

DPLSM: Imaging anisotropy in biological samples

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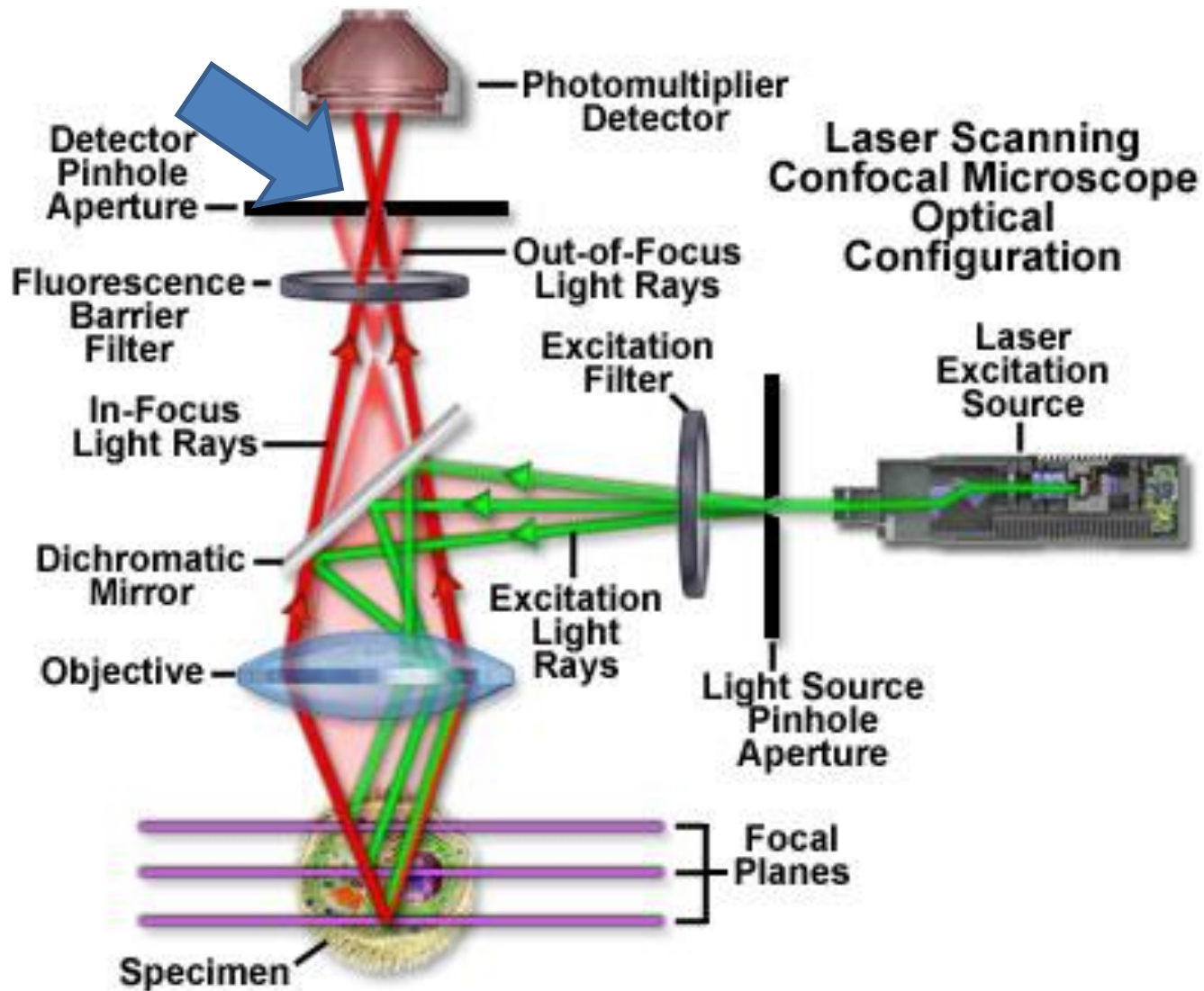
20.07.2017

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DPLSM: Imaging anisotropy in biological samples

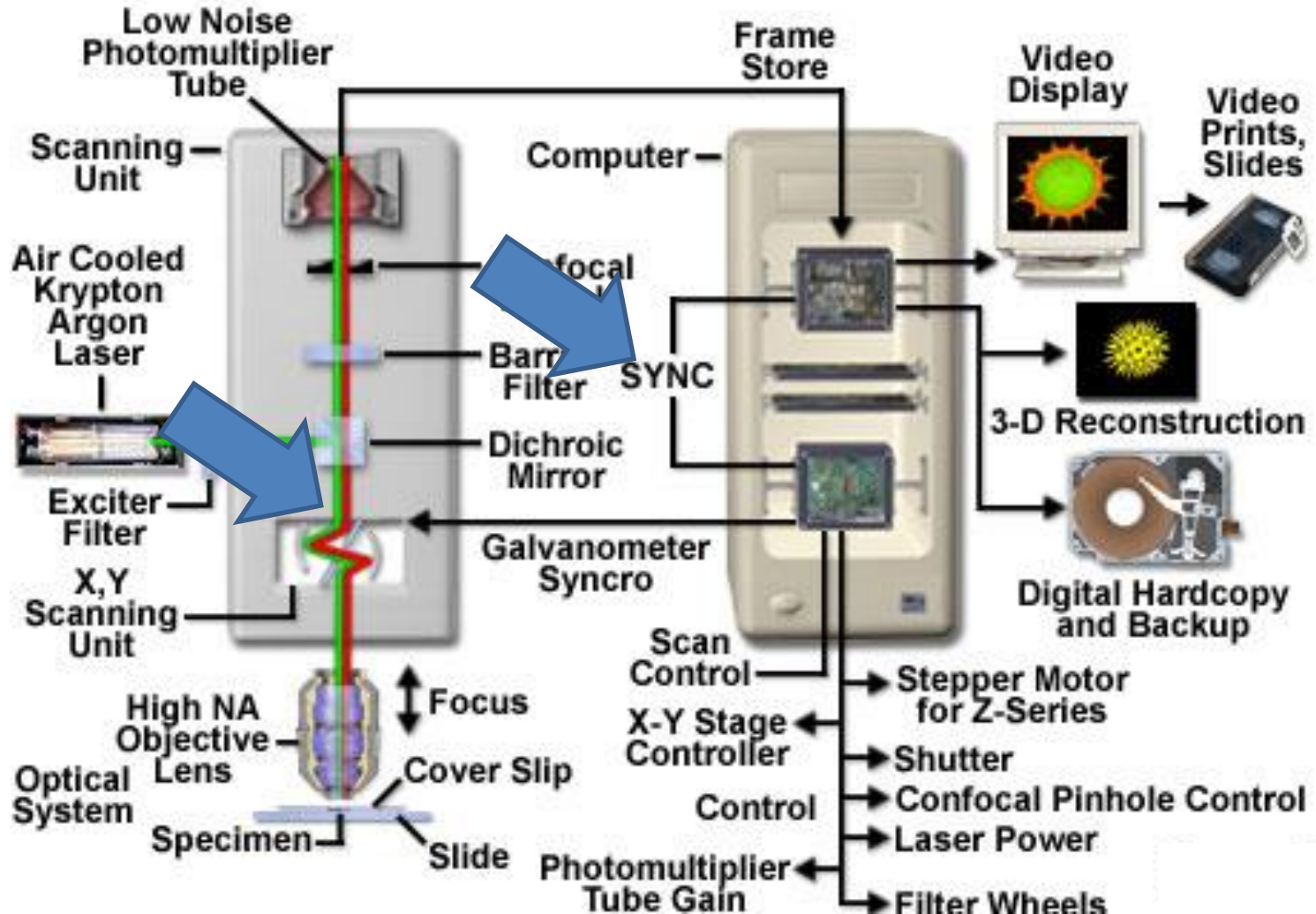
- Based on CLSM
- Using the advantages of laser excitation
- Provides additional parameters due to the pixel-by-pixel imaging

Confocal microscopy



Confocal microscopy

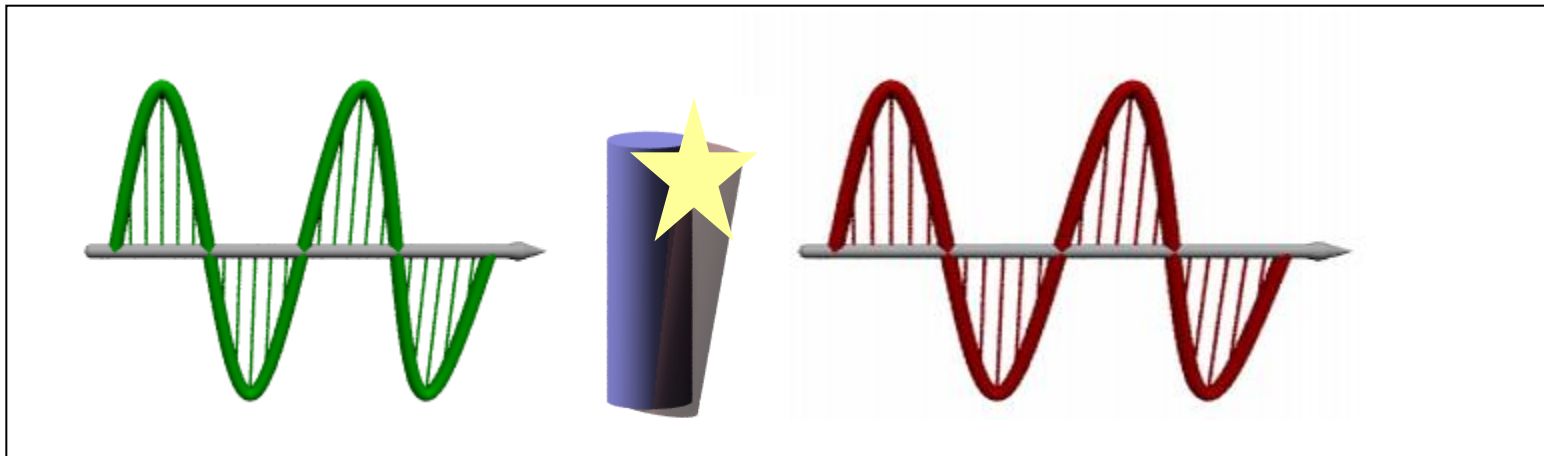
Confocal Microscopy Information Flow Schematic Diagram



Interactions with Polarized Light

Linearly polarized light:

Transition dipoles will interact with the linearly polarized light



... it provides new parameters, independent from the absorbance

DP values

CONFOCAL

	Modulated polarization of the laser beam:	Analysis of the polarized fluorescence emission:
lin. pol.	[Fluorescence detected] linear dichroism (LD and FDLD), Optical phase shift (LB)	Anisotropy (r) and Degree of polarization of the fluorescence (P)
cir. pol.	[Fluorescence detected] circular dichroism (CD and FD CD)	Circularly polarised luminescence (CPL)

DP values

$$P = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}} \quad r = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + 2I_{\perp}} \quad CPL = \frac{I_{right} - I_{left}}{I_{right} + I_{left}}$$

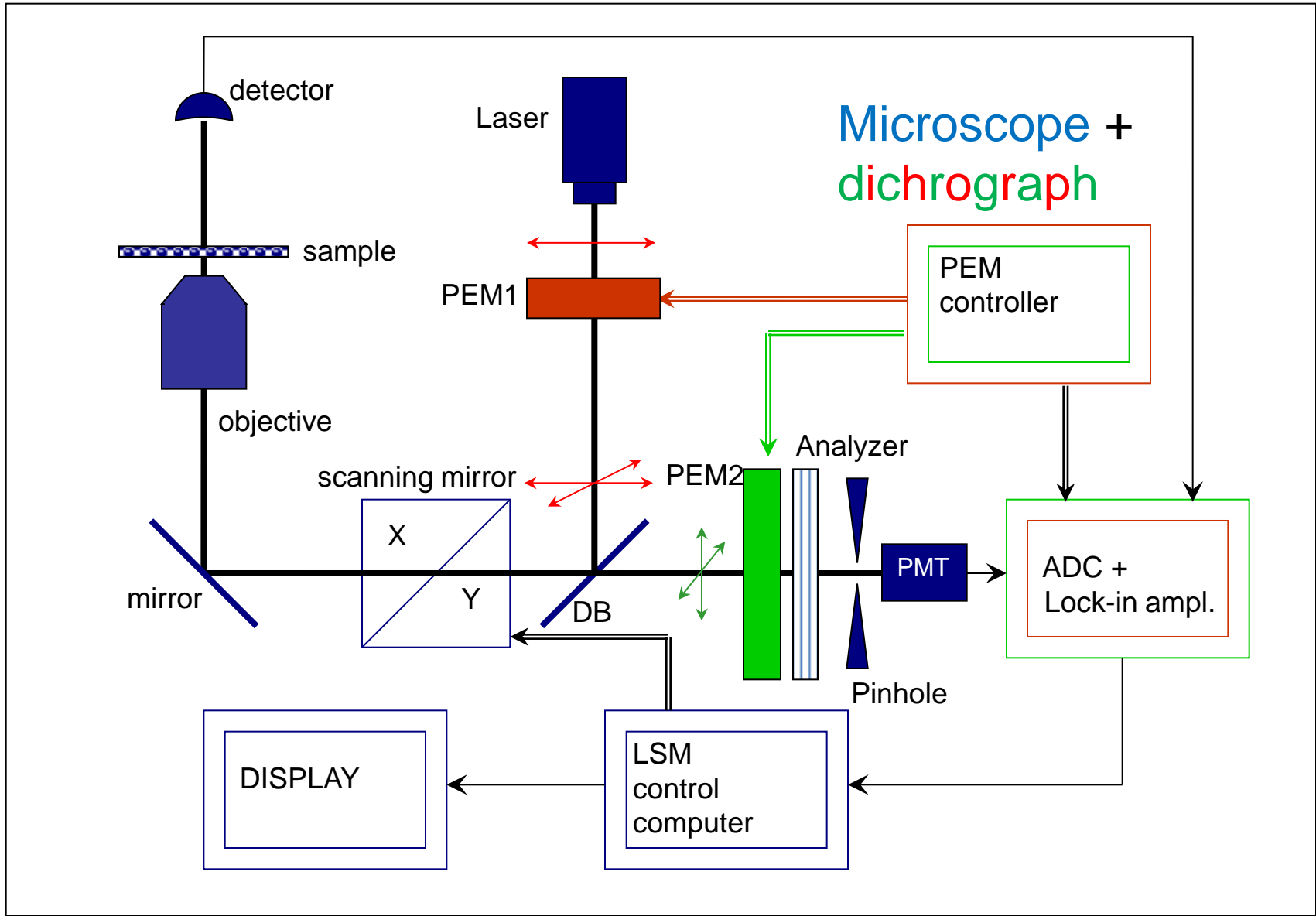
$$LD = A_{\parallel} - A_{\perp} = -\frac{1}{\ln(10)} \frac{I_{\parallel} - I_{\perp}}{I_0} \quad FDL D = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}}$$

$$CD = A_{left} - A_{right} = -\frac{1}{\ln(10)} \frac{I_{left} - I_{right}}{I_0} \quad FD CD = \frac{I_{left} - I_{right}}{I_{left} + I_{right}}$$

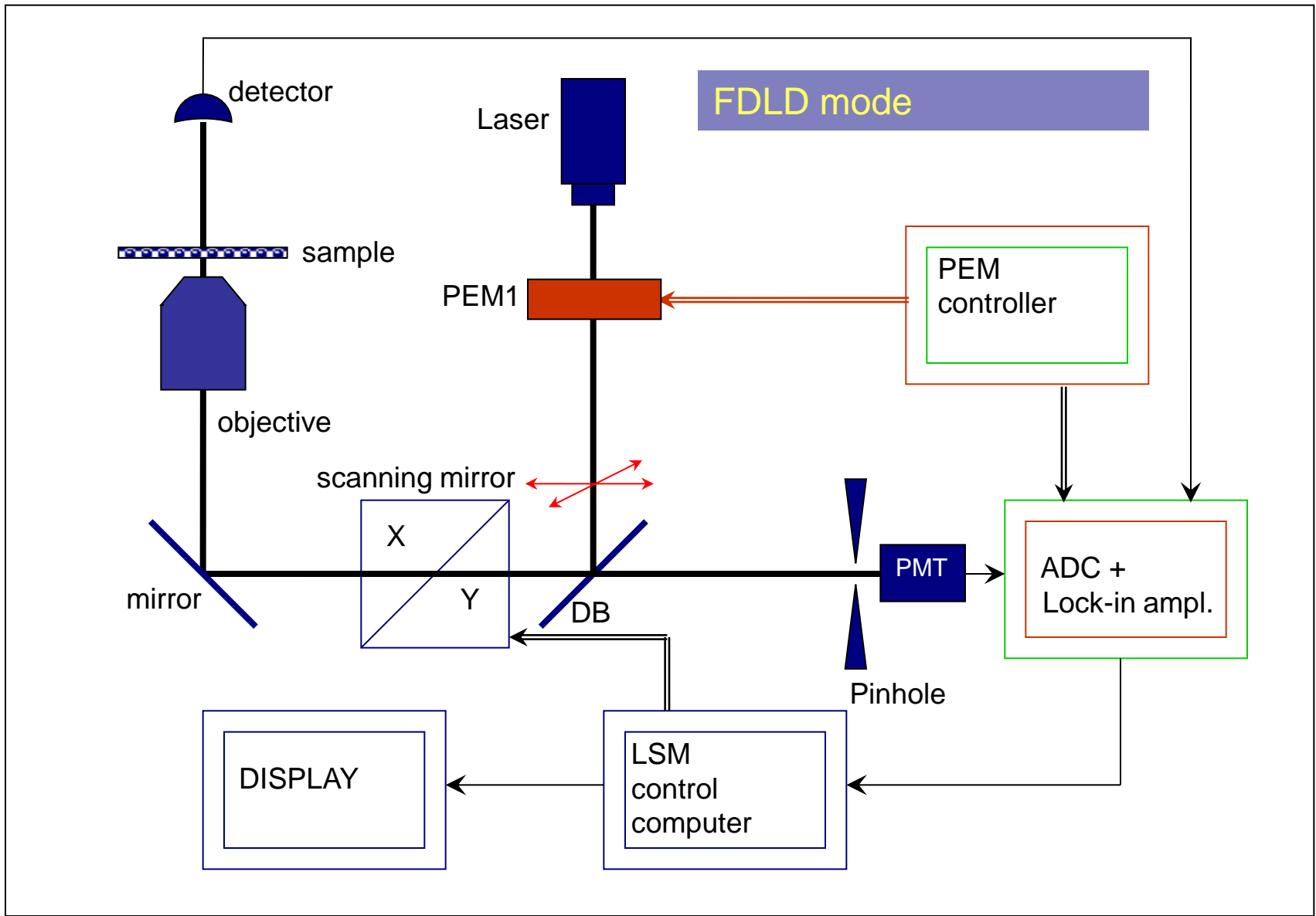
$$DP = \frac{I_1 - I_2}{\bar{I}}$$

DPLSM attachment

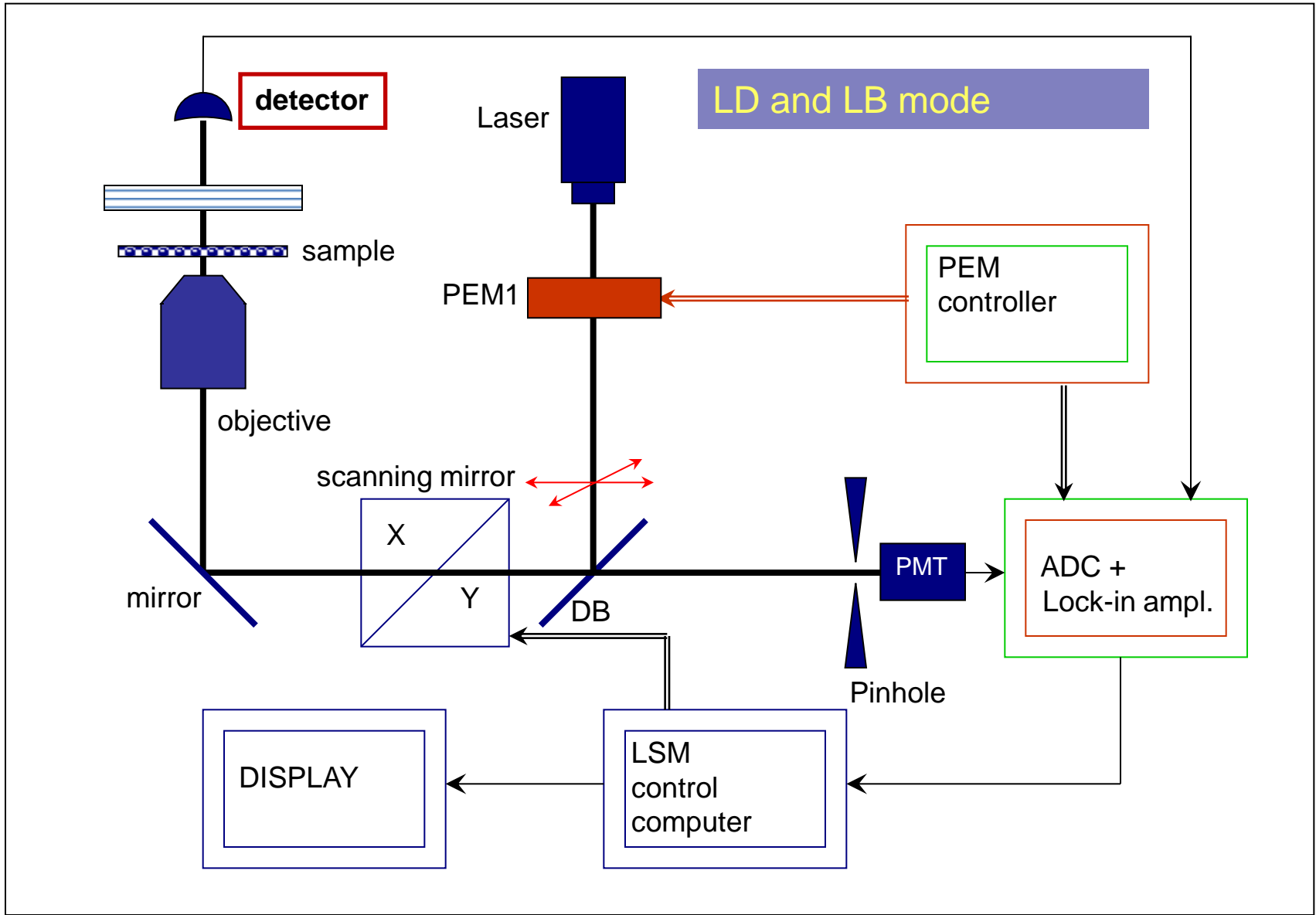
Scheme of DP-LSM (LSM410)



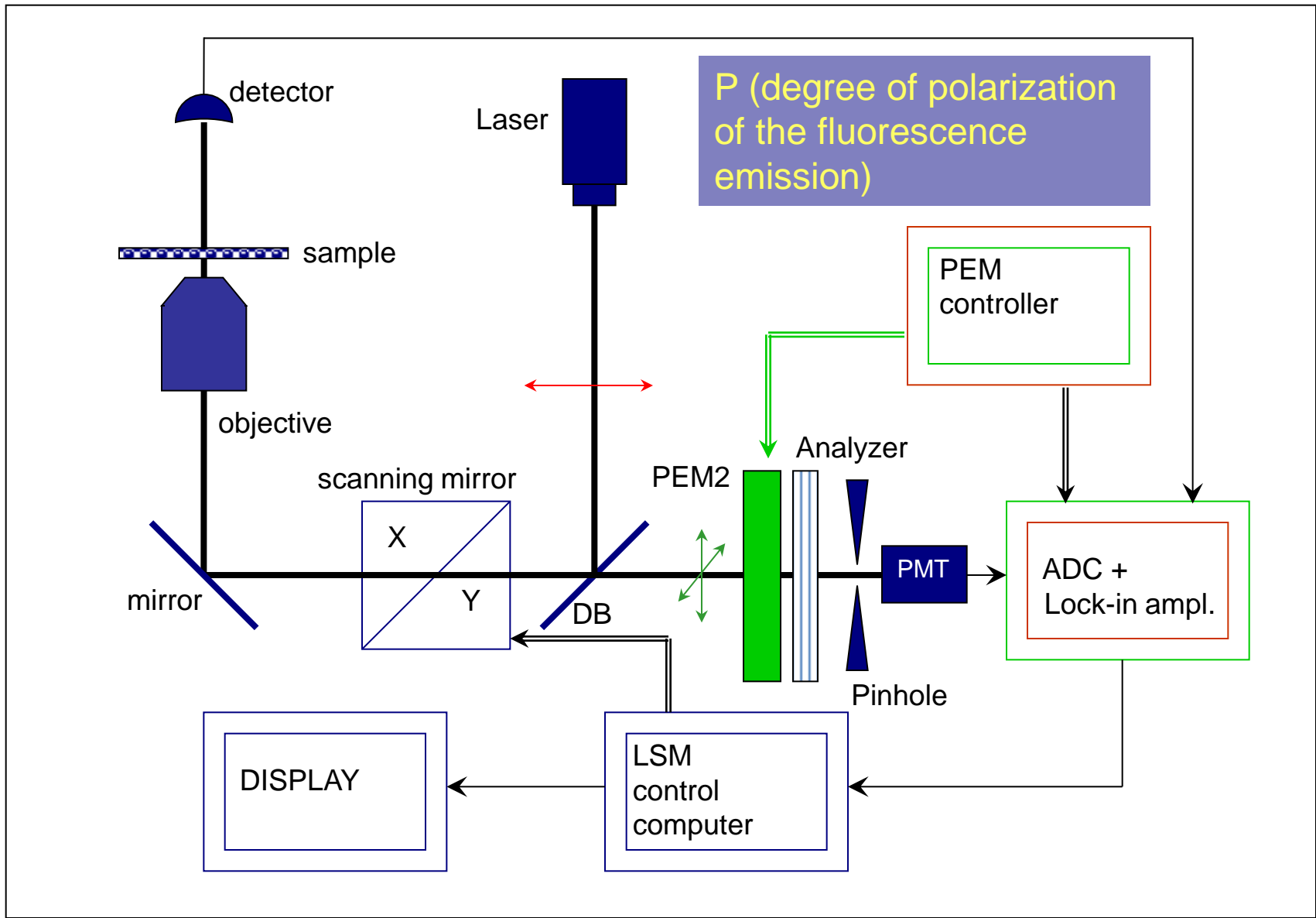
Scheme of DP-LSM (LSM410)



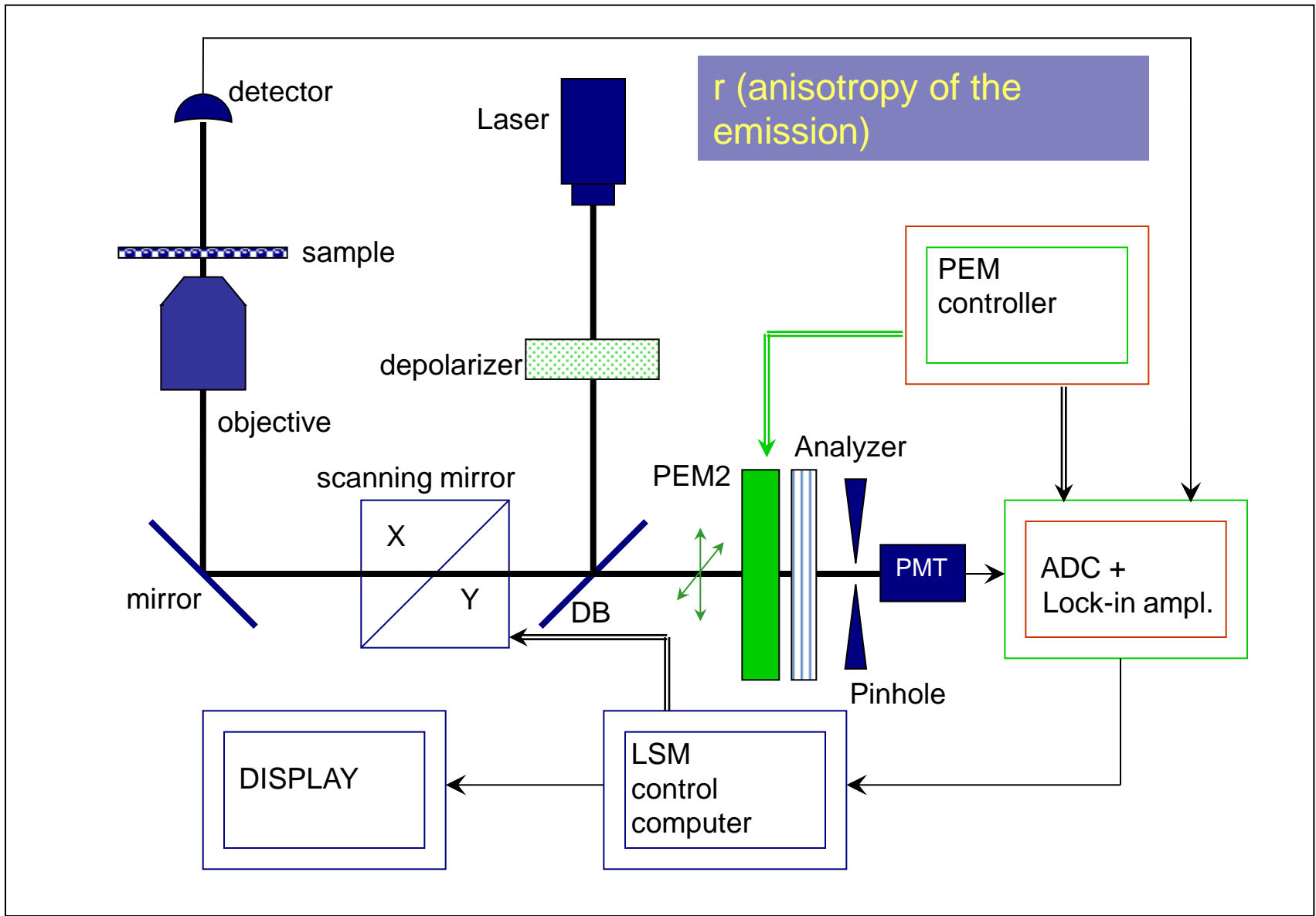
Scheme of DP-LSM (LSM410)



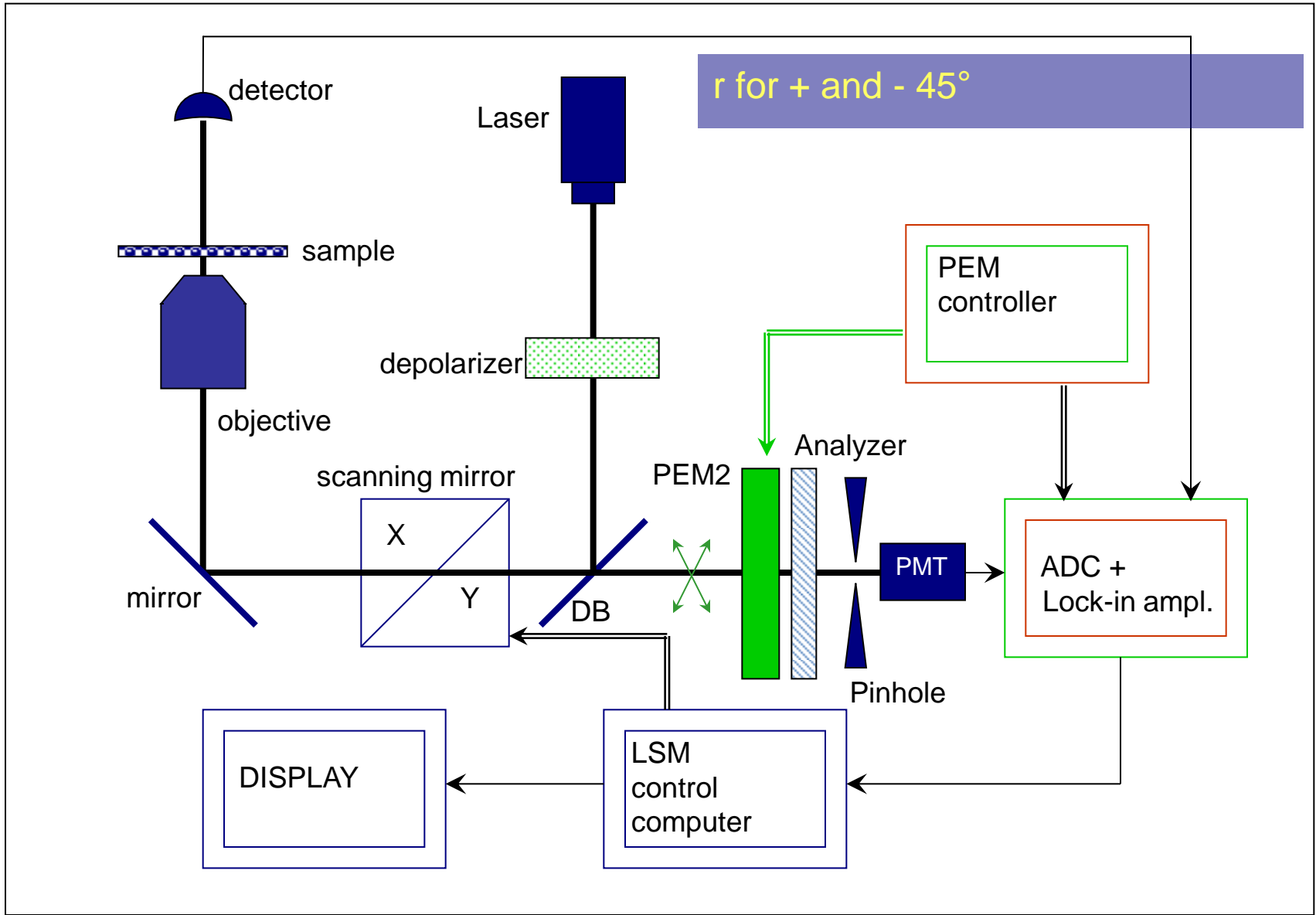
Scheme of DP-LSM (LSM410)



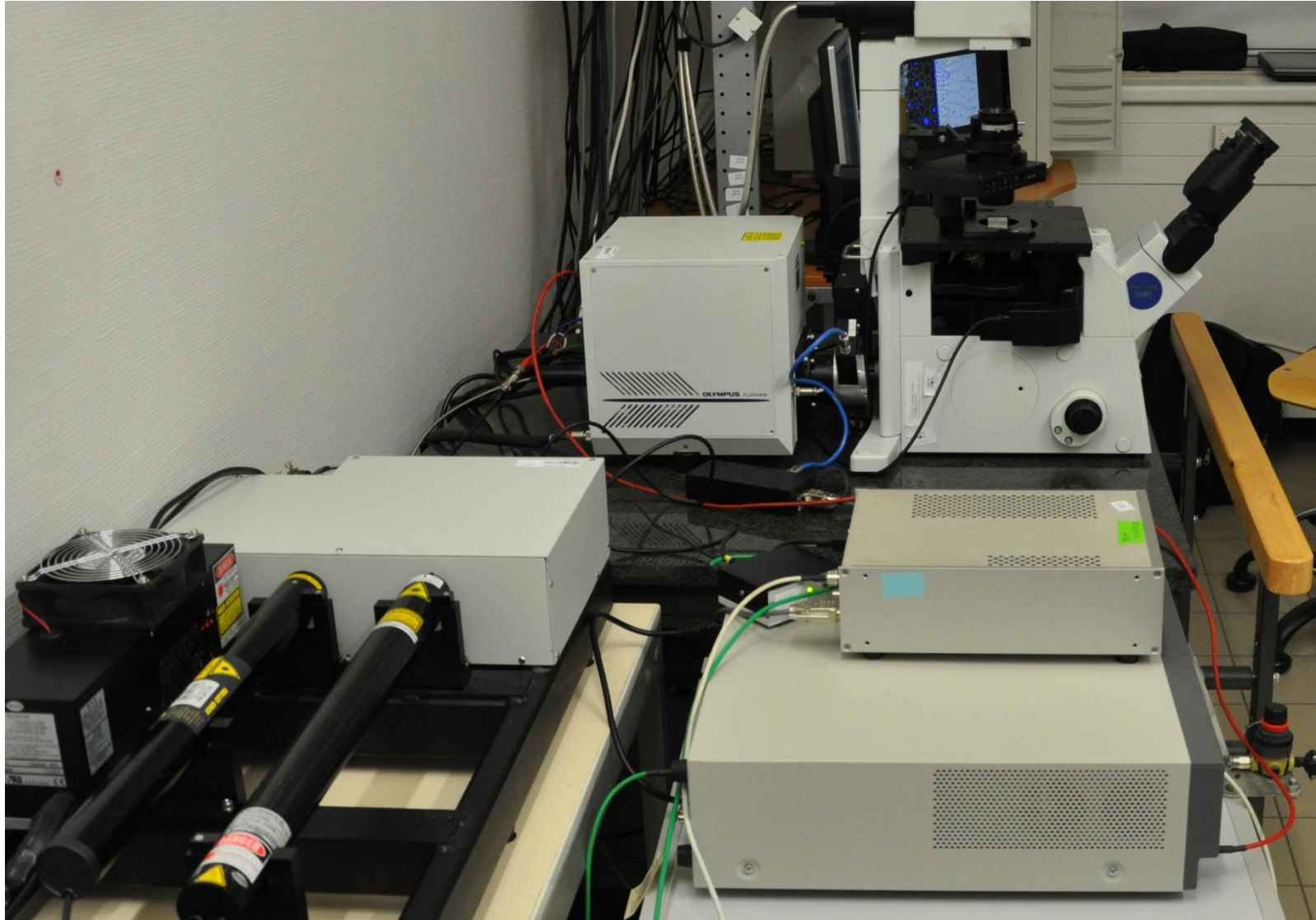
Scheme of DP-LSM (LSM410)

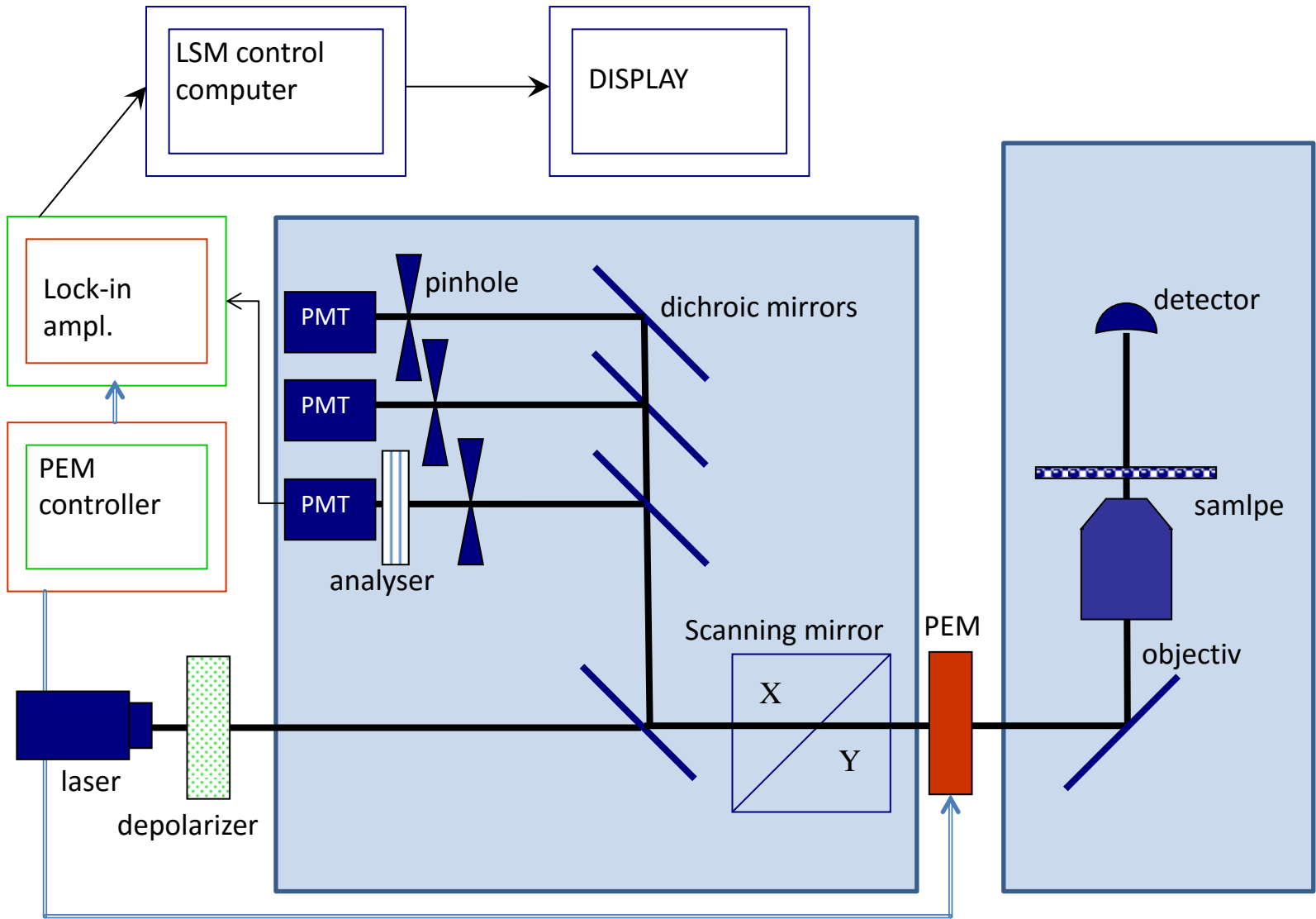


Scheme of DP-LSM (LSM410)



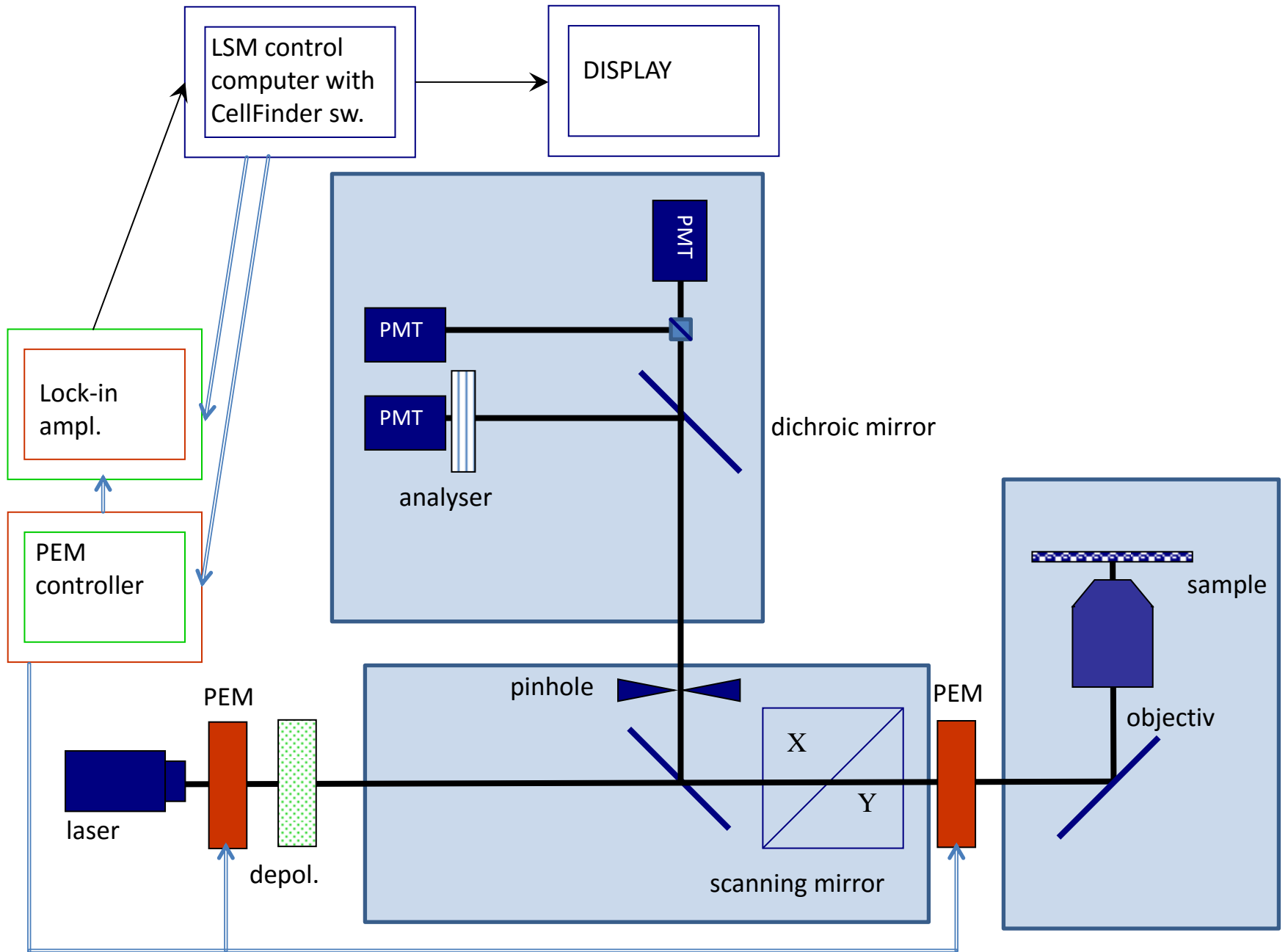
Scheme of DP-LSM (FV500)



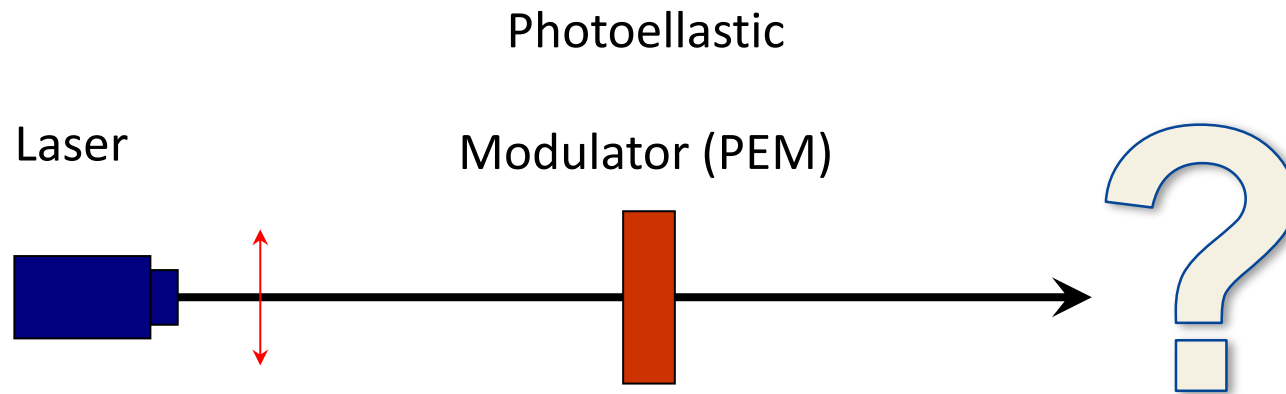


Scheme of DP-LSM (C1)



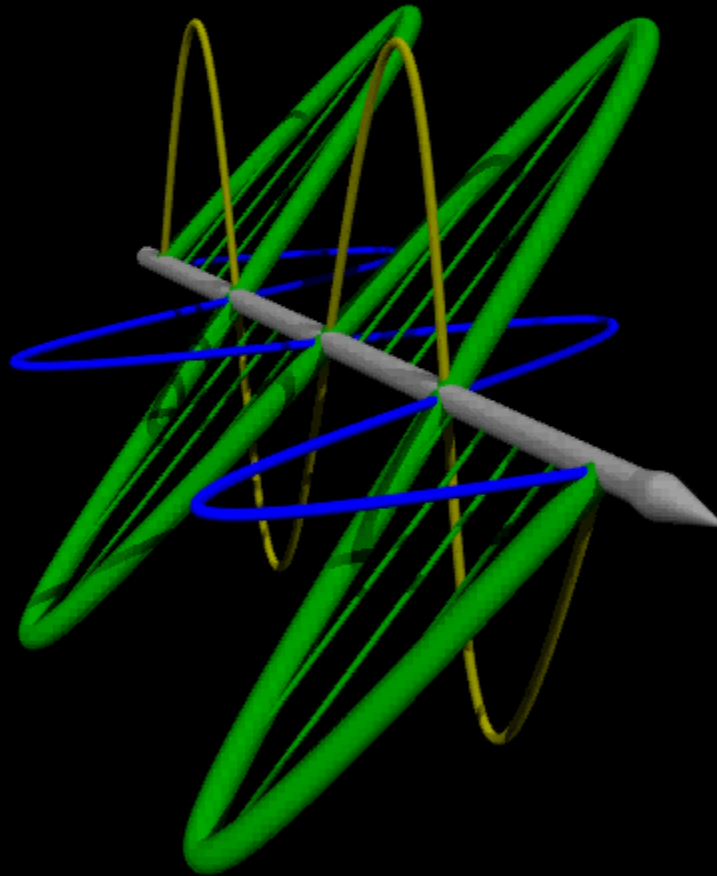


Modulation



Polarized light

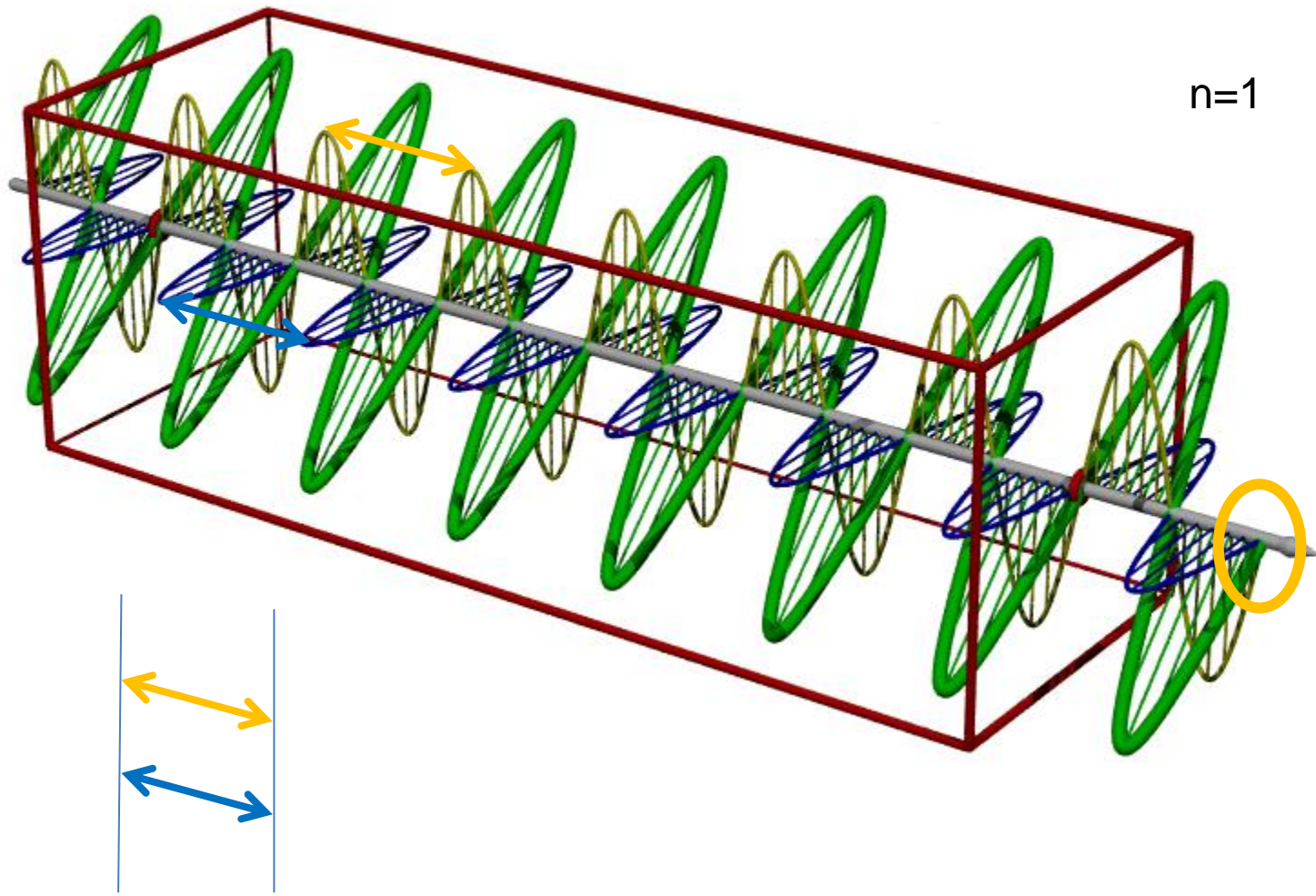
Linear polarization



Phase shift

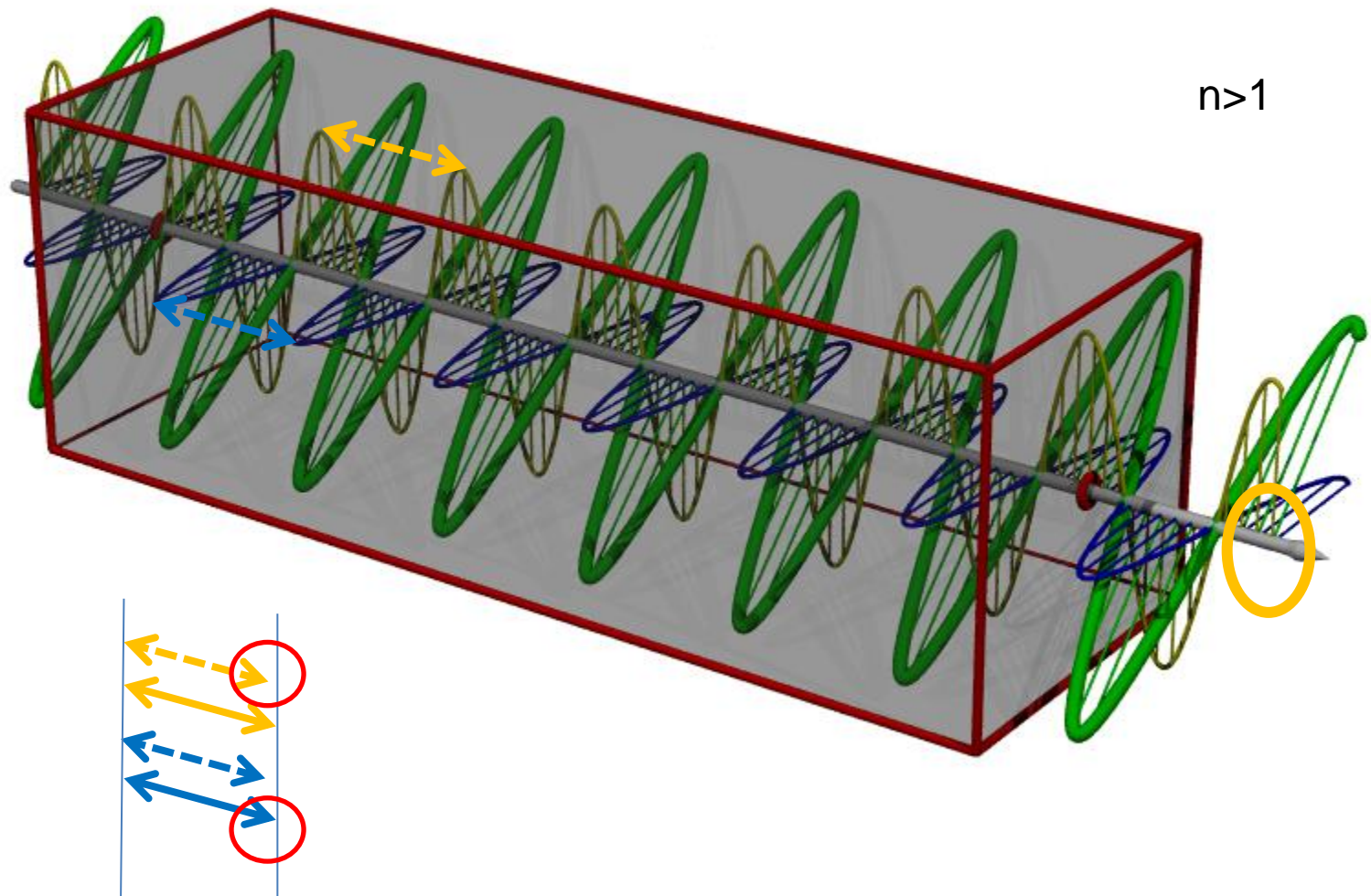
Air

$n=1$



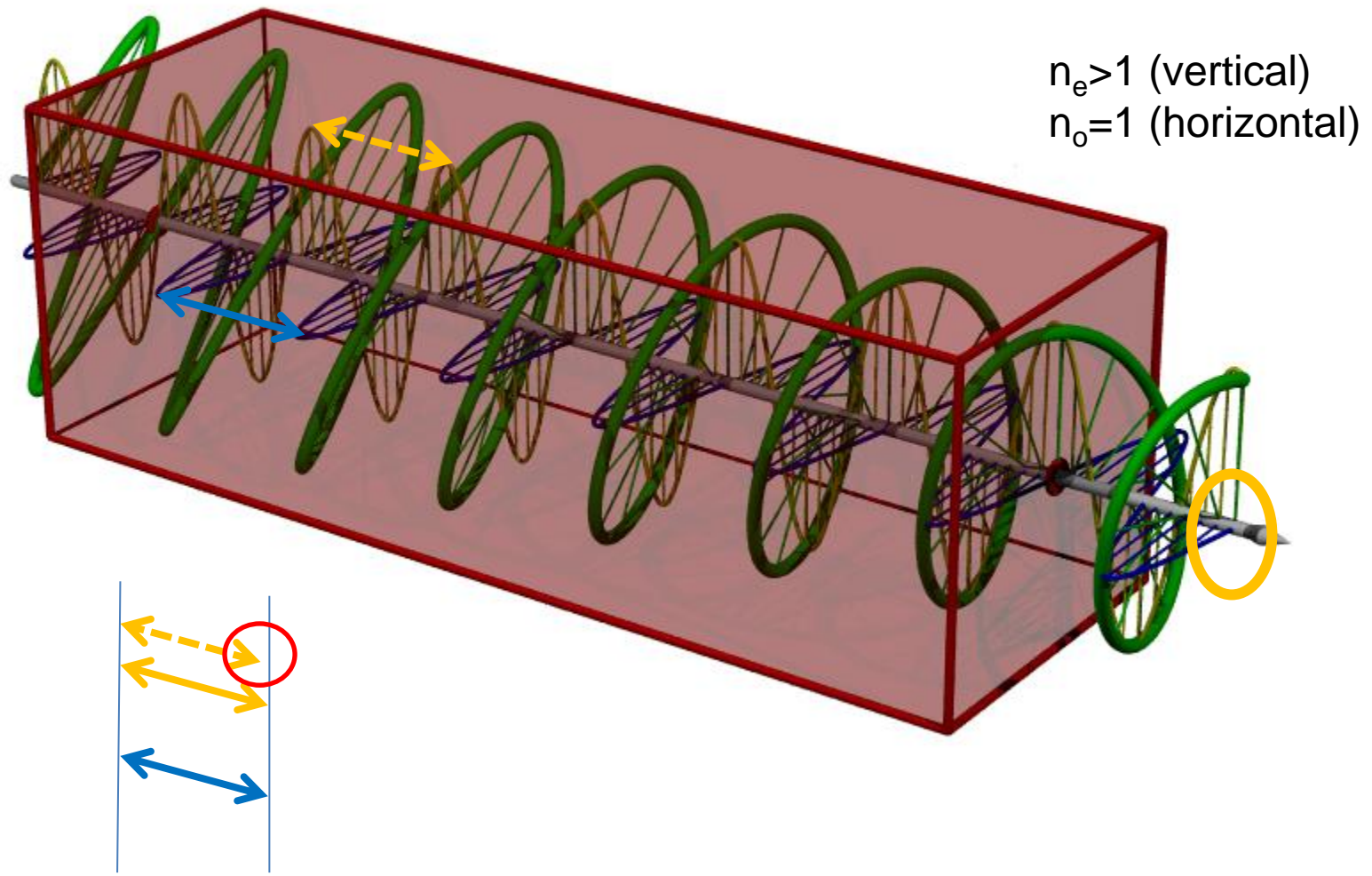
Phase shift

Glass

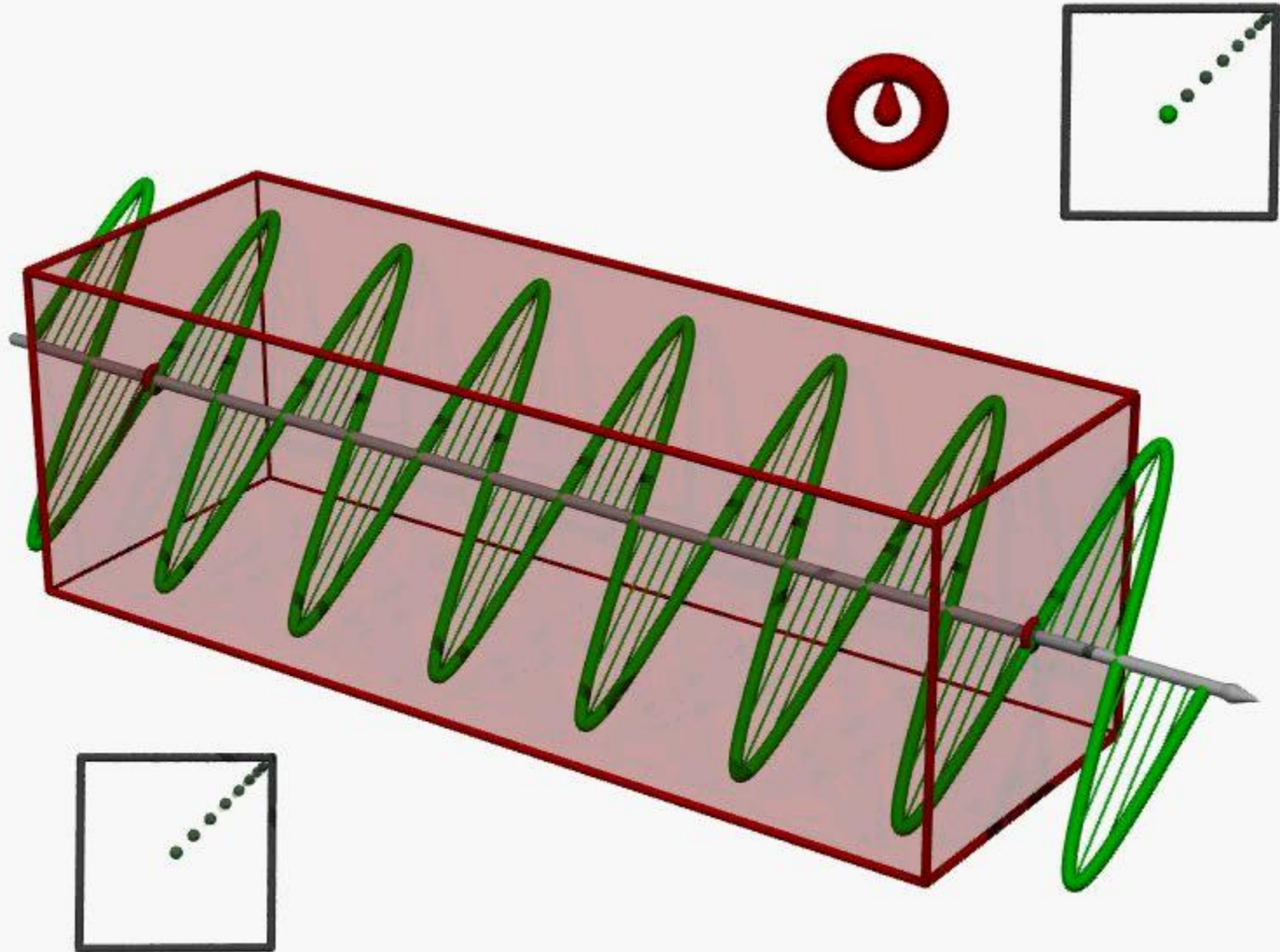


Phase shift

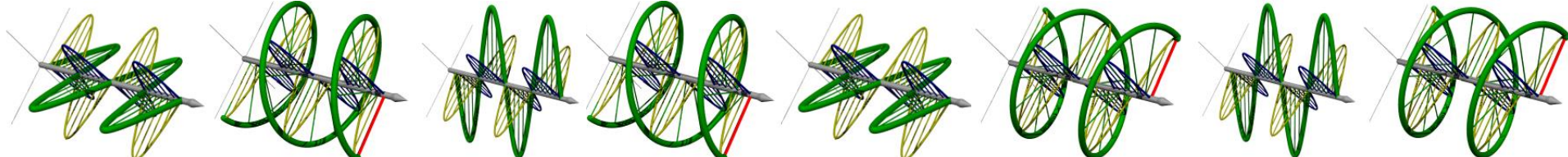
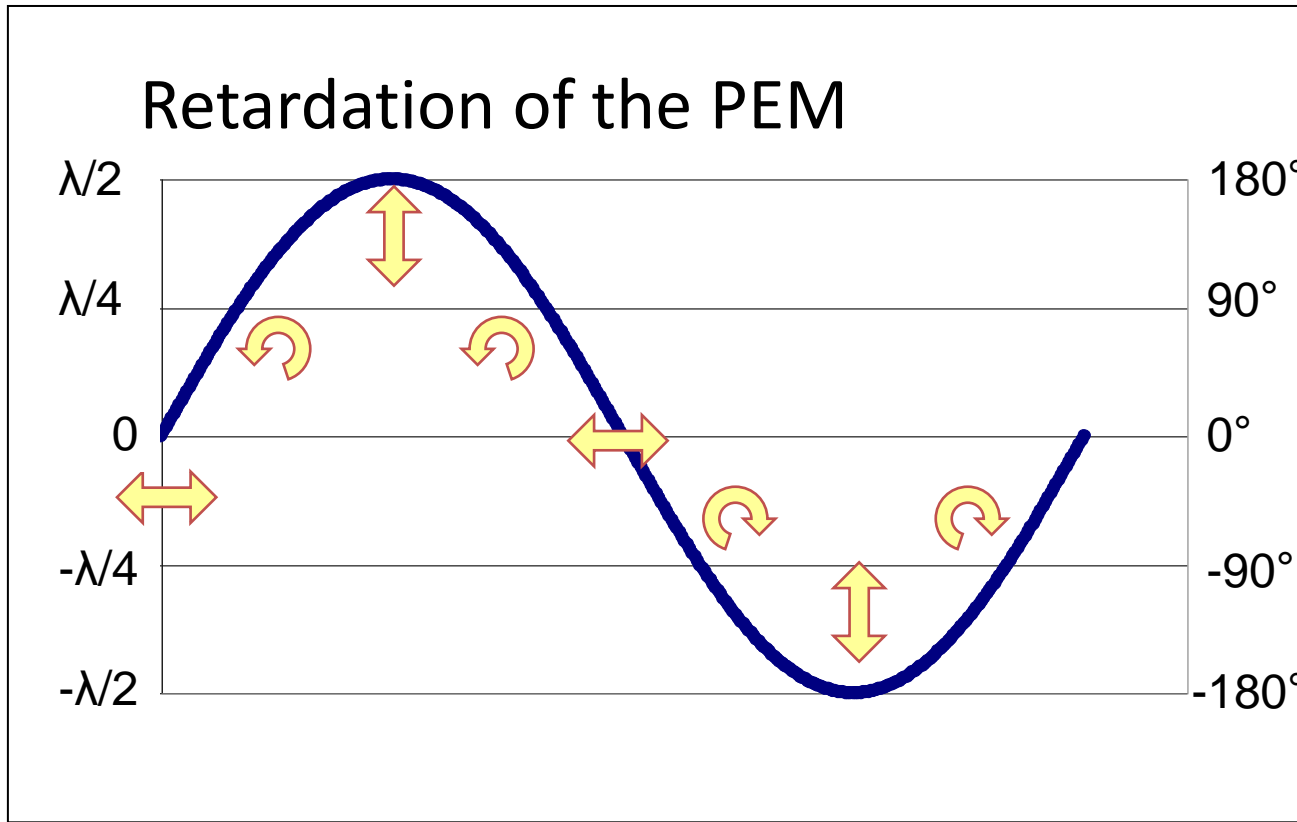
Birefringent crystal



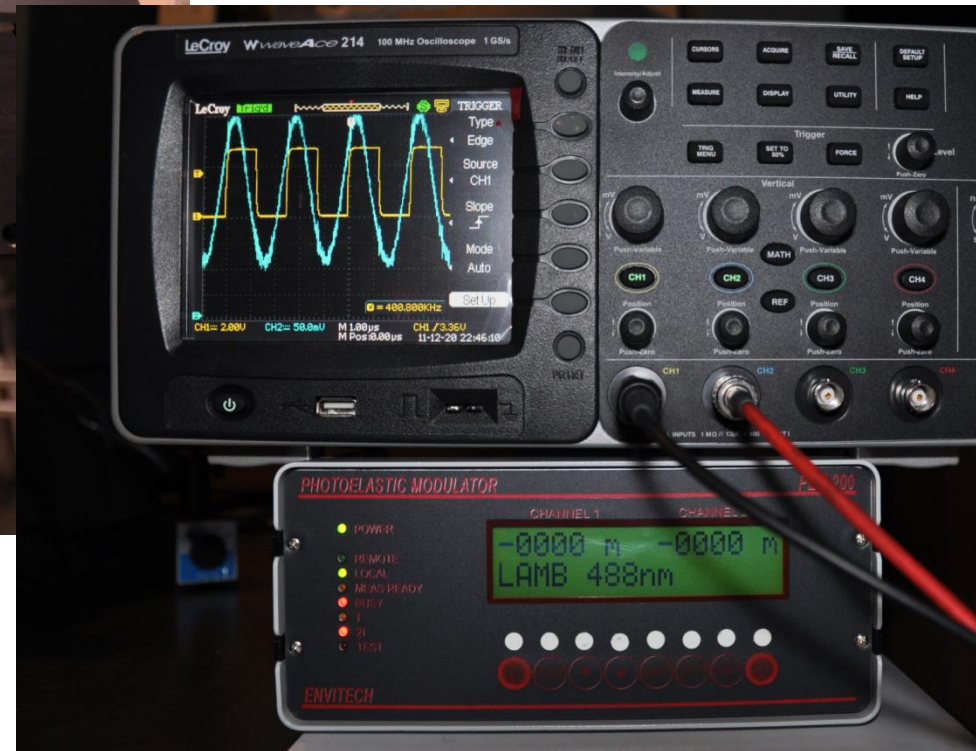
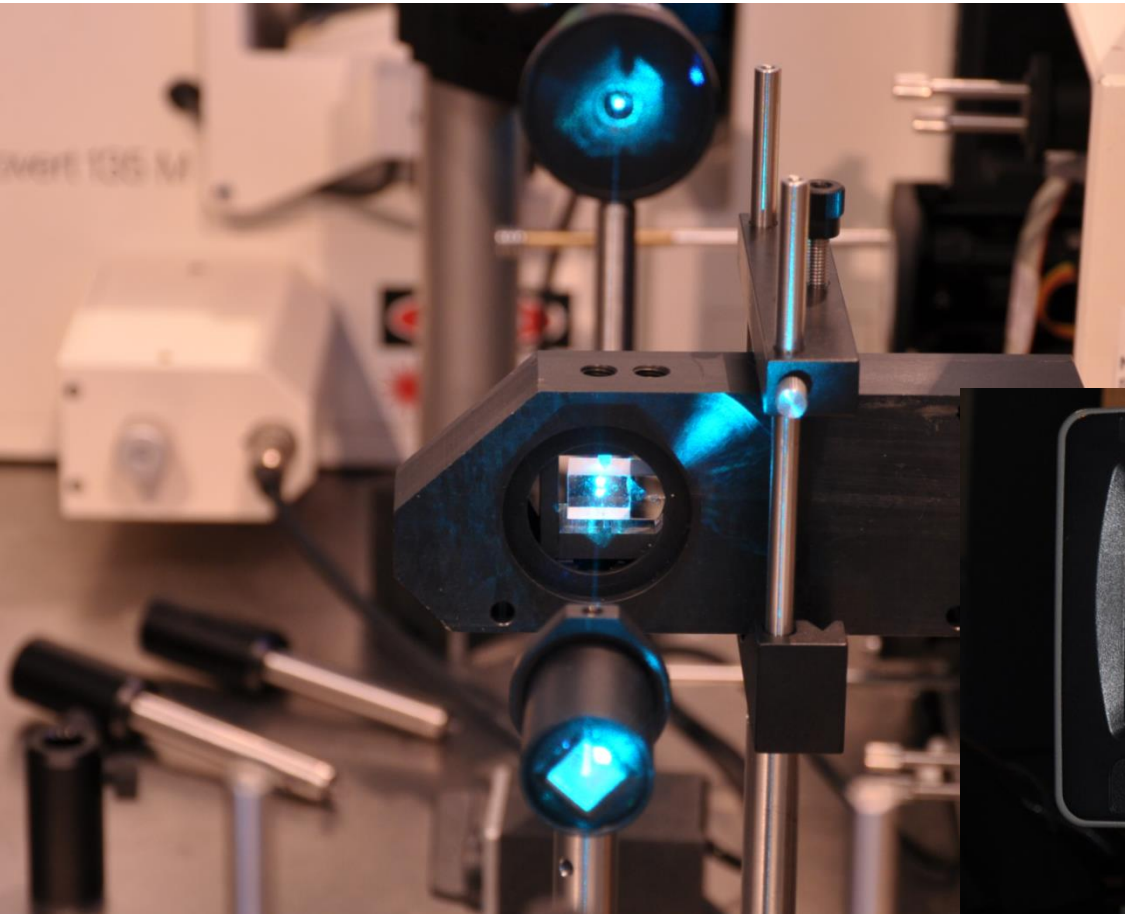
Phase shift



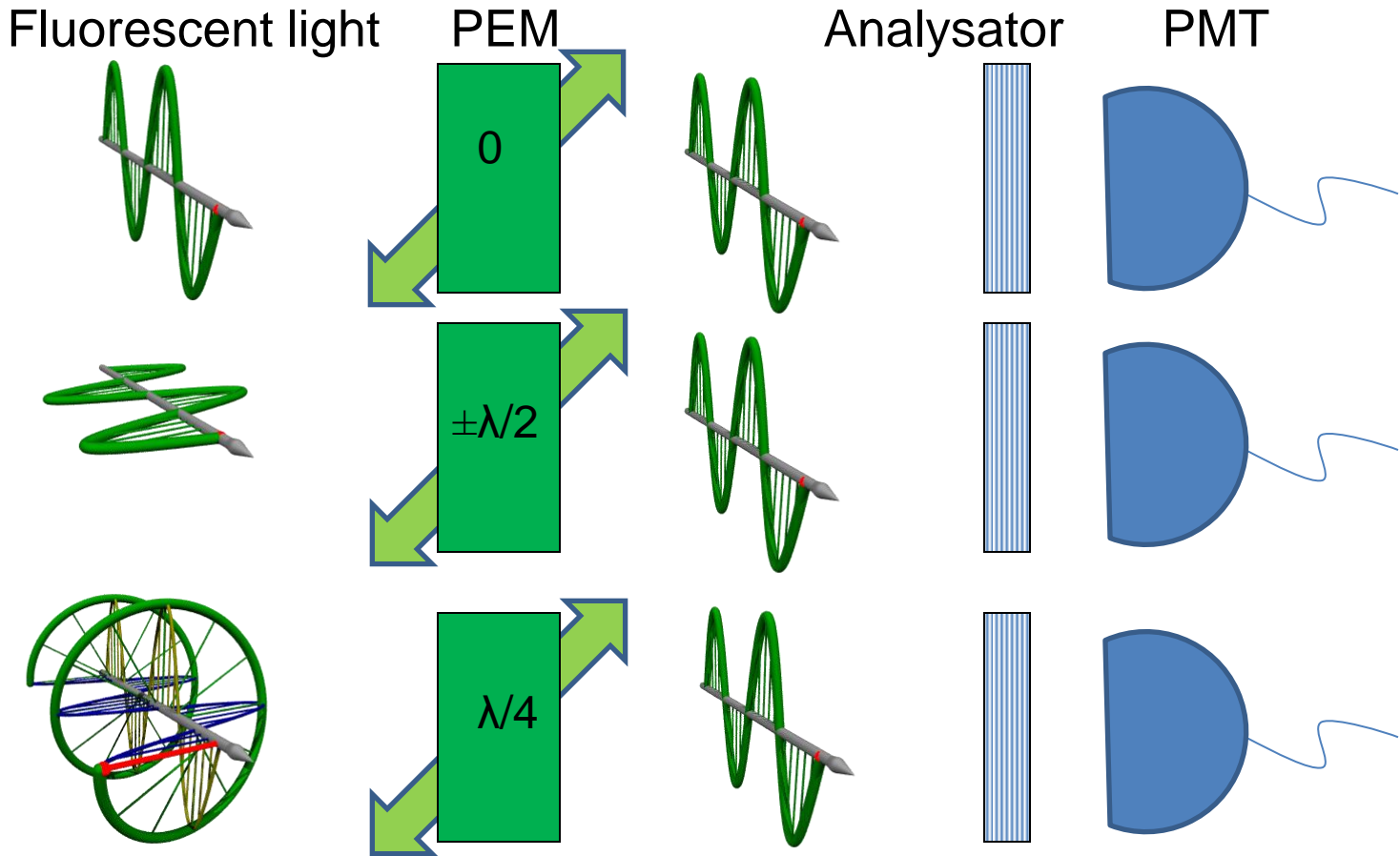
Modulation



PEM in use



Analyse the fluorescence



FDLD rotation

Vertical (yellow):

$$0.27 \pm 0.06^1$$

Diagonal (gray):

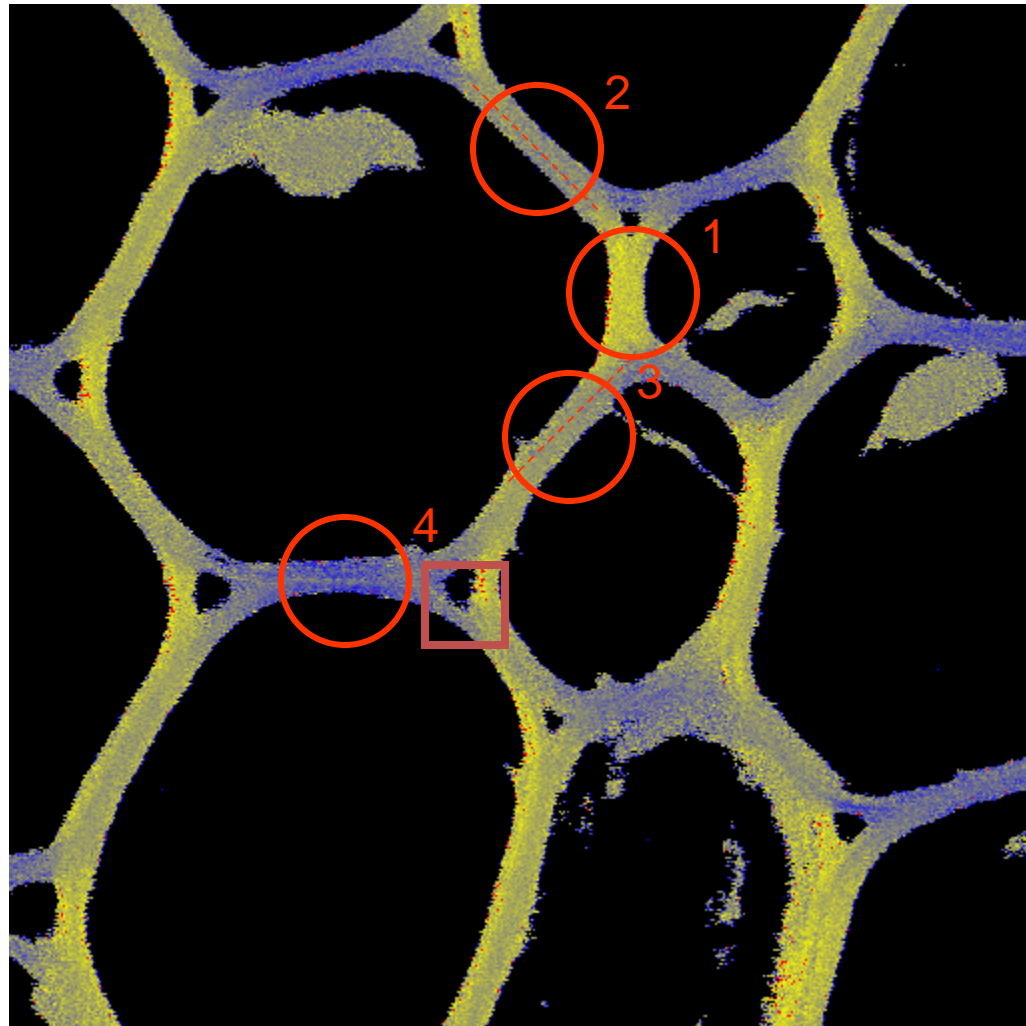
$$0.01 \pm 0.002^2,$$

$$0.06 \pm 0.01^3$$

(steeper than 45°)

Horizontal (blue):

$$-0.23 \pm 0.08^4$$



FDLD rotation

Vertical (yellow):

0.24 ± 0.05^3 ,

Diagonal (gray):

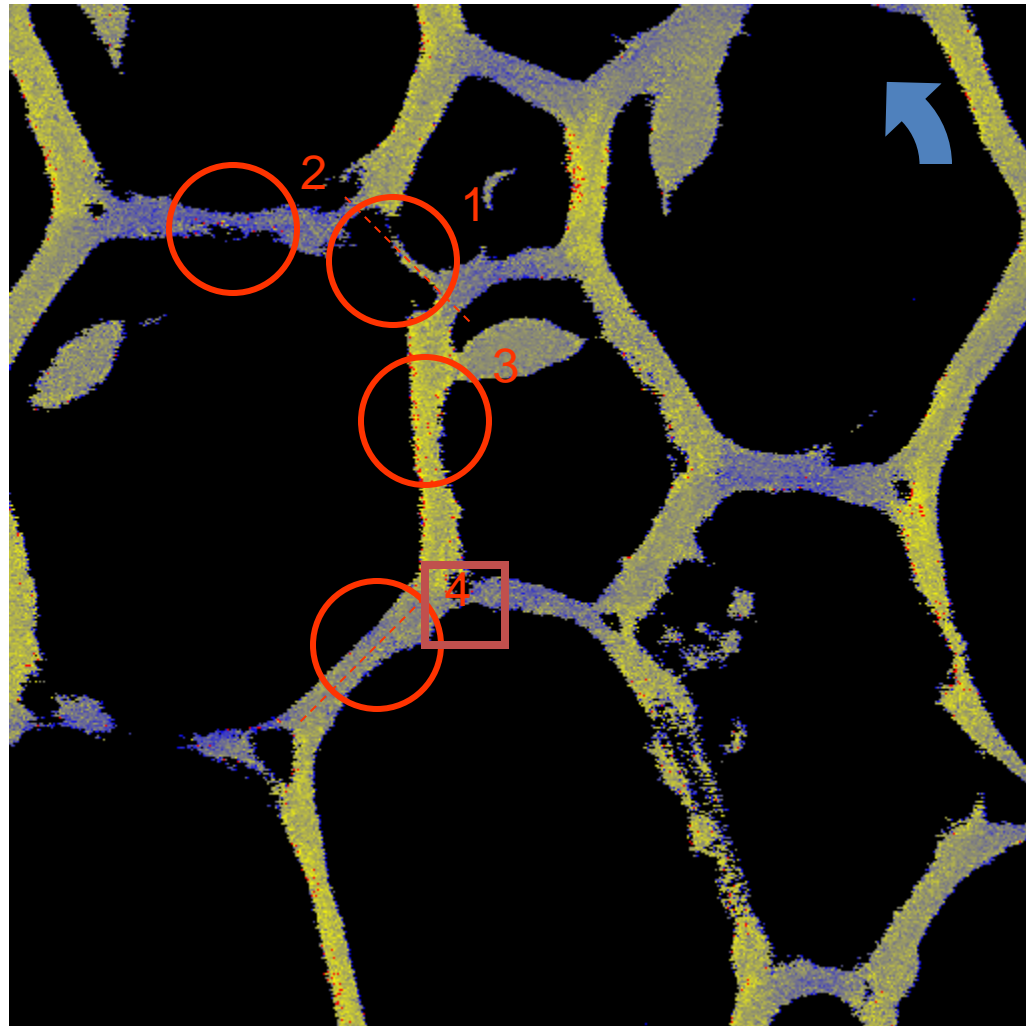
0.034 ± 0.008^1 ,

(very few points)

0.004 ± 0.001^4

Horizontal (blue):

-0.21 ± 0.1^2



FDLD rotation

Vertical (yellow):

$$0.23 \pm 0.05^4,$$

Diagonal (gray):

$$-0.024 \pm 0.006^2,$$

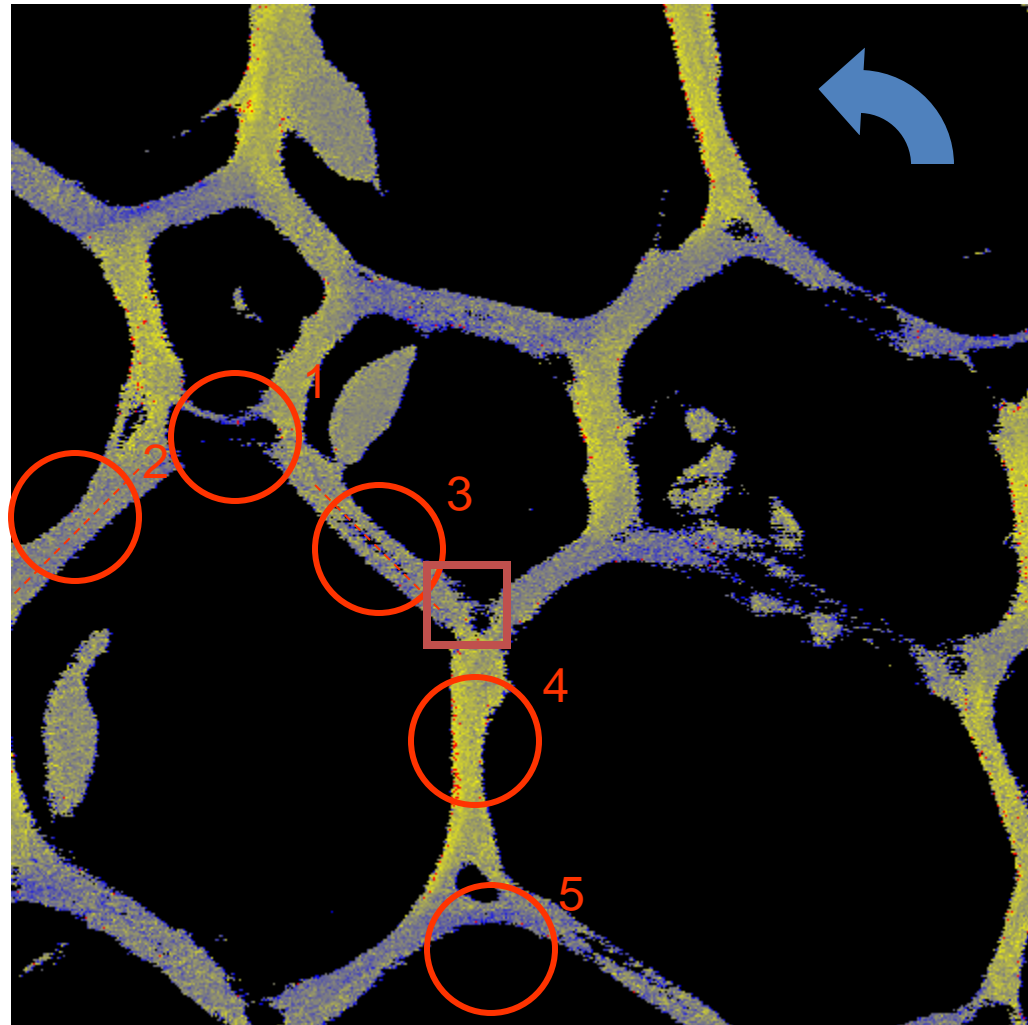
$$-0.005 \pm 0.002^3$$

Horizontal (blue):

$$-0.2 \pm 0.1^1$$

(very few points),

$$-0.20 \pm 0.07^5$$



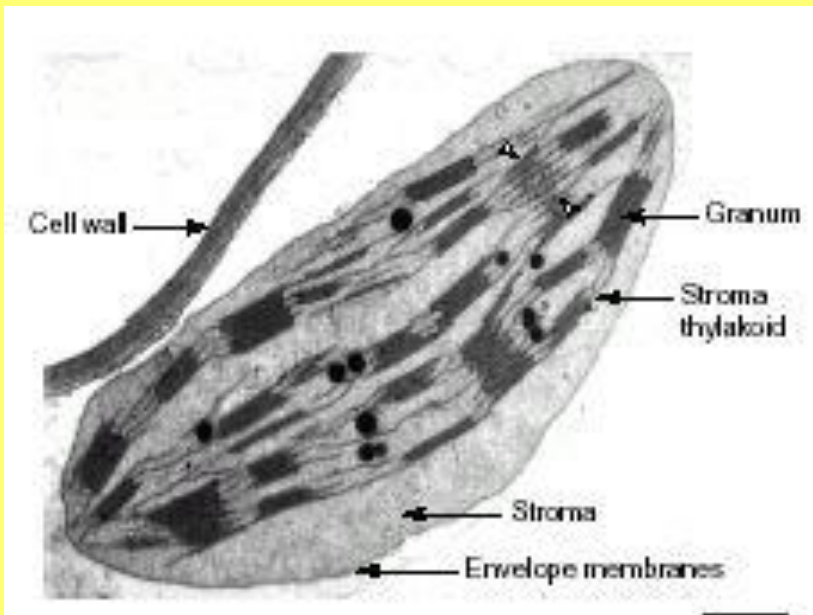
Applications

Different biological systems:

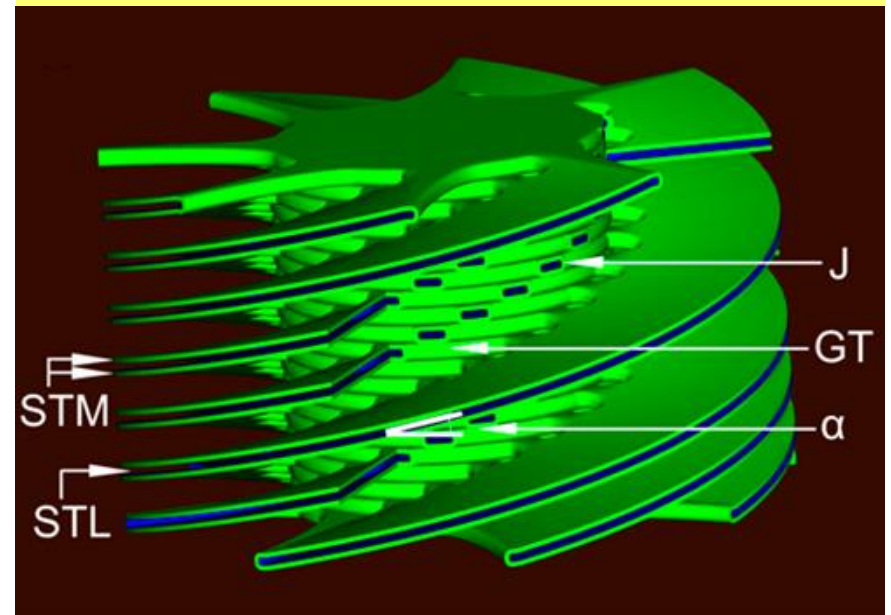
- Actin based cell-cell connections
- Amyloid filaments
- Artificial light harvesting macro-assemblies

- Cellulose (and other cellwall-components)
- Membrane-structures (limphocytes, chloroplast thylakoids membranes)

Linear Birefringence of chloroplasts

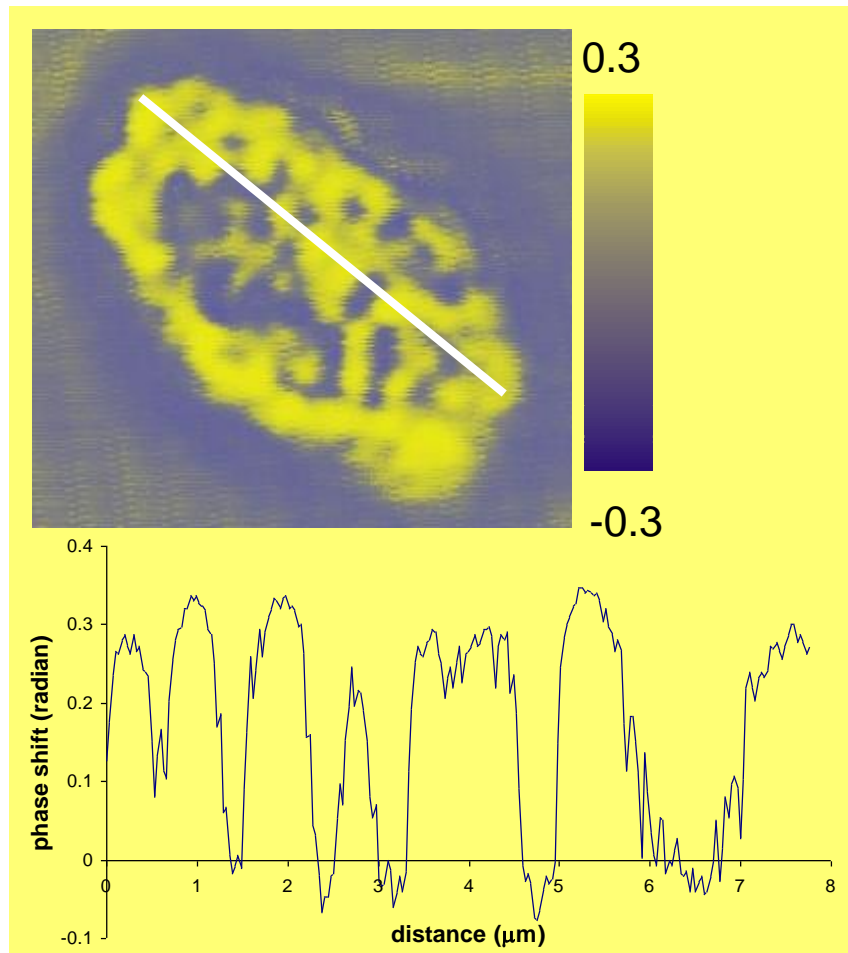


Grana & stroma thylakoids
in the chloroplasts



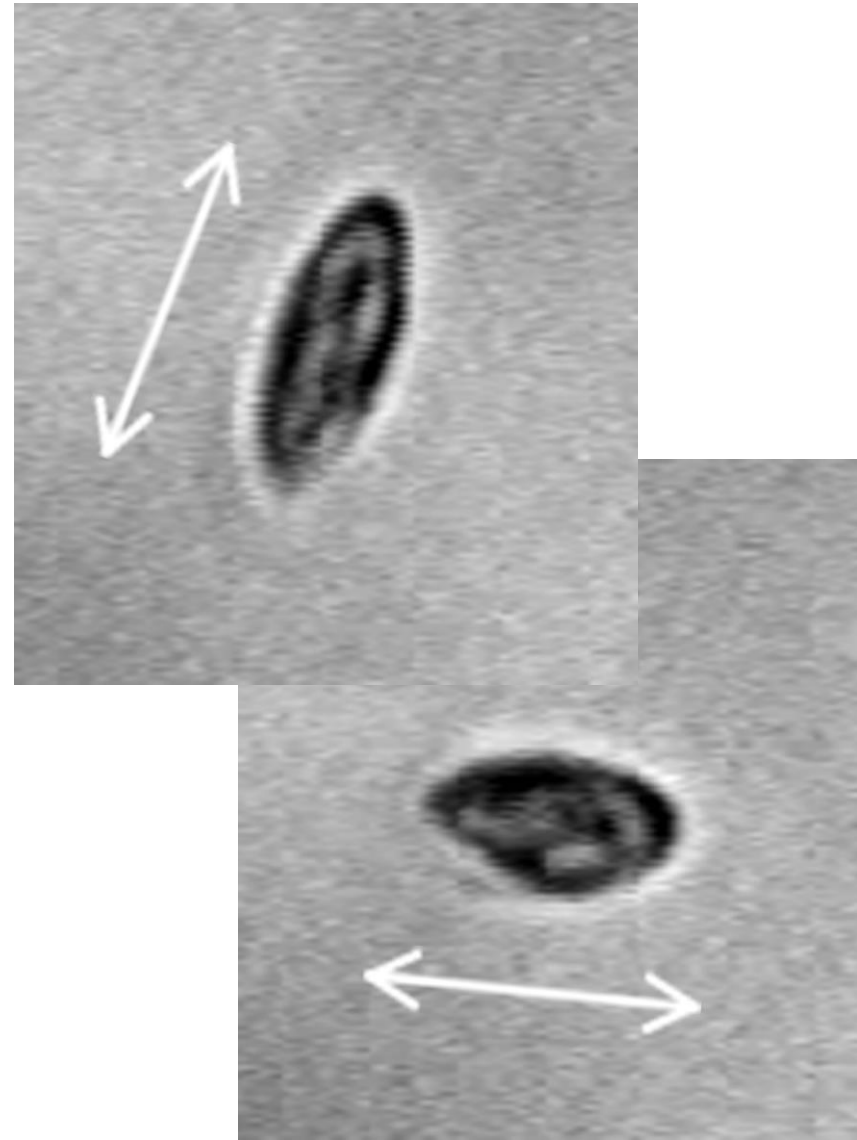
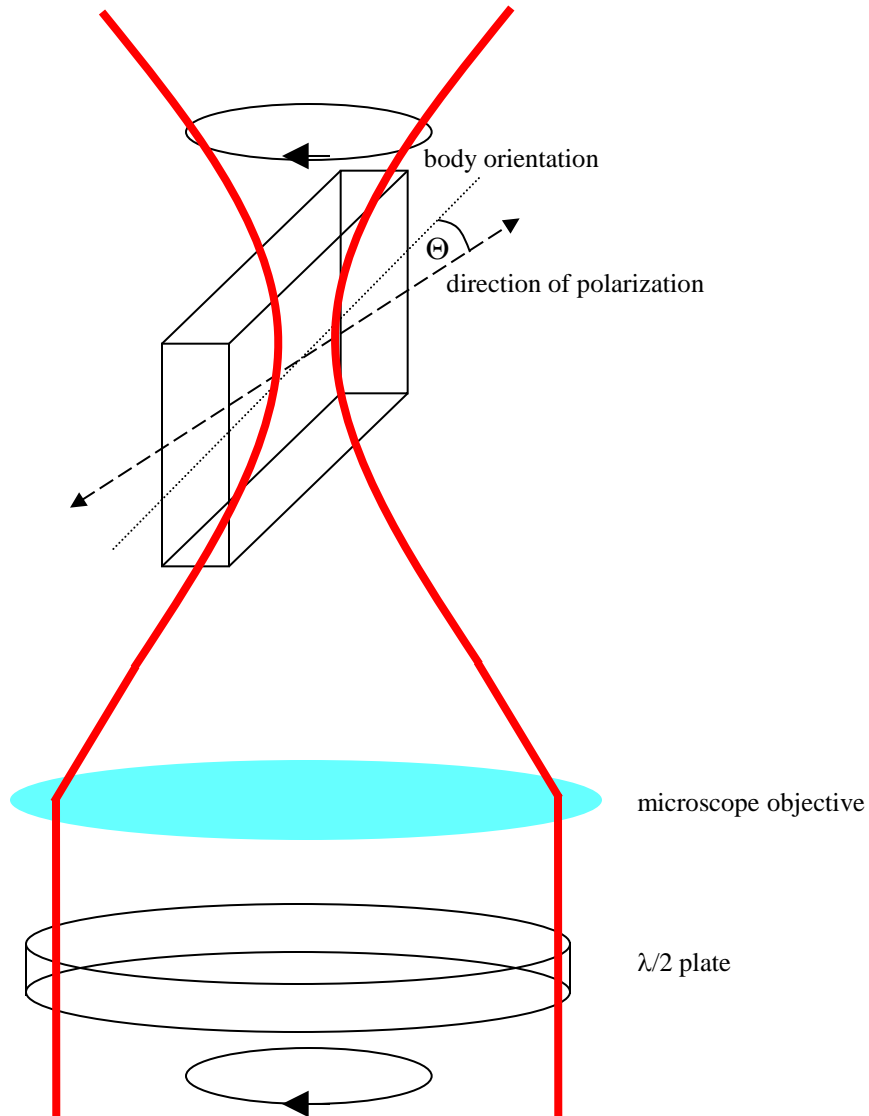
Stacked and single
membranes

Linear Birefringence of chloroplasts



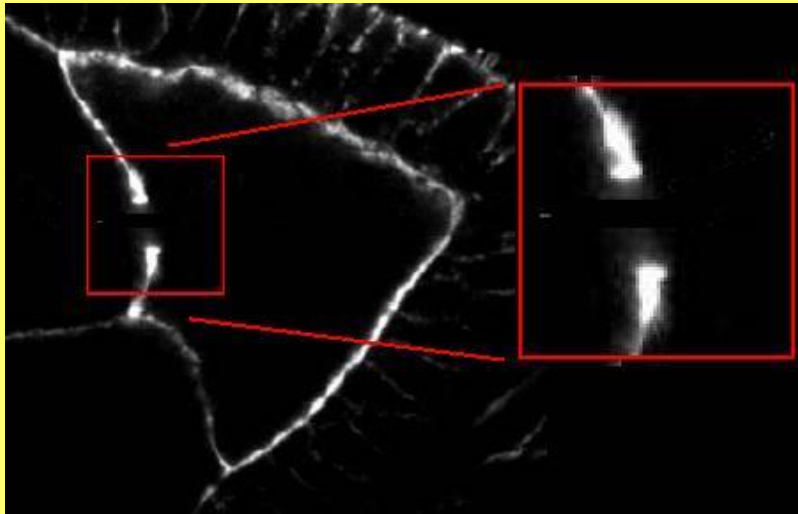
- High birefringence of densely packed grana: grana size quasi-periodic pattern in the chloroplast
- Birefringence allows micromanipulation in optical tweezers

Isolated chloroplast in laser tweezers



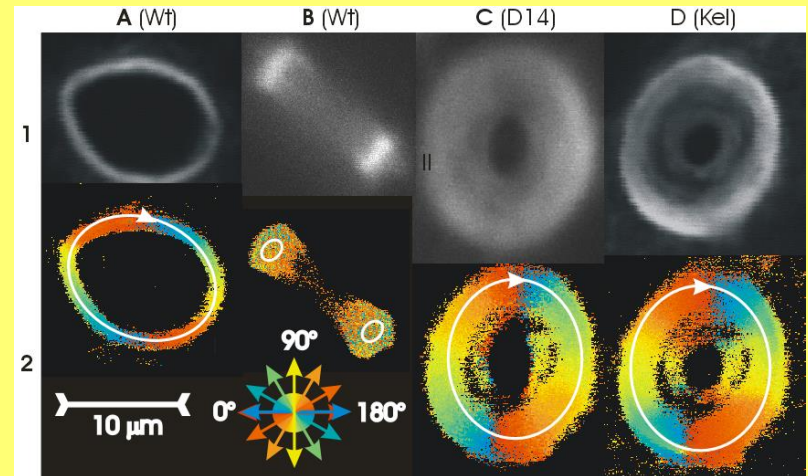


Anisotropy of O-ring canals



Anisotropic architecture of the ring canals of *Drosophila* nurse cells

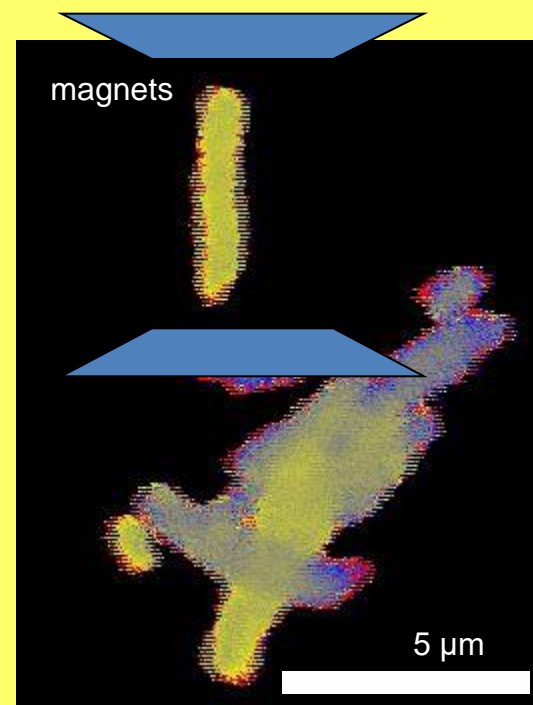
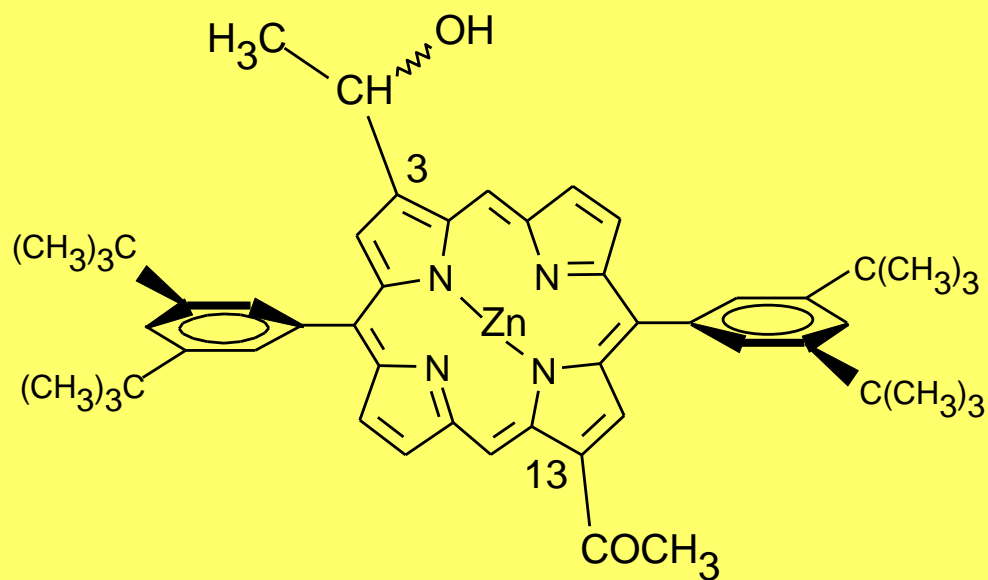
- Wild type and mutants



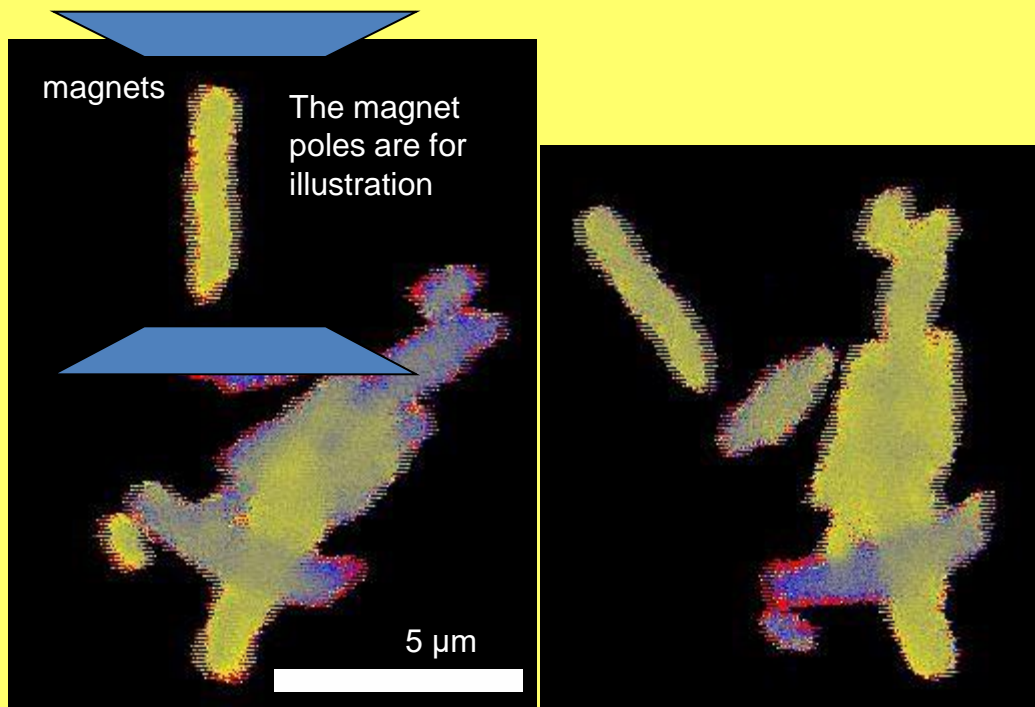
Sample	r , anisotropy mean value \pm S.E.	δ , fluctuation angle calculated for the mean
wt	0.42 ± 0.006	17.4°
kel	0.31 ± 0.003	22.9°
d14	0.31 ± 0.003	23.6°

Artificial porphyrin nanorods

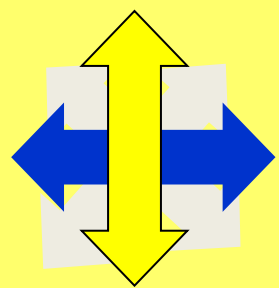
- Artificial light harvesting complex



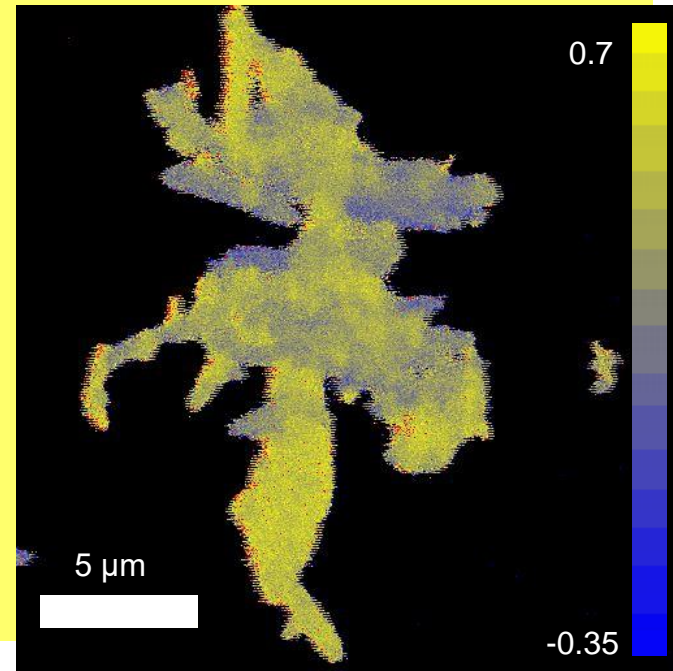
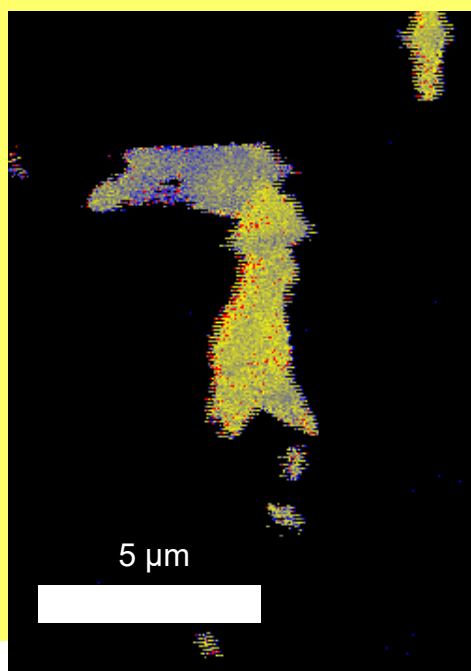
1
-1

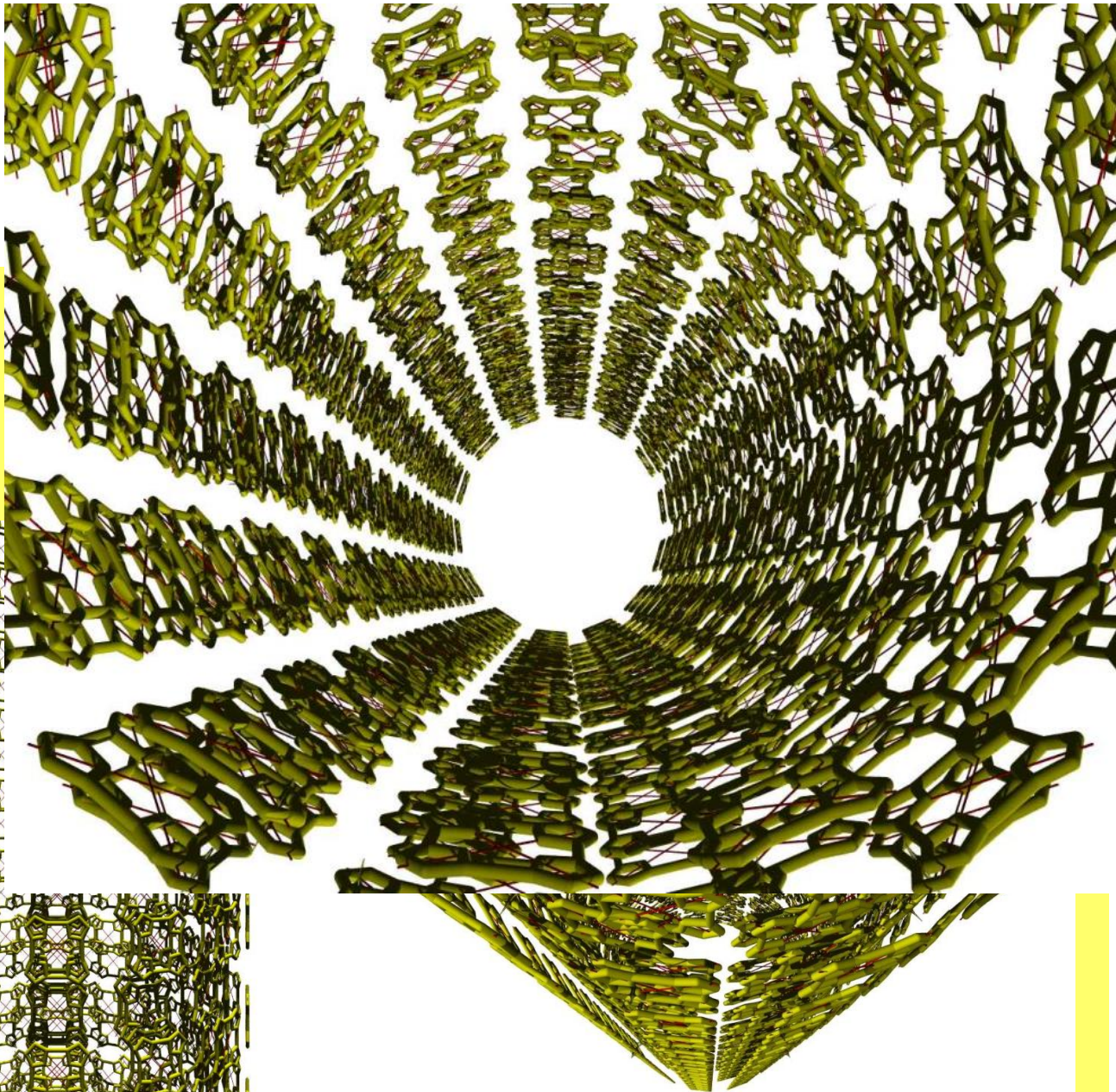


Modulated polarized excitation, intensity analysis of the emission;

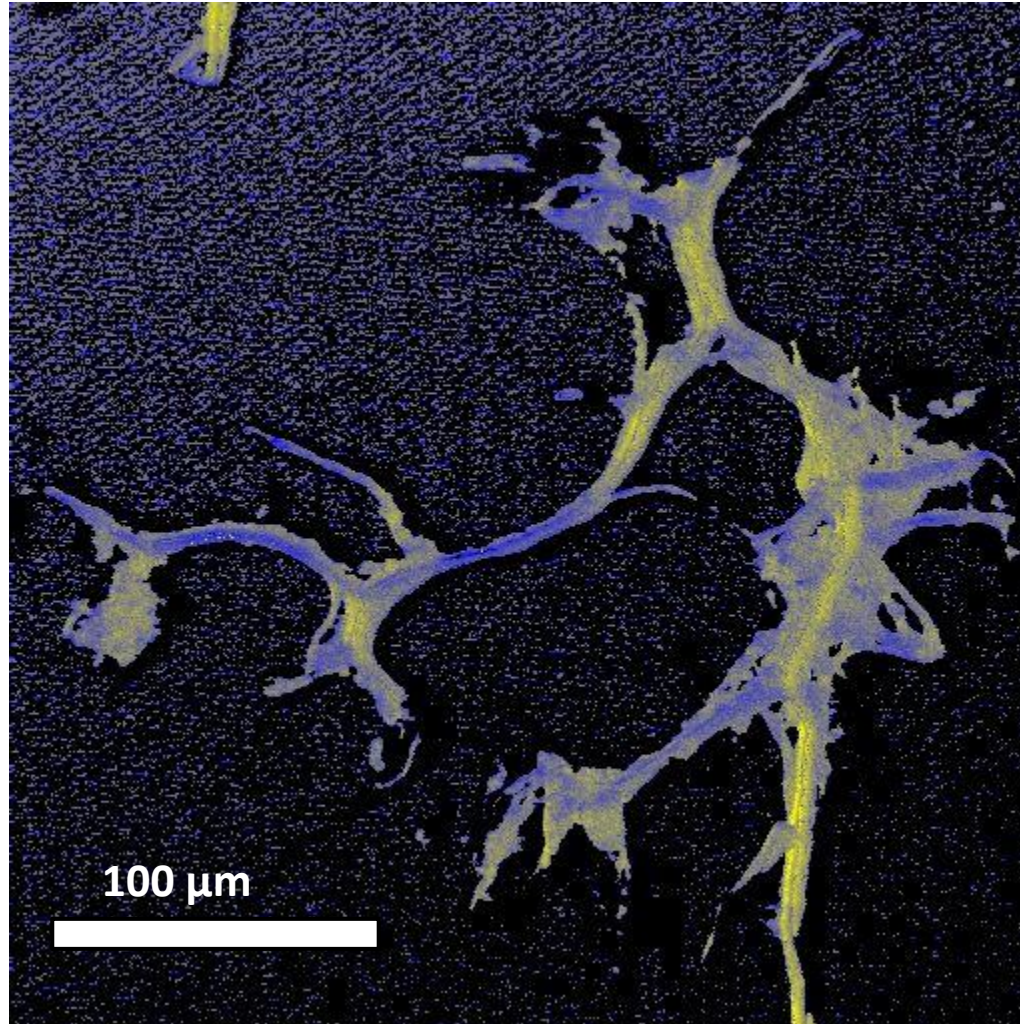


Non-polarized excitation, analysis of the polarization status of the emission;

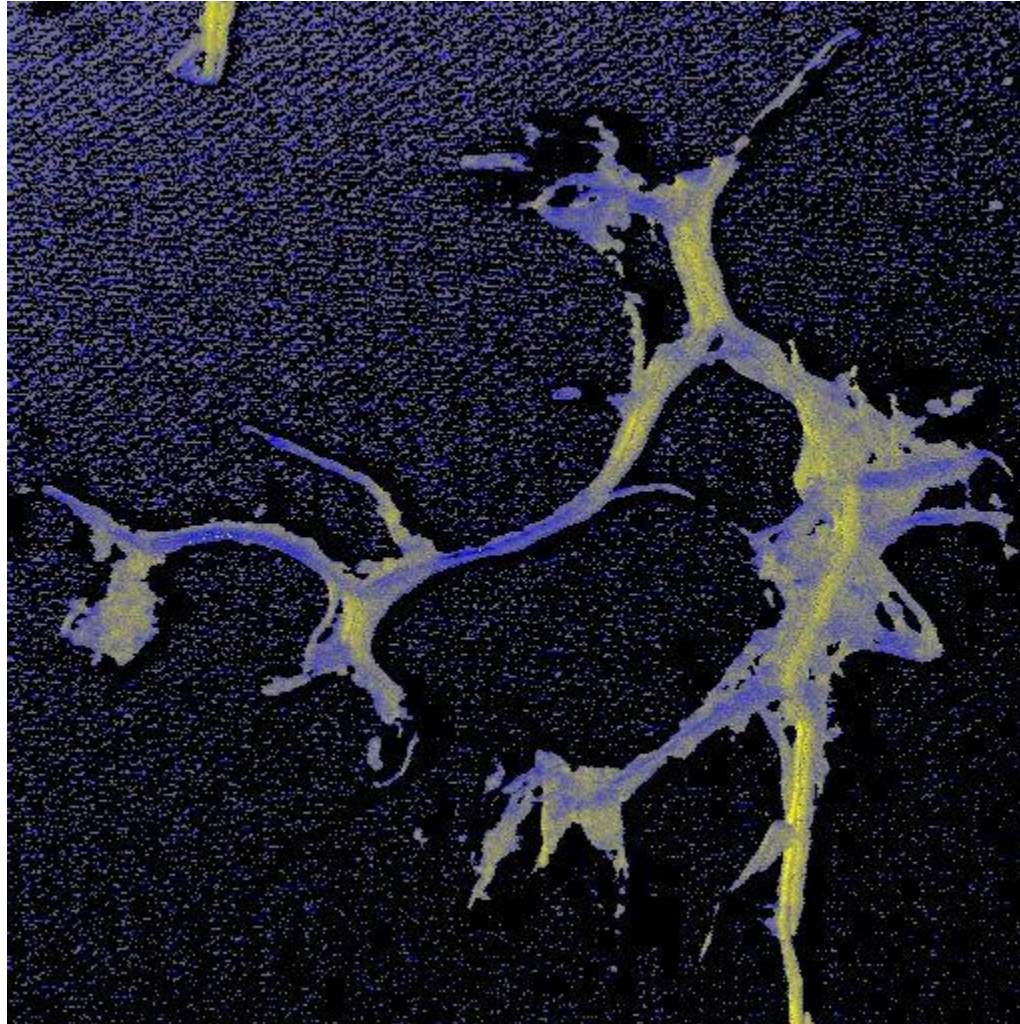




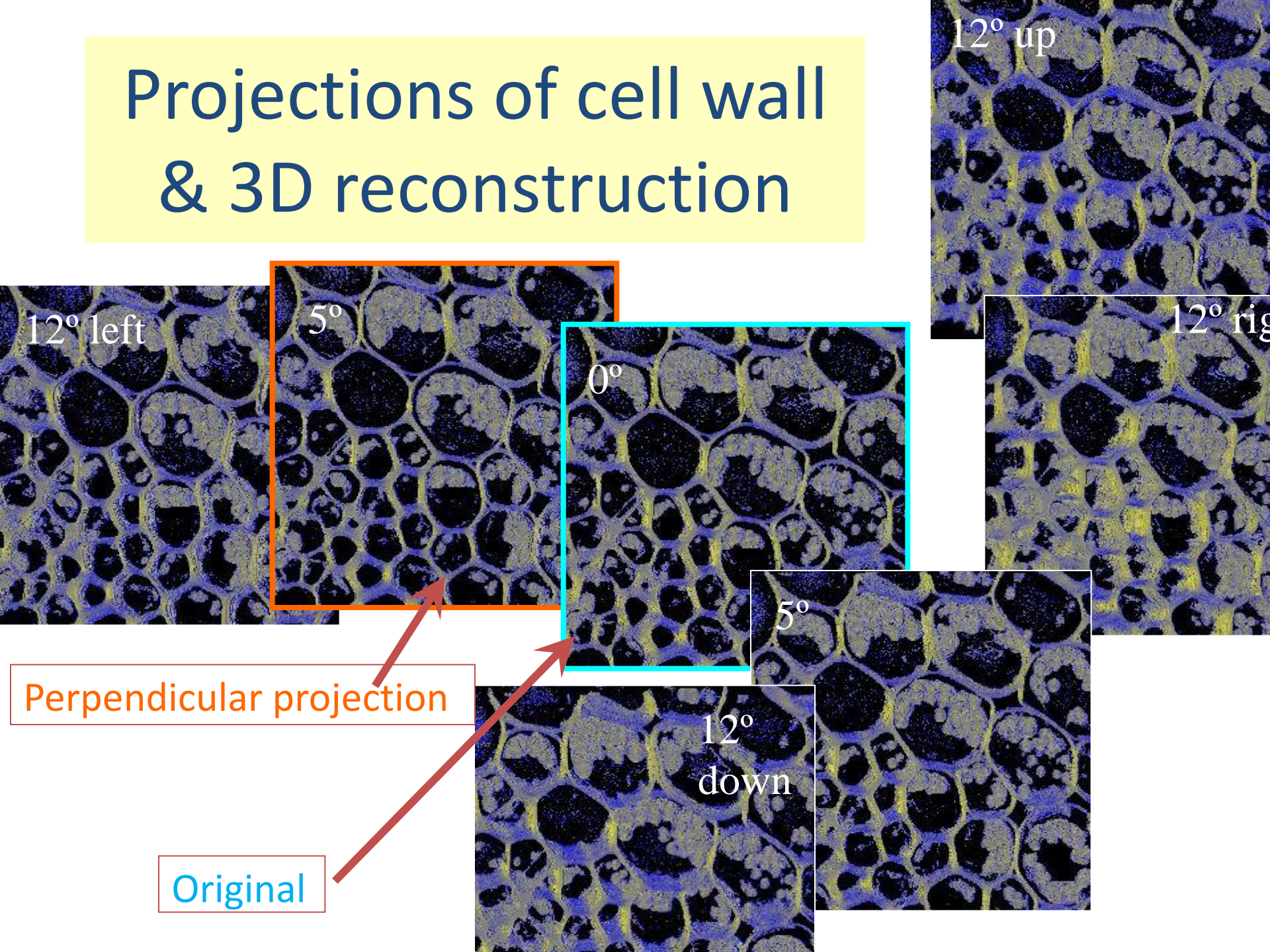
Amyloid filaments



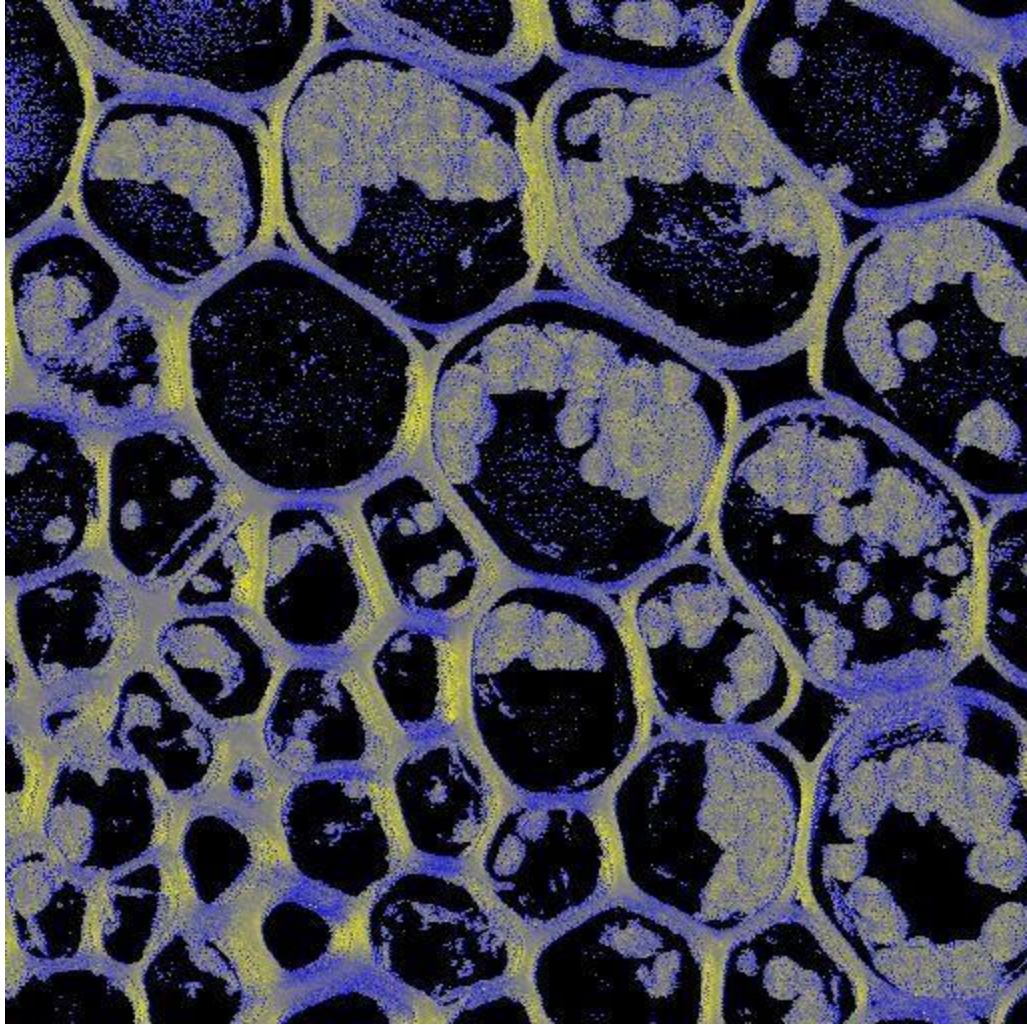
Amyloid filaments



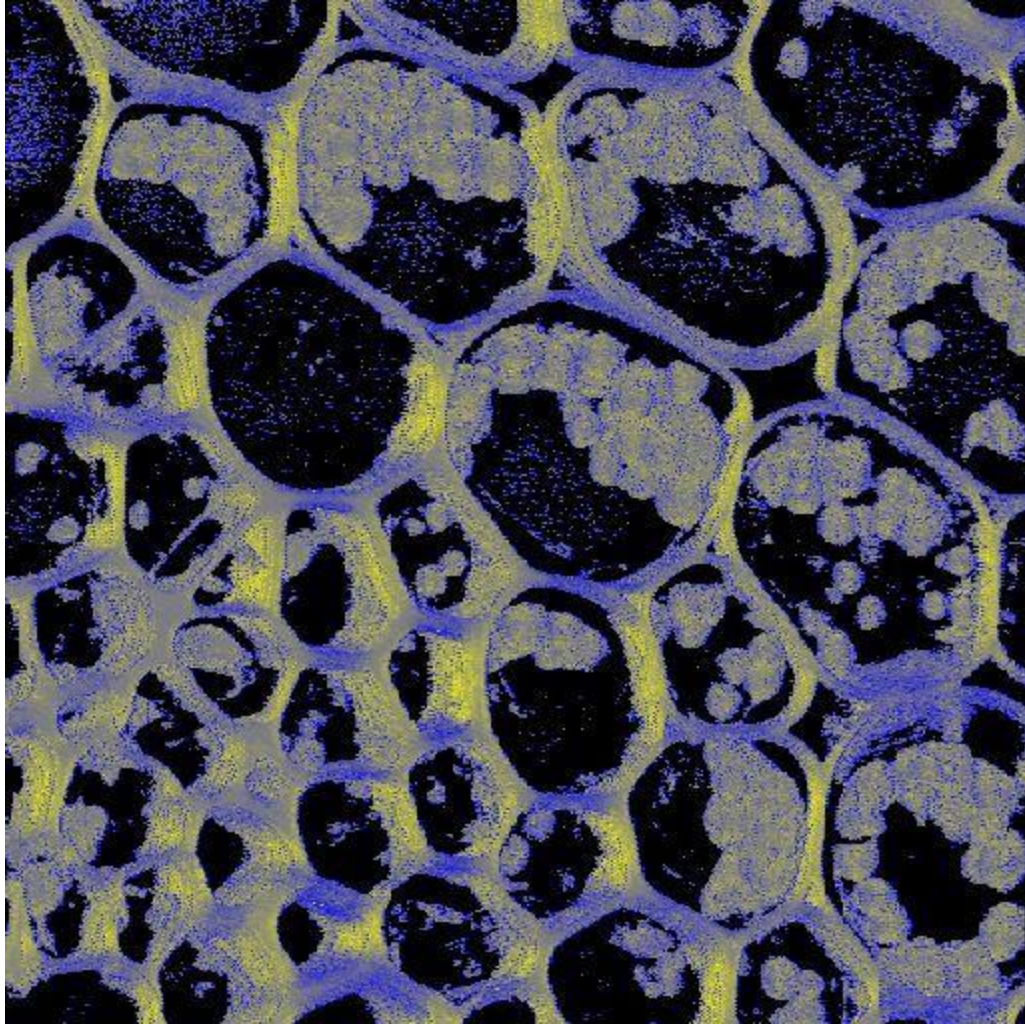
Projections of cell wall & 3D reconstruction



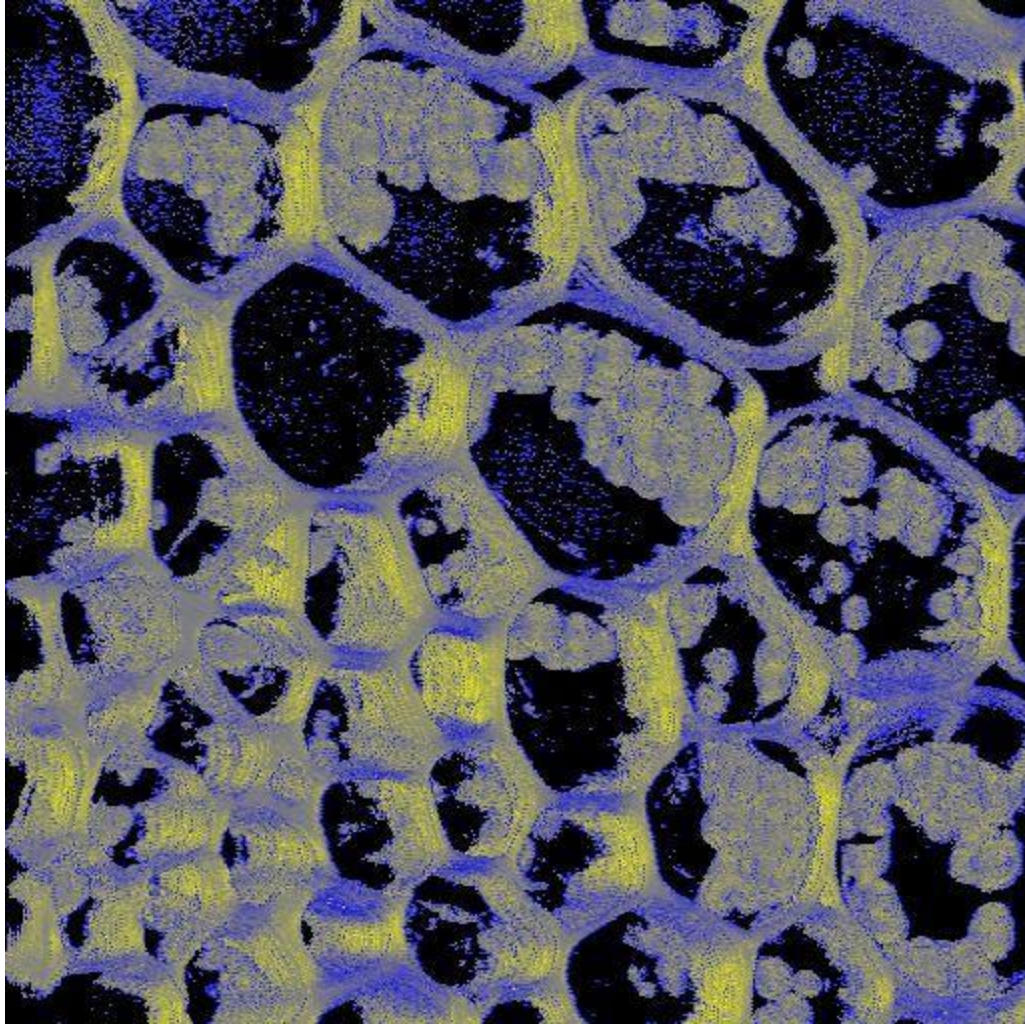
0°



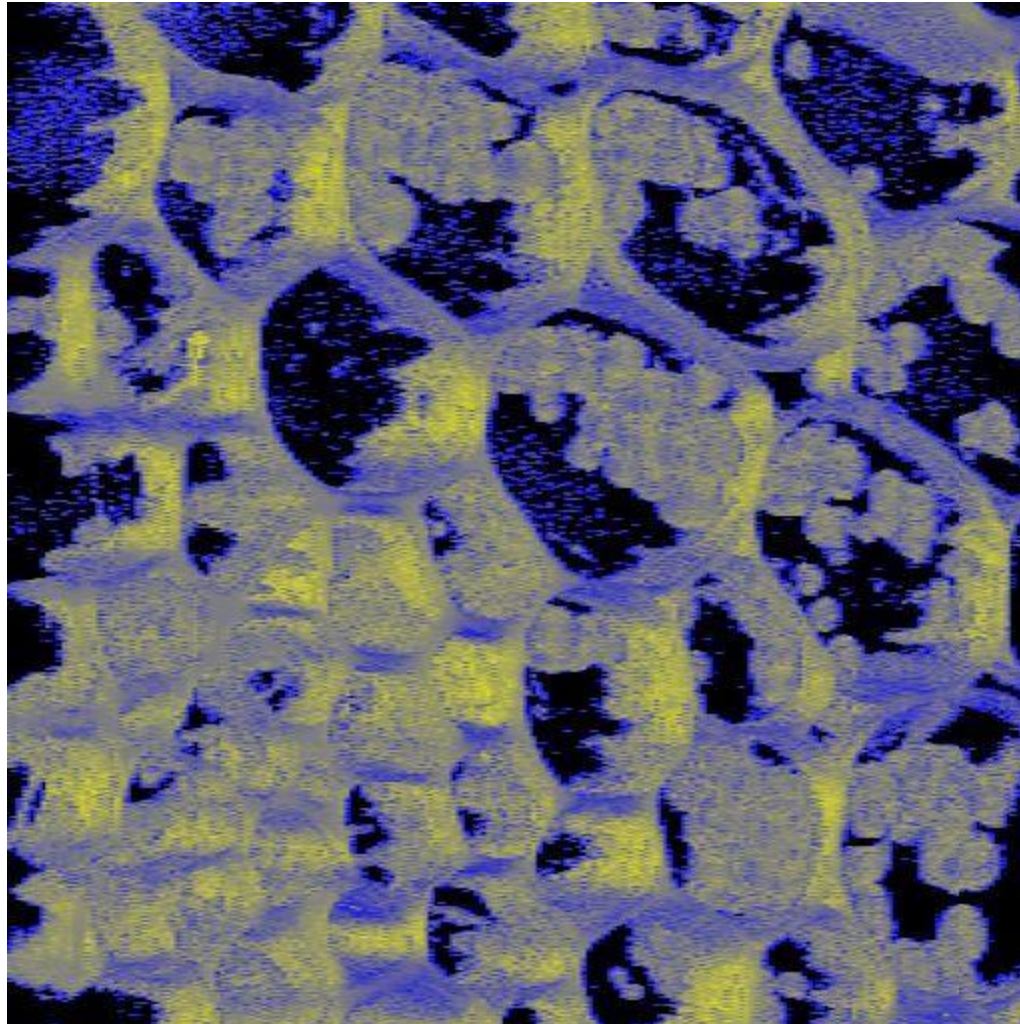
5°



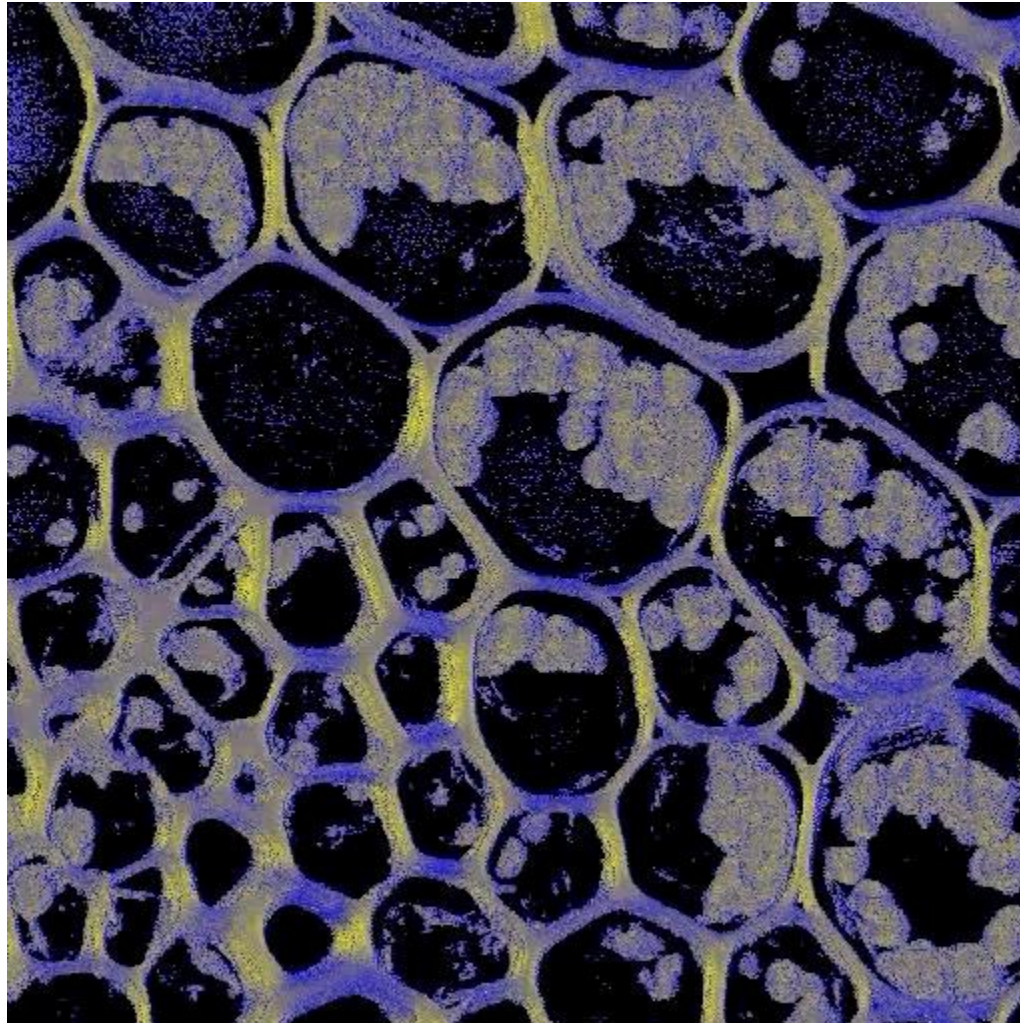
12°

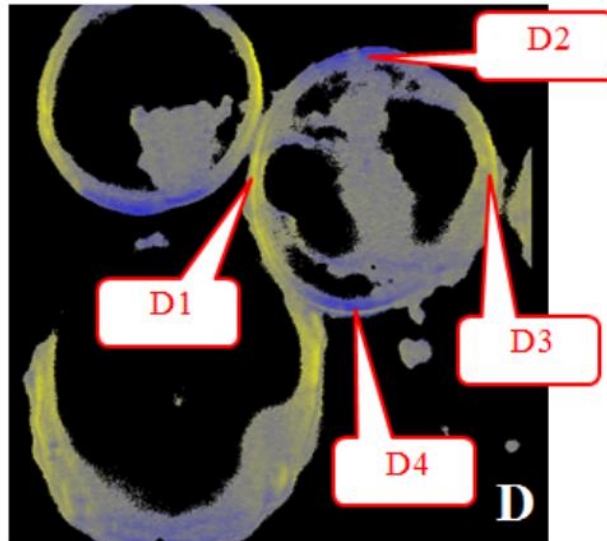
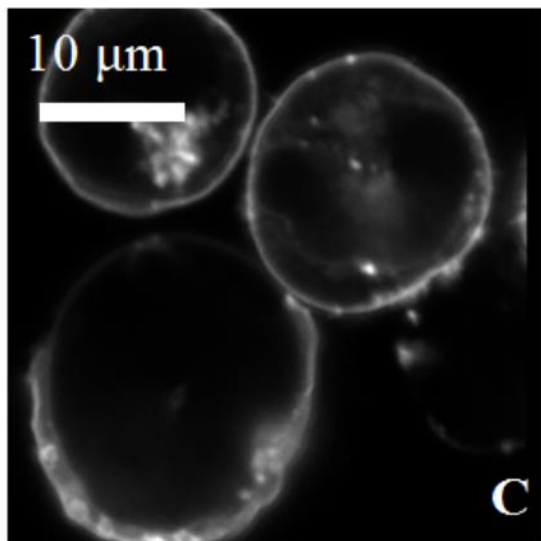
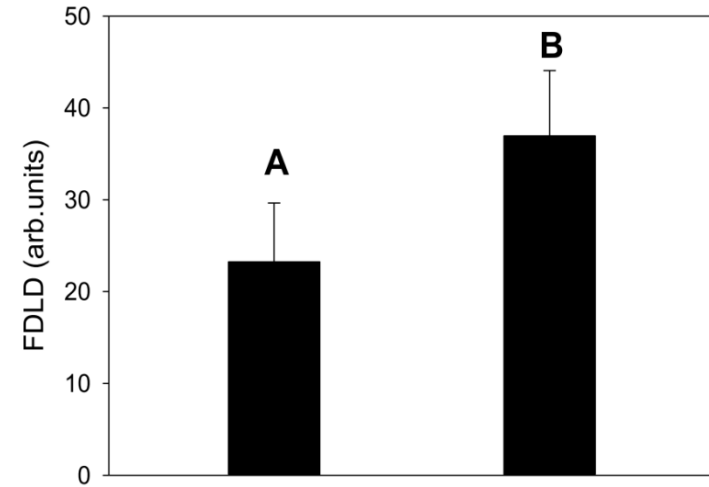
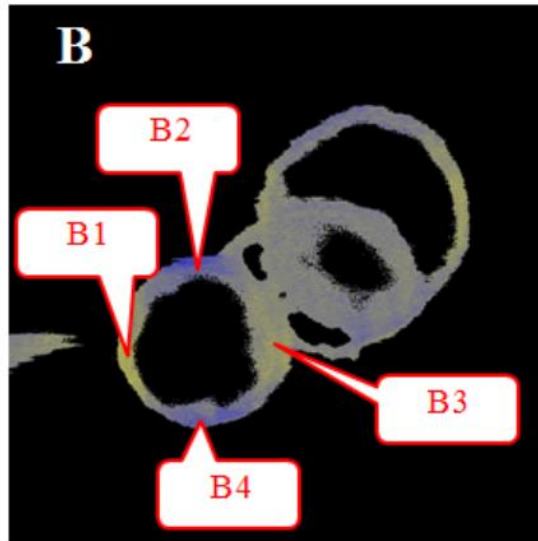
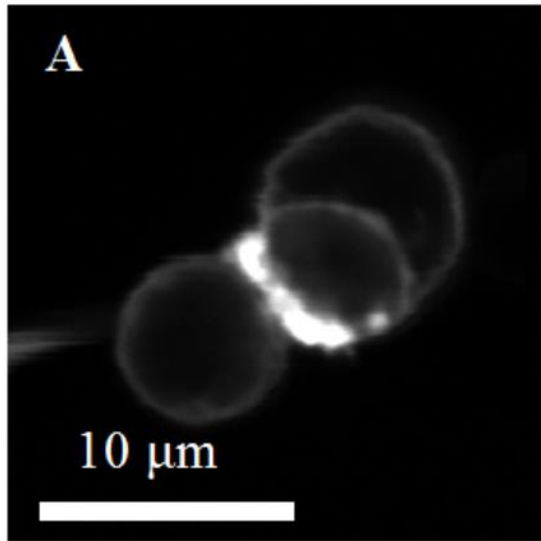


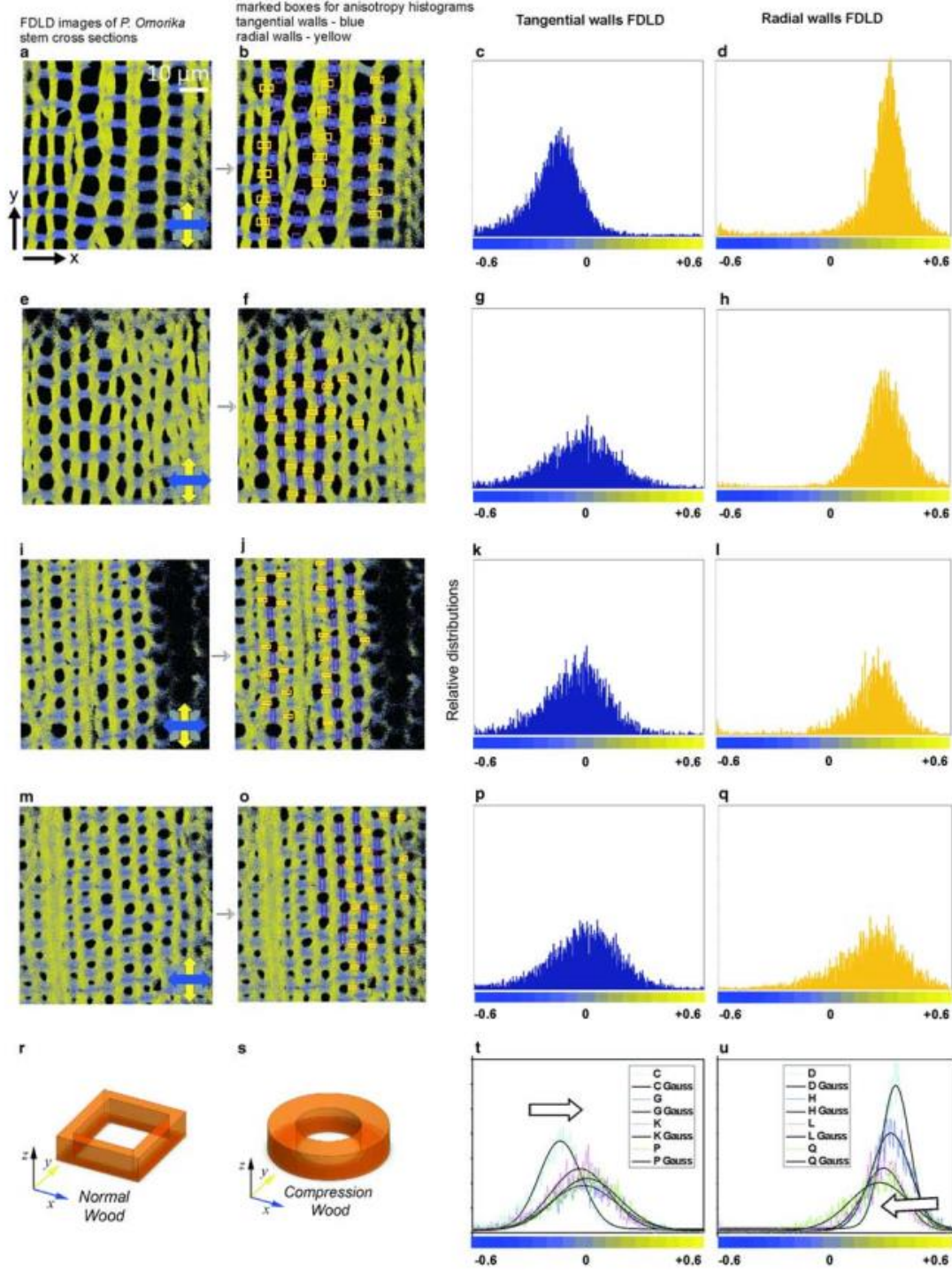
20°



3D reconstruction







Publication based on DP-LSM

- Steinbach G., Besson F., Pomozi I., Garab G. (2005) Proc SPIE 5969:566–575.
- Gorjanacz M., Torok I., Pomozi I., Garab G., Szlanka T., Kiss I., Mechler B.M. (2006) J Struct Biol 154 (2006) 27–41
- Steinbach G., Pomozi I., Zsiros O., Páy A., Garab G. (2008) Cytometry A, 73A: 202-208.
- Gombos I., Steinbach G., Pomozi I., Balogh A., Vámosi G., Gansen A., László G., Garab G., Matkó J. (2008) Cytometry A 73A:220-229.
- Steinbach G., Pomozi I., Zsiros O., Menczel L., Garab G. (2009) Acta Histochem 111:317-326.

Publication based on DP-LSM

- Steinbach G., Pomozi I., Jánosa D. P., Makovitzky J., Garab G. (2011) *J Fluoresc* 21:983-989.
- Chappaz-Gillot C., Marek P. L., Blaive B. J., Canard G., Bürck J., Garab G., Hahn H., Jávorfí T., Kelemen L., Krupke R., Mössinger D., Ormos P., Malla Reddy C., Roussel C., Steinbach G., Szabó M., Ulrich A. S., Vanthuyne N., Vijayaraghavan A., Zupcanova A. and Balaban T. S. (2012) *J Am Chem Soc* 134:944-954.
- Steinbach G, Pawlak K, Pomozi I, Tóth EA, Molnár A, Matkó J, Garab G (2014) *Methods and Applications in Fluorescence Methods Appl. Fluoresc.* 2: 015005 (9pp)
- Djikanović D, Devečerski A, Simonović J, Matović B, Garab G, Steinbach G, Kalauzi A, Radotić K (2016) *Wood Science and Technology*, 50: 547-566.
- Savić A, Mitrović A, Donaldson L, Simonović Radosavljević J, Bogdanović Pristov J, Steinbach G, Garab G, Radotić K (2016) *Microscopy and Microanalysis*, 22: 361-367.

*Thank you for
your attention!*