Applied NanoFluorescence
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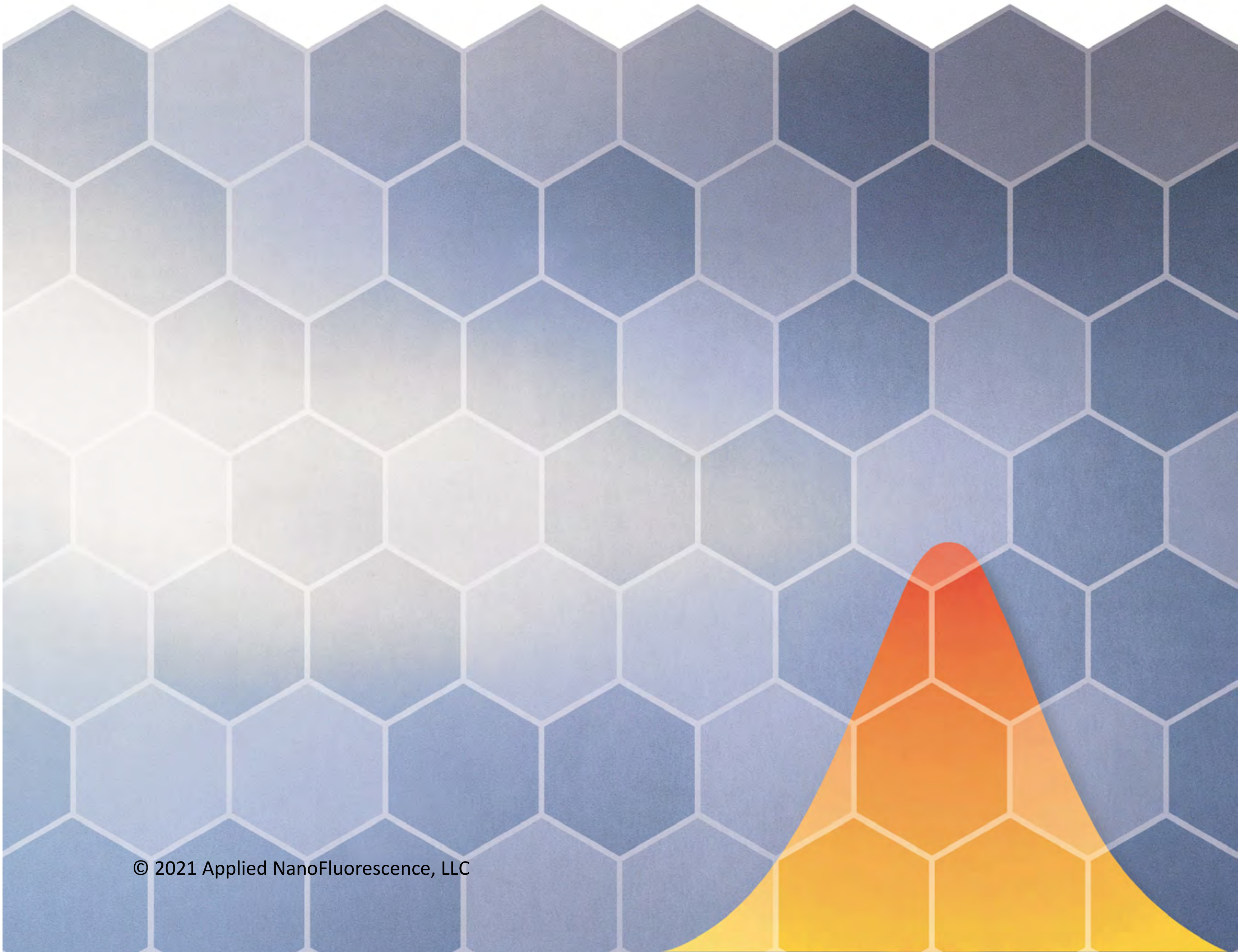
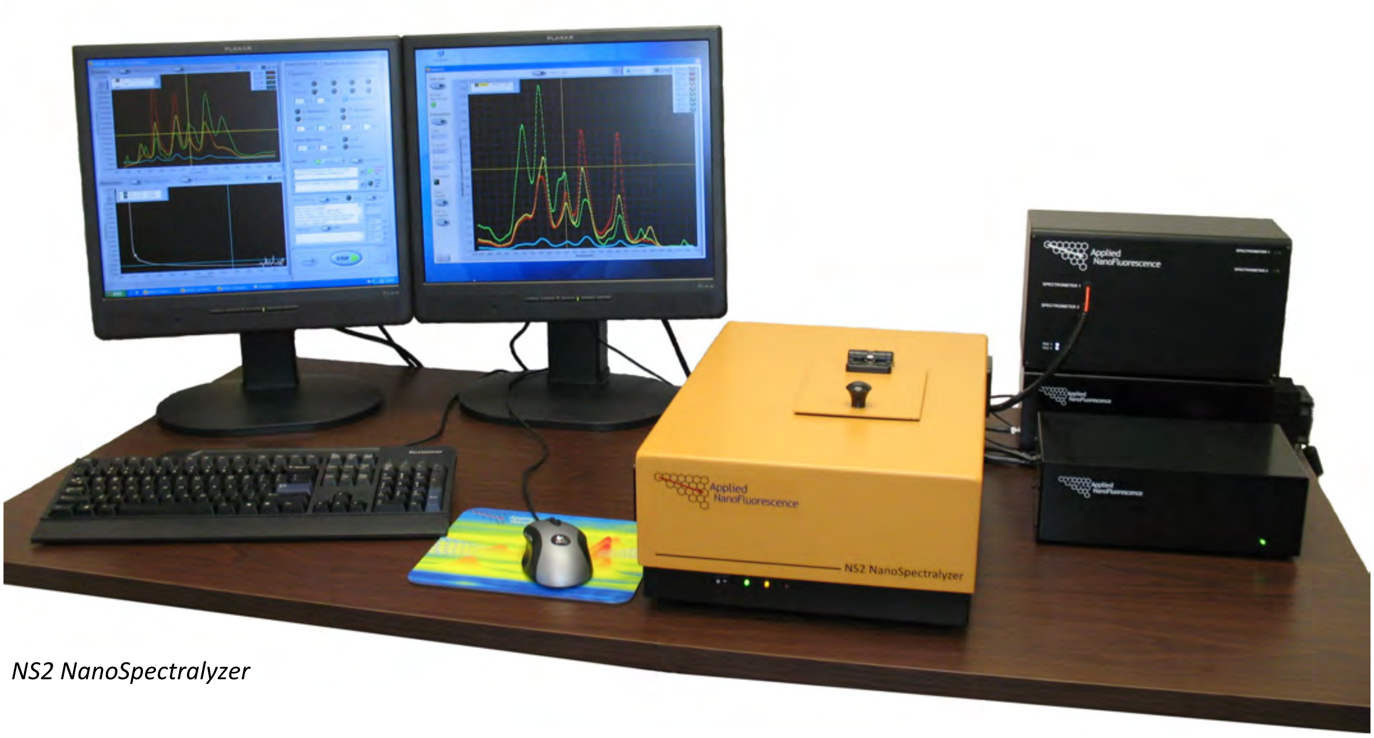
*Models: NS1*
*NS2*
*NS3*
*NS MiniTracer*

# The NanoSpectralyzer®

*for Nanomaterial and Carbon Nanotube Characterization*



# The NanoSpectralyzer®




*NS2 NanoSpectralyzer*

## Introduction

The NS1, NS2, and NS3 NanoSpectralyzers® from Applied NanoFluorescence (ANF) are unique laboratory instruments designed to characterize carbon nanotube (CNT) and other nanoparticle samples. They contain specialized multi-mode spectrometers integrated with sophisticated computer control and analysis software. The NS1 sensitively captures near-infrared (NIR) fluorescence spectra induced by four excitation lasers, and measures sample absorbance throughout the visible and NIR regions. The NS2 has all of the NS1 functionality plus Raman spectroscopy. The NS3 is the most versatile addition to the innovative line of NanoSpectralyzer instruments. Its modular design allows easy customization to meet specific customer needs for a variety of nanomaterial characterization.

In all instruments, CNT NIR fluorescence data are automatically analyzed using the latest research findings to give detailed information on carbon nanotube composition.

## Background

NIR band-gap fluorescence from single-walled carbon nanotubes (SWCNTs) was discovered at Rice University in 2001. Subsequent research at Rice deciphered the complex pattern of absorption and emission peaks seen in mixed samples. Each distinct spectral feature was securely assigned to a specific nanotube structure. These structures differ in diameter and roll-up angle, and are uniquely labeled by pairs of integers denoted (*n*,*m*). Thus, the NIR emission spectra provide compositional "fingerprints" that allow rapid and convenient (*n*,*m*) analysis of bulk SWCNT samples.

Near-infrared fluorescence is not emitted by the one-third of SWCNT species that have metallic character, or from multi-walled carbon nanotubes (MWCNTs). However, these nanotubes can be detected and characterized by their optical absorption and Raman spectra. Absorption and Raman also offer valuable information about SWCNT samples and a variety of other nanoparticles such as gold, graphene, and quantum dots.

General-purpose spectrofluorometers are not optimized for nanotube analysis. Although versatile, they are bulky, slow, insensitive, and give raw data that need careful manual interpretation. In addition, they cannot measure absorption or Raman spectra. ANF has developed the NanoSpectralyzer line of instruments specifically for nanotube analysis. In addition, these instruments are capable of measuring spectra from a variety of nanomaterials. These integrated systems efficiently measure and interpret multimode spectral data to provide the best available optical characterization.

**Relevant Literature:**


M. O'Connell et al., **Science 297**, 593 (2002).

S. M. Bachilo et al., **Science 298**, 2361 (2002).

R. B. Weisman and S. M. Bachilo, **Nano Letters 3**, 1235 (2003).

R. B. Weisman, **Analytical and Bioanalytical Chemistry 396**, 1015 (2010).

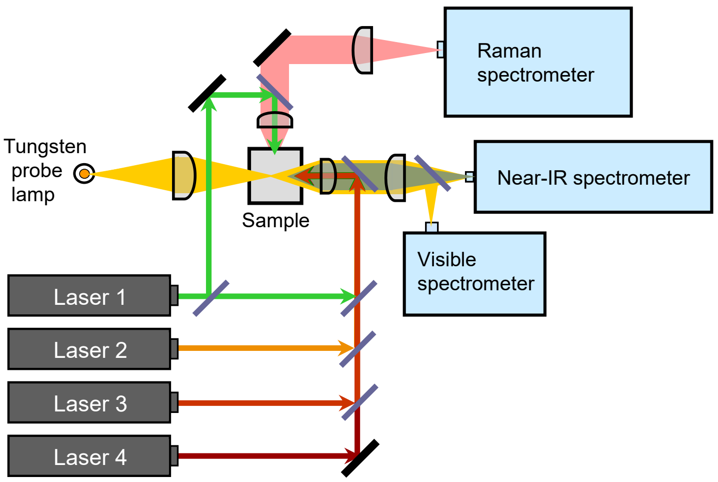
J. R. Rocha et al., **Analytical Chemistry 83**, 7431 (2011).


## Applications

- **Quality Control:** these instruments can be used to monitor changes in CNT product composition while exploring different growth process conditions. Later, during routine production, the NanoSpectralyzers allow rapid analysis of batch-to-batch consistency and quality. Because the instruments are highly automated, this QC/QA function can be performed by technicians with limited scientific training.
- **Nanoparticle Characterization:** the versatility of the NS3 NanoSpectralyzer can be used to collect optical spectra from a variety of different nanoparticles including gold, graphene, and quantum dots, with the full carbon nanotube analysis capabilities of the other NanoSpectralyzer models.
- **Biomedical & Environmental Research:** the NanoSpectralyzers' very high NIR fluorescence sensitivity can be used to detect and quantify trace concentrations of SWCNTs in complex surroundings. Major applications include biomedical research involving SWCNTs in tissue specimens, and environmental research that must track the movement and fate of SWCNTs in nature.
- **Nanotube Basic Research:** the NS1, NS2, and NS3 are versatile laboratory tools. They allow SWCNT starting materials to be properly characterized, avoiding experimental pitfalls caused by inconsistent or unknown sample properties. The NanoSpectralyzers also guide and check the sorting of mixed samples into specific structural forms, for example by chromatography, density gradient centrifugation, or two-phase aqueous separation methods. And they enable sophisticated structure-resolved studies of many chemical reactions and physical processes.

*Please visit our website for a current list of research publications by NanoSpectralyzer users.*

# How it Works: Carbon Nanotube Characterization




*NS2 NanoSpectralyzer optical schematic*

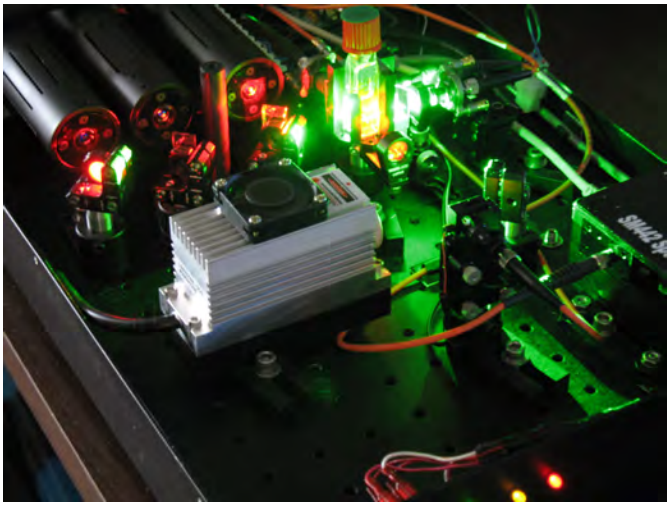
A small sample of raw carbon nanotubes is first ultrasonically dispersed into an aqueous surfactant solution to individually suspend a portion of the nanotubes. Then a few drops of the dispersion, containing as little as nanograms of carbon, are placed into a sample cell.

The NanoSpectralyzer uses multiple lasers to excite near-infrared fluorescence from semiconducting SWCNTs. These laser wavelengths are chosen to match the absorption range of nanotubes in samples grown by common processes such as HiPco or CoMoCAT. As each laser irradiates the sample, SWCNT emission is captured by an efficient optical system, spectrally dispersed in a near-infrared spectrometer, and detected by a cooled InGaAs array with 512 elements.

The resulting set of fluorescence spectra is quickly analyzed by sophisticated fitting software based on the latest research to deduce an inventory of (*n*,*m*) species in the sample and their relative concentrations. These results are automatically compiled into tables and publication-ready graphs.

The fluorescence spectra are complemented by absorption spectra covering the NIR and visible. All CNTs in the sample contribute to these spectra, including those that are non-emissive because of aggregation, functionalization, defects, metallic character, or multi-walled structure.

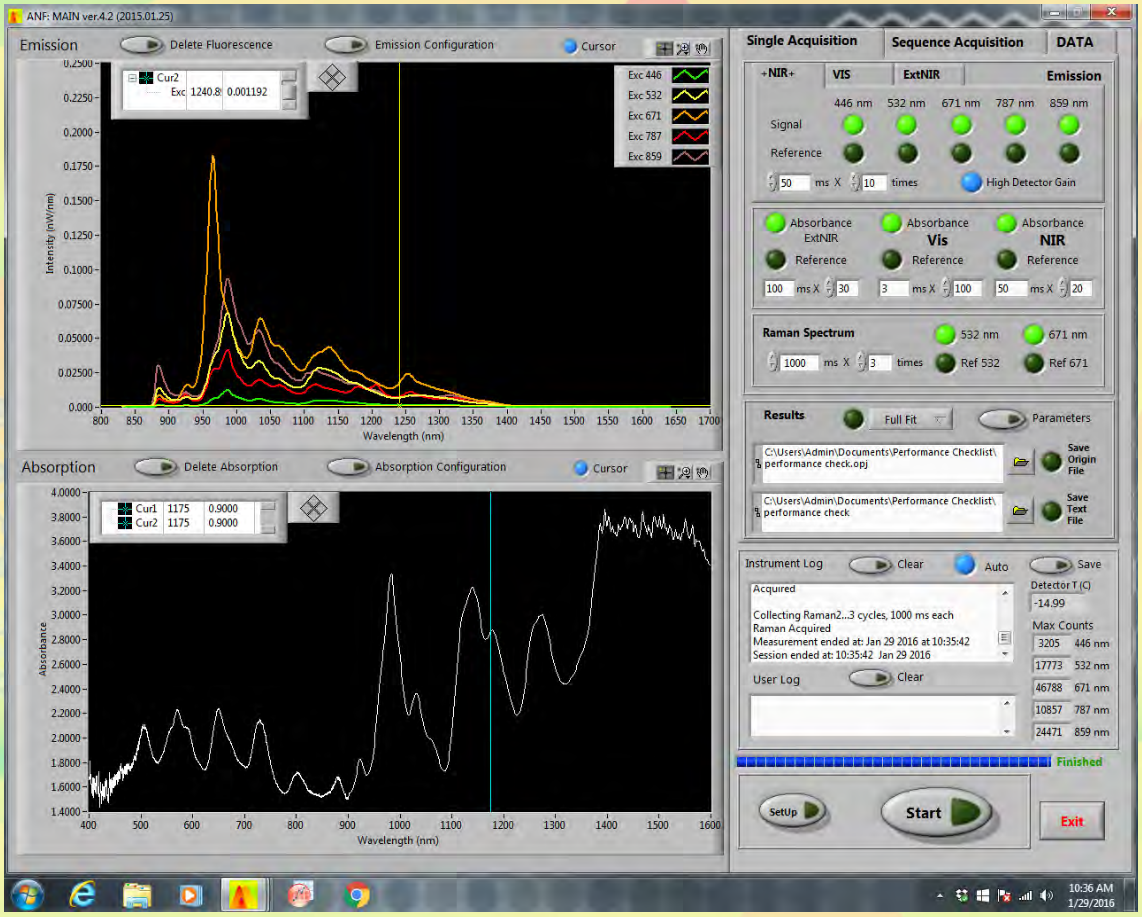
The data analysis software automatically divides the total emitted fluorescence by the absorption of the excitation beam to display a fluorescence efficiency index. This index is an incisive measure of relative quality for SWCNT samples, reflecting nanotube perfection and disaggregation.



The NS2 and NS3 NanoSpectralyzers also measure Raman scattering, using an excitation wavelength selected when the instrument is ordered. Raman spectra may show contributions from nanotubes that are semiconducting or metallic, aggregated or disaggregated, pristine or functionalized, single, double, or multi-walled. They capture RBM, D, G, and 2D bands, which arise from different vibrational modes and reflect aspects of sample structure and condition. Raman data complement the fluorescence and absorption spectra to give more complete sample characterization.

# Why use a NanoSpectralyzer instead of separate instruments?

- The multi-mode NanoSpectralyzers save expense, lab space, and time
- Each sub-system of the NanoSpectralyzer is designed for CNT spectroscopy, but can also obtain spectra of other nanomaterials that have an optical response to the characterization method
- All spectra are automatically and quickly measured on a single undisturbed sample
- Integrated analysis software correlates data from different spectral modes, combining all results into a unified Origin project file
- Applications support is provided by expert nanotube and nanomaterial scientists




*Main control screen*

## NanoSpectralyzer Interface

The main control screen of an NS3 NanoSpectralyzer shows some of the possible modules that can be combined to create a highly customized spectrometer for any nanomaterial research application. Some modules shown as separate tabs on the main control screen include Emission in the Visible, NIR, and Extended NIR with up to 5 different excitation wavelengths; Absorption in the Visible, NIR, and Extended NIR; and Raman with two different excitation wavelengths.

# NanoSpectralyzer Model Overview

## NS1 Measurement Features

- Records NIR fluorescence, NIR absorption, and visible absorption spectra
- Four lasers for excitation of SWCNT fluorescence emission
- Sensitive NIR fluorescence detection from 900 to 1600 nm
- Rapid absorption spectroscopy from 410 to 1600 nm
- Flexible, integrated data acquisition and analysis software
- Small sample volume capability
- Flow cell compatibility
- Sequence mode for monitoring eluent streams or chemical reactions

## NS2 Additional Measurement Features

- Raman spectra covering RBM, D, G, and 2D bands

## The NS3 Measurement Features

The NS3 is a modular multi-mode spectrometer. The base system includes NIR emission and absorption (900 to 1600 nm) with five discrete excitation laser wavelengths from 405 to 830 nm. Your NS3 can be custom-configured with your choice of the following additional spectral capabilities:

### Fluorescence measurements

- Fluorescence spectra in the Visible (410 to 900 nm) and/or Extended NIR ranges (to 1900 nm)

### Absorption measurements

- Absorption spectral coverage seamlessly expanded to include the UV (210 to 430 nm), Visible (410 to 900 nm), and/or Extended NIR (to 1900 nm)

### Raman measurements

- Single or dual Raman spectrometers, with excitation at 532 and/or 660 nm and 6 $cm^{-1}$ spectral resolution from 150 to 3000 $cm^{-1}$ shift

## NanoSpectralyzer Convenience Features

- Compact footprint
- Low power consumption
- No cryogen use
- Eye-safe CDRH Class 1 laser device
- Low maintenance
- Turn-key system includes computer, software, installation, training, warranty, application support
- Free software updates for three years

# NanoSpectralyzer Model Comparison and Customization Options

| | NS1 | NS2 | NS3 |
|---|---|---|---|
| NIR emission and absorption | ✓ | ✓ | ✓ |
| Visible absorption | ✓ | ✓ | ✓ |
| Raman (1 excitation wavelength) | | ✓ | ○ |
| Raman (2 excitation wavelengths) | | | ○ |
| Visible emission | | | ○ |
| Extended NIR emission/absorption | | | ○ |
| UV absorption | | | ○ |
| External signal input port | | | ○ |
| Laser output port | | | ○ |
| Vertical sample scanning | ○ | ○ | ○ |
| Reduced sample volume | ○ | ○ | ○ |

○ = Optional

NS1

NS2

NS3

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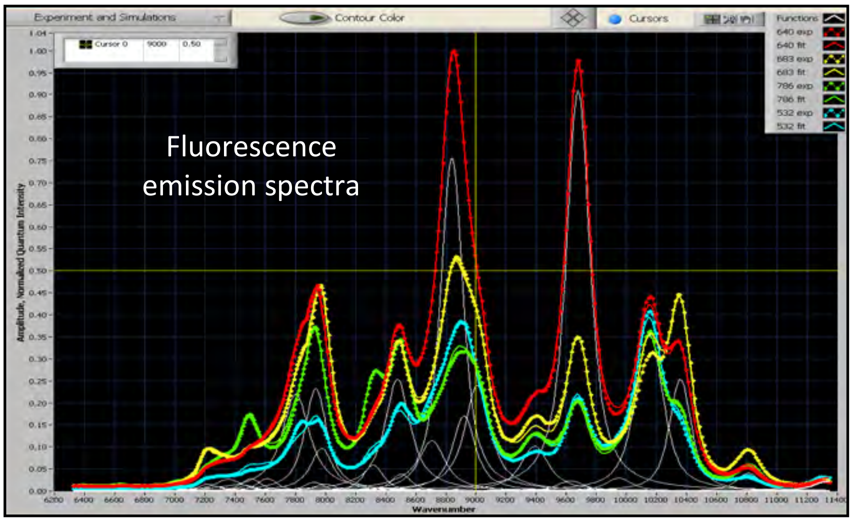
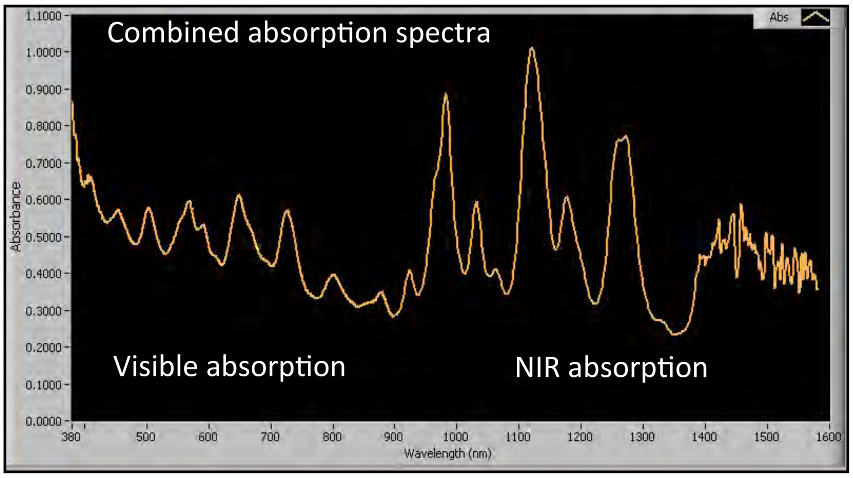
# Spectral Modes

Users can select measurements using any combination of the following spectral modes. All data are acquired under program control with a single placement of the sample cell.

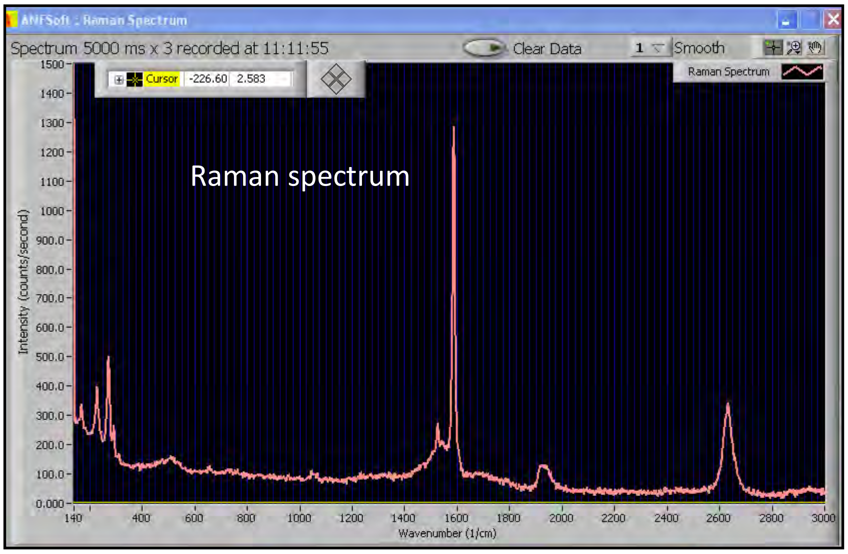
**Near-Infrared Fluorescence** spectra are measured using four or five excitation lasers. Very weak sample emission can be quickly recorded with high signal-to-noise ratios because of the high excitation powers, focused excitation beams, high aperture collection optics, and efficient emission spectrometer and array detector.




**Absorption** spectra are measured in single-beam mode using broadband probing light from a stabilized tungsten-halogen lamp. A 2 mm beam diameter permits the use of small sample volumes. The seamless spectra cover 410 to 1600 nm in all NanoSpectralyzers, with optional UV extension to 210 nm in the NS3.

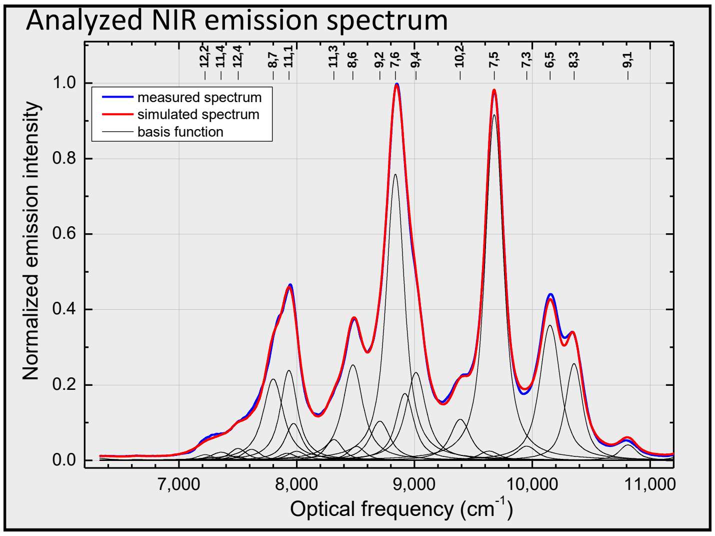



**Raman** spectra are available only with models NS2 and NS3. The Raman excitation wavelength(s) may be selected (on ordering) as 532 and/or 660 nm, according to customer preference. The optical configuration has been designed to provide a spectral resolution and range appropriate for spectroscopy of carbon nanomaterials (CNTs, graphene, carbon quantum dots).



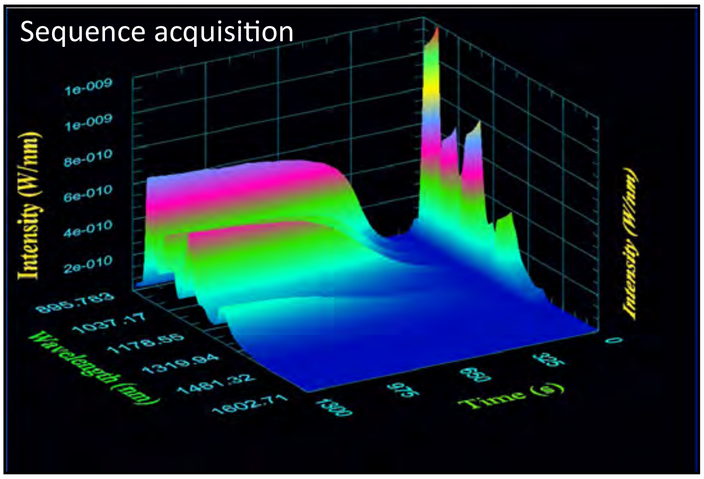
# Data Acquisition Modes

All NanoSpectralyzers offer the following selection of data acquisition modes.

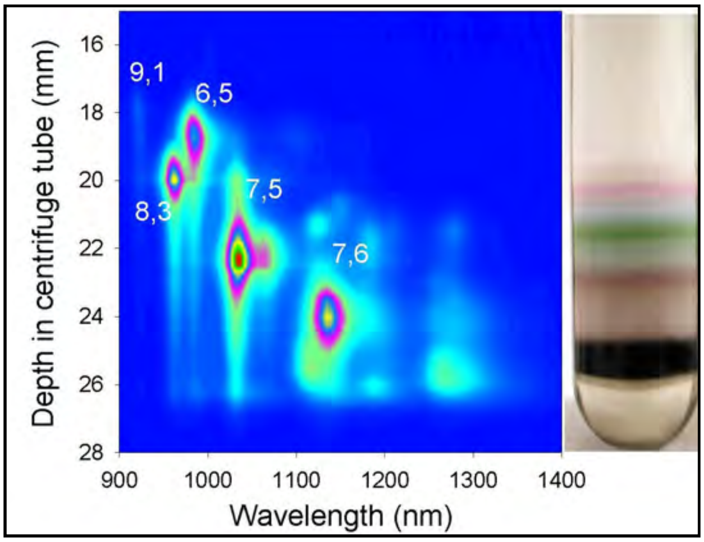



**Setup Mode** allows full manual control of all instrument parameters and displays raw spectra in real time. This is useful for exploratory research applications and for estimating acquisition parameters for new samples.

**Single Acquisition Mode** provides automated, accurate sample characterization. The user selects the desired spectral modes and acquisition parameters and directs the results to the built-in data analysis module and/or to an Origin project file.



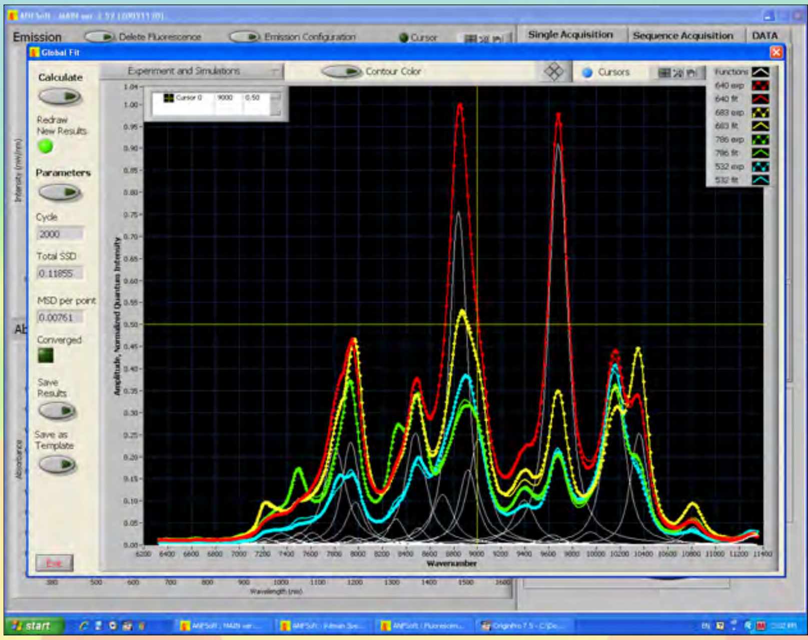

**Time Sequence Acquisition Mode** allows any spectral measurements to be recorded as kinetic sequences to monitor changes in sample composition or condition. This mode is ideal for studying nanotube aggregation or chemical reactions. If a flow cell is installed, sequence mode also allows the user to monitor nanotubes in eluent streams. Up to 10 spectra per second can be recorded.




**Spatial Profiling Mode** is used with the vertical translation option to quickly identify and visualize the layers containing sorted SWCNTs in density gradient centrifuge tubes. After an unfractionated centrifuge tube is placed into the NanoSpectralyzer, spectra are automatically measured as a function of depth in the tube. The spatially resolved spectral data are displayed as contour or surface plots.

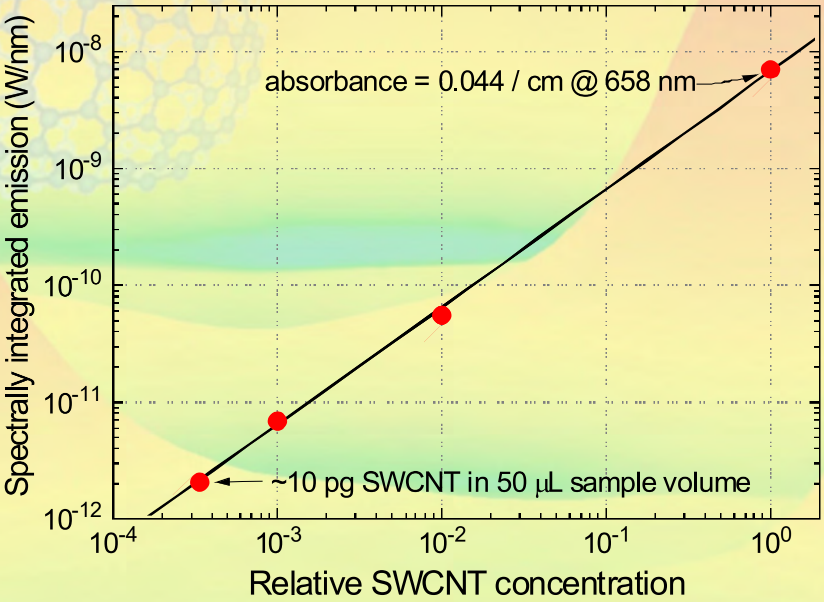
## Spectral Analysis

Sets of near-infrared (NIR) fluorescence spectra measured from SWCNT samples are semi-automatically interpreted by a sophisticated software module (NanoSpectralyze) that is part of the NanoSpectralyzer system. This program uses templates containing detailed spectral data on dozens of semiconducting (*n*,*m*) species. It accurately simulates the measured spectra to find the set of (*n*,*m*) concentrations that account for the observed fluorescence data. This analysis is based on current research findings in carbon nanotube spectroscopy and photophysics.



*Spectral fitting screen*

Although the NanoSpectralyzer system captures emission spectra using several discrete excitation wavelengths rather than a conventional continuous excitation scan, it provides equivalent analyses with far higher speed and sensitivity. The NanoSpectralyze software interprets the emission data with the aid of built-in analysis templates that constitute a detailed database of SWCNT spectral properties. The system includes multiple spectral templates with parameters tuned for different surfactant coatings to allow efficient analysis of a variety of SWCNT samples.




## Quantitation

High NIR fluorescence sensitivity makes the NanoSpectralyzer particularly well suited for SWCNT trace detection. Spectrally integrated NIR emission is automatically computed and displayed along with the sample's fluorescence efficiency index (described in How it Works). As illustrated in the adjacent graph, the NanoSpectralyzer provides a dynamic range of $\sim 10^5$ and detection limits in the picogram range.

See Schierz et al, *Environmental Science and Technology* ***46***, 12262-12271 (2012)

ANFSoft : Fluorescence Power and Efficiency

| Excitation (nm) | 640 nm | 785 nm | 532 nm | 671 nm |
|---|---|---|---|---|
| Total Power (nW) | 38.10093 | 20.37163 | 22.45301 | 13.38291 |
| Efficiency (nW cm) | 70.11062 | 58.57185 | 49.83078 | 26.84586 |

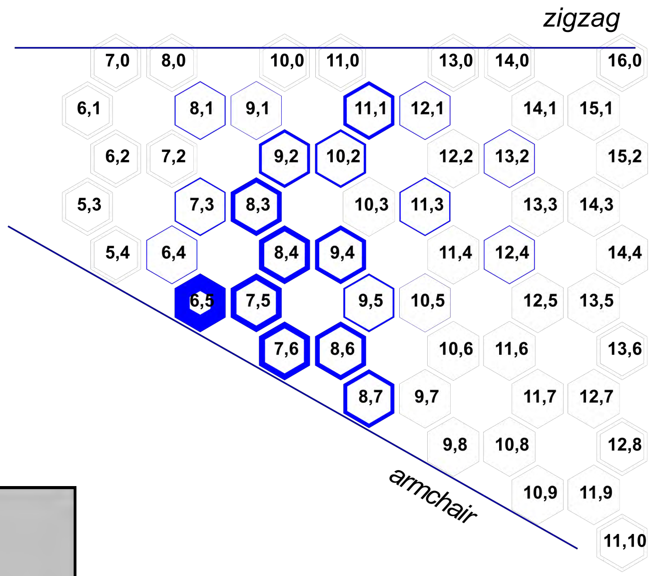
*Calculated Fluorescence power and efficiency results*

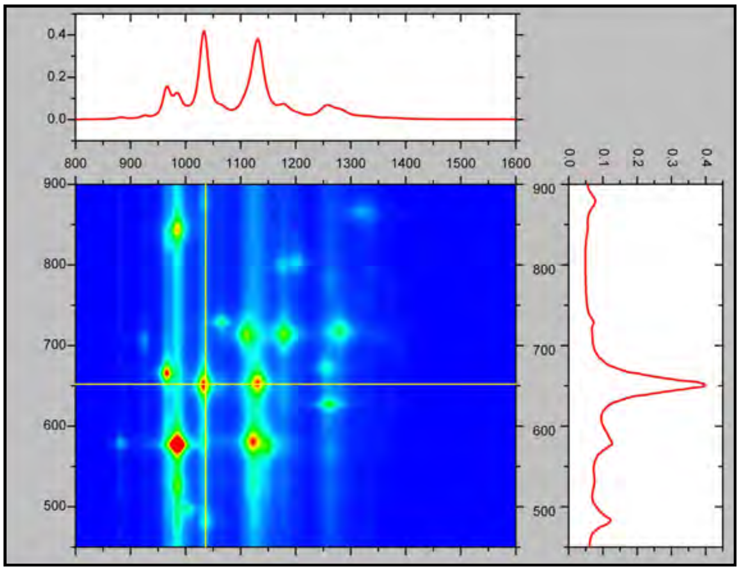
*NanoSpectralyzer Models NS1, NS2, and NS3*

# Presentation-ready Graphs

Spectral data and analysis results are automatically compiled into an Origin project file and converted into publication-quality graphs.

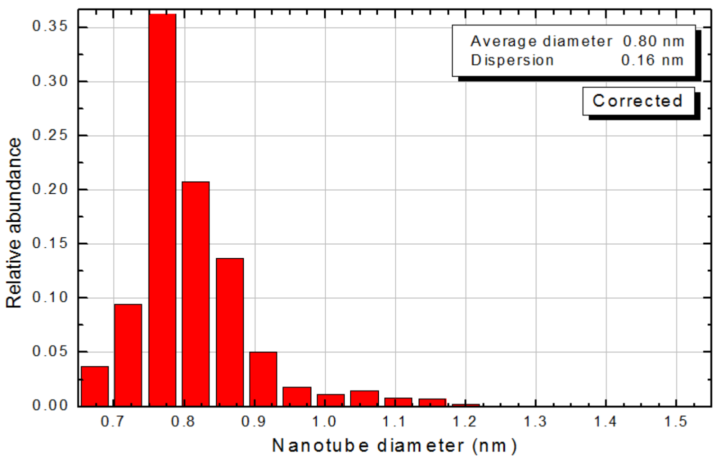
The graphene sheet plot shows the abundance distribution of different ($n$,$m$) species in an SWCNT sample. The relative abundance deduced for each species is proportional to the thickness of its hexagonal border. Results are provided with and without fluorimetric efficiency corrections.




The deduced ($n$,$m$) distribution is also displayed in the form of an excitation-emission contour plot. This plot is synthesized from the discrete set of measured emission spectra and the parameters deduced from spectral analysis.

The deduced diameter distribution of a SWCNT sample is automatically computed from the ($n$,$m$) distribution and converted into a histogram plot. The plot also displays the first and second moments of the diameter distribution. Results are provided with and without corrections for ($n$,$m$)-dependent fluorimetric efficiencies.



# Nanomaterial characterization by NS models

| | **NS1** | **NS2** | **NS3**<br>with Raman, UV absorption, visible absorption, and visible fluorescence options |
|---|---|---|---|
| SWCNTs | ✓ | ✓ | ✓ |
| Other CNTs | | ✓ | ✓ |
| Gold Nanoparticles | ✓ | ✓ | ✓ |
| Graphene | | ✓ | ✓ |
| Quantum Dots | | | ✓ |
| General Spectroscopy | | | ✓ |

# Specifications

| | |
|---|---|
| Fluorescence excitation laser $\lambda$ | 532, 638, 660, and 785 nm (or customized) |
| Fluorescence geometry | High numerical aperture epifluorescence |
| Fluorescence spectral range | 900-1600 nm |
| Near-infrared detector type | 512 element TE-cooled InGaAs array |
| *Raman excitation laser $\lambda$ | 532 or 660 nm (select when ordering) |
| Raman spectral range | 150 to 3000 $cm^{-1}$ shift |
| Raman spectral resolution | 6 $cm^{-1}$ |
| Raman detector type | 2048 pixel TE-cooled Si CCD |
| Absorption light source | Stabilized tungsten-halogen lamp |
| Absorption spectral range | 410-1600 nm |
| Absorption spectral resolution | 6 nm (NIR), 1 nm (vis) |
| Absorption ceiling | 3 AU (NIR and vis) |
| Visible detector type | 2048 pixel Si CCD |
| Absorbance noise (rms), NIR | < 2 x $10^{-4}$ AU at 0 AU for 10 s integration |
| Absorbance noise (rms), visible | < 5 x $10^{-4}$ AU at 0 AU for 10 s integration |
| Minimum sample volume | 120 $\mu$L (or 50 $\mu$L, optional) |
| Data acquisition time (typical) | 2 minutes for full set of spectra |
| Power consumption | 75 W (excluding computer) |
| Main Optical Module dimensions | NS1: 12.3" W x 18.3" D x 7.7" H (310 x 465 x 195 mm)<br>NS3: 22" W x 22" D x 7.5" H (560 x 560 x 190 mm) |
| System weight | NS1: 48 lbs/22 kg (excluding computer) |

*Raman not available with NS1

# What’s included with the NanoSpectralyzer?

NanoSpectralyzers are complete turn-key instruments built from stable, high quality components to provide reliable long term operation without user adjustment.

Each NanoSpectralyzer comes with:

- An integrated measurement system containing multiple lasers and multiple spectrometers
- Full custom software for instrument control, data acquisition, and spectral analysis
- A licensed copy of OriginLab Origin, a leading program for data analysis and scientific graphics
- An interfaced desktop computer system with pre-loaded software
- On-site installation and training
- A one-year parts and labor warranty
- Free software upgrades for three years
- Expert applications support

# Product List

## NS1 NanoSpectralyzer

includes 4 excitation lasers; 900-1600 nm range for fluorescence; 410-1600 nm range for absorption

## NS2 NanoSpectralyzer

includes all functions of NS1 plus Raman spectroscopy with choice of 532 nm or 660 nm excitation

## NS3 NanoSpectralyzer

modular, customizable multi-mode system with 5 lasers for versatile nanomaterial characterization

## NS MiniTracer

includes 1 excitation laser; 900-1600 nm range for fluorescence; optional NIR absorption

## NM1 Near-Infrared Fluorescence Microscope

designed to image individual SWCNTs through their intrinsic NIR fluorescence

## Options

**Vertical sample translation stage**
allows rapid, automated fluorescence mapping of DGU centrifuge tubes

**Reduced sample volume capability**
permits measurements on 50 microliter samples

**Extra license for NanoSpectralyze software**
allows spectral data analysis on a second stand-alone computer

**Flow cells**
for spectral monitoring of eluent streams

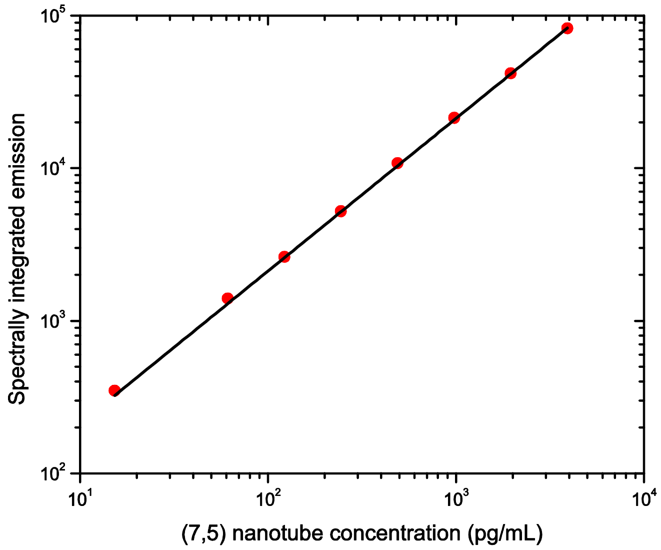
*Introducing the*

# NS MiniTracer

*For quick and easy trace analysis of single-walled carbon nanotubes*

The NS MiniTracer is the newest and most affordable member of the innovative line of NanoSpectralyzer instruments from Applied NanoFluorescence, LLC. Its design is optimized for fast, easy, and highly sensitive analytical measurements of fluorophores in the near-infrared biological window (900-1600 nm), with optional absorption measurements over the same wavelength range.

The NS MiniTracer is ideal for detecting and quantifying a variety of near-infrared fluorophores such as single-walled carbon nanotubes (SWCNTs) or quantum dots (QDs) in biological and environmental specimens!

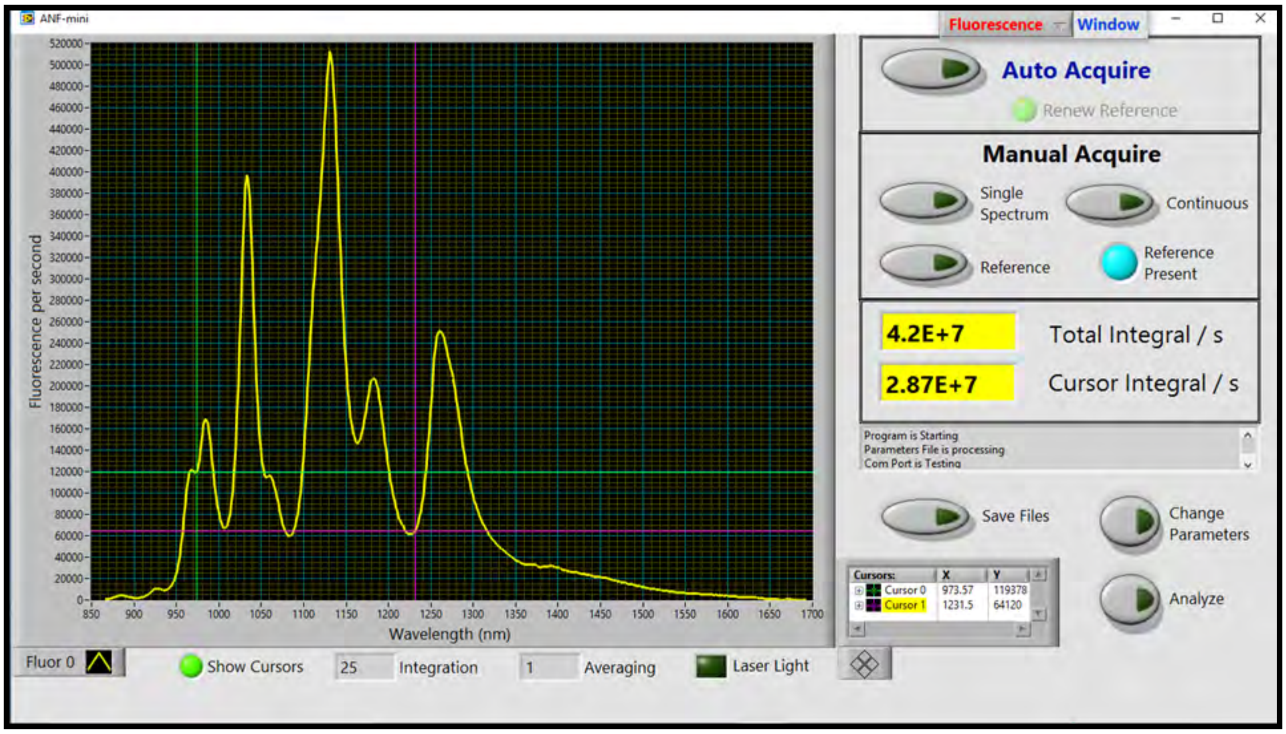




*Trace detection down to picogram/mL levels; lowest LOD (15 pg/mL) and LOQ (60 pg/mL) for (7,5) nanotubes with 638 nm excitation*

## Features:

- Most affordable fluorometer for single-walled carbon nanotubes
- Sensitive fluorescence measurements
- Optimized for the NIR biological window
- Trace detection down to picogram/mL levels of SWCNTs; lowest LOD and LOQ
- Wide dynamic range
- Robust and compact design: footprint only 165 x 215 mm (6.5 x 8.5”)
- NIR emission spectra from 900 to 1600 nm
- Option to add NIR absorption spectra from 900 to 1600 nm
- Choice of one excitation wavelength

*Easy-to-use software interface*




The NS MiniTracer has a robust, compact design that includes one excitation laser and optics for capturing near-infrared fluorescence from dispersed SWCNTs, QDs, and other near-infrared fluorophores. Optimized for the lowest possible optical backgrounds, the NS MiniTracer offers long integration times to provide exceptional detection sensitivity.

Operation of the NS MiniTracer is also exceptionally easy. Custom software prompts the user to load blank and sample specimens, and automatically selects optimal data acquisition parameters. After acquisition is complete, a full fluorescence spectrum is displayed along with the total integrated fluorescence signal. This convenient numerical result is used to create project-specific calibration curves for quantitative measurements of any NIR fluorophore.

## Software Highlights:

- Quick measurements with user-friendly software
- Auto acquire function that will automatically find the optimum integration time for your sample
- Manual acquire  functions for user defined acquisition parameters
- Includes a single numerical total integral value that allows easy direct comparison between samples
- Simply integrate a selected peak with the cursor integral function
- Rapid sequence acquisition for kinetic studies or eluent monitoring
- Calibration curve function to use a set of standards to measure unknown samples
- Files saved in text format for straightforward import into external software

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## About ANF

***Founded by Pioneers in Nanotube Research***

Applied NanoFluorescence, LLC was founded in Houston in 2004 by Rice University's Prof. R. Bruce Weisman, who serves as its President. The primary mission of ANF is to provide the SWCNT community with advanced spectral analysis capabilities through easy-to-use, sophisticated instruments that are based on the latest research findings. ANF's Chief Scientific Officer is Dr. Sergei M. Bachilo.

Weisman and Bachilo are internationally recognized as the pioneering researchers who led the discovery and interpretation of fluorescence spectra from single-walled carbon nanotubes. The products offered by Applied NanoFluorescence embody these scientists' expertise in spectroscopic instrumentation and analysis.