By Barbara Foster and Robert Mulligan

Focus on microscopy

INTEGRATING FLUORESCENCE AND FTIR MICROSCOPY

WHILE FLUORESCENCE microscopy is usually thought of as a biological technique, it is becoming increasingly valuable to materials science and the semiconductor industry. In failure analysis, the research laboratory, or production support, it can be combined with FTIR microscopy for the combined impact of detection/location (fluorescence) and identification (FTIR).

A key example is the electronics industry. As electronics move toward ever-smaller structures, even minute amounts of contaminant present increasingly serious problems, interfacing with basic processes such as electrical contacts or soldering of surface-mounted components. Fortunately, many common contaminates such as photoresists, solder flux residue, skin flakes, and cosmetics are naturally fluorescent, undergoing excitation and emission with off-the-shelf fluorescent accessories. Once the contaminant is located, a variety of FTIR microscopy techniques can be used for chemical analysis.

The basics of fluorescence

As seen in *Figure 1b*, fluorescence images show bright objects against a dark background. The technique involves the excitation of valence electrons with high-energy light, followed by relaxation, and an energy release. Since a small amount of the original excitation energy is absorbed in the form of heat, the emitted light is lower in energy and, therefore, has a longer wavelength: an ideal situation for separation of the incoming and emitted light.

A three-part optical filter set, typically mounted in a cube format for easy handling, is used in the microscope to generate fluorescence: an excitation filter narrowly defines the incoming light, a dichroic beam splitter reflects the shorter wavelength excitation light to the sample then passes the longer wavelength fluorescence to the viewing system, and a barrier filter eliminates any extra excitation illumination or secondary fluorescence. The result is a bright signal from the features of interest and a clean, black background. A typical filter cube for blue excitation/apple green emission includes a 450–490 nm excitation filter, a 510-nm dichroic beam splitter (reflects light lower than 510 nm, then transmits light higher than 510 nm), and a 520-nm barrier filter, while the set for green excitation/red emission includes a 515–560 nm excitation filter, a 580-nm beam splitter, and a 590-nm barrier filter.

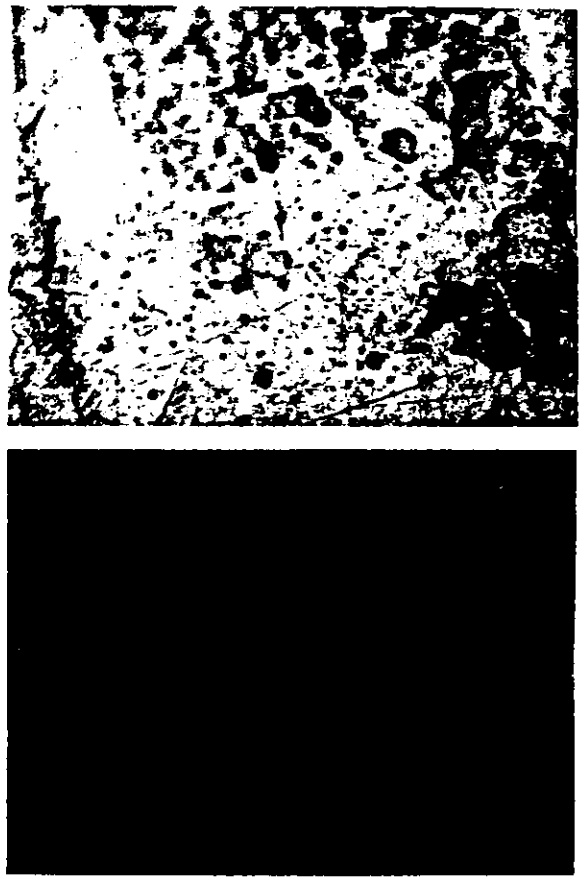

Figure 1 *Image of a contaminated region of a gold-plated test probe, (a) brightfield, (b) fluorescent.*

Since the ultimate goal of fluorescence is efficient collection of a clean but dim signal, care must be taken to choose high-numerical-aperture objectives that have no fluorescing elements and a light source that has strong output in the excitation region. Fluorescence images can be photographed using conventional cameras with high-speed film or collected with sensitive

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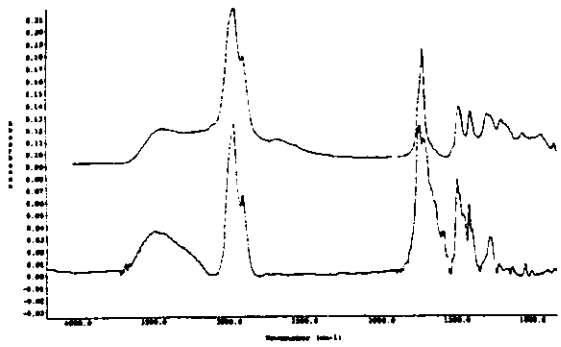

Figure 2 *FTIR spectrum of contamination, identifying it as flux residue.*

CCD cameras and fed into a computer for later analysis and direct inclusion in reports.

Adding FTIR microscopy

Once the location of the contaminant is noted on a finder stage, the slide is moved to an FTIR microscope to collect the IR spectra. Instead of using spectrally limited refracting or glass objectives, the FTIR microscope uses reflecting objectives that can reach into the standard infrared region, with a standard refracting objective for locating the area for examination. To eliminate spurious effects from scatter or adjoining features, the area of interest is masked using adjustable apertures. The standard infrared reflectance system is very effective for routine analysis of particles on reflective metal surfaces while grazing angle and ATR (attenuated total reflectance) objectives extend the applications to thin films and low-reflectance materials.

Collected spectra can be compared to standard libraries of known materials for identification, as well as used to fingerprint the exact type and source of contamination. For example, *Figure 2* shows the spectrum of pine resin, traceable to solder flux residue.

Sources of further information

Fluorescent detection of contaminants is quick, easy, and unambiguous enough for minimally trained operators, but a more complete understanding of traditional microscopy, fluorescence, and FTIR microscopy is critical for successful, valid instrument setup and image and spectral interpretation. Microscopy/Microscopy Education provides expert advice and instruction in the laboratory to walk the operator through principles and techniques relevant to his/her particular application. McCrone Research Institute (Chicago, IL) offers a broad range of courses on microscopy and particle analysis, while some of the FTIR manufacturers, notably, Nicolet Madison, WI)/SpectraTech (Stamford, CT), offer short courses within their own training facilities, emphasizing the breadth and depth of FTIR microscopy. New this year is a traveling chemistry road show from Spectros Associates (Mendon, MA) featuring one- to three-day courses on FTIR interpretation as well as a one-day introductory course on "Microscopy for Spectroscopists."

Whatever the source of information, integrating fluorescence and FTIR microscopy provides a powerful battery of tools for rapid contaminant detection and chemical analysis.