European Commission

MiNaSens

mIniaturized biochemical SEnsiNg devices & systemS for health/food/enviromental monitoring

MiNaSys CoE

National Centre of Scientific Research "Demokritos",
Athens, Greece
7 - 8 March 2013

imel NCSR Demokritos

IPC

Welcome

"MINASENS- MINiAturized biochemical SEnsiNg devices & systemS for health/food/environmental monitoring"

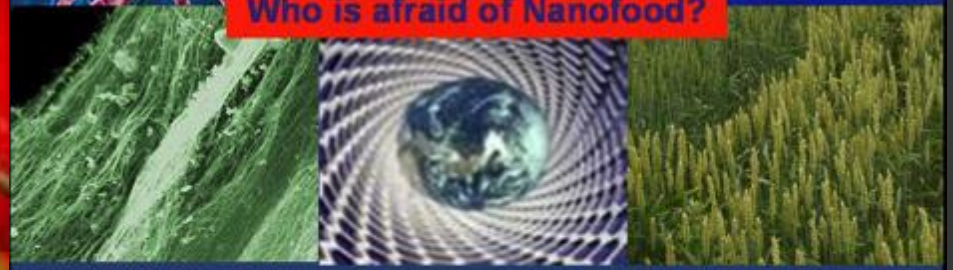
The Institute of Microelectronics of NCSR "Demokritos", (IMEL), now part of the Institute of Advanced Materials, Physicochemical Processes, Nanotechnology and Microsystems, organizes the "MINASENS- MINiAturized biochemical SEnsiNg devices & systemS for health/food/environmental monitoring" workshop. MINASENS is a part of the activities of the Micro & Nano Systems Centre of Excellence (MiNaSys-CoE) project, funded through the Regional Potential Programme of EU.

Nano food 2040

Nanotechnology in Food, Food Processing, Agriculture, Packaging and Consumption

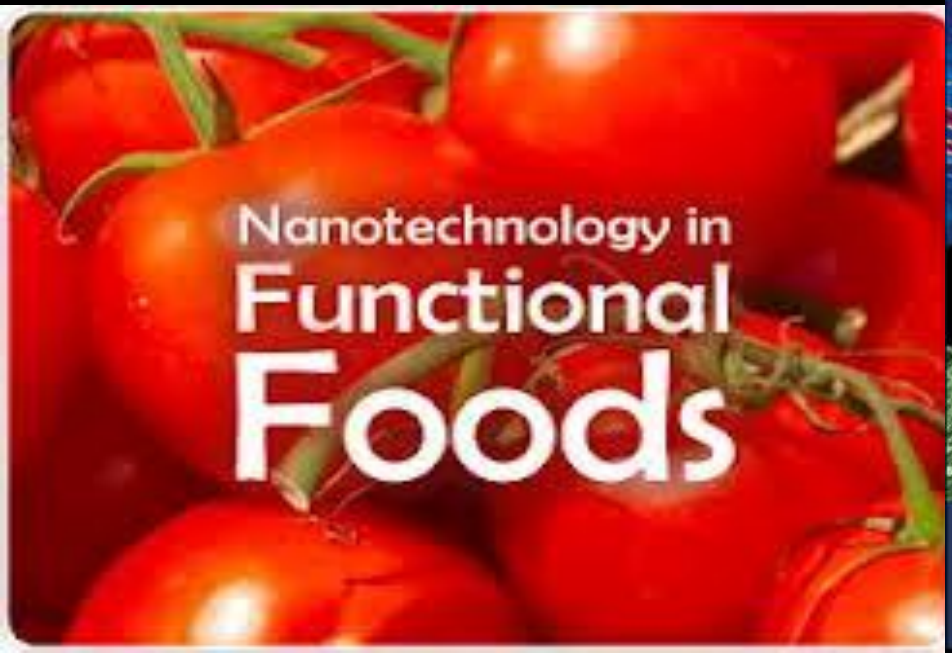
State of Science, Technologies, Markets, Applications and Developments to 2015 and 2040

Who is afraid of Nanofood?



Helmut Kaiser

HKC22



FOOD vs NANOTECHNOLOGY:
Can NANOTECH be a solution to current (economical) crisis ?

Application of Vibrational Spectroscopy and Multispectral Imaging in Food Spoilage

E. Z. Panagou, & G.-J. E. Nychas

Lab of Microbiology & Biotechnology of Foods, Dept of Food Science & Technology, Iera Odos 75, Athens 11855, Greece,Tel +30-210-5294938,
e-mail; gjn@aua.gr

INTRODUCTION

- What is Quantitative Food Microbiology?
- How (microbes) spoilage can be measured?
- What is Food Spoilage?

METHODS/RESULTS/TOOLS

- Case Study; Beef
- FTIR/RAMAN/IMAGING ANALYSIS

GENERAL CONCLUSIONS

INTRODUCTION; Quantitative Food Microbiology (Where?)

Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs; 3rd article

As necessary, the food business operators responsible for the manufacture of the product shall conduct studies (i, ii) to investigate compliance with the criteria throughout the shelf-life e.g. to ready-to-eat foods that are able to support the growth of Listeria monocytogenes and that may pose a risk for public health.

INTRODUCTION; Quantitative Food Microbiology (Where?)

Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs; 3rd article

STUDIES (for)

i- specifications for physico-chemical characteristics of the product, such as pH, aw, salt content, concentration of preservatives and the type of packaging system,

ii- predictive mathematical modelling established for the food in question, using critical growth or survival factors for the micro-organisms of concern in the product,

INTRODUCTION: How Spoilage(microbes) Can Be Measured?

Conventional microbiology

Method	Pre-treatment	Time required for the results
Microbial population counts	Sampling	Depended on tested microbiota e.g.Total viable counts 48h, enterobacteria 18-24h, yeast and molds 72-120h
Direct microscopy count of organism		<1 hour
Optical Density (OD)		<1 hour

Enumeration

INTRODUCTION: How bacteria can be measured?

Molecular tools

Molecular

Technique	Pre-treatment	Time required for the results
Species -specific PCR	Isolation, culture, DNA extraction	1 day
PCR-DGGE	DNA extraction	2 days
T-RFLP	DNA extraction	1 day
Real-Time PCR	RNA/DNA extraction	1 day
SDS-PAGE	Protein extraction	1-3 days
RAPD	Isolation, culture, DNA extraction	1 day
rep-PCR	Isolation, culture, DNA extraction	1-2 days
RFLP	Isolation, culture, DNA extraction	1 day
PFGE	Isolation, culture	4-7 days
2-DE	Protein extraction	3-4 days
Microarrays	DNA/RNA extraction	2-3 days
FISH		1-3 days

INTRODUCTION; How (bacteria) spoilage can be measured?

Conventional vs Molecular tools in measuring microorganisms in food microbiology

The use of microbiological methods either conventional or advance molecular methods in the Food Industry is under consideration

Both are time consuming, (results from 18 H to 72H)

Retrospective results, Few samples, Limited reproducibility,

measurements requires sophisticated procedures,

highly educated personnel, time and equipment

Detection limits (e.g. insufficient for the reliable detection of low levels of organisms)

INTRODUCTION: (What is) Food Spoilage

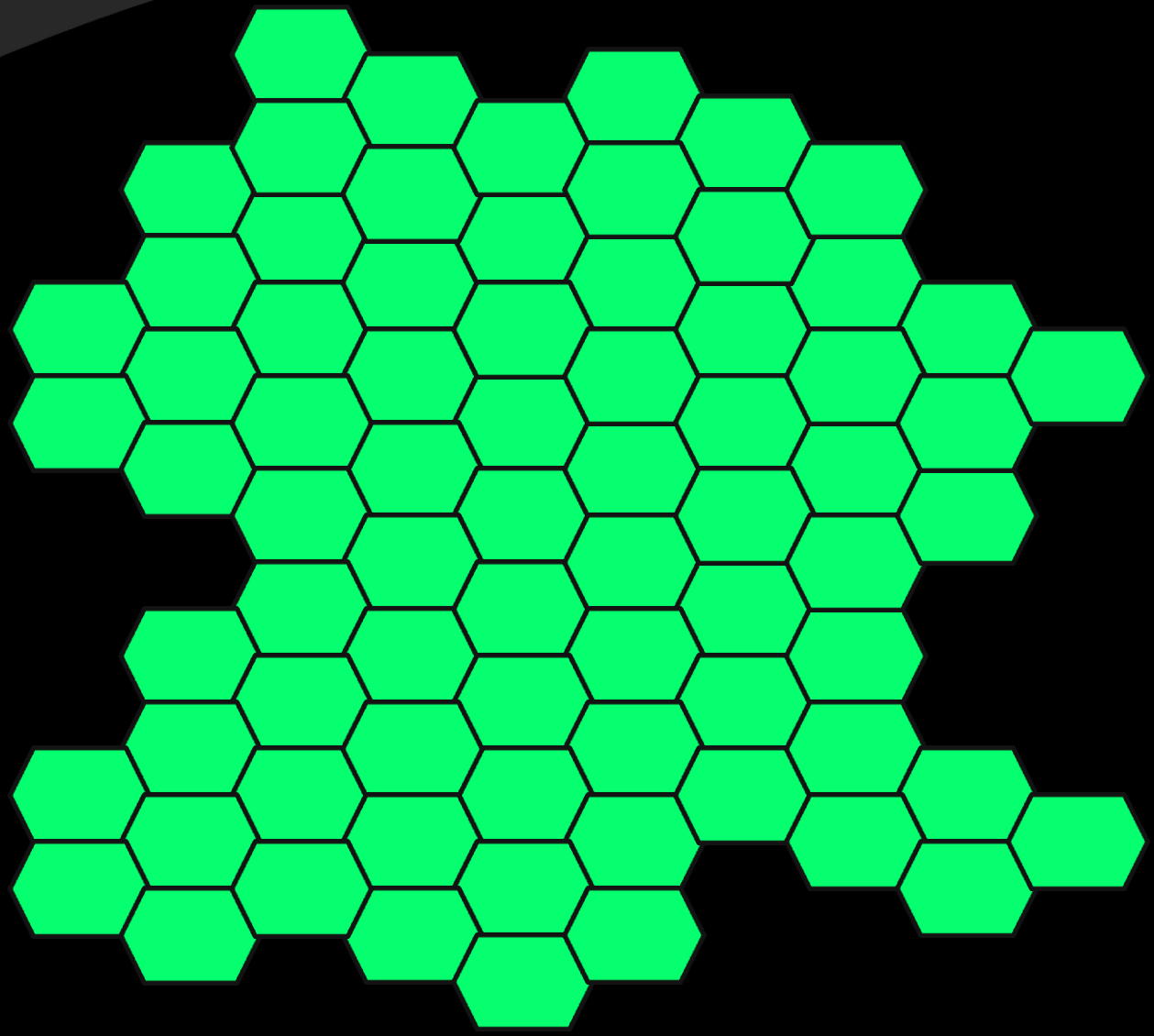
- Spoilage of food can be considered as an ecological phenomenon that encompasses changes in the available components (e.g., low molecular weight compounds) during proliferation of bacteria present in the microbial association of product.

- The correlation between microbial growth and chemical changes during spoilage has been continuously recognised as a means of revealing indicators that may be useful for quantifying the degree of spoilage.

Ellis & Goodacre (2001) Trends in Food Science & Technology 12, 414

Nychas, G.-J.E., et al. (2008) *Meat Science,* 78: 77-89.

INTRODUCTION; Food Spoilage



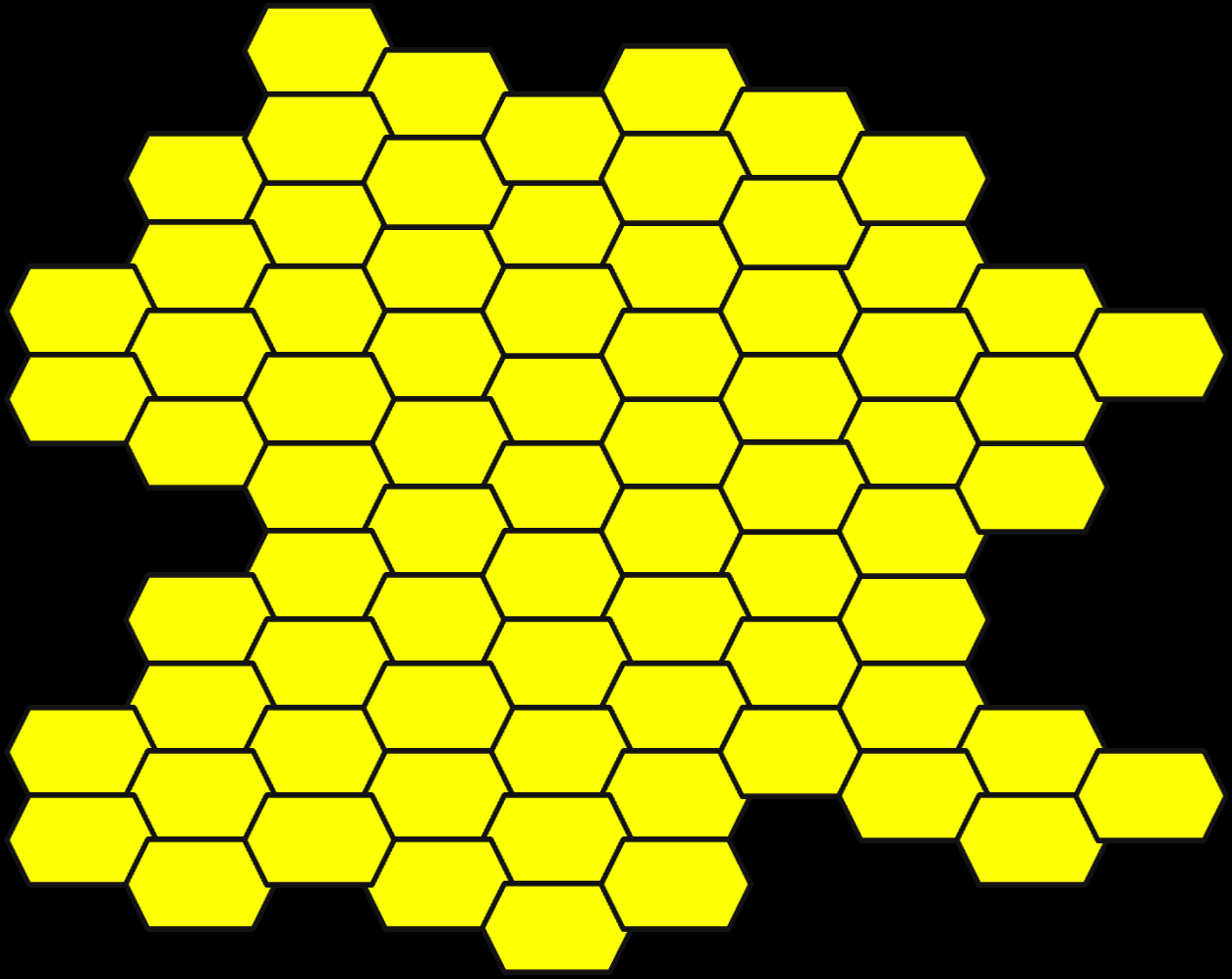
Food Spoilage

Fresh food

INTRODUCTION: Food Spoilage

Food Spoilage

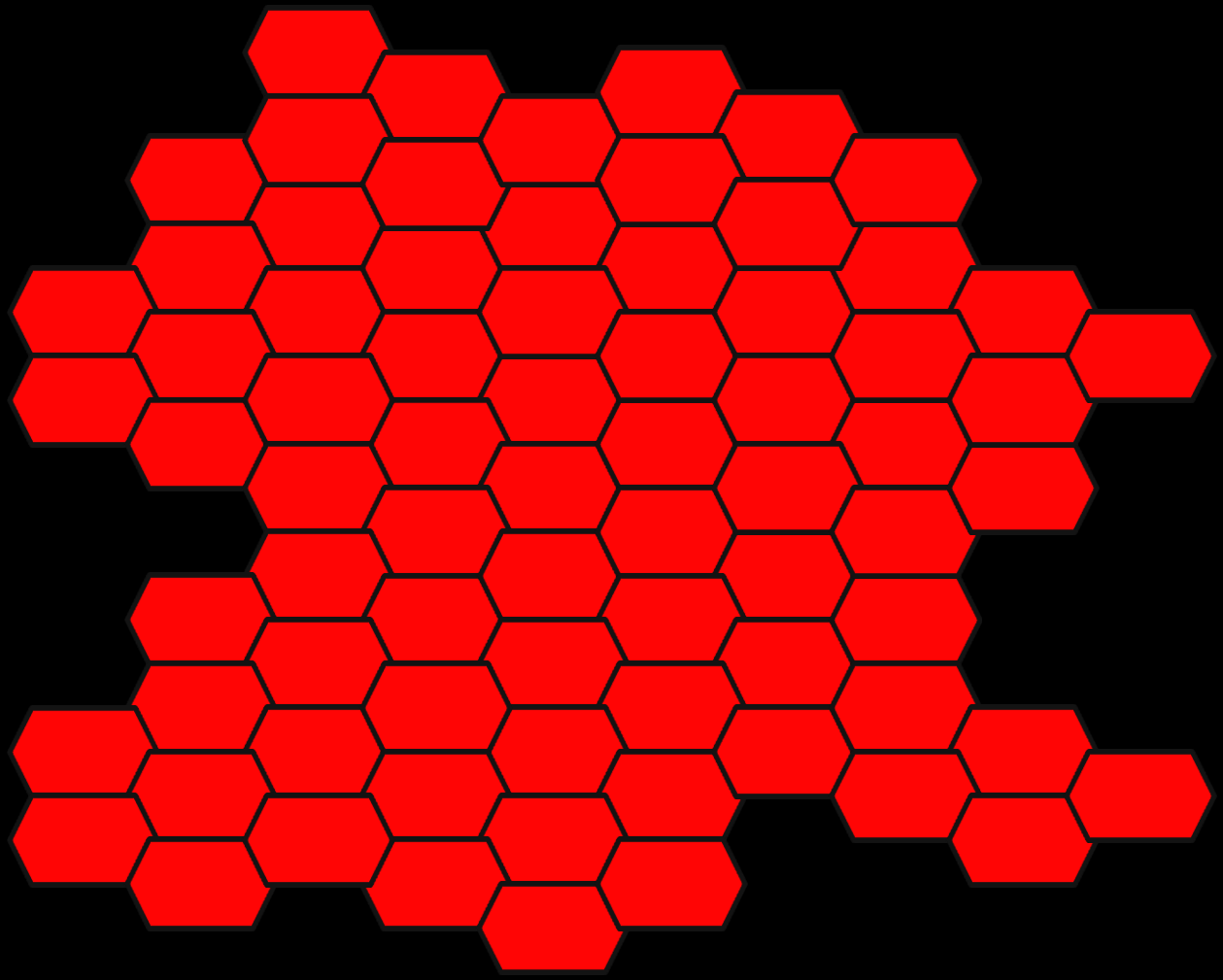
Soon will spoil



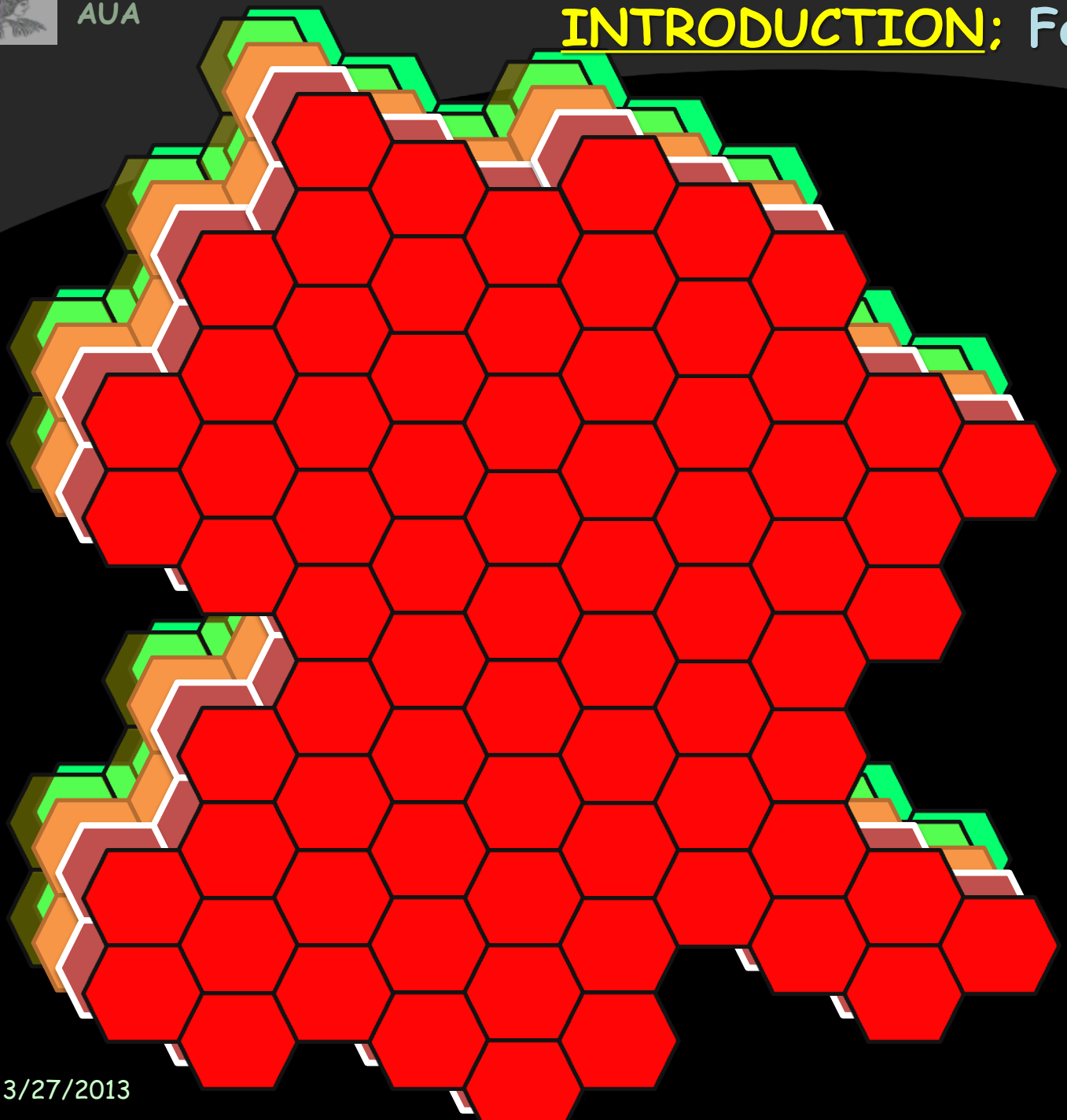
INTRODUCTION: Food Spoilage

Food Spoilage

spoiled food



INTRODUCTION; Food Spoilage



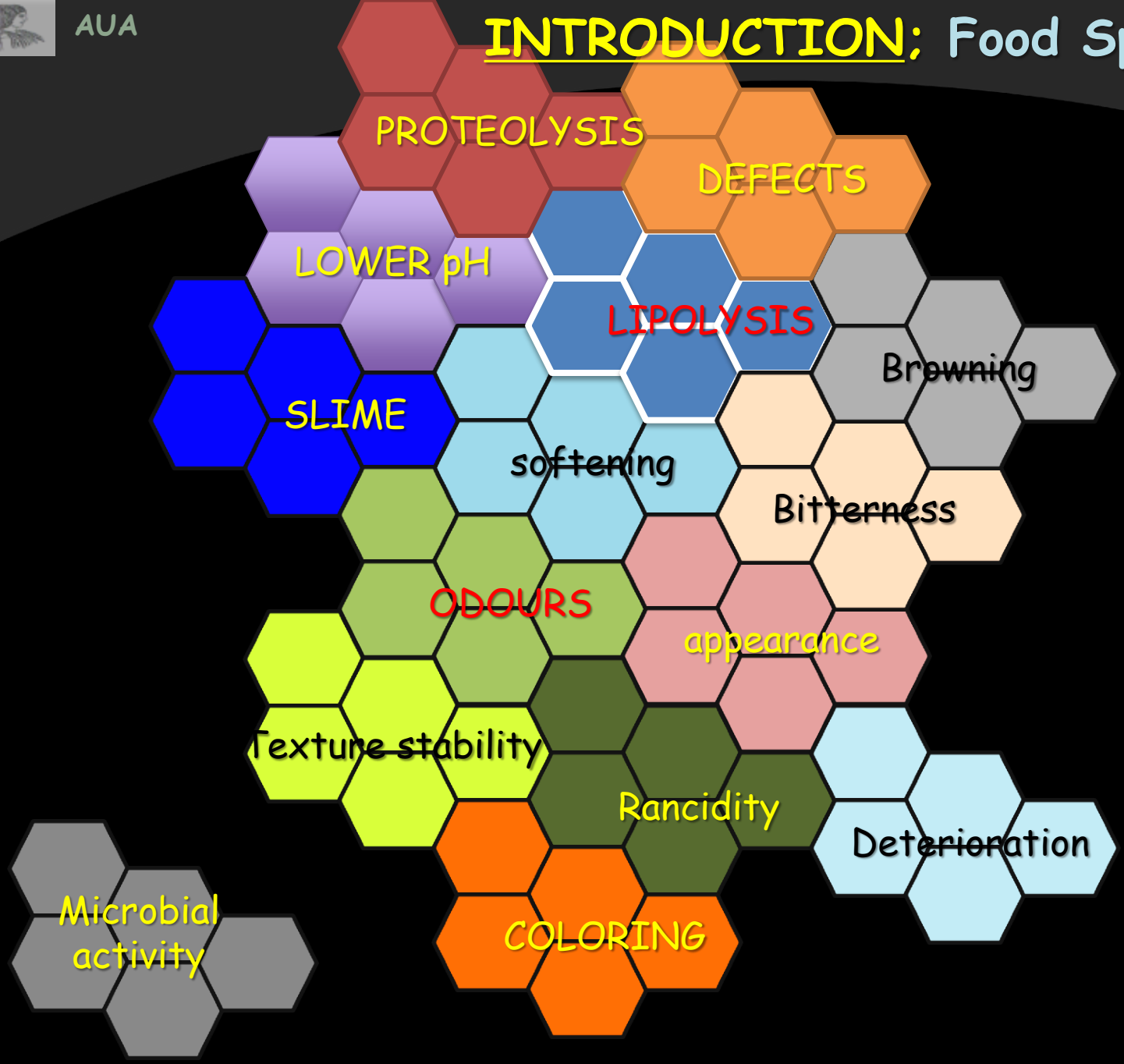
Food Spoilage

Fresh

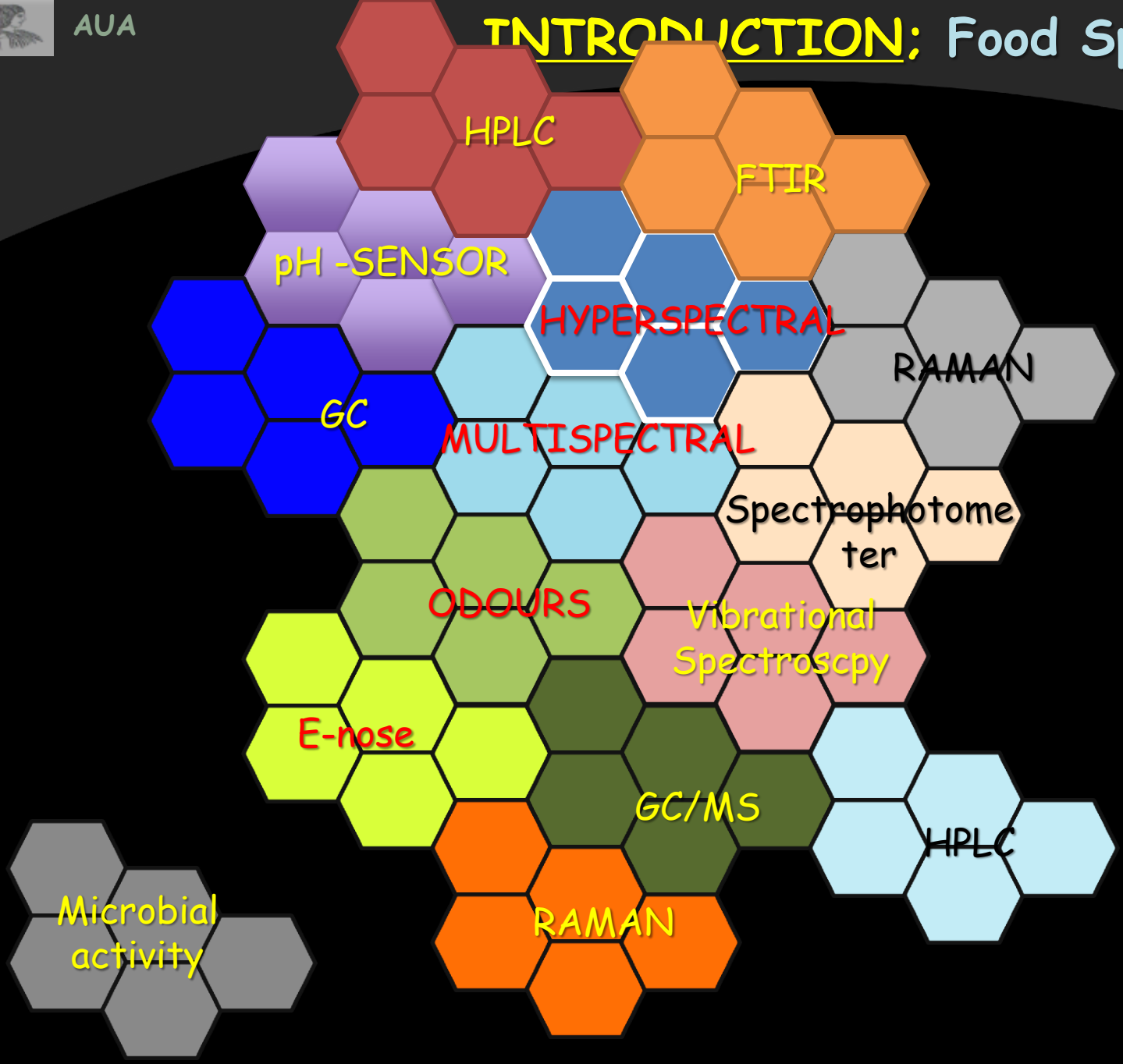
Spoilage in progress

Spoiled

INTRODUCTION: Food Spoilage



INTRODUCTION: Food Spoilage



Food Spoilage

LIPOLYSIS

PROTEOLYSIS

DEFECTS

Browning

Bitterness

LOWER pH

SLIME

Deterioration

rancidity

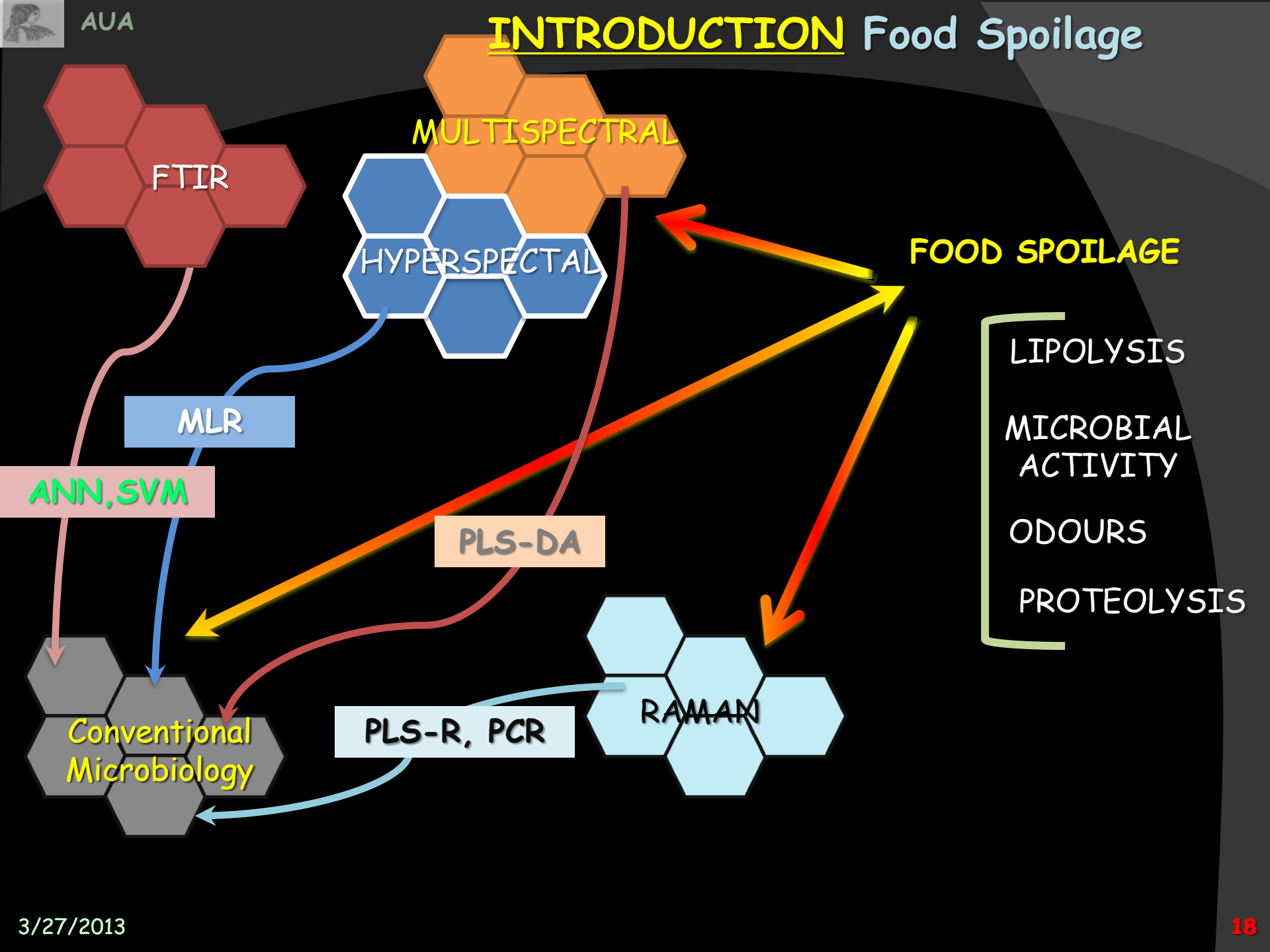
COLORING

Off-Odours

softening

appearance

INTRODUCTION Food Spoilage



Meat quality assessment – Current situation

- Today, more than 50 methods have been used for the detection of microbiologically spoiled or contaminated meat, including chemical and instrumental analyses, sensory evaluation, and molecular screening techniques (Jay, 2000).

- However, these methods are invasive, time consuming, labor intensive, demand highly trained personnel, and they are thus unattractive for routine analysis (Nychas *et al.*, 2008).

Jay, J.M. (2000) Modern Food Microbiology, 6th edition, Aspen Publishers Inc., Maryland, USA.

Nychas, G.-J.E., Skandamis, P.N., Tassou, C.C., Koutsoumanis, K.P. (2008) Meat spoilage during distribution. *Meat Science,* 78: 77-89.

Meat quality assessment – Alternative approach

- Recently the concept based on qualitative and/or quantitative determination of multiple metabolites which represent a fingerprint (metabolic snapshot), providing information about the type and rate of spoilage is developed (Nychas et a. 1988; Ellis and Goodacre, 2001).

- To this end, alternative analytical approaches have been forwarded for the rapid and quantitative monitoring of meat spoilage, based on biosensors, e-noses, vibrational spectroscopy methods (FT-IR, Raman)[Nychas et al. 2008].

.

Ellis, D.I. and Goodacre, R. (2001) Rapid and quantitative detection of the microbial spoilage of muscle foods: *Current status and future trends. Trends in Food Science and Technology,* 12: 414-424.

Nychas, G.-J.E., Drosinos, E.H., Board, R.G. (1988) Chemical changes in stored meat. In R.G. Board & A.R. Davies (Eds.), The Microbiology of Meat and Poultry, Blackie Academic and Professional, London, UK.

Nychas, G.-J.E., Skandamis, P.N., Tassou, C.C., Koutsoumanis, K.P. (2008) Meat spoilage during distribution. *Meat Science,* 78: 77-89.

Materials and methods

Meat samples: Fresh deboned beef fillets (80 mm x 10 mm x 5 mm).

Packaging: Aerobic conditions.

Storage temperatures: 0, 5, 10, 15 and 20°C in high precision (± 0.5 ° C) incubation chambers for an overall period of 350 hours.

Microbiological analyses: Total viable counts (PCA – incubation 30° C/48h)

Organoleptic assessment: Spoilage detection based on changes in colour, odour and taste based on a five member taste panel.

Scoring scale: range 1-3; 1=Fresh, 1.5 Semi-Fresh, 2-3 Spoiled.

Sampling frequency: 24h at 0 and 5° C; 8, 6, 4 h at 10, 15, and 20° C.

FT-IR analysis: Spectra collected in parallel to monitor biochemical changes in meat during storage.

Objectives of the work

- Investigate the meat Spoilage stored under different storage conditions (i.e. Packaging and Temperature)

(a) Imaging analysis (MULTISPECTRAL)

(b) using the vibrational spectroscopy (FTIR) and

as a quick analytical method for monitoring

Hyper/Multispectral

Imaging analysis

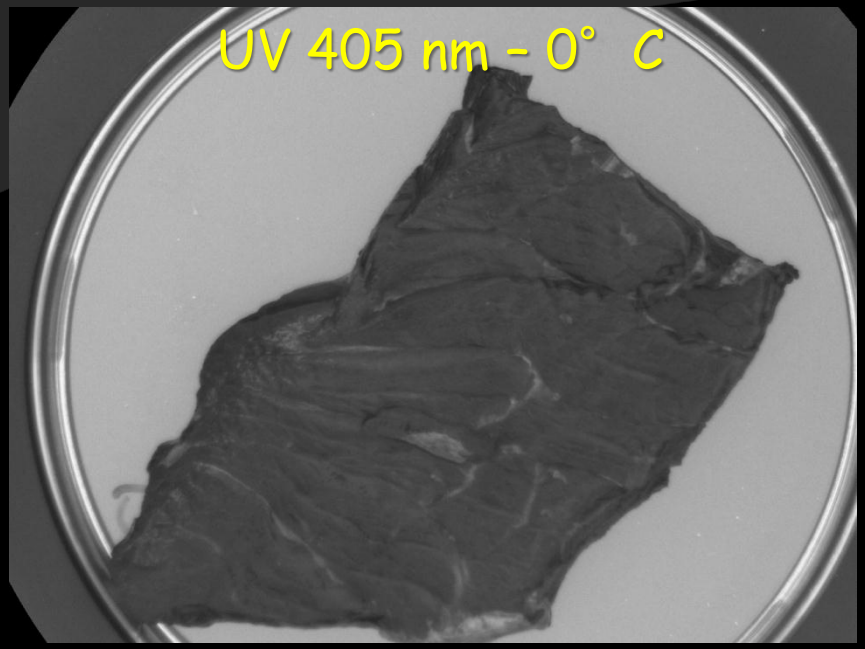
Surface Chemistry

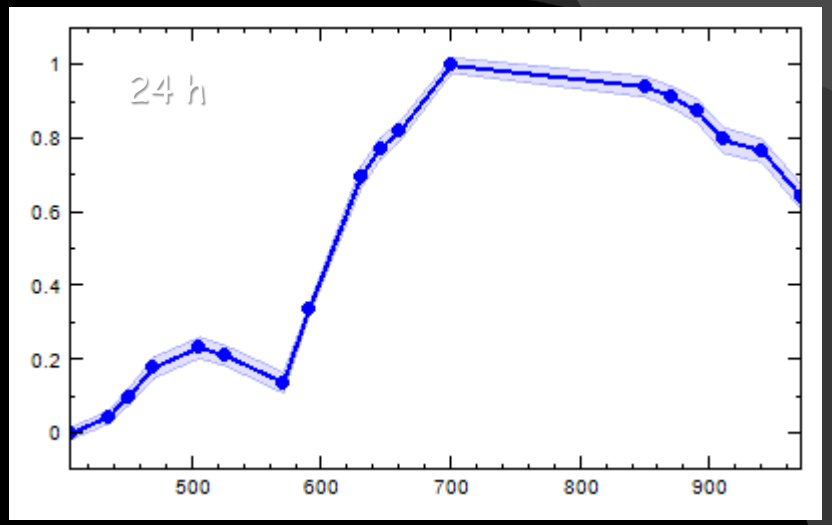
VideometerLab

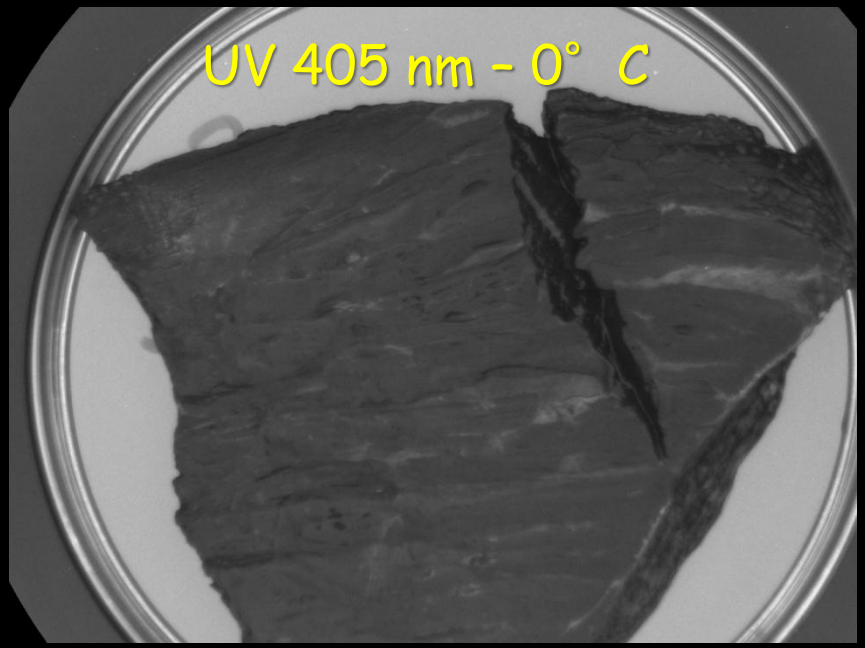
Ζώνη	Μήκος κύματος (nm)	Χρώμα
1	405	Υπεριώδες ακτινοβολία
2	435	Υπεριώδες ακτινοβολία τύπου B
3	450	Μπλε
4	470	Μπλε
5	505	Μπλε - Πράσινο
6	525	Πράσινο
7	570	Κίτρινο
8	590	Πορτοκαλί
9	630	Κόκκινο
10	645	Κόκκινο
11	660	Κόκκινο
12	700	Κόκκινο
13	850	Εγγύς Υπέρυθρο
14	870	Εγγύς Υπέρυθρο
15	890	Εγγύς Υπέρυθρο
16	910	Εγγύς Υπέρυθρο
17	940	Εγγύς Υπέρυθρο
18	970	Εγγύς Υπέρυθρο

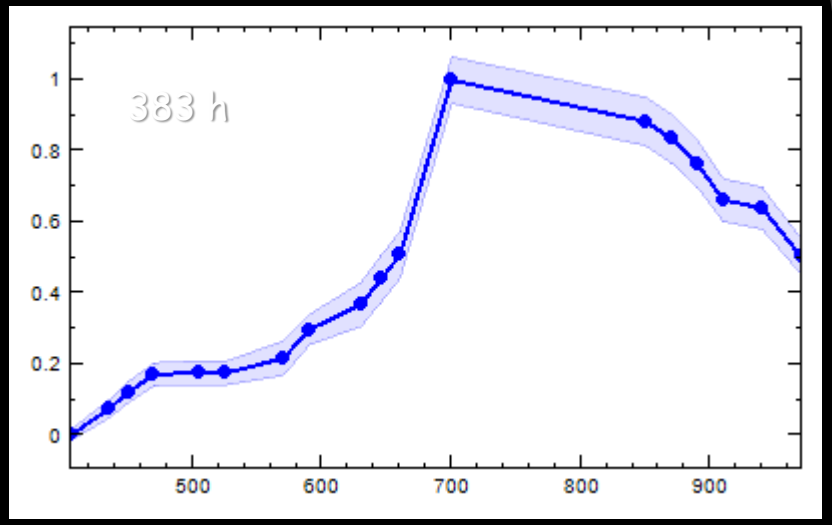
AUA

Beef fillets – aerobic packaging



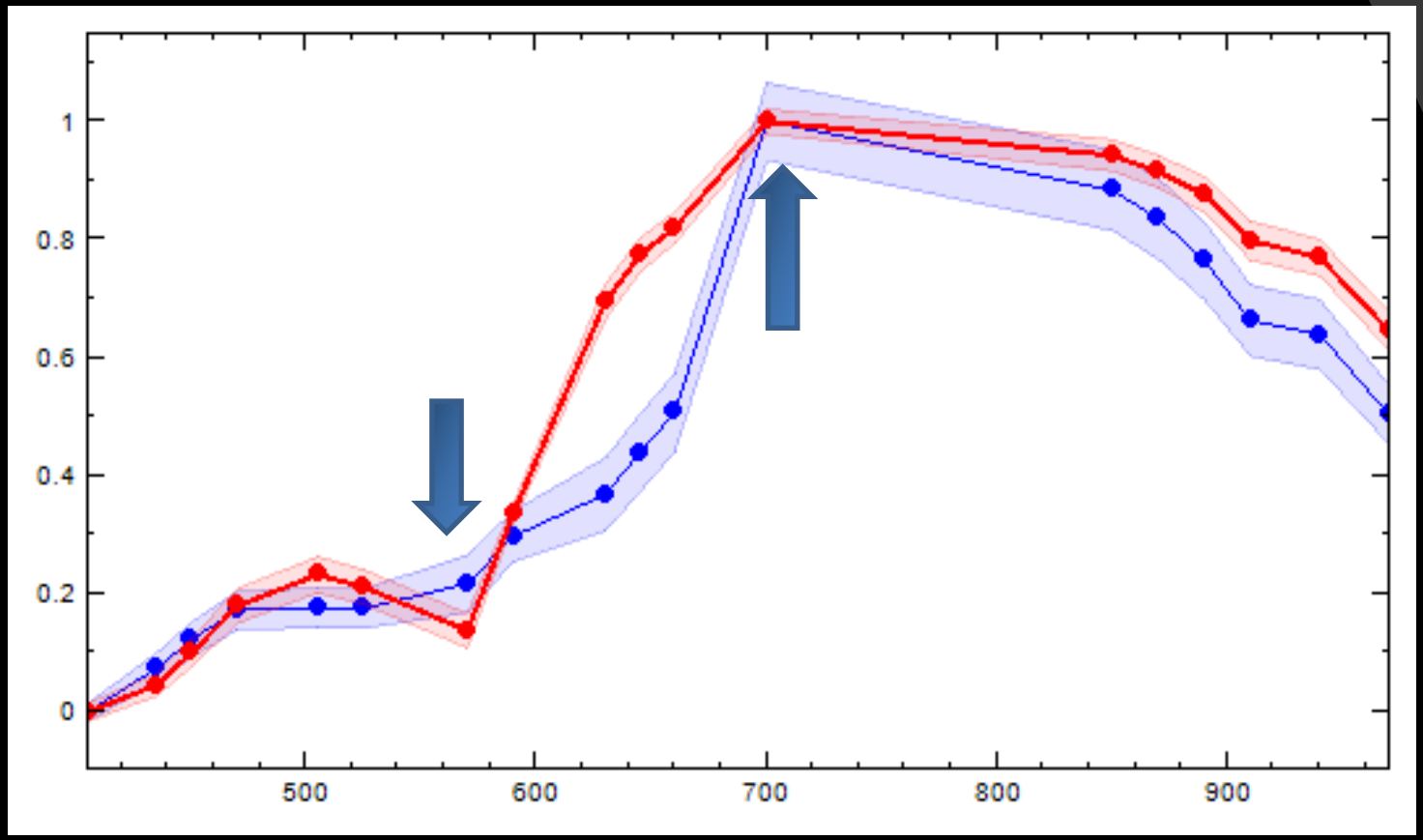






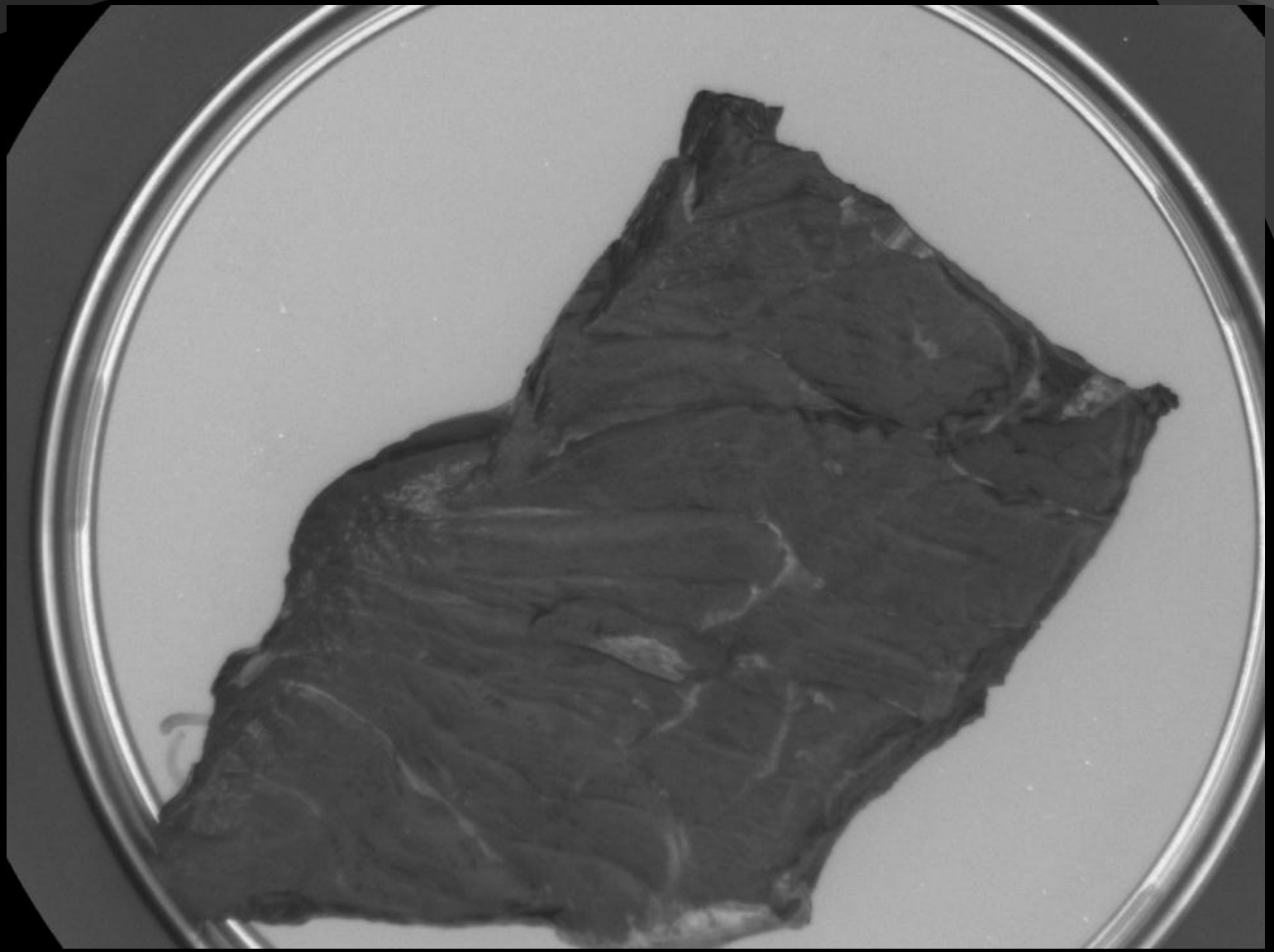
AUA

Beef fillets – aerobic packaging



590 nm (orange) – 700 nm (red)

Image processing pipeline – raw image



AUA

Image processing pipeline – raw image

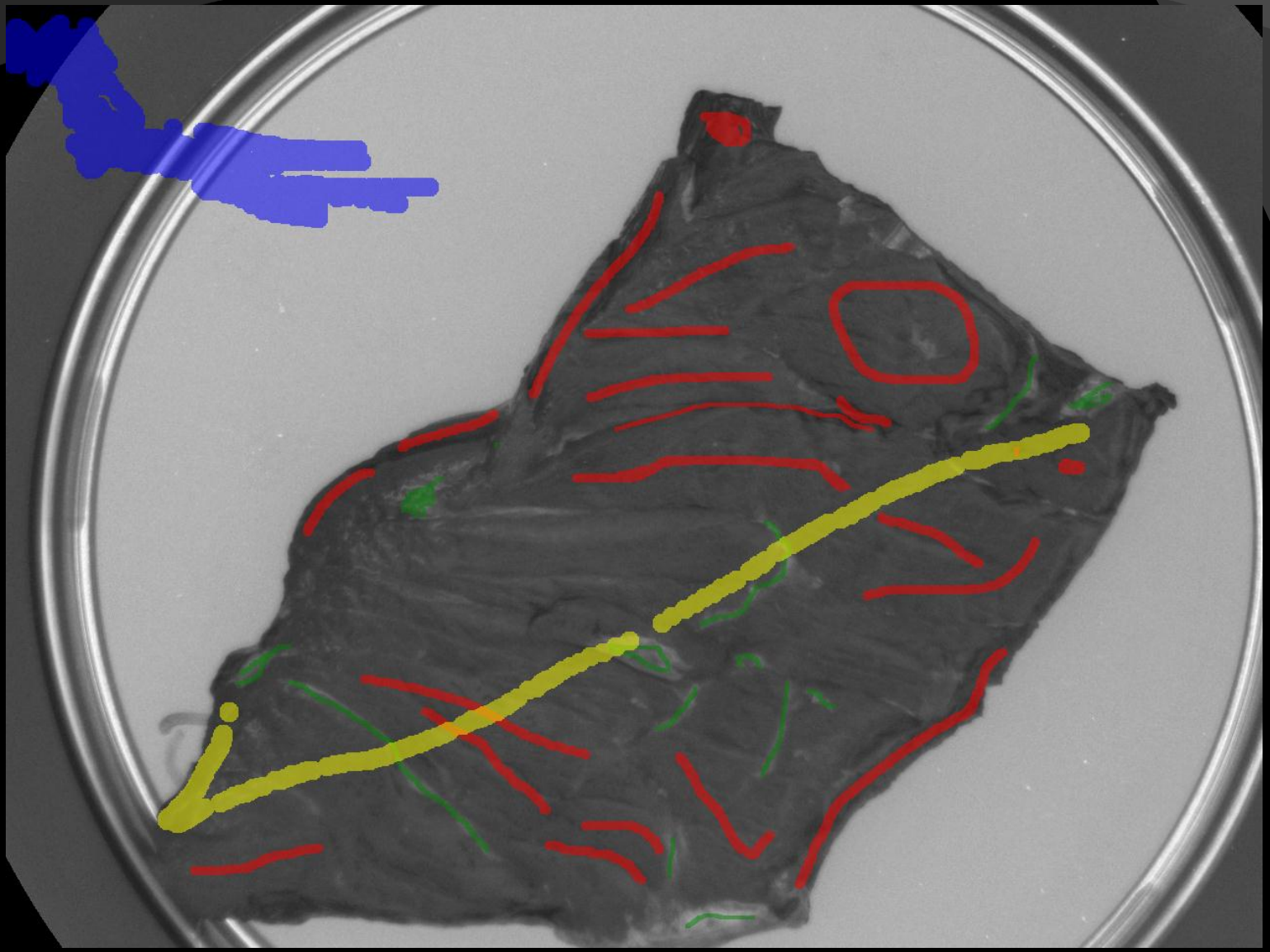


Image processing pipeline – transformation

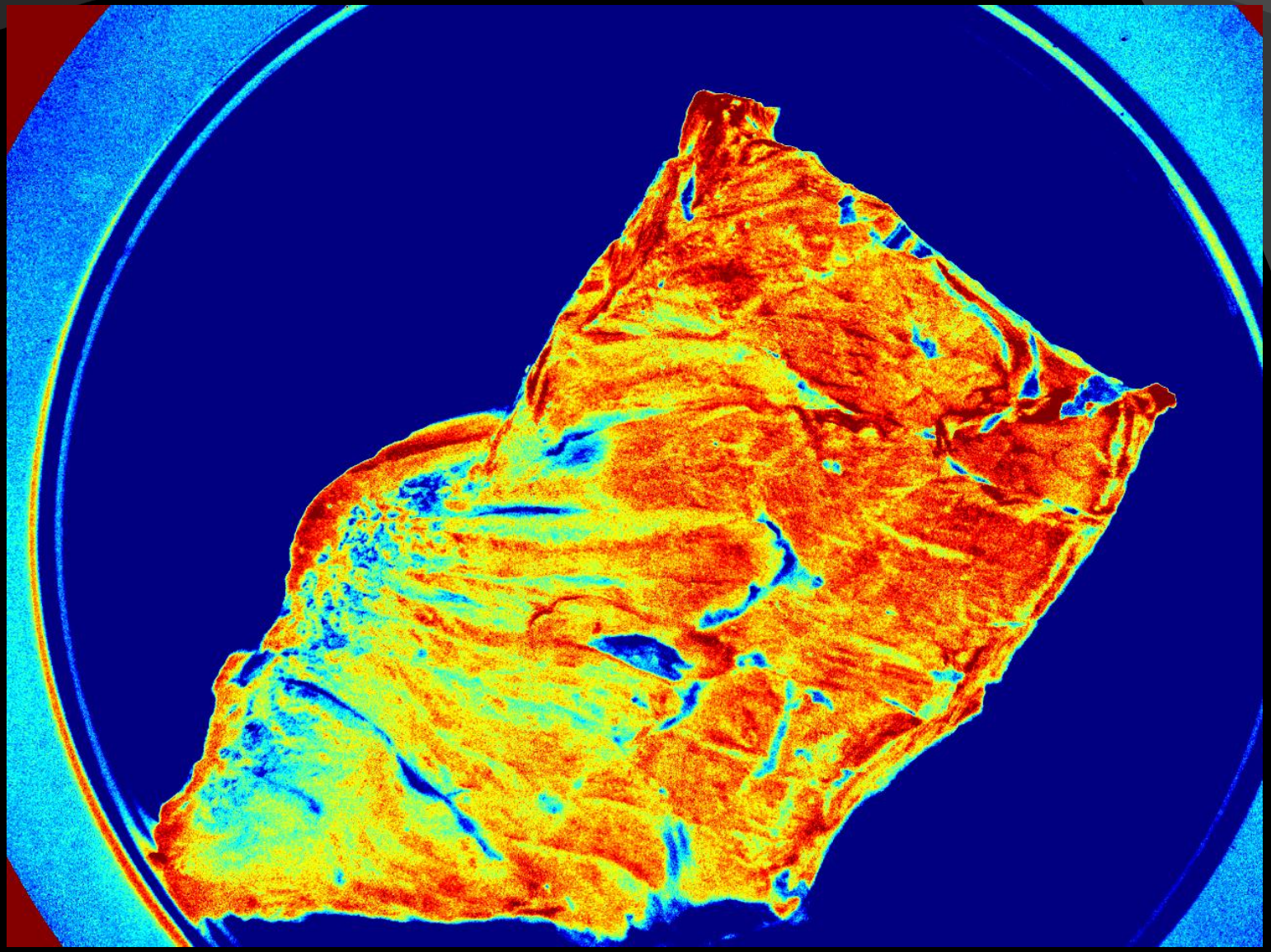
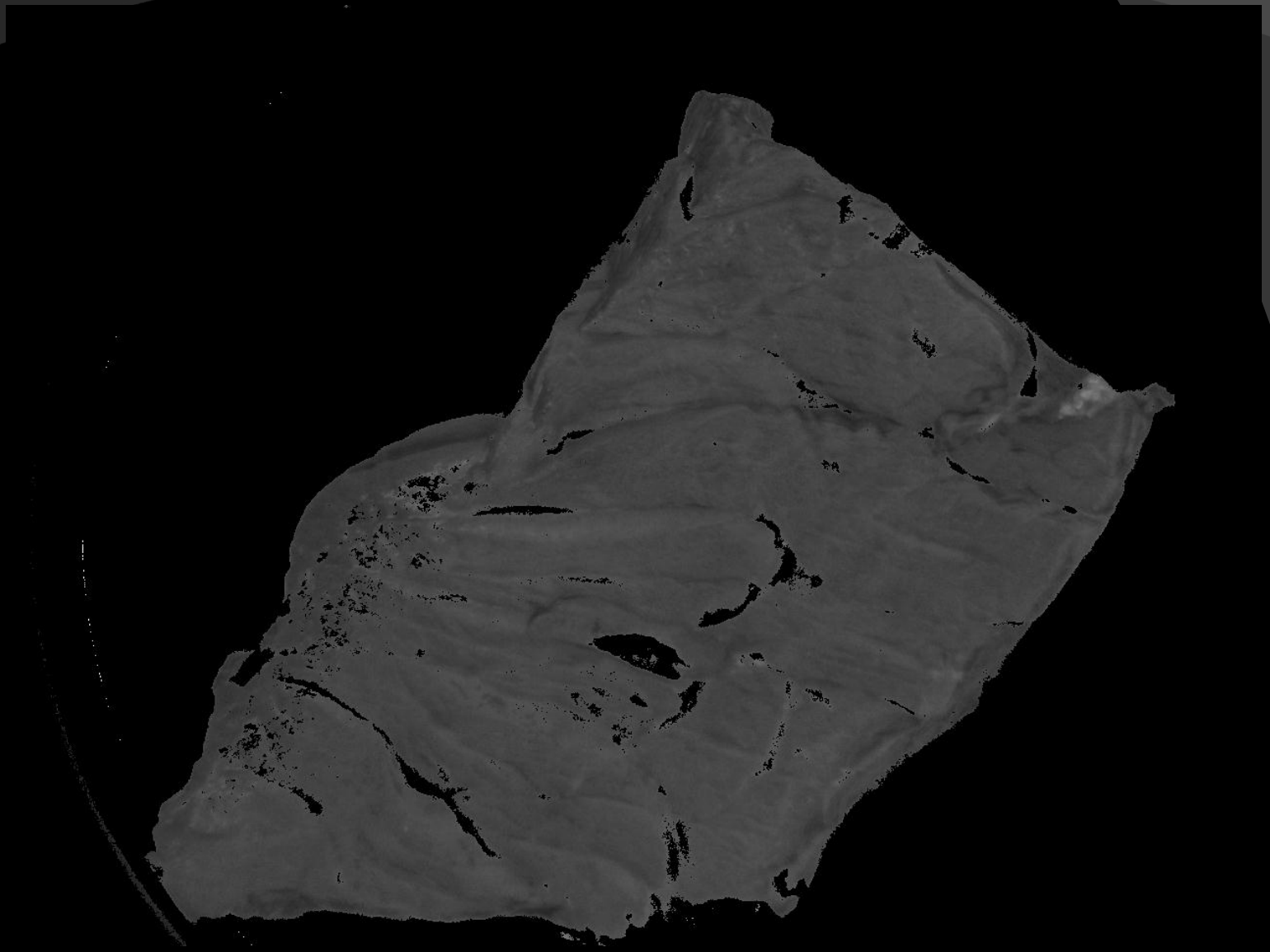
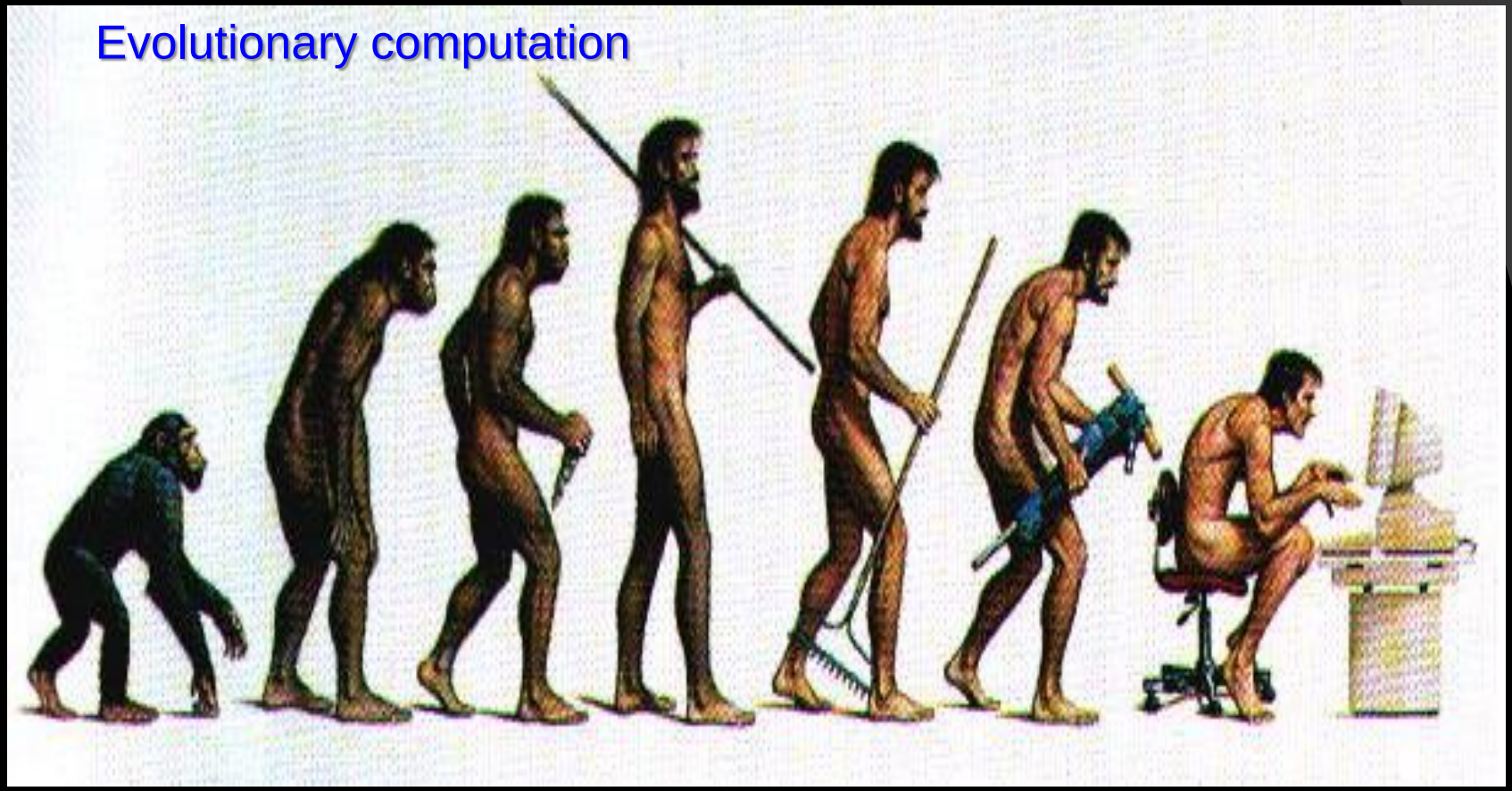


Image processing pipeline – segmentation



Evolutionary Computation



Classification performance of beef fillets using PLS regression

True class	Predicted class			Sensitivity (%)
	Fresh	Semi-fresh	Spoiled	
Fresh (*n* = 18)	**12**	6	0	66.7
Semi-fresh (*n* = 20)	3	**17**	0	85.0
Spoiled (*n* = 48)	0	5	**43**	89.6

Overall correct classification (accuracy): 83.7%

We are not just looking for the

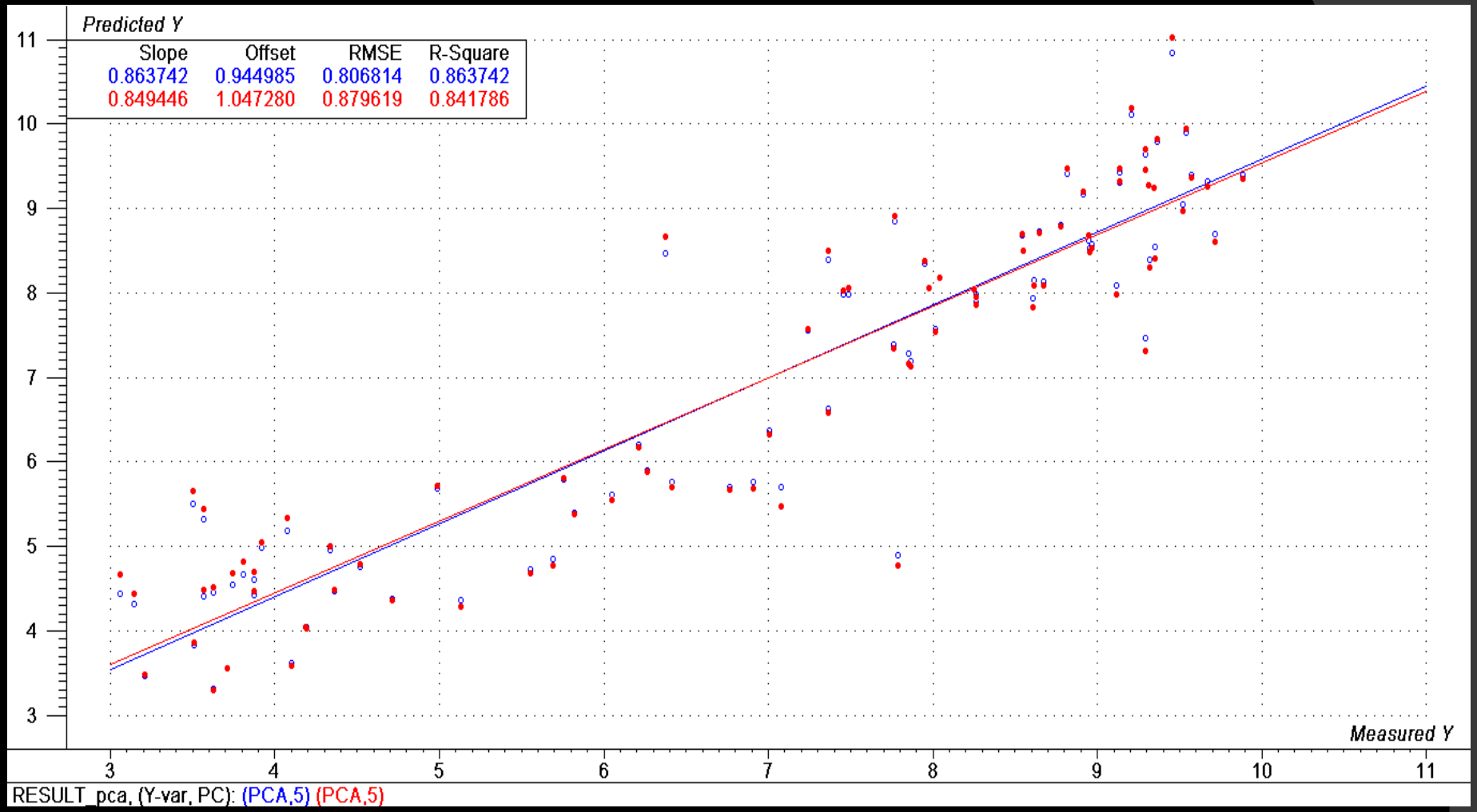
NEXT top model

Classification performance of beef fillets using ANNS (36 input – 40 hidden neurons-1 output)

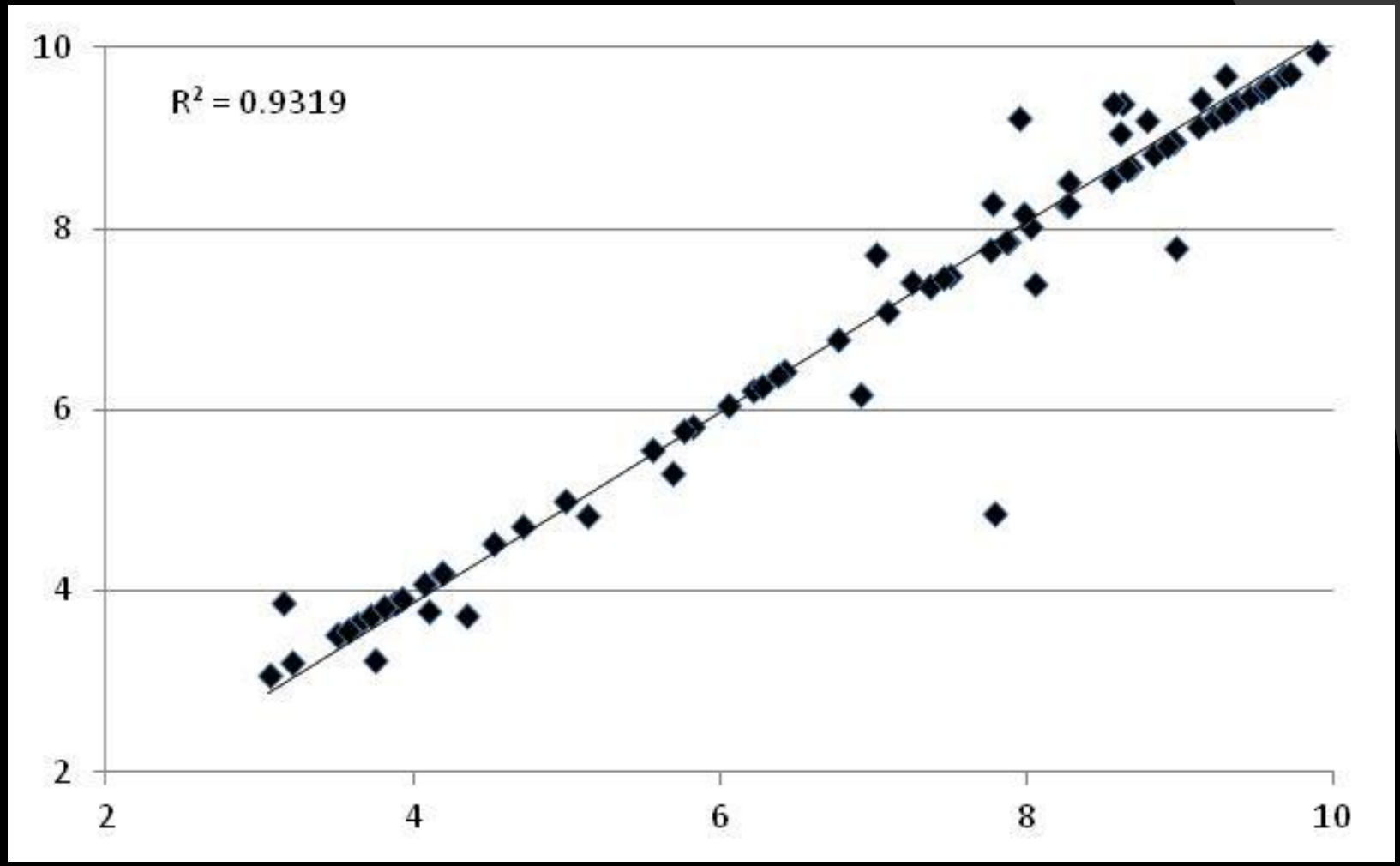
	Predicted class			Sensitivity (%)
	Fresh	Semi-fresh	Spoiled	
Fresh (*n* = 18)	**17**	0	1	94.4
Semi-fresh (*n* = 20)	1	**18**	1	90.0
Spoiled (*n* = 48)	0	2	**46**	95.8

Overall correct classification (accuracy): 94.2%

Comparison between observed total viable counts (TVC) and predicted counts by PLS regression on beef fillets



Comparison between observed and predicted total viable counts (TVC) by ANNs on beef fillets



(Vibrational Spectroscopy)

FTIR

FT-IR Analysis

Nicolet 6700 FT-IR

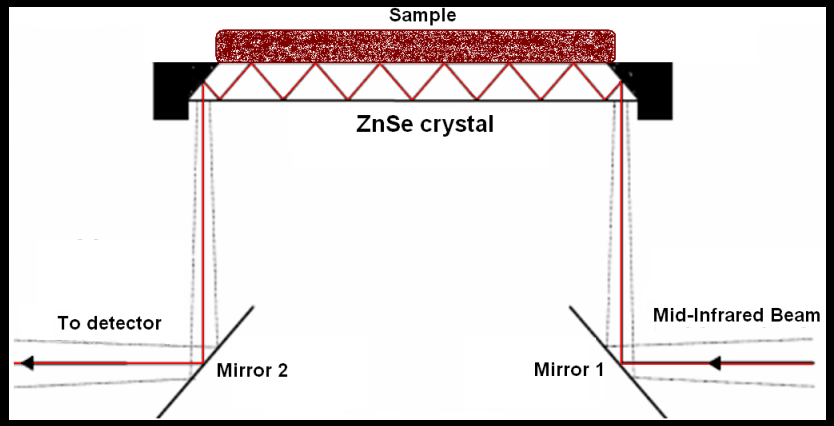
Detector: DLaTGS with KBr Beam splitter

Crystal: Zinc Selenite (ZnSe) 45° ATR (Attenuated Total Reflectance)

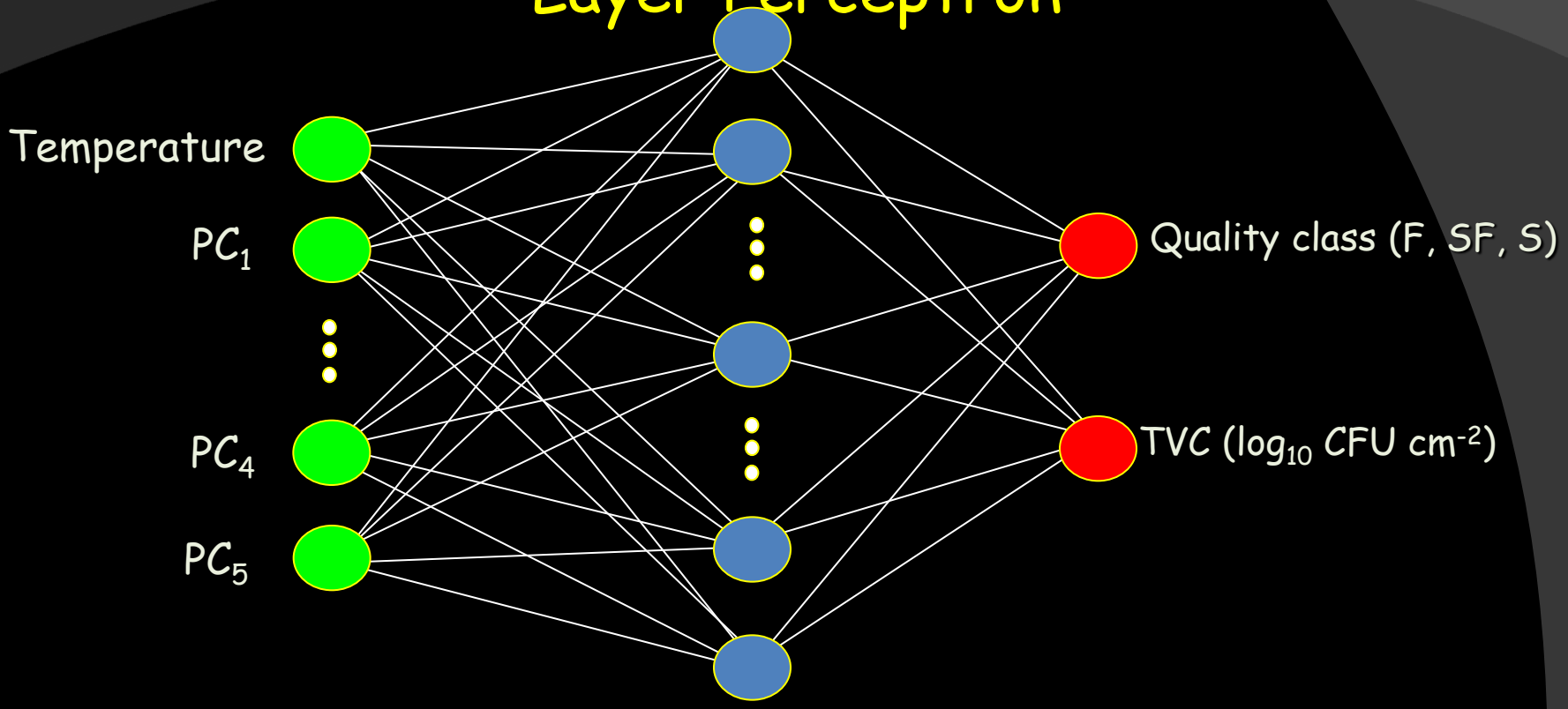
Wavenumber range: 4,000 – 400 cm^{-1}

Scanning frequency per sample: 100 scans with a resolution of 4 cm^{-1}

Collection time for each spectrum: 2 min

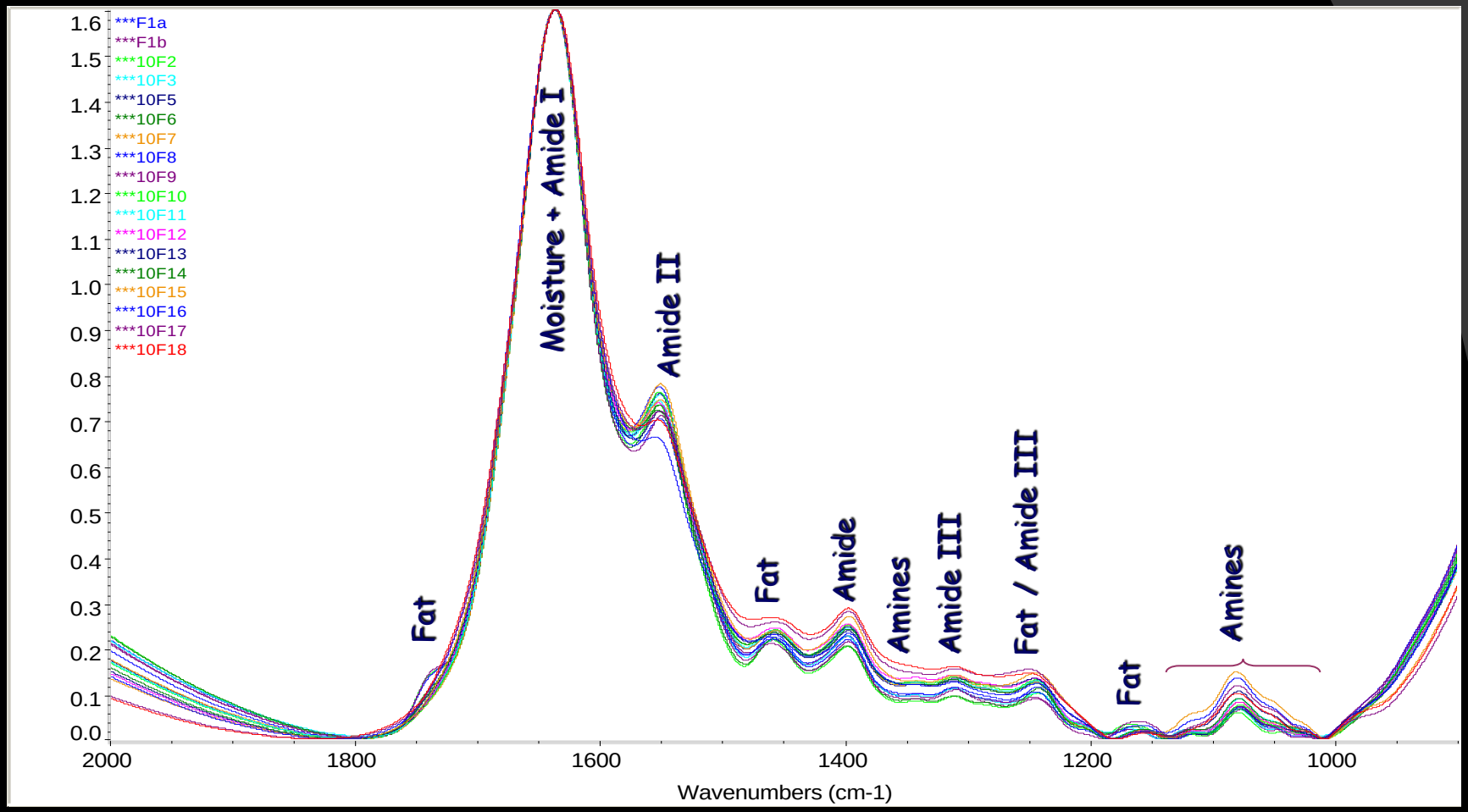


Neural network development – Multi Layer Perceptron



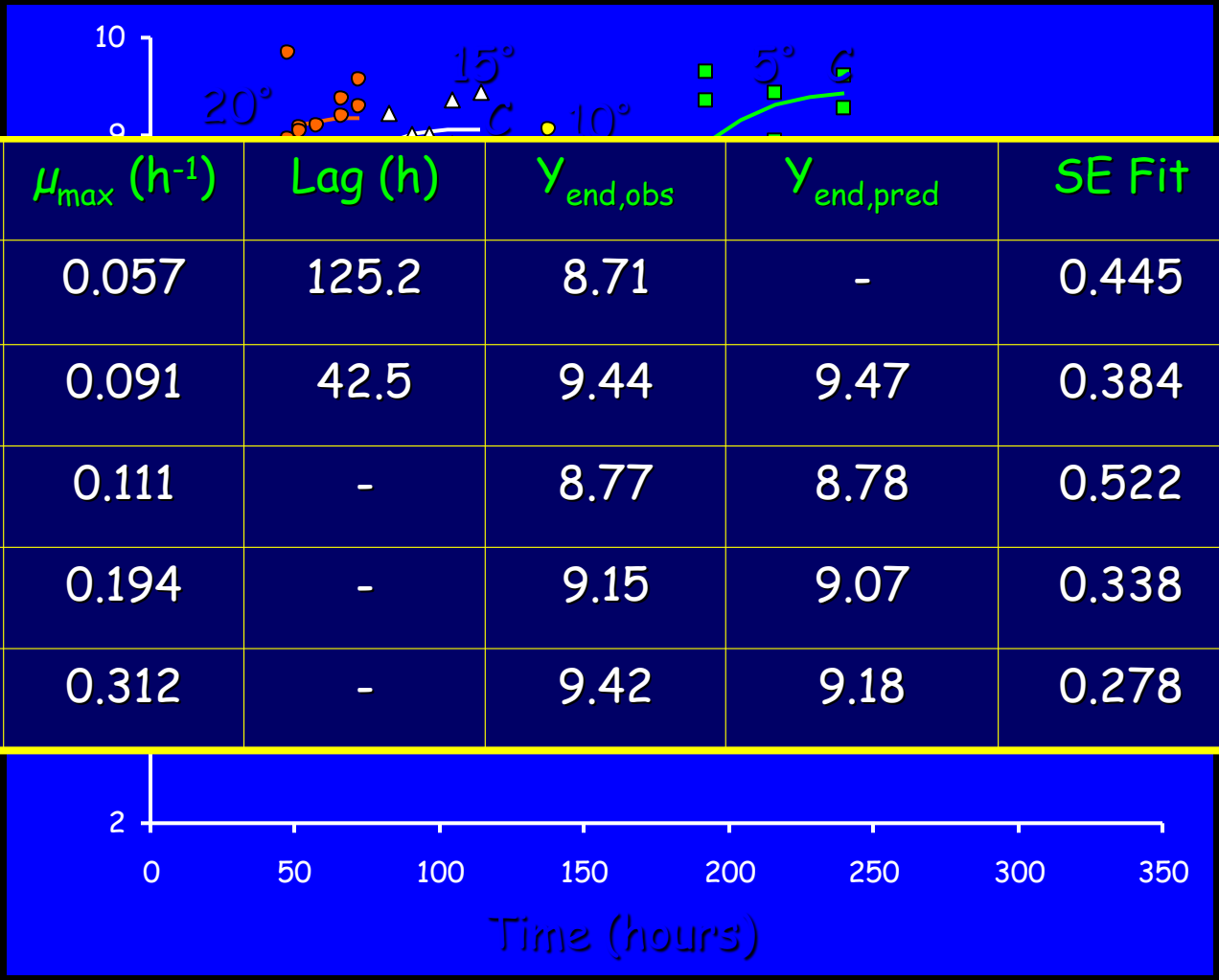
Transfer functions: (a) logistic sigmoid, and (b) hyperbolic tangent
Network training: Steepest-descent gradient algorithm
Network testing: Leave-1-out cross validation
Network Performance: % classification accuracy in each sensory class

Typical FTIR spectra in the range of 1800 to 1000 cm^{-1} collected from beef fillets stored at 5° C for 10 days



Different colours represent different sampling days.

Changes of total viable counts (TVC) obtained from beef fillets stored under aerobic conditions at different temperatures



T (°C)	μ_{max} (h^{-1})	Lag (h)	$Y_{end,obs}$	$Y_{end,pred}$	SE Fit	R^2
0	0.057	125.2	8.71	-	0.445	0.953
5	0.091	42.5	9.44	9.47	0.384	0.974
10	0.111	-	8.77	8.78	0.522	0.924
15	0.194	-	9.15	9.07	0.338	0.975
20	0.312	-	9.42	9.18	0.278	0.982

AUA

Confusion matrix of the 7-10-2 Multi Layer Perceptron classifier performing the task of discrimination of beef fillets based on leave-1-out cross validation method

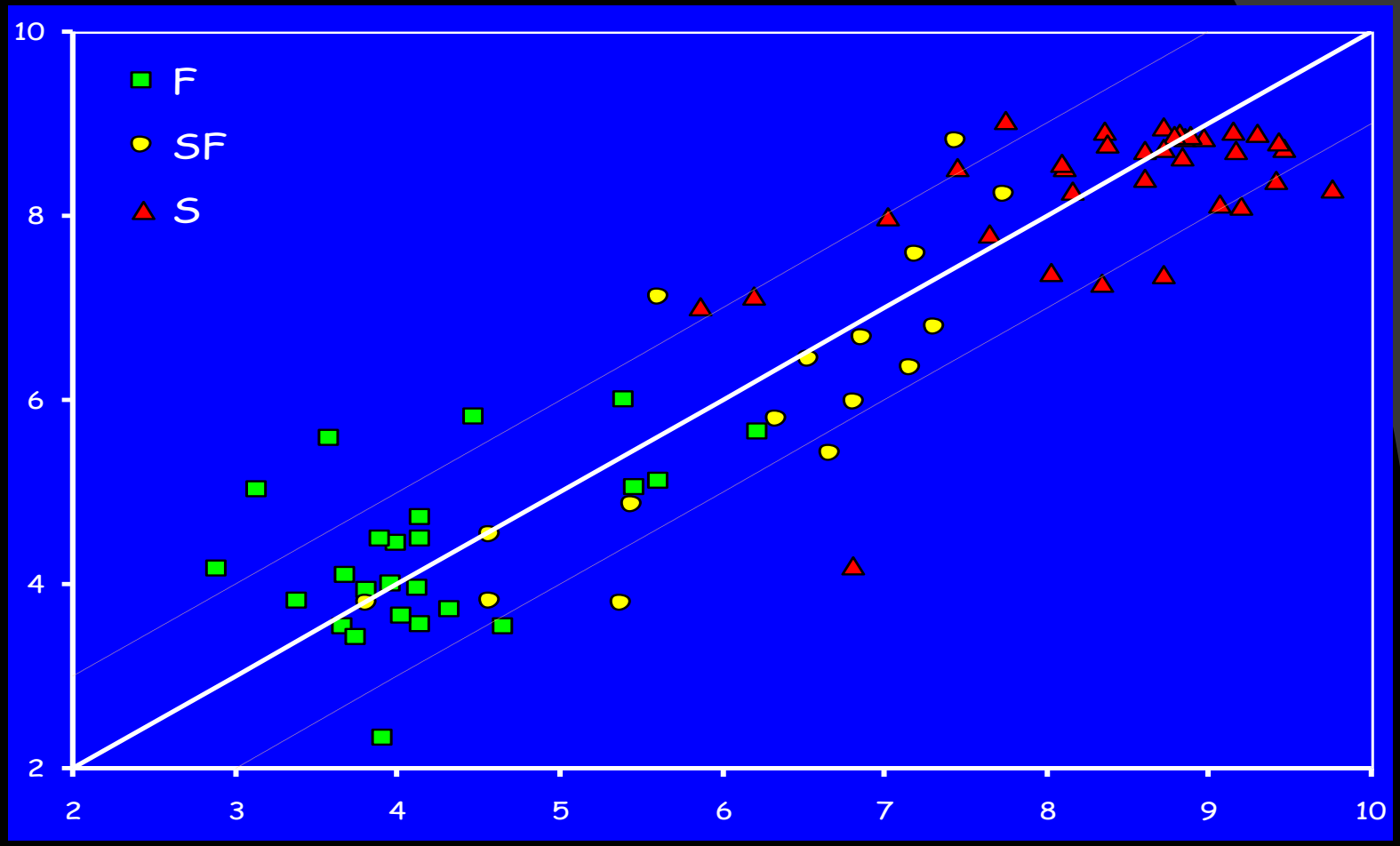
True class	Predicted class			Sensitivity (%)
	Fresh	Semi-fresh	Spoiled	
Fresh (*n* = 24)	**22**	2	0	91.7
Semi-fresh (*n* = 16)	1	**13**	2	81.2
Spoiled (*n* = 34)	0	2	**32**	94.1

Overall correct classification (accuracy): 90.5%

Forecasting and optimization are complex

AUA

Comparison between observed total viable counts (TVC) and predicted counts by the MLP classifier



ca. 78% of the data are included in the ± 1 log unit area

In fact we are looking what is the performance of this model

Performance of the 7-10-2 MLP classifier for the prediction of total viable counts in meat samples analyzed by FT-IR

Index	Fresh	Semi-fresh	Spoiled	Overall
Bias factor (B_f)	1.031	0.951	0.982	0.991
Accuracy factor (A_f)	1.181	1.122	1.084	1.123

- Bf < 1 under-estimation of TVC
- Bf > 1 over-estimation of TVC

Concluding remarks

- FTIR Spectral and imaging analysis spectral data, in combination with an appropriate machine learning strategy could become an effective tool to monitor beef fillets spoilage during aerobic storage at different temperatures.
- Further training of the taste panel and/or development of a common protocol for meat sensory evaluation will increase the efficacy of the method.

Concluding remarks

- As machine learning strategies are data driven approaches, additional analysis of a higher number of meat samples is necessary to improve the accuracy of the method.

- The microbial load (as total viable counts) could be satisfactorily predicted directly from the collected spectra, although some over-prediction was observed for meat samples stored at 0° C and short storage times.

Panagou, et al.(2008) Table olives volatile fingerprints and machine learning: potential of an electronic nose for quality discrimination. Sensors and Actuators B 134, 902-907

Argyri et al. (2009) The potential of end-products metabolites on predicting the self life of minced beef stored under aerobic and modified atmosphere with or without the effect of essential oils 6ICPMF – Washington 8-12 Sept 2009 USA

Ammor, et al.(2009)– "Rapid Monitoring of the Spoilage of Minced Beef Stored Under Conventionally and Active Packaging Conditions Using Fourier Transform Infrared Spectroscopy in Tandem with Chemometrics" Meat Science 81, 507-515

Argyri, et al., (2010) Rapid qualitative and quantitative detection of beef fillets spoilage based on Fourier transform infrared spectroscopy data and artificial neural networks, Sensors and Actuators B 145, 146-154

Argyri, et al.(2011) Potential of a simple HPLC based approach to quantify spoilage of minced beef stored at different temperatures and packaging systems. Int. J. Food Microbiol. 150, 25-33

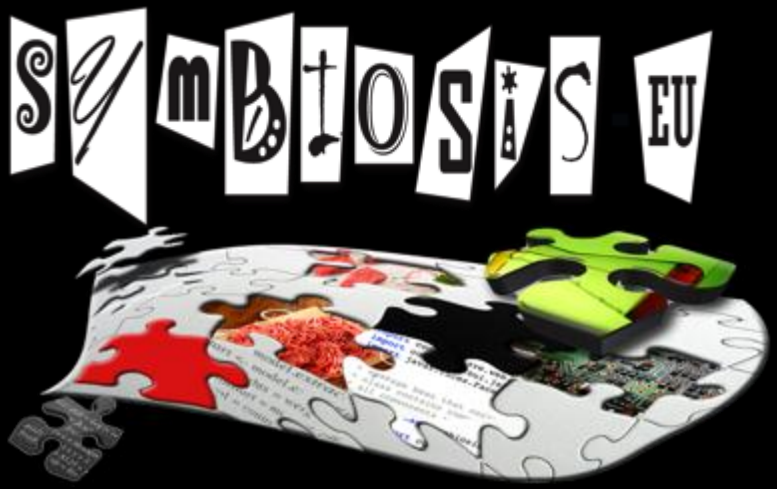
Papadopoulou, et al.(2011) Contribution of Fourier transform infrared (FTIR) spectroscopy data on the quantitative determination of minced pork meat spoilage Food Research Int. 44, 3264-3271

Dissing et al. (2012). Using multispectral imaging for spoilage detection of pork meat. Food and Bioprocess Technology (Accepted)

Argyri, et al. (2012) A Comparison of Raman and FT-IR Spectroscopy For The Prediction of Meat Spoilage. Food Control (in press)

Acknowledgements

This work was supported by the EU projects Symbiosis [7th Framework Programme (Con. No 21638)] and ProSafeBeef [6th Framework Programme (ref. Food-CT-2006-36241)].



Cranfield University UK
F. MOHAREB
C. BESSANT

UMAN
R.JARVIS
X. YUN
R. GOODACRE

Agricultural University of Athens
A. ARGYRI
B. BLANA
A. DOULGERAKI
A. MALOUCHOS
B. E. PANAGOU
S. HAROUTOUNIAN

Nano-MEGA-FOOD Project

Thank you for your attention