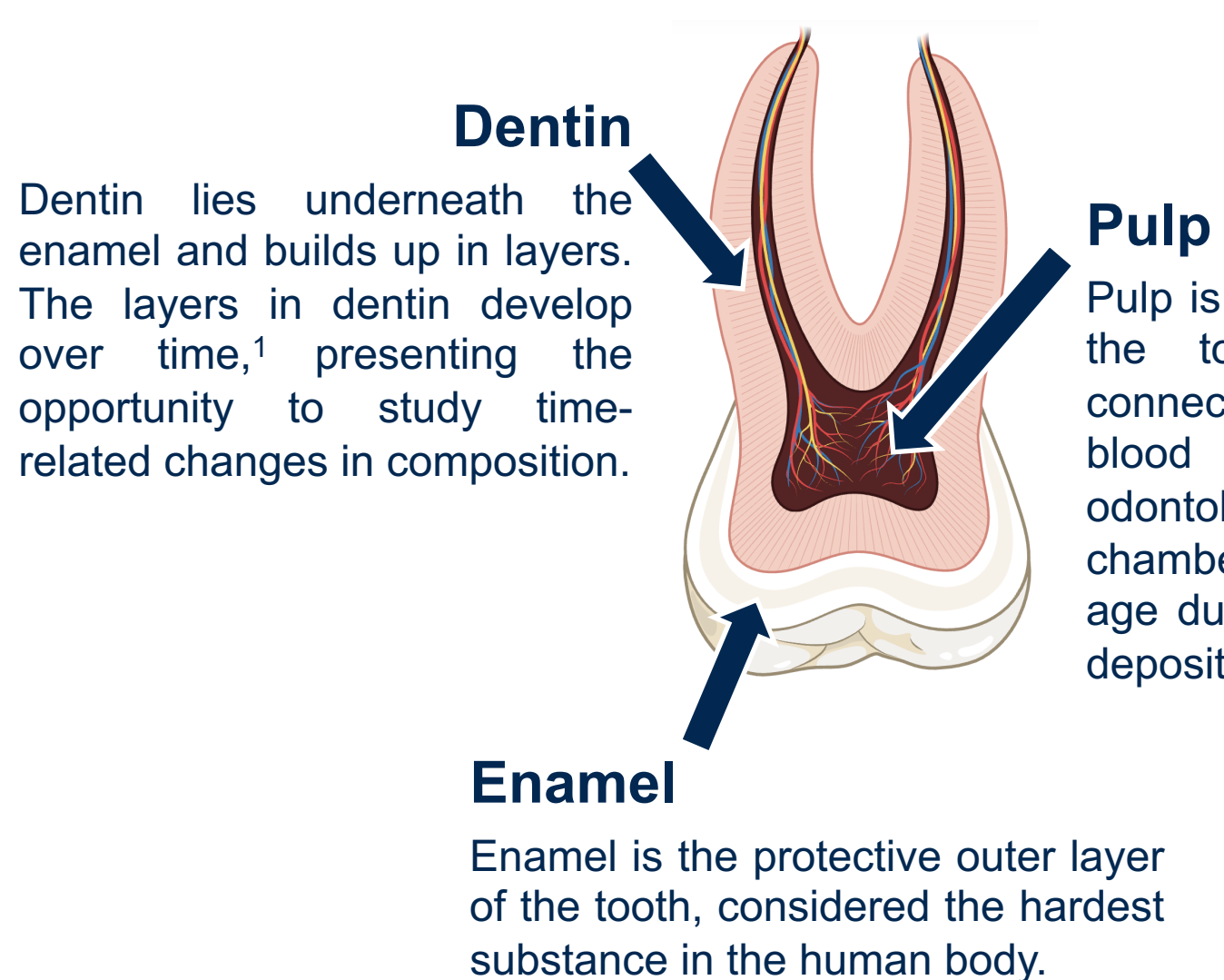


INTRODUCTION



- Environmental exposures and their associated metabolites can be found in teeth, especially in layers of dentin.^{2, 3}
- MALDI MSI requires sample preparation that maintains spatial integrity of the sample, making methods for sample preparation an important area of research in the field.⁴⁻⁸
- Previously, sample preparation that maintains spatial integrity has been a challenge due to the hard, brittle nature of human teeth.
- Commonly, hard tissues such as bone or teeth are decalcified before sectioning to soften the sample, but decalcification interferes with the endogenous metabolites.⁹ As such, we have aimed to use MALDI MSI to image non-decalcified deciduous and permanent human teeth.

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CONFLICTS OF INTEREST

Joanna Ellis and Manish Arora are employees of Linus Biotechnology, Inc. Linus Biotechnology, Inc. also funded portions of this work.

RESULTS

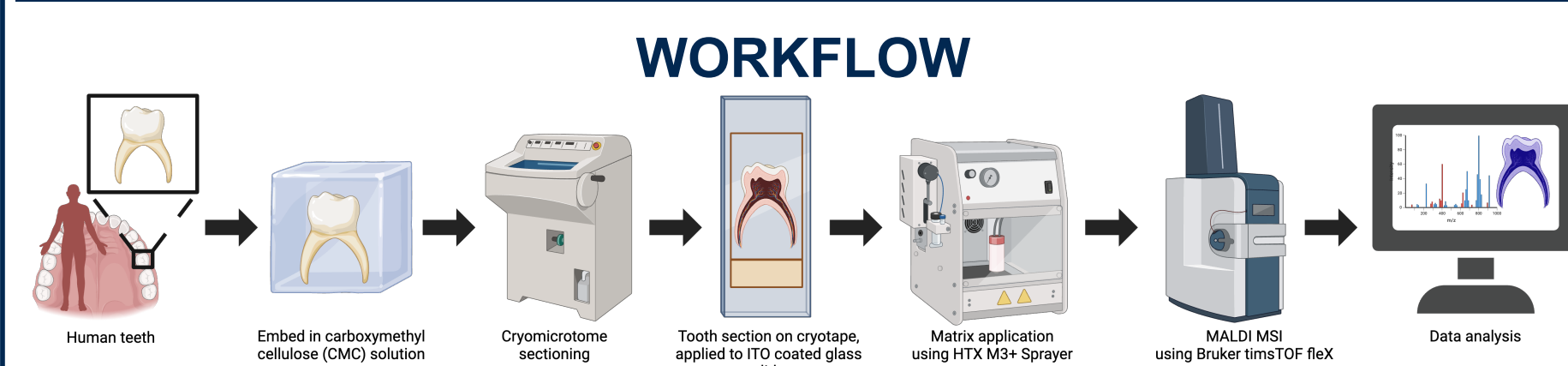


FIGURE 1. Workflow for MALDI MSI analysis of non-decalcified human teeth.

CRYOSECTIONING: Embedding Material

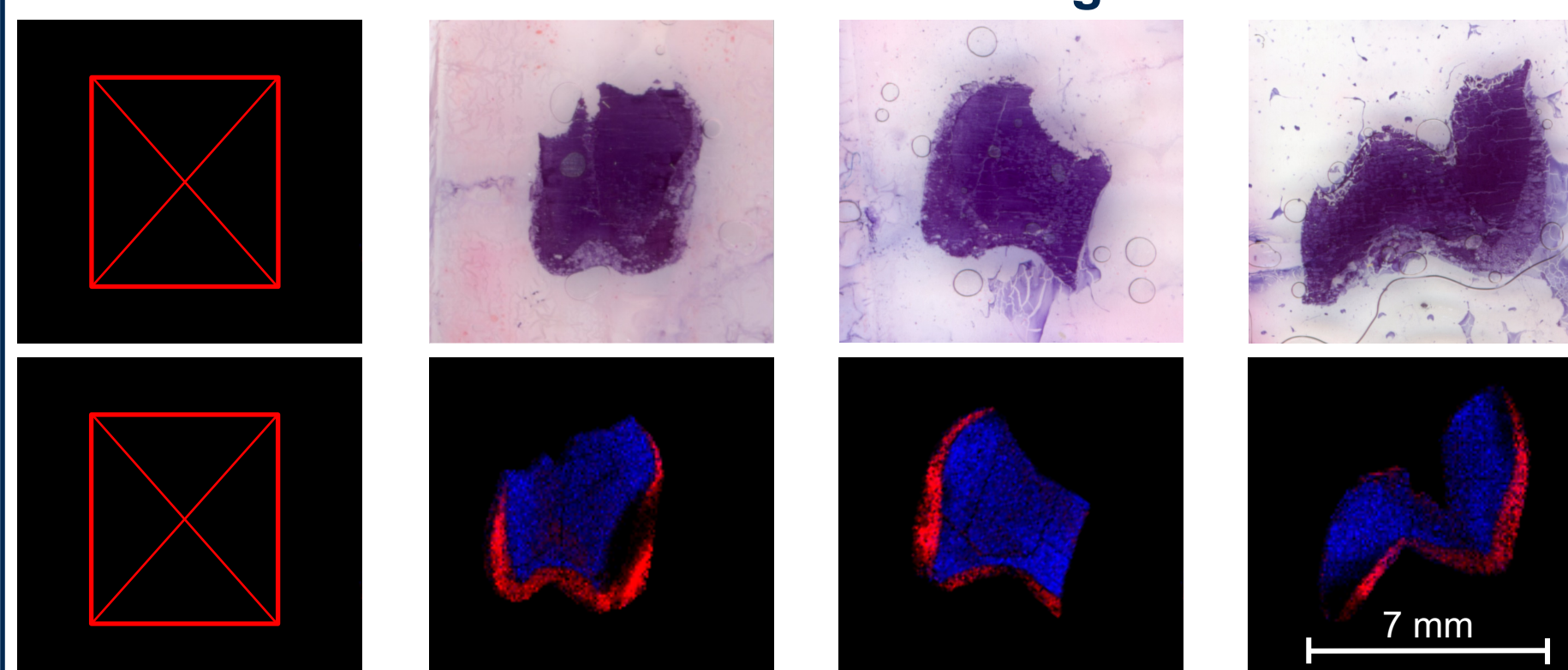


FIGURE 2. Carboxymethyl cellulose (CMC) is an embedding material used to provide structural support to the tooth during sectioning. In the absence of embedding material, sectioning with a tungsten-carbide blade often resulting in tooth breakage, or the blade bends around the smooth surface of the tooth. Ion images represent relative abundance of m/z 889.0405 (enamel, red) and m/z 532.2541 (dentin, blue).

CRYOSECTIONING: Blade Angles

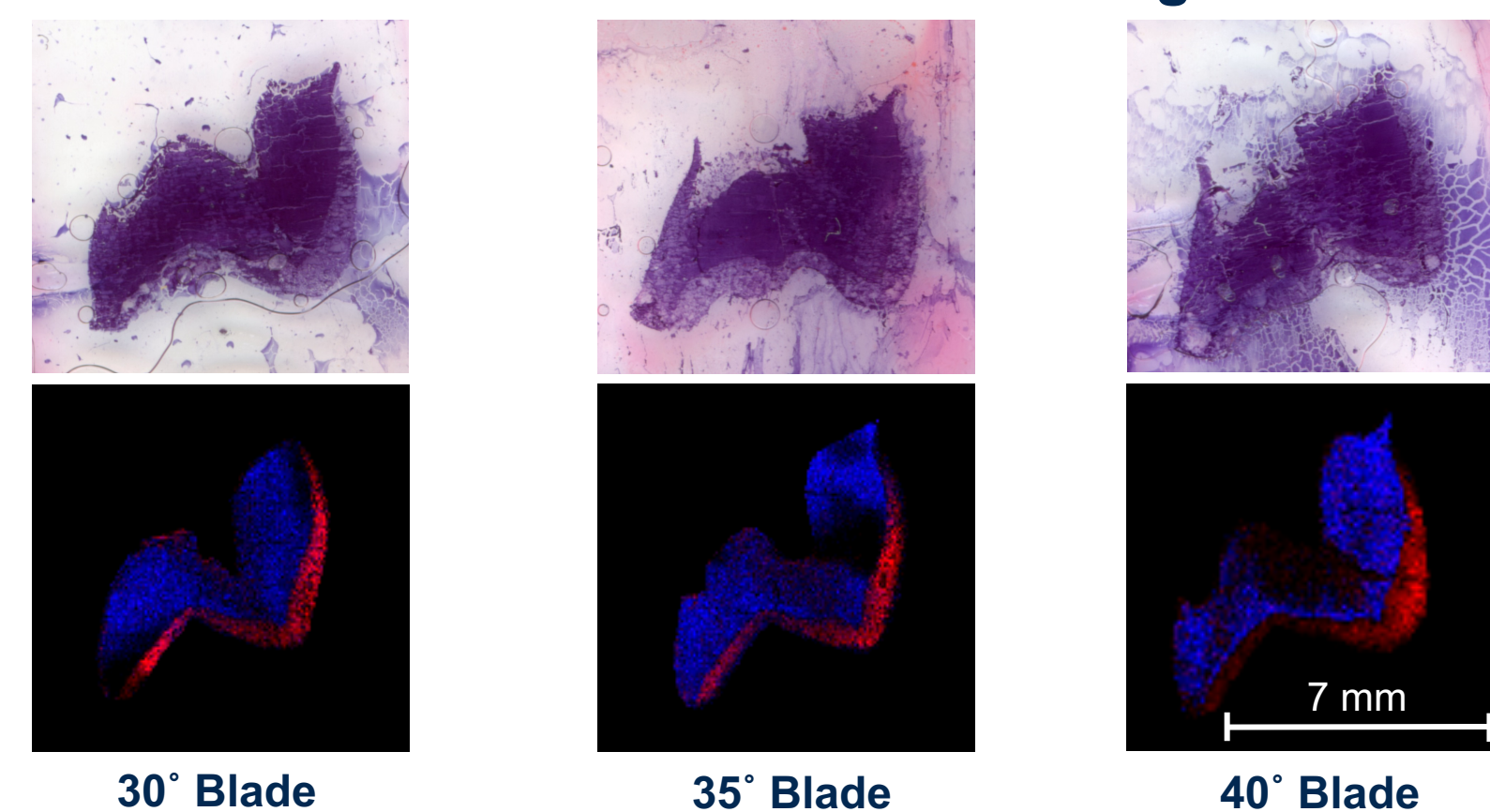


FIGURE 3. Cryosectioning non-decalcified human teeth with 30° or 35° tungsten-carbide blades results in less tooth fracturing than with 40° blades. Ion images represent relative abundance of m/z 889.0405 (enamel, red) and m/z 532.2541 (dentin, blue).

MATRICES FOR SMALL MOLECULE DETECTION

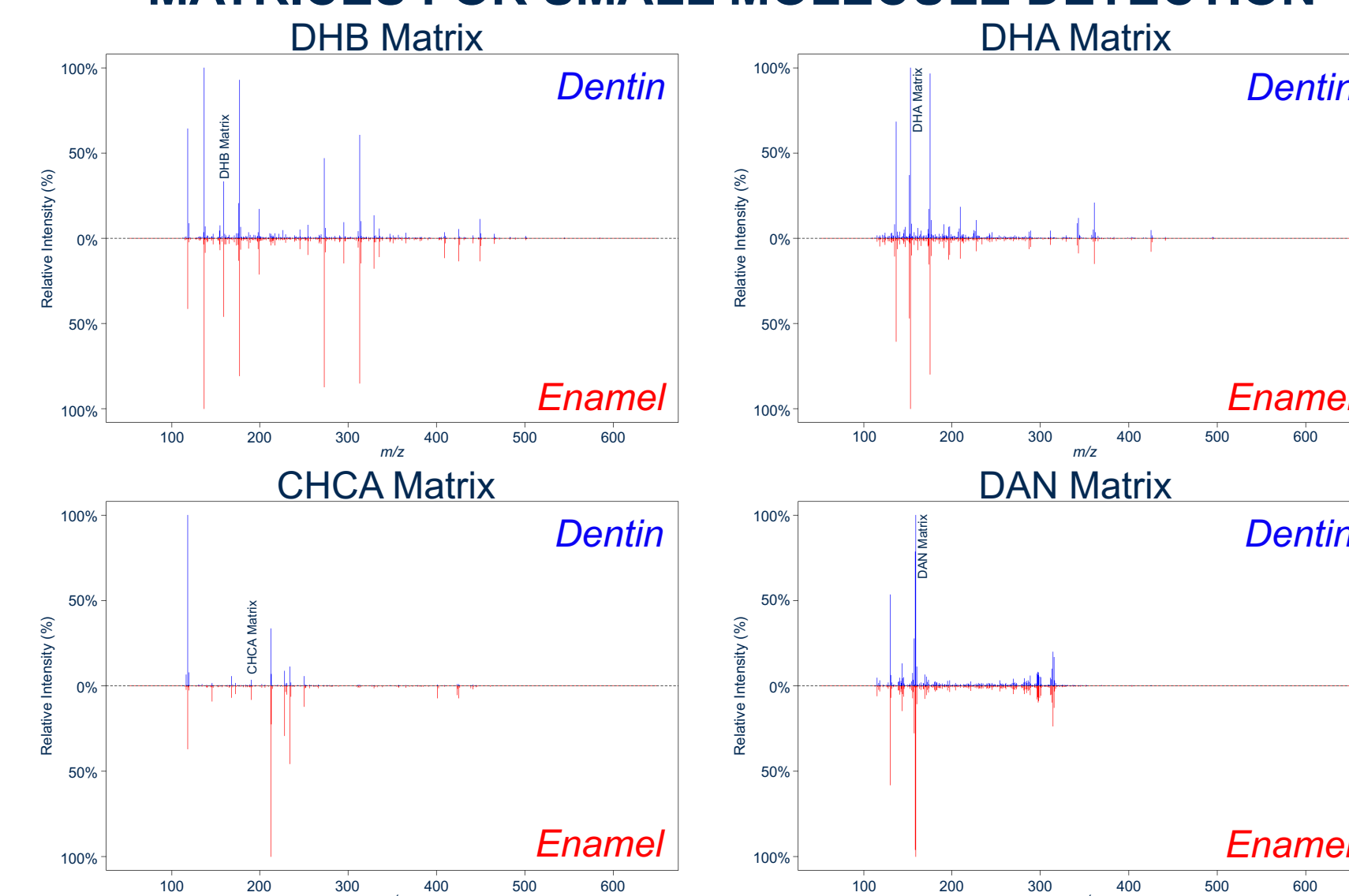


FIGURE 4. Small molecules found in teeth using α -Cyano-4-hydroxycinnamic acid (CHCA), 1,5-Diaminonaphthalene (DAN), 2,5-dihydroxyacetophenone (DHA), or 2,5-dihydroxybenzoic acid (DHB) matrix in positive mode. Blue spectra represent dentin regions, and red spectra represent enamel regions.

DECIDUOUS vs. PERMANENT HUMAN TEETH

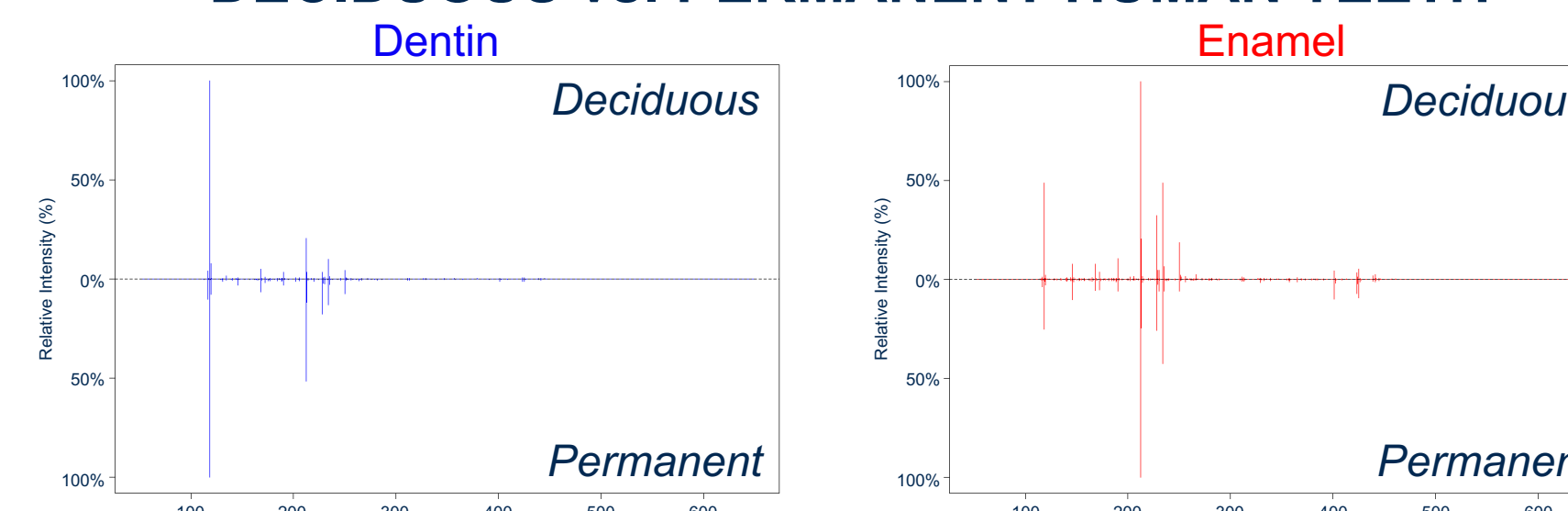


FIGURE 5. Deciduous ("baby") teeth and permanent ("adult") teeth have similar mass spectral peaks in both dentin and enamel comparisons.

PHOSPHOLIPIDS IN HUMAN TEETH

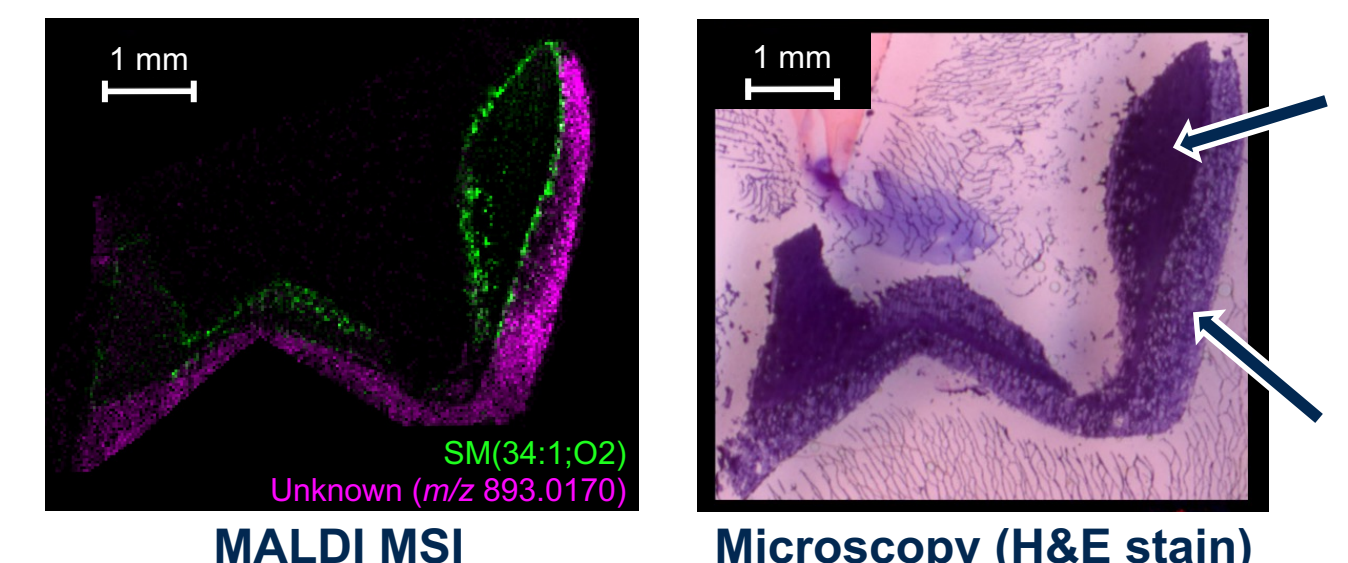
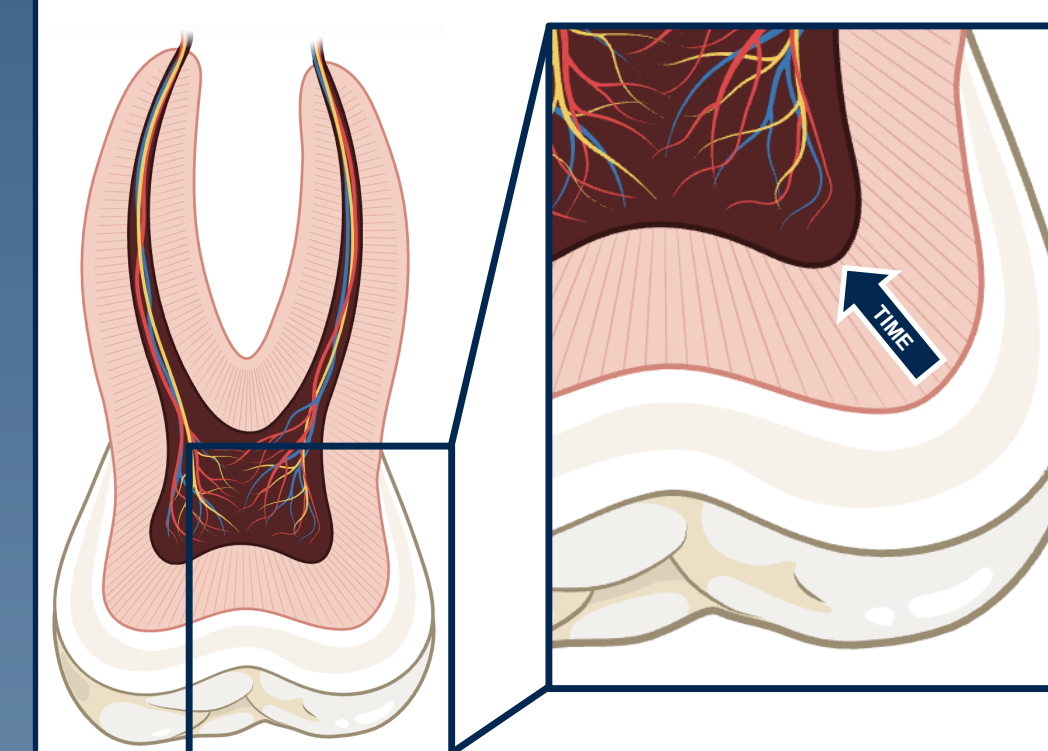


FIGURE 6. Mass spectrum peaks consistent with lipids have been observed at the dentin-enamel junction and at the interface of dentin and pulp.¹⁰

CONCLUSIONS & FUTURE DIRECTIONS

- Using MALDI mass spectrometry imaging (MSI) on non-decalcified human teeth allows us to see the differences in chemical composition across enamel or dentin layers without the assistance of labels, staining, etc.



This method could be applied to study environmental exposures and human health, by analyzing the present exposure-related molecules or metabolites in dentin layers, which continue to develop throughout life.¹

- The ability to section non-decalcified human teeth could allow for spatial analysis of native or exposure-related molecules without the interaction or degradation associated with decalcification.
- This is especially relevant to childhood diseases or exposures, as childhood teeth are naturally shed by the body to make room for permanent teeth.
- This is not only applicable to modern human health, but could be extended archeological specimens as well.

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