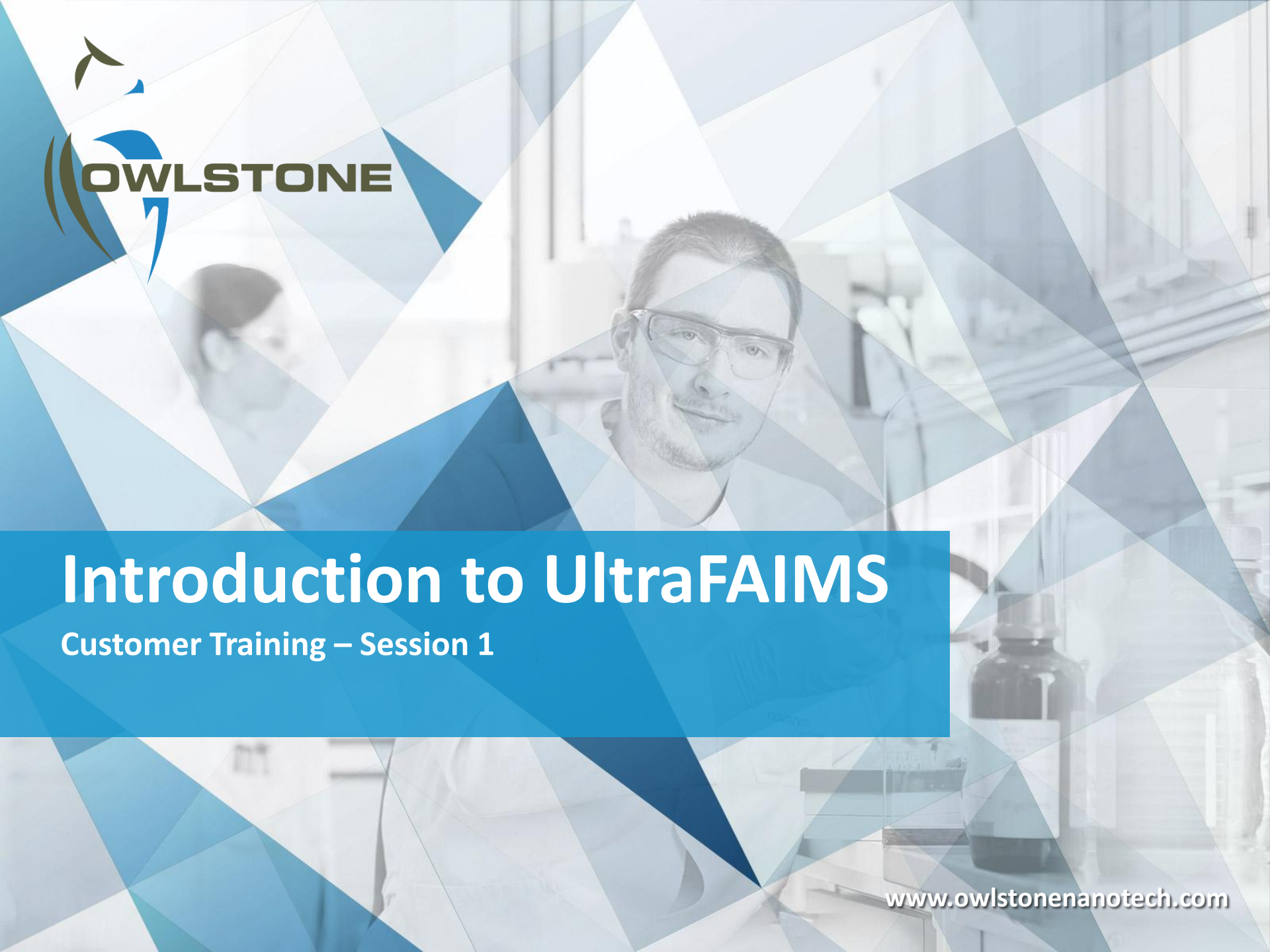


Introduction to UltraFAIMS

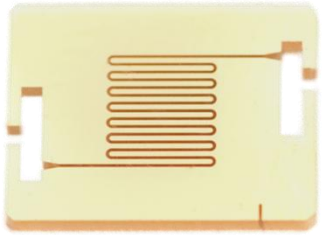
Customer Training – Session 1

www.owlstonenanotech.com

UltraFAIMS is....

a fast

easy to use



orthogonal separation stage

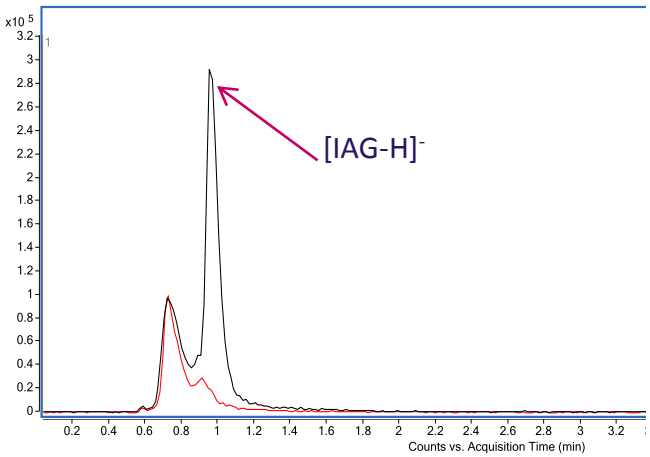
to enhance MS performance

for less than the cost of a new source

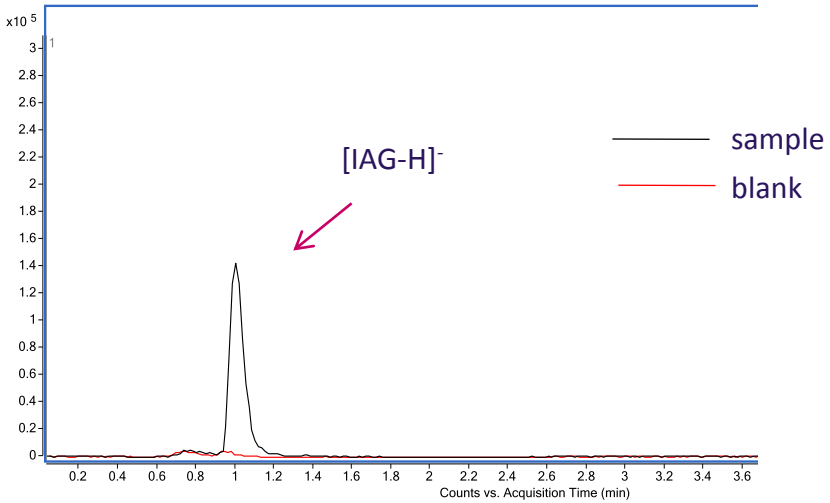
Why use ultraFAIMS with MS?

1. To improve LOQ when you have isobaric (and sometimes isomeric) interference

FAIMS Off



FAIMS On



S:N = 54:1
LOQ = 0.018µg/ml

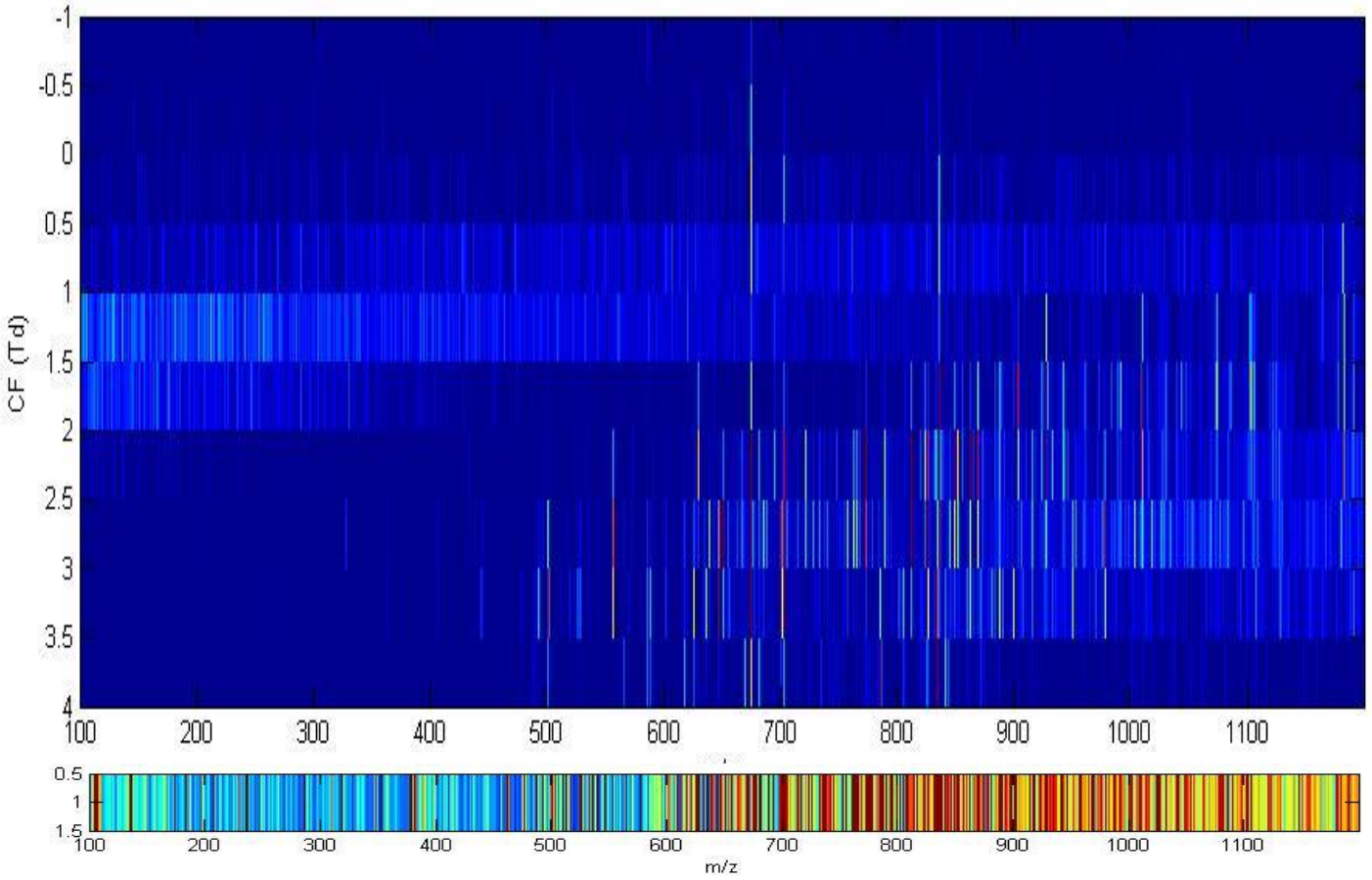
S:N = 93:1
LOQ = 0.010µg/ml

Why use ultraFAIMS with MS?

2. To add an extra dimension of fast separation
   - e.g. to distinguish charge states, conformers, different classes of compound
   - allowing user see more of what's there or speed up LC

(Low mass polyamide, direct infusion)

WITH FAIMS



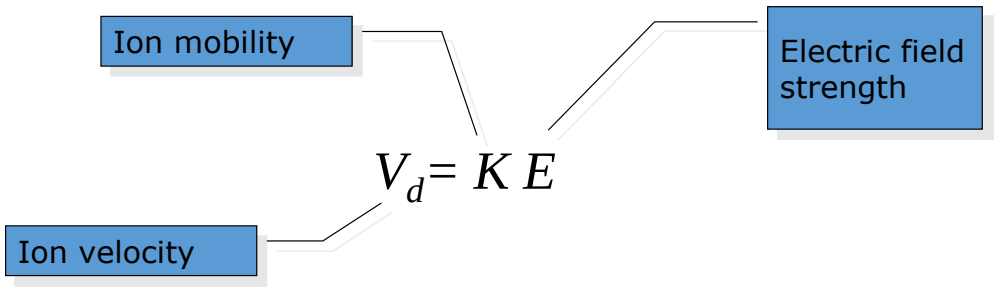
NO FAIMS

- Understanding FAIMS separation

What is FAIMS?

- FAIMS = High-**F**ield **A**symmetric-waveform **I**on **M**obility **S**pectrometry
  - Also known as Differential Mobility Spectrometry (DMS)

- It is a form of Ion Mobility Spectrometry, in which ions are separated on the basis of differences in the rate at which they drift through a buffer gas when driven by an electric field



- At low fields, the drift velocity is proportional to the field strength – and the constant of proportionality is called the ***(reduced) mobility***

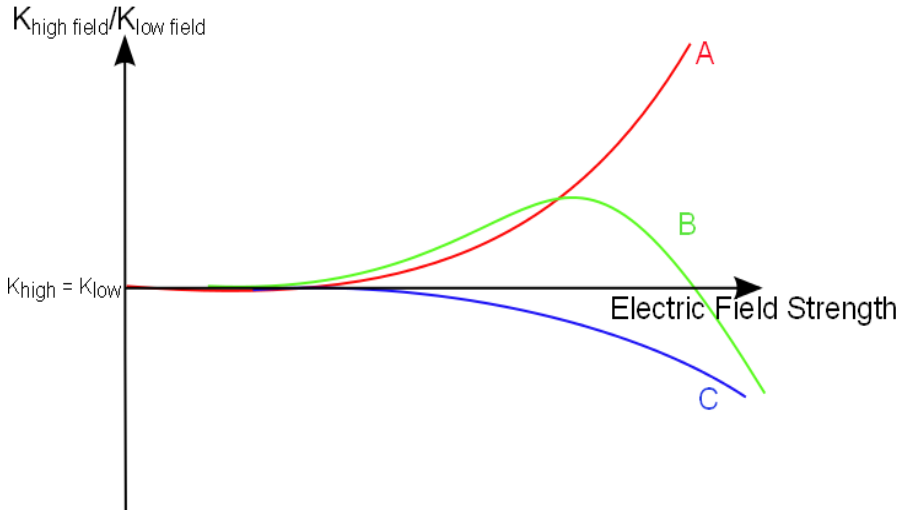
Low-field IMS

- Low-field Ion Mobility Spectrometers separate ions on the basis of their low-field mobility
- Ions are carried through the instrument by a gas flow
- A field of constant magnitude is applied (can be DC or sinusoidal) in the direction the ions are travelling
- If a packet of ions is released, the time at which the various ions reach the end of the drift region depends on their mobility
- This approach is analogous to Time-of-Flight MS
- A measurement of mobility allows the calculation of the ion's collision cross section (Ω)

$$K_0 = \frac{3ze}{16N_0}\left(\frac{2\pi}{\mu k_B T}\right)^{\frac{1}{2}}\frac{1}{\Omega}$$

Why FAIMS?

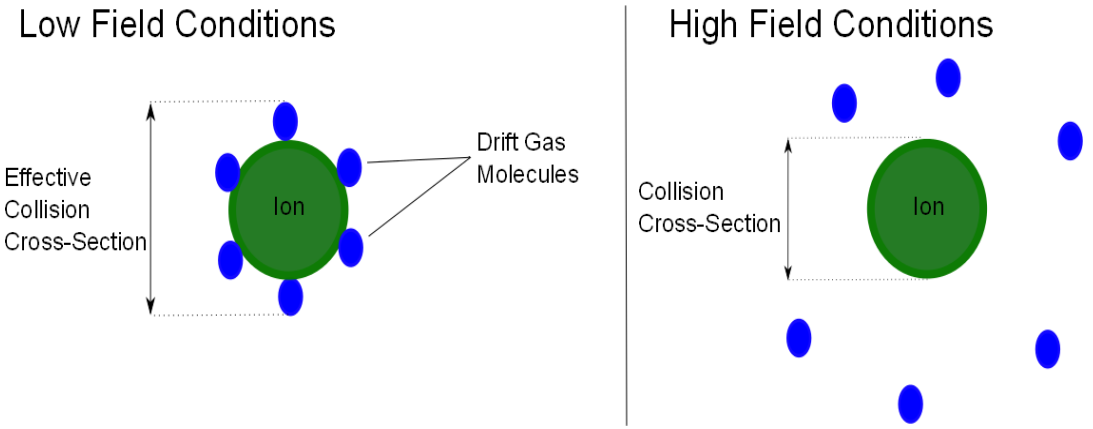
- IMS provides a fast separation, but there are some limitations
  1. IMS is a pulsed technique – ions must be release in packets, which can limit dynamic range & speed
  2. Resolution increases with length of drift region – but this leads to longer drift times and hence slower separation
- FAIMS emerged as a way to overcome these limitations
- It makes use of the fact that above a certain field strength, mobility is no longer constant – it becomes a function of the field strength



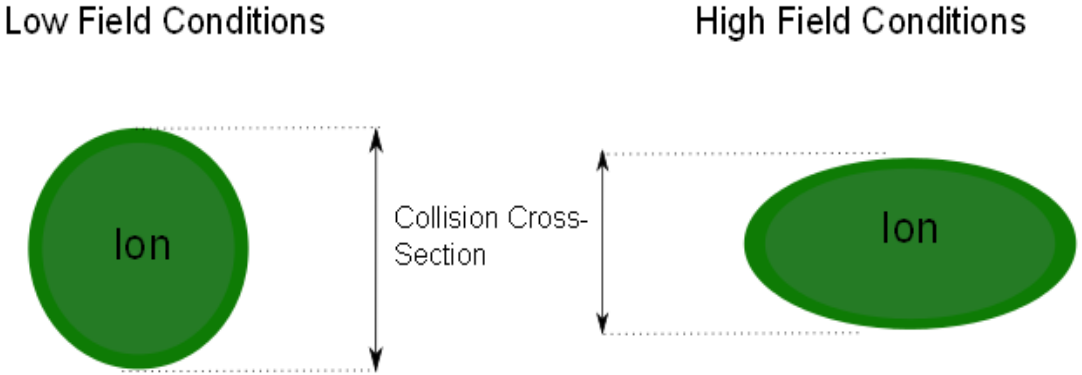
Why does mobility vary with field?

- At high fields, various effects can alter the ion cross-section – in particular clustering/declustering and structural changes due to field interactions
- This results in a “differential mobility” i.e. a difference between high & low field mobility

$K_{high} > K_{low}$

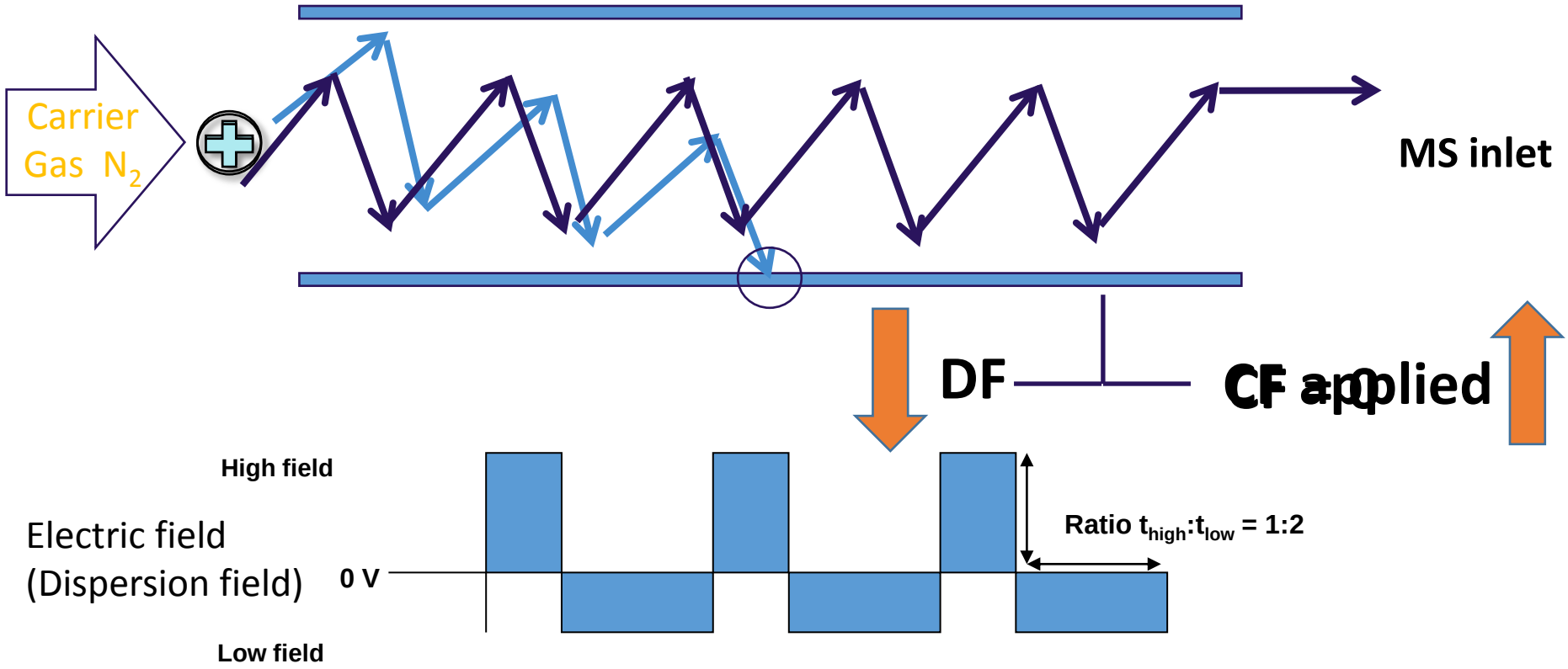


$K_{high} < K_{low}$



Inside the FAIMS device…

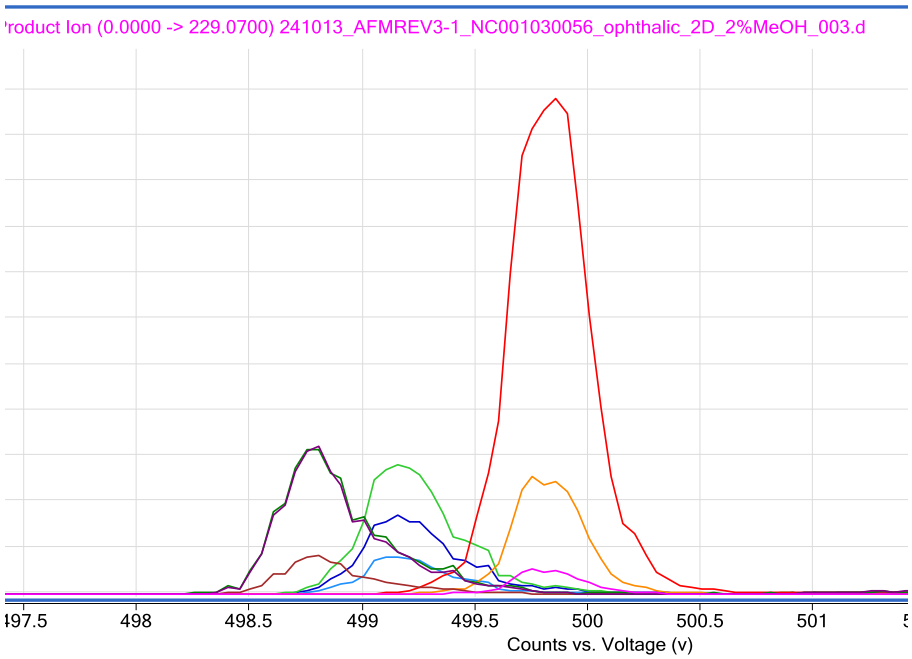
- Ions are carried between a pair of electrodes by the gas flow
- An alternating asymmetric field (dispersion field, DF) is applied perpendicular to the flow direction

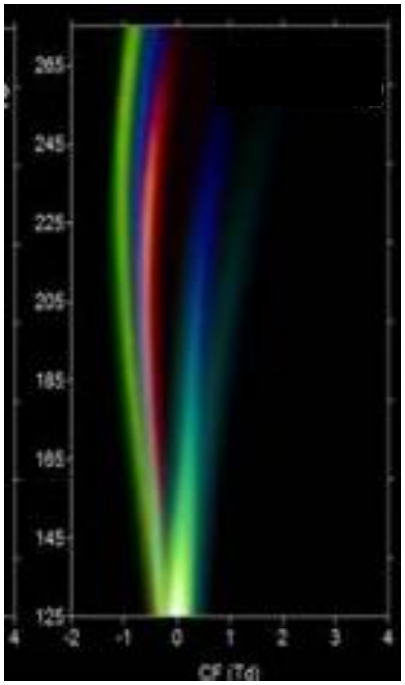


- The ions follow a zig-zag path as the field alternates
- Because the high field mobility is different to the low field mobility, there is a net drift towards the sidewall
- This sideways drift can be cancelled out by applying an opposing DC compensation field (CF)

The FAIMS device is a tunable filter

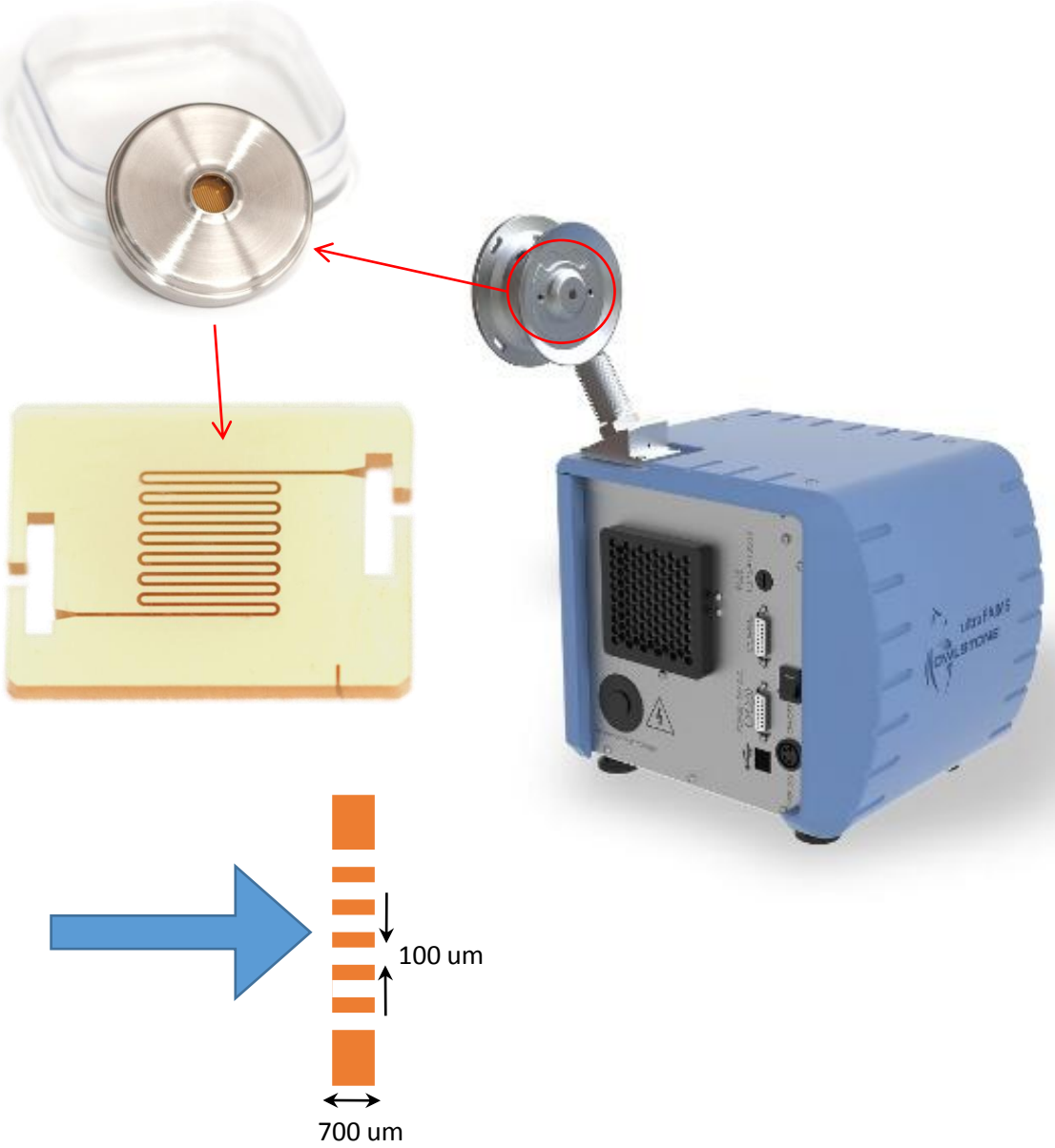
- Applying different CFs allows different ions to travel through the device
- Using a fixed CF, the device acts as a filter = **static mode**
- Scanning the CF across a range produces a FAIMS spectrum = **sweep mode**
- The magnitude of the DF can be altered to find the best separation





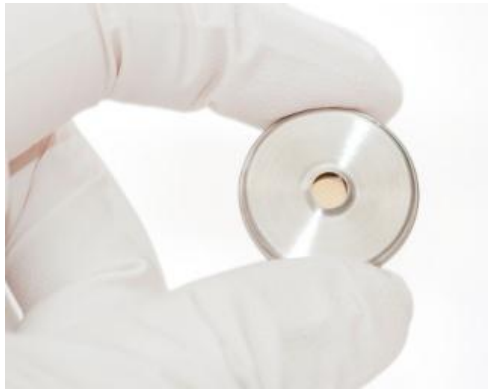
- Understanding FAIMS separation
- The ultraFAIMS difference

What is UltraFAIMS?



- Owlstone have developed a miniaturised version of FAIMS in which the electrodes are formed from a micro-manufactured “chip”
- Each device consists of a set of parallel gaps in a metal substrate that forms the electrodes
- The key dimensions that have been reduced are the electrode gap (now 100um) and the channel length (700um)
- Chip modules are replaceable

Why the chip-based approach?

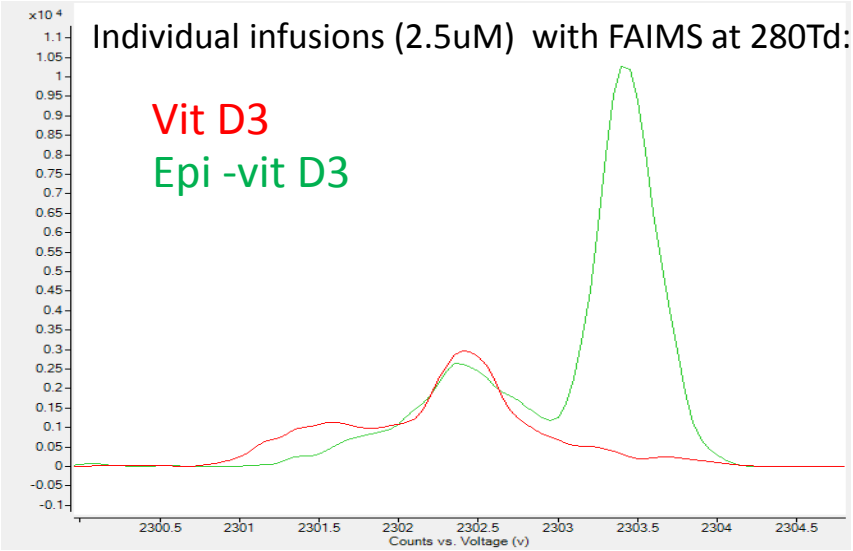


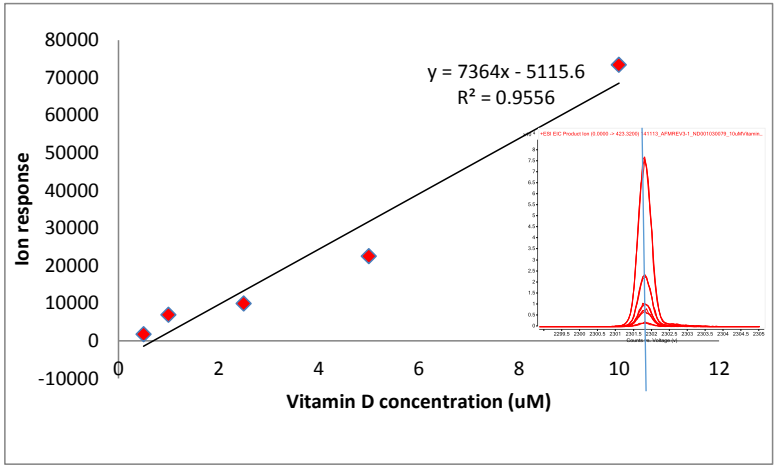
1. Fastest separation of any mobility stage
   - Compatible with UPLC or direct ionization timescales
2. Reaches separation fields twice as high
   - Allows greater scope for separation
   - Scope for investigating previously unexplored ion behaviour
3. Robust and easy to use
   - Chips can be quickly removed for cleaning or replacement
   - No additional gases required for standard operation
   - Chip will transmit all ions simultaneously during non-FAIMS operation
4. Good dynamic range
   - Use of multiple channels ensures that total ion capacity is not reduced
   - Splitting ions between multiple channels reduces space charging effects
5. Low up-front cost and running costs

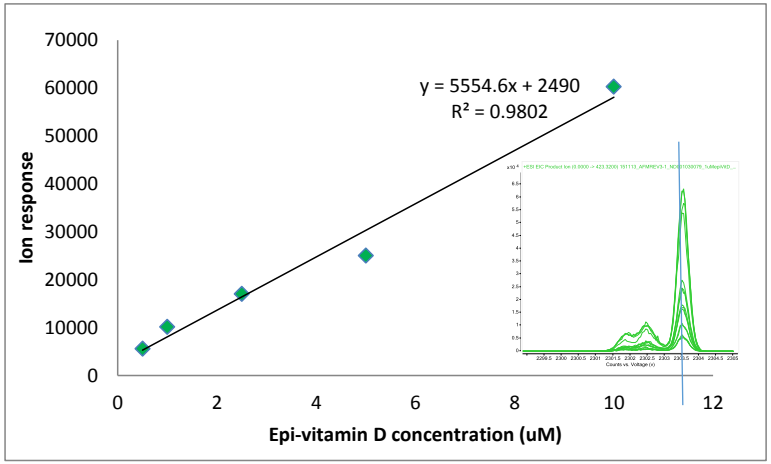
- Understanding FAIMS separation
- The ultraFAIMS difference
- Example applications

Vitamin D3 metabolite isomers

- Measurement of 25-hydroxy vitamin D3 is used clinically to help diagnose Vitamin D deficiency
  - The biologically inactive epimer, 3-epi-25-hydroxy vitamin D3, may be present in clinical samples, and if not accounted for, can causes inaccurate measurements/diagnoses
  - MS alone, or MS/MS cannot distinguish these ions – the gold standard currently is LC-MS/MS, which is accurate but relatively slow



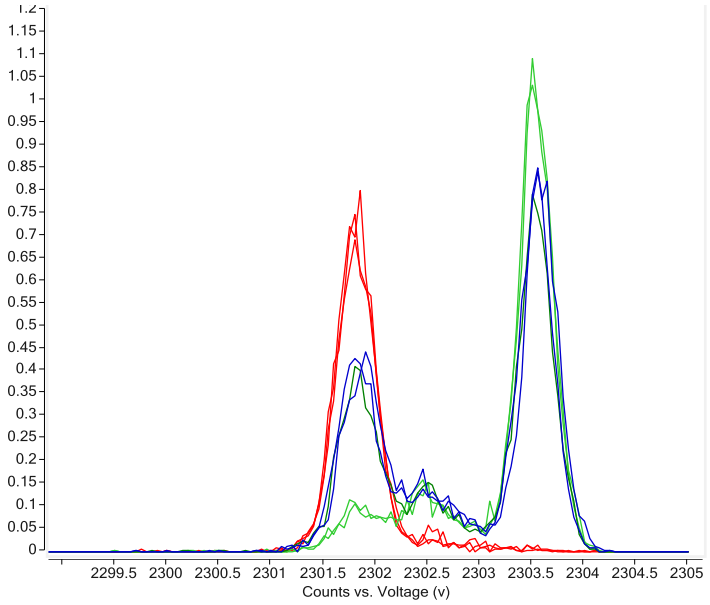


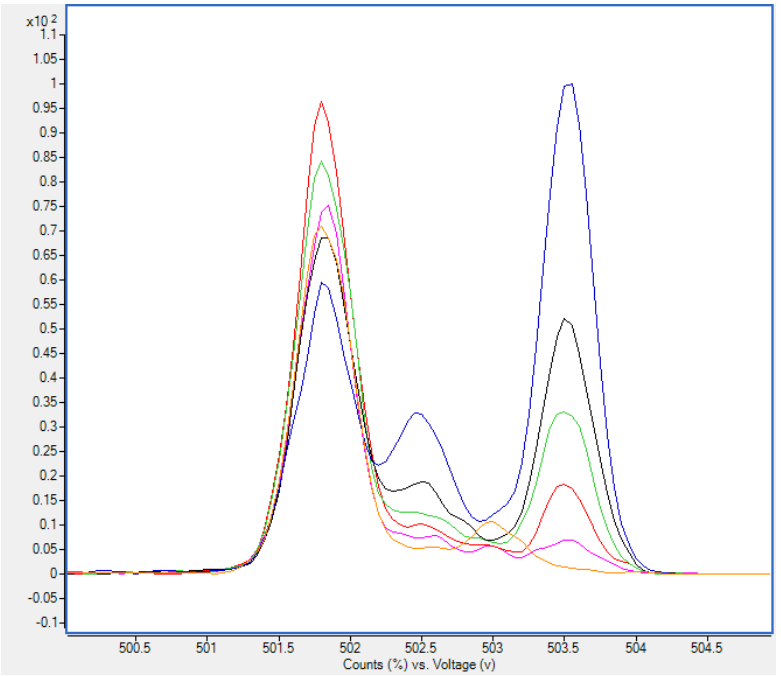


- The 2 isomers are transmitted at different CFs (though epimer secondary peak overlaps with vitamin D main peak – unclear whether this is contamination in sample or other effect)
- Peak height is proportional to concentration

Vitamin D3 metabolite isomers

- Experiments on mixtures confirmed that the epimer could be separately observed down to a ratio of at least 20:1
- Epimer concentration can be determined, and contribution to Vit D concentration subtracted



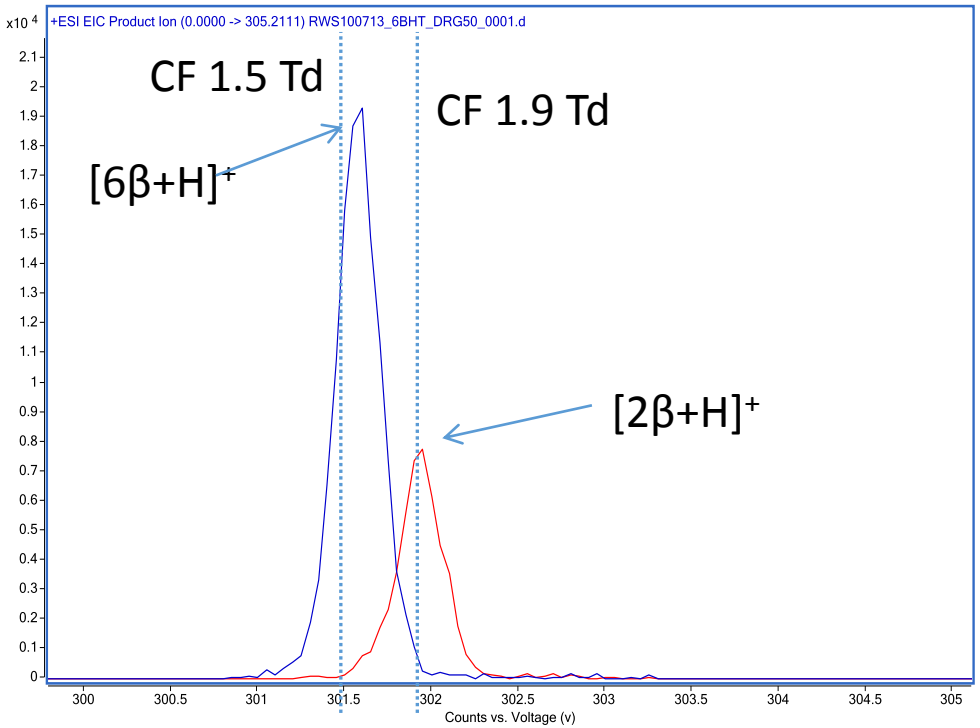


1uM Vit D
1uM Epi-Vit D
1:1 mix of 2.5uM Vit D and 2.5uM epi-Vit D

2.5uM Vit D + No Epi-Vit D
2.5uM Vit D + 0.125uM Epi-Vit D
2.5uM Vit D + 0.25uM Epi-Vit D
2.5uM Vit D + 0.5uM Epi-Vit D
2.5uM Vit D + 1uM Epi-Vit D
2.5uM Vit D + 2.5uM Epi Vit D

6β and 2β hydroxytestosterone

- When evaluating new drug candidates for potential drug-drug interactions, 6β-hydroxytestosterone (6β-HT) is typically monitored to quantify the functional activity of the enzyme CYP3A4
- In some cases, candidate drug compounds can inhibit 6β-HT formation while promoting formation of 2β-hydroxytestosterone (2β-HT)
- LC separation is currently used in these cases – customer would like to be able to continue to use RapidFire-MS, if separation can be achieved with FAIMS



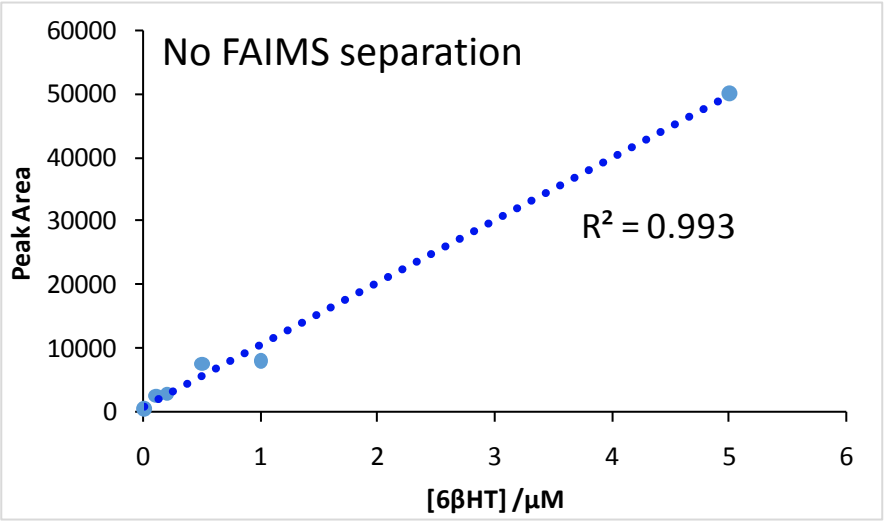
Compensation Field

Carrier Gas: Dry Nitrogen
Dispersion Field = 232 Td

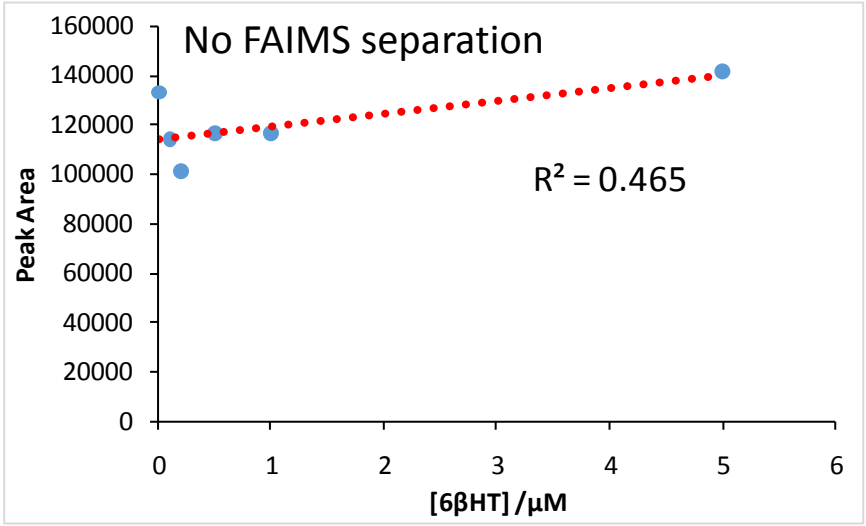
[2β-HT] = 5µM
[6β-HT] = 5µM

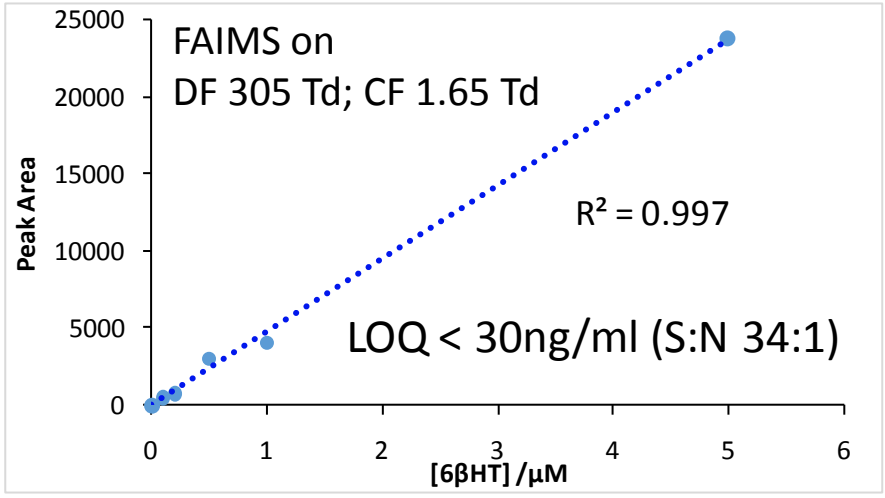
Quantitative Performance - 6β-HT Calibration curve

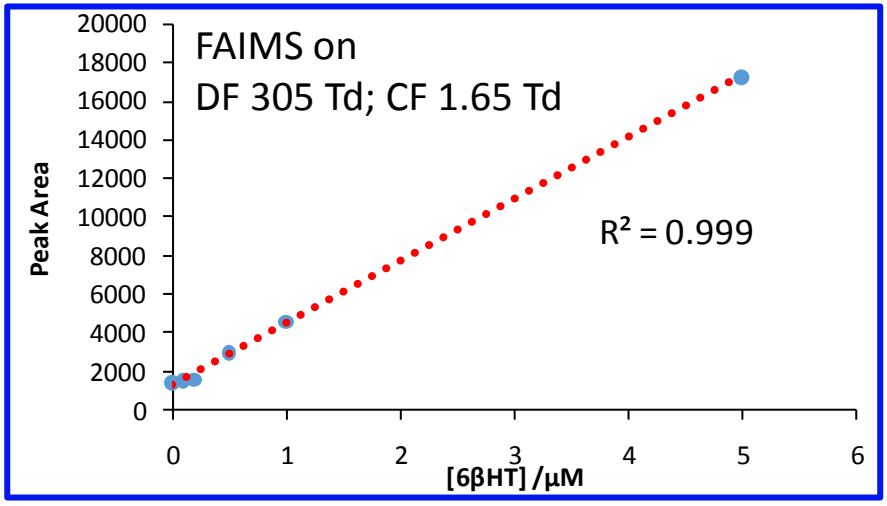
6β-HT only



6β-HT with 2β-HT interference (1µM)



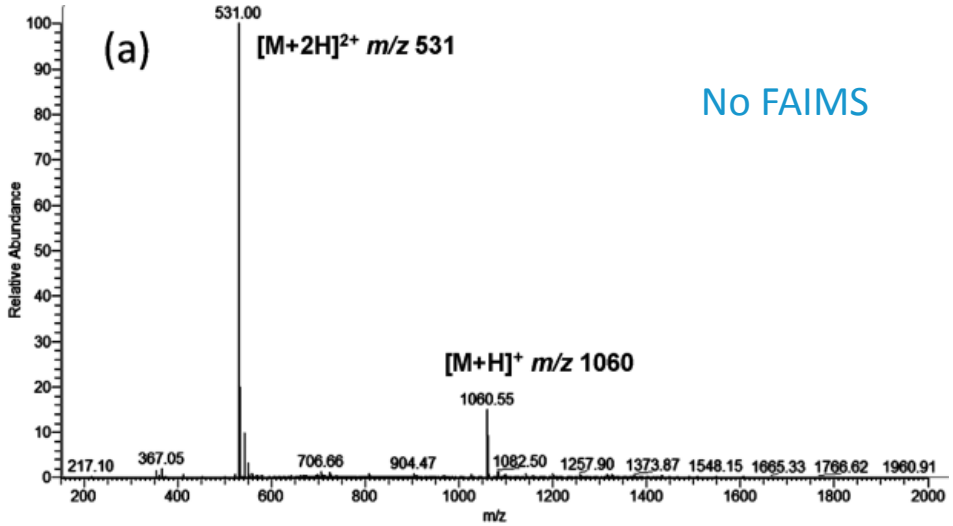




(Agilent 6460 QQQ, MRM mode)

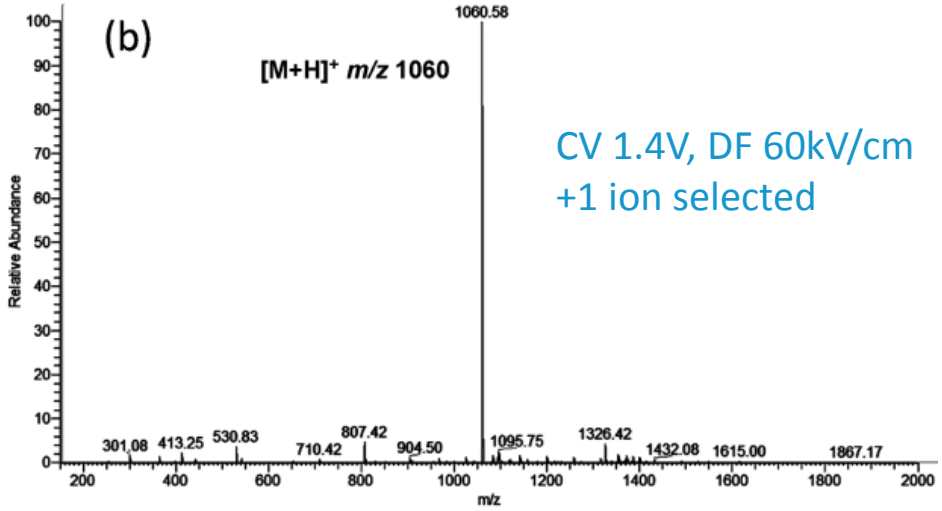
Separation of peptide charge states

- Different charge states tend to separate well in ultraFAIMS, e.g. Bradykinin

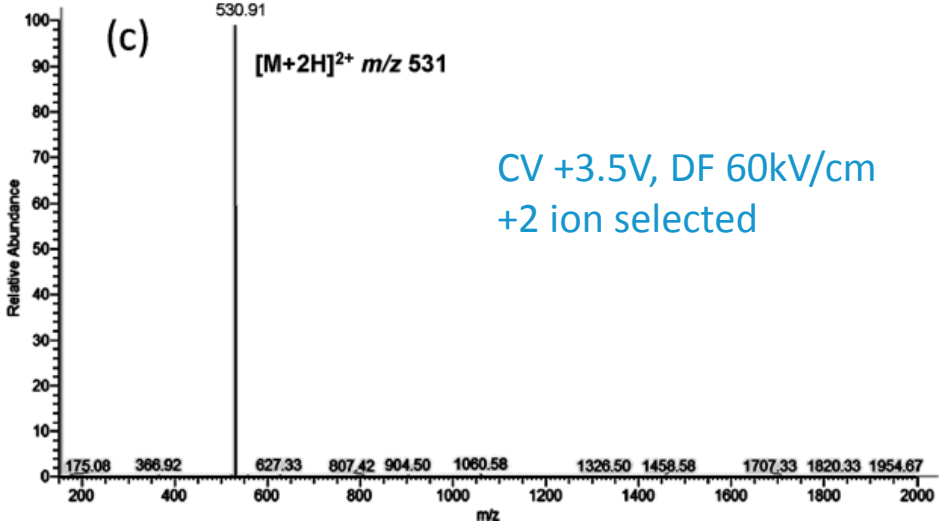


No FAIMS

(Brown LJ et al., Anal. Chem., 2010, 82, 9827-9834)



CV 1.4V, DF 60kV/cm
+1 ion selected

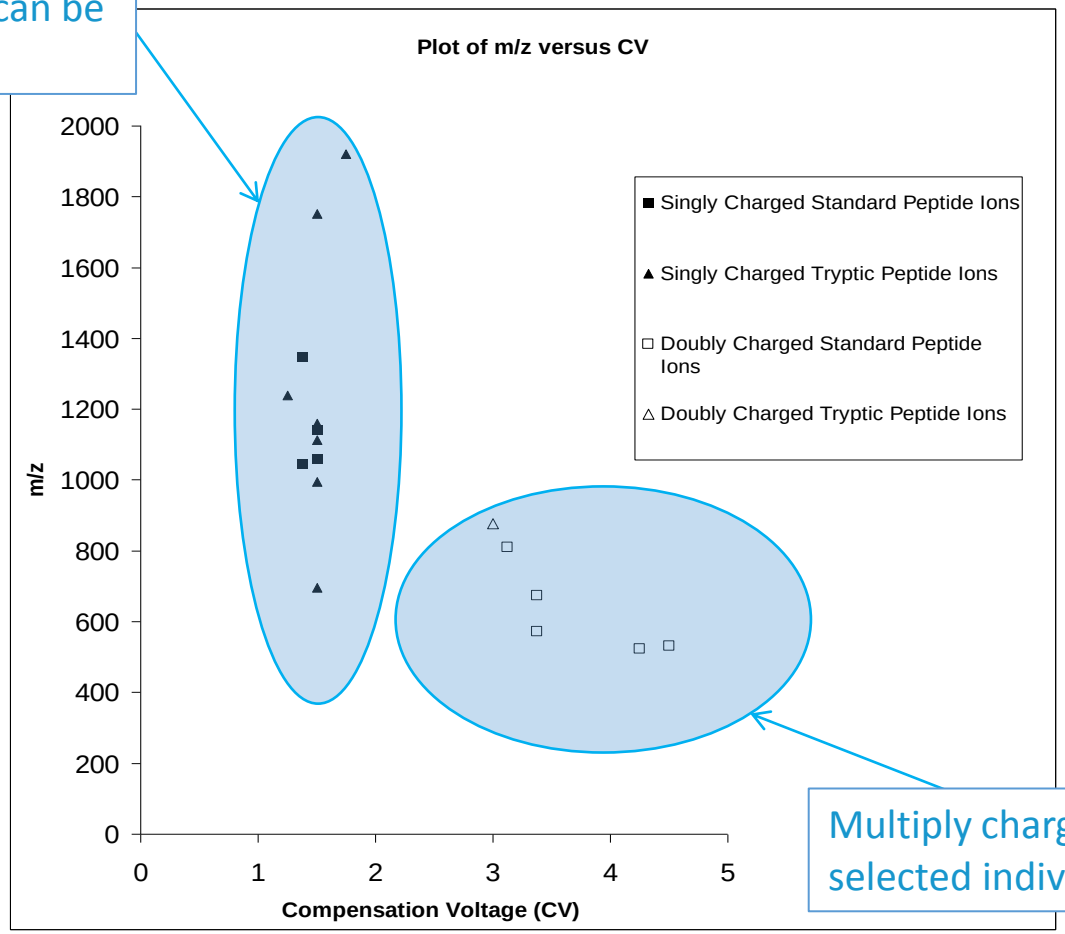


CV +3.5V, DF 60kV/cm
+2 ion selected

Separation of peptide charge states

- Other peptides (standards and from tryptic digest) behave similarly

Singly charged ions can be selected as a group



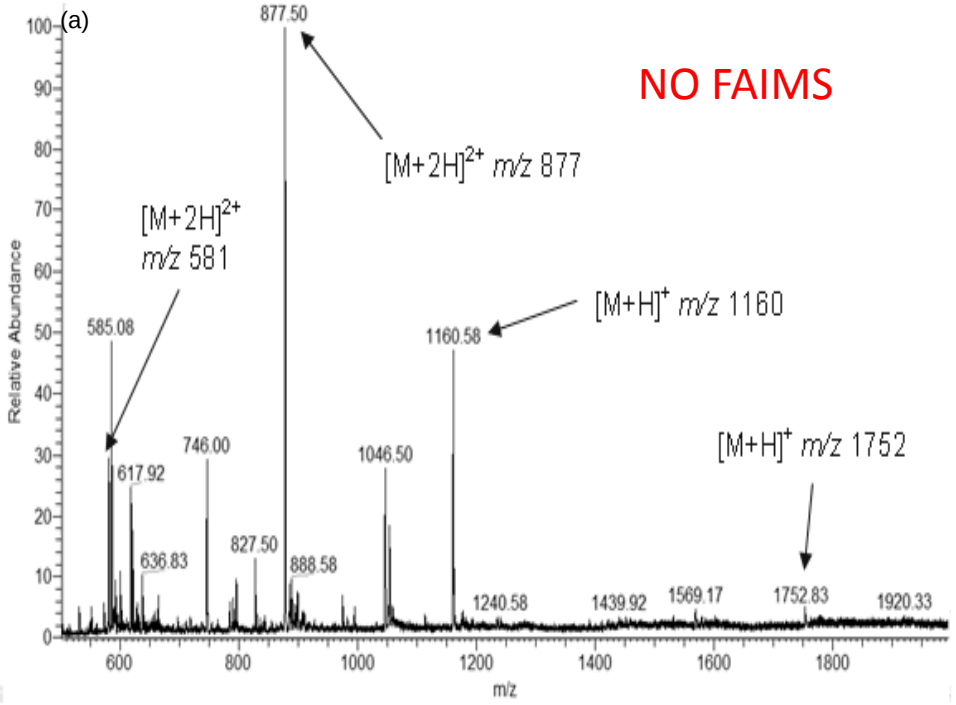
Multiply charged ions can be selected individually

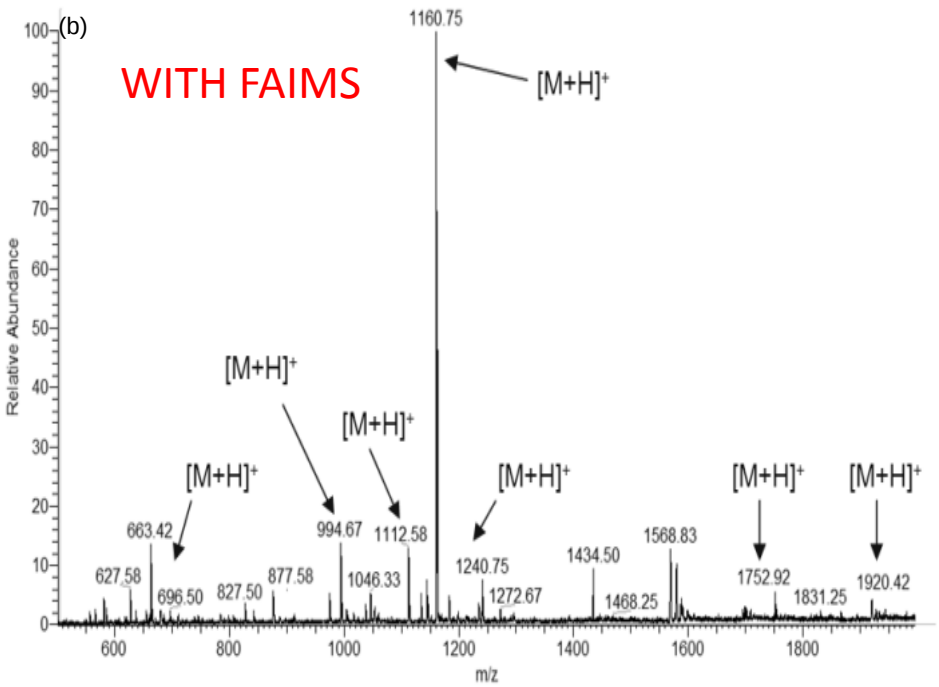
(Brown LJ et al., Anal. Chem., 2010, 82, 9827-9834

Loughborough University

Selection of singly charged ions for protein id

- Ion trap mass spectra alpha-1-acid glycoprotein (AAG) tryptic digest (73pmol µl-1) – without FAIMS both 1+ and 2+ charge states are present
- Doubly charged ions can be filtered out, leaving spectrum of singly charged ions





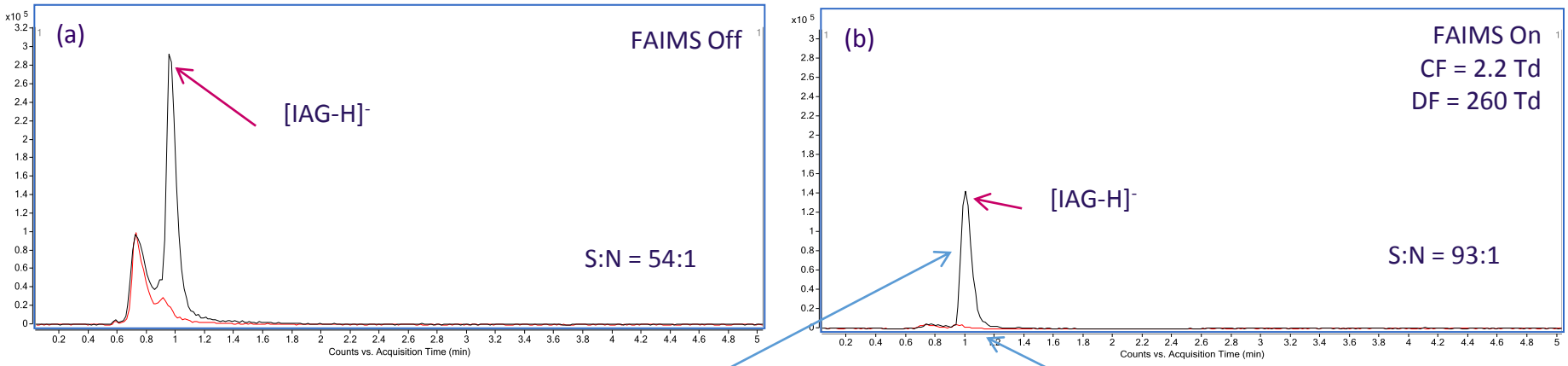
- With FAIMS filtering, submitting top 20 ions from Thermo software spectra list to Mascot results in significant ID, at 95% certainty

(Brown LJ et al., Anal. Chem., 2010, 82, 9827-9834)

Loughborough University

Improved metabolite quantitation

LC-MS analysis of ibuprofen 1-β-O acyl glucuronide (IAG) spiked into urine



Addition of FAIMS separation significantly reduces chemical interference from urine

Absolute intensity of the $[IAG-H]^-$ peak is reduced ~50% because of lower FAIMS transmission, but is compensated by an improvement in S/N, which reduces LOQ from 0.018 to 0.010 µg/ml

	FAIMS off	FAIMS on
LOQ (µg/ml)	0.018	0.010
LDR (µg/ml)	0.018-11	0.010-11
R^2	0.9991	0.9987
Intra-day (% RSD)	5.0	2.7

- Intra-day reproducibility with FAIMS pre-selection of the $[IAG-H]^-$ ion is improved
- LDR is higher with FAIMS separation

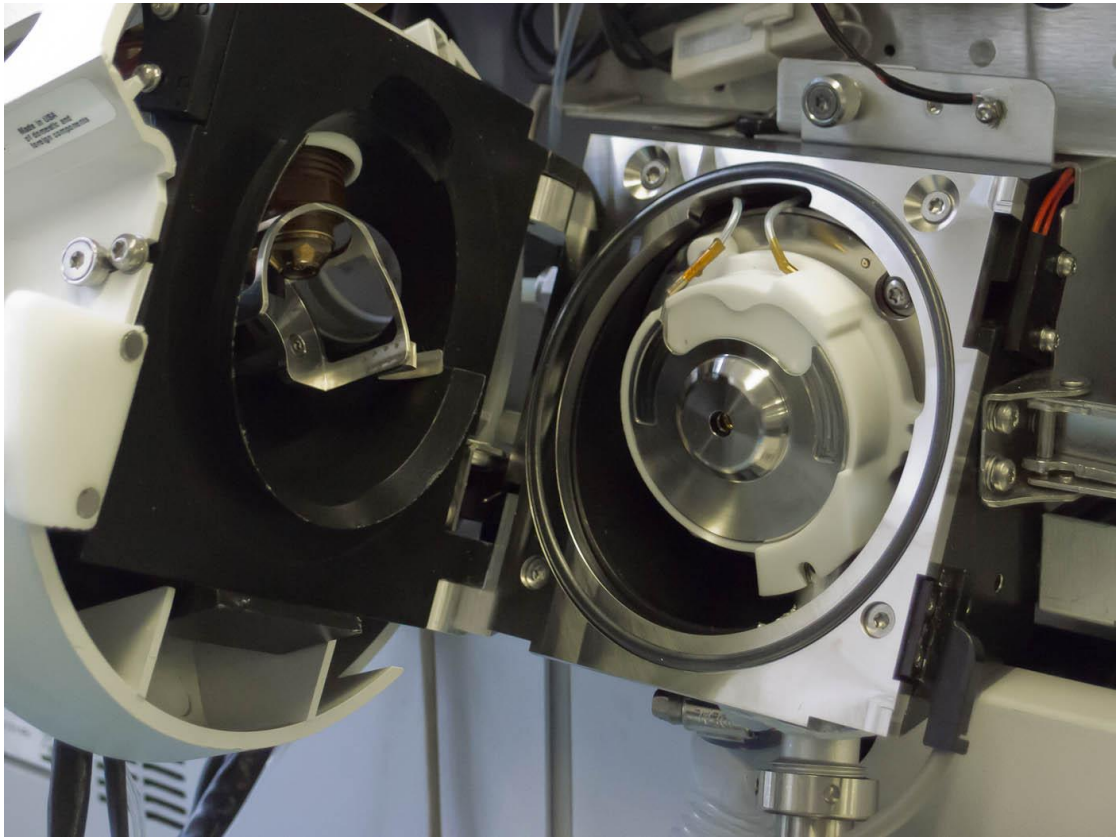
(Smith RW et al., J Chromatog. A, 2013, 1278:76–81)

Loughborough University

- Understanding FAIMS separation
- The ultraFAIMS difference
- Example applications
- Lab session 1 – installation & set-up

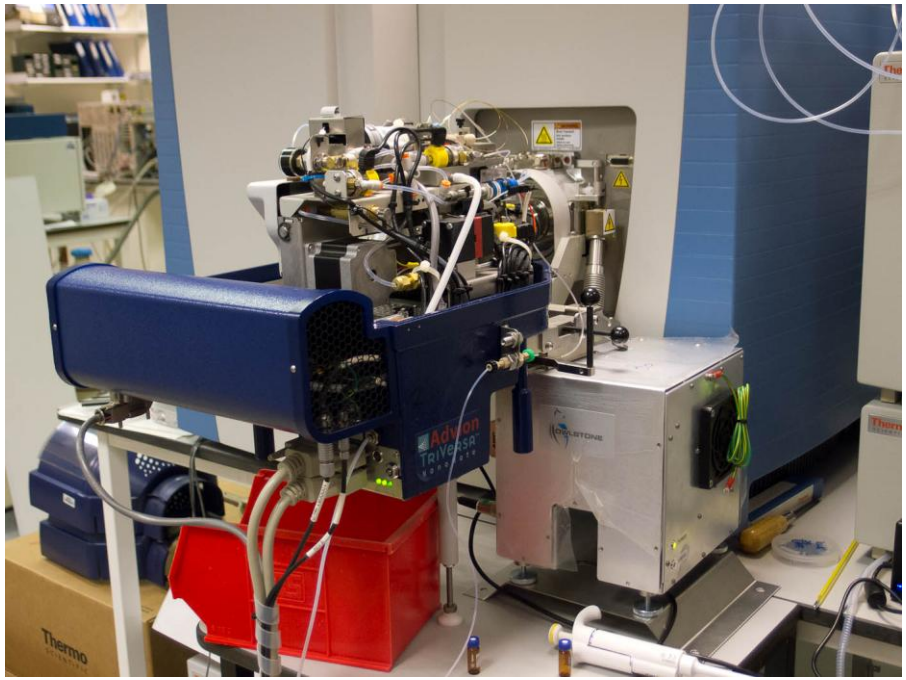
Installing ultraFAIMS – A1

- On initial installation, Agilent spray chamber must be upgraded to ultraFAIMS-compatible version – requires venting
- A1 interface can then be installed and removed in ~10 mins (by user)
- When ultraFAIMS is removed, MS can be converted back to non-FAIMS configuration without venting
- Agilent soon to release a version of Mass Hunter that can control ultraFAIMS (to be initially launched for 6460 QQQ). Stand-alone software also provided



Installing UltraFAIMS - T1

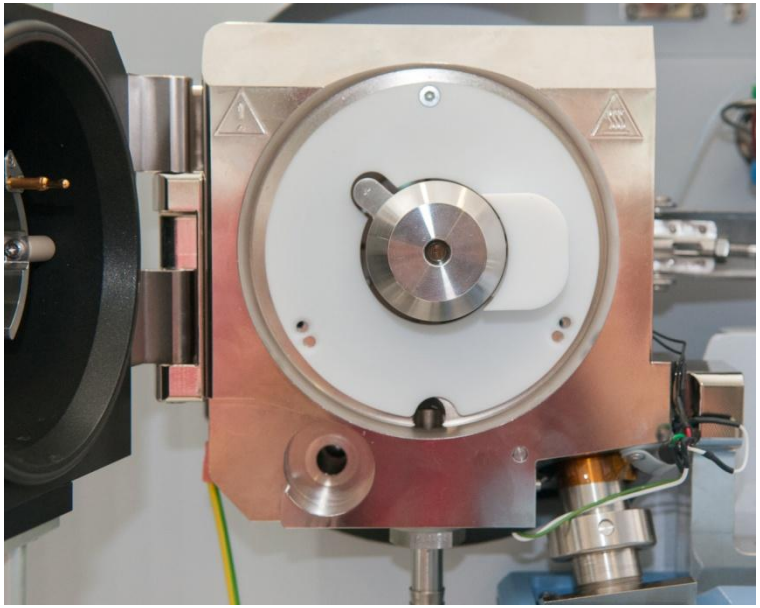
- "T1" interface is compatible with Thermo Scientific LTQ-Orbitrap and Exactive Series Instruments
- No modifications to the MS are necesssary
- Standalone software provided, and Thermo will be releasing integrated software for Exactive series later this year
- Installation guide: http://support.owlstonenanotech.com/entries/56471555-UltraFAIMS-T1-User-Guide

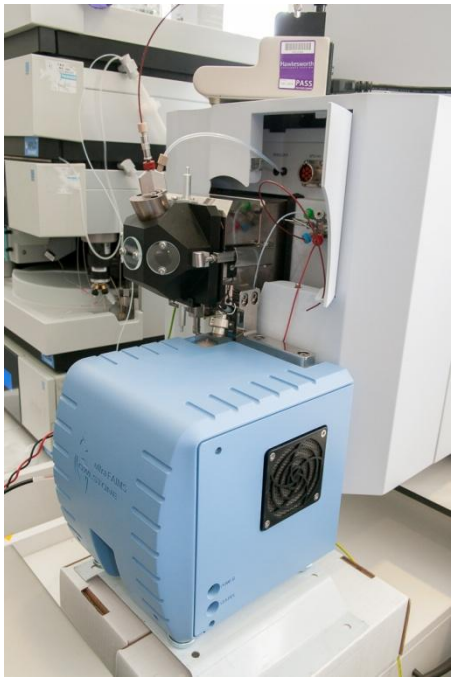


UltraFAIMS-T1 mounted on Thermo Exactive with Advion Nanomate source

Installing UltraFAIMS - B1

- “B1” interface is compatible with a number of Bruker Mass Spectrometers
- No modifications to the MS are necesssary
- Standalone software is provided for controlling the UltraFAIMS device
- Installation guide: http://support.owlstonenanotech.com/entries/82736899-UltraFAIMS-B1-User-Manual





UltraFAIMS-B1 mounted on Bruker Impact HD with Apollo II electrospray source