Localization Tattoos: An Alternative Method Using Fluorescent Inks

Jennifer E. David, B.S., R.T.(T); Steven K.B. Castle, M.B.A., R.T.(T); Karla M. Mossi, Ph.D.

In radiation therapy, patient markings are used for target localization to ensure accurate and precise treatment set up. The typical treatment plan consists of many consecutive treatments that require minimal variations from one daily set up to the next. Ink tattoos are the most common type of permanent markings for patients. Dark pigmented ink, or India ink, is injected beneath the skin at selected points—generally along the treatment axis. India ink originally was used for writing and printing and now is more commonly used for drawing. These tattoos are visible and reliable localization points during the prescribed treatment course, while also serving as a reference point later. There are 3 main issues in current tattoo practice. The first is mobility. On some elderly and obese patients, the skin tends to be looser; accuracy is lost because the external tattoo setup points are not necessarily consistent with internal structures. Some methods to correct this include increasing port film frequency; using bony anatomy structures, such as the suprasternal notch and pelvic crest, to verify correct field placement; or placing tattoos in more stable areas and shifting for treatment. The second challenge is locating and identifying the tattoos. With darker-skinned patients, it sometimes is difficult to locate the black India ink tattoos. Another daily issue for therapists is the identification of tattoos from moles or regions of dense hair. Hair follicles, moles and tattoos may be similar in appearance, leading to possible setup error. The third issue with patient tattooing is that the points remain highly visible. It is important to see the tattoo during daily set up and as a reference in future treatment planning; however, permanent tattoos remind cancer survivors daily of their disease and treatment. Historically, tattoos were used to localize past treatment ports during cancer recurrences. Today, general practice is to confirm earlier treatment volumes via imaging of bony anatomy in simulation.

Like scars, visible tattoos are an open doorway to a past experience many patients may prefer to forget. Tattoos are a physical tie to an emotional and difficult time in life and can lead to psychological challenges. Scars and permanent markings remind a patient of why the marks are there. For example, not only do breast cancer survivors have to cope with mastectomy or lumpectomy scars, but they also are marked with the small black tattoo dots. Commonly, breast and lung cancer patients are left with 1 or more tattoos on their neckline where the marks are easily seen. Institutions not equipped with thermoplastic masks or similar devices also may mark head and neck fields directly on the patient’s head, face and neck area. Again, these tattoos are visible and difficult to hide. Figure 1 illustrates the use of a head cast device and setup marks not using a head cast.

One possible solution to these problems is to use a tattoo ink that is less visible in normal light. However, the tattoo must be highly visible for daily treatment set up when room lights are dimmed to illuminate the lasers. An ideal solution would be a tattoo that only is visible in the treatment setting.

The purpose of this study was to investigate the feasibility of using black light-responsive tattoos as an alternative for radiation therapy localization. The technique uses fluorescent ink and black lights installed in the treatment room. Fluorescent ink is extremely black light-responsive and is commonly found in pen highlighters. It is more likely than dark India ink to blend with skin tones, making it virtually invisible under normal light. Additionally, because highlighters are available in multiple colors, therapists may select a fluorescent ink to stand out against hair and on darker-skinned patients. According to the American Society for Testing and Materials (ASTM) and the Art and Creative Materials Institute (ACMI), Sanford highlighter ink (Sanford Corporation, Oak Brook, Ill) is certified nontoxic, making it safe to use for dermal and subdermal applications.
Methods and Design

A randomized experimental design was chosen in which a control group (India ink tattoos) was compared to equally distributed treatment units (fluorescent ink tattoos) in 2 light settings: normal and black light.

Measurements of relative intensity, diameter and relative visibility were performed to investigate the efficacy of India ink and fluorescent ink in different light settings: normal light and a treatment room with black light. Four raw chicken breasts were used to simulate human tissue. Both ink types were injected under the skin using an 18-gauge needle. Three different colors of fluorescent ink were tested for maximum black light responsiveness and minimum visibility in normal light. The ink was extracted from yellow, pink and orange highlighters (Sharpie Accent Liquid Highlighters, Sanford Corporation, Oak Brook, Ill). Each chicken breast was tattooed with each of the fluorescent ink colors as well as India ink.

The tattoos were examined over a period of 5 additional days in which the 4 samples were identically washed with a soap and water mixture once a day to test for durability and longevity of the tattoo. Tattoo size measurements and ink intensity were recorded daily for each of the 16 tattoos. Intensity was measured according to the theoretical inverse of the law of light intensity, which states that the further the distance an object is seen, the higher intensity the object has. Hence the intensity, in units of energy per unit area, is directly proportional to the distance, as shown in equation 1:

\[ I_f = I_o / d^2 \]

For the study, the tattoos were assigned an arbitrary intensity \( I_o \) of 1 at 30.5 cm (12 inches). On the first day and each consecutive day after that, the distance at which each tattoo still could be seen was measured and put into equation 2:

\[ I_R = d / 30.5 \]

In equation 2, \( I_R \) is the relative intensity of the tattoo, which is dimensionless.

The value of relative intensity, \( I_R \), is very limited and is used for relative comparison only, since the sensitivity of the measurement depends on the visibility of the tattoo as determined by the observer and the device used for recording the distance to the target. To reduce some of the variability, measurements were taken of each ink type (4) on each of the 4 different samples during a total period of 6 days, for a total 96 observations.

The relative visibility of the tattoos in both a simulated treatment room with black light and normal light setting also was recorded. The observer, standing at a distance of 61 cm (24 inches) from the chicken breasts, recorded whether the tattoo could be identified. Two nominal values were used to tabulate visibility. A “1” denoted visible, while a “0” represented not visible. The meat was kept refrigerated between testing. Other factors such as toxicity of India ink and fluorescent ink were researched and compared, as well as the associated cost of each method.

Hypothesis and Assumptions

We hypothesized that fluorescent ink tattoos would have better relative intensities than India ink in the treatment room with normal light vs black light.

We made the following assumptions in conducting the study:

- Human skin is similar to raw chicken breasts, which can serve as a suitable substitute for human tissue with respect to tattoos.
- The chicken breast remained the same consistency and texture throughout the experiment.
- Each of the tattoo inks used for the study would be durable, permanent and equal in size.
- The researcher’s visual acuity was consistent under different lighting conditions.

We recognize that refrigeration of the samples may preserve some qualities of the ink that would not occur in normal skin, like in the case of oily human skin.
Results

The average intensity for the India ink tattoos in normal light (8.26) was 54% less intense than in the black light (3.82). For the fluorescent inks, however, the differences were reversed. For pink, orange and yellow ink, the intensities were 52%, 66% and 86%, respectively, more intense in the black light than normal light. The yellow highlighter ink tattoo had the greatest variation in intensity measured in normal light, while the India ink tattoo had the greatest deviation in intensity in the black light. (See Fig. 2.)

To further analyze and validate the data, a 2-way analysis of variance (ANOVA) with replication was performed on the relative intensity data. An ANOVA assumes a normal distribution and helps eliminate the possibility that the differences in the results are random within a designated confidence level. Such analysis is shown in Table 1, where the probability of the differences in lighting method and the type of ink are less than 5% ($p < 0.0001$). In addition, results show a strong possibility of interaction between the type of ink and the type of illumination used.

According to the binary data representing visibility, all the tattoos had an average visibility factor of 1 under the black light. However, under normal light conditions, the yellow ink tattoo had an average visibility factor of 0, making it difficult to identify.

Figures 3 and 4 show the tattoos on 1 chicken breast sample in normal lighting and black lighting. Only the fluorescent ink tattoos appear in Figure 4 because the India ink tattoo is not visible. Both images were taken at a distance of 61 cm.

All the tattoos appeared to be durable and long-lasting because no observable change in diameter size was noted during the 6-day study period. To ensure that the differences observed were not significant, a 2-way ANOVA was performed. Results of the analysis are shown in Table 2. Contrary to the type of lighting of the room, the differences were not significant ($p > 0.05$). The analysis verifies that the tattoo diameter did not fade over the test period and that there are no significant differences in diameter among the different ink types, as illustrated in Figure 5. In this figure, the tattoo diameter, $D$, with respect to the initial tattoo diameter, $D_i$, is plotted over the 6-day period.

Extraneous variables such as room and refrigerator temperature were noted, with standard deviations of 1.26° and 1.21°, respectively. These variables were relatively constant and did not appear to have an effect on the experiment.

As part of the experiment, toxicity certifications were reviewed to determine the safety of each type of ink. Toxicity of India ink and highlighter ink proved nonsignificant; both types of ink are certified nontoxic.\textsuperscript{10} While India ink is certified by the ACMI, both the ACMI and ASTM certify highlighter ink as nontoxic.

![Fig. 2. Relative intensity of the India and fluorescent ink tattoos in normal and black light.](image-url)

<table>
<thead>
<tr>
<th>Table 1</th>
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<tr>
<td><strong>Two-way ANOVA for Ink Type and Lighting Method</strong></td>
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<tr>
<td>Source of Variation</td>
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<tr>
<td>Lighting Method</td>
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<tr>
<td>Ink Type</td>
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<tr>
<td>Interaction</td>
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<tr>
<td>Within</td>
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<tr>
<td>Total</td>
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Discussion

The results of the study support the use of a black light-responsive tattooing technique. Relative intensity proved to be the most significant measurement of tattoo ink magnitude due to the differences in measurements between black light and normal light settings. As mentioned before, an important characteristic of a tattoo used in cancer treatment is its visibility for treatment set up and invisibility at other times. The yellow highlighter ink tattoo had the most significant deviation in normal light and relatively small change in black light. Pink highlighter ink, or other variations of red, should not be used due to the color of the laser alignment lights used in the treatment room.

The binary value of visibility was not beneficial in a study of this size. More measurements obtained over a longer time would better represent the visibility of the tattoo. However, because a tattoo must be visible to have intensity, the intensity measurements summarized both intensity and visibility. A longer study with human subjects is necessary to better evaluate the longevity and durability of the tattooing method. In addition, a more permanent ink would enhance the durability and longevity of the tattoo.

Invisible fluorescent ink would be a suitable ink. The ink is nontoxic and is safe for skin. Another safe ink is black light UV-reactive tattoo ink, which is used solely for tattooing purposes and is approved by the U.S. Food and Drug Administration. Cost of the UV-reactive tattoo ink is roughly twice ($20 per bottle) that of India ink ($10-12 per bottle).

Implementing this tattooing method on a clinical level would require minor, inexpensive modifications to the radiation therapy equipment. Figure 6 illustrates a proposed treatment room modification that includes retrofitting black lights on the treatment table and gantry head. As an option, a hand-held battery-powered black light can be supplied for each treatment room ($10 per unit).

A track of embedded black light light-emitting diodes (LEDs) running along the left and right sides of the treatment couch would provide close range intensity without inhibiting the patient or therapist. Embedding the lights would prevent breakage and reduce the need for repair. The gantry head may be retrofitted with a black light for anteroposterior tattoo visualization of supine patients.

It should be noted that an experiment using more sophisticated and precise techniques of measuring tattoo intensity would improve the accuracy of this study’s results. The current study did not test the tattooing method on patients and was only conducted over 6 days.

<table>
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<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P value</th>
<th>F critical</th>
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<tr>
<td>Ink Type</td>
<td>2.84</td>
<td>3</td>
<td>0.947</td>
<td>1.871</td>
<td>0.1421</td>
<td>2.732</td>
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<tr>
<td>Days</td>
<td>4.36</td>
<td>5</td>
<td>0.871</td>
<td>1.722</td>
<td>0.1405</td>
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<td>Interaction</td>
<td>3.86</td>
<td>15</td>
<td>0.257</td>
<td>0.509</td>
<td>0.9281</td>
<td>1.808</td>
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<td>Within</td>
<td>36.44</td>
<td>72</td>
<td>0.506</td>
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<tr>
<td>Total</td>
<td>47.50</td>
<td>95</td>
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Summary
Existing tattooing techniques neglect to address the long-term psychological effects on the patient, the challenges associated with areas of hair follicles and difficulty in identifying tattoos on darker-skinned patients. This study suggests that fluorescent highlighter ink is a viable way of overcoming these limitations. Although the methods used in this experiment are simple, the results should provoke further investigation on the topic using human subjects in the clinical environment and equipment to directly measure visibility. This study lays the baseline for future work in the area.

References

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