

Quantitative assessment of Zn in the outer enamel surface in relation to total enamel thickness

Dean et al. *Biology*

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Methods

For each specimen, the Zn map was recorded as a 32-bit tiff file in Geopixe for Proposals I-20160686 and I-20180047 (Maia detector), and in HDIP for Proposals I-20190203 and I-20210133 (Vortex EM silicon drift detectors).

The Zn map was then opened in ImageJ (version 1.53t), the image scale was set according to the scan resolution (e.g., 1 pixel equals 10 μm), and the contrast and brightness adjusted to best visualize the Zn variations.

Then for each of the enamel areas of interest (cuspal, mid-lateral and cervical), a segmented line going in the direction of the prisms was drawn from beyond the enamel-dentine junction (EDJ) in the dentine, to in the air, beyond the outer enamel surface (OES). To make sure the ROIs respect the direction of the prisms, the line started deeper in the dentine than the EDJ, and ended in the air, beyond the OES.

These 3 regions of interest (ROIs) were saved using the ROI manager, and an image (see below) was saved as a record. The raw data along the 3 ROIs were saved as a csv file, and a multiplot was drawn and saved as a pdf for easy visualization of the data and detection of mistakes.

For relative enamel thickness, the measurements were restricted to start from the EDJ and end on the OES, and Zn contents were plotted against a percentage of distance from the EDJ (0%) to the OES (100%).

Summary graphs were then build in Excel by compiling the raw data of all specimens.

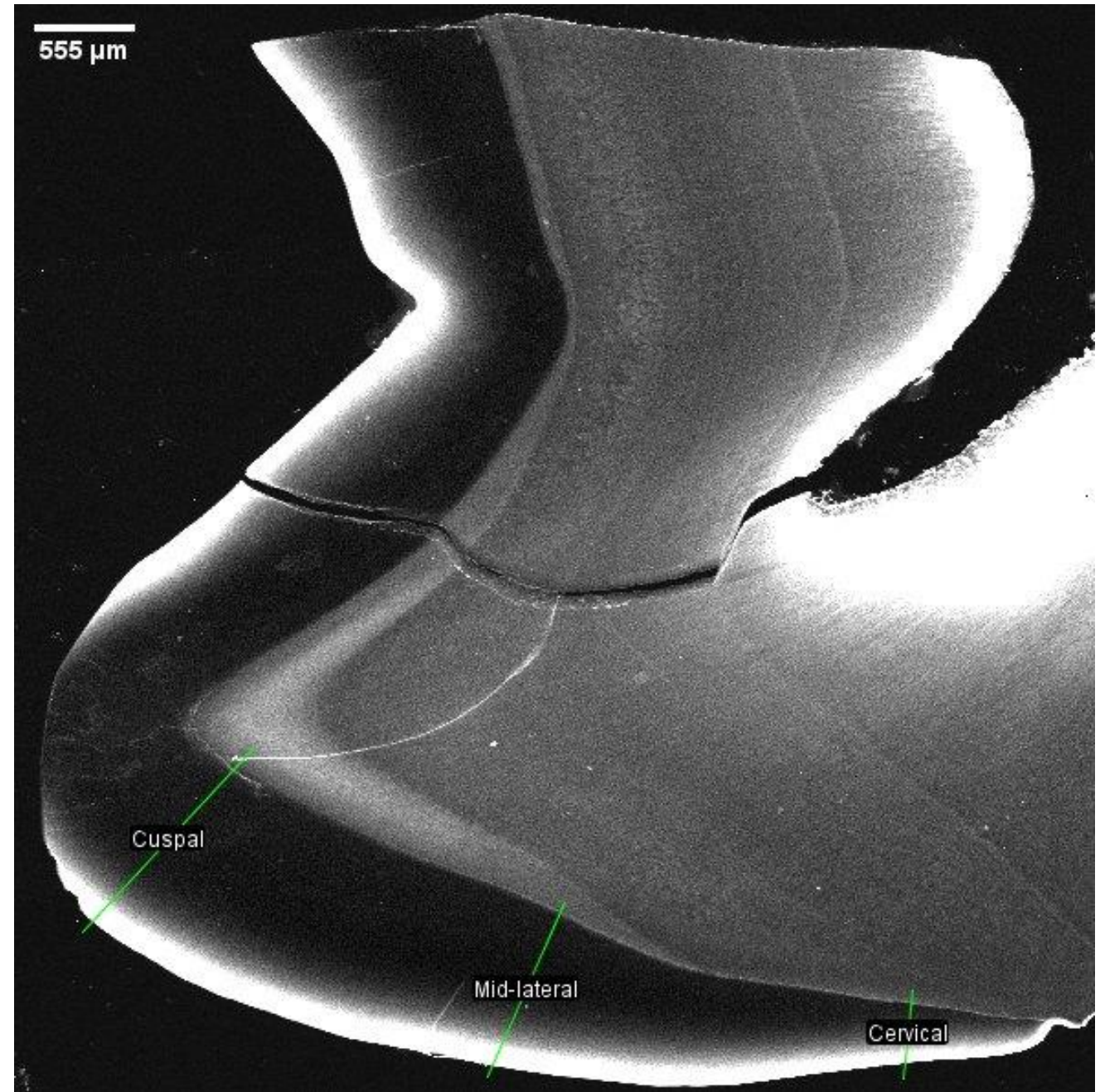
From the subset of values for relative enamel thickness, the maximum Zn value (Zn peak) was computed in Excel, as well as the width of the Zn-enriched band, the distance between “take-off” and the Zn peak, and the distance between the Zn peak and the OES, all in relation to total enamel thickness (absolute and relative [percentage] values reported hereafter).

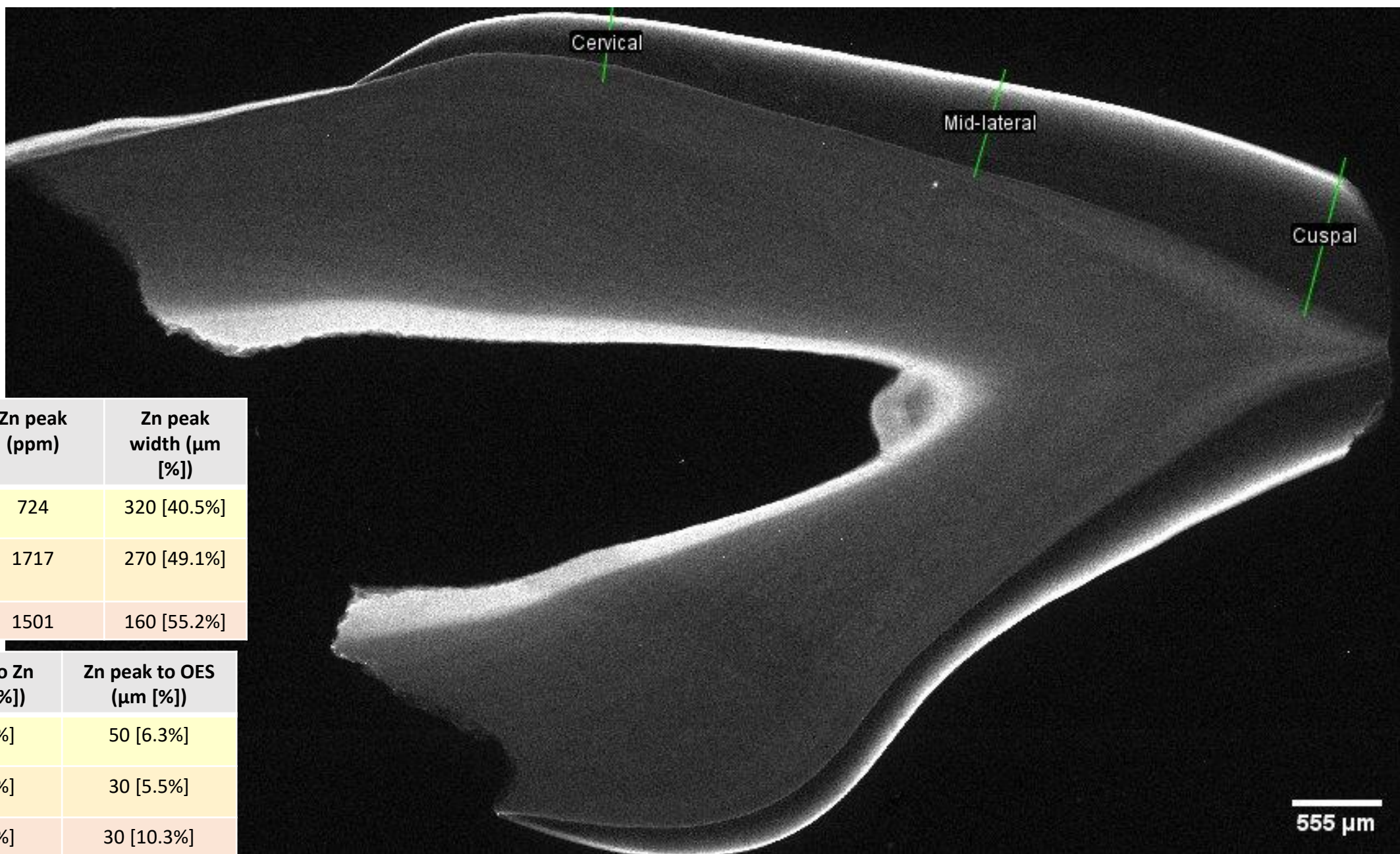
Table - Descriptive statistics in enamel thickness, maximal Zn concentration (peak) and width of the Zn-enriched band of enamel in the three anatomical regions (cuspal, mid-lateral, and cervical).

	Enamel thickness [μm]	Zn peak (ppm)	Width of Zn-enriched band (μm)
Cuspal	Min = 44 Max = 2820 Mean = 962.2 SD = 614.1	Min = 434 Max = 1743 Mean = 879.2 SD = 334.3	Min = 16 Max = 950 Mean = 388.7 SD = 219.6
Mid-lateral	Min = 38 Max = 1970 Mean = 806.28 SD = 484.1	Min = 197 Max = 1717 Mean = 915.9 SD = 412.4	Min = 17 Max = 950 Mean = 412.9 SD = 203.6
Cervical	Min = 14 Max = 840 Mean = 370.4 SD = 208.8	Min = 393 Max = 1602 Mean = 821.6 SD = 351.3	Min = 13 Max = 560 Mean = 272.2 SD = 123.5
Overall	Min = 14 (cervical) Max = 2820 (cuspal)	Min = 197 (mid-lateral) Max = 1743 (cuspal)	Min = 13 (cervical) Max = 950 (cuspal)

	Enamel thickness [μm]	Zn peak (ppm)	Zn peak width (μm [%])
Cuspal	1120	896	400 [35.7%]
Mid-lateral	910	872	490 [53.8%]
Cervical	330	798	320 [97.0%]

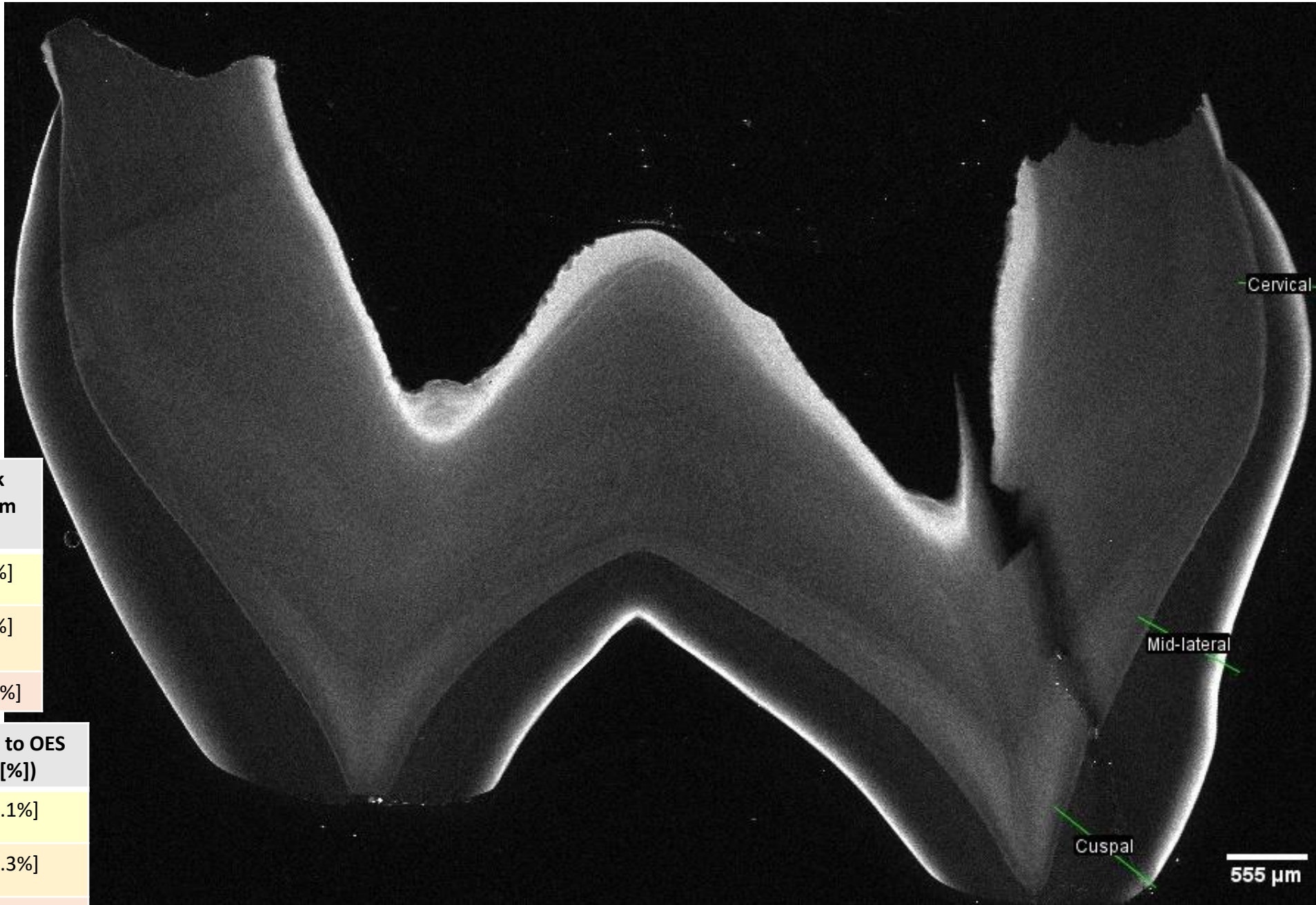
	"Take-off" to Zn peak (μm [%])	Zn peak to OES (μm [%])
Cuspal	360 [32.1%]	40 [3.6%]
Mid-lateral	440 [48.4%]	50 [5.5%]
Cervical	300 [90.9%]	20 [6.1%]





	Enamel thickness [μm]	Zn peak (ppm)	Zn peak width (μm [%])
Cuspal	790	724	320 [40.5%]
Mid-lateral	550	1717	270 [49.1%]
Cervical	290	1501	160 [55.2%]

	“Take-off” to Zn peak (μm [%])	Zn peak to OES (μm [%])
Cuspal	270 [34.2%]	50 [6.3%]
Mid-lateral	240 [43.6%]	30 [5.5%]
Cervical	130 [44.8%]	30 [10.3%]

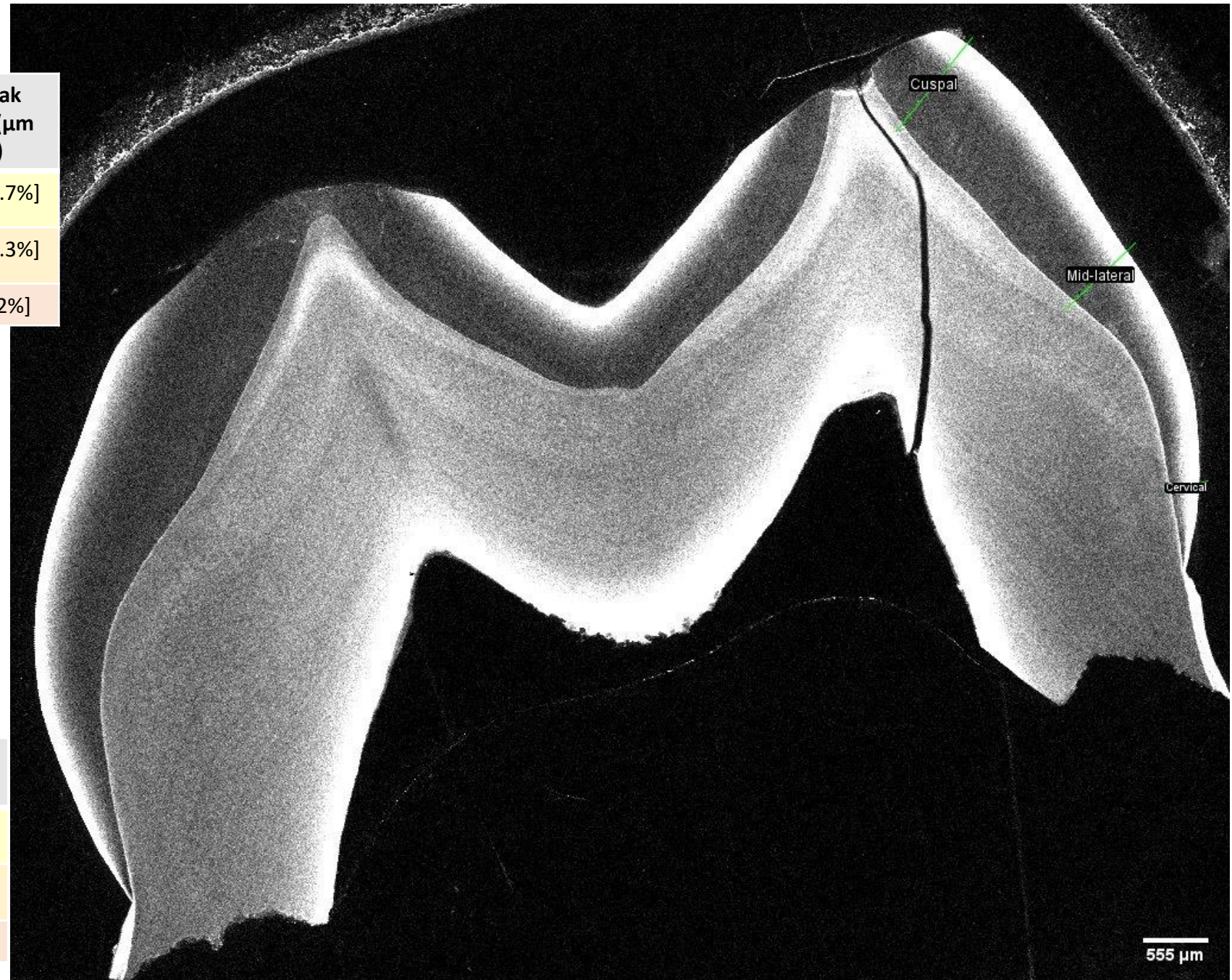


	Enamel thickness [μm]	Zn peak (ppm)	Zn peak width (μm [%])
Cuspal	740	451	200 [27%]
Mid-lateral	480	313	240 [50%]
Cervical	270	574	210 [77.8%]

	"Take-off" to Zn peak (μm [%])	Zn peak to OES (μm [%])
Cuspal	170 [23%]	30 [4.1%]
Mid-lateral	200 [41.7%]	40 [8.3%]
Cervical	160 [59.3%]	50 [18.5%]

555 μm

	Enamel thickness [μm]	Zn peak (ppm)	Zn peak width (μm [%])
Cuspal	870	1050	380 [43.7%]
Mid-lateral	600	1289	320 [53.3%]
Cervical	250	1179	230 [92%]



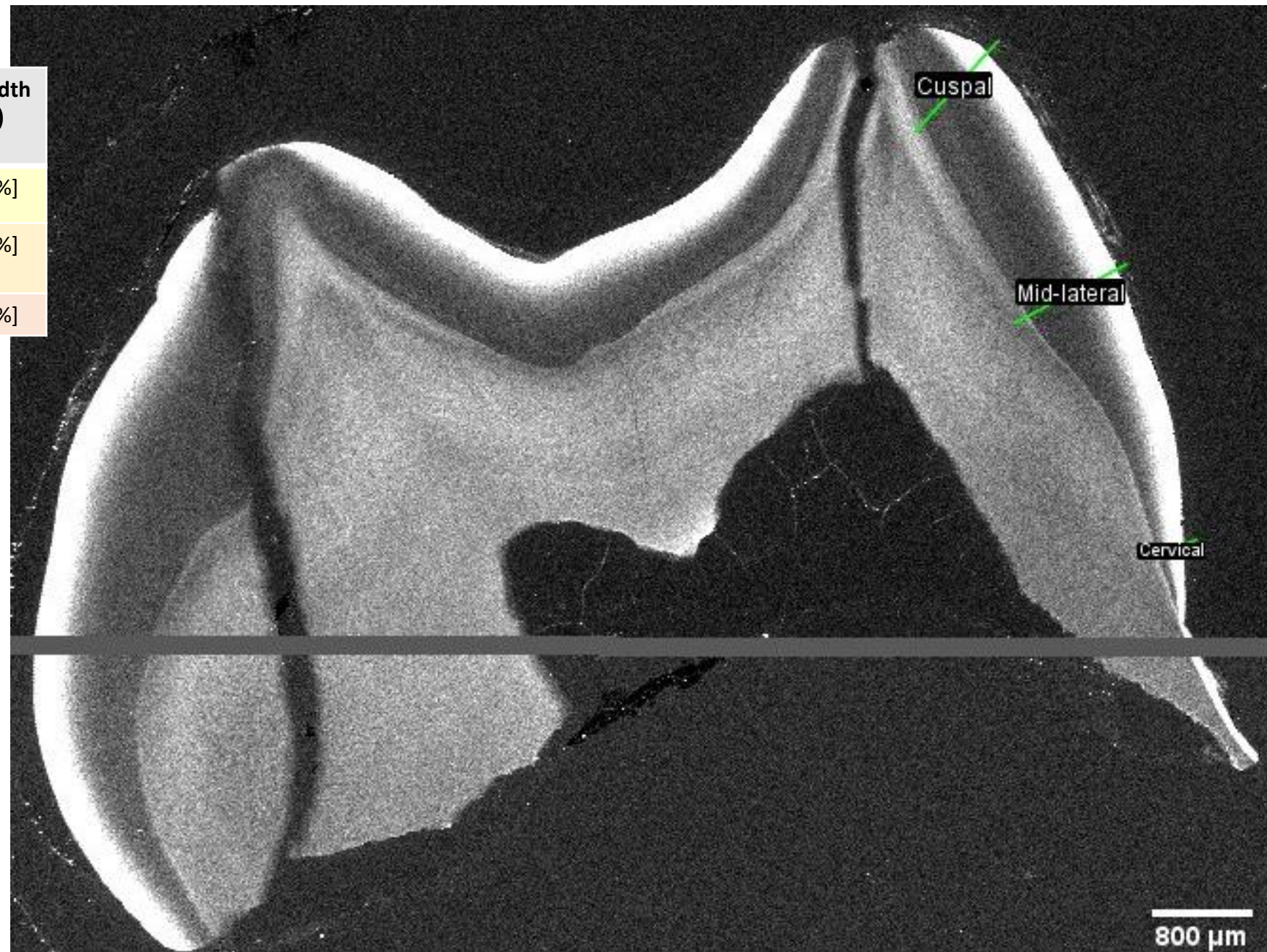
	"Take-off" to Zn peak (μm [%])	Zn peak to OES (μm [%])
Cuspal	350 [40.2%]	30 [3.4%]
Mid-lateral	300 [50%]	20 [3.3%]
Cervical	200 [80%]	30 [12%]

555 μm

	Enamel thickness [μm]	Zn peak (ppm)	Zn peak width (μm [%])
Cuspal	735	904	345 [46.9%]
Mid-lateral	795	1314	375 [47.2%]
Cervical	240	1002	210 [87.5%]

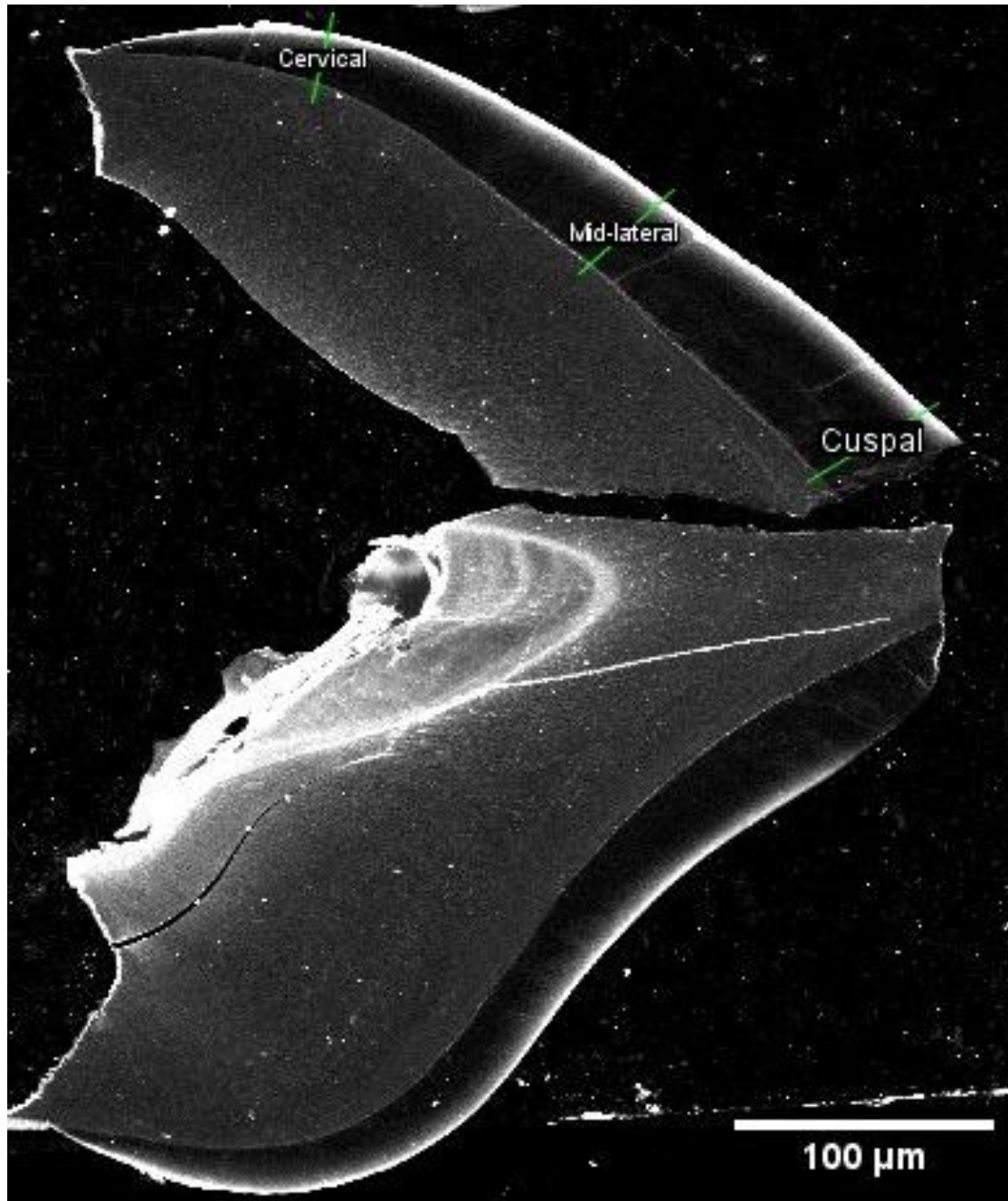
	"Take-off" to Zn peak (μm [%])	Zn peak to OES (μm [%])
Cuspal	315 [42.9%]	30 [4.1%]
Mid-lateral	315 [39.6%]	60 [7.5%]
Cervical	165 [68.8%]	45 [18.8%]

N.B.: The horizontal grey line is a scanning artifact: a few lines of data are missing, but this does not affect the measurements.



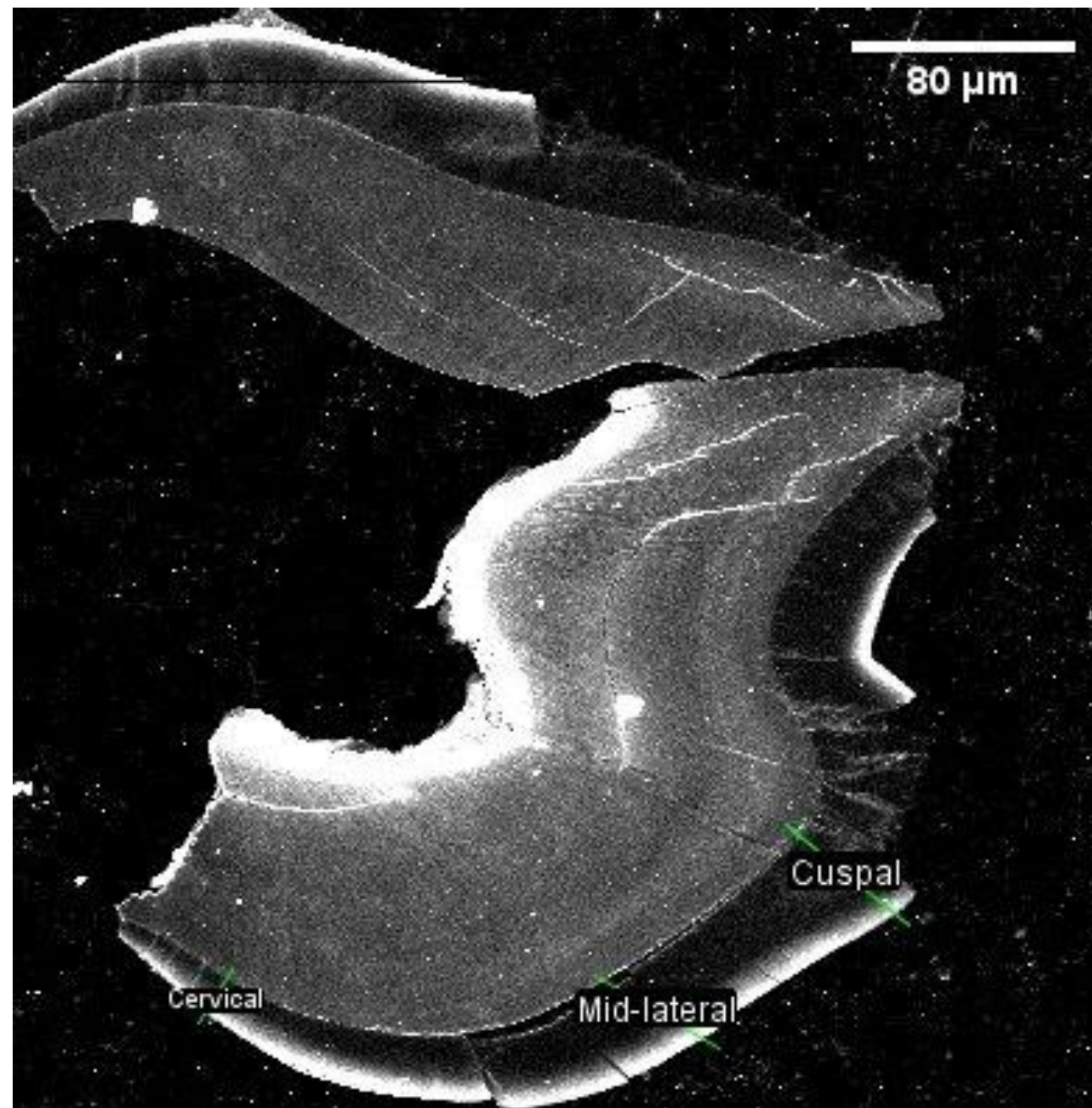
	Enamel thickness [μm]	Zn peak (ppm)	Zn peak width (μm [%])
Cuspal	49	1320	17 [34.7%]
Mid-lateral	38	1437	18 [47.4%]
Cervical	20	1140	15 [75%]

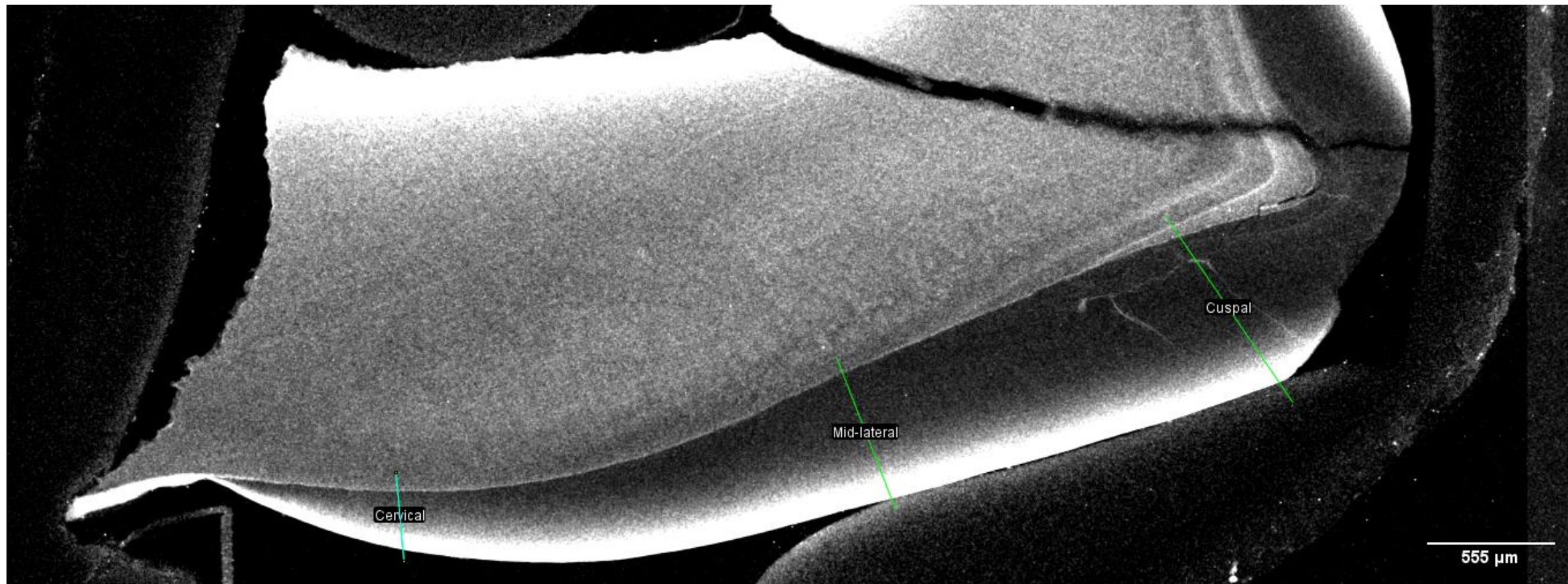
	"Take-off" to Zn peak (μm [%])	Zn peak to OES (μm [%])
Cuspal	14 [28.6%]	3 [6.1%]
Mid-lateral	15 [39.5%]	3 [7.9%]
Cervical	14 [70%]	1 [5%]



	Enamel thickness [μm]	Zn peak (ppm)	Zn peak width (μm [%])
Cuspal	44	434	16 [36.4%]
Mid-lateral	42	885	17 [40.5%]
Cervical	14	528	13 [92.9%]

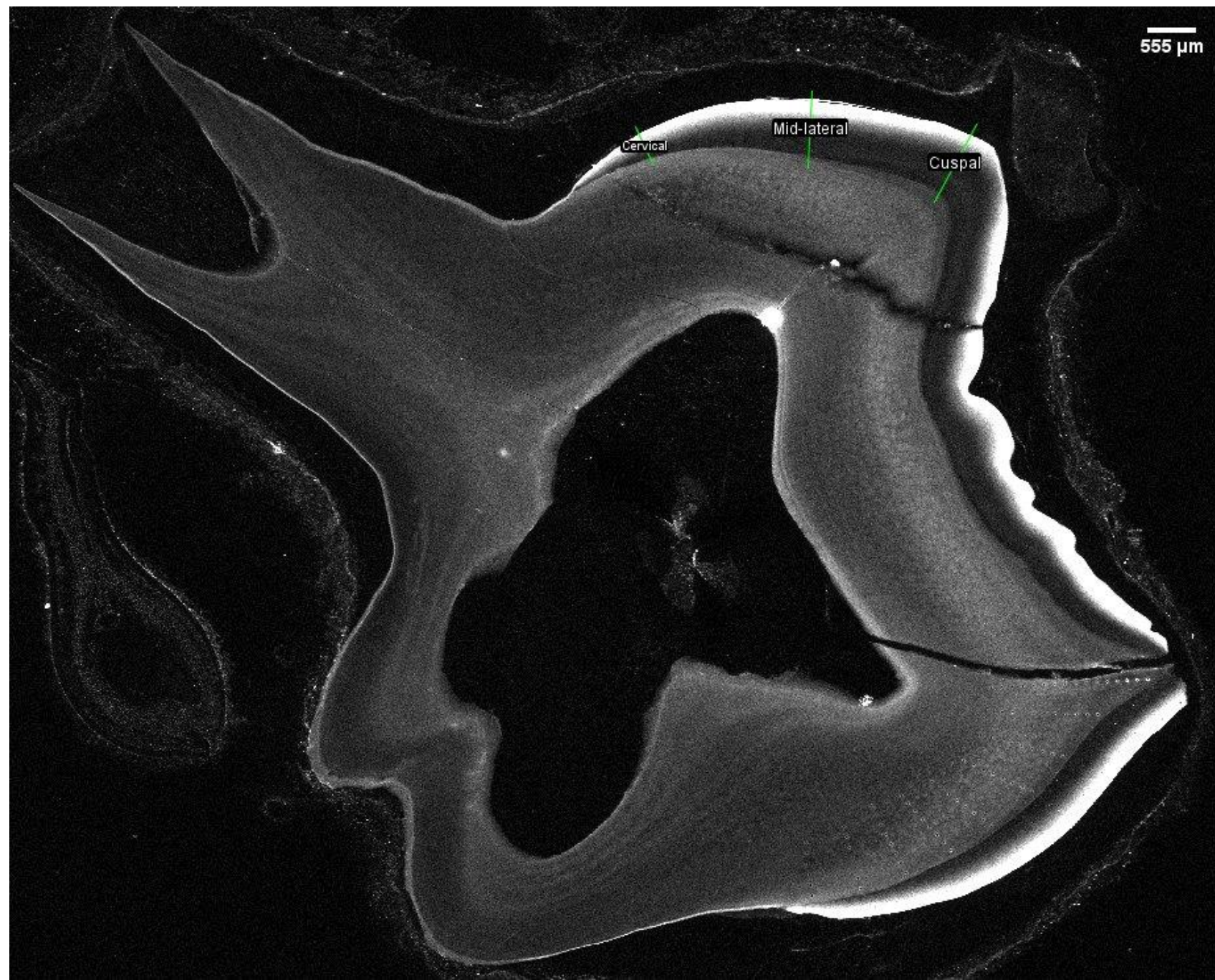
	"Take-off" to Zn peak (μm [%])	Zn peak to OES (μm [%])
Cuspal	11 [25%]	5 [11.4%]
Mid-lateral	14 [33.3%]	3 [7.1%]
Cervical	9 [64.3%]	4 [28.6%]





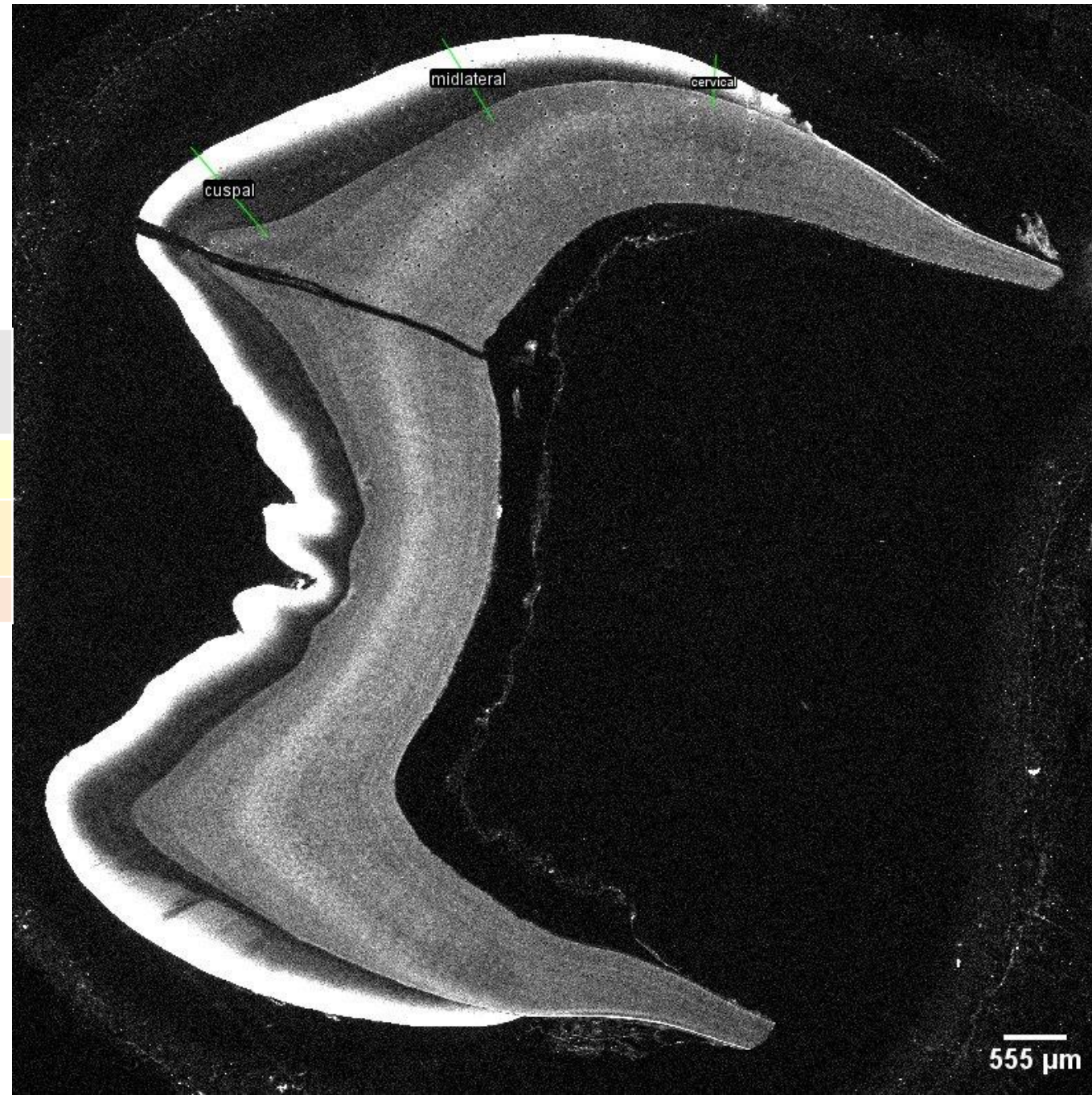
	Enamel thickness [μm]	Zn peak (ppm)	Zn peak width (μm [%])	“Take-off” to Zn peak (μm [%])	Zn peak to OES (μm [%])
Cuspal	770	986	282.5 [36.7%]	267.5 [34.7%]	15 [1.9%]
Mid-lateral	590	871	317.5 [53.8%]	305 [51.7%]	12.5 [2.1%]
Cervical	260	981	240 [92.3%]	220 [84.6%]	20 [7.7]

	Enamel thickness [μm]	Zn peak (ppm)	Zn peak width (μm [%])
Cuspal	750	1456	390 [52%]
Mid-lateral	660	1383	390 [59.1%]
Cervical	300	1000	255 [85%]
	"Take-off" to Zn peak (μm [%])	Zn peak to OES (μm [%])	
Cuspal	360 [48%]	30 [4%]	
Mid-lateral	330 [50%]	60 [9.1%]	
Cervical	225 [75%]	30 [10%]	



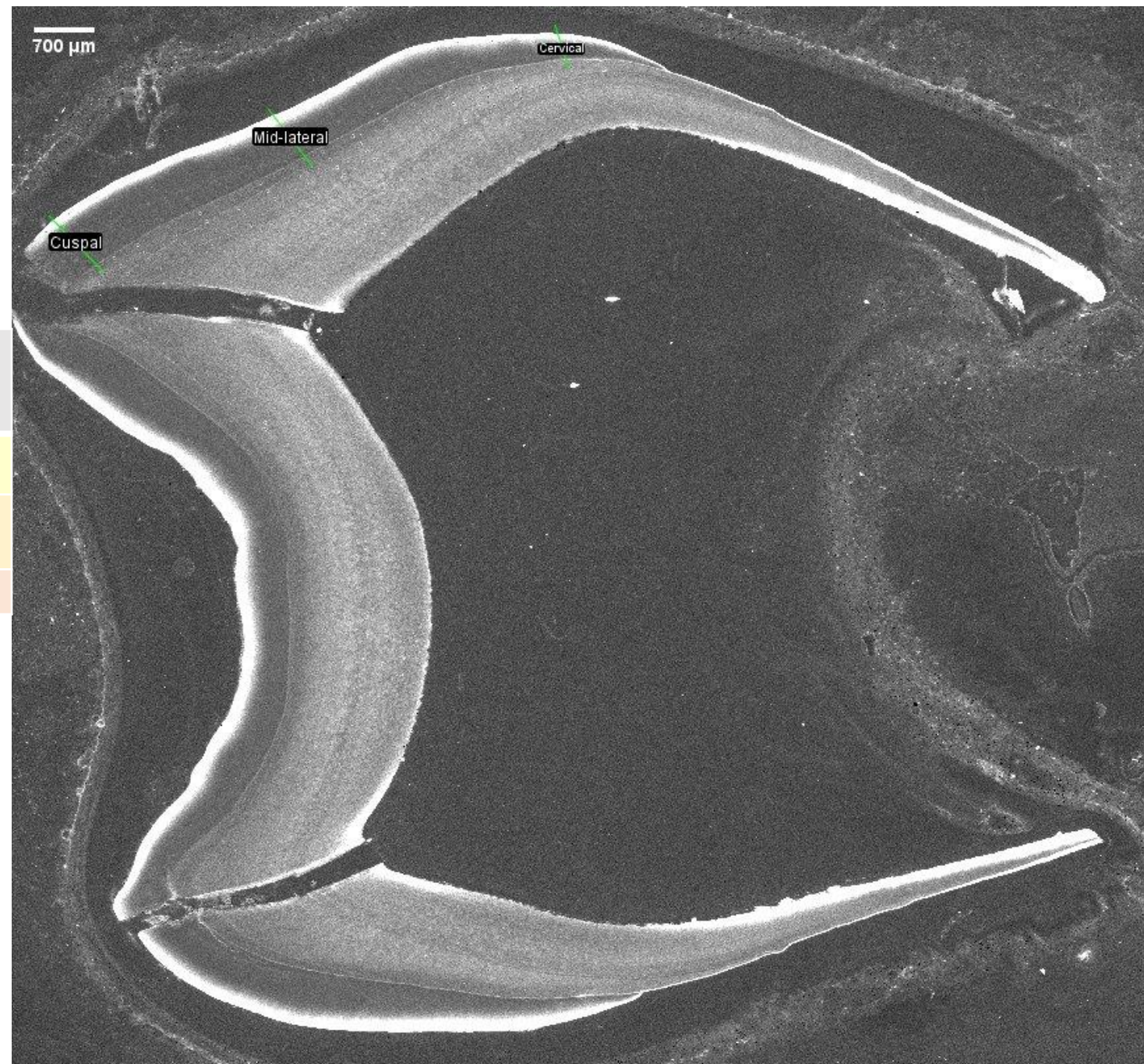
	Enamel thickness [μm]	Zn peak (ppm)	Zn peak width (μm [%])
Cuspal	792	1743	336 [42.4%]
Mid-lateral	612	1222	348 [56.9%]
Cervical	252	539	240 [95.2%]

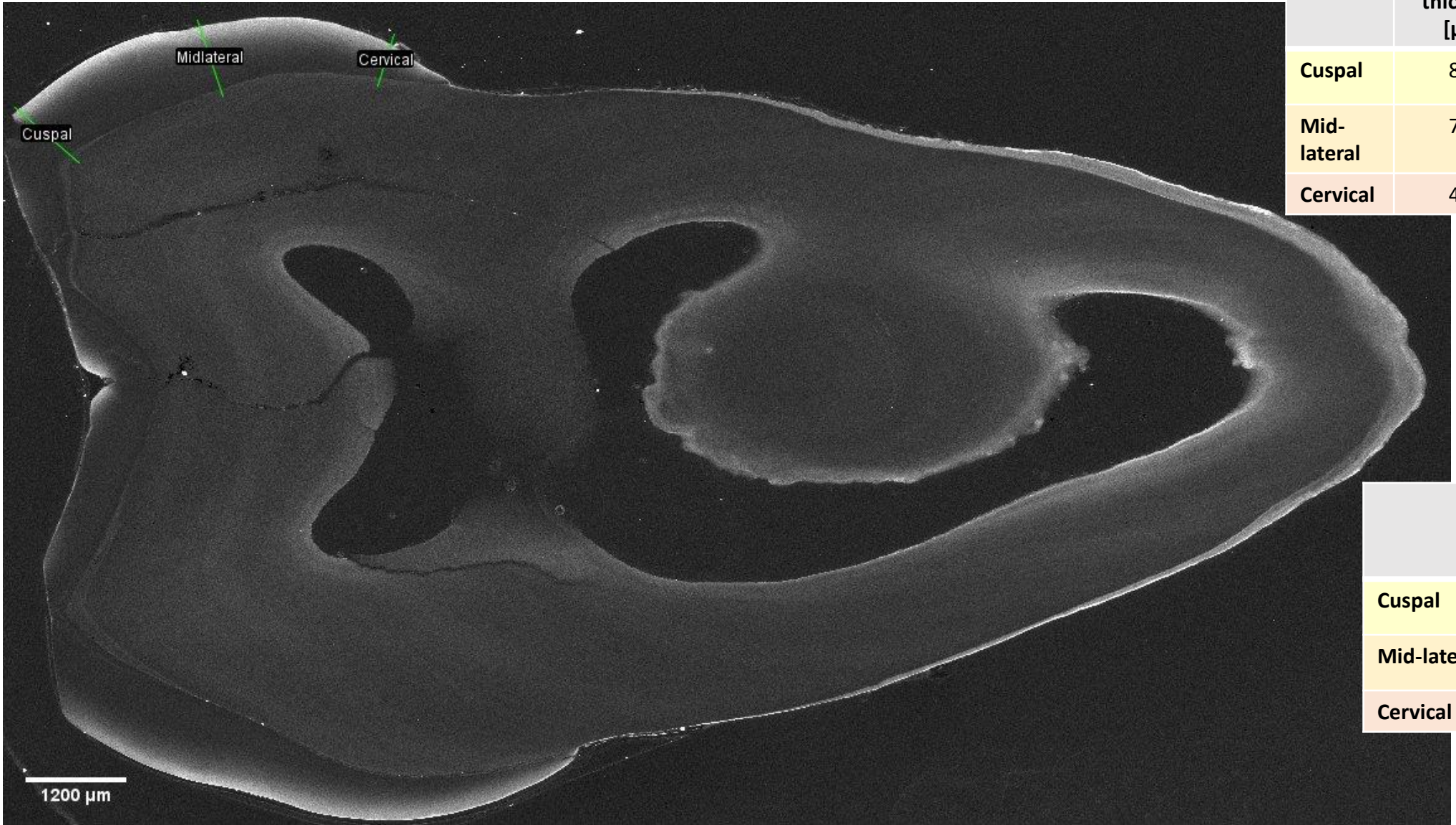
	"Take-off" to Zn peak (μm [%])	Zn peak to OES (μm [%])
Cuspal	300 [37.9%]	36 [4.5%]
Mid-lateral	312 [51%]	36 [5.9%]
Cervical	156 [61.9%]	84 [33.3%]



	Enamel thickness [μm]	Zn peak (ppm)	Zn peak width (μm [%])
Cuspal	690	733	255 [37%]
Mid-lateral	435	197	300 [69%]
Cervical	300	1602	240 [80%]

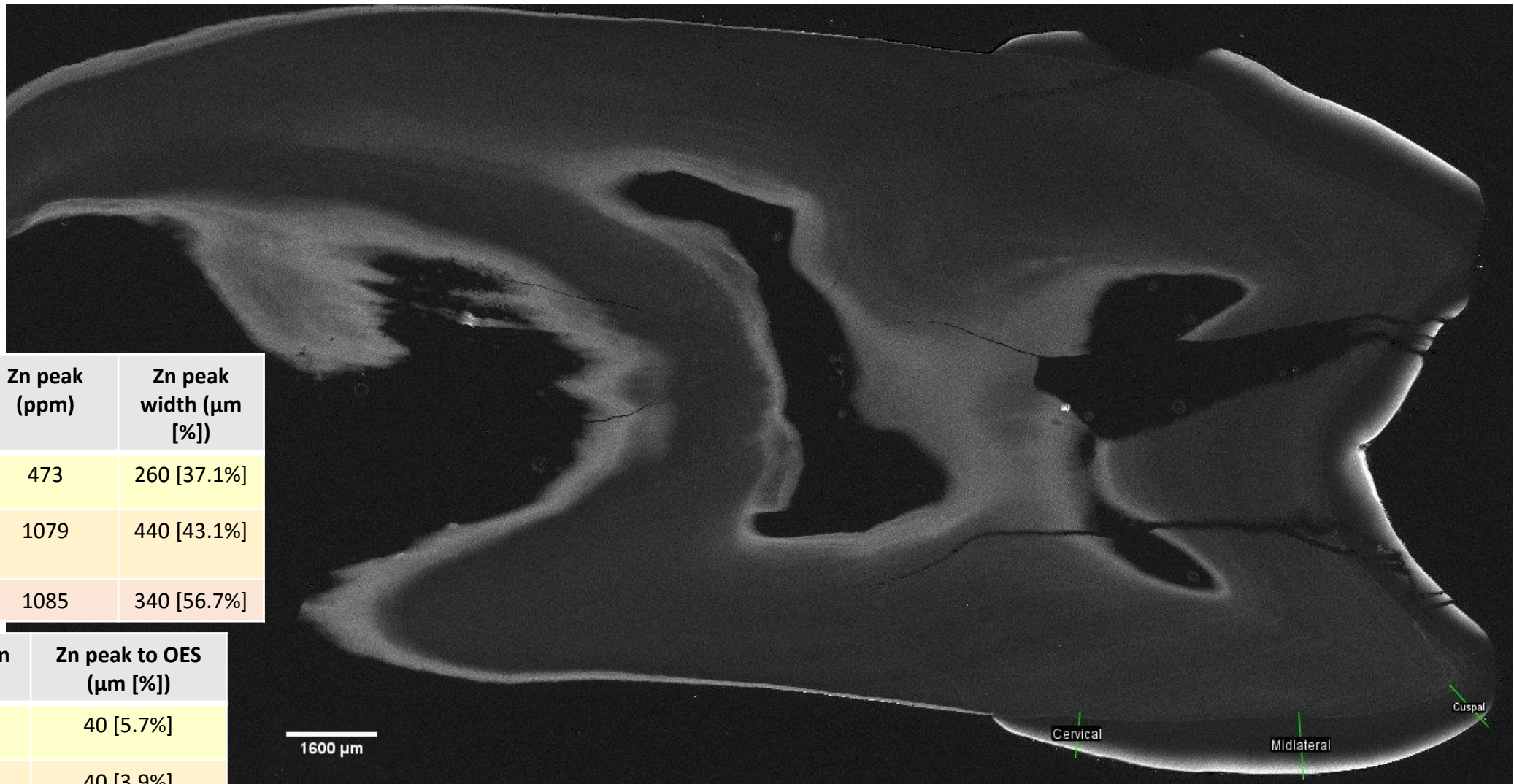
	"Take-off" to Zn peak (μm [%])	Zn peak to OES (μm [%])
Cuspal	210 [30.4%]	45 [6.5%]
Mid-lateral	255 [58.6%]	45 [10.3%]
Cervical	210 [70%]	30 [10%]





	Enamel thickness [μm]	Zn peak (ppm)	Zn peak width (μm [%])
Cuspal	810	463	345 [42.6%]
Mid-lateral	780	562	510 [65.4%]
Cervical	420	462	330 [78.3%]

	"Take-off" to Zn peak (μm [%])	Zn peak to OES (μm [%])
Cuspal	300 [37%]	45 [5.6%]
Mid-lateral	450 [57.7%]	60 [7.7%]
Cervical	285 [67.9%]	45 [10.7%]

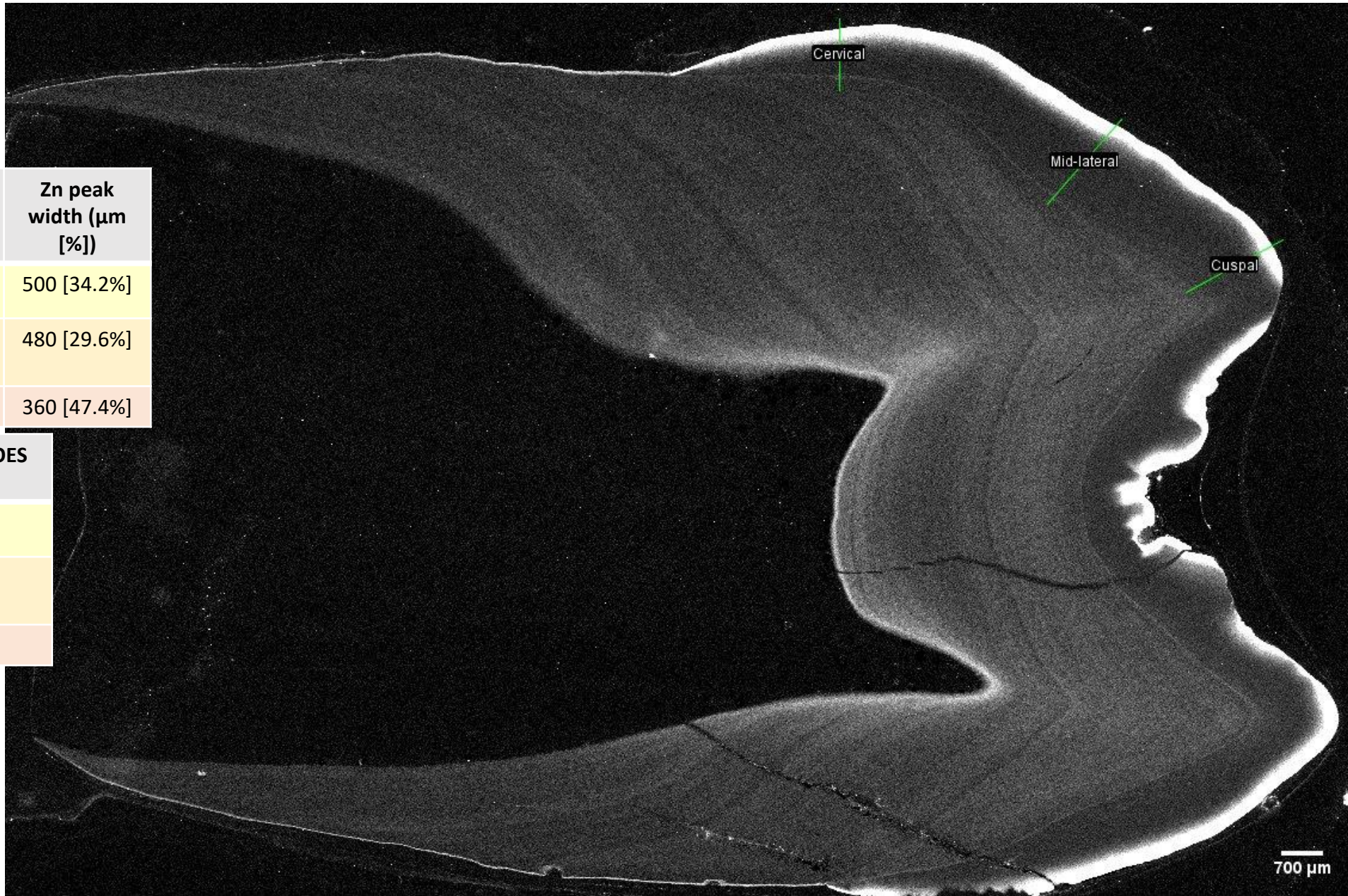


	Enamel thickness [μm]	Zn peak (ppm)	Zn peak width (μm [%])
Cuspal	700	473	260 [37.1%]
Mid-lateral	1020	1079	440 [43.1%]
Cervical	600	1085	340 [56.7%]

	"Take-off" to Zn peak (μm [%])	Zn peak to OES (μm [%])
Cuspal	220 [31.4%]	40 [5.7%]
Mid-lateral	400 [39.2%]	40 [3.9%]
Cervical	280 [46.7%]	60 [10%]

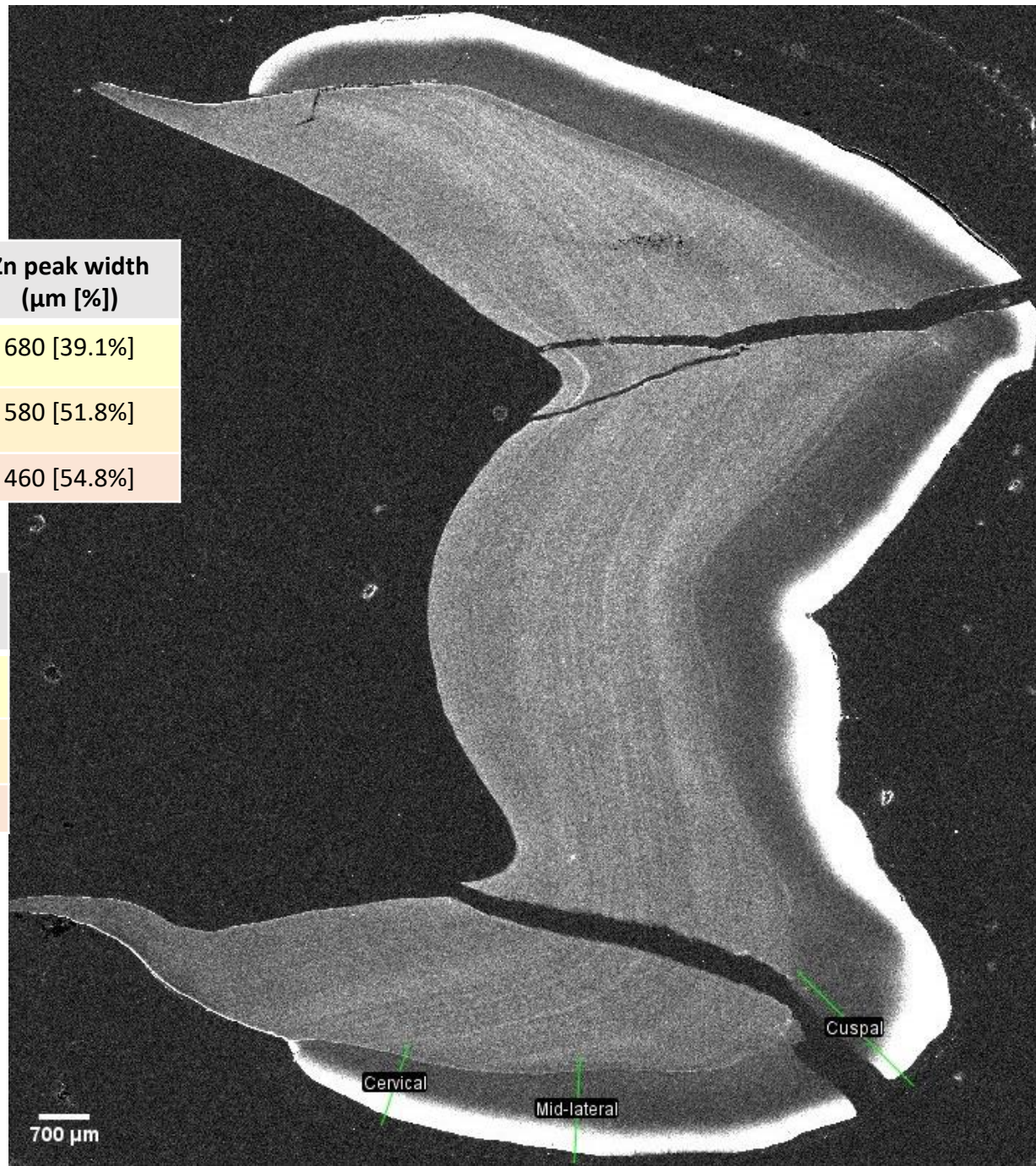
	Enamel thickness [μm]	Zn peak (ppm)	Zn peak width (μm [%])
Cuspal	1460	1120	500 [34.2%]
Mid-lateral	1620	1507	480 [29.6%]
Cervical	760	699	360 [47.4%]

	“Take-off” to Zn peak (μm [%])	Zn peak to OES (μm [%])
Cuspal	440 [30.1%]	60 [4.1%]
Mid-lateral	440 [27.2%]	40 [2.5%]
Cervical	320 [42.1%]	40 [5.3%]



	Enamel thickness [μm]	Zn peak (ppm)	Zn peak width (μm [%])
Cuspal	1740	790	680 [39.1%]
Mid-lateral	1120	417	580 [51.8%]
Cervical	840	509	460 [54.8%]

	“Take-off” to Zn peak (μm [%])	Zn peak to OES (μm [%])
Cuspal	640 [36.8%]	40 [2.3%]
Mid-lateral	380 [33.9%]	200 [17.9%]
Cervical	340 [40.5%]	120 [14.3%]



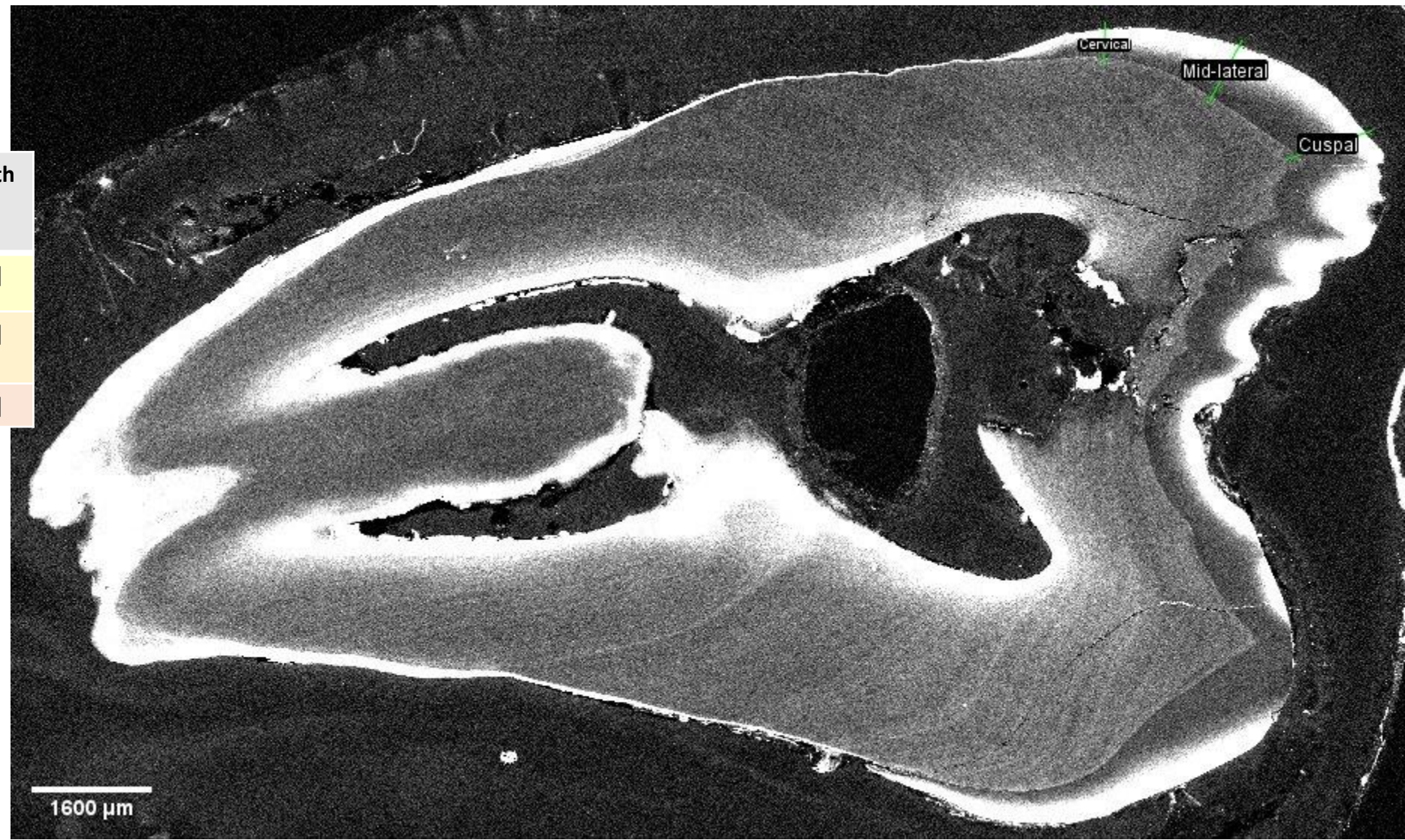


	Enamel thickness [μm]	Zn peak (ppm)	Zn peak width (μm [%])
Cuspal	800	622	500 [62.5%]
Mid-lateral	680	400	580 [85.3%]
Cervical	260	556	260 [100%]

	“Take-off” to Zn peak (μm [%])	Zn peak to OES (μm [%])
Cuspal	440 [55%]	60 [7.5%]
Mid-lateral	540 [79.4%]	40 [5.9%]
Cervical	200 [76.9%]	60 [23.1%]

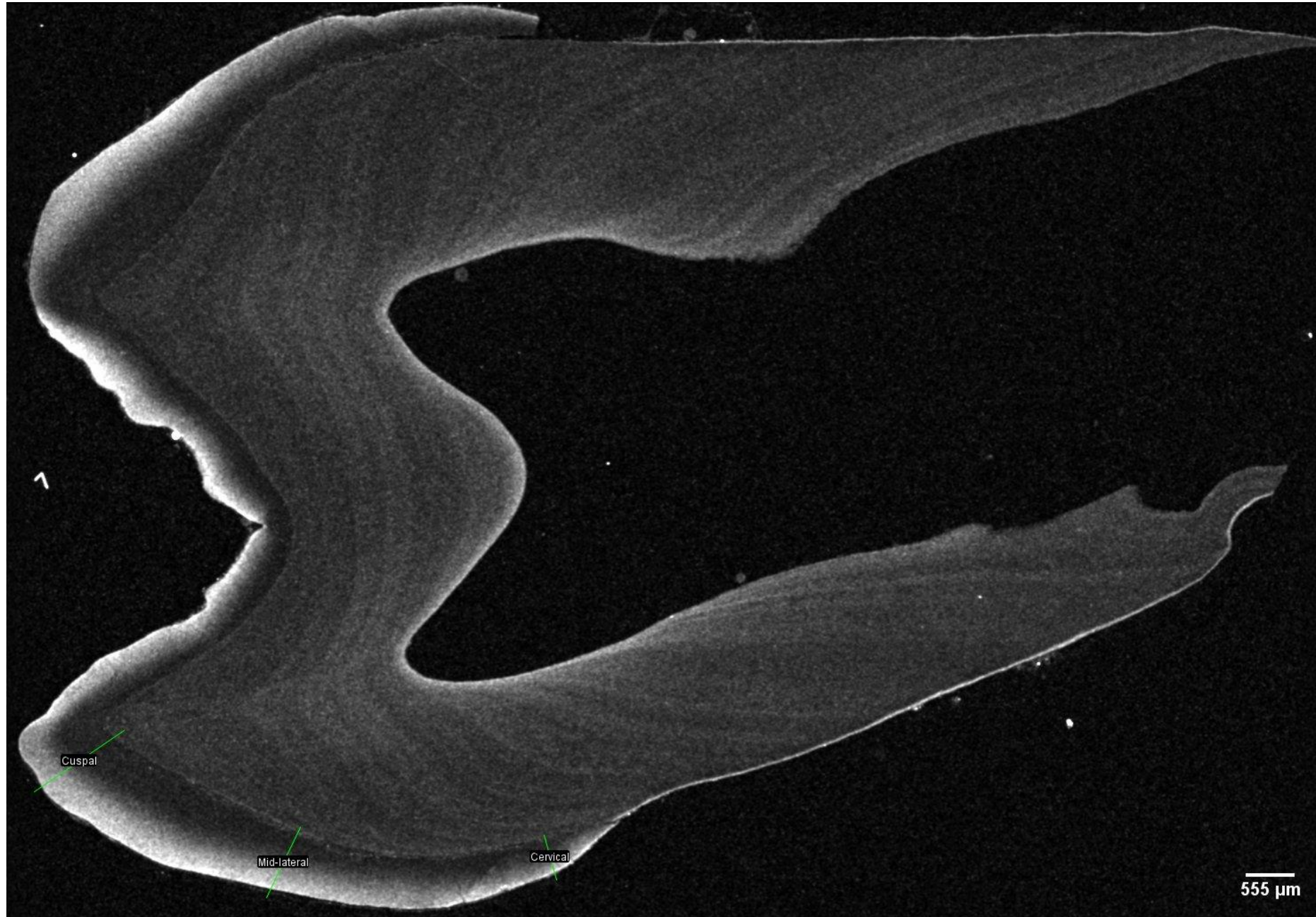
	Enamel thickness [μm]	Zn peak (ppm)	Zn peak width (μm [%])
Cuspal	960	752	460 [47.9%]
Mid-lateral	760	696	540 [71.1%]
Cervical	380	776	320 [84.2%]

	"Take-off" to Zn peak (μm [%])	Zn peak to OES (μm [%])
Cuspal	400 [41.7%]	60 [6.3%]
Mid-lateral	480 [63.2%]	60 [7.9%]
Cervical	300 [78.9%]	20 [5.3%]



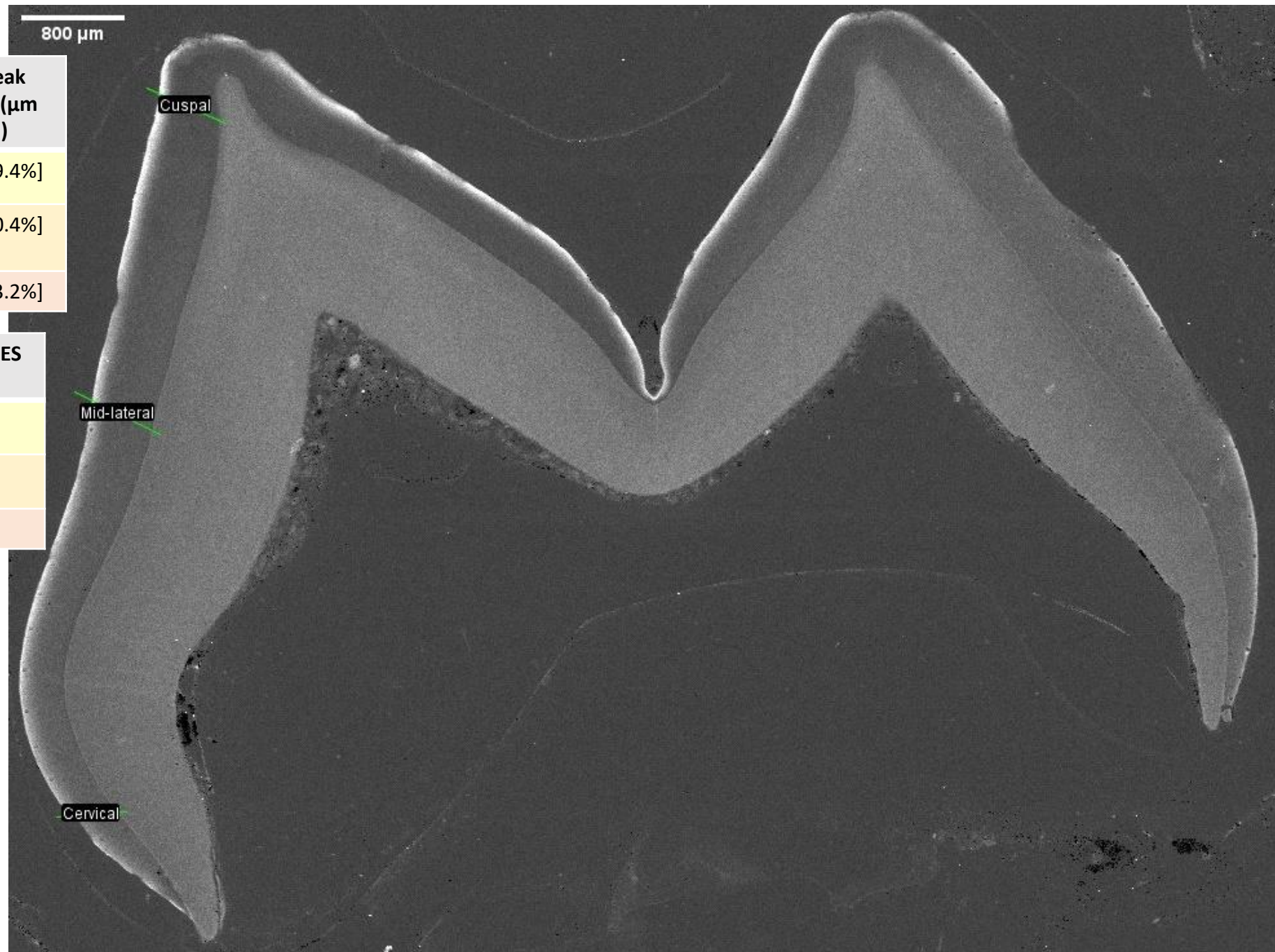
	Enamel thickness [μm]	Zn peak (ppm)	Zn peak width (μm [%])
Cuspal	990	571	560 [56.6%]
Mid-lateral	710	727	500 [70.4%]
Cervical	400	691	360 [90%]

	"Take-off" to Zn peak (μm [%])	Zn peak to OES (μm [%])
Cuspal	510 [51.5%]	50 [5.1%]
Mid-lateral	450 [63.4%]	50 [7%]
Cervical	290 [72.5%]	70 [17.5%]



	Enamel thickness [μm]	Zn peak (ppm)	Zn peak width (μm [%])
Cuspal	510	779	150 [29.4%]
Mid-lateral	520	535	210 [40.4%]
Cervical	370	421	160 [43.2%]

	“Take-off” to Zn peak (μm [%])	Zn peak to OES (μm [%])
Cuspal	120 [23.5%]	30 [5.9%]
Mid-lateral	180 [34.6%]	30 [5.8%]
Cervical	140 [37.8%]	20 [5.4%]



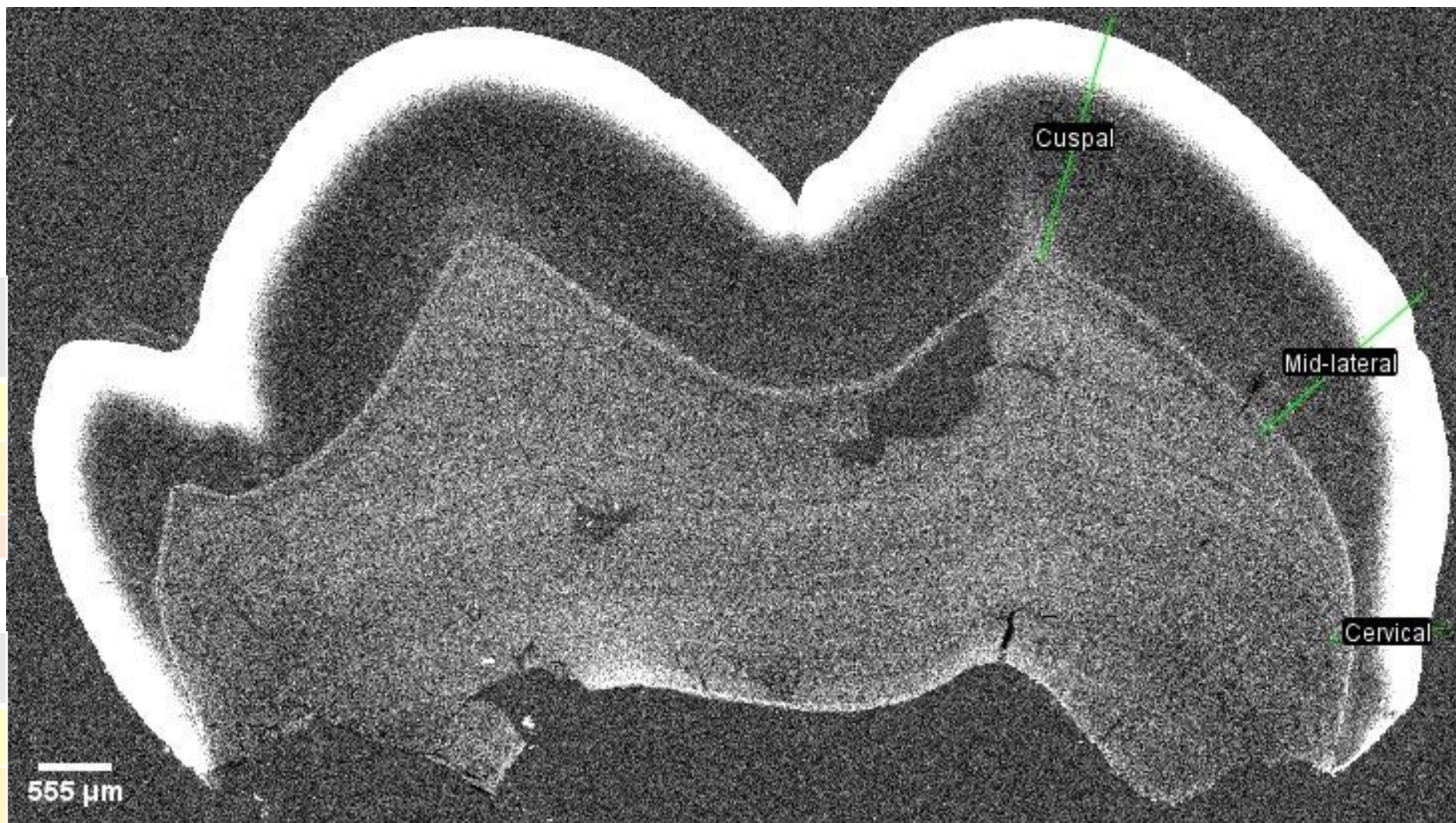
	Enamel thickness [μm]	Zn peak (ppm)	Zn peak width (μm [%])
Cuspal	450	1192	190 [42.2%]
Mid-lateral	290	1147	170 [58.6%]
Cervical	170	744	150 [88.2%]

	"Take-off" to Zn peak (μm [%])	Zn peak to OES (μm [%])
Cuspal	140 [31.1%]	50 [11.1%]
Mid-lateral	120 [41.4%]	50 [17.2%]
Cervical	110 [64.7%]	40 [23.5%]



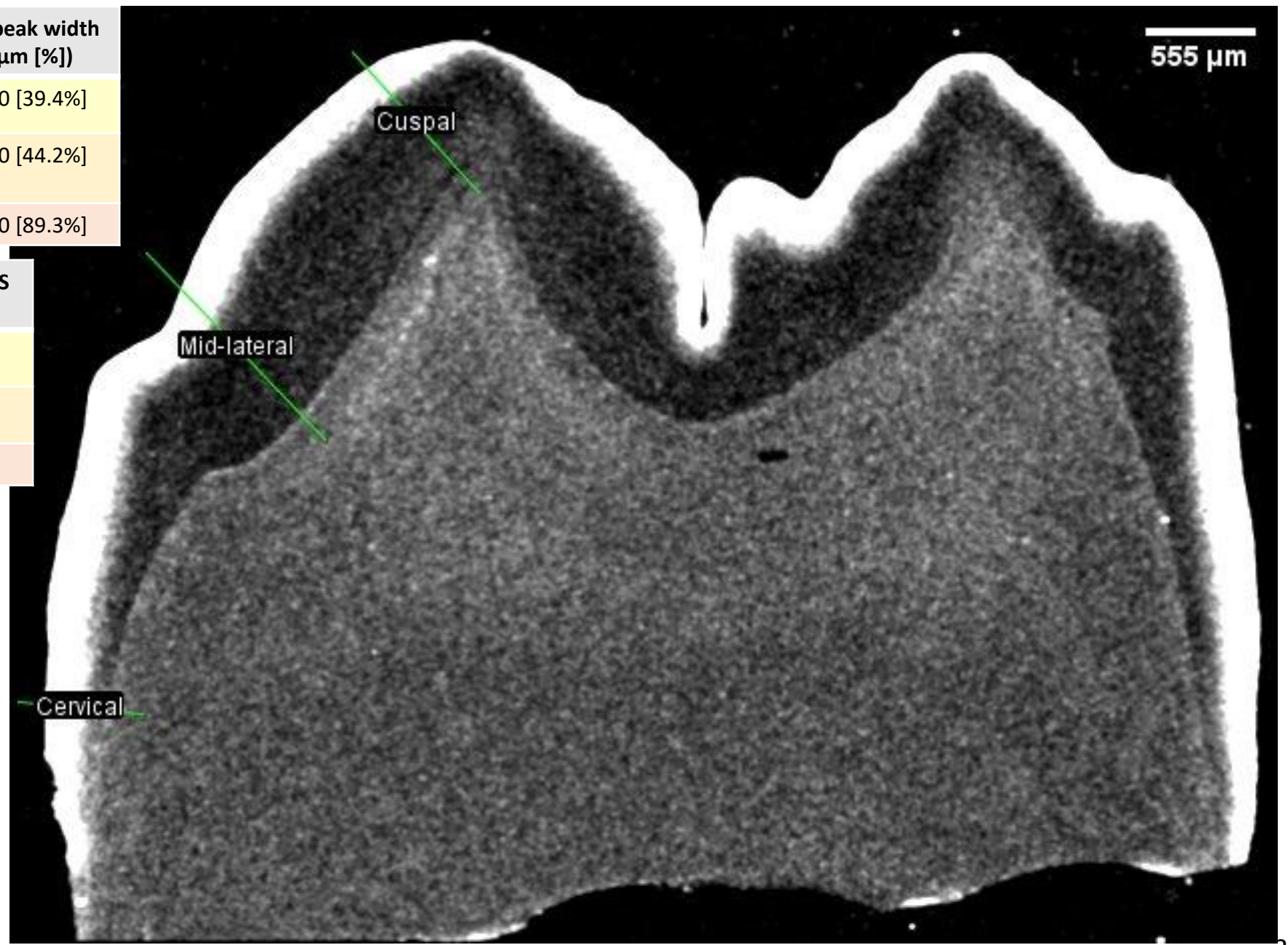
	Enamel thickness [μm]	Zn peak (ppm)	Zn peak width (μm [%])
Cuspal	1755	885	645 [36.8%]
Mid-lateral	1395	667	600 [43.0%]
Cervical	570	536	480 [84.2%]

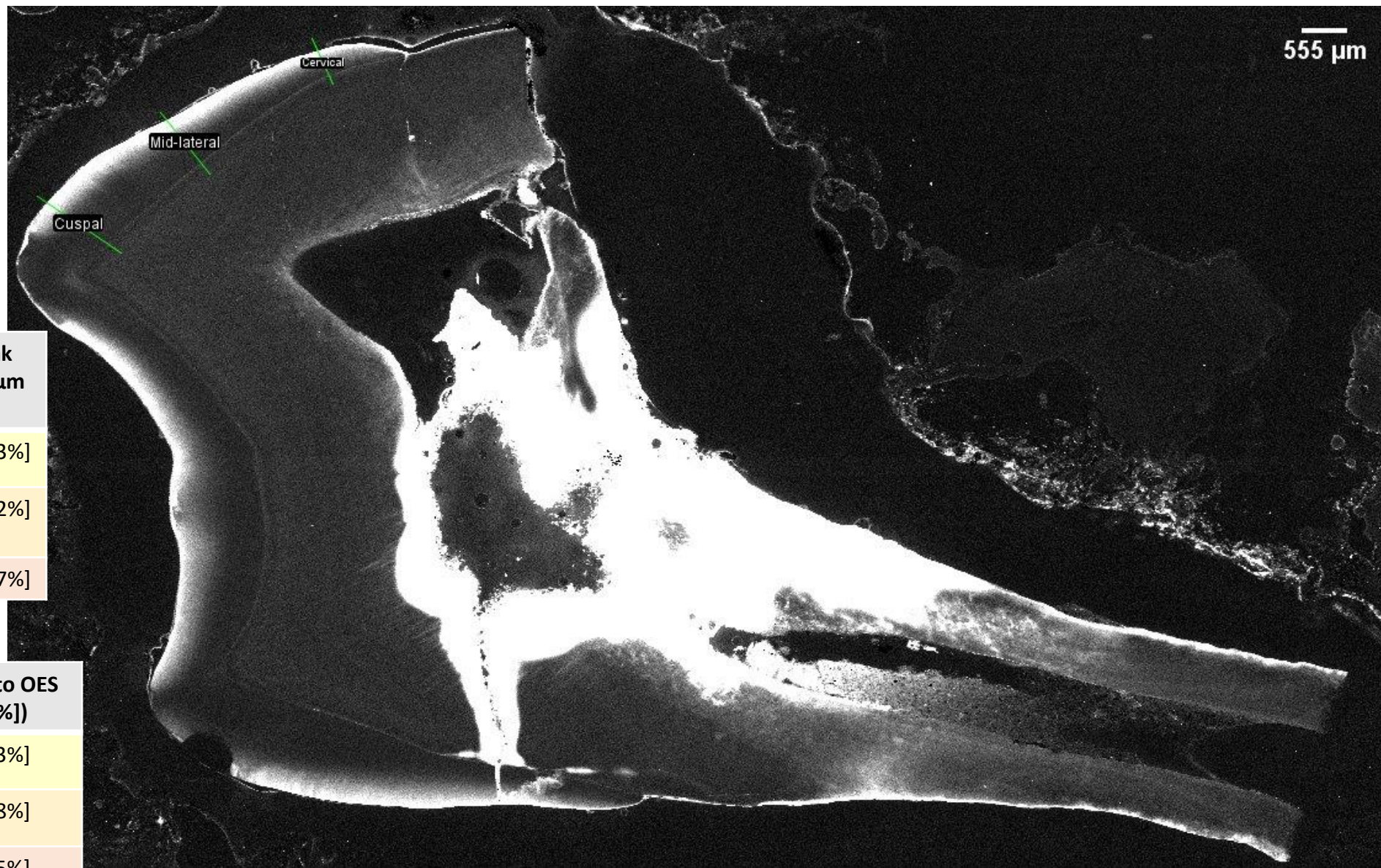
	"Take-off" to Zn peak (μm [%])	Zn peak to OES (μm [%])
Cuspal	585 [33.3%]	60 [3.4%]
Mid-lateral	555 [39.8%]	45 [3.2%]
Cervical	420 [73.7%]	60 [10.5%]



	Enamel thickness [μm]	Zn peak (ppm)	Zn peak width (μm [%])
Cuspal	660	1241	260 [39.4%]
Mid-lateral	860	1253	380 [44.2%]
Cervical	280	706	220 [89.3%]

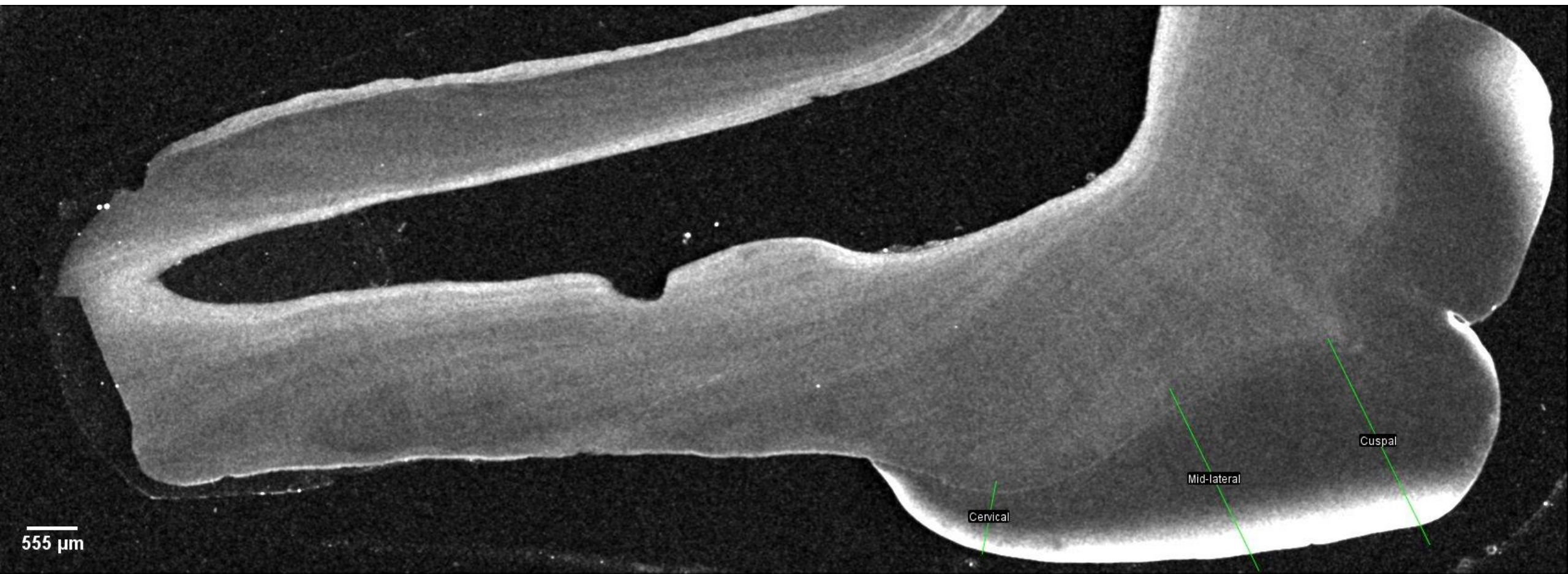
	"Take-off" to Zn peak (μm [%])	Zn peak to OES (μm [%])
Cuspal	220 [33.3%]	40 [6.1%]
Mid-lateral	320 [37.2%]	60 [7.0%]
Cervical	200 [71.4%]	50 [17.9%]





	Enamel thickness [μm]	Zn peak (ppm)	Zn peak width (μm [%])
Cuspal	900	971	525 [58.3%]
Mid-lateral	660	995	450 [68.2%]
Cervical	315	739	270 [85.7%]

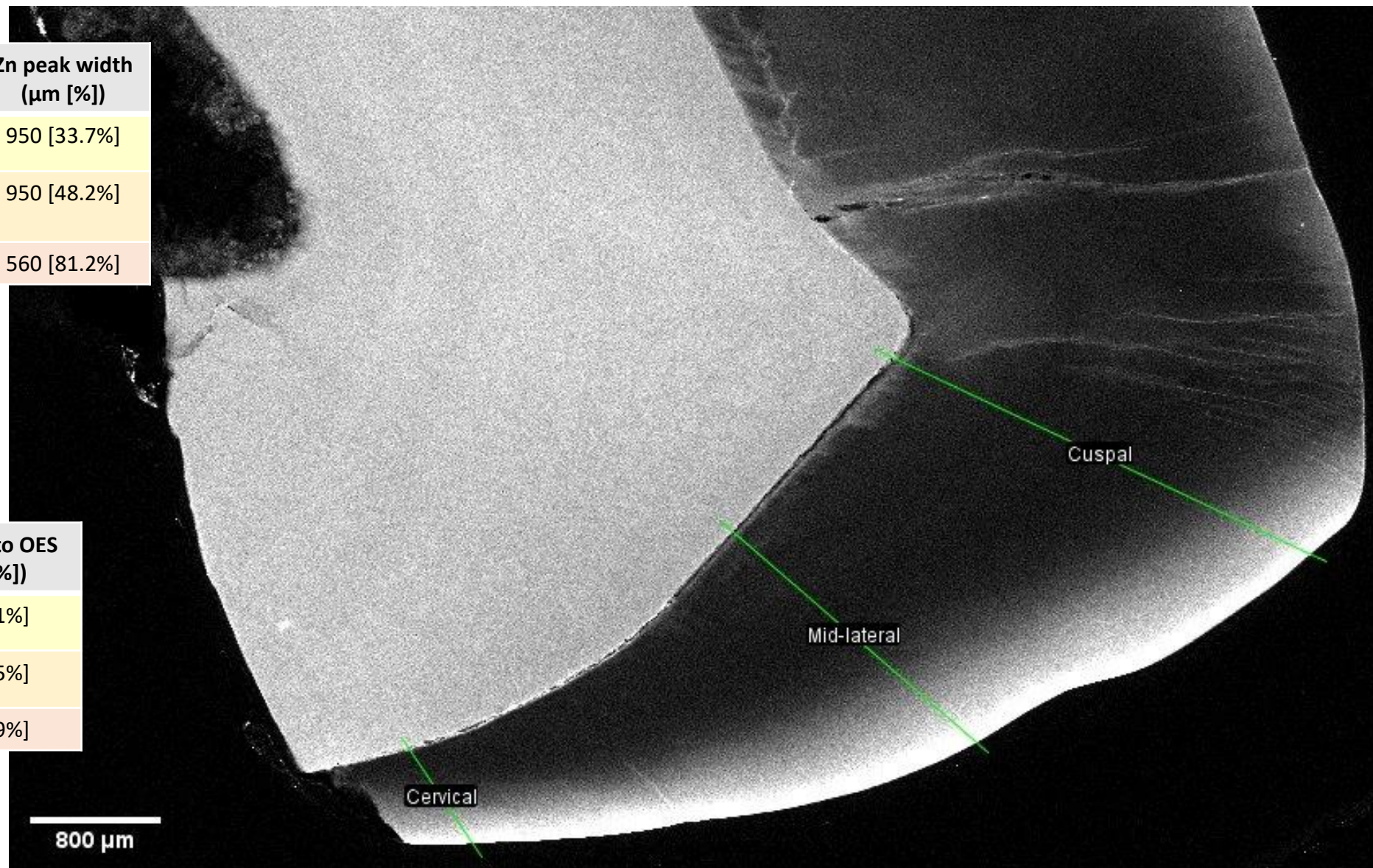
	"Take-off" to Zn peak (μm [%])	Zn peak to OES (μm [%])
Cuspal	495 [55%]	30 [3.3%]
Mid-lateral	405 [61.4%]	45 [6.8%]
Cervical	240 [76.2%]	30 [9.5%]

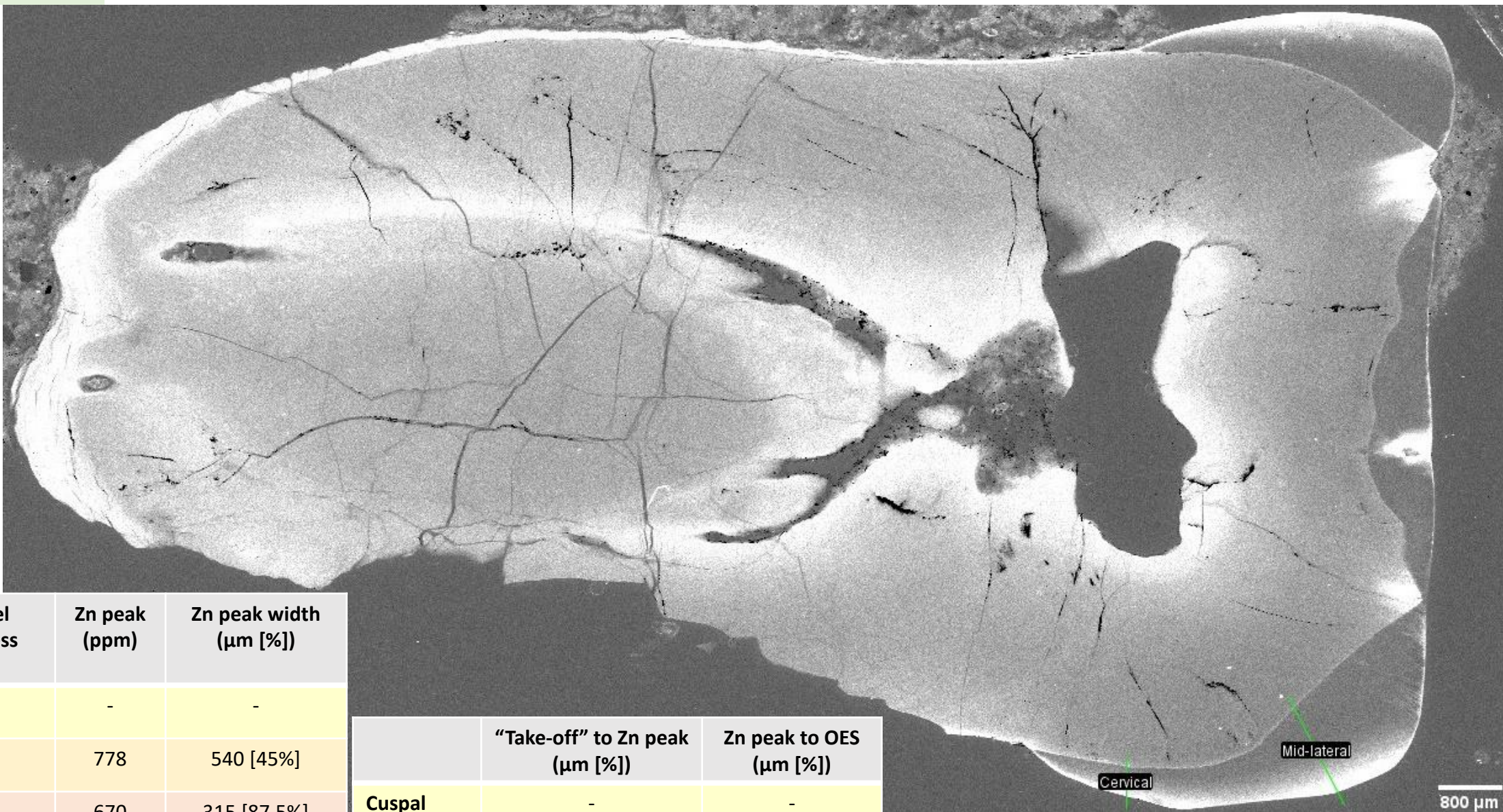


	Enamel thickness [μm]	Zn peak (ppm)	Zn peak width (μm [%])	"Take-off" to Zn peak (μm [%])	Zn peak to OES (μm [%])
Cuspal	2150	544	750 [34.9%]	670 [31.2%]	80 [3.7%]
Mid-lateral	1770	506	720 [40.7%]	650 [36.7%]	70 [4%]
Cervical	650	393	330 [50.8%]	280 [43.1%]	50 [7.7%]

	Enamel thickness [μm]	Zn peak (ppm)	Zn peak width (μm [%])
Cuspal	2820	879	950 [33.7%]
Mid-lateral	1970	1000	950 [48.2%]
Cervical	690	1506	560 [81.2%]

	"Take-off" to Zn peak (μm [%])	Zn peak to OES (μm [%])
Cuspal	930 [32.6%]	30 [1.1%]
Mid-lateral	900 [45.7%]	50 [2.5%]
Cervical	540 [78.3%]	20 [2.9%]





	Enamel thickness [μm]	Zn peak (ppm)	Zn peak width (μm [%])
Cuspal	Worn	-	-
Mid-lateral	1200	778	540 [45%]
Cervical	360	670	315 [87.5%]

	"Take-off" to Zn peak (μm [%])	Zn peak to OES (μm [%])
Cuspal	-	-
Mid-lateral	495 [41.3%]	45 [3.8%]
Cervical	285 [79.2%]	30 [8.3%]