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# Histomorphometry of spherical tumors using holoptical cross-sections

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**Summary.** A new morphometric method for quantifying tumour regression on holoptical cross-sections is described which permits assessment of the extent and distribution of viable tumour tissue and necrosis in spherical tumours. The value of the method is demonstrated by comparing tumour regression in the liver metastases of a colorectal adenocarcinoma before and after regional chemotherapy. The practicability of the method for clinicopathological therapeutic studies is emphasized.

**Key words:** Histomorphometry – Tumour regression – Holoptical cross-section – Therapy control

## Introduction

Histomorphometric methods are increasingly important in experimental and clinical tumour pathology. They make it possible to measure tumour growth and the effect of factors which influence tumour cell proliferation. Numerous studies have been devoted to angioarchitectural changes (Vogel 1965; Tannock and Steel 1969; Yamaura and Sato 1974; Gabbert et al. 1982), the structure of tumour cords (Thomlinson and Gray 1955) and, to a lesser extent, the distribution of tumour necrosis. The results of these studies have helped to explain the specific metabolic conditions present in tumours (Vaupel 1974 and 1979) and provided fundamental knowledge for understanding chemo- and radiosensitivity (Hilmas and Gillette 1974; Brammer et al. 1979). In contrast to these mainly experimental studies performed on small tumours or on segments of larger ones, by point counting methods on the basis of special stereological models (Chalkley 1943; Delesse 1847; Henning 1958; Weibel 1963; Dunnil 1968; Oberholzer 1983), morphometric methods are only rarely applied to large tumours in clinical biopsy material. An exact quantification of tumour architecture and tumour regression is

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necessary to evaluate the efficacy of cytostatic and radiological treatment. For this purpose a practicable morphometric method has been developed which permits a quantification of tumour regression on holoptical cross-sections.

## Material

The application of the method is demonstrated, by way of example, on 24 complete liver metastases of colorectal adenocarcinomas from 9 patients. 23 metastases had been removed before therapy. In one patient an additional metastasis was excised 10 weeks after isolated cytostatic liver perfusion (Aigner et al. 1982) with 400 mg 5-fluorouracil. The metastases measured between 1.0 cm and 3.5 cm in diameter.

## Method

*Preparation of holoptical cross-sections.* Tumours which are nearly spherical, or conglomerates from spherical tumours with a radially symmetric structure are suitable material for morphometric evaluation. A complete cross-section with a maximal cross-sectional area is mounted on a 5 × 5 cm glass slide. In larger tumours the slices are divided and mounted on several slides in such a way that the total area is reconstructable afterwards. The sections are 5 μ thick. Haematoxylin-eosin staining with strong visualization of the nuclei is a suitable staining method.

Morphometric evaluation proceeds in three steps:

*Step one.* Projection of the sections and plotting of the vital tumour compartments.

The tumour cross-sections, magnified ×10, are projected onto the radial measuring grid of a projector screen. The grid has 20 radiants. With this magnification glandular structures can be visualized, but evaluation of individual cells is not possible. After the tumour has been centered in the measuring grid, its vital compartments arranged along the measuring radiants are plotted on a transparent foil (Fig. 1a). The distances between center and tumour margin which are covered by the measuring radiants are measured as tumour radii $r_n$, and the average tumour radius $\bar{r}$ is computed from them. The index $n$ indicates the number of the measuring radiants and the individual number of the respective tumour radius.

*Step two.* Plotting of standardized marking points on the tumour radii. Starting from the margin, marking points are drawn on the tumour radii on a square scale. From the periphery towards the center these points are consecutively numbered with number $a=0, 1, 2 \ldots$. The marking points situated on the tumour margin have the number $a=0$. The distances $s_n(a)$ of the marking points from the tumour periphery are determined for each tumour radius $r_n$ separately according to the square function

$$s_n(a)=c_n a^2 \quad [\text{mm}]. \tag{1}$$

The variable $c_n$ is defined as the quotient from the length of the respective tumour radius $r_n$ and the amount of the mean length of the radius:

$$c_n=\frac{r_n}{|r|} \quad [\text{mm}]. \tag{2}$$

The square scale in which the marking points are plotted is adjusted to the varying length of the radii by the quotient $c_n$: as the radius $r_n$ increases, $c_n$ also increases, and the distance between the markings becomes therefore greater. In this way, the tumour cross-section, which is not completely round, is related to an ideally round slice having the radius $\bar{r}$. On the basis of these considerations a template was developed on which the marking points are drawn in relation to the quotient $c_n$ (Fig. 2). They are directly transferred from this template to the tumour radii of the transparent foil. By connecting the markings having the same

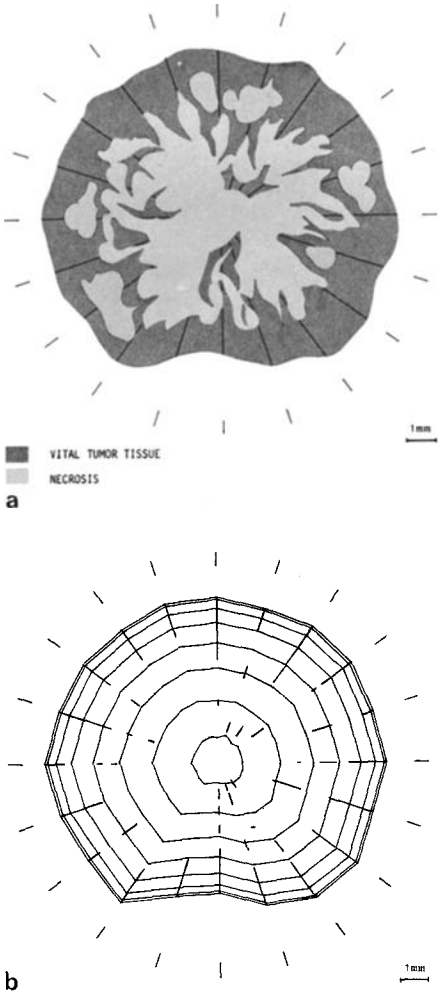


**Fig. 1 a, b.** Histomorphometry of a tumour cross-section (schematic drawing). **a** 1st measuring step: plotting of the measuring radiants covering the vital tumour compartments (dark grey). **b** 2nd measuring step: plotting of concentric lines in squarely increasing distance from the tumour periphery. From the number of intersections of a concentric line the viable tumour content for the respective distance from the tumour margin can be computed

number $a$, concentric lines are formed. On the ideally round tumour slice standardized to the mean radius $\bar{r}$, these lines cover all points which are equidistant from the periphery (Fig. 1 b).

*Step three.* Demonstration of the distribution of vital tumour tissue and necroses in the standardized tumour cross-section.

Buy counting the intersections m of such a concentric line with the tumour radius segments situated on viable tissue and relating them to the number $n$ of the measuring radii, one arrives at the percentage of vital tissue $T_{rel}$ in the distance $s_a$ from the periphery:

$$T_{rel}(s_a) = \frac{m_a}{n} \times 100 \quad [\%]. \tag{3}$$

By plotting $T_{rel}$ against the distance $s_a$ of the markings from the tumour margin, the percent distribution of viable tumour tissue in a slice from the periphery towards the center can be demonstrated. The curve shows the percentage of viable tissue in the maximal tumour cross-section.

From the percentage of vital tumour tissue $T_{rel}$ $(s)$, the absolute area content and the

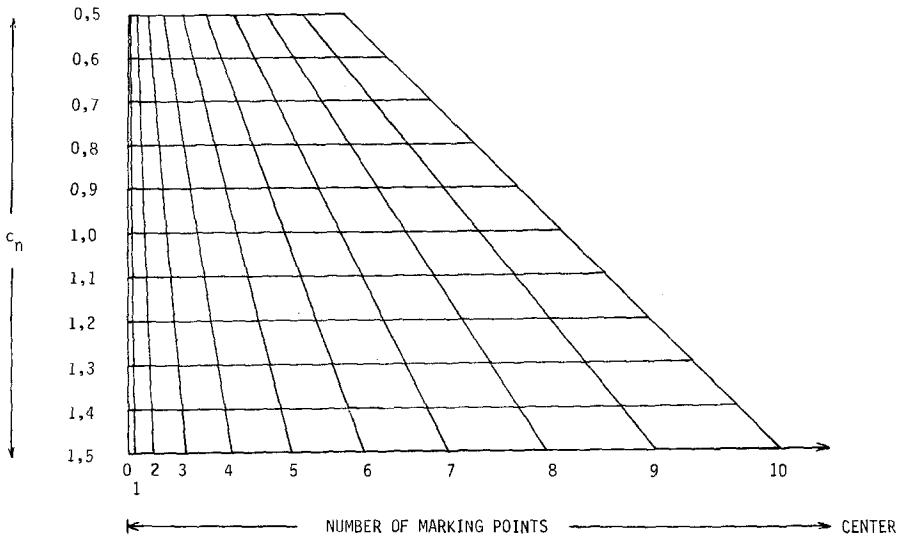


**Fig. 2.** 2nd measuring step: template for the determination of standardized marking points. Each tumour radius $r_n$ drawn on the transparent foil is brought into register with the horizontal line of the appropriate quotient $c_n$. The grid points of the horizontal line are then directly transferred to the foil as marking points

distribution of viable tissue are computed. For this purpose, $T_{rel}$ $(s)$ is set in relation to the circumference $2\,n(\bar{r}-s_a)$ of the respective concentric line according to the equation

$$T_{abs}(s_a)=\frac{T_{rel}}{100}\times 2\,\pi(r-s_a)=\frac{m_a}{n}\times 2\,\pi(r-s_a)\quad [\text{mm}]. \tag{4}$$

$T_{abs}(s_a)$ corresponds to the total length of the circumference segments of a concentric line which cover vital tumour tissue in the distance $s_a$ from the tumour periphery. Plotting $T_{abs}$ against the distance $s_a$ of the markings from the margin, the distribution of the actual viable tumour area in a cross-section from the periphery towards the center is obtained. The area under the curve path, which can be determined planimetrically, equals the total area of viable tumour tissue in the cross-section. The area of necrosis is computed analogously.

*Measuring errors*

The accurracy of measurement depends on the number of measuring radiants and on the density of the marking points. The relation between measuring error and the number of the measuring radiants is established as follows: Each tumour radius is assigned a circular segment. The percentage of viable tumour tissue of the respective circular segment is determined from the number of intersections of each tumour radius with viable tumour tissue. Thereafter the average percentage of viable tissue is calculated for an increasing number of circular segments. The difference between the average percentages of viable tumour tissue tends towards 0 with an increasing number of circular segments considered. This difference in mean value corresponds to the extent of variation in the evaluated areas and thus to the measuring error in dependence on the number of measuring radiants employed.

The radial symmetric distribution of viable tumour is checked by means of a series of 10 parallel cross-sections which are spaced 0.2 cm apart in a lesion having a diameter of 2.5 cm. To prove the validity of the method these cross-sections are comparatively measured by a point counting method. In addition, the percentage of viable tissue is determined by comparison with maximal cross-sectional areas of another 10 tumours.

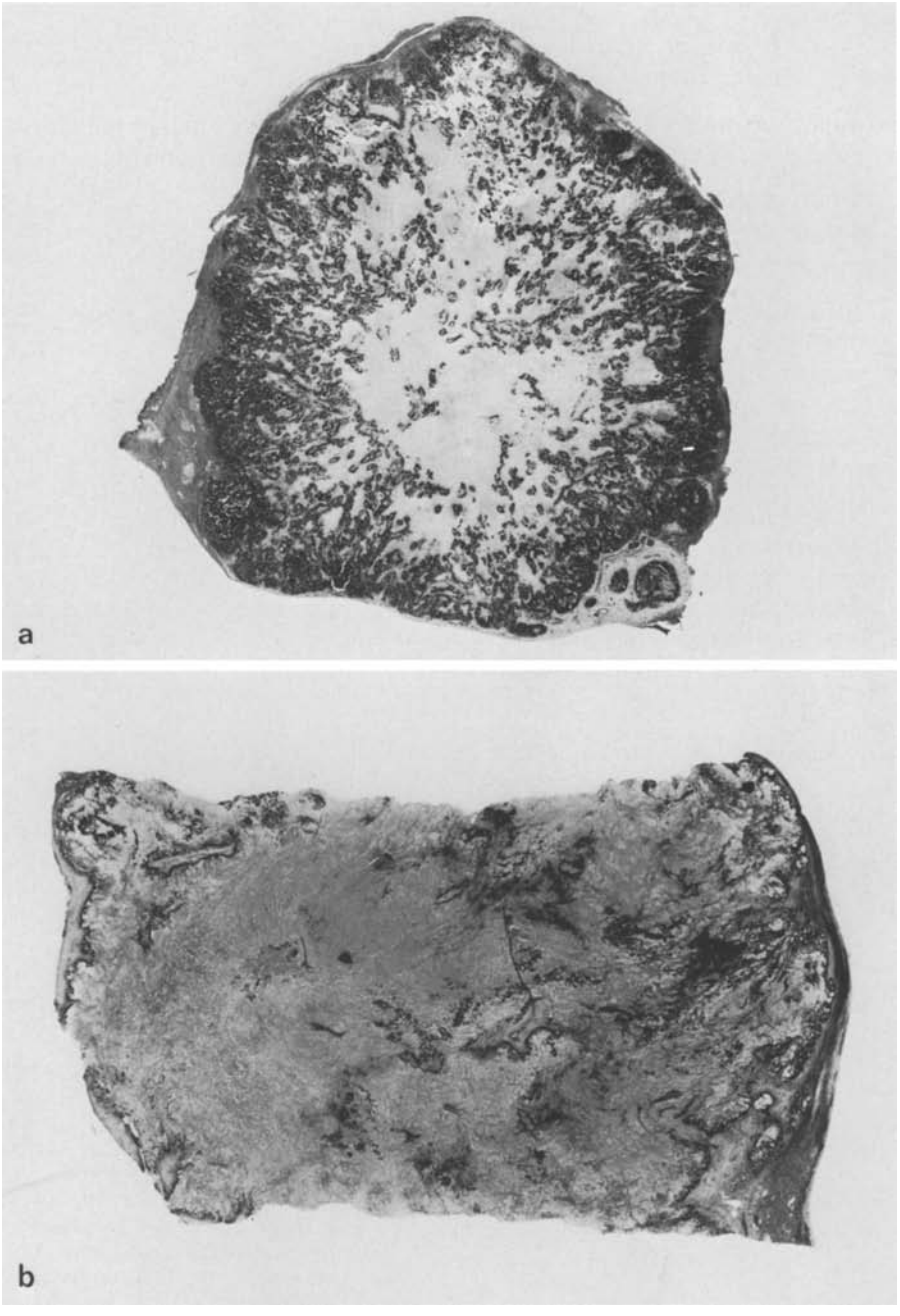


**Fig. 3a, b.** Liver metastases of a moderately differentiated colorectal adenocarcinoma: **a** before regional cytostatic therapy (H-E, × 3.2) **b** after regional cytostatic therapy (H-E, × 3.2)

## Results

*Quantification of tumour regression*

The quantification of tumour regression performed on maximum cross-sectional areas of colorectal adenocarcinomas yielded the following results:

- Group of untreated metastases ($n=23$) (Fig. 4):

| | | |
|---|---|---|
| Distance from tumour margin with 50% vital tumour tissue | 1.80 mm | |

- Untreated metastasis (intraindividual comparison) (Figs. 3a, 4)

| | | |
|---|---|---|
| Maximum cross-sectional area | 2.84 $cm^2$ | |
| Area of viable tumour tissue | 1.15 $cm^2$ | 40.5% |
| Necrotic area | 1.69 $cm^2$ | 59.5% |
| Distance from tumour margin with 50% vital tumour tissue | 2.01 mm | |

- Treated metastasis (intraindividual comparison) (Figs. 3b, 4)

| | | |
|---|---|---|
| Maximum cross-sectional area | 3.53 $cm^2$ | |
| Area of viable tumour tissue | 0.47 $cm^2$ | 13.2% |
| Necrotic area | 3.06 $cm^2$ | 86.8% |
| Distance from tumour margin with 50% viable tumour tissue | 0.25 mm | |

*Examination of the described measuring method*

- Measuring error in dependence on the number of measuring radiants. Relative to a standardized tumour slice with the radius $\bar{r}$, the extent of variation in the evaluated areas was $\pm 0.5$–1% for liver metastases of colorectal adenocarcinomas up to 3.5 cm in diameter with 20 measuring radiants and the employed square scale.

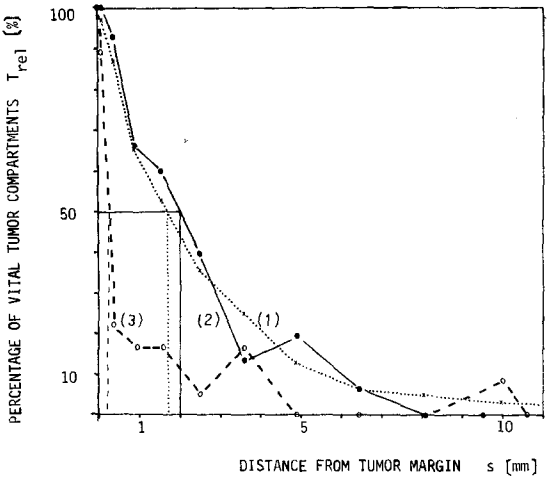


**Fig. 4.** Percent distribution of vital tumour tissue in cross-sections of liver metastases from colorectal adenocarcinomas: (*1*) untreated metastases ($n=23$), (*2*) metastasis of Fig. 3a, (*3*) metastasis of Fig. 3b (same patient after isolated liver perfusion with 5-fluorouracil)

- Comparative control measurement using a point counting method.
  Comparative evaluation with the described method and with a point counting method showed that the difference in the area percentage of vital tumour tissue in relation to the cross-sectional area is 3% (mean) for cross-sectional areas of a completely stepsectioned tumour and 4.0% (mean) for maximum cross-sectional areas from 10 different tumours.
- Demonstration of radial symmetry.
  As expression of the radial symmetric distribution of vital tumour tissue there is a symmetric increase of necrotic compartments from the periphery towards the center in the cross-sections of a completely step-sectioned metastasis.

## Discussion

The morphometric method described has been developed for quantifying radial symmetric area compartments which are present in the cross-sectional areas of many spherical tumours. In contrast to the conventional point counting methods which make use of a set line grid (Delesse 1847; Oberholzer 1983), the new method uses a radial symmetric measuring grid which is constructed in relation to the relevant circular surface. By choosing a scale that increases quadratically from the periphery towards the center for the distances of the marking points from the tumour margin, the measuring accuracy achieved corresponds to the distribution of viable and necrotic tumour compartments. This accuracy is greatest in the peripheral segments where the decrease of vital compartments is greatest for many tumour types.

The validity of the method was checked by comparative control measurements with a conventional point counting procedure on maximum cross-sections of different tumours, and on cross-sections from a step-sectioned tumour. The results obtained for the areas evaluated were nearly identical.

The advantage of the measuring principle is evidenced by the evaluation of liver metastases from colorectal adenocarcinomas, which was performed as an example. The percental and absolute area of vital tumour tissue and necroses in addition to their distribution from the periphery towards the center can be determined in holoptical tumour cross-sections. The method was used to measure tumour regression in liver metastases before and after regional cytostatic therapy (Fischer et al. 1984). It permits assessment of the extent of increasing necrosis as an important indication of effective therapy and localisation of areas where there is no increased necrosis. Although the distribution of vital tumour compartments – especially in the periphery – is an important prognostic criterion after therapy, it is not considered or is inadequately considered, in numerous regression gradings.

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