

Quantitative Age Estimation Of The Fingerprints Donor Through Atomic Force Microscopy: Nanoscale Analysis Of Morphological Variations

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Abstract

This research studies the extensive application of the atomic force microscope (AFM), which is a non-invasive and non-destructive method of analysis to estimate the age of the latent fingerprint donor. 200 latent fingerprints (consisting of subjects of 5 age groups between 10 and 60 years of both male and female) were analyzed using the non-contact mode under AFM, which enabled the nanoscale reading of the morphometric characteristics of the sample surface upon the interaction of the cantilever tip over the sample surface. The deflection in the laser beam focused on the cantilever tip is captured with the help of a photodiode, which in turn is depicted in the form of a graph. The study analyzed the variation in the deflection range of the samples of varying age groups and further used statistical analyses such as ANOVA, correlation, and a regression model to prove the significance of the study with forensic relevance. The study showed a strong correlation between sex, age groups, and the morphometric characteristics of the ridges, making the age estimation of the fingerprint donor and the sex determination equally possible, ascertaining that the AFM is a novel approach for the latent fingerprint analysis and criminal profiling.

Keywords: Atomic Force Microscope; latent fingerprints; forensic science; age estimation.

Introduction

Dactyloscopy defines the use of fingerprints for the purpose of identifying an individual based on ridge characteristics such as pattern shapes, ridge density, ridge dimensions, and Galton details, etc. Firstly, concerning the modern era of crimes and criminals, it is difficult to identify criminals based on Ridgeology as the experts hardly find 5% of the intact fingerprints without overlapping, or smudging. Secondly, in certain cases where the criminal(s) is unknown (eg, Serial killer cases), identifying an individual just from the class characteristic and Galton details is a difficult task. The fingerprints hold information beyond just the ridge. For instance, various researchers have worked on determining the sex of the fingerprint donor from the inked fingerprints (Nayak et al, 2010). On reviewing the literature, a research gap was found as most of the research works focus on sex determination with the help of microscopy and other scientific instrumentation, which gives rise to the further question of whether it is possible to ascertain or estimate the age of the fingerprint donor with similar significant results as the sex determination studies.

Countable and minimal studies have been done on atomic force microscopy (AFM) and its capacity in forensic aspects; similarly, the potential of the latent fingerprints and its residue in identifying information about an individual, beyond just comparing the pattern characteristics. In a study (Ricci & Caputo, 2007), the researchers explain the importance of AFM as a non-destructive method of analysis and its advantages over the traditional optical profiling methods, such as the SEM. In another study (Costa et al., 2012), the researchers demonstrated the AFM's ability to accurately profile and map the 3D topography of the latent fingerprint ridges. AFM's capacity to detect the surface differences through the roughness parameter of the samples between lipid-rich and

sweat-rich fingerprint regions that effectively aided in the deposition time estimation in a study (Kasas et al, 2005). Moreover, another study (Bal-Price et al, 2015) positively portrays the roughness parameter studied under the AFM helped to differentiate between the fresh and the older prints, which is a very essential milestone in the crime scene investigation and served as a base for this research.

AFM has advantages over traditional imaging methods, such as optical and electron microscopy, as optical microscopy lacks the resolution to capture nanoscale fingerprint details, while electron microscopy requires vacuum conditions that can disrupt delicate fingerprint residues. Whereas AFM operates under ambient conditions, providing non-destructive, high-resolution images. The scientific capacity of AFM as a complementary technique, coupled with other conventional methods, provides both chemical and structural details of the samples that other instrumentations failed to (Bendell & Jenkins, 2014). Considering the real crime scene cases, profiling an individual from the available evidence while preserving the original state of the evidence is tedious and difficult. The fingerprint sciences is a branch of forensics that still relies on the traditional powder and chemical development methods, which are destructive in nature. The preservation of a latent fingerprint's original state is very close to impossible and needs techniques that are non-destructive and non-invasive. This research studies the importance of the AFM as a non-destructive and non-invasive method of analysis, which can be coupled with the multispectral imaging method to locate the latent fingerprints at the scene of the crime and analyze them under the instrumentation to retrieve the maximum potential information such as sex and age group of the fingerprint donor.

Materials and Methods:

Instrumentation: Atomic Force Microscopy (Model Name: A100-AFM, Pace Analytics)

The Atomic Force Microscope relies on the interactive force between the cantilever tip and the atoms on the surface of the sample. To study the interaction between the cantilever and the sample surface, a laser beam is directed onto the top of the cantilever tip, which is reflected onto the position-sensitive photodetector. As the beam moves over the surface of the sample, the variation in the deflection is recorded through the detector to give real-time feedback through the instrument-specific software and mapped through Gwyddion software. (Margenau, 1939), (Aizenberg et al, 2000), & (Blumberger et al, 2020). There are three AFM Imaging Modes, (a) Contact mode, in which the tip interacts with the surface of the sample, (b) Tapping mode in which the cantilever tip oscillates in a very near resonance making the cantilever tip tap on the sample surface, and (c) Non-Contact mode, in which a constant tip-to-sample distance is maintained through a piezoelectric scanner. The non-contact mode was preferred to analyze the samples in this research, as this study focuses on a non-destructive method of analysis to study the fingerprint exhibits and prioritizes the preservation of the exhibits.

Samples Collected: On obtaining the ethical committee certificate, the participants were notified in advance of the sample collection and the analysis procedures, and their consents were collected. 200 samples were obtained using a stratified approach, ensuring an equal proportion of male and female samples. The subjects were requested to wash their hands and were asked to deposit their fingerprints onto the glass slides, which were cleaned with ethanol before deposition. The surface chosen here for the sample collection is a non-porous surface (i.e., Glass slides), as fingerprints are majorly found on non-porous surfaces in the scene of the crime.

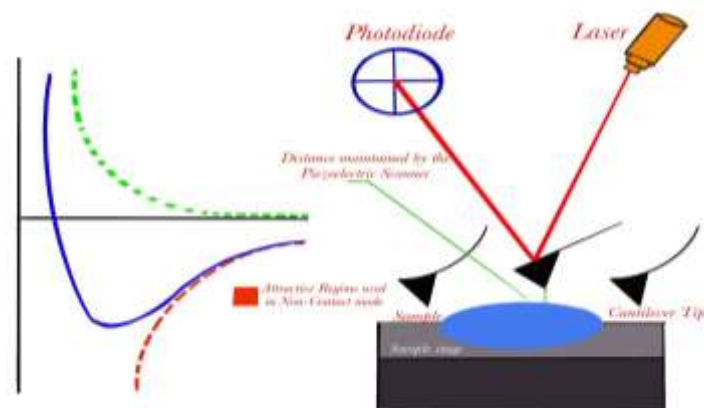
Sample collection requirements: The 200 samples were gathered and categorized into five distinct categories (C1 to C5), of equal proportion of both male and female samples (1:1) under each category (Table 1).

Categories	Sex	Code	Age Group	Number of Samples Collected
Category 1	Male	M:C1	10-20 years old	20 samples
Category 1	Female	F:C1	10-20 years old	20 samples
Category 2	Male	M:C2	21-30 years old	20 samples
Category 2	Female	F:C2	21-30 years old	20 samples
Category 3	Male	M:C3	31-40 years old	20 samples
Category 3	Female	F:C3	31-40 years old	20 samples
Category 4	Male	M: C4	41-50 years old	20 samples
Category 4	Female	F:C4	41-50 years old	20 samples
Category 5	Male	M:C5	51-60 years old	20 samples
Category 5	Female	F:C5	51-60 years old	20 samples

(Table 1: Number of samples collected under the age categories (C1-C5) and their designated codes.)

Procedure: The glass slides were cleaned with ethanol, and the fingerprint samples were collected on glass slides. The undeveloped latent fingerprint samples were examined under the Non-contact mode of Hanging Atomic Force Microscopy (AFM) for morphological investigations and examination of ridge conditions. The laser is first pointed at the cantilever tip (Fig. 1), and the resonance is tracked with a magnified view until a smooth curve is obtained. The piezoelectric scanner is then arranged to maintain the appropriate distance between the sample and the cantilever tip by identifying the peak of the curve. The precise region of the sample to be examined is chosen by using the XYZ movement of the stage, and the PID (Proportional-Integral-Derivative) parameter is set to 10.0% after the system has been correctly configured. After selecting the sample size for analysis, the scanning speed is lowered to half of the designated sample size (i.e., analysis region 10,000nm sq area from the core to the first ridge on the distal ulnar region, and hence the speed is 5000). The sample is prepared for analysis with these parameters. The PID settings can be further changed to lessen background noise during the oscillation.

The retrieved data from the samples were stored in a (.dat) file and subsequently processed with the Gwyddion software program. In-depth analysis was conducted to evaluate the morphological features, statistical data, and 3D structure of the samples.



(Figure 1. Atomic Force Microscopy: Non-Contact Mode)**Results and Discussions:**

The cantilever tip moves over the sample's selected region for the morphometric analysis, such as identifying the presence of particles, particles' diameter, depth of the furrows, surface roughness, waviness, and texture. The noise in the sample analysis was avoided by removing any vibration or sound interference around the instrumentation while the analysis was performed, as the AFM is extremely sensitive to such interferences. Throughout the analysis, the laser was made sure to be pointed at the tip of the cantilever to retrieve a precise analysis of the surface of the samples. The region selected for the latent fingerprint analysis was a 10,000nm sq area from the core to the first ridge in the distal ulnar region of the fingerprint pattern area, considering that the core region is always found in the crime scene sample. The resolution was selected as 240x250p (which takes 17 to 19 mins for the scan). The same setting was utilized for all the samples analyzed under AFM to apply the forensic law of comparison, which states that only like things can be compared.

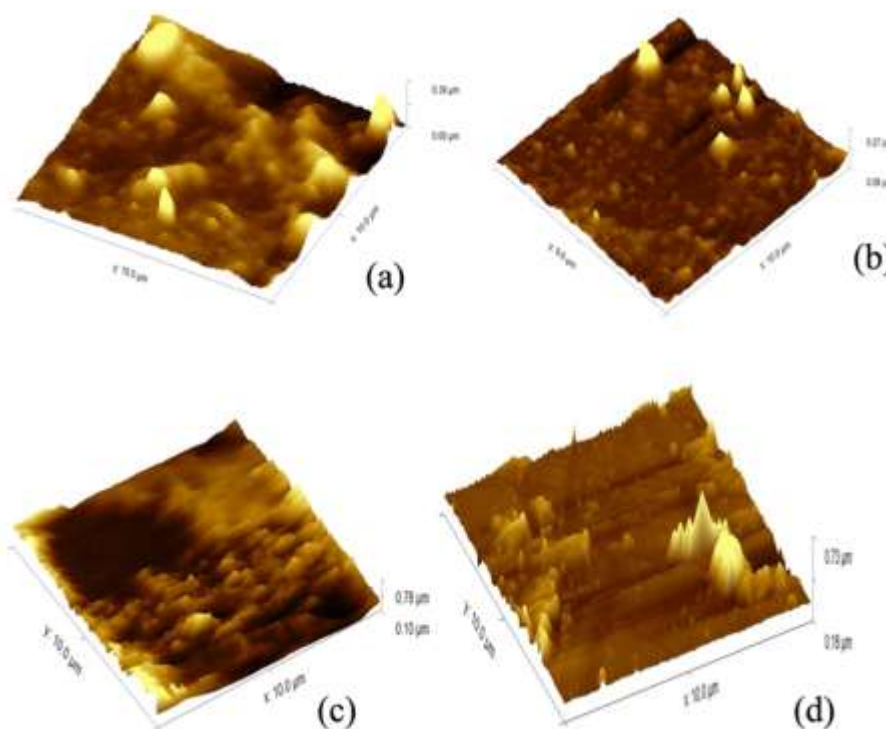
The software Gwyddion was used for annotation and to retrieve the data and graphs of the sample surface and the 3D image of the surface, which are discussed below. A profile was created for all the samples from the core to the first ridge that follows in the distal ulnar region as the region for comparison. The number of particles present in the region, surface roughness, texture, and waviness of the samples belonging to various age groups were measured. Analyzing all the data, it was found that the roughness of the fingerprint ridges have the potential to become an age marker in the latent fingerprint exhibits. Hence, it was calculated using the annotation and statistical tools from the software and presents comprehensive data on the average roughness value (ARV) of latent fingerprints on non-porous surfaces measured in nanometres scale in the following table. (Table 2)

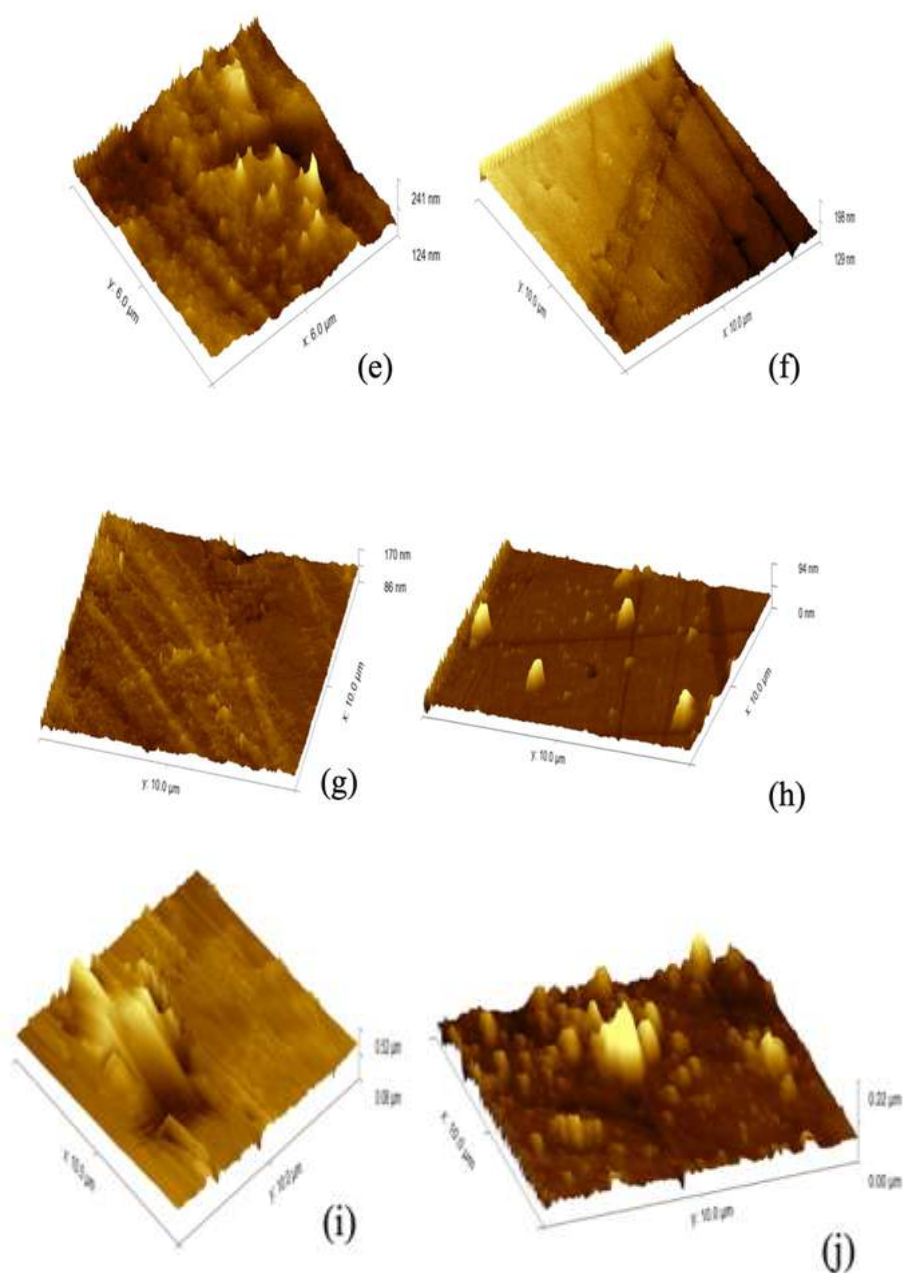
M:C1	M:C2	M:C3	M:C4	M:C5	F:C1	F:C2	F:C3	F:C4	F:C5
1.21	1.87	2.5	1.9	0.8	0.95	1.32	1.76	1.46	0.49
1.22	1.87	2.7	1.94	0.89	0.96	1.39	1.79	1.46	0.5
1.25	1.88	2.75	1.95	0.89	0.96	1.49	1.79	1.51	0.55
1.26	1.89	2.78	1.95	0.9	0.96	1.49	1.82	1.52	0.55
1.29	1.9	2.86	1.97	0.91	0.97	1.49	1.84	1.59	0.56
1.3	1.91	2.87	1.99	0.95	0.98	1.5	1.84	1.59	0.59
1.31	1.92	2.88	1.99	0.98	0.99	1.52	1.85	1.59	0.59
1.31	1.92	2.88	1.99	0.99	0.99	1.53	1.86	1.62	0.61
1.32	1.93	2.89	1.99	0.99	0.99	1.55	1.87	1.62	0.62
1.32	1.98	2.9	2	1.09	0.99	1.56	1.88	1.65	0.65
1.33	1.99	2.9	2.01	1.13	1	1.62	1.89	1.66	0.66
1.35	2	2.91	2.01	1.15	1.01	1.64	1.9	1.69	0.66
1.36	2	2.91	2.02	1.16	1.01	1.66	1.91	1.7	0.67
1.36	2.1	2.93	2.03	1.18	1.01	1.66	1.92	1.71	0.67
1.41	2.1	3	2.1	1.19	1.02	1.69	1.92	1.71	0.69
1.42	2.2	3.01	2.1	1.19	1.02	1.69	1.94	1.75	0.69
1.44	2.2	3.01	2.1	1.2	1.04	1.7	1.94	1.77	0.71
1.45	2.22	3.01	2.2	1.21	1.06	1.71	1.95	1.77	0.71
1.46	2.23	3.1	2.2	1.32	1.07	1.72	1.96	1.81	0.73
1.46	2.3	3.12	2.23	1.33	1.1	1.72	2.01	1.82	1.02

(Table 2. Average Roughness values of the latent fingerprint ridge (first ridge near the core) analyzed under the Atomic Force Microscopy (unit: nm) arranged in the ascending order for comparison.)

Quantitative analyses of these nanoscale parameters identified notable variations that correlate with age and sex, offering insights into the forensic application of AFM for age estimation of the latent fingerprint donor.

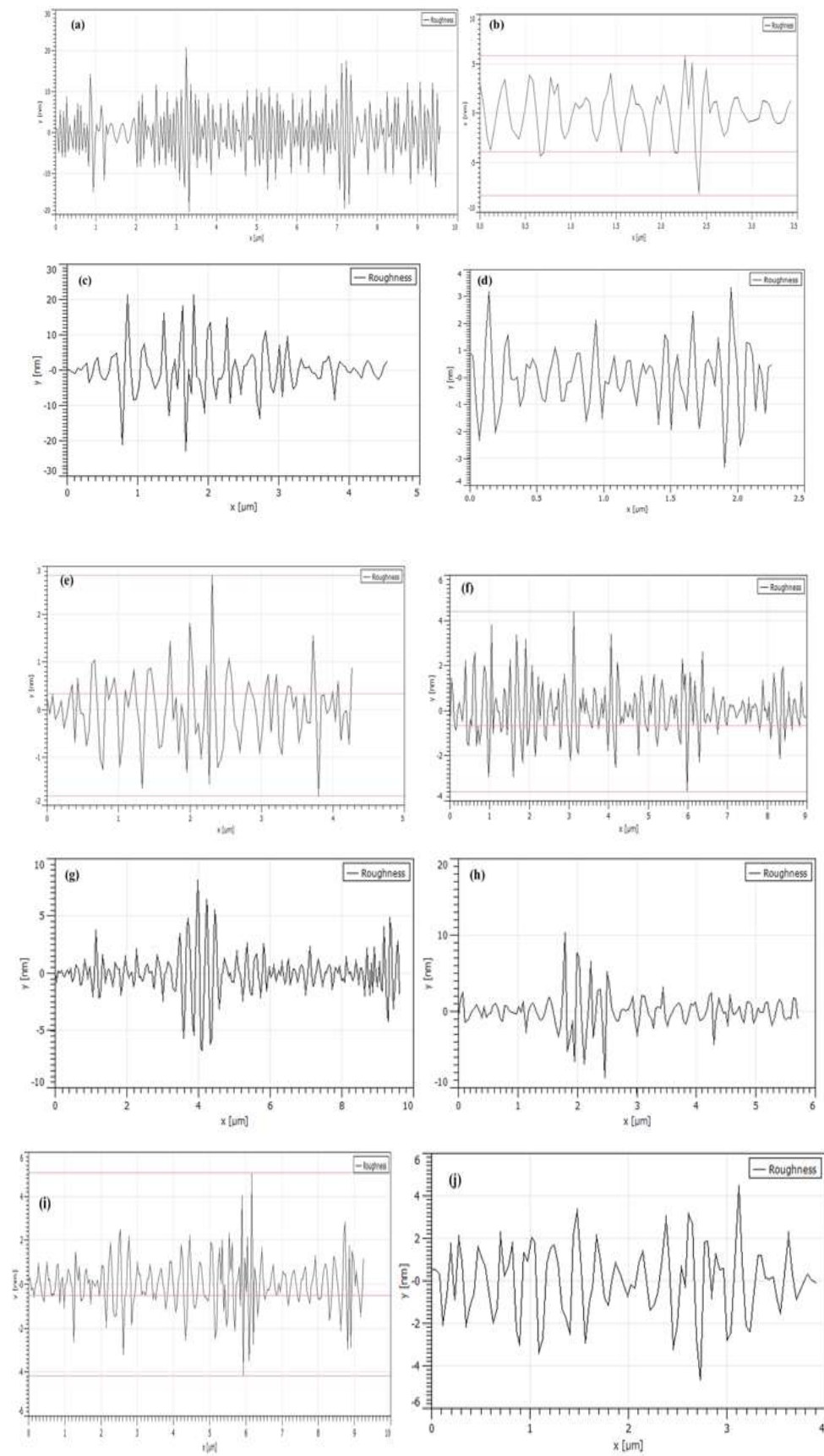
Surface Roughness and Ridge Morphology: The samples examined using atomic force microscopy exhibited distinct disparities across the various age groups and associated fluctuations in fingerprint residues. The 3-D figures (Fig.2) depict the changes in fingerprint residue seen in undeveloped fingerprint samples, as observed using AFM. Upon analyzing all the samples and examining the 3D images, it is evident that the age group C3 (30-40 years) exhibits the largest concentration of fingerprint residue. Conversely, the age category C1 (10-20 years) displays the lowest quantity, followed by category C5 (50-60 years). While the research conducted (Khare and Singla, 2022) suggests that volatile ester levels are greater in children compared to adults, the total amount of fingerprint residue in children remains lower than that seen in adults. This might also be attributed to lifestyle factors, occupation type, and behaviours such as the use of cosmetics, smoking, dietary choices, etc. Figure 2 illustrates the variations in fingerprint residues across different age groupings.





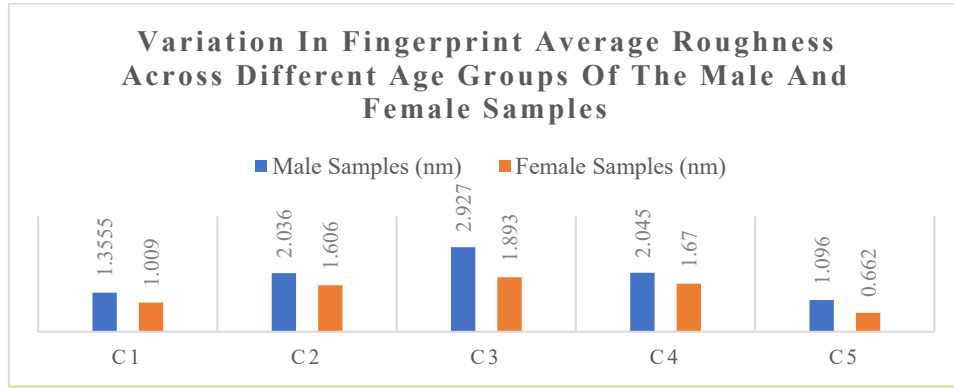
(Figure 2: 3D Representation of the morphology of the male and female subjects' sample surfaces analyzed under Atomic Force Microscopy. (a and b: male and female) Category 1: age 10-20, (c and d: male and female) Category 2: age 20-30, (e and f: male and female) Category 3: age 30-40, (g and h: male and female) Category 4: age 40-50, (i and j: male and female) Category 5: age 50-60.)

The profiles were established to delineate the regions where a significant concentration of fingerprint residues is detected, and subsequently, the graphs are generated based on these profiles. The region exhibiting the highest concentration of fingerprint residue in the latent fingerprint samples from various age groups is then analyzed to examine their exact roughness. (Graphs 1- 10)

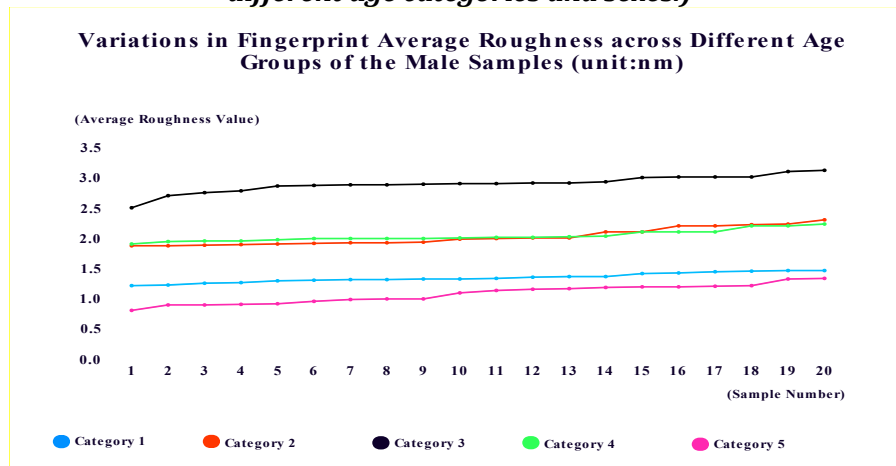


(Graph 1-10: Surface roughness of the sample analyzed under Atomic Force Microscopy. (a) Category 1 - age 10-20, (b) Category 2- age 10-20, (c) Category 3 - age 30-40, (d) Category 4 - age 40-50, (e) Category 5 - age 50-60.)

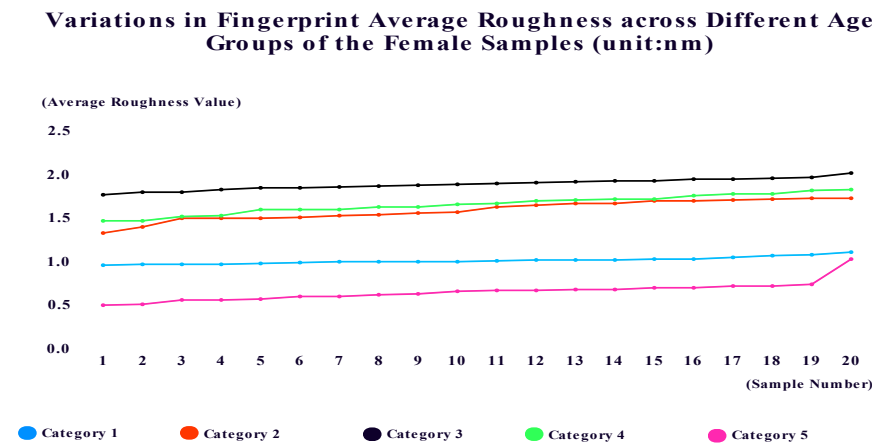
The analysis of latent fingerprint samples using AFM revealed a clear, age-dependent distinction in surface morphology, with statistically significant differences across age groups and between sexes, as shown in the following graph no. 11.



(Graph 11 shows the changes in the ridge average roughness with respect to the different age categories and sexes.)



(Graph 12 shows the difference between the ridge average roughness (unit: nm) with respect to the different age categories of the male samples.)



(Graph 13 shows the difference between the ridge average roughness (unit: nm) with respect to the different age categories of the female samples.)

The above graphs (graphs no. 12 and 13) display the difference in average roughness values between the categories C1 to C5 of both male and female samples and their potential in being a marker for sex determination and in estimating the age of the fingerprint donor. The hypothesis's statistical analyses confirmed the relationship between nanoscale morphological features and the age of the fingerprint donor. Correlation analysis identified strong positive correlations between age and roughness of the sample surface (i.e., $r > 0.75$), particularly in male samples. (Table No. 3)

	M:C1	M:C2	M:C3	M:C4	M:C5	F:C1	F:C2	F:C3	F:C4	F:C5
M:C1	1.000									
M:C2	0.953 *	1.000								
M:C3	0.931 *	0.834	1.000							
M:C4	0.950 *	0.955 *	0.877	1.000						
M:C5	0.949 *	0.921 *	0.904 *	0.899	1.000					
F:C1	0.949 *	0.947 *	0.879	0.974 *	0.934 *	1.000				
F:C2	0.215	0.231	0.301	0.233	0.152	0.166	1.000			
F:C3	-	-	-	-	-	-	0.083	1.00 0		
F:C4	-	-	-	-	-	-	-	0.16 7	1.000	
F:C5	-	-	-	-	-	-	-	0.13 9	-	1.00 0
	†	0.240	†	0.379	0.395	0.347	0.239	0.061		

(Table 3: Correlation test performed on the overall Average Roughness Data of the age categories and sexes. (-1 as a strong negative correlation and *as a denote, 0 as no correlation, and +1 as a strong positive correlation and † as a denote).)

The statistical analysis indicates a strong positive correlation in the male population across several age categories, including M-C1: M-C2, M-C1: M-C3, M-C1: M-C4, M-C1: M-C5, M-C2: M-C4, M-C2: M-C5, and M-C3: M-C5. This suggests that as age increases, roughness also increases. However, a weaker correlation is observed after the C3 category, both in males and females, indicating that the increase in roughness begins to reverse post-adulthood. Notably, a distinct difference in roughness is observed between the male age groups and the corresponding female age categories. Additionally, a weak correlation was found between M-C1:F-C5 and M-C3:F-C5, highlighting the disparity in average roughness between the male and female populations in category 5.

Furthermore, to assess the significance of these findings, a Student's t-test and regression analysis were conducted on the overall dataset to identify factors contributing to the observed variance. The results of the t-test and regression analysis are provided in Tables 4 and 5. Regression models supported the observed trend, showing a significant effect of age on roughness and residue distribution. An ANOVA test further validated significant

differences in roughness across age groups, with P-values indicating strong within-group variance ($p < 0.05$). These statistical findings support the hypothesis that AFM can distinguish age-based variations in fingerprint morphology.

Two-Sample Assuming Equal Variances		
	Male	Female
Mean	1.8727	1.3529
Variance	0.4223	0.2189
Observations	100	100
Pooled Variance	0.3206	
Hypothesized Difference	Mean	0
df	198	
t Stat	6.490	
P(T<=t) one-tail	0.000	
t Critical one-tail	1.6525	
P(T<=t) two-tail	0.000	
t Critical two-tail	1.9720	

(Table 4: T-test performed on the overall average roughness data.)

Regression Statistics	
Multiple R	0.2689
R Square	0.0723
Adjusted R Square	0.0532
Standard Error	14.112
Observations	100

(Table 5: A regression statistics performed on the overall average roughness data.)

The above tables reveal a progressive increase in roughness from early adolescence to adulthood, followed by a subsequent decline in roughness from adulthood to older age, when observed under AFM. To further investigate the intra-group and inter-group variations, statistical analyses – The Analysis of Variance (ANOVA) was conducted on the samples that revealed a significant p value, indicating substantial variance both within and between the groups. The results of these analyses are summarized in Table 6.

Groups	Count	Sum	Average	Variance
Age	100	3550	35.5	210.353
Male	100	187.27	1.872	0.422
Female	100	135.29	1.352	0.218

ANOVA						
Source of Variation	SS	df	MS	F	p-value	F crit
Between Groups	76569.664	2	38284.832	544.347	0.000	3.026
Within Groups	20888.489	297	70.331			
Total	97458.153	299				

(Table 6: ANOVA performed on the overall samples.)

To study the difference between and within the means of the various groups through regression, ANOVA through regression was again performed, and ρ values of the intercept, male, and female populations are stated in Table 7.

	df	SS	MS	F
Regression	2	1506.632	753.316	3.782
Residual	97	19318.367	199.158	
Total	99	20825		
	Coefficients	Standard Error	t Stat	P-value
Intercept	40.694	4.386	9.279	0.000*
Male	14.733	6.629	2.222	0.028*
Female	-24.233	9.207	-2.632	0.009*

(Table 7: Analysis of Variance through the Regression model performed on the overall average roughness data. (*ANOVA through Regression))

The Variance Inducing Factor (VIF) is calculated from the regression analysis performed between the comparison categories, as presented in the following table. The raw data on average roughness, obtained from the Atomic Force Microscopy analysis, was used for this study. Each category was compared with every other category, and the R-squared (R^2) values were derived through regression analysis. The VIF, which quantifies how much the variance of a regression coefficient is inflated due to multicollinearity among the predictor variables, was then calculated using the formula below. The resulting VIF values are summarized in the following Table 8.

$$\text{VIF Value} = 1 / (1 - R^2)$$

Where: R^2 is the R-squared value obtained from the regression of one independent variable on all other independent variables in the model. A higher VIF indicates more multicollinearity, suggesting that the independent variable is highly correlated with other predictors in the model.

Comparison Category*	Sex	R Sq	VIF	Comparison Category*	Sex	R Sq	VIF
C1:C2,C3,C4,C5	Male	0.970	34.010	C1:C2,C3,C4,C5	Female	0.192	1.239
C2:C1,C3,C4,C5	Male	0.958	23.983	C2:C1,C3,C4,C5	Female	0.115	1.130
C3:C1,C2,C4,C5	Male	0.915	11.804	C3:C1,C2,C4,C5	Female	0.116	1.132
C4:C1,C2,C3,C5	Male	0.937	16.082	C4:C1,C2,C3,C5	Female	0.087	1.096
C5:C1,C2,C3,C4	Male	0.918	12.143	C5:C1,C2,C3,C4	Female	0.183	1.224
C1:(C1,C2,C3,C4,C5)	M:F	0.413	1.703	C1:(C1,C2,C3,C4,C5)	F:M	0.140	1.163
C2:(C1,C2,C3,C4,C5)	M:F	0.377	1.606	C2:(C1,C2,C3,C4,C5)	F:M	0.130	1.149
C3:(C1,C2,C3,C4,C5)	M:F	0.362	1.569	C3:(C1,C2,C3,C4,C5)	F:M	0.083	1.091
C4:(C1,C2,C3,C4,C5)	M:F	0.418	1.720	C4:(C1,C2,C3,C4,C5)	F:M	0.351	1.541

C5:(C1,C2,C3,C4,C 5)	M:F	0.48	1.944	C5:(C1,C2,C3,C4,C 5)	F:M	0.34	1.537
		5				9	

Table 8. Variance-Inducing Factor data derived from the Regression model studied between the comparison categories. (*Categories that are compared.)

A Variance Inflation Factor (VIF) greater than 10 indicates strong collinearity among multiple individual variables within a multiple regression model. VIF values between 5 and 10 suggest moderate collinearity, while values between 0 and 5 indicate weaker collinearity, although it still persists. The latent fingerprint samples, analyzed under Atomic Force Microscopy (AFM) across different sexes and age categories, were compared to assess the degree of collinearity between them. The analysis revealed that male samples across all age categories exhibited strong collinearity with other categories when analyzed using a regression model. Whereas the female samples in all categories had VIF values below 2, showing a very weak collinearity. The lowest collinearity was observed when the F-C4 category was compared with all male age categories, while the highest collinearity was found between the M-C1 category and the M-C2, M-C3, M-C4, and M-C5 categories. Additionally, collinearity was examined between the samples grouped by sex, and the corresponding variance inflation factor (VIF) values are presented in Table 9.

Variance Inducing Factor	R Sq	VIF
Age: Male	0.006	1.006
Age: Female	0.025	1.0257
Male: Female	0.891	9.226

Table 9. Variance-inducing factor data derived from the regression model studied between the sexes.

A significantly low collinearity was observed between the groups, as mentioned in the Tables 8 and 9. The AFM data was thoroughly analyzed from all possible perspectives to assess the significance of this research and its potential effectiveness in the analysis of actual crime scene samples. The weaker the collinearity is, the higher the samples' potential to be the age and sex-based markers.

The application of AFM in this study enabled a very precise and non-invasive detection of age-related differences in latent fingerprints by capturing nanoscale surface features. The underlying physics of AFM, including van der Waals force interactions, cantilever deflection, and piezoelectric scanner adjustments, allowed for highly accurate measurements of the surface roughness of the ridges. These results indicate that AFM can provide reliable morphological markers for age estimation, with significant forensic potential for profiling unknown individuals based on fingerprint analysis. This study supports AFM's role in advancing forensic and fingerprint science through age determination, offering a novel dimension to latent fingerprint evaluation.

Conclusion

This study validates the application of atomic force microscopy (AFM) for forensic age estimation of latent fingerprints, leveraging AFM's high-resolution, non-destructive imaging capabilities to examine nanoscale morphological features. By analyzing 200 fingerprint samples across five age groups (10-60 years) using AFM in non-contact mode, significant age-dependent variations in ridge roughness were identified, with peak residue concentration observed in the 30-40 age group. Through statistical analyses—

including ANOVA, t-tests, and correlation studies—a strong positive correlation was confirmed between specific surface characteristics and age, with further variations noted between male and female samples. These findings highlight AFM's potential to distinguish age-specific fingerprint patterns, supporting its utility as a precise, non-invasive forensic tool for sex and age profiling. AFM's ability to capture both the surface roughness without damaging the sample provides an invaluable tool for latent fingerprint analysis and in forensic sciences. This capability enables fingerprint experts to go beyond pattern recognition, accessing molecular-level detail that traditional methods cannot resolve. In latent fingerprint analysis, AFM's non-invasive profiling offers new dimensions in forensic science, particularly for estimating donor age, adding a strategic advantage in criminal profiling and suspect identification.

To expand on this work, future studies could investigate the impact of environmental factors on AFM fingerprint analysis, such as humidity and temperature variations affecting residue stability. Additionally, integrating AFM data into automated fingerprint databases could enhance forensic matching capabilities. This study establishes AFM as a transformative tool in forensic science, merging physics and material science principles to unlock detailed insights into fingerprint residue morphology, thereby extending the evidentiary value of latent fingerprints in forensic investigations.

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