# Using UltraFAIMS

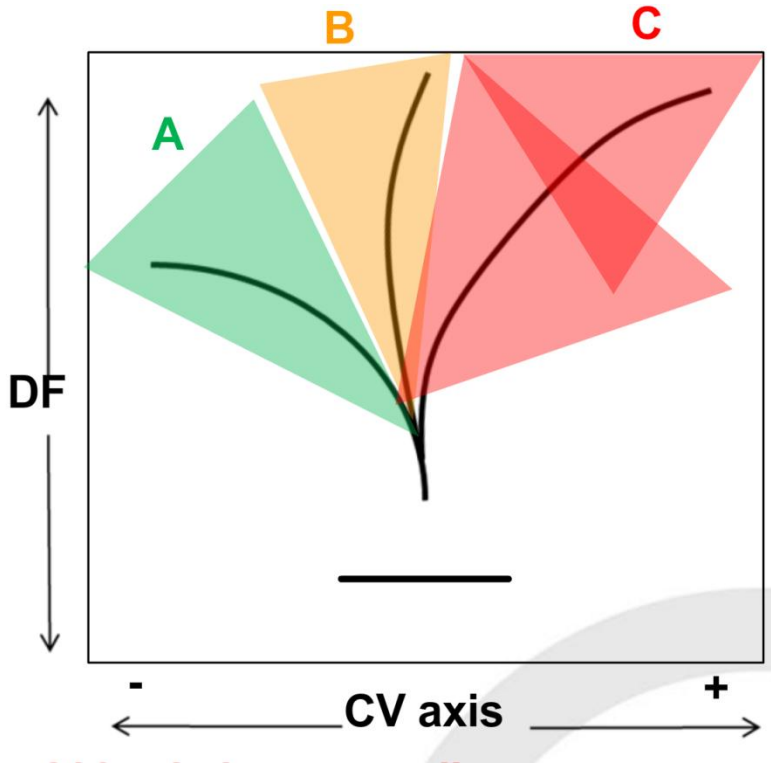
## Customer Training – Session 2

www.owlstonenanotech.com

- Method development for UltraFAIMS

# FAIMS performance optimization

- A DF/CF sweep (known as a 2D sweep) categorises ions into one of 3 groups.
- Type A are ions that de-cluster in high field conditions, making them smaller, with a higher mobility.
- Type C ions are polarised by high fields, becoming larger and moving slower due to increased interactions with the buffer gas molecules. Type C ions are typically observed using ultraFAIMS.




- Type B ions experience an extent of both effects.
- The exact DF/CF required for an ion's transmission is dependent on many experimental parameters which must be optimised and is often a balance between separation and sensitivity.

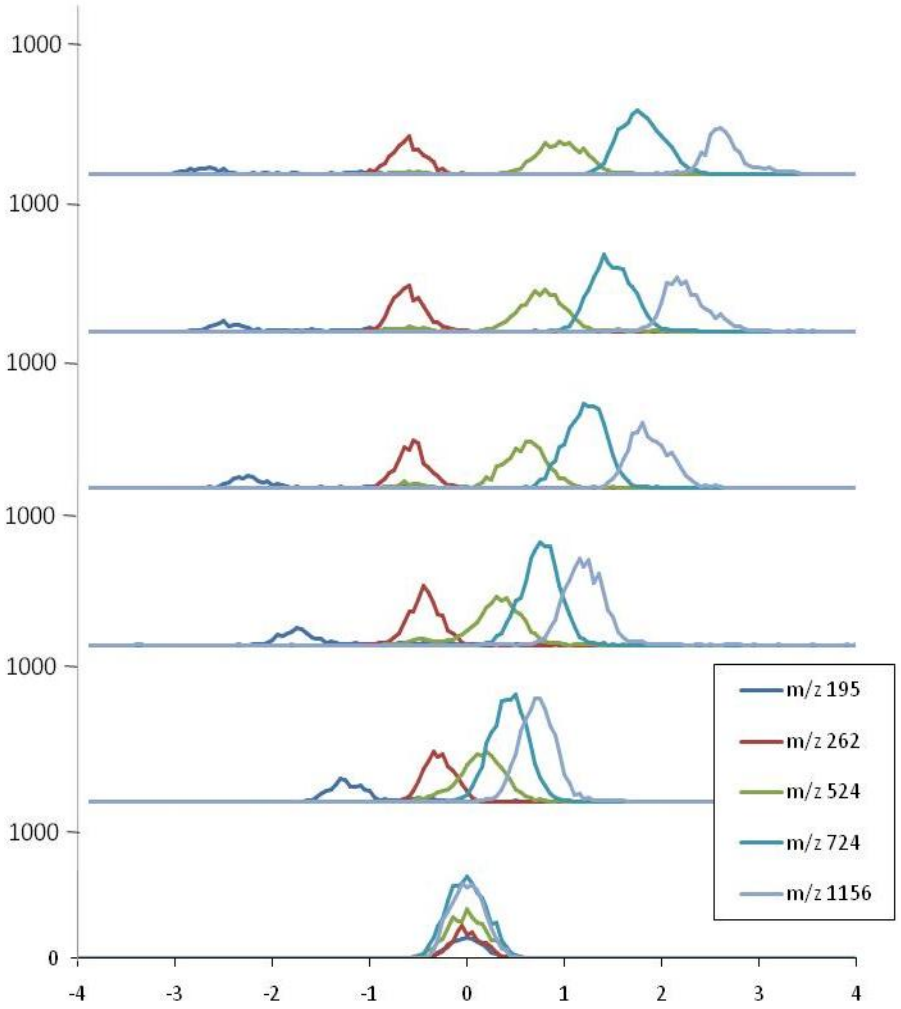
# DF optimisation

- In low electric fields, the ion will oscillate equally in both directions with no overall drift. All ions will be transmitted simultaneously, with no additional CF required.

- In high electric fields there is a net drift towards a sidewall due to differences in the low and high field mobility, requiring the addition of the CF to transmit ions of interest.

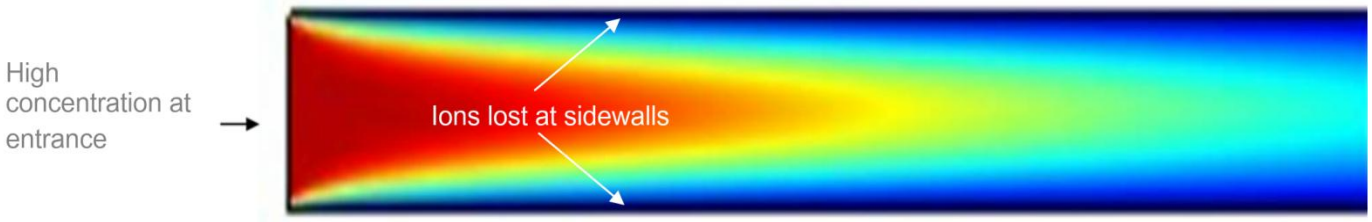
    - A mixture of ions carried by a gas flow can be resolved into several peaks by scanning CF.

    - The CF required is dependent on the magnitude of the DF. Increased separation can be observed with increased electric fields.



# Sensitivity

- Diffusion losses do occur whilst ions move though the device.
- Ions with higher mobility will suffer higher diffusion losses (for a given residence time)
- A reduction in ion residence time reduces diffusion losses




- Carrier gas flow rate through the ultraFAIMS chip is fixed, relying on what is pumped from the mass spectrometer so there is no scope to increase flow rate to reduce diffusion losses
- However, different chip designs have different flow path cross-sectional areas – a smaller area leads to shorter ion residence time & hence lower diffusion losses – the penalty is less time for separation, hence lower selectivity
- The RF electric field also causes field heating of ions. This additional heating is added to the thermal energy of the ions, so higher fields further increase diffusion losses, resulting in a sensitivity drop.

- Therefore the optimal electric field & chip choice is a balance between selectivity and sensitivity.

# DF - Townsends

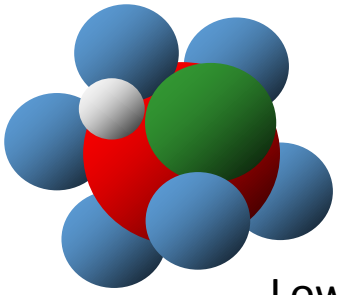
- DF settings are specified in Townsends (Td), which are units of reduced field.
- The reduced field (E/N) in Townsends equivalent to a given field strength can be calculated as follows:
- Reduced field in $Vm^2$ is: $$E/N = \frac{Ek_BT}{P}$$

  where $k_B$ is Boltzman's constant ($1.38 \times 10^{-23}$), T is carrier gas temperature in Kelvin, P is pressure at the chip (assumed to be ambient pressure) in Pa, and E is the dispersion field strength in V/m.

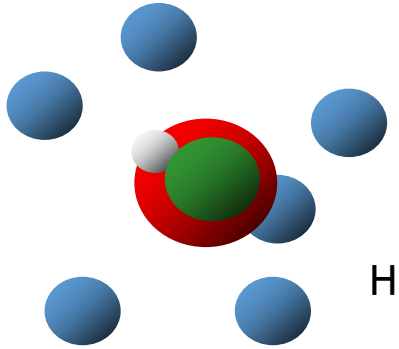
- The conversion of reduced field from $Vm^2$ into Townsends, is $1Td = 1 \times 10^{-21}\ Vm^2$.
- **Pressure cannot be controlled using UltraFAIMS, however daily ambient fluctuations are measured by the control unit & corrected for.**
- **Chip region temperature must be input into the software correctly (see later).**

# Using gas modifiers

- Ions clustered with neutral molecules dissociate due to temperature increases in high field conditions
    - Forms ‘bare’ ions with a reduced cross section - increased mobility in high field.



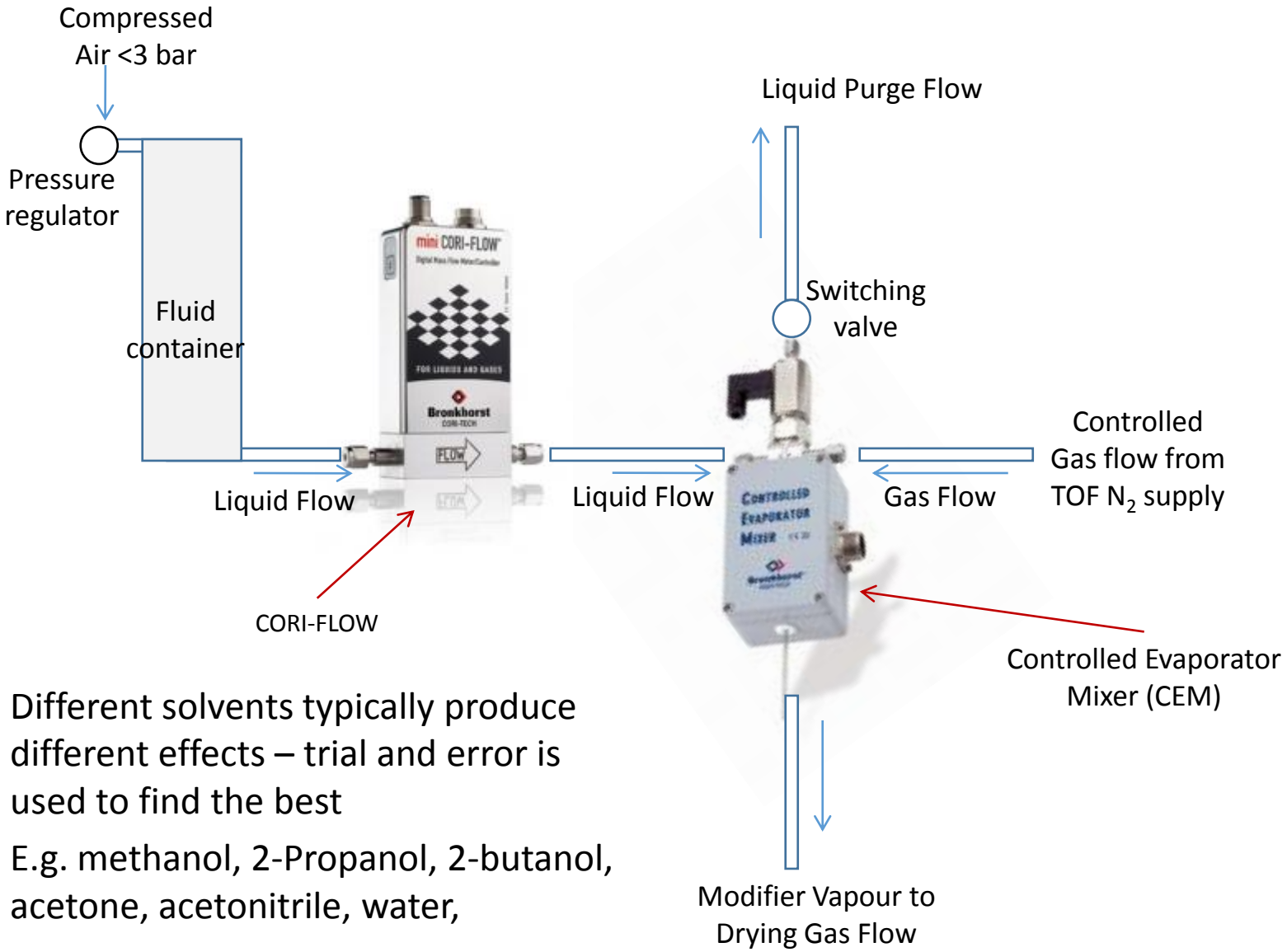
Low field



High field

- Volatile reagents can be introduced to modify how the ions interact with curtain gas during FAIMS separation.
- Different species have different affinities to form clusters in the low field
    - Dependent on hydrogen-bonding potential, electrostatic attraction, steric repulsion and favourable conformations of ions.
- Has the potential to increase separation capacity of FAIMS.

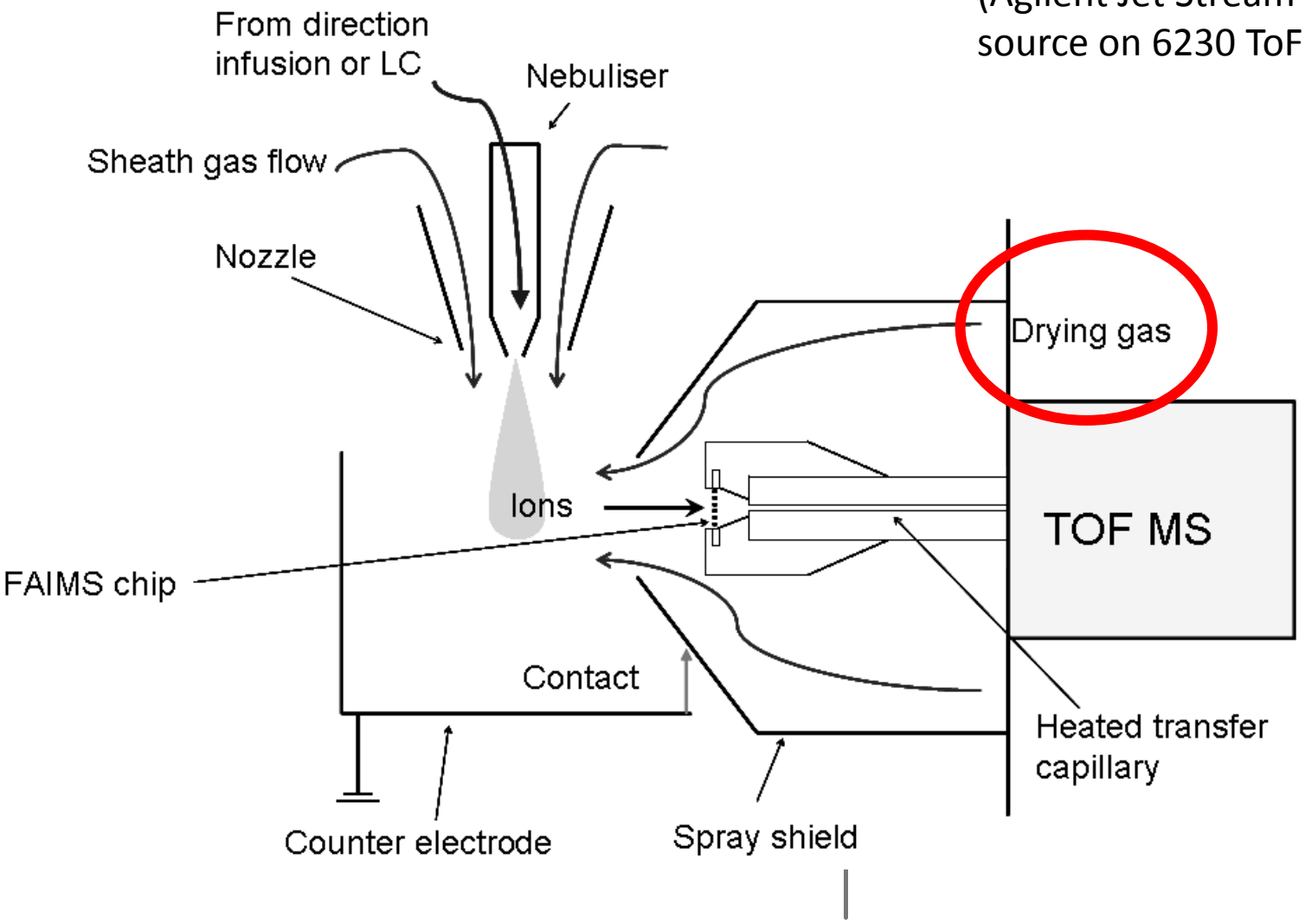
# Modifier rig set up – Bronkhorst CEM




- Different solvents typically produce different effects – trial and error is used to find the best
- E.g. methanol, 2-Propanol, 2-butanol, acetone, acetonitrile, water,

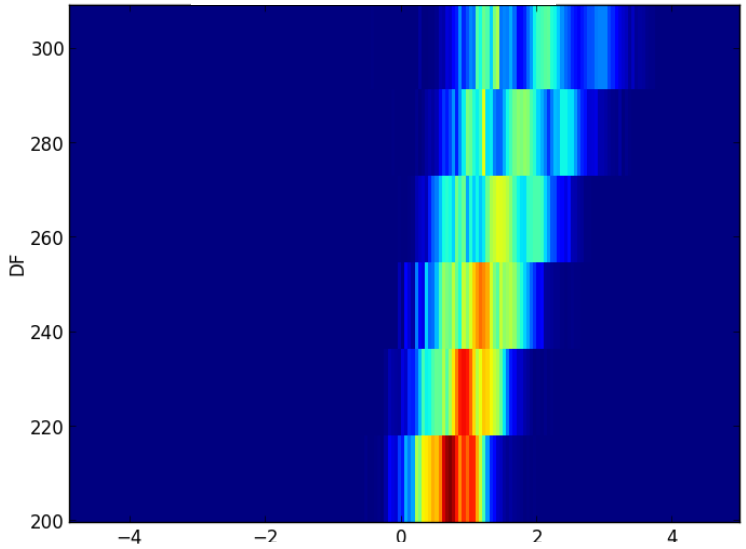
# Modifiers introduction

(Agilent Jet Stream ESI source on 6230 ToF)

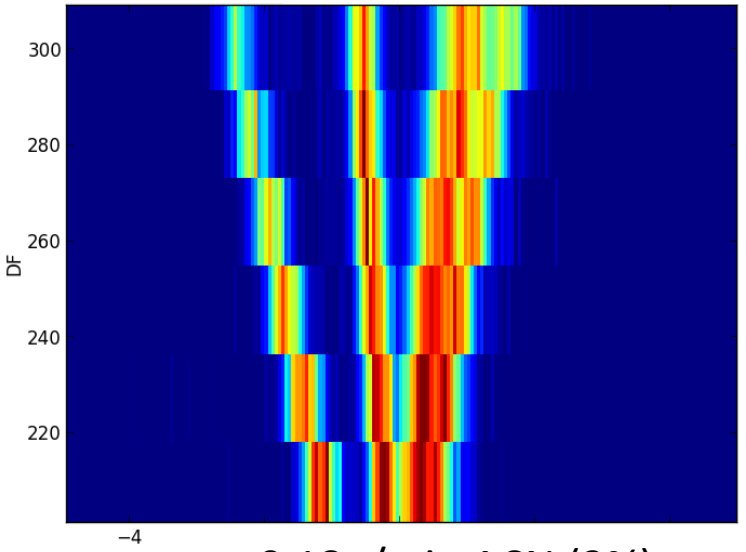


# Modifiers example – o,p,m phthalic acids

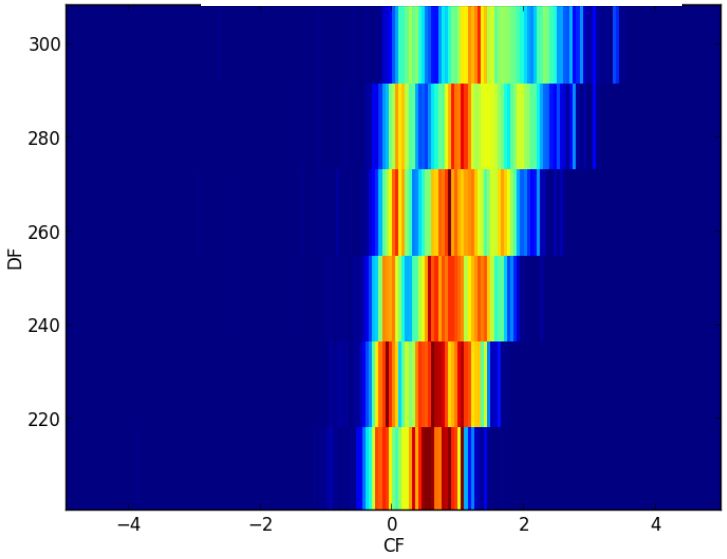
0.00g/min ACN



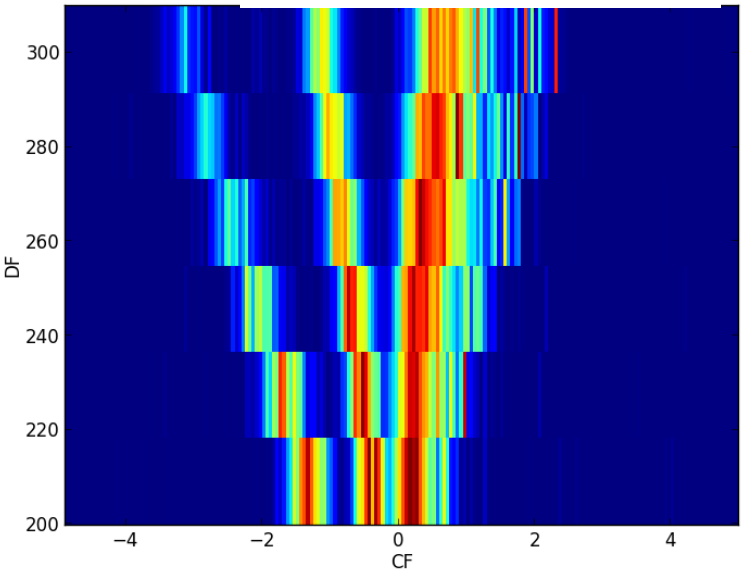

0.12g/min ACN (2%)




0.06g/min ACN (1%)




0.18g/min ACN (3%)



# Sweep mode *vs* static mode

The software provided allows three modes of operation, as follows:

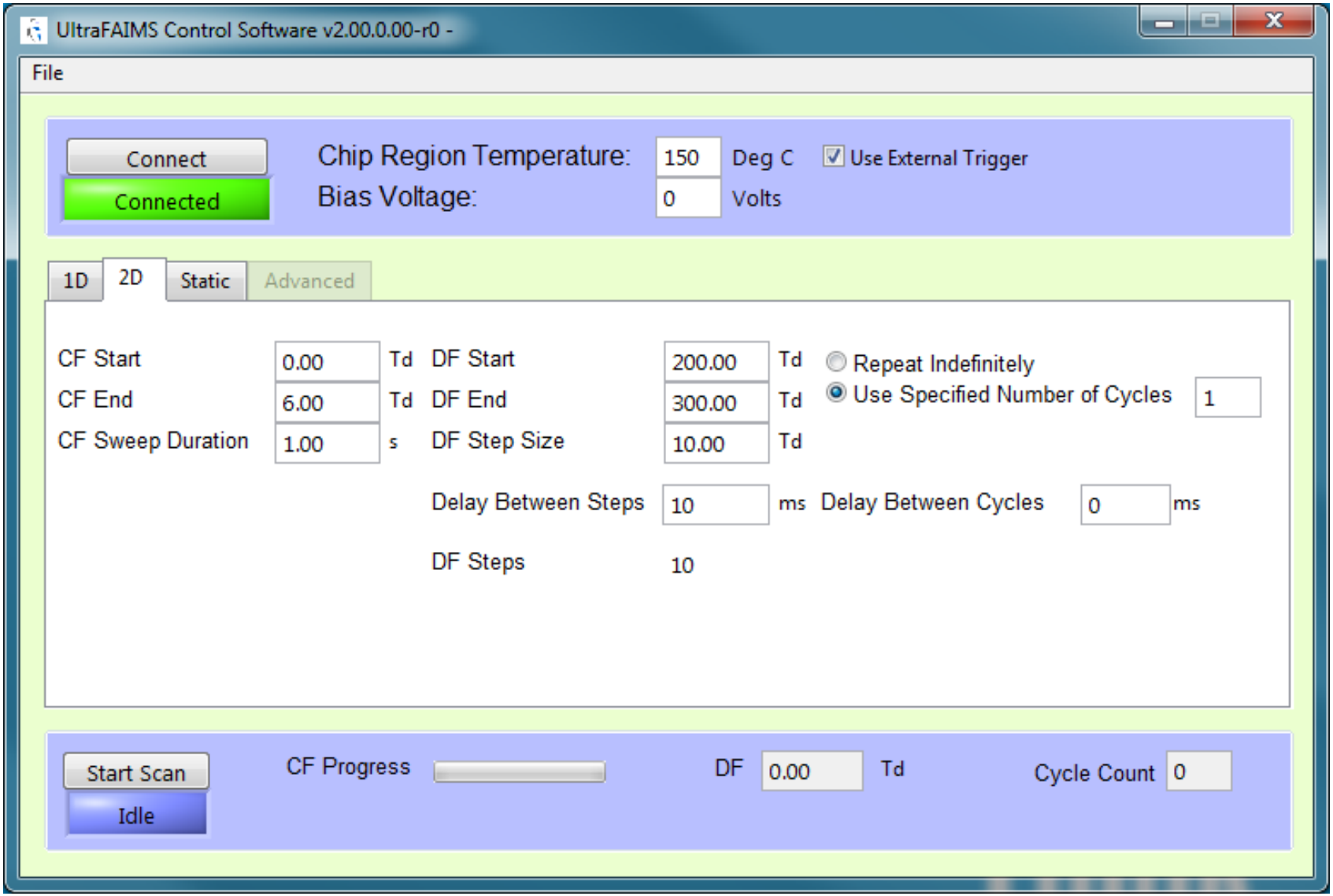
1. 1D sweep
   A single dispersion field value is chosen and the system repeatedly sweeps over a set CF range to form a spectrum of ions passing through the filter. Both DF value and CF range can be set by the user.

2. 2D sweep
   As the 1D sweep but in this mode the system carries out a two-dimensional sweep by building up a series of CF sweeps at gradually increasing DF levels. The maximum and minimum DF levels and number of DF steps can be set by the user, as can the CF range.

3. Static/Hop table
   DF and CF parameters are set at fixed values, to allow a single subset of ions through the filter.

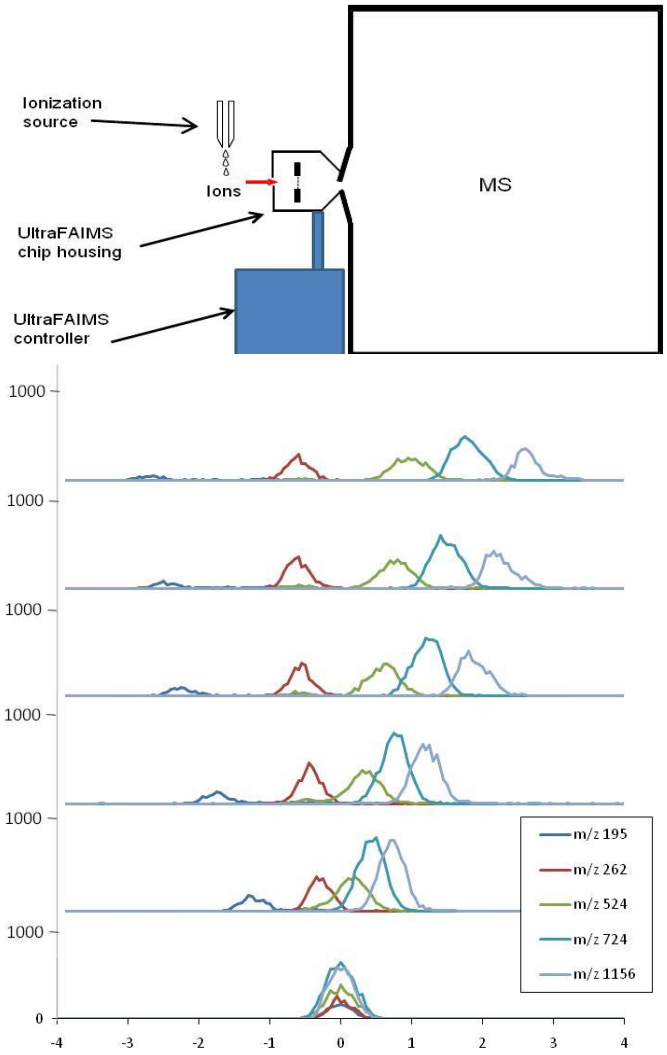
Different modes of operation may be required depending on the type of analysis being carried out.

# Example software set up



# Method development with FAIMS

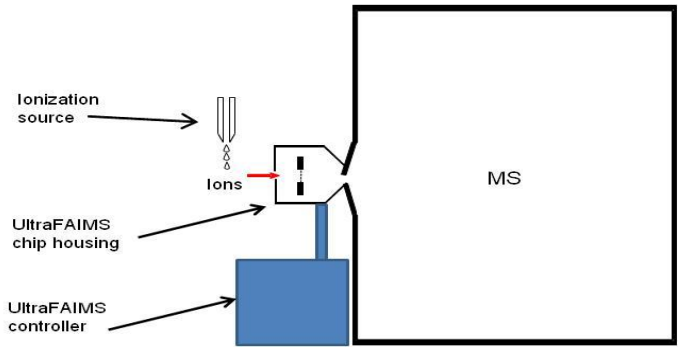
1. Targeted analysis

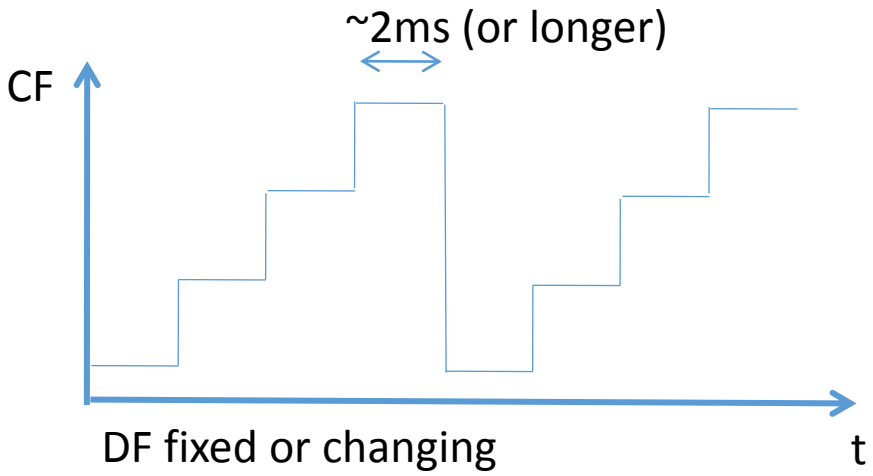



- Infuse each analyte separately (if possible)
- Run in “sweep mode” – producing a FAIMS spectrum at several DF levels
- Repeat for each analyte
- Overlay spectra
- Select the DF that gives best separation
- If necessary, repeat above steps with different conditions or with modifiers
- Identify optimum CFs for each analyte & apply using “static” mode during the measurement runs.

# Method development with FAIMS

2. Untargeted analysis




- Ideally, run preliminary infusion experiments with some representative standards, using FAIMS sweep mode, to identify good conditions for separation – i.e. best DF and CF range
- Divide CF range into segments
- Then set up FAIMS in static mode to hop through each CF segment
- Run using normal sample introduction method
- (Can also continue to run in sweep mode, if preferred)

- Method development for UltraFAIMS
- Case study example

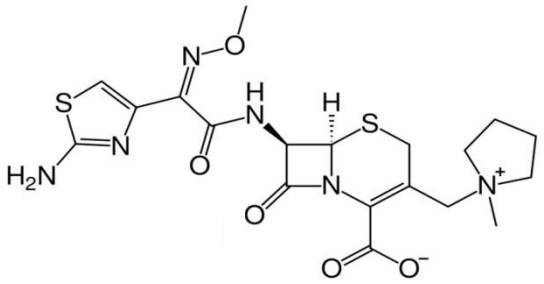
# Case study: separation of cefepime and NMP

- Cefepime hydrochloride is a common antibiotic
- N-methylpyrrolidine (NMP) is the principal hydrolysis product
- NMP is considered harmful and is limited in cefepime hydrochloride by current regulations to not more than 0.3% w/w
- Existing methods for the LC analysis of this impurity fail to separate NMP from the drug substance prior to analysis
- NMP continues to be formed by hydrolysis of cefepime in the final analytical solution and so the starting concentration cannot be accurately determined

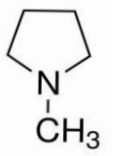
**Can FAIMS help?**

# Case study: can FAIMS help?

- We have seen that ions that have structural differences or different clustering affinity for neutrals may separate using FAIMS
- Will cefepime and NMP separate?



Cefepime

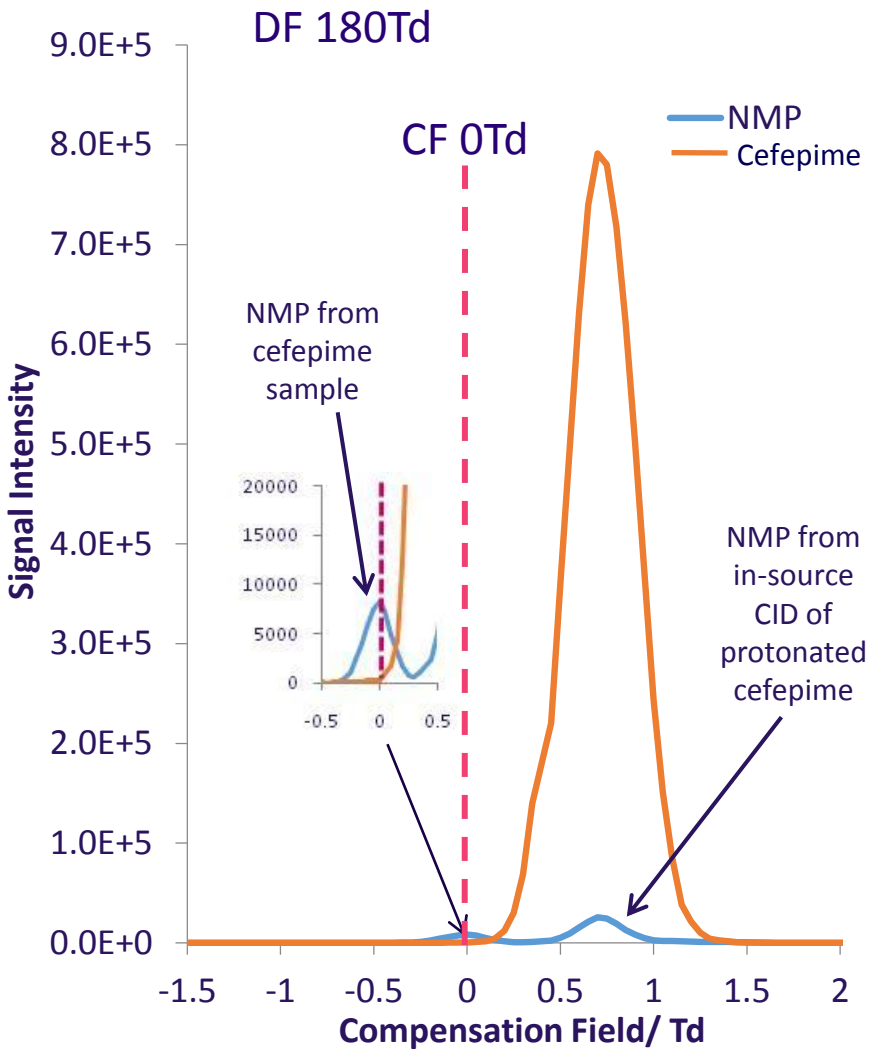


NMP

- In this case, there is a big difference in structure, so fairly good chance of separation
- But this is hard to predict *a priori* – especially with small structural differences
- Must be tested by experiment

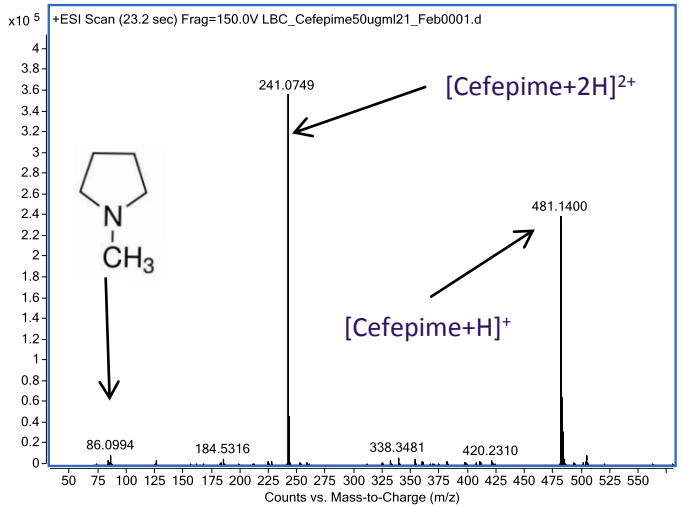
# Case study: Method development stage

- Solutions of NMP and Cefepime prepared in methanol/water (50:50) + 0.1% formic acid were directly infused (10µL/min) into the ESI source, which was operated in positive ion mode
- Dispersion fields (DF) of 180-300Td were used for optimisation phase
- NMP trace shows two peaks:
  - At CF ~0Td, arising from NMP in the Cefepime sample
  - At CF 0.7Td, arising from the transmission of intact Cefepime, followed by in-source CID to form NMP
- FAIMS selection at 180Td allows NMP in Cefipime to be separated from NMP formed by in-source CID

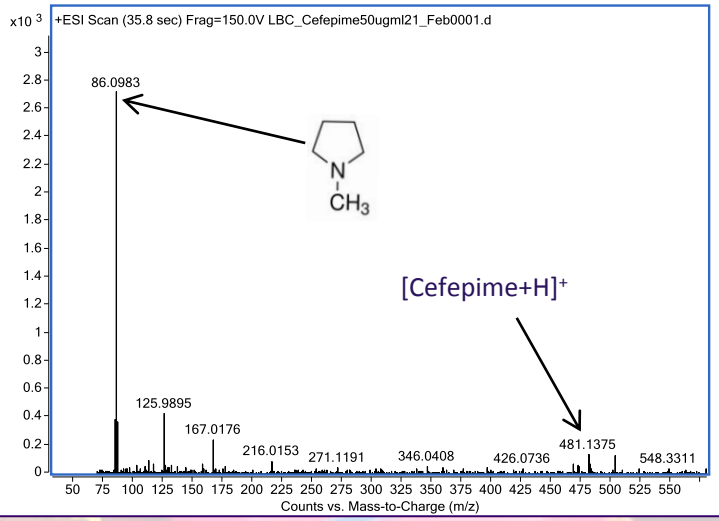


# Case study: Quantitation stage

(a) without FAIMS separation




(b) with the FAIMS set to CF 0 Td, DF 180 Td to transmit NMP




Mass spectra of NMP (0.05 µg/ml) in cefepime (50 µg/ml)

- UltraFAIMS was operated in static mode for quantitation, enhancing S:N of NMP
- The LOQ was 0.013µg/ml, equivalent to 0.027% (w/w) in the Cefepime sample, which is well below the 0.3% threshold

| LOD /µg.ml$^{-1}$ | LOQ /µg.ml$^{-1}$ | LDR /µg.ml$^{-1}$ | $R^2$ | %RSD |
|---|---|---|---|---|
| 0.004 | 0.013 | 0.005-0.5 | 0.9979 | 3.85 |

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- Case study example
- Lab session 2: Demo & practical examples

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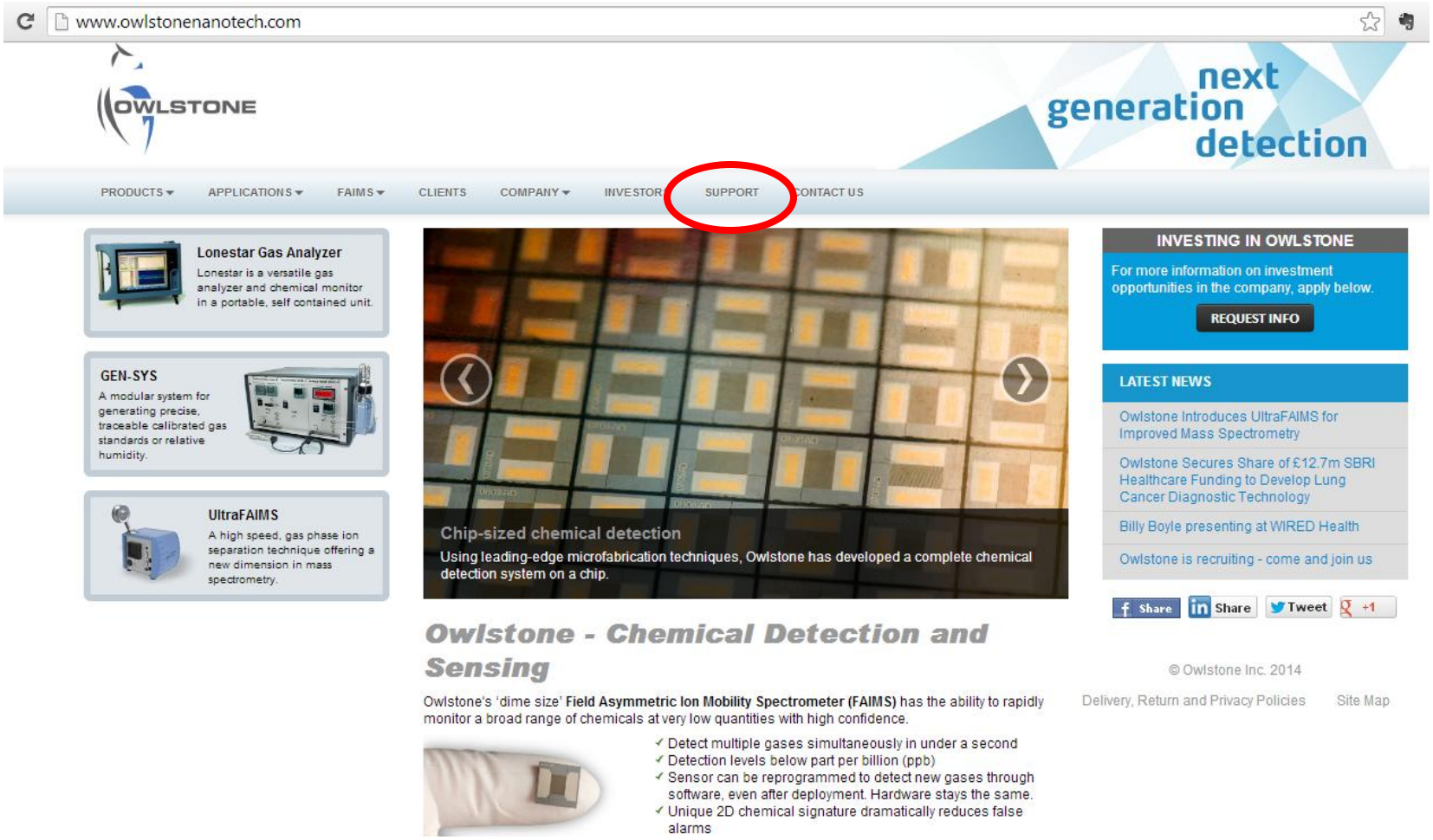
- Technical support & resources

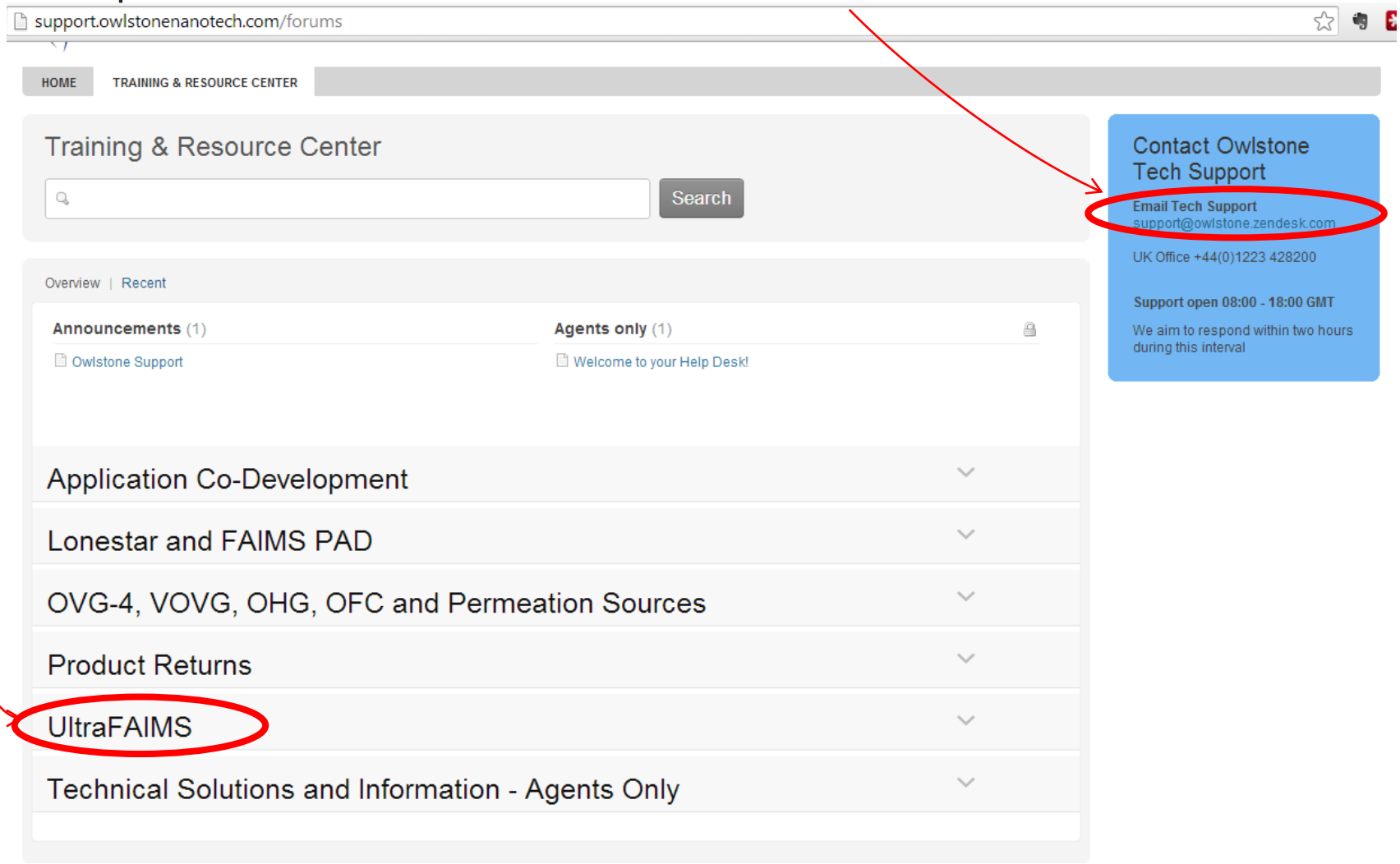
# Technical support

- For technical support, follow the “support” link on our website:



# Technical support

- The support site contains some reference information
- For other queries, please use the support email to ensure a fast response



# IM-MS contacts

- LinkedIn IM-MS group

  https://www.linkedin.com/groups/Ion-Mobility-Mass-Spectrometry-4404523

- ASMS IMS SIG

  http://www.asms.org/member-center/interest-groups/ion-mobility-mass-spectrometry

  Matt Bush (mattbush@uw.edu)

  Erin Baker (Erin.Baker@pnnl.gov)

- BMSS SIG

  http://www.bmss.org.uk/SIG_ion-mobility.shtml

  Perdita Barran (perdita.barran@manchester.ac.uk)
  Justin Benesch (justin.benesch@chem.ox.ac.uk)
  Claire Eyers (claire.eyers@liverpool.ac.uk)

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- Q & A / Wrap up