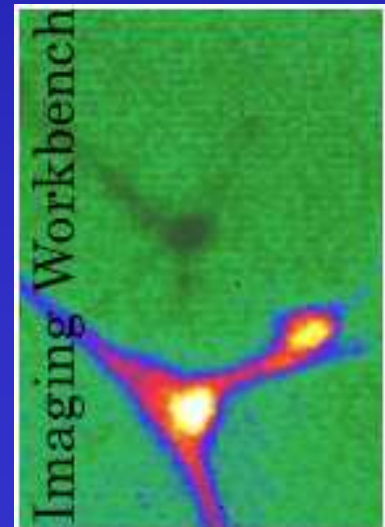


Cooperative Electrophysiology and Multiwavelength Imaging with Live Cells

Imaging Workbench 5

INDEC BioSystems



Bases for Electrophysiological Recordings

- Cell membrane separates intracellular from extracellular space
- Cell metabolism maintains ion (Na^+ , K^+ , Ca^{++} , Cl^-) concentration gradients across membrane
- Changes in voltage across membrane, or binding of agonists to membrane receptors, can modify (open, close, block, ...) the states of ion channels
- Ion channel state changes regulate short and long term cell behavior

Varieties of Electrophysiological Recordings

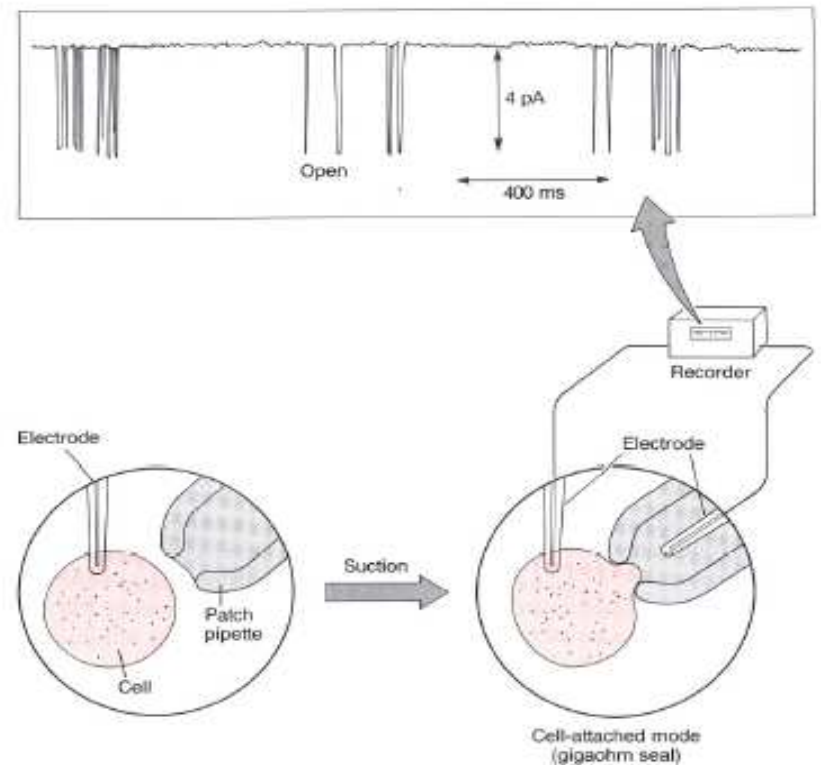
Cellular/Subcellular Recordings



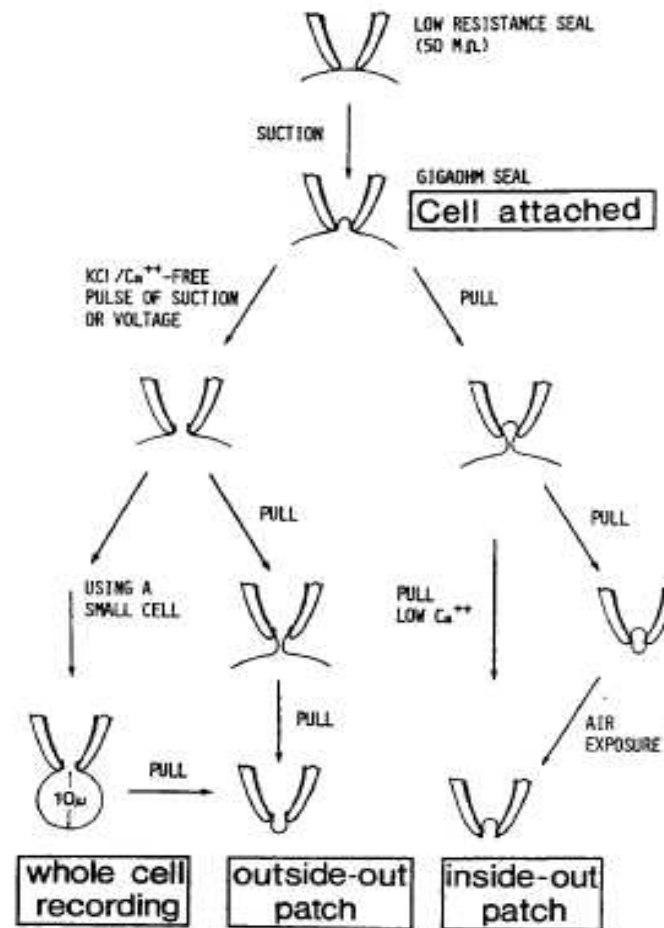
- Patch clamping – measurements from a small patch of cell membrane, containing a single ion channel.
- Voltage clamping – measurements from an entire cell with voltage fixed at a predefined level.
- Microelectrode recordings – transmembrane potential from a single cell within multicellular preparation. Gold standard for action potential recordings

Patch Clamp Recording

Under most circumstances, ion channels are either open or closed and they switch very rapidly from one state to the other. Thus the effect of a single ion channel opening is to cause an abrupt increase in the conductance of the patch of membrane beneath the pipette which may be 'visualised' using the patch clamp technique as a step-like increase in current.



Patch Clamp Recording Types



Why Combine Ephys and Imaging?

- The combination is the gold standard for assessing behavior in electrically excitable cells
 - Checking a transformed stem cell for correct physiology
 - Front-end assay development at pharma
- Not limited to wide-field microscopy
 - Also used on 1- and 2-photon scopes
 - PhysioLink add-on to FLUOVIEW

Some Useful Fluorescence Measurements Associated with Electrophysiological Recording

- Ratiometric ion indicators (fura-2, indo-1)
 - Ratio of two images at distinct excitation or emission wavelengths - related to ion concentration
 - Excitation ratio requires device control (e.g. monochromator), precludes use of frame transfer mode
 - Emission ratio with Optical Insights image splitters
- Single-wavelength indicators (fluo-4)
 - $\Delta F/F(t=0)$ eliminates confounding factors such as cell thickness, indicator concentration
 - Frame transfer modes ok
- FRET

Key Features of IW 5

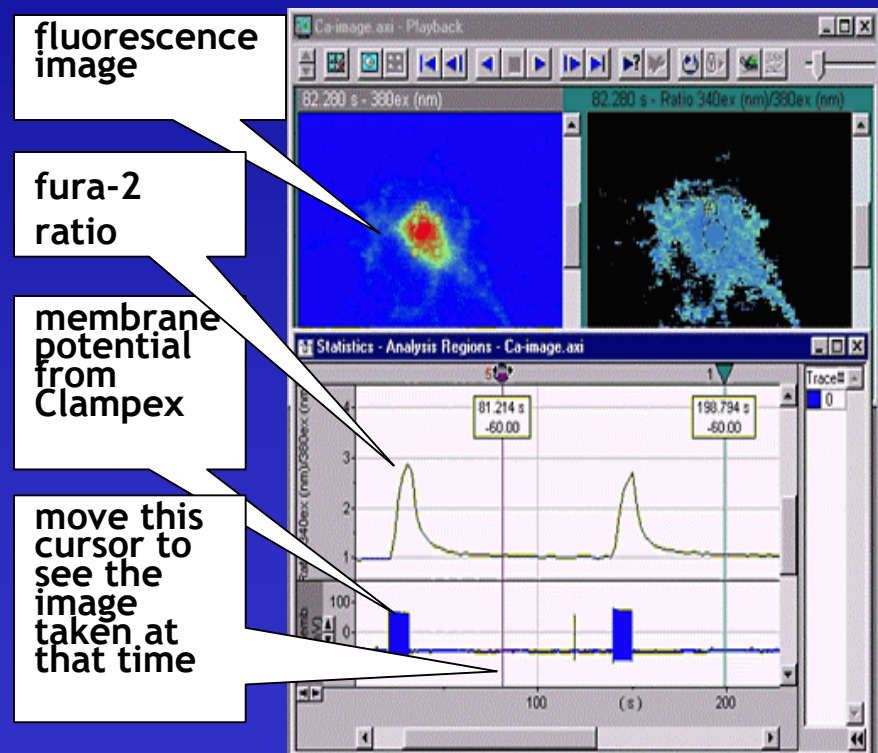
- IW 5 focuses on multiwavelength time series imaging
- Controls wavelength switching devices (monochromators, galvo devices, filter wheels), shutters, z steppers and more
- Two independent imaging channels
 - Each channel can be ratiometric or up to 3-wavelength nonratiometric
- Calculates intensities, ratios and ion concentrations over time, averaged over ROIs or LOIs

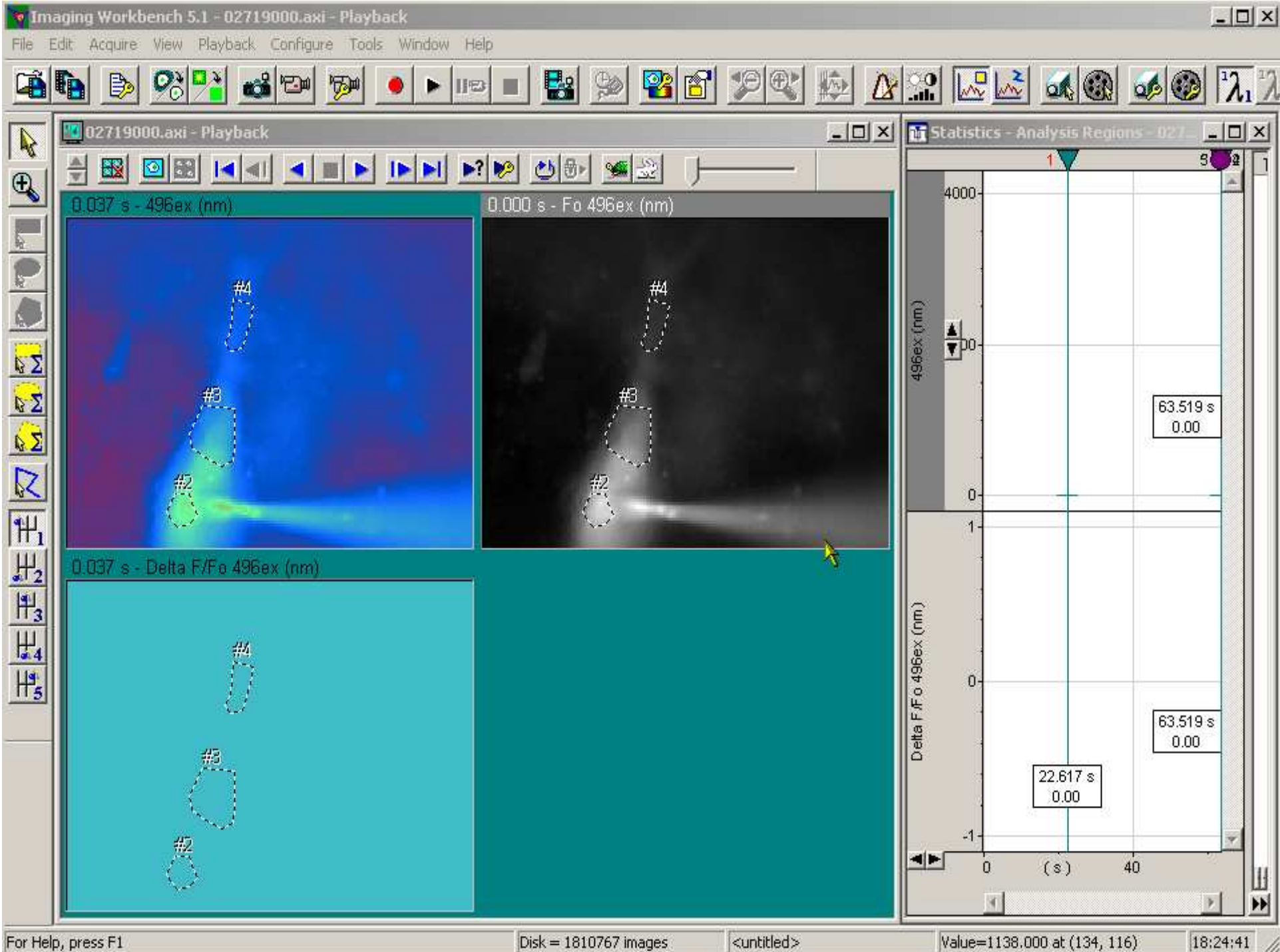
Key Features of IW 5

- Full camera speeds supported
- Free-form, on-line control of acquisition parameters
- Online calculation and display of ratio, $\Delta F/F_0$, background subtraction, shading correction
- Cooperates with Axon Instruments' pCLAMP
- Analyzes acquired image data online and offline
- Exports data to movies, multi-image TIFF stacks and spreadsheets

Cooperative Imaging and Electrophysiology in IW 5

- Synchronization
 - wireless triggering
 - start and stop
 - master-slave relationship
- Compatible protocol structures
 - episodic or continuous modes
- Compatible data
 - IW 5 automatically imports Clampex data into graph
 - IW 5 can export time-course data to be analyzed in Clampfit





Adaptations of IW 5 to Ephys- Imaging Experiments

- Reduce overillumination as much as possible
- Optimize for best timing fidelity without modifying Windows
 - Below 1 ms accuracy - but not much below
- Reduce all camera latencies to minimum
- Strong collaborations with users

Benefits of EMCCD Cameras in Ephys-Imaging Experiments

- Usually get low to moderate light levels in these experiments, so modest EM gain appropriate
- Increased signal-to-noise in images means shorter exposures
- Shorter exposures means less bleaching and phototoxicity ...
- ... and longer, more productive, experiments
- EMCCDs are evolving to be the best choice for multi-purpose experimental setups

Recent Improvements in iXon Operation

- Shorter cleaning cycles
 - Reduces overillumination
 - Improves response time to start exposure
- Baseline clamping
 - Reduces or eliminates the low level of initial baseline drift
- Run Till Abort mode allows indefinitely long frame transfer mode operation in sufficiently fast PCs

Wish List re iXon Operation

- More flexible frame transfer mode operation
 - Variable exposure time
 - Output synchronization info for wavelength switch devices