Energy-sensitive calorimetric detectors that operate at low temperatures ("cryogenic detectors") have recently been applied for the first time as ion detectors in time-of-flight mass spectrometry. Compared to conventional, ionization-based detectors, which rely on secondary electron formation or the charge created in a semiconductor, cryogenic detectors measure low-energy solid state excitations created by a particle impact. This energy sensitivity of cryogenic detectors results in several potential advantages for TOF–MS. Cryogenic detectors are expected to have near 100% efficiency even for very large, slow-moving molecules, in contrast to microchannel plates whose efficiency drops considerably at large mass. Thus, cryogenic detectors could contribute to extending the mass range accessible by TOF–MS and help improving detection limits. In addition, the energy resolution provided by cryogenic detectors operates at cryogenic temperatures (''cryogenic detectors'') have recently been applied for the first time as ion detectors in time-of-flight mass spectrometry. Compared to conventional, ionization-based detectors, which rely on secondary electron formation or the charge created in a semiconductor, cryogenic detectors measure low-energy solid state excitations created by a particle impact. This energy sensitivity of cryogenic detectors results in several potential advantages for TOF–MS. Cryogenic detectors are expected to have near 100% efficiency even for very large, slow-moving molecules, in contrast to microchannel plates whose efficiency drops considerably at large mass. Thus, cryogenic detectors could contribute to extending the mass range accessible by TOF–MS and help improving detection limits. In addition, the energy resolution provided by cryogenic
detectors can be used for charge discrimination and studies of ion fragmentation, ion-detector interaction, and internal energies of large molecular ions. Cryogenic detectors could therefore prove to be a valuable diagnostic tool in TOF–MS. Here, we give a general introduction to the cryogenic detector types most applicable to TOF–MS including those types already used in several TOF–MS experiments. We review and compare the results of these experiments, discuss practical aspects of operating cryogenic detectors in TOF–MS systems, and describe potential near future improvements of cryogenic detectors for applications in mass spectrometry. © 1999 John Wiley & Sons, Inc.,* Mass Spec Rev 18: 155–186, 1999

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I. INTRODUCTION

Over the last decade, mass spectrometry has become an increasingly important biomolecular research tool (Senko & McLafferty, 1994; Siuzdak, 1994; Burlingame, Boyd, & Gaskell, 1998). A large portion of this success is the result of the development of new ionization methods. Matrix-assisted laser desorption/ionization (MALDI) (Karas & Hillenkamp, 1988; Bahr, Karas, & Hillenkamp, 1994) and electrospray ionization (ESI) (Fenn et al., 1990) provide relatively “mild” desorption and ionization conditions for large biomolecules. Only a decade ago, ions weighing more than a few thousand Daltons were considered “large”. Today, MALDI and ESI can generate ions with masses well above 100,000 Da. During the same time, the resolving power of mass analyzers was improved considerably. It is now common to obtain a mass resolution of several thousand for time-of-flight techniques that employ orthogonal acceleration and reflectron techniques (Verentchikov, Ens, & Standing, 1994; Krutchinsky et al., 1998) and up to a million for reflectron techniques (Verentchikov, Ens, & Standing, 1993; Westmacott, Ens, & Standing, 1996). In this detection scheme, light (~1–200 Da) secondary ions created by surface-induced dissociation or sputtering upon impact of a large primary ion onto conversion electrode are post-accelerated over a short distance and detected e.g., by an MCP or secondary electron multiplier. For the detection of high-mass primary ions, the additional time spread introduced by the distribution of secondary ion masses is small compared to the time spread caused by the spread of primary ion velocities and can be tolerated. Using IR MALDI and a detector based on secondary ion emission, the Münster group has shown that singly charged protein molecules with masses exceeding 500 kDa and multiply charged species with masses approaching 2 MDa can be measured with MALDI TOF–MS (Berkenkamp et al., 1997). Also, large gold clusters in the mass range of 40 kDa to 2 MDa with kinetic energies of tens of keV have been detected using a secondary ion detection scheme (Nguyen et al., 1996).

Another potentially useful group of candidates for advancing ion detector technology are cryogenically cooled, calorimetric detectors also known as cryogenic detectors or low-temperature detectors (LTD-6, 1995; Booth, Cabrera, & Fiorini, 1996; Booth & Goldie, 1996; Twerenbold, 1996b; LTD-7, 1997). Cryogenic detectors are very sensitive, energy-resolving, low-threshold particle detectors which have been developed over the last decades for a variety of applications in particle physics and astrophysics. First used as infrared detectors and detectors for molecular beams about 30 years ago, cryogenic detectors are now also used as energy-resolving, photon-counting detectors for high-resolution X-ray spectroscopy and for searches for dark matter in the Universe in the form of Weakly Interacting Massive Particles (WIMPs). More recently, cryogenic detectors have also been applied as ion detectors in biomolecule TOF–MS with encouraging results. The cryogenic detectors used in these preliminary TOF–MS experiments were basically the same types of detectors as the ones
presently used by the physics community for X-ray detection, and did not require special adaptation for the use in TOF–MS.

We have gained practical experience of using cryogenic ion detectors in several TOF–MS experiments. In this review, we wish to provide a general overview of cryogenic detector technology, summarize the recent results from TOF–MS experiments using cryogenic ion detectors, and describe practical benefits and limitations associated with this detector technology. We hope this review will help potential users decide whether cryogenic ion detectors could be beneficial to their applications.

Conventional ion detectors, such as microchannel plates (MCPs), currently used in most TOF–MS applications rely on ionization or secondary electron formation in their detection mechanism. In contrast, cryogenic detectors are energy-sensitive and energy-resolving ("calorimetric") detectors. When applied to TOF–MS, cryogenic detectors measure low-energy solid-state excitations, called phonons, created by a particle impact. Phonons are quantized crystal lattice vibrations and can be measured, in the simplest case, as the heat created by the impact. The energy of these phonons is typically less than a few meV which is much smaller than the energy in the electronvolt range needed to produce secondary electrons or electronic excitations in conventional ionization detectors.

The fact that cryogenic detectors respond to low-energy crystal lattice excitations is the reason why cryogenic detectors are more sensitive to weakly ionizing, slow-moving particles than ionization detectors. Although the kinetic energy of large ions in a TOF–MS is typically tens of thousands times larger than the energy required to excite a secondary electron, the actual energy transferred in a single collision is very small. This is a result of the vastly different masses of incident ions and electrons and constraints from momentum conservation. As a result, the concept of a “threshold velocity” for this “kinetic” electron emission for a given incident ion type has been adopted (Beuhler & Friedman, 1980; Baragiola, Alonso, & Oliva, 1993). Many authors have noted that, in practice, there is no real velocity threshold for electron emission. Electron emission for ion impacts below the so-called threshold velocity is observed, although generally at a significantly reduced probability (Westmacott, Ens, & Standing, 1996; Brunelle et al., 1997), and this emission is attributed to “collective” effects. In contrast, the energy transfer from incident ions to atoms in single collisions on the surface of a detector is more efficient and leads to the local generation of strong lattice vibrations (phonons) among the atoms at the surface. A single impact of a large ion with 10 keV kinetic energy may ultimately produce hundreds of thousands of these phonons, which can be detected in a cryogenic detector. (Also as a result of the ion impact, some atoms and light ions are sputtered off the surface and can then be used in a secondary ion detection scheme).

Detectors relying on measuring low-energy phonon excitations must be operated at low temperatures, typically below 2 K, hence the name “cryogenic detectors”. This low operating temperature is required because at higher temperatures typical thermal energies become comparable or exceed the energy of the phonons to be measured. At higher temperatures, the impact signal would, therefore, be buried in a large background of thermally excited phonons.

Cryogenic detectors are energy-resolving detectors because the number of excitations created by an ion impact is proportional to the energy deposited by the ion. Most types of cryogenic detectors are designed to produce a current pulse in response to a particle impact with a pulse height proportional to the particle energy and a pulse onset corresponding to the particle arrival time. Measuring ion energy independently of ion arrival time adds another dimension to time-of-flight data and provides additional information on the ions.

Generally speaking, the energy sensitivity and energy resolution provided by cryogenic detectors applied to TOF–MS could prove valuable for the following reasons.

i. Cryogenic detectors should not exhibit any decrease of sensitivity for large mass. Any impacting particle will create a signal as long as the energy deposited exceeds the low noise level of the detector, typically tens to hundreds of electronvolts. Cryogenic detectors are, therefore, expected to detect large, slow-moving ions or neutrals with ~100% efficiency.

ii. The energy resolution provided by cryogenic detectors may be used for charge discrimination. A doubly charged ion carries twice the kinetic energy than a singly charged ion and will result in a pulse with twice the height. Such a charge measurement based on energy is independent of time-of-flight. It could help to resolve m/z ambiguities and, for example, distinguish between doubly charged dimers of a molecule and singly charged monomers, even though they both have the same flight time.

iii. The energy resolution of cryogenic detectors may also be useful for studies of ion fragmentation and internal energies. A cryogenic detector may be used to discriminate between fragments and intact ions of the same charge state, because fragments carry less kinetic energy than intact ions.

It should be noted here that other ion detectors may offer some of these three features, although combining all
three in a single instrument seems unique to cryogenic detectors. Because MCPs are the most common type of detector used in TOF–MS, the comparisons made here are directed primarily at them. Large-mass ions can be detected with MCPs; and by switching from techniques based on secondary electron emission to secondary-ion-based techniques, the ion detection efficiency at large mass improves. MCPs can provide some ion energy or charge information under certain operating conditions, because the secondary electron yield is dependent on the velocity (i.e., kinetic energy) and mass of an incident ion (Beuhler & Friedman, 1980; Geno & Macfarlane, 1989). When an MCP is used to detect single ions in pulse-counting mode and the MCP is not run in saturation mode, the pulse amplitude can be used to distinguish between different m/z states or to reject low-mass fragments (Axelson, Reimann, & Sundqvist, 1994; Bondarenko, Grant, & Macfarlane, 1994; Chernushevich, Ens, & Standing, 1997). The Uppsala group gave this technique a name: secondary electron resolved mass spectrometry (SERMS) (Axelson, Reimann, & Sundqvist, 1994). Compared to that technique, cryogenic detectors may offer better energy resolution and energy measurements with cryogenic detectors may be more straightforward. While in secondary electron resolved measurements the pulse height is dependent not only on ion energy, but also mass and composition of ions, cryogenic detectors should provide a more direct way to measuring the energy deposited by an impacting molecule including the possible contributions from potential or internal energy.

Ion energies have also been measured with a silicon surface barrier detector (Bae et al., 1996), although only for ions with relatively high kinetic energy. Bae et al. detected large water cluster ions and cytochrome c molecules with energies of 500–600 keV/charge and showed that pulse height can be used to distinguish between monomers, dimers, and trimers of cytochrome c molecules with the same mass-to-charge ratio.

As will be described in more detail below, the potential of cryogenic detectors to measure the total energy of molecules and ions was recognized already in the 1960s, and several experiments involving molecular beams have been suggested or performed. Their use in mass spectrometry was considered several years ago by Jaklevic, Benner, and Katz (1991) but was not pursued at that time mostly because the detectors considered then had a response time too slow for time-of-flight measurements. The idea to use cryogenic detectors in TOF–MS was developed independently more recently by D. Twerenbold and presented at the 6th International Workshop on Low Temperature Detectors (LTD-6) in 1995 (Twerenbold, 1996a). Shortly thereafter, Twerenbold and collaborators reported the first results of a TOF demonstration experiment with a cryogenic detector (Twerenbold et al., 1996). Other feasibility experiments followed promptly (Frank et al., 1996b) and already in 1997 a first short review article by N. Booth, describing the general idea and the results of these initial experiments was published (Booth, 1997). Since then, several other mass spectrometry experiments have been performed with encouraging and enticing results that warrant a more detailed review. The general interest by the mass spectrometry community in this new detector technology for TOF–MS has grown, although skepticism about the practicality of using small detectors operated at ultralow temperatures for “real-life” mass spectrometry applications remains. In this review article, we not only summarize recent experimental results, but also attempt to provide some context and background information about these new detectors and to discuss practical aspects and potential limitations.

We begin with an overview of cryogenic detectors in section II followed by a more detailed description of those types of cryogenic detectors that have already been used or might be considered for use in mass spectrometry applications in near future. In section III, we give some historical context to the development and use of cryogenic ion detectors and summarize the TOF MS experiments with cryogenic detectors performed during the last three years. This section ends with a comparison and discussion of the results obtained with different types of cryogenic detectors. In section IV, we discuss important practical aspects for extending the technology to a wider range of applications. These aspects include detector operation at cryogenic temperatures within a MS system at room temperature, detector size, and obtainable mass resolution. This section is followed by conclusions and an outlook in section V.

II. CRYOGENIC DETECTORS

A. Overview

There are various types of cryogenic detectors that have in common operation at low temperature, high sensitivity and energy resolution, but that are quite distinguishable by the details of their detection mechanism. In general, every cryogenic detector consists of an absorber coupled to a sensitive sensor and linked to a cold bath. The sensors and often the absorbers are made of thin metal films fabricated by thin-film deposition technology onto a carrier substrate, which in most devices also serves as the cold bath. In the simplest devices, sensor and absorber are the same, i.e., the sensor is used also as absorber. An incoming photon or particle is stopped by the absorber creating solid-state excitations that are measured by the sensor. For a particle stopped on the absorber surface, the
predominant initial excitations are phonons. Phonons are quantized crystal lattice vibrations that behave as particle-like energy packets and propagate through the material at the speed of sound. Depending on the absorber type, some or most of the created phonons may rapidly be converted to other excitations, such as quasiparticles (electron and hole-like excitations) in a superconductor or so-called “hot electrons” in a normal metal absorber.

Cryogenic detectors fall into two general classes: non-equilibrium devices and equilibrium devices. In non-equilibrium devices, such as superconducting tunnel junctions (STJs), the excitations are measured before they thermalize and the particle energy is determined by “counting” the number of excitations. In equilibrium devices, such as hot-electron microcalorimeters, the excitations more or less thermalize and result in a temperature rise proportional to the deposited energy before the excitations are measured by the sensor. In both classes of detectors, the link to the cold bath allows the detector to relax back to equilibrium conditions shortly after the particle interaction.

Detector speed and energy resolution depend on the detector class and strongly on the operating temperature. In general, better energy resolution can be achieved with a detector operating at lower temperature, but the trade-off is response time. Faster response time can be achieved with detectors that operate at a somewhat higher temperature. For a given operating temperature, non-equilibrium devices offer a higher speed, whereas equilibrium devices exhibit better intrinsic energy resolution and lower noise. When applied to mass spectrometry, both classes of cryogenic detectors have 100% detection efficiency for ions independent of their mass or velocity as long as the energy deposited by the ion is above a (small) threshold determined by the noise in the detector. The noise depends on detector type and operating temperature and ranges typically from a few eV to hundreds of eV.

Some of the first cryogenic detectors developed were bolometers used primarily for infrared radiation detection. For a recent review see Richards (1994). Bolometers are energy-sensitive “equilibrium devices” that measure the heating power resulting from the flux of impinging photons or particles onto the detector. Bolometers are generally not designed to detect individual particles or to measure the energy of single particles. Detectors more appropriate for detecting single ions are “cryogenic calorimeters” or “low-temperature microcalorimeters” (not to be confused with the microcalorimeters often used in chemistry to measure chemical reaction energetics, which generally operate at room temperature). The underlying physical detection principle of bolometers and low-temperature microcalorimeters are the same. Thus, the distinction is often blurred and X-ray microcalorimeters are sometimes called bolometers.

Most of the older bolometers used thermistors based on doped silicon or germanium crystals as temperature sensors. Thermistors are resistors whose resistance is strongly temperature-dependent. Infrared bolometers were first built by attaching thermistors to a variety of radiation absorbers and were operated at low temperature, typically ~4 K. The first X-ray microcalorimeters, which were developed mainly for X-ray astrophysics, also employed semiconductor thermistors. Semiconductor-based X-ray microcalorimeters operated at a temperature of ~100 mK or below have achieved excellent energy resolution of 5–8 eV FWHM for 5.89 keV X-rays (McCammon et al., 1993; Silver et al., 1996; Alessandrino et al., 1999). But semiconductor-based X-ray microcalorimeters are slow, equilibrium devices. Their response times are of the order of a millisecond, which is too slow for most TOF applications. Therefore, semiconductor-based calorimetric detectors are not considered further here.

Cryogenic detectors, both bolometers and microcalorimeters, with faster response time and potentially better sensitivity are now being built using sensors based on superconducting films. A superconducting film can be used as a very sensitive thermometer when it is operated as a “transition edge sensor” (TES) within its phase transition from the superconducting state (zero resistance) to the normal conducting state. In this narrow transition region, the electrical resistance of the film increases rapidly with temperature.

Superconducting bolometers based on superconducting thermometers were first introduced many years ago and are now becoming the IR bolometers of choice (see review by Richards, 1994). One of the first applications of superconducting bolometers to detect particles involved ion beams aimed directly at the films (Cavallini, Gallinara, & Scoles, 1969). More recently, single particle detectors with large, dielectric absorber crystals and transition edge sensors have been developed for particle physics applications (Ferger et al., 1994; Cabrera et al., 1996). In these detectors, the particle interactions take place in the dielectric absorber, and the TES films measure the phonons created by the interaction. Smaller versions of such calorimeters have also been applied to measurements with heavy atomic ions (Kienlin et al., 1996).

Very sensitive X-ray microcalorimeters can be built by coupling superconducting thermometers to small, normal-metal absorbers. Recently, such “hot-electron microcalorimeters” have surpassed the performance in both energy resolution and speed of semiconductor-based microcalorimeters and will soon be applied to X-ray fluorescence microanalysis in semiconductor industry (Frank, 1997; Wollman et al., 1997; Ladbury, 1998).
Energy-resolved detection of single optical photons with a TES-based detector has been demonstrated (Cabrera et al., 1998). A hot-electron microcalorimeter has also been applied to TOF–MS (Hilton et al., 1998) and the results are discussed further below in Section III C.

Superconducting tunnel junction (STJ) detectors are another type of cryogenic detectors that are based on superconducting films and explained in detail in the next section. The development of these non-equilibrium detectors was also motivated initially by X-ray astrophysics (Kurakado, 1982; Twerenbold, 1986; Kraus et al., 1989; Zehnder, Hagen, & Rothmund, 1990). They have recently been applied to X-ray measurements at synchrotron beam lines (Frank et al., 1998a, b), energy-resolving detection of single optical photons (Peacock et al., 1996), and were the first cryogenic detectors to be applied to MALDI–TOF–MS (Frank et al., 1996b; Twerenbold et al., 1996) as described further below in Section III B.

We now discuss in more detail the two cryogenic detector types already used in TOF–MS applications: STJ detectors and hot-electron microcalorimeters. In this context, we also describe some variations of these types, which might be applied to MS in the future.

B. Superconducting Tunnel Junction (STJ) Detectors

Superconductor–insulator–superconductor (SIS) tunnel junctions, commonly called superconducting tunnel junctions or STJs, are a family of non-equilibrium cryogenic detectors. Simple STJ detectors, in which the sensor and absorber are the same film structure, were used for the first TOF–MS demonstration experiments. STJs consist of two layers of superconductors (S) separated by a thin insulating barrier (I), for example Nb–Al2O3–Nb, and are typically fabricated by thin-film deposition onto a substrate such as silicon, sapphire, or glass (Fig. 1).

An STJ detector operates best at a temperature T well below the critical temperature Tc of its superconducting films, typically, T ~ 0.1 Tc or below. At this low temperature, all of the conduction electrons of the superconducting layers form weakly bound pairs, called Cooper pairs, which are responsible for the superconductivity. The binding energy of a Cooper pair is 2Δ, where Δ is the superconducting energy gap and is typically of the order of 1 meV or less. At the operating temperature of an STJ detector far below Tc, the energy gap Δ becomes practically temperature-independent and approaches its low temperature limit Δ(T = 0). Table 1 lists several materials commonly used to fabricate STJs along with their critical temperatures and energy gaps. Note that Tc and Δ(T = 0) are roughly proportional to each other. In the standard theory of superconductivity by Bardeen, Cooper and Schrieffer, the BCS theory, this proportionality is predicted to be Δ(T = 0) = 1.76 kBTc, roughly in agreement with the experimental values for most materials (Ashcroft & Mermin, 1976).

When a particle, such as an energetic biomolecular ion, strikes the surface of an STJ detector, the kinetic energy of the particle is converted into heat, which in turn excites the conduction electrons of the superconducting layers. This excitation causes the formation of a small number of Cooper pairs, which in turn create a measurable change in the electrical resistance of the detector. The energy resolution of an STJ detector is determined by the temperature of the detector, the energy gap of the superconducting films, and the duration of the excitation process. The lower the temperature of the detector, the higher the energy resolution of the detector. The energy gap of the superconducting films and the duration of the excitation process are determined by the properties of the superconducting films and the properties of the incident particle, respectively.

FIGURE 1. Schematic cross section (a) and top view (b) of an STJ detector. The detector shown is diamond-shaped (magnetic field aligned with detector diagonal). Other common STJ detector shapes are square (magnetic field parallel to one side) and football-shaped (magnetic field along the axis of the football shape). Typical sizes range from several 10 μm to 200 μm.
energy of the ion creates many non-thermal phonons. A biomolecule impact creates phonons presumably with a wide distribution of energies. Phonons whose energy is greater than $2 \Delta$ can subsequently be absorbed by Cooper pairs in the superconducting films. In this process, Cooper pairs are broken and so-called quasiparticle excitations, or quasiparticles, are created, which can quantum-mechanically tunnel through the thin insulating barrier that separates the two superconducting films. Because only a few meV are required to break a Cooper pair, the impact of a biomolecular ion with kinetic energy of tens of keV produces millions of quasiparticles. When a small bias voltage of the order of 1 mV is applied to the tunnel junction, tunneling of the quasiparticles results in a measureable current pulse. This current pulse lasts until the quasiparticles have recombined back to Cooper pairs, typically a few microseconds. This process is illustrated in Fig. 2. The magnitude of the tunneling current pulse is proportional to the number of quasiparticles produced, which in turn is proportional to the energy deposited into the detector by an impacting ion. STJ detectors measure only a fraction of the total deposited energy, because only phonons with energy equal to or larger than $2 \Delta$ can break Cooper pairs and contribute to the signal. Nevertheless, the STJ signal is approximately proportional to the total energy. The pulse onset corresponds to the ion arrival time and can typically be measured to a precision of ~100 ns or better depending on details of the electronic readout circuit.

A variation of the simple STJ detector type shown in Fig. 1 is an STJ detector fabricated with superconducting quasiparticle trapping layers added on one or both sides of the tunnel barrier. Their function is to concentrate quasiparticle excitations near the tunnel barrier, and thus to increase the tunneling probability and the signal (Booth, 1987). Such STJ detectors have not yet been applied to TOF–MS, but are discussed here briefly because they show superior performance in X-ray measurements and therefore might also find TOF–MS applications in the near future. Trapping layers are made with a superconductor whose energy gap is lower than that of the other superconducting layers. One example of such a device would be a Nb–Al–Al$_2$O$_3$–Al–Nb STJ (Fig. 3a), in which the Al trapping layers have a smaller energy gap than the Nb layers (see Table 1). The operating principle is illustrated in Fig. 3b. In this device, quasiparticles created in the Nb can diffuse to the Al, where they can relax energetically by emitting a phonon. With the correspondingly lower energy, they cannot return into the Nb; they are “trapped” in the Al and thus concentrated near the tunnel barrier. Typically, STJs with trapping layers have larger signal and better energy resolution, but must be operated at a lower temperature to avoid thermal quasiparticle excitation in the lower-gap trapping layers. Such devices have been operated very successfully as high-resolution X-ray detectors (Mears, Labov, & Bark- knecht, 1993; Frank et al., 1998a). When operated as a biomolecule detector, STJ detectors with trapping layers could capture a larger fraction of the impact energy because the lower gap superconductor can absorb phonons with correspondingly lower energy. Thus, the quasiparticle trapping and the lower phonon threshold would both enhance the signal.

STJ detectors are usually relatively small, with single pixel detectors rarely exceeding areas of $\sim 200 \times 200$ $\mu$m$^2$. Increasing the size of single detector pixels much beyond that size seems impractical because this increase would also increase the detector capacitance and the noise. More
practical ways of increasing the area of STJ detectors are discussed in section IV B.

The STJ detector bias and electronic readout can be provided by simple electronics. Because STJ detectors have a relatively large impedance ($\sim k\Omega$) under typical operating conditions, the readout can be provided by relatively simple and inexpensive FET-based preamplifiers, which can be coupled to conventional analog or digital nuclear detector signal processing electronics. In addition to the bias voltage, a small magnetic field ($\sim 100$ Gauss) must be applied parallel to the plane of the tunnel junction for proper operation (see Fig. 1). The magnetic field helps to suppress the dc Josephson effect, a large supercurrent flowing even at zero bias voltage, which is caused by Cooper pair tunneling, and other detrimental effects, such as Fiske resonances caused by the ac Josephson effect (Fiske, 1964). In practice, this suppression is easily achieved by placing the STJ detector in a small magnetic field coil.

C. Hot-Electron Microcalorimeters

Another type of cryogenic detector that has also been applied to TOF–MS measurements is the hot-electron microcalorimeter (Fig. 4). It consists of a normal metal absorber film coupled to a very sensitive thermometer such as a normal metal–insulator–superconductor (NIS) tunnel junction or a transition edge sensor, both explained further below in this section. These detectors are a family of equilibrium devices. They are true calorimetric detectors because they measure the temperature rise ultimately generated in the detector by a molecule’s

FIGURE 3. Schematic cross section (a) and operating principle (b) of a Nb STJ detector with Al quasiparticle trapping layers near the tunnel barrier. The basic principle is similar to that shown in Fig. 2, except that the trapping layers provide signal enhancement. Quasiparticles created in the Nb by phonons from a particle impact can diffuse to the Al, which has a lower superconducting energy gap energy, and relax energetically by emitting a phonon. With the resulting lower energy, they cannot return to the Nb; thus, they become “trapped” near the tunnel barrier. As a result the overall tunneling rate increases, and the current signal is enhanced.

FIGURE 4. Schematic cross section (a) and top view (b) of a hot-electron microcalorimeter. The cross section is from Hilton et al., 1998. The top view is a simplified sketch. The temperature rise caused by an ion impact onto the absorber is proportional to the deposited energy, and is measured with the sensitive thermometer attached to the absorber. Commonly used thermometers are NIS tunnel junction sensors and transition edge sensors, read out with a SQUID-based current preamplifier. To maximize the temperature signal, the absorber is often placed on a thin membrane as shown here to prevent phonons from escaping the detector before they contribute to the heating of the electrons in the metal absorber. A cooled aperture not shown in (b) limits the detector’s exposure to infrared radiation, and also prevents ions from directly striking the thermometer. (Figure 4a reprinted from Hilton et al., 1998, with permission from Nature.)
impact. Initially, the impact of an energetic molecule onto the normal metal absorber creates a broad range of non-thermal phonons with a broad range of energies similar to what was described above for the STJ detectors. These phonons are quickly absorbed by the electrons in the metal, which in turn rapidly share the energy with the other electrons by electron–electron scattering. As a result, the whole electron system in the metal heats up creating a measurable temperature rise, which is proportional to the deposited energy. After the particle event, the detector cools back to its initial temperature with a time constant, which is given by the detector heat capacity and the detector’s thermal coupling to the substrate, and is typically tens to hundreds of microseconds. Except for a very short time right after a particle impact, the detector is near thermal equilibrium during the measurement of the heat pulse.

The temperature signal of hot-electron microcalorimeters is often maximized by placing them on very thin membranes made of a mechanically strong insulator, such as Si3N4. This configuration ensures that nearly all of the phonons are absorbed in the normal metal absorber and do not escape to the substrate before absorption occurs.

As opposed to STJ detectors, there is no phonon energy threshold for the absorption process in a normal metal, because a normal metal, in contrast to a superconductor, does not have an energy gap. Therefore, hot-electron microcalorimeters can, in principle, capture all the deposited energy and not just a fraction, as STJ detectors do. This fact is one of the reasons why hot-electron microcalorimeters generally have a better energy resolution than STJ detectors. On the other hand, because they are near-equilibrium detectors, their response is somewhat slower than that of STJs. Generally, the pulse durations of thermal detectors are determined by their cooling times, which are in the range of tens of microseconds or more for typical hot-electron microcalorimeters. Moreover, microcalorimeters must be operated at very low temperature (~0.1 K or below) for best performance. The lower the operating temperature of a thermal detector (and the smaller the detector) the smaller is its heat capacity and the larger is the detector signal.

A crucial part of a microcalorimeter is the sensitive thermometer used to measure the particle-induced temperature rise of the absorber. Commonly used thermometers are NIS tunnel junctions and transition edge sensors. Although microcalorimeters with NIS sensors have already been used in TOF–MS, microcalorimeters with transition edge sensors may prove advantageous for this application in the near future. Both types are briefly described here.

NIS tunnel junctions consist of one layer of normal conducting metal (N) and one layer of superconductor (S) separated by a thin insulating barrier (I); for example, Cu–Al2O3–Al or Ag–Al2O3–Al (Fig 5a). Typical bias voltages are of the order of mV or below, similar to those for SIS tunnel junctions. Under proper bias conditions, the tunneling current in an NIS tunnel junction is a very sensitive function of the temperature of the normal metal electrode and of the microcalorimeter absorber attached to it (Nahum, Martinis, & Castles, 1993; Nahum, Richards, & Mears, 1993). The operating principle is illustrated in (Fig. 5b). Following a particle impact onto the absorber, an NIS tunnel junction sensor measures the absorber temperature rise by producing a tunneling current pulse whose height is proportional to the temperature rise and thus to the deposited energy.

Transition edge sensors (TESs) are another type of sensitive thermometers used in microcalorimeters. A TES consists of a thin film of superconductor and is operated within its transition region between the superconducting and the normal conducting states. In this narrow transition region, the electrical resistance of a TES is a very sensitive function of temperature (Fig. 6). The short temperature rise caused by the impact of a particle onto an absorber connected to a TES briefly changes the resistance of the TES, and this change is easily measured with the proper readout circuit, usually as a current pulse. TES sensors can be made either of pure superconductors, such as Nb, Ta, Al, Ti or Ir, or of bilayers or multilayers of normal metals and superconducting metals, e.g., Ag/Al, Cu/Al or Au/Ir. The deposition of a normal metal film onto a superconducting film lowers the superconducting transition temperature because of the so-called proximity effect. This technique is often used to tune the critical temperature and thus the operating temperature of a TES-based microcalorimeter to a lower value to increase its sensitivity. (Most of the pure superconductors have too high or too low transition temperatures to be useful.) In recent years, very sensitive hot-electron microcalorimeters with TES thermometers operating at ~100 mK have been developed very successfully for high-resolution X-ray spectroscopy in materials analysis by the group of J. Martinis at NIST, Boulder, CO (Wollman et al., 1997).

Some of the general characteristics of hot-electron microcalorimeters are summarized and compared to STJ detectors in Table 2. As opposed to STJ detectors based on SIS tunnel junctions, microcalorimeters with NIS tunnel junction sensors or TESs do not need an applied magnetic field for proper operation. In fact, a magnetic field may actually be detrimental to the operation of a TES because magnetic fields can broaden the superconducting phase transition and thus render a TES less sensitive. On the other hand, because microcalorimeters measure signals based on temperature rise, they are sensitive to temperature fluctuations of the cryostat in which they are operated. Hot-electron microcalorimeters, therefore, require good temperature stabilization, a feature not needed during STJ
detector operation. Because NIS tunnel junctions and TESs used for hot-electron microcalorimeters are typically low-impedance devices, they are poorly matched to conventional, FET-based preamplifiers. Instead, current amplifiers based on Superconducting Quantum Interference Devices (SQUIDs) are commonly used for the readout of hot-electron microcalorimeters. In practice, SQUID-based amplifiers are viewed as somewhat more complicated and expensive than their FET-based counterparts; that view probably results mostly from the fact that they are not as commonly used.

D. Energy Calibration

The signal from a cryogenic detector is usually a current pulse with a pulse height proportional to the energy deposited by the impacting particle. Like other energy-dispersive radiation detectors, cryogenic detectors must be calibrated to relate the measured pulse height to the energy. Radioactive sources that emit X-ray photons of known energy, for example, $^{55}$Fe emitting predominantly X-rays with an energy of 5.89 keV (often loosely called “6 keV X-rays”), are commonly used for detector calibration. In addition, hot-electron microcalorimeters can also be calibrated by passing a short current pulse of known heating power through the detector. TES-based microcalorimeters offer yet another, absolute way of energy calibration, if operated in the so-called extreme electro-thermal feedback mode. This mode is not further discussed here. For more details see Irwin, (1995).

When the pulse heights from X-ray or heater calibration pulses measured with a cryogenic detector are compared to pulses from ion impacts onto the detector, it is important to keep in mind that the mechanisms of energy deposition and signal creation are different. Biomolecule impacts take place on the surface of a cryogenic detector and create a non-thermal phonon shower which is subsequently absorbed by free electrons or Cooper pairs. On the other hand, X-ray absorption events or temperature pulses created by heating take place in the bulk of an absorber film and transfer energy directly to the electron system of the detector.

This difference is most relevant for STJ detectors because of their phonon energy threshold discussed above. In STJ detectors, the signal from a biomolecule impact is created only from a fraction of its energy; specifically, the part of its energy which is converted into non-thermal phonons with energy greater than twice the superconducting gap energy. Thus, the signal heights from biomolecules are likely to be smaller than those of X-rays of the

![FIGURE 5. Schematic cross section (a) and operating principle (b) of a normal metal/insulator/superconductor (NIS) tunnel junction sensor attached to a normal metal absorber of a hot-electron microcalorimeter. The energy distribution of electrons on the normal metal side of the tunnel barrier is indicated by the solid lines on the left side of the tunnel barrier in (b). $E_F$ is the Fermi energy. On the superconducting side, practically all the states below the energy gap are filled, and tunneling is allowed only into the states above the energy gap $\Delta$. Thus, only the “hot electrons”, i.e. the electrons with high enough energy, can tunnel from the normal metal to the superconducting side. The fraction of electrons that can tunnel increases rapidly with the temperature of the normal metal, rendering the NIS tunnel junction a very sensitive thermometer. The largest temperature sensitivity of the tunneling current is obtained when the NIS junction is biased such that the bias voltage $V_{bias} \sim \Delta/e$.](image1.png)

![FIGURE 6. Operating principle of a transition edge sensor (TES). When the sensor is operated in the narrow phase transition region between the superconducting state (zero resistance) and the normal conducting state (normal resistance $R_n$), the resistance is a very sensitive function of temperature. A small temperature change $\Delta T$ causes a relatively large resistance change $\Delta R$, which is measured.](image2.png)
same energy. Nevertheless, the pulse heights for biomolecules are proportional to the energy, as long as the number of these phonons created upon the particle impact is proportional to the energy; in practice, that proportionality seems to be the case.

For hot-electron microcalorimeters, the comparison between biomolecule impacts and X-rays is less problematic because most of the deposited energy will ultimately be converted to heat, which is measured as a temperature rise of the detector. It does not matter whether the initial excitation is an energetic photoelectron created by an X-ray and subsequently sharing its energy with other electrons in the normal metal absorber or the initial excitations are phonons created by molecular impact, which are then absorbed by the electrons. Of course, this statement is only true as long as the phonons cannot leave the absorber film and carry energy away; preventing phonon loss is usually assured by placing the absorber on a thin membrane.

Note that although hot-electron microcalorimeters can be considered the most efficient detectors for the energy of an impinging particle, some of the impact energy may not result in heating and, therefore, not contribute to the microcalorimeter temperature signal. It is plausible for some of the impact energy to be lost via other channels (for experimental evidence see Section IV D). Energy loss mechanisms include plastic deformation of the absorber film, breaking of molecular bonds in the impacting biomolecule, and ejection or rebounding of parts of the molecule, all of which will absorb or carry away energy and thus reduce the observed heating. A better microscopic understanding of the detailed energy transfer processes that occur during the impact of a large molecule onto a detector surface should be obtainable when responses from different cryogenic detector types to the same molecules are compared. Microcalorimeters are especially suited to study these processes, because they are capable of measuring total deposited energies without phonon threshold. It may also be possible to sort out the various effects when the responses of STJ detectors made of different superconducting materials with different energy gaps and different phonon thresholds are compared.

E. Detector Signal and Signal Processing Compared to Microchannel Plates

Starting with the MALDI laser pulse and extending for some time period (typically hundreds of microseconds), the output signal of a cryogenic detector applied to TOF–MS contains pulses caused by impacting molecular ions (for an example see Fig. 8 below). Compared to the signal from microchannel plates, where detector pulses are very short (~10 ns) and pulse height is independent of ion energy (when operated in saturation mode), the pulses measured with cryogenic detectors are relatively long (equivalent to microseconds) and their pulse height contains energy information. The onset of each pulse in such a cryogenic detector record corresponds to the arrival time of one or more ions and the height of the pulse is proportional to the deposited energy. Because of the small size of cryogenic detectors, most pulses are typically caused by individual ions that impact the detector and the pulse height corresponds to the energy of individual ions. Only when the ion flux is large will more than one ion with the same mass arrive at the same time, causing a single pulse with correspondingly larger height. Because the pulse height is proportional to the deposited energy, it is proportional to the ion charge and the number of ions that impact simultaneously onto the cryogenic detector. A cryogenic detector cannot distinguish on an event-by-event basis the simultaneous arrival of two identical, singly charged ions from the arrival of a doubly charged dimer. Such a distinction can, however, be made on a statistical basis when singly charged multimers are also observed at other flight times.

The cryogenic detector signal can, in principle, be recorded and processed in the same modes used for MCP signals: 1) the transient mode, i.e., using a transient recorder to digitize and simply average single-shot records, and 2) the pulse counting mode, i.e., performing
single pulse counting using a combination of analog and digital electronics to determine ion arrival times, which can be histogrammed to obtain a TOF spectrum. In the second mode, the pulse height for every ion impact pulse can also be obtained (energy-resolved pulse counting).

The first mode, simple averaging or co-adding of digitized transients, as is commonly done with signals from MCPs, is not ideal for processing data from a cryogenic detector because the energy information contained in the pulse height of individual pulses is lost by averaging. In the worst case, one single, very large pulse caused by an ion with high charge and thus high energy may give rise to a deceivingly tall peak in a spectrum obtained by averaging single-shot records measured with a cryogenic detector. When creating a TOF spectrum by averaging single-shot records from a cryogenic detector, one must also keep in mind that the pulses from individual ion impacts are relatively wide. On the one hand, the wider cryogenic detector pulses accumulate more readily on top of each other than the narrow MCP pulses and thus seem to enhance the peaks in the averaged cryogenic detector mass spectrum. (Of course, this enhancement could also be achieved with stretching the pulses from MCPs). On the other hand, a more subtle but important consequence of the relatively large width of cryogenic detector pulses is that the pulse maximum is noticeably delayed compared to the actual arrival time of the ion, which causes the pulse. TOF peaks in averaged spectra correspond to pulse maximum times and not to the pulse onset times, which are the true ion arrival times.

A more appropriate and meaningful way to analyze cryogenic detector pulses in TOF–MS is the energy-resolved pulse counting mode, where both the onset time of each pulse and its height for all the individual ion impact pulses are obtained and recorded. One can imagine at least two implementations of this method. One implementation is equivalent to the combination of analog and digital electronics previously employed, e.g., by the Orsay group (Brunelle et al., 1993) for recording MCP pulses in the secondary electron resolved mode. Brunelle et al. used a constant-fraction discriminator coupled to a time-to-digital converter (TDC) to determine ion arrival times in combination with a charge-to-digital converter (CDC), which recorded the pulse heights. In such a scheme, the processing of the detector signal is fast and could easily be performed at kHz shot rates. The other implementation is based on recording many individual digitized single-shot records (such as the one shown in Fig. 8) and on applying a digital post-processing procedure to extract the pulse onset times and corresponding pulse heights from all the stored digital single-shot records. Such a scheme has been used by all of the TOF–MS experiments with cryogenic detectors discussed in this review. Note that digital post-processing requires full recording of complete transients in fine time steps during the data acquisition. Even with modern digitizers this is a relatively slow process limiting the repetition rate during the data acquisition presently to ~10 Hz. Also the digital post-processing is relatively slow, because it requires considerable computing power, and is usually performed off-line after the data has been taken.

If very accurate pulse height information is desired, pile-up rejection procedures can be applied in order to process only cryogenic detector pulses unaffected by pulse pile-up. (Pulse pile-up occurs when too many ions hit the detector within a short time interval and the pulses from individual ion impacts overlap). Such pile-up cuts can be added to both implementations of the energy-resolved pulse counting mode.

For both implementations of the energy-resolved pulse counting mode the processed data consist of a set of pairs of arrival time and pulse height. From these data, one can easily generate one-dimensional time-of-flight spectra by histograming all of the measured pulse onset times exactly as for MCP data obtained in the single-pulse counting mode. In addition, with the cryogenic detector pulse height data, one can also generate energy spectra of impact events by histograming the measured pulse heights or, more informatively produce two-dimensional time-of-flight vs. ion energy scatter plots (for examples see Section III below). When pulse heights and onset times are extracted on an event-by-event basis in a pulse-counting mode, the final TOF data are practically free of electronic noise because the thresholding eliminates most of the noise (for both cryogenic detectors and MCPs). In contrast, in spectra obtained by averaging transients the noise is merely reduced by averaging but not completely suppressed.

III. TOF–MS EXPERIMENTS WITH CRYOGENIC DETECTORS

A. History

1. Molecular Beam and Ion Detection with Cryogenic Detectors

Although the practical application of cryogenic detectors to time-of-flight mass spectrometry is fairly new, such detectors have already been suggested and used for measuring atomic and molecular beams and their internal and kinetic energy distributions and for measuring the energy of heavy atomic ions.

In 1967, Cavallini, Gallinaro, and Scholes demonstrated that a commercially available, semiconductor-based infrared bolometer could be used to measure neutral
molecular beams with energies down to the thermal (300 K, or ~25 meV) range with a performance comparable to that of more complicated systems based on bulky electron bombardment ionizers and mass filters (Cavallini, Gallinaro, & Scoles, 1967; Cavallini et al., 1971). In their pioneering experiments, they measured the heating power that resulted from the flux of impinging particles onto the Ge bolometer operated at ~2 K and achieves a sensitivity of $2 \times 10^8$ molecules s$^{-1}$. The bolometer was not sensitive enough, though, to detect individual molecules, in part because of the relatively low energy of the molecules (~eV). Cavallini et al. pointed out the advantages of this bolometric measurement technique for detection of molecular beams and, in particular, scattering experiments in the energy range of 0.1–10 eV. In subsequent years, several scattering experiments were performed using the bolometric detection technique (Cantini et al., 1968; Dondi et al., 1969; Bassi et al., 1976). Semiconductor-based bolometers were also used to measure the energy released by the adsorption and recombination of hydrogen on low-temperature surfaces (Marenco et al., 1972; Schutte et al., 1976).

Shortly after introducing semiconductor-based bolometers for molecular beam detection, Cavallini et al. also explored the use of superconducting bolometers in this application (Cavallini, Gallinaro, & Scoles, 1969). In 1969, using a superconducting tin bolometer, they achieved a detection limit of $7 \times 10^6$ atoms s$^{-1}$ for Ar atoms, a significant improvement of the sensitivity compared to the semiconductor-based bolometers. The superconducting bolometer consisted of a tin film on a mylar foil operated as a transition edge sensor at the superconducting transition of tin at 3.7 K. More details on the construction and operation of superconducting tin and indium bolometers used for molecular beam detection can be found in (Gallinaro & Varone, 1975; Gallinaro, Roba, & Taterek, 1978). Although superconducting bolometers were shown to provide a sensitivity advantage over semiconductor-based bolometers operating at the same temperature, they were not widely used in the following years because their sharp superconducting phase transition severely limited the operating temperature range and required good temperature stabilization. On the other hand, it was recognized that superconducting tin bolometers could provide a much higher speed than semiconductor-based bolometers. Tin bolometers could be designed to have a response time of ~1 μs, rendering them useful for time-resolved studies.

In 1981, Bassi et al. demonstrated the feasibility of performing time-resolved laser-bolometric spectroscopy on molecular beams using a superconducting tin bolometer (Bassi et al., 1981). With this detector, Bassi et al. measured the kinetic energy distributions of SF$_6$ molecules in selected internal states created by an infrared laser beam. The laser excitation was pulsed, and the time structure of the signal measured with the bolometer was used to obtain additional information on the kinetic energy spread of the SF$_6$ molecules. The intrinsic bolometer response time was ~1 μs. However, in their experimental setup the actual time resolution was limited to ~50 μs by the electronic read-out circuit. Molecular beam experiments using bolometers continued throughout the years. For another more recent molecular beam application see, e.g., Sanna, Nardi, & Tomassetti (1990).

Cryogenic detectors can also be used for energy-sensitive detection of heavy (atomic) ions. Small cryogenic calorimeters with sapphire absorbers have been used by a group at the Gesellschaft für Schwerionenforschung (GSI) Darmstadt, Germany, to measure the energy of individual ions with GeV energies from cooled heavy ion beams in storage rings. They used two types of calorimetric detectors, both with sapphire absorbers with a volume of several mm$^3$ but different thermometers: semiconducting Ge thermistors (Kienlin et al., 1991, 1996) and superconducting aluminum transition edge sensors (Meier et al., 1993, 1997). The pulse decay time for these detectors was in the range of 500 μs–4 ms, due to the relatively large heat capacity of the absorber; however, time resolution was not required for the ion energy measurements. With one of their TES-based calorimeters operating at 1.5 K they achieved an energy resolution of $\Delta E/E \sim 1.1 \times 10^{-3}$ for $^{238}$U ions with an energy of ~87 GeV (360 MeV/nucleon), better than the resolution achievable with conventional, ionization-based detectors.

Another type of cryogenic ion detector for heavy ions was suggested by A. Benoit et al. in 1993. They explored the idea of using a microcalorimeter based on a normal metal–insulator–superconductor (NIS) tunnel junction thermometer coupled to a micrometer-thick gold absorber for the energy measurement of MeV ions; e.g., from Rutherford backscattering (Benoit, Martin, & Pannetier, 1993). They estimated that an energy resolution of $\Delta E/E \sim 1/1000$ and a time constant of ~50 μs should be obtainable with such a detector.

2. Cryogenic Detectors Proposed for TOF–MS

Expecting that detector performance (i.e., a velocity threshold imposed by ionization-based detectors) might ultimately impose an upper mass limit on MALDI–TOF–MS, Jaklevic et al. in 1991 anticipated the need to develop better ion detectors for the large biomolecular ions produced by MALDI (Jaklevic, Benner, & Katz, 1991). The cryogenic detectors they considered at that time were mainly bolometers with semiconductor thermistor sensors. These bolometers were too slow for practical TOF applications and the idea was not further pursued.
In 1995, D. Twerenbold introduced the idea of using cryogenic particle detectors in MALDI–TOF–MS to the cryogenic detector community at the 6th International Workshop on Low Temperature Detectors, LTD-6 (Twerenbold, 1996a). At this meeting, Twerenbold described the detection efficiency problem encountered in TOF–MS of large molecules and outlined how TOF–MS and, in particular, DNA sequencing by mass spectrometry may benefit from the use of cryogenic detectors. Soon thereafter, Twerenbold and collaborators performed the first experiment that demonstrated the feasibility of using cryogenic detectors in MALDI–TOF–MS, as described below. Twerenbold now holds a patent for application of cryogenic detectors in mass spectrometry (Twerenbold, 1997).

### B. Applications of STJs to TOF–MS

#### 1. First Results with Sn-Based STJ Detectors

Twerenbold and collaborators performed the first MALDI–TOF–MS experiments with cryogenic detectors (Twerenbold et al., 1996a). The detectors were Sn/Sn-oxide/Sn SIS tunnel junctions with a sensitive area of $20 \times 80 \mu m^2$ operated at a temperature of $\sim 0.4$ K in a $^3$He cryostat attached to a custom-made MALDI–TOF–MS system. The sample consisted of lysozyme (MW $\approx 14,300$ Da) in sinapinic acid matrix. In this first experiment, Twerenbold et al. observed detector pulses that corresponded to the impact of individual lysozyme molecules at the expected flight time. An energy calibration of the Sn STJ detector was performed with a $^{55}$Fe radioactive source. The observed detector pulses caused by 5.89 keV X-rays were reported to have about the same pulse height and pulse shape as the pulses produced by the protein ions with a somewhat larger kinetic energy of 10 kV observed some tens of microseconds after the laser shots. This observation confirmed that the pulses measured with the protein sample were, indeed, caused by the impact of individual protein ions. (As discussed in Section II E above, the relatively smaller detector response for molecules stopped at the surface compared to X-rays absorbed in the bulk of the thin-film structure is expected.)

They obtained time-of-flight spectra by determining the onset time of each ion pulse in the digitized records of single laser shots and histograming the arrival times. In Fig. 7a, the time-of-flight histogram measured with an acceleration voltage of 20 kV is shown for events between $t = 0$ and $t = 70 \mu s$. The first peak at $\sim 8 \mu s$ corresponds to sinapinic acid molecules, which had this flight time for $U = 20$ kV and $d = 105$ cm. The second peak is due to lysozyme proteins with a time-of-flight of 63 $\mu s$. (b) Same TOF spectrum for events between 20 and 170 $\mu s$. (Reprinted from Twerenbold et al., 1996, with permission by the American Institute of Physics.)

![Time-of-flight spectra obtained with a Sn STJ detector from a lysozyme sample in the first demonstration experiment (Twerenbold et al., 1996). The acceleration voltage was 20 kV. (a) TOF spectrum for events between $t = 0$ and $t = 70 \mu s$. The first peak at $\sim 8 \mu s$ corresponds to sinapinic acid molecules, which had this flight time for $U = 20$ kV and $d = 105$ cm. The second peak is due to lysozyme proteins with a time-of-flight of 63 $\mu s$. (b) Same TOF spectrum for events between 20 and 170 $\mu s$. (Reprinted from Twerenbold et al., 1996, with permission by the American Institute of Physics.)](image)

#### 2. First Experiments with Nb-Based STJ Detectors

Shortly after Twerenbold reported his initial results, we began to evaluate a superconducting Nb–Al$_2$O$_3$–Nb tunnel junction detector that operated at 1.3 K as ion detector in our MALDI–TOF–mass spectrometer (Frank et al., 1996b; Benner et al., 1997a). We modified our mass spectrometer and attached a small $^4$He cryostat at the end of the flight tube, used to cool our STJ detector. We also added a microchannel plate ion detector mounted slightly off-axis. Deflection plates allowed ions to be directed towards either the STJ detector or the MCP. Our STJ detector was mounted in the cryostat vacuum in good thermal contact with the pumped liquid helium reservoir at a temperature of $\sim 1.3$ K. A pair of 2 mm diameter...
collimators, cooled to 1.3 K, was placed in front of the STJ detector to limit its exposure to infrared radiation from the flight tube at room temperature. A radioactive \(^{55}\)Fe calibration source could be inserted in front of the STJ detector. The active area of the STJ detector was 0.04 mm\(^2\), about 25 times larger than the area of the Sn STJ detectors used in Twerenbold’s experiments.

Figure 8 shows the digitized response of the STJ detector to ions generated from a MALDI sample that contained human serum albumin (HSA, MW \(\approx 66,300\) Da) with a single laser shot. The pulses rising above the baseline were due to the impact of ions. Individual ions striking the STJ generate STJ detector pulses that have a rise time of 500 ns and a decay time of about 1.5 \(\mu\)s. Even though the STJ detector had an active area of only 0.04 mm\(^2\), about 25 times larger than the area of the Sn STJ detectors used in Twerenbold’s experiments.

Figure 8 shows the digitized response of the STJ detector to ions generated from a MALDI sample that contained human serum albumin (HSA, MW = 66,300 Da) with a single laser shot. The pulses rising above the baseline were due to the impact of ions. Individual ions striking the STJ generate STJ detector pulses that have a rise time of 500 ns and a decay time of about 1.5 \(\mu\)s. Even though the STJ detector had an active area of only 0.04 mm\(^2\), many ions hit the detector every time the laser was fired. Pulse pileup obscured the arrival of individual ions at short flight times, but the pulses after about 40 \(\mu\)s in this figure were attributed to the impacts of individual ions. Pulses occurring at the expected flight times for HSA\(^{3+}\), HSA\(^{2+}\), HSA\(^+\), or 2HSA\(^{2+}\), and 2HSA\(^+\) are labeled. The pulse heights of the 3+ ion and the 2+ ion, respectively, are about three times and two times as large as singly charged ion pulses, as expected for the output of an energy-responding detector in this application.

Figure 9a shows a scatter plot of STJ signal pulse height vs. flight time that was derived from the digitized records of the STJ detector output from 500 single shots at the MALDI HSA sample. Every point in this plot corresponds to a detector pulse whose onset time and height were extracted from the single-shot records by a simple digital data analysis procedure. When these points were histogrammed into time bins, a typical-looking TOF mass spectrum (Fig. 9b) was obtained. By histogramming ion pulses with heights less than 250 mV, i.e., those below the horizontal line in Fig. 9a, a TOF spectrum that is comprised primarily of singly charged ions was obtained (Fig. 9c). When Fig. 9b and Fig. 9c were compared, it was concluded that the events in the “dark band” of points near the bottom of Fig. 9a correspond to singly charged ions. Clusters of points lying above the dark band in Fig 9a correspond to multiply charged ions, as was revealed by the histogram presented in Fig. 9d. This type of pulse height analysis of the data showed, for the first time, that the energy-dependent response of a cryogenic detector provides a way to discriminate ions of different charges. A similar data analysis procedure was applied to ion data.
obtained from a mixture containing HSA and immunoglobulin G (IgG, MW $\approx 150,000$ Da). It was shown that the energy-resolving capability of a cryogenic detector is useful for sorting out TOF peaks from a variety of singly and multiply charged ions.

The charge discrimination demonstrated with the STJ detector in these experiments was not perfect. For example, the pulse height cut at 250 mV in Fig. 9a did not discriminate perfectly singly charged from multiply charged ions, as evidenced, for example, by the presence of HSA$^{2+}$ peaks in Fig. 9c. Imperfect discrimination may have occurred for several reasons. First, the STJ detector appeared to have a much poorer energy resolution for biomolecular ions than expected from the measured X-ray resolution. A second reason for poor charge discrimination could have been the result from the way the pulse height cut was made. The top of the dark bands comprising presumably mostly singly charged ion events is not flat, and perhaps the height of the cut should increase with flight time. Possible explanations for the apparent poor energy resolution for biomolecular ions are discussed below in Section III D, where the observed energy responses of the different cryogenic detector types used in the experiments reviewed here are compared.

During these experiments with our Nb–Al$_2$O$_3$–Nb STJ detector, we also attempted to compare the detection efficiencies of the STJ detector and the MCP detector. Ions were alternatively guided to strike either the STJ detector or the MCP detector in our MALDI–TOF–MS system. Our data from this initial experiment indicated that the

FIGURE 9. Scatter plot of pulse height vs. TOF for an albumin sample measured with the Nb STJ detector by our LLNL/LBNL collaboration (Benner et al., 1997a) (a). Acceleration voltage was 25 kV. The pressure during the measurement ranged from $1 \times 10^{-6}$ Torr to $2 \times 10^{-7}$ Torr. (b) TOF spectrum derived from the data shown in (a). Charge discrimination was demonstrated by applying a pulse height cut to the data indicated by the line in (a), resulting in a TOF spectrum of mostly singly charged ions (c) for pulse heights smaller than 250 mV and a TOF spectrum of mostly multiply charged ions (d) for pulse heights larger than 250 mV. (Reprinted from Benner et al., 1997a, with permission by the American Society of Mass Spectrometry.)
detection efficiency per unit area of the STJ detector for 25 keV HSA ions was about two to three orders of magnitude higher than that of the MCP (Frank et al., 1996b). We are currently in the process to improve these experiments by using an MCP with an annular aperture and an STJ detector mounted behind the aperture. The use of an annular MCP detector avoids potential errors introduced by beam distortions that might be caused by deflecting the ion beam and will allow us a better comparison between STJ detector and MCP. (Note: In such a more careful measurement performed with an annular MCP operated in pulse counting mode we recently determined the efficiency of the MCP when constrained to the same effective area as the STJ detector for detecting 66 kDa ions with 20 keV energy to be about 3% of that of the STJ (Westmacott et al., 1999a, 1999b submitted to Rapid Commun in Mass Spectrum)—less than 2 orders of magnitude, but not too far from our initial crude determination. Note also that typical ion beam diameters at the ion detector are much larger than the present size of STJ detectors and that MCPs (when unconstrained) intercept more ions because of their larger size. Therefore, the overall probability for MCPs detecting a signal at 66 kDa can still be near 100%).

3. 750 kDa Protein Molecules Measured with an STJ Detector

In a more recent experiment, we have studied macroglobulin ions, which consist of four identical subunits with a total mass of 700 kDa, with an STJ detector (Benner et al., 1997b; Labov et al., 1997). Initial MALDI–TOF–MS measurements of macroglobulin showed a mass peak that corresponded to 350 kDa, but very little of the intact molecule appeared to survive the MALDI process. We then used chemical cross-linking to stabilize the full molecule. The measured time-of-flight spectrum obtained with the crosslinked macroglobulin (Mac), shown in Fig. 10, contains a small signal that corresponds to 750 kDa ions. The higher than expected mass associated with the peak at 638 µs is most likely due to the addition of two units of a 25 kDa protein, known to be present in the original sample, by the cross-linking process. No signals from 750 kDa ions were observed with an MCP detector operating slightly off-axis at the same time in our MALDI–TOF system.

Although the results obtained with cross-linked macroglobulin clearly demonstrated that STJ detectors are in fact sensitive to very massive, slow-moving molecules, these results also helped to elucidate the extent to which fragmentation broadens TOF peaks and decreases mass resolution. The peaks for Mac2+ and Mac4+ in Fig. 10 are broad and appear to be skewed towards short flight times; that phenomenon may be considered indicative of fragmentation. A better understanding of the spread in arrival times can be obtained when the scatter plot of measured pulse height (proportional to ion energy) vs. flight time for the same ion data is examined (Fig. 11). Every point in this plot corresponds to one ion striking the detector. In this plot, several slanted or slightly curved clusters of data points are noticed that correspond to the TOF peaks seen in Fig. 10. The vertical range of the clusters indicates a variation in energy for a particular ion and its width indicates the variation in ion arrival times. In the absence of fragmentation, one would expect fairly well-localized clusters; for example, one concentrated cluster of points at ~638 µs and ~200 mV, corresponding to the singly charged, full macroglobulin molecules that contain all four subunits and another concentrated cluster at ~450 µs and ~200 mV, corresponding to the singly charged, “half” macroglobulin molecules that contain two of the four subunits. The large extent of these clusters in the data is indicative for significant fragmentation. The slanting and curved appearance of the clusters reveals that the ions that carry less kinetic energy than the full 30 keV actually travel at a faster speed and have shorter flight times. The fact that the clusters slant or curve can not be explained by metastable decay in free flight or ion energy straggling. Metastable decay and in-free-flight fragmentation would lead to relatively narrow vertical clusters in the scatter plot, as seen in data obtained by the NIST group and described below. The cause for cluster curvature seen in Fig. 11 is

![FIGURE 10. TOF spectrum obtained by our LLNL/LBNL collaboration using an Nb STJ detector to measure a sample that contained chemically cross-linked macroglobulin to demonstrate the high-mass capability of the STJ detector. Acceleration voltage was 30 keV. The intact molecule (“Mac”) is a tetramer of four identical subunits. Also contributing to the peaks in this spectrum are “half” molecules (“1/2Mac”) consisting of only two subunits, as is revealed by the corresponding scatter plot pulse height vs. TOF shown in Fig. 11. (Reprinted from Labov et al., 1997, with permission by the Max Planck Institute for Physics.)](image)
due to in-source fragmentation. The shape of the clusters can be reproduced with a model that we recently developed (Benner et al., 1997b; Frank et al., 1999, manuscript in preparation). As an unexpected result of the modeling work, we were able to identify a region near the grounded grid in our MALDI source, where most of the fragmentation occurs. Fragmentation occurring late in the accelerating process can cause the observed vertical, slightly curved patterns in the scatter plot of Fig. 11. Fragmentation earlier during the acceleration would lead to a larger curvature. The reason why these ions tended to fragment as they approach the grid is not yet understood and is under investigation.

4. DNA Oligonucleotides Measured with an STJ Detector

D. Gerber et al. (1997) and D. Twerenbold et al. (1997) reported the first detection of DNA oligonucleotides with cryogenic detectors at the 7th International Workshop on Low Temperature Detectors (LTD-7) in Munich (Gerber et al., 1997) and at the 9th International Genome Sequencing and Analysis Conference in Hilton Head (Twerenbold et al., 1997). Their Sn STJ detectors had an area of $50 \times 50 \, \mu m^2$. The operating temperature was 0.6 K. The experimental setup was similar to the one used by these authors before, with additional improvements that included better ion focusing and the use of delayed extraction of the ions.

Gerber et al. used oligomers with three different sizes, a 40-mer, a 70-mer, and a 120-mer for their measurements. Figure 12a shows the TOF spectrum of a sample with an equimolar concentration of the 40-mer and the 70 mer, and Fig. 12b shows the TOF spectrum of a sample with the 120-mer. Figure 12c shows a control spectrum taken with just the hydroxypicolinic acid (HPA) matrix used to prepare the MALDI samples. All these measurements were performed at an acceleration voltage of $-12 \, kV$. The intensity of the mass peaks of the 40-mer and the 70-mer in Fig. 12a are about equal. Also, the peak height of the 120-mer in Fig. 12b is comparable to the 40-mer and 70-mer when the different number of laser shots is taken into account. The observation of comparable peak heights for 40-mer, 70-mer, and 120-mer seems to indicate that the launch, ionization, and detection efficiencies are equal and independent of the oligomer length up to 120-mers. Gerber et al. concluded that the current limitation of TOF–MS for DNA analysis may be a problem of detection sensitivity and that cryogenic detectors will have an impact on the use of TOF–MS for DNA analysis. (The authors’ conclusion that the current upper mass limit for the MALDI method stems from a lack of detection efficiency is not supported by results obtained by the Münster group, who obtained considerably higher mass limits with MALDI–TOF–MS for proteins (Berkenkamp et al., 1997) compared to DNA (Berkenkamp, Kirpekar, & Hillenkamp, 1998) presumably using similar detectors in both measurements.)

In the same papers Gerber et al. and Twerenbold et al. described their investigation of the STJ detector response and pulse height distributions using an IgG sample. They showed that doubly charged IgG molecules carrying 12 keV kinetic energy cause events with pulse heights about twice as large as singly charged IgG molecules carrying 6 keV kinetic energy. As in the case with the measurements with Nb-based STJs described above, Gerber et al. found that the measured energy spread of the molecules was larger than the “intrinsic” energy resolution of the Sn STJ detector, which was about 180 eV for 6 keV X-rays. More details on the observed energy response of the Sn STJ detector are discussed below in Section III D.

C. TOF–MS Experiments with a Hot-Electron Microcalorimeter

Hilton et al. performed the first experiments using a hot-electron microcalorimeter in time-of-flight mass spectrometry. Their results were presented at the 7th International Workshop on Low Temperature Detectors, in Munich, July 1997 and later published (Hilton et al., 1998). Hilton and collaborators used a microcalorimeter consisting of a $200 \times 200 \, \mu m^2$ silver absorbed on a Si$_3$N$_4$
membrane coupled to a NIS tunnel junction thermometer. This microcalorimeter was operated at a temperature of 100 mK, cooled by a liquid helium cryostat equipped with an adiabatic demagnetization refrigerator stage, and coupled to a custom-built MALDI–TOF system. A schematic cross section of their microcalorimeter is shown in Fig. 4a. A radioactive $^{55}$Fe source was mounted in the detector line-of-sight and used to calibrate the detector energy scale.

Figure 13a shows a single-shot record measured with the microcalorimeter and a bovine serum albumin (BSA, MW = 66,430 Da) sample. The two prominent pulses in this record were identified to be due to a single BSA$^{2+}$ and a single BSA$^+$ ion striking the detector. The pulse rise times are 1.2 $\mu$s, the decay time is 17 $\mu$s. Compared to pulses in the single-shot record measured with a Nb-based STJ detector (see Fig. 8 above), these microcalorimeter pulses are slower but show a better signal-to-noise ratio. The better signal-to-noise ratio may largely be attributable to the fact that the hot-electron microcalorimeter was operated at a lower temperature (100 mK vs. 1.3 K), reducing noise and providing an increased detector response. In addition, the hot-electron microcalorimeter can capture a larger fraction of the ion energy (because it has no phonon threshold), also increasing the signal.

Several thousand digitized single-shot records were obtained with the BSA sample for an accelerating voltage of $U = 20$ kV. Pulse times and heights were extracted by an off-line analysis procedure based on an edge-finding algorithm (for TOF extraction) and numerical fits of theoretical pulse shapes to events in single-shot records (for pulse height extraction). Using the energy calibration with 6 keV X-rays, the pulse heights were converted to energy. The resulting scatter plot of measured impact energies vs. arrival times is shown in Fig. 13b. The plot shows a clear energy banding, caused by the discrete ionization states of the particles. The clusters of events associated with BSA$^+$ and BSA$^{2+}$ are indicated in this figure. Additional clusters in this figure include sinapinic acid (MW = 224 Da, TOF = 10 $\mu$s) and an unidentified fragment (MW $\approx$ 14 kDa, TOF = 67 $\mu$s). In analogy to the pulse height cuts described for the STJ detector data above, pulse height cuts were made to demonstrate the charge discrimination (not shown here). Because of the better energy banding observed with the microcalorimeter, this discrimination was very efficient.

One of the most interesting results of these measurements was that the thermal energy $E_d$ deposited in the microcalorimeter by a protein particle impact was only about half of the particle’s kinetic energy $zU$. This result can be seen in Fig. 13b, where the band of events caused by singly charged ions (carrying 20 keV kinetic energy) is centered roughly around a pulse height of 10 keV. (Note that the arrival of BSA$^+$ at the flight time of 146 $\mu$s ...
expected for intact BSA and 20 keV confirms that the kinetic energy is, indeed, 20 keV and not 10 keV). This difference implies that a significant fraction of the particle’s kinetic energy is not converted to thermal energy and thus not detected. Even more exciting was the observation that the fraction of detected energy depended on the molecule type. This dependence is illustrated in Fig. 14, which is a plot of the ratio of observed impact energy (measured as thermal energy) to the kinetic energy vs. the kinetic energy for different ion types. Two general trends can be observed in this figure. As molecular mass increases, large molecules seem to deposit less of their kinetic energy as heat in the detector and, as kinetic energy is increased, the fraction of kinetic energy converted to thermal energy seems to decrease. For heavier ions, such as lysozyme and BSA, this fraction approaches 0.54 at large kinetic energies, whereas for the lighter SA ion the asymptote is about 0.72.

These results show that the impact of a molecular ion cannot be modeled as a rigid molecule striking and sticking to the detector. Hilton et al. listed several processes which may account for the “missing” energy, including fragmentation of the molecule hitting the detector (with the breaking of molecular bonds absorbing energy) and possible ejection of molecular fragments (carrying part of the energy away). Because there are many more molecular bonds in large molecules, more of their kinetic energy might be lost in those processes, partially explaining the smaller measured energy fraction observed for larger molecules. From the observation that the total bond energy of SA is less than the impact energy deficit, Hilton et al. further concluded that fragment ejection appears to be the dominant energy loss mechanism.

Hilton et al. also used their microcalorimeter to explore the effect of background gas pressure in the mass spectrometer on the ion energy distributions. The effect of pressure was clearly demonstrated, as can be seen in Fig. 15a, which contains two scatter plots of energy vs. flight time measured with a lysozyme sample at different pressures. The measurement at a low pressure of \(4 \times 10^{-5}\) Pa \((3 \times 10^{-7}\) Torr\) is shown on top in this figure. Using an estimate of 15 nm\(^2\) for the cross-sectional area of lysozyme ions, Hilton et al. calculated the mean free path of these molecules at this pressure to be \(\sim 6\) m, corresponding to about 6 times the flight tube length. In contrast, as can be seen at the bottom of Fig. 15a, a significant increase in the number of low energy \(<5\) keV\) fragments is observed when the pressure was increased to \(8 \times 10^{-4}\) Pa \((6 \times 10^{-6}\) Torr\) corresponding to a mean free path of only 0.3 m. The large number of low-energy ions that arrived at the predicted time for lysozyme was attributed to collision-induced fragmentation in the free-flight region. When fragments are produced in free flight,
after acceleration is completed, they travel at velocities nearly equal to that of intact analyte molecules (and arrive at the “right” time) but carry less kinetic energy. Fragmentation that occurs during acceleration, on the other hand, additionally affects the velocity and may explain some of the other events found in these scatter plots. The effect of this fragmentation on the measured impact energy spectrum is shown in Fig. 15b. The data at higher pressure show a low-energy peak around 2 keV and an enhanced low-energy tail of the 11 keV peak, corresponding to the intact lysozyme molecule with 20 keV kinetic energy. Hilton et al. noted that although there is evidence for in-flight fragmentation due to residual gas collisions, the energy straggling due to these collisions alone cannot explain their observed mass resolution. They concluded that additional effects contribute to broadening of flight times and that those effects must be sought in launch and acceleration.

D. Comparison of the Energy Response

In this section, we compare the energy response and energy resolution for biomolecular impacts measured with the three different types of cryogenic detectors used in the TOF–MS experiments described above: the niobium-based STJ detector; the tin-based STJ detector; and the hot-electron microcalorimeter. The experimental results are summarized in Table 3. First, we discuss the measured signal height from biomolecular impacts compared to X-rays. Second, we discuss the measured energy resolution and charge resolution achieved for biomolecules with these detectors.

As described above in detail in Section II D “Energy Calibration”, when the cryogenic detector response to X-rays is compared to response to biomolecular impacts, it is important to keep in mind that the initial mechanisms of energy deposition are different for X-rays and molecules. X-rays are absorbed by electrons throughout the absorber film, whereas biomolecules are stopped at the surface of the absorber and create phonons, which are then absorbed by the electrons. In STJ detectors, only phonons with an energy greater than twice the superconducting gap energy can interact with the Cooper pairs and contribute to the signal. Hot-electron microcalorimeters do not exhibit this phonon energy threshold and should capture all of the impact energy converted to phonons.

Given these facts, one would naively expect that the signal heights for X-rays and biomolecules of the same energy are about equal when measured with a hot-electron microcalorimeter. For Sn-based STJ detectors, one would expect a somewhat smaller signal for biomolecules relative to X-rays because of their phonon threshold. And, for Nb-based STJ detectors, the biomolecule signal would be expected to be even smaller, because due to their higher phonon threshold they capture an even smaller fraction of the energy.

The data for the measured signal heights summarized in Table 3 are interesting in two respects. First, the microcalorimeter response for biomolecules is clearly smaller than for X-rays, and second, the relative response of the Sn-based STJ detector seems larger than that of the microcalorimeter. Only the small relative response of the Nb STJs is, at least qualitatively, expected. As mentioned above, Hilton et al. considered the breaking of molecular bonds upon impact and ejection of molecular fragments as potential effects that could reduce the fraction of biomolecular kinetic energy actually converted to the heat signal in their microcalorimeter. Other processes,
such as metastable decay of ions during free flight and plastic deformation of the absorber film upon ion impact, may also reduce the detected energy and contribute to the “missing energy”. All of these processes should occur to a similar extent for all three detector types considered here, and the response of the Sn-based STJ detector to biomolecules should be similarly affected by such effects. In this context, the relative response reported for the Sn-based STJ detector seems unexpectedly large and certainly warrants further investigation.

The effects that are possibly responsible for reducing the overall signal height should also increase the spread in measured energy; i.e., degrade the observed energy resolution. In fact, all three detector types show much better energy resolution for X-rays than for biomolecules (see Table 3). A small degradation of energy resolution for biomolecules compared to X-rays would be expected as a result of the relatively smaller signal height for biomolecules as can be seen from the row labeled “extrapolated signal height” in Table 3. These values were obtained by extrapolating from the energy resolution obtained for X-rays at 6 keV to the energy of the biomolecules, taking the reduced signal into account. In this extrapolation, we assumed that the energy resolution scales proportionally to the square-root of the energy. Such a square-root dependence is expected when the signal height varies and the noise is constant. The observed energy resolution for biomolecules is about ten times worse than predicted by the extrapolation. Interestingly, it is similarly worse for all three detector types. This observation is another indication that the reasons for the large spread in measured impact energy are not primarily caused by the properties of the detectors, but are caused by other factors, such as metastable decay, in-flight fragmentation in residual gas collisions, and fragmentation upon impact with ejection of parts of the molecules.

Another interesting aspect of the energy response for ions measured with the three different cryogenic detector types is that the fractional energy deposition seems to be mass-dependent. As was first noticed by Hilton et al. with the microcalorimeter, small molecules such as sinapinic acid matrix molecules seem to deposit a larger fraction of their kinetic energy in the detector than larger molecules such as lysozyme. We recently found a similar effect with our Nb-based STJ detectors (G. Westmacoff et al., submitted to Rapid Commun Mass Spectrom, 1999a).

We are still far from a microscopic understanding of the details of molecule fragmentation in a MS system and of biomolecular impacts and the transfer of energy into a detector. The original premise that cryogenic detectors may provide a way to directly and precisely measuring the total energy of ions has not been fulfilled yet. It has been found that interpretation of ion energy data obtained with cryogenic detectors is not as straightforward as naively expected. The experimental findings that the energy actually deposited may be smaller than the total ion energy and may also depend on molecular mass, size, and other details of the impact confound the interpretation and warrant further studies. Nevertheless, the results discussed here should give a glimpse of the potential for improving our understanding by using cryogenic detectors and the energy information they provide. Although understanding biomolecule impacts and energy transfer processes on a microscopic level may seem a bit esoteric and interesting only to physicists, measuring and understanding molecule fragmentation during acceleration and free flight may result directly in developing better TOF–MS systems with better sensitivity and improved mass resolution and thus be appealing to the general mass spectrometry community.

### Table 3.

Comparison of the energy response for the three cryogenic detectors applied to TOF–MS so far. Quoted resolutions are FWHM.

<table>
<thead>
<tr>
<th>Operating temperature</th>
<th>Nb STJ</th>
<th>Sn STJ</th>
<th>Hot-electron Microcalorimeter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phonon threshold</td>
<td>~1.3 K</td>
<td>~0.5 K</td>
<td>~0.1 K</td>
</tr>
<tr>
<td>Signal height relative to X-rays</td>
<td>~0.2</td>
<td>yes, lower than Nb</td>
<td>no</td>
</tr>
<tr>
<td>Energy resolution for X-rays</td>
<td>300 eV at 6 keV</td>
<td>180 eV at 6 keV</td>
<td>92 eV at 6 keV</td>
</tr>
<tr>
<td>Extrapolated resolution for biomolecules</td>
<td>~1.5 keV for 30 keV ions</td>
<td>180 eV for 6 keV ions</td>
<td>~215 eV for 20 keV ions</td>
</tr>
<tr>
<td>Measured energy resolution for biomolecules</td>
<td>~15 keV for 30 keV ions</td>
<td>~1.5 keV for 6 keV ions</td>
<td>1.7 keV for 20 keV ions</td>
</tr>
<tr>
<td>Obtained charge resolution q/Δq</td>
<td>~2</td>
<td>~4</td>
<td>~10</td>
</tr>
</tbody>
</table>
hesitant and skeptical, and remain so today, about the small detector size and the requirement for cryogenic technology. Some of these concerns are addressed in this section.

**A. Detector Operation at Cryogenic Temperatures**

Cryogenic detectors operate at a very low temperature, typically 1 K or below; that factor may be viewed, at best, as inconvenient compared to room-temperature operation and, at worst, may hinder wider dissemination of this new detector technology for mass spectrometry applications. The question arises how low an operating temperature is really needed for sufficient sensitivity, and whether the necessity of liquid cryogens can be avoided in future detector generations. These questions, together with some other practical problems arising from low-temperature operation, are addressed in this section.

Cryogenic detectors are extremely sensitive because they detect low-energy excitations such as crystal lattice vibrations caused by the impact of ions. Such low-energy excitations can not be measured at much higher temperature, because the excitations would be buried in a huge background of thermally created excitations. It is conceivable that detectors that operate at somewhat higher temperature, say 5 K or even 10 K, may have sufficient sensitivity to be useful in mass spectrometry, and this path will certainly be explored. Detectors that operate in the 5 to 10 K range, for example based on Nb films with a critical temperature of 9.2 K, have the potential to be much faster than current cryogenic detectors, but probably at the cost of vastly diminished energy resolution. In this context, it seems unlikely that cryogenic detectors based on high-temperature superconductors operating at the temperature of liquid nitrogen (77 K) or above will offer sufficient sensitivity.

Although detector operation at low temperature is essential, the use of liquid helium is not. It should be noted that the use of liquid helium is not a large obstacle nor does it contribute significantly to the operation costs. For our Nb STJ detector, we typically use less than 50 liters of LHe a week at a cost of ~$4 per liter. The STJ detector is operated in a small liquid 4He cryostat that costs less than $10,000. Nevertheless, the additional complexity introduced by the use of liquid helium may hinder the wide dissemination and routine use of this technology in the future. To avoid the need for liquid helium, mechanical coolers such as closed-cycle refrigerators can be used. In fact, in the past few years several companies have been developing closed-cycle refrigerators that can provide temperatures as low as 3 K. Combined with adiabatic demagnetization refrigerator units, such refrigerators will be able to achieve sub-Kelvin temperatures without supply of any liquid cryogens, not even liquid nitrogen.

Currently, high-resolution cryogenic detector systems that operate without liquid cryogens are under development for X-ray fluorescence microanalysis applications in the semiconductor industry. As the semiconductor industry begins to apply this new cryogenic detector technology for improving the sensitivity of X-ray fluorescence microanalysis over the next few years, such closed-cycle systems are expected to become readily available at moderate cost. Such systems will also easily be usable for cooling cryogenic detectors in TOF–MS systems. Depending on the type of cryogenic detector and the required temperature, the price for a cryogenic detector system with closed-cycle cooling may range from $40,000–200,000.

Implementing a cryogenic system into a MS system requires only moderate cryogenic engineering. Typical cryostats do not take up any significant additional space when attached to a TOF–MS system. For example, the cryostat that was used in our experiments at LBNL to cool the Nb STJ detector to ~1.2 K was approximately 20 cm in diameter and 40 cm tall; the NIST group’s cryostat provides an operating temperature of ~100 mK and is only about twice that size in each dimension. Pumps needed for our 1.2 K cryostat or for future closed-cycle systems can usually be placed away from the actual system where convenient.

Another practical problem to consider when operating a cryogenic detector in the environment of a room-temperature MS system is the need to limit the amount of thermal infrared radiation reaching the detector. Too large a flux of infrared photons onto the detector may diminish the detector performance by increasing the noise (infrared shot noise), or, in the worst case, heat up the detector too much and render it non-operational. The lower the operating temperature of the detector the smaller the tolerable infrared radiation load; thus, more care is needed to avoid this problem. X-ray spectrometers that employ cryogenic detectors are usually equipped with thin, aluminized windows mounted at low temperature in front of the detector. These windows block infrared photons but allow most of the X-rays to be transmitted to the detector. For a mass spectrometry application, of course, the use of windows is not an option, because windows would also block biomolecules from reaching the detector. Instead, a series of cooled collimators with small holes placed in front of the detector can be used, as was shown by the demonstration experiments described above. When larger detectors or detector arrays with a larger total area become available in the future (see below), the size of the collimator holes must be correspondingly larger, and some cryogenic engineering may be required to keep the IR flux tolerably low. In case the cooled-collimator technique proves too impractical for large cryogenic detectors, one could consider using metal grids with micron-sized meshes to attenuate IR radiation at the cost.
of blocking a small fraction of ions from reaching the detector.

Another potential problem may arise from the accumulation of biomolecular fragments, matrix material and frozen-out residual gases on the cryogenic detector surface during operation in a mass spectrometer. The extent to which this accumulation affects detector performance is not known. A simple estimate reveals that the accumulation of molecular fragments and matrix material is negligible and only the residual gases may contribute significantly to the material buildup. The following order-of-magnitude calculation is quite instructive. We assume for simplicity that most of the sample material in a MALDI sample is carbon, and that a carbon atom covers an area of about $2 \times 2 \, \text{Å}^2$ when adsorbed on the surface of a detector. A single monolayer of carbon on top of a $200 \times 200 \, \text{µm}^2$ detector pixel consists of $\sim 10^{12}$ atoms. We assume further that, with each laser shot, $\sim 100$ molecular ions with an average weight of 12 kDa ($\sim 1000$ carbon atoms) hit the detector pixel and stick to it. This hit rate is probably an overestimate given the small detector size and our experience from the demonstration experiments. If the laser fires at an average rate of 1 per sec for 8 h every day it will take $\sim 3 \times 10^9$ s, or about 1 year, to cover the detector with one monolayer of carbon. Granted that this calculation may be naive, it illustrates that the buildup of material from the MALDI sample on the detector will be very slow. Residual gas molecules that condense on the surface of the detector, on the other hand, may give a larger contribution to the buildup. At a background pressure of $10^{-7}$ Torr, for example, residual gas molecules impinging onto the detector could build up one monolayer of frozen gas in as little as $\sim 10$ seconds, if their sticking coefficient were 100%. Fortunately, because their detection mechanism is based on phonons, cryogenic detectors will tolerate some accumulation of material on their surface without degrading their sensitivity significantly. This is verified by experiment. The STJ detectors used in some of our previous measurements were covered by a protective layer of $\sim 5000 \, \text{Å}$ of SiO$_2$, and exhibited only a small decrease in pulse height compared to the uncovered STJ detectors. Some of our experiments were performed at higher pressure, and no adverse effects on detector sensitivity from freezing out of residual gas were observed. Typically the detectors were operated for periods of $\sim 1$ day, and warmed up in between experiments. Experiments with longer periods of cooled operation will reveal whether material buildup might be a detrimental factor for long-term operation and whether additional baffles or detector cleaning procedures need to be introduced. For example, additional cooled apertures could be installed in front of the detector to intercept and condense residual gas from the mass spectrometer before it reaches the detector.

### B. Increasing the Size of Cryogenic Detectors

For all types of the cryogenic detectors used in the demonstration experiments discussed above, the detector area of the prototypes was small, about 0.05–0.2 mm on a side. This small size is certainly not ideal for routine MS applications. In the following, we discuss possibilities and practical limitations for increasing the size of individual detector pixels. We also discuss the alternative to increasing the effective area by grouping many individual detector elements into larger arrays, in which each individual detector element is read out by its own electronic channel. Some earlier discussions on both approaches can be found in (Frank et al., 1996b; Booth, 1997).

#### 1. Increasing the Size of Individual Detector Pixels

Increasing the size of an individual detector is possible but will most likely result in a degradation of sensitivity, energy resolution, and speed for all cryogenic detector types discussed in this review.

In the case of a hot-electron microcalorimeter, increasing the size of the detector increases proportionally the heat capacity; this increase results in a corresponding decrease in signal. In principle, this heat capacity increase could be mitigated by lowering the operating temperature. However, a practical lower limit for the operating temperature of a cryogenic detector for use in a TOF–MS system may be encountered at around 50–100 mK; not much below the operating temperature previously used by the NIST hot-electron microcalorimeter. This temperature range is still easily accessible by relatively simple, compact adiabatic demagnetization refrigerators. To achieve much lower temperatures, one would have to turn to more complicated and expensive refrigerators, for example, dilution refrigerators. Also, the infrared heating of the detector, which is still manageable around 100 mK as the NIST demonstration experiments have shown, will become a much larger problem at lower temperatures. Another undesired effect associated with lowering the operating temperature of a microcalorimeter is that the detector signal tends to become slower. This slowdown is due to a decrease in thermal coupling between the detector and the cold bath, resulting in longer thermal relaxation and pulse decay times. Although lowering the operating temperature to well below 100 mK may turn out to be impractical, other options that could be explored are the use of an absorber material with low specific heat capacity, such as the semimetal Bi, or to improve the temperature sensitivity by using a TES instead of an NIS tunnel junction as temperature sensor. As the NIST group has shown in X-ray measurements, with hot-electron microcalorimeters considerable increase in
sensitivity and speed can be achieved using a TES operated in the so-called extreme electrothermal feedback mode (Irwin, 1995). With those improvements, hot-electron microcalorimeters for TOF–MS with detector areas of ~1 mm² or more could be obtainable, possibly trading off some loss in energy resolution and speed for detector size.

For STJ detectors, an increase in detector area is accompanied by a corresponding increase in detector capacitance and leakage current and a decrease in resistance, resulting in additional electronic noise when operated with conventional, FET-based preamplifiers. Currently, STJ detectors with sizes up to 200 × 200 μm² and conventional electronics show good noise performance in X-ray applications (Frank et al., 1998a) and in the TOF–MS measurements by our collaboration described above. This size may be improved a bit further in the future. With SQUID-based current preamplifiers, which are not as sensitive to input capacitance, it may be possible to read out much larger STJ detectors with areas as large as ~1 mm² (Frank et al., 1996a; Mears et al., 1997). Other practical limitations may prevent an increase in STJ detector size much beyond that size even when SQUID-based amplifiers are used. Above ~1 mm² the STJ detector capacitance may become so large that parts of the signal current may be shunted through the capacitance itself instead of being measured. Also, as a more practical aspect, the undesired leakage current of an STJ detector generally becomes larger with detector size, in part due to some practical difficulties in fabricating larger-area, high-quality, pin-hole-free tunnel barriers.

Two other approaches to increase the size of STJ-based detectors have been pursued by various groups. First, two or a few more small STJ sensors can be coupled to larger superconducting absorber films made of higher superconducting gap material. Quasiparticle excitations created by particle impacts onto the absorber can diffuse to the STJ sensors placed at different parts of the absorber film, where they are trapped and measured. From relative signal heights and timing, the energy and impact location in the absorber film can be extracted (Kraus et al., 1989; Friedrich et al., 1997; Hettl et al., 1997). This approach would not be ideal for TOF–MS, because the quasiparticle diffusion through the absorber and the generation of current pulses at the sensors can take several microseconds. This effect would add an impact-position-dependent delay to the “true” ion arrival time. The second approach is to cover the surface of a dielectric absorber substrate with many STJ sensors connected in series. Such a series array can have a small capacitance (note that the total capacitance of n capacitors C connected in series is C/n) and can be read out by a single channel FET-based amplifier (Kurakado et al., 1991). The dielectric substrate acts as the particle absorber. The phonons produced by particle impacts are absorbed in the STJ sensors on the surface and measured as a tunneling current pulse. Detector sizes ranging from a few mm² to 1 cm² have been realized with charge pulse rise times ranging from 10 to 100 μs (Goldie et al., 1994; Gaitskell et al., 1996; Kurakado et al., 1997).

Although some of the methods to increase the size of individual detector pixels described above are appealing and may be employed in future cryogenic detectors for TOF–MS, it seems doubtful that a single-channel detector can be made fast and at the same time large enough to cover the entire cross-sectional area of an ion beam with typical diameter of several mm in a mass spectrometer. In addition to a decrease in sensitivity and increased response time associated with larger-area cryogenic detectors, another undesired effect is pulse pile-up. Pulse pile-up occurs when too many ions hit the detector within a short time interval, and the pulses from individual ion impacts overlap. As the count rate increases, pulse height and energy information are usually compromised before pulse counting and timing are seriously affected (moderate pile-up situation). As long as the overlap of two pulses is not too large, good pulse timing and counting is still possible, while pulse height determination, which usually involves some low-pass filtering, may already be affected. When the count rate is very high, i.e., the overlap of the two pulses from two ions hitting the detector at nearly the same time is too large (extreme pile-up), then it is difficult to electronically separate the two impact events anymore. Instead, they are counted as a single event with a pulse height corresponding to the sum of both ion energies. In this case, information on the energy of each individual ion and on the fact that two ions have hit the detector is completely lost. Because in most cases the energy information provided by the cryogenic detector will be considered useful, it will generally be desired to avoid even moderate pile-up situations.

Generally, the decay time τ of the detector pulses determines the hit rate that a detector can tolerate. To keep pile-up effects negligible, this rate should be kept well below 1/τ (Leo, 1994). As opposed to X-ray events from a radioactive source, the ion impacts in a TOF-MS measurement are usually not randomly distributed in time, but clustered around the times of the TOF peaks. Therefore, it is not surprising that already with the relatively small STJ detectors (200 × 200 μm²) used by our collaboration we could observe some pulse pile-up in our MALDI experiments (Frank et al., 1996b) although the pulse decay time was relatively fast (~1.5 μs) and the average hit rate relatively low (~10–20 hits in a 400 μs interval). Based on this experience, we expect the maximal useful size of a STJ detector pixel to be around ~0.5 mm on a side, maybe smaller for a hot-electron
2. Increasing the Effective Area with Detector Arrays

The obvious solution to avoid detector performance degradation and limit pulse pile-up when a larger-area detector is desired is to use a detector composed of a closely packed array of relatively small detector pixels, each read out by its own electronic channel. (The other solution would be to reduce the ion flux, e.g., by turning down the laser power in MALDI, but this solution would increase the total number of laser shots and the time it takes to perform a measurement.)

Another possible advantage of using parallel arrays of detectors (for both, small conventional detectors or cryogenic detectors) is that such an arrangement enables multiplexing time-of-flight measurements in applications where high throughput is required; e.g., for fast DNA sequencing using TOF–MS. A multiplexing approach was suggested by D. Twerenbold at the 6th International Workshop on Low Temperature Detectors, LTD-6 (Twerenbold, 1996a) for speeding up a TOF measurements of a large number of DNA samples. In this multiplexing scheme, a laser beam with a high repetition rate could be rapidly stepped across an array of oligomer samples such that it fires one shot per step and sample in the array at a time. Using appropriately switched deflection plates, the ions generated from each sample in the array could be guided to a corresponding element of a detector array. The detector array contains as many elements as the array of samples. Thus, each sample has a corresponding detector. In this scheme, the laser repetition time could, in principle, be much faster than the ion flight time, and the measurement of the whole array of samples could be performed in nearly the same time as the measurement of a single sample with a single-element detector resulting in a potentially large reduction in overall measurement time. Of course, such a multiplexing scheme would require significant effort in developing suitable ion optics to precisely guide the ions generated by one laser shot to the desired detector.

Because most cryogenic detectors can be fabricated by photolithographic techniques, fabricating large arrays of detector pixels is almost as simple as fabricating a single detector. Cryogenic detector arrays with many pixels read out by individual electronic channels are already under development by various groups. STJ detector arrays are currently being developed at Lawrence Livermore National Laboratory (Friedrich et al., 1998) and by a group at the European Space Research and Technology Centre (ESTEC) in The Netherlands (Schilling, 1996). At the same time, arrays of hot-electron microcalorimeters are planned for applications in microanalysis (Ladbury, 1998) and X-ray astrophysics. The development of larger cryogenic detector arrays useful for TOF–MS will certainly profit from these projects.

A practical limitation of the overall detector size and number of pixels is given by the number of electronic channels that can be afforded. Although most of the electronic components, such as shaping amplifiers and other signal-processing electronics, are commercially available, the preamplifiers are non-standard and different for each cryogenic detector type. The preamplifiers for STJ detectors are usually based on FETs, and are presently less expensive (equivalent to several hundred US$ per channel) than the SQUID-based preamplifiers used for hot electron microcalorimeters (~$2000 per channel). These costs are expected to drop as the use of cryogenic detectors increases. Also interesting in this context is the development of a fast multiplexing SQUID-based amplifier at NIST (Chervenak et al., 1999), which may allow the readout of several microcalorimeter pixels in an array by one SQUID channel.

C. Time Resolution and Energy Resolution Achievable with Cryogenic Detectors

1. Time Resolution and Mass Resolution

When considering the time resolution and mass resolution achievable with cryogenic detectors, we need to distinguish between the accuracy of the timing obtainable with the detectors and the overall TOF resolution obtainable with a TOF–MS system, which determines its mass resolution.

The accuracy of timing, or “intrinsic” time resolution, achievable with any detector is given by the accuracy with which the onset of an ion impact pulse can be determined. This accuracy depends on the pulse rise time, baseline noise, and the signal-to-noise ratio. The accuracy of timing (in pulse counting mode) is independent of the pulse length except for cases of pulse pile-up, where this accuracy may be affected to some extent. Ion arrival times obtained with cryogenic detectors for individual pulses can be quite good in spite of their relatively long pulse decay times of microseconds or more. When the noise is low, the pulses from two ion impacts closely following each other can be recognized as two events, even when the time between the two impacts is not much longer than the detector’s intrinsic time resolution. In practice, i.e., in the presence of noise, the pulses must be spread out more, in order to be recognizable as two events. With noise present, it may be difficult to separate the pulses from two ions unless the second ion arrives after the peaking time of the first pulse. The pulse peaking time sets a practical limit to the TOF resolution. (Of course, when a good pulse height and energy measurement in addition to the arrival time
measurement is desired, ion arrival times and detector pulse onsets must be separated by more than just the pulse peaking time).

For small-mass ions with short flight times, the relatively slow response of cryogenic detectors and their long pulse peaking time of ~1 μs limits their usefulness for precise TOF measurements compared to MCPs, which have pulse peaking times of only a few nanoseconds. In this low-mass range, the only important benefit of cryogenic detectors is their good energy resolution.

For higher-mass ions, system-intrinsic factors other than the speed of the detectors used degrade the obtainable TOF resolution and, ultimately, TOF resolution does not depend anymore on whether an MCP or a cryogenic detector is used. This fact was already pointed out above in the discussion of the TOF–MS demonstration experiments with cryogenic detectors. For example, the observed TOF resolution of ~50 for molecules such as human serum albumin is clearly worse than the resolution of several hundred expected from the speed of the cryogenic detectors alone. This low resolution was presumably due to spreads of flight times introduced by the MALDI process. MALDI ions with exactly the same mass can acquire slightly different final velocities because of different initial velocities obtained during laser desorption and ion drag in the MALDI plume in the early stages of acceleration (Juhasz, Vestal, & Martin, 1997). In addition, ion fragmentation can contribute significantly to the broadening of TOF peaks.

It is certainly desirable to increase mass resolution obtainable with TOF–MS at high mass, and significant progress has recently been made by the implementation of delayed extraction (see, e.g., Bahr et al., 1997 and references therein). Measuring ion energy simultaneously by with flight time may help to reveal where the present system-intrinsic limitations come from. Using cryogenic detectors, the causes for spreads in ion arrival times can be studied in better detail (Labov et al., 1997; Hilton et al., 1998; Frank et al., 1999) and cryogenic detectors may thus contribute to developing improved mass spectrometers in the future.

Although the TOF resolution of MS systems for high-mass ions is presently not limited by the speed of detectors, it is interesting to consider how far the speed of cryogenic detectors can be pushed because this speed sets an ultimate limit to the theoretically achievable TOF resolution. The intrinsic time resolution reported so far for the cryogenic detectors used in the TOF–MS experiments described above was around ~100 ns. Faster detector response time and better time resolution should be obtainable with cryogenic detectors optimized for more speed, but probably at the cost of a decrease in energy resolution. It is conceivable that superconducting films made from superconductors that have a critical tempera-

## 2. Energy Resolution and Charge Resolution

It remains unclear what are the practical limits for the energy resolution for molecular impacts achievable with cryogenic detectors. Naive extrapolation of the energy resolution achieved in X-ray measurements can only be used to calculate theoretical limits for the energy resolution achievable for molecular ions under ideal circumstances. The experimental results indicated that not all the kinetic energy of an ion is converted to a signal. What seems to confound energy measurements are large variations in deposited energy introduced possibly by molecule fragmentation during ion launch, acceleration, and free flight, and the breaking of chemical bonds and ejection of fragments upon impact onto the detector. These effects, and not the intrinsic detector performance, seem to dominate the spread in measured ion energies. The charge resolution for molecular ions obtainable with a cryogenic detector is directly related to the spread of measured energies, and the charge resolution is presently limited to about 2–4. Already such a moderate resolution can be useful for investigating fundamental aspects of TOF–MS. For example, it allows resolving $M^+$ from $2M^{2+}$ ions, both arriving at the same TOF. Better energy resolution and charge resolution are certainly desirable and might be obtainable in the future when the reasons for the large spread in measured energies will be understood better.

## V. CONCLUSIONS AND OUTLOOK

It has been only little more than four years since Twerenbold (1996a) inspired the cryogenic detector community to consider applying cryogenic detectors as energy-sensitive ion detectors in TOF–MS. Since then, three research groups have studied the performance of three different types of cryogenic detectors as ion detectors in MALDI–TOF–MS. Proof-of-principle experiments were
performed, and it was shown that cryogenic detectors can indeed detect single, large biomolecular ions. A comparison of the response of a cryogenic detector with that of an MCP indicated a much higher sensitivity per area of the cryogenic detector at large molecular mass. Cryogenic ion detectors not only improve signal strength due to their high efficiency, but also provide a direct way to simultaneously measure ion energy and flight time. With the three cryogenic detector types used so far in TOF–MS, it was shown that the energy-sensitive response can be used to discriminate the charge of MALDI ions. The energy-sensitive response was also shown to be useful to reveal in-source and in-flight ion fragmentation. At the same time, the first TOF–MS measurements with cryogenic detectors raised new questions about ion properties, ion fragmentation, and ion-detector interaction, which are far from being understood. The study of these microscopic details using energy-resolving cryogenic detectors is just at its beginning.

The experimental results discussed here show that cryogenic detectors could be a powerful tool in TOF–MS with interesting measurement capabilities. Simultaneous measurement of ion energy and arrival time has the potential to reveal ion properties not previously evident and hard to quantify with ionization-based detectors. The capabilities provided by cryogenic detectors are likely to help increase our understanding of ion energy distributions and issues related to ion fragmentation. A better understanding of these issues may help in developing softer launching techniques and better TOF–MS systems with improved mass resolution and possibly increased mass range. Thus cryogenic detectors may ultimately help in improving mass spectrometry.

Although the application of cryogenic detectors to mass spectrometry has been demonstrated, further development of cryogenic detector systems will be needed for widespread use. Most importantly, simplification of the cryogenics are needed to make cryogenic detector systems more user-friendly. Closed-cycle coolers will provide the greatest step in this direction. Also, a significant increase in detector size seems necessary to increase the usefulness of cryogenic detectors for TOF–MS.

Even given these improvements, it seems unlikely that cryogenic detectors will replace microchannel plates in routine TOF–MS applications. MCPs work very well at room temperature and will continue to provide larger area and faster timing than cryogenic detectors. In our opinion, cryogenic detectors should, therefore, not be viewed as competition to MCPs, but as additional tools. Most likely, in the near future, cryodetectors will be most valuable as diagnostic tools in MS research applications. It is conceivable that cryogenic detectors may also be implemented as an auxiliary detector for special applications, such as TOF–MS measurements involving very high mass ions. If their charge resolution can be improved, they may also be used to measure directly the charge state of electrospray ions and thus greatly facilitate the deconvolution of ESI spectra. One important future high-mass application of TOF–MS with cryogenic detectors could be DNA sequencing. Because cryogenic detectors allow single particle counting of DNA fragments of arbitrary high mass, one could analyze large sequencing ladders and thus greatly increase the DNA sequencing rate compared to standard gel-electrophoresis DNA sequencers. Before this can be done the problems of ionizing mixtures of large DNA fragments in TOF–MS need to be solved and mass resolution needs to be improved. These issues could be addressed using the diagnostic capabilities of cryogenic detectors which may thus contribute in various ways to speeding up sequencing efforts in the future.

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