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Standardization method, testing scenario, and accuracy of the infrared prediction model affect the standardization accuracy of milk mid-infrared spectra

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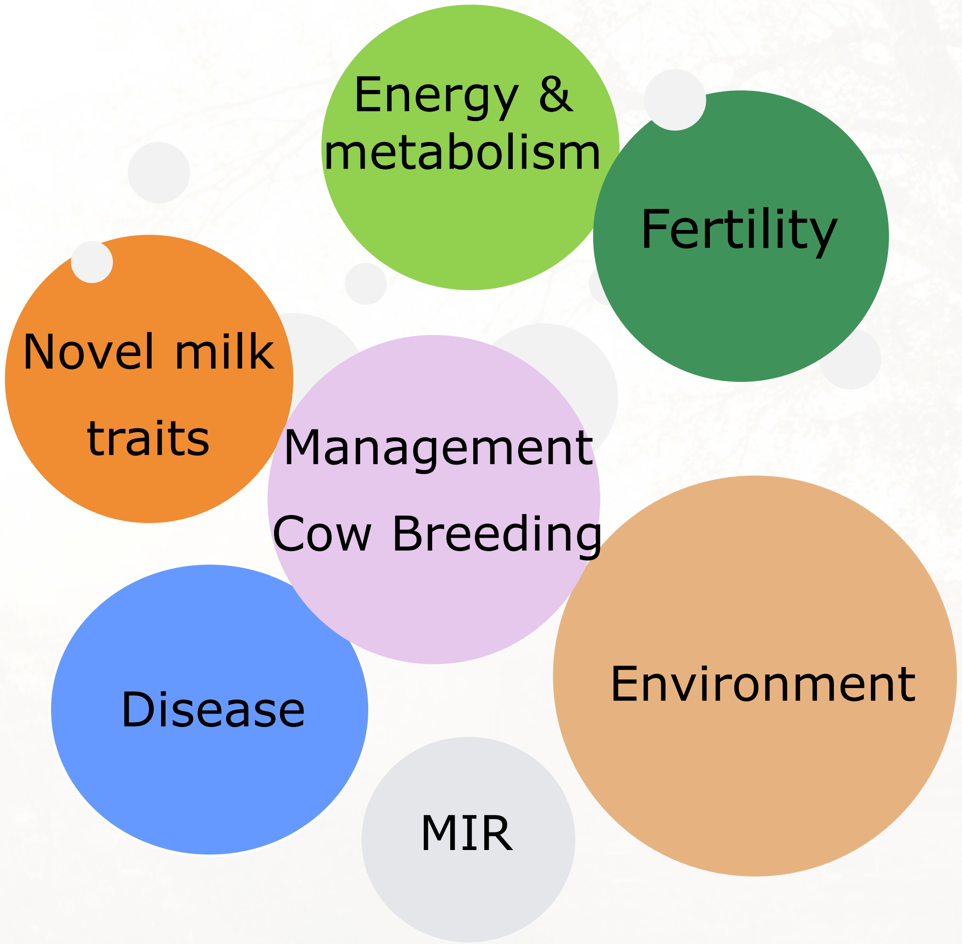
Introduction of mid-infrared (MIR) spectrometry

Lambert-Beer's Law $A = -\log_{10} \frac{I_t}{I_0} = \log_{10} \frac{1}{T} = K \cdot l \cdot c$

- Different organic chemical bonds absorb the MIR light → **MIR spectra**
- Advantages: non-destructive, high sensitivity and throughput, and low-cost

Introduction of applications in dairy cows

- Dairy Herd Improvement (DHI) program
- Novel milk components and cow phenotype
    - i.e., fatty acids and methane
- Applications
    - Farm management
    - Dairy cow breeding

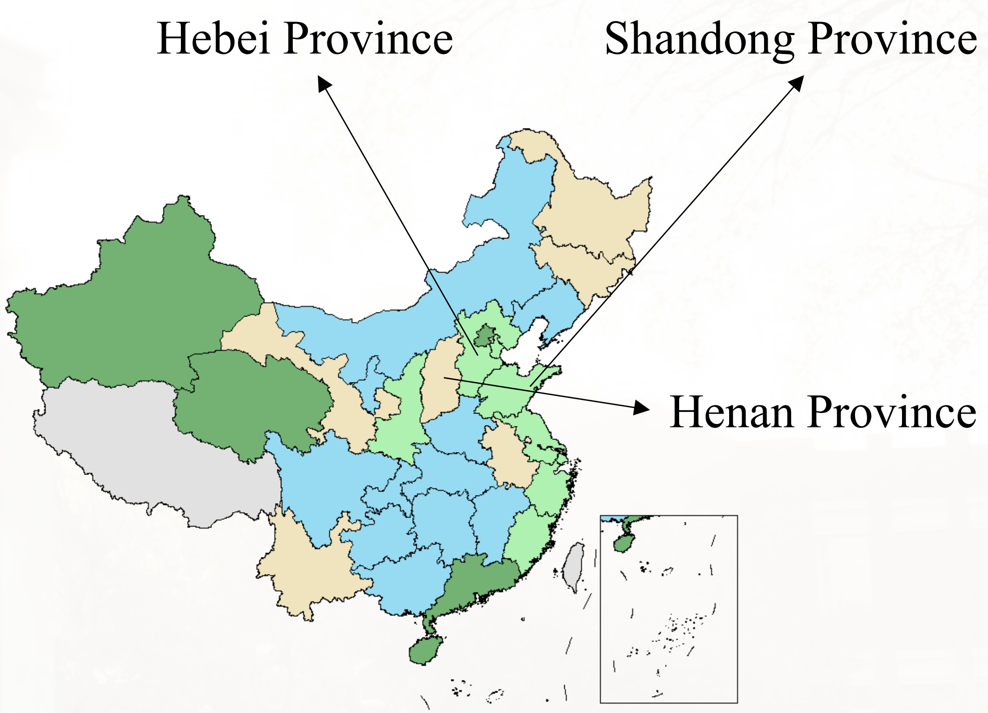


(De Marchi, M. 2014; Tiplady, K. M. 2020)

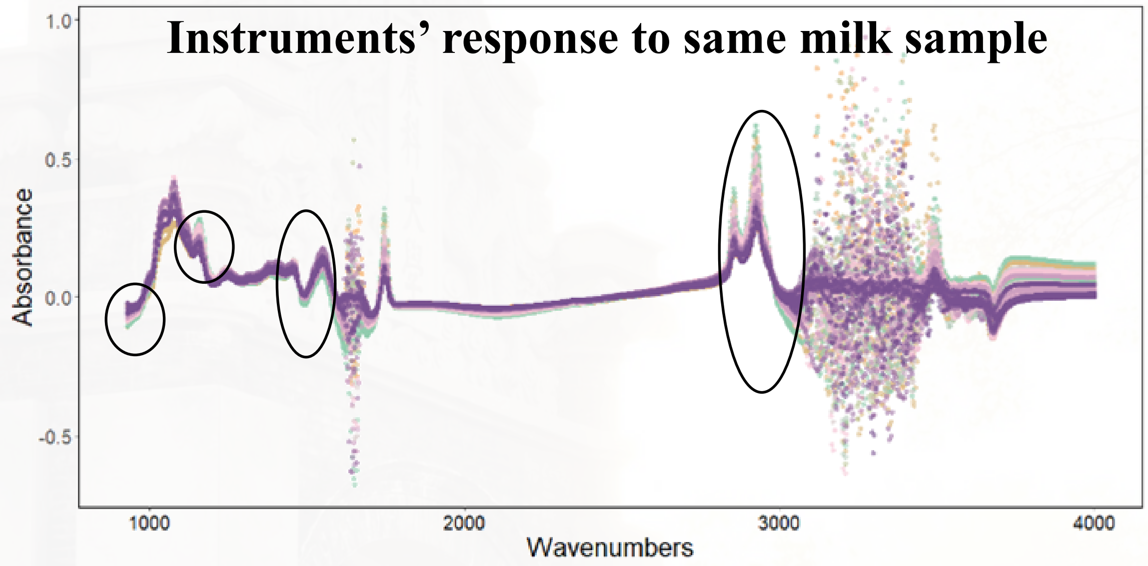
Situation in China (2023 year)

- 39 Dairy center
- 1,300 farms + 2.00 millions cows
- 11.00 millions observations

Top laboratories in the work



Issue



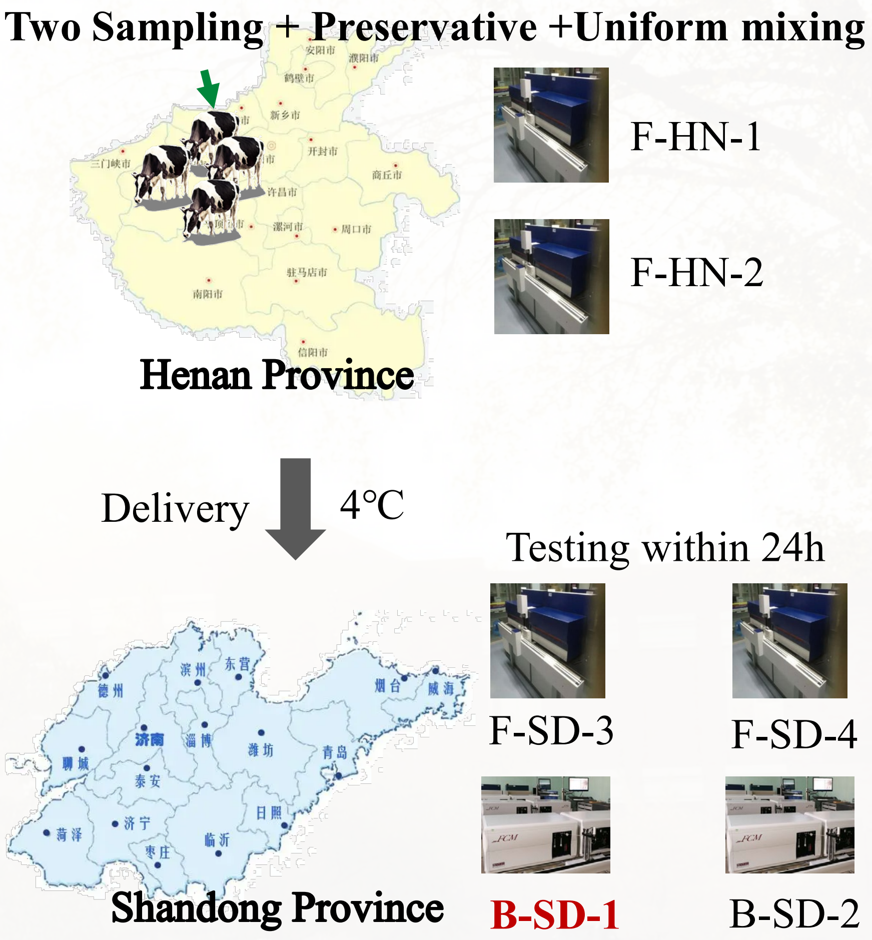
- Transfer models on different instruments or regions is crucial
  - Wear, operation, environment, and working time cause unique instrumental responses
- No one else has done it before in China → standardization

Aim

- Investigate spectra standardization to improve the transfer of prediction model across instruments

Experiment and data

- Six instruments in Henan and Shandong Provinces
- Two milk samplings in Mar and Oct 2023
- 30 MIR spectra collected per instrument each time
- Primary instrument (**B-SD-1**)
- Secondary instrument (others)



Methods

1. Harmonization of units and ranges between Bentley (*A*) and Foss (*T*) :

- $A = -Log_{10}^{T}$ → Absorbance; Linear interpolation → 928.77 to 3998.59 cm^{-1}

2. Spectral preprocessing:

- Centering + removal of water regions (1600.18~1689.70, 3010.13~3998.59 cm^{-1})

3. Prediction model: high (FP, %) and low (C8:0, g/L) accuracy

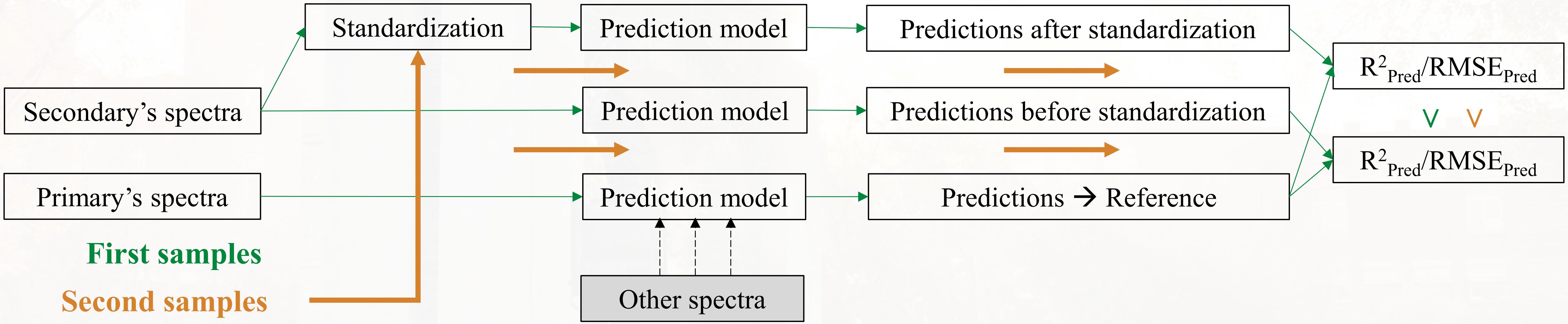
4. Standardization:

- Piecewise direct standardization (PDS) → making secondary spectra **close** to primary

Validation of standardization effect

Interval between two samplings = 6 months

- First → Internal validation (Standardization of spectra collected on the same day)
- Second → External validation (Standardization of spectra collected after long time)



Results: Prediction models' performance

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Parameters	FP (%)	C8:0 (g/L)
Data size	5,277	155
Mean (SD)	3.93 ± 0.70	33.28 ± 11.88
Range	2.01 ~ 6.46	11.67 ~ 76.35
R^2_{CV}	**0.9997 ± 0.00001**	**0.6630 ± 0.0192**
$RMSE_{CV}$	**0.0126 ± 0.0002**	**6.9714 ± 0.2222**

✓ PLS regression + 10-fold random cross-validation procedure + repeated 10 times

Results: Internal validation before and after standardization

First sampling (Internal validation)	Before standardization				After standardization			
	FP (%)		C8:0 (g/L)		FP (%)		C8:0 (g/L)	
	R^2_{Pred}	$RMSE_{Pred}$	R^2_{Pred}	$RMSE_{Pred}$	R^2_{Pred}	$RMSE_{Pred}$	R^2_{Pred}	$RMSE_{Pred}$
F-HN-1	0.9924	0.4163	0.9191	21.2984	0.9999	0.0466	0.9999	0.0457
F-HN-2	0.9928	0.3566	0.9113	28.6549	0.9999	0.0387	0.9999	0.0184
F-SD-3	0.9902	0.3694	0.9451	16.3846	0.9999	0.0382	0.9999	0.0118
F-SD-4	0.9939	0.3062	0.8886	16.9127	0.9999	0.0185	0.9999	0.0308
B-SD-2	0.9965	0.0667	0.7846	13.9158	0.9999	0.0028	0.9999	0.0009
Average	**0.9932**	**0.3030**	**0.8897**	**19.4333**	**<0.9999**	**0.0290**	**0.9999**	**0.0215**

- ✓ After standardization, the average R^2 increased to 0.99 and RMSE was about 0.02
- ✓ The least accurate model showed the greatest improvement
  - ✓ Increased by 11% (C8:0) vs 0.7% (FP) in R^2
  - ✓ Decreased by 99% (C8:0) vs 90% (FP) in RMSE

Results: External validation before and after standardization

Second sampling (External validation)	Before standardization				After standardization			
	FP (%)		C8:0 (g/L)		FP (%)		C8:0 (g/L)	
	R^2_{Pred}	$RMSE_{Pred}$	R^2_{Pred}	$RMSE_{Pred}$	R^2_{Pred}	$RMSE_{Pred}$	R^2_{Pred}	$RMSE_{Pred}$
F-HN-1	0.9715	0.5045	0.8661	26.3714	0.9801	0.1819	0.9568	3.3346
F-HN-2	0.9730	0.4139	0.8973	31.8572	0.9788	0.1910	0.9475	4.0378
F-SD-3	0.9835	0.3912	0.8181	19.8612	0.9899	0.1949	0.9393	3.1612
B-SD-2	0.9913	0.1077	0.8277	15.2051	0.9948	0.0562	0.9607	2.7544
Average	**0.9798**	**0.3543**	**0.8523**	**23.32 <**	**0.9859**	**0.1560**	**0.9511**	**3.3220**

- ✓ After standardization, the average R^2 increased to 0.98 and RMSE was 0.16 to 3.32
- ✓ Standardization is less effective after long time (6 months)

Results: Statistical test

Effects	R^2_{Pred}		Absolute errors
	t-value	*P*-value	*P*-value
Standardization method			
PDS versus non-standardization	**7.1299**	**< 0.01**	**< 0.01**
Application scenarios			
Internal versus External validation	**29.2090**	**< 0.01**	**< 0.01**
Prediction model			
FP versus C8:0	**27.5190**	**< 0.01**	**< 0.01**
Secondary instruments	0.0015	> 0.05	> 0.05

*Hotelling Williams t-test: R^2
*ANOVA: absolute prediction errors

✓ Standardization method, application scenarios, and model accuracy significantly affected transfer performances ($P < 0.05$)

Take home messages

Standardization greatly improve model transferability on unfamiliar instrument

Model with low prediction accuracy requires standardization during transfer

Shorter intervals of standardization are more effective (i.e., one or two months)

- It offers opportunities for MIR model transfer and spectra cooperation at laboratory, region, and country levels

Acknowledgement

Team members:

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(Dairy Center in Shandong Province)	**Xiuxin Zhao**

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THANKS FOR YOUR ATTENTION
Q&A

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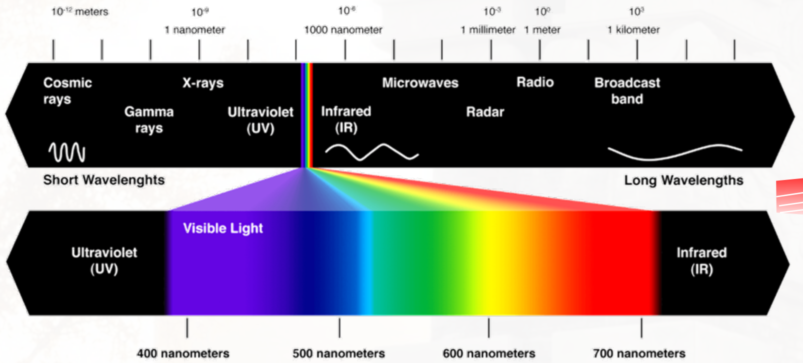
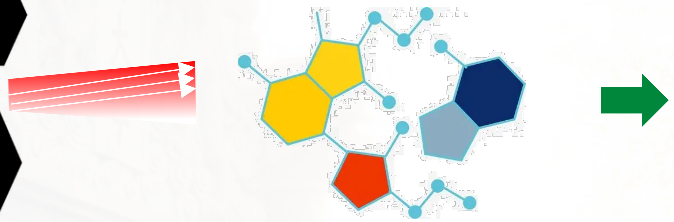
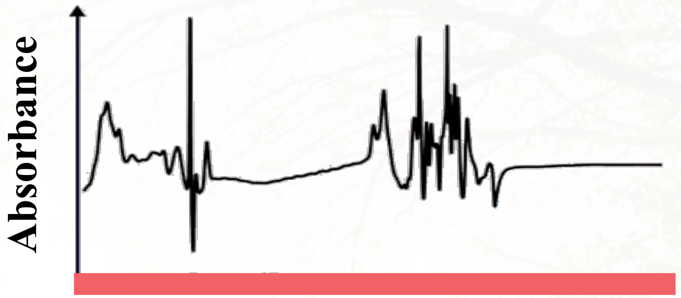
Mid-infrared (MIR) spectra





Chemical bonds/groups

MIR spectra



Wavenumbers

- Different organic functional groups/chemical bonds will absorb the MIR light → **MIR spectra**
  - Wavelength: 2.50~25.00 μm or wavenumber: 400~4,000 cm^{-1}
  - Fingerprint region: 600~1,400 cm^{-1}
  - Unit: Absorbance (*A*) or Transmittance (*T*)

(Subramanian, A., & Rodriguez-Saona, L. 2008)

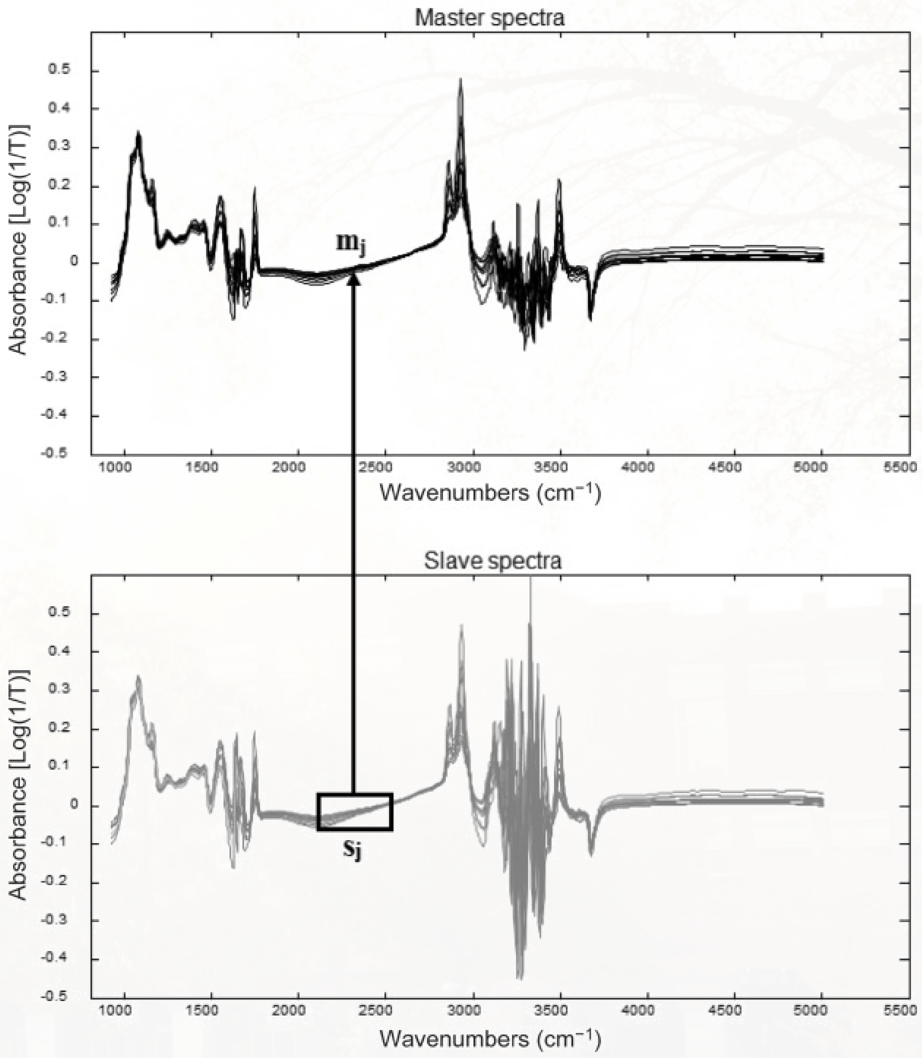
PDS

PDS:

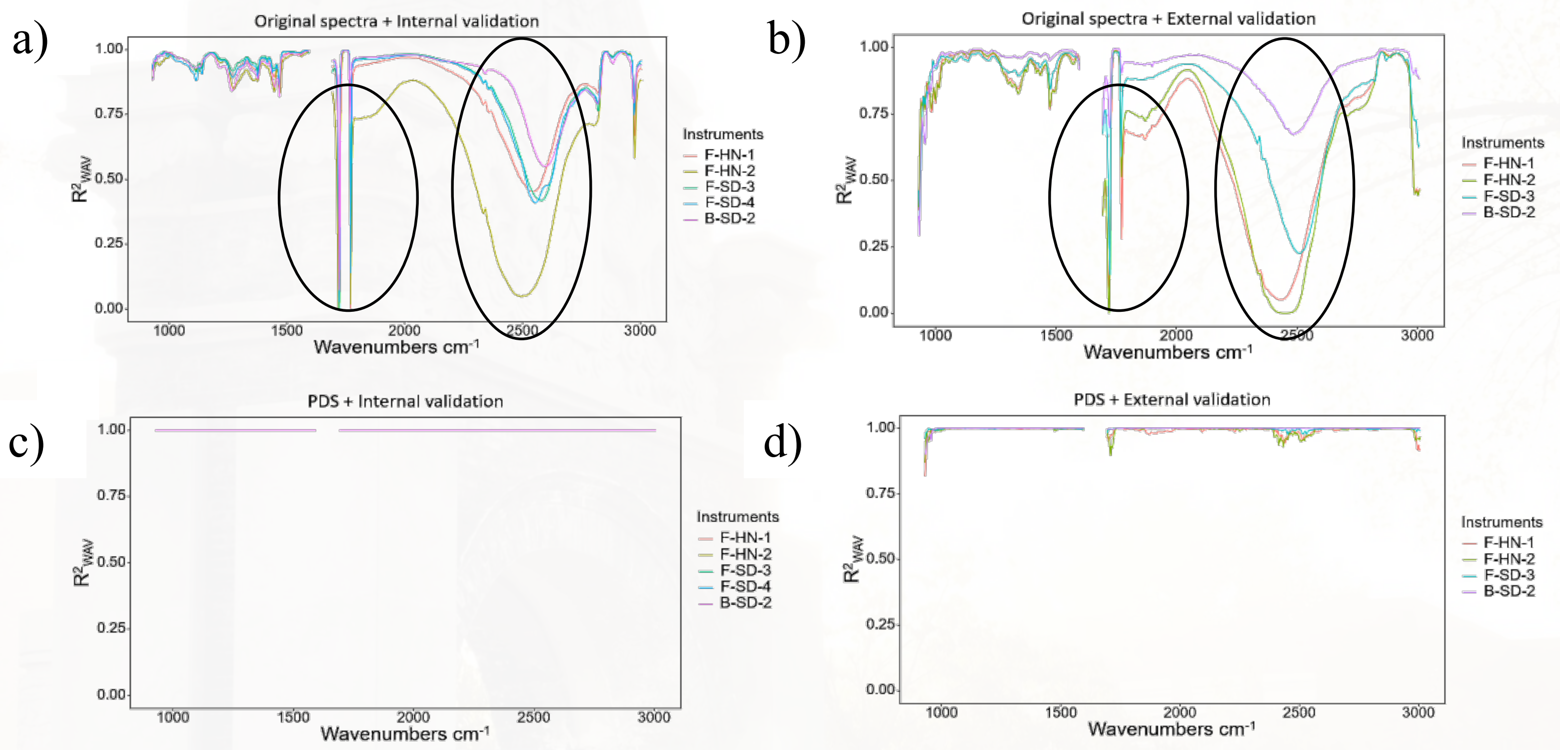
$$\mathbf{s_j} = \left[S_{(j-n)},\ldots,S_{(j)},\ldots,S_{(j+n)}\right]$$

$$m_j = \mathbf{s_j}\mathbf{b_j} + b_{0j}$$

$$(\mathbf{S_{new}})_{\mathbf{std}} = \mathbf{S_{new}}\mathbf{F} + \mathbf{b}_0$$



Results: MIR spectra before and after standardization



Y-axis is R^2_{Wav}: The coefficient of determination between primary and secondary instruments' spectra

- ✓ Some regions showed significant differences before standardization
  - ✓ Prediction models utilize the spectra from this region, standardization may be even more necessary
- ✓ PDS can effectively reduce the variations in spectra among instruments