# Label-free detection of micro- and nanoplastics using dark-field hyperspectral and atomic force microscopies

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**Abstract**

The potential toxicity of nano- and microsized plastic particles necessitates the development of efficient methods for plastics detection in environmental samples and living organisms. Dark-field hyperspectral and atomic force microscopies are non-destructive and label-free techniques that can be successfully applied to study the uptake and distribution of micro/nano- plastics in cells and whole organisms. Commercially-available polystyrene particles (100 nm, 200 nm, 500 nm, 1 $\mu$m), and microspheres of polymethacrylate (1 $\mu$m) and melamine formaldehyde (2 $\mu$m) were visualized and spectrally identified in vivo in Caenorhabditis elegans nematodes using dark-field hyperspectral microscopy (DFM) in visible-near infrared (400-1000 nm) wavelength range. Particle quantification and differentiation between chemically-different ingested microplastics were possible. Using atomic force microscopy in nanomechanical PeakForce Tapping mode, the internalization and distribution of polystyrene spherical microplastics sized down to 500 nm in human skin fibroblasts was shown. Internalized and cell surface attached plastics could be differentiated based on their nanomechanical characteristics. Application of deep learning algorithms allowed classification of pigmented polystyrene microparticles in human skin fibroblasts using enhanced dark-field microscopy and a residual neural network. The dataset consisting of 11,528 particle images has been used to train and evaluate the neural network model. The accuracy of the obtained classification algorithm in cell samples achieved 93%. The ability of DFM to detect enviromnental micro/nano- sized plastic particles emerging during the UV-induced degradation of polypropylene face mask was tested. The spectral profile of intact outer blue spunbond mask layer differed from both middle meltblown and internal spunbond white mask layers. After UV (254 nm) treatment the spectra of all mask layers became similar. After 192h of UV exposure, differently shaped micro- and nanosized plastic fragments appeared. The spectral features allowed ascribing some microfragments to a source mask layer. The works were supported by the Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030).

**Keywords:** nanoplastics, dark, field microscopy, atomic force microscopy, in vivo, in vitro

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