



Titel: Effect of Isolated Protein from Whey and Green Pea on Erosive Tooth Wear Assessed by 3D Laser Scanning Microscopy

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Aim

This *in vitro* study evaluated the protective effects of protein supplements against erosive tooth wear (ETW) using three-dimensional (3D) laser scanning microscopy.

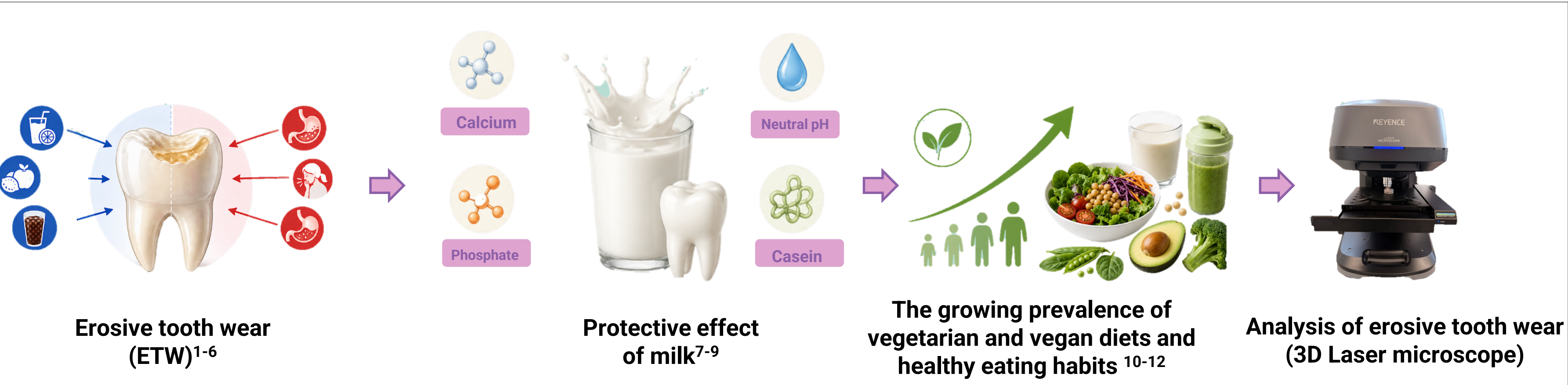


Fig. 1 Study overview.

Material and Methods

Sixty polished bovine enamel specimens were randomly allocated to five experimental groups (n = 12). Over seven days, specimens were subjected to six daily erosive cycles using 0.05 M citric acid (pH 2.3, 2 min), interspersed with a remineralizing solution (pH 6.5). Post-acid preventive measures consisted of immersion in solution Skimmed Milk, (positive control), tin-containing fluoride solution (500 ppm Fluorid und 800 ppm Sn²⁺ (als Zinn(II)-chlorid), pH 4,5) , or Mineral Water (negative control) for 3 min after the first and last erosive challenges. Enamel surface loss was measured after 7 days using three-dimensional laser scanning microscopy, the profilometric analysis was performed with 25 horizontal lines spaced 15 µm apart to calculate the mean enamel loss.

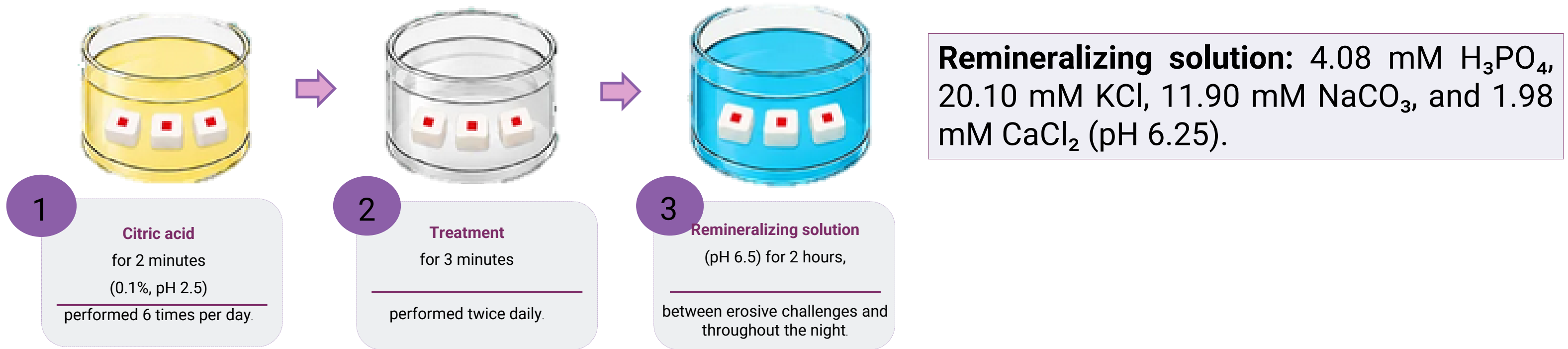


Fig. 2 Preparation of the samples.

Table 1. Composition of the products used in the experiment and nutrition information of experimental products.

Product / Commercial Brand	Composition	Nutrition Information
Whey Protein Isolate (Growth supplements®)	Isolated whey protein, cocoa powder, maltodextrin, chocolate flavoring, emulsifier (lecithin), sweeteners (acesulfame potassium, sucralose), anti-caking agent (silicon dioxide).	Per 30 g: energy value 116 kcal (6% DV); carbohydrates 2 g (1% DV), of which total sugars 0 g and added sugars 0 g (0% DV); protein 27 g (54% DV); It also contains L-alanine (1389 mg), arginine (516 mg), aspartate (3201 mg), L-cysteine (651 mg), L-glutamic acid (5049 mg), glycine (477 mg), L-histidine (384 mg), L-isoleucine (1854 mg), L-leucine (2838 mg), lysine (2682 mg), L-methionine (768 mg), L-phenylalanine (816 mg), L-proline (1755 mg).
100% Pea Protein (Growth supplements®)	Isolated pea protein (<i>Pisum sativum</i> L.), cocoa powder, maltodextrin, flavoring, sweeteners (sucralose, acesulfame potassium), anti-caking agent (silicon dioxide).	Per 30 g: energy value 121 kcal (6% DV); carbohydrates 2 g (1% DV), of which total sugars 0 g and added sugars 0 g (0% DV); protein 22 g (44% DV); total fat 2.8 g (4% DV), of which saturated fat 0 g (0% DV) and trans-fat 0 g (0% DV); dietary fiber 1.6 g (6% DV); sodium 263 mg (13% DV).
Skimmed Milk (Piracanjuba®)	Skimmed milk, stabilizers (sodium triphosphate, sodium citrate, sodium monophosphate, diphosphate).	Per 200 mL: energy value 68 kcal (3% DV); carbohydrates 9.2 g (3% DV), of which total sugars 9.2 g and added sugars 0 g (0% DV); protein 6.2 g (12% DV); total fat 0.7 g (1% DV), of which saturated fat 0.4 g (2% DV) and trans-fat 0 g (0% DV); dietary fiber 0 g (0% DV); sodium 124 mg (6% DV); calcium 243 mg (24% DV).
Natural Mineral Water (Crystal®)	Mineral Water	
Erosion Protection Solution (Elmex®)	Aqua, Glycerin, Sodium Gluconate, Polyglyceryl-4 Caprate, Oleaminopropylamineth-3 HCl, Aroma, Poloxamer 407, Sodium Saccharin, Stannous Chloride, Sodium Fluoride, Anethole, Eucalyptus Globulus Oil, and Menthol. Sodium Fluoride (500 ppm).	

Erosive cycle: 7-day duration, 6 times per day



3D laser scanning microscopy



VK-X3000, Keyence®, Osaka, Japan

Statistical analysis

The data were analyzed using GraphPad Prism software. Normality and homogeneity of variances were assessed.
• **3D microscopy:** ANOVA followed by Tukey's multiple comparisons test ($p < 0.05$).

Results

Significant differences were observed among groups (ANOVA/Tukey, $p < 0.0001$). Tin-containing fluoride solution showed the lowest enamel loss (Mean $\pm$ SD: $10.76 \pm 1.64 \mu\text{m}$), whereas Mineral Water had the highest ($43.41 \pm 2.15 \mu\text{m}$). Whey Protein Isolate ($37.53 \pm 2.89 \mu\text{m}$) and Skimmed Milk ($35.28 \pm 1.88 \mu\text{m}$) significantly reduced enamel loss compared to Mineral Water, while Pea Protein ($40.03 \pm 3.08 \mu\text{m}$) provided limited protection.

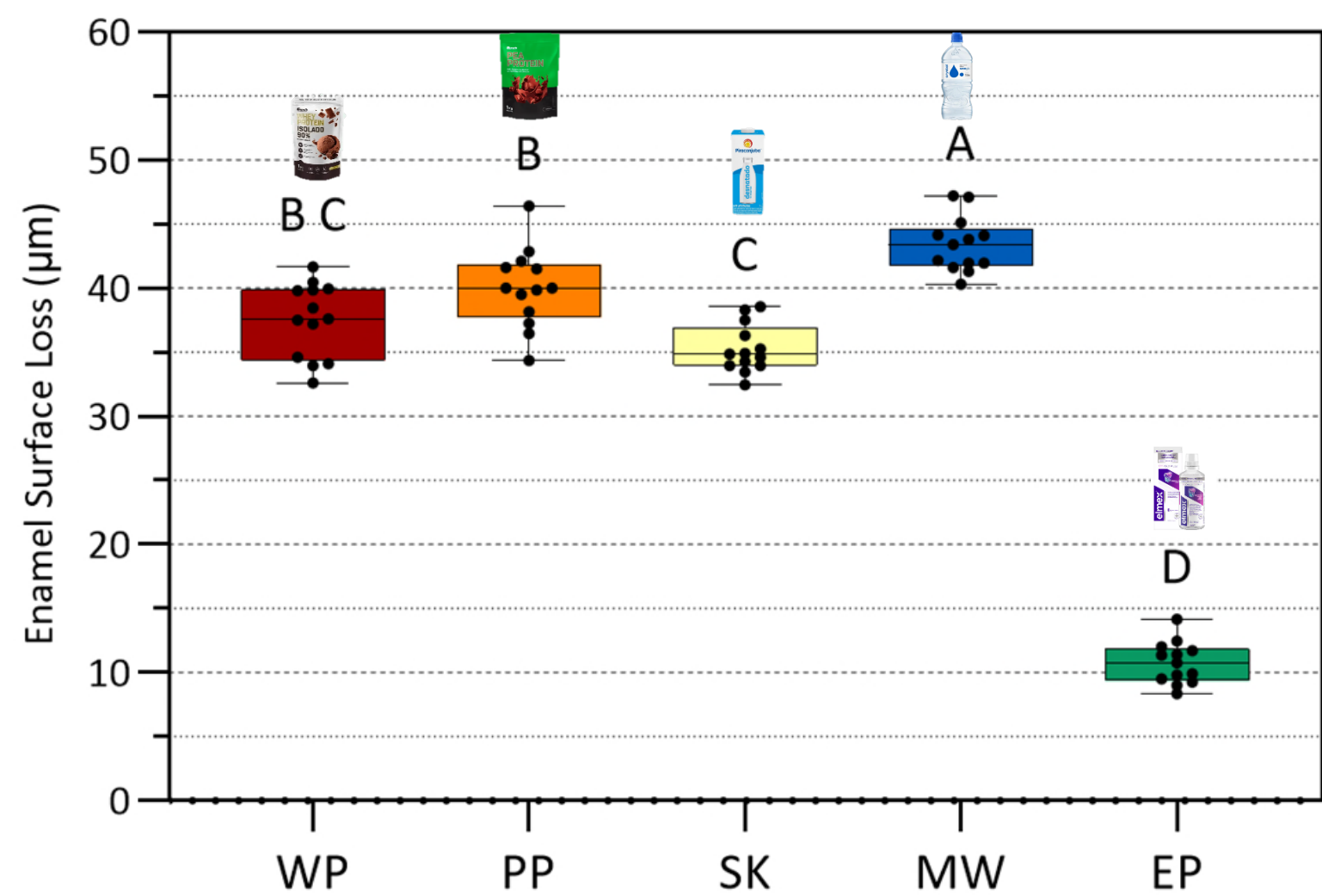


Fig. 3 Erosive enamel surface loss measured by 3D laser scanning microscopy (Mean $\pm$ SD, μm). Different letters indicate statistically significant differences between groups (ANOVA followed by Tukey's test, $p < 0.0001$).

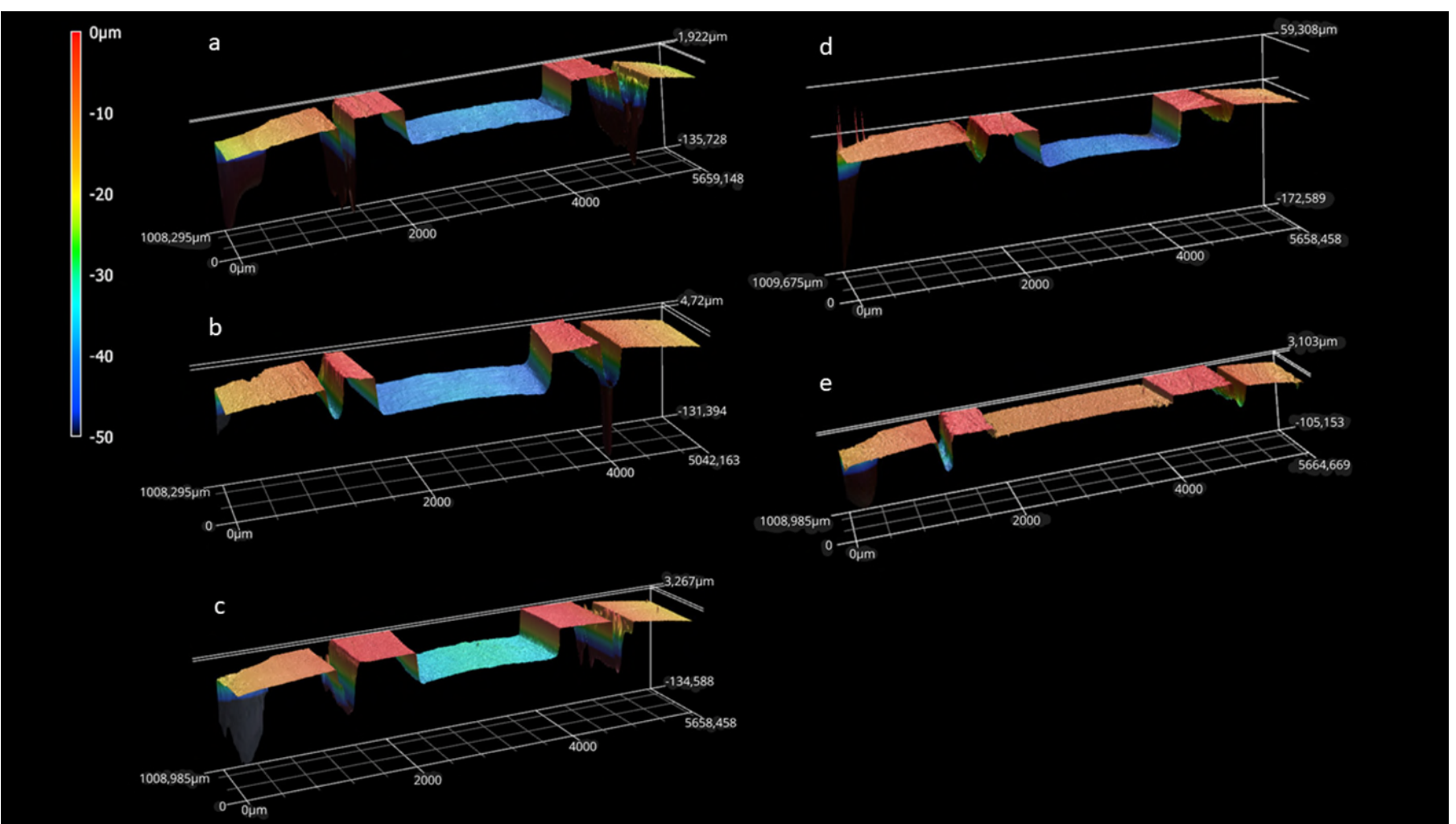


Fig. 4 Representative images per group of the final aspect of the analysis by 3D laser scanning microscopy. a. Whey Protein Isolate; b. Pea Protein; c. Skimmed Milk; d. Mineral Water; e. Elmex® Erosion Protection.

Conclusion

Protein-based products provided limited to moderate protection against erosive tooth wear, with skimmed milk and whey protein performing better than pea protein but remaining markedly less effective than the fluoride-based solution.

References/ Funding



Coauthor: L. G. T. F.
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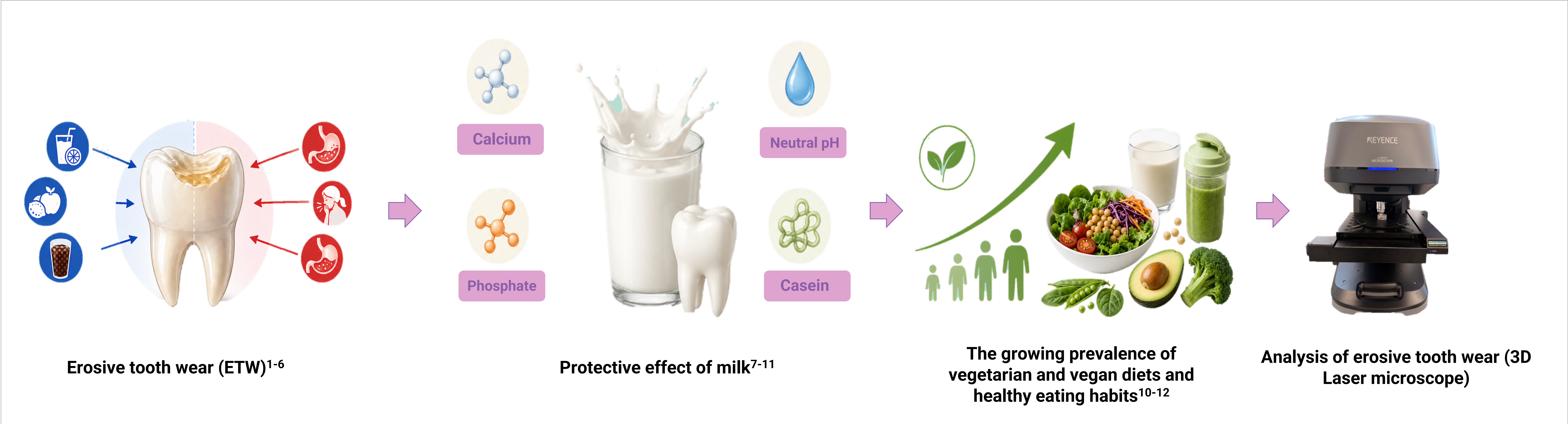


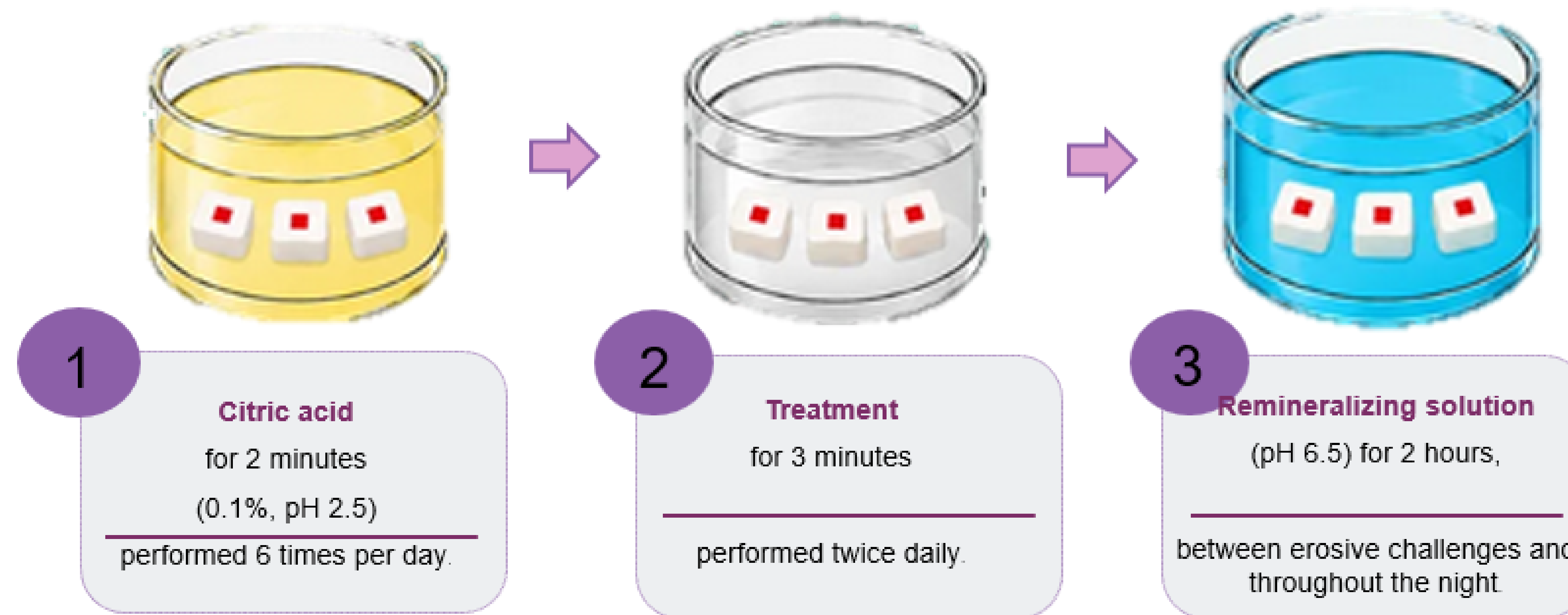
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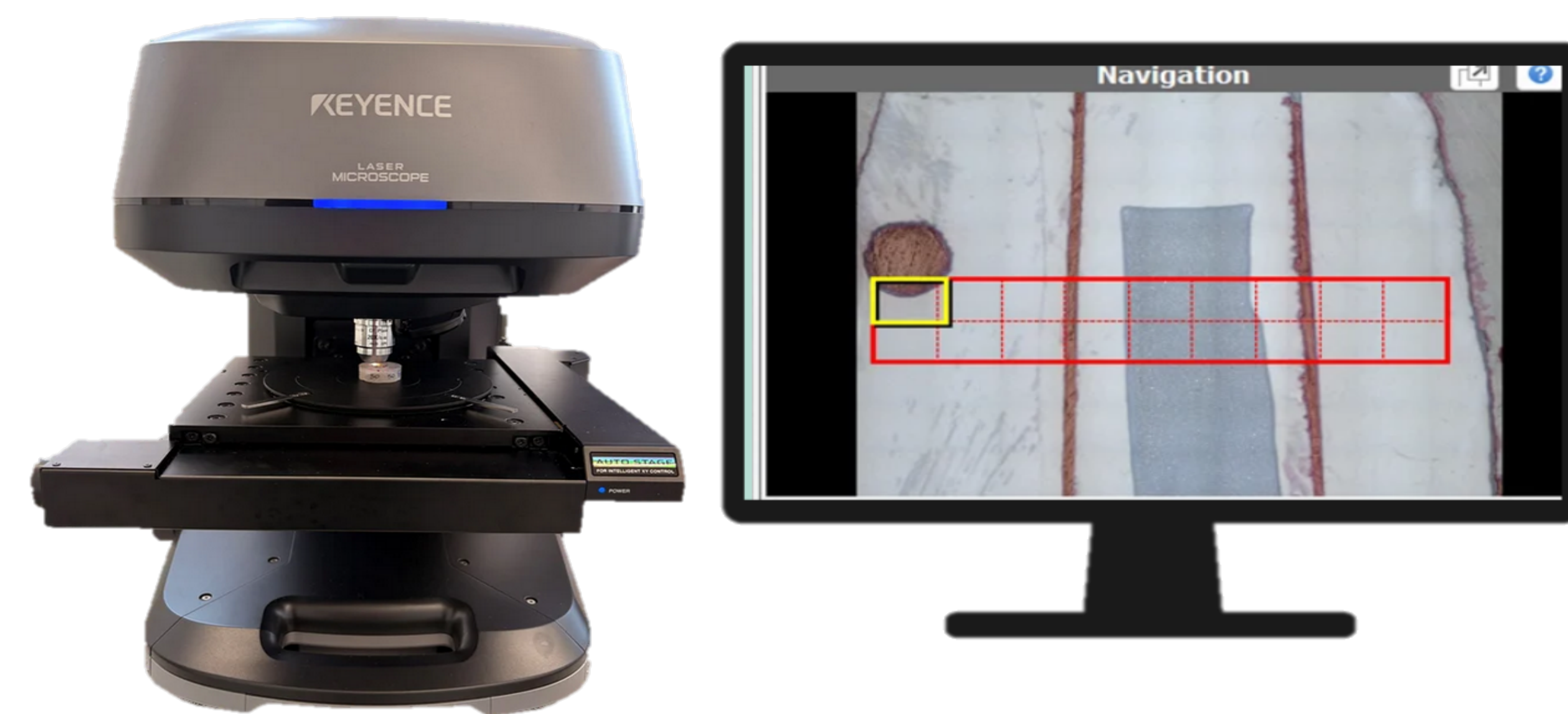
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Erosive cycle: 7-day duration, 6 times per day



Remineralizing solution: 4.08 mM H_3PO_4 , 20.10 mM KCl, 11.90 mM NaCO_3 , and 1.98 mM CaCl_2 (pH 6.25).

3D laser scanning microscopy



VK-X3000, Keyence®, Osaka, Japan

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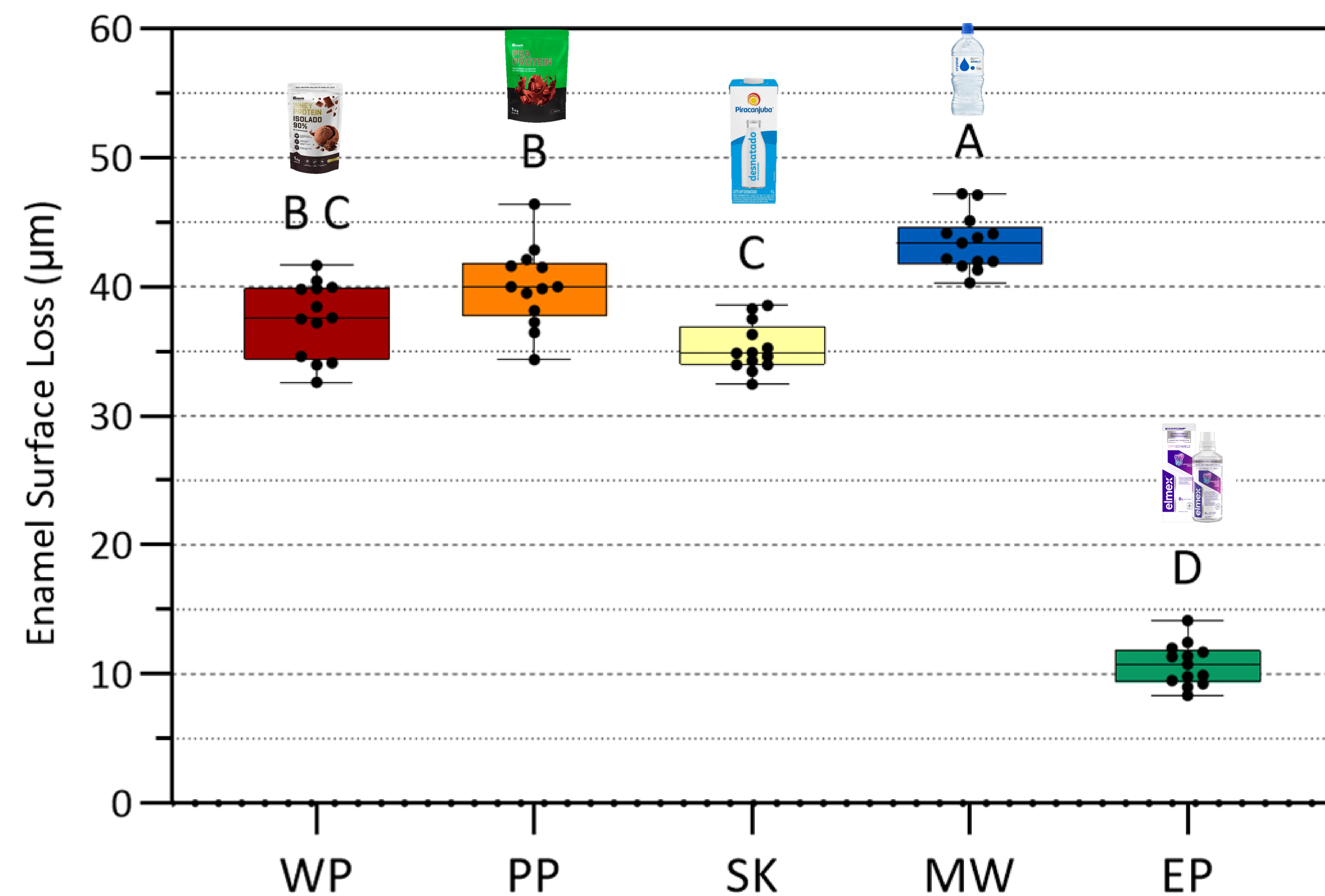


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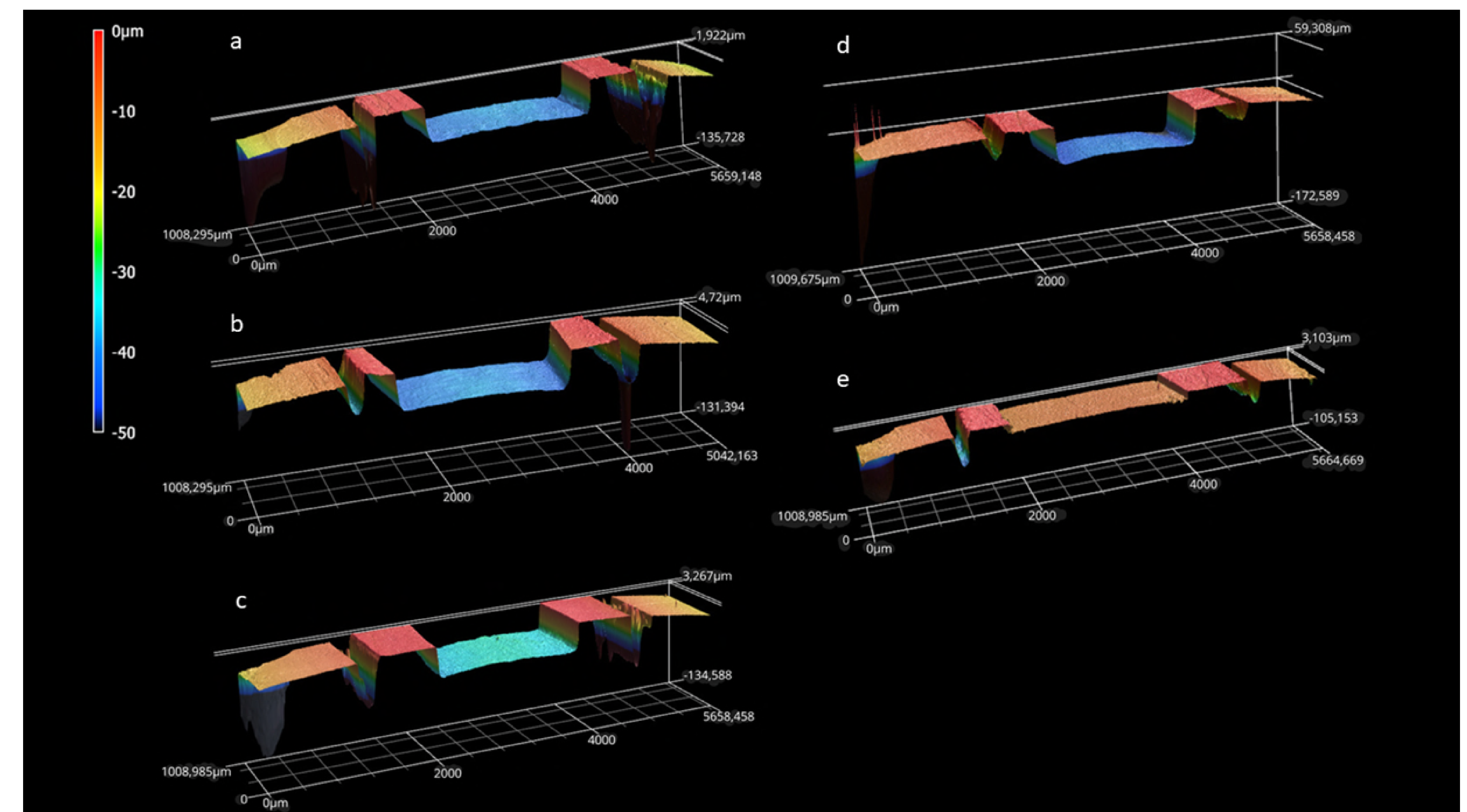


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