

DISCUSSION

It has been well documented that the aetiology of root resorption is multifactorial.⁹ Not only has the magnitude of applied force,^{15,11,32} the duration of force application,^{11,33,34,35,7,36} the type of orthodontic appliance^{37,38} and the type of applied force^{39,5,40} has been advocated, individual variations^{40,41,32,42,36,16,12} play a major role in root resorption as well. To restrict these variations, a specific selection criterion for samples has been imposed on the present study.²⁶ Any external and systemic factors that may predispose root resorption were excluded. Teeth from the same patient served as control on one side and experimental on the contra-lateral side. This also eliminated the amount of inter-individual variability. Brackets were bonded on the control teeth to simulate the environment of the experimental teeth.

In previous reports,^{40,41,32,42,36} the active wires have been ligated to the brackets on the premolars using ligature ties. During this process of ligation, there would be different levels of distortion on the spring and a varied force distribution on the tooth. Although the original force levels measured on the springs could be accurate, ligature tying may alter the resultant force acting on the experimental teeth. Moreover, the two-point contact between the ligature wire (or modules) and the springs would introduce complex and undesired moments. In the present study, Speed (Strite Industries, ON, CA) self-ligating brackets were used. The built-in self-ligating Nickel-Titanium spring clips are elastic in nature and do not undergo plastic deformation when activated within its working range. A constant force was applied onto the tooth through the wire and spring clip. A one-point contact was also

maintained and undesirable mesio-distal forces and moments are not actively generated.⁴³

It has been documented that the uncalcified mineral tissues, osteoid, precementum, and predentine are resistant to resorption and may initially prevent loss of root tissue.⁴⁴ However, continuous pressure will eventually lead to resorption of these areas.^{44,45} It has also been reported that a continuous force was not necessary to produce tooth movement. Tooth movement has been previously shown to be effectively performed with functional appliances^{46,47} as well as headgear^{48,49} with interrupted wear. It has also been demonstrated that an interrupted continuous force with periods of rest may favorably enhance cell proliferation in the supporting tissues, which in turn might promote further tooth movement,⁴⁴ and subsequently result in less root resorption^{23,50,51} or equals the amount of root resorption observed under continuous forces.⁴⁰ However with a closer examination, these studies may be flawed in one way or another. Acar *et al.*²³ applied buccal forces with elastics from the buccal button bonded to the sample tooth, to a passive intrusion arch. Strict buccal tipping forces were not achieved; some level of intrusion would be expected in this setup. Proximal contacts of the experimental teeth were also reduced to eliminate interferences. This may be an external traumatic insult to the pulp and periodontal ligament of the experimental teeth, which may induce or promote some form of resorption. It has been noted that the control teeth were not approximated. This issue of variability not excluded between the control and experimental groups would not form a good basis for comparison. The other issue brought up by King²⁴ about compliance of the patients in Acar's study has been debated as well. In the other study by Owman-Moll⁴⁰ and Lundgren *et al.*,²⁷ the spring used in the continuous group

was not strictly continuous and the accuracy of serial sectioning, identifying and measuring all craters present was questionable.

Force decay has been an issue with most studies on root resorption.^{40,32,42,36} While some setups allowed uncontrolled force decay, others reactivated the springs weekly. Using elastics has its inherent problems of decay and patient compliance as well.²³ Owman-Moll *et al.*⁴⁰ prescribed a continuous force applied to premolars with a 50g force reactivated weekly. However, it has been observed that with the Sentalloy wires used, there was up to 22% force decay at the end of the week just prior to re-activation. Hence continuous as described by the authors in this case could not be strictly continuous as commented by King.⁵²

It has been demonstrated that β -Titanium Molybdenum Alloy (TMA) wires performs very well when long range of activation was required with almost insignificant force decay over a prescribed active range.⁵³ These wires have long been demonstrated to be very successful in controlling precise tooth movement in sectional mechanics in orthodontics.⁵⁴ In the present study, all TMA springs in the experimental groups were custom made and calibrated to allow for maximum force deflection with minimum decay.²⁶ Force decay was not significant and the setup in the present study does not require re-activation of force. The light (25g) and heavy forces (225g) were maintained throughout the 28 days of experimentation. Glass-ionomer cement (GIC, Transbond, 3M Unitek) was placed as occlusal stops on the lower first permanent molars to dis-occlude the occlusion.

Furthermore, the implicated studies ^{40,32,42,36,23} do not seem to have a strict selection criteria on the premolars used. Previous fillings, decay, previous trauma, bruxism, attrition, periodontal disease, root maturity/ formation, medical history (endocrinal disorders, asthma) have not been excluded. All these factors have been reported to be linked to root resorption and should not be overlooked. ^{9,17}

The optimum force necessary for physiologic tooth movement has long been debated. In-vitro experiments, no matter how accurately mimicking the biological system, could only be estimates. ²⁷ As early as 1932, Schwartz ⁵⁵ proposed the pressure-tension theory in the biology of tooth movement. Although Schwartz's range of optimal and detrimental forces was later challenged by Miura ⁵⁶ to be higher, he suggested that the optimal force level for tooth movement should be between 7 and 26g/cm² of root surface area. Darendeliler *et al.* ⁵⁷ have noted, with drum spring application of 50g constant and continuous force, that ideal tooth movement suggested by Roberts *et al.* ⁵⁸ could not be achieved even though the 'optimal physiologic forces' suggested by Schwartz was used. However, exceeding this force can cause periodontal ischaemia leading to root resorption. ⁹ On the other hand, when pressure decreases below force levels of 20 to 26 g/cm², root resorption ceases. ⁵⁹

Again in controversial with Schwartz's paper, the "optimal force" theory introduced by Storey and Smith ⁶⁰ proposed that a range of pressure 150-200g (equivalent to 150-200 cN) activated on the tooth-bone interface would produce the maximum rate of tooth movement for distalization of maxillary canines in man. For pressures below this range, movement was limited due to the ability of the soft tissue

to function as a shock absorber. If the force was increased beyond this optimum, hyalinization and undermining resorption occurs.

Assuming the hypothesis proposed by Schwartz ⁵⁵ to be correct, the ideal optimal force would fall within 7 to 26g/cm² of root surface area. As reported by Jespen, ⁶¹ the amount of surface area of a premolar tooth would lie between 2.34 ± 0.33 cm². Hence in the present study, 25 grams was selected as the light force. A triple exponential increase was selected for the heavy force at 225 grams.

The total duration of force, often equivalent to treatment time, is considered in some investigations to be a more critical factor than the magnitude of force with regards to adverse tissue reactions during orthodontic treatment. ^{33,11} It has been reported that after application of force, it can take between 10 and 35 days for resorption craters to appear. ^{15,22,11,33} Clinically, this degree of resorption cannot be detected with radiographs, especially when occurring on the buccal and lingual surfaces. ^{10,62,63} After 10 days of force application (50g) Kvam ²¹ recorded small, round root resorption cavities in humans using a scanning electron microscope. The amount of root resorption increased with time. After 25 days the resorption had reached the dentine and showed a characteristic honeycomb appearance. This positive association between duration of force and root resorption has later been confirmed in rats ⁶⁴ as well as in humans again. ^{1,65} However, other reports have not been able to verify these findings. ^{66,34,38,35} This may be due to the lack of control of the forces. Lilja and Odenrick ³⁴ measured root resorption induced by slow maxillary expansion with quadhelix appliances. However, as the applied force was distributed among teeth, surrounding tissues and elastic sutures, force measurements were hard to

perform. Assuming that force decay was significant, the forces would then have to be re-activated more often and measurements updated accordingly. In the paper presented by Stenvik and Mjör, ³³ forces were measured at the beginning and end of the experiment while Kvam ²⁰ measured the forces only at the beginning of the experiment even though experimental periods were 35-76 days.

In the present study, twenty-eight days was selected as the experimental duration. This allowed for noticeable resorption to occur, insignificant levels of force decay, and also for practical reasons like avoiding breakages. In the beginning of the present study, there were more than thirty-six teeth earmarked for experimentation. However, some patients reported breakages and those teeth were omitted.

Inter-operator variability was negated in the present study with one operator bending and calibrating the TMA sectional wires, one operator placing all brackets and wires and removing the appliances. The same surgeon also did the extraction of teeth at the end of the experimental period. Extreme care was ensured during extraction of teeth and it was only done with elevators to avoid damage to the cementum; as would if performed with the usual forceps delivery.

Specimen preparation has been varied in the previous papers presented on root resorption. 5% sodium hypochlorite, ²³ 4% formalin, ⁴⁰ 4% formaldehyde ³² were used in separate studies either as disinfectants or for storage. The study of physical properties and surface characteristics of cementum is very technique sensitive as cementum is thin and soft tissue debris could be difficult to remove. The ideal storage media for these specimens should preserve the hard tissue structure and physical

characteristics of the cementum. Milli-Q (de-ionized water) was the storage medium of choice in the present study, as it has been demonstrated to perform well in the areas described.⁶⁷

Previous studies studied root resorption craters using confocal, light or scanning electron microscopes.^{2,11,23,40,19,20} Depending on the technique selected, specimens could be demineralized, kept dry, coated or embedded and sectioned. It has been noted that scanning electron microscope provided enhanced visual and perspective assessment of root surfaces, particularly when recorded in stereo pairs, has resolution and detail not attainable with histological models reconstructed from serial sections.¹⁵ It has been demonstrated in the present study that the resorption craters could be shallow, deep, small or large and very irregularly shaped. The root morphology of the first premolars studied was variable as well. In the papers presented by Owman-Moll *et al.*,^{40-42,59,32} three serial sections along the long axis of the tooth were performed bucco-lingually for one half of the tooth. The other half was further sectioned into another 3 parts mesio-distally, longitudinally. It would then be reasonable to assume that some irregularly c-shaped craters could be totally miscalculated. Craters, which were deeper at one end and shallower at the other, may have only part of it measured. Smaller craters may be missed as well. If the roots were curved and/or rotated apically, apical or even some mid-root craters may be totally missed. The micrometer mounted on the eyepiece of the microscope used in the study would encourage parallax errors and measurements might again be distorted. Furthermore, only arbitrary units were used. In the papers presented previously,^{2,11,23} composite low-magnification electron micrographs of the root surfaces were taken. These composite micrographs were then pieced together and resorption craters

measured with a digitizer. The micrographs obtained could only provide a straight-on 2-dimensional view of the root surfaces and resorption craters. There would also be some inherent error in measurements if the craters were along the edges of the micrographs that were pieced together. In the present study, the whole buccal and lingual surfaces of the control and experimental teeth were scrutinized and all existing craters were imaged in stereo pairs. The expanded version of a commercial software (*Analysis Pro 3.1*) was written specifically for this study to allow volumetric measurement of these craters. Due to the surface irregularities of the craters and the innate curvature of the tooth surface, grayscale adjustments had to be performed prior to image analysis.³¹ The motorized rotating stent designed and employed in this study allowed the buccal and lingual surfaces of the teeth, and also any mesio-buccal, mesio-lingual, disto-buccal, disto-lingual craters to be imaged without constant and time-consuming pumping and venting of the SEM chamber. The Phillips XL30 SEM used in the present study has an internal calibration for measurement. Once the images were saved digitally as TIFF images, the dimensions of the craters were automatically transferred to the commercial software. This digital transfer of measurements allowed a high degree of accuracy in measurements. Calibration of this commercial software for volumetric measurements with known pyramidal indentations on different metallic rods matched to the size of premolar roots was highly reliable. It was demonstrated that there was 96.4% and 98.2% accuracy for the brass and stainless steel rods respectively.³⁰

As would be expected in data collection in any studies, several levels of error would be evident. In the present study, the level of error was isolated by calculating the variability of the results. The variance component between different images and

within each image was 0.098 and 0.051 respectively. As the variance between images was about twice the variance between processes within images, each crater was imaged four times with four stereo pairs of images and each pair of image processed twice with the analysis software. Thus for every crater present, eight volumetric recordings were obtained. This negated the innate degree of error in the study.

The present investigation demonstrated that predictable root resorption could occur under controlled circumstances. The repair of the resorption craters^{68,25,59} was not within the scope of this study.

In the present study, it was clearly demonstrated that the heavy force group had significantly greater volumetric resorption than control and light groups ($p = 0.000$). Although there was more volumetric resorption recorded in the light group, control and light force groups demonstrated no statistical significant difference in the amount of resorption (Fig. 14). The mean volume of the resorption crater in light force group was 3.49-fold greater than control, and the heavy force group 11.59-fold than control group. The heavy force group had 3.31-fold greater resorption volume than light force group. However, this outcome was not in concert with several other studies. A series of studies by Owman-Moll *et al.*^{40,41,32,42} performed on 144 adolescents and 200 premolar teeth reported that tooth movements and severity of root resorption were not significantly affected by doubling the force magnitude from 50cN to 100cN. It was also noted that the amount of root resorption was greater after 7 weeks with 50cN compared with 100cN. Although the authors could not explain this phenomenon, they found their study in agreement with Stenvik and Mjör,³³ who found that an increased force caused a decrease in frequency of root resorption when

premolars were intruded. They observed that root resorptions increased after application of light forces, 35g when compared to heavy forces, 250g. They all contributed this phenomenon to other idiopathic individual variations *e.g.* the metabolic responses of the subjects. It was concluded that root resorptions do not seem to be very force-sensitive. Although Owman-Moll *et al.* found that when continuous force increased 4-fold to 200cN, tooth movement increased 50% without any significant increase in root resorption; they cautioned that the results should not mislead clinicians into using heavy forces. The technique described in the study: selective serial histologic sections may not be accurate enough and many of the resorption craters may have been missed. Selection criteria for the premolars were not strict and external factors that may predispose root resorption did not seem to be excluded. Ligature wires used to ligate the wires could have distorted the force system by introducing undesired moments.

Extreme use of heavy forces has been demonstrated to cause total hyalinization of the tissues in the pressure zone.⁵ This phenomenon demonstrates a cell free zone that does not contain the osteoblastic and osteoclastic cells that propagate root resorption. This could be another explanation behind the less resorption seen in the heavy force group in the previous two studies.

On the other hand, Reitan^{5,15,44} had always advocated the use of light orthodontic forces in treatment in order to increase the cellular activity in the surrounding tissues and reduce the risk of root resorptions. This was later confirmed by King and Fischlschweiger.⁶⁹ They found, in an investigation in rats, that light forces produced insignificant root resorptions, whereas intermediate or heavy forces

resulted in substantial cratering. This result was in agreement with earlier findings, both in animals^{20,71} and in humans,¹¹ as well as the findings of the present study.

Due to the fact that different types of orthodontic appliances were utilized, *e.g.* frictionless sectional arch,^{60,72} Kloechn-type headgear,⁴⁸ full fixed appliance,⁷³ latex elastics,^{74,23} sliding mechanics⁷⁵ and different magnitudes of forces were applied in the above mentioned studies (25g to 1515g), the comparison of the effects on root resorption has been difficult. Moreover, the direction of tooth movement varied in different investigations. Some investigators moved teeth mesio-distally,^{60,75} while other authors used techniques for tooth intrusion³³ or extrusion.¹⁵ Reitan also investigated the effect of root tipping, applying torque,⁵ or crown tipping.¹⁵ Therefore a direct comparison of these results could not be performed.

It has been reported that resorbed craters appear mainly on the pressure side,^{5,44,15,76,22} and rarely on the tension side.⁶⁴ In the present study, it was observed that greater amounts of root resorption were consistently found on the buccal cervical and lingual apical regions of the root surface than any other regions ($p = 0.000$) (Fig. 11). Table IV demonstrated that at baseline (control group), buccal cervical regions had no resorption recorded while some resorption was already evident at the lingual apical regions. As forces increased to 25g (light force group), 11 and 23 regions increased significantly when compared to the other regions. When forces increased further to 225 g (heavy force group), resorption at 11 regions increased 2.66 times more while resorption at 23 remained the same. Resorption at the other regions (except 12 in the light group) remained almost at baseline throughout the increase in forces.

In the present study, the springs were directed buccally; tipping the experimental teeth crown buccal, apex lingual. The compression between the cementum and alveolar bone at these regions would have brought about cellular changes contributing to cemental as well as bony changes (Fig. 17). Although the springs were directed buccally, absolute buccally directed vectors should not be expected. Some degree of unpredictable intrusion or extrusion forces should be expected.²⁷ It should also be noted that meticulous control of magnitude and direction of force is no guarantee against variations in the individual effects of the force, which are likely to be highly dependent on variations in the individual anatomy of the tooth *e.g.* tooth length, number of roots, initial tooth position and amount of surrounding supporting tissues.

The biology of tooth movement and bone physiology has been previously described.⁵⁸ However, the individual variations of cellular reactions at cortical and spongy bone regions have not been isolated. It could then be postulated that at baseline (control group), due to normal bone physiology and cellular turnover at the apical regions of the teeth, some degree of baseline root resorption at the apex of the teeth could be expected.⁸ The cortical bone has reported to have higher density and hardness values than spongy bone in the alveolus. As force level increases, there was greater pressure expressed on the buccal cervical and lingual apical regions of the experimental teeth. At light force levels, the degree of resorption at these two regions seemed to be equivalent; however it is an exponential increase at the buccal cervical regions while at the lingual apical regions, the increase was only 3.83 times. However, at even higher force levels (heavy force group), the buccal cervical regions showed increase in resorption of a further 2.66 times while the lingual apical regions

remained constant as before. The buccal cervical region being moved against the harder cortical bone of the dento-alveolus would have greater reactive changes as compared to the lingual apical region being moved against the softer spongy bone of the dento-alveolus.

According to Schwartz ⁵⁵ and Roberts *et al.*, ⁵⁸ the 225g of force used in the present study would indeed be high. It has been reported that the post-lag phase for hyalinization of tooth movement in human teeth was 1-2 weeks. ⁷⁷ The experimental period in the present study being four weeks would mean that the post-lag phase of hyalinization was surpassed. The extreme root resorption demonstrated in the heavy force group would thus be assumed to be caused by undermining resorption. During total hyalinization, a cell-free zone would be present and would not provide any cementoclastic activity and root resorption may not occur at that stage. It could then be postulated that the resorption craters observed in this heavy force group was formed mostly during the third and fourth week of experimentation.

It has been noted by Schroeder ⁷⁸ that acellular cementum dominates the cervical region of the root while cellular cementum dominates the apical regions. Microradiographs of cementum have shown that cervically located acellular extrinsic fibre cementum (AEFC) was more radiodense, and therefore, more mineralized than the apically positioned cellular intrinsic fibre cementum (CIFC) and the variably located cellular mixed fibre cementum (CMFC). ⁷⁹ This difference could be explained by the presence of uncalcified spaces, such as lacunae in CIFC and by an uncalcified core of extrinsic Sharpey's fibres in the CMFC. ⁸⁰ It was also postulated by Rex ⁸¹ that the apical regions are less mineralized than the cervical regions that may

therefore be predisposed to more root resorption. In the present study, although there was more volumetric root resorption per unit area at the apical regions of the experimental teeth, there was no significant difference between the buccal cervical and lingual apical regions.

In the present study, even though the premolars were carefully selected to exclude any external or systemic predisposition to resorption, resorption craters were still evident in small quantities in the control group. This demonstrates that resorption could be a naturally occurring physiologic phenomenon.⁸

Several other previous studies on root resorption measured the area of root resorption craters and their relation to types of forces.^{23,2} These studies could only obtain a 2-dimensional qualitative analysis of the craters. Craters with large surface areas may be shallow while those with smaller surface areas may be deep. Unless a 3-dimensional qualitative analysis of these craters is performed, the true value of root resorption may not be totally understood. Hence the results of previous studies measuring root resorption areas should be taken with reservation.

CONCLUSIONS

Histomorphologic studies on root resorption in the past had focused on 2-D evaluation of the craters and any attempts at 3-D were poor estimates with low degrees of accuracy. With the introduction of new software and technology, we could understand root resorption from an improved perspective. 3-D replicas of root

resorption craters allow us to visualize the true extent of the crater and study its morphology and topography in greater detail and also from all directions.³¹

In the past, volumetric measurements could be done by displacement techniques or by using light bodied dental impression materials to measure the size of these craters. These methods are very technique-sensitive, highly inaccurate and hence were not very much embraced.

In the present study, we demonstrated that (1) heavy forces caused more root resorption than light forces and controls. (2) Control and light force groups demonstrated no significant statistical difference in resorption although there was more resorption recorded in the light group. (3) Buccal cervical and lingual apical regions demonstrated significantly more resorption than the other regions suggesting that high-pressure zones on the root are more susceptible to resorption. (4) There was no significant difference in the volume of root resorption per unit area between the buccal cervical and lingual apical regions; although there was more resorption per unit area in the lingual apical region.

This study allowed a more accurate measurement of root resorption in a controlled human study, which compared light and heavy forces in orthodontics. Although the true aetiology of root resorption still remains elusive, we are more aware of the undesirable effects of heavy forces in orthodontics.

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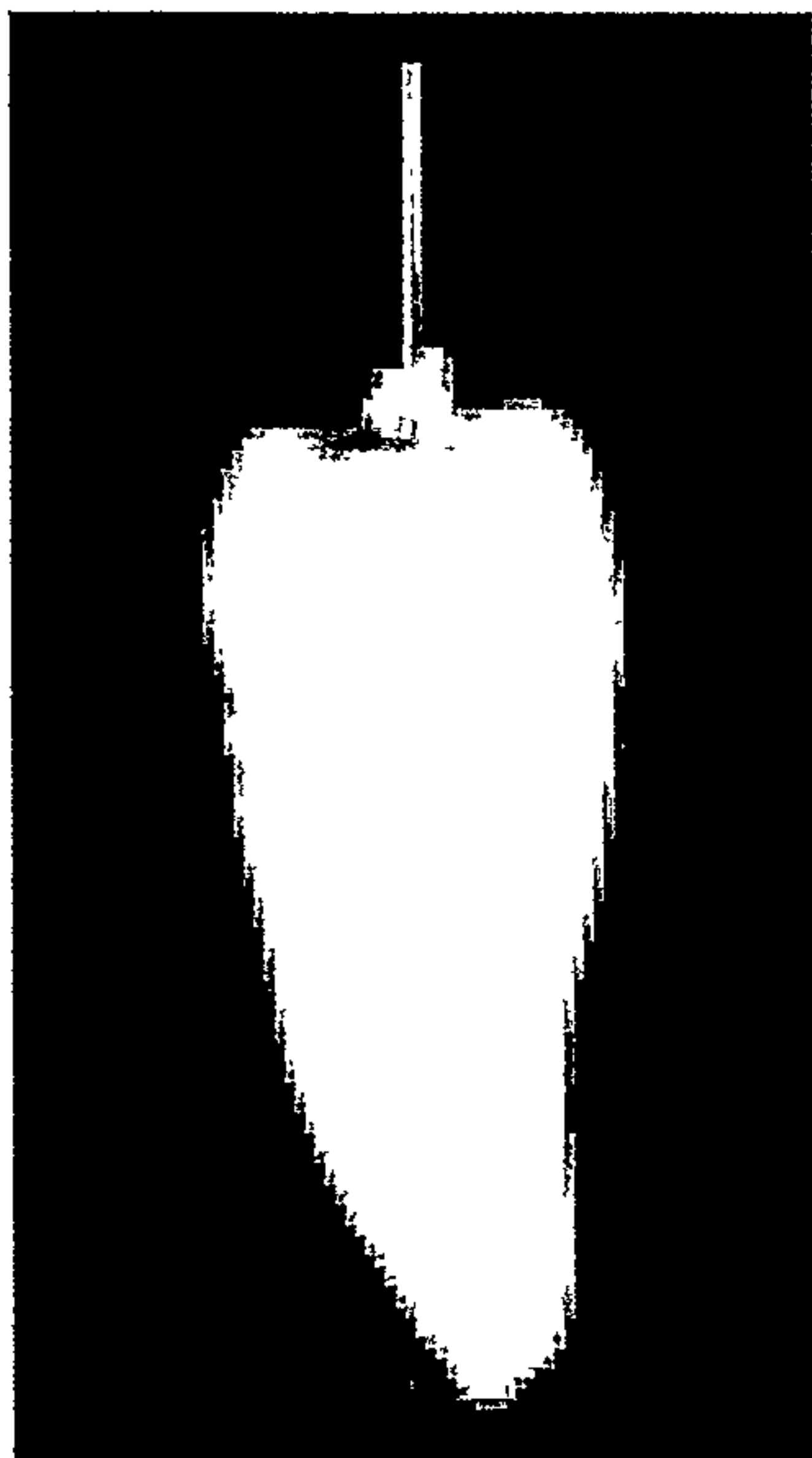


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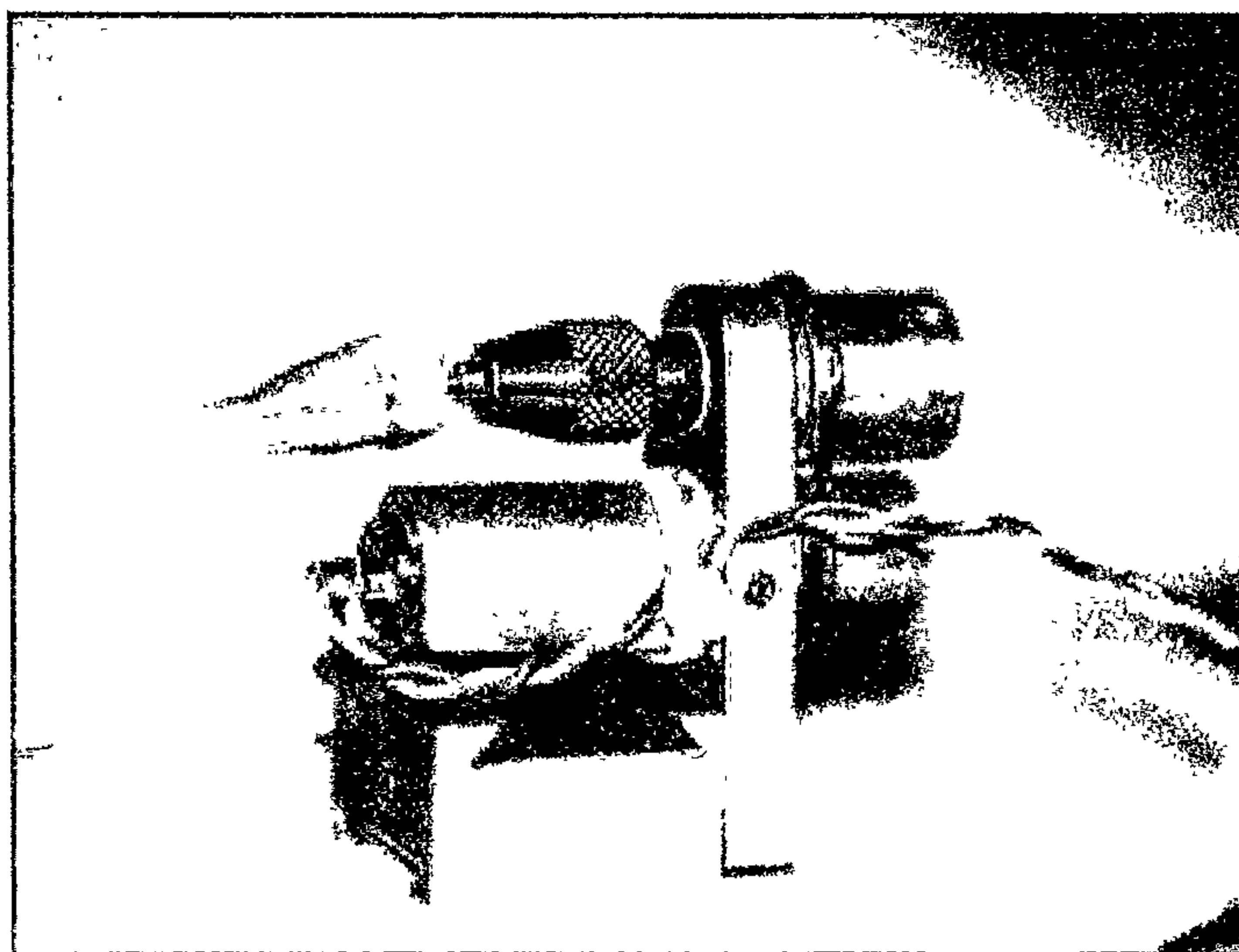


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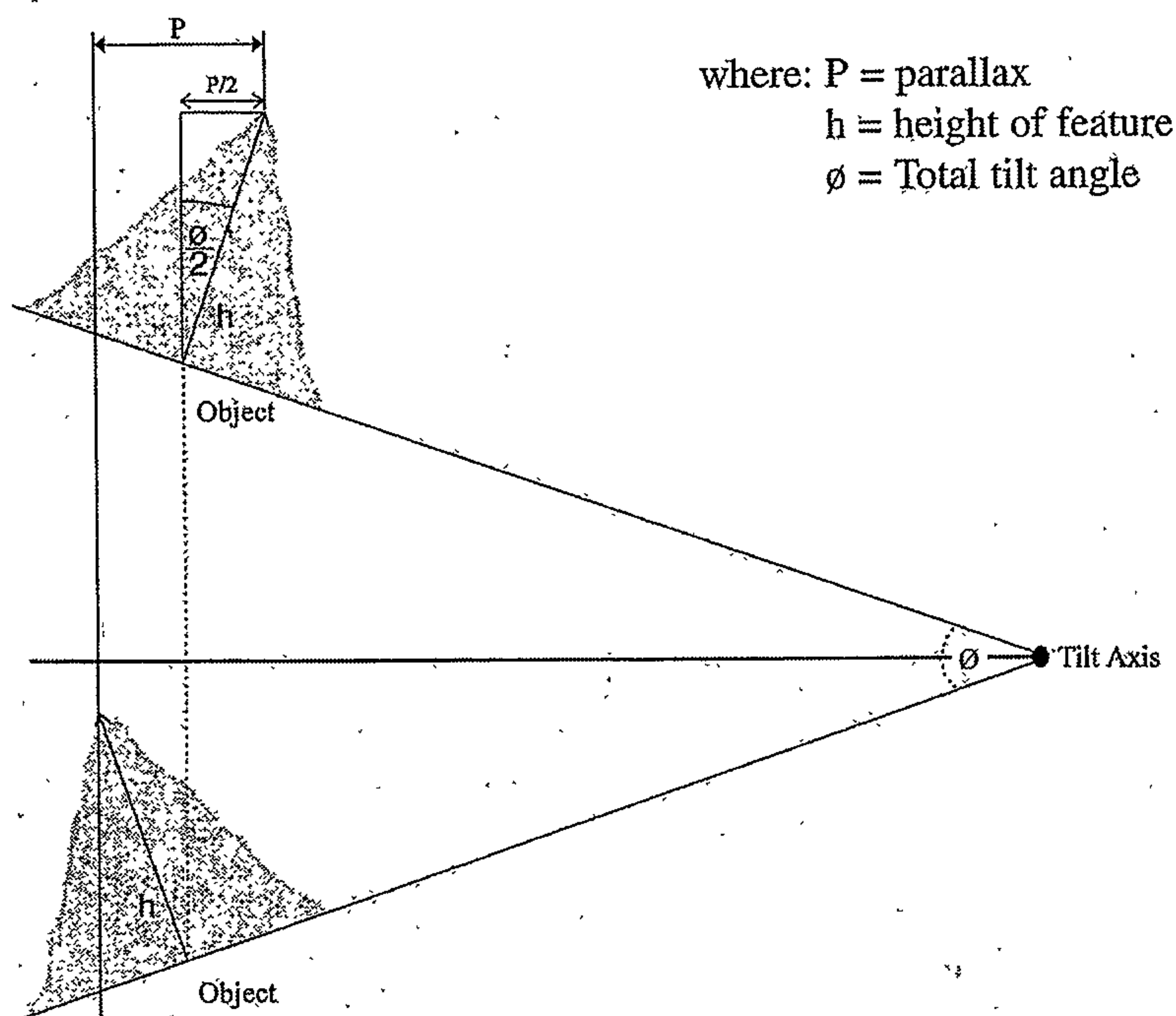


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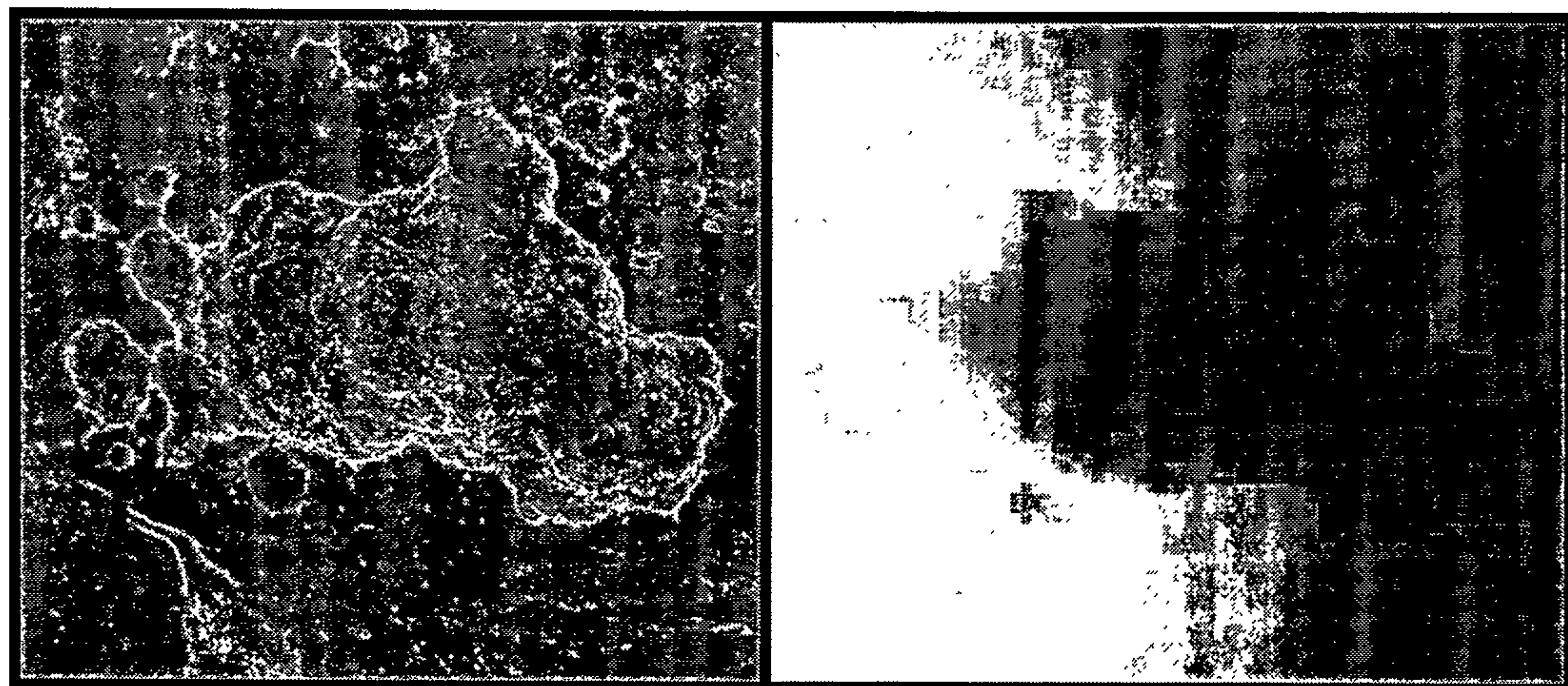


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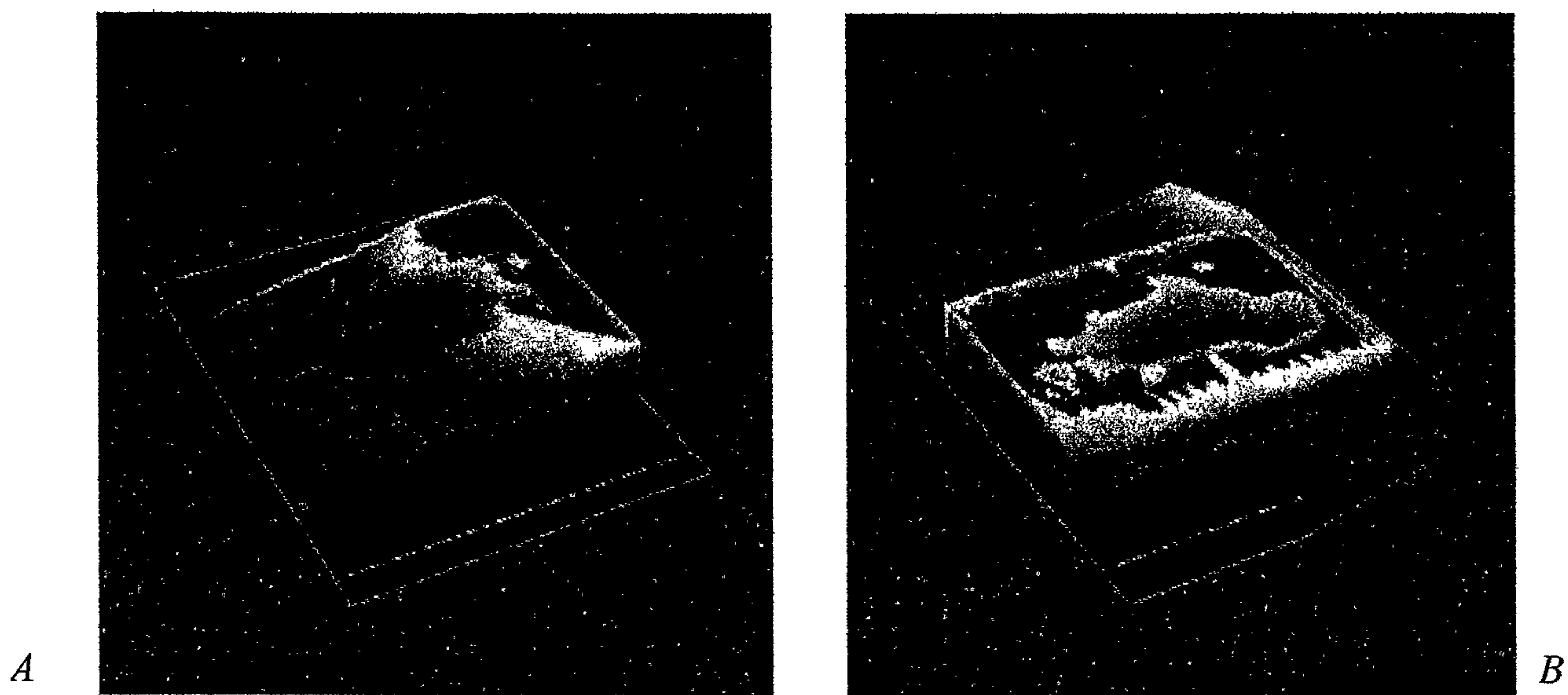


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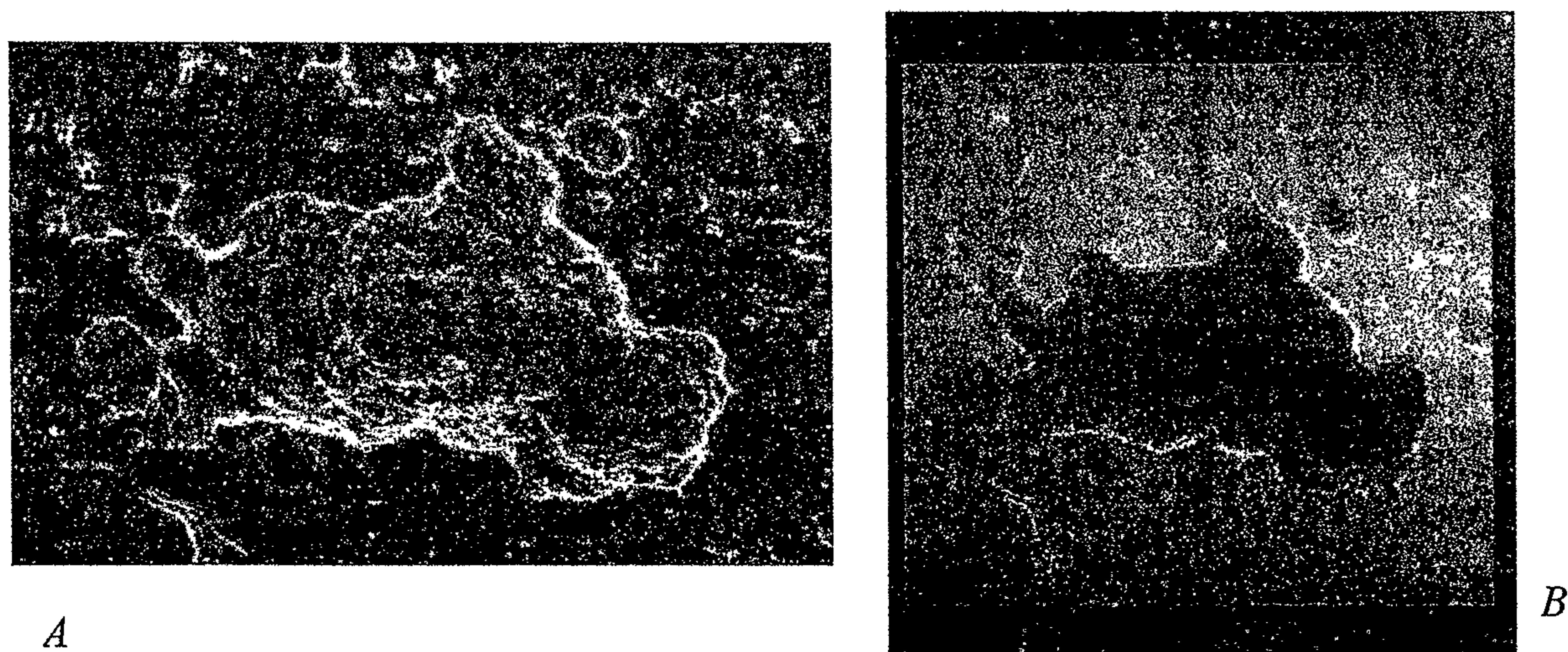


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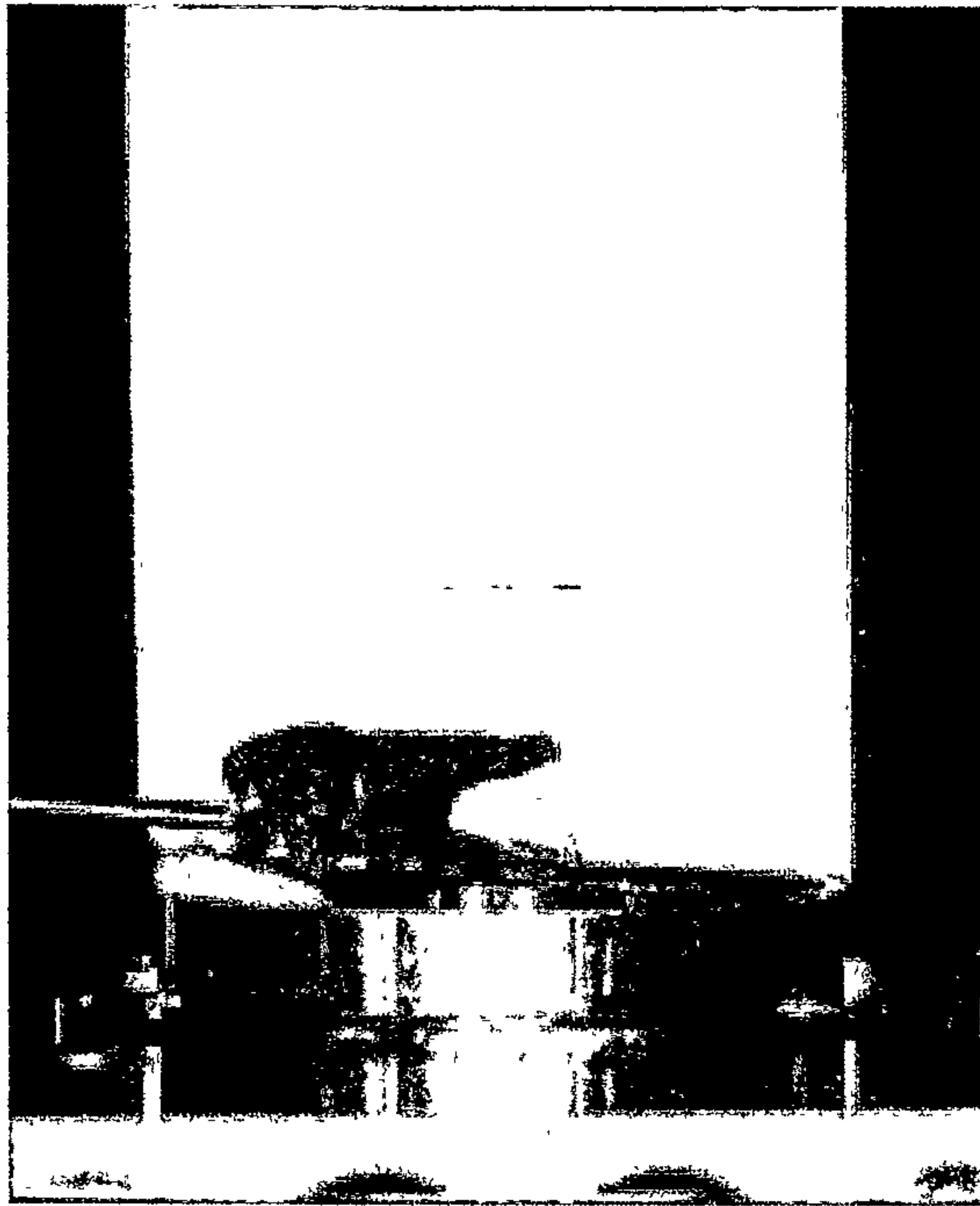


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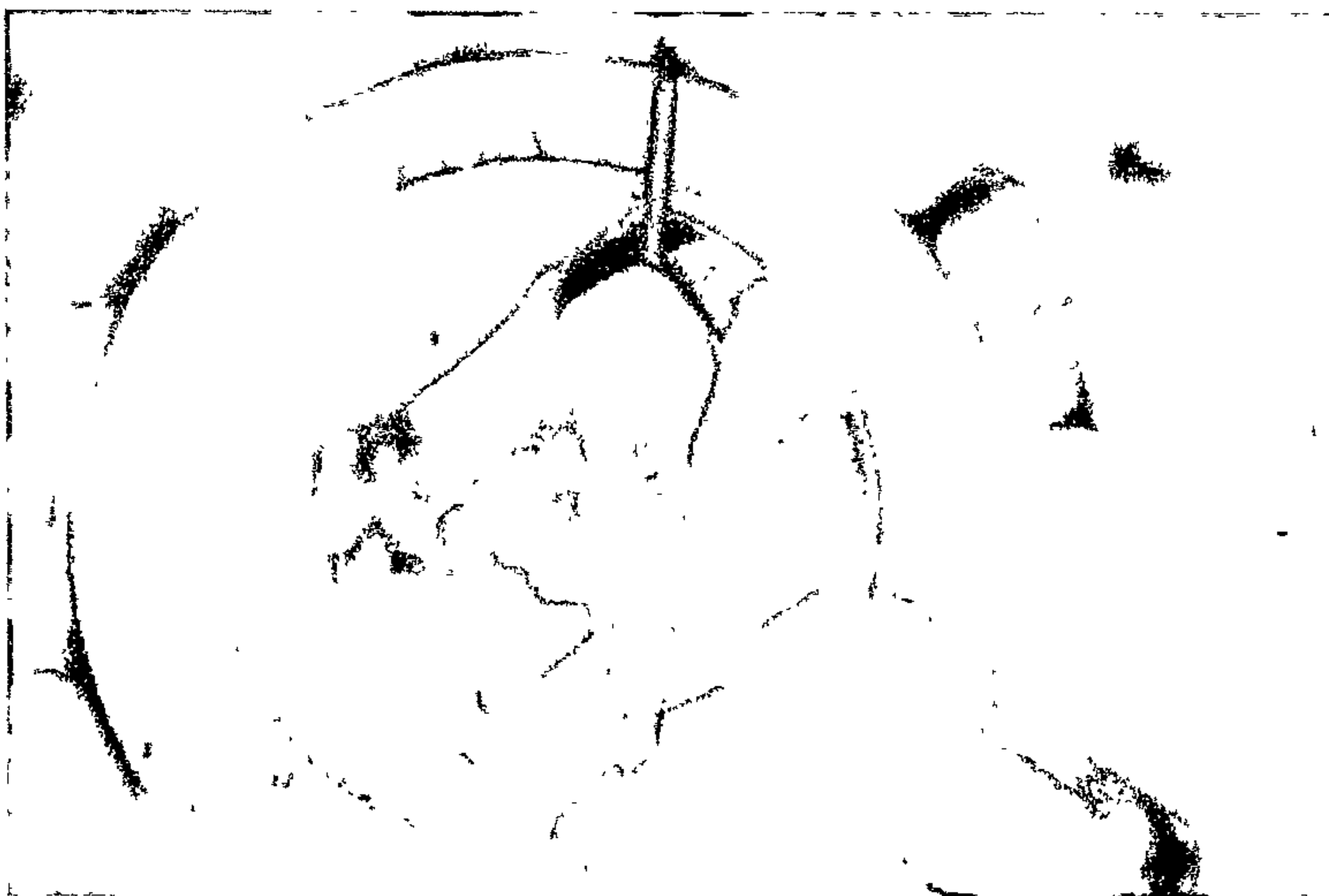


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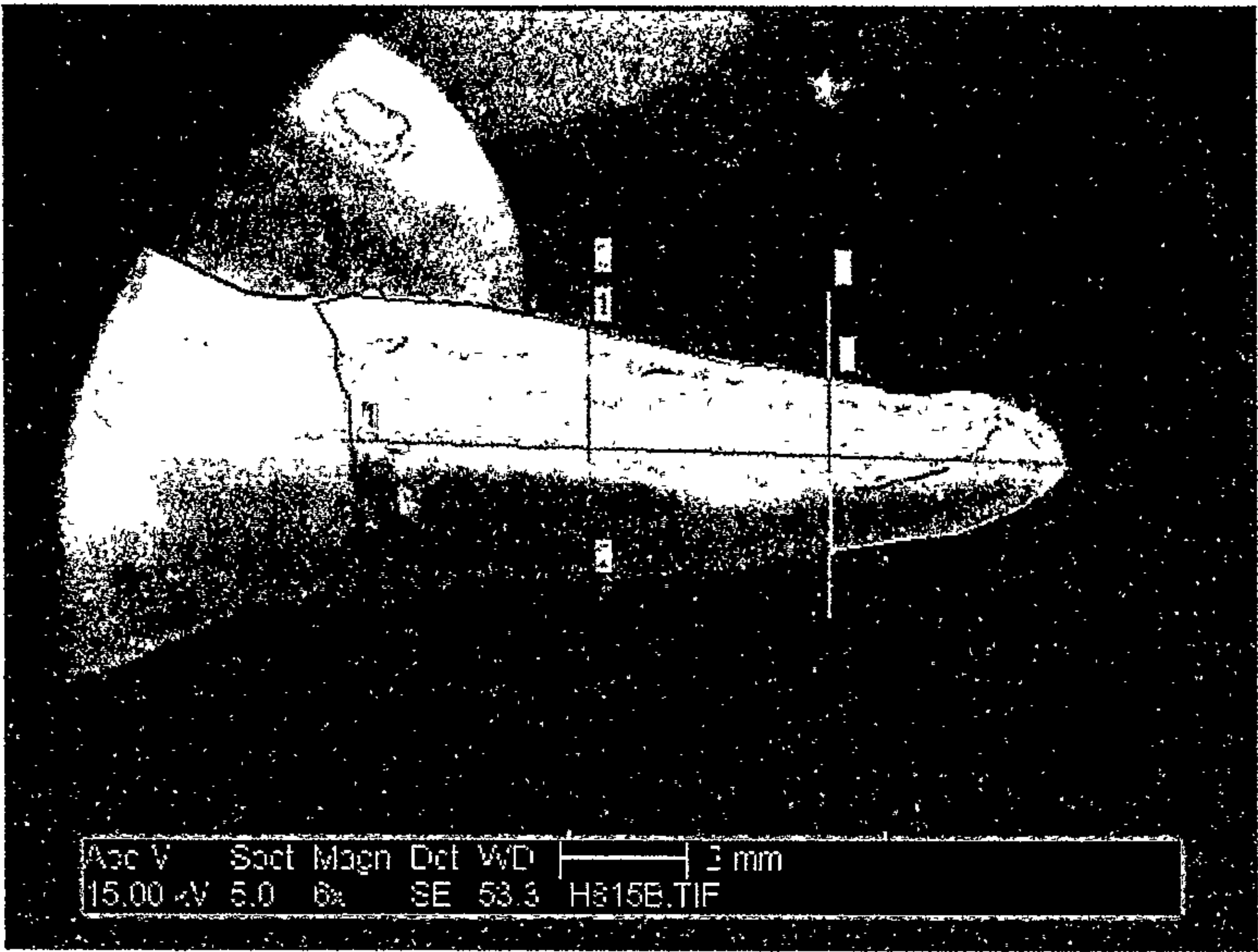


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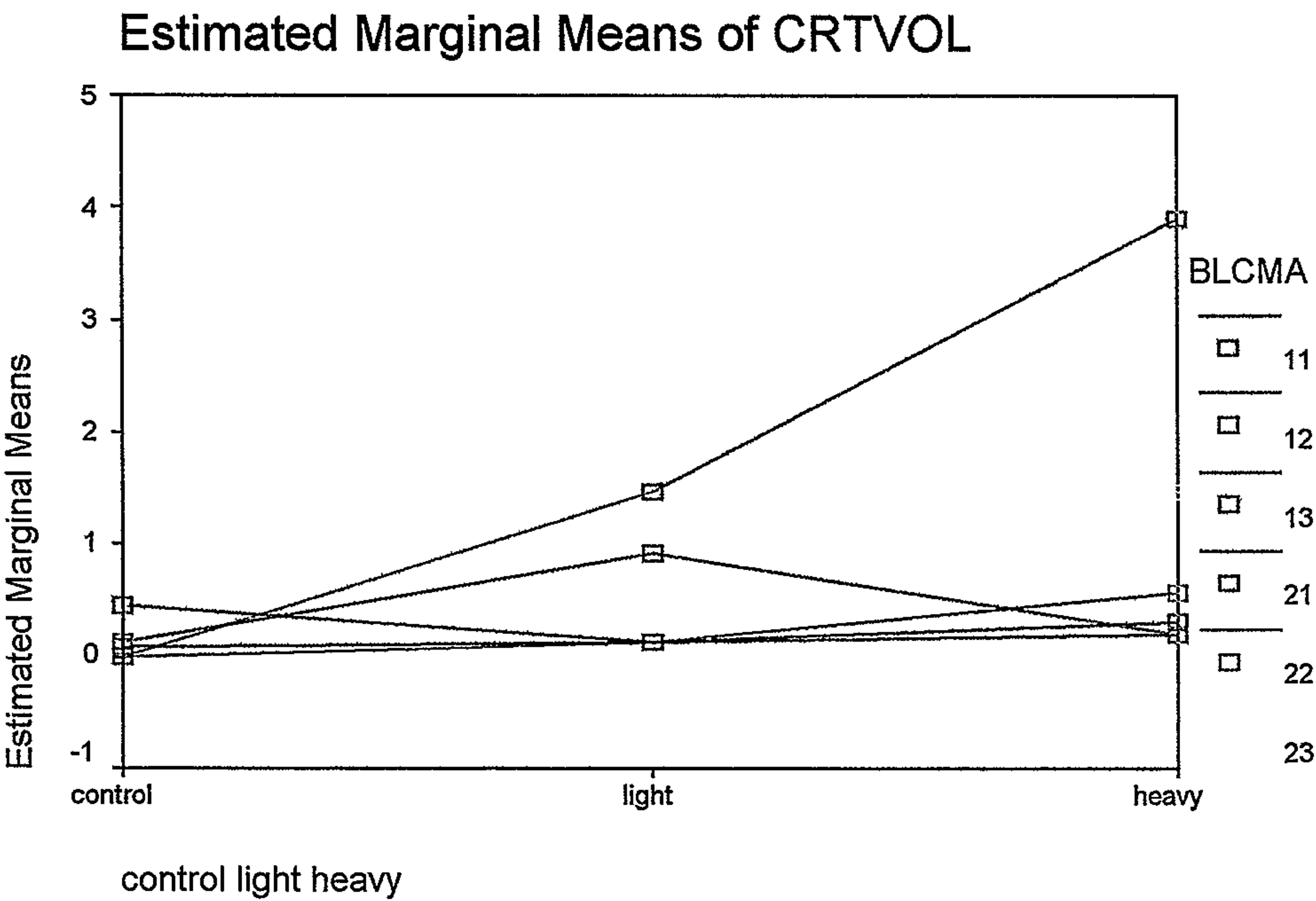


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CRTVOL: Cube root values of volumes.

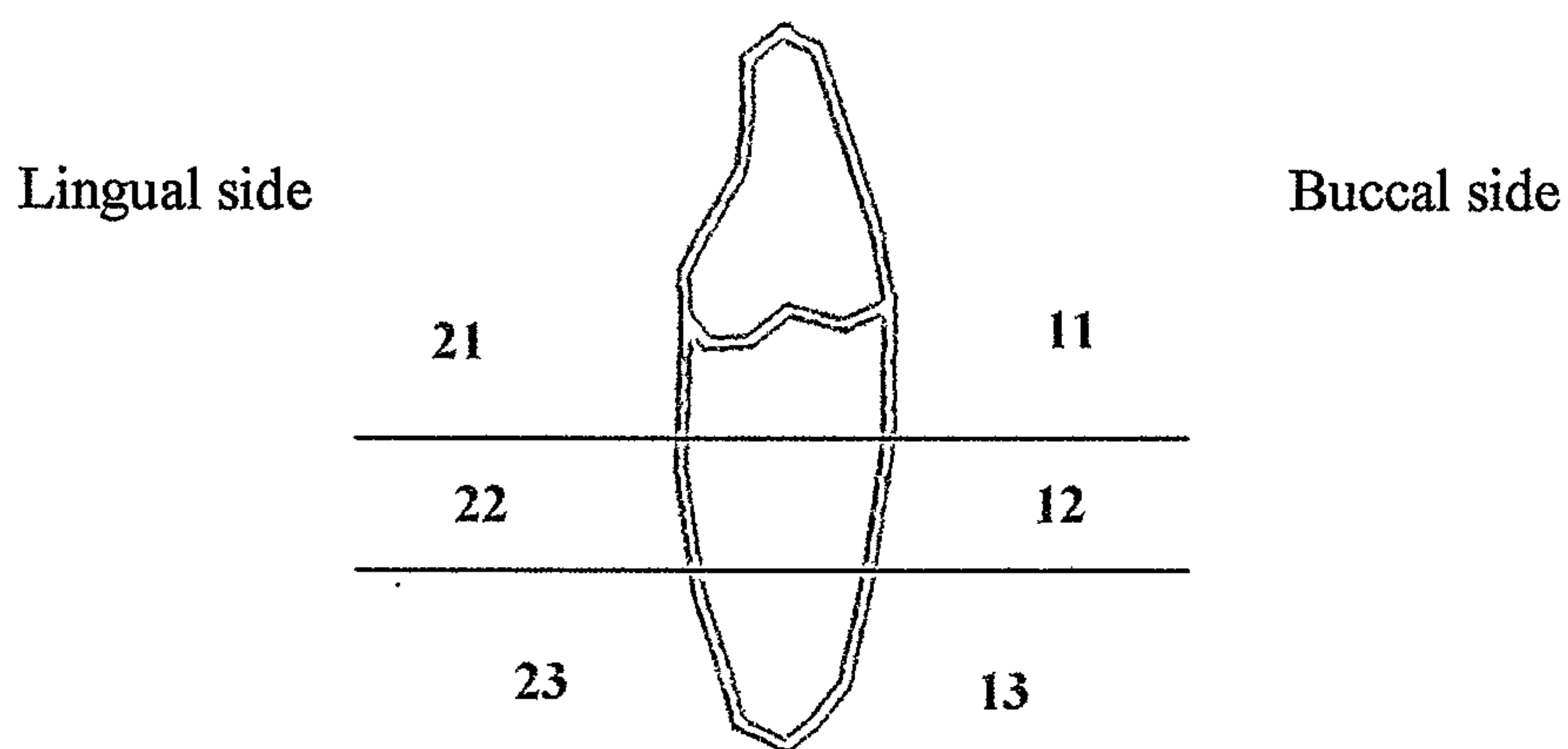


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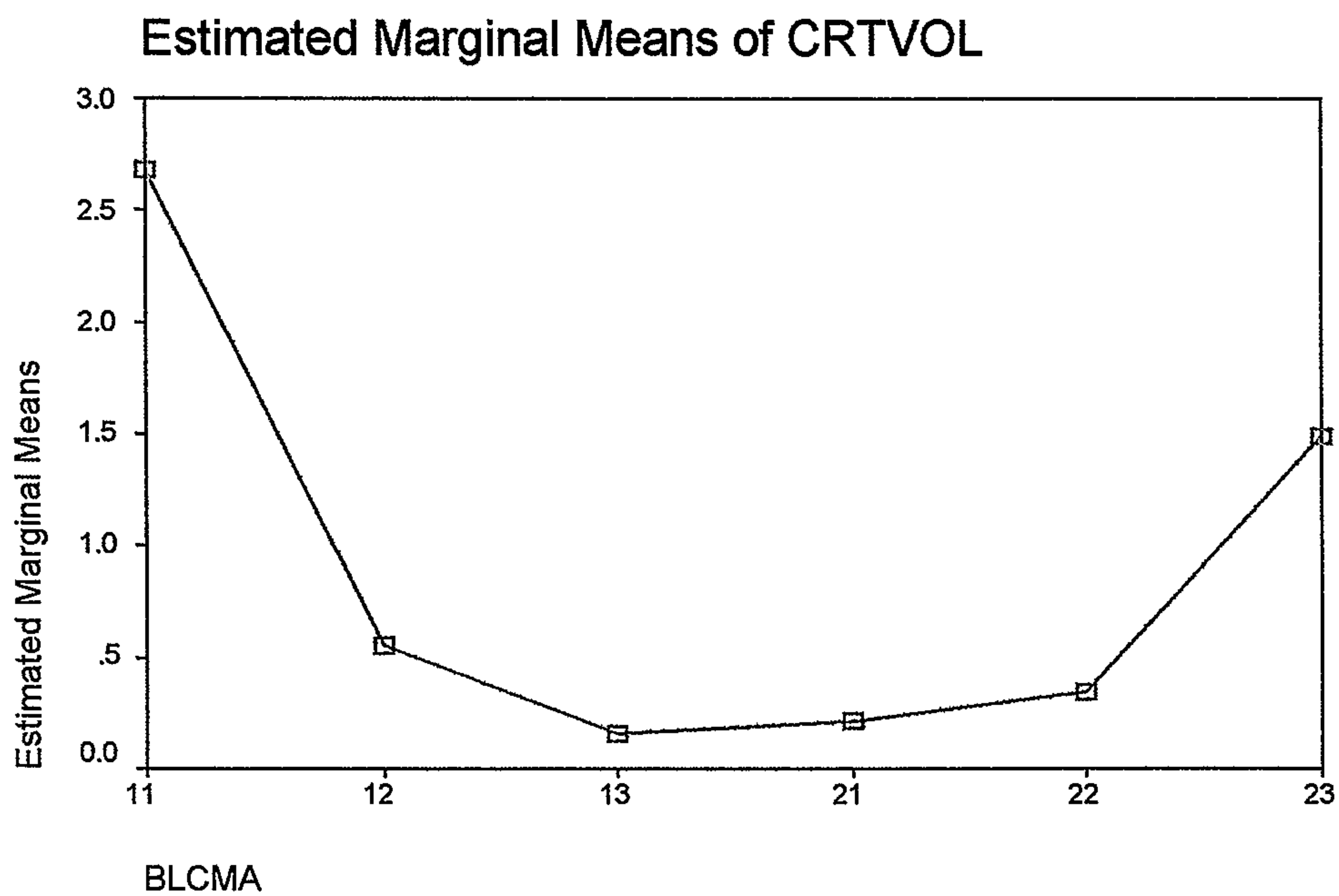


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BLCMA: buccal, lingual cervical, middle apical of root regions.

CRTVOL: Cube root values of volumes.

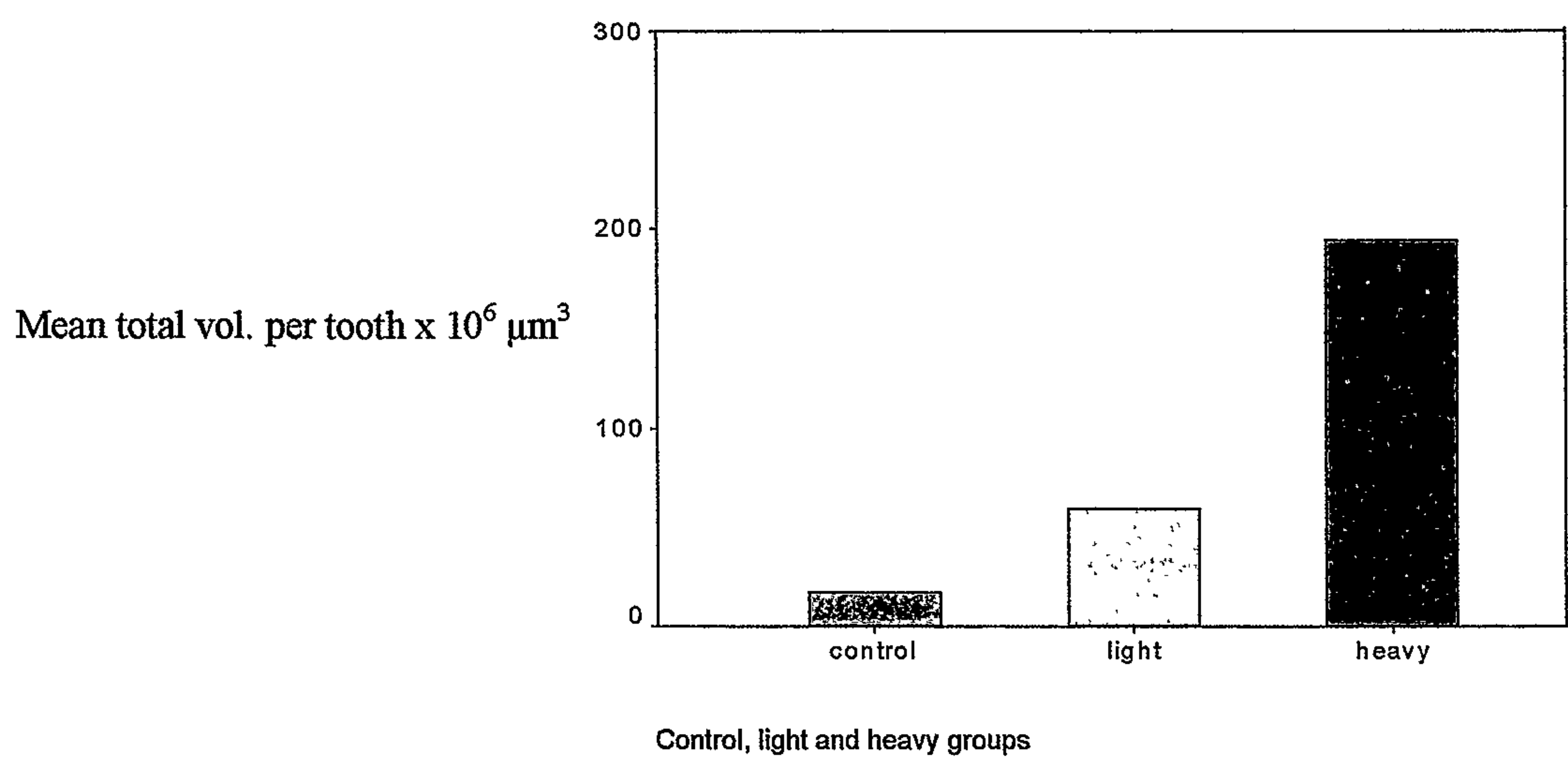


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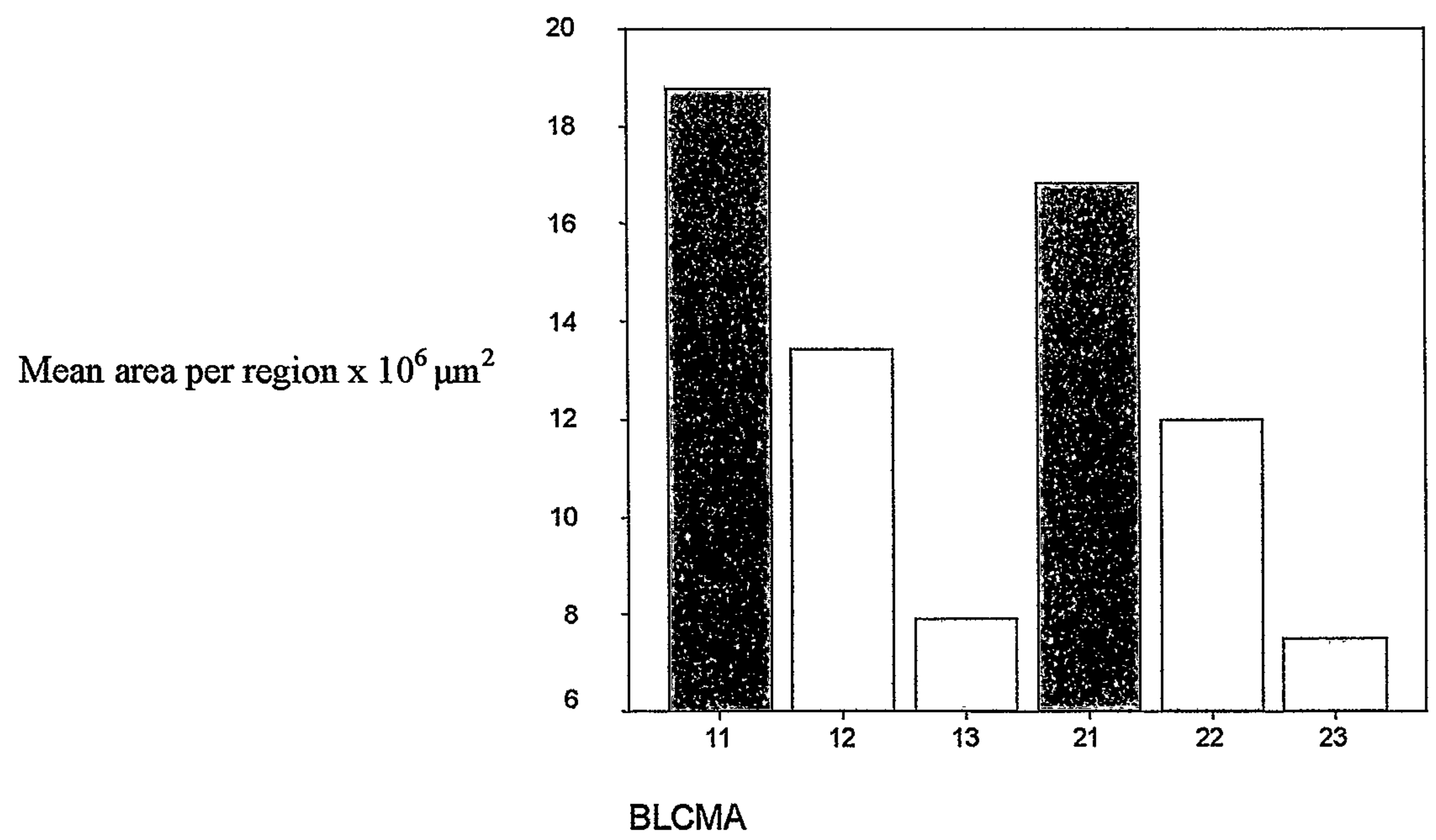


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BLCMA: buccal, lingual cervical, middle apical of root regions.

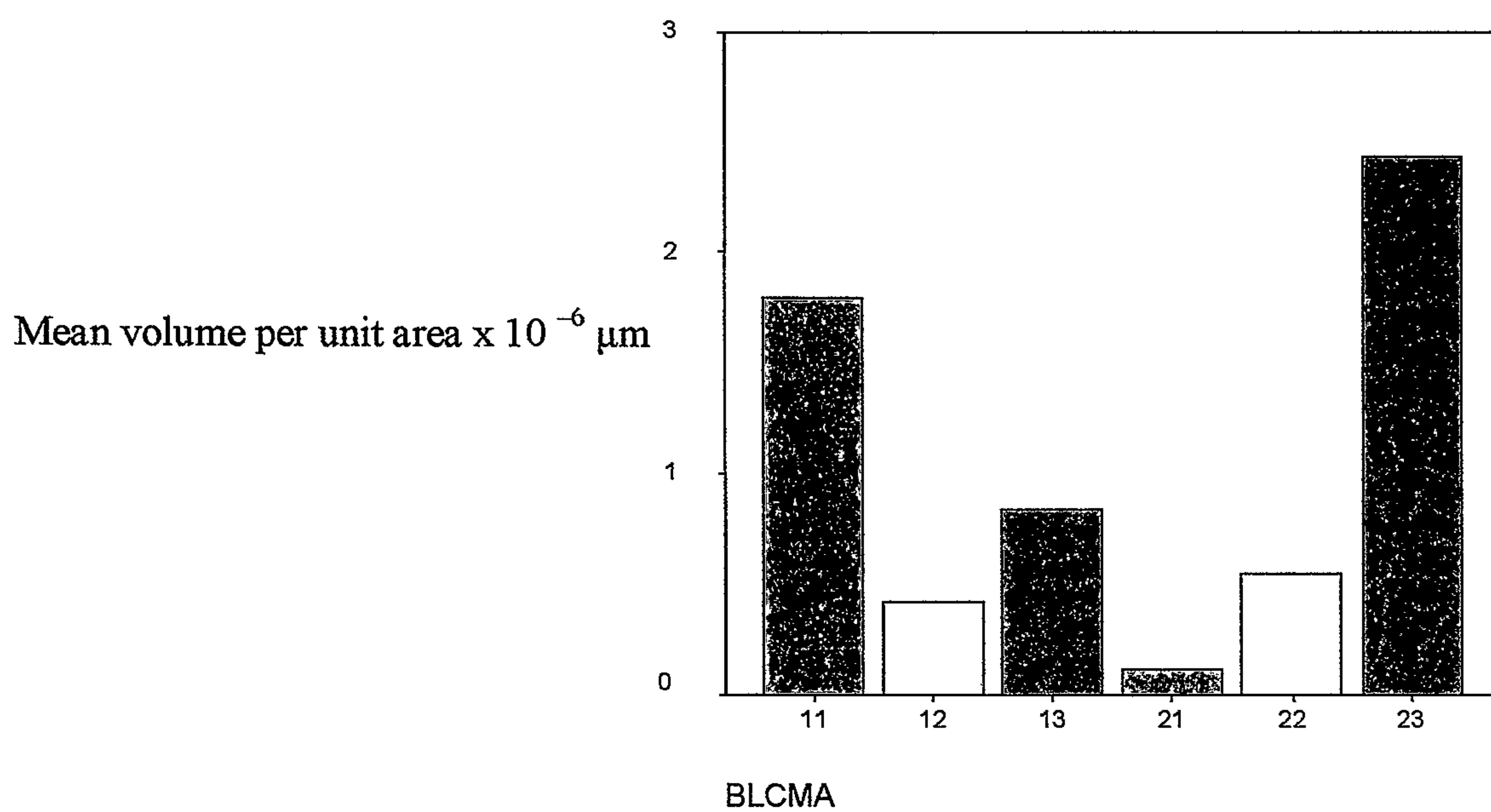


Figure 16. Graph demonstrating mean volume of root resorption per unit area of root surface.

BLCMA: buccal, lingual cervical, middle apical of root regions.

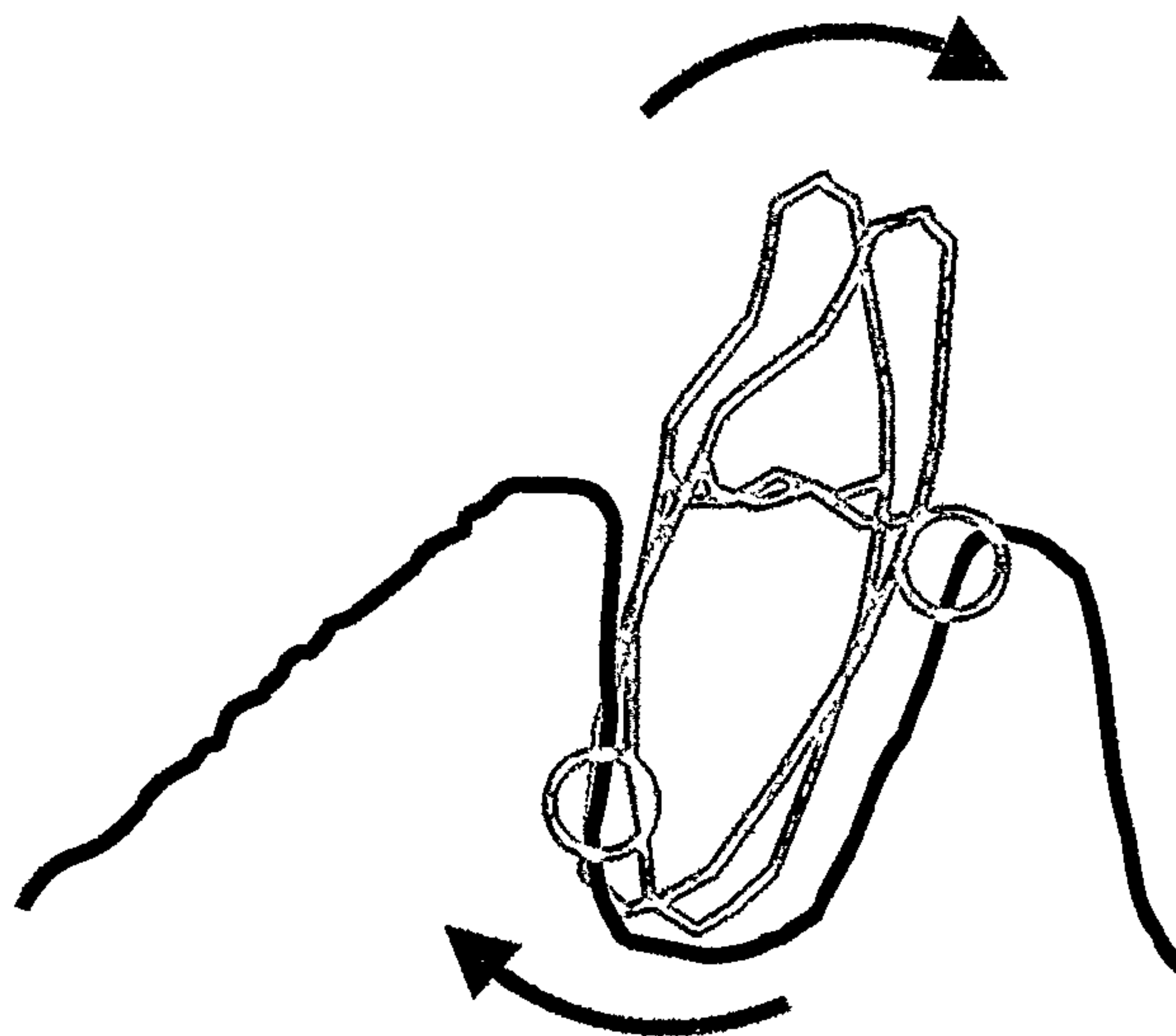


Figure 17. Diagrammatic representation of pressure zones.

TABLES

Table I. Contrast Results (K Matrix) demonstrating linear trend being significant.

Contrast Results (K Matrix)			Dependent Variable
control light heavy Polynomial Contrast ^a			CRTVOL
Linear	Contrast Estimate		.658
	Hypothesized Value		0
	Difference (Estimate - Hypothesized)		.658
	Std. Error		.157
	Sig.		.000
	95% Confidence Interval for Difference		.348
	Lower Bound		.968
	Upper Bound		
Quadratic	Contrast Estimate		-5.37E-02
	Hypothesized Value		0
	Difference (Estimate - Hypothesized)		-5.37E-02
	Std. Error		.203
	Sig.		.791
	95% Confidence Interval for Difference		-.454
	Lower Bound		.346
	Upper Bound		

a. Metric = 1.000, 2.000, 3.000

CRTVOL: Cube root values of volumes.

Table II. Tests of Between-Subjects Effects showing df at various levels of analysis.

Tests of Between-Subjects Effects

Dependent Variable: CRTVOL

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	85.109	1	85.109	33.824	.000
	Error	46.265	18.387	2.516 ^a		
CLH	Hypothesis	31.021	2	15.511	11.639	.000
	Error	241.205	181	1.333 ^b		
SUBJECT	Hypothesis	44.354	17	2.609	1.958	.016
	Error	241.205	181	1.333 ^b		
BLCMA	Hypothesis	69.740	5	13.948	10.467	.000
	Error	241.205	181	1.333 ^b		
CLH * BLCMA	Hypothesis	76.471	10	7.647	5.738	.000
	Error	241.205	181	1.333 ^b		

a. .927 MS(SUBJECT) + 7.273E-02 MS(Error)

b. MS(Error)

CLH: Control, light and heavy groups.

BLCMA: Buccal and lingual surfaces at cervical, middle and apical regions.

CRTVOL: Cube root values of volumes.

Table III. Tests of Between-Subjects Effects demonstrating df and p values for regions.

Tests of Between-Subjects Effects						
Dependent Variable: CRTVOL						
Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	88.527	1	88.527	30.933	.000
	Error	48.653	17	2.862 ^a		
SUBJECT	Hypothesis	48.653	17	2.862	1.137	.334
	Error	213.956	85	2.517 ^b		
BLCMA	Hypothesis	90.066	5	18.013	7.156	.000
	Error	213.956	85	2.517 ^b		

- a. MS(SUBJECT)
- b. MS(Error)

CRTVOL: Cube root values of volumes.

Table IV. BLCMA control light heavy, showing the trend of resorption at different regions as forces increase from control to light to heavy.

Dependent Variable: CRTVOL

		Mean	Std. Error	95% Confidence Interval	
control light heav BLCMA				Lower Bound	Upper Bound
control	11	.000	.272	-.537	.537
	12	.115	.272	-.422	.652
	13	.448	.272	-.088	.985
	21	.087	.272	-.450	.624
	22	.000	.272	-.537	.537
	23	.395	.272	-.142	.932
light	11	1.466	.401	.676	2.256
	12	.910	.401	.120	1.700
	13	.115	.401	-.676	.905
	21	.115	.401	-.676	.905
	22	.115	.401	-.676	.905
	23	1.513	.401	.723	2.304
heavy	11	3.905	.401	3.115	4.695
	12	.195	.401	-.595	.985
	13	.184	.401	-.607	.974
	21	.308	.401	-.483	1.098
	22	.574	.401	-.216	1.365
	23	1.466	.401	.676	2.256

BLCMA: Buccal and lingual surfaces at cervical, middle and apical regions.

CRTVOL: Cube root values of volumes.

Table V. Table showing mean volume per root surface region.

Report

volume total

BLCMA	Mean	N	Std. Deviation
11	33.1209	36	86.2046
12	6.0467	36	30.7685
13	6.4628	36	34.8948
21	1.6319	36	9.1478
22	6.6214	36	39.7283
23	17.8170	36	51.7411
Total	11.9501	216	48.8131

BLCMA: Buccal and lingual surfaces at cervical, middle and apical regions.

Table VI. T-test for volume total between regions 11 and 23.

Group Statistics

BLCMA		N	Mean	Std. Deviation	Std. Error Mean
volume total	11	36	33.1209	86.2046	14.3674
	23	36	17.8170	51.7411	8.6235

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
volume total	Equal variances assumed	1.830	.180	.913	70	.364	15.3039	16.7567	-18.1164	48.7241
	Equal variances not assumed			.913	57.321	.365	15.3039	16.7567	-18.2468	48.8546

BLCMA: Buccal and lingual surfaces at cervical, middle and apical regions.

Table VII. Table demonstrating mean area per region.

Report

average area per region

BLCMA	Mean	N	Std. Deviation
11	18783821.6275	36	2813615
12	13447103.2216	36	2006832
13	7898326.0746	36	1329606
21	16840002.3542	36	3346727
22	11992014.4485	36	2515556
23	7477977.8655	36	2196406
Total	12739874.2653	216	4851882

BLCMA: Buccal and lingual surfaces at cervical, middle and apical regions.

Table VIII. Table showing mean volume per unit area.

Report

volume per unit area

BLCMA	Mean	N	Std. Deviation
11	1.80E-06	36	4.7E-06
12	4.26E-07	36	2.1E-06
13	8.40E-07	36	4.5E-06
21	1.11E-07	36	6.1E-07
22	5.53E-07	36	3.3E-06
23	2.43E-06	36	7.3E-06
Total	1.03E-06	216	4.3E-06

BLCMA: Buccal and lingual surfaces at cervical, middle and apical regions.

Table IX. Table showing t-tests for volume per unit area.

Group Statistics

BLCMA		N	Mean	Std. Deviation	Std. Error Mean
volume per unit area	11	36	1.8E-06	4.7E-06	7.9E-07
	23	36	2.4E-06	7.3E-06	1.2E-06

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
volume per unit area	Equal variances assumed	1.299	.258	-.439	70	.662	-6.35E-07	1.45E-06	-3.5E-06	2.2E-06
	Equal variances not assumed			-.439	59.890	.662	-6.35E-07	1.45E-06	-3.5E-06	2.3E-06



MANUSCRIPT II

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A new method for volumetric measurement of irregularly shaped open craters.

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ABSTRACT

The purpose of this paper was to introduce a new method for volumetric measurement of irregularly shaped open craters on an uneven surface. This method was designed to study root resorption craters on the surface of human premolar tooth cementum induced by mechanical orthodontic forces. These craters could be physiologically occurring or induced by other means. They usually range between 20 - 200 μ m across and could be as deep as 100 μ m. A Stereo pair of images ($\pm 3^\circ$) was captured with Philips XL30 scanning electron microscope (SEM) focused at the eucentric point. The electron beam was set at 15kV, corresponding to a diameter of approximately 200nm. The sample was mounted at approximate zero degrees horizontal and this was controlled by using a 360 degrees motorized rotating stent. The images were saved as tagged image file format (TIFF). They were then imported as a pair into a commercial image analysis software package (*AnalySIS Pro 3.1*, Soft Image System, Germany). The image pairs were aligned by a cross-correlation technique, which used a sub-region of the images. This sub-region was defined by selection of a reference point that was clearly visible in both images. Once aligned, a grayscale depth map of the crater was generated based on the parallax difference and the known tilt angle at which image acquisition was made. This depth map was subsequently corrected for errors due to residual tilt and curvature of the cementum surface using shading correction based on sampling of three areas of the background. Following this correction, thresholding was used to segment the crater extent from the background, and to subsequently obtain a measure of both the cementum surface height (x) (*i.e.* surface level) and the average depth of the crater (y). The depth of the crater relative to the cementum surface was then calculated as the difference in these values (y-x)

with the crater volume then being obtained by multiplication of the average of this difference with area of the crater (a). A flight simulation program allowed us to study the morphology and topography of the crater in all directions.

KEY WORDS: root resorption, scanning electron microscope, shading correction, volume measurement.

INTRODUCTION

Root resorption is an idiopathic phenomenon associated with orthodontics that results in the active removal of mineralized cementum and dentine. It is often unpredictable and if destruction has extended into dentine, irreversible (Brudvik and Rygh, 1994). The true aetiology of root resorption is multi-factorial and has not been isolated. Although iatrogenic root resorption may seem to compromise the benefits of successful orthodontic outcome, most root loss due to orthodontic treatment does not decrease the longevity or the functional capacity of the teeth involved (Brezniak and Wasserstein, 1993). Under normal circumstances, root resorption caused during orthodontic treatment is reversible and repair occurs once the force applied or orthodontic treatment ceases. However, apical root resorptions have been reported to be detrimental to total root length and could show up as decreased crown-root ratio in a radiograph (Fig. 1). This biologic phenomenon can sometimes result in the total loss of the tooth or several teeth.

The literature has associated root resorption in human teeth to the magnitude and duration of the force (DeShields, 1969; Kennedy and Joondeph, 1983; Reitan, 1964; Harry and Sims, 1982; Reitan, 1974), type and mechanics of force delivery (DeShields, 1969; Reitan, 1964; Goldson and Henrikson, 1975), bone density (Reitan, 1964; Wainwright, 1973), systemic factors (*e.g.* endocrine disorders, asthmatics) (Becks, 1939; McNab *et al.*, 1999), vitality of the pulp (Spurrier and Hall, 1990), history of previous trauma (Ohm Linge, 1983) and gender (DeShields, 1969).

Untreated permanent teeth have also demonstrated sites of resorption, particularly on surfaces facing the direction of physiologic movement (Henry and Weinmann, 1951). The most commonly affected teeth (in decreasing frequency) are: maxillary laterals, maxillary canines, mandibular incisors, mandibular first molars, mandibular second premolars, maxillary second premolars (Brezniak and Wasserstein, 1993).

A previous study was carried out with a sample of thirty-six human premolars to compare the effects of light and heavy orthodontic forces on root cementum (Chan *et al.*, 2002a). The aim of this study was to develop a protocol to allow volume measurements of root resorption craters (Fig. 2) on this sample of specimens and to reproduce digital 3-dimensional (3-D) replica of root resorption craters, so that this phenomenon could be studied in greater detail.

MATERIALS AND METHODS

Pre-imaging sample preparation

Samples were obtained from patients requiring at least bilateral first premolar extraction as part of their orthodontic treatment (Ethical approval:- Project 5/98 CSAHS Human ethics review committee UDH). A buccally directed force was applied to the teeth with a β -titanium molybdenum alloy (TMA,Ormco, CA, USA) cantilever spring spanning from first molar to first premolar on Speed brackets (Strite Industries, ON, CA) (Srivicharnkul *et al.*, 2002).

The teeth were extracted by one operator after a test period of 28 days. They were then soaked for 10 minutes in Milli-Q (de-ionized water) ultrasonic bath to remove all

traces of soft tissue fragments. The teeth were then carefully cleaned with a gauze swab to remove fragments of periodontal ligament (PDL). They were then disinfected in 70% alcohol for 30 minutes and each sample was stored separately in Milli-Q at 23 ± 1 °C until time of experimentation (Malek *et al.*, 2001). All samples were mounted on a stainless steel diamond coated high-speed long shank chamfer bur secured with Glass ionomer cement (Transbond, 3M, CA, USA) (Fig. 3). All samples were bench dried for at least 48 hours prior to carbon coating.

The samples were carbon coated using Edwards Coating System E306A (Fig. 4A). A sputter technique (Echlin, 1975) was employed to coat the samples to a thickness of between 250Å to 300Å. A gold-coated button was used to ensure that a specified even thickness of coating was achieved. A reddish-blue tinge suggests the thickness prescribed (Fig. 4B).

The Philips XL30 SEM was used (Fig. 4C). A 360° motorized rotating jig was created (Fig. 4D) to enable all aspects of the samples to be studied without constant venting and pumping of the SEM chamber. This jig also allowed total 3-D control of the teeth and enabled image capture at approximate zero degrees to the horizontal plane. In this way, the mesio-buccal, mesio-lingual, disto-buccal as well as the disto-lingual surfaces could be easily studied.

The side cap of the Philips XL30 was modified to allow the lead from the stent out of the chamber (Fig. 4E). This stent has a motor and was connected to an alternating current (AC) adaptor (Fig. 4F). When the current is positive (+ve) the motor rotated clockwise and when negative (-ve), anti-clockwise. The voltage of the current

determined the speed of the rotation. All images were focused at the eucentric point with the SEM operated at 15kV, which corresponds to a diameter of approximately 200nm and saved as TIFF images (tagged image file format).

Stereo imaging principle

To produce a stereo pair, 2 SEM images of the specimen were collected. The difference in perspective was introduced by tilting the specimen. One image was collected with the specimen tilted to a positive angle and one to a negative angle; *e.g.* $\pm 3^\circ$ (Fig. 5A).

The parallax (P) of an object measured from a stereo pair is directly related to the height (h) of the object where $h = P/[2\sin(\varnothing/2)]$ (Fig. 5B), where $\varnothing$ is the degree of tilt of the specimen. The amount of tilt required will depend on the roughness of the surface. The smoother the surface the greater the tilt required, and the greater the tilt the greater the 3-D effect. The maximum practical tilt is typically $10 - 15^\circ$. For the optimal stereo imaging effect in this study, $\pm 3^\circ$ tilt was used.

Crater Visualization

The root resorption craters were initially visualized using 3-D red-green stereo anaglyph coding to obtain a quick and simple qualitative assessment. This visualization was produced using the SIStereo imaging module of the *AnalySIS Pro 3.1* software. Images were imported into the software as a matched pair (*i.e.* $+3^\circ$ Tilt and -3° Tilt) for each crater under examination (Fig. 6). One image was rendered in a

red intensity scale whilst the other image in the pair was rendered in a green intensity scale. A single color composite image is then formed that superimposes the data from both images. This so called anaglyph image was then viewed with red-green glasses so that each eye received data from only one of the images in the pair. This results in visual depth cueing and subsequent generation of a 3-D visualization effect (Fig. 7).

Crater Analysis

Quantitative measurement of the volume of the root resorption craters was undertaken using a MACRO (macroinstruction, *i.e.* application specific programming language) program specifically developed for this project by Soft Imaging Systems (Münster, Germany). This program uses standard commands from the SIStereo module. Using the stereo image pair and the known angle of tilt the magnitude of the parallax was used to generate a depth image for each crater from which a volume estimate could be made as detailed below.

Alignment and Depth Coding

Using the image pair imported for anaglyph generation, a point of reference was selected in one of the images that would also be clearly visible in the second image. This point was usually a high contrast particle at the edge of the crater. The software uses this reference point to define a small region and then uses correlation to accurately align the images (Castleman, 1996). Once aligned the parallax difference allows generation of a new 8-bit grayscale image in which depth differences are encoded as different gray values (Fig. 8).

Curvature Correction

Although great care was taken to ensure that samples were mounted horizontally and that images were collected from directly above each crater by axial rotation using the motorized stent, additional spatial corrections were required to ensure accuracy of the measurement data. For the larger craters in particular, some residual tilt was inherent in the images of the craters due to the fact that the tooth surface was not totally flat, but in fact had a curvature, which varied over both the horizontal and vertical dimensions (Fig. 15). This curvature of the cementum if uncorrected in the depth image would have resulted in inaccuracies in subsequent volume estimates.

To overcome this phenomenon, a technique known as shading correction was applied. This technique is typically used in microscopy to correct uneven illumination due to the curvature of the microscope field that optical lens systems sometimes induce (Russ, 1990). In this instance the same method could be used to correct for the curvature of the surface of the sample rather than for the image itself. On the grayscale depth image, three widely spaced “hot-spots” 20 pixels in diameter were selected from the tooth surface (Fig. 16A). The average pixel values in these three selected areas was then used to define a correction plane for the entire image that removed the majority of the residual tilt bias due to curvature while maintaining the difference in the gradient of the pixels which encoded the crater depth information (Fig. 16B). In this way, the tilt of the image was corrected and the depth profile could be generated (Fig. 17).

At this point a 3-D mapping of the resorption crater could be generated that allowed interactive viewing from all directions. This allowed the morphology and topology of the craters to be examined in detail in a natural context without having to use red-green anaglyph glasses. As a further enhancement of this visualization process the crater could also be viewed in a continuous motion format using the “flight simulation” module of the software (Fig. 18).

Volume Estimation

After pre-processing and curvature correction, the grayscale depth image could be used to obtain accurate quantitative information. The extent of the crater was determined by segmenting the corrected depth image using thresholding. Border particles were removed and holes filled using binary processing to ensure that the entire crater was included but that no extraneous material was included. An initial threshold level was set so that surface height (x) of the tooth cementum could be defined (Fig. 19). A subsequent inverted threshold was then applied to obtain the depth of the crater (y), which was defined as the average of the depth encoded gray values within the extent of the crater. The volume of each crater was determined by obtaining the difference between the background height and the crater depth, and multiplying this difference by the planar area of the crater (Table I).

In some instances, as in the example shown in the above figures, the topology of a single crater resulted in a main crater area being defined by the thresholding process with smaller associated crater areas, which were considered to be part of the single

crater. In order not to under estimate craters in these instances volumes of all of the contributing craters were summed to give a total volume as shown in Table I.

Calibration of this commercial software for volumetric measurements with known pyramidal indentations on different metallic rods was highly reliable. It was demonstrated that there was 96.4% and 98.2% accuracy for the brass and stainless steel rods respectively (Chan *et al.*, 2002b).

DISCUSSION

Volumetric analysis of solid objects has long been discussed in the literature. Especially in recent times, magnetic resonance imaging (MRI) and axial computer tomography (CT) scans have allowed accurate volumetric analysis of solids within biologic samples. (Convit *et al.*, 2001; Kopelman *et al.*, 2001). However, volumetric analysis of irregularly shaped open craters on biologic samples is still very technique sensitive and inaccurate. Volume displacement methods and using light bodied dental impression materials to take impressions of craters and measuring those impressions has been suggested. However with small samples, these methods would be difficult and highly inaccurate.

The topography of tooth root surfaces has been examined using: radiographs (Ketcham, 1927), light microscope (Reitan, 1974) and scanning electron microscope (SEM) (Jones and Boyde, 1972; Kvam, 1972a, 1972b). It has been reported that enhanced visual and perspective assessment of root surfaces, particularly when

recorded in stereo pairs, has resolution and detail not attainable with histological models reconstructed from serial sections (Reitan, 1974).

Previous root resorption studies done with SEM focused on: anatomic description of the craters (Rygh, 1977; Harry and Sims, 1982; Barber *et al.*, 1981; Kvam, 1972a), observations on presence or absence of resorption craters (Malueg *et al.*, 1996), resorption and repair patterns of the craters (Sismanidou and Lindskog, 1995; Hellsing and Hammarström, 1996) and surface area of craters studied and measured with composite micrographs (Acar *et al.*, 1999). No previous studies have made attempts at quantifying the extent of root resorption by volumetric measurement of the resorption craters.

The shading technique applied in this instance to correct for residual tilt and surface curvature proved to be simple and effective without requiring undue complexity of processing. By using the average of the pixels in three selected background areas a simple plan was defined which allowed the gray-scale depth values to be corrected for gross errors, particularly along the axial length of the tooth surface where curvature was higher. A further improvement in measurement accuracy could be obtained by use of a non-linear correction surface rather than a simple plan, however this would require substantially more complex image processing to define the exact curvature at any given background point. Given the relatively minor gain in accuracy that such a method would achieve the added complexity does not in our opinion seem warranted for our particular studies. It would however be worthy of consideration for studies where the surface topography was more complex.

In the past, 3-D reconstruction of an irregularly shaped open crater would involve embedding the specimen in resin. Careful thin sections were then made and subsequently imaged with a transmission electron microscope (TEM). The images could then be pieced together with software to study the sample in three dimensions. This process is tedious and technique sensitive. There exist innate problems, such as some samples not being suitable to embed in resins. In addition, physical sectioning also has problems associated with loss of material due to the thickness of the cutting kerf, which is usually of the order of 300µm. Furthermore, once embedded and sectioned, the sample would not be intact for any further experimentation.

In this investigation, after volumetric analysis was performed on these samples, they were then embedded and sectioned. A further study with these samples looked at the mineral content of the cementum with an electron probe. These results were then correlated. This would not be possible with the old technique.

With embedding, sectioning and then imaging with TEM, volumetric measurements of these craters are still only approximates. Working with such small samples, volume displacements are highly inaccurate and should not be attempted.

CONCLUSION

The technique presented in this paper has greatly facilitated ongoing studies of root resorption in relation to orthodontic procedures. It has overcome the limitations associated with physical sectioning and has the advantage of being rapid and accurate (Chan *et al.*, 2002). In addition, the combination of stereo-imaging and modern digital

analysis has resulted in a method that has a highly visual context that allows researches to have visual checking against quantitative data. The initial stereo pair images could be viewed as red-green anaglyphs that demonstrated the 3-D nature of the craters. The craters could then be measured for quantitative comparison, and finally the 3-D nature of this data allowed a dynamic visualization to be generated from which the morphology of the craters to be examined in detail.

This type of imaging has a great future in modern orthodontic research and certainly elevates the problems of sectioning which to date, have been the main impediment to more extensive study of root resorption processes.

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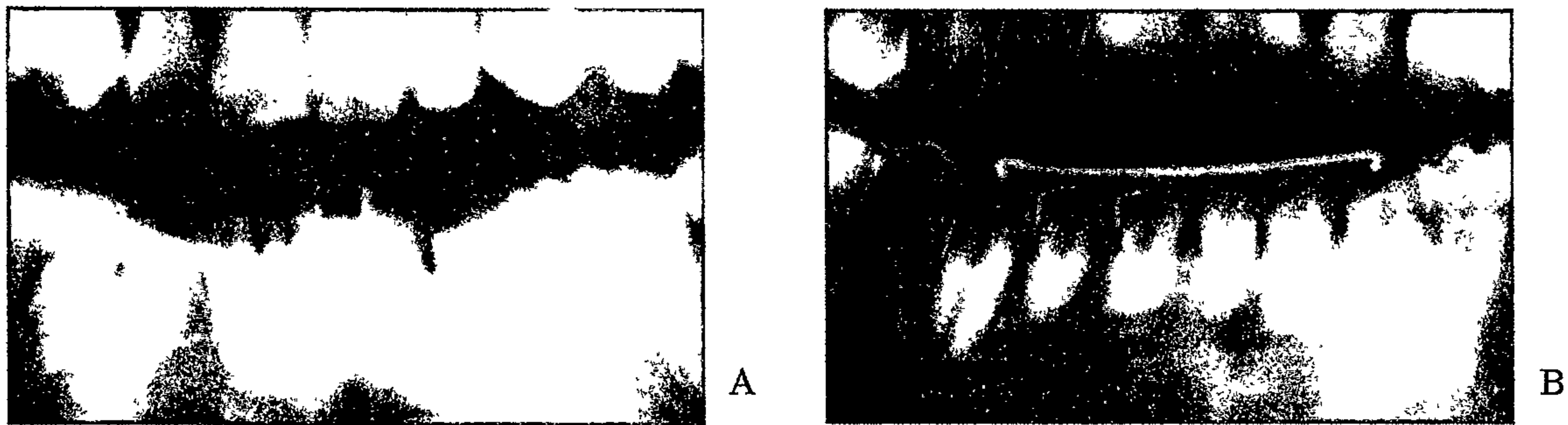


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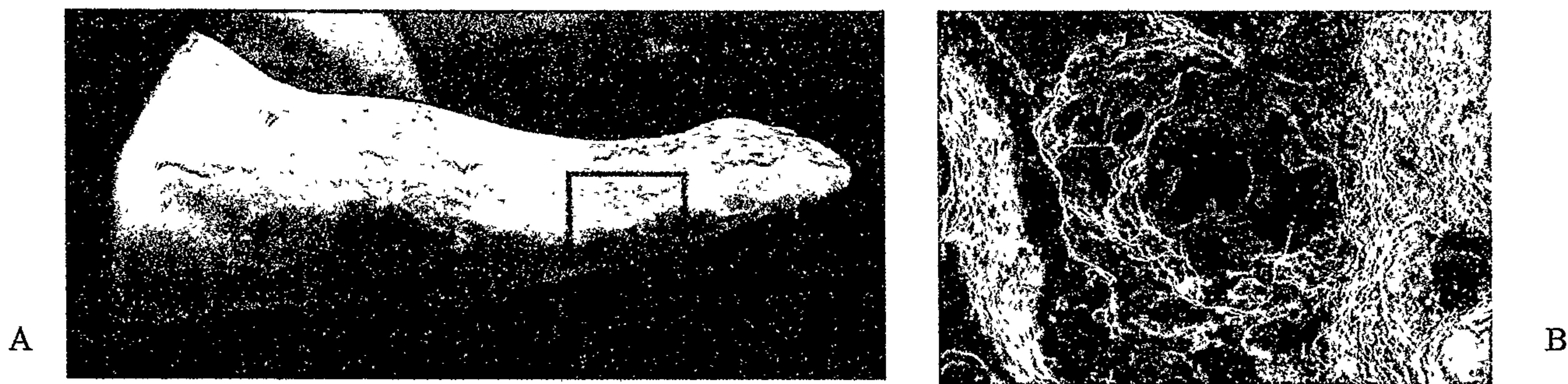


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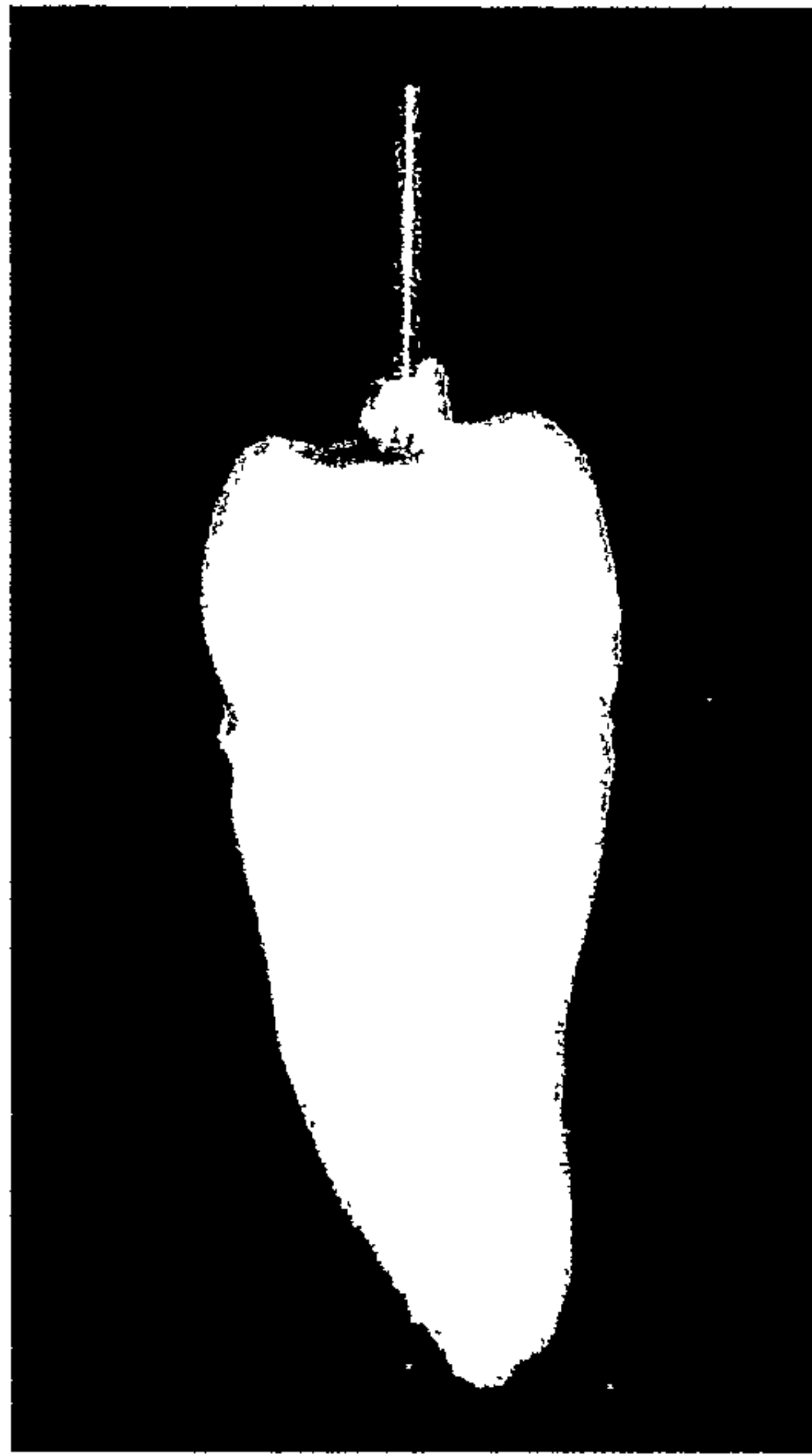


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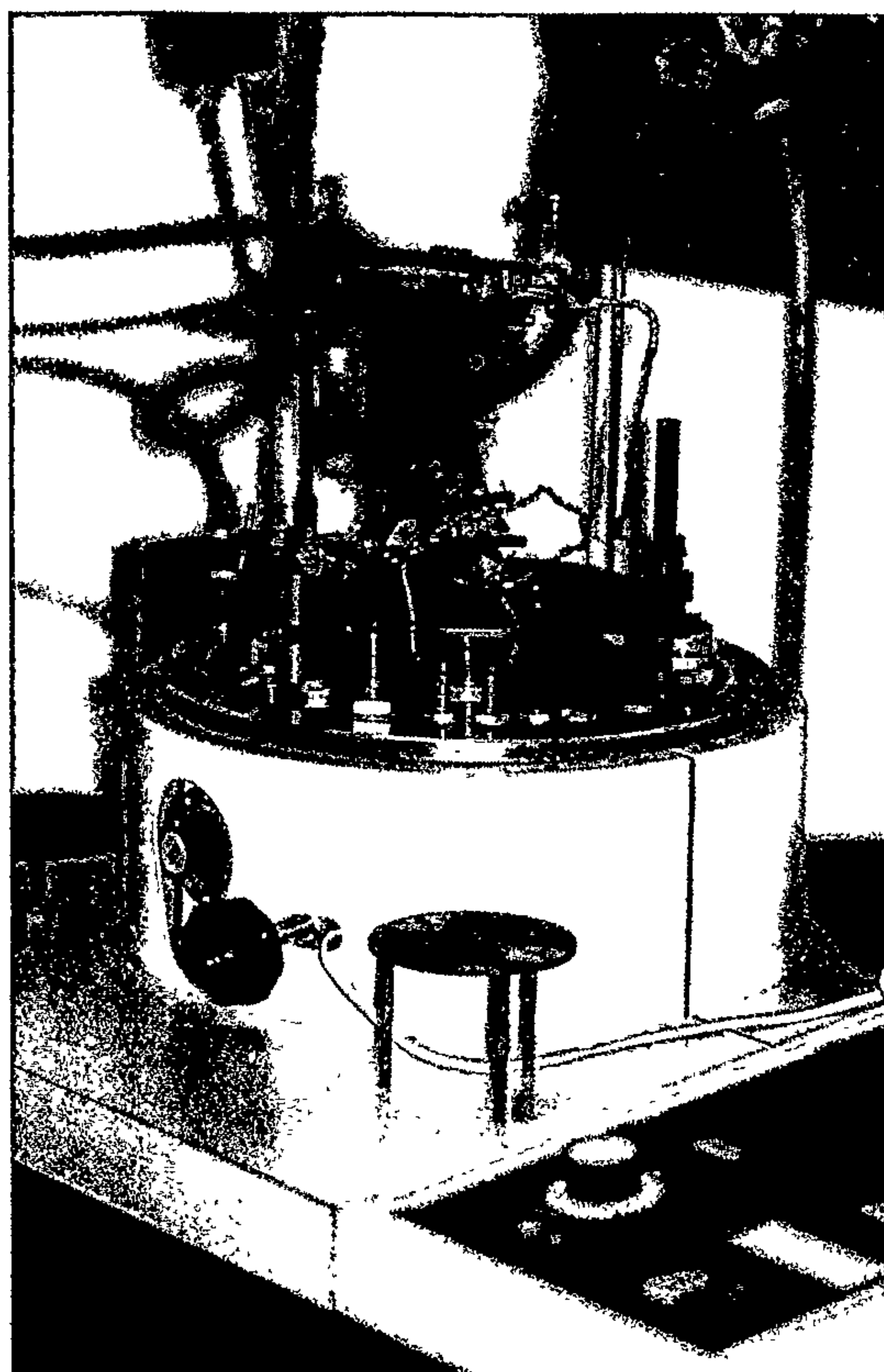


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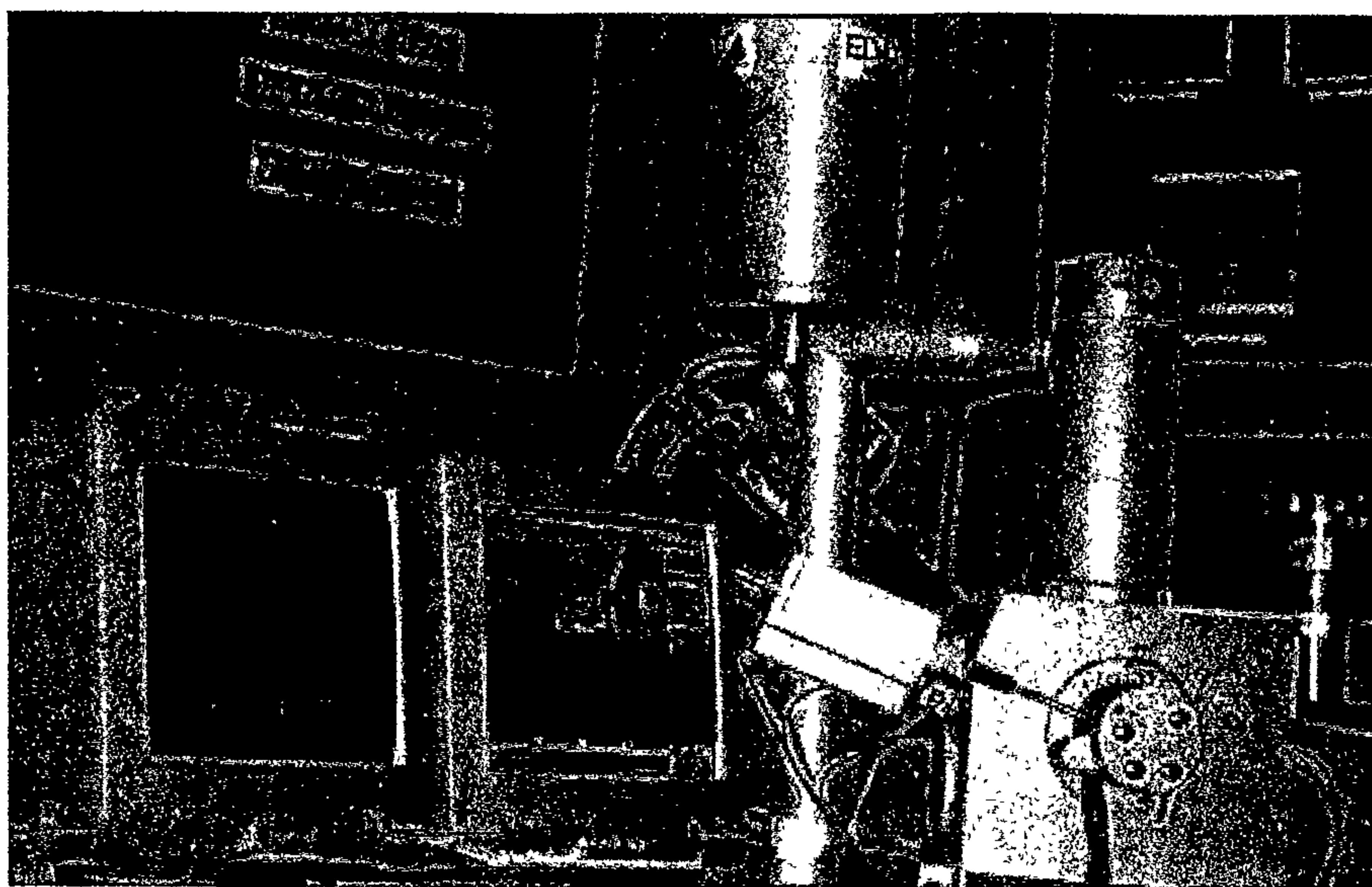


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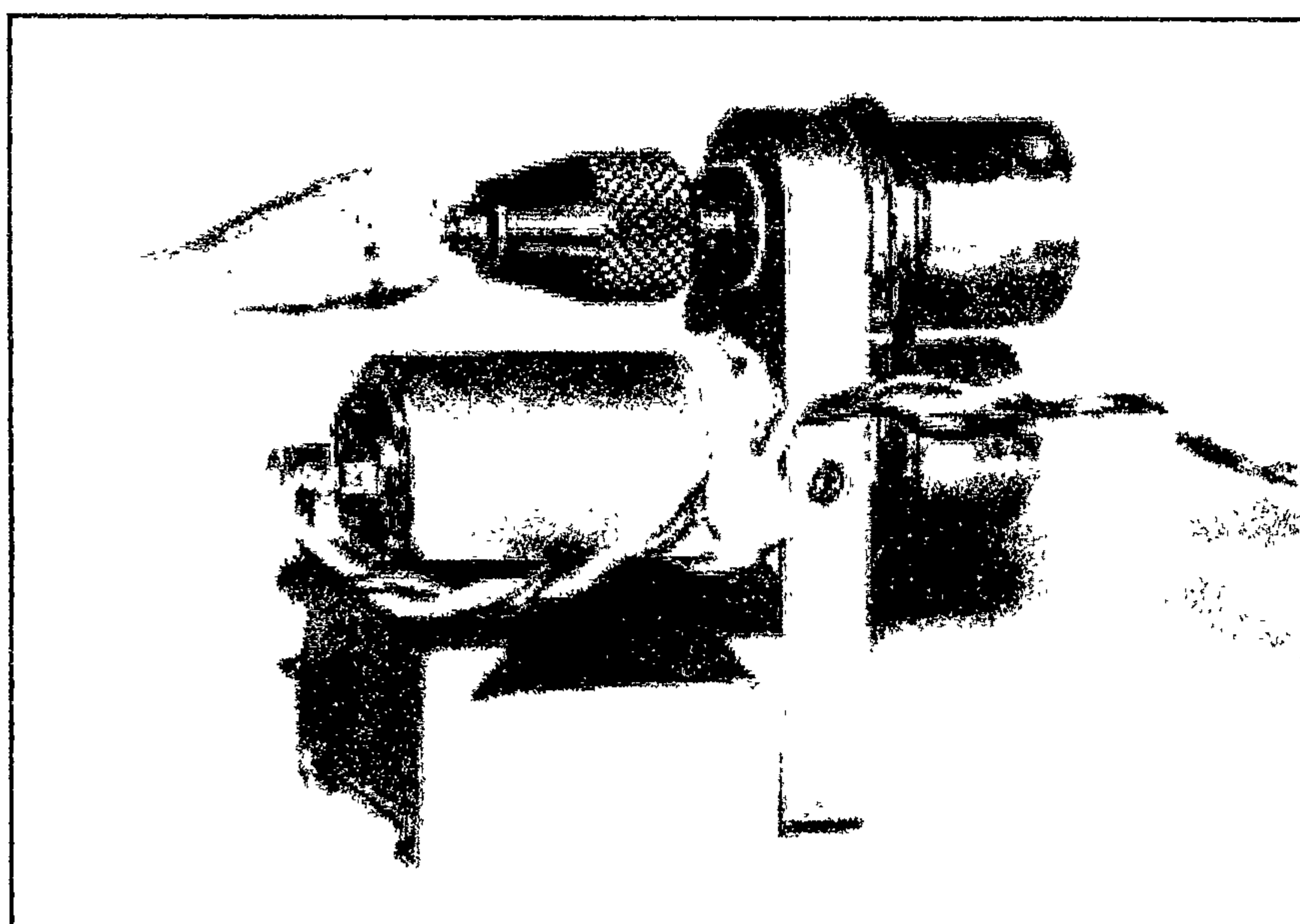


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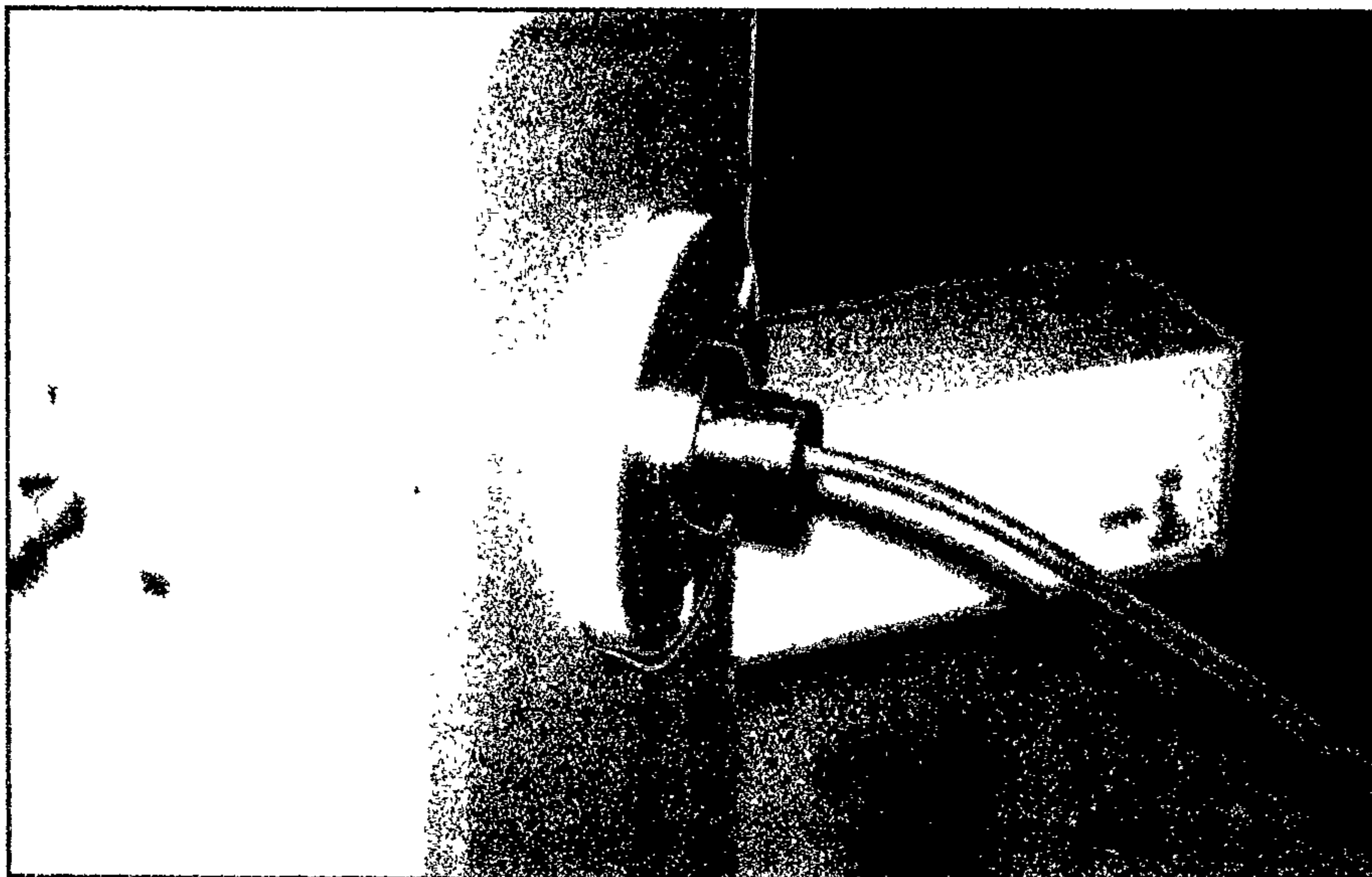


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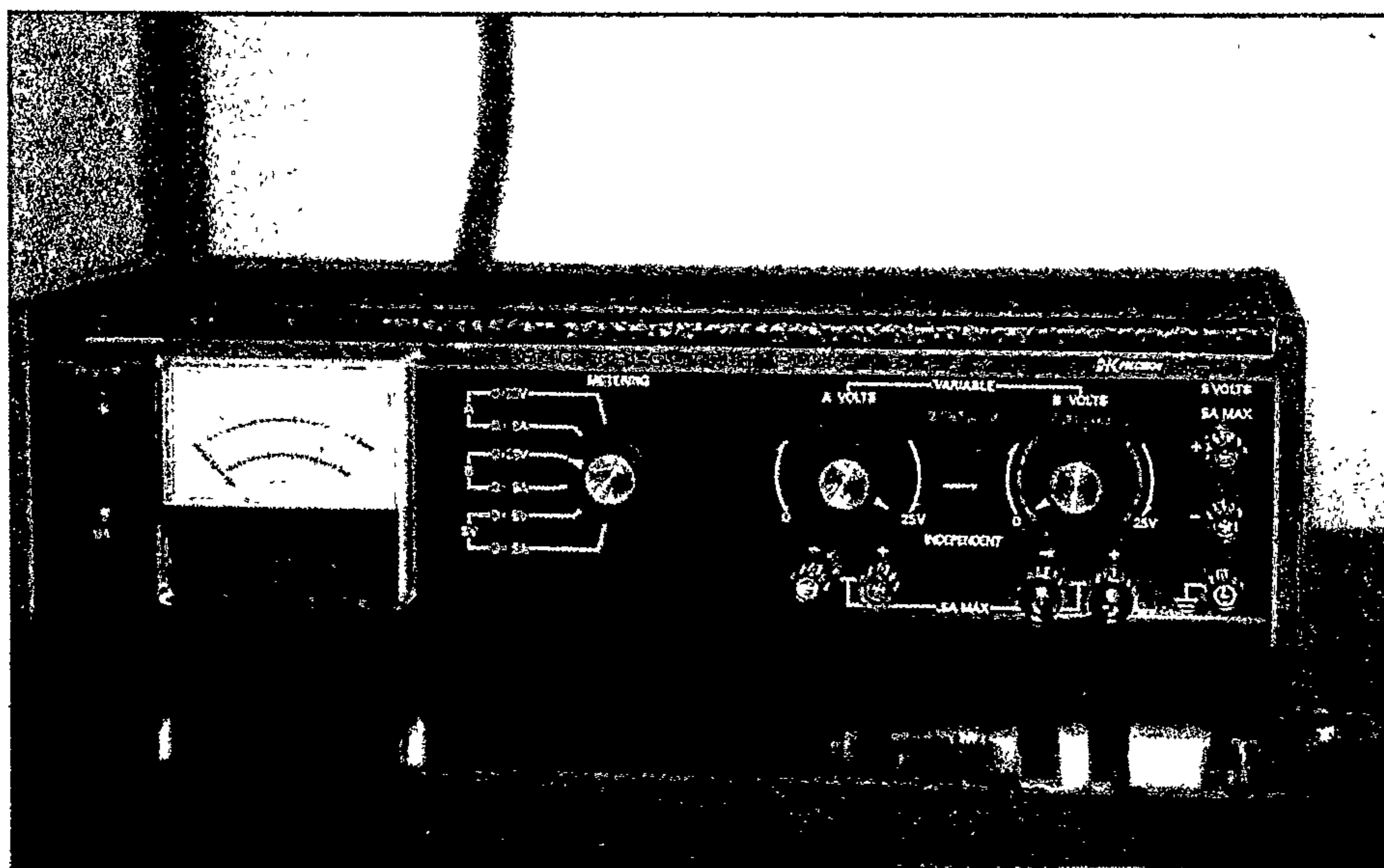


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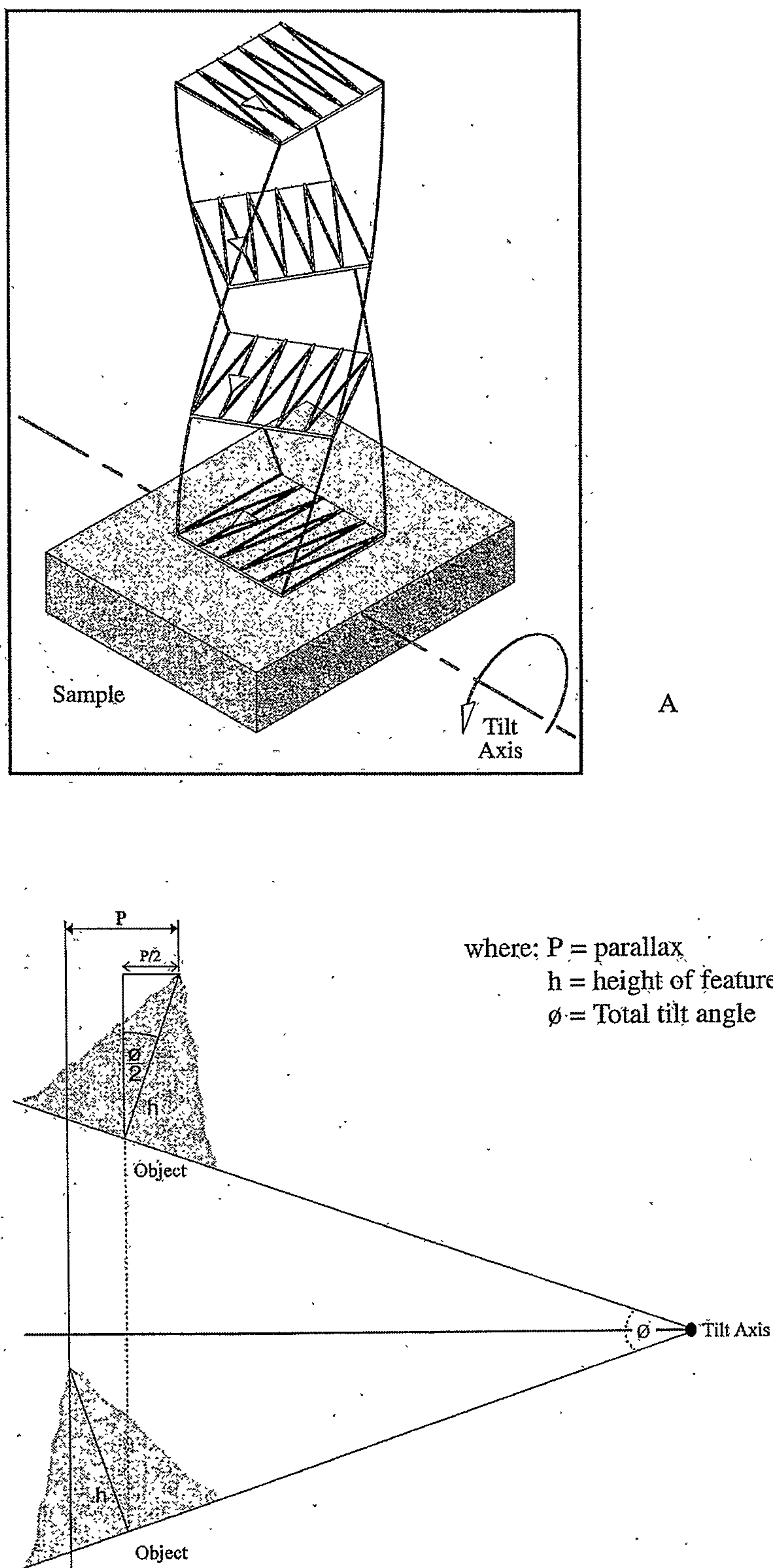


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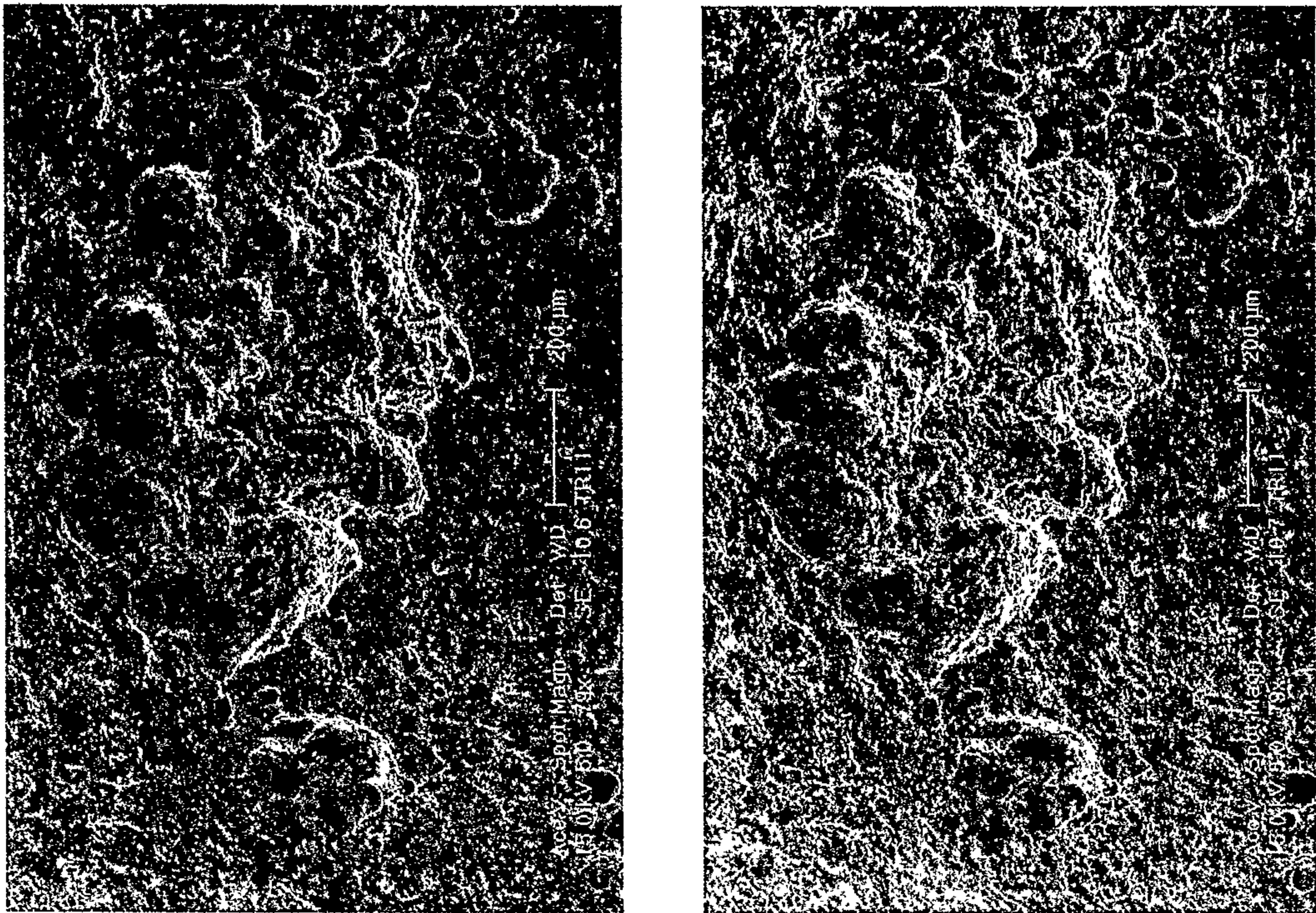


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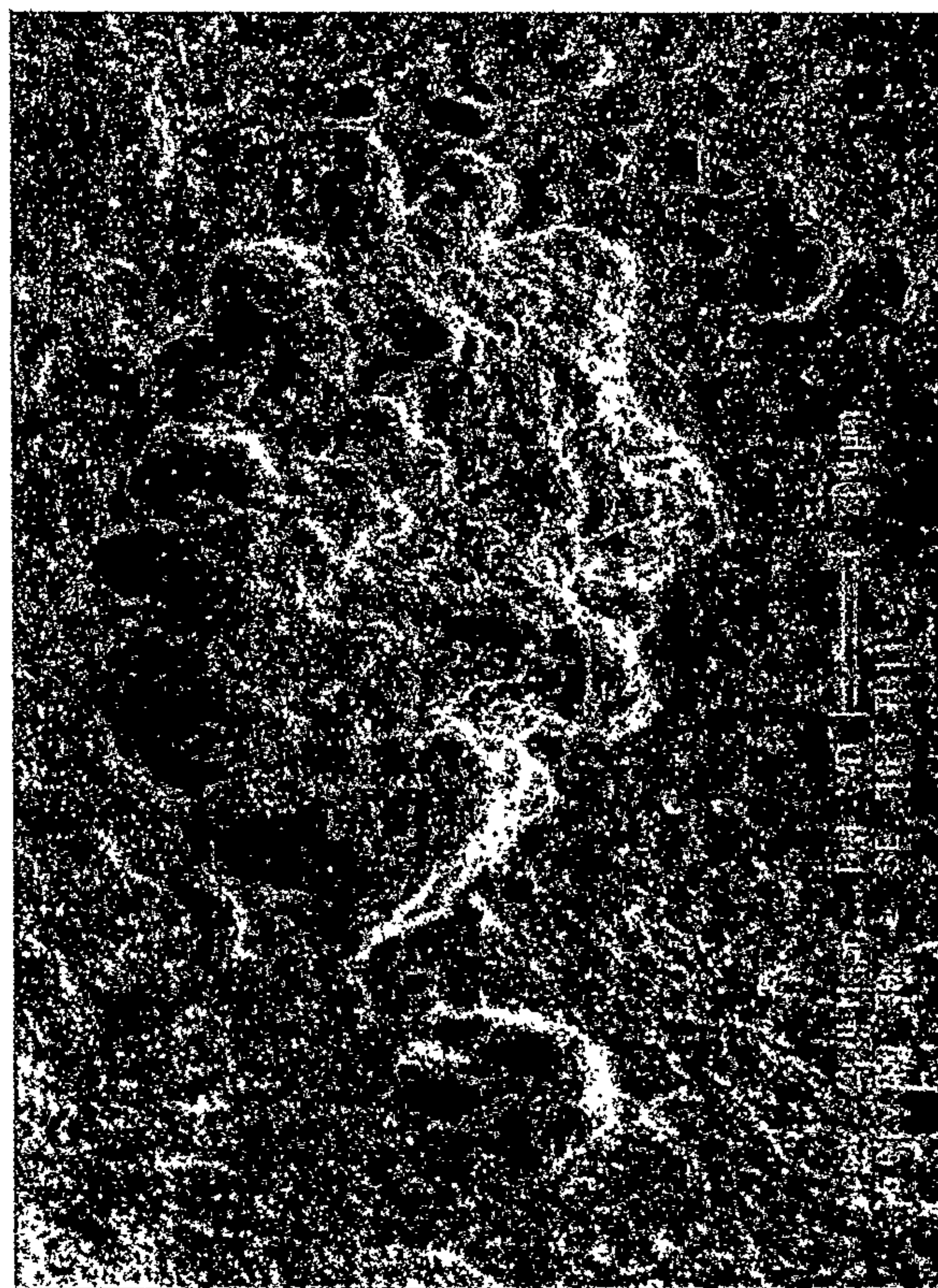


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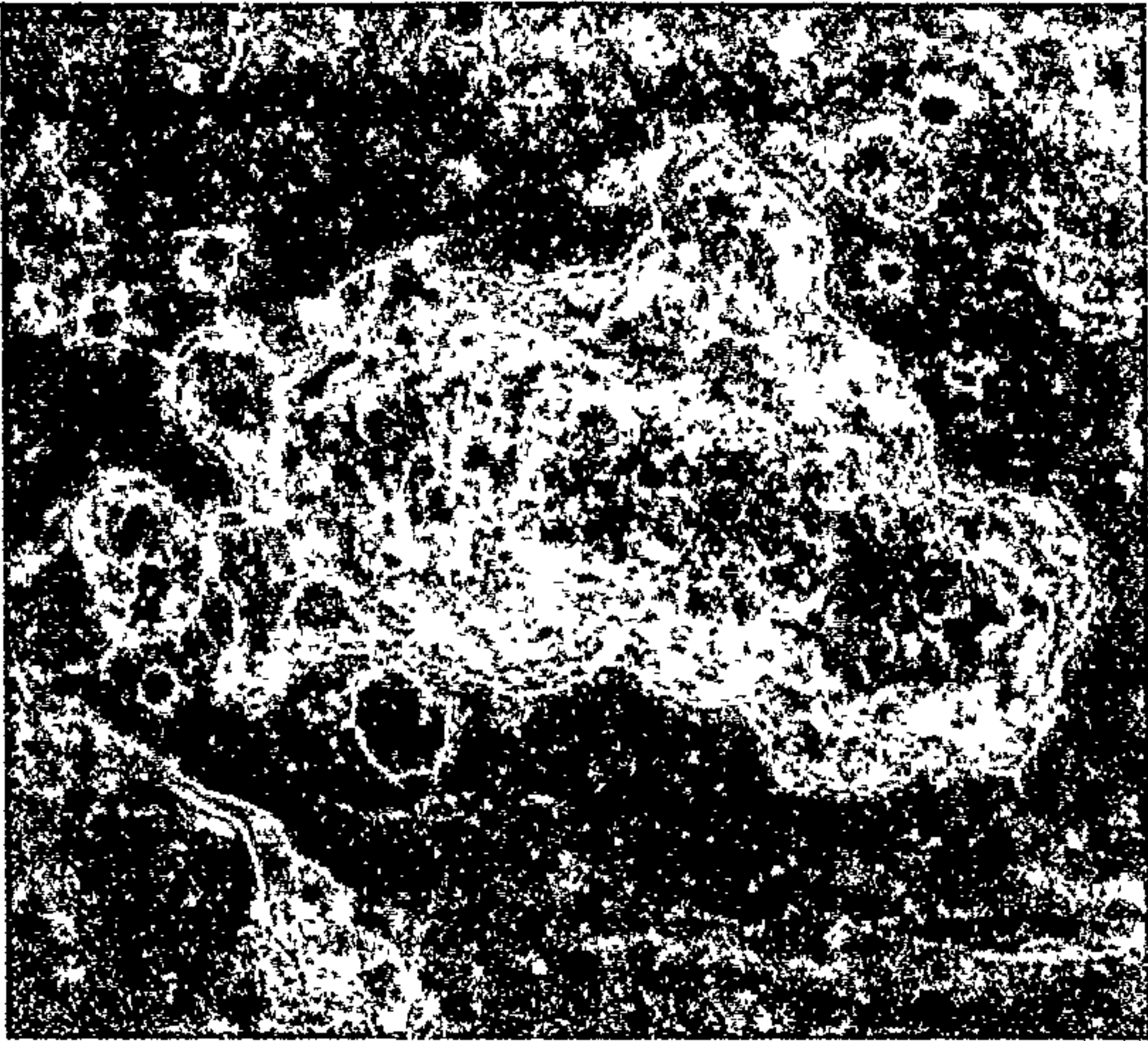
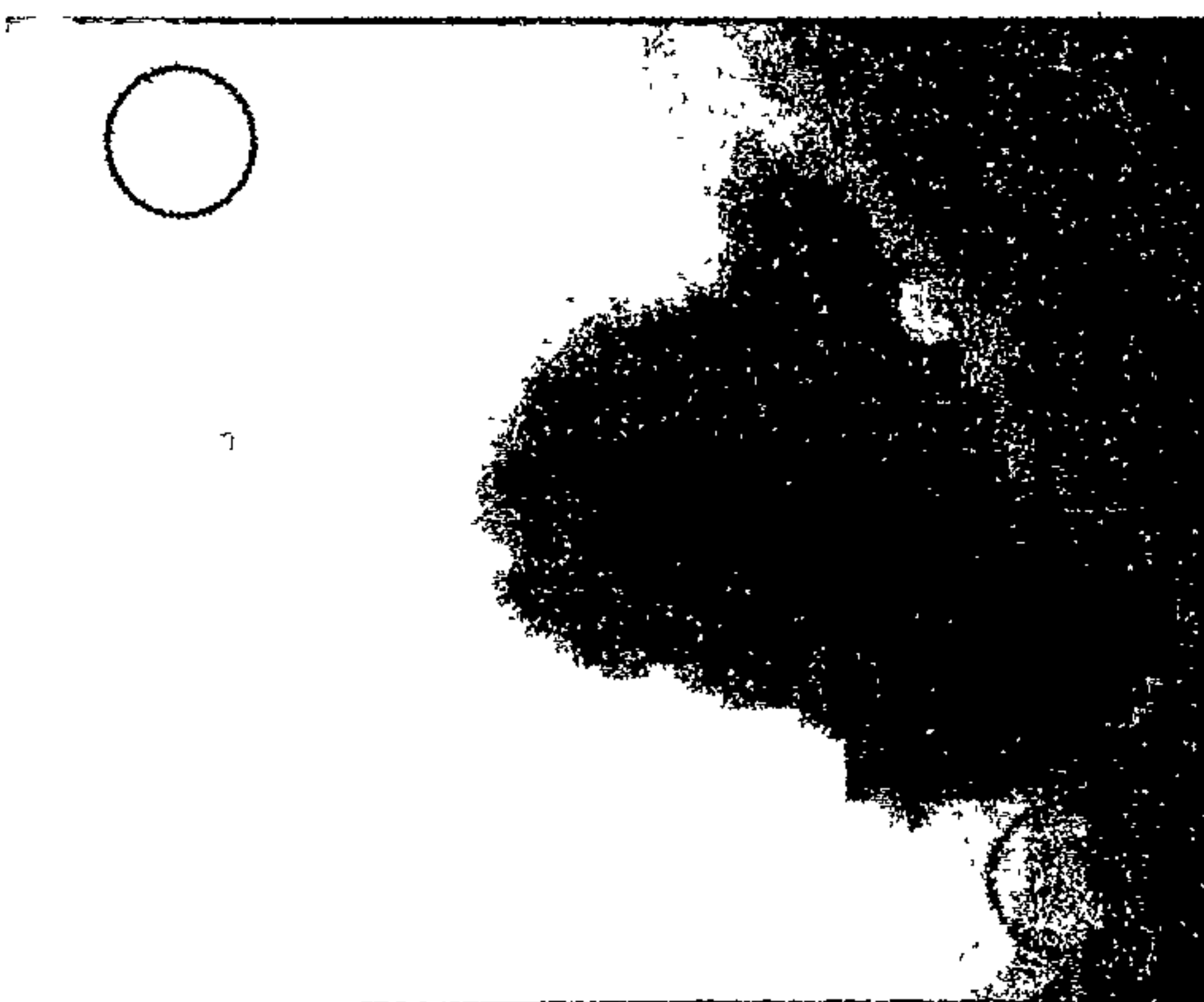
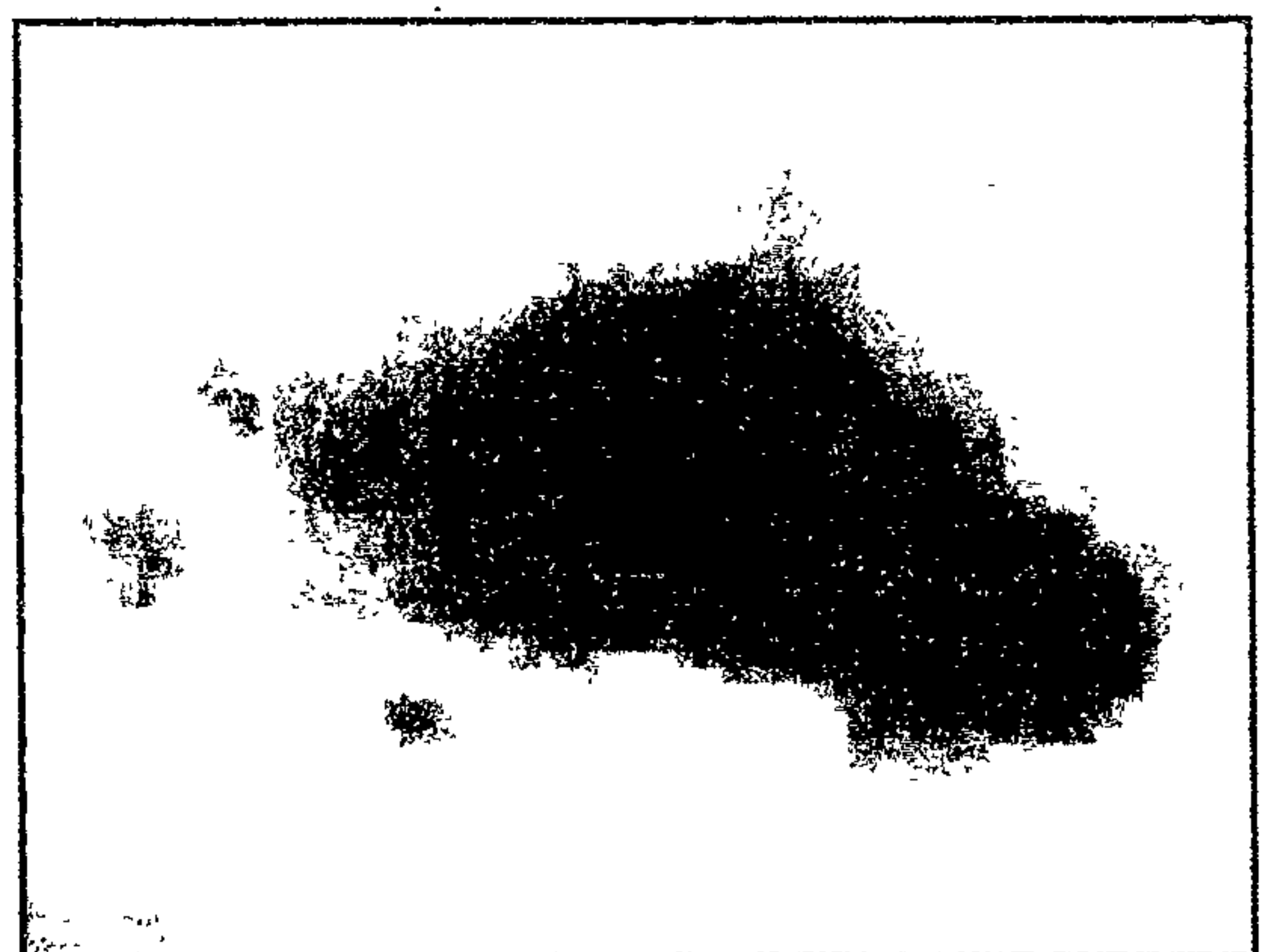


Figure 8. Image converted to an 8-bit grayscale image for further processing.



A



B

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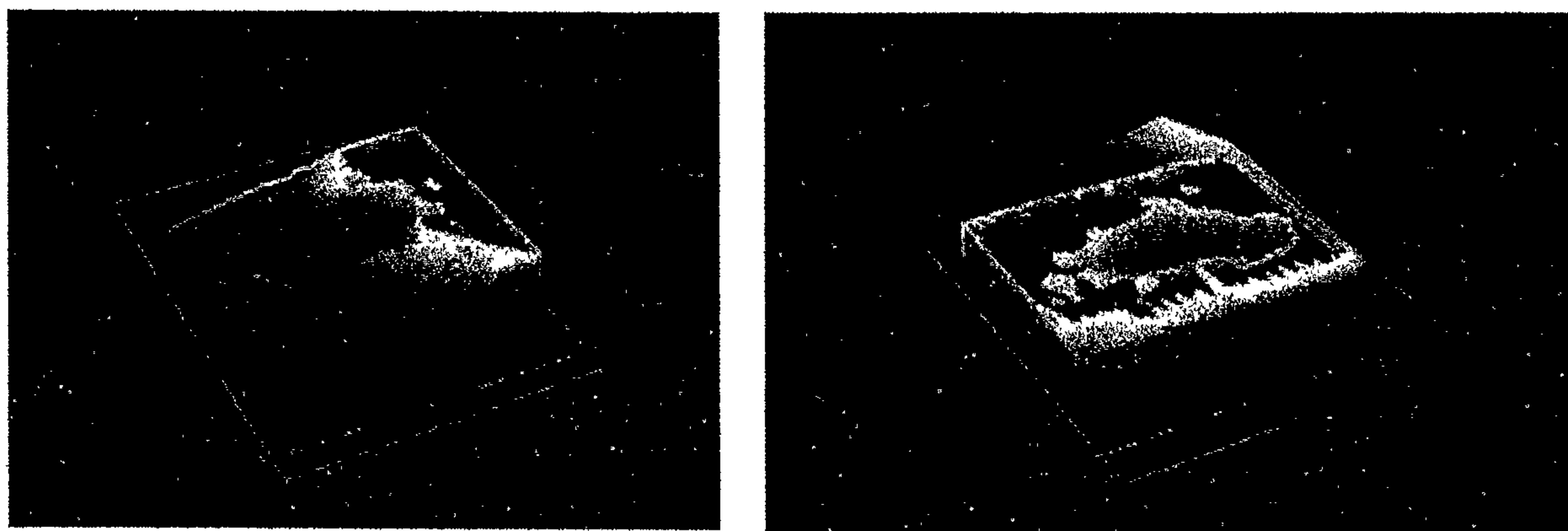


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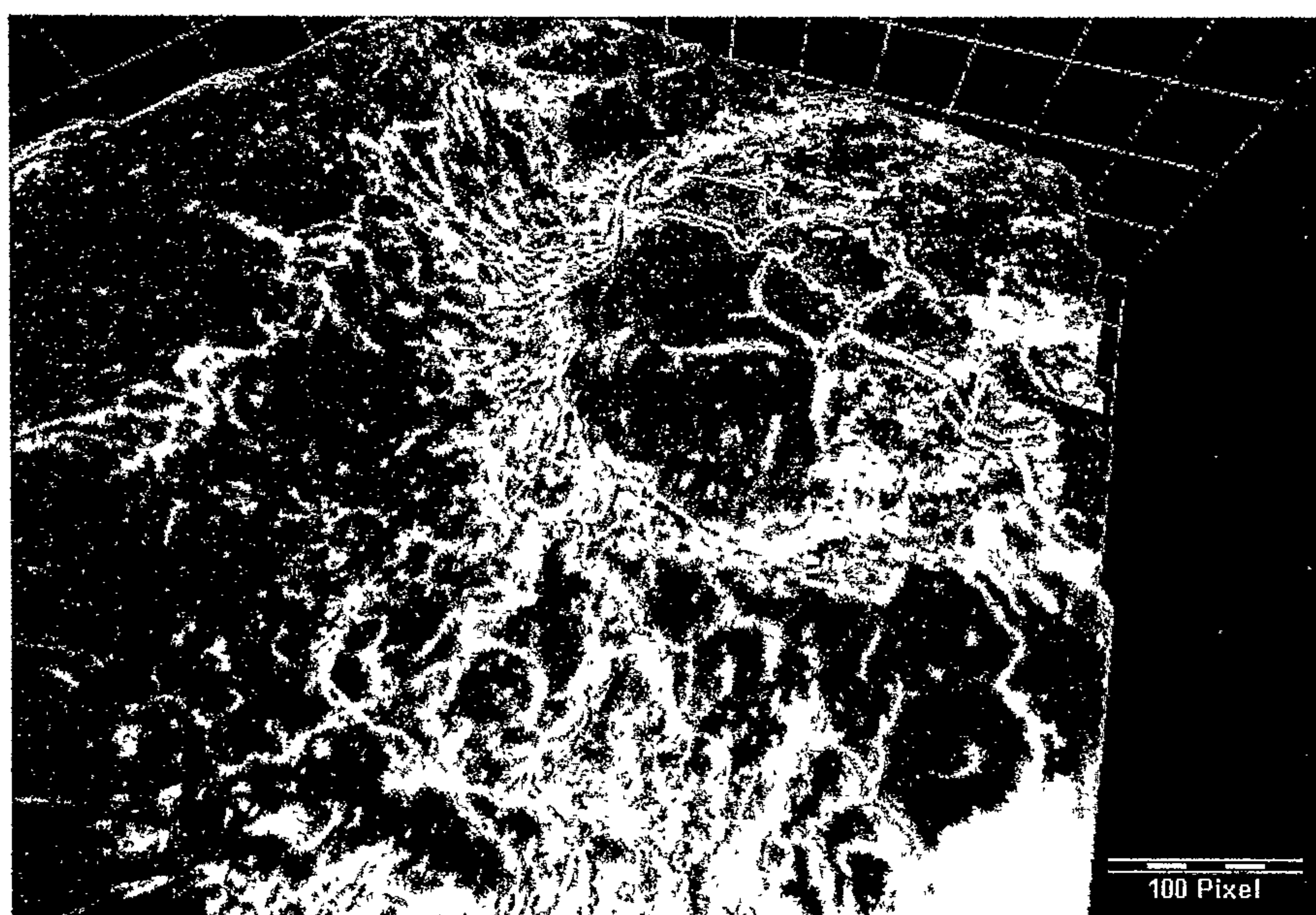


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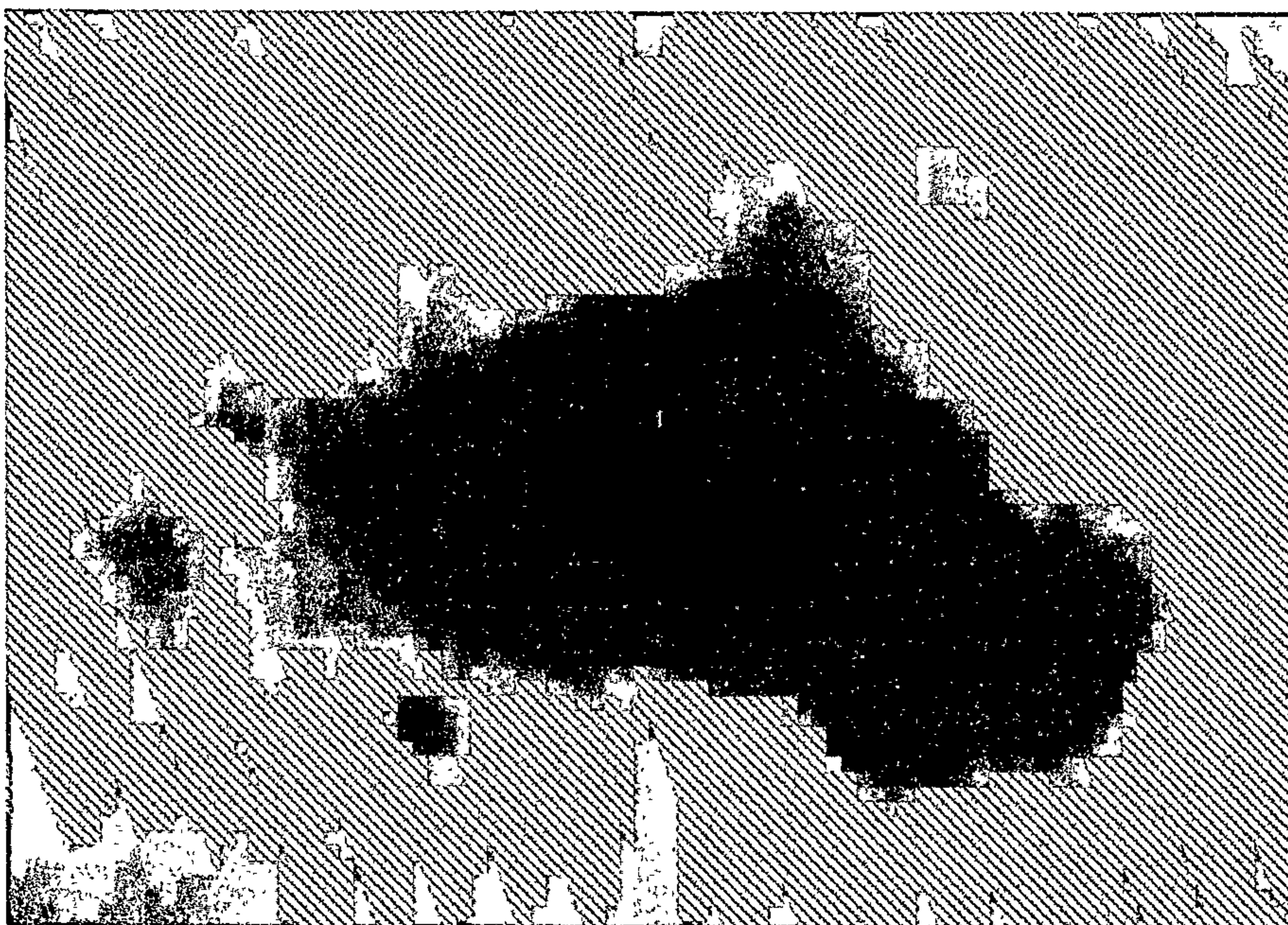


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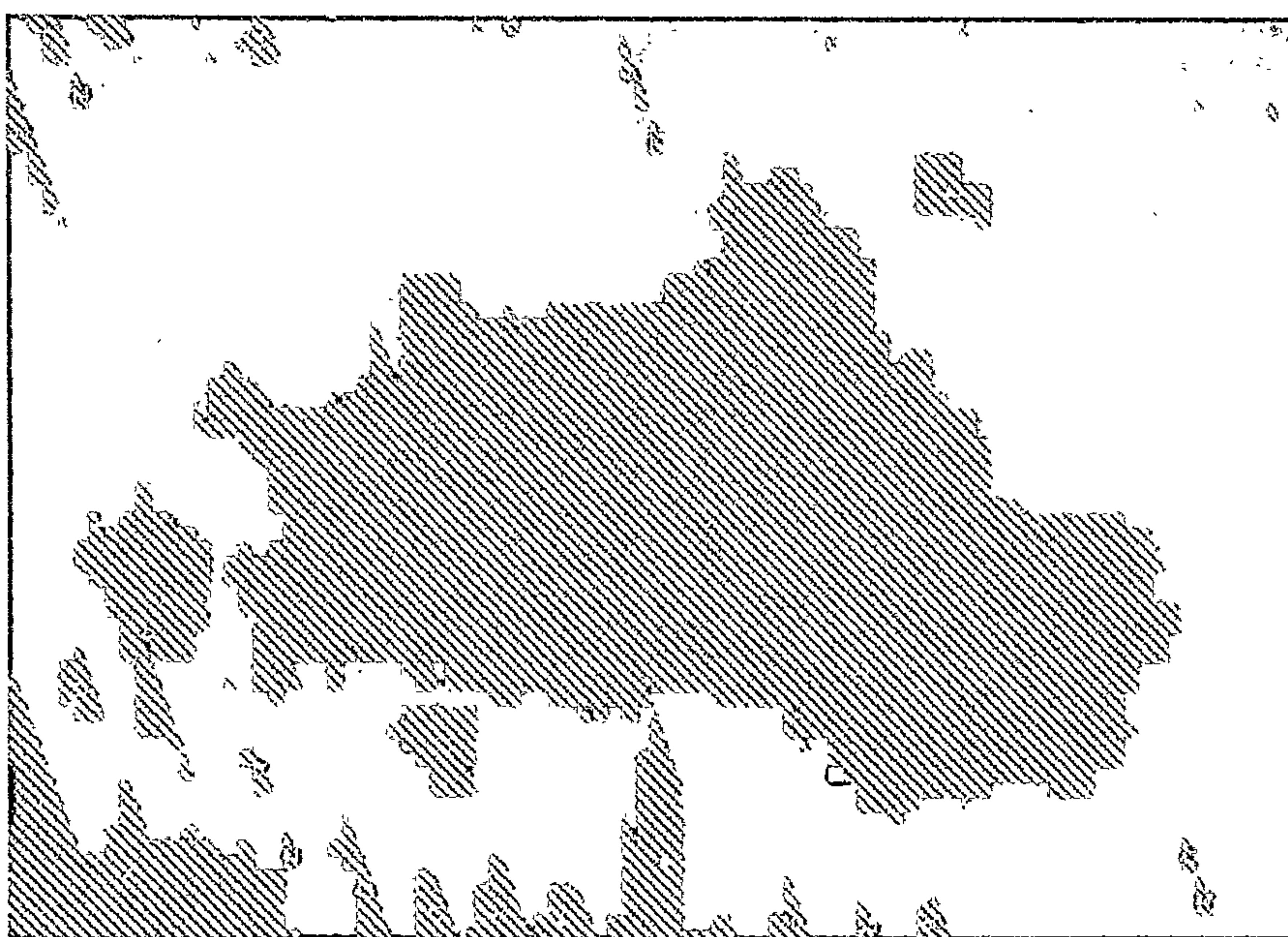


Figure 12. (B) Inverted thresholding.

TABLES

Table I. Table showing method of obtaining volume of 4 craters of interest.

Crater no.	Height <i>y</i> (μm)	Height <i>x</i> (μm)	True height (Height <i>y</i> – basic height <i>x</i>)	Area (μm ²)	Volume (x 10 ⁶ μm ³)
22	19.13	66.36	19.13- 66.36 = -47.23	144577.94	6.83
23	48.91	66.36	48.91- 66.36 = -17.45	1552.74	0.027
29	46.19	66.36	46.19- 66.36 = -20.17	7228.44	0.15
35	40.14	66.36	40.14- 66.36 = -26.22	2343.72	0.061
Total volume					7.07



MANUSCRIPT III

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Calibration of software used for volumetric measurements in the Scanning Electron Microscope.

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Summary

Volumetric measurements of open craters have been a difficulty in the past. With the advent of new technology and computing software, this difficulty has been overcome. The commercial software package *AnalySIS Pro 3.1** (Soft Image System, Munich, Germany) has previously been used to perform volumetric measurements of root resorption craters on human root cementum. The aim of this study was to calibrate this software with known pyramidal indentations for accurate volume measurements. Four solid metallic cylindrical rods (brass, copper, stainless steel and aluminum) matched to the dimensions of human premolars were used. Eight standardized pyramidal indentations were made on each rod using the Vickers microhardness tester Type M (Shimadzu, Tokyo, Japan) with a pyramidal indenter of 136° inclusion angle. To eliminate the variance component of the measurements, 4 pairs of stereo images ($\pm 3^\circ$) for each indent were taken with a scanning electron microscope (SEM) (Philips XL 30) focused at the eucentric point, operated at 15kV, corresponding to a diameter of approximately 200nm. Each pair of images was processed twice to obtain 8 sets of readings per indentation. Images were saved in tagged image file format (TIFF) and imported as a pair into the image analysis program. The images were aligned and a grayscale image generated. A shading correction was performed to eliminate the errors of volume measurement due to the mild curvature of the rod surface. Following this correction, thresholding was used to segment the indentation extent from the background, and to subsequently obtain a measure of both the rod surface height (x) (*i.e.* surface level) and the average depth of the indentation (y). The depth of the indentation relative to the rod surface was then calculated as the difference in these values (y-x) with the indentation volume then

being obtained by multiplying this difference by the area of the indentation (a). This gave the estimated volume of the indentation. Using the same software, the mean base length (d) of the pyramid was obtained. As the tangent of $136^\circ/2$ equates to $(d/2)/h$, where h is the height of the pyramidal indentation, the h value could be calculated. The volume of a square pyramid is $v = 1/3ah$ and the 'true' volume of the pyramidal indentations could be calculated. This calculated volume was then compared to the volume estimates performed by the software. The percentage error was found to be 3.5%, 18.9%, 1.7% and 18.2% for brass, copper, stainless steel and aluminum respectively. The software has a high level of accuracy for brass and stainless steel but overestimates the volumes for copper and aluminum. Creep of the materials during indentation plays a major role in volume measurement in this study. True calculated volumetric measurement of pyramidal indentations ($v = 1/3ah$) should not be used for soft materials with high levels of creep. It appears that the overestimated volumes obtained by the *Analysis Pro 3.1* software for copper and aluminum could be the true representation of the actual volume of the indentations made.

Key words: Vickers microhardness tester, pyramidal indentations, scanning electron microscope (SEM), grayscale, and volumetric measurements.

PACS: 06.30.Bp., 06.60.Mr., 07.78.+s.

* The authors have no commercial interest in Soft Imaging Systems (SIS).

Introduction

Three-dimensional (3-D) measurements of biological samples have always been challenging. It is easy to obtain 3-D rendering using various computer software to observe and study specimen morphology. However, the accuracy of these 3-D renderings has been questionable.

Macro- and microanalysis with 3-D volumetric measurements have been previously attempted on a myriad of specimens using different modalities.

Magnetic resonance imaging (MRI) has been used to measure cerebral venous volume (An *et al.* 2002). A 3-D high-resolution gradient echo sequence was employed to obtain DeltaB maps by two algorithms. The DeltaB maps were then used to recover the signal loss in images acquired by a two dimensional (2-D) multiecho gradient echo/spin sequence. Finally both quantitative estimates of the cerebral oxygen extraction fraction (MR_OEF) and venous blood volume (vCBV) were obtained from the DeltaB-corrected 2-D multiecho gradient echo / spin echo images. The author's ability to simultaneously obtain MR_OEF and vCBV noninvasively may have profound clinical implications for studies of cerebrovascular disease. MRIs have also been used extensively to measure brain volumes (Chard *et al.* 2002), volume and thickness of knee joint cartilages (Westhoff *et al.* 1997) and volumes of hypointense objects (Gadeberg *et al.* 2002).

Computed tomographic (CT) volumetric measurements have been performed on the aorta to assess healing after endovascular aortic aneurysm repair (Czermak *et*

al. 2001). The aneurysm sac, intra-aneurysmal vascular channel (IAVC), the thrombus and the stent graft were studied. The authors concluded that CT volumetric analysis was indeed an effective tool for evaluating the outcome of endovascular aortic aneurysm repair. In patients in whom contrast agents were contraindicated, volume measurements could also be obtained without the use of contrast. CT volumetric measurements have also been successfully used to study pharyngeal airway before and after maxillofacial surgery in obstructive sleep apnea (Metes *et al.* 1993), measure orbital volumes (Bite *et al.* 1985) and mastoid air spaces in temporal bones (Colhoun *et al.* 1988).

Disler *et al.* (1994) looked at the accuracy of volume measurements of CT and MRI phantoms by 3-D reconstruction. They placed round, cylindrical and irregularly shaped high-contrast phantoms of known volumes in a water bath for imaging. They concluded that under certain circumstances, 3-D reconstructive volume estimation could be a convenient and accurate method for volume determination.

Ultrasound techniques have been employed to study 3-D endometrial volume measurements in patients (Yaman *et al.* 2002). 3-D volume and 2-D thickness measurements were made and compared. The authors noted that 3-D ultrasound measurements of endometrial volumes demonstrated good reproducibility and were better than the 2-D measurements. 3-D endoluminal ultrasound (ELUS) has also been used to measure pseudotumours in dog's oesophageal specimens (Liu *et al.* 2000). The images were downloaded for 3-D reconstruction. Volume measurements were made and compared to spiral computed tomographic (CT) images. Results were found to be favorable.

3-D echocardiography has been used to measure ventricular volumes (Rusk *et al.* 2001). Real-time 3-D echocardiography avoids geometric assumptions in volume analysis but it has also been noted that accurate measurements are limited by image quality. This study compared volumes from a balloon model mimicking the left ventricle, scanned with and without harmonic imaging, using real-time 3-D echocardiography. It was concluded that the enhanced resolution provided by harmonic imaging improves accuracy of volume analysis by real-time 3-D echocardiography.

3-D volumetric measurements seem to be successful in measuring enclosed volumetric dimensions. No commercially available software has been able to measure volumes of open craters/holes.

Analysis Pro 3.1 (Soft Imaging System, Munich, Germany) is an expanded version of a soft-imaging software package. It was employed in a previous comparative study to measure the volume of craters induced by different levels of forces produced orthodontically on human premolar roots (Chan *et al.* 2002a). The aim of this study was to investigate the accuracy of this new software for volumetric measurements.

Materials and methods

In order to match the morphology of the curved human premolar root, four solid metallic cylindrical rods (copper, brass, aluminum, stainless steel) (Fig. 1) matched to the dimensions of human premolars were selected. Eight standardized

pyramidal indentations were made on each rod using the Vickers Microhardness tester Type M (automatic loading) (Shimadzu, Tokyo, Japan) (Figs. 2A-C). The indenter had a 136° inclusion angle and due to the different hardness of the materials selected, the size of the indentations was standardized by varying the loads without changing the loading cycles.

To eliminate the variance component of measurements, 4 pairs of stereo images ($\pm 3^\circ$) for each indentation were taken with a SEM (Philips XL 30) operating at 15kV and the specimen focused at the eucentric point, corresponding to a diameter of approximately 200nm. Each pair of images was processed twice. Images were saved in tagged image file format (TIFF) (Fig. 3). These images were then imported as a pair (Fig. 4) into the *Analysis Pro 3.1* for image processing and analysis. The image pairs were aligned by a cross-correlation technique, which used a sub-region of each image. The sub-region was defined by selection of a reference point that was clearly visible in both images. Once aligned, a grayscale depth map (Fig. 5) of the indentation was generated based on the parallax difference and the known tilt angle at which images were acquired. This depth map was subsequently corrected for errors due to residual tilt and curvature of the rod surface using shading correction (Figs. 6A-B) based on sampling of three areas of the background (Fig. 7). Following this correction, thresholding was used to segment the indentation extent from the background, and to subsequently obtain a measure of both the rod surface height (x) (*i.e.* surface level) and the average depth of the indentation (y) (Figs. 8A-B). The depth of the indentation relative to the rod surface was then calculated as the difference in these values (y-x) with the indentation volume then being obtained by multiplying this difference by the area of the indentation (a). This gave the estimated

volume of the indentation. This methodology has been previously described by Chan *et al.* 2002b.

Using the same software, the mean base length (d) of the pyramid was obtained. As the tangent of $136^\circ/2$ equates to $(d/2)/h$, where h is the height of the pyramidal indentation, the h value could be calculated (Fig. 9). The volume (v) of a square pyramid being $v = 1/3ah$, the 'true' volume of the pyramidal indentations could be obtained. This calculated volume was then compared to the volume estimates performed by the software.

Results

The individual results obtained for the rods were as follows (Table I) (Fig. 10).

(i) Brass:

The mean calculated and mean estimated volume per indentation was $8663.47\mu\text{m}^3$ and $8973.12\mu\text{m}^3$ respectively.

The percentage error was 3.59% with a standard deviation of 4.57.

(ii) Copper:

The mean calculated and mean estimated volume per indentation was $9564.64\mu\text{m}^3$ and $11377.72\mu\text{m}^3$ respectively.

The percentage error was 18.86% with a standard deviation of 4.47.

(iii) Stainless steel:

The mean calculated and mean estimated volume per indentation was $8160.49\mu\text{m}^3$ and $8281.02\mu\text{m}^3$ respectively.

The percentage error was 1.79% with a standard deviation of 9.27.

(iv) Aluminum:

The mean calculated and mean estimated volume per indentation was $8436.85\mu\text{m}^3$ and $9981.88\mu\text{m}^3$ respectively.

The percentage error was 18.27% with a standard deviation of 3.21.

Discussion

Four metallic rods of different hardness were selected for this study. They were matched to the cross-sectional diameter of human premolars that was used in the previous study (Chan *et al.* 2002a).

In the present study, the Vickers Microhardness tester was used with a pyramidal diamond indenter. The indenter being a square pyramid of a 136° inclusion angle could have the volume calculated using the formula: $v = 1/3ah$, where v is the volume and h is the height of the pyramid.

The rods selected were of different hardness and indentations made with the same load and loading cycle, would yield indentations of different sizes. As the surfaces of the rods are curved, indentations of different sizes would occupy different extent of the curved surface. This would result in greater variations during volumetric

estimations. Thus the size of the indentations was standardized by varying the load during indentation without changing the loading cycle.

From the initial observations, it was unclear why the software gave accurate volumes for brass and stainless steel but not copper and aluminum. But closer scrutiny revealed the mathematical calculations to be at fault.

The materials used in this study were of different hardness. During indentation, a softer material would tend to distort and deform more under loading. This phenomenon could be viewed as a form of 'shock absorption' or creep of the material. This creep would result in a distorted pyramidal indentation. Furthermore, the creep may also be expressed differently at different depths of the indentation. Hence for such materials, it would be erroneous to calculate the volumes from the formula $v = 1/3ah$.

On the other hand, the *Analysis Pro 3.1* software uses the grayscale depth map and thresholding for volumetric measurements and would not be affected by the creep of the material expressed during indentation. In fact the larger estimations obtained by the software may more accurately reflect the true volume of the distorted indentation of the two softer materials (copper and aluminum).

Conclusions

The *Analysis Pro 3.1* software was successful in estimating the volumes of pyramidal indentations in metallic rods. However the creep that occurred in softer

materials during indentation may distort calculated results. The estimates obtained by the software even for distorted indentations caused by creep have high degrees of reproducibility and accuracy.

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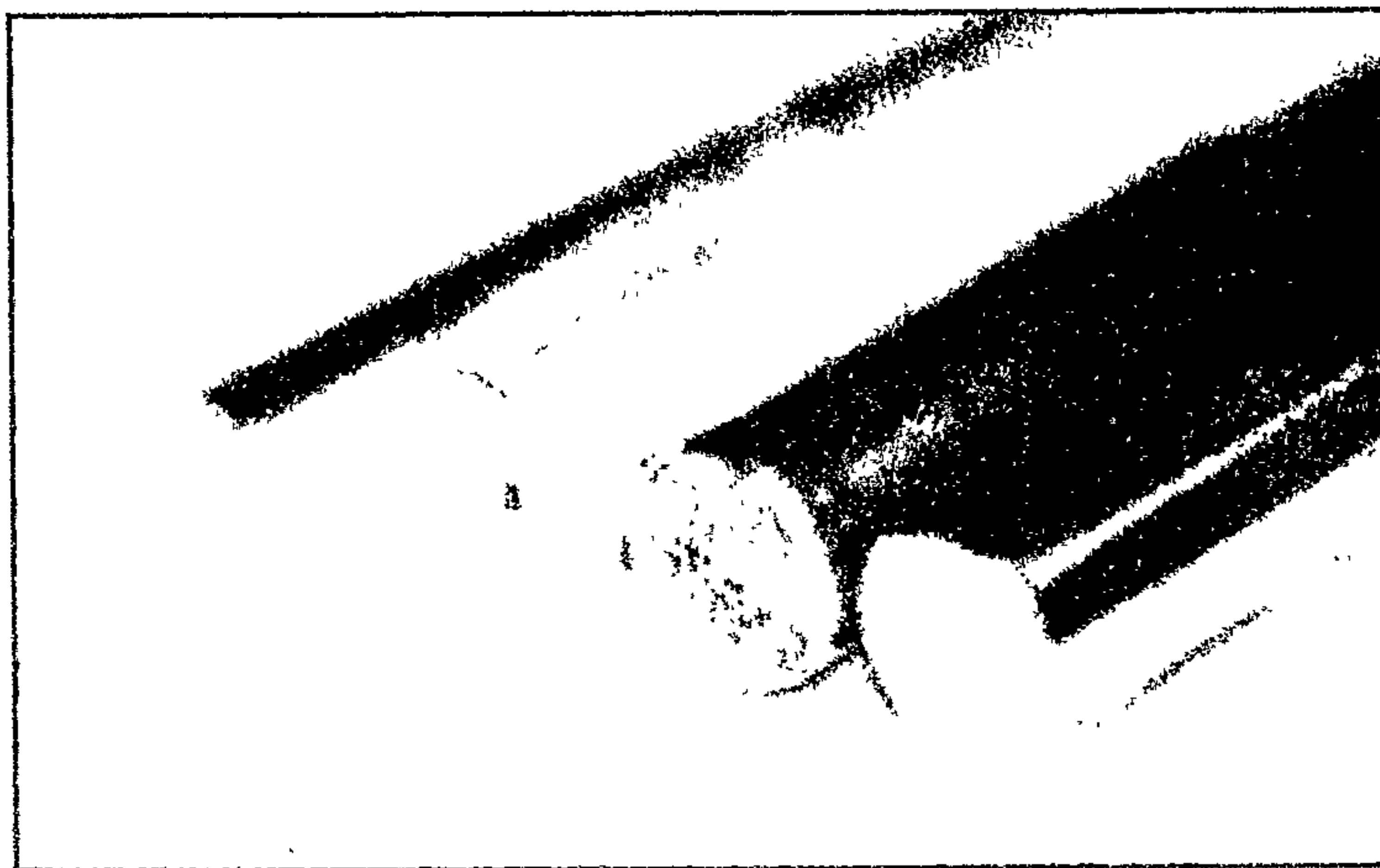


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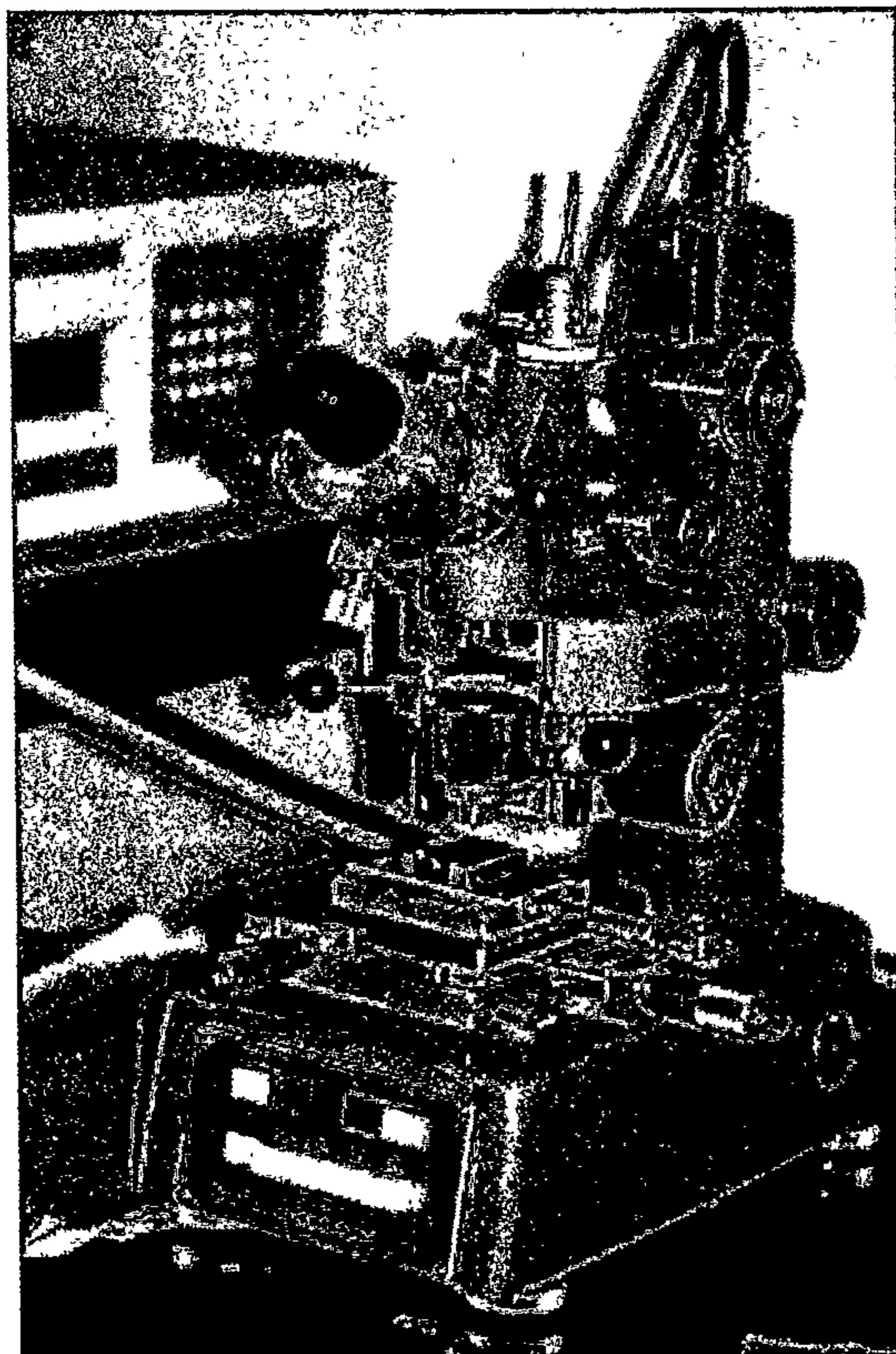


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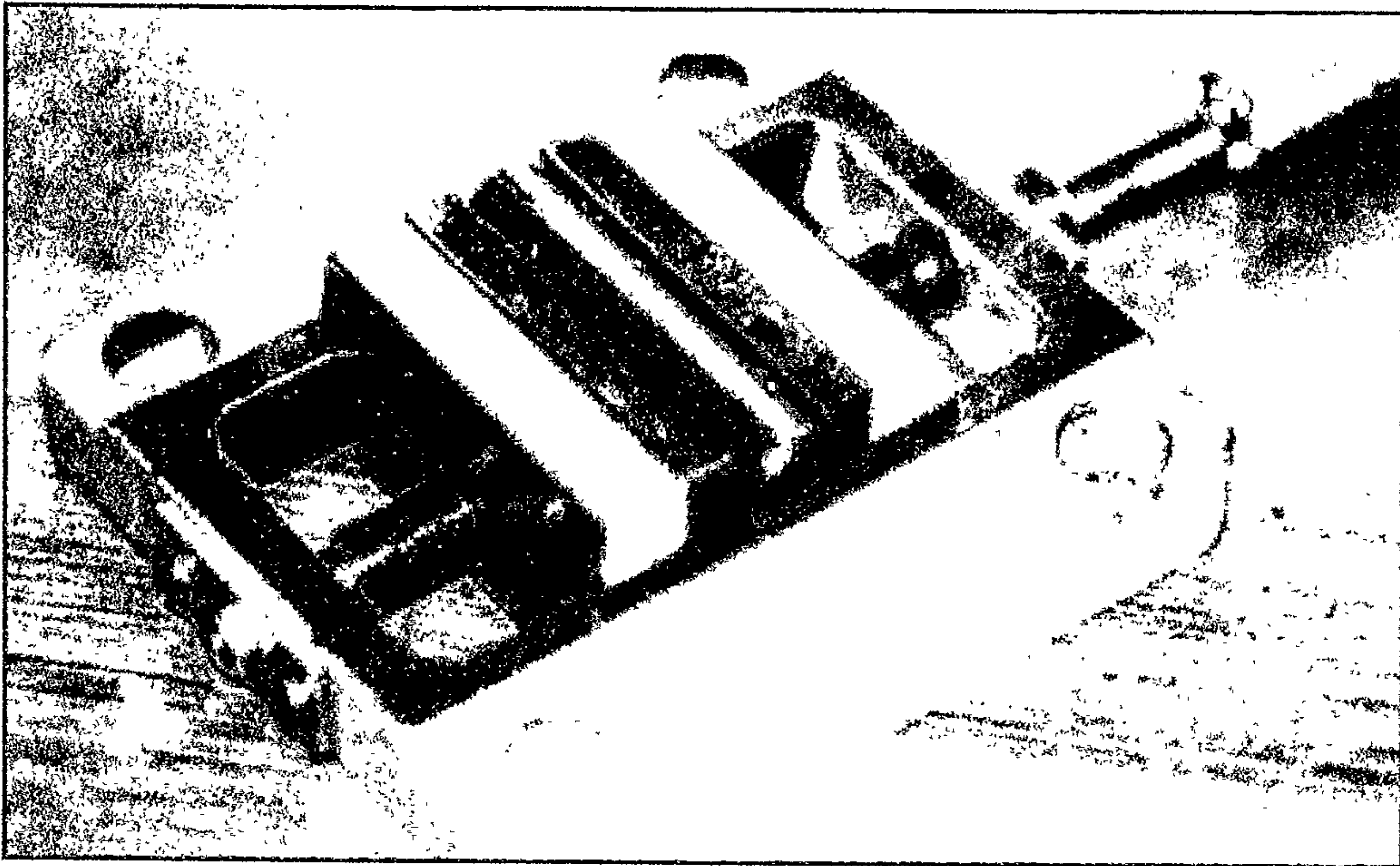


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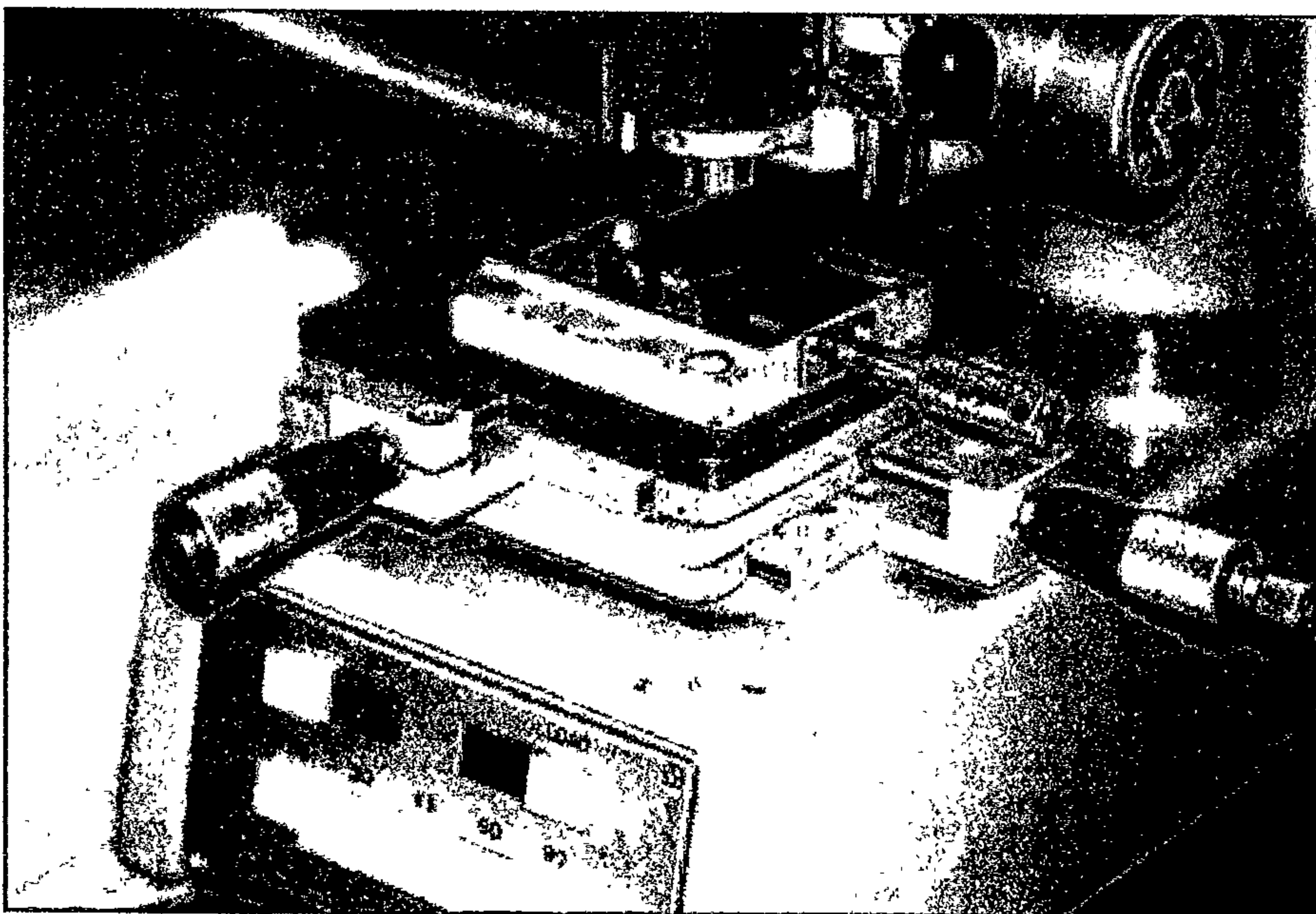


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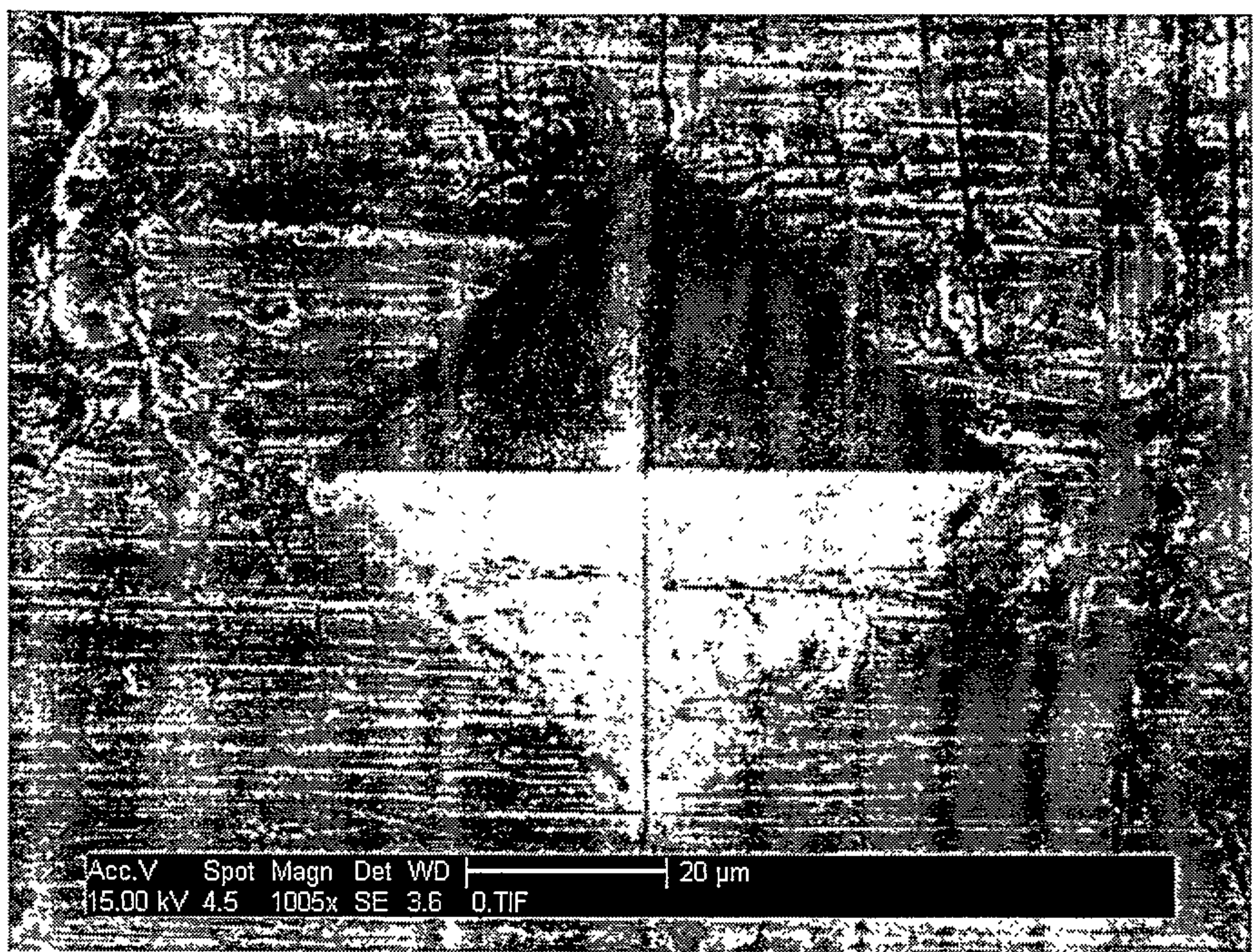


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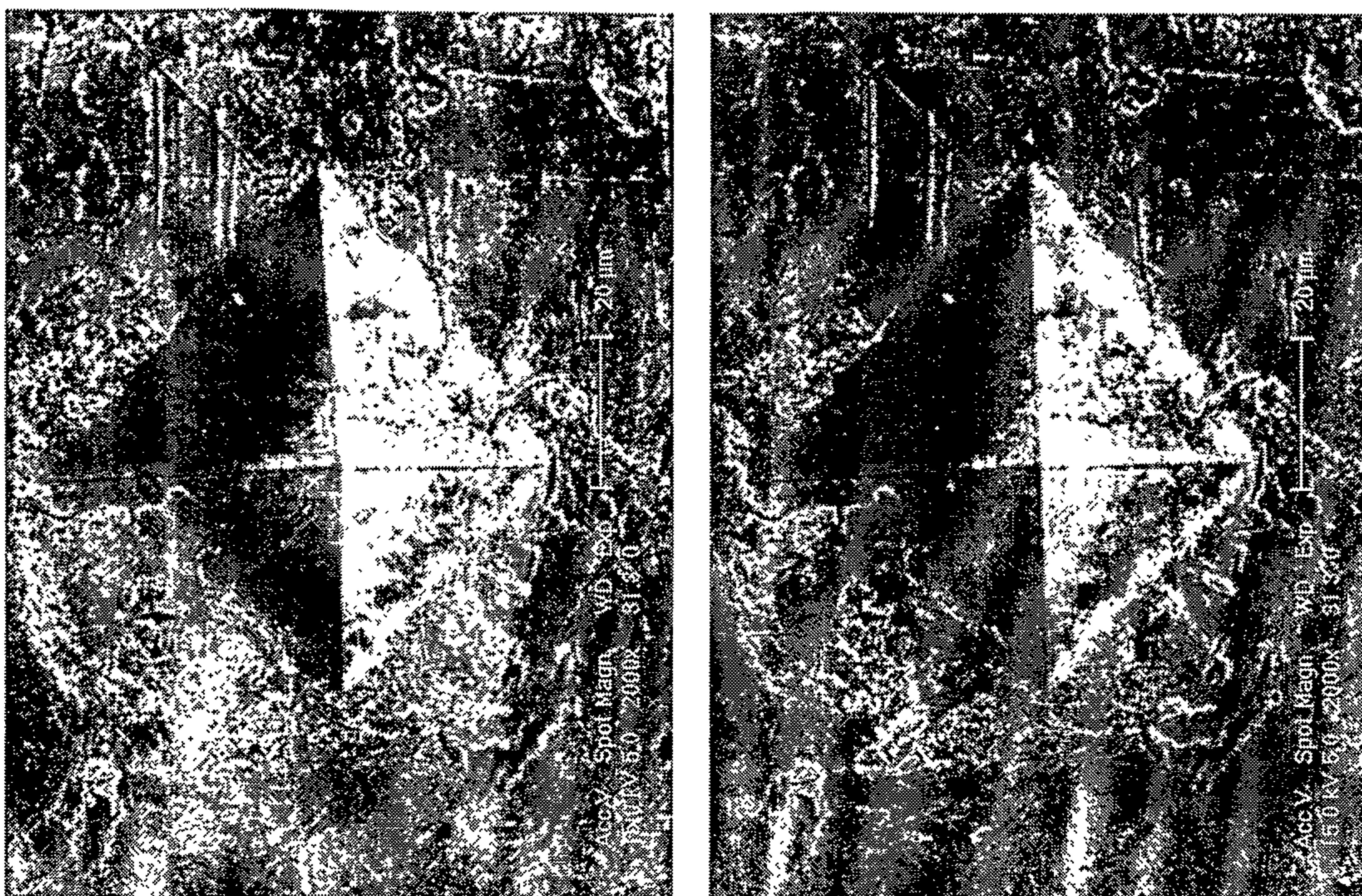


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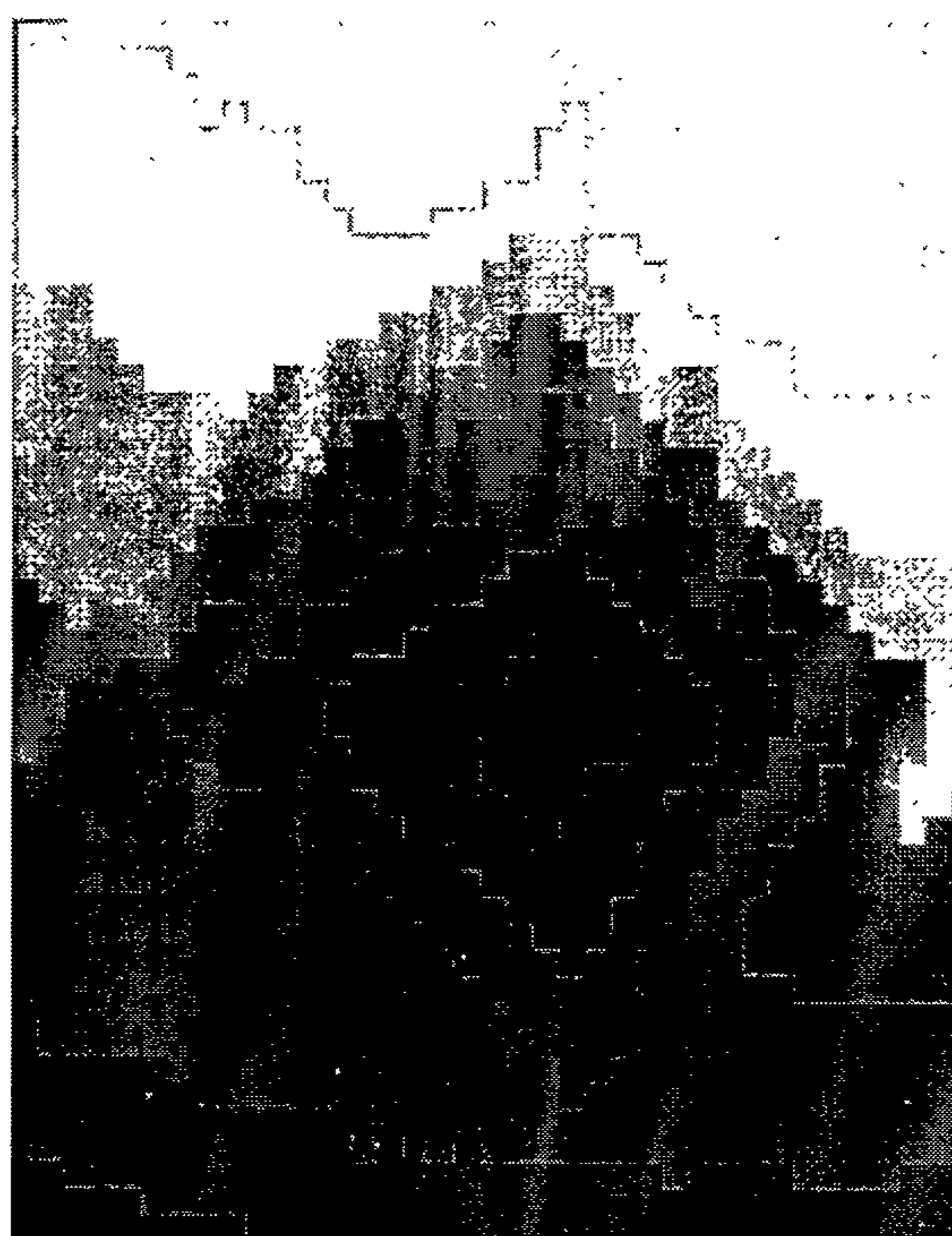


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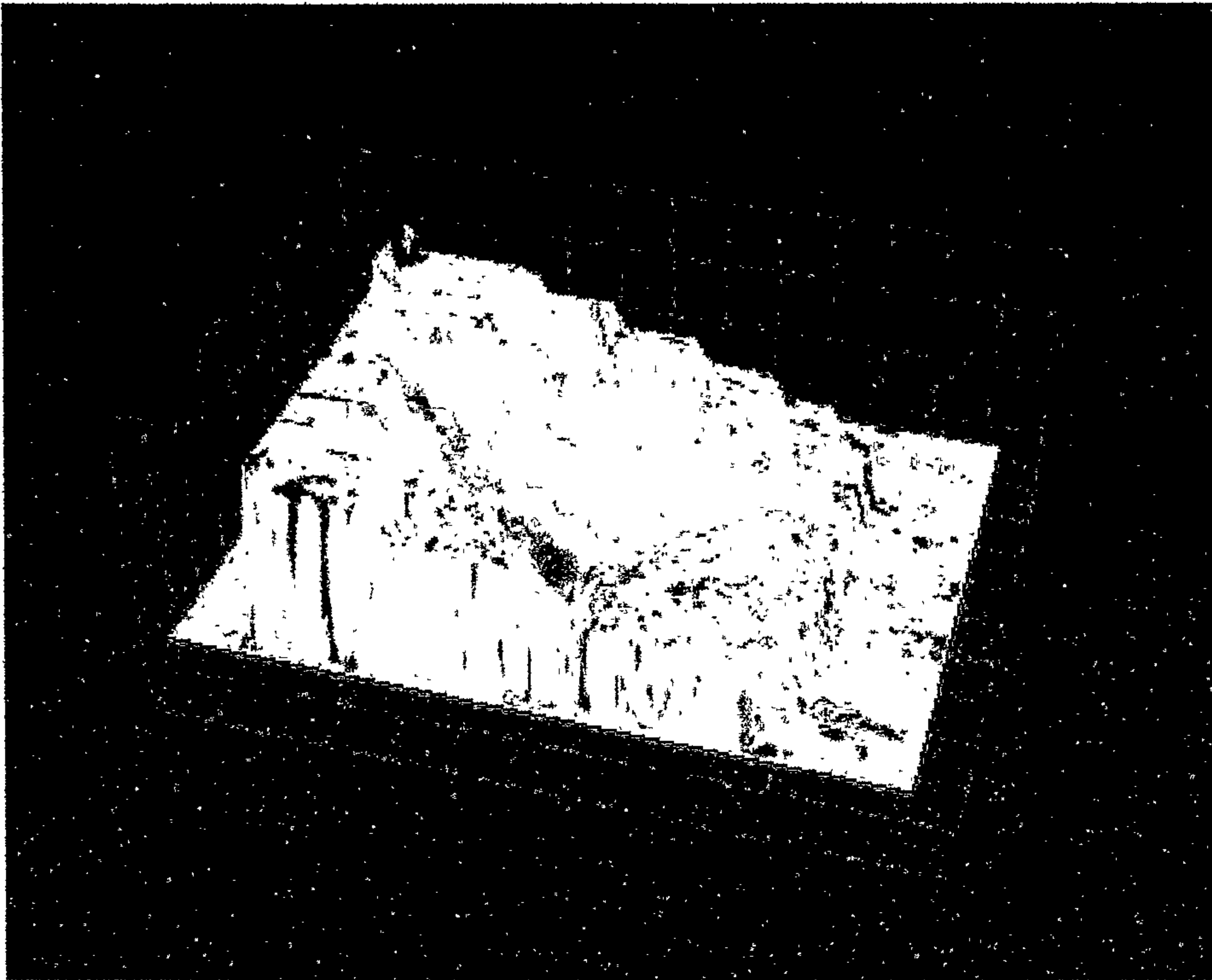


Figure 6. (A) 3-D reconstruction of indentation before tilt correction.

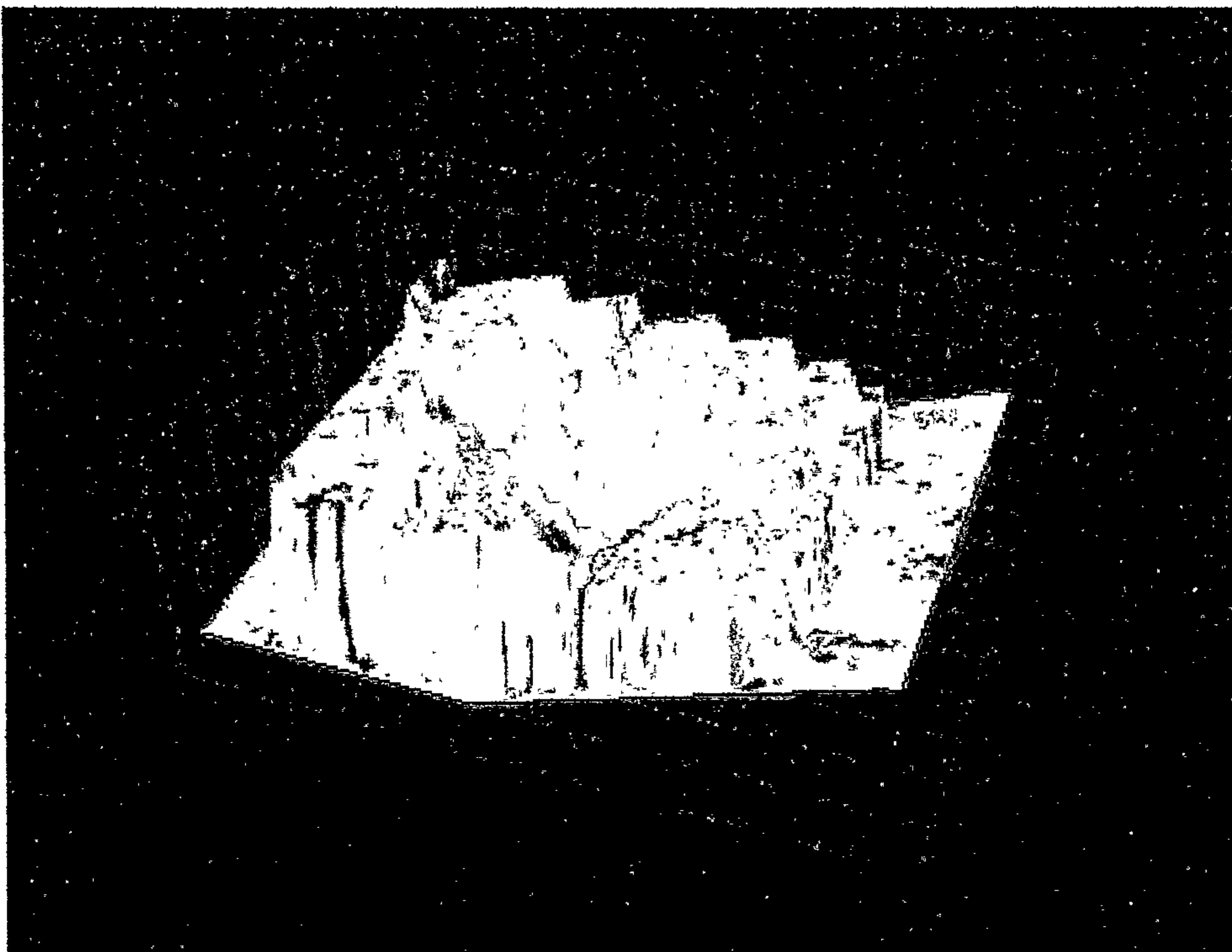


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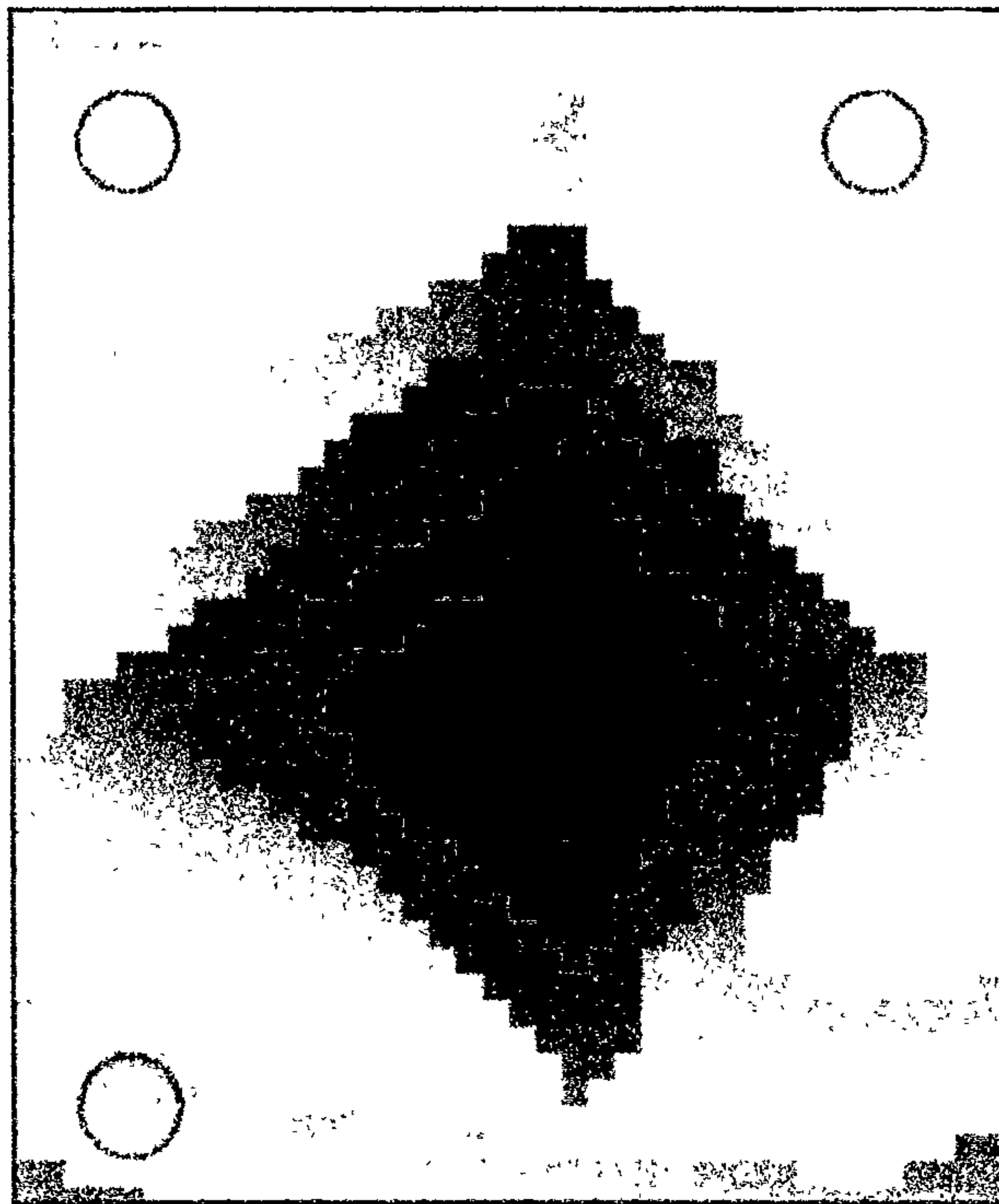


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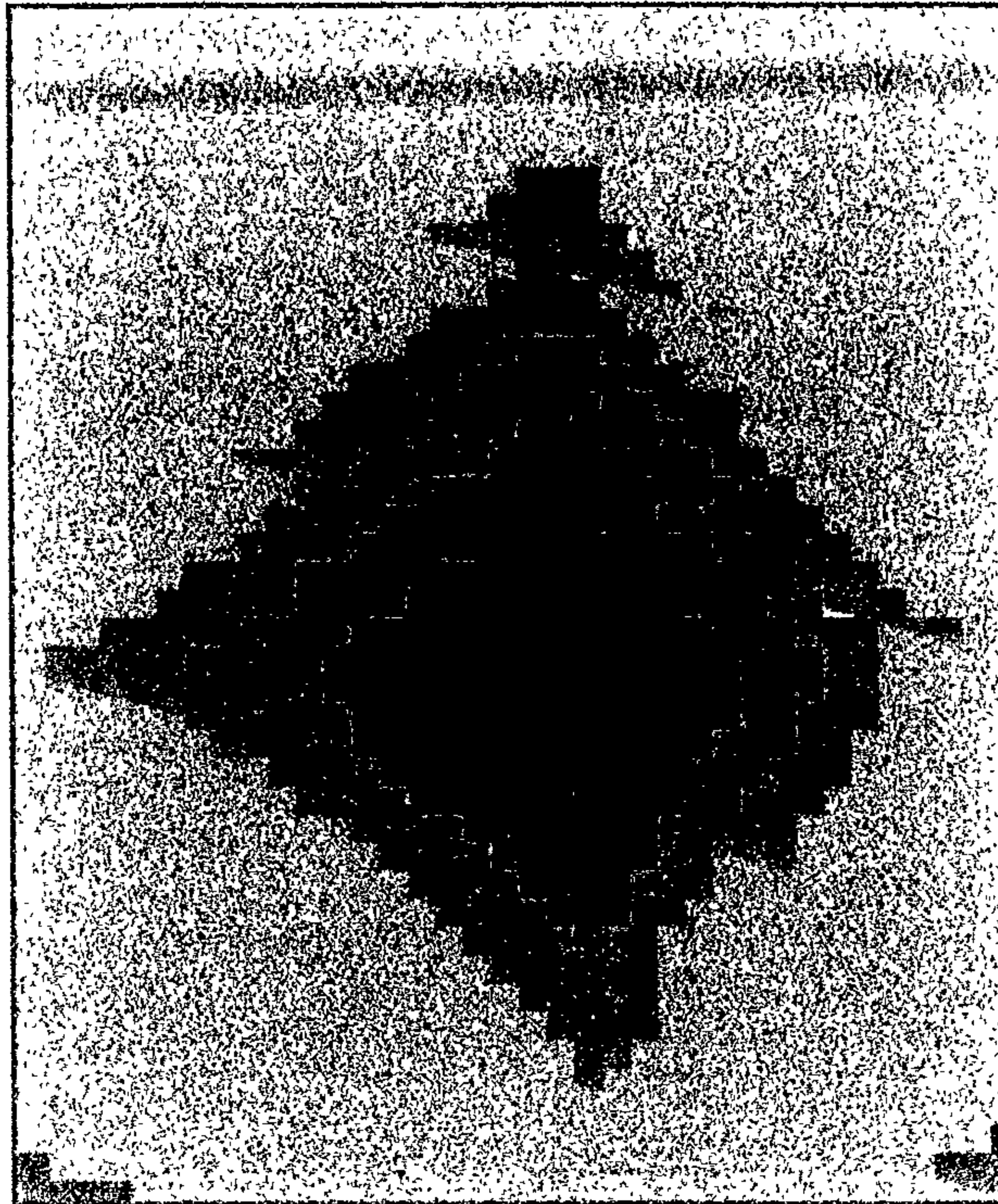


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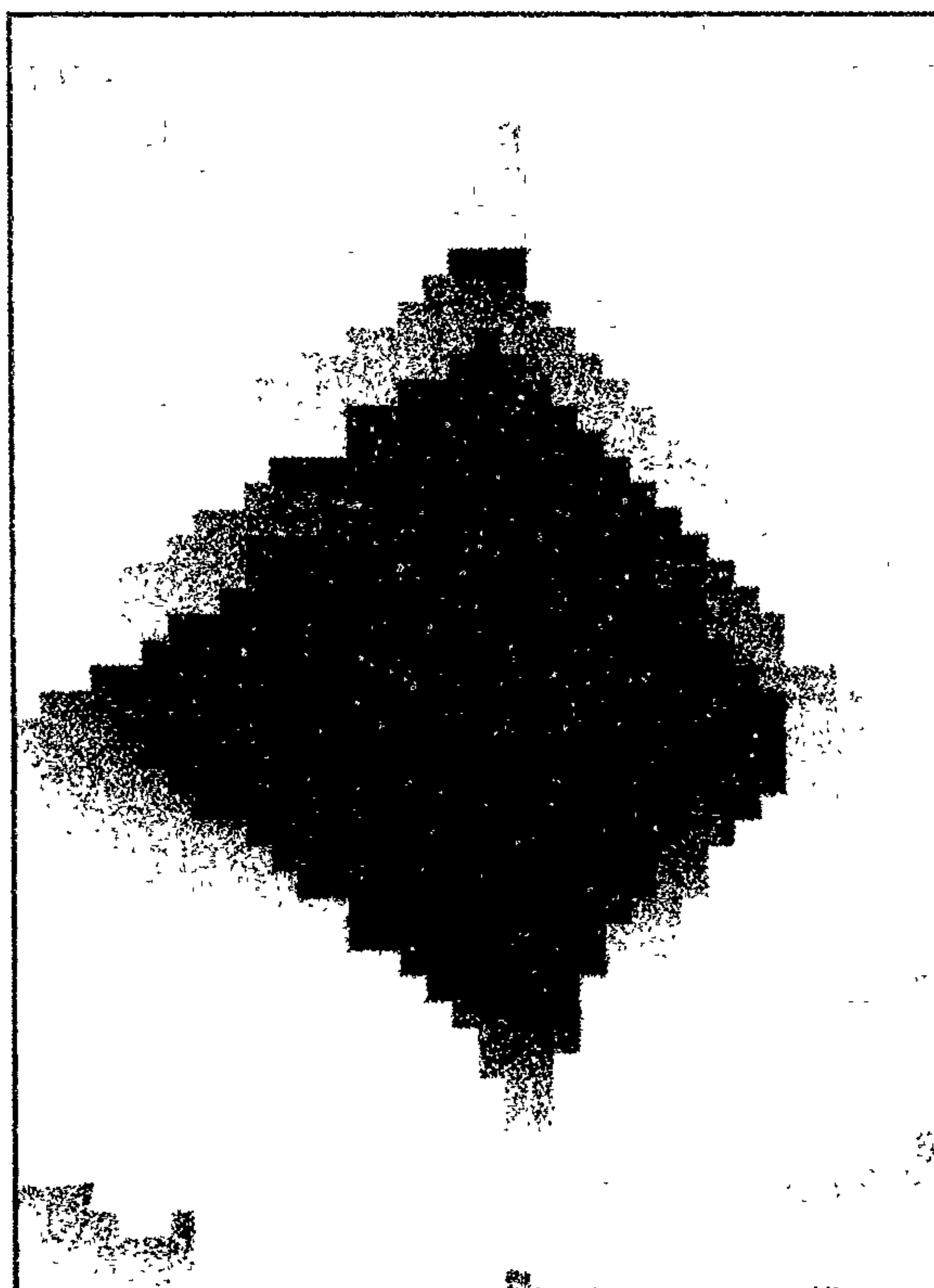


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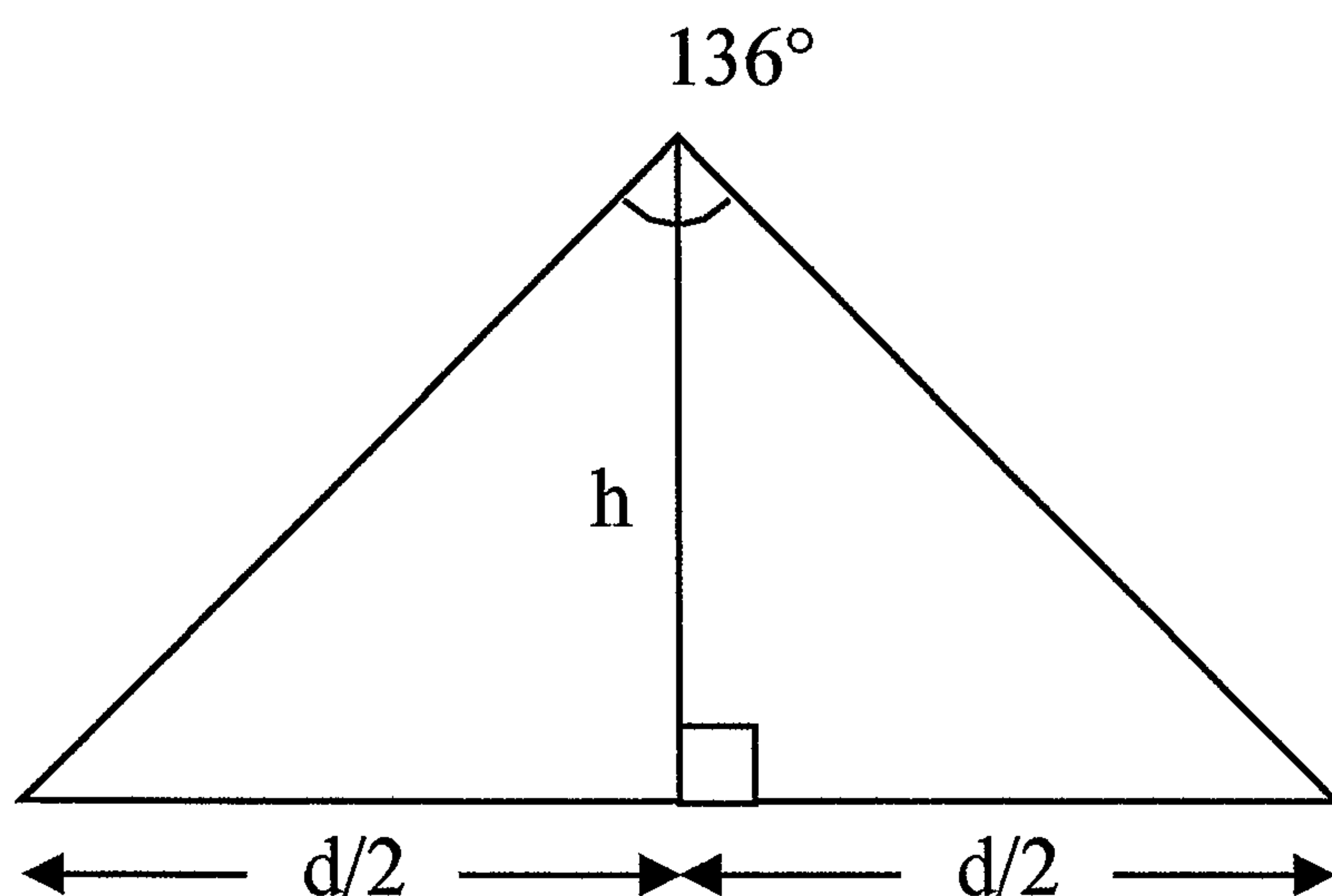


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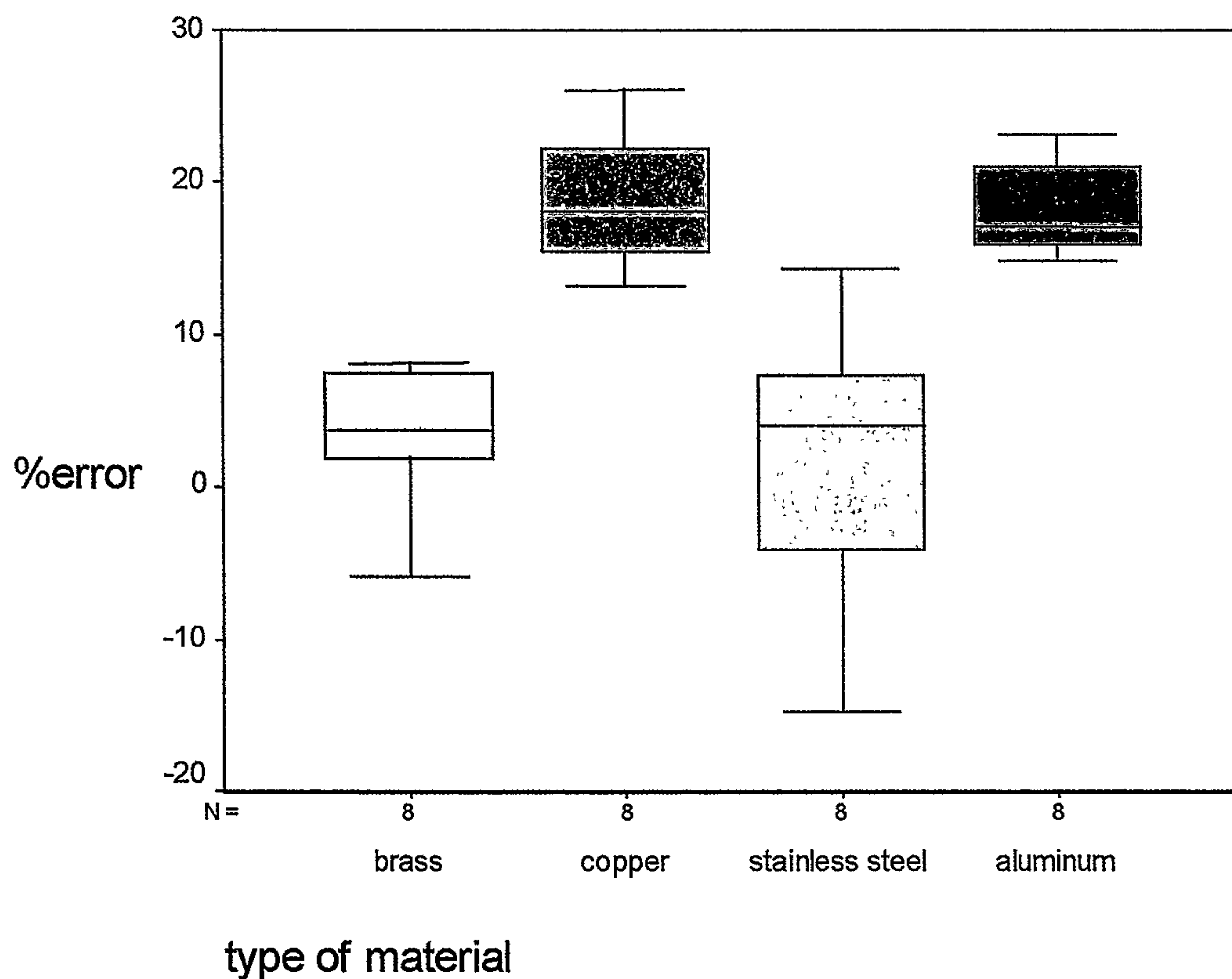


Figure 10. Graph demonstrating percentage errors observed for different materials.

TABLES

Table I. Statistical table showing percentage errors for different materials.

type of material		calculated volume	estimated volume	%error
brass	Mean	8663.4741	8973.1271	3.5890
	N	64	64	8
	Std. Deviation	441.1708	745.5986	4.5659
copper	Mean	9564.6367	11377.7244	18.8616
	N	64	64	8
	Std. Deviation	454.8746	1480.6510	4.4653
stainless steel	Mean	8160.4887	8281.0155	1.7909
	N	64	64	8
	Std. Deviation	414.5463	1355.1401	9.2658
aluminum	Mean	8436.8502	9981.8806	18.2686
	N	64	64	8
	Std. Deviation	479.2521	1328.4061	3.2145
Total	Mean	8706.3624	9653.4369	10.6275
	N	256	256	32
	Std. Deviation	690.4825	1711.9654	9.8199

Future Directions

Research performed on the topic of root resorption has been vast and extensive. With the introduction of new computing technology, software and improved methodology, recent findings have been able to shed much light on this subject. However, there remain areas where root resorption is still a big uncertainty.

- (i) Intrusive and torquing forces have been reported to cause significant amounts of root resorption. However these studies are still flawed. 3-D quantitative measurements of resorption could not be ascertained due to technical difficulties. These studies could be replicated with an improved methodology.
- (ii) Light and heavy forces could also be applied on contralateral premolars in the same human subjects to eliminate any form of intra-individual variability.
- (iii) Experimental duration could be extended to study the quality and quantity of repair after the termination of orthodontic forces.

It has been proposed that there could be a genetic predisposition to root resorption. Other systemic factors may also be isolated to further understand the true aetiology of this phenomenon.

- (iv) Cellular and molecular control of root resorption following tooth movement.
- (v) Negative Control of Root resorption: Assessing the role of apoptosis.
- (vi) Role of systemic calcium, phosphate and fluoride and their effects on root resorption.
- (vii) *In-vitro* organ/cell culturing and observation of live samples with Micro CT to document root resorption and repair at tissue/cellular levels.

The End