Multidimensional Imaging with the Opterra Multipoint Scanning Confocal System

Atomic Force Microscopy
3D Optical Microscopy
Fluorescence Microscopy
Tribology
Stylus Profilometry
Nanoindentation
Outline

- Opterra design overview
- Light Path
- Scan Modes
- Performance
- Application data
Opterra allows you to pick your position in the Iron Triangle of Imaging

Figure courtesy of Paul Maddox
Concept originally developed by Jonas Dorn and Gaudenz Danuser
Two modes of operation
- Variable pinhole, linear array field scanner.
- Supravideo rate, high speed slit scanner.
- EMCCD camera-based detector.
- High frequency scanning minimizes phototoxicity and photobleaching
- Multiple scan modes allow for optimization of resolution, speed and light delivery
- Linear pinholes and separate light paths for emission and excitation minimize emission crosstalk
Overhead View of Opterra Imaging Scanner Showing Lightpath
Galvanometer and Piezos

Camera side piezo

Galvanometer

Scope side piezo
Aperture plate
Opterra Aperture Positions

Slit scanning positions:
- 22 μm slit
- 35 μm slit
- 50 μm slit
- 70 μm slit

Pinhole scanning positions:
- 30 μm pinholes (x32)
- 45 μm pinholes (x32)
- 60 μm pinholes (x32)
Opterra Imaging Scanner
Light Path
Galvanometer and Piezo Movement

Piezos move a fixed distance

Time to move depends on exposure time

It takes 2 frames for one complete “piezo sweep”

Galvo moves a fixed distance

Time to move depends on exposure time

Galvo sweeps multiple cycles/frame, and piezos sweep only once per frame
Field-scanning combines multiple galvo sweeps with bidirectional piezo movement.
Scan pattern timing

Sample Piezo/Galvo/Blanking Waveforms For Single Frame

- piezo offset
- piezo half period
- piezo scanning time
- piezo rest time
- piezo swing
- piezo back porch
- piezo front porch
- galvo offset
- galvo rest time
- galvo swing
- blanked
- unblanked

(R)est, (A)ccelerate, (F)ront porch, (S)canning, (B)ack porch, (D)eaccelerate
Cross talk

- Linear pinhole arrangement: < one half of the crosstalk of 2-D array scanners.
  - Provides improved axial resolution.
Performance

- Standard Opterra camera (Evolve Delta)
  - 512 x 512 full frame acquisition.
  - Up to 40 full frames per second pinhole mode.
  - Faster acquisition from smaller region of interest.
  - Slit scan imaging at 66 fps full frame (512x512), 500 fps with ROI, up to 1000 fps with specialized detector
Opterra benefits for Multidimensional Imaging

- Less cross talk provides improved axial resolution and imaging depth
- High acquisition speed provides excellent temporal resolution
- Shorter exposure times results in lower phototoxicity and photobleaching
  - Sea urchin embryos, 50 plane Z series every 2 minutes, experiments for over 2 hours compare to 1 hour on spinning disc
  - Generally across a number of samples exposures 5 – 10 times shorter than spinning discs
Zebrafish Neurons
100 uM Z Range
Tumor Slice

CFP and GFP label

100 um Z range
Z Series Collected on Timed Intervals
Max projections of Z series timelapse, Rab5-GFP vesicles in axons within spinal cord of intact Zebrafish embryo.

12 section z series
Less than 1 second per Z stack
Z every 5 seconds
Z Series Timelapse

Green: EGFP labelled EB3, Red H2B mCherry
Max projections, 5 seconds intervals, 21 plane stacks, 0.5 um spacing
Max projection of high speed z series
YFP mitochondria, mCherry peroxisomes

11 Planes, 0.3 micron spacing
2 Color
1.2 seconds / Z stack
Z every 2 seconds
Atlas Imaging for Stage Montages
Moving using the Atlas Window
Changing Focus

- Focal plane can be changed using:
  - Click and drag on Z slice (shown)
  - Mouse scroll wheel
  - Prairie View’s existing Z controls
  - Wheels on motor controlling hardware
Atlas Imaging
Pruning the Grid
Arabidopsis 2x4 montage, 60x objective
Tissue section, 2.4 mm by 1.2 mm area at 60 x 18x9 montage
Opterra Multipoint Scanning Confocal Summary

• Flexibility in aperture selection to meet experimental needs, allows user to balance speed, resolution and sensitivity. Allows the user to determine where they want to be in the “Iron Triangle of Imaging”

• Pinholes in 1-D array minimizes cross talk and improves axial resolution compared to 2-D arrays

• Scanning pattern with pinholes minimizes phototoxicity

• Speed provides high temporal resolution with 4-D imaging

• Higher throughput on grid collection applications
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