Dipartimento di Fisica
Università degli studi di Genova

ISTITUTO ITALIANO
DI TECNOLOGIA
OPTICAL
NANOSCOPY

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Italian Physical Society

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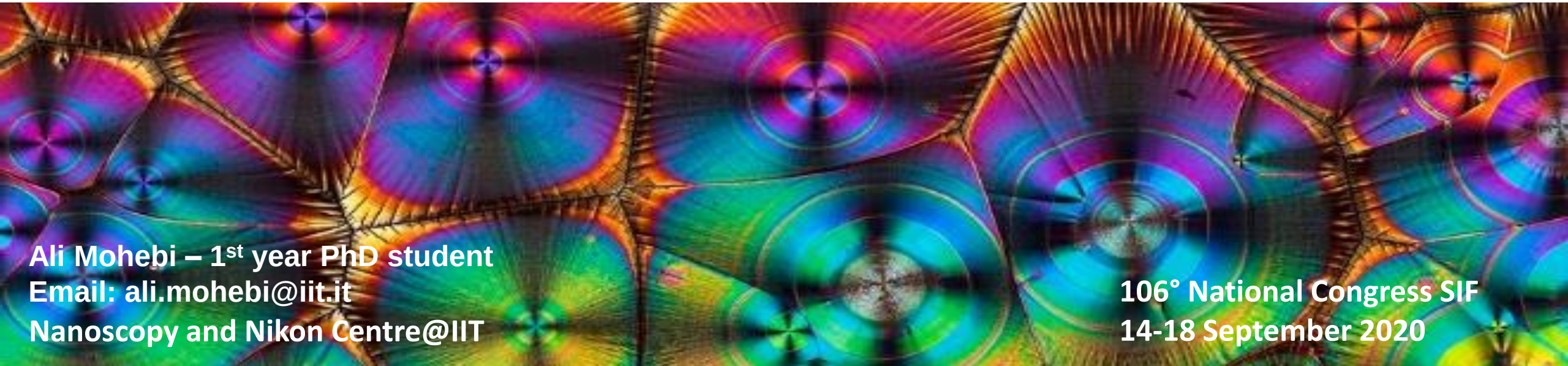
Resolution improvement in circular intensity differential scattering scanning microscopy integrated with two photon fluorescence microscopy using a phasor plot approach



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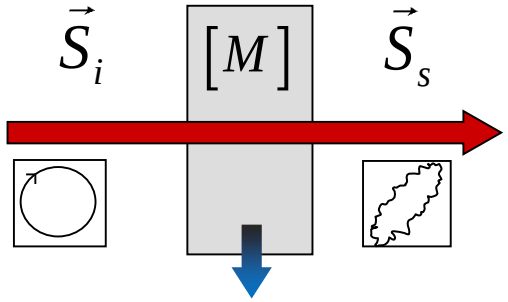
106° National Congress SIF
14-18 September 2020

Outline

- Label-free microscopy
- Circular intensity dichroism scattering (CIDS)
- Two photon microscopy
- Integration of the modalities
- Phasor approach for a better interpretation of the sample image
- Conclusion

Light matter interaction: label-free characterization of a sample

$$\vec{S} = \begin{bmatrix} S_0 \\ S_1 \\ S_2 \\ S_3 \end{bmatrix} = \begin{bmatrix} I_0 \\ I_x - I_y \\ I_{+45°} - I_{-45°} \\ I_R - I_L \end{bmatrix}$$



Mueller matrix

(4x4) real coefficients

Incident light ⇒  ⇒ **Polarization**

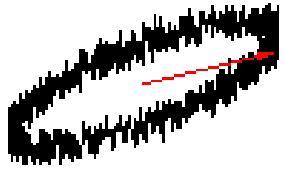
Sample

(imaging example: Polarimetry/Ellipsometry**)**

Polarization state before interaction

$$\begin{pmatrix} m_{00} & m_{01} & m_{02} & m_{03} \\ m_{10} & m_{11} & m_{12} & m_{13} \\ m_{20} & m_{21} & m_{22} & m_{23} \\ m_{30} & m_{31} & m_{32} & m_{33} \end{pmatrix}$$

CIDS

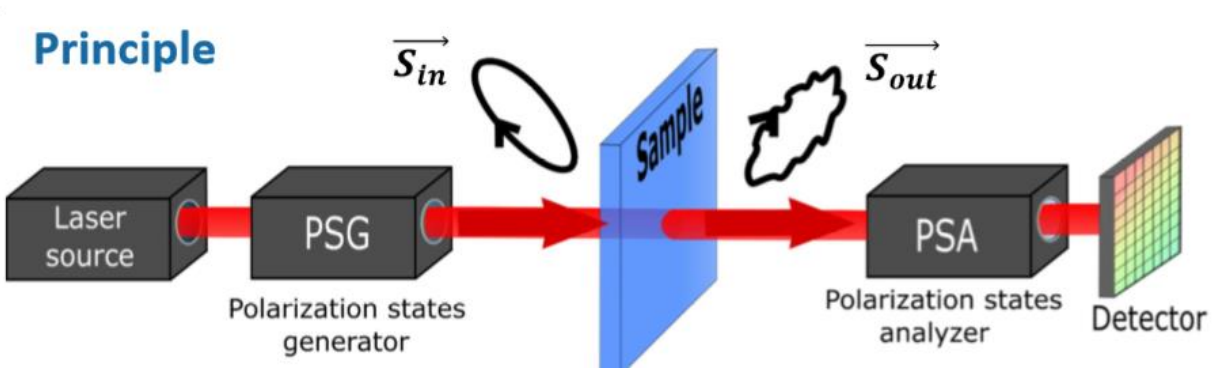


Polarization state after interaction

- **Absorption**
- **Scattering**
- **Dichroism**
- **Birefringence**
- **Depolarization**

S.Y. Lu & R.A. Chipman (1996)

Circular Dichroism (& CIDS) microscopy: temporal modulation states



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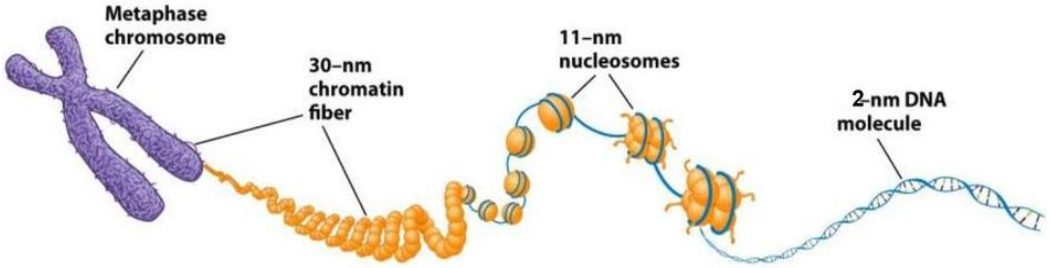
PhotoElastic Modulator (PEM)

Fast modulation of the polarization states

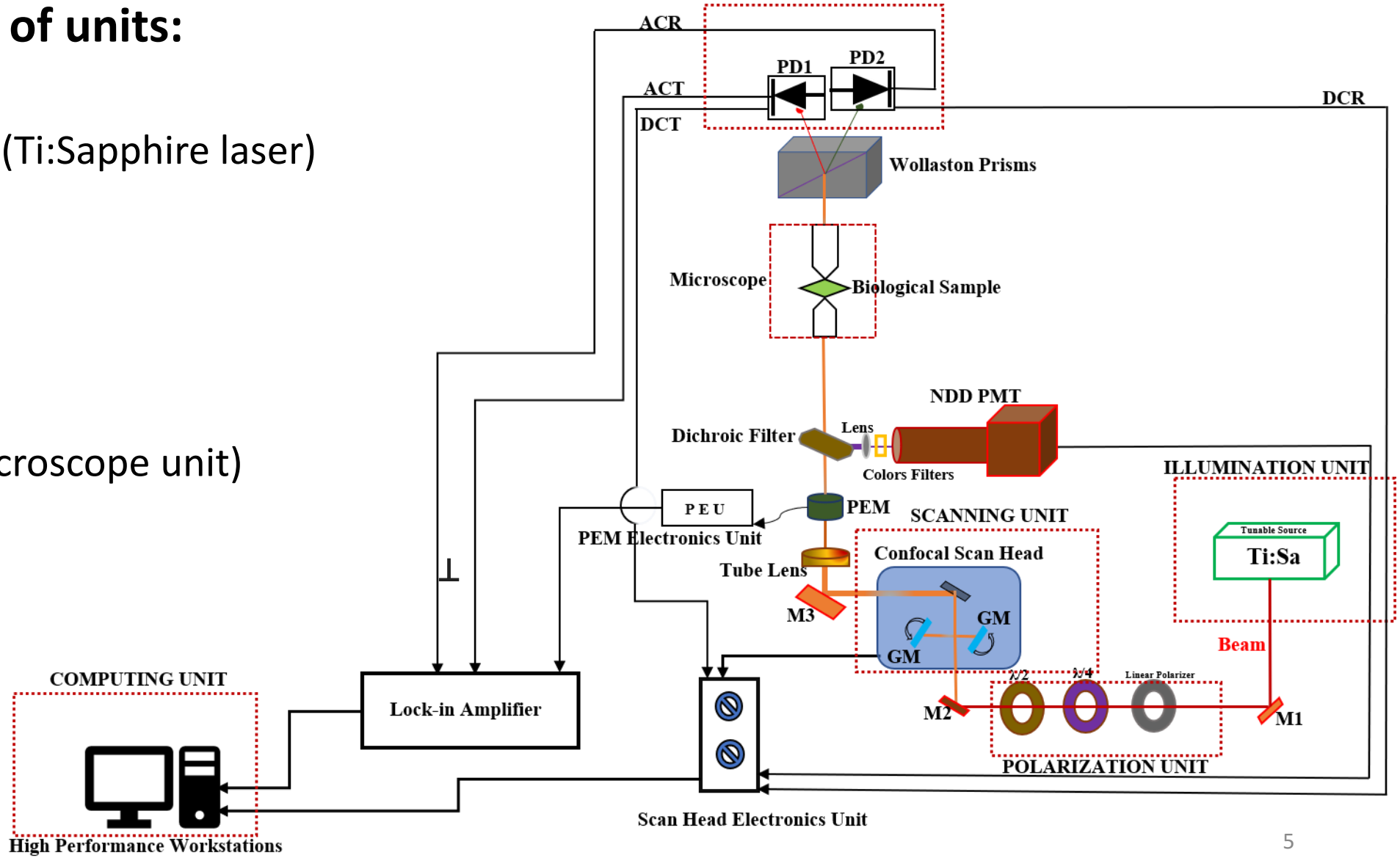
- ✓ 50 kHz & 100 kHz modulations **Circular & Linear dichroism**
- ✓ Tunable resolution: 10X, 40X, 60X, 100X objectives
- ✓ Multimodality with **epi-fluorescence**
- ✓ Far from the absorption band: **CIDS Sensitivity to λ/10 - λ/20**
- ✓ Sensitivity to study **anisotropy for biopolymers organization**
  e.g. Levels of DNA Packing

$$m_{03} = CIDS = \frac{I_L - I_R}{I_L + I_R}$$

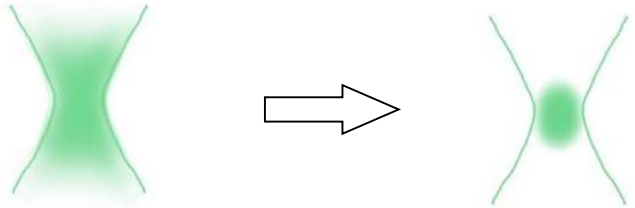


Schematic layout of units:

1. Illumination unit (Ti:Sapphire laser)
2. Polarization unit
3. Scanning unit
4. Imaging unit (microscope unit)
5. Detection unit
6. Computing unit

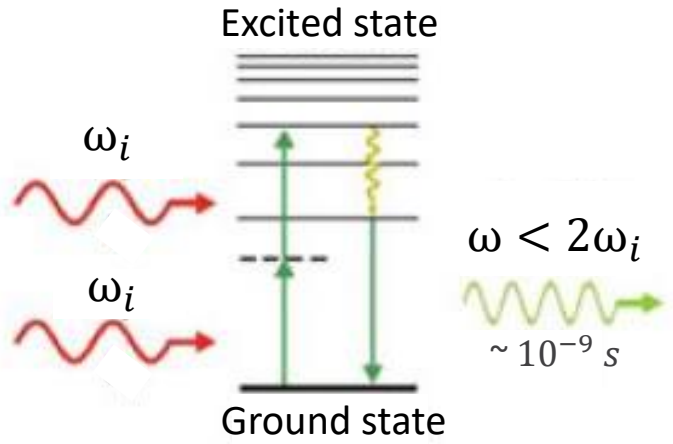


Two-photon microscopy

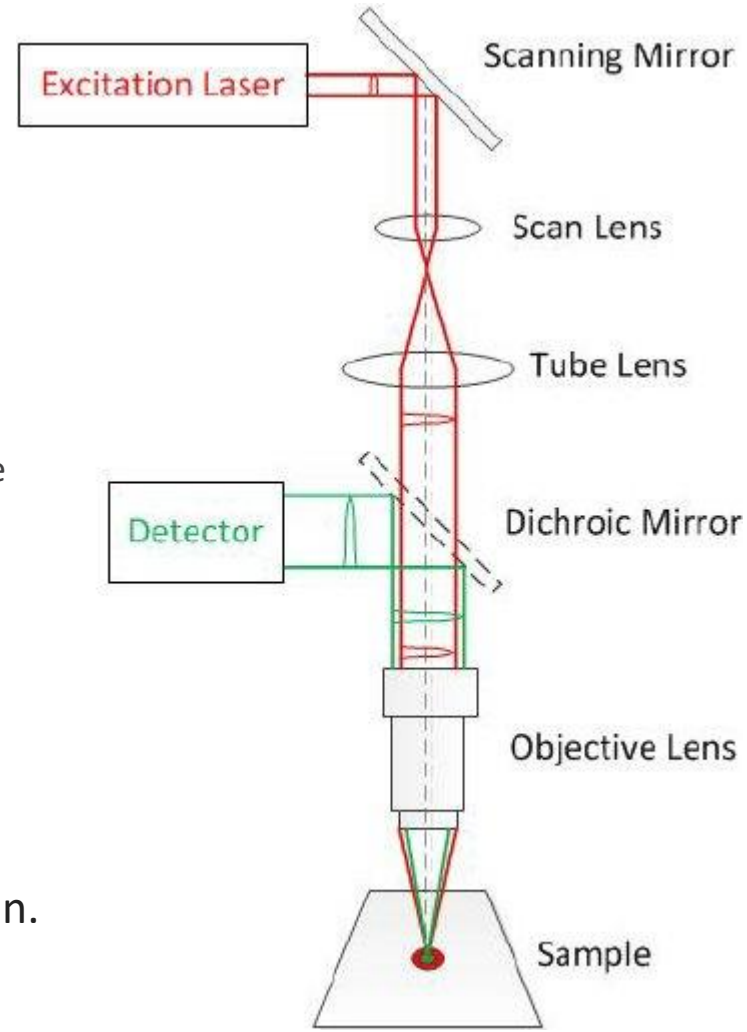


single-photon microscopy
CW laser

two-photon microscopy
fs pulsed laser



two photon meet each other at the focal plane



- ✓ Eliminates secondary fluorescence out of focus signals without using pinhole
- ✓ Deeper view into the tissue with higher precision and better spatial resolution (less auto-fluorescence and photobleach)
- ➢ The DNA stains Hoechst and DAPI were found to display two-photon excitation. These probes can be used for either two-photon imaging of DNA or chromatin.

Multiphoton excitation of the DNA stains DAPI and Hoechst, Ignacy Gryczynski; etc., 0966-9051/96/030138+11$19.50,NOV1996

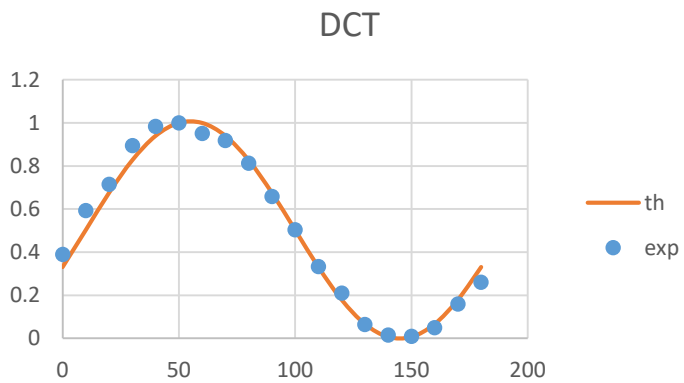
Simulation provides intelligent laser choice for two-photon microscopy-February 2015-Laser Focus World 51(2):34-36

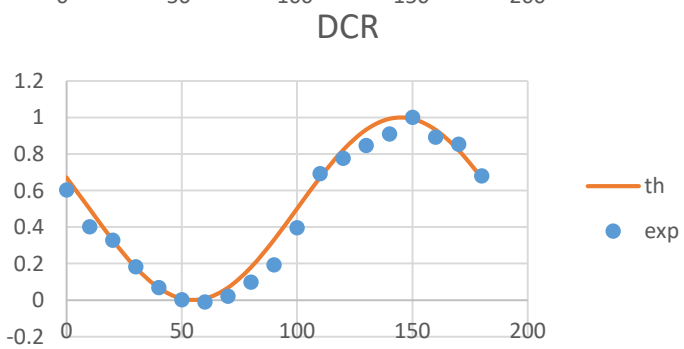
Our CIDS setup:

Characterization of Linear Polarizer and Half Wave-Plate using oscilloscope

LP

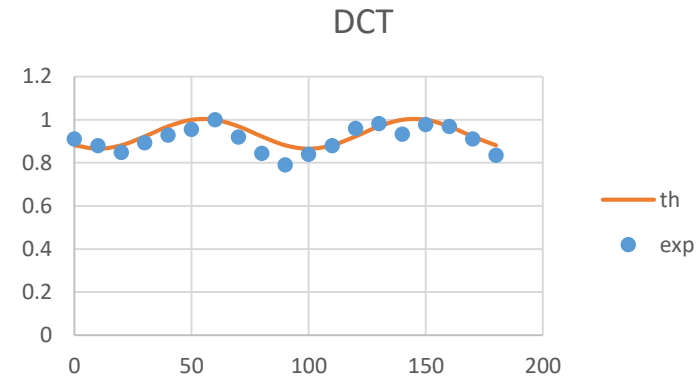
$cos^2(\theta)$

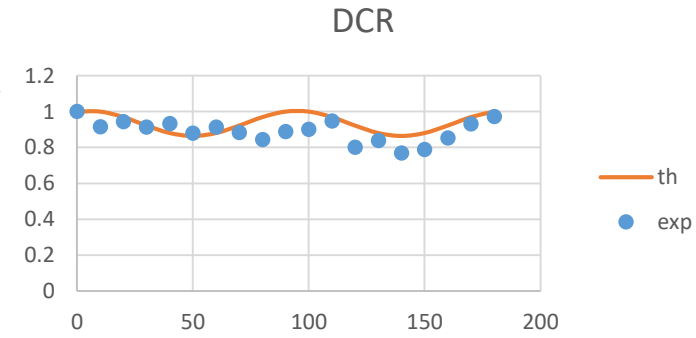




HWP

$A\ cos^2(\theta).\ sin^2(\theta)$ + DC



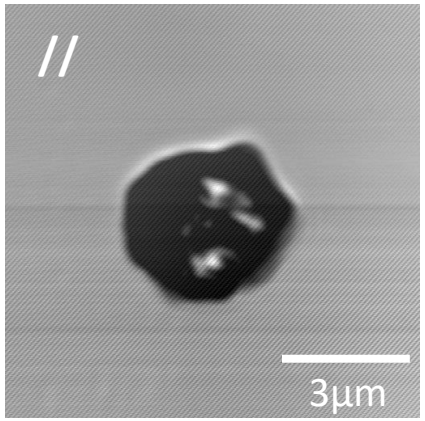


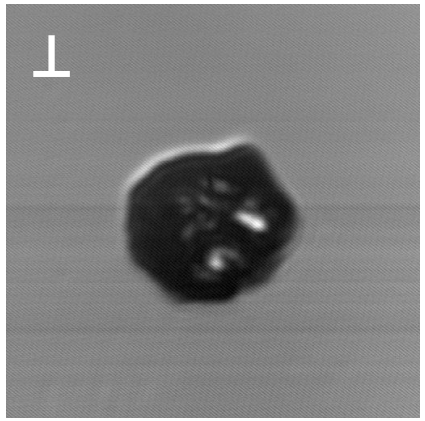
rotation from 0^o to 180^o with the steps of 10 degrees

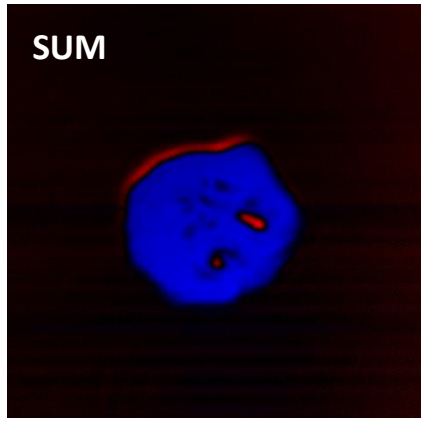
Experimental validation

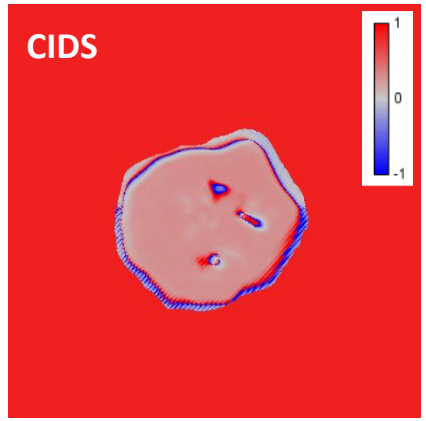
Starch granules

10x zoom
40X/0.6 NA
dry Objective



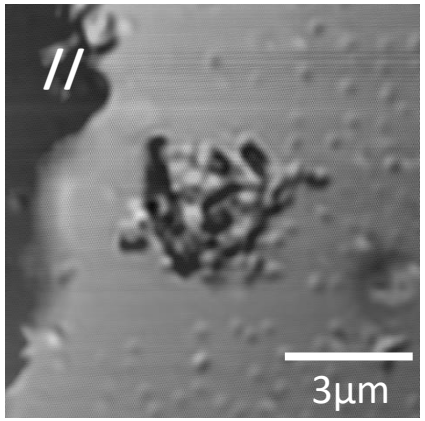


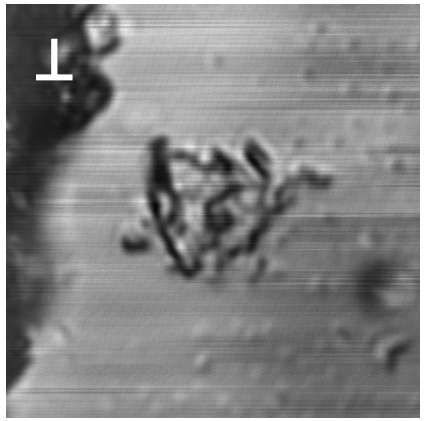


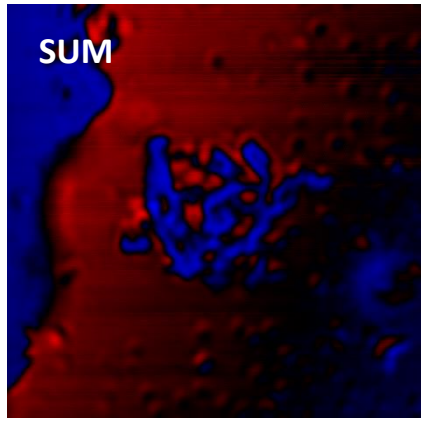


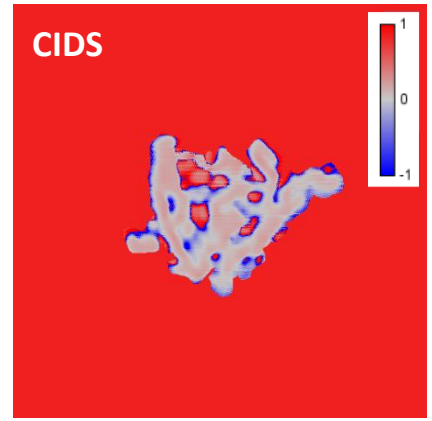
Cellulose

10x zoom
40X/0.6 NA
dry Objective



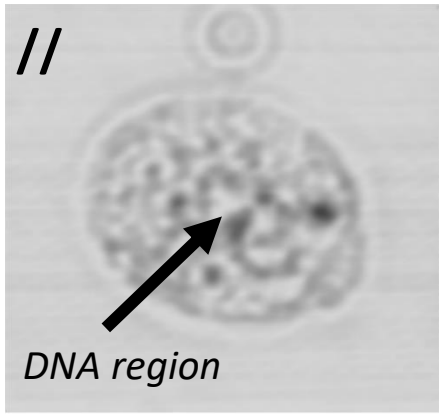


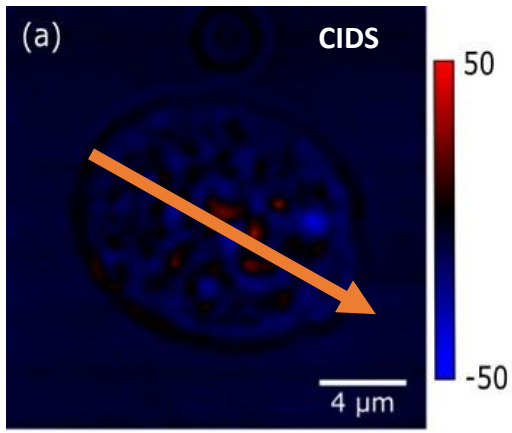


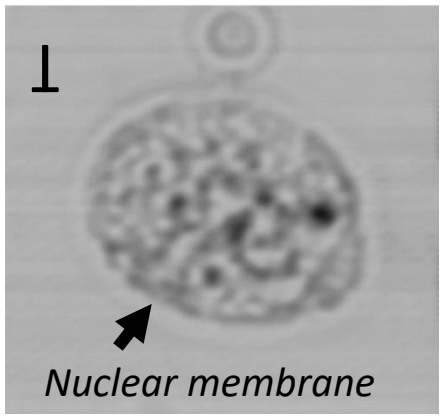


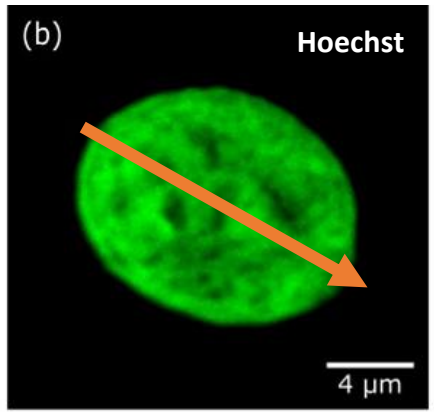
Nuclear organization under polarized illumination

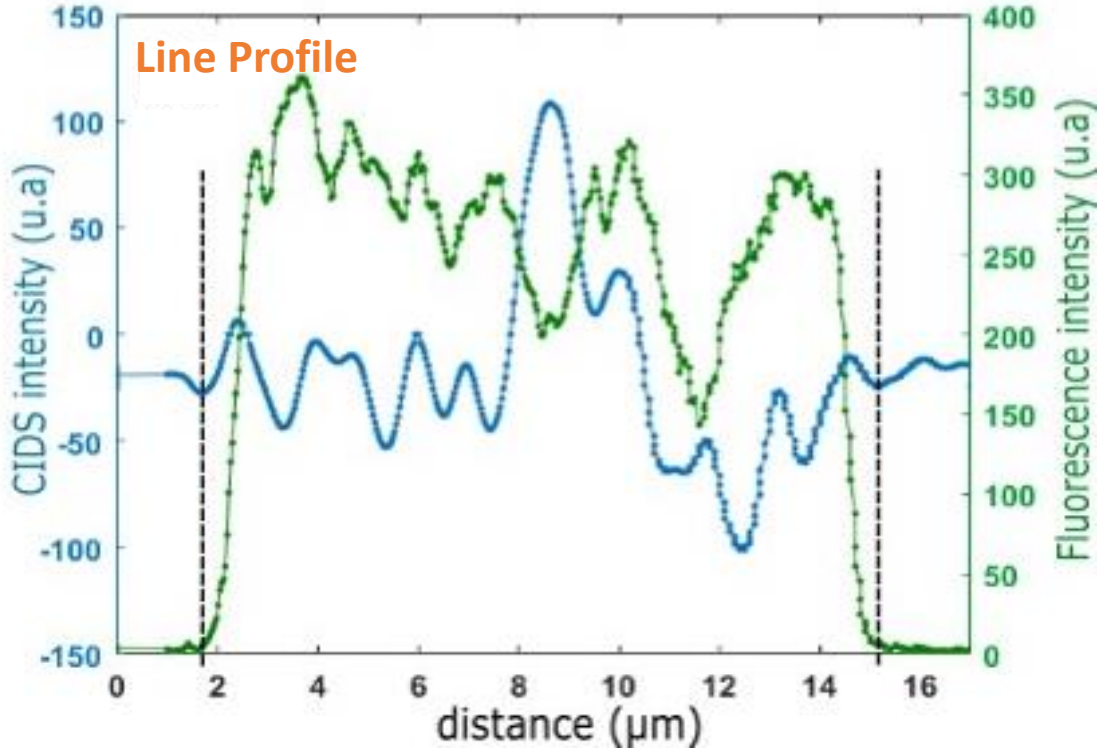
Isolated nuclei (hek cells), 100X/1.4NA oil Objective







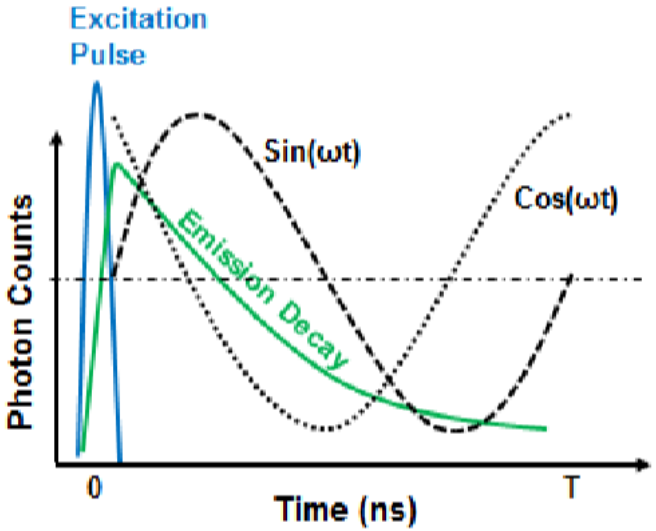


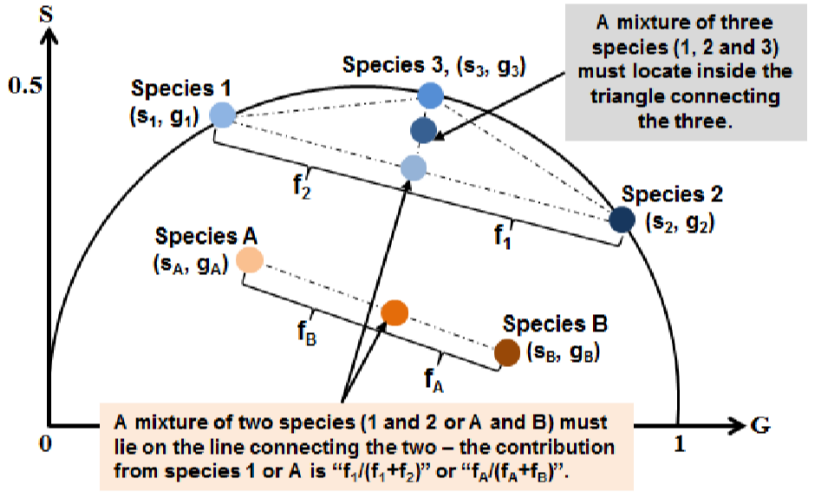


CIDS vs Fluorescence Microscopy

Le Gratiet, et al., OSA Continuum (2018)

The phasor plot analysis for fluorescence data





The phasor linear decomposition.

$$I(t) = I_0\, e^{-t/\tau}$$

$$\tau = \frac{1}{\omega}\left(\frac{s}{g}\right)$$

$$g(\omega) = \frac{\int_0^\infty I(t)\cos(\omega t)}{\int_0^\infty I(t)}$$

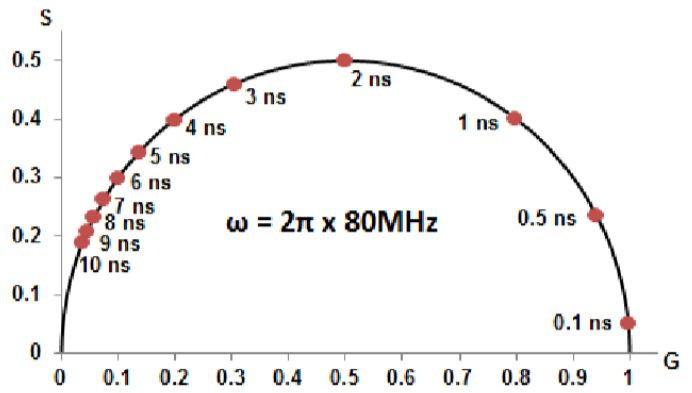
$$s(\omega) = \frac{\int_0^\infty I(t)\sin(\omega t)}{\int_0^\infty I(t)}$$

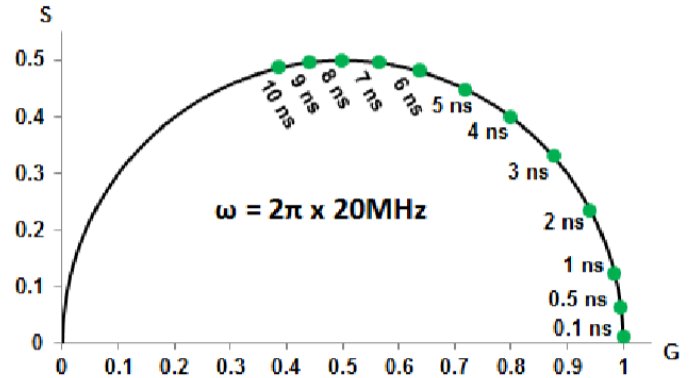
ISS

TECHNICAL NOTE

FLIM Analysis using the Phasor Plots

Shih-Chu Liao, Yuansheng Sun, Ulas Coskun
ISS, Inc.





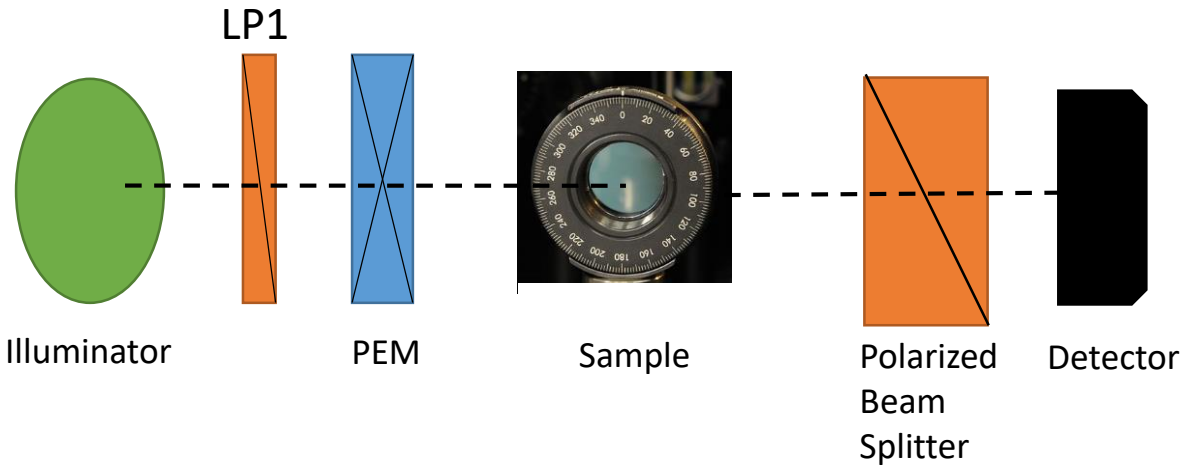
Comparison of the phasor plot scales of two different modulation frequencies.

The phasor plot analysis for CIDS data

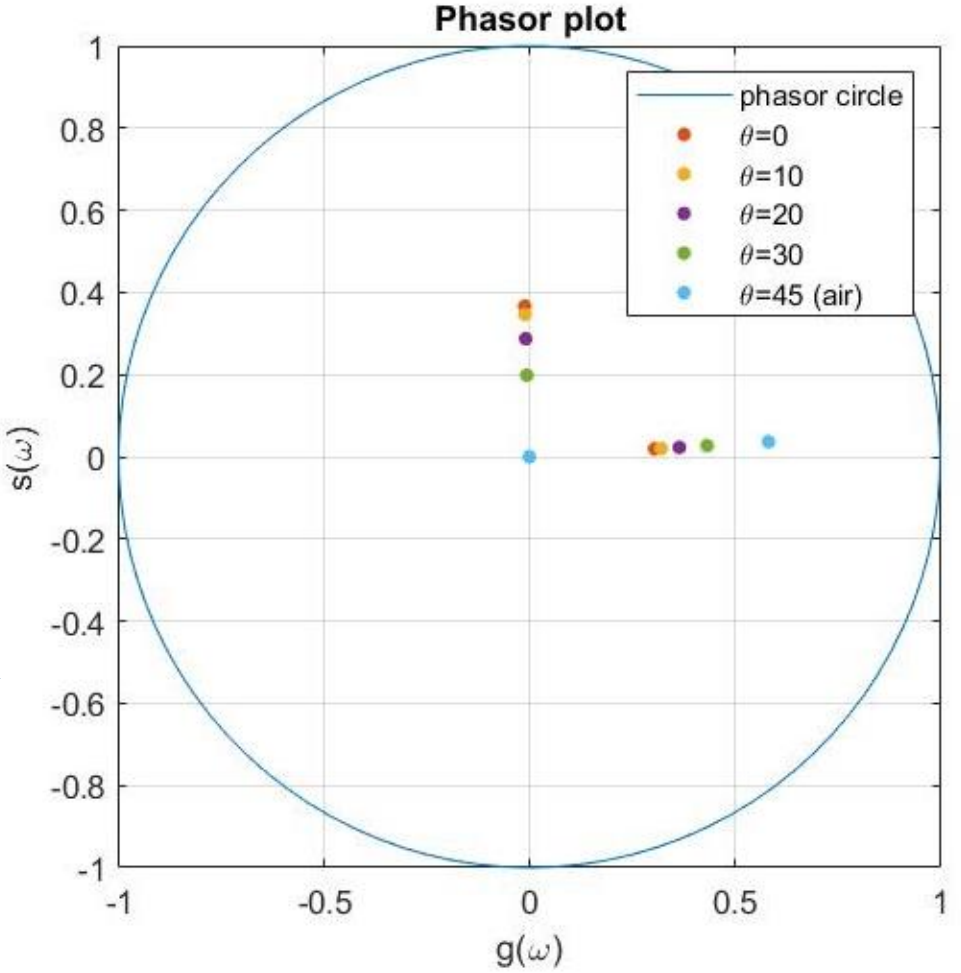
- **Phasor approach** of the simulated output intensity of light in our CIDS setup

$$I(t) = I_{DC} + I_{\omega} \cdot \cos(\omega t)$$

  - **Example:** Quarter Wave-Plate simulation



FT



- The points along vertical axes are corresponding to ω and the horizontal ones are linked to 2ω

Conclusion

- Higher spatial resolution and quantification of highly packed chiral molecules
- Time resolved microscopy based on ultra-short laser pulses
- Molecular view of the sample using Phasor approach
- Fast interpretation of huge image data in terms of Birefringence, Polarizability, etc..
- Platform for Multimodal Microscopy Imaging
- Multi Messenger Microscopy to unraveling dark side of knowledge on sample

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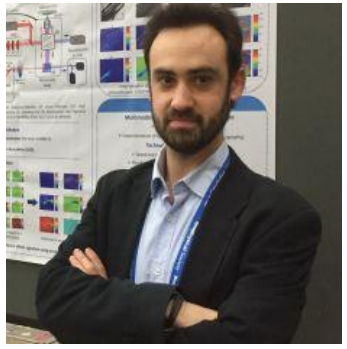
Prof. A. Diaspro

Dr. P. Bianchini

Thank you for your attention

Dr. A. Le Gratiet



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