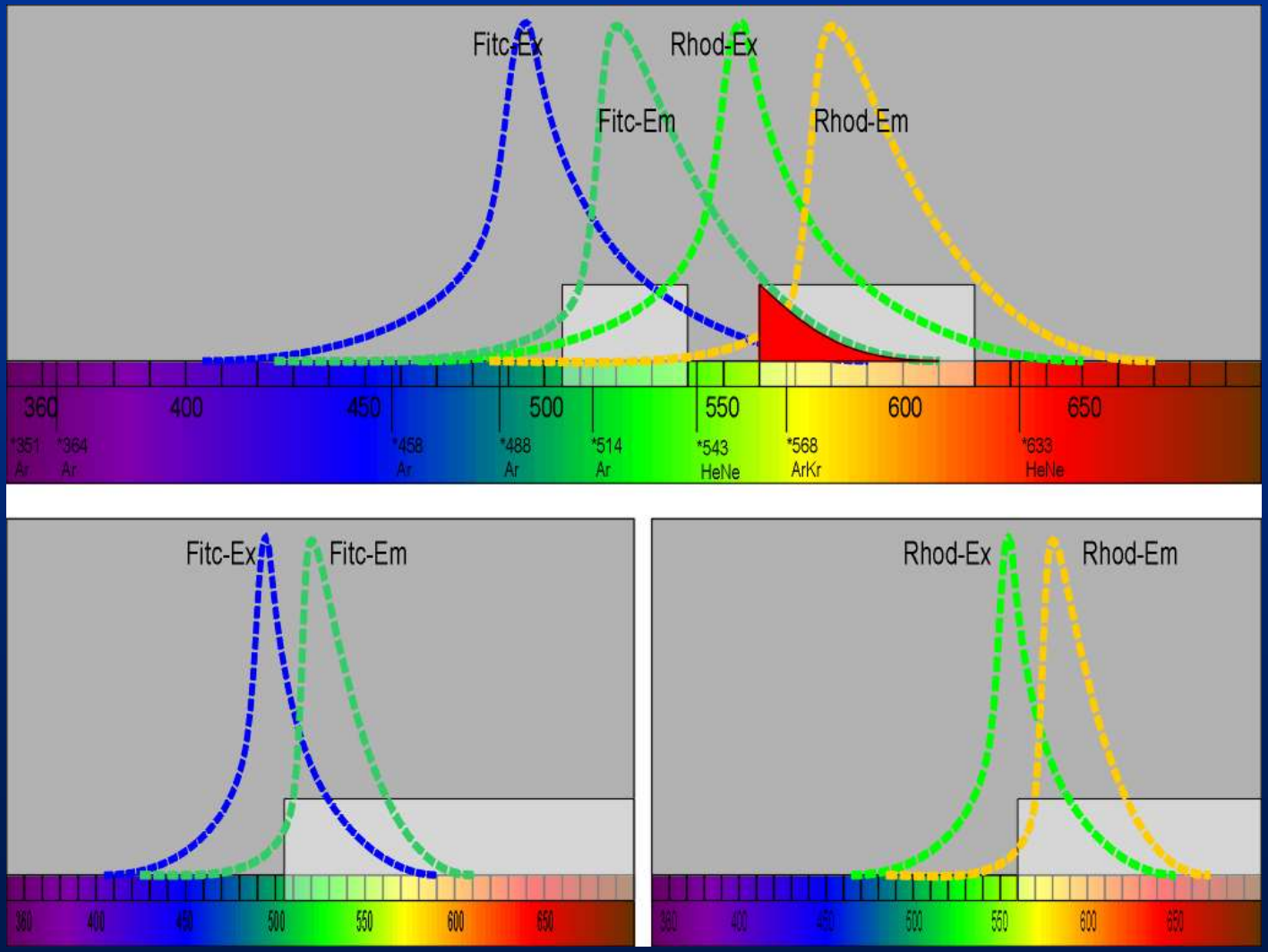


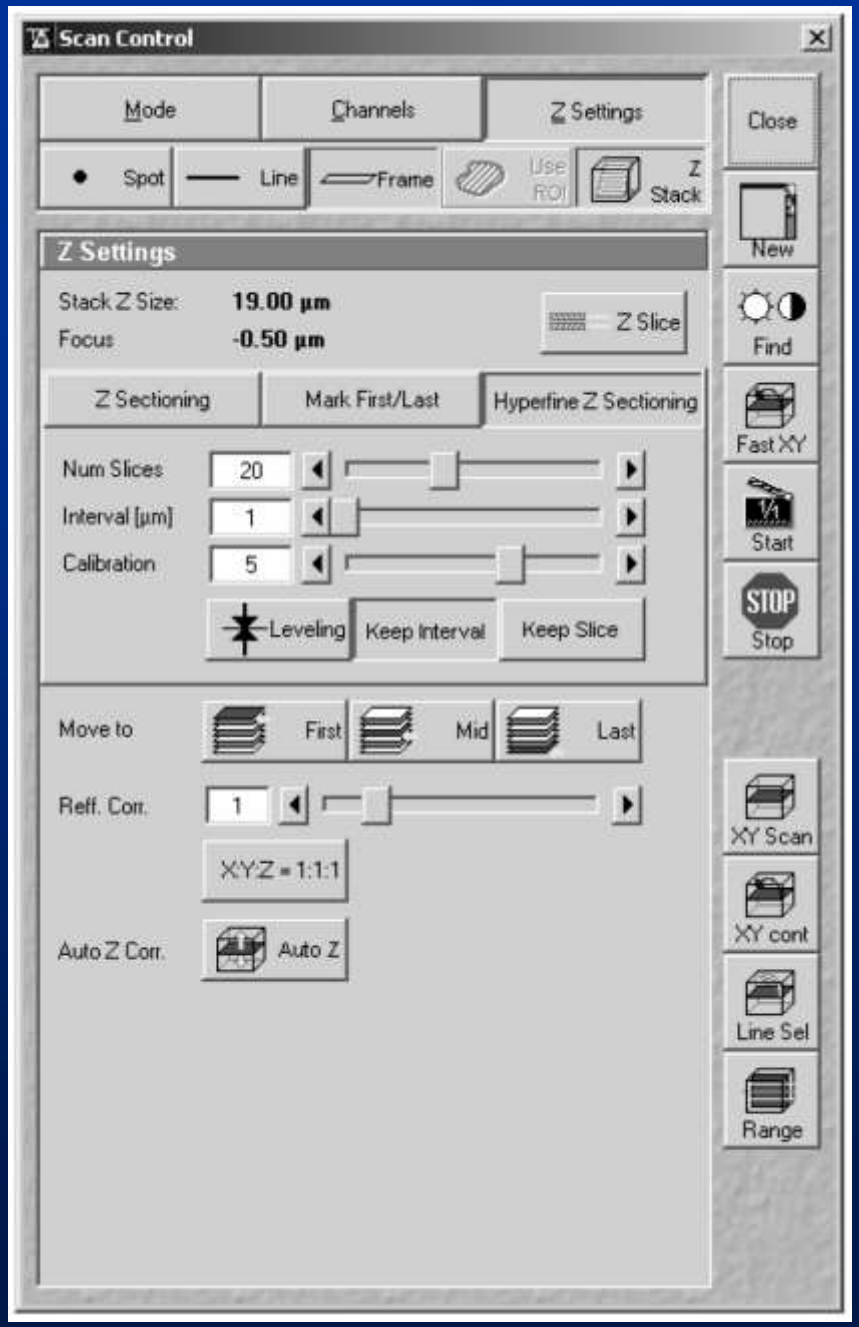
$$\Delta Z_{сл} = ([0{,}88 \times \lambda_{фл} / (n - (n^2 - A^2)^{0{,}5})]^2 + 2 \times n^2 \times \phi^2 / A^2)^{0{,}5}$$

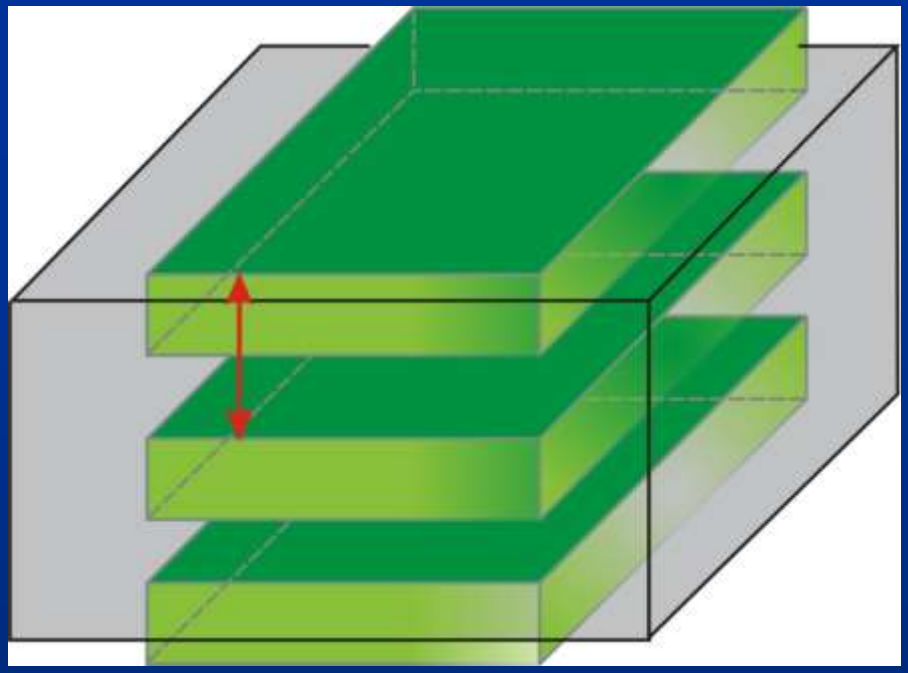

Configuration Control
Channel Mode
Lambda Mode
Close
Single Track
Multi Track
Ratio
Spectra
List of Tracks
Switch tracks after each
Line
Frame
Frame Fast
Laserline
Name
Channels
Light (nm)
Track
Ch2
488
Track
Ch3
543
Add Track
Remove
Store/Apply Single Track
Config
Beam Path and Channel Assignment - Track
Descanned
Non Descanned
ChS
Plate
BP 505-530
Ch2
Mirror
BP 560-615
Ch3
NFT 545
HFT 488/543
Excitation
ChM
Specimen
None
ChD

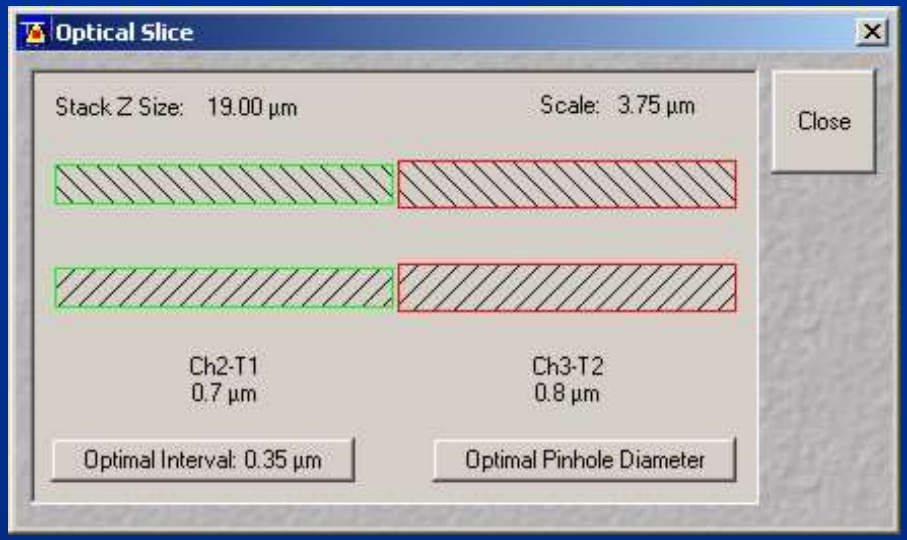

Detection Spectra & Laser Lines Track
Close
Laser
ChS1
ChS2
ChS3
ChS4
ChS5
ChS6
ChS7
ChS8
Ch2
Ch3
400
500
600
700

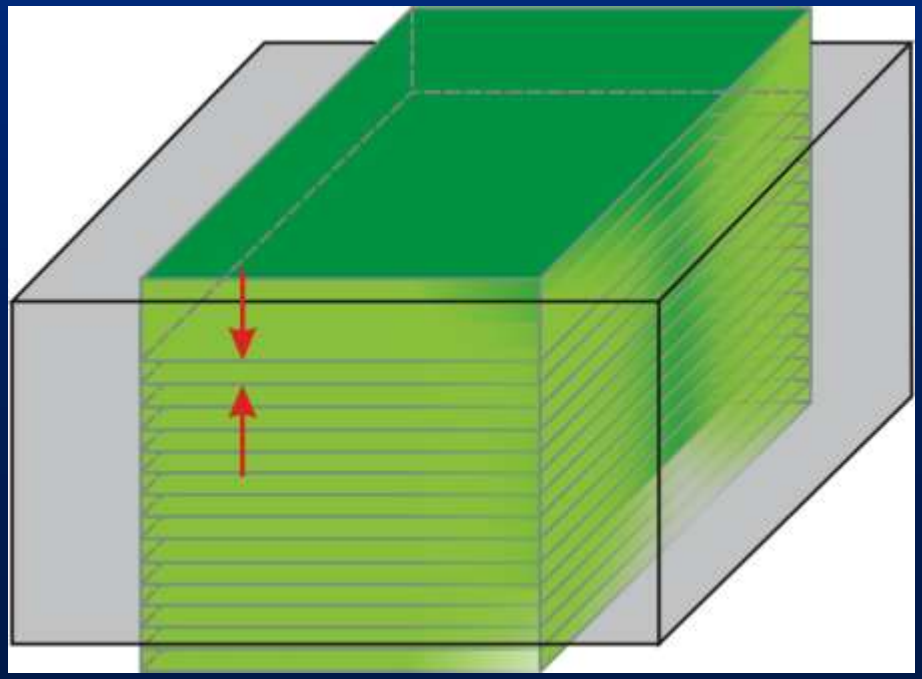

Detection Spectra & Laser Lines Track
Close
Laser
ChS1
ChS2
ChS3
ChS4
ChS5
ChS6
ChS7
ChS8
Ch2
Ch3
400
500
600
700

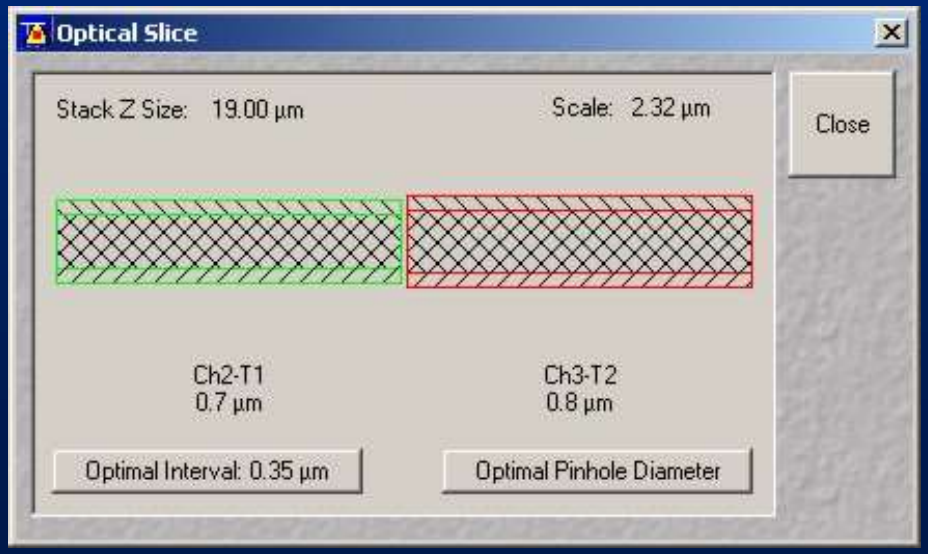

Fitc-Ex
Rhod-Ex
Fitc-Em
Rhod-Em
360
400
450
500
550
600
650
*351 Ar
*364 Ar
*458 Ar
*488 Ar
*514 Ar
*543 HeNe
*568 ArKr
*633 HeNe
Fitc-Ex
Fitc-Em
Rhod-Ex
Rhod-Em

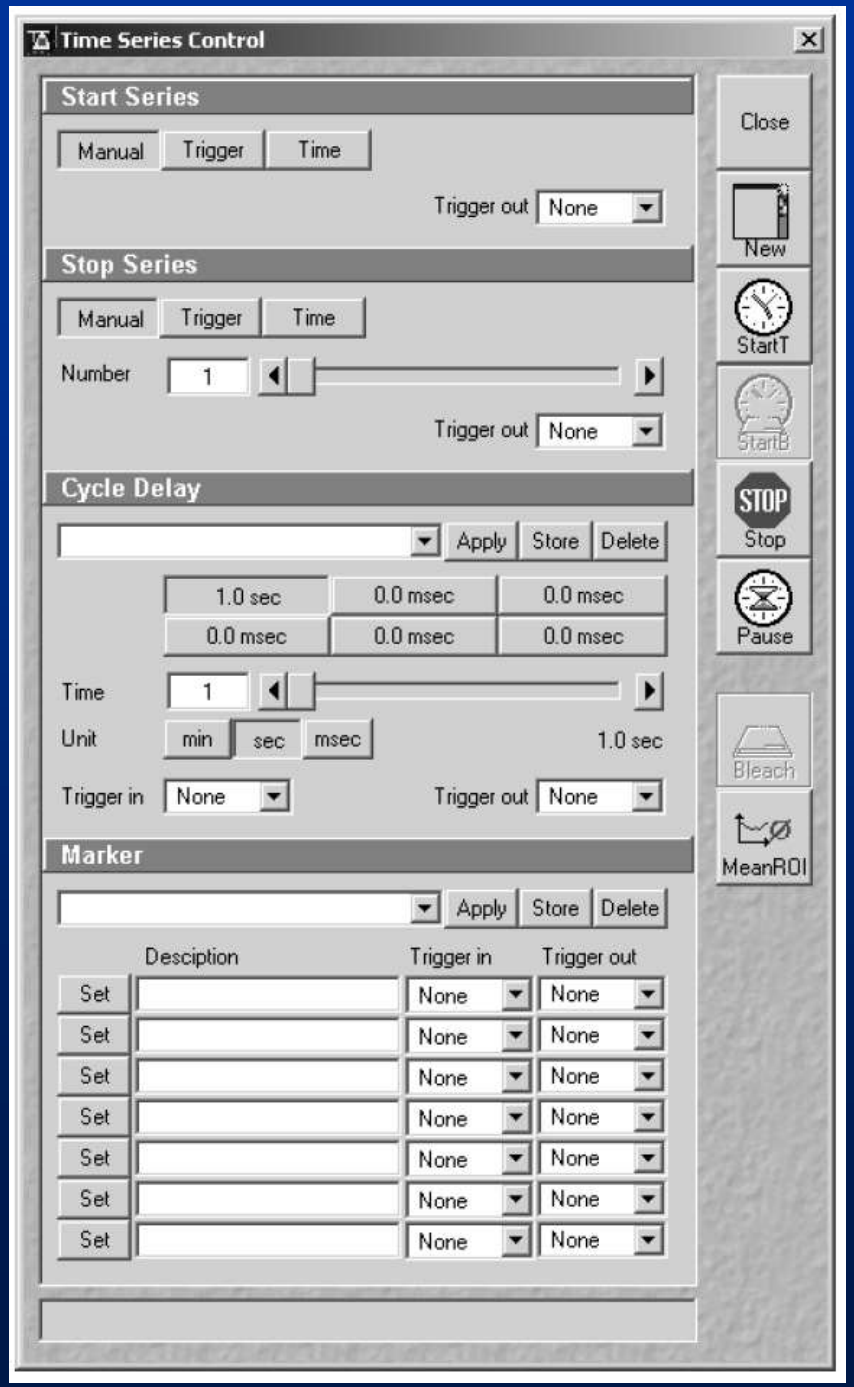

Scan Control
Mode
Channels
Z Settings
Spot
Line
Frame
Use ROI
Z Stack
Close
New
Find
Fast XY
Start
Stop
Z Settings
Stack Z Size:
19.00 µm
Focus
-0.50 µm
Z Slice
Z Sectioning
Mark First/Last
Hyperfine Z Sectioning
Num Slices
20
Interval [µm]
1
Calibration
5
Leveling
Keep Interval
Keep Slice
Move to
First
Mid
Last
Reff. Corr.
1
XYZ = 1:1:1
Auto Z Corr.
Auto Z
XY Scan
XY cont
Line Sel
Range




Optical Slice
Stack Z Size: 19.00 µm
Scale: 3.75 µm
Close
Ch2-T1
0.7 µm
Ch3-T2
0.8 µm
Optimal Interval: 0.35 µm
Optimal Pinhole Diameter




Optical Slice
Stack Z Size: 19.00 µm
Scale: 2.32 µm
Close
Ch2-T1
0.7 µm
Ch3-T2
0.8 µm
Optimal Interval: 0.35 µm
Optimal Pinhole Diameter


Time Series Control
Start Series
Manual
Trigger
Time
Trigger out
None
Stop Series
Manual
Trigger
Time
Number
1
Trigger out
None
Cycle Delay
Apply
Store
Delete
1.0 sec
0.0 msec
0.0 msec
0.0 msec
0.0 msec
0.0 msec
Time
1
Unit
min
sec
msec
1.0 sec
Trigger in
None
Trigger out
None
Marker
Apply
Store
Delete
Desciption
Trigger in
Trigger out
Set
None
Close
New
StartT
StartB
STOP
Stop
Pause
Bleach
MeanROI