Detection of carbon monoxide from biological tissue using difference frequency generation in periodically-poled lithium niobate near 4.6 μm

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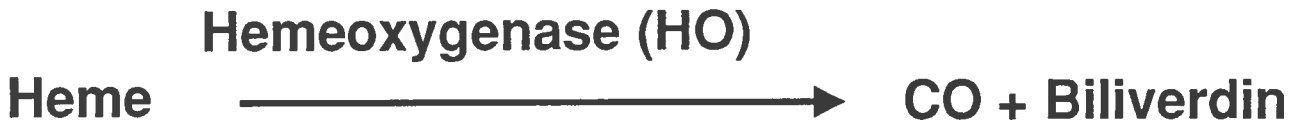
Biology of CO

Primary mechanism for production of cellular CO



Biological role of CO

- **Modulator of cyclic GMP** (Science 259:1993, 381)
- ~~**Endothelial derived relaxation**~~ **of vascular smooth muscle** (Circulation 91:1995, 2306)

  Relaxation factor
- **Inhibition of platelet reactivity** (J. Clin. Invest. 100:1997, 589)
- **Intercellular messenger in the brain** (Nature 364:1993, 147)

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Current available techniques for sub-ppb level detection of CO

Technique	Advantage	Disadvantage
Gas chromato-graphy	• High sensitivity (~ 20 ppb) • Relatively fast (~10 min) • Requires small sample (~100 μl) • Highly accurate quantitative (1- 5%)	• Easily interfered by humidity and ambient air • Relatively complicated procedure • Indirect measurement
Radioisotope using ^{14}C	• Extremely high sensitivity (sub-ppb)	• Slow analysis (~ hours) • Complicated procedure • Difficult for continuous detection

Principle of DFG-based mid-infrared spectroscopy

Difference Frequency Generation (DFG)

$$\omega_i = \omega_1 - \omega_2$$

Advantages of DFG-based spectroscopy

- High sensitivity (~20 ppb) and accuracy (~ 5%)
- Not affected by humidity and ambient air including CO_2
- Fast analysis (~secs)
- Simple and easy analysis because of direct absorptiometry
- Direct detection without chemical reactions

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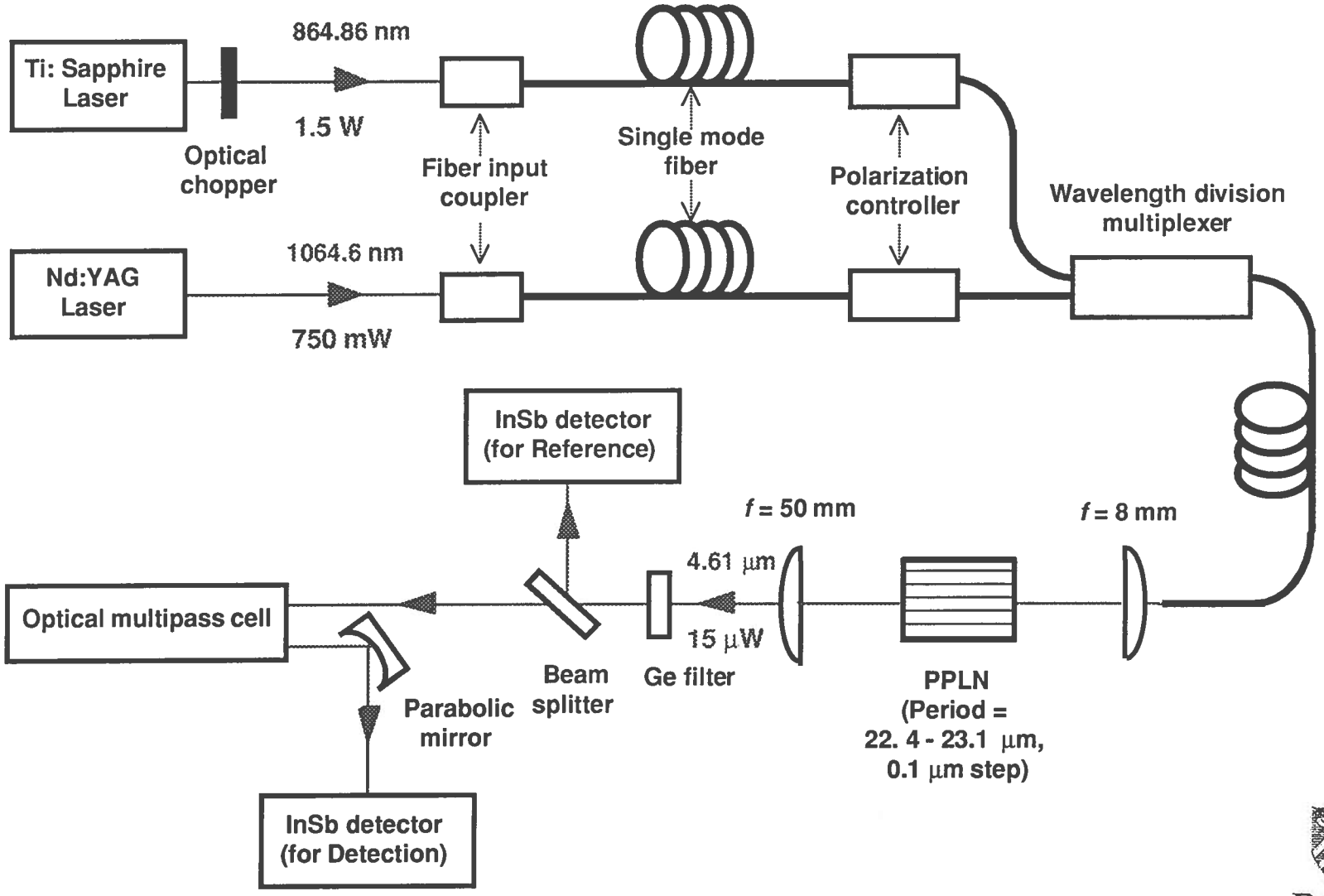
Optical arrangement of DFG-based sensor



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Cell culture and its treatment

- Material: Rat vascular smooth muscle cells (10^7)
- Treatment: Hemin (20 μM)
- Kinetics:

$$\text{Heme} \xrightarrow{\text{Hemeoxygenase (HO)}} \text{CO} + \text{Biliverdin}$$

- Preparation: Two 250 ml-Flask; Cells + Medium

Cells + Medium + Hemin

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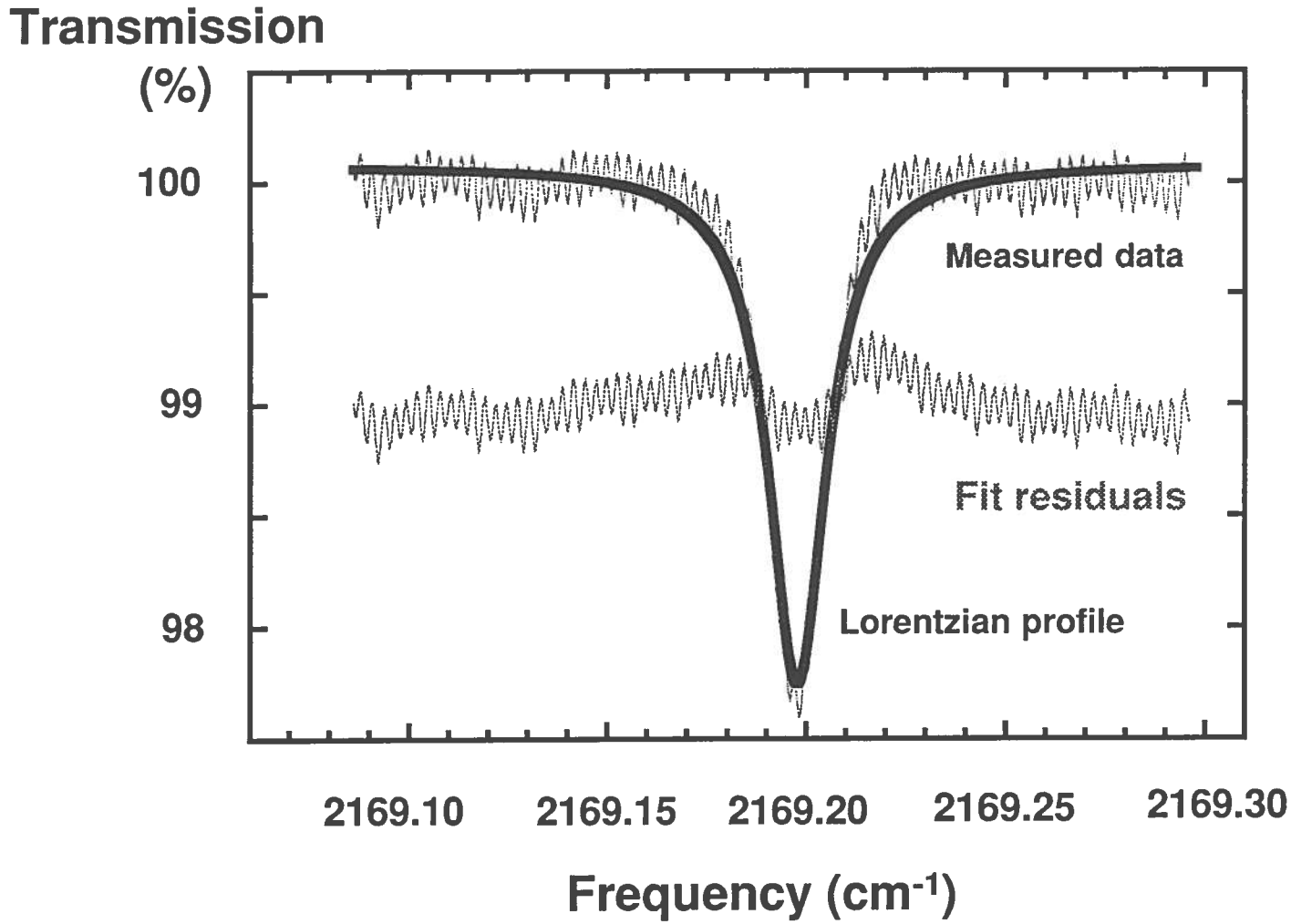
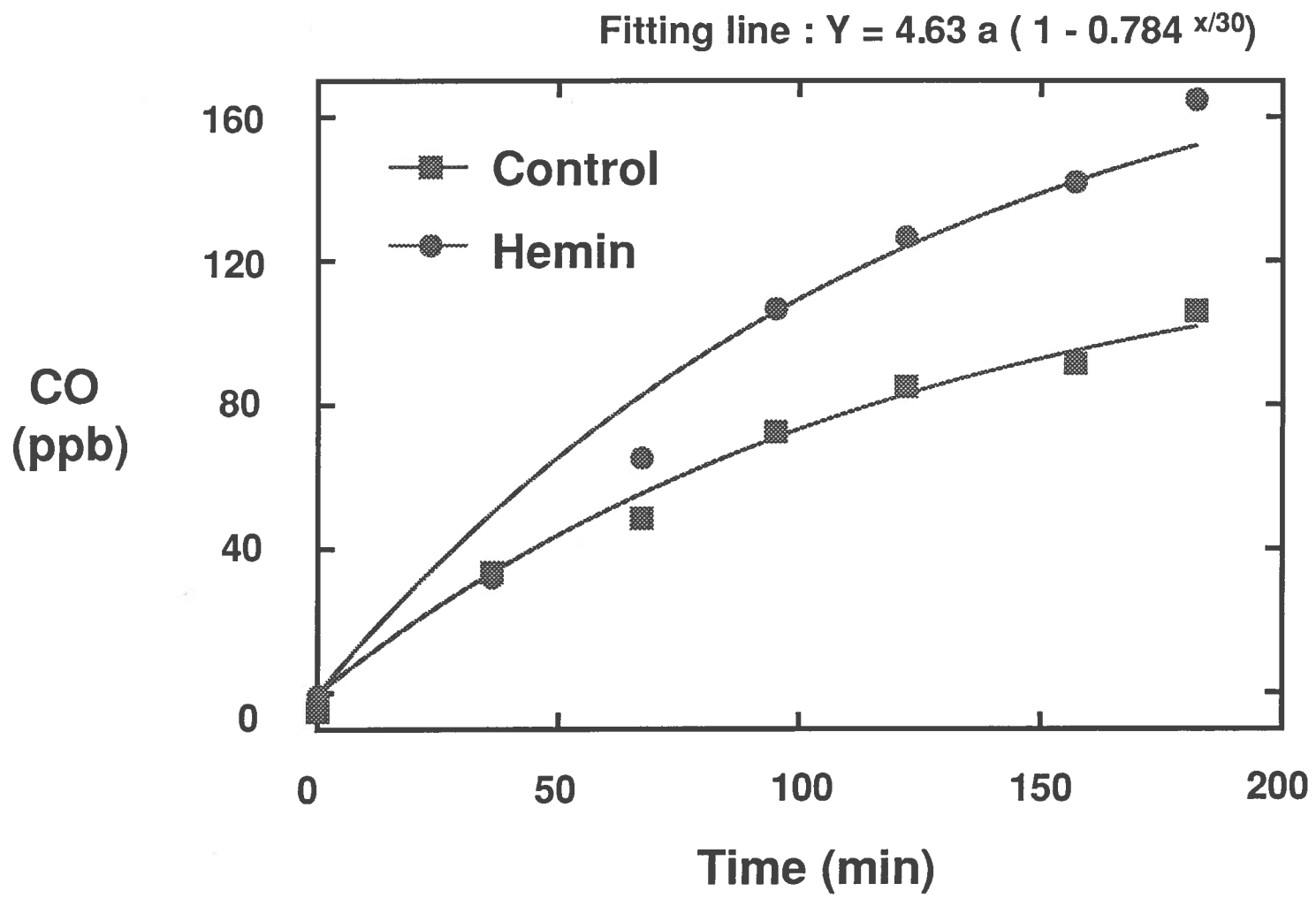
CO spectrum at 2169.2cm^{-1} at 100 Torr



CO production from vascular smooth muscle cells



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CO production rates from biological materials

CO production rate ($x10^2$ pmol/ 30 min)/ Treatment	Tissue/ Material / Amount	Method	Reference
4.4 / with Heme	Rat aorta/ homogenate/ 1 mg protein	Gas chromatography with photometric detector	Cook, 1995
11 / with Heme + NADPH	Rat aorta/ homogenate/ 1 mg protein	Gas chromatography with flame-ionization detector	Grundemar, 1995
8.6 / (-)	Rat olfactory bulb/ culture cells/ 33 mg protein (presumably 10^7 cells)	Radioisotope	Ingi, 1996
0.75-2.8 / with Heme	Guinea pig brain/ homogenate/ 1 mg microsome protein	Gas chromatography with photometric detector	Cook, 1996
2.8 / (-) 4.2 / with Heme	Rat vascular smooth muscle/ culture cells/ 10^7 cells	DFG-based mid-infrared spectroscopy	Present results

Future directions

Enhanced sensitive and faster detection

A CO sensitivity of 1 ppb, and detected response time of 10 s

Measurement of nitric oxide (NO) from biological cells

Measurement of NO (5.3 μm) is an expertise challenge. Present sensitivity for NO is ~0.5 ppm level, because of low DFG efficiency (< 5%) at 5.3 μm and reduced NO absorption strength. However, DFB-QC laser will access NO.

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Conclusion

Using a mid-infrared gas sensor based on difference frequency generation, we detected the CO generation at levels of 280 pmols/ 30 min from vascular smooth muscle cells (10^7) in the basal state.

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