# Quantitative phase microscopy as a novel *in vitro* assay to assess potential hazard of medical nanoparticles

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## Introduction

In the field of medical nanotechnology cytotoxicity evaluation is a key issue for preclinical development and patient safety, but also in hazard classification for medical staff and the environment[1]. Exposure scenarios are possible in everyday clinical practice when handling medical nanosuspensions and by release into the environment. The reliability of established *in vitro* biochemical assays depends on possible interactions between the test nanomaterial and assay chemicals[2]. To address this issue of interference, we developed a time-resolved cell proliferation assay based on label-free quantitative phase imaging (QPI). QPI, a minimally invasive and label-free microscopy method, allows quantitative access to multiple biophysical cell parameters[3]. We demonstrate that the QPI method described here allows precise and reliable measurement of cell proliferation affected by chemicals as well as nanoparticles, and thus facilitates assessment of possible hazards of medical nanomaterials.

## Experimental setup for temporal determination of dry mass

RAW 264.7 mouse macrophages were imaged using a digital holographic microscopy (DHM) system that was equipped with a stage-top incubator. The cells were cultivated and seeded into 96-well imaging plates and exposed to medical nanoparticles or digitonin as cytotoxicity control for 24 hours (Figure 1A). Every 30 minutes, digital off-axis holograms were recorded (Figure 1B) and reconstructed numerically (Figure 1C). The changes in dry mass of the cell populations were calculated from the average phase shifts observed in the quantitative phase images captured by DHM (Figure 1D).

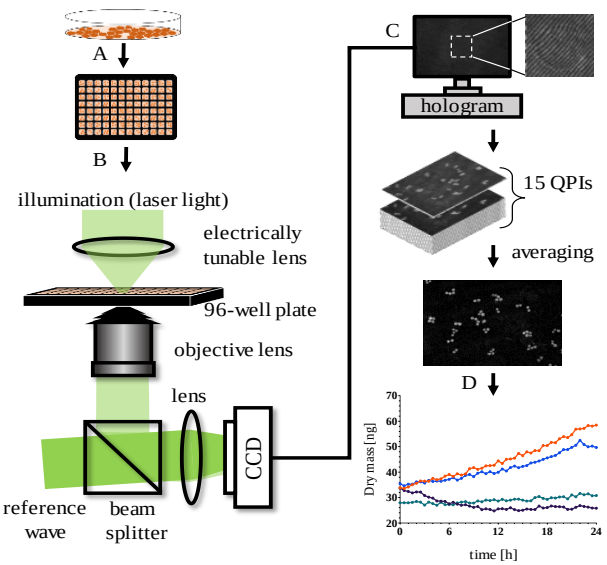


*Figure 1: Experimental setup of the DHM imaging process for dry mass dynamics observation of RAW 264.7 macrophages after incubation with medical nanoparticles and control agents. (A) Cultivation and seeding of cells into 96-well imaging plates (B) Recording of digital off-axis holograms (C) Numerical reconstruction and averaging of the QPI images to reduce coherence induced image disturbances. (D) Calculation of cellular dry mass from the average phase shift induced by the cell populations.*

## Results

Figure 2 shows the dry mass dynamics of RAW 264.7 mouse macrophages incubated with LipImage 815 lipidots (CEA-Leti, Grenoble France) (Figure 2A) and empty Poly(Alkyl Cyanoacrylate) nanoparticles (PACA, SINTEF Industry, Trondheim Norway) (Figure 2B) and corresponding representative QPI images (Figure 2D). Medium control cells grew constantly and doubled their dry mass within 24 hours, while 32 µg/mL of digitonin significantly reduced the rate of dry mass increment. Exposure of cells to LipImage 815 lipidots did not affect dry mass dynamics at concentrations up to 128 µg/mL. Empty Poly(Alkyl Cyanoacrylate) nanoparticles induced moderate inhibition of cellular growth at 2 µg/mL, while 32 µg/mL showed effects similar to the cytotoxicity control digitonin.

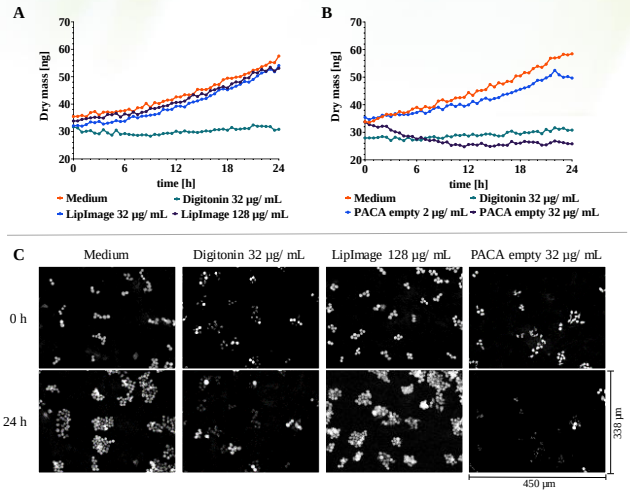


*Fig 2: Medical nanoparticle effects on macrophages in vitro quantified by quantitative phase imaging with digital holographic microscopy. A, B): Dry mass dynamics of RAW 264.7 mouse macrophage populations upon incubation with medium viability control, digitonin cytotoxicity control agent, and two medical nanoparticles over 24 h (time dependently plotted dry mass data represent average values from N=3 independently performed experiments and n=4 positions). LipImage 815 lipidots (A) show no effect on dry mass dynamics in comparison to the medium control, while empty Poly(Alkyl Cyanoacrylate) nanoparticles (B) reduce the dry mass increment of the macrophages in a dose-dependent manner. D): Representative quantitative phase images obtained by DHM at time point 0 h and 24 h of the control and medical nanoparticle incubation.*

## Conclusions

Quantitative monitoring of dry mass dynamics of macrophages with QPI allowed time-resolved assessment of cellular proliferation. Cytotoxicity effects as response to exposure of empty medical nanoparticles and medical nanoparticles loaded with dye could be clearly detected and a discrimination between non(low)-toxic and harmful nanomaterials was demonstrated. LipImage 815 lipidots, which were reported as non toxic showed dry mass increments similar to medium incubated cells, while empty Poly(Alkyl Cyanoacrylate) nanoparticles inhibited dry mass increment in a dose-dependant manner.

## Summary

The presented novel quantitative phase microscopy-based *in vitro* cell proliferation assay provided quantitative data to measure cytotoxic effects of medical nanomaterials. Clear advantages are that this assay is non-invasive, time-resolved, and label-free. In this study dry mass dynamics were evaluated, however QPI would also allow access to further parameters like cell number, motility and morphology. Thus, it can be regarded as promising new tool to improve evaluation of medical nanoparticles for potential human health and environmental hazards.

## References

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