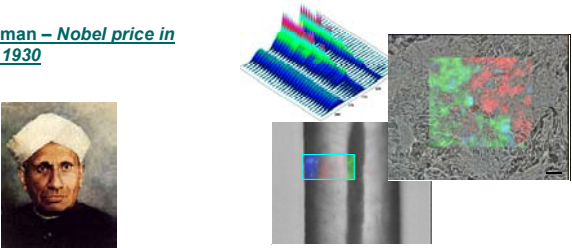


Diapositive 1

1

Raman Spectroscopy and imaging to explore skin and hair

Sir C.V. Raman – Nobel price in physics in 1930



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Diapositive 2

2

What is the Raman scattering effect?

Incident mono-chromatic beam $\bar{\nu}_0 = 1/\lambda_0$

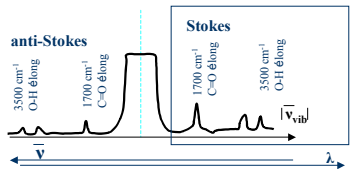
Scattered light is polychromatic

$$\bar{\nu}_0 + \bar{\nu}_{vib}; \bar{\nu}_0; \bar{\nu}_0 - \bar{\nu}_{vib}$$
$$+ \bar{\nu}_{vib}; 0; -\bar{\nu}_{vib}$$

Échantillon opaque

Échantillon transparent

Spectrum of bands shifted compared to the excitation position, cm^{-1}



anti-Stokes

Stokes

3500 cm^{-1} O-H elong

1700 cm^{-1} C=O elong

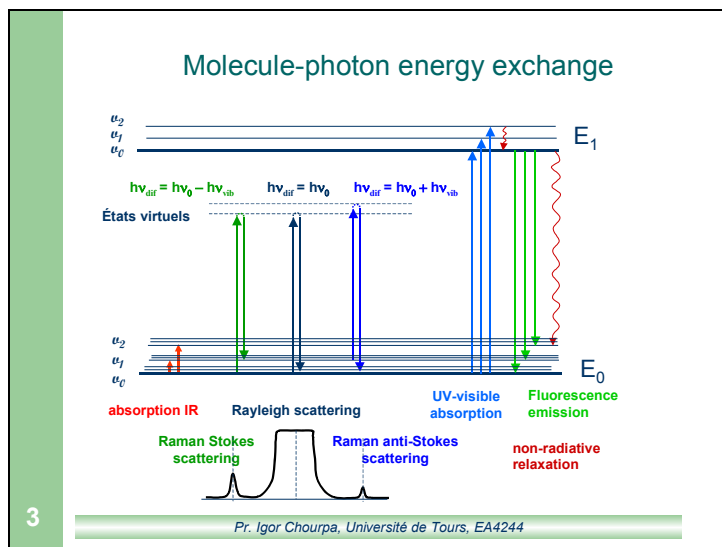
1700 cm^{-1} C=O elong

3500 cm^{-1} O-H elong

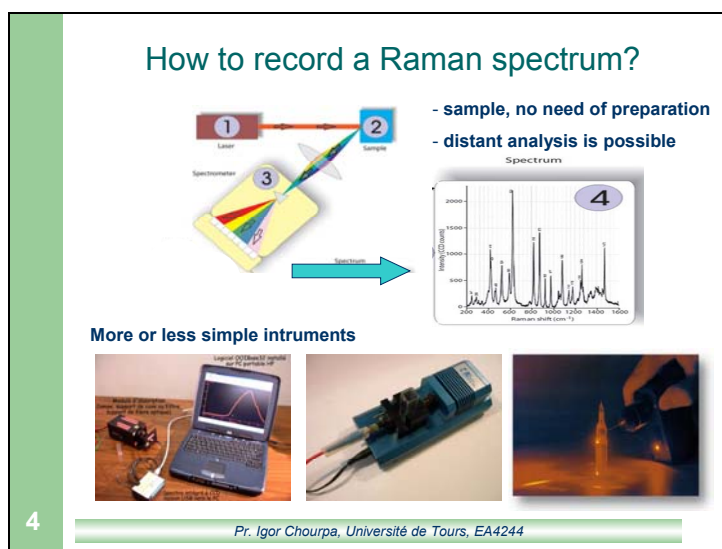
$|\bar{\nu}_{vib}|$

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Diapositive 3

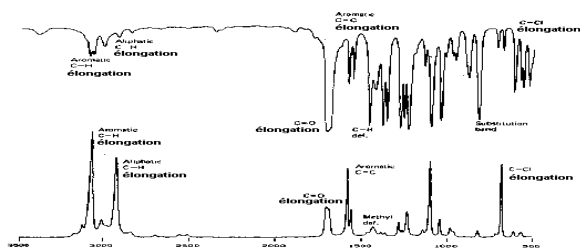


Diapositive 4



Diapositive 5

- **Raman spectroscopy** is complementary to IR, both being structure-specific methods



The figure displays two Raman spectra. The top spectrum shows peaks labeled 'Aromatic C=C elongation', 'Aliphatic C-C elongation', 'C-H def.', and 'C-D elongation'. The bottom spectrum shows peaks labeled 'Aromatic C=C elongation', 'Aliphatic C-C elongation', 'C-H def.', 'Aromatic C=C', and 'C-D elongation'. The x-axis represents wavenumber in cm⁻¹.

Advantages in Raman :

- no sample preparation (*non destructive analysis, in situ*)
- distant analysis and *in vivo* analysis is allowed (*optical fiber*)
- better spatial resolution (*mixtures*) and spatial resol-n (*microscopy*)
- low frequencies are analysable (*inorganic substances*)

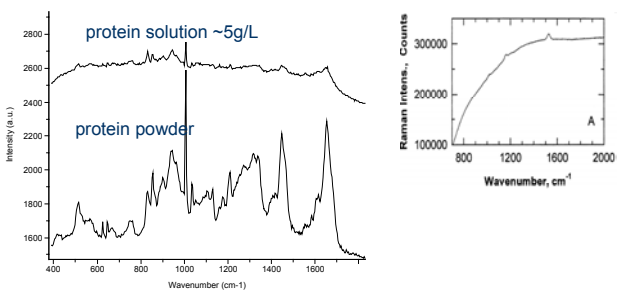
5

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Diapositive 6

Difficulties in Raman

- Poor sensitivity: 10^{-1} M (RR : 10^{-4} M)
- interference with emissions



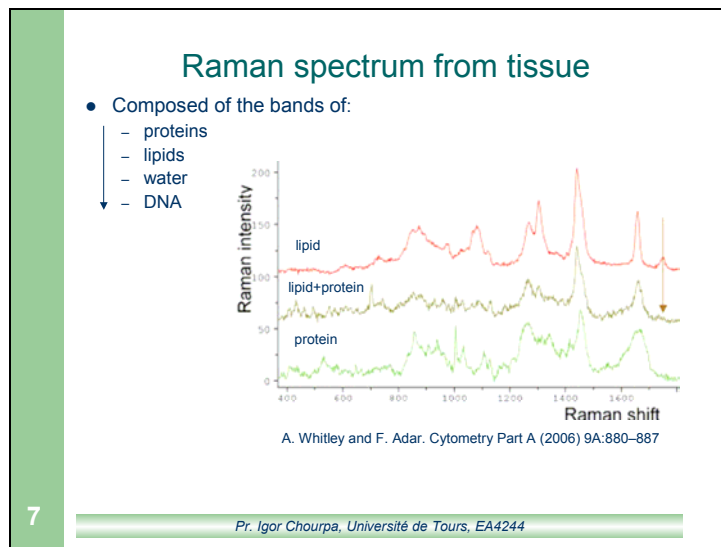
The figure contains three plots. On the left, two Raman spectra are shown: 'protein solution ~5g/L' (top) and 'protein powder' (bottom). The y-axis is 'Intensity (a.u.)' and the x-axis is 'Wavenumber (cm⁻¹)'. On the right, a plot shows 'Raman Intense., Counts' vs 'Wavenumber, cm⁻¹' with a curve rising from 100,000 to 300,000 counts between 800 and 1600 cm⁻¹.

- Low signal from low density samples like cells (*progres of instrumentation goes on*)
- Use of powerful lasers emitting in red and near-IR

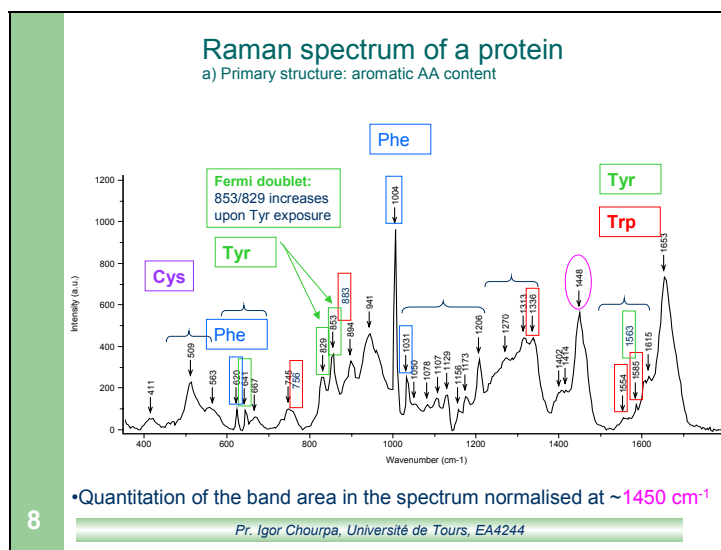
6

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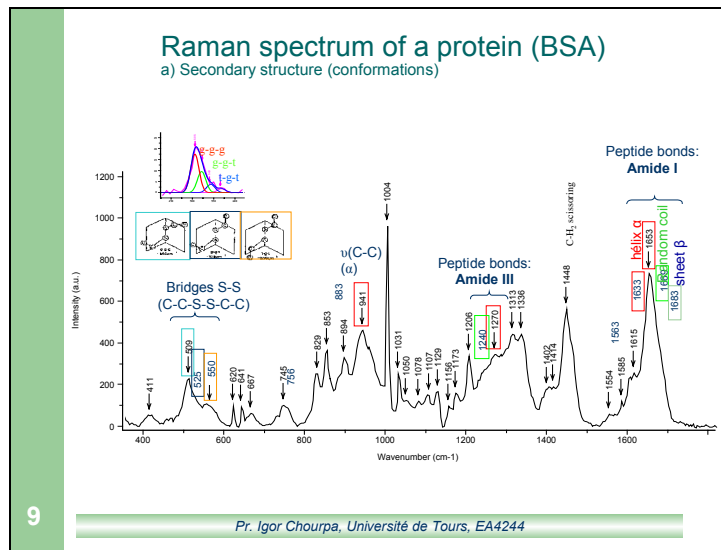
Diapositive 7



Diapositive 8



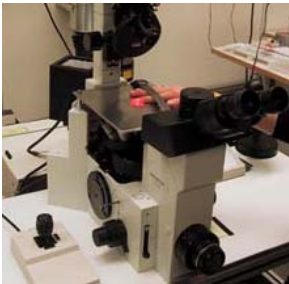
Diapositive 9



Diapositive 11

Raman measurements of skin *in vivo*

Application note : horiba.com



- Laser 633,785 or 830 nm, 5-10 mW; obj 20X LWD or 50x LWD;
- Resolution in Z (Si): 6 to 30 μm
Depth max $\sim 300 \mu\text{m}$
- Inversed microscope (immobilisation against a quartz window d. $\sim 2 \text{ mm}$)
- Otherwise: optical fiber with focus/collect head
Ex: Skrebova N. et al., J. Biomed. Opt. 2005: 10, 014013

LabRam INV, Horiba JY
Institute of Analytical and Marine Chemistry
University of Chalmers, Göteborg, Sweden at

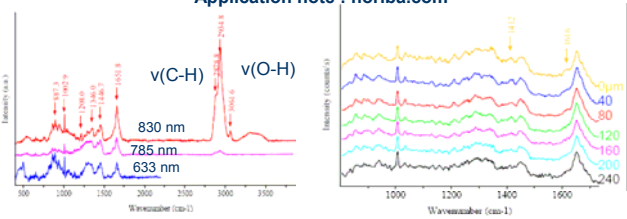
11

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Diapositive 12

Raman spectra of skin *in vivo*

Application note : horiba.com



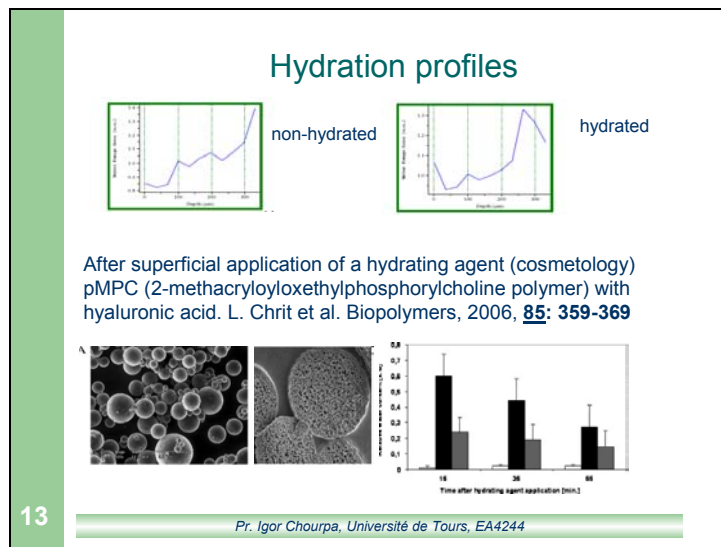
Peak Position (cm ⁻¹)	Protein Assignments	Lipid Assignments	Others
1745		v(C=O)	
1655	v(C=O)Amide I		
1445	$\delta(\text{CH}_2)$, $\delta(\text{CH}_3)$, δ	$\delta(\text{CH}_2)$ scissoring	
1301		$\delta(\text{CH}_2)$ twisting, wagging	
1209	v(CN), $\delta(\text{NH})$ Amide III		
1080		v(CC)skeletal	v(C-C), $\nu_2(\text{PO}_4)$
1030		v(CC) skeletal	Nucleic acids
1002	v(CC) Phenyl ring		
838	v(CC) proline, valine		
855	$\delta(\text{CCH})$ aromatic, olefinic		polysaccharide
822	$\delta(\text{CCH})$ aliphatic		

Table 1: Summary of major vibrational bands identified in skin: v, stretching mode; ν_s , symmetric stretch; δ , bending mode.

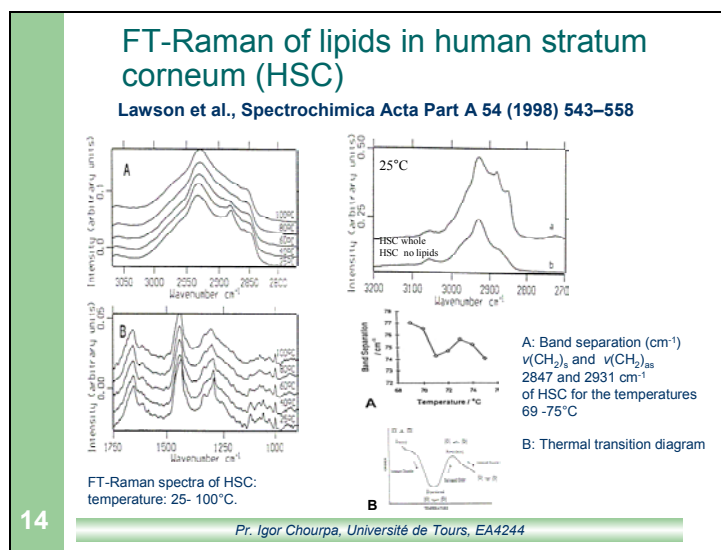
12

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Diapositive 13



Diapositive 14



Diapositive 15

Carotenoids in human skin

Hata TR et al. *J Invest. Dermatol.* (2000) 115, 441–448

Exc. 488 nm: strong fluorescence: correction

- Lutein, zeaxanthin, lycopene and its Z-isomers, and -α, -β, -γ, and ξ-carotene conc. quantitated by HPLC, compared with the 1524 cm⁻¹ Raman intensity (counts).
- Carotenoid concentration in the skin correlates with the presence or absence of skin cancer and precancerous lesions

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Diapositive 16

Human tissue : pathologic or not?

FIGURE 1 Mean Raman spectra of oral tissues: (a) normal, (b) malignant, (c) inflammatory, and (d) premalignant.

FIGURE 3 Plot of spectral residual against sample number with mean spectrum of malignant set as reference. ○-normal, △-inflammatory, + -pre-malignant, * -malignant.

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Diapositive 17

Raman spectroscopy of human hair: effect of chemicals (1) or of aging (2)

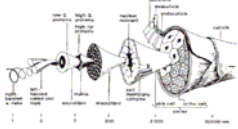


FIGURE 3 The hierarchical structure of a fine wool fiber.

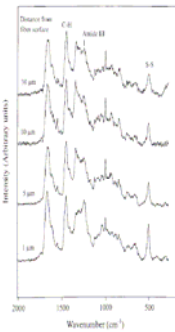


FIGURE 4 Raman spectra of the untreated human hair fiber (Sample 1) at depths of 1.5, 5, 10, and 30 μm .

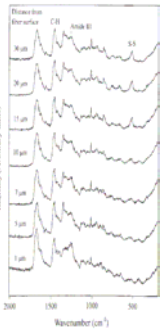


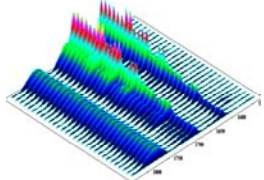

FIGURE 5 Raman spectra of the human hair fiber resulting from the reduction process (Sample 2) at depths of 1.5, 7, 10, 15, and 30 μm .

- (1) Kuzuhara A., Biopolymers, Vol. 77, (2005) 335-344
- (2) Kuzuhara et al., Biopolymers Volume 87, (2007) 134-140

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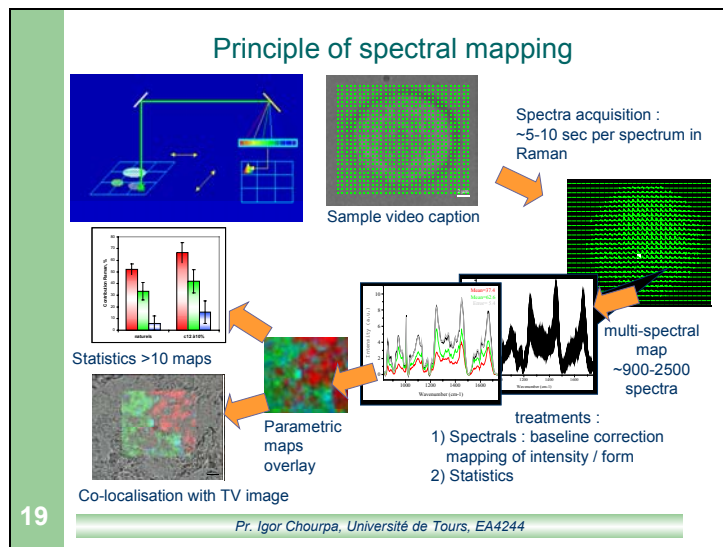
Diapositive 18

Molecular imaging by Raman spectral mapping

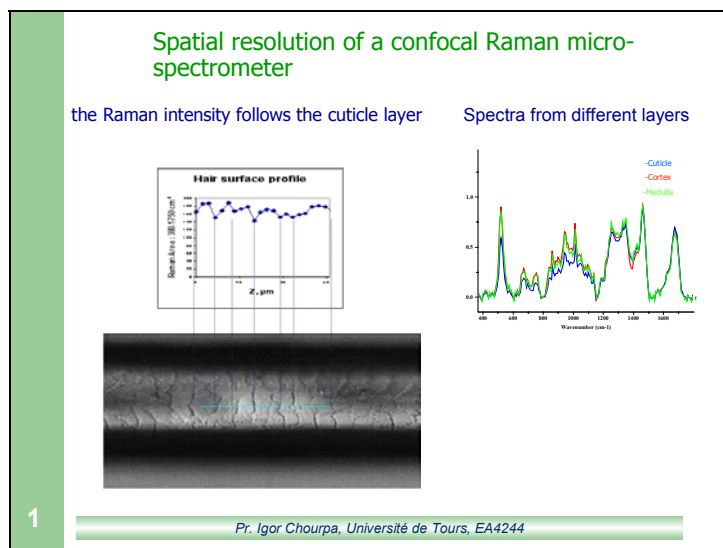


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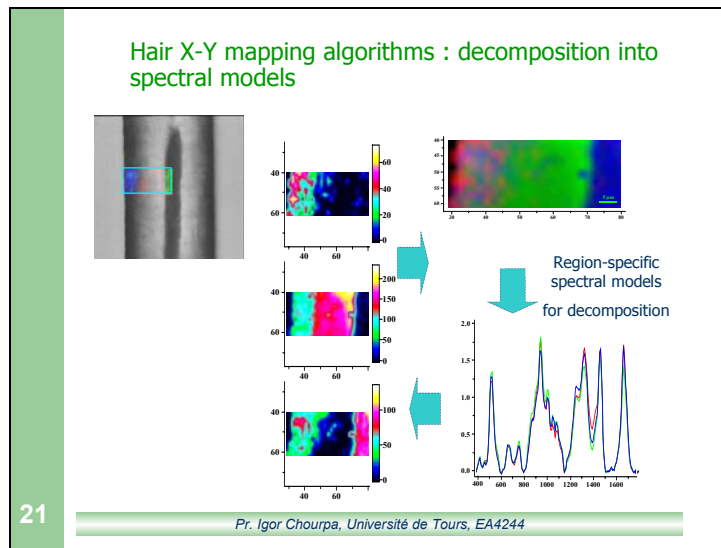
Diapositive 19



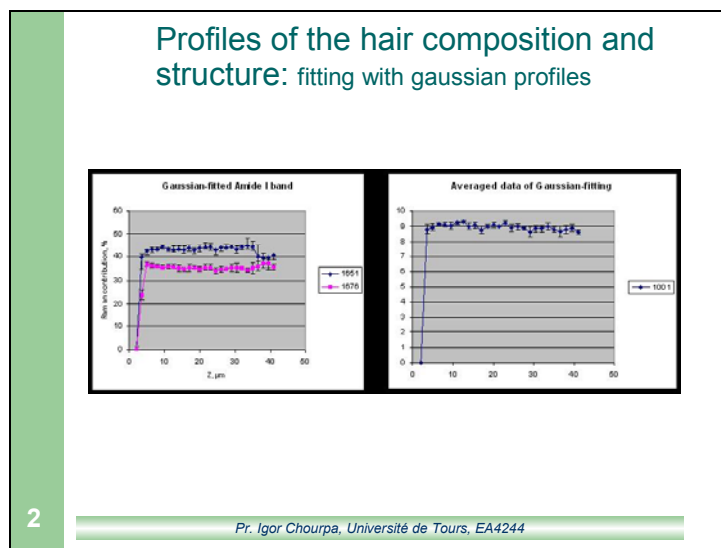
Diapositive 20



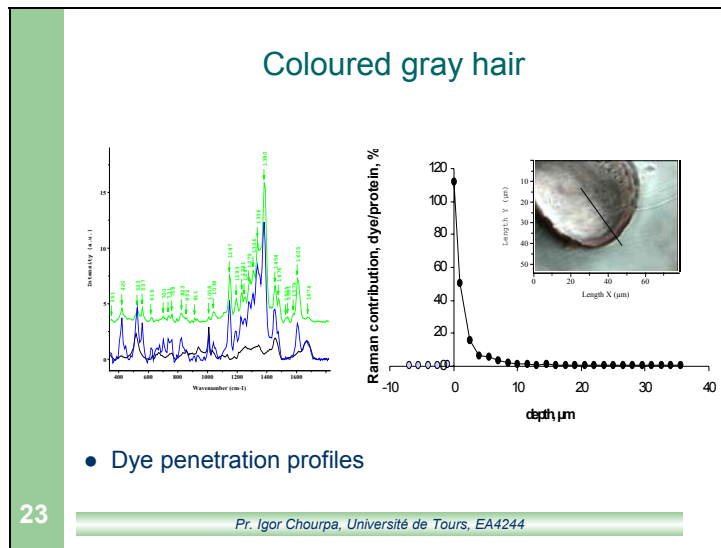
Diapositive 21



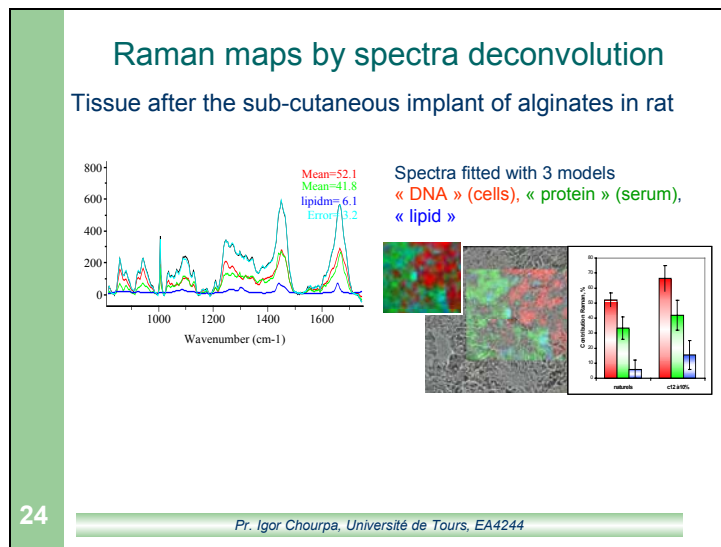
Diapositive 22



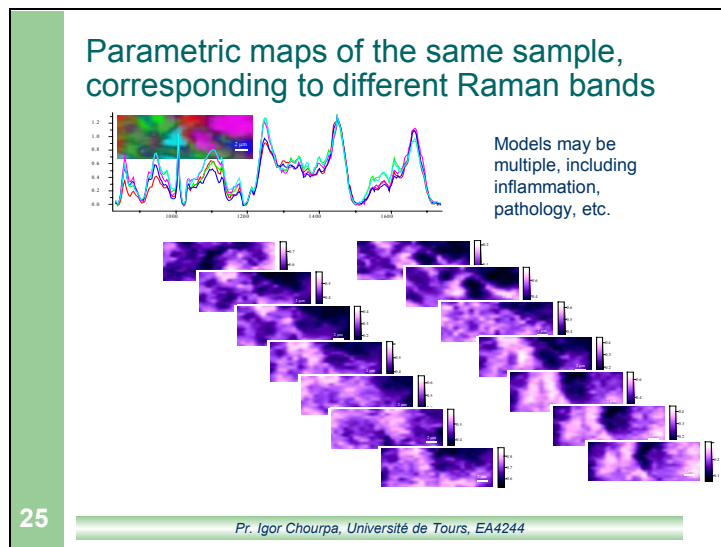
Diapositive 23



Diapositive 24



Diapositive 25



Diapositive 26

Acknowledgments

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