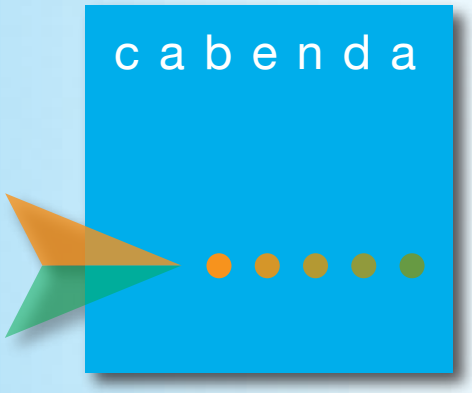


innovative tools for drug discovery

- Map microregional activity of anticancer drugs
- Assess the effects of drugs on cells relative to vasculature
- Screen for drug penetration using proprietary *in vitro* assays
- Discover more potent anticancer drugs

Overview

Cabenda provides services for evaluation of drug activity in the context of the tumour microenvironment. Advanced in-house methodologies allow us to conduct high volume *in vivo* tumour and *in vitro* 3-D tissue model screening for quantitative assessment of drug activity.

whole tumour immunostaining

Advanced microscopy and immunohistochemical staining methodologies that bypass conventional bottlenecks allow for high throughput *in vivo* studies.

intelligent image analysis

Whole tumour analysis and measurement of drug activity in relation to other markers such as vasculature or hypoxia allows for comprehensive, advanced drug evaluation.

3-D tissue based screening

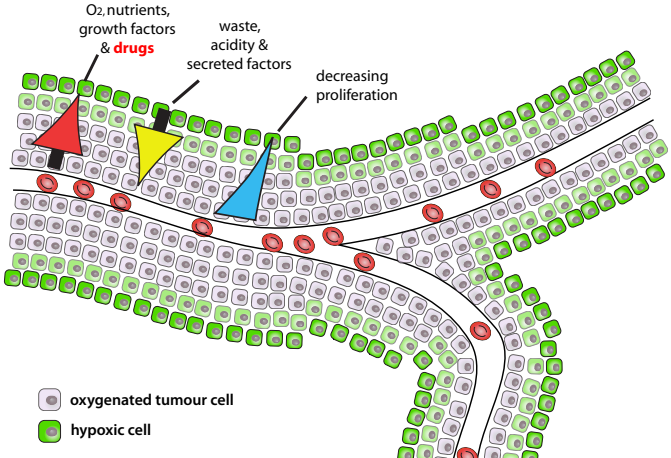
In vitro screening using 3-D tissue discs allows for evaluation of drug penetration and activity under controlled conditions in the context of the tumour microenvironment

Assessing the microregional activity of drugs

The histological architecture of solid cancers plays a decisive role in determining response to therapy. Vascular density and variabilities in perfusion and leakiness of vessels are key determinants of drug access to the parenchymal compartment of cancers.

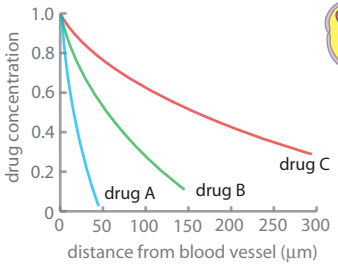
Gradients in oxygen, nutrients, growth factors and waste products determine proliferation and apoptosis and, in turn, have consequences for drug efficacy.

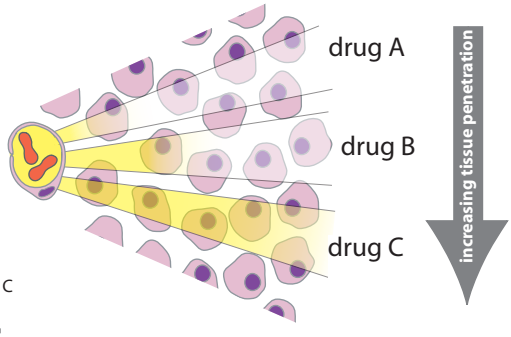
Tumour tissue mapping allows the ability of drugs to induce apoptosis or halt proliferation to be assessed quantitatively on a microregional basis. For example the effects of a drug as a function of proximity to the vasculature can be assessed.



Gradients in diffusible molecules supplied and removed by the blood result in gradients in proliferation, quiescence and survival that vary with distance from vasculature.

The ability of a drug to distribute within the extravascular compartment of tumour tissue is a critical determinant of efficacy



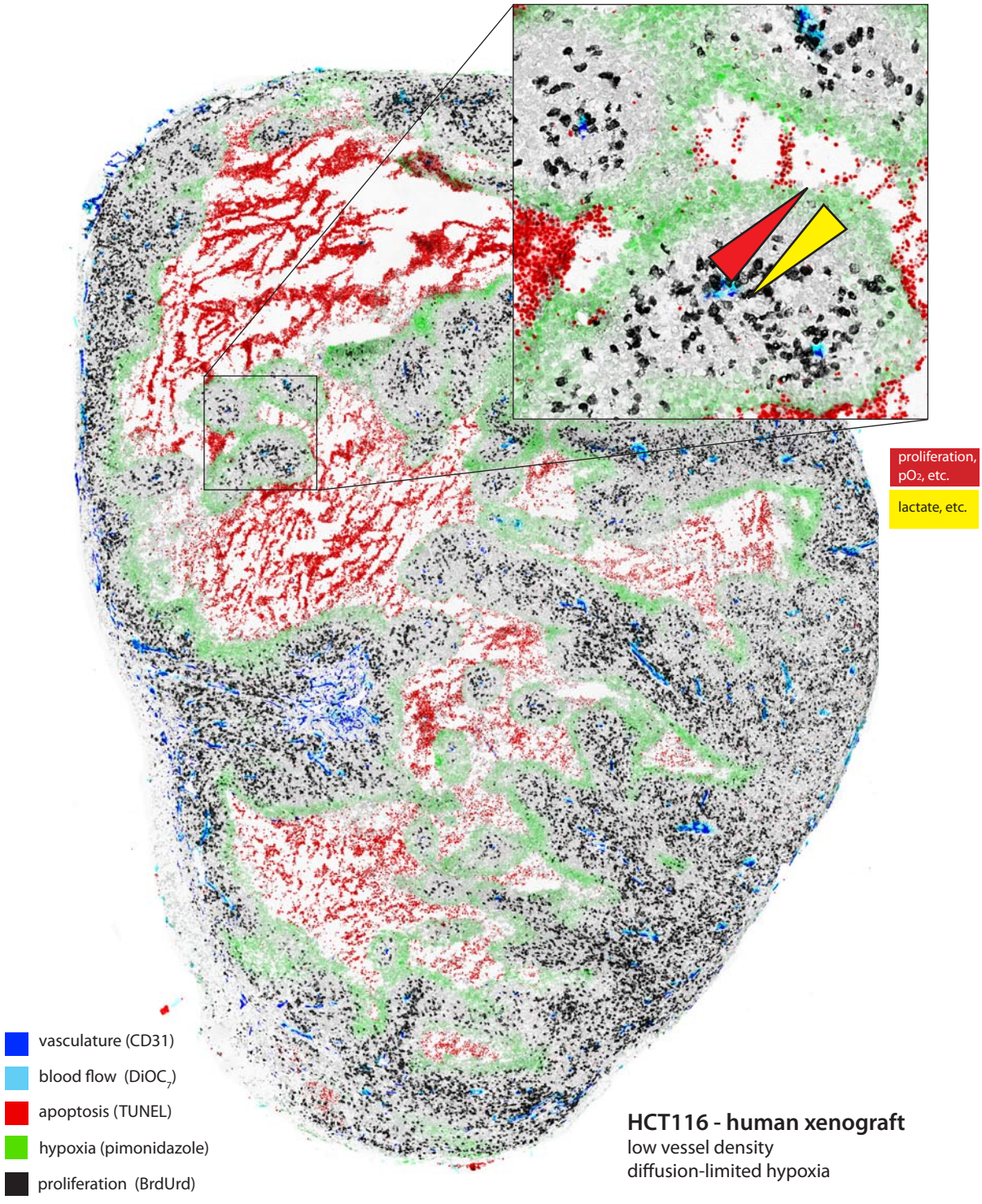


Diagrammatic representation of drug diffusion showing three drugs with different capacities to penetrate the extra-vascular compartment.

Examining whole tumour sections

whole tumour immunostaining

Different tumour types may be characterized by their patterns of growth. For example, **corded tumours** typically exhibit low vascular density with tumour cells existing up to ~150 µm from perfused vessels. Those cells nearest to the vessels are found to be proliferating, while further away the tumour cells become quiescent, hypoxic, and eventually apoptotic and necrotic. Diffusion of oxygen, nutrients and growth factors determine the maximum distance that cells survive and proliferate in corded tumours. Diffusion derived gradients in proliferation status create a sub-population of quiescent tumour cells that will be resistant to anti-proliferative based therapies, and consequently present a barrier to drug efficacy. **Intermittently perfused tumours** are characterized by higher microvessel density, where hypoxia and tumour cell proliferation are found to coexist, as are perfused vessels in hypoxic areas and unperfused vessels in well oxygenated zones. This architecture implies better access of drugs to all cancer cells.



HCT116 - human xenograft
low vessel density
diffusion-limited hypoxia

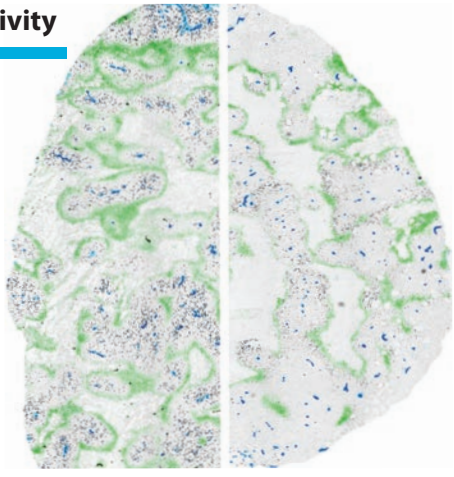
Map drug activity in whole sections

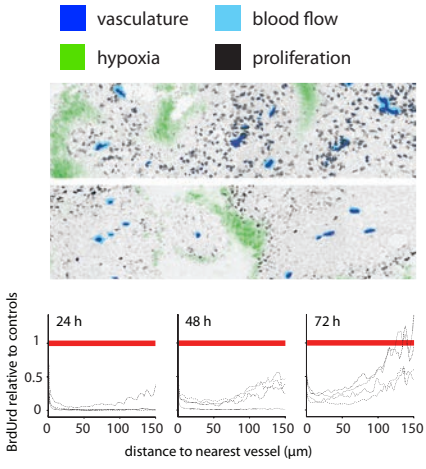
intelligent image analysis

Visualize microregional drug activity

Using whole tumour mapping techniques drug activity can be measured in relation to vasculature. e.g. Gemcitabine effectively inhibits tumour cell proliferation throughout the tumour at early timepoints after administration. However, cells distal to blood vessels, adjacent to necrosis, are least affected and later resume proliferation.

Huxham *et al* (2004)
Cancer Res. **64** 6537

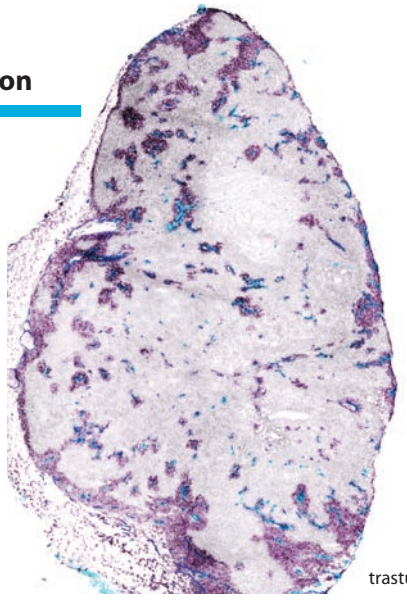


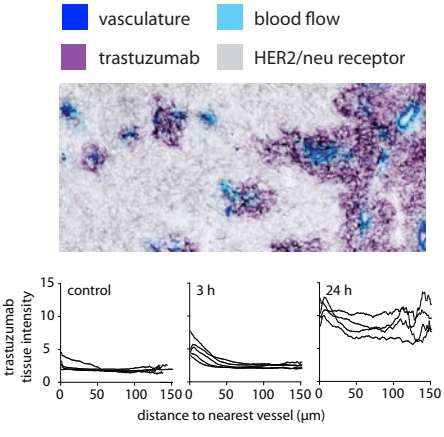


Map extravascular drug distribution

For drugs that can be directly visualized, tumour mapping allows determination of drug distribution from vasculature. e.g. Systemically delivered trastuzumab (Herceptin) is seen binding proximal to vasculature 3 h after dosing but later reaches cells up to 150 μm away.

Baker *et al* (2008)
Clin. Cancer Res. **14** 2171

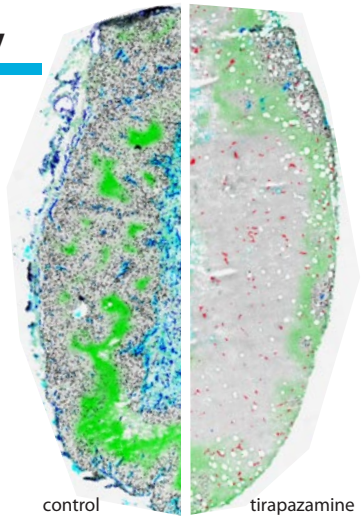


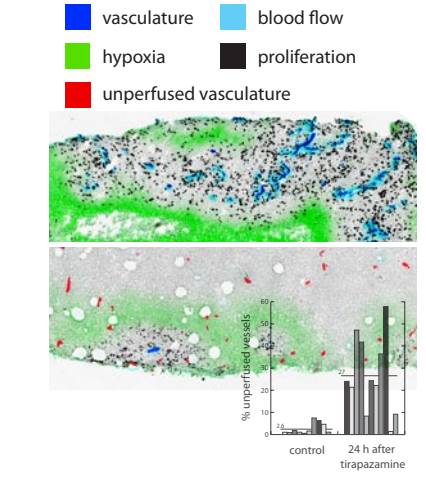


Quantify anti-vascular drug activity

By monitoring perfusion, proliferation and hypoxia in whole tumour sections it is possible to detect unexpected mechanisms of drug activity. e.g. The hypoxic cytotoxin tirapazamine was unexpectedly discovered to exert an effect through vascular shutdown of central blood vessels causing loss of tissue perfusion and cell death.

Huxham *et al* (2006)
Radiother. Oncol. **78** 138



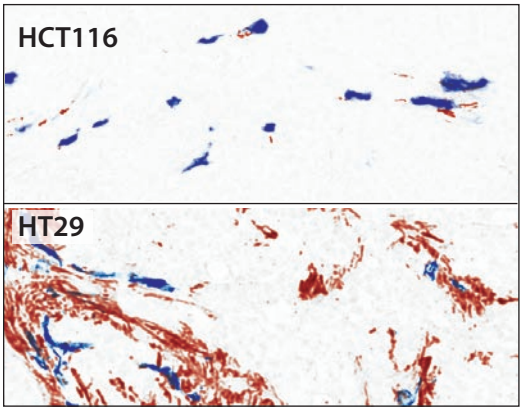


Assess anti-vascular therapies in whole sections

intelligent image analysis

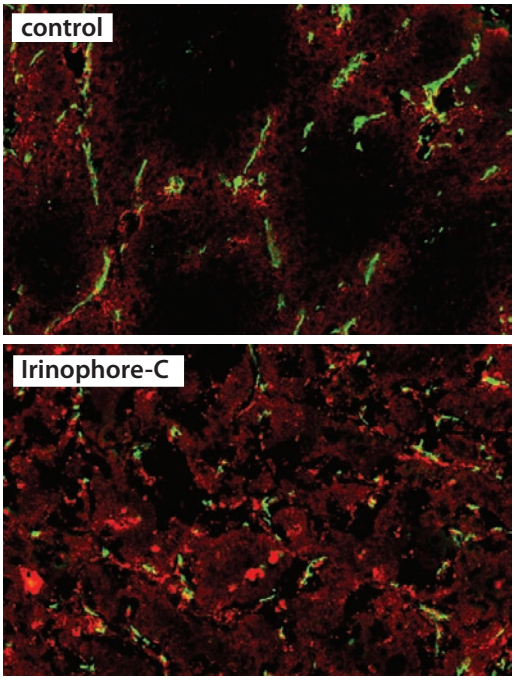
Anti-vascular effects of drugs are notoriously difficult to characterize and quantify, largely due to the **inadequacy of available assays**, particularly for *in vivo* studies. Microvessel density has great utility and prognostic significance in the clinic, but is routinely criticized for being subjective and inadequate. These studies are labour intensive, and **lack any functional determination**.

We have developed multiplex immunohistochemistry staining procedures that, in combination with our **high throughput staining and imaging** systems, enable comprehensive, **quantitative, vascular specific** data to be generated from tumours grown and treated *in vivo*. This more broad approach provides both **functional and morphological vascular data** that can be further characterized in the context of tumour microenvironmental features such as proliferation, apoptosis and hypoxia *all in the same section*.



Changes in vascular morphology and maturity

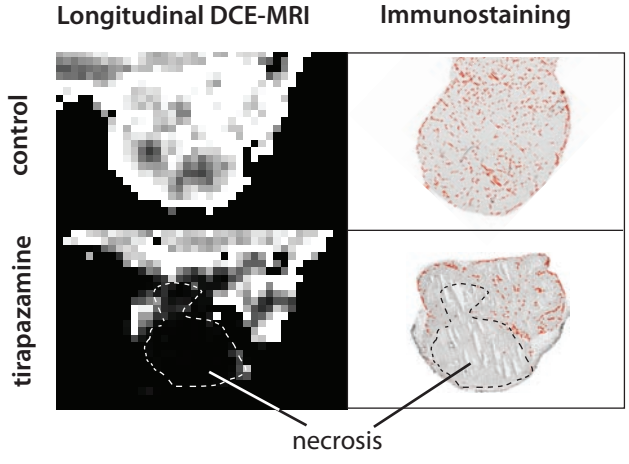
Anti-vascular therapies may result in changes to vascular support cells such as pericytes, which may consequently impact vascular function and tumour progression. For example, vascular normalization is a proposed phenomenon whereby immature vessels are selectively pruned by therapy. As a sample illustration two colorectal carcinoma xenograft models (right) exhibit dramatically different vascular smooth muscle covering; multiple markers for maturity may be co-stained with vascular markers to assess morphology.



Changes in vascular function

In addition to assessing MVD and morphology, functional vasculature data is critical in assessing antivascular effects. Shown here are portions of whole tumour sections imaged for vasculature (CD31) and the native fluorescence of systemically administered doxorubicin in untreated or Irinophore-C treated tumours.

A decrease in vascular density was found in the treated tumours, however this coincided with improved perfusion and consequently increased distribution of doxorubicin.



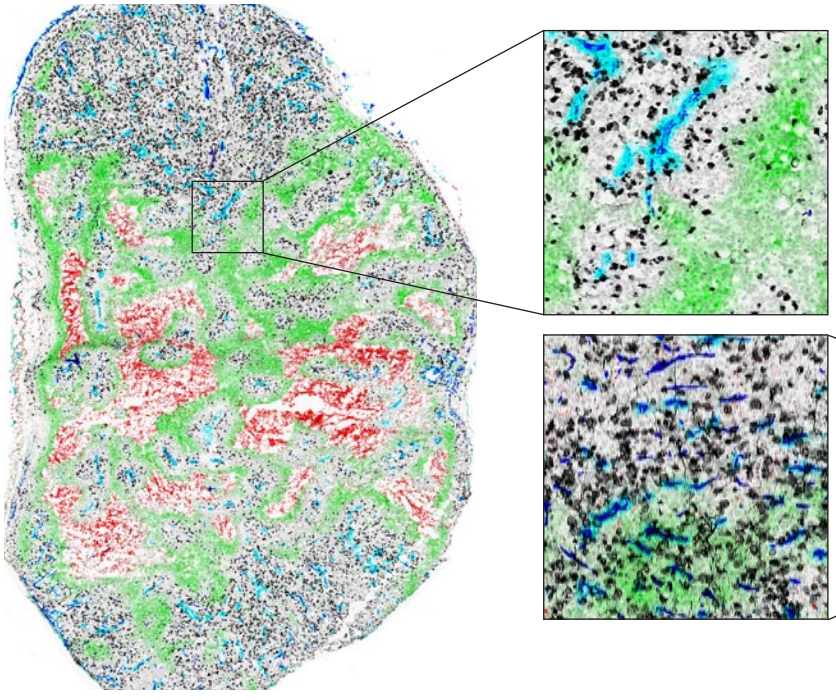
DCE-MRI and histological mapping

Using a 7-Tesla MR scanner, novel fiducial markers and our histological mapping technologies, the progression of anti-vascular treatment response can be tracked *in vivo* using longitudinal DCE-MRI imaging combined with high resolution tumour mapping data (perfusion shown in red).

Tumour architectures

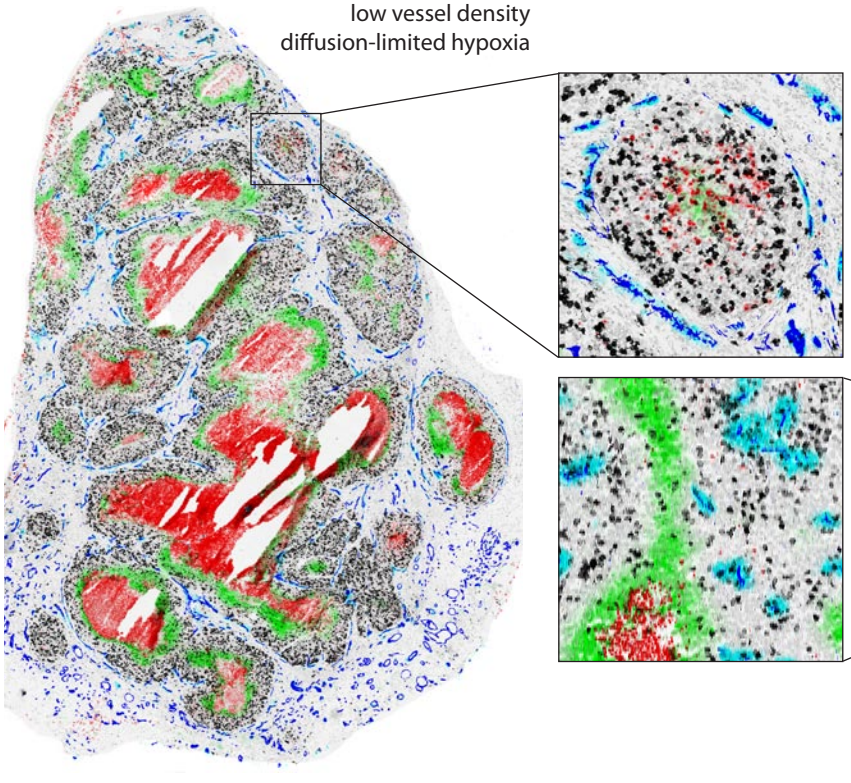
whole tumour immunostaining

HT29 - human xenograft
low vessel density
diffusion-limited hypoxia

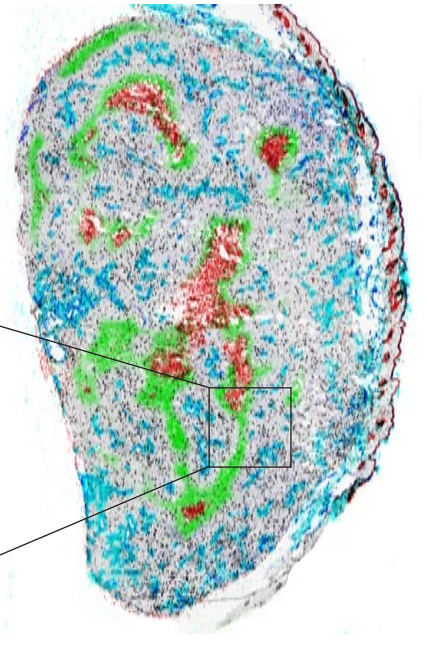


SCCVII - mouse syngeneic
high vascular density
hypoxia derived from intermittent flow

HeLa - human xenograft
compartmentalised growth
low vessel density
diffusion-limited hypoxia



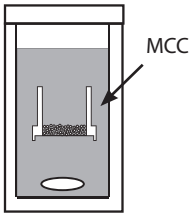
MDA-MB-435 LCC6 - human xenograft
intermediate vessel density
diffusion-limited hypoxia



vasculature (CD31) blood flow ($DiOC_7$) apoptosis (TUNEL) hypoxia (pimonidazole) proliferation (BrdUrd)

Multilayered cell culture (MCC-discs)

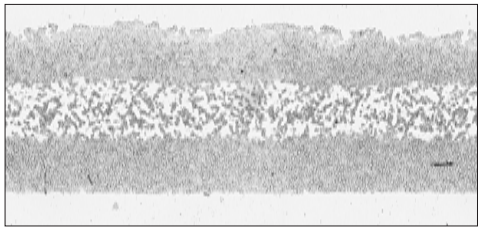
3-D tissue based screening

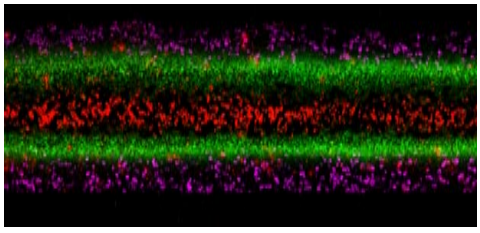


Growth apparatus

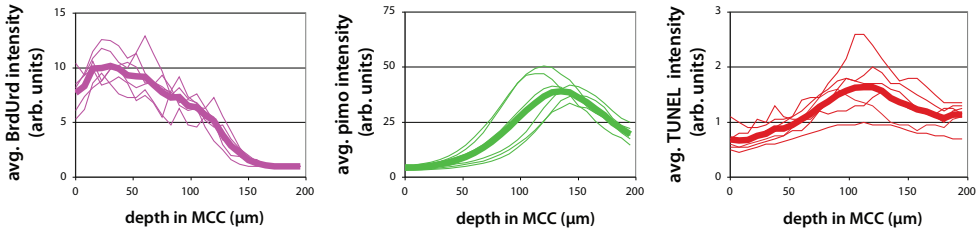
MCC is a 3-D tissue culture which models the extravascular compartment of tumours and can be used to study drug penetration in a controlled environment. Similar in some ways to spheroids, MCCs are grown as discs of tissue under controlled conditions.

Necrosis

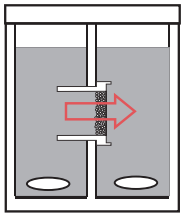




proliferation, oxygen and apoptosis gradients formed in MCC-discs

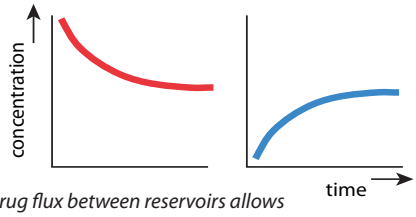


Flux-based drug screening assay



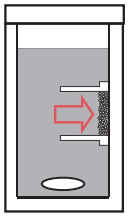
Flux assay

MCC-discs can be used to separate two media filled reservoirs to monitor the rate of flux of drugs through tissue. Mass balance calculations then allow measurement of rate of diffusion and reaction within the tissue.



drug flux between reservoirs allows for mass balance quantification of drug reaction within tissue

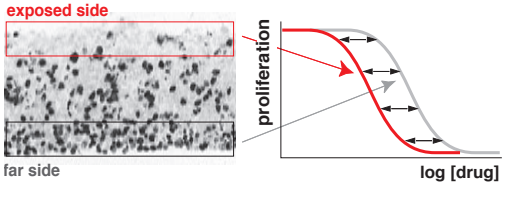
Effect-based screening assay



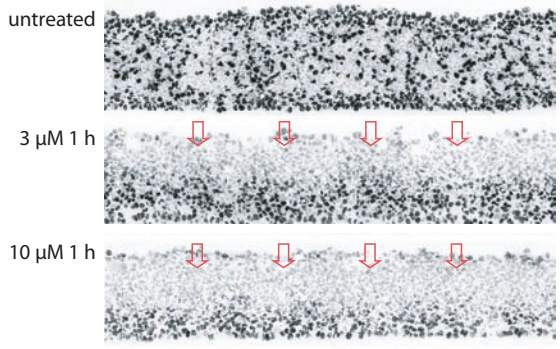
Penetration assay

In addition to allowing direct visualisation of labelled drugs, the symmetrical growth that occurs in MCCs allows drug penetration to be evaluated by comparing a drug's effect on the cells on either edge of the cultures following exposure to one side.

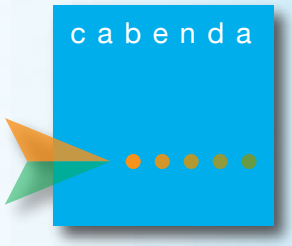
During growth the cultures have access to oxygen and nutrients from top and bottom and both of these edges exhibit high proliferation rates. During drug exposure cultures are temporarily closed-off to allow drug build up.



Shift in dose response between edges indicates magnitude of concentration gradient across the tissue.



Gemcitabine - effect on proliferation in an MCC-disc exposed from one side to 3 & 10 µM for 1 h compared with untreated controls. Increasing gemcitabine exposure causes inhibition of proliferation deeper into tissue.



- Intelligent image analysis of drug activity within the tumour microenvironment
- Multiplex quantitative immunostaining of tissue sections
- 3-D tissue culture assessment of drug distribution and penetration
- Comprehensive assessment of antivascular and antiangiogenic activity
- Histologically correlated MRI analysis of anticancer effects
- Consulting

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