Standard Operating Procedure:

Multiple Breath Nitrogen Washout

Exhalyzer® D (Eco Medics AG, Duernten, Switzerland)

Signal Re-Alignment 3.3.2

Signal Synchronization using DDC

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January 26, 2023

Contents

1. What is Signal Alignment?

Signal alignment or synchronization refers to the temporal alignment of the flow, oxygen and carbon dioxide traces. The lag time between flow and gas signals may vary between Exhalyzer D units and may differ depending on which DSR is used. This is mainly due to differences in sample flow rate between systems but also results from subtle variances in Nafion tubing length and characteristics of the gas analyzers. Therefore, the default delay values in Spiroware will not always result in optimal signal alignment.

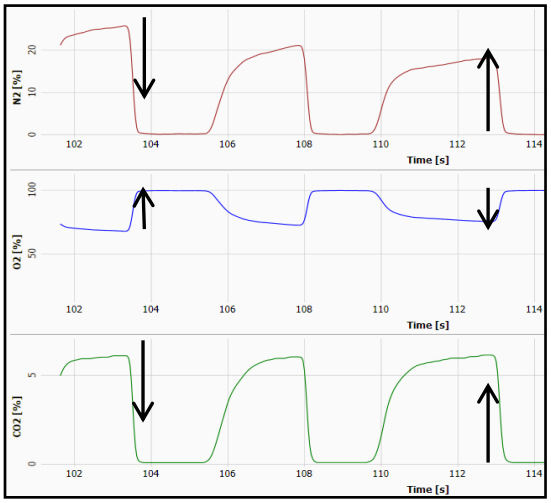
Recall that:

$[N_2]$ is calculated from $[O_2]$ and $[CO_2]$

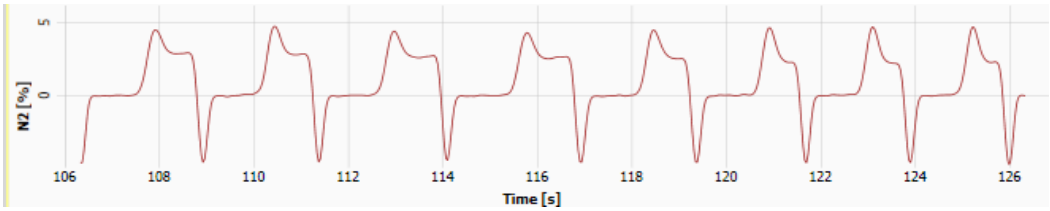
$[N_2] = 100-[0_2]–[CO_2]$

During the breathing cycle $[O_2]$ and $[CO_2]$ travel in opposite directions.

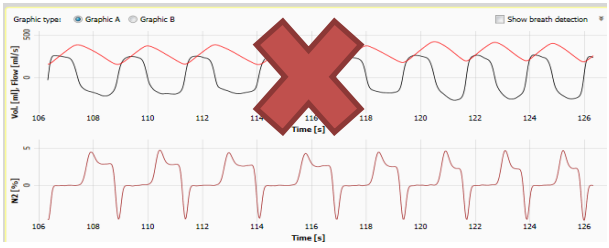
Inspiration: $[O_2]$ increases ↑ and $[CO_2]$ decreases ↓
Exhalation: $[O_2]$ decreases ↓ and $[CO_2]$ increases ↑

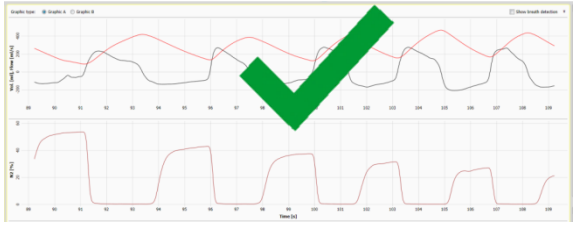


Temporal alignment of flow, oxygen and carbon dioxide signals results in proper alignment that appear as smooth waveforms. Signal misalignment of $[O_2]$, $[CO_2]$, and flow results in a repeating pattern of peaks and dips in $[N_2]$ signal that are NOT apparent in the $[O_2]$ and $[CO_2]$ trace. These deflections can alter FRC calculation and measured CetN_2, which in turn affects determination of end of washout and LCI measurements.



Gas and flow signals must be accurately aligned with one another in time to ensure precise estimation and accurate calculation of gas concentration and volume.





2. Signal alignment using Spiroware 3.2 and higher - Dynamic Delay Correction

Spiroware Software versions 3.2 and higher include a dynamic delay correction (DDC) algorithm that works in conjunction with the fixed delay correction, and results in improved signal synchronization.

The **fixed O_2 and CO_2 flow-to-signal offsets** (delay times) represent how much the O_2 and CO_2 signals must be shifted in time in order to align with the flow signal. These fixed values are established during the DDC Signal Synchronization maneuver.

In addition to the fixed offset values, the built-in algorithm uses the nafion tube volume determined during the synchronization maneuver to compensate for the change in gas transit time as the washout progresses. Travel (transit) time through the nafion tube increases as gas becomes **more viscous** with **higher O_2 concentrations.**

Washout	Approximate Gas Composition	Gas Viscosity	Travel Time
Start Washout	78% N_2 / 17% O_2 / 5% CO_2	Low	Fast
End Washout	2% N_2 / 93% O_2 / 5% CO_2	High	Slow

As gas viscosity increases, and the gas sample moves more slowly through the nafion tube, the **required flow-to-signal offset** to align the gas and flow signals **increases.** DDC corrects for the change in gas sample transit time over the course of the washout.

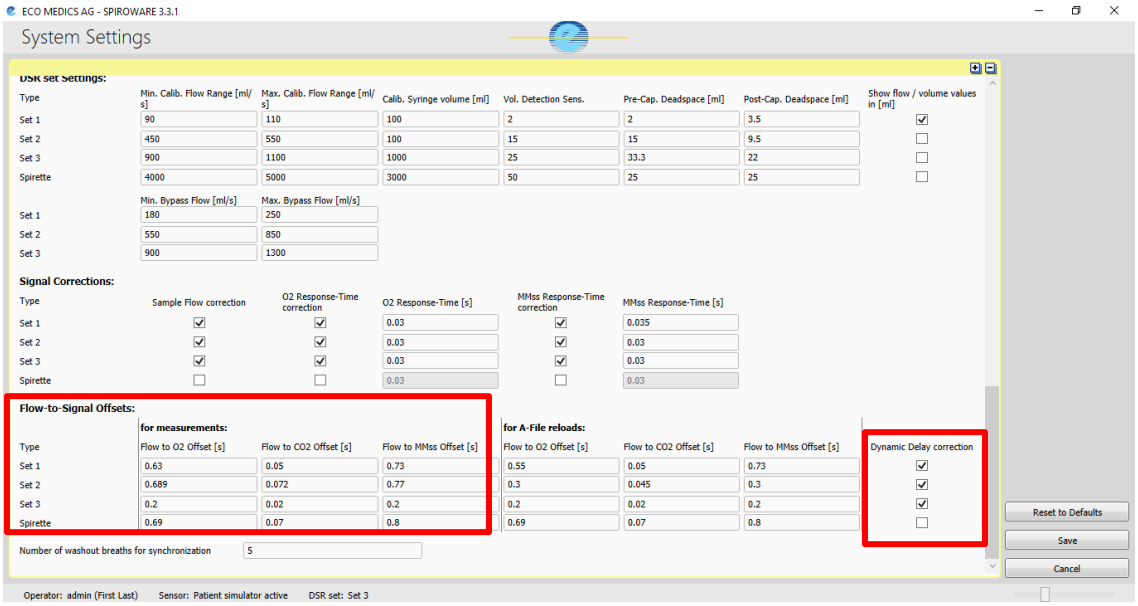
Please Note: DDC Synchronization maneuver replaces the Fixed Delay Correction maneuver

DDC Synchronization Maneuver

The synchronization procedure using DDC is very similar to the previous fixed delay correction maneuver. The maneuver still requires stable breathing within volume targets and must be performed for each DSR Set. However, the DDC is performed **during pre-phase** while breathing **medical air** and the synchronization values are determined over **5 breaths instead of 10**.

Similarities	Differences
Requires **stable breathing** within targets	Performed during pre-phase while breathing **medical air**
Synchronization performed for **each DSR Set**	Synchronization values are determined over **5 breaths instead of 10**

Fixed flow-to signal offsets and dynamic delay correction option are found under Flow-to-Signal Offsets in System Settings.

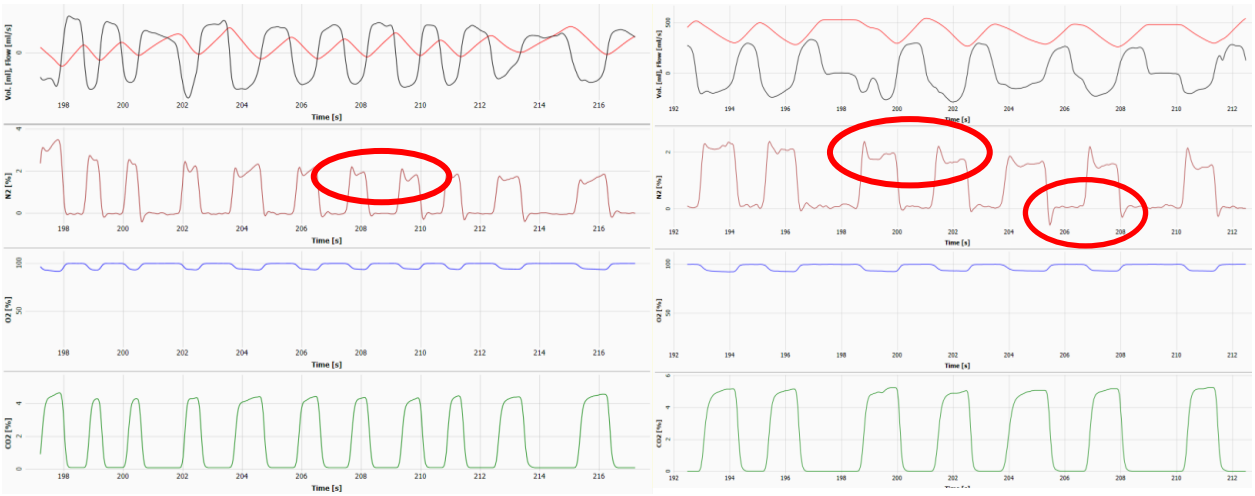


<u>Prior to beginning all testing</u>, a trained operator should have performed several signal alignment maneuvers to determine the "characteristic" delay of the machine, which is then saved in the system's settings. These values are found in the **Flow-to-Signal Offsets** section within the **Calibration** tab of the **System Settings**.

Signal alignment should then be repeated (values recorded but not necessarily saved!) every week to verify that results are within +/-10 ms of previous characteristic delay times **(See section Appendix 1)**.

2.1 Examples of Poor Signal Alignment

Poor signal alignment can be identified by repetitive deflections in the N_2 concentration trace.



The N_2 signal should return to zero between breaths, and provided gas calibration has been performed, should not be a negative value (dip below zero).

3. Why is it Necessary to Correct Signal Misalignment?

Signals can occasionally become misaligned during testing. This may occur for a variety of reasons, someone may have unintentionally saved a poor synch calibration to the system settings, or the subject being tested may have a breathing pattern that is outside the range of values generated by operator synchronization.

Signals must be aligned to generate accurate MBW outcomes, therefore signal realignment must be performed before analysis can occur.

4. Determining Correct Synchronization Values (delay times).

Determining correct delay times depends on why the original delay values were incorrect. Sometimes the correct delay settings are known but the incorrect values accidentally saved in system settings. Sometimes the delay values in system settings just need to be updated, for example nafion tube was changed so old values are no longer accurate. Sometimes, the subject being tested has a breathing pattern that is outside the typical range of values generated by operator synchronization.

Synchronization Values Known - See Section 5

Correct delay values are known but incorrect synchronization times were accidentally saved to system software settings need to be set back to usual settings. Correct values can be found in recorded in calibration log and used to re-run data (See Section 5).

Updated Synchronization Values Needed - See Section 5

Nafion tube was changed so usual settings are no longer correct. A new synchronization calibration needs to be performed to determine new characteristic delay values (See Appendix 1- How to perform Flow Channel Synch using DDC). Once correct values are known they can be used to re-run data (See Section 5).

Synchronization values Unknown – See Section 6

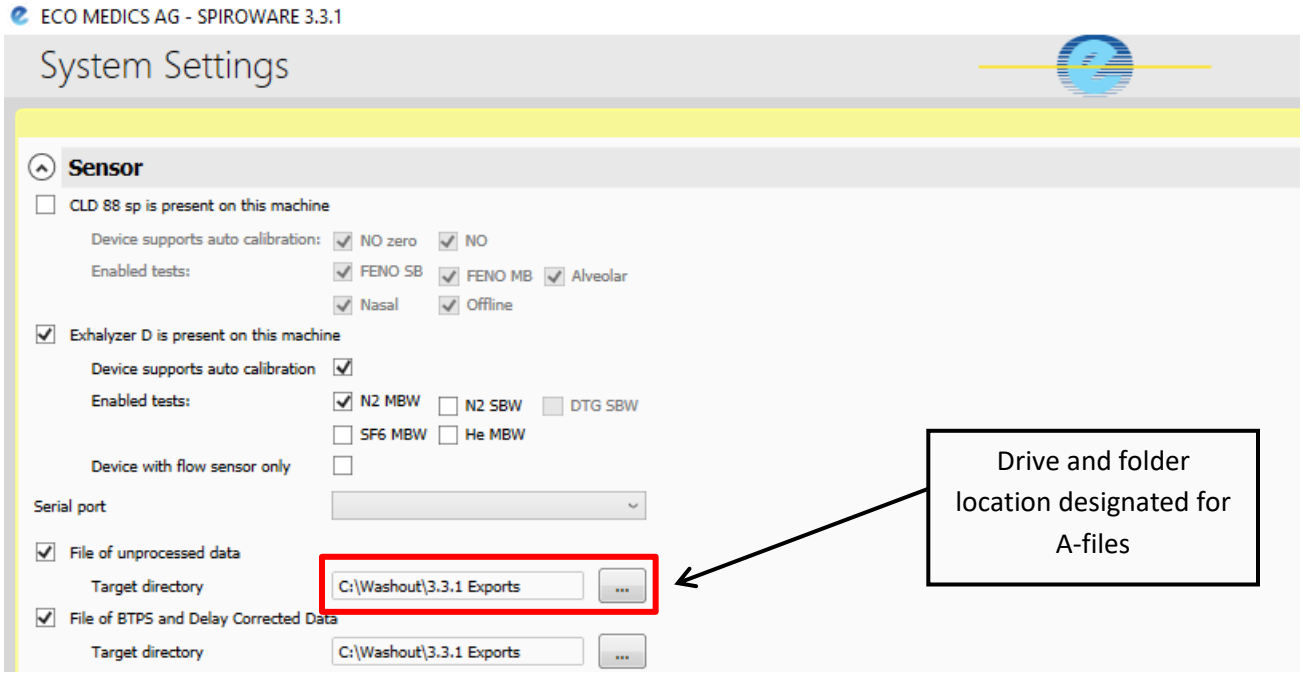
Trials were collected using the usual synchronization settings, but the signals were still misaligned. Generate subject specific delay values by **performing a *Flow/Channel Signal Synchronization*** with the subject's data (A-files) and use to re-run data (See Section 6).

5. Synchronization Values Known

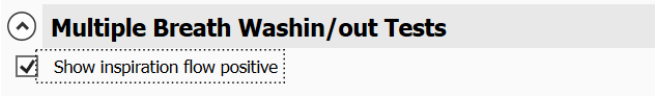
If correct synchronization values are known, follow the following steps to re-run data.

5.1 Set General System Settings

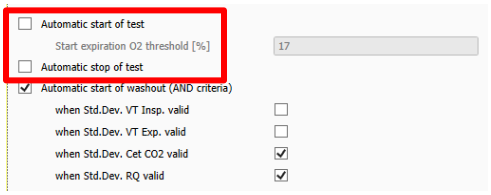
1. Location of A-files (unprocessed data)
    - A-files can be found in the target directory indicated for 'File of unprocessed data' under the **Sensor** tab in **System Settings**.
    - **New A-files will not be created** by re-running data, but 'File of unprocessed data' should ALWAYS be selected.
    - If desired, B, C or Breath Table data re-calculated with new settings can be saved to a folder on your local hard drive *provided the following settings are correct:*
        - Ensure that the **data type** is selected
        - Ensure that the data is mapped to an **existing** folder on the C:\ Drive



2. Ensure orientation of the flow signal is correct and automatic start and stop are disabled.
    - Select the **Multiple Breath Washin/out Tests** tab in **System Settings** to confirm **Show inspiration flow positive** is selected.



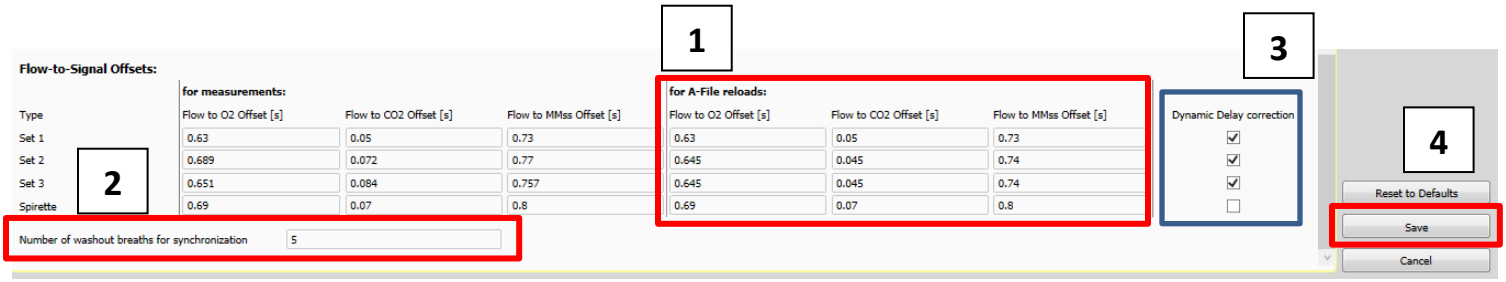
- Click on the **N2 Multiple Breath Washout** tab in **System Settings** to confirm **Automatic start** and **Automatic stop** of the test are **NOT selected.**



5.2 Enter Correct *Flow to O_2 Offset* and *Flow to CO_2 Offset* values (synchronization values/delay times).

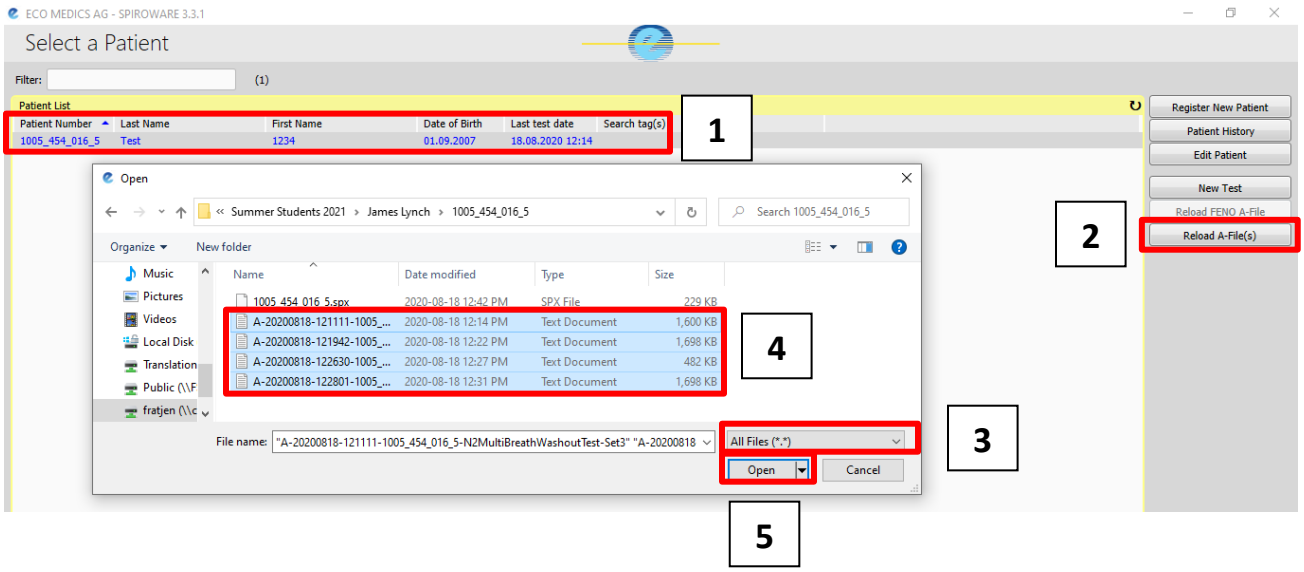
From the **Administration** menu navigate to **System Settings**. Scroll to flow-to-signal offsets under the **Calibration** tab.

1. Enter the correct fixed delay values (not necessarily the values shown here) corresponding to DSR (Set 2 or 3) in the Flow to O_2 Offset, Flow to CO_2 Offset, and Flow to MMss Offset fields in '**for A-File reloads'**.
   - *Note: If signal misalignment occurred during the test occasion, DON'T use the values from the A-Files.*
2. Ensure that "Number of washout breaths for synchronization" is set for 5 breaths.
3. Ensure Dynamic Delay Correction is selected for all DSR Sets.
4. Press **SAVE** before returning to the main menu

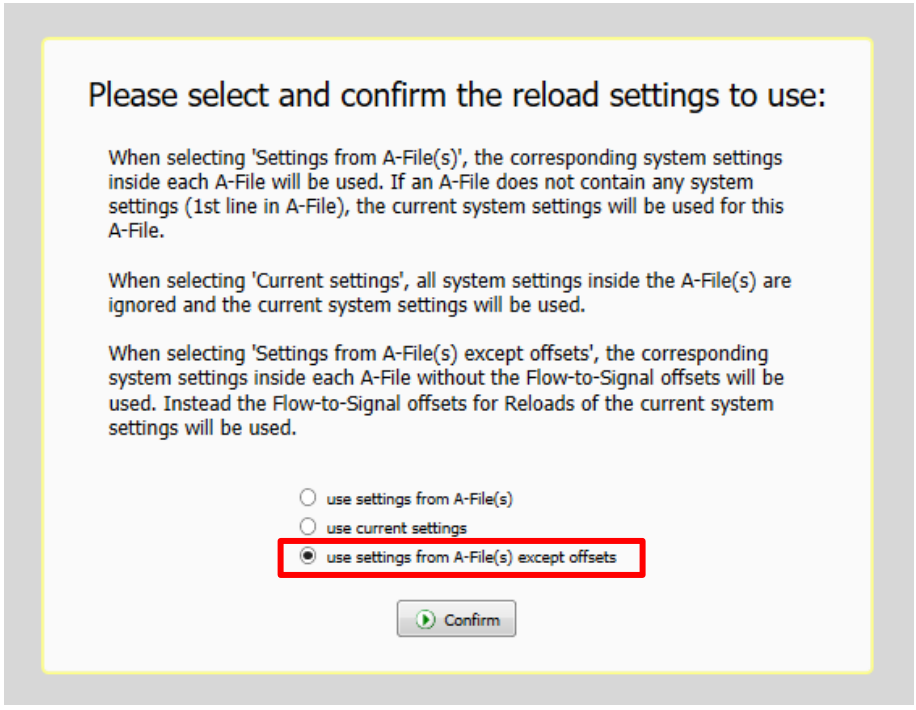


5.3 How to Re-Run A-Files to Correct Signal Misalignment.

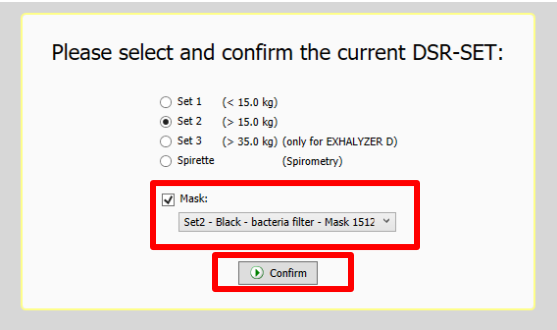
1. On the Select a Patient page, **highlight** the subject whose data needs to be re-run.
2. Once highlighted, press "**Reload A-file(s)"**
3. Make sure that All Files is selected.
4. Find the A-files to be re-run. All A-files can be selected together.
5. Press "**Open"**.



6. Select **Use settings from A-File(s) except offsets** and press **Confirm.**



7. Select the appropriate **DSR set** (same set as used at time of test) and press **Confirm** . If a mask set-up was used on the day of test, ensure that the correct mask is chosen before clicking confirm.



- The rerun will then begin, and the A-files will automatically re-play in sequence, once the re-run is complete the software will stop automatically.

- **Once the re-run is complete, navigate to the Analysis Page (exactly the same as during a live test) and be sure to SAVE AS DRAFT before leaving the test occasion or the results will not be saved.**

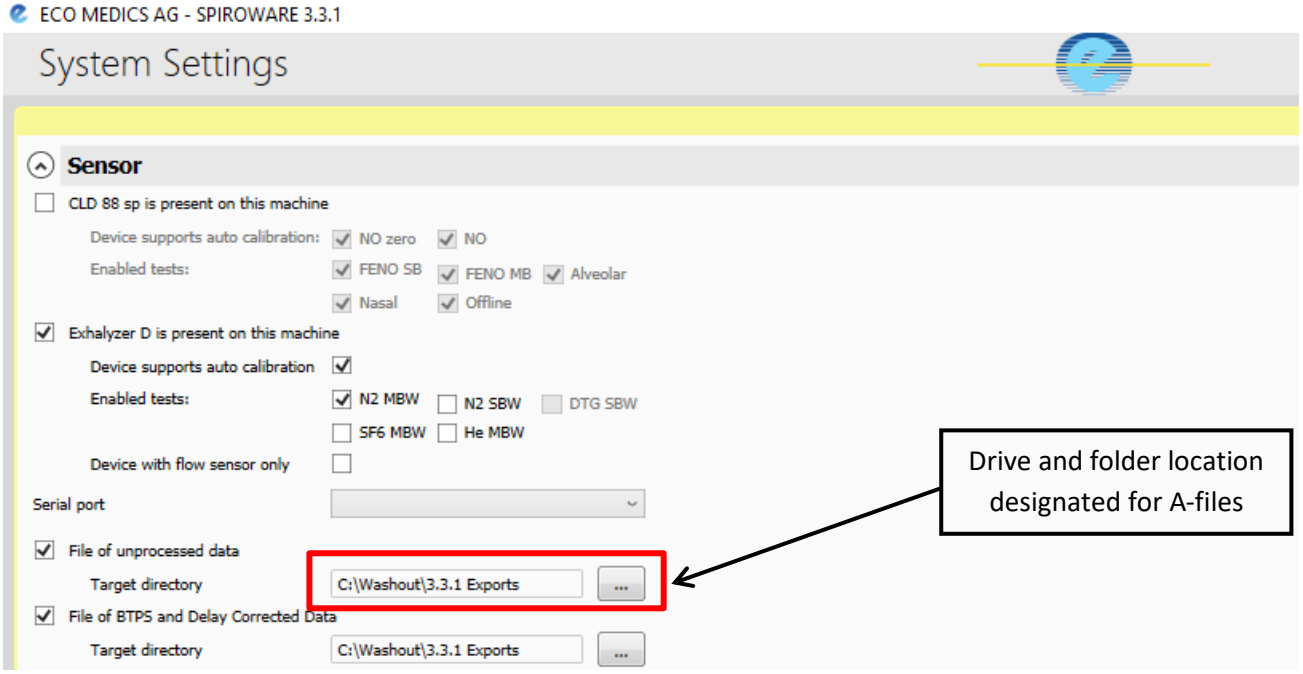
- In addition to the draft file saved at the time of test, a second draft file will now be visible in the subject file. **DO NOT DELETE THE ORIGINAL DRAFT FILE!**
    - Prior to submitting the corrected file for analysis check that the signals have now been aligned by opening the draft file and scrolling through each trial inspecting for evidence of signal misalignment.

- If alignment is still not corrected proceed to Section 6

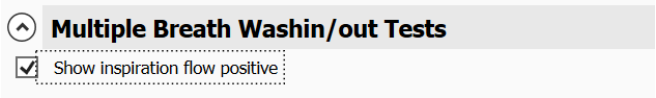
6. Synchronization Values Unknown

6.1 Set General System Settings to Perform Flow/Channel Signal Synchronization

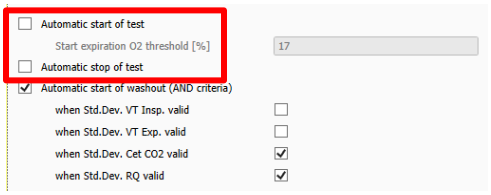
1. Location of A-files (unprocessed data)
   - A-files can be found in the target directory indicated for 'File of unprocessed data' under the **Sensor** tab in **System Settings**.
   - **New A-files will not be created** by re-running data, but 'File of unprocessed data' should ALWAYS be selected.
   - If desired, B, C or Breath Table data re-calculated with new settings can be saved to a folder on your local hard drive *provided the following settings are correct:*
     - Ensure that the **data type** is selected
     - Ensure that the data is mapped to an **existing** folder on the C:\ Drive



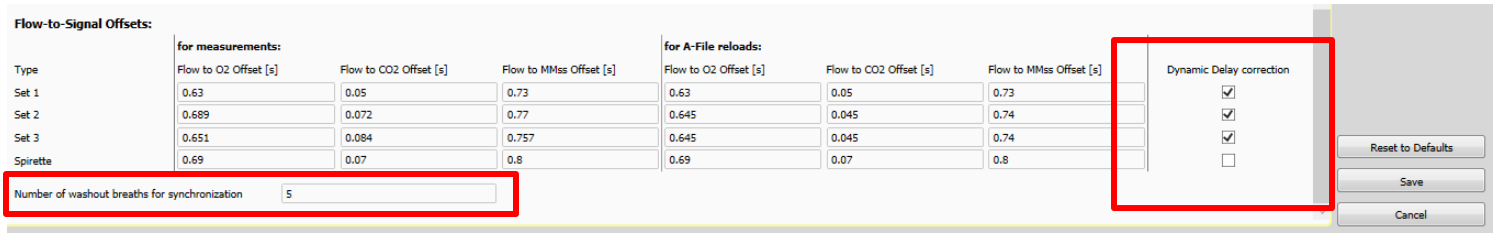
2. Ensure orientation of the flow signal is correct and automatic start and stop are disabled.
   - Select the **Multiple Breath Washin/out Tests** tab in **System Settings** to confirm **Show inspiration flow positive** is selected.



- Click on the **N2 Multiple Breath Washout** tab in **System Settings** to confirm **Automatic start** and **Automatic stop** of the test are **NOT selected.**

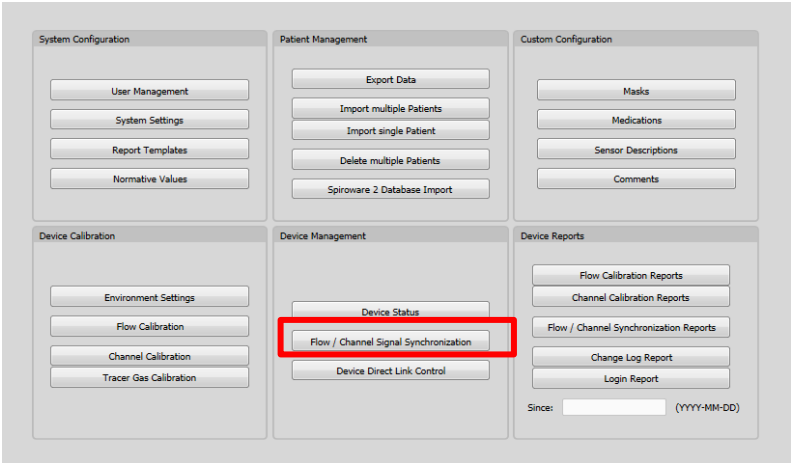


3. From the **Administration** menu navigate to **System Settings**. Scroll to flow-to-signal offsets under the **Calibration** tab.
    - Ensure Dynamic Delay Correction is selected for all DSR Sets.
    - Ensure Number of breaths for synchronization is set to **5 breaths**.

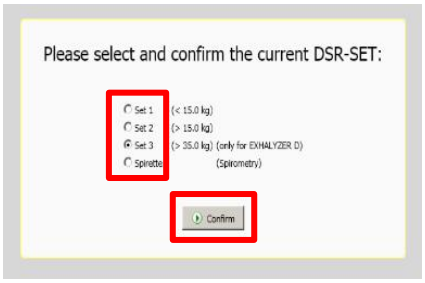


6.2 Perform Flow/Channel Signal Synchronization Using Existing Data.

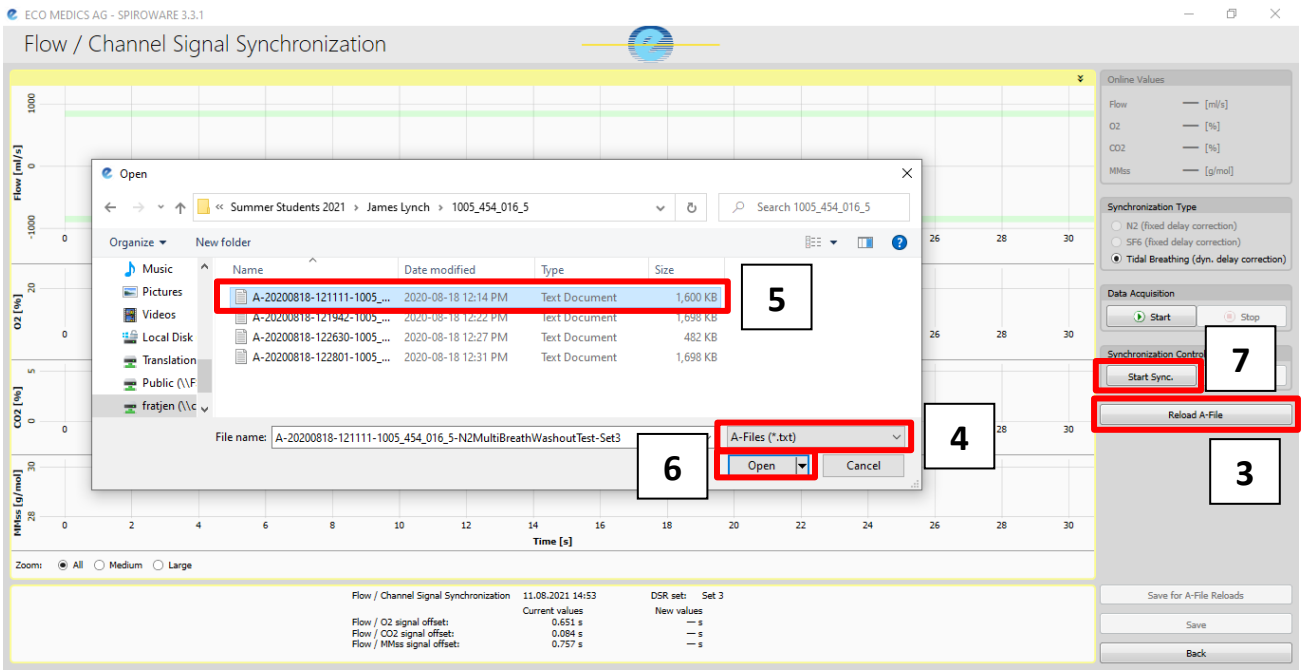
1. From the **Administration** menu, select **Flow/Channel Signal Synchronization**



2. Select Set 2 or Set 3 depending on which DSR Set was used for testing – press **Confirm**



3. Select "**Reload N_2 A-File**"
4. Make sure that A-Files is selected.
5. Find the A-file to be used to generate new delay values.
6. Press "**Open"**.
7. The synchronization will begin automatically once you click "Open." When you believe the subject is in a comfortable breathing pattern during the pre-phase, click **"Start Synch."**



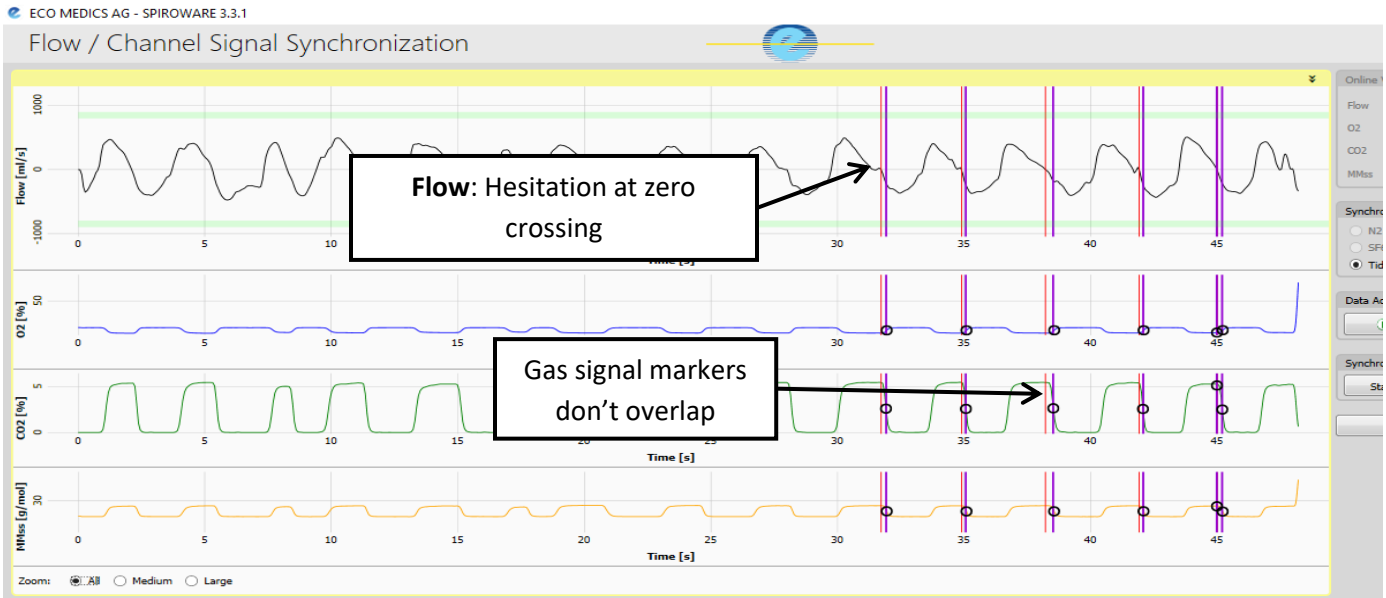
6.2.1 How Do I Know if the Synchronization is Acceptable?

Acceptable:

- ✓ Flow tracing clearly crosses zero during transition between inspiration and expiration for 5 breaths of the pre-phase; no hesitations (see below).
- ✓ All gas signal marker lines (blue, red) overlap for 5 breaths of the pre-phase
- ✓ 5 tidal pre-phase breaths with no irregular breaths

Example of Acceptable Synchronization

Example of Unacceptable Synchronization



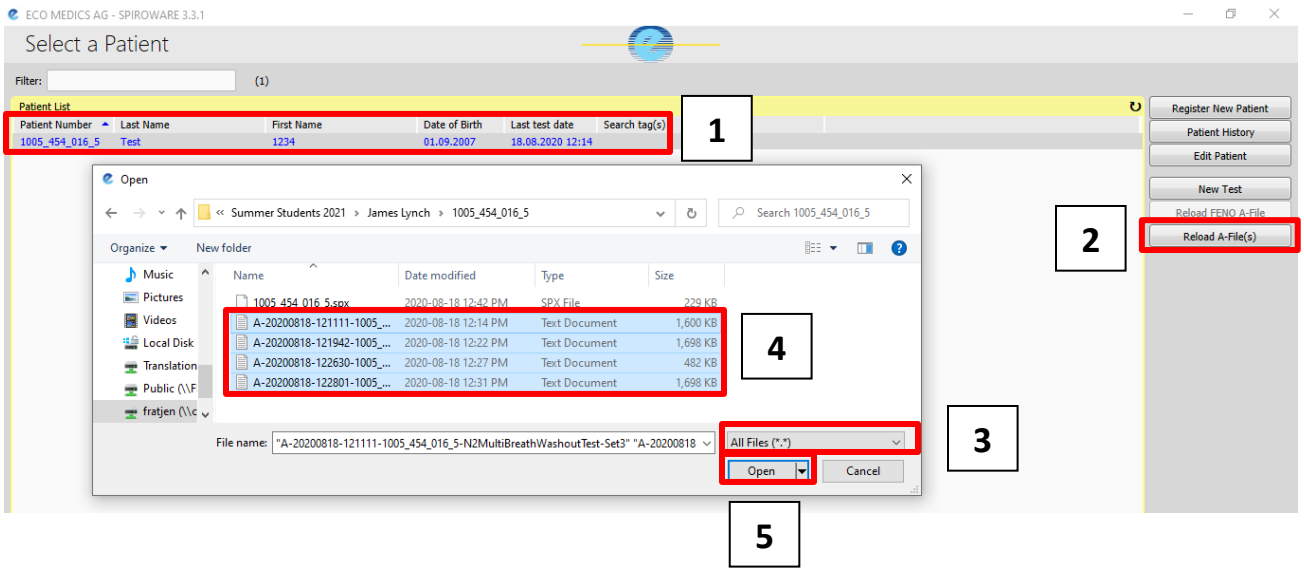
Only values generated from acceptable synchronization should be saved. If the first A-file is unacceptable select **"Reload N2 A-file"** again to load the subject's next A-file. You can do this as many times as needed until an acceptable trial is found.

Once an acceptable trial is found, press save for A-file Reloads; the newly generated numbers will then be saved in System Settings. These values will be used for A-file re-run but will not overwrite settings used for data collection**.**

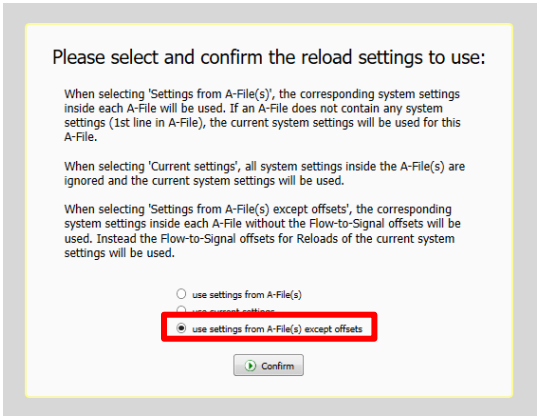
6.3 How to Re-Run A-Files Once New Delay Times Have Been Generated.

Now that the new delay values have been saved to system settings, the operator may proceed with re-running files to correct signal misalignment.

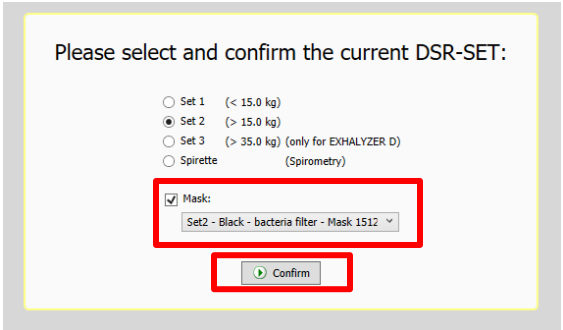
1. On the Select a Patient page, **highlight** the subject whose data needs to be re-run.
2. Once highlighted, press "**Reload A-file(s)"**
3. Make sure that All Files is selected.
4. Find the A-files to be re-run. All A-files can be selected together.
5. Press "**Open"**.



6. Select **Use settings from A-files except offsets** and press **Confirm.**



7. Select the **DSR set** to be used and press **Confirm**. Use the same set as the time of test. If a mask set-up was used on the day of test, ensure that the correct mask is chosen before clicking confirm.



8. The rerun will then begin and the A-files will automatically re-play in sequence, once the re-run is complete the software will stop automatically.

9. **Once the re-run is complete, navigate to the Analysis Page (exactly the same as during a live test) and be sure to SAVE AS DRAFT before leaving the test occasion or the results will not be saved.**

10. In addition to the draft file saved at the time of test, a second draft file, with the date of the re-run, will now be visible in the subject file. **DO NOT DELETE THE ORIGINAL DRAFT FILE!**
    - Prior to submitting the corrected file for analysis check that the signals have now been aligned by opening the draft file and scrolling through each trial inspecting for evidence of signal misalignment.

11. If alignment is still not corrected go back to Section 6.2, select a new A-file from that participant for the signal synchronization, and try to Re- Run the test occasion again. Continue to go back and try different A-files for the participant until mis-alignment has been corrected.

7. Appendix 1 – How to perform flow/channel synch using DDC

7.1 Flow/Gas Signal Synchronization (MBWN2 SOP section 5.4)

To be performed weekly by Supervisor.

Please Note: Flow and channel calibrations must be performed prior to Signal Synchronization.

- The lag time between flow and gas signals may vary between Exhalyzer D units and may differ depending on which DSR is used. This is mainly due to differences in sample flow rate between systems but also results from subtle variances in Nafion tubing length and characteristics of the gas analyzers. Therefore, the default delay values in Spiroware will not result in optimal signal alignment.
- The supervisor should perform several signal alignment maneuvers to determine the "characteristic" delay of their machine.
- Results within +/- 10 ms of previous or characteristic delay times are acceptable. Results outside this limit should prompt investigation into technique and equipment (see section 2.1, Signal Synchronization Troubleshooting).

7.2 Set up equipment for Flow/Gas Signal Synchronization (MBWN2 SOP section 5.4.1)

1. Ensure analyzer has been on for at least **5 minutes** to ensure temperature stabilization of internal oxygen analyzer.
2. Ensure flowhead, appropriate DSR+ SPIRETTE, CO_2 cuvette, and CAPNOSTAT sensor are in place with Nafion tubing connected and bias flow on (if applicable).

- Flow/Channel Signal Synchronization should be performed using the DSR Set to be used for testing.

3. The supervisor will require filter, mouthpiece, and nose clips to perform calibration.

7.3 Perform Signal Synchronization (MBWN2 SOP section 5.4.2)

- To ensure accurate calculation of results, gas and flow signals must be aligned in time.
- The algorithm used to synchronize gas and flow signals is dependent upon a step change in gas signals produced when post gas sampling-point dead space volume is re-inspired.

1. Navigate to the **Administration** menu, **System Settings** and select the **Calibration** tab.
2. Confirm that the correct post gas sampling-point (Post-Cap.) dead space volume is listed in the DSR Set Settings for the appropriate DSR Set to be used for synchronization and testing (Figure 1).
3. Confirm number of washout breaths for synchronization is set to **5** (Figure 1).
4. Confirm Dynamic Delay Correction is enabled (checked)

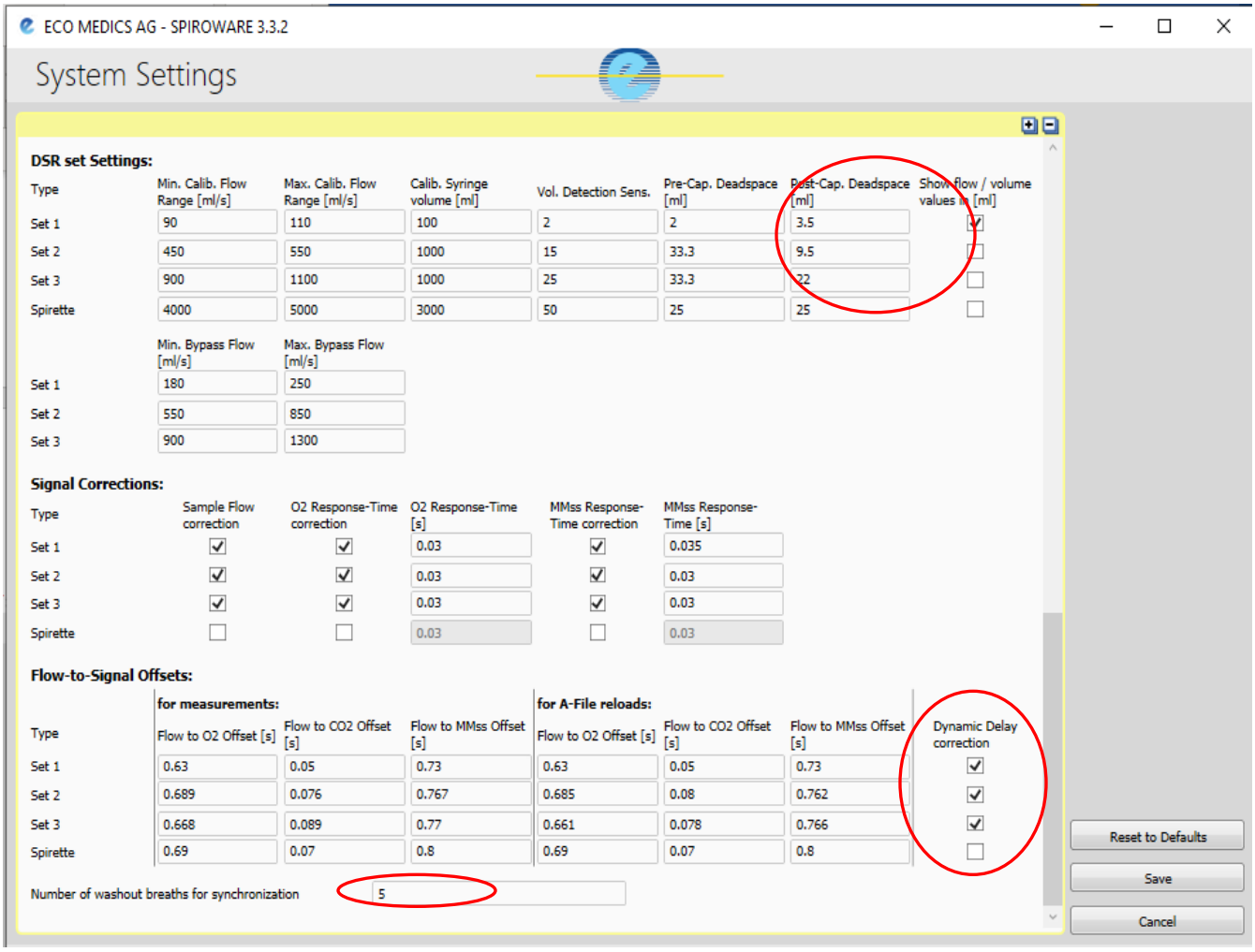


Figure 1. Post gas sampling-point dead space values, values entered here will be applied to raw data.

6. From the **Administration** menu, select **Flow/Channel Signal Synchronization.**

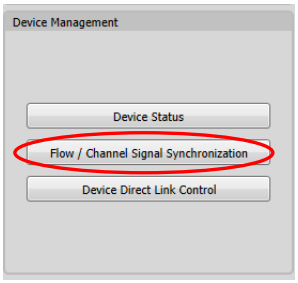


Figure 2. Administration Menu; Flow/Channel Signal Synchronization

7. Wearing nose clips, place mouth on mouthpiece and begin tidal breathing through the system; press **Start** (Figure 3).

8. As soon as breathing pattern is stable, select **Start Sync.** (Figure 3).

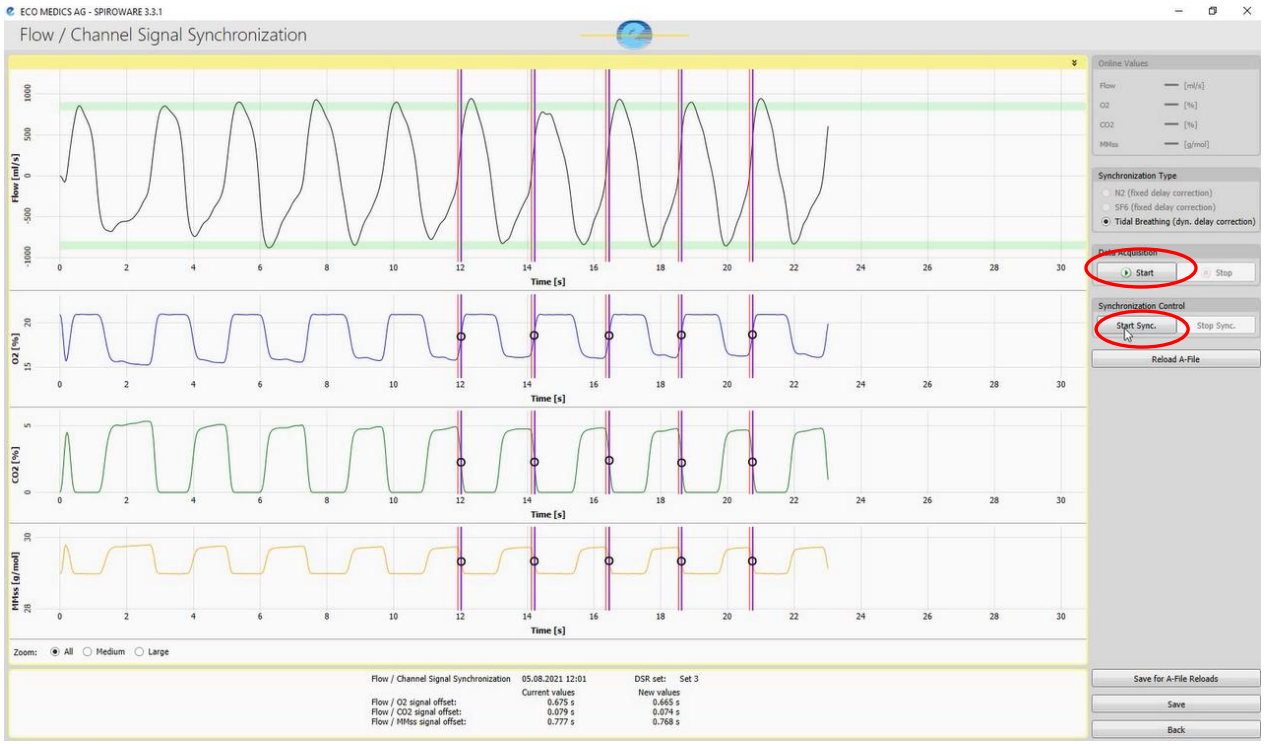


Figure 3. Flow/Channel Signal Synchronization; Set 3, 10 breaths

> ➢ Inhalation must be sharp enough to depict a clear zero crossing but not forceful enough to exceed bias flow and entrain room air. Exhalation must not be too slow or hesitant.

9. The supervisor should aim to achieve peak flows in the target range indicated by green bands along the flow tracing of the synchronization screen. The target range will vary with DSR Set # (Set 2 peak flow = 500 mL/s; Set 3 peak flow = 1000 mL/s).

- The system will then collect the **pre-determined number** of washout breaths and will stop automatically.
- The resulting Flow/ O_2 and Flow/CO_2 signal offsets **(delay times)** represent how much the CO_2 and O_2 signals will be shifted in time during recording to align with flow. Values are displayed at the bottom of the screen.

10. Confirm that the v**alues for O_2 are between 0.500 and 0.800s and that the values for CO_2 are between 0.040 and 0.070s**

- O_2 and CO_2 delay times are influenced by sample flow rate and to some extent properties of the respective gas analyzers and, in the case of O_2, the state and length of the Nafion sampling tube (see Exhalyzer D operator's manual for further information on sampling rate and gas analyzers).
- As such, providing the equipment set up is correct, the post gas sampling-point dead space volume is entered properly in system settings and the breaths used for calibration clearly cross zero flow the delay times should remain within +/- 10 ms.

11. Atypical delay times (see above) should prompt investigation.

12. Record delay times and ambient conditions, these values may be required to review data at a later date.