Advanced Light Microscope Techniques 7

Deconvolution
SPIM
STED
SI
PALM, STORM
CARS

Edited by: Zvi Kam, Weizmann
For Advance Light Microscopy course

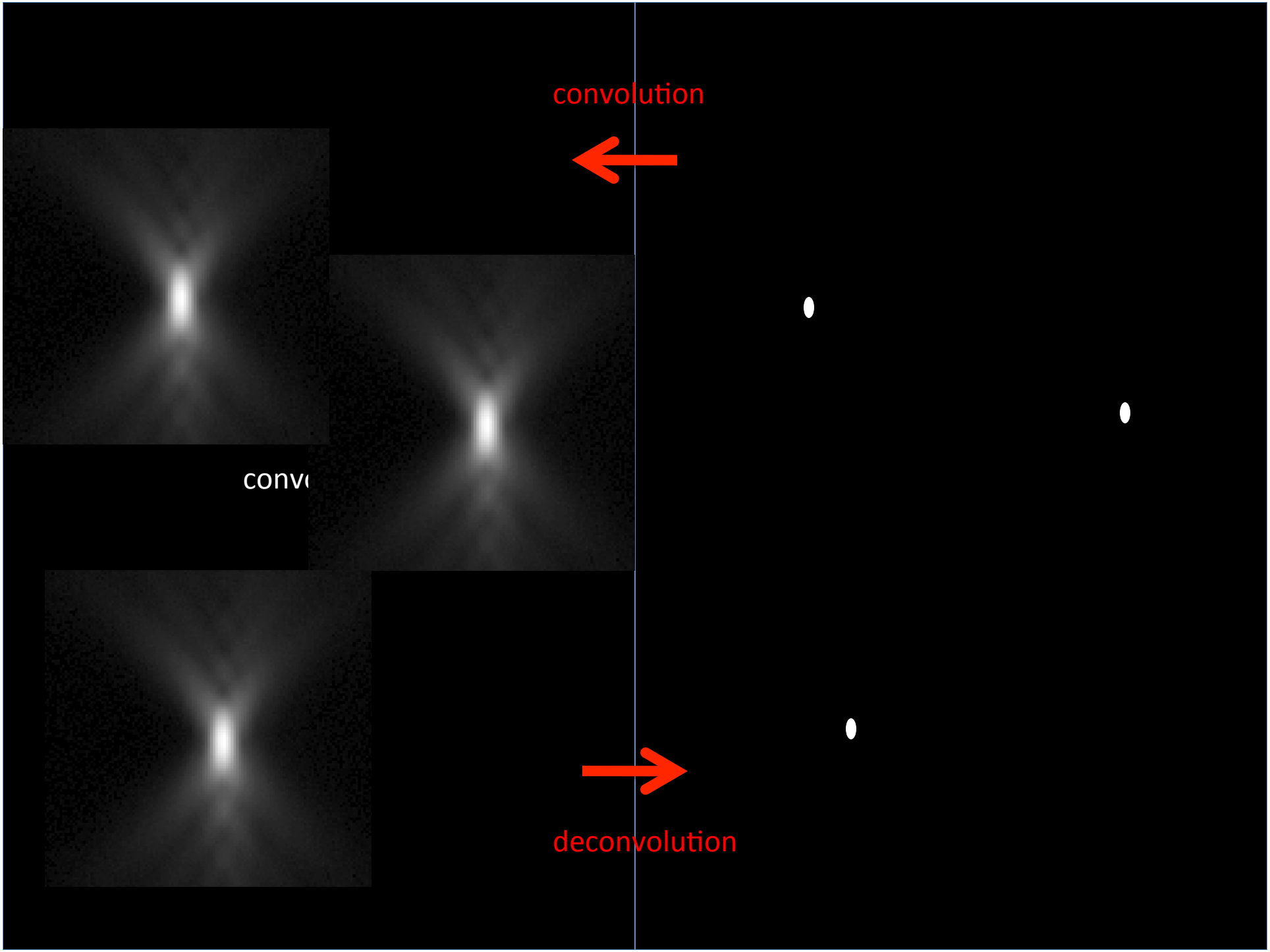
ADVANCED TECHNIQUES

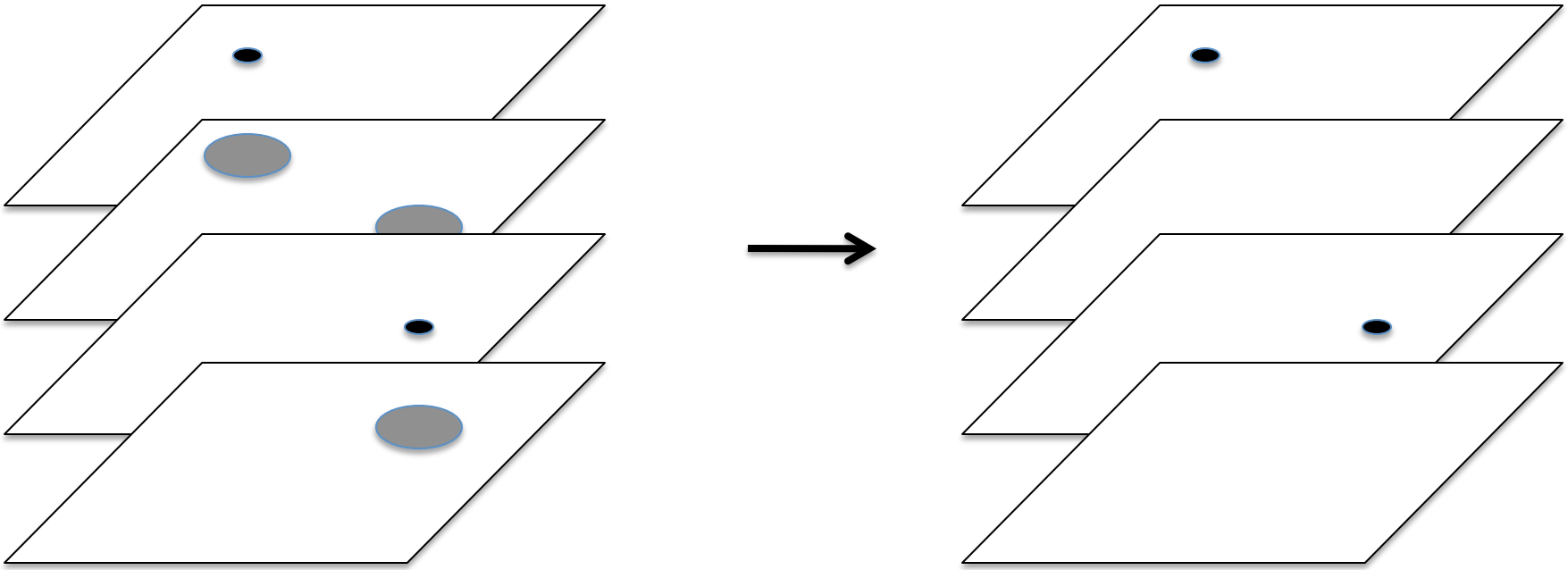
- Deconvolution
- SPIM
- STED
- SI
- PALM, STORM [G. Haran]
- CARS

Three-Dimensional Deconvolution

3D DECONVOLUTION

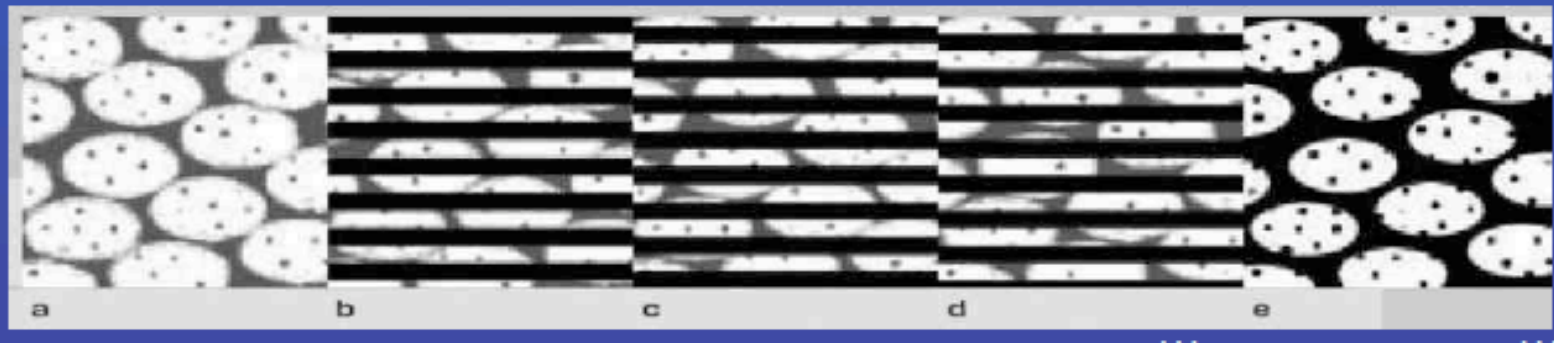
- Acquires wide field images at various heights, and uses a mathematical model to calculate the 3D distribution of light from the object.
- Blind deconvolution estimates the instrument parameters.
- Non-blind deconvolution requires measurement of the PSF for the system (or a reasonable guess thereof)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


convolution
deconvolution

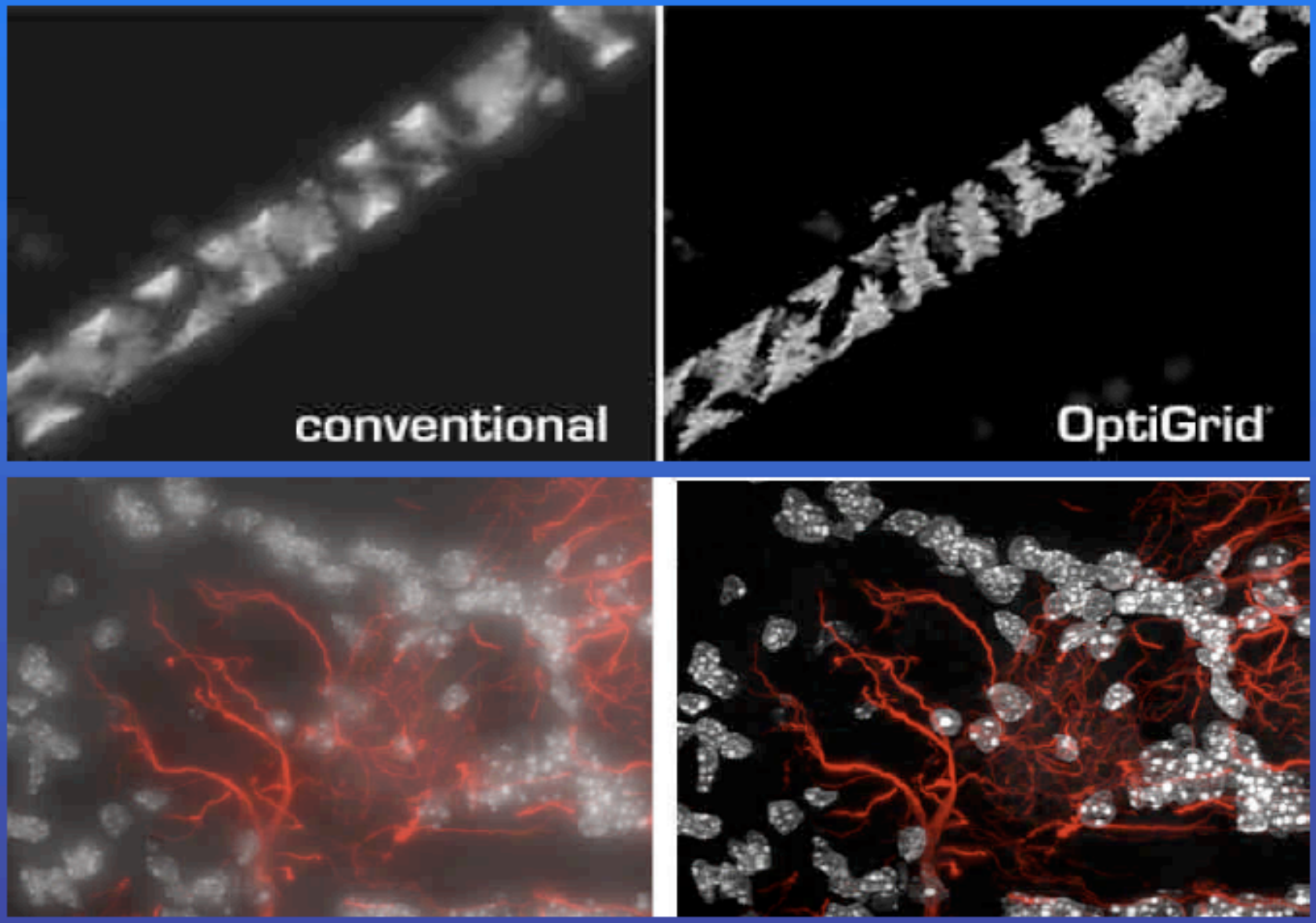


Structured Illumination (SI)

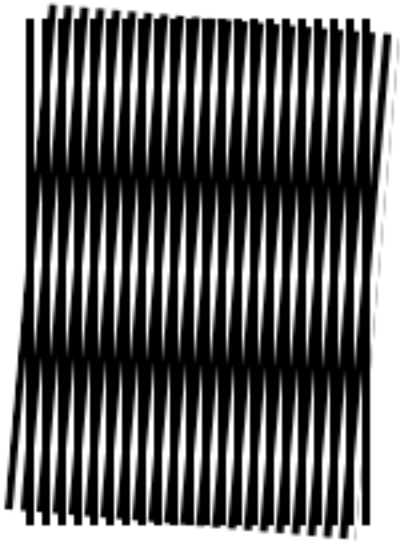
Structured light systems

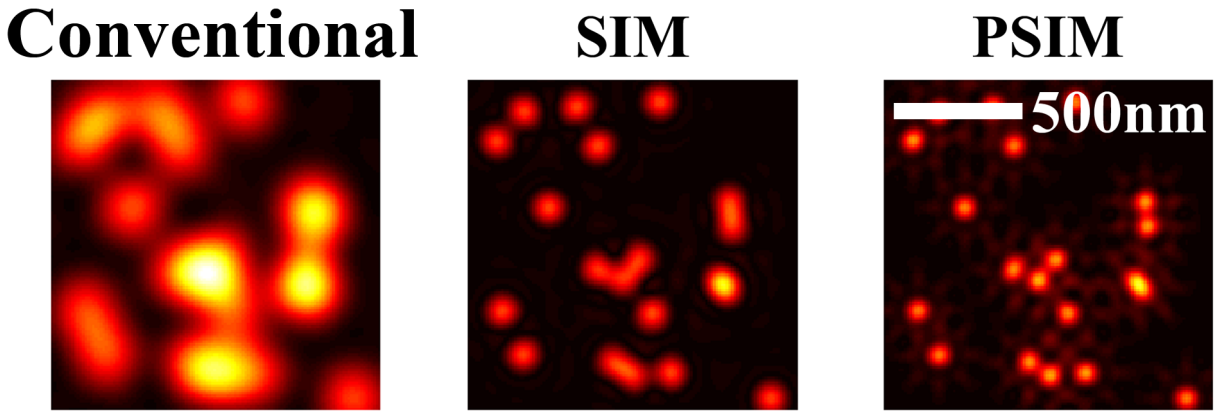


Structured light examples



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)

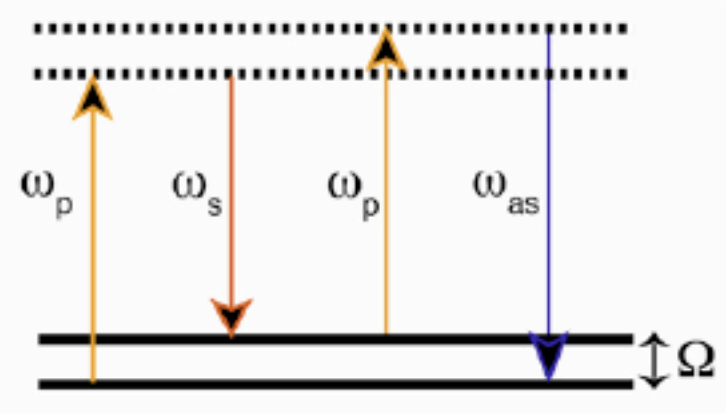
CARS microscopy

Coherent Anti-Stokes Raman Scattering

Intrinsic and *specific* contrast (?)

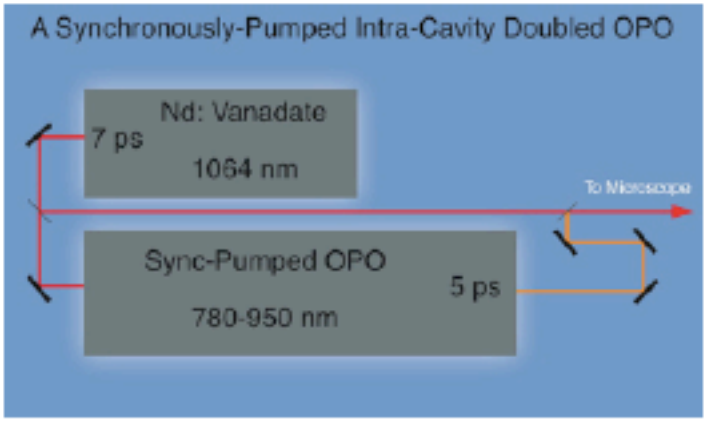
Output depends on molecular vibration spectrum

Exploit characteristic molecular spectra
in "fingerprint region" (1000-1700 cm^{-1})?



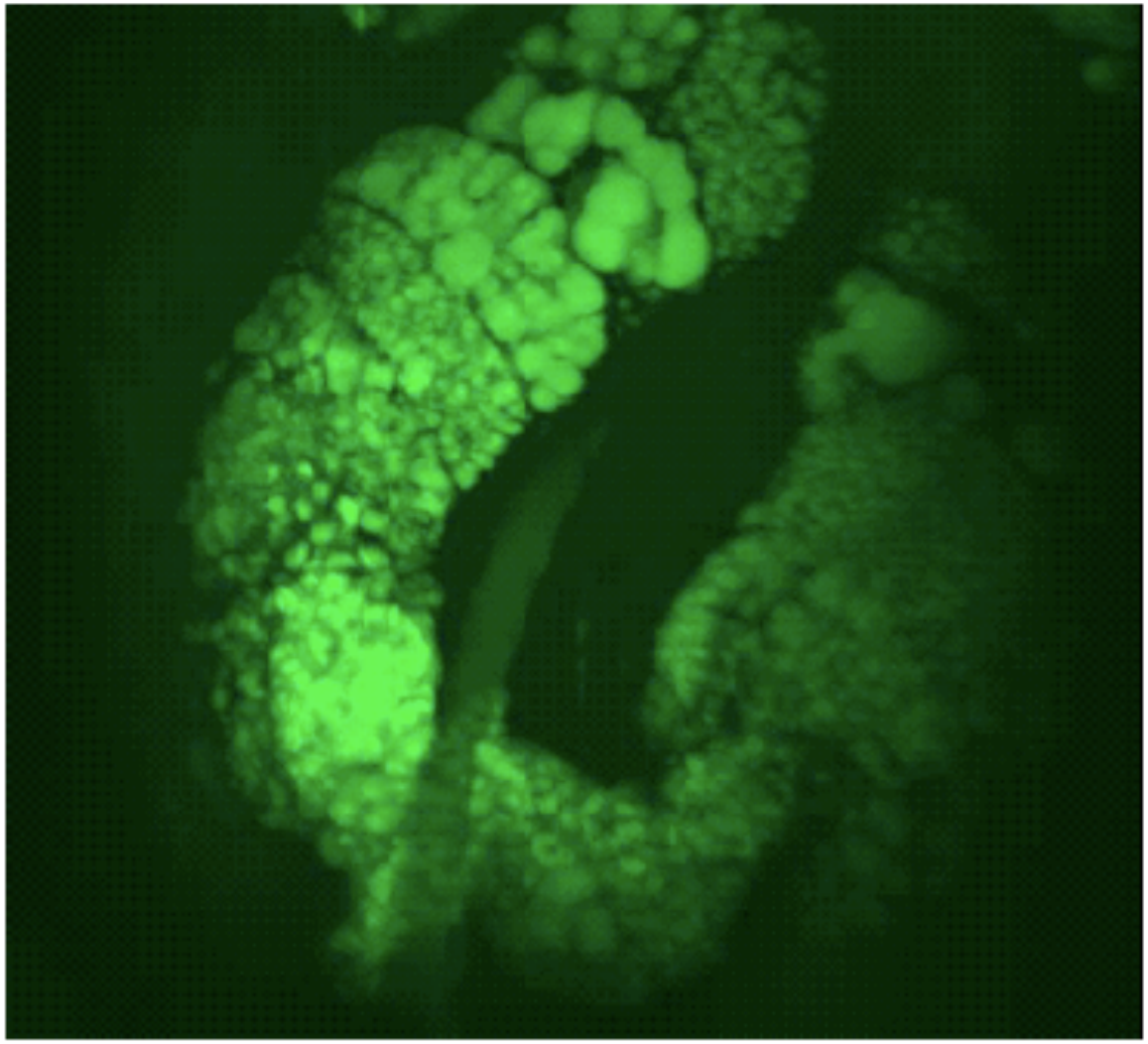
Pro:
+ Natural chemical contrast
+ Get chemical contrast of IR spectroscopy
and spatial resolution of visible light
+ No bleaching -> can image "forever"

Con:
- Only natural contrast
- Complex laser system



Sunney Xie group, Harvard

CARS microscopy

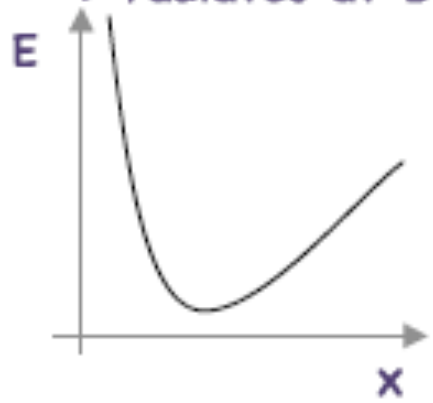


Mouse sebaceous gland imaged with CARS using the CH2 symmetric stretching vibration, which is abundant in lipids

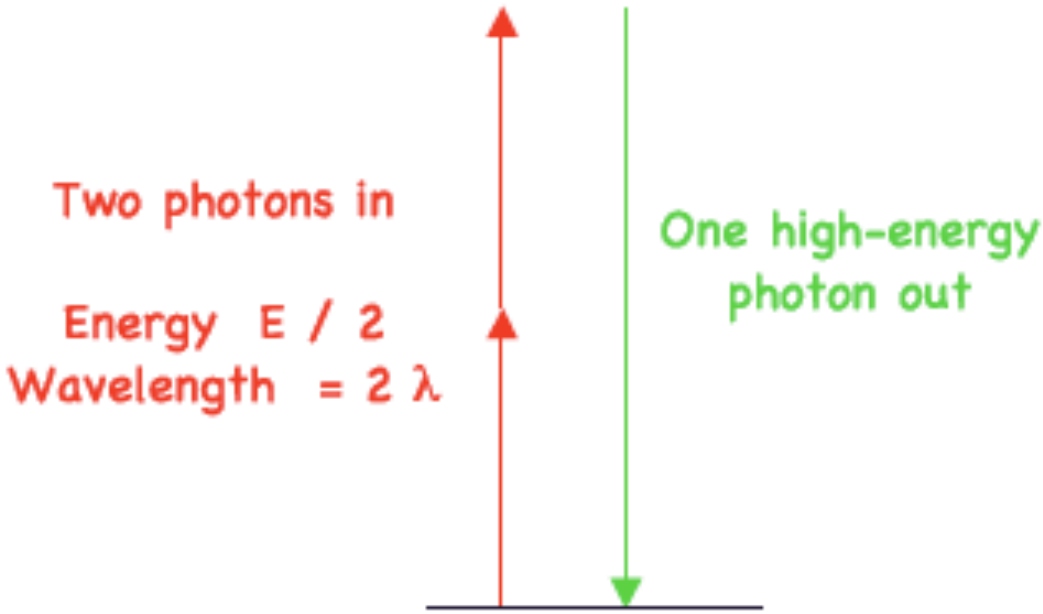
Sunney Xie group, Harvard

Other nonlinear microscopy: Second and third harmonic generation

Asymmetric potential well
⇒ non-harmonic oscillation
⇒ radiates at 2 ω



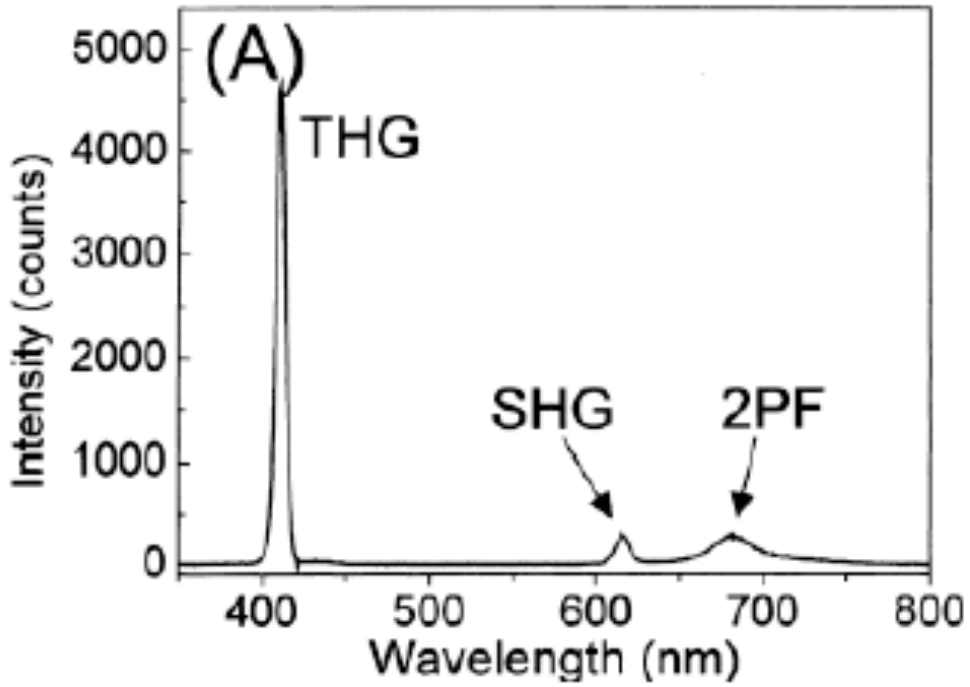
Second harmonic generation



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



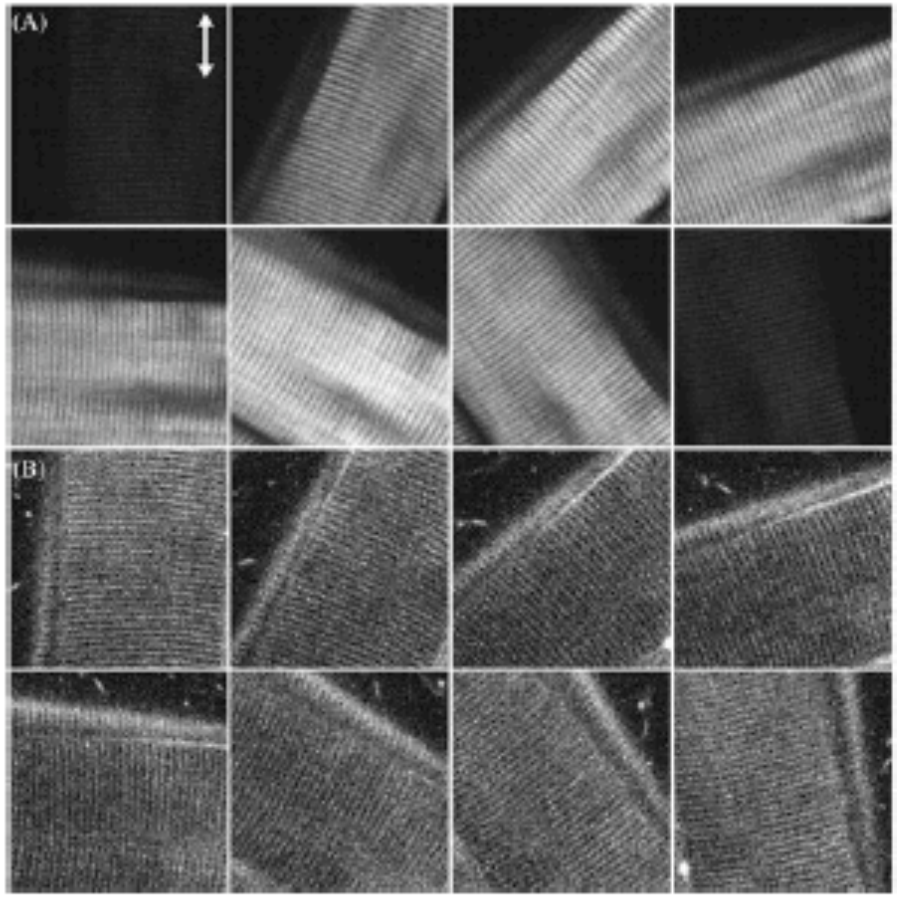
Chu et al., J. Microsc. 208, 190 (2002)

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

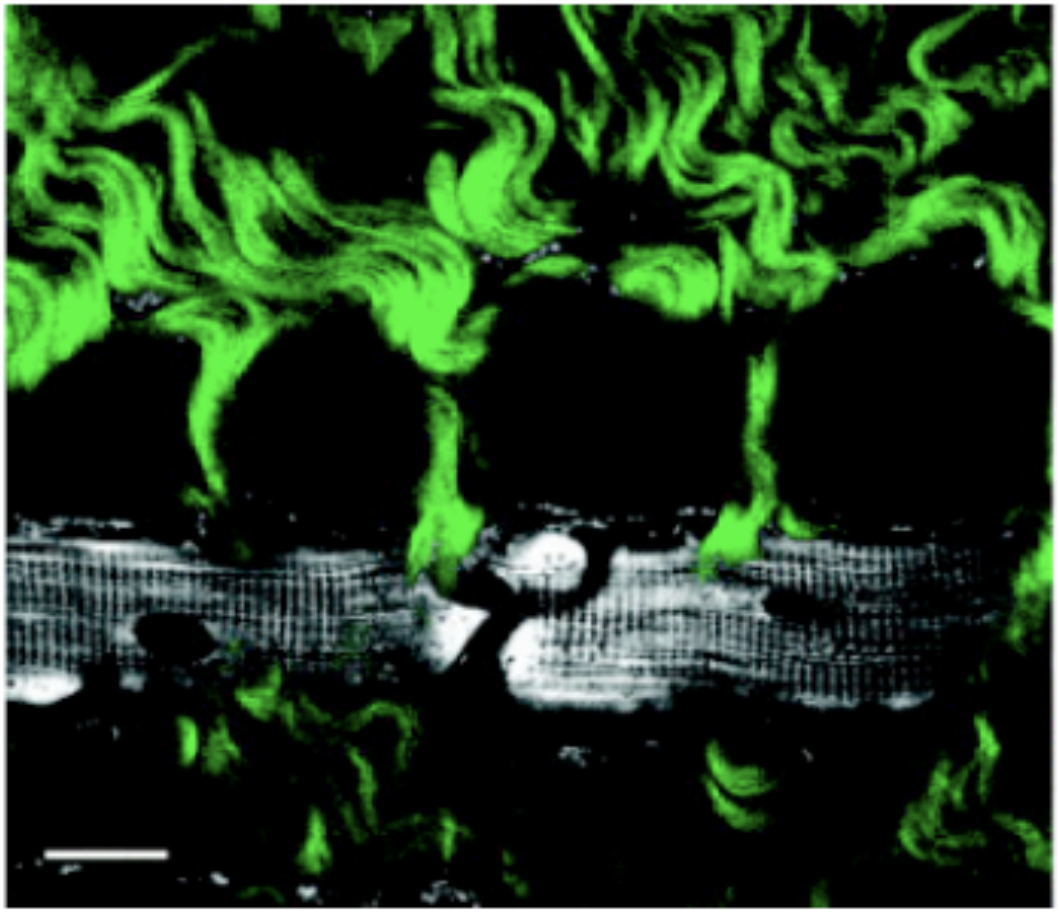
SHG

THG



Chu et al., Biophysical Journal 86:3914–3922 (2004)

Harmonic generation *with* multi-photon fluorescence



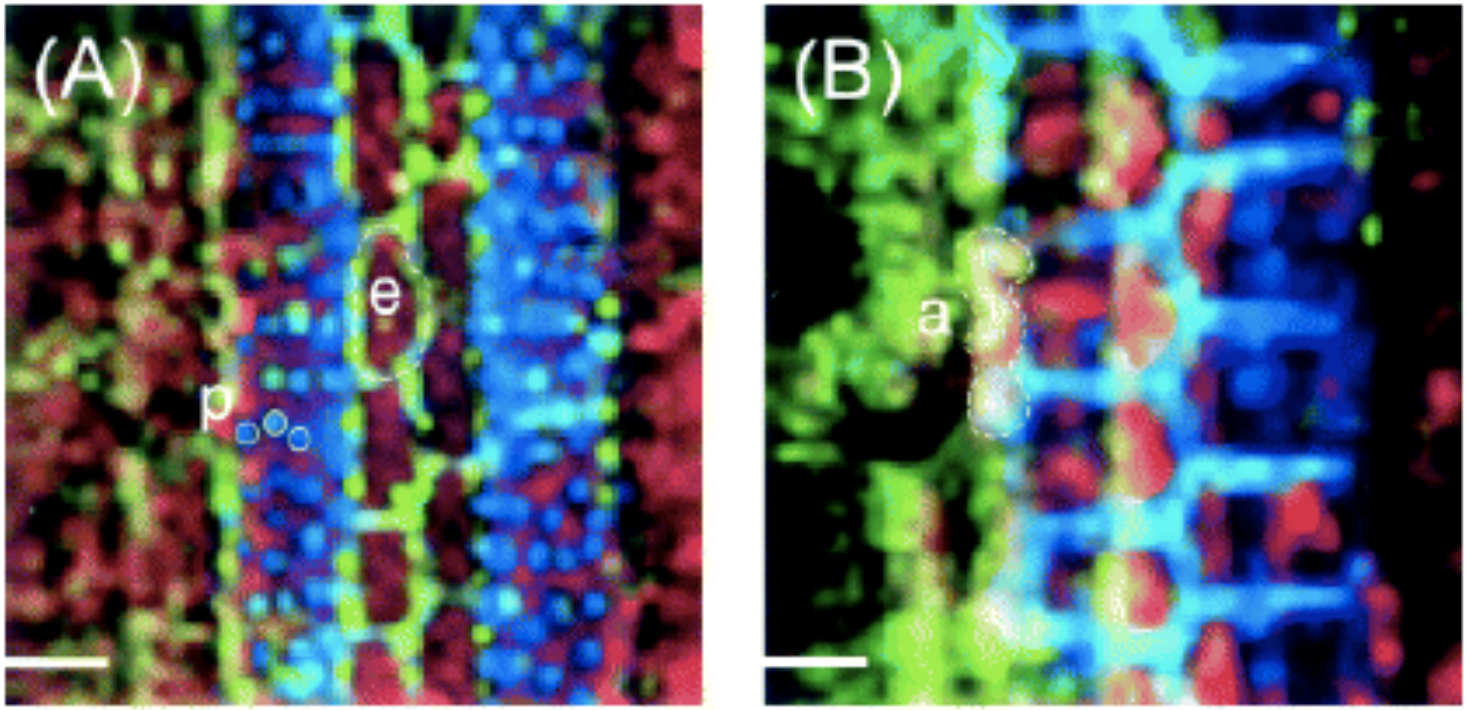
Mouse heart tissue (740 nm excitation)

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



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