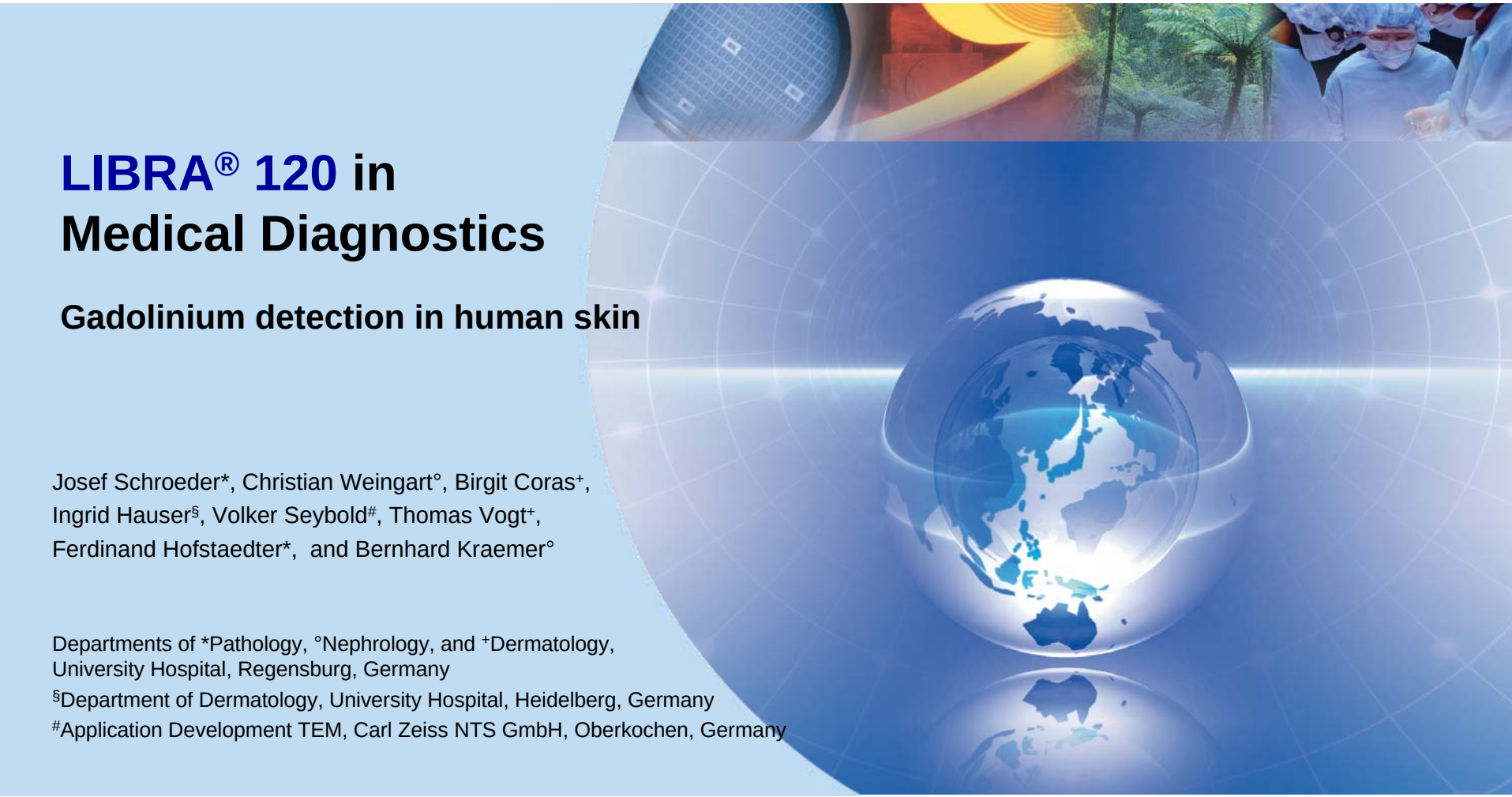
Carl Zeiss SMT – NTS Division



LIBRA® 120 in Medical Diagnostics

Gadolinium detection in human skin

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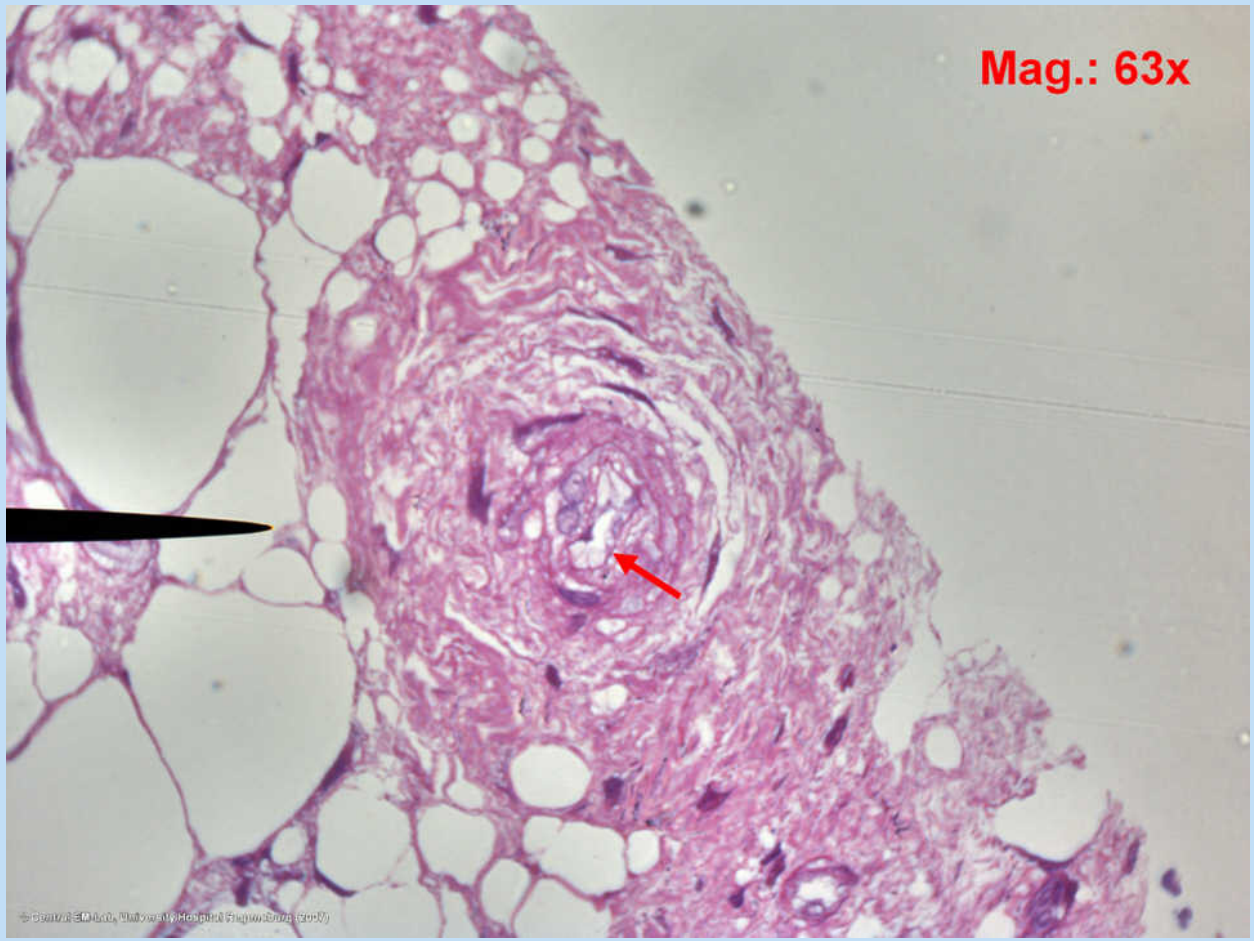
LIBRA® 120 in Medical Diagnostics: Gd detection in human skin

Market:	Life Science, Medical Diagnostics
Samples:	Human skin biopsies of a renal dialysis patient (epoxy re-embedded from paraffin blocks)
Purpose of investigation:	• A male patient with end-stage renal insufficiency had developed a severe skin inflammation with ulcerous lesion at his left bunion due to a peripheral artery disease • The inflammation was conventionally treated with antiphlogistics but could not be stopped • A few months earlier, awareness of impaired renal function had led to the decision of performing MRT scans, to avoid renal toxicity of conventional iodinated CT contrast agents. A total of 49.5 mmol Gadopentetate had been applied to improve contrast and resolution of MRTs. ➔ To confirm the suspicion that incorporated free Gd^{3+} ions, which are very toxic for tissues, could be the inflammatory trigger, high resolution electron spectroscopic images of biopsies were recorded. The findings were confirmed with Parallel EEL spectra.

LIBRA® 120 in Medical Diagnostics: Gd detection in human skin

Solution:



LM survey of the area of interest

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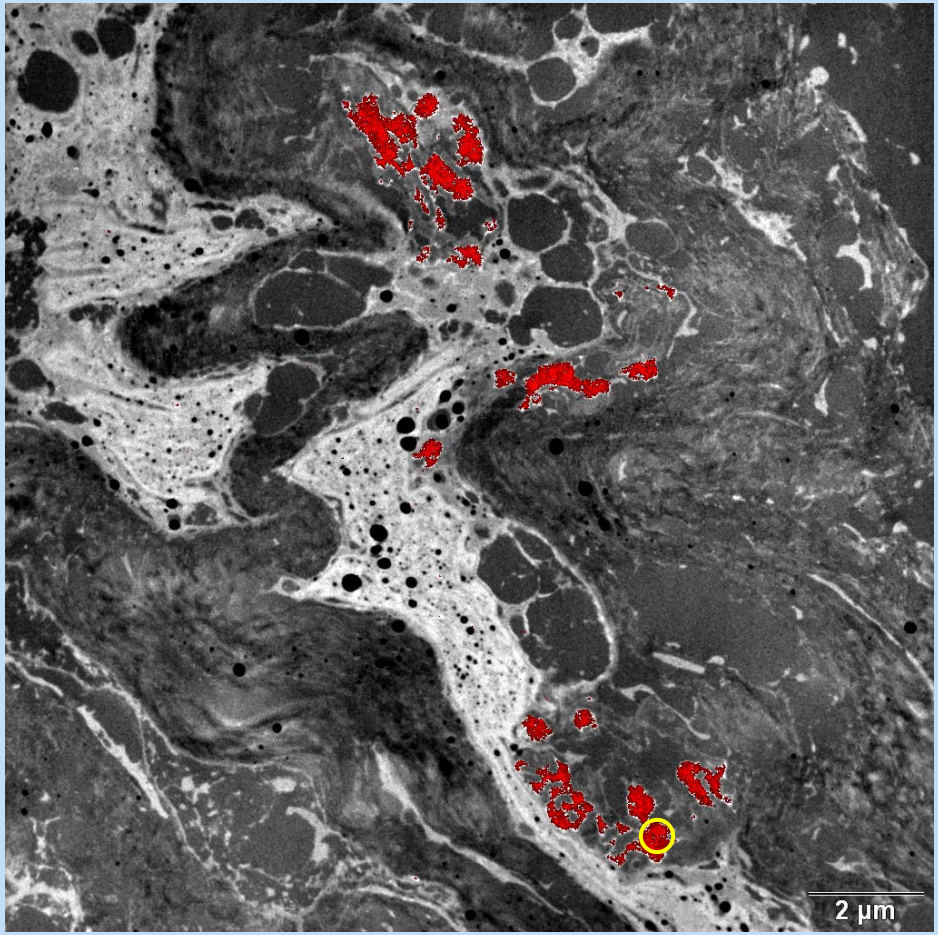
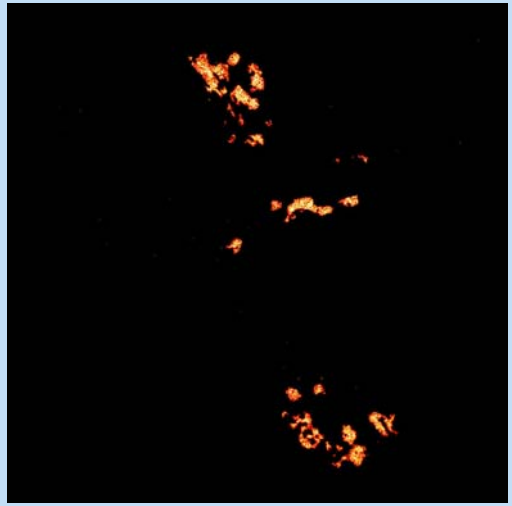
Solution:





Above: ESI Gd $M_{4,5}$ concentration profile (3-window power law method)

Left: Ultrastructural image (HCI)

Gd distribution overlaid to HCI

Selected spectrum area (see next page)

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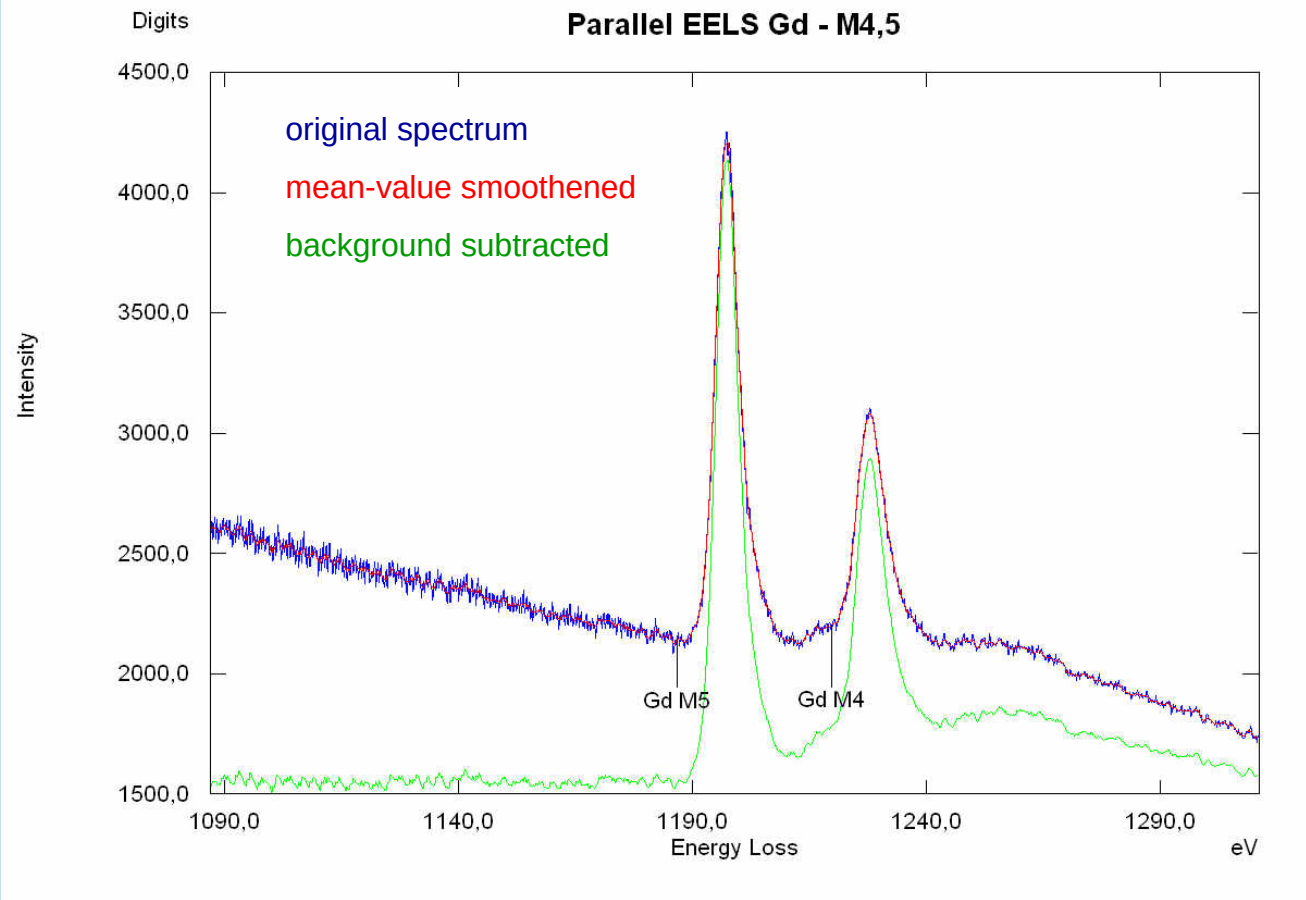
Solution:



Parallel EELS of the area marked in the previous image (to confirm presence of Gd)

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Conclusion:	▪ The implication of Gadolinium in triggering the breakout of Nephrogenic Systemic Fibrosis (NSF) could be shown ▪ In contrast to common opinions, that MRT with Gd as paramagnetic ligand in linear or macrocyclic chelates is relatively save, it is now evident that there is a high risk for renal dialysis patients to develop NSF after treatments with such contrast agents ▪ For the actual case history, clinical responsibilities could be identified, being of utmost importance to clarify insurance compensations. However, an adequate treatment is not available. ▪ Consequently, contraindication of Gd chelates as contrast agents to improve MRT signal and resolution has to be heeded for renal dialysis patients.
System:	LIBRA® 120 with in-column Omega spectrometer 2k bottom-mount SSCCD with YAG scintillator (TRS) iTEM image analysis system (OSIS) with EFTEM plugin
Parameter settings:	120kV, LaB6 filament (6-8 µA emission current), 1575-5000x mag, 15 mrad (90µm) objective aperture, 0.04-2.0 mrad illumination angle operation modes: HCI (250eV, 20eV slit), ESI (20eV slit), Parallel EELS (100x)

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Benefits:	Investigation needs an energy filtering system, elemental analysis by EDS is not sufficient in resolution, and too time consuming (mapping). Imaging of unstained samples only possible with HCI mode. Gatan post-column filter (GIF) not favourable, because: - Too expensive (400,000 US$ for 1k camera version, and more) - Needs regular alignment of at least 6 hexapoles and 6 quadrupoles (in-column Omega filter of LIBRA 120 is factory aligned) - GIF has a post-magnification factor of at least 20x (tubus!), relatively low magnifications as required here are not possible (magnification of Omega filter is 1x)
Summary:	Incorporation of Gd into skin of a renal dialysis patient, following gadopentetate treatment to enhance MRT sensitivity and resolution, could be shown. The Gd conglomerates are very small (a few nm to less than 2 µm). Therefore, energy filtering TEM in ESI mode is the only method to demonstrate the fine structural distribution of Gd and to directly correlate it with the ultrastructure of the skin tissue. EDS as an alternative method is not sufficient in spatial resolution, and very time consuming. Post-column energy filters are expensive, need regular alignment skills, and do not allow to record images at magnifications as low as required here (due to their high tubus magnification).

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Special Comments:

Here are some comments of a referee of the original paper, which was submitted for publication in the "Clinical Journal of the American Society of Nephrology":

This paper is technically excellent. It…emphasizes very important points that I have been repeatedly saying are true, but I have been drowned out to some degree by others using very crude electron microscopy that is being passed off as possessing more sensitivity and being more telling than it actually is…

These fine authors make several points I have been repeatedly making:

1. These conglomerations are very very very small and people claiming that they are 1-3 microns (Abraham et al.) are misled because these are the only particles they can "see" with crude ESEM technology employed. Note how small the lesions actually are using TEM in the fashion of these authors !!!!!
2. These authors note…, that there is a lot of iron around. Too much lip service has been made to calcium and phosphorus and not enough attention has been paid to iron since my original work.
3. They also note…, that the material is inside of cells and also rarely in the interstitial areas between collagen, just as I had observed and predicted.

This paper serves as an important counterbalance to a lot of lesser science that has been hastily published since my last work in the area.

I commend the authors for this work. It is needed.

We make it visible.