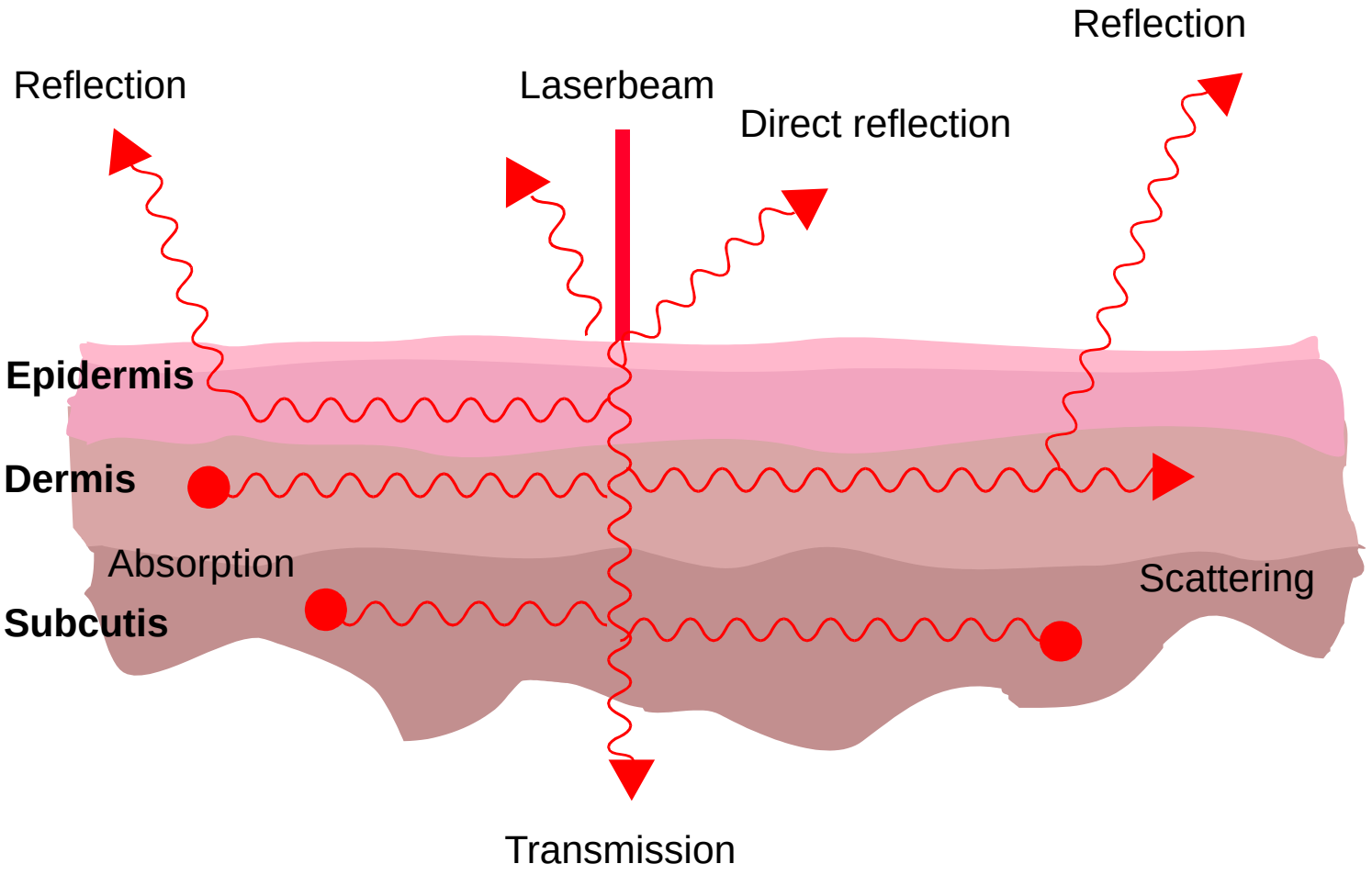
# Principals of MALDI

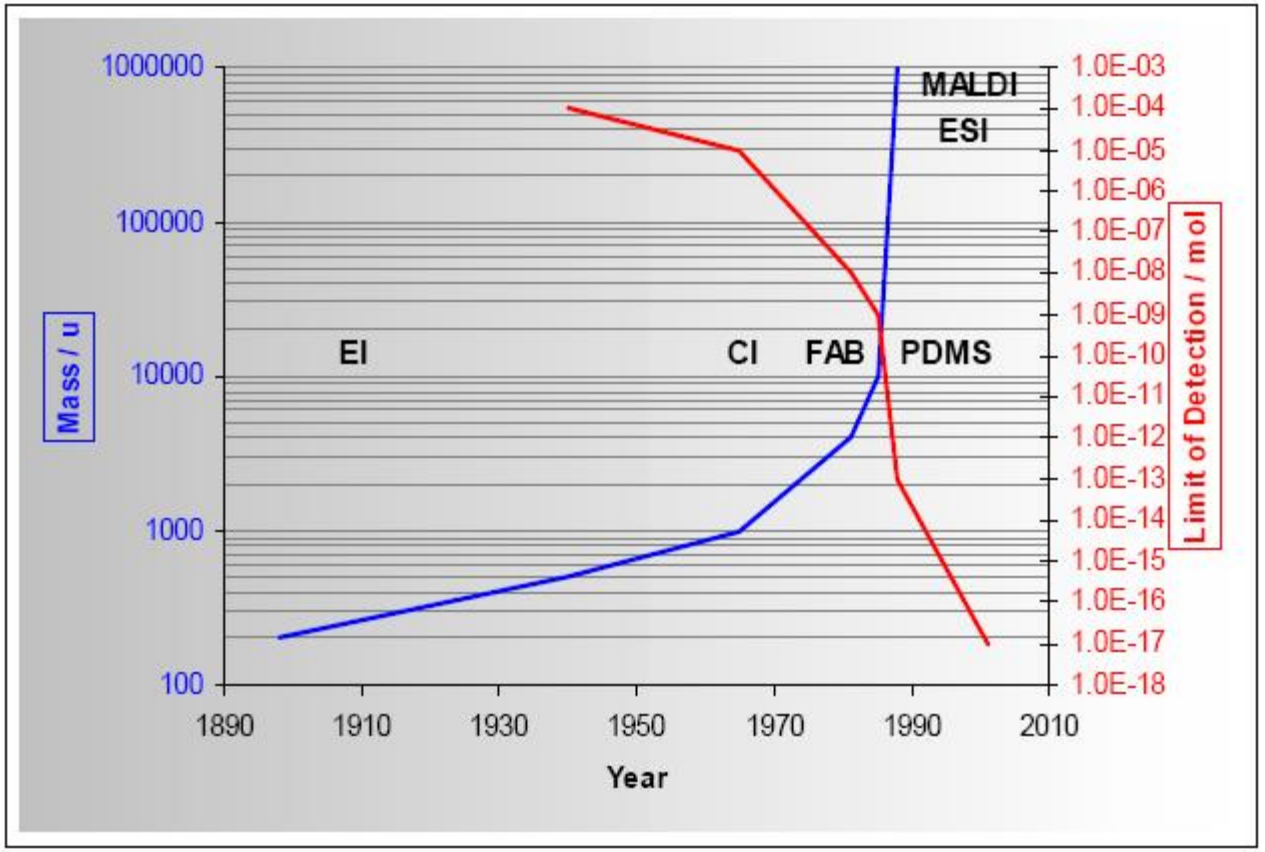
# Laser radiation in tissue




General illustration of laser radiation - tissue interaction.

# Laser Application

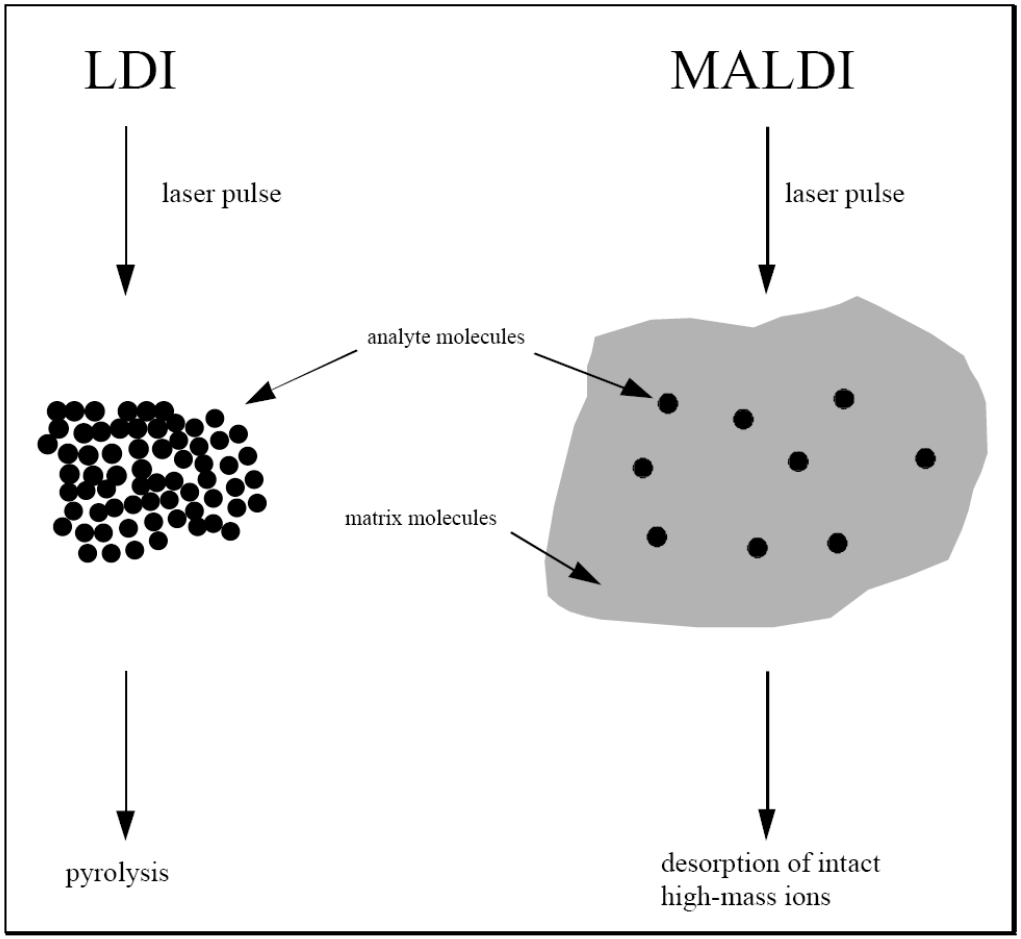
- Laser ablation
- Laser light scattering
- IR, FI, Raman, NIR …. Laser source
- Laser desorption
- ……



Although the non-absorbing amino acid alanine as a neat sample was not accessible by LDI, it gave clear signals in the mass spectrum when mixed with the highly absorbing amino acid tryptophan. Tryptophan was acting here as a matrix for the analyte alanine by taking up the energy of the laser pulse and providing for desorption and ionization of both components.

This observation led to the development of the technique of matrix-assisted LDI (MALDI) by using a standard highly-absorbing material in a considerable excess to the analyte under investigation.

# Principle of Matrix-assisted Laser Desorption Ionization (MALDI).




1. As laser energy absorbent and for energy transport.

2. The matrix also separates the analyte molecules from each other, preventing cluster formation and

3. Avoiding direct laser “hits” on the analyte that would lead to intense fragmentation.

MALDI MS process:

1. Sample preparation,
2. Excitation of sample and disintegration of the condensed phase:

a. The role of the different relevant irradiation (laser wavelength, pulse duration and fluence (laser energy per pulse and unit area)),
b. The matrix and the preparation protocol (crystallization and the matter of the incorporation of analyte molecules into matrix crystals for the material side).
c. The dynamical parameters of the expanding MALDI particle; the initial kinetic energies and energy distributions of molecules and ions, and the composition of the plume (ion-to-neutral ratio and ejection of particles and
d. Clusters versus the emission of molecular constituents.

3. Generation and separation of charges and ionization of analyte molecules,
4. Extraction, separation according to the mass to-charge ratio of the ions in the mass spectrometer
5. and Detection.

A useful matrix is believed to provide a number of different essential functions:

1. The first is to isolate analyte molecules by dilution within the preparation, to prevent analyte aggregation.
2. Second, the matrix has to absorb the laser energy via electronic [ultraviolet (UV-) MALDI] or vibrational [infrared (IR-) MALDI] excitation.
3. Third, disintegration of the condensed phase has to take place without excessive destructive heating of the embedded analyte molecules.
4. Last, but not least, an efficient ionization of analyte molecules has to be provided.
5. Other more technical aspects are a sufficient solubility in a suitable (analyte-compatible) solvent and a sufficient vacuum stability.

Two closely related techniques have, moreover, been developed.

1. The first is based on the use of suspensions of highly absorbing inorganic "nanoparticles", e.g., cobalt, silicon, or titanium-nitride with diameters in the low nm to low µm range, in a suitable liquid (typically glycerol).

2. In comparison to common MALDI with chemical matrixes, laser desorption from particle suspension matrixes is less soft, however, and accompanied by a higher degree of analyte fragmentation. The mass range in which suspension matrixes can be reasonably employed is, therefore, limited to about 10 kDa for peptides/proteins. The limitations result essentially from the different ways compared to MALDI with chemical matrixes-of energy deposition and phase transition, mediated by a heated surface. This is believed to result in a too destructive heating of the analyte molecules and an insufficient desorption rate. "Two-phase matrix-","graphite-assisted-", and "surface-assisted" laser desorption ionization (SALDI) have been introduced as alternative notations for this approach.

Another modification of “surface-MALDI” is the preparation of neat analyte samples on highly porous silicon surfaces. This desorption ionization on silicon (DIOS) technique was introduced by Wei et al. in 1999 and has been shown meaningful for molecules in the low (matrix) mass range.

The upper mass limit of DIOS is in the low kilodalton range. A so far not satisfactorily resolved problem of the technique is a poor reproducibility from chip to chip. Because of the different mechanisms and their minor analytical relevance, compared to conventional MALDI with chemical matrixes, the surface-MALDI approaches will not be further addressed.

LASER

In by far the most cases, lasers with wavelengths in the near-ultraviolet are today employed for MALDI. Most commonly, either

1.N2 lasers, emitting at 337 nm, or

2. Frequency-tripled Nd:YAG lasers with a wavelength of 355 nm (employing four discrete laser wavelengths of 266, 308, 337, and 355 nm).

Pulse durations of these lasers are typically in the range of 0.5 to ∼10 ns. Whereas matrix compounds have to exhibit a sufficiently high absorption at the laser wavelength, most analyte compounds will be nonabsorbing in the near-UV. Therefore, direct photoexcitation of labile analyte molecules is prevented.

A second advantage of the use of the near UV-laser wavelengths is that they induce a relatively low degree of photochemical products such as matrix-analyte adduct formation. These processes have been found to be considerably more prominent if lasers with shorter wavelengths, e.g., 308, 266, and 248 nm (excimer and frequency quadrupled Nd:YAG lasers), are employed.

Pulsed infrared (IR) lasers, energy deposition is achieved via absorption by vibrational modes of the matrix molecules. Typically, either O-H or N-H stretch vibrations of the molecules are excited at wavelengths around 3 µm.

Pulsed erbium solid-state lasers (Er:YAG, ì ) 2.94 µm, or Er:YSGG, λ=2.79 µm) with pulse durations between 50 and 100 ns or wavelength tunable optical parametric oscillator (OPO) laser systems with pulse durations of 5-10 ns are commonly used for IR-MALDI.

The applicability of pulsed CO2 lasers with emission wavelengths around 10 µm. has moreover been demonstrated. In this case, O-H bending and C-H stretch vibrations of the molecules are excited.

A free-electron laser (FEL) with a picosecond pulse substructure has, finally, been employed.

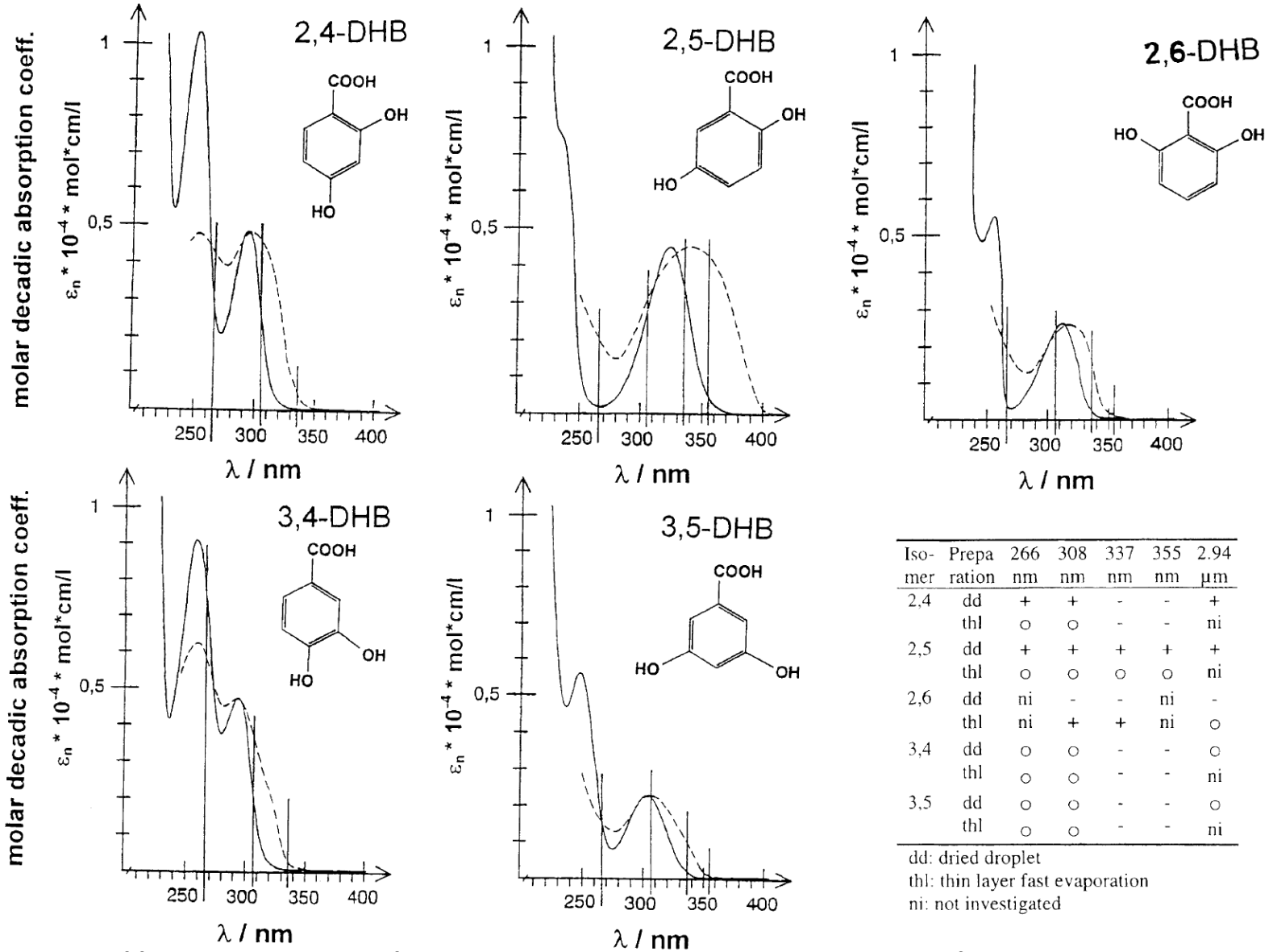
# A. Laser Wavelength and Matrix Absorption

**Table 1. Definition of the Relevant MALDI Irradiation (laser) Parameters, Range of Values Meaningful for UV-MALDI, and Typical Values for Common (axial-TOF) MALDI Conditions at a Laser Spot Size of ~100 μm in Diameter**

| parameter | range | typical values for common matrixes and a laser spot diameter of ~100 μm |
|---|---|---|
| laser wavelength, $\lambda$ | 193−430 nm | (266), 337, 355 nm |
| laser penetration depth into the sample, $\delta$ (= $\alpha^{-1}$; $\alpha$: absorption coefficient) | ~ 50−300 nm | 50−200 nm |
| photon energy at laser wavelength, $E_v$ | 2.9 eV (430 nm) − 6.5 eV (193 nm) | 4.71 eV (266 nm), 3.72 eV (337 nm), 3.53 eV (355 nm) |
| laser pulse duration, $\tau$ | 0.5 − ~20 ns, (560 fs [a]) | 0.5−10 ns |
| diameter of laser spot on sample surface | 1 μm − ~1 mm | 50−200 μm |
| energy per single laser pulse as applied to the sample, E | 10 nJ − 10 μJ [b] | 1−10 μJ |
| mean power of a single laser pulse, averaged over the pulse duration ($1/e^2$-definition)[c], $P_{avg}$ | 1−5 × $10^3$ W [b] | $10^2$ W − 5 × $10^3$ W |
| fluence (energy per laser pulse and unit area), $H$ [d] | 30 − ~10,000 $Jm^{-2}$ [b] | 30−600 $Jm^{-2}$ |
| irradiance (fluence divided by the laser pulse duration), $I$ | 3 × $10^5$ − 1 × $10^9$ $Wcm^{-2}$ [b] | 1 × $10^6$ − 5 × $10^7$ $Wcm^{-2}$ |

[a] Ref 53. [b] Values strongly depend on the focal laser spot size. [c] For near Gaussian pulse shape the peak power is about two times the average power. [d] Often denoted as F or Φ, instead.

Klaus Dreisewerd, Chem. Rev. 2003, 103, 395-425.




| Isomer | Preparation | 266 nm | 308 nm | 337 nm | 355 nm | 2.94 µm |
|---|---|---|---|---|---|---|
| 2,4 | dd | + | + | - | - | + |
| | thl | o | o | - | - | ni |
| 2,5 | dd | + | + | + | + | + |
| | thl | o | o | o | o | ni |
| 2,6 | dd | ni | - | - | ni | - |
| | thl | ni | + | + | ni | o |
| 3,4 | dd | o | o | - | - | o |
| | thl | o | o | - | - | ni |
| 3,5 | dd | o | o | - | - | o |
| | thl | o | o | - | - | ni |

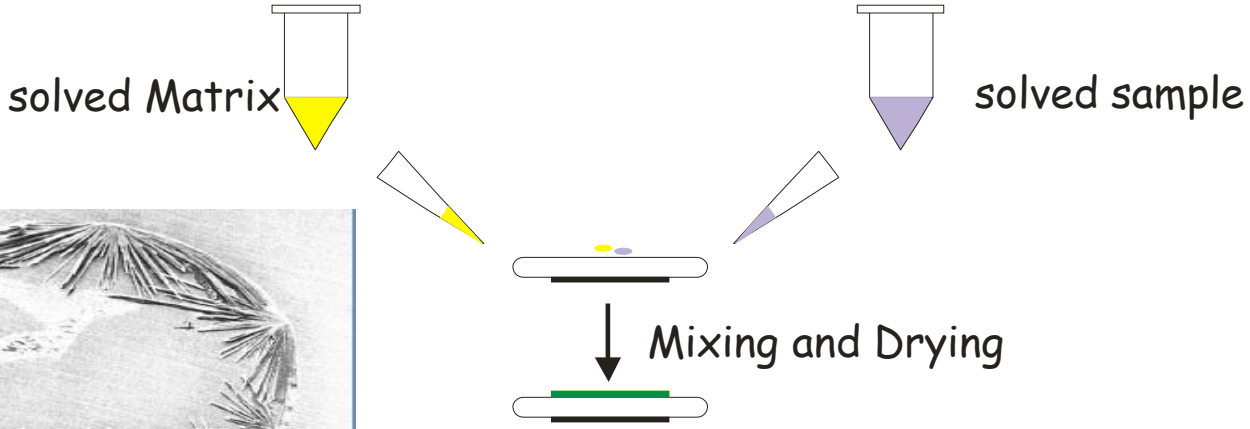
dd: dried droplet
thl: thin layer fast evaporation
ni: not investigated

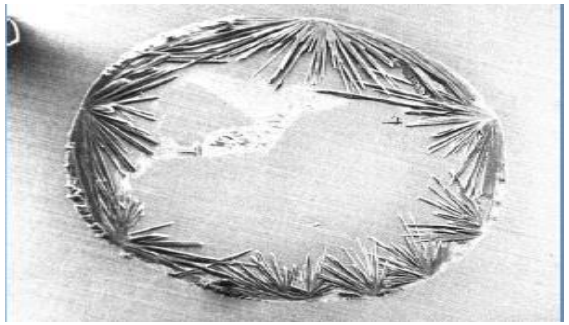
UV-absorption spectra of five positional isomers of dihydroxybenzoic acid. Solid lines: spectra obtained from a water/ethanol solution (9:1, v/v); dashed lines For the diffuse reflection spectra log(1/R) is plotted (R: reflectivity), normalized to the value of the absorption coefficient in solution at the long wavelength maximum.

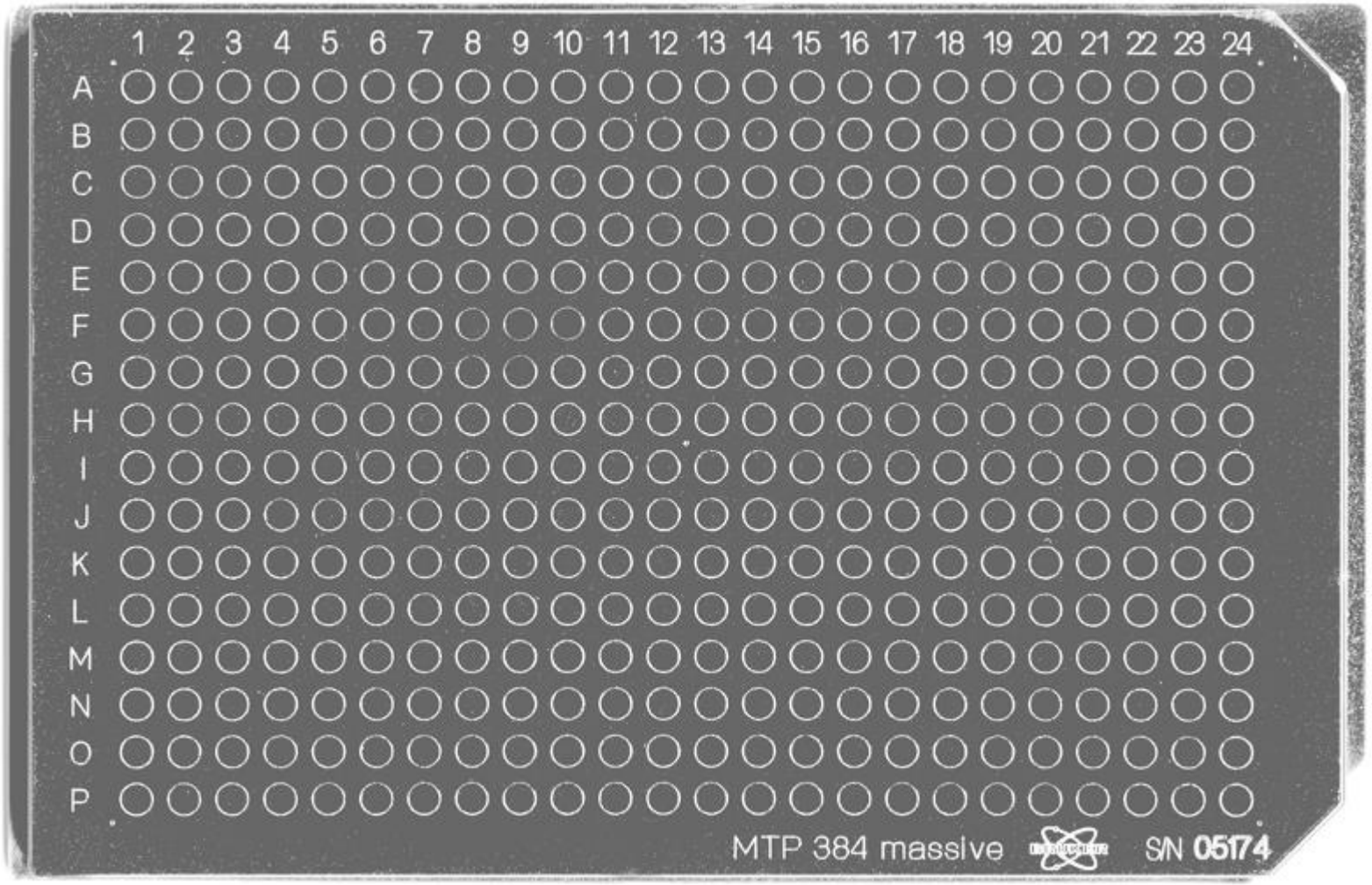
The table summarizes the mass spectrometric quality of cytochrome C ion signal detection when desorbed from the different DHB positional isomer matrixes and at the four UV-wavelengths of 266, 308, 337, and 355 nm. The right column shows data for IR-MALDI, acquired with a 2.94 ím-Er:YAG laser, for comparison. +: good protein signals; O: weak protein signals; -: no protein signals.

# Analyte Incorporation

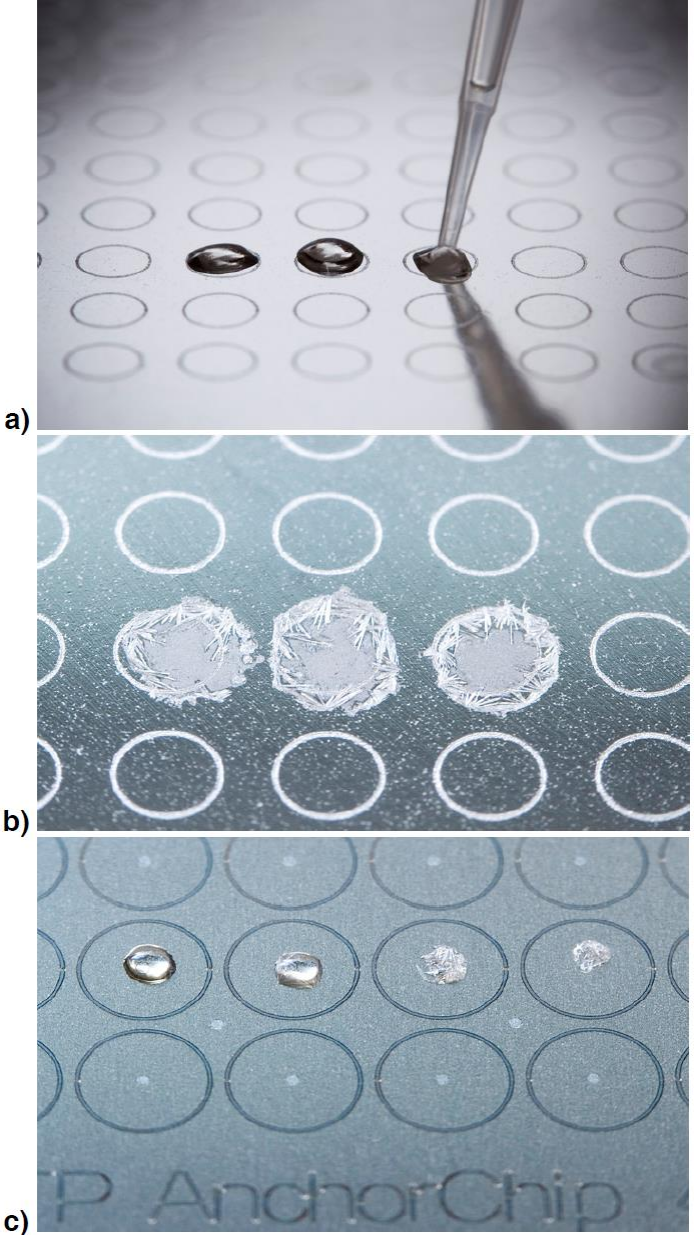
In a typical UV-MALDI sample preparation small volumes of an about $10^{-6}$ M solution of the analyte and a near-saturated (ca. 0.1 M) solution of the matrix are mixed; the solvent is then evaporated before the sample can be introduced into the vacuum of the mass spectrometer.





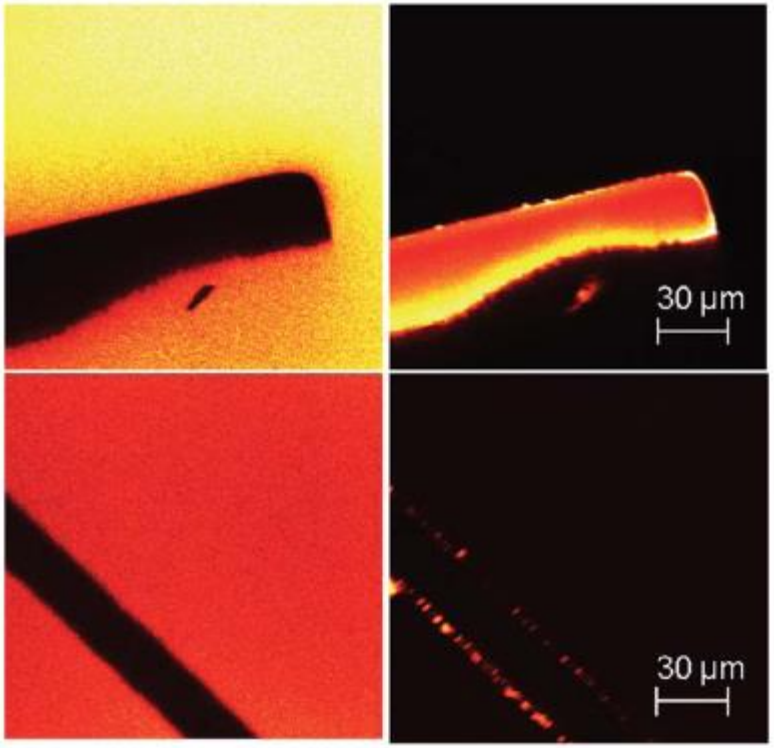

Typical MALDI target. The Bruker Scout384™ target offers a 16 × 24 spot array with 384 positions for sample preparation. Here, a standard nickel-coated massive aluminum version is shown; its dimensions are 84 × 128 mm with marks of 3 mm in diameter.




Sample preparation for MALDI. (a) Pipetting of 1 µl of sample–matrix solution onto a standard MALDI target; (b) same spots after DHB has crystallized show large crystals on the rim and evenly distributed small crystals in the center; (c) cummulative effect of hydrophilic spots (bright areas in circles) present on a hydrophobic surface of an Anchor-Chip™ target on crystallizing DHB matrix.

The mechanisms and driving force for this incorporation are still largely unknown.

Horneffer et al. have shown in a systematic study of different position isomers of dihydroxybenzoic acids that only 2,5-DHB incorporates homogenously and quantitatively, whereas other isomers such as 2,6-DHB do not incorporate at all, while some others incorporate only randomly




Confocal laser scan fluorescence images of single crystals of 2,5- dihydroxybenzoic acid (top panels) and 2,6-dihydroxybenzoic acid (bottom panels). Both matrices were doped with the protein avidin, labeled with a Texas red (TR) fluorescent dye. The images were recorded at an x,y-plane 12µm into the crystals. The left panels show dark shadowgraphs of the shape of the crystals against the bright green Bodipy 493/503 nm fluorescence of the immersion liquid. The right panels show the red TR fluorescence of the labeled protein.

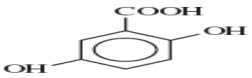
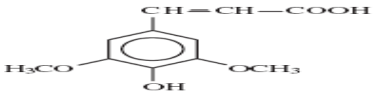
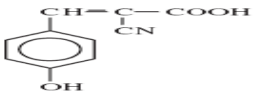
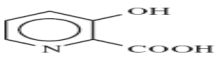
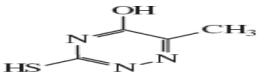
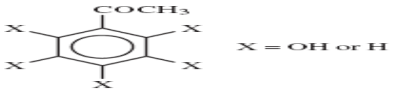
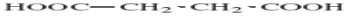
# 1. Absorption

$$H = H_o e^{-\alpha z}$$

H: Laser fluence at depth z into the sample

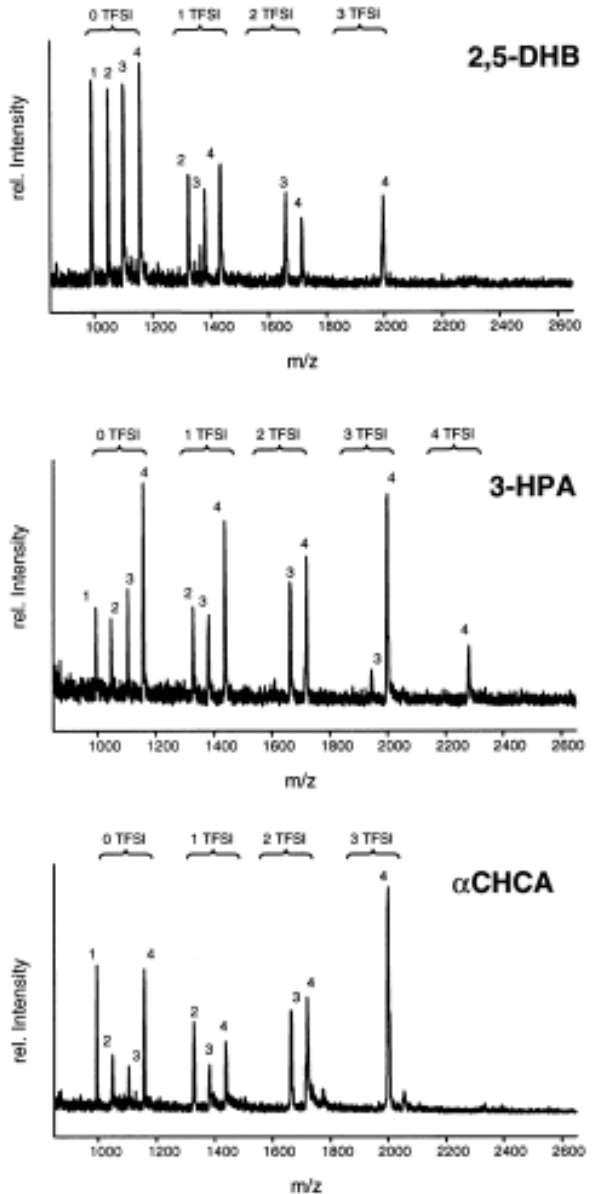
$H_o$: Laser fluence at the sample surface

$\alpha$: The absorption coefficient

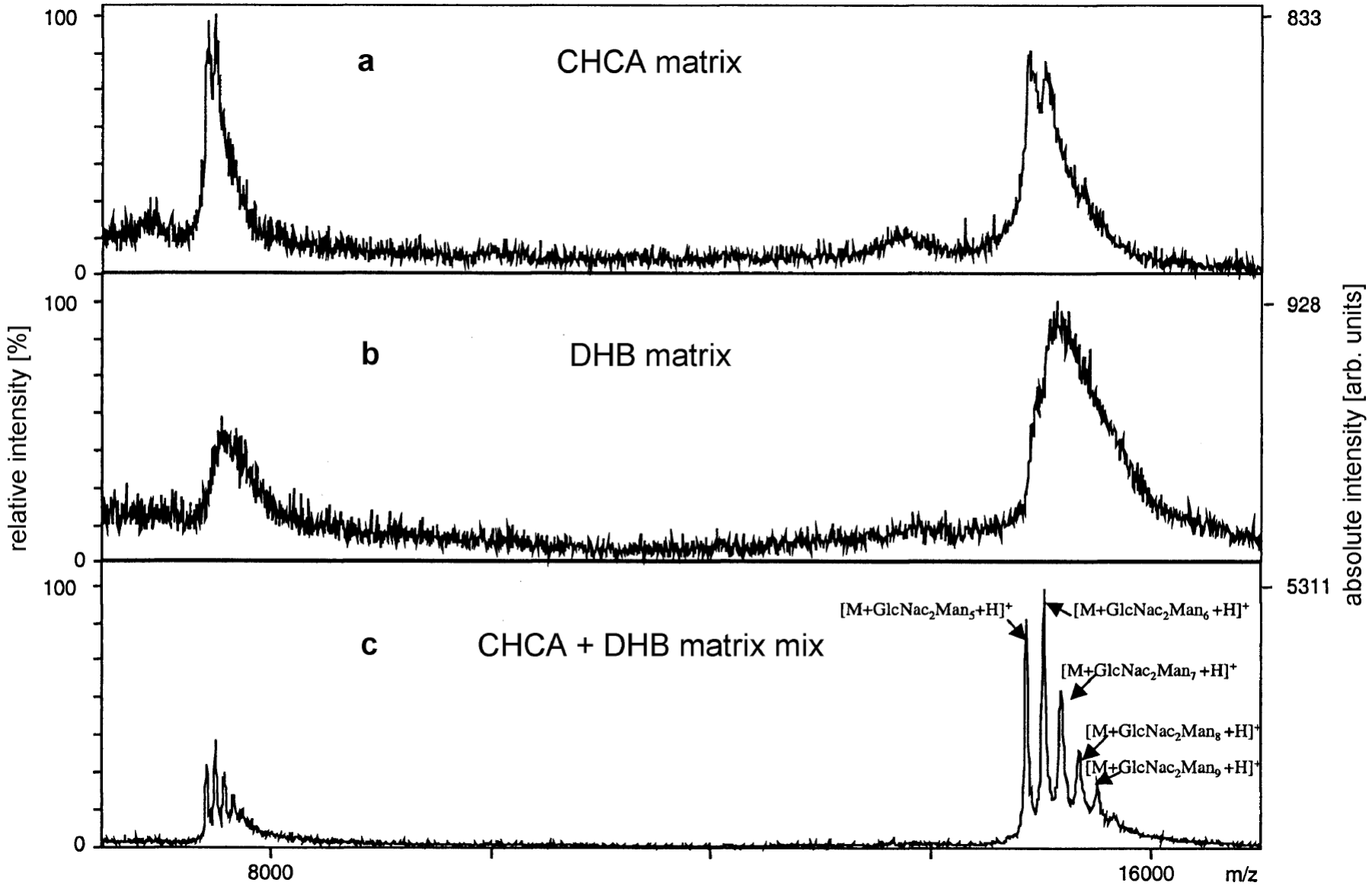
| *Matrix* | *Structure* | *Wavelength* | *Major applications* |
|---|---|---|---|
| Nicotinic acid |  | UV<br>266 nm | Proteins, peptides, adduct formation |
| 2,5-Dihydroxybenzoic acid (plus 10% 2-hydroxy-5-methoxybenzoic acid) |  | UV<br>337 nm, 353 nm | Proteins, peptides, carbohydrates, synthetic polymers |
| Sinapinic acid |  | UV<br>337 nm, 353 nm | Proteins, peptides |
| α-Cyano-4-hydroxycinnamic acid |  | UV<br>337 nm, 353 nm | Peptides, fragmentation |
| 3-Hydroxy-picolinic acid |  | UV<br>337 nm, 353 nm | Best for nucleic acids |
| 6-Aza-2-thiothymine |  | UV<br>337 nm, 353 nm | Proteins, peptides, non-covalent complexes; near-neutral pH |
| k,m,n-Di(tri)hydroxy-acetophenone |  | UV<br>337 nm, 353 nm | Protein, peptides, non-covalent complexes; near-neutral pH |
| Succinic acid |  | IR<br>2.94 µm, 2.79 µm | Proteins, peptides |
| Glycerol |  | IR<br>2.94 µm, 2.79 µm | Proteins, peptides, liquid matrix |

IR = infrared; UV = ultraviolet.

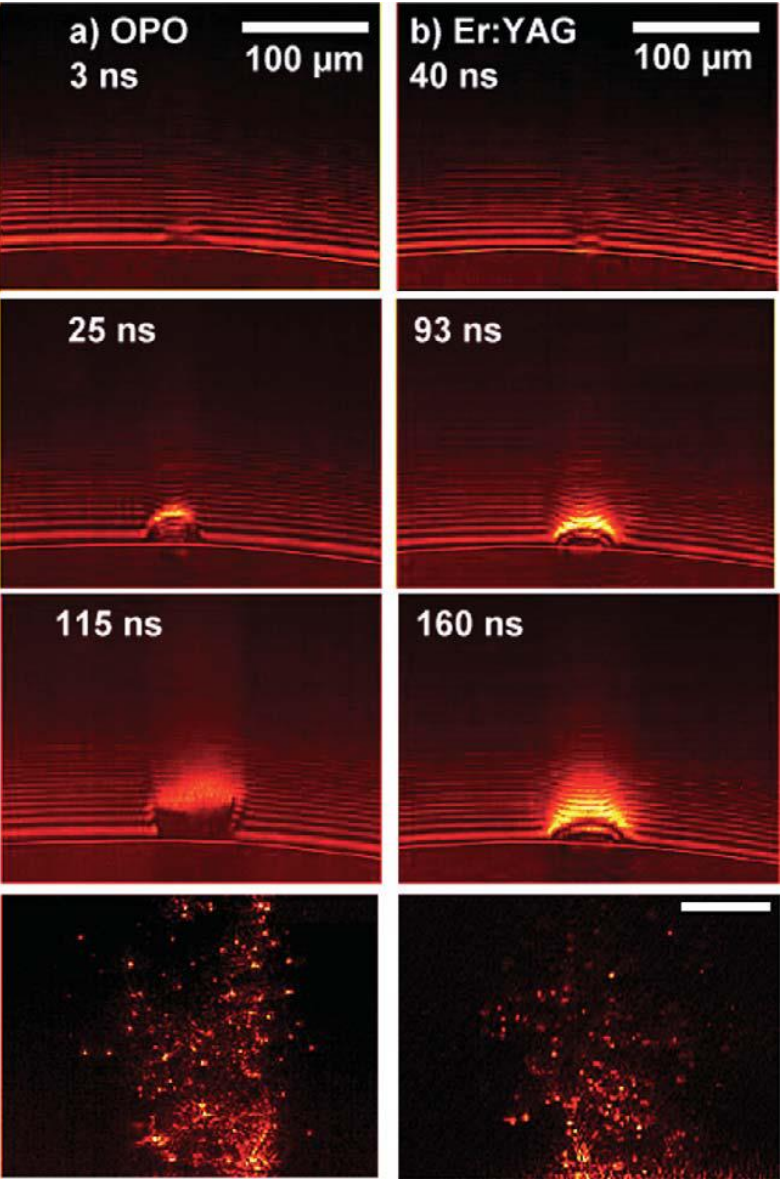
Positive mass spectra of a peptide mixture obtained with different matrixes and addition of HTFSI (bis(trifluoromethylsulfonyl)imide). (2,5-DHB ) 2,5-dihydroxybenzoic acid, 3-HPA ) 3-hydroxypicolinic acid, RCHCA) hydroxy-R-cyanocinnamic acid.




Chem. Rev. 2003, 103, 427−439




Linear mode positive-ion MALDI-TOF spectra of ribonuclease B in 80 mM urea. a) 300 fmol in CHCA, (b) 600 fmol in DHB, and (c) 300 fmol in CHCA/DHB matrix mix.




High-speed time-lapse photographs of IR-MALDI plumes generated with an optical parametric oscillator (OPO) laser with 6-ns pulse width and an Er:Yag-laser with 100-ns pulse width. Both lasers were operated at 2.94 µm wavelength. Matrix: glycerol; time resolution 8 ns; spatial resolution 4 µm.

In *IR-MALDI* (ER:YAG-laser), the absorbing volume is superheated to a temperature that is substantially **above** the *boiling temperature*, followed by a volume *ejection* of material through boiling by heterogeneous nucleation.

The *longer time* course of material ejection in the IR as compared to the UV is caused by a *deeper penetration* of the radiation into the sample.

The *ablated* mass per laser exposure in IR-MALDI exceeds that of UV-MALDI by at least a factor of 10, and the sample *consumption rate* is accordingly higher.

# 2. Ionization

**Two models:**

1.The older model assumes neutral analyte molecules in the matrix crystals and a photoionization of the matrix molecules as the initial step, followed by charge transfer to the analyte molecules in the plume (M:matrix).

(1) $M^{**} \rightarrow M^{\bullet +} + e^-$

(2) $M^{\bullet +} + M \rightarrow [M + H]^+ + [M - H]^\bullet$

2. The more recent “lucky survivor” model assume that protein are incorporated into the matrix as charge species, most of which become reneutralized within desorbed clusters of matrix and analyte.

# Problems of MALDI

1. One critical problem is that the suppression effects between analyte molecules still frequently arise during MALDI analysis, especially in the analysis of the mixtures (for analysis of trace compounds).

2. The presence of sodium and potassium in the sample solution, cation adduct clusters are commonly detected in MALDI-MS spectra; these reduce sensitivity by partitioning the signal intensity arising from one analyte into different adduct cluster peaks.

3. The serious background arising from organic matrix ions also makes characterization of small molecules (<500 Da) difficult.

4. Contaminants like buffers, salts and detergents brought in by the samples are an important factor influencing the results in MALDI MS.

5. A strong variation in intensity and resolution of the signals at *different* ***positions*** of a sample. This so-called hot spot or sweet spot formation leads to poor shot-to-shot and spot-to-spot reproducibility.

6. The mass resolution for a MALDI linear TOF mass spectrometer can reach to ~2000. The mass resolution of a reflectron TOF mass spectrometer can be higher than 10,000. Nevertheless, a reflectron TOF mass spectrometer is difficult to apply to molecules with mass-to-charge ratio (m/z) larger than 20,000.

7. Inconvenience in detecting small biomolecules due to the interference of ions produced by matrix molecules. Direct ionization on silicon (DIOS) and direct ionization on metal (DIOM) have been developed to reduce this concern. In these approaches, biomolecules are placed on surfaces with porous structures or sharp metal needles to achieve ionization without the need of matrices.

# To circumvent these limitations

1. Explore suitable matrices or matrix additives to improve the sensitivity of MALDI detection.

2. The develop proper sample-pretreatment methods to remove sample contaminants and to enrich trace analytes;

3. Using internal standard.

4. However, good results were obtained with the method called surface-activated laser desorption ionization (*SALDI*) which uses graphite as the surface. But the use of porous silicon as a new surface is more promising and has led to the development of a new method called desorption ionization on silicon (*DIOS*). Unlike the other matrix-free laser desorption ionization methods, DIOS allows ion formation from analyte with little or no degradation. DIOS allows the analyte molecules to be retained in porous silica and ionization of the sample under UV laser irradiation without using a organic matrix. DIOS mass spectra do not present interference in the low-mass range, while signals due to the matrix are observed in MALDI. The deposition of the sample in aqueous solution is uniform and the preparation of the sample is simplified. Furthermore, DIOS is equivalent to MALDI in sensitivity, but is more tolerant of the presence of salts or buffers.

C.Pan, et al. Anal Bioanal Chem (2007) 387:193–204

***The major advantages of MALDI-TOF include:***

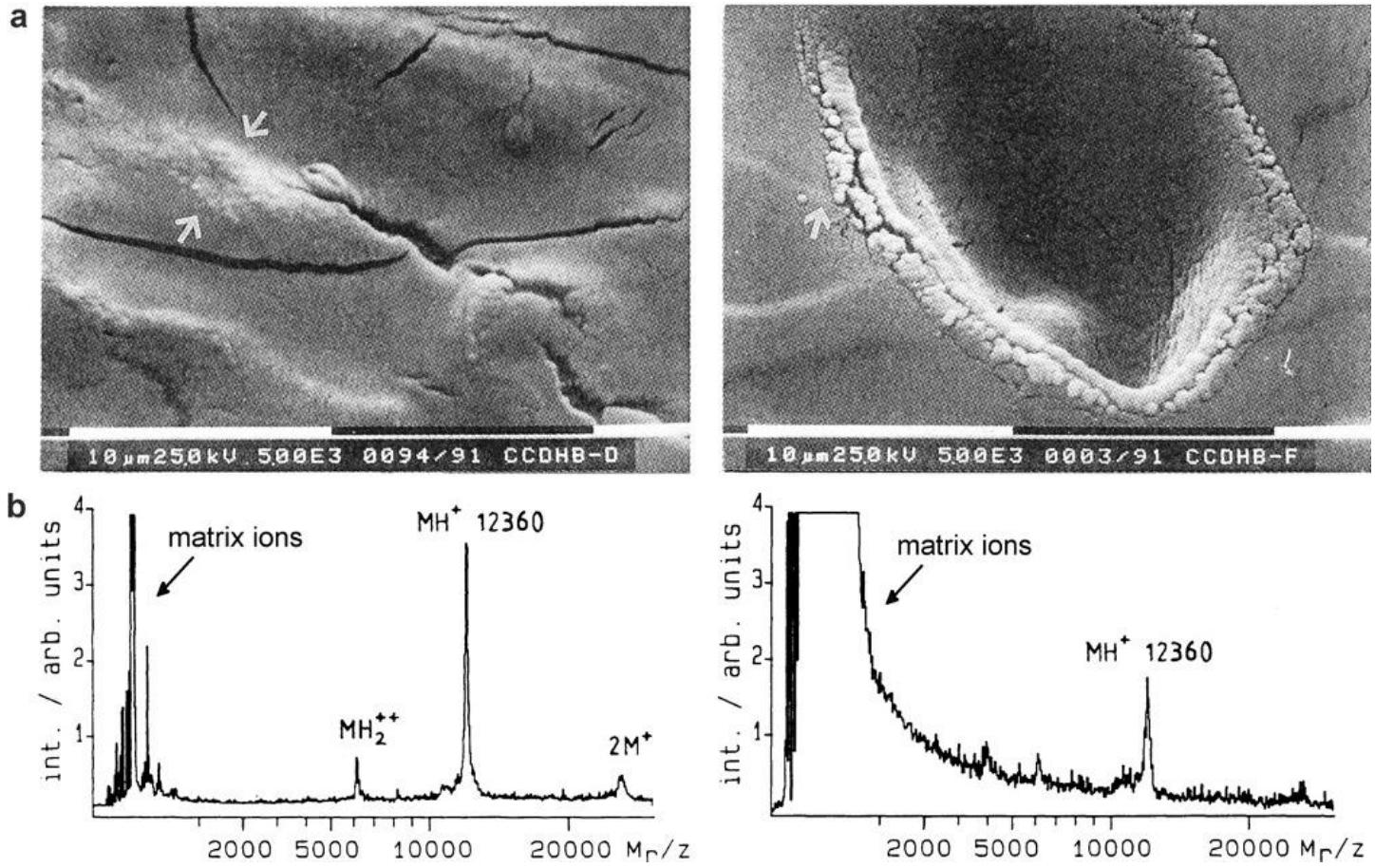
(1) Fast analysis speed: some commercial MALDI-TOF-MS can finish 100 samples in less than 10 min.

(2) No mass range limitation: It can measure from short peptides to a very large antibody (>100,000Da).

(3) Simple mass spectrum for analysis: most ions are with one charge and only a small percentage of ions are doubly charged. Ions with triply charges are seldom observed.

(4) Molecular imaging: since the laser beam can be focused to a tiny spot (~5 μm) depending on the focal lens used and the beam divergence, MALDI-TOF has been successfully used for tissue imaging. It makes the imaging of proteomic feasible.

(5) High detection sensitivity: the detection sensitivity can reach to a few attomoles for short peptides.




Effect of focused and defocused laser beam. (a) SEM micrographs of DHB single crystals after exposure to 10 laser shots (337 nm) under focused (right column) and defocused (left column) irradiation with corresponding sum spectra of horse heart cytochrome C (Mr = 12,360 u); black and white bars correspond to 10 µm. (b) Resulting MALDI spectra.

# The application of *liquid matrices* for improved sample homogeneity and do not exhibit hot spot formation

1.The first group comprises compounds that work analogously to solid matrices and are thus capable of absorbing UV light leading to desorption and ionization of the analytes. Substances like nitrobenzyl alcohol belong to this class.

2. The second group comprises particle-doped matrices. For example, fine metal or graphite particles are suspended in non-volatile solvents not self-absorbing in UV region, but playing an active role in protonation or deprotonation of the analyte. Examples are the systems graphite-glycerol  or the combination of nanomaterials (e.g., nano-TiN) with glycerol.

3. The third class comprises the chemically doped liquids. Representatives of this class are mixtures of classical crystalline matrices, e.g., DHB or CCA, dissolved in glycerol. For application of atmospheric pressure (AP)-MALDI, solvents such as ethanol, acetonitrile water and diethanolamine were applied.

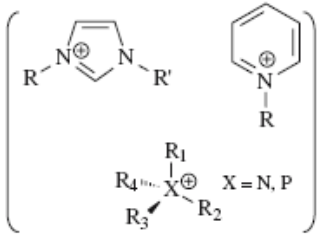
# Limitation of liquid matrices

Extensive *adduct* formation, peak broadening leading to reduced resolution, low sensitivities and practical problems like the detachment of liquid matrices from *vertical* targets or *volatility* under high vacuum conditions.

A more widespread application of liquid matrices can be found in IR-MALDI.

**Ionic Liquids**

## ionic liquids (IL/RTIL)




$[PF_6]^-$
$[(CH_3)_2PO_4]^-$
$[BF_4]^-$
$[OctSO_4]^-$
$[(CF_3SO_2)_2N]^-$

- molten salts (below 100°C)
- non-inflammable
- low vapor pressure
- „designer solvents“, used for
  - chemical synthesis/catalysis
  - biocatalysis
  - analytical chemistry
- not suited as MALDI matrices

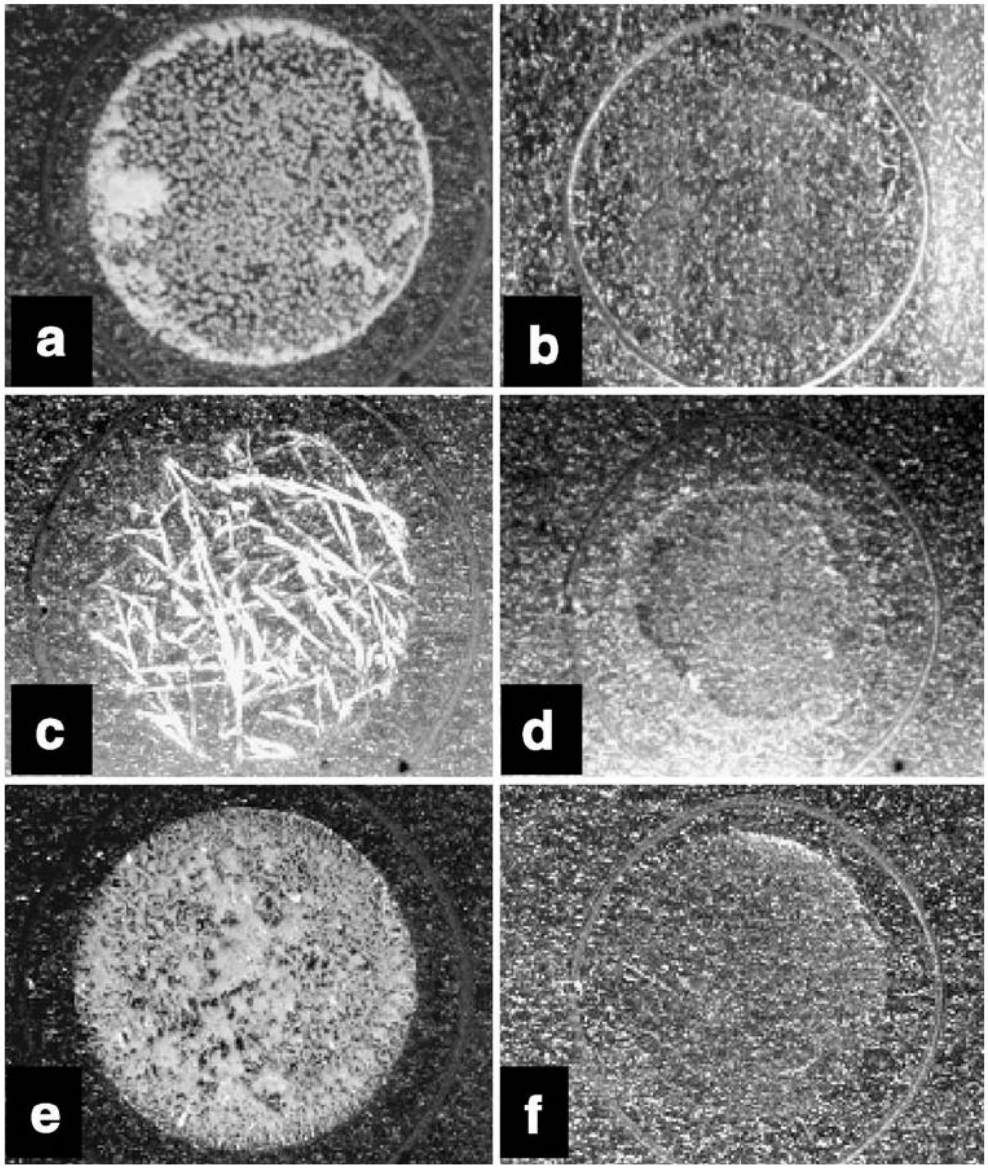
## ionic (liquid) matrices (ILM/IM)

| base \ acid | SA | CCA | DHB |
|---|---|---|---|
| TBA | viscous liquid | viscous liquid | viscous liquid |
| MI | viscous liquid | viscous liquid | viscous liquid |
| Py | solid | solid | viscous liquid |

- equimolar mixtures of MALDI matrices and organic bases
- suited as MALDI matrices

a–e Photographs of preparations of crystalline matrices and corresponding ionic liquid matrices. a CCA, b CCA-1-methylimidazole, c DHB, d DHB-pyridine, e sinapinic acid (SA), f SA-N,N-N,Ndimethylethylendiamine.

The diameter of all spots shown is about 2 mm. All samples were prepared on a polished steel target, using the dried-droplet method. Solvent: 50% acetonitrile/ 0.1% TFA.



# MALDI MS Instrumentation

# MALDI contrast to electrospray

Advantage

1. MALDI ions carry only a few charges with the singly charged ions generally predominant.
2. In principle an unlimited mass range.

Disadvantage

1. High initial axial velocity
2. Broad kinetic energy

Overview of commercial mass spectrometers designed for MALDI-MS with their technical specifications provided by individual manufacturers.[a]

| Mass analyzer type | Instrument, manufacturer | Resolving power, FWHM (defined at $m/z$) | Resolution ($\Delta m/z$) | Mass accuracy (ppm) | | $m/z$ range | Laser (wavelength, frequency) |
|---|---|---|---|---|---|---|---|
| | | | | Internal calibration | External calibration | | |
| LIT | LTQ XL, Thermo Scientific | 15,000–30,000 (depending on scan speed) | – | – | – | 15–4000 | $N_2$ (337 nm, 60 Hz) |
| TOF | Autoflex Speed, Bruker Daltonics | 26,000 ($m/z$ 3147) | 0.1 | <2 | <10 | 100–500,000 | Nd:YAG (355 nm, 1000 Hz) |
| | Axima Confidence, Shimadzu | 15,000 ($m/z$ 3660) | 0.2 | <10 | <100 | 1–500,000 | $N_2$ (337 nm, 50 Hz) |
| IT-TOF | Axima Resonance, Shimadzu | 8000 ($m/z$ 2465) | 0.3 | 3 | 5 | 100–12,000 | $N_2$ (337 nm, 10 Hz) |
| Q-IMS-TOF | MALDI Synapt G2-S HDMS, Waters | 32,000 ($m/z$ 3495) | 0.1 | <1 | – | 20–100,000 | Nd:YLF (349 nm, 1000 Hz) |
| TOF/TOF | Axima Performance, Shimadzu | 20,000 ($m/z$ 3660) | 0.2 | <5 | <50 | 1–500,000 | $N_2$ (337 nm, 50 Hz) |
| | JMS-S3000 SpiralTOF, Jeol | 60,000 ($m/z$ 2093) | 0.03 | 1 | 10 | 10–30,000 | Nd:YLF (349 nm, 250 Hz) |
| | TOF/TOF 5800 System, AB SCIEX | 33,000 (for $m/z$ range 1200–3700) | 0.07 | <1 | <5 | – | Nd:YLF (345 nm, 1000 Hz) |
| | UltrafleXtreme, Bruker Daltonics | 40,000 ($m/z$ 3147) | 0.08 | <1.5 | <5 | 100–500,000 | Nd:YAG (355 nm, 1000 Hz) |
| LIT-Orbitrap | MALDI LTQ Orbitrap XL, Thermo Scientific | 100,000 ($m/z$ 400) | 0.004 | <2 | <3 | 50–4000 | $N_2$ (337 nm, 60 Hz) |
| Q-ICR | SolariX 15T, Bruker Daltonics | 2,500,000 ($m/z$ 400) | 0.0002 | <0.25 | <0.6 | 100–10,000 | Nd:YAG (355 nm, 1000 Hz) |

[a] If manufacturers have more instruments in particular series, then only the instrument with the best performance is listed here. Individual manufacturers take the full responsibility for the correctness of technical specifications. Instruments in individual classes are sorted alphabetically according to the instrument name. This list contains only main manufacturers and may not be comprehensive.

*M. Holčapek et al. / J. Chromatogr. A 1259 (2012) 3–15*

MALDI source generates a pulse of ions of at most a few nanoseconds in duration at a rate of 1 to 100 pulses per second; thus, they are most compatible with mass spectrometers which are effectively pulsed-ion detectors, including instruments which trap the ions for later analysis.

Mass spectrometers which operate on a continuous beam of ions, such as magnetic sector and quadrupole instruments, tend to suffer substantial problems in terms of sensitivity and *are generally not suitable* for a pulsed-ion source.

The important feature of MALDI is that it can generate ions of very large masses of greater than 1 MDa.

In contrast to electrospray, MALDI ions carry only a few charges with the singly charged ions generally predominant.

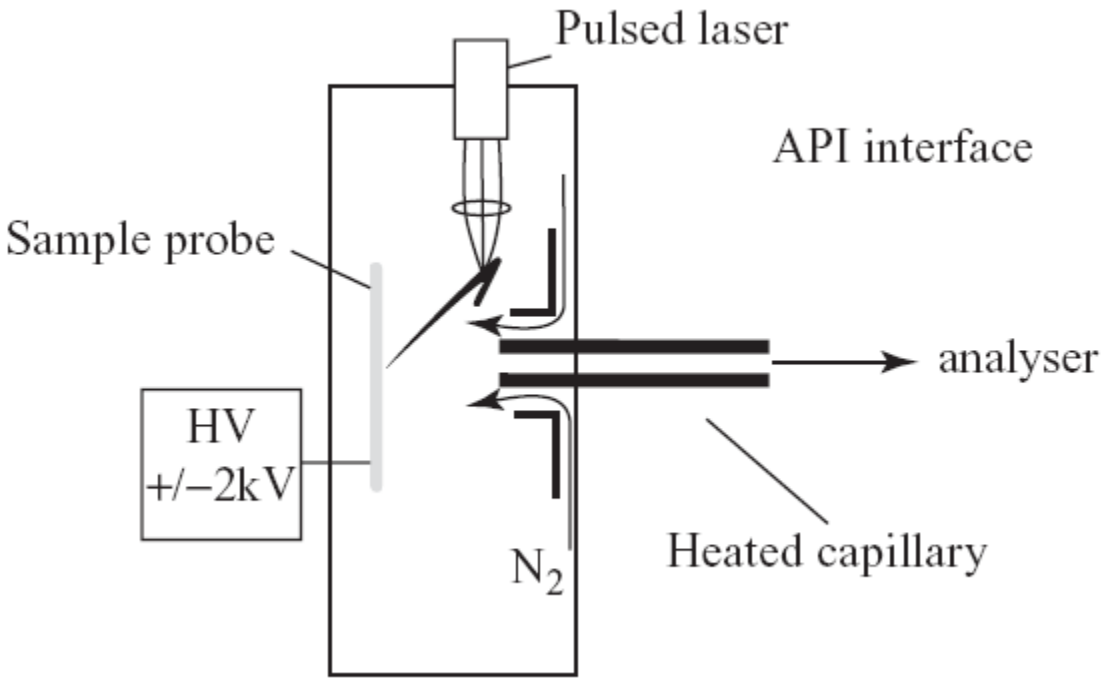
Again, the TOF is by far the most suited analyzer for such large ions, because it has, in principle, an unlimited mass range.

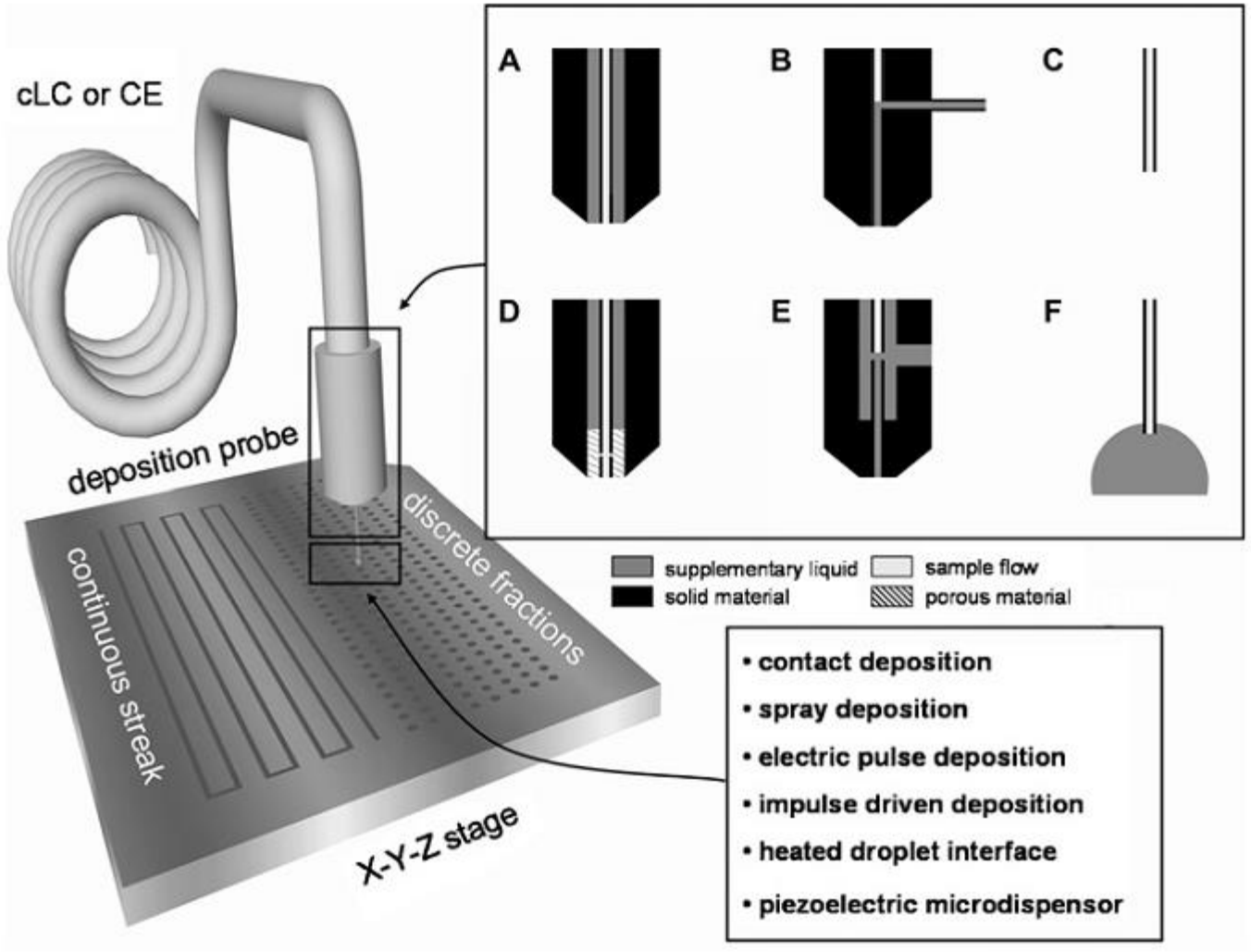
However, the high initial axial velocity of MALDI ions and their broad kinetic energy distribution causes problems for all mass analyzers.

# Fragmentation of MALDI Ions

1. Ions absorb some of the laser energy directly and are also heated by collisions with the matrix molecules which absorb the laser light very efficiently. For MALDI, because the ions are generally metastable, a "Pseudo-MS/MS" technique exists where the parent ions are fragmented, but isolation of the precursor is not performed. This method is often (and incorrectly) called tandem mass spectrometry, but is correctly called simply metastable decay and, in TOF instruments, can be classified as either "in-source" decay or "post-source" decay.
2. An electric field in the source will accelerate the ions and drag them through the matrix plume, causing collisional activation. A few millibars of inert gases such as He, Ar, or N2 in the ion source, can cool the ions and thereby increase their stability. If the MALDI ions collide with these thermal gas molecules, some of their vibrational energy gets removed. Without cooling, the ions could barely be detected.
3. Chemical reactions such as hydrogen and proton transfer occur inside the plume and have certain exothermicities associated with them.
4. Metastable decay

# Atmospheric Pressure MALDI (AP-MALDI)






Scheme of off-line LC–MALDI-MS coupling with the supplementary liquid addition and deposition mechanisms. Junctions used for coupling of microcolumn techniques include the following: (A) coaxial sheath flow, (B) T-junction, (C) sheathless interface, (D) porous junction, (E) liquid junction, and (F) droplet electrocoupling. A, B and C are typical for capillary LC–MS, while D, E and F are common in capillary zone electrophoresis–MS

# List of Ambient MS Techniques

| abbreviation | name | first report | figure | abbreviation | name | first report | figure |
|---|---|---|---|---|---|---|---|
| | | | Solid–Liquid Extraction-Based | | | | |
| *DESI* | *desorption electrospray ionization* | 2 | 1, 2 | DICE | desorption ionization by charge exchange | 96 | |
| EASI | easy ambient sonic spray ionization | 100 | 16 | LMJ-SSP | liquid micro junction-surface sampling probe | 5 | 3 |
| DAPPI | desorption atmospheric pressure photo-ionization | 138 | 4 | *LESA* | *liquid extraction surface analysis* | 125a | |
| | | | Plasma-Based | | | | |
| *DART* | *direct analysis in real-time* | 161c | 5 | DCBI | desorption corona beam ionization | 250 | |
| FAPA | flowing atmospheric pressure afterglow | 225b | 8 | PADI | plasma assisted desorption ionization | 146 | |
| ASAP | atmospheric solids analysis probe | 150 | | APTDI | atmospheric pressure thermal desorption/ ionization | 148 | |
| LTP | low-temperature plasma probe | 233 | 9 | HAPGDI | helium atmospheric pressure glow discharge ionization | 149 | |
| DAPCI | desorption atmospheric pressure chemical ionization | 168 | | PPAMS LTP | plasma pencil atmospheric mass spectrometry LTP | 234 | |
| DBDI | dielectric barrier discharge ionization | 232 | | ambient MHCD | ambient microhollow cathode discharge ionization | 160 | |
| | | | Two-Step Thermal/Mechanical Desorption/Ablation (Non-Laser) | | | | |
| ND-EESI | neutral desorption extractive electrospray ionization | 363 | | AP-TD/SI | atmospheric pressure thermal desorption-secondary ionization | 260 | |
| BADCI | beta electron-assisted direct chemical ionization | 259 | | PESI | probe electrospray ionization | 263 | |
| | | | Two-Step Laser-Based Desorption Ablation | | | | |
| ELDI | electrospray-assisted laser desorption ionization | 4 | | LEMS | laser electrospray mass spectrometry | 282 | |
| MALDESI | matrix-assisted laser desorption electrospray ionization | 284 | | LD-APCI | laser desorption atmospheric pressure chemical ionization | 278 | |
| *LAESI* | *laser ablation electrospray ionization mass spectrometry* | 275 | 10 | IR-LAMICI | infrared laser ablation metastable-induced chemical ionization | 276 | 11 |
| LADESI | laser-assisted desorption electrospray ionization | 279 | | PAMLDI | plasma assisted multiwavelength laser desorption ionization | 186 | |
| LDESI | laser desorption electrospray ionization | 280 | | LAAPPI | laser ablation atmospheric pressure photoionization | 314 | 12 |
| | | | Acoustic Desorption | | | | |
| RADIO | radio-frequency acoustic desorption and ionization | 324 | | LIAD/ APCI | laser-induced acoustic desorption/atmospheric pressure chemical ionization | 323 | |
| LIAD/ESI | laser-induced acoustic desorption-electrospray ionization | 321a | 13 | SAWN | surface acoustic wave nebulization | 325 | 14 |
| | | | Multimode | | | | |
| DEMI | desorption electrospray/metastable-induced ionization | 332 | | | | | |
| | | | Other Techniques | | | | |
| REIMS | rapid evaporative ionization mass spectrometry | 333 | | SwiFerr | switched ferroelectric plasma ionizer | 335 | |
| LDI | laser desorption ionization | 334 | 15 | LSI | laserspray ionization | 364 | |

[a]Techniques in italics are available commercially.

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# Direct analysis in real time (DART)

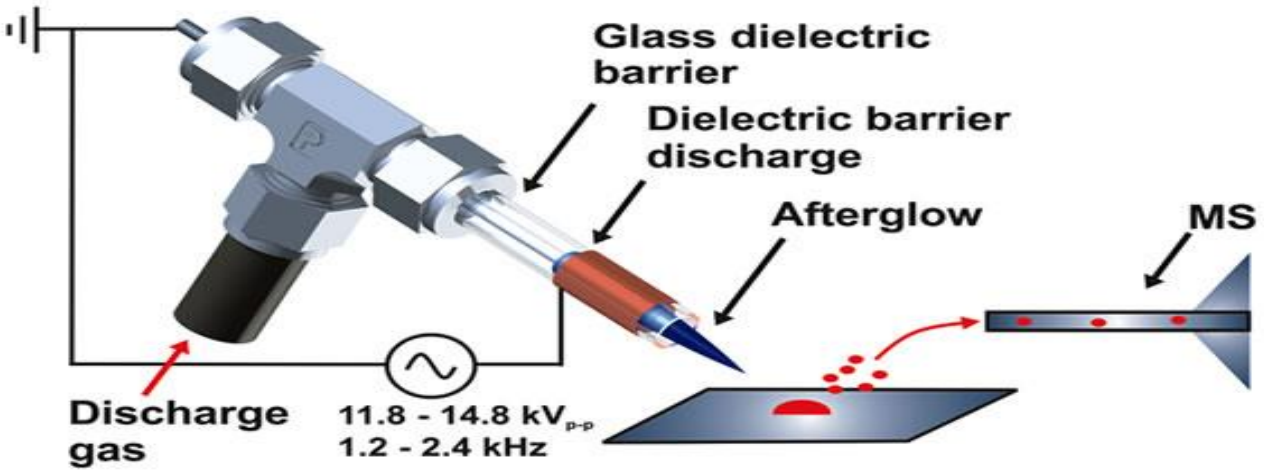
DART MS is ionization of low-molecular-mass compounds present on the surfaces of solids or liquids in a gas stream without sample preparation.

A DART source was patented in 2005 and the first DART-MS study was also reported in that year. However, a prototype of the commercially available DART source was used in the US Army since 2002.

# How It works

- The two main techniques used in ambient ionization are known as DESI and DART

- These techniques desorb and ionize the analytes so they can be analyzed by the Mass Spectrometer



# DART

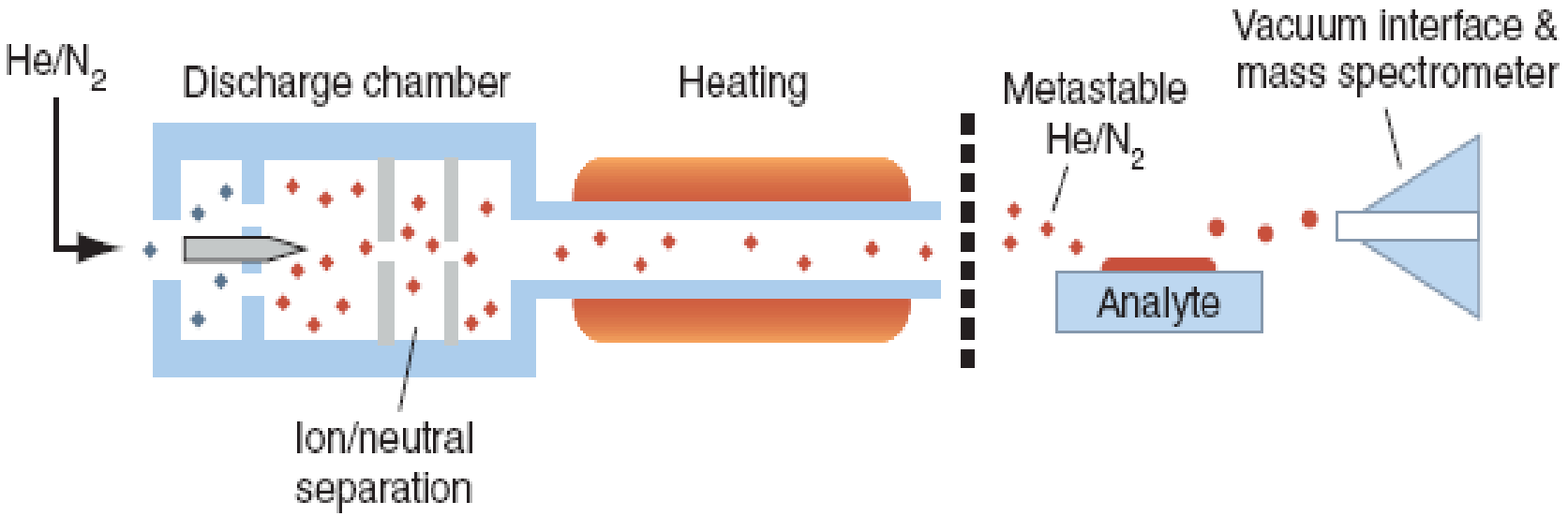
**Very similar to DESI (gas solvent instead of liquid)**

**The original experiments picked up contaminants (glue) “far removed from the laboratory”**

**Shoots a beam of charged metastable gas, such as helium or nitrogen**

**This beam then collides with the sample, desorbing and charging the sample upon impact**

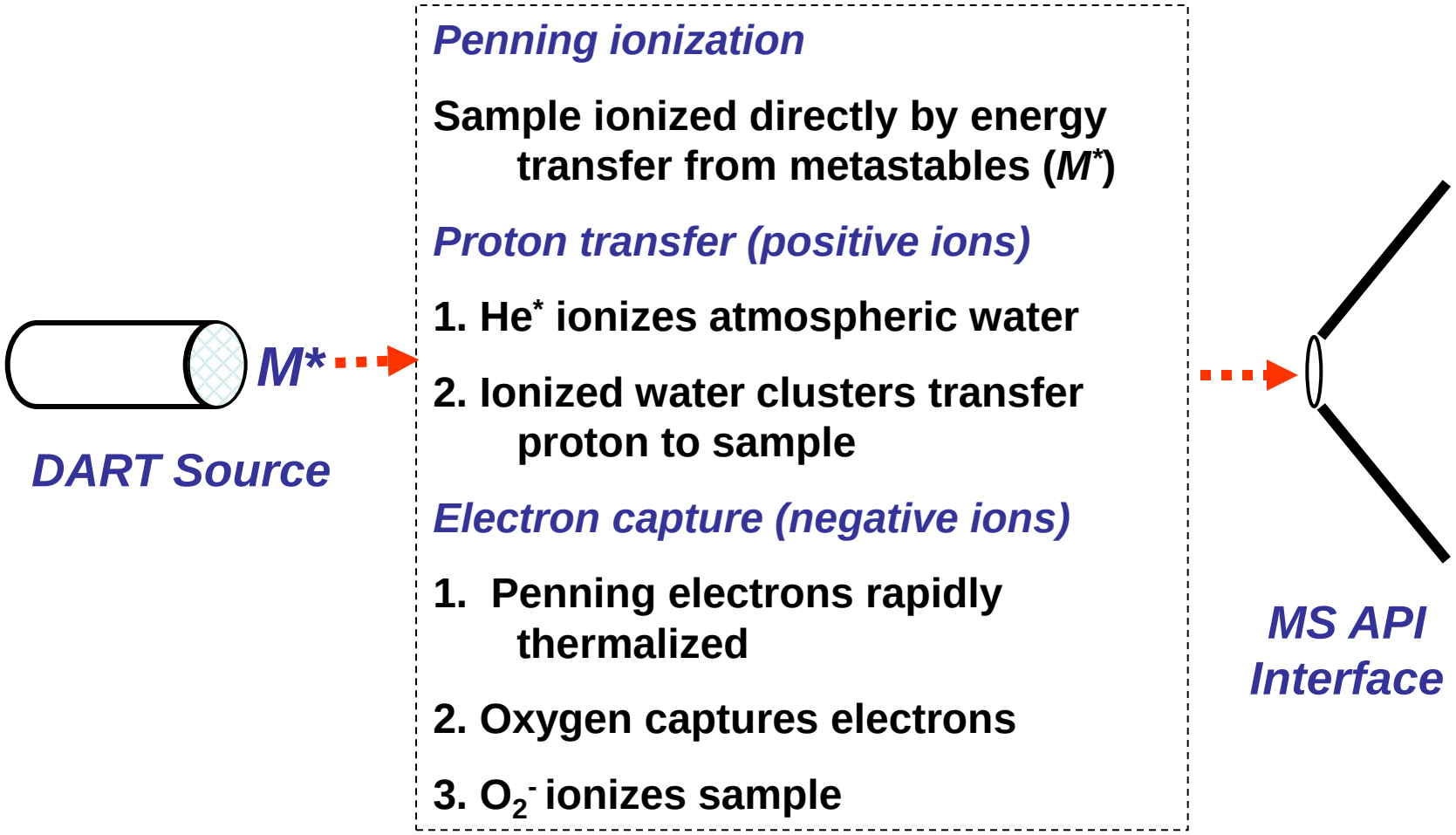
**The charged particle is then drawn into the Mass Spectrometer via vacuum**




**Opinions are different as to the nature of the discharge in the DART source. Earlier, Cody and Laramee (inventors of DART-MS) used the term `electrical discharge‘ without specifying its nature.**
**Hieftje and co-workers stated that a corona discharge is used in the DART source.**

This was also confirmed by classification of discharges depending on the potential difference applied to electrodes:

Tens of volts correspond to the ***arc*** discharge, hundreds of volts correspond to the ***glow*** discharge and a few kilovolts correspond to the ***corona*** discharge.



This process dominates if the ionization energy of the molecule M is lower than the internal energy of the species N*.

$$N^* + M \longrightarrow M^{+\bullet} + N + e^-.$$

The energy of an excited (triplet) state of a helium atom is 19.8 eV. The ionization energies of atmospheric gases and organic molecules are usually lower than 19.8 eV. When using helium, the following reaction dominates

$$He(2^3S) + n\,H_2O \longrightarrow [(H_2O)_{n-1} + H]^+ + OH^- + He(1^1S).$$

If the proton affinity of an analyte molecule is higher than that of a water cluster, transfer of a proton occurs:

$$[(H_2O)_n + H]^+ + M \longrightarrow [M + H]^+ + n\,H_2O;$$

If the proton affinity difference is large, fragmentation of ions is possible.

Shortening of the distance between the DART source exit and the mass spectrometer sampling orifice to 5 mm and an increase in the grid electrode potential from 250 to 650 V caused changes in the mass spectrum of the background, viz., the appearance of strong signals of $O_2^{+\bullet}$. radical cations.

Under the conditions of production of $O_2^{+\bullet}$ species, the ionization mechanism changed. Namely, signals of $M^{+\bullet}$ and other odd-electron species, e.g., [M-CO]+. appeared in the mass spectra of nonpolar analytes, whereas signals of protonated molecules and other even-electron species are mainly observed in the mass spectra.

Presumably, the mechanism of production of odd-electron ions involved a charge exchange between $O_2^{+\bullet}$ and the analyte molecule provided that the ionization energy of the analyte was lower than the ionization energy of $O_2^{+\bullet}$ (12.07 eV):

$$He^* + O_2 \longrightarrow O_2^{+\bullet} + e^- + He,$$

$$O_2^{+\bullet} + M \longrightarrow M^{+\bullet} + O_2,$$

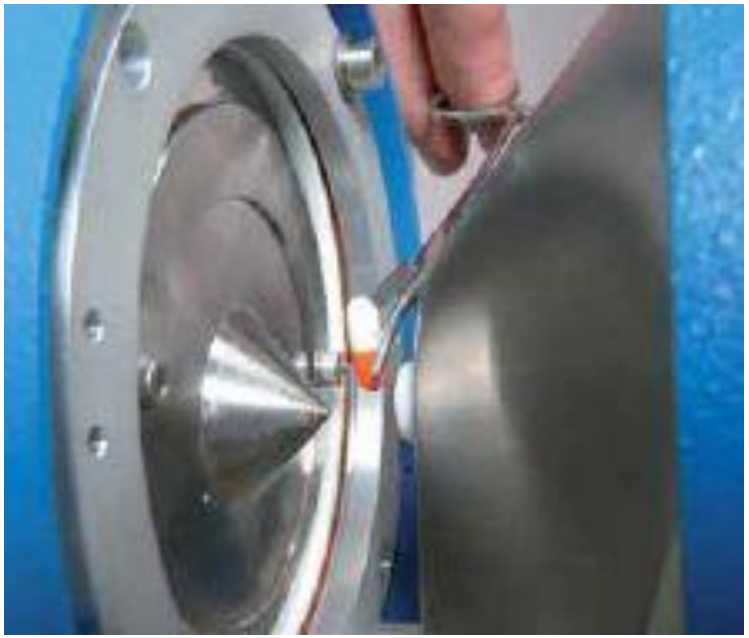
$$O_2^{+\bullet} + M \longrightarrow [\text{fragment ion}]^+ + O_2 + R^{\bullet},$$

$R^{\bullet}$ is an organic radical or a molecular fragment.

Parameters affecting the DART mass spectra

1. The nature of analytes
2. The nature of the matrix
3. Chemical composition of reagent gas in the DART source and the environment
4. The temperature and the gas flow rate in the DART source
5. Voltage at electrodes of the DART source and at the inlet cone of mass spectrometer

# Advantages



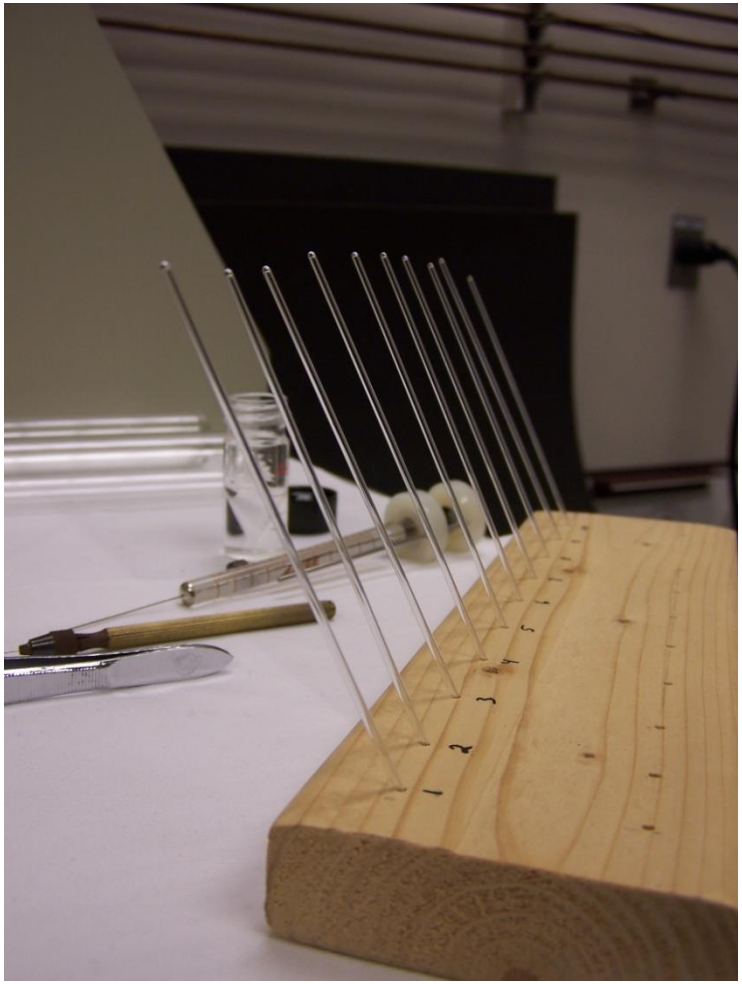
- Little to no sample preparation
- Non-destructive
- Continuous sample collection
- Rapid Results

# HEADSPACE SAMPLING

1. Start acquisition
2. Open container near DART gas stream
3. Remove container

Can’t get simpler than that!!!!!!

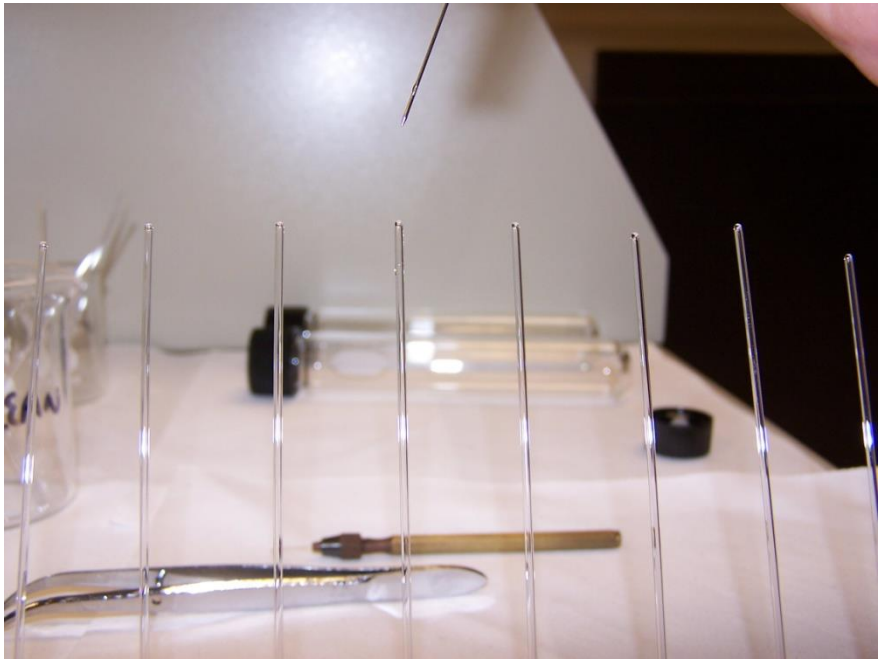
# “DRIED ON TUBE” METHOD



Deposit sample onto tubes with syringe

Can measure how much sample is deposited

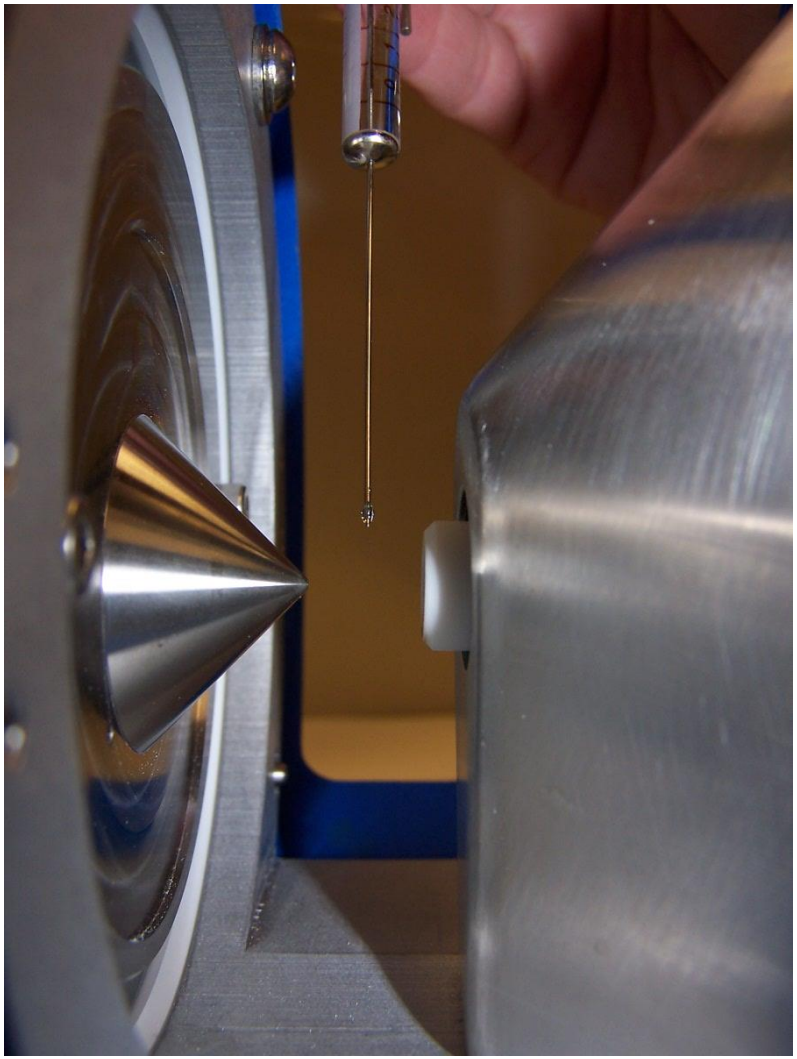
Turned out not so good!




Loading cap tubes with sample

Even 1 uL “runs down” tube

Inconsistent results obtained



# Injecting sample into gas stream with syringe

Draw up samples from ALS vials

Can measure amount injected

Turned out not so good!

# LIQUID SAMPLING

Dissolve sample into suitable solvent

Dip capillary tube in liquid/hold “wand” in DART gas stream

Advantages:

1. EASY!!!
2. Can control concentration of sample
3. Homogeneous sample

Preferred method at DFS!!!

# Excellent signal to noise ratio

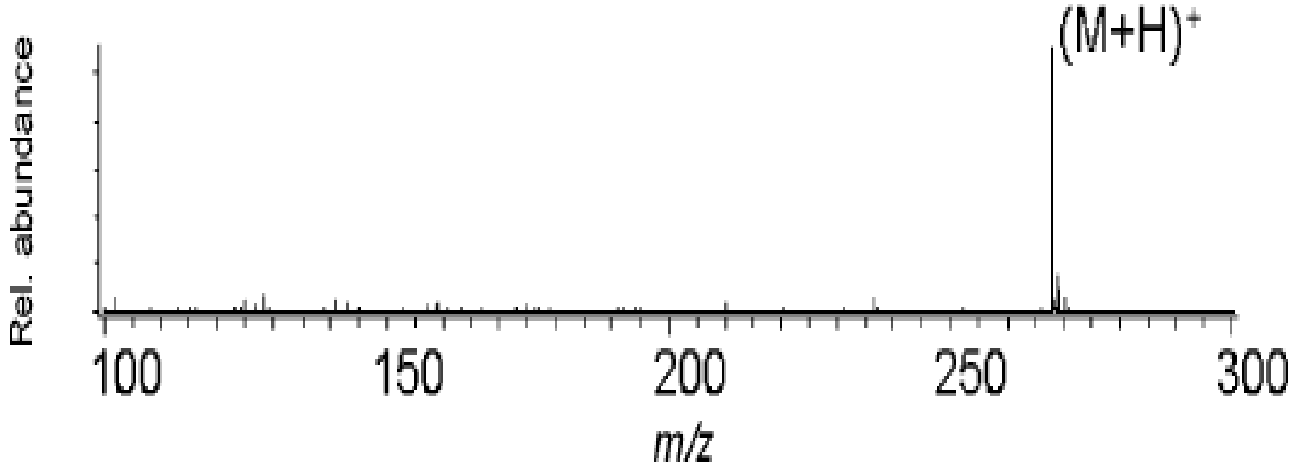



**Figure 7.** Detection of 788 ng American VX on a porous concrete sample.

# Practical Use

- Identification of traces left behind by explosives, toxic industrial compounds, chemical warfare agents, illicit drugs, some foods, inks, fingerprints and skin.

  - This can provide valid evidence to convict  someone in court.

- Can be used by pharmaceutical companies to rapidly analyze compounds in their natural form

- Able to analyze illicit drugs within biological fluids

  - this is valuable because most other methods require sample extraction before analysis

- Identifying contaminants in foods such as melamine, which can make the product appear to have a higher protein content.

- DESI can be used to produce images of latent fingerprints

# AccuTOF-DART MS

- The DART is the first open air, ambient ion source for a mass spectrometer
- Coupled to a time of flight instrument exact mass measurements can be used in the putative identification of compounds

Direct Analysis in Real Time (DART™)
US Patent Numbers 6,949,741 and 7,112,786

http://www.jeolusa.com/Portals/2/prodshots/AI/accutof-dart-tm.jpg

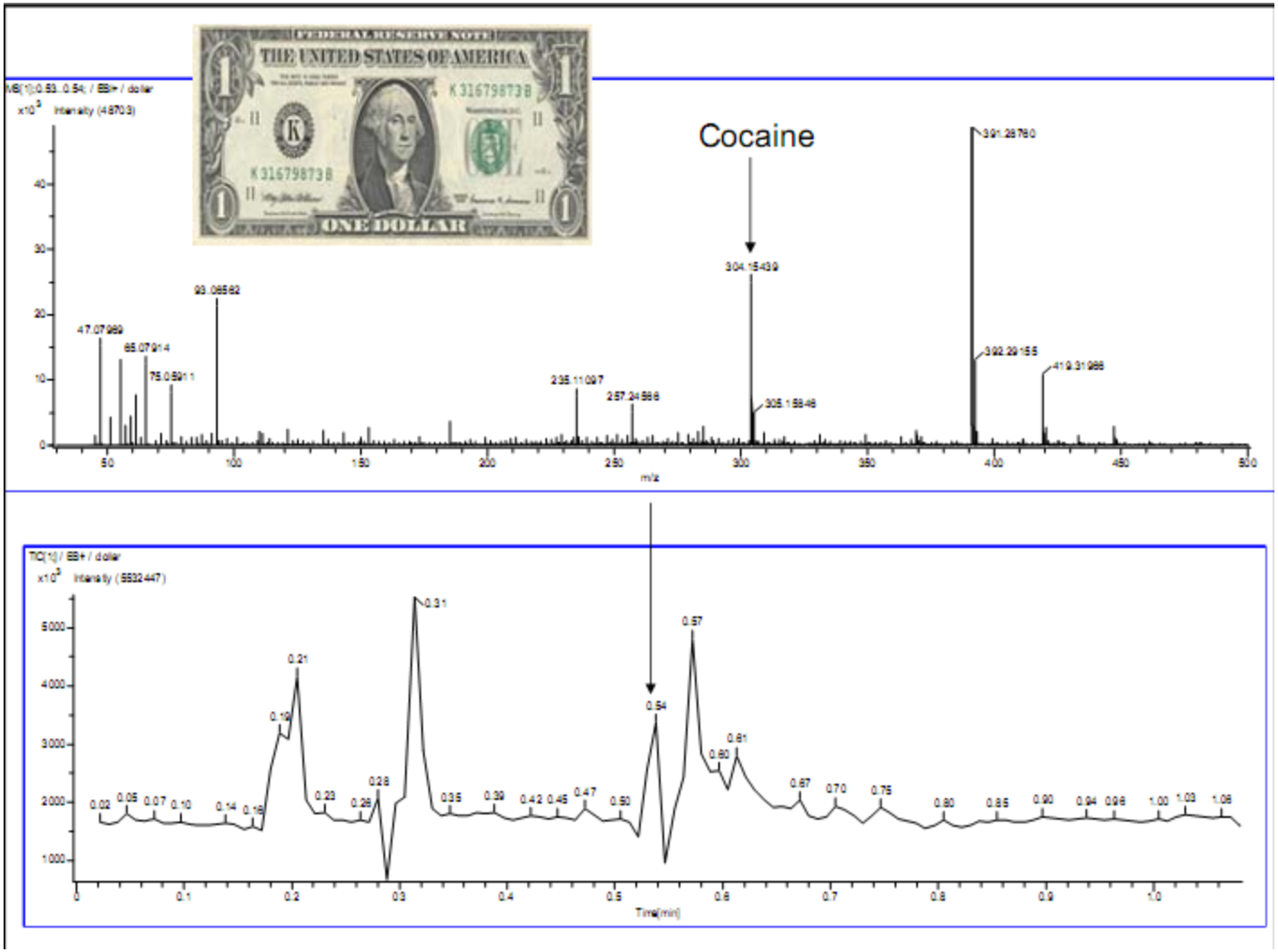
# Types of Samples Analyzed with the AccuTOF-DART MS

http://www.ecstasy2.com/img/ecstasy_pill_collage1.jpg



# Sample Introduction with the AccuTOF-DART MS

www.jeoluk.com/images/DART01.jpg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93.08582
47.07989
65.07914
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305.15846
392.29155
419.31988
m/z
TIC(1) / ESI+ / dollar
x10^3 Intensity (5532447)
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0.21
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0.61
0.60
0.67
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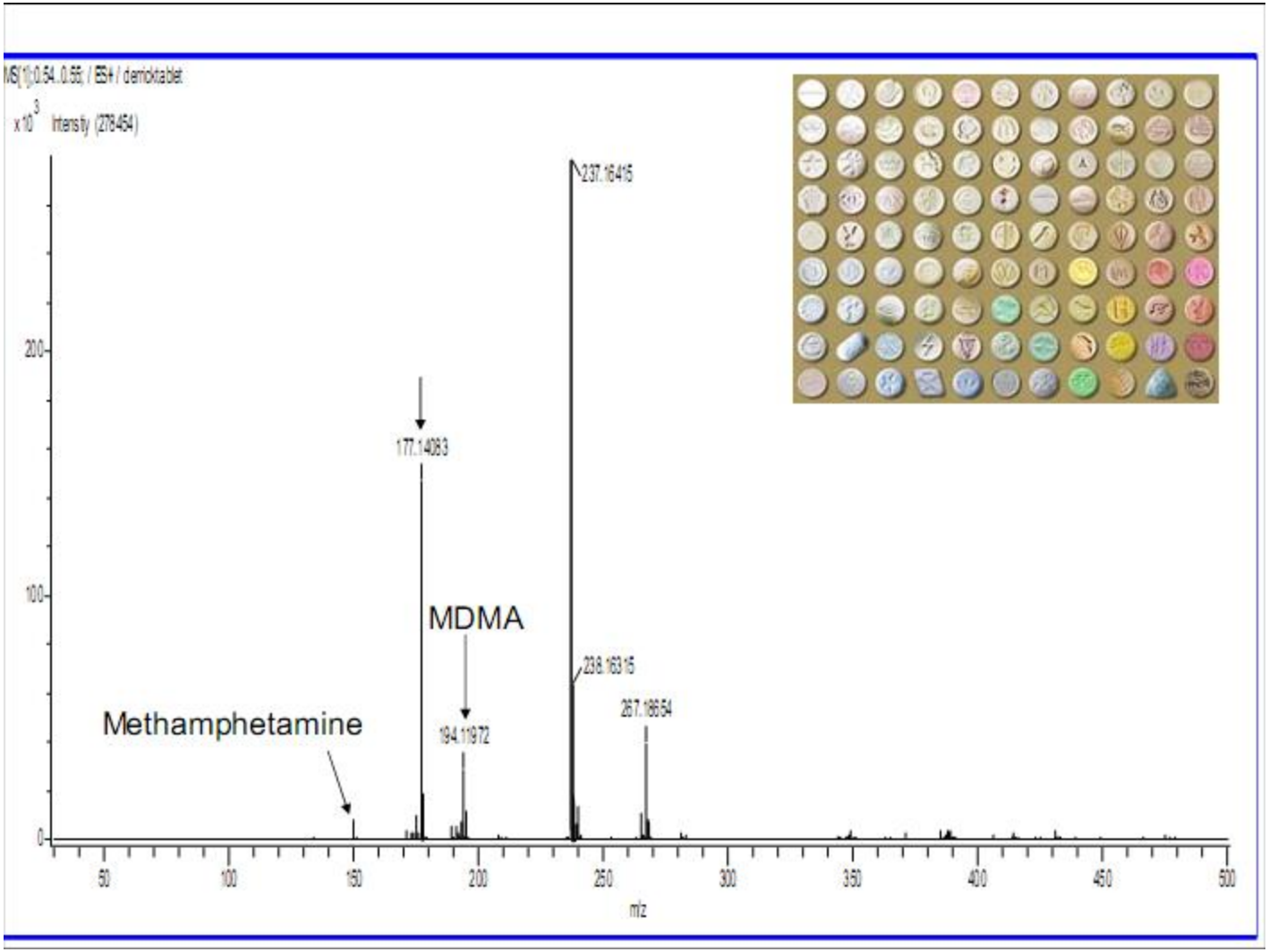
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