Fourier analysis of differential light scattering by cell populations for the demonstration of their activation

In this work, both theoretical and experimental tools were developed for the quantification of morohological changes which take place in biological cells (or other small scatterer) , when exposed to changing environmental conditions.
The motivation for this work is based on the need to extract as much morphlogical information as possible regarding living cells ,since, this information may be exploited both in medical research and in diagnostics field.
Looking for a method that will best quantify morphlogical parameters , I have discovered ,that such device is not easily found. Miss leading results are to be expected when using any existing device for the measurement of living cell.
Theoretical approach I developed , based on stationary phase approximation , enabled the analysis of the sources of inaccuracies and errors of these different device . Also, i was able to point out the characteristics of a measuring system that will function with reduced theoretical error.
The main source i identified for errors , is the fact ,that two of the main features of a living cell is mixed when analyzed by existing measuring tools.
The absorption map of the cell - that is the distribution of absorbing mollecules on it , and the refraction map -that is the amount of optical path locally made by a ray of light , both affect the output of the device in a way , which makes it virtually impossible to tell the features of each of them alone. Moreover, it is expected , that in some cases , both parameters will , yet no reaction will be detected , because while one parameter increase the reading while the other parameter might decrease it.
In the theoretical background I developed a tool for the analysis of scattering pattern created by spherical object in a way overcoming te mix mentioned above , in addition, a method is introduced to deduce the shape of a non-spherical objects when illuminated as a population . Both are applied using stationary phase approximation.

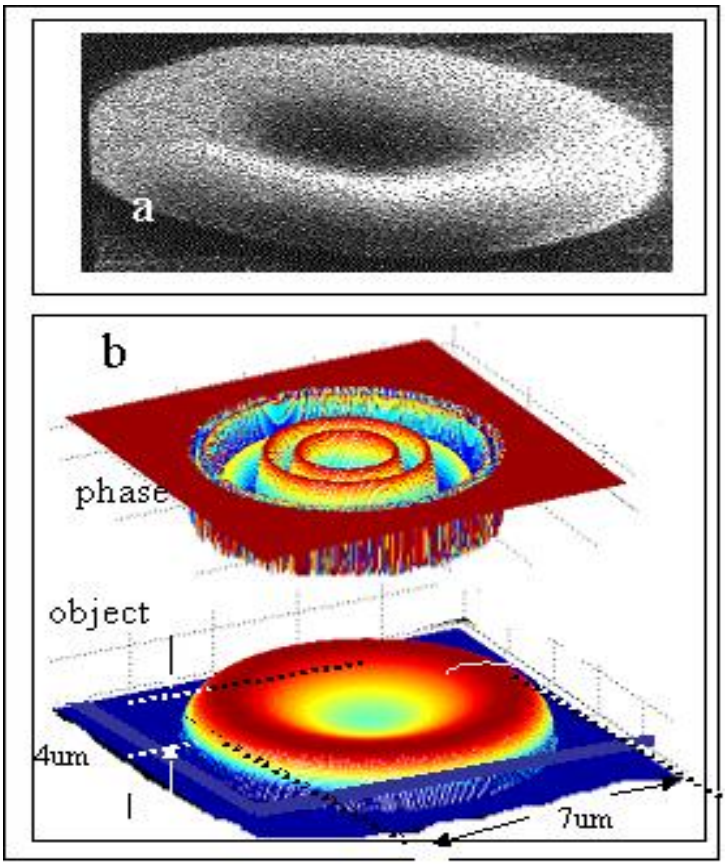
Following , in the theoretical chapter, I develop expressions for the novel analysis on particle sizing accuracy concerning the use of microscope image.

The shortcoming of the method so far described , is the fact , that only simple profile is allowed for the scatterer in order to be analyzed with stationary phase method . By simple we mean , profile which has only on minimum in particle thickness function.
In case the profile is more complicated ., the degree of complexity of the expression becomes intolerably difficult to deal with.

For the purpose of analyzing the morphology of cells having complex profile , according to the above definition , a diffractive propagation technique called angular spectrum was used.
Using this software I wrote , many simulations were made including that of extracting data from scatterers. The complete mapping of the object from intensity pattern only was recovered , this result was one that strongly motivated the composition of the measuring device.
In the measuring system chapter , I describe the components of the device , algorithms and computational methods by which theoretical expressions derived earlier are applied on practice for the extraction of full, accurate information regarding leaving cell.

First experimental results described , are those connected to the calibration of the system .For this purpose polystyrene beads were used , having a well known shape and index of refraction.
These were suspended in solutions having also different known refractive index.
A comparison was made between results obtained with microscope and flow aided cell analyzer (FACS) and the experimental system , showing good agreement with predictions regarding miss leading results. It was also proved that the system is capable of measuring both simple and complicated objects , separating absorption map from refraction map.

In the biological section , spherical cells were checked (lymphocytes) using , again , three different device among which measuring system.
Cells were swollen , by suspending them in salt solution , of osmolarity higher than the natural values . Comparison between experimental results indicate the fact that theoretical predictions regarding biological cells are also valid.
Presented in this chapter as well are cells undergoing apoptotic process, in that case , the kinetics of their reaction was recorded , using the same three measuring device. The most sensitive to cellular reaction detection was the DLS measuring system .also elongated cells reacting to minute amounts of hormone are investigated, showing the ability of the method in analyzing a spherical cells
As a conclusion, in this work it was shown , that improvement of measuring device for the quantification of morphological changes can be achieved when using DLS approach . My hope is , that this statement will serve as a starting point for the composition of a device capable for routine analysis of cells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