Optimization of an Optical Magnetic Twisting Cytometry system for the study of cell mechanics

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Abstract— In this work the development and optimization of an Optical Magnetic Twisting Cytometry (OMTC) system specifically designed to study the mechanical properties of living cells is presented. The system allows the precise synchronization of the magnetic field signal, used to apply a known stress on beads bound to the cell surface, and the image acquisition to track the bead movement. We present a detailed procedure to accurately validate the system in order to standardize its calibration procedure and to obtain quantitative and reproducible data. First results obtained on human airway smooth muscle cells after consecutive challenges of a constrictive and relaxant stimuli support the accuracy and sensitivity of the system.

I. INTRODUCTION

High precision measurements of viscoelastic parameters of cells are of great practical value for the quantification of the effect of drugs, mutations, or diseases on the cell structure. Viscoelastic measurement techniques must allow local tests on micrometer-nanometer scales and reproducible and reliable measurements [1]. Techniques based on magnetic field fulfill these requirements; an external magnetic field is used to apply stress on ligand-coated magnetic microbeads bound to membrane receptors; mechanical properties of the cell can be derived from measurement of resulting bead rotation. Even if the measurement of the decay of the magnetic field [2] and the cell creep answer to stimulation led to interesting implementations [1], [3] and results [3-5], a common method to separate elastic behavior from dissipative for any material is to measure responses to oscillatory loads [6] and to measure the mechanical properties in terms of the complex elastic modulus. This led Maksym et al. 2000 to implement a new technique based on twisting ligand-coated beads on the surface of a living cell, known as Magnetic Twisting Cytometry (MTC).

MTC with optical detection of the bead movement [7], known as Optical Magnetic Twisting Cytometry (OMTC) reduces the intrinsic variability in the results obtained with the previous systems [8], since it allows to discharge unbound beads and increases the frequency range, up to 1kHz. OMTC has a good time and frequency resolution, allows to probe a high number of cells simultaneously, and beads can be functionalized. All these features made it a suitable technique to measure changes in cell mechanics due to biochemical stimulations.

At the moment all the OMTC systems in use are self-made prototypes, since it is not a commercially available technique. Moreover, there are no detailed procedures to design OMTC systems and, most important, a standard protocol to calibrate and evaluate their accuracy in order to make OMTC measurements reproducible between different prototypes and centres.

We built an OMTC system optimizing its components to simplify and improve the synchronization between magnetic signal generation and image acquisition. We also made an in-depth validation of each component of the system, in order to point out which are the real advantages of this technique and which are its intrinsic limits that have to be taken in account for applications. The device has been finally tested by evaluating the response of human airway smooth muscle cells subjected to consecutive constrictive and relaxant stimuli.

II. METHODS

A. System development

The system was designed to fit on the Leica DMIRB inverted microscope modified stage, it consists of two pairs of orthogonal coils, the magnetization and twisting coils, driven by a voltage controlled current source and a magnetizer circuit. The microscope is equipped with a progressive scan camera (C4742-95-12ER, Hamamatsu- Photonic K.K, Japan) connected to a camera controller. System architecture was designed in order to completely separate it from the computer to guarantee the synchronization of the camera signal and the magnetic field; this cannot be assured by a computer driven controller.

Fig. 1. System architecture.

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The software controls the operations of the entire system, using high level functions to communicate with the frame grabber and camera controller to manage image acquisition, and uses image analysis algorithms to process the acquired data. It also communicates with the signal generation board, specifying the frequency and amplitude of the sinusoidal wave signal used to drive the twisting coils through the voltage controlled current source. The signal generation board is responsible for generating the requested signals and synchronizing image acquisition by means of a trigger.

**Magnetic actuator.** The OMTC magnetic actuator consists of two orthogonal pairs of coaxial coils designed to reduce the overheating in the stage and to guarantee a constant gain factor at all frequencies. The magnetizing coils are oriented in the vertical direction and receive a large (30A) current impulse (<20ms) resulting from the discharge of a 60μF capacitor. This generates an horizontal magnetic field of 1300G between the coils and it is sufficient to permanently magnetize the ferromagnetic beads in the sample [2]. The stage is heated and a thermostat is responsible for maintaining a constant temperature (30°C-60°C). This is necessary to eliminate any thermal effects. The twisting coils are oriented in the horizontal direction and are responsible for producing a weak vertical oscillatory magnetic field (<92G) over the sample area. The coils are mechanically uncoupled from the stage and set up to carry the same current in the same direction at all times. The twisting coils are driven by a voltage controlled current source characterized by a sensitivity of 1A/V and a maximum input of 2.1A, and equipped with a filter to compensate the phase lag over 100Hz.

**Signal generation board.** We chose a microcontroller based dedicated solution to satisfy the timing constraints of the system. Additional advantages are its low cost and ease of implementation. The board produces the sinusoidal analog voltage signal used to generate the oscillatory magnetic field in the sample (0.01Hz-1000Hz, +/- 2.1V) and the digital trigger signal used by the camera to acquire images at specific time instants. The sinusoidal signal is generated by a sequence of 100 values per period; these are rescaled according to the amplitude of the desired signal and sequentially sent to the DAC which converts the digital information received into its analog counterpart. The software responsible for controlling all the operations of the system was designed as a single application and developed in C++. It manages the communication between the frame grabber and the camera, allows to set the hardware and software parameters of the acquisition and the image analysis.

**Image acquisition and analysis.** The microscope was placed on a vibration isolation table (LW 3036B-opt, Newport corporation, Irvine, CA.) Images were acquired with a high quality monochrome area scan digital camera with square pixels of 6.45μm. The interline-transfer architecture allows very short exposure times (138.75μs) and eliminates the need for a mechanical shutter. Camera operation is managed by a dedicated controller which is also responsible for transferring the image data from camera to framegrabber.

A real limit of the system is the frame transfer time, in fact when the sample rate is higher than the frame transfer rate it is not possible to follow the bead movement. A possible solution is an heterodyne image acquisition [7] but this can lead to long acquisition time. To reduce the acquisition time a new method for image acquisition was implemented. The software calculates the maximum number of samples per period allowed by the frame transfer time, the minimum number of period, n, to satisfy the requested frames, m, per period, and the proper camera trigger. The acquisition time is reduced by a factor equal to (m-n)/m.

The image analysis is divided into two main tasks, bead localization and bead tracking. The first is responsible for identifying all valid beads present in the frame, while the second follows the movement of the bead from one frame to the next and it was implemented as already published in a previous work [7].

**B. System validation**

As first step of system validation we tested the ability of the electronic board to synchronize the signal for the field generation and the trigger for image acquisition, and the ability to do it every time a single measurement parameter is changed. We proceeded with the evaluation of distortions of the generated magnetic field both in static and dynamic conditions caused by ferromagnetic material. Then we characterized the system in terms of gain and phase between 0.01 and 1000Hz to correctly calculate the specific torque on the beads. Finally we tested the system simulating the beads movement with a piezoelectric translator (P-611.3S, Physik Instrument, Lederhose, Germany).

**Signal synchronization.** The microcontroller was instructed to generate a set of signals spanning the 0.01-1000Hz frequency range. For each of these, both the wave and corresponding trigger signals were acquired with a 12-bit National Instruments DAQCard-6062E. The exact wave frequencies and trigger periods were calculated from the values set in the microcontroller timer registers. Then a set of signals subjected to some predefined parameter changes, were generated by the microcontroller and acquired by the National Instruments DAQCard-6062E. The acquisition rate in both protocols was chosen to sample the highest frequency wave 100 times per period, the number of frame per period was set to 16 in all cases, and the minimum time between two consecutive active edges was set to 0.5s.

**Influence of ferromagnetic material.** Coils were fed with constant voltage and the magnetic field without objective and with both the 10 X and 20 X objectives was measured. Then a sample of beads was immobilized on a petri dish with a styrene-based fiberglass resin, the sample was placed on the OMTC microscope stage and a twisting magnetic field of amplitude 90G was applied (0.01-1000Hz). The tracking software was used to record the bead displacement and the Fourier analysis was performed on the signals.

**Magnetic field measurement and system transfer function.** Coils were fed with a constant voltage between +/-36V to
generate magnetic field at different intensity. A Hall effect analog sensor (SS941A, Honeywell International Inc., US) with high sensitivity (25mV/G) and a range between +/100G was used to measured the field. The Hall sensor was positioned on the microscope stage. Both the current flowing in the coils and the output voltage of the sensor were simultaneously acquired by a National Instruments DAQCard-6062E. The expected magnetic field was calculated according to the geometrical parameters of coils and was compared with the actual measured values. Subsequently, a twisting magnetic field was generated by using the same set up used for OMTC experiments on cells. The output voltage from the DAC of the signal generation board and the output of the Hall sensor were acquired with a National Instruments DAQCard-6062E, and the transfer function of the system was estimated in terms of gain and phase with a Fourier analysis.

**System test.** The whole system was tested by producing a known displacement oscillation, 0.2Hz, with a piezoelectric translator. We simulated a bead displacement between 60nm and 4µm as the average of bead movement in standard OMTC experiment.

**C. In vivo test**

**Materials.** Human airway smooth muscle cells (HASMC) and human smooth muscle grown medium (HsmGM) were purchased from Lonza (Milan, Italy). The synthetic peptide containing arginine-glycine-aspartic acid (RGD) was obtained from the American Peptide Company (Sannyvale, CA). The ferromagnetic beads were provided by Dr J. Fredberg unit (Harvard Scholl of public health, Boston, MA). Unless otherwise marked, all other compounds were purchased from Sigma (Milan, Italy).

**Cell culture.** HASMC were grown until confluence in HsmGM medium in humidified and controlled air (5%CO₂) at 37°C. HASMC (3rd-7th passage), were then plated (20×10³ cells/well, n=3) on collagen I-coated (500ng/cm²) well plates (6.4mm diameter, Costar, Corning Life Sciences, USA) and serum was removed 24h before the experiment.

**Cell-Beads preparation.** Ferromagnetic beads (Fe₃O₄, 4.5µm diameter) were coated with a synthetic peptide containing the RGD sequence by an overnight incubation at 4°C. Then beads were washed, suspended in serum-free medium, and 20,000 beads were added to each well. After 20min incubation at 37°C to allow the bead binding to the cell surface, the well was washed twice with serum-free medium to remove unbound beads.

**Protocol.** The beads were horizontally magnetized and then vertically twisted in a 20G sinusoidal magnetic field varying the frequencies between 1-20Hz. Measurements were repeated at baseline, after 10min incubation with charbacol (Che) (10⁻⁴M) and after further 10min incubation with isoproterenol (Iso) (10⁻⁵M).

**Data Analysis.** Bead trajectory was calculated and drift was eliminated with a moving average filter, then the high frequencies were cut with a low pass filter. Only beads with average displacement lower than 1µm and coherence between the specific torque and the displacement higher than 0.80 were considered valid. The elastic complex modulus was calculated with the Fourier analysis, it is defined at each radiant frequency as \( G^* (\omega) = T^* (\omega) / d^* (\omega) \), where \( T^* \) and \( d^* \) are the Fourier coefficients of the complex specific torque (torque per unit bead volume) and the complex bead displacement. As defined here, \( G^* \) has units of Pa/µm. This is equivalent to computing the components of bead displacement both in phase and out of phase with the applied specific torque, \( G (\omega) = g (\omega) + i g (\omega) \), where the real part is called elastic modulus, the imaginary part, loss modulus and the ratio \( g''/g' \) is called histeresivity.

### III. RESULTS

**Signal synchronization.** The trigger signal is adequately synchronized with the wave signal at all tested frequencies and the first trigger active edge correctly fires on the wave’s rising edge, when the voltage value is equal to 0V. The board adequately handles both frequency and amplitude transitions resynchronizing the trigger signal with the wave each time the parameters were changed.

**Influence of ferromagnetic material.** Fig.2 shows that in static situation there is no significant difference between the measured magnetic field with or without the microscope objective, only the 10x condition is reported; the 20x had similar results.

![Comparison between magnetic field with and without 10x objective.](image)

In dynamic situation the Fourier analysis highlights that there is no distinct peak appearing at the corresponding applied magnetic field frequency. This confirms that the forces experienced by the objective during an OMTC experiment are negligible and do not influence the measurements.

![Power spectrum of a bead displacement subjected to a twisting field of an immobilized bead (a) and of a bead bound on the cell surface (b).](image)

**Magnetic field measurement and system transfer function.** As shown in Fig.4 there is a linear relationship between the theoretical magnetic field and the measured magnetic field.
The computation of the transfer function of the system shows a small attenuation at high frequencies to take into account during data analysis and a null and constant phase at all frequencies.

A rank test for non normally distributed population was performed. A significant increase in both real and imaginary part of the complex elastic modulus was observed at all frequencies ($P<0.047$) after Chc incubation. After the subsequent Iso incubation both the real and the imaginary part of the complex elastic modulus returned to basal conditions and there is no statistically significant difference ($P>0.32$) at all frequencies.

**System test.** The results in Fig.6 show a good agreement between the theoretical displacement and the displacement that the system was able to detect.

**In vivo test.** A rank test for non normally distributed population was performed. A significant increase in both real and imaginary part of the complex elastic modulus was observed at all frequencies ($P<0.047$) after Chc incubation. After the subsequent Iso incubation both the real and the imaginary part of the complex elastic modulus returned to basal conditions and there is no statistically significant difference ($P>0.32$) at all frequencies.

The developed system is able to generate known low forces and to detect bead displacements in a wide range of frequencies. A detailed protocol to characterize and validate the system has been defined and it can be proposed as standard procedure to guarantee reproducible data between different laboratories. Finally, the in vivo test confirmed the sensitivity of the device showing that Chc induces a contraction and Iso a relaxation [4] and shows that β-adrenoceptor stimulation is able to reverse the mechanical changes induced by muscarinic stimulus in HASMC.

**IV. CONCLUSION**

We thank Andrea Bernasconi for his help in developing the OMTC set up.

**REFERENCES**


