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REMOTE QUANTITATION IN DNA IMAGE CYTOMETRY

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ABSTRACT

Remote Quantitation (RQ) is the application of telematics technologies to the quantitation procedures used in morphology for research and diagnostics. It can be differentiated in Active Expertise, Passive Expertise and Remote Quality Control. All these fields share the common methodological approach that a quantitation of a slide at a local site is done remotely, either in total or in single components of quantitation procedures.

Diagnostic DNA image cytometry will serve as an example to demonstrate the feasibility and usefulness of remote quantitation of images grabbed from cytological or histological specimens. On the one hand, a European consensus already exists concerning standardization of diagnostic DNA image cytometry and further European efforts are on the way for ongoing standardisation and quality control. On the other hand, the method urgently needs further standardisation to become more widely applied and accepted. The goal is to transform data of DNA measurements obtained from different machines into a uniform format by a Quantitation Server and to derive standardised parameters, algorithms, statistical tests and diagnostic classifications. As the diagnostic results depend on high quality measurements the technical performance of the instruments should be tested regularly. Remote quantitation will offer the facilities to perform these tests by telematics or to send data of measurements on test slides for remote quality control to that server.

Tests of the diagnostic performance of individual machines and pathologists as well as a remote service for quantitation tasks will be offered by telematics, too. Finally, the reliability and consistency of data on tumour markers, directed to European Databases, could and should also be guaranteed by this telematics approach.

This paper was aimed to show the validation of Remote Quantitation by using the quantitation server for the analysis of different aspects of remote DNA ploidy analysis.

Keywords: remote quantitation, DNA image cytometry, standardisation, quality control.

INTRODUCTION

Remote Quantitation (RQ) is the application of telematics technologies to the quantitation procedures used in morphology for research and diagnostics (Haroske et al. 1997b).

Generally, three different scenarios can be visualised, which will determine the user needs, the technological demands, and the acceptance by pathologists:

(i) *Active expertise* (AE) in RQ, where a specimen at a *local site* is scanned under remote control, and the images are sent together with method and patient data to a *remote station* for segmentation, quantitation, feature extraction and interpretation of results. The results of all these steps are sent back to the local site. This scenario requires a robotic microscope at the users site.

(ii) *Passive expertise* (PE) in RQ, where a specimen is scanned, (segmented, quantified, and featured) at a local site under local control, whilst the images and/or quantitation data of measured nuclei (which are marked and classified by the requester at the local site) are submitted to the remote station for result interpretation, validation and/or discussion prior to diagnostic/prognostic evaluation, entrance in reference image data bases, multi-center trials etc. This approach is also called *Remote Consultation (RC)*. In a wider sense the RC is also aimed at discussion of measurement results or troubleshooting.

(iii) *Remote Quality Control* by RQ, where the measurements and their interpretations at the local site are validated and accredited by the remote station.

Because DNA ploidy analysis already has an impact on diagnostics, it has been selected for further study by the techniques of Remote Quantitation. In future, other currently-used techniques such as quantitative immunohistochemistry and quantitative molecular pathology will also benefit from the solutions devised for Remote Quantitation of DNA ploidy.

For practical solutions in Remote Quantitation the server concept has been defined (Haroske et al., 1997c). Procedures and protocols have been developed for interoperability between the server and various cytometry workstations as well as for the evaluation of measurement and diagnostic performance in static DNA cytometry (Haroske et al., 1997c).

This paper evaluates Remote Quantitation by using the quantitation server for the analysis of different aspects of remote DNA ploidy analysis.

MATERIALS AND METHODS

The quantitation server EUROQUANT (http://euroquant.med.tu-dresden.de) is based on a PC-system with INTEL Pentium® 200 CPU, 64 (max. 512) MB RAM and 4 GB HD capacity, equipped with the following software components:

- WINDOWS NT ®4.0 with MS-IIS included (Microsoft **I**nternet **I**nformation **S**erver) and with WWW, FTP and GOPHER-services.
- Database-software MS-ACCESS® 7.0
- DNA ploidy analysis software package, derived from an OPTIMAS® based self-written prototype.

By means of these tools a system of functional modules has been constructed for:

- Data exchange via Internet technology.
- Databases for data on
  - the user,

  - the users´s laboratory environment,
  - raw measurement data,
  - images,
  - results,
  - test specimens from scientific boards;
- DNA ploidy analysis;
- Performance control by the server;
- Teaching and training with definitions, explanations and examples for measurement conditions, quality assurance protocols, and diagnostic problems.

Table 1. Technical equipment for the image cytometry systems.

User code	User 1	User 2	User 3
Cytometry workstation	Axioplan / OPTIMAS	Axioplan / CIRES	Axiolab / CAS160
Microscope	Axioplan®	Axioplan®	Axiolab®
Objective	Plan-Neofluar x 63/1.25	Plan-Neofluar x 40/0.75	Plan-Achromat x 40/0.65
Condenser	Condenser 0.9	Condenser 0.9	Condenser 0.9
Filter	„green" filter 570 +/- 10 nm	„green" filter 570 +/- 10 nm	„green" filter 540 +/- 10 nm
xy-scanning-stage	with MCU 26	none	none
TV camera	XC77CE (Sony) pixel size : 11 µm * 11 µm; 0.03 µm ² in the object plane	XC77CE (Sony) pixel size : 11 µm * 11 µm; 0.076 µm ² in the object plane	XC77CE (Sony) pixel size : 11 µm * 11 µm; 0.076 µm ² in the object plane
Frame grabber	MFG®	Matrox® Meteor	not specified
Image analysis basic software	Optimas 5.2®	CIRES®	CAS160 SI®
Correction for	glare, diffraction [10]	glare, diffraction [10]	shading

Three sets of DNA measurement data on Feulgen stained imprints and FNAB preparations were used for the analyses in this paper. Two came from two user's scans of 105 breast cancers (User 1 and 3). The third came from 19 pleural effusions, 5 ascites samples, 8 cervical smears, 2 bladder washings, 2 broncho-alveolar lavages, and 2 thyroid adenomas (User 2). For calibration purposes 2 x 44 external reference specimens from rat liver imprints were used additionally (User 1 and 3). The preparation and Feulgen staining of the specimens were done at different laboratories following the recommendations of the ESACP (Giroud et al, 1997a).

The measurement data have been obtained by three different cytometry workstations, described in detail in Table 1.

The data sets exported from the cytometry devices in ASCII format have been transferred interactively via Internet technology (FTP) to the EUROQUANT server.
The verification of the server functions was performed in two ways:

- test of all server functions by means of DNA measurements performed at one cytometry workstation with known results,
- test of all server functions by means of DNA measurements performed on other commercial DNA cytometry devices. Both comparisons between original results and server results (User 2 and 3) and between server results from double measurements on two workstations (User 1 and 3) have been performed.

The users were asked to give short reports and comments of their test experiences, which have been analysed for immediate modifications or future extensions of the server.

RESULTS

So far 28 users have been registered and authorised at the quantitation server EUROQUANT. They contacted the server for information, training, and analysis of measurements. Meanwhile 2268 measurement data sets (specimens) have been analysed and stored in the databases of the server. In this paper only selected data from three of the users were studied.

The individual steps of the following two scenarios out of the three mentioned in the introduction have been analysed:

Passive expertise (Remote consultation)

- Check-in into the server

The check-in is possible for authorized users only. Each user can access only his own database domain via the server directly or via FTP.

Each new user is asked for information about his cytometry device(s) and his laboratory protocols. The structure of the device-protocol-measurement-hierarchy in the server´s databases is shown in Fig. 1.

So far, the three users analysed in this study have defined 3 devices and 167 protocols for their concrete measurement conditions. A typical protocol is given in Table2.

- outward transfer of IOD and area measurement data from the user to the server

The outward transfer of data sets and their import into the servers databases seems to be the most complicated task for the user. It needs some training and the understanding of the server`s database hierarchy. The user can build up several (different) database records from a

single data set of measurement data, e.g. using different types of reference cells (such as lymphocytes, rat hepatocytes, etc.). Due to the interactive mode of the server functions in this task the user can see whether the data structure used by himself is the appropriate one in terms of the databases.

In principle, cytometric DNA data from images transferred by the user can also be extracted by the server (not yet published). The biggest difficulty when transferring many (uncompressed) images per case is the low transfer rate using Internet connections.

*Table 2. Exemplary protocol information for a series of measurements (*specific parameters for correcting glare and diffraction (Haroske et al., 1997a)).*

Type of variable	Specification
reference cell type	Lymphocytes
numerical coding of ref.cells	2
preparation	Imprint
prestained with	None
fixative	4% buffered formalin
time of fixation	900 min
hydrolysis with	5.0 m HCl
hydrolysis at	22 ^{0}C
hydrolysis for	35 min
Schiff's reagent	Pararosanilin
colour index	42500
self prepared on	01/06/97
manufacturer of the dye	Merck
SO_2 rinsing	50 min
negative controls	yes
mounting medium	neutral balsam
manufacturer of the cytometry system	Zeiss, OPTIMAS
type	self-tailored
software release	5n
objective x	63
numerical aperture	1.25
filter	570 nm
+/-	10 nm
software correction for	glare and diffraction
by method	0.028; 1.17*

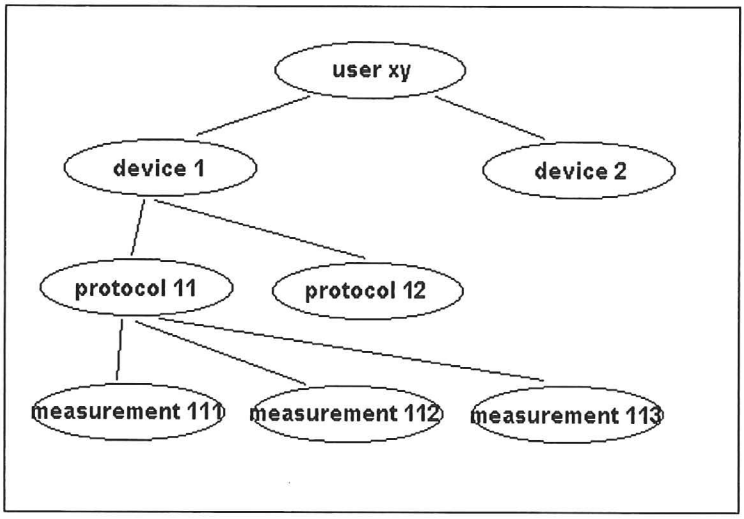

Fig. 1. Schematic database structure of the quantitation server.

- analysis of general process quality of the actual measurement

In Fig. 2 an example of the result display is shown. In addition to the usual DNA histograms of reference and analysis cells, calibrated by the reference cells, additional graphics and figures are displayed, aimed at detecting deviations from an optimal performance or indicating departures from normality in analysis cells. Most of these variables are not available on recent DNA cytometry devices. The user can simply print out this result page for his archives. More conveniently, the diagrams and the numerical results are stored for each result page as GIF- and ASCII-files, respectively, under the special name of the dedicated database record. The user can download these files via FTP for further computations and demonstration purposes.

If „classical" DNA histogram parameters are compared between the server and the cytometry station results, a high degree of consistency is shown. In Fig. 3 the correlation between the local results and server results of the cv of reference cells and the DNA ploidy of the G0/1-phase-fraction of DNA-stemlines is demonstrated for measurements of User 3. There is a high correlation between the results of the server and and the local cytometry device. The minor deviations are caused by different algorithms for peak finding as well as by parametric versus non-parametric calculations in the local workstation and in the server, respectively.

For the actual process quality of the measurement among others the following variables are computed

- cv of reference cells (cv_ref)
- relative error of the mean for reference cells (rem_ref)
- correlation coefficient of DNA vs nuclear area in reference cell peaks (corr_coeff_ref),
- error probability for the measurement data to be homogenously sampled (p_ homo_all),
- error probability for the data of each peak to be homogenously distributed (p_ homo_ana1, p_ homo_ana2)

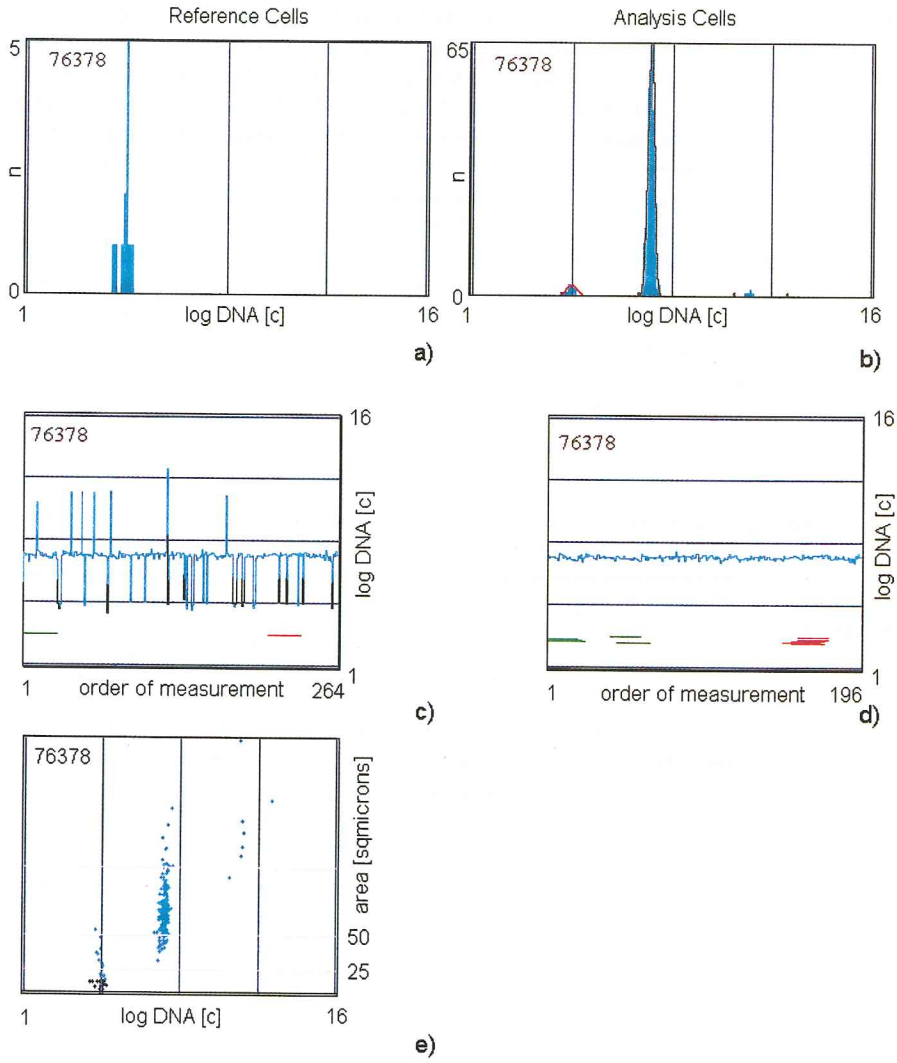

Fig. 2: DNA histogram displays of breast tumour "76378".
a) classical DNA histogram (logarith. scale) of the internal lymphocytes: n = 14; cv = 3.2%.
b) classical DNA histogram (log. scale) of the tumour cells: n = 250; DNA- stemline at 3.45c; cv = 3.76%.
c) sequential DNA diagram of reference (black) plus tumor cells (blue). The bars demonstrate parts in the sequence deviating significantly (p=0.0472) from other parts to higher (red) or lower (green) DNA values, i.e. the spatial distribution of DNA values is inhomogeneous.
d) sequential DNA diagram of G0/G1 tumour cells (blue). The red and green bars have the same meaning as in c).
e) DNA vs area scattergram of reference (black) plus tumor cells (blue); r = 0.1134 for reference cells; r = 0.2343 for tumour cells of the DNA-stemline.

The two latter variables are based on the assumption that the sampling of cells for the DNA cytometry should be a stochastic one (Giroud et al., 1997a). This holds true for the sequence between reference and analysis cells as well as for the sequence of analysis cells in each histogram peak. If those sequences of DNA values are divided in several (here up to ten) parts, and each part is statistically compared with each other, in a stochastic sampling procedure no differences between those parts should be observed. Statistical differences indicate in that approach either an inhomogeneous sampling (p_ homo_all), or inhomogeneities of analysis cells due to systematically increased or decreased DNA values in certain parts of the sequence (p_ homo_ana1, p_ homo_ana2). The most frequent cause for such deviations are preparation artifacts on the sample. A bilogical non-uniformity of the sample will also affect those variables if cell groups are studied not being stochastically distributed, e.g. by touch preparations. Finally, a drift of the instrument setting will also be indicated by those variables.

The median values and the appropriate 15%- and 85%-quantiles for the quality control related variables are listed in Table 3 for all three users.

Table 3. Median values and the appropriate 15%- and 85%-quantiles of variables indicating the process quality in all clinical specimens analysed. Values exceeding the ESACP consensus guidelines or appropriate statistical thresholds are printed in bold letters. VOG...percentage of values out of ESACP guidelines.

	User 1		User 2		User 3	
QC variable		VOG		VOG		VOG
cv_ref	1.56 2.50 3.52	1.55	1.56 2.76 3.91	5.1	2.69 3.91 **5.11**	16.3
rem_ref	0.48 0.85 1.42	13.1	0.30 0.59 0.91	0	0.66 1.13 **1.58**	19.4
corr_coeff_ref	-0.19 0.28 **0.58**	41.1	-0.01 0.24 **0.49**	23.0	0.05 0.40 **0.62**	50.0
p_homo_all	**0.001 0.04** 0.72	51.9	**0.00 0.006** 0.14	64.1	**0.00 0.001** 0.16	75.2
p_homo_ana1	**0.001** 0.24 0.99	35.6	**0.00 0.002** 0.17	79.5	**0.00** 0.07 0.99	48.1
p_homo_ana2	**0.03** 0.59 0.99	13.1	0.27 0.89 1.0	5.1	**0.01** 0.51 0.99	17.8

If one of these variables exceeds the ESACP consensus guidelines, the respective values are marked. Although for the p_homogeneity no ESACP consensus exists, the appropriate guideline value should be regarded as $p > 0.05$. It becomes evident from Table 3 that in all of the three cytometry systems studied a considerable proportion of measurements violate the guidelines for a „good measurement practice".

The most critical variables in the measurements analysed are the inhomogeneity indicators.

The correlation between DNA content and nuclear area for the reference cell peak, indicating problems with glare and diffraction effects and leading to an unpredictable shift of the DNA values of analysis cells to the right, is also important.

If those quality control variables, shown above, are within the agreed limits in an individual case, then the similar variables in analysis cells (cv of each peak of the analysis cells correlation coefficient of DNA vs. nuclear area) can be used as indicators for slight departures from normality in the analysis cells, too subtle as to be found as separate peaks.

- *comparison of the process quality from previous analyses stored at the server*

The process quality of the actual measurement is analysed concerning the IOD [AU] of the reference cells and a series of modal peak values [c], if the peak(s) has (have) been known

to be euploid, either by their non-pathological origin or by independent methods (flow cytometry). By means of „running values" of the variables above (special variants of Levey-Jennings charts) the user can see whether the actual measurement fits into the pattern created by previous measurements performed under the same methodological conditions. Those running values are computed non-parametrically. The mean values, their standard errors, and the 95% confidence limits are demonstrated graphically to the user (Figs. 4 and 5). Measurements outside the 95% confidence limits are listed for the user, too.

Additionally, by such running values a very updated cv-calculation of the corrective factors is possible (and demonstrated to the user, Fig.6). These statistical figures are the decisive basis for diagnostic decisions.

So far, two comprehensive series of measurements from the three users were analysed concerning those cv-statistics mentioned above. The results are listed in Table 4.

Table 4. Means and cv's of modal peak values from two series of measurements by User 1 and 3 on the same specimen set (28 rat liver imprints, 105 breast cancer imprints and FNAB; 76 diploid and 16 tetraploid stemlines were analysed). For comparison, corresponding figures of 13 pleural effusions measured by User 2 are shown. Dashed fields indicate values above ESACP basic performance standards.

	User 1		**User 3**		**User 2**
	euploid tumours	reference slides	euploid tumours	reference slides	nontumourous pleural effusions
mean 2c/ref	1.026	1.012	1.210	1.153	1.020
cv 2c/ref	2.024 %	1.767 %	6.188 %	3.670 %	4.401%
mean 4c/ref	2.001	2.022	2.392	2.355	2.031
cv 4c/ref	2.652 %	1.655 %	6.955 %	5.719 %	2.182%
mean 4c/2c	1.970	1.988	2.005	2.035	1.965
cv 4c/2c	2.370 %	0.343 %	1.190 %	0.871 %	1.608%

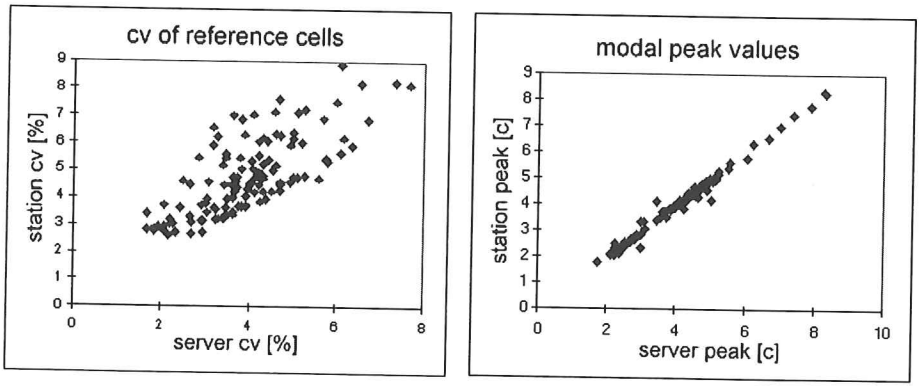

Fig.3. Scattergram of selected DNA histogram variables between the original data of User 3 and the appropriate server results: a) cv of the reference cells, r = 0.7448; based on 128 histograms; b) modal values of the peaks in the DNA histogram, r = 0.9961; based on 217 peaks;

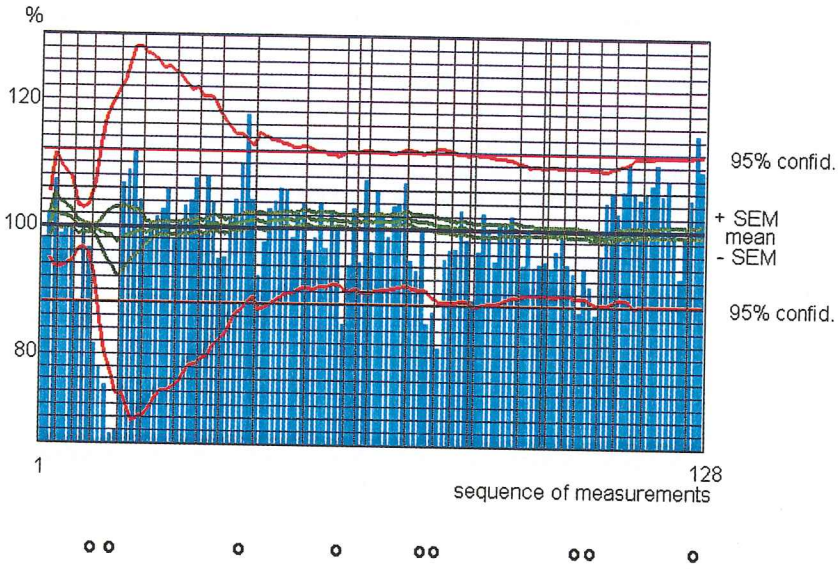

Fig. 4. Running IOD values [AU] of the reference cells of a large series of measurements in User 1. The circles beneath the diagram indicate individual cases outside the 95% confidence limits of the running value.

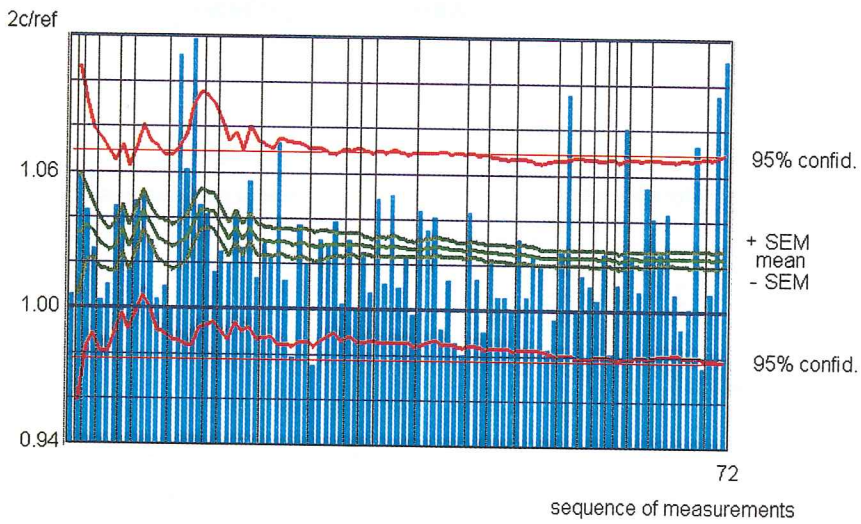

Fig. 5. Running means of the modal diploid peak values [c] of a large series of measurements by User 1.

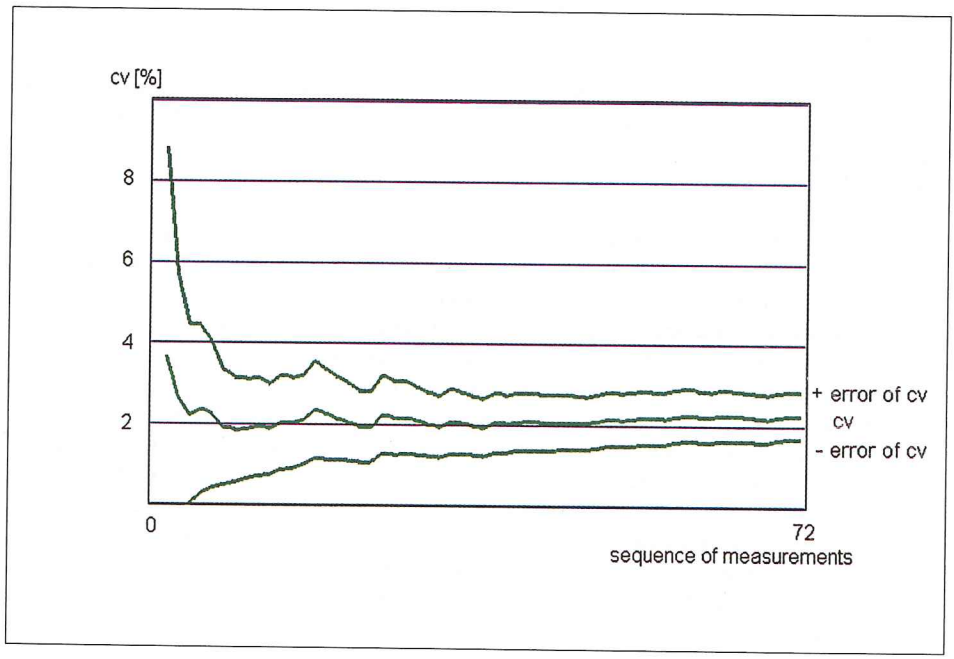

Fig. 6. Running cv [%] of the ratio between modal peak values of diploid analysis and reference cells (so-called corrective factor) of a large series of measurements by User 1.

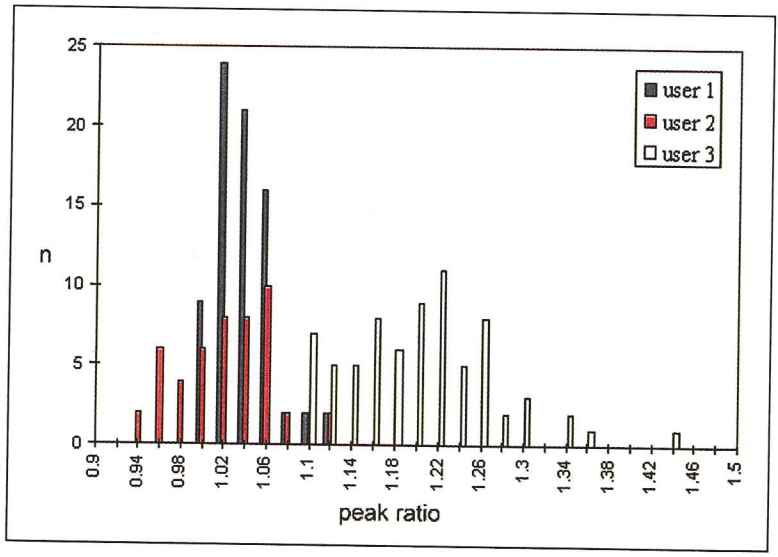

Fig. 7. Frequency distribution of ratios between modal peak values of diploid analysis and reference cells of large series of measurements by all three Users.

- *analysis of the accuracy of the actual measurement and proposal of a diagnosis (euploid vs. aneuploid, DI of stemlines).*

The modal values of the peaks detected were quite differently distributed in the two series of measurement (see Table 4). In Fig. 7 the distributions of modal DNA peak values for euploid peaks in tumour specimens from all three users are shown. Note that the peridiploid peaks of the User 3 measurements have much broader distributions than the same peaks of User 1.

Based on the means of modal peak values (from which the so-called corrective factor is derived) and their cv's, shown above, diagnostic conclusions concerning DNA aneuploidy (euploid vs. aneuploid) of each of the stemlines detected are possible. The strategy for those conclusions has been described in detail previously (Haroske et al., 1997a).

For that purpose classifiers can be constructed interactively by the user. The calculation of classifier requires a series of measurements on non-pathological conditions (e.g. normal cells or tissues) or with non-pathologic DNA peaks (confirmed by independent methods).

The means and cv's of the corrective factors for each peak selected (diploid and/or tetraploid, and/or octoploid) are displayed to the user. All numbers are flagged which are not based on a statistically sufficient number of euploid peaks, or which exceed consensus thresholds. If data measurements relating to tetraploid and/or octoploid peaks are insufficient, default values are proposed having been interpolated from the sufficient values.

The user can substitute any values by his own limit suggested by his experience in the material under investigation. However, all values indicated in red or substituted by the user are flagged for further evaluations.

In Fig. 8 the running values of those very important cv's of the corrective factors for diploid peaks are shown for the three different users.

By summarizing the results for each stemline a case diagnosis is formed (see also Fig.9).

Using the non-tumourous effusion specimens from User 2 a classifier was constructed with test characteristics listed in Table 5. That classifier was designed to detect aneuploid stemlines with an error probability of $p < 0.01$.

Table 5. Characteristics of a classifier for discrimination euploid from aneuploid effusion specimens.

ratio tested	mean	cv [%]
2c / ref	1.008	4.216
4c / ref	1.984	4.2
8c / ref	3.99	4.2
4c / 2c	1.984	2.0
8c / 2c	3.99	2.0
8c / 4c	1.95	2.0

The application of this classifier to all pleural and ascites specimens measured by User 2 resulted in a 100% coincidence between the classification results of the server and of the experienced User 2 itself. One of those classified DNA histograms from User 2 is shown in Fig. 9.

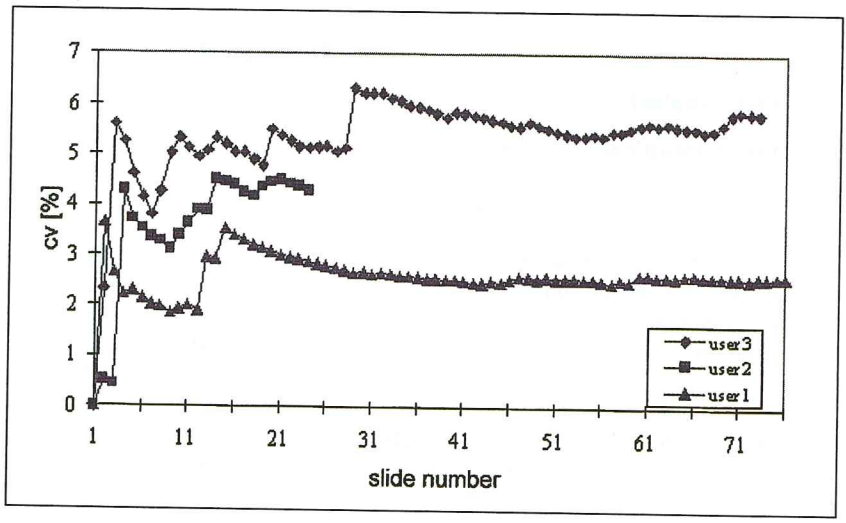

Fig. 8. Running cv [%] of the ratio between modal peak values of diploid analysis and reference cells (so-called corrective factor) by all three users.

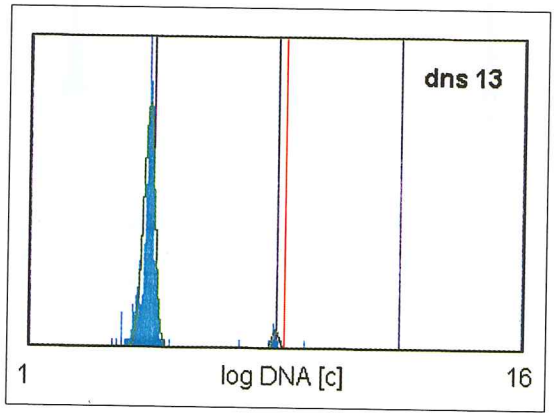

Fig. 9. Classified and rescaled DNA histogram (User 2). The specimen „dns13" (pleural effusion) shows two histogram peaks at 1.94 c and 4.00 c (G0/1- phase and G2/M phase of a stemline). That histogram does not differ significantly (p = 0.2329) from the euploid DNA histgrams used for construction of the classifier. The red vertical line indicates the threshold above that a cell does not belong to the G2/M peak of the DNA stemline.

On the other hand, the series of breast tumour specimens, mentioned above, was classified twice (by specific classifiers for User 1 and 2, respectively). Among those tumours 45 were classified as euploid and 60 as aneuploid by User 1 measurements. The same tumour specimens measured by user 3 resulted in 71 euploid and 34 aneuploid cases. The confusion matrix is as follows:

	user 1 euploid	**user 1 aneuploid**
user 3 euploid	41	30
user 3 aneuploid	4	30

classification efficiency: 67.6 %

In Fig. 10 two classification results on one of the breast cancer specimens are demonstrated. Whereas the specimen is classified aneuploid by User 1, the measurement of User 3 leads to a euploid result. The leading causes for the discrepant results on one and the same material are in the low measurement precision by User 3, leading to high cv's of the corrective factors. This broad variance prevents the detection of near-euploid DNA stemlines in measurements of User 3. The lack of precision need not be due to defects only. A different sampling strategyfor the visual selection of reference and tumour cells might have also influenced the overall precision, or might have led to an undersampling of aneuploid cells.

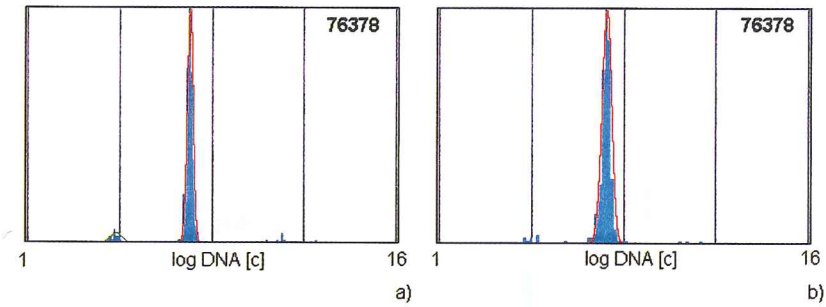

Fig. 10. Classified and rescaled DNA histograms of the specimen „76378" (invasive ductal breast cancer). a) User 1: a small diploid peak at 1.96 c and an aneuploid stemline ($p < 0.001$) with G0/1 at 3.41 c and with a few G2/M events; b) User 3: a few diploid events and a peritetraploid (euploid) stemline ($p = 0.0365$) with G0/1 at 3.528 c and with a few G2/M events.

3.2. Remote quality control

In most current DNA cytometry devices the evaluation of quality control tests is difficult, especially if those tests require rather comprehensive additional computations. Previously a special kind of test slide was designed with four imprints from one rat liver on it.

Four of such test slides should be stained together in one staining bath (PRO-4 experiment, Giroud et al. 1997b, Giroud et al., 1996). Fig. 11 demonstrates graphically exemplary outcomes of the PRO-4 test by Users 1 and 3. The numerical test values are given in Table 6.

Table 6. PRO-4 experiment data for cytometry devices of User 1 and 3. The shaded values are beyond ESACP consensus thresholds.

Parameter	User 1	User 3
within each imprint		
cv of IOD of lymphocytes	2.04 +/- 0.64 %	3.37 +/- 1.05 %
cv of IOD of diploid hepatocytes	1.69 +/- 0.59 %	4.72 +/- 2.13 %
cv of IOD of tetraploid hepatocytes	1.67 +/- 0.67 %	3.73 +/- 1.94 %
between all 16 imprints		
cv of modal IOD [AU] of lymphocytes	1.58 %	6.42 %
cv of modal IOD [AU] of diploid hepatocytes	2.79 %	6.44 %
cv of modal IOD [AU] of tetraploid hepatocytes	2.75 %	6.28 %
cv of modal IOD [c] of diploid hepatocytes	1.77 %	3.96 %
cv of modal IOD [c] of tetraploid hepatocytes	1.66 %	4.42 %
cv of modal IOD ratio between dipl. & tetrapl. hepatocytes	0.32 %	0.97 %

The results of the whole experiment confirm the sufficient precision of the laboratory processes in the tests of User 1. The differences in the test results (with identical specimens) are clearly due to differences in the measurement process in both devices, e.g. the systems have different methods of correction for shading, glare and diffraction.

The PRO-4 test is a valuable test for getting quick information about the methodological competence of the laboratories. However, it reflects the competence in highly optimised specimens only.It is imperative that the measurement precision is maintained for clinical specimens throughout the daily routine. Therefore the tests for the stability of the routine settings shown above, inevitable for diagnostic decisions, are of high importance for any user with clinical background.

DISCUSSION

Among the application fields of telepathology, remote quantitation is comparatively recent (Haroske et al., 1997b, Kayser et al., 1997). Telepathology is almost exclusively based on the exchange of digitised images (Eide et al., 1997, Oberholzer et al., 1993), and the parallel use of cytometric or similar techniques together with an image transfer seems to be the logical consequence for solving special problems in clinical diagnostic pathology. However, the quantitation of digitised images is faced with several difficulties, most caused by the lack of interoperability and methodological comparability of the broad spectrum of existing

cytometry and morphometry devices. Therefore, a server concept was designed to overcome those constraints (Haroske et al., 1997c). The server concept is based on the technological state of the art for existing cytometry workstations spread over university institutes, hospital departments, and private institutes of pathology within Europe. It is derived from the logistic requirements of remote measurements in cytometry (active and passive expertise), as well as from those of remote consultation in cytometric problems, especially where quality control procedures for image cytometry measurements and diagnostic data interpretation are concerned.

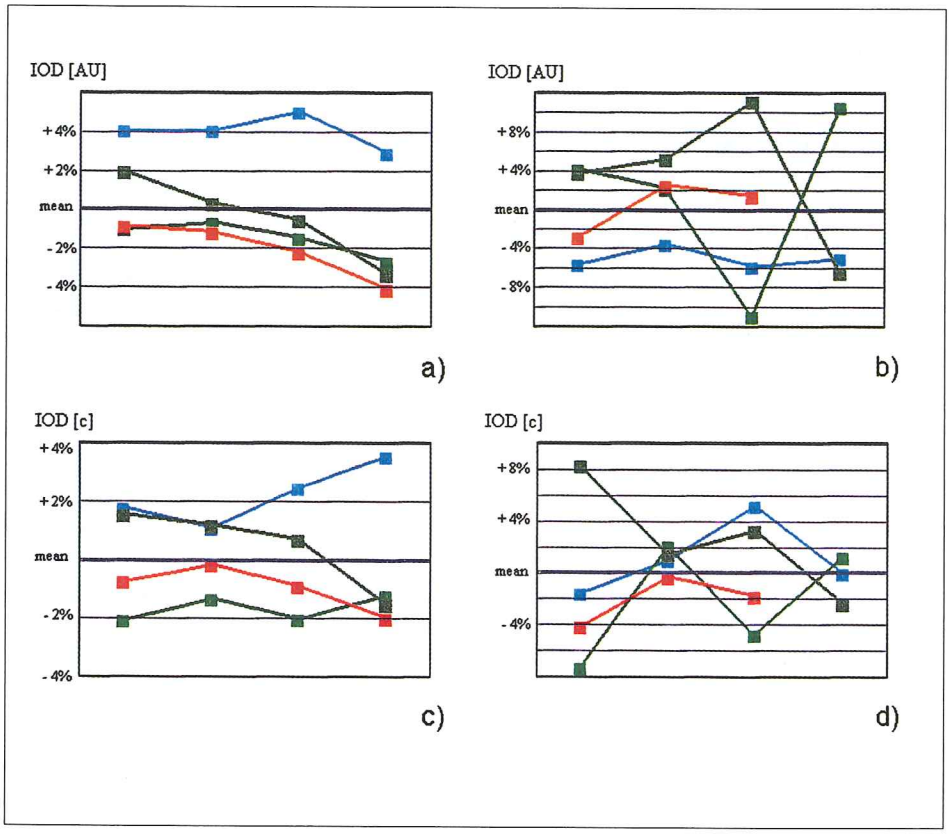

Fig. 11. PRO-4 experiment with an identical set of test slides by User 1 (a+c) and by User 3 (b+d). The IOD values of all (16) diploid peaks of rat liver cells in the four imprints are shown in arbitrary units (a+b) and in c after rescaling by internal lymphocytes (c+d).The imprints from each test slide are coded by different colours.The order of data points from left to right corresponds with the imprint position on the slide.

Recently, the main aim of Remote Quantitation in DNA cytometry has been the improvement of precision and accuracy, as well as the standardised interpretation of the results according to the most recent knowledge. The server concept is therefore an integral part of Remote Quantitation. Concerning the three application environments mentioned in the introduction, the question is how a server has to be designed to make it function as a cytometry workstation with an appropriate data base and consultation system, remotely tusable to a requester. The goal is to analyse a specimen, to check the measurement performance, and to confirm or revise the diagnostic interpretation of a user. Furthermore, the server should be able to act as an interface for contributors of data to centralised tumour marker data bases, and for an editorial board evaluating the entries to this marker database.

Being a „neutral" platform a quantitation server may enable a very practical application of the concepts in quality control, quality assurance, and good laboratory practice in pathology, especially in diagnostic DNA cytometry (Bacus et al, 1994, 2, 16, 20, 21].

The results of this first analysis of the functionality of EUROQUANT show that a „Passive Expertise" remote DNA ploidy analysis is feasible at a highly automated level, fitting to the client-server-philosophy. The quantitation server allows not only a remote quantitation of DNA data, but it also facilitates those analyses without any human interaction, i.e. in an objective way. To our knowledge there has been no comparable service for remote performance analysis and diagnostic assistance in DNA cytometry so far. In addition, the functions of a robotic microscope can easily be implemented in the current server options.

The use of Internet technology has proven to be advantageous and successful in telepathology (DiGiorgio et al., 1994, Gombas et al., 1997). The same holds true for remote quantitation and the corresponding technology. Its main advantage is the compatibility to all hardware platforms, thus enabling any user to contact the server by means of any HTML browser (Della Mea et al., 1997). It is not mandatory to rely on the Internet itself, but point-to-point ISDN- or ATM-connections can also be used. In that way three problems of remote quantitation will be overcome: The interoperability between different hardware solutions is guaranteed by the use of Internet browsers, the transfer rate is speeded up by fast media, e.g. ISDN or ATM, and the data security is enhanced by point-to-point connections.

At a first glance it seems to be a drawback that a direct, remote measurement on microscopic images, the remote quantitation in the strict sense, is not provided by the server. However, although this type of measurement is possible with only a little additional effort, we refrained so far from implementing this function for several reasons. Firstly, a clinical pathologist interested in performing telepathology needs a technical equipment very similar to a cytometry workstation (Hufnagel et al., 1997). If she or he wants to perform DNA cytometry, a comparatively cheap software upgrading should be sufficient. Secondly, the transfer of many images, necessary for the DNA cytometry of a diagnostic sample, will often be hindered by low transfer rate. It has been shown previously (Phillips et al., 1995, 1996) that image data compression is possible to a very limited extent without substantial impairment of the precision of the measurements. Finally, diagnostic DNA image cytometry should not be practised occasionally only, because a considerable amount of DNA measurements is necessary for getting a stable methodology. But for certain tasks in quality control of the machinery as well as for sampling attitudes a remote measurement could be very helpful. Those aspects will be analysed in the near future.

The results of this paper indicate that the discrepancies in the diagnostic classifications of the tumour series measured twice were caused by a low degree of precision. Those discrepancies may be crucial, because DNA aneuploidy when not recognised or erroneously declared may lead to wrong therapeutic implications. As the appropriate quality parameters

suggest, the technology of User 3 could be improved. However, the differences can also be caused by attitude differences in sampling or the sampling applied. Only a perfectly automatic procedure can be expected to be comparable.

As mentioned above, the comprehensive testing of remote quantitation in DNA image cytometry needs more effort and time. The number of measurements analysed by different users at the EUROQUANT server is growing. Therefore, the functions of the server have still to be adapted to several demands from the users. For detailed instruction of the use of EUROQUANT a manual can be downloaded directly from the server. At present, for some of the DNA histogram variables with a quality control background only little experimental data exist. In further studies with a broader spectrum of users those variables have still to be analysed concerning their statistical distributions and their correlation with diagnostically relevant characteristics of the DNA histograms. This should include an updated proposal for the consensus guidelines.

However, the most important benefit of this type of remote quantitation is already obvious: After classifiers have been calculated interactively by the users a rescaling of their measurement data is possible, leading to DNA ploidy data that can be directly compared with other data due to their standardised quality control procedures. Any user can have this standardised evaluation for certification purposes. But the most important precondition for a practical acceptance of a quantitation server is the preservation of the user's privacy under all conditions. Any research in the server databases is possible through the users only. We aim to provide a service, not a police, to the cytometrist's community.

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