REMOTE MONITORING
OF
CELLULAR NETWORK ASSEMBLY AND FUNCTION

Vijay Noronha[2], Can Evren Yarman[2] , J. Yasha Kresh[1], Banu Onaral[2]

[1]Depts. of Cardiothoracic Surgery / Medicine, Medical College of Pennsylvania/Hahnemann University, PA, USA
[2]School of Biomedical Engineering, Science and Health Systems, Drexel University, PA, USA

Abstract- **A video microscopy based collaboratory has been developed to study cellular network dynamics, in particular, to monitor live-cell spatio-temporal organization in real-time. The aim is to investigate the effects of intercellular communication on tissue genesis, differentiation and cell survival.**

The platform enables multiple researchers to remotely access a digital microscopy system consisting of very high-resolution CCD-imaging technology coupled with real-time (~1Gbps) data transfer throughput. Remote control and image acquisition facilitates collaboration between cellular biologists, tissue engineers and computational scientists studying complex cellular organization dynamics and assembly.

The remote control microscope is connected to a local 'Server' such that a 'Client' using any web browser that supports Java can access it. The 'Server' software is written in Java 2 (jdk 1.3). Any Internet user can control the microscope and interact with other users who are on-line or are directly connected. This interactive environment does not impose any hardware or software limitations on the 'Clients'.

A remote user can control the movement of the stage in X-Y axes, control focus (Z axis), change magnification, change excitation and light emission filters and their corresponding shutters, acquire topographic and fluorescent images from the microscope and process theses images.

The tele-microscopy based environment serves as a focal point for investigators with different expertise to collaborate synergistically on projects that require multi-disciplinary approaches.

Keywords - **Telemicroscopy, Collaboratory, Biocomplexity, Live-Cell Observatory**

I. INTRODUCTION

The 'Cellular Network Dynamics' project aims to monitor cellular networks in real-time in order to gain insight into their biological organization. The specific aims are: (i) to study the live-cell spatio-temporal organization, the role of intercellular communication and its effects on cell culture 'function' and 'survival'; (ii) to develop computational models of cellular network signaling and adaptability. A microscope-based live video system (cellular observatory) is implemented to enable tissue visualization, image analysis and cellular network modeling.

This paper describes the implementation of a distributed high-resolution digital microscopy system for visualizing cellular network assembly and function. The "Telemic" remote-control microscopy system [1,2] enables a shared (client-server mode) observatory (Fig. 1) to study the evolution and communication in cellular networks.

Cellular Network: Interaction and Pattern Formation

Networks are ubiquitous in the biological world. Multi-cellular organisms develop by transforming a collection of undifferentiated cells into intricately organized groups of cells that coalesce to form functional structures. This process known as pattern formation is responsible for structural differentiation, including rhythm formation in cardiac tissue. The ability to dynamically detect cellular and sub-cellular network dependent events is a first step in developing models of cellular organization. Video monitoring of cellular activity in tissue culture using immuno-fluorescence localization of inter- and intracellular integrity (gap junctions, integrins, actin fibers) makes possible studies of cellular network dynamics (signaling complexity, self-organization). As the numbers of attachment points (nodes) in these cellular networks increase and the connectivity becomes more efficient, one would expect the emergence of global, self-organized, coherent tissue structures with new and complex phenotype expression (i.e., network adaptation and stress tolerance). The common problem in many highly coupled systems is how the network structure (e.g. neurons; societies and Internet) facilitates and constrains its own behavior and/or mediates the propagation of stress/failure [3].

Cellular Observatory

Existing methods monitor singular cellular / sub-cellular events and or produce only a static 'snap-shot' of a specific moment of a dynamic process of interest. Conventional 'fixed-cell' assays are inherently deficient in elaborating the dynamic links between the inter- and intracellular processes that define cellular homeostasis. In contrast, simultaneous tracking of a set (e.g. three or more) of cell specific functions in each of the 'units' of the cellular assembly can help unravel some of the critical links that are involved in the dynamic re-organization of cellular network behavior.

The specific methodological approach under development in our labatory monitors cellular organization by binding fluorescent markers to specific structures within the living cell and recording real-time images of cellular culture activity, spanning many hours of observation. The use of multiple fluorescent markers help inter-relate cell function (e.g. cytoskeletal meshwork, gap junction distribution) to the functional inter-connectivity of the cellular network, as well to its viability. The acquired images form the basis for the time-lapse mapping of processes and modeling of the emergent intercellular communication patterns. The sequential frames (pattern formation) are used as a baseline to study the role of spatio-temporal organization in the

Report Documentation Page

Report Date	Report Type	Dates Covered (from... to)
25OCT2001	N/A	-

Title and Subtitle	Contract Number
Remote Monitoring of Cellular Network Assembly and Function	Grant Number
	Program Element Number

Author(s)	Project Number
	Task Number
	Work Unit Number

Performing Organization Name(s) and Address(es)	Performing Organization Report Number
Depts. of Cardiothoracic Surgery / Medicine, Medical College of Pennsylvania/Hahnemann University, PA	

Sponsoring/Monitoring Agency Name(s) and Address(es)	Sponsor/Monitor's Acronym(s)
US Army Research, Development & Standardization Group (UK) PSC 802 Box 15 FPO AE 09499-1500	Sponsor/Monitor's Report Number(s)

Distribution/Availability Statement
Approved for public release, distribution unlimited

Supplementary Notes
Papers from the 23rd Annual International Conference of the IEEE Engineering in Medicine and Biology Society, October 25-28, 2001, held in Istanbul, Turkey. See also ADM001351 for entire conference on cd-rom., The original document contains color images.

Abstract

Subject Terms

Report Classification	Classification of this page
unclassified	unclassified

Classification of Abstract	Limitation of Abstract
unclassified	UU

Number of Pages
4

adaptation of the formed cellular network to the imposed environmental changes.

The distinguishing features of the live-cell telemicroscopy system is its modularity and universal applicability, and in particular, its ability to remotely acquire and control fluorescent and brightfield images in real-time, spanning hours to days of observation.

In addition, the concept of a distributed 'collaboratory' i.e., a multidisciplinary environment without walls ("virtual center"), assembled and constituted by the research areas and expertise of its members is exemplified by this initiative. A major rationale advanced for this approach is that "It is easier to move information around than the requisite technology or people."
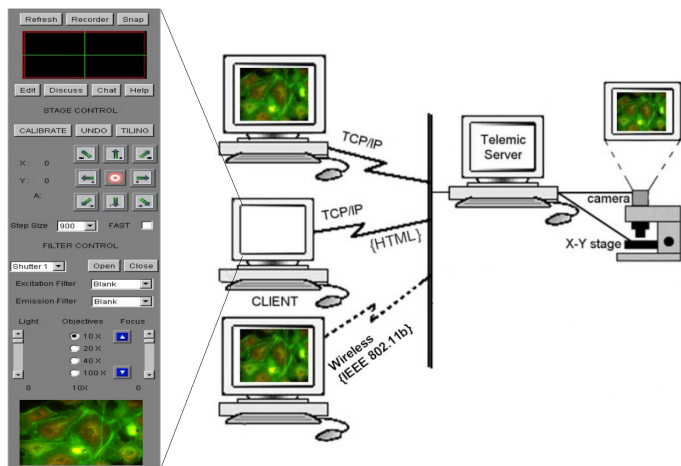

Fig 1. *Telemicroscopy (Client-Server Architecture)*

II. METHODOLOGY

The software for remote control microscopy was initially developed utilizing a very high-speed optical link with 1 Gbps IP infrastructure (gigabit per second Ethernet + 2.5 Gbps SONET), ultra-high capacity router and ultra-high speed non-linear optical regeneration, between Drexel main campus and MCP-Hahnemann University for controlling the Telemic microscope and associate components:

1) Microscope (Nikon Diaphot 300): The Diaphot 300 is a research level inverted optical microscope with multiple optical output ports and epi-fluorescence capability

2) Digital Controller (Ludl MAC 5000 with RS232 and USB communication): This is a high performance modular control system designed to automate and remote control the microscopy functions (X-Y and Z movement, Filter and shutter control). This module automates the control of:

a) <X-Y Movements>: This module controls the movement of the stage in the X-Y plane and has the following features:
- Incremental (0.2 micron / step) movements in the any X-Y directions.
- Ability to return back to the set origin from any given position
- Calibrate the stage by prompting its movement to the respective X-Y limits.
- Ability to move the stage to any specified X-Y coordinates, within the available field of excursion. This functionality is graphically emulated on the screen that maps the area available for stage movement and then provides cursors to mark the x-y coordinates.
- Ability to undo stage movements.
- Ability to control the speed of the stepper motors controllable by the 'Client' -the stage can be directed to move at a prescribed speed.

b) <Focus Control> (Z axis): The focus control system provides reproducible, high-resolution automated control of the microscope focus using a micro-stepping motor 1 (0.02 micron/step -step size.)

c) <Filters Wheels>: This module allows the changing of filters at Excitation (illumination end) and at the Emission (camera end) with 30 msecs between switched positions.

d) <Shutter Control>: This module controls the opening and closing of shutters of the excitation and emission filter wheels. Closing the shutters between image acquisitions prevents light bleaching (e.g. fluorescent marker) of cell culture during prolonged observation (time-lapsed motion studies).

3) Digital Camera:
Camera 1) Roper CoolSnapFX (12-bit dynamic range @ 1300 x 1030) A cooled (-30°C) digital CCD camera designed for fluorescence imaging
Camera 2) Sony DFW-V300 (30 fps @ (640 x 480) VGA) is a digital video camera which uses the "IEEE-1394" (Firewire) computer interface.

The remote control microscope is connected to a local 'Server', which can be accessed by a 'Client' using any web browser that supports Java. The 'Server' software is written in Java 2 (jdk 1.3). Any Internet user can control the microscope and interact with other users who are on-line or are directly connected. This interactive environment does not impose any hardware or software limitations on the 'Clients'.

The Telemicroscopy 'Server' runs on a standard Windows NT workstation with a fixed IP address. The 'Server'

automates functions such as image acquisition and stage, focus, filter and shutter control.

The image acquisition module interfaces with the camera and captures images at a specified rate (e.g. 10 fps) and writes them to disk in jpeg (compressed) format.

The 'Server' also provides a <Chat> function, which allows on-line users to converse with other Telemic subscribers.

A <Discuss> mode was implemented to enhance the collaboration functionality to enable users to exchange information about a captured image among each other. In addition, a dynamic pointer is invoked such that it can be "surrendered" to any one of the users in the <Discuss> Mode. If a user moves the arrow around and repositions it, then a corresponding arrow on all of the active screens will be repositioned accordingly.

The Telemicroscopy 'Client' is a Java applet (Fig. 2) that emulates the functions of a microscope and can be viewed in any java-enabled browser. The java 'Client' provides an interface to facilitate the following functions – <X-Y stage movement control> <filter wheel> and <shutter control>, <focus control>, <image update>, <image snapping>, <image editing> (filtering), <chat client>, <discuss client>, <video recorder> to display images at user-specified rates from the 'Server', <illumination control> and <changing objectives>.

The 'Client' provides an <EDIT> feature, which allows editing of captured images. Image processing functions currently available are <Grayscale>, <Invert>, <Blur>, <Sharpen> and <Contrast>. This module can be expanded to include various nonlinear filtering algorithms and user-specific image processing routines such classification counting, size distribution and analysis

The Java 'Client' updates its image after every successful 'Server' command execution. The "virtual" <Video Recorder> feature allows users to view real time video at user specified frame rates. (Default-10 fps)

The <Tiling> feature allows a user to create a montage (topographic map) of a specific region of the cell culture. This allows automatic control of the stage to facilitate capturing sequential "tiles" of the desired area and then reassembling them into a composite representation.

To observe live cell phenomenon (e.g. making and breaking of communication channels) brightfield imaging is combined with fluorescence imaging. Brightfield imaging using a digital video camera (Sony DFW-V300- 30 fps) camera provides a topographic view of the specimen under observation. Fluorescence imaging enables monitoring of cellular organization by binding fluorescent markers (GFP) to specific intra- or inter-cellular structures and recording the real-time images of cellular network activity, spanning many hours of observation to make time-lapsed movies. The use of fluorescent markers helps relate specific cell phenomenon to the network emergent function. The live-cell imaging system combined with a regulated micro-environment (temperature, media composition, atmospheric gases) control is a basic requirement for long-term observation of the dynamic organization of cellular networks.
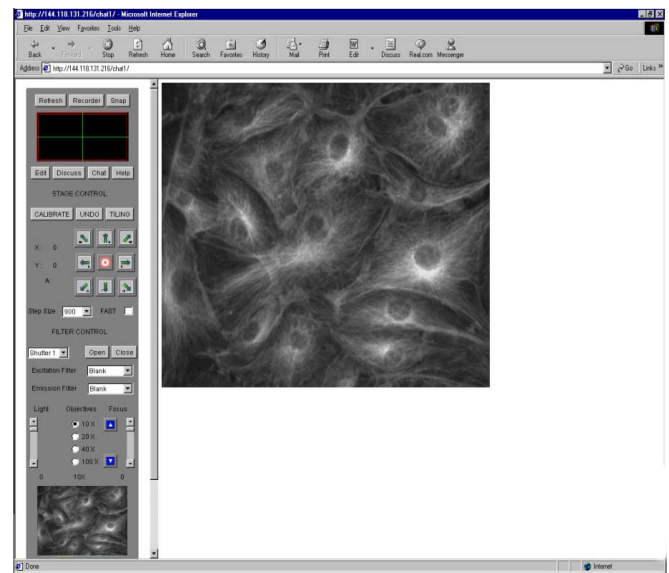

Fig 2. *Telemicroscopy Applet viewed by Microsoft Internet Explorer (Left - The microscope control and image acquisition interface. Center – updated image from the microscope)*

III. ABBREVIATIONS AND ACRONYMS

1) Telemicroscopy: Remote Control Live Cell Virtual Observation Platform
2) Telemic: Refers to Telemicroscopy 'Client' or 'Server' or component module
3) Biocomplexity: Study of complex systems occurring within organisms
4) GFP- Green Fluorescent Protein

IV. CONCLUSION

The imaging platform presented in this paper is being used in studies of cellular network dynamics, in particular, the monitoring of live-cell spatio-temporal organization and the role of intercellular communication and its effects on tissue genesis, differentiation and survival.

The distinguishing features of the live-cell telemicroscopy system are 1) its modularity and universal applicability, 2) ability to remotely acquire and control fluorescent and brightfield images in real-time spanning hours to days of observation.

The digital microscopy collaboratory environment serves as a focal point for investigators with different expertise to collaborate dynamically on projects that require multi-disciplinary approaches.

The long-term objective of this research is to reverse-engineer biological principles and help inspire the design and engineering of robust and adaptive communication networks and intelligent systems. The advantages gained in mimicking biological organization and function are particularly intriguing. The cellular network models may have applicability in areas such as emergent communication networks, evolutionary/adaptive optimization of networks, and distributed memory/"smart agents". In addition, the "biocomplexity" inspired concepts of self-regulation [3] and assembly can be explored "in-silico" and generalized to study autonomous agent models; 2-D cellular automata; "flocking" self-organized/coalition behavior; search pattern evolution problems; evolutionary optimization problem; spatial genetic algorithms among others.

V. ACKNOWLEDGMENT

VI. REFERENCES

[1] Wolf G, Petersen D, Dietel M, Petersen I: *Telemicroscopy via the Internet,* Nature, 391:613, 1998.

[2] Onaral, B., Kresh, J.Y., Luzuria, E. "Complex Biological Systems" Plenary Lecture: IEEE-EMBS Asia-Pacific Conference on Biomedical Engineering, September 26-28, 2000, China

[3] Kresh, J.Y., Izrailtyan, I. "The Heart as a Complex Adaptive System". In: *Unifying Themes in Complex Systems*, Y. Bar-Yam, Ed., Perseus Books, 2001.