FULL PAPER

A randomized control trial evaluating fluorescent ink *versus* dark ink tattoos for breast radiotherapy

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Objective: The purpose of this UK study was to evaluate interfraction reproducibility and body image score when using ultraviolet (UV) tattoos (not visible in ambient lighting) for external references during breast/chest wall radiotherapy and compare with conventional dark ink.

Methods: In this non-blinded, single-centre, parallel group, randomized control trial, patients were allocated to receive either conventional dark ink or UV ink tattoos using computer-generated random blocks. Participant assignment was not masked. Systematic ($\sum$) and random (σ) setup errors were determined using electronic portal images. Body image questionnaires were completed at pre-treatment, 1 month and 6 months to determine the impact of tattoo type on body image. The primary end point was to determine that UV tattoo random error (σ_{setup}) was no less accurate than with conventional dark ink tattoos, *i.e.* <2.8 mm.

Results: 46 patients were randomized to receive conventional dark or UV ink tattoos. 45 patients completed treatment (UV: $n = 23$, dark: $n = 22$). σ_{setup} for the UV tattoo group was <2.8 mm in the u and v directions ($p = 0.001$ and $p = 0.009$, respectively). A larger proportion of patients reported improvement in body image score in the UV tattoo group compared with the dark ink group at 1 month [56% (13/23) *vs* 14% (3/22), respectively] and 6 months [52% (11/21) *vs* 38% (8/21), respectively].

Conclusion: UV tattoos were associated with interfraction setup reproducibility comparable with conventional dark ink. Patients reported a more favourable change in body image score up to 6 months following treatment.

Advances in knowledge: This study is the first to evaluate UV tattoo external references in a randomized control trial.

INTRODUCTION

The number of breast cancer (BC) survivors is rising with most recent estimates suggesting there are over 500,000 such females in the UK alone.[1] Consequently, an acceptable cosmetic outcome following treatment is of importance to females in this increasing population.[2]

Dark ink tattoos (1–3 mm diameter) are routinely used in the majority of radiotherapy (RT) departments to reproduce the patient planned position and ensure precise radiation delivery.[3] Furthermore, the use of external references, immobilization and image guidance in modern RT practice mitigates the risk of geographic miss, ensuring compliance with International Commission on Radiation Units 50 guidelines.[4] The Royal College of Radiologists (RCR) cite a survey by Dobbs et al[5] (2003) suggesting

systematic errors ($\sum_{setup}$) and random errors (σ_{setup}) of 3.2 and 2.9 mm, respectively, for breast RT.

Despite the clear advantages of dark ink RT tattoos, they are associated with the following limitations:

(a) It has been reported that 15–30% of patients with BC experience body image concerns that persist into survivorship[6] and a number of other authors have suggested that RT tattoos may contribute to body image dissatisfaction.[2,6–8] Changes to physical appearance and body function are associated with poorer psychosocial outcomes including anxiety and depression.[8,9] A UK survey of RT departments suggested that patients undergoing breast RT do not want a permanent reminder of treatment and are most likely to decline

permanent tattoos for cosmetic reasons (Townend 2014, national audit results, unpublished).

(b) Melanocytic lesions or hair follicles can be mistaken for tattoos, potentially causing errors in treatment delivery.[7,10]

(c) Religious or cultural beliefs may prohibit or make patients feel uncomfortable about receiving tattoos.[7]

(d) It has also been reported that dark ink tattoos can be difficult to localize when patients have a dark skin tone.[7]

Alternatives to dark ink tattoos include semi-permanent marking methods; however, these have been found to be inferior to dark ink tattoos in terms of patient comfort, durability and longevity.[2,11] In this study, we test the use of "ultraviolet (UV) ink" tattoos. This ink is a light-coloured tattoo pigment combined with a UV responsive fluorescent dye. This ink is observable under UV light when the wavelength of emission from the fluorophore is Stokes shifted into the visible region of the electromagnetic spectrum. However, once the excitation source is removed, the dye does not fluoresce and therefore, the tattoo becomes "invisible" in ambient lighting.

In this study, we focus on a commercially available tattoo ink whose active component is the non-toxic fluorescent compound 7-diethylamino-4-methylcoumarin (coumarin 1) dispersed in a melamine formaldehyde toluenesulfonamide polymer matrix

(Figure 1a). It was found that the ink had an excitation maximum of approximately 390 nm (dashed in Figure 1b) and a peak emission at approximately 435 nm (red in Figure 1b). This light is readily observed as a blue/green fluorescence (Figure 1c). Excitation is facilitated by the use of a handheld wavelength-matched UV torch with a peak emission of 375 nm (± 5 nm, full width at half maximum). Further spectral filtering was not required to directly visualize the tattoo on the skin surface.

Several preliminary investigations using chicken and pig skin suggest that UV ink tattoos may offer superior RT marking than conventional dark ink tattoos.[7,10] However, further investigation with human subjects has been recommended. A dermatology study proposed that UV ink tattoos could be used as a discrete method to aid the correct identification of cutaneous biopsy sites.[12] This single patient study indicated that UV ink may have sufficient longevity to provide a record of RT throughout the patient life, although this has not been verified, and may be dependent on a number of variables such as UV exposure.[13]

These studies indicate that UV ink tattoos offer a viable alternative to dark ink. In addition, they may ameliorate body image dissatisfaction and improve the patient experience of breast RT. Indeed, a patient advocate group who were consulted about the patient experience of RT tattoos confirmed that the negative

Figure 1. Principle of invisible tattoos: (a) a wide-field fluorescence micrograph of a 10^6 dilution of tattoo ink in Phosphate-buffered saline (PBS) (excited with 405 nm light) is demonstrating a dye molecule dispersed in the polymer; scale bar is 3 µm. (b) Spectral properties of ultraviolet (UV) tattoo ink: excitation (Exc.) (dashed red) and emission (Em.) (solid red) spectra of the UV ink and the emission spectrum of the handheld torch are used to visualize the dye (blue). (c) Manufactured sample skin tattooed with standard dark (left) and UV ink (right) under ambient (top) and UV light (bottom): UV is invisible under ambient light and clearly visible under UV illumination with a handheld UV torch; scale bar is 25 mm.
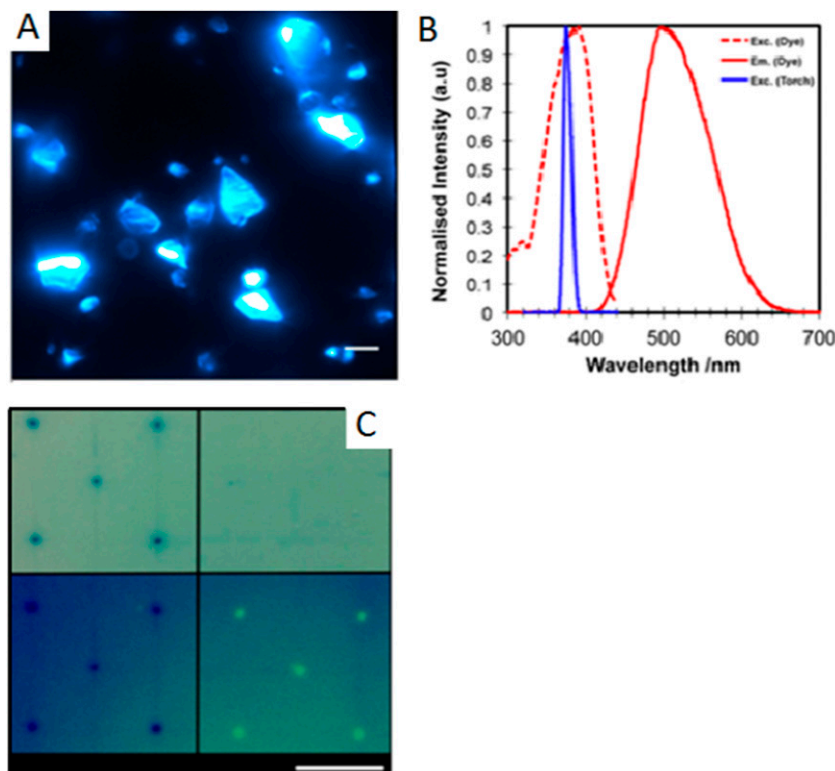
experience may impact upon survivorship for some females. This study investigated the use of UV ink in the RT treatment of BC. The primary aim was to evaluate interfraction reproducibility using UV ink tattoos. Secondary aims were to assess body image satisfaction, radiographer satisfaction, tattoo visibility in dark skin tone and the time burden at the treatment unit.

METHODS AND MATERIALS
This study was approved by The Royal Marsden Committee for Clinical Research and a National Health Service Research Ethics Committee. All females had undergone breast-conserving surgery or mastectomy for early-stage invasive ductal or lobular carcinoma (pT1-3b N0-1 M0) and had been recommended adjuvant RT to the whole breast or chest wall (with or without nodal irradiation or tumour bed boost). Dose prescription was 40 Gy (Gy) in 15 fractions (±13.35 Gy/5 tumour bed boost) over 3–4 weeks.

The absorption and emission characteristics of two UV tattooing ink products were determined using a Cary Eclipse Fluorescence Spectrophotometer© (Agilent Technologies, Santa Clara, CA) (Figure 1). A selection of UV torches was also tested to determine peak emission wavelengths using a compact charged coupled device spectrometer (Thorlabs, CCS175). This analysis revealed that the selected dye (Nuclear Fallout©; Millenium colorworks, CA) is well suited to the light emitted by the UV torch (INOVA X5©; Nite Ize Inc., Boulder, CO), and the primary emission wavelength of the dye is in the visible region of the electromagnetic spectrum. Consultation with a professional tattoo artist demonstrated that round liner (Size 3) professional tattooist needles were the most effective for manually administering UV ink into the dermis. The UV ink was available in 1 oz bottles and decanted into sterile receptacles for each patient before administration with a sterile lance.

All radiographers were trained in safe and accurate operation of UV handheld torches, adhering to the International Commission for Non-ionizing Radiation Protection 2004.[14] UV torch emission was measured to ensure exposure limit values for the skin and eyes would not be exceeded for the patient or user during clinical use (exposure limit values defined by International Commission for Non-ionizing Radiation Protection 2004).[16] Pre-treatment radiographers were trained in the safe and effective administration of UV ink tattoos.

All patients were positioned on a breast board (Medtec, Indiana) and CT scan images were acquired using 3 mm slice thickness/spacing. Tattoos were marked bilaterally and medially with the addition of an anterior supraclavicular tattoo if required. A hypodermic needle was used to administer dark ink tattoos as per standard departmental practice. Measurements from anatomic landmarks and photographs were taken to record the location of UV tattoos. The handheld UV torch was used during treatment sessions to locate and mark (using a fine marker pen) the centre of UV tattoos to facilitate daily setup.

The primary end point was interfraction reproducibility measured using electronic portal images (EPIs) acquired from the tangential treatment fields, for Fractions 1–3, and a minimum of once weekly thereafter. Template matching was used to register chest wall and contour as visualized on EPIs with digitally reconstructed radiographs to quantify displacements from the planned position. Displacements were recorded in the u–v plane in millimetres (Figure 2).

Secondary end points included patient body image, radiographer satisfaction, time taken at CT simulation and for treatment delivery and ease of visualization of UV tattoos in patients with a darker skin tone. The influence of tattoo type on body image was measured using a 10-item validated body image scale (BIS). Patients were asked to complete questionnaires at baseline (before the RT planning CT scan) and at 1 and 6 months post-CT simulation. Opportunity for verbatim responses was also provided. Radiographers were asked to complete satisfaction questionnaires at CT simulation and once weekly during treatment. Questions were related to ease of administration and visualization of tattoos. All questions had a response on a scale of 0–3 with space for comments. The time-on and time-off CT or treatment couch was recorded as well as the beam-on and beam-off times.

A Felix von Luschan chromatic scale was modified and used by CT simulation radiographers to record patient skin tone. The scale was simplified into three distinct groups [White European (1), East Asian (2) and Sub-Saharan skin tone (3)].

Statistical considerations
To rule out σ_{setup} of >2.8 mm when using UV tattoos, assuming a σ_{error} of 2 mm with dark ink tattoos and a standard deviation of 1.0 mm, 21 patients were required in each group (42 patients in total) based on a two-sample t-test with 80% power and a one-sided 5% significance level.

Patients were randomized using a 1:1 ratio by a telephone call to the local clinical trials and statistics unit (Clinical Trials and Statistics Unit, Institute of Cancer Research).

Figure 2. The right anterior oblique tangential field digitally reconstructed radiograph to illustrate the u and v directions (arrows) in the imaging plane.
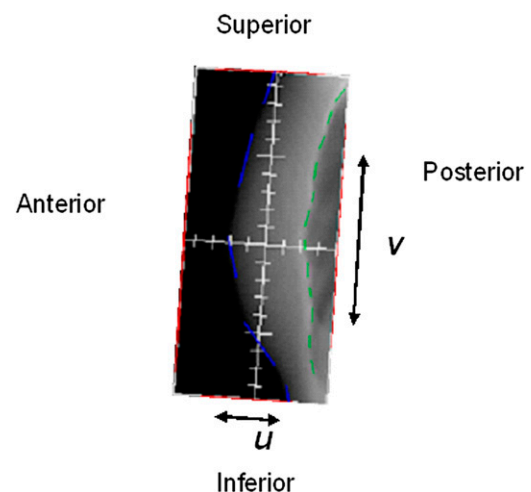
Computer-generated random blocks were used and allocation was non-blinded.

Statistical analyses were performed using SPSS® Statistics v. 19 (IBM Corp., New York, NY; formerly SPSS Inc., Chicago, IL). EPI displacements were quantified for anterior and posterior oblique beams. These displacements were averaged to determine daily errors in u and v directions for each imaged session. The RCR (2008)[15] guidelines were used to calculate individual and population random (σ_{setup}, σ_{error}) and systematic ($\sum_{setup}$, $\sum_{error}$) errors in both directions. Descriptive statistics were reported and formal statistical comparisons between groups were made using t-tests. A one-sided, one-sample t-test was used to determine whether σ_{setup} calculated for the UV tattoo group were <2.8 mm in both u and v directions.

The changes in BIS from baseline to 1 month and from baseline to 6 months were computed and compared between groups using a Mann–Whitney U-test. Changes from baseline at each time point were also categorized as no change, improvement or worsening of score. Participant verbatim responses were analyzed and salient themes reported.

Radiographer satisfaction scores (RSS) were calculated and compared between conditions for CT simulation and treatment stages using descriptive statistics and Mann–Whitney analysis. Scores ranged from 0 (no satisfaction) to 9 (complete satisfaction). Verbatim responses were analyzed and comments representing salient themes reported.

Total session times and treatment setup durations were reported using descriptive statistics and Mann-Whitney U-test for comparison.

Analyses were conducted on an intention to treat basis once the last patient had reached the 6-month follow-up.

RESULTS

46 patients (23 patients in dark ink group, 23 patients in UV ink group) were randomized from a single UK centre between April and July 2014. The median age of participants was 57 years (range: 30–70 years) and the majority of patients were White European (Table 1). There were no significant differences in baseline characteristics between the two groups.

One patient was consented and randomized to the dark ink group but did not commence treatment owing to a change in clinical management. 45 patients completed RT. All patients treated within the study have now been followed up for 2 years and there have been no reports of tattoos becoming visible in ambient lighting or any tattoo-related skin toxicity for either group.

Random setup error (σ_{setup}) for patients receiving UV tattoos measured in the u and v directions was statistically less than the pre-specified 2.8 mm ($p = 0.001$; $p = 0.009$, respectively). No statistically significant differences between groups were found in σ and Σ errors in any direction (Table 2).

100% (45/45) and 96% (43/45) of participants completed the body image questionnaires at 1 month and 6 months, respectively, post-CT simulation. 56% (13/23) of patients with UV tattoos reported improved body image as compared with only 14% (3/22) of those with dark ink at 1 month compared with baseline. Worsening body image score was reported by 22% (5/23) of patients with UV tattoos compared with 50% (11/22) of patients with dark ink at 1 month compared with baseline. A similar distribution was seen at the 6-month stage with

Table 1. Baseline characteristics for each group

Patient characteristics		UV ink tattoos $N = 23$ n (%)	Dark ink tattoos $N = 22$ n (%)	p-value
Age (years)	Mean (SD)	58 (12.73)	56 (8.83)	0.618
	range	30–79	43–71	
Surgery	Breast conservation	19 (83)	20 (91)	0.413
	Mastectomy	4 (17)	2 (9)	
Nodal irradiation	Yes	3 (13)	1 (5)	0.317
	No	20 (87)	21 (95)	
Tumour bed boost	Yes	4 (17)	8 (36)	0.150
	No	19 (83)	14 (64)	
Chemotherapy received	Yes	6 (26)	4 (18)	0.524
	No	17 (74)	18 (82)	
Skin tone	White European	16 (70)	13 (59)[a]	0.261
	East Asian	5 (22)	5 (23)	
	Sub-Saharan	2 (9)	4 (18)	

SD, standard deviation; UV, ultraviolet.
Statistical comparisons have been made using the t-test for age, χ^2 test for trend for skin tone and χ^2 tests for all other baseline characteristics.
[a]Baseline data were not available for the patient who did not receive radiotherapy.

Table 2. Setup accuracy data [in millimetres (mm)] in *u* and *v* directions (in mm)

Direction		UV ink group	Dark ink group	Significance. (two-tailed)
v	MD	−0.3	−0.3	–
	Σ	1.5	1.1	0.865
	σ	2.1	1.5	0.068
u	MD	−0.3	−0.8	–
	Σ	2.0	1.7	0.337
	σ	2.0	1.8	0.469

Σ, population systematic error; σ, population random error; MD, population mean displacement; UV, ultraviolet.

a worse score reported by 26% (6/23) and 50% (11/22) of patients, respectively, compared with baseline.

Median BIS was consistent for the UV tattoo group with a median score of 7 at both baseline and 1 month. However, median BIS scores showed deterioration for the dark ink group with scores of 5.5 and 6.5 at baseline and 1 month, respectively. At 6 months, however, median scores had improved (decreased) from the baseline by 1.0 for the UV group and 0.5 for the dark ink group. No statistical difference in score change was found between groups. Comments suggested that some patients had concerns about the visibility of dark ink reference marks as shown below:

"I feel much better without tattoos being visible. Much more confident"

(patient comment, UV ink group).

Some participants may associate visibility of dark ink tattoos with cosmetic concerns or negative feelings, as illustrated below:

"I don't really have a problem with the tattoos but yes they do serve to remind you of a particularly traumatizing experience" (patient comment, dark ink group).

Median CT simulation time was 16 min [interquartile range (IQR): 8 min; range: 9–45 min] *vs* 20 min (IQR: 8 min; range: 15–35 min) for the dark and UV ink groups, respectively. Median treatment setup time increased from 5 min (IQR: 2 min; range: 2–16 min) to 6 min (IQR: 3 min; range: 1–24 min) for dark ink and UV ink groups, respectively. Total treatment session median times were increased from 9 min (IQR: 5 min; range: 4–48 min) to 10 min (IQR: 5 min; range: 4–48 min) for dark and UV ink groups, respectively (Table 3). Differences in CT simulation, setup and total treatment times were found to be statistically significant.

Table 3. Timing data

Radiotherapy stage	Descriptive statistics	Dark ink type	UV ink type	Mann–Whitney *p*-values
CT simulation	Median	16	20	0.0203
	Q1	14	17	
	Q3	22	25	
	Minimum	9	15	
	Maximum	45	35	
Treatment setup time (min)	Median	5	6	<0.0001
	Q1	4	5	
	Q3	6	8	
	Minimum	2	1	
	Maximum	16	24	
Treatment total time (min)	Median	9	10	0.0138
	Q1	7	8	
	Q3	12	13	
	Minimum	4	4	
	Maximum	48	48	

UV, ultraviolet.
Q1 = Quartile 1.
Q2 = Quartile 2.

Median RSS were lower when using UV tattoos compared with dark ink at CT simulation and Week 2 time points (8 *vs* 9, respectively). Median scores were equivalent for Weeks 1 and 3 (9 *vs* 9, respectively), but the range in RSS was greater for UV tattoos (Figure 3). Lower scores observed for the UV tattoo group were found to be statistically significant for all stages of the treatment pathway except Week 1.

Radiographer UV tattoo comments (CT simulation $n = 12$; treatment $n = 28$) revealed that difficulty in administering tattoos and poor visibility on some Patient's skin were likely responsible for reduced satisfaction. Radiographers were not able to locate all UV tattoos in both patients with Sub-Saharan skin tone (Category 3 skin tone). These patients were retattooed with standard dark ink; however, one comment suggested there was further difficulty in locating the dark ink tattoos for one of these participants, and the anterior UV tattoo was still used for setup.

DISCUSSION

This study has shown that setup accuracy using UV ink tattoos is comparable with that using standard dark ink. Moreover, the use of UV ink is associated with a more favourable change in patients' body image score compared with conventional dark ink.

The study sample captured a broad age range of female patients and was representative of a South-West London population. The absence of any reported tattoo ink skin reactions was consistent with other authors' assertions.[7,16] Furthermore, this finding implies that there is great potential for the clinical use of UV ink in RT treatment setups.

Setup accuracy data indicate that UV tattoos are associated with clinically acceptable interfraction reproducibility and therefore may be used as an alternative to dark ink tattoos. The lack of a statistically significant difference between setups with the two marking methods is reassuring, although the study was not powered to detect small differences in σ_{error} and $\sum_{error}$ between the two groups. Overall, UV tattoo setup accuracy was within RCR (2008) recommendations (<3 mm).[15]

Figure 3. Radiographer satisfaction scores for ultraviolet (UV) (left) and dark (right) ink tattoos at CT simulation and each week of treatment.
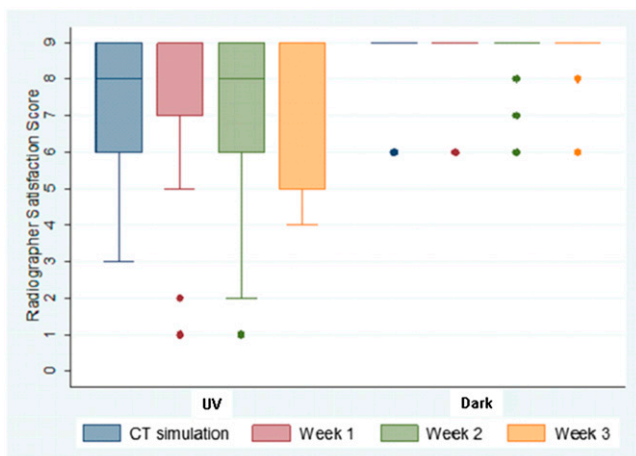
BIS comparison can only be made between the time points captured, as many variables known to influence body image could not be controlled for in this small sample, *e.g.* type of surgery;[17,18] however, baseline characteristics showed no significant differences between the groups. Some authors found that body image is sensitive to time since surgery[17] and so, BIS cannot be solely attributable to tattoo type; however, differences in the direction or degree of change between groups may indicate an effect.

Results suggest that invisible tattoos have a less negative impact on body image compared with dark ink. However, such a large difference between the groups is perhaps unexpected and could be the result of anticipation bias; *i.e.* as the study was non-blinded, patients may have been influenced by their randomization, with patients with UV tattoo scoring more favourably compared with the dark ink group.

It is difficult to know whether such differences between the groups are a result of tattoo type, bias or other variables that could not be controlled for. However, comments suggest that a proportion of patients value having invisible markings.

By offering UV tattoos, departments are likely to improve the patient experience of breast RT by offering choice and addressing the cosmetic and psychological concerns associated with conventional dark ink tattoos.

Radiographers reported greater satisfaction using conventional dark ink tattoos. Comments indicated that difficulty in administering and increased time to locate UV tattoos were partly responsible. Comments suggested that radiographer training and exposure to this new tattooing technique is important to deliver consistent, viable markings. Despite lower radiographer satisfaction, UV tattoos were visible in all participants except in those with Sub-Saharan skin tone (91%, $n = 21/23$). However, because of the small number of patients with Category 3 skin tone recruited ($n = 2$), it is not possible to comment on the role of UV tattoos to enhance visibility in patients with a darker skin tone and further investigation is required.

Timing analysis suggested there was an increase in CT simulation time. This is likely attributable to time spent measuring and documenting UV tattoo location and taking additional photos. Setup time and overall treatment time were also marginally increased in the UV tattoo group. This could be partly accounted for by the need to use a UV light source to highlight markings, which constitutes an additional task within the workflow. Difficulty in visualizing tattoos in some participants may also contribute to the protracted setup and treatment times recorded.

UV tattoos offer clinically acceptable interfraction reproducibility compared with conventional dark ink when used to set up patients with white European and East Asian skin tones. A difference in change of BIS between the two groups suggests improved satisfaction with UV tattoos. Patient comments further support the hypothesis that a significant proportion of females are likely to derive benefit from not having dark ink RT markings.

CONCLUSION

UV tattoos offer setup accuracy comparable with that of conventional dark ink and may improve patient experience of breast RT. UV tattoos are also associated with an improvement in BIS compared with standard dark ink.

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