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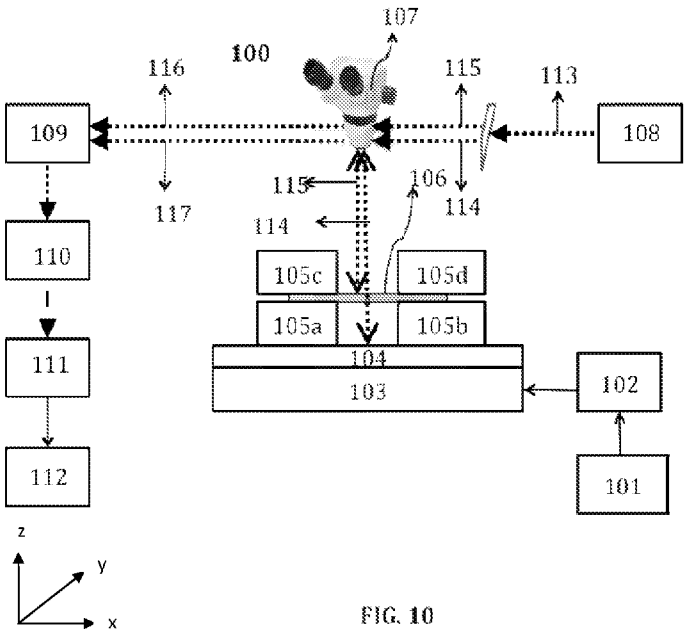

FIG. 10

(57) Abstract: The present invention relates to a method for an in vitro indication of muscular disorders, based on vibration signature of muscle fibers and cells, where suspended control and blind muscle fibers or cells are subjected to vibrations to measure the frequency responses and the frequency responses are compared to indicate a muscular disorder. The present invention also relates to a system for indication of muscular disorders comprising a vibrating base member with holders, to suspend a vibrating control muscle and blind muscle fibers or cells between the holders. A non-contact probe with a beam emitter and a receiver disposed to direct incident beams to the base member and the control and blind muscle fibers or cells and to receive the backscatter of the incident beams. A vibrometer is connected to the non-contact probe to demodulate the change in frequency and phase of the backscattered incident beams and a processing member to capture and compare the frequency response of control and blind members or cells to indicate a muscular disorder or a disease.

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METHOD AND SYSTEM FOR INDICATING MUSCULAR DISORDERS

Technical Field

[001] The present invention relates to a method and system for an i*n vitro* indication of muscular disorders, based on vibration signature of muscle fibers.

Background of the invention

[002] The myopathies and muscular dystrophies are a diverse group of muscle diseases presenting themselves as common complaints of muscle weakness and muscle degeneration, often leading to physical signs such as motor delay and respiratory abnormalities. Myopathies, hereditary or acquired, are characterized by a range of distinctive abnormalities in the muscle biopsy. There are different ways to diagnose muscle diseases using invasive and non-invasive techniques. Most myopathies are diagnosed using optical microscopy. Investigations other than muscle biopsy are rarely specific for myopathies, but are widely used to exclude other possible diagnoses. In recent years, muscle ultrasound and magnetic resonance imaging (MRI) have been used frequently to differentiate among different forms of myopathies. Most of the studies conducted till today are about the chemical basis of the disease. Identifying such diseases at a very early stage can save many patients from suffering due to muscle related problems.

[003] Mammalian muscles are categorized into three major muscle types viz., skeletal, cardiac and smooth muscles. The muscles are made up of multinucleated cells called myofibers, which are composed of several myofibrils, which in turn are made up of myofilaments. Sarcomeres, the smallest contractile functional units of the skeletal muscle, are composed of thick and thin myofilaments, interspaced by Z-discs.

[004] The thick filaments are made up of myosin and myosin binding proteins, while the thin filaments are composed of actin and actin binding

proteins, which includes tropomyosin, troponin I (TnI), troponin C (TnC) and troponin T (TnT) protein subunits. The interaction of actin and myosin is required for muscle contraction. In resting muscles, this interaction is hindered by the tropomyosin-troponin complex, which prevents the force production. However, when TnC binds with Ca^{2+}, the inhibitory actions of TnI and TnT are alleviated and tropomyosin slides, exposing the actin on the thin filament, which enable the cross bridge formation between actin and myosin to produce force. Any defect during the development or assembly of the structural proteins and regulation of actin and myosin interaction lead to muscle disorders. These disorders are heterogeneous in nature and timely identification will help in management and therapeutic interventions.

[005] Cells have the ability to sense the mechanical environment and adjust their behaviour accordingly. Clearly, the mechanical properties of cells are affected by and reflect their pathological condition that results from biochemical changes. Mechanical probing of cells for diagnostic parameters, such as change in adhesion properties and stiffness, are studied using techniques such as atomic force microscopy (AFM) and magnetic twisting cytometry (MTC). The mechanical assessment of muscle activity has generally focused on contraction and force production along the longitudinal axis, although there are additional dimensional changes during a contraction. Such measurements are generally limited to static state of a cell and probing is generally carried out with tools such as an AFM. However, such probing involves an invasive procedure.

[006] The known methods involving the usage of optical tweezers and MTC for detecting muscular disorders require cells to be attached with beads.

[007] In case of AFM and other cell-poking techniques, which involve poking with a sharp tip, often altering the cell response due to the invasive nature of probing. In addition, selection of appropriate tips is laborious and

expensive.

[008] An electron microscopy method of detecting muscular disorder requires that the selected samples are to be dried using critical point drying machine, which alters the response of the samples. Similarly, histo-pathological methods also involve extensive laborious protocols, which may alter the samples. In both the methods, a trained person is required to derive a conclusion.

[009] The biochemical assays that are currently used for detecting muscular disorders are time consuming and expensive.

[010] In known methods, sizes of muscular fiber samples for detecting muscular disorders are larger in size i.e., 1.0 cm x 1.5 cm, involving surgical biopsy by a clinician.

[011] *Drosophila melanogaster* is considered a good model system to study the genetic and molecular mechanisms of many diseases, including muscular dystrophies, due to its short life span, easy maintenance, cost effectiveness, high fecundity, well-sequenced genome and a high degree of conservation of developmental pathways. Significantly, 70% of human disease genes have counterparts in *Drosophila melanogaster.* Muscular mutations with known molecular lesions and heterogeneous phenotypes also exist in *Drosophila melanogaster.* In particular, indirect flight muscles (IFMs) of *Drosophila melanogaster*, provide a good genetic system to investigate muscle structure and functions. In *Drosophila melanogaster*, IFMs form the majority of the muscles present in the thorax and these muscles are physiologically similar to mammalian cardiac muscles and structurally similar to mammalian skeletal muscles.

[012] Indirect flight muscles (IFMs), which are the largest group of muscles in the body of *Drosophila melanogaster* with normal striations as seen in control fibers are as shown in **FIG.1(a)**. However, these normal striations of the control fibers are lost during the course of mutations, as

shown in **FIG.1(b-c)**. It is, therefore, difficult to relate these mutations to specific muscular disorders, based on the sarcomeric structure as many of the mutations bear similar phenotype, for instance, sarcomeric structure of **FIG.1(c)** is similar to the control fiber **FIG.1(a)** even though **FIG.1(c)** represents a mutant condition. Therefore, it is advantageous to have method to detect muscular disorders, which is not based on mutations.

[013] It is also desirable to provide a method and a system, which can indicate diseased conditions of cells (mammalian), based on the change in mechanical properties of the diseased cells, as against the indication based on the change in optical, chemical or biochemical properties of the diseased cells.

Objects of the present invention

[014] Accordingly, the primary object of the present invention is to provide a method for an *in vitro* indication of muscular disorders, based on vibration signature of muscle fibers.

[015] An object of the present invention is to provide a method where a single muscle fiber obtained from a needle biopsy of the muscular tissue is used in conjunction with a non-invasive probe to indicate the presence of muscular disorders.

[016] Another object of the present invention is to provide a method where the frequency responses of the vibrated muscle fiber are obtained at various focal points of the muscle fiber.

[017] Yet another object of the present invention is to provide a method, which is a non-optical assay, to determine the difference between a normal muscle fiber and the muscle fiber with a muscular disorder.

[018] It is also an object of the present invention to provide a method where the blind and control muscular fibers are not subjected to any downstream processing such as drying, staining, microscopy or purification.

[019] Still another object of the present invention is to provide a method where a very small sample of muscle fiber is used.

[020] Further object of the present invention is to provide a method for an *in vitro* indication of a diseased condition, based on vibration signature of the cell.

[021] It is also an object of the present invention to provide a system for an *in vitro* indication of muscular disorders, based on vibration signature of muscle fibers.

Brief description of the drawings

[022] FIG.1(a) depicts a known scanning electronic microscope (SEM) and confocal images of the normal muscular cells.

[023] FIG.1(b) depicts a known scanning electronic microscope (SEM) and confocal images of nemaline myopathy of muscular cells.

[024] FIG.1(c) depicts a known scanning electronic microscope (SEM) and confocal images of cardiac myopathy of muscular cells.

[025] FIG.2 is a schematic illustration of the steps of the method of the present invention to indicate the presence of muscular disorders, based on vibration signatures of muscle fibers of a subject.

[026] FIG.3 is an exemplary image obtained from laser Doppler vibrometer (LDV), depicting the position of a control muscle fiber in a normal state and in a vibrated state when the fiber is suspended between the muscle fiber holders in a fixed-fixed configuration.

[027] FIG.4 is an illustration of backscatter obtained from a base member and a muscle fiber with a change in frequency (Δf) and phase ($\Delta \phi$).

[028] FIG.5(a) is an exemplary plot as obtained from a laser Doppler Vibrometer (LDV) depicting a measurement of frequency responses from a base member.

[029] FIG.5(b) is an exemplary plot as obtained from a laser Doppler

Vibrometer (LDV) depicting a measurement of frequency responses from a control muscle fiber taken from indirect flight muscles (IFMs) of *Drosophila melanogaster*.

[030] FIG.6(a) an exemplary plot as obtained from a laser Doppler Vibrometer (LDV) depicting a measurement of frequency responses from a base member.

[031] FIG.6(b) is an exemplary plot as obtained from a laser Doppler Vibrometer (LDV) depicting a measurement of frequency responses from a blind muscle fiber taken from indirect flight muscles (IFMs) of *Drosophila melanogaster*.

[032] FIG.7(a) an exemplary plot as obtained from a laser Doppler Vibrometer (LDV) depicting a measurement of frequency responses from a base member.

[033] FIG. 7(b) is an exemplary plot as obtained from a laser Doppler Vibrometer (LDV) depicting a measurement of frequency responses from a blind muscle fiber taken from indirect flight muscles (IFMs) of *Drosophila melanogaster*.

[034] FIG.8 depicts frequency responses of control and blind muscle fibers in a fixed-fixed beam configuration.

[035] FIG.9 depicts statistical analysis of natural frequencies of control and blind muscle fibers in a fixed-fixed configuration.

[036] FIG.10 is an illustrative architecture of the system of the present invention.

[037] FIG.11 is an image from an LDV depicting the arrangement of muscle fiber between the holders of a base member.

[038] FIG.12 is an exemplary wave forms of response of base member and muscle fiber as measured by an LDV

Summary of the invention

[039] Accordingly, the present invention provides a method for an *in vitro* indication of muscular disorders, comprising the steps of suspending a control muscle fiber of an effective length, between holders of a base member and vibrating the base member and the control muscle fiber with a sweeping frequency. Thereafter, incident beams with desired wavelengths are directed, from a non-contacting probe to the vibrating base member and to the control muscle fiber and the corresponding backscatter of the incident beams with a change in frequency and phase is received. The change or shift in the frequency and the phase of the backscatter is demodulated into velocity and displacement, respectively and frequency responses of the base member and the muscle fiber are obtained from the demodulated backscatter. Subsequently, natural frequencies of the base member and the control muscle fiber are obtained from the frequency responses of the base member and the control muscle fiber, from respective peak amplitudes to select only the natural frequencies of the control muscle fiber. The natural frequency of a blind muscle fiber is obtained by substituting the control muscle fiber with the blind muscle fiber. Finally, the natural frequency of the blind muscle fiber is compared with the natural frequency of the control muscle fiber, to indicate a muscular disorder, where the natural frequency of the blind muscular fiber is less than the natural frequency of the control muscle fiber and greater than the natural frequency of the base member. The present invention also provides a method for an *in vitro* indication of a diseased condition or a disorder of a cell, based on vibration signature of the cell. The present invention further provides a system to indicate the presence of muscular disorders and diseases based on the vibration signatures of the muscle fibers and cells.

Description of the invention

[040] The present invention provides a method for an *in vitro* indication of

muscular disorders, based on the vibration signatures of muscle fibers of a mammalian subject.

[041] As an illustrative embodiment, in the present invention, *in lieu* of the muscle samples of a mammalian subject, IFMs from *Drosophila melanogaster* are used to demonstrate the implementation of the method of the present invention, since IFMs of *Drosophila melanogaster* are structurally similar to mammalian skeletal muscles (striated muscles) and they are functionally similar to mammalian cardiac muscles. Significantly, mutations affecting the structural proteins of IFMs demonstrate phenotypes, which are similar to mammalian counterparts and hence IFMs are selected as suitable candidates, for detecting muscular disorders. The contraction of IFMs of *Drosophila melanogaster* is regulated by both stretch and Ca^{2+} induced thin filament (actin-tropomyosin-troponin complex) activation, which is similar to the mammalian cardiac contractions.

[042] In addition, the results of muscular disorders of *Drosophila melanogaster* are found to be substantially similar to mammalian muscular fibers. The pathophysiology resulting from unregulated acto-myosin interactions in the IFMs of *Drosophila melanogaster* leads to the development of hyper contraction muscle phenotype, which is similar to cardiomyopathy in higher vertebrates, including human (Nongthomba et al., 2003; Camarato et al., 2004). Even the molecular signature produced from such phenotype also shows similarities (Montana and Littleton, 2006). Many structural protein disorders also produce protein aggregate structures called “nemaline bodies” in human, which are also seen in the IFMs of *Drosophila melanogaster* (Nongthomba et al., 2007, Haigh et al., 2010). Even the chemical signature produced by both human and muscle disorders of *Drosophila melanogaster* are quite similar (Gautam et al., 2015), suggesting that IFMs *Drosophila melanogaster* can be used as surrogate for mammalian muscle disorders for initial studies.

[043] In an aspect of the present invention, initially, adult *Drosophila melanogaster (Canton-S)* flies of 3-days old are anaesthetized and collected in a centrifuge tube containing ethyl alcohol (70%) and stored at a temperature of -20 degrees C. The selected fly is then dissected to obtain biopsy samples of the indirect flight muscles (IFMs), by carefully bisecting the thorax into two halves. The fascicles, which are designated as control muscle fascicles, are removed and stored at a temperature of about -20^0C, in a medium, preferably ethyl alcohol (70%). These fascicles are of the type striated muscles. It is understood here that since the muscle fibers of *Drosophila melanogaster* are substantially smaller in size, the muscle fascicles of IFMs are used, as control muscle fibers, which are comparable to mammalian muscular fibers.

[044] In a significant aspect of the present invention control muscular fibers thus obtained are not subjected to any downstream processing such as drying, staining, microscopy or purification, thereby preventing any possible physiological and structural changes, which can affect the mechanical properties of the selected control muscle fiber. Accordingly, in the present invention, the control muscle fiber, which is in its natural form, is used to obtain vibration signatures, which are free from any possible errors. In other words, any suitable beam structure, which can allow a suspension of the control muscle fiber to vibrate, can be suitably adapted for use.

[045] In another aspect of the present invention, a single control muscle fiber, preferably of the length of 0.5 to 0.8mm is obtained from the sample and the control muscle fiber is suspended between holders of a movable base member with its ends, in a fixed-fixed beam configuration, to prevent a possible length variation across the selected muscle fiber during the course of in vitro indication of muscular disorders. The suspension arrangement between the holders is also to facilitate an intervening gap between the top surface of the movable base member and the control

muscular fiber so as to facilitate vibratory movement of the control muscle fiber.

[046] The single control muscle fiber thus selected is adapted for use with a preferred aspect ratio, having an effective length of 500-800 microns and a diameter of about 50 microns. In other words, the indicated effective length is the length that is used which permitted to vibrate and from where the backscatter of the incident light beams is captured.

[047] However, it is also within the purview of this invention to use a control muscle fiber, having larger aspect ratios, than the ones as specified here, since in a mammalian system as muscle fibers can grow by hypertrophy condition or by weight training. Whereas, in *Drosophila melanogaster*, the size of the muscle fiber corresponds directly to the body size and once fully formed there further change in the muscle fiber is expected unlike muscle fibers of mammals.

[048] The single control muscle fiber is then suspended between holders of a base member, thereby providing an effective length in the range of 100-500 microns, preferably 300-350 microns. In an exemplary aspect, in the method of the present invention, the control muscle fiber is arranged in a fixed-fixed configuration, to obtain vibration signatures. Alternately, other configurations such as cantilever, simply supported structure, can be suitably adapted for use, to suspend the control muscle fiber.

[049] The base member is then vibrated by means of an actuator, preferably a mechanical actuator such as a piezoelectric actuator. It is also within the purview of the invention to use electrical or optical actuators for actuating the base member. When mechanical actuators are used, the base member is actuated by providing a base excitation from a piezoelectric actuator. Whereas, in case of use of electrical actuators, patterned electrodes are connected to muscle fibers and a sinusoidal voltage is applied to the muscle fiber through the patterned electrodes. Likewise, in case of an optical actuation, the control muscular fiber is

coated with nanoparticles such as silver and gold nanoparticles, which are optically active and actuation is attained using a light source of required wavelength. It is understood here that control muscle fiber can also be coated with several other nanomaterials including magnetic materials such as ferrous nanoparticles, to facilitate an electromagnetic actuation.

[050] The selected actuator (piezoelectric actuator) is used to vibrate the base member with sweeping frequencies in the range of 0.5 to 200 kHz. The control muscle fiber, which is suspended from the holders of the base member, also vibrates along with the vibration of the base member. This range of sweeping frequencies, both in narrow and wide ranges is used to capture the frequency response of the base member and the muscle fiber. The sweeping frequencies can be suitably altered in case a desired mammalian muscle fiber is used. The resting and vibrating positions of the control muscle fiber are as shown in **FIG.3**.

[051] Incident beams with desired wavelengths (λ), preferably from a visible spectrum, in the range of 600-630 nm are directed from a beam emitter of a non-contact probe such as a laser Doppler vibrometer (LDV) to the base member and the control muscle fiber respectively, to obtain the corresponding backscatter of the incident beams.

[052] In an exemplary aspect, in the present invention, laser beams are used as incident beams. The incident beams are emitted by LDV. Alternately, a position-sensing detector can also used to emit incident beams. The incident beams are directed to the base member and the control muscle fiber. The incident beams are used to scan the base member, holders and the control muscle fiber. The scanning of the incident beam is performed to differentiate the frequency response of the control muscle fiber from the base member. In other words, during the course of vibration where the base member and the control muscle fiber are displaced vertically from their resting positions, it is important to segregate the vertical displacement of the muscle fiber from the base

member, so as to select only the frequency response of the muscle fiber.

[053] The backscatter thus obtained is with a change in frequency (Δf) and phase ($\Delta\phi$) as shown in **FIG.4**. The change in frequency and phase is demodulated to change in velocity (ΔV) and displacement (ΔZ) of the base member and control muscle fiber as shown in the formula (I).

Formula (I)

$$\Delta f = \frac{2V}{\lambda} \quad \Delta\varphi = \frac{4\prod}{\lambda}\Delta Z$$

[054] Thereafter, the natural frequencies of the backscatter of the base member and the control fiber are obtained from the demodulated backscatter as shown in **FIG.4**, in conjunction with sweeping frequencies and monitoring the amplitude of vibration based on change in phase of the backscattered laser light.

[055] The natural frequencies of the control muscle fiber and the base member are extracted from the frequency response using peak picking amplitude as shown in **FIG.5**, to select only the natural frequencies of the muscle fiber, by distinguishing the natural frequency of the control muscle fiber from that of the base member. In an exemplary aspect, the control muscle fiber is a wild type *Drosophila melanogaster (A-Canton-S),* which is free from muscular disorders.

[056] For instance, in this exemplary embodiment as shown in **FIG.5**, the natural frequency of the base member is 5 Hz, and of the control muscle fiber is 122 kHz, as obtained from the respective peak amplitudes as shown in **FIGs.5,6,7 & 8**. The natural frequencies of the muscle fiber thus obtained are as shown in **Table 1**.

TABLE 1

Control	sp^3	sp^{2N}	$3\omega_{BP}^{Diode}$	$3\omega_{RC}/\omega_{ref}$	$\omega_{ref}/4$	$\omega_{ref}^{25}/4$	A Vs B	A Vs C	A Vs D	A Vs E	A Vs F	A Vs G	C < B	D < C	E < D	F < E	G < F
A (kHz)	B (kHz)	C (kHz)	D (kHz)	E (kHz)	F (kHz)	G (kHz)											
123.05	73.54	67.98	67.77	31.28	36.78	44.80	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
122.91	73.13	67.41	63.34	31.68	37.52	45.27	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
122.99	71.98	66.77	61.15	32.58	38.99	43.92	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
122.94	73.60	65.82	61.56	39.19	44.12	44.12	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
123.00	73.44	67.94	62.88	31.78	37.94	43.88	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
122.93	69.58	66.04	65.50	29.64	37.31	43.41	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
123.02	76.73	67.31	61.90	30.61	37.90	44.60	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
123.04	74.03	70.51	62.87	32.99	37.41	44.04	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
123.12	74.19	68.83	66.33	32.07	37.59	42.81	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
122.96	73.99	67.49	64.87	33.43	38.46	44.07	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
122.81	74.59	69.14	61.47	32.68	38.52	44.85	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
122.91	75.40	68.94	62.48	30.02	36.52	43.97	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
123.09	71.90	67.58	65.33	33.88	38.77	44.49	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
122.89	76.33	65.57	62.45	33.26	38.96	43.98	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
123.06	74.31	71.92	62.25	31.93	37.34	42.71	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
122.99	74.55	68.47	59.50	32.25	37.29	43.74	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
123.09	72.46	71.63	56.75	32.67	39.20	43.98	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
122.82	74.29	68.28	60.58	34.14	38.53	43.07	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
122.96	74.93	70.82	65.62	33.51	38.94	42.86	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
122.88	74.54	69.55	61.43	33.64	37.33	44.61	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
123.11	75.13	68.56	60.64	32.68	39.50	44.36	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
123.05	72.43	65.20	61.74	33.53	38.53	43.57	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
123.09	70.78	66.91	64.04	32.91	37.34	42.94	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
122.90	74.23	67.68	60.81	32.32	37.21	44.83	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
122.94	72.59	70.33	57.28	32.03	37.24	44.75	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
122.96	74.19	70.18	60.92	32.93	37.53	43.68	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
123.07	74.95	68.91	64.35	31.39	38.23	44.05	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
122.86	72.07	68.22	65.43	35.19	38.74	44.43	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
123.04	71.53	69.39	65.04	32.00	38.74	43.92	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
122.92	72.84	67.29	60.53	32.03	36.59	44.21	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
122.95	72.88	70.41	62.80	31.53	37.10	45.38	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
122.91	74.04	65.94	60.17	32.49	37.01	44.19	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
122.85	73.93	65.45	64.07	32.28	37.29	44.98	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
123.02	73.94	68.12	60.83	30.78	36.32	44.72	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
123.00	74.13	65.41	63.55	33.43	38.98	43.59	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
122.98	75.54	68.98	59.51	31.06	38.96	44.96	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
122.87	73.95	70.85	62.25	30.79	38.30	43.86	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
123.07	72.09	65.58	65.36	30.74	38.19	44.40	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
123.01	73.48	65.04	61.83	31.38	36.74	44.08	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
122.96	73.20	67.83	63.40	31.64	37.58	43.86	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
122.98	71.85	68.20	62.36	29.98	37.12	44.87	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
122.96	73.13	70.68	61.59	31.14	38.54	44.45	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
122.84	73.32	69.00	63.07	32.98	35.64	43.99	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
122.96	73.10	69.22	61.41	31.74	37.91	44.67	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
122.91	73.13	65.83	63.76	31.99	39.23	44.53	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
122.90	74.40	68.34	60.98	30.62	38.68	44.60	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
122.83	73.13	70.47	62.75	29.63	38.42	44.19	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
122.94	74.53	69.75	62.57	30.58	37.42	42.84	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
122.82	73.64	67.85	64.65	31.29	40.91	44.34	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO
123.06	75.46	67.74	63.58	31.43	37.71	44.98	B < A	C < A	D < A	E < A	F < A	G < A	Yes	YES	YES	NO	NO

[057] Once the natural frequencies of the control muscle fiber are obtained, the control muscle fiber is substituted with a blind muscle fiber and the corresponding natural frequencies are obtained by following the aforementioned procedure and shown in **Table 1**. In this exemplary aspect, the blind muscular fibers, which are selected from the mutants of *Drosophila melanogaster are viz., B -up^{1}, C -up^{101}, D -Act88F^{KM88}, E -Mhc^{7}/Mhc^{7}*, F -*Mhc^{7}*/+ and G -*Mhc^{2B}*/+.

[058] *up^{1}* is a Troponin T (TnT) splice site mutation, where an adult-specific TnT isoform fails to accumulate in the IFMs. *up^{101}* is a TnT hypomorph mutant, leading to disregulation of Actin and Myosin interaction.

[059] *Act88F^{KM88}* is an IFM specific actin isoform null, which removes all the actin from the IFMs, leading to loss of thin filaments from the fiber.

[060] Whereas, *Mhc^{7}/Mhc^{7}* is an IFM-specific myosin null mutation, which removes all the myosin from the IFMs resulting in the loss of thick filament from the fiber.

[061] *Mhc^{7}*/+ is a mutant form, where half of the normal myosin is present but the condition of the muscle fiber is not normal.

[062] *Mhc^{2B}*/+ is a mutation in actin binding loop (Proline 401 residue changed to Serine) hampering with the myosin interacting with actin, leading to malfunctioning of fiber.

[063] In **Table 1**, the natural frequencies (control-A) are tabulated along with natural frequencies of the blind muscle fibers (B-G).

[064] Subsequent to the determination of the natural frequencies of the control muscle fiber and the blind muscle fibers, the natural frequencies of the control muscle fiber are compared with the natural frequencies of the blind muscle fibers as shown in **Table 1**. Thereafter, as shown in **Table 1**, the natural frequencies of the blind muscle fibers are compared *inter se,* to indicate the nature of the myopathies, prevailing in these blind muscle

fibers.

[065] For instance, as shown in **Table 1**, in case of the blind Sample B, the natural frequency of the Sample B is greater than the natural frequencies of the other blind samples viz., C, D, E, F and G. However, the natural frequency of the Sample B is less than that of the control muscle fiber A, thereby indicating the presence of a myopathic condition.

[066] Now, the natural frequencies of the samples of the blind muscle fibers (B, C, D, E, F and G) are compared with the control A and samples of the blind muscle fibers (B, C, D, E, F and G) are grouped into two groups viz., Group I and II, as shown in **Table 2**.

Table 2

Blind Sample	*Frequencies* (kHz)	*No. of samples (n)*		Genotype	Disorder
A	122.98 ± 0.08	50		*Canton-S*	
B	74.37 ± 1.59	50	Group I	up^{1}	Thin filament
C	68.37 ± 2.38	50		up^{101}	
D	62.51 ± 2.79	50		$Act88F^{KM88}$	
E	32.06 ± 1.44	50	Group II	Mhc^{7}/Mhc^{7}	Thick filament
F	37.80 ± 0.92	50		$Mhc^{7}/+$	
G	44.10 ± 0.68	50		$Mhc^{2B}/+$	

[067] It can be seen from the above **Table 2**, that the natural frequencies of Group I and II are less than the natural frequencies of control muscle fiber (A) and greater than the natural frequency of the base member (5 Hz). It is also observed here that the natural frequencies of the samples of Group I are more than natural frequencies of the samples of Group II.

[068] When the corresponding mutations are matched with the natural frequencies of the blind muscle fibers, the natural frequencies of Group I and II, it is indicated that they relate to thin and thick filament disorders, respectively. Accordingly, by this method it is possible to indicate molecular nature of the muscular disorder irrespective of the histological

defects.

[069] It can be seen from the above **Tables 1** and **2** that mutants with muscular disorders, show natural frequencies less than wild type control (*Canton-S*) but more than the base frequencies. Muscles of mutants with nemaline phenotype show natural frequencies which are less than wild type but higher than frequency of mutants with cardiac type.

[070] In an aspect of the present invention, natural frequencies of plurality of control and blind muscle fibers can also be measured, by suspending these fibers from the holders of the base member and obtaining backscatter from the respective incident beams.

[071] Accordingly, the present invention provides a method for an *in vitro* indication of muscular disorders, comprising the steps of suspending a control muscle fiber of an effective length, between holders of a base member and vibrating the base member and the control muscle fiber with a sweeping frequency. Thereafter, incident beams with desired wavelengths are directed, from a non-contacting probe to the vibrating base member and to the control muscle fiber and the corresponding backscatter of the incident beams with a change in frequency and phase is received. The change or shift in the frequency and the phase of the backscatter is demodulated into velocity and displacement, respectively and frequency responses of the base member and the muscle fiber are obtained from the demodulated backscatter. Subsequently, natural frequencies of the base member and the control muscle fiber are obtained from the frequency responses of the base member and the control muscle fiber, from respective peak amplitudes to select only the natural frequencies of the control muscle fiber. The natural frequency of a blind muscle fiber is obtained by substituting the control muscle fiber with the blind muscle fiber. Finally, the natural frequency of the blind muscle fiber is compared with the natural frequency of the control muscle fiber, to indicate a muscular disorder, where the natural frequency of the blind muscular

fiber is less than the natural frequency of the control muscle fiber and greater than the natural frequency of the base member.

[072] In the method of the present invention the control muscle fiber is a healthy muscle fiber.

[073] The control and blind muscle samples as used in the method of the present invention are advantageously selected from a striated muscle, non-striated muscle and a cardiac muscle, which are obtained from control and blind muscle biopsy samples through a needle biopsy in case of a mammalian subject.

[074] The control and blind muscle fibers are suspended between the holders by gripping, capillary adhesion or nanoclipping.

[075] The effective length of the suspended control and test muscular fibers is in the range 100-500 microns, preferably in the range of 300-350 microns.

[076] The sweeping frequency of the base member and muscle fiber in the method of the present invention is preferably in the range of 0.5-200 kHz.

[077] The wavelength of the incident beams as used in the method of the present invention is in the range of 600-630nm.

[078] In the method of the present invention, natural frequencies of the control muscle fiber and the blind muscle fiber are in the range 20-200kHz and the natural frequency of the control muscle fiber is preferably equal to or greater than 110kHz.

[079] In yet another aspect of the present invention, the natural frequencies of the plurality of control and blind muscle fibers are measured, to indicate the presence of respective muscular disorders.

[080] The method of the present invention is now illustrated in the form of the following non-limiting examples.

Example 1

[081] Three-day-old adult flies *Drosophila melanogaster (Canton-S)* are anaesthetized and the indirect flight muscles are dissected, by carefully bisecting the thorax into two halves in 70% alcohol. A single fascicle without any deformities is removed and stored in ethyl alcohol (70%) in a freezer at a temperature of -20 degree C. The single fascicle is suspended as fixed-fixed beam, by using the holders, so that the effective length of the fascicle is 300 µm and the holders are mounted on the base member. The holders and base member are connected to a piezoelectric actuator. The piezoelectric actuator is initially provided with sweeping excitation frequency initially of 0.005 kHz and gradually increased to a sweeping frequency of 200 kHz so that the fascicle is vibrated, along with the vibration of the base member. Thereafter, incident beams with wavelength of 630 nm, are directed from a LDV through a beam emitter to the vibrating base member and the fascicle. The LDV receives the backscatter of the incident beams from the base member and the fascicle, where the backscatter having a change in frequency and a phase is received. The change in the frequency and phase is demodulated by the vibrometer to convert the frequency into a velocity (60 µm/sec) and the phase into a displacement (80 nm) of the fascicle. Thereafter, frequency responses of the base member and the fascicle are obtained as shown in **FIG.5(a)** and **FIG.5(b)**. The corresponding natural frequencies of the base member (0.05 kHz) and of the fascicle (122.98 kHz), as shown in Control column of **Table 1** are obtained from the respective peak amplitude of 3nm and 80 nm respectively. It is factored here that the natural frequency of *Canton-S* is greater than 110 kHz. The natural frequency of (122.98 kHz) of the fascicle is found to be greater than 110 kHz and hence this fascicle is selected as control fascicle. The control fascicle is then substituted with a blind fascicle and the natural frequency (75.44 kHz), as shown in **Table 1** up^1 column is obtained. The natural frequency (75.44 kHz) is compared with the natural frequency of the base member (0.05 kHz) to infer the

natural frequency of the blind fascicle, which is 75.44 kHz. Since, the obtained natural frequency of the blind fascicle is significantly less than 110 kHz and is more than the natural frequency (0.05 kHz)of the base member as shown in **FIG.6**, thereby demonstrating the presence of Nemaline myopathy in the blind sample.

Example 2

[082] The procedure of Example 1, is repeated for 49 more sample fascicles of *Canton-S* and the same is repeated for other mutant samples, namely *B -up^{1}*, *C -up^{101}*, *D -Act88F^{KM88}*, *E -Mhc^{7}/Mhc^{7}*, F *-Mhc^{7}/+* and *G -Mhc^{2B}/+*. Thereafter, frequency responses of the base member and all the experimental fascicles are obtained as shown in **FIG.7(a)** and **FIG.7(b)**. The corresponding natural frequencies of the base member (0.05 kHz) and the fascicles are obtained from the respective peak amplitude respectively. The natural frequency of the *Canton-S* fascicle (122.98 kHz ±0.08) is found to be greater than 110 kHz, and the rest of the mutant frequencies are specified in **Table 1**. While comparing the natural frequency of control to natural frequency of other mutants, the natural frequencies of all the mutants were less than that of the Canton-S. Inter se comparison of mutant frequencies revealed that all the myosin mutants (Cardiac myopathies) were grouped together, and TnT mutants and actin null mutants (nemaline myopathies) were grouped into other regime based on natural frequency database (**Table 2**). The natural frequency data thus obtained are able to categorize mutations into two distinct muscle disorder groups, such as cardiac and nemaline myopathies. Though **FIG.1(a)** (Control) and **FIG.1(c)** (Cardiac) have shown similar sarcomeric structures, by adopting the method of the present invention it is made possible to differentiate these two myopathies, based on the natural frequencies of the respective muscle fibers.

[083] In the method of the present invention, in case a mammalian muscle fiber is desired, a needle biopsy of a selected muscle tissue is

preferred, since it is a minimally invasive technique, to remove a smaller piece of desired muscle tissue from a subject, to minimize the trauma to the subject and is a an alternate procedure to other open muscle biopsies. The incision of the needle biopsy is about 3-5 mm, which primarily depends on the selection of a needle with a desired gauge. Therefore, the biopsy samples thus obtained are smaller in size, which are obtained from a tissue of about 300 mg, depending on the muscle bulk. The preferred types of muscular samples include striated, non-striated and cardiac muscles.

[084] The present invention also provides a system for an *in vitro* indication of muscular disorders, based on the vibration signatures of muscle fibers of a mammalian subject.

[085] The system **100** of the present invention is now described by particularly referring to **FIG.10.** The system **100** comprises a signal generator **101**, which can be an external or an inbuilt signal generator, to generate an excitation signal **102**, which can be a sine wave, a sweep wave, a periodic chirp wave or the like. In the present invention, advantageously an inbuilt signal generator (Polytec) is adapted for use, to generate the required signals, which serve as input signals, to vibrate the muscle fiber and the base member. Typically, the input frequencies are in the range of 0.005 Hz to 20 MHz, so as to facilitate excitation of control, blind muscle fibers (diseased or normal muscle fiber) and the base member.

[086] A vibrator **103** is arranged to receive the excitation signal **102** and to facilitate holding of muscle fibers. In the present system, as an exemplary aspect, a mechanical actuator such as a piezoelectric actuator is used as a vibrator to receive the excitation signal **102**. It is also within the purview of the invention to use electrical or optical actuators. In electrical actuators, patterned electrodes are connected to muscle fibers and a sinusoidal voltage is applied to the muscle fiber through the

patterned electrodes. In case of an optical actuation, the muscle fibers are coated with nanoparticles such as silver and gold nanoparticles, which are optically active. Actuation can be attained using a light source of required wavelength. The muscle fiber can also be coated with several other nanomaterials including magnetic materials, such as ferrous nanoparticles, to facilitate actuation using other means such as electromagnetic.

[087] A base member **104**, which is movable, is connected to a vibrator **103**. The base member **104** is advantageously made from dielectric materials such a glass, PDMS, aluminum and tungsten. The material for the base member **104** is preferably a light-reflecting material, which is responsive to light beams having a wavelength 600-630nm. The shape of the base member **104** is exemplarily shown as rectangle. However, it is understood that any other suitable shapes such as circular and polygonal can also be adopted for use. The size of the base member **104** is variable and commensurate with the effective length of muscular fibers that are to be used in the system **100**.

[088] At least a first pair of holders **105a** and **105b** are integrally connected to the base member **103**. The holders **105a** and **105b** are made of biocompatible materials. In the present invention, the holders **105a** and **105b** are made of glass material. However, other dielectric materials such as glass, PDMS, aluminum and tungsten can be suitably used. The holders **105a** and **105b** are typically used to hold or grip the ends of muscular fiber. Accordingly, arrangements such as grippers, adhesive holders, including capillary adhesive holders and nanoclippers, can also be suitably used. The total number of holders can be suitably varied considering the number of muscle fibers that are to be tested. A second pair of holders **105c** and **105d** are mounted on the first pair of holders **105a** and **105b** with an intervening gap of 100-500 µm is arranged between the holders **105a** and **105b**; **105c** and **105d**, to permit the

passage of muscle fiber. The holders **105a** and **105b**; **105c** and **105d** can be cover slips, tungsten wires and aluminum grippers. The holders can be polymeric grippers, which are biocompatible, such as PDMS, which can hold the muscle fiber, preferably, in a fixed-fixed configuration.

[089] In yet another aspect of the present invention, the number of holders can be suitably increased so as to suspend the desired number of muscle fibers.

[090] At least a selected muscular fiber **106** (control or blind) is suspended in between the holders **105a** and **105b**; **105c** and **105d** of the base member **104** with an intervening space between the top portion of the base member **104** and the muscle fiber **106**.The intervening gap is provided to permit the vibration of the muscle fiber **106** during actuation.

[091] A beam emitter **108**, preferably a monochromatic laser beam emitter with a preferred wave length of about 630nm, is used to emit a beam **113**, which is split into at least a pair of laser beams **115** and **116** (measurement beams), through a beam splitter **114** and these beams **115** and **116** are directed to the muscle fiber **106** and the base member **104**, respectively, through a microscope **107**.

[092] The microscope with an objective lens, advantageously having a working distance of 33 mm, field of view of 0.6 mm x 0.97 mm and a pixel resolution of 0.65 μm **107**, preferably a movable one, is arranged above the muscle fiber **106** and is positioned perpendicular to the parallel axis of the muscle fiber **106**. The microscope **107** is also enabled to move along x, y and z axes so as to focus on the muscular fiber and a base member. In the event, more than a single muscle fiber is used the position of the microscope can be moved along the z axis as shown in **FIG.10**.

[093] In an exemplary aspect, at least two light (laser) beams laser beams **114** and **115**, preferably in visible frequency range, one each for the muscle fiber **106** and the base member **104** are fed through the

microscope **107** of the system, to reduce the focused spot area of the light beams, since the velocity of the light beams are averaged over the spot area and the vibratory motion of the muscle fiber can only be measured within the small spot area.

[094] A non-contact probe such as a laser Doppler vibrometer (LDV) **109** is connected to the microscope **107** to measure out-of-plane velocities by interferometrically measuring the change in frequency and phase of the laser beams **116** and **117** that are reflected from the muscle fiber **106** and the base member **104**, respectively.

[095] In yet another aspect of the present invention, a position-sensing detector or high-speed camera, can also be used in place of the LDV to record the motion and measure the natural frequency of the muscle fiber.

[096] A demodulator **110** is disposed to receive backscatter light beams from LDV **109** where the shift in frequency and phase of backscattered laser beams are demodulated to shift in velocity and displacement. The shift (Doppler) in the frequency and the phase of the measurement beams **115** and **116**, is directly proportional to the velocity of the object. Acousto-optic modulator generates a modulation frequency of 40 MHz when the object (base member or muscle fiber) is at rest. If the object moves towards the microscope, this modulation frequency is reduced and if the object moves away from the microscope, the detector receives a frequency higher than 40 MHz, thereby enabling to detect not only the amplitude of movement but also to clearly define the direction of movement of the object.

[097] A processor **111** is connected to the demodulator **110.** The recorded frequency response is stored by the processor, which is the natural frequency of the muscle fiber **106** using peak picking approach. The processor **111** is disposed to compare natural frequency responses across the muscle to indicate a muscular disorder. The processor **111** is also equipped to categorize the myopathies into nemaline and cardiac

based on the natural frequency values.

[098] A display unit **112** is connected to the processor **111** to indicate the measurement of muscular disorder.

[099] Accordingly, the system of the present invention comprises a base member with at least a pair of holders, connected to a vibrator, where the at least pair of holders are disposed to suspend at least a control muscle fiber and at least a blind muscle fiber between the holders, with an intervening space between the top portion of the base member and at least the control and blind muscular fibers. A non-contact probe with a beam emitter and a receiver disposed to direct incident beams to the base member and the control and blind muscle fibers and to receive the backscatter of the incident beams. A vibrometer is connected to the non-contact probe to demodulate the change in frequency and phase of the backscattered incident beams. A processing member connected to the vibrometer and configured to capture the frequency response and the natural frequencies of the movable base member and the muscular fiber and to compare the natural frequencies of the base member with the muscle fiber to select the natural frequencies of the muscular fiber, which are greater than the natural frequency of the base member. The processing member is connected to the display unit and is also configured to compare the natural frequency of the blind muscle fiber with natural frequency of the control muscle fiber to indicate a muscular disorder where the natural frequency of the blind muscular fiber is less than the natural frequency of the control muscle fiber and greater than the natural frequency of the base member.

[0100] The material for the movable base member is preferably a dielectric material, preferably, glass, PDMS, aluminum and tungsten.

[0101] In the system of the present invention, the interspatial distance between the first movable base member and the muscular fiber is in the range of 0.5 to 1 mm.

[0102] The muscular fiber holders as used in the system of the present invention are preferably nanoclippers, tungsten wires, elastic polymers or capillary adhesive holders.

[0103] The non-contact probe as used in the system of the present invention is preferably a laser Doppler vibrometer (LDV) or a position sensing detector.

[0104] It is also within the purview of the system of the present invention to use an arrangement of plurality of muscle fibers, which are disposed between the holders, to indicate muscular disorders.

[0105] Therefore, the method and system of the present invention provides means for indicating muscular disorders in muscular fibers, based on vibration signature of the muscle fibers.

[0106] The system and method of the present invention enables an indication of segregation of myopathic conditions based on the measured natural frequencies of the muscle fibers.

[0107] The system and the method of the present invention provides a robust, cost-effective and a high-speed tool for the indication of muscular disorders in muscle fibers with a minimal sample requirement.

[0108] The system and method of the present invention can indicate very small changes in the natural frequency of muscle fibers in response to the internal structural changes, which may occur due to onset of pathologies.

[0109] The system and method of the present invention can also be used for an *in vitro* indication of a diseased condition of a cell such as cancerous cells, based on vibration signature of the cell.

[0110] It is also understood that the following claims are intended to cover all the generic and specific features of the invention herein described and all statements of the scope of the invention, which as a matter of language might be said to fall there between.

We claim:

1. A method for an *in vitro* indication of muscular disorders, comprising the steps of:

   - suspending a control muscle fiber of an effective length, between holders of a base member and vibrating the base member and the control muscle fiber with a sweeping frequency;

   - directing incident beams with desired wavelengths, from a non-contacting probe to the vibrating base member and to the control muscle fiber and receiving backscatter of the incident beams with a change in frequency and phase;

   - demodulating the change in the frequency and the phase of the backscatter into velocity and displacement, respectively and obtaining frequency responses of the base member and the muscle fiber from the demodulated backscatter;

   - obtaining the natural frequencies of the base member and the control muscle fiber from the frequency responses of the base member and the control muscle fiber, from respective peak amplitudes to select only the natural frequencies of the control muscle fiber;

   - obtaining a natural frequency of a blind muscle fiber by substituting the control muscle fiber with the blind muscle fiber; and

   - comparing the natural frequency of the blind muscle fiber with the natural frequency of the control muscle fiber, to indicate a muscular disorder where the natural frequency of the blind muscular fiber is less than the natural frequency of the control

muscle fiber and greater than the natural frequency of the base member.

2. The method as claimed in claim 1, wherein the control muscle fiber is a healthy muscle fiber.

3. The method as claimed in claim 1, wherein the control and blind muscle samples are from a striated muscle, non-striated muscle and a cardiac muscle.

4. The method as claimed in claim 1, wherein the control muscle fiber and blind muscle fiber are obtained from control and blind muscle biopsy samples through a needle biopsy.

5. The method as claimed in claim 1, wherein the control and blind muscle fibers are suspended between the holders by gripping, capillary adhesion or nanoclipping.

6. The method as claimed in claim 1, wherein the effective length of the suspended control and test muscular fibers is in the range 100-500 microns, preferably in the range of 300-350 microns.

7. The method as claimed in claim 1, wherein the sweeping frequency of the base member and muscle fiber is preferably in the range of 0.5-200 kHz.

8. The method as claimed in claim 1, wherein the wavelength of the incident beams is in the range of 600-630nm.

9. The method as claimed in claim 1, wherein the natural frequencies of the control muscle fiber and the blind muscle fiber are in the range 20-200kHz.

10. The method as claimed in claim 1, wherein the natural frequency of the control muscle fiber is equal to or greater than 110kHz.

11. The method as claimed in claim 1, wherein natural frequencies of plurality of control and blind muscle fibers are measured.

12. The method as claimed in claim 1, wherein at least a mammalian cell is used *in lieu* of the muscle fiber for detection of cell disease.

13. A system for an *in vitro* indication of muscular disorders, the system comprising:

- a base member with at least a pair of holders, connected to a vibrator, where the at least pair of holders are disposed to suspend at least a control muscle fiber and at least a blind muscle fiber between the holders, with an intervening space between the top portion of the base member and at least the control and blind muscular fibers;
- a non-contact probe with a beam emitter and a receiver disposed to direct incident beams to the base member and the control and blind muscle fibers and to receive the backscatter of the incident beams;
- a vibrometer is connected to the non-contact probe to demodulate the change in frequency and phase of the backscattered incident beams;
- a processing member connected to the vibrometer and configured to capture the frequency response and the natural frequencies of the movable base member and the muscular fiber and to compare the natural frequencies of the base member with the muscle fiber to select the natural frequencies of the muscular fiber, which are greater than the natural frequency of the base member; and
- the processing member connected to a display and is also configured to compare the natural frequency of the blind muscle

fiber with natural frequency of the control muscle fiber to indicate a muscular disorder where the natural frequency of the blind muscular fiber is less than the natural frequency of the control muscle fiber and greater than the natural frequency of the base member.

14. The system as claimed in claim 13, wherein the material for the movable base member is a dielectric material, preferably, glass, PDMS, aluminum and tungsten.

15. The system as claimed in claim 13, wherein the interspatial distance between the first movable base member and the muscular fiber is in the range of 0.5 to 1 mm.

16. The system as claimed in claim 13, the muscular fiber holders are nano clippers, tungsten wires, elastic polymers, or capillary adhesive holders.

17. The system as claimed in claim 13, wherein the non-contact probe is a laser Doppler vibrometer or a position sensing detector.

18. The system as claimed in claim 13, wherein a plurality of muscle fibers are disposed between the holders.

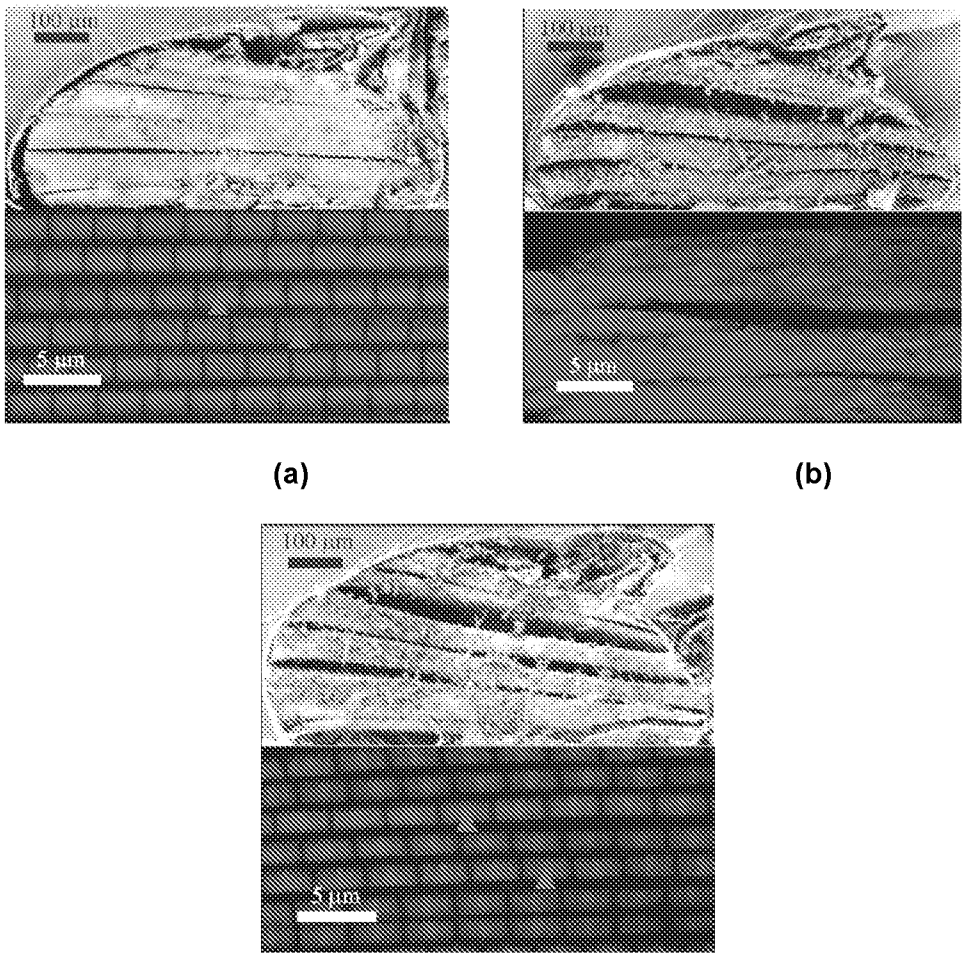


FIG.1 (Prior art)

FIG.1 (Prior art)

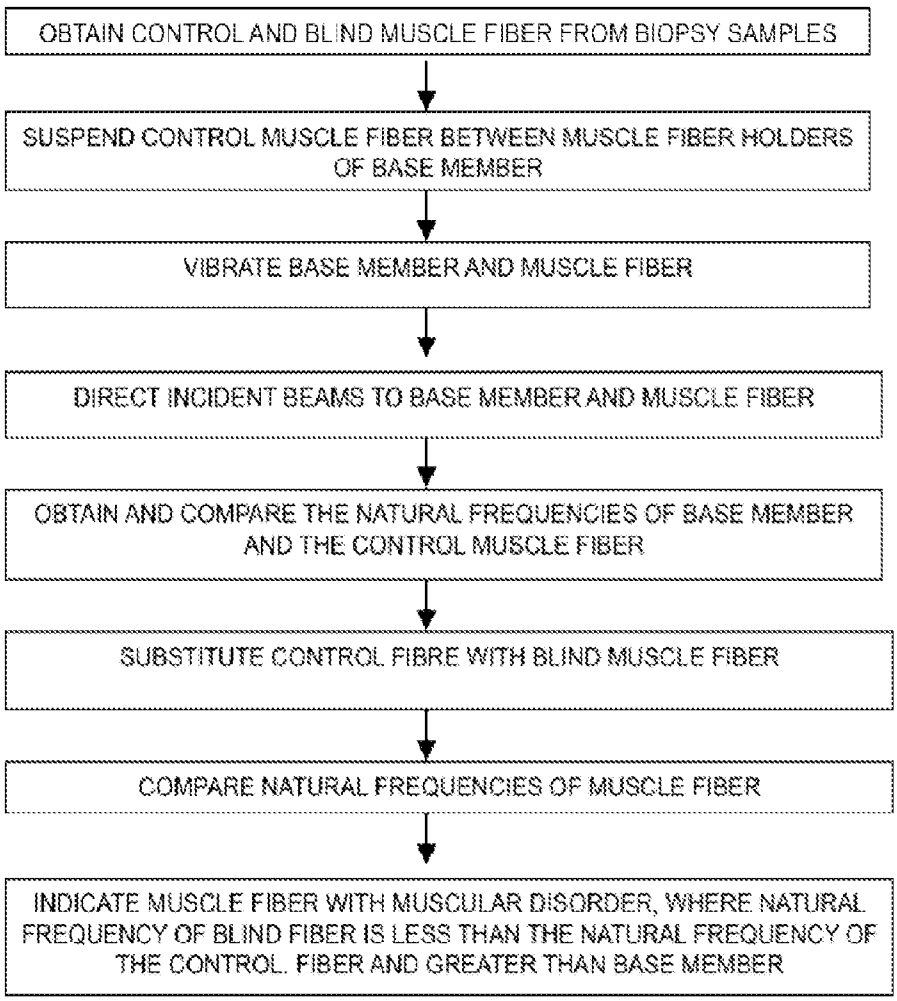


FIG.2

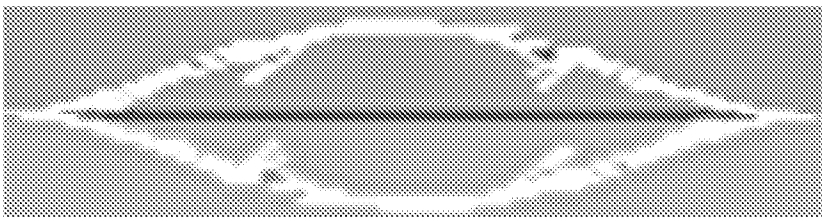
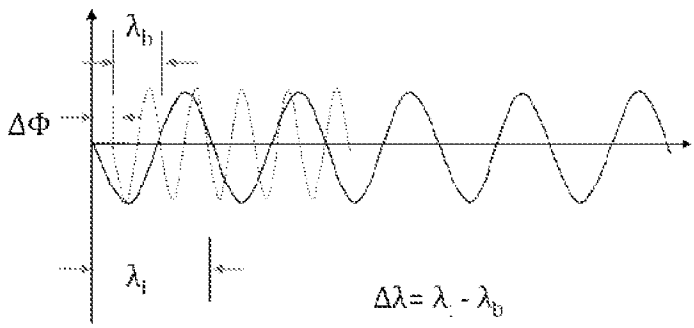
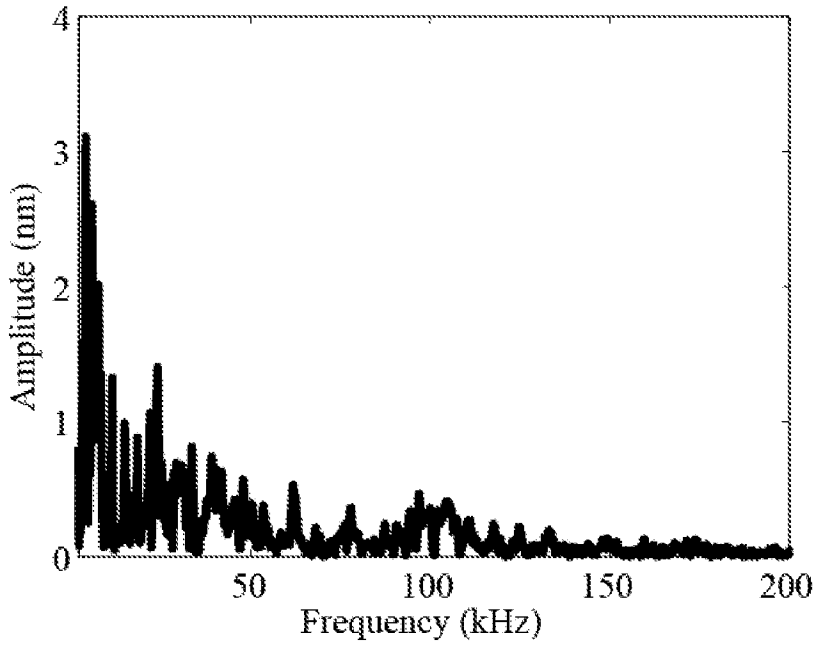


FIG.3

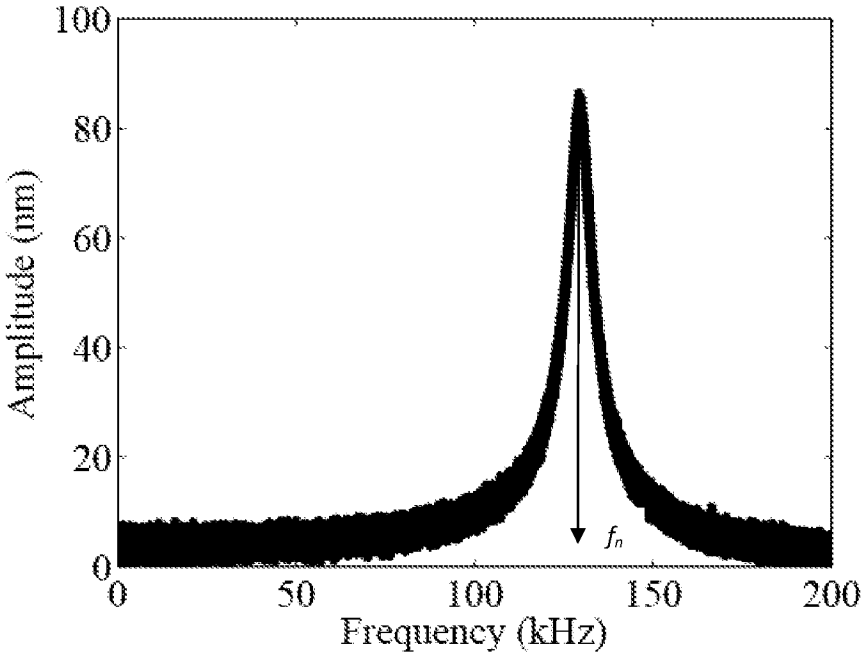


$$\Delta f = \frac{2V}{\lambda} \qquad \Delta\varphi = \frac{4\prod}{\lambda}\Delta Z$$

FIG.4

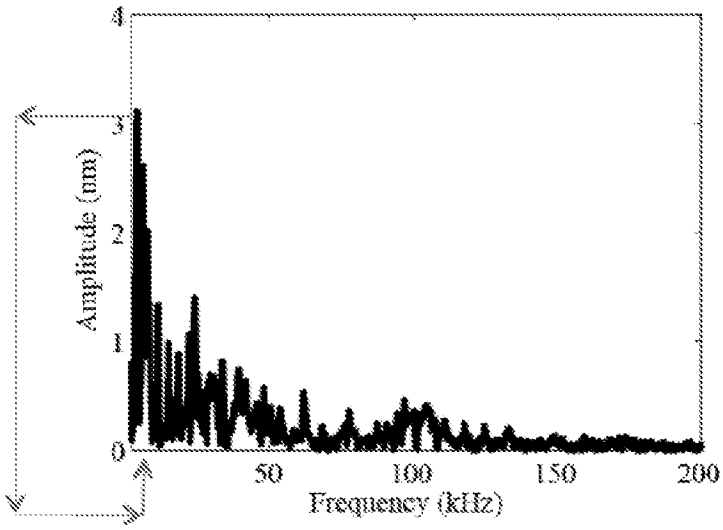


(a)

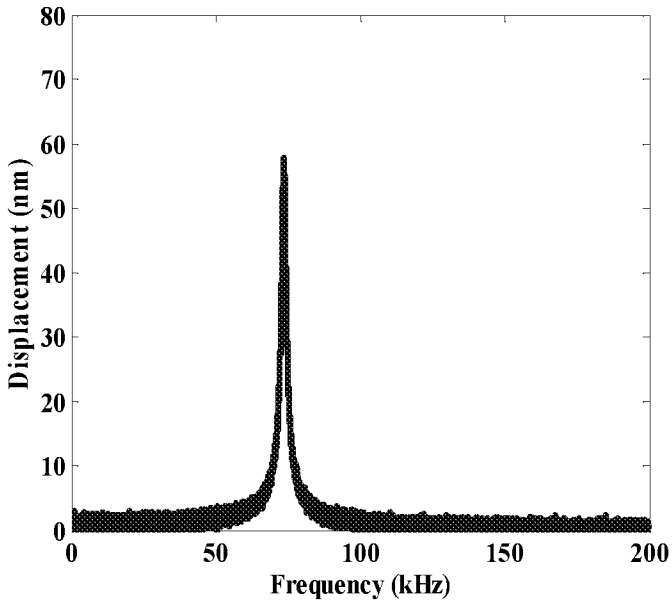


(b)

FIG.5

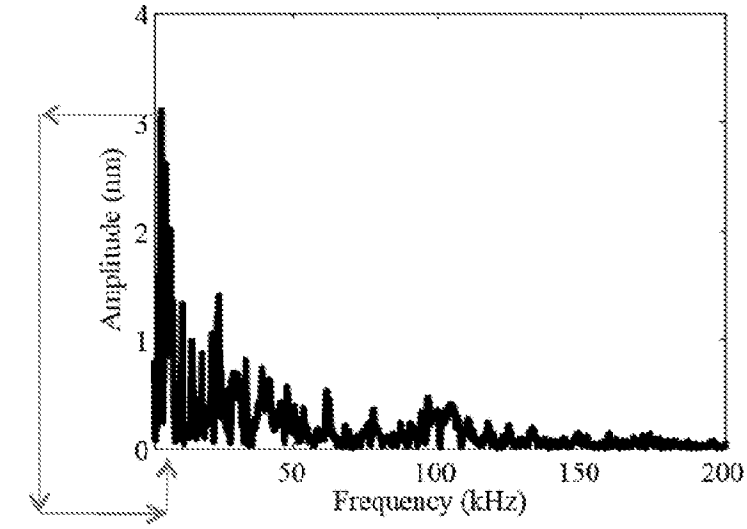

Amplitude (nm)
Frequency (kHz)
0
1
2
3
4
50
100
150
200

(a)


Displacement (nm)
Frequency (kHz)
0
10
20
30
40
50
60
70
80
0
50
100
150
200

(b)

FIG.6


Amplitude (nm)
0
1
2
3
4
50
100
150
200
Frequency (kHz)

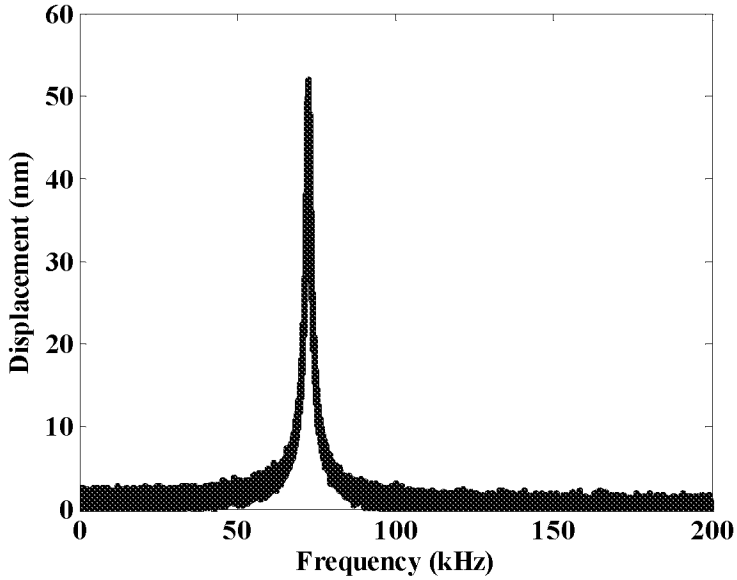

Displacement (nm)
0
10
20
30
40
50
60
0
50
100
150
200
Frequency (kHz)

FIG.7

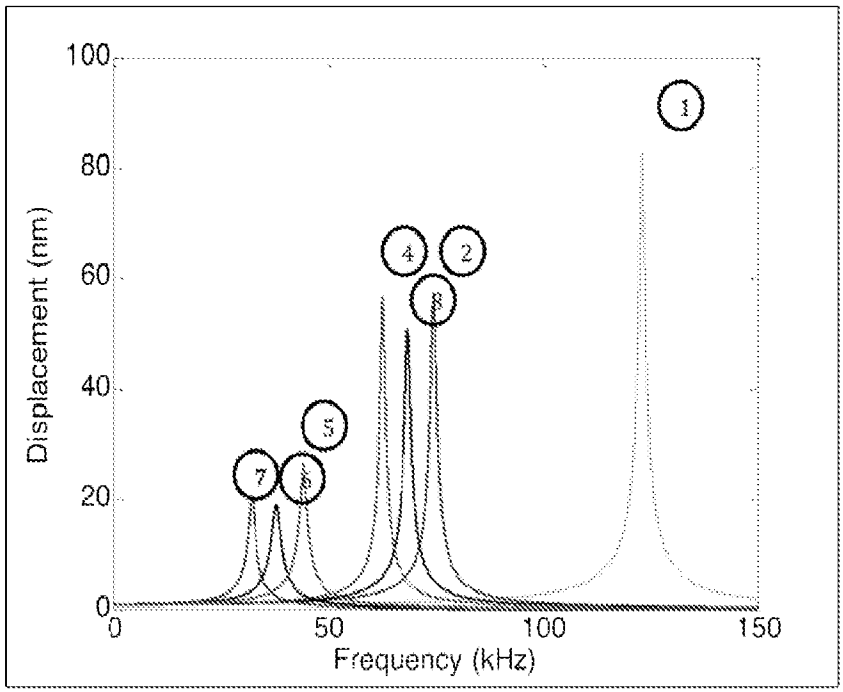

100
80
60
40
20
0
Displacement (nm)
0
50
100
150
Frequency (kHz)
1
2
3
4
5
6
7

FIG.8

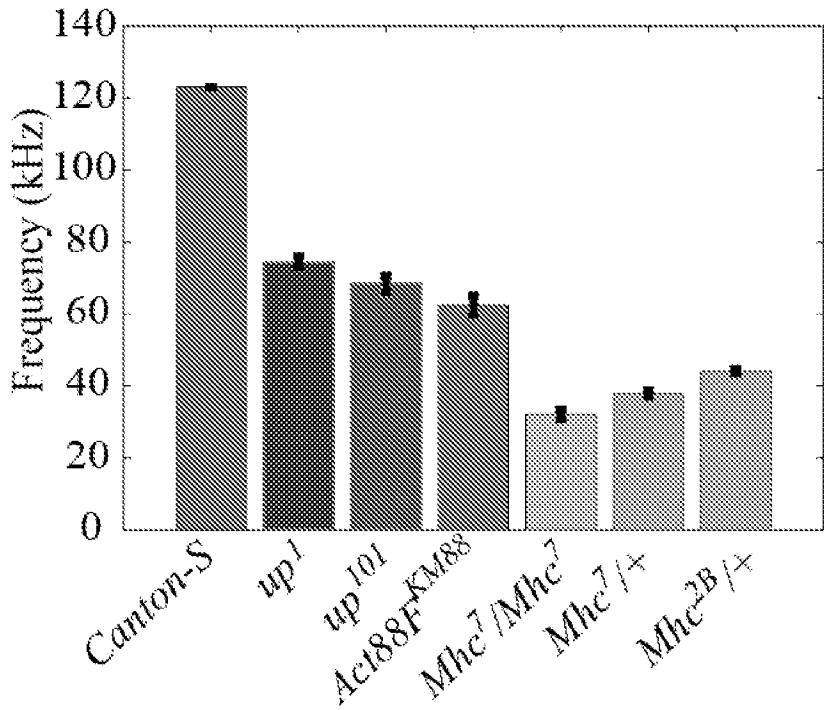

Frequency (kHz)
140
120
100
80
60
40
20
0
Canton-S
up1
up101
Act88FKM88
Mhc7/Mhc7
Mhc7/+
Mhc2B/+

FIG.9

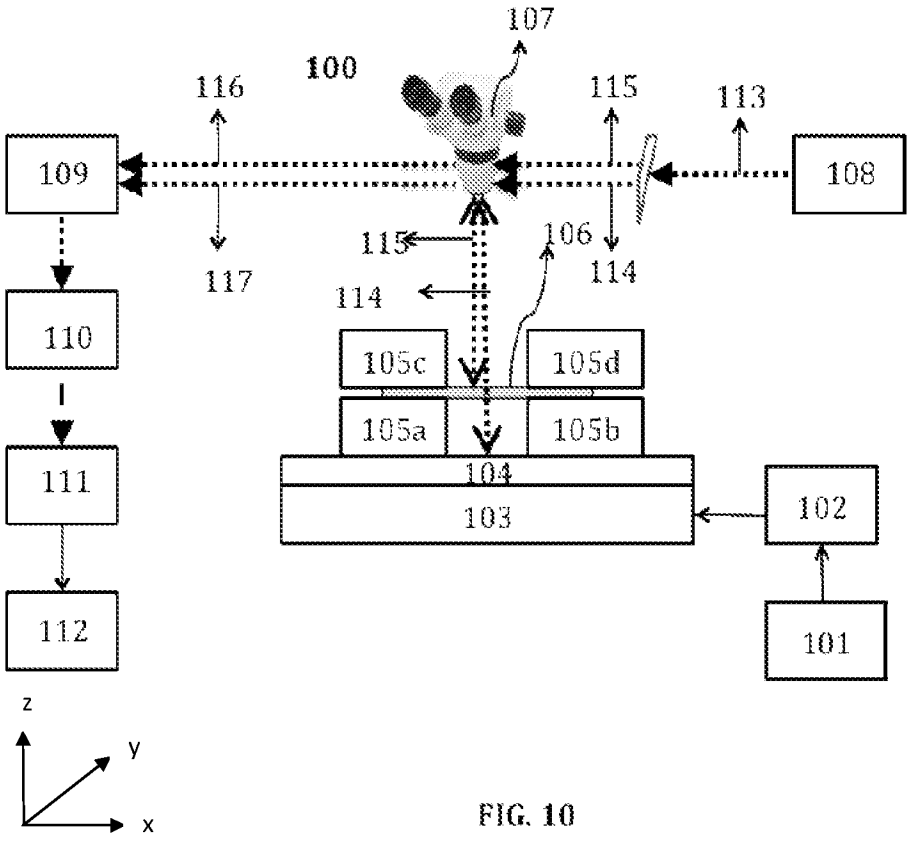

107
100
116
115
113
109
108
115
106
117
114
114
110
105c
105d
105a
105b
111
104
103
102
112
101
z
y
x

FIG. 10

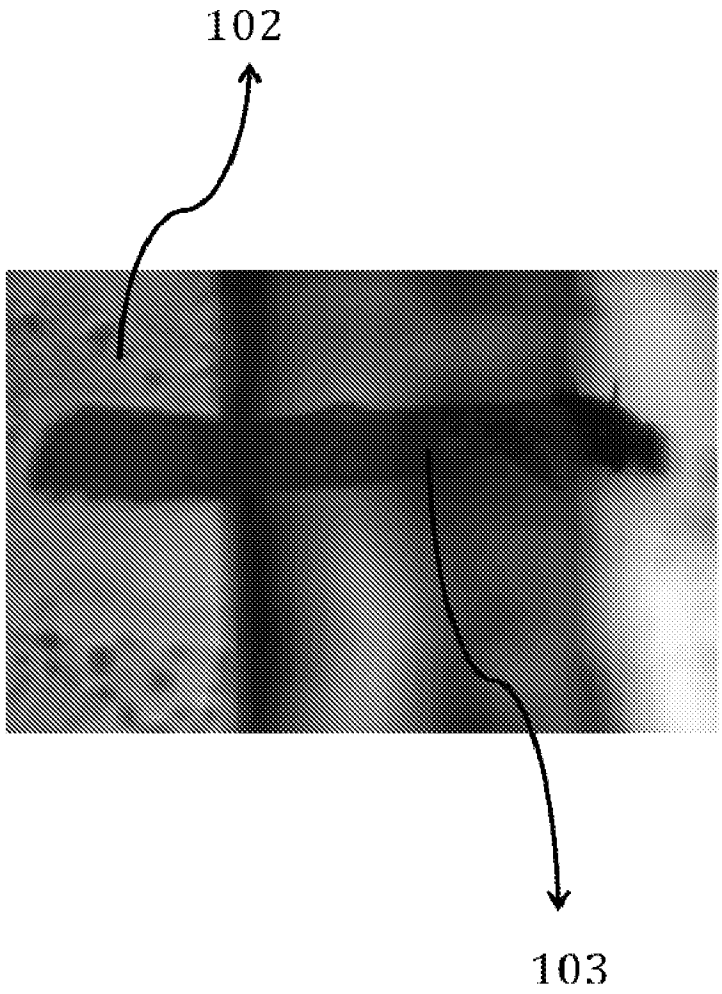


FIG.11

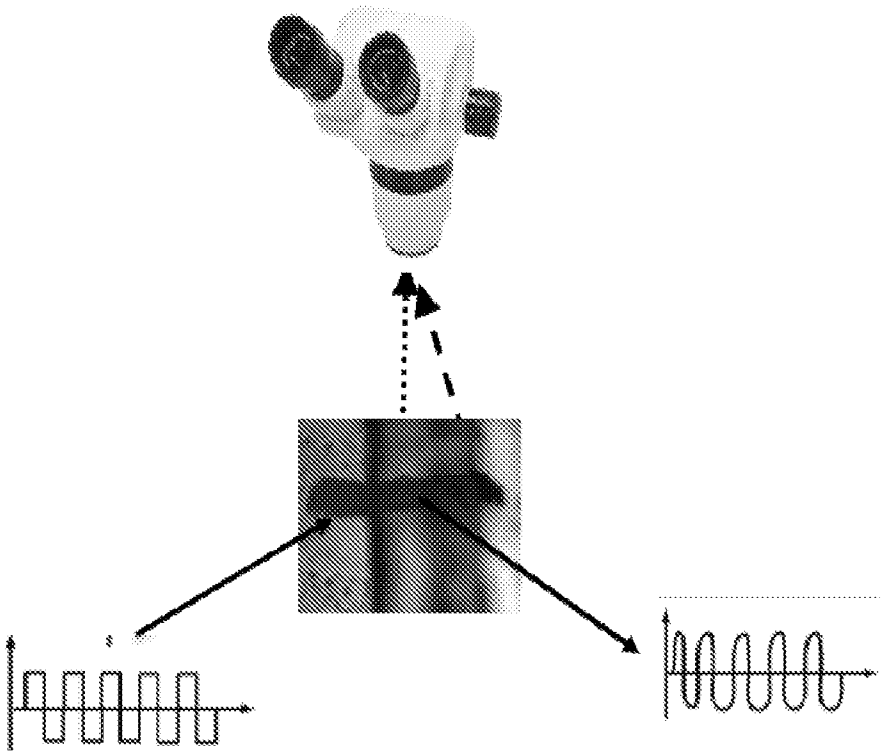


FIG.12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB 16/56424

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01N 21/00 (2017.01)
CPC - G01N 21/00
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)
IPC (8): G01N 21/00 (2017.01);
CPC: G01N 21/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
IPC (8): A61B 5/00, G01J 9/02, G01N 21/00 21/01 21/47 29/036 29/24, G03B 42/06, G01H 9/00 (2017.01);
CPC: A61B 5/00, G01J 9/02, G01N 21/00 21/01 21/47 29/036 29/24, G03B 42/06, G01H 9/00 9/002

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Google (Web, Patents); PatBase; Search Terms Used: muscle, disorder, indication, suspending, fiber, vibration, vibrometer, sweeping, frequency, backscatter, demodulation, beam, natural, comparison, non, contact, probe, laser, Doppler, vitro, clamp, holder, diagnosis, biopsy, processing, emitter, base, member, plate, help, clasp, resonance, LDV

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2006/0225509 A1 (Haupt et al.) 12 October 2006 (12.10.2006), entire document, especially fig 2; para [0005]-[0006], [0021], [0026], [0035], [0041], [0043]	1-17
A	US 2014/032599 A1 (Hickman et al.) 9 October 2014 (09.10.2014), entire document, especially para [0012]	1-17
A	US 2013/0332115 A1 (Pratt et al.) 12 December 2013 (12.12.2013), entire document, especially para fig 2; [0100]-[0101]	1-17
A	"Targeting the Limits of Laser Doppler Vibrometry" (Johansmann et al.) (2005), entire document, especially pg 1, INTRODUCTION, MAIN SECTION, Laser Doppler Vibrometer Principle of Operation	1-17
A	US 6,186,004 B1 (Kaduchak et al.) 13 February 2001 (13.02.2001), entire document, especially fig 3a; col 8, ln 66 - col 9, ln 31	13-17
A	US 8,210,044 B1 (Maleki et al.) 3 July 2012 (03.07.2012), entire document	1-17
A	US 2004/0252587 A1 (Melese et al.) 16 December 2004 (16.12.2004), entire document	1-17

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"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)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"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
"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
"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
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 February 2017	27 FEB 2017
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774