Polarized Imaging

A Journey Towards

Circular Dichroism Imaging

---

and what to do if you cannot afford it!

Cedric Dicko, Pure and Applied Biochemistry

Outline

- Circular Dichroism spectroscopy
  - What is it?
  - Information content?
  - In proteins?
- Polarization Imaging
  - Birefringence
  - Linear dichroism
  - Circular birefringence and dichroism
- Circular Dichroism Imaging
- Structured illumination microscopy

Circular Dichroism

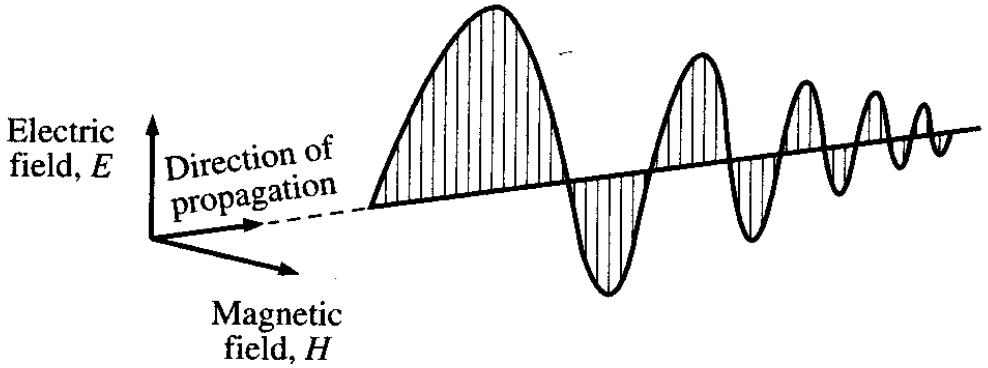
Theory and Applications

- Circular Dichroism (**CD)** is a type of absorption spectroscopy that can provide information on the structures of many types of biological macromolecules
- It measures the difference between the absorption of left and right handed circularly-polarized light by proteins. CD is used for;
- Protein structure determination.
- Induced structural changes, i.e. pH, heat & solvent.
- Protein folding/unfolding.
- Ligand binding
- Structural aspects of nucleic acids, polysaccharides, peptides, hormones & other small molecules.

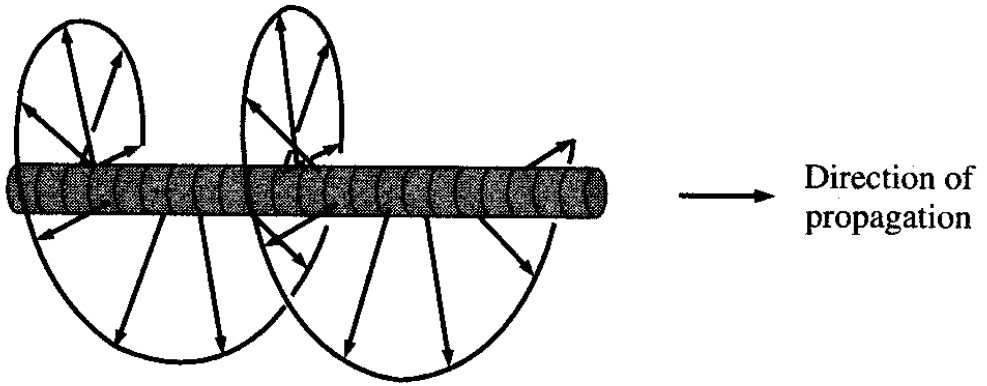
Unpolarized light

Linearly polarized or plane-polarized light

Circularly polarized light



Linearly (or plane-) polarized light



Right-circularly polarized light

Absorption spectroscopy

- CD measures the difference between the absorption of left and right handed circularly-polarized light:

$$\Delta A(\lambda) = A_R(\lambda) - A_L(\lambda) = [\varepsilon_R(\lambda) - \varepsilon_L(\lambda)]lc$$

or

$$\Delta A(\lambda) = \Delta\varepsilon(\lambda)lc$$

- $\Delta\varepsilon$ is the difference in the extinction coefficients
- typically < 10 $M^{-1}cm^{-1}$
- typical ε around 20 000 $M^{-1}cm^{-1}$
- So **the CD signal is a very small difference between two large originals**.

Characteristics of CD spectroscopy

- CD is only observed at wavelengths where absorption occurs, in absorption bands.

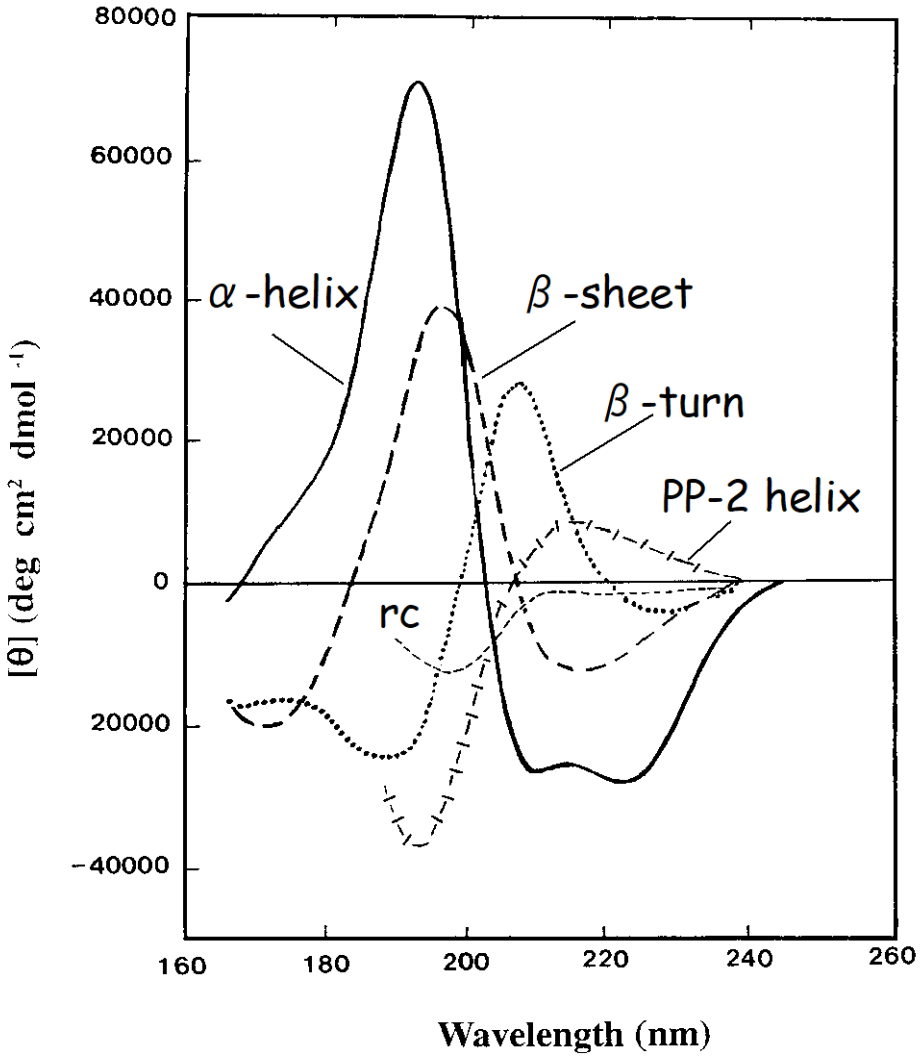
- CD arises because of the interaction between different transition dipoles doing the absorption. As this depends on the relative orientation of different groups in space, the signal is very sensitive to conformation.

- So in general $\Delta\varepsilon$ is much more conformation dependent that ε.

- We will concentrate on the "electronic CD" of peptides and proteins below 240nm. This region is dominated by the absorption of peptide bond and is sensitive to changes in secondary structure.

- Can also do CD in near UV (look at trp side chains), visible (cofactors etc.) and IR regions.

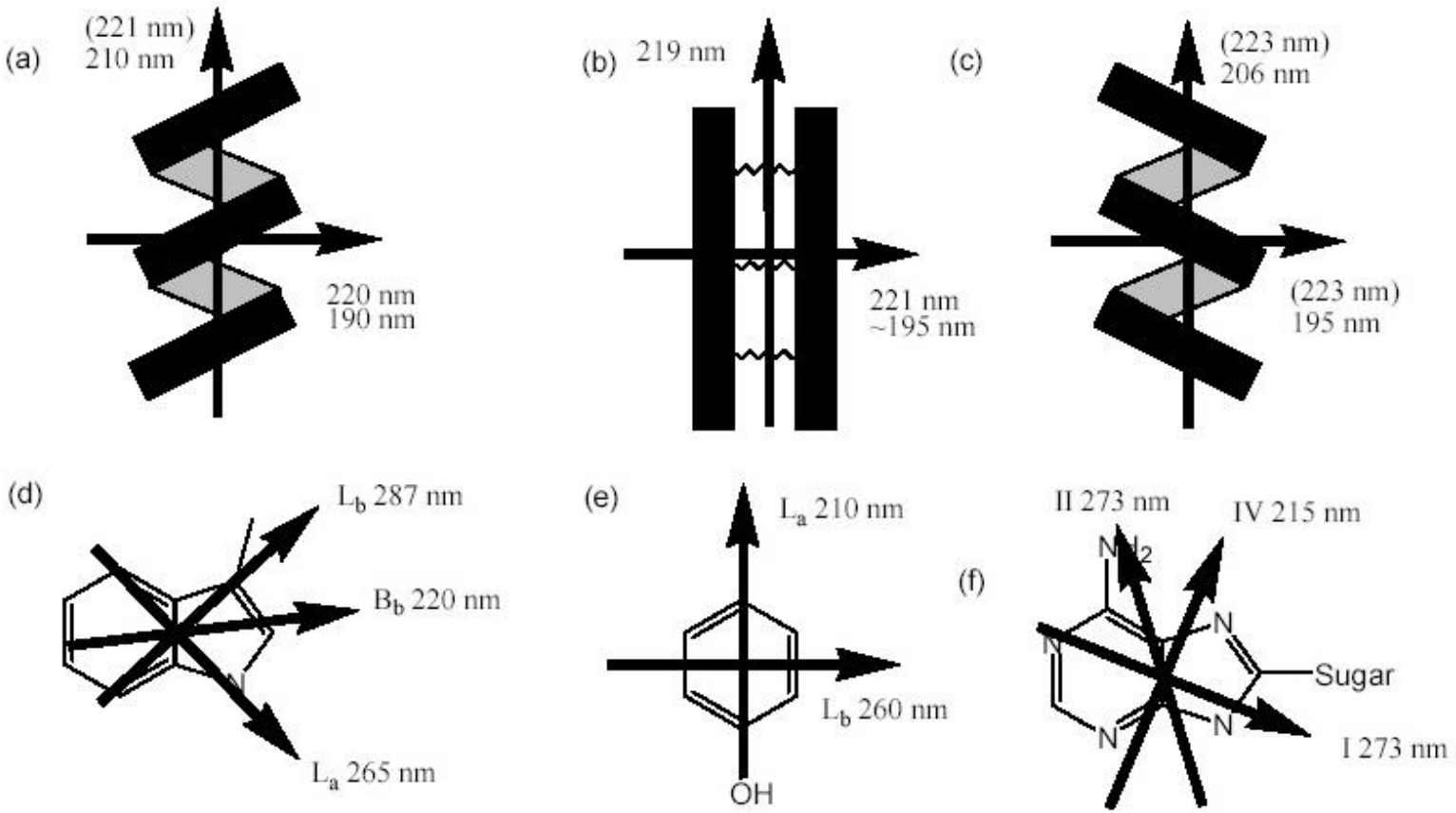
Canonical spectra



Features of CD spectra

Secondary structure element	*Signal*	*Electron transition*	*Position of minimum or maximum*	*Molar ellipticity of minima and maxima* [deg·cm^2 dmol^{-1}]
α-helix	positive	π->π*	190-195 nm	60.000 to 80.000
	negative	π->π*	208	-36.000 ± 3.000
	negative	n->π*	222	-36.000 ± 3.000
β-sheet	positive	π->π*	195 - 200	30.000 to 50.000
	negative	n->π*	215 - 220	-10.000 to –20.000
random	negative	π->π*	ca. 200	-20.000
	positive	n->π*	220	

Transition dipoles



Information content

SRCD vs. CD information content

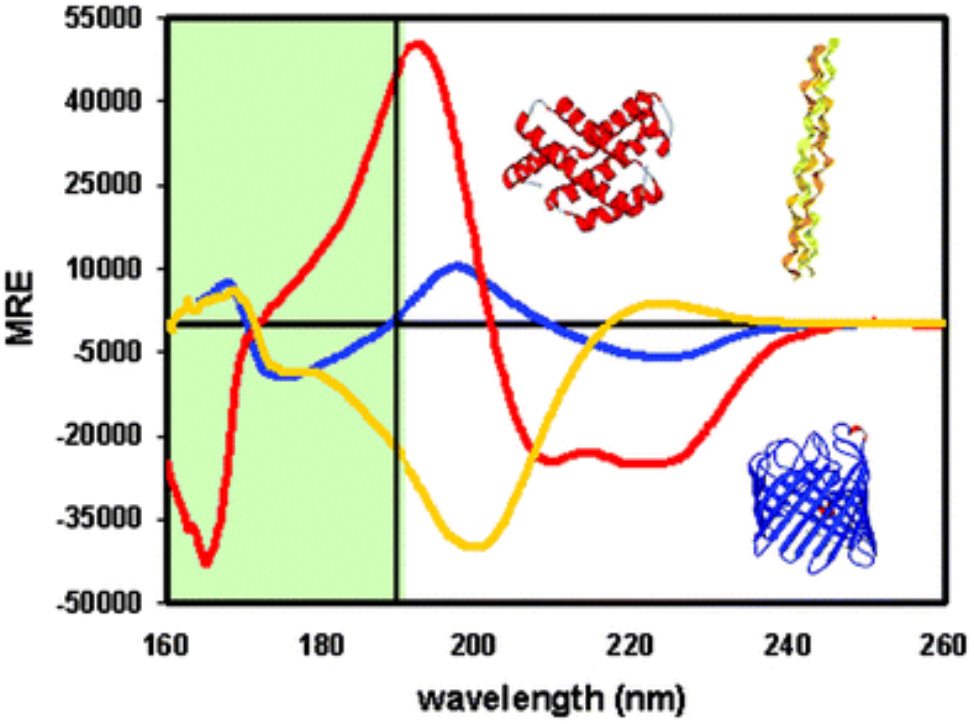
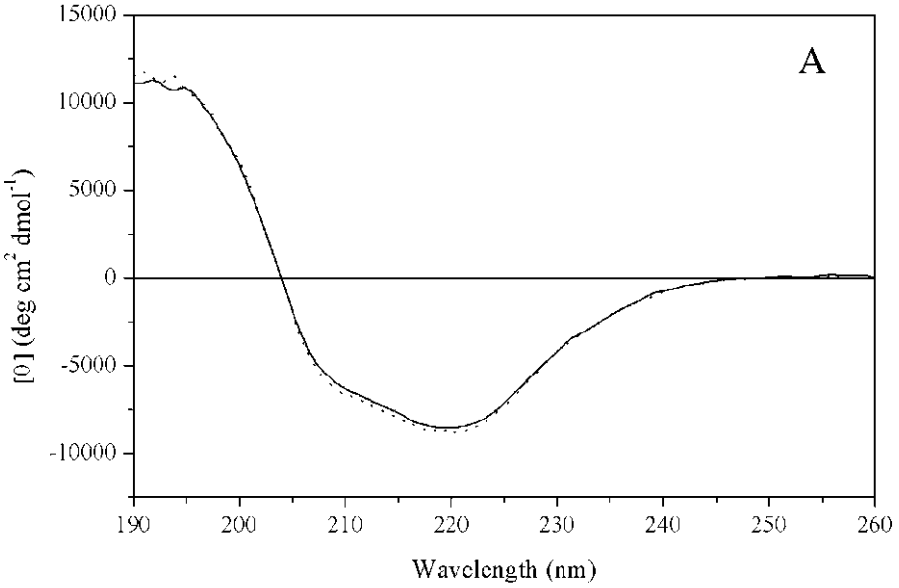


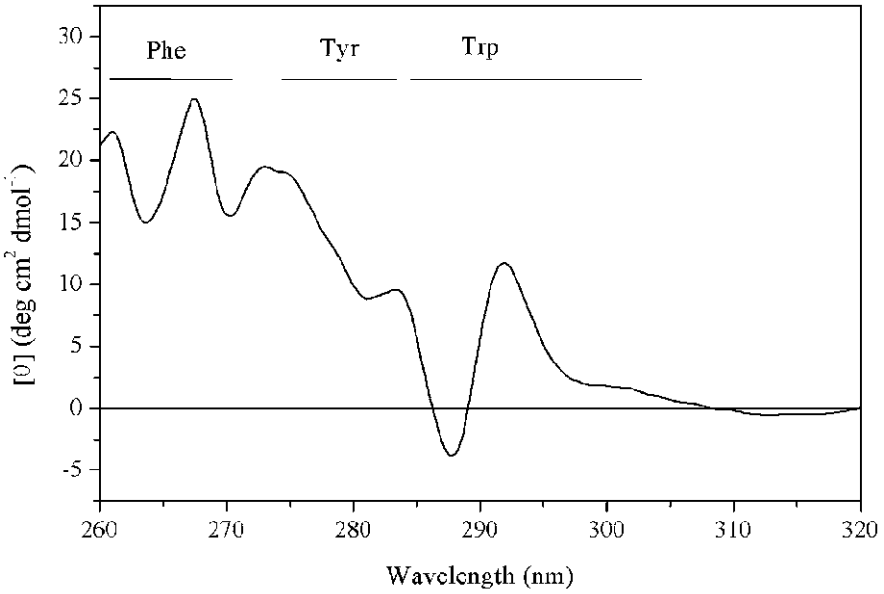
Table 2.1. Typical information content in CD spectrum of proteins from singular value decomposition method [a]

Wavelength	Eigenvectors	Number of secondary structure
260 to 200 nm	2	2
260 to 190 nm	3-4	4
260 to 178 nm	5	8
260 to 168 nm	6	10
260 to 160 and below	7-8	12

See Bonnie Wallace compiled work, Birbeck College

Far UV and Near UV

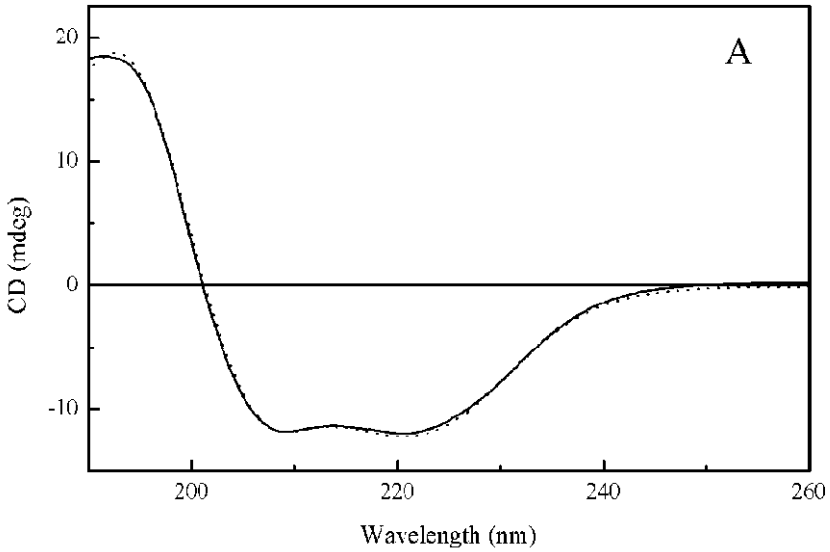


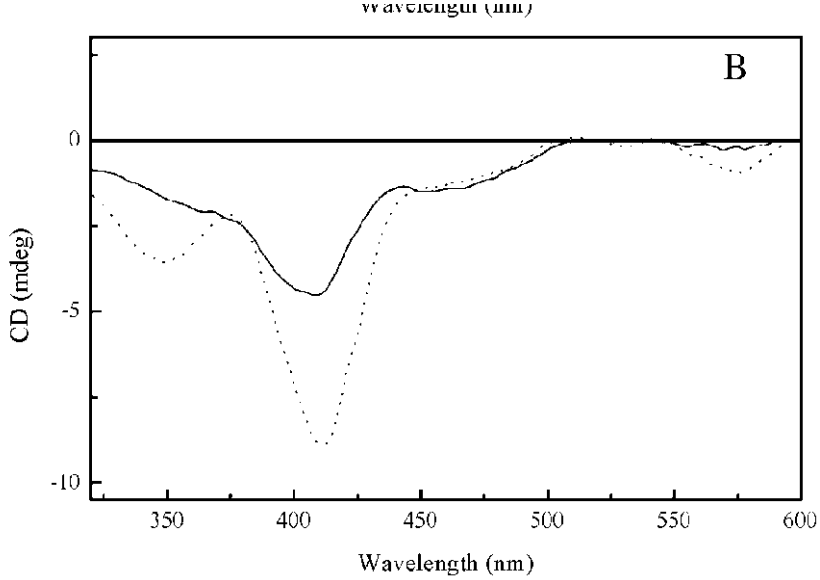


Far UV: < 260
Sensitive to secondary structure

Near UV: 260 – 390
Sensitive to tertiary structure

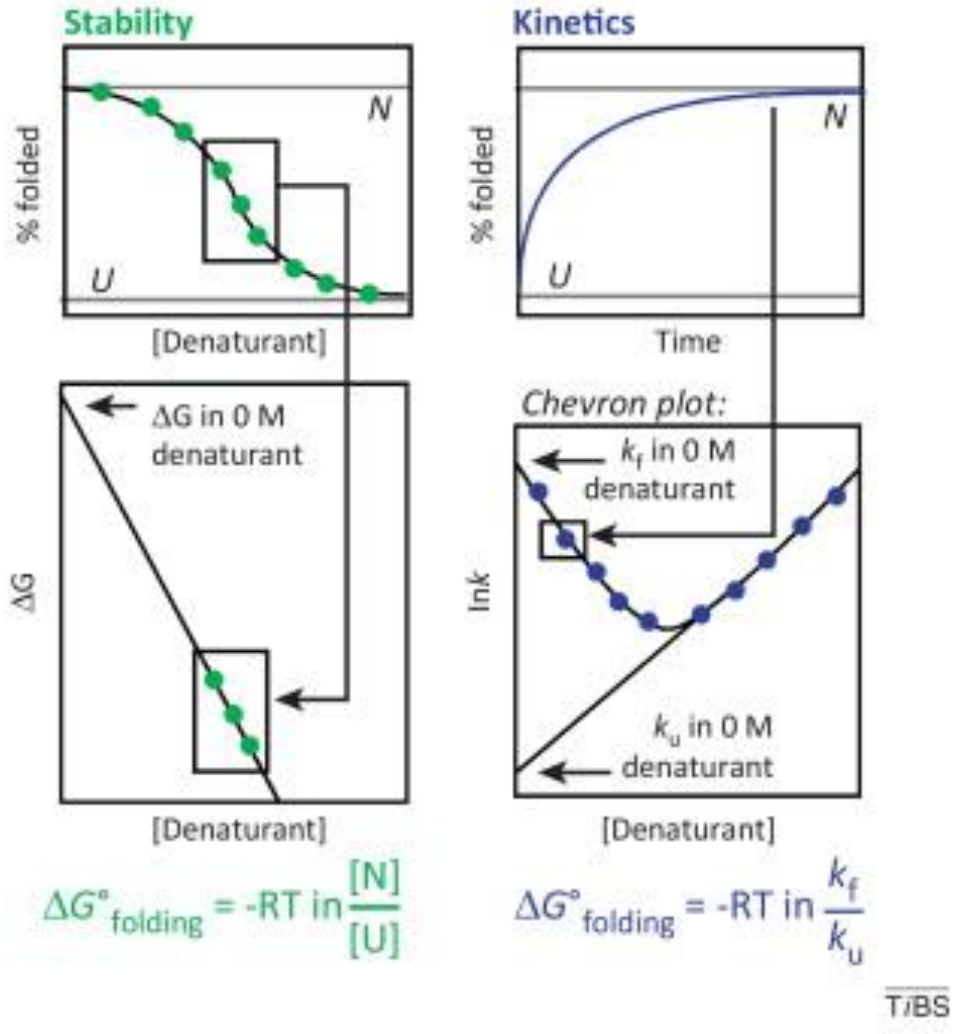
Far UV and Near UV
effect of conformational change





Exotic techniques: Vibrational CD or Raman Optical Activty

Equilibrium unfolding and kinetic



More “Protein Spectroscopy” course for PhD students in PCLS
(Post graduate Course in Life Science)

Can we do Circular dichroism imaging?

Challenges

- Camera frame rate of 30fps and modulator at 50kHz
- Birefringence
- linear dichroism
- Raleigh scattering (circular intensity differential scattering CIDS)
- Anomalous circular extinction (ACE)
- Imaging vs. scanning

A journey through polarization

imaging

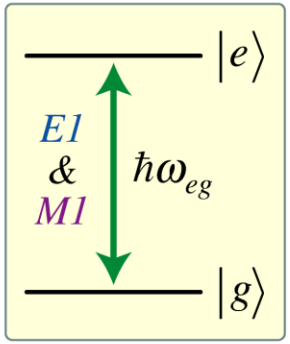
Wave optics parameters – Jones formalism

Effect	Description	Definition
Refraction	n is the (real part of the) mean of the refractive indices of the two eigen modes	$\eta = \frac{2\pi}{\lambda} n$
Absorption	k is the mean extinction coefficient (imaginary part of the mean refractive index)	$\kappa = \frac{2\pi}{\lambda} k$
Linear birefringence	n_y-n_x is the difference between the principal refractive indices for linear polarization states	$\delta = \frac{\pi}{\lambda}(n_y - n_x)$
Linear dichroism	k_y-k_x is the difference between the principal extinction coefficients for linear polarisation states	$\varepsilon = \frac{\pi}{\lambda}(k_y - k_x)$
Circular birefringence (optical activity)	n_L-n_R is the circular birefringence (positive for dextro-rotatory crystals)	$\rho = \frac{\pi}{\lambda}(n_L - n_R)$
Circular dichroism	k_L-k_R is the difference between the extinction coefficients for the Left and Right circularly polarised light	$\sigma = \frac{\pi}{\lambda}(k_L - k_R)$

Linear Chiro-Optical Response

TRANSITION ROTATORY STRENGTH

$$ {}^{eg}R = \mathrm{Im}\left\{ \langle g|\hat{\boldsymbol{\mu}}^{(E1)}|e\rangle \cdot \langle e|\hat{\boldsymbol{\mu}}^{(M1)}|g\rangle \right\} $$



DIFFERENTIAL REFRACTIVE INDEX

$$ \Delta\eta(\omega) = \Delta n(\omega) + i\Delta\kappa(\omega) $$

Measured Properties

Polarization Rotation

$$ \Phi(\omega) = \phi(\omega)\ell = \frac{\omega\ell}{2c}\Delta n(\omega) $$

Polarization Ellipticity

$$ \Theta(\omega) = \theta(\omega)\ell = \frac{\omega\ell}{2c}\Delta\kappa(\omega) = \frac{[C]\ell\ln 10}{4}\Delta\varepsilon(\omega) $$

Chiro-Optical Response

Circular Birefringence

$$ \Delta n(\omega) = n_L(\omega) - n_R(\omega) = \frac{4N\omega}{3\hbar c\varepsilon_0}\sum_{e\neq g}\frac{{}^{eg}R}{\omega_{eg}^2 - \omega^2} $$

Circular Dichroism

$$ \Delta\kappa(\omega) = \kappa_L(\omega) - \kappa_R(\omega) = \frac{4N\omega}{3\hbar c\varepsilon_0} L\left(\omega;\omega_{eg},\Gamma_{eg}\right) {}^{eg}R $$

From Shawn Wilson, Yale, 2006

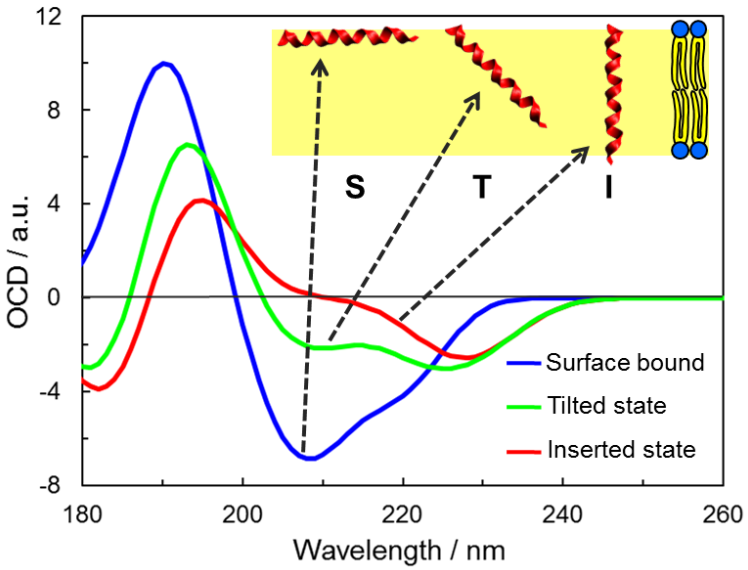
Removal or at least control of anisotropy
for circular dichroism imaging

But

at the same time
spatial organization is critical to function

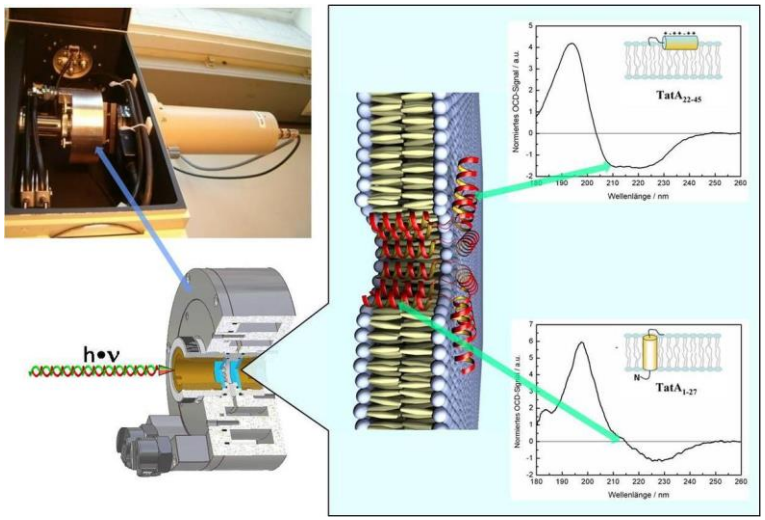
The problem of anisotropic samples in solution CD: Oriented CD

Removal of LD and Birefringence from Oriented structures in solution



Wu, Biophysical Journal 1990, 57:797
Miles & Wallace Chem. Soc. Rev. 2016, 45:4859
Bürck et al. Acc. Chem. Res. 2016, 49:184

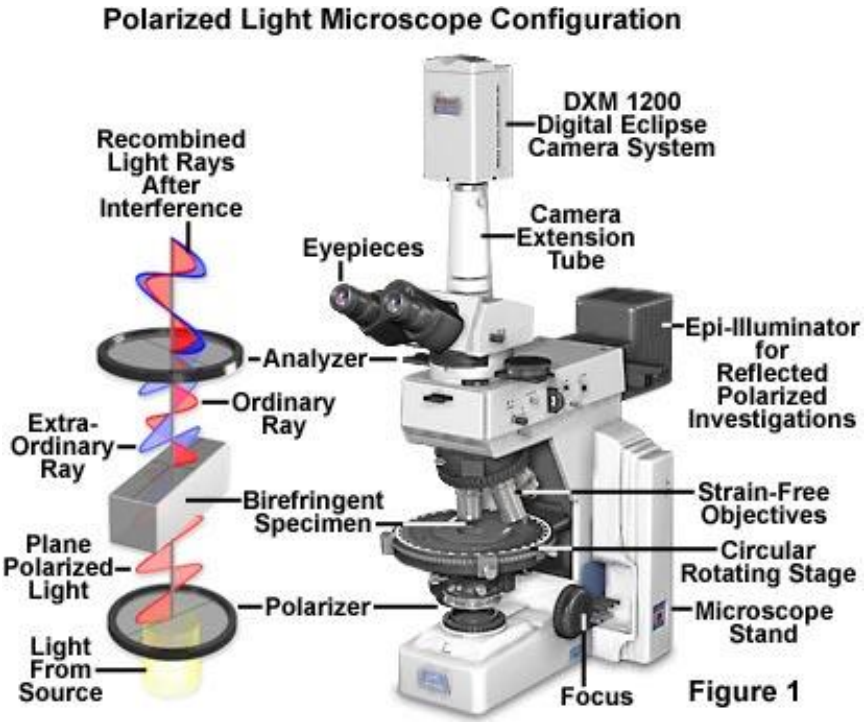
Utilizing anisotropy to study anisotropic events

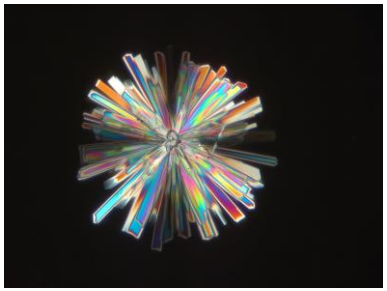


Bürck et al. Acc. Chem. Res. 2016, 49:184

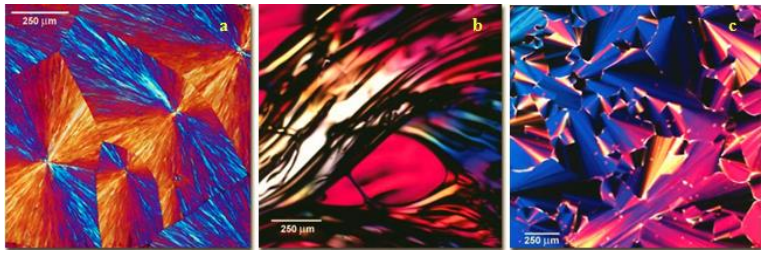
How about imaging/microscopy?

Polarization microscopy:

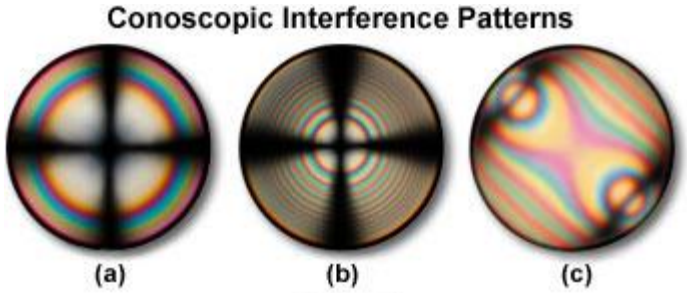




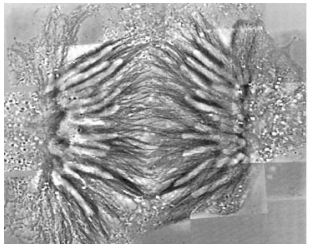
Honey crystal (Zeiss)
(crystalline objects in general)



Polymer and liquid crystalline phases (Zeiss)



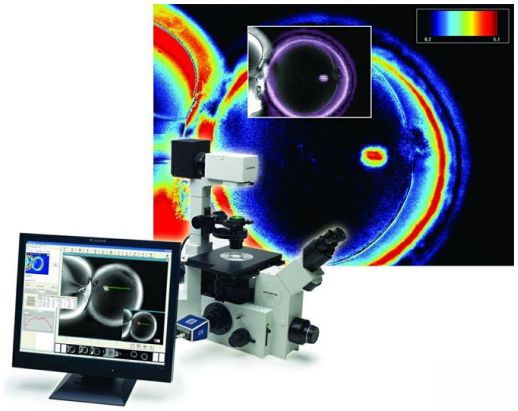
Conoscopy



Life science (microtubules)

Polarization imaging

PolScope



Metripol

Rotopol

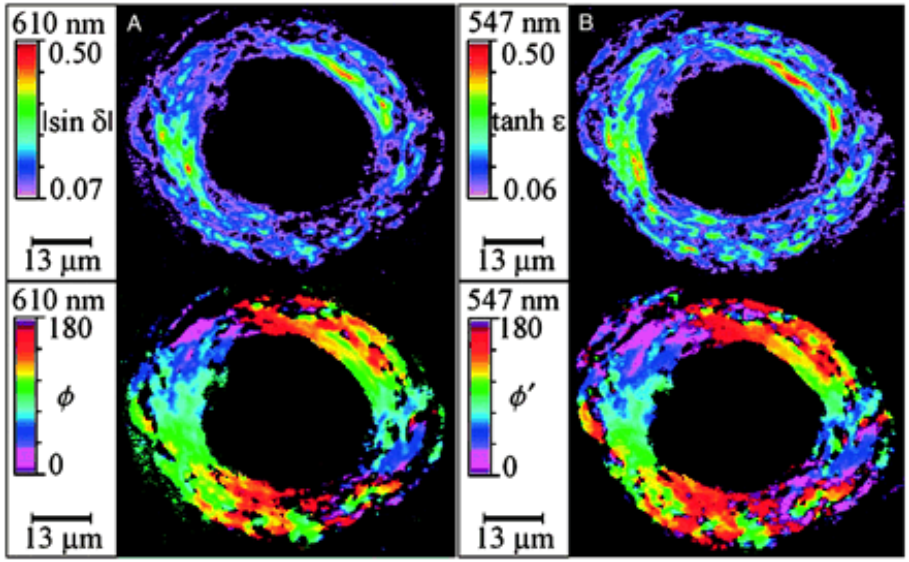
Polarization imaging

- PolScope / Metripol / Rotopol

- Aim to separate the contributions from
  - Birefringence
  - Linear Dichroism
  - Circular dichroism (more generaly circular extinction)

- Algorithm is based (more or less) on rotating polarizer method

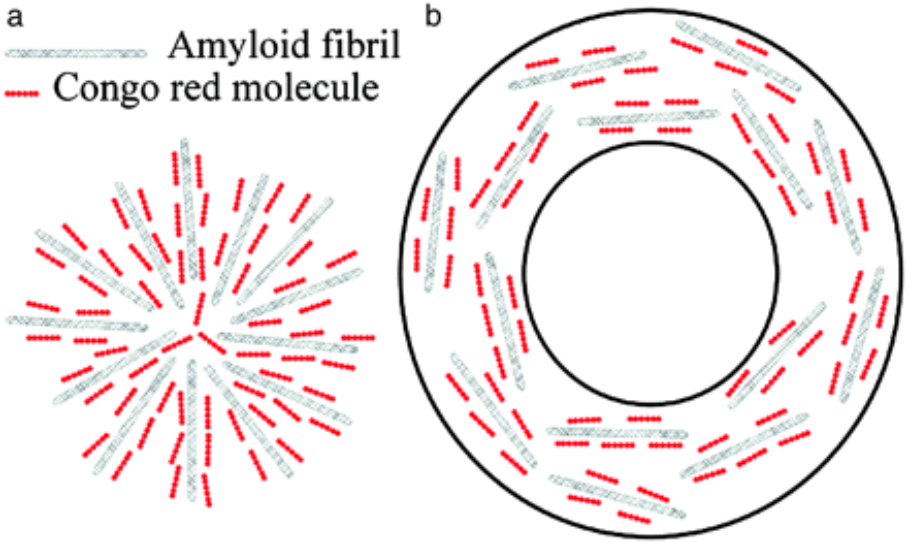
Birefringence and linear dichroism in amyloid plaques



Birefringence of elongated amyloids fibrils (left)

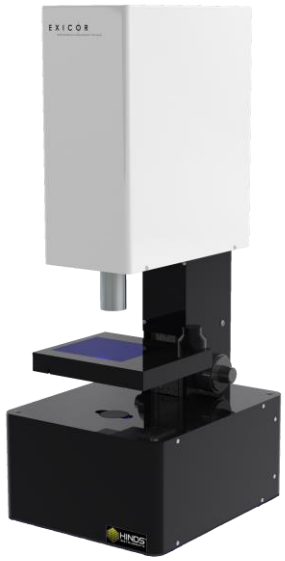
Dichroism of the oriented congo red (right)

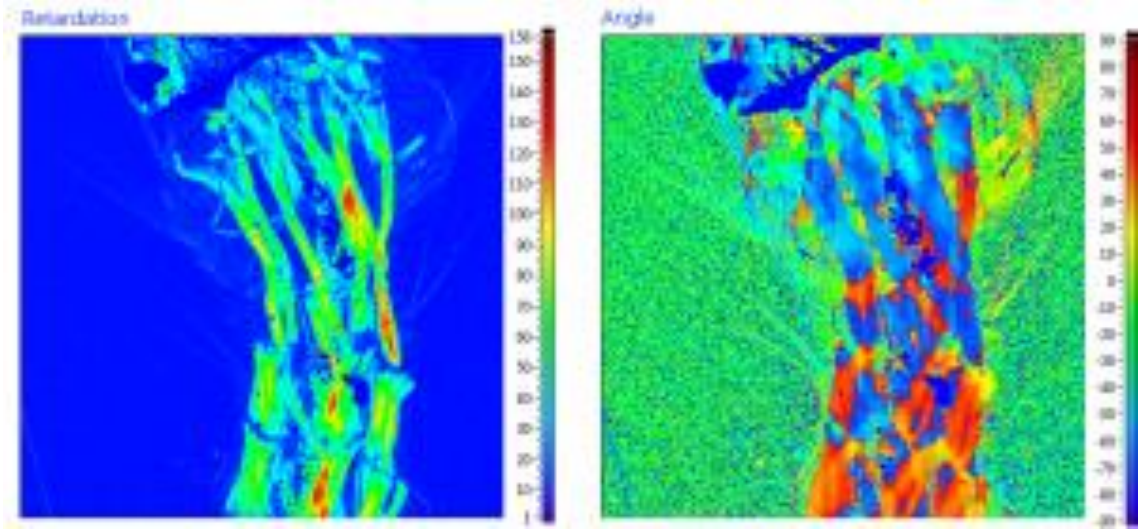
Putative model



Jin et al. PNAS 2003, 26:15294

Modulated Polarization





Drosophila larvae

Exicor from HINDS
Note: HINDS is the main manufacturer of photo-elastic modulator

PolScope (Cambridge Research Instr) Abrio (Perkin Elmer) Exicor from Hinds

What next?

Problem with birefringence

Problem with linear dichroism

Problem with scattering induced CD

Not full spectra CD

Weak CD signals

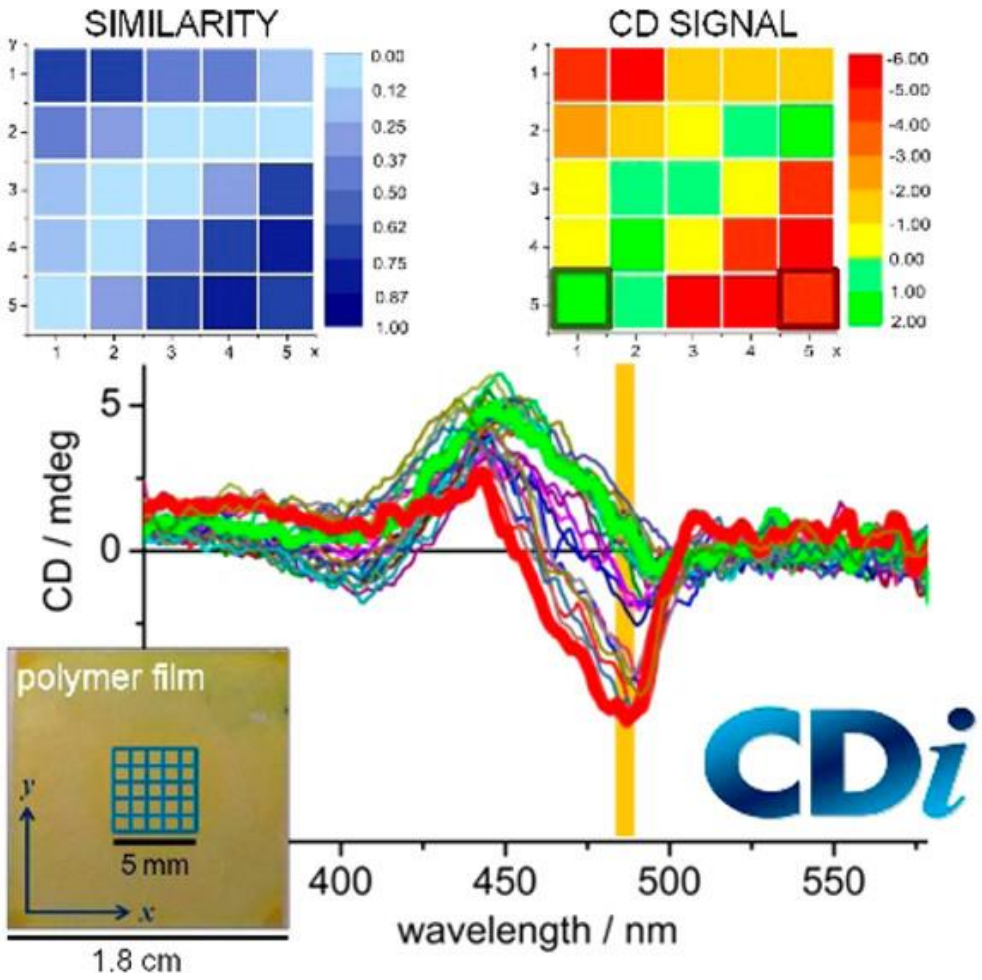
Two approaches

- Circular dichroism scanning of thin samples at synchrotrons
- Modulated circular dichroism
- High resolution microscope

Circular dichroism scanning

Cdi (circular dichroism imaging)
- thin films
- spatial resolution 1mm2
- removal of LD and LB by sample rotation

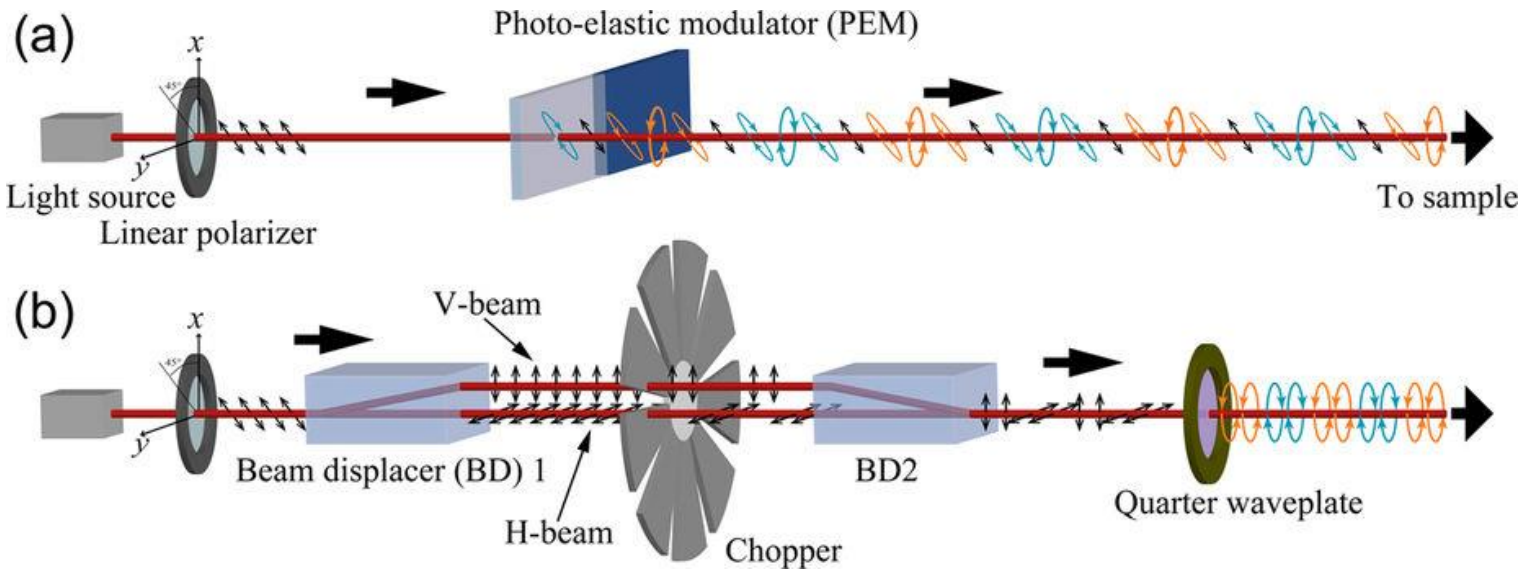
•Problems
- sample thickness
- spatial resolution
- artifacts



Zinna et al. Macromolecules 2017, 50:2054

Modulated circular dichroism imaging

Main idea is to illuminate the samples with a “clean” circularly polarized light

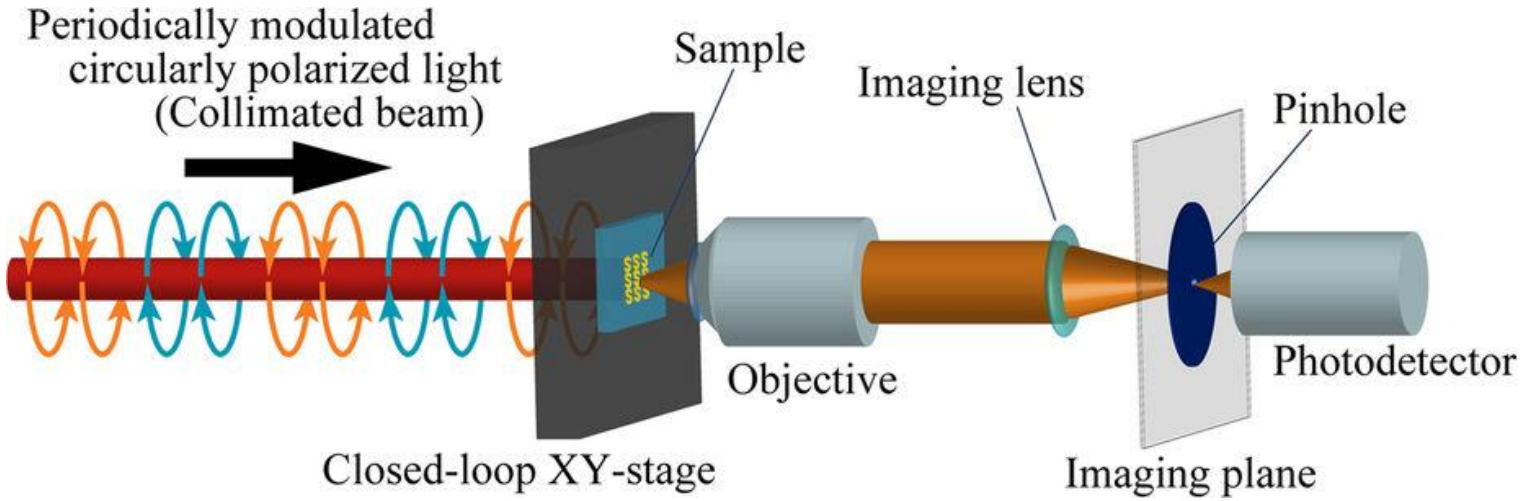


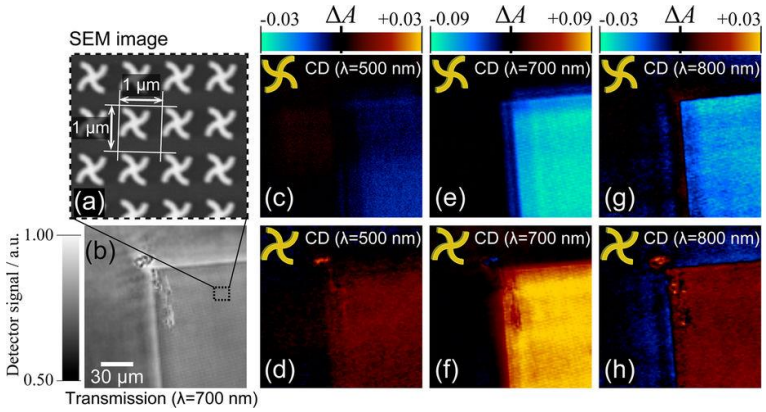
(a) Standard method

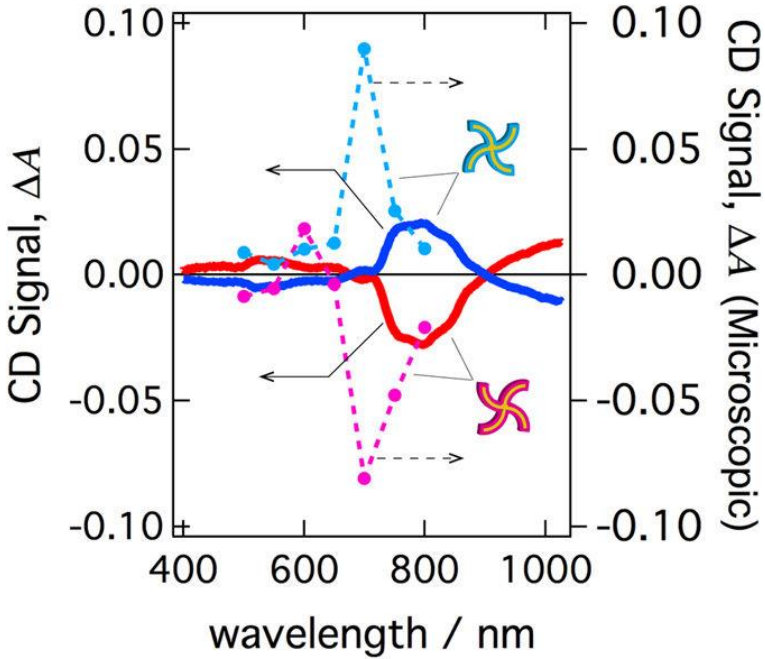
(b) modulated method

Narushima & Okamoto, Scientific Reports 2016, 6:35731

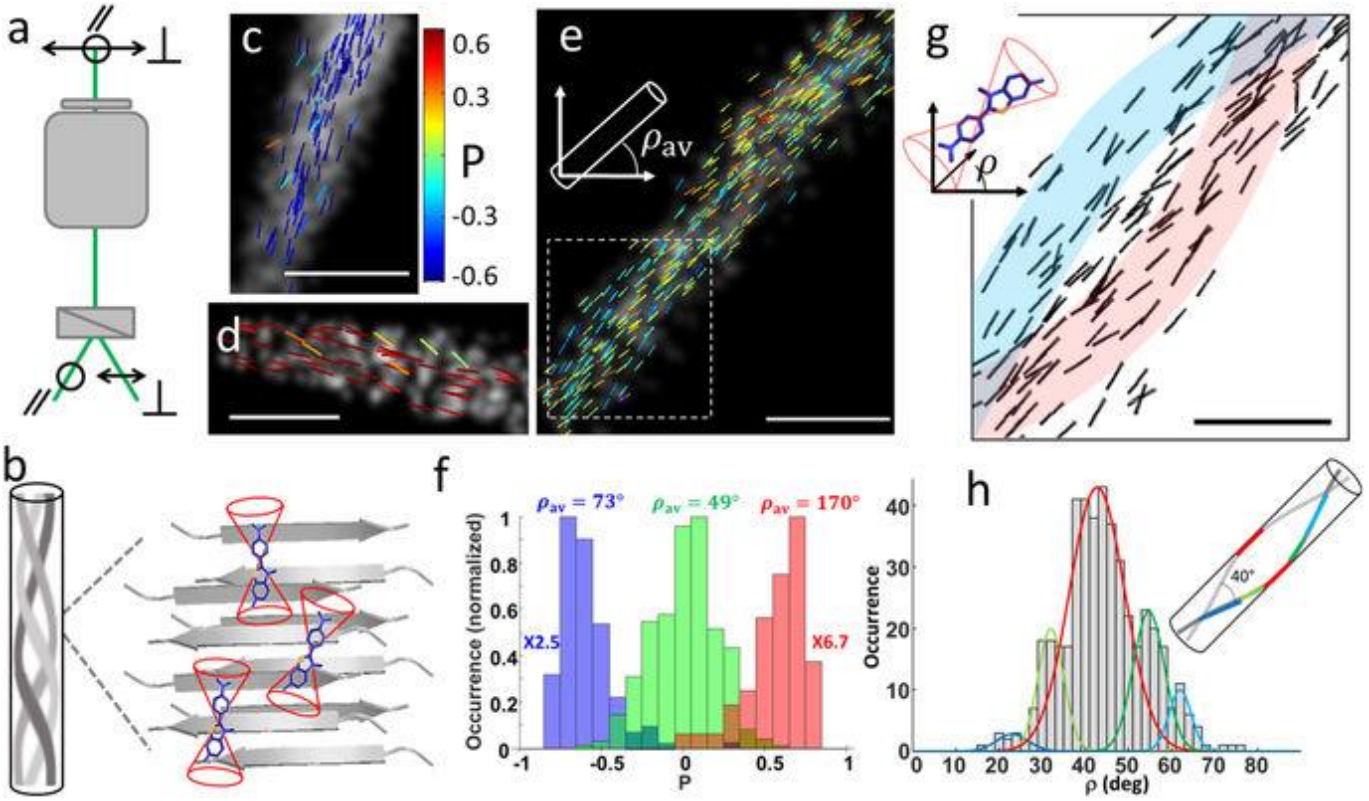
Modulated circular dichroism imaging





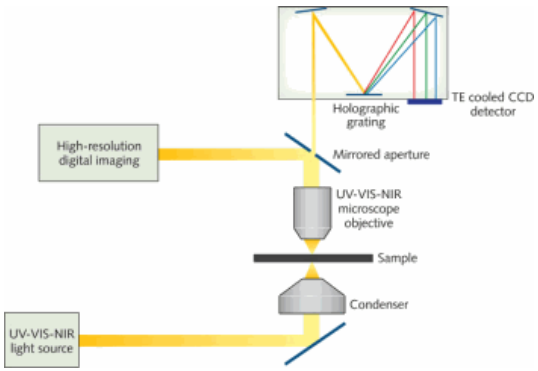


Polarized super-resolution structural imaging inside amyloid fibrils using Thioflavine T



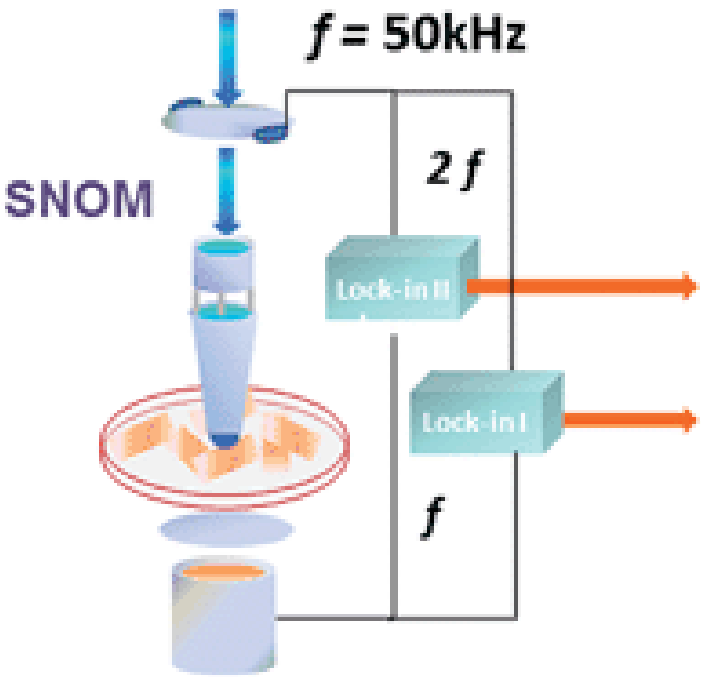
Shaban et al. Scientific Reports 7, 12482

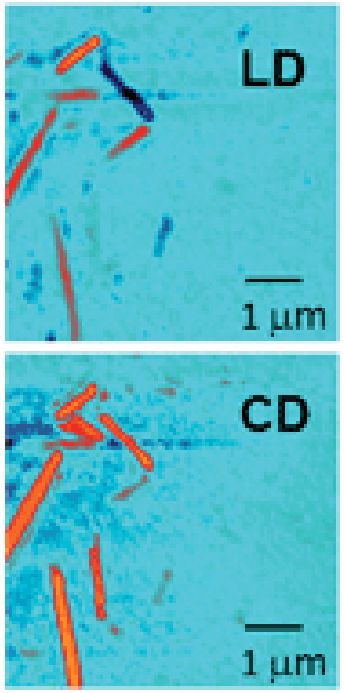
Microspectrometry (may be) – SNOM (may be)



Microspectra /CRAIC

Polarization modulated Scanning near field optical microscopy





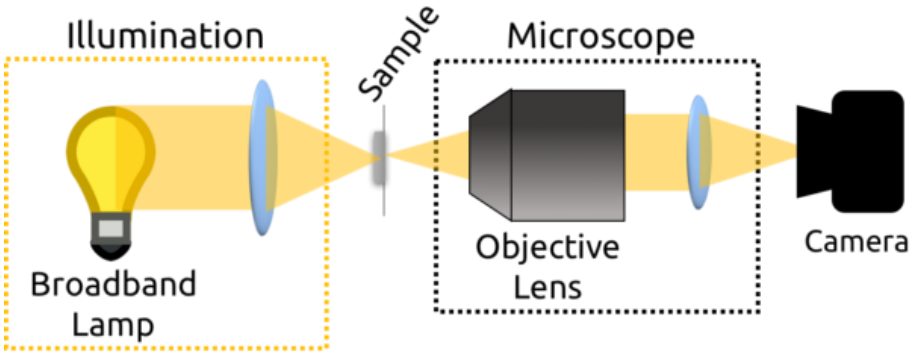
Tantussi et al. Nanoscale 2004, 6:10874

What next?

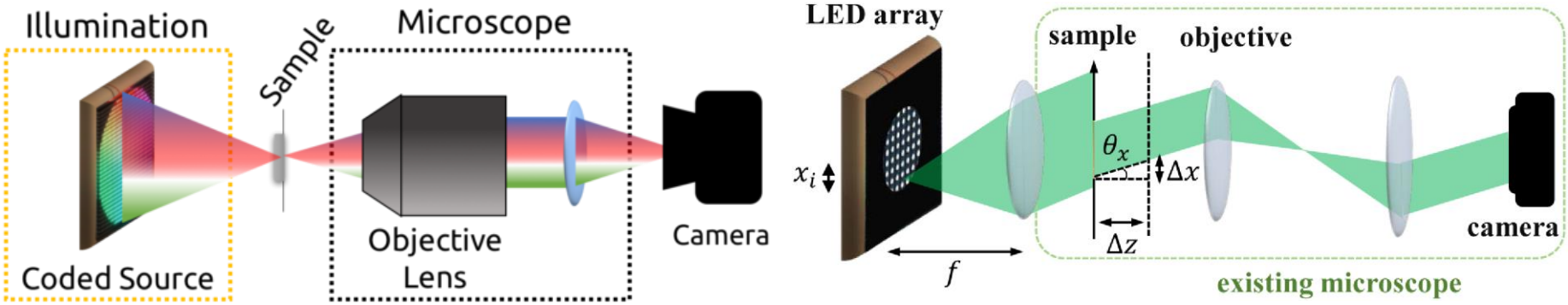
You do not have that much money!!!

Structured illumination using LED panels

Standard microscope

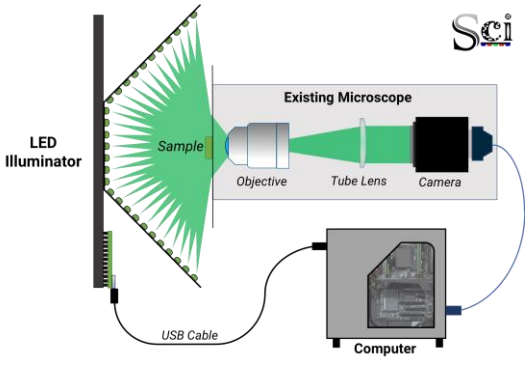


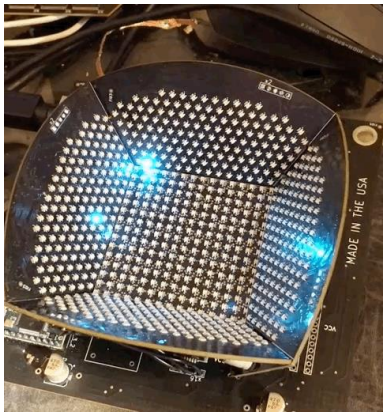
LED microscope

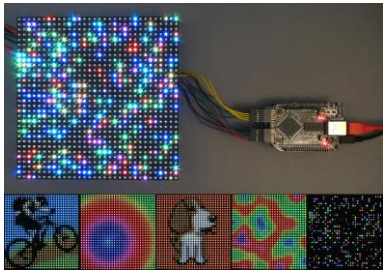


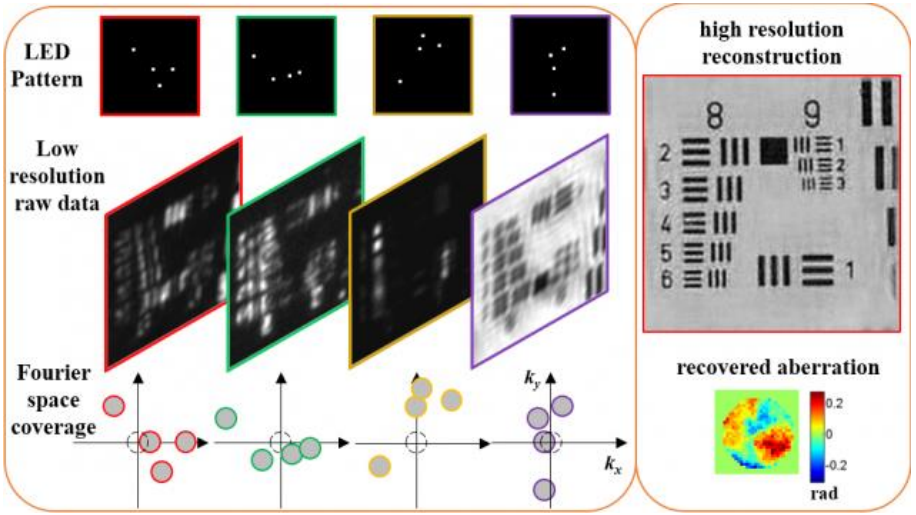
Source Spectral Coded Illumination, Berkeley; see Laura Waller compiled work

Structured illumination a bit more expensive



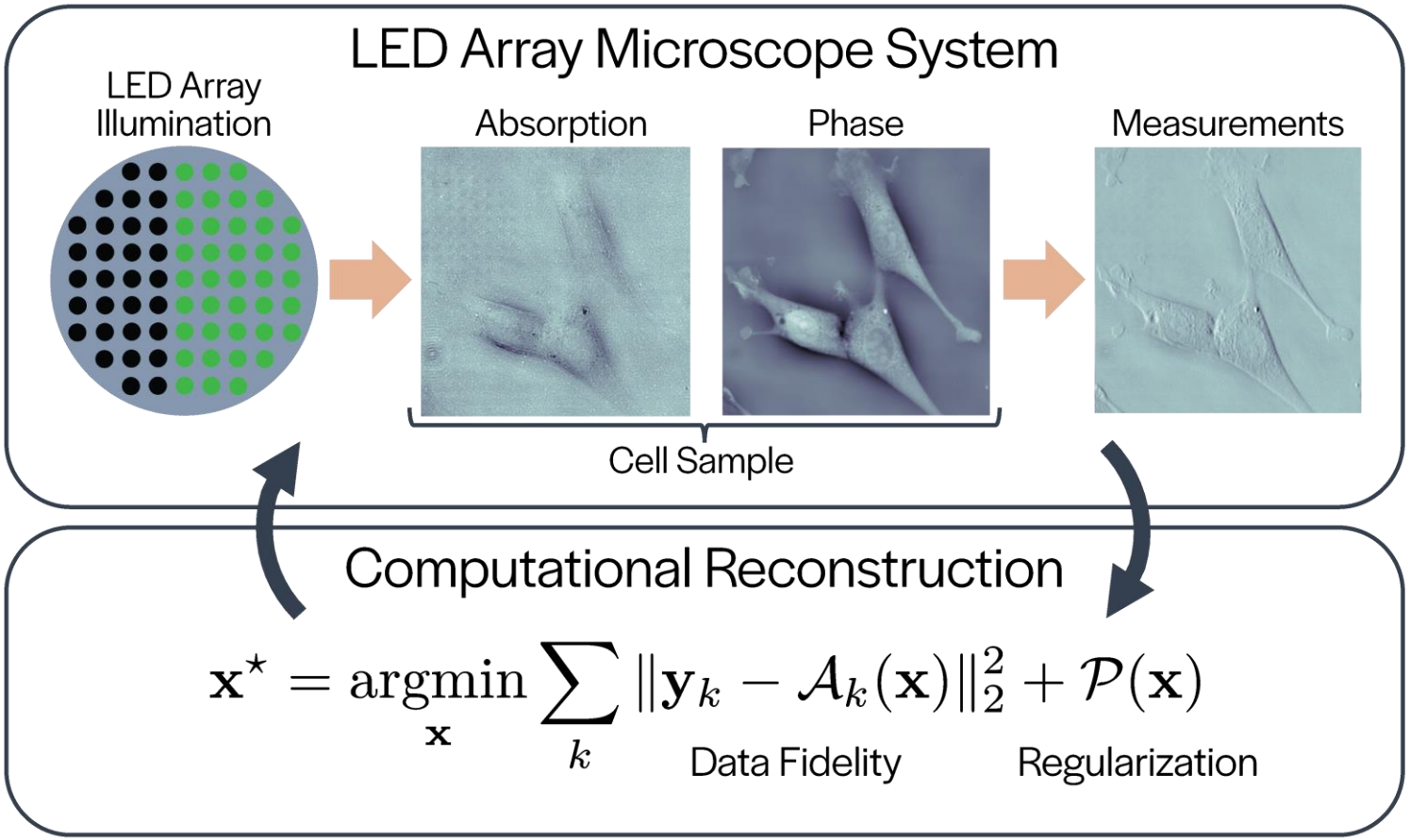




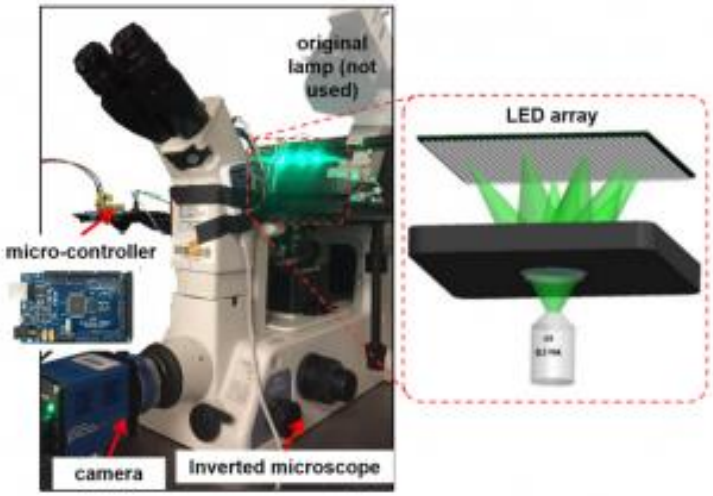


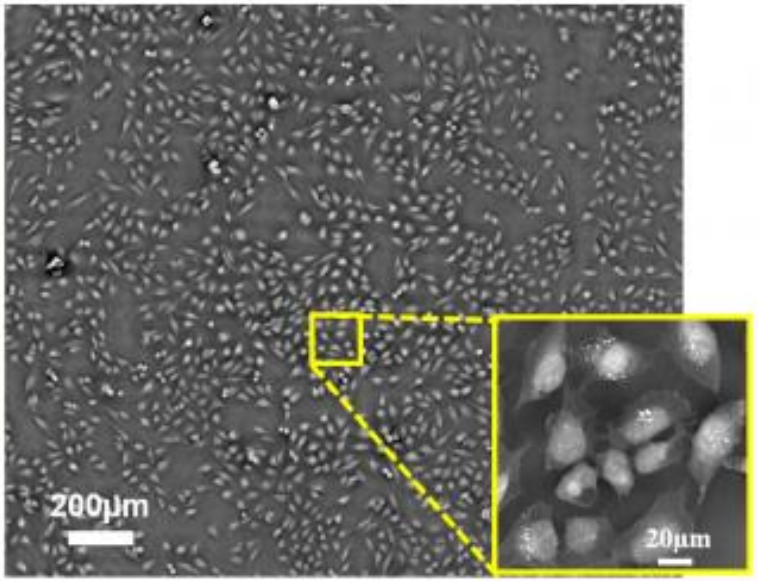
Source Spectral Coded Illumination, Berkeley

Quantitative phase imaging



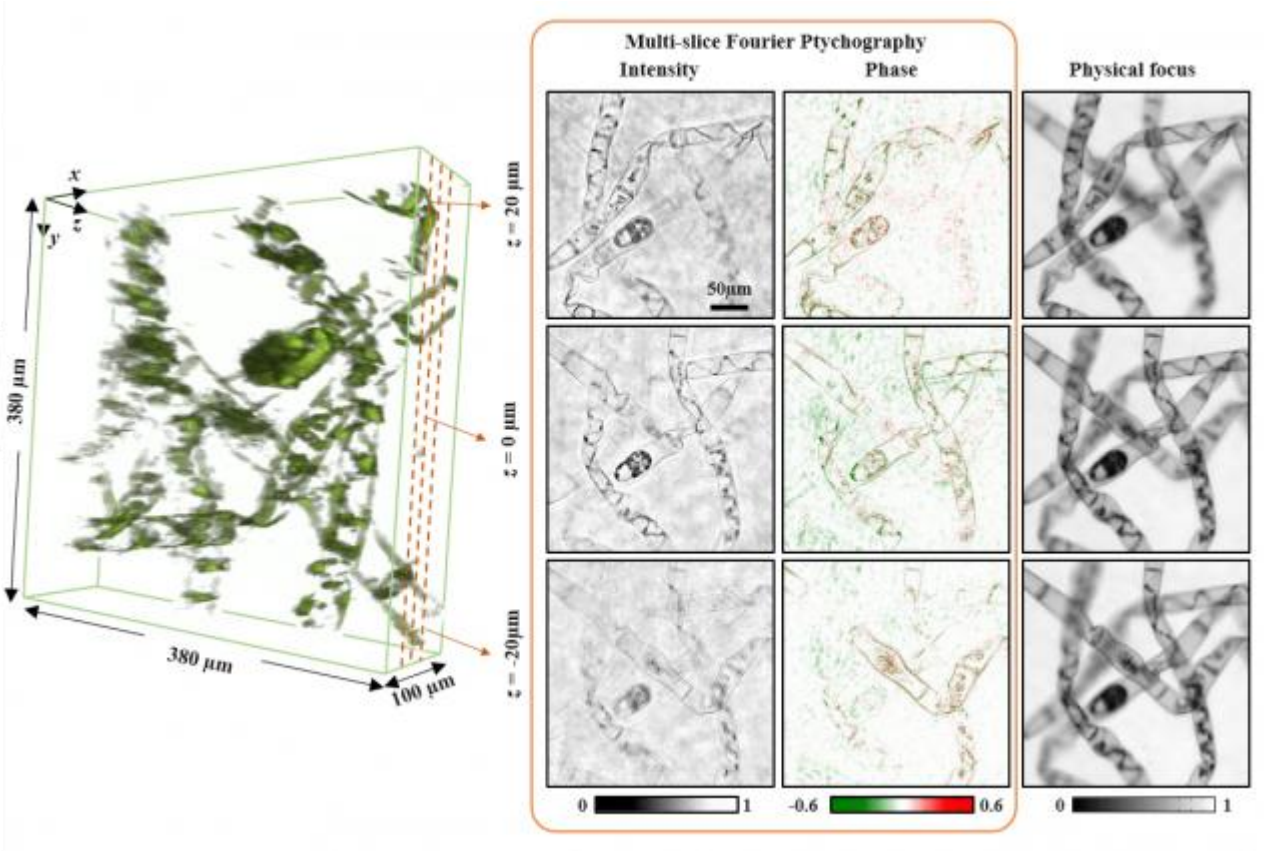
Giga-pan





Source Spectral Coded Illumination, Berkeley; see Laura Waller (Berkeley) and Lei Tian compiled work (Boston)

Tomography



see Lei Tian compiled work (Boston University)

Conclusion

- Circular dichroism imaging may be !?
- Issues with
  - Birefringence
  - Linear dichroism
  - Scattering
- Scanning methods and high resolution microscopy as a possible alternative
- Improved polarization microscopes (“polarizations”) may provide a solution
- A good alternative is fluorescence polarization
- Another alternative is structured LED illumination

Thanks