Transmission SIMS: A novel approach to achieving higher secondary ion yields of intact biomolecules

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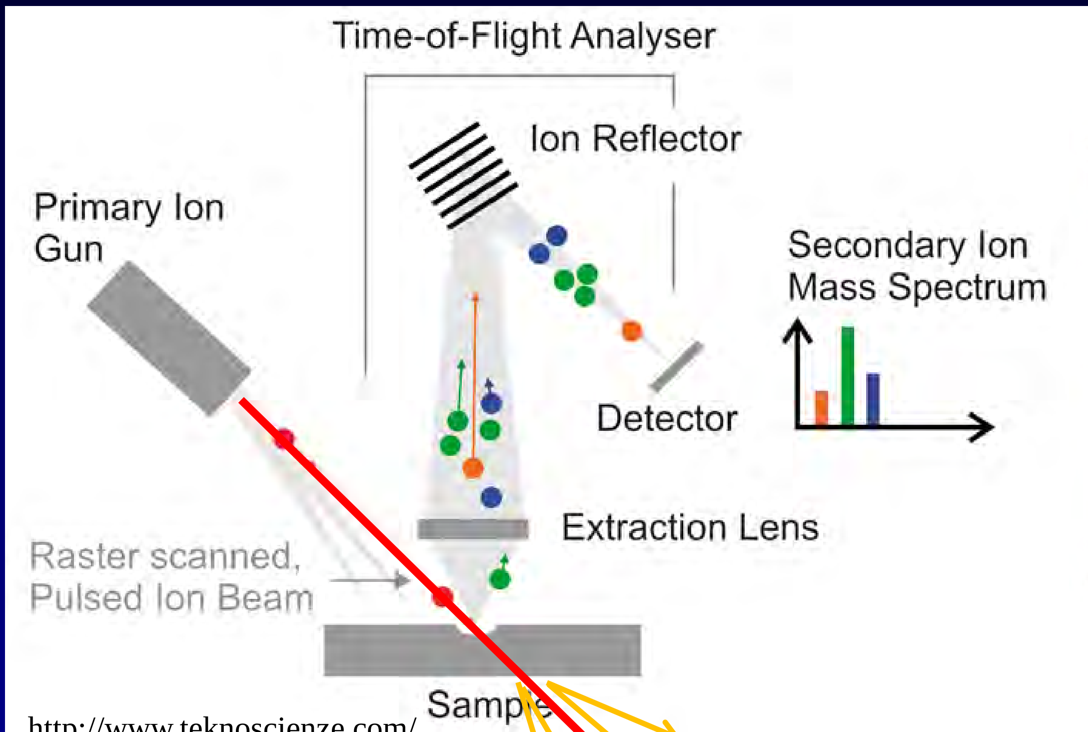
K. Nakajima *et al*, APL 104 (2014) 114103.

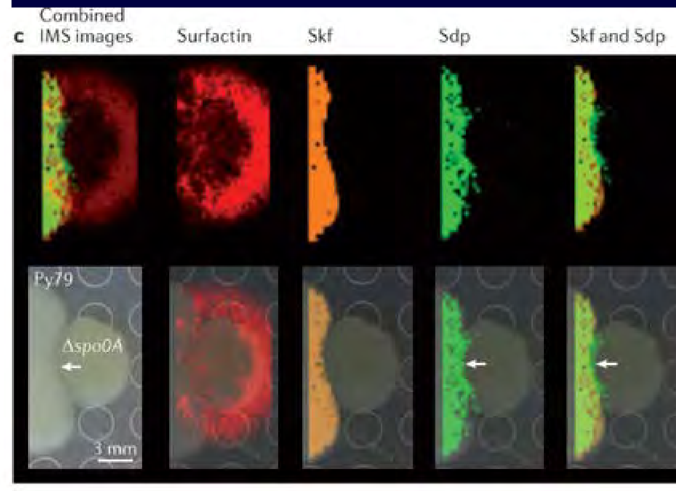
SIMS: secondary ion mass spectrometry

Excellent lateral and depth resolution
Sensitivity is extremely high



http://www.teknoscienze.com/

Often used for analysis and imaging of biomolecules



D. Jeramie et al, Nature Reviews Microbiology 9, 683.

Sensitivity for large biomolecules is rather poor because biomolecules are easily destroyed by ion impact

Transmission SIMS

in combination with high energy cluster ions, secondary ion yield of intact biomolecule is enhanced and fragment ions are suppressed

OUTLINE

1. Introduction

Analysis and imaging of biomaterials using SIMS

2. Transmission SIMS using 6 MeV Cu^{4+}

In the forward direction, yield of intact amino acid ion is enhanced but fragment ions are more enhanced

3. Transmission SIMS using 5 MeV C_{60}^{+}

Yield of intact amino acid ion is enhanced by one order of magnitude and fragment ions are suppressed in the forward direction

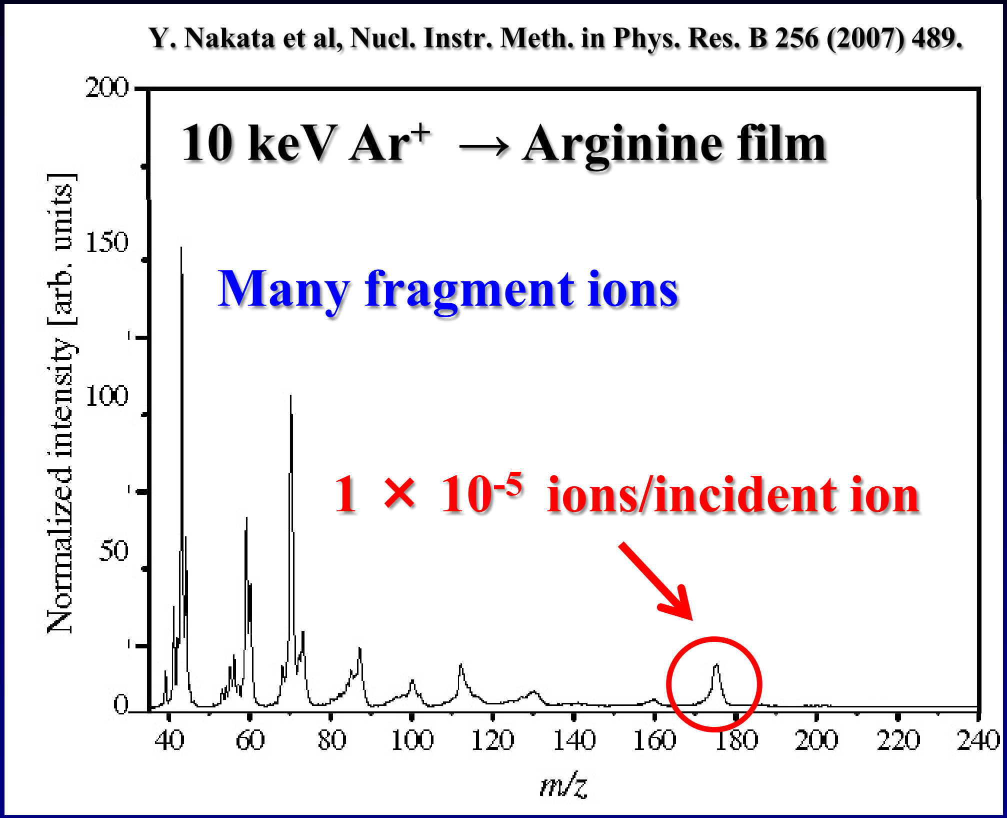
The mechanism of the enhancement and suppression is discussed in terms of deposited-energy distribution at the surface

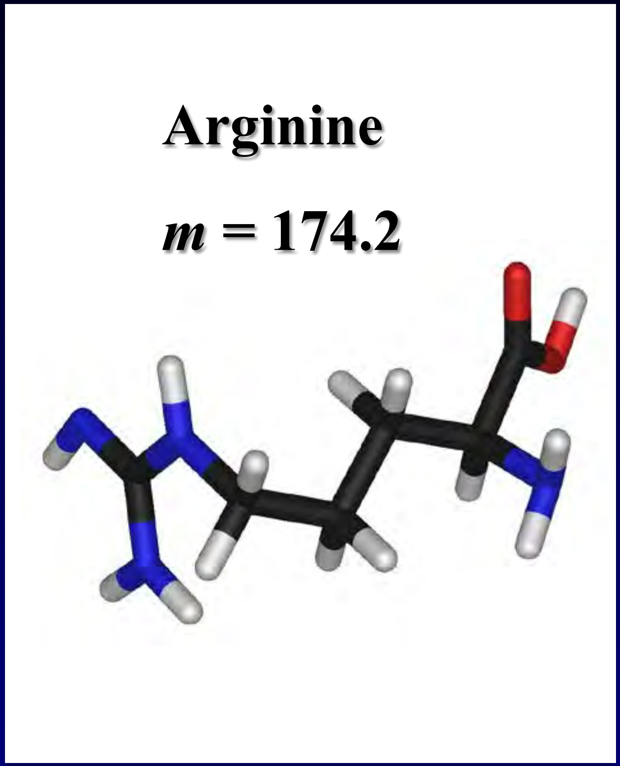
4. Development of new molecular imaging technique

Transmission SIMS + PEEM

5. Summary

Sensitivity of conventional SIMS is poor for biomolecules





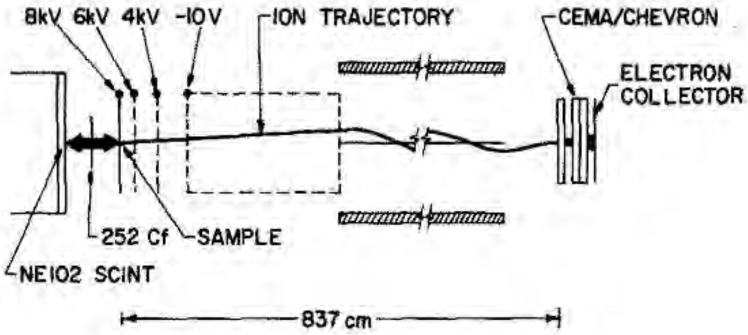
Yield of intact molecular ion is very low
Fragment ions are dominant

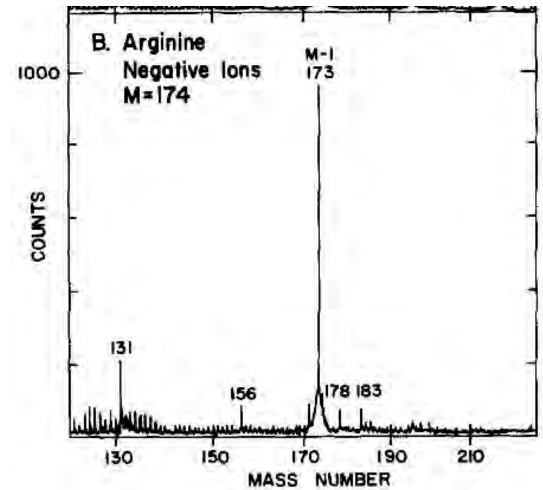
To improve the sensitivity of biomolecules

1. Use of high energy ions → PDMS, MeV-SIMS
2. Use of cluster ions → Cluster SIMS

Plasma Desorption Mass Spectrometry (PDMS)

Fission fragments → arginine



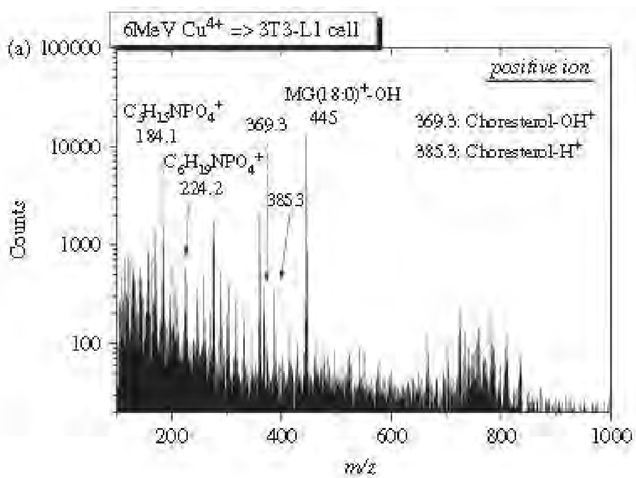


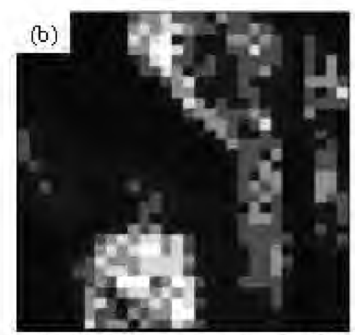
D.E. Torgerson et al, Biochem. Biophys. Res. Commun. 60 (1974) 616.

MeV-SIMS: becomes popular for the analysis of biomaterials

6 MeV Cu^{4+} → animal cells

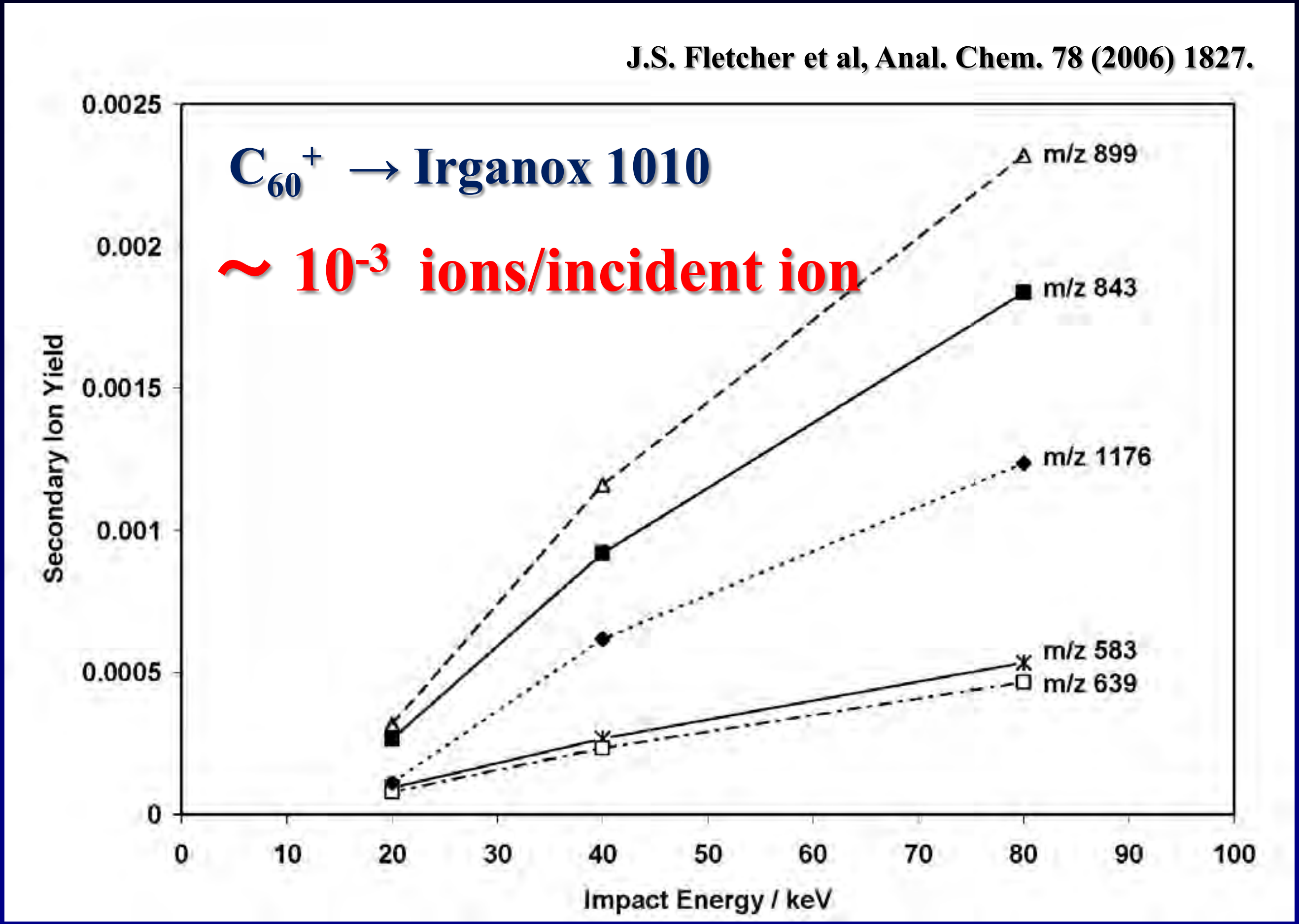
H. Yamada et al, Surf. Interface Anal. 43 (2011) 363.





m/z 79 (PO_3^-) Max counts : 247

Cluster SIMS: C_{60} ion



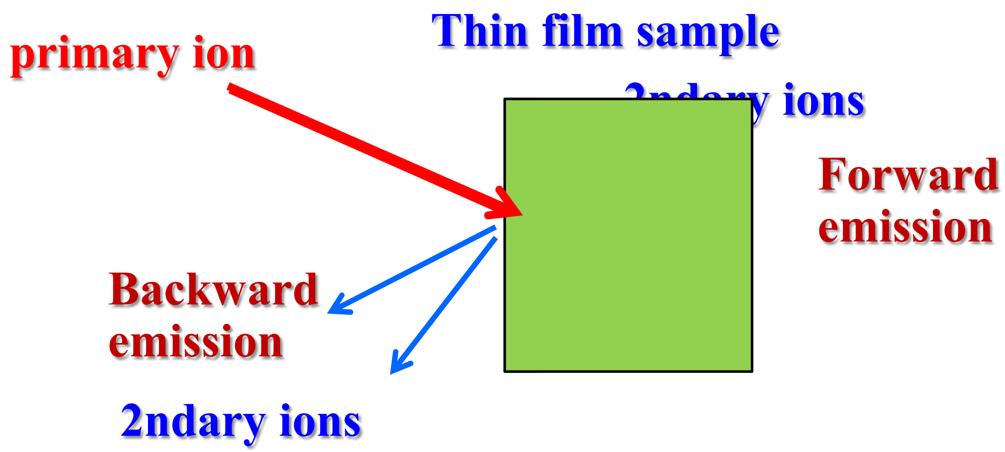
Large yields compared to conventional SIMS

Yield increases with impact energy

→ further enhancement with MeV C_{60}^{+} ?

Another possibility to enhance the yield in SIMS

In the conventional SIMS, 2ndary ions emitted in the backward direction are measured

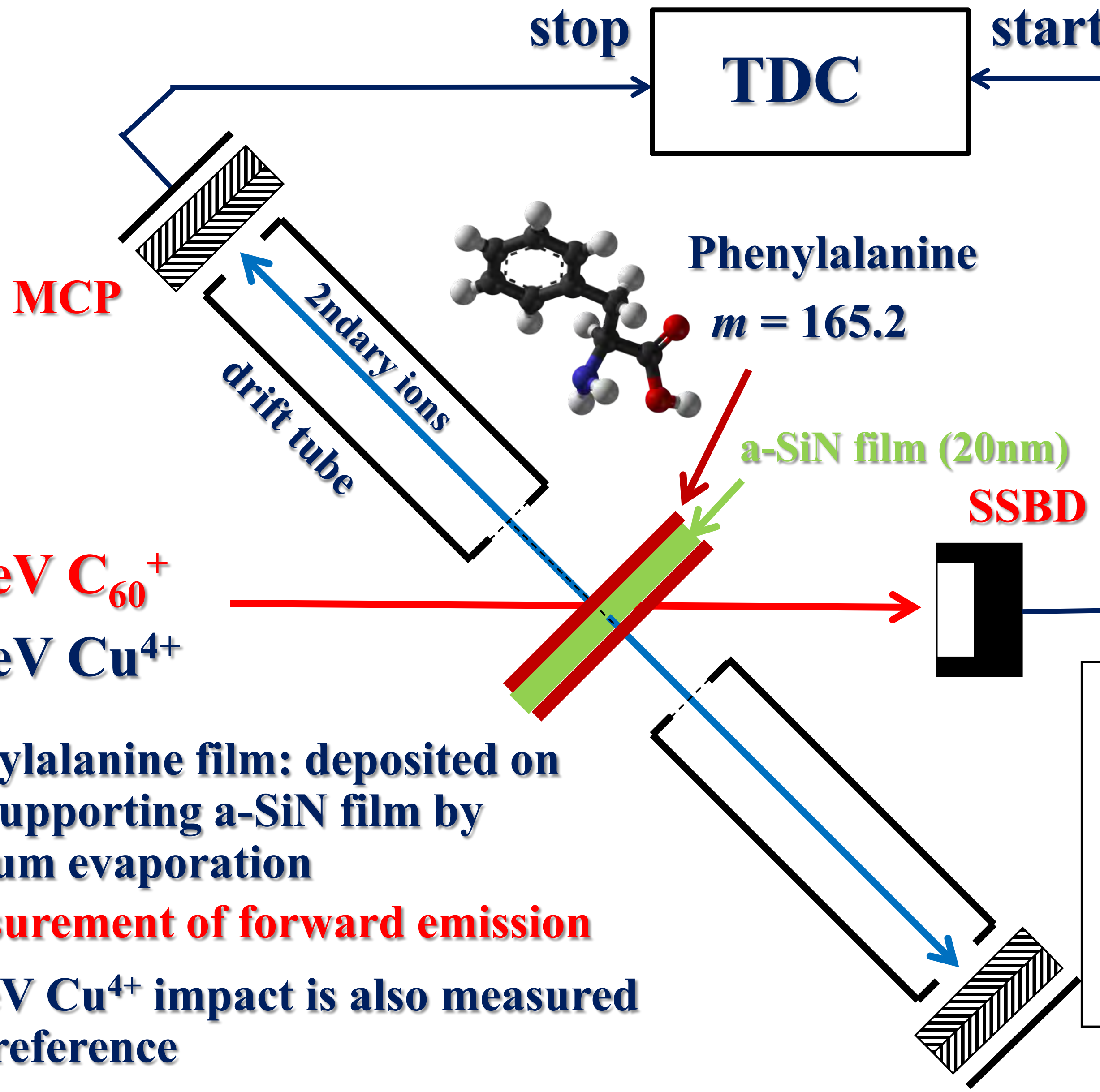


Primary ions can directly transfer their momentum to the target atoms in the forward direction → Enhancement of secondary ion yield is expected in the forward direction

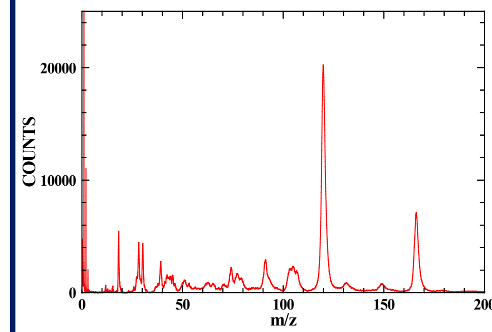
MeV ions, cluster ions, transmission geometry

→ Transmission SIMS using MeV C_{60} ion

Setup: TOF-SIMS with 5 MeV C_{60} ion



Mass spectrum



5 MeV C_{60}^{+}

6 MeV Cu^{4+}

Phenylalanine film: deposited on self-supporting a-SiN film by vacuum evaporation

Measurement of forward emission

6 MeV Cu^{4+} impact is also measured as a reference

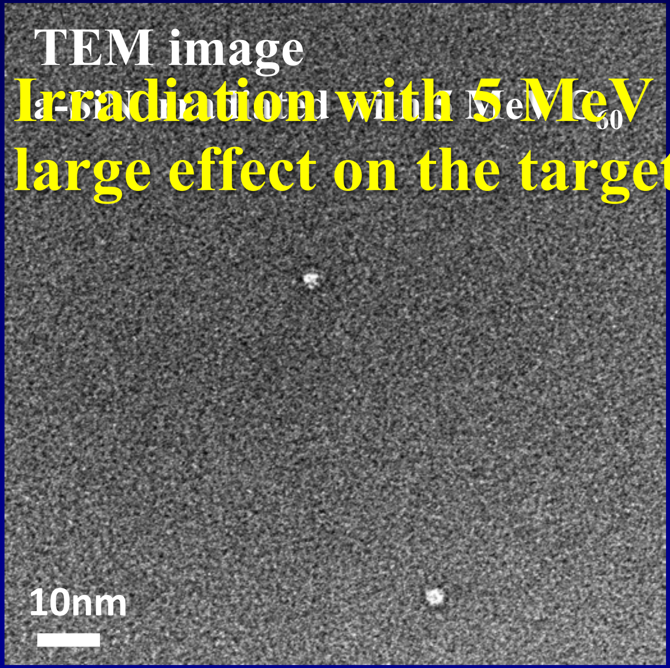
6 MeV Cu^{4+}
- Forward emission
- Backward emission

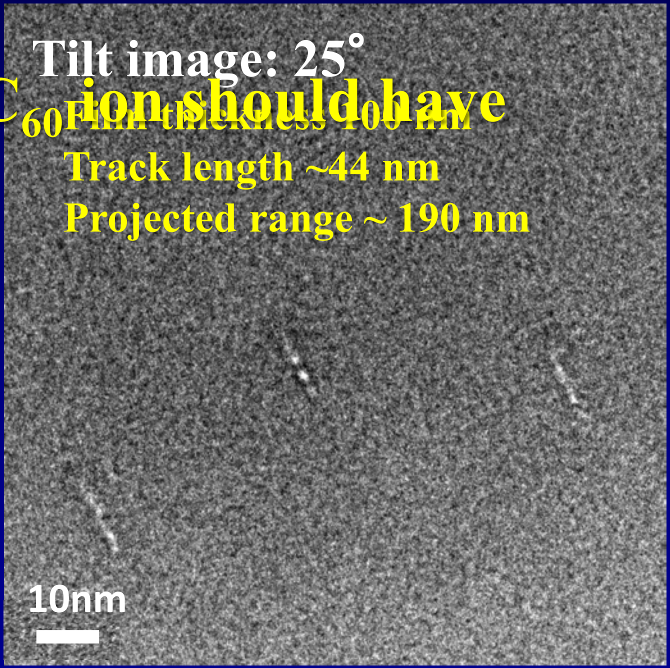
5 MeV C_{60}^{+}
- Forward emission
- Backward emission

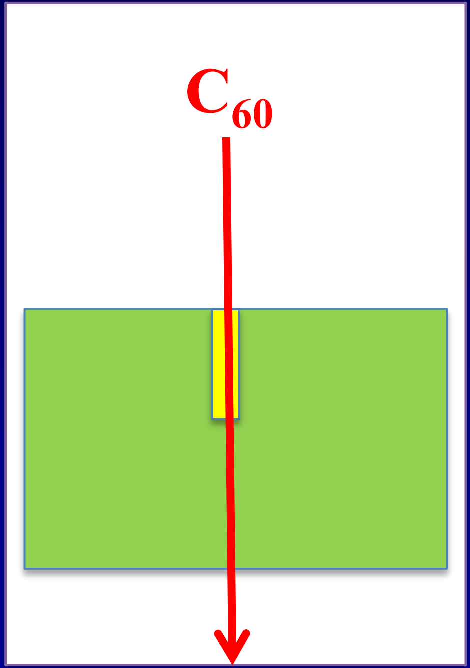
What happens when 5 MeVC_{60} → a-SiN

	5 MeV C_{60}	30 keV Ga (Conventional SIMS)	6 MeV Cu (MeV-SIMS)
S_{total}	25.9 keV/nm	1.71 keV/nm	3.52 keV/nm
S_{elec}	21.9 keV/nm	0.16 keV/nm	3.33 keV/nm
S_{nucl}	4.0 keV/nm	1.55 keV/nm	0.19 keV/nm

Irradiation with 5 MeV C_{60} ion should have large effect on the target





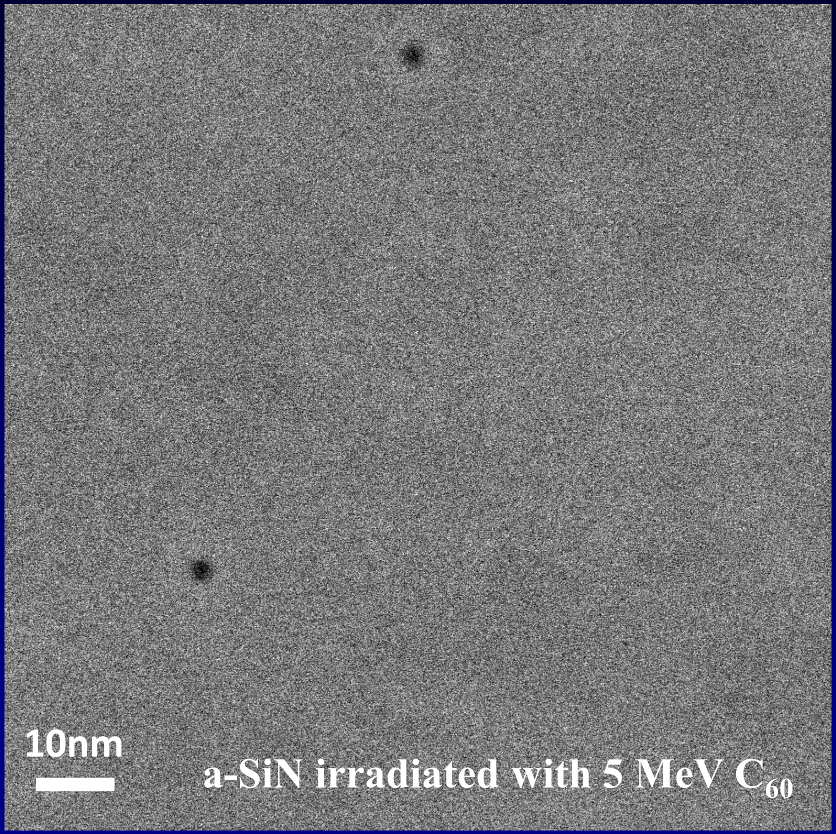


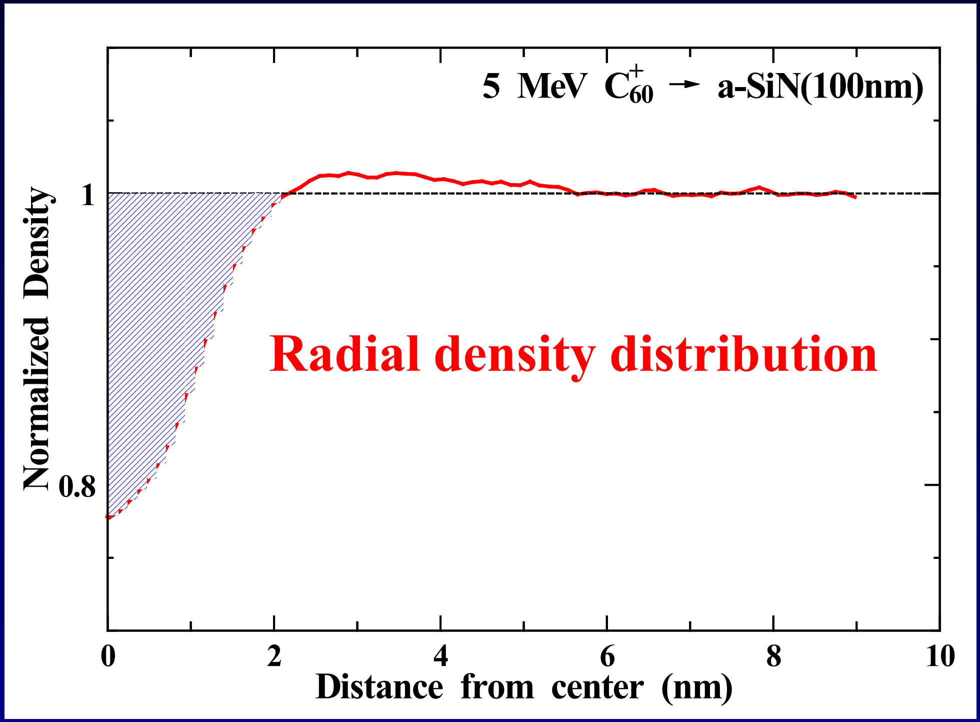
Cylindrical ion track is produced

Density is reduced inside the ion track? → HAADF-STEM

HAADF-STEM: semi-quantitative analysis is possible

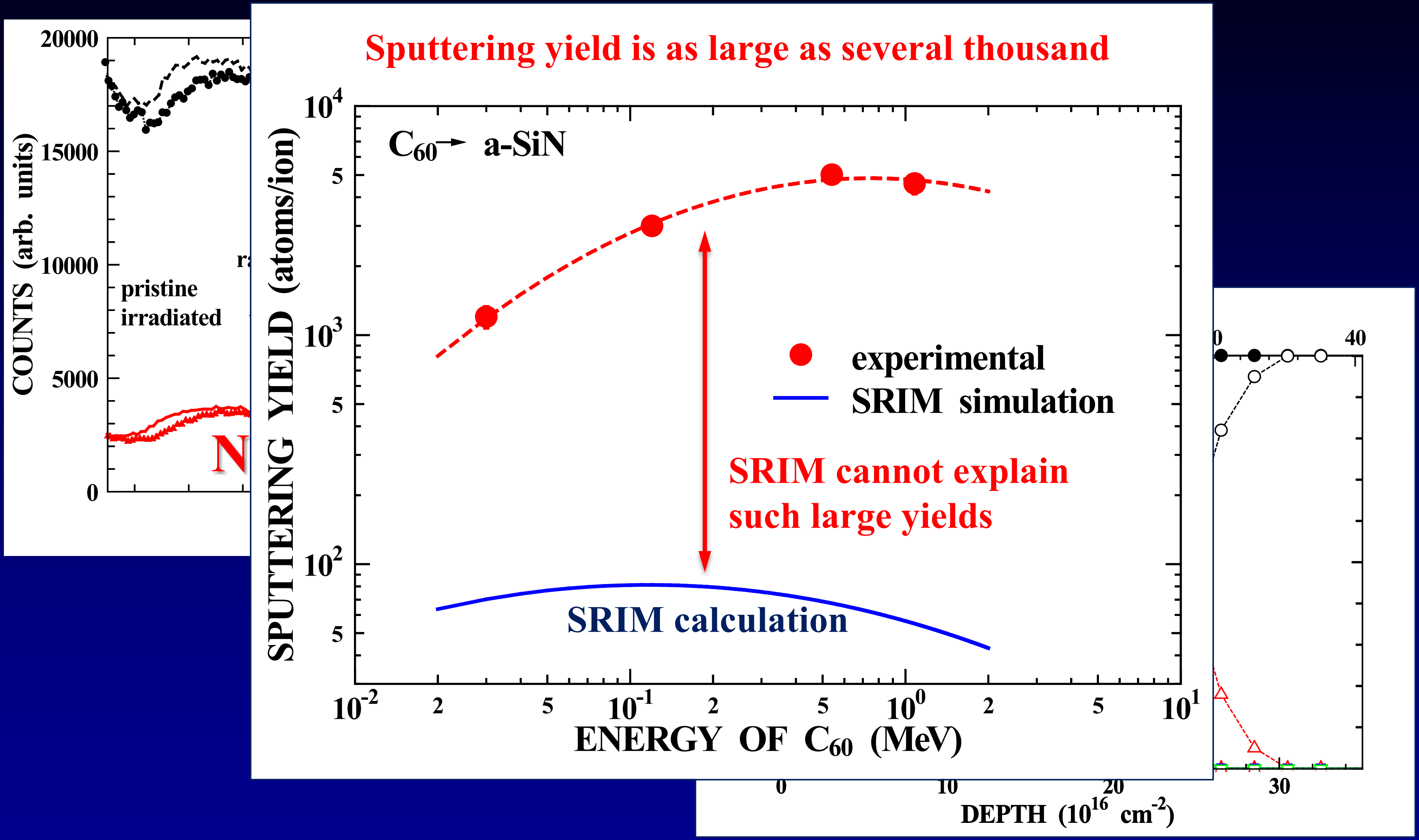
HAADF-STEM image





Missing volume ~400 nm^3 → 3000 atoms!
This suggests very large sputtering yield

Sputtering yield measurement using high-resolution RBS



Ballistic collision cascade is negligible → Thermal spike plays an important role.
Sputtering yield is estimated using unified thermal spike (u-TS) model

Unified thermal spike (u-TS) model

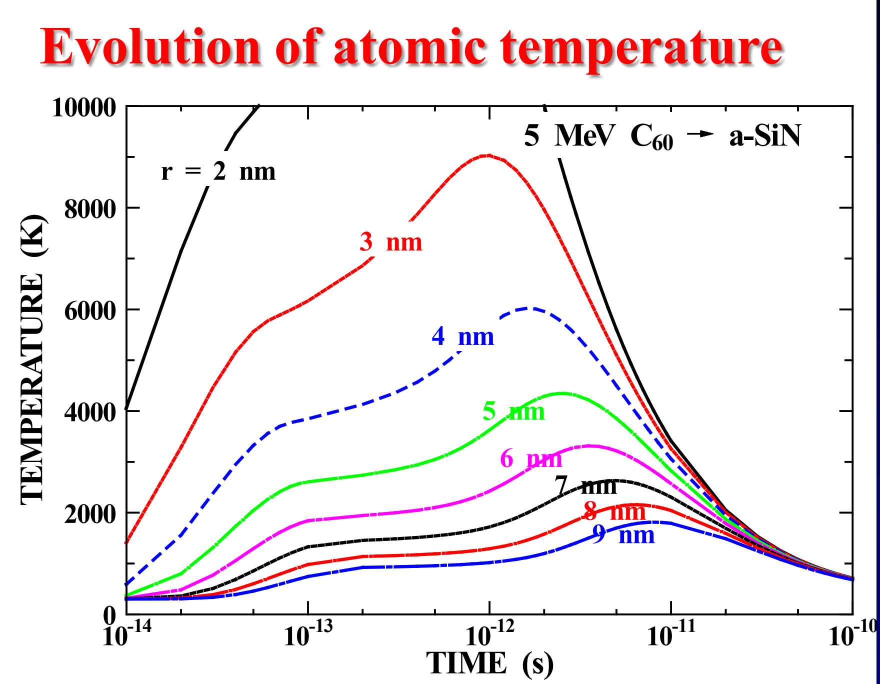
M. Toulemonde *et al*, Phys. Rev. B 83, 054106 (2011).

The u-TS model describes temperature evolution using two thermal diffusion equations.

$$C_e \frac{\partial T_e}{\partial t} = \frac{1}{r}\frac{\partial}{\partial r}\left[rK_e \frac{\partial T_e}{\partial r}\right] - g(T_e - T_a) + A(r,v,t)$$

$$C_a \frac{\partial T_a}{\partial t} = \frac{1}{r}\frac{\partial}{\partial r}\left[rK_a \frac{\partial T_a}{\partial r}\right] + g(T_e - T_a) + B(r,t)$$

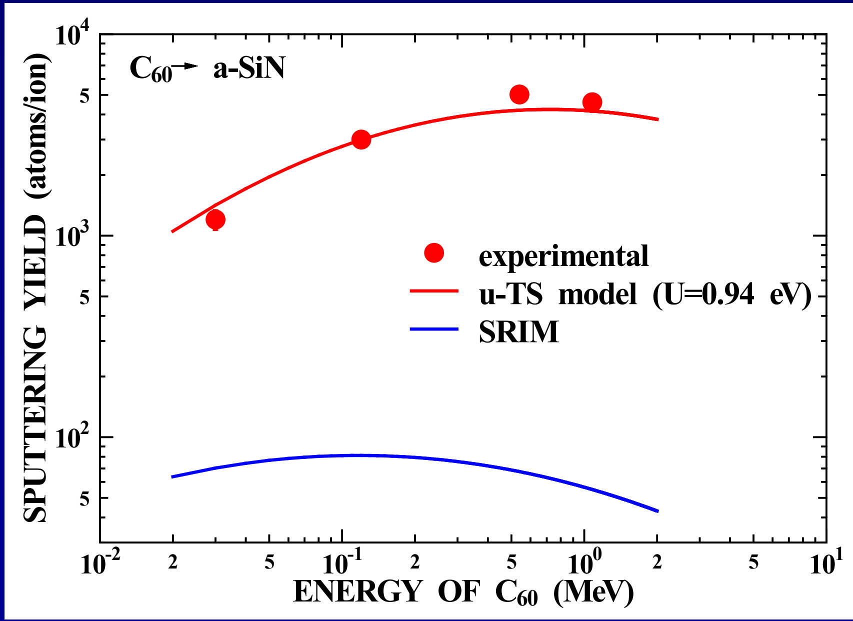
Both S_e and S_n are included



Thermal evaporation mechanism

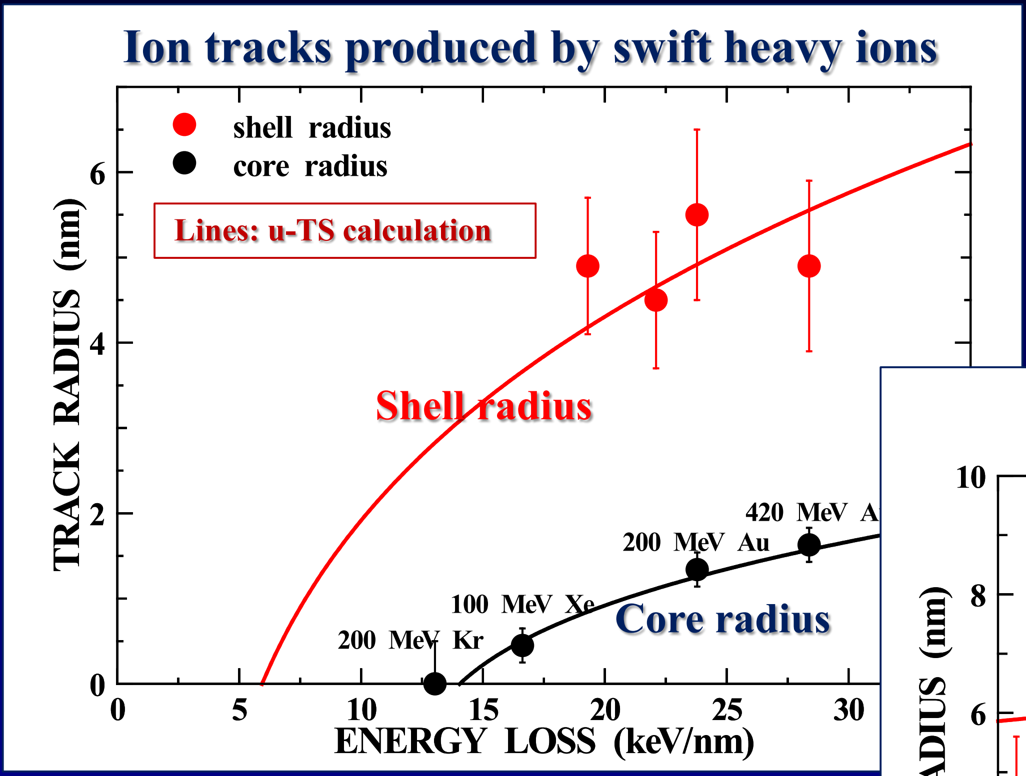
P. Sigmund, C. Claussen, J. Appl. Phys. 52 (1981) 990.

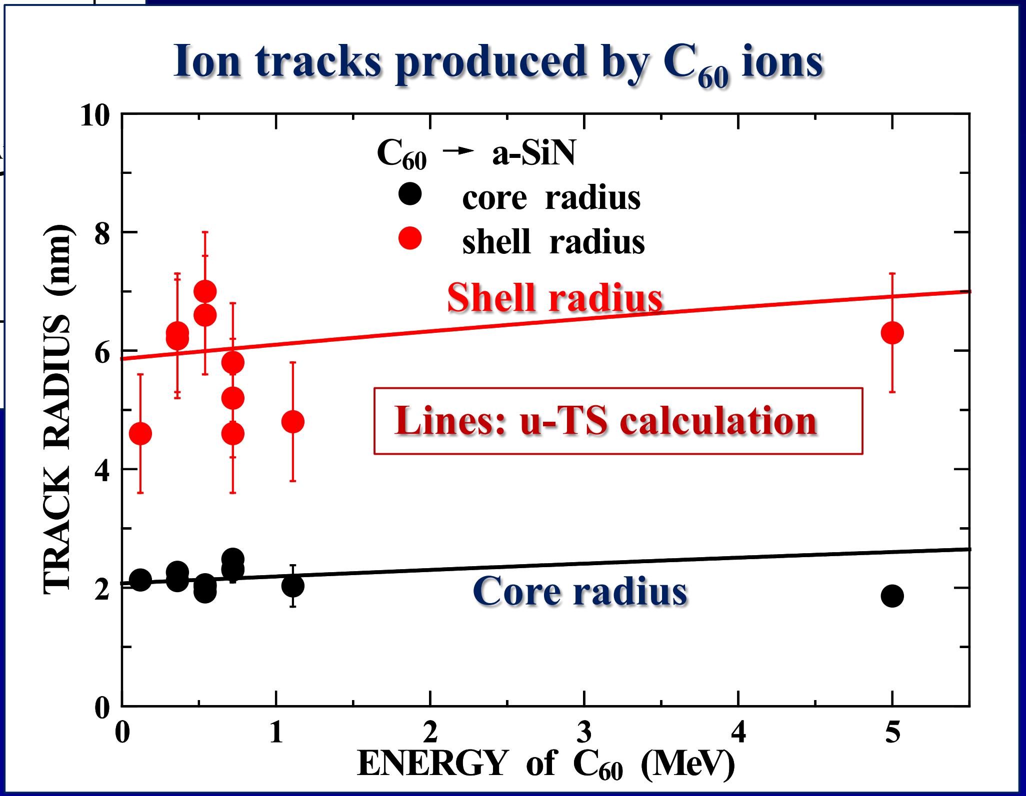
$$\Phi_i(T_a(r,t)) = N_i \sqrt{\frac{k_B T_a(r,t)}{2\pi M_i}}\, exp\left(\frac{-U_i}{k_B T_a(r,t)}\right)$$



u-TS model reproduces observed sputtering yield

u-TS model also reproduces observed track radius produced by swift heavy ions as well as C_{60} ions

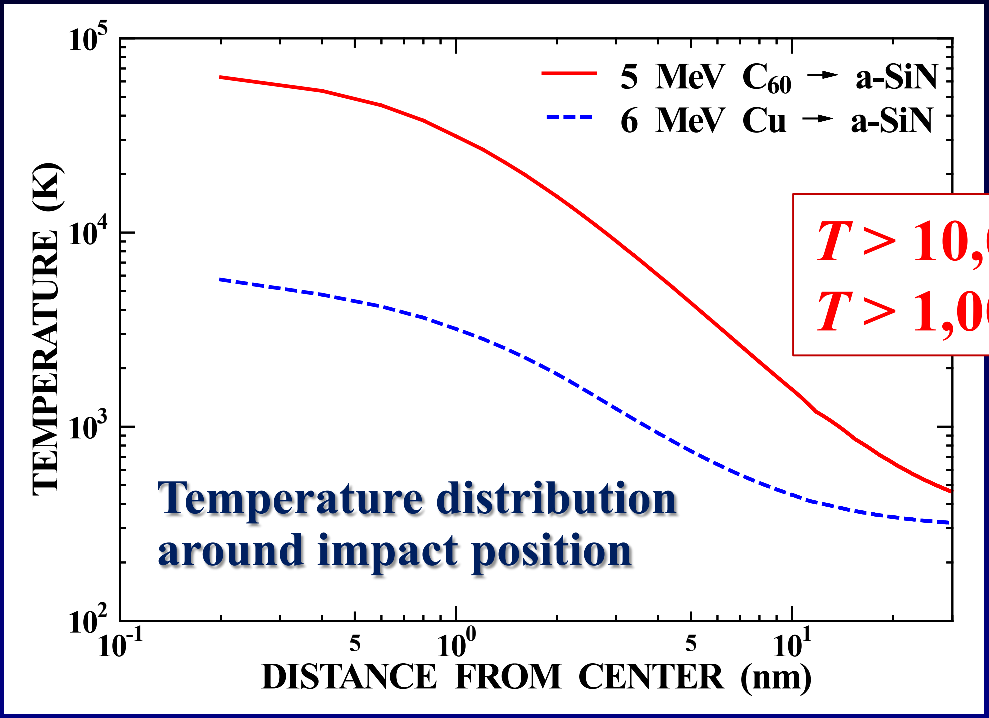




u-TS model is a reliable model to describe and understand the ion-solid interactions, including MeV C_{60} ions

Summary: effect of MeV C_{60} ion impact on materials

A very intense thermal spikes are produced

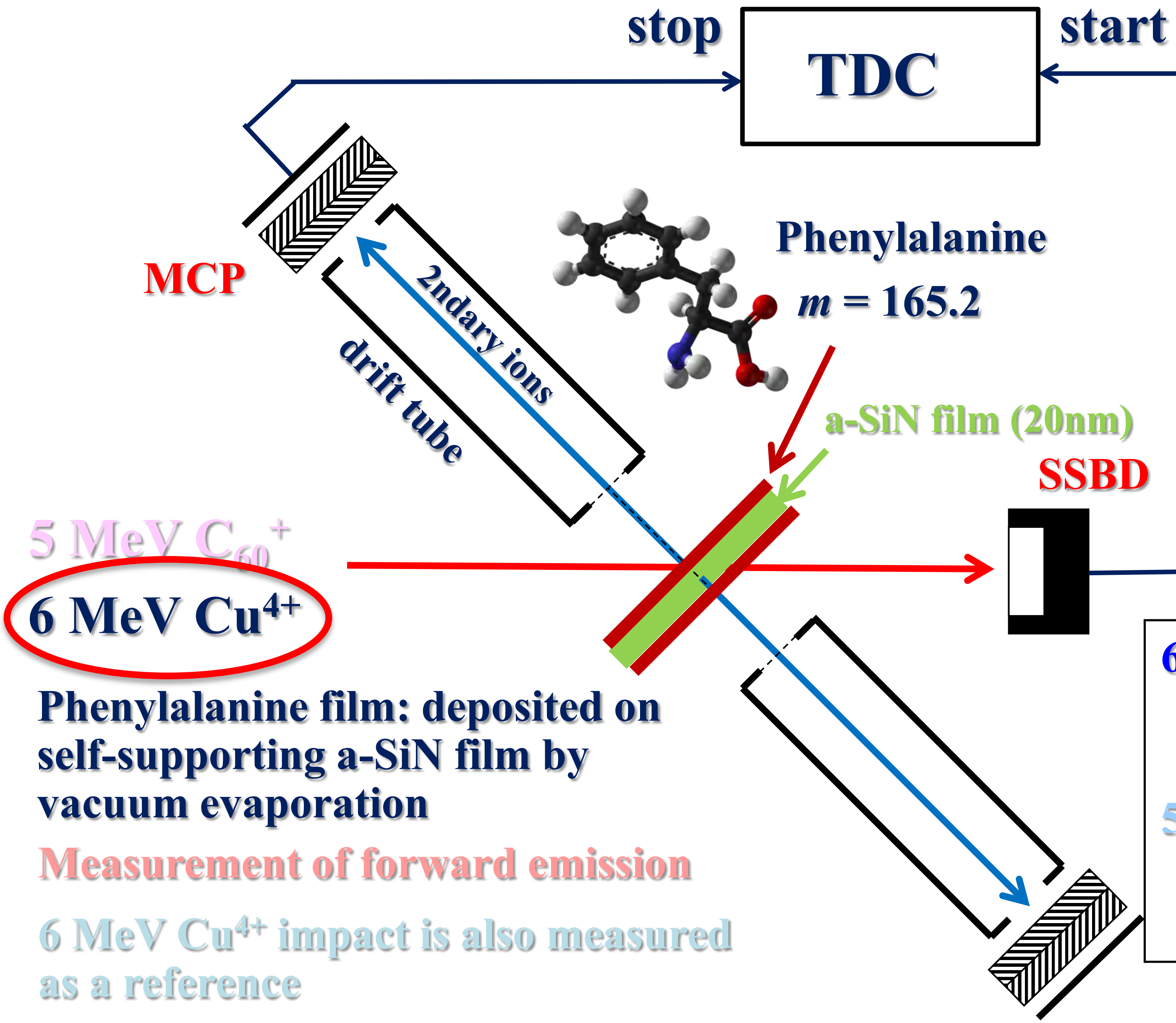


$T > 10{,}000$ K at $r < 3$ nm
$T > 1{,}000$ K at $r < 13$ nm

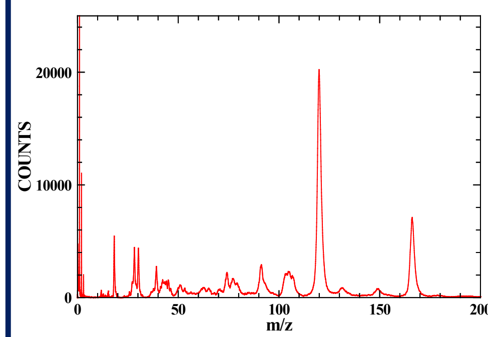
As a result of such a very intense thermal spike,

1) Cylindrical ion tracks are produced:
   diameter a few nm length several tens nm
2) Extremely high sputtering yield is observed:
   several thousand atoms/incident ion → large 2ndary ion yield?

SIMS measurement with 6 MeV Cu^{4+} ion



Mass spectrum



Phenylalanine film: deposited on self-supporting a-SiN film by vacuum evaporation

Measurement of forward emission

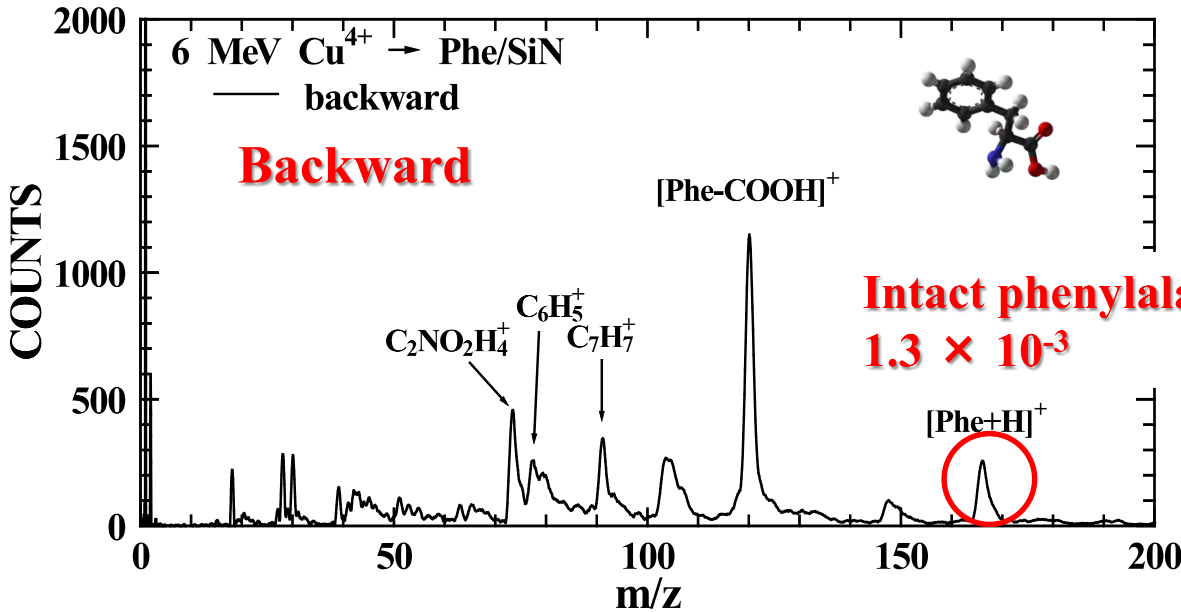
6 MeV Cu^{4+} impact is also measured as a reference

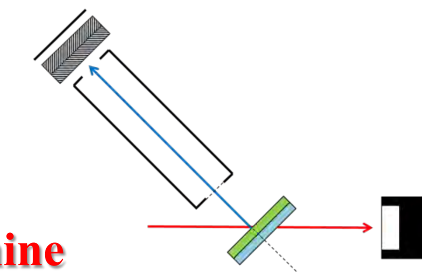
6 MeV Cu^{4+}
- **Forward emission**
- **Backward emission**

5 MeV C_{60}^{+}
- **Forward emission**
- **Backward emission**

6 MeV Cu^{4+} → phenylalanine

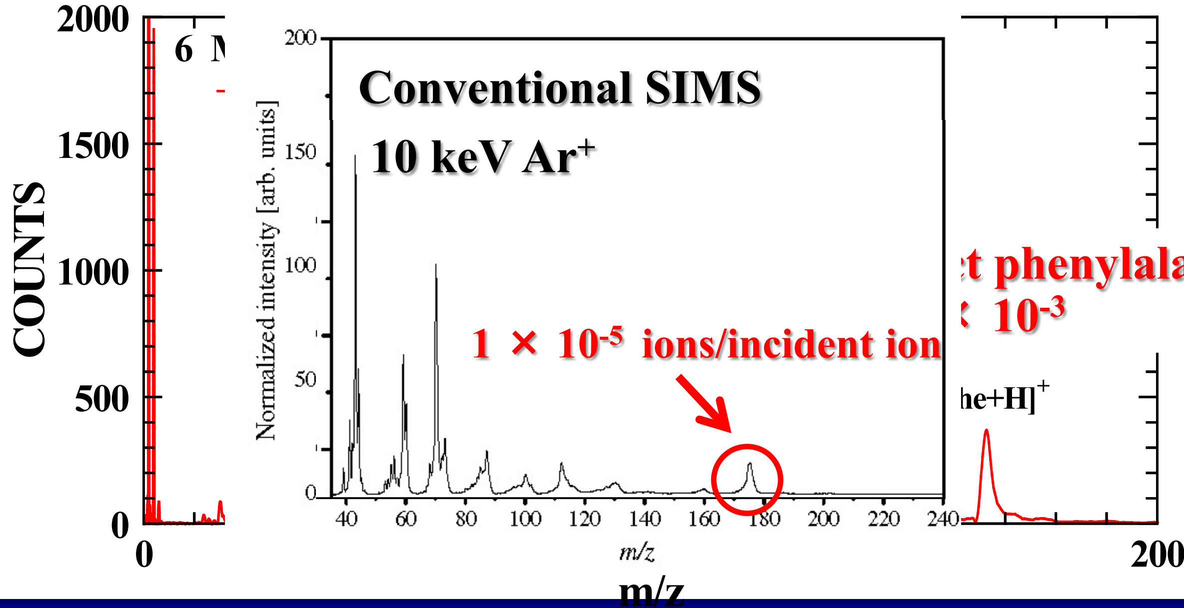
Comparison with conventional SIMS
Comparison between forward & backward emission

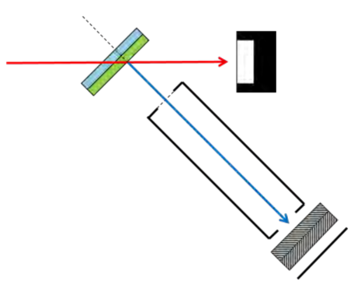




Intact phenylalanine
1.3×10^{-3}

About 2 orders of magnitude larger than the conventional SIMS





Yield is enhanced in the forward direction (×1.2)

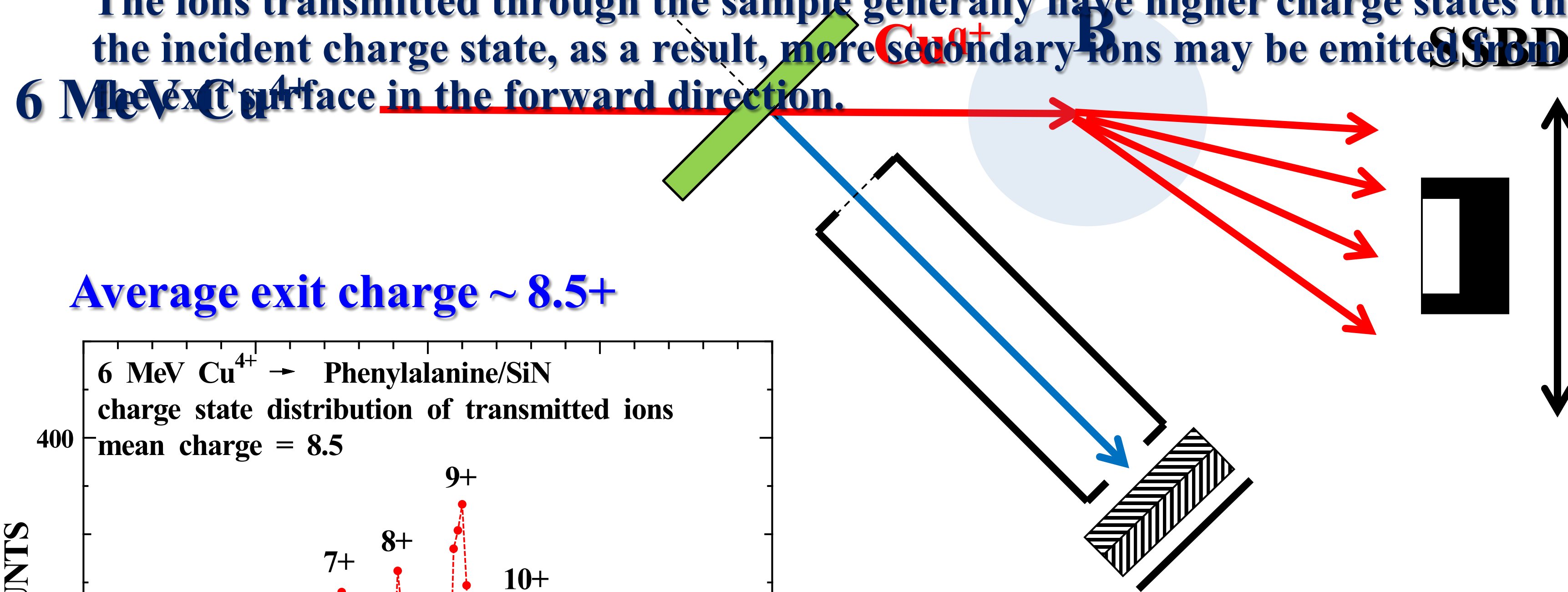
What is the mechanism?

What is the mechanism of enhancement in the forward direction?

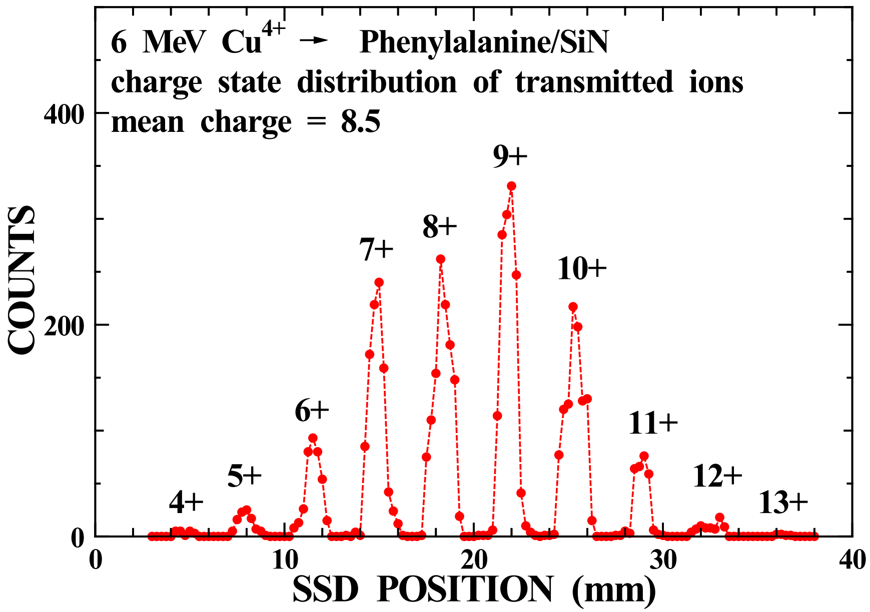
① Preferential momentum transfer in the forward direction

② Charge state dependence of secondary ion yield

The ions transmitted through the sample generally have higher charge states than the incident charge state, as a result, more secondary ions may be emitted from the exit surface in the forward direction.

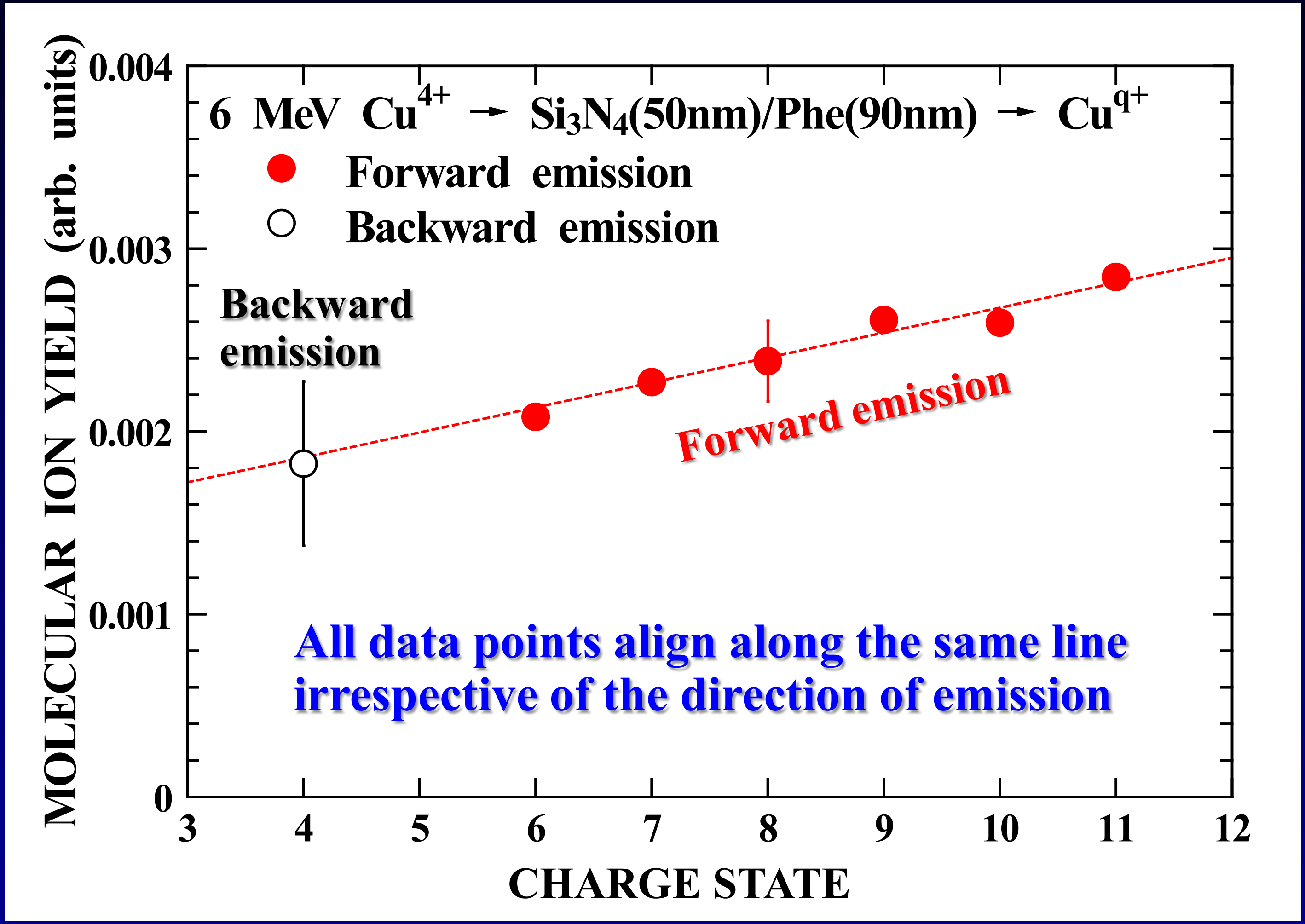


Average exit charge ~ 8.5+



In order to see if the ions of higher charge states really produce more secondary ions, we measured secondary ions in coincidence with the exit charge state.

Charge state dependence of secondary ion yield

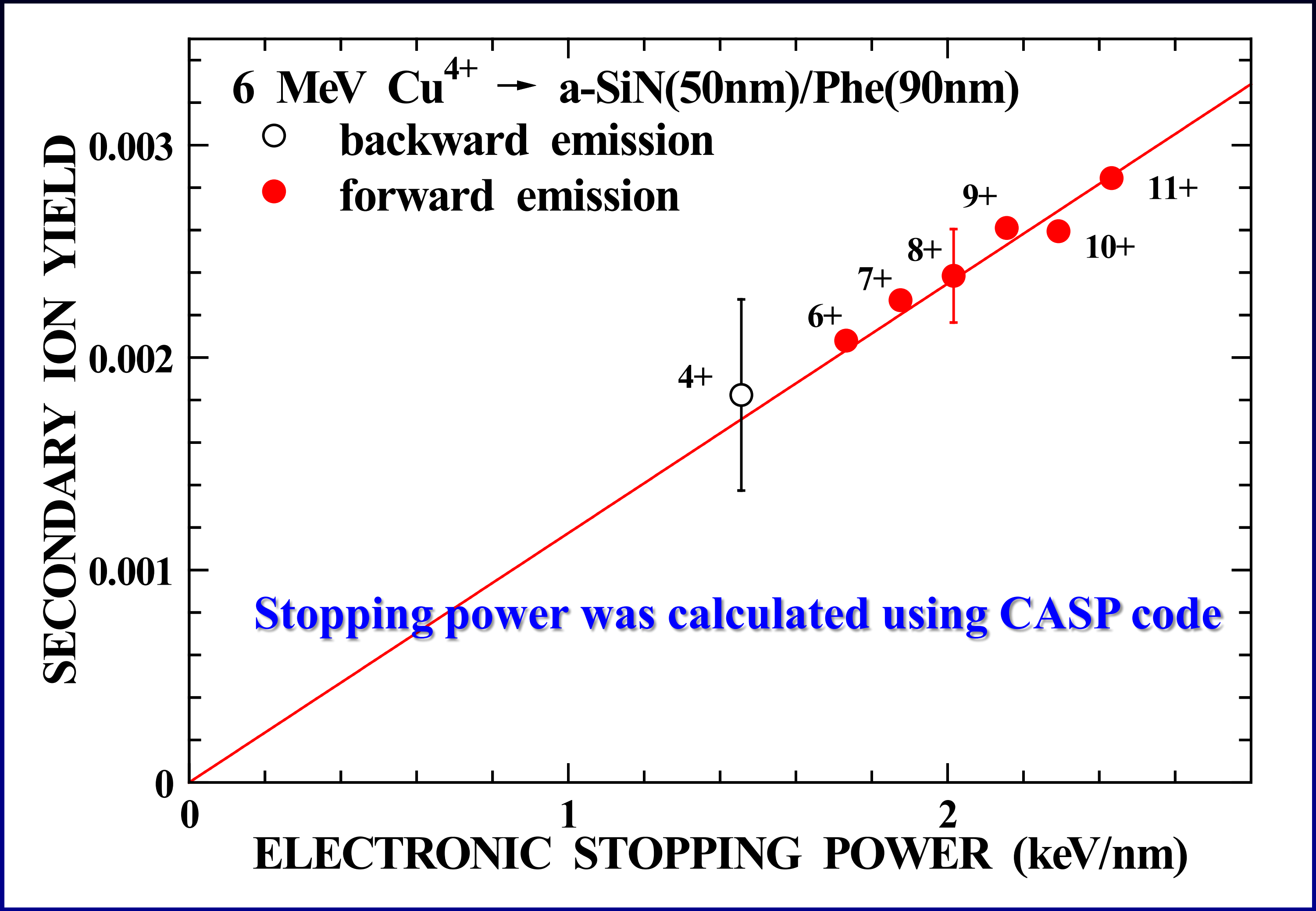


Enhancement in the forward direction can be explained by the charge state dependence.

Preferential momentum transfer does not play a role.

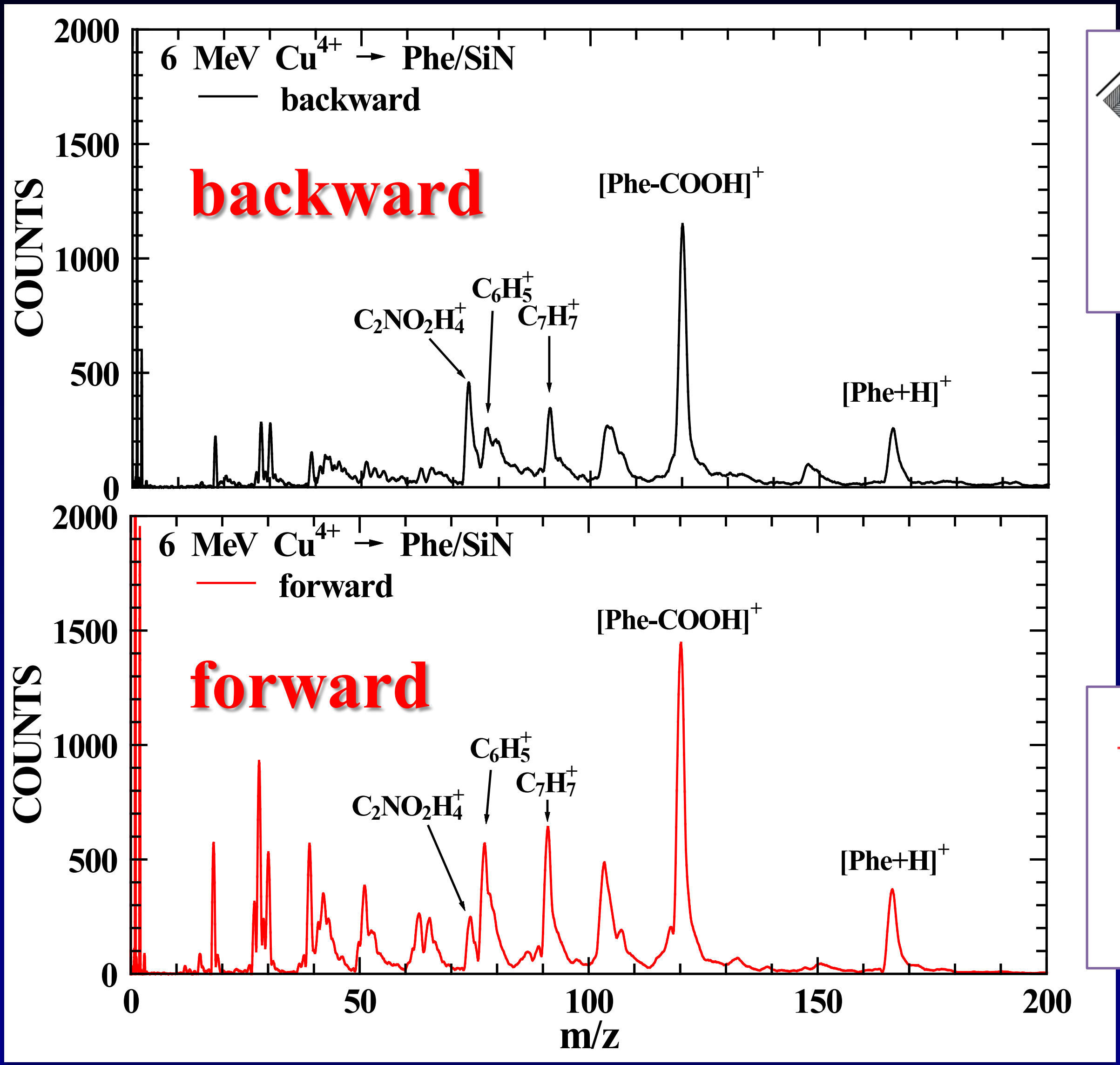
The origin of charge state dependence → Stopping power dependence

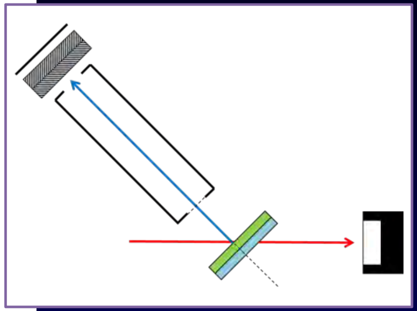
Stopping power dependence of 2ndary ion yield

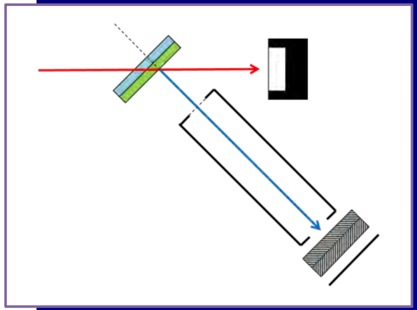


In the present case, primary ions lose energy mainly by electronic excitation. Excited electrons transfer their energy to target atoms/molecules and eventually secondary ions are emitted.

6 MeV Cu^{4+}: forward *vs* backward (Fragment Ions)

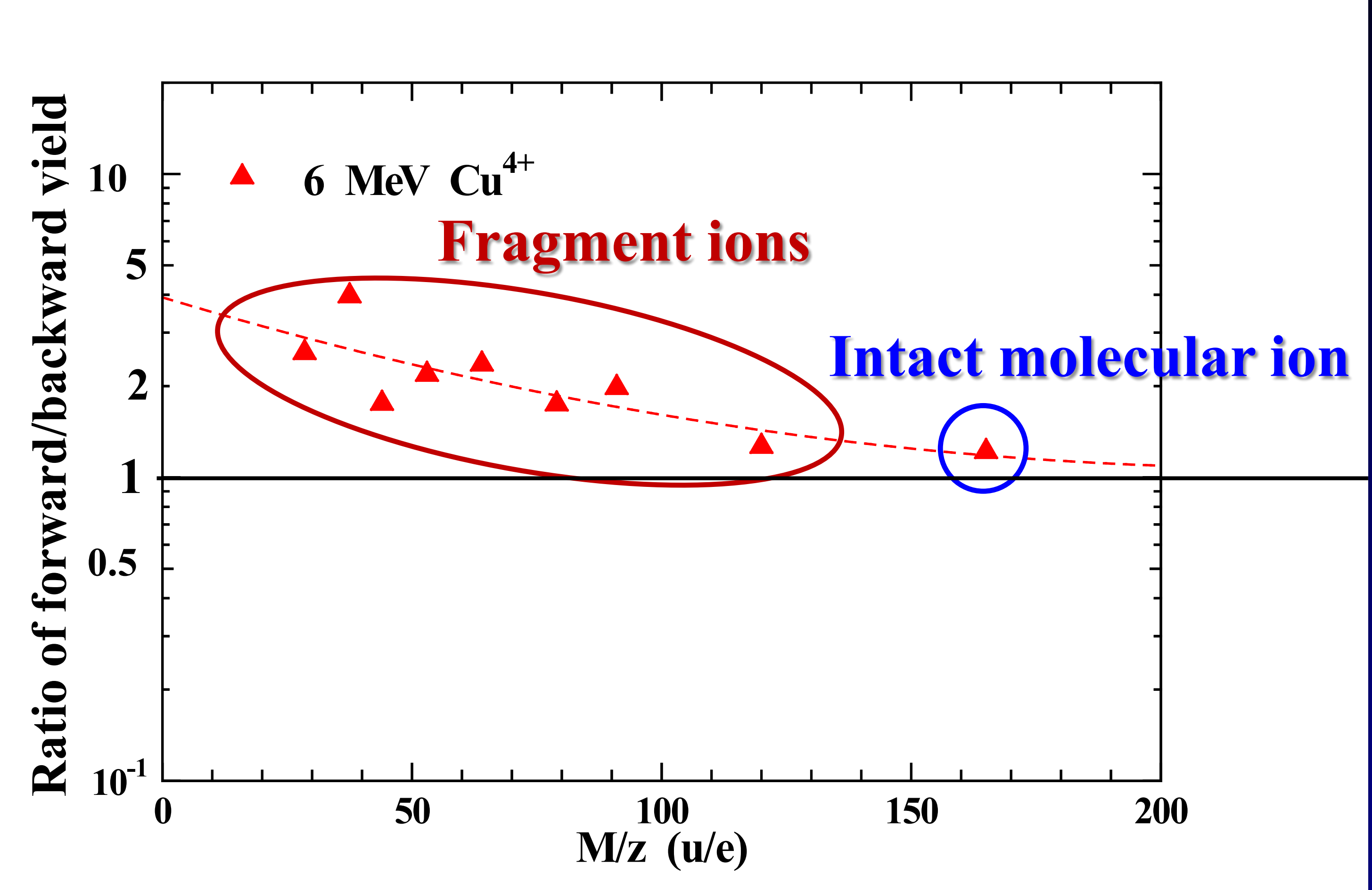






Yield enhancement of fragment ions in the forward direction looks more pronounced than the intact molecular ion

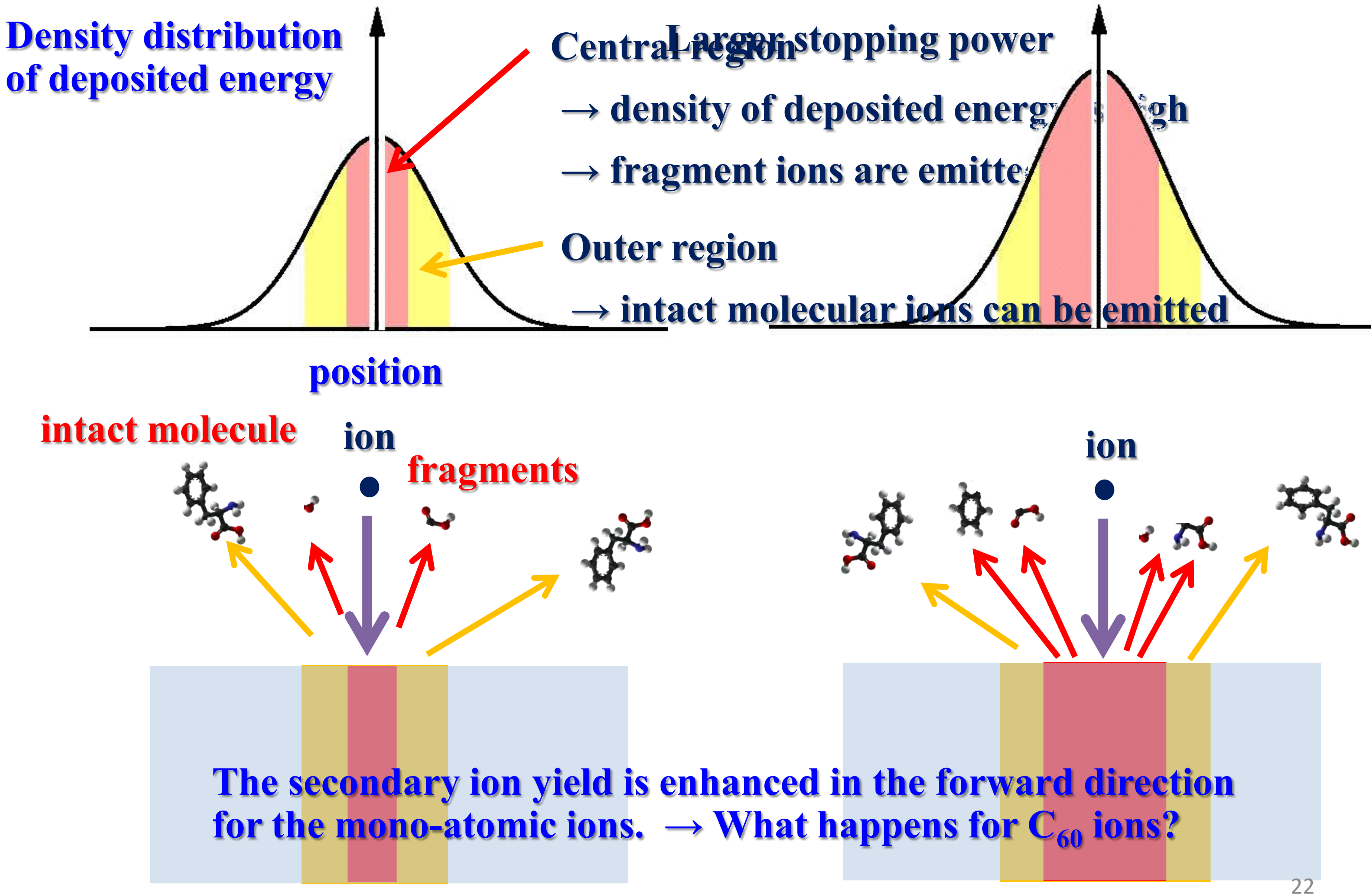
Ratio of forward/backward yield: 6 MeV Cu^{4+}



Yield enhancement in the forward direction increases with decreasing mass number

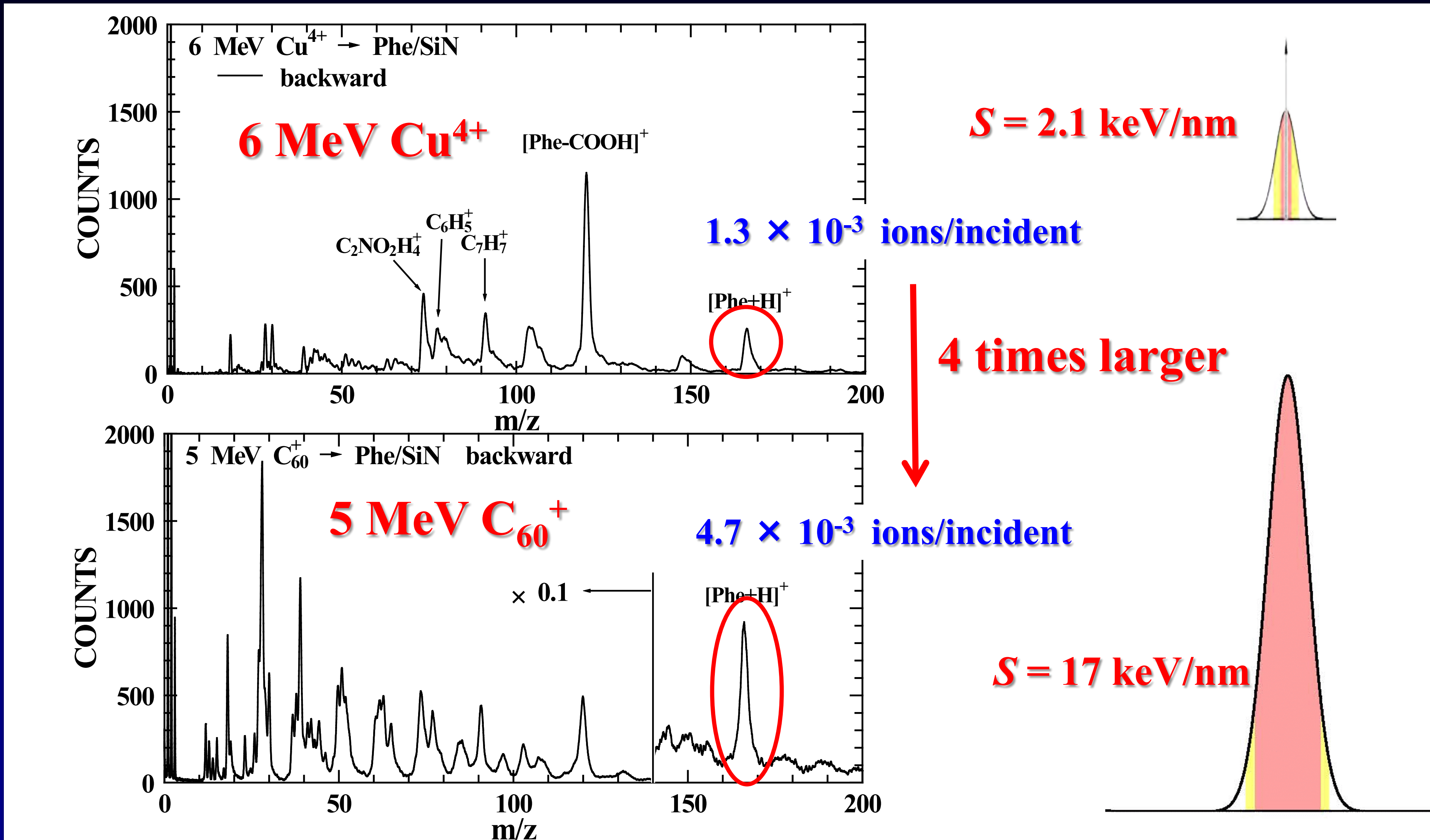
This behavior can be explained by a simple model

Why fragment ions are more enhanced ?



The secondary ion yield is enhanced in the forward direction for the mono-atomic ions. → What happens for C_{60} ions?

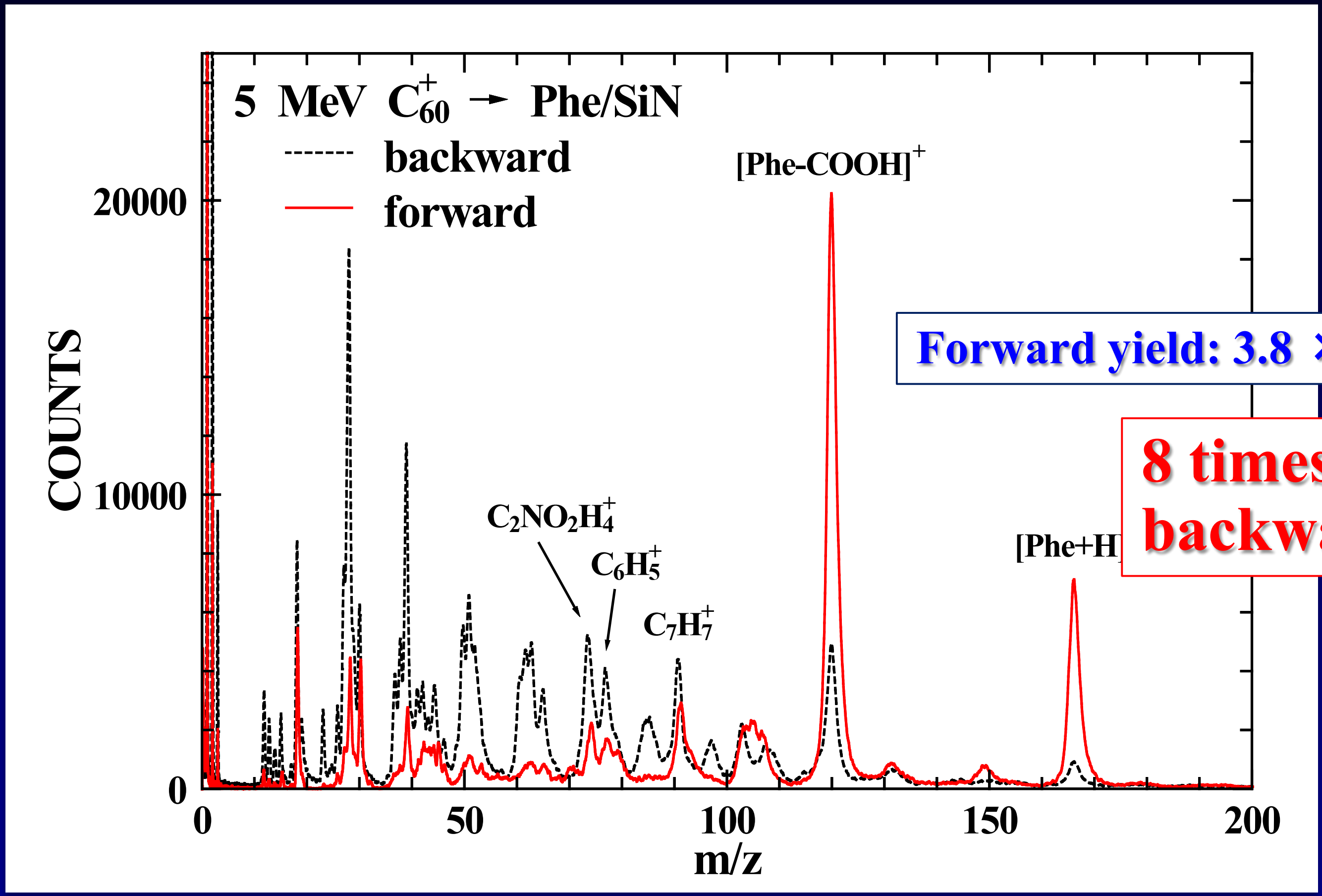
Backward emission: 6 MeV Cu^{4+} *vs* 5 MeV C_{60}^{+}



○ Yield of intact molecular ion is enhanced by using C_{60} ion

× Yields of fragment ions are much more enhanced

5 MeV C_{60}^{+} : forward *vs* backward

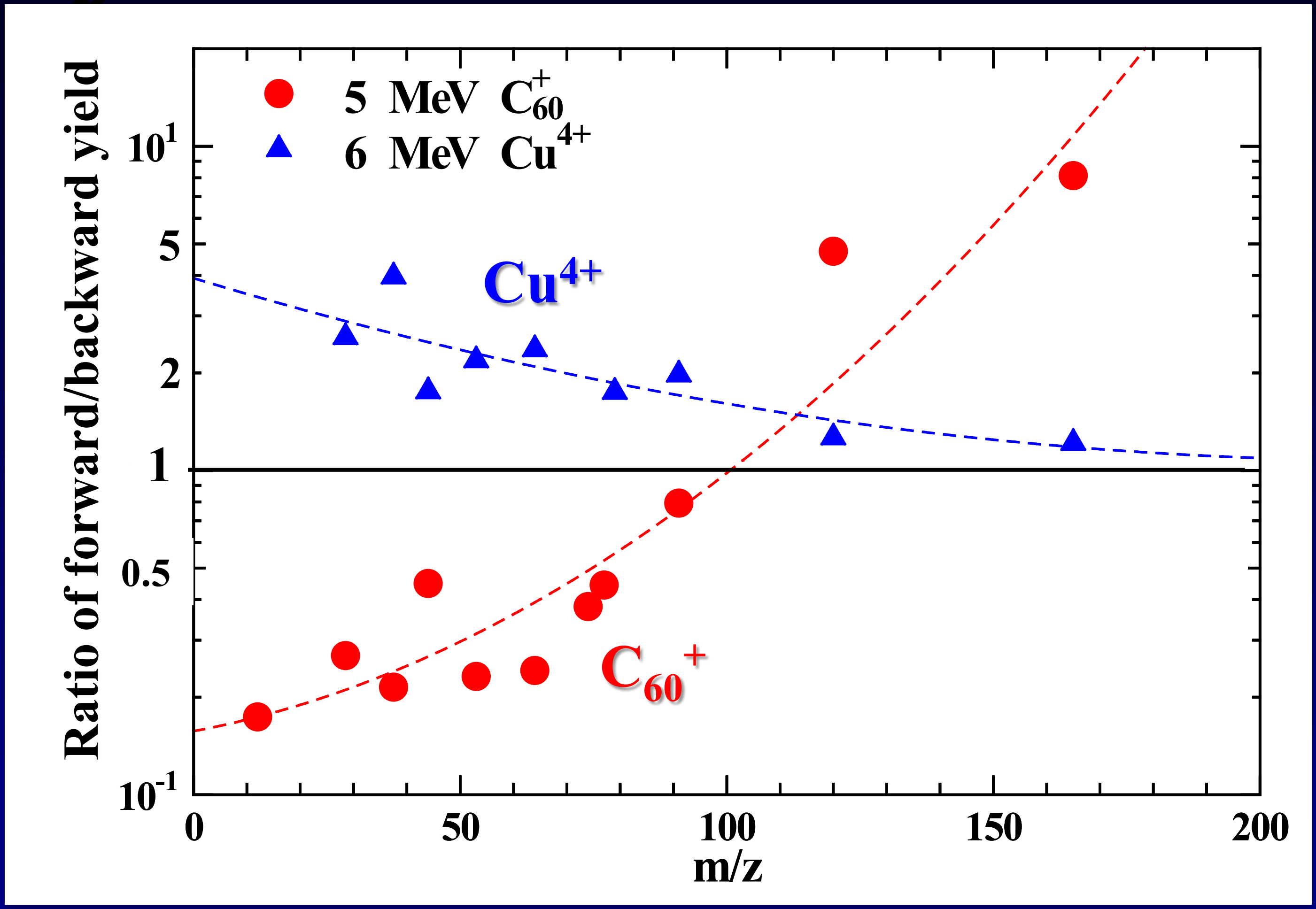


Forward yield: 3.8 × 10^{-2} ions/incident

8 times larger than the backward emission

- Yield of intact molecular ion is enhanced by a factor of 8 in the forward direction
- Yields of fragment ions are suppressed

Ratio of forward/backward yield: 5 MeV C_{60}^{+} *vs* Cu^{4+}

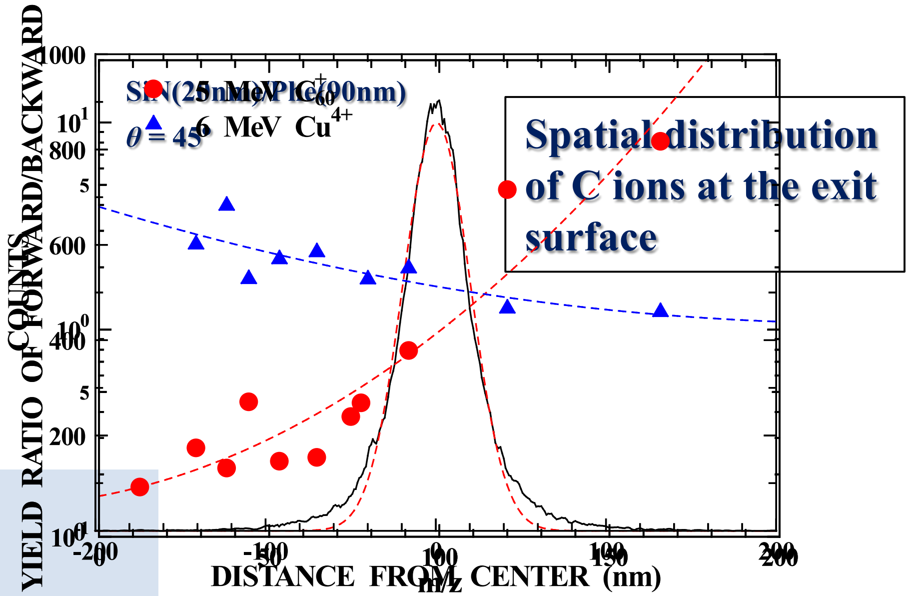


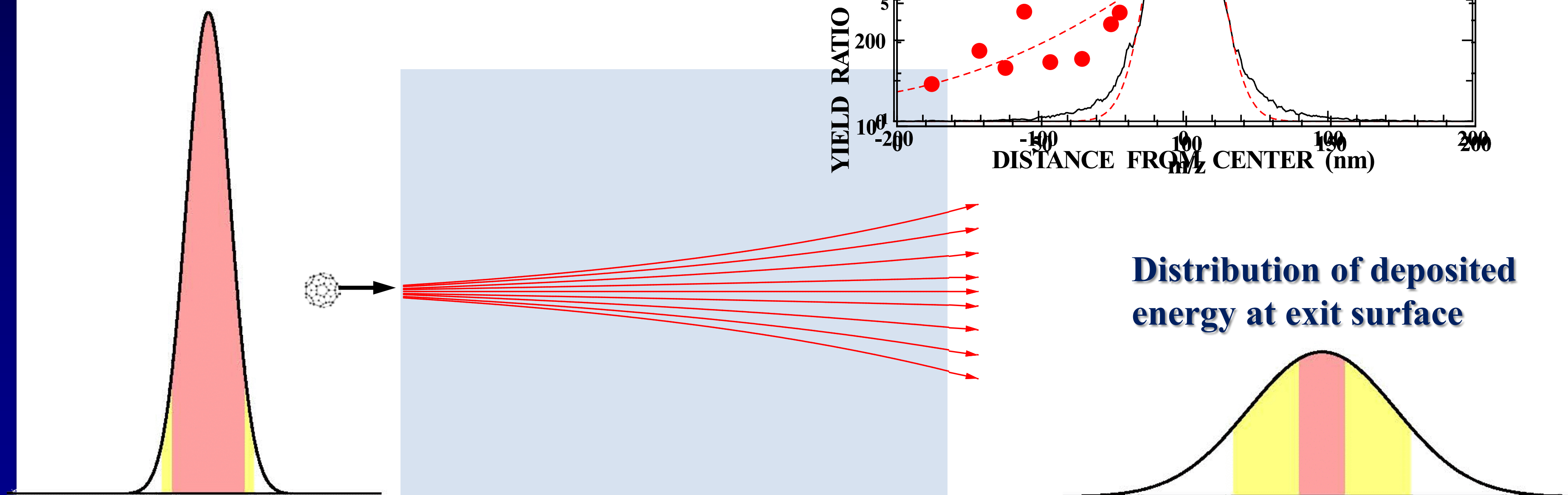
In the case of C_{60} ion bombardment, the ratio of forward/ backward yield decreases with decreasing mass number contrary to the monoatomic ion bombardment.

Passage of C_{60} ion in the sample

Due to multiple scattering, the spatial distribution of constituent C ions becomes broader along the ion path.

Width becomes several tens nm at the exit surface

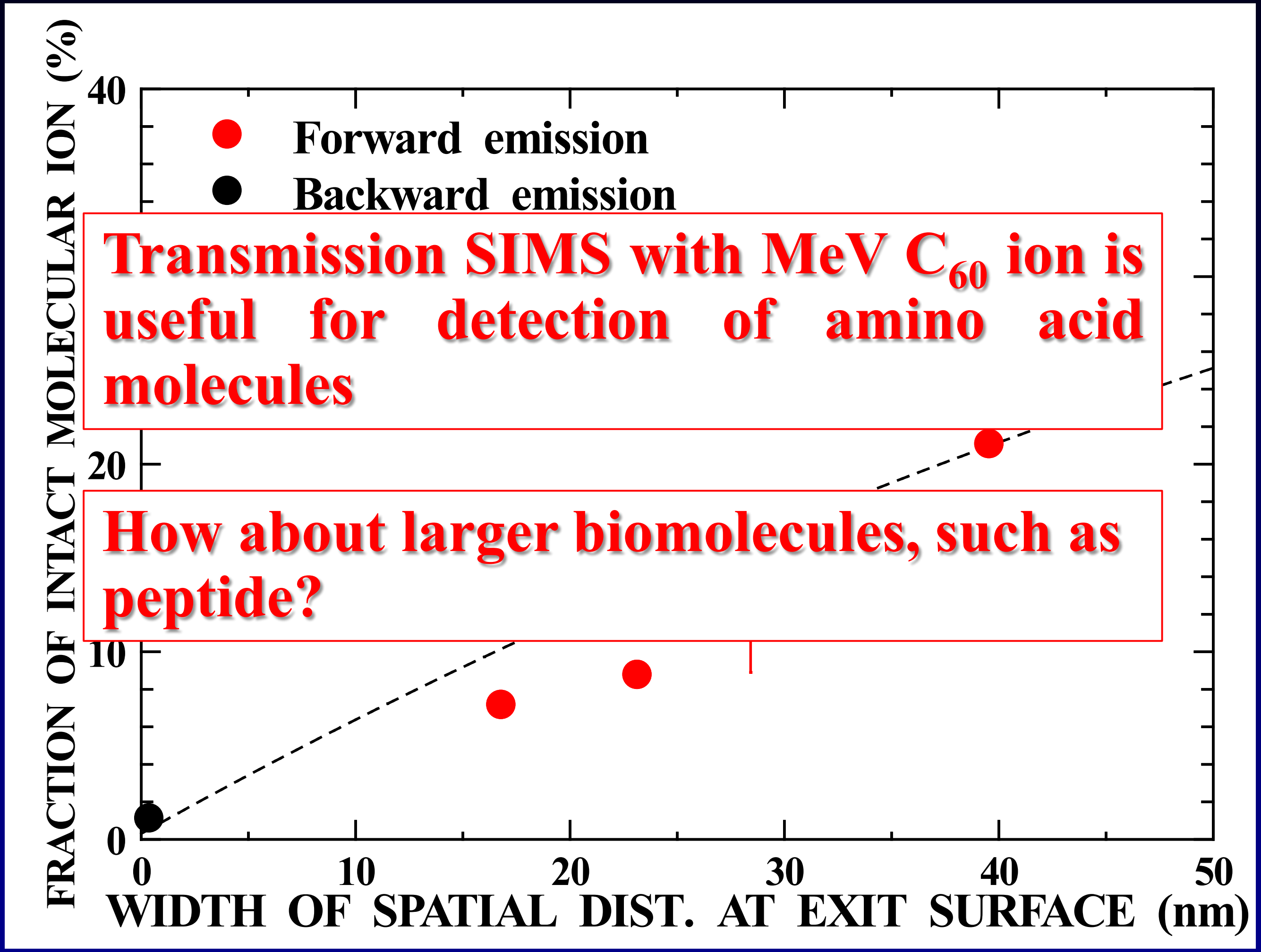




For thicker samples, the distribution is wider and so larger enhancement of intact molecular ion and larger suppression of fragment ions are expected.

→ Measurements with samples of various thicknesses were performed.

Fraction of intact molecular ion vs distribution width



Transmission SIMS with MeV C_{60} ion is useful for detection of amino acid molecules

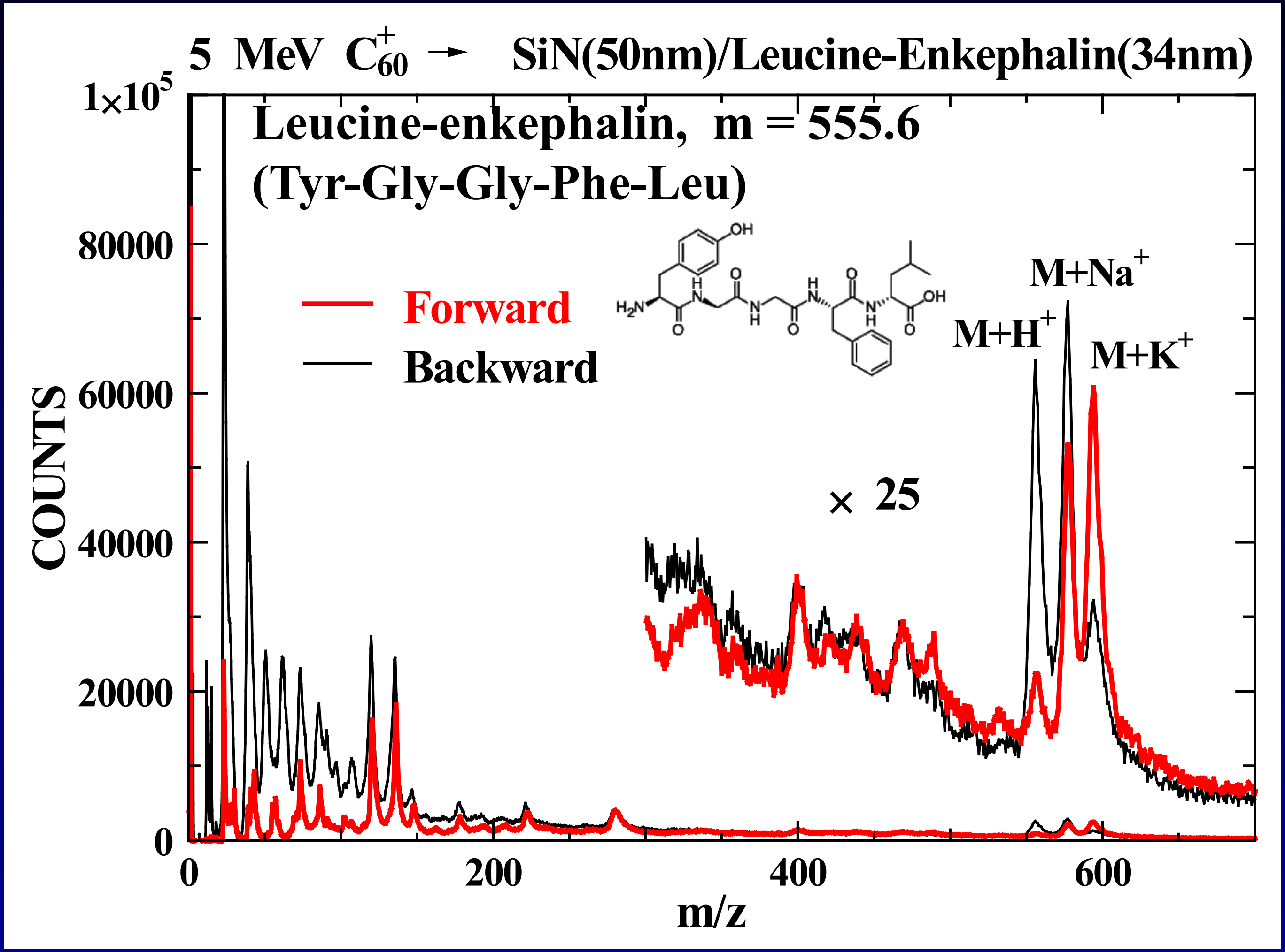
How about larger biomolecules, such as peptide?

Yield of intact ion increases with increasing width as is expected

Maximum yield of intact molecular ion obtained so far is ~ 0.2 ions/incident C_{60} ion

Can be improved by choosing optimal conditions

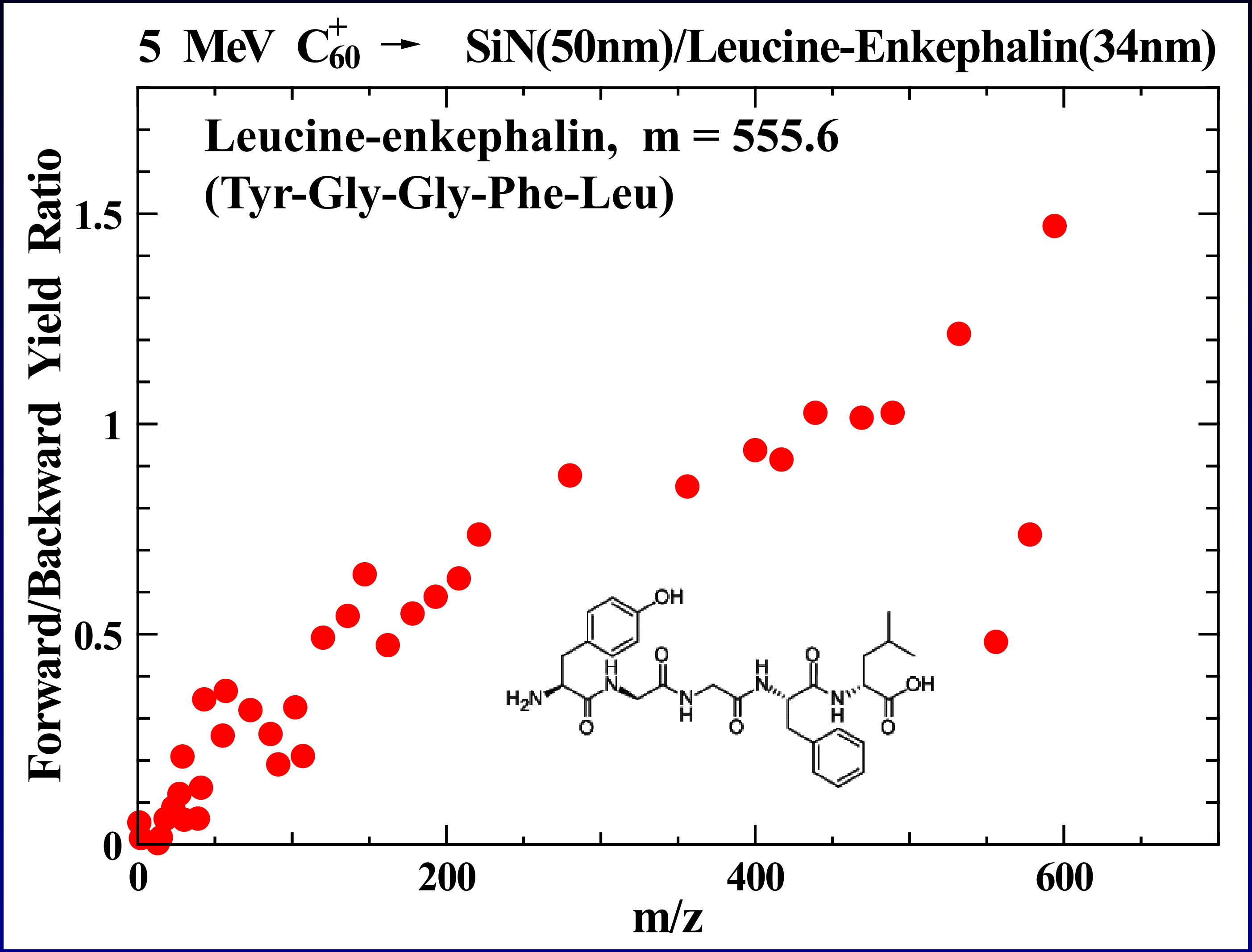
Result of peptide: prepared by drip-dry of water solution



Intact peptide molecules are observed.

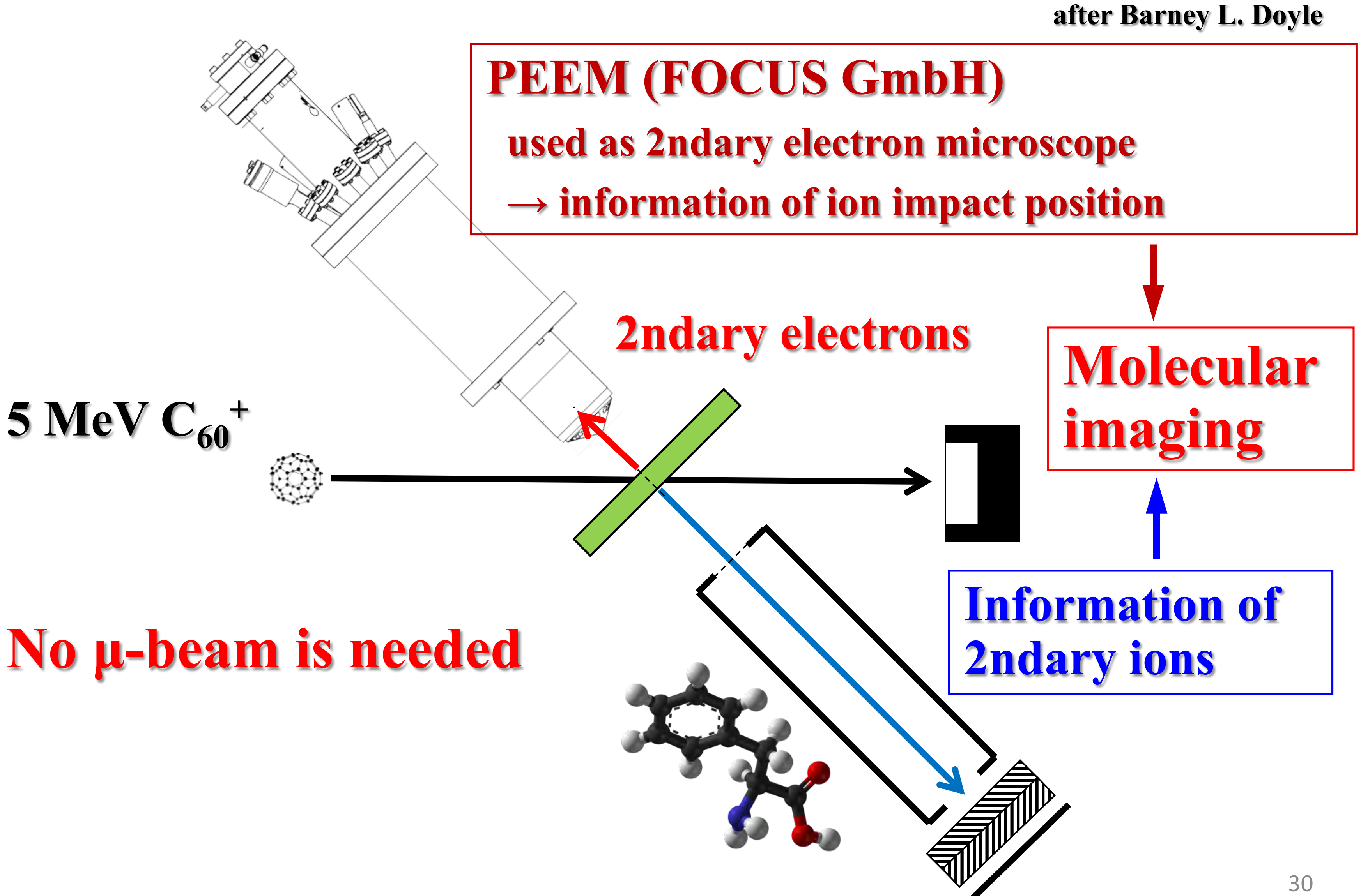
The yield of intact molecular ion is ~ 0.1 ions/incident ion

Ratio of forward/backward yield: 5 MeV C_{60}^{+} *vs* Cu^{4+}

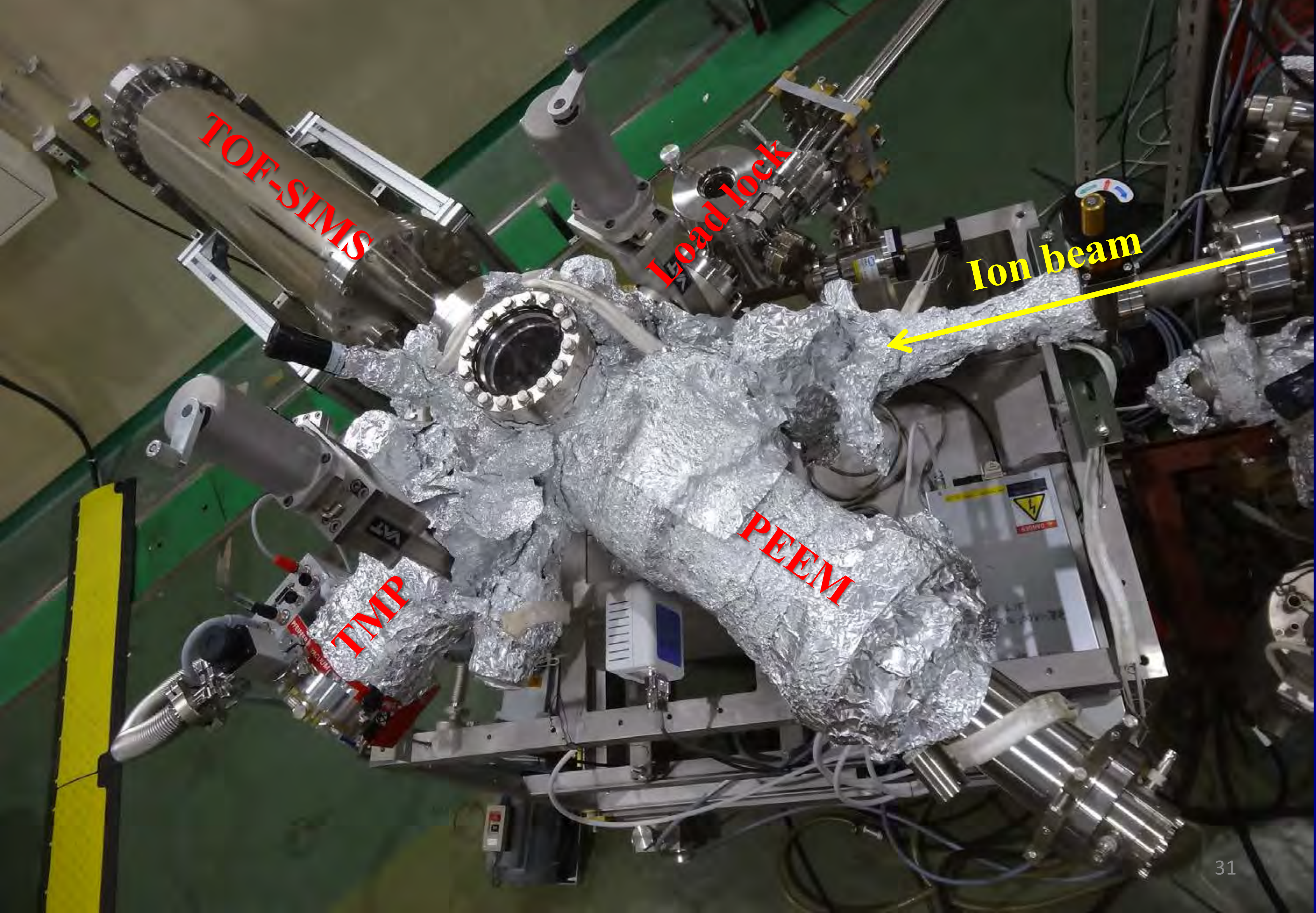


Fragmentation is largely suppressed in the forward direction

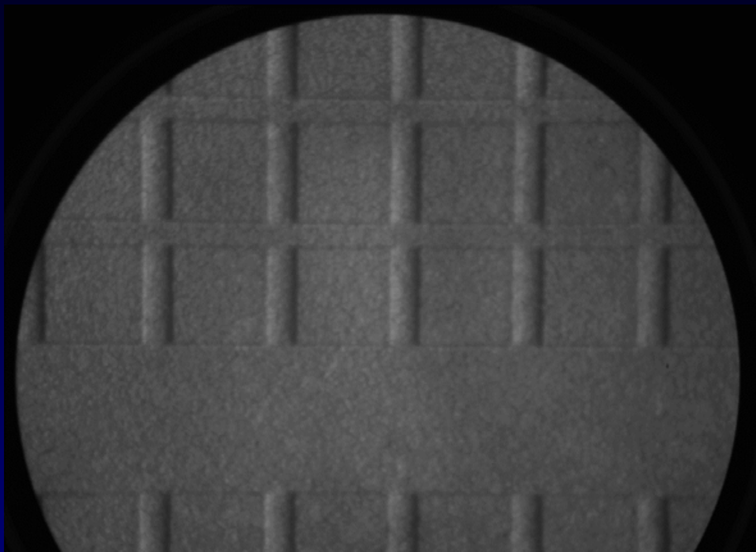
Molecular imaging using transmission SIMS

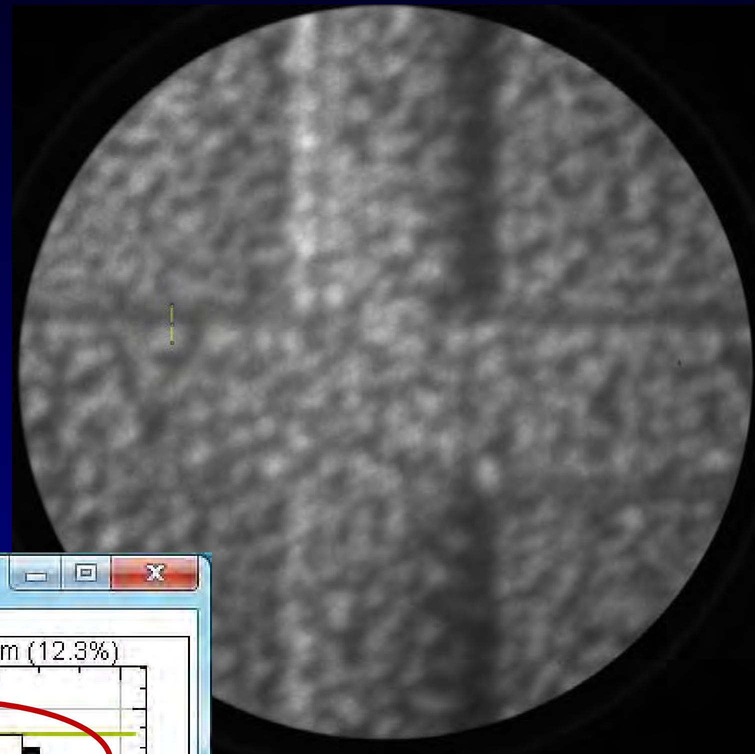


Just installed in the beam line last week



PEEM image: test sample





In principle, molecular imaging can be performed with a resolution of 74 nm

Summary

- Transmission SIMS measurements were performed using 6 MeV Cu^{4+} and 5 MeV C_{60}^{+} ions.
- Under the bombardment of 6 MeV Cu^{4+} ions, secondary ion yields of both intact molecules and fragment ions are enhanced in the forward direction.
- Under the bombardment of 5 MeV C_{60}^{+} ions, secondary ion yield of the intact molecules is enhanced in the forward direction while the yield of fragment ions is reduced.
- The observed yield enhancement and reduction can be qualitatively understood by a simple model of secondary ion emission.
- The maximum yield of intact phenylalanine ion is ~ 0.2 ions/incident ion so far and may be improved by choosing optimal conditions.