Background
The purpose of this study was to compare the effects of nJ versus μJ pulse-energy laser cutting in a number of parameters, of which this summary specifically focuses on the initiation of a pro-fibrotic response and apoptosis in the cornea. The FEMTO LDV model Z6 (nJ pulse-energy) and VisuMax 500kHz (μJ pulse-energy) femtosecond laser systems were used. There are several differences between FEMTO LDV and VisuMax femtosecond lasers, namely the scanning pattern, laser spot distance, pulse energy level, and interface of suction cone. These differences may have different implications on post-operative effects such as inflammatory reactions and visual recovery.

Methods
Corneal flaps were created by either VisuMax laser or FEMTO LDV. To investigate the tissue responses to femtosecond laser incision, 8 rabbits were used (three in each laser treatment group and two controls). Corneas were removed at 4 and 24 hours post-operatively, fixed and sectioned for staining. Immunofluorescence staining for fibronectin using mouse monoclonal antibodies was performed on the sections. In addition, TUNEL assay was performed to detect apoptotic cells. To assess the flap bed smoothness, six cadaveric human corneas (two in each group) from donors aged 50 to 74 years were cut then imaged with a scanning electron microscope (SEM). Mean death to enucleation was 6.7 ±1.5 hours and mean death to experiment was 8.0 ± 2.4 days.

Results
Fibronectin was not detected in the central cornea at 4 hours after treatment with VisuMax but was present along the flap interface 24 hours post-operatively. The staining intensity was, however, substantially weaker than that seen in the positive control cornea. In the positive control cornea, weak staining could also be seen posterior to the keratotomy plane. In the FEMTO LDV group, fibronectin was absent in the cornea at 4 and 24 hours after flap preparation. The positive control cornea received a 6-D excimer stromal ablation after the flap was lifted. Here, fibronectin was observed along the excimer ablated stromal plane and in weaker intensity posterior to the ablation plane 24 hours after surgery (Figure 1).

TUNEL positive cells signaling apoptosis were present along the laser incision plane in the central corneal stroma at 4 and 24 hours after treatment with VisuMax, but were largely absent at both time points after flap preparation with FEMTO LDV. At post-operative hour 4, there was a significant difference between the VisuMax (17.33 ± 1.53 cells) and LDV (0.67 ± 0.58 cells) groups (P < 0.001). A significant difference was also found at post-operative hour 24 between the VisuMax (12.67 ± 1.53 cells) and LDV (0.33 ± 0.58 cells) groups (P = 0.002). Apoptotic cells could be observed along the excimer ablated stromal plane at 24 hours after surgery in the positive control cornea.

The smoothness of human corneal central flap beds visualized by SEM appeared similar for both VisuMax and LDV groups. Observer 1 scored the VisuMax group 8.00 ± 1.00 and the LDV group 7.33 ± 0.58 (P = 0.387); observer 2 scored the VisuMax group 8.33 ± 0.58 and the LDV group 7.67 ± 0.58 (P = 0.230). Both observers agreed that there was no significant difference in the mean irregularity score of the flap bed comparing the VisuMax and LDV groups.

Figure 1. Expression of fibronectin in the central cornea after flap creation with VisuMax and FEMTO LDV femtosecond lasers. A) Fibronectin staining 4 h and, B) 24 h after VisuMax treatment. C) Fibronectin staining 4 h and, D) 24 h after LDV treatment. E) Positive control. Scale bar: 100 μm.
Conclusions

The pro-fibrotic protein fibronectin was expressed along the incision plane with cuts from the VisuMax laser but not the LDV. In addition, the LDV also produced significantly less cell death in the corneal stroma when compared to the VisuMax incision as assessed by TUNEL assay. It could be possible that the lower energy pulses (nJ range) produced by the FEMTO LDV attributed to the above observations. Finally, the smoothness of the flap was not found to be significantly different between the LDV and VisuMax groups as assessed by two independent observers.

In conclusion, nJ energy lasers such as the LDV may be a better choice for ophthalmic operations which require corneal incisions as they may result in reduced cell death and reduced pro-fibrotic protein expression in the corneal stroma.

References:

Fig 2. Apoptotic cells in cornea following laser treatment.