# Photonic Science

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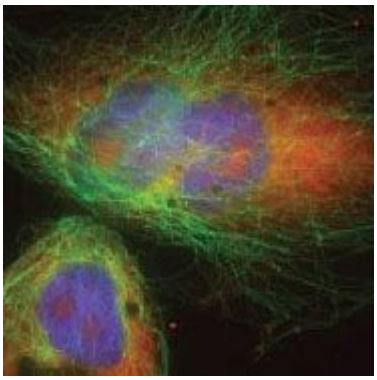
## T1000 CAMERA

## Confocal Fluorescence Imaging.

In a confocal microscope, the detector aperture obstructs the light that is not coming from the focal point. The out-of-focus light is blocked by the pinhole and in order to produce crisp image with haze contribution introduced by the depth of field from the objective. The smaller the pinhole, the sharpest the image will be as it will block effectively the fluorescence from the nearest neighbouring planes, but also the less light will be caught by the detector. Therefore there is a requirement for a very sensitive camera in order to capture the fluorescence arising from the confocal plane.

Typical integration time must be kept as short as possible in order to avoid cell damages under extended periods of digital recording. Typically 100ms to 1 second is used per image, depending on sensitivity settings chosen on the camera.

Three dimensional re construction is achieved by acquiring multiple images at different Z positions using a piezo / stepper motor driven z axis on a motorized microscope. Z stacks acquired over time allows to build multidimensional data sets taking into account multiples variables such as wavelengths, x,y,z positions and time. Multiple cameras can be synchronized as one single detector in order to produce parallel acquisition.



Confocal Fluorescence Imaging

## TIRF

A Total Internal Reflection Fluorescence Microscope uses evanescent waves to selectively illuminate and excite fluorophores in a restricted region of the sample. Evanescent waves are generated only when the incident light is totally reflected. The evanescent electromagnetic field decays exponentially and thus penetrates to a depth of only approximately 100 nm into the sample.

Thus the TIRFM enables a selective visualization of surface regions such as plasma membrane. TIRF can also be used to observe the fluorescence of a single molecule. Large magnification objectives are used routinely: typically 63 up to 100x objectives are combined with x2 projective optics in order to reach less than 100nm resolution.

As the volume probed is very small, the amount of fluorescence recorded is small and hence requires very high sensitivity cameras. Exposure time from 10ms to less than 1sec are also necessary in order to record live data from biological samples.

TIRF

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## T1000 CAMERA

## High Throughput Cell Screening.

Green Fluorescent Protein (GFP) has many applications as a marker in living cells, and has become widely used as a reporter gene in microbial, plant and animal cells.

Screening microbial colonies for GFP expression enables various types of assays (e.g. for mutations).

Fast automated imaging data collection routines enable discrimination between colonies, based on the level of fluorescence activity and the picking function automatically transfers cells to microplate wells.

Measuring fluorescent activity allows quantification of fluorescent tag concentration/expression. Fast acquisition requires a high sensitivity camera to collect images at high speed with as little as 1ms integration time.

Gated cameras can be used with pulsed fluorescence sources in order to reduce noise and synchronise accurately exposure to excitation sequences.

High Thoughput Cell Screening

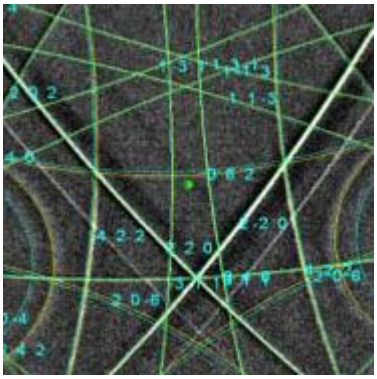
## Scanning Electron Microscope EBSD / KOSSEL Imaging.

EBSD can be used for crystal orientation mapping, defect studies, phase identification, grain boundary, morphology studies, regional heterogeneity investigations, material discrimination, microstrain mapping, and using complimentary techniques, physico-chemical identification.

Experimentally EBSD is conducted using a SEM equipped with a backscatter diffraction camera that records faint Kikuchi bands. This corresponds to each of the lattice diffracting planes and can be indexed individually by the Miller indices of the diffracting plane which formed it.

The bands formed can also be analysed to show the deformation present within the material: pattern blurring gives an indication of the plastic strain within the crystal and small rotations of the pattern (compared to a perfect crystal at this orientation) indicate elastic strain.

Cameras with good sensitivity are required for performing a fast acquisition duty cycle over 100 fps. Reduction in beam current / increased duty cycle can be achieved with optimized camera coupling and scintillator absorption. EBSD cameras can be upgraded to digitize Kossel diffraction patterns that will lead to subsequent structural / strain analysis.



Scanning Electron Microscope EBSD / KOSSEL Imaging

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## T1000 CAMERA

## EUV / DUV Lithography, Source, Optics and Resin Characterization.

The semiconductor industry roadmap uses shorter wavelength light sources to produce smaller feature sizes on processors as well as on memory components. Wavelength ranging from 248 nm to 193 nm are currently used to produce feature sizes < 100 nm. The next generation includes EUV sources which use 13.5 nm for printing feature size as small as 32 nm.

A source with very good brightness is needed for maintaining production throughput similar to that of DUV techniques. Therefore, EUV and UXV CCD detectors with good UV sensitivity and good dynamic range are necessary to cope with pulsed sources that are used to characterize resin, prior to mask manufacturing.

EUV sources can produce an important amount of debris so it important that that the CCD detectors withstand over exposures without saturation / bleeding artefacts as well as potential contamination from debris coming from the plasma generation.

Large area cameras from 13x13mm up to 24x36mm can be used with frame rate up to 5ps at full resolution.

EUV / DUV Lithography, Source, Optics and Resin Characterization

## Astronomy / Diffraction limited CCD Imaging.

Recent technological changes in CCD cameras now permit short exposures to be taken with negligible read out noise, allowing a high speed stream of images to be captured. This can result in thousands of quick short exposures being saved for subsequent post processing.

This technique uses a bright star nearby the object to be observed, and calculation of the Strehl number of the reference star in each image taken. A selection algorithm drops images that fall below the minimum and those images that meet the selection criteria are used and this may be as little as 1% to 10% of the data stream.

These selected images are combined by shifting and co-adding the sequence to produce diffraction limited image of the object being observed. The resultant image has been corrected for the turbulence of the atmosphere. This technique has also been called ” LUCKY IMAGING”.

Associated techniques are “speckle interferometry”.

Astronomy / Diffraction limited CCD Imaging

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## T1000 CAMERA

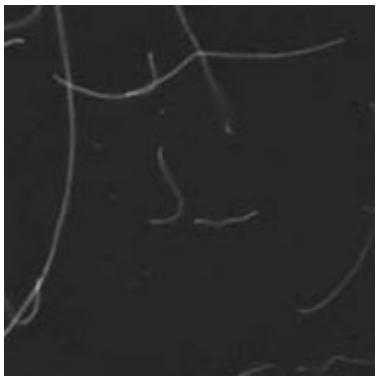
## Single Molecule Fluorescence Imaging.

Single Molecule emission spectra, lifetime, and intensity deliver specific information about the molecule location. The physical properties of materials such as lateral or rotational diffusion, conformational studies including protein folding can be studied using single molecule fluorescence technique.

Combined with a confocal microscope set up, SMF is capable of mapping both the location and orientation of single molecules, observing orientation and intensity changes over time down nanometer range.

It is important for the detector to record fast sequences at low intensity as the emission from single fluorescent molecules could be weak and changing rapidly over time.

Subtle intensity changes are usually recorded over a large background, this translates into good dynamic range requirements for the camera on top of high sensitivity, fast shuttering capability and rapid image transfer to a host PC.



Single Molecule Fluorescence Imaging

## Forensics Imaging.

UV sensitive CCD detectors are used for recording UV reflectance of untreated fingerprints, as well as fingerprints that have only been processed with cyanoacrylate fuming (superglue evaporation).

Good sensitivity is often required for detecting faint luminol treated stains. The cameras are used with a narrow band pass filter which will select the UV response only above the visible background.

High resolution sensors, typically over 1 megapixel, are required for sampling specific finger print details. Image enhancement tools like Fast Fourier Transform and image subtraction that allow you to quickly identify your images.

The cameras are incorporated into portable equipment, which includes a UV source, a digital recorder (labtop), a specific high transmission UV lens and a real time UV sensitive camera.

Laboratory equipment will use more specific UV optics, mounted onto a microscope for extensive research, comparative analysis.

Forensics Imaging