Advanced Light Microscope Techniques 7

Deconvolution
SPIM
STED
SI
PALM, STORM
CARS

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For Advance Light Microscopy course
ADVANCED TECHNIQUES

- Deconvolution
- SPIM
- STED
- SI
- PALM, STORM [G. Haran]
- CARS
Three-Dimensional Deconvolution
3D DECONVOLUTION

- Acquires wide field images at various heights, and uses a mathematical model to calculate the 3D distribution of light from the object.
- Blind deconvolution estimates the instrument parameters.
- Non-blind deconvolution requires measurement of the PSF for the system (or a reasonable guess thereof)
- Makes maximal use of sample exposure (good for living cells).
convolution

deconvolution
Structured Illumination (SI)
Structured light systems
Structured light examples

conventional

OptiGrid™
Moiré pattern: two superimposed high resolution patterns create low resolution pattern

Information from multiple images illuminated with periodic striated patterns in 5 orientations and 5 “phases” is combined to doubles the lateral resolution of Wide-field microscopes. 3D interference can be used to increase also the axial resolution. Using non-linear effects, the resolution can be even higher.  
Reference: M. Gustafsson
Coherent Anti-Stokes Raman Scattering (CARS)
CARS microscopy
Coherent Anti-Stokes Raman Scattering
Intrinsic and specific contrast (?)

Output depends on molecular vibration spectrum

Exploit characteristic molecular spectra in “fingerprint region” (1000-1700 cm⁻¹)?

Pro:  
+ Natural chemical contrast
+ Get chemical contrast of IR spectroscopy  
  and spatial resolution of visible light
+ No bleaching -> can image “forever”

Con:  
- Only natural contrast
- Complex laser system

Sunney Xie group, Harvard
Mouse sebaceous gland imaged with CARS using the CH2 symmetric stretching vibration, which is abundant in lipids.

Sunney Xie group, Harvard
Other nonlinear microscopy:
Second and third harmonic generation

Asymmetric potential well
⇒ non-harmonic oscillation
⇒ radiates at $2 \omega$

Second harmonic generation

Two photons in
Energy $E / 2$
Wavelength $= 2 \lambda$

One high-energy photon out

Pro:
+ Natural geometric contrast (edges, fibers (collagen!))
+ No bleaching → can image “forever”
+ Can do together with two-photon fluorescence

Con:
- Only natural contrast
Simultaneous imaging of SHG, THG and multi-photon fluorescence

![Graph showing intensity vs. wavelength for SHG and THG with 2PF peaks.]

Chu et al., J. Microsc. 208, 190 (2002)

Easy to separate spectrally by filters. Directionality also helps separation: can detect 2PF in epi, but SHG and THG in forward direction.
Polarization Dependence of Harmonic Generation Microscopy

Harmonic generation with multi-photon fluorescence

Mouse heart tissue (740 nm excitation)

Green: Second Harmonic Generation in extracellular collagen scaffolding
Grayscale: 2-photon excitation of NAD(P)H intrinsic fluorescence in a cardiac myocyte

Zipfel et al. (Watt Webb group), PNAS 2003
THG, SHG & multi-photon fluorescence

(A) Rice leaf

Blue: Third Harmonic Generation
Green: Second Harmonic Generation
Red: 2-photon excitation
• X-Ray Microscopy
• Electron microscopes
• AFM
• Correlative OM & SEM
• Correlative OM & TEM/tomography