The work of the food microscopist encompasses a wide range of different activities. These range from a fundamental understanding of the structure and function of food raw materials and final products, through studies of machinery and equipment used to manufacture food and the various materials used to package food, to troubleshooting problems such as foreign bodies, faults in the food itself, or poorly performing food packaging materials.

This may involve a combination of long-term research work on one day, using more traditional microscopical methods, followed the next day by a rapid investigation of a problem requiring an immediate solution, using rapid methods such as temporary light microscope mounts of hand sections, smears or squashes in a stain mountant such as Toluidine Blue. The cryostat usually sees a great deal more use than the bench microtome in a food microscopy laboratory. There may be no time for a perfect tissue section, but a wedge-shaped hand section will often provide one area which is suitable for study and a resolution of the problem, even if the photographs taken would not be of the quality required for publication in a journal!

The food microscopist will usually work in conjunction with other people in the food industry. These may be new product development or sensory analysis staff when dealing with food structure, quality assurance and technical managers in connection with foreign body investigations, or packaging technologists when trying to solve a packaging problem. A wide range of different microscopical techniques may be employed, including light microscopy of various types, scanning electron microscopy, confocal microscopy and various methods of analysing or interpreting images, as well as microanalytical techniques such as energy-dispersive X-ray microanalysis. A constant requirement is the need to present results in a way that can be readily appreciated by non-microscopists or even non-scientists.
Food Structure and Function

The microscopic structure of any food is fundamental to its function, and Vaughan (1979) summarised the state of the art at that time for the microscopy of a range of different foods. However, most of that excellent book deals with basic foods such as meat, fish, eggs, fruit and vegetables etc. Much of the work of the food microscopist involves supporting the work of food technologists in developing complex new food products. For example, there is a wide range of thickeners that can be used in sauces, gravies and dessert products. These include gelatine, starches, pectins, and various gums such as xanthan or locust bean gum. Moreover, the requirements for a commercial food product may be quite different to those in a domestic kitchen. Whilst a home-cooked food may be made, cooked and eaten within hours, a commercial equivalent of the same food may have to withstand cooking, chilling or freezing, transport, long-term storage and then a second cooking process before being eaten. At the same time, it will still have to imitate, as far as possible, the properties of the home-cooked version.

For this reason, the new food product technologist has to choose one of a wide range of different thickeners or stabilisers to produce the required texture in the final product. The microscopist may be involved in the examination of the structure in a range of different trial products to help determine the most suitable stabiliser or thickener for that particular application – which may not necessarily be the most obvious first choice. In another investigation, the ability of the microscopist to differentiate between a wheat starch added at one stage in a process, and a modified maize starch added at a different point in the process, and in addition to distinguish between gelatinised and ungelatinised starch granules, led to an explanation for a puzzling loss of viscosity in a sauce product. The book by Olga Flint (1994) provides a useful practical guide to this kind of work.

One of the most popular thickeners for use in dessert products is gelatine, because of its great versatility, but its use in any product means that the market for that product necessarily excludes vegetarians. It would therefore be very useful for a manufacturer to be able to avoid the use of gelatine in a product. A recent research project carried out at CCFRA examined the potential for the use of various other thickeners as replacements for gelatine in dessert products. Part of this project included a light and scanning electron microscope study of some of the structures produced by different thickeners, in an attempt to understand how they work, and why some are unsuitable in certain situations. Environmental Scanning Electron Microscopy (ESEM) appeared to show small pools of liquid water within the gel structure in some commercial mousse products. Because most food materials contain large amounts of water or fat or both, Scanning Electron Microscope (SEM) systems that enable control of both variable pressure and moisture within the sample chamber have great advantages for the study of food systems without the need for either freezing or drying, both of which may change the structures being observed. The ability to add or remove moisture whilst a specimen is under the electron beam opens the possibilities for dynamic experiments to study the role of water in food systems.

Biscuits are relatively dry foods which are much more amenable to SEM study. However, they are not the easiest of materials from which to prepare thin sections, limiting other techniques that can be employed. A multidisciplinary study of the fundamentals of biscuit manufacture involved co-operation between biscuit technologists, sensory analysts and microscopists in developing an understanding of the interactions between recipe formulation, the manufacturing process, and appreciation of biscuit texture in the mouth. Part of the microscopy work involved the use of confocal microscopy to construct three-dimensional images of biscuit structures made in different ways. Polarsised light microscopy was also used in conjunction with differential scanning calorimetry (DSC) to understand the effects of tempering (holding at specific temperatures for several hours) and ageing on fat crystal structure. Scanning electron microscopy was used to develop an understanding of the intimate relationship between starch granules and fat in biscuit crumb structure, and also demonstrated the presence of glasy areas of melted sugar when coarse sugar crystals are used, giving a very firm “crunch” as found in, for example, ginger biscuits.

Much of the work of the food microscopist involves supporting the work of food technologists in developing complex new food products.

Despite its relatively high fat content, chocolate can be examined without prior preparation in the SEM, provided a little care is taken to keep the probe current low so as to avoid melting the chocolate surface. Combined with x-ray microanalysis, this has provided an elegant way to examine the efficiency of mixing in the production process, by using calcium and phosphorus as markers for the milk solids (Brooker, 1990). More recently, SEM has proved to be an excellent way to evaluate the efficiency of systems designed to introduce minute bubbles of air into chocolate, simply by examining a fracture surface.

Food Packaging

Food packaging has a number of different functions. It contains and protects the food, and prevents contamination by chemicals or foreign bodies. It also prevents microbiological deterioration or reaction with atmospheric oxygen. Packaging is used increasingly as part of the marketing of a food product, but environmental and commercial pressures also mean that as little material as possible must be used in the construction of the package, and that after use the package should ideally be either recyclable or biodegradable. All of these aspects will affect the microscopist’s work.

Traditional forms of packaging such as glass or tinplate are well established and reliable, but occasionally problems occur in which microscopy and microanalysis can form an important part in finding a solution. When tinplate is affected by corrosion and/or leakage of the can, it is important to be able to establish whether this was caused by failures during manufacture or sealing of the can, by an interaction between the can and the product, or by external mechanical or chemical damage. The
A microscopist can work together with the canning technologist and other analysts to help determine the cause of the problem. For example, many food cans are coated with an internal lacquer to prevent the food reacting with the tinplate surface. Because the surface layer of tin is covered with lacquer and is therefore unable to offer the anodic protection of the metal surface found in a plain unlacquered tinplate can, small gaps in the lacquer surface may result in pitting corrosion of the internal can wall and rapid subsequent leakage. Such lacquer holes may form around bubbles or lumps in the lacquer. These lacquer faults are readily identified by either light or scanning electron microscopy.

Plastic packaging materials often rely on an effective heat seal at the edge of the pack. Where failures occur, a compound light microscope study of cryo-sections of the failed seal will often identify where it has failed and why – possibly a misalignment of the sealing jaws resulting in a narrower seal than specified, for example.

It is important to exclude oxygen as far as possible from many food packs in order to avoid oxidation and rancidity. However, many plastics that are commonly used for food packaging are permeable to oxygen, so it is necessary to include an oxygen barrier layer within the structure. This may sometimes become too thin to perform its function adequately, for example due to stretching of the material during formation of a plastic food tray or pot. A light microscope study of cross-sections of the material at the critical points may indicate how the design may be modified to avoid the problem.

Where a food packaging material fails to perform as expected, a combination of microscopy and microanalysis may help to identify the cause of the problem. For example, there is a wide range of different lidding films available to the food manufacturer, intended to be heat-sealed onto the edges of a plastic tray. Where the seal fails, a first check is usually to ensure that both tray and lidding film are those originally specified. Light microscope sections of multi-laminate materials, combined with FT-IR microscopy to check the composition of the various layers, will often identify the cause of the problem.

The development of novel forms of packaging is an important part of the food industry. For example, new oxygen barrier materials present an important challenge to the microscopist, because materials such as AlOx and SiOx are used in much thinner layers than the traditional EvOH, making them much harder to detect. The performance of some clear films used to pack fresh herbs relies upon the presence of micro-holes to allow controlled gas exchange across the plastic film, but these are usually invisible to the naked eye. Microscopy can be used to confirm their presence and measure their diameter, but finding a few small holes in a large area of packaging under the microscope can be a difficult task unless one understands that such holes are not usually randomly distributed, but are generally produced at regular intervals in straight lines using a laser. The development of biodegradable and compostable packaging materials presents yet another challenge in understanding the interactions between the food product and its packaging, in which microscopy will play a valuable role.
Foreign Body Identification

The first occasion on which a food technologist comes across a microscopist is often when a foreign body needs to be identified, and this is often an opportunity for the microscopist to impress! Virtually all food and drink companies have to cope with foreign body complaints at some time, and the range of items that get reported as foreign bodies is almost limitless. Because of the wide range of samples that may be encountered, a broad spectrum of identification methods may have to be employed, culled from many different scientific disciplines (Edwards, 2006). Sometimes the work takes on the character of a forensic investigation, looking for minute traces of deposits on the surface of a foreign body that can supply clues as to what it has been in contact with. A useful definition of a foreign body is “something that the consumer perceives as being alien to the food”. This may include things like glass fragments, metals, plastics and stones, which are clearly alien to the food. However, it may also include fragments from the food itself, such as bits of vegetable matter or stalks in plant products, or fragments of bone or cartilage in animal or fish products. Very often foreign bodies derived from the food itself will be a familiar problem for the food manufacturer and therefore will be unlikely to require the services of a microscopist. It is the more difficult samples that are more likely to require detailed investigation.

The key to foreign body identification is very often an understanding of the structure of the sample, with chemical analysis being used to confirm the structural observations. Initial examination is often carried out at low magnifications under a light stereomicroscope, and the sample will usually be photographed at this stage for evidential purposes. Scrapings of surface deposits may then be taken for compound microscope examination, or hand sections or cryo-sections taken of biological material. Compound light microscope examination is often crucial to understanding the structure of a sample, and may provide definitive evidence to make an identification, in, for example, the identification of animal hair. A range of histochemical stains familiar to biologists may well be employed to confirm the composition of the sample. The distribution of lignified tissue, as shown by staining with phloroglucinol-hydrochloric acid, may be important in distinguishing different types of woody tissue. Woody fragments may come from wooden boxes or pallets, usually made of softwood from shrubs or trees in hedgerows at the edge of fields, or from woody tissue in plants such as carrots, peas or cabbage stems. Polarised light microscopy is invaluable in examining crystalline materials and also many fibres, such as those found in rope or sacking. Differential Interference Contrast (DIC) or Nomarski optics will sometimes demonstrate the presence of unsuspected microscopic structures in unstained material. Fluorescence microscopy is also valuable, for example in demonstrating the presence of bacteria on surfaces.

Energy-dispersive X-ray microanalysis in the scanning electron microscope is a very quick, non-destructive way to identify the main elements present in a sample, and is often valuable where other methods of identification have failed completely. Once the analyst knows what the sample is made up from, it may be possible to reinterpret other evidence in a new light. However, X-ray analysis is particularly useful in identifying a range of inorganic materials such as glass, metal or stone, enabling distinctions to be drawn between different glass types or different types of stainless steel. The ability to map the distribution of elements across a sample can be very useful in understanding its structure. For example, whilst the presence of zinc, tin or nickel plating on the surface of a steel item may be quite obvious in an undamaged item, in an unidentified and damaged item, the distribution of the different metals may be crucial to understanding its structure and hence the identity of the item.

Another useful microanalytical technique is FTIR microscopy. FTIR spectroscopy complements X-ray microanalysis in analysing organic materials whilst X-ray microanalysis is used for inorganic materials. Whilst larger samples can be readily analysed in a normal benchtop FTIR spectrometer, the addition of a microscope enables much smaller items to be analysed, together with the ability to analyse small portions of a sample in isolation. One example might be in demonstrating that a fragment of plastic is in fact a piece of food packaging material composed of several different layers including an oxygen barrier layer.

It is often important to be able to establish whether or not a foreign body has been processed with the food. In the case of insects and other foreign bodies that were once living, it may be possible to employ the alkaline phosphatase test. The alkaline phosphatase enzyme is found in virtually all living organisms. It remains active for a long period after death, but activity is destroyed by heating, so a test for alkaline phosphatase activity can be used as a means of determining whether or not an insect has been heat-processed. There are, however, many difficulties with this test, so very often other means...
**Allergies**

With increasing public concern regarding allergies and intolerances to particular food ingredients, the microscopist is often called upon to help identify suspected allergenic material. This requires a detailed knowledge of the structure of these materials so that the presence of diagnostic cells or tissues can be determined in the suspect sample. For example, peanut tissue is characterised by tissues which can be determined in the suspect sample. This means that the internal surfaces must be very smooth, so as not to provide crevices for food material to accumulate or for bacteria to grow. Whilst tight corners can be designed out of pipework and particular finishes can be specified for stainless steels used in machinery manufacture, the rubber gaskets needed to effect a seal between adjacent pieces of machinery must also have very smooth finishes. Scanning electron microscopy of the food contact surfaces of such gaskets during the design stage can help to avoid surfaces with grooves produced by the lathe machining the mould for the gasket, or holes in the gasket produced by air pockets or mineral fillers.

Failures in food manufacturing equipment sometimes result from poor work carried out during modification or repair, perhaps caused by the employment of sub-contractors not fully conversant with the particular needs of the food industry. For example, a weld failure around a repair to a tubeplate was examined using X-ray microanalysis in the Scanning Electron Microscope. The large areas of titanium found around the weld indicated that the wrong type of welding rod had been used in the repair, which then failed under pressure during subsequent use.

**Food Manufacturing Equipment**

Good design of food manufacturing equipment is essential to the modern food industry. Such equipment must be made of appropriate materials, it must perform efficiently and it must be designed to avoid contamination by micro-organisms or by foreign objects. Much food manufacturing equipment is designed to be Clean In Place (CIP); in other words, to be cleaned periodically by passing suitable cleaning solutions through it rather than being regularly dismantled for cleaning. This means that the internal surfaces must be very smooth, so as not to provide crevices for food material to accumulate or for bacteria to grow. Whilst tight corners can be designed out of pipework and particular finishes can be specified for stainless steels used in machinery manufacture, the rubber gaskets needed to effect a seal between adjacent pieces of machinery must also have very smooth finishes. Scanning electron microscopy of the food contact surfaces of such gaskets during the design stage can help to avoid surfaces with grooves produced by the lathe machining the mould for the gasket, or holes in the gasket produced by air pockets or mineral fillers.

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**REFERENCES**


**Dr Mike Edwards**

Microscopy Section,
Campden & Chorleywood Food Research Association
Chipping Campden, Gloucestershire, GL55 6LD UK
m.edwards@campden.co.uk

Mike trained as a plant pathologist and has headed the Microscopy Section at Campden & Chorleywood Food Research Association (CCFRA) since 1987. The prime responsibility of the Microscopy Team at CCFRA is the identification of foreign bodies that have been reported in food products. This work uses a wide range of microscope techniques, including light and scanning electron microscopy, X-ray microanalysis and FT-IR microscopy.

Research and development work is also carried out on food structure and texture, product development problems, problems with food packaging of all kinds, and on micro-organisms on food and machinery surfaces. Mike has also worked on surveys of tin and canned food products and on lead and cadmium in food. Mike is Technical Secretary to the RA’s Food Science Panel.

Prior to joining CCFRA, Mike carried out microscopy research in plant pathology and plant physiology at the University of Aberdeen.