manner and used to study interrelationships of different cellular constituents and processes. The feasibility of these improvements relies not only on an increased number of probes or a larger set of fluorophores but also on advances in the hardware components. We are now incorporating spectral analysis into the coherent multiprobe approach by means of a Sagnac interferometer. The application of spectral analysis in this setup will increase the resolution of emission peaks separated by as little as 10–20 nm, which will avoid the spectral overlap caused by wide bandpass effects[21]. This approach will also allow the development of a larger number of multiple coherent probes. A hardware limitation of the multimode microscope is the time delay caused by the mechanical positioning of the filters in the microscope beam path. This limitation can be obviated by replacing the glass filters and carriers with acousto-optical tunable filters with resulting increased speed of the system[22]. This continuous solid-state tunability will permit switching between multiple fluorescence modes within milliseconds or less, allowing examination of events at closer time intervals than the current system could possibly attain. These increased efficiencies will facilitate monitoring functional probes for enzyme inhibition or induction, membrane or organelle synthesis and cytoplasmic motion.

Methods

Multimode microscopy. We used a microscope system modified from that previously described[8] (Fig. 1). The multimode microscope used here consists of a robotic stage fitted with a controlled environmental chamber, a series of dichroic filter sets housed within a motorized carrier, supplemental high-speed fluorescence and neutral density filter wheels, a high-sensitivity CCD camera (Photometrics 12-bit Series 300 CH250, Tucson, Arizona) and a video-rate camera (C2400 CCD; Hamamatsu, Hamamatsu City, Japan), all attached to an inverted microscope (Zeiss Axiovert 135) equipped with 100-W mercury and halogen light sources and high-numerical aperture objective lenses.

Primary liver cell isolation and culture. Primary hepatocyte cultures were prepared from rat livers perfused with collagenase as described[7]. Hepatocytes were purified by differential centrifugation through a Percoll gradient, washed and suspended in hepatocyte culture medium (HCM): Leibovitz-15 with L-glutamine (Life Technologies) supplemented with 7.5% BSA (Life Technologies), 1% penicillin/streptomycin, 3 mg/ml proline, 50 mg/ml galactose, 0.1% insulin-transferrin-selectin (ITS; Collaborative Biomedical Products, Bedford, Massachusetts), 0.4 mg/ml dexamethasone (Sigma), 8.4% sodium bicarbonate, and 0.1% trace elements ($CuSO_4$, $Fe(NO_3)_3$, $ZnSO_4$ and $MnCl_2$). Hepatocyte viability was assessed by Trypan Blue exclusion. Hepatocytes were plated into four-chambered glass slides to a density of approximately 75,000 cells per cm^2, and incubated overnight at 37 °C in an environment of 5% CO_2. Fresh media was added to each chamber before the cells were exposed to the fluorescence probe coherent combination.

Fluorescent probe loading. The fluorescent probes were loaded by 'spiking' each chamber with an appropriate volume of probe stock solution. A 1-mM stock solution of Fura-2 and a 1-μM stock solution of MitoTracker Green were prepared in DMSO. A stock solution of Texas-Red-conjugated phalloidin (200 unit per ml) was prepared in methanol. For single-probe experiments, media with the final probe concentration was added to the culture chamber. After incubation at 37 °C, the cells were washed once with fresh media and 0.5 ml was added to each chamber. Preliminary experiments identified optimal probe concentrations and loading times (not shown). For Fig. 4, Fura-2 was loaded for 30 min at a final concentration of 5 μM, MitoTracker Green was loaded for 15 min at a final concentration of 5 nM, and 1 unit of Texas-Red phalloidin per ml of media was loaded for 15 min. For the coherent probe combinations, MitoTracker Green and Texas-Red phalloidin were added 15 min after the addition of Fura-2. All other parameters and steps were identical to those just described for single-probe loading.

Image acquisition and analysis. Under multimode microscopy, digital images were captured with sequential exposures of 0.05 s, 0.2 s, 1.0 s and 1.0 s for Texas-Red phalloidin, MitoTracker Green, Fura-2 (380 nm) and Fura-2 (340 nm), respectively. Illumination was provided by a 100-W mercury lamp and 560/645, 480/530, 340 or 380/510 excitation/emission filters for the same sequence of probes. These exposures consistently saturated greater than 75% of the linear range of the 12-bit CCD detector. In coherent labeling experiments, the order of acquisition for discrete field images was transmitted/DIC, Texas-Red phalloidin, MitoTracker Green and Fura-2 at 340 nm and then at 380 nm (Fig. 1). A series of images using this filter combination was sequentially acquired at each stage location every 5 min over 2 h using the high-resolution, liquid-cooled CCD camera mounted on the multimode microscope. Four fields from each chamber of a four-chamber slide were photographed a total of 25 times in a single run, generating 400 images over the 2-h sampling period. For digital image analysis, the average pixel intensities from the discrete fields were calculated using the raw 12-bit images as sources and applying image analysis software (Media Cybernetics, Silver Spring, Maryland).

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CORRECTION

In the New Technology *article "Gene expression profiles of laser-captured adjacent neuronal subtypes,"* which appeared in the January issue (*Nature Medicine* **5**, 120; 1999), a company name was misspelled. The correct spelling is Arcturus Engineering.