Electron Microscopy at SCRI

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As you read this, it is sobering to realise that there has been a continuous electron microscopy service at SCRI for over 43 years, making it a research presence that started before most of the current staff were born. It also means that, because this was a new technology in its infancy, this Institute was at the forefront of the development of electron microscopy in biology, particularly in the study of plant viruses. Initially SHRI, as it was then called, borrowed time on an old EM 2 electron microscope that was the first EM in Scotland, (Fig. 1). This was supplanted in 1958 by a second-hand Metropolitan Vickers EM3A microscope that boasted a 'fitted' binocular for focusing, and a large luminous faced clock, just in case you were so engrossed with the work that you forgot that it was time to go home, or, more likely, unable to see your watch in the complete darkness! This electron microscope had a few unusual features. Its first user

Figure 1 EM 2 electron microscope (c.1958).

here was a Dundonian called Jimmy Cathro (of whom more later), and he, like almost all early electron microscopists, topped six feet in height. Unfortunately, the console of this machine was rather too low to the ground, either to sit comfortably or to view the screen through the binoculars. His answer…. raise the whole microscope to a more suitable height, which he did by standing it on layers of wooden disks, actually the 'holes' cut from lavatory seats by a local manufacturer, as shown in the accompanying picture (Fig. 2). The other 'unusual' feature

Figure 2 Metropolitan Vickers EM 3A electron microscope (1960).

was that this EM had two massive radiation leaks, one through the gun, and the other through the camera chamber that effectively bathed the operator's lap in unmentionable amounts of radiation that was thought to be damaging to the male reproductive organs. However, since its last operator went on to father two healthy children, this seems to have been an unfounded fear.

Such was the impact of the EM on the Virology research, that in 1961, the A.R.C Visiting Group authorised the purchase of a new EM which was installed in 1962, a German machine called the Siemens Elmiskop I which cost £11,000 (Fig. 3). At

Figure 3 Siemens Elmiskop I electron microscope (1962 – 1980).

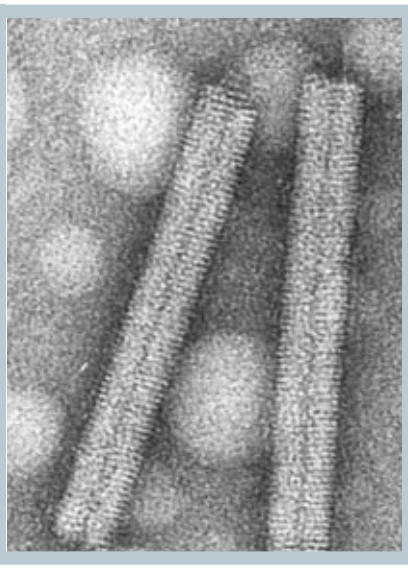

Figure 4 *Tobacco rattle virus (TRV)* particles in uranyl formate negative stain, imaged using the Siemens Elmiskop, and showing the 2nm helical periodicity.

the time, this was the 'Rolls Royce' of electron microscopes. It was capable of resolving 2nm routinely and some of the early pictures taken by Jimmy Cathro on this microscope are still classics and were used in text books. To do justice to this fabulous new equipment, an old pea shed was converted to form the first SHRI Electron Microscopy suite. Not long after the installation of this electron microscope, the National Radiological Protection Board (NRPB) visited the site to establish the safety (or otherwise) of this new radiation source. Imagine, therefore, the considerable concern when they found that a focused beam of X-rays was being emitted from a flaw in the electron gun casing, and was passing uncomfortably close to the Farm Manager's chair in his office next door. Lead sheeting was immediately glued on to the gun, thus permanently curing the problem. One interesting aspect to the purchase of this EM was that, in those far off days, considerable pressure was put on scientists to 'buy British'. At that time, the only UK manufacturer of electron microscopes was the AEI company whose microscopes were 'Morris Minors' compared to this German 'Rolls Royce', and realistically were not serious opposition. To actually purchase this foreign machine, the Institute therefore had to pay a 30% cost penalty to Customs and Excise, before it could be imported. So much for scientific freedom! Nonetheless, the fact that you had a 'Siemens' meant that you were envied by everyone else for the next ten years or more. Time would, however, prove that this was a wise investment, because it performed to its original specification for the 18 years it served the Institute. It comfortably resolved the 2nm helix of *Tobacco rattle virus (TRV)* (Fig. 4), kept SCRI at the forefront of electron microscopy, and helped to establish its international reputation for plant virus research under Bryan Harrison. The Siemens Elmiskop I, which moved into the old Laboratory Service workshops in 1968 when they were converted into the present EM laboratories, left SCRI in 1980 in bits, as spares for another 20 year old machine operating at the Moredun Institute, Edinburgh, complete with part of the then service engineers thumb, the legacy of a mishap with a high voltage valve and an earthing strip.

When Colin Cadman became the second Director of SHRI in 1965, one of his first changes was to split Virology and create the fledgling Zoology section under Charles Taylor, thus establishing a co-operation and rivalry which continued for the next 30 years or so. This resulted in the acquisition of the Institute's second EM, a Jeol Superscope for Zoology in 1967, thus setting the precedent of having two Departments, each with its own EM unit and technicians. This new EM was unusual in that the column was angled like the barrel of a cannon and you sat in front looking down the 'muzzle' (Fig. 5), and the electron beam was so poorly screened that it could sometimes melt the copper support grids of inexperienced operators. One wonders what it did to the operator, but like the present incumbent, he not only survives, but thrives. Its poor performance and difficulty of use thus led to the purchase of a new EM for Zoology, the

Figure 5 Jeol Superscope electron microscope (1967 – 1970).

Figure 6 Hitachi HS 8 electron microscope (1970 – 1985).

Hitachi HS8 (Fig. 6) in 1970, and this gave good service for 15 years until its replacement in 1985, when this microscope was put into an electron microscope collection in an Edinburgh museum. Prior to the arrival of the Hitachi microscope, Zoology made use of the Siemens Elmiskop, usually after normal working hours, and this EM produced the first ever micrographs of spherical virus particles located at a specific retention site within its nematode vector (Fig. 7). Such was the rivalry in the quest for scientific scoops in those early days, that, because we (Virology) had the only darkroom used to develop the photographic plates, the 'opposition' would sometimes develop

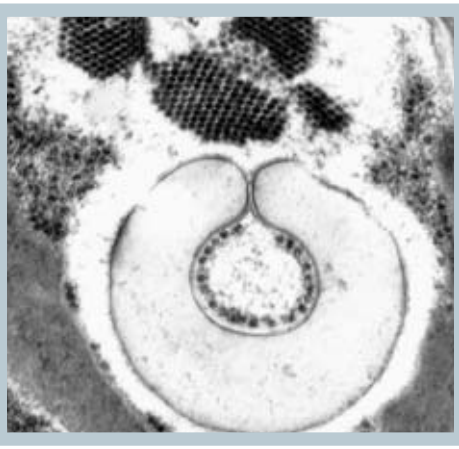

Figure 7 Spherical particles of *Raspberry ringspot nepovirus (RRV)* attached to the lumen of the odontostyle of the vector nematode *Longidorus macrosoma.* Small crystalline clumps of virus lie free in the foregut.

theirs after hours, in case sensitive results became common knowledge!

Along with the rest of the biological science community, electron microscopy moved ahead swiftly with new developments in staining, ultramicrotomy and imaging, nowhere more rapidly than in the field of virology. A second Virology EM was therefore bought, ostensibly as a replacement for the existing microscope, but this was reviewed in the light of the volume of work. This brought the number of EMs to three, thus making SCRI unrivalled in Scotland and only surpassed by the John Innes Institute, Norwich, which had four. This latest acquisition was a Philips EM 301G, (Fig. 8) the new 'leader of the pack' and it was installed in 1973 in rooms specially constructed for it. In those halcyon days, it was common Institute

Figure 8 Philips EM 301 G electron microscope (1973 – 1990).

policy to allow you to choose your own colour scheme, and the new Philips was surrounded by dull yellow and deep purple, ideally suited to rapid dark-adaption of the eye. However, this colour scheme did not meet with universal approval, notably by one Tony Murant who maintained he 'wouldn't be seen dead in there', thus evoking the instant retort by one of the female technicians, 'that's why we chose it!'. This microscope was fitted with the first eucentric goniometer stage (hence the 'G' in the name, and now a standard stage for all new EMs) which allowed the operator to examine a specimen under tilt conditions, and thus permitting 3D reconstruction from a 2D image. To mark the auspicious occasion of its installation, a cocktail party was held in the new refur-

bished EM suite, hosted and supplied by Bryan Harrison, and enjoyed by all (except Zoology). At the time, this electron microscope had the highest routine performance specification using a conventional specimen stage, and was guaranteed to give a resolution of 0.34nm on site using graphitised carbon as a test specimen. In fact, our new microscope did considerably better, actually achieving 0.17nm, the half-lattice spacing of graphite, as can be seen from the micrograph in Figure 9.

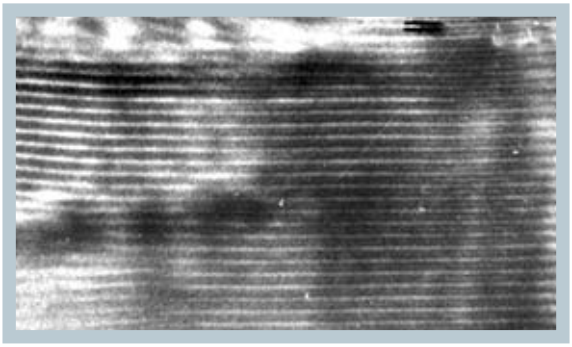

Figure 9 Graphitised carbon test specimen for electron microscope resolution, showing the 0.17nm half-lattice spacing of the crystals.

Throughout this period of often frenetic and competitive EM activity, additional equipment essential to the units was purchased. This included new photographic enlargers, printing equipment and also new ultramicrotomes, bringing the number of these machines to three. However, these new microtomes were not without their problems. Manipulating these minute sections required very fine points, so it became (and remains) a common practice to 'acquire' eyelashes from the ladies in the department. It soon became clear, however, that not all eyelashes were of the same standard and so the girls with dark hair were the most sought after, one instance when natural blondes were not the preferred choice! Another common problem was static electricity which created charges on the sections and the specimen block. This showed the true dedication of the female technicians who removed (or should I say changed) their nylon underwear and stockings, in an attempt to cure the problems. A new vacuum coating unit, somewhat unkindly called 'the black coffin' by some members of staff, also came into use. This was designed and built on site in 1970 and for many years sported a small white label inscribed 'manufactured by Roberts, Robertson & Co.' The company referred to was one Aileen K. who supplied Ian and Walter with coffee and biscuits during the

Figure 10 Jeol 100 S electron microscope (1980 – 1997).

construction period, and who smilingly ignored the strong language when things did not always go according to plan. This vacuum coating unit was in constant service until October 2000, when it was replaced by something more visually appealing.

When the Siemens microscope reached the end of its working life the intention was to replace it with a new Philips microscope. At that time there was intense rivalry between Philips and Jeol as manufacturers, and all sorts of enticements were offered to prospective buyers, primarily by Jeol. Thus it was that in 1980 SCRI got two electron microscopes for the price of one, a Jeol 100S transmission microscope like the previous ones (Fig. 10), and the Jeol T200 our first (and only existing) scanning electron microscope (Fig. 11),

Figure 11 Jeol T 200 scanning electron microscope (1980 – present).

Figure 12 Jeol 1200 EX electron microscope (1985 – present).

thus bringing the total to four. This SEM was improved by the purchase of a cryo-transfer stage in 1985, which allowed examination of fully hydrated specimens with minimum preparation, at temperatures as low as –140°C, and expanded its usefulness for the examination of specimens such as fungi, nematodes, mites, and other soft tissues. The Jeol 100S transmission microscope was particularly easy to use, and thus was ideally suited to training new users or visitors. It gave good service until 1997, when the electron microscope suite was completely refurbished, at which time it was decommissioned to leave only the two better transmission microscopes, and the scanning microscope.

Figure 13 Philips CM 10 electron microscope (1990 – present).

In 1985, the Zoology department replaced its aging Hitachi with a Jeol 1200EX (Fig.12), which initially had a STEM (scanning transmission and analysis) unit attached. This EM was extremely versatile and was suitable to biological and physical applications. The Philips 301G was sold to one of the Oxford colleges, and replaced, in 1990, with a Philips CM10, a dedicated biological electron microscope (Fig. 13). Thus, together with the 1200EX and the SEM, SCRI had an impressive electron microscope facility in terms of equipment and its versatility. With the renovation of the microscopy laboratories, the disposal of one 'spare' electron microscope in 1997, and the unification of the facility as an Institute service under the Cell Biology banner, SCRI is extremely well placed to extend its expertise and reputation in this field for many years to come.

Figure 14 Jimmy Cathro, Electron microscopist 1960-1968.

A review of the electron microscopy facilities at the Institute would be incomplete without some mention of staff. The first electron microscopist at SHRI, as it was then, was Jimmy Cathro, as mentioned earlier. He was a professional glass blower and had been in charge of Laboratory Service before moving to Virology to operate the electron microscope. He was a true perfectionist and having taught you how to make the perfect Pasteur pipette, proceeded to teach you how to make glass animal and bird ornaments, or, seasonally, coloured glass baubles for the Christmas tree. In his search for perfection, Jimmy Cathro also found a unique solution to the problem of obtaining suitable glass for making ultramicrotome knives.

Since the best knives were achieved with 'strain free' glass, and this was not yet commercially available, we made several trips to the Dundee tram's graveyard in the mid 60's to collect the remains of the windows in large metal pails. This glass, having rattled around the streets of Dundee for several decades was essentially strain free and supplied the unit with excellent knives for some considerable time. His genius, however, was sometimes flawed, and on one notable occasion, he reluctantly sought out the Director to ask for a replacement anglepoise lamp, because he 'couldn't get the bulb out'. In truth, this piece of equipment gave him an electric shock, whereupon he threw the offending article against a wall (accompanied by a few well chosen words), and then proceeded to systematically kick it around the EM room until it was in bits, the lamp cover buckled but with the bulb still intact inside! He could also be slightly eccentric at times, sometimes sitting at the microscope wearing a crash helmet lined with lead, ' to protect him from stray radiation'. As mentioned earlier, Jimmy was also over 6 feet tall, a feature common to many of the early breed. The reasons for this was that the early models of electron microscopes were mechanically aligned, thus necessitating the adjustment of the electron gun c.6 feet off the ground while sitting down and looking through the binoculars. This was only possible if you were above average height, or possessed gorilla-like arms. The aforementioned Aileen K., who was all of 5 feet tall, had to stand on a stool, reach the high controls with one hand while bending and peering down one ocular from the side! The prerequisite for tall operators disappeared in the mid seventies, with the advent of electrical alignment controls. Jimmy Cathro was electron microscopist for 10 years, and since he left in 1968, there have been only five staff changes in the Units. Following the recent early retirement of Walter Robertson after 30 years in Zoology there remain two 'dinosaurs' who have accumulated over 60 years between them. This 90 year continuing service record of electron microscopy is probably unequalled anywhere. Much of this long service has to do with the nature of the job, experience counting perhaps for more than anything else, because by nature it is highly skilled, labour intensive, and generally slow with regard to the time required to produce results. It takes a minimum of 2 years to become proficient in this line of work, and the learning curve continues steeply thereafter. Like the real dinosaurs, the present incumbents are of course irreplaceable, but the Institute should be looking forward to alternatives to ensure the future of this facility.

It is difficult to put into context the contributions of electron microscopy to the scientific research of the Institute, in the UK, and also world wide. SCRI has been responsible for training scientists from all corners of the globe, and staff have set up electron microscope laboratories in countries such as Peru, Pakistan, and India, thus establishing fruitful collaborations, many of which exist still. Although for most of their working lives the microscopes have remained within only two Departments, they have contributed significantly to the scientific reputation of SHRI and SCRI. It is also probably fair to say that no other single piece of scientific equipment has been so influential to the science of the Institute. Within Virology alone, more than 20 percent of publications since 1962 have had some electron microscopy input and, including those from Zoology and external collaborations, this

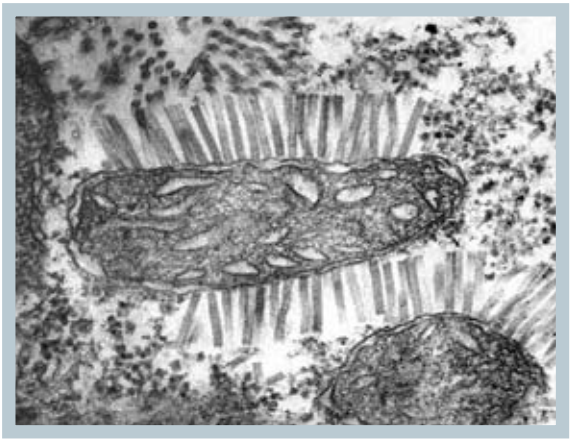

Figure 15 Particles of *Pepper ringspot tobravirus (PRSV)* attached to the outer membrane of a mitochondrion in a leaf cell.

amounts to c. 200 publications, some of which have been scientific landmarks. As a science in its own right, many papers were themselves milestones, either in technical innovation or in scientific discovery, and set the pattern of recognition and reputation which exists to this day. Among the many notable discoveries were the first association of a plant virus with mitochondria (Fig. 15), and the first detection of a plant virus at specific sites within the food canal of vector nematodes (Fig. 7). Complementary studies of virus/vector combinations provided much new information on the ultrastructure of the feeding apparatuses of certain nematodes, aphids and mites, subtle differences in the structure of the cuticle between different species of nematodes within the same family, and the first evidence of a chemoreceptor gustatory

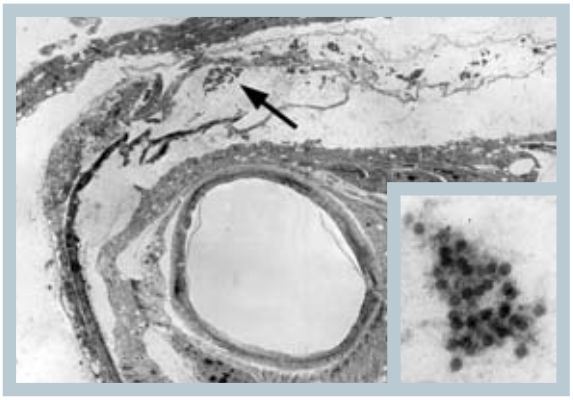

Figure 16 Median section through the head of the aphid *Cavariella aegopodii* showing the oesophagus and food contents. Arrow indicates the specific site of location of virus particles (inset).

organ in a nematode. Numerous other studies of ultrathin sections of virus infected leaf, plant or insect material gave us *Rhabdoviruses, Badenaviruses, Closteroviruses* and others in diseases of soft fruits and their vectors, mycoplasmas in potatoes infected with potato witches broom disease, and *Nepoviruses* in tubules passing through plasmodesmata. Of major significance was the discovery for the first time of the location of two semi-persistent viruses, *Parsnip yellow fleck (PYFV)* and *Anthriscus yellows (AYV)* at specific sites within the feeding apparatus of their vector aphid *Cavariella aegopodii* (Fig. 16). Elsewhere, studies on the leaf architecture and the types, numbers and the location of plasmodesmata have also helped in our understanding of the role plasmodesmata play during leaf development, and in virus movement and cell-to-cell signalling, complementing other studies using green fluorescent protein (GFP) and the confocal laser scanning microscope (CLSM).

In other work with GFP, we were the first to illustrate that this protein could be incorporated into the capsid of a plant virus, *Potato virus X (PVX),* without adversely affecting its assembly or replication in cells (Fig. 17), thus demonstrating its potential for protein

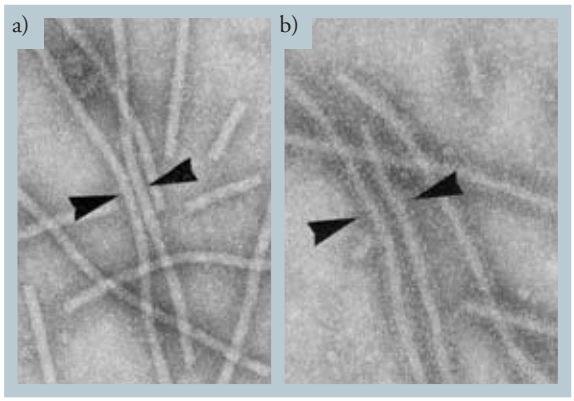

Figure 17 Negatively stained particles of *Potato virus X (PVX)* (a) and the additional coat of GFP protein (b) which does not affect virus assembly. Arrowheads illustrate the marked difference in the centre-centre spacing of adjoining particles.

overcoat technology and its implications for the production of therapeutics. These discoveries, together with those mentioned elsewhere, illustrate the wide range of techniques which are available, and which have done much to help our understanding of the interactions between plants, virus pathogens, and their vectors. Several new technical advances also had their origins in SCRI, including the Heat Pen for removing the cutting compression from ultrathin sections, a grid holder which later formed the basis of the grid block for modern automatic staining modules, and a method for coating glass knives with a thin film of tungsten, an advantage which had to wait almost 20 years for the new technique of cryo-sectioning to make full use of it. Aspects of virus architecture were also unveiled in the determination of the structure of the disk aggregates of *Tobacco rattle virus (TRV)* and the handedness of its helix, and in the first illustration of the surface structure of a *Nepovirus viz.* the T=1 capsid morphology of particles of *Tomato ringspot virus (TRSV)* by freeze drying and high resolution shadowing with uranium metal (Fig. 18). This latter discovery resulted from the design and construction, and ultimately the commercial production, of a freeze-drying module. This Institute was also at the forefront of the developments in immunoelectron

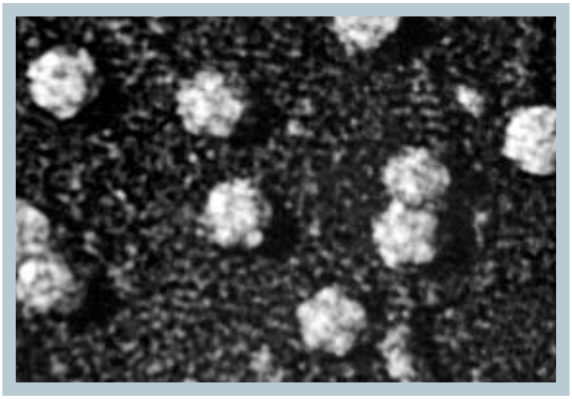

Figure 18 Particles of *Tomato ringspot nepovirus (TRSV),* after freeze-drying and high resolution shadowing with uranium metal. The T=1 capsid structure is clearly seen.).

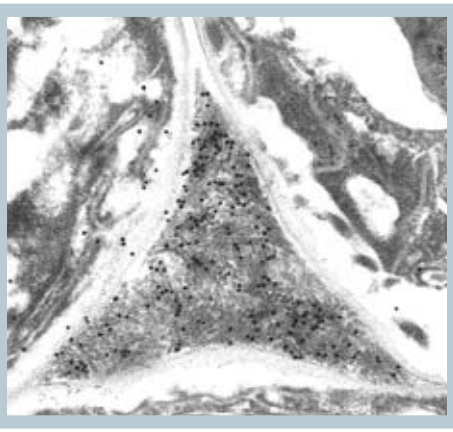

Figure 19 Immunogold labelling of particles in the oesophageal lumen of the vector nematode *Paratrichodorus anemones* confirming the identity of the tobravirus *Tobacco rattle (TRV).*

microscopy, where serology and electron microscopy were combined to aid the detection and identification of viruses in extracts of leaves, roots, petals etc. and in insect vectors such as nematodes and aphids. Variations of these techniques therefore gave 'firsts' for the detection of *Potato leafroll luteovirus (PLRV)* in a single aphid, *Raspberry ringspot nepovirus (RRV)* in a single nematode, as well as determining more precisely the serological relationships between *Luteoviruses* and between *Geminiviruses.*

Complementary to these immuno-techniques for viruses in extracts is immunogold labelling (IGL). This technique involves conjugating or tagging specific antibodies with small (5-20nm) gold particles, thus allowing the identification and/or localisation of

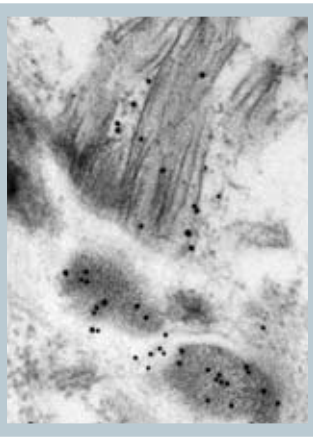

Figure 20 Immunogold labelling of *Pea seed-borne mosaic virus (PSbMV)* infected tissues, confirming the presence of the virus coat protein in the plasmodesmal cavity and on the cylindrical inclusions (CI).

viruses or viral proteins in negatively stained extracts, or in ultrathin sections of cells. An application of this technique allowed us to prove for the first time that the rod-shaped (putative) virus particles found in sections of vector nematodes (Fig. 19), were indeed those of the tobravirus they transmitted. In collaborative work with the John Innes Institute, IGL was a key feature in the studies of the location and distribution of *Pea seed-borne mosaic virus (PSbMV)* at the infection front in peas. In this study, IGL confirmed the role of the cylindrical inclusion (CI) in cell to cell movement, and further showed that, while both virus coat protein and that of the CIs could be found associated with the cell walls and plasmodesmata, only virus coat protein was present in the plasmodesmal cavity (Fig. 20). IGL was also instrumental in determining specific epitope sites on the surface of the

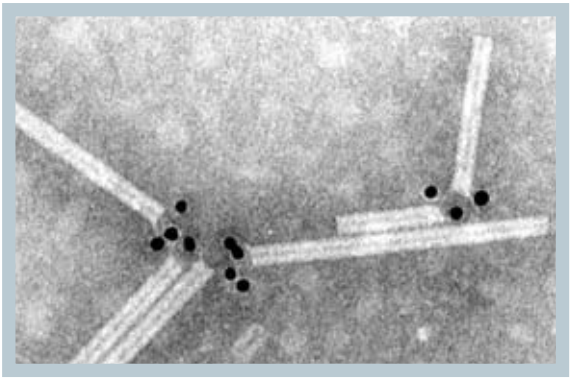

Figure 21 Immunogold labelling of negatively stained particles of *Potato mop-top virus (PMTV).* The gold particles have been conjugated to a monoclonal antibody that recognises an epitope at one end of the virus particle.

virus capsid of *Potato mop-top virus (PMTV),* by direct conjugation of monoclonal antibodies to a gold probe (Fig. 21), and convincingly demonstrated that different monoclonal antibodies recognised epitopes at different sites on the virus particles. A particularly effective application of this technique was in studies of the expression of the coat protein gene of *Potato leaf roll virus (PLRV).* In these studies, IGL conclusively demonstrated that the quasi-crystalline structures found in the nuclei of insect cells expressing this gene consisted of isometric particles, indistinguishable from those of PLRV. A fundamental part of the *in situ* hybridisation (ISH) technique to detect RNA in sections also makes use of antibodies conjugated to gold particles. In very recent work, this has shown that the nucleic acid of a plant virus (*Tobacco mosaic virus, TMV*) co-localises in leaf cells with the non-structural

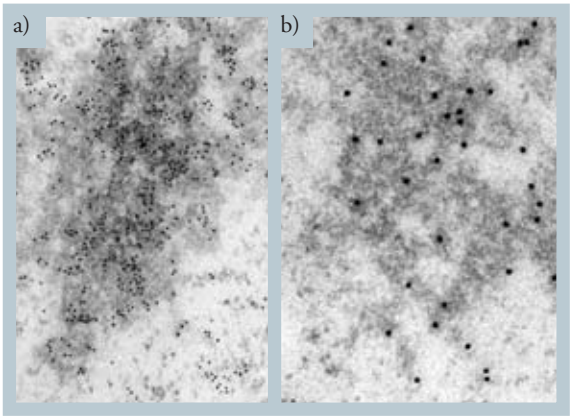

Figure 22 Co-localisation of the movement protein of *Groundnut rosette virus (GRV)* with RNA of *Tobacco mosaic virus (TMV)* by (a) conventional immunogold labelling of the coat protein, and (b*) in situ* hybridisation (ISH) of the RNA..

long-distance movement protein encoded by the unrelated *Groundnut rosette virus (GRV)* (Fig. 22).

Scanning electron microscopy (SEM) has also played a significant role in the Institute's research and has led to some novel findings. In particular, the low temperature studies of mites which infest blackcurrant species, and are responsible for the transmission of blackcurrant reversion virus, has provided new information on the taxonomy of these creatures, and showed that subtle differences in the pattern of folds on the insect's head shield could be used to give unequivocal identification (Fig. 23). Other notable SEM observations include the finding that dry *Botrytis* fungal spores dusted on the surface of rose petals (Fig. 24) could germinate and penetrate the surface, in the absence of water droplets, and that sporulating colonies of powdery mildew (*Blumeria graminis* f.sp. *hordei*) often originated from more than one conidium, even at low inoculum density (Fig. 25).

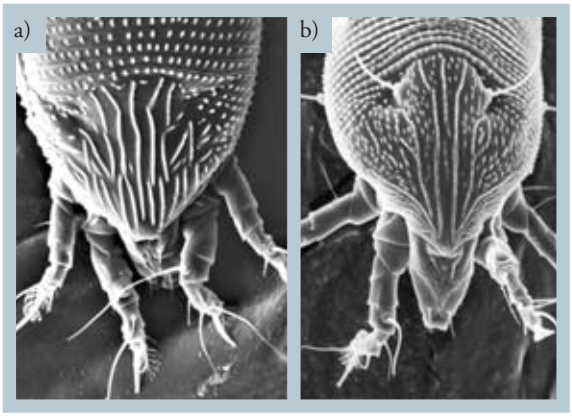

Figure 23 Scanning electron micrographs of (a) *Cecidophyopsis selachodon* and (b) *Phylocoptus gracilis*, showing the differences in the head shield structure which can be used for identification purposes.

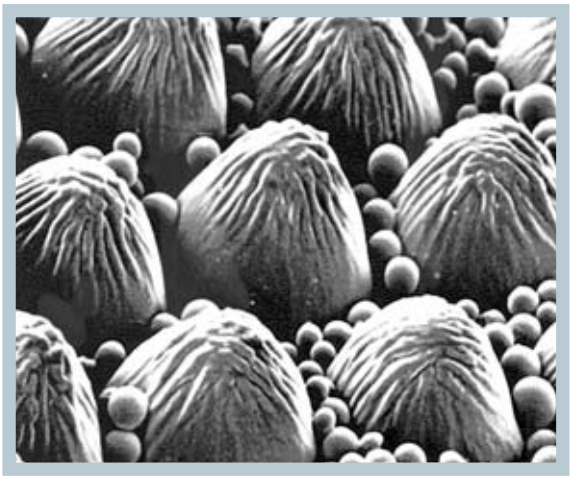

Figure 24 Scanning electron microscope image of the surface of a rose petal which has been dusted with the dry fungal spores of *Botrytis* (small spheres).

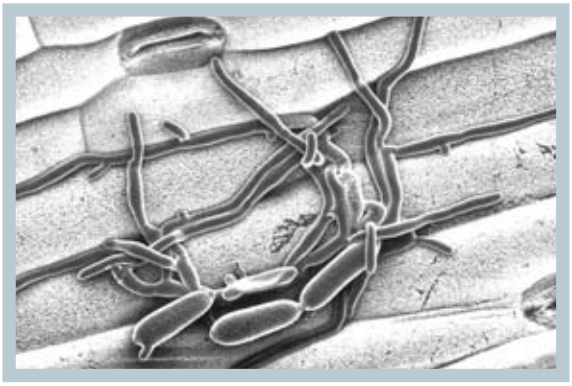

Figure 25 Powdery mildew (*Blumeria graminis* f.sp. *hordei*) colony on the surface of a barley leaf..

As an Institute, SCRI is rare in having, as well as the TEM and SEM microscopes under the same collective 'roof', a wide range of imaging facilities including NMR microscopy, FTIR microscopy and also state-of-the-art CLSM microscopes, so creating possibilities for many research areas as yet unexplored. Electron microscopy is not the dying art that some would have us believe, but an expanding, vibrant and versatile science that, in addition to its own specialist field, plays a complementary role to many other imaging techniques now available. To plagiarise a current rather well known advertising slogan, 'the future is bright, the future is electron microscopy!'.