# Detection of ultrafine particles in living cells

Michael Edetsberger


# Aims of the project

- **Study of effects caused by the interaction of nanoparticles with eukaryontic cells**
- **For a better understanding of immunological, cellular or heritable effects**
- **A fast screening method to detect interactions and uptake mechanisms is essential**
- **Usage of Fluorescence Correlation Spectroscopy (FCS) and Photon Counting Histogramming (PCH)**
- **Application of FCS and PCH under physiological conditions is possible**

## Abstract

**If humans are exposed to nanoparticles or nanostructures they can generate general health problems. Our study is directed towards a better understanding of the basic interactions of nanoparticles with cells. The attention should be turned to the general uptake mechanisms. Using different advanced fluorescence methods like Fluorescence Correlation Spectroscopy (FCS) or Photon Counting Histogramming (PCH), allowing the detection of single fluorescent molecules in a defined focus-volume, we could show that nanoparticles of 20nm size occur very fast in the cytoplasm and with a time-delay of about 15 minutes also in the nucleus.**

## Introduction

Nanotechnology is widely accepted as a future key technology with a variety of issues for medical treatment and in life sciences. If humans are exposed to nanoparticles, accumulating in the environment from various technological sources as e.g. the exhaust of modern combustion processes, general health problems can be generated.

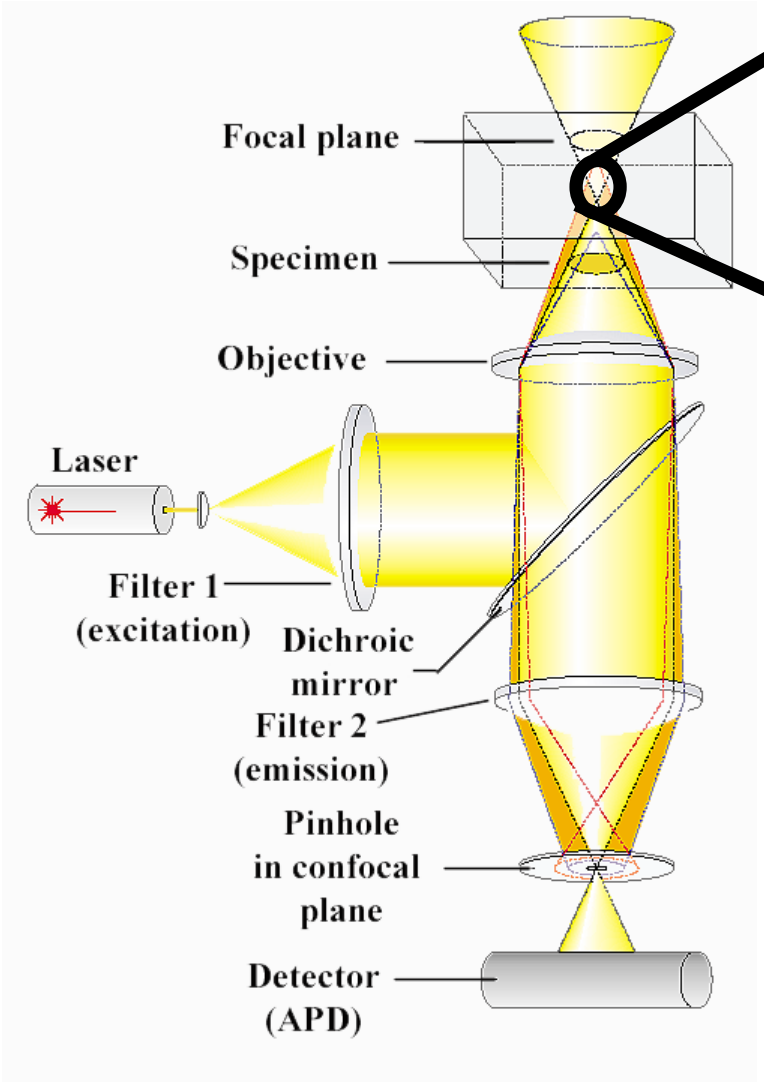
Our methods allow to follow the time course of the appearance of fluorescent particles in specific compartments in the cell in comparison to the distribution of the particles obtained from confocal scanning microscopy images. The principle for Fluorescence Correlation Spectroscopy (FCS) and Photon Counting Histogramming (PCH) are fluctuations, caused by the passage of fluorescent labeled particles through the focus volume.

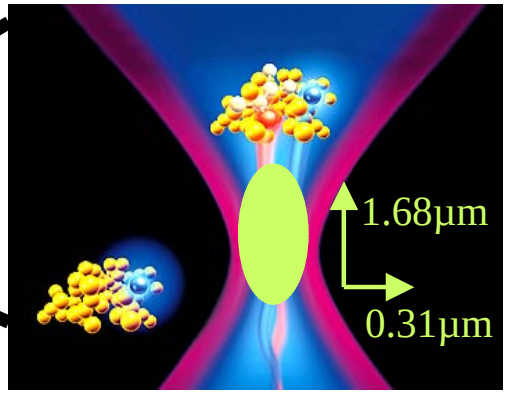
## Results

It could be shown that 20nm carboxy-labeled nanoparticles can be detected in the cytoplasm after a few minutes. The diffusion time of these particles imply that these particles are singular, because of a single diffusion-time and a homogenious distribution of the brightness. After about 30 minutes particles, showing different diffusion-times and brightness distribution, occur. These signals result from aggregation of particles or from particles included in endosomes or vesicles.

Albeit in the nucleus with a time-delay an increase of fluorescence intensity is observed which levels off after aprox. 15 minutes. This signal can be attributed to high concentrations of single diffusing particles.  After aprox. 60 minutes additional fluctuations arise which could result from particles attached to macromolecules.

# Schematic set-up of the FCS Microscope



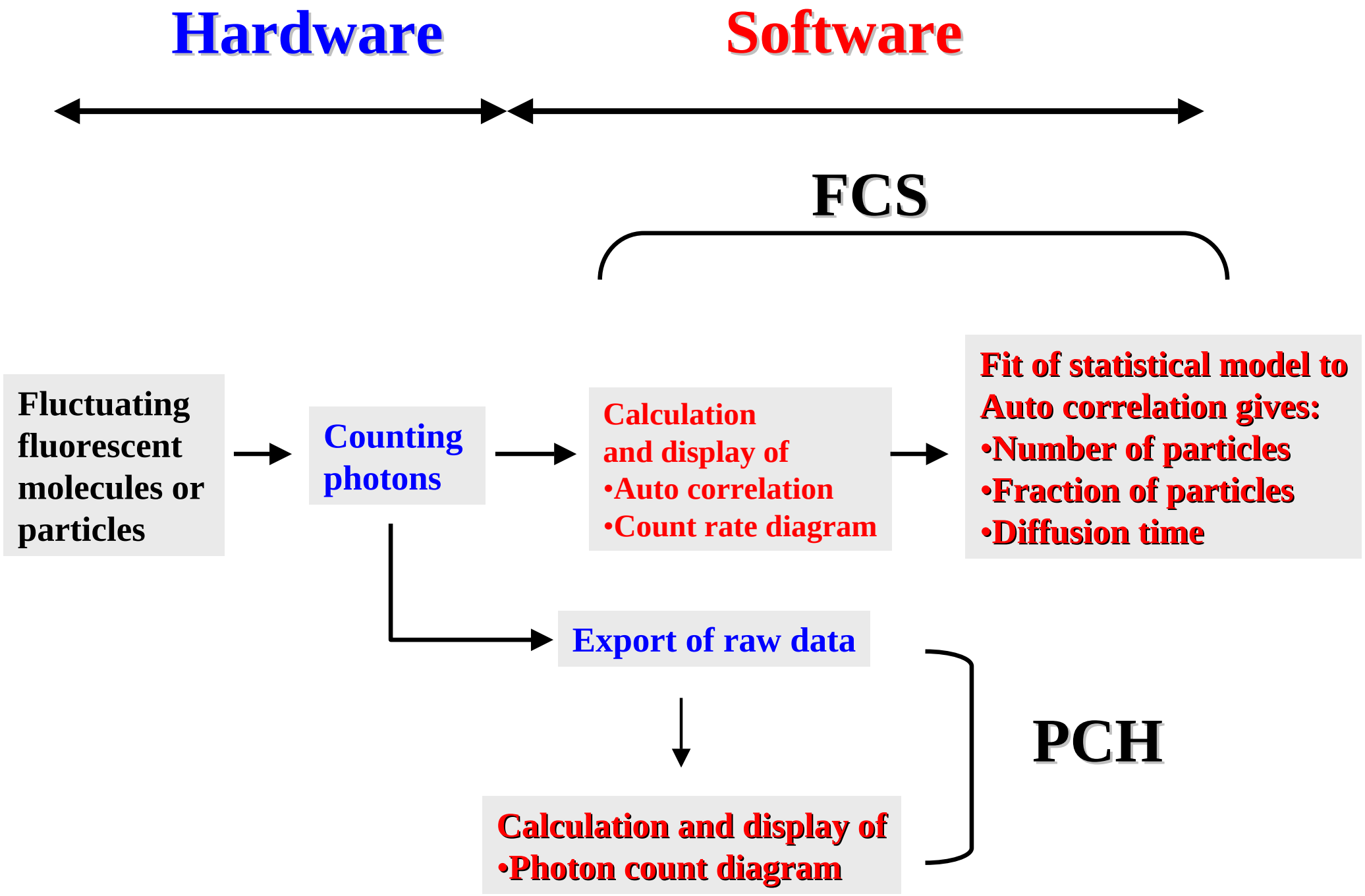




**Different particle size and environment conditions influence diffusion time**

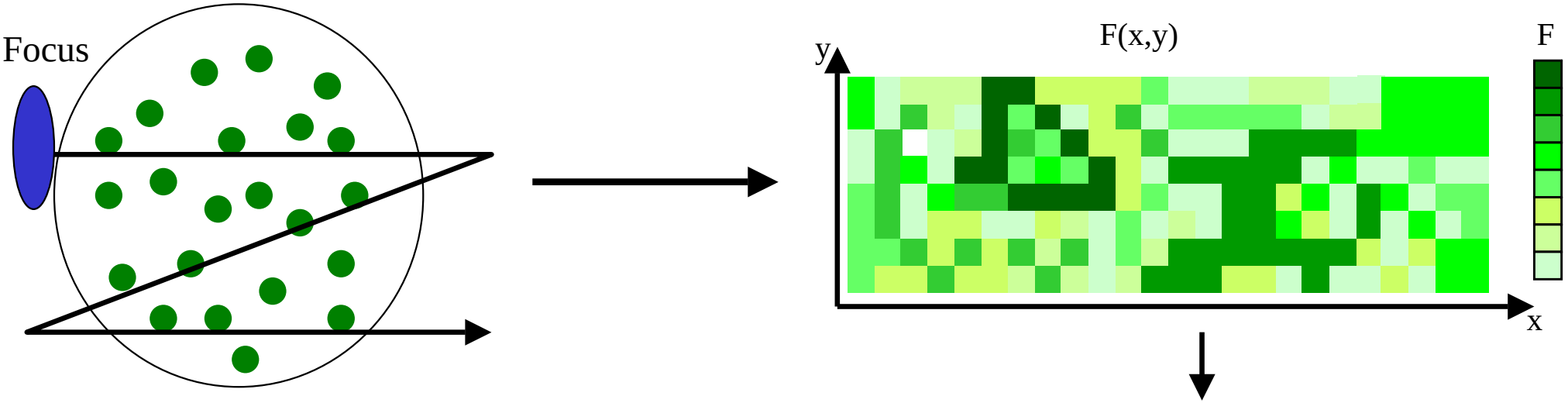
→ **Counting of emitted photons**

**Fit of statistical model gives:**

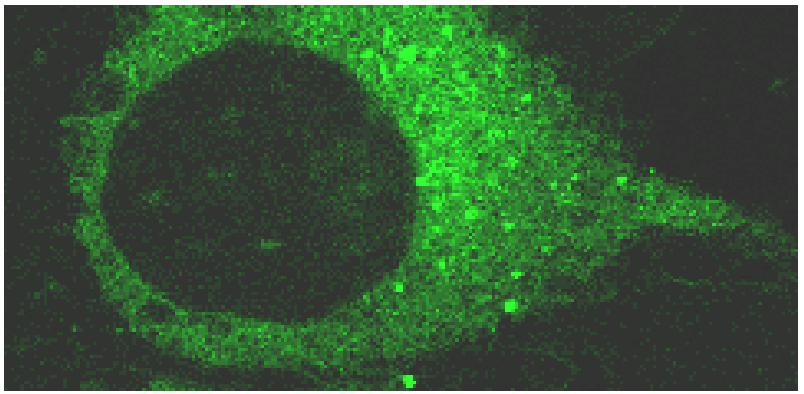
- **Number of particles**
- **Brightness of particles**
- **Diffusion time**


Hardware
Software
FCS
Fluctuating fluorescent molecules or particles
Counting photons
Calculation and display of
•Auto correlation
•Count rate diagram
Fit of statistical model to Auto correlation gives:
•Number of particles
•Fraction of particles
•Diffusion time
Export of raw data
Calculation and display of
•Photon count diagram
PCH

# Laser Scanning Microscopy (LSM)

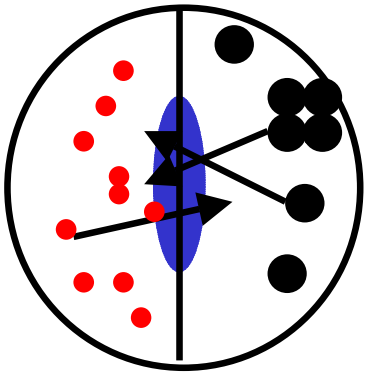


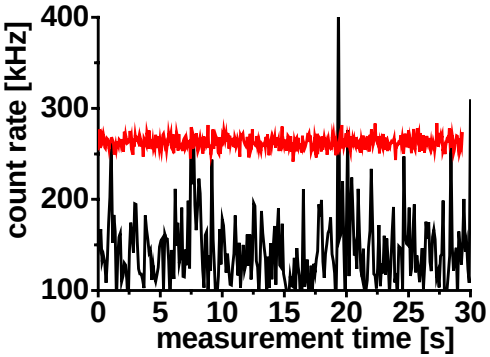

- **Sample is read pixel per pixel**
- **Fluctuation in intensity results in increase of noise**
- **For a better signal-noise ratio many labeled molecules should be in the focus volume**

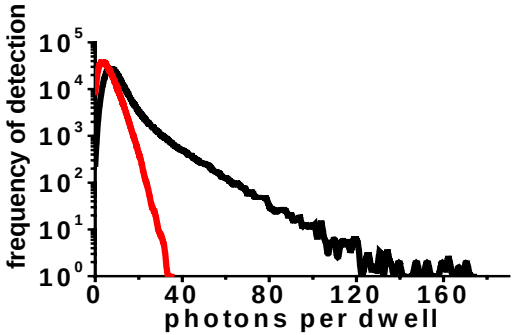


HeLa cells with 20nm green fluorescent polystyrene particles

# Fluorescence Correlation Spectroscopy (FCS) and Photon Counting Histogramming (PCH)

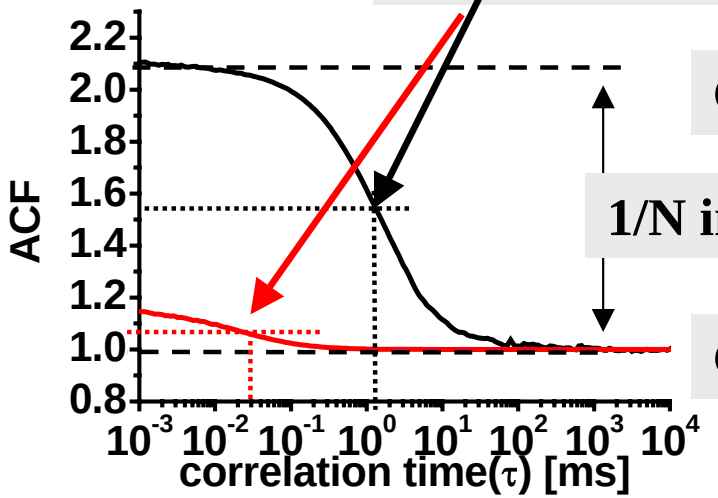





$$G_{(\tau)} = \frac{\frac{1}{N}}{\frac{1+\tau}{\tau_D}} x\left(\sqrt{\frac{1+\tau}{K^2\tau_D}} + 1\right)$$

Point of inflection ($\tau_D$)




$G_{(\tau=0)}$

1/N in detection volume

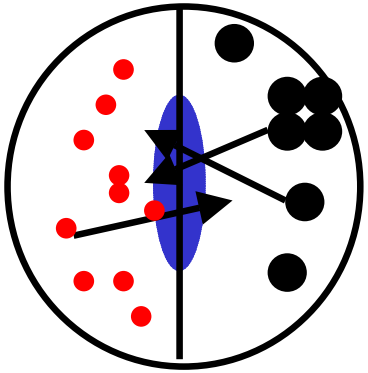
$G_{(\tau\to\infty)}$

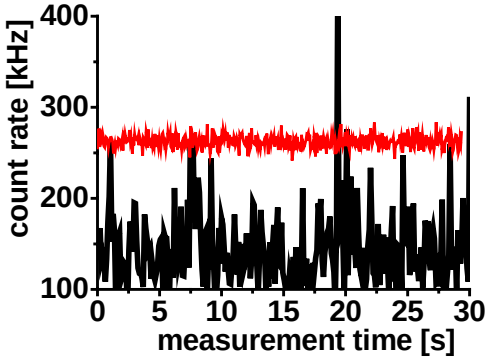
$\tau_1$= 0.03ms

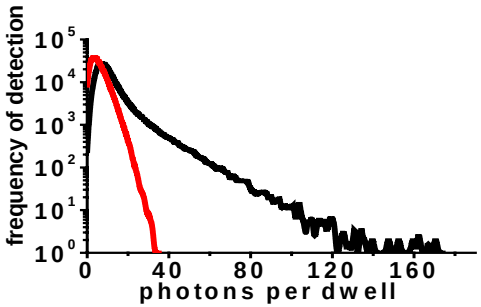
$\tau_2$= 1.4ms

$\tau_3$= *70ms*

# Fluorescence Correlation Spectroscopy (FCS) and Photon Counting Histogramming (PCH)

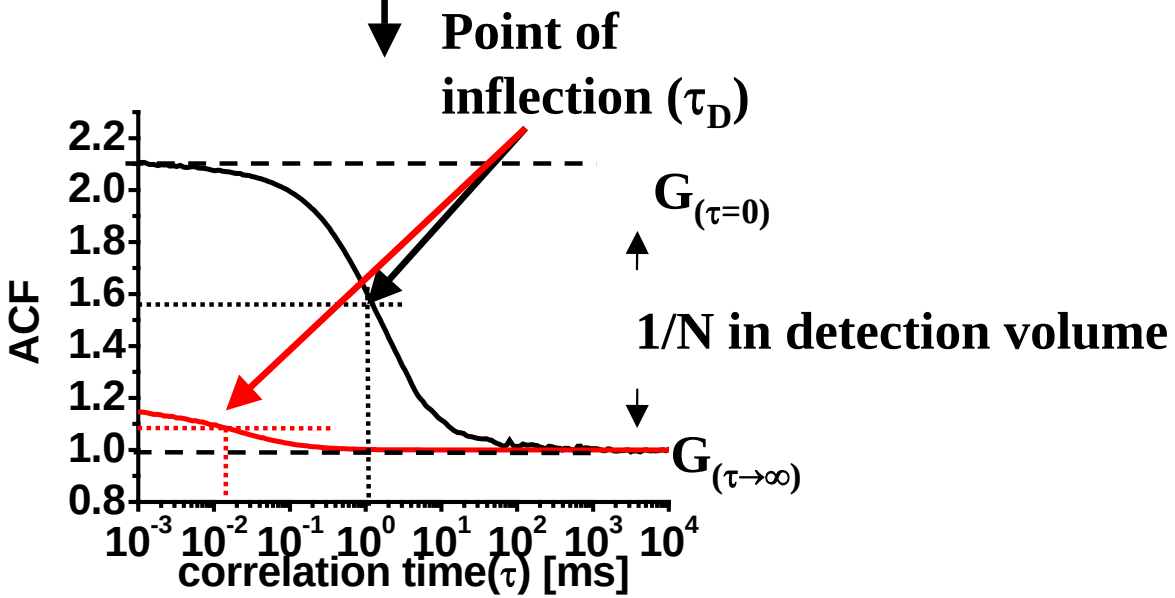





$$G_{(\tau)} = \frac{\frac{1}{N}}{\frac{1+\tau}{\tau_D}} x\left(\sqrt{\frac{1+\tau}{K^2\tau_D}} + 1\right)$$

- Fluctuations are the base for FCS and PCH
- For a better signal-noise-ratio a small number of particles should be in the focus element



# Conclusions and outlook

- 20 nm particles can be detected in the cytoplasm after a few minutes.
- Initially isolated particles are observed.
- After about 30 minutes larger and brighter particles can be detected, probably included in vesicles.
- Accumulation of 20 nm particles can also be observed in the nucleus.

- For the uptake of particles, viable cells are necessary.
- FCS together with PCH can be used as a fast sreening-method to study inter-actions of fluorescent labeled particles with cells under physiological conditions.

# Detection of ultrafine particles in living cells

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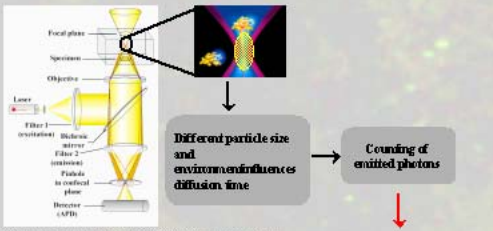
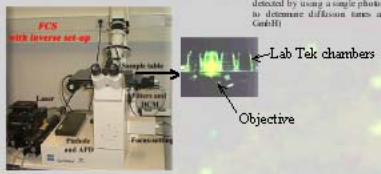
## Abstract

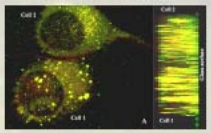
When humans are exposed to nanoparticles or nanostructures they can generate general health problems. Our study is directed towards a better understanding of the basic interactions of nanoparticles with cells. The attention should be turned to the general uptake mechanisms. By using different advanced fluorescence methods like Fluorescence Correlation Spectroscopy (FCS) or Photon Counting Histogramming (PCH), which allows the detection of single fluorescent molecules in a defined focus-volume, we could show that nanoparticles of 20nm size occur very fast in the cytoplasm and with a time-delay of about 40 minutes also in the nucleus.




**Schematical drawing of the FCS/PCH setup.** The laserbeam is focused and sent through a dichroic mirror and an excitation filter (Filter1). After passing the objective (63x, NA1.32, water) the laser excites particles inside the foctuse. The fluorophore-labeled particles emitt light at a specific wavelength, which passes the dichroic mirror, the emission filter (Filter2) and the pinhole. The emitted photons are detected by using a single photon detector (APD). Raw datas are used to determine diffusion times and brightness (picture source: Zeiss GmbH)

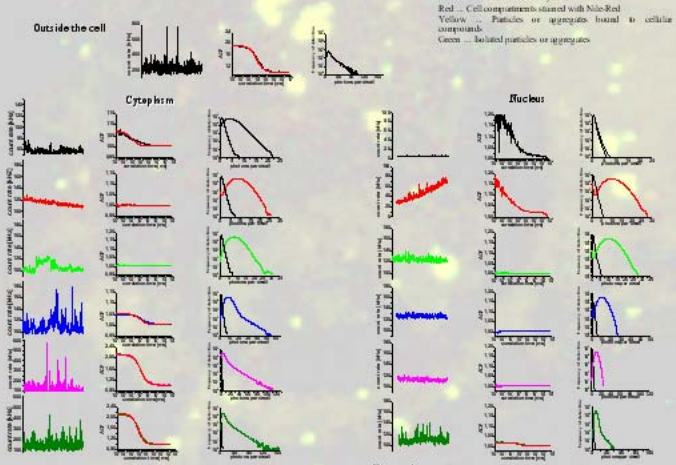



## Confocal imaging




**Confocal view of the uptake of 20nm particle by HeLa cells:** Picture A shows a compressed view of 6 nuclear sections (100 nm per layer with a gap of 400nm). On the right a 3D-reconstruction of the layers can be seen.
Red ... Cell compartments stained with Nile-Red
Yellow ... Particles or aggregates bound to cellular compounds
Green ... Isolated particles or aggregates

## FCS and PCH




| Time-point after addition of particles | $\tau_1$ [ms] | $\tau_2$ [ms] | $\tau_3$ [ms] | kHz |
|---|---|---|---|---|
| 1 min | 0,06 (53%) | 1,8 (41%) | - | 57 |
| 5 min | - | - | - | 112 |
| 10 min | - | - | - | 104 |
| 15 min | - | 1,4 (70%) | 23,4 (17%) | 107 |
| 35 min | 0,001 (13%) | 1,4 (70%) | 75 (9%) | 113 |
| 60 min | - | 1,4 (100%) | - | 145 |
| *outside the cell* | *0,7 (52%)* | - | *3 (48%)* | *631* |

| Time-point after addition of particles | $\tau_1$ [ms] | $\tau_2$ [ms] | $\tau_3$ [ms] | kHz |
|---|---|---|---|---|
| 1 min | - | - | - | 3 |
| 5 min | - | - | - | 46 |
| 10 min | - | - | - | 123 |
| 15 min | - | - | - | 125 |
| 35 min | - | - | - | 112 |
| 60 min | - | 1,05 (60%) | 7 (34%) | 109 |
| *outside the cell* | *0,8 (83%)* | - | *9,5 (23%)* | *200* |

Measurement of FCS and PCH in two cellular compartments using 20nm polystyrene particles:

Top: Measurement outside the cell. Fluctuations in intensity of up to 400kHz can be detected. The diffusion times range between 0.7 and 3ms. By analysing the PCH particles emitting 40 to 80 photon can be detected.

Bottom left: Measurement in the cytoplasm. At the beginning fluctuation in intensity of 200Hz can be observed, which disappear after 5 minutes. These particles the with diffusion times of 1.8 ms and brightnes of 20-35 photons. After 15 minute fluctuations up to 500kHz appear again. Now two species of particles can be detected. The diffusion times range from 1.4ms for the smaller particles and 23 to 75ms for larger particles. These species show brightness up to 150 photons.

Bottom right: Measurements in the nucleus. At the beginning no significant fluorescence intensity can be detected. But after 10 minutes a significant increase in intensity can be observed. After 40 minutes fluctuations of 20 – 30kHz can be observed, resulting in diffusion times of 1.05 to 7ms and brightness of 50 photons.

## Introduction

Nanotechnology is widely accepted as a future key technology with a variety of areas for medical treatment and in life sciences. When humans are exposed to nanoparticles, accumulating in the environment from various technological sources as e.g. the exhaust of modern combustion processes, general health problems can be generated.
The base for Fluorescence Correlation Spectroscopy (FCS) and Photon Counting Histogramming (PCH) are fluctuations, caused by the passing of fluorescent labeled particles through the focus volume. Therefore our method allow to follow the time course of the appearance of fluorescent particles in specific compartments in the cell in comparison to the distribution of the particles obtained from confocal scanning microscopy images.

## Method

HeLa cells ($10^5$ cells/well) are seeded in LabTek-chambers, using DMEM, supplemented with 4,5mM L-Glutamin, 4500mg/L Glucose, 10% FCS, 10 units/ml penicillin/streptomycin and 3,7g/L Na-bicarbonate and cultivated over night under standard conditions. At the next day the cells are washed with PBS (PBS supplemented with 0,01M glucose) and carboxy-coated, green fluorescent particles (Fluospheres, Molecular Probes) in the size of 20nm were added (0.075 w%). The measurement starts immediately after adding the particles.
For the measurement the following parameters are used:
Pinhole: 35 µm
Focus: 1.48x0.31 µm
Aquisition-time: 30s for PCH and 42s for FCS
Dwell-time for PCH: 100 µs

## Results

It could be shown that 20nm carboxy-labeled nanoparticles can be detected in the cytoplasm after a few minutes. The diffusion time of these particles imply that these particles are singular, because diffusion-time and brightness show a homogenic picture. After about 30 minutes particles, showing different diffusion-time and brightness, occur. These signals are results from aggregates or particles, actively taken up by the cell

Albeit with a time-delay of 40 minutes a similar picture can be seen in the nucleus. First just an increase of fluorescence can be observed and after 60 minutes a signal, implifying particles, can be detected. Even after 80 minutes no heterogenic species of particles can be detected.

## Conclusions and outlooks

- 20 nm particles can be detected in the cytoplasm after a few minutes
- First arrising particles seem to be isolated
- After about 30 minutes bigger and brighter particles can be detected
- 20 nm particles can also arrive in the nucleus
- For the uptake of particles, viable cells are necessary
- FCS together with PCH can be used as a very fast screening-method to investigate interaction of fluorescent labeled particles with cells under physiological conditions

# Acknowledgement


- **The project is funded by AUVA, Allgemeine Unfallversicherungsanstalt, Adalbert-Stifterstrasse 65, A-1201 Vienna**
- **E.Waigmann, M.F. Perutz Laboratories, University Departments at the Vienna Biocenter, Dept. For Medical Biochemistry, Medical University of Vienna for providing the Confocal Microscope**