

ASSESSING THE HAZARD OF MULTI- WALLED CARBON NANOTUBES (MWCNT).

A critical step to safe implementation of
nanotechnologies

- International Collaboration on Nanotubes Safety – Project
 - A joint European-U.S. collaborative project to investigate toxicity of carbon nanotubes

- ERA-NET Safe Implementation of Innovative Nanoscience and Nanotechnology



ICONS

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- ERA-NET Safe Implementation of Innovative Nanoscience and Nanotechnology



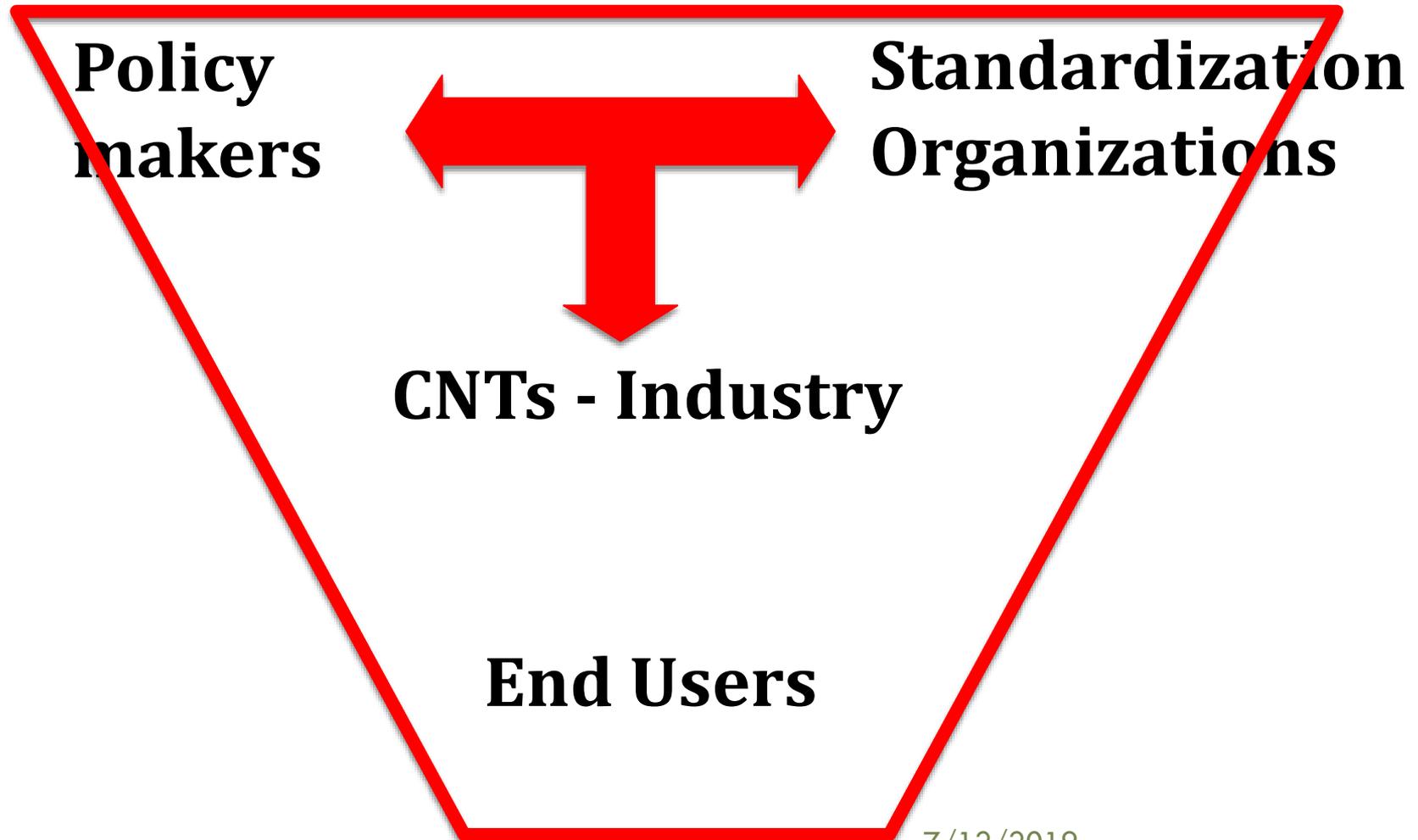
ICONS

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- ERA-NET Safe Implementation of Innovative Nanoscience and Nanotechnology
- ***“promotes the safe and rapid transfer of European research results in nanoscience and nanotechnology (N&N) into industrial applications”***

ICONS

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ICONS

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TRL			DRL
9	Authorization/certification.	„Something is missing...” or “Would be cool to...”	1
8	Quality control, reliability, security, etc.	Identifying a specific need/problem	2
7	Industrial prototype (supply chain, etc.)	Defining the required features	3
6	Demonstrated in operational conditions	Market segmentation – quantifying required features	4
5	Robust design	Intra/Entrepreneur selection	5
4	Lab prototype	Define capabilities needed to develop solution	6
3	Proof of concept (“it works!”)	Quantify resources and skills needed	7
2	Applied research („I know what I want”)	Identify technical experts with the required skills	8
1	Basic research (curiosity)	Develop the solution that the market needs.	9

ICONS

8

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ICONS

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- A joint European-U.S collaborative project to investigate toxicity of carbon nanotubes

UCL
Université
catholique
de Louvain

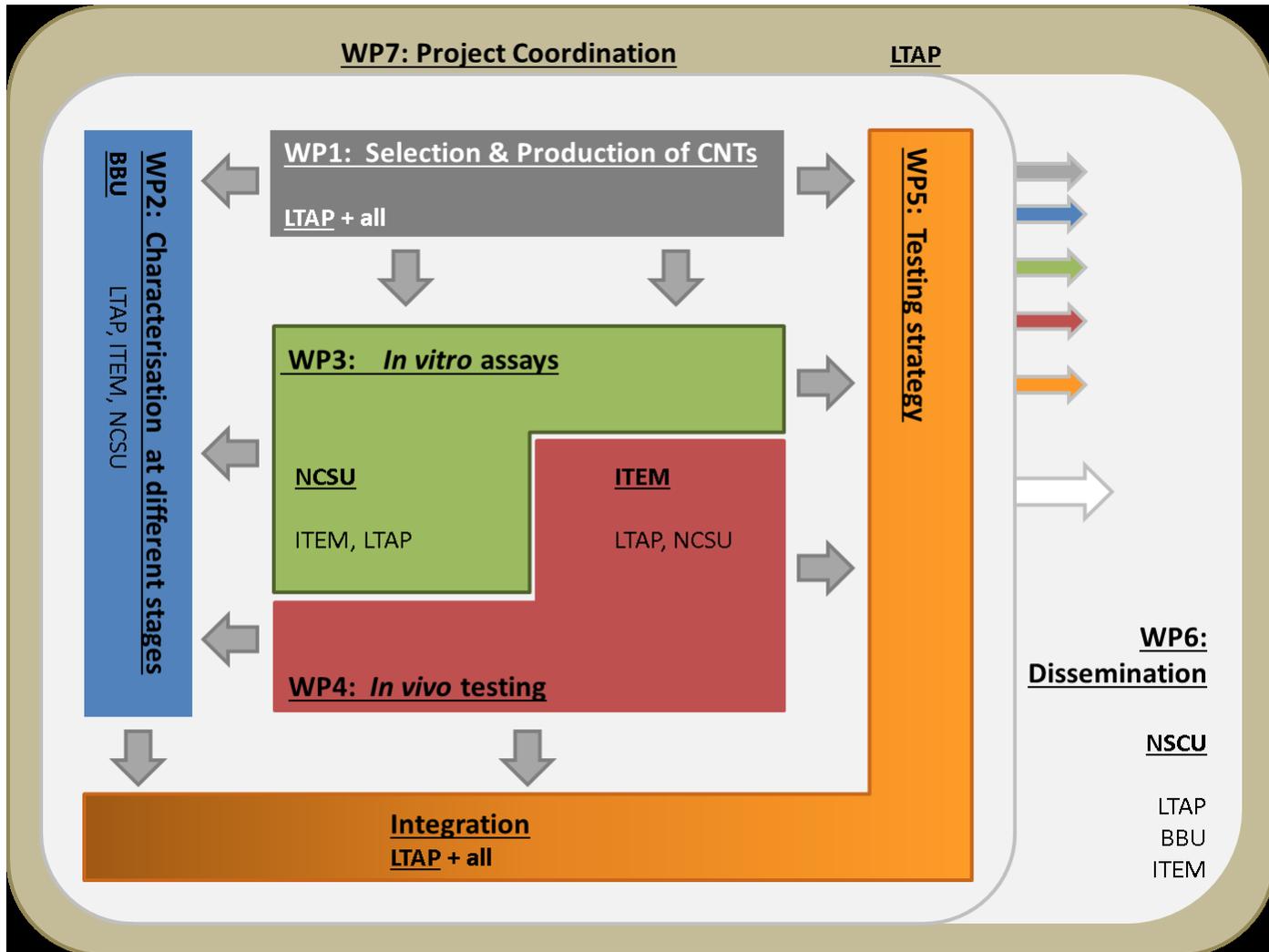


 **Fraunhofer**
ITEM

NC STATE
UNIVERSITY

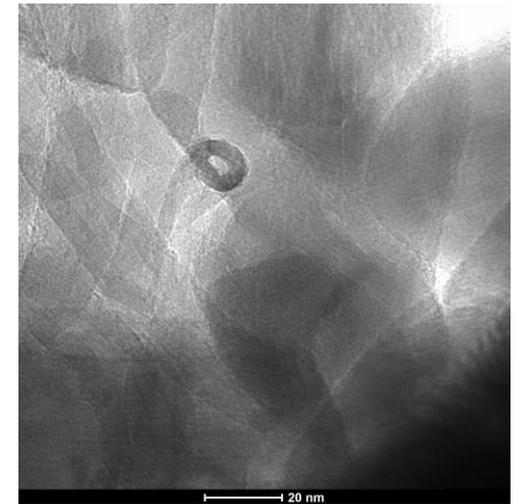
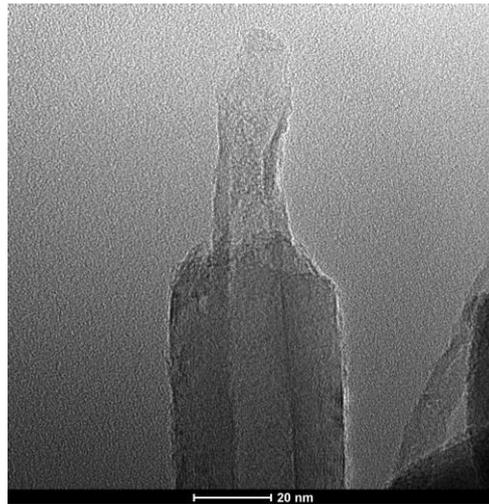
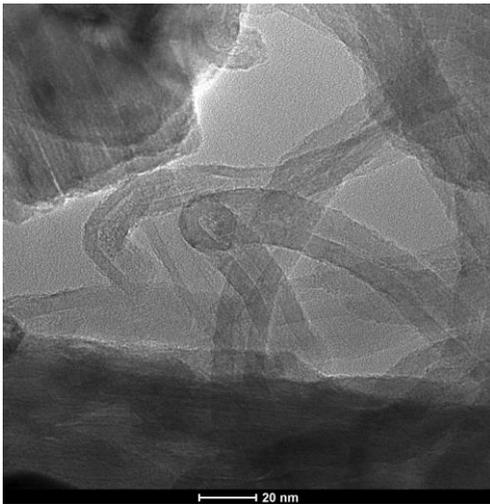
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- ❑ WP2 - Characterization at Different Stages
 - ❑ Physical-Chemical Properties
 - ❑ Biological Activities
 - ❑ Before
 - ❑ After functionalization
 - ❑ After *In Vitro*
 - ❑ After *In Vivo* ???

□ WP2 - Characterization at Different Stages



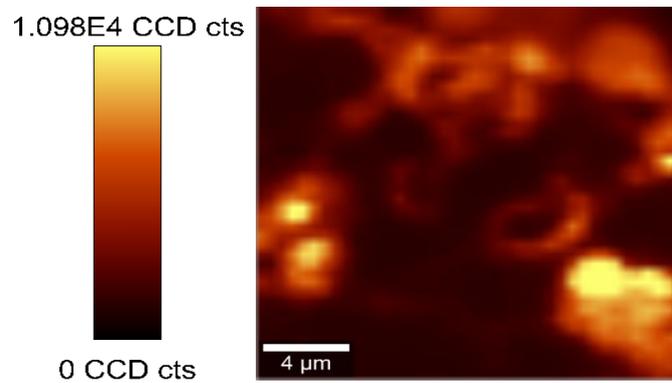
ICONS

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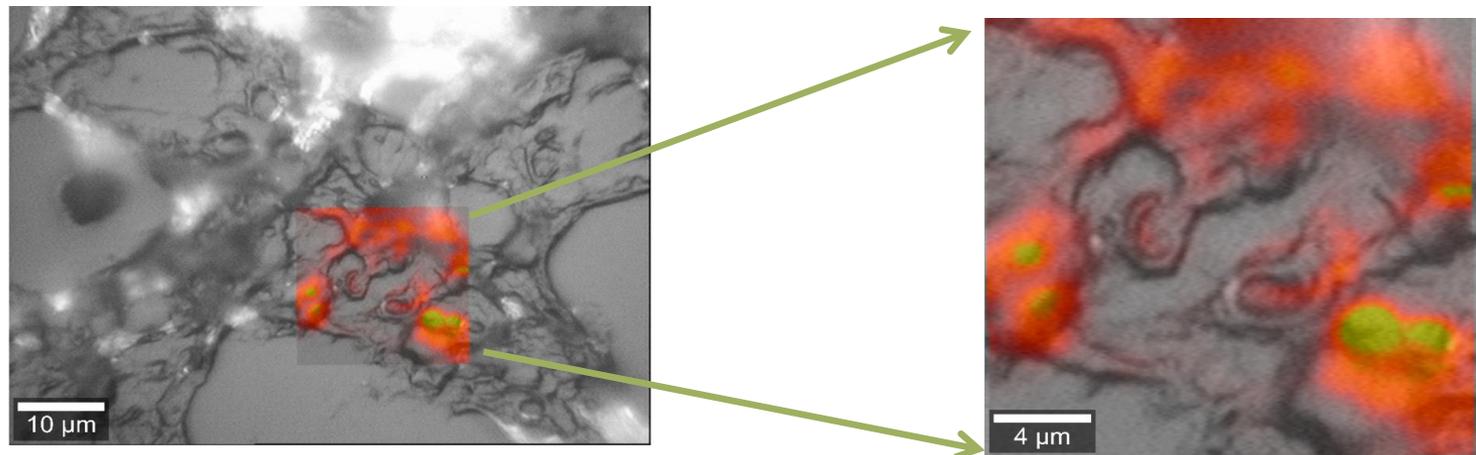
- ❑ WP2 - Characterization at Different Stages
 - ❑ TGA
 - ❑ Microscopy
 - ❑ SEM
 - ❑ TEM
 - ❑ EDX & XPS
 - ❑ AFM
 - ❑ Raman & FTIR
 - ❑ NMR & EPR & MRI

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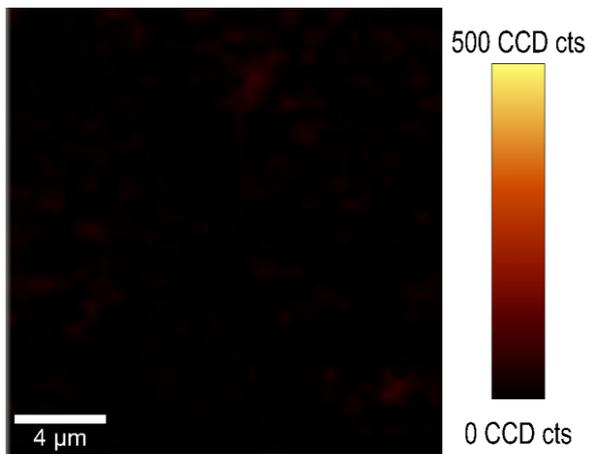


Raman spatial map generated by plotting the intensity of the peaks at 2800 -3100 cm^{-1} (C-H stretching vibrations of lipids)



Overlay of the optical image of the tissue and the Raman map

CONTROL- statistical analysis



Raman spatial map generated by plotting the intensity of the peak at 1343 cm^{-1}

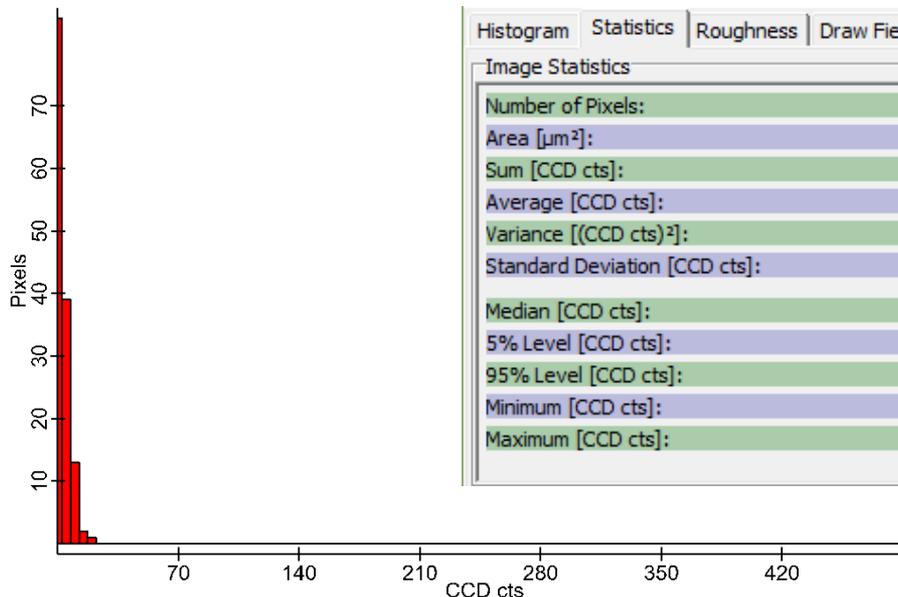
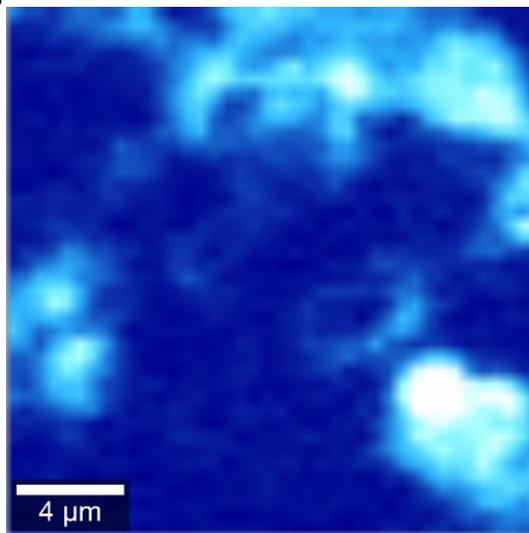
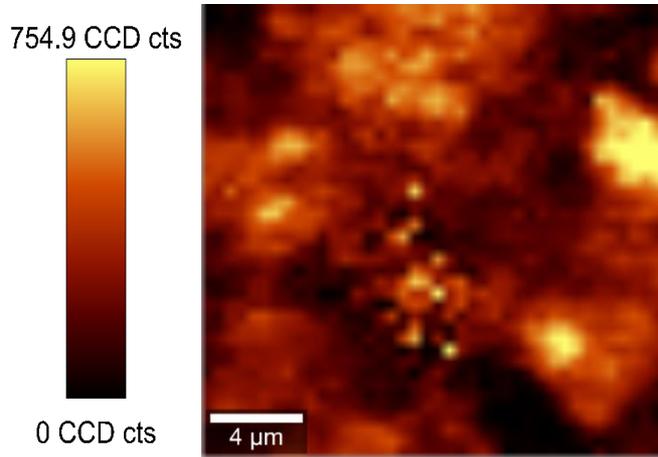


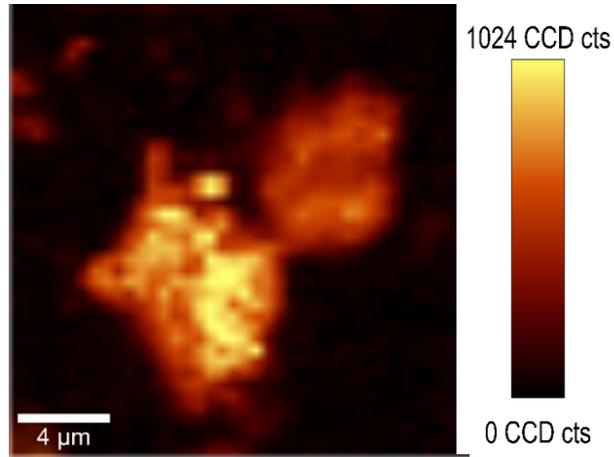
Image Statistics	
Number of Pixels:	1600
Area [μm^2]:	400
Sum [CCD cts]:	-135955
Average [CCD cts]:	-84.9717
Variance [(CCD cts) 2]:	14763.6
Standard Deviation [CCD cts]:	121.505
Median [CCD cts]:	-29.7173
5% Level [CCD cts]:	-322.776
95% Level [CCD cts]:	3.67321
Minimum [CCD cts]:	-1021.84
Maximum [CCD cts]:	20.7185

Image histogram and statistics related to the intensity of the peak at 1343 cm^{-1} (D band of CNTS)

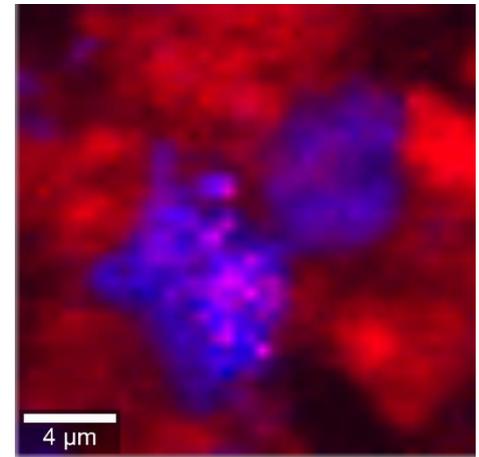
CNT NM-400 N° 7



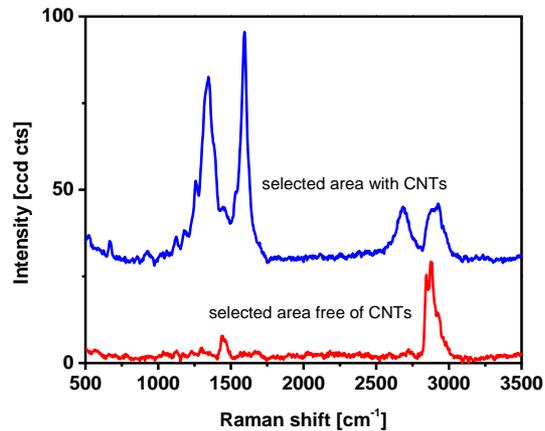
Raman spatial map generated by plotting the intensity of the peaks at 2800 -3100 cm^{-1} (C-H stretching vibrations of lipids) **Shows the tissue**



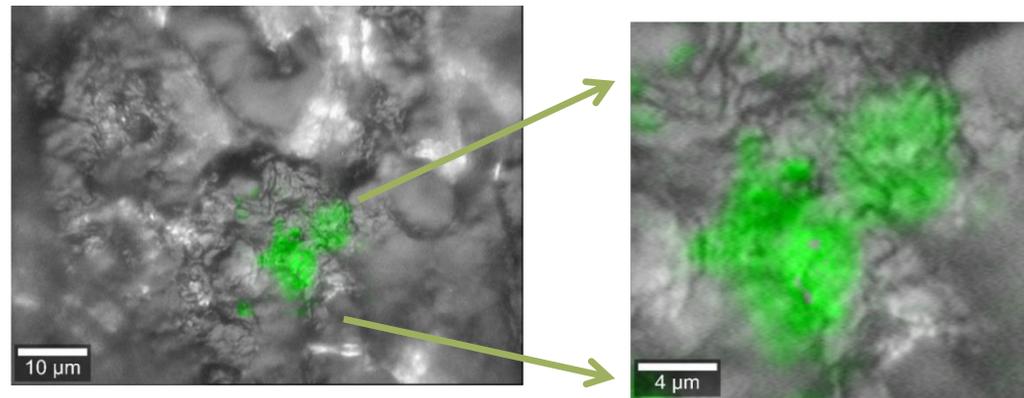
Raman spatial map generated by plotting the intensity of the peak at 1343 cm^{-1} (D band of CNTs) **Shows the distribution of CNTs**



Overlay of the Raman map of the tissue (red) and the Raman map of CNTs (blue)

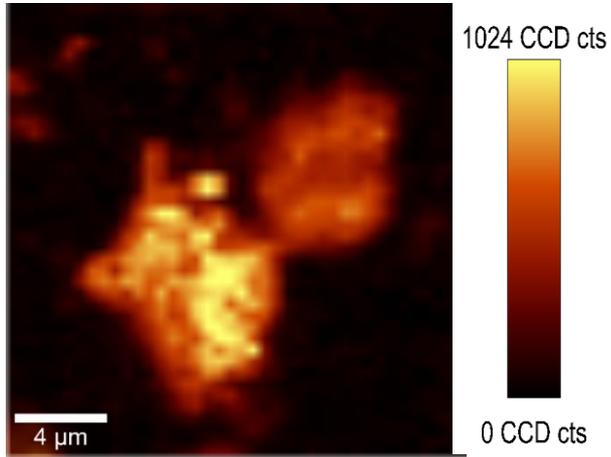


Extracted spectra from Raman map. The spectrum extracted from the area with CNTs exhibits composite bands of tissue and of CNTs



Overlay of the optical image of the tissue and the Raman map of CNTs

CNT NM-400 N° 7- statistical analysis



Raman spatial map generated by plotting the intensity of the peak at 1343 cm^{-1} (D band of CNTs) **Shows the distribution of CNTs**

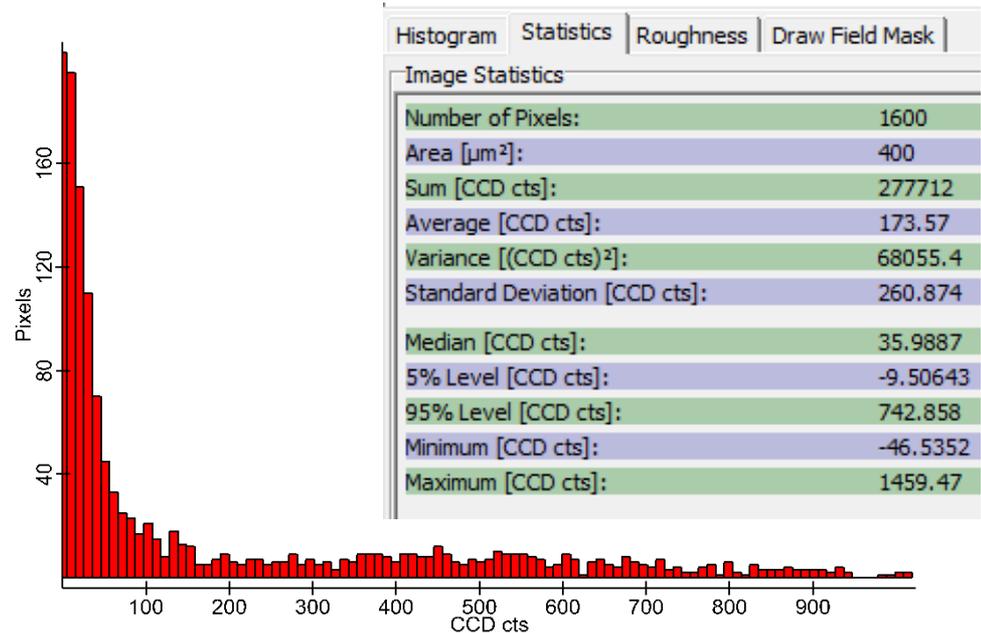
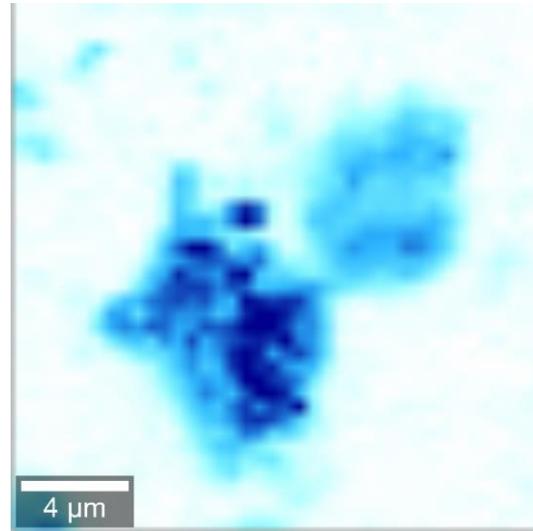
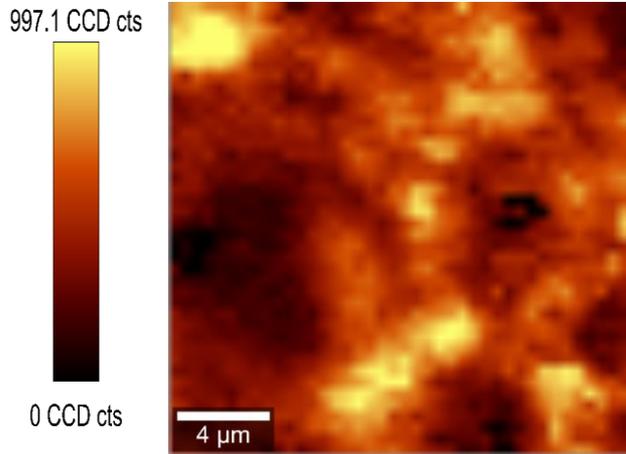
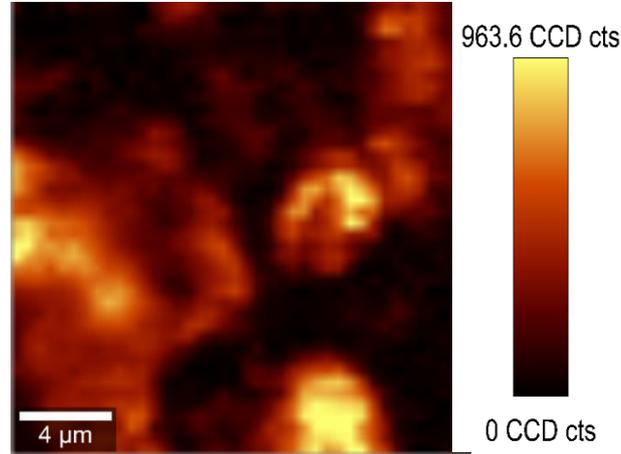


Image histogram and statistics related to the intensity of the peak at 1343 cm^{-1} (D band of CNTs)

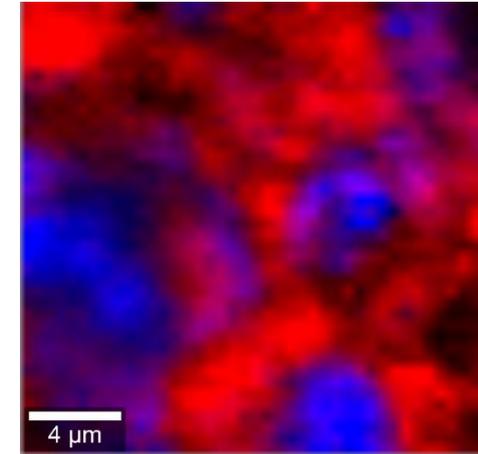
CNT NM-400 N° 7



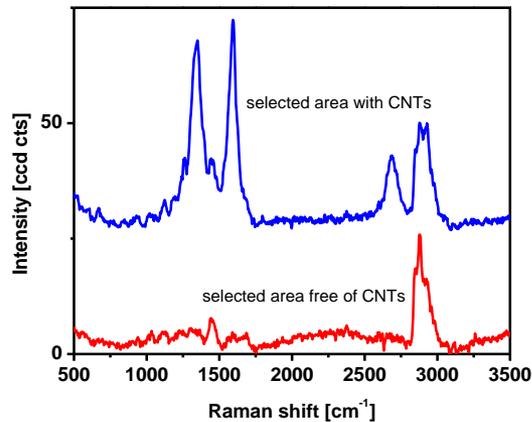
Raman spatial map generated by plotting the intensity of the peaks at 2800 -3100 cm^{-1} (C-H stretching vibrations of lipids) **Shows the tissue**



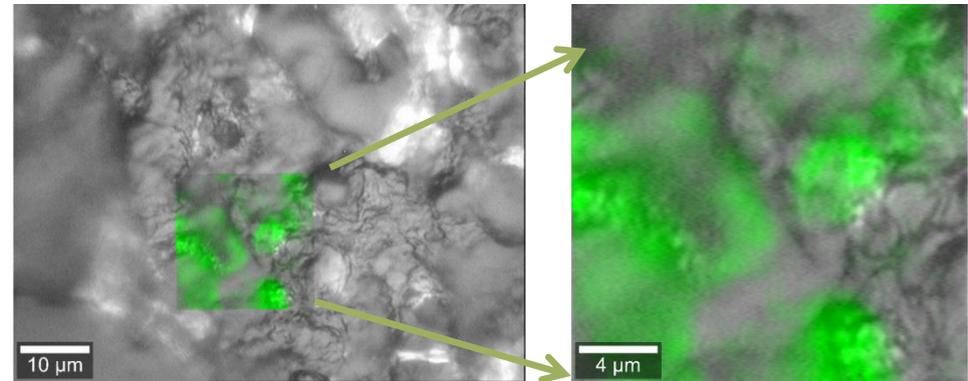
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Overlay of the Raman map of the tissue (red) and the Raman map of CNTs (blue)

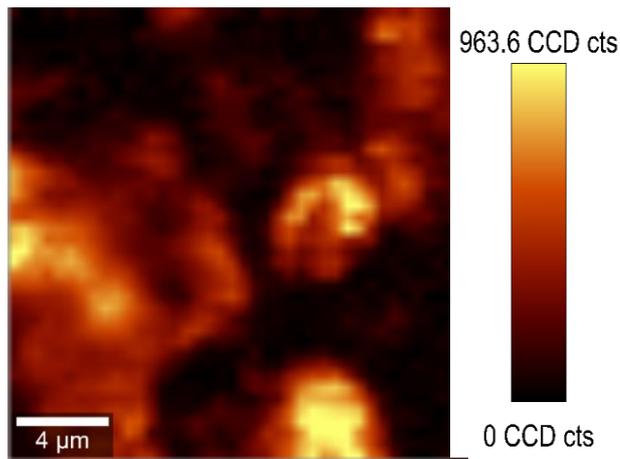


Extracted spectra from Raman map. The spectrum extracted from the area with CNTs exhibits composite bands of tissue and of CNTs



Overlay of the optical image of the tissue and the Raman map of CNTs

CNT NM-400 N° 7 –statistical analysis- using the Raman software



Raman spatial map generated by plotting the intensity of the peak at 1343 cm^{-1} (D band of CNTs) **Shows the distribution of CNTs**

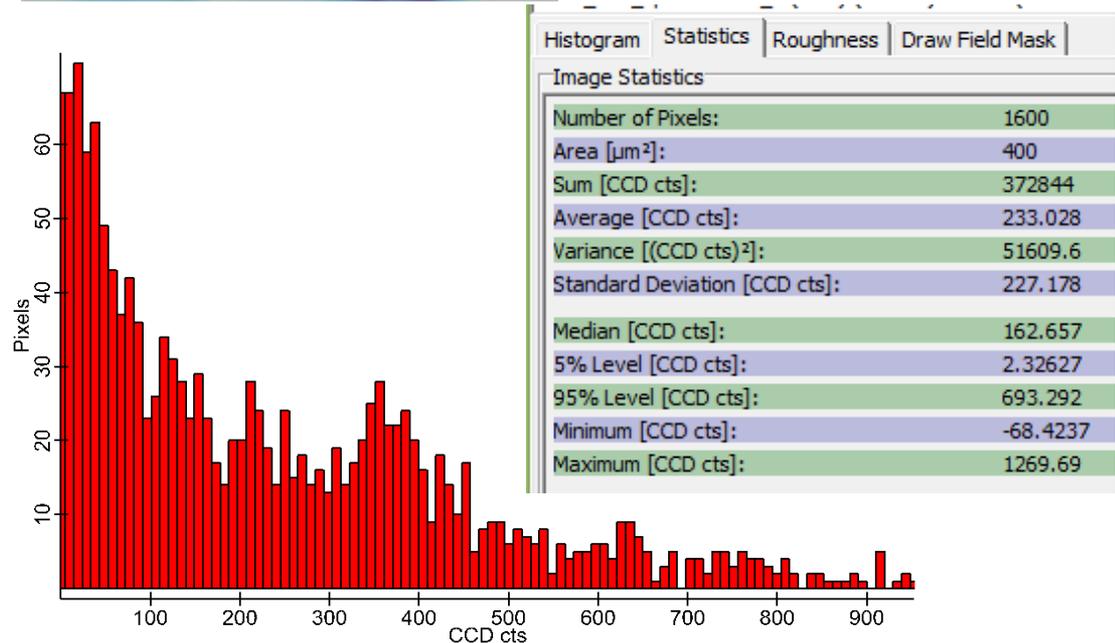
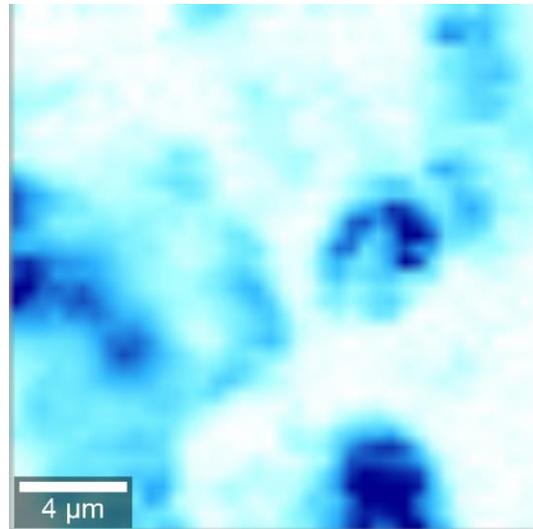


Image histogram and statistics related to the intensity of the peak at 1343 cm^{-1} (D band of CNTs)

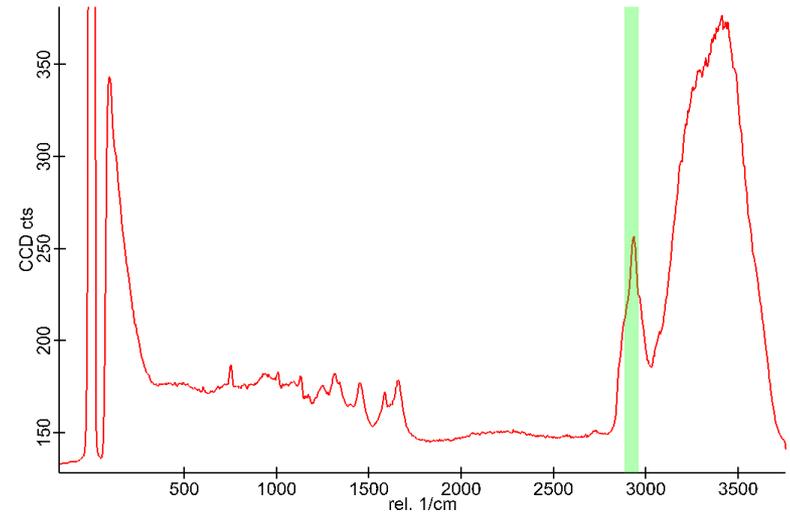
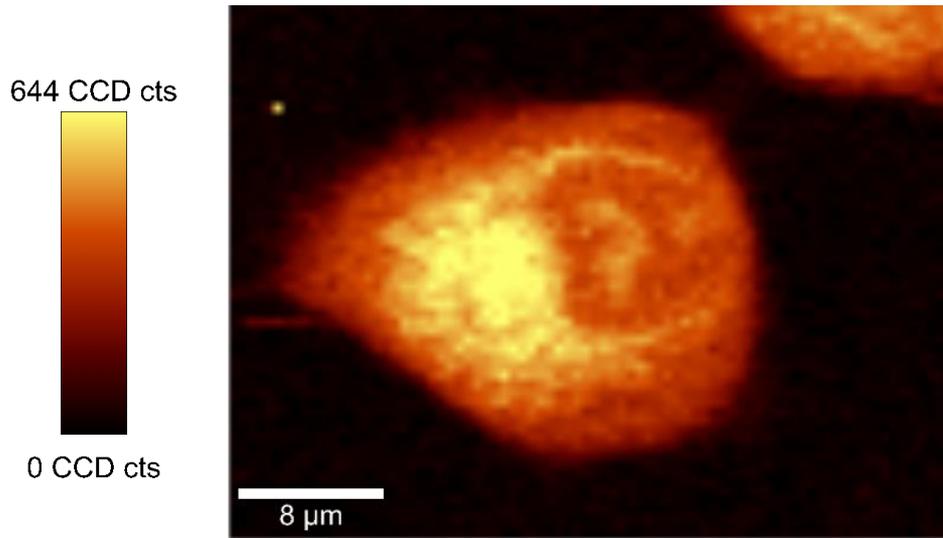
Raman measurements – detection of CNTs inside A549 cancer cells

Confocal Raman microscope (CRM alpha 300R from WITec GmbH, Germany)

532 nm excitation line of a Nd:YAG laser

63 x water immersion objective (NA = 1)

A549 Human lung carcinoma cells -control



Raman spatial map generated by plotting the intensity of the peaks at 2800 -3100 cm^{-1} (C-H stretching vibrations of lipids) -shows the whole cell

A549 Human lung carcinoma cells incubated with sample 7 (NC3151 SHORT THIN MWCNT 95% C PURITY & SURFACE MODIFIED COOH)

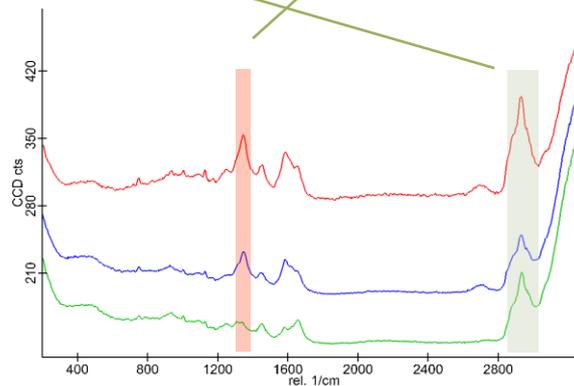
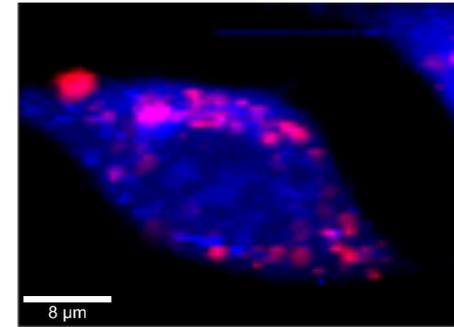
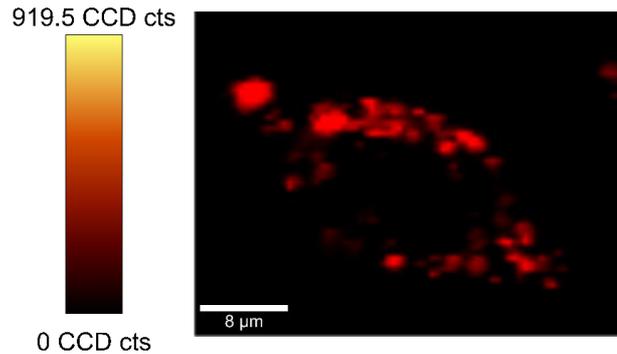
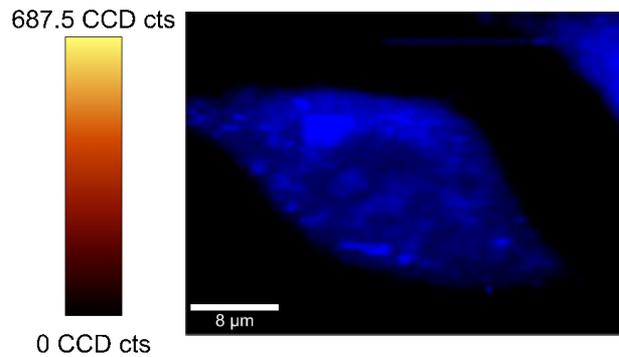
Sample 7: easy dispersible in water; stable aqueous solution
Massive internalization inside A549 cancer cells
Extranuclear internalization

Observation: negative Zeta potential NC3151

Raman spatial map generated by plotting the intensity of the peaks at 2800 -3100 cm^{-1} (C-H stretching vibrations of lipids) -shows the whole cell

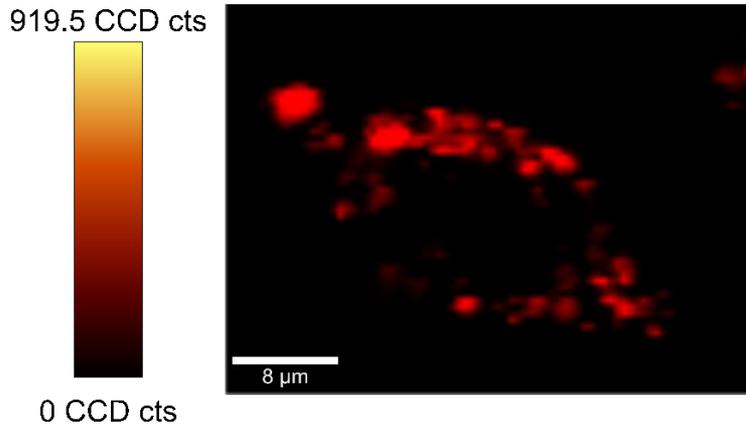
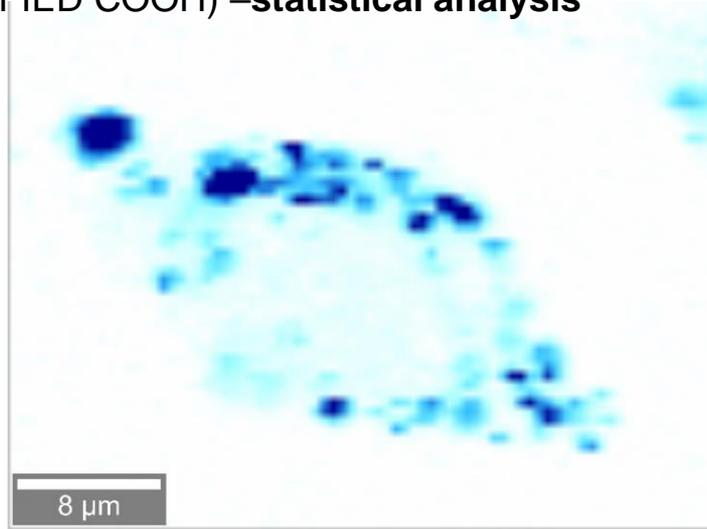
Raman spatial map generated by plotting the intensity of the peak at 1343 cm^{-1} shows the distribution of CNTs

Overlay



Extracted spectra from the Raman map featuring the characteristic Raman vibrational bands of cell and CNTs

A549 Human lung carcinoma cells incubated with sample 7 (NC3151 SHORT THIN MWCNT 95% C PURITY & SURFACE MODIFIED COOH) – **statistical analysis**



Raman spatial map generated by plotting the intensity of the peak at 1343 cm^{-1} shows the distribution of CNTs

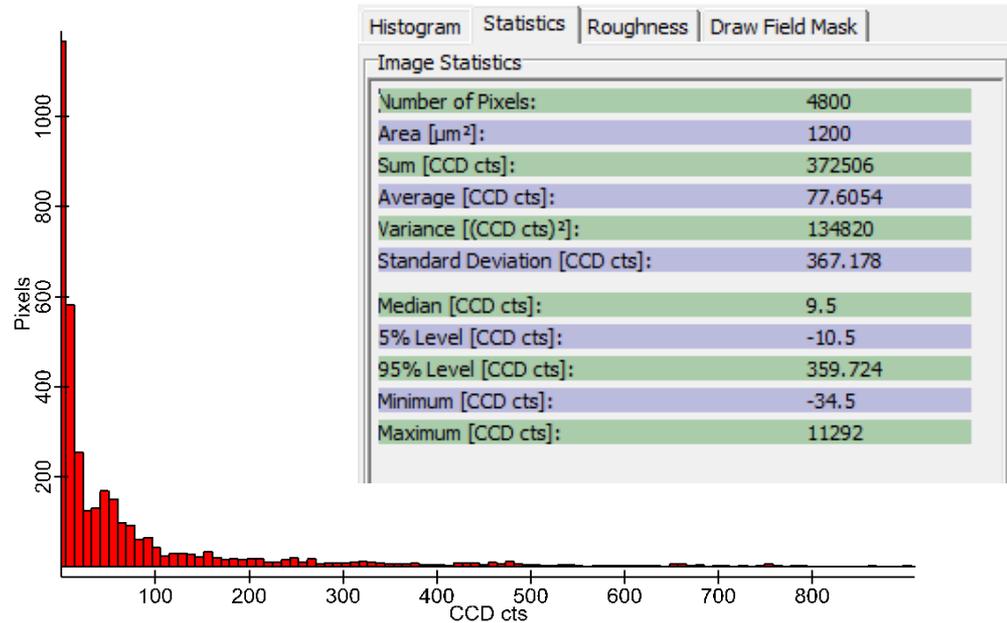


Image histogram and statistics related to the intensity of the peak at 1343 cm^{-1} (D band of CNTS)

A549 Human lung carcinoma cells incubated with sample 6 (NC3100 THIN MWCNT
95+% C PURITY)

Observation: negative Zeta negative NC3150

Sample 6: hardly dispersible in water; unstable aqueous solution

Most of CNTs were bound on the substrate or on the cell membrane; small amount of internalized CNTs; extranuclear internalization

Raman spatial map generated by plotting the intensity of the peaks at 2800 -3100 cm^{-1} (C-H stretching vibrations of lipids) -shows the whole cell

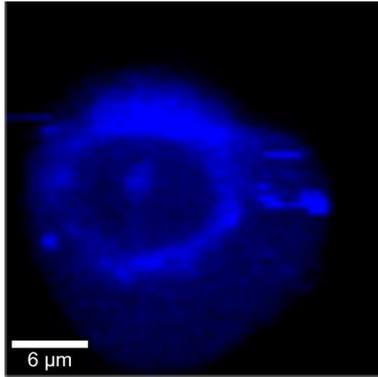
Raman spatial map generated by plotting the intensity of the peak at 1343 cm^{-1} shows the distribution of CNTs

Overlay

816 CCD cts



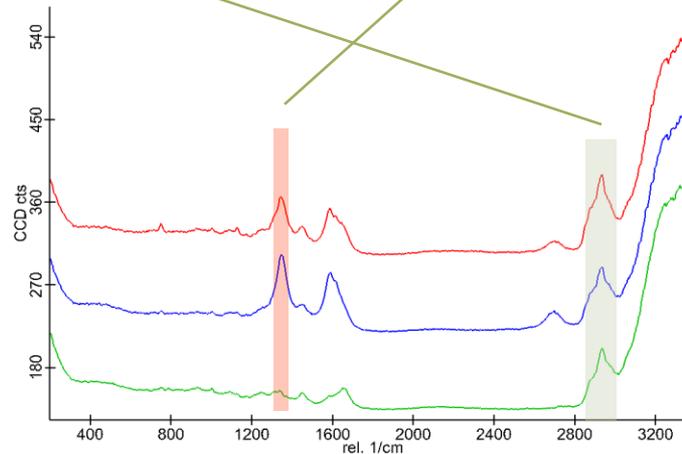
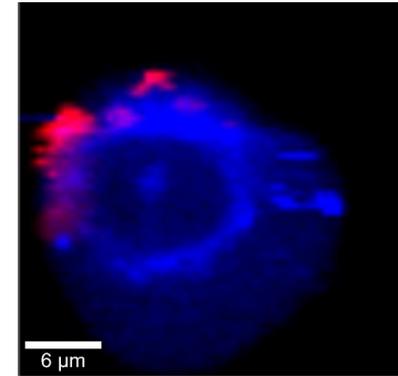
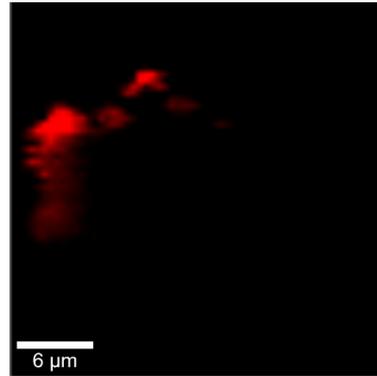
0 CCD cts



1495 CCD cts

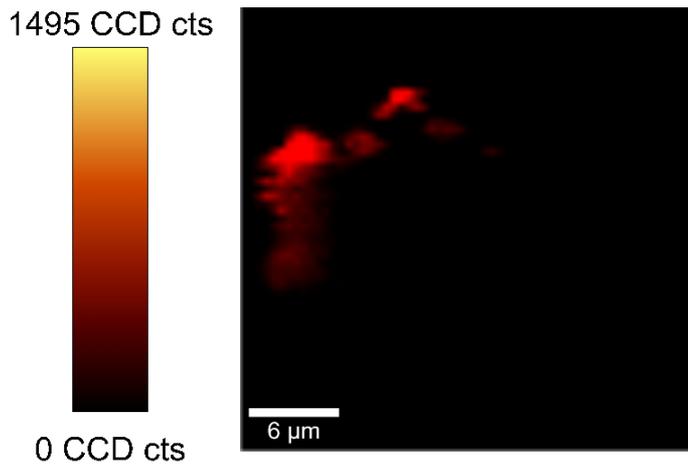


0 CCD cts



Extracted spectra from the Raman map featuring the characteristic Raman vibrational bands of cell and CNTs

A549 Human lung carcinoma cells incubated with sample 6 (NC3100 THIN MWCNT 95+% C PURITY) –**statistical analysis**



Raman spatial map generated by plotting the intensity of the peak at 1343 cm^{-1} shows the distribution of CNTs

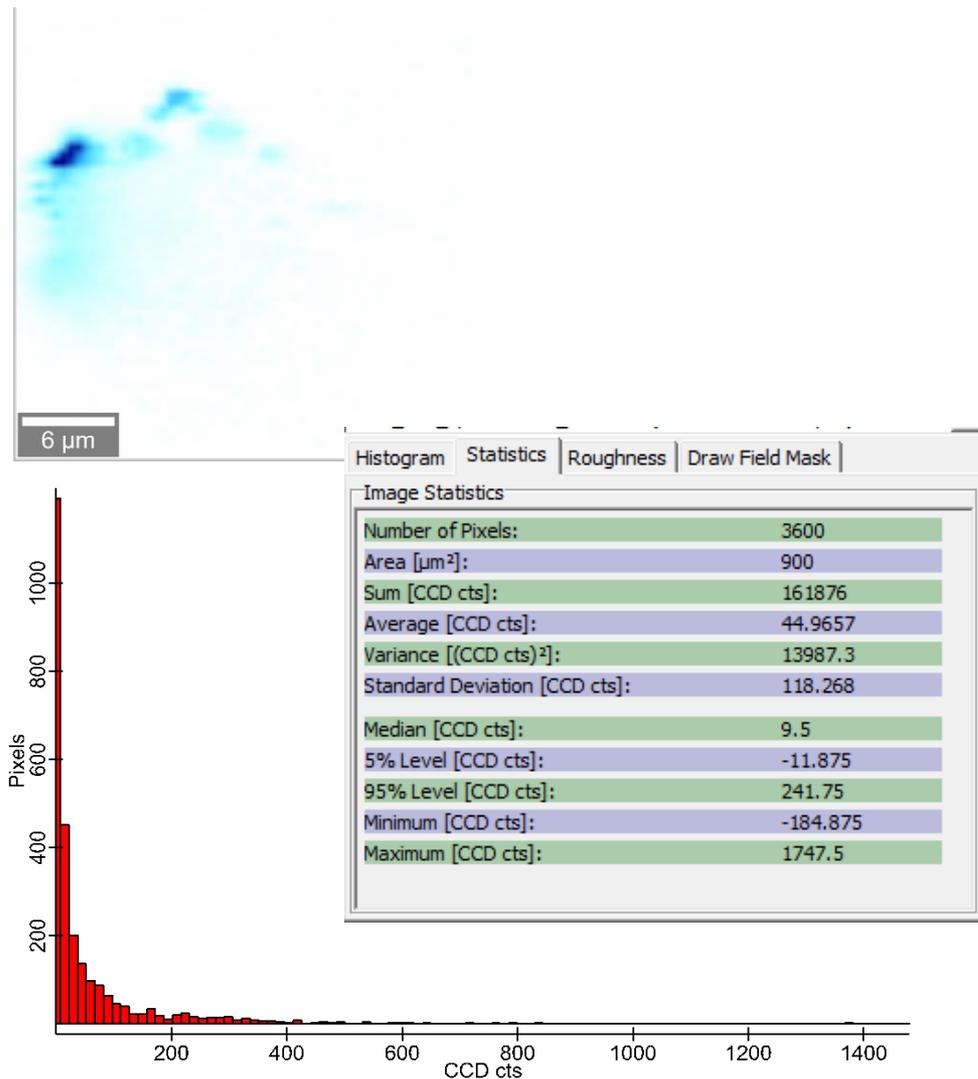
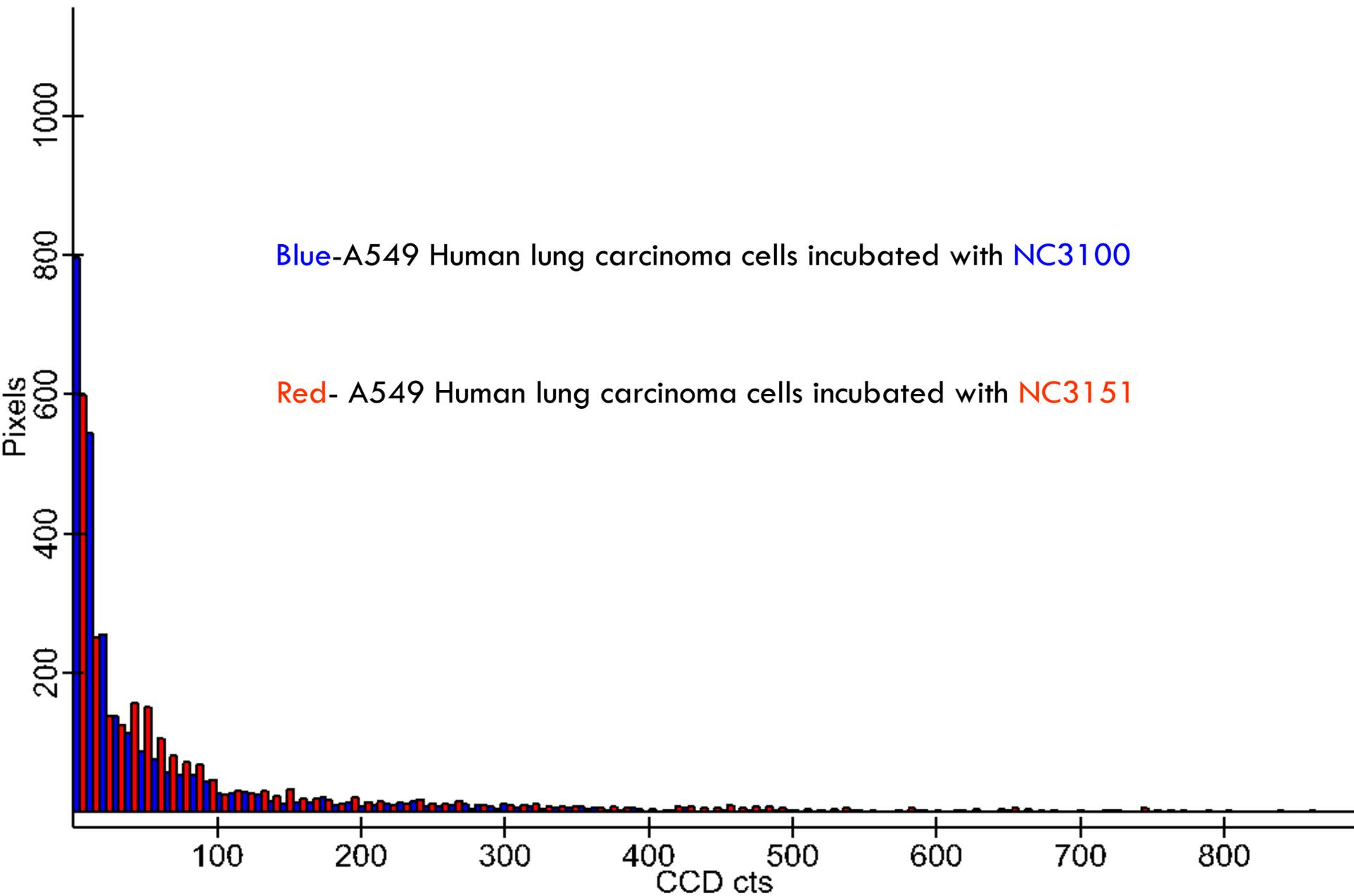
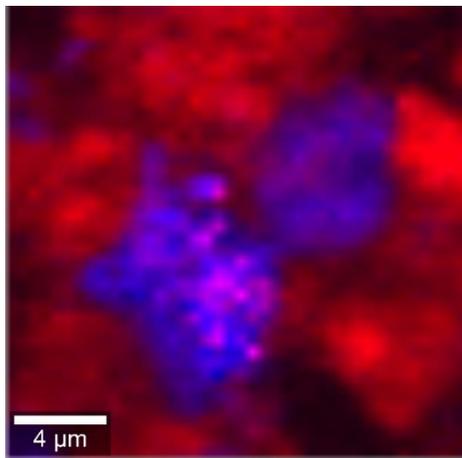


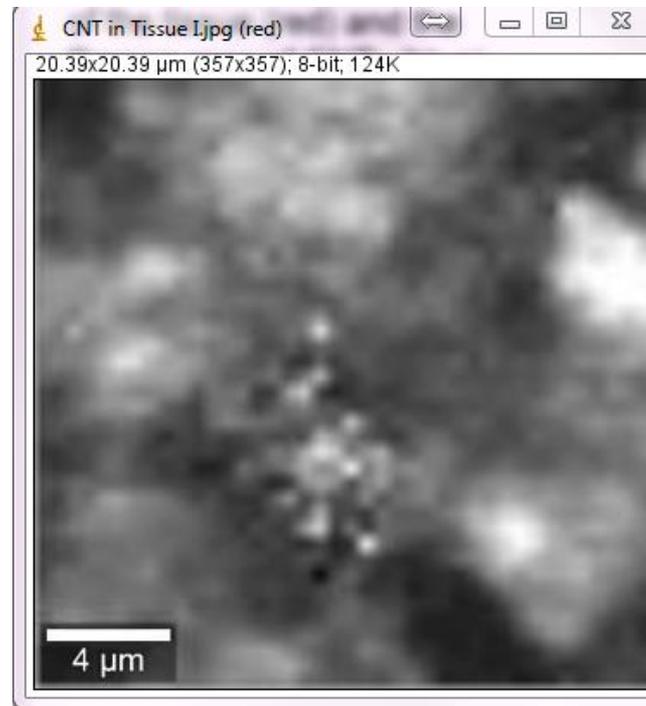
Image histogram and statistics related to the intensity of the peak at 1343 cm^{-1} (D band of CNTS)



Quantification trials using ImageJ software

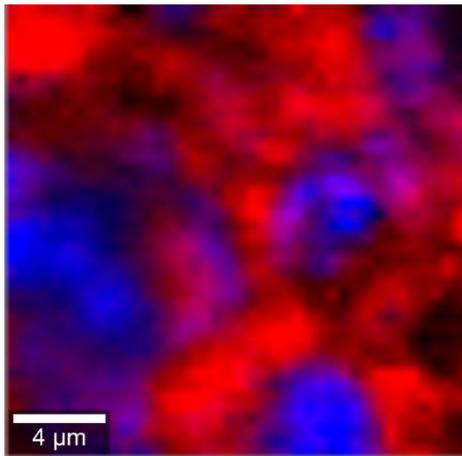


Overlay of the Raman map of the tissue (red) and the Raman map of CNTs (blue)



Label	Area	%Area	MinThr	MaxThr
CNT in Tissue I.jpg (red)-1	415.552	87.806	0	255
CNT in Tissue I.jpg (blue)	415.552	37.063	0	255

Quantification made with ImageJ software



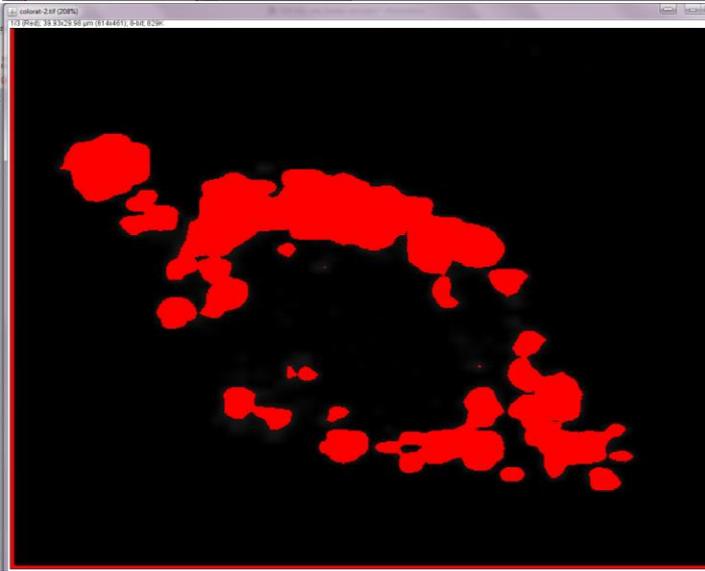
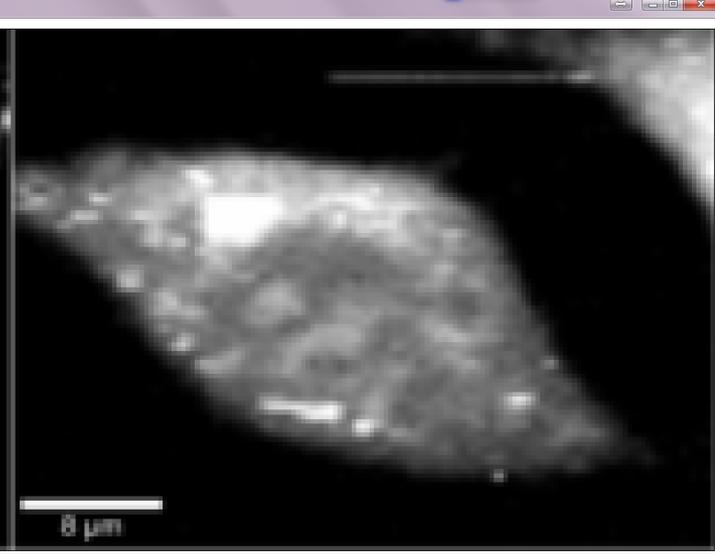
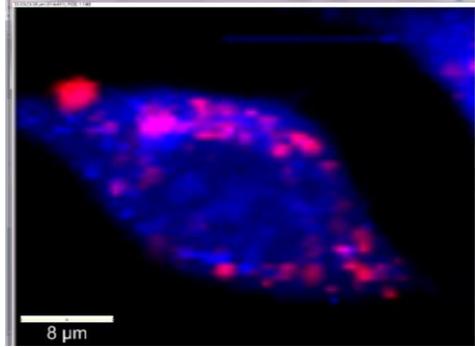
Overlay of the Raman map of the tissue (red) and the Raman map of CNTs (blue)



Results

File	Edit	Font	Results		
	Label	Area	%Area	MinThr	
1	CNT in Tissue II .jpg.png (red)-1	127449.000	85.834	0	
2	CNT in Tissue II .jpg.png (blue)-2	433.357	51.138	0	

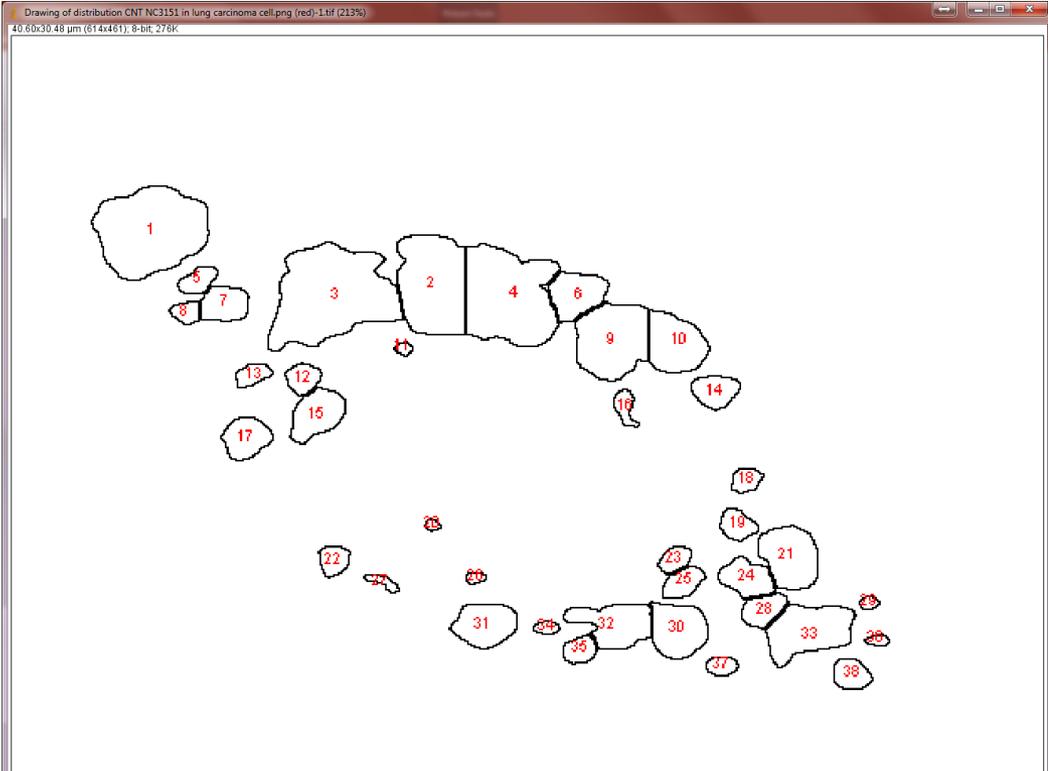
Quantification made with ImageJ software



File Edit Font Results					
	Label	Area	%Area	MinThr	MaxThr
1	colorat-2.tif.Red	154.761	12.925	24	255

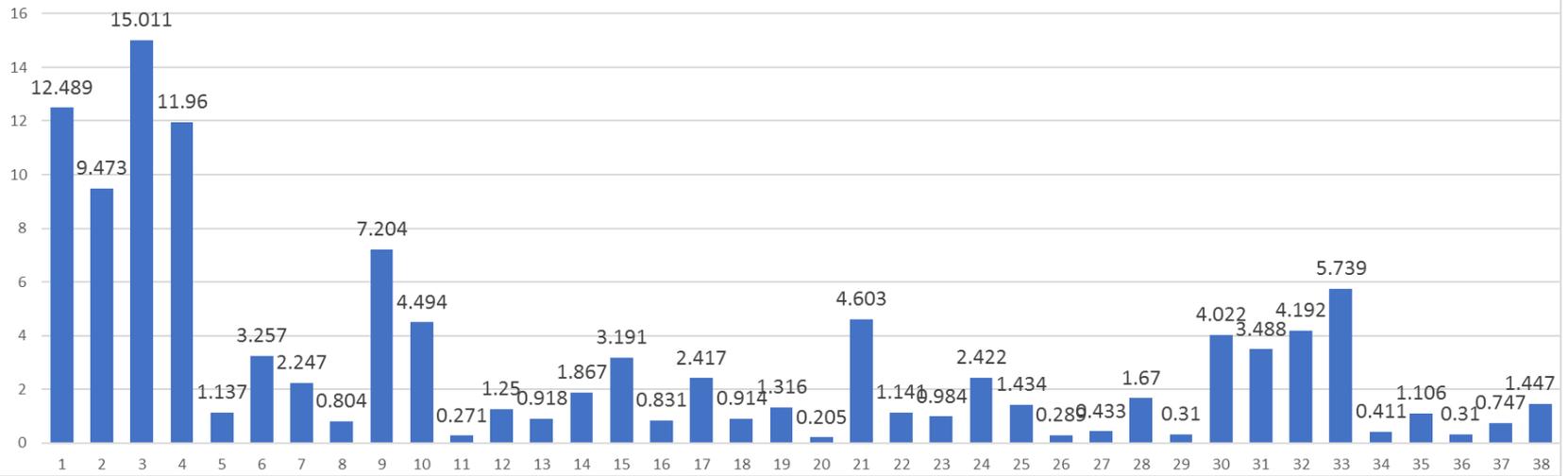
File Edit Font Results					
	Label	Area	%Area	MinThr	MaxThr
1	colorat-1.tif.Blue	1217.028	38.286	0	255

Quantification made with ImageJ software



Area distribution CNT NC3151 in lung carcinoma cell

■ Area



ICONS

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- 2016 – 2019
- MWCNTs
- UEFISCDI – 27/2016
 - ▣ Integrated Testing Strategy for mechanistic assessment of the respiratory toxicity of functionalized multiwalled carbon nanotubes (MWCNT)