Bacteria remaining in the root canal (RC) space after treatment can lead to a persistent or secondary infection, leading to treatment failure and the need for re-intervention in around 24% of cases [1-2]. Currently, no standard method exists for detection of bacterial presence within the RC space. Using in situ (scouting the RC with a fibre-probe) and ex situ (RC sampling with paper points) approaches, we aim to develop bench side diagnostics to optically detect and quantify the amount of remnant bacteria (Fig. 1a-b).

Primarily and as a proof of concept for detection, fluorescent beads were used to simulate live (green) and dead (red) bacteria (Fig. 2). For the ex situ principle, paper points were immersed in fluorescent beads, which were resolved and distinguished from each other and the paper point using two-photon microscopy (Fig. 2a-b). For the in situ approach, fluorescent beads where added directly to RCs of extracted teeth and detected with an endoscope (Fig. 2c).

Secondly, fluorescent dyes for bacterial staining were evaluated and optimised for incubation time, sensitivity and specificity. Using confocal microscopy, we identified that calcein AM positively stains a mixed species oral biofilm at a clinically relevant incubation time, with minimal background staining. It was further confirmed that both ex situ and in situ approaches were able to perform detection of the in vitro grown bacterial biofilms.

Preliminary studies have focused on developing and optimising the ex situ approach. Real-time detection of stained bacteria was achieved using a spectrometer coupled to a wide field fluorescence microscope. Spectral unmixing was used to distinguish between the distinct calcein AM emission and paper point autofluorescence (Fig. 3a-b). The methodology was validated in vivo by detection of bacteria from samples acquired during RC treatments (Fig. 3c).

This work has identified the potential for our technique to be applied as a powerful tool in dental clinics for the efficient and effective detection of remnant bacterial bio-burden, minimising failure and future need for root canal re-intervention.


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Fig. 1: Diagram showing the a) in situ incubation and detection and b) ex situ incubation and detection.

Fig. 2: Two-photon microscopy image of a paper point tip immersed in green and red fluorescent 1 µm beads. Paper fibers were visualised using SHG (blue). a) Front view and b) side view of a rendered 3D stack. c) Endoscopic recording of the root canal: green fluorescent beads (yellow arrow) are visible on reflected blue background (red arrow).

Fig. 3: Spectral readings of a) comparison of the normalised spectra of calcein emission and paper point autofluorescence. b) Detection of calcein peaks on an in vitro paper point sample. spectra taken every 500 µm starting from the tip. c) Detection of calcein peaks on an in vivo sample taking during a RC treatment.