

**Thermographic Assessment of Tumour Growth in Mouse Xenografts**

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Statement: We believe the finding in the paper is significant as it could lead to new protocols and measurement methods for human tumour xenografts. We consider the International Journal of Cancer as the most appropriate journal to publish the unexpected findings of this research.

## Summary

In human breast tumours, a 1-2°C increase in skin surface temperature is usually observed at the periphery; it has been proposed that this change is due to the hypervascularity and increased blood flow resulting from tumour-associated angiogenesis. Here we tested the hypothesis that thermal imaging might represent a useful adjunctive technique in monitoring the growth dynamics of human tumour xenografts.

Xenografts were established in immunocomprised nude mice using MDA-MB-231 or MCF7 breast cancer cells. We exploited the inherent non-contact and non-invasive advantages of infrared thermography to detect skin surface temperature changes. Continuous thermographic investigation was performed to detect and monitor tumour growth *in vivo* and high resolution digital images were analysed to measure the tumour temperature dynamics.

In contrast to the skin temperature increases associated with human breast cancer, a consistent temperature decrease was found in the xenograft mice. In one case, a smaller secondary tumour, otherwise undetectable, was clearly evident by thermal imaging. The tumours were cooler than the surrounding tissue with a maximum temperature reduction of 1.5°C for MDA-MB-231 tumour and 3°C for MCF7 tumours observed on day 14. In addition, the temperature of the xenograft tumours decreased progressively as they grew throughout the observation period.

It was demonstrated that thermographic imaging could detect temperature changes as small as 0.1°C on the skin surface at an early stage of tumour development. The findings of the study indicate that thermographic imaging might have considerable potential in monitoring human tumour xenografts and their response to anti-cancer drugs.

## **Introduction**

In current cancer research, human tumour xenografts in immunocompromised (nude) mice have been established as an effective method to study the growth of many tumour types and the activity of anti-cancer agents. The nude mouse accepts human cancer cells and can develop solid tumours in days<sup>1-2</sup>. These *in vivo* animal studies provide essential pathological and biological information on the anti-cancer effects and toxicity of novel drug agents before studies on humans can be undertaken. Tumour size, vascularity and angiogenesis are evaluated *in vivo* during these studies to reveal tumour progression and regression.

There are few objective methods to assess tumour development in this animal model prior to sacrifice. Some improved techniques may be available in the form of animal CT, MRI or PET scanning, but they are expensive and not widely available. Currently, most measurements are carried out manually, using mechanical callipers, which have low accuracy and poor reproducibility. The measurement is achieved by compressing the tumour between the calliper blades until the experimenter subjectively detects no further movement due to tumour resistance. The tumour size is then obtained from the calliper display. In addition to the inherent errors introduced by this process, there is concern that the attendant mechanical disruption may disturb tumour development.

Widely available, accurate, objective measurement methods and tools for xenograft evaluation are therefore needed. Of the many choices, thermal imaging is an excellent candidate which offers unique advantages such as accurate, non-contact, non-invasive and real-time measurement of surface temperature. With calibration, thermal images are also capable of detecting the actual size of the thermally-changing foci. Advances in modern thermal imaging technology have resulted in enhanced overall performance with improved functionality, better portability and lower cost; all of which make thermal imaging an ideal candidate for small animal studies.

Thermal imaging has been well established in clinical medicine since the early 1960s<sup>3-6</sup>. It has been used in the evaluation of patients suspected of having breast cancer, where an abnormal thermal pattern can be detected prior to clinical or mammographic changes. A typical infrared image of a breast tumour reveals a 1-2°C elevation in skin temperature at the periphery of the tumour, and lesions with a rapid growth rate and less favourable prognosis can be identified when thermographic studies are used in conjunction with conventional physical and radiological examinations<sup>7</sup>. Dynamic thermal imaging has been used intraoperatively during neurosurgical interventions, including real-time assessment of cerebral vessel patency and cerebral perfusion and determination of tumour margins during resectional surgery<sup>8-9</sup>. Thermography has also been applied intra-operatively to detect high-risk unstable plaques in human coronary arteries by thermal heterogeneity and this technique may prove useful in identifying plaques prone to rupture or thrombosis<sup>10-11</sup>. Recently, an infrared laparoscope measuring 10 mm in diameter and 300 mm in length has been incorporated into a thermal camera system. This enabled the monitoring of surface temperature during radiofrequency ablation<sup>12</sup> and during energised laparoscopic dissections involving cutting and coagulation<sup>13</sup>.

Thermal imaging has rarely been used to monitor tumour growth in experimental animals. The aim of this paper is to report on observed temperature contrasts in xenograft tumours and to provide a framework for using thermal imaging as a research tool for objective evaluation of tumour xenografts in mice. We show how thermal images evolve during xenograft tumour development and examine how thermal imaging measurement of tumour growth relates to mechanical calliper measurements. In future, we aim to integrate this approach with pathological studies and mathematical modelling techniques in order to gain a better understanding of the mechanism of human tumour xenograft development.

## Materials and Methods

### *Human Tumour Xenografts*

Two human breast cancer cell lines, oestrogen receptor-negative MDA-MB-231 and oestrogen-receptor positive MCF7, were acquired from ATCC (Rockville, MD, USA) and cultured according to their instructions. All animal experimental protocols used in this study were approved by the Tayside Research Ethics Committee, and the xenograft procedures were carried out in accordance with the guidelines of the UK Co-ordinating Committee on Cancer Research and under license. Mice were inspected daily and their well-being and body weight monitored.

Female nude mice (Harlan UK Ltd) were injected subcutaneously in both flanks with MDA-MB-231 (5 mice) or MCF7 (6 mice). In both cases,  $10^8$  cells were combined with 100  $\mu$ l DMEM and matrigel (1:1) medium (BD Biosciences, Oxford, UK). Mice receiving MCF7 cells had  $17\beta$ -estradiol pellets (0.72 mg/pellet, Innovative Research of America, Sarasota, FL, USA) implanted at least 2 days before injection. No pellets were required for the growth of oestrogen receptor-negative MDA-MB-231 xenografts. A control group (5 mice) was established and subjected to the same protocol, the only difference being that the injectant carried no tumour cells.

Both MDA-MB-231 and MCF7 xenografts have been used as tumour models for developing novel anti-cancer drugs<sup>1-2</sup>. The time for tumour development depends on the cell line, with drug treatment starting after 2-3 weeks, when individual tumours have reached a size of 40-50 mm<sup>3</sup>. Both MDA-MB-231 and MCF7 tumours exhibit growth of up to 500% in volume within 14 days. They represent excellent animal models for examining the effects of subcutaneous tumour growth on skin temperature.

The xenograft tumours develop volumetric shapes that are approximately elliptical. The widths,  $w$ , and lengths,  $l$ , of these tumours were measured with callipers (without applying compression) twice a week. Volume approximations were then calculated using the formula:  $V = \pi(w + l)^3 / 48$ .

### *Thermal imaging of tumours*

Thermal imaging experiments were carried out in a closed room at a constant temperature of 20°C with tolerance of 1°C, while the environment of the cages where the mice were kept was controlled by an integrated unit, the temperature of the cages was also at 20°C. During the experiments, lighting was kept minimal to eliminate any effect on the performance of the thermal camera. Temperature variations inside the room were therefore minimised and, in addition, the mice were kept in a sterilized hood during imaging to further restrict environmental influences. A Merlin thermal camera (FLIR Systems Ltd, UK) was used to examine the mouse body temperature. The camera contains a 320×256 pixel focal plane array detector with a spectral response range of 3-5  $\mu$ m. The thermal sensitivity is 0.025°C, the spatial resolution is 0.2 mm at a distance of 30 cm and the frame rate is 50 Hz. The thermal camera was calibrated using a black body source from the National Physical Laboratory<sup>14</sup>. The difference between the thermal camera measurement and the standard reference temperature was no more than 0.1°C.

Mice were handled in familiar conditions by expert animal license holders and thermal images were taken while the mice were awake and without any sedation. The camera was positioned 1m from the mouse and used a standard 25 mm lens for image formation. The MCF7 mice were also imaged using an infrared

transmitting microscope lens for a closer view of the tumours. To serve as a reference for the injection site, digital infrared images were taken immediately after injection of tumour cells. The injected medium was at room temperature (20°C) and produced a reduction in mouse skin temperature which lasted for about 3 hours. The injection sites were imaged every day at the same time (11am) until the tumours reached a size of 1.44 cm<sup>2</sup>, at which point the animals were sacrificed. To verify the accuracy of thermal imaging temperature measurements, a thermocouple was used to measure directly both the xenograft tumour and mouse body temperatures after image capture.

Digital thermal images were analysed using the software of ThermaCAM Researcher 2.7 (FLIR Systems Ltd, Kent, UK), the software was able to perform advanced thermal analysis within any chosen region of interest. The temperature reduction of tumour was calculated using measurements at the centre of tumour site and the surrounding body part of each mouse. This value was independent of day-to-day and animal-to-animal variations during experiments. The data from thermal imaging was periodically compared to the thermocouple measurement data to ensure that calibration did not vary.

## Results

During the initial period after injection, there was no visible sign of tumour or any skin temperature change in the mice. However, thermal images showed a skin temperature reduction around the injection site 3 days before any visible or palpable signs of a tumour. Once the MDA-MB-231 xenograft tumours were visible and measurable they underwent exponential growth in volume to reach a mean value of 380 mm<sup>3</sup> within 17 days. The tumours continued growing bigger while the temperature kept decreasing during growth, both curves was showed in Fig. 1 to illustrate corresponding relationship between them. With the control group, there was no temperature change for any mouse.

With continuous growth in size, the tumour temperature continued decreasing. On day 10, the average size of tumours was 120 mm<sup>3</sup>, while the average temperature drop was 1.2°C (Fig. 1), with a maximum temperature reduction of 1.5°C in one mouse. This temperature reduction was considered significant (Student t-test,  $P = 0.001$ ). With the fastest growing tumour in one mouse, the size reached 650 mm<sup>3</sup> on day 21 while its corresponding temperature was dropped even further at 1.8°C below the surrounding normal tissue.

A typical thermal image of a mouse on day 6 after cancer cell injection (Fig. 2a) reveals cooler areas that are invisible to the naked eye at this stage. A line drawn through the cooler areas with its temperature profile is illustrated in Fig 2b. A 2°C temperature reduction occurs at the injection site and a 1.4°C reduction occurs at the second point 18 mm away. The latter was a tumour resulting from cell migration after injection.

The growth rate and temperature reduction for MCF7 tumours (Fig. 3) demonstrated the average tumour volume reached 78 mm<sup>3</sup>, and the average temperature reduction was 2.2°C on day 14, with a maximum temperature reduction of 3°C in one mouse. Further fine details of one MCF7 tumour were revealed under a microscopic view (Fig. 4). The average body temperature (Fig. 4a) was 36.1°C, while the average tumour temperature of the circled area (Fig. 4b) was 33.9°C, significantly lower than the normal body temperature (Student t-test,  $P = 0.0003$ ). It was interesting to note that even with slower growth rate, the MCF7 tumours induced a higher temperature reduction than the MDA-MB-231 tumours.

## **Discussion**

This study suggests that thermography was able to monitor the presence and progression of xenograft tumours with high sensitivity; it could be used conveniently to monitor tumour development in xenografts. Small temperature changes at injection sites were identified by thermal imaging much earlier than any visible sign. As the tumours grew bigger in size, their temperature decreased to become colder than surrounding tissue. Tumour size was inversely related to the temperature reduction during the experimental period. These results contrasted strongly with those from the control group, where there was no observable temperature change (at the 0.1°C sensitivity level) at the injection site in any mouse.

The observation that the tumours were cooler than the surrounding tissue requires explanation, particularly since it contrasts with the expectations based on human tumour temperature measurements. Such an explanation is fundamental in considering the possibility of using thermal imaging to detect tumours and monitor their progress *in vivo*.

In general, the reasons for reduced tumour temperature must lie in their biological environment, i.e., the immune-compromised mice. In xenograft research, tumours grow very rapidly and their vasculature is significantly different from that of normal tissue in several respects<sup>15-16</sup>. Unlike normal blood vessels, the tumour vessels are small, poorly organised and hyperpermeable. Thus, even though the tumours had greater vascularity than normal tissue, they had impaired blood supply<sup>17-18</sup> due to the structural and functional abnormalities. In addition, vascular hyperpermeability means that tumour vessels are unable to maintain gradients between vascular and interstitial pressures, contributing to interstitial hypertension. This lack of pressure gradient also impairs the flow of fluid and macromolecules. Thus, reduced blood flow, and therefore temperature, seems to be a possible adjunct to tumour development.

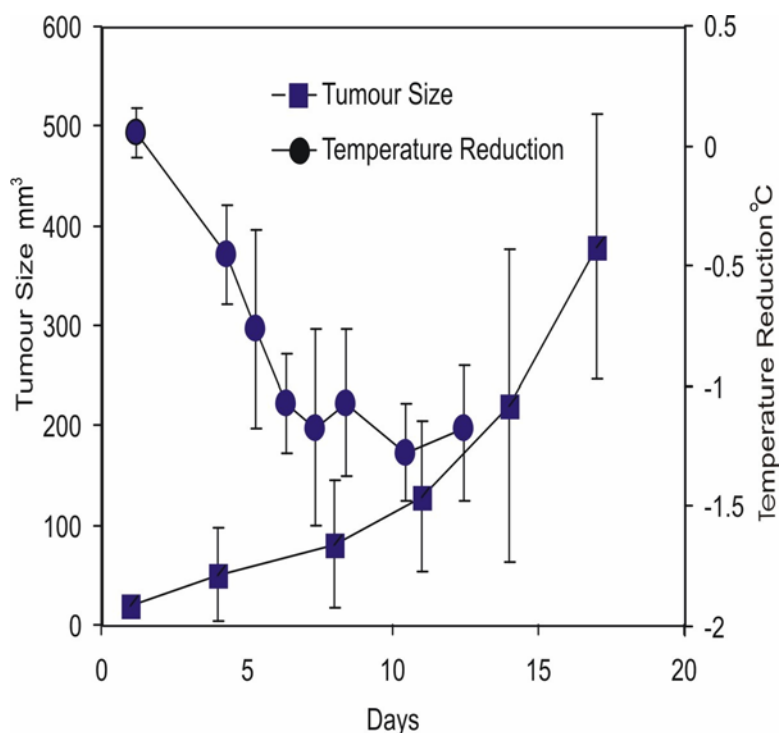
If reduced temperature can be regarded as a significant biomarker of xenograft tumours, thermographic imaging may be very useful for monitoring tumour development and it could also be used for monitoring response of anti-tumour drugs during xenograft trials. Potentially, fewer animals might then be required for *in vivo* drug development work. Further studies are required to understand the biological environment of the xenograft tumour, including the correlative relationships between their physical (temperature and stiffness) and physiological (blood flow and oxygenation) changes. Facilities, such as thermal imaging, for quick and accurate identification of any physiological changes represent considerable enhancements to scientific research in the areas of cancer diagnostics and novel anti-cancer drug development.

## **Acknowledgements**

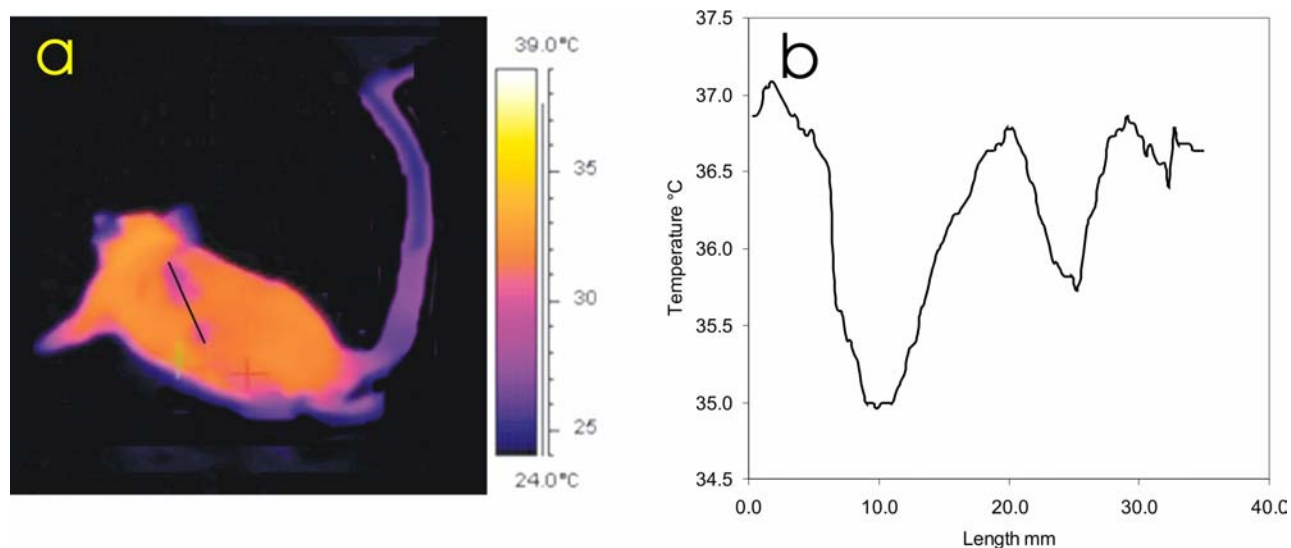
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## References

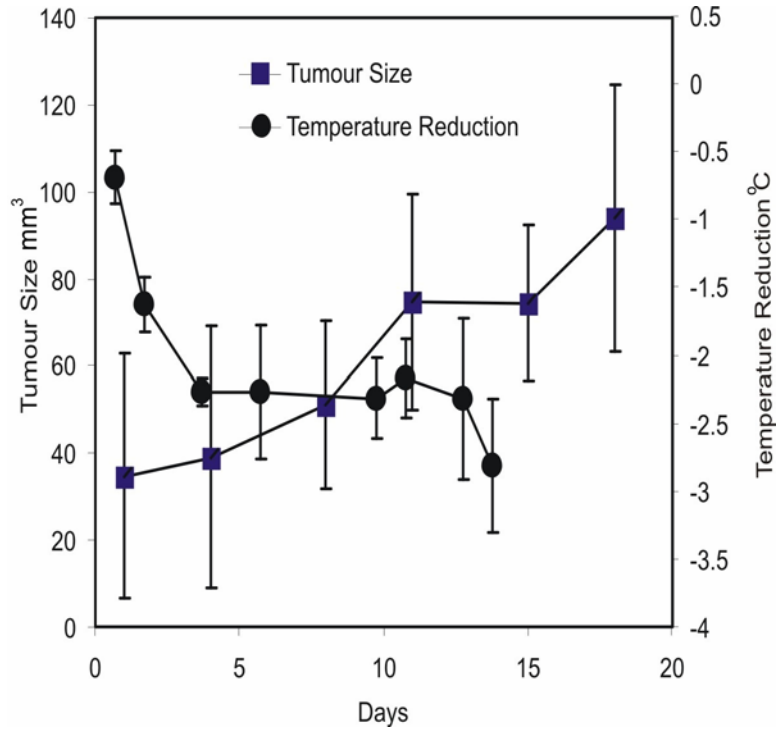
1. V Appleyard, M A O'Neill, K E Murray, S E Bray, G Thomson, A M Thompson, 2004. Activity of MDI-301, A Novel Synthetic Retinoid, in Xenografts. *Anti-Cancer Drugs*, 15, pp. 991-996.
2. B A Spruce, L A Campbell, N McTavish, V L Appleyard, M O'Neill, A M Thompson, S Eccles, 2004. Small Molecule Antagonists of the  $\sigma$ -1 Receptor Cause Selective Release of the Death Program in Tumour and Self-Reliant Cells and Inhibit Tumour Growth in Vitro and in Vivo. *Cancer Research*, 64, 4875-4886.
3. L J Jiang, E Y K Ng, A C B Yeo, S Wu, F Pan, W Y Yau, J H Chen, and Y Yang, 2005. A Perspective on Medical Infrared Imaging. *Journal of Medical Engineering and Technology*, 29(6), 257-267.
4. J F Head, F Wang, CA Lipari, RL Elliott, 2000. The Important Role of Infrared Imaging in Breast Cancer. *IEEE Eng Med Biol Mag*, 19, 52-57.
5. J R Keyserlingk, PD Ahlgren, E Yu, N Belliveau, M Yassa, 2000. Functional Infrared Imaging of the Breast. *IEEE Eng Med Biol Mag*, 19, 30-41.
6. B F Jones, 1998. A Reappraisal of the Use of Infrared Thermal Image Analysis in Medicine. *IEEE Trans. Med Imaging*, 17(6), 1019-1027.
7. M Gautherie, C M Gros, 1980. Breast Thermography and Cancer Risk Prediction. *Cancer*, 45, 51-56.
8. R D Ecker, S J Goerss, F B Meyer, A A Cohen-Gadol, J W Britton, J A Levine, 2002. Vision of the Future: Initial Experience with Intraoperative Real-time High-resolution Dynamic Infrared Imaging. *Journal of Neurosurgery*, 97, 1460-1471.
9. A M Gorbach, J D Heiss, L Kopylev, 2004. Intraoperative Infrared Imaging of Brain Tumour. *Journal of Neurosurgery*, 101, 960-969.
10. C Stefanadis, L Diamantopoulos, C Vlachopoulos, E Tsiamis, J Dernellis, K Toutouzas, E Stefanadi, P Toutouzas, 1999. Thermal Heterogeneity within Human Atherosclerotic Coronary Arteries Detected In Vivo. *Circulation*, 99, 1965-1971.
11. M Madjid, J T Willerson, S W Casscells, 2006. Intracoronary Thermography for Detection of High Risk Vulnerable Plaques. *Journal of the American College of Cardiology*, 47, C80-85.
12. K Ogan, W Roberts, D Wilhelm, L Bonnell, D Leiner, G Lindberg, L Kavoussi, J Cadeddu, 2003. Infrared Thermography and Thermocouple Mapping of Radiofrequency Renal Ablation to Assess Treatment Adequacy and Ablation Margins. *Urology*, 62(1), 146-151.
13. C Song, 2006. Seeing the Heat – Development and Evaluation of an Infrared Endoscope. *Congress of European Association of Endoscopic Surgery*, Berlin, 12-16 September 2006.
14. Report on the Thermal Imaging Calibration System, National Physical Laboratory, 2006.
15. W Xie, P McCahon, K Jakobsen, and C Parish, 2004. Evaluation of the ability of digital infrared imaging to detect vascular changes in experimental animal tumours. *International Journal of Cancer*, 108, 790-794.
16. Jain R K, 2001. Normalizing Tumor Vasculature with Anti-Angiogenic Therapy: A New Paradigm for Combination Therapy. *Nature Medicine*, 7, 987-989.
17. Padera T P, Stoll B R, Toorredman J B, Capen D, Tomaso E, Jain R K, 2004. Cancer Cells Compress Intratumor Vessels. *Nature*, 427, 695.
18. Song C W, 1984. Effect of Local Hyperthermia on Blood Flow and Microenvironment: a Review. *Cancer Research*, 44, 4721s-4730s.



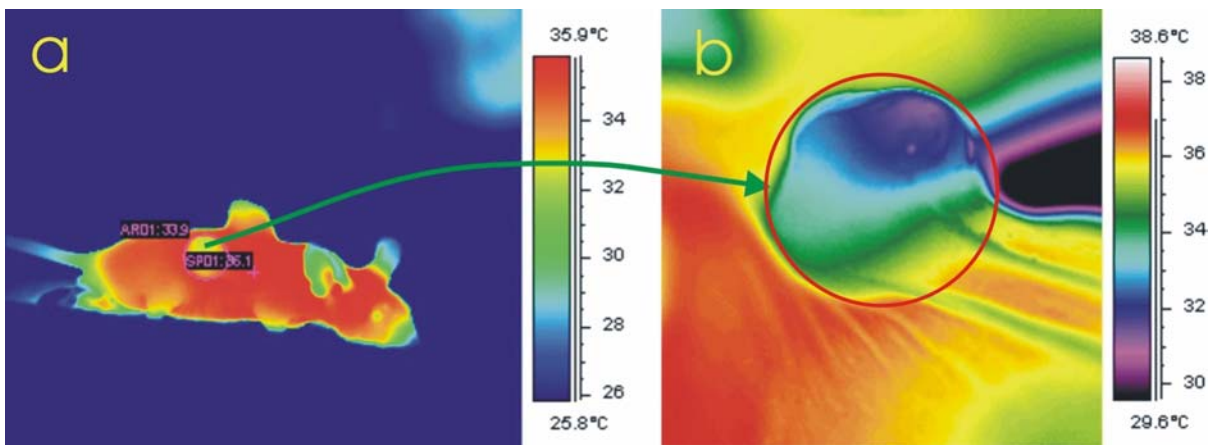
**FIGURE 1** MDA-MB-231 tumour size development and temperature reduction. Day one was considered to be the day when the tumour volume was 10 mm<sup>3</sup>, data are expressed as mean ± SD of values for five tumours in the experimental group.



**FIGURE 2** (a) Thermal image of a mouse and (b) the corresponding temperature profile through the tumour site. The tumour was identified by thermography at day 6 after cancer cell injection and this image was unusual in that it revealed a smaller second cooler area.



**FIGURE 3** MCF7 tumour size development and temperature reduction. Day one was considered to be the day when the tumour was 10 mm<sup>3</sup>, data are expressed as mean ± SD of values for six tumours in the experimental group.



**FIGURE 4** (a) Whole body thermal image and (b) microscopic view of an MCF7 tumour. The average temperature of the circled area is 33.9°C; 2.2°C below the surrounding body temperature.