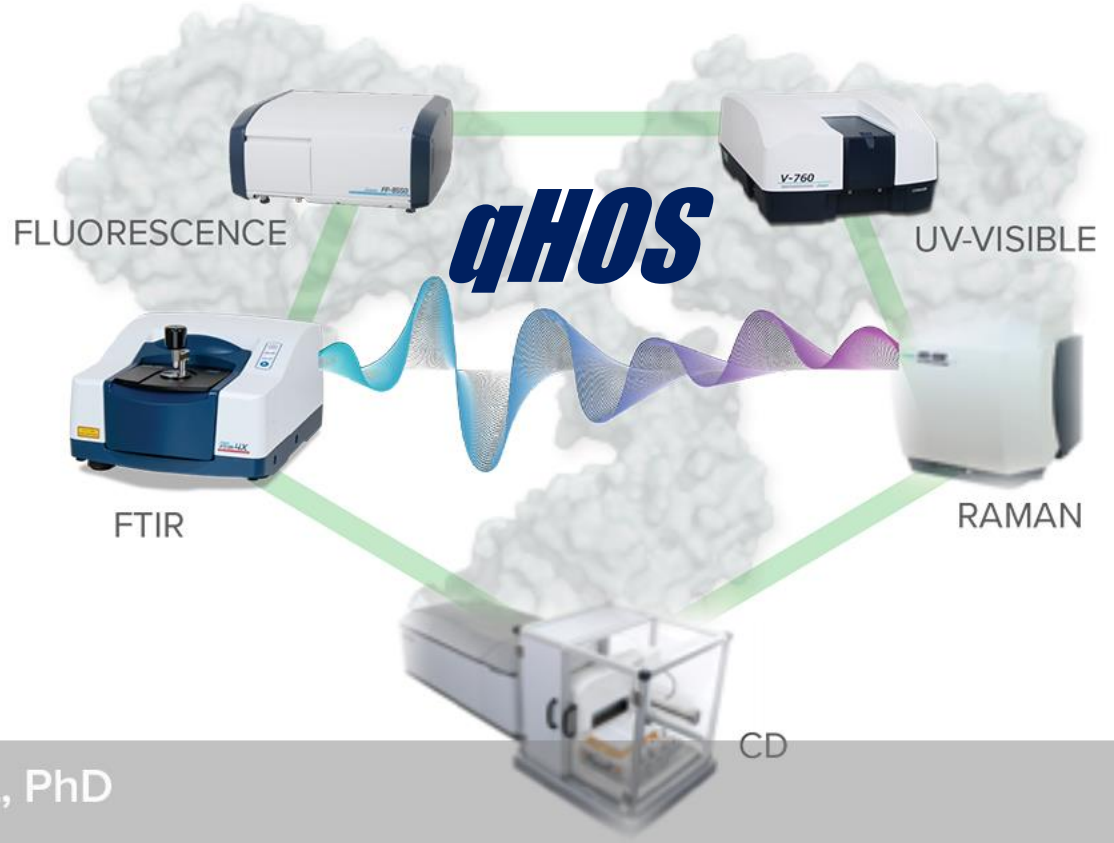
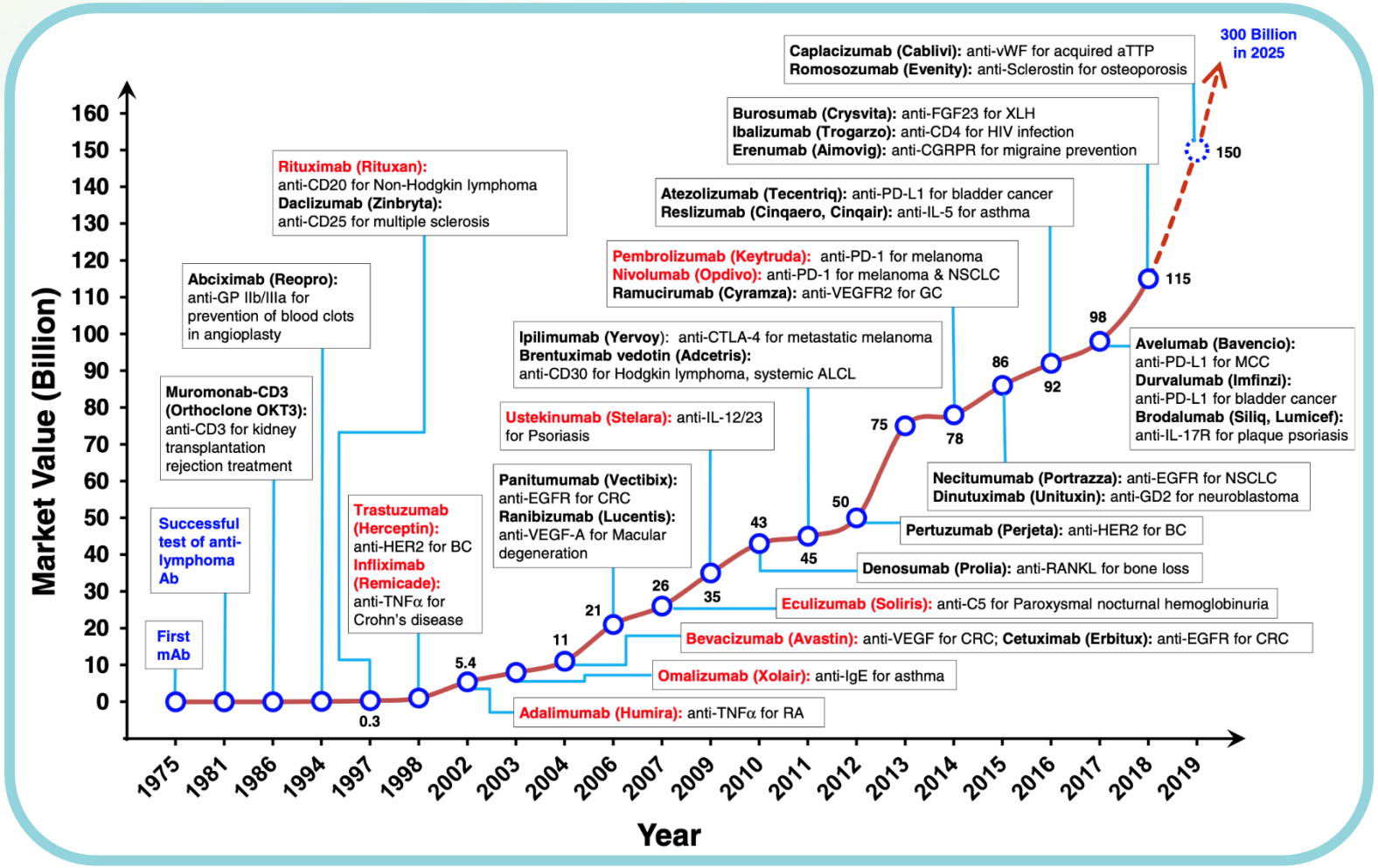
Orthogonal Similarity Assessment of Monoclonal Antibodies Using **CIRCULAR DICHROISM, FTIR** and **RAMAN**



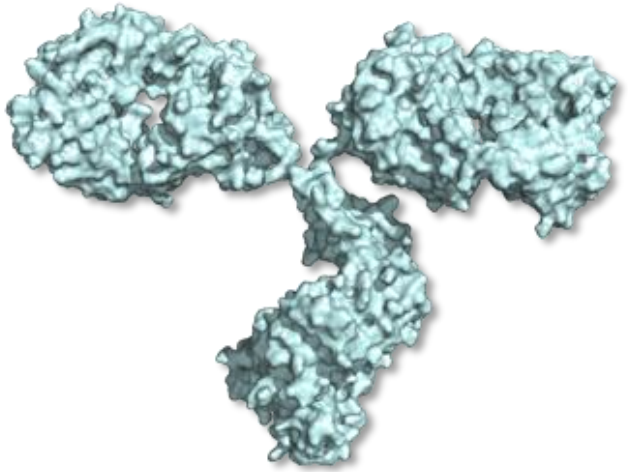
WEBINAR WITH **FORREST KOHL, PhD**

JASCO

Antibody therapeutics market



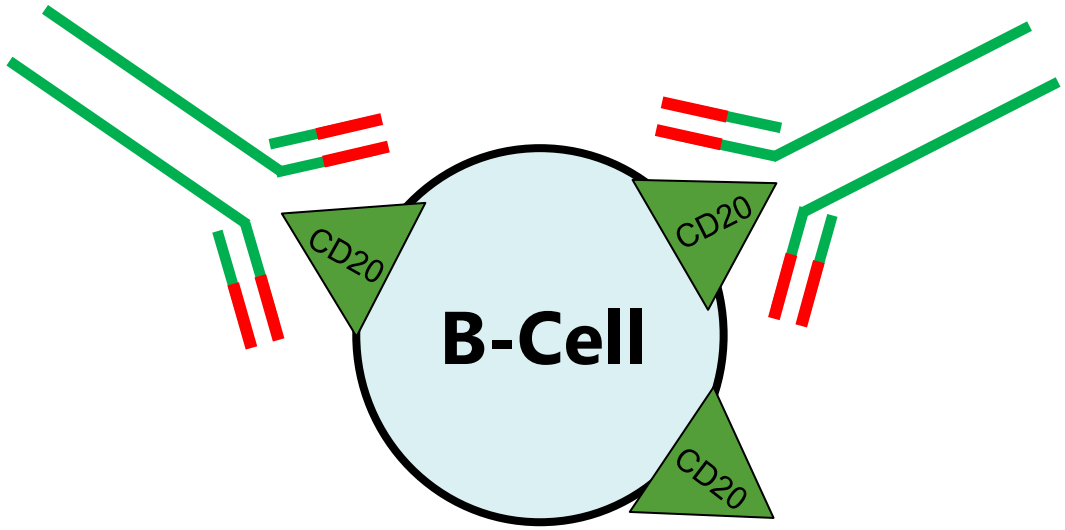
CGCR of therapeutic monoclonal antibody is nearly 10% !!



Lu et al. *J. Biomed. Sci.* (2020) 27:1. https://doi.org/10.1186/s12929-019-0592-z

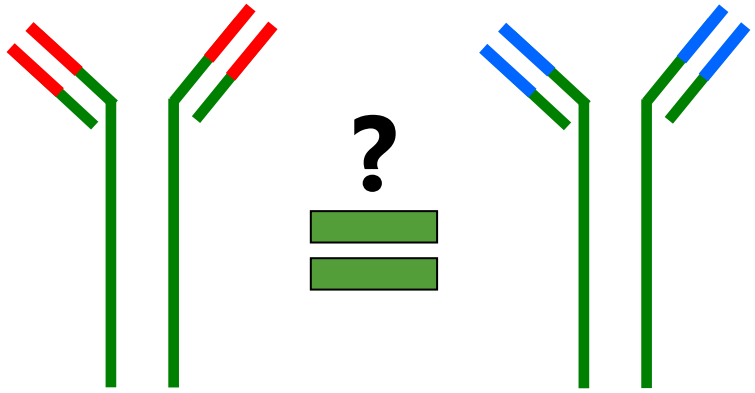
Rituximab

Rituximab (Rituxan):
anti-CD20 for Non-Hodgkin lymphoma
Daclizumab (Zinbryta):
anti-CD25 for multiple sclerosis



Rituximab binds to CD20 protein in B-cell membrane

Structure needs to be correct to bind effectively



Necessity of objective assessment

INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED TRIPARTITE GUIDELINE

COMPARABILITY OF BIOTECHNOLOGICAL/BIOLOGICAL PRODUCTS SUBJECT TO CHANGES IN THEIR MANUFACTURING PROCESS

Q5E

INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE

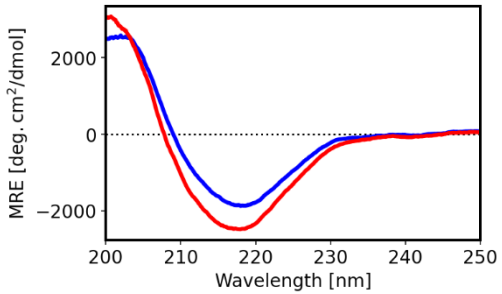
ICH HARMONISED TRIPARTITE GUIDELINE

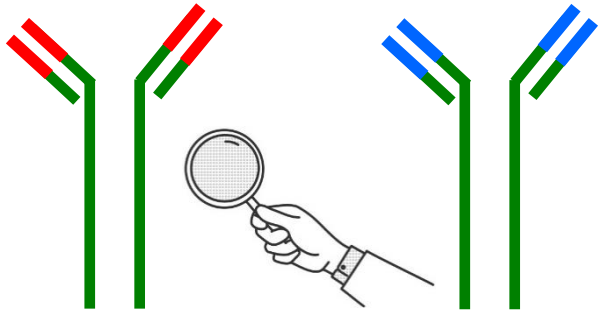
SPECIFICATIONS: TEST PROCEDURES AND ACCEPTANCE CRITERIA FOR BIOTECHNOLOGICAL/BIOLOGICAL PRODUCTS

Q6B

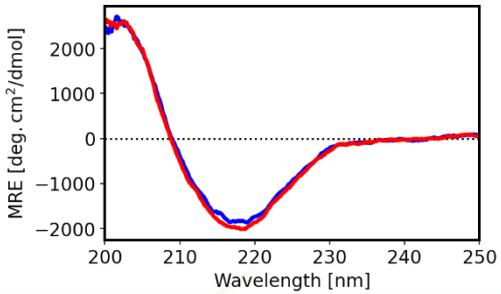
Development of Therapeutic Protein Biosimilars: Comparative Analytical Assessment and Other Quality-Related Considerations

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)





Are there significant differences in these spectra ?



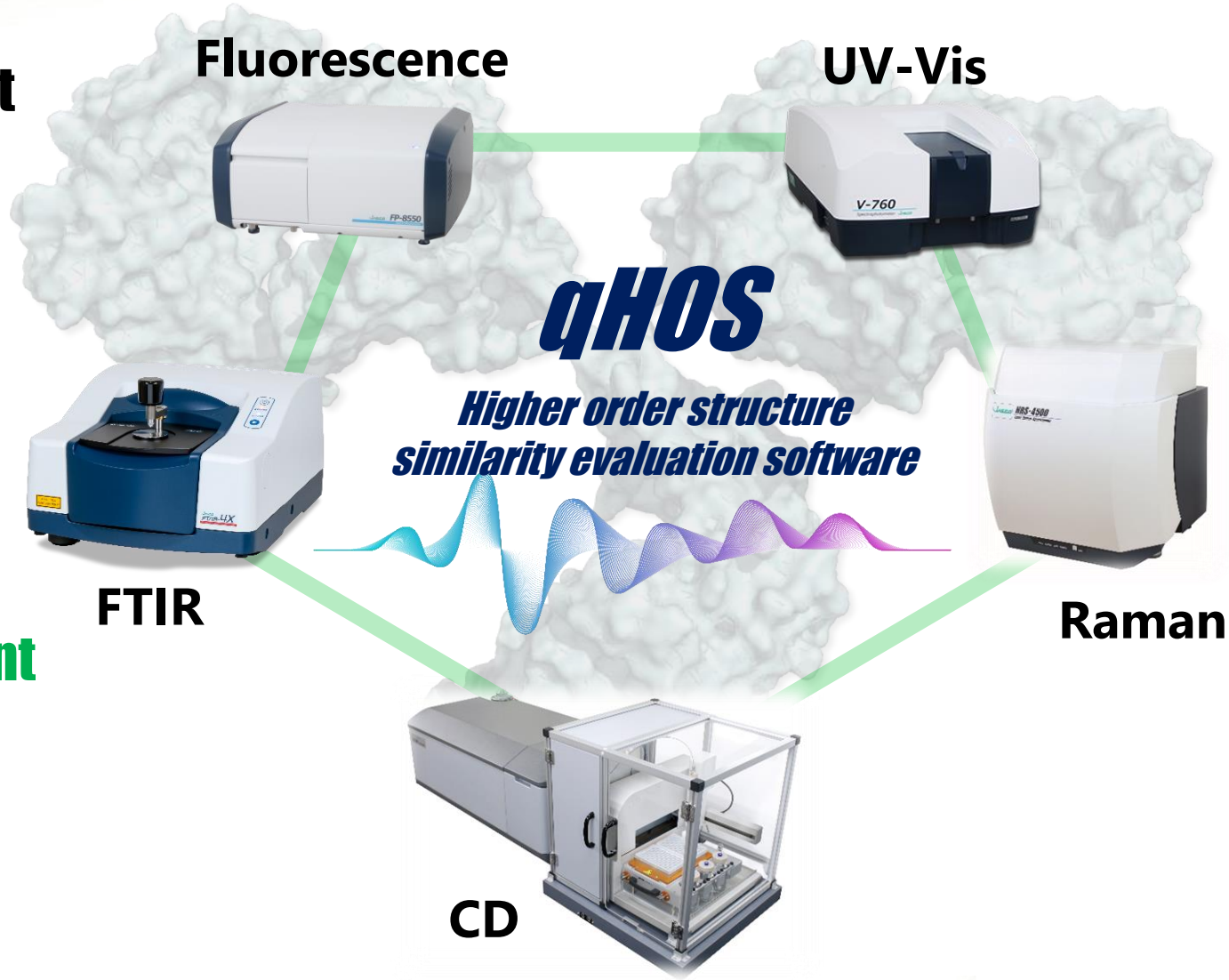
Necessity of objective assessment

"Emphasis should be placed on developing orthogonal quantitative methods to definitively identify any differences in product attributes."

From FDA Guidance (*Development of Therapeutic Protein Biosimilars: Comparative Analytical Assessment and Other Quality-Related Considerations)*

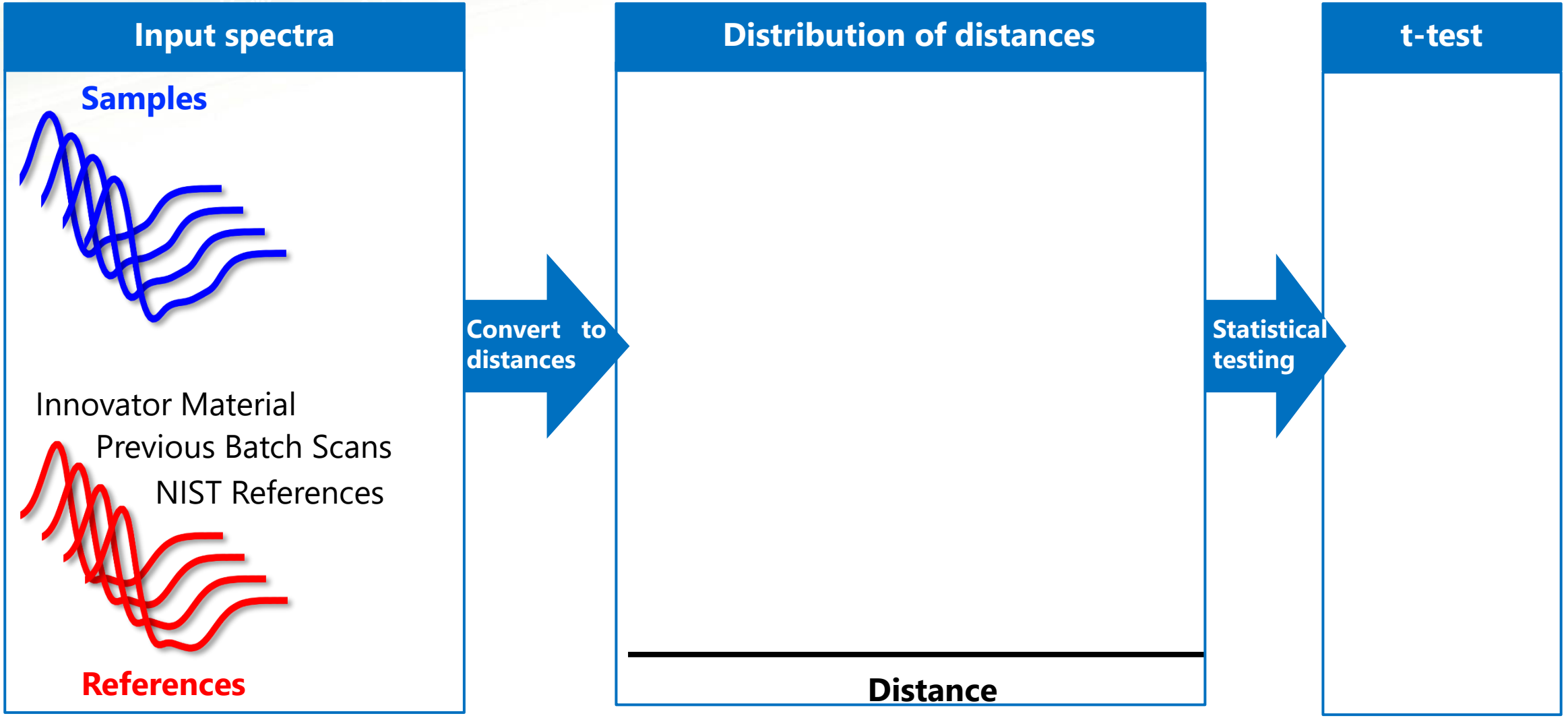
qHOS Concept and strong point

- Statistical similarity assessment
- Robust evaluation using noise weighting method
- Student, Welch, TOST t-test implementation
- Auto concentration correction
- Orthogonal similarity assessment
- Regulatory compliance with spectra manager CFR™

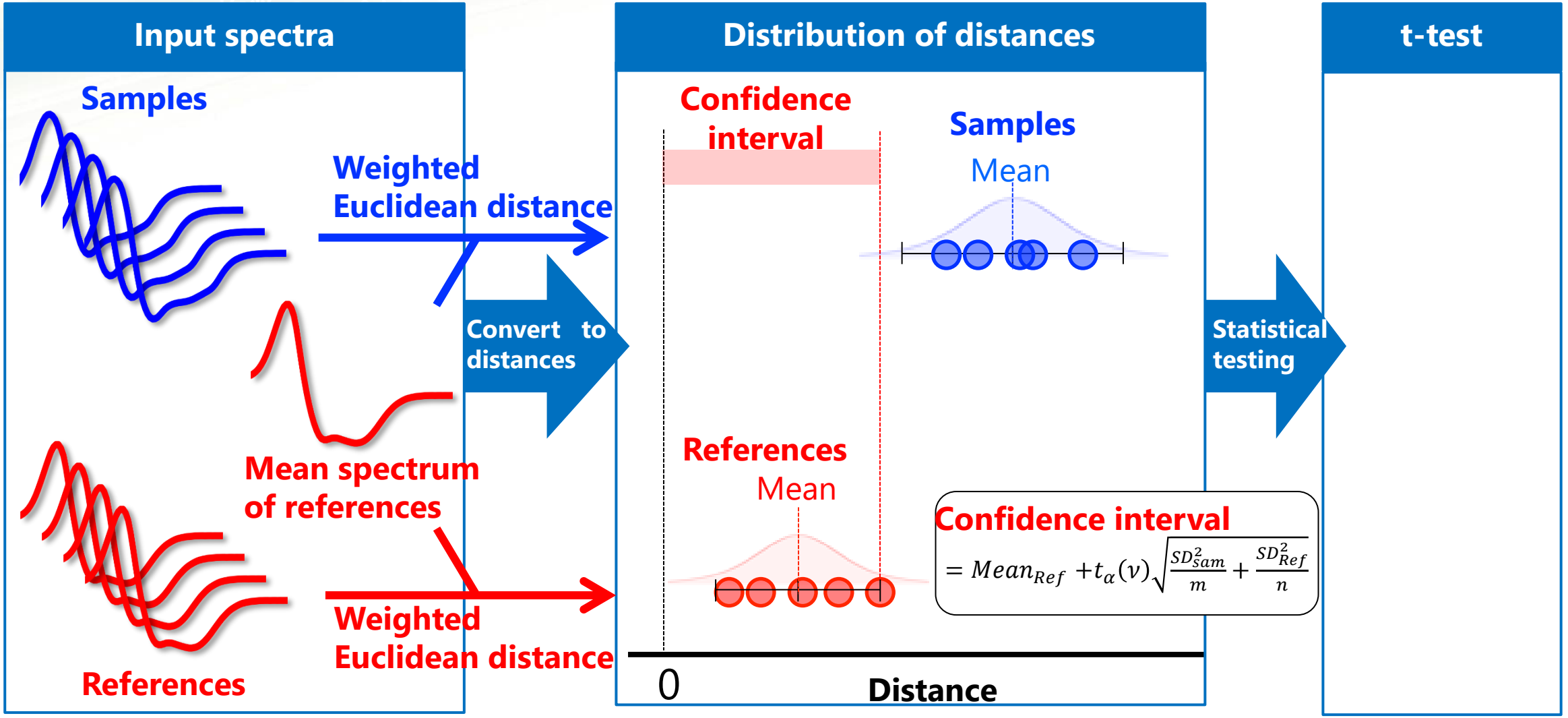


Scheme of *qHOS*

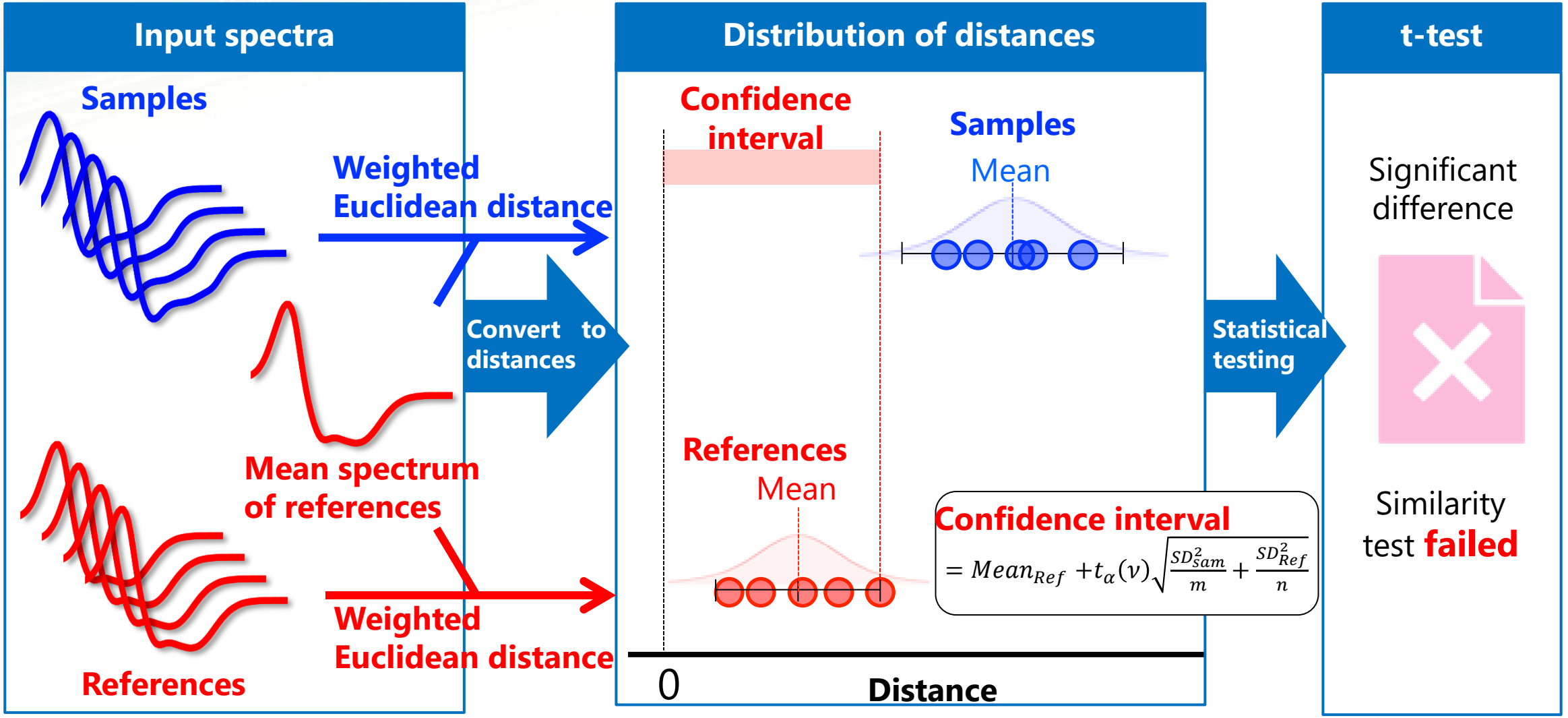
Scheme of *qHOS*



Scheme of *qHOS*



Scheme of *qHOS*

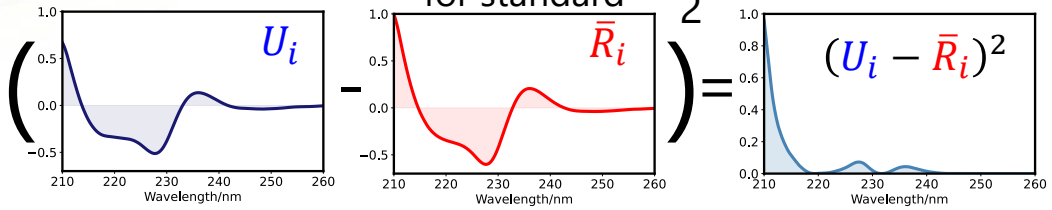


Similarity testing scheme of the qHOS

Calculation of distance between standard and sample spectra

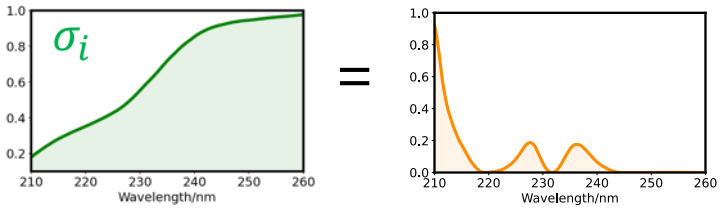
1

Sample spectrum U_i − Average spectrum for standard $\bar{R}_i$ $)^2 = (U_i - \bar{R}_i)^2$



2

$(U_i - \bar{R}_i)^2$ **x** Weighting function σ_i = $(U_i - \bar{R}_i)^2\sigma_i$



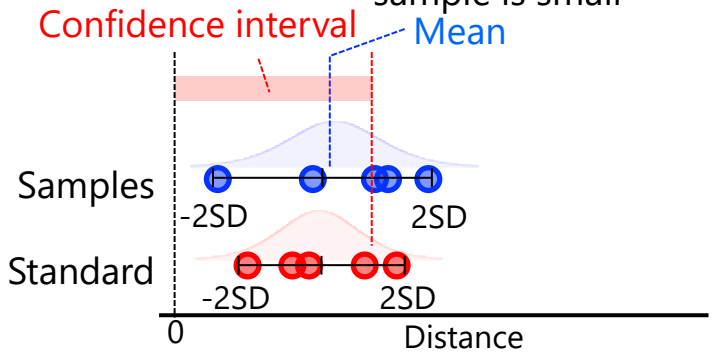
3

Weighted Euclidean distance:

$$E = \sqrt{\frac{1}{n}\sum_{i=\lambda_1}^{\lambda_n}(U_i - \bar{R}_i)^2\sigma_i}$$

Evaluate difference in distance between standard and sample

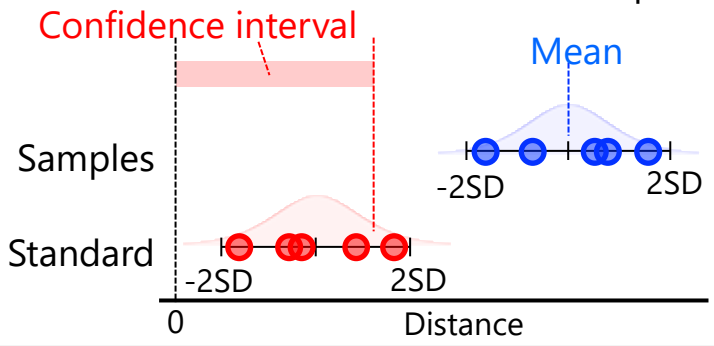
The difference in distance between the standard and the sample is small



No difference

Similarity test **passed**

There is a large difference in the distance between the standard and the sample



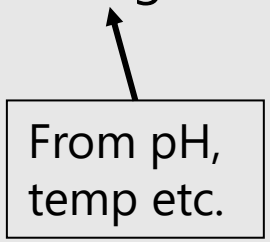
Significant difference

Similarity test **failed**

Confidence interval $= Mean_{std} + t_\alpha(\nu)\sqrt{\frac{SD_{sam}^2}{m} + \frac{SD_{std}^2}{n}}$

$t_\alpha(\nu)$: t-value at significance level α
m : Number of sample
n : Number of standard

Weighting method for Euclidean distance

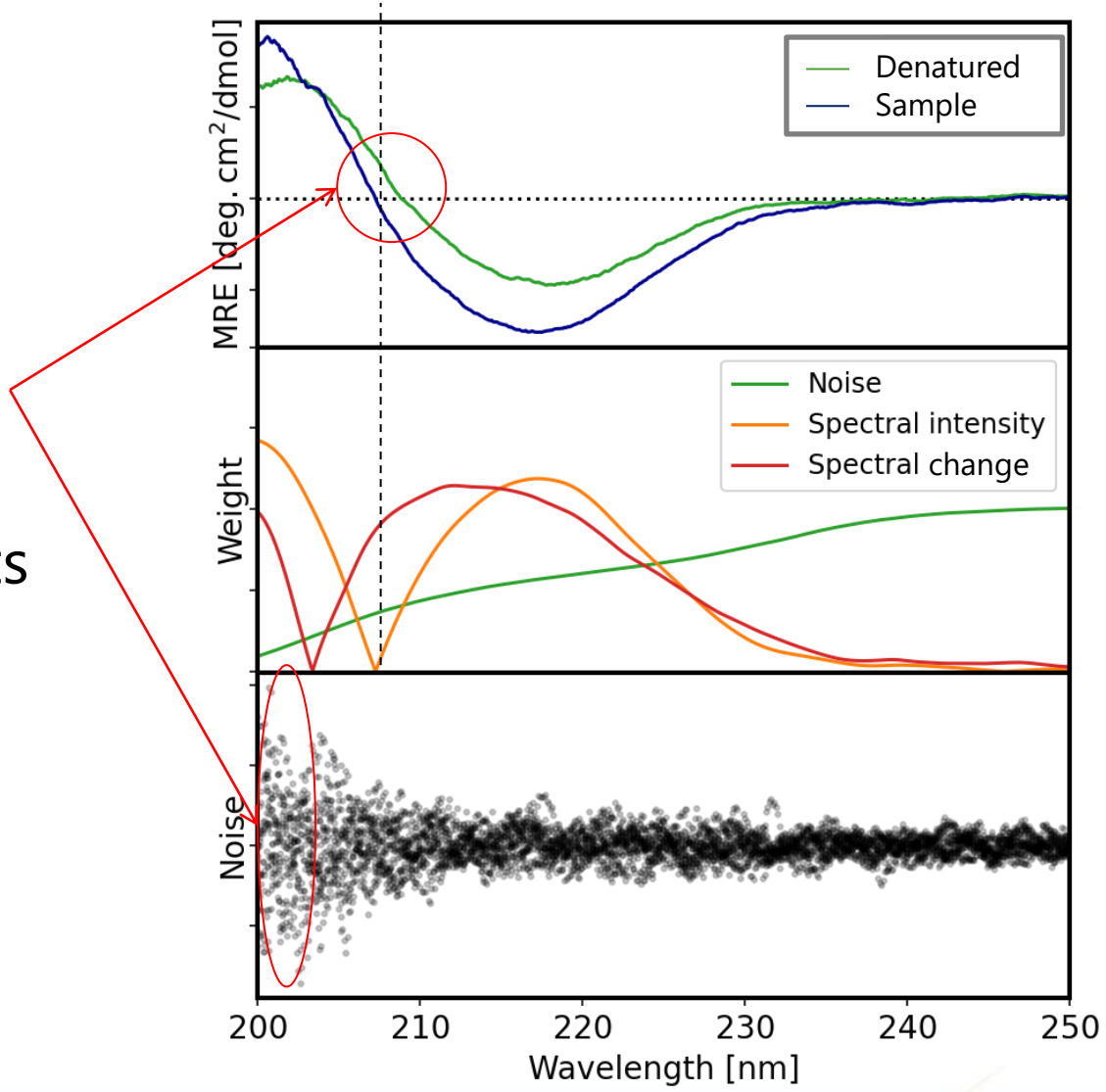
Method	Feature	Details
Noise **JASCO**	Weighting to spectral regions with low noise	Uses the noise (light level) in the spectrum and use it as a weighting function
Spectral intensity (Weighted spectral distance; WSD)	Weighting to spectral regions with high spectral intensity	Uses the absolute value of the intensity of the spectrum as a weighting function
Spectral change **JASCO** 	Weighting the regions where the spectrum changes	Perturbs the reference, examines the region where the spectrum changes, and use it as a weighting function

Problem of spectral intensity weighting

Example of similarity assessment

Spectral intensity weighting erases inherent spectral differences and weights noisy regions.

Spectral intensity weighting weights regions of high intensity, but since CD spectra are created by a combination of positive and negative Cotton effects, actual changes do not always occur in regions of high intensity.



Three types of t-test

Test	Description
Student's t-test	This method tests for significant differences in spectral distances, taking into account the variance in reference spectral distances.
Welch's t-test	This method tests for significant differences in spectral distances, taking into account the variances in both standard and sample spectral distances. Data for multiple reference and unknown samples are used.
TOST (Equivalence t-test)	This method tests for significant differences in spectral distances, taking into account the variances in both reference and sample spectral distances. In this method, a range (equivalence margin)is set in which the distance of each reference and the distance of each sample are equivalent. This test is based on the guidelines for similarity tests by the FDA and ICH.

TOST vs Welch's T-test

If we run **Samples** and compare to
a limit based on a **Reference**

T-Test asks: "Are the samples different"

Fail → Products are different

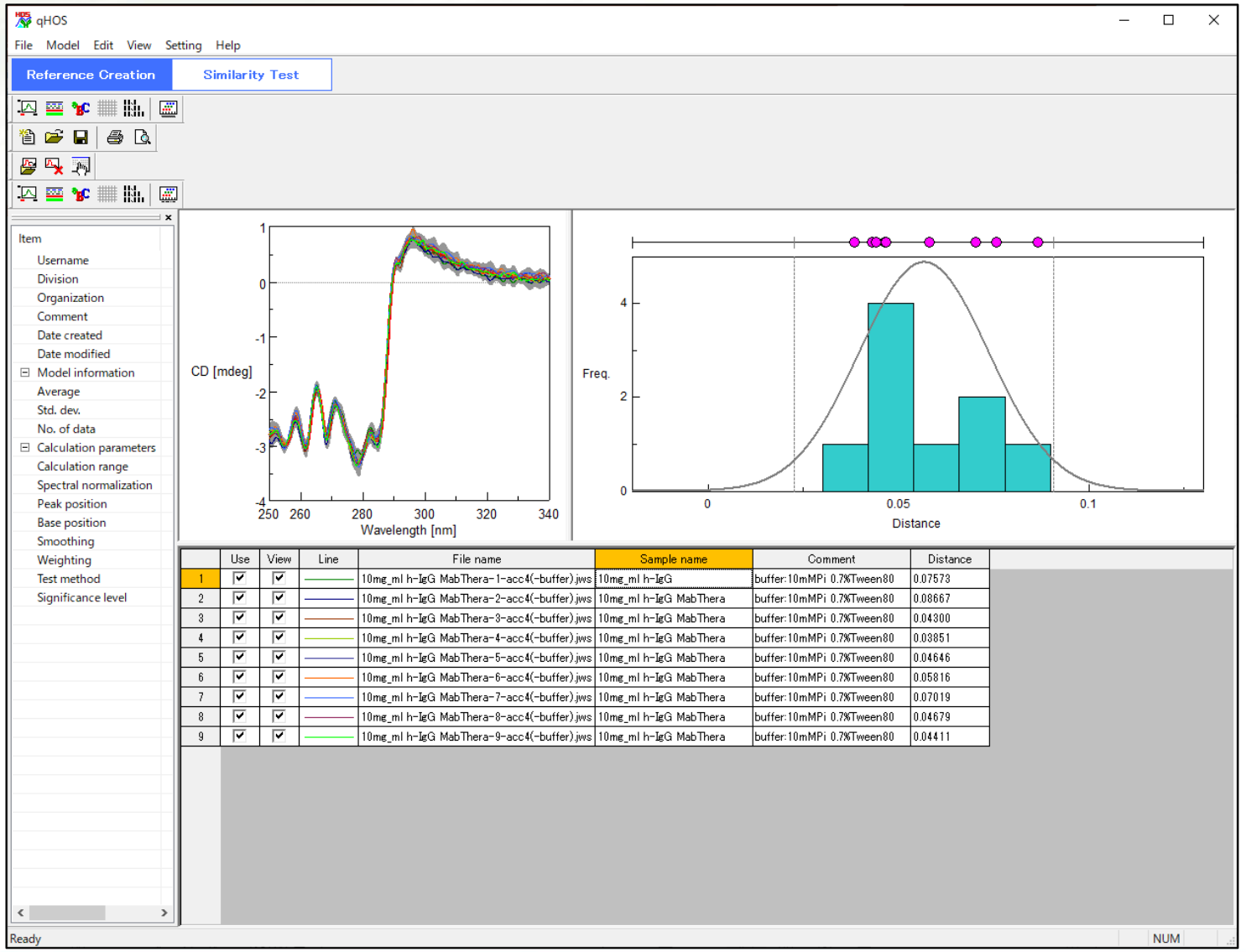
Pass→ Products are not necessarily the same, we just can't prove they are different.

TOST asks: "Are the samples the same?"

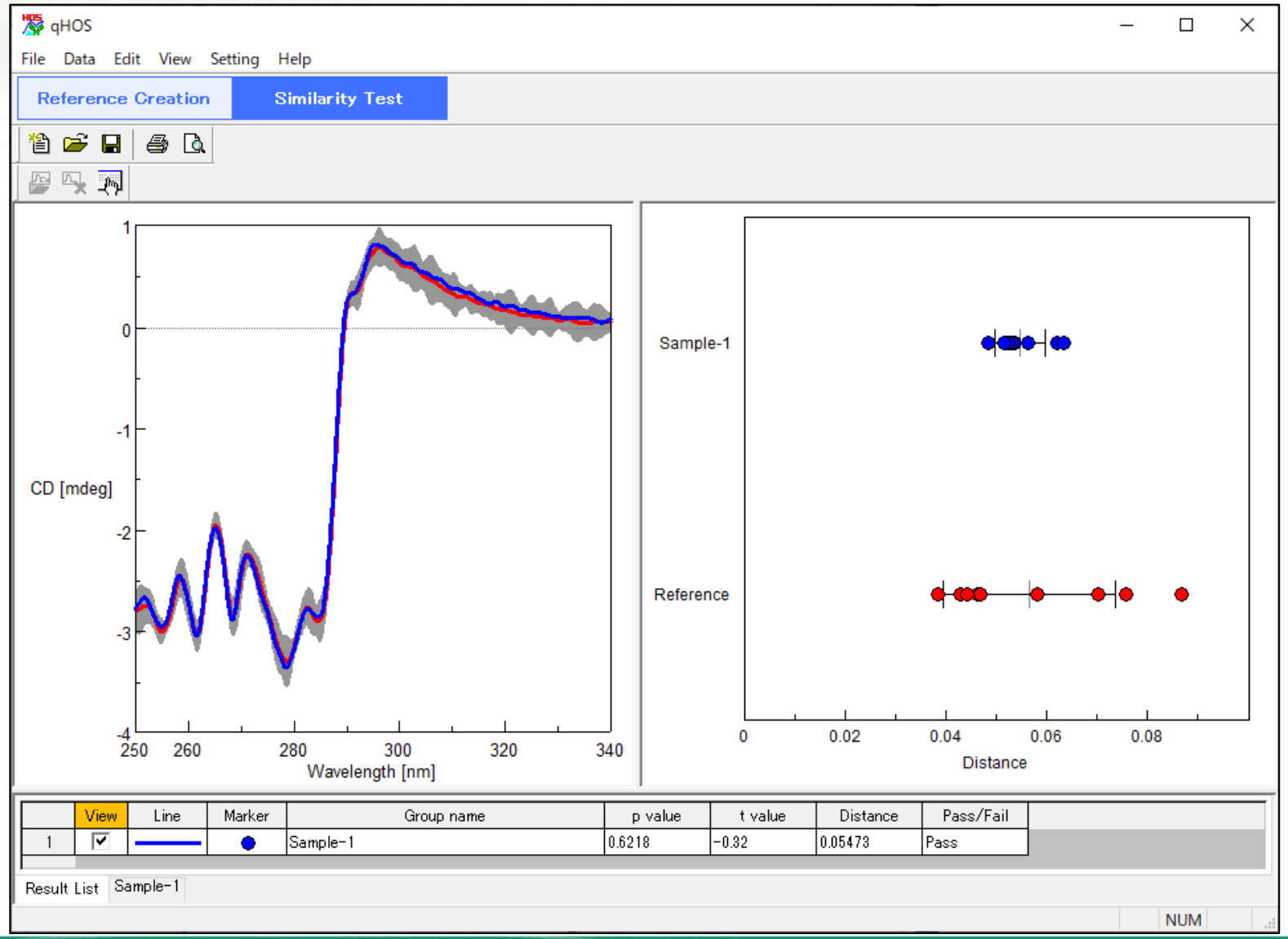
Fail → Products are not the same.

Pass→ Products are equivalent

User interface: Reference Creation



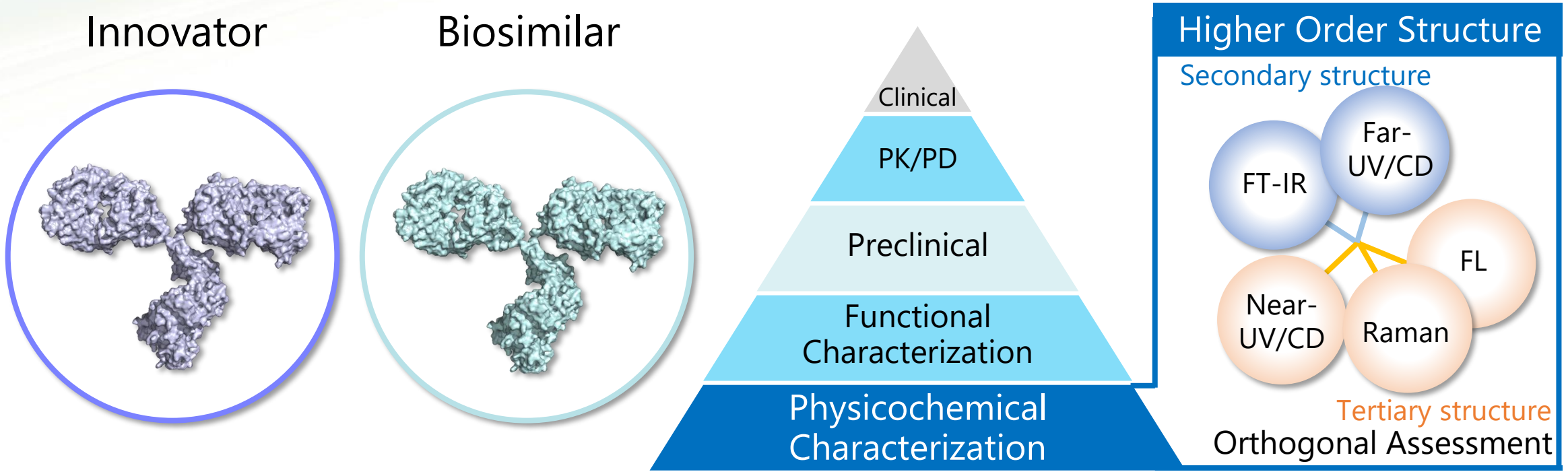
User interface: Similarity Test



qHOS Application

Raman, FTIR, and CD of Monoclonal Antibodies

HOS similarity assessment for biosimilar



Increasing the importance of analytical characterization of biosimilars.
Regulatory authorities recommend orthogonal assessment of biosimilar quality attributes using multiple instruments based on different principles.

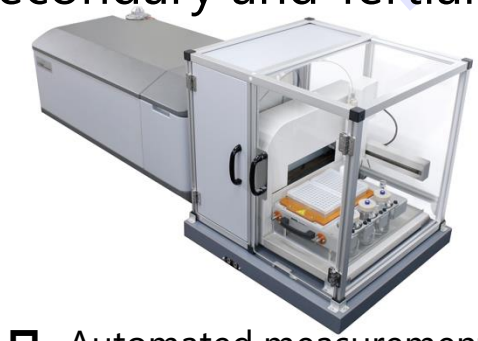
System for comprehensive orthogonal assessment

JASCO products

qHOS
Higher order structure similarity evaluation software

- Statistical significance testing
- Support for multiple instruments

HTCD Plus
Circular Dichroism
Secondary and Tertiary



- Automated measurement
- High throughput and high sensitivity

FT/IR-4X
Infrared spectrometer
Secondary

- High S/N and resolution with small body
- One drop ATR measurement

NRS-4500
Raman microscope
Tertiary

- Microanalysis
- Chemical imaging

Multiple spectroscopic techniques provide orthogonal similarity assessments of secondary and tertiary structures, and data can be statistically analyzed using the single qHOS platform.

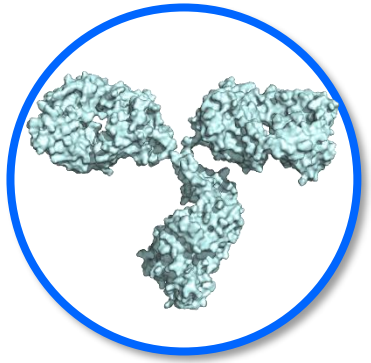
Materials and Methods

Materials

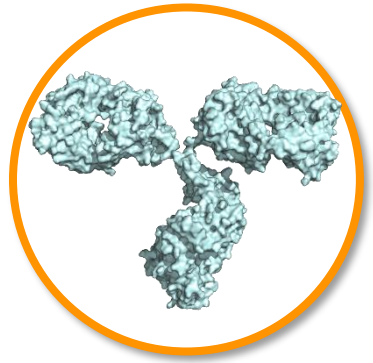
Rituximab

MabThera® (Innovator)
RIABNI™ (Biosimilar)
Anti CD20 monoclonal antibodies
Both samples were prepared to 10 mg/mL
Additive : Sodium citrate dihydrate 7.4 mg/mL,
Sodium chloride 9.0 mg/mL, Sodium hydroxide 9.0 mg/mL,
Polysorbate 80 0.7 mg/mL

MabThera®



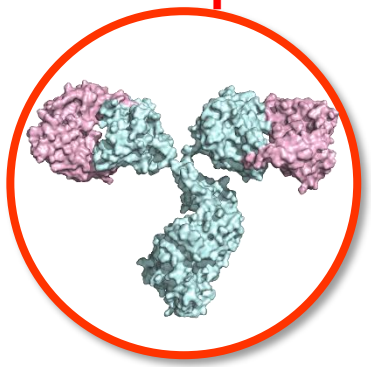
RIABNI™



Trastuzumab

Herceptin® (Innovator)
Anti HER2 monoclonal antibody
The powder was dissolved in H_2O to a concentration of 10 mg/mL
Additives: trehalose hydrate 4.7 mg/mL,
L-histidine hydrochloride hydrate 0.11 mg/mL,
L-histidine 7.4 x 10^{-2} mg/mL, polysorbate 2.1 x 10^{-2} mg/mL

Herceptin®



Materials and Methods

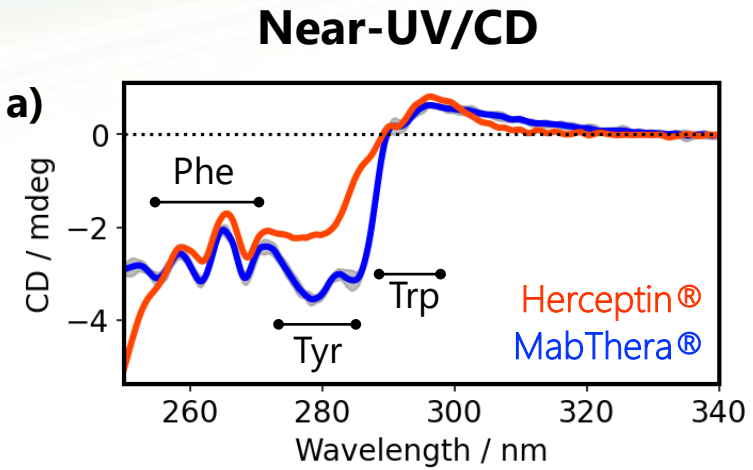
Methods

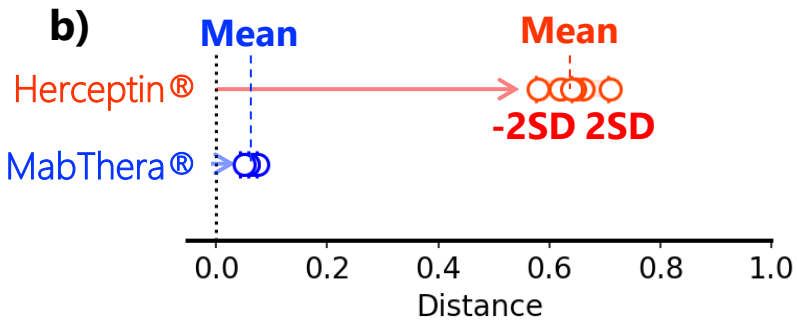
Far-UV/CD	
Optical path length : 0.2 mm	Auto washing : On
Band width : 1.0 nm	Scanning speed : 20 nm/min
Near-UV/CD	
Optical path length : 0.5 mm	Auto washing : On
Band width : 1.0 nm	Scanning speed : 50 nm/min
ATR FT-IR	
Detector : TGS	ATR crystal : Diamond
Resolution : 4cm^{-1}	Number of scans : 128
Raman	
Laser : 532 nm	Grating : 900 gr/mm
Exposure : 45 sec	Number of scans : 32
Analysis	
Weighting : Noise	
Significance level : 0.05 (95% confidence Interval)	

4 measurements

1 Method

Similarity of tertiary structure for different antibody drugs





Result of t-test
t-value = 49
p-value = 0.0
Similarity **failed**

Figure 1. Similarity assessment of the tertiary structure of MabThera® and Herceptin®, antibody drugs with different targeting and formulation conditions.

The shapes of the near-UV/CD spectra of MabThera® and Herceptin® differ significantly (Fig. 1a). Similarly, the distribution of distances between MabThera® and Herceptin calculated from the CD spectra show a significant difference (Fig. 1b). The p-value obtained from the t-test is below the significance level of 0.05, indicating that Herceptin® has a different tertiary structure to MabThera®.

Similarity assessment for secondary structure of biosimilar

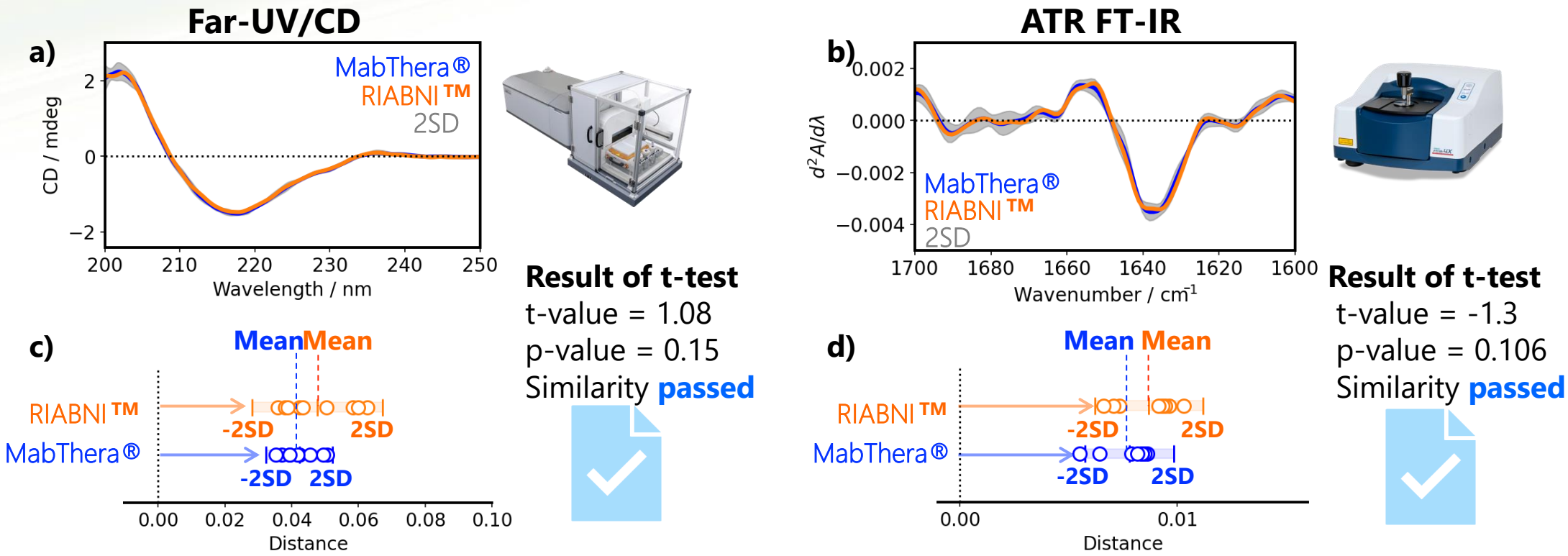


Figure 2. Orthogonal similarity assessment for the secondary structure of MabThera® and RIABNI™

The far-UV/CD and FTIR spectra of the biosimilar RIABNI™ are in excellent agreement with those of the innovator MabThera® (Figs. 2a and 2b), and the distributions of the distances between MabThera® and RIABNI™ are close to each other (Figs. 2c and 2d). The p-value is larger than the significance level of 0.05.

Similarity assessment for tertiary structure of biosimilar

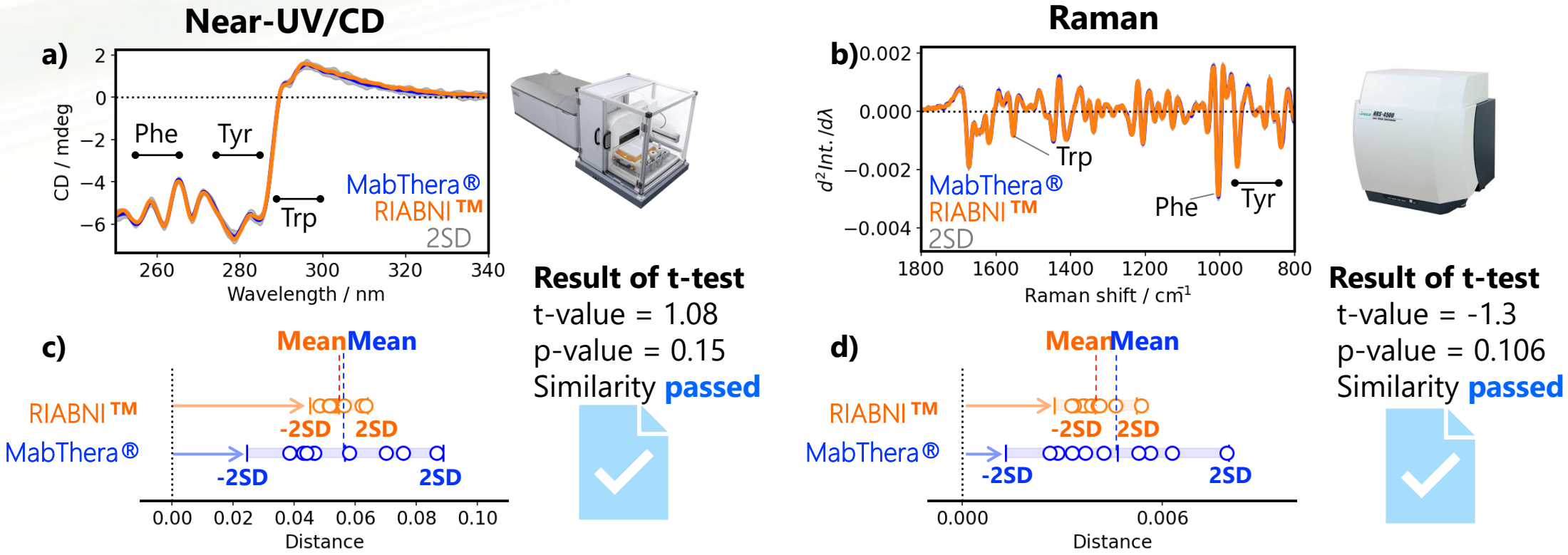
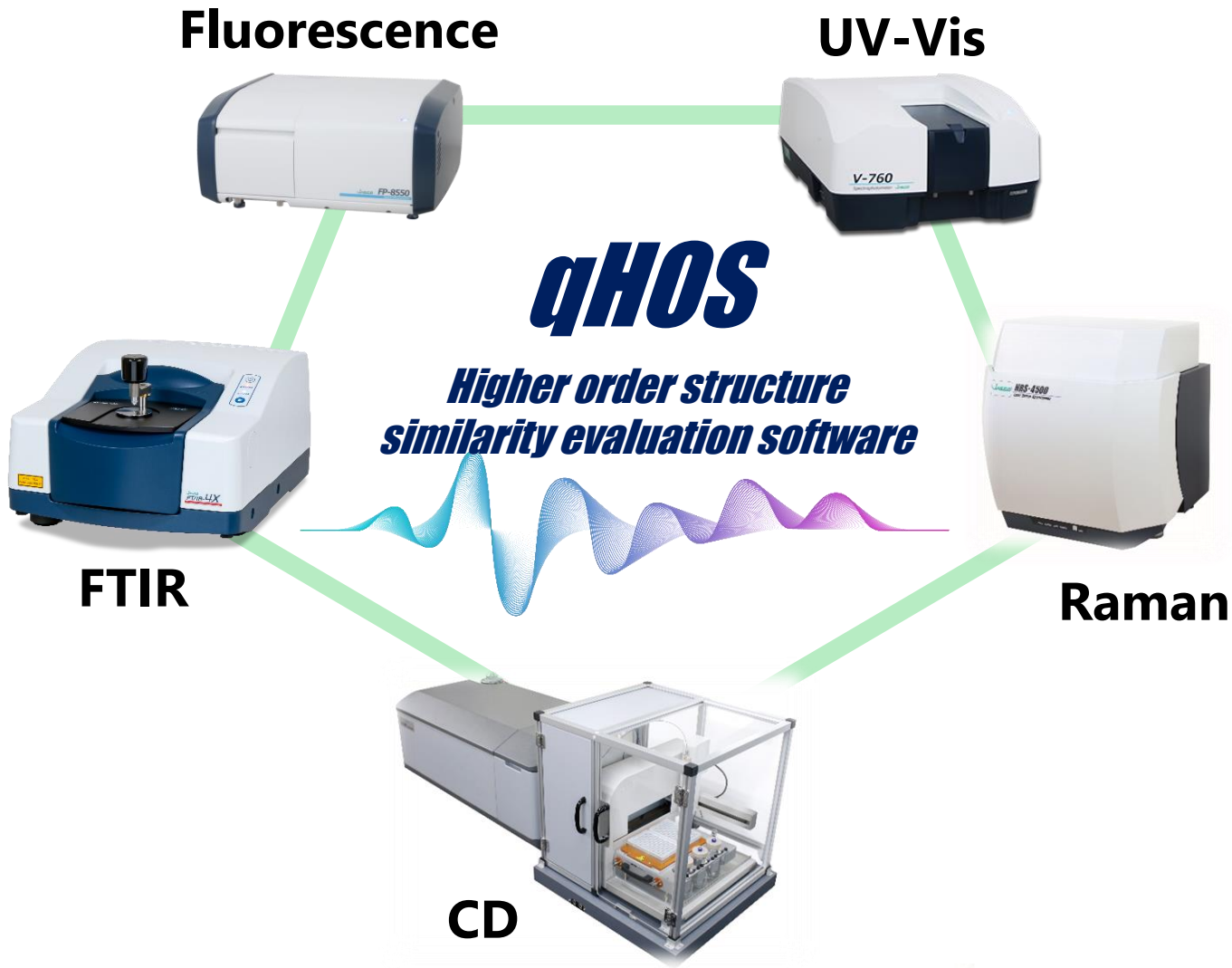


Figure 3. Orthogonal similarity assessment for the tertiary structure of MabThera® and RIABNI™

Similar to the secondary structure, the tertiary structure of MabThera® and RIABNI™ show excellent agreement in the near-UV/CD and Raman spectra (Figs. 3a and 3b), and the distribution of the distances between MabThera® and RIABNI™ are close to each other (Figs. 3c and 3d). The p-value is larger than the significance level of 0.05.

qHOS

- Statistical similarity assessment
  - For any sample
- Robust evaluation using noise weighting method
- Student, Welch, TOST t-test implementation
- Auto concentration correction
- Orthogonal similarity assessment
- Regulatory compliance with spectra manager CFR™



JASCO Educational Resources

Many Webinars:

- Circular Dichroism
- FTIR Theory, Instrumentation, and Techniques
- Raman Microscopy and Imaging
- SFC Theory and Applications
- VCD

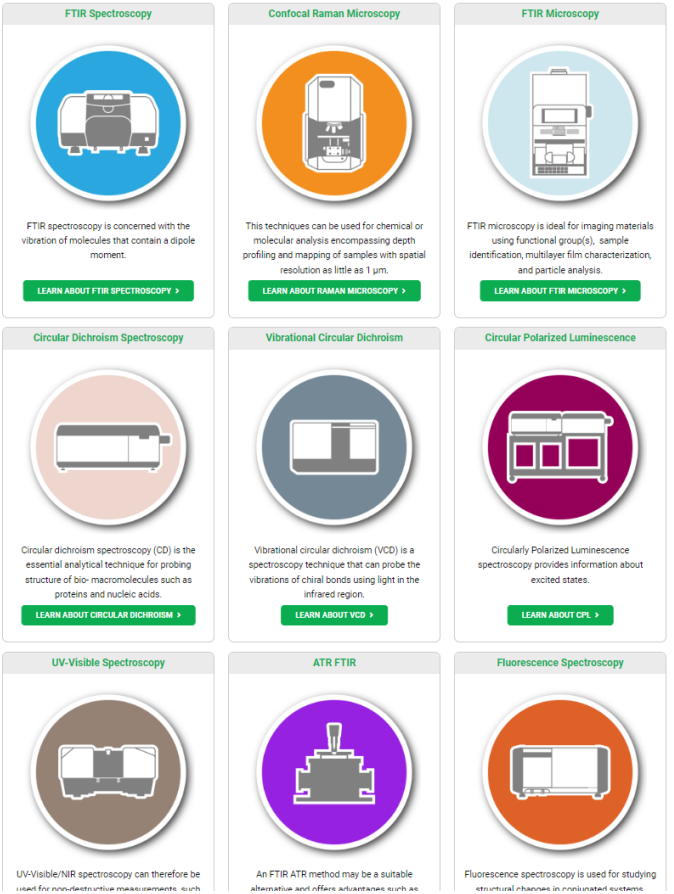
E-books and Posters

- Raman
- Fluorescence
- FTIR
- CD

KnowledgeBase

Repository of literature categorized by technique and field.

Theory explanation:



Thank You For Attending!

Questions?