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# Clinical imaging of cancer metastasis

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**Abstract**

Tumour imaging is an essential part of the practice of oncology, with a crucial role in screening programmes and in diagnosis and staging of established disease. Furthermore, the assessment of tumour size by imaging, usually with computer tomography (CT) scanning, is a key component in determining the tumour response to therapy both in clinical trials and in daily oncology practice. Techniques such as CT, ultrasound (US) and magnetic resonance imaging (MRI) provide high resolution anatomical images with detailed structural information. However, these imaging modalities yield limited functional information on the tumour tissues and often cannot distinguish residual disease from non-viable or necrotic tumour masses, nor can they detect minimal residual disease. In contrast, radiopharmaceutical imaging and, in particular, positron emission tomography (PET) can give some functional information about the underlying tissues. The possibility of refining these techniques and also the emergence of newer imaging modalities that can detect changes in cancers at the physiological, cellular or molecular levels, gives rise to the notion that these methods will have implications for drug development strategies and also future clinical management. In this review, we briefly discuss the current role of imaging in clinical practice, describe some of the advances in imaging modalities currently undergoing evaluation, and speculate on the future role of these techniques in developmental therapeutics programmes. 

*Keywords:* Imaging; PET scan; MRI; Fluorescence spectroscopy; Nuclear medicine; Cancer; Optical coherence tomography

## 1. The role of imaging in clinical practice

Imaging is fundamental to the practice of oncology from screening programmes through to diagnosis, staging, response assessment and subsequent tumour surveillance during follow-up. Mammography has made population-based screening for breast cancer feasible, and a national screening programme is now in place in the UK. Colonoscopy is currently the investigation of choice for those patients identified as being at high risk from colon cancer but it is time consuming, relatively invasive and the number of trained colonoscopists is limited. Virtual colonoscopy by computed tomography (CT) and magnetic resonance imaging (MRI), is a promising technique which in combination with colonoscopy, may make widespread screening for colon cancer a more realistic goal [1].

To be effective a screening investigation must identify disease at a stage where intervention can influence overall survival. Chest X-ray screening for lung cancer was unsuccessful for this reason. Spiral CT scanning is more promising, detecting lesions at a stage when they are still amenable to surgical resection. A pilot study is now underway assessing the use of low-dose CT scanning in the early detection of lung cancer [2]. A number of newer imaging techniques such as fluorescence spectroscopy have the potential to detect early or even pre-invasive lesions and may ultimately translate into effective screening programmes.

The first objective evidence of malignancy is often an abnormality on plain X-ray, CT scan or ultrasound scan (USS) instigated as a result of a clinical sign or symptom. Ultimately diagnosis can only be made on histological assessment but here too imaging has an important role as image guided biopsy, usually with CT or USS, can avoid the need for more invasive surgery in order to establish a histological diagnosis. Appropriate management of a patient requires accurate information as to the location of their tumour, its extent and nature.

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Staging provides important prognostic information and is the basis on which patients are selected for radical treatments with curative intent. The most commonly used modality for staging is CT and it is the method of choice for assessing pulmonary metastases and for imaging liver metastases of greater than 1 cm. Ultrasound has the advantage that it is quick, cheap, and involves no exposure to radiation. Its scope has been increased with the development of endoluminal USS, which is now increasingly used in the staging of oesophageal and rectal tumours [3].

Magnetic resonance imaging produces multiplanar images with superior soft tissue resolution, and so is particularly valuable in the imaging of the central nervous system, the pelvis and of soft tissue sarcomas. Furthermore recent advances have enhanced the quality of MR intestinal imaging which is likely to provide complementary information to that of other methods of imaging the intestine [4]. CT and MRI have made a significant contribution to the staging and management of apparently limited disease. However, a large percentage of patients who undergo surgery with curative intent develop recurrent disease, highlighting the fact that detection of presumed micrometastatic disease remains a significant challenge.

The majority of tumours are not easily accessible to accurate clinical assessment. Consequently serial assessment of tumour size by imaging modalities remains the cornerstone of disease response assessment which is essential not only for the management of individual patients but also for consistent reporting of the results of clinical trials. Traditionally, response assessment criteria have been based on changes in bi-dimensionally measurable lesions [5,6]. However, the recently published Response Evaluation Criteria in Solid Tumours (RECIST) criteria [7] have recommended that response assessments should be based on changes to uni-dimensional lesions and also made recommendations on the use of imaging techniques in response assessments with CT scanning, the method of choice given the variability of repeated measurements by USS and MRI.

None the less, assessments of disease status based on anatomical measurements alone have a number of drawbacks. Many patients will not have measurable or evaluable disease, particularly at certain anatomical sites. Furthermore tumour size may not always equate to viability of tumour cells within a tumour deposit (e.g. in necrotic tissue or scarring). Changes in size in solid tumours are frequently only seen over a period of weeks to months following several courses of chemotherapy. Consequently, the development of imaging tools that could detect early functional changes in tumours soon after starting treatment and which could be used to predict clinical outcome, is particularly attractive. Indeed, such techniques would be particularly pertinent in the clinical assessment of those novel therapeutic agents which have a cytostatic rather than cytotoxic mechanism of action, and with which objective tumour response, as determined by conventional criteria, may not occur even though the desired biological effect is produced. Imaging modalities which give functional information as well as structural images have the potential to vastly improve the processes of staging, response assessment and follow-up, as well as the assessment of new agents, and some of these techniques are discussed below.

## 2. Advances in imaging modalities

### 2.1. Nuclear medicine

Nuclear medicine encompasses a variety of techniques that use radiopharmaceuticals. These consist of either gamma or positron emitting radionuclides bound to ligands which causes differential accumulation in malignant or diseased tissue compared with normal tissue. Differences in tumour biology such as blood flow, metabolism, concentration of specific receptors or differences in antigen expression, are exploited in order to target radionuclides to the tumour tissue. Some techniques such as the $^{99m}$[Tc]-diphosphonate bone scan are well established in clinical practice. Tc-diphosphonate accumulates preferentially in areas of increased bone turnover which occurs in response to the presence of metastatic tumour deposits, and is the most sensitive method of detecting metastatic bone disease though its specificity is low [8]. Other examples of radioisotope imaging and their clinical indications are described in Table 1.

The efficacy of any imaging agent depends on its specificity for the target tumour. Some tumour types have unique biological features that can be targeted such as uptake of norepinephrine by neuroblastomas and phaeochromocytomas which is exploited in metaiodobenzylguanidine (MIBG) scans [10]. Unfortunately the majority of common tumours lack such specific differentiating features.

Advances in the field of antibody technology have made radioimmunoscintigraphy (RIS) feasible. RIS uses radiolabelled monclonal antibodies raised against various tumour antigens. For example, several studies have demonstrated improved sensitivity for the combination of RIS with antibodies to carcinoembryonic antigen (CEA) in addition to conventional techniques in the detection of liver metastases and extra-hepatic disease from colorectal cancer [20–26].

Another promising targeting ligand is folic acid which enters cells either by means of a carrier protein or via receptor-mediated endocytosis facilitated by the folate receptor (FR) [27]. Drug–folate conjugates are not substrates for the former so they enter the cells exclusively

Table 1
Clinical applications of radioisotope scans

| Radioisotope | Clinical application |
|---|---|
| Bone scan — [$^{99m}$Tc]-methylenediphosphonate | Staging of bony disease particularly in prostate, breast and lung cancers [8,9] |
| MIBG scan – $^{131}$I or $^{123}$I-labelled MIBG | Localisation of neuroendocrine tumours that take up norepinephrine (e.g. phaeochromocytoma, paraganglionoma, neuroblastoma) [10] |
| Octreotide scan — [$^{111}$In]-octreotide | Localisation of tumours with somatostatin receptors (e.g. pancreatic tumours, carcinoid tumours, medullary thyroid cancer, neuroblastoma) [11–13] |
| Sestamibi scan — [$^{99m}$Tc]-sestamibi | Localisation of active disease in thyroid cancer in iodine-blocked patients or those with non-iodine avid metastases and in breast cancer cases which remain undiagnosed after conventional diagnostic tests [14,15] |
| Thallium scan — [$^{201}$Tl] chloride | Localisation of viable tissue, particularly in brain tumours and osteosarcoma [16] |
| Gallium scan — [$^{67}$Ga] chloride | Staging and assessment of treatment response in Hodgkin's and non-Hodgkin's lymphoma [17,18] |
| Monoclonal antibody scans — $^{111}$In or $^{99m}$Tc-labelled tumour antibodies | Staging of tumours that express specific antigens (e.g. colorectal and prostate cancers) [19] |

MIBG, metaiodobenzylguanidine.

via FR-mediated endocytosis [27–30]. The folate receptor is significantly overexpressed in a large proportion of human cancers and FR expression is correlated with tumour grade in ovarian cancer [31]. High tumour to non-tumour ratios for uptake of folate-based radiopharmaceuticals have been demonstrated in animal models [32,33]. Folate-targeted imaging has many advantages over techniques that use monoclonal antibodies, in that the FR is expressed more strongly on dedifferentiated tumours (which typically tend to lose the expression of tumour-specific antigens), folate can diffuse more rapidly into tumours, is relatively easy to conjugate to a wide variety of molecules and is stable in a number of different storage conditions. Furthermore it is cheap, non-immunogenic and non-toxic, making it a potential ligand for targeting of therapeutic as well as imaging agents [34].

[$^{99m}$Tc]-sestamibi is taken up by tumours including lung and breast carcinomas, lymphomas and sarcomas [14,35–37] and it may have a role in the early detection of tumours which are likely to be resistant to chemotherapy. [$^{99m}$Tc]-sestamibi is a transport substrate for P-glycoprotein [38] and a correlation has been demonstrated between [$^{99m}$Tc]-sestamibi efflux and P-glycoprotein expression in untreated breast carcinomas [39]. Furthermore, a correlation between rapid tumour clearance of [$^{99m}$TC]-sestamibi and lack of tumour response to neoadjuvant chemotherapy for breast cancer has been shown [40], suggesting that [$^{99m}$Tc]-sestamibi scanning could be used to identify patients unlikely to respond to chemotherapy with drugs for which resistance is mediated, at least in part, by the P-glycoprotein drug-efflux pump.

Nevertheless, there are some limitations to the use of this technique in that not all tumours take up [$^{99m}$Tc]-sestamibi, necrotic tumours have low tumour to background ratios of [$^{99m}$Tc]-uptake and small clusters of resistant cells within tumours would not be detected as uptake and rates of clearance would be determined by the predominant number of sensitive cells. In addition, [$^{99m}$Tc]-sestamibi is cleared by the liver into the biliary ducts, gallbladder and intestinal tract which produces high activity in the abdomen and makes scans of this area difficult to interpret.

### 2.2. Positron emission tomography

PET was initially developed in the 1960s but has largely been used as a research tool. (See Table 2 for an explanation of the principles of PET.) However, PET can provide useful information for clinical practice. The images generated by PET represent the metabolic activity of the underlying tissue and can therefore distinguish benign from malignant lesions on the basis of differences in metabolic activity. Consequently, the technique can be used to identify lesions not seen on conventional imaging, allowing the distinction of benign from malignant tissue, and identification of areas of viable tumour, as distinct from necrosis, after therapy. Similarly, it can identify recurrent disease in areas in which conventional scans are difficult to interpret because of prior treatment. Specific examples of the clinical applications of PET scanning are listed in Table 3 and discussed below.

Radiopharmaceutical agents commonly used in PET scanning include the glucose analogue [$^{18}$F]-fluorodeoxyglucose (FDG) [56–58] and [$^{11}$C]-thymidine [59,60]. FDG exploits the increased rate of glycolysis seen in tumour cells both as a result of increased glucose uptake and increased activity of the glycolytic enzymes. FDG is taken up into the cell by glucose transporter proteins and, once in the cell, it is phosphorylated in the same way as glucose by hexokinase. In contrast to glucose, FDG-6-phosphate cannot be further metabolised and it remains trapped within the cell. Thus, the intracellular concentration of FDG is proportional to the glucose utilisation of the tissues. [$^{11}$C]-thymidine, is incorporated into DNA during S phase and thus provides a means of measuring cell proliferation. It has an advantage

Table 2
Principles of PET

1) In positron decay a proton is converted to a neutron and a positron is emitted.
2) The positron traverses a few millimetres before combining with an electron.
3) The mass of positron and electron is converted into energy with the production of a pair of photons travelling at 180° to each other with an energy of 0.511 MeV.
4) The opposed photons can be detected by pairs of co-linearly aligned detectors which are arranged in multiple rings around the patient. Photons that interact with these detectors within a predefined time-window are registered as decay events.
5) The radioactivity detected along lines at a series of angles is subsequently used to reconstruct tomographic images of regional radioactivity distribution.
6) For quantitative measurements images must be corrected for photon attenuation. This is achieved by rotating an external radioactive source around the patient and measuring the attenuation of this radiation by the body of the patient.

over FDG when measuring response to treatment as cells may remain energetically active even after their replicative machinery has been damaged.

A number of small studies have evaluated the ability of PET to assess response to treatment [61,62]. The results of these studies suggest that changes in uptake after as little as one course of therapy can predict for ultimate response. However, these findings need to be confirmed in larger studies in different tumour types to establish the validity of using PET scanning as an accurate method of detecting early changes with therapy that can predict for eventual outcome, thereby avoiding unnecessary morbidity.

### 2.3. Magnetic resonance imaging

The use of MRI is now well established in clinical practice but MRI has the potential for much wider applications. At a practical level, the development of open MRI scanners, which allow access to patients during scanning, opens up the possibility of MRI-guided interventions [63].

A number of different MRI contrast agents are currently under investigation. Dextran-coated ultrasmall superparamagnetic iron oxide (USPIO) agent (known as AMI 227) is one of a new class of MRI contrast agents specifically designed for the evaluation of the reticuloendothelial system [64]. After intravenous (i.v.) injection, small iron oxide particles are taken up by macrophages within normal functioning lymph nodes. As a result, they reduce the signal on postcontrast MRI because of the magnetic susceptibility effects of the iron oxide. Metastatic nodes that are partially or completely replaced by tumour do not have the same levels of phagocytic activity as normal nodes, do not take up the iron oxide particles to the same extent and therefore maintain the same signal intensity on postcontrast MRI images.

AMI 227-contrast enhanced MRI had a sensitivity of 95% and specificity of 84% in detecting malignant

Table 3
Clinical applications of PET scanning

| Tumour type | Clinical application |
|---|---|
| Brain | Detection of tumour recurrence |
| | Planning of stereotactic biopsies |
| | Indication of grade of tumour [41] |
| Head and neck | Lymph node staging [42] |
| | Detection of recurrence |
| | Detection of unknown primary |
| Non-small-cell lung | Differentiation of benign and malignant solitary pulmonary nodules [43] |
| | Staging of the mediastinum [44,45] |
| | Detection of local recurrence [46,47] |
| Pancreatic | Differential diagnosis of chronic pancreatitis and pancreatic cancer [48] |
| Colorectal cancer | Detection of extra-hepatic metastases and recurrent disease [49] |
| | Investigation of rising CEA with normal conventional imaging |
| Germ cell tumours | Detection of residual disease after therapy and differentiation of malignant from mature teratoma or scar tissue [50–52] |
| | Investigation of rising markers with normal conventional imaging |
| Melanoma | Detection of lymph node and distant metastases [53,54] |
| Hodgkin's and non-Hodgkin's lymphoma | Staging of initial disease |
| | Identification of residual disease after therapy [55] |

PET, positron emission tomography; CEA, carcinoembryonic antigen.

lymph nodes in one small study ($n = 12$) in patients with head and neck cancer [65]. However, false positives occurred due to necrotic nodes and reactive follicular hyperplasia and further studies are warranted to evaluate the usefulness of this technique.

Angiogenesis is essential to tumour growth and metastasis and a number of therapies aimed at inhibiting this process are now in clinical trials [66–71]. By analysing the intratumoral kinetics of MRI contrast agents of different molecular weights, dynamic contrast enhanced MRI (DCE-MRI) can provide information on several aspects of tumour vascularity including blood volume, perfusion and permeability. Since MRI is non-invasive, tumours can be monitored over time and changes in vascularity with tumour growth and in response to treatment can be studied. For example Brasch and colleagues were able to demonstrate that macromolecular contrast media-enhanced MRI can detect and quantify changes in tumour microvasculature permeability in response to antiangiogenesis intervention in rat models of human breast cancer [72]. Consequently, this method can potentially be used to determine if putative angiogenesis inhibitors produce the desired biological endpoint within tumours, which may be a more relevant endpoint than objective tumour response in early clinical trials of these agents.

Magnetic resonance spectroscopy (MRS) can detect differences in cellular metabolites and as a result can distinguish malignant tissue from adjacent normal tissue [73–75]. Marked spectral differences have been observed in MRS imaging studies of the brain and the prostate, and these can distinguish viable normal tissue from necrosis and from cancer and can also be used to monitor response to therapy [76]. This approach is particularly attractive as MRI and MRS techniques can be combined within a single examination without introducing additional technology. MR can also be used to study other features of tumour pathophysiology including cell shrinkage, changes in tumour water, intra and extracellular pH and tumour oxygenation [77,78].

### 2.4. Fluorescence spectroscopy

Fluorescence spectroscopy is a non-invasive diagnostic technique with the potential to detect early neoplastic lesions and also to guide surgical procedures by defining appropriate resection margins.

It is based on the principle that when a molecule is illuminated at an excitation wavelength, which lies within the absorption spectrum of that molecule, it will absorb this energy and be activated from its ground state to an excited state. The molecule then relaxes back from the excited state to the ground state, at the same time emitting energy in the form of fluorescence [79]. The wavelength of emission energy is longer than that of the excitation wavelength.

A number of molecules exhibit endogenous fluorescence including amino acids, structural proteins, enzymes and co-enzymes, vitamins, lipids and porphyrins [80]. Differences in the proportions of these molecules and differences in structure between normal and neoplastic tissues result in different fluorescent spectra [81–84].

The ability of fluorescence spectroscopy to detect neoplastic growth *in vivo* has been investigated in the colon, cervix, bronchus, bladder, brain, oesophagus, head and neck, skin and bile duct (reviewed in [85]). A commercial laser-induced fluorescence endoscopy (LIFE) device has been developed [86,87]. This can improve the detection of areas suspicious of dysplasia for biopsy when used as a adjunct to white light bronchoscopy [88]. Use of the LIFE device is currently being evaluated in other tissue types and further clinical applications of this rapid non-invasive technique are likely to be explored.

### 2.5. Optical coherence tomography (OCT)

OCT has potential as a screening modality as well as in interventional procedures. The principles of the technique are similar to B mode ultrasound. Briefly, when a beam of light is directed onto tissue, it is reflected or scattered back from structures which have different optical properties as well as from boundaries between structures. The dimensions of different structures can be determined by measuring the "echo" time it takes for light to be reflected or scattered from the different structures at varying longitudinal distances. The main difference from ultrasound is that the velocity of light is approximately a million times faster than that of sound. As a result, the echo time delay cannot be measured directly by electronics and instead it is necessary to employ interferometry techniques [89].

OCT can create images with resolutions of between 1 and 15 μm, that is one to two resolutions higher than conventional ultrasound. This high resolution permits imaging of tissue architecture and morphology and, unlike ultrasound, imaging can be performed directly though air without requiring direct contact with the tissue or a transducing medium. Light is highly scattered in most biological tissues so image penetration is limited to depths of 2–3 mm but this is similar to a biopsy specimen taken for histological analysis however, unlike a biopsy, OCT can be performed *in situ*. As well as avoiding the potential hazards of biopsy, analysis can be performed at multiple sites reducing sampling errors. OCT is fibre optically based and can therefore be incorporated into a wide range of instruments such as endoscopes and laparoscopes. Furthermore, real-time imaging can be performed potentially allowing real-time diagnosis and guidance of surgical procedures.

OCT has been used in imaging of the eye [90–92] but *in vitro* studies of gastrointestinal, urinary, respiratory

and female reproductive tracts suggest that OCT can detect changes in epithelial and glandular structure associated with early neoplastic change [93–97]. Studies are in progress to determine the feasibility of using OCT as a diagnostic and screening tool [98].

## 3. Imaging and cancer: future directions

During the past decade, there has been a rapid development in the understanding of the molecular basis of cancer. Associated with this development in the molecular and cellular biology of cancer have been remarkable advances in imaging technology and methodology [99]. It is now possible to image at a resolution of less than 1 mm, and the development of specific ligands and probes has allowed *in vivo* investigation of metabolic pathways and specific cellular functions. Indeed, there is now evidence that metabolic imaging can provide relevant clinical information that can influence patient management.

Several single-institution studies with small patient numbers have shown that FDP–PET can distinguish malignant from benign solitary pulmonary nodules with a sensitivity of 80–100% [100–112]. Similarly several single-institution studies with small patient numbers have compared PET with standard CT in assessing mediastinal disease in the staging of non-small cell lung cancer with subsequent histological confirmation. A meta-analysis of 514 patients evaluated with PET and 2226 patients evaluated with CT in 14 published studies has demonstrated superior sensitivity (79% compared with 60%) and specificity (91% compared with 77%) for FDG–PET over CT in demonstrating nodal metastases [113]. Indeed the sensitivity and specificity of PET are almost comparable to those of mediastinoscopy. In addition, several studies have shown that FDG–PET can detect distant metastases not seen with conventional imaging techniques in approximately 10% of patients, which could potentially influence decisions regarding operability in some patients [114–116].

Other tumours for which metabolic imaging by PET has demonstrated promise are malignant melanoma, lymphoma and colorectal cancer. PET has been used for the detection of occult regional nodal metastases at the time of presentation [117,118], and a sensitive and specific technique of detecting regional nodal involvement could avoid the need for elective lymph node dissection or sentinel node mapping and also yield useful information that could influence adjuvant therapy. Several studies have also shown that PET can detect occult distant metastases at initial presentation, with one study ($n = 100$) concluding that PET can detect some metastases up to 6 months earlier than conventional imaging and PET specifically influenced surgical or medical management in 22% of cases [119]. CT scanning is conventionally considered the standard imaging method for staging and assessment of response in both Hodgkin's and non-Hodgkin's lymphoma. However, PET can often identify additional regional or occult nodal involvement not identified on CT imaging and several studies have suggested that PET is superior to CT in staging lymphoma [119,120], and significantly can also detect involvement of the bone marrow [121]. Interestingly, there are preliminary data to suggest that patients who have a significant reduction in FDG uptake with chemotherapy have a decreased rate of relapse [122], raising the possibility that metabolic tumour imaging may give information that influences patient management beyond the increased sensitivity in detecting occult disease.

Comparison has also been made between conventional CT scanning and whole-body PET imaging in detecting recurrent colorectal carcinoma, with PET consistently having sensitivity of greater than 90% with CT sensitivity in the range of 55–75% [49,123–126], further highlighting the potential impact that metabolic imaging can make in the diagnosis, staging, response assessment and clinical management of solid tumours.

One of the most intriguing possibilities to arise from the advances in imaging technology is the use of this technology for the non-invasive analysis of reporter gene expression. This has implications not only for evaluating gene expression during cellular processes but will also permit *in vitro* and *in vivo* evaluation of genetic-based therapies [127,128]. Furthermore, this technology can also be used to assess pharmacokinetic and pharmacodynamic endpoints, which will be relevant in the early evaluation of novel cancer therapies.

Recent advances in reporter gene technologies allow us to image gene transcription at the single cell level using either absorbance, fluorescence or luminescence microscopy, thereby allowing gene expression to be followed dynamically and in real-time during complex cellular processes (reviewed in [17]). All of these methods, including B-lactamase, green fluorescent protein (GFP) and luciferase imaging have advantages and disadvantages [129]. For example, only luciferase imaging currently allows rapid, quantitative dynamic imaging of gene expression and has been proven to allow simultaneous detection of multiple reporter genes in the same cell. GFP is the least invasive of these methods, but is also probably the least sensitive, with background autofluorescence an inherent problem. All the methods are applicable to high-throughput screening and could be applied as the basis for expression, cloning or functional genomics strategies [130] to identify and validate novel targets for drug discovery.

Several strategies have evaluated non-invasive imaging of tumours in living animals including protease-activated near-infrared fluorescent probes which allow the detection of tumours with submillimetre sized diameters [131] which mimics minimal disease states.

Similarly, bioluminescent indicators can be used for real-time measurements [132] and can also detect small numbers of tumour cells in living animals non invasively, again suggesting that this approach could be used to evaluate the molecular steps in the metastatic process and to monitor therapy of micrometastatic disease [133]. Simple, non-invasive, whole body optical images can also be obtained with either a trans-illuminated epifluorescence microscope or a fluorescence light box and thermoelectrically cooled colour charge-coupled device camera and with GFP-expressing tumours and metastases [134]. This allows real-time measurements of tumour growth and metastasis formation in intact animals which should facilitate the rapid evaluation of novel therapeutic strategies in malignant disease.

The advances in imaging technology raise the intriguing possibilities of monitoring the molecular and cellular processes in the development of cancer and its metastases, and the impact of anticancer treatments on these processes *in vivo*.

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