Journal of Thermal Biology 36 (2011) 209–218

Contents lists available at ScienceDirect

Journal of Thermal Biology

journal homepage: www.elsevier.com/locate/jtherbio

Review

Thermal microdevices for biological and biomedical applications

Ankur Jain*, Kenneth E. Goodson

Microscale Heat Transfer Laboratory, Department of Mechanical Engineering, Stanford University, Stanford, CA 94305, USA

ARTICLE INFO

Article history:
Received 7 October 2010
Accepted 25 February 2011
Available online 21 March 2011

Keywords:
MEMS
Biomedical microdevices
PCR
TGF
Temperature

ABSTRACT

Temperature strongly influences the form and function of biologically important macromolecules and cells. Advances in microfabrication technology have enabled highly localized and accurate temperature control and manipulation, allowing the investigation of thermal effects on biological microsystems. This paper reviews progress in this field, with emphasis on techniques and microdevices with biomedical applications. Recent advances in the study of thermal effects on cellular behavior, enabled by MEMS-based structures are reported. These studies focus on investigating thermal interactions between the cell and its microenvironment. Thermal-based tools for concentration and purification of biologically important macromolecules like DNA and proteins are summarized. These tools address common issues in protein/DNA research, like concentration, separation and purification of samples. With the increasing research focus on the integration of biomedicine with engineering technologies and the several incentives of miniaturization, MEMS-based devices are likely to become increasingly prevalent in biology and medicine. Thermal engineering is expected to continue to play an important role in the improvement of current microdevices and the development of new ones.

Contents

1. Introduction

The past few decades have witnessed the advent of Micro-ElectroMechanical Systems (MEMS) (Petersen, 1982; Kovacs, 1984; Trimmer, 1996). Improvements in microfabrication technology have enabled the realization of novel devices on the micron length scale. While early work in MEMS focused on Silicon and Silicon-related materials, a large variety of new materials, including soft polymers (McDonald et al., 2000), novel organic compounds (LaBianca and Delorme, 1998) and plastics (Martin et al., 1999) are increasingly becoming popular. Microfabricated structures allow precise control of mechanical forces, temperature fields and fluid motion at the same spatial scale as typical cells, making possible investigations that traditional experimental tools were incapable of. In addition, the precise control of physical parameters by MEMS-based devices make them an attractive choice for probing and manipulating biologically important macromolecules like DNA and proteins. These capabilities have led to the application of MEMS in both basic biological research (Folch and Toner, 2000) and as well as clinical applications (Polla et al., 2000; Singh and Kim, 2009). MEMS devices have been used to better understand the nature of cell-surface interactions and the influence of the microenvironment on the growth and proliferation of cells (Folch and Toner, 2000). The effect of thermal gradients on neurite outgrowth in nerve cells is being studied (Jain et al., 2005). On the clinical side, Silicon-based microneedles and neural probes offer several advantages compared to

* Correspondence to: Advanced Micro Devices Inc. Austin, TX, USA. Tel.: +1 512 419 8327.
E-mail address: ankurjain@stanfordalumni.org (A. Jain).

0306-4565/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jtherbio.2011.02.006

traditional devices (McAllister et al., 2000; Kewley et al., 1997; Coulman et al., 2009). MEMS-based microsurgery is expected to become increasingly important in the future (Rebello, 2004). In another set of applications, MEMS devices promise to significantly miniaturize bioanalytical procedures, the best example of which is the miniaturization of Polymerase Chain Reaction (PCR), which is used to amplify the quantity of specific DNA in a given sample. Among the several advantages offered by MEMS-based bioanalytical tools include fast assay times, reduced costs due to small reagent volumes and the possibility of integration of several assays on a single chip to create what is popularly known as micro-Total-Analysis-System (μTAS) (van den Berg and Lammerink, 1998; Arora et al., 2010).

Temperature is one of the most important components of the cellular microenvironment. While thermal effects on biological microsystems have traditionally not been accorded as much importance as, say, chemical effects, it is nevertheless clear that temperature may be a key tool with which to control and manipulate microsystems like single cells, DNA and proteins. Temperature control at the same scale as typical cells, made possible by microfabrication technology has enhanced our capability to investigate thermal phenomena in biological microsystems and develop a better understanding of biothermal effects.

This paper presents an engineering review of temperature and temperature gradient effects on biological microsystems. Special emphasis is given to techniques for control and manipulation using microfabricated structures. In the next section, several temperature measurement techniques being used in and of potential use in biological microsystems are reviewed. These include traditional methods as well as some newly developed ones. Subsequent sections review techniques and microdevices for the manipulation of biological macromolecules like proteins and DNA using temperature. The paper concludes with our perspective about the future of BioMEMS and the future role of thermal engineering in studying biological microsystems.

2. Temperature measurement techniques

Temperature is one of several physical parameters that play a key role in tissue form and function. In addition, temperature also plays an important role in thermal-based therapeutics like tumor treatment using laser irradiation (Roemer, 1999). As a result, a variety of temperature measurement techniques continue to be developed for biological systems (Vyazovkin, 2010). Biological systems provide new challenges for temperature measurement compared to more traditional applications. Among the new constraints brought about are biocompatibility of the measurement technique, spatial accuracy in three dimensions and the usually small size of the measurement space. Since physiological conditions span only a very narrow temperature range around the body temperature, a temperature measurement system for tissue does not usually need to operate in a very large range. The temperature range for human beings is only a few °C around the normal value of 37 °C. For other species, the temperature range may be different, and in some limited cases, certain species are known to be able to withstand a very high temperature (Zeng et al., 2009). While the temperature range is often quite small, the measurement accuracy can often be very important. In addition, cost is almost always a major concern for biomedical applications.

The brain is known to be extremely sensitive to temperature, and even very small temperature changes inside the brain can lead to significant short-term and long-term effects (Dietrich, 1992; Minamisawa et al., 1990). Consequently, brain temperature measurement has been widely worked on. Traditional temperature measurement techniques like thermocouples (Brambrink et al., 1999) and thermistors (Childs and Machin, 2009) have been used for temperature measurement in brain-related experiments. Intraventricular temperatures have also been measured and used as a parameter for studying the health of the brain (Mellergård, 1994). While most of these measurements measure the brain temperature only indirectly, for example through intraventricular (Mellergård, 1994) or tympanic membrane measurements (Brambrink et al., 1999), direct brain temperature measurements have also been recently reported using intracerebral probes (Stone et al., 1997; Iatrou et al., 2002). It is to be noted, however, that the use of this technique has been explored only in limited regions of the brain and it is unlikely to be able to probe the entire brain.

In addition to the use of thermocouples and thermistors, IR thermography has also been widely used for a variety of biomedical diagnostic applications (Welch and van Gemert, 1995; Goff and Clark, 1985; Kateb et al., 2009; Gorbach et al., 2004). A pyroelectric thermal imaging system has also been developed (Black et al., 1990). While IR and pyroelectric based diagnostic methods tend to be simple and relatively cheap, they provide only surface temperature information and may provide an accuracy of only around 1–2 °C, even with extensive calibration.

Intraventricular temperature measurements have been performed using microfabricated Fabry-Perot sensors (Wolthius et al., 1991; Saxena and Hui, 2010) for application in cardiovascular interventions using catheters. The use of in-fiber Bragg gratings (FBG) has also been investigated (Rao et al., 1997; Li et al., 2009). Resolution of up to 0.1 °C has been reported. These methods require microfabricated sensors as well as extensive signal processing techniques.

Another interesting temperature measurement technique used in a biological microdevice is liquid crystal thermometry, which uses the isothermal phase change of a thermotropic liquid crystal for temperature detection in a microdevice (Gillot et al., 2007).

The advent of new imaging and diagnostic techniques has also led to the development of novel temperature measurement methods in biomedicine. These techniques usually work on the same principles that are used for imaging. For example, magnetic resonance temperature imaging has been developed for aiding thermotherapy (Kickhefel et al., 2009; de Zwart et al., 1996). This method relies on the temperature dependent magnetic resonance properties of water molecules inside the tissue of interest. In particular, the variation with temperature in the proton diffusion constant (MacFall et al., 1995) and the proton resonance frequency (de Zwart et al., 1996) has been used for temperature measurement of the tissue of interest. This method produces accurate, three-dimensional temperature data with excellent linearity and sensitivity (Maswadi et al., 2004). NMR spectroscopy has also been used for temperature monitoring in capillary electrophoresis with excellent spatial and temporal accuracy of the order of 1 mm and 1 s, respectively (Lacey et al., 2000). Such measurements help in the optimization of capillary electrophoresis, which is known to be strongly affected by temperature (Knox and McCormack, 1994; Gobie and Ivory, 1990).

Optoacoustic methods for temperature measurement have also been developed (Larina et al., 2005; Pramanik and Wang, 2009). These methods rely on laser-induced optoacoustic waves and the temperature dependence of acoustic properties of tissue. Good agreement of optoacoustic measurements with more traditional methods has been reported. This method can be easily integrated in thermal therapeutics where real time temperature measurement of the irradiated tissue is critical to the efficacy of the treatment. Time shifts in ultrasound echo signals reflected by tissue has also been shown to be a function of the tissue temperature, and has been used as a method for temperature

measurement of muscle tissue (Maass-Moreno et al., 1996), and the fundus of the eye (Schüle et al., 2004). However, evidence for successful *in vivo* application of this technique is lacking.

In another interesting work, telecommunications and MEMS technology have been integrated into a medical telesensor (Ferrell et al., 1998) that is small enough to be placed in the tympanic membrane. The sensor measures the body temperature and transmits it to an outside receiver. While this work is currently aimed at military applications only, the widespread use of such integrated microdevices for health monitoring is quite possible in the future. The medical telesensor shown in Fig. 1 indicates the typical size of microfabricated sensors.

With the advent of miniaturized bioanalytical devices for separation, purification and identification of biologically important macromolecules like DNA and proteins, temperature measurement within microfluidic flows and other microfabricated structures has become increasingly important. The use of liquid crystals for temperature measurement in microfabricated PCR chambers has been reported (Chaudhari et al., 1998). Optical temperature detection has also been found to be very effective in microfluidic flows (Ross et al., 2001; Ryu et al., 2009). Fluorescence thermometry makes use of molecules that absorb light of a specific wavelength and emit back at a different wavelength. The quantum efficiency of the emission is known to be a function of temperature. Hence, with proper calibration, the amount of emission may be used as a measure of the local temperature. Temperature measurement based on fluorescence provides excellent spatial resolution and is practically non-interfering with the flow conditions. The spatial and temporal resolution of fluorescence-based measurements depends primarily on the characteristics of the CCD camera. For typical devices, this could be as good as micrometer spatial resolution and millisecond time resolution (Ross et al., 2001). Rhodamine B has been used recently to measure temperature profiles in electrokinetically driven microfluidic flows (Ross et al., 2001). Tris(bipyridine)ruthenium(II) has been used in conjunction with micro-PIV for temperature measurements in convective mixing in microchannels (Sato et al., 2003), an application of potential interest for future lab-on-a-chip devices.

Fig. 1. A medical telesensor capable of transmitting body temperature measurements, indicating the typical size of microfabricated sensors. (From http://ornl.gov/info/ornlreview/rev29_3/text/biosens.htm, accessed 12/31/2008.)

3. Thermally driven biological and biomedical microdevices

One of the most promising applications of microfabrication technology and microfluidics is the miniaturization of bioanalytical tools. It has been expected that microfluidics-enabled Lab-On-A-Chip or Micro-Total-Analysis-Systems (μTAS) will significantly improve assay performance, while reducing analysis time and cost (Polla et al., 2000; Rebello, 2004). While truly integrated analytical microsystems are far from prominence in end-applications, several techniques for purification, separation and concentration of biologically important macromolecules like DNA and proteins have been demonstrated. The temperature sensitivity of these macromolecules has been made use of in designing some of these new techniques, and hence manipulation of the temperature field plays an important role in these microdevices. In this section, some of the recently developed techniques aimed at processing of DNA, proteins and cells using spatial and temporal thermal gradients are reviewed.

3.1. Polymerase chain reaction (PCR)

Polymerase Chain Reaction (PCR), a technique for DNA amplification developed by Kary Mullis in the 1980s (Mullis and Faloona, 1987; Saiki et al., 1988) is one of the most significant advances in DNA biochemistry in recent times. The rapid amplification of DNA samples enabled by PCR is a significant improvement over previous techniques and has played a significant role in the unraveling of the human genome, besides being useful in a large variety of investigations across several research fields. The underlying principle of PCR, shown schematically in Fig. 2 is surprisingly simple. The DNA of interest is mixed with an ample supply of DNA bases, oligonucleotides, also known as primers that are specific to the DNA to be amplified and a polymerase protein. The mixture is heated to a high temperature, around 95 °C, causing the denaturing of the DNA double helix. The mixture is then cooled down to 45–55 °C, when primers bind to specific sites on the single strand. In the final step, the solution is heated up to 72 °C, at which point the polymerase begins to attach nucleotides to the primers, resulting in extension of the primer, and thus creation of two double stranded DNA molecules identical to the original DNA. Rapid thermal cycling of the mixture between the three temperatures causes an exponential increase in the concentration of the DNA of interest, so that after around 30 cycles, up to a billion-fold amplification may be observed. By fluorescently labeling the PCR primers, it is possible to monitor reaction progress in real time.

The impact of PCR on biological research in the past couple of decades cannot be understated. It is now an integral part of the molecular biologist's toolbox (Heller et al., 2000). Because of its capability to amplify minute quantities of DNA, PCR has led to quicker, cheaper and more efficient detection of organisms causing infectious diseases, and identification of gene mutations that may cause abnormalities and disease. PCR has also influenced basic biological research across nearly all disciplines. For example, most mapping techniques in the Human Genome Project (HGP) have relied on PCR.

PCR is ideally suited for miniaturization. Apart from reduction in cost and reaction times, miniaturization makes it possible to develop a highly parallelized system by running several PCR assays together. Other sample preparation and analysis steps could also be combined with PCR in a single microfabricated device (Heller et al., 2000; Woolley et al., 1996). A large number of microfabricated PCR devices have been presented in the literature (see, for example, (Taylor et al., 1997; Kopp et al., 1998; Khandurina et al., 2000; Northrup et al., 1993)). Some of the earliest works in miniaturization of PCR recognized Silicon as an ideal material because of its high thermal conductivity and small

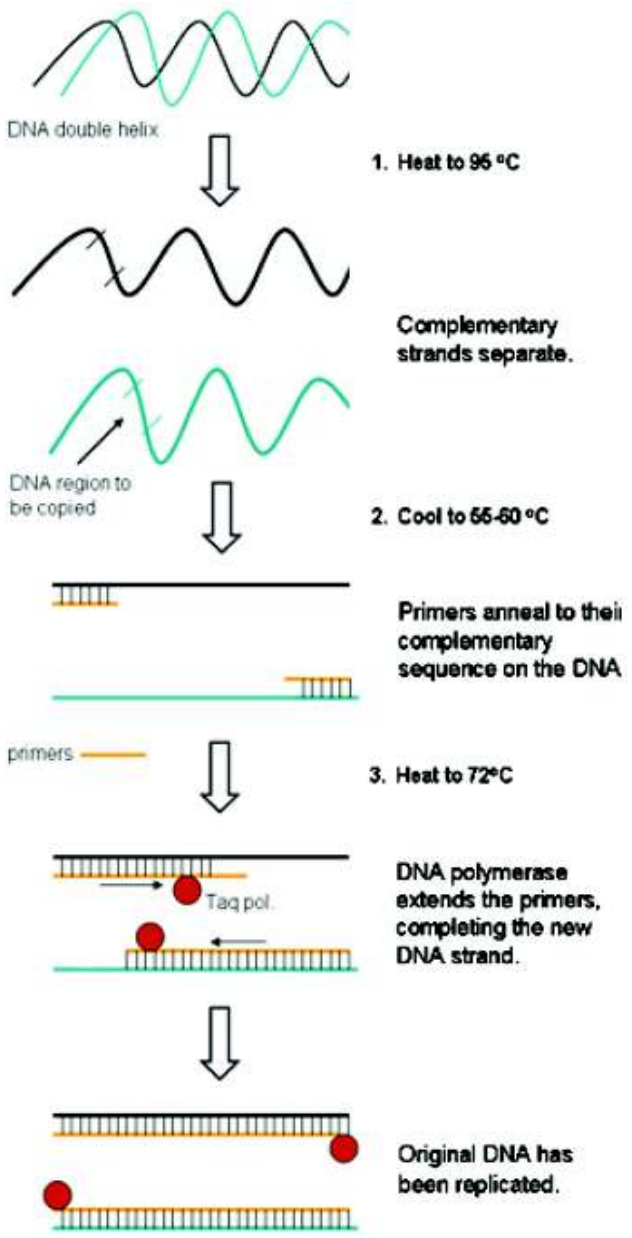

Fig. 2. Schematic of the mechanism behind Polymerase Chain Reaction (PCR). (From http://www.virtualmedicalcentre.com/healthinvestigations.asp?sid=60&title=PCR-%28Polymerase-Chain-Reaction%29, accessed 2/21/2011).)

thermal mass (Northrup et al., 1993; Wilding, et al. 1994). This improves reaction yield by ensuring a more uniform temperature field, especially for the first and third steps. Moreover, the small thermal mass of microfabricated structures ensures faster cooling down between the first and second steps. With the increased surface-to-volume ratio in microfabricated structures, the importance of surface chemistry has been recognized and efforts have been made to optimize microfabricated surfaces for PCR (Shoffner et al., 1996). Active cooling based on Peltier thermoelectric elements has been investigated in an effort to increase cycling rates (Khandurina et al., 2000).

Flow-through PCR has also been demonstrated on microdevices. In this variation, the fluid containing the PCR mixture flows through regions where constant temperature conditions as required by the PCR chemistry are maintained (Kopp et al., 1998; Zhang and Xing, 2010). This increases throughput, while introducing new thermal optimization challenges. For example, it is important to design the microdevice to not only maintain three zones under tight temperature control, but also ensure rapid transition between temperature zones. The temperature control requirement is seen to be most stringent in the first and third steps of PCR. Further, the flowing PCR mixture must spend enough time in each zone for completion of each PCR step (Wittwer and Garling, 1991). While this is not a big problem for static PCR, flow-through PCR microdevices must be designed to ensure long enough residence in each temperature zone. This involves control of microchannel dimensions, including lengths, flowrates and parameters relating to the mechanism that drives the flow, which could be either external pressure-driven (Sadler et al., 2003), electrokinetically driven (Chen et al., 2005) or simply by natural convection (Wheeler et al., 2004). While Silicon is the preferred material for microfabricating static PCR devices, glass is preferred for continuous-flow PCR microdevices because of better thermal isolation of the three temperature zones owing to the low thermal conductivity of glass.

Droplet-based PCR involving picoliter droplet generation and PCR amplification within the droplets has been of much recent interest (Beer et al., 2007; Markey et al., 2010; Wang and Burns, 2009). The reduction in volume and the inherent parallelization of droplet-based microfluidics is expected to increase the PCR throughput even higher. Application of droplet microfluidics based PCR for large-scale targeted sequencing has been reported (Tewhey et al., 2009).

Since temperature control and manipulation is at the heart of the PCR mechanism, several interesting papers have investigated both the thermal modeling as well as experimental measurement of temperature fields in PCR microdevices. While preliminary PCR investigations did not focus much on thermal optimization of the process, finite-element modeling has recently been used as a tool for thermal design of PCR microdevices (Sadler et al., 2003). In a recent work, genetic algorithms were used for speeding up thermal optimization of a convective PCR microdevice (Lee et al., 2005). Experimental measurement of temperature in PCR microdevices has been performed using a variety of techniques including a thermocouple embedded in the external heater (Wilding, et al. 1994), a thin strip of paper with embedded temperature-sensitive liquid crystals (Wilding, et al. 1995) and liquid crystals directly in the PCR microchambers (Chaudhari et al., 1998). Integration of temperature sensing and feedback elements into microfabrication of PCR microstructures has not been investigated much and is indeed an area requiring much work. Also, the commonly used fluorescence method for real-time monitoring for PCR can potentially be used for temperature monitoring as well.

PCR has come a long way since its inception about two decades ago. Aided by miniaturization, it now influences several facets of biological research. Parallelization and integration are expected to play key roles in determining the direction of micro-PCR research. The trend of performing sample preparation, separation, detection and other assays on the same chip is likely to continue. At the same time, requirements for faster, cheaper and more accurate PCR are also likely to increase. Thermal optimization of microfabricated PCR structures is likely to play a major role in the realization of these goals.

3.2. *Temperature gradient focusing (TGF)*

Temperature Gradient Focusing (TGF) is a recently developed bioanalytical technique with excellent potential for application in

biomedical microdevices (Ross and Locascio, 2002; Kim et al., 2006). TGF has been used for separation and concentration of DNA and proteins in microchannels. Greater than 10,000 fold concentration has been reported with relatively little design optimization. This technique relies on the temperature-dependent electrophoretic mobility of common analytes. As a result of this temperature dependence, an axially varying temperature field in a microchannel causes an axial variation in the electrophoretic velocity. As shown schematically in Fig. 3, this results in a non-uniform total velocity, which reduces to zero at the location where the electrophoretic and bulk velocities exactly balance each other. The bulk velocity could be either electro-osmotic or pressure-driven or both. Fluid properties like viscosity and electrical conductivity are also strong functions of temperature, and hence must be accounted for in TGF models. In practice, as shown in Fig. 4, temperature gradient for TGF may be obtained by controlling temperature of two copper blocks on either ends of a microchannel. A high voltage power supply is used for applying the desired electric field. Visualization and temperature measurement is performed using fluorescence microscopy. Joule heating within the microchannel may be used to generate the temperature gradient needed for this technique (Ross and Locascio, 2002).Several papers in the recent past have focused on theoretical and numerical analysis of TGF. (Tang and Yang, 2008; Huber and Santiago, 2007, 2008; Sommer et al., 2007). The shape of the focused band in TGF depends on the balance of the

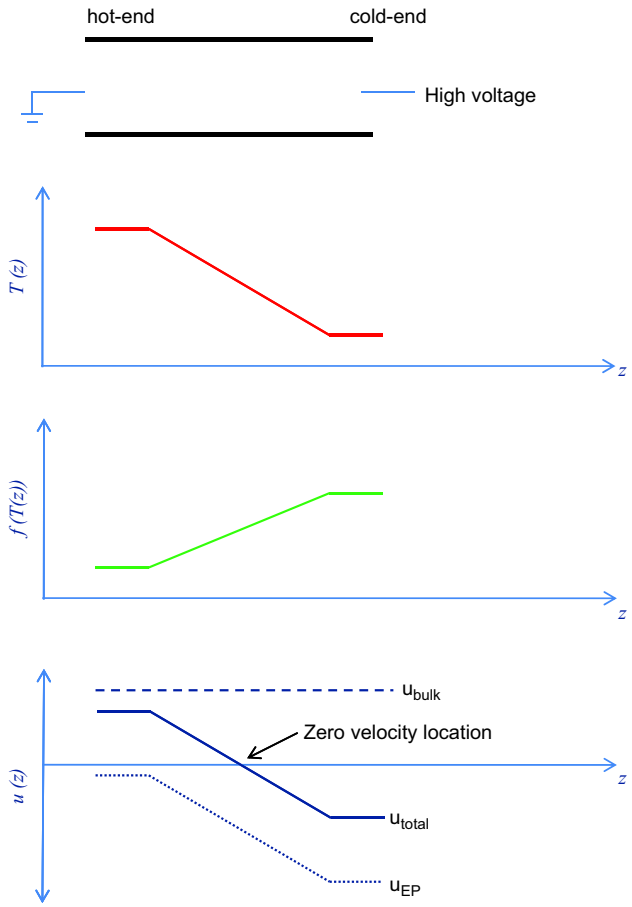

Fig. 3. Schematic diagram showing the basic principle behind TGF (from Ross and Locascio, 2002). Sample focusing occurs at the point where u_z is zero. Note that f is a function that accounts for temperature dependence of fluid viscosity and electrical conductivity.

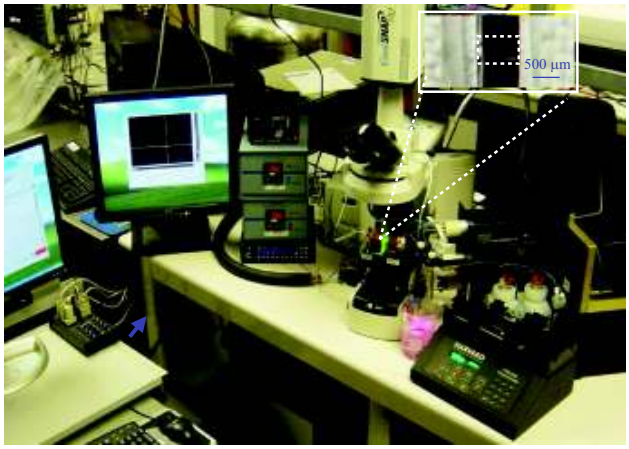

Fig. 4. A photograph of a TGF setup, showing an image of the microchannel in the inset (Ness, 2005).

convective and diffusive forces. In uniform temperature gradient conditions, the governing equation for microfluidic dispersion has an analytical solution, which has been shown to agree well with experimental results (Huber and Santiago, 2007). In more complicated situations, where the temperature gradient is not uniform due to Joule heating, numerical modeling has been used to solve all governing equations and the results compare well with experimental data (Tang and Yang, 2008). The effect of large sample concentration, which induces strong non-linear interactions with buffer ions, has been analyzed (Lin et al., 2008). Non-linear temperature gradient conditions have also been experimentally investigated (Shah et al., 2010)

A significant advantage of TGF over more traditional concentration techniques is that TGF requires a very short microchannel length for concentration/separation. Microchannels as short as 30 mm, with a working length of only 2 mm have been used (Ross and Locascio, 2002; Ness, 2005). Moreover, TGF can be completely run using on-chip controls. This makes TGF an attractive option for integration on micro-Total-Analysis Systems (μTAS).

Following the first TGF experiments by Ross, this technique has been applied for several biological assays, including concentration and separation of chiral compounds (Blass et al., 2004a; Vreeland and Locascio, 2003), DNA hybridization assays (Blass et al., (2004b); Ross et al., 2001), trace biomarker analysis using scanning mode TGF (Danger and Ross, 2008; Hoebel et al., 2006), characterization of biomolecular binding (Munson et al., 2008) development of free-flow electrophoresis (Becker et al., 2009), etc. High throughput TGF on a re-usable microfluidic platform consisting of a number of parallel microchannels has been demonstrated (Mao et al., 2002). The platform design in this work enabled subjecting each microchannel to a different temperature, and thus run high throughput, two variable experiments. We have been working on using microfabrication to integrate heaters and temperature sensors with the microchannel, in order to gain better control over the local temperature field (Ness, 2005). Previous works have only used linear temperature profiles, obtained by controlling the temperature at the microchannel ends. The realization of non-linear temperature profiles by localized non-uniform heating may provide better control over the focusing process and may enable the simultaneous focusing of more than one analyte. The major challenge to be overcome in this task is to passivate the metal heaters/sensors from the analyte and the strong electric field inside the microchannel.

While TGF is a relatively new technique, it provides several advantages over traditional separation and concentration

techniques. The future work in this field is expected to continue to apply TGF to new applications as well as focus on integration of TGF with sample preparation as well as detection techniques.

3.3. Thermotaxis

The nervous system in mammals consists of billions of nerve cells intricately connected to each other by neurites. The precision with which these neuronal connections are made in the central nervous system during early development has been the focus of much research (Lockerbie, 1987). Nerve regeneration and growth following injury to the peripheral nervous system has also been widely studied (Schmidt and Leach, 2003; Rogers et al., 1983), with the goal of developing devices and therapies to aid in recovery (Miller et al., 2001). At a fundamental level, neurite growth is believed to occur as a result of chemical interactions between the cell and its microenvironment. In particular, the sensing of gradients in specific chemical species around the cell determines the rate and direction of growth of neurites. This widely studied phenomenon is known as chemotaxis (Cajal, 1982; Mueller, 1999). The equivalent thermal effect, thermotaxis – motion or growth due to thermal gradients – is known to exist, at least at the organismal level, but has not been studied as widely. Organisms are known to be surprisingly sensitive to temperature. For example, fish cultivated in a large tank have been observed to avoid regions where the temperature is more than 0.3 °C different from their acclimatization temperature (Davis and Kleerekoper, 1971). Similarly, *C Elegans* are known to migrate on a surface with a temperature gradient to a region where the temperature matches the temperature at which they were cultivated (Ryu and Samuel, 2002; Hedgecock and Russell, 1975). The individual neurons responsible for temperature sensing in *C Elegans* have been identified indirectly (Mori and Ohshima, 1995), but the temperature effects on the individual neurons have not been studied. It is difficult to use traditional biological research tools for such a study due to the lack of temperature control down to the scale of typical cells. Advances in microfabrication have enabled the realization of microstructures capable of generating an accurately controlled temperature field (Jain et al., 2009). In conjunction with techniques for cell culture on Silicon and Silicon-related surfaces, this enables the study of temperature gradient effects of cell behavior. Several microdevices aimed at temperature control over a surface have been developed, with applications in chemical sensing and catalysis (Simon et al., 2001). These microdevices take advantage of the large thermal conductivity of Silicon to achieve a uniform temperature field. On the other hand, use of low thermal conductivity Silicon Oxide and/or Silicon Nitride leads to the generation of large temperature gradients, making them suitable for studying thermotaxis at the cellular level (Jain et al., 2009). Such thin film microheater structures are usually microfabricated using silicon substrates and microfabrication tools like photolithography, chemical vapor deposition (CVD) and deep reactive ion etching (DRIE). The thin film structure ensures thermal isolation and hence good control over the temperature distribution on the membrane. Fig. 5 shows an SEM image of one such microheater device, which is currently being used to study the effect of temperature gradients on neurite outgrowth in nerve cells. Aluminum heater and temperature sensors are visible on the silicon nitride membrane. Fig. 6 shows a fluorescence image of Retinal Ganglion cells cultured on the microheater device. The heater and temperature sensors can also be seen.

In a related work (Pearce et al., 2004), a MEMS-based system consisting of microelectrode arrays and photopolymer-based microfluidic channels has been developed. Fluid entering the microdevice is pre-heated, and thermocouples are used for

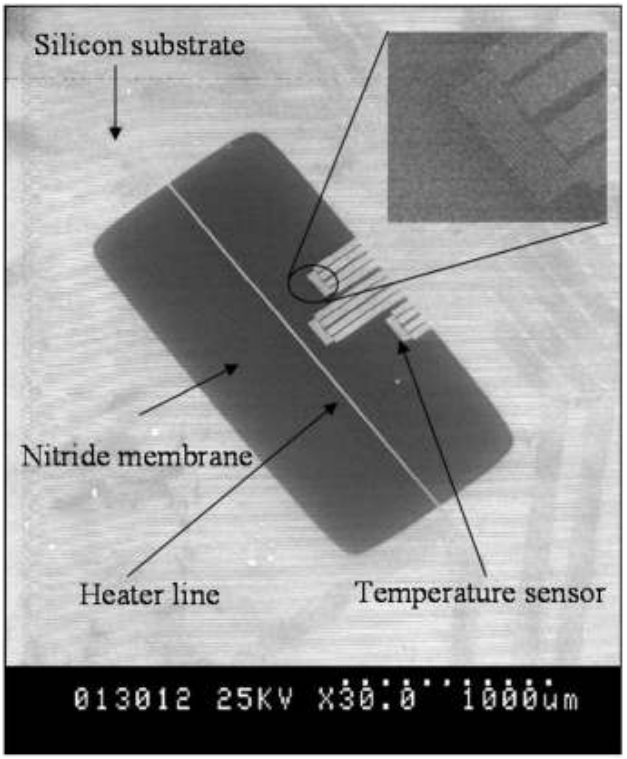

Fig. 5. SEM of a microheater device used for studying the effect of thermal gradients on nerve cells (Jain et al., 2009).

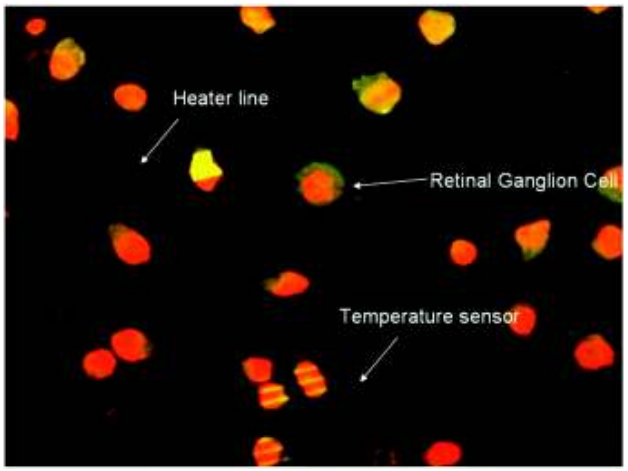

Fig. 6. Fluorescent image of nerve cells cultured on the microheater device (Jain et al., 2009).

temperature measurement. Confirmation of localized temperature control is obtained by electrically monitoring neural activity. While it is possible to further refine temperature control and sensing as presented in this work, for example by integrating the heating and sensing functions within the microfabricated device, this work nevertheless demonstrates the possibility of using MEMS-based devices to alter and manipulate cellular activity using thermal signals.

In conjunction with recent experimental work on thermotaxis, some advances in the theoretical understanding of the effect of temperature on the cellular sensory processes have been reported (Jain et al., 2005). The theoretical model is based on the effect of

the temperature gradient on the chemical equilibrium of the ligand binding reactions that are important in the chemical sensory process of the cell. Using equilibrium biochemistry and the van't Hoff equation to model the temperature-dependence of the equilibrium constant, the model predicts the minimum temperature gradient required to observe thermotactic effects in cells. The synergistic effect of combining chemical and thermal gradients is also explained by the model. The results of the model indicate that a moderate temperature gradient of 1 °C across the cell body may be sufficient to trigger cellular response traditionally associated with chemical gradients. It is not clear whether a temperature gradient of such a magnitude may be expected in the cellular microenvironment. Thus, while it is difficult to say whether temperature gradients play a significant role in cell-microenvironment interactions *in vivo*, it is still instructive to study thermal gradient effects *in vitro* using biocompatible microfabricated structures.

3.4. Other bioanalytical techniques based on thermal gradients

Temperature-gradient gel electrophoresis (TGGE) is another application of temperature gradients to manipulate biomolecules. First introduced about two decades ago (Rosenbaum and Riesner, 1987; Wartell et al., 1990), this method involves the application of a temperature gradient across an electrophoretic gel. The presence of the temperature gradient is seen to encourage migration of biomolecules perpendicular to the direction of the gradient. Hence, if the temperature gradient is applied perpendicular to the electric field used to drive electrophoresis, the two effects – electrical and thermal – can be combined synergistically to enhance separation speeds. TGGE has been used extensively in DNA mutation detection (Buch et al., 2004; Wong et al., 2004), microbiology (Vasquez et al., 2001; Monstein et al., 2000; Fouratt et al., 2003), food biotechnology (Ogier et al., 2002; Hernán-Gómez et al., 2000), etc.

The reduced size and power requirements of TGGE compared to other traditional gel electrophoresis methods makes it much easier to integrate into microfluidic systems, like a Lab-on-a-Chip microanalytical device. TGGE offers real-time, high throughput, highly sensitive DNA detection.

Apart from the original idea of applying the thermal field by heated Copper blocks on two ends of a gel, TGGE has also been demonstrated in microfluidic devices, both by an externally applied temperature gradient (Schell et al., 1999; Gao and Young, 2000) and through Joule heating of the buffer (Gao and Young, 2000; Gelfi et al., 1997). While Joule heating of the buffer eliminates the need for an external temperature control system, it couples the applied voltage and temperature gradient to each other, causing practical difficulties. The true potential of TGGE is revealed when integrated in a parallelized, multi-capillary system. For example, a high throughput TGGE system consisting of 96 capillaries in parallel has been demonstrated (Gao and Young, 2000). A microfluidic device containing integrated microheaters and sensors for generation and detection of the thermal field has also been presented (Buch et al., 2004).

Due to the application of large electric fields and the usually low thermal conductivities of substrates used in microdevices for TGF and TGGE, distortion of the temperature field due to Joule heating becomes an important issue (Gobie and Ivory, 1990; Erickson et al., 2003). Though Joule heating could be leveraged for generating the required temperature gradients (Ross and Locascio, 2002), it increases band broadening in microsystems where concentration of a particular molecule is desired (Gobie and Ivory, 1990). With careful simulation and comparison with experimental data, it is possible to develop guidelines for accounting for Joule heating and for improving chip design from the thermal point of view.

Thermophoresis is another example of the application of temperature gradients at the microscale as an effective force for manipulating biomolecules. Thermophoresis, also known as the Soret effect (Soret, 1879; Maxwell, 1890; de Groot and Mazur, 1969), has been used to trap and concentrate DNA samples (Braun and Libchaber, 2002) and to develop protein-binding assays in biological liquids (Wienken et al., 2010). DNA has been shown to be repelled from a region that is locally heated by an infra-red source. This phenomenon can be carefully used to increase DNA concentration in unheated regions. Temperature differences as small as a few K have been shown to produce significant thermophoretic effect (Duhr et al., 2004). While thermophoresis is still far from being proved to be commercially viable, it has been shown to have several advantages over standard gel electrophoresis methods. For example, thermophoresis is much easier to miniaturize and interface with microanalytical systems. Unlike gel electrophoresis, there is no need to separately prepare gel phases, which is usually a tedious process. Since thermophoresis has been shown to be size-dependent, it may also be developed as a method for DNA separation and concentration. Moreover, unlike electrophoresis, thermophoresis can be carried out in complex fluids such as blood serum (Reineck et al., 2010).

Due to reduced sample sizes and enhanced temperature controllability, microfluidic calorimetry offers an exceptional means of thermodynamic characterization of biochemical reactions of interest (Lee et al., 2009; Torres et al., 2010). The development of flow-through calorimeters (Hany et al., 2010; Lee et al., 2009) is particularly interesting due to the possibility of its on-chip integration with other microfluidic assays.

Thermally triggered liposomes have recently been used for controlled delivery and rapid mixing of reagents in a microfluidic device (Vreeland and Locascio, 2003). The reagent of interest is encapsulated in liposomes, which release the reagent when provided a thermal trigger. By careful application of a spatially varying temperature field, it would be possible to obtain fine control over the spatial distribution of the reaction.

A better understanding of heat transfer and temperature control and manipulation issues in each of the microdevices presented above will lead to improved performance. Though the development of micro-Total-Analysis Systems (μTAS) has been slower than was widely expected, integration is very much the need of the day in order to develop effective analytical tools that can run a variety of assays and reduce analysis times and costs.

4. Concluding remarks

Thermal effects play an important role in determining the behavior of biological microsystems. Although the traditional understanding of the cell has not accorded as much importance to its temperature microenvironment as to other factors, the importance of thermal factors is slowly being recognized. Several bioanalytical techniques based on thermal effects have been developed in the recent past. These techniques make use of the strong temperature dependence of the form and function of biologically important macromolecules like DNA and proteins. Advances in microfabrication have enabled the miniaturization of bioanalytical tools, creating the distinct discipline of BioMEMS. The progress in microfabricated device technologies will play an important role in shaping the nature of basic biological research as well as the medical care industry.

There are several examples of the innovative use of thermal engineering principles to improve biological assays or devices. A successful application of thermal engineering principles to

biological problems not only requires a good understanding of the basics of thermal engineering, but also an appreciation of how thermal effects interact with biological microsystems. For instance, it is important to recognize that biocompatibility is one of the foremost requirements of the design of a microdevice that will interact with cells or tissue.

Biological research has become increasingly multi-disciplinary in the past few decades. With the involvement of physicists and engineers, several innovative techniques and methodologies to analyze biological systems have been developed. Not only has this interaction resulted in improvement in research methods, but it has also led to the development of tools that enable biologists to perform investigations that were simply not possible with traditional tools. The interaction between biology and engineering can only be expected to increase in the future.

Acknowledgments

The authors would like to acknowledge useful discussions with Kevin Ness. Stanford Graduate Fellowships have provided financial support to one of the authors.

References

Arora, A., Simone, G., Salieb-Beugelaar, G.B., Kim, J.T., Manz, A., 2010. Latest developments in micro total analysis systems. Anal. Chem. 82 (12), 4830–4847.

Becker, M., Mansouri, A., Beilein, C., Janasek, D., 2009. Temperature gradient focusing in miniaturized free-flow electrophoresis devices. Electrophoresis 30 (24), 4206–4212.

Beer, N.R., Hindson, B.J., Wheeler, E.K., Hall, S.B., Rose, K.A., Kennedy, I.M., Colston, Bill W., 2007. On-chip, real-time, single-copy polymerase chain reaction in picoliter droplets. Anal. Chem. 79 (22), 8471–8475.

Black, C.M., Clark, R.P., Darton, K., Goff, M.R., Norman, T.D., Spikes, H.A., 1990. A pyroelectric thermal imaging system for use in medical diagnosis. J. Biomed. Eng. 12 (4), 281–286.

Blass, K.M., Vreeland, W.N., Phinney, K.W., Ross, D., 2004a. Simultaneous concentration and separation of enantiomers with chiral temperature gradient focusing. Anal. Chem. 76, 7243–7249.

Blass, K.M., Ross, D., Begley, H.C., Olsen, K.G., Tarlov, M.J., 2004b. DNA hybridization assays using temperature gradient focusing and peptide nucleic acids. J. Am. Chem. Soc. 126, 13474–13479.

Brambrink, A.M., Kopacz, L., Astheimer, A., Noga, H., Heimann, A., Kempski, O., 1999. Control of brain temperature during experimental global ischemia in rats. J. Neurosci. Methods 92, 111–122.

Braun, D., Libchaber, A., 2002. Trapping of DNA by thermophoretic depletion and convection. Phys. Rev. Lett. 89 (18), 1–4.

Buch, J.S., Kimball, C., Rosenberger, F., Highsmith Jr., W.E., DeVoe, D.L., Lee, C.S., 2004. DNA mutation detection in a polymer microfluidic network using temperature gradient gel electrophoresis. Anal. Chem. 76, 874–881.

Cajal, S.R., 1982. Degeneration and Regeneration of the Nervous System. Oxford University Press, London (translated R.M. May).

Chaudhari, A.M., Woudenberg, T.M., Albin, M., Goodson, K.E., 1998. Transient liquid crystal thermometry of microfabricated PCR vessel arrays. J. MEMS 7 (4), 345–355.

Chen, J.F., Wabuyele, M., Chen, H.W., Patterson, D., Hupert, M., Shadpour, H., Nikitopoulos, D., Soper, S.A., 2005. Electrokinetically synchronized polymerase chain reaction microchip fabricated in polycarbonate. Anal. Chem. 77 (2), 658–666.

Childs, C., Machin, G., 2009. Reliability issues in human brain temperature measurement. Critical Care 13, 106.

Coulman, S.A., Anstey, A., Gateley, C., Morrissey, A., McLoughlin, P., Allender, C., Birchall, J.C., 2009. Microneedle mediated delivery of nanoparticles into human skin. Int. J. Pharmaceutics 366, 190–200.

Danger, G., Ross, D., 2008. Development of a temperature gradient focusing method for *in situ* extraterrestrial biomarker analysis. Electrophoresis 29 (15), 3107–3114.

Davis, S., Kleerekoper, H., 1971. Observations on the response of goldfish to temperature gradients. Am. Zool. 11 (4), 695.

de Groot, S.R., Mazur, P., 1969. Non-Equilibrium Thermodynamics. North-Holland, Amsterdam.

de Zwart, J.A., van Gelderen, P., Kelly, D.J., Moonen, C.T., 1996. Fast magnetic-resonance temperature imaging. J. Magn. Reson. Ser. B 112 (1), 86–90.

Dietrich, W.D., 1992. The importance of brain temperature in cerebral injury. J. Neurotrauma 9, 475–485.

Duhr, S., Arduini, S., Braun, D., 2004. Thermophoresis of DNA determined by microfluidic fluorescence. Eur. Phys. J. E 15, 277–286.

Erickson, D., Sinton, D., Li, D.Q., 2003. Joule heating and heat transfer in poly(dimethylsiloxane) microfluidic systems. Lab Chip 3 (3), 141–149.

Ferrell, T.L., Crilly, P.B., Smith, S.F., Wintenberg, A.L., Britton, C.L., Morrison, G.W., Ericson, M.N., Hedden, D., Bouldin, D., Passian, A., 1998. Medical telesensors. Proc. SPIE 3253, 193–198.

Folch, A., Toner, M., 2000. Microengineering of cellular interactions. Annu. Rev. Biomed. Eng. 2, 227–256.

Fouratt, M.A., Rhodes, J.S., Smithers, C.M., Love, N.G., Stevens, A.M., 2003. Application of temperature gradient gel electrophoresis to the characterization of a nitrifying bioaugmentation product. FEMS Microbiol. Ecol. 43 (2), 277–286.

Gao, Q., Young, E.S., 2000. High-throughput detection of unknown mutations by using multiplexed capillary electrophoresis with poly(vinylpyrrolidone) solution. Anal. Chem 72, 2499–2506.

Gelfi, C., Righetti, P.G., Travi, M., Fattore, S., 1997. Temperature-programmed capillary electrophoresis for the analysis of high-melting point mutants in thalassemias. Electrophoresis 18 (5), 724–731.

Gillot, F., Morin, F.O., Arata, H.F., Guégan, R., Tanaka, H., Fujita, H., 2007. On-chip thermal calibration of 8 CB liquid crystal of micro-thermal device. Lab Chip 7, 1600–1602.

Gobie, W.A., Ivory, C.F., 1990. Thermal model of capillary electrophoresis and a method for counteracting thermal band broadening. J. Chromatogr 516 (1), 191–210.

Goff, M.R., Clark, R.P., 1985. Computerised infra-red thermography in medicine and physiology. J. Photogr. Sci. 33 (2), 60–67.

Gorbach, A.M., Heiss, J.D., Kopylev, L., Oldfield, E.H., 2004. Intraoperative infrared imaging of brain tumors. J. Neurosurg. 101 (6), 960–969.

Hany, C., Lebrun, H., Pradere, C., Toutain, J., Batsale, J.-C., 2010. Thermal analysis of chemical reaction with a continuous microfluidic calorimeter. Chemical Eng. J. 160 (3), 814–822.

Hedgecock, E., Russell, R., 1975. Normal and mutant thermotaxis in the nematode *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. U. S. A. 72, 4061–4065.

Heller, C., Sadava, D., Orians, G.H., Purves, W.K., 2000. Life: The science of biology 6th Ed. W.H. Freeman.

Hernán-Gómez, S., Espinosa, J.C., Ubeda, J.F., 2000. Characterization of wine yeasts by temperature gradient gel electrophoresis. FEMS Microbiol. Lett. 193, 45–50.

Hoebel, S.J., Balss, K.M., Jones, B.J., Malliaris, C.D., Munson, M.S., Vreeland, W.N., Ross, D., 2006. Scanning temperature gradient focusing. Anal. Chem. 78 (20), 7186–7190.

Huber, D., Santiago, J.G., 2007. Taylor–Aris dispersion in temperature gradient focusing. Electrophoresis 28, 2333–2344.

Huber, D., Santiago, J.G., 2008. Ballistic dispersion in temperature gradient focusing. Proc. R. Soc. A 464, 595–612.

Iatrou, C.C., Domaingue, C.M., Thomas, R.D., Nye, D.H., 2002. The effect of selective brain cooling on intracerebral temperature during craniotomy. Anaesth. Intensive Care 30 (2), 167–170.

Jain, A., Ness, K.D., Goodson, K.E., 2005. A theoretical model for temperature gradient effects on cells. In: Proceedings of the ASME Summer Heat Transfer Conference, San Francisco.

Jain, A., Ness, K.D., McConnell, A.D., Jiang, L., Goodson, K.E., 2009. Theoretical and experimental investigation of spatial temperature gradient effects on cells using a microfabricated microheater platform. Sensors Actuators B 143, 286–294.

Kateb, B., Yamamoto, V., Yu, C., Grundfest, W., Gruen, J.P., 2009. Infrared thermal imaging: a review of the literature and case report. NeuroImage 47, 154–162.

Kewley, D.T., Hills, M.D., Borkholder, D.A., Opris, I.E., Maluf, N.I., Storment, C.W., Bower, J.M., Kovacs, G.T.A., 1997. Plasma-etched neural probes. Sens. Actuators, A 58 (1), 27–35.

Khandurina, J., McKnight, T.E., Jacobson, S.C., Waters, L.C., Foote, R.S., Ramsey, J.M., 2000. Integrated system for rapid PCR-based DNA analysis in microfluidic devices. Anal. Chem. 72 (13), 2995–3000.

Kickhefel, A., Roland, J., Weiss, C., Schick, Fritz, 2009. Accuracy of real-time MR temperature mapping inthe brain: a comparison of fast sequences. Phys. Med. 26, 192–201.

Kim, S.M., Sommer, G.J., Burns, M.A., Hasselbrink, E.F., 2006. Low-power concentration and separation using temperature gradient focusing via Joule heating. Anal. Chem. 78, 8028–8035.

Knox, J.H., McCormack, K.A., 1994. Temperature effects in capillary electrophoresis. 1. Internal capillary temperature and effect upon performance. Chromatographia 38 (3–4), 207–214.

Kopp, M.U., de Mello, A.J., Manz, A., 1998. Chemical amplification: continuous-flow PCR on a chip. Science 280 (5366), 1046–1048.

Kovacs, G.T.A., 1984. Micromachined Transducers Sourcebook second ed. McGraw-Hill, New York.

LaBianca, N., Delorme, J., 1998. High aspect ratio resist for thick film applications. Proc. SPIE 2438, 846–852.

Lacey, M.E., Webb, A.G., Sweedler, J.V., 2000. Monitoring temperature changes in capillary electrophoresis with nanoliter-volume NMR thermometry. Anal. Chem. 20, 4991–4998.

Larina, I.V., Larin, K.V., Esenaliev, R.O., 2005. Real-time optoacoustic monitoring of temperature in tissues. J. Phys. D: Appl. Phys. 38, 2633.

Lee, H.W., Azid, I.A., Seetharamu, K.N., 2005. Optimization of temperature control in BioMEMS. In: Proceedings of the ASME InterPACK, San Francisco.

Lee, W., Fon, W., Axelrod, B.W., Roukes, M.L., 2009. High-sensitivity microfluidic calorimeters for biological and chemical applications. Proc. Natl. Acad. Sci. U. S. A. 106, 15225–15230.

Li, Chenglin, Chen, Na, Chen, Zhenyi, Wang, Tingyun, 2009. Fully distributed chirped FBG sensor and application in laser-induced interstitial thermotherapy. In: Proceedings of the Asia Communications and Photonics Conference and Exhibition (ACP), pp. 1–2.

Lin, H., Shackman, J.G., Ross, D., 2008. Finite sample effect in temperature gradient focusing. Lab Chip 8, 969–978.

Lockerbie, R.O., 1987. The neuronal growth cone: a review of its locomotory, navigational and target recognition capabilities. Neuroscience 20 (3), 719–729.

Maass-Moreno, R., Damianou, C.A., Sanghvi, N.T., 1996. Noninvasive temperature estimation in tissue via ultrasound echo-shifts. Part II. In vitro study. J. Acous. Soc. Am. 100 (4), 2522–2530.

MacFall, J., Prescott, D.M., Fullar, E., Samulski, T.V., 1995. Temperature dependence of canine brain tissue diffusion coefficient measured in vivo with magnetic resonance echo-planar imaging. Int. J. Hyperthermia 11 (1), 73–86.

Mao, H.B., Holden, M.A., You, M., Cremer, P.S., 2002. Reusable platforms for high-throughput on-chip temperature gradient assays. Anal. Chem. 74 (19), 5071–5075.

Markey, A.L., Mohr, S., Da, P.J.R., 2010. High-throughput droplet PCR. Methods 50 (4), 277–281.

Martin, P.M., Matson, D.W., Bennett, W.D., Lin, Y., Hammerstrom, D.J., 1999. Laminated plastic microfluidic components for biological and chemical systems. J. Vac. Sci. Technol. A 17 (4), 2264–2270.

Maswadi, S.M., Dodd, S.J., Gao, J.-H., Glickman, R.D., 2004. Temperature mapping of laser-induced hyperthermia in an ocular phantom using magnetic resonance thermography. J. Biomed. Opt. 9 (4), 711–718.

Maxwell, J.C., 1890. Collected Papers II. Cambridge University Press, pp. 681–712.

McAllister, D.V., Allen, M.G., Prausnitz, M.R., 2000. Microfabricated microneedles for gene and drug delivery. Annu. Rev. Biomed. Eng. 2, 289–313.

McDonald, J.C., Duffy, D.C., Anderson, J.R., Chiu, D.T., Wu, H., Schueller, O.J.A., Whitesides, G.M., 2000. Fabrication of microfluidic systems in poly(dimethylsiloxane). Electrophoresis 21, 27–40.

Mellergård, P., 1994. Monitoring of rectal, epidural, and intraventricular temperature in neurosurgical patients. Acta. Neurochir. Suppl. 60, 485–487.

Miller, C., Jeftinija, S., Mallapragada, S., 2001. Micropatterned Schwann cell-seeded biodegradable polymer substrates significantly enhance neurite alignment and growth,. Tissue Eng. 7 (6), 705–715.

Minamisawa, H., Mellergård, P., Smith, M.L., Bengtsson, F., Theander, S., Boris-Moller, F., 1990. Preservation of brain temperature during ischemia in rats. Stroke 21, 758–764.

Monstein, H.-J., Tiveljung, A., Kraft, C.H., Borch, K., Jonasson, J., 2000. Profiling of bacterial flora in gastric biopsies from patients with Helicobacter pylori-associated gastritis and histologically normal control individuals by temperature gradient gel electrophoresis and 16S rDNA sequence analysis. J. Med. Micobiol. 49, 817–822.

Mori, I., Ohshima, Y., 1995. Neural regulation of thermotaxis in *Caenorhabditis elegans*. Nature 376, 344–348.

Mueller, B.K., 1999. Growth cone guidance: first steps towards a deeper understanding. Annu. Rev. Neurosci. 22, 351–388.

Mullis, K.B., Faloona, F.A., 1987. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. Methods Enzymol. 155, 335–350.

Munson, M.S., Meacham, J.M., Locascio, L.E., Ross, D., 2008. Counterflow rejection of adsorbing proteins for characterization of biomolecular interactions by temperature gradient focusing. Anal. Chem. 80 (1), 172–178.

Ness, K. D., 2005. Microfabrication of microchannels in glass with integrated temperature sensors and heaters for temperature gradient focusing, private communication.

Northrup, M.A., Ching, M.T., White, R.M., Watson, R.T., 1993. DNA amplification with a microfabricated reaction chamber. In: Proceedings of the 7th IEEE Conference on Solid State Sensors and Actuators, New York.

Ogier, J.-C., Son, O., Gruss, A., Tailliez, P., Delacroix-Buchet, A., 2002. Identification of the bacterial microflora in dairy products by temporal temperature gradient gel electrophoresis. Appl. Environ. Microbiol. 68 (8), 3691–3701.

Pearce, T.M., Wilson, J.A., Oakes, S.G., Chiu, S.-Y., Williams, J.C., 2004. Integrated microelectrode array and microfluidics for temperature clamp of sensory neurons in culture. Lab Chip 5, 97–101.

Petersen, K.E., 1982. Silicon as a mechanical material. Proc. IEEE 70 (5), 420–457.

Polla, D.L., Erdman, A.G., Robbins, W.P., Markus, D.T., Diaz-Diaz, J., Rizq, R., Nam, Y., Brickner, H.T., Wang, A., Krulevitch, P., 2000. Microdevices in medicine. Annu. Rev. Biomed. Eng. 2, 551–576.

Pramanik, M., Wang, L.V., 2009. Thermoacoustic and photoacoustic sensing of temperature. J. Biomed. Opt. 14, 054024.

Rao, Y., Webb, D.J., Jackson, D.A., Zhang, L., Bennion, I., 1997. In-fiber Bragg-grating temperature sensor system for medical applications. J. Lightwave Tech. 15 (5), 779–785.

Rebello, K.J., 2004. Applications of MEMS in surgery. Proc. IEEE 92 (1), 43–55.

Reineck, P., Wienken, C.J., Braun, D., 2010. Thermophoresis of single-stranded DNA. Electrophoresis 31, 279–286.

Roemer, R.B., 1999. Engineering aspects of hyperthermia therapy. Annu. Rev. Biomed. Eng. 1, 347–376.

Rogers, S.L., Letourneau, P.C., Palm, S.L., McCarthy, J., Furcht, L.T., 1983. Neurite extension by peripheral and central nervous system neurons in response to substratum-bound fibronectin and laminin. Dev. Biol. 98 (1), 212–220.

Rosenbaum, V., Riesner, D., 1987. Temperature-gradient gel electrophoresis: thermodynamic analysis of nucleic acids and proteins in purified form and in cellular extracts. Biophys. Chem. 26, 235–246.

Ross, D., Locascio, L.E., 2002. Microfluidic temperature gradient focusing. Anal. Chem. 74, 2556–2564.

Ross, D., Gaitan, M., Locascio, L.E., 2001. Temperature measurement in microfluidic systems using a temperature-dependent fluorescent dye. Anal. Chem. 73, 4117–4123.

Ryu, S., Yoo, I., Song, S., Yoon, B., Kim, J.-M., 2009. A thermoresponsive fluorogenic conjugated polymer for a temperature sensor in microfluidic devices. J. Am. Chem. Soc. 131 (11), 3800–3801.

Ryu, W.S., Samuel, A.D., 2002. Thermotaxis in Caenorhabditis elegans amalyzed by measuring responses to defined thermal stimuli. J. Neurosci. 22 (13), 5727–5733.

Sadler, D.J., Changrani, R., Roberts, P., Chou, C.-F., Zenhausern, F., 2003. Thermal management of BioMEMS: temperature control for ceramic-based PCR and DNA detection devices. IEEE Trans. Comp. Packag. Technol 26 (2), 309–315.

Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B., Erlich, H.A., 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 4839, 487–491.

Sato, Y., Irisawa, G., Ishizuka, M., Hishida, K., Maeda, M., 2003. Visualization of convective mixing in microchannel by fluorescence imaging. Meas. Sci. Technol. 14, 114–121.

Saxena, I.S., Hui, K., 2010. Polymer coated fiber Bragg grating thermometry for microwave hyperthermia. Med. Phys. 37, 4615–4619.

Schell, J., Wulfert, M., Riesner, D., 1999. Detection of point mutations by capillary electrophoresis with temporal temperature gradients. Electrophoresis 20, 2864–2869.

Schmidt, C.E., Leach, J.B., 2003. Neural tissue engineering: strategies for repair and regeneration. Annu. Rev. Biomed. Eng. 5, 293–347.

Schüle, G., Hüttmann, G., Brinkmann, R., Framme, C., Roider, J., 2004. Noninvasive optoacoustic temperature determination at the fundus of the eye during laser irradiation. J. Biomed. Opt. 9, 173.

Shah, J.J., Geist, J., Gaitan, M., 2010. Microwave-induced adjustable nonlinear temperature gradients in microfluidic devices. J. Micromech. Microeng. 20, 10.

Shoffner, M.A., Cheng, J., Hvichia, G.E., Kricka, L.J., Wilding, P., 1996. Chip PCR 1. Surface passivation of microfabricated silicon-glass chips for PCR. Nucleic Acids. Res 24 (2), 375–379.

Simon, I., Barsan, N., Bauer, M., Weimar, U., 2001. Micromachined metal oxide gas sensors: opportunities to improve sensor performance. Sensors Actuators, A 73, 1–26.

Singh, K., Kim, K.C., 2009. Clinical bio-micro-electro-mechanical systems: technology and applications. Sensor Lett. 7 (6), 1013–1024.

Sommer, G.J., Kim, S.M., Littrell, R.J., Hasselbrink, E.F., 2007. Theoretical and numerical analysis of temperature gradient focusing via joule heating. Lab Chip 7, 898–907.

Soret, C., 1879. Arch. Sci. Phys. Nat. Genéve 3, 48.

Stone, J.G., Goodman, R.R., Baker, K.Z., Baker, C.J., Solomon, R.A., 1997. Direct intraoperative measurement of human brain temperature. Neurosurgery 41 (1), 20–24.

Tang, G., Yang, C., 2008. Numerical modeling of Joule heating-induced temperature gradient focusing in microfluidic channels. Electrophoresis 29 (5), 1006–1012.

Taylor, T.B., Winn-Deen, E.S., Picozza, E., Woudenberg, T.M., Albin, M., 1997. Optimization of the performance of the polymerase chain reaction in silicon-based microstructures. Nucleic Acids Res. 25, 3164–3168.

Tewhey, R., Warner, J.B., Nakano, M., Libby, B., Medkova, M., David, P.H., Kotsopoulos, S.K., Samuels, M.L., Hutchison, J.B., Larson, J.W., Topol, E.J., Weiner, M.P., Harismendy, O., Olson, J., Link, D.R., Frazer, K.A., 2009. Microdroplet-based PCR enrichment for large-scale targeted sequencing. Nat. Biotechnol. 27, 1025–1031.

Torres, F.E., Recht, M.I., Coyle, J.E., Bruce, R.H., Williams, G., 2010. Higher throughput calorimetry: opportunities, approaches and challenges. Curr. Opin. Struct. Biol. 20 (5), 598–605.

Trimmer, W., 1996. Micromechanics and MEMS: Classic and Seminal Papers to 1990. IEEE Press, New York.

Vasquez, A., Ahrne, S., Pettersson, B., Molin, G., 2001. Temporal temperature gradient gel electrophoresis (TTGE) as a tool for identification of *Lactobacillus casei*, *Lactobacillus paracasei*, *Lactobacillus zeae* and *Lactobacillus rhamnosus*. Lett. Appl. Microbiol. 32 (4), 215–219.

Vreeland, W.N., Locascio, L.E., 2003. Using bioinspired thermally triggered liposomes for high-efficiency mixing and reagent delivery in microfluidic devices. Anal. Chem. 75 (24), 6906–6911.

Vyazovkin, S., 2010. Thermal analysis. Anal. Chem. 82 (12), 4936–4949.

van den Berg, A., Lammerink, T.S.J., 1998. Micro total analysis systems: microfluidic aspects, integration concept and applications. In: Manz, A., Becker, H. (Eds.), Topics in Current Chemistry, vol. 194. Springer Verlag, Berlin, pp. 21–49.

Wang, F., Burns, M.A., 2009. Performance of nanoliter-sized droplet-based microfluidic PCR. Biomed. Microdevices 11 (5), 1071–1080.

Wartell, R.M., Hosseini, S.H., Moran Jr., C.P., 1990. Detecting base pair substitutions in DNA fragments by temperature-gradient gel electrophoresis. Nucleic Acids Res. 18 (9), 2699–2705.

Welch, A.J., van Gemert, M.J.C., 1995. Optical-Thermal Response of Laser-Irradiated Tissue. Plenum Press, New York.

Wheeler, E.K., Benett, W., Stratton, P., Richards, J., Chen, A., Christian, A., Ness, K.D., Ortega, J., Li, L.G., Weisgraber, T.H., Goodson, K., Milanovich, F., 2004. Convectively driven polymerase chain reaction thermal cycler. Anal. Chem. 76 (14), 4011–4016.

Wienken, C.J., Baaske, P., Rothbauer, U., Braun, D., Duhr, S., 2010. Protein-binding assays in biological liquids using microscale thermophoresis. Nature Communications.

Wilding, P., Shoffner, M.A., Kricka, L.J., 1994. PCR in a silicon microstructure. Clin. Chem. 40 (9), 1815–1818.

Wilding, P., Shoffner, M.A., Cheng, J., Hvichia, G., Kricka, L.J., 1995. Thermal cycling and surface passivation of micromachined devices for PCR. Clin. Chem. 41 (9), 1367–1368.

Wittwer, C.T., Garling, D.J., 1991. Rapid cycle DNA amplification: time and temperature optimization. BioTechniques 10 (1), 76–83.

Wolthius, R., McCrae, D., Hartl, J.C., Saaski, E., Mitchell, G.L., Garcin, K., Willard, R., 1991. Development of medical pressure and temperature sensors employing optical spectrum modulation. IEEE Trans. Biomed. Eng. 38 (10), 974–981.

Wong, L.-J.C., Chen, T.-J., Tan, D.-J., 2004. Detection of mitochondrial DNA mutations using temporal temperature gradient gel electrophoresis. Electrophoresis 25, 2602–2610.

Woolley, A.T., Hadley, D., Landre, P., DeMello, A.J., Mathies, R.A., Northrup, M.A., 1996. Functional integration of PCR amplification and capillary electrophoresis in a microfabricated DNA analysis device. Anal. Chem. 68 (23), 4081–4086.

Zeng, X., Birrien, J.-L., Fouquet, Y., Cherkashov, G., Jebbar, M., Querellou, J., Oger, P., Cambon-Bonavita, M.-A., Xiao, X., Prieur, D., 2009. Pyrococcus CH1, an obligate piezophilic hyperthermophile: extending the upper pressure-temperature limits for life. The ISME J. 3, 873–876.

Zhang, C., Xing, D., 2010. Microfluidic gradient PCR (MG-PCR): a new method for microfluidic DNA amplification. Biomed. Microdevices 12, 1–12.