Supporting Information
Quantification of carbon nanotubes in environmental matrices: Current capabilities, case studies, and future prospects

Elijah J. Petersen[1*], D. Xanat Flores-Cervantes[2], Thomas D. Bucheli[3], Lindsay C. C. Elliott[1], Jeffrey A. Fagan[1], Alexander Gogos[2,3], Shannon Hanna[1], Ralf Kägi[2], Elisabeth Mansfield,[1] Antonio R. Montoro Bustos[1], Desiree L. Plata[4], Vytas Reipa[1], Paul Westerhoff[5], Michael R. Winchester[1]

[1] Material Measurement Laboratory, National Institute of Standards and Technology, Gaithersburg, Maryland 20899, United States

[2] Eawag, Swiss Federal Institute of Aquatic Science and Technology, Überlandstrasse 133, CH-8600 Dübendorf, Switzerland

[3] Agroscope, Institute of Sustainability Sciences ISS, 8046 Zurich, Switzerland

[4] Department of Chemical and Environmental Engineering, Yale University, New Haven, Connecticut 06520, United States

[5] School of Sustainable Engineering and The Built Environment, Arizona State University, Box 3005, Tempe, Arizona 85278-3005, United States

*Corresponding author: Elijah J. Petersen: Telephone (301)-975-8142 Email: Elijah.Petersen@nist.gov

Current address for D. Xanat Flores-Cervantes is 2042 Dwight Way, Berkeley, CA 94704, United States.

Summary of contents

15 pages

4 tables

Method	**Natural Waters**	**Cell Media**	**Biological Tissues**	**Soil/Sediment**	**Release from polymer nanocomposites**
Spectroscopic					
Absorbance[65,109,150,151]	Scattering and absorbance of other components from the matrix may interfere with CNT quantification				
Near infrared fluorescence (NIRF) [15,29,31,152]	Scattering and absorbance of other components may interfere with CNT quantification; background fluorescence of the matrix may also impact measurements				
Raman[13,122,123,153-164]	Humic acid, other Raman active organic contaminants, and suspended particles (e.g., clays) could impact the detection method as could background fluorescence	This matrix may have background fluorescence	This matrix may have auto-fluorescence and may limit light penetration	Light scattering by large particulate material, may require separation prior to Raman analysis	Presence of the aromatic compounds at high concentration could influence the signal as could fluorescence
Spectrometric					
Inorganic Elemental Analysis[29,32]	The background metallic content in the matrix should be clearly defined before ICP-MS analysis and compared to the metals present in the CNTs; the elemental specificity and the high matrix tolerance, makes ICP-MS based techniques practically independent to most common environmental and biological interferences				
Single particle inductively coupled plasma-mass spectrometry (spICP-MS)[22]	Difficult to distinguish CNTs from other particulates containing the same metals, this is most likely for soils/sediments; extraction may be needed first				
Microscopic					
Atomic Force Microscopy[109,165]	For all matrices the presence of any other particulates depositing on the measurement substrate will require protocols for selective removal of all non-nanotube components; calculation of length distributions can be hindered by resolution issues (for short nanotubes) and observation bias (undercounting of long nanotubes) and/or the presence of aggregates				
Hyperspectral Imaging[166,167]	For all matrices, soot and other black particles could impact the detection of CNTs				

Photoacoustic (PA) and photothermal (PT)[24, 168-170]	Water is a good PA/PT solvent, anything else in the sample that absorbs or scatters the beam(s) would decrease signal or increase background		PA/PT works well in tissues transparent to beam(s); PT sensitivity drops in non-transparent tissue	No reports in literature; would be a difficult matrix to detect CNT with a lot of scattering and absorption of the beam(s)	A polymer matrix does not inhibit CNT detection; as long as there is a thermal response in the matrix, PT/PA can detect a signal
Scanning Electron Microscopy, Scanning Transmission Electron Microscopy, Transmission Electron Microscopy (TEM)[27,66]	Biopolymers, low concentration of CNTs compared to other particles leading to overlapping particles on the samples holder		Other fibrillar particles; low contrast between CNTs and biological tissue	Other fibrillar particles; low concentration of CNTs compared to other particles leading to overlapping particles on the samples holder	
Thermal					
CTO-375[18]	Very little interference in this matrix except for high N, organic carbon, or black carbon content waters	Matrices with high N or organic C content can char and form higher stability materials that, together with high BC concentrations, can interfere with the analysis of CNTs; conversely some matrices can produce "catalytic" effects that reduce the oxidation temperature of recalcitrant carbons		Sample specific oxidative strength (protective or catalytic) leading to variable recoveries of spiked CNTs; high organic C can char and high BC content can interfere with the CNT analysis	Some tested polymers (e.g., gamma-poly caprolactone) have lower thermal stability than CNTs which makes this a promising approach
Thermal Gravimetric Analysis (TGA) [20,171,172]	Very little interference expected except for waters with levels of BC that are approximately equal in concentration to the CNTs, NOM can stabilize CNTs	Isolated test materials show little interference, full matrix testing needed, peptone also binds to CNTs and may change oxidation temperature		Isolated test materials show little interference; full matrix testing needed, major	Interferences are unclear, tested epoxies have overlapping thermal stabilities with CNTs, and seem to influence the

				challenge in sample size (typically > 10 mg) and overlap of oxidation temperatures may hinder detection of CNTs	burn temperature of one another; overlap of oxidation temperatures or changes in thermal stability of CNTs in this matrix can hinder CNT quantification; in some cases, CNTs can be extracted from the matrix to give clear ratios of matrix:CNT composition
Thermal Gravimetric Analysis-Mass Spectrometry (TGA-MS) [14]	Very little interference; interference with BC can be reduced by MS peak deconvolution	Interferences with this matrix are unknown, but the unique chemical composition of cell media is promising for lower interferences	Isolated test materials show little interference and the unique chemical composition of biological tissues suggests low interferences, major challenge in sample size and overlap of oxidation temperatures may hinder detection of CNTs	Few direct interferences, but can raise background levels and raise the detection limit	The unique chemical compositions of most polymers suggests low interferences, evolved gases should be distinct for CNTs as compared to the polymer matrix
Total Organic Carbon (TOC) Analysis[71]	Interference exists from any organic matter (natural organic matter, soil organic matter, cellular material, serum or other organic compounds, organic polymers, etc.)				

Thermal Optical Transmittance (TOT) [16,23]	Typically little interference	Interferences should be minimal, but may arise if cell material chars into optically absorptive or thermally stable material	Interferences can be minimized by preparatory digestions (demonstrated for mouse lung)	Few direct interferences, unless the soil or sediment has a high non-CNT organic load	Potentially interfering, as many polymers will exhibit poor degradability under the inert atmosphere utilized in the first phase of this method; This could cause charring and confound the measurement of EC once the oxidative atmosphere is introduced
Isotopic labeling					
Carbon-13 Labelling[21,32,78]	Very little interference expected	Very little interference expected	Separation of CNTs from tissues is advised as accumulation of CNTs may be tissue dependent; background $\delta^{13}C$ signatures are necessary for each tissue type	Sulfates may interfere with the preparation of pure CO_2; CNT-free background required for comparative $\delta^{13}C$ signatures	CNT-free background require for comparative $\delta^{13}C$ signatures
Carbon-14 Labelling[15,26,30,99,124,152,173-181]	The potential biases depend on how the carbon-14 is quantified; some compounds may interfere with scintillation cocktails adsorbing beta emissions and could lead to autofluorescence; these issues would not be expected if the sample is combusted using biological oxidation		It may be possible to sonicate the tissue in liquid scintillation fluid, but there may be incomplete dispersion of the CNTs or quenching of the radioactivity; interferences are	Interferences have not been observed in previous studies with biological oxidation of the samples; good recovery was also found when sonicating SWCNTs with sodium dodecyl sulfate and using	There may be interference from quenching if the sample is added to liquid scintillation cocktail, but interferences would not be expected for biological oxidation

			unlikely with biological oxidation	liquid scintillation counting
Other radioactive isotopes[96-98]	This would depend to some extent on the radioactive isotope added and quantification used but generally interferences would not be expected for these different matrices; however, the stability of the radioactive tracer may be impacted by the dispersion process in the matrix or metabolic processes in tissues			
Additional Techniques				
Analytical Ultracentrifugation (AUC) [60,165,182-184]	Measurement of sedimentation requires homogenous dispersions with measureable viscosities and densities; significant light scattering from suspended particles from the matrix will additional likely complicate all but the most rigorous experimental protocols			
Gravimetric[185]	Measurement of CNTs in these matrices would encounter significant biases depend on the mass of other compounds that would be deposited when drying samples except at very high CNT concentrations		This technique is not applicable for these matrices	
Microwave Method[62,125,186,187]	Other carbon forms such as soot may cause interferences; this interference would be most likely for soils and sediments			
aF4-MALS[46]	No known interferences, but theoretically other low density fibre-like/high aspect ratio particles may interfere; if these particles exhibited lower thermal stability compared to the CNTs, oxidation could potentially be used to selectively remove them			

Table S1: Potential Matrix Interferences for Selected Techniques for CNT Quantitation

Abbreviations: Asymmetric flow field flow fractionation with multi-angle light scattering (aF4-MALS), analytical ultracentrifugation (AUC), black carbon (BC), carbon nanotube (CNT), chemothermal oxidation at 375 °C (CTO-375), inductively coupled plasma-mass spectrometry (ICP-MS), near infrared fluorescence (NIRF), photoacoustic (PA), photothermal (PT), single particle inductively coupled plasma-mass spectrometry (spICP-MS), single-wall carbon nanotube (SWCNT), thermal gravimetric analysis (TGA), thermal gravimetric analysis-mass spectrometry (TGA-MS), total organic carbon (TOC), thermal optical transmittance (TOT).

Method	Impact of CNT agglomeration	Impact of CNT Oxidation/Degradation	Wrapping with Organic Molecules (proteins, NOM)
Spectroscopic			
Absorbance[65,109,150,151]	Measured absorbance signal per unit mass typically decreases above a threshold level of nanotube aggregation, especially for the intrinsic nanotube optical transitions, although apparent absorbance in the UV and visible regions may broadly increase due to increased light scattering by larger particles; for intrinsic optical transitions, the transition wavelength will typically red shift and peak intensities will decrease with any reduction from individualized dispersion	Absorbance of intrinsic optical transitions typically decreases monotonically above very low levels	In the absence of changes in agglomeration state, the adsorption of material to the nanotube interface generally will affect the absorbance mostly through red/blue shifts in intrinsic optical transition wavelengths by modification of the local dielectric environment; changes to the surface accessibility of the bulk solvent can also affect optical transition intensities
Near infrared fluorescence (NIRF) [15,29,31,152]	Peak shifts and intensity decrease for SWCNTs could occur for either of these changes		Variable
Raman[13,122,123,153-164] Absorbance[65,109,150,151]	Not a significant factor but G and D band ratio may be sensitive to sample agglomeration	Raman spectra are very sensitive to oxidation or degradation	Vibrational features are sensitive to structural stress which may be caused by wrapping with organic molecules or polymers
Spectrometric			
Inorganic Elemental Analysis[29,32]	Minimum impact on elemental analysis when a complete sample digestion is performed	Any loss of metals intercalated in CNTs before the elemental analysis would lead to biased results	Minimum impact when the wrapping does not alter the elemental composition of CNT
Single particle inductively coupled plasma-mass spectrometry (spICP-MS)[22]	Severe undercounting effect on actual CNT concentrations since each agglomerate may only be counted as one single pulse depending on the dwell time	Important influence on sizing and counting results because of the increasing contribution of smaller CNTs containing metal masses below instrument detection limit	Wrapping would affect physical transport of the CNT in introduction system, increasing the uncertainty on the size and number concentration determination
Microscopic			
Atomic Force Microscopy[109,165]	Agglomeration or oxidation/degradation may impact apparent size distribution and hinder analysis		Variable

Hyperspectral Imaging[166,167]	Better optical visibility due to enhanced scattering from agglomerates	Potential changes in spectral profiles from oxidation/degradation or wrapping with organic molecules	
Photoacoustic (PA) and Photothermal (PT)[24, 168-170]	Will follow the same changes that affect Absorbance; anything that changes the absorption of the CNTs would affect the PT/PA signal causing shifts in peak wavelength and changes in absorption cross-section; degradation would certainly affect the PT/PA signal; the effect of agglomeration, oxidation, and the addition of physisorbed or chemisorbed ligands would be case-by-case		
Scanning Electron Microscopy, Scanning Transmission Electron Microscopy, and Transmission Electron Microscopy[27,66]	CNTs will still be detected if investigated manually, but automated analysis may fail to identify CNT in agglomerates	Change in the size distribution, depending on the extent of degradation	May reduce the image resolution due to contamination effects (resulting from the volatilization and redeposition of the organic material under the electron beam)
Thermal			
CTO-375[18]	CNT agglomeration will slightly increase thermal stability, but not to an extent discernable by CTO-375	Oxidation and degradation reduce CNT thermal stability, which would enhance separation from BC but require a different cut off temperature to quantify SWCNTs; MWCNTs will still be interfering with BC	Organic coatings should be resolved (e.g., more labile than CNTs) by CTO-375 and not affect the measurement; however, proteins can char and cause interference

Thermal Gravimetric Analysis (TGA) [20,171,172]	CNT thermal stability will increase measurably; SWCNTs may no longer be resolved from BC or soot; MWCNTs should still have higher thermal stability than BC; higher temperature shoulders on oxidation peaks occurs with bundling, changes in oxidation temperature of material when bundled vs not	Oxidation and degradation of CNTs will reduce the thermal stability, which should help resolve SWCNTs from soot, but will likely not change the MWCNT thermal stability to such an extent that it interferes with BC	Organic coatings can influence the thermal stability of the CNTs, where lower onset temperatures and broader mass loss events have been observed, increasing potential interferences; proteins can char and cause interference
Thermal Gravimetric Analysis-Mass Spectrometry (TGA-MS) [14]	CNT thermal stability will increase measurably, no anticipated change in MS signal	Oxidation of CNTs will reduce the MS-derived advantages, which leverage the low-oxygen content of CNTs; potential for changes in decomposition products	Organic coatings can change CNT thermal stability and should increase the O and/or N content of the diluting matrix; thus, CNT-derived depletions in O would become easier to observe with organic matter coatings
Total Organic Carbon (TOC) Analysis[71]	Unlikely to be impacted by aggregation	Oxidation of CNTs would likely improve detection, as TOC analysis relies upon complete conversion to gaseous carbon mon- or di-oxides	Any organic surface coating (citrate, amine, etc.) contributes to the carbon detected from the CNT
Thermal Optical Transmittance (TOT) [16,23]	Sample on the filter won't be uniform, the split point of organic carbon/elemental carbon needs to be manually chosen instead of by optical information	Oxidation/degradation decreases the thermal stability and causes peak position shift; no issue with quantifying CNTs unless the sample has huge amount of organic carbon and the peak position of CNT after shifting is getting close to organic carbon	Having too much organic carbon may affect the thermal stability of CNT, and the signal from organic carbon will overlap that of elemental carbon; organic carbon should be resolved as much as possible
Isotopic labeling			
Carbon-13 Labelling[21,32,78]	Comprehensive oxidation of the CNTs required to prevent isotopic fractionation; agglomeration may affect thermal stability in closed-tube-combustion approaches, and efforts should be made to ensure complete combustion	Pre-analysis CNT oxidation may have slight impacts on CNT δ^{13}C signature (by virtue of reactive fractionation); these should be small depending on the extent of surface oxidation and/or if that process removes CNT-C from the CNT matrix	Wrapping with organic molecules will affect δ13C; the effect will depend on the δ13C of the molecule, and measures to separate the coating from the CNT are critical

Carbon-14 Labelling[15,26,30,99,124,152,173-181]	The impact of agglomeration would depend on the quantification procedure used; interference from self-quenching has been reported in some studies with agglomerates of CNTs, but this would not be expected for quantification using biological oxidation	This is not expected to impact this approach; carbon-14 analyses of released carbon dioxide has been used to quantify CNT degradation	This may impact measurements with liquid scintillation counting of dispersed CNTs but should not impact samples combusted using biological oxidation
Other radioactive isotopes[96-98]	This would not be expected to impact most isotopes unless self-quenching occurs	Oxidation or degradation may render this technique unusable if these processes lead to substantial separation of the radioactive isotope from the CNT	This would not be expected to impact most isotopes unless quenching occurs
Additional Techniques			
Analytical Ultracentrifugation (AUC)[60,165,182-184]	Aggregates rather than primary particle would be measured, data analysis potential decreased	Interpretation of results will become suspect due to differences in actual sample with respect to expected behavior	Variable effects, will likely bias size analysis
Gravimetric[185]	No impact	No impact unless there is complete degradation to CO_2	This can limit the accuracy of this approach since the concentration of these organic molecules will need to be assumed to be homogeneously distributed
Microwave Method[62,125,186,187]	These potential interferences have not been tested for this technique		
aF4-MALS[46]	Enhances material losses on the membrane and hinders accurate shape factor determination; thus agglomeration has to be avoided	Modifies CNT interactions with the membrane which can lead to higher or lower losses depending on carrier solution and membrane material	

Table S2: Potential Interferences for CNT Quantitation from Changes to CNTs for Selected Techniques

Abbreviations: Asymmetric flow field flow fractionation with multi-angle light scattering (aF4-MALS), analytical ultracentrifugation (AUC), black carbon (BC), carbon nanotube (CNT), chemothermal oxidation at 375 °C (CTO-375), multiwall carbon nanotube (MWCNT), near infrared fluorescence (NIRF), photoacoustic (PA), photothermal (PT), single particle inductively coupled plasma-mass spectrometry (spICP-MS), single-wall carbon nanotube (SWCNT), thermal gravimetric analysis (TGA), thermal gravimetric analysis-mass spectrometry (TGA-MS), total organic carbon (TOC), thermal optical transmittance (TOT).

Method	**Relevant reference materials or standard methods**	**Key steps in instrument calibration**	**Challenges to standardization (i.e., traceability to the SI)**
Spectroscopic			
Absorbance	Informational values RM8281, ISO/TS 10868:2011	Calibration of wavelength and intensity performed at 100 % transmittance. Usable wavelength range should be established by testing the absorbance of a blank sample and considering regions of high absorbance, scattering interference and Beer's law considerations.	Chemical environment can affect intensity and peak positions. Absorbance of water in NIR wavelengths for cell path lengths > 1mm.
Near infrared fluorescence	Informational values RM8281, ISO/TS 10867:2010	Traceable lamp detector train calibration	Chemical environment can affect intensity and peak positions, in-filter effects
Photoacoustic and photothermal	CNTs with well characterized absorbance of narrow size distribution in a pure solvent could be used for calibration	A standard sample with material similar to the sample CNTs (that absorbs the same wavelength) can be used to tune the setup. Laser power, sensitivity, and time constant can be adjusted for the sample as needed.	No standards published or referenced to date. Short shelf life for samples if in situ CNTs degrade or change over time. Difficult traceability to SI.
Raman	Frequency (x-axis) calibration standards ASTM E1840, Intensity (Y-axis) E2911, E2529, NIST SRM series 224X	Choose the appropriate standard for frequency and intensity depending on the excitation wavelength. Alternatively, a series of standard solutions (dilution series) of the pure analyte in combination with the internal standard can be used	
Spectrometric			
Inorganic Element Analysis	Dissolved standards of the monitored elements are required to determine the instrument sensitivity for the elemental quantification. Potential influences from residual carbon content and dissolved solids can be accounted for by suitable calibration techniques, including isotope dilution, matrix matched standards and the method of additions. SRM 2483 (single-wall carbon nanotubes (raw soot)) could be used to test instrument performance	ISO/TS 13278:2011E, This Technical Specification provides reference standard methods for the determination of elemental impurities in CNTs using ICP-MS. Results traceable to the SI can be readily achieved using traceable high-purity calibration standards. Calibration is performed with solutions having known concentrations of the metallic analytes of interest and matrix-matched to the composition of the prepared samples.	Lack of control environmental and biological matrices. Guarantee that sample digestion is quantitative prior to elemental analysis.

Single particle inductively coupled plasma-mass spectrometry (spICP-MS)	Reference Materials: Single element standard solutions available from numerous reference materials producers; NIST RM 8013 Gold Nanoparticle, Nominal 60 nm Diameter; Standards: ISO TS13278 Determination of metal impurities in samples of carbon nanotubes using inductively coupled plasma mass spectrometry	Calibration of ICP-MS instrument sensitivity is performed with solutions having known concentrations of the metallic analytes of interest and matrix-matched to the composition of the prepared samples. Calibration of sample transport efficiency is performed using metallic nanoparticles having known size (metal does not need to be the same as the trace metal analytes). Sample transport efficiency may also be calibrated using the waste collection method, but this method is generally less reliable.	Reference CNT samples with homogeneous size and controlled metal impurities contents are required to address the standardization.
Microscopic			
Atomic Force Microscopy	ASTM E2859-11 Standard guide for size measurement of nanoparticles Using atomic force microscopy, NIST RM8281, NRC Canada SWCNT-1	In-plane resolution, i.e. distance/pixel should be selected to enable identification of smallest expected particles of interest.	Surface roughness of deposition substrates varies significantly with preparation methodology. Polydisperse samples may require measurements at multiple resolutions to identify small particles, and to locate larger particles.
Hyperspectral Imaging		A representative spectral library is generated from the parent material. The spectral library is then used to detect the same material in a sample (e.g. cell) using a mapping algorithm	Unspecific absorption in the VNIR spectral range
Scanning Electron Microscopy	ISO/TS 10798:2011 Nanotechnologies -- Charaterization of single-wall carbon nanotubes using scanning electron microscopy and energy dispersive X-ray spectrometry analysis	Reference materials (regarding CNTs number concentrations) must be use to evaluate instrumental losses during sample preparation	Reference CNT suspensions with certified number concentrations must be developed; the shelf life of these suspensions maybe limited due to CNT agglomeration
Transmission Electron Microscopy	ISO/TS 10797:2012 Nanotechnologies -- Characterization of single-wall carbon nanotubes using transmission electron microscopy	Reference materials (regarding CNTs number concentrations) must be use to evaluate instrumental losses during sample preparation	Reference CNT suspensions with certified number concentrations mus be developed. The shelf life of these suspensions maybe limited due to CNT aggregation.
Thermal			
CTO-375			
Thermal Gravimetric Analysis	ISO/TS 11308:2011 Nanotechnologies -- Characterization of single-wall carbon nanotubes using thermogravimetric analysis	Temperature and mass calibration required	No reference materials for temperature calibration; traceable mass standards available
Thermal Gravimetric Analysis-Mass Spectrometry		Temperature and mass calibration required. MS peak identification database needed.	No reference materials for temperature calibration; traceable mass standards available
Total Organic Carbon Analysis			
Thermal Optical Transmittance	NIOSH, Elemental Carbon (Diesel Particulate): Method 5040. In NIOSH, Manual of Analytical Methods, 4th ed.; 2003.	Sucrose solution and methane gas carbon standandards are often used for mass calibration	Each CNT has slightly different peak position depending on defect, purity, functional group etc.; also it differs by the temperature program

Isotopic labeling			
Carbon-13 Labelling	Standards include calcium carbonate (commonly used Vienna PeeDee Belemnite and NBS ^{13}C standard), barium carbonate	Ensuring accuracy of standards is key to reliable measurements. Also, running standards throughout sample analysis is required to understand measurement drift.	Samples containing sulfate cause contamination in the final product. Small samples may not release enough gas for the analysis. Nanotubes will differ in their 13C ratios based on original source of C.
Carbon-14 Labelling	Standards include NIST 4222C (carbon-14 hexadecane radioactivity standard solution)	Calibration depends on the method (liquid scintillation counting, autoradiography, biological oxidation) used to quantify the radioactivity. For all methods, it may be necessary to calibrate the instrument using other radioactive carbon-14 chemicals or elements.	
Other isotopes	Multiple radioactivity standards are available from NIST (e.g., 4915F cobalt-60 radioactivity standard solution) and from other organizations	Calibration depends on the instrument used to measure the radioactivity	One principal challenge is the stability of the radioactive isotope onto the carbon nanotube.
Additional techniques			
Analytical Ultracentrifugation	SRM under development for ensuring radial measurement precision; sedimentation of Bovine Serum Albumin (BSA) frequently used as an unofficial standard.	External evaluation of temperature calibration and bulk solution viscosity and density properties are critical for correct measurements.	Requires unique absorbance or refractive index signals from solute differentiable from media.
Gravimetric	A broad range of mass RMs and protocols are available for gravimetric measurements	Balances can be calibrated using device-specific procedures, reference masses are readily available	Works only for a limited number of conditions and matrices
Microwave Method	CNT material used in the exposure experiment, (reference) control material (e.g., CNT-free biological tissue such as NIST SRM 1573 Tomato leaves)	The very same CNT material that is to be quantified must be used to calibrate the instrument; a calibration curve is generated using the thermal response as a function of known CNT amounts spiked into tissue samples	A main limitation to standardization is that the instrument used to make these measurements is not readily available
Field flow fraction/asymmetric flow field flow fraction/asymmetric flow field flow fraction-multi-angle light scattering	Certified polystyrene (PS) beads (Single or mix, available from NIST or other sources). Bovine serum albumin (BSA). Any other certified particle standard (e.g., Au, Ag, and SiO2) that can be dispersed in the carrier solution	PS beads dispersed in the used carrier solution are used for retention time calibration (hydrodynamic diameter). An isotropic scatterer is used for normalization of the MALS detector angles (e.g. 20nm PS beads). BSA is used for molecular weight calibration of the MALS detector	Reference CNT samples with homogeneous size and controlled particle impurities (e.g. soot) would be required for aF4-MALS quality assurance. Changes in the chemical environment of the CNTs as well as changes of the CNTs themselves (e.g., surface functionalizations, length distributions) can affect retention time in aF4; standardized methods must include extensive methodological details to ensure reproducibility

Table S3: Standards and calibration of selected carbon nanotube quantification techniques

Material	**NIST SRM 2483 Single-wall carbon nanotube soot**	**NIST RM8281 Single-wall carbon nanotubes (dispersed, three length-resolved populations)**	**NRC Canada: SWCNT-1 Single-wall carbon nanotube certified reference material**	**JRC Multiwall carbon nanotube representative test materials**
Reference Material (Y/N)	Yes	Yes	Yes	No
Are certified, reference, or information values provided for these characteristics?				
AFM imaging	No	Information values (length distributions)	Information values	No
Elemental composition	Certified, reference, and information values	No	Certified and reference values	Yes
NIR fluorescence spectra	No	Information values	Information values	No
Raman ratio	Reference values	Reference values	Reference values	Yes
Raman spectra	Information values	Information values	Reference values	Yes
SEM imaging	Information values	No	Information values	No
Specific surface area	No	No	Reference values	Yes
TEM imaging	Information values	Information values	Information values	Yes
Thermogravimetric analysis (residual mass and oxidation temperature)	Reference values	No	Reference values	Yes
UV-vis-NIR absorbance spectra	Information values	Information values	Information values	No
X-ray diffraction	No	No	Reference values	Yes

Table S4: Characterization of carbon nanotube reference materials, standard reference materials, and representative test material.