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DIGITAL CORRELATION OF ION AND OPTICAL MICROSCOPIC IMAGES : APPLICATION TO THE STUDY OF THYROGLOBULIN CHEMICAL MODIFICATION

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Abstract

A method has been developed in order to digitally correlate ion and optical microscopic images of the same sample areas. Serial cross-sections of human thyroid tissue were analyzed by secondary ion mass microscopy and by light microscopy. The resulting chemical and immunochemical map images were superimposed and correlated by means of a two-pass registration algorithm which allows to correct for geometrical distortions introduced by the ion microscope. Results are presented for the study of thyroglobulin chemical modification in pathological thyroid tissue that demonstrates heterogeneous molecular activity.

Key Words : analytical ion microscopy, light microscopy, digital correlation, registration, geometrical distortions, thyroglobulin, thyroid

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Introduction

Analytical ion microscopy (AIM) has been used to map the elemental chemical distribution of biological tissues and is one of the most powerful microanalysis techniques as it allows direct imaging with isotope discrimination even at low element concentrations (< 10 ppm) (Castaing and Slodzian, 1981; Burns and File, 1986; Chandra and Morrison, 1985; Kahn et al., 1988). In thyroid tissue, it provided the way to directly study iodine ^{127}I distribution, which indicates the functional activity of the thyroid gland (Fragu et al., 1989). It now appears of interest to relate this elemental distribution of iodine to that of thyroglobulin (Tg) in which ^{127}I is stored. However, as direct molecule mapping is not yet well assessed, biochemical interpretation of AIM images is difficult. Here we present the result of a digital correlation method which combines AIM images with optical microscope images of thyroid tissue sections treated by immunohistochemistry. This new approach allows to relate elemental chemical distributions (^{127}I, ^{32}S, ^{31}P) of organic compounds to the molecular distribution of Tg. It is used to assess the heterogeneous iodination and sulfuration of this protein within human pathological tissue.

Material and Methods

Negative secondary ion images were obtained on a CAMECA IMS-3F ion microscope using a caesium primary ion beam of 10 keV energy and 30 nA current intensity. A mass resolution of 2000 was used in order to eliminate mass interferences between cluster ions and the specifically studied elements. Image recording was done by means of a LHESA (Pontoise, France) LH-4036 SIT camera connected to an IMAGING TECHNOLOGY (Woburn, Massachussets, USA) Series 151 image processor. The field of view is 400 mm in diameter. Samples were cut into small pieces and fixed in a solution containing 1 g/L glutaraldehyde and 20 g/L paraformaldehyde in cacodylate buffer (0.1 mol/L; pH 7.4). Fragments were dehydrated in ethanol and embedded in methacrylate resin (Historesin, Pharmacia, Uppsala, Sweden). This method preserves localization of iodine bound to macromolecules. Serial semithin sections were deposited on glass slides for histological examination (1.5 µm) and on ultrapure

gold holders for ion analysis (3 μm). The section observed in light microscopy by a LEITZ microscope was treated with a specific antithyroglobulin antibody (Schlumberger et al., 1979) and eosine stained. The immunoperoxydase reaction coupled to eosine staining reveals Tg distribution and cell nuclei positions within the tissue.

Results and Discussion

The main advantage of AIM for thyroid analysis is its ability to preserve ^{127}I and ^{32}S distributions in relation to the histological structure which is given by the ^{31}P distribution (Fig. 1). The iodine ^{127}I distribution in a 400 μm diameter thyroid tissue section is shown (Fig.2a, green) digitally superimposed, according to a previously presented method (Olivo et al., 1989), to the phosphorus ^{31}P distribution (red). The ^{31}P distribution is distributed in the cells surrounding the follicular lumina and appears to be characteristic of nucleic acids in the nucleus (DNA) and of the organic phosphorus in the cytoplasm (Olivo et al., 1989). In this image, iodine distribution appears to be highly heterogeneous and demonstrates, *in situ*, the well-known iodine heterogeneity (Ekholm, 1990). The cellular presence (cytoplasm) of iodine can also be noticed. It shows that iodine is not restricted to the colloidal compartment. Sulfur ^{32}S distribution, shown in green in Fig.2b, superimposed on the phosphorus distribution (red), maps the distribution of sulfur-containing proteins in the tissue, and particularly the Tg distribution within follicular lumina. The upper most vesicle (1) shows a major sulfur signal, while the lowermost ones (5 and 6) are nearly empty (see Table 1). However, it is remarkable that the two distributions of iodine and sulfur do not have the same heterogeneity. One could expect that the absence of iodine corresponds to the absence of sulfur, which in turn should indicate the absence of Tg. The presented images show that in fact the iodination degree of Tg is not dependent on sulfur content of this thyroid protein.

Table 1

Variation of elemental local concentration within thyroid follicles.

Vesicle	^{32}S sulfur	^{127}I iodine	Thyroglobulin
1	1.0	1.0	1.0
2	0.53	5.95	0.71
3	0.61	15.82	0.68
4	0.75	22.10	0.82
5	0.56	21.10	0.78
6	0.12	4.99	0.62

Results are given in arbitrary units and are expressed by reference to the local concentration of vesicle 1. The local concentrations were computed by dividing the mean grey level over each follicle by its surface.

In order to confirm this fact and to relate the modifications of chemical distribution to molecular maps, a digital correlation method was developed which allows us to superimpose ion and optical microscopic images (Bryan et al.,1985; Olivo et al., 1990a). Two serial cross-sections are obtained from a chemically fixed thyroid sample which has been embedded in a methacrylate resin. One of the two sections is analysed by AIM and produces a series of images showing different chemical distributions. The second one is treated by immunohistochemistry in order to reveal thyroglobulin. Figure 3a shows Tg distribution and cell nuclei positions within the tissue. Ion and optical images are correlated by means of a registration algorithm based on cell position. Details of the algorithm, which combines an affine global transformation and an elastic local transformation (Bajcsy and Kovacic, 1989) are published elsewhere (Olivo et al., 1990a-b), but here it can be pointed out that this two-step procedure is necessary to correct for geometrical distortions introduced by the ion microscope.

Figure 3b, where the optical image appears in red, the iodine distribution in green and the sulfur distribution in blue , gives an image resulting from the correlation between the different microscopic images. Vesicles where both iodine and sulfur are present clearly correspond to vesicles where Tg is revealed by immunohistochemistry. This is the case with vesicles 2-3-4-5. The case of vesicles 1 and 6 is of great interest because they show a high content of Tg. However, within vesicle 1 there is very little iodine, while the sulfur content is very high. We can therefore assume that this vesicle has produced a Tg molecule that is not able to incorporate iodine. In the case of vesicle 6, we have a similar situation, that is to say, presence of Tg and absence of iodine, but, in addition, the sulfur is also undetectable. This seems to correspond to a major modification of the protein chemical composition. By means of more classical biomolecular methods, it will now be interesting to investigate if this chemical modification is due to a pathological replacement of sulfur-containing amino-acids by non-sulfur-containing amino-acids, and, if it is the case, if there is an alteration of mRNA. Due to the unique and original methodology we have presented, we can already combine different microscopic *in situ* approaches of a tissue and, in addition, display structural and functional information in the same image.

Conclusion

The purpose of the proposed digital image correlation method is to combine corresponding chemical maps revealed by ion microscopy with molecular maps revealed by immunochemical light microscopy. The most significant advantage of this approach is to display, on the same composite image, structural and functional information in such a way that

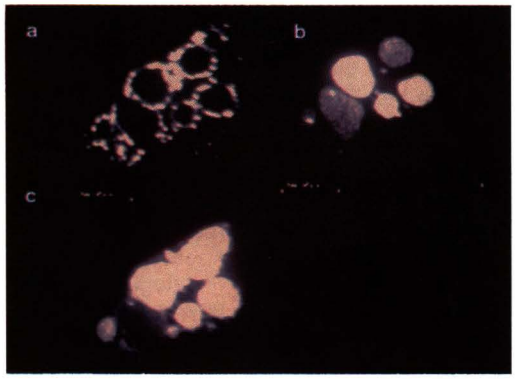

Figure 1 : AIM images of human thyroid tissue (field of view : 400 µm) : a) phosphorus ^{31}P distribution; b) iodine ^{127}I distribution; c) sulfur ^{32}S distribution.

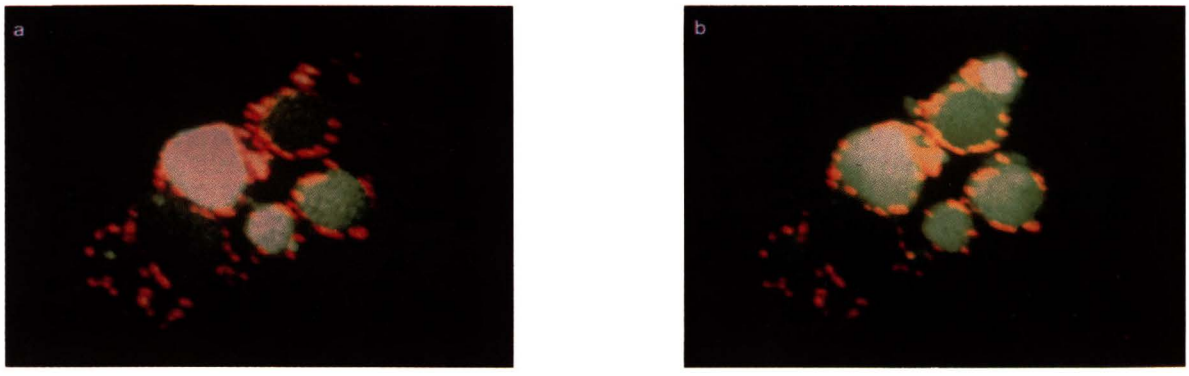

Figure 2 : AIM images of human thyroid tissue (field of view : 400 µm) : a) superimposition of iodine ^{127}I distribution (green) onto phosphorus ^{31}P distribution (red) which gives the histological structure of the tissue. ^{127}I is visualized in follicular lumina and inside thyroid cells (yellow) and shows a highly heterogeneous distribution; b) superimposition of sulfur ^{32}S distribution (green) onto phosphorus ^{31}P distribution (red) reveals the sulfur-containing proteins distribution in the tissue and heterogeneous Tg distribution within lumina.

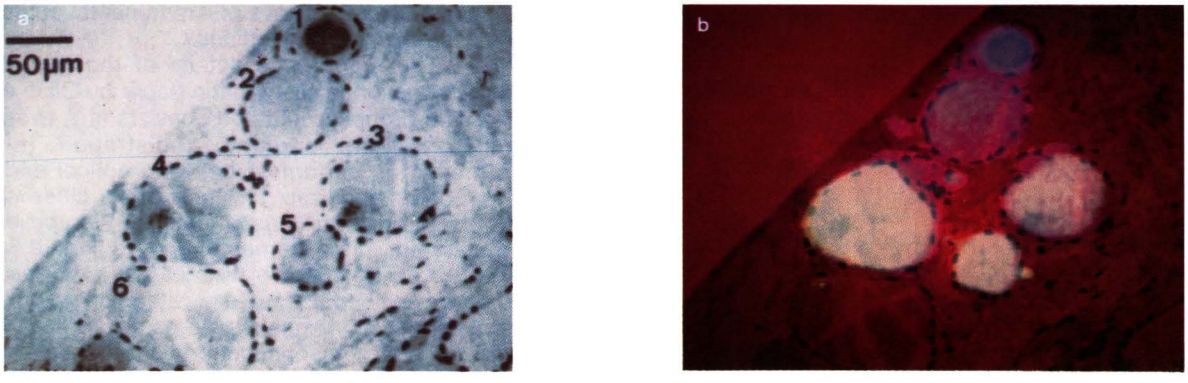

Figure 3 : light and ion microscopic images of the same 400 µm diameter field of view obtained from two serial cross-sections : a) section observed by light microscopy. It can be seen the vesicle structures formed by the thyroid cells surrounding the follicular lumina. Thyroglobulin (Tg) was evidenced by immunohistochemistry in follicular lumina; b) superimposition of optical (red), iodine ^{127}I distribution (green) and ^{32}S sulfur (blue, images gives an image resulting from the correlation between the different microscopic images and allows us to assess the chemical modification of Tg composition.

complementary data from different analysis techniques can be optimally exploited. In addition, this approach allows to correct for the distortions produced by the ion microscope, which would have otherwise resulted in ambiguous topography of chemical map features.

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References

Bajcsy, R. and Kovacic, S. (1989) Multiresolution elastic matching. *Comp. Vision, Graphics and Im. Processing* 46, 1-21

Bryan, S.R., Woodward, W.S., Griffis, D.P. and Linton, R.W. (1985) A microcomputer based digital imaging system for ion microanalysis. *J. Microscopy* 138, 15-28

Burns, M.S. and File, D.M. (1986) Quantitative microlocalization of diffusible ions in normal and galactose cataractous rat lens by SIMS. *J. Microscopy* 144, 157-182

Castaing, R. and Slodzian, G. (1981) Analytical microscopy by secondary ion imaging techniques. *J. Phys. E: Sci. Instrum.* 14, 1119-1127

Chandra, S. and Morrison, G.H. (1985) Imaging elemental distribution and ion transport in cultured cells with ion microscopy. *Science* 228, 1543-1544

Ekholm, R. (1990) Biosynthesis of thyroid hormones. *Int. Review of Cytology* 120, 243-288

Fragu, P., Briancon, C., Noel, M. and Halpern, S. (1989) Imaging and relative quantification of ^{127}I in human thyroid follicles by analytical ion microscope : characterization of benign thyroid epithelial tumors. *J. Clin. Endocrinol. Metab.* 69, 304-309

Kahn, E., Olivo, J.C., Halpern, S. Larras-Regard, E., Fragu, P. and DiPaola, R. (1988) Image processing in ion microscopy by the PC-AT computer. *Secondary Ion Mass Spectrometry, SIMS VI (John Wiley and Sons, Chichester, 1988)*, 381-384

Olivo, J.C., Kahn, E., Halpern, S., Fragu, P. and DiPaola, R. (1990a) Ion and light microscopic images superimposition and correlation. *SPIE, Image Processing III*, 1135, 56-61

Olivo J.C., Kahn E., Halpern S., Fragu P. and DiPaola R. (1990b) Ion-to-optical images registration : a tool for correcting distortions in ion microscopy images. *SIMS VII,J.Wiley &Sons Inc.*, 925-928

Olivo, J.C., Kahn, E., Halpern, S., Briancon, C., Fragu, P. and DiPaola, R. (1989) Microcomputer system for ion microscopy digital imaging and processing. *J. Microscopy* 156, 105-114

Schlumberger, M., Fragu, P., Vignal, A. and Tubiana, M. (1979) Dosage de la thyroglobuline humaine dans le sérum et le tissu thyroidien. *Ann. Endocrinol. (Paris)* 40, 439

Discussion with Reviewers

G.M. Roomans : Although the Introduction suggest that the work was carried out on human pathological tissue, the Material and Methods section tells us nothing about the source of the material, and if indeed was human pathological tissue, the disease involved. Also, it is not made clear in the Discussion, whether the authors believe that the differences in distribution between Tg, S and I bear any relationship to the pathological process involved. Please comment.

Authors : This preliminary study which is also a feasibility study was performed on human thyroid specimen obtained from surgery. The tissue was histologically a macrofollicular adenoma. Our purpose now is to apply this methodology to different groups of patients with different thyroid diseases and groups of adenoma (hypo and hyperfunctioning nodules, goiter) in order to emphasize and explain systematically changes in the chemical composition of Tg. Our previous study on ^{127}I mapping in human thyroid disease (Fragu et al., 1989) has clearly demonstrated that the distribution of ^{127}I concentrations between thyroid follicular cells and lumina was different in the different pathological conditions : goiter, hypo or hyperfunctioning nodules.

R.W. Linton : I question the statement that the iodination degree of Tg is not dependent upon the sulfur composition of this thyroid protein. This assumes that the I^- and S^- images reflect only the distribution of Tg molecules. All that can be concluded is that the variability in the I^-/S^- intensity ratio suggests chemical modifications of Tg in different vesicles. Please comment.

Authors : The biosynthesis of Tg molecule involves incorporation of sulfur-containing amino-acids. The decrease of S^- suggests a defect in the Tg molecule which is unable to incorporate iodine even if the enzymatic mechanism of iodination (peroxydase and H_2O_2 generating system) are present. When sulfur is present within thyroid follicle, we can assume that the biosynthesis of Tg is normal. The absence of iodine suggests a defect in enzymatic systems while simultaneous presence of iodine and sulfur implies normal Tg.

R.W. Linton : Discuss details of experimental approach to correct for geometric distortions.

Authors : The algorithmic details of the geometric distortion correction is given in the paper by Olivo et al. (1990a). The proposed method consists in a two-step procedure : first, a global registration (affine transformation) is performed between optical and ion images in order to correct for geometrical differences. Then a local transform (elastic matching) is applied to compensate for displacements and to match local properties and structures of the images. Images are registered on the basis of pairs of reference points that are characteristic of images structures. An evaluation of this method on a semi-conductor misaligned chemical maps is presented in the paper by Olivo et al. (1990b).