Molecular Spectroscopy for Food Analysis

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In this talk:

- Mid-infrared spectroscopy
- Low-field NMR spectroscopy
- The food industry
- Case studies

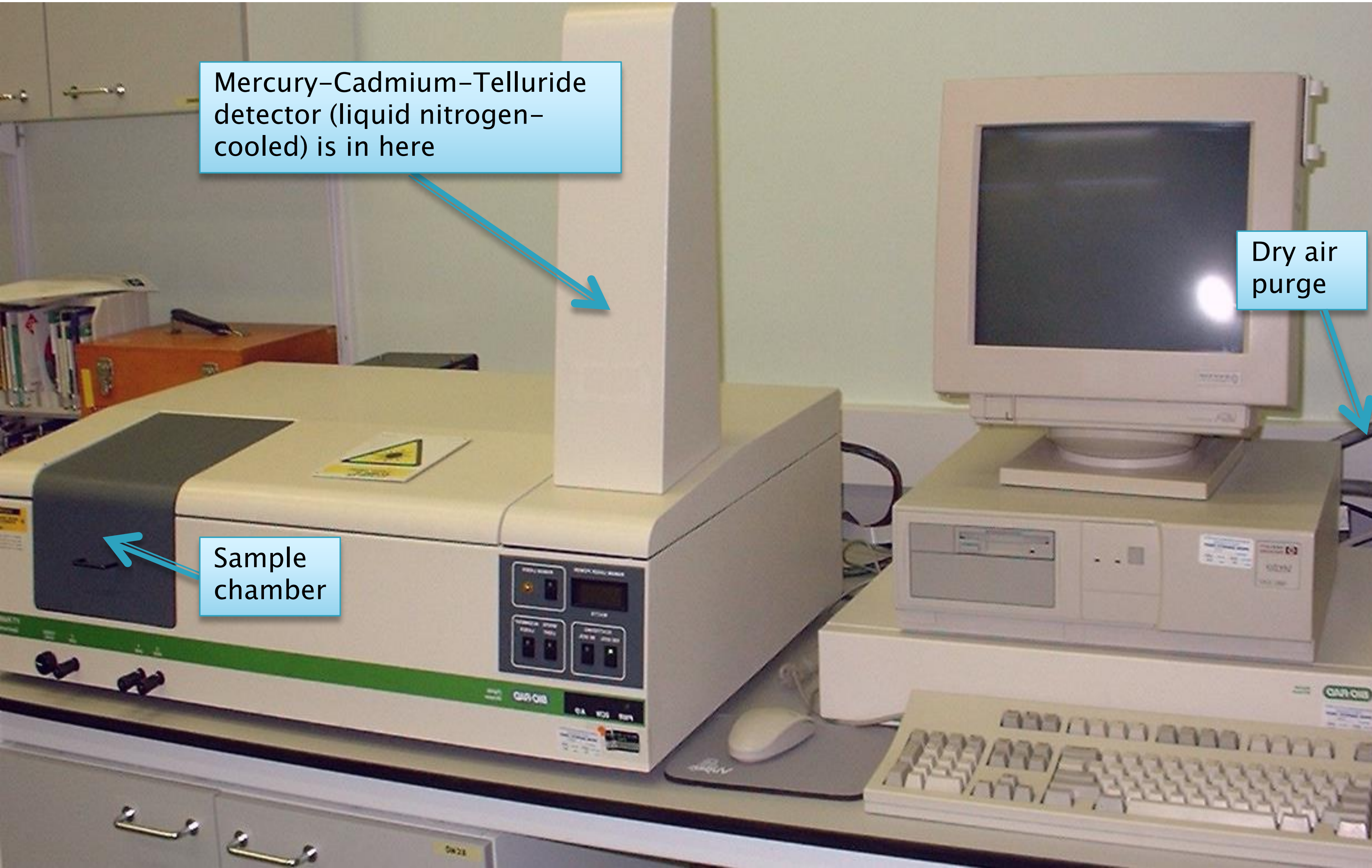
Mid infrared spectroscopy

- Wavelength range is 400 – 4000 cm^{-1} (wavenumbers, or 2.5 – 25 microns)
- Electromagnetic radiation is absorbed due to **fundamental vibrations** of molecular bonds
- Absorption are very chemically specific – especially in the “fingerprint” region

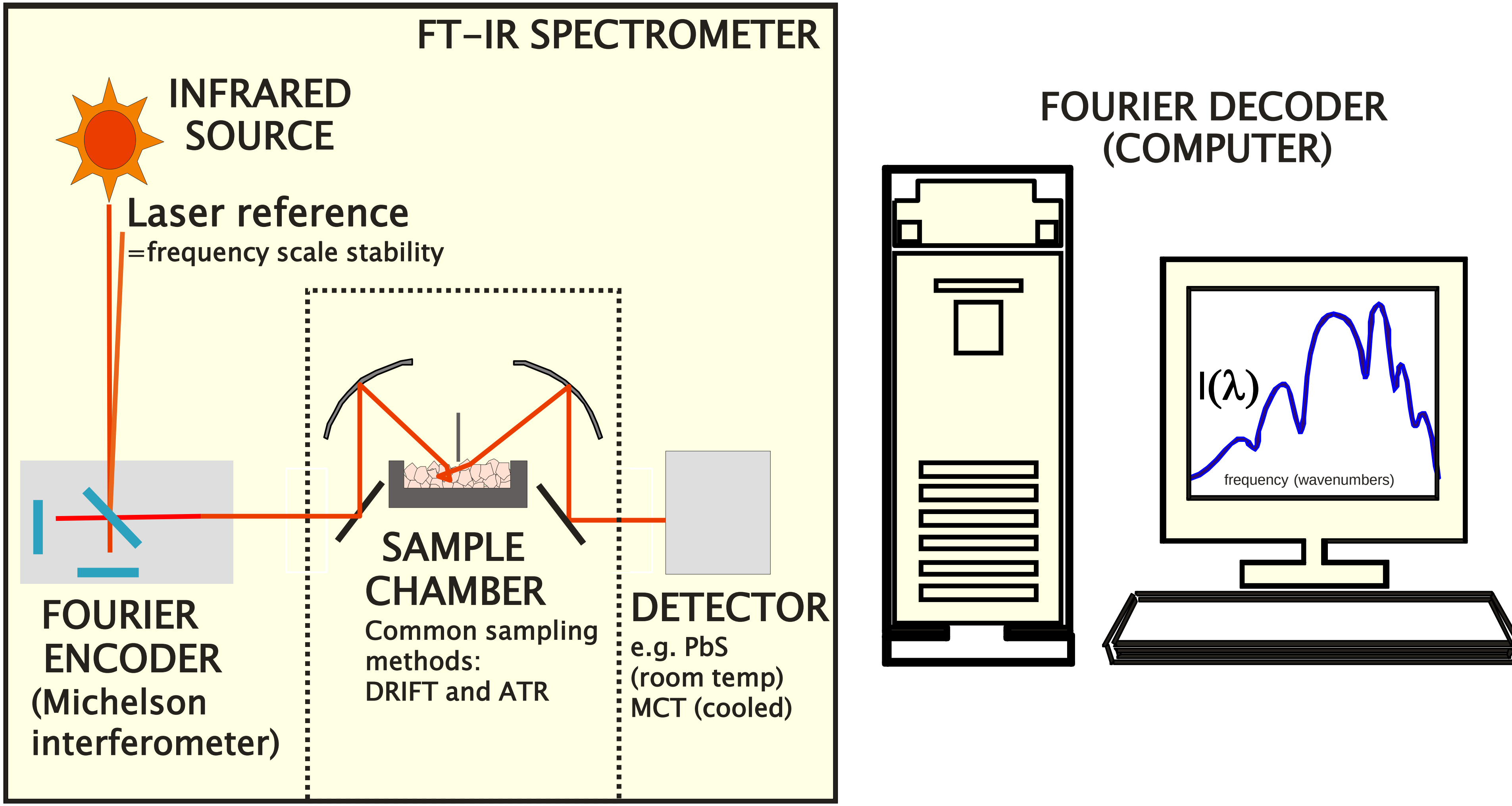
Mid infrared spectroscopy

- Modern laboratory instruments are Fourier transform spectrometers (FTIR)
  - Broad-band radiation goes through the sample, individual frequency information extracted via Fourier transformation
  - This gives a “multiplex” advantage with regards signal-to-noise – high optical throughput
  - Laser reference beam means frequency axis is exceptionally accurate
    - Allows digital co-addition of repeated scans to improve signal-to-noise (“signal-averaging”)
    - Comparison across different samples without further alignment

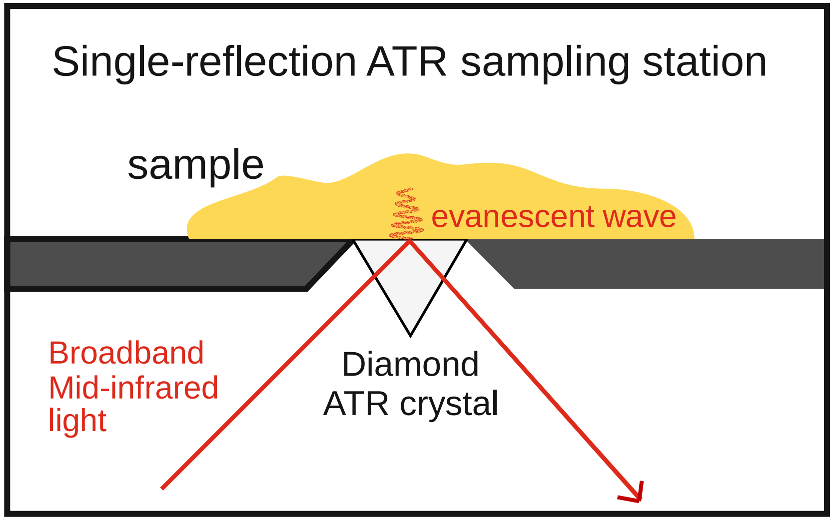
A Fourier transform infrared spectrometer



Schematic of Fourier transform infrared spectrometer system



ATR Sampling methods



- ATR is very effective for analysing wide variety of samples (liquids, semi-solids, solids, powders)
- Evanescent wave penetrates sample at crystal surface – requires good contact between sample and crystal
- Diamond ATR accessories use a clamp to ensure contact between the sample and crystal
- Effective pathlength is refractive-index dependent, and difficult to calculate

Mid infrared spectrometers

- FT-IR spectrometers are "**single-beam**" instruments

- Analysing a sample involves:
  - Collecting a "single-beam background" of the empty sampling accessory. *Call this* $I_0(\lambda)$

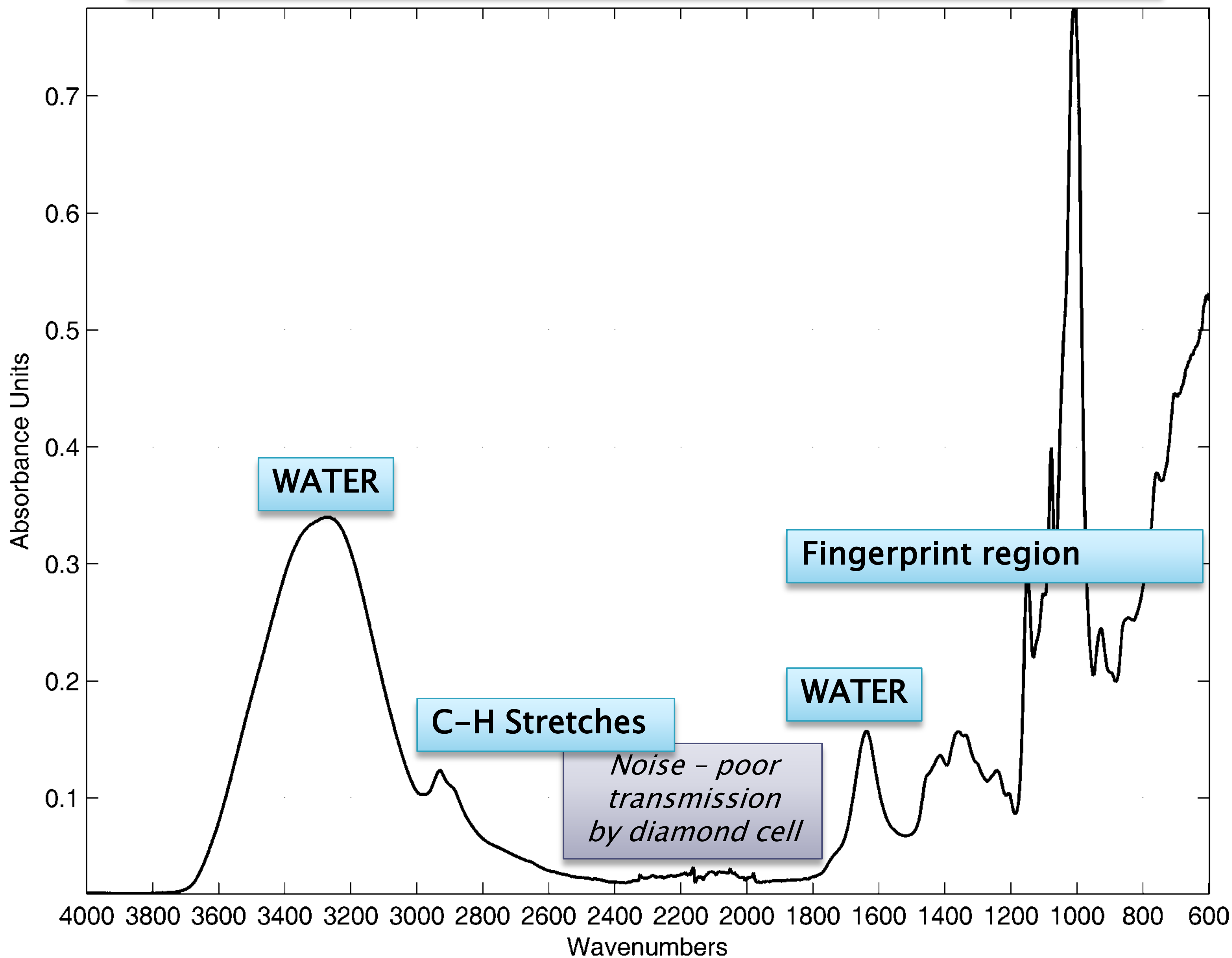
  - Collecting a single-beam spectrum with the sample in place. *Call this* $I(\lambda)$

- The absorbance at any given wavelength is given by:

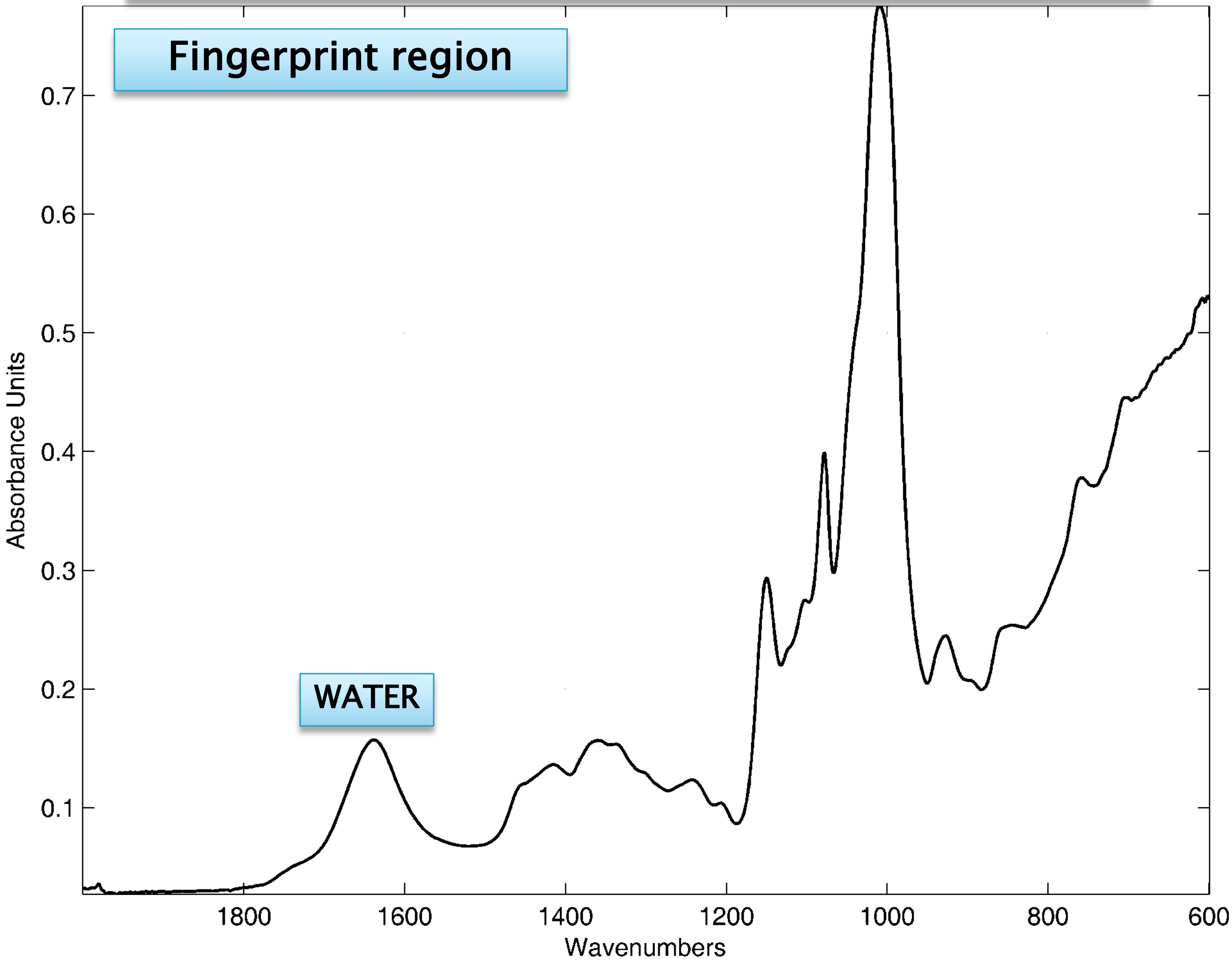
$$A(\lambda) = -\log_{10} (I(\lambda) \; / \; I_0(\lambda))$$

LINEAR WITH CONCENTRATION: Beer-Lambert law

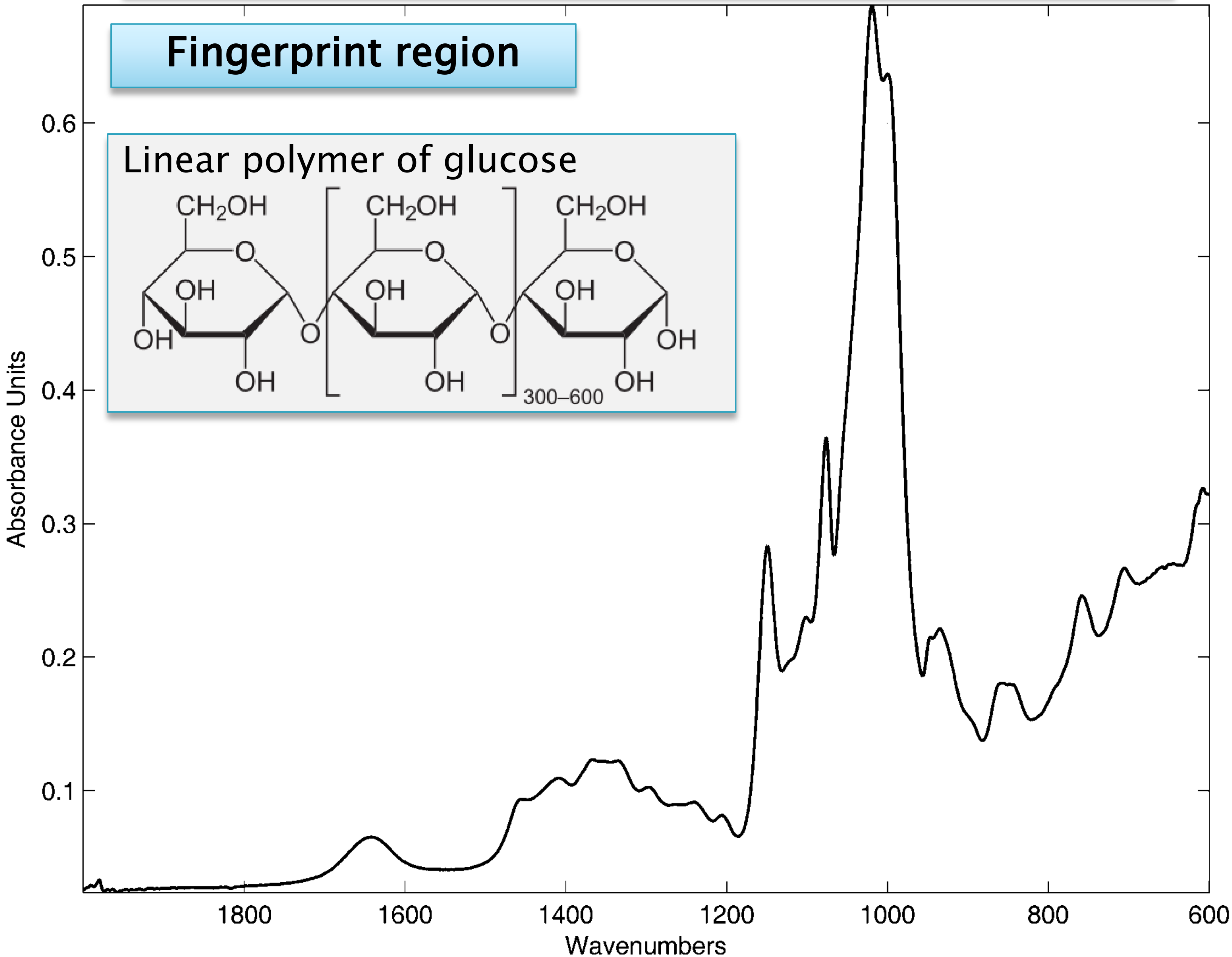
Mid-Infrared spectrum of raw potato



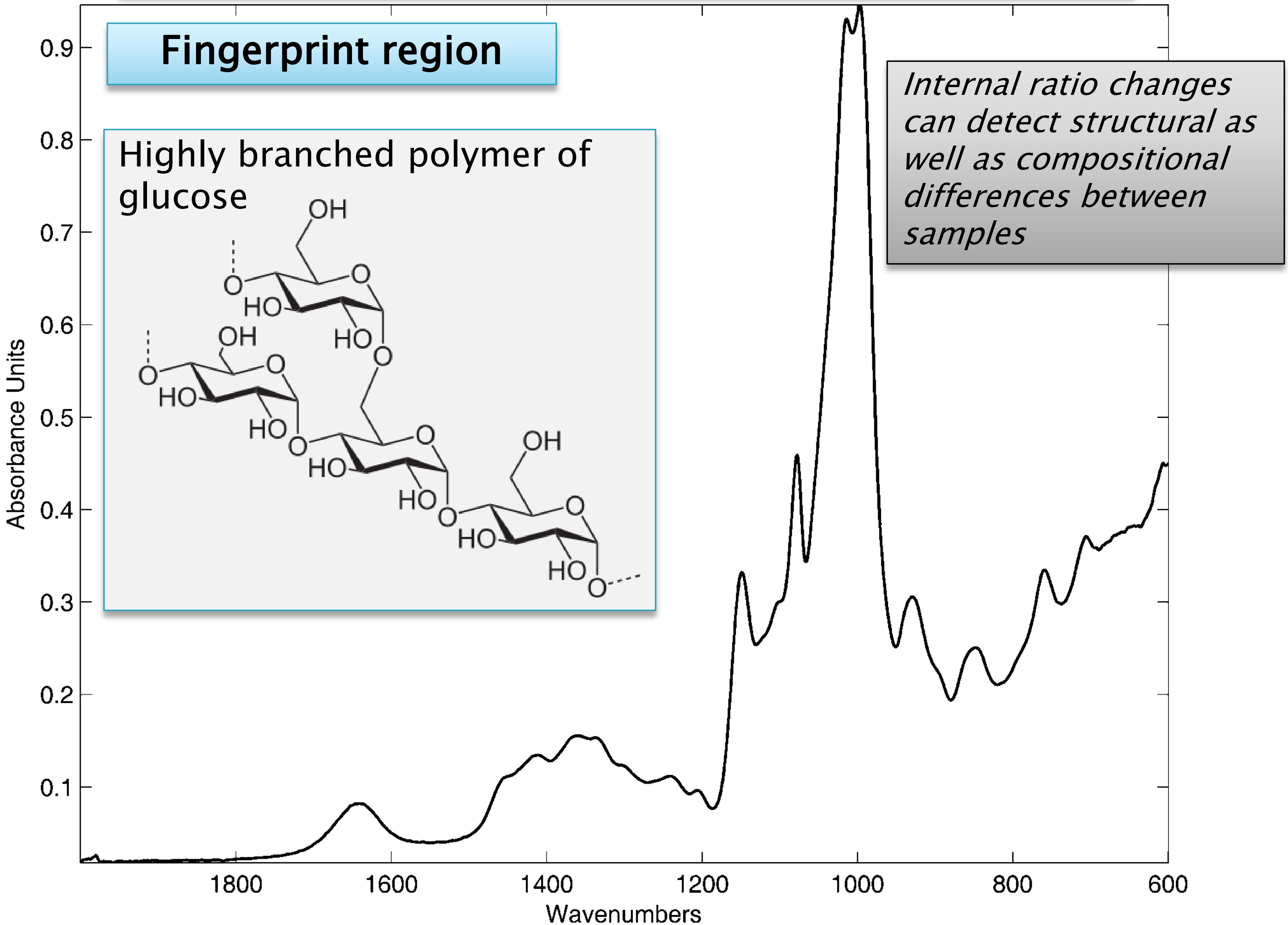
Mid-Infrared spectrum of raw potato



Potato Amylose



Potato Amylopectin



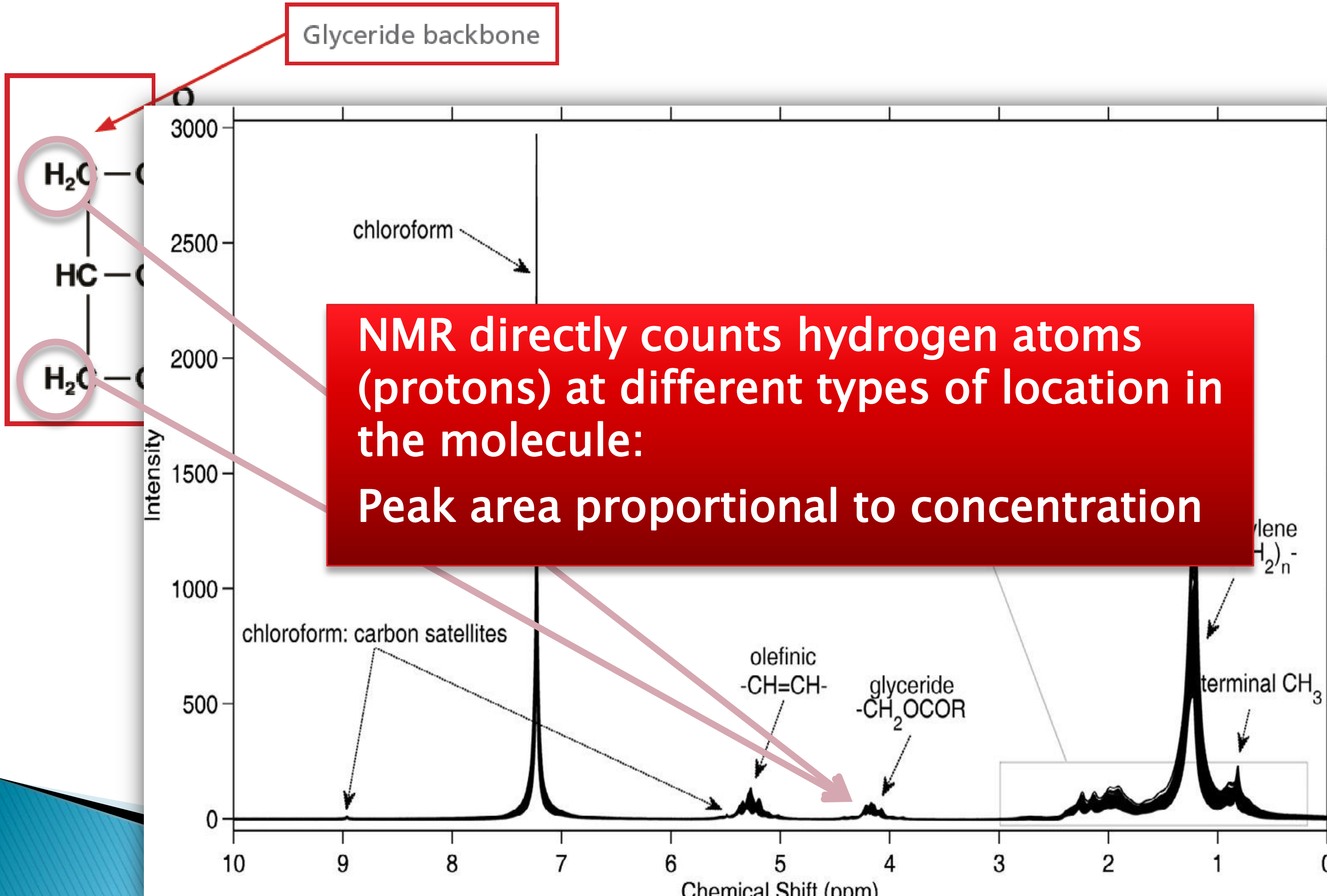
Low-field (60MHz) NMR

- **Nuclear Magnetic Resonance spectroscopy**
  - gives detailed information on chemical composition
- **Low-field (60Mhz "bench-top") instruments** are a recent development
- Compared with high-field spectrometers, these are:
  - Very low-cost (1/10th the capital cost)
  - Virtually no running costs (no cryogens, no air-conditioning)
  - Robust and easy-to-use by non-specialists

Low–field (60MHz) NMR

Triglyceride NMR spectra

- Most fat in foods is present as triglycerides:



NMR directly counts hydrogen atoms (protons) at different types of location in the molecule:

Peak area proportional to concentration

The Food Industry

The UK agri-food sector….

- …is the largest industry in the country, worth >£96 billion in 2011
- …employs ~500,000 people
- …high turnover, low profit margins
- …resilient to economic downturn (people always need to eat)
- …resistant to economic growth (there is a limit to consumer intake)

Sector economic growth relies upon savings made from process control, quality improvement, waste reduction – this is where technology comes in.

Food fraud – widespread and increasing

Food fraud – widespread and increasing

Some much more recent headlines

2008 Chinese milk scandal

From Wikipedia, the free encyclopedia

The **2008 Chinese milk scandal** was a food safety incident in th

By November 2008, China reported an estimated 300,000 vi

have been added to milk to cause it to appear to have a

The scandal broke on 16 July, after sixteen infants

initial focus on Sanlu—market leader in the bu

Yashili.[5]

16 October 2012 Last updated at 09:04

Food fraud tackled by forensic scientists

By Anna-Louise Taylor

BBC Food

Olive oil food fraud: pressing truths

Not everything labelled 'extra virgin' is immaculately conceived; it seems there are some very slippery customers in the olive oil trade, ...blem is spreading

Food Fraud: 8 Hoaxes To Look Out For At The Grocery Store

Posted: 04/20/2012 12:51 pm Updated: 04/20/2012 12:54 pm

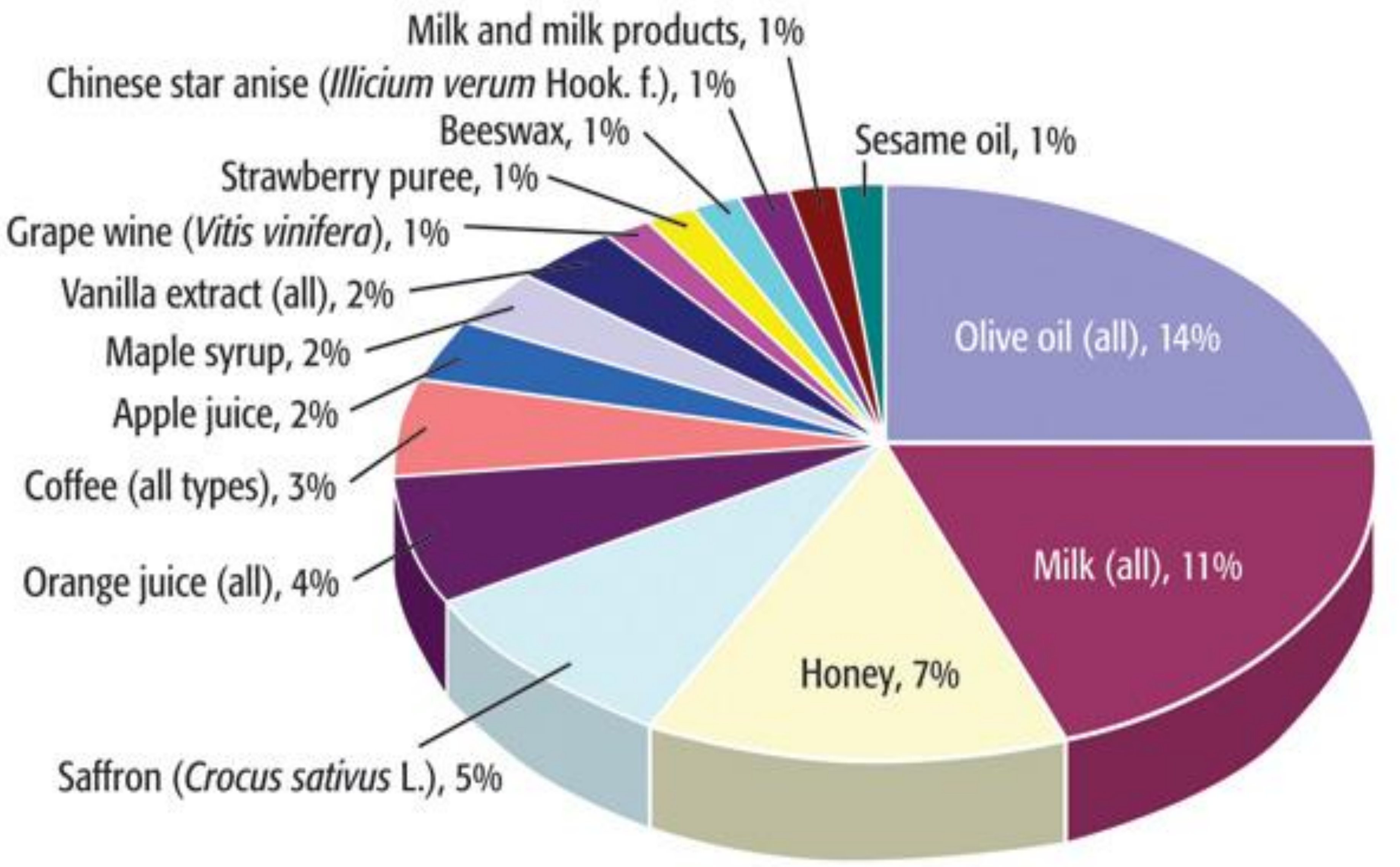
Horse meat scandal 'breathtaking'

Beef products contaminated as a result of criminal activity may pose a risk to human health, MPs warn for first time

Italian olive oil scandal: seven top brands 'sold fake extra-virgin'

The investigation, the latest scandal to hit Italy's lucrative food and drink sector, involves seven well-known producers accused of passing off virgin olive oil as extra-virgin

Most frequent food frauds – by economic value



Source: Food safety Magazine/World Customs Organization

Molecular spectroscopy for food analysis

- The food industry wants **robust, rapid and low-cost methods**
  - **a big ask!**

- Near-IR devices are commonly used for measurement of gross composition – fast, cheap

- Mid-IR and NMR more useful for examining subtle parameters relevant to fraud
  - Edible oils, fats
  - Fruit juices, purees
  - Meat, poultry
  - Ground and instant coffee

Case study 1:
Mid-infrared analysis of instant coffees

- Coffee – a very important economic commodity
- Over 2,250,000,000 cups of coffee are consumed in the world every day
- 25,000,000 farmers worldwide are involved in producing coffee beans
- High-value product with large range of qualities and prices, **and a long commodity chain…**

PRODUCERS (farmers, collectives) → MIDDLEMEN EXPORTERS ("coffee coyotes") → IMPORTERS → ROASTERS → RETAILERS

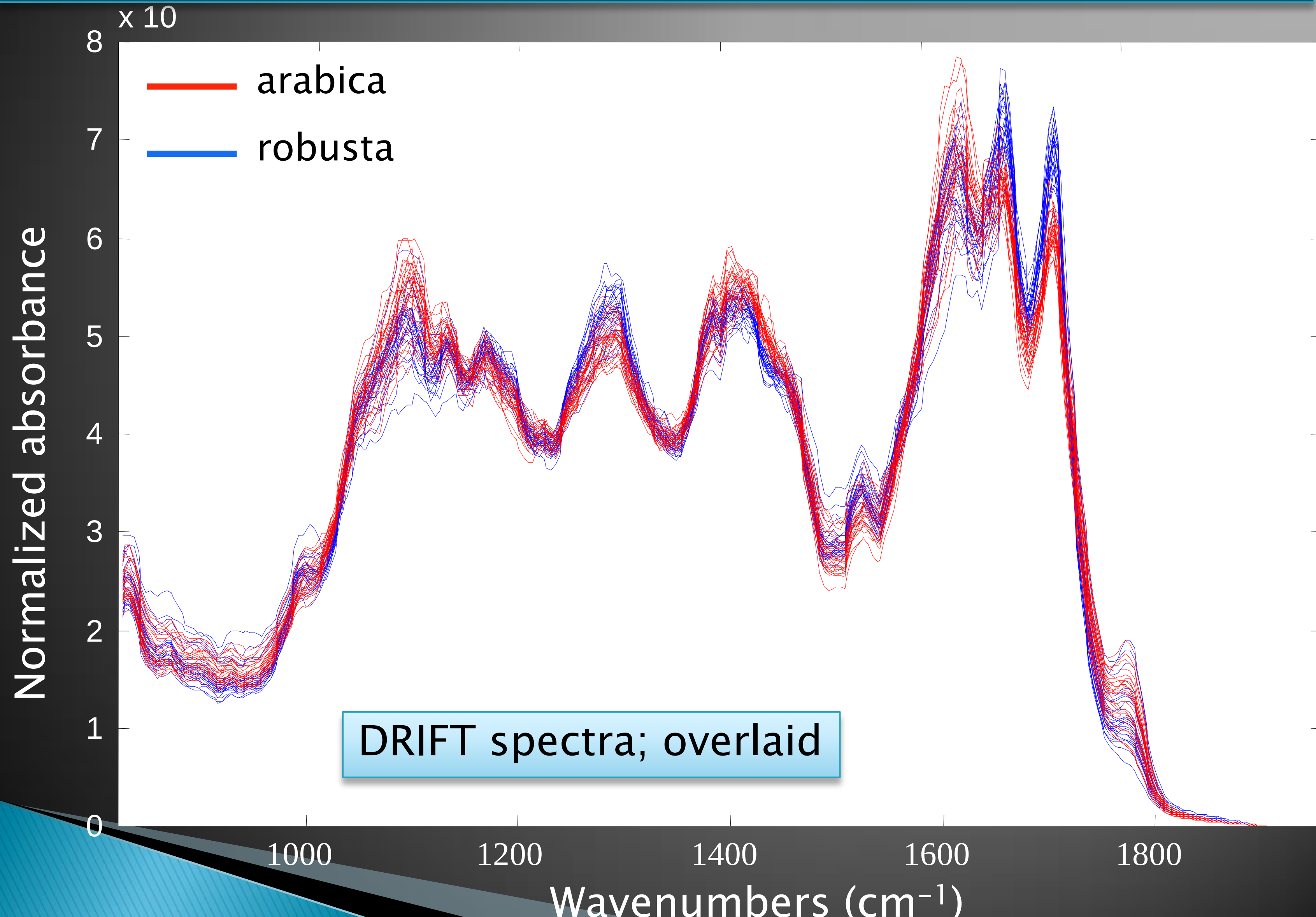
…fraudulent substitution and adulteration is commonplace.

Case study 1:
Mid-infrared analysis of instant coffees

Can MIR and chemometrics be used to rapidly screen and identify ground roast coffees?

- Pure, <u>authenticated</u> freeze-dried samples, of the two main commercially grown coffee species:
  - 29 “Arabica” – *these are “high-value” beans*
  - 23 “Robusta” – *these are low-value, rougher-tasting beans of a different subspecies*
- Substitution of Robusta into Arabica and sale of the blend as “pure Arabica” is economic fraud worth £millions, and disadvantages honest producers

Case study 1:
Mid-infrared analysis of instant coffees

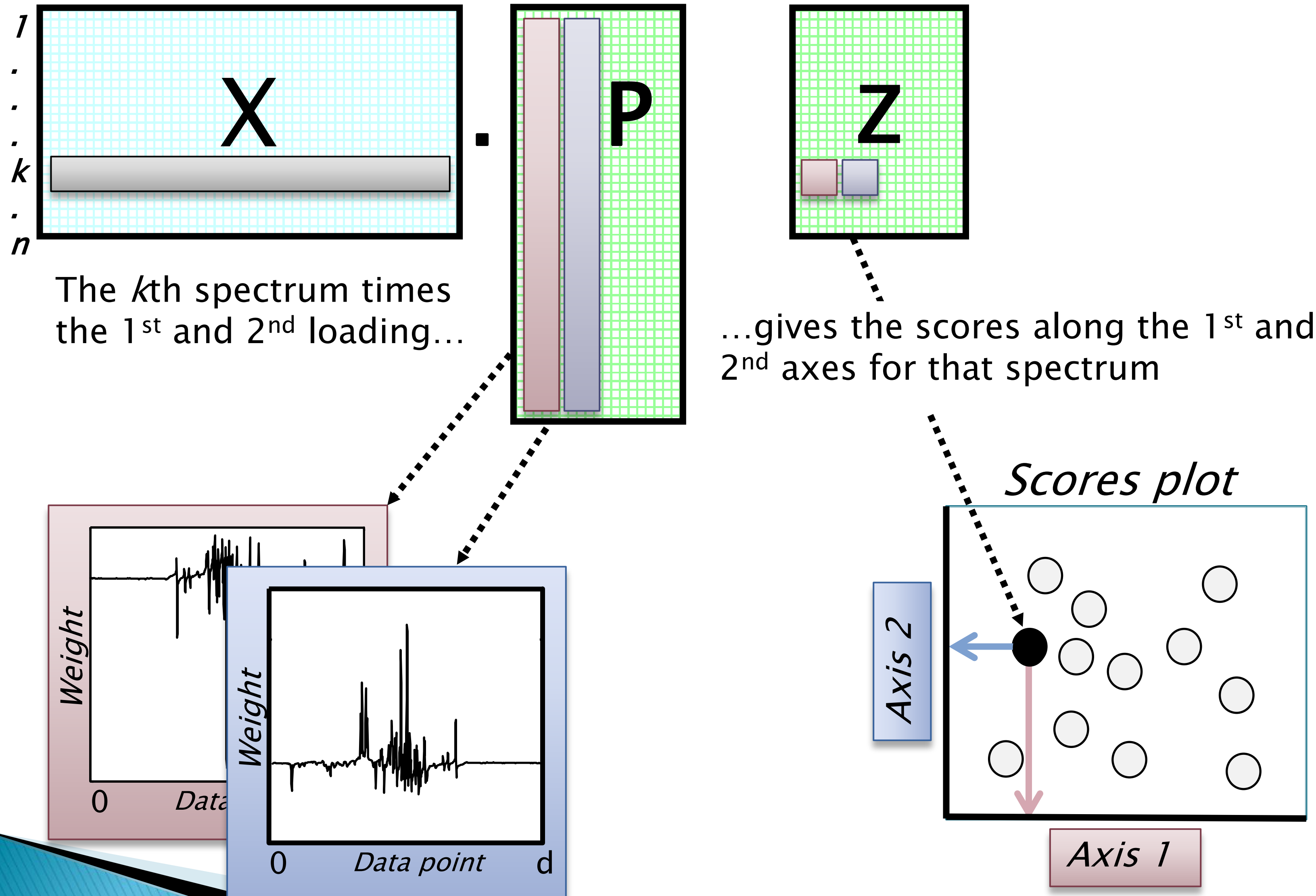


Case study 1:
Mid-infrared analysis of instant coffees

- Data analysis:

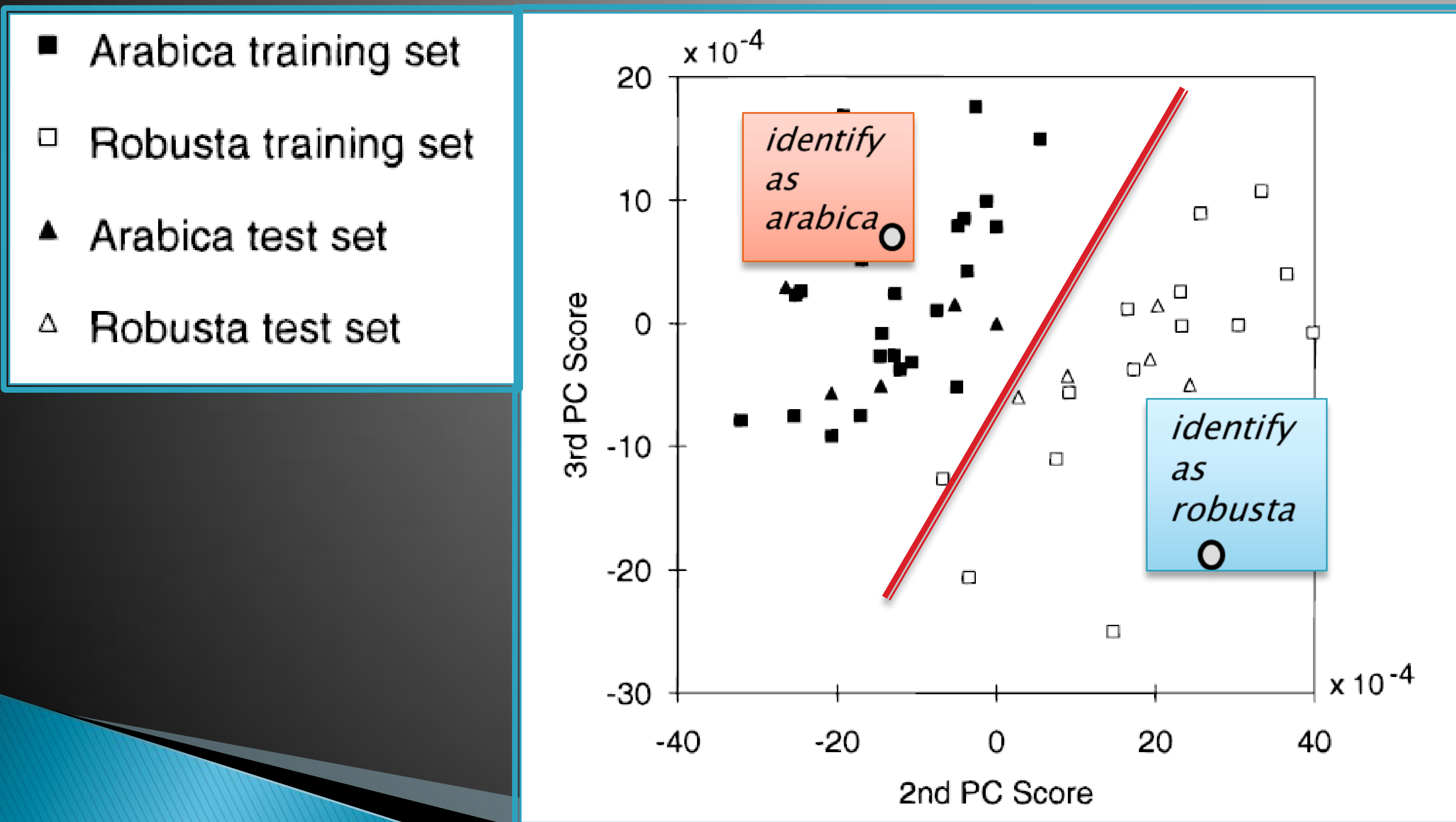
1. Divide data at random into training and test sets
2. Perform PCA on the training set
3. Look for any clustering in the scores
4. Apply the PCA compression to the test data
5. Check that the test data follow the same pattern of clustering

PCA and PLS – Both are data compression methods



Case study 1:
Mid-infrared analysis of instant coffees

PCA results for DRIFT spectra, freeze-dried coffees

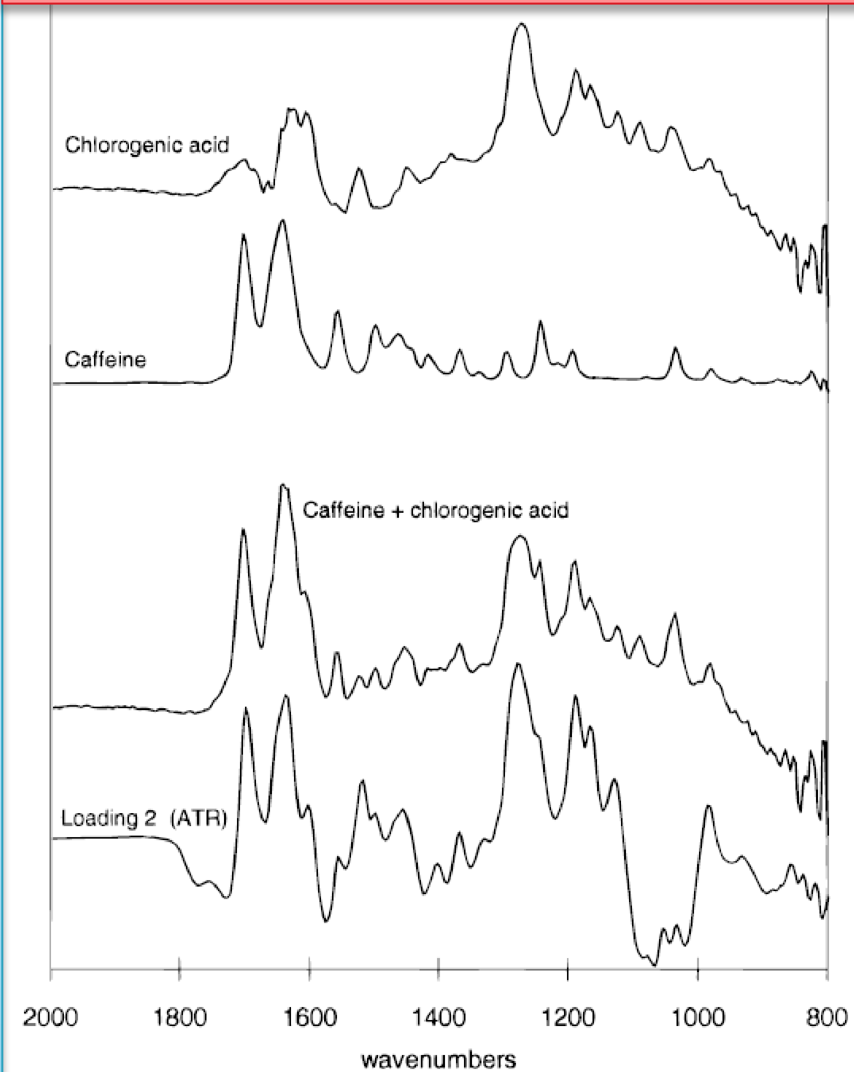


Case study 1: Mid-infrared analysis of instant coffees

Understanding the basis of the discrimination between species

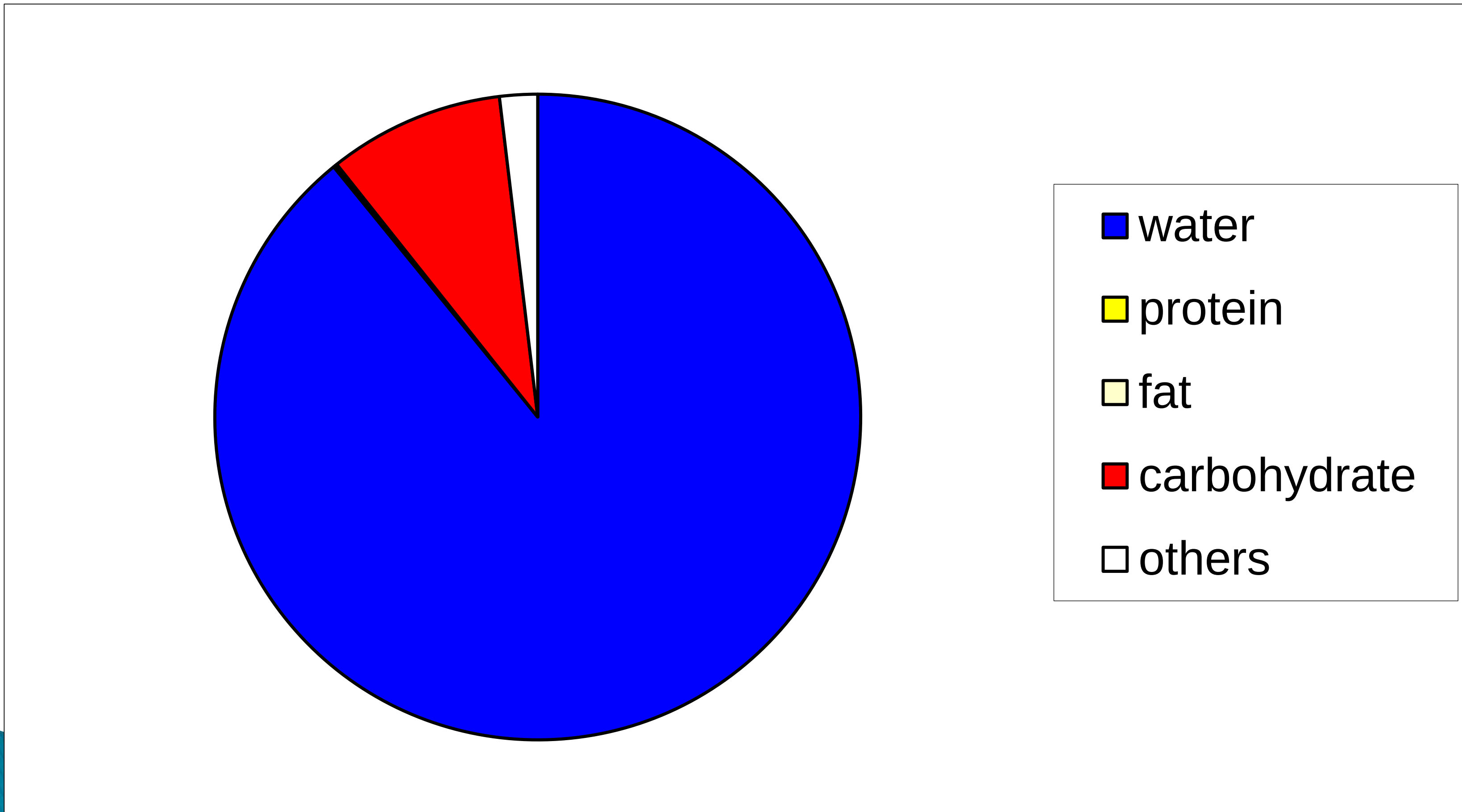
- In the ATR analysis, PC loading 2 is the most important for discriminating species
- This loading shares many features with a mixture spectrum of chlorogenic acid and caffeine
- Robusta coffees score higher with respect to this loading – consistent with the known difference between species

ATR spectra of pure compounds and a mixture spectrum, compared with PC Loading 2

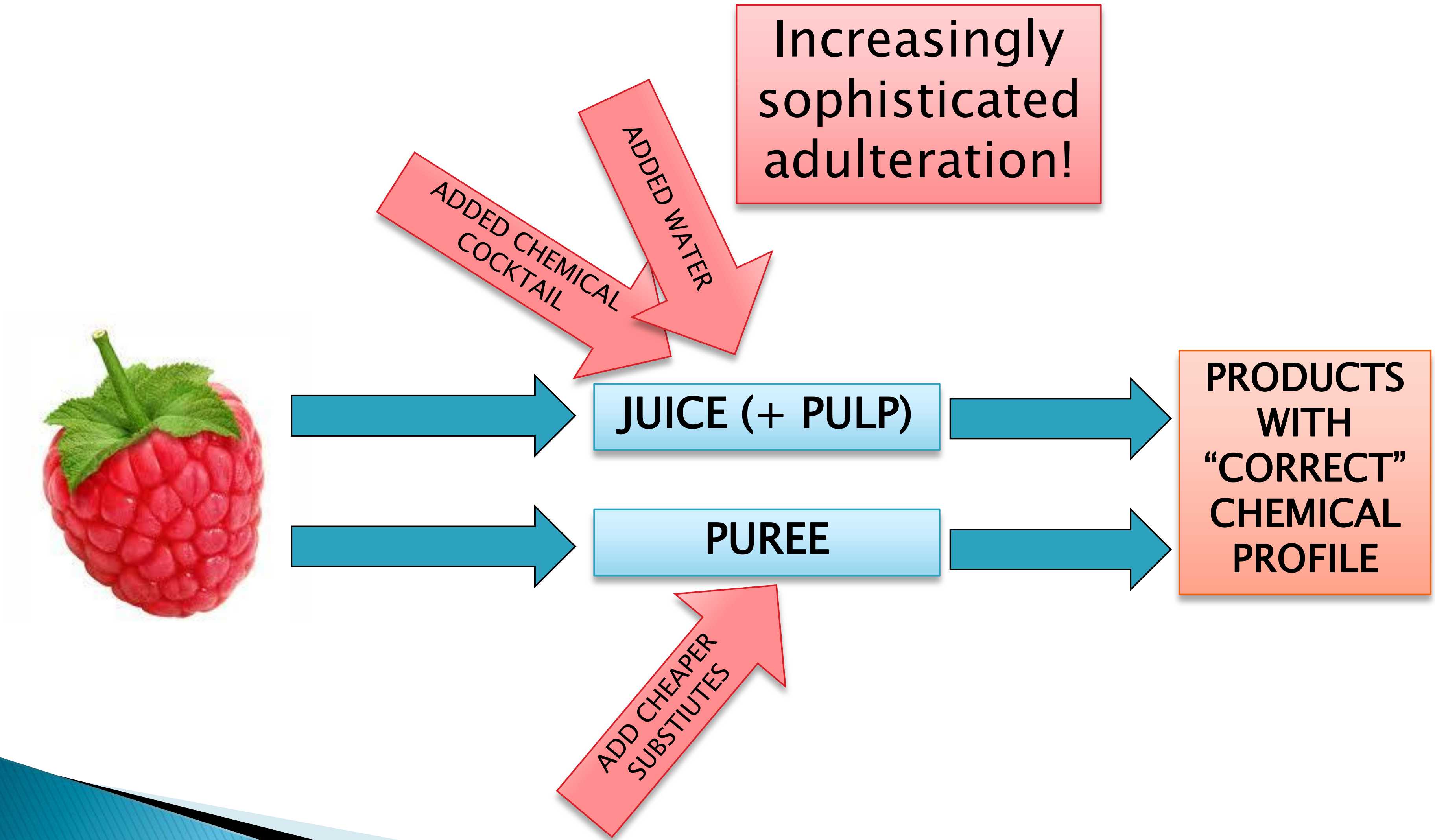


Case study 2: detecting adulteration in fruit purees

Composition of a typical fruit juice or puree:



Case study 2: detecting adulteration in fruit purees



Case study 2: detecting adulteration in fruit purees

Fruit purees are imported in large quantities into the UK for a wide range of processing needs (jams, jellies, yoghurts, etc)

Products arrive pureed, frozen or sulphited (preserved)

Main authenticity issues:

- Addition of chemicals e.g sugars, organic acids
- Addition of local ground water (very difficult to detect this!)
- **Addition of other (cheaper) fruits**
  - **High value soft fruits extended with other, cheaper local produce (rhubarb, apple,…)**

Case study 2: detecting adulteration in fruit purees

Aim: to develop a rapid MIR-based method for testing strawberry and raspberry purees for authenticity

details	training set samples	tuning set samples	test set samples
raspberry	1–55	1–53	1–51
blackberry	56–59	54–57	52–55
plum	60–64	58–62	56–59
strawberry	65–89	63–87	60–83
black currant	90–97	88–95	84–90
strawberry	98–135	96–132	91–127
apple	136–158	133–155	128–150
apricot	159	156	151
cherry	160		
mixtures of strawberry and apple	161–163	157–159	152–153
raspberries adulterated with 10, 30, 50, 70 90% w/w apple	164–171	160–167	154–161
mixtures of strawberry and apple	172–178	168–173	162–168
mixtures of strawberry and plum	179–181	174–177	169–171
strawberry	182–227	178–223	172–216
blackberry	228–232	224–228	217–220
black currant	233–236	229–231	221–223
cherry	237–244	232–238	224–230
plum	245–247	239–241	231–232
strawberry	248–257	242–250	233–241
apple	258	251	
apricot	259	252	
raspberries adulterated with 2, 4, 6, 8% w/w sucrose	260–299	253–292	242–280

Case study 2: detecting adulteration in fruit purees

- Spectral analysis of large number of independent, authentic fruits
- Compare new "unknowns" with the database of authentic spectra, to detect anomalies in profiles
- Around 900 samples analysed over three harvest years

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mixtures of strawberry and apple	172–178	168–173	162–168
mixtures of strawberry and plum	179–181	174–177	169–171
strawberry	182–227	178–223	172–216
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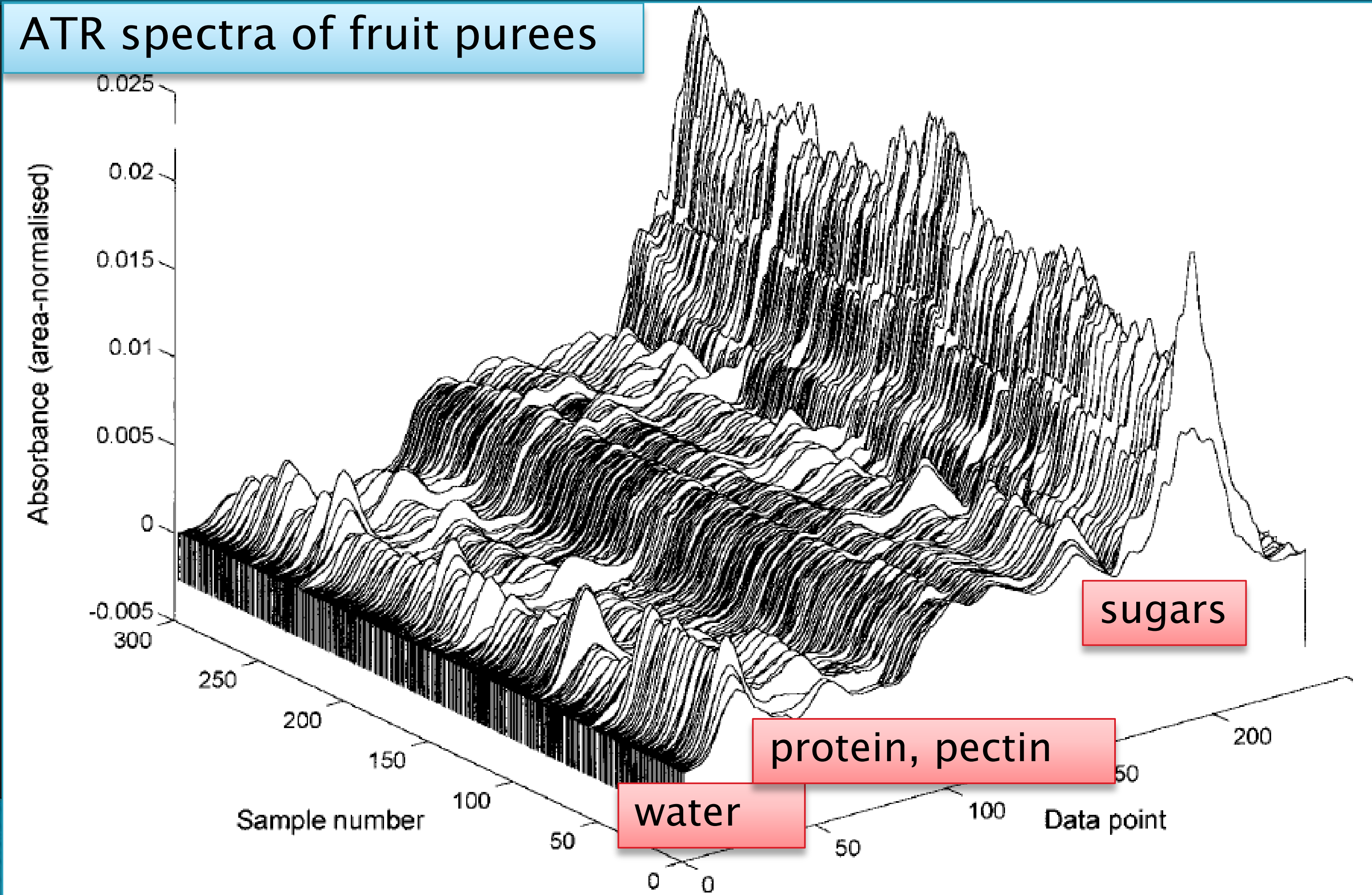
Case study 2: detecting adulteration in fruit purees

ATR spectra of fruit purees



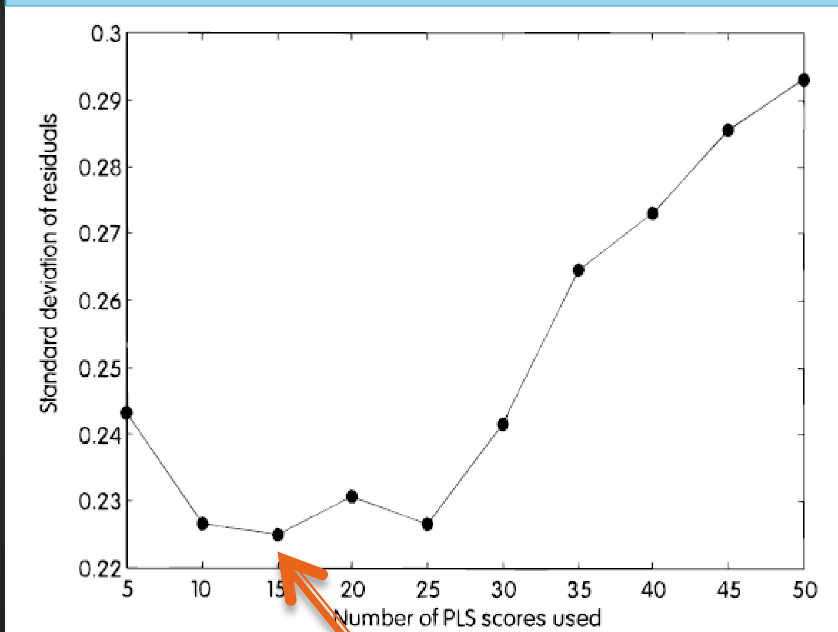
Case study 2: detecting adulteration in fruit purees

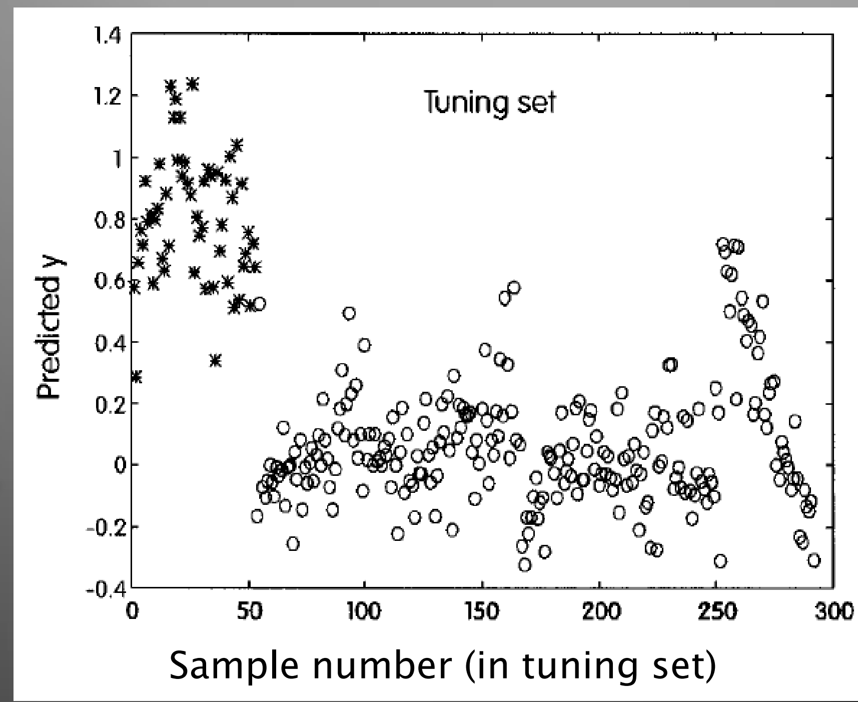
- Data analysis:

1. Divide data at random into training, tuning and test sets
2. Perform PLS on the training set – y is a "dummy" variable indicating raspberry (=1) or non-raspberry (=2)
3. Apply each regression model (different numbers of PLS scores) to the tuning set
4. Make decision on which is the optimum model
5. Finally, apply to test set data

Case study 2: detecting adulteration in fruit purees

Regression results from applying different models to tuning set



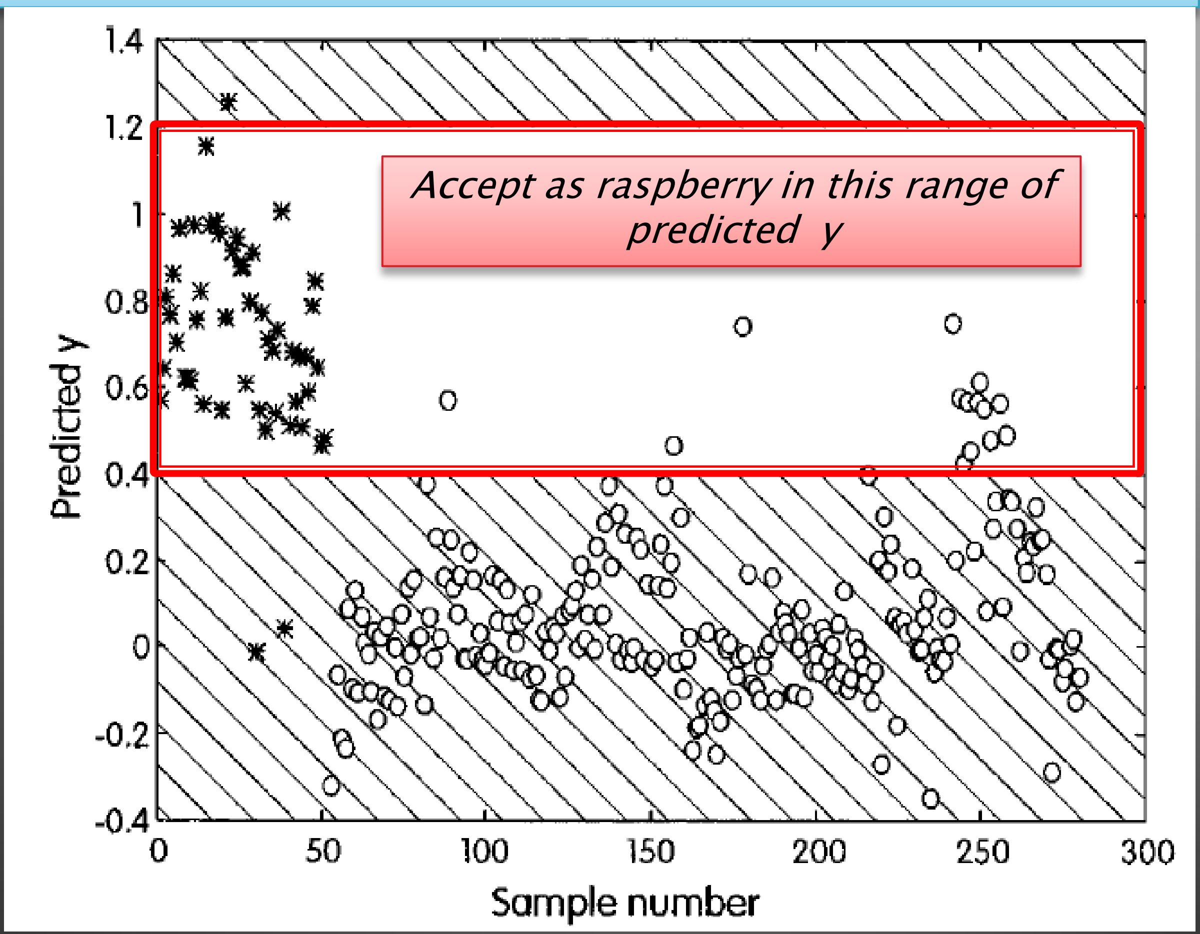


Optimal PLS model

Case study 2: detecting adulteration in fruit purees

Final test of the model: application to “unknowns” (test data

Detection limit: ~10% by weight of adulterant fruits present in a raspberry puree



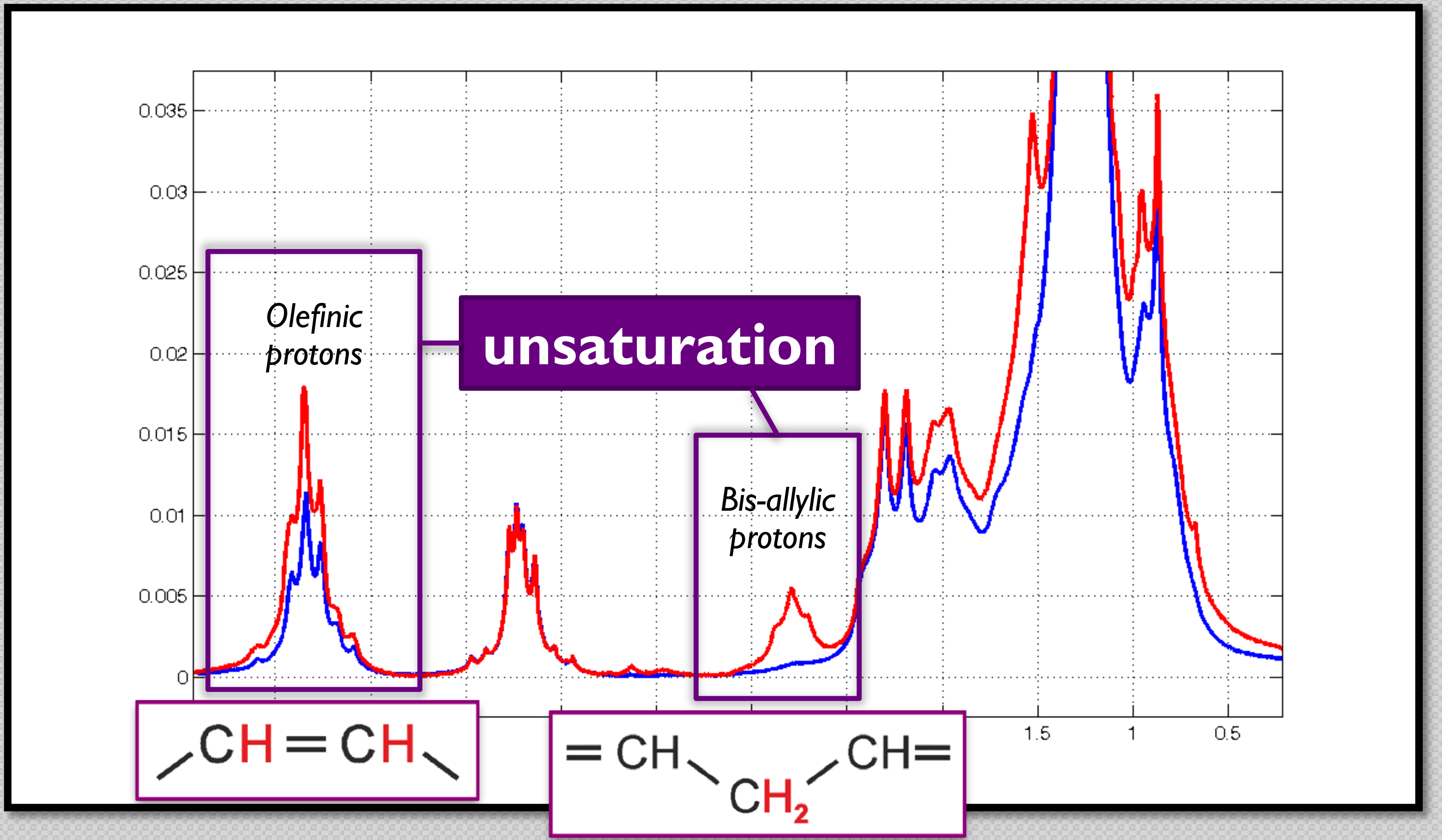
Case study 3: authenticating beef using low-field NMR

- Horsemeat scandal of 2013 was largest European incident of food fraud

- Food sector has scaled up its internal testing, but to date…
  - No regulatory changes
  - No increased funding for independent testing/policing

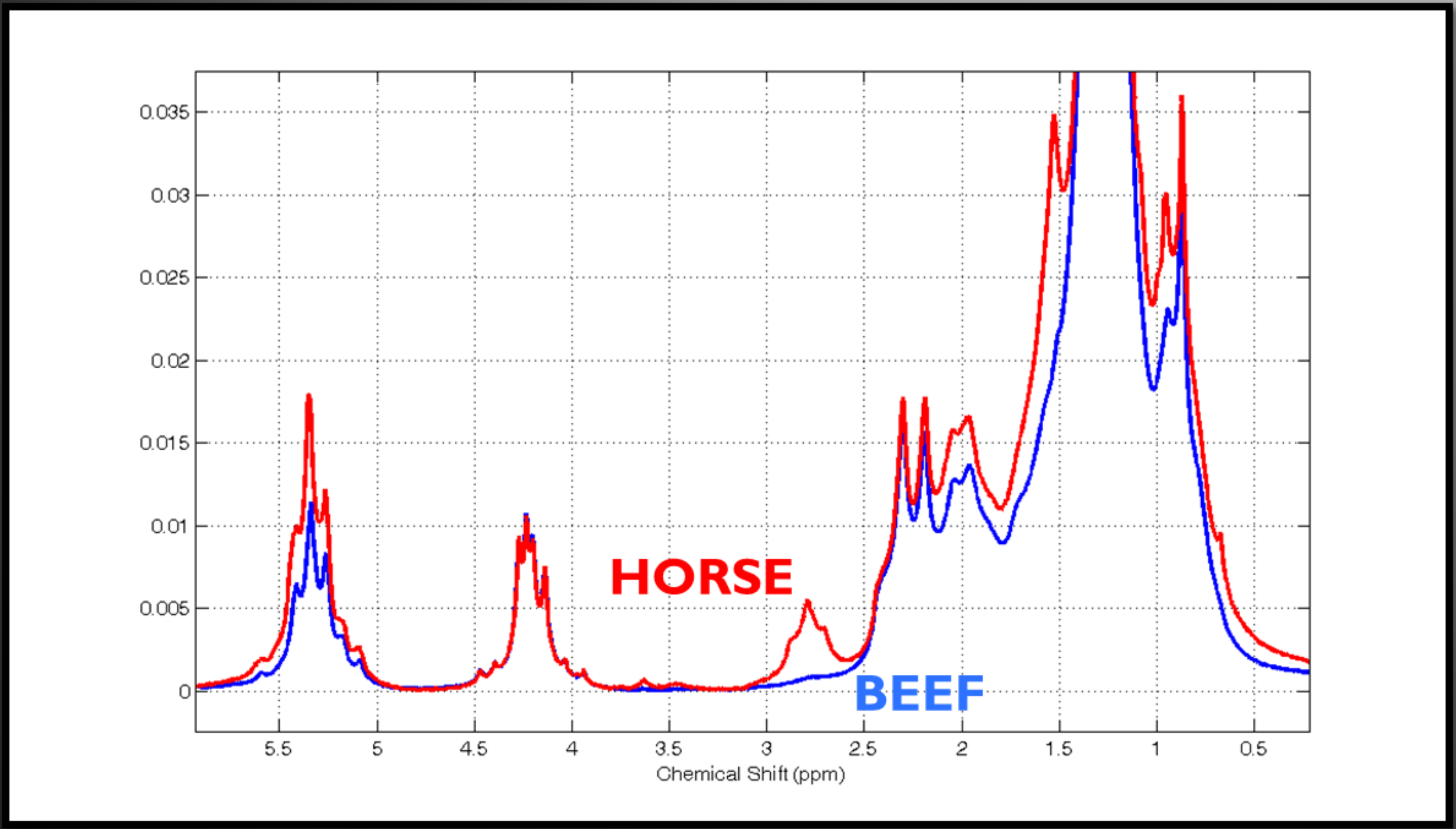
- Elliott review on food fraud has led to:
  - New Food Crime unit
  - New Network of Centres of Excellence in Authenticity

Case study 3: authenticating beef using low-field NMR



Different meats have different fatty acid "fingerprints"

Case study 3: authenticating beef using low-field NMR

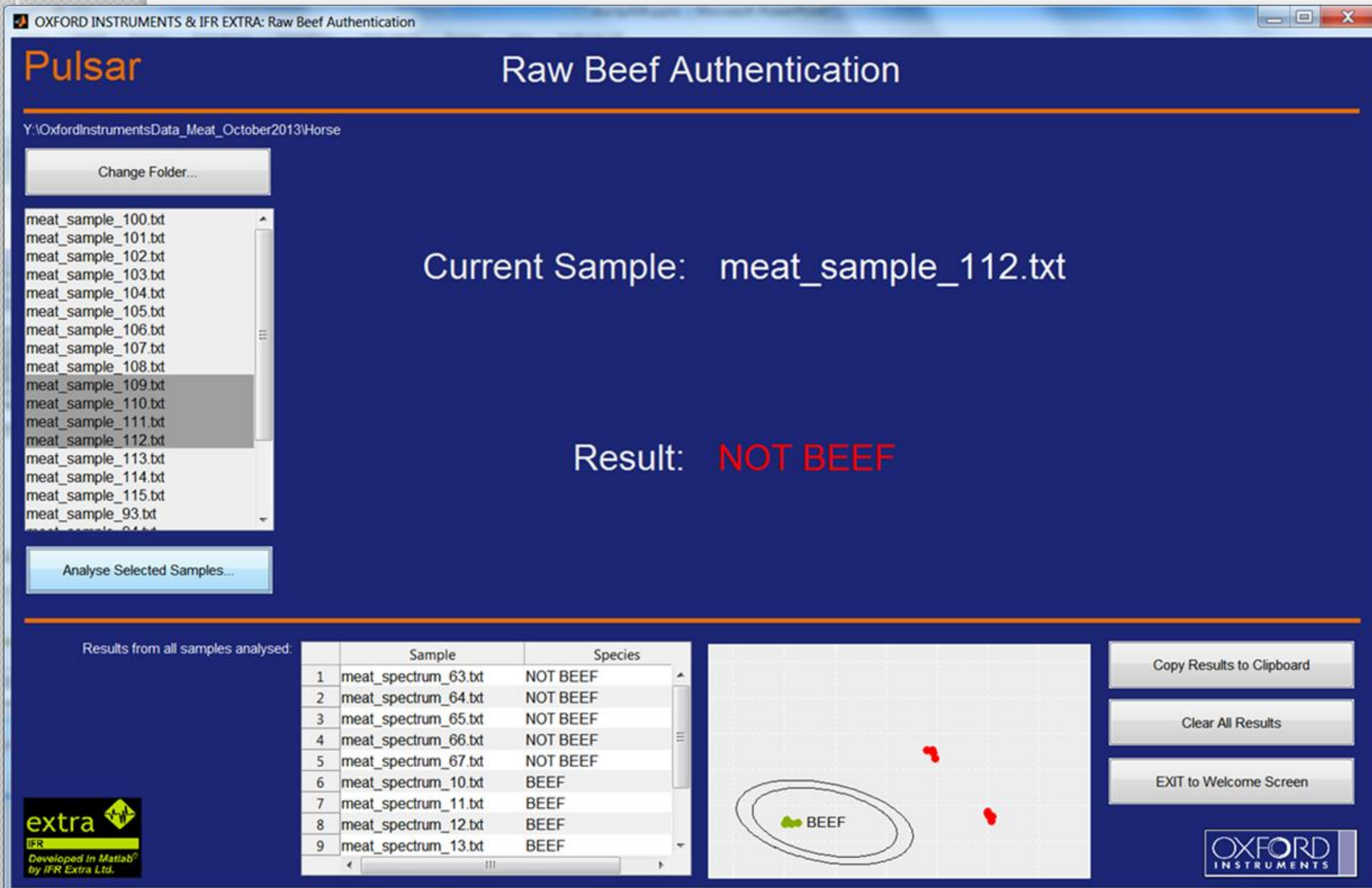


British Patent Application 1315962.9 (6 Sept 2013)

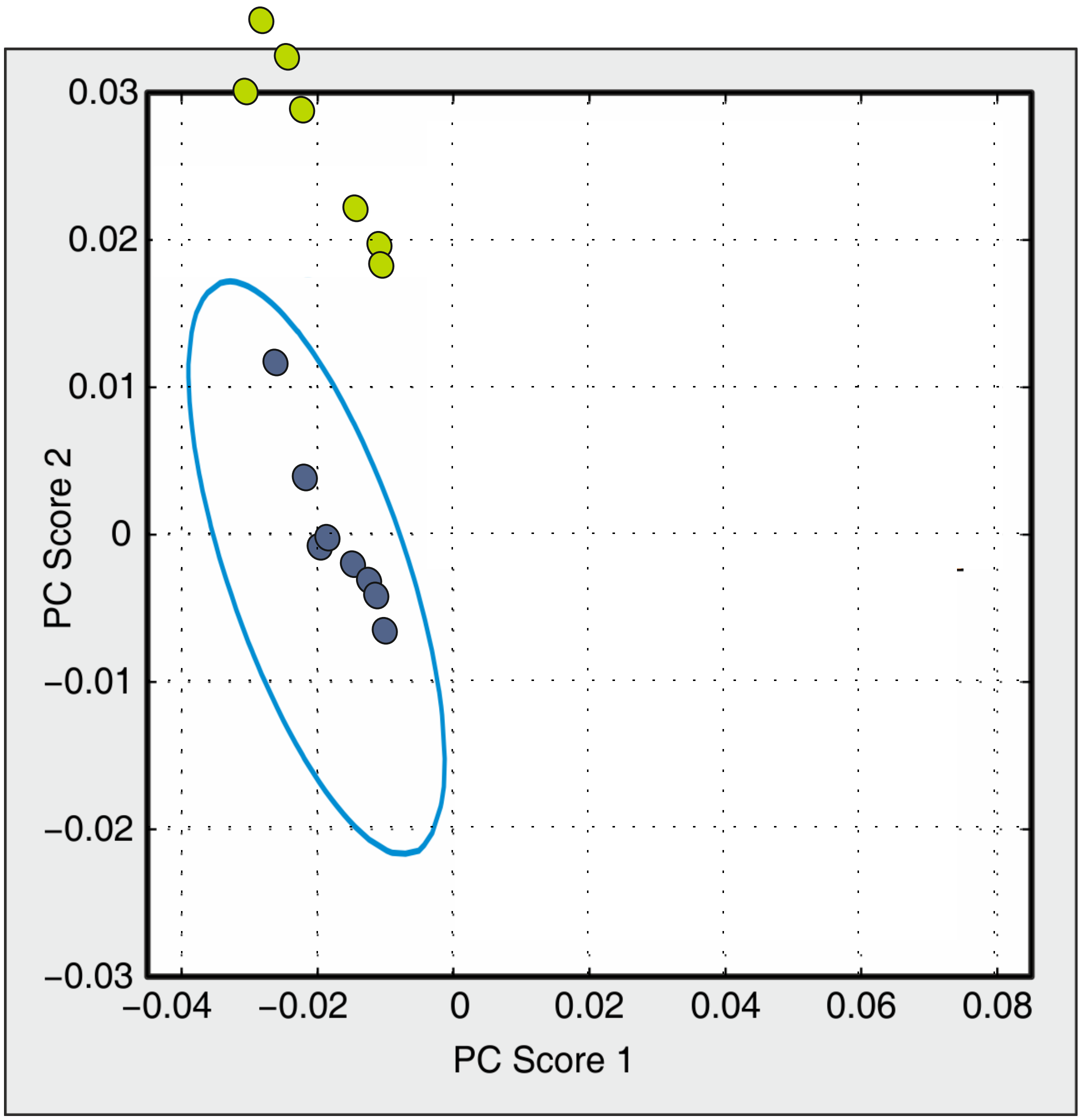
“NMR Sample Analysis” – protects the idea of using low-field NMR spectroscopy to speciate meat via its fatty acid fingerprint

Case study 3: authenticating beef using low-field NMR

Speciation of meats



Speciation of meats



First results from commercial installation:

Test PORK Samples

Test BEEF Samples

IFR Institute of Food Research

Key points

- Molecular spectroscopy techniques useful for analysis of a wide range of foodstuffs
- Not as sensitive as high-end techniques (MS, GC-MS, LC-MS, high-field NMR) – **but ideal for rapid screening needs**
- In combination with chemometrics, can provide quick methods for detecting adulteration at gross levels
- Instrumentation is relatively cheap and robust
  - can be placed in food production environments

Further information

- My team webpages
  - http://asu.ifr.ac.uk/
- On-line library of FTIR spectra of foods
  - http://www.ifr.ac.uk/ASU/FTIRLibrary.html
- Book (not science literature, but good read):
  - “Swindled: The Dark History of Food Fraud, from Poisoned Candy to Counterfeit Coffee”, by Bee Wilson (2008)
- This talk (and previous, on multivariate stats) will be uploaded to:
  - http://asu.ifr.ac.uk/resources/lecture-notes/