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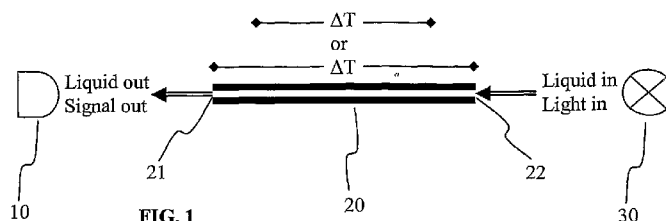
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(54) Title: METHOD AND SYSTEM FOR THERMAL MELT ANALYSIS



(57) Abstract: The present invention is directed at a system for carrying out a thermal melt analysis of an organic compound, wherein the system comprises: a microchannel having a first opening and a second opening at opposite ends of the microchannel, wherein the temperature at the opposite ends of the microchannel is different thereby creating a temperature gradient between the opposite ends of the microchannel; a light source emitting light which is coupled into the microchannel through the first opening; a light detector arranged to detect a light signal exiting the microchannel through the second opening; and wherein the first opening of the microchannel is in fluid communication with at least one fluid source providing at least one organic compound. The present invention is also directed to a method of using this device.

METHOD AND SYSTEM FOR THERMAL MELT ANALYSIS

FIELD OF THE INVENTION

[001] The present invention relates to microfluidic devices, in particular to the field of
5 microfluidic devices.

BACKGROUND OF THE INVENTION

[002] Detecting DNA melting temperature (melting curve analysis – MCA) is a standard
technique in the art which is used to verify the specificity of the real-time PCR or real time
10 RT-PCR product using a fluorescent intercalator, such as SYBR[®]-GREEN. The temperature
(T) of the sample is gradually increased while amplitude of fluorescent signal (F) is recorded.
This operation is typically performed after the PCR is completed in the same equipment (real-
time PCR machine); this is very convenient as the sample manipulation is eliminated. As the
temperature increases, the amplitude of the fluorescence signal gradually decreases. Around
15 the DNA melting temperature, there is sharp drop in the amplitude, due to separation of the
two strands. Local maxima from $-dF/dT$ determines the melting temperature of the DNA. The
same equipment can be used to detect the melting curve of proteins (Zheng Zhou & Yawen
Bai, 15 February 2007, Nature, vol.445, pp. E16).

[003] A protein is labelled with a fluorescent marker and a quencher, thus non-fluorescent
20 when correctly folded (native state). When the protein melts, the quencher and the marker are
separated and the fluorescence amplitude increases. In drug discovery, a change of the
melting temperature when a drug is applied would indicate the drug influence on the protein
unfolding.

[004] The melting curve analysis of both DNA as well as proteins is currently performed
25 in costly real-time PCR systems. A real-time PCR system consists of a temperature controlled
part, a set of capillary tubes or wells, a light source, a filter set and a fluorescence detection
system, typically a photo multiplier tube or a CCD camera. Unfortunately, as it is of now the
systems are bulky, costly and the melting curve is detected from rather bulky samples with
volume of 5 μ l or more. Performing statistics to increase the confidence would require large
30 amount of protein thus increasing the cost of drug discovery. Therefore, further developments
of tools for conducting thermal melt analysis are desired.

SUMMARY OF THE INVENTION

[005] In a first aspect, the present invention is directed to a system for carrying out a thermal melt analysis of an organic compound, wherein the system comprises:

- a microchannel having a first opening and a second opening at opposite ends of the microchannel, wherein the temperature at different portions of the microchannel is different thereby creating a temperature gradient between the different portions of the microchannel;
- light from a light source which is coupled into the microchannel through the first opening;
- a light detector arranged to detect a light signal exiting the microchannel through the second opening; and
- wherein the first opening or the second opening of the microchannel is in fluid communication with at least one fluid source providing at least one organic compound.

[006] In another aspect, the present invention is directed to a method of carrying out a thermal melt analysis of an organic compound using a system as described herein, wherein the method comprises:

- feeding a fluid stream comprising an organic compound into the microchannel;
- detecting a signal generated by the organic compound upon excitation of the organic compound with the light emitted by the light source.

DETAILED DESCRIPTION OF THE INVENTION

[007] In a first embodiment, it is referred to a system for carrying out a thermal melt analysis of an organic compound. The system can comprise any of the following components:

- a microchannel or channel having a first opening and a second opening at opposite ends of the microchannel, wherein the temperature at different portions of the microchannel is different thereby creating a temperature gradient between the different portions of the microchannel;
- light from a light source which is coupled into the microchannel through the first opening;
- a light detector arranged to detect a light signal exiting the microchannel through the second opening; and
- wherein the first opening or the second opening of the microchannel is in fluid communication with at least one fluid source providing at least one organic compound.

[008] This system allows carrying out a thermal melt analysis of any organic compound or organic compounds that is/are fed through the microchannel of the system. The organic

compound can be injected into a liquid stream which is fed through the microchannel of the system. The temperature gradient over the microchannel subjects the organic compound to a temperature change. Any signal from the organic compound generated due to any conformational change while passing through the microchannel can be measured photometrically through the light detector located at end of the microchannel opposite the end of the microchannel with the light source. The arrangement of the light source and the detector at the ends of the microchannel allows to generate and to measure signals from the entire microchannel rather than generating and measuring signals only at a specific point or portion of the microchannel.

[009] The microchannel referred to herein fulfils two functions. Firstly, the microchannel serves to transport the fluid stream comprising the organic compound through the microchannel, i.e. it serves as fluid conduit. Secondly, the microchannel serves as light conduit that transmits the light from the light source and the signals generated by the organic compounds in the microchannel through the entire microchannel to the light detector arranged at or near the end of the microchannel.

[010] Thus, in one embodiment, the microchannel is made of a reflective material that allows transmitting light within the microchannel. In another embodiment, the inside wall or inner wall of the microchannel is coated with a reflective material. Reflective material means that the microchannel is made of a material or is coated with a material which can transmit information signals in the form of pulses of light. The use of reflective materials can help to avoid or minimize the loss of signal that exits the microchannel along the length of it before being detected by the light detector located at the end of the microchannel.

[011] The reflective material that can be used is glass, a polymer, a metal (which is not a semiconductor material), or a semiconductor material. Examples of reflective materials include, but are not limited to glass, polyethylene, polypropylene, polyvinyl chloride, polystyrene, nylon, polyester, acrylics, polyurethane, polycarbonate, epoxy-based polymers and fluorene derivative polymers. Further examples of reflective materials include, but are not limited to nickel, copper, zinc, aluminium, silver, gold, chromium, an alloy or a composite thereof. Other examples include titanium oxide, zinc oxide, barium oxide and silicon oxide.

The reflective material can be applied in form of layers. Thus, in one embodiment, the reflective material is applied as a single layer or multiple layers, such as double layers. Using multiple layers can also be used to achieve total internal reflection.

[012] In another embodiment, a reflective material is coated around the outside of the

microchannel. In that case the microchannel can be made of a transparent material. The outside coating of reflective material has the effect that any light passing the transparent wall of the microchannel is redirected into the microchannel and towards the detector at the end of the microchannel.

5 [013] The microchannel can be between about 1 to about 200 mm long. In one embodiment, the microchannel is at least 1 mm or 10 mm long. In another embodiment, the microchannel is between about 10 to 100 mm long, or between about 20 to 200 mm long, or between about 50 to 200 mm long, or between about 10 to 70 mm long, or about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 150 or 200 mm long. The microchannel can also be bent or
10 comprise another shape which is different from a straight shape as long as the light is directed within the microchannel so that it reaches the end where the light detector is positioned.

[014] The microchannel is hollow to allow the fluid stream comprising the organic compound to pass through the microchannel. The word tube or microtube can be used instead of microchannel. "Micro"channel means that the inner diameter of the channel can be in the
15 range as indicated in the following. The inner diameter defining the hollow portion of the microchannel can be at least 10 μm or 1 mm. In another embodiment, the inner diameter can be between about 10 μm to about 10 mm, or between about 10 μm to about 1 mm, or between about 10 μm to about 500 μm , or between about 100 μm to about 1 mm, or between about 100 μm to about 500 μm .

20 [015] The light source and the light detector are positioned to allow coupling light into the microchannel through the first opening and detecting a light signal exiting the microchannel through the second opening, respectively. The light source and the light detector can be positioned either in front of the first or the second opening of the microchannel. In one embodiment, the light source is positioned in front of the first opening through which the fluid
25 stream comprising the organic compound enters the microchannel while the light detector is positioned in front of the second opening through which the fluid stream exits the microchannel. That means that the direction of flow of the fluid stream and the direction in which the light from the light source is coupled into the microchannel is the same. In another embodiment, the light detector is positioned in front of the first opening through which the
30 fluid stream comprising the organic compound enters the microchannel while the light source is positioned in front of the second opening through which the fluid stream exits the microchannel. Thus, in the latter embodiment, the direction of light from the light source and the direction of flow are different. The position of light source and light detector at the

openings of the microchannel is adapted to allow a fluid stream to flow into the microchannel and out of the microchannel through the first and second opening of the microchannel.

[016] With respect to the light source, positioned or located in front of the first opening of the microchannel means that the light is coupled into the microchannel at this point. The light source as such, e.g. the laser can be located somewhere else as long as the light from the light source is coupled into the microchannel through an opening of the microchannel, either the first opening or the second opening. The light from the light source can be directed to a light tube, such as a waveguide to the respective opening of the microchannel. Thus, the light source does not need to be located directly in front of the first opening. Only the light from the light source needs to be coupled into the microchannel through the respective opening. The same applies to the light detector which needs to be positioned to be able to measure a light signal(s) exiting through the respective opening of the microchannel.

[017] The light source can be, for example, a laser, a laser diode and LEDs. The light source emits light of a specific wavelength or different specific wavelengths which is/are suitable to excite the organic compounds which respond by emitting a signal to be measured by the detector at the opposite opening of the microchannel. The light detector is adapted to measure a light signal of an organic compound within the microchannel of the system described herein. The detectors can be adapted to measure the spectral characteristics, for example, such as fluorescence, chemiluminescence of the organic compound(s) within the microchannel. Thus, detectors can also detect light emitted by reporter molecules which are bound to organic compounds within the microchannel.

[018] Detectors can include spectrophotometers, photodiodes, avalanche photodiodes, PIN (P-i-N) photodiodes, photomultiplier tubes, photon counting devices, scanning detectors as well as combinations thereof. Those devices convert light (photons) into an electrical signal. For example, the detection of each photon by the PMT is amplified into a larger more easily measurable pulse of electrons. Photo diodes can also be used herein to detect, e.g., fluorescence. Photodiodes absorb incident photons that cause electrons in the photodiode to diffuse across a region in the diode thus causing a measurable potential difference across the device. The potential can be measured and is directly related to the intensity of the incident light.

[019] The detectors can use various algorithms for the evaluation of fluorescence signals from individual compounds based on changes in, e.g., brightness, fluorescence lifetime, spectral shift, FRET, quenching characteristics, to name only a few.

[020] The light source and the light detector can be an integral part of the system or can be separate units. Using the light source and/or the detector as separate units allows exchanging these units depending on the application and the organic compounds to be analyzed.

[021] In the system described herein a temperature gradient is generated along the length of the microchannel or at least along a portion of the microchannel in which the organic compound is to be subjected to the temperature gradient. The temperature gradient between the opposite ends or between different portions of the microchannel can be created by a heating arrangement. The temperature at one end of the length of the microchannel can be controlled to a first selected temperature, and the temperature at the other end of the length can be controlled to a second selected temperature, thus creating a continuous temperature gradient spanning the temperature range between the first and second selected temperatures. Once the liquid stream comprising the organic compound(s) flows through the microchannel or the portion of the microchannel with the temperature gradient, a temperature gradient will be established within that liquid stream or fluid.

[022] The temperature gradient along the microchannel of the system described herein can be generated either by resistive heating, non-resistive heating, or both resistive heating and non-resistive heating. When resistive heating is used, a temperature gradient can be established along the length of a microchannel by fabricating the channel so that it continuously changes in cross-sectional area along its length, and then applying a single electric current through that length. In one embodiment, the micro channel changes monotonically in cross-sectional area along its length. One method of establishing a temperature gradient along the length of a microchannel when non-resistive heating is employed is to place a thermal block in contact with the microchannel, and to establish a temperature gradient across the block in the direction corresponding to the length direction of the microchannel using heating or cooling elements.

[023] In one embodiment, resistive heating is performed by flowing a selectable electric current through the microchannel, thereby elevating the temperature. Resistive heating can occur over the entire length of the microchannel or over a selected portion of the microchannel. Resistive heating can be applied to selected portions of microchannels by flowing a selectable electric current through a first section and a second section of a microchannel wherein the first section comprises a first cross-section and the second section comprises a second cross-section. Furthermore, the first cross-section is of a greater size than the second cross-section, which causes the second cross-section to have a higher electrical

resistance than the first cross-section, and therefore a higher temperature than the first cross-section when the selectable electric current is applied. By generating different temperatures at opposing ends of the microchannel a temperature gradient can be established for the thermal melt analysis.

5 [024] The level of resistive heating can be controlled by changing the selectable current, the electrical resistance, or both the current and the resistance. The selectable current used for resistive heating can include direct current, alternating current or a combination of direct current and alternating current.

10 [025] In another embodiment, generating a temperature gradient over the microchannel can be achieved by heating the microchannel via non-resistive heating methods, e.g., through application of an internal or an external heat source. In one embodiment, the internal or external heat source includes a thermal heating block. Just as for resistive heating, non-resistive heating optionally occurs over the entire length of the microchannel or over a selected portion of the microchannel. For example, one or more regions of the microchannel
15 can be proximal to one or more heating element. In one embodiment a thermal heating block is located at the opposite ends of the microchannel. To generate a temperature gradient over the entire length of the microchannel the temperature of the two heating blocks is different.

[026] In one embodiment, the heating arrangement comprises temperature controlled heating blocks, wherein a first heating block is located near the first opening of the
20 microchannel while a second heating block is located near the second opening of the microchannel.

[027] The magnitude of the temperature gradient is determined by the temperature difference between the first and the second thermal heating blocks which are located at the opposite ends of the microchannel.

25 [028] In one embodiment, the heating arrangement comprises a heating block and a cooling block. Using a combination of a heating element or block and a cooling element or block also allows creating a temperature difference between the end or a section of the microchannel. The cooling element can be located at or near one of the ends of the microchannel, such as at or near the end of the first opening or the second opening of the
30 microchannel. In that case the heating element is located at or near the respective opposite end.

[029] The temperature gradient depends on the application and the organic compounds to be analyzed. In case of analyzing proteins the temperature gradient the upper temperature

limit can be about 70°C or 80°C or 90°C or 100°C. In case of analyzing nucleic acids, the upper temperature can be about 90°C or about 95°C or about 100°C.

[030] The lower limit of the temperature gradient can be about 30°C or down to about 0°C or even lower when using a cooler instead of a heater. In other embodiments, the lower limit of the temperature gradient can be about 35°C, or about 37°C, or about 40°C, or about 45°C, or about 50°C or about 55°C. Thus, the temperature gradient can be between about 0°C or 30°C to about 100°C or about any range between the lower and upper ends just defined.

[031] To feed the liquid stream and couple the light into and out of the microchannel through the respective same opening, the microchannel can be connected either at one end or at both ends to a T-junction or a cross-junction.

[032] Thus, in one embodiment, the first opening of the microchannel can be connected to a T-junction comprising a first, a second and a third end; wherein

- the first end of the T-junction has an opening which is in fluid communication with the first opening of the microchannel;
- the second end of the T-junction located directly opposite the first end of the T-junction is connected to the light source; and
- the third end of the T-junction comprises an opening which is in fluid communication with the first opening of the microchannel.

[033] On the other side of the microchannel a further T-junction can be positioned. Thus, in another embodiment, the second opening of the microchannel can be connected to a T-junction comprising a first, a second and a third end; wherein

- the first end of the T-junction has an opening which is in fluid communication with the second opening of the microchannel;
- the second end of the T-junction located directly opposite the first end of the T-junction is connected to the light detector; and
- the third end of the T-junction comprises an opening which is in fluid communication with the second opening of the microchannel.

[034] One option to feed different liquid streams into the microchannel is by connecting the microchannel to a cross-junction which provides two openings which fluidly connect the microchannel with liquid sources connected to the microchannel via the openings of a cross-junction.

[035] Therefore, in one embodiment, the first opening of the microchannel of the system can be connected to a cross junction comprising a first, a second, a third and a fourth end;

wherein

- the first end of the cross junction has an opening which is in fluid communication with the first opening of the microchannel;
- the second end of the cross junction located directly opposite the first end of the cross junction is connected to the light source; and
- the third and fourth end of the cross junction comprise an opening which is in fluid communication with the first opening of the microchannel;

wherein when liquid is flowing through either the third or the fourth end of the cross junction into the microchannel the respective other end is blocked, for example through a valve, so that liquid can flow into the microchannel only either through the third end or the fourth end of the cross junction.

[036] The end of the T-junction or cross-junction leading to the light source or the detector is comprised of a transparent material which allows passage of light but does not allow the passage of liquid flowing through the T-junction or cross-junction. The transparent material can be glass, a transparent polymer, or an immiscible transparent liquid such as oil of fluoropolymers.

[037] The system described herein permits easy integration of additional operations into the system. For example, the system described herein can further include structures, reagents and devices for performing any number of operations both upstream and downstream from the operations specifically described herein. Such upstream operations include sample handling and preparation operations, e.g., cell separation, extraction, purification, amplification, cellular activation, labeling reactions, dilution, aliquotting, and the like.

[038] For example, the microchannel can be fluidly connected to an array of reservoirs which include different organic compounds or mixtures of organic compound. The microchannel can also be connected to a compound library. Thus, the system described herein can be used for high-throughput analysis of multiple organic compounds.

[039] Further examples of other components which can be included in the system described herein include for example a fluid transport system that directs fluid movement within the microchannel. The fluid transport system could conceivably employ any fluid movement mechanism known in the art (e.g., fluid pressure sources for modulating fluid pressure in the microchannels, electrokinetic controllers for modulating voltage or current in the microchannels, or combinations thereof).

[040] The system described herein can also employ multiple microchannels which are

operating in parallel.

[041] Therefore, the system described herein can also include fluid manipulation elements such as a parallel stream fluidic converter. For example, the systems herein optionally include a valve manifold and a plurality of solenoid valves to control flow switching between channels, reservoirs and/or to control pressure/vacuum levels in the microchannels. Another example of a fluid manipulation element includes, e.g., a capillary optionally used to sip a sample or samples from a microtiter plate and to deliver it to a microchannel.

[042] The amount of organic compound needed for the thermal melt analysis can be greatly reduced by providing the organic compounds in smaller volumes instead of simply injecting the organic compound into the liquid stream constantly flowing through the microchannel. In one embodiment, the organic compound can therefore be comprised within a sample section having a volume of between about 1 nl to about 5 μ l. In another embodiment, the organic compound can be comprised within a sample section having a volume of at least one 1 nl, or at least 50 nl, or between about 1 nl to about 150 nl, or between about 1 nl to about 100 nl, or between about 1 nl to about 50 nl, or between about 5 nl to about 200 nl, or between about 10 nl to about 150 nl, or between about 10 nl to about 70 nl, or about 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 nl.

[043] Concentrating the organic compound to be analyzed in smaller volumes allows reducing the amount of organic compound used for the analysis which can reduce the costs for carrying out of the thermal melt analysis. The sample section can be transported to and through the microchannel by a carrier stream of a fluid, i.e. a gas or a liquid. The sample section can be injected into this carrier stream which is constantly flowing through the system. Depending on how fast different sample sections are injected into the carrier stream or how fast the carrier stream flows it is possible to vary the distance of different sample sections within the carrier stream. This option to vary the distance between the sample sections allows supplying several sample sections into the microchannel to be analyzed at the same time. Therefore, in one embodiment the system described herein is adapted to measure multiple sample sections at the same time wherein the multiple sample sections in the microchannel at a specific time are separated from each other by an inert fluid, i.e. the carrier stream, which separates the multiple sample sections from each other. Such an inert fluid or liquid can be a gas or a water immiscible liquid, such as mineral oil or non mineral oil. An example of a mineral oil would be M5904 from Sigma-Aldrich or a FluorinertTM (from 3M), such as FC-72 or FC-75.

[044] The different sample sections can comprise the same organic compound or organic compounds to be tested to allow repeating the analysis several times or the sample sections comprise different organic compounds or different concentrations of an organic compounds or mixtures of organic compounds. Thus, in one embodiment the microchannel of the system is fluidly connected to multiple reservoirs comprising different organic compounds or mixtures of organic compounds.

[045] The organic compound to be analyzed can include, but is not limited to a protein or a nucleic acid. Examples of proteins include, but are not limited to polypeptides, enzymes, antibodies, antigens, ligands, cofactors or receptors. Examples of nucleic acids include, but are not limited to double-stranded (ds) DNA, double-stranded (ds) RNA, single-stranded (ss) DNA and single-stranded (ss) RNA or portions of chromosomal DNA.

[046] The system described herein can also be used for organic compounds that comprise a complex of two or more molecules, e.g., an enzyme complexed to a second enzyme, a ligand, a peptide nucleic acid, a cofactor, a receptor, a substrate, or other such combinations.

The interaction of organic compounds with other organic compounds, such as lipids, phosphate groups, oligosaccharides or prosthetic groups can also be analyzed. Using the system described herein allows determining different properties of the organic compounds via the thermal melt analysis as will be described further below. The system referred to herein also allows analyzing the influence of pharmaceuticals or potential pharmaceuticals, i.e. small molecules which can be either inorganic compounds, organic compounds or a combination of both on the organic compounds to be examined in the microchannel of the system described herein. Those small molecules can influence the stability of the organic compound(s) to be tested in the present system and thus lead to conformational changes of the organic compound to be tested which can be detected with the system described herein.

[047] Using the system and the method described herein allows for rapid characterizing of a variety of organic compounds or interactions of organic compounds with pharmaceuticals via the generation of organic compound melt curves.

[048] For example, the precise melting behaviour of double-stranded DNA (ds-DNA) is characteristic of physical properties such as their size, sequence, and molecular composition.

A primary factor is the ratio of guanine-cytosine bonds to adenine-thymine bonds, or G-C%. This denaturing of DNA occurs in less than one second over a range of temperatures depending on the G-C content. Analyzing the melting behaviour allows to differentiate between sequence variations of only a single base pair.

[049] On the other hand, the melting behaviour of a protein is characteristic of physical properties of proteins such as their stability which depends on the stability of protein folding. At elevated temperatures protein folding changes, i.e. the protein denatures. Protein denaturation can include loss of their secondary, tertiary or quaternary structure by uncoiling, untwisting, unfolding, dissociation of nucleic acids from the protein.

[050] The melt-curve of an organic compound can also be used to indicate the degree of binding between one or more organic compounds and another organic compound or test molecule, such as between an enzyme and its cofactor or a receptor-target binding or the interaction of a protein with a pharmaceutical.

[051] The detection of a change(s) in a physical property of the organic compounds via a thermal melt analysis can be detected in different ways depending on the specific organic compounds and reactions involved. For example, the denaturation of a protein can be tracked by following fluorescence or emitted light from proteins in the assay. The degree of, or change in, fluorescence is correlational or proportional to the degree of change in conformation of the proteins being assayed. The methods and the system described herein allow for various methods of exciting the organic compounds involved in the assay, through use of, e.g., lasers, lights, to name only a few. The fluorescence can be intrinsic to the organic compound being assayed, e.g., from tryptophan residues in proteins, or extrinsic to the molecules being assayed, e.g., from dye molecules, such as fluorophores added to the assay mixture in the microfluidic device. The change(s) in fluorescence or emitted light can optionally be detected in a number of ways according to the specific needs of the assay desired.

[052] The change in fluorescence of emitted light indicates a change in conformation of the organic compound from which the thermal melt curve is constructed. Displacement or shift of the thermal melt curve when the organic compound is in the presence of another organic compound allows detection and quantification of binding between the two compounds.

[053] The unfolding, disassociation or denaturing of an organic compound(s) in response to changes in temperature can be used in many applications, e.g., in determining the stability of a specific protein under specified conditions, or in the identification of a nucleic acid, the detection of SNPs in a nucleic acid, to name only a few. The measurement of the molecular denaturing, disassociation or unfolding of the organic compound can be used to construct a thermal melting curve.

[054] In constructing a thermal melting curve, a physical property of the organic compound to be tested must be measured in order to determine the denaturation/unfolding or dissociation (in case of nucleic acids) of the organic compound. The change in this physical property is measured as a function of changing temperature and is proportional/correlative to the change in conformation of the organic compound. For example, a change in calorimetric analysis, heat capacity, can be measured to indicate the temperature induced denaturation of organic compounds. Additional physical properties which can be measured to indicate a change in molecular folding/conformation include, e.g., various spectral phenomena, such as presence of fluorescence or emitted light, changes in fluorescence or emitted light, or changes in polarization of fluorescence or emitted light. These properties can be measured over a range of temperatures and correlated to changes in the unfolding/denaturation or dissociation (in case of nucleic acids) of organic compound(s) to be tested in the system and method described herein.

[055] In one embodiment, calorimetry is used to measure changes in thermodynamic parameters as the organic molecule(s) is subjected to changes in temperature. For example, differential scanning calorimetry (DSC) is optionally used to measure the relative stability of organic compounds. Using DSC in the current invention, a sample containing the organic compound is heated over a range of temperatures in the microchannel of the system. At some point during the heating process the organic compound undergoes a physical or chemical change, e.g., denaturation that either absorbs or releases heat. The thermal change(s) during the process is then plotted as a function of temperature with the area under the curve representing the total heat or enthalpy change (ΔH) for the entire process. Those skilled in the art can use the resulting plots to determine, e.g., heat capacity change (ΔC_p), the T_m (or midpoint temperature where the denaturation or unfolding reaction is half complete), or the like.

[056] The denaturation of a protein can be influenced by interaction with other molecules, such as pharmaceuticals which can influence the stability of the organic compound. Such changes can also be detected and are used by the pharmaceutical industry to examine the influence of a specific drug on the stability, for example of a protein or the interaction of a protein and its receptor which is targeted by this pharmaceutical.

[057] Thus, the above described procedure can be repeated with the addition of a binding partner of the organic compound, e.g., a ligand. The thermal melting curve generated by heating the organic compound and its putative binding partner is then compared with the

thermal melting curve generated by heating the organic compound by itself. Comparison of the two thermal melting curves can disclose, e.g., whether the binding partner actually binds to the organic compound. If the molecules do bind to each other then the thermal melting curve of the organic compound assayed in the presence of the binding partner will be 'shifted' in comparison to the thermal melting curve of the organic compound by itself. This shift in the thermal melting curves is due to a binding-dependent change in the thermal denaturation of the organic compound.

[058] Conformational changes can be monitored via spectroscopic methods. Therefore, in another embodiment spectroscopy is used to measure changes in fluorescence or light to track the denaturation/unfolding of an organic compound that is subjected to changes in temperature. Spectrometry, e.g., via fluorescence, is a method of detecting thermally induced denaturation/unfolding of organic compounds. Many different methods involving fluorescence are known for detecting denaturation of molecules (e.g., intrinsic fluorescence, numerous fluorescence indicator dyes or molecules (i.e. use of a reporter molecule), fluorescence polarization, fluorescence resonance energy transfer, etc.). These methods make use of either internal fluorescent properties of organic compounds or external fluorescence, i.e. the fluorescence of additional reporter molecules involved in the analysis.

[059] For example, when measuring the intrinsic fluorescence, the method of detecting can lead to exciting aromatic amino acid residues, such as tryptophan in a protein, via the light emitted from the light source thereby creating excited tryptophan residues. Discerning and measuring an emission or quenching event of the excited tryptophan residues is used to detect a property change of the organic molecule(s) being assayed. The quantum yield of the emission from an aromatic amino acid either decreases or increases depending on the sequence and conformation of the organic compound. Upon unfolding of a protein, there is in general a red shift in the intrinsic emission of the organic compound, which can also be used to detect conformational changes. The changes in intrinsic fluorescence observed with this method are measured as a function of temperature and can be used to construct thermal melting curves. Binding of a binding partner to the organic compound can for example shift the thermal melting curve and is used to determine and quantify/qualify the binding event.

[060] Measuring of the external fluorescence refers to methods in which the organic compound is bound to a reporter molecule emitting a signal upon excitation with light from the light source used in the system described herein. The reporter molecule can bind to the organic compound(s) either once the organic compound is unfolded or denatured or before the

organic compound undergoes the conformational change by, e.g. denaturing and which emits a light signal, such as fluorescence energy after it is excited by light of a specific wavelength. Such reporter molecules can include, but are not limited to organic dyes, such as fluorophores or quantum dots, such as Qdot605 or Qdot655. Examples of fluorophores include for example fluorescein and its derivatives or coumarins, such as AlexaFluor 350, or rhodamines, such as TRITC, TMRE' or Rhodamine 123. For example, fluorescein is efficiently excited by the light source from 460 – 490 nm such as 488 nm argon-ion or 471 GaN laser resulting in a green emission with maximum amplitude at about 520 nm. Other examples include AlexaFluor dyes, such as AlexaFluor 488, AlexaFluor 568, AlexaFluor 594, AlexaFluor 647, BOPIDY dyes, such as BOPIDY FL or BOPIDY TR, and cyanine dyes, such as Cy3 or Cy5. Further examples include phycobiliproteins, styryl dyes or fluorescent proteins, such as ECFP, EGFP, EYFP, or dsRed.

[061] Some organic dyes consist of fluorophores that bind specifically to hydrophobic areas of molecules. An illustrative, but not limiting, example of a dye in that group is 1-anilino-8-naphthalene sulfonate (ANS). ANS has a low fluorescence in polar environments, but when it binds to apolar regions, e.g., such as those found in interior regions of natively folded proteins, its fluorescence yield is greatly enhanced. As organic compounds are denatured, e.g., as happens with increasing temperature in the microfluidic device, they become denatured thereby allowing solvent, e.g., water, to reach and quench the fluorescence of the ANS.

[062] Another optional dye type that can be used in the system described herein is one that intercalates within strands of nucleic acids. The classic example of such type of dye is ethidium bromide. An example of use of ethidium bromide for binding assays includes, e.g., monitoring for a decrease in fluorescence emission from ethidium bromide or due to binding of test molecules to nucleic acid target molecules.

[063] Dyes that bind to nucleic acids by mechanisms other than intercalation can also be employed. For example, dyes that bind the minor groove of double stranded DNA can be used to monitor the molecular unfolding/denaturation of the nucleic acid due to temperature increase. Examples of suitable minor groove binding dyes are the SYBR[®] Green family of dyes sold by Molecular Probes, Inc. SYBR[®] Green dyes will bind to any double stranded DNA molecule. When a SYBR[®] Green dye binds to double stranded DNA, the intensity of the fluorescent emissions increases. As more double stranded DNA are denatured due to increasing temperature, the SYBR[®] Green dye signal will decrease. Other popular dyes are

LCGreen[®] or EvaGreen[®].

[064] Other embodiments utilize fluorescence polarization. Fluorescence polarization (FP) provides a suitable method to detect hybridization formation between organic compounds of interest. This method is especially applicable to hybridization detection between nucleic acids, e.g., to monitor single nucleotide polymorphisms (SNPs). In general, fluorescence polarization operates by monitoring the speed of rotation of fluorescent dyes, e.g., before, during and/or after binding events between organic compounds. In short, binding of an organic compound to another organic compound ordinarily results in a decrease in the speed of rotation of a bound reporter molecule on one of the organic compounds, resulting in a change in fluorescence polarization. For example, when a fluorescent molecule is excited with a polarized light source, the molecule will emit fluorescent light in a fixed plane, e.g., the emitted light is also polarized, provided that the molecule is fixed in space. However, because the molecule is typically rotating and tumbling in space, the plane in which the fluoresced light is emitted varies with the rotation of the molecule (also termed the rotational diffusion of the molecule). Restated, the emitted fluorescence is generally depolarized. The faster the molecule rotates in solution, the more depolarized it is. Conversely, the slower the molecule rotates in solution, the less depolarized, or the more polarized it is. The polarization value (P) for a given molecule is proportional to the molecule's "rotational correlation time," or the amount of time it takes the molecule to rotate through an angle of approximately 68.5°. The smaller the rotational correlation time, the faster the molecule rotates, and the less polarization will be observed. The larger the rotational correlation time, the slower the molecule rotates, and the more polarization will be observed. Rotational relaxation time is related to viscosity (η) absolute temperature (T), molar volume (V), and the gas constant (R). The rotational correlation time is generally calculated according to the following formula: Rotational Correlation Time (θ) = $\eta V/RT$. As can be seen from the above equation, if temperature and viscosity are maintained constant, then the rotational relaxation time, and therefore, the polarization value, is directly related to the molecular volume. Accordingly, the larger the molecule, the higher its fluorescent polarization value, and conversely, the smaller the molecule, the smaller its fluorescent polarization value.

[065] In the context of the present invention, fluorescently labeled organic compound, e.g., a ligand, antigen, etc., having a relatively fast rotational correlation time, can be used to bind to a much larger organic compound, e.g., a receptor protein, antibody, etc., which has a much slower rotational correlation time. The binding of the small labeled organic compound to the

larger organic compound significantly increases the rotational correlation time (decreases the amount of rotation) of the labeled species, namely the labeled complex of organic compounds over that of the free unbound labeled organic compounds. This has a corresponding effect on the level of polarization that is detectable. Specifically, the labeled complex of organic compounds presents much higher fluorescence polarization than the unbound, labeled organic compound.

[066] In another embodiment fluorescence resonance energy transfer (FRET) can be used to monitor the conformational changes of an organic compound to be tested (and interactions with other binding partners which can bind with the organic compound) as a function of temperature. Fluorescence resonance energy transfer relies on a distance-dependent transfer of energy from a donor fluorophore to an acceptor fluorophore. If an acceptor fluorophore is in close proximity to an excited donor fluorophore then the excitation of the donor fluorophore can be transferred to the acceptor fluorophore. This causes a concomitant reduction in the intensity of the donor fluorophore and an increase in the emission intensity of the acceptor fluorophore. Since the efficiency of the excitation transfer depends, inter alia, on the distance between the two fluorophores, the technique can be used to measure extremely small distances such as would occur when detecting changes in conformation. This technique is particularly suited for measurement of binding reactions, protein-protein interactions, e.g., such as a protein of interest binding to an antibody, and other biological events altering the proximity of two labeled organic compounds. Many appropriate interactive reporter molecules are known. For example, fluorescent labels, dyes, enzymatic labels, and antibody labels are all appropriate. Examples of interactive fluorescent label pairs include terbium chelate and TRITC (tetrahydroamine isothiocyanate), europium cryptate and Allophycocyanin, DABCYL and EDANS and many others known to those of skill (e.g., donor fluorophores such as carboxyfluorescein, iodoacetamidofluorescein, and fluorescein isothiocyanate and acceptor fluorophores such as iodoacetamido eosin and tetramethylrhodamine). Similarly, two colorimetric labels can result in combinations which yield a third color, e.g., a blue emission in proximity to a yellow emission provides an observed green emission.

[067] With regard to fluorescent pairs, there are a number of fluorophores which are known to quench one another. Fluorescence quenching is a bimolecular process that reduces the fluorescence quantum yield, typically without changing the fluorescence emission spectrum. Quenching can result from transient excited state interactions, (collisional quenching) or, e.g., from the formation of non-fluorescent ground state species. Self

quenching is the quenching of one fluorophore by another; it tends to occur when high concentrations, labeling densities, or proximity of labels occurs. FRET is a distance dependent excited state interaction in which emission of one fluorophore is coupled to the excitation of another that is in proximity (close enough for an observable change in emissions to occur). Some excited fluorophores interact to form excimers, which are excited state dimers that exhibit altered emission spectra (e.g., phospholipid analogs with pyrene sn-2 acyl chains).

[068] In another embodiment molecular beacons can be used in monitoring the conformation changes of organic compounds as a function of temperature. Molecular beacons are reporter molecules that can be used to report the presence of specific nucleic acids. They are especially useful in situations where it is either undesirable or not possible to isolate the nucleic acid hybrids being analyzed. Structurally, molecular beacons are hairpin-shaped nucleic acid molecules having a center 'loop' section of a specific nucleic acid sequence flanked by two complementary end regions (annealed together), one of which has a fluorescence moiety and the other a quencher moiety. The loop region is complementary to a target or specific nucleic acid sequence. When the molecular beacon is not in the presence of the nucleic acid to which it could bind and is in its hairpin conformation, the fluorescence moiety and quencher moiety are in close enough proximity that the fluorescence is quenched and the energy is emitted as heat. However, when the molecular beacon encounters its proper binding partner, i.e. the nucleic acid comprising a complementary sequence to the one provided by the molecular beacon, it changes conformation so that its internal loop region binds to the target nucleic acid sequence. This forces the fluorescence moiety to move away from the quencher moiety, which leads to a restoration of fluorescence. Through use of different fluorophores, molecular beacons can be made in a variety of different colors. DABCYL (a non-fluorescent chromophore) usually serves as the universal quencher in molecular beacons. Molecular beacons can be very specific and thus be used to detect, e.g., single nucleotide differences between nucleic acids.

[069] In another embodiment circular dichroism (CD) is used to monitor the conformational changes of the organic compounds as a function of temperature. Circular dichroism is a type of light absorption spectroscopy which measures the difference in absorbance by a molecule between right-circularly polarized light and left-circularly polarized light. Circular dichroism is sensitive to the structure of polypeptides and proteins. In order to construct molecular melt curves, circular dichroism can be used to follow the conformational changes in the organic compound(s) caused by changes in temperature.

[070] In another aspect, the present invention is directed to a method of carrying out a thermal melt analysis of an organic compound using a system as defined herein. The method comprises:

- feeding a fluid stream comprising an organic compound which is to be subjected to the thermal melt analysis into the microchannel;
- detecting a signal generated by the organic compound upon excitation of the organic compound with the light emitted by the light source.

[071] Feeding of a fluid stream through the system comprises in general flowing or transporting of a liquid stream through the microchannel of the system and through any other microchannel or tubes connected to the system. This feeding can be done electrokinetically, by use of positive or negative pressures, by both electrokinetics and positive or negative pressure or through displacement of fluid by expanding membranes.

[072] Instead of mixing the organic compound into the fluid stream, the organic compound can be dissolved in a separate fluid which is injected as a plug (i.e. sample section) into the flow of the fluid stream. Thus, different sample sections comprising the organic compound are fed in sequence through the microchannel. Depending on the distance of the sample sections to each other within the fluid stream, it is possible that more than one sample section flows through the microchannel at the same time. Thus, it is possible to measure multiple sample sections in the microchannel at the same time. The sample section can have a volume of between about 1 nl and 200 nl. In case the organic compound is delivered in confined sample sections through the system, the fluid stream functions as carrier stream separating the different sample sections from each other. In this case the fluid stream comprises a fluid, such as a liquid or gas, which is not miscible with the sample sections comprising the organic compound. The liquid can be an oil, such as a mineral oil.

[073] The direction of the fluid stream through the microchannel can be reversed. Thus, a sample section can enter the microchannel through an opening of the microchannel and flow through the microchannel while it is subjected to the temperature gradient in the microchannel (e.g. warm to cold). Once the sample section reaches the other end of the microchannel the flow direction can be reversed and the organic compound in the sample section can be subjected to the temperature gradient in the opposite direction (cold to warm).

[074] The flow rate within the system can be selected to be between about 100 nl/s to about 100 μ L/s or between about 10 μ l/min to about 500 μ l/min depending on the tube diameter, its length and the temperature gradient applied. Thus, the number of sample sections

analyzed in the system and method described herein can be influenced by varying the flow rate within the system, the sequence in which the sample sections are injected into the fluid stream, the dimensions of the microchannel as well as the inner diameter of the microchannel. Considering the above flow rates, it is possible that a sample section flows through the microchannel and thus is subjected to the temperature gradient for only about 1 second. Considering this throughput it becomes obvious that the present system allows measuring many different samples within a very short time. As more measurements can be carried out as more statistically significant the results. It also allows analyzing many different organic compounds within a short time. Thus, the present system can be used to analyze the effect of a large number of compounds, such as compounds from a compound library, on, e.g. a protein.

[075] The present invention will now be described with reference to the non-limiting Figures. **Figure 1** shows a microfluidic channel 20 with a temperature gradient (ΔT) spanning from one end of the microchannel 20 to the other end of the microchannel 20 or from one portion of the microchannel to the other portion. That means that the temperature gradient can be applied over the whole length of the microchannel or only over a short portion/section of the microchannel, such as from one end to a section ending before but not at the other end of the microchannel. The microchannel comprises a first opening 22 (in this case the inlet) and a second opening 21 (in this case the outlet) at the opposite ends of the microchannel. The light source 30 is positioned in front of the first opening 22 (inlet) while the detector 10 is positioned in front of the second opening 21 (outlet). Thus, the light source 30 and the detector 10 are positioned at opposite ends of the microchannel. The position of the light source 30 and the detector 10 is adapted to allow a fluid stream comprising the organic compound to flow through the microchannel 20 entering through the first opening 22 (inlet) and exiting the microchannel 20 flowing through the second opening 21 (outlet). In **Figure 1** the direction of the light from the light source 30 shining into the microchannel 20 and the direction of the fluid stream flowing into the microchannel 20 is the same. As illustrated in **Figure 2**, it is however also possible that the flow direction and the direction of the light from the light source 30 shining into the microchannel 20 is different. In **Figure 2**, the detector 10 is positioned before the first opening (inlet) while the light source 30 is positioned before the second opening (outlet).

[076] **Figure 3** illustrates a system in which a heating arrangement using two heating blocks 40a and 40b is used to generate the temperature gradient between the one end of the

microchannel 20 and the other end of the microchannel. The heating block can be located near or at the end of the microchannel 20. In Figure 3 the first heating block 40a is positioned near the outlet of the microchannel 20 while the second heating block 40b is positioned at the inlet of the microchannel 20. From Figure 3 it becomes obvious that the temperature gradient can be adjusted by changing the distance between the heating elements, in this case heating blocks, or by adjusting the temperature difference between the first and the second heating element. As higher the resolution of the thermal melt analysis needs to be as finer the transition from one temperature to a second temperature needs to be. This can be achieved by either decreasing the temperature difference within the heating arrangement for a given length of the microchannel or by increasing the length of the microchannel and thus the distance of, e.g. the heating elements located at opposite ends of the microchannel.

[077] **Figure 4** illustrates a microchannel 20 which is connected at both ends to a T-junction. Figure 4 indicates also the path of the light and the path of the fluid stream through the system. The dotted line indicates the path of the light while the solid line indicates the path of the fluid stream through the system. In Figure 4 the T-system at the inlet of the microchannel is in fluid connection with a further microchannel or tubing (not shown) through which the fluid stream is fed into the system. The fluid stream exiting the microchannel through the outlet of the T-junction can be discarded or subjected to a further treatment or measurement if desired.

[078] **Figure 5** shows an embodiment in which the inlet of the microchannel is connected to a cross-junction. The opposite openings of the cross-junction through which a fluid stream can flow into the microchannel allows to feed two different fluid streams from different sources into the microchannel. In case the organic compound is comprised in sample sections it is possible to feed different sample sections injected into a carrier fluid through the microchannel.

[079] **Figure 6** illustrates an embodiment in which two microchannels 20 are operated in parallel. In general it is possible to operate multiple microchannels in parallel. In Figure 6 the two microchannels 20 are connected to each other via their respective cross-sections which are connected to the inlet of every microchannel 20. In this configuration the same organic compound can be analyzed in different microchannels 20 at the same time. Such a configuration can further increase the throughput of the system.

[080] **Figure 7** shows a diagram of a possible configuration of a system described herein. In Figure 7 two thermal blocks 40 comprising each a temperature sensor 42 are positioned at

the inlet and the outlet of the microchannel 85. The fluid stream is flowing through the line 60 via a T-junction 70 into the microchannel 85 and exits the microchannel 85 via the T-junction 70 connected to the microchannel at the outlet and through line 61. The direction of the light from the light source is indicated by 50 and 51. Light is coupled from the light source 50 into the microchannel 85 coming from the light source via the T-junction 70 and exits the microchannel via the T-junction 70 into the detector 51. Figure 7 also shows light filters 55 which separate the light from the light source from the signal generated by the organic molecules in the microchannel.

[081] **Figure 8** shows a system for high throughput melting curve analysis which can be made by silicon micromachining. Figure 8 shows further components of the system which can be located upstream or downstream of the microchannel. As in Figure 7, the microchannel 85 is connected to two T-junctions. The fluid flows from one end 60 through the microchannel towards the other end 61. In Figure 8 the microchannel is connected via the T-junction to fluid reservoirs which can comprise different organic compounds which are to be analyzed in the system. In Figure 8 the organic compound is injected into a carrier fluid in form of sample sections or plugs 80. The lower right picture of Figure 8 shows that multiple plugs are measured at the same time in the microchannel. The analyzed samples are discarded by flowing through line 61 into the fluid waste container 90. Locating the reservoirs at some distance from the microchannel allows cooling those reservoirs which might comprise temperature sensitive materials.

[082] **Figure 9** illustrates the actual set-up of a device as it was illustrated and described in detail in Figure 7.

[083] **Figure 10** shows the results of measurements which have been carried out using a system as shown in Figure 9. A nucleotide dimer obtained from a PCR has been injected in a volume of 1 nl into a mineral oil flowing through the device. The temperature gradient has been generated by two block heaters located at the ends of the microchannel as illustrated in Figure 9. The heating block with the lower temperature was located at the first opening while the heating block with the higher temperature was located at the second opening. The nucleotide dimer was pumped through the first opening into the microchannel, thus flowing from the colder end to the warmer end, thereby warming up gradually. After flowing through the microchannel in one direction the flow direction was reversed and the nucleotide dimer sample was flowing through the device in the reverse direction, i.e. from the warmer end to the colder end, thereby cooling gradually. The upper diagram in Figure 10 shows the

denaturation curve of the nucleotide dimer while the lower diagram shows the renaturation curve which mirrors the denaturation curve indicating the consistency of the results obtained with this system.

[084] **Figure 11** shows an image taken with an infrared camera of a system as described herein. The square-like structures at the end of the microchannels illustrate the heaters. The square-like heaters pertaining to the channel on the left-hand side were connected to a PCB for control and set at 2 different temperatures. The square-like heaters pertaining to the channel on the right-hand side were not operating. On the left hand side, the infrared image illustrates the temperature gradient between the heaters while on the left-hand side no temperature gradient could be recorded by the infrared camera. It shows that the temperature gradient can be created on a beam, such as a beam made of silicon, where the microchannel for the melting curve analysis is positioned.

[085] By "consisting of" is meant including, and limited to, whatever follows the phrase "consisting of". Thus, the phrase "consisting of" indicates that the listed elements are required or mandatory, and that no other elements may be present.

[086] By "comprising" it is meant including, but not limited to, whatever follows the word "comprising". Thus, use of the term "comprising" indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present.

[087] The inventions illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms "comprising", "including", "containing", etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention, in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the inventions embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention.

[088] The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or

negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[089] Other embodiments are within the following claims and non-limiting examples. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

EXPERIMENTS

[090] For the experiment described herein a product of a PCR from Light Cycler system containing double stranded DNA with SYBR[®]Green intercalating dye was used. A testing system as per Figure 9 was assembled from 70 mm long glass capillary tube with inner diameter of 0.4 mm connected by a cube-based T-junction to both an inlet and an outlet. The entire system was filled with a mineral oil M5904 (Sigma-Aldrich). 1 μ L of a real-time PCR product (DNA sample stained with SYBR[®]Green) was added into the mineral oil to form a droplet and pumped through the system using a syringe pump at a rate of 200 μ L/min from the colder end (55°C) to the warmer part (95°C), warming up gradually. At a flow rate of 200 μ L/min it took about 6 – 7 s for the sample to pass from one end to the other end of the capillary tube (microchannel). Once the droplet with the DNA passed the capillary and entered the exiting tube after the second T-junction, the pumping direction was reversed and the droplet was cooled from 95°C to 55°C. Light from metal halide source (EXFO 120) passed through band pass filters with transmission band from 470 – 490 nm (Chroma, Inc.) and entered the capillary. There the light interacts with the sample and green light is emitted with peak amplitude at 517 nm. All light coming out from the capillary, which is blue light used for excitation as well as the emitted green enters the detection filter system. It is a set of other band pass filters, this time with bandwidth from 510 – 540 nm blocking blue light and letting only green to pass. The light is detected by a photomultiplier tube and its output voltage is recorded by an oscilloscope. Data in a text format (ASCII) were transferred into a PC and processed by Origin Software (MicroCal, Inc.).

[091] The results of this experiment (fluorescence as function of time; PMT (V)) are shown in Figure 10. Interesting phenomena could be observed at 2 s (about 60°C) on Figure 10, when a nonlinearity of the fluorescent decay occurred. It corresponds to the drop of the fluorescent amplitude of the melting curve as tested by a LightCycler. It should also be noted that the renaturation curve mirrors the denaturation curve. In Figure 10, the dotted line

indicates the temperature.

REFERENCE LIST

- 5 10, 51 Detector
- 20, 85 Microchannel
- 21 First opening of the microchannel
- 22 Second opening of the microchannel
- 30, 50 Light source
- 10 40, 40a, 40b Heating block
- 55 Filter
- 60 Fluid stream fed into the microchannel (liquid in)
- 61 Fluid stream flowing out of the microchannel (liquid out)
- 42 Sensor
- 15 70 T-junction
- 80 Fluid comprising organic compound
- 81 Connecting line from reservoir
- 84 Fluid reservoir(s)
- 90 Fluid waste
- 20 ΔT Temperature gradient

CLAIMS

1. A system for carrying out a thermal melt analysis of an organic compound, wherein the system comprises:
 - 5 ▪ a microchannel having a first opening and a second opening at opposite ends of the microchannel, wherein the temperature at different portions of the microchannel is different thereby creating a temperature gradient between the different portions of the microchannel;
 - 10 ▪ a light source emitting light which is coupled into the microchannel through the first opening;
 - a light detector arranged to detect a light signal exiting the microchannel through the second opening; and
 - wherein the first opening or the second opening of the microchannel is in fluid communication with at least one fluid source providing at least one organic compound.
- 15 2. The system of claim 1, wherein the temperature at the opposite ends of the microchannel is different thereby creating a temperature gradient between the opposite ends of the microchannel.
- 20 3. The system of claim 1, wherein the light is coupled into the microchannel through the second opening and the light signal to be detected by the detector is exiting the microchannel through the first opening.
- 25 4. The system of any one of claims 1 to 3, wherein the microchannel is made of a reflective material or the inside of the microchannel is coated with a reflective material suitable to guide a light beam emitted by the light source through the microchannel.
- 30 5. The system of claim 4, wherein the reflective material is selected from the group consisting of glass, polyethylene, polypropylene, PVC, polystyrene, nylon, polyester, acrylics, polyurethane, polycarbonate, epoxy-based polymers, fluorene derivative polymers, nickel, copper, zinc, aluminum, silver, gold, chromium, an alloy or a composite of the aforementioned metals, titanium oxide, zinc oxide, barium oxide and silicium oxide.

6. The system of any one of the preceding claims, wherein the length of the microchannel is between about 1 to about 200 mm.
- 5 7. The system of claim 6, wherein the length of the microchannel is between about 10 to 70 mm.
8. The system of any one of the preceding claims, wherein the microchannel has an inner diameter of between about 10 μm to about 10000 μm .
- 10 9. The system of any one of the preceding claims, wherein the first opening or the second opening or the first and the second opening of the microchannel is/are connected to a T-junction or a cross-junction.
- 15 10. The system of claim 9, wherein the first opening of the microchannel is connected to a T-junction comprising a first, a second and a third end; wherein
- the first end of the T-junction has an opening which is in fluid communication with the first opening of the microchannel;
 - the second end of the T-junction located directly opposite the first end of the T-junction is connected to the light source; and
 - the third end of the T-junction comprises an opening which is in fluid communication with the first opening of the microchannel.
- 20
11. The system of claim 9, wherein the second opening of the microchannel is connected to a T-junction comprising a first, a second and a third end; wherein
- 25
- the first end of the T-junction has an opening which is in fluid communication with the second opening of the microchannel;
 - the second end of the T-junction located directly opposite the first end of the T-junction is connected to the light detector; and
 - the third end of the T-junction comprises an opening which is in fluid communication with the second opening of the microchannel.
- 30

12. The system of claim 9, wherein the first opening of the microchannel is connected to a cross junction comprising a first, a second, a third and a fourth end; wherein

- the first end of the cross junction has an opening which is in fluid communication with the first opening of the microchannel;
- the second end of the cross junction located directly opposite the first end of the cross junction is connected to the light source; and
- the third and fourth end of the cross junction comprise an opening which is in fluid communication with the first opening of the microchannel;

wherein when fluid flows through either the third or the fourth end of the cross junction into the microchannel the respective other end is blocked so that fluid flows into the microchannel only either through the third end or the fourth end of the cross junction.

13. The system of any one of the preceding claims, wherein the inlet of the microchannel is in fluid communication with multiple reservoirs comprising different organic compounds or mixtures of organic compounds or reservoirs comprising different concentrations of an organic compound.

14. The system of any one of the preceding claims, wherein the temperature gradient is created by a heating arrangement.

15. The system of claim 14, wherein the heating arrangement comprises temperature controlled heating blocks, wherein a first heating block is located near the first opening of the microchannel while a second heating block is located near the second opening of the microchannel.

16. The system of claim 14, wherein the heating arrangement comprises a temperature controlled cooling block and a temperature controlled heating block.

17. The system of claim 16, wherein the heating block is located near the first opening of the microchannel while the cooling block is located near the second opening of the microchannel.

18. The system of any one of the preceding claims, wherein at least one organic compound is located within a sample section having a volume of between about 1 nl to about 5 μ l.
19. The system of claim 18, wherein at least one organic compound is located within a sample section having a volume of between about 1 nl to about 200 nl.
20. The system of claim 18 or 19, wherein the system is adapted to measure multiple sample sections at the same time wherein the sample sections comprised in the microchannel at a specific time are separated from each other by an inert fluid which separates the multiple sample sections from each other.
21. The system of any one of the preceding claims, wherein the light source is a laser, a laser diode or an LED.
22. The system of any one of the preceding claims, wherein the organic compound is selected from the group consisting of a nucleic acid, mixtures of different nucleic acids, a protein and mixtures of different proteins.
23. The system of any one of the preceding claims, wherein the organic compound is bound to a reporter group emitting a signal upon excitation with light from the light source.
24. The system of any one of the preceding claims, wherein the organic compound is bound to a fluorescence label and a quencher.
25. The system of any one of the preceding claims, wherein the light detector detects a light signal emitted by at least one of the organic compounds flowing through the microchannel.
26. The system of any one of the preceding claims, wherein the light signal is a fluorescence signal.
27. The system of any one of the preceding claims, wherein the system is a continuous flow system.

28. A method of carrying out a thermal melt analysis of an organic compound using a system as defined in any one of claims 1 to 27, wherein the method comprises:
- feeding a fluid stream comprising an organic compound into the microchannel;
 - detecting a signal generated by the organic compound upon excitation of the organic compound with the light emitted by the light source.
29. The method of claim 28, wherein the fluid stream located in the microchannel comprises a sample section with a volume of between about 1 nl to about 200 nl in which the organic compound is comprised.
30. The method of claim 29, wherein the fluid stream in the microchannel comprises more than one sample section with a volume of between about 1 nl to about 200 nl comprising the organic compound.
31. The method of claim 30, wherein the more than one sample section located in the microchannel are separated by an inert fluid which is not miscible with the sample section comprising the organic compound.
32. The method of claim 31, wherein the inert fluid is a liquid or a gas.
33. The method of claim 32, wherein the liquid is a mineral oil.
34. The method of any one of claims 28 to 33, wherein the flow direction of the fluid stream fed through the microchannel is changing.
35. The method of any one of claims 28 to 33, wherein the flow rate within the microchannel is between about 100 nl/s to about 100 μ l/s.
36. The method of any one of claims 28 to 34, wherein the organic compound comprises a reporter molecule emitting a signal upon excitation with light from the light source.

37. The method of any one of claims 28 to 36, wherein the organic compound is fed through the microchannel together with a further inorganic or organic compound.

38. The method of claim 37, wherein the further organic or inorganic compound is a drug candidate or a drug.

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FIG. 1

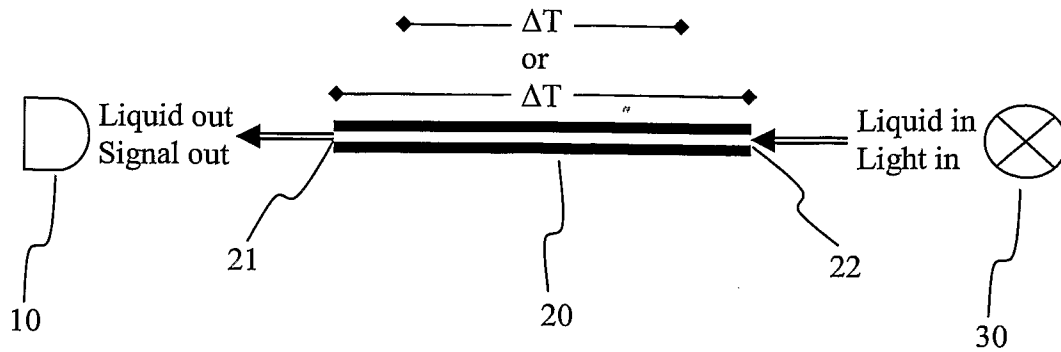


FIG. 2

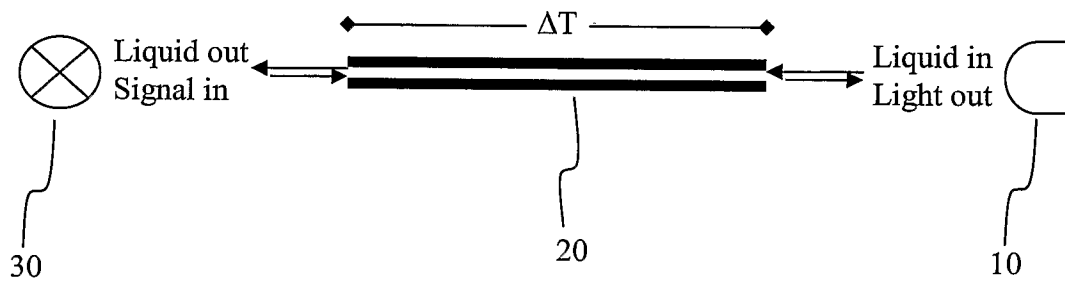
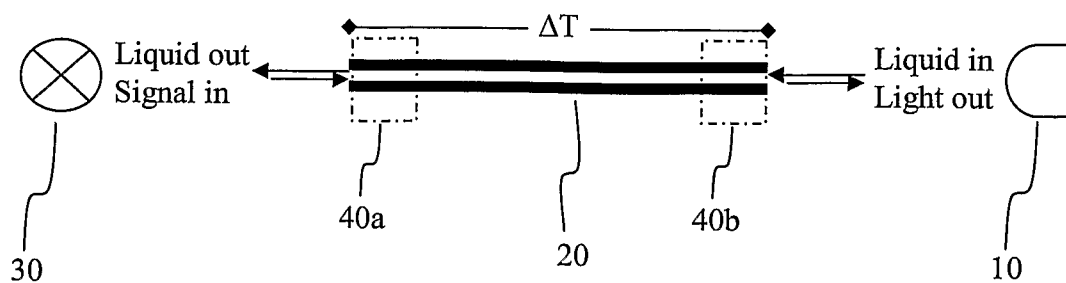


FIG. 3



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FIG. 4

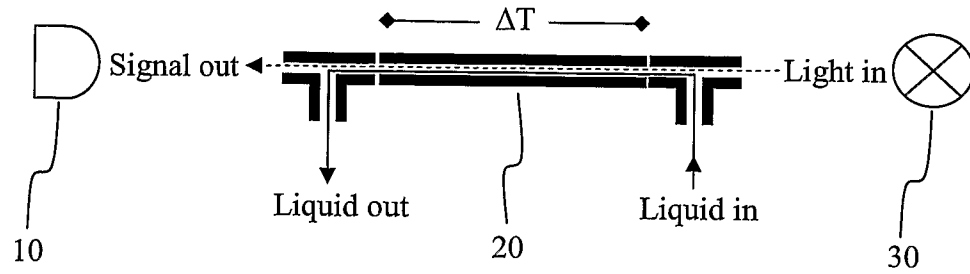


FIG. 5

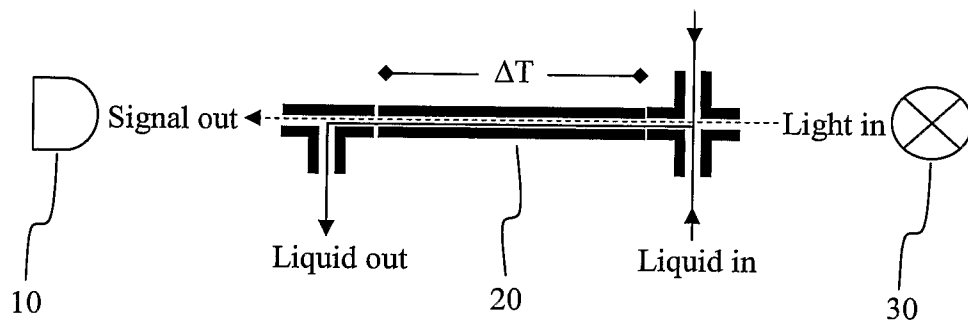


FIG. 6

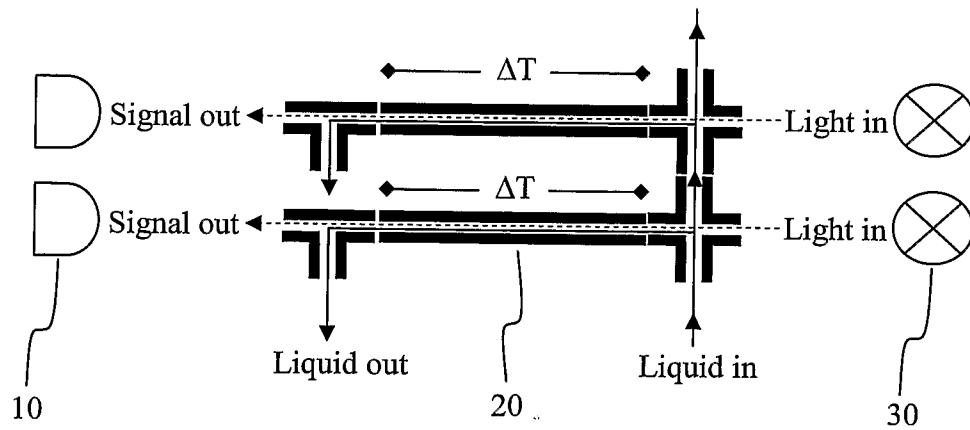


FIG. 7

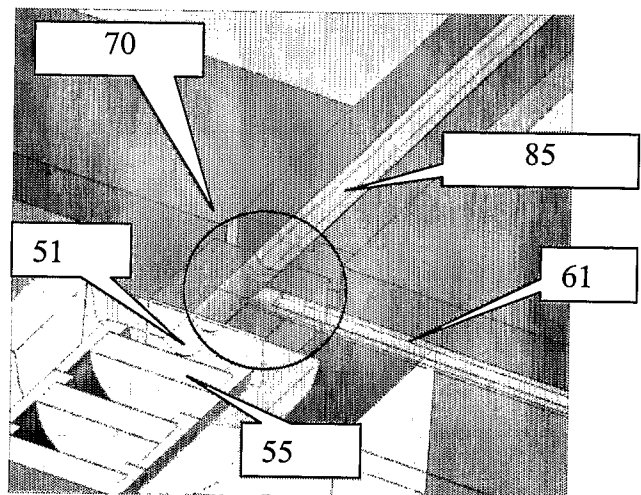
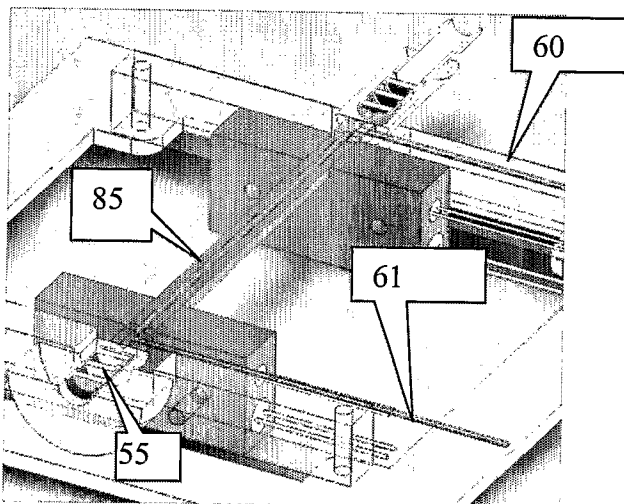
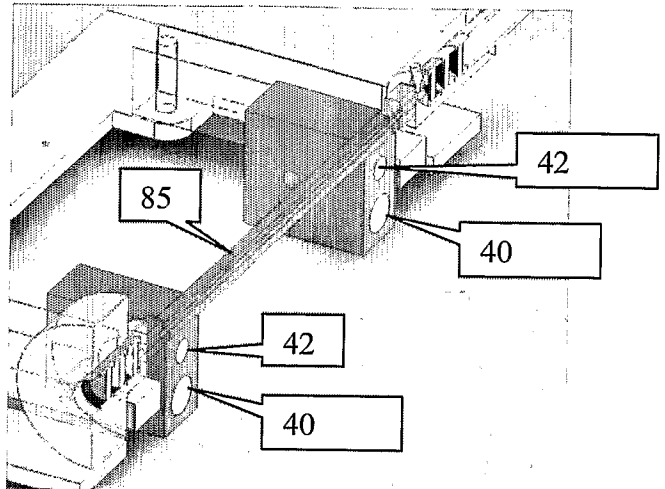
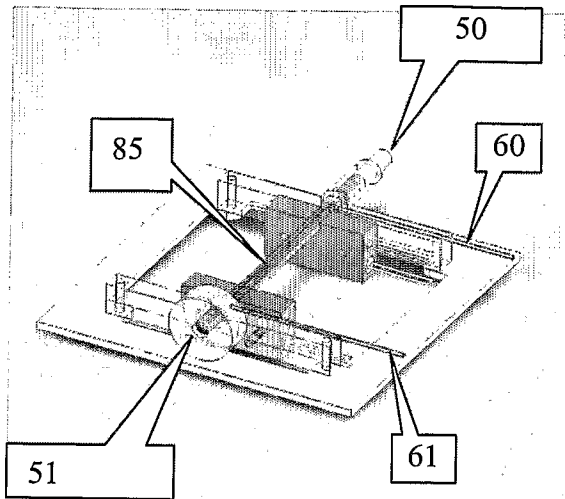


FIG. 8

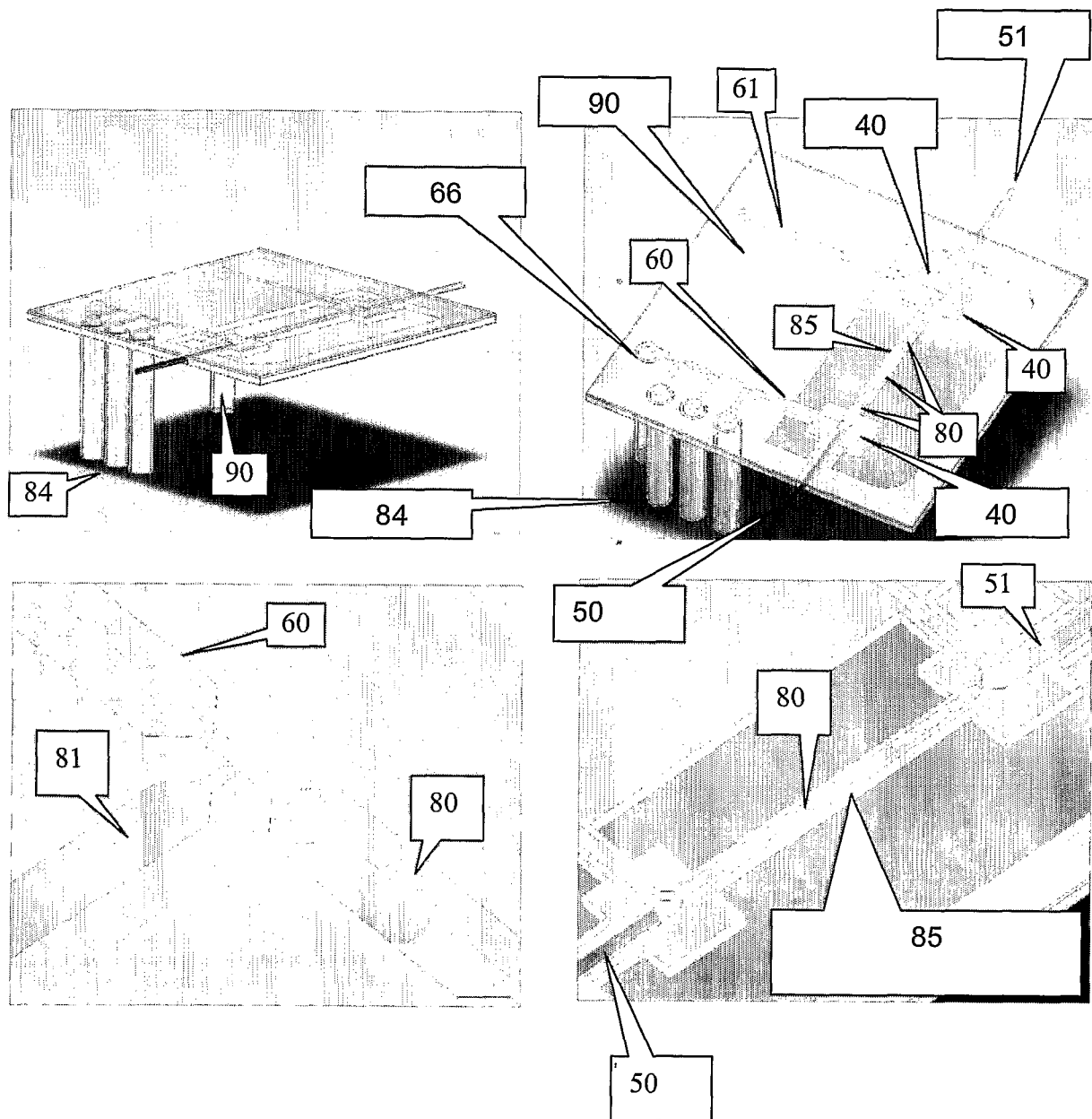
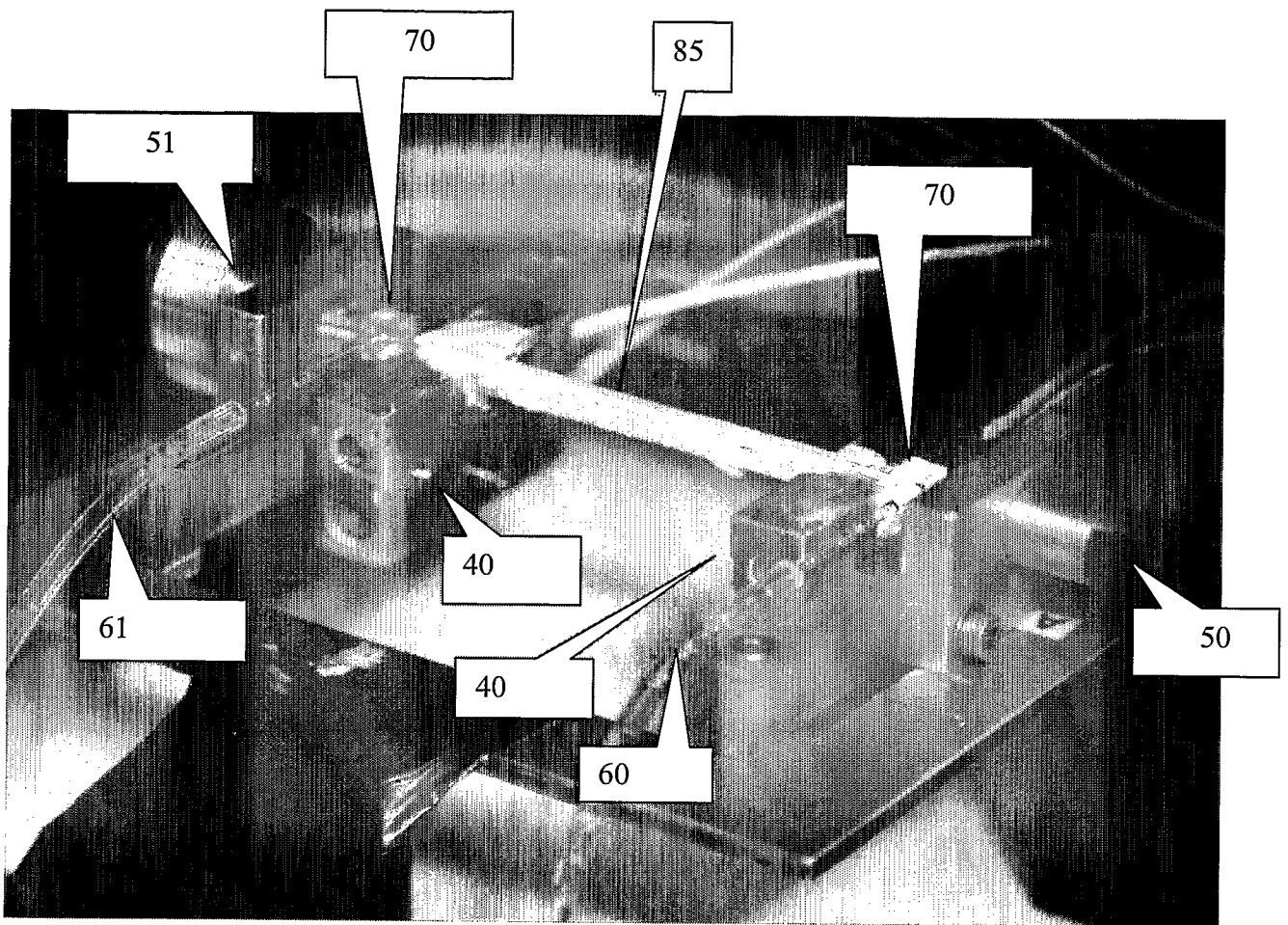
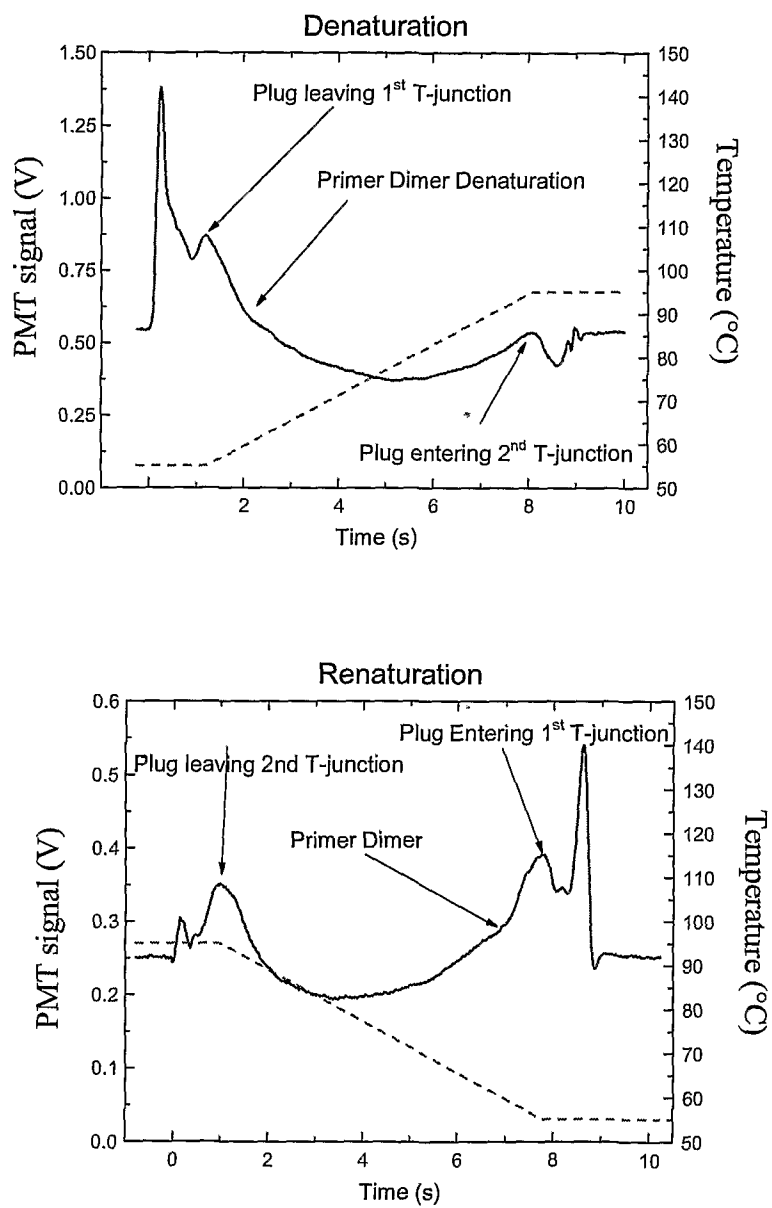


FIG. 9



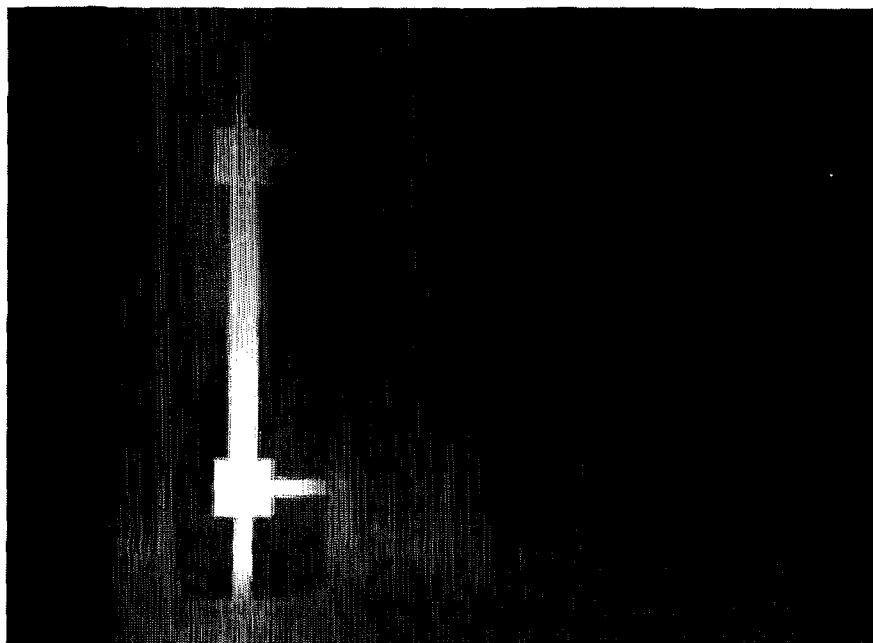
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FIG. 10



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FIG. 11



INTERNATIONAL SEARCH REPORT

International application No.

PCT/SG2009/000323

A. CLASSIFICATION OF SUBJECT MATTER		
Int. Cl.		
<i>G01N 21/64</i> (2006.01) <i>G01N 21/85</i> (2006.01) <i>C12Q 1/68</i> (2006.01) <i>G01N 25/06</i> (2006.01)		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols)		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) WPI/EPODOC/INSPEC & keywords (mca, dna, fluorescence, microchannel, denature, waveguide and similar terms); GOOGLE and GOOGLE PATENTS & keywords (waveguide, mca, capillary, US Class 435/6 and similar terms)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DE 10309526 A1 (KLAPPROTH) 16 September 2004 See figure 1 and abstract	
A	US 6106777 A (FUJITA et al) 22 August 2000 See cols 10 and 11 and figure 14	
A	US 2007/0026421 A1 (SUNDBERG et al) 1 February 2007 See paragraphs 0015-0034 and figures 1 and 9A-9C	
A	WO 2008/005241 A2 (CANON US LIFE SCIENCES, INC) 10 January 2008 See abstract and figure 1	
☒ Further documents are listed in the continuation of Box C ☒ See patent family annex		
* "A"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed	
Date of the actual completion of the international search 12 October 2009		Date of mailing of the international search report 15 OCT 2009
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustalia.gov.au Facsimile No. +61 2 6283 7999		Authorized officer STEPHEN CLARK AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No : +61 2 6283 2781

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SG2009/000323

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2008/038259 A1 (STOKES BIO LIMITED) 3 April 2008 See abstract and figure 1	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/SG2009/000323

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report			Patent Family Member		
DE	10309526	NONE			
US	6106777	EP	0711840	JP	8187098
US	2007026421	CN	101426933	EP	1987163
		WO	2007092643	US	2001036637
WO	2008005241	EP	2038401	EP	2041260
		EP	2044220	EP	2046988
		US	2008003593	US	2008003594
		US	2008176230	US	2008176289
		WO	2008005246	WO	2008005248
		WO	2008005322	WO	2008005323
WO	2008038259	EP	2069070	EP	2044191
				US	2008003588
				US	2008124723
				US	2009053726
				WO	2008005321
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.					
END OF ANNEX					