Electronic Supplementary Material (ESI) for RSC Advances

Nanoscale infrared absorption imaging permits subwavelength non-invasive intracellular gold nanoparticle conjugate localization: Supporting Information.

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Contents

TBOGNP particle characterization

An FEI Tecnai G2 20 TEM microscope was used to measure TBOGNP particle diameters on a carbon coated copper grid. Conjugate diameters were calculated on the assumption that the particles were approximately cylindrical in cross section and thus the diameters were calculated as $D = 2\sqrt{(V/\pi)}$ i.e. the unidirectional particle diameter. The MATLAB® code for automated diameter measurement is shown below, which accounted for single pixel errors via filtering and required a pixel to nanometer calibration. Objects below 0.5 nm diameter were considered below our ability to resolve and discounted in the histogram.

```
Raw = imread('TEM.png');
J = rgb2gray(Raw); %if image is not in black and white
I = medfilt2(J); %remove single pixel errors
[junk threshold] = edge(I, 'sobel');
tolerance = .65;
BWs = edge(I,'sobel', threshold * tolerance);
angle1 = strel('line', 3, 90);
angle2 = strel('line', 3, 0);
BWs_a = imdilate(BWs, [angle1 angle2]);
BWdfill = imfill(BWs_a, 'holes');
BWint = imclearborder(BWdfill, 4);
diamondmap = strel('diamond',1);
BW = imerode(BWint,diamondmap);
BW = imerode(BW,diamondmap);
BWoutline = bwperim(BWfinal);
L = bwlabel(BW);
rgb = label2rgb(L, 'jet', [.95 .95 .95],'shuffle');
a = regionprops(BW,L,'PixelValues');
b = size(a);
for i = 1 : b(1)
c = size(a(i).PixelValues);
d(i) = c(1);
end
d(d<5) = [];
diammeters = 2*(d/3.14).^(1/2); %assuming circular objects
ratio = 282/20; %pixels per nm calibration  - depends on image
diammeters = diammeters/ratio;
```

Fig.1 Code used for TBOGNP size assessment.

Choice of Substrate

Glass, Mica, Calcium Fluoride and Sapphire were used as substrates in order to ascertain their influence on the cell spectra. Substrates influence the cell spectra and thus the wavelength specific IR imaging intensity due to their partial absorption of the IR source. Therefore the absorption co-efficient of the substrate material is the property of interest. Specifically, a linear absorption co-efficient over the laser range precludes spectral de-convolution. Due to the aqueous nature of the culture and ambient environment, CaF_2 was ruled out due to its solubility (0.0015 g/100 mL). Mica and glass were not spectrally linear over the laser region and so sapphire was chosen as the primary substrate.

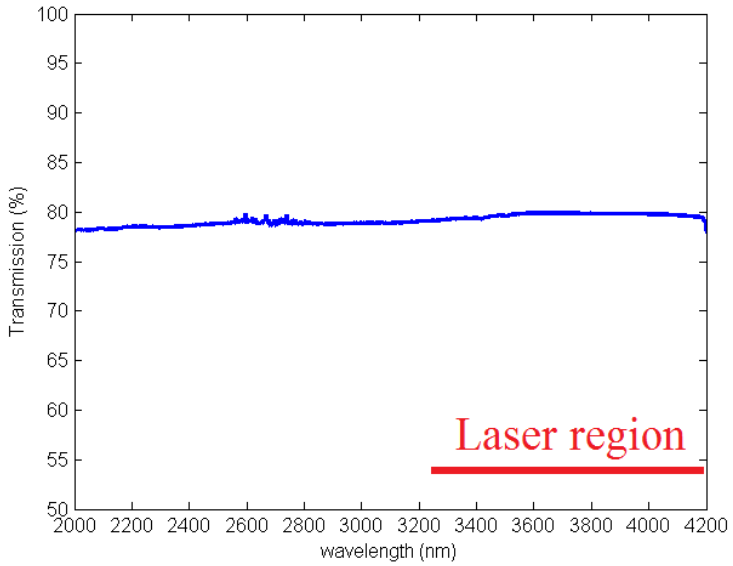

Fig.2 Transmission spectrum of the sapphire substrate with the laser region of interest shown in red.

Cell Spectra

Only a small subsection of the cell spectra is shown in the main paper in order to clearly explain the ratio maps. A broader section of the absorption profile is shown here in Fig.3. The spectra were acquired of the culture post fixation on a substrate which was held at the FTIR sample stage via a custom built mount. This method was used so that the same cells could be characterized by FTIR and nanoscale IR on the same day where necessary. The data obtained is in good agreement with cancer cell FTIR spectra [1]. The dominant mid-IR carbon (2800-300cm^{-1}) and nitrogen (3100-3400cm^{-1}) peaks are all present with the expected intensities, although some noise is evident on the nitrogen peak.

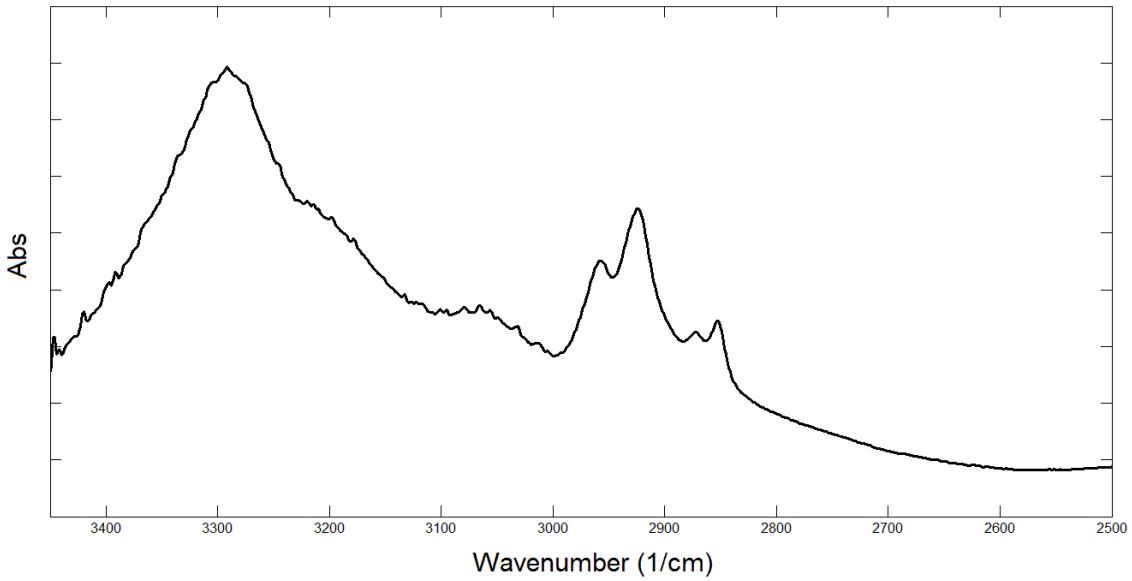

Fig.3 The IR absorption spectrum for the colon cancer cells post fixation on sapphire.

Data handling

When reading in nanoscale point source IR data, the influence of background absorption and laser intensity are experimental considerations. The absorption on the substrate was used a

background reference to be subtracted from the cell absorption values. The monitor efficiency was used to normalize the data in order to differentiate variation in laser intensity from variation in absorption from changes of source wavelength. The code used to find absorption 'abs' is shown in Fig.4.

```
fname = input('enter filename: ' ,'s') ;
data = load(fname) ;

Is = data(:,2); %intensity reading at each wavelength
Ib = 14; % intensity when laser is off
nm = data(:,1); %wavelength
MonEff = 0.00022*nm + 0.12; %good linear fit to monitor efficient curve
Vs = data(:,3); %background dB
Vb = data(:,4); %on sample dB

Abs = (Vs - Vb)*MonEff./(Is - Ib);
```

Fig.4 Matlab code for output data handling.

For imaging, the same process was used but was instead applied to the data stream as a whole. Because the AFM raster scans the image area, the readout is a 1D vector that needs to be reshaped into a square matrix in order to be visualized. Time synced masked raster regions were employed in order to correlate IR and AFM images, as in Figure 5.

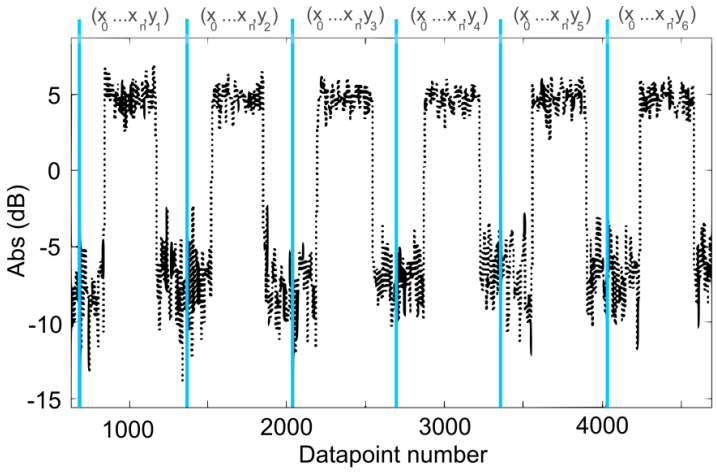

Fig.5 Absorption values while raster scanning for high laser intensity with the new line reshape positions shown vertically in cyan.

Choice of IR image contrast

Image coloring was performed using the Gwyddion software package. Choice of color and contrast was used to highlight surface features on the cell and also to highlight the cell perimeter outline, both experimentally relevant attributes in order to assess TBOGNP localization. The AFM tip, which relaxes by mechanical expansion, is a forced resonance system and so operates as a Cauchy distribution of responses. The cells showed local IR hotspots, broadly varying IR intensity in line local volume and other features on the surface. An example is given in Fig.6 where the cyan histogram values in (a) are masked in the image in (c). This allows on cell surface features in good contrast detail but gives little information about the noise and signal from the substrate. These can be assesed in (b) and (d) where the higher intensity, and thus cell absorption readings are masked in cyan.

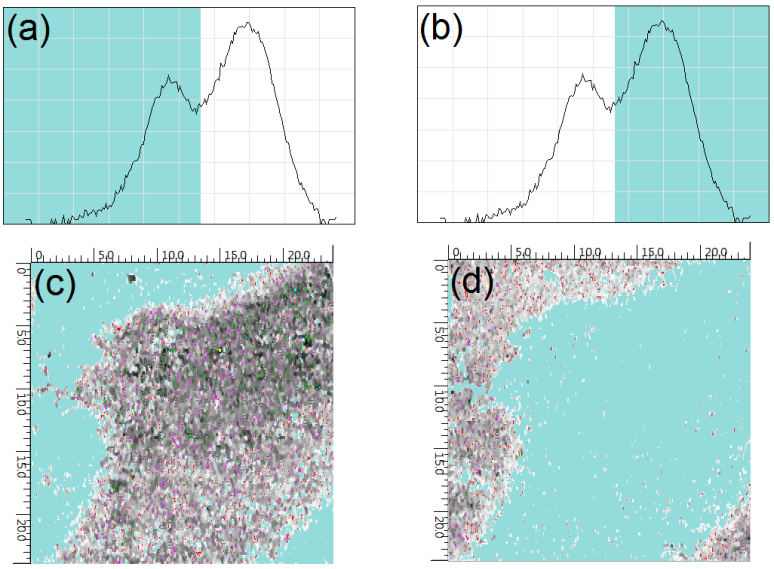

Fig.6 Choice of image contrast. (a) Histogram of absorption intensities in the image with the corresponding masked regions shown in (c). (b) masks the values above the substrate level, and so masks the cell in the image in (d).

Infrared nanospectroscopy and imaging of TNOGNP aggregates

For completeness, the TBOGNP conjugates were analysed exclusively on a cleaved mica substrate using AFM-IR. Comparison of the bulk FTIR and nanoscale spectra is shown in Fig.7 along with a typical absorption map taken of the nanoparticles. Good agreement exists between both spectral methods and the AFM topographic outline is reproduced in the IR image. The TBOGNP nanoparticles here have clearly aggregates compared to their size in TEM imaging, primarily due to line broadening. However, the FTIR spectral features are still reproducible from a 50nm point source.

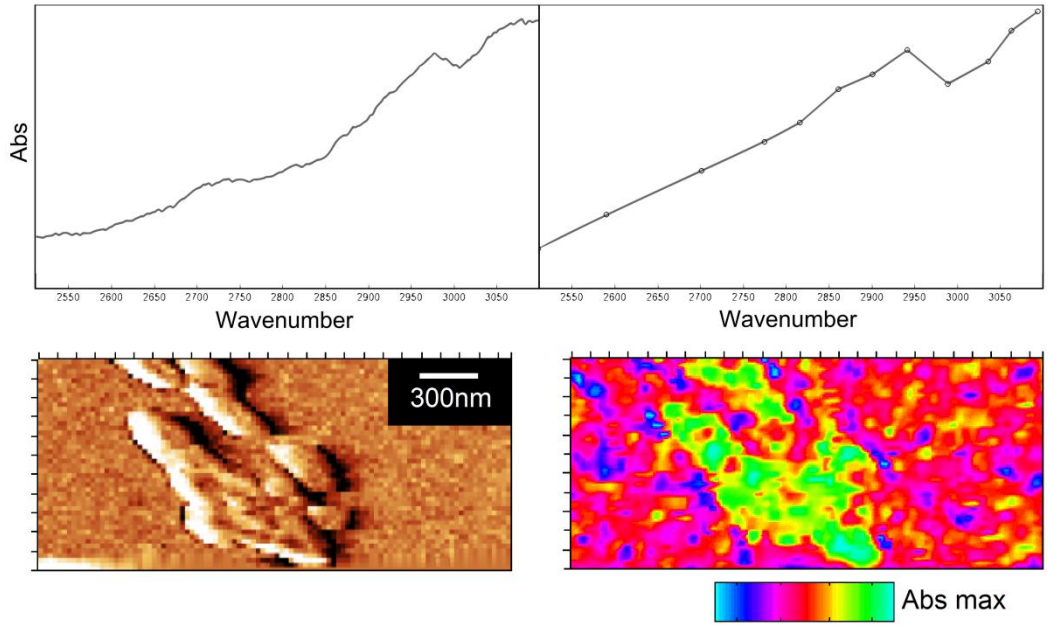

Fig.7 The FTIR (top left) and nanoscale IR (top right) spectra for TBOGNPs. The overall shape is reproduced although the nanoscale IR has a low frequency resolution. AFM of TBOGNP aggregates and the corresponding absorption map are shown below, indicating increased tip deflection via light absorption in the areas close to the nanoparticles.

[1] Jacek K. Pijanka et al. 'FTIR microspectroscopy of stained cells and tissues. Application in cancer diagnosis.' Spectroscopy 24 (2010) 73–78