MODELLING OF FOCUSED ION BEAM INDUCED INCREASES IN SAMPLE TEMPERATURE: A CASE STUDY OF HEAT DAMAGE IN BIOLOGICAL SAMPLES

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SUMMARY

Ion beam induced heat damage in soft materials and biological samples is not yet well understood in Focused Ion Beam systems (FIBs). The work presented here discusses the physics behind the ion beam – sample interactions and the effects which lead to increases in sample temperature and potential heat damage. A model by which heat damage can be estimated and which allows parameters to be determined that reduce/prevent heat damage was derived from Fourier's law of heat transfer and compared to finite element simulations, numerical modelling results and experiments. The results suggests that ion beam induced heat damage can be prevented/minimized by reducing the ion beam current (local dose rate), decreasing the beam overlap (reduced local ion dose) and by introducing a blur (increased surface cross-section area, reduced local dose) while sputtering, patterning or imaging soft material and non-resin-embedded biological samples using FIBs.

INTRODUCTION

FIB/SEMs, which combine a Scanning Electron Microscope (SEM) and a Focused Ion Beam (FIB) in a single device, are now an established and common tool for failure- and micro-analysis, nano structuring, region specific cross-sectioning and *in-situ* TEM lamella preparation (Drobne *et al.*, 2007). Today, these instruments are predominantly used in material science and the semiconducting industry (Drobne *et al.*, 2007). Throughout the past decade, FIB/SEMs have sparked great interest in the life sciences (Drobne *et al.*, 2007; Leser *et al.*, 2010; Earl *et al.* 2010; Milani *et al.*, 2007; Villinger *et al.*, 2012; Schneider *et al.*, 2011; Stokes *et al.*, 2005) due to the unique capability of this technique in revealing subsurface structures at high resolution and to prepare TEM-lamellas (sections) at precisely selected points within the sample that cannot be achieved by other techniques.

Heat damage, which easily occurs for soft materials and biological samples, is increasingly being reported (Drobne et al., 2007; Schneider et al., 2011; Bassim et al., 2011; Diez-Escudero *et al.*, 2007). A commonly presented solution that allows work to be performed at room temperature is embedding the biological sample in resin (Villinger et al., 2012; Schneider *et al.*, 2011), but this can induce undesirable artefacts. Working on non-embedded biological samples is, therefore, advantageous and several studies have explored this approach and have offered sets of parameters which achieved acceptable results for the specific sample (Drobne *et al.*, 2007; Bassim et al., 2011; Diez-Escudero et al., 2007). However, a basic understanding on how the ion beam – sample interaction in FIBs leads to heat damage, and how the choice of parameters can help to overcome this issue has not yet been fully discussed in literature. A recent study discussed ion beam induced heat by looking at the ion beam power (Volkert et al., 2007). However, the effect of ion beam parameters on the heat damage was not considered. A further study offers an equation to estimate the local heating which is induced by the ion beam. The physical principles behind the equation were not discussed and the causes of heat, which were outlined in the paper, were not included directly in the equation (Bassim et al., 2011).

To gain a better understanding of the ion beam induced heat damage, the choice of parameters that can limit heat induced artefacts, due to the focused ion beam – sample interactions, need to be considered. The broader results are true for any type of instrument which includes a FIB such as gallium FIBs, plasma FIBs, HIM-FIB as well as LoTIS and MOTIS FIBs, despite the focus on gallium ion-solid interactions discussed in this work. This work, however, looks at the basic FIB operating principles in FIB/SEMs and the physics behind the ion-solid interactions with a special emphasis on those ion-solid interactions which induce heat.

The interactions of gallium ions with copper, silicon and skin were studied using the Monte

Carlo simulation program SRIM (Ziegler et al., 2015). The program COMSOL (finite element analysis platform) was used to evaluate the heat transfer of a single ion into skin. A numerical analysis using the forward-time central-space method to solve the 3D heat equation was additionally used to understand the effect of heat accumulation due to multiple gallium ion impacts in collagen and the effect of different ion beam parameters. A model, based on Fourier's law was adapted for FIBs to estimate the ion beam induced changes in sample temperature in dependence of various FIB parameters. The theoretical results for the well-studied materials copper and silicon were then compared to literature. The results obtained for this new model were then applied for skin, a soft biological material, compared to the finite element simulation as well as numerical modelling results and are experimentally tested on purified collagen. Purified collagen was selected as the test material as it is the principle component of skin and other body tissues. Also, it is readily purified and prepared as reproducible samples adapted to the present experiment protocols. As a material, purified collagen is frequently used in a variety of biomedical applications, including for skin repair, where reconstituted purified collagen materials are normally stabilized to enhance their durability (Ramshaw, 2016; Gorell *et al.*, 2015). Collagen also forms a fibrillar structure that is visible by microscopy, but is denatured by heat to give gelatine that lacks any fixed structure (Zeugolis et al., 2008; Privalov *et al.*, 1989).

Focused ion beam principles

During the patterning process in FIBs a focused ion beam is scanned across the sample surface. The ion beam remains in a single scan point for a specified time (dwell time) before it moves on to the next scan point. To achieve a smooth cut, the beam conventionally overlaps between two scan points to create a smooth pattern edge (50% default for the FEI

Quanta 200 3D). The overlap is determined by the beam diameter overlap between two scan points. The process is illustrated in Figure 1. This process is repeated a specific number of times (passes) until the desired pattern size (global dose) is achieved.

Focused ion beam description

The focused ion beam can best be described by using the following two concepts:

$$1. \text{ Dose: } \frac{N}{A} = \frac{\frac{I [A] \times t [s]}{q [C]}}{A [\text{cm}^2]} \quad [\text{Eq 1}]$$

The dose $\left[\frac{\text{ions}}{\text{cm}^2}\right]$ is here defined as the number of ions N which enter through a specified surface A area (scanned area) into the sample. This is occasionally referred to as areal dose or dose per area in literature. The number of ions can be calculated by multiplying the ion beam current $I [A]$ with the time $t [s]$ divided by the ion charge $q [C]$. In the case of a single scan point, the time is equivalent to the dwell time t_{dwell} and the area A which is the larger of either the focused/defocused beam or the width of the sub-surface sample interaction. The width of the sub-surface sample interaction can be determined using SRIM simulations, the area underneath the beam can be calculated from the ion beam radius via $A = \pi * r_{\text{beam}}^2$. This is illustrated in Figure 1. This local dose can be set by either the choice of aperture or condenser lens strength, the exposure time or the size of the scan/patterning area.

A specific global dose is required to pattern or sputter a specific volume (or to image a specific area). This global dose only depends on the total pattern size. The total patterning time increases when using lower ion beam currents.

$$2. \text{ Dose rate: } = \frac{\frac{I [A]}{q [C]}}{A [\text{cm}^2]} \quad [\text{Eq 2}]$$

The dose rate is the number of ions entering the sample through a specific area per during a given time. This parameter is set by the choice of aperture/beam current and the scanned area A . The local dose rate corresponds to the number of ions which enter the sample per scan point per unit of time. The global dose rate corresponds to the number of ions entering the sample through the pattern area per unit of time.

Ion-solid interactions

Each incident ion interacts with the sample atoms and loses its energy in a multitude of collisions with sample atoms until it comes to rest in or exits the sample. The projected range for the ions is the distance underneath the sample surface which is perpendicular to the incident ion beam where the majority of ions come to rest in the sample. The ion-solid interactions are statistical in nature (Giannuzzi et al., 2010): for 3 nA and 1 μ s dwell time, in average approximately (18700 ± 140) ions interact with the sample. This needs to be considered when evaluating and describing ion beam parameters. For ease of illustration, a single ion impact is illustrated in Figure 2 to describe the ion-solid interactions that can occur. Most common is an inelastic collision with sample electrons or an elastic collision with sample atom nuclei (Giannuzzi et al., 2010), as shown in the inset of Figure 2. Elastic collisions with electrons and inelastic collisions with nuclei are not common in the FIB energy range and are therefore not considered here. The elastic collisions with the sample atom nucleus can lead to sputtering, sample atom displacements including the creation of vacancies and interstitials, collision cascades or it can produce replacement collisions, backscattered ions as well as phonons. The inelastic collisions with the sample atom electron shell can lead to the emission of secondary electrons, to polymerization (Milani et al., 2007) or photon excitation. Heat is generated in these interaction types: electronic excitation and

relaxation, emission and recapturing of electrons and photons as well as direct energy losses to phonons in nuclear collisions. Phonons are lattice atom oscillations which are induced in multiple processes during the ion-solid interactions. Phonons are created when insufficient energy is transferred to the sample atom to dislocate it from its lattice position. In addition, the lattice binding energy for every created vacancy is deposited into phonons (Ziegler et al., 2015). Only a small fraction of the phonons are created by the primary ions directly, the majority of phonons are induced by the recoiling sample atoms. The interactions which induce heat are illustrated in the inset of Figure 2.

EXPERIMENTAL SETUP

Collagen sample preparation

Preparation of pepsin soluble, porcine collagen (UAEC no.1700000190) followed standard methods (Miller and Rhodes, 1982). Briefly, porcine skin from an abattoir was solvent degreased, and then rehydrated in water and minced (4 mm). This material was suspended at 5 g per liter in 100 millimolar acetic acid, adjusted to pH 2.4 by HCl and containing 1 mg/ml pepsin (Sigma). After 48 h digestion at 4°C, coarse solids (> 1 mm) were removed by a sieve and fine material then removed by centrifugation, 6000 x g for 45 min. NaCl solution (4.4 M) was then added to the supernatant slowly with stirring to take it to 0.7 M NaCl. After 24 h at 4°C, the resulting precipitate of type I and III collagens was collected by centrifugation, as above. Collagen fractionation also followed a standard procedure (Trelstad et al., 1976). Precipitated collagens were redissolved in 50 mM acetic acid containing 0.2 M NaCl and then adjusted to pH 7.2 with 1 M Tris (tris(hydroxymethyl)aminomethane) solution. The NaCl was then increased to 1.7 M NaCl and the precipitate stirred for 18 h at 4°C. The precipitate, which contains type III collagen and partially solubilized oligomers, was separated by centrifugation, as above. The supernatant was taken to 2.4 M NaCl and stood,

with intermittent stirring, for 18 h. The precipitate, which is type I collagen was also collected by centrifugation, and was then dialyzed exhaustively against 20 mM acetic acid and then freeze dried.

For collagen sponge formation, a 5 mg/ml collagen solution in 20 mM acetic acid was made. In order to stabilize the sponges, immediately prior to use, the collagen solution was adjusted to pH 7.2 with 2 M NaOH, and 10% w/v glutaraldehyde immediately added to a final concentration of 0.5% w/v followed by thorough mixing. Within 2 min of these additions, 0.4 ml aliquots of this solution were dispensed into well of a 24-well tissue culture plate. After a further 15 min these were frozen at -20°C and subsequently freeze dried.

Stopping and Range of Ions in Matter (SRIM) simulations of 30 keV gallium ions (Ga^+) in copper, silicon and skin

The program SRIM (Monte Carlo simulation) was used to determine the heat flux that 30 keV gallium ions (Ga^+) induce in copper, silicon and human skin. The heat flux was later used as input parameter for the COMSOL simulations and the numerical modelling of the heat equation. Skin was used to estimate the ion-solid interactions for collagen, as literature values for skin are readily available (Kreith, 2000) and collagen is an essential part of skin. 50000 ions were simulated for each material. The methods “surface sputtering/monolayer collision steps” was selected as the calculation type. All basic plots were selected for the simulation and were used to evaluate the volume in which the ion-solid interactions occur and to confirm that all ions trajectories in the simulation were completely followed. The calculation parameter output was evaluated to determine the energy losses of each ion for the different interactions. The exact contribution to heat in the inelastic losses (ionization) cannot be determined from the program directly. The ionization value is the combined value for heat and energy required for secondary electron creation. The following values were used to

describe collagen: specific heat $c_p = 3540 \left[\frac{\text{J}}{\text{kg} \cdot \text{K}} \right]$; density $\rho = 1079 \left[\frac{\text{kg}}{\text{m}^3} \right]$; thermal conductivity $k = 0.29 \left[\frac{\text{W}}{\text{m} \cdot \text{K}} \right]$; thermal diffusivity $\alpha = 1.36 \text{ E-}7 \left[\frac{\text{m}^2}{\text{s}} \right]$.

COMSOL simulations for 30 keV gallium ions (Ga^+) in collagen

The program COMSOL (finite element simulation) was used to simulate the interaction of a single and then ten 30 keV gallium ion (Ga^+) impacts with collagen using the time dependent differential equation of heat conduction for a stationary, homogeneous, isotropic solid

$$\rho \cdot c_p \frac{\partial T(\vec{r}, t)}{\partial t} = \nabla \cdot [k \nabla T(\vec{r}, t)] + g(\vec{r}, t) \quad [\text{Eq 2a}]$$

As COMSOL, does not yet support the inclusion of ion cascade induced sample heating, two heat source simulations were performed. The first assumed a single ion energy deposition, $0.9 \cdot E_{\text{ga}}$ into a volume of $\pi \cdot r_{\text{phonon}}^2 \cdot z_{\text{sample_range}}$ in a time, t_{range} with units of W/cm^3 , for a total of $3.1 \text{ W}/\text{cm}^3$ per ion. The second simulation included the heat contribution of the electronic stopping power, S_e of ten ions and although we show pictorially the ion trajectory paths modified by a correct nuclear scattering term, S_n , the heat contribution of the resulting collision cascade induced heat was not passed back into the thermal model.

3D numerical solving of the heat equation by the forward-time central-space method

A numerical modelling approach was chosen to solve the time dependent differential heat equation [Eq 2a]. A forward-time central-space method as a finite-difference method has been implemented for three dimensions in Python to simulate the heat accumulation of multiple impacts on a time-scale of several nanoseconds, similar to (Isaacson and Keller 2012; Langtangen 2003). A sample volume of $600 \text{ nm} \times 600 \text{ nm} \times 400 \text{ nm}$ (depth) has been simulated with a voxel size of $\Delta x = 5 \text{ nm}$. According to a von Neumann stability analysis (Crank et al., 1947, Charney et al., 1950) the differential time step has been calculated to $\Delta t \leq \Delta x^2 / (8\alpha) = 23 \text{ ps}$. The boundary voxels has been fixed to room temperature with exception of

the sample surface, since no significant heat dissipation into the vacuum is expected. Ion impacts are generated in equal time intervals specified by the average time between two ion impacts. The ions are randomly placed with a uniform spatial distribution on a circular beam profile (specified as the diameter that was used in the experiments). A heat input of 0.9×30 keV for each ion was used which corresponds to the total heat loss of the primary ion. The ion track was simulated by a 10 nm x 10 nm x 20 nm cuboid and the temperature raise was calculated according to the volume, density and specific heat of collagen. The simulation results have been stored with mK precision in timesteps of 0.1 ns and have been post processed with ImageJ (Schindelin et al., 2012).

FIB cross-sectioning: 30 keV gallium ions (Ga^+) in collagen

The FEI Quanta 200 3D at the Queensland University of Technology, Brisbane, Australia was used for cross-sectioning experiments. Multiple cross-sections ($15 \mu\text{m} \times 10 \mu\text{m} \times 200 \text{ nm}$) were cut into the non-resin embedded collagen sample. The first cross-section was cut using 30 kV acceleration voltage with 3 nA, 50% overlap and 81 nm beam diameter. A second cross-section was cut with heat reducing parameters of 30 kV acceleration voltage with 50 pA, 20% overlap and 200 nm blur. The blur was achieved by overfocusing the beam. Several additional cross-sections were cut to determine the effect of the different ion beam parameters independently. The effect of the beam current was evaluated by comparing cross-sections that were cut using 3 nA and 50% overlap; 0.1 nA with 50% overlap with 23 nm beam diameter as well as 50 pA 20% overlap and 50 nm blur. The 20% overlap and 50 nm blur were required to allow patterning: an excessive amount of points would have resulted otherwise which could not be computed by the Quanta 200 3D. The blur was chosen to be smaller than the width of the interaction volume, so that the latter remained the larger parameter. The effect of a blur/overlap combination was evaluated by cutting cross-sections

using 3 nA, 0.1 nA as well as 50 pA with 20% overlap and 200 nm blur. Two additional larger cross-sections ($30\ \mu\text{m} \times 20\ \mu\text{m} \times 200\ \text{nm}$) were cut using 50 pA, 20% overlap and 200 nm blur to evaluate the effect on an increased global dose on heat damage.

SEM images of the prepared cross-sections were recorded with 5 kV acceleration voltage, 90 pA beam current, 1 μs dwell time, 128 frames integration filter and 1024*882 pixel resolution with an Everhart-Thornley detector.

FIB TEM-lamella preparation and TEM analysis

To verify that the collagen was not heat damaged in the FIB process, a TEM lamella was prepared using the Quanta 200 3D and compared to a conventionally prepared TEM resin section using an ultra-microtome. For the FIB prepared TEM lamella, a protective platinum layer was deposited first onto the desired area. The cross-sections were cut using 30 kV acceleration voltage with 50 pA ion beam current, 20% beam overlap and 200 nm beam blur. The lifted-out lamella was pre-thinned at 50.5° (front side) and 53.5° (backside) using 30 kV, 30 pA and 20% beam overlap. The final thinning was performed using 30 kV, 10 pA and 20% beam overlap at 50.5° (front side) and 53.5° (backside). The lamella was afterwards polished at 58° (front side) and 46° (backside) using 5 kV acceleration voltage and 10 pA ion beam current. 2 kV polishing is not available for the Quanta 200 3D.

The FIB prepared TEM lamella and the conventionally prepared ultra-microtome TEM section were analyzed using a Jeol 1400 TEM at 100 kV to allow comparison of results. In addition, the FIB prepared TEM lamella was analyzed using a Jeol 2100 TEM at 200 kV.

Conventional ultra-microtome TEM- section preparation

Collagen samples were additionally fixed in 2.5% w/v glutaraldehyde at 4°C for 1 h, postfixed in osmium tetroxide, dehydrated through a series of graded ethanol dilutions,

treated with acetonitrile, and finally infiltrated with a Quetol-based resin. Embedded samples were polymerized at 60°C for 24 h, sectioned (80 nm) using Leica EM UC7 ultra-microtome and collected on bare 300 mesh copper TEM grids followed by post-stained with uranyl acetate and lead citrate.

The prepared TEM lamella was analyzed using a Jeol 1400 at 100 kV. Ultra-microtomed samples could not be imaged using the Jeol 2100 due to the sample damage induced by the higher energy electrons.

RESULTS AND DISCUSSION

SRIM simulations

SRIM simulations were carried out to evaluate the difference in gallium ion interactions for different materials. The 30 keV gallium ion trajectories for the different materials are displayed in Figure 3. The difference in projected range for metals such as silicon (Figure 3a) or copper (Figure 3b) and soft materials such as skin (Figure 3c) can easily be seen in the ion trajectories plot. This distance is highlighted in the ion trajectories plot in Figure 3. The projected range is a statistical value: some ions penetrate deeper into the material, as evident in the trajectories plots. The created cascades (xy longitudinal) extend farther than the ion trajectories for samples with lighter elements such as skin, (see insets of Figure 3). The simulated energy losses, given in Table 1, show that the ions lose the majority of their energy (> 90%) to phonons and ionization and therefore heat. Phonons are the primary cause of the heat damage. This result is in good agreement with literature (Volkert et al., 2007; Bassim et al., 2011). The heat due to the inelastic interactions (ionization), however contributes a significant amount. The majority of phonons and ionization are not induced by the ions directly but by the recoiling sample atoms in the collision cascade. The phonon and ionization distributions maximum for the different materials are given in cascade plots in the

inset of Figure 3. The simulations were additionally used to determine the heat flux into skin for various ion beam settings (refer to Table 2).

COMSOL simulations

The heat, induced by a single gallium ion in collagen, was studied first using two COMSOL simulations. The first COMSOL simulation, shown in Figure 4a shows the temperature cross section profile produced by a line heat source of $3.1 \times 10^{15} \text{ W/cm}^3$, equivalent to a single ion track volume represented by a 10 nm by 100 nm cylinder for the time it takes the gallium ion to stop in the sample, about 300 fs. The 10 nm diameter corresponds to the phonon heating FWHM (Figure 4a). Next, as an approximation to the actual ion cascade situation, the scattering of ten incident 30 keV gallium ions spaced 60 picoseconds (i.e. a 1.6 nA beam) apart, we modelled the temporal implication of these ten consecutive incident ions. Here, ion cascade heating was omitted, but the ions' electronics stopping power term heating was included. From line source heating, the increase in temperature from a single ion impact was determined to be $\Delta T_{\text{single ion}} \sim 250 \text{ K}$. The cool down time constant τ of about 1 ns was derived from the second simulation (Figure 4c) which is orders of magnitude longer than the heating time. The line heat source simulation suggests that the sample is irreversibly damaged within an envelope of about a Debye phonon's range (5 nm) around each ion track. The area around each ion track will be sputtered away during the milling process and the irreversible heat damage from the individual ion impact can be neglected if heat damage does not extend into the surrounding tissue. The simulation shows that the heat damage only extends 5 nm into the material. The heat from single ion impacts does not affect the material for a TEM lamella or cross-section.

To understand the effect of multiple ion impacts in the same area, COMSOL was used to evaluate the changes in sample temperature for 10 consecutive ion impacts within 600 ps,

which corresponds to a point irradiation with 1.6 nA ion beam current. The resulting change in sample temperature, (a ΔT per ion ~ 80 K) is plotted in Figure 4c and is considerably lower than what one would expect realistically had the ion cascade heating been included. Note that the impact of a second ion occurs before the sample had time to cool down. The trajectories and temperature isosurfaces are shown in Figure 4b. The plot in Figure 4c shows an accumulative temperature increase from the sequence of ion impacts raising the bulk temperature of the material and inducing thermal damage in an extended area surrounding the ion tracks. This would result in heat damage within TEM lamella's or cross-sections. The difference of an initial impact maximum temperature in the first 'line heat source' COMSOL simulation ($\Delta T_{\text{single_ion}} = 250$ K) and that of the second ion collision cascade simulation ($\Delta T_{\text{single_ion}} = 80$ K) is the result of COMSOL's present inability to transfer heat from the ion collision cascade to COMSOL's multiphysics thermal modeler.

3D numerical heat transfer approach

A 3D numerical heat transfer approach was used for more complete analysis of heat transfer and to better understand the effect of multiple ion impacts. The specific heat and a time dependent heat equation was used to fully describe and anticipate the effects of global and local heat damage for different ion beam settings. Figure 5 shows the surface and a cross section through the middle of the ion beam spot for a simulated sample volume of $600 \text{ nm} \times 600 \text{ nm} \times 400 \text{ nm}$ at different irradiation times. The simulations show that the samples heat up and heat damage is expected for 3 nA (Figure 5 case A and D) where temperatures in excess of 400 K are reached. For this setting, subsequent ion impacts occur faster than the sample can cool down, leading to bulk heating. The cross-section at 1000 ns (Figure 5 case A and D) shows that the heated area extends several hundred nanometers beyond the ion impact area, suggesting that heat damage extends into the surrounding tissue. For 100 pA, 23 nm

beam size (Figure 5 case B), a temperature raise of ~ 50 K can be observed around the irradiated area. The simulations for the case C and E in Figure 5 show a slight temperature increase of 10 to 20 Kelvin, but do not allow a clear statement about the heat damage. For the 50 pA and 200 nm blur ion beam setting (Figure 5 case F), no significant temperature raise (below 5 Kelvin) beyond the ion tracks can be observed. The lower ion dose rate of this setting allows a sufficient temporal and spatial separation of subsequent ion events and bulk heating should not occur at all. The results suggesting that this setting is suitable for biological samples. The consequences of the ion beam overlap and different dwell time settings will be studied in future.

Proposed model to estimate changes in sample temperature

The simulation results suggest that the sample heating process can be described by a simple 1D approach. The irradiation volume is mainly a flat disc with the diameter of the beam and a thickness of the penetration depth of the beam. Since the beam diameter of the disc is much larger than the penetration depth of the gallium ion beam, the heat dissipation to the side of the irradiation volume can be neglected. Most of the heat will be dissipated downwards into deeper layers of the sample.

To estimate the ion beam induced heat and associated changes in sample temperature for a single scanpoint, the conduction of heat transfer model can be applied to the ion beam sample interactions. Conduction heat transfer can be described via Fourier's law $\bar{q} = -k\nabla T$ with $\bar{q}$ as the local heat flux density [$\frac{W}{m^2}$], k as the thermal conductivity [$\frac{W}{mK}$], and ∇T as the temperature gradient [$\frac{K}{m}$]. The heat transfer for a homogeneous material with constant temperature at the endpoints in 1D for an equilibrium case can be described via:

$$\frac{\Delta Q}{\Delta t} = -k \times A \frac{\Delta T}{\Delta x} \text{ [Eq 3]}$$

with $\frac{\Delta Q}{\Delta t}$ as the heat flux, k as the thermal conductivity, A as the cross-sectional surface area, Δx as the distance and ΔT as the temperature gradient between the end points.

To estimate the ion beam induced increase in temperature in a single scan point, equation 3 must be adapted to take the ion beam – sample interactions into account. First, the number of ions N which interact with the sample per scanpoint need to be determined from the ion beam current I and the dwell time $t = t_{\text{dwell}}$:

$$N = \frac{I [A] \times t [s]}{q [C]} \quad [\text{Eq 4}]$$

The positive elementary charge 1.602×10^{-19} C can be used to describe the charge q under the assumption of single charged gallium ions. Each ion loses a specific amount of energy to phonons ΔE_{phonon} and in inelastic collisions ΔE_{el} , both of which induce heat. The heat flux can therefore be described by the total energy losses to ionization and phonons (obtained from the SRIM simulations) per dwell time t . The cross-sectional surface area A in equation 3 corresponds to the larger of either the focused/defocused beam or the width of the sub-surface sample interaction. The width of the interaction volume can be determined using SRIM. The area underneath the beam is instrument specific. It can be calculated via $A = \pi \times r_{\text{beam}}^2$, with r_{beam} as half the ion beam diameter (device dependent). The probe is here presumed to be circular with a uniform distribution. The distance Δx in equation 3 corresponds to the ion projected range into the sample. Changes in sample temperature due to the ion beam sample interaction can now be estimated via

$$\Delta T [K] = \frac{(\Delta E_{\text{el}} [J] + \Delta E_{\text{phonon}} [J]) * N}{t [s] * A [m^2]} * \frac{R_{\text{proj}} [m]}{k \left[\frac{W}{mK} \right]} \quad [\text{Eq 5}].$$

The derived equation suggests that the following parameters directly allow the ion beam induced temperature increases per scan point to be minimized:

1. reducing the local dose rate (set by aperture/ion beam current) $\frac{N}{A \times t}$
2. reducing the local dose (set by aperture or beam overlap or beam blur) $\frac{N}{A}$
3. increasing the area (introducing a beam blur) A
4. advanced scanning strategies (multiple frames)

These suggestions are in good agreement with experimental findings in literature (Bassim et al., 2011)

For conventionally used ion beam parameters such as 3 nA and 50% overlap, the increases in temperature after the irradiation of a single scanpoint for Si which has a thermal conductivity of $148 \left[\frac{W}{mK} \right]$ (Volkert et al., 2007) and copper which has a thermal conductivity of $400 \left[\frac{W}{mK} \right]$ (Kreith et al., 2000) are negligible with $\Delta T_{Si} = 3$ K and $\Delta T_{Cu} = 0.4$ K according to equation 5. This is in good agreement with literature (Volkert et al., 2007).

For soft materials or biological samples such as skin, which exhibit a low thermal conductivity of $0.29 \left[\frac{W}{mK} \right]$ (Kreith et al., 2000) the same ion beam parameters lead to excessive increases in temperature per single scanpoint. According to the proposed model, the increase in temperature, calculated using equation 5, is $\Delta T_{Skin} = 2544$ K which is far above the melting point of organic material. Heavy heat damage is expected which is in good agreement with previous literature reports (Diez-Escudero et al., 2007).

A heat reduced approach using 30 keV gallium ions with a reduced ion beam current of 50 pA, 20% overlap and 200 nm blur (as suggested by the model), is expected to lead to a locally increase in temperature of $\Delta T_{Skin} = 5.7$ K. The model predicts that heat damage will not occur for the heat reduced set of parameters.

Comparison of simulations and experimental data

To verify the obtained results from the simulations and the proposed model, cross-sections were cut into collagen using the conventional set of parameters (3 nA, 50% overlap) as well as the suggested heat reduced parameters 50 pA, 20% overlap and 200 nm beam blur. The results are shown in the SEM images in Figure 6. The unnaturally smooth and curled up surface, visible in Figure 6a, indicates that heat damage occurs when using higher ion beam current (larger local dose rates) for collagen. This result agrees well with the COMSOL simulations and the numerical modelling results. Both approaches suggest that the high frequency of ion impacts does not allow sufficient time for the sample to cool down between subsequent ion impacts for this setting, leading to bulk heating and extended heat damage. The heat damage is not visible when using a reduced ion beam current, reduced beam overlap and applied beam blur as suggested by the simulations, modelling and the proposed model (Figure 6f). The results match the predicted outcomes.

To better understand the effect and magnitude of the individual ion beam parameters on heat damage, cross-sections were prepared with different independently set parameters. The resulting changes in temperature were calculated using the SRIM simulations and the proposed model.

The effect of the ion beam current (local dose rate) on heat damage was evaluated by cutting cross-sections using the same conventional beam overlap of 50% and different currents ranging from the nA to the pA range. A similar approach was chosen by (Kizilyaprak et al., 2015) to understand the effect of ion beam parameters on resin embedded biological samples and to determine stable resins in the ion beam. The SEM micrographs in Figure 6 (a) - (c) of the cross-sections cut with 3 nA, 0.1 nA and 50 pA all show heat damage to the sample. The extent of the heat damage increases with increasing ion beam current. For 3 nA, the heat damage is so extensive that the collagen curls upwards within the cross-section area. The inset shows that the heat damage extends into the surrounding areas of the cross-section. A 30-times lower ion beam current (0.1 nA) leads to a reduction in heat damage as

evident in Figure 6(b). The reduced dose rate, when using 0.1 nA (Figure 6 (b)) still creates unnaturally smooth areas and holes which are typical for heat damage, however, the collagen has not curled up and the extent of the heat damage could be slightly reduced. The calculated change in locally increased sample temperature was calculated to $\Delta T_{\text{skin}} = 1233 \text{ K}$. Despite reducing the beam current by 30 times, the increase in local sample temperature could only be dropped by half according to the calculation. This result can be understood when considering that setting smaller ion beam currents lead to reduced final beam diameters. As a result of the decreased final beam diameter (area underneath the beam), the resulting local ion dose per scanpoint is reduced by a factor of 2 from $3.6 \times 10^{14} \frac{\text{Ions}}{\text{cm}^2}$ for 3 nA (50% overlap) to $1.5 \times 10^{14} \frac{\text{Ions}}{\text{cm}^2}$ for a 30 times smaller ion beam current of 0.1 nA (50% overlap). Fourier's law 1D likely overestimates the temperature in this case since beam diameter is not much larger than ion range.

Heat damage is still visible in the SEM micrograph showing a cross-section that was cut with a low ion beam current of 50 pA (Figure 6 (c)). The heat damage has been significantly reduced, as evident by the smaller areas of molten collagen, however, the experiment shows that reducing the ion beam current (local dose rate) is not sufficient to avoid heat damage for collagen. The calculated increase in local temperature is $\Delta T_{\text{skin}} = 91 \text{ K}$. The results suggest that heat damage cannot be avoided by reducing the ion beam current alone but the beam overlap and blur combination (see cross-section in Figure 6(f)) plays a significant role. Furthermore, a comparison of the 50 pA cut cross-sections indicates that an estimation of the beam power and resulting heat damage, using the milling time and the beam voltage is not able to figure out the ion beam induced heat damage. Additional ion beam parameters, like the overlap and blur play a significant role and need to be considered.

To better understand the effect and impact of the blur/overlap combination, cross-sections were cut using 20% overlap and 200 nm blur for different ion beam currents (3 nA, 0.1 nA, 50 pA) and the results compared to the previous experiment. SEM micrographs of the cross-section are displayed in

Figure 6 (d) - (f). The corresponding numerical modelling results are displayed in Figure 5 case D -F.

Heat damage could be avoided when using 50 pA in combination with 200 nm blur (Figure 6 (f)) indicating that setting a blur, which increases the area underneath the beam plays a crucial role in preventing heat damage once the ion beam diameter, is larger than the width of the interaction volume which is in good agreement with the simulations and modelling. For 0.1 nA, and 200 nm blur (Figure 6 (e)), some heat damage occurs. The sample areas in the bottom left edge of the cross-section retained some of the native sample structure while thinner areas show heat damage, indicating that the characteristic of the sample plays into heat damage as well. The observed can be explained since thinner samples could not cool down into deeper layers and heat can only be transported to the surrounding material at the sides. The amount of heat flux that can be transported away is reduced and could explain a higher local temperature increase. The overlap/blur combination (heat reduced approach), however, leads to a significant reduction in heat damage for 0.1 nA, as evident when comparing the size of molten collagen areas for the conventional approach in Figure 6 (b) and the heat reduced approach (e). This result is in good agreement with the numerical modelling results (see Figure 5 case B and E. The 20% overlap and 200 nm blur combination also significantly reduces the induced heat for 3 nA, up to the point where rupturing and curling of the collagen can be prevented. However, heat damage could not be prevented. This in good agreement with the results of the COMSOL simulations and the numerical modelling results which both suggest that the high frequency of ion impacts leads to bulk heating. The calculated increase in temperature is $\Delta T_{\text{skin}} = 342 \text{ K}$. The effect of the beam blur/overlap combination is stronger than the effect of a reduced beam current as evident from the calculations and SEM micrographs in Figure 6 (a, b, d).

Heat damage could not be prevented but significantly reduced using the blur/overlap combination for 0.1 nA and 3 nA while heat damage could be avoided when reducing the overlap and blurring the beam for 50 pA. These measurements indicate that the area underneath the ion beam, and therefore the resulting local ion dose and dose rate both play a significant part in heat damage. Only a

combination of a reduced ion beam current, blur/overlap combination can prevent heat damage which is in good agreement with the COMSOL simulations, the numerical modelling results and the proposed model. Reducing the ion beam current reduces the amount of ion impacts per unit of time, allowing more time for the sample to cool down between impacting ions. Increasing the beam blur (in combination with reducing the overlap) increases the area underneath the beam and therefore reduces the probability of an ion impact in close proximity to the previous impact. The probability of subsequent ion impacts, which leads to accumulative increase in local temperature, is reduced by a factor of r^2 , with r as the ion beam radius. For 3 nA, the time in between ion impacts is too short for the sample to cool down, leading to a large accumulative increase in sample temperature. The calculated dose rate for the different ion beam settings shows the same trend as the experimentally observed heat damage suggesting that heat damage is therefore likely a dose rate effect which is in good agreements with the simulations.

When preparing cross-sections using reduced ion beam currents, the amount of passes is increased (for a constant dwell time) to achieve the desired pattern depth. As each point is scanned several times, the resulting heat damage to the sample can occur via two mechanisms: local heat damage which is induced for each single scanpoint as well as the global heat damage which can occur because the ion beam scans over the pattern area several times (passes). Later can cause a slow but gradual build-up of heat in the sample leading to extended heat damage once a critical threshold is reached as evident in the numerical modelling results (Video 1 in the supporting information). The locally introduced heat is the dominant factor in heat damage and needs to be controlled before the global heat damage can be minimized. The effect of global damage, however, can be seen in Figure 7 where a larger cross-section (larger global dose) was cut using the heat reduced approach 50 pA, 20% overlap, 200 nm blur. A small amount of heat damage is visible in the cross-section on the right. This heat damage could be avoided by introducing a 1 s refresh time. The refresh time corresponds to the time for which the beam is blanked into the column after each pass. This time was sufficient for the

introduced heat to dissipate and to prevent a build-up of heat in the sample allowing to cut a larger cross-section without introducing heat damage. A reduced ion current (local dose rate) per scan point leads to a total increased patterning time since the total global dose, which is required to cut the pattern, remains the same for a given pattern size. A drastic reduction in ion beam current can therefore make large area cross-sectioning unfeasible. To overcome this issue, approaches which reduce the local beam energy dose rate and the global beam energy dose rate could be tried as they would result in reduced ΔE_{phonon} and ΔE_{el} .

To further verify that no heat damage occurs, a TEM lamella was prepared using the heat reduced approach (50 pA, 20% overlap, 200 nm blur) and compared to a conventional microtome section. Both results are shown in Figure 8 (c) - (d). The collagen fibres can clearly be observed in both the FIB/SEM prepared lamella as well as in the microtome prepared section, indicating that heat damage did not occur. The FIB/SEM prepared lamella offers several additional advantages over the conventional microtome section: the area of interest, here collagen fibres, can easily be located, is more readily visible and can be analyzed in a HRTEM (see Figure 8 (a) - (b). The protective platinum layer on top, the back sputtered copper layer at the bottom from the thinning process as well as the large platinum side walls which secure the lamella in the V-shape) form a metallic matrix which appears to have a stabilizing effect. The experimental results obtained from the cross-sectioning and the TEM lamella comparison are in good agreement with the predictions from the simulations.

Conclusions

The underlying principles of ion-solid interactions in focused ion beam systems leading to heat damage were analyzed. SRIM results show that gallium ions lose more than 90% of their initial energy to phonons and ionization, both of which induce heat in the sample. The dominant contributing factors are phonons which are predominantly created by the recoil

cascade and not by the primary ion directly. The COMSOL simulations show that the sample around the ion tracks are heat damaged. These areas, however, are sputtered away and do not represent a problem unless the heat damage extends into the surrounding areas. Global heat damage, which extends into surrounding tissue can be avoided if sufficient time in between subsequent ion impacts is given which allows the sample to cool down as shown in the numerical modelling results. This can be achieved by reducing the ion beam current in addition to blurring the beam. Later creates a spatial spread which reduces the probability of subsequent ion impacts within a small area. The resulting increase in time between ion impacts in the same spot prevents accumulative temperature increases by giving the sample enough time to cool down. For low thermally conductive materials such as soft- and biological materials, the induced changes in sample temperature can be excessive, leading to heat damage when using conventional ion beam parameters. The same set of parameters only produces an insignificant increase in sample temperature for most metals and semiconductors. A model, based on Fourier's law of conductive heat transfer, which incorporates several ion beam parameters was applied to estimate the overall increases in temperature and produces the same qualitative results as the simulations. The theoretically obtained results for silicon and copper were compared to literature and the results for collagen were experimentally verified in cross-sectioning and TEM-lamella experiments. The good agreement with literature for silicon and copper as well as the experimental results for skin/collagen demonstrate that the proposed model allows to estimate the temperature increase and the potential of resulting heat damage for various sample types.

The rise in temperature due to ion solid interaction was evaluated using different approaches, including finite element simulations and 3D, time dependent numerical modelling approaches. The same trends and similar temperatures were calculated using a simple proposed model that was adapted

from Fourier's law. Samples are in general more complex and can be anisotropic. In addition anisotropic materials and different sample topographies are likely to play a crucial role. The effect of sample morphology on heating was observed and further work should be conducted to evaluate this in more detail. Future work should be conducted to allow modelling of more complex heat transfer mechanisms and to evaluate further ion beam parameters which reduce the energy losses to phonons and ionization in the samples. Heat damage is an important subject for focused ion beam work, which needs to be addressed in more detail, especially with the increase in biological and soft materials.

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Table 1: SRIM calculation parameters showing the total energy loss to phonons and ionization per incident ion for 30 keV gallium ions for silicon, copper and skin.

Material	Energy losses to phonons per ion [keV]	Energy losses to ionization per ion [keV]	Energy losses to ionization and phonons per ion [keV]	Ion Range [Å]
Si	16.2	12.3	28.5	279
Cu	22.5	5.2	27.6	103
Skin	19.2	9.6	28.8	515

Table 2: Summary and comparison of ion beam parameters, the predicted increase in temperature and experimentally observed heat damage in Figure 6.

Figure Nr.	Ion beam setting	Dose [$\frac{\text{Ions}}{\text{cm}^2}$]	Heat Flux [μW]	Ion impacts per ns	Dose rate [$\frac{\text{Ions}}{\mu\text{s} \cdot \text{nm}^2}$]	ΔT_{Skin} [$^{\circ}\text{C}$]	Observed heat damage
6(a)	3 nA 50% overlap	3.6×10^{14}	90	18	3.6	2544	yes
6(b)	0.1 nA 50% overlap	1.5×10^{14}	3	0.6	1.5	1233	yes
6(c)	50 pA 20% overlap 50 nm blur	1.1×10^{13}	1.5	0.3	0.16	91	yes

6(d)	3 nA	20%	4.2× 10 ¹³	90	18	0.6	342	yes
	overlap 200 nm blur							
6(e)	0.1 nA	20%	1.4× 10 ¹²	3	0.6	0.02	11.3	Thickness dependent
	overlap 200 nm blur							
6(f)	50 pA	20%	7.0 × 10 ¹¹	1.5	0.3	0.01	5.7	no
	overlap 200 nm blur

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