Studii asupra celulelor utilizand senzori optici in domeniul UV-Vis

Mircea Bercu[1] D.Poenar[2]

[1]Universitatea Bucuresti
[2]Nanyang Technological University-Singapore

OPTICAL SENSORS AND SIGNALS TREATMENT FOR RAPID BIO-SAMPLE INVESTIGATION - MULTIPLE PRINCIPLES DETECTION

Cell- cell interaction and clusters formations

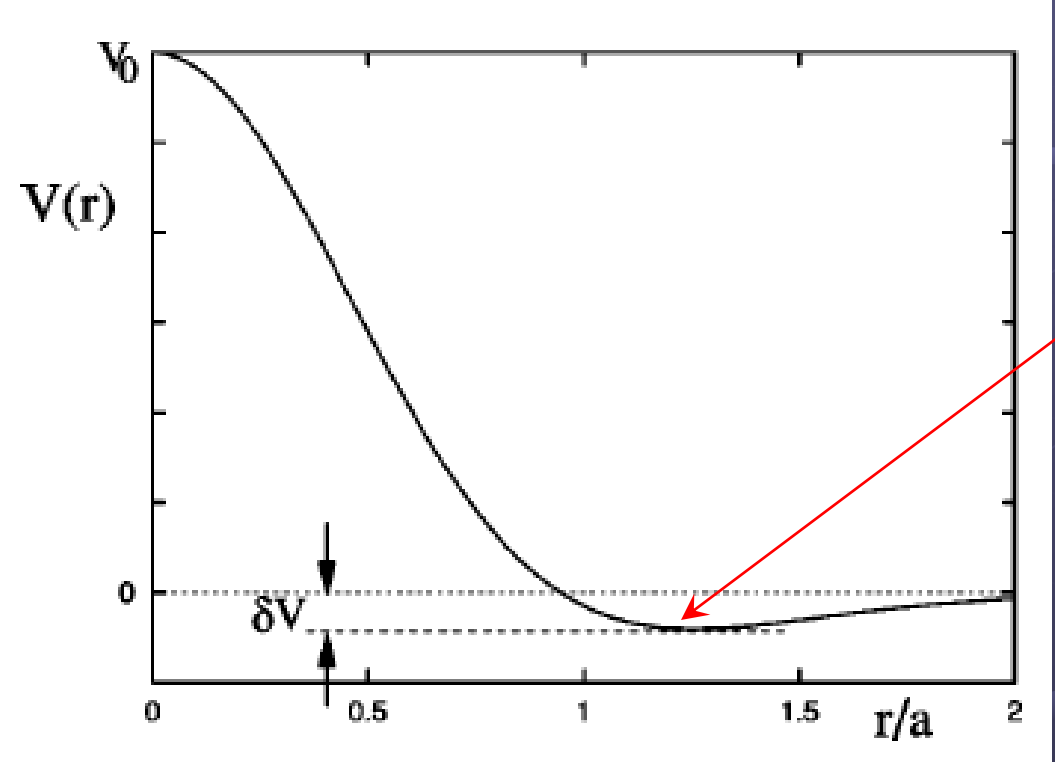
Description and characterisation of an optical sensor of high sensitivity for bio-cells characterisation

Aggregation of cells investigated by using the optical sensor

On cip spectrometry and rapid cell characterisation

Individual cell diffraction pattern and parameters extraction by modelling of light scattering pattern.

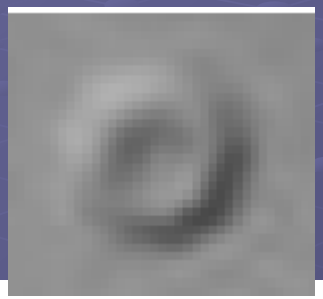
Cell-cell interaction



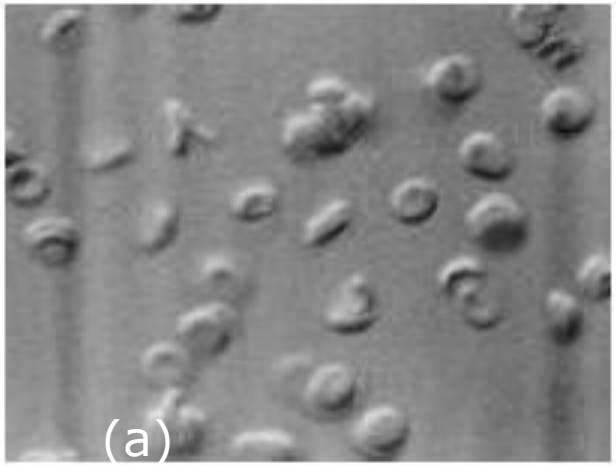
Many-body theory of chemotactic cell-cell interactions
T. J. Newman1,2 and R. Grima1, PHYSICAL REVIEW E **70**, 051916 (2004)

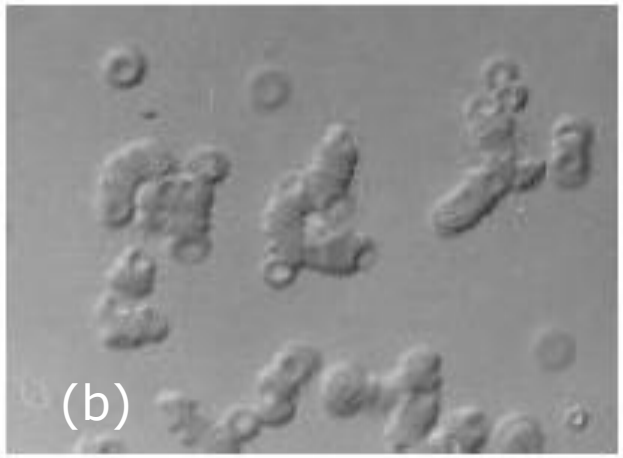
Human blood cells aggregation

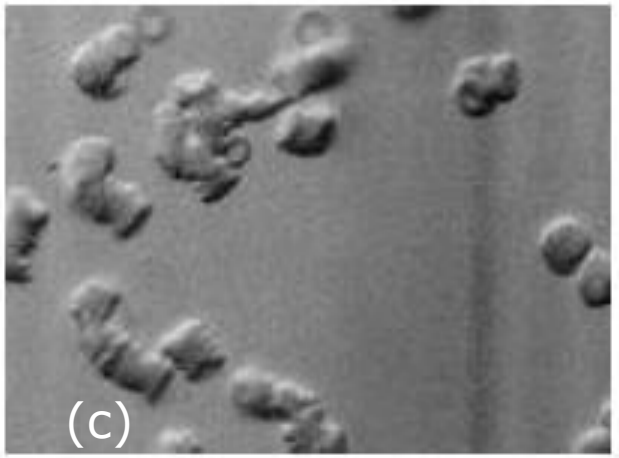
Single cell



Five cells cluster







Typical red blood cell aggregates with increasing degree of aggregation. (a) mild aggregation (b) moderate aggregation (c) severe aggregation.

MEASUREMENT SCIENCE REVIEW, Volume 7, Section 2, No. 5, 2007, 43
Assessment of Human Red Blood Cell Aggregation A. Kavitha, S. Ramakrishnan
Department of Instrumentation Engineering, MIT Campus, Anna

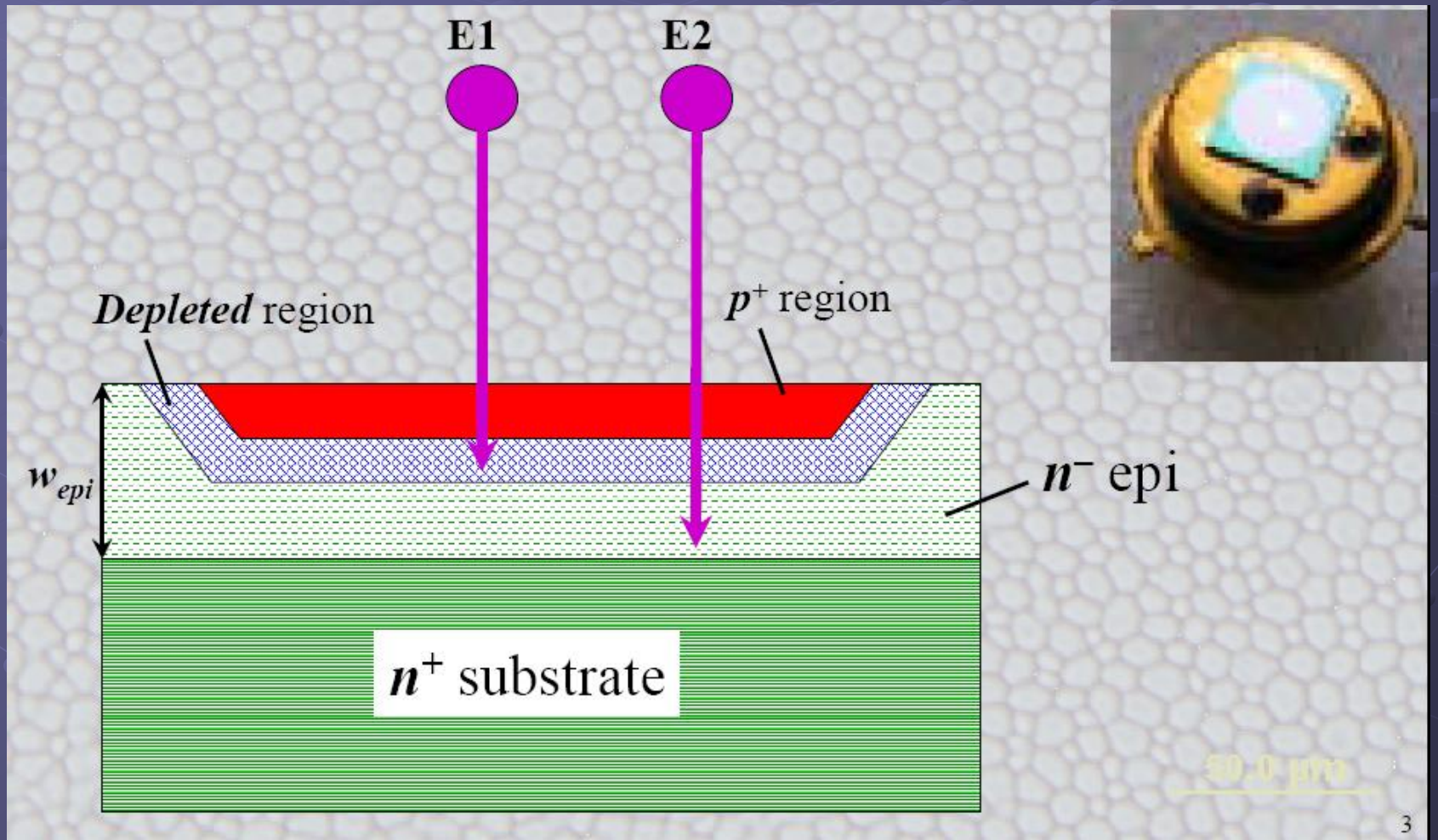
The importance of quick monitoring of blood cells aggregation

Potential applications

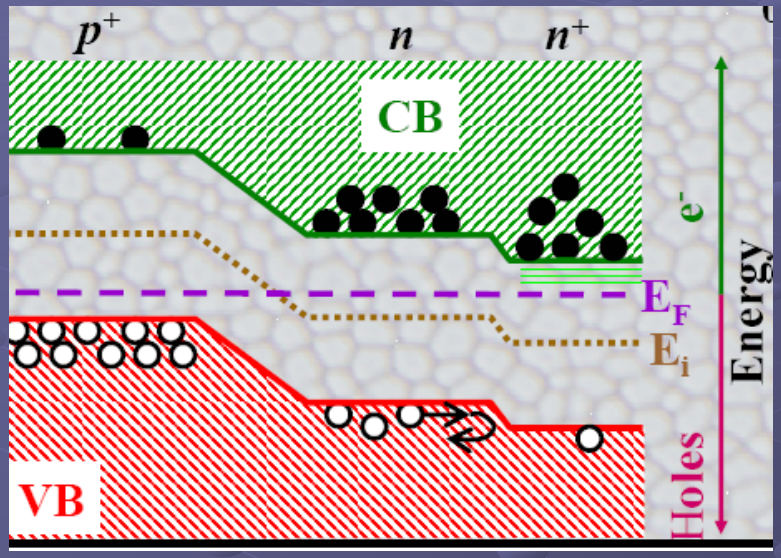
- Enhanced red blood cell (RBC) aggregation has an adverse effect on microcirculatory blood flow and tissue oxygenation.
- It has been previously shown that obesity is associated with increased RBC aggregation.
- It is of high interest to examine whether the enhanced aggregation is a plasma- or cellular-dependent process.
- Abnormal red cell aggregation has been found to be associated with several diseases and conditions, which include:
  - **diabetics**, -**malaria**, -**cardiovascular malfunction**, -**lacunar brain infarcts**, -**essential hypertension**, and **hematological disorders**, **local anesthesia** etc.

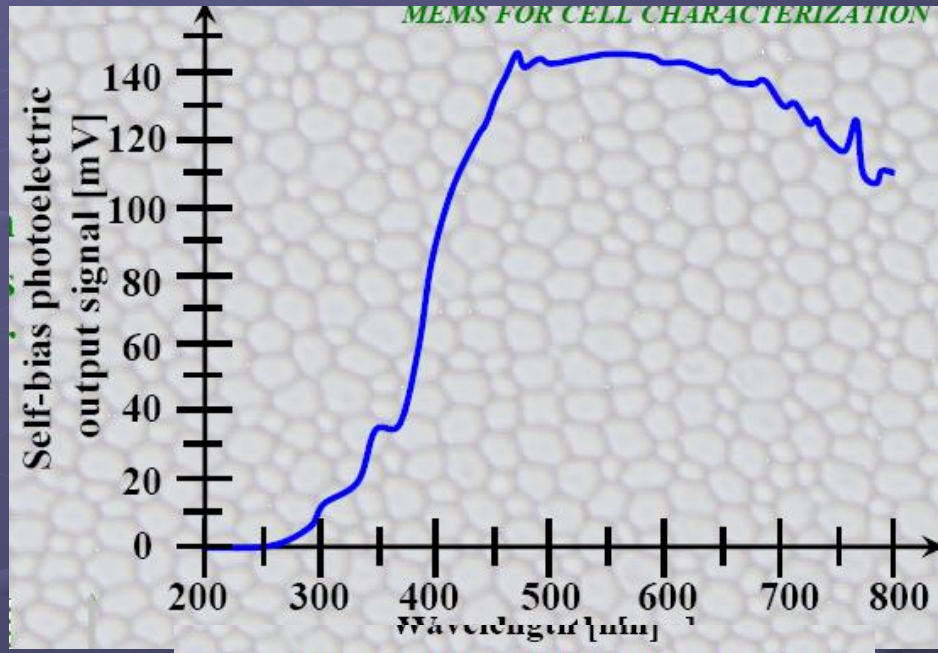
Mchedlishvili G., Varazashvili M., Mamaladze A. and Momtselidze N., **Bloodflow structuring and its alterations in the capillaries of the cerebral cortex**, Micro Vasc. Res., 53, 201-210,1997.

Optical sensor for bio-cells characterisation



Optical sensor for bio-cells characterisation





Low doped epi =>

A1) Large diffusion length (496 μm) of minority carriers copared to the epi layer thickness (7 μm): $L_D >> w_{epi}$

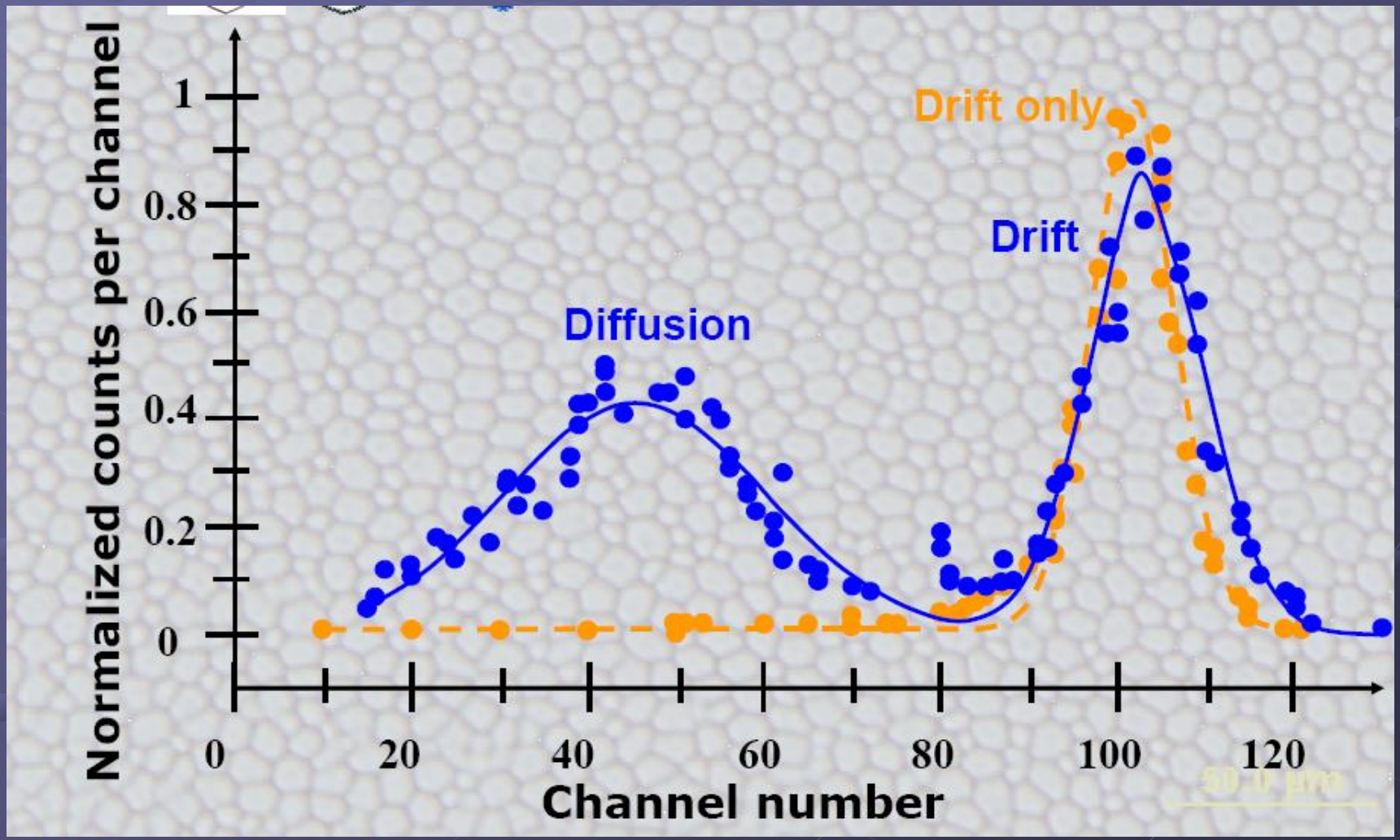
A2) Small potential barrier at the n-n^+ 'junction' =>

=> B1) Easy diffusion of photo-generated minority carriers;

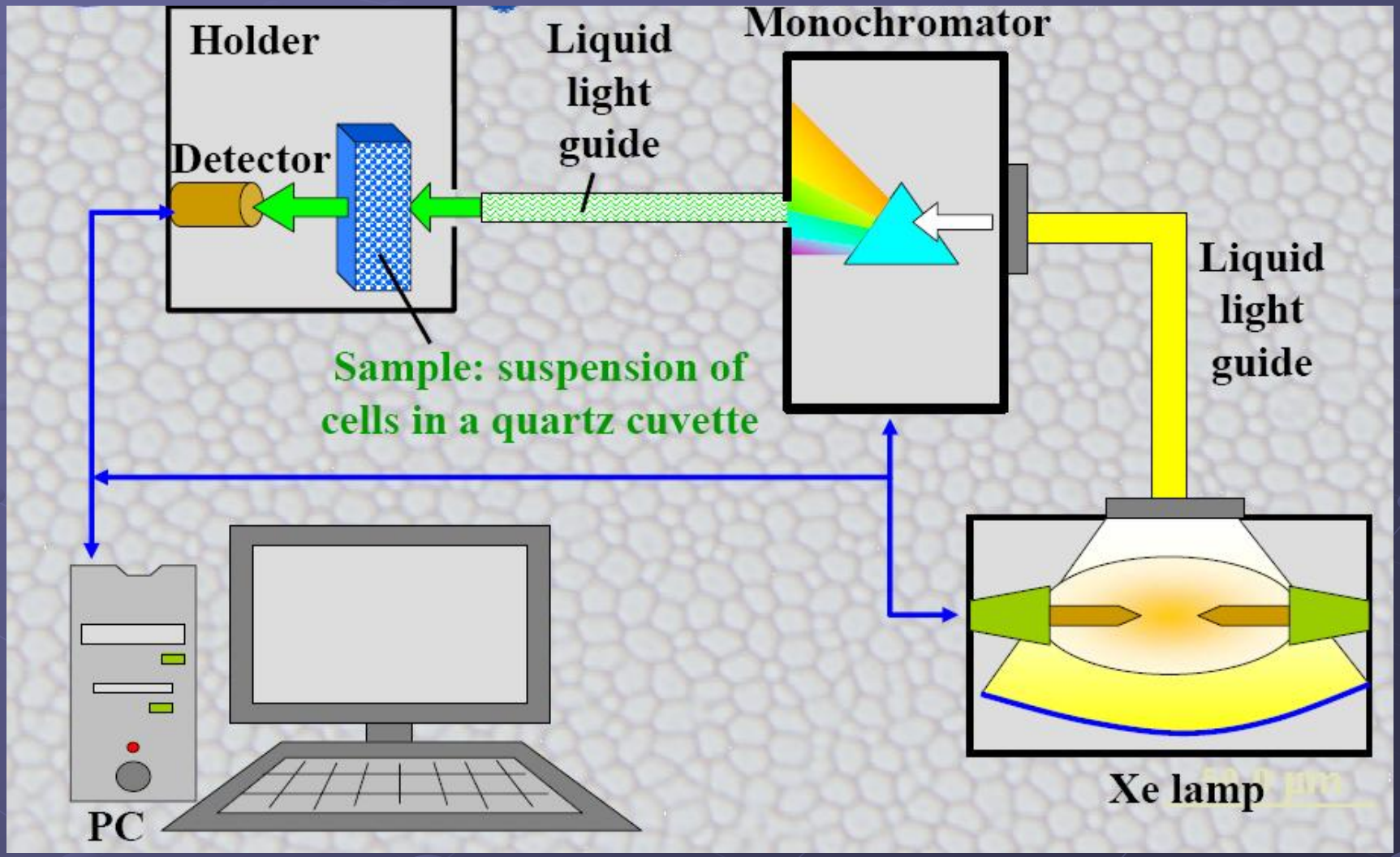
B2) Reflection of holes at the n-n^+ interface.

All these factors enable an ↑ photocollection by both diffusion & drift => ↑ photosensitivity.

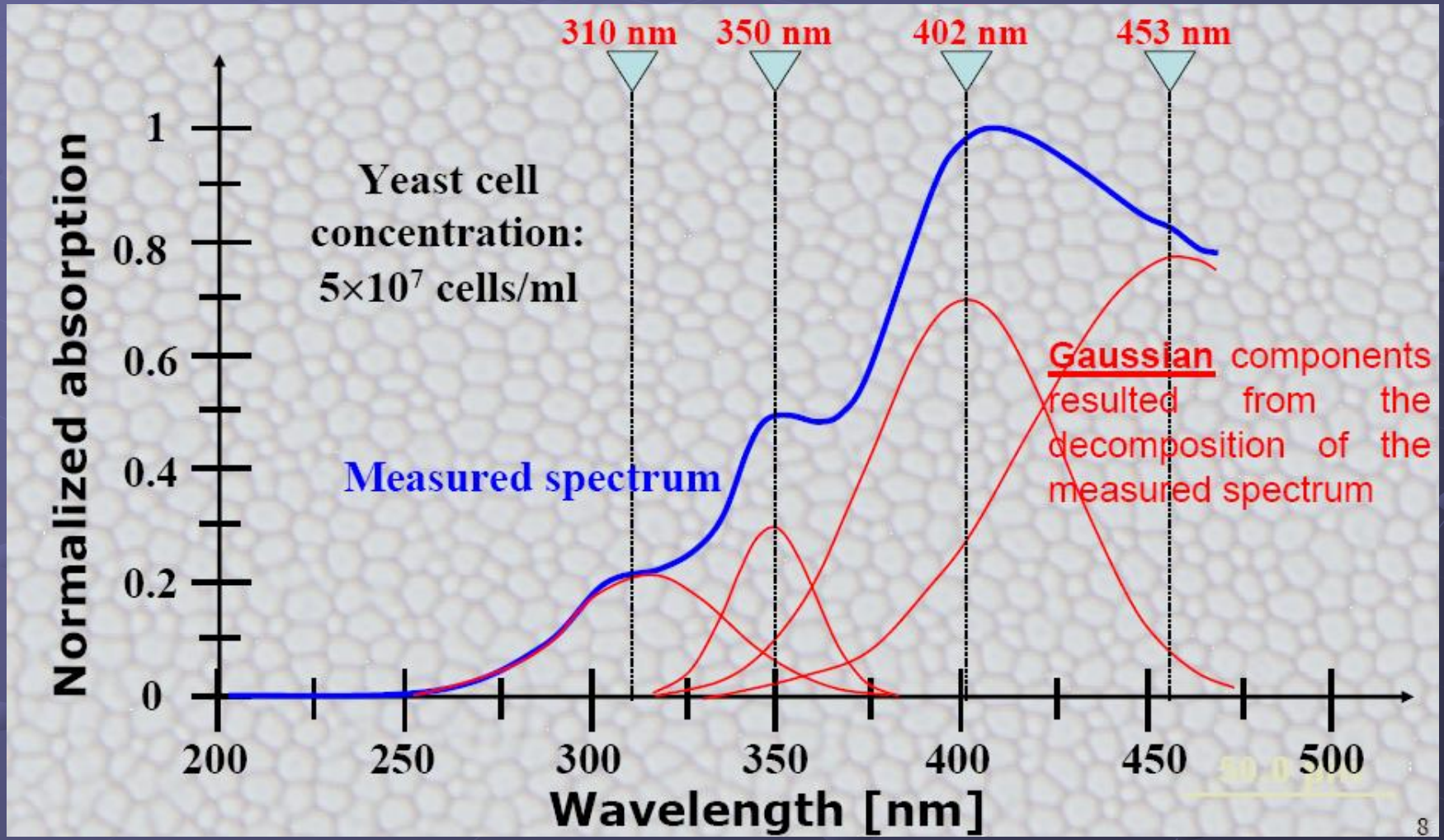
Amplitude electric impulses spectrum produced by alpha particle (Test for extra charge collection in optic sensor)



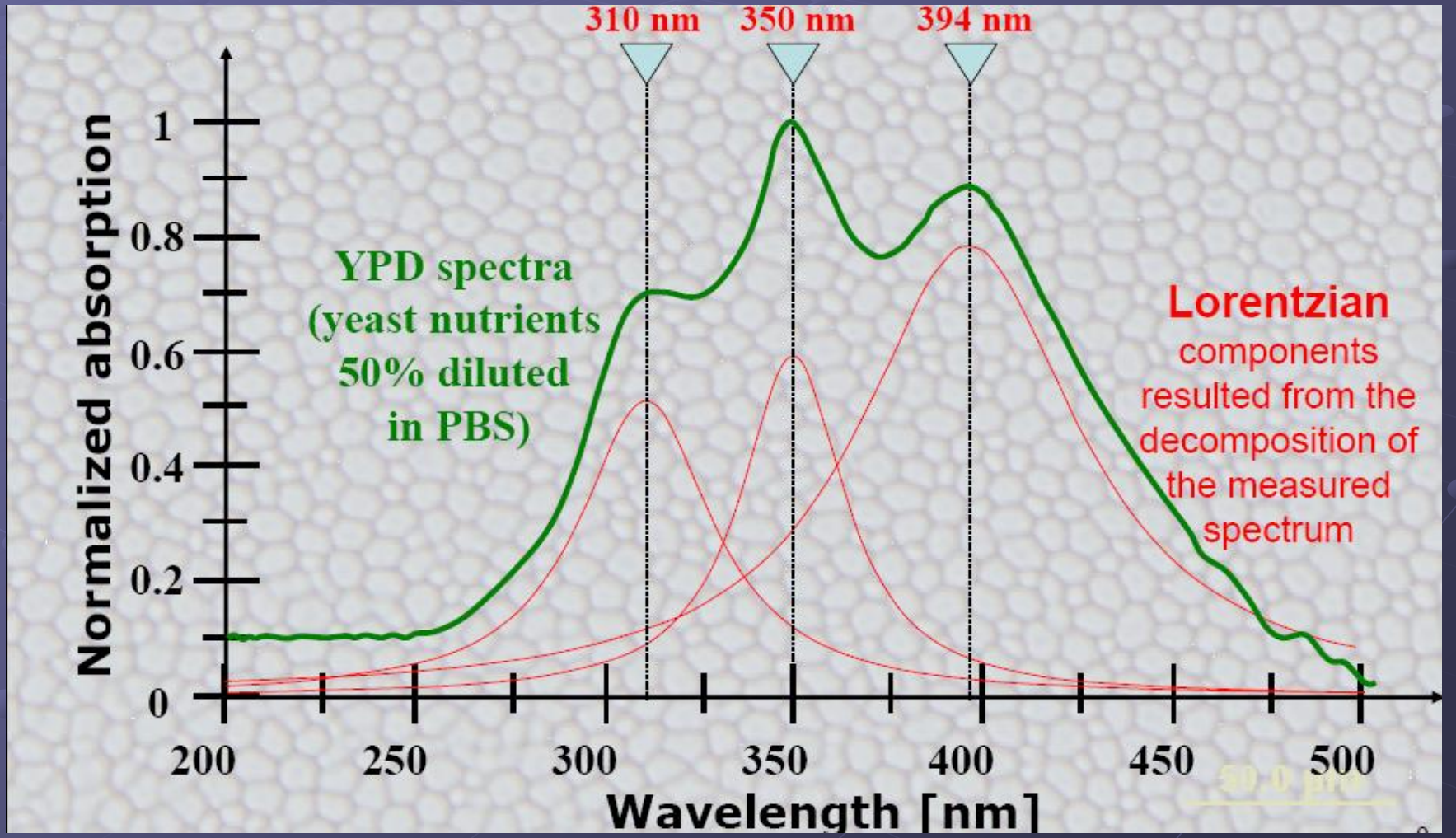
Experimental set-up



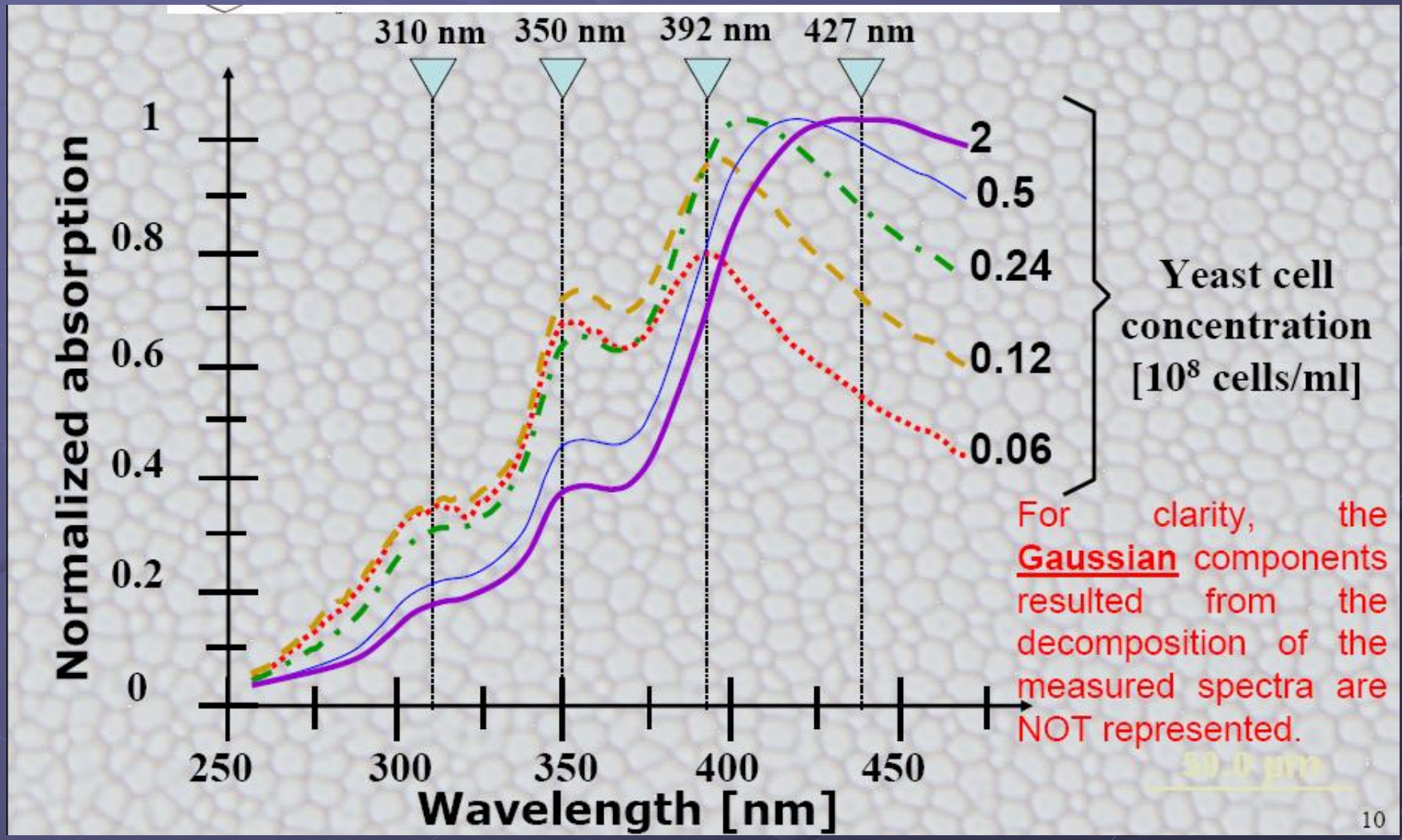
Spectral data obtained on Yeast cells and absorption lines extraction



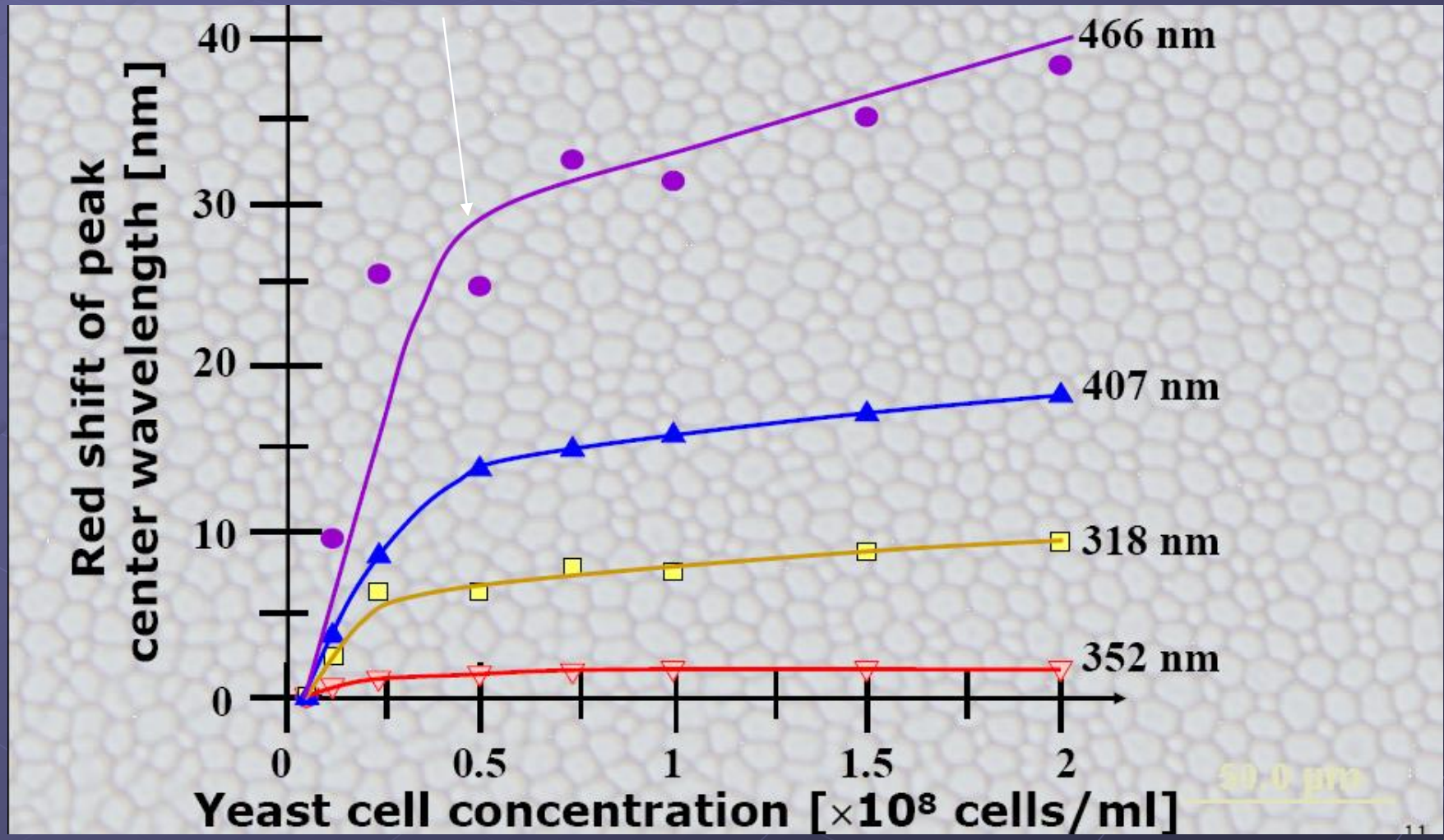
Absorption bands assignation.



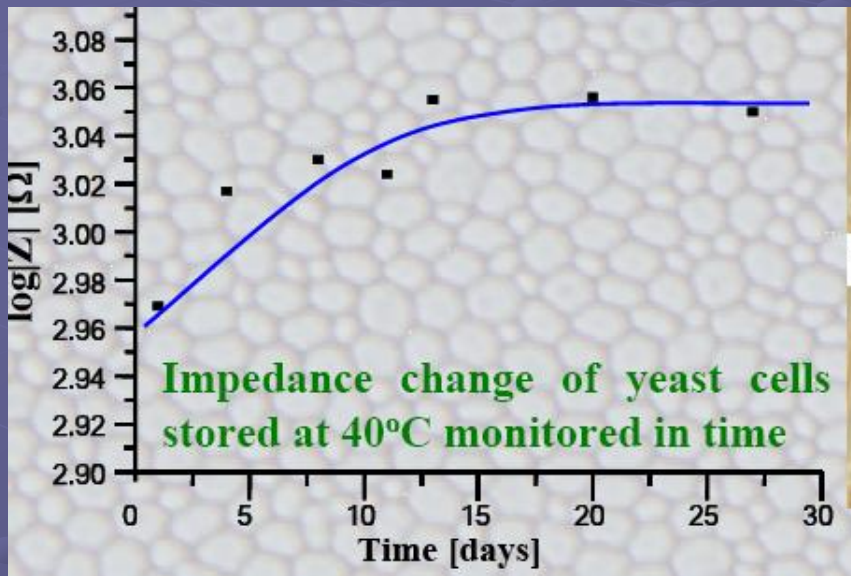
UV-Vis spectra at different concentrations of cells

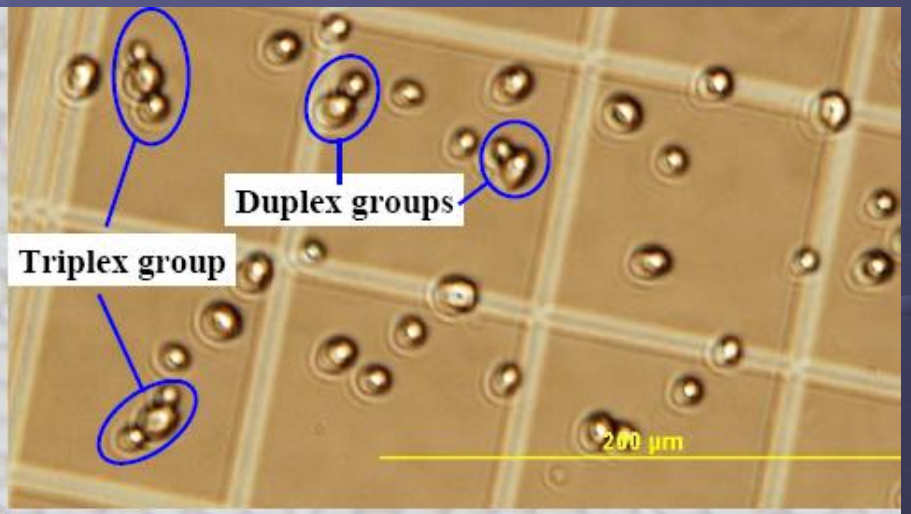


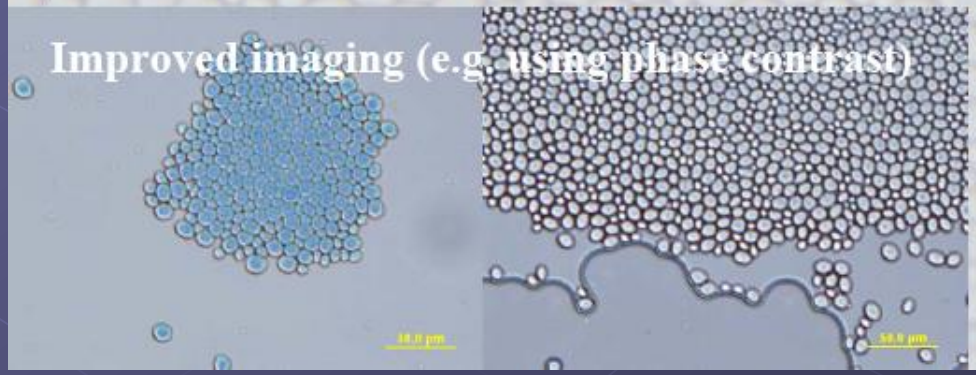
The shift of the absorption bands versus cells concentration



Views on large cells assembly

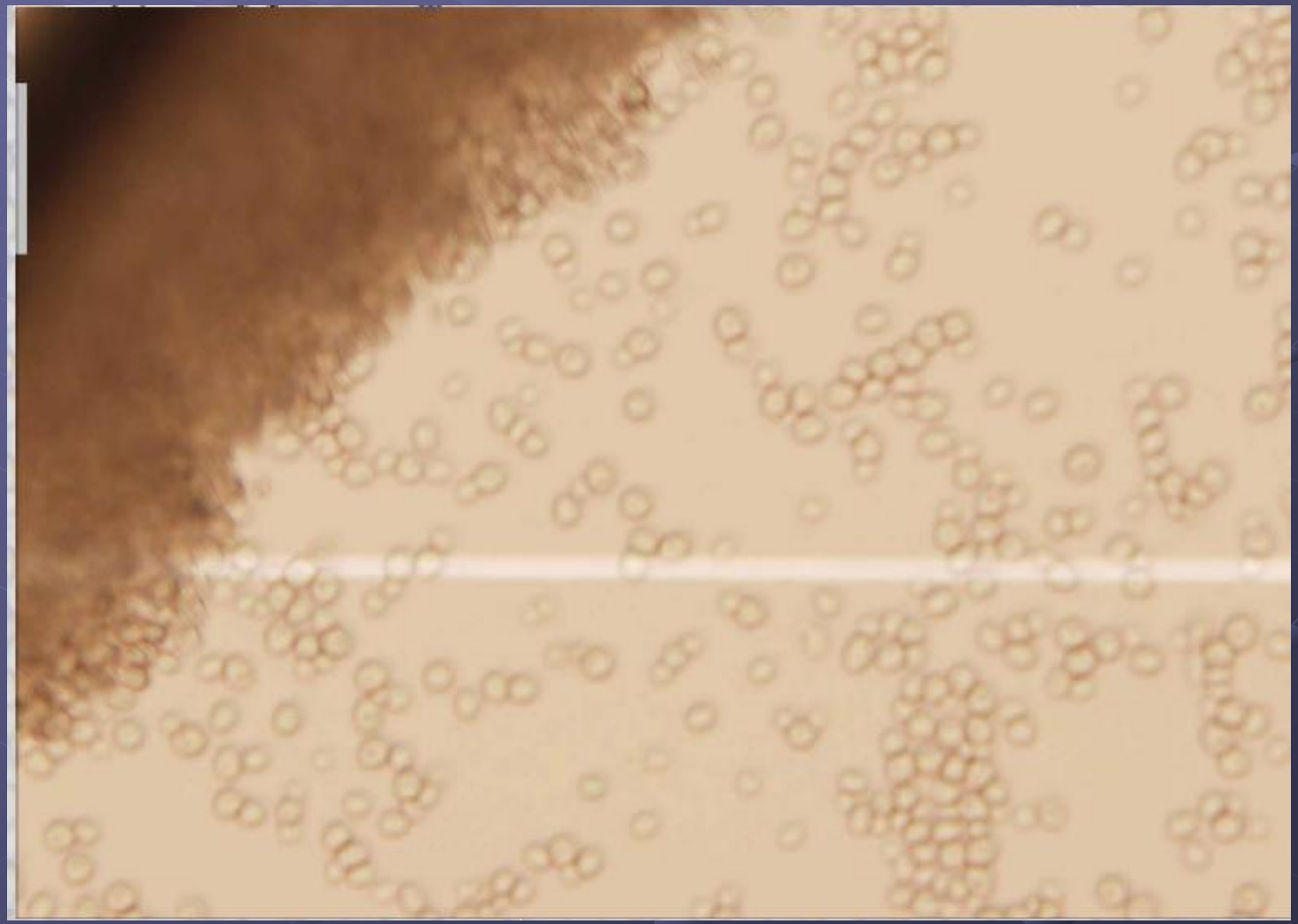




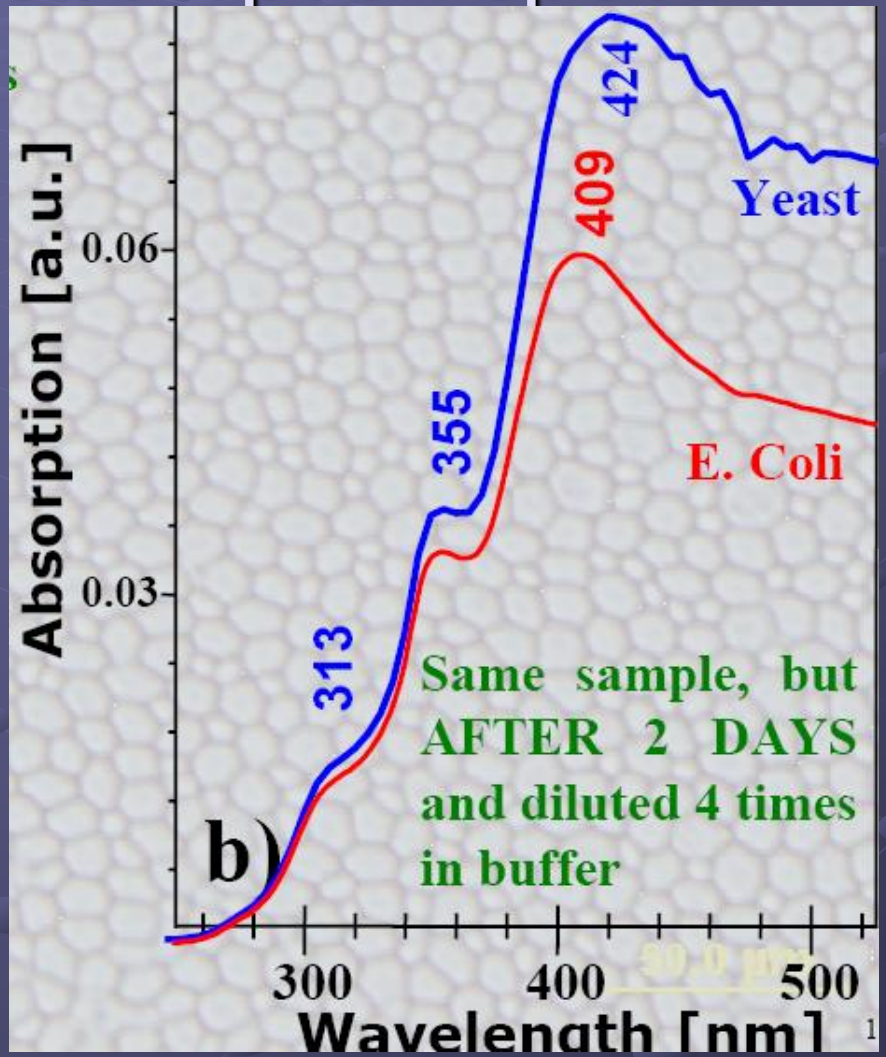


a) Heat-killed yeast cells stained with methylene blue dye, and
b) Without staining

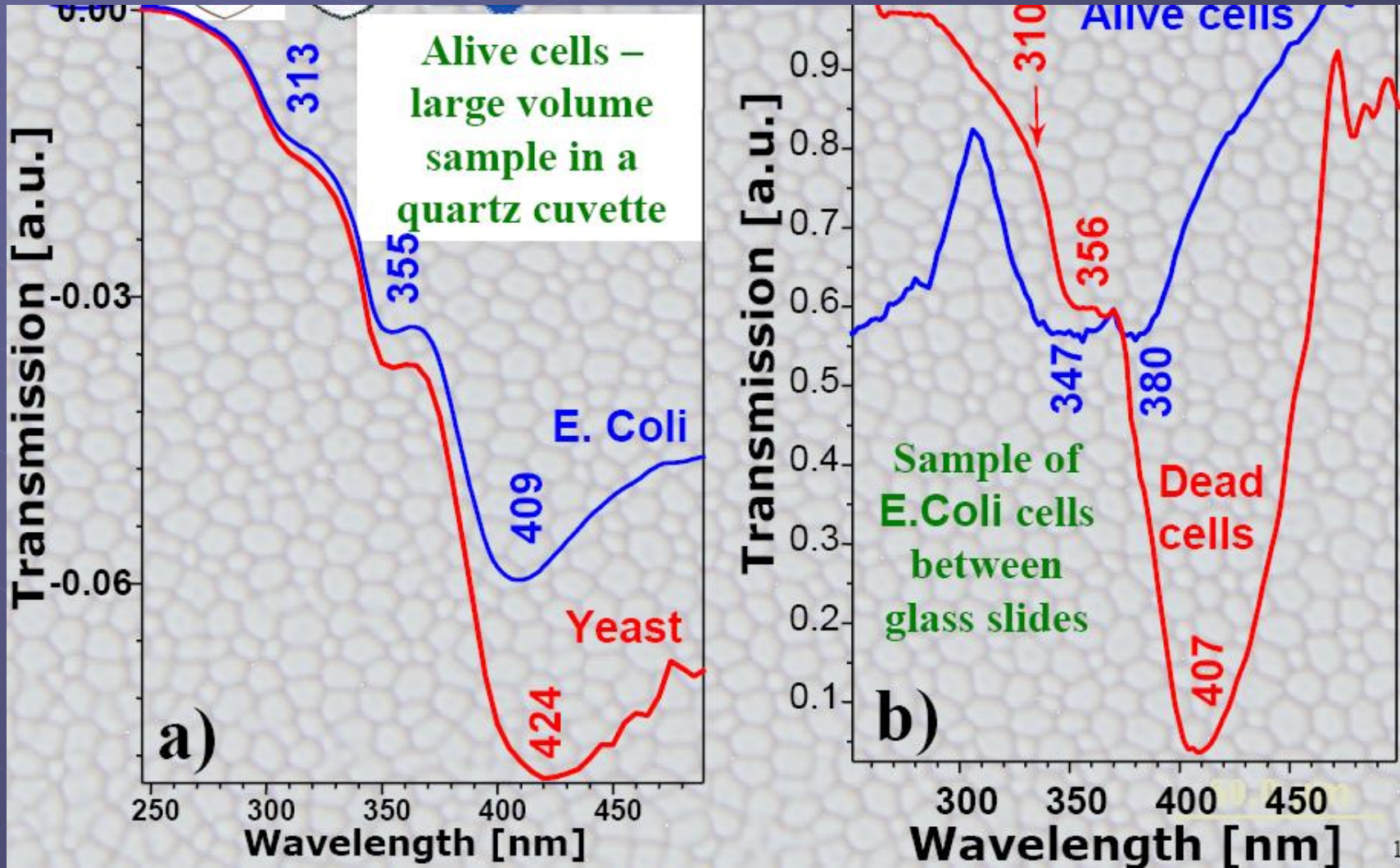
Cells clustering inside a water droplet



Data obtained by using “on chip absorption spectrometry”.

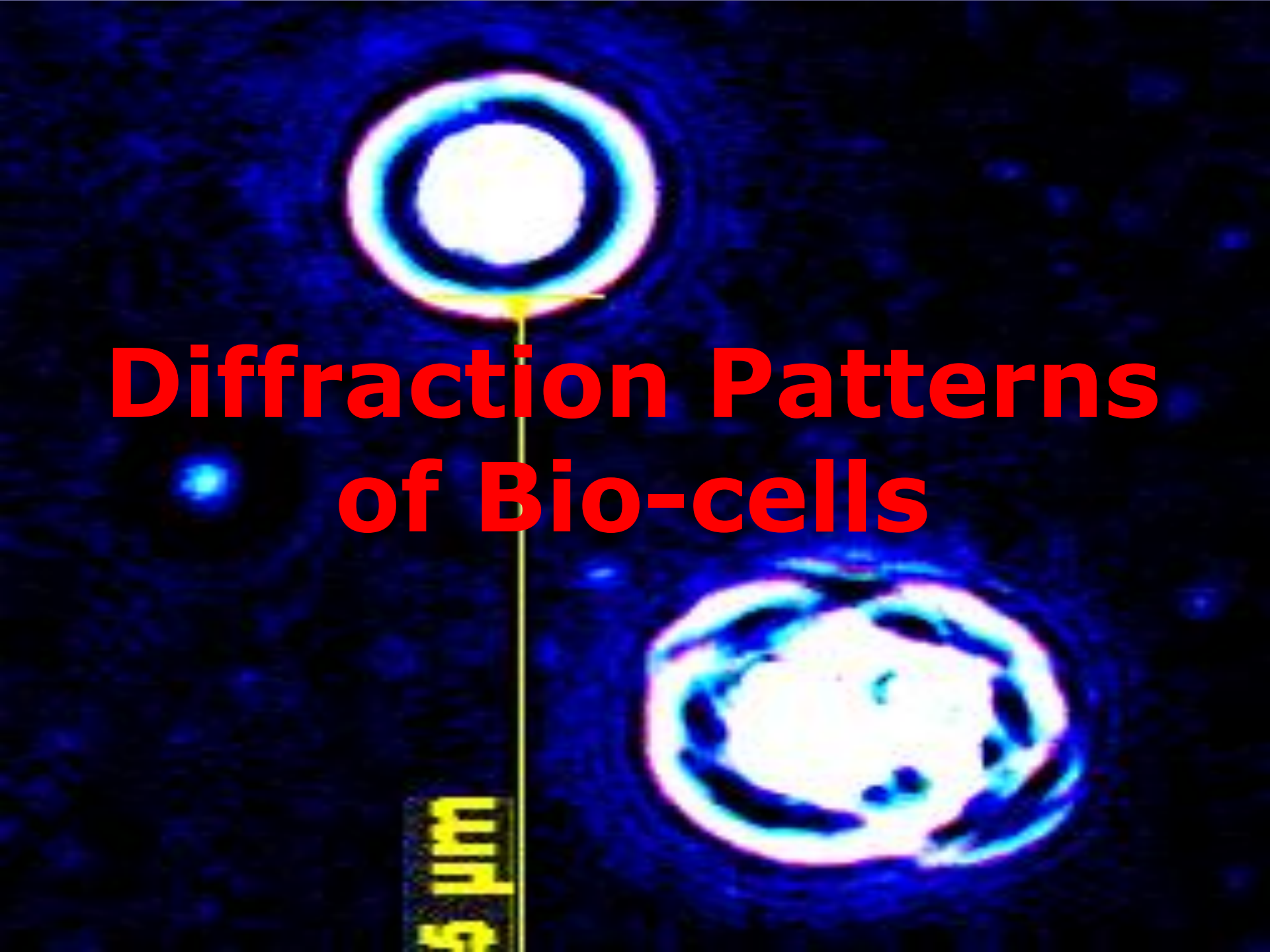


“On chip spectrometry” data of spectra collected in transmission.



Conclusions about bio-sample characterisation by on-chip spectroscopy.

- A simple but very effective optical sensor was realized an used successfully for bio-sample characterisation.
- The concentration of any type of bio-cells can be determined from integral adsorption bands corresponding to electronic transitions in uv-vis domain.
- The cell-cell interaction determined the read shift of the absorption bands especially for those above 500nm.
- This change of electronic transitions (read shift and the increasing of absorption bandwidth) were determined by the interactions between the membranes of the coupled cells.
- The saturation of the absorption band shift was found to be related to cells aggregation being propose as a new technique for bio-sample characterisation.
- The extension of this technique to other type of cells as red blood cells is given by the same quantum effects determined by local interaction between electronic structures of the cells membranes.


Diffraction Patterns
of Bio-cells

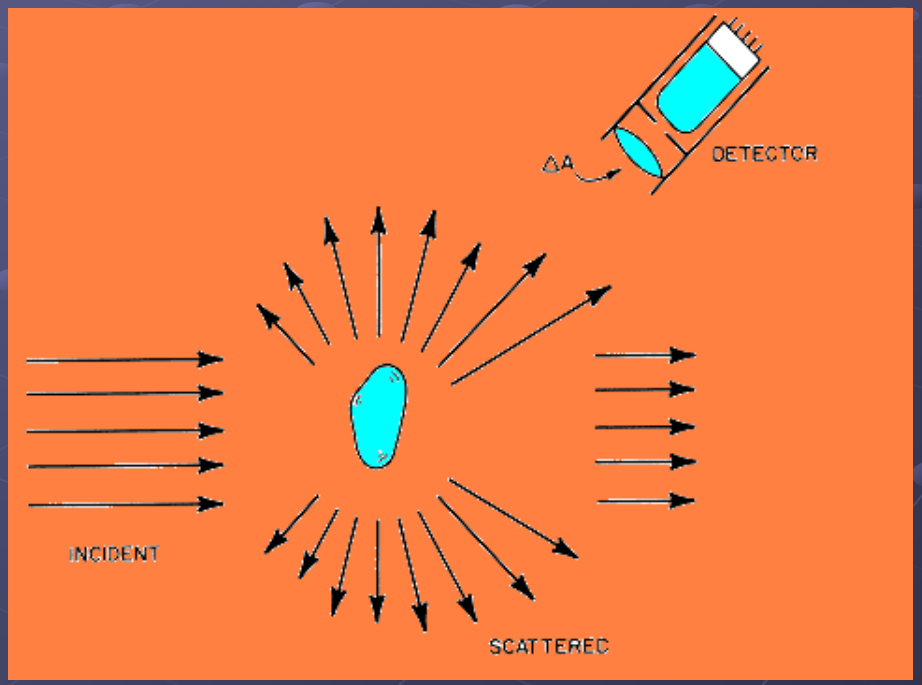
Matter and photons interaction

- **Magnetic fields H and the Electric field E interact through the material parameters as permitivity and permeability.**
  - Maxwell's equations describe light propagation.
- **Propagation in matter**
  - the charge carriers of the material oscillate and radiate as dipoles,
  - in a homogeneous medium the dipoles emission cancel each other by interference except in the forward direction,
  - Defects or non homogeneities scatter the light because the dipoles cannot cancel each other.

Geometry of a scattering experiment

Cross sections
absorption,
scattering,
extinction
= scattering + absorption.

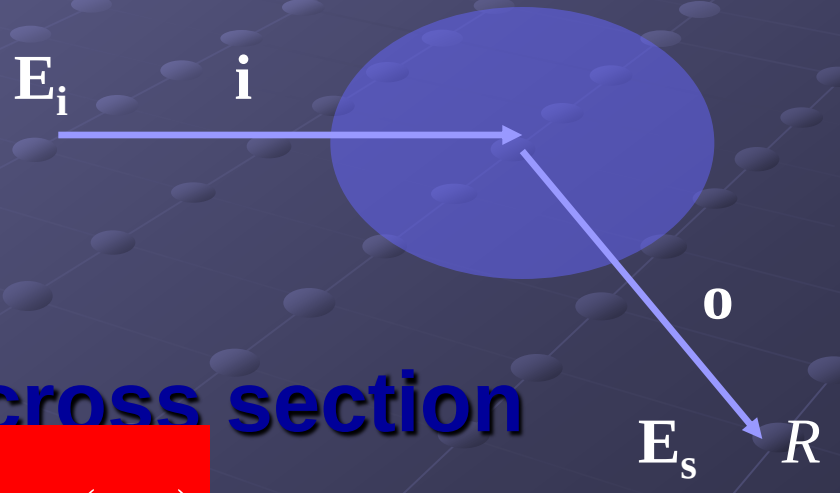
Angular dependence
scattering phase function.



Cross sections

- **Far-field limit: $R > D^2/\lambda$**

$$\mathbf{E}_s \quad \mathbf{E}_s = \mathbf{f}(\mathbf{o},\mathbf{i})\frac{\exp\{ikR\}}{R}$$

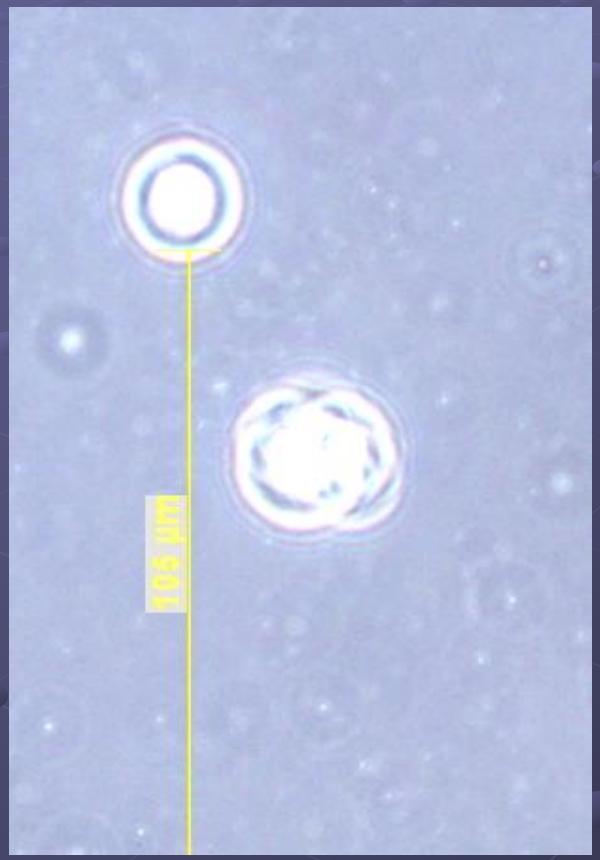


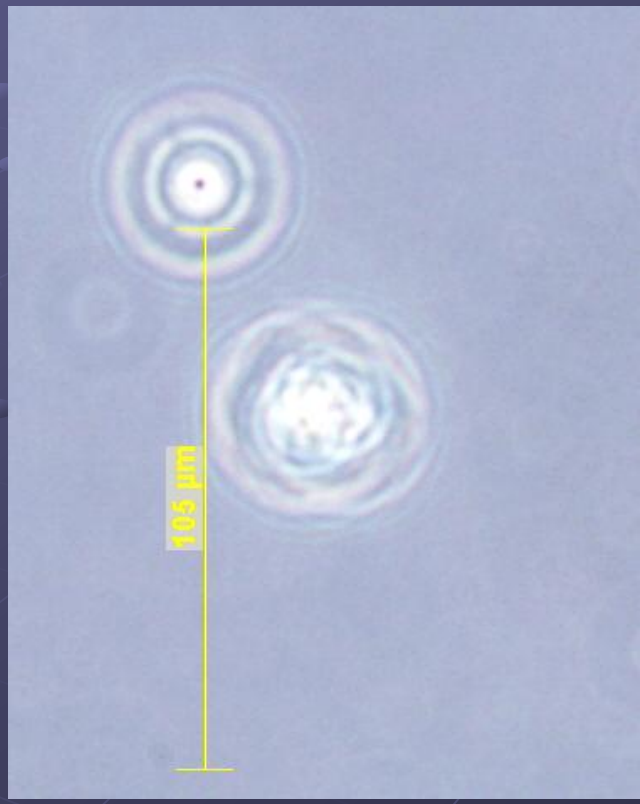
- **Differential scattering cross section**

$$\sigma_d(\mathbf{o},\mathbf{i}) = \left.\frac{R^2 S_s}{S_i}\right|_{R\to\infty} = \left|f(\mathbf{o},\mathbf{i})\right|^2 = \frac{\sigma_t}{4\pi}p(\mathbf{o},\mathbf{i})$$

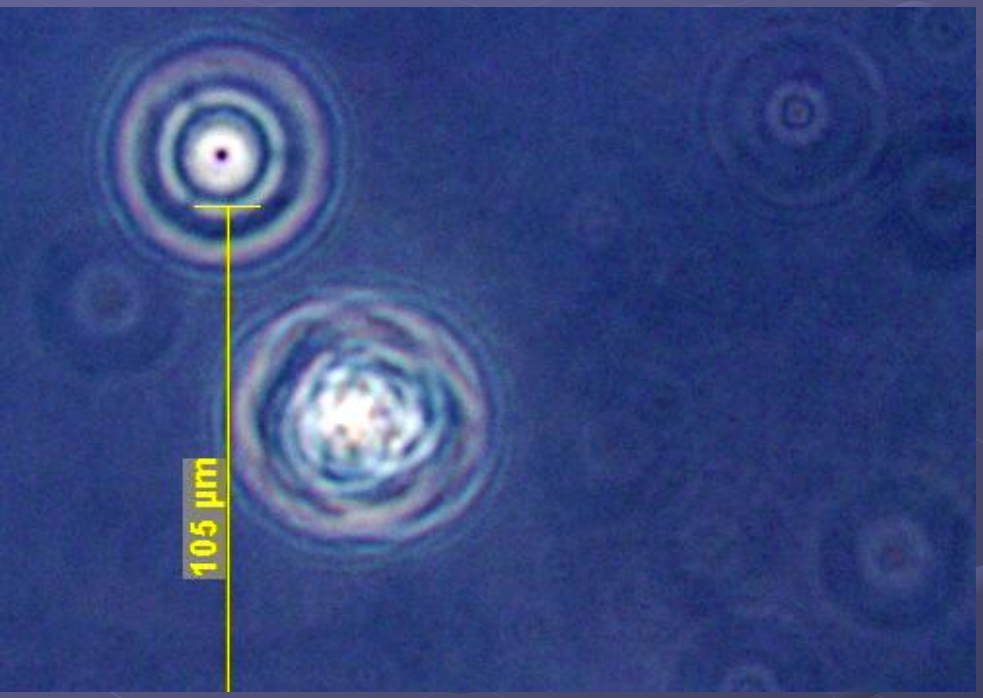
- **p(o,i) is the scattering phase function**

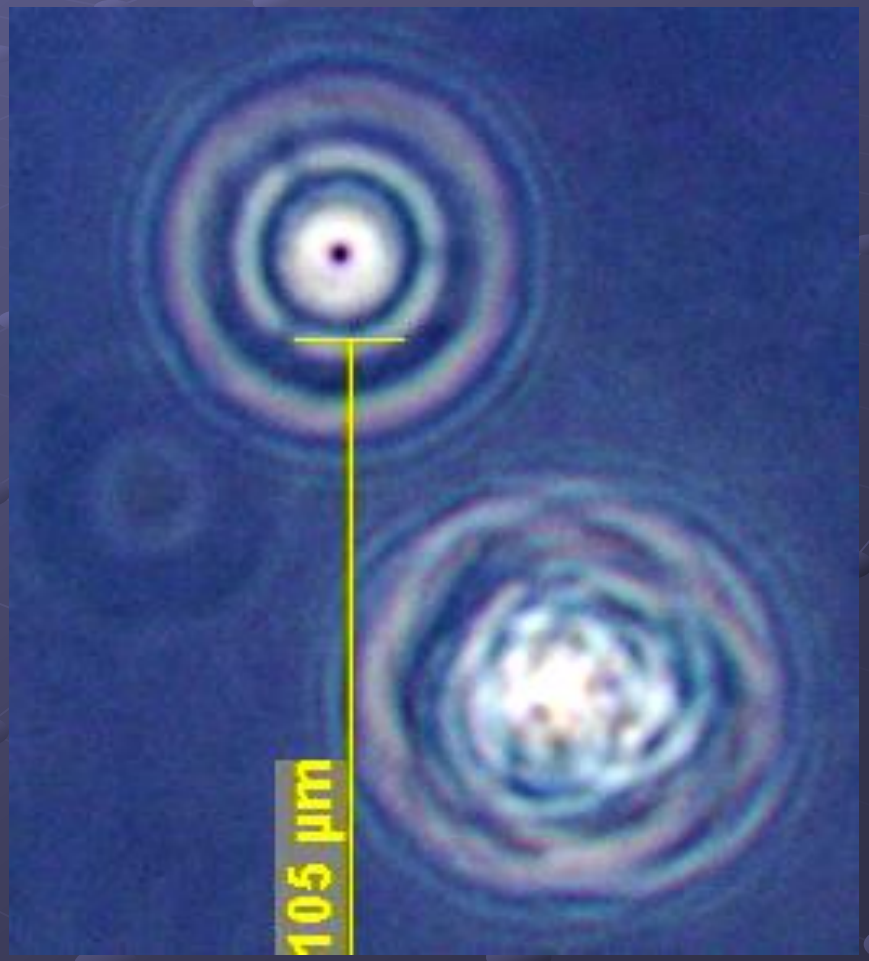
3D diffraction patterns of dead yeast cells taken at difference distances from the focus plan.





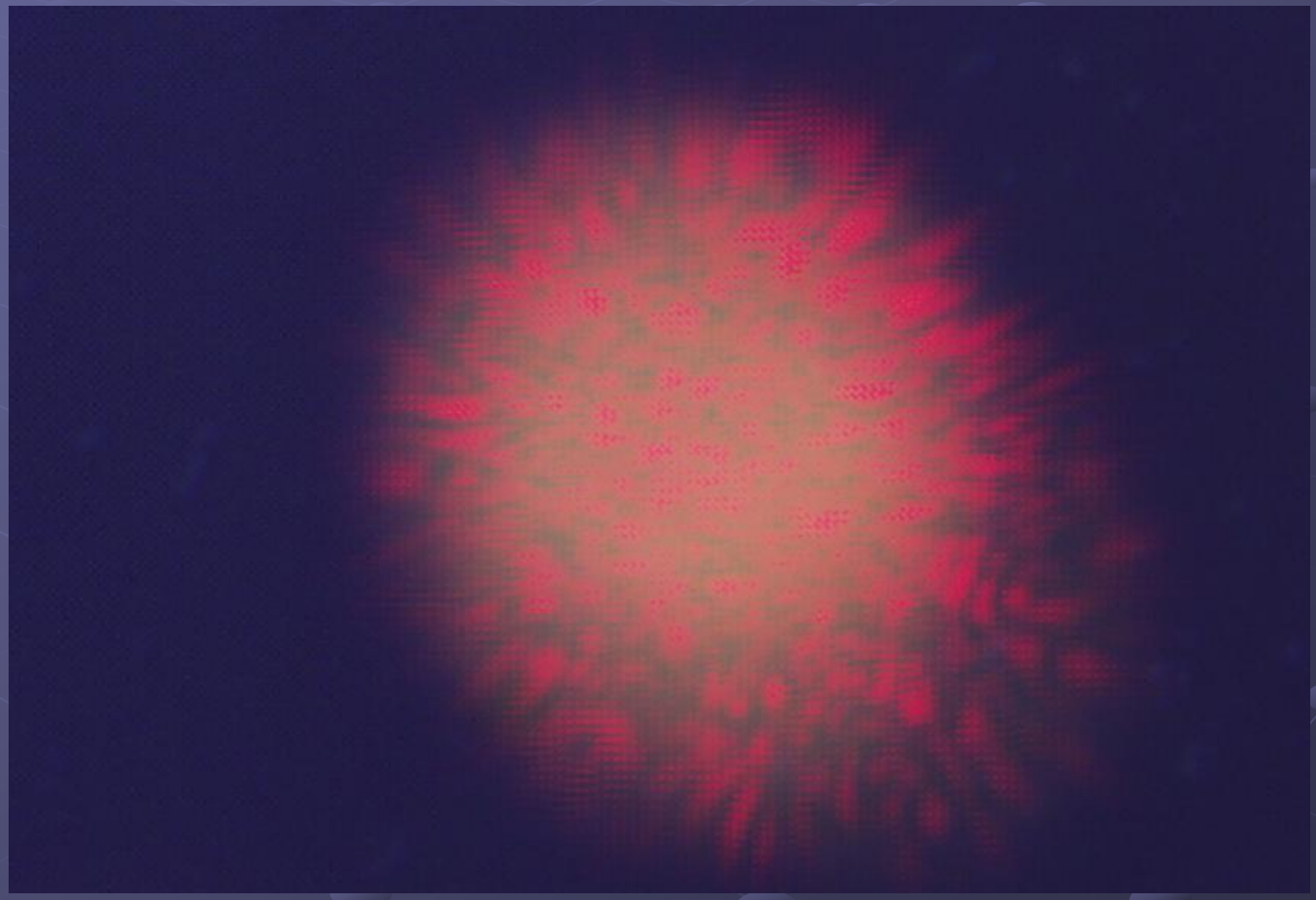
An isolated cell, a cluster of five cells and other. Diffraction in background.



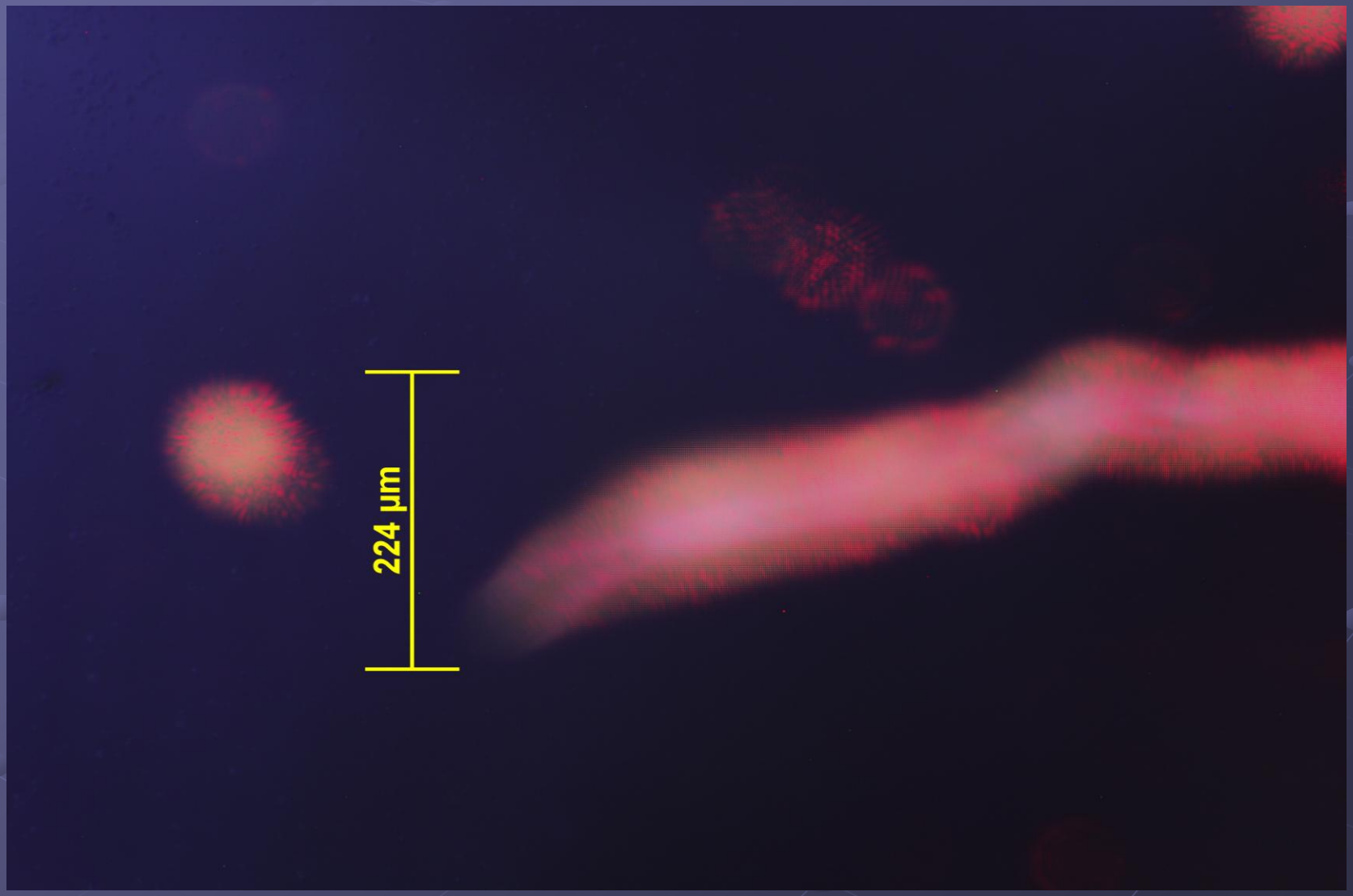


2D diffraction pattern outside focal plane of a single e-coli cell

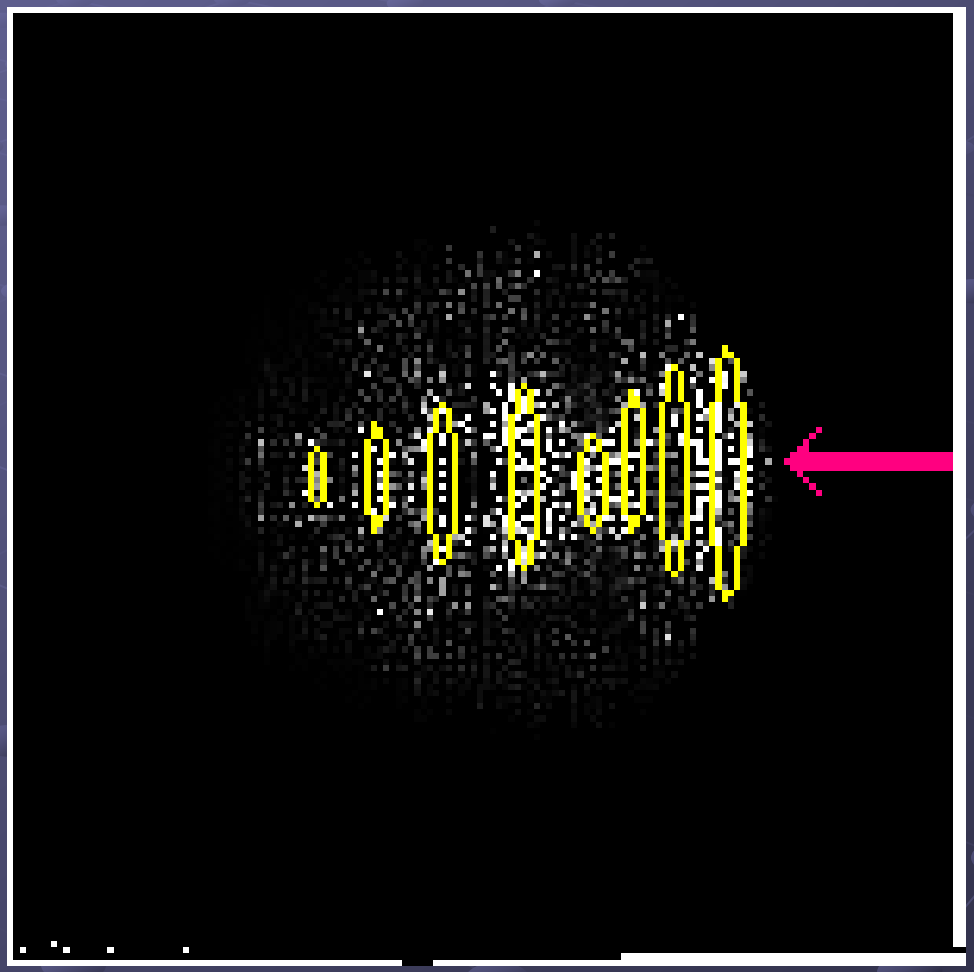
(diameter 500nm)



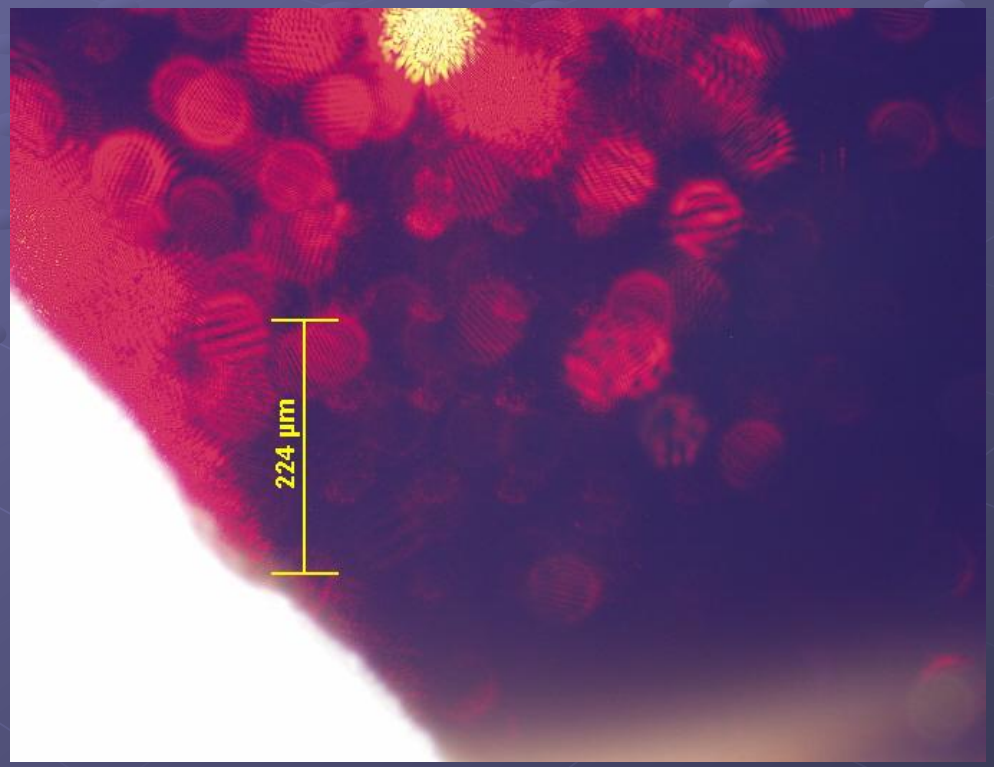
Particles (500nm) interaction revealed by 3D scattering pattern projection above the focal plane. (long distance field)

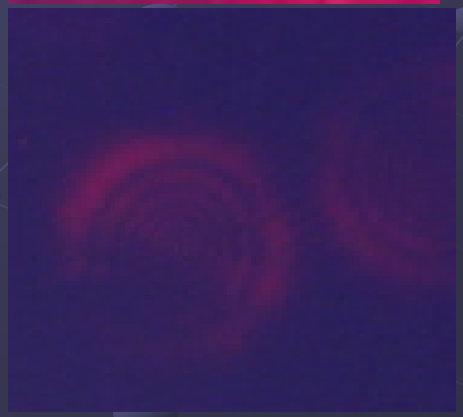


3D diffraction pattern calculation

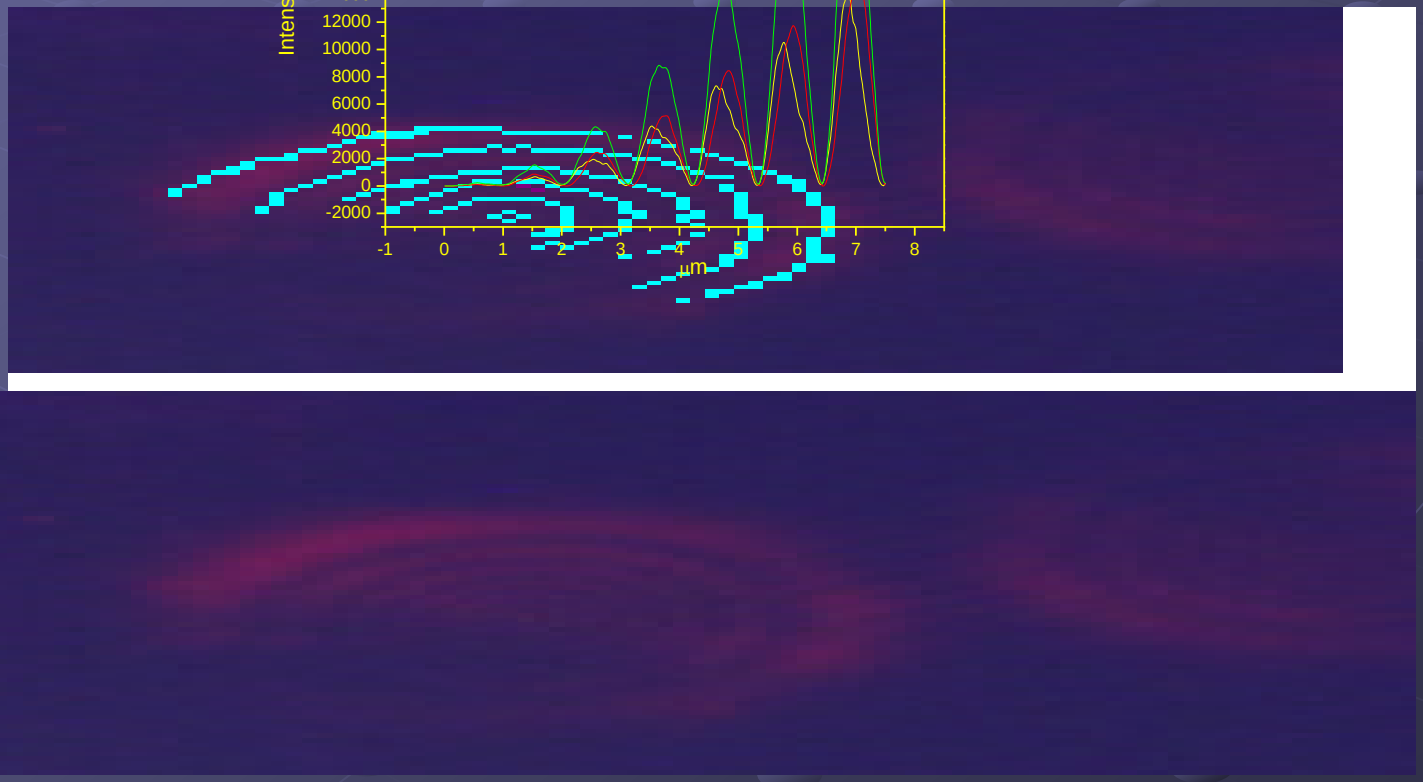


Yeasts cells at the border of a drop placed on $Si_3N_4/SiO_2/Si$ substrate showing mixed pattern of LASER light diffraction at 633nm





Images of isolated Yeasts cells in LASER Scattered light out from focal plane



CONCLUSIONS about individual bio-cells characterisation by parameters extraction from their diffraction pattern

- The diffraction pattern of individual cells obtained by LASER beam scattering contains implicitly the data about their size, geometry and structure (dimension of nucleus, average dimension of the organelle, external radius, eccentricity, average refractive index,…)

- The 3D diffraction patterns are collected by an optical system build around a CCD (Charge Coupled Device).

- The bio-cell parameters mentioned above are extracted by fitting the experimental data using theoretical approach for diffracted pattern calculation.

- The technique we use gives encouragement results on both individual cells and on small cell clusters.

GENERAL CONCLUSIONS

•These studies contribute to the development of *on chip spectrometry* techniques based on absorption and scattering of photons by bio-cells, concerning data about:

- rapid measurement of cells concentration

- investigation on cells aggregation

- cell characterisation by parameters extraction from light diffraction patterns