SWIM user guide

Sliding Windows in Ion Mobility (SWIM): a New Approach to Increase the Resolving Power in Trapped Ion Mobility-Mass Spectrometry Hyphenated with Chromatography

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This short manual is intended to help users to implement the SWIM method on their timsTOF instruments using timsControl (V3.1).

1. **$1/K_0$ vs RT plot**. First, create a plot of inverse reduced ion mobilities ($1/K_0$) versus retention time for the compounds of interest (Figure 1). If these values are not available, they can be determined by first performing a pre-scan GC/LC-TIMS-MS run on a sample containing the analytes of interest.
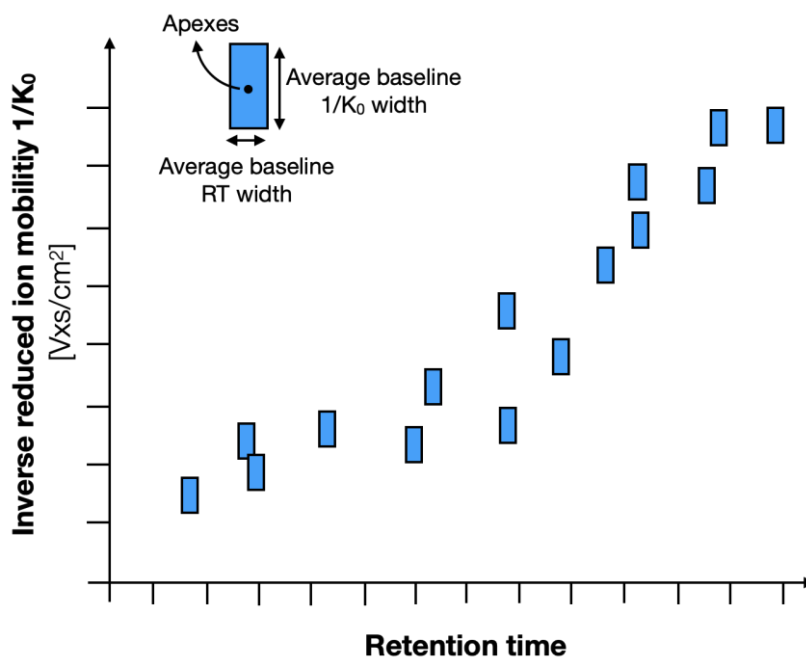

Figure 1 - Schematic plot of $1/K_0$ vs RT. Analytes are represented by the blue rectangles.

2. **Ion mobility windows configuration**. Superimpose rectangles (representing the ion mobility windows) on the plot of $1/K_0$ versus RT to cover the desired range of ion mobilities as a function of retention time (Figure 2). This step can easily be done using software such as PowerPoint. Note the following points.
   - The length of the IM windows in the $1/K_0$ dimension must all equal, since the IM calibration in TIMS is only valid for a given IM range (i.e., scan rate β). Smaller lengths result in higher resolving powers. In the example shown in Figure 2, the length of all five IM windows was set to 3 arbitrary units.
   - IM window lengths in the RT dimension can be set to different values and don't need to be equal.
   - Each analyte of interest must be completely included in the corresponding IM window (taking into account their average baseline width in both $1/K_0$ and RT dimensions).
   Note: Analytes can overlap two different IM windows as long as they are completely contained in both windows.
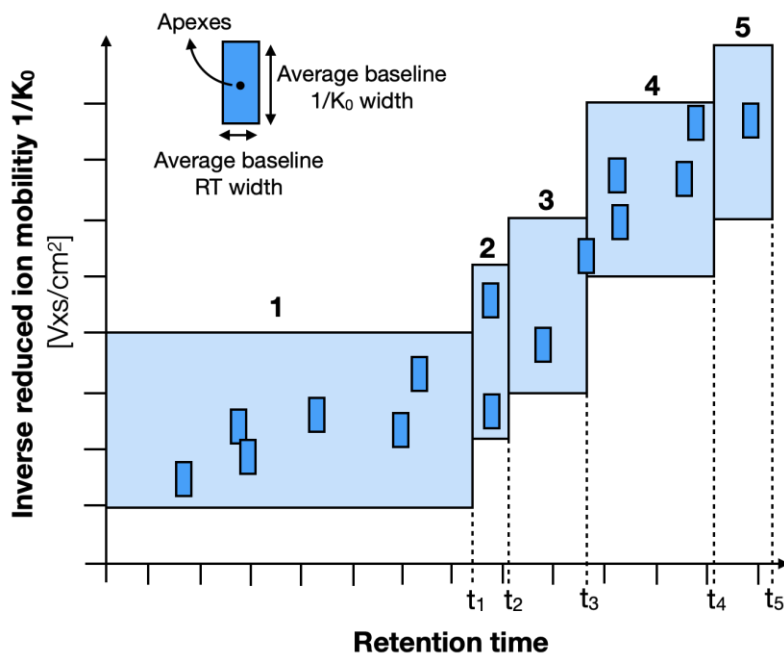
Figure 2 – Adding ion mobility windows (light blue rectangles) on the plot of $1/K_0$ vs RT. In this example, five ion mobility windows were defined to cover all the analytes.

3. **Setting the ion mobility windows in the TIMS instrument software**. Open the desired method (.m file) in timsControl. In the "Tuning" panel (Figure 3 - arrow 1), maximize the "Chromatogram view" (Figure 3 - arrow 2). To add an ion mobility window, right click anywhere on the chromatogram and select "insert new segment limit" (Figure 3 - arrow 3).
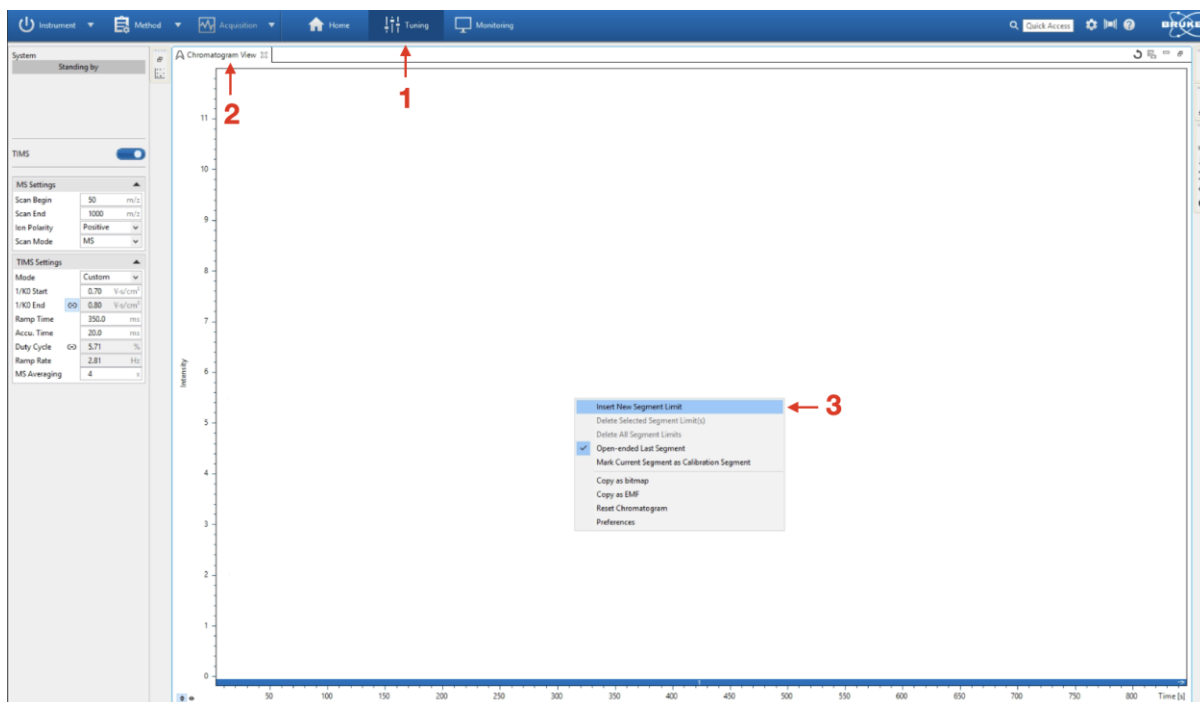

Figure 3 – Creating an IM window in timsControl.

The vertical line that appears in the "Chromatogram view" sets the chromatographic time limit of the first IM window. To change this time limit to match that set in the $1/K_0$ vs RT plot (i.e., t_1 in Figure 2), simply place the cursor on the vertical line limit and drag it to the desired retention time (Figure 4 – arrow 4). Then, set the IM range limits of the corresponding IM window (Figure 2) in the "TIMS Settings" box (Figure 4 – arrow 5). At this point, the first IM window is configured.
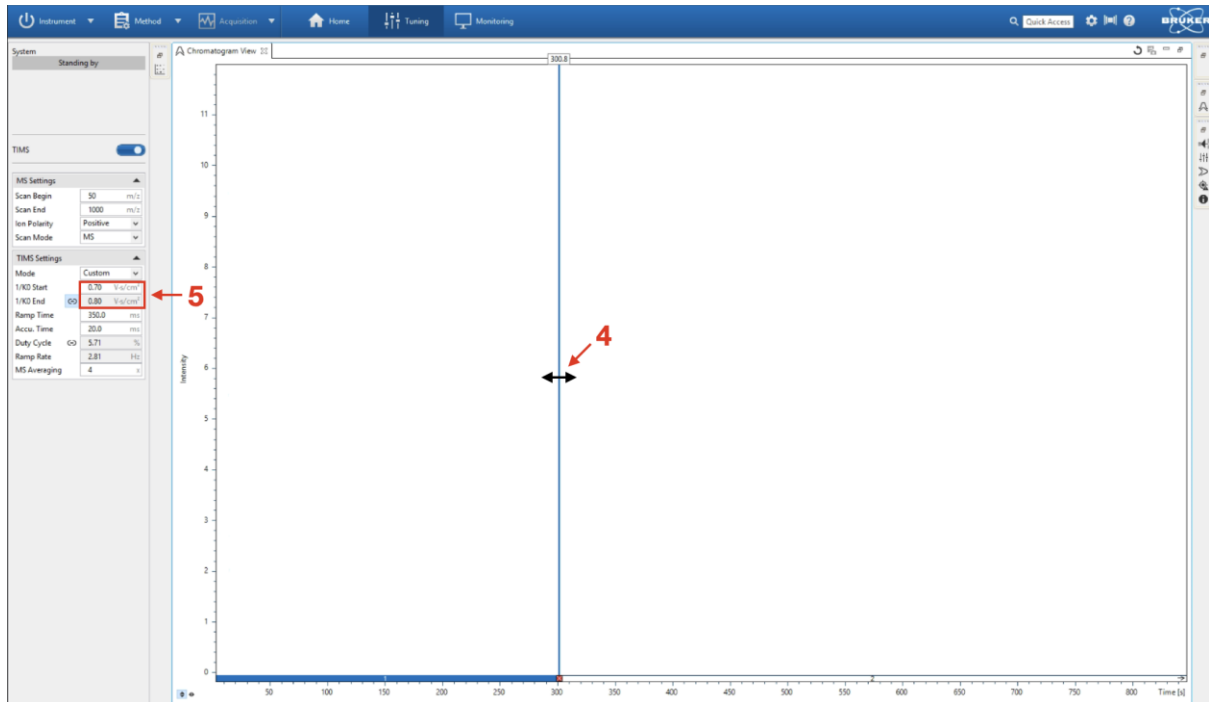

Figure 4 – Setting the retention time and the IM range limits of the first IM window.

These three steps of
- Inserting a new segment limit (arrow 3)
- Setting the retention time limits (arrow 4)
- Setting the IM range limits (arrow 5)

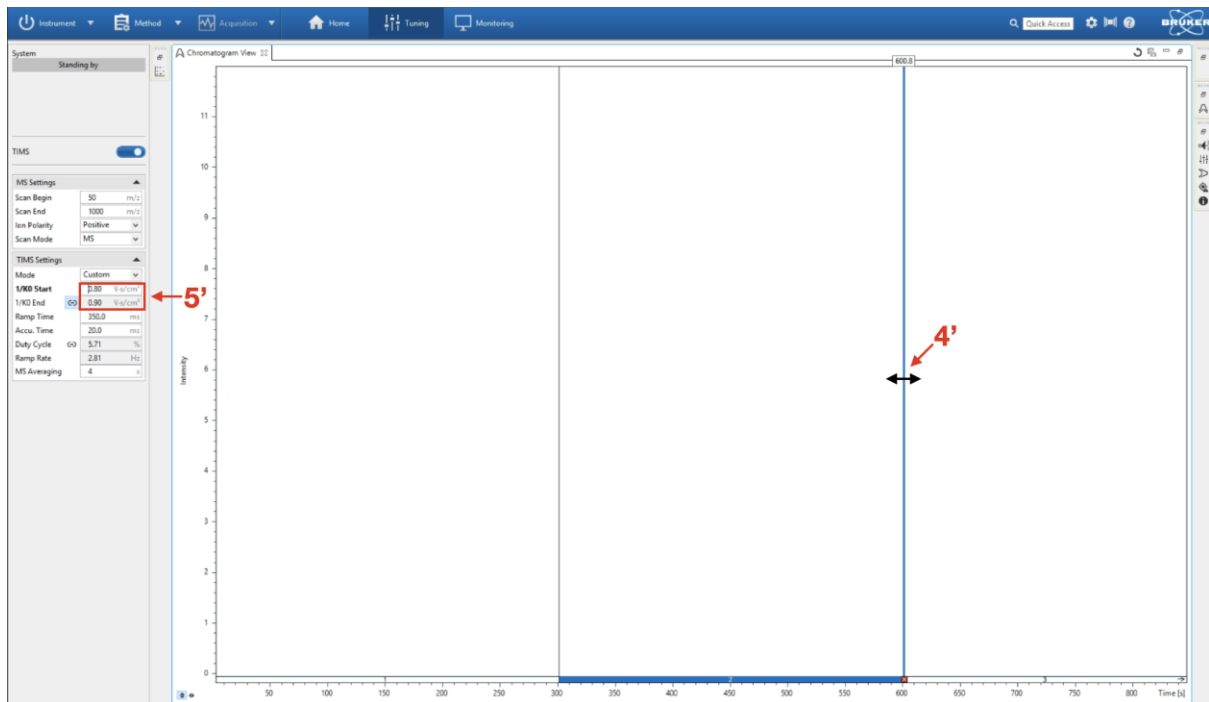can then be repeated to create the remaining IM windows (Figure 5).

Figure 5 – Setting a second IM window.

4. **Performing a SWIM analysis**. Once all the IM windows have been created, the method can be saved and used to perform TIMS analysis in SWIM mode. Before performing an analysis in SWIM mode, make sure to have a system that is well calibrated in the IM dimension. The IM calibration must be performed at the same scan rate β as that used for the SWIM mode.