aurora spectral technologies

ADVANCED SYSTEMS FOR SCANNING MICROSCOPY

Giving Color to Life

Widefield, Confocal and Two-Photon Microscopy Systems with Exquisite Spectral Resolution

Aurora Spectral Technologies is pleased to offer state-of-the-art optical microscopy and micro-spectroscopy (OptiMiS) technology capable of generating unparalleled data for biological and biophysical research. AST products and services range from standard confocal and multiphoton (two-photon) microscopes and accessories to our powerful optical micro-spectroscopy technology, OptiMiS TruLine. The OptiMiS can be attached to the customer's microscope or sold as a complete system.

OPTIMIS SYSTEM

This is our proprietary product, which may be attached to existing research microscopes that incorporate a side port and a femtosecond laser. Includes: OptiMiS Electronics Module, OptiMiS Optics Module, OptiMiS Scanning Module, OptiMiS Control Software, a Computer with all necessary boards, and a User Manual. Spectral unmixing software for data analysis is also provided.

OptiMiS is the only scanning system that permits real-time quantitative analysis of single molecules and molecular complexes and their spatial distribution in living cells. The system is capable of producing a full set (up to 200 wavelength channels over 425-650 nm) of spectrally resolved images with a single excitation scan. In addition, it features sensitivity much higher (by over 100×) than any point-scan confocal or two-photon (multiphoton) microscope in the market.

OPTIMIS INTEGRATION PACKAGE

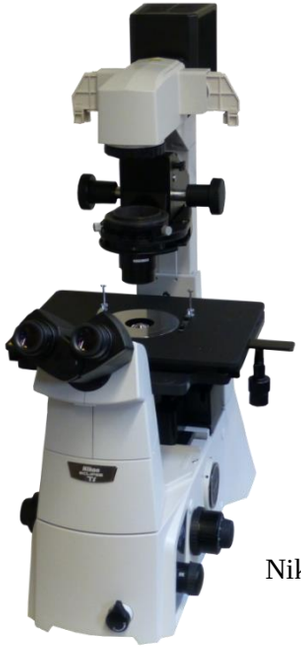
This package includes all of the components required to configure our system with a laser, microscope, and camera. It comprises a periscope system to align

the laser beam, a computer controlled laser power modulator, and all of the elements needed for complete assembly of the system. Importantly, the package also includes precision alignment by a skilled instrument specialist and two days of hands-on training.

ANDOR iXON™ EMCCD CAMERA

The Andor iXon Ultra and iXon3 electron multiplying charge-coupled device (EMCCD) camera is an ultrasensitive imaging solution ideally suited for the types of low-light bioimaging applications OptiMiS is designed for.

OptiMiS Provides Flexibility in the Integration of Confocal and Two-Photon Microscope Components



Nikon Eclipse Ti-U

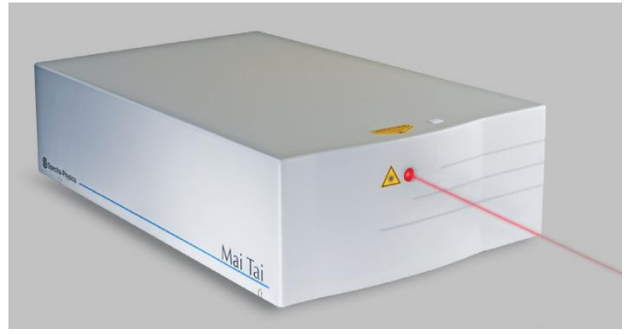
Zeiss Axio Observer D1

MICROSCOPES:

We integrate our systems with a wide range of microscopes sold by any of the major manufacturers. We can also offer specific microscopes as part of our complete system package. We recommend the Carl Zeiss Axio Observer™ and the Nikon Eclipse Ti™, equipped with fluorescence and either brightfield or phase contrast. These instruments, with their legendary optics, robust design and rock-solid stability, are ideally suited for use with OptiMiS systems.

Important Note: *In order for the above products to properly function, an adequate optical table is also necessary. Although we cannot be responsible for the customer's choice, we are happy to provide suggestions on an appropriate table.*

For further information contact Vali Raicu at (414) 333-9973 or vraicu@auroraspectral.com



LASERS:

Pulsed: The Spectra-Physics MaiTai XHP™ femtosecond laser was exclusively designed for use with the OptiMiS system and is only available from Aurora Spectral Technologies.

- Completely automated one box mode-locked Ti:Sapphire laser.
- Wide Tuning Range 750nm–1040nm.
- High Average Power: >1.35 W at 750nm; > 2.0 W at 800nm; > 300mW at 1040 nm
- Unique mode locking
- Completely CPU controlled.
- On-board diagnostic package.
- Outstanding beam pointing stability eliminates the need to realign external routing optics and guarantees optimum microscopeoutput at every wavelength.
- Incorporates new, ultra reliable Prolite diodes in an ultra-compact package (23x14x6 inches).
- Includes rack mountable power supply, chiller, and rack accessory.

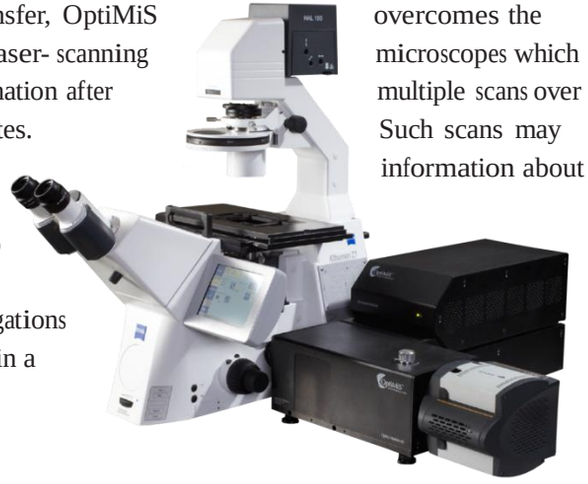
Continuous wave: For confocal microscopy, a variety of Ar-ion, solid-state, and diode lasers exist for the customer to choose from.

OptiMiS: Delivers Pixel-Level Spectral Resolution

OptiMiS is a state-of-the-art imaging tool that uses a single scan of multiphoton excitation to deliver never-before-seen pixel level spectral resolution of complex, multi-color fluorescence samples.

Based on technology developed during ten years of research by Dr. Valerica Raicu and his research group at the University of Wisconsin-Milwaukee, OptiMiS provides a unique blend of speed, sensitivity, and spatial and spectral resolution.

OptiMiS can upgrade the imaging capability of almost any microscope, enabling it to achieve multi-photon microscope (MPM) grade three-dimensional spatial resolution with the addition of a femtosecond laser and a camera. When applied to the study of, e.g., protein-protein interactions via resonant energy transfer, OptiMiS overcomes the limitations of existing laser-scanning microscopes which obtain multicolor information after multiple scans over a period of many minutes. Such scans may provide misleading information about complex intracellular dynamics with respect to protein trafficking and transient structural aggregations which may happen within a fraction of a second.



OptiMiS Advances Current Toolkit of Microscopists by Separating Fluorescence Signals from Co-Localized Molecules.

Typically, two-photon microscopes build three-dimensional images from planar sections of a sample, and laser scanners rely on dichroic beam splitters with a particular threshold wavelength to separate emitted light of a particular wavelength. However, when the emission spectra of fluorescent dyes overlap appreciably in co-localized molecules, existing technology is unreliable in separating the different signals.

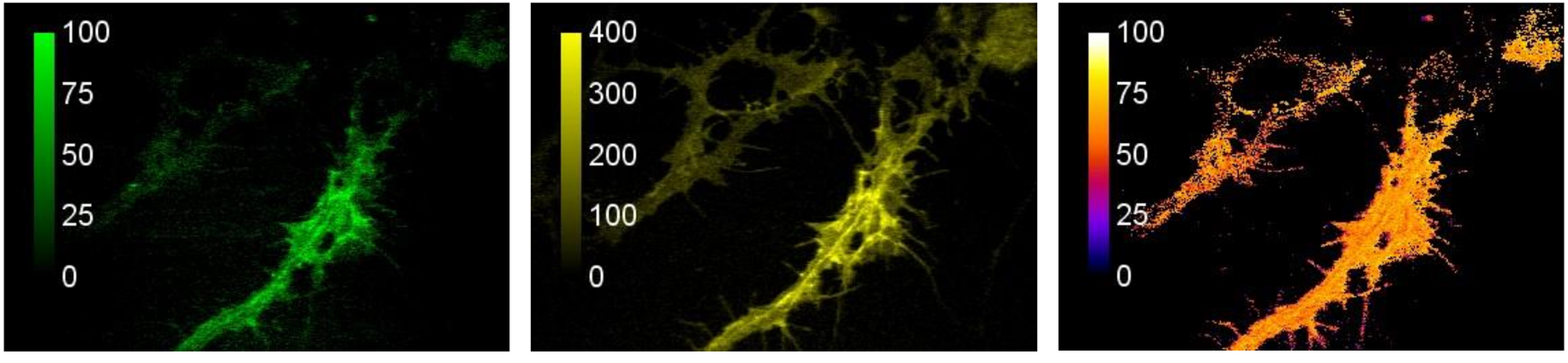
Consequently, unambiguous detection of multiple co-localized fluorescent dyes (indicating tagged, co-localized molecules) becomes dependent on the dye's emission characteristics and choice of emission spectra. This limits the range of dyes which can be employed and the types of molecular phenomena that can be reliably studied. In addition, filters may reject useful signal along with noise, and can only be changed sequentially, hence, slowly. As a result, the spectral information about dynamic molecular complexes is scrambled before all the spectral information is acquired. OptiMiS elegantly solves this problem by parallel detection of hundreds of wavelengths using an EMCCD with single photon sensitivity.

Unrivaled Combination of Superior Sensitivity and Superior Resolution

OptiMiS delivers nanometer spectral resolution with high efficiency capture of dispersed light over wide spectral ranges within seconds.

OptiMiS delivers superbly resolved intracellular images. In OptiMiS, an excitation beam is raster-scanned across the sample using galvanometric mirrors. Fluorescence emitted from the excited sample passes through a transmission diffraction grating, which separates the light into its spectral components prior to their detection via an extremely sensitive EMCCD camera.[1,2]

The OptiMiS software then reconstructs the data acquired from the excitation scan into a set of spatial maps of the emission intensity at various wavelengths, which may range from 425 nm to 650 nm, with spectral resolution as high as 1 nm per channel and as low as broadband. This range of wavelengths is acquired for each pixel in the reconstructed images within mere microseconds. For maximum field-of-view, a full set of spectrally resolved 512 pixel x 300 pixel images (up to 200 wavelength channels; wavelength range 425-650 nm) can be scanned in as little as 5 seconds; this scan time can decrease further for lower or no spectral resolution.

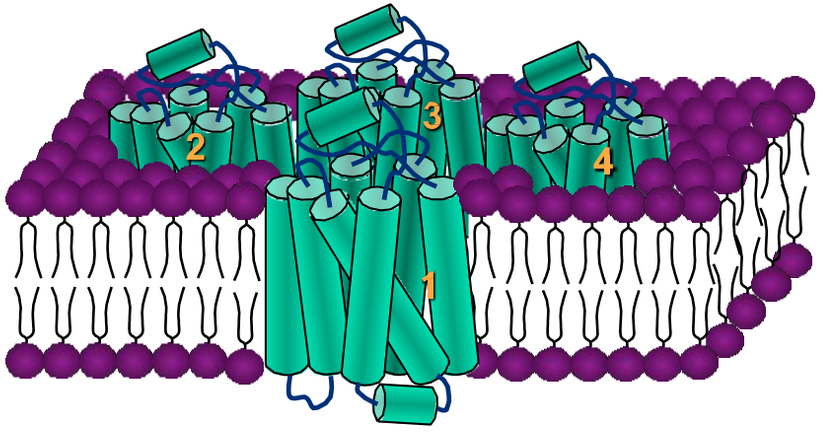


Monitoring intramolecular interactions. *Xenopus* spinal neuron cell expressing the RhoA biosensor. This single-chain biosensor consists of a full length RhoA protein and a domain which binds to RhoA upon activation.[3] Sandwiched between these two molecules are two fluorescent proteins, CFP and YFP, which participate in FRET when in close proximity to one another. A byproduct of the binding domain contacting the RhoA protein is the movement of the two fluorescent proteins closer to one another, hence causing an increase in the amount of FRET occurring. **Left:** Spatial maps of the CFP emission. **Middle:** Spatial maps of the YFP emission. **Right:** FRET efficiency map of the biosensor. *Image courtesy of LOCI, UW-Madison and Raicu Lab, UW-Milwaukee. Samples provided by the Gomez lab, UW Madison.*

Unsurpassed Flexibility to Utilize FRET in Probing Protein Interactions

OptiMiS allows choice between strongly coupled donor/acceptor fluorophore pairs while eliminating the need for photobleaching or corrections for spectral bleed through.

Förster (or Fluorescence) Resonance Energy Transfer (FRET) is a distance-dependent, non-radiative transfer of energy from an excited donor molecule to an unexcited acceptor molecule which occurs when the two molecules are in close proximity (typically <10nm) to one another. When FRET is used as a contrast mechanism, quantitative evaluation of intra- and inter-molecular distances between macromolecules can be determined with spatial resolution far beyond the limits of conventional optical microscopy.



Several different types of GPCRs appear to form tetramers with their monomers arranged in the corners of a parallelogram (or rhombus).[1,4] While other proteins, e.g., IRE1 (an enzyme) in yeast[5] and bacterial ABC transporters in CHO cells,[6] form different structures. *Image provided by Dr. Raicu of UW Milwaukee.*

Because of its nanometer spectral resolution and high-efficiency capture of dispersed light over large spectral ranges, OptiMiS is perfectly suited to utilize FRET to study macromolecular interactions. The highly accommodating 425 to 650 nm wavelength range acquired by OptiMiS sidesteps the concept of filter "bleed through" and allows users many choices for easily resolvable donor/acceptor fluorophore pairs. Because of this enhanced flexibility, donor/acceptor fluorophore pairs can be chosen to eliminate direct excitation of the acceptor and hence eliminate the need for the acceptor photobleaching scans in donor quenching FRET applications.

Using OptiMiS, Dr. Raicu's group, for the first time in published literature, successfully determined the stoichiometry and quaternary structure of protein complexes in living cells by FRET. In their work with G protein-coupled receptors (GPCRs), the instrument revealed a clear molecular fingerprint with relative disposition of macromolecules showing four monomers arranged as a parallelogram, which may dissociate into dimers.[7]

Quantify Interactions Between a Few or Even Single Molecular Complexes

When attached to a two-photon microscope, the OptiMiS device takes advantage of the low excitation probability inherent in the two-photon effect. By allowing the excitation of only the fluorescent markers in the focus of the excitation beam, TPM essentially delivers image sections similar to confocal microscopes. Crisp images with three-dimensional resolution are obtained and the need for pinhole geometries is eliminated.

Additionally, the exquisite sensitivity of the associated EMCCD detector makes it feasible to detect just a few and even single molecular complexes at one time, while the use of near infrared excitation light makes it possible to separate emission from excitation light, which dramatically increases signal-to-noise ratio.

By combining this first-in-class resolution with an ultra-sensitive detection device, an EMCCD, OptiMiS revolutionizes the depth and scope of real-time molecular information that can be acquired by scientists.

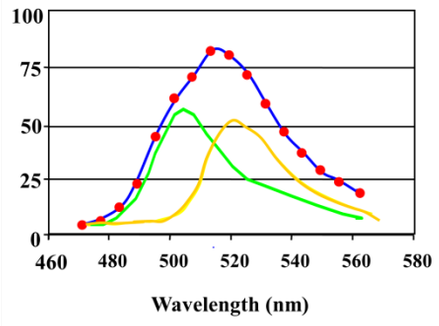
Any stoichiometric or structural permutations to the "average" molecular configuration occurring within the cell is no longer "washed out" by molecular diffusion, but distinctly contained within this distribution of spectral profiles. This type of pixel-level FRET information allows researchers to determine whether intermolecular interactions are occurring, visualize supramolecular structure of molecular complexes (proteins and nucleic acids), and detect macromolecular conformational changes (i.e., intramolecular FRET) in subcellular regions.

Parallel Acquisition of Spectral Information Avoids Complications Arising from Molecular Diffusion

OptiMiS avoids the "averaging" effect inherent to approaches requiring multiple excitation scans to acquire spectral information.

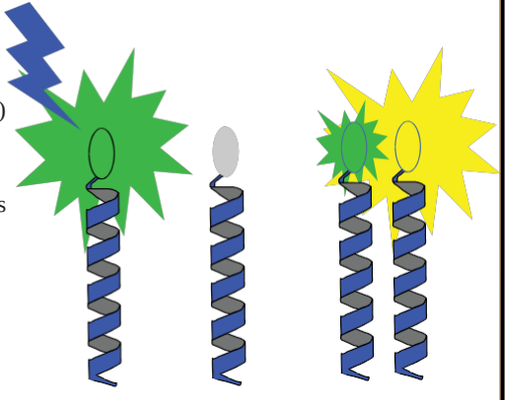
Because natural molecular diffusion changes the makeup of every excitation voxel from scan to scan, the same molecular complex is never sampled in successive scans. This essentially negates any pixel-level information and, by necessity, relegates the quantification of the obtained FRET efficiency values to averages over the entire cell.

From a single shot of the excitation beam, OptiMiS provides a full spectral profile of the molecules residing in the focal volume of the excitation beam. By scanning the laser beam across the entire sample, the unique spectral profiles of hundreds or even thousands of molecular complexes can be obtained.



Pixel level deconvolution of measured emission spectra. Fluorescent molecules with overlapping emission spectra can be uniquely characterized even when localized in the same pixel. The measured composite emission spectrum from a single pixel (red circles) is deconvoluted to uncover the fluorescence intensity emanating from each of the constituent molecules (green and yellow lines) comprising the signal.[1]

Illustration of Förster Resonance Energy Transfer (FRET). The two proteins on the left are not in close enough proximity for the excited donor molecule (green oval) to transfer energy to the neighboring fluorophore. This is in contrast to the two proteins on the right. A transfer of energy is occurring between them, resulting in fluorescence of the acceptor (yellow glowing oval). This detection of the signal emanating from the right-most protein signifies that there is indeed interaction occurring.[8,9]



OptiMiS Applications

Live cell imaging techniques are providing the first detailed look at intracellular interactions, with fresh insights into molecular functions and dynamic structural associations. It has the potential to revolutionize intelligent drug design based on biochemistry and macromolecular structural analysis, as opposed to random high throughput screening. A wide range of standard FRET and non-FRET-based imaging applications can be technically enhanced by the unique capabilities offered by OptiMiS.

FRET-Based Applications:

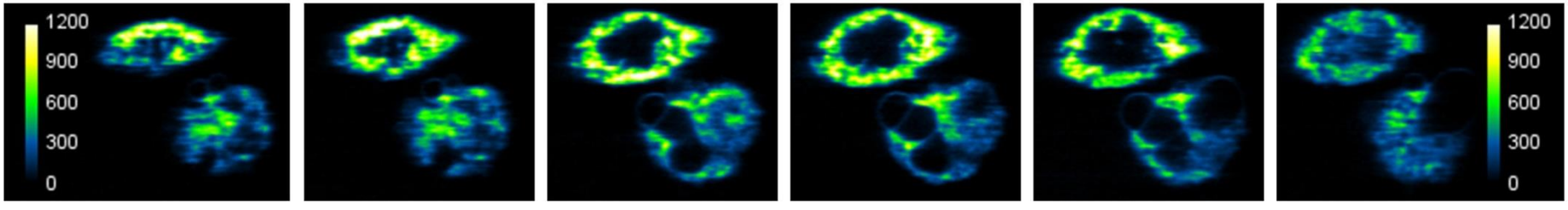
1. Detecting Protein-Protein Interactions:

Protein-protein interactions may lead to stable (or constitutive) as well as transient associations, collectively called 'oligomers.' In addition, even stable oligomers, once formed, may move around the cell. OptiMiS is especially well-suited to study both of these interactions. For whole samples, OptiMiS can complete multi-color data collection for a full-set of spectrally resolved images within seconds. A full spectrum (up to 200 wavelength channels; wavelength range 425 nm-650 nm) is acquired for each individual line (512 pixels) in as little as 15 milliseconds. That is less time per voxel than it takes molecular diffusion to cause a change in the molecular composition of the sample.

Although current two-photon microscopes provide crisp images of a single slice from a cell, they cannot capture the full array of colors needed to discriminate between the spectral fingerprints of different fluorescent proteins.

2. Studying Conformational Changes of Macromolecules (Proteins and Nucleic Acids)

Due to the technological inability to visualize dynamic interactions in real-time, the specific role of protein internal motions in enzyme catalysis is not well understood. Researchers can hurdle this challenge using OptiMiS. By attaching D and A tags to different parts of the same molecule, internal motions in the protein cause changes in distances between the two fluorescent tags, thereby causing a change in FRET. Using OptiMiS, changes in FRET efficiency are easily resolved, thereby giving information on the dynamics of protein conformation.



Example of OptiMiS optical sectioning capability. Fluorescence intensity maps of Chinese Hamster Ovary (CHO) cells expressing rhodopsin labeled with SCFP3A. The microscope objective is translated along the optical axis and scans of the cells are taken at various stage positions, giving the spatial distribution of the proteins for various sections of the cells. These sections may be stacked to form three dimensional distributions of the fluorescence in the cell. *Image courtesy of LOCI, UW-Madison and Raicu Lab, UW-Milwaukee; DNA provided by Paul Park, Case* Western Reserve University.

3. Measuring Intracellular Concentrations

A wide variety of biological processes are affected by the extent of ion transport through various ion-exchange channels. For example, calcium regulates enzyme activities, metabolic processes, replication, gene expression, etc. Using OptiMiS, researchers can measure the emission from fluorescence- and FRET-based sensors (e.g., "cameleons" for calcium sensing),[10,11] and quantify concentration changes of free ions of calcium, magnesium, sodium, potassium, and others – in large numbers of single living cells.

Non-FRET-Based Applications:

1. Quantifying Biomolecular Relationships: Protein Co-Localization

By quantifying the spatial overlap between multiple biomolecules tagged with spectrally distinct fluorescent labels, researchers can monitor the proximity of two biomolecules inside the cell and determine if the spatial relationship has biological significance. Measurements of this nature are dependent on two critical features of the imaging instrument: the spatial resolution must be high, and the spectral resolution must be of good quality. An MPM system incorporating OptiMiS overcomes these technical barriers.

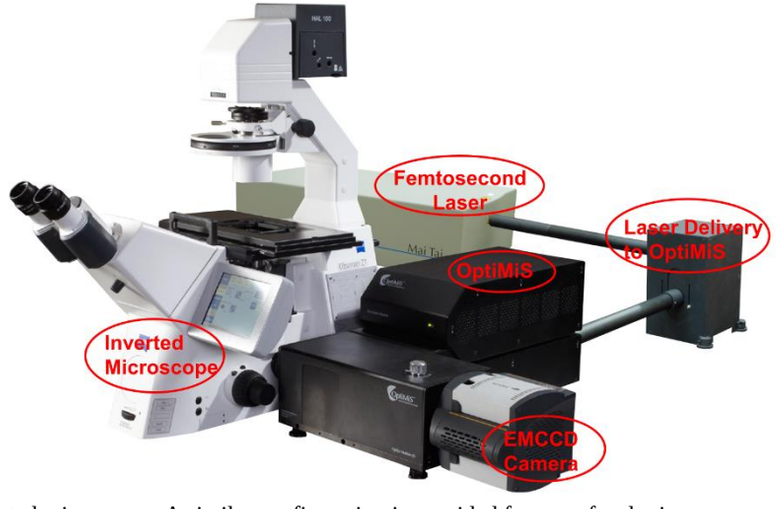
2. Localization of Specific DNA/RNA Sequences: Fluorescence in situ Hybridization (FISH)

FISH uses hybridization to detect and localize specific sequences of DNA on chromosomes. In cells, FISH can be utilized to pinpoint specific mRNAs in order to clarify spatial-temporal patterns of gene expression. With its combination of high spatial and spectral resolution, OptiMiS helps identify the location and level of expression of desired nucleic acid sequences.

Flexible Customized Solutions for Your Research Projects

OptiMiS can enhance the functionality of new or existing multiphoton microscopes, as well as almost any other microscope with a side port (left or right) with the purchase of an appropriate femtosecond laser. Microscopists are provided with recommendations for lasers and microscopes, as well as direction on building customized systems that will best suit specific imaging applications.

The overall design simplicity of OptiMiS makes it easy to increase reliability and robustness in your system's operations.



Sketch of a complete microscope setup. OptiMiS integrates a femtosecond laser, an inverted microscope, and an EMCCD camera to create a working two-photon microscope with spectral resolution. The end user can decide from a number of available options regarding the type of femtosecond laser and inverted microscope. A similar configuration is provided for a confocal microscope set-up, except that the femtosecond laser is replaced by a battery of cw lasers.

OptiMiS Specifications

Excitation and Detection

Microscope Coupling: Capable of direct coupling to side ports (both left and right) of various microscope bodies (Zeiss, Nikon, Leica, Olympus, and others).

Detection Hardware: The Andor iXon X3 or iXon Ultra 897 EMCCD can used for nondescanned detection of emitted fluorescence and can be purchased together with OptiMiS.

Scanners: There are two laser excitation scanning modes available, a point scan and line scan mode. In the point scan mode, two galvanometric scanning mirrors are used for raster scanning the excitation beam across the sample. In the line scan mode, one galvanometric scanning mirror works in conjunction with a cylindrical mirror which spreads the excitation beam into a line shape, allowing for simultaneous excitation of multiple voxels along a line.

Computer Control

Computer: Dell OptiPlex 990 running Windows 7 Operating System. The processor is an Intel Core i7 running at 3.40 GHz, with 16 GB of RAM. The Computer is equipped with a 1TB Hard Drive. A National Instruments 6221 M Series DAQ PCI Card is installed.

Screenshot of the **OptiMiS Control Software GUI** with representations of data obtained from various acquisition modes.

Software: User-friendly instrument-control software is included and pre- installed on user's PC. The powerful processor makes the computer suitable for on-site image analysis. The computer/software manages the scanner and collects all spectral data transforming it into data that can be exported.

Software Functionality

Acquisition Modes: Maximum field-of-view spectral acquisition, small region of interest spectral acquisition, time series spectral acquisition, broadband acquisition, and wide-field transmitted light acquisition.

Acquisition Time per Pixel: In the point scan mode, the whole spectrum of individual pixels (up to 200 wavelength channels; wavelength range 425-600 nm) is acquired as fast as 30 microseconds, less time than it takes molecular diffusion to cause a change in the molecular composition of an excitation voxel along the line.

Maximum Field of View Scanning Speed: Full set of spectrally resolved 512 pixels x 300 pixels images (up to 200 wavelength channels; wavelength range 425-650 nm) obtained in as little as 5 seconds.

Small Region of Interest Scanning Speed: Full set of spectrally resolved images (up to 200 wavelength channels; wavelength range 425-650 nm) with a variable image size (field of view). The acquisition speed is dependent on the user-selected size of the field of view, reaching as fast as 100 ms per complete frame, depending on region of interest size and spectral resolution.

Spectral Resolution: Software controllable variable spectral resolution as high as 1 nm per channel and as low as broadband. The range of wavelengths detected by the hardware ranges from 425-650 nm.

Unique Features and Benefits of the OptiMiS Module

- **Single Scan Image Capture:** No need for acceptor photobleaching or multiple scans
- **Ultra-fast Data Acquisition:** Collects whole spectrum for each image line within milliseconds
- **Superbly Resolved Images:** Delivers extremely high spatial and spectral resolution
- **Unsurpassed Detection Capability:** Provides spectral information with 100 × the sensitivity and speed of competing systems, and similar to that of broadband microscopes.
- **Highly Accommodating Wavelength Range:** Arbitrarily large numbers of fluorescent markers are easily resolvable spectrally
- **Flexibility to Upgrade Present Systems:** Upgrade any microscope with a side port
- **Freely Available Analysis Software:** Spectral unmixing and FRET efficiency calculations at single pixel level
- **Proven Technology:** Quantitative FRET imaging utilized in several publications for determining binding stoichiometry and geometry of protein complexes

References:

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