

# Method Development and Validation: RMID for Regulated Industries

**Dwight Walker, Melissa J. Gelwicks, & Adam J. Hopkins**

Traditional time-consuming analytical techniques for Raw Material Identification and Verification (RMID) are being replaced by spectroscopic methods such as handheld Raman. In-situ RMID methods must accommodate new types of users, warehouse conditions, and faster sampling strategies without compromising the success of the method. This 4-part series guides Mira P users through method building, method validation and implementation.

Part II recommends how to collect top quality spectra followed by detailed instructions for building Operating Procedures and Training Sets.

Part I: General Considerations

**Part II: Method Development**

Part III: Method Robustness and Validation

Part IV: Method Implementation

## Part II – Developing RMID Methods

Part II of this series guides development of methods for verification of raw materials with Raman. Successful development of a method relies on the inclusion of exemplary spectra in library/training sets used for RMID. Special focus is given to defining, creating, and evaluating Training Sets (TS) and Operating Procedures (OP).

### Detailed Considerations Before Beginning

#### System Suitability Tests (SST) and Calibration

To reiterate, the conditions used to build a method must reflect actual testing conditions. In terms of calibration, Metrohm Raman recommends that Mira be calibrated upon install. Once calibrated, SST [1] will be performed regularly: every hour, or between lot changes, or minimally at the beginning and middle of a workday, for example. *Recalibration is not recommended until the system fails SST.* The Calibrate-Verify Attachment (CVA) for Mira makes this simple. A full calibration and SST routine utilizes sides 1 and 2 on CVA, but regular SST verification uses only side 2. Also, if multiple instruments are to be used for testing, then multiple instruments must also be used when building the method.



#### Training Set Size

All TS should consist of spectra from a minimum of **3 distinct lots of material**, with each lot measured multiple times. Metrohm Raman recommends that each lot be tested **3 times over 3 days**, regardless of sample type or form. Lots should be sourced from distinct vendors/sources. This is the *minimum variation* for a robust TS for synthesized APIs. When working with natural products (e.g. sodium alginate), more samples across several vendors/sources/lots will be required to provide adequate robustness within the TS. In these cases, it may require **5 lots x 5 tests x 5 days** by more than one user to account for greater variance in natural products. Similarly, colored samples may require more spectral input into the TS to account for variances in Raman intensity due to absorbance of incident laser radiation or fluorescence.



#### Sample Configuration

It is essential that TS spectra resemble expected sample spectra as closely as possible. All acquisitions for TS should include repeat measurements to capture sample placement variances. For example, acquiring through-container spectra at the top, middle, and bottom of the container helps to account for any variance in wall thickness and density. Also note, TS spectra for materials received in amber bottles must be collected in appropriate amber bottles. For powders in bags, collect TS samples through appropriate bags, accounting for thickness, opacity, and possible surface treatments to ensure the most robust TS. In the special instance of colored bags, accounting for the effect of the bag color is necessary to ensure a robust TS.





## User-Based Variance

Adequate training with Mira P must be conducted to ensure that significant user-based sampling variance is avoided. The same qualities that make handheld Raman so flexible and convenient also provide opportunities to incorporate undesirable variance, including poor sampling practices, into a model. For bottled samples, this variance could be the angle at which the Smart Tip is held against the bottle. For bagged samples, this variance can include the sampling angle and degree of force between Mira and the bag.

However, there are nuances that can be captured in a model when more than one user is included in the initial data collection. For example, two different users operating at different times of the day would provide a more representative data set.



## Sampling Optic

The sampling optic, called a **Smart Tip** in Mira Cal, which is used for collection of TS spectra, should be the same tip used while implementing the method. When acquiring sample spectra through packaging, the choice of Short, Long, or Extra Long Working Attachment will be dependent on the container thickness. In practice, Mira should be held with the Smart Tip perpendicular to the sample, and pressed firmly against the sample or packaging material. Accordingly, when sample aliquots are removed to a secondary container before testing, TS spectra must be collected in a manner consistent with the secondary container. Ideally, sample aliquots would be placed into vials compatible with Mira's Vial Holder, further simplifying measurements.



## Summary

Generation of representative data in the form of Libraries, Training Sets, and Operating Procedures is critical for successful implementation of Mira. Model data must represent adequate sampling and material variance, respecting that:

- natural products may exhibit more spectral variance than synthetic products
- spectra of solids may exhibit more variance than liquids
- user effects must be avoided with adequate training

Good standard practices include all sampling configurations that might be expected in routine operation (e.g. plastic bags, number of bag layers, glass bottle, clear and/or amber, etc.). Note that, minimally, it is best practice to reposition Mira for each spectrum.



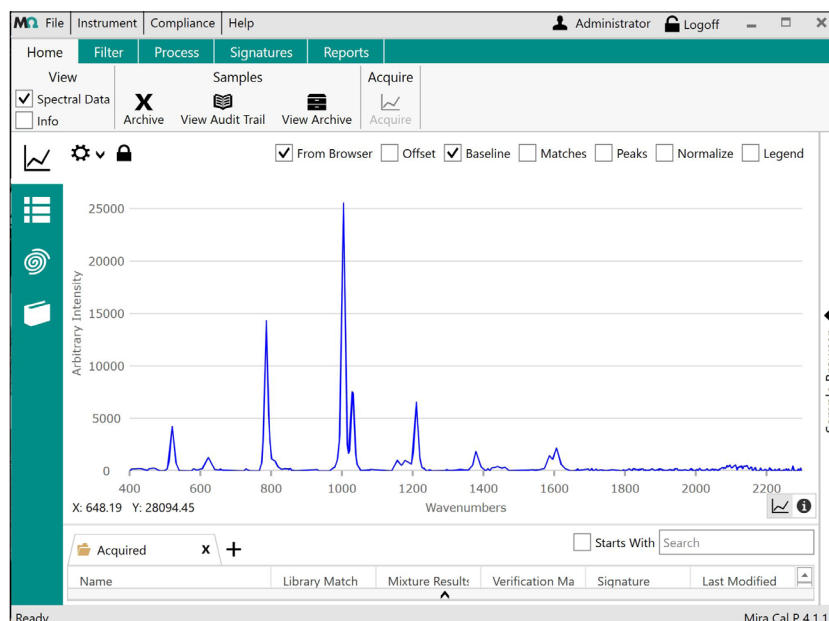
## Instructions for Development of a RMID Method – Data Collection

*Real testing conditions must be incorporated into RMID methods.* Do not perform all data acquisition in a laboratory if the method will be implemented at receiving. Method development is limited to Laboratory Manager and Administrator level access. Specifics of Mira Cal P use can be found in the Tutorial. [2]

1. Connect Mira to a computer via USB cable.
2. Open Mira Cal P software, and log into your predefined account.
3. Select **Instrument > Connect** in the top menu to connect Mira, specify correct USB port in popup window.
4. Select the **Operating Procedures** icon in the vertical menu to display current operating procedures.
5. Select **New** and fill in **Name**, **Sample Name**, and **Description**.
6. Select **Acquisition** to access acquisition parameter options used to generate spectra for the TS and, ultimately, for sampling. The **Auto Integration** feature optimizes data collection based on measured signal to noise (s/n). Auto Integration can be used for method development and implementation; it is rare that specifying integration time will improve overall results. All fields (**Laser Power**, **Integration Time**, **Averages**, and **Smart Tip**) will be determined depending on real sampling conditions and initial spectral data, and may require iterations to optimize.



Operating Procedures



Mira Cal P Home Screen

# Metrohm Tutorial

7. Select **Barcodes** to facilitate barcode selection of the OP. Select **Scan Barcode** and scan the barcode to enter an example of your barcode content. Contact support for specific barcode settings.
8. Select **Save** to store the initial OP.
9. Attach a Smart Tip (sampling optic or sample holder) to the accessory port on Mira. Again, the choice of Smart Tip must be consistent for method development and implementation.
10. Select **Synchronize** to synchronize Mira.
15. Review the spectra, individually or in groups, for fingerprint peaks, sharpness, intensity, adequate s/n, and consistency. It is recommended that each spectrum be compared with other spectra of the same material or with reference spectra.
16. This step is repeated to generate the minimum number of spectra necessary to build a calibration data set (refer to **Training Set Size** on page 2). **We recommended including a minimum of 40–60 spectra to develop a robust method.**

**You are now ready to begin acquiring data to build the method.**

11. In Mira Cal, select **Samples** in the vertical menu. This displays current sample data (note that the initial screen displays the latest sample spectra collected).



**Samples**

The United States Pharmacopeia (USP) specifies that the spectrum of the unknown material must exhibit maxima only at the same wavelengths/wavenumbers as that of a similar preparation of the corresponding reference standard. [3] This is commonly performed using a referenced spectral library, which is populated by reference spectra of authentic materials.

This is a critical step in ensuring the samples used to generate the model are truly representative of ALL possible sample presentations and natural variations to the instrument as discussed previously. The number of samples required for developing a robust model (which will be successful upon implementation) will vary depending on such factors as the strength of the Raman signal, influences from the packaging, and natural variances in the sample.

12. Select **Acquire** to choose an OP. The **Name** field will be automatically populated.
13. Fill in appropriate **Lot ID**, **Batch ID**, and **Container** fields, either manually or with Mira's barcode reader.
14. Select **Acquire** button to begin data acquisition. The laser illumination icon displays during acquisition. After data is collected, the spectrum name will appear in the samples list at the bottom of the screen.

## Instructions for Development of a RMID Method – Library or Training Set (TS) Construction

1. In Mira Cal select **Libraries** in the vertical menu to display current libraries. Select **New**, and populate the **Name** and **Description** fields.
2. Select **Samples > Add Samples** to display a list of samples available to add to the Library. Click individual spectra or use the click and hold function to select desired samples. Select **Save** to store the Library.
3. Select **Training Sets** in the vertical menu to display the current TS. Select **New** and populate the **Name**, **Sample Name**, and **Description** fields.
4. Select **Samples > Add Samples** to display a list of samples available to add to the TS. Click individual spectra or use the click and hold function to select desired samples. Select **Save** to store the TS.
5. Select **Operating Procedures** in the vertical menu to display current OPs, and select the OP under development.
6. To select evaluation type:

Choose **Identification** and select a **Match Threshold**. Generally, a value of 0.85 is sufficient, but if tighter control is needed, this value can be increased. Double click to select **Libraries** at right.

Click **Verification** and select a **Confidence Interval**. A value of 0.9 is usually sufficient. **Container Mode** can be specified here. Double click to select **Training Sets** at right.

Mixture Identification, along with associated libraries can also be specified.

**Note:** Verification will be the primary evaluation type used in RMID. However, there may be times when Identification is useful. Refer to the tutorial RMID IV: Method Implementation for examples.

7. Select **Save** to store this OP. The method is now ready for use.
8. To transfer the new OP to the instrument, select **Synchronize** from the top menu to synchronize Mira. Click **Disconnect** in order to use Mira in standalone mode.



**Libraries**



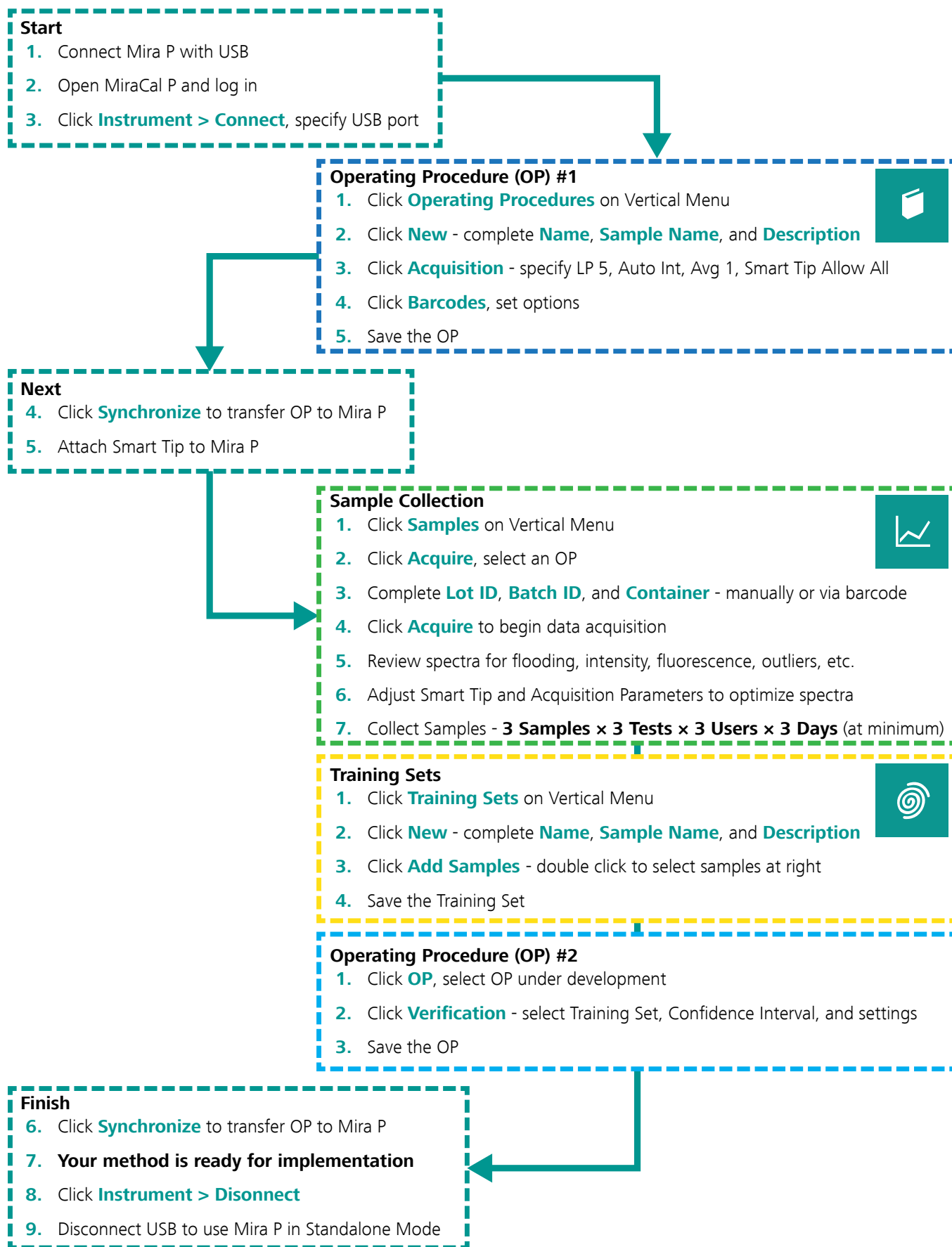
**Training Sets**



**Operating Procedures**

**On the final page, find a summarized workflow for the development of a method.**

# Metrohm Tutorial



# Metrohm Tutorial

## References

- [1] Metrohm White Paper **043** Instrument Calibration, System Verification, and Performance Validation for Metrohm Instant Raman Analyzers (Mira)  
<https://www.metrohm.com/en/applications/WP-043EN>
- [2] Tutorial Mira Cal P  
<https://www.metrohm.com/en/documents/801058004>
- [3] United States Pharmacopeia **USP 29 <1120>** Raman Spectrophotometry  
[http://www.pharmacopeia.cn/v29240/usp29nf24s0\\_c1120.html](http://www.pharmacopeia.cn/v29240/usp29nf24s0_c1120.html)