

On some recognition problems for biomedical specimens

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Abstract

This paper presents a method for extraction of "good" mitoses from biomedical specimens.

Our filter, the C-Filter, which functions according to the rules of the C-Calculus and uses the properties of the C-Transform, already gave good results in classification and extraction of textures. The C-Calculus and C-Transform have been formulated in our previous works.

Making use of different textures, which obviously are present in the specimens of mitoses, our filter permits good results to be obtained and, thus, encourages the further application of the method.

Introduction

After a rather painful and difficult beginning the use of a digital computer in the field of biomedicine now enjoys a complete recognition and wide application.

The use of the computer not only gives undisputably reliable results, but also opens new vistas for further and a more profound research.

Indeed the application of the computer for white-corpuses analysis, for chromosomes classification, for detection of different kinds of cancer such as cancer of the cervix, breast, abdomen, brain, stomach, and of other organs and parts of the human body,

not only supplies absolutely unambiguous results, but also these are obtained much faster than the diagnoses of a pathologist and moreover, are less costly [1].

The studies of the mechanisms of different cell diseases, whether the causes of them are known or not, and whether they are congenital or acquired later in life, are carried out all over the world.

The modern pathology subdivides the diseases into two categories: hereditary and acquired. For the latter it is thought that they are caused by an unfavorable environment.

Let us discuss briefly the first category: a genetic basis of a disease can be viewed in the light of Mendelian laws of heredity. It is now established, without any doubt, that certain traits in an individual are determined by the genes he inherited, and in replication of these genes.

We also know that the cause in many hereditary diseases can be a defective gene, or an altered one, or the absence of certain genes.

However there is another cause for a genetic anomaly, which has nothing to do either with a mutation of a gene, or with the absence of certain ones, but deals with some anomaly in chromosomes. The anomaly can be in the number, or form, or size of the

chromosomes. A classic example here is the disease called Mongolism (Down's syndrome). It occurs as often as once in each 700 newborn babies.

Finally there is a group of diseases in which the genetic anomaly is restricted to somatic cells, or rather, to the cells "corporee". These cells, figuratively speaking, appear on the stage after the fertilization took place; they are distinct from the germinal cells responsible for the initiation of the foetus formation.

It can also happen that a carcinogenous cell, whether a somatic one or of some other kind, becomes anomalous as result of mutation in one or several of its genes. From this moment on the entire progeny of this cell can also be carcinogenous [2].

With the new techniques and the use of the computer, new methods, simple and accurate, for the determination of numbers and configurations of the chromosomes have been developed, accelerating the research of today and enabling further progress.

These methods led to the discovery of new diseases, and they continue to lead to the discovery of new ones, the causes of which should be sought in the mutations of genes, as above mentioned.

There is, thus a great need for further studies in this direction, as well as in the studies concerned with the classification of the human chromosomes, or, in brief, there is a great need of research in the field of "good" mitoses.

In these studies we propose to use a C-Calculus and a filtering procedure with a C-Filter. We have introduced these methods and applied them to many cases of "image processing," in particular the search for a "good" mitosis in the biomedical specimens.

C-Calculus

The central idea of the C-Calculus [3] was born with the observation that in a numerical system each of its numbers has a double value: one of the order (significance in relation to other numbers) and the other, of the position in the system.

The C-Calculus, first introduced some years ago, can be subdivided into three essential parts.

If one would think to apply the rules similar to those for the numbers in a numerical system to some sets, the calculus thus obtained would operate not on single elements, members of the system, but on the members which now will consist of strings of sets.

When each set in the string, so to say, acquires a certain value for its order in the string, this value will be determined by the position of the set in this string.

Such a string we named as a "Composite set," or, briefly, a C-set, whence the name C-Calculus. Then, if

$$a_n, a_{n-1}, \dots, a_0$$

is a mapping of some set U , the corresponding C-set will be represented by a string:

$$a_n a_{n-1} a_{n-2} \dots a_0$$

If we now use the formal rules of the arithmetic for operations on these strings and from the operations take only two—that of a product and of the sum (the operations of division and of subtraction, however simple they may be, do not allow themselves to be defined)—and if, further, we define the product and the sum of the sets, composing the strings, as intersection and union of sets of ordinary set calculus, we obtain a commutative semi-ring, the elements in which can be generated in an obvious manner.

The C-Calculus which is also somehow related to the theory of measurements and the physiology of certain neurons (we cannot go deeper into these relationships in this paper) suits extremely well for a variety of applications.

Among these applications we will discuss some related to pattern recognition, and in particular, the applications to texture analysis and to the filtering of biomedical specimens [4, 5].

Suppose we have a digitized image, and let us represent it, in the customary way, as a square matrix of size n

$$A = [y_{i,j}]$$

then a C-set for this matrix can be easily formed.

Suppose we have a device, a "reader", with a window "w". This reader can read only a maximum and a minimum value of the function y , seen through window "w", that is, in the area "w" of the matrix.

This area "w" can be thought of as a submatrix of the size "w", of the matrix A .

Now if we scan the matrix A in some direction, applying to successive windows, with the only condition that the application of these "windows" is contiguous, then we will obtain a set-mapping of A .

This scanning, will be equivalent to the partitioning of the matrix A into submatrices of the area "w", with additional information on the maximum and minimum values of y inside of each such square submatrix.

If now all these squares, components of the matrix,

are ordered in some way, then each square can be represented by a quadruple of values. One pair of values will indicate the position of the square in the matrix, and the other pair, the information on the variation of y inside of this square.

An alignment of all these "quadruples" into a string will give a C-set, for example, a C_0 . Thus the C_0 is nothing more than a representation of the original image. This representation will be more or less accurate depending on the size of the window "w": the smaller the size, that is, the higher the number of the squares, the finer is the partitioning and, thus, the representation.

If we now rigidly translate this "grid", we will obtain a second C-set, C_1 . And if, after that, we operate on them according to the rules of the C-Calculus to obtain the product, the result will be not only a finer partitioning of the original matrix, but also a description of the function y in greater detail than before.

This, then is the next characteristic of the C-calculation; it allows the reconstruction of an image to the degree of precision of the instrument with which the image has been obtained.

Now we state the conditions necessary such that the above procedure converges to each of its respective points in matrix. For a unidimensional case, which interests us here, it is obvious from [6] that this condition is:

$$w \leq \frac{D}{2} + 1 \quad (1)$$

where D is the length of the smallest interval where the "y" is monotonous.

C-Filter

The existence of the condition (1) makes the C-Calculus suitable for the filtering of the images—this is another characteristic of this calculus.

In order to illustrate this characteristic let us consider a signal. This signal shows some periodicity in some of its regions, and in other regions either some other periodicity or no periodicity at all.

The condition (1) enables us to separate the regions of signal where it has different behaviors.

Indeed our method of filtering, with the C-Filter, allows us to reconstruct point-by-point these regions: one where the signal is periodic, and the other where it is nonperiodic, that is, where the signal is absent.

The principle which forms the basis of our filtering method can be explained, as follows: for a window,

the size of which is larger than the period of the signal, the scanning gives the components of the C-set with the same maximum and minimum values of the function. Hence, as the condition (1) shows, with this size of window we are unable to reconstruct the periodic region of the signal. However in the non-periodic region of the signal, the maximum and the minimum values differ and hence this region can be reconstructed.

Consequently, by choosing, the appropriate size of the window we can extract from our signal just those parts that we are interested in.

From the above it is obvious that the dimensions of the window play a decisive role in filtering of the components, namely, they ensure the convergence only in those parts of the image where the distance D in the zone of monotonicity satisfies the condition (1).

The choice of the window required for extraction of a desired feature from an image seems to be arbitrary, or needs to be determined empirically from case to case. How to choose a proper size will become clearer from the next section.

C-Transform

Let us return once more to our matrix with the image and let us scan the entire matrix with a window of size "w".

At this point of our discussion it is very appropriate to state most definitely that the C-Transform does not depend on the manner of scanning, that is, whether the scanning is done along some line point-by-point ("row-by-row" as we call it), or area-by-area along the line. Below we will refer only to scanning "row-by-row" in order to be consistent with our previous representation and not to impair the generality of the C-Transform.

After the scanning let us compute all differences between the extreme values of the grey tones ($M - m$) in the regions of monotonicity of the signal.

Let us plot these values on an axis (u) of a three-dimensional space—C-space [7]. Let us plot on the second axis (v), of the C-space the value of

$$w = \frac{D}{2} + 1$$

where D is as defined in the condition (1).

And on the third axis (t) let us plot the frequency with which points with equal v and u are encountered over the entire image (matrix).

Let us once more recall the property of the C-space: there a signal with a constant period but with a varying

amplitude will be represented by a straight line parallel to the axis (u); the signal with a constant amplitude, but with a variable period will be represented by a straight line parallel to the axis (v).

Thus a sine curve will be given just by a single point in the C-space; however, any other signal of the same period and amplitude as this sine curve will also be represented by the same point.

Thus, the C-space does not possess a unique inverse transform.

The reasons which led to such a representation of a signal should be looked for in the significance which the variation of amplitude in the zone of monotonicity of a signal and the size of the zone have for a given signal.

Indeed, the value on the axis (v) of the C-space correlates with the spectral properties of a signal, while the choice of u seems to have its origin in the observation—now a firmly established fact in the physiology of vision—that a human eye is capable of comparing the brightnesses of light sources, but not of determining their absolute values. The parameters (u, v, t) we have chosen thus correlate with the fundamental concepts in vision physiology: that of the contrast, and the other of the resolving power.

Application

(a) *Texture*

A concept of texture is paramount for the discrimination of objects in a visual field. Each object has its texture, hence to discern the textures means to be able to distinguish the objects. Moreover the texture gives the information on the depth of the background, the distances of the objects from the observer [8]. Hence texture analysis is vitally important. This analysis presents also a very complex problem, and therefore it is imperative to do it on a digital computer.

To use a digital computer in the texture analysis presents in its turn a very complex task. There have been attempts [9, 10] to analyze the texture on a digital computer using certain models. These models however are such that they could not be described in exact terms. As a result difficulties arise when they are used.

For the purpose of analysis and classification one can think of a texture as a repetitive layout of some subpatterns, some subareas. Such a notion of a texture suggests two approaches: a structural and a statistical one.

From the point of view of the structural approach a texture can be imagined as if assembled

of "patches" (small areas of similar size) of subpatterns. These areas (patches) then repeat themselves periodically over the entire visual field [11].

From the point of view of statistics a texture is a set of statistical parameters evaluated from numerous measurements carried out over a picture, or texture.

At this point it is necessary to observe that for the grey tones in the patterns the first order of statistics (i.e., the average and the standard deviation) is not sufficient. The second order of statistics here is necessary, so Julesz [12], for instance, has used the transition probability between the different tones—levels of greyness. Other techniques with the transition probability have also been developed [13, 14].

There is still another approach for describing a texture. It is based on the probabilities of the distribution of certain features in the pattern: e.g., frequency of angles, of straight line segments, etc. Such features can be considered as useful parameters for the texture description [15, 16].

The standard procedure in "looking for the angles" in a pattern or texture consists of comparing the degrees of greyness of the adjacent points in the picture. In this procedure we recommend that measurements over pairs of points should not be used, but rather a pair of averages, each average taken for a pair of neighbouring points. Such a procedure would reduce the noise in measurements.

The difference between the values depends on the length of the interval between the points. Indeed the intervals for which the differences are high correlate with the elements of a pattern so that the size of the interval can itself be used as a texture parameter [17,18].

One can think of textures as of the models with the following characteristics:

- a. They consist of "patches" or "pieces," roughly uniform, whether in size, or in variation in the tones of grey.
- b. The number of such "pieces" is very high as compared to the occurrences of other characteristics which can be extracted from the patterns.

For such a description of a texture we obtained a C-Transform with the points extremely clustered in the C-space.

Already in our previous paper [20] we presented several experimental results, those of classification of textures and of extraction of the object outlines from a textured background; finally, we gave examples of the filtering of overlapping textures.

(b) Biomedical specimens

Form, size, and density in a biomedical specimen can be thought of as sufficient parameters for a human observer to distinguish different types of cells.

To some observers, however, the idea occurred to think of a specimen as a complex texture and make use of the difference in their textures for the classification of the cells [21, 22, 23].

The good results we have obtained with the C-Transform for discrimination and classification of textures prompted us to apply this method to biomedical specimens in order to filter the "good" metaphase.

We worked with specimens of the peripheral blood and of the spinal cord. They had been given to us in two different forms:

- a. film taken through a microscope, using a 24 x 36 mm camera;
- b. glass-slides for a microscope.

For the input of specimens (b) a telecamera was optically linked to a microscope, then this camera was connected to a computer, giving there a matrix of 256 x 256 with 16 levels of grey.

We applied the C-Transform to this input using the well-known algorithm of clusterization [24, 25].

Two distinct spikes were noticeable in the C-space, one much higher than the other. It also had low values of u 's and v 's, corresponding to the high level of noise in the specimen.

The second spike had not only much higher values of u than the first, but the values of v in it were much more consistent than in the first spike.

The latter peculiarity prompted us to modify the manner of operation of our C-Filter.

Indeed, until now we not only worked on modifying the frequency by choosing the size of the window u^* but we also imposed constraints that the variations of the signal within the window had to exceed a certain value v^* .

The selection of u^* and v^* has been done automatically by the program; in fact, these values have been determined by the algorithm of clusterization from a relationship between the accuracy and the dimensions of the spikes in the plane u, v .

It is necessary to point out that, for the input of the film, so long as the magnification of the microscope remained constant, and for the routine input by a human operator, the same values of u and v have been obtained.

The constant values of u^* and v^* for the numerous images (more than 100) we have examined, induced

us to think about the application of special hardware for our filter.

In conclusion let us examine the results statistically.

As already described the pictures of specimens of the peripheral blood and of the spinal cord have been fed into the computer in the way discussed above.

The picture-output of the computer contained human cells (some of them in metaphase) and different types of noise. The noise of the background, exhibiting a definite periodicity, was nearly always present; in other cases, the noise had form and periodicity similar to those of mitoses.

As the first operation we counted the cells in our pictures (about 100 of them). The cells were very small. There were in total about 1700 of them from which 176 were in metaphase.

In all specimens 10 cells (3%, on average of the total number of cells) were in metaphase.

As the standard deviation was small we considered that the specimens were representative of the whole population.

After that we analyzed 820 cells, among them 95 in metaphase. These samples represented well the population, because the ratio mitoses/cells remained unchanged.

Let us consider the results after our output has been filtered out. We counted:

- a. Nine cells not in metaphase that is, either the filter retained some, or they were so small that the periodicity of their texture was compatible with that of mitoses. However, note that in each case we could see clearly whether a cell was in a metaphase, or not.
- b. Ninety-seven mitoses, from which 93 were true, and 4 not, the latter being that of noise, whose periodicity was very similar to that of mitoses.

Of the 95 cells in metaphase we obtained, perhaps only 2 did not interest us, because they were not "good" mitoses. Exactly that fact can serve as the proof of the efficiency of our filter.

We can see in our output that only 1.2% of the cells not in metaphase have been "lost" (i.e., retained by the filter) while more than 98% of such cells have been properly filtered.

If now we compare the input-pictures with those of the output, the errors in this output clearly show that the input-pictures in this case were too complex in comparison to the usual input.

Fig. 1 gives a photograph of a specimen of the peripheral blood taken through a microscope (input-picture).

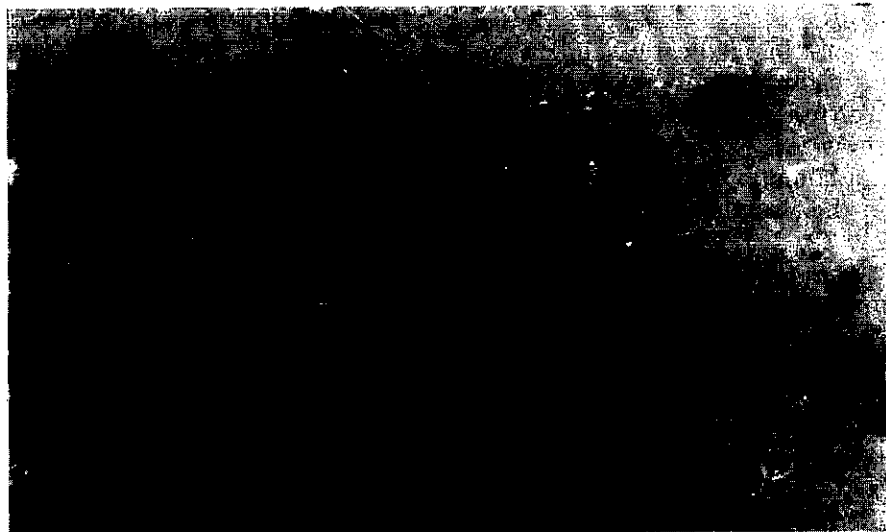


Fig. 1

Fig. 2 is the same photograph, but resolved into 64 levels of grey on a 512 x 512 matrix (output-picture). In this picture one can see 6 cells, from which 4, close to the edge of the picture, are in metaphase.

Fig. 3 shows the result of filtering. In it, one can notice that the noise has been completely eliminated, leaving only mitoses.

Fig. 4 gives four parts of the processing: the photograph, the input-picture, the output-picture, and, finally, the filtered image. In part 3 one can see how well the mitoses have been isolated.

In Fig. 5 one can see the type of error when the noise has been filtered as mitoses.

Finally, Fig. 6 shows the opposite type of error, namely, the "loss" of a "poor" mitosis.

Conclusions

The results we have obtained prompt us to the next stage of application of our method; the extraction of the mitoses has been only the first stage of its application.

In the future we intend to apply our method to chromosome classification, profiting there by bands or strips typical for the textures of chromosomes.

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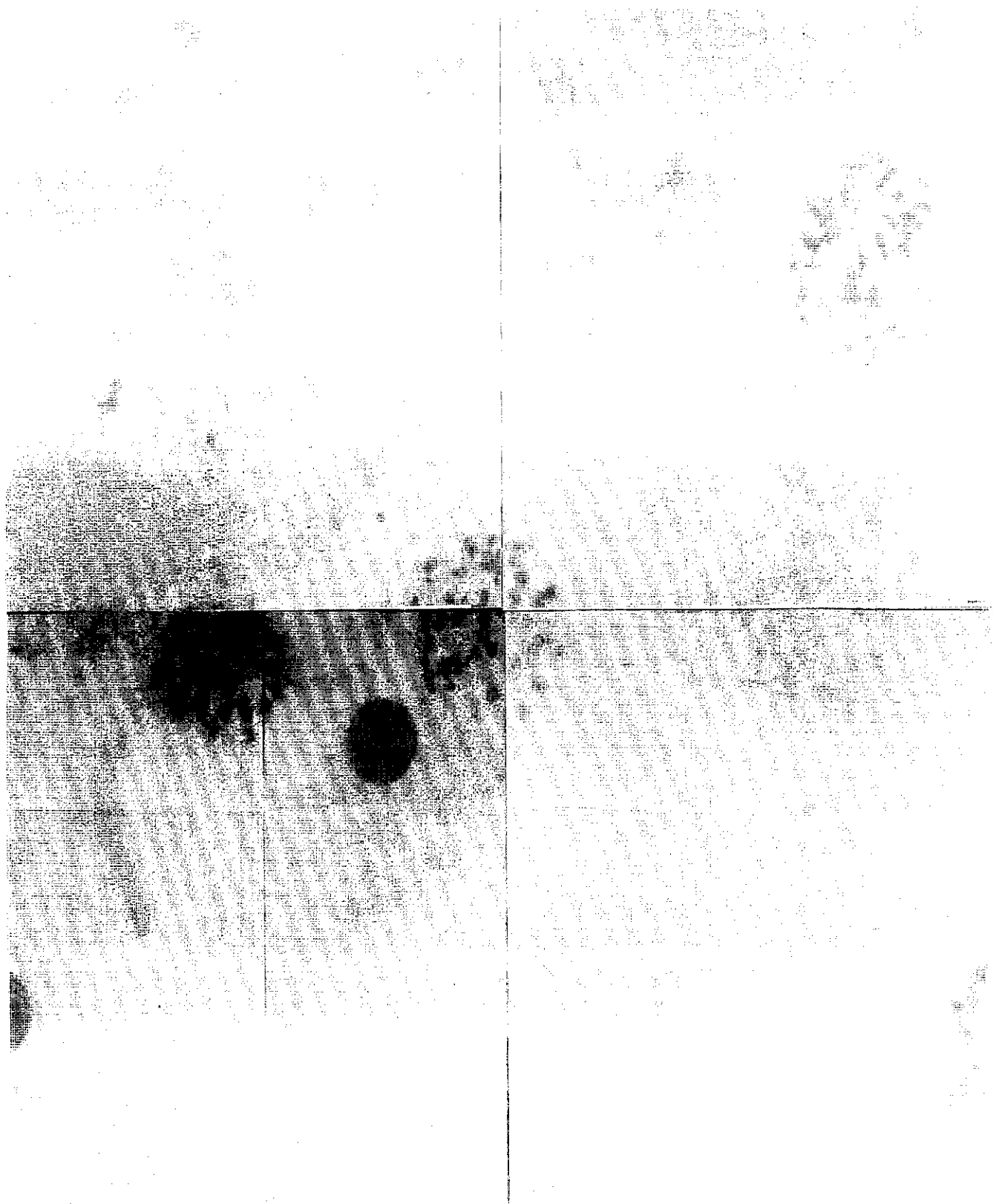


Fig. 2

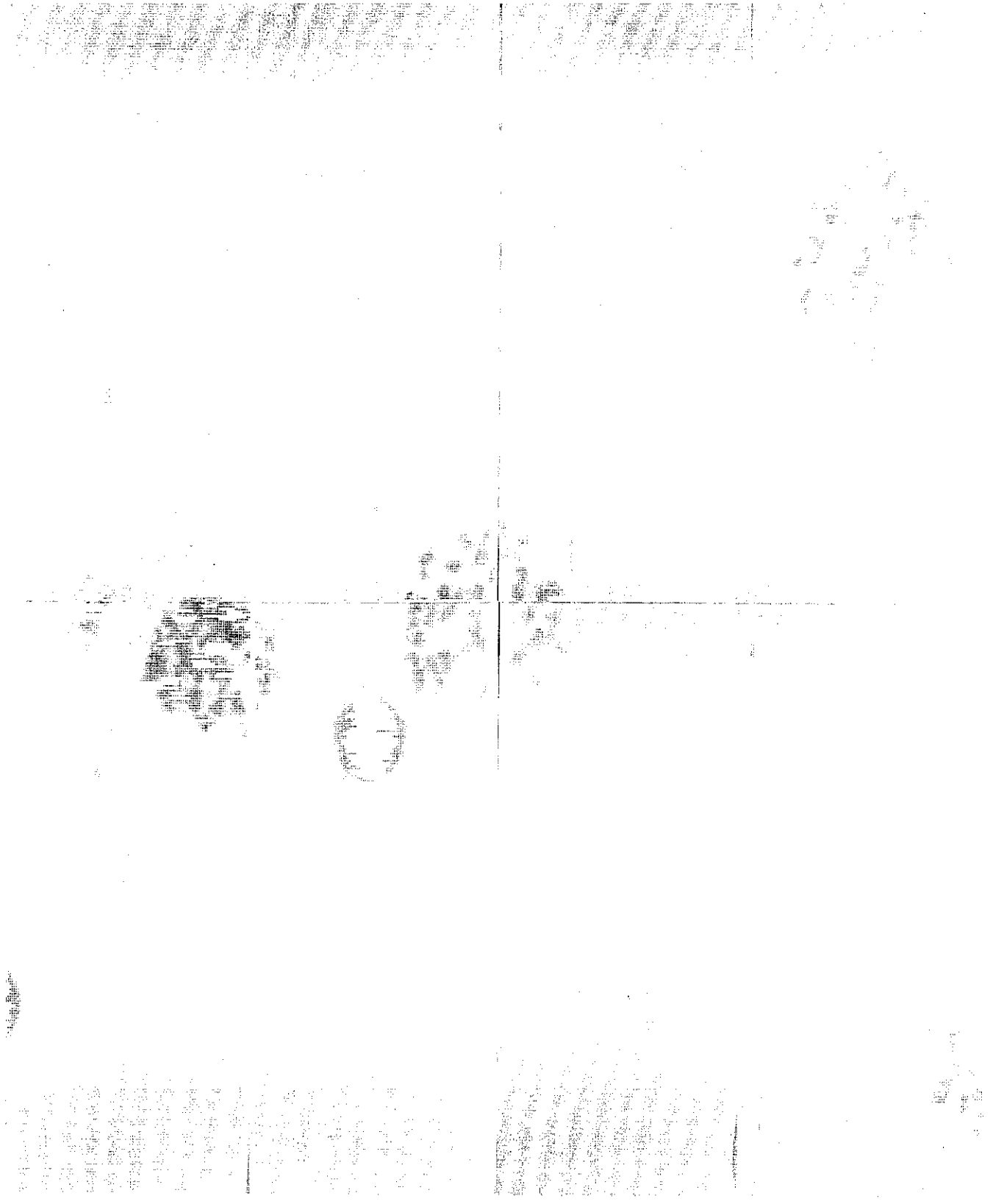


Fig. 3

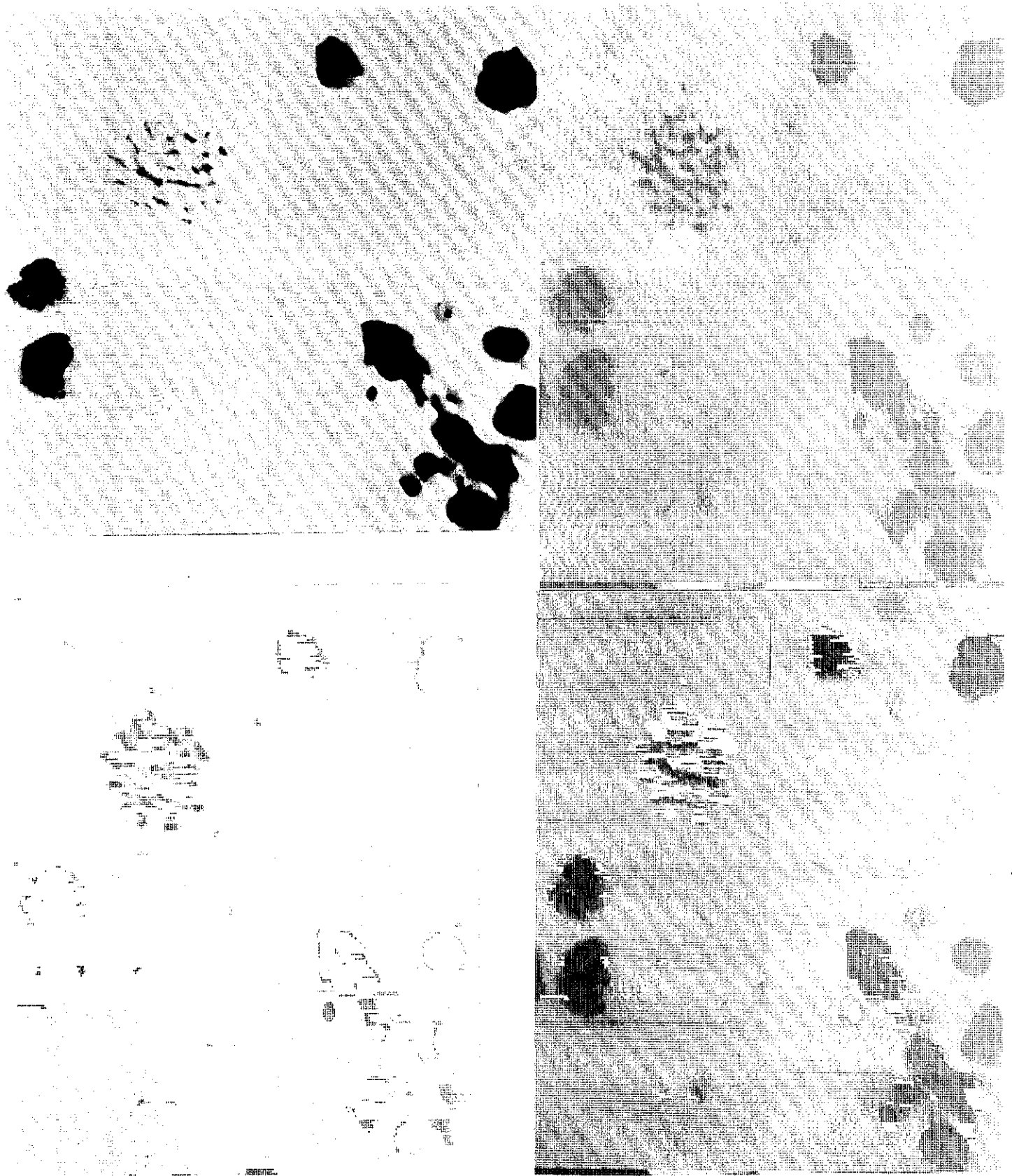


Fig. 4

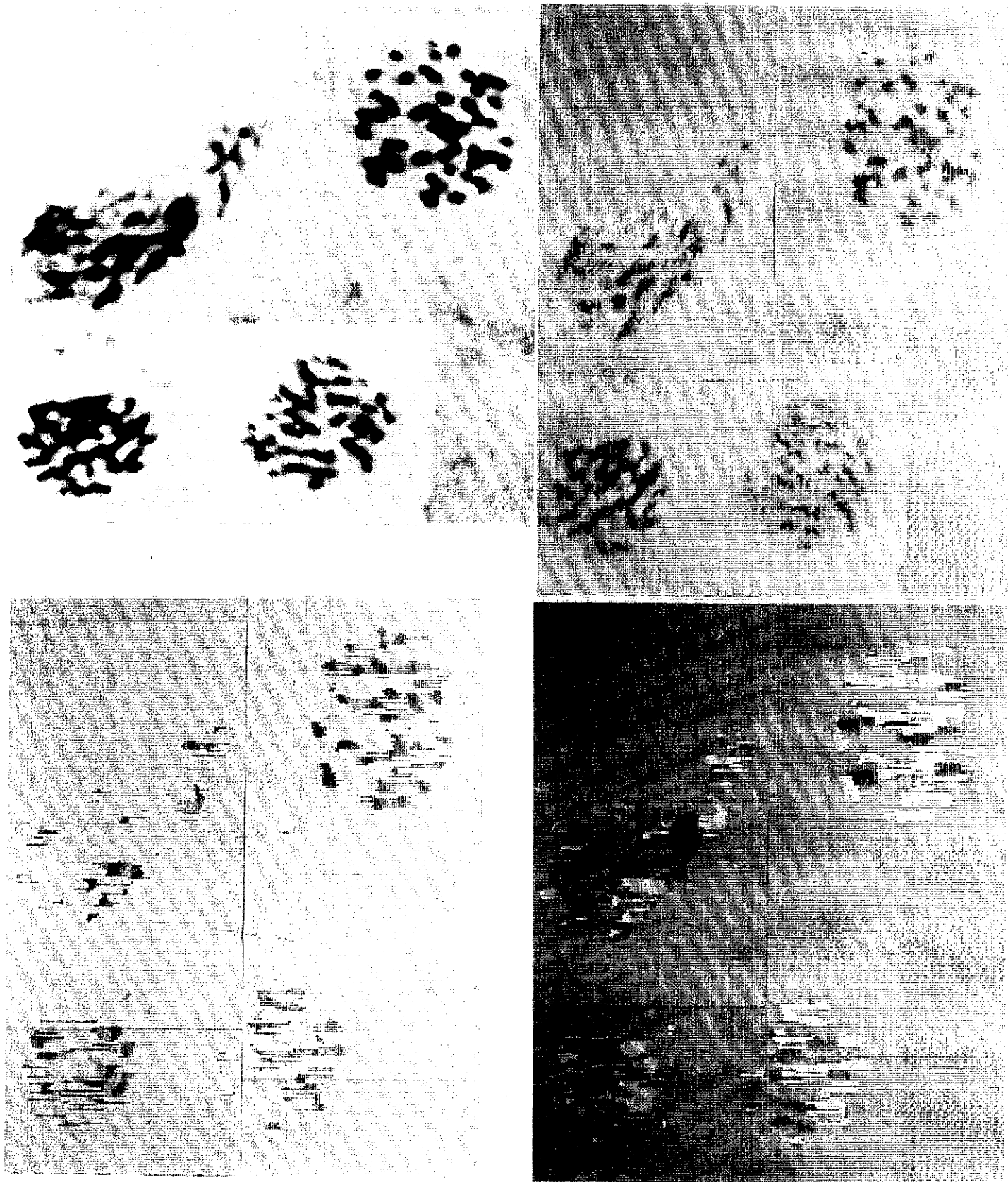


Fig. 5

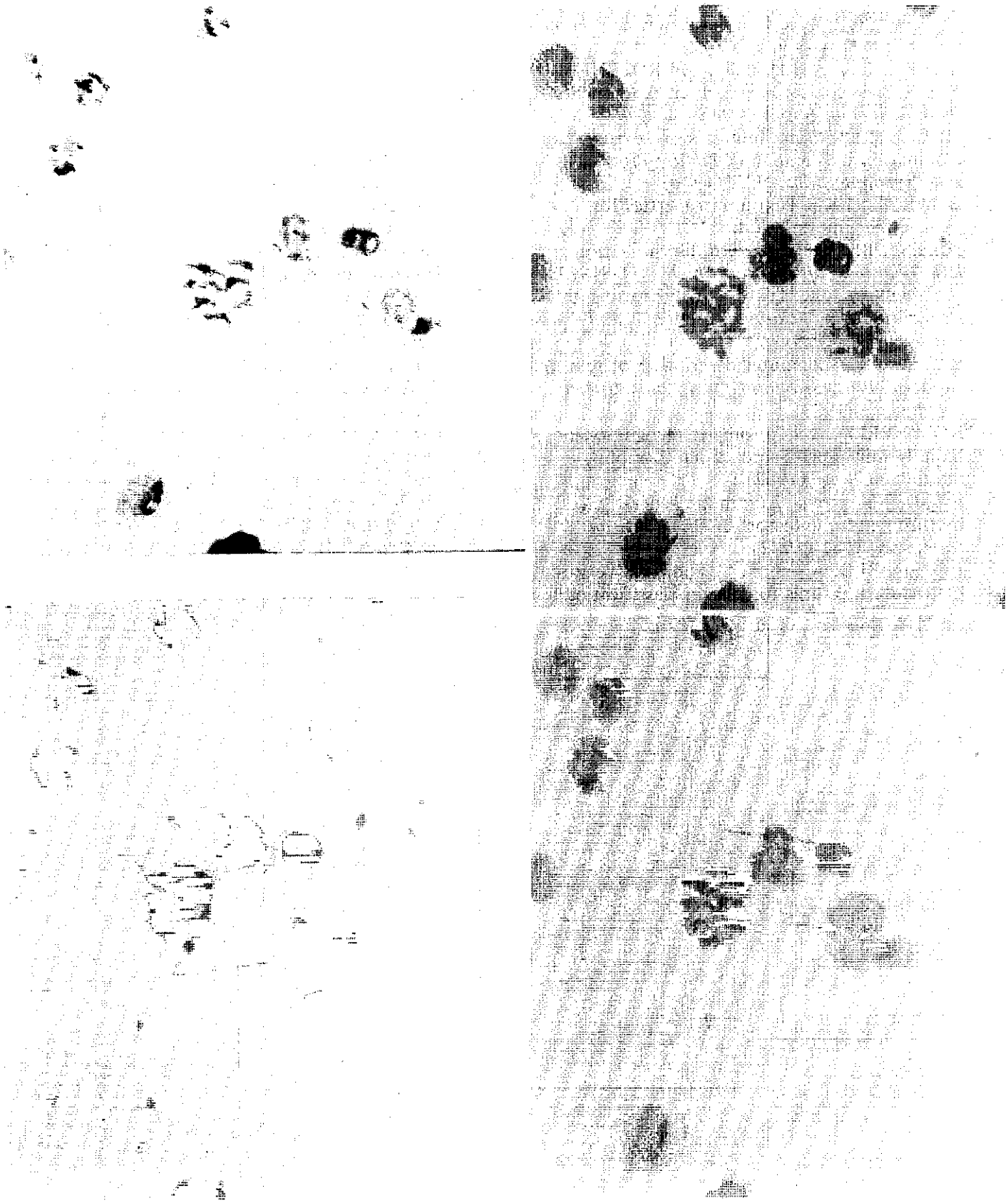


Fig. 6

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