

Vytrus Biotech, S.L.
C/ Sant Gaietà, 121, 2nd Floor
Terrassa (08221)
Barcelona, SPAIN
Tel.: +34 931 278 106
e-mail: info@vytrus.com



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**EVALUATION OF THE
EFFECTIVENESS
OF A HAIR LOTION IN REDUCING
HAIR LOSS**

VYTRUS BIOTECH



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OF A HAIR LOTION IN REDUCING HAIR LOSS**

VYTRUS BIOTECH

Efficacy study no.: ID004-15

Realised by: Centro de Tecnología Capilar, S.L.

Study Managers: Encarnación Gómez
Bsc. Biology
Alfonso Fernández
PhD Chemistry

Technician: Danna Millán

Customer: VYTRUS BIOTECH
C\ Sant Gaietà 121
08221 Terrassa
Barcelona

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I. EVALUATION STUDY

1. TITLE

In vivo clinical trial on human volunteers to assess the effectiveness of a hair lotion in reducing hair loss

2. OBJECTIVE

2.1. PRIMARY OBJECTIVE

To investigate whether the daily application of a lotion produces a reduction in hair loss as well as hair regeneration for 5 months of treatment.

2.2. SECONDARY OBJECTIVE

- ✓ To evaluate the effect produced by the lotion treatment on hair growth.
- ✓ To evaluate the number of hairs in anagen phase for the individuals who participated in the study.
- ✓ To evaluate the number of hairs in telogen phase for the individuals who participated in the study.
- ✓ To evaluate the effect of the treatment under study at different times through the self-perception of characteristics such as increased hair strength, increased vitality, reduced hair loss, increased number of new hairs, as well as issues related to the organoleptic characteristics and tolerance of the lotion

3. EXPERIMENTAL DESIGN

3.1. TYPE OF STUDY

This study was single-center, with 2 intervention group that included 40 subjects in total with hair loss. The study was conducted according to the standard operating procedure of Centro de Tecnología Capilar, S.L. and in compliance with the regulations established in "Guía para investigaciones con seres humanos" (Guidelines for Research on Human Beings) and the guidelines of the Scientific Committee on Consumer Safety (SCCS).

4. LENGTH OF THE STUDY

150 days (5 months)

The next tests were carried out at the following times:

- ✓ T₀
 - General photograph of the hair
 - Trichoscan®
 - Trichogram
 - Combing test

- ✓ T₄₅
 - Combing test

- ✓ T₉₀
 - Trichoscan®
 - Combing test

- ✓ T₁₅₀
 - General photograph of the hair
 - Trichoscan®
 - Trichogram
 - Combing test

5. ATTRIBUTES UNDER STUDY

The following attributes were evaluated in this study:

- ✓ General appearance of the hair
- ✓ Anagen/telogen ratio (Trichogram)
- ✓ Number of hairs extracted during the combing test

6. EQUIPMENT AND MATERIAL

The following items were required for conducting this study:

- ✓ Canon EOS 600D digital camera
- ✓ TrichoScan®, Tricholog GmbH, Germany
- ✓ Dino-Lite Pro Digital Microscope (microcámara), Naarden, Holland
- ✓ Light microscope
- ✓ Pean clamp
- ✓ Disposable plastic capes
- ✓ Slides and coverglasses
- ✓ Canada balsam, Panreac
- ✓ Comb
- ✓ Towels
- ✓ Envelopes

7. VOLUNTEERS

Number of volunteers:	40
Hair type:	Varied
Sex:	31 women and 9 men
Ages:	18 to 65 years

Table 1: Description of the volunteers according to sex and age

Group 1

Volunteer	1	2	3	4	5	6	7	8	9
Sex	M	M	W	W	W	W	W	M	W
Age	30	38	44	58	56	26	53	27	28
Volunteer	10	11	12	13	14	15	16	17	18
Sex	W	W	W	W	W	W	W	W	M
Age	46	43	57	65	52	44	26	55	47
Volunteer	19	20							
Sex	M	W							
Age	44	41							

Group 2

Volunteer	21	22	23	24	25	26	27	28	29
Sex	M	M	M	M	W	W	W	M	W
Age	43	55	54	61	55	27	24	53	23
Volunteer	30	31	32	33	34	35	36	37	38
Sex	W	W	W	W	M	W	W	W	W
Age	42	27	53	51	25	52	52	30	28
Volunteer	39	40							
Sex	W	W							
Age	58	28							

7.1. SELECTION CRITERIA

7.1.1. Inclusion criteria

- healthy volunteers
- Aged 18 to 65
- A/T ratio < 4 (hair loss from different causes)
- Men: degrees II-IV on the Hamilton scale; women: degrees I-II on the Ludwig scale
- Having signed the written consent form and being informed of the study objective
- Enough motivation and availability

7.1.2. Non-inclusion / exclusion criteria

- Capilar treatments in the last three months
- Correction of hair loss by surgery
- Scalp diseases: psoriasis, dermatitis, etc.
- Allergy or hypersensitivity to any of the ingredients of the products tested
- Treatment with medication that may cause alopecia: fluoxetine, anticoagulants, retinoids, etc.
- Use of nutricosmetics for reducing hair loss
- Modification of the normal diet: hypocaloric diet, Atkins diet, etc.

7.2. CONSENT

Each volunteer was informed orally and in writing about the characteristics and objectives of the study. Each volunteer signed a written consent form and received a copy, with another copy kept on file at C.T.C

7.3. INCIDENTS AND WITHDRAWALS

Group 1: Volunteer 10 has withdrawn the treatment.

Group 2: Volunteers 32 and 40 have withdrawn the treatment.

8. FORMULAS

8.1. PRODUCT UNDER STUDY

Product 1:

Ref. 151002iD1-SH

INCIs: Aqua; Alcohol Denat; Curcuma Longa Callus Culture Conditioned Media;
Phenoxyethanol

Product 2:

Ref. 151058iD1-SH

INCIs: Aqua; Alcohol Denat

Manufactured: Vytrus Biotech

8.2. ASSIGNMENT OF SAMPLES

Group 1: Product 1 (Ref. 151002iD1-SH)

Group 2: Product 2 (Ref. 151058iD1-SH)

9. PROTOCOL

9.1. BEFORE THE START OF THE STUDY (T₋₂)

The volunteers did not wash their hair 48 hours prior to the first visit to C.T.C., and they did not comb their hair 24 hours prior to this visit (T₀).

9.2. T₀

During the first visit to C.T.C., one specialist carried out the following:

- ✓ General photograph of the hair
- ✓ Trichoscan®
- ✓ Trichogram
- ✓ Combing test

Each volunteer received:

- ✓ 250 mL of anti-hair loss lotion and product application instructions
- ✓ An appointment for the next visit to C.T.C. (T₄₅)

9.3. IN THE VOLUNTEERS' HOMES

The volunteers applied the lotion according to the indicated guidelines:

- ✓ Apply on the product daily, preferably at night.
- ✓ Gently massage in with fingertips to aid absorption.
- ✓ Not rinse.

9.4. T₄₅

The volunteers did not wash their hair 48 hours prior to the visit to C.T.C., and they did not comb their hair 24 hours prior to this visit (T₄₅).

In this visit to C.T.C., the following tests were carried out

- ✓ Combing test on each volunteer

Each volunteer received:

- ✓ 250 mL of anti-hair loss lotion and product application instructions
- ✓ An appointment for the next visit to C.T.C. (T₉₀)

9.5. T₉₀

The volunteers did not wash their hair 48 hours prior to the visit to C.T.C., and they did not comb their hair 24 hours prior to this visit (T₉₀).

In this visit to C.T.C., the following tests were carried out

- ✓ Trichoscan®
- ✓ Combing test

Each volunteer received:

- ✓ 250 mL of anti-hair loss lotion and product application instructions
- ✓ An appointment for the next visit to C.T.C. (T_{150})

9.6. T_{150}

The volunteers did not wash their hair 48 hours prior to the visit to C.T.C., and they did not comb their hair 24 hours prior to this visit (T_{150}).

In this visit to C.T.C., the following tests were carried out

- ✓ General photograph of the hair
- ✓ Trichoscan®
- ✓ Trichogram
- ✓ Combing test

10. TESTS CARRIED OUT

10.1 GENERAL PHOTOGRAPH OF THE HAIR

In the visits at the start of the study (T_0) and at the end (T_{150}) several photographs were taken of the upper part of the scalp. The images show the temporal and vertex areas (crown) of all the volunteers.

The objective was to see whether the treatment under study visually improves the quality and appearance of the hair.

10.2 TRICHOGRAM

This involves extracting a certain number of hairs by plucking them from the scalp for the purpose of determining which phase of the hair growth cycle they are in as well as the initial and final appearance of the hair bulbs.

In general, the trichogram is useful for studying the physiology and the growth phase of the hair roots before the treatment in order to compare it with the end of the study to see whether the growth phases have increased.

The trichogram is a “snapshot” of the condition of the hair roots or bulbs, and therefore, of the scalp at a certain time. Depending on the results, it shows whether the treatment has had a positive effect on the hair bulbs.

This study attempted to discover whether the regeneration treatment is capable of improving the initial trichogram of the scalp and whether an improvement is made in the physiology of the hair bulbs and sheaths.

10.2.1 Protocol

This was carried out at the start (T_0) and at the end of the study (T_{150}).

As a precondition, the volunteers were neither to have washed nor treated their hair with any cosmetic product at least 48 hours before the trichogram was carried out, in order to maintain the hairs which are near the end of telogen phase and avoid the artificial reduction in the percentage of telogenic hairs observed in the trichogram. The left parieto-occipital area was selected for extracting hairs.

Plucking was repeated as many times as necessary to obtain the number of hairs needed for the trichogram – between 15 and 25 hairs in a cosmetic study, approximately. Immediately after epilation, the hair bulbs were mounted on glass slides for microscopic study.

10.2.2 Expression of results

The following were calculated:

A_0	Number of hairs in anagen phase at T_0 (before the treatment)
T_0	number of hairs in telogen phase at T_0 (before the treatment)
$\%A_0$	Percentage of hairs in anagen phase at T_0 (before the treatment)
$\%T_0$	Percentage of hairs in telogen phase at T_0 (before the treatment)
A_0/T_0	Ratio between hairs in anagen phase and in telogen phase at T_0
A_{150}	Number of hairs in anagen phase at T_{150} (after 150 days of treatment)
T_{150}	Number of hairs in telogen phase at T_{150} (after 150 days of treatment)
$\%A_{150}$	Percentage of hairs in anagen phase at T_{150} (after 150 days of treatment)
$\%T_{150}$	Percentage of hairs in telogen phase at T_{150} (after 150 days of treatment)

A_{150}/T_{150} Ratio between hairs in anagen phase and in telogen phase at T_{150}

10.2.3 Criteria for evaluating results

In order to evaluate the results obtained in the study, the trichogram values considered “normal” in the literature consulted were used as a reference. According to Camacho and Montagna, normal trichogram values for a healthy adult are as follows:

Phase of the hair cycle	% of hairs
Anagen	86
Catagen	1
Telogen	13

These values provide an anagen/telogen ratio of approximately 6.

However, it is very possible that these values should be revised. Indeed, as confirmed in recent dermatology conferences and through our experience, nowadays it is not frequent to find such a high proportion of hairs in anagen phase. Currently, the most common trichograms show a definite increase in hairs in telogen phase and a slight increase in hairs in catagen phase, which is why the anagen/telogen ratio is usually lower than 6.

This observation is already being applied in efficacy studies of cosmetic products for hair loss. For example, the efficacy study on the active Prodhyhair concentr  , from Laboratoires Prod'Hyg, accepts an anagen/telogen ratio value greater than or equal to 4 as “normal.” Consequently, this study will consider the anagen/telogen ratio normal if $A/T \geq 4$.

10.3 HAIR DENSITY

This is defined as the number of hairs present in a unit of area (generally cm^2). Increased hair density at the end of the study is invaluable proof of the efficacy of the regeneration treatment.

10.3.1. Protocol

The number of hairs present by unit of area was calculated from images of the scalp taken with the TrichoScan[®] microcamera at T₀, T₉₀ and T₁₅₀.

The computer program associated with TrichoScan[®] was used for this, permitting manual counting of the number of hairs present in an area equaling 0.25cm² of scalp. For comparison between the number of hairs present at the end and at the start of the study, the increase in the number of hairs in that area was established and hair density could be calculated at the end of the study.

10.4 PHOTOGRAPH OF BULBS

The photograph of the hair bulbs plucked for the trichogram:

Confirmed the results of the trichogram by viewing the hair bulbs at the start and at the end of treatment, and thus ascertained its efficacy in the appearance and development of the bulbs.

10.5 COMBING TEST

The number of hairs falling out during hair combing under standardized conditions was determined.

After the regeneration treatment under study, the goal was for the number of hairs falling out during combing to be lower than the initial value

10.5.1. Protocol

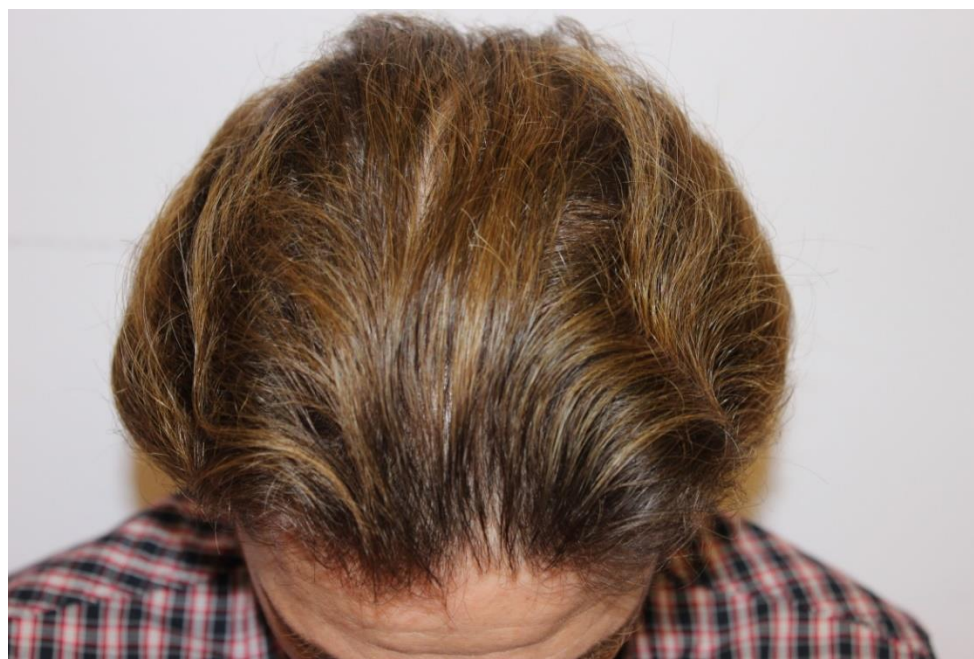
This was carried out at T₀, T₄₅, T₉₀ and T₁₅₀. As a precondition, the volunteers came to the visit without having washed their hair at least 48 hours before and without having combed their hair at least 24 hours before, in order to maintain the hairs which are near the end of telogen phase and avoid artificial reduction in the percentage of hairs in telogen phase.

The technician combed each volunteer's hair. The hair falling out – in the comb and on the cape – was collected for later counting.

II RESULTS

1. GENERAL PHOTOGRAPHY

1.1 GROUP 1



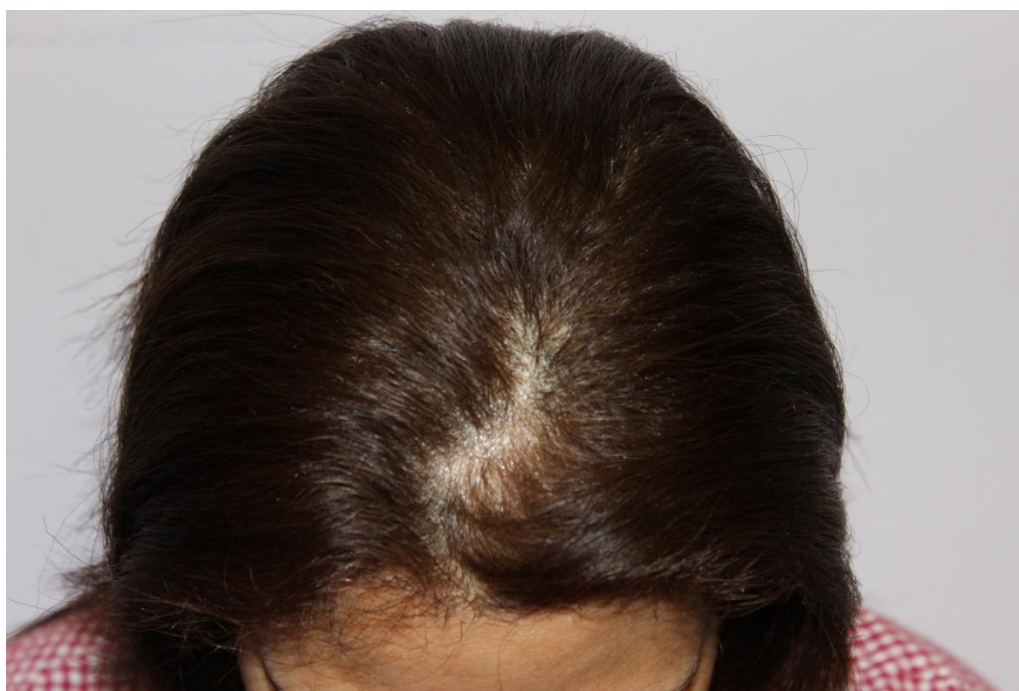
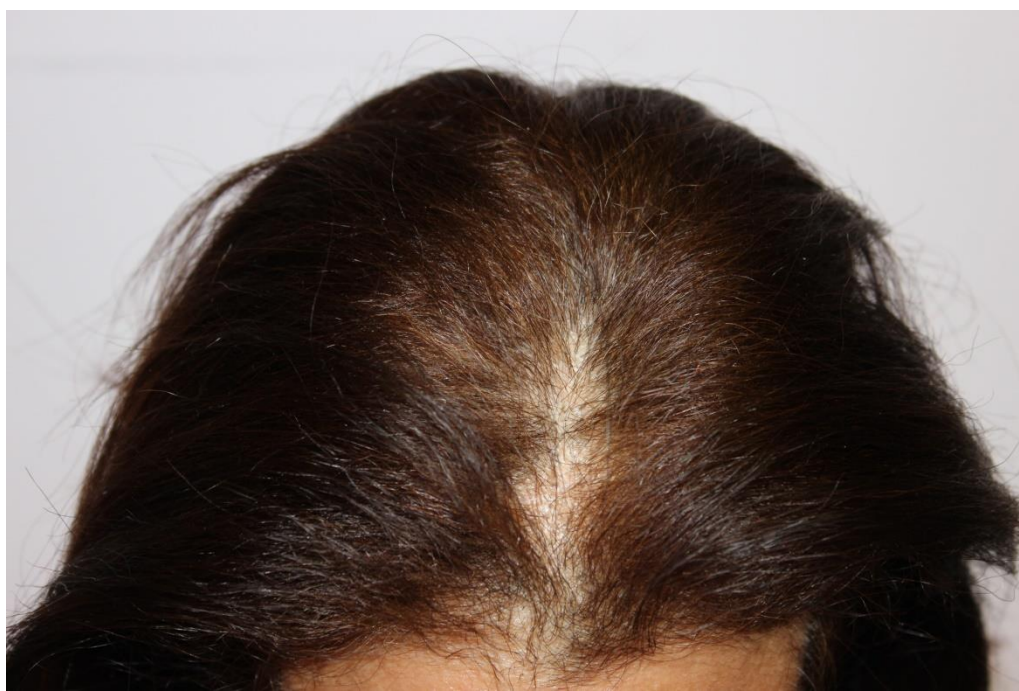
Volunteer 2

General photography at T_0 and T_{150}



Volunteer 19

General photography at T₀ and T₁₅₀



Volunteer 20

General photography at T₀ and T₁₅₀

1.2 GROUP 2



Volunteer 22

General photography at T₀ and T₁₅₀



Volunteer 25

General photography at T₀ and T₁₅₀



Volunteer 28

General photography at T₀ and T₁₅₀

2. TRICHOGRAM

2.1. RATIO BETWEEN HAIRS IN ANAGEN PHASE AND IN TELOGEN PHASE

2.1.1. Group 1

VOLUNTEERS	T ₀		RATIO A ₀ / T ₀	T ₁₅₀		RATIO A ₁₅₀ / T ₁₅₀
	N°HAIRS ANAGEN PHASE	N°HAIRS TELOGEN PHASE		N°HAIRS ANAGEN PHASE	N°HAIRS TELOGEN PHASE	
1	15	9	1,7	15	8	1,9
2	7	4	1,8	12	6	2,0
3	9	4	2,3	11	2	5,5
4	7	3	2,3	10	5	2,0
5	11	5	2,2	11	5	2,2
6	12	5	2,4	14	6	2,3
7	12	4	3,0	15	6	2,5
8	11	3	3,7	16	6	2,7
9	11	5	2,2	14	2	7,0
10	10	3	3,3	-	-	-
11	11	4	2,8	15	7	2,1
12	9	4	2,3	14	5	2,8
13	20	5	4,0	25	4	6,3
14	11	3	3,7	19	4	4,8
15	15	7	2,1	14	6	2,3
16	19	7	2,7	15	5	3,0
17	18	6	3,0	21	4	5,3
18	11	4	2,8	17	6	2,8
19	16	10	1,6	16	5	3,2
20	11	5	2,2	13	3	4,3
		Average	2,6 ± 0.6		Average	3,3 ± 1.4

The values in red (highest and lowest value) are not taken into account for the mean value

2.1.2. Group 2

VOLUNTEERS	T ₀		RATIO A ₀ / T ₀	T ₁₅₀		RATIO A ₁₅₀ / T ₁₅₀
	N°HAIRS ANAGEN PHASE	N°HAIRS TELOGEN PHASE		N°HAIRS ANAGEN PHASE	N°HAIRS TELOGEN PHASE	
21	14	5	2,8	16	7	2,3
22	16	6	2,7	16	9	1,8
23	11	7	1,6	8	5	1,6
24	13	6	2,2	17	9	1,9
25	16	8	2,0	15	8	1,9
26	12	6	2,0	17	8	2,1
4 27	12	5	2,4	13	5	2,6
28	13	6	2,2	17	9	1,9
29	12	5	2,4	14	6	2,3
30	13	3	4,3	15	6	2,5
31	12	6	2,0	14	8	1,8
32	11	5	2,2	-	-	-
33	11	5	2,2	13	7	1,9
34	10	5	2,0	9	6	1,5
35	14	7	2,0	14	6	2,3
36	12	5	2,4	14	6	2,3
37	13	7	1,9	15	7	2,1
38	4	1	4,0	7	4	1,8
39	11	5	2,2	13	6	2,2
40	7	1	7,0	-	-	-
		Average	2,4 ± 0.7		Average	2,0 ± 0.3

The values in red (highest and lowest value) are not taken into account for the mean value

2.2. INCREASE OF ANAGEN PHASE (ΔFA) AND REDUCTION OF TELOGEN PHASE (ΔFT)

2.2.1. Group 1

VOLUNTEERS	ANAGENIC PHASE			TELOGEN PHASE		
	FA_0	FA_{150}	ΔFA_{150}	FT_0	FT_{150}	ΔFT_{150}
1	62,5%	65,2%	4,3%	37,5%	34,8%	-7,2%
2	63,6%	66,7%	4,8%	36,4%	33,3%	-8,3%
3	69,2%	84,6%	22,2%	30,8%	15,4%	-50,0%
4	70,0%	66,7%	-4,8%	30,0%	33,3%	11,1%
5	68,8%	14,0%	-79,6%	31,3%	6,0%	-80,8%
6	70,6%	70,0%	-0,8%	29,4%	30,0%	2,0%
7	75,0%	71,4%	-4,8%	25,0%	28,6%	14,3%
8	78,6%	72,7%	-7,4%	21,4%	27,3%	27,3%
9	68,8%	87,5%	27,3%	31,3%	12,5%	-60,0%
10	76,9%	-	-	23,1%	-	-
11	73,3%	68,2%	-7,0%	26,7%	31,8%	19,3%
12	69,2%	73,7%	6,4%	30,8%	26,3%	-14,5%
13	80,0%	86,2%	7,8%	20,0%	13,8%	-31,0%
14	78,6%	82,6%	5,1%	21,4%	17,4%	-18,8%
15	68,2%	70,0%	2,7%	31,8%	30,0%	-5,7%
16	73,1%	75,0%	2,6%	26,9%	25,0%	-7,1%
17	75,0%	84,0%	12,0%	25,0%	16,0%	-36,0%
18	73,3%	73,9%	0,8%	26,7%	26,1%	-2,2%
19	61,5%	76,2%	23,8%	38,5%	23,8%	-38,1%
20	68,8%	81,3%	18,2%	31,3%	18,8%	-40,0%
Average	71,3± 0.1%	74,6± 0.1%	5,1± 0.1%	28,7± 0.1%	24,1± 0.1%	-16,0± 0.2%

The values in red (highest and lowest value) are not taken into account for the mean value

2.2.2. Group 2

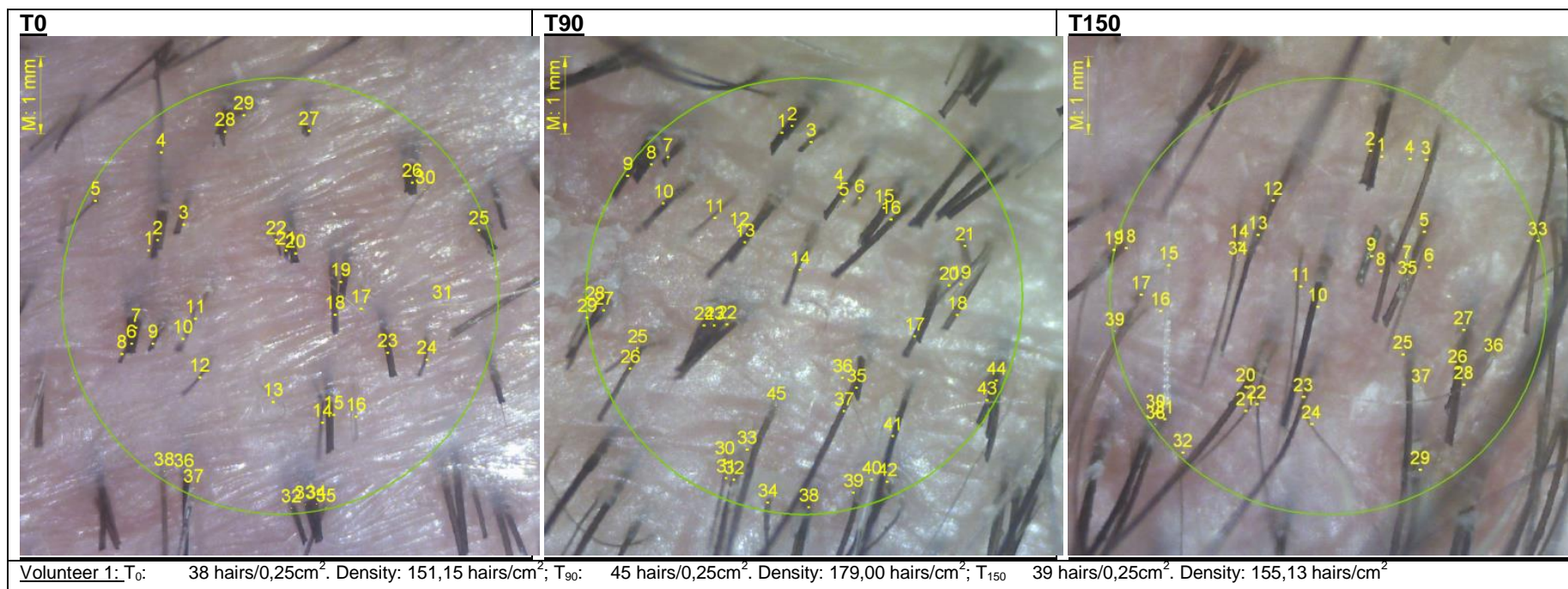
VOLUNTEERS	ANAGENIC PHASE			TELOGEN PHASE		
	FA ₀	FA ₁₅₀	ΔFA ₁₅₀	FT ₀	FT ₁₅₀	ΔFT ₁₅₀
21	73,7%	69,6%	-5,6%	26,3%	30,4%	13,5%
22	72,7%	64,0%	-12,0%	27,3%	36,0%	24,2%
23	61,1%	61,5%	0,7%	38,9%	38,5%	-1,1%
24	68,4%	65,4%	-4,4%	31,6%	34,6%	8,8%
25	66,7%	65,2%	-2,2%	33,3%	34,8%	4,2%
26	66,7%	68,0%	2,0%	33,3%	32,0%	-4,2%
27	70,6%	72,2%	2,3%	29,4%	27,8%	-5,9%
28	68,4%	65,4%	-4,4%	31,6%	34,6%	8,8%
29	70,6%	70,0%	-0,8%	29,4%	30,0%	2,0%
30	81,3%	71,4%	-12,1%	18,8%	28,6%	34,4%
31	66,7%	63,6%	-4,5%	33,3%	36,4%	8,3%
32	68,8%	-	-	31,3%	-	-
33	68,8%	65,0%	-5,5%	31,3%	35,0%	10,7%
34	66,7%	60,0%	-10,0%	33,3%	40,0%	16,7%
35	66,7%	70,0%	5,0%	33,3%	30,0%	-11,1%
36	70,6%	70,0%	-0,8%	29,4%	30,0%	2,0%
37	65,0%	68,2%	4,9%	35,0%	31,8%	-10,0%
38	80,0%	63,6%	-20,5%	20,0%	36,4%	45,0%
39	68,8%	68,4%	-0,5%	31,3%	31,6%	1,0%
40	87,5%	-	-	12,5%	-	-
Average	70,1± 0.0%	66,8± 0.0%	-3,3± 0.1%	29,9± 0.0%	33,2± 0.0%	7,1± 0.1%

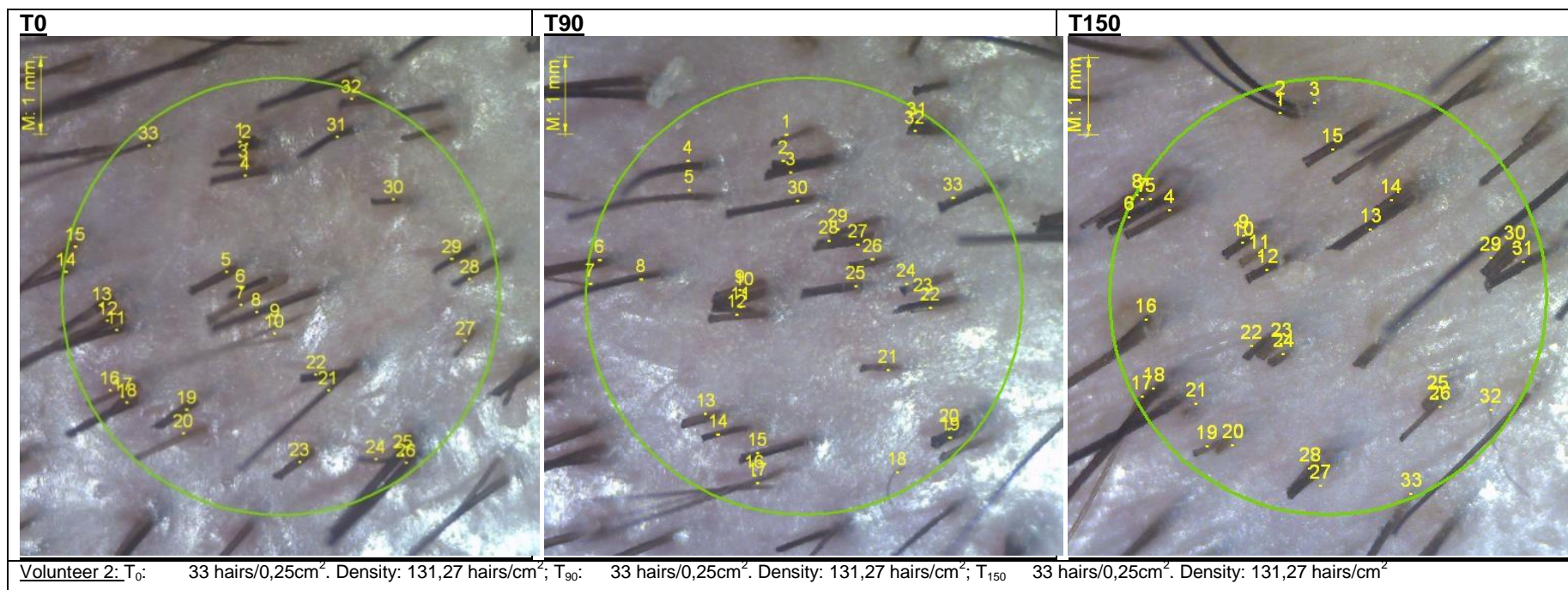
The values in red (highest and lowest value) are not taken into account for the mean value

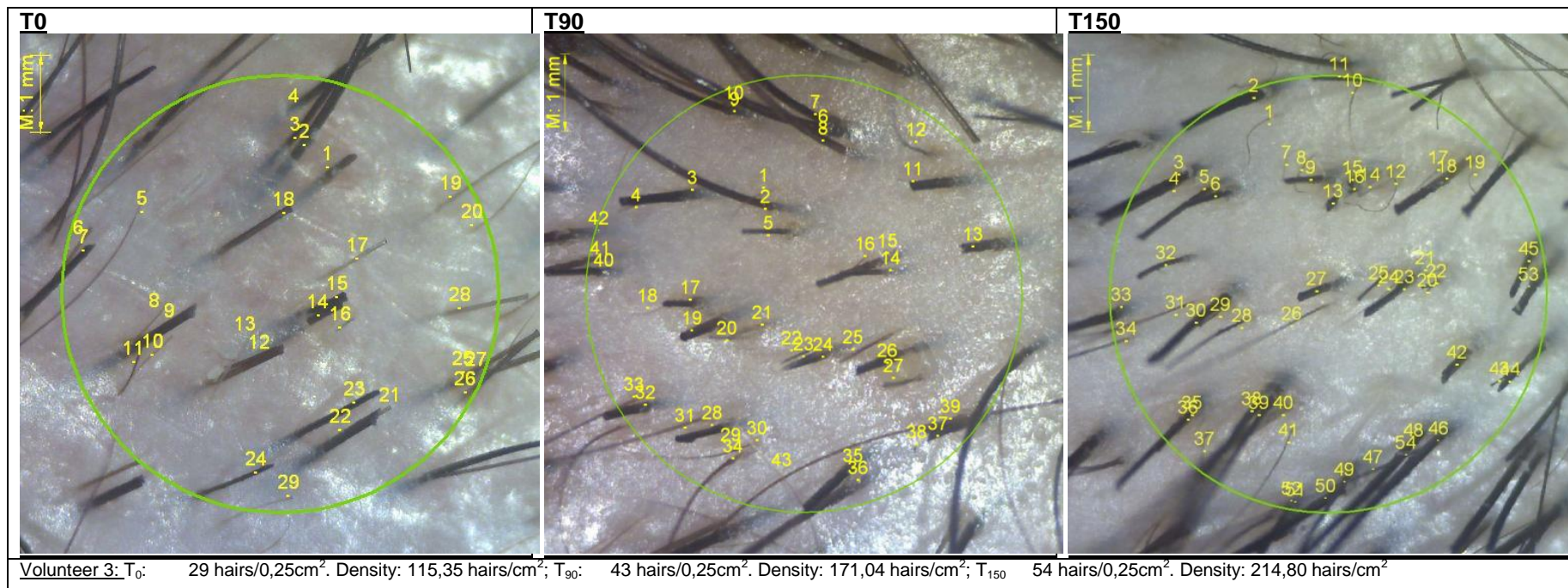
3. HAIR DENSITY

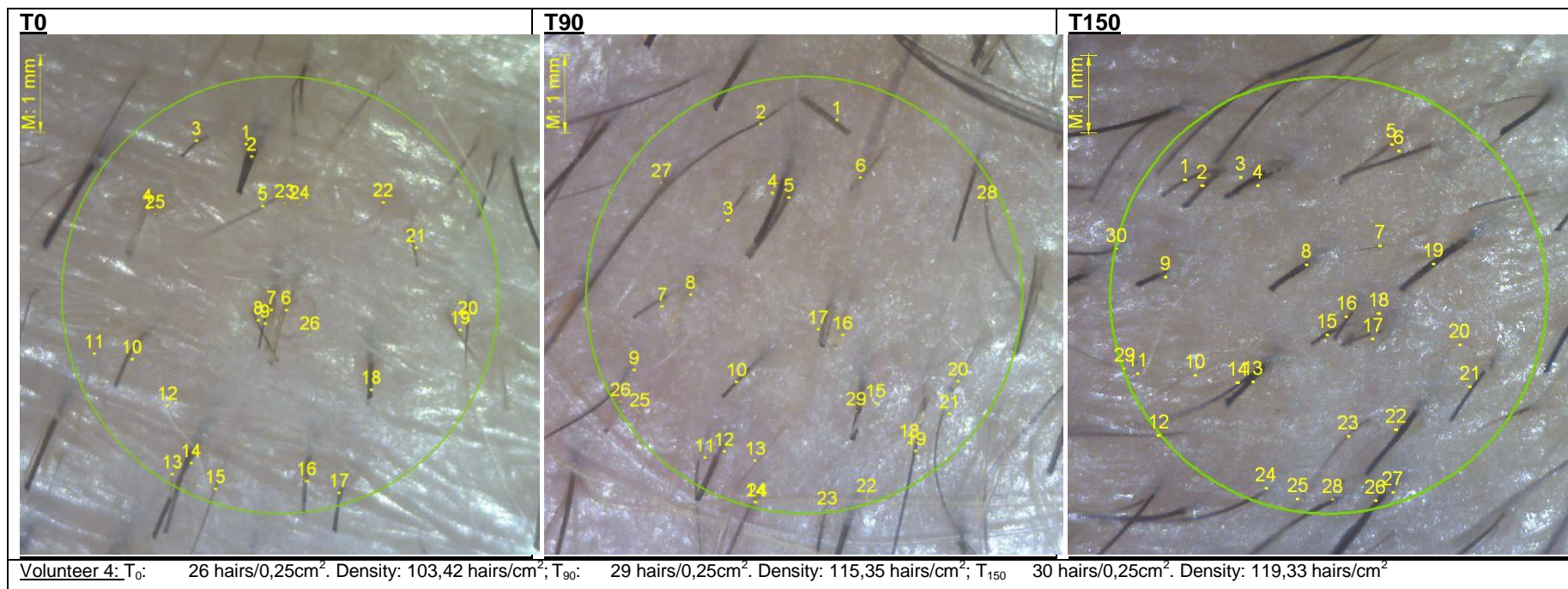
3.1. HAIR DENSITY IMAGES

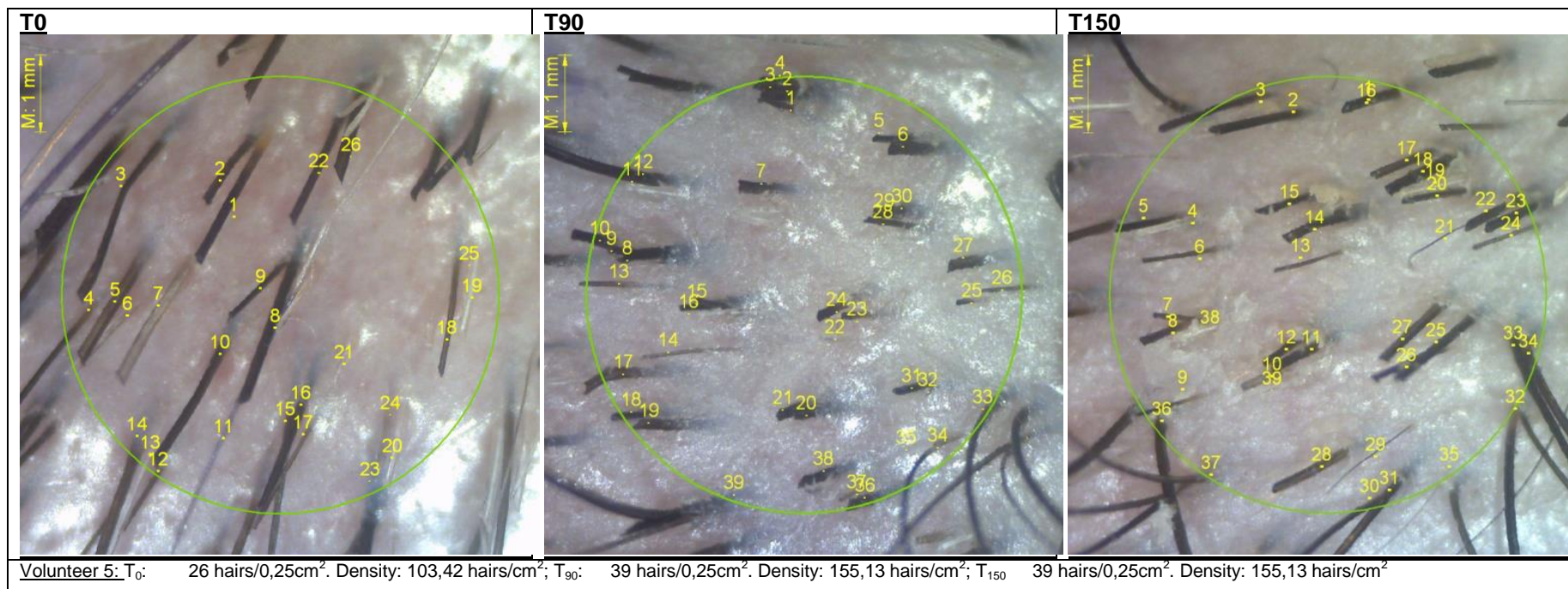
3.1.1. Group 1

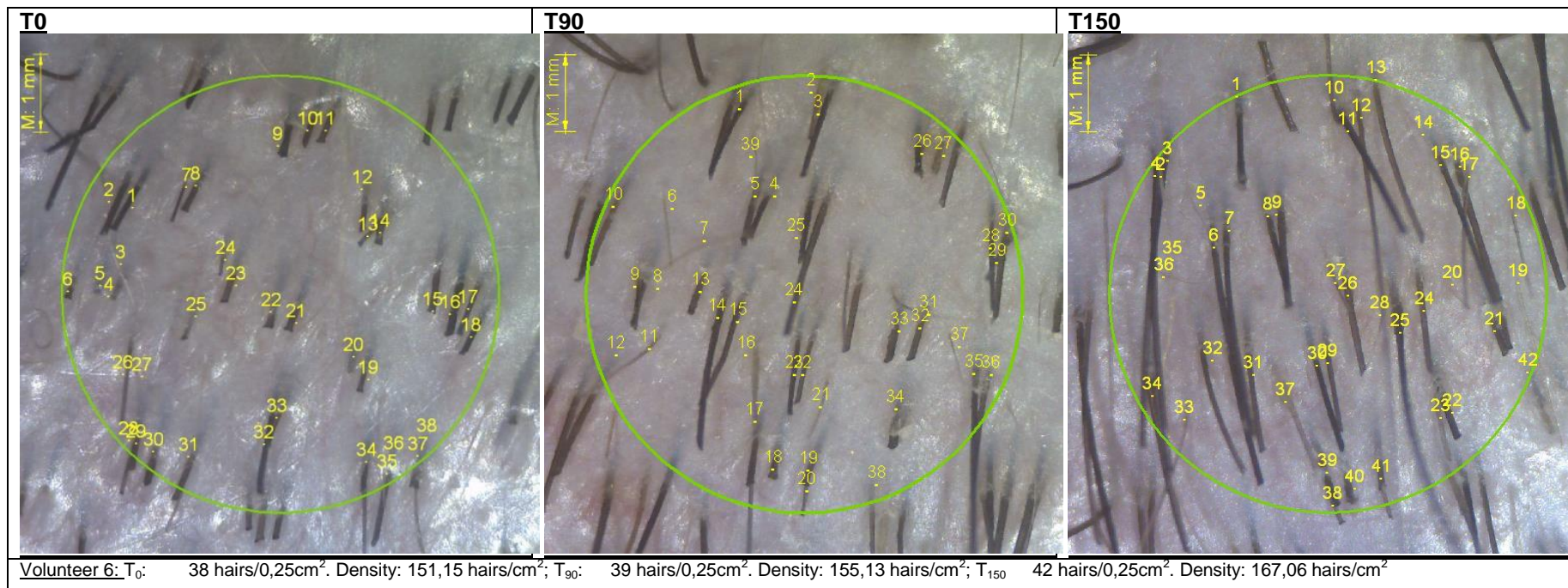


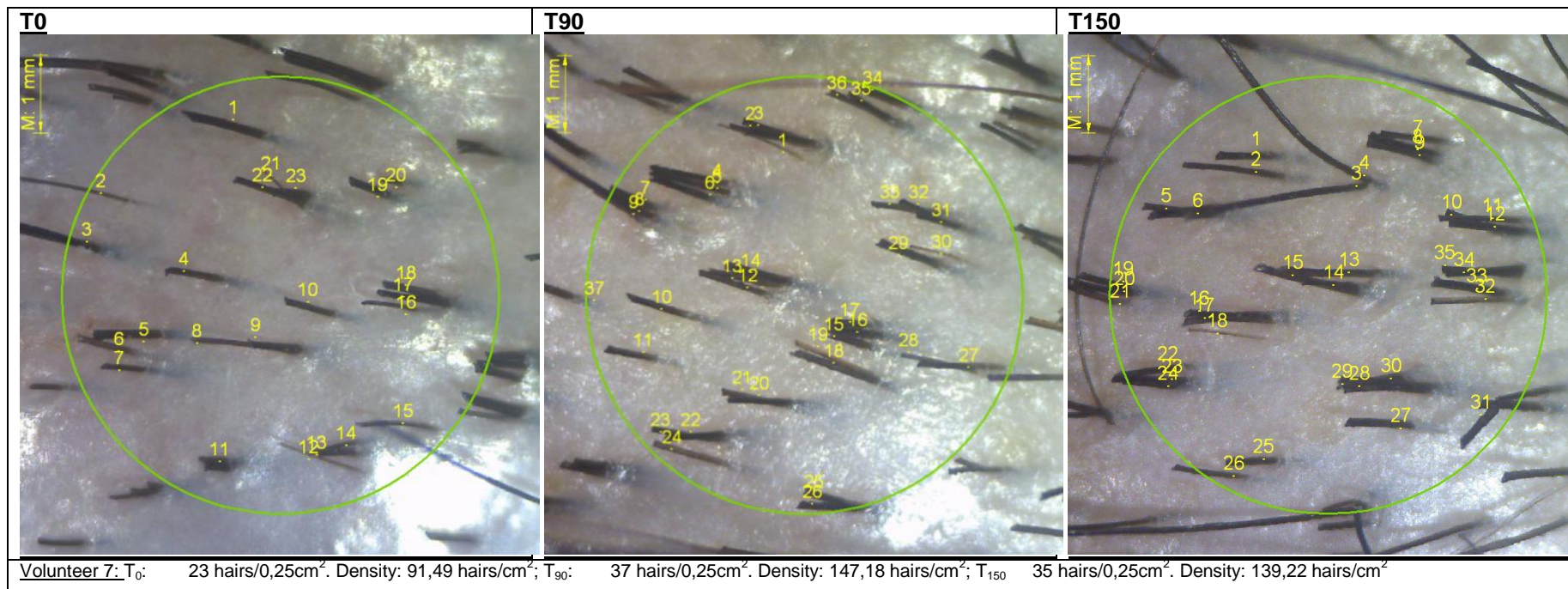


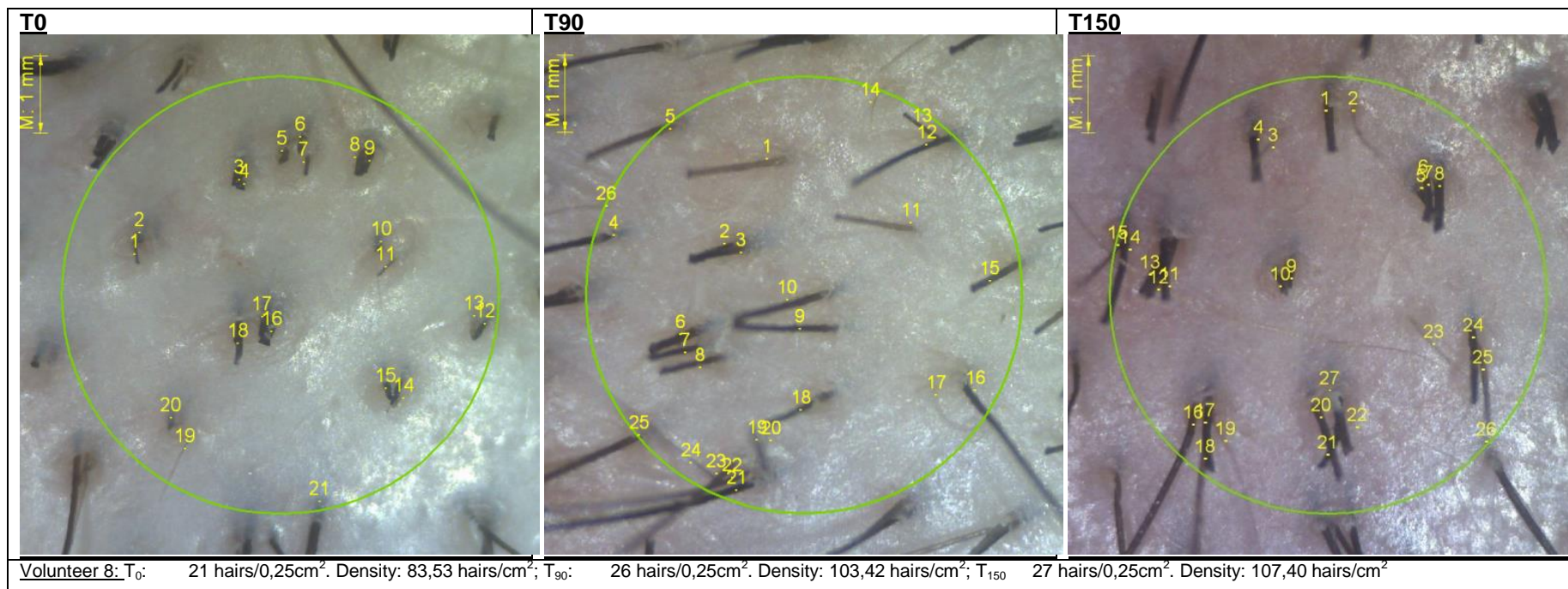


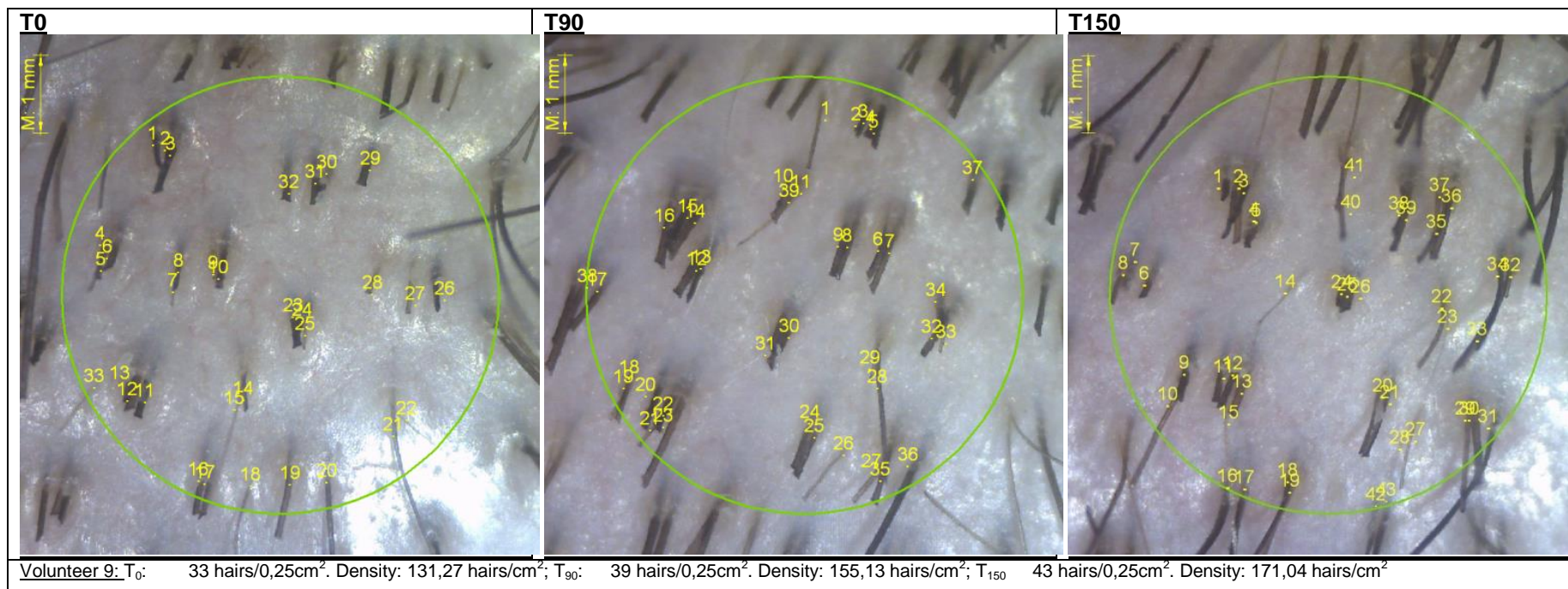


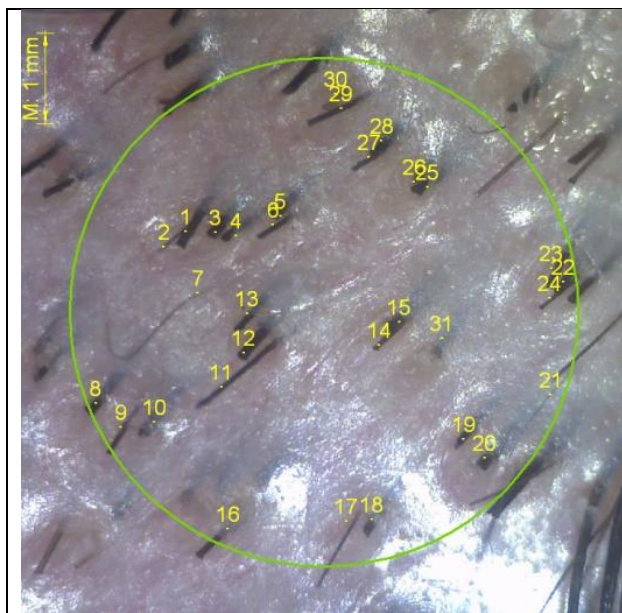












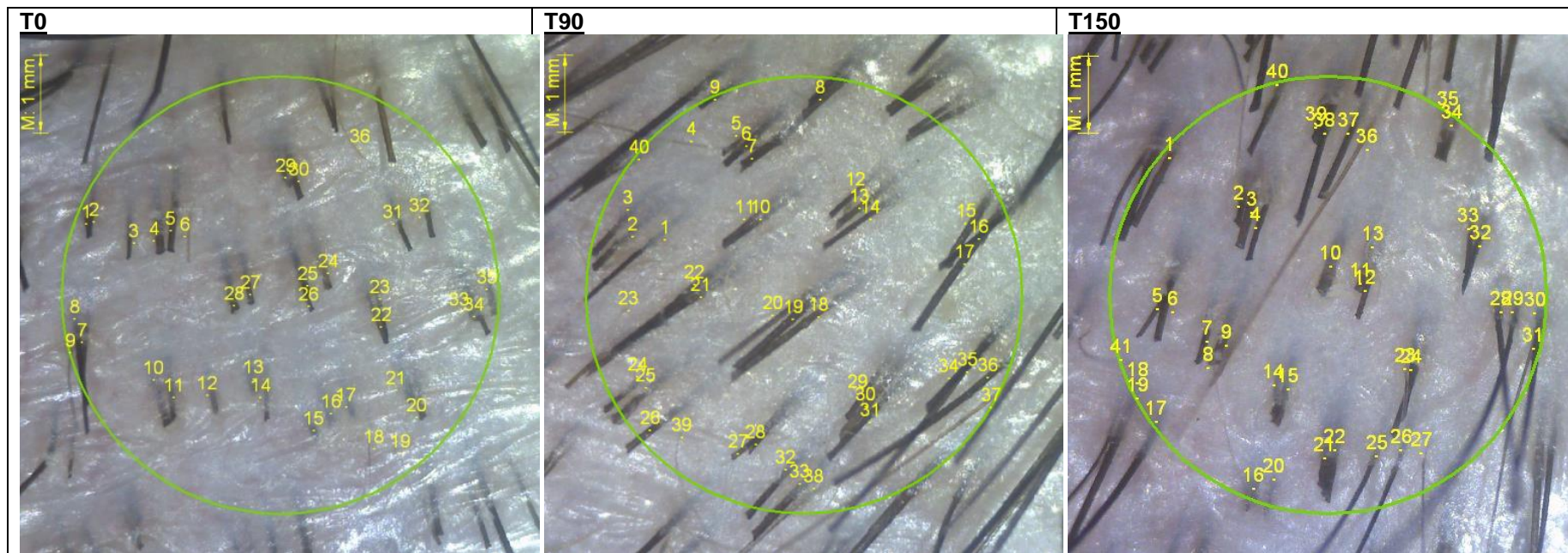
T90

Volunteer has withdrawn the treatment

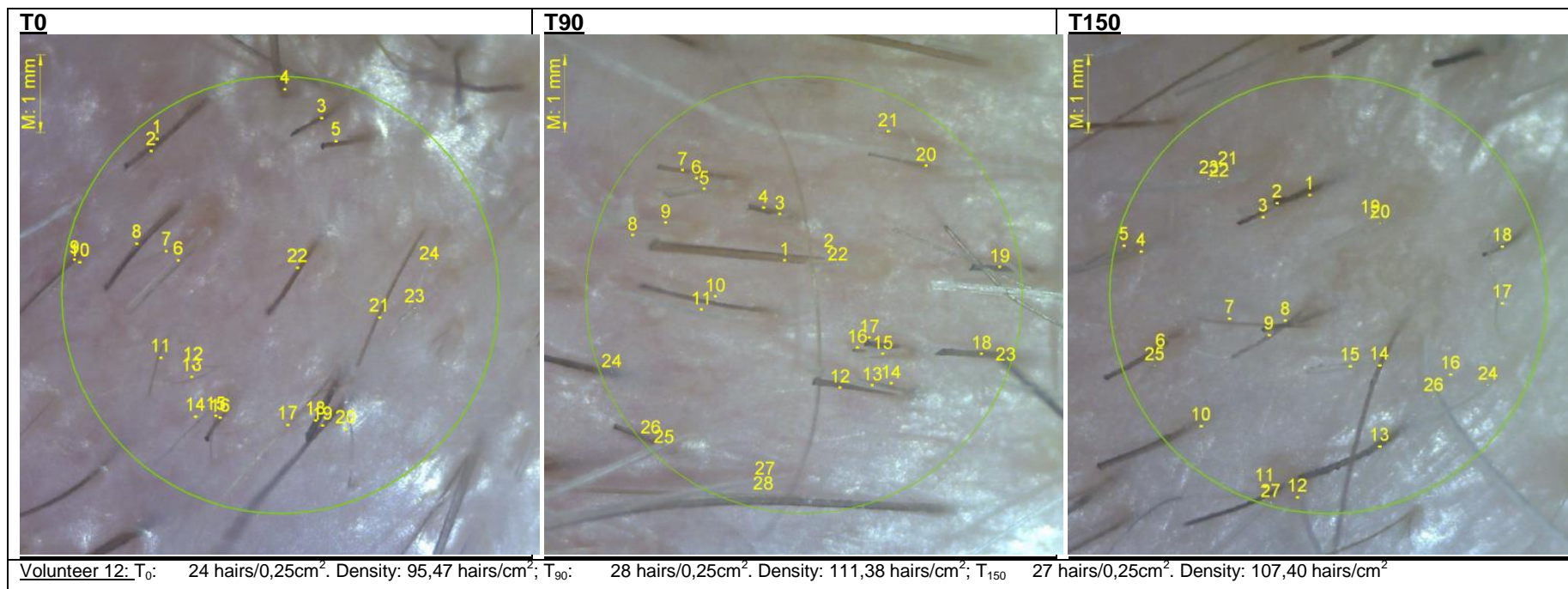
T150

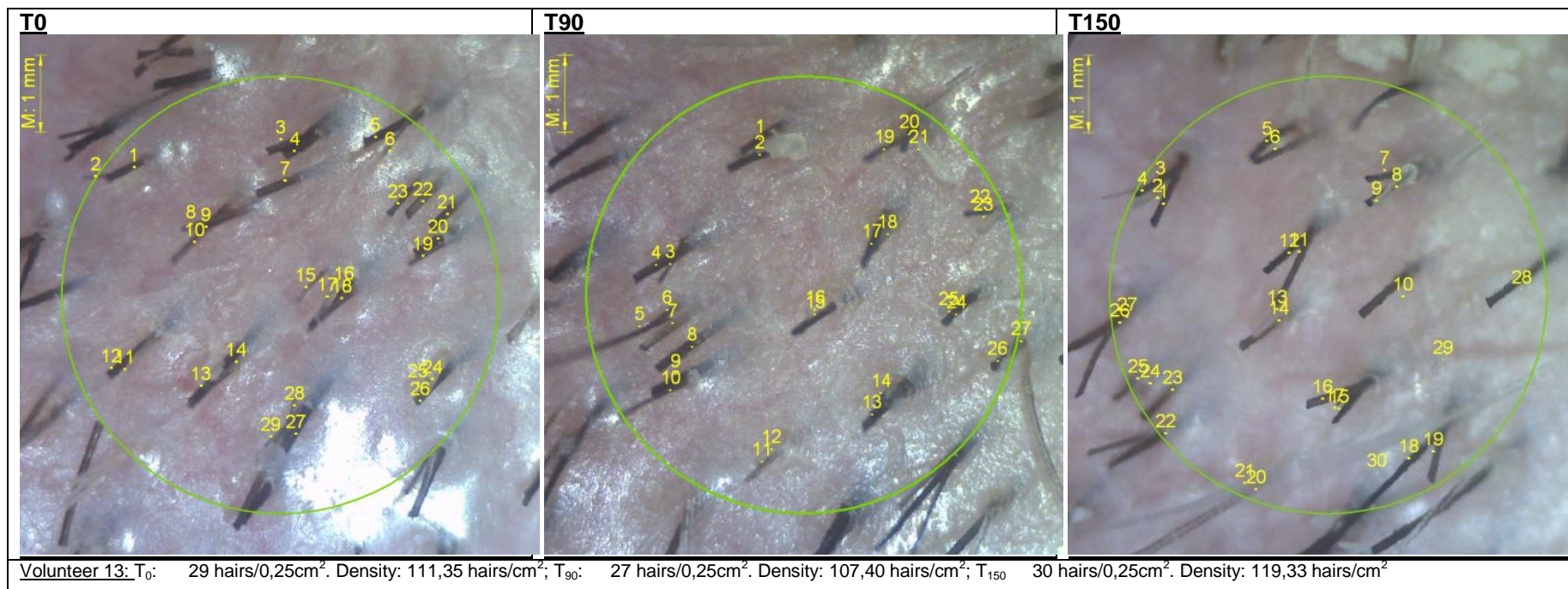
Volunteer has withdrawn the treatment

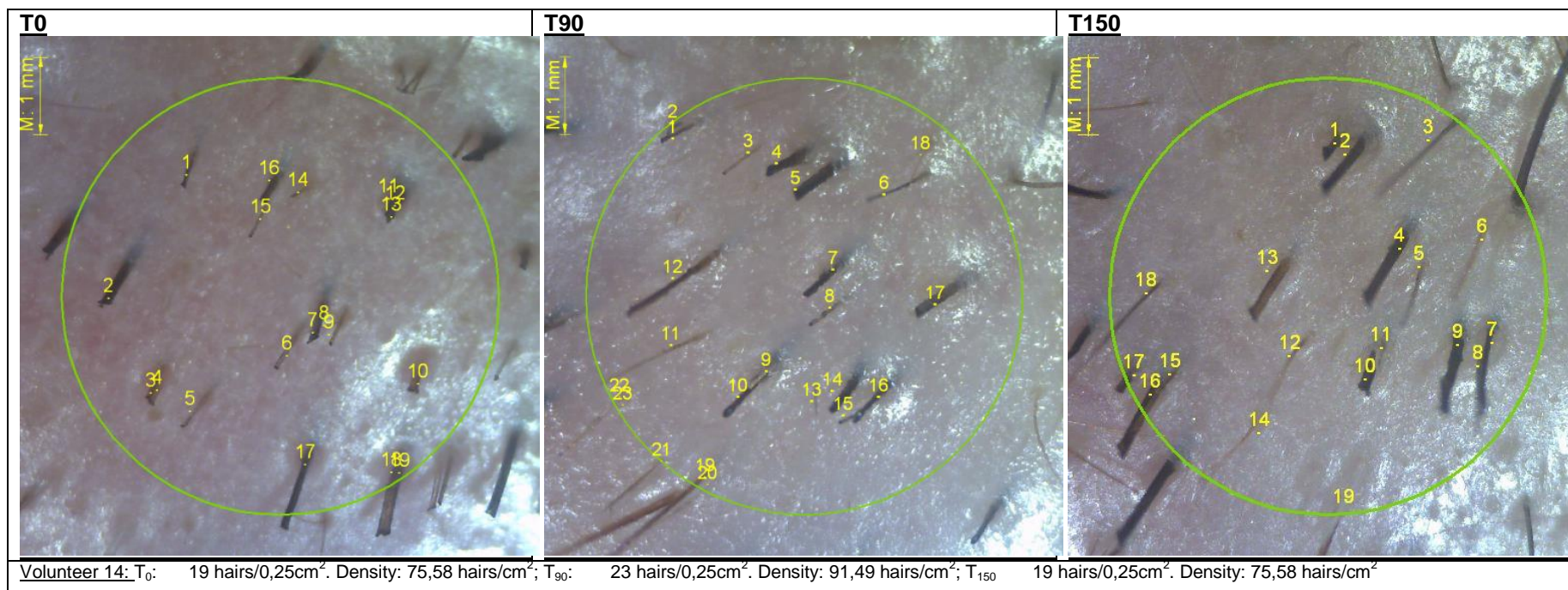
Volunteer 10: T₀: 31 hairs/0,25cm². Density: 123,31 hairs/cm²

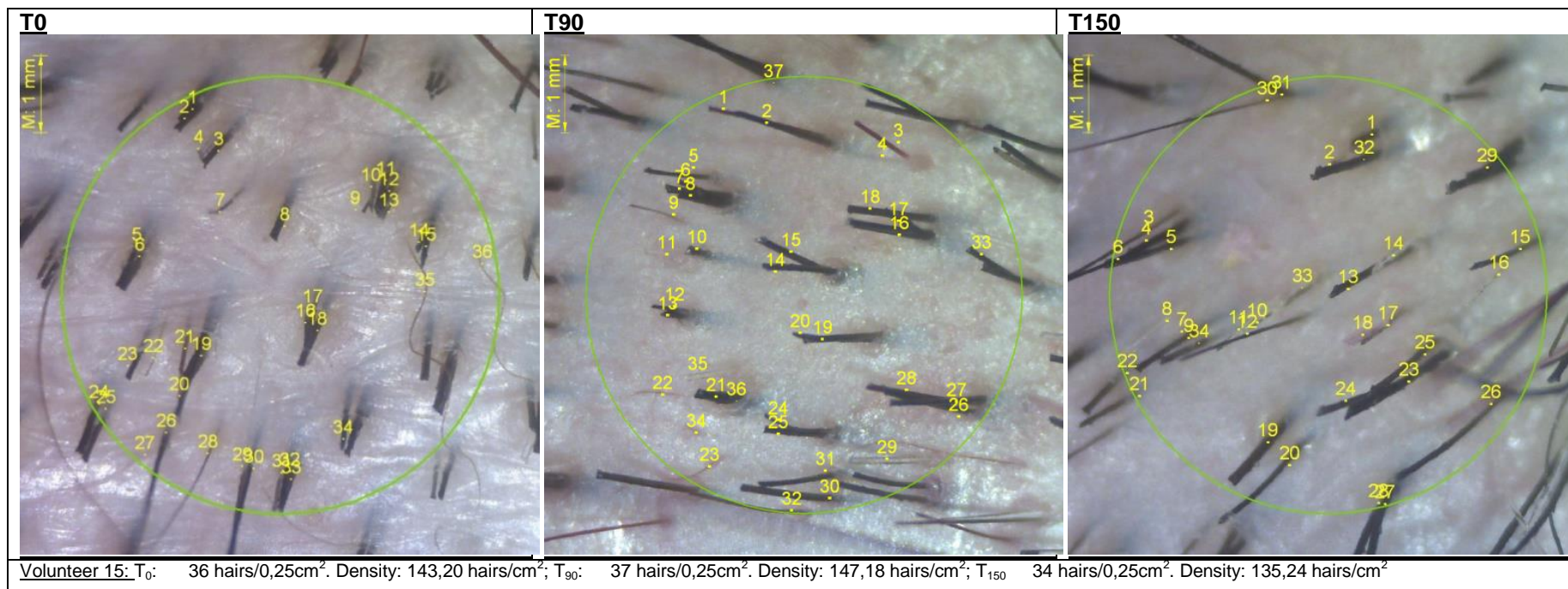


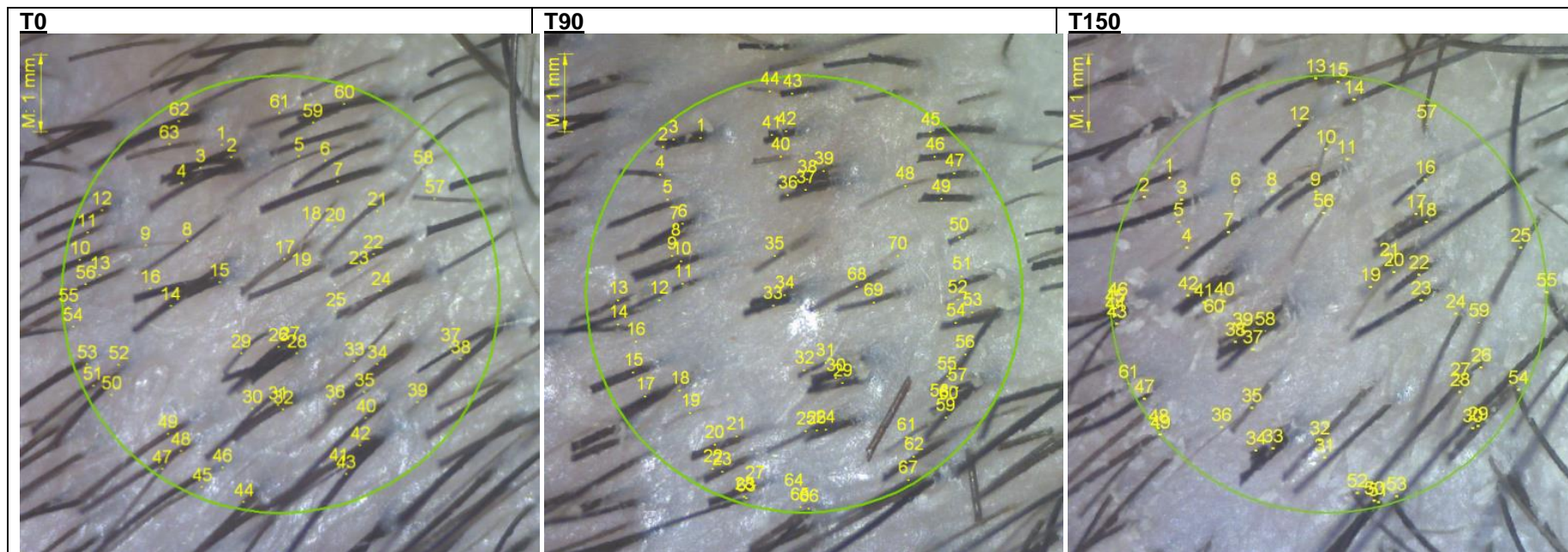
Volunteer 11: T₀: 36 hairs/0,25cm². Density: 143,20 hairs/cm²; T₉₀: 40 hairs/0,25cm². Density: 159,11 hairs/cm²; T₁₅₀: 41 hairs/0,25cm². Density: 163,09 hairs/cm²



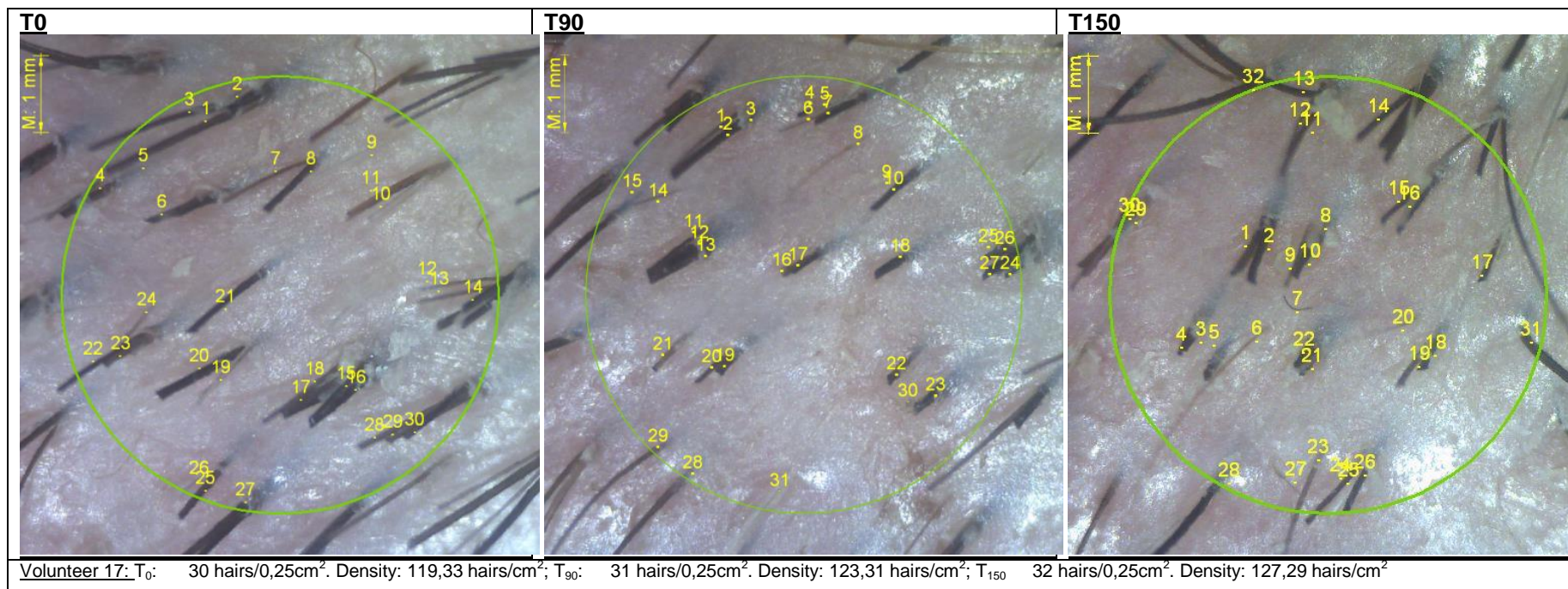


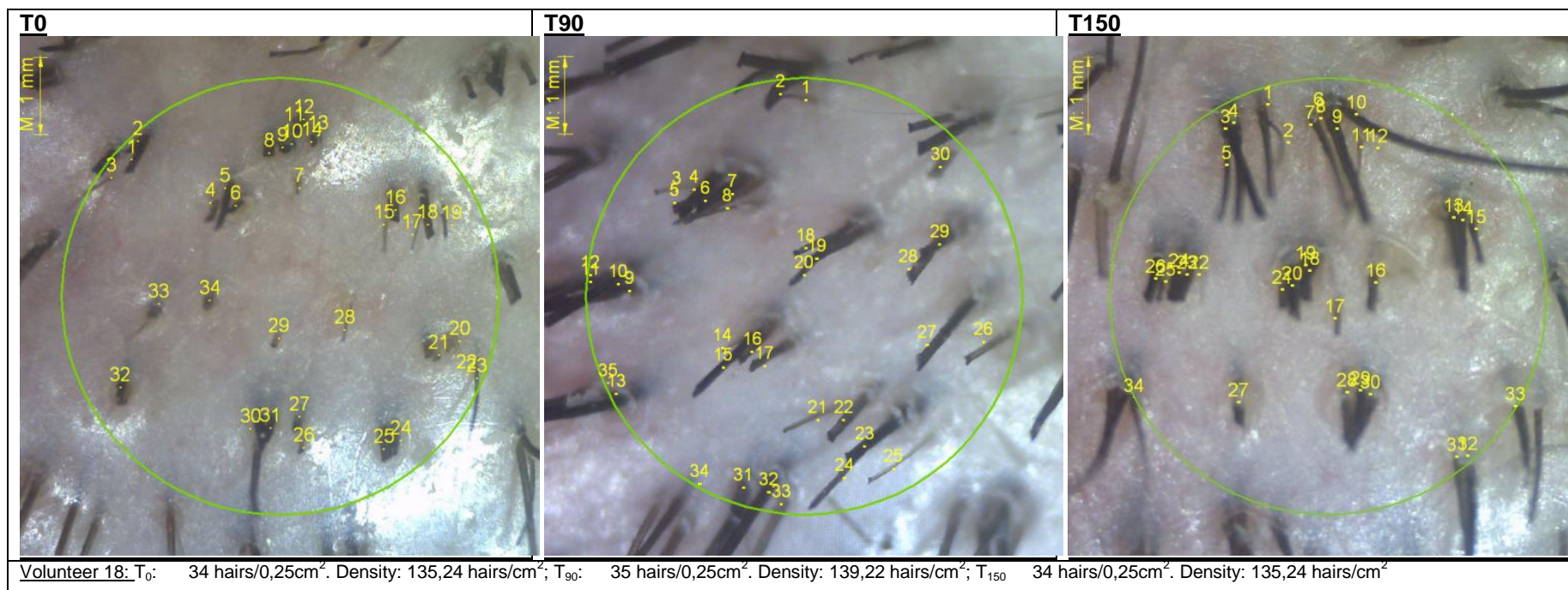


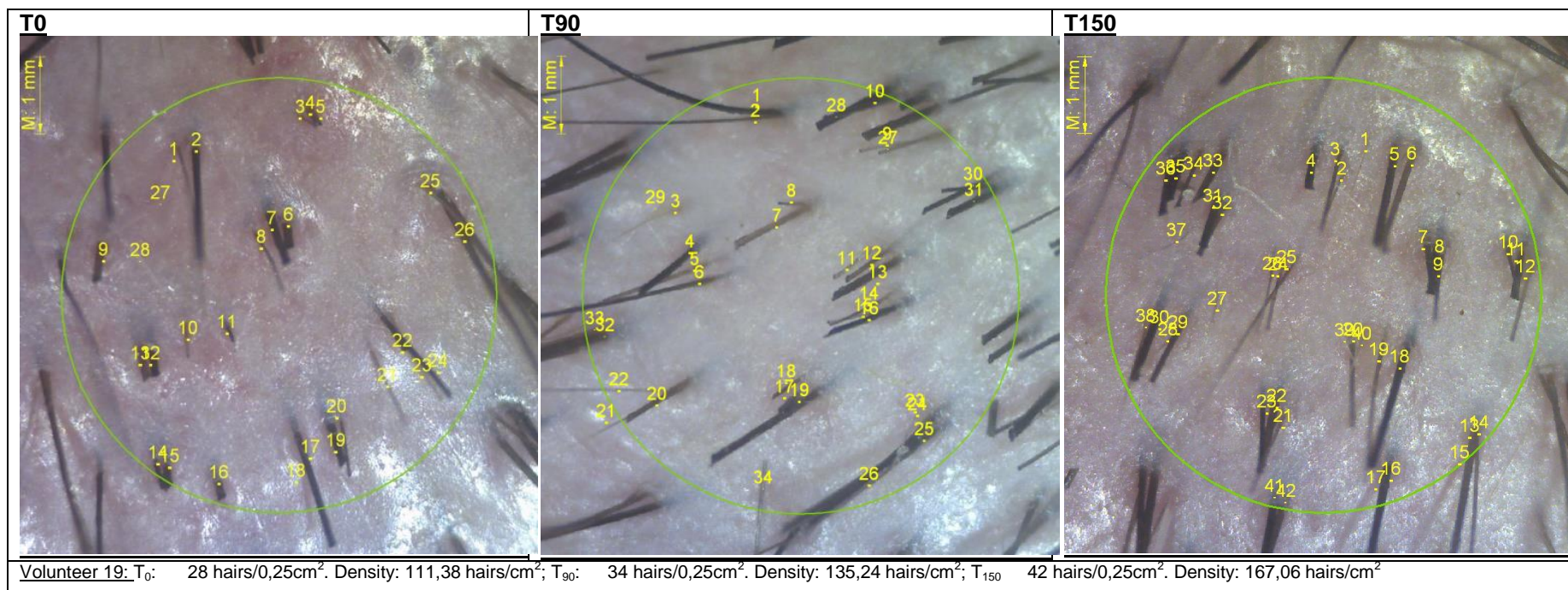


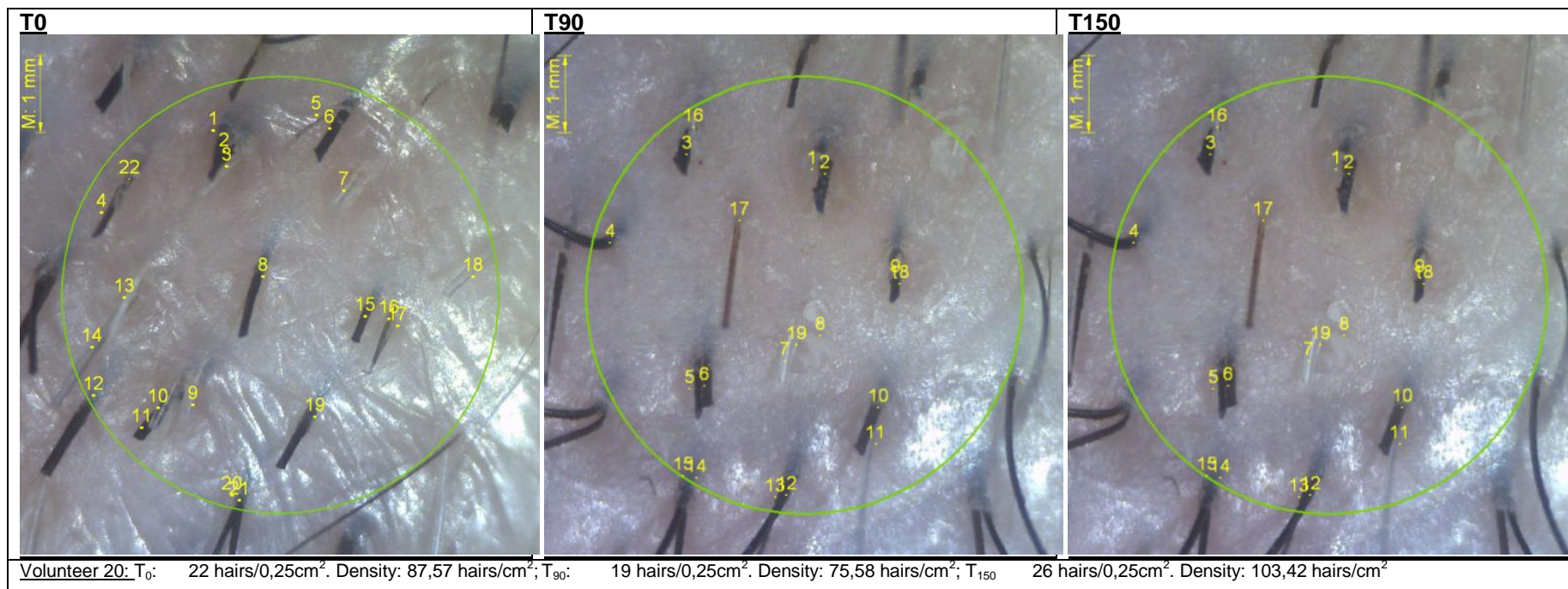


Volunteer 16: T₀: 63 hairs/0,25cm². Density: 250,60 hairs/cm²; T₉₀: 70 hairs/0,25cm². Density: 278,44 hairs/cm²; T₁₅₀: 61 hairs/0,25cm². Density: 242,64 hairs/cm²

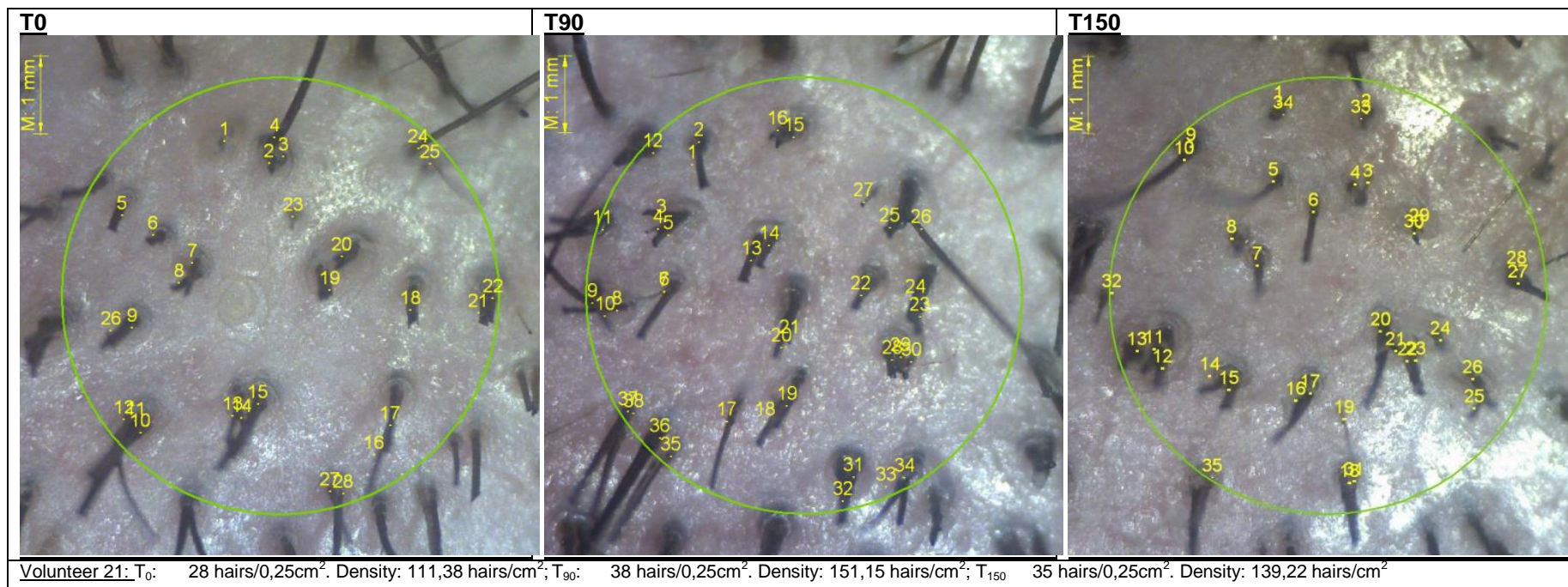


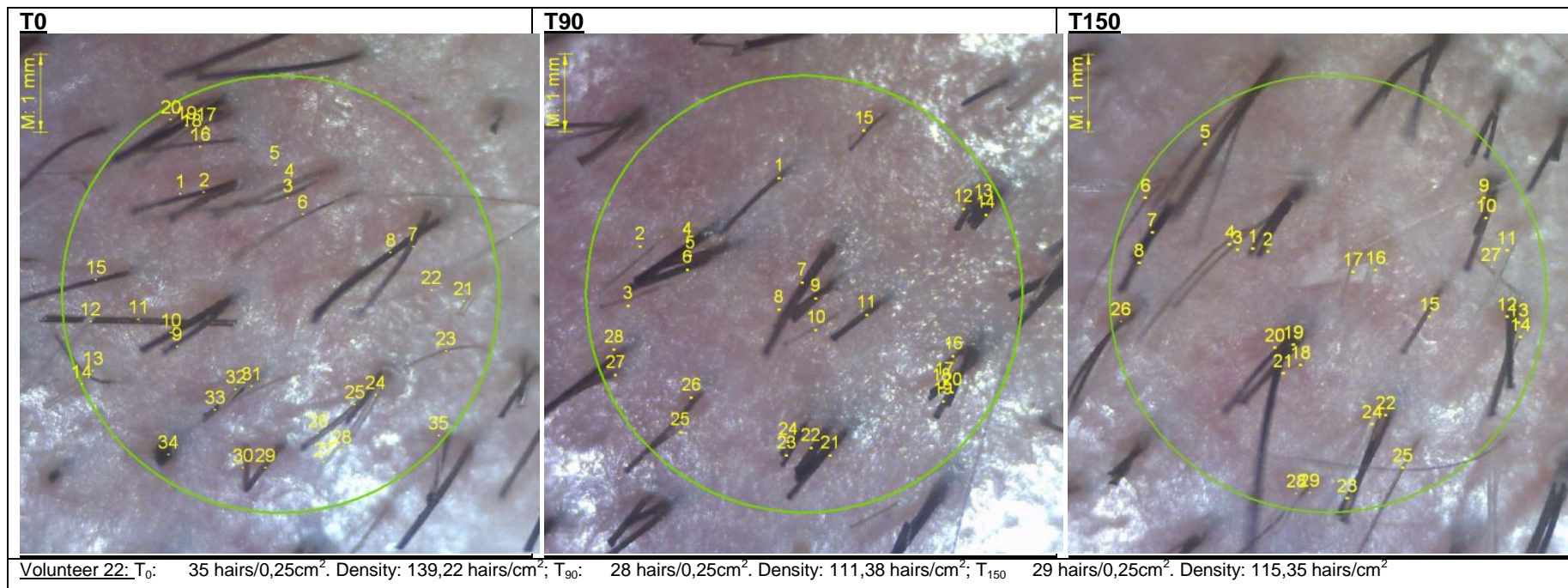


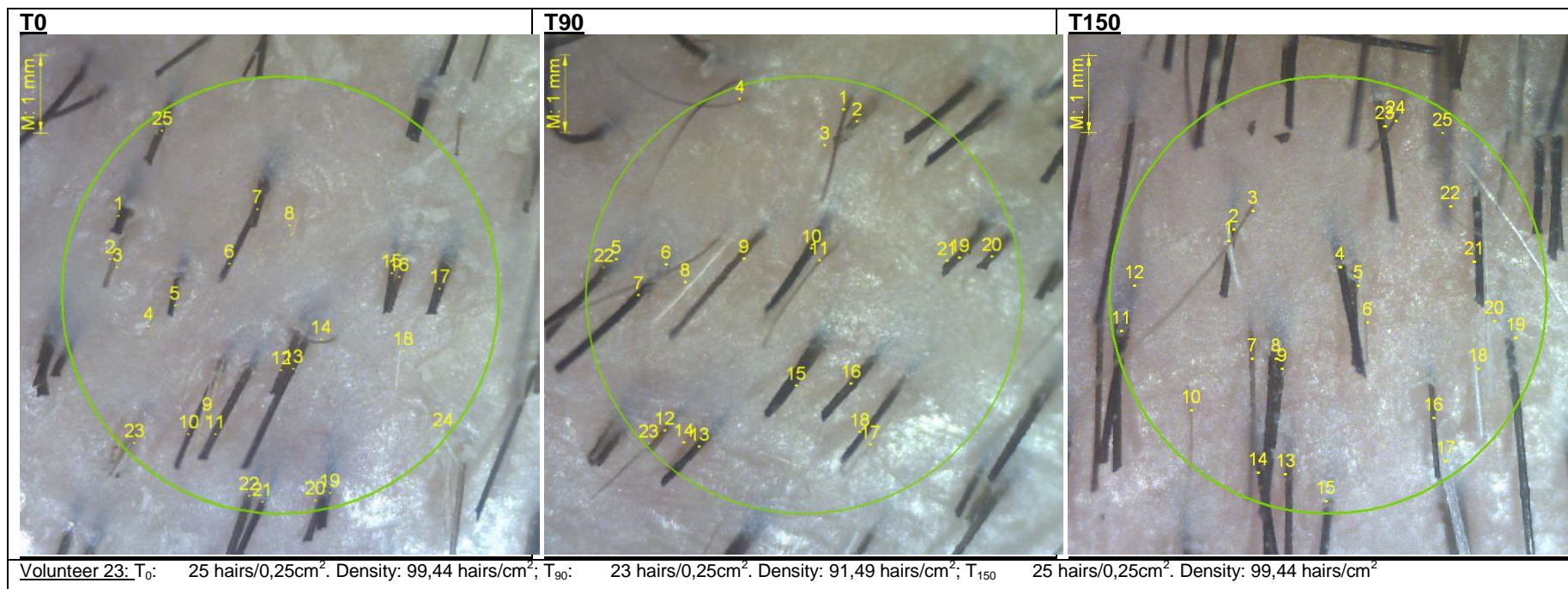


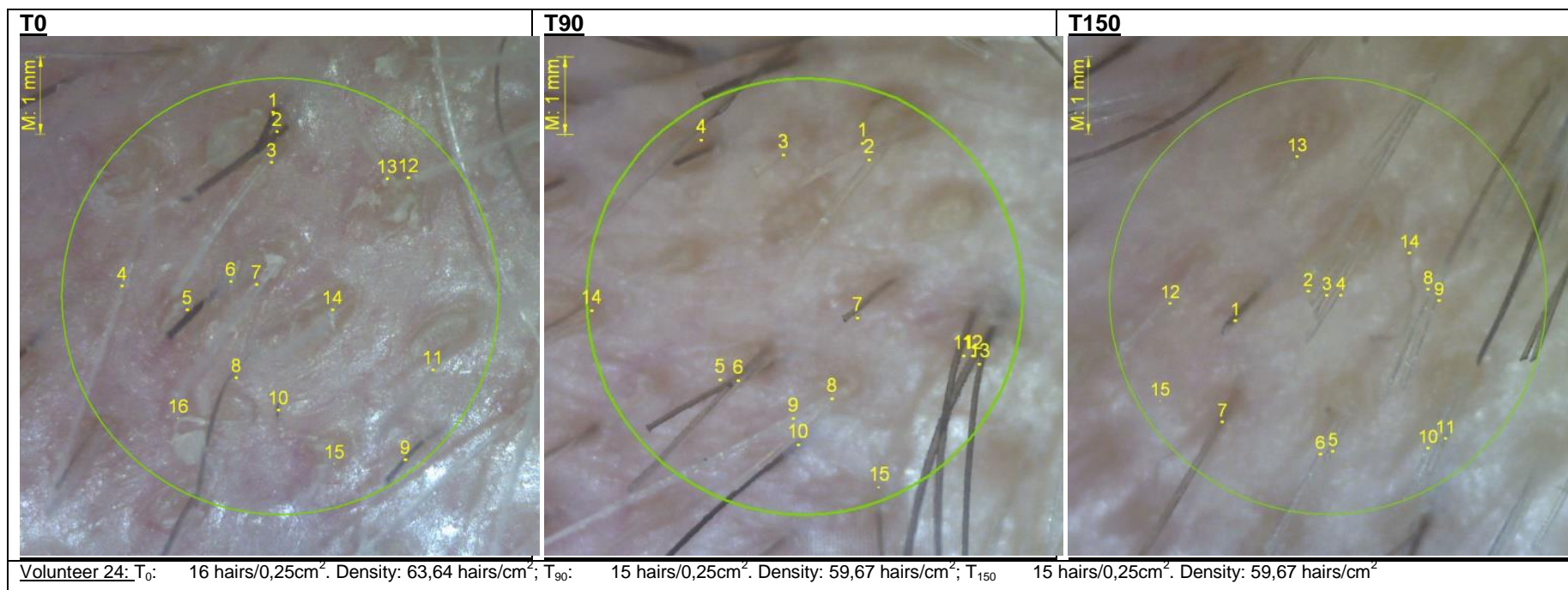


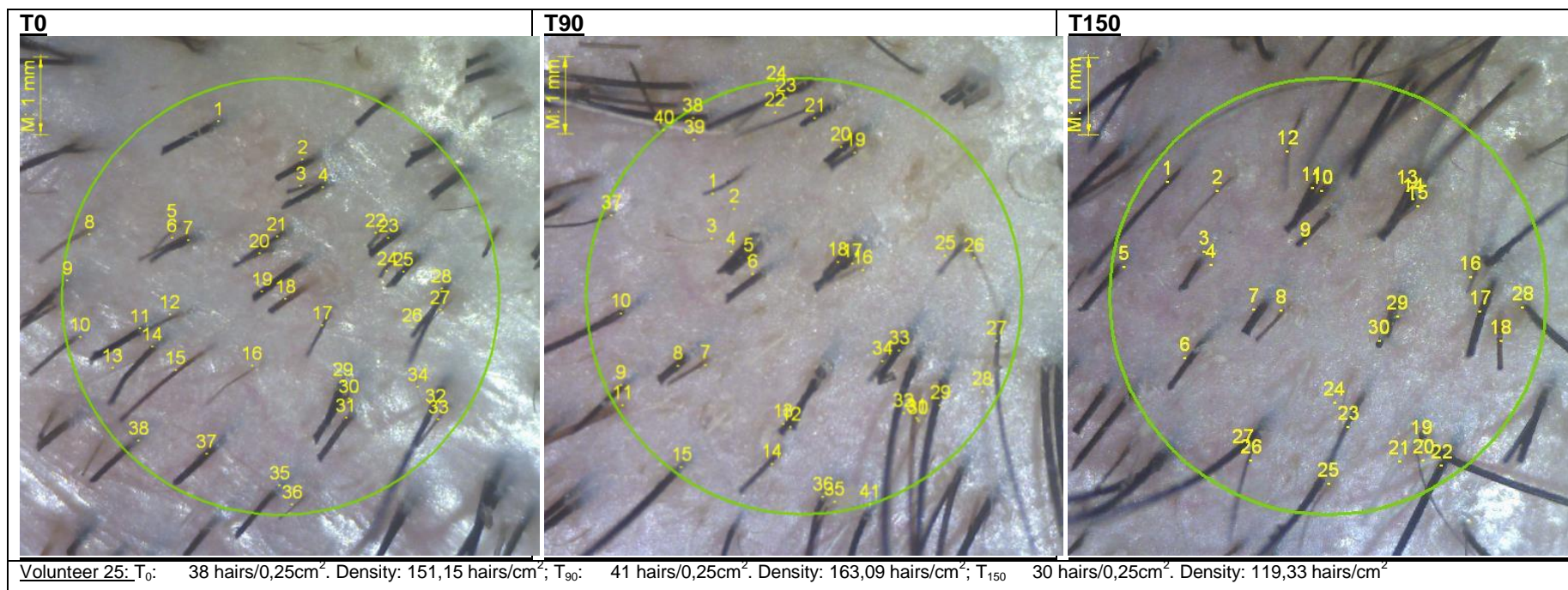
3.1.2. Group 2

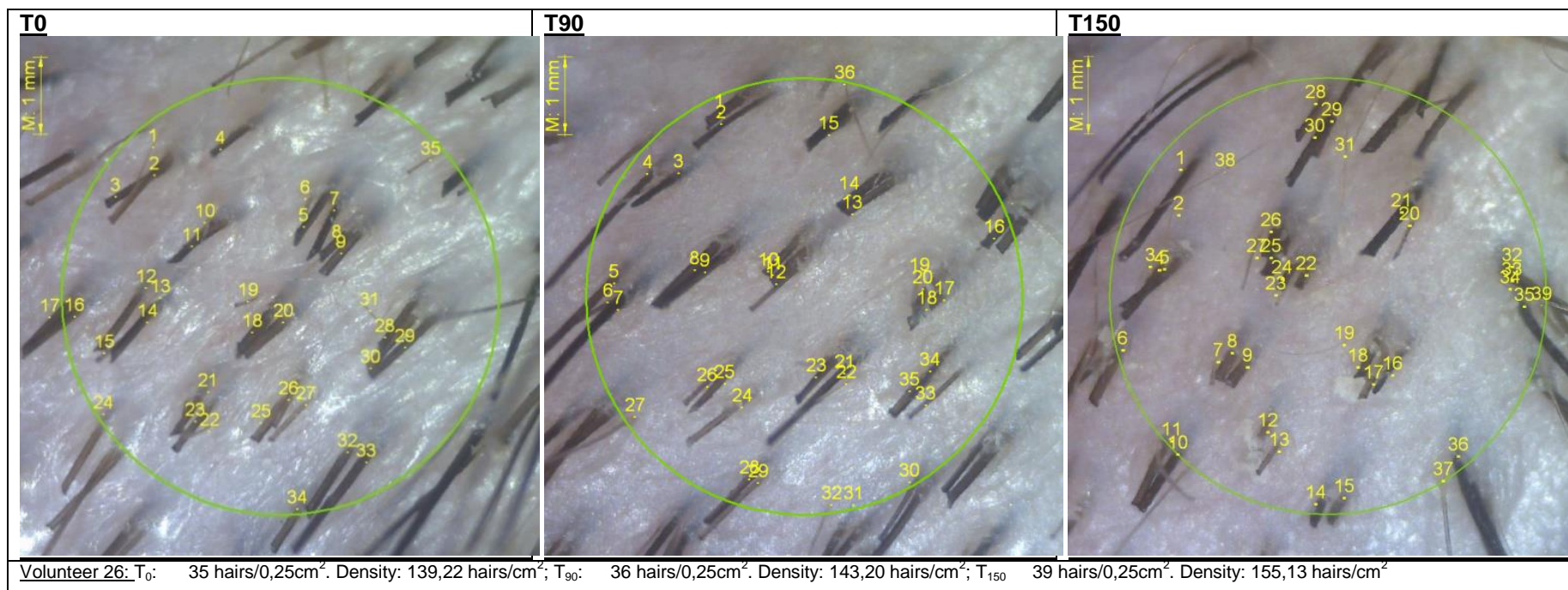


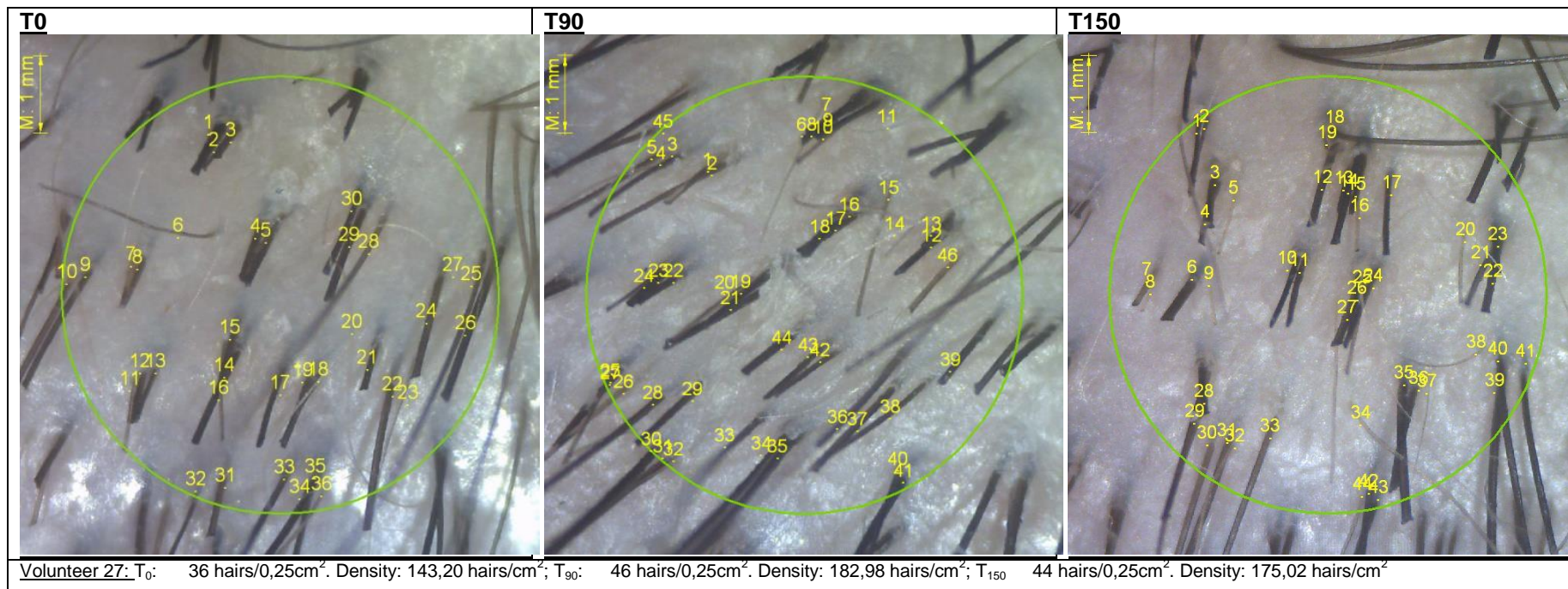


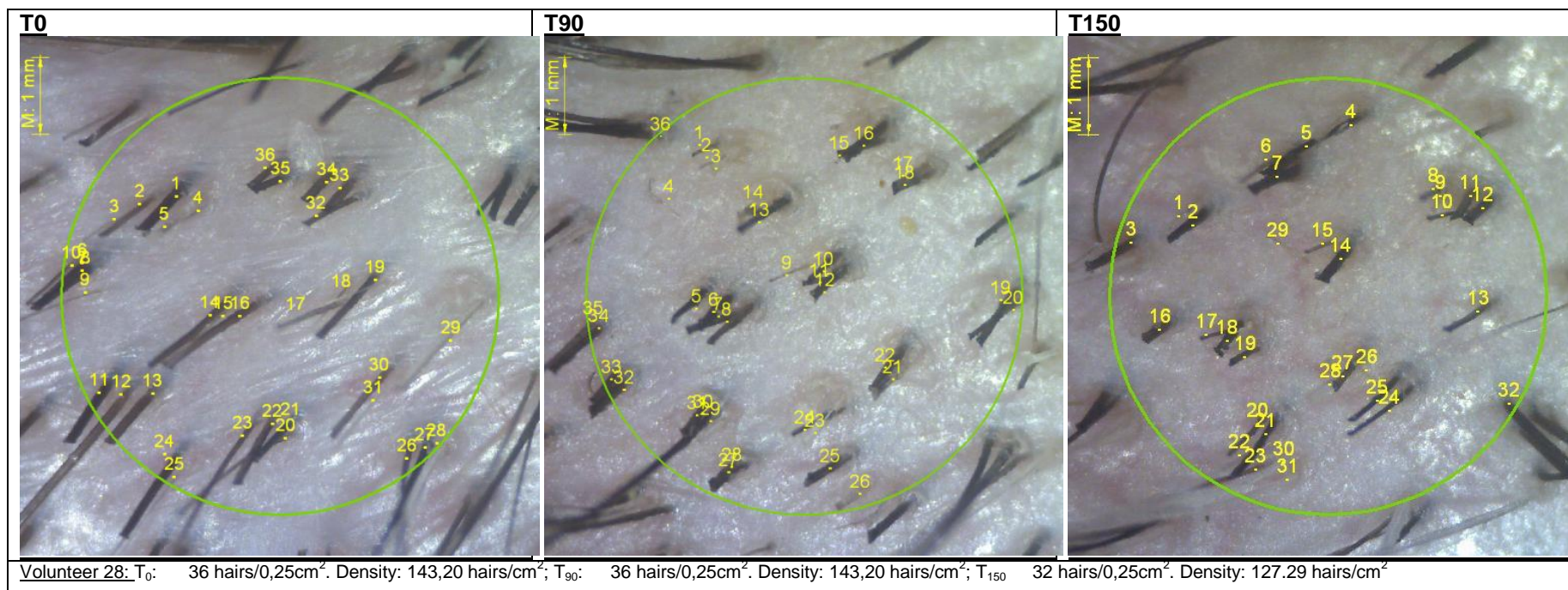


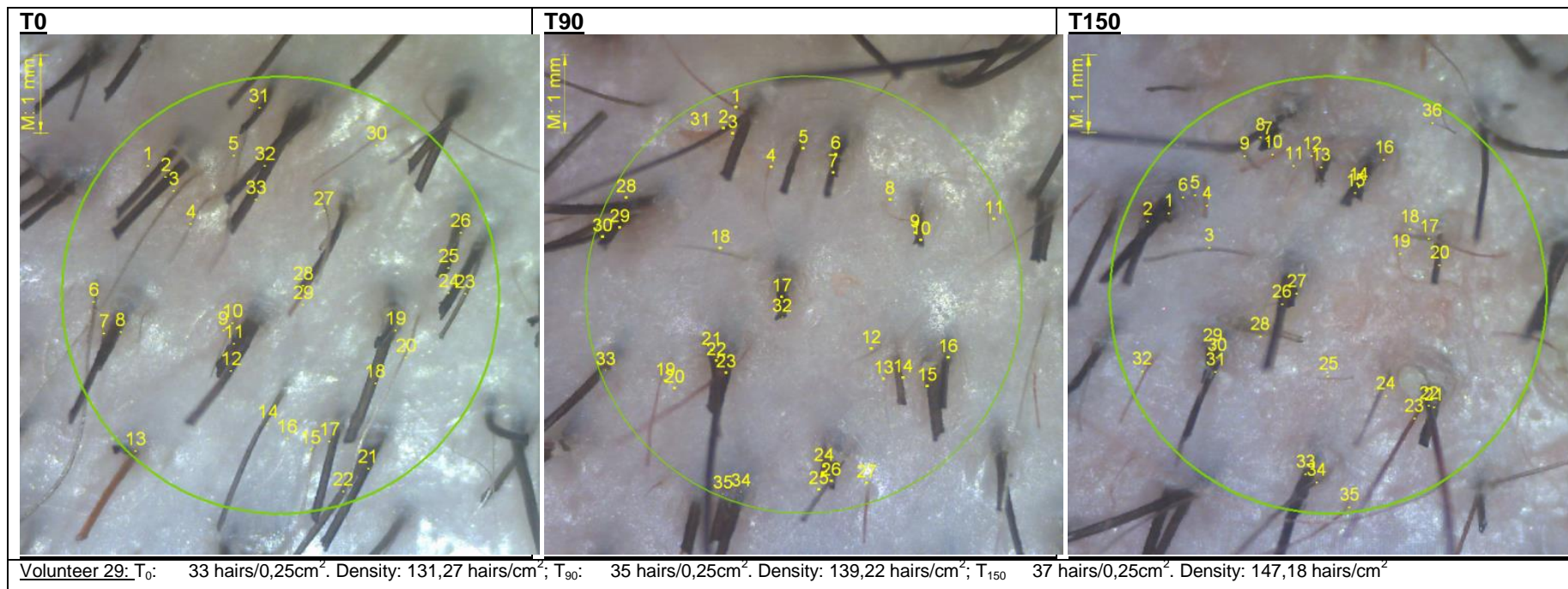


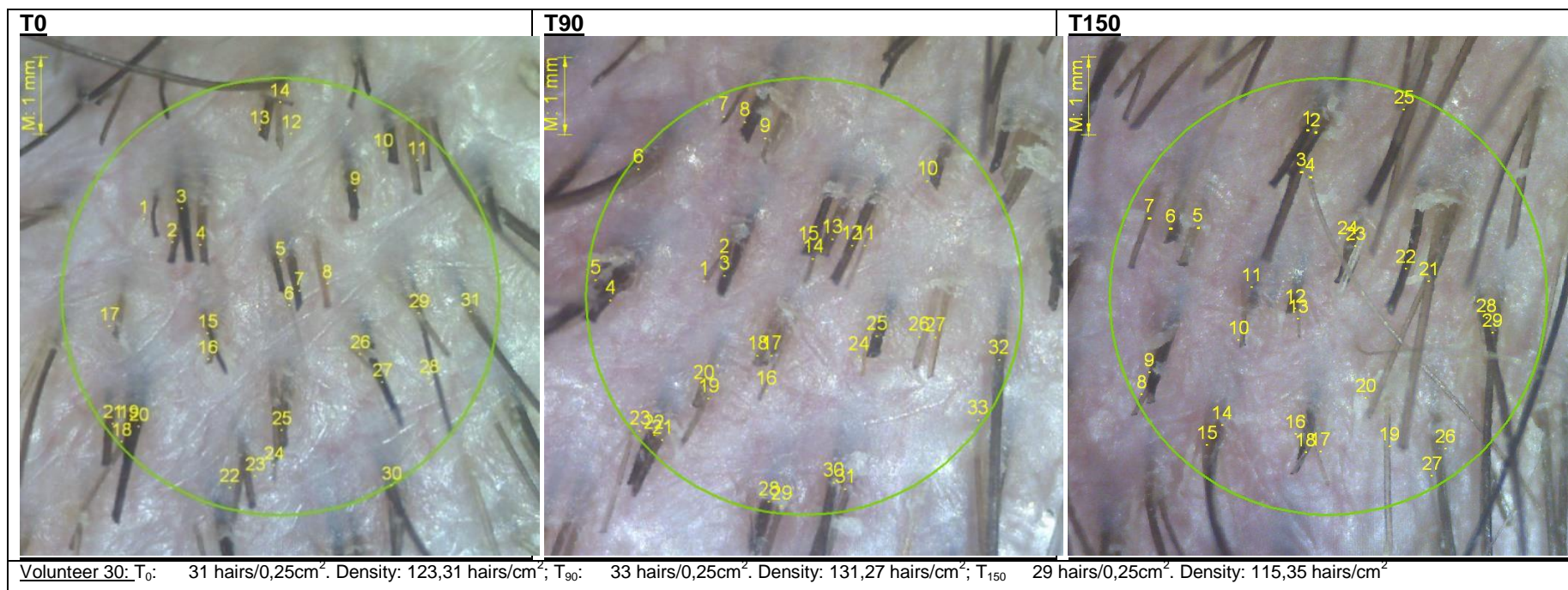


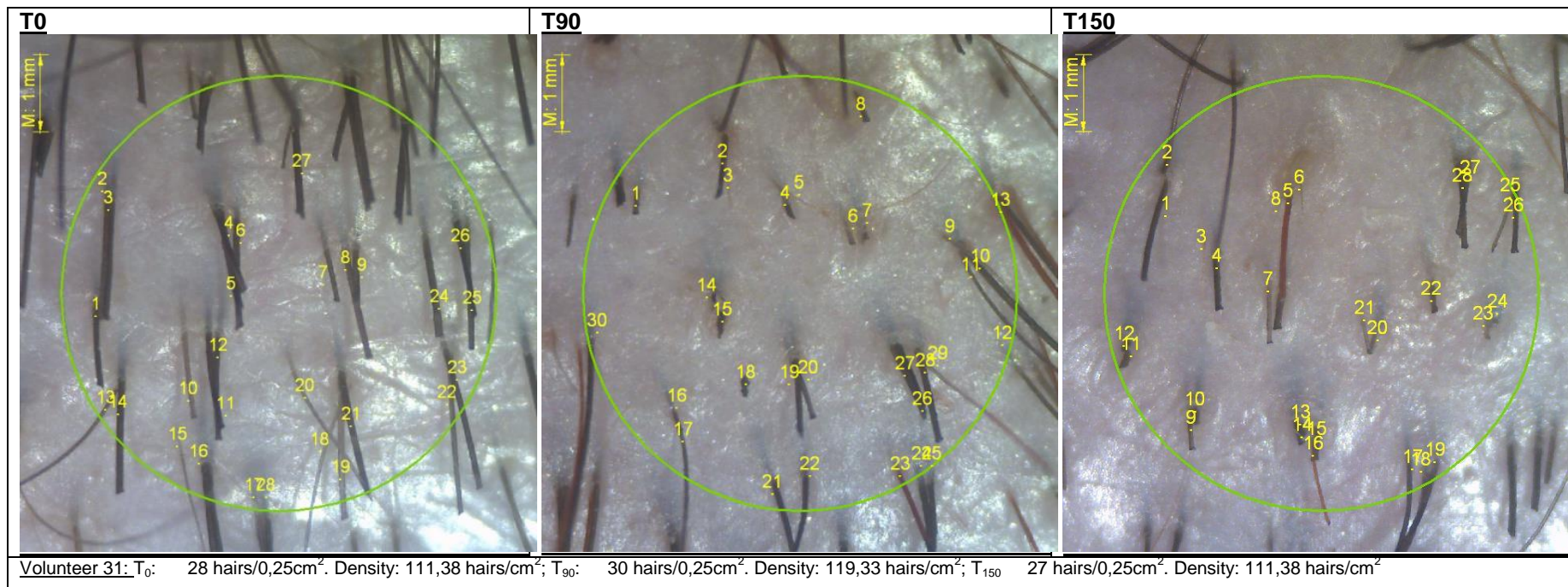


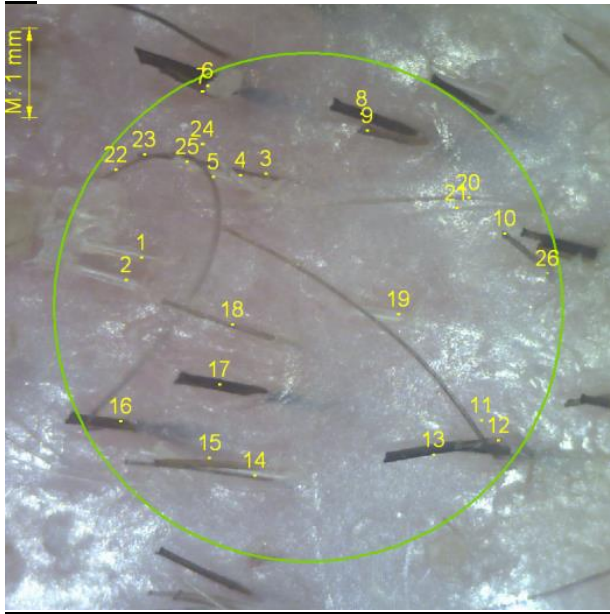
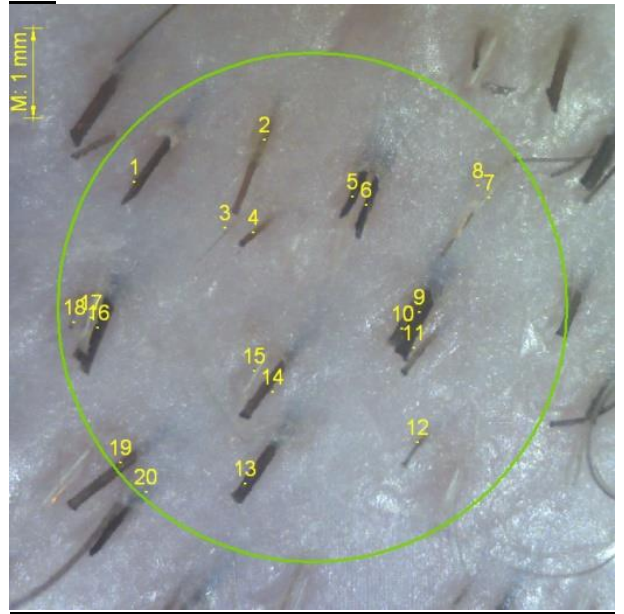


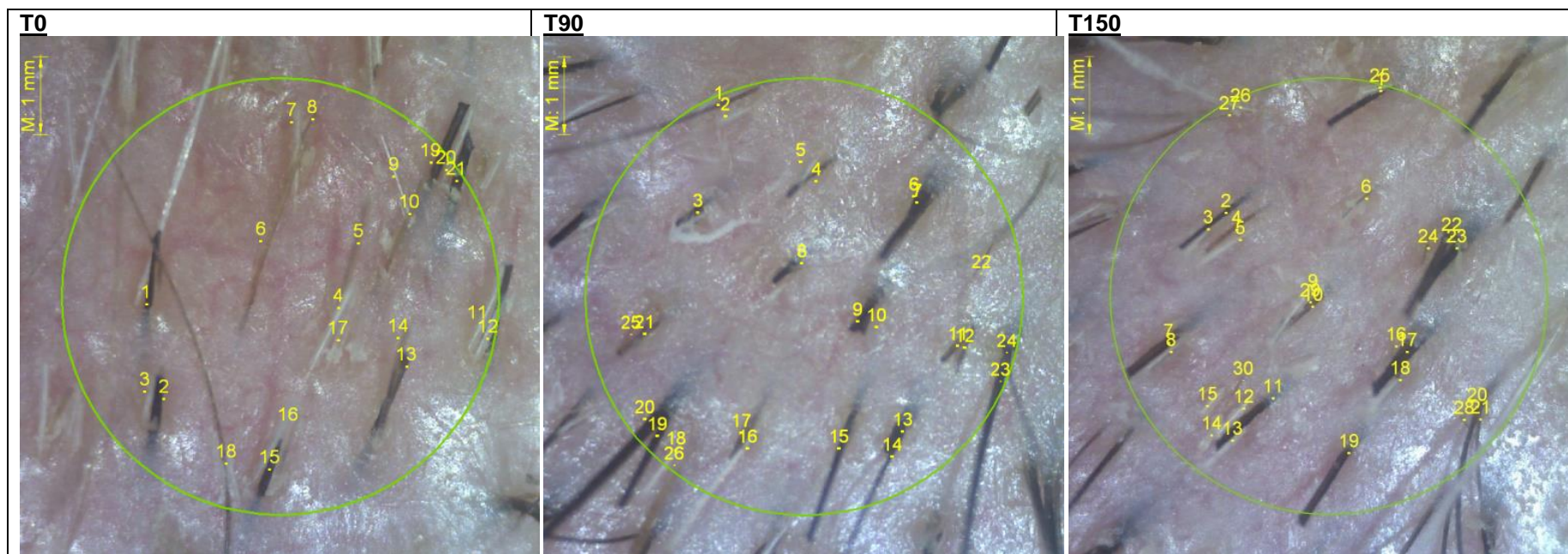




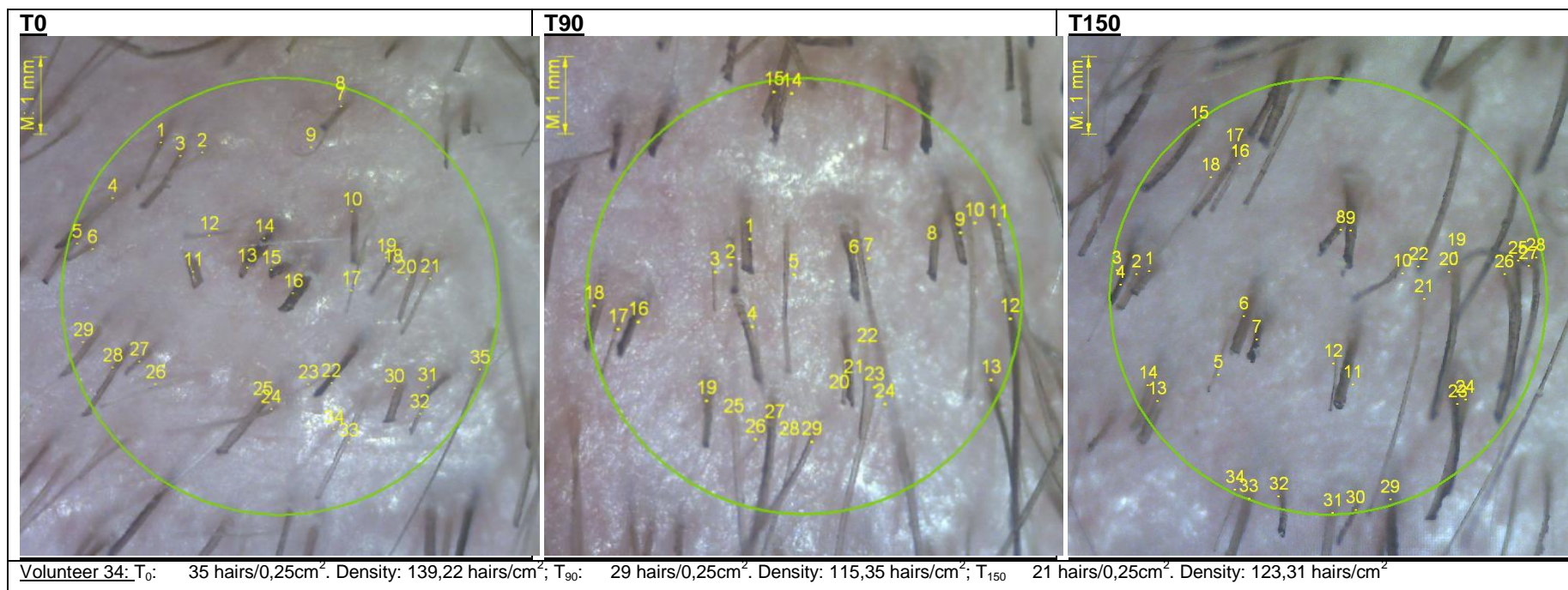


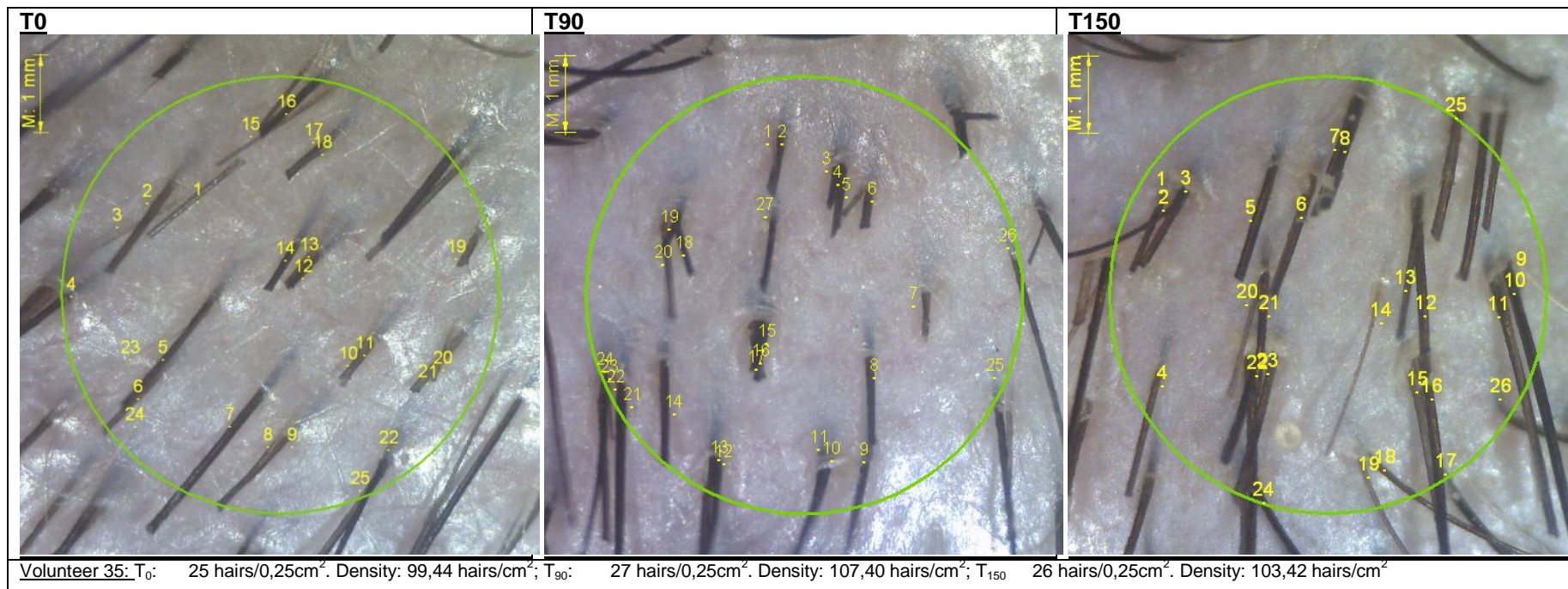


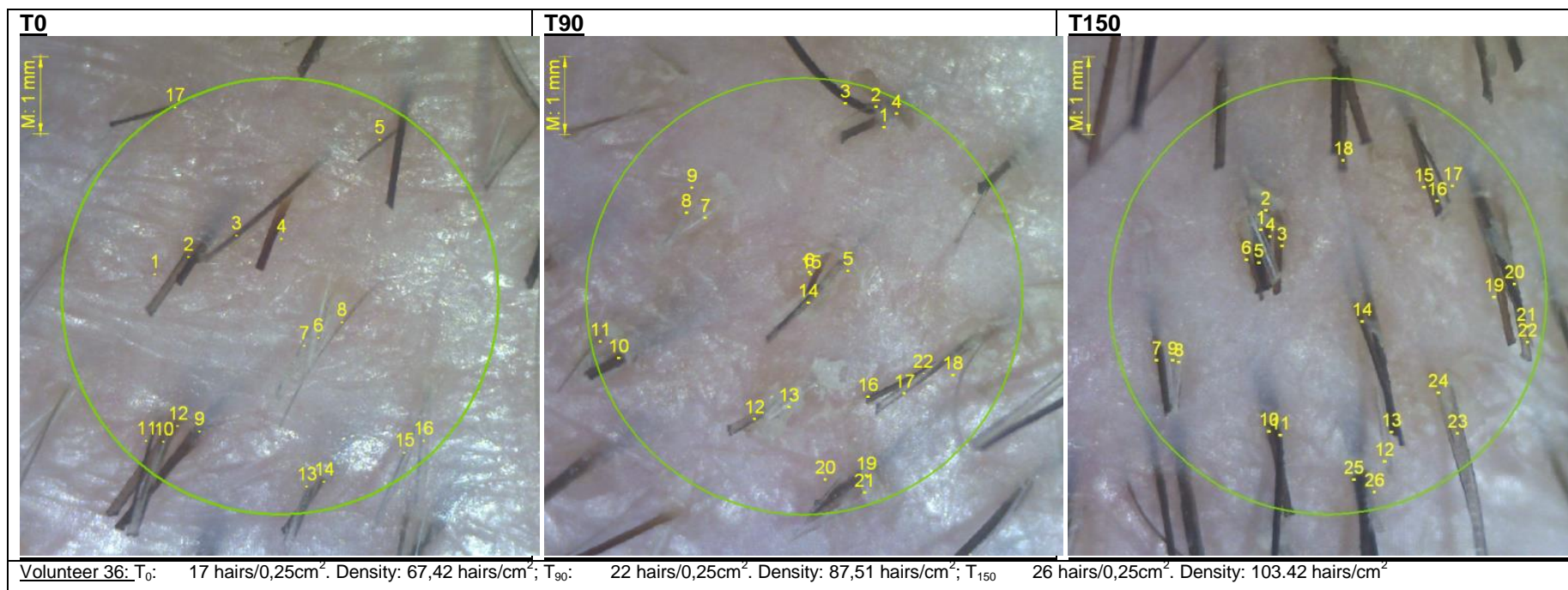
T0	T90	T150
		<p data-bbox="1512 363 1973 395">Volunteer has withdrawn the treatment</p>
<p data-bbox="248 887 1339 924">Volunteer 32: T₀: 26 hairs/0,25cm². Density: 103,42 hairs/cm²; T₉₀: 20 hairs/0,25cm². Density: 79,55 hairs/cm²</p>		

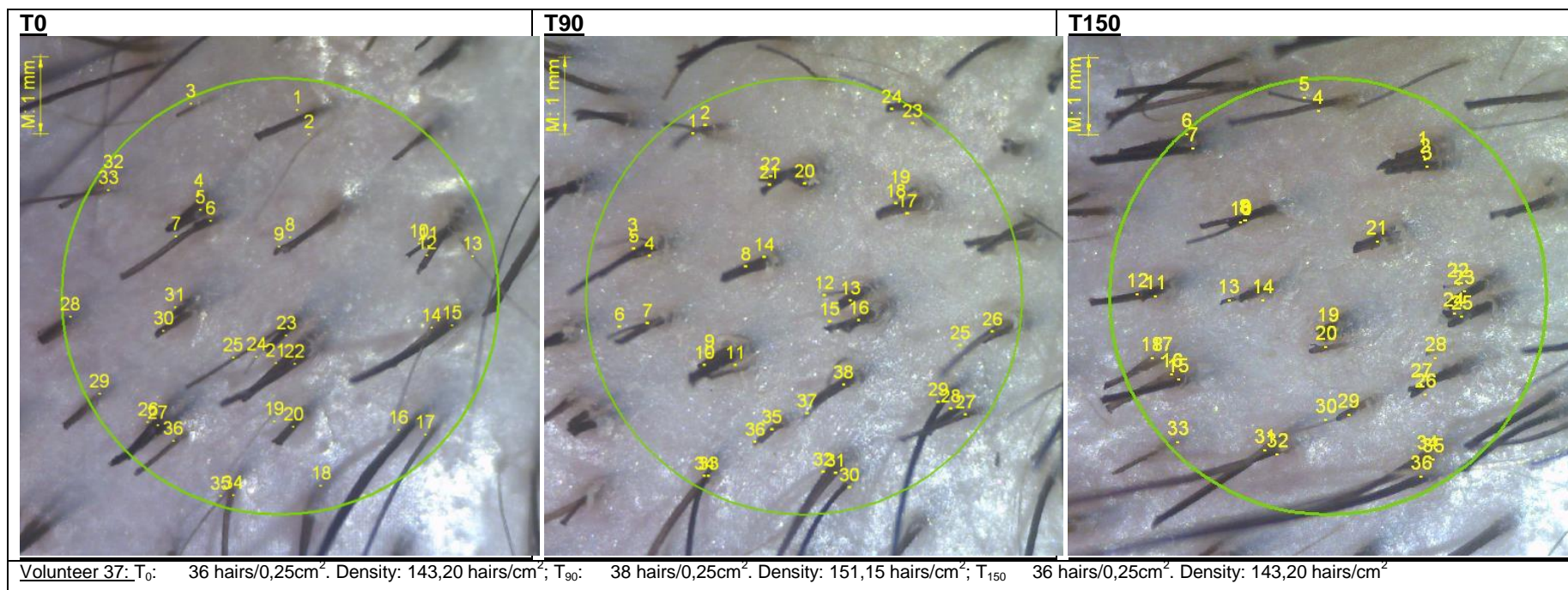


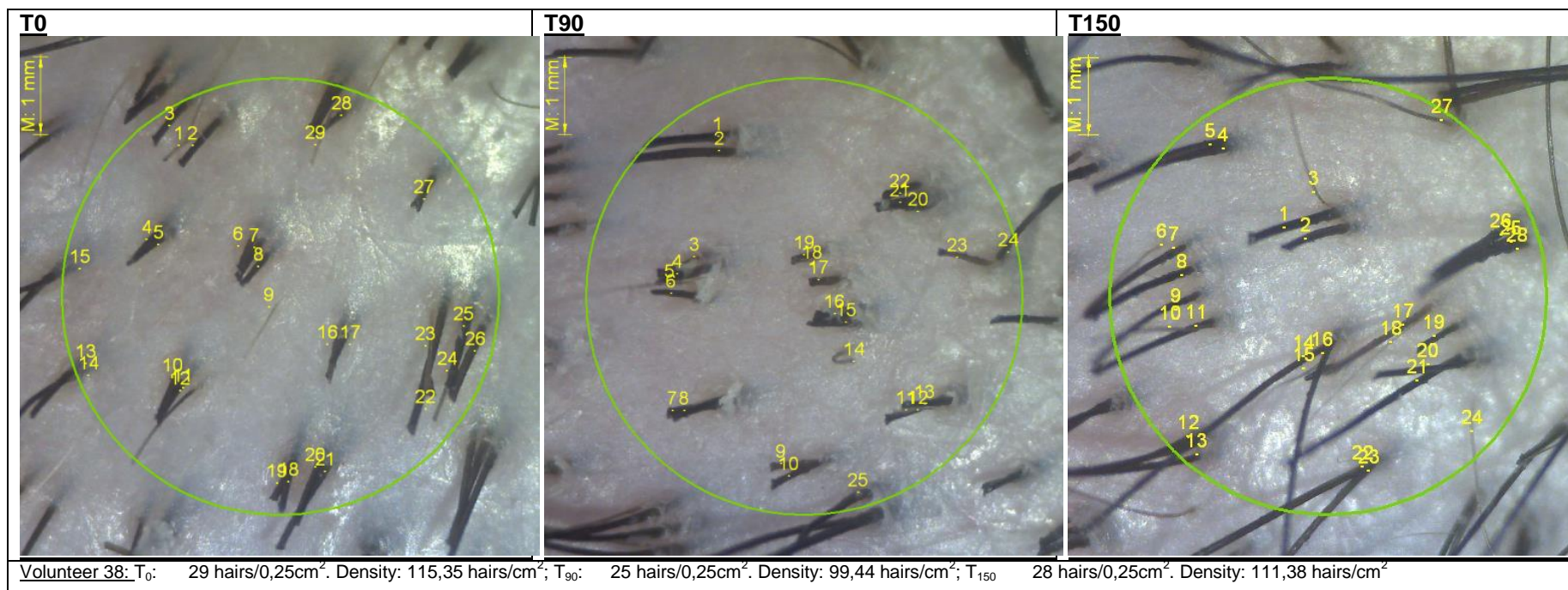
Volunteer 33: T₀: 21 hairs/0,25cm². Density: 85,53 hairs/cm²; T₉₀: 26 hairs/0,25cm². Density: 103,42 hairs/cm²; T₁₅₀: 30 hairs/0,25cm². Density: 119,33 hairs/cm²

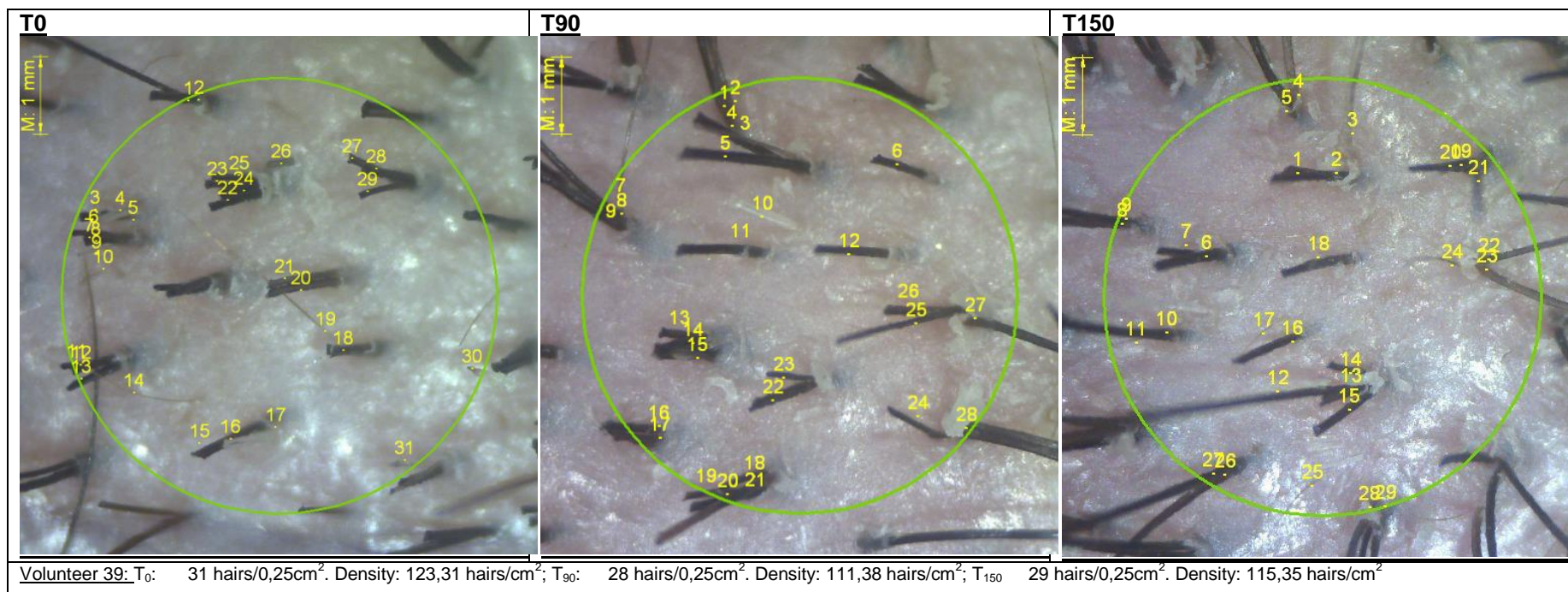


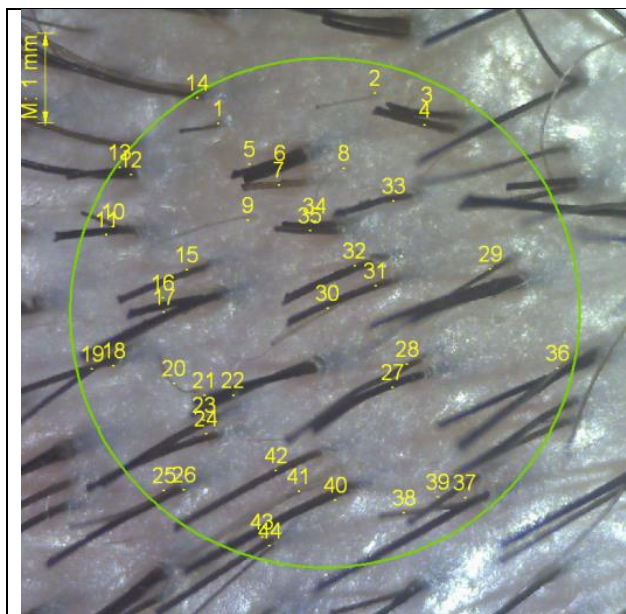












Volunteer 40: T₀: 44 hairs/0,25cm². Density: 175,02 hairs/cm²

T90

Volunteer has withdrawn the treatment

T150

Volunteer has withdrawn the treatment

3.2. HAIR DENSITY VALUES

3.2.1. Group 1

VOLUNTEER	Nº HAIRS/ 0,25cm ²			HAIR DENSITY 1/cm ²			INCREASE IN HAIR DENSITY	
	T ₀	T ₉₀	T ₁₅₀	T ₀	T ₉₀	T ₁₅₀	T ₀ VS T ₉₀	T ₀ VS T ₁₅₀
1	38	45	39	151,15	179	155,13	18,43%	2,63%
2	33	33	33	131,27	131,27	131,27	0,00%	0,00%
3	29	43	54	115,35	171,04	214,8	48,28%	86,22%
4	26	29	30	103,42	115,35	119,33	11,54%	15,38%
5	26	39	39	103,42	155,13	155,13	50,00%	50,00%
6	38	39	42	151,15	155,13	167,06	2,63%	10,53%
7	23	37	35	91,49	147,18	139,22	60,87%	52,17%
8	21	26	27	83,53	103,42	107,40	23,81%	28,58%
9	33	39	43	131,27	155,13	171,04	18,18%	30,30%
10	31	-	-	123,31	-	-	-	-
11	36	40	41	143,20	159,11	163,09	11,11%	13,89%
12	24	28	27	95,47	111,38	107,40	16,66%	12,50%
13	29	27	30	111,35	107,40	119,33	-3,55%	7,17%
14	19	23	19	75,58	91,49	75,58	21,05%	0,00%
15	36	37	34	143,20	147,80	135,24	3,21%	-5,56%
16	63	70	61	250,60	278,44	242,64	11,11%	-3,18%
17	30	31	32	119,33	123,31	127,29	3,34%	6,67%
18	34	35	34	135,24	139,22	135,24	2,94%	0,00%
19	28	34	42	111,38	135,24	167,06	21,42%	49,99%
20	22	19	26	87,57	75,58	103,42	-13,69%	18,10%
						Average	15,30± 0.15%	17,34± 0.19%

The values in red (highest and lowest value) are not taken into account for the mean value

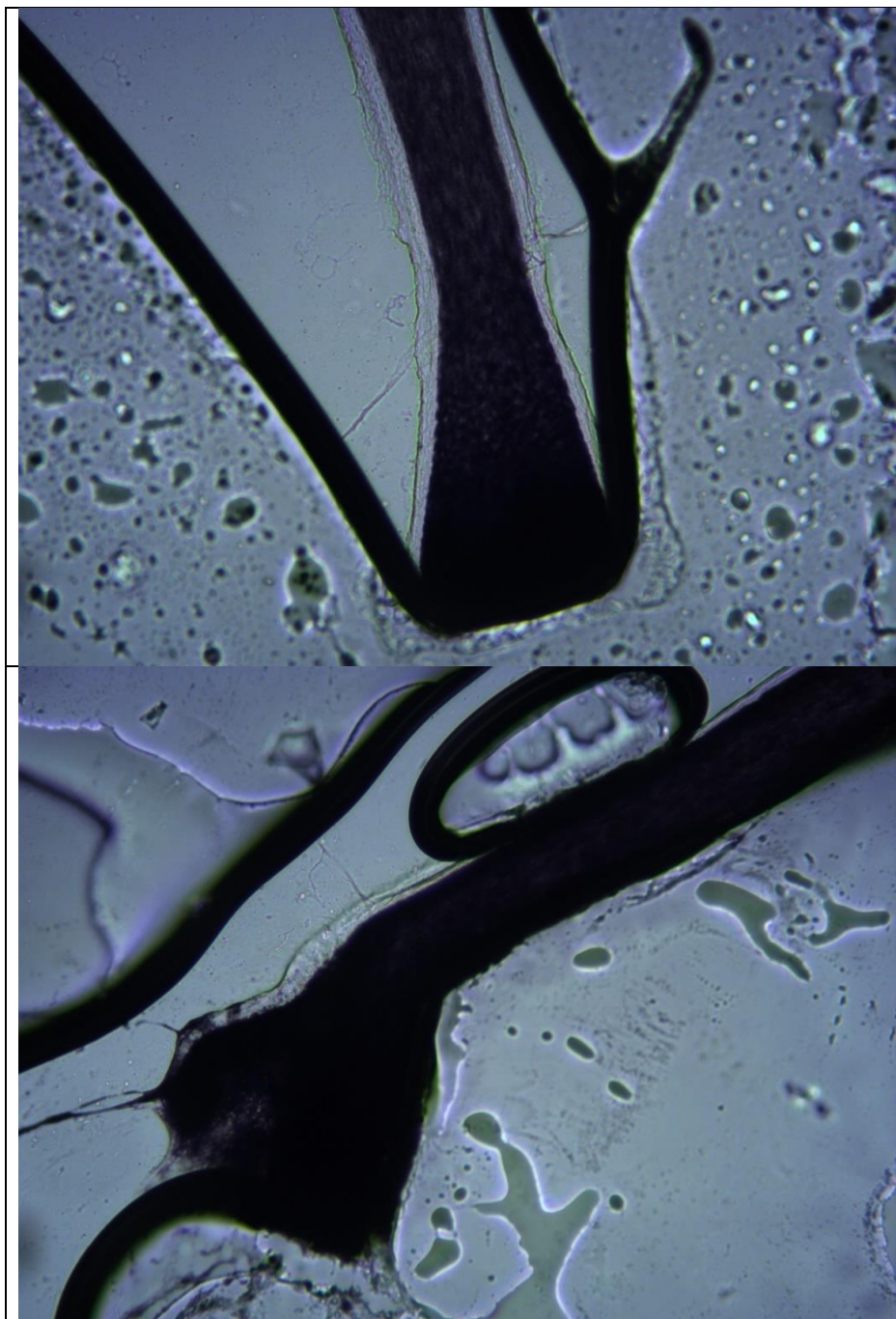
3.2.2. Group 2

VOLUNTEER	N° HAIRS/ 0,25cm ²			HAIR DENSITY 1/cm ²			INCREASE IN HAIR DENSITY	
	T ₀	T ₉₀	T ₁₅₀	T ₀	T ₉₀	T ₁₅₀	T ₀ VS T ₉₀	T ₀ VS T ₁₅₀
21	28	38	35	111,38	151,15	139,22	35,71%	25,00%
22	35	28	29	139,22	111,38	115,35	-20,00%	-17,15%
23	25	23	25	99,44	91,49	99,44	-7,99%	0,00%
24	16	15	15	63,64	59,67	59,67	-6,24%	-6,24%
25	38	41	30	151,15	163,09	119,33	7,90%	-21,05%
26	35	36	39	139,22	143,20	155,13	2,86%	11,43%
27	36	46	44	143,20	182,98	175,02	27,78%	22,22%
28	36	36	32	143,2	143,2	127,29	0,00%	-11,11%
29	33	35	37	131,27	139,22	147,18	6,06%	12,12%
30	31	33	29	123,31	131,27	115,35	6,46%	-6,46%
31	28	30	27	111,38	119,33	111,38	7,14%	0,00%
32	26	20	-	103,42	79,55	-	-23,08%	-
33	21	26	30	85,53	103,42	119,33	20,92%	39,52%
34	35	29	34	139,22	115,35	135,24	-17,15%	-2,86%
35	25	27	26	99,44	107,40	103,42	8,00%	4,00%
36	17	22	26	67,42	87,51	103,42	29,80%	53,40%
37	36	38	36	143,2	151,15	143,20	5,55%	0,00%
38	29	25	28	115,35	99,44	111,38	-13,79%	-3,44%
39	31	28	29	123,31	111,38	115,35	-9,67%	-6,46%
40	44	-	-	175,02	-	-	-	-
						Average	2,80± 0.14%	4.04± 0.16%

The values in red (highest and lowest value) are not taken into account for the mean value

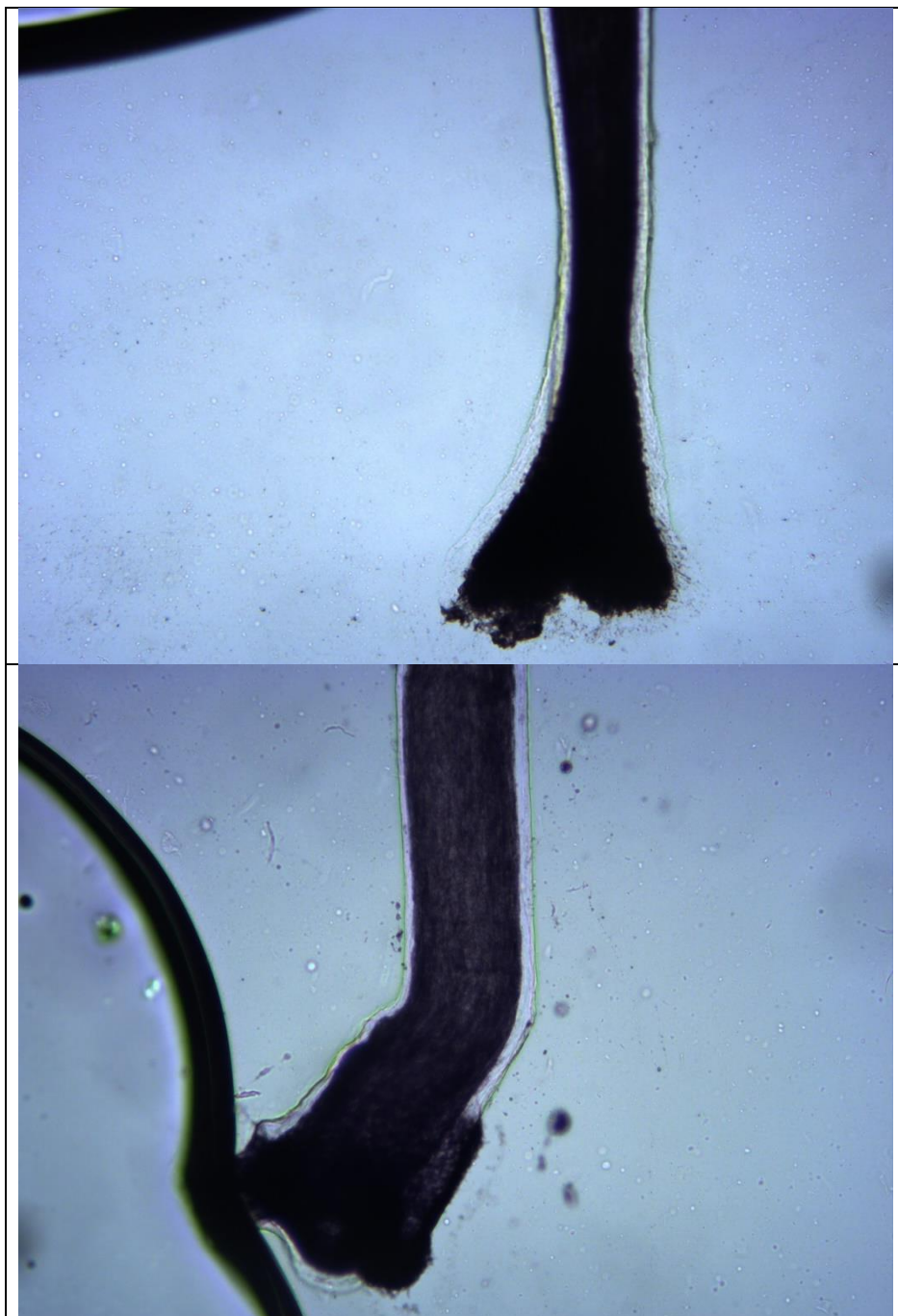
4. PHOTOGRAPHING HAIR ROOTS

4.1. GROUP 1



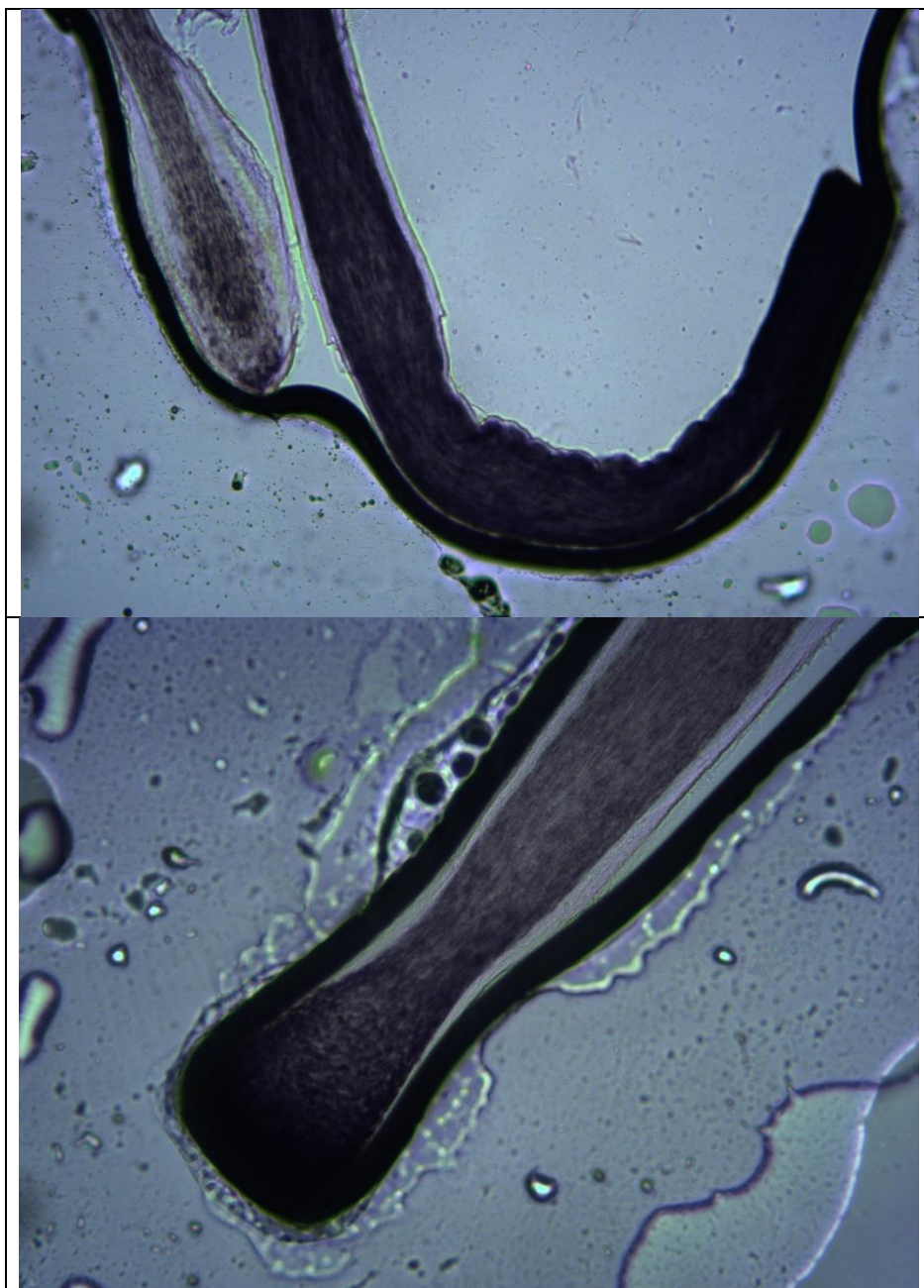
Volunteer 3

Photographs of hair roots at T_0 and T_{150}



Volunteer 5

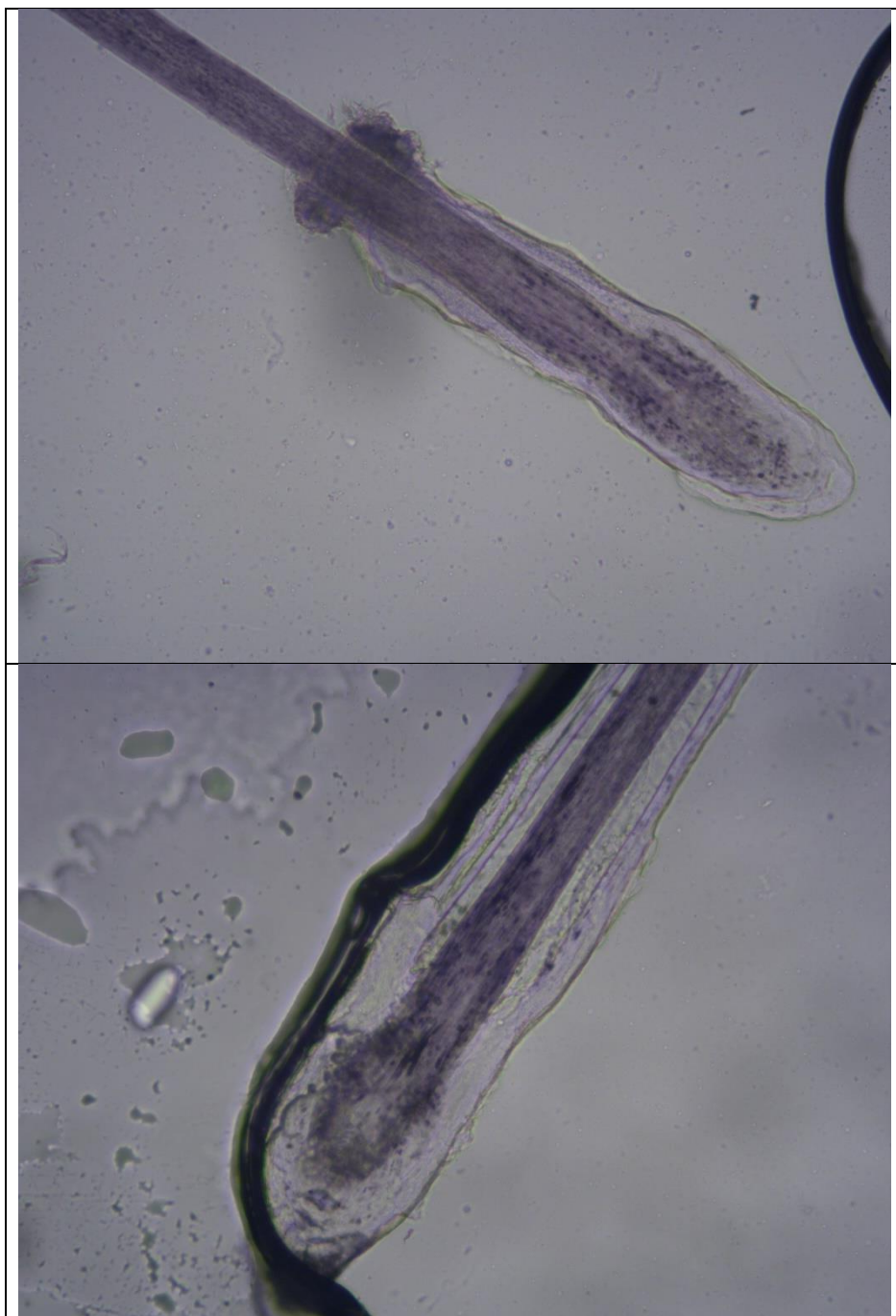
Photographs of hair roots at T_0 and T_{150}



Volunteer 19

Photographs of hair roots at T_0 and T_{150}

4.2. GROUP 2



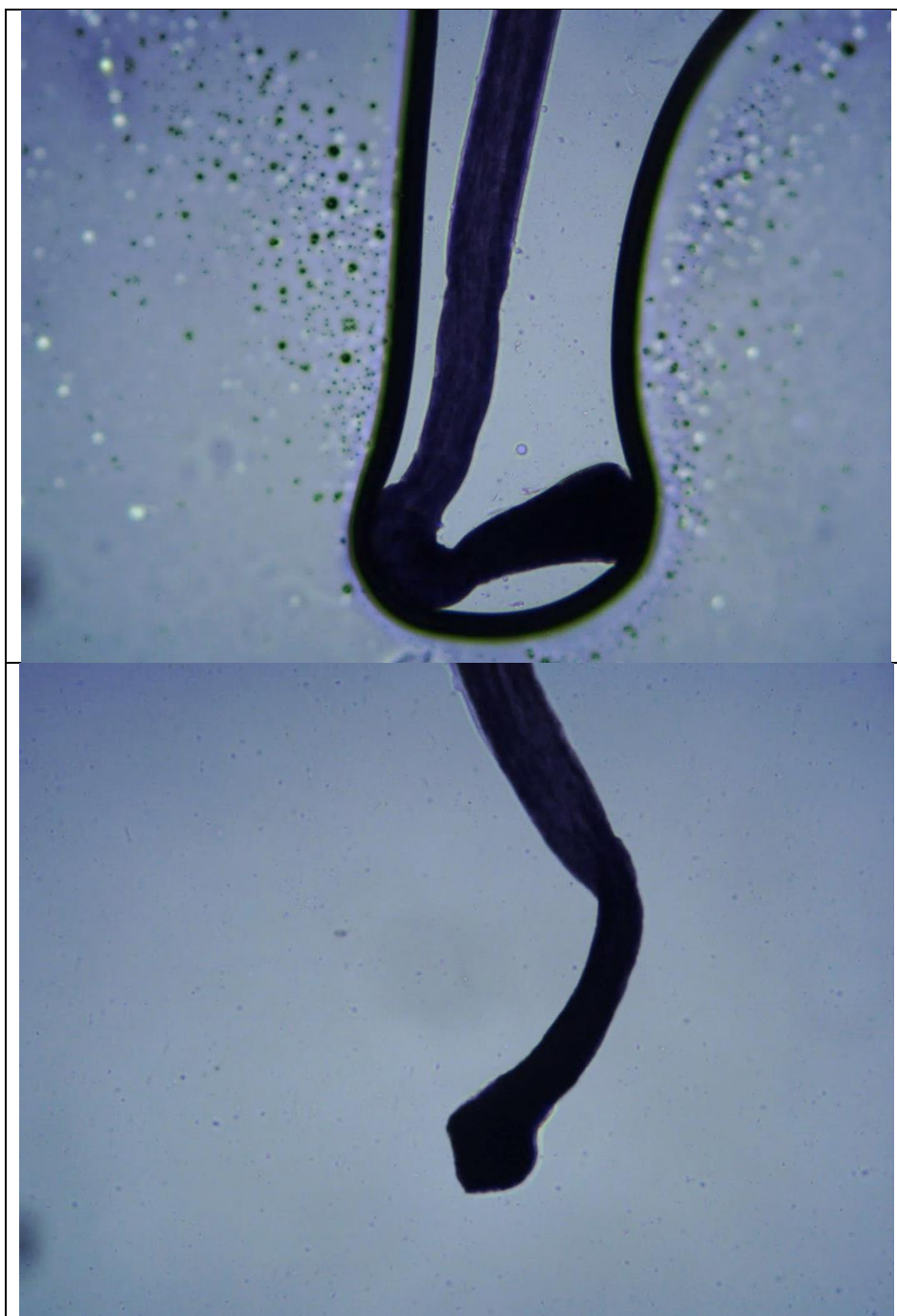
Volunteer 22

Photographs of hair roots at T_0 and T_{150}



Volunteer 34

Photographs of hair roots at T_0 and T_{150}



Volunteer 38

Photographs of hair roots at T_0 and T_{150}



5. COMBING TEST
5.1. GROUP 1

VOLUNTEER	T ₀	T ₄₅	%REDUCTION OF HAIR LOSS	T ₉₀	%REDUCTION OF HAIR LOSS	T ₁₅₀	%REDUCTION OF HAIR LOSS
			T ₀ vs.T ₄₅		T ₀ vs.T ₉₀		T ₀ vs.T ₁₅₀
1	38	46	21,05	90	136,84	35	-7,89
2	41	11	-73,17	20	-51,22	37	-9,76
3	160	12	-92,50	53	-66,88	26	-83,75
4	108	19	-82,41	31	-71,30	28	-74,07
5	83	75	-9,64	61	-26,51	51	-38,55
6	82	12	-85,37	18	-78,05	9	-89,02
7	107	19	-82,24	11	-89,72	16	-85,05
8	39	33	-15,38	21	-46,15	31	-20,51
9	86	35	-59,30	46	-46,51	38	-55,81
10	67	-	-	-	-	-	-
11	122	24	-80,33	19	-84,43	4	-96,72
12	142	121	-14,79	99	-30,28	167	17,61
13	110	21	-80,91	22	-80,00	17	-84,55
14	59	27	-54,24	21	-64,41	55	-6,78
15	54	12	-77,78	19	-64,81	23	-57,41
16	105	64	-39,05	45	-57,14	26	-75,24
17	58	58	0,00	36	-37,93	14	-75,86
18	92	66	-28,26	63	-31,52	43	-53,26
19	58	23	-60,34	33	-43,10	20	-65,52
20	81	35	-56,79	12,00	-85,19	19,00	-76,54
		Average	52,94 ± 29.36%		56,79 ± 19.44%		56,45 ± 29.12%

The values in red (highest and lowest value) are not taken into account for the mean value

5.2. GROUP 2

VOLUNTEER	T ₀	T ₄₅	%REDUCTION OF HAIR LOSS	T ₉₀	%REDUCTION OF HAIR LOSS	T ₁₅₀	%REDUCTION OF HAIR LOSS
			T ₀ vs. T ₄₅		T ₀ vs. T ₉₀		T ₀ vs. T ₁₅₀
21	108	34	-68,52	47	-56,48	75	-30,56
22	34	21	-38,24	26	-23,53	26	-23,53
23	37	67	81,08	77	108,11	14	-62,16
24	31	40	29,03	16	-48,39	23	-25,81
25	90	117	30,00	124	37,78	186	106,67
26	103	52	-49,51	58	-43,69	37	-64,08
27	82	33	-59,76	29	-64,63	16	-80,49
28	66	74	12,12	64	-3,03	82	24,24
29	50	19	-62,00	17	-66,00	32	-36,00
30	52	32	-38,46	54	3,85	26	-50,00
31	76	13	-82,89	17	-77,63	50	-34,21
32	82	61	-25,61	46	-43,90	-	-
33	181	54	-70,17	30	-83,43	108	-40,33
34	51	74	45,10	50	-1,96	84	64,71
35	127	69	-45,67	59	-53,54	24	-81,10
36	94	81	-13,83	133	41,49	157	67,02
37	85	18	-78,82	4	-95,29	30	-64,71
38	62	34	-45,16	62	0,00	16	-74,19
39	35	84	140,00	60	71,43	43	22,86
40	89	-	-	-	-	-	-
		Average	23,44 ± 46.62%		24,22 ± 45.15%		25,45 ± 46.41%

The values in red (highest and lowest value) are not taken into account for the mean value

III. DISCUSSION

GENERAL PHOTOGRAPH

Certainly when an efficacy study is conducted on a hair regeneration treatment, it is desirable for the results to be seen with the naked eye, although it is not always possible to distinguish the differences. When the treatment increases the number of hairs, the general photograph is a simple test to clearly demonstrate the success of the treatment.

For all volunteers, a difference was observed in the number of hairs between T₀ and T₁₅₀. The differences are, however, more appreciable in group 1 (151002iD1-SH) than in group 2 (151058iD1-SH).

TRICHOGRAM

Ratio between hairs in anagen phase and in telogen phase

In Figure 1 the average values of A/T ratio at T₀ and T₁₅₀ are shown

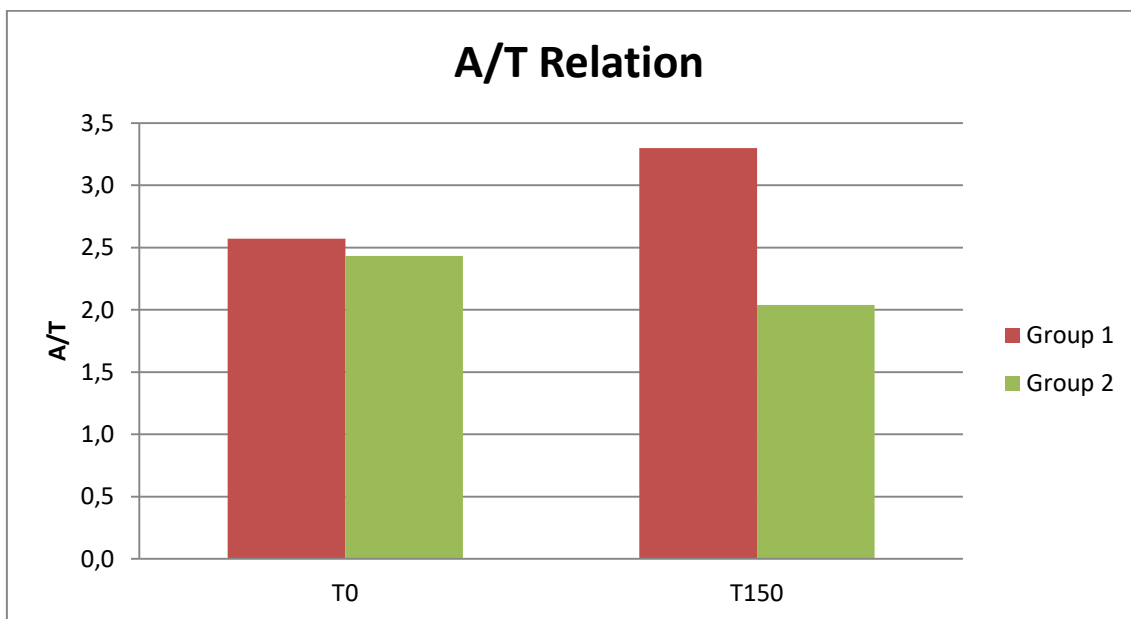


Figure 1. A/T ratio at T₀ and T₁₅₀.

An A/T ratio greater than or equal to 4 is considered normal. After 5 months of treatment, although the ratio is below 4, it has increased for group 1 (2.6 at T₀ compared with 3.3 at T₁₅₀); while for group 2 (placebo) the ration is seen to have fallen (2.4 at T₀ compared with 2.0 at T₁₅₀),

Since an increase in the A/T ratio is related to an increase in the number of hairs in the anagen phase and/or a reduction in the number in the telogen phase, it is clear that the product 151002iD1-SH result in an improvement in the state of the hair (increase the A/T), while the 151058iD1-SH product causes it to worsen (the A/T ratio drops).

The statistical results are according with the previous paragraph. At T_0 the differences between both groups are not statistically significant (Wilcoxon test: $W = 238.5$, $p\text{-value} = 0.175$, t test $t = 0.966$, $df = 36.897$, $p\text{-value} = 0.340$). However, at T_{150} the differences between both groups are statistically significant (Wilcoxon test: $W = 289$, $p\text{-value} = 0.0003$, t test $t = 3.6807$, $df = 19.362$, $p\text{-value} = 0.0015$, IC95% confidence interval for the difference of means (0.595, 2.158)).

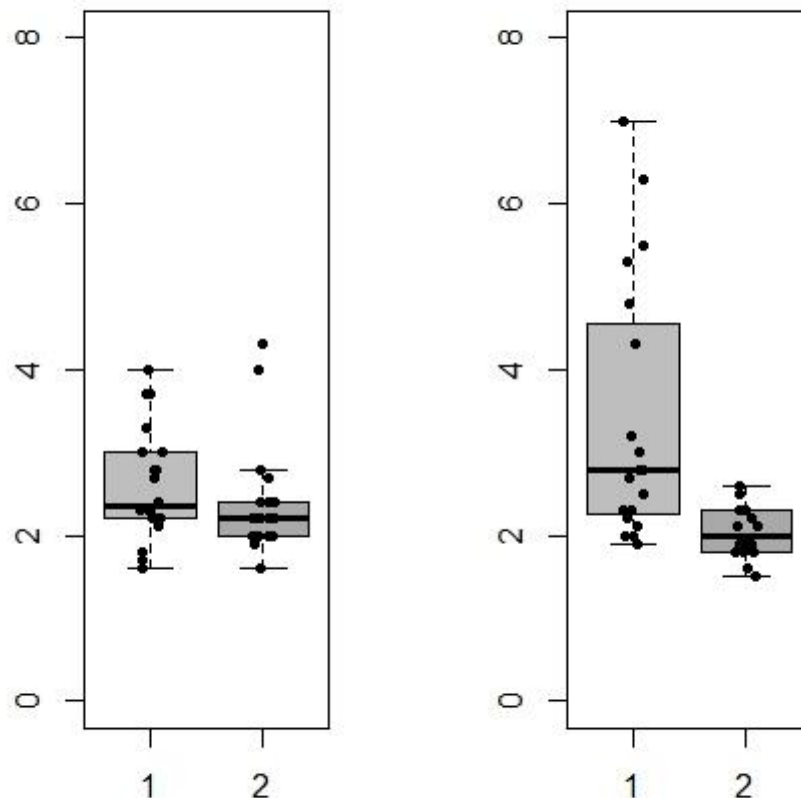


Figure 2. Left panel ratio AT at 0. Right panel ratio AT at 150. Treatment group (1), Control group (2).

Increase of anagen phase and reduction of telogen phase

Once the change in the A/T ratio has been determined for the different groups, it would be interesting to know the average percentages by which the anagen and telogen phases change, since this percentage is important in determining the success of the treatment. Figure 3 shows the average values of the increases in these phases for the 2 groups in the study.

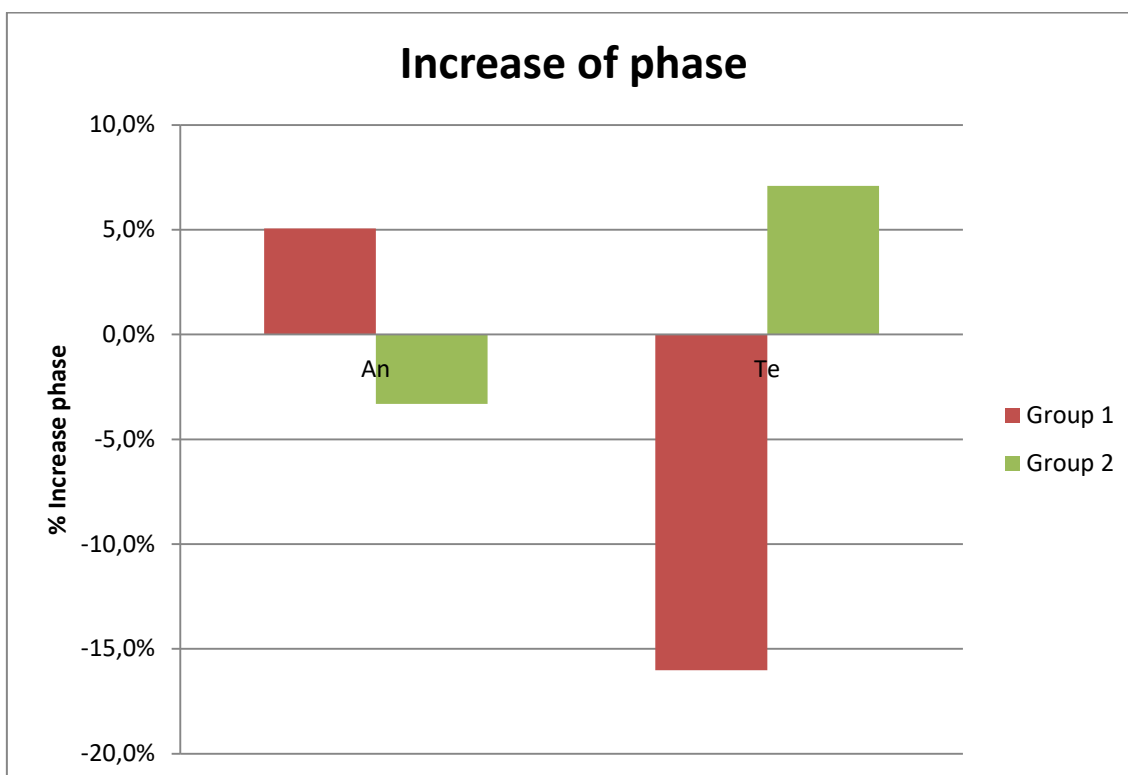


Figure 3. Increase of anagen phase and reduction of telogen phase

It is clearly observed that the products applied by group 1 (151002iD1-SH) cause an increase in the number of hairs in the anagen phase and a reduction in the number of hair is the telogen phase, indicating that the product not only leads to new hair growth, it also increases the length of the life of that phase. In the case of group 2 (151058iD1-SH), however, it is observed that there is a reduction in the number of hairs in the anagen phase and an increase in the number in the telogen phase, i.e. the placebo cannot stop hair loss nor increase the length of the life of the hair the volunteer already has.

The statistical results are according with the previous paragraph and in both cases, anagen and telogen phases, statistically significant differences are observed:

Anagen: Wilcoxon test: $W = 254$, $p\text{-value} = 0.012$), but not accept using t test $t = 1.052$, $df = 21.345$, $p\text{-value} = 0.304$

Telogen: Wilcoxon test: Wilcoxon test: $W = 76.5$, $p\text{-value} = 0.004$, t test $t = -3.4453$, $df = 27.295$, $p\text{-value} = 0.002$, IC95% confidence interval for the difference of means $(-0.406, -0.103)$.

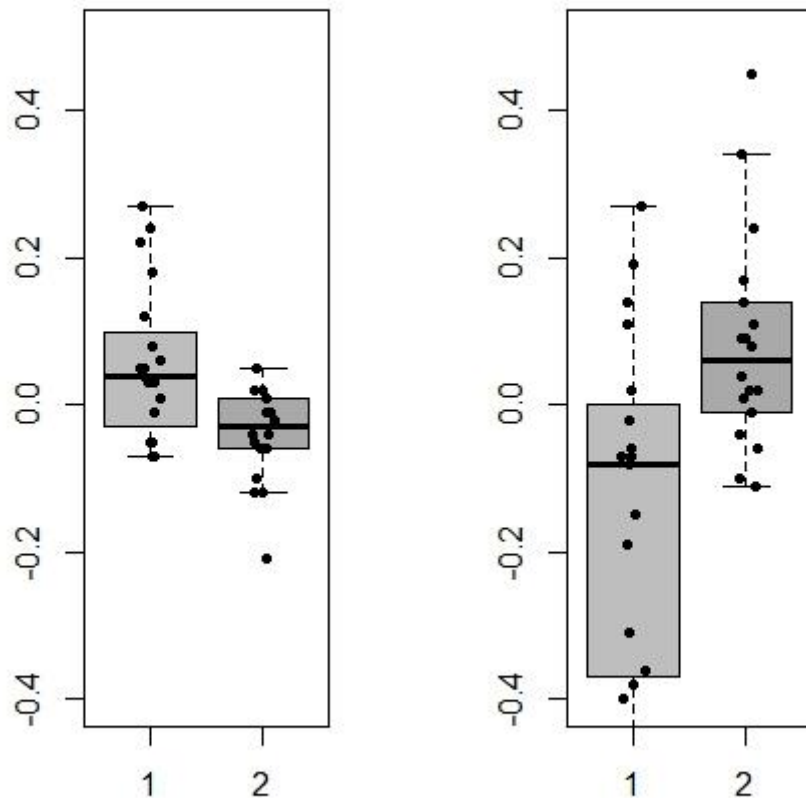


Figure 4. Left panel comparative anagenic phase. Right panel comparative telogen phase. Treatment group (1), Control group (2).

HAIR DENSITY

In Figures 5 and 6 the evolution of hair density for each volunteers of different groups at 90 and 150 days of starting treatment is shown.

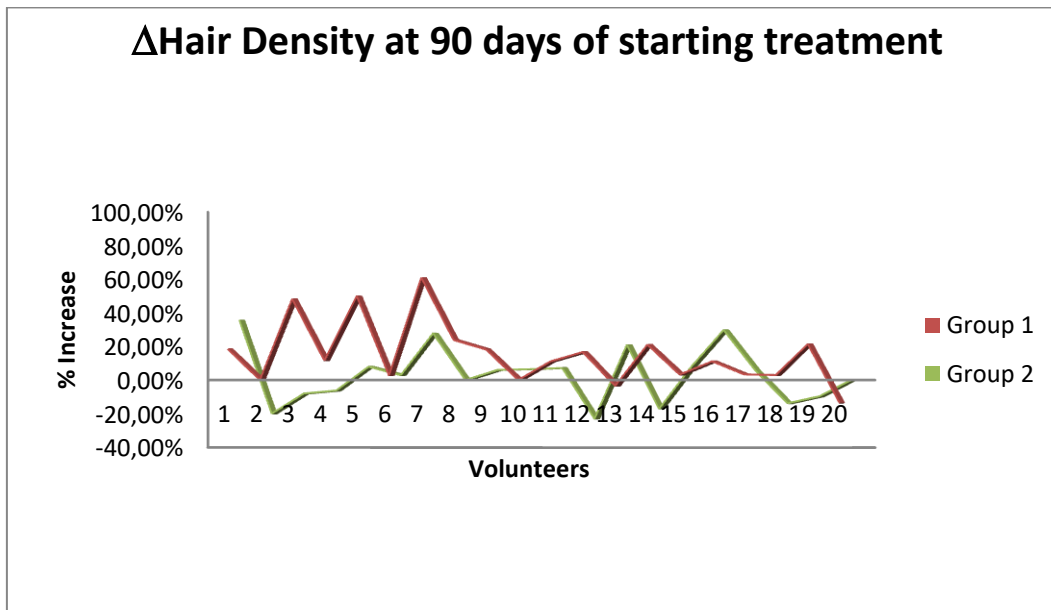


Figure 5: Hair density increased for each volunteers at 90 days of starting treatment

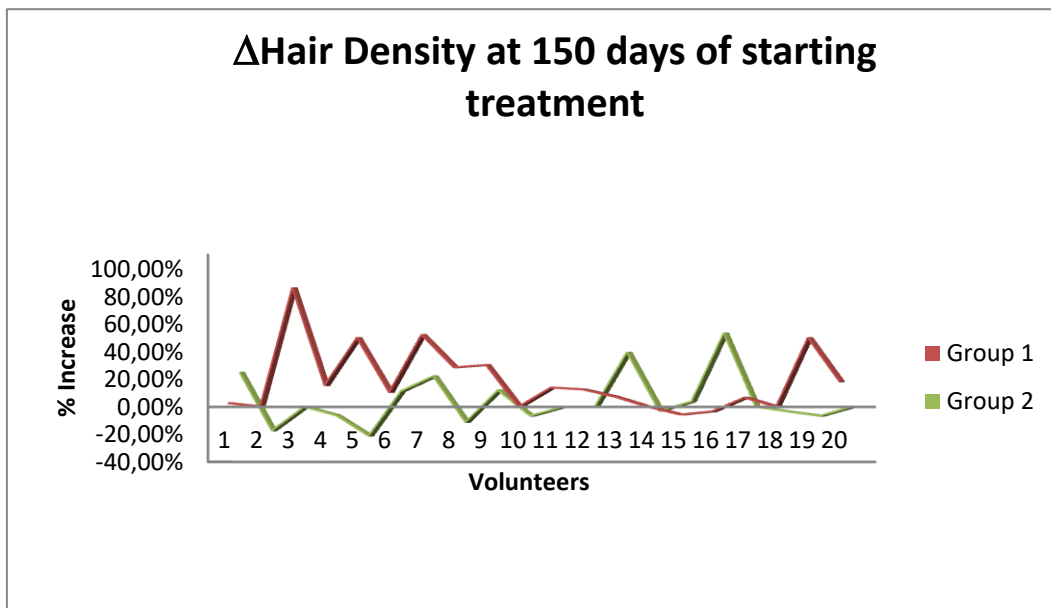


Figure 6: Hair density increased for each volunteers at 150 days of starting treatment

If we compare T0 hair density values with T90 hair density values, no significant differences between group are observed Wilcoxon test: $W = 246$, $p\text{-value} = 0.057$, but accept using , t test $t = 2.1991$, $df = 35.429$, $p\text{-value} = 0.03449$, IC95% confidence interval for the difference of means (0.009, 0.248). However, if we compare T0 hair density values with T150 hair density values significant differences between groups are observed (Wilcoxon test: $W = 244.5$, $p\text{-value} = 0.026$, t test $t = 2.03$, $df = 33.974$, $p\text{-value} = 0.05$. IC95% confidence interval for the difference of means (-0.0001,0.292).

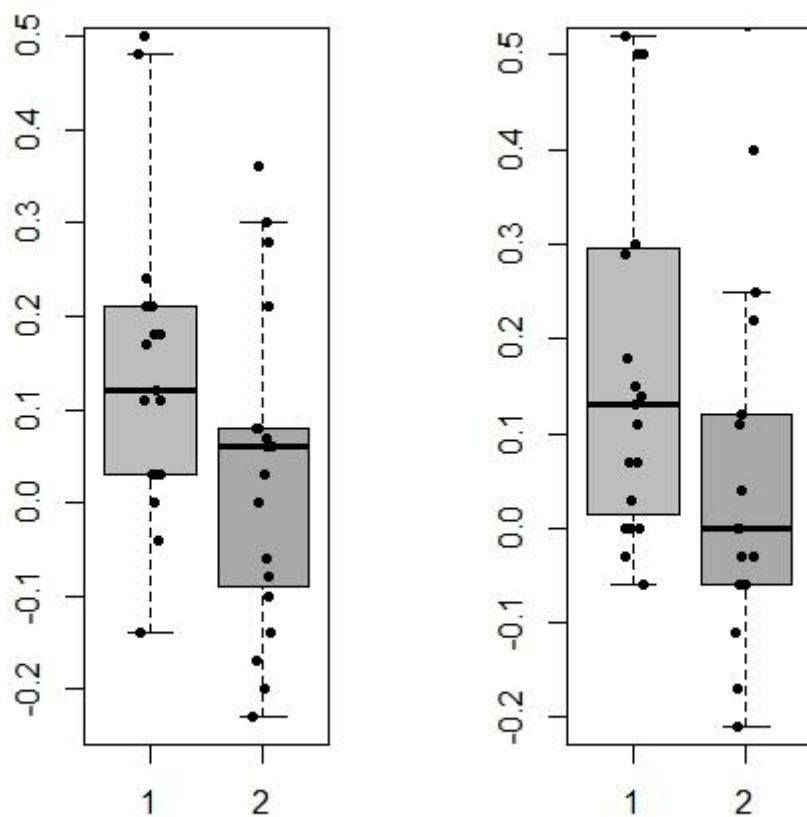


Figure 7. Left panel comparative at 90. Right panel comparative at 150. Treatment group (1), Control group (2)

Figure 8 shows the average values of the increases in density for the 2 groups, 90 and 150 days after the start of treatment.

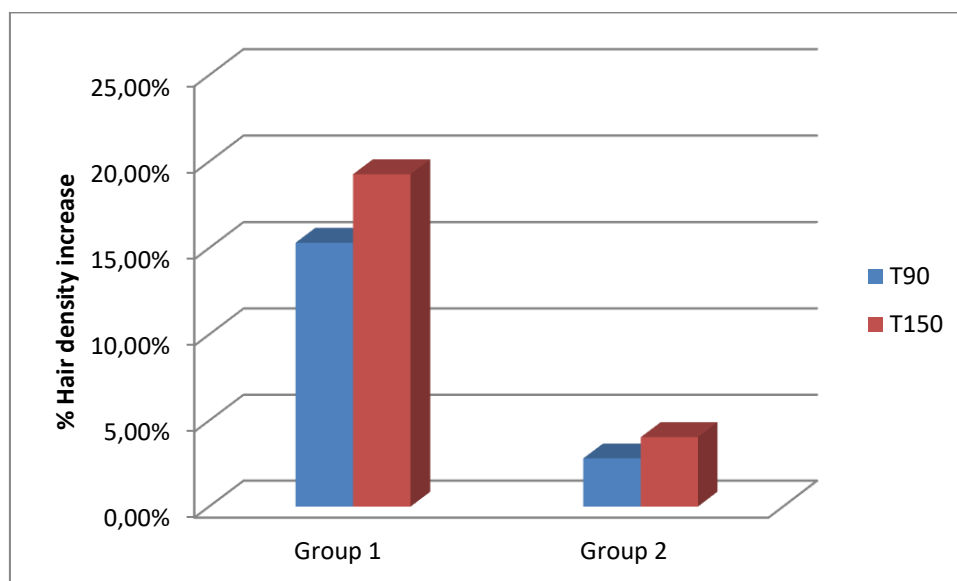


Figure 8: Average of hair density increased

An increase in hair density is observed for group 1 when compared with group 2 (placebo). It is also observed that hair density continued to increase between days 90 and 150 of the treatment, which indicates that these products have an effect that is maintained over time.

PHOTOGRAPHING HAIR ROOTS

GROUP 1

Volunteer 3

The selected image of this volunteer shows a bulb in the anagen phase, with well-developed sheaths present. If we compare this same phase at T_{150} , we observe that the matrix area is thicker, which may be indicative of an increased number of keratinocytes in the area where germination occurs, which could translate over time into thicker hair.

Volunteer 5

In volunteer 5 of this group, we observe a hair in the active anagen phase at T_0 , with sheaths present and abundant active melanocytes that give the hair an intense colour. At T_{150} the regenerating area is seen to have increased with respect to the start, the bulb is broader and more developed than at the start of the treatment.

Volunteer 19

In the trichogram of volunteer 19, at T_0 roots with little activity are observed, as is usual in a period of hair loss. We observe two roots that are starting the telogen phase in which the hair is

expelled, and in both, the area where reproduction takes place is thin and has little activity, the sheaths are poorly developed and look dehydrated. At T_{150} , for this same volunteer, a visible increase in the area where germination occurs is observed, with active and well-developed keratinocytes. The sheaths are visible and well formed.

GROUP 2

Volunteer 2

In this case, not many differences are seen between the images observed at T_0 and T_{150} . In both cases, the roots correspond to the late anagen phase, the sheaths are small, as are the areas where germination occurs, and are typical of periods when hairs are replaced.

Volunteer 14

The selected images of this volunteer show few differences between the roots at the start of the treatment and at the end. No differences are seen between the sizes of the areas where germination occurs or of the sheaths, which in both cases are seen to be dehydrated and rounded (which could result in the hair being poorly anchored to its structure). There are no significant differences in the morphologies of roots observed at T_0 from those observed at T_{150} .

Volunteer 18

In this cases, the two roots shown from this volunteer, taken at the start and end of the treatment, are in the catagen-telogen phase in which roots are replaced. In both cases, the hair bulb is seen to be inactive, the sheaths are not present, and the hair will fall within a few days. The fact that the bulb looks sclerotic (hardened) and small, shows that the treatment followed has not resulted in an improvement in the morphology of the follicle.

COMBING TEST

In order to confirm the efficacy of the treatment under study, we only need to add up the hairs removed in the combing test at the start, at mid-study and at the end of the treatment.

The results obtained in regard to the reduction of hair loss (expressed in %) in comparison with the start of the treatment are the following:

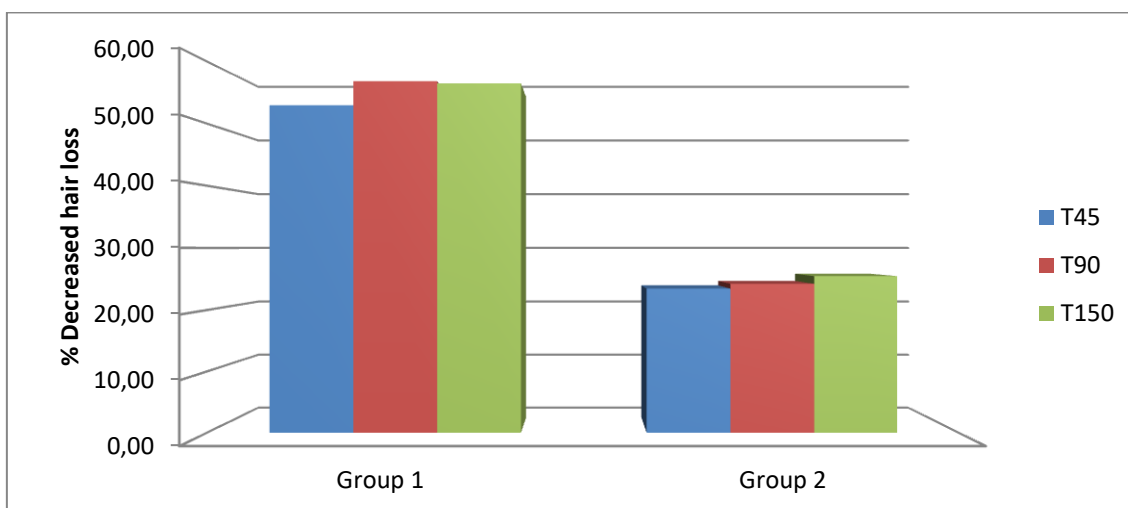


Figure 9. Reduction in hair loss (expressed in %) for different groups at different time.

Differences are observed between the placebo group (group 2) and group 1, which used products with active ingredients, at T45, T90 and T150. From the results obtained, it can be concluded that products 1 (151001iD1-SH), slow down hair loss by the different times studied. However, at T45 and T90 these differences are not statistically significant (T45: Wilcoxon test: $W = 115$, $p\text{-value} = 0.05711$), but accept using t test $t = -2.101$, $df = 28.511$, $p\text{-value} = 0.0446$, IC95% confidence interval for the difference of means $(-65.474, -0.856)$; T90: (Wilcoxon test: $W = 119$, $p\text{-value} = 0.075$, t test: $t = -1.6131$, $df = 35.495$, $p\text{-value} = 0.1156$). At T150 these differences are statistically significant (Wilcoxon test: $W = 103$, $p\text{-value} = 0.046$, t test: $t = -2.1971$, $df = 27.796$, $p\text{-value} = 0.036$, IC95% confidence interval for the difference of means $(-64.672, -2.255)$).

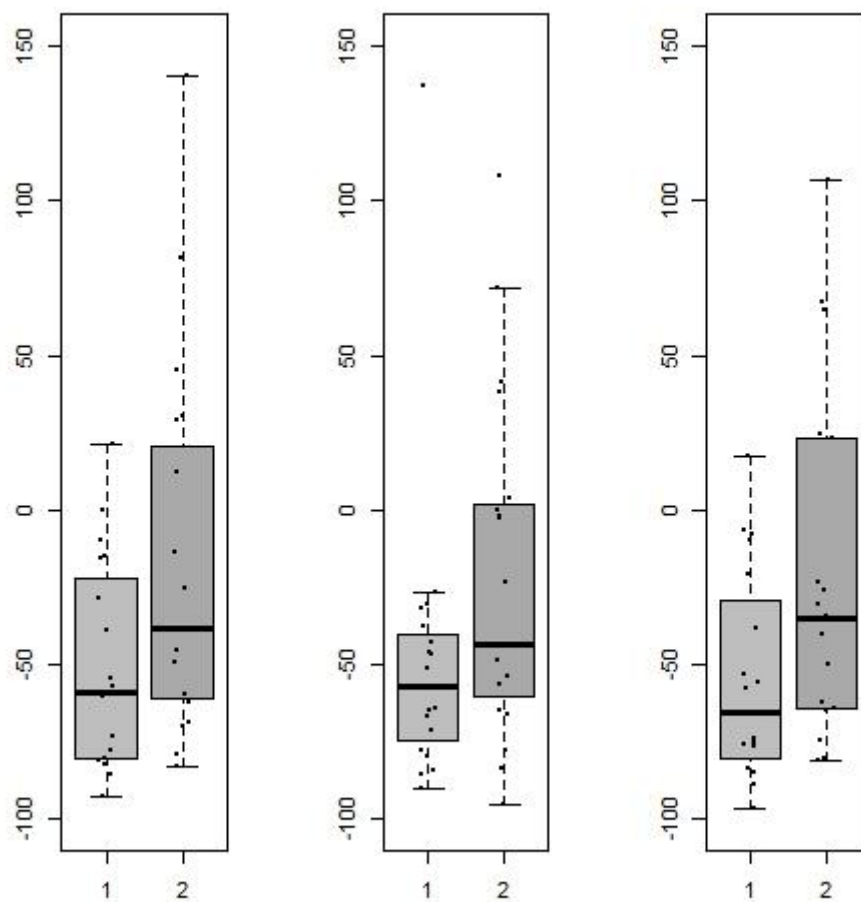


Figure 9. Left panel comparative at 45. Middle panel comparative at 90. Right panel at 150. Treatment group (1), Control group (2).

IV. CONCLUSION

According to the results the following conclusions were obtained

- ❖ **At the end of the experiment, the effect of the product 151002iD1-SH is appreciable visually.**
- ❖ **Treatment with the product 151002iD1-SH causes an increase in the A/T ratio. The differences between both groups are statistically significant.**
- ❖ **The increase in the percentage of the hair in the anagen phase, for product 151002iD1-SH is statistically significant compared with placebo**
- ❖ **After applying the product 151002iD1-SH an increase in hair density was observed at all the control points. This differences are statistically significant at T₁₅₀ (compared with placebo).**
- ❖ **At T₁₅₀ the results obtained in regard to the reduction of hair loss are statistically significant (compared with placebo).**

- ❖ **In the photographs of the bulbs, in the case of product 151002iD1-SH, as a result of the application of treatment the regenerating area is seen to be more developed and the sheaths are more extensive, which ensures that the hair is better anchored to its structure. A visible increase in the area where germination occurs is also observed, with active and well developed keratinocytes. For the placebo group there are no significant differences in the morphologies of the roots observed.**

V. CERTIFICATE AND SIGNATURE

Efficacy study no.: ID004-15

Start date: 27th October 2015

Completion date: 04th April 2016

Date report handed over: 09th May 2016

This document is filed for a period of 2 years at CENTRO DE TECNOLOGÍA CAPILAR, S.L.

C.T.C. no se hace responsable de las afirmaciones o conclusiones que no sean estrictamente las indicadas en el presente estudio.

Head of Product assessment:



Alfonso Fernández
PhD. Chemistry

Director of the study:



Eni Gómez
BSc Biology