Imaging – The Challenges in Objectively Evaluating Spatial Distribution in 2 and 3 Dimensions

Imaging – The Challenges in Objectively Evaluating Spatial Distribution in 2 and 3 Dimensions

Steve Hammond, Pfizer Global Technology, USA.

Mark J. Henson, Ph.D. // Pfizer, Inc.

Aspects of Chemical Image Validation

- **Spectral Validation**
  - **Information content**
  - **Spectral sampling volume**
  - **Calibration**
- **Sampling Validation**
  - **# of pixels required**
  - **# of images required**
  - **# of samples required**
- **Spatial Validation**
  - **Spotsize**
  - **Lateral precision/reproducibility**
  - **Spectral focal point vs. optical focus**

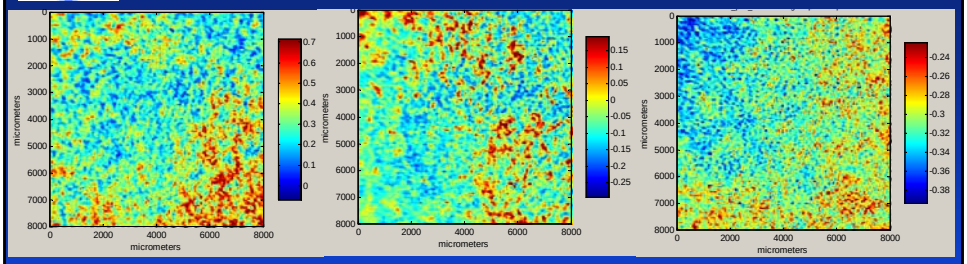
Mark J. Henson, Ph.D. // Pfizer, Inc.

Spectral Validation - Introduction

- Chemical imaging provides full vibrational spectra at all pixels in an image
- While "pretty pictures" are easy to generate, understanding of spectral content behind images necessary for full interpretation
- Pixels with "pure" spectra are easy to classify but only observed when:
  - Materials are severely segregated or agglomerated
  - Large particles/domains exist (>*n*×pixel size; *n* = ?)
- Most pixels in NIR images of "good" pharma samples are not pure
  - Most crucial components (*e.g.*, API, lubricant) small (< 50 μm) and/or well-distributed
- PLS models often built on pure component spectra alone
  - Challenging to model all components at various concentrations
  - Potentially give rise to calibration error when nonlinearities exist

Mark J. Henson, Ph.D. // Pfizer, Inc.

PEO/Lactose Blend NIR Images

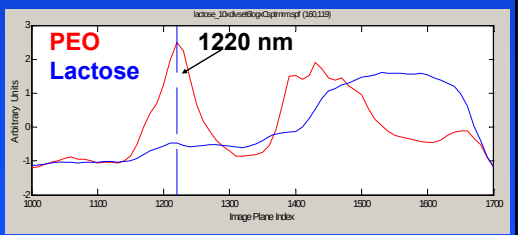


75% PEO
25% Lactose

50% PEO
50% Lactose

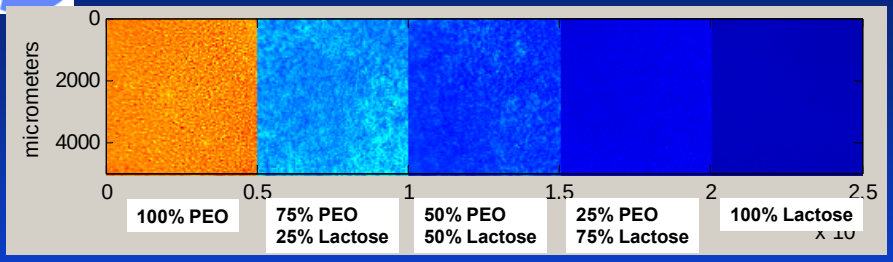
25% PEO
75% Lactose

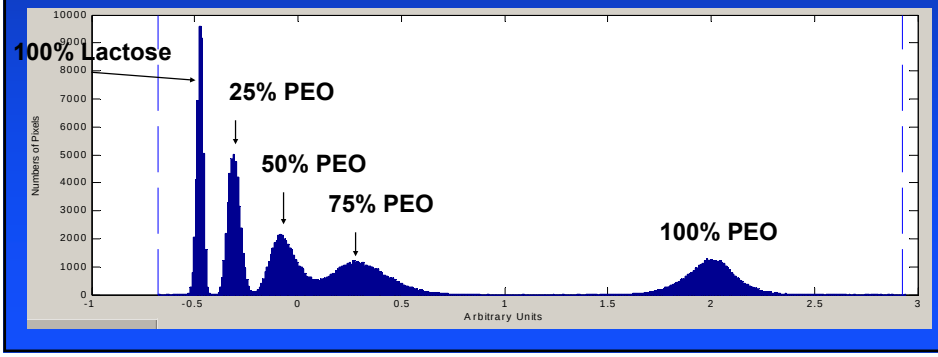
- Image spectra SNV normalized
- Red points = PEO domains
- Separate color scales



Mark J. Henson, Ph.D. // Pfizer, Inc.

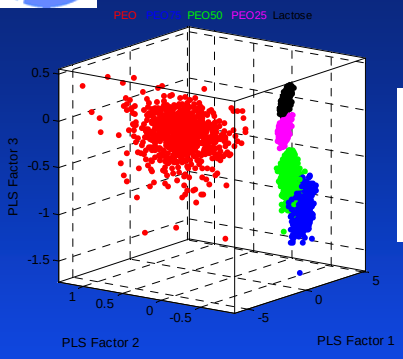
Common Color Scale



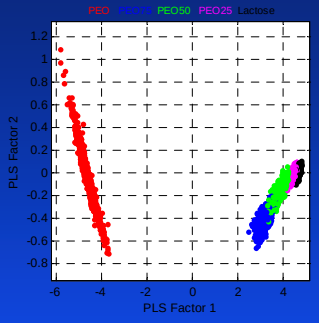


Mark J. Henson, Ph.D. // Pfizer, Inc.

NIR Image Pixel Trends



100% Lactose
75:25 Lact:PEO
50:50 Lact:PEO
23:75 Lact:PEO
100% PEO

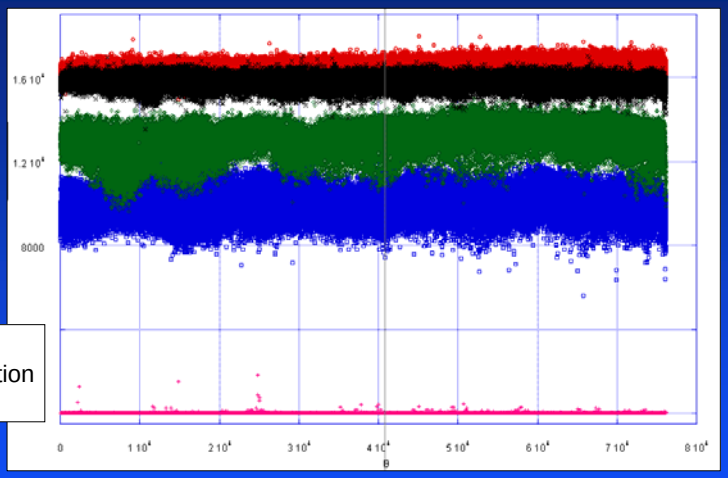


- Spectra normalized, PLS library containing only pures
- Projection of prediction set onto space defined by 1st 3 PLS vectors
- Ideally should need only 1 PLS factor to model concentration
- Distribution indicates dramatic nonlinearity present in spectral system

Mark J. Henson, Ph.D. // Pfizer, Inc.

NIR: Mahalanobis Distances From Pure PEO

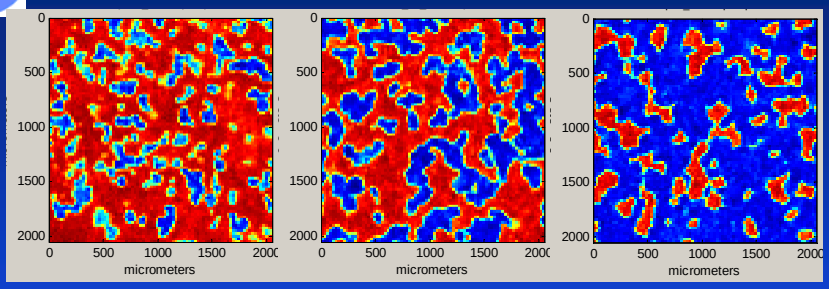
Lactose
25% PEO
50% PEO
75% PEO
PEO



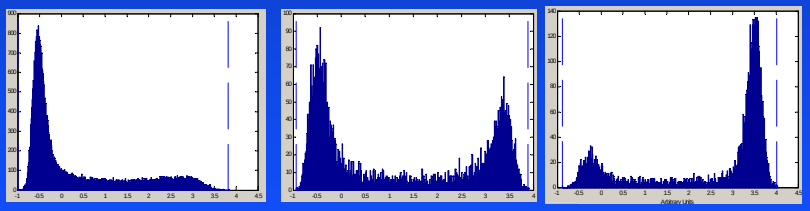
normalized NIR
2-image calibration
3 lv

Mark J. Henson, Ph.D. // Pfizer, Inc.

FT-IR Imaging = Low Penetration Depth



75% PEO | 50% PEO | 25% PEO

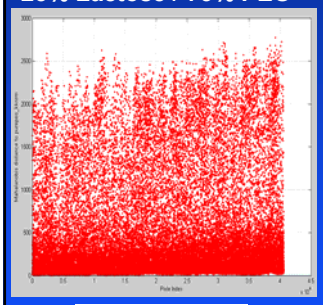


Mark J. Henson, Ph.D. // Pfizer, Inc.

IR: Mahalanobis Distances From Pure PEO

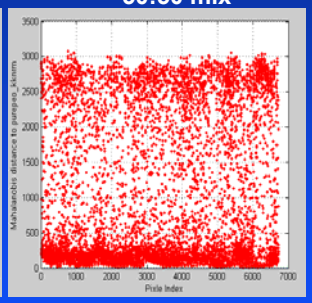
FT-IR
normalized only
2 pure image calibration set
3LV*

25% Lactose / 75% PEO



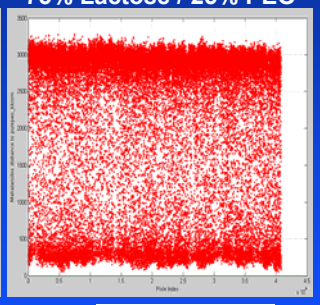
mean = 223
median = 38.9
max = 1632

50:50 mix



mean = 518
median = 201
max = 1852

75% Lactose / 25% PEO

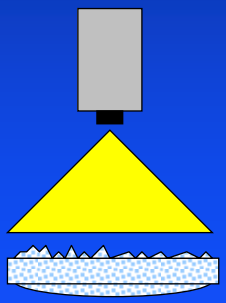


mean = 1062
median = 1342
max = 2036

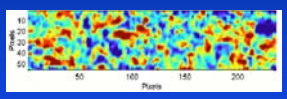
Mark J. Henson, Ph.D. // Pfizer, Inc.

Depth Penetration vs. Wavelength

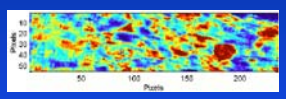
- Depth penetration increases at lower λ (*eg*, depth @ 1130 > 1400 > 1660)
  - Thus, penetration varies as: Raman (most) > NIR > IR (least)
- Penetration depth @ 1100nm = 3x depth at 1675 nm (Clarke *et al., Appl. Spectr.* 2002, 56, 1475)
- Different sampling volume at each end of NIR spectrum



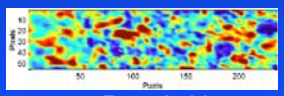
Increasing penetration



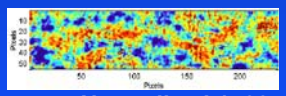
Raw 1660



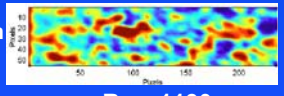
Normalized 1660



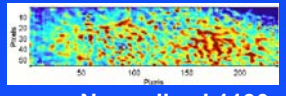
Raw 1400



Normalized 1400



Raw 1130



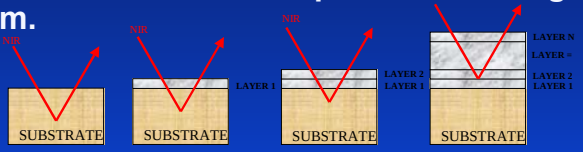
Normalized 1130

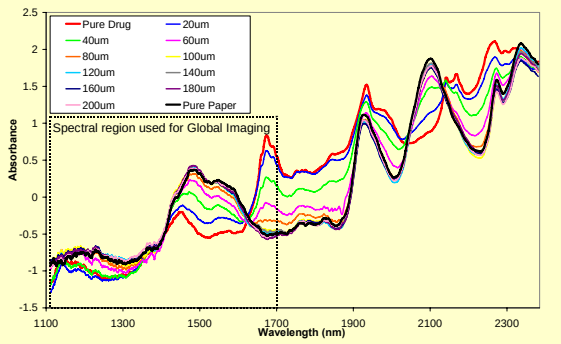
Mark J. Henson, Ph.D. // Pfizer, Inc.

Experimental NIR Penetration Depth

Slide courtesy of Fiona Clarke

- Measurement of DoP was performed using a layer system.



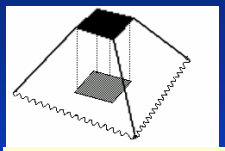


Mark J. Henson, Ph.D. // Pfizer, Inc.

Varying NIR Sampling Volume w/ Wavelength

Slide courtesy of Fiona Clarke

- If x and y are ~25 µm
- Know z is
  - –109 µm at 2380nm
  - –777 µm at 1100nm



$$Volume = \frac{1}{3} ID(A_1 + A_2 + \sqrt{A_1 \times A_2})$$

- At 2380 nm
  - –A1 = 6.25 x 10^{-8} m^2
  - –A2 = 118.81 x 10^{-8} m^2
  - –Volume = 5.534 x 10^{-10} m^3
- At 1100 nm
  - - A1 = 6.25 x 10^{-8} m^2
  - - A2 = 6038.3 x 10^{-8} m^2
  - - Volume = 1615.6 x 10^{-10} m^3
- Mass of Sample (presuming þ= 0.8g/m^3)
  - –At 2380nm = 0.443 ng
  - –At 1100nm = 129.2 ng
- Using an analysis area of 5 x 5 mm area have 40,000 spectra
  - –At 2380nm = 0.018 mg
  - –At 1100nm = 5.2 mg
- Typical Dosage Form 300mg
  - –At 2380nm sampling 0.006%
  - –At 1100nm sampling 1.72%

Mark J. Henson, Ph.D. // Pfizer, Inc.

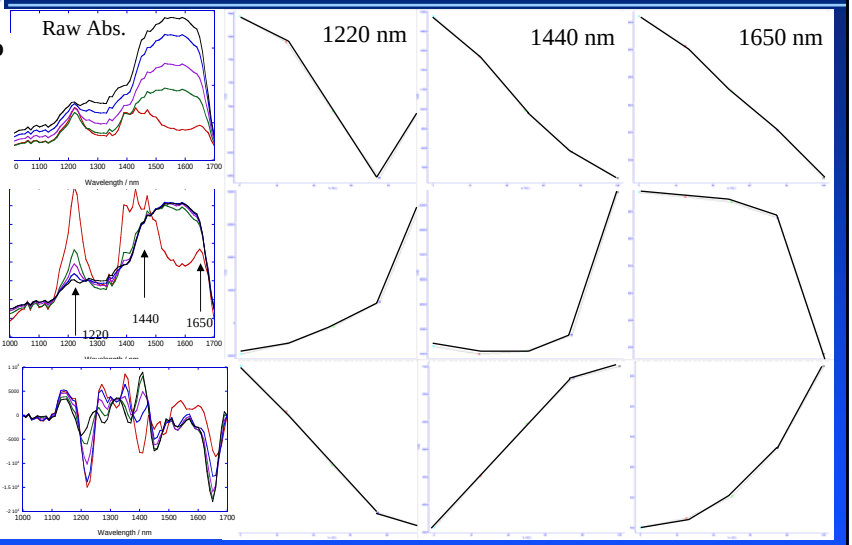
Linearity vs. wavelength, preprocessing

Lactose:PEO
100:0
75:25
50:50
25:75
0:100

Raw Abs.

normalized

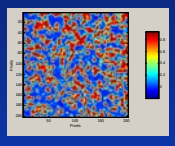
9SG2
vnorm



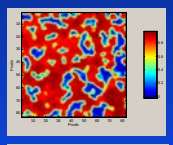
- **Spectra generated by summing 200x200 pixel images to a single spectrum**
- **Significantly different intensity trends w/ wavelength**

Mark J. Henson, Ph.D. // Pfizer, Inc.

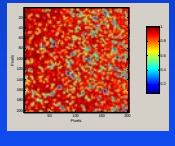
FT-IR 2-point PLS Model Results



25% PEO Sample
5 mm x 5 mm
~41,000 pixels
PEO prediction = 0.36



50% PEO Sample
2 mm x 2 mm
~6700 pixels
PEO prediction = 0.68

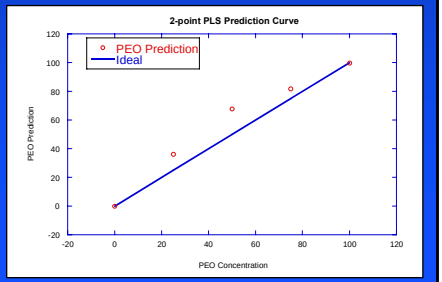


75% PEO Sample
5 mm x 5 mm
~41,000 pixels
PEO prediction = 0.82

IR still nonlinear, but PEO is overpredicted (vs. lactose in NIR)

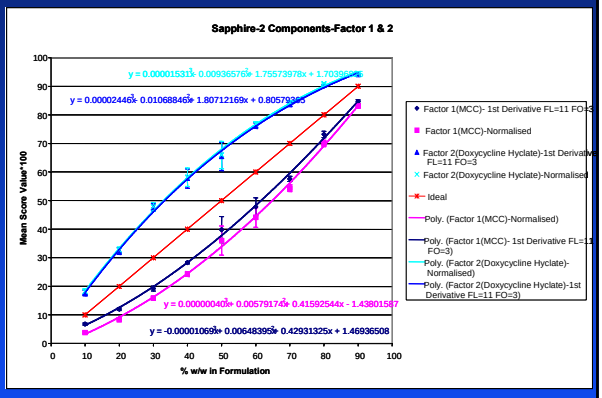
PEO PLS Score Images
Model built on pures only

Preprocessing:
1) KK Transform
2) 13-point SG2
3) Vector Normalization



Pure Calibration: API/MCC NIR Images

% w/w MCC	% w/w Doxycycline Hyclate
90	10
80	20
70	30
60	40
50	50
40	60
30	70
20	80
10	90



- Spectral nonlinearity also observed for API/MCC blends pressed into wafers and imaged w/ NIR
- 3 wafers at each concentration produced and imaged
- Mean PLS score for each image used to estimate concentration

Lisa Makein Ph.D. Thesis, 2007

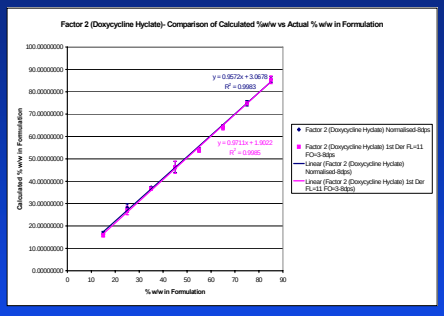
Full Calibration Approach

Calibration Set

% w/w MCC	% w/w Doxycycline Hyclate
90	10
80	20
70	30
60	40
50	50
40	60
30	70
20	80
10	90

Validation Set

% w/w MCC	% w/w Doxycycline Hyclate
15	85
25	75
35	65
45	55
55	45
65	35
75	25
85	15

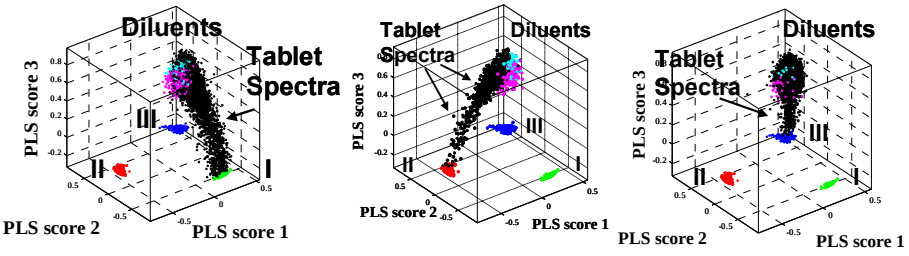


- When mixtures used to calibration, quantitative predictions are possible
- Challenge then becomes definition of sampling strategy

Lisa Makein Ph.D. Thesis, 2007

Mark J. Henson, Ph.D. // Pfizer, Inc.

Raman Mapping

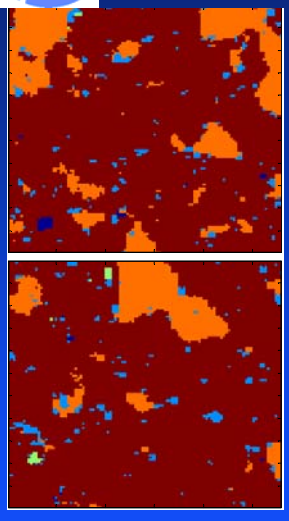


Tablet w/ 0.5% API (polymorph I)	Tablet w/ 0.5% API (polymorph II)	Tablet w/ 0.5% API (polymorph III)

Multivariate classifier based on smallest Euclidean distance results in 50x over-prediction of API content

Mark J. Henson, Ph.D. // Pfizer, Inc.

Raman Limit Test Validation



API form I
API form II
API form III
Excipient 1
Excipient 2

- Test samples were manufactured containing 0.05% API form I; 0.05% form II; 0.40% form III
- Represents a limit check of 10% API polymorph conversion
- Several images acquired over multiple surfaces per tablet
- Several tablets of each dosage level tested

Mark J. Henson, Ph.D. // Pfizer, Inc.

Raman Classification Results

Sample	Surface	# pixels	I	II	III	% I	% II	% III	Ratio I	Ratio II	Ratio III
0.5 mg (1)	1	4961	36	21	388	0.73	0.42	7.8	8	5	87
0.5 mg (1)	2	4960	26	25	411	0.52	0.50	8.3	6	5	89
1 mg (2)	1	2501	13	17	181	0.52	0.68	7.2	6	8	86
1 mg (3)	1	1679	8	6	95	0.48	0.36	5.7	7	6	87
1 mg (3)	2	1681	2	7	115	0.12	0.42	6.8	2	6	93
1 mg (2)	2	12099	4	51	371	0.03	0.42	3.1	1	12	87
1 mg (2)	3	12099	37	12	394	0.31	0.10	3.3	8	3	89
									6	**6**	**88**
AVE											

- Demonstrates that homogeneous impurity can reliably be detected for each image
- Not defined: edge of failure (minimum pixels needed to detect)

Mark J. Henson, Ph.D. // Pfizer, Inc.

Conclusions

- Pharmaceutical chemical images contain primarily mixture spectra
  - Understanding how to deconvolute signals is key to interpretation
- Sampling depths vary between images or even in the same image
  - Wavelength dependence
  - Scattering efficiency dependence on particle sizes
  - Raman scattering cross sectional differences
- Absolute concentration or particle size determinations possible, but require full calibration models
  - Intensities may vary nonlinearly with concentration
  - Standard approach in bulk spectroscopy, but challenging for imaging
  - Definition of sampling strategy crucial for effective calibration
- Many applications may not require full quant
  - Can be validated semi-quantitatively or qualitatively

Acknowledgments

- Fiona Clarke
- Lisa Makein
- Lin Zhang
- Sonja Sekulic
- Steve Hammond