Tech note

Automated high-throughput polymorph/crystallization screening through Kapton plates

A powerful application in pharmaceutical development using Raman spectroscopy and microscopy

Authors

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Thermo Scientific DXR3 Raman Microscope

Thermo Scientific DXR3 SmartRaman Spectrometer

Active pharmaceutical ingredients (APIs) are most often produced via crystallization. During the crystallization process, an organic molecule can adopt more than one crystalline form. Morphology and particle size distribution are important solid-state characteristics; however, uncontrolled occurrences of multiple physical forms—polymorphs, solvates cocrystals or amorphous—of APIs can have significant effects on the performance of the material during processing, manufacturer storage, and administration of the drug. For instance, solubilities can differ by as much as a factor of four between some polymorphs of the same API.

Crystallization screening is the process of evaluating methods, reagents, and other chemical and physical variables with the objective of identifying the variables which are positively or negatively associated with crystallization of the sample. In the early stages of development, only a few hundred milligrams of compound may be available for solid form screening; in such cases, small-scale, high-throughput approaches using multi-well plates can prove to be very useful.

Analysis and characterization of solid forms

Regardless of the method used to generate crystallized samples of the API after the formation of the crystallized solid, the next step is to identify the forms produced. There are several analytical techniques used to unambiguously identify novel polymorphs or mixtures of solid forms. There are also several techniques which can be used to distinguish between different crystalline or amorphous forms of API. Raman spectroscopy and Raman microscopy are among the most useful.

When crystallization is done using high throughput methods, a small amount of recrystallized material is deposited nonuniformly around the base or on the walls of the vessel. Locating the position of a crystal within each well can be sometimes challenging. However, using the Thermo Scientific™ DXR3 SmartRaman Spectrometer and Thermo Scientific™ DXR3 Raman Microscope with OMNIC™ Array Automation Software, one can locate the particles distributed nonuniformly on the base with ease.

In cases where metastable polymers have been formed that may quickly change to other forms, speed of analysis is particularly important. Here also Raman spectroscopy and microscopy are advantageous, as crystallized samples in the Kapton well plates can be directly transferred to the DXR3 Raman Microscope stage or to the DXR3 SmartRaman Spectrometer sampling accessory for analysis. This ensures direct sample measurement while reducing the risk of sample damage.

The figure below shows the spectrum of an API acquired through Kapton well plate and the spectrum of and empty well plate. The distinct lack of overlap between the two spectra shows that all the spectral features of the API (blue) are observed even when measuring through the well plate (red).

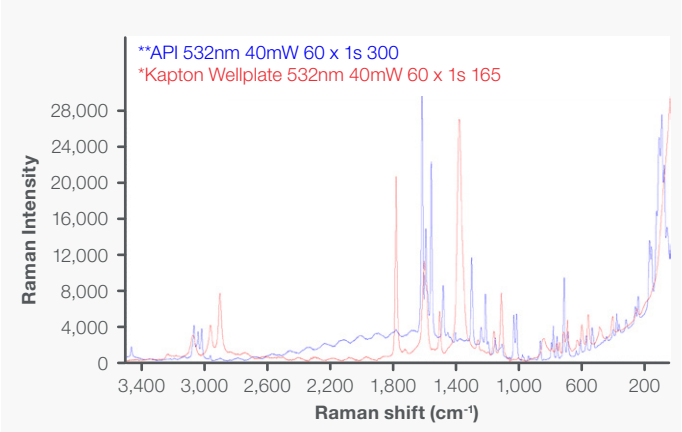


Figure 1. Spectrum of an API (blue) overlaid with spectrum of a Kapton well plate (red).

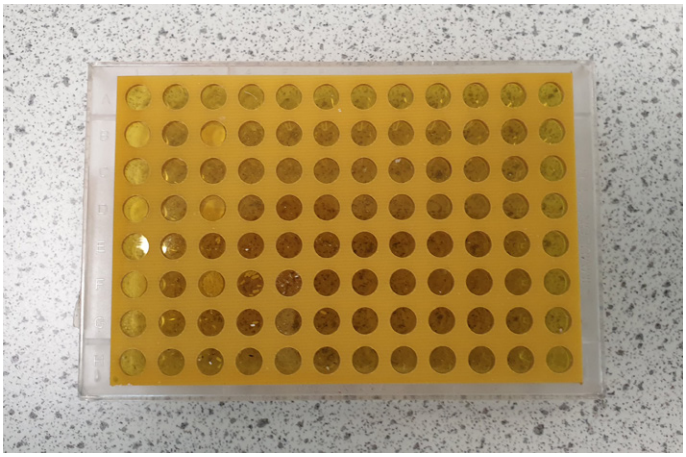


Figure 2. Kapton Plate.

There are several important parameters to consider when choosing Raman for high-throughput automated polymorph screening:

1. Sampling accessories—12-, 96-, 384-, and 1536-well formats
2. Ability to measure at multiple locations within the base of the well plate
3. Ability to measure through Kapton and other well plates without sampling
4. Automated data collection, analysis and two-way communication with LIMS
5. Compatibility with sampling robots

In this tech note we demonstrate the workflow for high-throughput polymorph screening using DXR3 Smart Raman.

There are four common polymorphs of TiO_2 including anatase, rutile, brookite and TiO_2-B. Anatase and rutile polymorphs were used to show the specific automated workflow. Raman spectra of anatase, rutile and a blank well (polystyrene) are shown below in Figure 3. Spectral features which help to decide the specific polymorph or form are present and are not obscured by measuring them through polystyrene well plate.

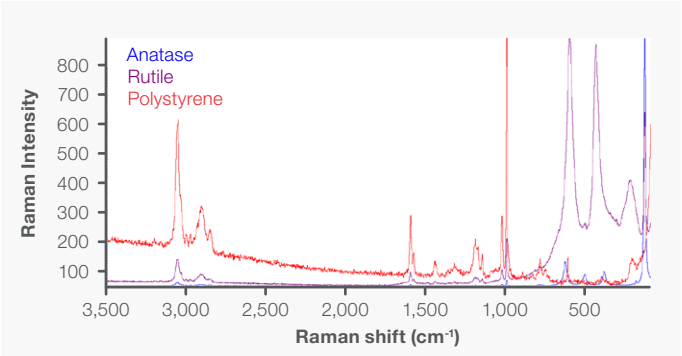


Figure 3. Raman spectra of anatase (blue), rutile (purple), and polystyrene (red).

After applying the Savitzky-Golay derivative to the spectra of rutile and anatase TiO_2, clear differences are seen in the spectra between the wavenumber range of 904-82 cm^{-1}.

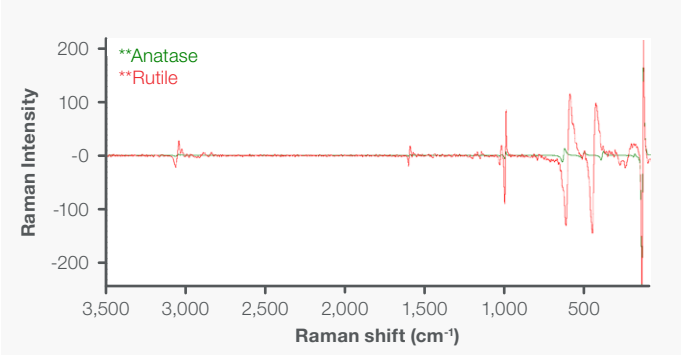


Figure 4. Savitzky-Golay derivative applied to the spectra of rutile (red) and anatase (green) TiO_2.

Preprocessed spectra can then be used for cluster analysis. Hierarchical cluster analysis is a procedure that non-subjectively clusters the spectra based on the similarities of their properties (spectral features). In this case cluster analysis is applied on the spectra acquired from samples in a 96 well plate; after the analysis well plates are color coded with each color representing a different form, the presence of different colors represents different types of forms.

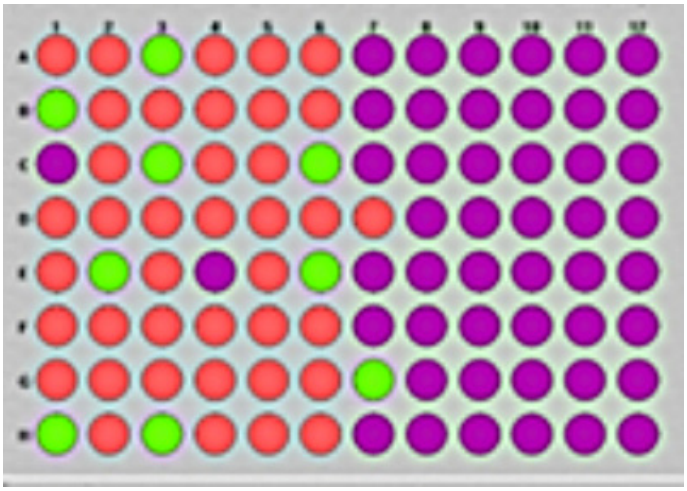


Figure 5. Well plates are color coded after analysis.

In this case three different colors are seen: orange and green represent the presence of one or the other type of polymorph, while purple indicates empty well plate positions. This type of analysis is a non-supervisory method and it quickly identifies the types of samples in different wells with clear indication using a color-coded map of the well plates.

A full report for all the wells is also available, which includes the well position and corresponding result according to the chosen method.

Title: WP1

Data file: C:\my documents\omnic\Array\WP1.srs

Sequence file: C:\my documents\omnic\VRParam\MicroTiter.ary

Comment:

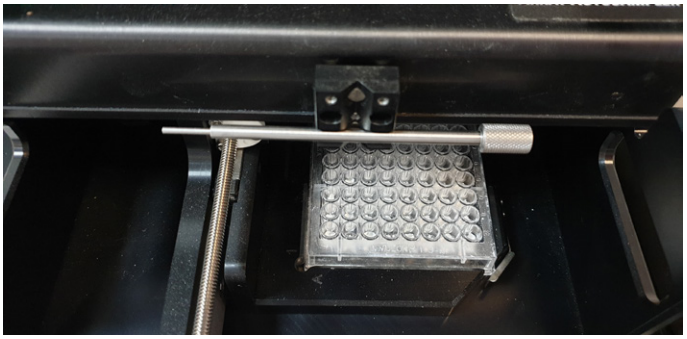
Method: Cluster analysis Region: 904.9 - 82.6

Similarity: 70.0

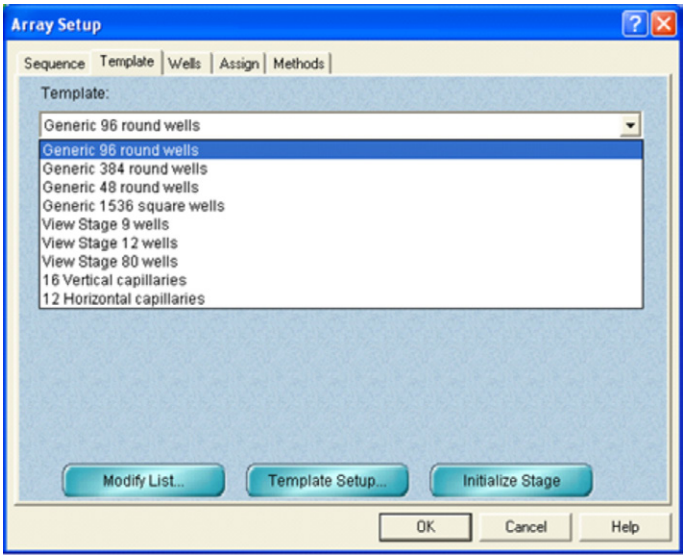
Well	Mode	X Pos	Y Pos	Z Pos	Cluster
A1	Center	-3	5	9220	2
A2	Center	5	5	9220	2
A3	Center	2	5	9220	1
A4	Center	0	5	9220	2
A5	Center	-3	5	9220	2
A6	Center	-5	5	9220	2

Workflow Steps:

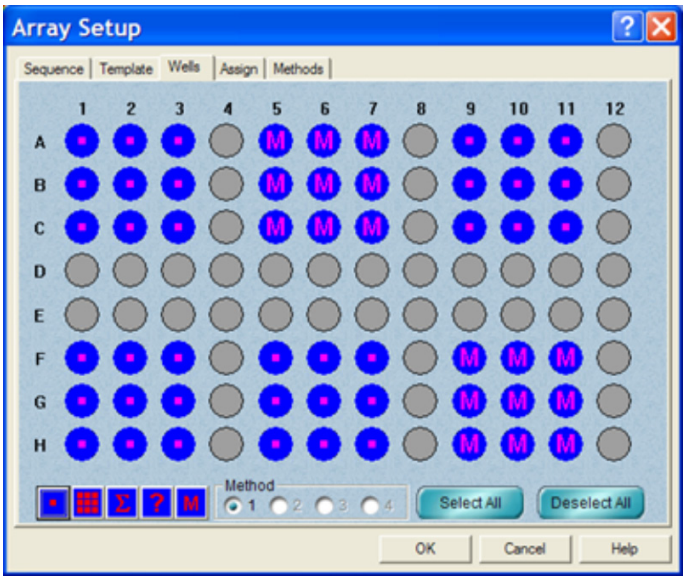
1. Load the plate on the DXR3 SmartRaman Spectrometer plate holder



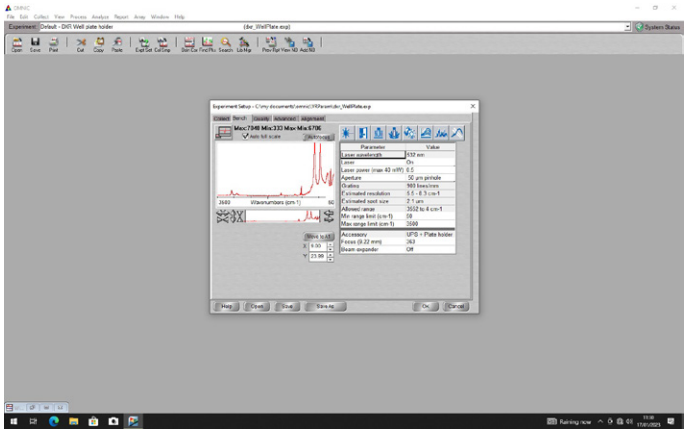
2. Select the predefined template



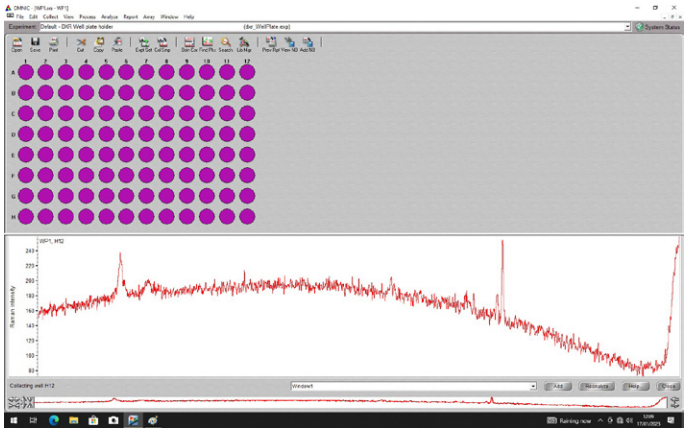
3. Specify which wells to sample and how



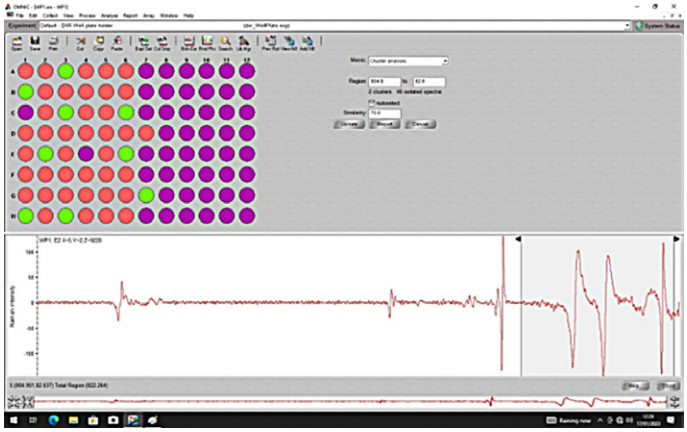
4. Set Data Collection parameters with live spectrum view for the ease of Optimization



5. Collect data for specified wells on the well plate



6. Apply analysis (Automated)



7. Extract data

8. Report

Title: WP1

Data file: C:\my documents\omnic\Array\WP1.srs

Sequence file: C:\my documents\omnic\VRParam\MicroTiter.ary

Comment:

Method: Cluster analysis Region: 904.9 - 82.6

Similarity: 70.0

Well	Mode	X Pos	Y Pos	Z Pos	Cluster
A1	Center	-3	5	9220	2
A2	Center	5	5	9220	2
A3	Center	2	5	9220	1
A4	Center	0	5	9220	2
A5	Center	-3	5	9220	2
A6	Center	-5	5	9220	2
A7	Center	3	5	9220	

Well	Mode	X Pos	Y Pos	Z Pos	Cluster
B1	Center	-3	2	9220	1
B2	Center	5	2	9220	2
B3	Center	2	2	9220	2
B4	Center	0	2	9220	2
B5	Center	-3	2	9220	2
B6	Center	-5	2	9220	2
B7	Center	3	2	9220	

Well	Mode	X Pos	Y Pos	Z Pos	Cluster
C1	Center	-3	-1	9220	
C2	Center	5	-1	9220	2
C3	Center	2	-1	9220	1
C4	Center	0	-1	9220	2
C5	Center	-3	-1	9220	2
C6	Center	-5	-1	9220	1
C7	Center	3	-1	9220	

Well	Mode	X Pos	Y Pos	Z Pos	Cluster
D1	Center	-3	-5	9220	2
D2	Center	5	-5	9220	2
D3	Center	2	-5	9220	2
D4	Center	0	-5	9220	2
D5	Center	-3	-5	9220	2
D6	Center	-5	-5	9220	2
D7	Center	3	-5	9220	2

Well	Mode	X Pos	Y Pos	Z Pos	Cluster
E1	Center	-3	2	9220	2
E2	Center	5	2	9220	1
E3	Center	2	2	9220	2
E4	Center	0	2	9220	
E5	Center	-3	2	9220	2
E6	Center	-5	2	9220	1
E7	Center	3	2	9220	

Well	Mode	X Pos	Y Pos	Z Pos	Cluster
F1	Center	-3	0	9220	2
F2	Center	5	0	9220	2
F3	Center	2	0	9220	2
F4	Center	0	0	9220	2
F5	Center	-3	0	9220	2
F6	Center	-5	0	9220	2
F7	Center	3	0	9220	

Well	Mode	X Pos	Y Pos	Z Pos	Cluster
G1	Center	-3	-4	9220	2
G2	Center	5	-4	9220	2
G3	Center	2	-4	9220	2
G4	Center	0	-4	9220	2
G5	Center	-3	-4	9220	2
G6	Center	-5	-4	9220	2
G7	Center	3	-4	9220	1

Well	Mode	X Pos	Y Pos	Z Pos	Cluster
H1	Center	-3	3	9220	1
H2	Center	5	3	9220	2
H3	Center	2	3	9220	1
H4	Center	0	3	9220	2
H5	Center	-3	3	9220	2
H6	Center	-5	3	9220	2
H7	Center	3	3	9220	