High Performance MALDI-TOF Mass Spectrometry

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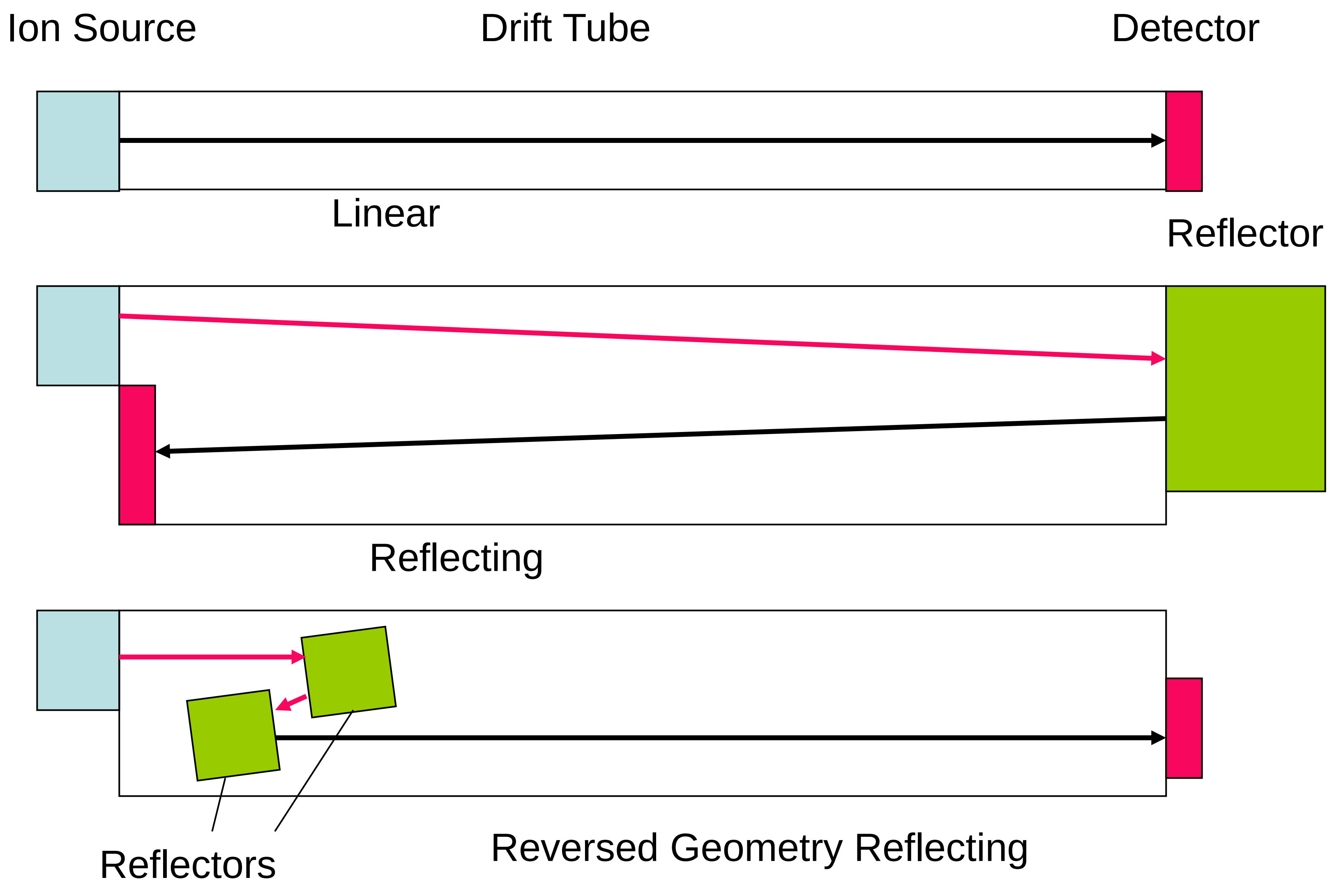
Outline

- **Goals:**

Design and build MALD-TOF Analyzers Optimized for Selected Applications

- **Approach:**
  - Modular construction using common elements
  - State-of-the-art technology, e.g. 5 khz laser
  - Focus on the needs of the application
  - Keep it small, simple, and reliable without sacrificing performance
- **Analyzer geometries**
  - Linear for broad mass range and sensitivity at high mass (to 500 kDa).
  - Reflector for high resolution, mass accuracy, and sensitivity at low mass (0.1-5 kDa)
  - Reversed geometry reflector for both resolution and sensitivity in intermediate mass range (1-50 kDa)
- **Optimization Strategy**
- **Results**

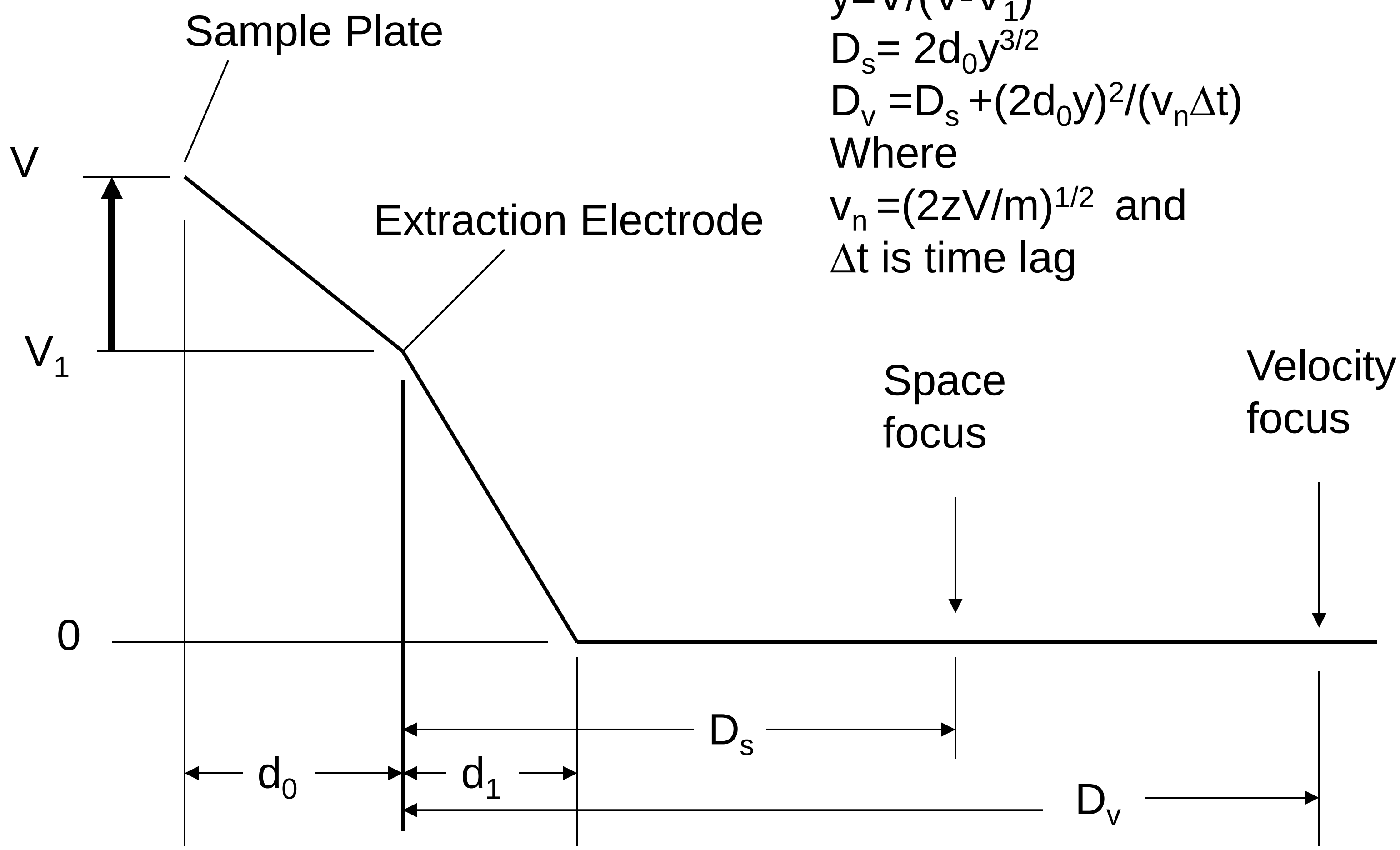
Time-of-flight analyzer geometries



Reflector MS for MALDI mass fingerprinting peptides, metabolites, and other small molecules

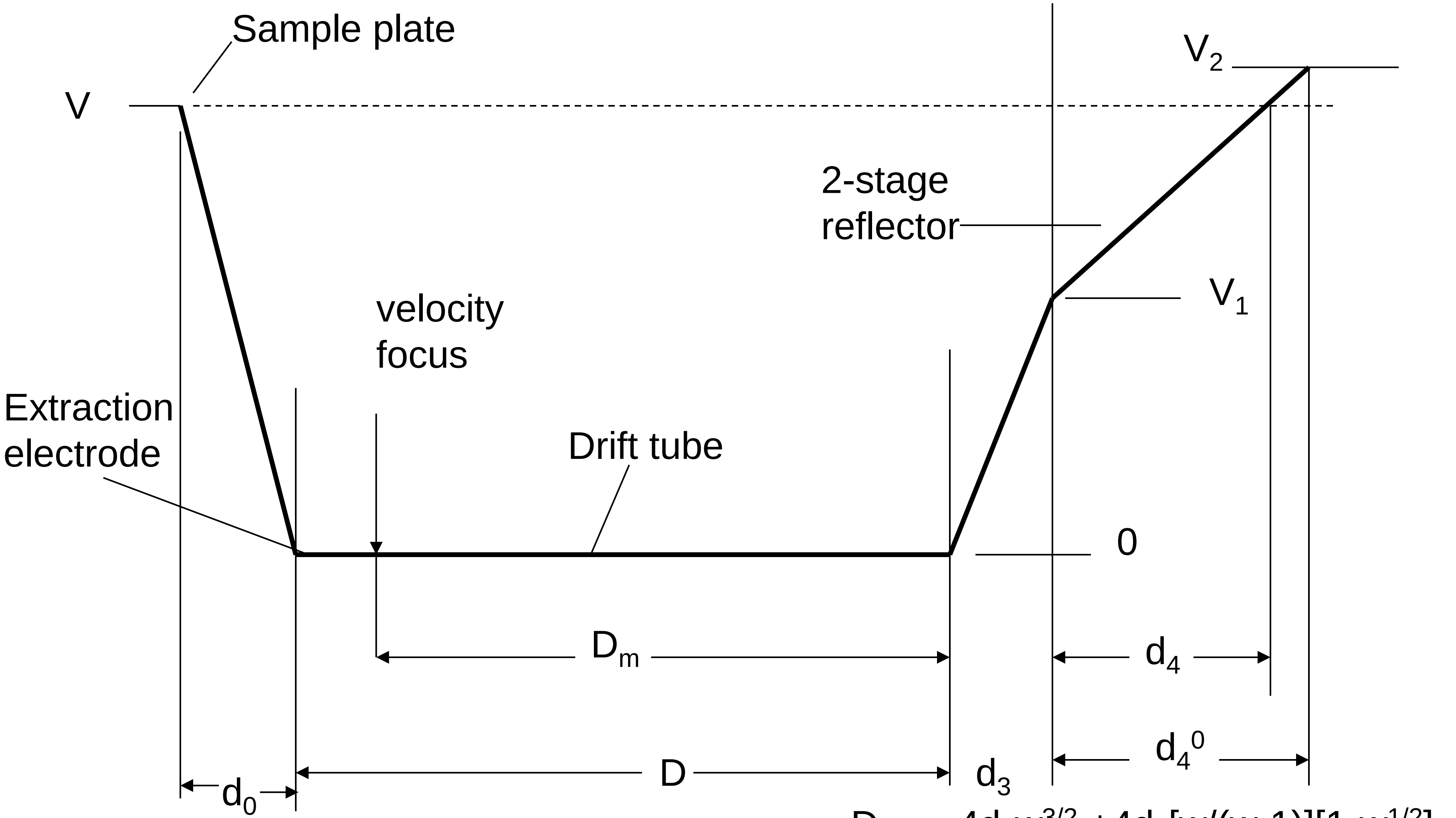
- High sensitivity and dynamic range in mass range 0.1-5 kDa
- Highest possible resolving power and mass accuracy (1 ppm RMS)
- High throughput
- Low cost, stable, reliable, and small
- No operator expertise required

$y = V/(V - V_1)$
$D_s = 2d_0 y^{3/2}$
$D_v = D_s + (2d_0 y)^2/(v_n \Delta t)$
Where
$v_n = (2zV/m)^{1/2}$ and
Δt is time lag



For single field source, $V_1=0$, $y=1$
Simultaneous 1st and 2nd order focus
$D_v=6d_0$, $v_n\Delta t=d_0$

Wiley and McLaren
RSI (1955) 26, 1150-1157
Vestal and Juhasz
JASMS (1998) 9, 892-911



$D_{m1} = 4d_4w^{3/2} + 4d_3[w/(w-1)][1-w^{1/2}]$
$3D_{m2} = 4d_4w^{5/2} + 4d_3[w/(w-1)][1-w^{3/2}]$

Simultaneous 1st and 2nd order focus

$4d_3/D_m=(w-3)/w$

$4d_4/Dm = w^{-3/2} + (4d_3/D_m)/(w + w^{1/2})$

$d_4 = d_4^0(V-V_1)/(V_2-V_1)$

$w=V/(V-V_1)$

Contributions to relative peak width, Δm/m, with 1st and 2nd order velocity focusing

Initial position, δx: $R_{s1} = 2\mathbf{[(D_v-D_s)/2d_0y](}\delta x/D_e)$

Initial velocity, δv_0: $R_{v1} = (4d_0y/D_e)(\delta v_0/v_n)[(1-(m/m^*)^{1/2}]$

$R_{v2} = 2\mathbf{[2d_0y/(D_v-D_s)]}^2 (\delta v_0/v_n)^2 = 0$

$R_{v3} = 2\mathbf{[2d_0y/(D_v-D_s)]}^3 (\delta v_0/v_n)^3$

$R_v = R_{v1} + R_{v3}$

Time error, δt: $R_t = 2\delta t/t = 2\delta t v_n/D_e$

Trajectory error, δL: $R_L = 2\delta L/D_e$

Voltage error, δV: $R_V = \delta V/V$

Resolving power:

$$R^{-1} = [R_{s1}^2 + R_v^2 + R_t^2 + R_L^2 + R_V^2]^{-1/2}$$

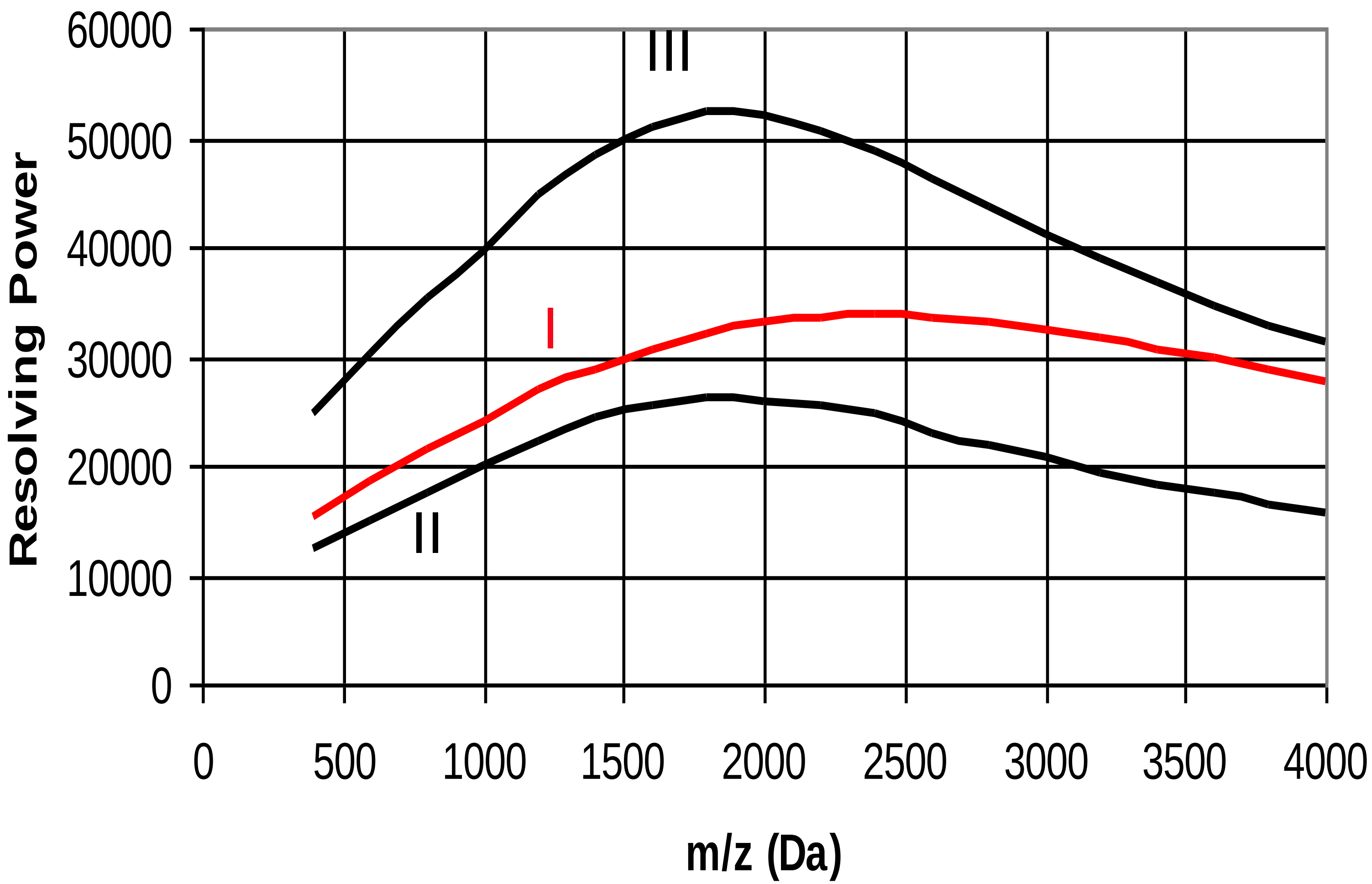
Instrument Parameters for Reflecting Analyzer (single-field source and two-field mirror)

D_e=3200, d_0=3, D=2264, d_3=114.2, d_4^0=127.7 all in mm
V=8.56, V_1=6.31, V_2=9.23 all in kV
δt=1.5 nsec (0.5 nsec bins, 1 nsec single ion pulse width)
Focus mass m^*=1.6 kDa
$D_v = 6d_0$ = 18 mm, v_n (for m^*)=0.03215 mm/nsec
Time lag $\Delta t = d_0/v_n$ = 93 nsec
$K = 2d_0/(D_v - D_s) = 0.5$

Initial Conditions for MALDI (typical)

δv_0=400 m/sec, δx=0.01 mm

Trajectory error and voltage error assumed to be small



Calculated resolving power for reflecting analyzer.
δv_0=400m/s, δx=0.01 mm for I and III and 800 m/s and 0.02 mm for II.
δt=1.5 nsec for I and II and 0.75 nsec for III,

Conditions for minimizing relative peak width, $\Delta m/m$

Initial position, δx: $R_{s1} = 2\mathbf{K}^{-1}(\delta x/D_e)$

Initial velocity, δv_0: $R_{v1} = (4d_0 y/D_e)(\delta v_0/\mathbf{v_n})[(1-(m/m^*)^{1/2}]$

$R_{v3} = 2\mathbf{K}^3 (\delta v_0/\mathbf{v_n})^3$

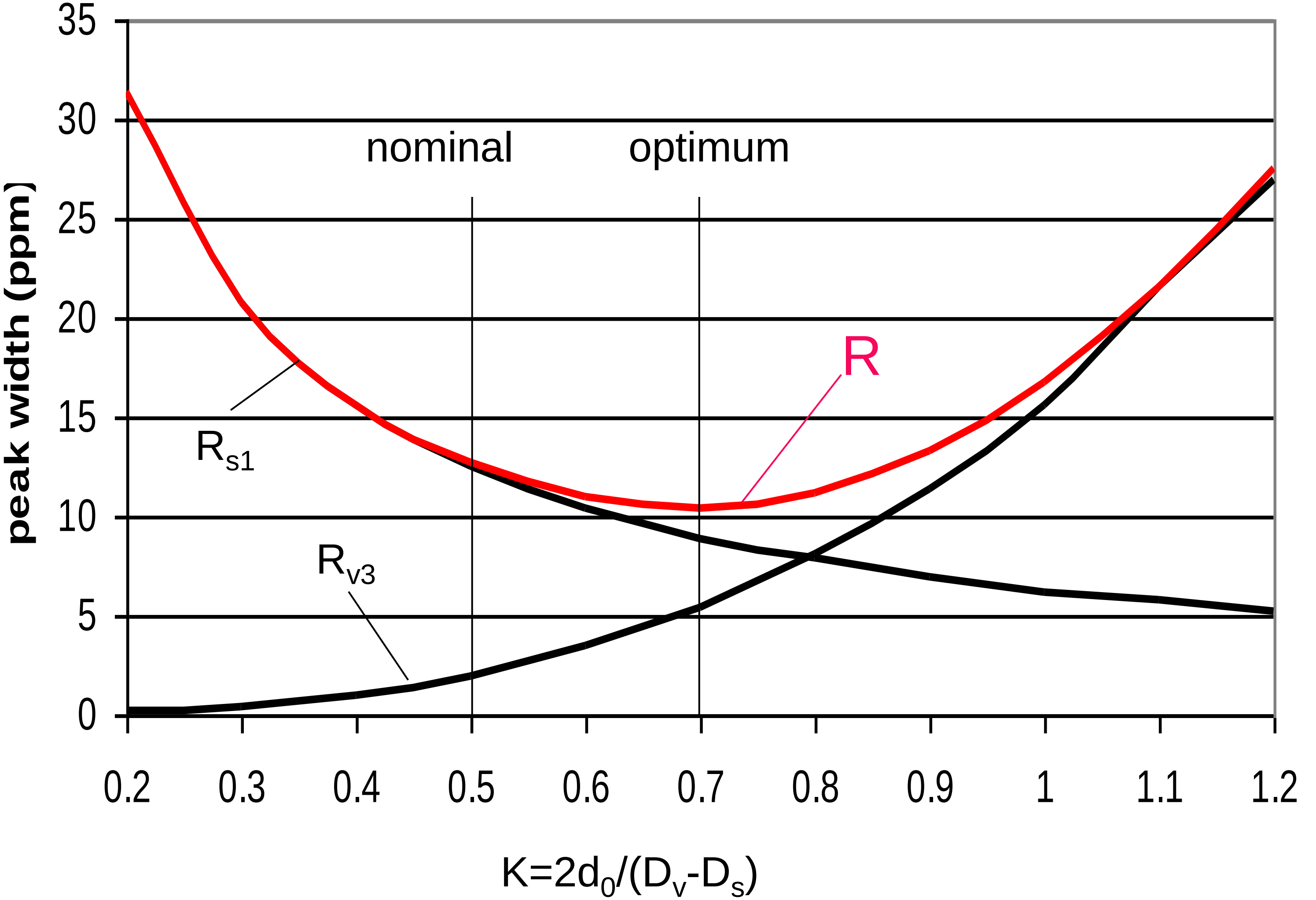
$R_v = R_{v1} + R_{v3}$

Time error, δt: $R_t = 2\delta t/t = 2\delta t\mathbf{v_n}/D_e$

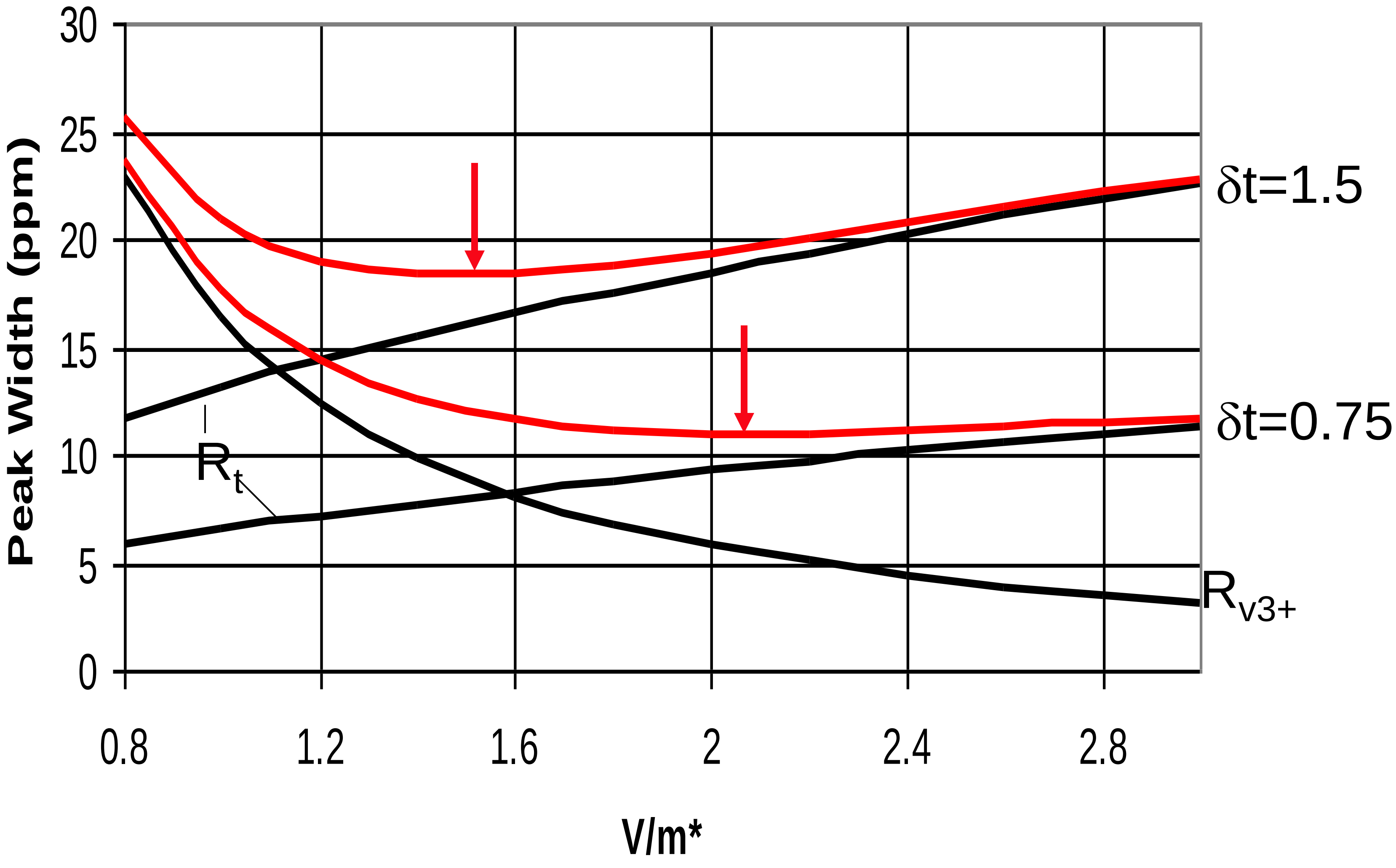
Peak Width: $R = [R_{s1}^2 + R_v^2 + R_t^2 + R_L^2 + R_V^2]^{1/2}$

Optimum focus: $dR/dK = 0$, $K = 2d_0/D_v - D_s$

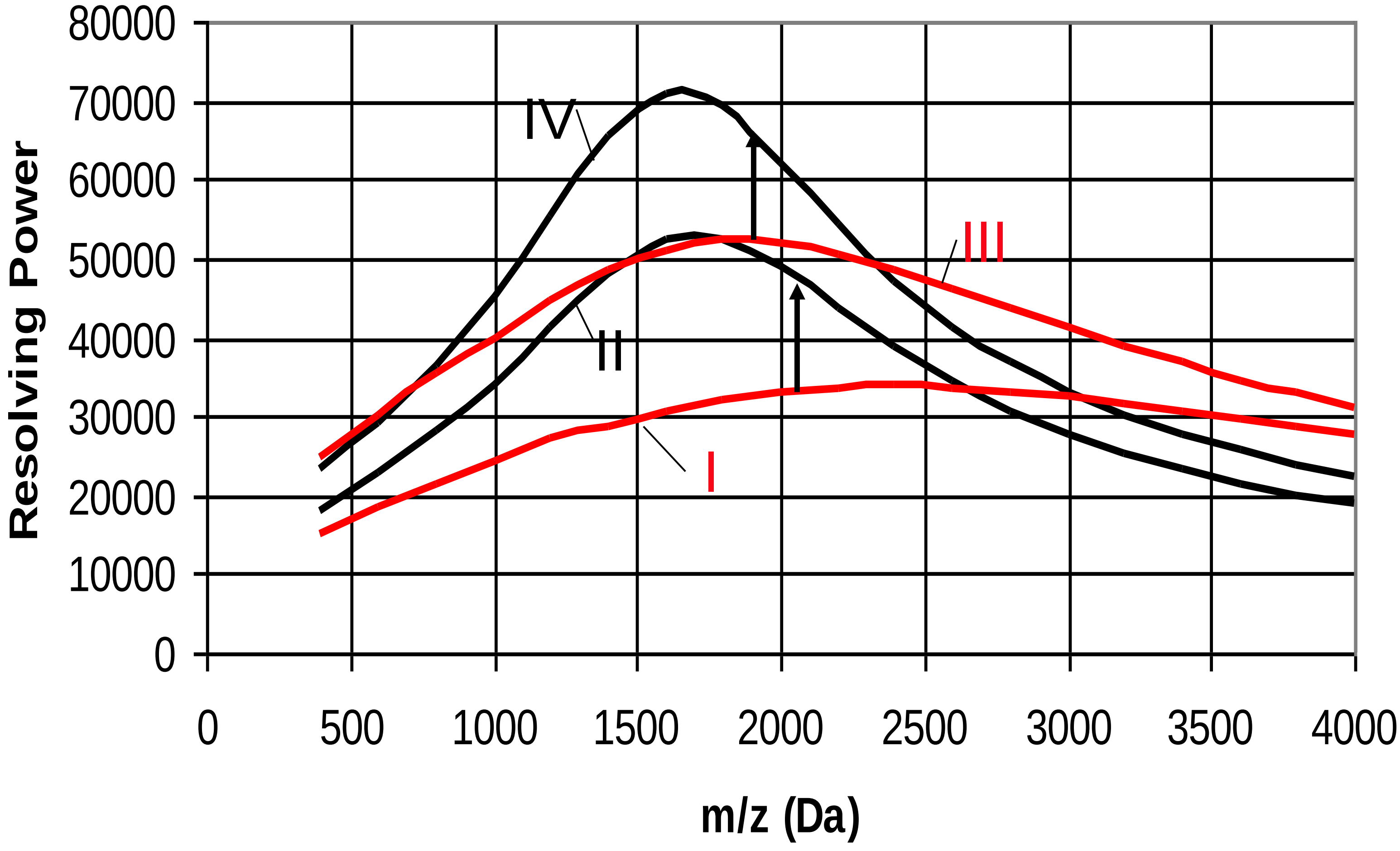
Optimum velocity: $dR/dv_n = 0$, $v_n = C(V/m^*)^{1/2}$
(can be optimized at m* or any other mass)



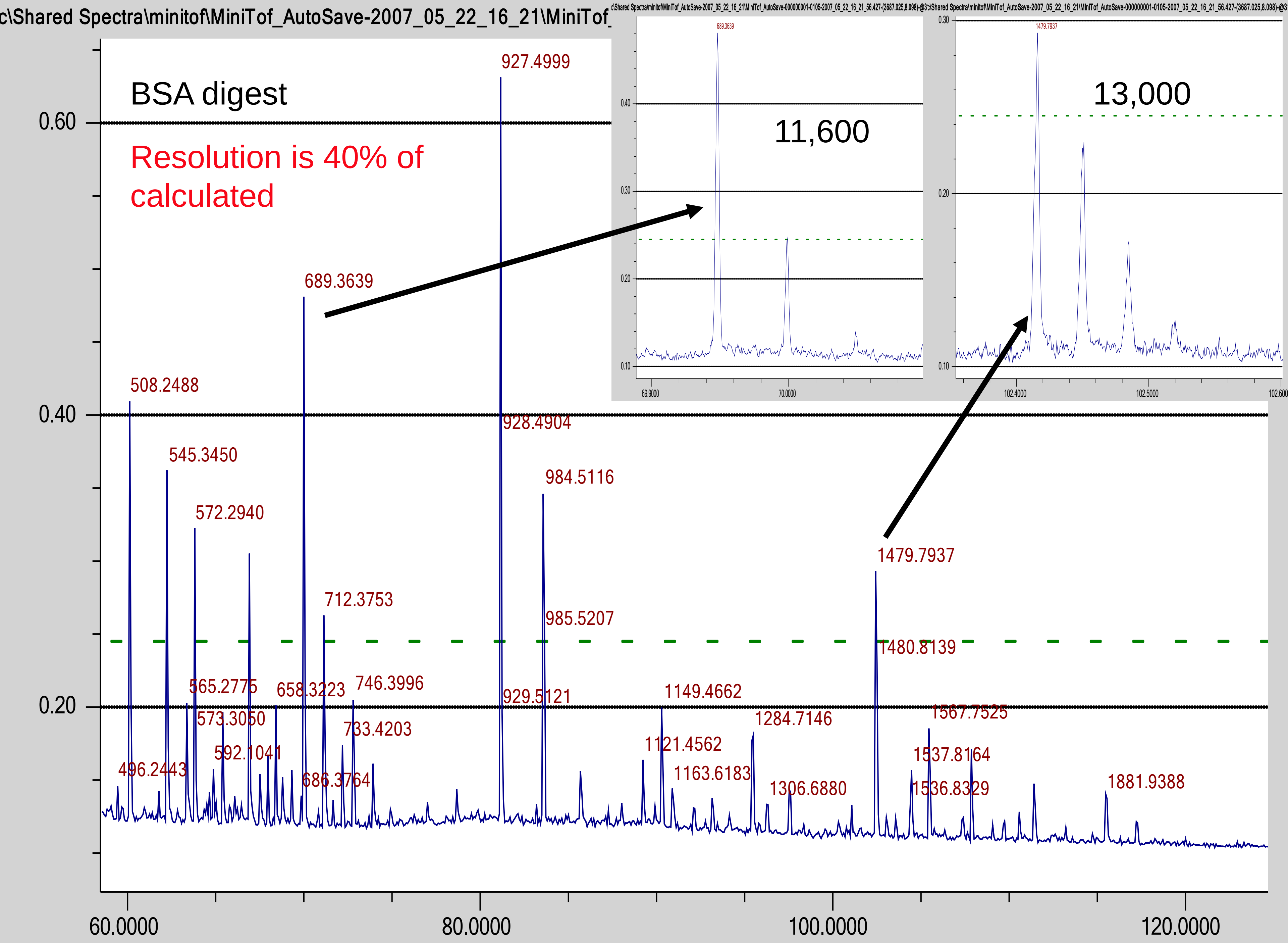
Minimum peak width as function of focusing parameter K.
Optimum value corresponds to dR/dK=0

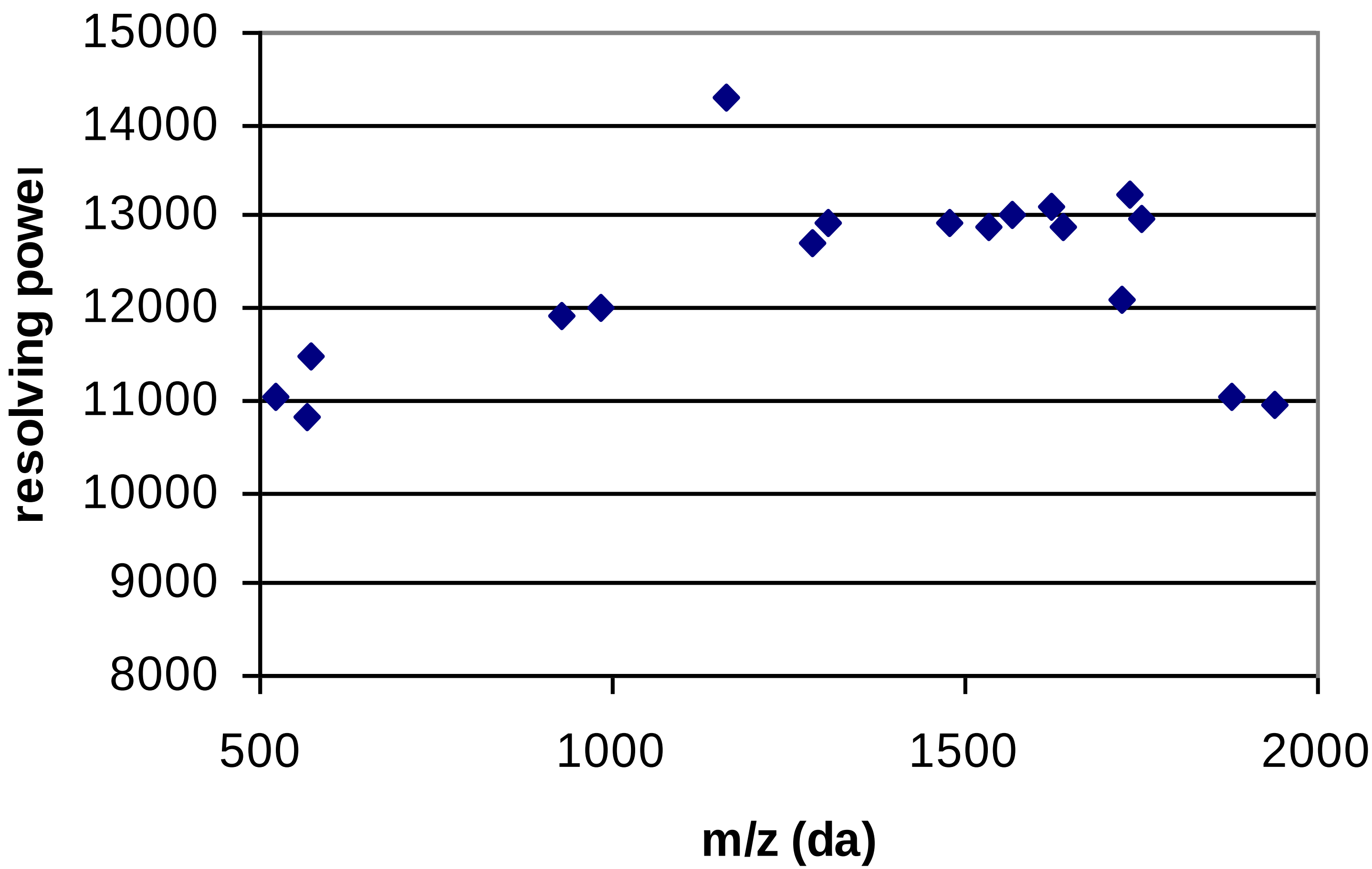


Minimum peak width as function of V/m*(indicated by arrows for δt= 1.5 and 0.75 nsec). For m*= 1600 Da optimum voltages are 2.4 and 3.4 kV, respectively.



Optimized resolving power for reflecting analyzer, δv_0=400m/s, δx=0.01 mm. δt=1.5 nsec for I and II and 0.75 nsec for III and IV. Red lines nominal, black at optimum for focus mass.


BSA digest
Resolution is 40% of calculated
11,600
13,000
927.4999
928.4904
689.3639
508.2488
545.3450
572.2940
984.5116
712.3753
985.5207
1479.7937
1480.8139
565.2775
658.3223
746.3996
929.5121
1149.4662
573.3050
733.4203
1284.7146
1567.7525
1121.4562
592.1041
496.2443
686.3764
1163.6183
1537.8164
1306.6880
1536.8329
1881.9388
0.60
0.40
0.20
60.0000
80.0000
100.0000
120.0000



Experimental resolving power for reflecting analyzer on BSA digest

Contributions to relative peak width, $\Delta m/m$

Initial position, δx: $R_{s1} = [(D_v - D_s)/D_e](\delta x/d_0 y)$

Initial velocity, δv_0: $R_{v1} = (4d_0 y/D_e)(\delta v_0/v_n)[(1-(m/m^*)^{1/2}]$

$R_{v2} = 2[2d_0 y/(D_v - D_s)]^2 (\delta v_0/v_n)^2$

$R_{v3} = 2[2d_0 y/(D_v - D_s)]^3 (\delta v_0/v_n)^3$

Time error, δt: $R_t = 2\delta t/t = 2\delta t v_n/D_e$

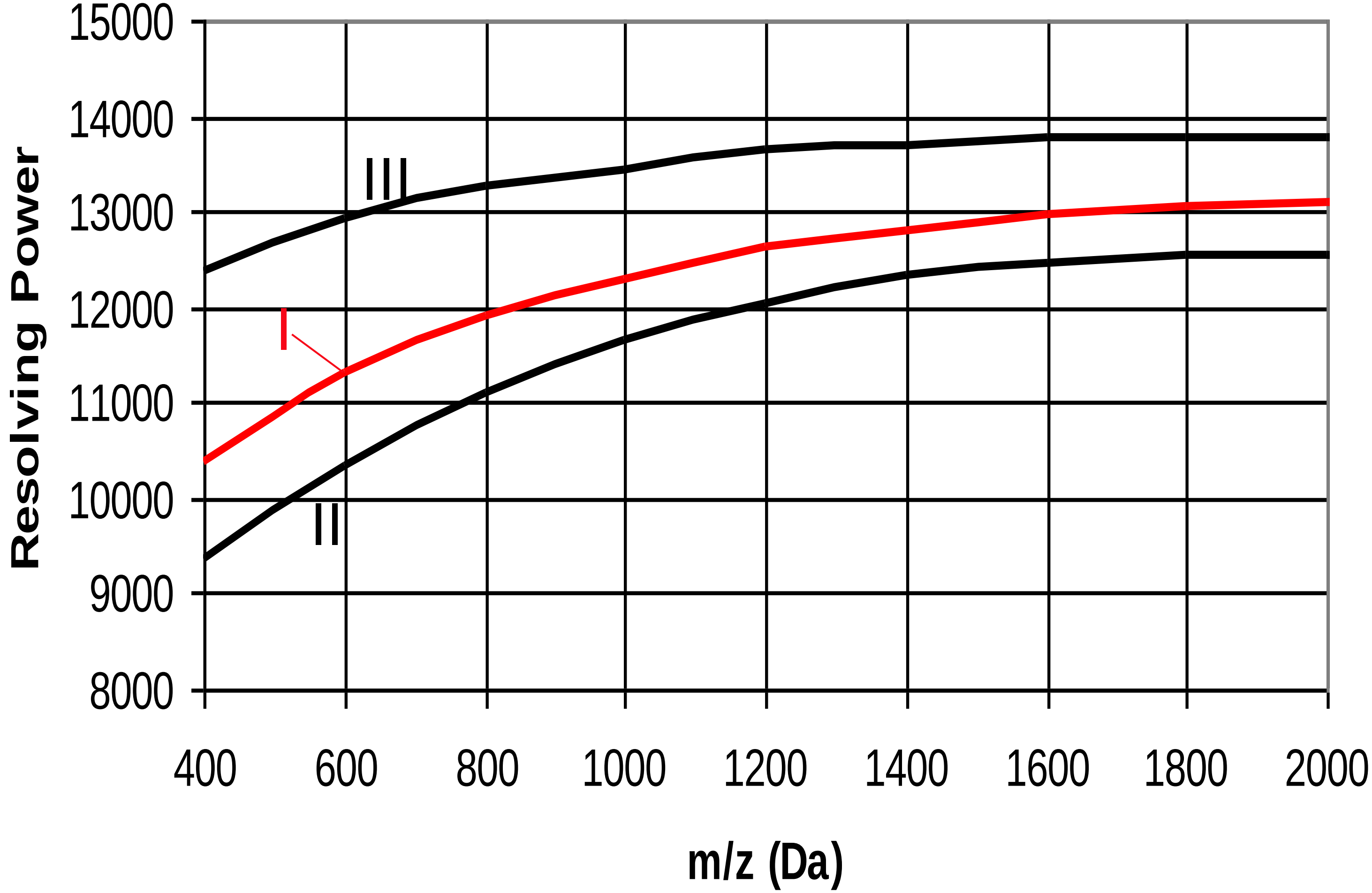
Trajectory error, δL: $R_L = 2\delta L/D_e$

Not small!!!

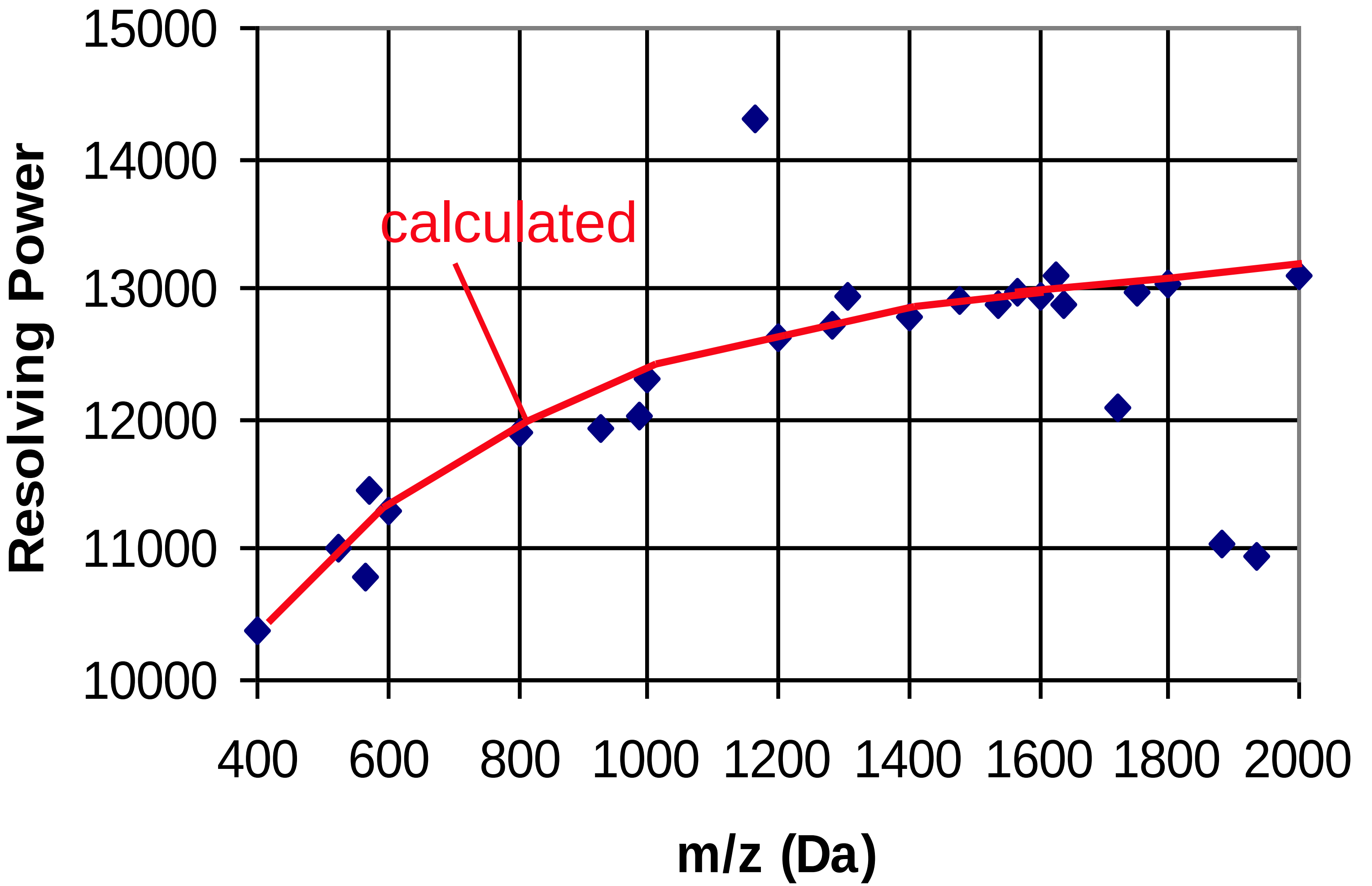
Voltage error, δV: $R_V = \delta V/V$

Resolving power:

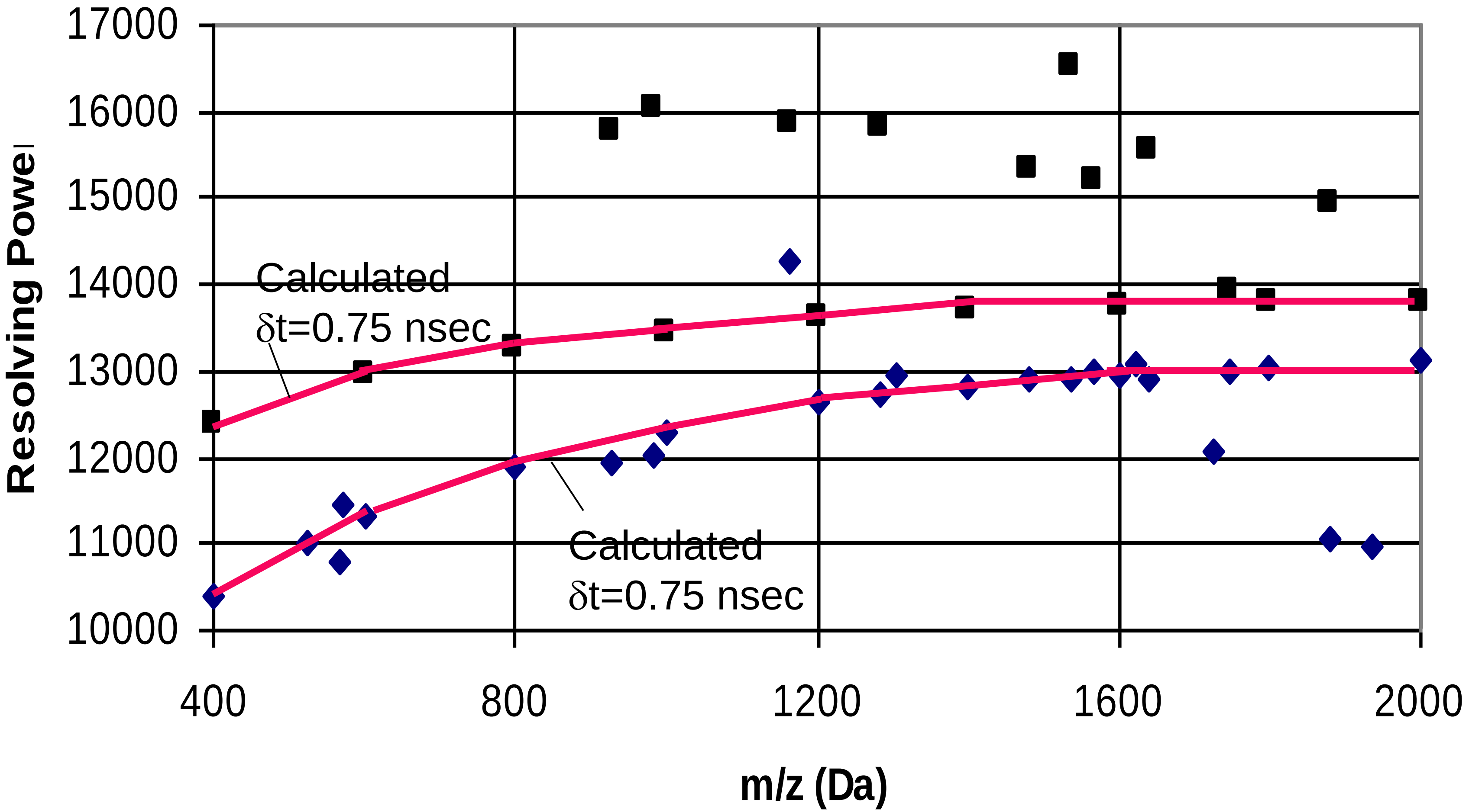
$$R^{-1} = [R_{s1}^2 + R_v^2 + R_t^2 + R_L^2 + R_V^2]^{-1/2}$$



Calculated resolving power for reflecting analyzer with $\delta V/V$ =70 ppm



Comparison of calculated resolving power with experimental results
With $\delta V/V$=70 ppm



Comparison of calculated resolving power with experimental results
With δV/V=70 ppm. Measured ♦ 5 μchannel plate ■ ETP MagnetTOF™

Contributions to relative peak width, Δm/m, with 1st and 2nd order velocity focusing (size matters)

Initial position, δx: $R_{s1} = 2\mathbf{[(D_v\text{-}D_s)/2d_0y](}\delta x/D_e)$

Initial velocity, δv_0: $R_{v1} = (4d_0y/D_e)(\delta v_0/v_n)[(1-(m/m^*)^{1/2}]$

$R_{v2} = 2\mathbf{[2d_0y/(D_v\text{-}D_s)]}^2\ (\delta v_0/v_n)^2 = 0$

$R_{v3} = 2\mathbf{[2d_0y/(D_v\text{-}D_s)]}^3\ (\delta v_0/v_n)^3$

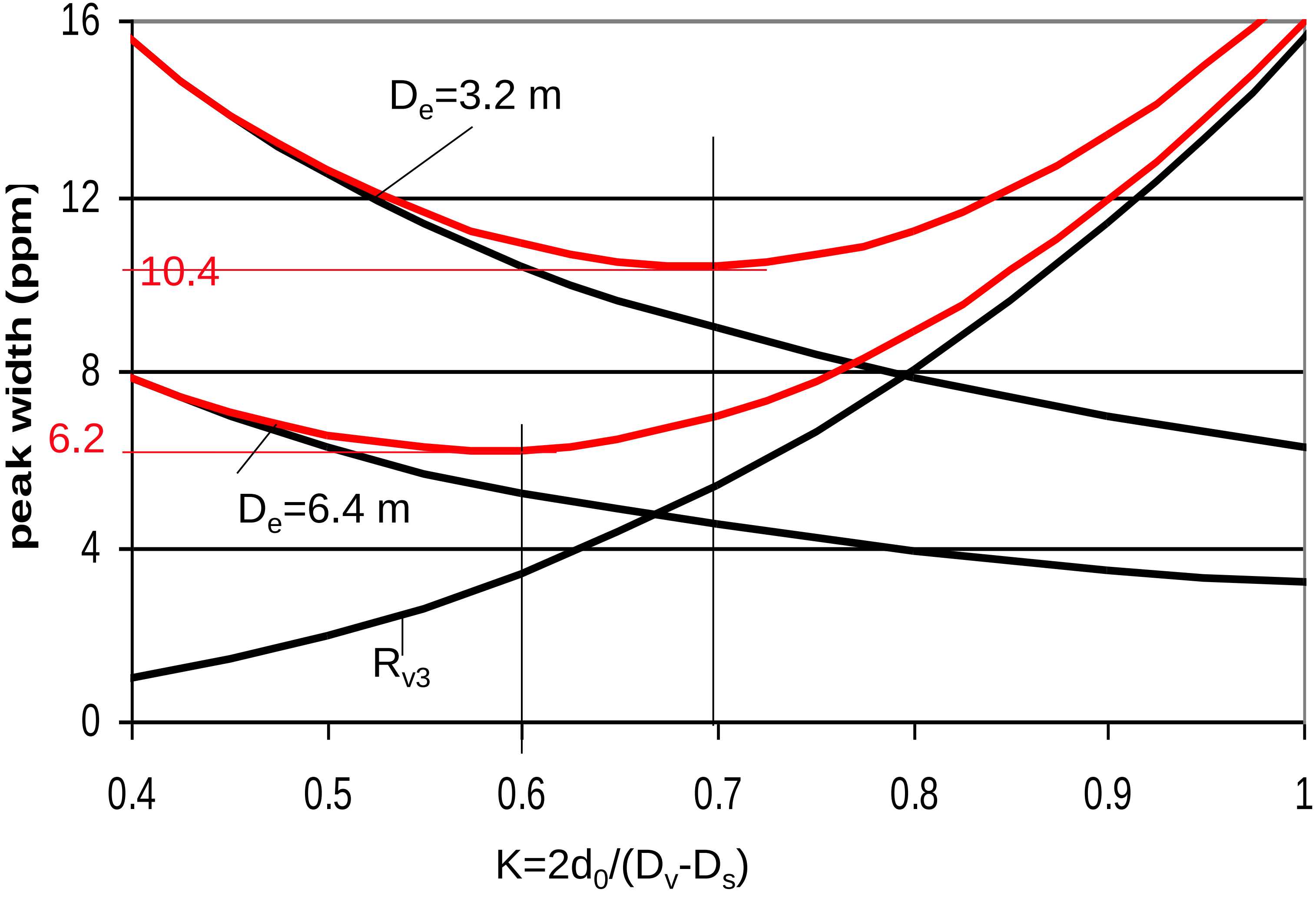
Time error, δt: $R_t = 2\delta t/t = 2\delta t v_n/D_e$

Trajectory error, δL: $R_L = 2\delta L/D_e$

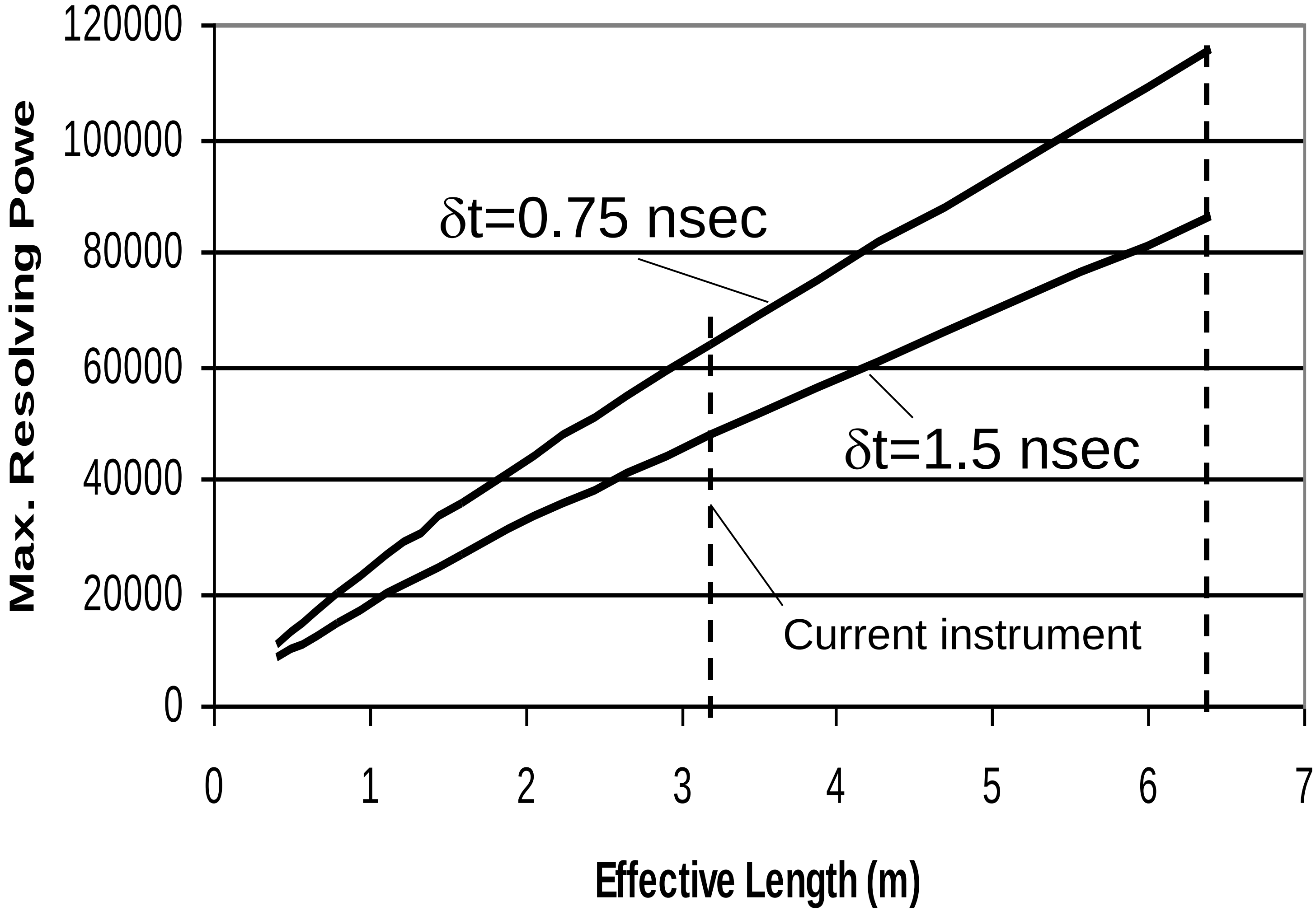
Voltage error, δV: $R_V = \delta V/V$

Resolving power:

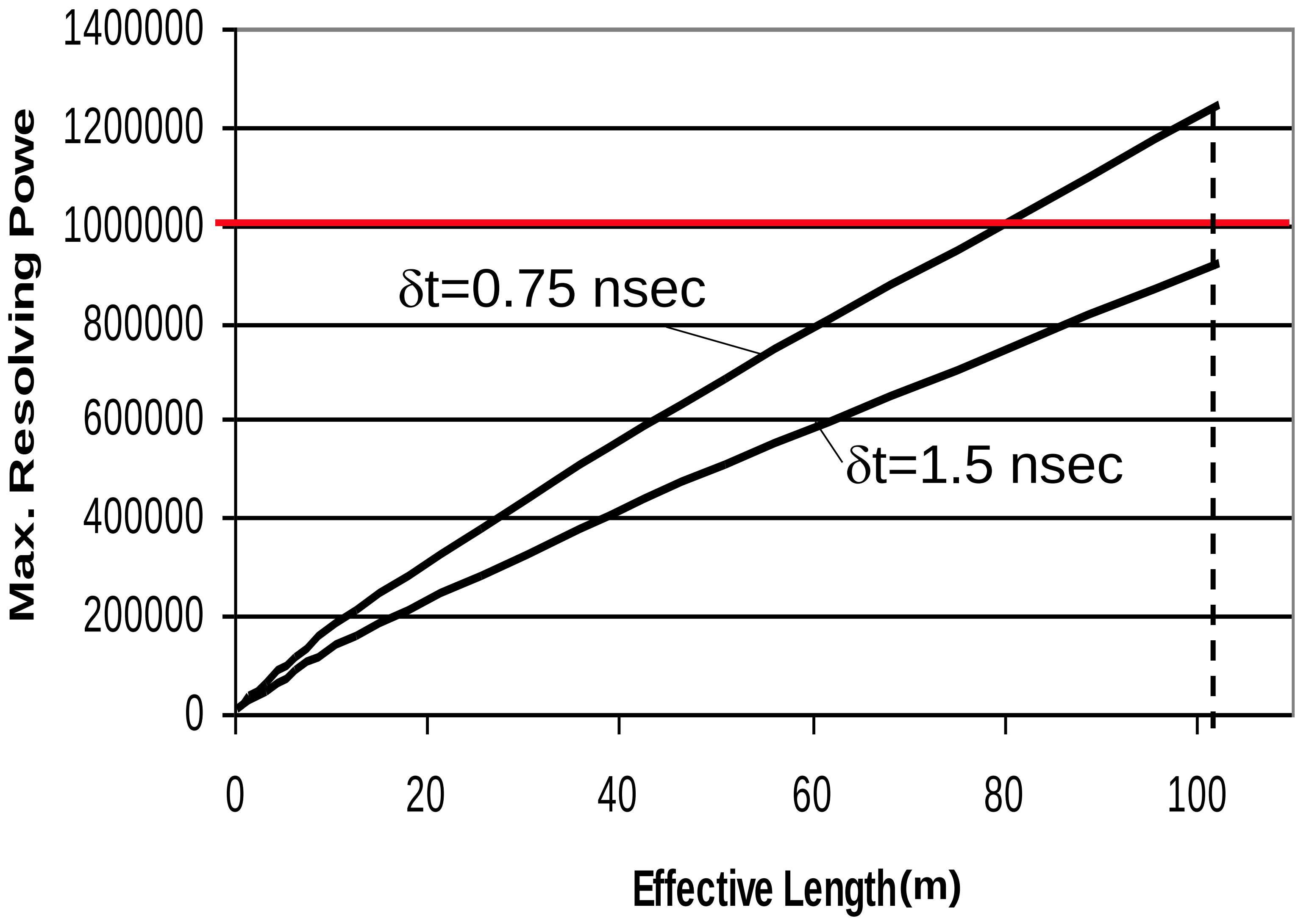
$$R^{-1} = [R_{s1}^2 + R_v^2 + R_t^2 + R_L^2 + R_V^2]^{-1/2}$$



Minimum peak width as function of focusing parameter K for effective Lengths of 3.2 and 6.4 m. Optimum values correspond to dR/dK=0, And is proportional to $D_e^{0.84}$ for these initial conditions.



Calculated maximum resolving power as function of effective length neglecting voltage error.



Calculated maximum resolving power as function of effective length neglecting voltage error.

Conclusions

- Resolving power of prototype reflecting analyzer is limited by voltage error or trajectory error
- If the total contribution of these two mass independent errors can be reduced to <10 ppm, then maximum resolving power of 50,000 is feasible with current prototype.
- Maximum resolving power is proportional to $D_e^{0.84}$
- 1,000,000 resolving power is theoretically feasible with an effective length of 100 m (physical length about 40 m)
- But!!!
- Errors independent of length must be less than 0.5 ppm (5 millivolts at 10 kilovolts)

The Virgins