Characterisation data and description of a characterisation experiment for Optofluidic Force Induction (OF2i®) technology used in NanoPAT

Overview of the Characterisation

1	**Sample**	Optofluidic Force Induction (OF2i®) technology can be deployed for online analysis of nanosuspensions, nanoemulsions and colloidal formulations with particles in the size range of nano- and micrometers. In NanoPAT, OF2i® technology was optimized for the real time characterization with hydroxyapatite and silicon carbamide (nano)particles production at lab and pilot-plant scale.
2	**Chain of methods**	Machine prep 1. Setup OF2i B1 PAT Sensor station which contains the high-precision pump, the Asynchronous Dilution System (AoDiSys), the OF2i *fluidic box* and the OF2i *sensor module* and the waste outlet.
		Machine prep 2. Switch OFF laser safety toggle and turn on the sensor station and the computer to open BRAVE K.A.R.I.N. (AoDiSys control) and BRAVE H.A.N.S (*Fluidic box* and *sensor module* control) and connect all the devices with the 2 softwares, and switch on the laser if detector module closed.
		Method 1. Set flowrates (dilution ratio) in the K.A.R.I.N. software and set valve position, flow rate and laser power in H.A.N.S. software for the performance check nanoparticle of suspensions.
		Measurement 1 - Perform Measurement for the performance check standard for the duration of interest.
		Data processing and calculation is performed during measurement. Output files and reports are generated. Raw-Data are stored.
		Method 2. Determining the optimal particle concentration for sample/process under investigation.
		Method 3. Continuous control of laser beam and adjustment the different parameters to obtain a clear output signal.
		Measurement 2 - Perform Measurement for the duration of interest.
		Processing and calculation is performed during measurement and output files and reports are generated; Raw-Data is stored.
		Machine prep 3 – Switch off the 2 software, computer and OF2i devises.
		Machine prep 4 – Disconnect sample/by pass and clean and store devises appropriately.
3	**Data publication**	Measurement and analysis files may be stored and made public if deemed relevant and not compromising intellectual property rights of NanoPAT partners.
4	**Access conditions**	Data management e.g. as data used for scientific publications, used PAT platform demonstration, or technique validation in standardization context, belongs to the data owner.
5	**Workflow of the characterisation**	Raw data and processed data are acquired by following the chain of methods described in point 2. It is then possible to deduce particle size distributions and concentrations in function of time and identify nano production process relevant information.

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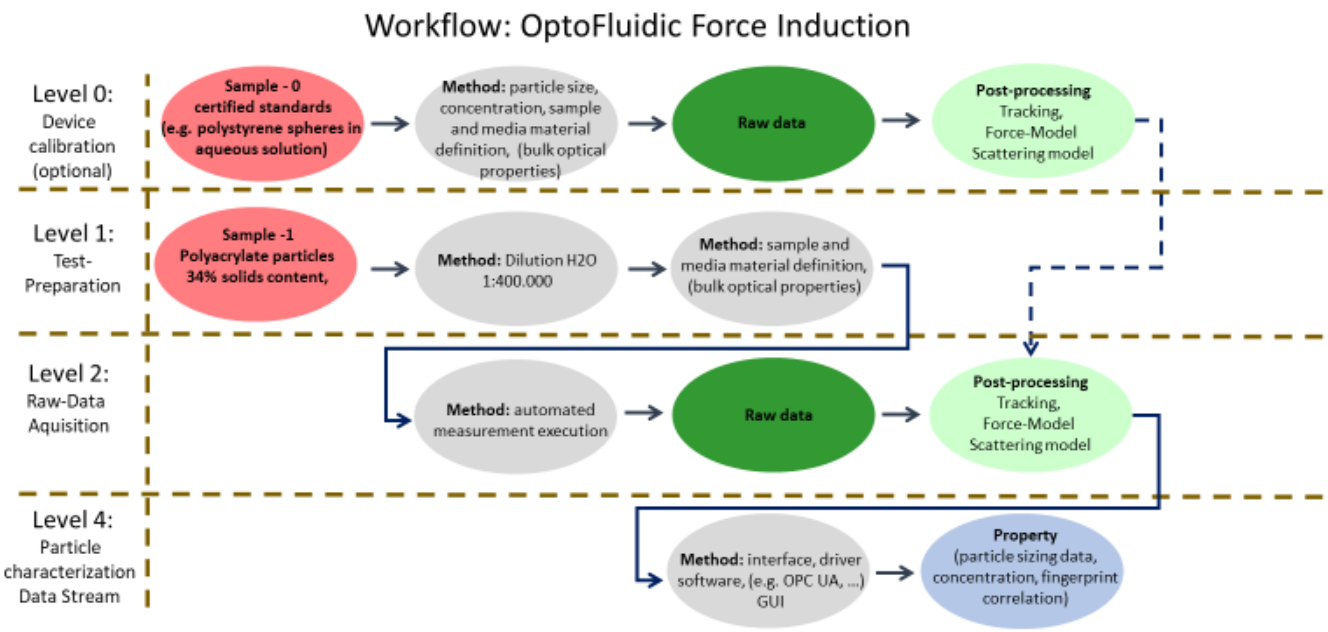

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1. SAMPLE		
1.1	**USER**	The OF2i device should be only operated by specifically trained staff and within the given technical specifications. Installation, Operational, and Performance Qualification documents are available to verify that the equipment is correctly installed, operates according to requirements and performs safely. User Guide including technical specifications available.
1.2	**Process under investigation**	Nano-production processes e.g. nucleation, gelation, polymerization using particles stability studies.
1.3	**Characterization environment**	• Environmental considerations for sampling point: Temperature range: +18°C to +25°C, Controlled humidity (non-condensing) • Chemical Consideration for Installation: pH range need to be checked according to used wetted materials: Wetted materials for the device are currently as follows, but can be subject to change on customer request: PEEK, PTFE, PCTFE, FKM (Viton), N-BK7 glass, fused silica glass, FEP. Additional materials can be added according to requirements. Stirred or flowing systems (i.e., 0,7 to 6 mL/min for sample preparation unit, or 4 to 1000 µl for direct measurement without sample preparation unit). Please refer to Installation requirement documentation.
1.4	**Sampling process**	Online sample is automatically taken with an initial extraction speed between 0,7 to 6 mL/min in an interval of 4 to 10 seconds via a bypass.
1.5	**Sample**	0,7 to 6 mL/min of sample is automatically taken via a bypass (online) and directed to the online dilution device and then analyzed in the flow chamber (minimal flow of 4 µL/min and minimum concentration of 10^4 object/mL depending on the particles' refractive index). Offline measurement are also possible but the sample must be homogenous and adequately diluted. A magnetic stirrer can be used if the sample tends to aggregate or agglomerate.
1.6	**Sample material properties**	Various (nano)particle compositions and sizes (approx. 50 nm to 5 µm (diameter)) can be analyzed by OF2i device depending on the particles' refractive index. To perform a valid measurement with OF2i which is a scattering based method, the optical contrast must be high enough. This means that the ratio of the refractive indices of the particle to the surrounding medium must be high enough in order to detect scattering signal. Secondly, being a single particle based method, OF2i requires low particle concentrations to avoid excessive scattering (optimal concentration of objects between 10^4 to 10^{10} objects/mL). The lower end can also be lower, higher scanning times are needed to get statistical relevant data.
1.7	**Sample preparation**	Automatic dilution of extracted sample is conducted online in 2 stages in order to reach an optimal concentration between 10^4 to 10^{10} objects/mL depending on the particles' refractive index. The diluted sample is then introduced (online) in the flow-chamber for analysis at a flow of 4 to 200 µL/min. For offline, sample is not diluted in the automatic dilution devise (i.e. Asynchronous Dilution System) and thus needs to be adequately diluted before its introduction in the flow chamber for analysis.

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1. SAMPLE		
1.8	**Material**	By measuring the velocity of the individual particles, OF2i® has a single-particle sensitivity for size and concentration characterization of all the particle populations, even at ultra-low concentrations. The measurement is continuous and therefore allows online process monitoring, monitoring of dynamic changes and evaluation of polydisperse substances in real time. Different particle populations can be measured in parallel and with a throughput of up to 3000 particles per minute. Validation with polystyrene nanoparticle (several sizes of Series NIST™ traceable Nanosphere™ Size Standards), NIST gold standards of several sizes. Tested at lab scale and plant pilot scale for Hydroxyapatite and SiC (nano)particles production in the context of NanoPAT.
1.9	**Hazard**	The B1 device is for prototype and beta-test usage only. Full product certification compliance (e.g. DIN EN 61010-1, VDE 0411-1:2020-03 Safety requirements for electrical equipment for measurement, control, and laboratory use, EN 60825-1 Safety of laser products - Part 1: Equipment classification and requirements, EN 61326-1 Electrical equipment for measurement, control and laboratory use – EMC requirements) is not guarantee. Enclosure cannot be opened without tools, no unintended leakage of laser radiation is possible. If any enclosures of the devise is open when the laser is on, the internal Class 4 Laser which operates with a wavelength of 532 nm and a max. power of 1650 mW can cause severe injuries to the body (especially skin and eyes) and the environment, especially dark and/or lightweight materials at close range. Potential leakage from fluid paths leading to electrical safety issues.

2. METHOD		
2.1	**Sample/probe physics of interaction**	Induced Forces from laser (e.g. with 532 nm wavelength) with Laguerre-Gaussian mode distribution (e.g. TEM01 optical charge of m=2) as well as a fluidic flow interact with a sample. Scattered light as well as position and velocity of particles are measured within an ultra-microscope setup. Intensity of laser beam is defined via the laser beam's output power as well as the optical setup. Fluidic forces are defined by the flow-chamber, by the particle containing media and pump setup. Following description: A method where particles are simultaneously trapped and accelerated by a laser beam in a liquid suspension and the change in positions of individual particles is used to determine the size of individual particles. NOTE 1 Analysis of the time-dependent position of trapped particles by means of scattered light can yield the optical force response due to their individual position within the defined optical field and hence the particle size as the hydrodynamic diameter using Mie-theory and Stokes´ - law relationships with corresponding equivalent sphere approximations. NOTE 2 The non-Brownian motion based counting method allows sizing in high flow rate particle streams. Analysis is sensitive to nanoparticles typically in the range of 50 nm to 5000 nm. The lower limit requires particles with high refractive index.

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2. METHOD		
		NOTE 3 OF2i equipped with Raman spectrometer can give particle based chemical composition through inelastic scattered light [see 5.25].
2.2	**Volume of interaction**	This is physically defined through the laser beam and is particle size dependent. It is described in: Šimi´ c M, Hill C, Hohenester U. Theoretical description of optofluidic force induction. Physical Review Applied. 2023;19(3):034041 And shown in M. Šimi´ c, D. Auer, C. Neuper, N. Šimi´ c, G. Prossliner, R. Prassl, C. Hill, U. Hohenester, Real-time nanoparticle characterization through optofluidic force induction. Physical Review Applied 18(2), 024,056 (2022)
2.3	**Equipment setup**	Fluidic connection to liquid-sample for continuous probing. Fluidic pump rate are fixed for adequate dilution ratio (2 step dilution) and optimal tracking of (nano)object depending on initial sample concentration. Laser beam power varies between 1000 mW to 1650 mW, depending on particle sizes, with highest power preferred for smaller size characterisation). For an optimal particle detection, the camera settings; Exposure and Gain can be adjusted in two sets, respectively. Increasing the gain will lead to a more intense signal (particles shine brighter) but will not influence the size measured due to only speed measurement.
2.4	**Calibration**	Does not require calibration other than that of the video - image scale and input laser intensity which are calibrated at the factory (i.e. permanent calibration).
2.6	**Detector**	Ultra-microscope setup with CCD/CMOS camera system. Fluidic flow measurements, temperature measurements at various positions within the device.
2.7	**Signal**	Localization and intensity of scattered light from particles in media.
2.8	**Time lapse (Measurement time)**	Continuous update of values 1x per second possible but delay due to bypass: from 20 sec to up to 10 minutes (sample-and plant-dependent) providing a live stream of data for complete of the production process 24/7.
2.9	**Testing Input parameters (Measurement parameters)**	The RI (Refractive Index) of the sample and media has to be set manually by the end user and depends on the sample. Equipment parameters have to be optimized beforehand. Flow rate and laser beam power are optimized for the sample through method development.

3. RAW DATA		
3.1	**Raw Data**	Hardware and software generate video streams with particle localization and particle scattering intensity information continuously and in “real-time”. An H264 encoded video stream, named with the time and other parameters (laser power, flow rate etc.). Particle trajectories (tracking algorithmics) and single particle scattering information, mean values and variance of signal over time is extracted.
3.2	**Unit**	Particle positions are obtained from the raw video data in pixel format and transformed according to a developed ray-tracing model into a local coordinate system. This conversion is done using a constant. Particle Velocity in mm/s are the calculated for a fixed laps of time (e.g. 5 or 10 s).
3.3	**Data acquisition rate**	Raw data (video tracking images) is processed 200times / sec, processed data output-stream is given 2x/seconds (e.g. plots).

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4. DATA PROCESSING		
4.1	**Level of expertise**	Little expertise: Person can read out particle sizes directly and plot particle size distributions from results. But Domain expertise required to interpret the particle stability profiles in terms of nano-production processed (e.g. gelation, nucleation).
4.2	**Data normalisation**	Absolute measurement technique, does not require calibration other than that of the video - image scale and input laser intensity,
4.3	**Data Processing reproducibility**	The non-Brownian motion based counting method allows sizing in high flow rate particle streams defined by the laser intensity. Hence, reproducibility is only limited by the accuracy of laser power stability and pumping stability and is defined within +/-10% of given sizing values.
4.4	**Data filtering processes**	Analysis of the stability of other parameters measurements during the time series; e.g. beam intensity distribution, flow rates, presence of bubbles during measurement, temperature anomalies, beam power, to filter out data.
4.5	**Data analysis procedures**	By monitoring the light scattered by the particles through a microscope objective, one obtains information of the scattering cross sections and via particle tracking the velocities and live visualization of individual particles. These signals are used to calculate particle size and time-dependent number-based size distributions in real-time. First, the scattering signal can be evaluated via the well-known Rayleigh-Scattering theory for particles of size well below the wavelength of the exciting laser. Second, we can use the information of particle position over time with the OF2i model which assumes spherical and dense particles. The particle size can then be obtained by solving Newton's equations of motion together with the measured particle velocity $v(r) = vfluid + (Fopt(r))/(6\pi\eta R)$, where vfluid is the flow velocity, Fopt are the position dependent optical forces, η is the viscosity of the fluid and R the particle radius.
4.6	**Main processed signals**	The hydrodynamic size of particles is measured by the introduction of optical forces altering particle pathways and the subsequent determination of resulting trajectories which contain size Software containing Mie theory, boundary element methods and particle tracking algorithms are used for converting particle data. Concentrations, size and size distributions (D-values) are extracted. For the velocity determination, we start with the waterfall diagrams, and analyze for a given position the image within the range of ±20 pixels. In a second image we plot a straight line with gradient angle θ and with a Gaussian profile in the transverse direction (σ = 2), and choose θ such that the overlap of the two images becomes maximal. From θ we can compute the particle velocity at a specific position. In our simulations, the fluid velocity v fluid is computed from the maximum of the parabolic velocity profile of Hagen Poiseuilles's equation for a given capillary. We always take the maximum velocity

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4. DATA PROCESSING		
		of the flow profile, since particles deviate only slightly from the capillary center along the beams profile compared to the capillary diameter when they are optically trapped. The flow velocity is calculated via the capillary's cross section together with the flow rate given in µL/s. The refractive index of (nano)particles and the surrounding background medium (water) at a wavelength of λ = 532 nm should be known. The effective focal length of the focused laser beam as well as the topological charge m = 2 are chosen in accordance with experiment.
4.7	**Data processing through calibrations**	OF2i works calibration-free.
4.8	**Properties (elaborated data)**	Particle size distribution, particle diameters in nm (D90, D50, D10), particle concentrations, correlation value to previously determined sample fingerprints. D values can be plotted as function of time when extracted from cumulative PSD accumulated for a certain number of seconds (e.g. 5 or 10 s).
4.9	**Quality of the data**	Performance check for size characterization before the analysis using NIST certified size standards (300 and 600 nm). Robustness of the data may be evaluated with offline size characterization technique for a sub-sample of the reaction suspensions such as DLS technique.
4.10	**Data management**	Data management is left to the discretion of the data owners.

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