

A COMPUTER-CONTROLLED MICROSCOPE STAGE INCUBATION SYSTEM FOR TIME-LAPSE IMAGING OF LIVING CELLS. APPLICATIONS TO CELL TRACKING

Vincenzo Sibillo, Sergio Caserta, Luigi Sabetta, Marino Simeone and Stefano Guido
Dipartimento di Ingegneria chimica
Università di Napoli "Federico II"
P.le V. Tecchio 80, 80125 Napoli, Italy
E-mail: steguido@unina.it

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In this work, we present a novel microscope stage incubation system allowing extended observations of living cells under controlled environmental conditions. The system is based on a water-jacketed chamber provided with optical windows for sample observation. Depending on system configuration, both single and multi-well Petri dishes can be accommodated in the chamber. Water temperature is set by a thermostated bath endowed with a pump for external water circulation and a serial port for remote operation through a PC. A dedicated PID software has been developed to control sample temperature based on the readings from a fine gauge thermocouple immersed in a well (filled with water) inside the chamber. The system is hence able to adapt to variations in room conditions by properly adjusting the temperature of the water bath. Temperature uniformity within the chamber has been tested by temperature sensitive liquid crystals. As far as environmental conditions are concerned, both humidity and CO₂ levels are set to appropriate values to ensure cell viability and proliferation. To this purpose, CO₂ from a tank and air from a compressed air line are mixed in the desired amounts by gas flow meters. The so obtained gas mixture is then circulated in the water bath for saturation and pre-heating before entering the chamber. Time-lapse studies have confirmed cell viability and proliferation for several days, with small differences with respect to duplicate samples grown in CO₂ bench incubators.

The above described microscope incubation system has been applied to investigate cell migration, both on 2D and 3D substrata. In the latter case, cells were suspended in a collagen solution with the usual supplements for cell culture. Upon incubation at physiological conditions, the solution turns into a soft gel due to the self-assembly of collagen molecules in a 3D network of fibrils. Such "tissue-equivalent" reconstituted collagen gels are a popular substratum to investigate cell motility, with a special emphasis on directional modes of cell migration, such as chemotaxis [1] and contact guidance [2]. In our approach, we integrated our incubation system in a video microscopy workstation provided with motorized stages for automated sample positioning and focussing. An especially designed software was used for iterative sample scanning and optical sectioning. Image analysis techniques were used to extract cell contours from the archived images and calculate motility parameters of the cell population investigated. Some results of cell migration in anisotropic environments will be also presented.

[1] W.J. Rosoff; J.S. Urbach; M.A. Esrick; R.G. McAllister; L.J. Richards, and G.J. Goodhill, "A new chemotaxis assay shows the extreme sensitivity of axons to molecular gradients," *Nat. Neurosci.*, **7**, 678-682 (2004).

[2] S. Guido and R. T. Tranquillo, "A methodology for the systematic and quantitative study of cell contact guidance in oriented collagen gels. Correlation of fibroblast orientation and gel birefringence," *J. Cell Sci.*, **105**, 317-331 (1993).