



Photometrics Customer Profile

Simultaneous Atomic Force Microscopy, TIRF, and FSD Confocal Microscopy for Live Cell Mechanotransduction Studies

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Andreea Trache, Ph.D., Assistant Professor at Texas A&M Health Science Center, Department of Systems Biology and Translational Medicine, and Texas A&M University, Department of Biomedical Engineering

BACKGROUND

Understanding the molecular mechanisms behind how cells remodel in response to mechanical stimulation is essential to develop therapies for many vascular diseases. Studies of how cytoskeletal proteins respond to mechano-chemical stimulation have traditionally relied on post-stimulation cell fixation and staining. Because this method offers only static information, researchers began investigating the dynamics of fluorescently tagged proteins using the latest low-light cameras.

An ideal experiment would mechanically stimulate and image live cells in real time. However, the technical requirements of optical microscopy and mechanical stimulation techniques conflicted. That is, until Dr. Andreea Trache at Texas A&M Health Science Center designed the first integrated microscopy system that can simultaneously stimulate and image live cell response in real-time.

Dr. Trache's integrated system uses an atomic force microscope (AFM) tip coated with fibronectin to mechanically stimulate the cortical actin fibers beneath the apical cell surface. Simultaneously, the mechanical-induced cytoskeletal reorganization throughout the cell is captured at high spatial and temporal resolution by either total internal reflection fluorescence (TIRF) microscopy or fast spinning disk (FSD) confocal microscopy.

CHALLENGE

“This setup is challenging because it's hyper sensitive to noise and vibrations. The AFM can measure nanometer displacements at picoNewton force. At that level, the rotation of the spinning disk or the camera fan would disturb the experiment. Slamming a door would end it,” explained Dr. Trache, who needed to isolate any vibration sources from the AFM and specimen.

“Also, a typical experiment lasts about 80 minutes, in which time 250 confocal images are acquired. We can't have photobleaching because it would misrepresent the biological process we're recording,” continued Dr. Trache. “We needed a highly sensitive camera that would allow us to minimize the intensity of the laser excitation. Critical, too, was a high signal-to-noise ratio to record dim fluorescence images with a dark background, in order to minimize post-processing of hard data.”

Dr. Trache also needed to synchronize the spinning disk of the confocal scanning head, which rotates at up to 5,000 rpm, with the low-light camera to obtain uniformly illuminated images. In turn, the field of view of the confocal camera needed precise alignment with the TIRF camera, the AFM tip, and the AFM video camera and eyepiece.

OVERVIEW

Andreea Trache, Ph.D. designed the first integrated system to use TIRF and fast spinning disk confocal microscopy to study protein dynamics in live cells in response to real-time mechanical stimulation by an atomic force microscope (AFM)

FACILITY

Texas A&M Health Science Center

PHOTOMETRICS EQUIPMENT

QuantEM™ EMCCD cameras
Off-camera air cooling fans

KEY FEATURES

- Capture clear images at low and high frame rates (low dark current and read noise)
High sensitivity to avoid photobleaching from the laser excitation (>92% QE, 16 μm x 16 μm pixels)
- Wide dynamic range for both fast spinning disk confocal and TIRF microscopy (16-bit digitization)
- Vibration elimination for simultaneous AFM

SOLUTION

Dr. Trache methodically isolated all sources of vibration by mounting the confocal scanner on a silicone damper pad, and isolating it from the microscope body. Vibrating equipment, such as external Photometrics camera fans, were mounted to adjacent structures.

Out of the cameras that Dr. Trache tested, Photometrics' provided "the best synchronization between the spinning disk and the camera." She used a pair of QuantEM:512SC EMCCD cameras for TIRF and FSD confocal microscopy. The camera's pixel-clock timing resolution is improved over ten-fold with ACE™ technology, which allows for better synchronization and superior signal-to-noise sampling.

Cooled to -30°C, the QuantEM cameras have greater than 92% quantum efficiency, sub-electron read noise, and larger 16 µm x 16 µm pixels to provide fast image capture at a low, laser excitation intensity. The QuantEM's 16-bit digitization also delivered the wide dynamic range that Dr. Trache needed to capture TIRF's high-contrast images.

"Everyone was looking at mechano-transduction as a before-and-after event. Currently, we obtain consistent data from studying real-time live cell remodeling."

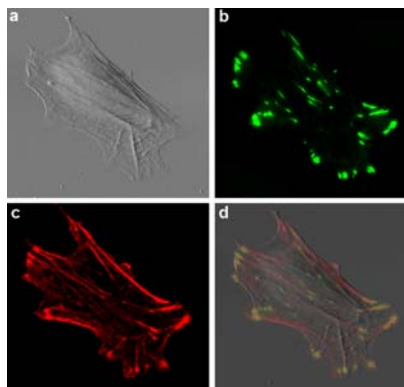
RESULTS

"Before, everyone was looking at mechanotransduction as a before and after event. Currently, we obtain consistent data from studying real-time live cell remodeling," said Dr. Trache. "We can image focal adhesion proteins recruitment at the basal cell surface as the cell remodels in response to the AFM's applied force. It's action and reaction."

As for minimizing laser intensity, there is "no photobleaching with time," Dr. Trache said. "We are able to capture images at 20 fps. If we need to, we can push it to 50 fps or more." The QuantEM has a 100% duty cycle that can continuously collect and transfer data to a computer at much faster frame rates.

"As I said, especially with TIRF microscopy that provides images within 100 nm from the basal cell surface, we want the signal-to-noise ratio as high as possible. We do post-processing, but our hard data gives us an excellent signal-to-noise ratio," she continued.

The TIRF microscopy images of the cytoskeleton and focal adhesions proximal to the basal cell surface can be directly overlaid and analyzed in conjunction with the FSD confocal microscopy data, which scans across the entire cell. The result is a 3-D capture of whole-cell adaptive responses to mechanical force at high spatial and temporal resolution.



A live vascular smooth muscle cell transfected with vinculin-GFP and actin-mRFP was imaged by AFM (a) to provide a general topographic view of the cell with actin cytoskeletal fibers immediately beneath the apical membrane. The same cell was then imaged by TIRF microscopy (b) to show the focal adhesions at the basal cell membrane marked by vinculin-GFP (green channel). A confocal image (c) was acquired as a 3D stack of 20 planes and presented as maximum projection to show the cytoskeletal actin structure (red channel) throughout the cell. The overlay (d) depicts the excellent registration between the AFM image and the fluorescence imaging techniques.

LOOKING FORWARD

Professor Trache's research will open novel investigation pathways to analyze cellular adaptive responses to the local mechanical microenvironment. This research will yield a deeper understanding of the structure-function relationship between contractile proteins involved in force transmission. Her goal is to provide the knowledge base needed to develop better therapies for diseases such as hypertension, atherosclerosis, or tissue edema, for which cellular changes due to mechanical force are key. Dr. Trache will also be pushing the imaging system to faster frame rates in order to investigate the effects of chemical stimuli, such as calcium ions on cellular structure.

PUBLISHED DIAGRAM OF THE IMAGING SYSTEM: Trache, Andreea and Lim, Soon-Mi. Integrated microscopy for real-time imaging of mechanotransduction studies in live cells. *Journal of Biomedical Optics*. 13(3), 034024 (May/June 2009).