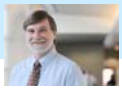
# PLM Validation of WSL Assessment by Photothermal Radiometry-Modulated Luminescence Technology

C.M. Carey and S.S. Coleman
University of Colorado, Anschutz Medical Campus, School of Dental Medicine, Aurora, CO, USA



## Abstract

Recently, a caries detection system based on photothermal radiometry - modulated luminescence (PTR-LUM) technology has become available from Quantum Dental Technologies, Inc. called the Canary Laboratory. This non-destructive caries detector has the capability to monitor small changes in the mineral density within the tooth over time. For this system to be useful it has to generate Canary numbers (Canary#) that correlate with lesion depth.

**Objective**: Correlate the Canary# to white spot lesion (WSL) depth as determined by polarized light microscopy (PLM).

**Methods**: The margins of 4x4mm areas of caries-free human molars were protected with nail polish to define a demineralization zone. The teeth were placed in demineralizing gel consisting (0.1 M lactic acid adjusted to pH 4.5 with NaOH and thickened with carboxymethyl cellulose sodium salt). The teeth were demineralized for 110d, removed from the gel and rinsed with DI-water. Nail varnish was removed with acetone and the sample, including the protected zone, was scanned with the Canary lab. The teeth were cut in half through the protected and exposed area and one half sectioned into slices which were further thinned to 110µm thickness for PLM imaging. Digitized images of the sections were analyzed for lesion depth using Image-J software. The cut surface of the larger section was scanned with the Canary lab to produce a cross-section profile.

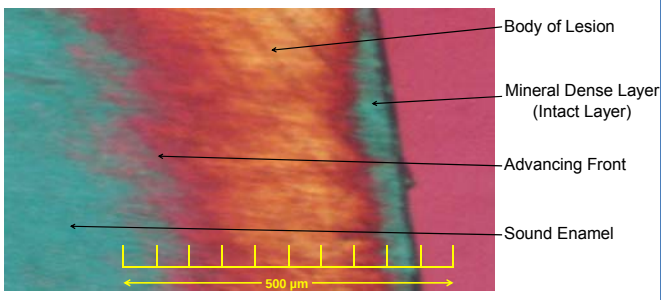
**Results**: (n≥25 pixels), Canary#s are significantly larger as a function of lesion depth 150>75>0µm ($p<0.05$, ANOVA, Duncans) for both the surface scans and the cross-sectional scans.

| Lesion Depth (µm) | Canary # (Cross Section) Mean ± Std Dev | Canary # (Surface) Mean ± Std Dev |
|---|---|---|
| 150 | 66.0 ± 8.1 | 42.3 ± 6.1 |
| 75 | 45.8 ± 3.7 | 29.3 ± 4.5 |
| 0 | 20.9 ± 3.3 | 23.8 ± 1.8 |

**Conclusion**: The Canary# can be correlated with the depth of the WSL as long as the lesion does not have a large intact zone. The intact zone at the surface causes the Canary# to be suppressed. Supported by NIH-NIDCR R01DE021391.

## Introduction - PLM

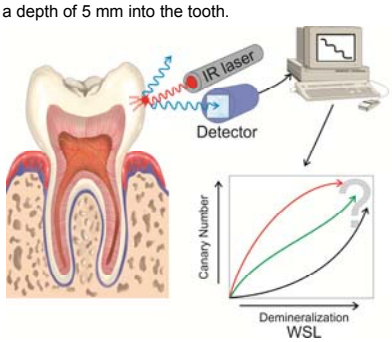
The earliest stage of enamel demineralization typically looks like a white spot on the tooth which are often referred to as white spot lesions (WSL) and are the earliest signs of demineralization. WSL are variable in the amount of enamel demineralization and depending on a number of factors may be reversible. In the 1970s, Dr. Leon Silverstone and others developed a histological method to evaluate the extent of WSL by use of polarizing light microscopy (PLM). The PLM requires that the tooth be sliced and the sections polished to a thickness of 80 to 120 µm. The samples are placed under a microscope with polarized light transmitted through the samples where the enamel crystals rotate the light (and demineralized crystals rotate the light to a lesser extent). The transmitted light then passes through another polarized filter set at 90 ° from the first filter resulting in a black background with various shades of grey of the tooth. This light is then passed through a red filter which allows for the colors of the light rotated by the enamel crystals to be generated, and a pink-ish background where there is no sample. Sound enamel looks blue, demineralized enamel has yellow-to-red colors, and extreme demineralization is dark grey or black. In the example below, the tooth with a WSL has been prepared according to the method.




## Introduction PTR-LUM

Photothermal Radiometry (PTR) and Modulated Luminescence (LUM) signatures are combined to detect demineralization within the tooth. The PTR signature is the result of a pulsed infrared laser beam focused on the tooth surface that generates a thermal wave absorbed by the tooth which in turn generates a Planck radiation emission out of the tooth. The LUM signature is the result of the absorbed infrared light causing light emission from the tooth at different wavelengths based on the crystal orientation and demineralization. These two signatures provide complimentary results that when combined can be used to detect very small changes in the mineral and specifically demineralization. The Canary System (Quantum Dental Technologies, Inc.) uses the PRT-LUM methods that have been refined to pulse the infrared light such that the resulting signatures have probed to a depth of 5 mm into the tooth.

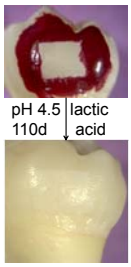
The resulting signatures are combined in a mathematical formula developed from repeated scans of teeth to generate a single number called the Canary Number which is diagnostic of mineral quality where the larger the number the more likely there is demineralization. What is not known is how the Canary Number relates to white spot lesion depth, or what features may alter the resulting Canary Number.



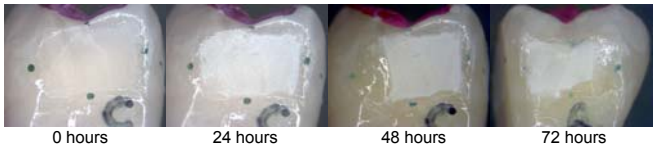

## Purpose

Correlate the PTR-LUM Canary Number generated by the Canary Lab System to white spot lesion (WSL) depth as determined by polarized light microscopy (PLM).

## Methods



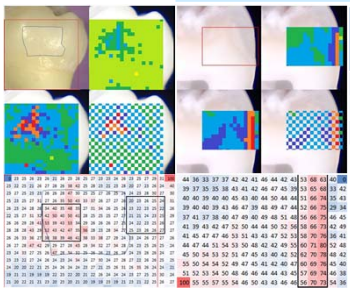

Experiment 1: The margins of 4x4mm areas of caries-free human molars were protected with nail polish to define a demineralization zone. The teeth were placed in demineralizing gel consisting (0.1 M lactic acid adjusted to pH 4.5 with NaOH and thickened with carboxymethyl cellulose sodium salt). The teeth were demineralized for 110d, removed from the gel and rinsed with DI-water. Nail varnish was removed with acetone and the sample, including the protected zone, was scanned with the Canary Lab. The teeth were cut in half through the protected and exposed area and one half sectioned into slices which were further thinned to 110µm for PLM imaging. Digitized images of the sections were analyzed for lesion depth using Image-J software. The cut surface of the larger section was scanned with the Canary Lab to produce a cross-section profile.





Experiment 2: Molars were masked and demineralized in 24 h increments to 120 h. Canary Lab scans were made at each increment and a portion was protected. Stepwise WSL depth was obtained.

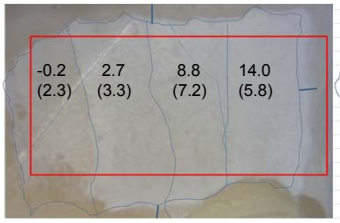
## Results – Exp #1



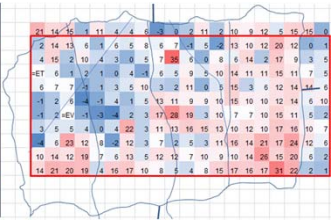
| Lesion Depth (µm) | Canary # (Surface) Mean ± SD | Canary # (Cross Section) Mean ± SD |
|---|---|---|
| 0 | 23.8 ± 1.8 | 20.9 ± 3.3 |
| 75 | 29.3 ± 4.5 | 45.8 ± 3.7 |
| 150 | 42.3 ± 6.1 | 66.0 ± 8.1 |

Canary numbers are significantly larger as depth 150>75>0µm ($p<0.05$, ANOVA, Duncans) increases for both the surface scans and the cross-sectional scans. Intact zone thickness tends to lower the observed Canary number.

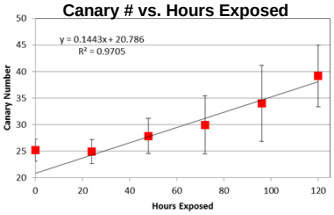
## Results – Exp #2




Demineralized sample in 4 steps (48, 72, 96, and 120 hours). Average difference of Canary # from scan at 0 hours shown in each area (std. dev.).



Canary Lab scan of demineralized sample in 4 steps (48, 72, 96, and 120 hours).




## Discussion

Exp #1 - The Canary# can be correlated with the depth of the WSL as long as the lesion does not have a large intact zone.

Exp #2 - The plot of Canary # vs. hours exposed is linear from the 24 h to 120h. It is possible that surface demineralization does not generate a higher Canary # because the depth of the scan is 5 mm.

## Conclusions

The Canary# can be correlated with the depth of the WSL as long as the lesion does not have a large intact zone. The intact zone at the surface causes the Canary# to be suppressed. More experiments need to be completed to understand this relationship.

## Funding

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